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A novel fluorescent chemosensor for Cu(II) in aqueous solution based on a β-aminobisphosphonate receptor

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Abstract—A novel β-aminobisphosphonate receptor has been combined with naphthalene in the fluorophore–spacer–receptor format of a typical photoinduced electron transfer (PET) based sensor. The sensor was synthesised in two steps by first reacting aminomethylnaphthalene with diethyl vinylphosphonate to produce the parent tetraester, followed by deprotection with bromotrimethylsilane to afford the desired sensor. The fluorescence emission of the sensor was observed to remain 'On' over a wide pH range (2–10). Cu(II) was found to bind strongly to the sensor resulting in an 'On–Off' fluorescent response with sensitivity in the μM range.

Derivatives of bisphosphonates have found many applications in the diagnosis and treatment of disease states. The aminobisphosphonates (NBP's), for example, are an important class of drugs used to treat bone metastases in patients suffering from breast and prostate cancer.[1] The strong metal ion chelating ability of these compounds is well known, particularly for divalent and trivalent ions.[2] Gummiena-Kontecka et al. have shown using electron paramagnetic spectroscopy (EPR) that β-aminobisphosphonate derivatives bind strongly to Cu(II).[3] Progressing from this we have developed a luminescent sensor incorporating β-aminobisphosphonate as a receptor using the well known photoinduced electron transfer (PET) design principle.[4] Naphthalene was chosen as fluorophore due to its established photophysical properties.[5] The receptor was connected to the fluorophore via a methylene unit in the conventional fluorophore–spacer–receptor format of PET systems to produce {2-[naphthalen-1-ylmethyl-(2-phosphono-ethyl)amino]ethyl}-phosphonic acid (**2**).

The desire to measure Cu(II) concentrations accurately at the sub μM level is inspired by biological and environmental importance of this ion. Cu(II) is involved in critical catalytic, biosynthetic and transport processes within the cell and can also contribute to algae growth in seawater.[6] The accurate measurement of transition metals such as Cu(II) using PET type sensors can prove difficult due to the acidic nature of their hydration shell. This is a particular problem in organic solvents when pH buffering is not possible.[7] Certain examples of PET sensors where a fluorescent enhancement was attributed to metal binding[8] have later been shown to be due to proton transfer from the coordinated water.[7] Therefore, sensors for monitoring Cu(II) that prove robust to changes in pH are advantageous.

This study investigated the fluorescence response of **2** and its parent **1** to variations in pH. We also examined the selectivity of **2** against common metal ions and propose **2** as a fluorescent chemosensor for Cu(II), operable over a wide pH range.

The synthesis of **2** was performed in two steps (Scheme 1). The first step involved a Michael addition reaction between aminomethylnaphthalene and diethyl vinylphosphonate to give (2-{[2-(diethoxy-phosphoryl)ethyl]-naphthalen-1-ylmethylamino}ethyl)phosphonic acid diethyl ester (**1**) in 77% yield.[9] The subsequent hydrolysis of **1** with bromotrimethylsilane resulted in the formation of **2** in 93% yield.[10]

However, the synthesis of **1** was not as straightforward as it appears. An attempt to synthesise it by alkylating aminomethyl naphthalene with diethyl 2-bromoethylphosphonate was unsuccessful. The basic conditions

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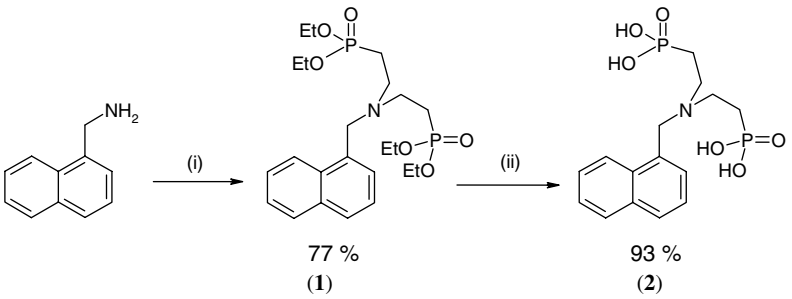


