A two-channel chemosensor for the optical detection of carboxylic acids, including cholic acid

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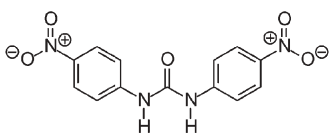
A neutral receptor, in which a urea fragment has been equipped with two naphthaleneimide subunits, on interaction with acetate, in a DMSO solution, undergoes deprotonation of one of the N–H fragments; an event which is signalled by a yellow-to-red colour change and by the quenching of the blue fluorescence of the naphthalneimide subunit, with no competition by a number of anions (phosphate, nitrate, sulfate, chloride or bromide). This procedure can be employed for the visual and spectroscopic detection of cholic acid, even in presence of the other competing bile acids, such as glycocholic and taurocholic.

Introduction

There is a current interest in the development of neutral optical chemosensors for anions to be used for real time and real space analyte detection in molecular physiology, medicinal chemistry and food science.[1] Classical design involves the covalent linking of a signalling unit (a chromophore or a fluorophore) to a specific receptor for the chosen anion.[2–4] It is expected that the receptor–anion interaction modifies the electronic properties of the reporter subunit in its ground and/or excited state, thus giving rise to an optical signal in the form of a colour change and/or modification of the fluorescent emission. Neutral receptors typically contain an N–H fragment (from amides,[5] sulfonamides[6] or pyrroles)[7] which acts as an H-bond donor. In particular, urea contains two rigidly positioned N–H fragments suitable for the interaction with Y-shaped anions, like carboxylates.[8]



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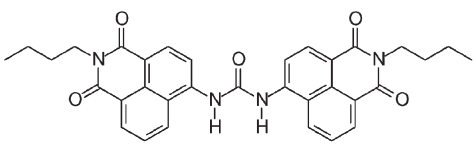

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We have recently investigated the interactions of the urea based receptor **1** with anions in MeCN solution. Presence of the 4-nitrobenzyl substituents enhanced acidity and H-bond donor tendencies of the N–H fragments of **1**, ensuring the formation of stable complexes with carboxylates. Moreover, it was observed that in presence of an excess of fluoride, receptor **1** underwent deprotonation at one N–H fragment, with formation of $[HF_2]^-$.[9] Receptor **1** behaved as a colorimetric sensor, since anion coordination altered the charge transfer transition from each N–H to the NO_2 group (colour change from pale yellow to bright yellow). We wish to consider now the urea based receptor **2**, 1,3-bis-(2-butyl-1,3-dioxo2,3-dihydro-1*H*-benzo[*de*]isoquinolin-6-yl)-urea, which contains two naphthaleneimide substituents; naphthaleneimide being a classical chromogenic group, displaying a charge transfer transitition that originates a yellow colour,[10–12] which could be red-shifted on anion interaction. In addition, molecule **2** displays blue fluorescence whose intensity can be altered on anion interaction, thus providing a further signalling mode. The capability of **2** to act as colorimetric and fluorescent sensor for anions has been investigated in DMSO solution. We anticipate that, in view of its high affinity towards the carboxylate group, **2** is able to selectively sense bile acids, with the events being signalled through a yellow-to-red colour change and quenching of the blue fluorescence.

Results and discussion

The interaction with anions studied by UV-vis spectrophotometry

A DMSO solution of **2** (5.00×10^{-5} M) was titrated with $[Bu_4N]CH_3COO$. On acetate addition, the yellow solution of **2** turned red (see the photograph in Fig. 1).

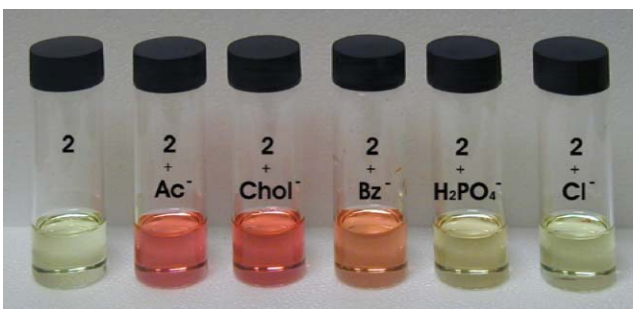

Fig. 1 Colour changes observed on addition of 1 eq. of anion (as a tetrabutylammonium salt; sodium for cholate) to a DMSO solution of receptor **2**.

