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Novel naphthalimide–aminobenzamide dyads as OFF/ON fluorescent supramolecular receptors in metal ion binding

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ABSTRACT

A series of novel naphthalimide–aminobenzamide (NAPIM-2ABZ) dyads **3** connected by different length polymethylene chains were synthesized and studied as fluorescent supramolecular receptors in metal ion binding. The photophysical properties were evaluated and compared with separated chromophores. The electronic absorption spectra of dyads **3** showed no interaction between chromophores in the ground state. The fluorescence quantum yields were lower in dyads **3** in comparison with *N*-propyl-2-aminobenzamide (**8**). The fluorescence quenching is attributed to a PET mechanism between fluorophores (from 2ABZ to NAPIM), which is dependent on the polymethylene chain length. In metal binding study was found a response towards transition metal ions such as Hg(II), Cu(II), Zn(II) and Ni(II). Dyad **3b** presented selectivity towards Cu(II). The UV-vis, IR and ¹H-NMR studies demonstrated the interaction with 2ABZ moiety in the ground state, and interestingly dyads with shorter polymethylene chains **3a** (n = 0), **3b** (n = 1) and **3c** (n = 2) exhibited an *OFF/ON* fluorescence behaviour due to the PET inhibition and the quenching of 2ABZ fluorescence. Dyads **3d** (n = 4) and **3e** (n = 6) presented opposite response *ON/OFF* in the complex with metal ions evidencing the absence of PET in these dyads.

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Introduction

2-Aminobenzamide (2ABZ) has been extensively studied as fluorescent label in order to improve the oligosaccharide detection and sequencing by high-performance liquid chromatography (1–6). The introduction of 2ABZ as fluorescent probe into saccharides occurs through a two-step reductive amination. The same strategy has been used in the label of monosaccharides with *N*-aminoethyl-2aminobenzamide (2AEAB) (7), which has the advantage of being a bifunctional molecule and allows to develop microarrays to study the protein–carbohydrates interactions (8). Fluorescent sensors for the detection of Zn(II) with a 2ABZ moiety as fluorophore were reported (9, 10).







Figure 1. Structure of NAPIM-DANS 1, NAPIM-PTZ 2 and NAPIM-2ABZ 3 dyads, *N*-propyl-2-aminobenzamide (8) and *N*-propylnaphthali mide (9).



Figure 2. (a) UV-vis spectra of **8**, **9** and **3b**. (b) UV-vis spectra of **3**(**a**-**e**). Solvent: MeCN. [**8**, **9** and **3**(**a**-**e**)] = 1×10^{-5} M.

Emission spectra appear in the visible region between 400 and 550 nm, and the detection is performed in HEPES buffer at pH = 7.4. A fluorescence enhancement is observed upon addition of Zn(II) to a ligand solution. The structure of 2ABZ, with amino and amide groups, forms an

electron donor-acceptor system with unique fluorescence properties due to an internal charge transfer process (ICT).

Furthermore, several groups have investigated the coordinating behaviour of 2ABZ and its derivatives. Lingaiah et al. (11) reported the formation of a light green distorted octahedral complex from *N*-phenyl-2ABZ to Cu(II) with a 1:2 metal–ligand ratio. The molar conductance was 67 ohm⁻¹cm² mol⁻¹ in DMF, which is in accordance to a 1:1 electrolyte. Recently, the mixed ligand complex synthesis with 2ABZ, α -amino acids and transition cations such as Cu(II) or Ni(II) was reported (12, 13). In these reports, the coordination of the cation with the amino and carbonyl groups of 2ABZ in an octahedral complex was proposed.

Recently, the immobilisation of *N*,*N*-alkyl-bis-2ABZs on Merrifield's resin and their labelling with a dansyl moiety were reported (*14*). The fluorescent properties were studied as a function of the alkyl length chain and the labelling with dansyl group. In the response with metal ions was found a selective recognition to Mg(II) based on the inhibition of PET when cation was combined with the ligand.

Additionally, bischromophoric dyads **1** containing a 1,8-naphthalimide (NAPIM) and dansylamide (DANS) fluorophores linked by a polymethylene chains were reported by Pischel et al. (*15*). In their studies, they found singlet-singlet energy transfer (SSET) and photoinduced electron transfer (PET) processes between NAPIM and DANS, and both processes were affected by the presence of protons quenching DANS and enhancing NAPIM fluorescence (Figure 1). Ramaiah et al. (*16*) found that the presence of Cu(II) and Zn(II) has the same



Figure 3. (a) Fluorescence spectra of **8**, **9** and **3b**, $\lambda_{ex}(\mathbf{8}) = 324$ nm, $\lambda_{em}(\mathbf{8}) = 394$ nm, $\lambda_{ex}(\mathbf{9}) = 332$ nm, $\lambda_{em}(\mathbf{9}) = 378$ nm, $\lambda_{ex}(\mathbf{3b}) = 328$ nm, $\lambda_{em}(\mathbf{3b}) = 398$ nm. [**8**] = [**9**] = 1 × 10⁻⁵ M (b) Emission spectra of dyads **3(a-e)**. Solvent: MeCN. [**3(a-e)**] = 1 × 10⁻⁵ M. $\lambda_{ex}(\mathbf{3a}) = 340$ nm, $\lambda_{ex}(\mathbf{3b-3c}) = 328$ nm.

effect in dyads with shorter spacing length due to the SSET and PET processes, instead a Förster resonance energy transfer (FRET) process occurs between NAPIM and DANS fluorophores in dyads with longer polymethylene chain (six and eight methylenes) (17). The radiometric response was influenced by the length of the polymethylene chain in both works.

It is well known that NAPIM derivatives have excellent electron–acceptor properties and have been used in many studies of PET involving the interaction with different donors (18–24). In addition, other NAPIM derivatives such as naph-thalimide–phenothiazine (NAPIM-PTZ) dyads **2** capable to form intramolecular exciplex and intermolecular excimer in non-polar solvents have been described (25). Nevertheless, 2ABZ acts as energy donor in fluorescence energy-transfer probes containing nitrotyrosine as energy acceptor in conformation studies of medium size peptides (26).

In this work were synthesised a series of novel naphthalimide–aminobenzamide (NAPIM-2ABZ) dyads **3** linked with different length polymethylene chains in order to evaluate their optical properties and the complex formation with transition metal ions in solution, as well as their effect on PET process between NAPIM and 2ABZ.



Figure 4. (a) UV-vis spectra obtained by titration of **8** with Hg(ClO₄)₂ in MeCN. (b) Absorbance profiles at 326 nm obtained by titration of **8** with different metal ions in acetonitrile. [**8**] = 1×10^{-5} M.

Experimental

General

All reagents were purchased in the highest quality available and were used without further purification. Nuclear magnetic resonance (NMR) spectra of ¹H and ¹³C were recorded in a Varian 200 MHz and in a Bruker 400 MHz Spectrometer in DMSO- $d_{c'}$ CDCl₃ or CD₃CN with TMS as internal standard. Chemical ionisation mass spectra and HRMS were recorded on a MStation JMS-700 JEOL. Electronic impact mass spectra were obtained by direct insertion in an Agilent 5975C mass spectrometer, and the intensities were reported as a relative percentage to the base peak after the corresponding m/z value. Infrared spectra were recorded on a Spectrum 400 FTIR-ATR spectrophotometer. Melting points were obtained on a Thermo Scientific apparatus model Mel-Temp, and the value is reported as an average of three separated experiments. Fluorescence spectra were measured on a Cary Eclipse spectrofluorometer, and the absorbance spectra were measured on a Cary 100 Conc UV-Visible spectrophotometer. The measurements were taken at room temperature in 1-cm quartz cuvettes.



