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Fluorescent anion sensors based on 4-amino-1,8-naphthalimide that employ the 4-amino N–H

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Abstract—The new charge neutral 4-amino-1,8-naphthalimide based anion sensors **2** and **3** bind to both acetate and dihydrogenphosphate with 1:1 stoichiometry through hydrogen bonding to both thiourea N–H atoms and in the case of dihydrogenphosphate, the naphthalimide 4 amino N–H group as well. This was clearly established from ^{1}H NMR titration experiments with $H_2PO_4^-$ in DMSO-d_6 where a substantial shift in the resonance for the naphthalimide N–H was observed concomitant with the expected migration of the thiourea N–H chemical shifts. The binding constants determined from the titration studies indicate that the new sensors bind $H_2PO_4^-$ more strongly than AcO^-. Fluorescence titrations with sensor **3** indicate quenching of 59% and 36% upon addition of acetate and dihydrogenphosphate, respectively.

In the field of supramolecular chemistry charge neutral anion receptors rely on hydrogen bonding as the dominant interactive force with anions.[1] A number of well placed hydrogen bond donors maximise the binding strength to a specific anionic species.[1,2] A naturally occurring example, the sulphate binding protein, contains a total of seven dedicated hydrogen bonds to ensure that binding of the target is both strong and selective.[3]

The rapid detection of anionic species is of great significance given the roles they play in the environment and in physiological systems.[1] Our interest in this field has led us to develop luminescent chemosensors for anions using both cationic and charge neutral receptors.[4,5] Recently we synthesised and evaluated the combined thiourea/naphthalimide host **1** as a photo-induced electron transfer (PET) chemosensor for anions[6] (Fig. 1). Receptor **1** was purposely constructed such that the acidic 'pseudo amide' N–H at position 4 of the naphthalimide ring could be employed as an additional H-bond donor (Fig. 1).[6] Whilst definite evidence of this H-bond donor participating in the binding of the tetrahedral dihydrogenphosphate anion was obtained using NMR titration

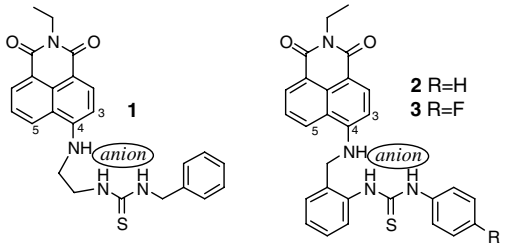

Figure 1. Structure of **1** and the new naphthalimide hosts **2** and **3**.

studies, to give strong binding (for **1**: $H_2PO_4^-$ $\log\beta = 3.4$), no quenching of the fluorescence of **1** was observed upon addition of any anion. The lack of any modulation in fluorescence was attributed to either, the purely aliphatic nature of the urea and its associated reduction potential, or that a nonconjugated *two*-carbon ethyl spacer was employed. It is known that ET requires the *donor* and *acceptor* to be in close proximity.[7]

In order to elicit PET quenching in the presence of anions whilst preserving the co-operative binding observed for **1** (with multiple H-bond donors) the new sensors **2** and **3** were designed. As can be seen (Fig. 1), the new design incorporates an *ortho* substituted aminobenzylamine spacer, with only one nonconjugated CH_2, and hence an element of conformational preorganisation has also been introduced.

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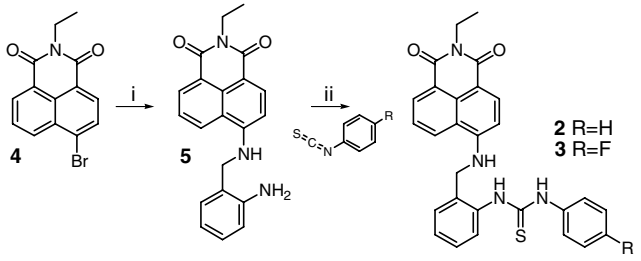

Scheme 1. Synthesis of hosts **2** and **3** from *N*-ethyl-4-bromonaphthalimide. Reagents and conditions: (i) *o*-aminobenzylamine, sealed tube, 130 °C, 12 h, 86%; (ii) DMF, rt, 12 h, **2** 33%, **3** 40%.

