Soft Matter pH Sensing: From Luminescent Lanthanide pH Switches in Solution to Sensing in Hydrogels

Colin P. McCoy*

School of Pharmacy, Queen's University Belfast, Belfast BT9 7BL, U.K.

Floriana Stomeo, Sally E. Plush, and Thorfinnur Gunnlaugsson

School of Chemistry, Centre for Synthesis and Chemical Biology, Trinity College Dublin, Dublin 2, Ireland

Received March 13, 2006. Revised Manuscript Received June 23, 2006

The synthesis, complexation, and photophysical properties of the Eu(III)-based quinoline cyclen conjugate complex **Eu1** and its permanent, noncovalent incorporation into hydrogels as sensitive, interference-free pH sensing materials for biological media are described. The Eu(III) emission in both solution and hydrogel media was switched reversibly "*on*−*off*" as a function of pH with a large, greater than order of magnitude enhancement in Eu(III) emission. The irreversible incorporation of **Eu1** into water-permeable hydrogels was achieved using poly[methyl methacrylate-*co*-2-hydroxyethyl methacrylate]-based hydrogels, and the luminescent properties of the novel sensor materials, using confocal laser-scanning microscopy and steady state luminescence, were characterized and demonstrated to be retained with respect to solution behavior. Water uptake and dehydration behavior of the sensor-incorporated materials was also characterized and shown to be dependent on the material composition.

Introduction

In recent years the use of ions and molecules to modulate fluorescence through the construction of fluorescent−receptor moieties has led to the development of luminescent sensors and switches.[1] This area is now well-established within the field of supramolecular chemistry.[2] Even though such fluorescent sensors are highly desirable and are now important tools in environmental, health, and biological monitoring, there are still significant drawbacks to the use of fluorescence.[3] This is particularly evident for on-line or field monitoring of biological media where many biological substrates are also fluorescent, emitting in the nanosecond time frame, and hence can mask or "pollute" the detection signal (the so-called autofluorescence).[4] Furthermore, light scattering in biological media can interfere with the measured signal. Means of overcoming these drawbacks have involved the use of long wavelength absorbing and emitting fluorophores, which can overcome the short wavelength emission, but not the short emission lifetimes. Using metal complexes, however, often overcomes both these drawbacks.[5] Such complexes have proved important in the study of nucleic acids as they can be selectively functionalized to intercalate, or bind, to DNA, with concomitant changes in their characteristic long wavelength emission bands. Alternatives to these metal complexes are those based on lanthanide ions, which emit at long wavelengths, with characteristic line-like emission bands and lifetimes ranging from sub-microseconds to sub-milliseconds, hence overcoming both of the above-mentioned drawbacks.[6−8] We have developed several examples of lanthanide-based luminescent sensors for ions and molecules, where the emission of Tb(III), Eu(III), Nd(III), and Yb(III) have been modulated.[9] As the lanthanides have low extinction coefficients, direct excitation is not feasible

* Corresponding author. Tel.: +44 28 9097 2081. Fax: +44 28 9024 7794. E-mail: c.mccoy@qub.ac.uk.

(1) (a) Feringa, B. L., Ed. *Molecular Switches*; Wiley-VCH: Weinheim, 2001. (b) Giordani, S.; Cejas, M. A.; Raymo, F. M. *Tetrahedron* **2004**, *60*, 10973. (c) de Silva, A. P.; McCaughan, B.; McKinney, B. O. F.; Querol, M. *Dalton Trans.* **2003**, 1902. (d) Balzani, V. *Photochem. Photobiol. Sci*. **2003**, *2*, 459. (e) Balzani, V.; Credi, A.; Venturi, M. *Pure Appl. Chem*. **2003**, *75*, 541. (f) Balzani, V.; Gómez-López, M.; Stoddart, J F. *Acc. Chem. Res.* **1998**, *31*, 405.

(2) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev*. **1997**, *97*, 1515.

(3) Faulkner, S.; Matthews, J. L. In *Application of Coordination Complexes*; Ward, M. D., Ed.; Comprehensive Coordination Chemistry, 2nd ed., Vol. 9; Elsevier: Amsterdam, 2003.

(4) Parker, D. *Coord. Chem. Rev*. **2000**, *205*, 109.

(5) (a) Gunnlaugsson, T.; Leonard, J. P. *Chem. Commun*. **2005**, 3114. (b) Leonard. J. P.; Gunnlaugsson, T. *J. Fluoresc.* **2005**, *15*, 585.

(6) (a) Pope, S. J. A.; Coe, B. J.; Faulkner, S. *Chem. Commun*. **2004**, 1550. (b) Bretonnière, Y.; Cann, M. J.; Parker, D.; Slater, R. *Org. Biomol. Chem*. **2004**, *2*, 1624. (c) Bobba, G.; Bretonnière, Y.; Frias, J.-C.; Parker, D. *Org. Biomol. Chem.* **2003**, *1*, 1870. (d) Li, C.; Wong, W.-T. *Chem. Commun*. **2002**, 2034. Faulkner, S.; Burton-Pye, B. P.; Khan, T.; Martin, L. R.; Wray, S. D.; Skabara, P. J. *Chem. Commun.* **2002**, 1668. (e) Govenlock, L. J.; Mathieu, C. E.; Maupin, C. L.; Parker, D.; Riehl, J. P.; Sligardi, C.; Williams, J. A. G. *Chem. Commun.* **1999**, 1699. (f) de Silva, A. P.; Gunaratne, H. Q. N.; Rice, T. E.; Stewart, S. *Chem. Commun.* **1997**, 1891. (g) Beeby, A.; Parker, D.; Williams, J. A. G. *J. Chem. Soc., Perkin Trans. 2* **1996**, 1565. (h) Dickins, R. S.; Gunnlaugsson, T.; Parker, D.; Peacock, R. D. *Chem. Commun.* **1998**, 1643.

