New fluorescent metal receptors based on 4,4′-carbonyl bis(carbamoylbenzoic) acid analogues with naphthalene fluorophore

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ABSTRACT
In this manuscript is described a research related with two ditopic receptors based on 4,4′-carbonyl bis(carbamoylbenzoic) acids bearing a naphthyl (2a) and naphthylmethyl fluorophores (2b), which interact with metal ions. The fluorescence properties of both receptors were influenced by the connectivity with the amide group. The non-spaced receptor 2a presents an electron delocalisation from the amide to the naphthyl group having a broad red-shifted emission band and high quantum yield. The spaced receptor 2b presented a photo-induced electron transfer (PET) process from the amide to the naphthyl group, and a structured emission band is present at the UV region with low quantum yield. The different chemical structure influenced the fluorescent response used to analyse the coordination with metallic ions. The 2a receptor has an ON–OFF response because of the inhibition of the ICT process. The 2b receptor shows an OFF–ON response because of the inhibition of the PET process. Additional analytical studies by 1H-NMR and FTIR demonstrated the strong interaction of the amide and carboxylic functional groups with the metallic ions. Competitive experiments with EDTA showed that a very stable complexes are obtained between metallic ions and the new receptors.

Introduction
The development of synthetic hosts for cations is one of the most active research fields of supramolecular chemistry (1). Fluorescent chemosensors have received considerable attention due to their potential use in biological and environmental fields (2–13). Among diverse fluorescent receptors the naphthalene 4,4′-carbonyl bis(carbamoylbenzoic) acids have attracted our attention by reason of their fluorescent properties and photo stability. As far as we know, there are no reports in literature about any supramolecular study of their interaction with metal ions base on the fluorescent properties of bis(carbamoylbenzoic) acid derivatives. Lingaiah et al. reported the coordination of monotopic ligands as 2-(aminocarbonyl)benzoic acid, 2-(phenylaminocarbonyl)benzoic acid, 2-(2-amino-phenylaminocarbonyl) benzoic acid and 2-(2-naphthaleneaminocarbonyl)benzoic with Cu(II) ions (14). In this study, the metal–ligand ratio was reported (1:2) and established an interaction between ligands and metal centre through a metal-heteroatom ionic bond with the carboxylate group and a coordination bond with the amide carbonyl group. More recently, Pirrung et al. reported the combinatorial synthesis of catalytic peptoids from secondary amines with aliphatic and aromatic anhydrides capable of complexing with metal cations by carbamoylbenzoic acid functional
groups (15). Some synthesised derivatives showed affinity towards Cu(II) and Fe(III) with a consequent change of colour in the solid support.

Recently, we report the synthesis and regioselectivity studies of a series of bis(carbamoylcarboxylic) acids (16). Continuing with our research, the first example of metal ions fluorimetric receptors based on 4,4′-carbonyl bis(carbamoylbenzoic) acids bearing naphthalene groups is reported. These receptors strongly interact with different divalent ions in submicromolar levels and change their fluorescence properties. The main goal of this research was to study the influence of a methylene spacer between naphthalene and amide group in analogues 2a and 2b in the interaction of metal ions and the fluorescence respond. The fluorescent behaviour of these derivatives as a function of pH and the addition of different metal ions were analysed, and competitive assays with EDTA demonstrate high stability in these complexes. These characteristics demonstrate the high potential of these receptors for the removal of metal ions in the wastewater treatment or in the coordination chemistry field.

Experimental

3,3′,4,4′-Benzenophenetetracarboxylic dianhydride, naphthylamine and naphthylmethylamine were purchased from Aldrich in highest available purity (>99%). All solvents were of spectroscopic or HPLC grade. Absorption spectra were recorded on a Varian UV-Visible Cary 300 spectrophotometer. Fluorescence spectra were taken on a RF-5301PC Shimadzu spectrofluorometer. The measurements were done at ambient temperature in 1 cm quartz cuvettes. Sample solutions for studying the pH dependence of the emission spectra were prepared adjusting at the desired pH, with solutions of 0.001 M NaOH and 0.001 M HCl. Fluorescence quantum yields were measured using anthracene in toluene as a standard with a known Φ of 0.30. The effect of the metal cations upon the fluorescence intensity was examined by adding a few microlitres of stock solution of the metal cations to a known volume of the receptor solution (3 mL). The addition was limited to 0.09 mL, so that dilution remained insignificant (5). Emission intensities were normalised against the most intense band of the corresponding free ligand. NMR spectra were obtained in a Bruker 400 MHz NMR Spectrometer at a probe temperature of 25 °C with TMS as internal standard.