Herein, the synthesis of the new 4-amino-1,8-naphthalimide based hosts, **2** and **3**, is presented along with an evaluation of their anion binding ability using ^{1}H NMR and fluorescence titration techniques.

The synthesis of **2** and **3** was achieved in three steps (Scheme 1) by first reacting 1 equiv of ethylamine in refluxing 1,4-dioxane with 4-bromo-1,8-naphthalic anhydride, which after aqueous work-up gave the imide **4** in ca. 90% yield as an off-white powder.[5,8] Nucleophilic aromatic substitution was accomplished by heating an excess of neat *o*-aminobenzylamine with **4** in a sealed tube to afford amine **5** as a yellow powder in 86% yield after recrystallisation. The final step involved stirring the requisite isothiocyanate with amine **5** in DMF to provide the desired compounds **2** and **3** in 33% and 40% yield after chromatographic purification. Both new compounds were highly fluorescent (Φ_F = 0.62 for **2** and 0.70 for **3**) and they were fully characterised by ^{1}H and ^{13}C NMR and elemental analysis and/or HRMS.[9]

The binding of the new hosts to a series of anions was first investigated by monitoring the changes in the ^{1}H NMR spectra of DMSO-d_6 solutions of **2** and **3** upon addition of AcO^-, $H_2PO_4^-$, F^- and Br^- as their tetrabutylammonium (TBA) salts.

The spherical halides were investigated first, however, the addition of Br^- afforded only minor changes in the ^{1}H NMR spectrum (after 6.0 equiv $\Delta\delta = 0.16$ ppm for the thiourea protons of **3**) and it was concluded that very weak, if any, binding of the bromide anion occurred.

In the case of F^-, however, the naphthalimide N–H signal became significantly broadened after the addition of only small quantities of the anion and completely disappeared after the addition of only 0.6 equiv. Concomitant with this disappearance was a distinct, visible colour change from fluorescent yellow/green to deep red/purple. This observation was in close agreement with previous experience with 4-aminonaphthalimide derivatives, as was the appearance of a new triplet at ca. 16.00 ppm—assigned to the formation of the bifluoride $[FHF]^-$ anion[10]—after 2.5 equiv of F^- had been added. These observations are consistent with our previous studies with 4-amino-naphthalimides and indicate that F^- mediated deprotonation of the naphthalic amine of **2** and **3** had occurred.[7,11]

The successive addition of the trigonal planar AcO^- anion to DMSO-d_6 solutions of both **2** and **3** resulted in significant changes in the chemical shifts of the protons of interest (Fig. 2). As anticipated, the largest shifts were seen for the two thiourea proton resonances, which experienced significant downfield shifts (ca. 3.5 ppm) for both **2** and **3**, indicative of strong hydrogen bonding between the thiourea anion receptor and the geometrically complementary acetate anion. Unfortunately, only a small shift (<0.5 ppm) in the naphthalimide amino N–H resonances of **2** and **3** (along with negligible shifts for the remaining naphthalimide ring protons) was observed and it was concluded that this N–H was not involved to any great extent in the binding of acetate. Both binding isotherms are consistent with a 1:1 host:guest stoichiometry and from this data binding constants, of $\log\beta = 2.8(\pm0.1)$ for **2** and $\log\beta = 3.4(\pm0.2)$ for **3**, were determined when fitting the data using WinEQNMR.[12] Such binding constants are slightly less than that of **1** against acetate ($\log\beta = 3.6$)[6] and it is possible that for the new sensors the introduced element of conformational preorganisation may slightly hinder the binding of anions such as acetate and the N–H at position 4 of the naphthalimide ring actually interacts unfavourably with the CH_3 of the bound acetate anion.