(7) (a) Parker, D. *Chem. Soc. Rev.* **2004**, *33*, 156. (b) Bünzli, J.-C. G.; Piguet, C. *Chem. Rev.* **2002**, *102*, 1897. (c) Tsukube, H.; Shinoda, S. *Chem. Rev*. **2002**, *102*, 2389. (d) Tsukube, H.; Shinoda, S.; Tamiaki, H. *Coord. Chem. Rev.* **2002**, *226*, 227. (e) Bünzli, J.-C. G.; Piguet, C. *Chem. Soc. Rev*. **1999**, *28*, 347. (f) Parker, D.; Williams, J. A. G. *J. Chem. Soc., Dalton Trans*. **1996**, 3616. (g) Sammes, P. G.; Yahioglu, G. *Nat. Prod. Rep*. **1996**, 1. (h) Sabbatini, N.; Guardigli, M.; Lehn, J.-M. *Coord. Chem. Rev.* **1993**, *123*, 201. (i) Georges, J. *Analyst* **1993**, *118*, 1481.

(8) Parker, D.; Dickins, R. S.; Puschmann, H.; Cossland, C.; Howard, J. A. K. *Chem. Rev.* **2002**, *102*, 1977.

10.1021/cm060603v CCC: $33.50
Published on Web 08/01/2006

in competitive media.[5,10] Hence, the use of macrocyclic or acyclic conjugates, furnished with chromophores (antennae) that have high extinction coefficients, has been employed by us and others to achieve indirect excitation of the lanthanide excited states by sensitization, also called the *antenna affect*.[11] By appropriately incorporating a recognition moiety into the antenna we have been able to achieve sensing through selective modulation of the lanthanide luminescence.[9,12]

In this paper we describe the synthesis of one such Eu(III)-based sensor for protons (**Eu1**), incorporating quinoline-derivatized cyclen (1,4,7,10-tetraazacyclododecane)[13] **1**. Ligand **1** has been designed to only have a single antenna. The Eu(III) emission is highly pH dependent can be easily and reversibly switched "*off*−*on*" in water. With the aim of developing such luminescent sensors with medical and pharmaceutical monitoring applications, we have also achieved the permanent, noncovalent incorporation of **Eu1** into water-permeable hydrogels and investigated several physical and photophysical properties of the resulting responsive materials.[14] The demonstration of retention of behavior in nonsolution environments is important for the development of soft matter real-time sensors which can be used in clinical monitoring environments. The choice of soft matter immobilization allows development of materials which are responsive to complex liquid analytes, such as blood, but can be employed reversibly, as the material is permeable to water but not complex biomolecules or cells, and can be returned to its initial state of switching by flushing with water.

Experimental Section

General Method. Starting materials were obtained from Sigma Aldrich, Strem Chemicals, and Fluka. Solvents used were HPLC grade unless otherwise stated. Water was purified with a Waters Milli-Q system to give a specific resistance of >15 MΩ cm and was then boiled for 30 min to remove CO_2 and allowed to cool in flasks fitted with soda lime tubes. ^{1}H NMR spectra were recorded at 400 MHz using a Bruker Spectrospin DPX-400, with chemical shifts expressed in parts per million (ppm or δ) downfield from the standard. ^{13}C NMR were recorded at 100 MHz using a Bruker Spectrospin DPX-400 instrument. Infrared spectra were recorded on a Mattson Genesis II FTIR spectrophotometer equipped with a Gateway 2000 4DX2-66 workstation. Mass spectroscopy was carried out using HPLC grade solvents. Mass spectra were determined by detection using Electrospray on a Micromass LCT spectrometer, using a Shimadzu HPLC or Waters 9360 to pump solvent. The whole system was controlled by MassLynx 3.5 on a Compaq Deskpro workstation. UV−visible spectroscopy was carried out on a Shimadzu UV-2401 PC UV−visible spectrophotometer. Studies were carried out on fast scan mode with slit widths of 1.0 nm, using matched quartz cells. Excitations were carried out at 300 nm unless otherwise stated. Solution fluorescence and luminescence measurements were made on a Perkin-Elmer LS 50B or Varian Carey Eclipse.

Synthesis of Ligand 2-{4,7-Bis-dimethylcarbamoylmethyl-10-[(2-methyl-quinolin-4-ylcarbamoyl)-methyl]-1,4,7,10-tetraaza-cyclododecayl}-*N*,*N*-dimethyl-acetamide, 1. 2-(4,10-Bis-dimethylcarbamoylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-*N*,*N*-dimethyl-acetamide (**3**) (0.272 g, 0.636 mmol), Cs_2CO_3 (0.228 g, 0.7 mmol), KI (0.116 g, 0.7 mmol), and chloro-*N*-(2-methyl-4-quinolyl)-ethanamide (**2**) (0.164 g, 0.7 mmol) were added to DMF (35 mL). The mixture was heated at 80 °C under inert atmosphere for 72 h. The solution was filtered and the solvent removed under reduced pressure. A dark orange viscous residue was obtained (0.359 g, 0.57 mmol) and then purified by precipitation from diethyl ether to give a brown solid in 53% yield. Calculated for $C_{32}H_{51}N_9O_4$, $(M + H)^+$ *m*/*z* (% ES^+): 626.4142; Found: 626.4163. δ_H (400 MHz, $CDCl_3$): 10.5 (1H, s, N*H*), 8.75 (1H, d, $J = 8.5$, Ar-*H*), 7.93 (1H, s, Ar-*H*), 7.89 (1H, d, $J = 8.5$ Hz, Ar-*H*), 7.57 (1H, t, $J = 6.0$ Hz, Ar-*H*), 7.43 (1H, t, $J = 6.0$ Hz, Ar-*H*), 3.86 (2H, s, HNCOCH_2), 2.70 (43H, m, N−CH_3, CH_2CON(CH_3)$_2$, cyclen CH_2). δ_c (100 MHz, $CDCl_3$): 172.1, 170.4, 170.3, 158.3, 148.0, 141.8, 128.7, 127.7, 124.6, 123.4, 119.7, 112.6, 57.6, 54.5, 54.4, 35.7, 35.6, 35.0, 28.8, 26.5, 25.2. *m*/*z*: 626.33 $(M + H)^+$, 313.67 $(M + 2H/2)^+$. υ(KBr)/ cm^{-1}: 3434, 2949, 2818, 2363, 1645, 1531, 1499, 1451, 1407, 1348, 1299, 1266, 1185, 1102, 1005, 769.