Synthesis

General procedure for the preparation of receptors. Naphthylamine (0.13 g, 3.0 eq) and triethylamine (0.16 g, 5.0 eq) was dissolved in THF (10 mL), and then, the mixture was reacted with the 3,3′,4,4′-benzenophenetetracarboxylic dianhydride (1) (0.1 g, 0.031 mol) with constant stirring. After 3 h a 5% hydrochloric acid was added and extracted with ethyl acetate. Then, the organic solvent was evaporated.

4,4′-carbonylbis(2-(naphthalen-1-ylcarbamoyl)benzoic acid) (2a). Purple solid. 95 mg, 98% yield. Mp 200–202 °C. IR (KBr): 3430, 3060, 2926, 1710, 1671, 1181, 1069 cm⁻¹. 1H NMR (DMSO-d₆, 400 MHz): δ 8.05 (d, J = 1.7 Hz, 2H), 8.04 (d, J = 7.0 Hz, 2H), 7.95 (dd, J = 7.9, 1.8 Hz, 2H), 7.84 (d, J = 7.9 Hz, 2H). 13C NMR (DMSO-d₆, 100 MHz): δ 193.3, 168.2, 167.3, 143.8, 137.7, 137.2, 134.1, 132.2, 132.0, 129.6, 128.6, 127.7, 126.5, 125.4, 123.7, 122.8, 122.2, 115.9, 108.0. MS (EI) m/z: 572 (M⁺–2H₂O, 5), 447 (100), 278 (20), 228 (25), 127 (40). HRMS (Cl⁻): Calculated for C₃₇H₂₀N₂O₅ (M–2H₂O)⁻: 572.1372. Found 572.1355.

4,4′-carbonylbis(2-(naphthalen-1-ylmethylcarbamoyl)benzoic acid) (2b). Pearl solid. 395 mg, >99% yield. Mp 198–200 °C. IR (KBr): 3048, 3005, 2925, 1658, 1628, 1084 cm⁻¹. 1H NMR (DMSO-d₆, 400 MHz): δ 8.53 (d, J = 2.0 Hz, 2H), 8.34 (d, J = 8.1 Hz, 2H), 8.14 (d, J = 8.4 Hz, 2H), 7.99 (dd, J = 7.8, 1.5 Hz, 2H), 7.96 (d, J = 8.2 Hz, 2H), 7.86 (dd, J = 8.1, 2.0 Hz, 2H), 7.65–7.56 (m, 6H), 7.55 (dd, J = 8.0, 7.2 Hz, 2H), 4.52 (s, 4H). 13C NMR (DMSO-d₆, 100 MHz): δ 194.6, 167.1, 167.0, 138.4, 137.8, 134.9, 133.8, 133.2, 132.9, 130.8, 130.7, 130.6, 128.8, 128.6, 126.9, 126.6, 126.1, 125.3, 123.3, 39.7. MS (EI) m/z: 600 (M⁺–2H₂O, 70), 472 (30), 141 (100). HRMS (Cl⁻): Calculated for C₂₅H₁₅N₂O₄ (M–2H₂O + 1) 399.1093. Found 399.1093.

Procedure for fluorimetric titrations

The effect of metal ions upon the emission was examined by adding 6 μL of a 0.001 M perchlorate salt solution to a known volume (3 mL) of a 1 x 10⁻⁵ receptor solution. The addition was limited to 0.060 mL; in this case, the dilution remained insignificant.