Figure 5. (a) Emission spectra obtained by titration of **8** with $Hg(CIO_4)_2$ in acetonitrile. (b) FI/FI_0 profiles at 394 nm obtained by titration of **8** with different metal ions in acetonitrile. [**8**] = 1×10^{-5} M.



Figure 6. (a) UV-vis spectra obtained by titration of dyad **3b** with $Hg(CIO_4)_2$ in acetonitrile. (b) UV-vis absorbance profiles at 328 nm obtained by titration of **3b** with different metal ions in acetonitrile. [**3b**] = 1×10^{-5} M.

General procedure for the synthesis of 1,8-naphthalimide alkylamines 6

In a round-bottom flask, the corresponding alkyldiamine (7 equiv. 17.64 mmol) was dissolved in 50 mL of distiled water, and the solution was stirred and heated at 80 °C. 1,8-naphthalenetetracarboxylic acid anhydride (**4**) (500 mg, 2.52 mmol) was added portion wise, and the mixture was heated for 20 min. The suspended solid was filtered and was cooled into an ice bath until a yellow solid was formed. The solid was separated by filtration and dry at room temperature.

2-(3-Aminoethyl)-1H-benzo[de]isoquinoline-1,3(2H)dione (6a)

Yellow solid, 304 mg, 1.27 mmol, 50% yield. Mp 129–130 °C. FTIR (KBr): 3340, 3450, 3063, 2929, 1693, 1609, 1388 cm⁻¹. ¹H-NMR (DMSO- $d_{6'}$ 400 MHz): δ 8.48 (dd, J = 7.3, 1.0 Hz, 2H), 8.44 (dd, J = 8.3, 1.0 Hz, 2H), 7.86 (dd, J = 8.3, 7.3 Hz, 2H), 4.1 (t, J = 7.0 Hz, 2H), 2.81 (t, J = 7.0 Hz, 2H). ¹³C-NMR (DMSO- $d_{6'}$ 50 MHz): δ 163.5, 134.0, 131.1, 130.4, 127.3, 127.0, 122.1, 42.7, 39.6.

2-(3-Aminopropyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (6b)

Yellow solid, 237 mg, 0.93 mmol, 37% yield. Mp 141–142 °C. FTIR (KBr): 3340, 3450, 3063, 2929, 1693, 1609, 1388 cm⁻¹. ¹H-NMR (CDCl₃, 200 MHz): δ 8.59 (d, J = 7.2 Hz, 2H), 8.21 (d, J = 8.0 Hz, 2H), 7.75 (t, J = 8.0 Hz, 2H), 4.19 (t, J = 7.3 Hz, 2H), 2.76 (t, J = 6.9 Hz, 2H), 1.8 (m, 2H). ¹³C-NMR (DMSO- d_{cr} 50 MHz): δ 164.1, 133.7, 131.5, 131.3, 126.9, 122.7, 41.8, 39.5, 28.5.

2-(4-Aminobutyl)-1H-benzo[de]isoquinoline-1,3(2H)dione (6c)

Yellow solid, 223 mg, 0.83 mmol, 33% yield. Mp 129–130 °C. FTIR (KBr): 3343, 3448, 3058, 2931, 1690, 1615, 1373 cm⁻¹. ¹H-NMR (CDCl₃, 200 MHz): δ 8.59 (dd, J = 7.3, 1.1 Hz, 2H), 8.21 (dd, J = 7.3, 1.1 Hz, 2H), 7.75 (t, J = 7.9 Hz, 2H), 4.21 (t, J = 7.5 Hz, 2H), 2.76 (t, J = 6.9 Hz, 2H), 1.77 (m, 2H), 1.57 (m, 2H). ¹³C-NMR (DMSO- $d_{6'}$, 50 MHz): δ 164.2, 133.9, 131.5, 131.2, 126.9, 122.5, 41.9, 40.1, 25.4, 31.2.

2-(6-Aminohexyl)-1H-benzo[de]isoquinoline-1,3(2H)dione (6d)

Yellow solid, 313 mg, 1.06 mmol, 42% yield. Mp 101–102 °C. FTIR (ATR): 3340, 3452, 3062, 2929, 1690, 1612, 1379 cm⁻¹. ¹H-NMR (CDCl₃, 200 MHz): δ 8.59 (d, J = 7.1 Hz, 2H), 8.2 (d, J = 7.9 Hz, 2H), 7.75 (t, J = 7.9 Hz, 2H), 4.2 (t, J = 7.4 Hz, 2H), 2.78 (t, J = 6.6 Hz, 2H), 1.69 (m, 2H), 1.59 (m, 2H), 1.39 (m, 2H), 1.31 (m, 2H). ¹³C-NMR (DMSO- $d_{6'}$ 50 MHz): δ 164.1, 133.8, 131.5, 131.2, 126.9, 122.6, 42.0, 32.2, 26.4, 26.4, 32.7, 40.4



Figure 7. Emission spectra obtained by titration of **3b** with Hg(ClO₄)₂ in acetonitrile (a), and its emission profiles obtained by titration with different metal ions. [**3b**] = 1×10^{-5} M. λ_{ex} (**3b**) = 328 nm.



Figure 8. (a) Emission spectra obtained in the titration of **3b**-Hg(II) complex with Cu(II). (b) Batch competitive assay with **3b**, Hg(II) and Cu(II).

2-(8-Aminooctyl)-1H-benzo[de]isoquinoline-1,3(2H)dione (6e)

Yellow solid, 261 mg, 0.81 mmol, 32% yield. Mp 71–72 °C. FTIR (ATR): 3342, 3452, 3062, 2930, 1682, 1610, 1382 cm⁻¹. ¹H-NMR (CDCl₃, 200 MHz): δ 8.58 (dd, *J* = 7.2, 0.9 Hz, 2H), 8.21 (dd, *J* = 8.3, 0.9 Hz, 2H), 7.74 (dd, *J* = 8.3, 7.2 Hz, 2H), 4.16 (t, *J* = 7,3 Hz, 2H), 2.63 (t, *J* = 6.6 Hz), 1.84–1.69 (m, 4H), 1.50–1.41 (m, 4H), 1.37–1.25 (m, 4H). ¹³C-NMR (DMSO-*d*₆, 50 MHz): δ 164.1, 133.8, 131.5, 131.2, 126.9, 122.7, 42.2, 40.5, 33.7, 29.3, 28.1, 27.1, 26.8.

General procedure for the synthesis of dyads 3

In a round-bottom flask, a naphthalimide derivative **6** (1 equiv.) was dissolved in 20 mL of dry THF or DMF depending on the solubility under argon atmosphere. Then, isatoic anhydride (44 mg, 0.27 mmol) was added portion wise, and the mixture was refluxed for 2 h. Finally, the solvent was removed under vacuum pressure, and the solid was washed with acetonitrile.