The successive addition of the tetrahedral dihydrogenphosphate anion to a DMSO-d_6 solutions of **2** and **3** also produced significant changes in the chemical shifts of several protons. Unfortunately, in this instance, after 3 equiv of the anion had been added the resonances

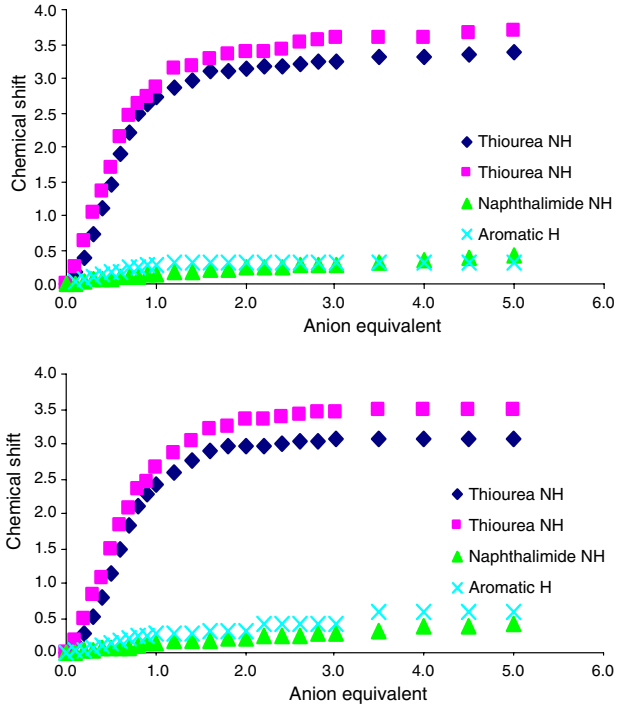

Figure 2. Changes in the chemical shift of relevant protons within **2** (top) and **3** (bottom) upon addition of AcO^- in DMSO-d_6.

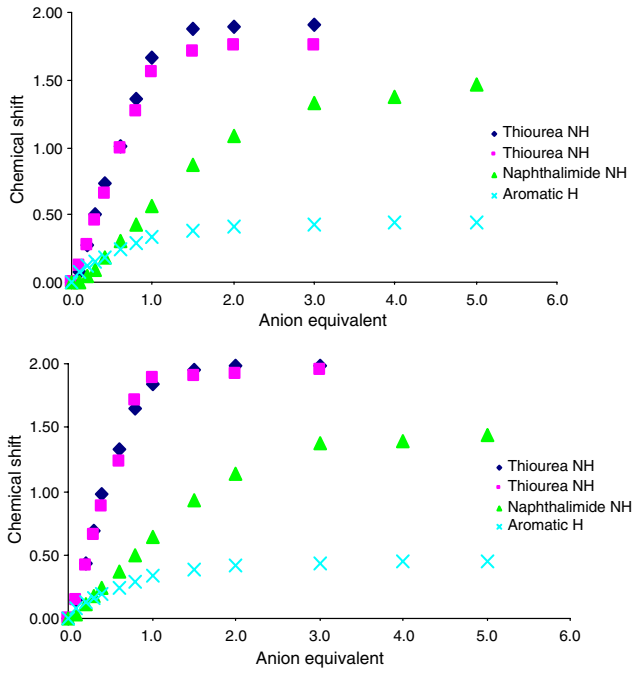

Figure 3. Changes in the chemical shift of relevant protons within **2** (top) and **3** (bottom) upon addition of $H_2PO_4^-$ in DMSO-d_6.

assigned to the thiourea protons became too broad to track. A plot of $\Delta\delta H$ for several resonances as a function of anion equivalents is presented in Figure 3 for sensors **2** and **3**. The thiourea N–H proton resonances experienced a significant downfield shift of ~1.9 ppm for **2** and ~2.0 ppm for **3**, indicative of strong hydrogen bonding of the receptor to the $H_2PO_4^-$ anion. The resultant isotherms were consistent with a 1:1 host:guest stoichiometry but unfortunately the N–H resonances completely disappeared after the addition of 3 equiv of the $H_2PO_4^-$ anion. As the titration curves were 'incomplete' the binding constants calculated from these titration curves had a high degree of uncertainty and are thus only estimates. Nevertheless the values of $\log\beta \sim 3.7$ for **2** and $\log\beta \sim 4.1$ for **3** are impressive and actually indicate that the new receptors **2** and **3** bind $H_2PO_4^-$ more strongly than AcO^-. This is the first 4-amino-naphthalimide based sensor where this 'reversal' of binding preference has been observed.