Procedure for NMR titrations

The effect of metal ions upon the chemical shift in the signal of receptors 2a and 2b was examined adding 5 μL of a 0.1 M perchlorate salt stock solution to a known volume (0.5 mL) of a 5 x 10⁻³ M solution contained in a NMR tube. The addition was limited to 0.050 mL, so that the dilution remained insignificant.

Potentiometric titration

The potentiometric titrations were carried out at 298.1 ± 0.1 K, using 0.1 M NaCl as the supporting electrolyte, using Metrohm 848 Titrino Plus. The reference electrode was an Ag/AgCl electrode in a saturated KCl solution.
The glass electrode was calibrated as a hydrogen-ion concentration probe by the titration of previously standardised amounts of HCl with CO₂-free NaOH solutions and the equivalent point determined by Gran’s method, which gives the standard potential, $E'$, and the ionic product of water [$pK_w = 13.77$].

The computer program HYPERQUAD 2008 v5.2.19 was used to calculate the protonation. The pH range investigated was 2.0–10.4, and the concentration of the Ligands was 4 mM.

**Results and discussion**

**Synthesis**

The synthesis of 4,4′-carbonyl bis(carbamoylbenzoic) acids 2a and 2b was readily achieved by the synthetic method outline in Scheme 1 [16]. Our synthetic strategy involves the initial reaction of 3,3′,4,4′-benzophenonetetracarboxylic dianhydride (1) with naphthylamine (a) or naphthylmethylamine (b) in THF with TEA at 0 °C, in order to obtain a bis(carbamoylbenzoic) acid analogues with a fluorophore directly attached in amide 2a, and with a methylene unit spacing the fluorophore to the amide 2b and compare their fluorescence and response in the complexation with metal ions.

**Photophysical properties**

In order to evaluate the influence of structural characteristics over the spectral properties in the receptors 2a and 2b, the UV–Vis and fluorescence spectra were measured (Figure S1). The electronic absorption spectra are compared in Figure 1. The receptor 2a has a continuous band with absorption peaks at 245, 268, 310 and 335 nm, while receptor 2b has a structured band with a relative maximum peak at 270 nm typical for polyunsaturated hydrocarbon π–π* transitions.

The excitation and emission fluorescence spectra of 2a and 2b in DMSO solution are presented in Figure 2. There are important differences in the excitation and emission fluorescence spectra of receptors 2a and 2b. The spectrum of 2a (λ_{exc} = 338 nm, λ_{em} = 428 nm) shows high fluorescence intensity, broad excitation and emission bands shifted to longer wavelength, in this receptor the lower energy absorption band in the ground state corresponds...
to the $S_0 \rightarrow S_1$ in the excited state. While $2b$ ($\lambda_{\text{exc}} = 283 \text{ nm}, \lambda_{\text{em}} = 329 \text{ nm}$) shows structured excitation and emission bands at lower wavelengths. In particular, emission bands showed a shift of 99 nm. These changes are due to the presence of amide group directly attached to naphthalene group in $2a$. The fluorescence properties may be attributed to the ability of the chromophore to delocalise charge over both carbonyl-amine–naphthalene groups in the molecular excited state (Figure 2). In receptor $2b$, the methylene unit between the naphthalene and amide group prevents electronic delocalisation over naphthalene amide group.

Moreover, a decrease in the fluorescent intensity is observed in receptor $2b$ because of the PET from the naphthalene to the carbamoyl benzophenone moiety (Figure 3). This mechanism of fluorescent quenching of tyrosine (fluorophore) and its derivatives by amide groups has been extensively studied (17–23).

On the other hand, the fluorescence emission bands are solvent dependent. Figure 4 shows that the excitation bands are shifted to lower wavelength, while emission bands are shifted to longer wavelength when compare acetonitrile (as less polar aprotic solvent) with DMSO (polar aprotic solvent) and water (polar protic solvent). Noteworthy, an increase in fluorescence intensity is also observed in DMSO. The solvent influence can be explained considering the formation of intermolecular hydrogen bonding between the protic solvent, the carboxylic and amide groups of the excited molecules, which affect the energy gap of the electronic transition and leads to a shift to longer wavelength (red shift).

