



HAL
open science

**A new class of fluorescent chemosensors based on the A
new class of fluorescent chemosensors based on the
 β -aminobisphosphonate receptor**

John Callan, Sukanta Kamila, Narinder Singh, Ray Mulrooney, Martha
Mackay, Maedhbh Cronin, John Dunn, David Durham

► **To cite this version:**

John Callan, Sukanta Kamila, Narinder Singh, Ray Mulrooney, Martha Mackay, et al.. A new class of fluorescent chemosensors based on the A new class of fluorescent chemosensors based on the β -aminobisphosphonate receptor. *Supramolecular Chemistry*, 2009, 21 (07), pp.643-649. 10.1080/10610270802709352 . hal-00520015

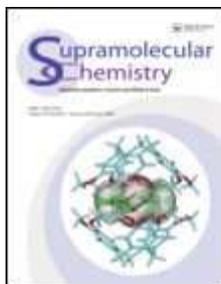
HAL Id: hal-00520015

<https://hal.science/hal-00520015>

Submitted on 22 Sep 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



A new class of fluorescent chemosensors based on the β -aminobisphosphonate receptor

Journal:	<i>Supramolecular Chemistry</i>
Manuscript ID:	GSCH-2008-0114.R3
Manuscript Type:	Full Paper
Date Submitted by the Author:	27-Nov-2008
Complete List of Authors:	Callan, John; Robert Gordon University Kamila, Sukanta; RGU Singh, Narinder; RGU Mulrooney, Ray; RGU MacKay, Martha; RGU Cronin, Maedhbh; RGU Dunn, John; RGU Durham, David; RGU
Keywords:	β -aminobisphosphonate , sensor, fluorescence
Note: The following files were submitted by the author for peer review, but cannot be converted to PDF. You must view these files (e.g. movies) online.	
Scheme 1.cdx	



Graphical Abstract

A new class of fluorescent chemosensors based on the β -aminobisphosphonate receptor.

John F. Callan^{1*}, Sukanta Kamila¹, Ray C. Mulrooney¹, Martha MacKay¹, Maedhbh C. Cronin¹, John Dunn¹, and David G. Durham¹.

School of Pharmacy and Life Sciences, The Robert Gordon University, Aberdeen, AB10 1FR, UK



A new class of fluorescent chemosensors based on the β -aminobisphosphonate receptor

John F. Callan,^a Sukanta Kamila,^a Narinder Singh,^a Ray C. Mulrooney,^a Martha MacKay,^a Maedhbh C. Cronin,^a John Dunn,^a David G. Durham^a

^a School of Pharmacy and Life Sciences, The Robert Gordon University, Aberdeen, AB10 1FR, UK

* corresponding author E Mail [.j.callan@rgu.ac.uk](mailto:j.callan@rgu.ac.uk) Ph: +44(0)1224 262546, Fax: +44(0)1224262555

Abstract

The photophysical properties of a range of fluorescent sensors with β -aminobisphosphonate receptors have been studied. The compounds were designed according to the Fluorophore-Spacer-Receptor format of Photoinduced Electron Transfer (PET) based sensors. The sensors displayed unusual fluorescence intensity-pH profiles that was attributed to a receptor – fluorophore H-bonding interaction that resulted in a quenching of fluorescence. The number of receptors and the nature of the fluorophore were both shown to have an effect on the pH profiles of the sensors. In addition, compound **1** was also shown to be selective for Cu^{2+} ions, in water at pH 7.4 with sensitivity in the μM range.

Keywords: Fluorescence, sensor, pH, copper.

Introduction

β -aminobisphosphonate derivatives, such as pamidronate (Aredia), are commercially used to treat bone resorption disorders such as osteoporosis and Pagets disease.¹ Their activity is thought to be due to the ability to strongly chelate certain metal ions, particularly Ca^{2+} .² In addition, it has been demonstrated using electron paramagnetic spectroscopy that β -aminobisphosphonates derivatives bind strongly to Cu^{2+} .³ Therefore, their incorporation as the ionophore component of luminescent sensing assemblies seems a logical progression.

We have previously developed a luminescent sensor that incorporated β -aminobisphosphonate as a receptor component.⁴ The sensor, designed according to the Fluorophore-Spacer-Receptor format of Photoinduced Electron Transfer (PET) based sensors,⁵ was shown to selectively bind Cu^{2+} ions in water at pH 7.4. However, the choice of naphthalene as fluorophore has obvious restrictions when considering intracellular applications, as UV excitation is necessary. Here, we improve on the design using anthracene as fluorophore by preparing {2-[anthracen-9-ylmethyl-(2-phosphono-ethyl)-amino]-ethyl}-phosphonic acid (**1**) and {2-[(10-[[Bis-(2-phosphono-ethyl)-amino]-methyl)-anthracen-9-ylmethyl)-(2-phosphono-ethyl)-amino]-ethyl}-phosphonic acid (**3**). Both sensors were again constructed according to the PET design principle and differ only by the number of receptor ligands they possess. Control compounds were also prepared (**2** and **4**) in which the phosphonate receptors were absent. The preparation of these compounds has enabled us to propose a mechanism for the unusual pH profile of the sensors **1** and **3**. In particular, it has allowed us to correct upon a postulated mechanism for the pH behavior of the aforementioned naphthalene based compound from an earlier communication.⁴ The

1
2
3 selectivity and sensitivity of **1** and **3** was also established against a range of common
4
5 metal ions to determine their potential use as chemosensors.
6
7
8
9

10 11 **Experimental**

12 13 14 15 **General**

16
17 All reagents used were of the highest grade obtainable and were purchased from
18 Aldrich. The compounds 5, 7-8 and 10 were prepared with literature methods.^{4,13,15}

19
20 Absorbance measurements were recorded on an Agilent UV-Vis spectrometer using
21
22 10 mm quartz cuvettes. Fluorescence measurements were recorded on a Shimadzu
23
24 RF-5301 luminescence spectrometer using 10 mm quartz cuvettes. Excitation slit size
25
26 was 10 nm and emission slit size was 10 nm. Scan speed was set at 500. NMR spectra
27
28 were recorded on a Bruker AVANCE 400 MHz spectrometer. Chemical shifts are
29
30 reported in parts per million, downfield of TMS. Details of solvents are provided in
31
32 the figure captions. Low resolution MS were obtained on an Agilent 6130 Quadrupole
33
34 LC/MS system. Accurate Mass data was provided by the EPSRC National Mass
35
36 Spectrometry Service, UK. Figure 3 was prepared on ArgusLab 4.0. The anthracene
37
38 aminobisphosphonate was energy minimized and the binding site optimized by
39
40 rotation of the side chains.
41
42
43
44
45
46
47
48
49

50 51 **Synthesis of (2-{anthracen-9-ylmethyl-[2-(diethoxy-phosphoryl)-ethyl]-amino}-** 52 53 **ethyl)- phosphonic acid diethyl ester (6)**

54
55 A mixture of 9-(aminomethyl)anthracene (0.97g, 4.72 mmol) and diethyl
56
57 vinylphosphonate (4.42 mL, 28.3 mmol) was heated at reflux in dry methanol (20 mL)
58
59 for 10 days. Following cooling of the reaction mixture the solvent was removed under
60

1
2
3 reduced pressure. The crude product was purified by flash chromatography using
4 silica gel eluted with methanol : dichloromethane (1:50). The product was separated
5 as an orange liquid (0.29g, 11%); δ_{H} (400 MHz, DMSO- d_6) 1.08 (12H, t, J 6.4 Hz, -
6 OCH₂CH₃), 1.95 (4H, m, -CH₂CH₂P(O)(OEt)₂), 2.81 (4H, m, -NCH₂CH₂-), 3.85 (8H,
7 m, -OCH₂CH₃), 4.59 (2H, s, -CH₂N-) 7.56 (4H, m, Ar-H) 8.09 (2H, d, J 8.0 Hz, Ar-
8 H), 8.55 (3H, m, Ar-H); δ_{C} (100 MHz, DMSO- d_6) 16.0, 21.4, 22.76, 45.7, 49.2, 123.4,
9 123.9, 125.0, 127.1, 129.2, 130.3, 130.9, 134.5; m/z (ESMS) 536 (60%) [M^+],
10 400 (100%), 191 (24%); HRMS calcd for C₂₇H₃₉NO₆P₂ 536.2325; found 536.2322.
11
12
13
14
15
16
17
18
19
20
21
22
23