Significantly, and in contrast to the results obtained upon addition of acetate to either **2** or **3**, a large downfield shift of ca. 1.4 ppm was observed for the 4-amino N–H proton. This change in chemical shift is only of a slightly smaller magnitude than that experienced by the thiourea N–H protons. Furthermore, of the remaining protons only that at position 5 of the naphthalimide ring experienced an appreciable shift (ca. 0.5 ppm, *see* Figure 3, green triangles). These results clearly indicate that the binding event involves the naphthalimide amino N–H—as was observed for compound **1**.[6] Of particular interest was the apparent 1:2 host:guest binding of this proton—the titration curves fit this model almost perfectly. The authors are unaware of any instances where a single H-bond donor binds to two acceptors and further experiments are in progress to elucidate this unusual behaviour.

To confirm that the new receptors bind $H_2PO_4^-$ more strongly than AcO^-, receptor **3** was investigated using low temperature titration studies (conducted in 50:50 $CDCl_3$–DMSO-d_6 at −10 °C). At this temperature the N–H resonance was clearly visible during the addition of up to 6 equiv of both AcO^- and $H_2PO_4^-$ anions. From these titration curves a binding constant of $\log\beta = 3.2(\pm0.2)$ for AcO^- and $\log\beta = 3.9(\pm0.3)$ for $H_2PO_4^-$ was determined. The binding constants for both acetate and dihydrogenphosphate are comparable to those determined and estimated in neat DMSO-d_6. Most importantly both results support the earlier conclusion that the new host **3** actually binds to $H_2PO_4^-$ more strongly than AcO^-.

When changes in the UV/vis absorption spectra were monitored upon successive addition of anions to sensors **2** and **3** (Fig. 4), only when $H_2PO_4^-$ was added to either **2** or **3** was a very weak hypsochromic shift observed. All other sensor and anion combinations afforded subtle bathochromic shifts. These results also suggest a unique mode of binding of **2** and **3** to $H_2PO_4^-$.

When DMSO solutions of anions were added to **2** and **3** and the fluorescence spectrum monitored ($\lambda_{ex} = 440$ nm), the fluorescence was quenched to varying degrees (Fig. 5 for compound **3** and Table 1 for both **2** and **3**). The best results were observed for additions of acetate to **3** where 59% quenching was observed after 5 equiv of anion had been added.

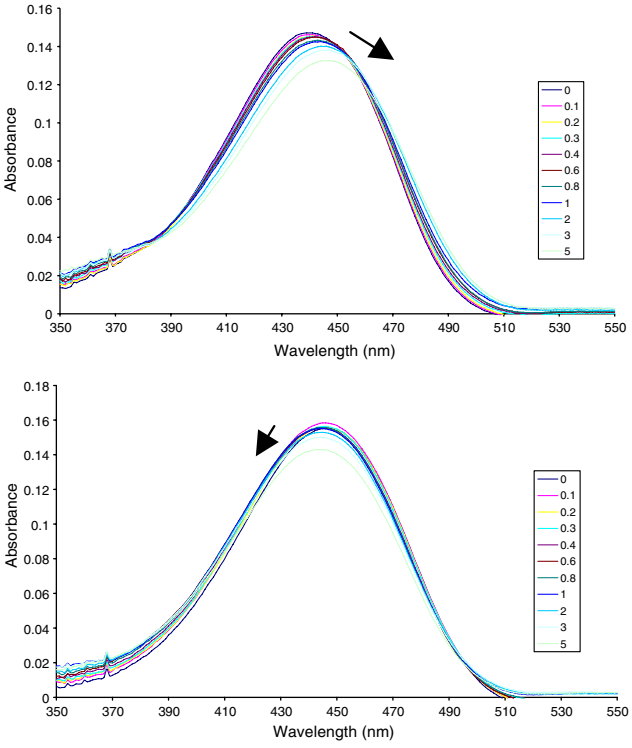

Figure 4. Changes in UV/vis absorbtion spectra upon addition of AcO^- (top) and $H_2PO_4^-$ (bottom) to a DMSO solution of **3**.