2-amino-N-(2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)ethyl)benzamide (3a)

Yellow solid, 36 mg, 0.10 mmol, 24% yield based on 100 mg (0.41 mmol) of **6a**. Mp 189–190 °C. FTIR (KBr): 3465, 3377, 3362, 3062, 2964, 1692, 1643, 1635, 1610, 1585, 1334, 1233 cm⁻¹. ¹H-NMR (DMSO- $d_{6'}$ 400 MHz): δ 8.48 (dd, J = 7.2, 1.1 Hz, 2H), 8.45 (dd, J = 8.3, 1.0 Hz, 2H), 8.27 (t, J = 6.0 Hz, 2H), 7.86 (dd, J = 8.3, 7.2 Hz, 2H), 7.28 (dd, J = 7.9, 1.4 Hz, 2H), 7.07 (ddd, J = 8.3, 7.2, 1.5 Hz, 1H), 6.62 (dd, J = 8.2, 1.0 Hz, 1H), 6.42 (ddd, J = 8.3, 7.2, 1.1 Hz, 1H), 6.17 (br, 2H), 4.26 (t, J = 5.8 Hz, 2H), 3.57 (dt, J = 6.0, 5.8 Hz, 2H). ¹³C-NMR (DMSO- $d_{6'}$ 50 MHz): δ 169.5, 164.2, 149.8, 134.6, 131.9, 131.8, 131.1, 128.5, 127.7, 122.9, 116.6, 115.7, 115.0, 40.1, 37.3. MS(EI) m/z (rel. int.): 359 (5) [M]⁺, 342 (2), 223 (100), 162 (9), 136 (3), 70 (45). HRMS (CI⁺): calculated for C₂₁H₁₈N₃O₃ [M + H]⁺, m/z = 360.1348, found for [M + H]⁺, m/z 360.1336.

2-amino-N-(3-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)propyl)benzamide (3b)

Yellow solid, 106 mg, 0.28 mmol, 72% yield based on 100 mg (0.39 mmol) of **6b**. Mp 177–178 °C. FTIR (KBr): 3340, 3261, 3063, 2929, 1693, 1609, 1338 cm⁻¹. ¹H-NMR (CDCl₃, 200 MHz): δ 8.62 (d, *J* = 7.3 Hz, 2H), 8.23 (d, *J* = 8.4 Hz, 2H), 7.77 (dd, *J* = 8.4, 7.3 Hz, 2H), 7.60 (d, *J* = 7.7 Hz, 1H), 7.26 (br, 1H), 7.20 (d, *J* = 7.7 Hz, 1H), 6.76–6.65 (m, 2H), 5.62 (br, 2H), 4.34 (t, *J* = 6.1 Hz, 2H), 3.41 (dt, *J* = 6.1, 5.8 Hz, 2H), 2.05 (qnt, *J* = 6.1 Hz, 2H). ¹³C-NMR (DMSO-*d*₆, 50 MHz): δ 169.2, 164.8, 149.0, 134.3, 132.1, 131.6, 128.2, 127.4, 122.3, 117.3, 116.7, 116.1, 37.4, 35.7, 27.8. MS(EI) *m/z* (rel. int.): 373 (12) [M]⁺, 253 (2), 236 (100), 198 (9), 137 (8), 120 (76). HRMS (CI⁺):



Figure 9. Reversibility study of the ion recognition using EDTA as competitive ligand with 3b-Hg(II). Inset: Fluorescence profile upon addition of EDTA.



Figure 10. ¹H-NMR spectra obtained in the titration of **3b** with $Zn(ClO_4)_2$ in CD_3CN . [**3b**] = 5 mM.

calculated for $C_{22}H_{20}N_3O_3[M + H]^+$, m/z = 374.1505, found for $[M + H]^+$, m/z 374.1501.

2-amino-N-(4-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)butyl)benzamide (3c)

Yellow solid, 62 mg, 0.16 mmol, 43% yield based in 100 mg (0.37 mmol) of **6c**. Mp 165–166 °C. FTIR (KBr): 3348, 3277, 3059, 2931, 1690, 1615, 1373 cm⁻¹. ¹H-NMR (CDCl₃, 200 MHz): δ 8.60 (dd, J = 6.0, 1.0 Hz, 2H), 8.21 (dd, J = 8.0, 1.0 Hz, 2H), 7.75 (dd, J = 8.0, 6.0 Hz, 2H), 7.38 (dd, $J = 7.4, 1.0 \text{ Hz}, 1\text{H}), 7.18 (ddd, J = 7.8, 7.4, 1.6 \text{ Hz}, 1\text{H}), 6.65 (dd, J = 7.6, 1.6 \text{ Hz}, 1\text{H}), 6.63 (ddd, J = 7.8, 7.6, 1.0 \text{ Hz}, 1\text{H}), 6.45 (s, 1\text{H}), 5.51 (br, 2\text{H}), 4.23 (t, J = 7.1 \text{ Hz}, 2\text{H}), 3.52 (dt, J = 6.5, 6.0 \text{ Hz}, 2\text{H}), 1.93-1.66 (m, 4\text{H}). ¹³C-NMR (DMSO-d_{6'}, 50 \text{ MHz}): \delta 169.3, 164.3, 148.7, 134.0, 132.1, 131.5, 131.3, 128.1, 127.3, 127.0, 122.5, 117.2, 116.5, 114.6, 39.6, 39.4, 26.8, 25.5. MS(EI): <math>m/z$ (rel. int.): 387 (88) [M]⁺, 267 (46), 222 (18), 197 (16), 136 (19), 120 (100). HRMS (CI⁺): calculated for $C_{23}H_{22}N_3O_3$ [M + H]⁺, m/z = 388.1661, found for [M + H]⁺, m/z 388.1682.



Figure 11. (Colour online) Infrared spectra of 3b and 3b-Zn(II) complex.



Figure 12. (Colour online) Optimised geometries by DFT for dyad 3a (a) and 3b (b).

2-amino-N-(6-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)hexyl)benzamide (3d)

Brown solid, 48 mg, 0.116 mmol, 43% yield based in 80 mg (0.27 mmol) of **6d**. Mp 161–162 °C. FTIR (KBr): 3351, 3281, 3058, 2931, 1696, 1614, 1377 cm⁻¹. ¹H-NMR (CDCl₃,

200 MHz): δ 8.57 (dd, J = 7.3, 0.8 Hz, 2H), 8.20 (dd, J = 7.9, 0.8 Hz, 2H), 7.74 (dd, J = 7.9, 7.3 Hz, 2H), 7.37 (dd, J = 7.4, 1.3 Hz, 1H), 7.18 (ddd, J = 8.3, 7.9, 1.5 Hz, 1H), 6.69–6.59 (m, 2H), 6.29 (br, 1H), 5.15 (br, 2H), 4.19 (t, J = 7.3 Hz, 2H), 3.40 (dt, J = 6.6, 6.1 Hz, 2H), 1.77 (qnt, J = 6.9 Hz, 2H), 1.66–1.56 (m, 2H), 1.50–1.46 (m, 4H). ¹³C-NMR (DMSO- $d_{6'}$ 50 MHz): δ 169.4, 164.3, 148.6, 133.9, 132.1, 131.6, 131.2, 127.2, 127.0, 122.7, 117.2, 116.6, 40.1, 39.4, 29.4, 27.9, 26.4, 26.4. MS(EI) m/z (rel. int.): 415 (84) [M]⁺, 295 (61), 198 (25), 150 (34), 120 (100). HRMS (CI⁺): calculated for C₂₅H₂₆N₃O₃ [M + H]⁺, m/z = 416.1974, found for [M + H]⁺, m/z 416.1963.