Synthesis of Eu(III) Complex of 1, Eu1. 2-{4,7-Bis-dimethylcarbamoylmethyl-10-[(2-methyl-quinolin-4-ylcarbamoyl)-methyl]-1,4,7,10 tetraaza-cyclododeca-yl}-*N*,*N*-dimethyl-acetamide (**1**) (0.095 g, 0.15 mmol) and Eu(III) trifluoromethane sulfonate (0.1 g, 0.16 mmol) were added to a 25 mL single-neck round-bottom flask that contained freshly dried MeCN (10 mL). After three freeze−pump−thaw cycles, the solution was placed under an argon atmosphere and left stirring at reflux for 24 h. The resulting solution was cooled to room temperature and then added dropwise to dry diethyl ether (100 mL) with stirring. The resulting precipitate was isolated to give **Eu1** as a pale green solid in 84% yield. δ_H (400 MHz, CD_3OCD_3): 12.7, 10.4, 8.0, 6.9, 3.1, 2.2, 1.6, −5.5, −6.2, −7.0, −8.5. *m*/*z*: 463.5 ($[M + CF_3SO_3]/2)^+$, 259.4 $(M/3)^+$, 388.6 $(M/2)^+$. υ(KBr)/ cm^{-1}: 3434, 2361, 2343, 1624, 1255, 1164, 1030, 639.

Ground and Excited State pK_a Determinations. Titration solutions were saturated with argon by passing a fine stream of bubbles (previously passed through aqueous 0.1 M KOH followed by 0.1 M NEt_4ClO_4) through them for at least 15 min before the commencement of titration. (CAUTION: Anhydrous perchlorates are potentially explosive and should be handled with caution.) During the titrations a fine stream of argon bubbles was passed through the titration vessel that was closed to the atmosphere except for a small vent for the argon stream. The titrations were carried out using a MOLSPIN titrimator, MOLSPIN potentiometer, and an Orion 8172 Ross Sureflow combination pH electrode. Values of E_0 and pK_w were determined using the program GLEE. At least three runs were carried out for each system, and at least two of these runs were averaged; the criterion for selection for this averaging being that χ^2 for each run was <12.6 at the 95% confidence level. Values for each pK_a and K were determined using the program HYPERQUAD. The ground state pK_as (S_0) where calculated from the following formula:[21]

(9) (a) Gunnlaugsson, T.; Leonard, J. P.; Sénéchal, K.; Harte, A. J. *J. Am. Chem. Soc.* **2003**, *125*, 12062. (b) Gunnlaugsson, T.; Leonard, J. P.; Sénéchal, K.; Harte, A. J. *Chem. Commun.* **2004**, 782. (c) Gunnlaugsson, T.; Leonard, J. P. *Chem. Commun.* **2003**, 2424. (d) Gunnlaugsson, T.; MacDónaill, D. A.; Parker, D. *Chem. Commun*. **2000**, 93. (e) Gunnlaugsson, T.; Parker, D. *Chem. Commun*., **1998**, 511.

(10) Bünzli, J.-C. G., Choppin, G. R., Eds. *Lanthanide Probes in Life, Chemical and Earth Sciences, Theory and Practice*; Elsevier: New York, 1989; p 219.

(11) (a) Merbach, A. E.; Tóth, E. *The chemistry of contrast agents in medical magnetic resonance imaging*; Wiley: West Sussex, England, 2001. (b) DeW Horrocks, W.; Sudnick, D. R. *Science* **1979**, *206*, 1194.

(12) Gunnlaugsson, T. *Tetrahedron Lett.* **2001**, *42*, 8901.

(13) Gunnlaugsson, T.; McCoy, C. P.; Stomeo, F. *Tetrahedron Lett*. **2004**, *45*, 8403.

(14) Gans, P.; Sabatini, A.; Vacca, A. *Talanta* **1996**, *43*, 1739.

(15) Gunnlaugsson, T.; Mac Dónaill, D. A.; Parker, D. *J. Am. Chem. Soc*. **2001**, *123*, 12866.

$$pK_a(S_0) = pH - \log\frac{(Abs_{AH} - Abs_A)}{(Abs_A - Abs_{A-})}$$

where Abs_{AH}, $Abs_{A,}$ and Abs_{A-} are the absorbances of every solution, the absorbance of the protonated species, and the absorbance of the deprotonated species.

The excited state pK_as (S_1) were determined from the Förster cycle as follows:[22]

$$pK_a(S_1) = pK_a(S_0) + \frac{0.625}{T}\Delta\nu$$

where $\Delta\nu$ is the difference between the energy of the protonated and deprotonated form in reciprocal centrimeters and T is the absolute temperature.

