DOI: 10.1002/ejoc.200900383

Fluorescent Cyclohexyl-Based Chemosensors for Selective Sensing of TMA Malonate in DMSO/Water

Ana M. Costero,*[a] Josep V. Colomer,[a] Salvador Gil,[a] and Margarita Parra[a]

Keywords: Host-guest systems / Molecular recognition / Fluorescent probes / Sensors / FRET (Fluorescence Resonance Energy Transfer)

Two new cyclohexyl-based fluorescent thioureas have been synthesized. The prepared ligands contain either a fluorescein or a rhodamine B moiety that are used as signalling units in dicarboxylate recognition. An equimolecular mixture of both ligands selectively recognize malonate in a competitive buffered medium. An inhibition of FRET as a result of the complex geometry might be proposed as a transduction mechanism in the sensing process.

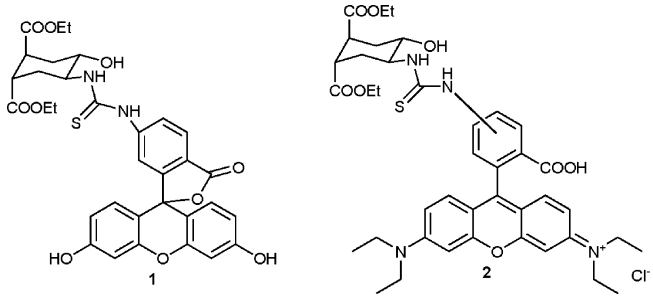
Introduction

The demand for chemosensors that are selective for specific anions is continuously increasing.[1] Especially important in this regard are sensors that monitor small organic anions of biological interest. Among these compounds α,ω-dicarboxylates can be included because of the different roles they play in different biochemical process.[2] Thus, whereas succinate participates in the Krebs cycle, malonate or oxalate can inhibit the action of succinyl dehydrogenase.[3] On the other hand, adipate and glutarate are intermediates in the degradation of nitrogen heterocyclic compounds.[4]

During the last years, we have been interested in the use of cyclohexane systems containing naphthylthiourea groups to design fluorescent chemosensors for anions and, more specifically, for dicarboxylates. Some of the prepared compounds exhibit sensing selectivity and are able to discriminate α,ω-dicarboxylates depending on their carbon chain length. With the studied ligands, complexes with a 2:1 stoichiometry are formed that induce intermolecular excimers with some specific α,ω-dicarboxylates.[5]

Results and Discussion

In order to increase sensing sensitivity and to explore new possible transduction mechanisms (for example, the FRET process), two new cyclohexyl derivatives have been prepared (Scheme 1). In these new compounds, the naphthyl moiety has been substituted by fluorescein and rhodamine B, respectively. Fluorescein is one of the most commonly used fluorophores because of its high molar absorptivity, large fluorescence quantum yield and high photostability. However, the photophysical properties of fluorescein are strongly dependent on pH, and for this reason anion recognition experiments must be carefully designed to distinguish between real complexation processes and mere acid–base reactions. Rhodamine derivatives have also been used in sensing and recognition processes.[6] Ligands containing rhodamine are less dependent on pH and present high quantum yield values.



Scheme 1.

In addition, the described ligands could be useful for exploring the use of resonance energy transfer (RET or FRET) as a transduction mechanism in a similar way to that described by Rudkeyich et al.[7] FRET relies on the distance-dependent transfer of energy from a donor fluorophore to an acceptor fluorophore. In FRET, the donor fluorophore is excited by incident light, and if the acceptor is in close proximity, the excited state energy from the donor can be transferred. This leads to a reduction in the fluorescence intensity of the donor and an increase in the emission intensity of the acceptor.[8] Even though this phenomenon has largely been used in biochemical applications,[9] there

[a] Instituto de Reconocimiento Molecular y Desarrollo Tecnológico, Centro Mixto Universidad Politécnica de Valencia – Universidad de Valencia,
Dr. Moliner 50, 46100 Burjassot, Valencia, Spain
Fax: +34-96-3543831
E-mail: Ana.Costero@uv.es
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.200900383.

are only a few number of publications related to cation sensing[10] and even less for anion species.[11] Here, we report on the utility of ligands **1** and **2** (Scheme 1) and their use to discriminate between malonate and its longer and shorter homologues in a very competitive medium [DMSO/water (2:1)] through inhibition of the FRET effect.