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Colour change resulted from the disappearance of the receptor's band at 400 nm and the development of the new band at 540 nm, as shown in the family of spectra in Fig. 2.

The presence of two definite isosbestic points indicates that two distinct chromophores are present at the equilibrium. On the other hand, the titration profiles, shown in the inset of Fig. 2, correspond to a process with a 1 : 1 stoichiometry, with an equilibrium constant, determined through non-linear least-squares treatment of titration data, $\log K = 4.97 \pm 0.01$. It has been previously mentioned that the absorption band of the plain receptor, centred at 400 nm, results from a charge transfer transition whose dipole is oriented from the N–H fragment to the carbonyl oxygen atoms of the naphthaleneimide moiety. It has been previously observed for the similar receptor **1** that the drastic red shift of the charge transfer absorption band ($\Delta\lambda$ = 130 nm, from 345 to 475 nm) results from the deprotonation of one of the urea N–H fragments. In particular, generation of a negative electrical charge increases the intensity of the transition dipole (or, in other words, stabilises the excited state), strongly reducing the energy of the absorption band. Thus, the remarkable red shift observed following the **2**–CH_3COO^- interaction ($\Delta\lambda$ = 140 nm, from 400 to 540 nm) in DMSO solution has to be described by the following acid–base neutralisation equilibrium (**2** = LH):

$$LH + CH_3COO^- \leftrightarrows L^- + CH_3COOH \qquad (1)$$

It should be noted that the constant of the equilibrium (eqn. (1)), K, corresponds to the ratio of the constant for the acidic dissociation of LH over the constant for the acidic dissociation of CH_3COOH: $K = K_A(LH)/K_A(CH_3COOH)$. Since in DMSO $pK_A(CH_3COOH) = 12.3$,[13] a value of 7.3 can be calculated for pK_A (LH). Notice that such a value is remarkably lower than that determined in the same medium for plain urea (pK_A = 27),[13] which reflects the substantial electron withdrawing effect exerted by naphthaleneimide substituents.

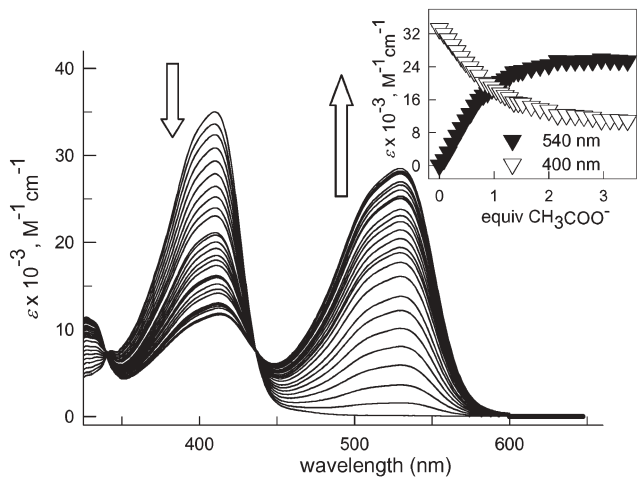


Fig. 2 Family of spectra taken in the course of the titration of a DMSO solution (5.00×10^{-5} M) of receptor **2** (LH) with a standard solution of $[Bu_4N]CH_3COO$, at 25 °C. The band that develops at 540 nm pertains to the deprotonated receptor $[L]^-$. Titration profiles (inset) indicate a 1 : 1 stoichiometry for the receptor-to-acetate proton transfer process, to which an equilibrium constant $\log K$ = 4.97 ± 0.01 corresponds.

It should also be noted that in an MeCN solution receptor **1** formed a genuine H-bond complex with acetate: $[L–H\cdots CH_3COO]^-$,[9] a circumstance which could be ascribed to the lower intrinsic acidity of **1** compared to **2** (and, ultimately, to the lower electron-withdrawing capability of the nitrobenzyl moiety compared to naphthaleneimide). However, the comparison is not correct due to the difference of the employed solvents (MeCN and DMSO). In particular, it is reasonable to assume that the most polar DMSO encourages deprotonation, stabilising the $[L]^-$ anion, while the less polar MeCN favours the formation of the H-bond complex.