Scheme 1. Synthesis of {2-[naphthalen-1-ylmethyl-(2-phosphono-ethyl)amino]ethyl}-phosphonic acid (**2**). Reagents and conditions: (i) diethyl vinylphosphonate/anhydrous MeOH, reflux 5 days; (ii) bromotrimethylsilane/anhydrous DCM, ambient, 60 min.

required for the reaction resulted in the conversion of diethyl 2-bromoethylphosphonate to diethylvinyl phosphonate due to the acidity of the methylene protons on the carbon α to the phosphorus atom (see Supplementary data). Since this reduced the amount of diethyl 2-bromoethylphosphonate available, the dialkylated product was not observed with only small amounts of the monoalkylated form, which was found to decompose due to the acidic nature of the work-up (see Supplementary data). Therefore a 'neutral' approach avoiding the presence of acids or bases was required, and the method outlined in Scheme 1 met this objective.

The fluorescent spectrum of tetraester **1** recorded in aqueous solution displayed an emission band centred at 333 nm, typical of the naphthalenic fluorophore, when excited at 283 nm (Fig. 1).[11] There was no evidence of intramolecular exciplex formation between the naphthalene excited state and the proximal amine, characterised by a broad longer wavelength emission that has been reported in other similarly constructed systems.[12] The emission was observed to be strongly dependent on pH, 'switching on' as the pH decreased, with no noticeable change in wavelength. This is typical of PET sensors where a basic amine forms part of the receptor.[13] A plot of fluorescent intensity against pH for **1** is shown in Figure 2. A large fluorescence enhancement (FE) over approximately 2 log units occurred with decreasing pH. The enhancement is due to an increase in the oxidation potential of the amine upon protonation, removing the thermodynamic driving force for PET, and switching fluorescence 'On'.[14] Using a plot of

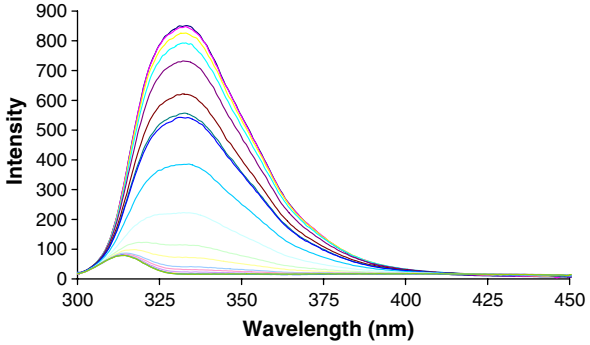


Figure 1. Emission spectra of **1** upon varying pH from 2 (high intensity) to 12 (low intensity).

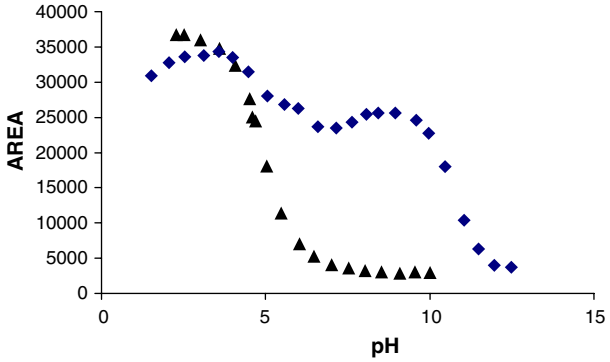
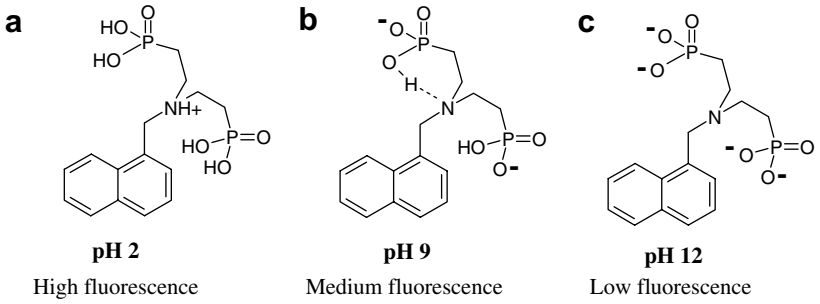


Figure 2. A plot of spectral area against pH for **1** (▲) and **2** (◆).