24 **Synthesis of {2-[anthracen-9-ylmethyl-(2-phosphono-ethyl)-amino]-ethyl}-** 25 **phosphonic acid (1)**

26
27
28 To a solution of **6** (0.12 g, 0.23 mmol) in dry CH₂Cl₂ (10 mL) was added
29 bromotrimethylsilane (0.82 mL, 6.31 mmol). The mixture was stirred overnight at
30 room temperature. Evaporation of the solvent along with excess bromotrimethylsilane
31 under reduced pressure afforded the crude product which was triturated with hexane.
32
33 The resulting solid was dissolved in DCM and the product obtained after precipitation
34 with ether (0.092 g, 98%); mp 172°C; δ_{H} (400 MHz, MeOD) 2.1 (4H, m, -
35 CH₂CH₂P(O)(OH)₂), 3.6 (4H, m, -NCH₂CH₂-), 5.5 (2H, s, -CH₂N-) 7.65 (4H, m, Ar-
36 H), 8.2 (2H, d, J 8.0 Hz, Ar-H), 8.45 (2H, d, J 8Hz, Ar-H), 8.85 (1H, s, Ar-H);
37
38 δ_{C} (100 MHz, MeOD) 22.7, 24.2, 51.8, 120.7, 124.0, 126.8, 129.4, 129.6, 130.9, 132.8,
39 133.0; m/z (ESMS) 423 (25%) [M^+], 232 (50%), 121 (100%); HRMS calcd for
40 C₁₉H₂₃NO₆P₂ (M-H)⁻ 422.0928; found 422.0923.
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56

57 **Synthesis of anthracen-9-ylmethyl-diethyl-amine (2)**

58
59 A mixture of 9-(chloromethyl)anthracene (1.080g, 4.41 mmol), diethylamine (1.37
60 mL, 13.23 mmol) and DIPEA (2.3 mL, 13.23 mmol) in anhydrous 1,4 dioxane (10

1
2
3 mL) was stirred at 100 °C overnight. The solution was allowed to cool and excess
4 solvent and un-reacted amine were removed by evaporation under reduced pressure.
5
6 The crude product was then washed with distilled water and extracted with ethyl
7 acetate. The organic layer was dried over Na₂SO₄ and the solvent removed under
8 reduced pressure to afford the product (0.89 g, 82.54%); mp 84°C; δ_H(400 MHz,
9 CDCl₃) 1.00 (6H, t, *J* 6.5 Hz, -NCH₂CH₃), 2.55 (4H, q, *J* 7.0 Hz, -NCH₂CH₃), 4.45
10 (2H, s, -CH₂NCH₂CH₃), 7.45 (4H, m, Ar-H), 7.9 (2H, d, *J* 8.5 Hz, Ar-H), 8.30 (1H, s,
11 Ar-H), 8.50 (2H, d, *J* 8.0 Hz, Ar-H); δ_C(100 MHz, CDCl₃) 10.9, 45.6, 49.2, 122.8,
12 123.9, 124.6, 126.4, 127.9, 130.4, 132.4, 132.9; *m/z* (ESMS) 264 (10%) (M⁺), 191
13 (100%); HRMS calcd for C₁₉H₂₁N 264.1747; found 264.1750.
14
15
16
17
18
19
20
21
22
23
24
25
26
27

28 **Synthesis of (2-([10-({Bis-[2-(diethoxy-phosphoryl)-ethyl]-amino)-methyl]-**
29 **anthracen-9-ylmethyl)-[2-(diethoxy-phosphoryl)-ethyl]-amino}-ethyl)-**
30 **phosphonic acid diethyl ester (9)**
31
32
33
34
35

36 The procedure for the preparation of **6** was followed using the following amounts of
37 reagents: **8** (1g, 4.23 mmol) and diethylvinyl phosphonate (8.58 mL, 55.0 mmol).
38 The product was obtained as an orange solid (0.54g, 16.7%); mp 115°C; δ_H(400MHz,
39 MeOD) 1.05 (24H, m, 8x-CH₃), 1.87 (8H, m, 4x-CH₂P-), 2.76 (8H, m, 4 x-NCH₂-),
40 3.70 (16H, m, 8x-OCH₂-), 4.51 (4H, s, 2 x Ar-CH₂), 7.42 (4H, dd, *J*₁ 3.5 Hz, *J*₂ 8.0 Hz
41 Ar-H), 8.47(4H, dd, *J*₁ 3.5 Hz, *J*₂ 3.0 Hz, Ar-H); δ_C(100MHz, MeOD) 16.6, 22.9,
42 24.3, 46.8, 51.2, 126.6, 127.9, 131.6, 132.4; *m/z* (ESMS) 894 (5%) [M⁺], 548 (10%),
43 447 (100%) [M²⁺], 346 (15%); HRMS calcd for C₄₀H₆₈N₂O₁₂P₄ 893.3795; found
44 893.3810.
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Synthesis of {2-[(10-{[Bis-(2-phosphono-ethyl)-amino]-methyl}-anthracen-9-ylmethyl)-(2-phosphono-ethyl)-amino]-ethyl}-phosphonic acid (3)

The procedure for the preparation of **1** was followed using the following amounts of reagents: **9** (0.54 g, 0.60 mmol) and bromotrimethylsilane (1.26 mL, 9.60 mmol). The product was obtained as an orange solid (0.12g, 30%). δ_{H} (400MHz, MeOD) 2.00 (8H, m, 4x-CH₂P-), 3.30 (8H, t, 4x-NCH₂-), 5.67(4H, s, 2 x Ar-CH₂), 7.77 (4H, dd, J_1 3.0 Hz, J_2 7.5 Hz, Ar-H), 8.48(4H, dd, J_1 3.0 Hz, J_2 7.5 Hz, Ar-H); δ_{C} (100MHz, MeOD) 20.8, 22.2, 48.1, 123.6, 123.8, 127.7, 130.5; m/z (ESMS) 668 (20%) [M^+], 588 (60%), 560 (60%), 349 (100%); HRMS calcd for C₂₄H₃₆N₂O₁₂P₄ (M-H)⁻ 667.1146; found 667.1139.

Synthesis of (10-Diethylaminomethyl-anthracen-9-ylmethyl)-diethyl-amine (4)

The procedure for the preparation of **2** was followed using the following amounts of reagents: **7** (1.00g, 2.75 mmol), diethyl amine (1.72ml, 16.54 mmol) and N-ethyl-diisopropylamine (2.88ml, 16.54 mmol). The product was obtained as an orange solid (0.72g, 75.2%). mp 227°C; δ_{H} (400MHz, DMSO) 1.23 (12H, t, 4 x-CH₃), 3.05 (4H, m, 2x-NCH₂-), 3.29 (4H, m, 2x-NCH₂-), 5.47 (4H, s, 2 x Ar-CH₂), 7.77 (4H, m, Ar-H), 8.54 (4H, m, Ar-H); δ_{C} (100MHz, DMSO) 8.7, 47.2, 48.4, 125.3, 125.5, 127.4, 130.9; m/z (ESMS): 276 (100%), 204 (6%), 175 (11%) [M^{2+}]. HRMS calcd for C₂₄H₃₂N₂ 349.2638; found 349.2634.