In Table 1, the photophysical parameters of $2a$ and $2b$ receptors in different solvent are listed. The quantum yields ($\Phi_F$) in acetonitrile (CH$_3$CN), H$_2$O, and DMSO at $1 \times 10^{-6}$ M were determined following the Equation (1) (24, 25).

$$\Phi_F = \Phi_R \times \text{Int} A_R n^2/\text{Int} A R^2$$

where $\Phi_R$ is the sample quantum yield, Int is the area under the emission peak (at a given wavelength scale), $A$ is the absorbance at the excitation wavelength, and $n$ is the refractive index of the sample. The subscript $R$ denotes the respective values of the reference substance. The reference substance was anthracene. As it was expected, the $\Phi_F$ were higher for $2a$ in the three solvents and follow the emission intensity pattern.

**Influence of pH in the emission of $2a$ and $2b$**

The influence of pH in the fluorescence intensity of $2a$ and $2b$ receptors was studied in a pH range of 1–8 (Figure 5). Receptors $2a$ and $2b$ showed different behaviour with pH variation. The fluorescence emission of receptor $2a$ increases considerably with the increase of pH in a pH range of 2–4. The fluorescence remains almost constant from 4 to 8. This process is reversible, then $2a$ is an efficient low pH range chemosensor. The pH dependence of fluorescence has been analysed using the experimental data and the p$K_a$ values $pK_{a1} = 3.05$ and $pK_{a2} = 3.15$ were calculated by a curve fitting in a program developed by Inoue based on a non-linear least-squares method (26).
In receptors 2a and 2b an intramolecular hydrogen bond is presented between the carboxylic acid hydrogen and the carbonyl amide group. This interaction has an effect over the electronic delocalisation and the molecular orbitals energy in the amide group. While upon full deprotonation of both carboxylic of 2a and 2b, an intramolecular hydrogen bond between the amide N-H and the carboxylate oxygen is observed.

In the deprotonated dicarboxylate 2a, the naphthyl groups connecting to the amide and the benzophenone core approach to co-planarity (naphthyl-amide torsional angle 1.9°). The planarisation and rigidity enhances the emission. On the contrary, the hydrogen bond interaction in the 2b dicarboxylate derivative enhances the PET process.

**Influence of metal ions in the emission of 2a and 2b**

The photophysical properties of 2a and 2b receptors as ligands in the presence of different metal ions [Li⁺, Na⁺, K⁺, Cs⁺, Ca(II), Mg(II), Ba(II), Cu(II), Pb(II), Ni(II), Hg(II), Zn(II) and Cd(II)] in CH₃CN solution were studied. Receptors 2a and 2b were titrated by successive increment of equivalent number of metal ions separately. The fluorescence emission changes were monitored during the course of the titration. The addition of cations such as Ni(II), Zn(II), Cd(II), Na⁺, Mg(II), Ca(II) and Ba(II) to receptor 2a only results in a variation of less than 10% of the fluorescence intensity (Figure S4). The addition of Pb(II), Cu(II) and Hg(III) ions cause total quenching in fluorescence intensity (Figure 6).

It is proposed the coordination of the metal ion with the amide and carboxylic groups inhibits the electron delocalisation. In addition, these transition metal ions exert their well-known heavy atom effect favouring the quenching of fluorescence.

According with the fluorescence profiles, the metal–ligand ratios were 1:1 with Hg(II) and Pb(II), and 3:2 with Cu(II). The Job’s plot confirmed the same metal–ligand ratio for these complexes (Figure S5). The association constants for Hg(II) and Pb(II) calculated with Equation (3) for a 1:1 metal–ligand ratio were 7.1 × 10⁶ and 4.1 × 10⁷, respectively.

\[
IF_{\text{obs}} = IF_s + 0.5IF_c \left[ \frac{|H| + |G| + \frac{1}{2} - \sqrt{(|H| + |G| + \frac{1}{2})^2 - 4|H||G|}}{|H|} \right]
\]

where \(IF_{\text{obs}}\) = Complex fluorescence intensity, \(IF_s\) = Free receptor fluorescence intensity, \(IF_c\) = Fluorescence intensity.
complexes with Mg(II), Ca(II), Cu(II) and Pb(II) showed a 1:1 metal/receptor ratio and with Ba(II) it was 1:2. Two distributions in the Job’s plot were obtained with Zn(II), Cd(II) and Ni(II) indicating the probable successive formation of two complexes (Figure S8).