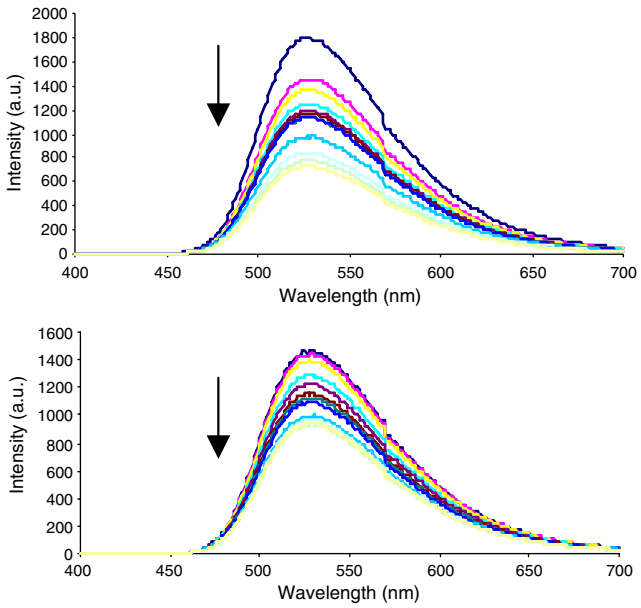

Figure 5. Quenching of fluorescence after addition of 0–5 equiv of AcO^- (top) and $H_2PO_4^-$ (bottom) to a DMSO solution of **3**.

Table 1. Binding constants and fluorescence quenching for **2** and **3** against $H_2PO_4^-$ and AcO^-

Compound	log β for AcO^-	Quenching for AcO^- (%)	log β for $H_2PO_4^-$	Quenching for $H_2PO_4^-$ (%)
2	2.8	31	3.7[a]	6.5
3	3.4	59	4.1[a]	36
	3.2[b]		3.9[b]	

Binding constants determined or estimated from ^1H NMR titration data using WinEQNMR software.[12]

[a] Values are estimates.

[b] Values determined at −10 °C in 50:50 $CDCl_3$–DMSO-d_6.

For the previously constructed sensor **1**, binding constants of $\log\beta = 3.8(\pm0.1)$ and $3.4(\pm0.1)$ were determined for AcO^- and $H_2PO_4^-$, respectively. The results for the new compounds indicate weaker binding of acetate (possibly based on steric reasons) and stronger binding for dihydrogenphosphate (as a result of both co-operation of the 4-amino N–H with the thiourea and the preorganised receptor site). Undoubtedly the more electron withdrawn thiourea receptor of **2** and **3** as compared to **1** also contributed to the increased binding strength. This factor also explains the results from the fluorescence quenching experiments which are in line with our previous findings where more electron deficient thiourea groups induce the greatest decrease in fluorescence intensity upon anion binding. Whilst the 4-amino N–H assists in overall binding strength it appears to play no part in fluorescence quenching and it is the more electron deficient thiourea for which the most effective quenching is observed.

In summary, we have synthesised two new fluorescent sensors **2** and **3** and again demonstrated that the naphthalimide N–H at position 4 in conjunction with the thiourea can cooperatively bind tetrahedral $H_2PO_4^-$ anions. A significant enhancement in binding affinity for $H_2PO_4^-$ was observed with the additional H-bond donor and indeed this anion is bound more strongly than AcO^-. The most significant result was that, unlike the previous compound **1**, the new sensors successfully combined this co-operative binding with fluorescence quenching. Further developments in the field of naphthalimide based sensor technology will be reported in due course.