Preparation of Sensor-Incorporated and Control Hydrogels. Complex **Eu1** was nonconvalently incorporated into three different hydrogel matrixes comprised of cross-linked poly[methyl methacrylate-*co*-2-hydroxyethyl methacrylate]. The control hydrogel matrixes were prepared using methyl methacrylate (MMA) and 2-hydroxyethyl methacrylate (HEMA) in three different ratios: 1:1 (MMA:HEMA, w/w), 1:3 (MMA:HEMA, w/w), and 100% HEMA. For all of these, monomers were stirred with ethylene glycol dimethacrylate (1% w/w) (used as a cross-linker) and azo-bis-isobutyronitrile as a radical initiator (1% w/w) until dissolved. In all cases, the total mass of polymer prepared was 10 g. Sensor-incorporated hydrogels were prepared in an analogous manner as control polymers, with Eu1 (0.05% w/w) being dissolved in the monomer mixture prior to polymerization. The mixture was injected into a mould using a 1 mm spacer between two glass places at 90 °C for 6 h, and the resulting polymer sheets were soaked in deionized water for 3 days.

Determination of Equilibrium Water Content of Hydrogel Sensor Materials. Complex-incorporated hydrogel films prepared with different ratios of HEMA and MMA were cut (1 cm × 1 cm) in their water-swollen state. The samples were dehydrated at 60 °C to constant weight. The samples were then immersed in deionized water and reweighed, following gentle removal of excess surface water with a tissue, at intervals until a constant weight was achieved. All measurements were made in triplicate, and values are quoted as the mean ± 1 standard deviation. The equilibrium water content (EWC), defined as a ratio of the mass of water taken up to the mass of dry sample, was calculated using the following equation:

$$\text{EWC (\%)} = \frac{\text{mass of hydrated sample} - \text{mass of dehydrated sample}}{\text{mass of dehydrated sample}} \times 100$$

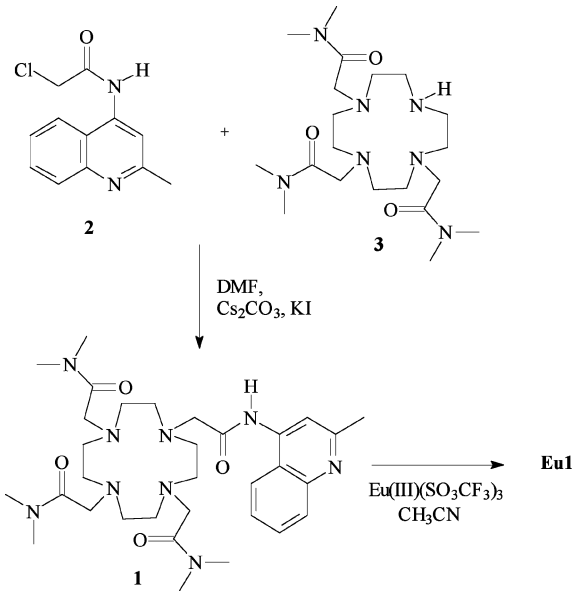

Figure 1. Synthesis of ligand **1** and the corresponding Eu(III) complex **Eu1**.

Luminescence Measurements of Hydrogel Sensor Materials. Confocal laser scanning microscopic (CLSM) examination of hydrogel samples was carried out with a Leica TCS SP2 confocal laser scanning microscope. After focusing, the sample surface was excited using the 458 nm line from a Ar/ArKr laser and luminescence data collected over the range 500−800 nm. Luminescence emission micrographs show summed photomultiplier intensities across the full wavelength range detected with a look-up table assigning color to overall intensity ranging from black (zero intensity) to bright red (highest intensity). Steady state luminescence measurements were made using a front-surface accessory to clamp hydrogel sections in a Perkin-Elmer LS 50B.

Results and Discussion

Synthesis of Sensor 1 and Its Eu(III) Complex, Eu1. The synthesis of ligand **1** is shown in Figure 1. The synthesis involved the formation of the α-chloroamide derivative of the corresponding quinoline amine. The α-chloroamide **2** was made in one step by reacting chloroacetyl chloride with 4-aminoquinoline at −10 °C in dry THF for 12 h. Filtration of the inorganic salts followed by acid−base extraction and trituration of the resulting off-white solid with DCM gave **2** in 65% yield. The trisubstituted ligand **3**, 2-(4,10-bis-dimethyl-carbamoylmethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-*N*,*N*-dimethyl-acetamide, which had been formed in a single step from cyclen, was then reacted with **2** in DMF at 85 °C in the presence of Cs_2CO_3 and KI for 3 days. The resulting ligand **1** was purified by precipitation from dry diethyl ether in a 53% yield. The Eu(III) complex of **1** was made by refluxing **1** and $Eu(CF_3SO_3)_3$ in dry MeCN under an inert atmosphere for 24 h, followed by precipitation from dry diethyl ether, and isolation of the desired off-white powder in 84% yield. The ^{1}H NMR of **Eu1** (CD_3COCD_3) showed the characteristic europium shifted axial and equatorial cyclen protons, confirming that in solution the complex adopts a square prismatic geometry. The ESMS also showed that complexation had occurred. An Eu(III) isotopic distribution pattern was observed for the complex which matched that of the calculated spectra.