Ligands **1** and **2** in their racemic form were easily prepared from (±) *trans*-*transoid*-*trans*-5-amino-1,2-bis-(ethoxycarbonyl)-4-hydroxycyclohexane and fluorescein 5-isothiocyanate and rhodamine B isothiocyanate, respectively. The reaction conditions were the same as those previously described for related compounds.[12] The fluorescent properties of ligands **1** and **2** were studied in 10^{-5} M buffered [10^{-2} M 2-morpholinoethanosulfonic acid (MES)] DMSO/water (2:1) solutions (Figure 1).

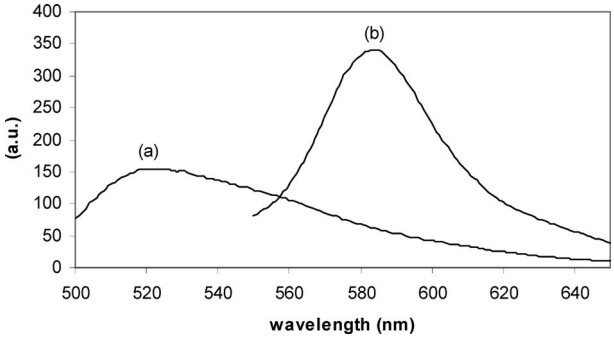


Figure 1. Fluorescence spectra of ligands (a) **1** and (b) **2** [10^{-5} M in 10^{-2} M MES DMSO/water (2:1) solutions] (λ_{exc} = 488 nm and 540 nm, respectively, slits = 5).

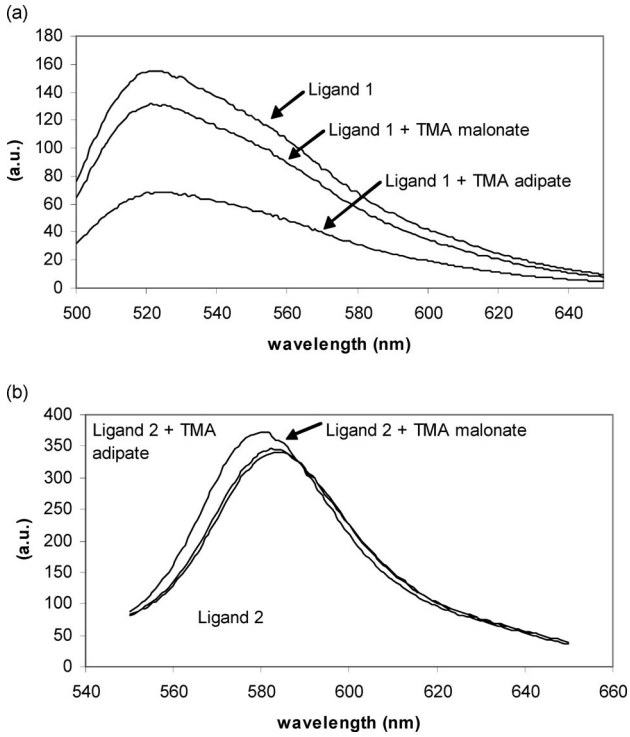


Figure 2. (a) Fluorecence spectra of ligand **1** and ligand **1** in the presence of 4 equiv. TMA malonate and adipate and (b) fluorescence spectra of ligand **2** and ligand **2** in the presence of 4 equiv. TMA malonate and adipate [in all cases 10^{-5} M in 10^{-2} M MES DMSO/water (2:1) solutions] (λ_{exc} = 488 nm for ligand **1** and 540 nm for ligand **2**, slits = 5).