Synthesis of Diethyl-naphthalen-1-ylmethyl-amine (11)

The procedure for the preparation of **2** was followed using the following amounts of reagents: 1-(chloromethyl)naphthalene (2.22 mL, 14.15 mmol), diethyl amine (4.40 mL, 42.45 mmol) and N-ethyl-diisopropylamine (7.39 mL, 42.46 mmol). The product was

1
2
3 obtained as dark viscous liquid (2.61g, 84.2%); δ_{H} (400MHz, CDCl_3) 0.99 (6H, t, J
4 6.2Hz, 2 x- CH_3), 2.49 (4H, m, 2x- NCH_2 -), 3.90 (2H, s, Ar- CH_2 -), 7.31 (4H, m, Ar-H),
5
6 7.50 (1H, d, J 7.5Hz, Ar-H), 7.66 (1H, d, J 7.5Hz, Ar-H). 7.72 (1H, d, J 7.5Hz, Ar-H);
7
8
9
10 δ_{C} (100MHz, CDCl_3) 11.8, 47.0, 56.3, 124.8, 125.6, 125.9, 127.4, 127.6, 127.8, 128.6,
11
12 132.7, 134.0, 135.9; m/z (ESMS) 214 (100%) [M^+], 141 (25 %); HRMS calcd for
13
14 $\text{C}_{15}\text{H}_{19}\text{N}$ 214.1590; found 214.1591.
15
16
17
18
19
20
21

22 Results and Discussion

23 Synthesis of Sensors (1 and 3) and Control Compounds (2 and 4).

24
25 The synthesis of the target and control compounds are shown in Scheme 1.
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
The synthesis of the target and control compounds are shown in Scheme 1. Compound **1** was formed by first converting 9-chloromethylanthracene to 9-aminomethyl anthracene (**5**) using the Delepine reaction.⁶ Following the Michael addition of **5** with diethylvinyl phosphonate and subsequent hydrolysis with bromotrimethylsilane, sensor **1** was isolated as a white solid. Control compound **2** was also formed from chloromethyl anthracene by direct reaction with diethyl amine.

A similar strategy was followed for the preparation of compound **3**. Bisbromomethyl anthracene (**7**) was obtained from anthracene after reaction with hydrobromic acid. This was then converted to **3** following similar chemistry as outlined above. The control compound **4** was also formed from 9,10-bisbromomethyl anthracene by reaction with diethyl amine.

56 pH titration of 1 and 2.

57
58
59
60
UV analysis of both compounds showed characteristic anthracene absorbance with a λ_{MAX} centered at ~ 370 nm. The emission spectra, when excited at 370 nm, were also

1
2
3 characteristic of anthracene based compounds, with three distinct bands observed at
4 401 nm, 422 nm and 444 nm. However, the dependence of their emission on solution
5 pH was markedly different. A plot of fluorescent intensity against pH is shown in
6 Figure 1. The sigmoidal profile of control compound **2** ($\phi = 0.16$) was typical of a
7 PET sensor with a tertiary amine as receptor, with fluorescence switching “On” over
8 two log units.⁷ The emission intensity was low at high pH due to PET from the
9 tertiary amine to the anthracene fluorophore. As pH was lowered, the tertiary amine
10 became protonated, its oxidation potential raised and PET cancelled, allowing
11 fluorescence to “Switch On”.⁸ Using a plot of $-\log (F_{\text{MAX}} - F) / (F - F_{\text{MIN}})$ against pH
12 (where F_{MAX} is the maximum fluorescence intensity, F_{MIN} the minimum fluorescence
13 intensity and F the measured fluorescence intensity) the pKa of the tertiary amine was
14 calculated as 9.43.⁹

15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33 The pH titration of sensor **1** ($\phi = 0.22$) also switches “On” fully at pH 9 (pKa = 10.24)
34 but then the intensity falls off between pH 8 – 5. This initial switching “On” can again
35 be attributed to protonation of the tertiary amine canceling PET as in the case of the
36 control, the pKa value being consistent with that found for the amino group in alkyl
37 aminobisphosphonates determined by potentiometric titration³.

38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
The reduction in fluorescence intensity between pH's 8 - 5 can be explained by
protonation of the phosphonate oxyanions and their hydrogen bonding with the π -
electron cloud of the anthracene. The intramolecular quenching of anthracene
emission by carboxylic acid groups has been demonstrated before.^{7,10} The flexibility
of the receptor unit in **1**, means that the approach of the phosphonic acid group with
the anthracene π -cloud is also feasible for **1**. The slight rise and then fall in

1
2
3 fluorescence intensity between pH 5-2, is probably due to the stepwise protonation of
4
5 the phosphonate groups before they interact with the anthracene π -system.
6
7

8 9 **pH titration of 3 and 4**

10
11 Figure 2 shows a plot of fluorescence intensity against pH for compounds **3** and **4**.
12
13 The control compound shows a typical sigmoidal profile with fluorescence switching
14
15 “On” over two log units. Both tertiary amines must be protonated before fluorescence
16
17 switches “On” as there are two PET channels present in the molecule. The calculated
18
19 pKa of 8.34 of **4** ($\phi = 0.12$) is approximately one log unit lower than in **2**. Again, the
20
21 profile of sensor **3** ($\phi = 0.09$) is markedly different than that of the control. The
22
23 fluorescence remains low until approximately pH 4 when it switches “On”
24
25 dramatically. There is also evidence of a small rise in intensity from ~ pH’s 12 - 9
26
27 (pKa 8.34) which falls off between pH’s 9 - 7. This can again be explained as in the
28
29 case of **1**, with the rise due to protonation of the tertiary amine, and the fall off due to
30
31 oxyanion protonation and subsequent hydrogen bonding with the anthracene π -cloud.
32
33 The much more effective quenching of fluorescence for **3** compared to **1** is most
34
35 likely due to a stronger interaction between the protonated oxyanions and the
36
37 anthracene π -cloud, due to the presence of the second receptor ligand. A second small
38
39 step is also evident between pH’s 7 and 5 (pKa = 5.99) which again may be explained
40
41 by a stepwise protonation of the phosphonate groups before interaction with the
42
43 anthracene π -cloud. But what causes the large enhancement in fluorescence on going
44
45 from pH’s 4 - 2? The hydrogen bonding effect of the phosphonate groups with the
46
47 anthracene π -cloud must be turned off in some manner.
48
49
50
51
52
53
54
55
56
57

58 We believe this can be best explained as follows: the full protonation of the
59
60 phosphonate groups, coupled with the flexibility of the receptor units enables a strong

1
2
3 hydrogen bonding interaction of the phosphonate groups with each other. That is, the
4 phosphonate groups on one side of the anthracene molecule (the 9-position) interact
5 strongly with those on the opposite side of the molecule (the 10-position) by bending
6 to one side of the π -system, as depicted Figure 3. This strong hydrogen bonding
7 interaction is at the expense of the hydrogen bonding with the anthracene π -cloud and
8 enables fluorescence to switch “On”. However, as observed in Figure 2, the intensity
9 maxima of the sensor is somewhat smaller than in the control, indicating that a
10 residual phosphonate – anthracene π -cloud quenching interaction still exists, even at
11 pH 1.0.
12
13
14
15
16
17
18
19
20
21
22
23
24

25
26 The mechanistic interpretation outlined above for the unusual pH profile of
27 compounds **1** and **3** is different to that previously postulated by us in an earlier
28 communication. For purposes of clarity we include the fluorescent intensity - pH
29 profile for {2-naphthalen-1-ylmethyl-(2-phosphono-ethyl)amino} ethyl}-phosphonic
30 acid (**10**) used in that study along with its control analogue (**11**) in Figure 4. The
31 profile of **10** is similar to **1** in that it switches “On” at high pH, switches “Off” slightly
32 at intermediate pH and then back “On” at low pH. However, the magnitude of the
33 decrease in intensity at intermediate pH is much smaller for **10** than for **1**. In addition,
34 the intensity of **10** at low pH (i.e pH 2) is about that of its control **11** (i.e it is fully
35 switched “On”) while the intensity of **1** at the same pH is about half that of the control.
36 We previously accounted for the intensity increase of **10** from pH’s 12-10 as being
37 due to intramolecular proton transfer from a protonated phosphonate group to the
38 tertiary amine of the receptor, resulting in a partial cancellation of the PET process.
39 The second stepped increase in intensity from pH’s 6 - 4 was attributed due to full
40 protonation of the tertiary amine and a complete cancellation of the PET process. We
41 now consider this to be an inaccurate description for the profile of **10** and believe the
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 same reasons as offered for **1** and **3** are also evident for **10**. That the reduction in
4
5 intensity between pH's 9 – 7 is much less dramatic for **10** than **1** is most likely due to
6
7 the much smaller π -cloud of naphthalene compared to anthracene, leading to a
8
9 reduced effectiveness of the phosphonate - π -cloud hydrogen bonding interaction
10
11 and a reduced quench.