2-amino-N-(8-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)octyl)benzamide (3e)

Yellow solid, 58 mg, 0.131 mmol, 42% yield based in 100 mg (0.31 mmol) of **6e**. Mp 197–198 °C. FTIR: 3452, 3342, 3062, 2930, 1682, 1610, 1282 cm⁻¹. ¹H-NMR (CDCl₃, 200 MHz): δ 8.56 (d, *J* = 6.3 Hz, 2H), 8.18 (d, *J* = 7.0 Hz, 2H), 7.73 (dd, *J* = 7.0, 6.3 Hz, 2H), 7.30 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.17 (ddd, *J* = 8.3, 7.9, 1.3 Hz, 1H), 6.67 (d, *J* = 8.2 Hz, 1H), 6.63 (ddd, *J* = 7.8, 7.4, 1.0 Hz, 1H), 6.13 (s, 1H), 5.46 (br, 2H), 4.16 (t, *J* = 7.5 Hz, 2H), 3.38 (dt, *J* = 6.8, 6.1 Hz, 2H), 1.76–1.66 (m, 2H), 1.62–1.52 (m, 2H), 1.43–1.25 (m, 8H). ¹³C-NMR (DMSO-*d*_{6'} 50 MHz) δ : 169.0, 164.2, 148.5, 133.9, 132.1, 131.5, 131.2, 127.1, 127.0, 122.6, 117.2, 116.6, 40.4, 39.7, 29.6, 29.2, 29.1, 28.0, 27.0, 27.0. MS(EI) *m/z* (rel. int.): 443 (8) [M]⁺, 323 (6), 295 (4), 198 (39), 120 (100). HRMS (CI⁺):



Figure 13. (Colour online) Optimised MOs for dyad 3a.



Figure 14. (Colour online) Optimised geometries of **3b-Hg(II**) (a) and **3b-Zn(II**) (b) complexes with the PM7 semiempirical method.

calculated for $C_{27}H_{30}N_3O_3 [M + H]^+$, m/z = 444.2287, found for $[M + H]^+$, m/z 444.2287.

N-propyl 2-aminobenzamide (8)

In a round-bottom flask, propylamine (1.1 equiv.) was dissolved in 20 mL of dry THF under argon atmosphere. Then, isatoic anhydride (200 mg, 1.23 mmol) was added portion wise, and the mixture was refluxed for 2 h. Finally, the solvent was removed under vacuum pressure, and the solid was washed with acetonitrile.

Pale brown solid, 110 mg, 0.617 mmol, 50% yield. Mp 83–84 °C. FTIR (ATR): 3420, 3296, 3189, 3063, 2960, 1633, 1617, 1532, 1309, 1258, 1152 cm⁻¹. ¹H-NMR (CDCl₃, 200 MHz): δ 7.30 (dd, J = 7.7, 1.5 Hz, 1H), 7.20 (ddd, J = 8.2, 7.2, 1.5 Hz, 1H), 6.67 (d, J = 8.2 Hz, 1H), 6.64 (ddd, J = 7.9, 7.2, 1.2 Hz, 1H), 6.11 (s, 1H), 5.21 (br, 2H), 3.37 (dt, J = 7.2, 5.8 Hz, 2H), 1.62 (sext, J = 7.2 Hz, 2H), 0.98 (t, J = 7.2 Hz, 3H). ¹³C-NMR (DMSO- $d_{6'}$ 50 MHz) δ : 169.4, 148.5, 132.1, 127.0, 117.2, 116.6, 41.4, 22.9, 11.5. MS(EI) m/z (rel. int.): 178 (50) [M]⁺, 120 (100), 92 (48), 65 (80). MS(CI⁺): 179 [M + H]⁺. HRMS (CI⁺): calculated for $C_{10}H_{15}N_2O$ [M + H]⁺, m/z = 179.1184, found for [M + H]⁺, m/z 179.1184.

N-propyl 1,8-naphthalimide (9)

In a round-bottom flask, propylamine (4 equiv. 10.08 mmol) was dissolved in 50 mL of toluene, and 1,8-naphthalenetetracarboxylic acid anhydride (4) (200 mg, 1.01 mmol) was added portion wise; the mixture was stirred for 30 min at room temperature. Then, the reaction mixture was stirred and refluxed for 4 h. The solvent was evaporated, and the solid was successively washed and filtered with CH_2CI_2 , Et₂O and petroleum ether.

Pale yellow solid, 130 mg, 0.54 mmol, 54% yield. Mp 151–152 °C. FTIR (ATR): 3077, 2950, 1691, 1654, 1624, 1588, 1346, 1237 cm⁻¹. ¹H-NMR (CDCl₃, 400 MHz): δ 8.59 (d, *J* = 7.3 Hz, 2H), 8.19 (d, *J* = 8.2 Hz, 2H), 7.73 (dd, *J* = 8.2, 7.3 Hz), 4.15 (t, *J* = 7.5 Hz, 2H), 1.77 (sext, *J* = 7.5 Hz, 2H), 1.02 (t, *J* = 7.5 Hz, 3H). ¹³C-NMR (DMSO-*d*₆, 50 MHz) δ : 164.2, 133.8, 131.6, 131.1, 128.2, 126.9, 122.8, 41.9, 21.4, 11.5. MS(EI) *m/z* (rel. int.): 239 (80) [M]⁺, 197 (100), 180 (50), 126 (40). MS(Cl⁺): 240 [M + H]⁺. HRMS(Cl⁺): calculated for C₁₅H₁₄NO₂ [M + H]⁺, *m/z* = 240.1025, found for [M + H]⁺, *m/z* 240.1021.

Determination of quantum yields

Fluorescence quantum yields was measured using anthracene in toluene as standard with a known ϕ_F of 0.30. Solutions of dyads **3(a-e)**, **8** and **9** were prepared at 1×10^{-6} M in spectroscopic grade acetonitrile. Absorbance and fluorescence spectra were measured, and the data required for Equation (1) were obtained from these spectra.

Procedure for spectroscopic titrations

The effect of metal cations upon the absorbance and fluorescence intensities was examined by adding 10 μ L of a 1 \times 10⁻³ M metal ion stock solution to a known volume (3 mL) of 1 \times 10⁻⁵ M ligand solution. The addition was limited to 0.09 mL, so that dilution remained insignificant.



Scheme 1. Synthesis of dyads 3(a-e).

Reversibility analysis

The reversibility analysis was examined by adding 6 μ L of 1×10^{-3} M EDTA stock solution to a known volume (3 mL) of 1×10^{-5} M ligand and 1.5×10^{-5} M Hg(ClO₄)₂ solutions. The dilution remained insignificant, as the addition was limited to 0.06 mL.