(16) (a) Special issue, "Recent Advances in Rare Earth Chemistry": Kobayashi, S. *Tetrahedron* **2003**, *52*, 10349. (b) Special issue, "Frontiers in Lanthanide Chemistry": Kagan, H. B., Guest Ed. *Chem. Rev.* **2002**, *102*, 1805.
(17) (a) Gunnlaugsson, T.; Harte, A.; Leonard, J. P.; Nieuwenhuyzen, M. *Supramol. Chem.* **2003**, *15*, 505. (b) Gunnlaugsson, T.; Harte, A.; Leonard, J. P.; Nieuwenhuyzen, M. *Chem. Commun.* **2002**, 2134.
(18) DeW Horrocks, W.; Sudnick, D. R *J. Am. Chem. Soc.* **1979**, *101*, 334.
(19) (a) Beeby, A.; Clarkson, I. M.; Dickins, R. S.; Faulkner, S.; Parker, D.; Royle, L.; de Sousa, A. S.; Williams, J. A. G.; Woods, M. *J. Chem. Soc., Perkin Trans. 2*. **1999**, 493 (b) Dickins, R. S.; Parker, D.; de Sousa, A. S.; Williams, J. A. G. *Chem. Commun.* **1997**, 697.
(20) Bruce, J. I.; Dickins, R. S.; Govenlock, L. J.; Gunnlaugsson, T.; Lopinski, S.; Lowe, M. P.; Parker, D.; Peacock, R. D.; Perry, J. J. B.; Aime, S.; Botta, M. *J. Am. Chem. Soc.* **2000**, *122*, 9674.
(21) Jaffé, H. H.; Orchin, M. *Theory and Applications of Ultraviolet Spectroscopy*; Wiley: New York, 1962.
(22) Förster, R.; *Z. Electrochem.* **1950**, *54*, 531.

Table 1. pK_a Values of the Protonated Ligand 1 at 298.2 K and I = 0.1 M (NEt_4ClO_4) in Water

equilibrium quotient	pK_a	designation
[**1**][H^+]/[H_1**1**$^{+}$]	10.65 ± 0.02	pK_{a1}
[H**1**$^{+}$][H^+]/[H_2**1**$^{2+}$]	8.96 ± 0.04	pK_{a2}
[H_2**1**$^{2+}$][H^+]/[H_3**1**$^{3+}$]	6.84 ± 0.05	pK_{a3}
[H_3**1**$^{3+}$][H^+]/[H_4**1**$^{4+}$]	4.95 ± 0.07	pK_{a4}
[H_4**1**$^{4+}$][H^+]/[H_5**1**$^{5+}$]	low	pK_{a5}

Potentiometric Measurements of 1 and Eu1. The pK_a values of the protonated ligand **1** were determined by potentiometric pH titration of the acidified ligand (L = 6.4 × 10^{-4} M, H^+ = 6.5 × 10^{-3} M) against 0.103 M NEt_4OH with I = 0.1 M (NEt_4ClO_4) in water. Four pK_a values were determined for ligand **1** using the program HYPERQUAD (Table 1).[15] The two larger pK_a values, pK_{a1} and pK_{a2}, and the smallest determined pK_a, pK_{a4}, of ligand **1** are assigned to the protonation of the macrocyclic ring amines. It is assumed that the fourth pK_a of the macrocyclic ring, pK_{a5}, is too small to be determined. The remaining pK_a value of 6.84 ± 0.05 (pK_{a3}) was assigned to the protonation of the quinoline nitrogen. For similar systems, large pK_a values for protoned quinoline nitrogens have also been observed.[16]

The complexation constants for ligand **1** were determined in an analogous manner to the protonation constants except that an equimolar quantity of $Eu(CF_3SO_3)_3$ was also present in solution, as shown in the titration curve in Figure 2. The derived complexation constants and pK_as for the protonated ligand complexes, determined using the program HYPERQUAD,[15] are shown in Table 2.

The pK_a of the MLH complex, 6.13 ± 0.09, can be assigned to the deprotonation of the quinoline nitrogen. This value is similar to the pK_{a3} value of the free ligand **1**. It was anticipated that the pK_a values would be similar because the quinoline nitrogen is not expected to be involved directly in metal ion coordination.

The deprotonation of the metal bound water was also observed with a pK_a of 7.47 ± 0.05 determined. This was expected because ligand **1** has only eight coordination sites and europium is capable of forming nine-coordinate complexes. The pK_a value of 8.77 ± 0.09 can be assigned to the deprotonation of the quinoline carboxylic amide. This value is lower than generally observed for carboxylic amides but this can be attributed to the effect of the strong Lewis acid europium metal ion center.

Determination of the Coordination Numbers of Eu1. The coordination numbers of lanthanides are usually high and hence require polydentate ligands.[17] Ligand **1** can provide eight such coordination sites, four from the nitrogens of the cyclen structure, and four from the carboxylic amides. Europium generally has a coordination number of nine in such tetrasubstituted cyclen complexes, with the axial ninth site being occupied by a coordinated solvent molecule or an anion.[18] The way of estimating q was first determined by Horrocks and Sudnick by measuring the excited state lifetimes of Eu(III) and Tb(III) in H_2O and D_2O, respectively.[19] This method was reviewed by Parker et al., which is the method of choice herein. In the case of **Eu1**, the presence of the coordinated water molecule was determined

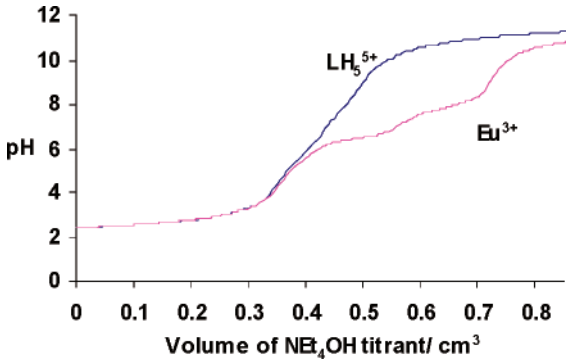

Figure 2. Titration curve for ligand **1** (LH_5^{5+}), formed in a solution, where [**1**] = 6.4 × 10^{-4} M and [H^+] = 6 × 10^{-3} M at 298.2 K and I = 0.1 M (NEt_4ClO_4). The titration curve is also shown for the same solution except that [Eu^{3+}] = 6.4 × 10^{-4} M was also present. Titrant [NEt_4OH] = 0.103 M.