12 13 14 15 16 17 **Selectivity and Sensitivity measurements of 1 and 3**

18
19 The nature of the pH - fluorescence profile of **1** means that its use as an Off-On sensor
20
21 for physiologically relevant analytes is limited, as its fluorescence remains “On” over
22
23 a wide pH range. However, as the fluorescence intensity is quite high at pH 7.4, **1**
24
25 may be effective as an “On – Off” sensor in buffered solution. We screened **1** against
26
27 a range of common metal ions, in 0.01 M HEPES buffer at pH 7.4. As shown in
28
29 Figure 5 , addition of Cu^{2+} leads to an ~ 80 % quenching of the original fluorescent
30
31 intensity, while Fe^{2+} and Zn^{2+} led to quenches of ~ 60 and ~ 20 % respectively, with
32
33 the other ions having virtually no effect. The quenching effect of Cu^{2+} is expected as
34
35 the open shell ion is known to quench fluorescence by electron and / or energy
36
37 transfer.^{5c} Also, its position in the Irving Williams series means it is a strong binder to
38
39 ligands, irrespective of the nature or number of ligands involved.¹¹ The quenching
40
41 effect from Fe^{2+} is most likely due to an inner filter effect,¹² but the quenching effect
42
43 from the closed shell Zn^{2+} , although minor, was not expected. In contrast, the addition
44
45 of these ions to a solution of the control produced negligible quenching, highlighting
46
47 the requirement of the aminobisphosphonate receptor for analyte binding. Figure 6
48
49 shows a plot of relative intensity against $-\log [\text{anion}]$ for Cu^{2+} and Fe^{2+} . Both ions
50
51 quench fluorescence over two log units indicating a 1:1 binding between sensor and
52
53
54
55
56
57
58
59
60

1
2
3 metal ion.¹³ The binding constants, Log β , calculated from equation 1 were 4.72 and
4
5 5.23 for Cu²⁺ and Fe²⁺ respectively.⁹
6
7

$$-\text{Log} (F_{\text{MAX}} - F) / (F - F_{\text{MIN}}) = \text{Log} [\text{Anion}] + \text{Log} \beta \quad (1)$$

8
9
10
11 This confirms that Fe²⁺ binds stronger to the sensor than Cu²⁺ as observed for **10** in a
12
13 previous study⁴. The sensitivity of **1** for Cu²⁺ is in the physiologically relevant range¹⁴
14
15 and the quenching effect caused by Fe²⁺ can be cancelled by the addition of fluoride
16
17 ion to produce the colorless FeF₆³⁻.¹² Therefore, **1** could be utilized as a water soluble
18
19 sensor for Cu²⁺, operable in the physiological range. The low fluorescence intensity of
20
21 **3** at pH 7.4 means it is potentially suitable as an “Off – On” sensor, provided analyte
22
23 binding is sufficient to disrupt the intramolecular hydrogen bonds between the
24
25 phosphonate groups and the anthracene π -cloud. Unfortunately when tested against
26
27 the same metal ions listed in figure 4, no fluorescence enhancements were observed
28
29 suggesting that the phosphonate - anthracene π -cloud interaction must be relatively
30
31 strong.
32
33
34
35
36
37
38
39

40 Conclusion

41
42 We have presented a new class of fluorescent sensors whose response to pH varies
43
44 considerably depending on both the number of receptors attached and the nature of
45
46 the fluorophore. The reason for the unusual pH profile for these compounds has been
47
48 attributed to a phosphonate - fluorophore π -cloud hydrogen bonding interaction that
49
50 quenches fluorescence. To the best of our knowledge, this is the first reported
51
52 example of such an effect with phosphonates on anthracene. In the case of compound
53
54 **3** this attraction can be broken at very low pH's when all the phosphonate oxyanions
55
56
57
58
59
60

1
2
3 are protonated. In addition, compound **1** showed good selectivity for Cu²⁺ ions in
4
5
6 water at pH 7.4 and its sensitivity was determined to be in the physiological range.
7
8
9

10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

References

1. British National Formulary (2005) Royal Pharmaceutical Society of Great Britain. 49:383
2. (a) Kiss, T.; Balla J.; Nagy, G.; Kozlowski, H.; Kowalik J.; Complexes of aminophosphonates. I. Transition metal complexes of aminophosphonic acid analogues of alanine, β -alanine, phenylalanine and tyrosine. *Inorg. Chim. Acta.*, **1987**, *138*, 2 (b) Kiss, T.; Farkas, E.; Kozlowski, H.; Complexes of aminophosphonates. II. Transition metal complexes of aminophosphonic acid analogues of aspartic acid and glutamic acid. *Inorg. Chim. Acta.* **1989**, *155*, 281 (c) Matczak-Jon, E.; Kurzak, B.; Sawka-Dobrowolska, W.; Kafarski, P.; Lejczak, B.; Zinc(II) complexes of phosphonic acid analogues of glutamic acid. *J. Chem. Soc. Dalton Trans.* **1996**, 3455 (d) Matczak-Jon, E.; Kurzak, B.; Sawka-Dobrowolska, W.; Lejczak, B.; Kafarski, P.; Zinc(II) complexes of phosphonic acid analogues of aspartic acid and asparagine. *J. Chem. Soc. Dalton Trans.* **1998**, 161 (e) Gumienna-Kontecka, E.; Jezierska, J.; Lecouvey, M.; Leroux, Y.; Kozlowski, H.; Bisphosphonate chelating agents Coordination ability of 1-phenyl-1-hydroxymethylene bisphosphonate towards Cu²⁺ ions. *J. Inorg. Biochem.*, **2002**, *89*, 13 (f) Kubicek, V.; Vojtíšek, P.; Rudovsky, J.; Hermann, P.; Lukes, I.; Complexes of divalent transition metal ions with bis

- (aminomethyl) phosphinic acid in aqueous solution and in the solid state. *Dalton Trans.* **2003**, 3927.
3. Gumienna-Kontecka, E.; Galezowska, J.; Drag, M.; Latajka, R.; Kafarski, P.; Kozłowski, H.; Coordination abilities of substituted beta-aminophosphonates towards Cu^{2+} and Zn^{2+} ions. *Inorganica. Chimica. Acta.*, **2004**, 357, 1632.
4. Kamila, S.; Callan, J.F.; Mulrooney, R.C.; Middleton, M.; A novel fluorescent chemosensor for Cu(II) in aqueous solution based on a β -aminobisphosphonate receptor. *Tetrahedron Lett.* **2007**, 48, 7756.
5. (a) Tsien, R.Y.; Intracellular signal transduction in four dimensions from molecular design to physiology. *Am. J. Physiol.* **1992**, 26, 723 (b) Bissell, R.A.; de Silva, A.P.; Gunaratne, H.Q.N.; Lynch, P.L.M.; Maguire, G.E.M.; McCoy, C.P.; Sandanayake, K.R.A.S.; Fluorescent PET (photoinduced electron transfer) sensors. *Top. Curr. Chem.* **1993**, 168, 233 (c) Czarnik, A.W.; Fluorescent chemosensors for metal and non-metal ions in aqueous solution based on the CHEF paradigm. *Adv. Supramol. Chem.* **1993**, 3, 131 (d) Czarnik, A.W.; Chemical communication in water using fluorescent chemosensors. *Acc. Chem. Res.* **1994**, 27, 302 (e) Fabbrizzi, L.; Poggi, A.; Sensors and switches from supramolecular chemistry. *Chem. Soc. Rev.* **1995**, 24, 197 (f) de Silva, A.P.; Gunaratne, H.Q.N.; Gunlaugsson, T.; Huxley, A.J.M.; McCoy, C.P.; Rademacher, J.T.; Rice, T.E.; Signalling Recognition Events with Fluorescent Sensors and Switches. *Chem. Rev.* **1997**, 97, 1515 (g) Fabbrizzi, L.; Licchelli, M.; Pallavicini, P.; Transition Metals as Switches. *Acc. Chem. Res.* **1999**, 32, 846 (h) Fabbrizzi, L.; The design of luminescent sensors for anions and ionisable analytes. *Coord. Chem. Rev.* **2000**, 205, 1 (i) Kojima, H.; Nagano, T.; Fluorescent Indicators for Nitric Oxide. *Adv. Mater.*