Voltamperometric analysis

Voltamograms were obtained in potentiostat–galvanostat station using Ag/AgCl as reference electrode, Pt as counter electrode and Pt as working electrode. The solutions of **8** $(3.4 \times 10^{-3} \text{ M})$, **9** $(6.4 \times 10^{-5} \text{ M})$ and **3b** were prepared in an electrolytic solution of KBF₄ (0.07 M).

Results and discussion

Synthesis and characterisation

The synthesis of dyads 3(a-e) started with the reaction of 1,8-naphthalenetetracarboxylic acid anhydride (4) with a series of dialkylamines (with n = 0, 1, 2, 4 and 6) at 80 °C in aqueous media. 1,8-naphthalimide alkylamines 6(a-e) were obtained in good yields and purity; these products reacted with isatoic anhydride (7) in THF at reflux providing the dyads 3 in moderate yields (Scheme 1) (27). In addition, *N*-propyl-2-aminobenzamide (8) and *N*-propylnaphthalimide (9) were synthesised under the same conditions, in order to compare the spectroscopic and photophysical properties of the individual chromophores with those for dyads 3(a-e).

Dyads **3(a-e)** were characterised by infrared spectroscopy (IR), NMR and electronic impact mass spectrometry MS(EI), and their chemical composition was verified by high-resolution mass spectrometry (HRMS). Dyad **3b** IR spectra showed the vibrations corresponding to N-H stretching at 3460 and 3400 cm⁻¹ for amine and 3345 cm⁻¹ for amide groups. The carbonyl vibrations in NAPIM moiety were at 1693 and 1653 cm⁻¹ for symmetric and asymmetric stretching, and at 1638 cm⁻¹ was identified as the stretching vibration for amide carbonyl (Figure S1). The rest of dyads presented the same vibration pattern.

In ¹H-NMR spectra obtained in CDCl₃, the typical patterns of signal for NIPAM and 2ABZ moieties were found (Table S1). It is noteworthy that there are significant differences in chemical shift for amide hydrogens H_a and the signals appear at 6.93, 7.28, 6.45, 6.29 and 6.15 ppm, respectively. In dyads with shorter alkyl chains, 3a and 3b, the amide hydrogens are unprotected, and as the polymethylene chain becomes longer, the signal shifts to up-field. In fact, in dyad 3e, the signal almost has the same chemical shift than in compound 8. In accordance with these results is convincing that the presence and proximity of NAPIM moiety affect the chemical environment of the amide hydrogens. Also, an interesting solvent influence over the chemical shift of **3a** signals was found when it was analysed in CD₃CN and DMSO- d_6 . The signal for H_f shifted to 7.05 ppm in CD₃CN and 8.27 ppm in DMSO-d₆. Another significant change as function of solvent was the chemical shift of hydrogens H_a , H_h and H_i . These hydrogen signals are in 8.59, 7.72 and 8.18 ppm in CDCl₃, but in DMSO- d_{6} , H_a shifted to up-field (8.48 ppm) and H, and H, shifted downfield (8.45 and 7.86 ppm, respectively). Additionally, all the aromatic hydrogens of 2ABZ moiety appear at slightly high field compared with the same signals in CDCl₂. These differences may be attributed to an interaction with the solvent, which increases with the polarity and viscosity.

Dyads **3(a-e)** were analysed by MS(EI), and the molecular ions M⁺ at 359, 373, 387, 415 and 443 amu were, respectively, detected. MS(CI⁺) analysis provided the corresponding $[M + H]^+$ ions at 360, 374, 388, 416 and 444 amu, respectively; and HRMS confirmed the chemical composition for all compounds.

| Compound | MeCN | | | |
|----------|------------------|----------------------------|--|------------|
| | λ_{A}/nm | $\lambda_{\rm F}/{\rm nm}$ | $\lambda_{\rm A} - \lambda_{\rm F} / \rm nm$ | Φ_{F} |
| 8 | 330 | 390 | 60 | 0.189 |
| 9 | 343 | 376 | 33 | 0.097 |
| 3a | 350 | 432 | 82 | 0.030 |
| 3b | 339 | 397 | 58 | 0.090 |
| 3c | 340 | 398 | 58 | 0.100 |
| 3d | 336 | 390 | 54 | 0.126 |
| Зе | 331 | 388 | 57 | 0.123 |

Table 1. Fluorescence quantum yields (ϕ F) of 8, 9 and dyads 3(a-e) in MeCN.

Photophysical properties of NAPIM-2ABZ dyads

In order to determine whether there is an interaction between the two chromophores in the ground state, the absorption spectra of **8**, **9** and **3b** were compared. The Figure 2(a) shows that the spectrum of dyad **3b** corresponds to the addition spectra of **8** and **9**. It has the absorption band at 230 nm and the structured band at 332 nm belonging to NAPIM chromophore and the absorption bands at 214 (π – π^*), 255 (n– π^*) and 326 nm (auxocrome n– π^*) belonging to aminobenzamide **8**. The same pattern in the rest of dyads was observed (Figure 2(b)). Based on these results, no ground state interaction between the two chromophores in dyads **3(a-e)** was observed.

As well as the absorbance, the fluorescence spectra of **8**, **9** and **3b** were obtained and are compared in Figure 3(a). Compound **8** exhibits a high-intensity broad emission band with a maximum peak at 394 nm, whereas **9** presents a less intense typical structured emission band with a maximum peak at 378 nm. Dyad **3b** has a broad emission band with a maximum peak at 398 nm, but it is twenty times less intensive than **8**. Dyads **3(c-e)** have the same shape emission band, and the quenching of fluorescence intensity for all of them was observed. Although it is interesting that the fluorescence intensity increases as the polymethylene chain becomes greater. Again, dyad **3a** presents singular fluorescent properties, and a low-intensity broad emission band with a maximum peak at 430 nm was detected.

The fluorescence efficiency of dyads **3(a-e)** was estimated by measuring the fluorescent quantum yield ϕ_F using Equation (1).

$$\phi_F = \phi_R \times \ln t A_R n^2 / \ln t_R A n_R^2 \tag{1}$$

where ϕ_F 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 quantum yields for **8**, **9** and dyads **3(a-e)** measured in acetonitrile at 1×10^{-6} using anthracene as reference substance are presented in Table 1.