Table 2. Complexation Constants, log K/dm^3 mol^{-1}, and pK_a Values for the Complexation of Eu^{3+} with Ligand 1 at 298.2 K and I = 0.1 M (NEt_4ClO_4)

equilibrium quotient	log K/dm^3 mol^{-1}
[Eu(**1**)$^{3+}$]/[Eu^{3+}][**1**]	9.59 ± 0.02
equilibrium quotient	**pK_a**
[Eu**1**$^{3+}$][H^+]/[Eu(H**1**)$^{4+}$]	6.13 ± 0.09
[Eu**1**OH^{2+}][H^+]/[Eu**1**$^{3+}$]	7.47 ± 0.05
[Eu**1**H_{-1}^{2+}][H^{-1}]/[Eu**1**$^{3+}$]	8.77 ± 0.09

by measuring the lifetimes of the Eu(III) emission in H_2O and D_2O, using the Parker modified equation:[20]

$$q_{Eu} = 1.2[(1/\tau_{H2O} - 1/\tau_{D2O}) - 0.25 - 0.075x] \ (\pm 0.3)$$

where x takes into account the effect of exchangeable amide N−H oscillators. The lifetime of the Eu(III) excited state was measured in neutral media. For **Eu1** τ_{H2O} was measured to be 429 μs whereas τ_{D2O} = 758 μs, yielding q = 0.91 (inner + outer sphere, after correcting for the amide NH oscillators). This indicates that the complex has a single bound water molecule, hence the complex is nine coordinate. This is in agreement with the potentiometric titrations.

Ground State Investigations. The ground, singlet excited state and the Eu(III) emission were evaluated for **Eu1** in water and in the presence of tetraethylammonium perchlorate (I = 0.1 M) to maintain a constant ionic strength. The ground state and the singlet excited state pK_a values were calculated according to the formulas described in the Experimental Section.[21,22]

For **Eu1**, the absorption spectra, when recorded first in acidic solution, displayed a maximum peak at 320 nm, with a shoulder at about 330 nm. Upon basification there was a significant hypsochromic and hypochromic shift to a broad absorption band due to the $\pi \rightarrow \pi^*$ transition centered at 305 nm, as shown in Figure 3. Two isosbestic points are observable in the spectra at ca. 263 and 303 nm, which suggests the presence of more than two species in equilibrium, as is reasonable with two deprotonation steps occurring across the range titrated.

The changes in the absorption spectra were plotted as a function of pH at 330 nm, as shown in Figure 4. This clearly shows that there is a significant decrease in the absorbance of the complex above pH 8. A pK_a of 8.82 ± 0.1 was

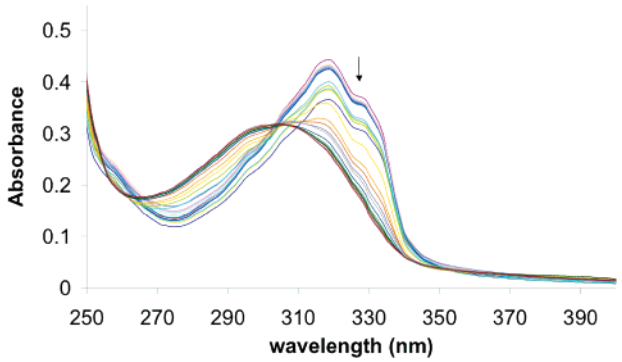

Figure 3. UV−visible spectra for the pH titration of **Eu1**. The arrow indicates the spectral trend with increasing pH between pH 3−11.5.

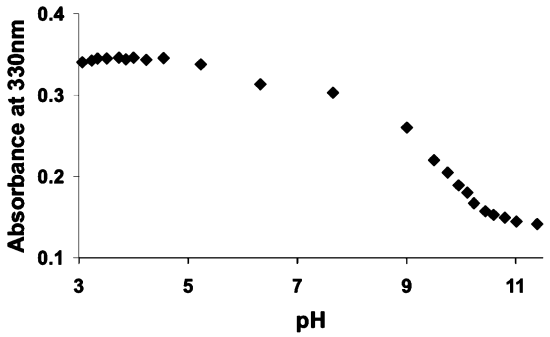

Figure 4. Changes in absorbance at 330 nm as a function of pH for **Eu1**.

calculated for this change. This coincides with the pK_a of 8.77 ± 0.09 determined for the deprotonation of the quinoline carboxylic amide. A slight decrease in absorbance is also noted above pH 5. A pK_a of 5.93 ± 0.1 was calculated. This change is therefore most likely due to the deprotonation of the quinoline nitrogen (pK_a 6.13 ± 0.09). As expected, deprotonation of the metal-bound water did not have an observable effect on the absorption of the complex. Therefore, only two pK_as where calculated as anticipated by the isosbestic points observed in Figure 3.

Singlet Excited State Investigations. The changes in the fluorescence emission spectra of the complex were investigated as a function of pH in water in the presence of tetraethylammonium perchlorate (I = 0.1 M). The changes were monitored following excitation at 330 nm, where there was a significant difference in the magnitude of the extinction coefficient in alkaline (low ϵ) and acidic solution (high ϵ). The changes in the emission spectra of **Eu1** when excited at 330 nm gave rise to spectral changes that mirror those seen in the absorption spectrum. In alkaline solution there was a structural band centered at 375 nm. Upon acidification there was a distinctive shift in the emission spectra from 375 nm to shorter wavelengths with the formation of a band with maximum at 357 nm, and two shoulders at 345 and 375 nm with concomitant enhancement of the emission intensity, as shown in Figure 5.