$-\log(F_{MAX} - F)/(F - F_{MIN})$ against pH (where F_{MAX} is the maximum fluorescence intensity, F_{MIN} the minimum fluorescence intensity and F the measured fluorescence intensity) the pK_a of the tertiary amine was calculated to be 4.99.[15]

As expected, the conversion of **1** to **2** resulted in no change in the absorption or emission maxima. A plot of fluorescence intensity against pH for **2** is again shown in Figure 2, but resulted in a markedly different profile to **1**, with evidence of two steps, one with a pK_a of 10.68 and the other at 4.98. In addition, fluorescence remained 'On' much longer and did not fully switch 'Off' until ~pH 12. The reason for this is best explained as follows: at high pH (>12) all the phosphate hydroxyls are deprotonated, as illustrated in Scheme 2c, and PET can happen in a similar manner as in **1**, from the tertiary amine to the fluorophore. As pH decreases, the phosphate oxyanions become protonated enabling the P–OH group to hydrogen bond with the amine lone pair through the formation of a 6-membered ring (Scheme 2b). This results in substantial proton transfer responsible for partially switching fluorescence 'On'. A similar effect has been reported by Czarnik et al. for an anthracene based PET sensor with a polyamine receptor used to sense for pyrophosphate.[16] At very low pH the amine becomes protonated and fluorescence is completely switched 'On' (Scheme 2a).

The wide pH window over which fluorescence remains 'On' for **2** does not lend itself to be an effective 'Off–On' sensor, as an extremely high pH (>12) is required



Scheme 2. Anticipated forms of **2** at different pH values and associated fluorescence intensity.

for operation. However, this does mean that if **2** is used as an 'On–Off' sensor then it can operate over a very wide range, as fluorescence is strong from pH 2 to pH 10. It is well known that transition metals can quench the excited state energy of organic fluorophores by energy and/ or electron transfer.[17] We determined the selectivity of **2** against a selection of common mono and divalent metal ions at a concentration of 2×10^{-5} M, in 0.01 M HEPES buffer (pH 7.4), the results of which are shown graphically in Figure 3.

The alkali and alkali earth ions have little effect nor do the closed shell Zn(II) and Hg(II). Ni(II), Co(II) and Mn(II) quench fluorescence by about 60% of the original intensity while Cu(II) and Fe(II) quench by over 95%. The sensitivity of **2** for Ni(II), Co(II), Mn(II), Cu(II) and Fe(II) was determined by investigating changes in the fluorescence intensity as a function of metal ion concentration. This is illustrated in Figure 4 where relative intensity is plotted against −log[anion] for Cu(II) and Fe(II). In each case a sigmoidal curve is observed with quenching occurring over ca. 2 log units suggesting a 1:1 binding between sensor and ion.[18]

From the equation $\log(F_{MAX} - F)/(F - F_{MIN}) = \log[\text{anion}] - \log\beta$[15] the binding constant ($\log\beta$) for each was calculated and is presented in Table 1.

These data suggest that Fe(II) binds more strongly to **2** than Cu(II), which in turn binds more strongly than Mn(II), Co(II) and Ni(II). As the quenching effect of

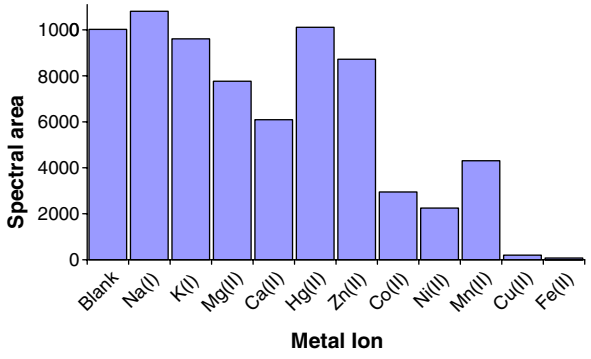


Figure 3. Selectivity data for **2** against commonly available metal ions as their chloride salts in 0.01 M HEPES buffered at pH 7.4. [Metal ion] = 2×10^{-5} M, [**2**] = 8.58×10^{-7} M.