In order to study the stability of the complexes 2a-Cu(II), 2a-Hg(II) and 2a-Pb(II) a competitive assay with EDTA were performed. In Figure 7, the emission spectrum of 2a, 2a-Cu(II) and 2a-Cu(II)-EDTA are compared. As can be seen, the fluorescence is partially recovered (~40%, relative to the free ligand) upon the addition of 2.0 eq of EDTA. This result indicates an equilibrium between complex 2a-Cu(II) and EDTA-Cu(II) and considerably stability of 2a-Cu(II) complex. In the assay with complexes 2a-Hg(II) and 2a-Pb(II), a similar behaviour was observed, but the recovered fluorescent intensity was around 70%. The higher fluorescence increase suggests lower stability for these complexes compared with 2a-Cu(II) (Figure S6).

On the contrary, the addition of metal ions increases the fluorescence intensity of receptor 2b in CH3CN solution. The fluorescence intensity enhancement $F_I/F_{I_0} = 2.2$ upon addition of Ca(II) ions (Figure 8). A lower enhancement was observed upon addition of other divalent cations as Mg(II), Ba(II), Zn(II), Pb(II), Cd(II) and Cu(II). The addition of other cations such as K+, Na+ and Cs+, resulted in a negligible enhancement of the fluorescence intensity.

Coordination of 2b with metal ions results in the inhibition of PET process and the fluorescence increase (28). The variation in the fluorescence emission observed upon metal ion addition depends on the nature of each metal ion. There is a preference to divalent metal ions.

The fluorescence intensity observed in the titration of 2b with Ca(ClO4)2 follows a 1:1 metal–ligand profile and it was verified by Job’s plot method. The emission profiles with other cations have a maximum fluorescence intensity enhancement $F_I/F_{I_0} = ~1.6$. The Job’s plot for the complexes with Mg(II), Ca(II), Cu(II) and Pb(II) showed a 1:1 metal/receptor ratio and with Ba(II) it was 1:2. Two distributions in the Job’s plot were obtained with Zn(II), Cd(II) and Ni(II) indicating the probable successive formation of two complexes (Figure S8).
The formation constants with Ca(II), Mg(II), Cu(II) and Pb(II) were determined using the Equation (1) and they are listed in Table 2.

To determinate the stability of the complexes with 2b, the competitive assay with EDTA was performed. In Figure 9, the emission spectra of 2b, 2b-Cu(II) and 2b-Cu(II)-EDTA are compared. As can be seen, the fluorescence remains constant upon the addition of 2.0 eq of EDTA. This result indicates that complex 2b-Cu(II) is more stable than EDTA-Cu(II) and EDTA does not competes for the cation. In the assay with the complex 2b-Ca(II), a slight decrease in fluorescence was observed upon the addition of EDTA (2.0 eq), but it did not reach the fluorescence intensity of the free ligand. Therefore, an equilibrium occurs between complex 2b-Ca(II) and EDTA-Ca(II) indicating considerably stability of 2b-Ca(II) complex.

In Table 3, the photophysical parameters of 2a and 2b metal complexes are listed. The quantum yields ($\Phi_F$) in CH$_3$CN at $1 \times 10^{-6}$ M of ligand with two molar equivalents of cation were determined following the Equation (1). The quantum yields values for the 2a complexes decreases considerably due to the quenching of fluorescence. The values for 2b complexes increases due to the fluorescence enhancement.