As was expected, compound 8 has higher guantum yield than 9, which is in accordance with the fluorescent intensity in Figure 3(a). In dyads 3(b-e), quantum yields are lower than 8 and comparable with 9, although the quantum yield dyad 3a is even lower. These results indicate that a fluorescence quenching of NAPIM and 2ABZ occurs by a PET process as in NAPIM-DANS dyads (15,16). According to these quenching mechanisms, irradiating at 328 nm, both fluorophores may be excited to form ¹(NAPIM)*-2ABZ and NAPIM-¹(2ABZ)*; if PET mechanism occurs from 2ABZ to NAPIM forms NAPIM[⊖]-2ABZ[⊕], but if it occurs from NAPIM to 2ABZ forms NAPIM[⊕]-2ABZ[⊖]; in either case, the energy emission is inactivated and the quantum yields are lower. Voltamperometric analysis of 8, 9 and 3b was performed in order to establish the direction of the electronic transfer. Compound 8 showed a reduction peak at -0.713 V and a guasi-reversible oxidation peak at -0.569 V, and 9 presented reduction and oxidation peaks at -1.250 V and -1.200 V, respectively. The dyad **3b** shows one reduction and oxidation peak at -1.230 and -1.180 V associated with the NAPIM moiety, and a quasi-reversible oxidation peak at -0.690 V corresponding to the 2ABZ (see Figure S2). These results indicate that 2ABZ moiety is easier to reduce than NAPIM when they are separated, but if they are linked 2ABZ, only oxidizes while the NAPIM behave a redox process. This is a clear evidence that the electronic transfer from 2ABZ to NAPIM is possible. The quenching of fluorescence by PET is more effective in dyads with shorter spacer as **3a**, but with the longest polymethylene chain the effect of PET is not so strong. In addition, the red shift (40 nm) and broadening emission of **3a** may suggest an intermolecular interaction between both fluorophores in the excited state.

Metal ion binding properties

Evaluation of binding properties, coordination of compound **8** with a different alkaline, alkaline earth and transition metallic ions was studied by electronic absorption (UV-vis) and fluorescence in acetonitrile. Nevertheless,

| Table 2. Binding constants | (log Kf) for some comp | lexes in MeCN. |
|----------------------------|------------------------|----------------|
|----------------------------|------------------------|----------------|

| Dyad | Metal ion | Log | g K _f |
|------|-----------|--------------------|-------------------------------|
| | | ML ₂ | M ₃ L ₂ |
| 3a | Hg(II) | 5.950 ± 0.240 | 10.048 ± 1.660 |
| | Cu(II) | 8.381 ± 0.044 | |
| | Zn(II) | 8.446 ± 0.029 | |
| | Ni(II) | 7.625 ± 0.132 | |
| 3b | Hg(II) | | 9.378 ± 1.520 |
| | Cu(II) | 10.689 ± 0.050 | |
| | Zn(II) | 9.428 ± 0.026 | |
| | Ni(II) | 7.748 ± 0.132 | |
| 3c | Hq(II) | 9.003 ± 0.043 | |
| | Cu(II) | 9.188 ± 0.029 | 7.974 ± 2.828 |
| | Zn(II) | 9.427 ± 0.026 | |
| | Ni(II) | 7.748 ± 0.132 | |
| 3d | Ha(II) | 9.004 ± 0.042 | 6.357 ± 0.088 |
| | Cu(II) | 9.188 ± 0.029 | 3.282 ± 0.174 |
| | Zn(II) | 8.997 ± 0.049 | |
| | Ni(II) | 8.229 ± 0.235 | |
| 3e | Ha(II) | | |
| | Cu(II) | 9.188 ± 0.028 | 12.184 ± 1.848 |
| | Zn(II) | 9.428 ± 0.025 | |
| | Ni(II) | 8.230 ± 0.026 | |

transition metal ions such as Cu(II), Hg(II) and Zn(II), as perchlorate salts, gave important spectroscopic changes. The absorption bands were most affected with Cu(II) and Hg(II). Several isosbestic points indicating the interaction of the ligand with the metal ions were observed. It is noteworthy that the corresponding $n-\pi^*$ transition absorption band at 326 nm due to the electron delocalisation from the amino group to the benzamide and other transitions in the carbonyl group at 255 $(n-\pi^*)$ and 214 $(\pi-\pi^*)$ nm were considerably diminished (Figure 4(a)). This behaviour indicates coordination of the metal ions with amino and carbonyl groups. The absorbance profile at 326 nm showed at least two different complexes, one with a 1:2 and other with a 3:2 metal–ligand ratio (Figure 4(b)).

The fluorescence of **8** was completely quenched by Cu(II) and Hg(II) in acetonitrile. The FI/FI₀ profile at 394 nm confirmed the formation of two complexes with a 1:2 and 3:2 metal–ligand ratio (Figure 5). The fluorescence of **8** is quenched in the complex due to the suppression of the ICT process of the 2ABZ. In other words, the excitation band at 326 nm ($S_0 \rightarrow S_1$) in the fluorescence spectra of **8** corresponds to the auxocrome $n-\pi^*$ absorption band in UV-vis. This transition disappears when the metal ion coordinates with the ligand; there is not excitation in the complex at this wavelength, and therefore, there is no emission of fluorescence (Figure S3).

The study of binding properties of **8** gives important information regarding this type of ligands: (a) the complexation with metal ions occurs in the ground state, (b) amino and carbonyl groups participate as coordination sites, (c) coordination with metal ion decreases the absorbance and quenches the fluorescence of 2ABZ and (d) there is selectivity towards Cu(II) and Hg(II) ions in a well-defined metal–ligand ratio. This information was important for interpreting the results obtained with dyads **3(a-e)**.

The binding of dyads 3(a-e) with different alkaline, alkaline earth and transition metallic ions was studied by UV-vis and fluorescence in acetonitrile. Again, only transition metal ions such as Cu(II), Hg(II), Zn(II) and Ni(II), as perchlorate salts, gave important spectroscopic changes. Interestingly in the titration of **3b** with Hg(II) in acetonitrile, the absorbance at 214, 253 and 334 nm decreases (Figure 6). These bands, belonging to the 2ABZ moiety, disappear during the titration. The final spectrum becomes equal to the spectrum of naphthalimide 9. This result indicates that the binding site is the 2ABZ and that the NAPIM moiety does not participate in the complexation in the ground state. The absorbance profile showed two successive complex formations with 1:2 and 2:3 metal-ligand stoichiometric ratio, as observed for compound 8. Similar results with the rest of dvad were observed.

The dyads with shorter oligomethylene chain **3a**, **3b** and **3c** presented a strong inhibition of PET process by the addition of metallic ions in the order Hg(II) >> Cu(II) > Zn(II) >> Ni(II). A semi-structured emission band at 388 nm increases in dyad **3a** as the amount of Hg(II) increases beyond 0.5 equivalents (Figure S4), and the emission turns from yellow to blue color. The fluorescence profiles showed higher sensibility for Hg(II) (FI/FI₀ = 13.5), and is evident the formation of two different complexes with 1:2 and 3:2 metal–ligand stoichiometric ratio, respectively.

In dyad **3b** with Hg(II) and Cu(II) increases, the florescence intensity and a band shift from 398 nm to 408 nm were observed upon the addition of 0.5 equivalents, evidencing the formation of a 1:2 metal:ligand complex. Continuing with the addition of metallic ion increases the intensity of a structured band at 380 nm (Figure 7 and S5) characteristic of NAPIM fluorophore. Dyads 3b and 3c were more sensitive towards Hg(II) (FI/FI₀ 10 and 4.6, respectively), followed by Cu(II) (FI/FI₀ - 6.05 and 2.6, respectively), and the stoichiometric ratio of these ligands is the same that of dyad **3a**. However, in dyads **3a**, **3b** and **3c**, the fluorescence increases to a maximum value upon the addition of 1.5 equivalents of Cu(II), and beyond this point the fluorescence decreases again. It is believed that Cu(II) guenches fluorescence by collisions with NAPIM fluorophore, and the paramagnetic nature of this ion may promote charge transfer from the ligand to metal centre or another energy deactivating mechanism. Additionally, in dyad **3b** is interesting the response profile with Zn(II), fluorescence increases two times when 0.5 equivalents of Zn(II) were added, and it remains constant until three equivalents. The Job's plot indicates a 1:2 metal-ligand ratio in the complex **3b**-Zn(II), and 1:2 and 3:2 in **3b**-Hg(II) (Figure S6).