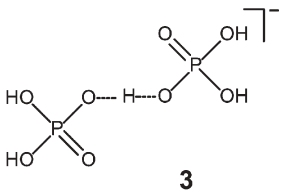
Benzoate anions display a similar behaviour. In particular, development of the band at 540 nm indicates the occurrence of the same neutralisation equilibrium observed for acetate. However, the titration profile is less steep than for acetate, to which a $\log K = 4.00 \pm 0.01$ corresponds. The lower value of the constant of the neutralisation equilibrium reflects the lower acidity of benzoic acid with respect to acetic acid in DMSO.

The $H_2PO_4^-$ anion displays a more complex behaviour. In fact, on titration of a DMSO solution 5.00×10^{-5} M of receptor **2** (LH) with a standard solution of $[Bu_4N]H_2PO_4$, firstly, a moderate shift of the band at 400 nm is observed. This feature is typically associated to the formation of a genuine H-bond complex.[9] Then, on further dihydrogenphosphate addition, the band at 540 nm begins to develop, which indicates receptors deprotonation. In particular, best fit of the spectral pattern, using a non-linear least-squares procedure, was obtained by assuming the occurrence of two stepwise equilibria, as represented below ($\log K_1 = 5.02 \pm 0.01$; $\log K_2 = 3.20 \pm 0.02$):

$$LH + H_2PO_4^- \leftrightarrows [LH\cdots H_2PO_4]^- \qquad (2)$$

$$[LH\cdots H_2PO_4]^- + H_2PO_4^- \leftrightarrows L^- + [H_3PO_4\cdots H_2PO_4]^- \qquad (3)$$

$H_2PO_4^{2-}$ is a weaker base than CH_3COO^- and is not capable of removing a proton from the receptor LH, thus an H-bond complex is formed as described by eqn. (2). However, on excess addition of anion, a molecule of H_3PO_4 is released by the complex, to give the deprotonated receptor L^- and the self-complex $[H_3PO_4\cdots H_2PO_4]^-$ as described by eqn. (3). The inset of Fig. 3 shows the distribution of the species at the equilibrium over the course of the titration, calculated from K_1 and K_2 values. Due to the relatively low stability of the self-complex $[H_3PO_4\cdots H_2PO_4]^-$, H_3PO_4 release and formation of $[L]^-$ requires the addition of a large excess of anion. A similar two-step pattern has been observed in the interaction of receptor **1** with fluoride.[9] A tentative sketch of the $[H_3PO_4\cdots H_2PO_4]^-$ self-complex is reported as **3**.



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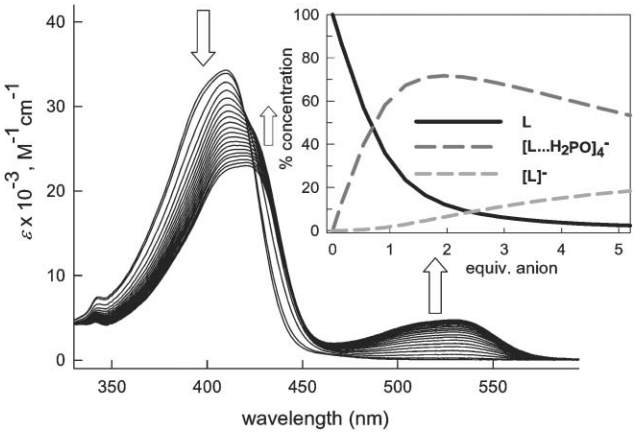


Fig. 3 Family of spectra taken in the course of the titration of a DMSO solution 5.00 × 10^{-5} M of receptor **2** (LH) with a standard solution of $[Bu_4N]H_2PO_4$, at 25 °C. The spectral pattern is interpreted by assuming the occurrence of two stepwise equilibria: first, formation of a genuine H-bond complex $[LH\cdots H_2PO_4]^-$ (band at 425 nm); then, H_3PO_4 release and formation of the deprotonated receptor $[L]^-$ (band at 540 nm). Inset: how the percent concentration of the three species at the equilibrium varies on $H_2PO_4^-$ addition.

Other investigated inorganic oxoanions (NO_2^-, NO_3^- and HSO_4^-) and halides (Cl^-, Br^- and I^-) do not interact at all with receptor **2** in DMSO solution (no spectral change even after large excess addition of the pertinent tetrabutylammonium salt), due to the relatively low basicity.[14] It derives that system **2** behaves as a selective colorimetric sensor for acetate. Recognition is not related to the formation of a stable H-bond complex, but instead to deprotonation of one of the N–H fragments of **2**; a feature reserved to the strong base acetate. Colour changes, observed or not, following the addition of a number of anions to a DMSO solution of **2** are shown in the picture in Fig. 1.