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
- 2000, 12: 763 (j) Rurack, K.; Resch-Genger, U.; Rigidization, preorientation and electronic decoupling—the magic triangle for the design of highly efficient fluorescent sensors and switches. *Chem. Soc. Rev.* **2002** 31, 116
- (k) de Silvia, A.P.; McClean, G.D.; Moody, T.S.; Weir, S.M. In Handbook of Photochemistry and Photobiology, Ed Nalwa HS. American Scientific Publishers, Stevenson Ranch, CA: **2003** (l) Martínez-Máñez, R.; Sancenón, F.; Fluorogenic and Chromogenic Chemosensors and Reagents for Anions. *Chem. Rev.* **2003**, 103: 4419 (m) Callan, J.F., de Silvia, A.P., Magri, D.C. (2005) Luminescent sensors and switches in the early 21st century. *Tetrahedron*, **2005**, 61, 8551.
6. Surrey, A.R. Named Reactions in Organic Synthesis. Academic Press Inc New York and London., **1961**.
7. Callan, J.F.; de Silva, A.P.; McClenaghan, N.D.; Switching between molecular switch types by module rearrangement: Ca²⁺-enabled, H⁺-driven Off–On–Off, H⁺-driven YES and PASS 0 as well as H⁺, Ca²⁺-driven AND logic operations. *Chem. Commun.* **2004**, 2048.
8. Magri, D.C.; Callan, J.F.; de Silva, A.P., Fox, D.P.; McClenaghan, N.D., Sandanayake, K.R.A.S.; The Anthracen-9-ylmethoxy Unit: An Underperforming Motif Within the Fluorescent PET (Photoinduced Electron Transfer) Sensing Framework. *J. Fluoresc.* **2005**, 15, 769.
9. (a) de Silva, A.P.; Gunaratne, H.Q.N.; Fluorescent PET (photoinduced electron transfer) sensors selective for submicromolar calcium with quantitatively predictable spectral and ion-binding properties. *J. Chem. Soc. Chem. Commun.* **1990**, 14 (b) de Silva, A.P.; Gunaratne, H.Q.N.; Lynch, P.L.M.; Luminescence and charge transfer.4. On-off fluorescent PET

- (photoinduced electron transfer) sensors with pyridine receptors-1,3-diaryl-5-pyridyl-4,5-dihydropyrazoles. *J. Chem. Soc. Perkin. Trans.* **1995**, 2, 685.
10. Eaton, D.F.; Reference materials for fluorescence measurement. *Pure Appl. Chem.* **1988**, 60, 1107.
11. Rurack, K.; Flipping the light switch 'ON' – the design of sensor molecules that show cation-induced fluorescence enhancement with heavy and transition metal ions. *Spectrochim. Acta. A.* **2001**, 57, 2161
12. Xie, H.Y.; Liang, J.G.; Zhang, Z.L.; Liu, Y.; He, Z.K.; Pang, D.W. Luminescent CdSe-ZnS quantum dots as selective Cu²⁺ probe. *Spectrochimica Acta. A.* **2004**, 60, 2527.
13. Gunnlaugsson, T.; Davis, A.P.; Hussey, G.M.; Tierney, J.; Glynn, M.; Design, synthesis and photophysical studies of simple fluorescent anion PET sensors using charge neutral thiourea receptors. *Org. Biomol. Chem.*, **2004**, 2,1856.
14. Gunnlaugsson, T.; Leonard, J.P.; Sénéchal, K.; Harte, A.J.; Eu(III)–cyclen–phen conjugate as a luminescent copper sensor: the formation of mixed polymetallic macrocyclic complexes in water. *Chem. Commun.* **2004**, 782.
15. Gunnlaugsson, T.; Davis, A.P.; O'Brien, J.E.; Glynn, M.; Synthesis and photophysical evaluations of charge neutral thiourea or urea based fluorescent PET sensors for bis–carboxylates and pyrophosphate. *Org Biomol Chem*, **2005**, 3, 48.

Acknowledgements

The authors would like to thank RGU and the Leverhulme Trust UK for financial support and Professor A.P. de Silva and Dr Bridgeen M^cCaughan for helpful

1
2
3 discussions. We also thank the EPSRC national Mass Spectrometry service for the
4
5 high resolution spectra.
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review Only

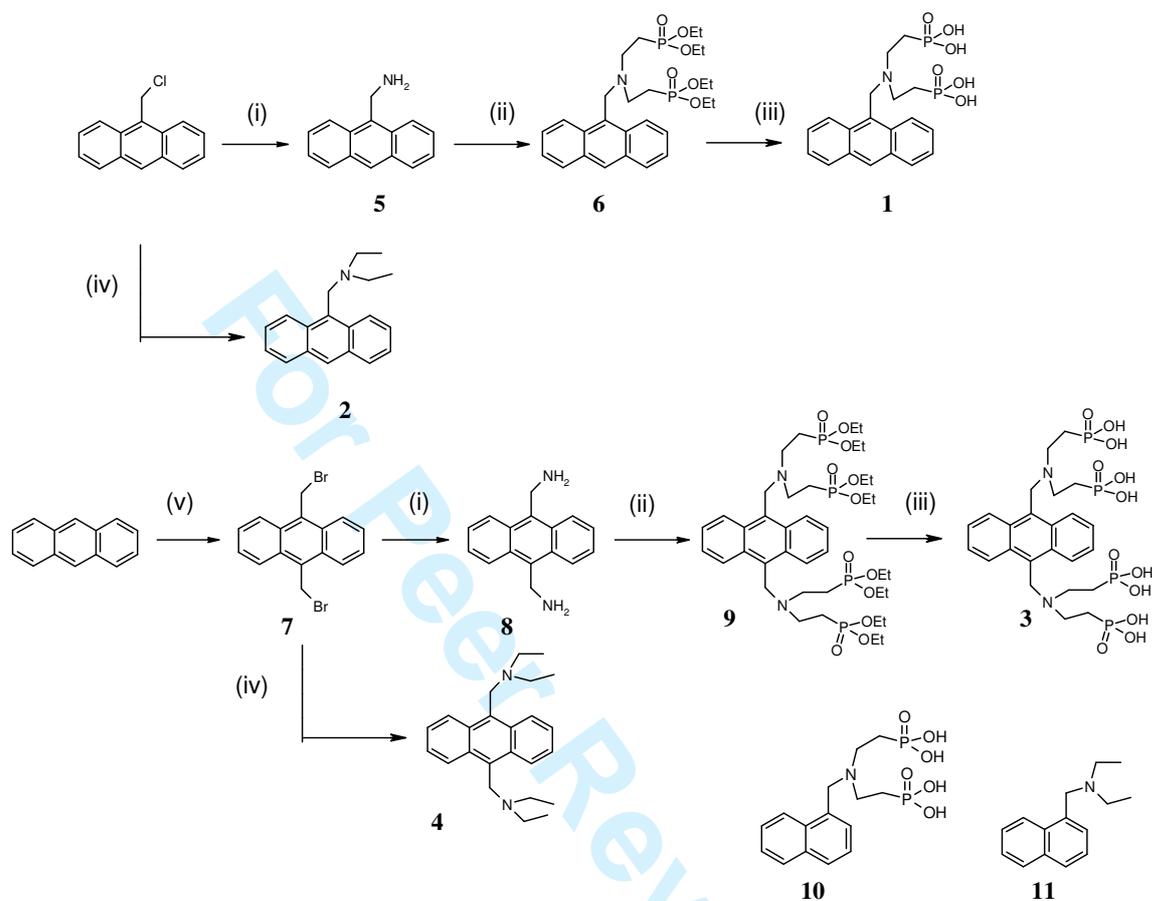
Tables

Table 1: Photophysical properties of **1-4** and **11**.

Property	λ_{MAX} ABS	λ_{MAX} EM	ϵ_{MAX} $\text{mol}^{-1} \text{dm}^3$ cm^{-1}	pKa (Amino)	pKa (Phos- phate)	$\Phi_{\text{FLU}}^{\text{a}}$	Log β
1	369	422	7485	10.24	-	0.22	-
2	368	421	9259	9.43	-	0.16	-
3	367	426	6601	8.34	2.80,5.99	0.09	-
4	368	428	8212	8.34	-	0.12	-
11	283	333	5610	9.91	-	0.08	-
1+Cu(II)	-	422	-	-	-	-	4.72
1+Fe(II)	-	421	-	-	-	-	5.23

^a Quantum yields (Φ) for **1-4** were calculated with reference to anthracene while **11** was calculated with reference to Tryptophan.¹¹ Φ for **1** and **3** were recorded in H₂O at pH 7.4 while **2** and **4** were recorded in EtOH.