**NMR studies**

The $^1$H NMR spectra for receptors 2a and 2b in DMSO-$d_6$ are shown in Figure 10. There are significant differences in chemical shifts for both benzophenone core and naphthalene hydrogens. In general, the signals for 2a appear upfield than signal for 2b. This is due to the electron enrichment in naphthalene group generated by delocalisation of an electron pair from the amide to the aromatic ring (ICT process). This effect is more evident in hydrogens.

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>Metal/receptor ratio</th>
<th>$K_f$</th>
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</thead>
<tbody>
<tr>
<td>Ca(II)</td>
<td>1:1</td>
<td>$6.5 \times 10^7$</td>
</tr>
<tr>
<td>Mg(II)</td>
<td>1:1</td>
<td>$1.2 \times 10^7$</td>
</tr>
<tr>
<td>Pb(II)</td>
<td>1:1</td>
<td>$6.8 \times 10^7$</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>1:1</td>
<td>$2.7 \times 10^7$</td>
</tr>
</tbody>
</table>

Figure 8. (Colour online) Relative fluorescence of 2b in CH$_3$CN solution ($1 \times 10^{-5}$ M) with different metal ions.
Notes: $\lambda_{ex}=295$ nm and $\lambda_{em}=332$ nm.

Figure 9. (Colour online) (a) Emission spectra for 2b, 2b-Cu(II) and 2b-Cu(II)-EDTA. (b) Emission spectra for 2b, 2b-Ca(II) and 2b-Ca(II)-EDTA.
H$_4$, H$_5$, and H$_6$ which are in the aromatic ring directly connected to the nitrogen atom. In fact, hydrogen H$_5$ displaces 1.20 ppm upfield in receptor 2a compared with 2b. In addition, the chemical environment of benzophenone core hydrogens changes due to the electronic delocalisation in receptor 2a. Interestingly, signal for H$_a$ and H$_c$ shifted upfield (~0.53 ppm) while H$_b$ shifted downfield (~0.1 ppm). The signal for amide hydrogen is not clearly observable in the NMR spectra of 2a indicating a probable chemical exchange with water contained in the solvent. Furthermore, a broad signal at 7.60 ppm underhanded with other aromatic signals was found in 2b spectrum and the signal for the methylene hydrogens appears as broad singlet at 4.50 ppm (Figure S9).

Table 3. Photophysical characteristics of metal complexes with 2a and 2b receptors.

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>Parameter (nm)</th>
<th>2a</th>
<th>2b</th>
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<tbody>
<tr>
<td>Ca$^{2+}$</td>
<td>$\lambda_L$</td>
<td>280</td>
<td>326</td>
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<tr>
<td></td>
<td>$\lambda_\lambda$</td>
<td>326</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>$\Phi_F$</td>
<td>0.075</td>
<td></td>
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<tr>
<td>Cu$^{2+}$</td>
<td>$\lambda_L$</td>
<td>330</td>
<td>280</td>
</tr>
<tr>
<td></td>
<td>$\lambda_\lambda$</td>
<td>420</td>
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<td></td>
<td>$\lambda_\lambda$</td>
<td>90</td>
<td>46</td>
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<tr>
<td></td>
<td>$\Phi_F$</td>
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<td>0.042</td>
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<tr>
<td>Pb$^{2+}$</td>
<td>$\lambda_L$</td>
<td>329</td>
<td>280</td>
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<td>$\lambda_\lambda$</td>
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<td>$\Phi_F$</td>
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<tr>
<td>Cd$^{2+}$</td>
<td>$\lambda_L$</td>
<td>280</td>
<td>327</td>
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<td>327</td>
<td>47</td>
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<td></td>
<td>$\Phi_F$</td>
<td>0.053</td>
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<tr>
<td>Ba$^{2+}$</td>
<td>$\lambda_L$</td>
<td>280</td>
<td>327</td>
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<td>327</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>$\lambda_\lambda$</td>
<td>0.051</td>
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<tr>
<td>Zn$^{2+}$</td>
<td>$\lambda_L$</td>
<td>280</td>
<td>327</td>
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<td>$\lambda_\lambda$</td>
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<td>$\lambda_\lambda$</td>
<td>416</td>
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<tr>
<td></td>
<td>$\Phi_F$</td>
<td>0.0021</td>
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</table>

The receptor 2b was analysed by 2D EXSY NMR in order to get more information about the chemical exchange of the amide hydrogens. The experiment was carried out in DMSO-$d_6$ and a cross peak between the signal at 7.60 ppm and the residual water (3.40 ppm) was observed (Figure S10). When 5 mL of D$_2$O was added to the sample, the broad signal in 7.60 ppm disappeared as well as the cross peak with the water signal (Figures S11–S12). This result indicates that the broad signal corresponds to the N-H hydrogens.