The interaction with anions studied by spectrofluorimetry

A solution of **2** in DMSO emits blue fluorescence, as shown in the photograph reported in Fig. 4.

On addition of acetate light emission is quenched, as shown in Fig. 4. Fig. 5 displays the family of emission spectra recorded in the course of the titration of a solution of **2** with tetrabutylammonium acetate.

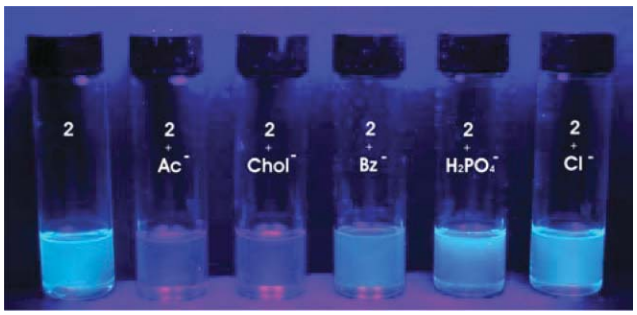


Fig. 4 Change of the fluorescent emission observed on addition of 1 eq. of anion (as a tetrabutylammonium salt; sodium for cholate) to a DMSO solution of receptor **2**.

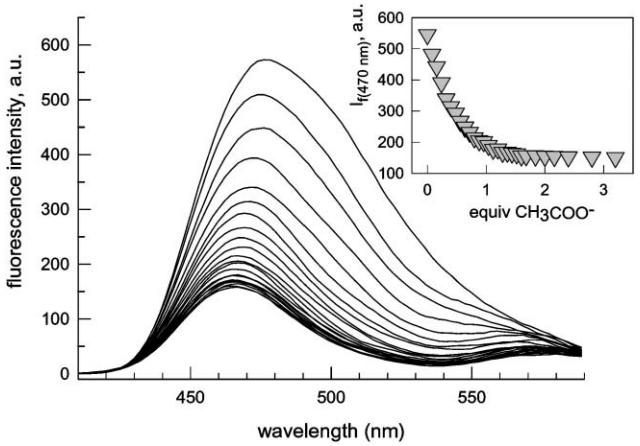


Fig. 5 Emission spectra taken in the course of the titration of a DMSO solution 5.00 × 10^{-5} M of receptor **2** (LH) with a standard solution of $[Bu_4N]CH_3COO$, at 25 °C.

The titration profile (inset of Fig. 5) shows that, on addition of the first eq. of acetate, the emission band, centred at 470 nm, decreases to about 20% of the pristine intensity, while λ_{max} undergoes a moderate blue shift. The emission band at 470 nm results from the radiative decay of the charge transfer excited state. On formation of the $[L]^-$ and delocalisation of the negative charge on the naphthaleneimide moiety, the excited state is drastically modified and, in particular, its lifetime substantially reduced, which results in a neat decrease of emission intensity.

Benzoate displayed a similar behaviour, the flatter titration profile (see Fig. 6) reflecting the lower concentration of the $[L]^-$ that forms on anion addition. Noticeably, in the case of the dihydrogenphosphate anion (see Fig. 6), quenching occurs also during the addition of the first anion equivalent, when the authentic H-bond complex, $[LH\cdots H_2PO_4]^-$, forms and the deprotonated receptor $[L]^-$ is not present. This indicates that even the partial donation of electron density from the anion to

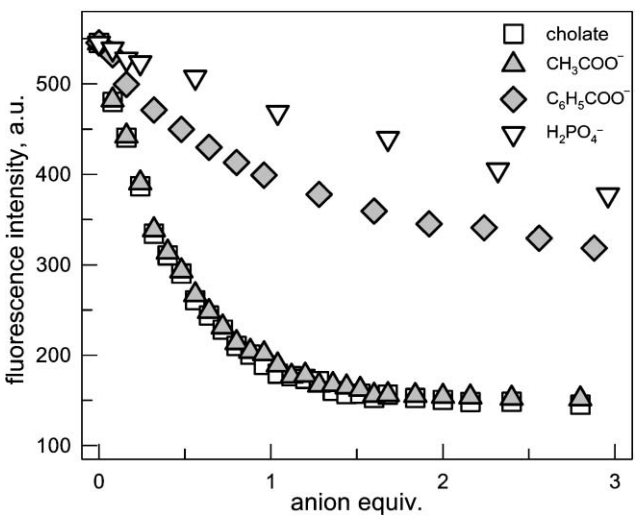
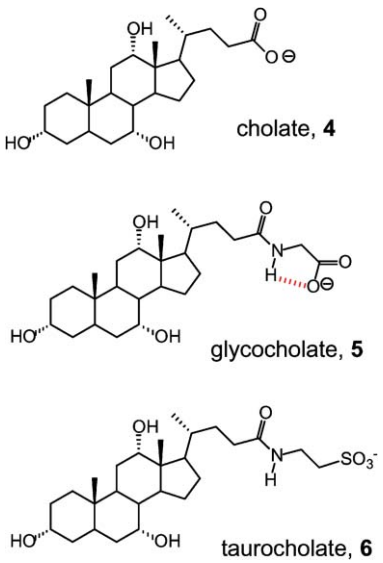