Figures and Diagrams



Scheme 1 Synthesis of compounds **1-4**. (i) $C_6H_{12}N_4$, dry $CHCl_3$, 80 °C, 5 h; (ii) $CH_2CHPO(OCH_2CH_3)_2$, dry MeOH, 80 °C, 10 days; (iii) $(CH_3)_3SiBr$, dry CH_2Cl_2 , 28 °C, 18h; (iv) $(CH_3CH_2)_2NH$, DIPEA, dry $C_4H_8O_2$, 100 °C, 18 h (v) HBr , CH_3COOH , $CH_3(CH_2)_{13}N(Br)(CH_3)_3$, $C_3H_6O_3$, 100 °C 18h

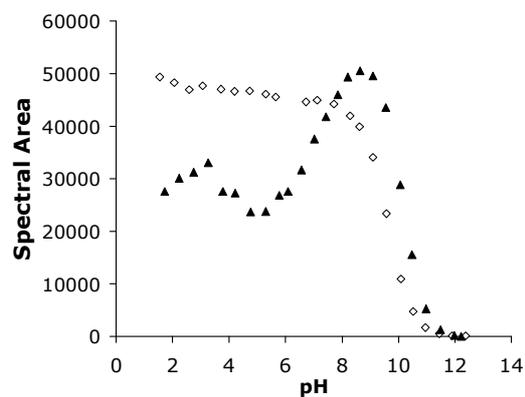


Figure 1. Plot of fluorescent intensity against pH for **1** (▲) and **2** (◇). $[1] = 4.40 \times 10^{-6} \text{ M}$, $[2] = 1.88 \times 10^{-6} \text{ M}$, $\lambda_{\text{ex}} = 370 \text{ nm}$.

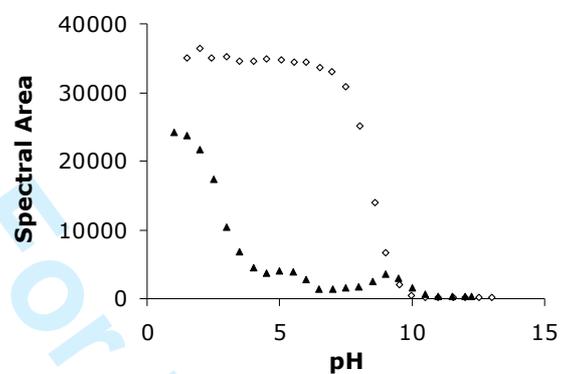


Figure 2 Plot of fluorescent intensity against pH for **3** (\blacktriangle) and **4** (\diamond). $[\mathbf{3}] = 2.25 \times 10^{-6}$ M, $[\mathbf{4}] = 2.87 \times 10^{-6}$ M, $\lambda_{\text{ex}} = 370$ nm.

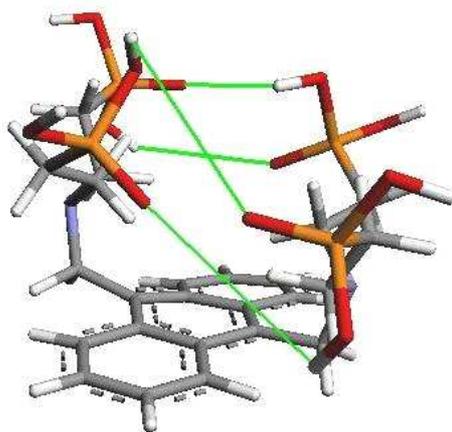


Figure 3 Illustration of potential intramolecular H-bonding between phosphonate groups on one side of **3** at low pH.

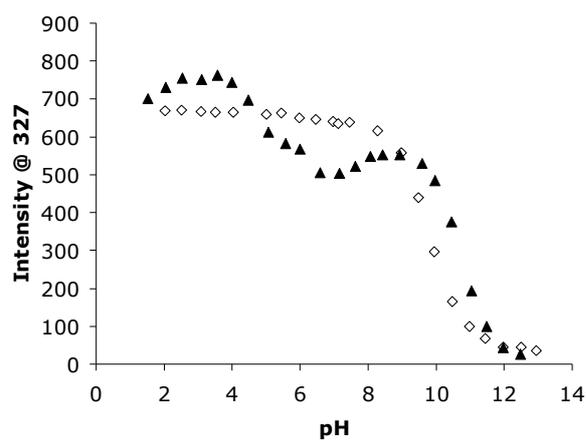


Figure 4 Plot of fluorescent intensity against pH for **10** (▲) and **11** (◇). $[10] = 8.57 \times 10^{-7} \text{ M}$, $[11] = 3.8 \times 10^{-7} \text{ M}$, $\lambda_{\text{ex}} = 283 \text{ nm}$.

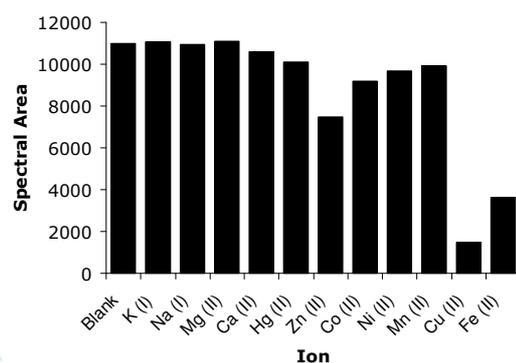


Figure 5 Selectivity Data for sensor **1** against a range of common metal ions as their chloride salts in 0.01 M HEPES buffer at pH 7.4. $[1] = 1.6 \times 10^{-7}$ M, $[\text{metal ion}] = 1 \times 10^{-4}$ M, $\lambda_{\text{ex}} = 370$ nm.

