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Synthesis and study of a cyclophane displaying dual fluorescence emission: a novel ratiometric sensor for carboxylic acids in organic medium

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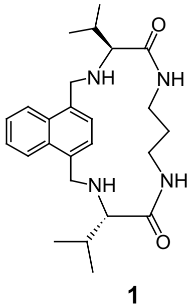
Abstract—The synthesis and fluorescent properties of a novel cyclophane containing two L-valine units and one naphthalene chromophore are described. Synthesis of the macrocycle **1** was accomplished without using high-dilution methods in moderate to high yields. The fluorescence spectrum of **1** in neutral dichloromethane shows a band at 390 nm attributable to emission from an exciplex formed between the naphthalene unit and the neighboring amine groups. Addition of trifluoroacetic acid restores the typical naphthalene emission at 330 nm. Due to the fact that both emissions have similar intensities under the working conditions, the ratio between them can be used to obtain a ratiometric response to carboxylic acids in organic medium.

In recent years the development of fluorescent chemosensors has received great attention due in part to the important applications found in the understanding of biological phenomena.[1] Such sensors consists basically of: (1) a binding unit, capable of recognizing selectively a target analyte, (2) a fluorescent chromophore that is sensitive to the presence or absence of the target, and (3) a suitable linker to modulate the interaction between the recognizing and signaling units. However, the general protocol for the utilization of a sensor requires a comparison of the intensity of the signal obtained from the analyzed sample with that of a known sample in order to take into account the experimental conditions (calibration). Thus, it is desirable that the information provided by the sensor should not depend on external factors but only on its intrinsic properties. In order to circumvent this inconvenience several ratiometric fluorescent probes have been developed in recent years for the sensing of calcium,[2] lead,[3] zinc,[4] hydrogencarbonate,[5] β-lactamase,[6] phosphodiesterase,[7] and others.[8,9] A ratiometric probe involves measurements at two different wavelengths, and thus, the ratio of both values becomes independent of the light source, the sample thickness, photobleaching of the sensor, etc.

Here we report the synthesis of a fluorescent cyclophane **1** based on naphthalene and the amino acid valine as building blocks, which is able to bind to carboxylic acids in organic media and to change its emission in a ratiometric fashion. This system displays dual emission at 330 and 390 nm when excited at 300 nm in dichloromethane, which makes it 'internally calibrated' for the study of the association with carboxylic acids by means of steady-state fluorescence spectroscopy.



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Keywords: Macrocycles; Amino acids; Amino amides; Fluorescence; Exciplex; Sensors; Supramolecular; Assembly; Ratiometric; Probes; Enantioselectivity.

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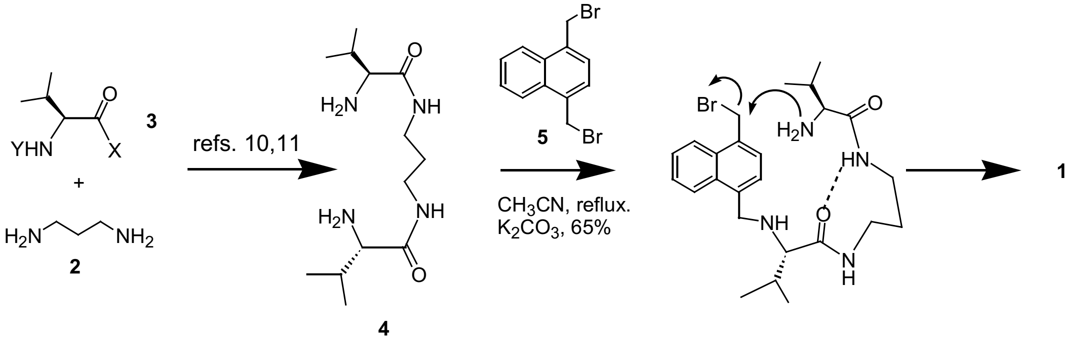


Scheme 1.