Fig. 6 Change of the intensity of the fluorescent emission observed on anion addition (as a tetrabutylammonium salt; sodium for cholate) to a DMSO solution of receptor **2**.

the receptor modifies enough the excited state to induce quenching of fluorescence. In a recent study, it has been shown that anion interaction with thiourea base receptors induced quenching of an appended anthracene subunit through an electron transfer mechanism.[15] Addition of NO_2^-, NO_3^-, HSO_4^-, Cl^-, Br^- or I^-, even in large excess, to a solution of **2** did not induce any modification of the fluorescent emission. Thus, it appears that fluorescence is a further visual way to signal the occurrence of the selective recognition of acetate by receptor **2**, based on an a Brønsted acid–base process.

Naked-eye and spectroscopic detection of bile acids

Cholic **4**, glycocholic **5** and taurocholic acid **6** are primary bile acids and play a fundamental role in cholesterol metabolism.[16] Their rapid an accurate detection is a pressing need of current medical analysis.[17] To the best of our knowledge, reported chemosensors for cholic acid all consist of a cyclodextrin subunit to which an aromatic fluorophore (*e.g.* naphtalene, pyrene or dansyl) has been covalently linked: cholic acid typically displaces the fluorogenic fragment from cyclodextrin cavity, an event which is signalled by a modification of the fluorescent emission.[18] We considered that the analytical procedure discussed in the previous sections could be conveniently applied to the spectrophotometric and spectrofluorimetric determination of carboxylic bile acids.



Indeed, chemosensor **2**, in a DMSO solution, interacts rather strongly with the cholate ion. In particular, on addition of 1 eq. of sodium cholate, the yellow DMSO solution of **2** turns red. Moreover, titration of a solution of **2** in DMSO with cholate produced the family of spectra displayed in Fig. 7.

Notice that the observed spectral pattern is very similar to that obtained on titration with acetate, with development of the band at 540 nm, indicating the occurrence of the receptor-to-anion neat proton transfer. In particular, a log$K$ value very close to that determined for acetate was obtained (log$K$ = 5.07 ± 0.05).

The anion of the related bile acid, glycocholic **5**, also induces deprotonation of the receptor, as indicated by the development of the band at 540 nm, but the titration profile (see Fig. 8) is much flatter than observed with cholate, to which a lower value of log$K$ corresponds (3.87 ± 0.01). Such a behaviour denotes a surprisingly lower basicity of the glycocholate ion with respect to cholate, which contains the same $-CH_2COO^-$ group. We suggest that such a lower basicity is due to the occurrence, in DMSO, of an intramolecular H-bond interaction, within the glycocholate ion, between one oxygen atom of the carboxylate group (detaining a partial negative charge) and the proximate N–H fragment of the amide group, as tentatively sketched in formula **5**. On the other hand, addition of the anion of the other bile acid, taurocholic **6**, does not induce any modification to the spectral feature of **2** in DMSO, indicating no interaction. This is due to the poor basicity of the $-SO_3^-$ group, which had also been observed for the parent anion HSO_4^-. Thus, it appears that **2** can discriminate cholic acid from other bile acids, especially on the basis of its affinity