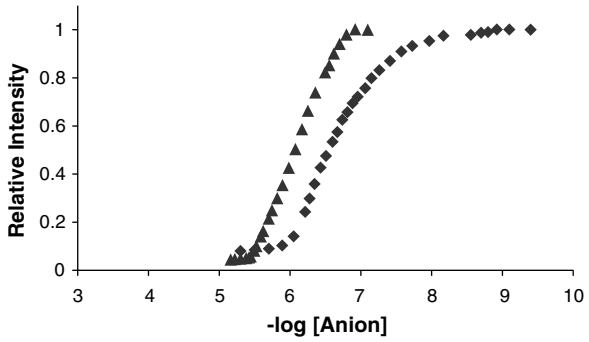


Figure 4. Plot of relative intensity against −log[anion] for **2** with (▲) Cu(II) and (◆) Fe(II) in 0.01 M HEPES buffered at pH 7.4. Metal ions added as chloride salts, [**2**] = 8.58×10^{-7} M.

Fe(II) can be cancelled by the addition of fluoride ion to produce the colourless FeF_6^{3-},[19] and as the sensitivity of **2** for Mn(II), Co(II) and Ni(II) is quite low (~20 mM), this sensor could be viewed as a probe for Cu(II) over a wide pH window in the μM range. The sensitivity of **2** for Cu(II) is in the physiological range meaning that it could potentially be used as an intracellular probe. As expected, the parent **1** does not respond to either Cu(II) or Fe(II) addition (see Supplementary data).

A more detailed look at the binding of Cu(II) to **2** is possible with ^{1}H NMR. Figure 5 shows the spectra of **2** with different concentrations of Cu(II). A significant downfield shift (0.7 ppm) was observed for the methylene protons adjacent to the phosphorus atom (b), with the other protons remaining essentially unaffected. This downfield shift suggests the involvement of this methylene group in the binding of the Cu(II) ion. A possible explanation is that a Cu(II) induced deprotonation of the methylene group occurs resulting in the formation of a carbanion that assists in chelation of the Cu(II), in a manner similar to that of sulfonamide based sensors.[20] More work is ongoing to fully understand this interaction.

In summary, we have incorporated a β-aminobisphosphonate receptor into a luminescent sensor according to the PET format for the first time. The fluorescence of the highly water soluble sensor remains 'On' over a wide pH range. Selectivity was demonstrated for Cu(II) over other common metal ions with sensitivity established at the μM level.

Table 1. Photophysical properties of **1** and **2**

Property	λ_{MAX}	λ_{EM}	ε_{MAX} (mol^{-1} dm^3 cm^{-1})	pK_a (amino)	pK_a (phosphonate)	Φ_{FLU}[a]	log β
1	283	333	5788	4.99	—	0.13	—
2 (unbound)	283	333	5524	4.98	10.68	0.15	—
2 + Cu(II)	283	—[b]	—	—	—	0.01	6.03
2 + Fe(II)	283	—[b]	—	—	—	0.02	6.58
2 + Mn(II)	283	333	—	—	—	0.06	3.75
2 + Ni(II)	283	333	—	—	—	0.07	3.60
2 + Co(II)	283	333				0.08	4.08

[a] Quantum yield calculated with reference to tryptophan.
[b] Accurate measurement not possible due to low intensity.