The cyclophane **1** was synthesized following a route similar to that developed by our group for analogous systems.[10,11] 1,3-Diaminopropane **2** was reacted with the N-protected L-valine through the formation of the *N*-hydroxysuccinimide ester (**3**, Y = Cbz, X = $ONC_4H_4O_2$) (Scheme 1). The yield of the intermediate peptidomimetic compound **4** was ca. 70%. The cyclization step was accomplished using 1,4-bis(bromomethyl)naphthalene **5** under mild conditions (refluxing acetonitrile as solvent and anhydrous K_2CO_3 as the base). The yield of the cyclization step to afford **1** was 65% (after purification by column chromatography), which can be considered as moderate to high taking into account that no high dilution conditions were employed (typical concentration of the reactants were ca. 10^{-3} M).[12]

This synthetic efficiency is in sharp contrast with the difficulties that has been described for the preparation of polyazanaphthalenophanes and anthracenophanes, which required the use of very specific protecting groups and reaction conditions.[13] The success in this kind of cyclization reactions has been shown to be associated with an appropriate preorganization of the open-chain intermediate via intramolecular hydrogen-bonding and solvophobic interactions.[11b]

The fluorescence emission of **1** was recorded in freshly distilled dichloromethane as solvent, exciting at $\lambda = 300$ nm.[14] Since the only emitting chromophore in **1** is the naphthalene unit, it would be expected, in principle, to emit at around 330 nm,[15] but the registered band was red shifted 60 nm (at 390 nm). The excitation spectrum corresponding to the emission at 390 nm showed that the origin of the band is the naphthalene chromophore. The addition of trifluoroacetic acid to the system decreased the intensity of the long-wavelength band and led to the appearance of a short-wavelength band at 330 nm (Fig. 1).

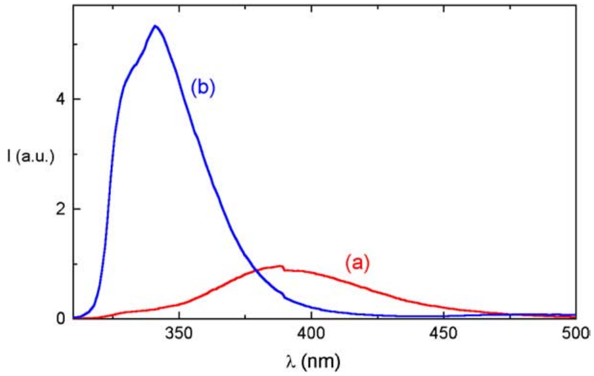
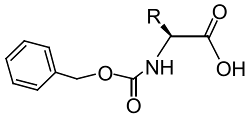


Figure 1. (a) Uncorrected emission spectra of **1** in dichloromethane (1.3×10^{-4} M) and (b) in the presence of 0.1 M CF_3COOH. Excitation wavelength: 300 nm.

The behavior in neutral conditions could be interpreted as photoinduced electron-transfer (PET) quenching of the fluorescence of the naphthalene by the lone pairs of the neighboring amines, preventing emission from the aromatic moiety at 330 nm. Addition of acid would lead to coordination of the amine and restoration of the typical emission of the naphthalene chromophore.[16] In fact, the recorded fluorescence spectrum of 1,4-dimethylnaphthalene in dichloromethane matched that of coordinated **1**. A remarkable feature of **1** is that the quenching of the fluorescence in neutral media does not lead to a complete elimination of emission, as commonly occurs in systems containing naphthalene and *secondary* amines,[17] but to emission from a new species in appreciable amount. Regarding the nature of this new species, it can be postulated that the formation of an intramolecular exciplex (excited state charge-transfer complex) occurs between the first singlet excited state of the arene subunit and the secondary amines, in accordance with some examples described in the literature.[18] In order to gain further insight into the nature of the exciplex, the fluorescence emission of **1** was recorded in other solvents. Displacement of the band to longer wavelengths with increasing polarity was observed. The following emission wavelengths were found (in nm): CH_2Cl_2 (390), $CHCl_3$ (390), THF (402), AcOEt (412), *i*-PrOH (415), CH_3CN (423), EtOH (430), MeOH (430). This stabilization with solvent polarity is in accordance with the polar nature of an excited charge-transfer complex.[19] Similar intramolecular exciplex species have also been observed in pyrene-derived thioureas and amides that have been used as sensors for cations and anions.[20] It must be said that, in this case, the semi-rigid macrocyclic structure of **1** could play an important role in the observed behavior. The emission of the open-chain amine *N*-(1-methylnaphthyl)propylamine in dichloromethane was recorded for comparison. In neutral dichloromethane a band was also observed at ca. 400 nm, but 5–6 times weaker than that observed for **1** under the same conditions. Again, addition of trifluoroacetic acid resulted in the restoration of the naphthalene fluorescence.