Ligand **1** shows an emission band with a maximum at 520 nm (λ_{exc} = 488 nm), which is consistent with spectral contributions arising from both the monoanion and the dianion species.[13] As was expected, the intensity of the emission is moderate because of the presence of the thiourea group.[14] On the other hand, ligand **2** shows an emission maximum at 585 nm (λ_{exc} = 540 nm); however when λ_{exc} = 488 nm, its emission intensity is practically negligible.

Complexation with different dicarboxylates (adipate, glutarate, succinate, malonate and oxalate), all as their trimethylammonium (TMA) salts, were carried out in buffered solutions to avoid acid–base reactions. The obtained results for malonate and adipate are shown in Figure 2(a) for ligand **1** and Figure 2(b) for ligand **2**.

In the presence of a dicarboxylate, ligand **1** suffers a quenching of the fluorescence that is higher for adipate than for malonate. This behaviour is not related to an acid–base reaction, because when we studied the sensing of carboxylates in nonbuffered solutions, we always observed a strong enhancement of the fluorescence. Figure 2(b) shows that the effect in ligand **2** is smaller, in any case, malonate induce a small enhancement of the fluorescence with a concomitant blueshift of the maximum ($\Delta\delta$ = 3 nm).

In order to explore the possible use of FRET as a transduction mechanism, solutions of equimolecular mixtures of ligands **1** and **2** were prepared, and their photophysical properties studied. Figure 3 shows the fluorescence spectrum of a DMSO/water (2:1) buffered solution (10^{-2} M MES) of both ligands **1** and **2** (10^{-5} M) [λ_{exc} = 488 nm]. As can be seen, the emission band at 520 nm, characteristic of ligand **1**, is in this case clearly smaller, whereas the emission at 585 nm, which corresponds to ligand **2**, appears even though the excitation was carried out at 488 nm. This behaviour could be related with a FRET process in which the energy absorbed by ligand **1** is transferred to ligand **2**, which emits at its characteristic wavelength. Energy transfer does not require physical contact of the interacting partners but a close proximity of both donor and acceptor is neccesary.[15] The strong tendency of thiourea derivatives to form aggregates could play an essential role in the observed behaviour for mixtures of ligands **1** and **2**.[16] By titration, a 1:1 stoichiometry ($\log\beta$ = 5.5 + 0.7)[17] was determined for the aggregation of these ligands in (10^{-2} M MES) DMSO/water (2:1) solutions.

To study the possible use of this system in the selective sensing of α,ω-dicarboxylates, studies with these species were carried out under the established conditions (MES, 10^{-2} M in DMSO/water, 2:1). Sensing studies were performed with oxalate, malonate, succinate, glutarate and adipate, all as their TMA salts, and a different behaviour was observed depending on the dicarboxylate used. Further, experiments with TMAOH were carried out to confirm that no acid–base reactions were operating under these conditions.

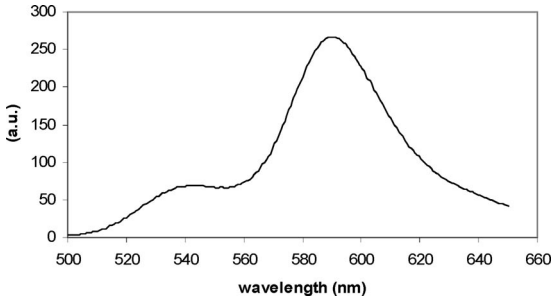


Figure 3. Fluorescence spectrum of a DMSO/water (2:1) buffered solution (10^{-2} M MES) of both ligands **1** and **2** (10^{-5} M) [λ_{exc} = 488 nm].

Oxalate, succinate, glutarate and adipate induced no appreciable changes in the fluorescence spectrum (Figure 4 for adipate and malonate), which suggests that the FRET process continued to be operative after anion complexation, unlike malonate, which gave rise to a completely different spectrum. With this anion, a spectacular recovery of ligand **1** emission (λ = 520 nm) was observed after excitation at 488 nm. Tritation experiments carried out with TMA