When the changes at 356 nm were plotted as a function of pH, a sigmoidal curve was observed, as shown in Figure 6. The changes observed show that the singlet state is only slightly affected below pH 8 and that the emission was switched "*off*" in alkaline solution. It is well-known that the acid−base behavior of many organic molecules can be strongly affected by electronic excitation.[23]

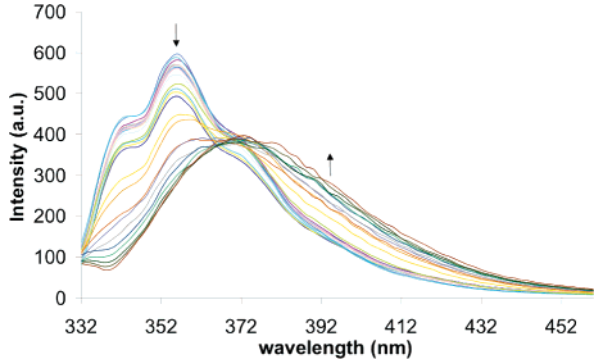

Figure 5. Overlaid fluorescence emission spectra for the pH titration of **Eu1**. Arrows indicate the spectral trend with increasing pH between pH 3−11.5.

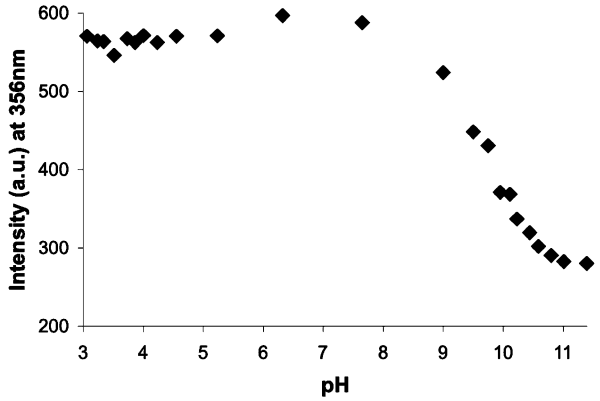

Figure 6. Changes in emission intensity at 356 nm as a function of pH for **Eu1**.

From the changes above pH 8, using the Förster equation given in the Experimental Section,[22] a pK_a of 9.47 ± 0.1 was calculated. This indicates that the basicity of **Eu1** is increased upon excitation to the first singlet state. There is a slight increase in the fluorescence intensity above pH 5; small changes in the absorption spectra were also noted above pH 5. The calculation of an excited state pK_a value using the Förster equation requires a change in wavelength; as this did not occur the excited state pK_a value for the changes between pH 5−8 cannot be determined in this manner. Excited state pK_a values and hence the assignment of the deprotonation steps cannot be determined with the same degree of accuracy as those for the ground state.[24] It is, however, expected that the changes between pH 5−8 are caused by the deprotonation of the quinoline nitrogen, while the changes above pH 8 are associated with the deprotonation of the quinoline carboxylic amide.

Eu(III) Excited State Investigations. The changes in the Eu(III) emission were investigated as a function of pH. In a similar manner as described above, the quinoline moiety of **Eu1** was excited at 330 nm in alkaline solution, and the changes in the Eu(III) emission were recorded as a function of pH.

In acidic solution, the Eu(III) emission, when excited at 330 nm (where the difference between the absorption in alkaline vs acid solution is the greatest), was clearly

(23) Tajima, S.; Tobita, S.; Shizuka, H. *J. Phys. Chem. A* **2000**, *104*, 11270.
(24) Sikorska, E.; Szymusiak, H.; Khmelinskii, I. V.; Koziolowa, A.; Spanget-Larsen, J.; Sikorski, M. *J. Photochem. Photobiol. A* **2003**, *158*, 45.

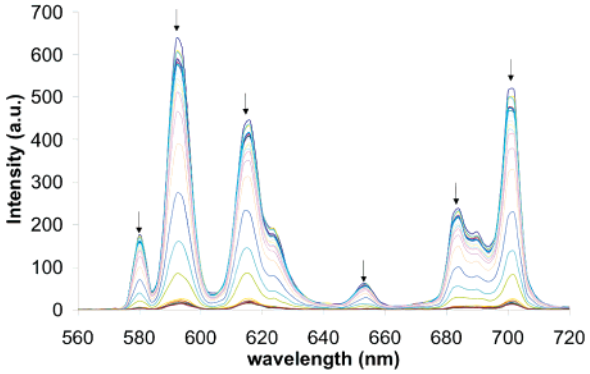

Figure 7. Changes in the Eu(III) emission of **Eu1** as a function of pH in solution, showing the deactivation of the $^5D_0 \rightarrow {}^7F_J$ ($J = 0$, 1, 2 (split), 3, and 4 (split)). Arrows indicate the spectral trend with increasing pH between pH 3−11.5. These changes are fully reversible.

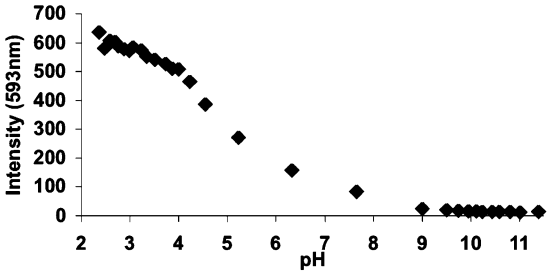

Figure 8. Changes in the Eu(III) emission of **Eu1** at 593 nm as a function of pH in solution.

"*switched on*" with large (up to a factor of 30) enhancements in the 580, 593, 615, 624, 654, 683, and 701 nm bands, representing the deactivation of the $^5D_0 \rightarrow {}^7F_J$ ($J = 0$ (580 nm), 1 (593 nm), 2 (split; 615 and 624 nm), 3 (654 nm), and 4 (split; 683 and 701 nm)),[7f] as shown in Figure 7.