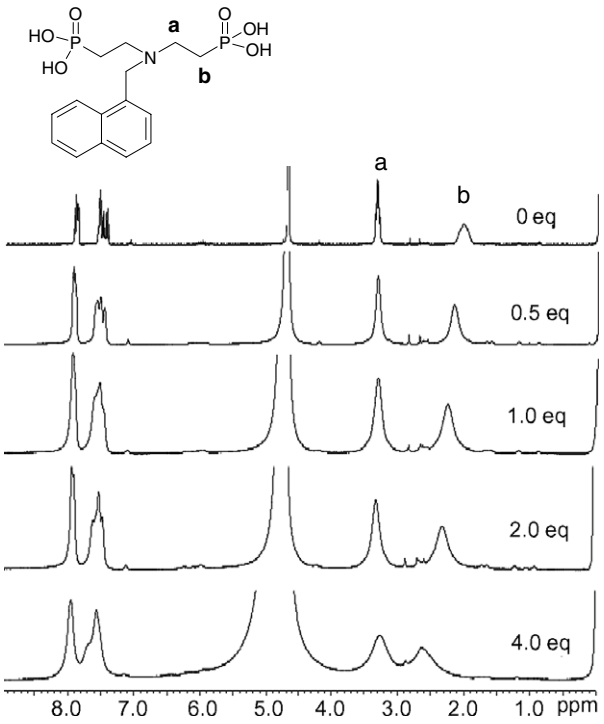


Figure 5. ^{1}H NMR spectra of **2** recorded in D_2O at 400 MHz after addition of increasing concentrations of Cu(II). The numbers on each plot represent the molar equivalence of Cu(II) added. [**2**] = 4.2×10^{-2} M.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.09.029.

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10. Analytical data for **1**. ^{1}H NMR ($CDCl_3$, chemical shift δ in ppm relative to TMS): 1.30 (t, $J = 6.4$ Hz, $-OCH_2CH_3$), 1.90–2.01 {m, $-CH_2CH_2P(O)(OEt_2)_2$, 4H}, 2.80–2.90 (m, $-N-CH_2-CH_2-$, 4H), 3.91–4.10 (m, $-CH_2-N$, $-OCH_2CH_3$, 10H), 7.35–7.50 (m, 4H, aromatic), 7.74 (d, $J = 8.0$ Hz, 1H, aromatic), 7.81 (dd, $J = 2.4$, 7.2 Hz, 1H, aromatic), 8.20 (dd, $J = 2.4$, 8.0 Hz, 1H, aromatic). ^{13}C NMR

($CDCl_3$, chemical shift δ in ppm relative to TMS): 16.32 (CH_3), 16.38 (CH_3), 25.08 (CH_2), 25.47 (CH_2), 42.6 (CH_2), 50.4 (CH_2), 61.8 (CH_2), 123.3 (CH), 125.3 (CH), 125.7 (CH), 126.3 (CH), 126.5 (CH), 128.2 (CH), 128.7 (CH), 131.6 (C), 133.8 (C), 134.0 (C), 175.2 (P═O). P^{31} NMR (with proton decoupling): δ 30.28. ESMS: Calculated for $C_{23}H_{37}NO_6P_2$ $[M^+] = 486.2169$; measured mass $[M^+] = 486.2170$. Analytical data for **2**. ^1H NMR (CD_3OD, chemical shift δ in ppm relative to TMS): 2.10–2.11 {m, $-CH_2CH_2P(O)(OEt_2)_2$, 4H}, 3.37–3.47 (m, $-N-CH_2-CH_2-$, 4H), 4.89 (s, $-CH_2N$, 2H), 7.47–7.53 (m, 3H, aromatic), 7.6 (dd, $J = 1.2$, 7.2 Hz, 1H, aromatic), 7.70 (d, $J = 7.2$ Hz, 1H, aromatic), 7.9 (d, $J = 8.0$ Hz, 1H, aromatic), 7.98 (d, $J = 8.0$ Hz 1H, aromatic), 8.11 (d, $J = 8.8$ Hz, 1H, aromatic). ^{13}C NMR (CD_3OD, chemical shift δ in ppm relative to TMS): 23.6 (CH_2), 24.96 (CH_2), 56.6 (CH_2), 125.01 (CH), 127.69 (CH), 128.99 (CH), 130.10 (CH), 131.49 (CH), 133.43 (CH), 133.65 (C), 134.22 (C), 136.68 (C), 175.3 (P═O).

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