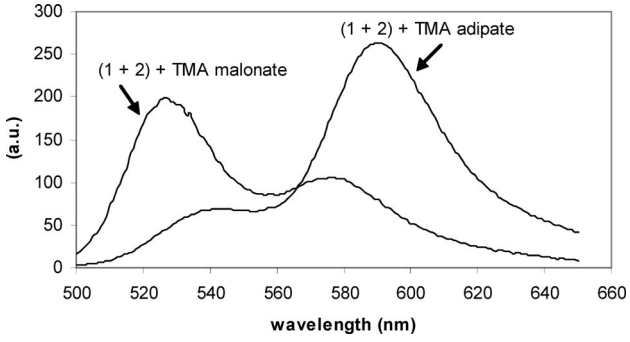


Figure 4. Fluorescence spectrum of a DMSO/water (2:1) buffered (10^{-2} M MES) solution of both ligands **1** and **2** (10^{-5} M) [λ_{exc} = 488 nm] with 4 equiv. TMA malonate and with 4 equiv. TMA adipate.

adipate and malonate demonstrated that the stoichiometry of the complexes is in both cases L_2A ($\log\beta$ 11.2 ± 0.5 for adipate and 8.7 ± 0.9 for malonate[17]). In addition, TMAOH did not induce any modification in the emission spectrum. This selective behaviour of TMA malonate could even be detected by the naked eye, as can be seen in Figure 5.

Figure 5. Equimolecular mixture of ligands **1** and **2** (10^{-5} M in each ligand) and 10^{-2} M in MES (DMSO/water 2:1), free and in the presence of the different anions.

The results obtained could agree with the inhibition of the FRET phenomenon after the addition of TMA malonate. The FRET process not only depends on the distance between the donor and acceptor moieties, but also on the spatial arrangement of the groups. A parallel disposition of the participant dipoles leads to the maxima energy transfer. This disposition can be adopted either in the fundamental or in the excited state.[18] Thus, if the formed mixed complex (**1**-anion-**2**) is rigid enough to preclude the appropriate orientation, the FRET process would be inhibited. Even though complexation induces a change in the donor–acceptor distance with any of the studied anions, malonate might give rise to a significant change in the relative spatial position of both fluorophores. As Figure 6 depicts, the thiourea groups from rhodamine and fluorescein in the **1**–**2** dimer are in the same plane. The same orientation is expected for those complexes formed with adipate, glutarate and suc-

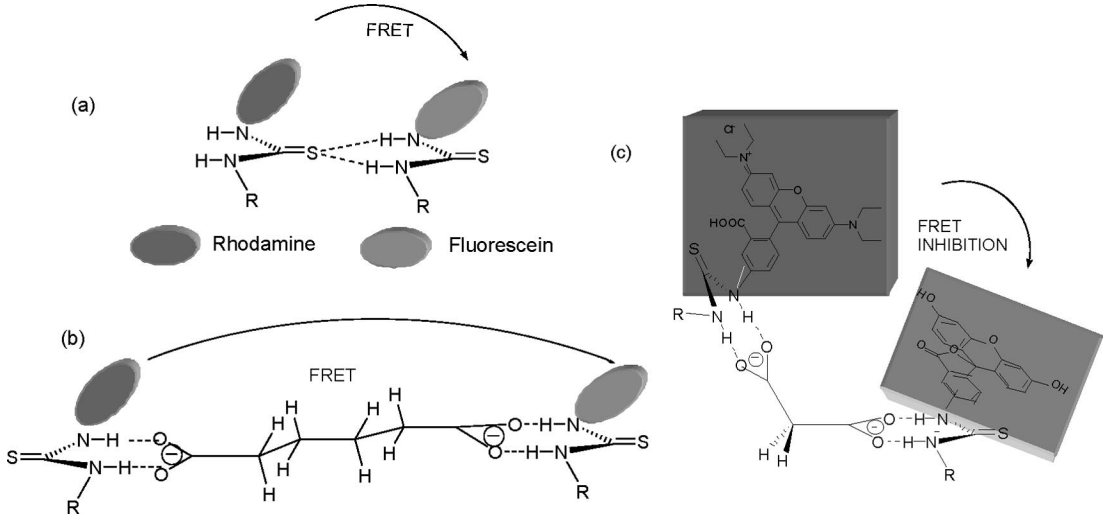


Figure 6. Optimized geometries for assembly of ligands **1** and **2** and their complexes with carboxylates (a) dimer **1**–**2**, (b) complex with adipate and (c) complex with malonate.