Dyads 3d and 3e present an opposite fluorescent response with the addition of metallic ions. Interestingly, the intensity of the broad emission band at 395 nm decrease and a structured band of NAPIM appears at 380 nm. In the titration of 3d with Hg(II), a minor fluorescence guenching occurs until the NAPIM structured emission band appears, and its intensity increases slightly upon the addition of more equivalents of Hg(II) (Figure S7). However, in the titration with Cu(II), the guenching of fluorescence is predominant due to the guenching of Cu(II) to the NAPIM fluorescence, as occurs in the other dyads. In dyad **3e** occurs a fluorescence guenching with both cations, but Cu(II) induces a strongest effect. Once the NAPIM emission band appears in the titration with Hg(II), it remains almost constant upon the addition of more Hg(II), while Cu(II) guenches the NAPIM fluorescence (Figure S8). This peculiar ON-OFF fluorescence response indicates that the distance of six and eight methylene groups between NAPIM and 2ABZ in 3d and 3e does not favour PET process (Figure S9). The fluorescence of 2ABZ is guenched by suppression of the ICT process in coordination with the metallic ion as occurs in the ligand 8, and there is no fluorescence enhancement of NAPIM.

Comparing the response profile with Hg(II) and Cu(II), dyads **3a-3c** exhibit an *OFF–ON* fluorescence behaviour due to the suppression of PET when the metallic ion coordinates to 2ABZ moiety (see Figure S10). It is noteworthy that dyad **3a** presents the highest enhancement of fluorescence for Hg(II) and **3b** for Cu(II) indicating that an alkyl spacer with two and three methylene groups give the optimal distance between NAPIM and 2ABZ fluorophores to inhibit the PET process upon coordination.

Based on the previous results, competitive assays were performed in order to establish the selectivity of dyad **3b** towards Cu(II) or Hg(II). First, a batch experiment

combining dyad **3b** with 1.5 M equivalents of each cation and with both Cu(II) and Hg(II) was done. Fluorescence of free ligand and complexes were measured, and the relative intensities with respect to the free ligand are presented in Figure 8. The relative fluorescence in the mixture with both cations is more comparable to the observed for the complex with Cu(II). In a second experiment, a solution of complex 3b-Hq(II) was titrated with Cu(II) from 0.0 to 3.0 M equivalents (Figure 8(b)). The quenching of the fluorescence was found as result from a $FI/FI_0 = 9.5$ to 3.8 indicating the displacement of Hg(II) by Cu(II). Interestingly, the emission band in the final addition of the titration is exactly the same as observed for the 1:2 metal-ligand complex obtained in the direct titration of **3b** with Cu(II). This demonstrates the complex 1:2 is more stable than 3:2. The results show that **3b** has binding preferences towards Cu(II) over Hg(II) ion. Competitive assays for the rest of dyads showed no selective response to any of these two cations (Figures S11 to S14). Therefore, selectivity of 3b towards Cu(II) ions can be explained based on the hardsoft acid base theory (28). Considering that Cu(II) ion and the coordination binding site of **3b** have been classified as borderline species (28-30), the formation of the adduct **3b**-Cu(II) will be favoured rather than the adduct **3b**-Hg(II). which can be described as a borderline-soft interaction.

In addition, the reversibility of the ion recognition using EDTA as competitive ligand was studied. In Figure 9, the emission spectra of **3b** (λ_{em} = 398 nm, full line) and the spectra obtained in the titration of complex 3b-Hg(II) with 0.0 eq (dashed line) to 2.0 eq (dotted lines) of EDTA in acetonitrile are compared. The structured fluorescence emission band at λ_{em} = 386 nm decreased as the EDTA solution was gradually added, but it remained constant from 1.0 to 2.0 equivalents. Nevertheless, the free ligand emission band was not recovered, and the shape and position of the emission corresponded to the NAPIM fluorophore. These results mean that EDTA competes by the Hg(II) in the 3:2 metal-ligand complex, but the complex 1:2 remains even in the presence of an excess of EDTA. In other words, 1.0 eq. of Hg(II) was combined with EDTA and the other 0.5 eq. with 3b. The same experiment with 3b-Cu(II) was performed, and in this case the emission band of 3b was recovered, and with the excess of EDTA the fluorescence was guenched (see Figure S15).

The formation of complex **3b**-Zn(II) was analysed by an NMR titration of a **3b** solution in CD₃CN with Zn(ClO₄)₂. A stacked ¹H NMR spectra is shown in Figure 10, which allows to compare how the signals of **3b** behave in the presence of 0.0, 0.2, 0.4, 0.6 and 1.0 M equivalents of Zn(II). The signals *g*, *h* and *i* correspond to the NAPIM hydrogens and after the addition of Zn(II) shift to up-field (0.11, 0.10 and 0.16 ppm, respectively, at 0.6 eq), probably due to a protecting effect over the NAPIM in the complex. The signals

| Dyad | $\Delta E[(HOMO-n)-HOMO]$ (eV) | ΔE(HOMO-LUMO) (eV) | $\Delta E[(HOMO-n)-LUMO)]$ (eV) | Distance (Å) |
|------|--------------------------------|--------------------|---------------------------------|--------------|
| 3a | 1.52 | 2.44 | 3.96 | 7.72 |
| 3b | 1.20 | 2.79 | 3.99 | 8.96 |
| 3c | 1.19 | 2.80 | 3.99 | 10.80 |
| 3d | 1.12 | 2.88 | 4.00 | 14.94 |
| 3e | 1.10 | 2.89 | 4.00 | 17.42 |

Table 3. Energy data of MOs and distance between fluorophores in dyads 3(a-e).

a, b, c, d, e and f correspond to the 2ABZ hydrogens and to all those shifted to downfield except signal e, which shifts to up-field. The most affected signals in the complex are the hydrogens e (0.51 ppm) and f (-0.84 ppm), which are located in the binding site with the metallic ion. The first one shifts to up-field probably because of a retro donating effect of the metallic ion in the complex. The second one shifts to downfield due to an increase in its acidity when carbonyl coordinates with metallic ion. The hydrogens located in orto (d) and para positions (b) also were significantly affected by the coordination of the amine group with Zn(II) because of the absence of electronic density in this positions, and the chemical shifts were -0.5 ppm and -0.66 ppm, respectively. In the graphs of chemical shift versus molar equivalents of Zn(II), there is an inflexion at 0.5 equivalents, corresponding to a 1:2 metal-ligand stoichiometry, which is in accordance with the data obtained by spectroscopic techniques (Figure S16).