The dual fluorescence from monomer (330 nm) and exciplex (390 nm) in **1** should allow the use of the ratio of intensities between those two bands to determine the H$^+$ concentration in organic solvents or for the study of the interaction in solution between **1** and coordinated carboxylic species.[21] One evident target for this purpose are amino acids.[22] On the one hand the carboxylic group would interact with the amino groups in **1** resulting in a decrease of the intensity of the band at 390 nm and the concomitant increase of the fluorescence at 330 nm. But most importantly, the side chain in the amino acid could play an important role by means of secondary interactions (solvophobic effects, π–π stacking, etc.) leading to selective binding to **1** depending on the nature of the amino acid.



6 R=Me
7 R=$CHMe_2$
8 R= CH_2CHMe_2
9 R=CH_2Ph

We have tested this hypothesis with four L-amino acids (N-protected with a benzyloxycarbonyl group to avoid formation of zwitterions and solubility problems): *Z*-L-alanine **6**, *Z*-L-valine **7**, *Z*-L-leucine **8**, and *Z*-L-phenylalanine **9**. As can be seen in Figure 2, all four amino acids change the intensities of both bands (decreasing at 390 nm, increasing at 330 nm) but those bearing an aliphatic residue (**6**–**8**) make the change much less pronounced than **9**.[23a] Although preliminary results, these findings point to possible π–π interactions between the aromatic residue of **9** and the naphthalene ring, and consequently it will be a key point to be considered for the future design of new receptors. In fact, the enantiomers of **7** and **9** were also tested under the same conditions. Rather interestingly, a small enantiodifferentiation was found for the phenylalanine derivatives, as can be seen in Figure 3.[23b]

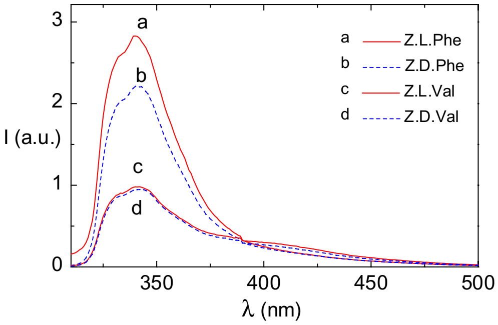


Figure 3. Uncorrected emission spectra of **1** in dichloromethane (1.3×10^{-4} M) in the presence of different N-protected amino acids (25 mM). Excitation wavelength: 300 nm. (a) *Z*-L-Phe, (b) *Z*-D-Phe, (c) *Z*-L-Val, and (d) *Z*-D-Val.