Figure 10. (Colour online) Partial $^1$H-NMR spectrum of 2a (a) and 2b (b) in DMSO-$d_6$. 
In order to investigate the nature of metal ion coordination, $^1$H-NMR titration experiments were conducted for 2a and 2b with different metal ions as Ca(II), Zn(II), Pb(II) and Hg(II) as perchlorate salts in CD$_3$CN:DMSO-$d_6$ (9:1) and DMSO-$d_6$, respectively. A partial $^1$H-NMR stacked spectra obtained in the titration of receptor 2a with Pb(ClO$_4$)$_2$ (0 to 2 M eq.) are shown in Figure 11. The signals for benzophenone hydrogens H$_a$, H$_b$ and H$_c$ remain unchanged, whereas all the signals belonging to the naphthalene were affected due to the coordination of the receptor with the metal ion. Hydrogens H$_d$, H$_e$ and H$_f$ lost the shielding effect of the amide electron pair, and their signals gradually shifted downfield (−0.19, −0.06 and −0.19 ppm, respectively) upon the addition of two molar equivalents of cation. The rest of signal are less affected. This is a clear evidence in the participation of the amide group in the complex formation in the ground state, as it was proposed in the fluorescence experiments. The effect of cations over the chemical shift of naphthalene signals were in the magnitude order Hg(II) > Pb(II) > Zn(II) > Ca(II) (see Figures S13–S15).

$^1$H-NMR studies with receptor 2b should be done in DMSO-$d_6$ due to the low solubility of complexes in the CD$_3$CN:DMSO-$d_6$ (9:1) mixture. Contrary to receptor 2a, in the titration of 2b with Pb(ClO$_4$)$_2$ (Figure 12), the most affected signals were those for benzophenone core H$_a$, H$_b$ and H$_c$. Interestingly, upon the addition of 0.8 equivalent of Pb(ClO$_4$)$_2$ (beyond this point the complex precipitates in DMSO solvent) the signals for H$_a$ and H$_c$ behave broadening and shifted upfield (0.15 and 0.11 ppm, respectively), while the signal for H$_b$ presented partially broadening and shifted downfield (−0.04 ppm). This behaviour indicates the coordination of Pb(II) with receptor 2b through the carboxylic and amide groups affecting the chemical environment and relaxation times of the nearest hydrogens to the binding site. This effect is according to the fluorescence response. The upfield shift may be possible because of a retrodonation mechanism from the metal centre to the receptor. The signals of naphthalene remain unchanged excepting the corresponding to H$_b$ which slightly shifted downfield since the first addition and then remained constant.

Ca(II), Zn(II) and Hg(II) showed interaction with the receptor affecting the same signals; however, the complexes formed in solution were completely soluble upon the addition of two molar equivalents of metallic salt. Among these cations, Hg(II) induced exceptional changes in the receptor shifting the signals H$_a$, H$_b$, H$_c$ in 0.50, −0.10 and 0.52 ppm, respectively. Curiously, the singlet signal for methylene groups at 4.50 ppm slightly shifted to downfield and becomes to a double of triplets indicating the diasterotopicity of these hydrogens in the rigid complex structure (Figure S18). Additional broad signal at 8.20 ppm was observed in the $^1$H-NMR spectra during the titrations with Pb(ClO$_4$)$_2$ and Hg(ClO$_4$)$_2$. The 2D EXSY NMR experiment of 2b-Hg complex showed a cross peak between this signal and the residual water signal, which indicates this is the N–H signal shifted to downfield due to the Hg(II) coordination with the amide group (Figure S20). The effect of metal ion over the chemical shift of benzophenone signals was in the magnitude order Hg(II) > Pb(II) > Zn(II) > Ca(II).
Ca(II) (Figures S16–S19). Also, the chemical shift profiles as function of the molar equivalents of Hg(ClO₄)₂ showed a 1:1 metal/receptor ratio for both receptors (Figure S21).