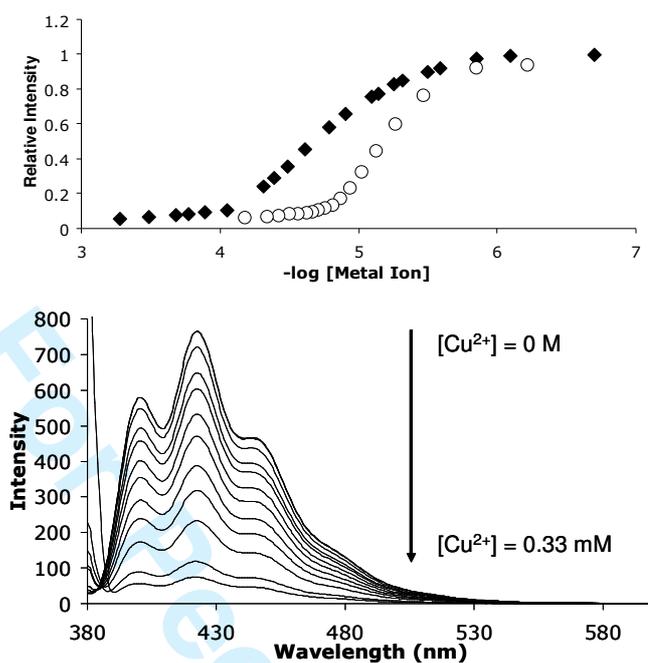
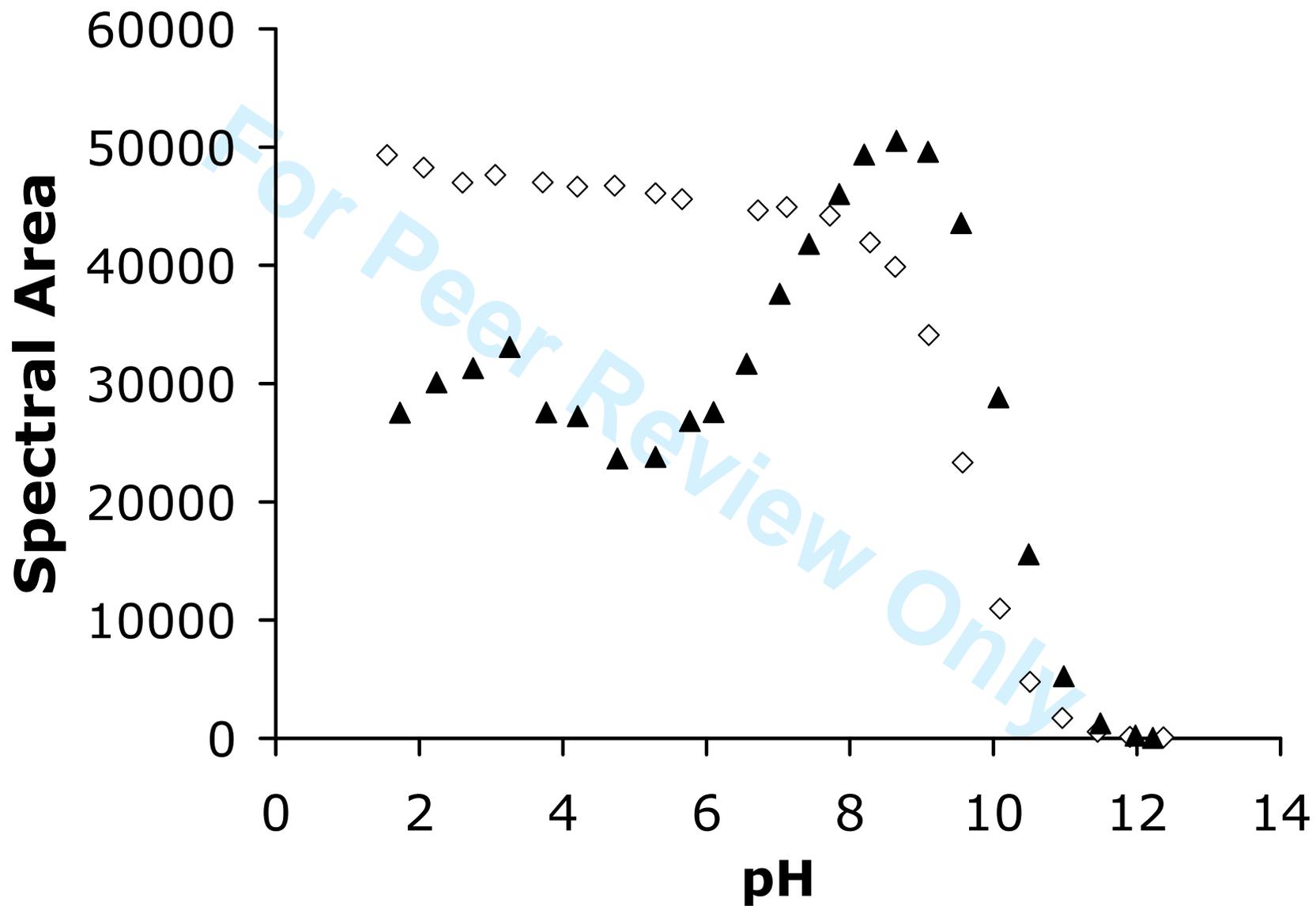
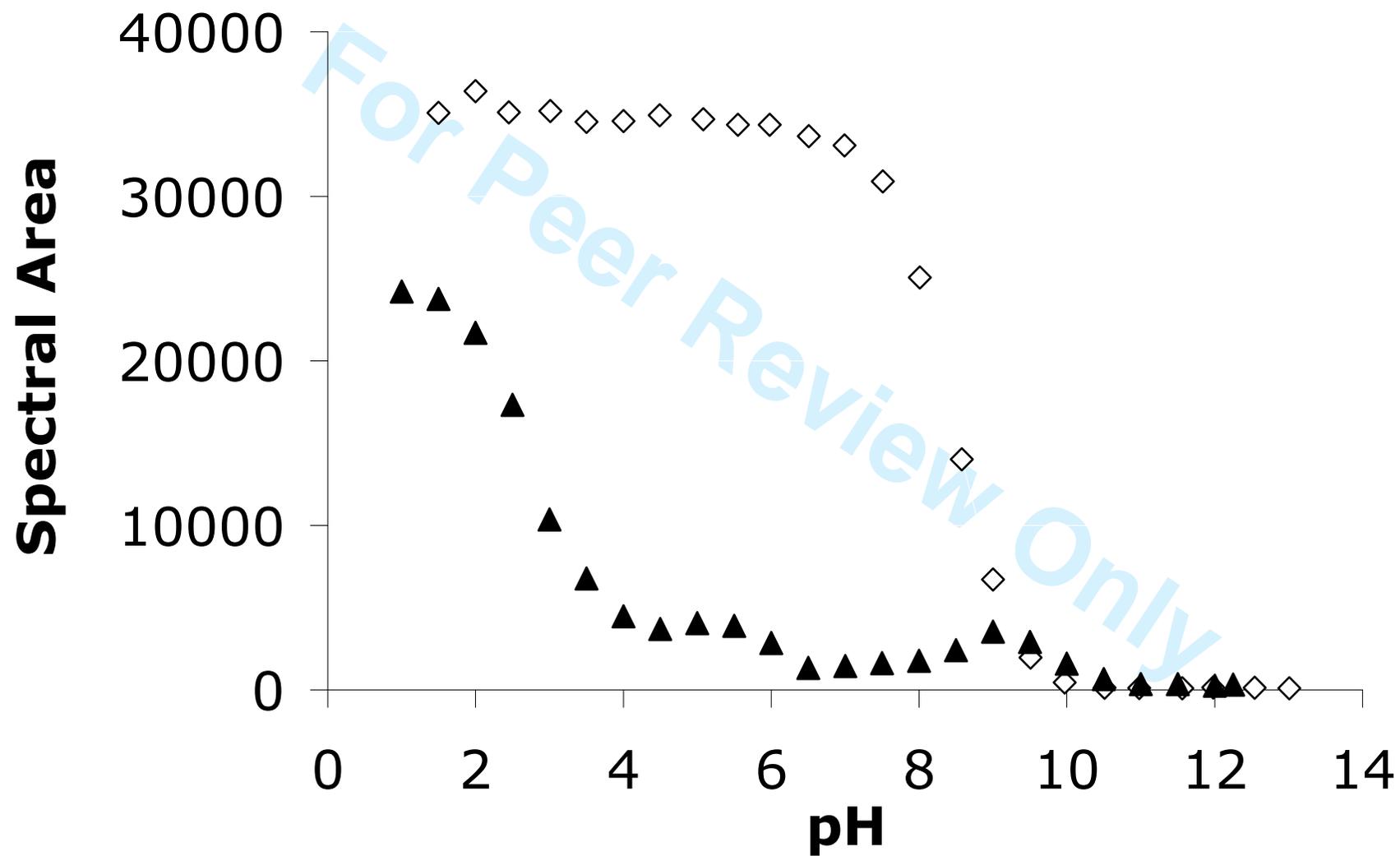
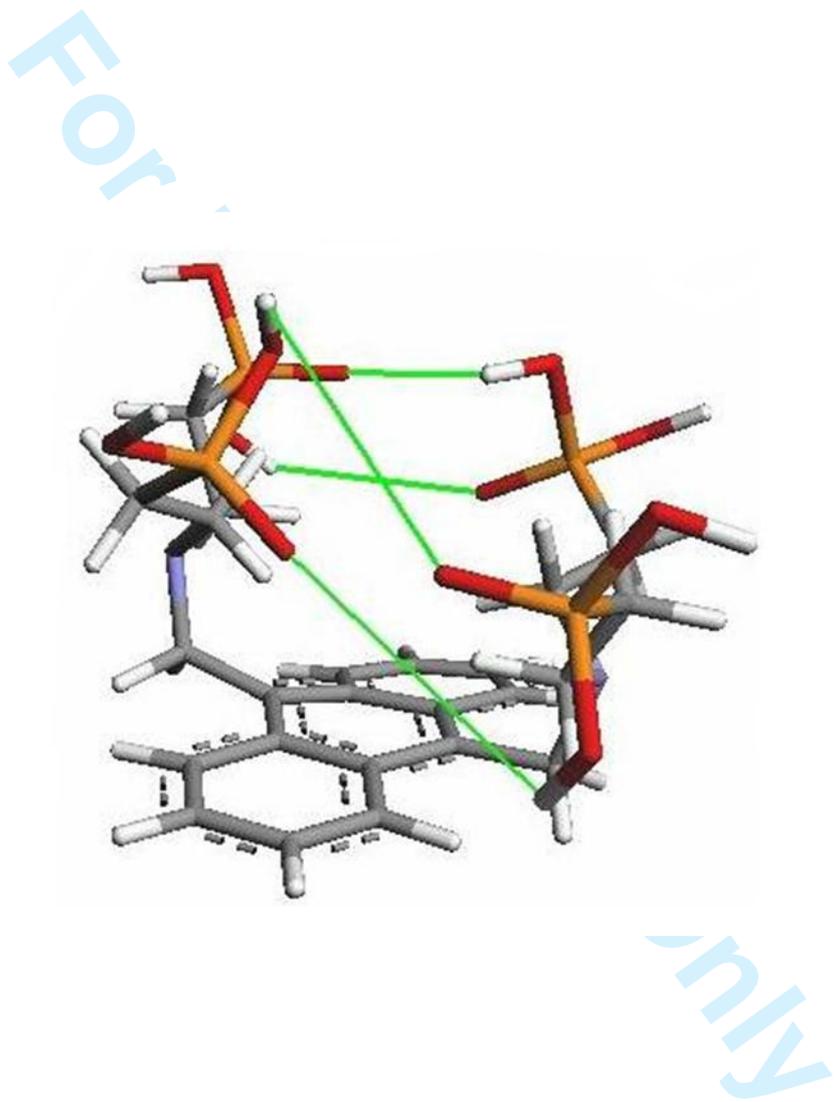