cinate; however, with malonate another geometry, one with both fluorophores in almost orthogonal planes (Figure 6), seems to be more favourable.[19] By taking into acount that the $S_0 \rightarrow S_1$ transition moments of both fluorescein[20] and rhodamine B[21] derivatives are parallel to the long axis of the xanthene ring system, it is expected that these transition moments are in orthogonal planes in the complex with malonate.

To confirm the feasibility of this hypothesis, studies of sensing with the 1,1-cyclobutanedicarboxylic TMA salt were carried out. With this anion, in which both carboxylate groups form a similar angle to that proposed for malonate, FRET inhibition was also observed.

Conclusions

The system formed by an equimolecular mixture of ligands **1** and **2** is able to act as a selective chemosensor for malonate rather than for its longer and shorter homologues. FRET inhibition, for geometrical reasons, seems to be a successful transduction process for designing chemosensors by following the binding-site–signalling-unit approach. Finally, the sensing process takes place in a very competitive and buffered medium (DMSO/water 2:1) and can be observed by the naked eye.

Experimental Section

Synthesis of Compounds 1 and 2 – General Procedure: Fluorescein isothiocyanate or rhodamine B isothiocyanate (available from Aldrich as mixtures of isomers ref. 283924) (4.5 mmol) were added dropwise to a solution of *trans*-*transoid*-*trans*-5-amino-1,2-bis-(ethoxycarbonyl)-4-hydroxycyclohexane (4.5 mmol) in THF (15 mL), and the resulting solution was heated at reflux for 16 h. The mixture was then cooled to room temperature and was poured over hexane (25 mL), to yield **1** or **2** (mixture of isomers) as an orange and dark red precipitates, respectively (47.4% for **1** and 89.7% for **2**). Ligand **1**: orange solid. M.p. 202–204 °C. IR (KBr): $\tilde{\nu}$ = 2982, 1738, 1592, 1464, 1208, 1180, 1109, 850 cm^{-1}. ^1H NMR (500 MHz, [D$_6$]DMSO, 25 °C): δ = 9.6 (br. s, 1 H, NH), 8.25 (br. s, 1 H), 7.75 (m, 1 H), 7.17 [d, ^4J(H,H) = 2.2 Hz, 1 H], 6.68–6.56 (m, 9 H), 5.26 (s, 1 H, OH), 4.47 (m, 1 H), 4.01 (m, 4 H), 3.92 (m, 1 H), 2.95 (m, 1 H), 2.93 (m, 1 H), 1.95 (m, 3 H), 1.88 (m, 1 H), 1.77 (m, 6 H) ppm. ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): δ = 173, 169, 160, 152, 147.5, 142, 130, 129.5, 126.5, 124, 118, 113, 110.2, 102.6, 68.8, 50.3, 46.1, 31.4, 24.4, 14.2, 14.0 ppm. UV/Vis (DMSO, 10^{-5} M): λ_{max} (ε, L mol^{-1} cm^{-1}) = 260 (22080), 272 (16550), 482 (6540), 521 (16550) nm. Fluorescence λ_{em} = 520 nm, λ_{exc} = 488 nm. FAB-HRMS calcd. for $C_{33}H_{33}N_2O_{10}S$ 649.1856; found 649.1854. Ligand **2**: purple solid. IR (KBr): $\tilde{\nu}$ = 3419, 2975, 2938, 1592, 1475, 1434, 1398, 1339, 1181, 1036 cm^{-1}. ^1H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 10.3 (br. s, 1 H), 10.1 (br. s, 1 H), 8.9 (m, 1 H), 7.9 (m, 1 H), 7.6 (m, 1 H), 6.7–6.6 (m, 3 H), 6.3 (s, 2 H), 6.0 (br. s 2 H), 4.0–4.2 (m, 5 H), 3.2 (m, 1 H), 2.9 (m, 8 H), 2.1 (m, 2 H), 1.9 (m, 2 H), 1.7 (m, 2 H), 1.3 (t, J = 7.2 Hz, 6 H), 1.1 (m, 12 H) ppm. UV/Vis (DMSO, 10^{-5} M): λ_{max} (ε, L mol^{-1} cm^{-1}) = 275 (17463), 555 (1664) nm. Fluorescence λ_{em} = 585 nm, λ_{exc} = 540 nm. FAB-HRMS calcd. for $C_{41}H_{51}N_4O_8S$ 759.9419; found 760.0126.