**FTIR studies**

The FTIR spectra of complexes in solid state were obtained. The receptor 2b and 2b-Hg(II) complex IR spectra are compared in Figure S22. According to the literature, the band at 2635 cm⁻¹ correspond to a ν(O–H) of the carboxylic group and it disappears in the complex. Also, the two bands at 1655 and 1620 cm⁻¹ corresponding to carbonyl stretching in the free receptor shifted to 1704 and 1653 cm⁻¹, respectively. These changes indicate the interaction of metal ion with the receptor through the carboxylic acid and the amide groups. Surprisingly, two well-defined bands at 1100 and 1072 cm⁻¹ were found in the FTIR spectrum, indicating coordination of perchlorate with the metallic centre in a monodentate fashion (29).

Finally, based on the symmetric structure and ditopic nature of receptors 2a and 2b with a 1:1 stoichiometric ratio with the most of the metal ions, it was expected a double set of signals in the ¹H-NMR spectra during titrations with metallic ions due to the molecular dissymmetry caused by the possible interaction in only one binding site. However, the titrations with two molar equivalents of a metallic ions do not show such differences in the signals, indicating that all hydrogens are simultaneously affected by coordination in both sites. These observations led us to propose the formation of a supramolecular structure, which is assembled when one metallic ion coordinates with the binding site of two different molecules and the free binding sites left of both molecules, then interact with another metal ions generating a coordination type polymer in solution as is shown in Figure 13. In this arrangement, the 1:1 metal/receptor ratio is conserved. In a batch experiment, the receptor solutions with an excess of Hg(ClO₄)₂ were prepared and it allows to reach the chemical equilibrium for 24 h. The ¹H NMR spectra showed the same changes observed in the titrations, but in this samples, additional signals with lower integration values were observed (Figures S23–S25). These signals presumably belong to the unbounded terminal moieties of the supramolecular structure. This arrangement is concise

**Figure 12.** (Colour online) Partial ¹H NMR spectrum of 2b in DMSO-d₆ upon addition of Pb(ClO₄)₂.
Notes: [2b] = 5 mM.

**Figure 13.** Plausible structure for supramolecular complexes formed with 2a and 2b and different metallic ions.
with the binding constants high values as well as the high stability against EDTA.

The ESI–MS spectra were obtained for receptors **2a** and **2b** and their metal complexes. In the analysis, the quasimolecular ions of 626 amu [M⁺H₂O]⁺ and 672 amu [M⁺2H₂O]⁺ were detected for **2a** and **2b**, respectively (Figures S26 and S28). In the receptors and Pb(ClO₄)₂ mixtures, several quasimolecular ions showing different metal ion isotopic contributions with different m/z values were found (Figures S27 and S29). These results indicate the formation of different size aggregates by supramolecular coordination assembly.

**Conclusions**

In conclusion, the two new fluorescent receptors based on 4,4'-carbonyl bis(carbamoylbenzoic) acid form very stable complexes with several metal ions because of the strong interaction with the amide and carboxylic acid functional groups present in their structures. The influence of a methylene spacer on the fluorescence properties was demonstrated. The receptor **2a** presents higher quantum yields associated to an ICT process between the naphthalene and the adjacent amide group, and receptor **2b** shows a PET mechanism. Both mechanisms are inhibited upon addition of metal ions. Competitive assays with EDTA exhibited the high stability of the metal–receptor complexes. The results described here demonstrated the potential practical application of these and other structurally related receptors in the environmental field and coordination chemistry. We are conducting our research in the removal of metal ion from wastewater effluents using carbamoyl carboxylic type receptors (30).

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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