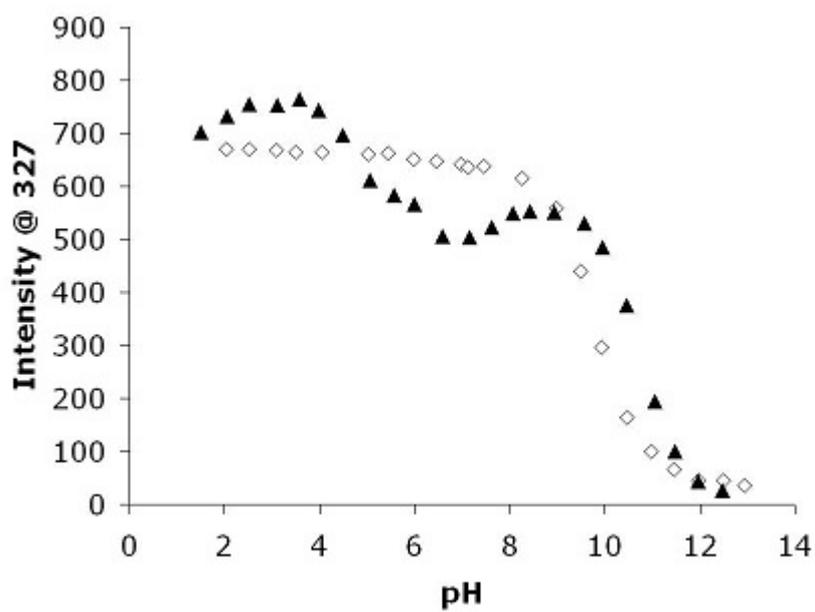
Figure 6 (a) Plot of relative intensity of **1** against $-\log [\text{metal ion}]$ for Cu (II) (\blacklozenge) and Fe (II) (\circ) as their chloride salts in 0.01 M HEPES buffer at pH 7.4. (b) Effect of Cu^{2+} ion concentration on the fluorescent intensity of **1**. $[\mathbf{1}] = 1.6 \times 10^{-7} \text{ M}$, $\lambda_{\text{ex}} = 370 \text{ nm}$.

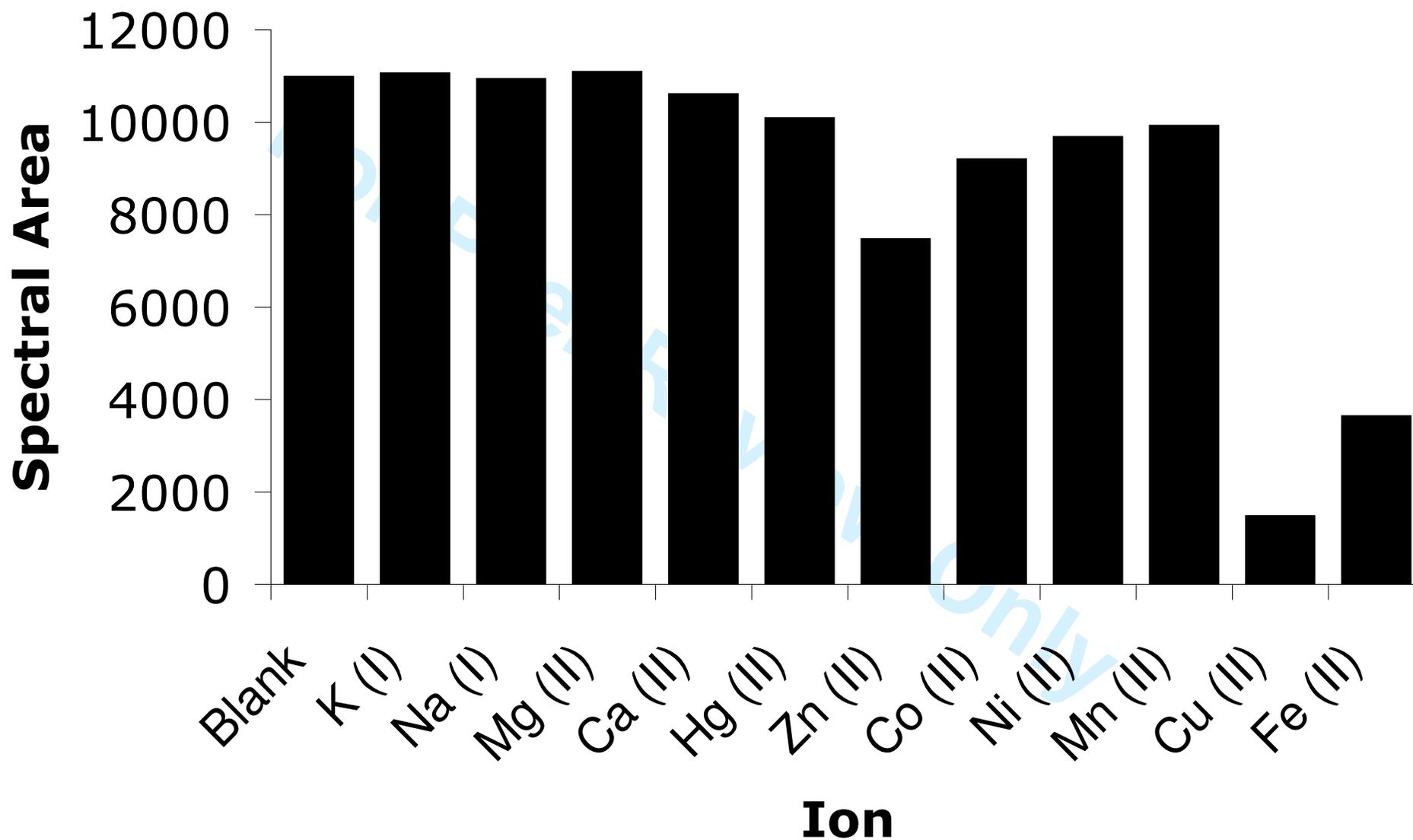




1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

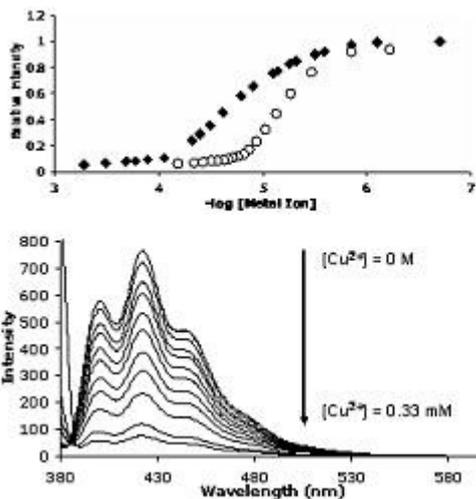






1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For P



Only