Acknowledgements

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9. *N-Ethyl-4-[2′-(N-phenylthioureido)-benzylamino]-1,8-naphthalimide* **2**
Yield 49 mg (33%) of fine yellow/orange crystals; mp 199–201 °C; δ_H (399.78 MHz; DMSO-d_6): 1.17 (3H, t, *J* 7.0 Hz, NCH_2CH_3), 4.04 (2H, q, *J* 7.0 Hz, NCH_2CH_3), 4.69 (2H, d, *J* 5.2 Hz, $ArCH_2NH$), 6.66 (1H, d, *J* 8.8 Hz, H3), 7.14 (1H, t, *J* 7.3 Hz, H18), 7.21 (1H, d, *J* 5.1 Hz, H14), 7.22 (1H, d, *J* 4.8 Hz, H11), 7.26 (1H, d, *J* 7.7 Hz, H19), 7.26 (1H, d, *J* 7.7 Hz, H20), 7.33 (1H, t, *J* 7.3 Hz, H17), 7.49 (1H, d, *J* 7.7 Hz, H16), 7.73 (1H, t, *J* 7.7 Hz, H6), 8.14 (1H, d, *J* 8.4 Hz, H2), 8.31 (1H, t, *J* 5.5 Hz, $ArNHCH_2$), 8.47 (1H, d, *J* 7.0 Hz, H5), 8.77 (1H, d, *J* 8.4 Hz, H7), 9.51 (1H, s, ArNHCS), 9.93 (1H, s, CSNHAr); δ_C (100.54 MHz; DMSO-d_6): 13.28, 34.25, 43.00, 104.84, 108.31, 120.23, 122.01, 123.85, 124.52, 124.67, 126.55, 126.93, 127.44, 128.52, 128.58, 128.95, 129.27, 130.65, 133.77, 135.10, 137.64, 139.21, 150.44, 162.67, 163.50, 180.81; LRMS *m*/*z* (ES) 515.4 $[M+Cl]^-$ $C_{28}H_{24}N_4O_2S + Cl$ requires 515.1; HRMS: calcd for $[C_{28}H_{24}N_4O_2S + Na]^+ = 503.1518$, found 503.1515.
N-Ethyl-4-[2′-(N-p-fluorophenylthioureido)-benzylamino]-1,8-naphthalimide **3**
Yield 67 mg, (40%) of fine yellow crystals; mp 193–195 °C. Found: C, 67.32; H, 4.53; N, 11.25. $C_{28}H_{23}FN_4O_2S$ requires C, 67.45; H, 4.65; N, 11.24; δ_H (399.78 MHz; DMSO-d_6): 1.17 (3H, t, *J* 7.0 Hz, NCH_2CH_3), 4.03 (2H, q, *J* 7.0 Hz, NCH_2CH_3), 4.68 (2H, d, *J* 5.1 Hz, $ArCH_2NH$), 6.65 (1H, d, *J* 8.4 Hz, H3), 7.15 (1H, d, *J* 8.8 Hz, H19), 7.18 (1H, d, *J* 8.8 Hz, H17), 7.22 (1H, t, *J* 4.4 Hz, H13), 7.26 (1H, t, *J* 7.3 Hz, H12), 7.31 (1H, d, *J* 3.3 Hz, H14), 7.31 (1H, d, *J* 3.3 Hz, H11), 7.47 (1H, d, *J* 8.8 Hz, H16), 7.48 (1H, d, *J* 8.8 Hz, H20), 7.73 (1H, t, *J* 8.1 Hz, H6), 8.14 (1H, d, *J* 8.4 Hz, H2), 8.31 (1H, t, *J* 5.1 Hz, $ArNHCH_2$), 8.46 (1H, d, *J* 7.3 Hz, H5), 8.76 (1H, d, *J* 8.4 Hz, H7), 9.50 (1H, s, ArNHCS), 9.87 (1H, s, CSNHAr); δ_C (100.54 MHz; DMSO-d_6): 13.28, 34.26, 42.99, 104.83, 108.32, 115.07, 115.30, 120.23, 122.01, 124.53, 126.42, 126.51, 126.60, 126.90, 127.48, 128.51, 128.93, 129.27, 130.65, 133.77, 135.08, 135.54, 137.53, 150.42, 159.31(d), 162.68, 163.50, 181.16; LRMS *m*/*z* (ES) 533.4 $[M+Cl]^-$ $C_{28}H_{23}FN_4O_2S + Cl$ requires 533.1.
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