Supporting Information (see footnote on the first page of this article): UV spectra and excitation spectra of ligands

Acknowledgments

The present research was financed by Spanish DGCYT (CTQ2006-15456-C04-02/BQU) and Generalitat Valenciana (Acciones Complementarias ACOMP07/080). SCSIE (Universidad de Valencia) is gratefully acknowledged for all the equipment employed.

[1] a) P. A. Gale, *Acc. Chem. Res.* **2006**, *39*, 465–475; b) E. A. Katayev, Y. A. Ustynyuk, J. L. Sessler, *Coord. Chem. Rev.* **2006**, *250*, 3004–3047; c) A. P. Davis, *Coord. Chem. Rev.* **2006**, *250*, 2939–2951; d) F. P. Schmidtchen, *Coord. Chem. Rev.* **2006**, *250*, 2918–2928; e) J. Yoon, S. K. Kim, N. J. Singh, K. S. Kim, *Chem. Soc. Rev.* **2006**, *35*, 355–360; f) K. Bowman-James, *Acc. Chem. Res.* **2005**, *38*, 671–678; g) R. Martínez-Máñez, F. Sancenón, *Chem. Rev.* **2003**, *103*, 4419–4476; h) C. Suksai, T. Tuntulani, *Chem. Soc. Rev.* **2003**, *32*, 192–202; i) J. L. Sessler, S. Camiolo, P. A. Gale, *Coord. Chem. Rev.* **2003**, *240*, 17–55; j) P. A. Gale, *Coord. Chem. Rev.* **2003**, *240*, 167–189; k) P. A. Gale, *Coord. Chem. Rev.* **2001**, *213*, 79–128; l) F. P. Schmidtchen, M. Berger, *Chem. Rev.* **1997**, *97*, 1609–1646.

[2] a) S. Carvalho, R. Delgado, N. Fonseca, V. Felix, *New J. Chem.* **2006**, *30*, 247–257; b) S.-Y. Liu, Y.-B. He, J.-L. Wu, L.-H. Wei, H.-J. Qin, L.-Z. Meng, L. Hu, *Org. Biomol. Chem.* **2004**, *2*, 1582–1586; c) N. Singh, G. W. Lee, D. O. Jang, *Tetrahedron* **2008**, *64*, 1482–1486.

[3] a) J. Stepinski, D. Pawlowska, S. Angielski, *Pol. Dev. Nephrol.* **1985**, *10*, 171–176; b) A. Gougoux, G. Lemieux, N. Lavoie, *Am. J. Physiol.* **1976**, *231*, 1010–1017.

[4] B. Combourieu, P. Besse, M. Sancelme, J.-P. Godin, A. Monteil, H. Veschambre, A.-M. Delort, *Appl. Environ. Microbiol.* **2000**, *66*, 3187–3193.

[5] a) A. M. Costero, M. Colera, P. Gaviña, S. Gil, *Chem. Commun.* **2006**, 761–763; b) A. M. Costero, M. Colera, P. Gaviña, S. Gil, M. Kubiny, K. Pál, M. Kállay, *Tetrahedron* **2008**, *64*, 3217–3224.

[6] a) R. L. Halterman, J. L. Moore, L. M. Mannel, *J. Org. Chem.* **2008**, *73*, 3266–3269; b) S. Kou, H. N. Lee, D. van Noort, K. M. K. Swamy, S. H. Kim, J. H. Soh, K.-M. Lee, S.-W. Nam, J. Yoon, S. Park, *Angew. Chem. Int. Ed.* **2008**, *47*, 872–876.