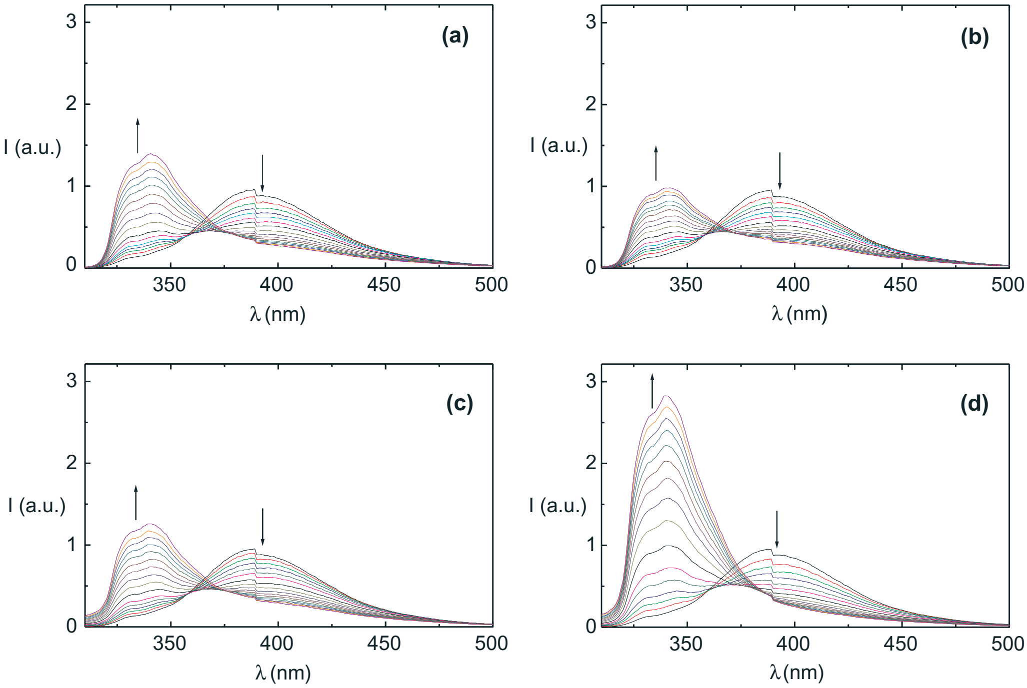


Figure 2. Uncorrected emission spectra of **1** in dichloromethane (1.3×10^{-4} M) in the presence of different N-protected amino acids at concentrations ranging from 0 M (first curve) to 25 mM (final curve). Excitation wavelength: 300 nm. (a) *Z*-L-Alanine **6**, (b)*Z*-L-valine **7**, (c) *Z*-L-leucine **8**, and (d) *Z*-L-phenylalanine **9**.

Other structures related to cyclophane **1** are currently being investigated, which could be used as first-generation probes for the ratiometric determination of amino acids in solution. Due to the modularity of their molecular architecture and their facile synthesis they could lead to a great variety of systems by changing the fluorophore, the amino acid or the polymethylene bridge. On the other hand, development of structures able to act in a similar way in water is required for general sensing purposes. Our work along this line is in progress.

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12. Characterization of compound **1**: $[\alpha]_D^{20}$ +89.8° ($c = 0.006$, CH_2Cl_2); ^1H NMR (300 MHz, CD_3OD) δ 8.26 (dd, 2H, $J = 3.3$ Hz, $J' = 6.1$ Hz), 7.56 (dd, 2H, $J = 3.3$ Hz, $J' = 6.1$ Hz), 7.39 (s, 2H), 4.62 (d, 2H, $J = 14.8$ Hz), 3.92 (d, 2H, $J = 14.3$ Hz), 2.96 (d, 2H, $J = 6.1$ Hz), 2.58 (m, 2H), 2.33 (m, 2H), 2.03 (m, 2H), 0.99 (d, 6H, $J = 7.2$ Hz), 0.95 (d, 6H, $J = 6.6$ Hz), 0.60 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 176.90, 137.21, 133.53, 127.10, 126.98, 125.44, 71.13, 37.17, 32.62, 28.91, 19.84, 18.54; IR (NaCl) 3322, 3075, 1651, 1520 cm^{-1}; ESI-MS $m/z = 425.3$ $(M + H^+)$, 447.3 $(M + Na^+)$.
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23. (a) For comparison purposes, the intensity ratios (monomer to exciplex) at a 10 mM concentration of the L-amino acid derivative are: $r = 1.4$ (for **6**), $r = 1.1$ (for **7**), $r = 1.2$ (for **8**), and $r = 3.4$ (for **9**); (b) At 10 mM the ratios for the D-derivatives are: $r = 3.1$ (for D-**9**) and $r = 1.1$ (for D-**7**).