The formation of complex with $Hg(CIO_4)_2$ was also analysed by ¹H-NMR, and similar changes were observed upon the addition of the metallic ion. However, the chemical shifts of the signals were higher with Hg(II) compared with Zn(II). Besides, the signal for amine hydrogens becomes broad since the first addition of the metal solution (Figure S17). Interestingly, in the graphs of chemical shifts versus molar equivalents, there is an inflexion at 0.5 equivalents indicating a metal–ligand ratio of 1:2 (Figure S18).

In Figure 11, the infrared spectra of 3b and 3b-Zn(II) complex obtained by ATR are compared. There are significant changes in the vibrations corresponding to the amino N-H (3461 and 3401 cm⁻¹) and amide C=O (1638 cm⁻¹) groups, the N-H stretching vibrations for amino group shifted lower frequencies 3326 and 3260 cm⁻¹, indicating coordination of this group with the metal ion. The amide N-H vibration at 3345 cm⁻¹ was not significantly affected, as well as the carbonyls of NAPIM, indicating these groups do not coordinate with metallic ion. The amide carbonyl shifted from 1638 cm⁻¹ to 1619 cm⁻¹ (~19 cm⁻¹). This result is in accordance with the data reported in the literature (11-13). The band at 1515 cm⁻¹ related with N–H bending vibration shifted to 1544 cm⁻¹. A strong broad vibration at 1059 cm⁻¹ corresponds to the perchlorate acting as counter ion in the complex. Interestingly, two well-defined bands at 1082 and 1043 cm⁻¹ in **3b**-Cu(II) and 1067 and 1034 cm⁻¹ in **3b**-Hg(II) were found, indicating the coordination of perchlorate with the metallic ion in a monodentate fashion (Figures S19 and S20) (*31*). These results indicate a different coordination number of Zn(II) with respect to Cu(II) and Hg(II) in the complexes; Zn(II) may be forming a tetrahedral complex with no vacancy for more ligands, whereas Cu(II) and Hg(II) may be octahedral including perchlorate ligands.

The binding constants K_f of the complexes were determined on the basis of fluorescence responses using the Hyperquad program considering the formation of 1:2 (ML₂) and 3:2 (M₃L₂) complexes (*32,33*). In Table 2 are reported the K_f values for several complexes. In general, the values are in agreement with the analytical responses, and the values of log K_f vary between 8 and 10 because the experiments were performed using acetonitrile as a non-competitive solvent.

Theoretical analysis

The geometries of dyads 3(a-e) were optimised with the PM6 semiempirical method and then by DFT using a non local correlation B3LYP and a 6-31G+(d,p) basis set in the gas phase with Gaussian 09 computational package (34). First, the structure of dyads 3 were optimised at semiempirical level, and then from these geometries, a scan calculation was performed by rotation of a carboncarbon bond in the oligomethylene chain. The relative minimum energy geometries found for each dyad were fully optimised using DFT and after their frequencies were calculated. Figure 12 shows the minimum energy geometries for dyads 3a and 3b. Interestingly, an intramolecular hydrogen bond between the amide N-H and a carbonyl group of NAPIM is present in both structures. The distance between the hydrogen and oxygen atoms is 2.154 and 2.140 Å, respectively. The hydrogen bond maintains closer the two fluorophores in the geometry of **3a** (7.72 Å centre to centre) in almost perpendicular position. In the **3b** optimised geometry, the centre-to-centre distance of both fluorophores is 8.96 Å, but they are almost in the same plane. For dyads 3c-e, any hydrogen bond is present in their minimal energy geometries, and the fluorophores should be as far apart as possible in order to minimise the repulsive interactions (Figure S21).

These theoretical results explain the unexpected chemical shifts in dyads **3a** and **3b** for amide hydrogens in ¹H-NMR; the presence of an intramolecular hydrogen bond shifts the signals and as shorter is the distance more the signal shifts to downfield. Furthermore, the proximity and position of both fluorophores in dyad **3a** favors an interaction between both fluorophores in the excited state. The latter may explain the unique broad emission band shifted to a higher wavelength in this dyad.

Moreover, important information comparing the molecular orbitals (MOs) in these geometries was found (Figure 13). The highest occupied molecular orbital (HOMO) in the dyad 3a is mainly located in 2ABZ fluorophore and the lowest unoccupied molecular orbital (LUMO) is in the NAPIM fluorophore. Comparing the energies of the MOs were observed that the HOMO is located between the HOMO-3 and the LUMO, and these MOs are involved in the excitation and emission of fluorescence in NAPIM. This electronic condition is determinant in the PET process as well as in the distance between the donor and the acceptor. Additionally, the HOMO-3 is located in both moieties and may be an evidence of the interaction of both fluorophores due to their proximity promoted by the intramolecular hydrogen bond. These characteristics are useful to explain the unique photophysical properties of dyad 3a.

In Table 3 are listed the $\Delta E[(HOMO-n)-LUMO]$, $\Delta E[(HOMO-n)-HOMO]$ and $\Delta E(HOMO-LUMO)$ energies and centre–centre distance between the two fluorophores obtained in the optimisation of these molecules under the same calculation procedures and theory level. The HOMO is located between the [(HOMO-n)-LUMO)], and technically PET process would be allowed in all dyads, but also depends on the distance between the donor and the acceptor. For these dyads, the maximum calculated distance in which the PET process is effective was around 10.8 Å.

Considering all the spectroscopic data of the complexes formed with dyads 3 and metallic ions, the geometries of complexes 3a-Hg(II), 3a-Zn(II), 3b-Hg(II) and 3b-Zn(II) were optimised with the PM7 semiempirical method (Figure 14 and S22). Figure 14a shows the plausible structure of complex **3b**-Hg(II) where two Hg(II) ions are coordinated with two ligands through the amino and carbonyl groups of 2ABZ, and another Hg(II) ion coordinates with a carbonyl group of NAPIM moiety. An octahedral configuration for Hg(II) and the six perchlorate units which are coordinated in a monodentade fashion were considered in the optimisation, and this represents a 3:2 metal-ligand ratio. In the structure of complex 3b-Zn(II) (Figure 14(b)), one tetrahedral Zn(II) ion coordinates with two ligands through the amine and the carbonyl groups, the hydrogen bond between the amide N-H and a NAPIM carbonyl remains in the complex, and the coordination of perchlorate units to the Zn(II) was not considered.

Conclusions

In this work, the binding ability of dyads 3 towards metallic ions, of clinical and environmental importance, such as Hg(II), Cu(II) and Zn(II) was demonstrated. Moreover, the ligand design with NAPIM and 2ABZ fluorophores separated by different length polymethylene chains induced versatility in the analytical response. The inhibition of PET in dyads with shorter spacing chain gave an OFF/ON response when 2ABZ moiety coordinates the metallic ion. Analytic studies by UV-vis, IR and ¹H-NMR showed that NAPIM does not participate in the complex. On the contrary, in dyads with longer spacing chains there is ON/OFF fluorescence response due to the absence of PET and the guenching of 2ABZ fluorescence. In addition, the energy of MOs calculated theoretically confirms the existence of PET as well as the optimisation of geometries, and the suitable distance between both fluorophores that favours this process.

Supplemental material

Supplemental data for this article can be accessed online here: http://dx.doi.org/10.1080/10610278.2016.1149180.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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