[7] H. Zhang, D. M. Rudkeyich, *Chem. Commun.* **2007**, 1238–1239.

[8] a) K. E. Sapsford, L. Berti, I. L. Medintz, *Angew. Chem. Int. Ed.* **2006**, *45*, 4562–4588; b) K. Okamoto, M. Terazima, *J. Phys. Chem. B* **2008**, *112*, 7308–7314; c) D. Magde, *Photochem. Photobiol.* **2002**, *75*, 327–334.

[9] a) M. Numata, K. Koumoto, M. Mizu, K. Sakurai, S. Shinkai, *Org. Biomol. Chem.* **2005**, *3*, 2255–2261; b) H. Takakusa, K. Kikuchi, Y. Urano, S. Sakamoto, K. Yamaguchi, T. Nagano, *J. Am. Chem. Soc.* **2002**, *124*, 1653–1657; c) I. Horsey, W. S. Furey, J. G. Harrison, M. A. Osborne, S. Balasubramanian, *Chem. Commun.* **2000**, 1043–1044.

[10] a) S. A. Ahmed, M. Tanaka, H. Ando, K. Tawa, K. Kimura, *Tetrahedron* **2004**, *60*, 6029–6036; b) S. H. Lee, S. K. Kim, J. H. Bok, S. H. Lee, J. Yoon, K. Lee, J. S. Kim, *Tetrahedron Lett.* **2005**, *46*, 8163–8167; c) F. He, Y. Tang, S. Wang, Y. Li, D. Zhu, *J. Am. Chem. Soc.* **2005**, *127*, 12343–12346; d) M. H. Lee, H. J. Kim, S. Yoon, N. Park, J. S. Kim, *Org. Lett.* **2008**, *10*, 213–216; e) A. B. Othman, J. W. Lee, J.-S. Wu, J. S. Kim, R. Abidi, P. Thuéry, J. M. Strub, A. Van Dosselaer, J. Vicens, *J. Org. Chem.* **2007**, *72*, 7634–7640.

[11] D. A. Jose, D. K. Kumar, P. Kar, S. Verma, A. Ghosh, B. Ganguly, H. N. Ghosh, A. Das, *Tetrahedron* **2007**, *63*, 12007–12014.

[12] A. M. Costero, M. Colera, P. Gaviña, S. Gil, *Tetrahedron* **2008**, *64*, 7252–7257.

[13] N. Klonis, W. H. Sawyer, *Photochem. Photobiol.* **2000**, *72*, 179–185.

[14] N. Klonis, W. H. Sawyer, *Photochem. Photobiol.* **2003**, *77*, 502–509.
[15] A. Kurian, S. D. George, V. P. N. Nampoori, C. P. G. Vallabhan, *Spectrochim. Acta A* **2005**, *61*, 2799–2802.
[16] J. Valdés-Martínez, S. Hernández-Ortega, M. Rubio, D. T. Li, J. K. Swearingen, W. Kaminsky, D. R. Kelman, D. X. West, *J. Chem. Crys.* **2004**, *34*, 533–540.
[17] *SPECFIT/32TM GLOBAL ANALYSIS SYSTEM v.3.0*, Spectrum Associates, Marlborough, MA, USA, www.bio-logic.info/rapid-kinetics/specfit.html.
[18] H. Langhals, S. Poxleitner, O. Krotz, T. Pust, A. Walter, *Eur. J. Org. Chem.* **2008**, 4559–4562.
[19] *PCModel 8.0*, Molecular modelling software for personal workstation, Serena Software, Bloomington, IN, http://www.serenasoft.com/pcm8.html.
[20] V. Tsukanova, A. Harata, T. Ogawa, *Langmuir* **2000**, *16*, 1167–1171.
[21] K.-I. Ishibashi, O. Sato, R. Baba, D. A. Try, K. Hashimoto, A. Fujishima, *J. Colloid Interface Sci. Langmuir* **2001**, *233*, 361–363.

Received: April 8, 2009
Published Online: June 22, 2009