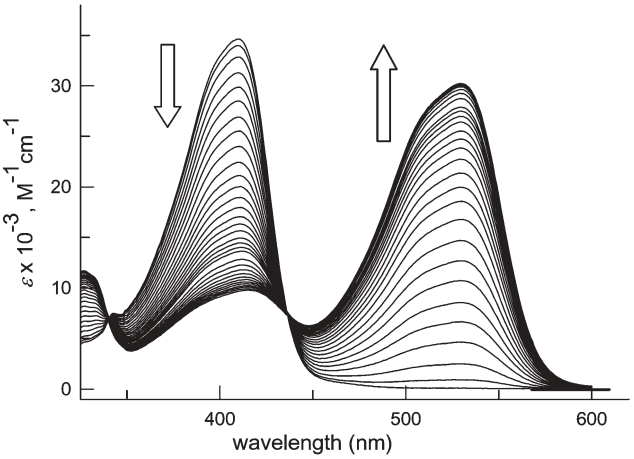


Fig. 7 Family of spectra taken in the course of the titration of a DMSO solution 5.00 × 10^{-5} M of receptor **2** (LH) with a standard solution of sodium cholate, at 25 °C. The band that develops at 540 nm pertains to the deprotonated receptor [L^-] and indicates the occurrence of a proton transfer process from **2**, to which an equilibrium constant log$K$ = 5.07 ± 0.05 corresponds.

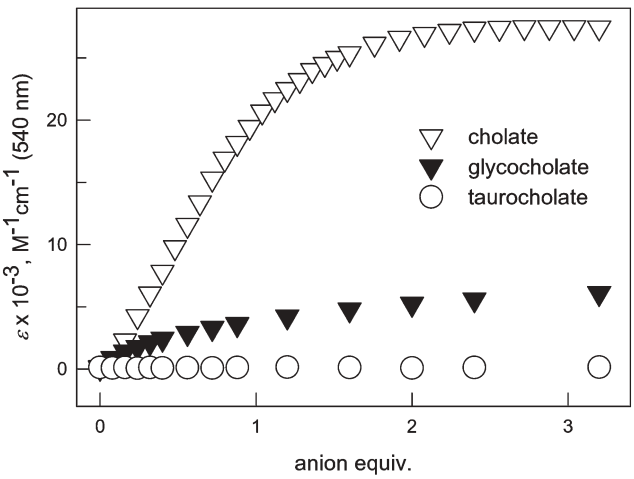


Fig. 8 Titration profiles at 540 nm obtained during the spectrophotometric titrations of **2** (5.00 × 10^{-5} M in DMSO) with sodium cholate, glycocholate and taurocholate.

towards the proton, in a DMSO solution. Such a discriminating effect can also be monitored by looking at the fluorescent emission, which is almost completely quenched by cholate (see Fig. 4), only slightly by glycocholate and not modified at all by taurocholate.

Conclusions

A chemosensor is a molecular system that, following the change of concentration of the investigated analyte, gives an optical signal in response. Such a behaviour usually results from the formation of a 1 : 1 complex between the receptor and the analyte (in the present case, an anion). As the receptor–anion complex is held together by non-covalent interactions (hydrogen bonding, electrostatic), the topic of anion recognition and sensing rightfully belongs to the discipline of supramolecular chemistry. In this work, we intended to investigate a receptor suitable for recognition and sensing of the carboxylate group and, in this sense, we chose the urea fragment, which can donate two hydrogen bonds in a parallel fashion and so is complementary to Y-shaped anionic groups, such as $–COO^-$. The urea subunit was equipped with naphthaleneimide substituents in order to provide two different optical signals: colour change and modification of the fluorescent emission. However, the urea based receptor **2**, made especially acidic by the strongly electron withdrawing substituents, does not form an H-bond complex with carboxylates, but rather transfers a proton of one N–H fragments to the anion. Thus, system **2** escapes from the realm of supramolecular chemistry and enters the classic domain of Brønsted acid–base equilibria. However, such a circumstance is not relevant from the point of view of signalling, because urea deprotonation induces a neat colour change and substantial quenching of the light emission in presence of most basic carboxylates, such as acetate and cholate.

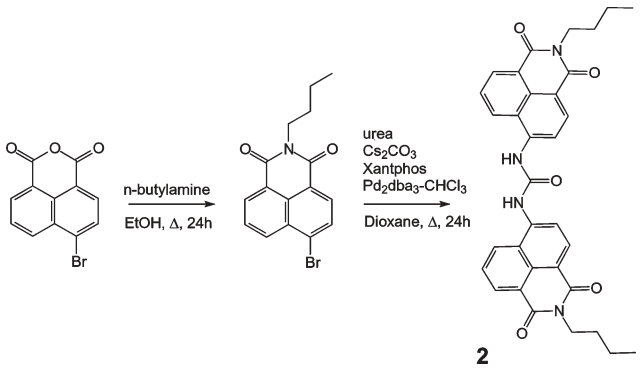
Experimental

Caesium carbonate was dried *in vacuo* at 150–200 °C. Dioxane was purified and dried by standard procedures. $Pd_2(dba)_3$–$CHCl_3$ was prepared as described in the literature.[19] 4,5-Bis(diphenylphosphino)-9,9-dimethylxanthene (xantphos), *n*-butylamine, 4-bromo-1,8-naphthalic anhydride and 4-amino-1,8-naphthalic anhydride are commercial products.

The chemosensor **2** was prepared according to the two-step pathway shown in Scheme 1.

4-Bromo-*N*-butyl-1,8-naphthalenimide

4-Bromo-1,8-naphthalic anhydride (0.24 g, 0.869 mmol) and *n*-butylamine (0.103 mL, 1.048 mmol) in ethanol (40 mL) were refluxed for 24 h. The solid product was isolated by concentration of solution and separated by filtration. The product was purified by column chromatography (hexane–ethyl acetate, 7 : 3) to give an off-white solid (0.21 g, 75%). IR spectrum (nujol mull), cm^{-1}: 1668, 1657 (C=O); 720 (C–Br). ^{1}H-NMR ($CDCl_3$, δ_H ppm): 8.70(d, 1H), 8.60(d, 1H), 8.45(d, 1H), 8,10(d, 1H), 7.90(t, 1H), 4.20(t, 2H), 1.75(quintuplet, 2H), 1.50(m, 2H), 1.00(t, 3H). *m*/*z* (negative ion mode), 331.4 ($M - H^+$).



Scheme 1 Synthetic route to chemosensor **2**.

N,N′-(Bis-*N*-butyl-1,8-naphthalenimide)-urea (2)

Into a reactor filled with argon, with 4-bromo-*N*-butyl-1,8-naphthalenimide (0.200 g., 0.603 mmol) in 4 mL of dioxane satured with argon, were added urea (0.0235 g, 0.392 mmol), Cs_2CO_3 (0.275g, 0.844 mmol), $Pd_2(dba)_3$–$CHCl_3$ (0.003 g, 0.5 mol%) and xantphos (0.0156 g, 3 mol%). The reaction mixture was degassed by evacuation then the reactor was filled with argon. The mixture was heated at 100 °C under magnetic stirring. The reaction progress was monitored by TLC (hexane–ethyl acetate, 7 : 3). On completion of the reaction (12 h) the mixture was cooled to rt and the content of the reactor was poured into 50 mL of saturated KCl solution. Then the products were extracted by ethyl acetate (3 × 20 mL), the combined extracts were washed with KCl solution and the solid product formed in the organic phase was separated by filtration to give a yellow solid (0.140 g, 83%) which is air stable in the solid state, soluble in dimethyl sulfoxide and insoluble in all other common solvents. (Found: C, 70.52; H, 5.39; N, 9.96%. $C_{33}H_{30}N_4O_5$ requires C, 70.45; H, 5.37; N, 9.96%). IR spectrum (nujol mull), cm^{-1}: 3292 (N–H); 1699, 1656 (C=O); 1553 (C–N). ^{1}H-NMR (DMSO-d_6, δ_H ppm): 9.35 (br, NH, 2H), 8.85(d, 2H), 8.55(m, 6H), 7.95(t, 2H), 4.00(t, 4H), 1.55(quintuplet, 4H), 1.35(m, 4H), 0.80(t, 6H). *m*/*z* (negative ion mode) 561.1 ($M - H^+$, 100%), 597.1 ($M + Cl^-$, 38%).

Physical measurements

Spectrophotometric and spetrofluorimetric grade solvents were used for spectroscopic measurements. UV/vis spectra were recorded on a Varian CARY 100 spectrophotometer, with a quartz cuvette; spectrofluorimetric measurements were carried out on a Perkin-Elmer LS-50 luminescence spectrometer, using quartz cells (path length: 1 cm). ESI-MS spectra were obtained by a Thermo Finnigan LCQ Advantage Max spectrometer. FTIR spectra were recorbed on a Perkin-Elmer Spectrum BX spectrophotometer. Binding constants were calculated through non-linear least-squares fitting of spectrophotometric titration curves by using the hyperquad package.[20]

Acknowledgements

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