These luminescence enhancements highlight the ability of the antenna to populate the lanthanide excited state by sensitization and that this process is highly pH-dependent. Moreover, the luminescence switching was fully reversible, since addition of strong base (pH ∼ 10) quenches the emission, which could be subsequently "*switched on*" again by the addition of acid (pH = 1.6). The changes to the Eu(III) emission when plotted as a function of pH gave rise to a sigmoidal curve, shown in Figure 8.

Above pH 4.5 the Eu(III) emission significantly decreased until it was completely quenched above pH 9. The pK_a of the quinoline nitrogen as determined by potentiometric titration (6.13), absorption, and fluorescence spectroscopy (5.93) is in the pH range of 4−8. Therefore the decrease in the Eu(III) emission above pH 4.5 can be associated with the deprotonation of the quinoline nitrogen. Above ca. pH 8, it would be expected that the effect due to the quinoline nitrogen deprotonation would be insignificant. However the Eu(III) emission is not fully quenched at this pH. It may be anticipated that the emission is affected by the deprotonation of either the metal bound water, the quinoline carboxylic amide, or a combination of the two. Deprotonation of the metal bound water molecule would affect the O−H harmonic quenching of the Eu(III) excited state. The small change in Eu(III) emission that occurs between pH 2.5−4 cannot be readily explained through ligand or complex deprotonation. Potentiometric titration, absorption, and fluorescence spectroscopy of the complex had shown no evidence of deprotonations in this pH range. The anion effect has also been discounted as a plausible explanation for the observed quenching between pH 2.5−4, as the luminescence titrations were repeated using a variety of counterions, i.e. ClO_4^-, $CF_3SO_3^-$, and Cl^-. The effect is therefore associated with the metal ion. We are currently exploring this low-pH Eu(III) emission quenching effect with different analogues.

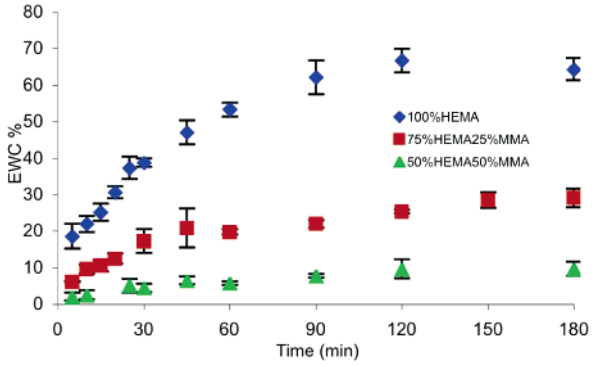

Figure 9. EWC (%) of the three different compositions of hydrogel with 0.1% (w/w) of **Eu1** during uptake from xerogels. Results are shown as the mean of three replicates ± one standard deviation.

From these results it is evident that the Eu(III) emission for **Eu1** is sensitive to changes in pH and that the changes shown in Figure 6 occur over a large pH range. Hence, **Eu1** can be used to monitor pH changes from ca. 2.5 to 8, mimicking pH electrodes.

Having established that the Eu(III) emission for**Eu1** was highly pH sensitive in competitive media, we choose to incorporate **Eu1** into water-permeable polymers.

Characterization of Eu1-Incorporated Water-Permeable Polymers. The Eu(III) complex **Eu1** was noncovalently incorporated into three different hydrogel matrixes comprised of poly[methyl methacrylate-*co*-2-hydroxyethyl methacrylate], denoted 1:1 (MMA:HEMA, w/w), 1:3 (MMA:HEMA, w/w), and 100% HEMA. The sensor was homogeneously incorporated at a concentration of 0.05% w/w.

Equilibrium Water Content. The water uptake kinetics and content of sensor-incorporated films was investigated using dehydrated samples of the materials (xerogels). When immersed into a hydrating medium, water enters and an equilibrium is eventually established between solvation of the copolymer and the forces holding the chains together. The degree of water uptake at equilibrium is a function of both the nature of the copolymer and the degree of cross-linking within that copolymer. Once this equilibrium is reached, the equilibrium water content (EWC) can be determined. The EWC of a material is an important parameter that has an influence on the physical properties of the hydrogels; the more hydrophilic the hydrogel the higher its EWC value. The EWC results for complex-incorporated films using the three different ratios of HEMA and MMA clearly demonstrate that increasing the HEMA content of the film significantly increased the equilibrium water content, as shown in Figure 9.

The EWCs for 1:1 MMA:HEMA, 1:3 MMA:HEMA, and 100% HEMA copolymers containing 0.05% (w/w) **Eu1** were determined to be 22.9 ± 1.7%, 39.6 ± 1.4%, and 58.2 ± 1.0%, respectively. All materials are fully equilibrated within

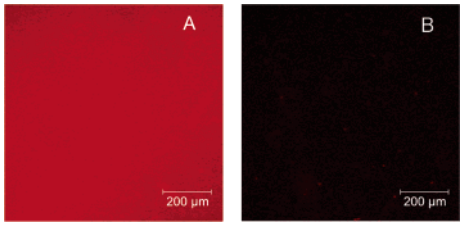

Figure 10. Confocal laser-scanning microscopy images of a section of hydrogel (MMA/HEMA, 1:3 w/w) incorporating 0.05% w/w **Eu1** in acidic (A) and basic (B) media.

90 min of immersion. An increase in EWC as the relative proportion of HEMA in the copolymers increases is expected due to the high hydrophilicity of HEMA relative to MMA, resulting from the pendant hydroxyl moiety of HEMA, which can participate in hydrogen bonding with water. The ability to control the EWC of the copolymer within which the complex resides is of considerable importance. High EWC hydrogels with immobilized sensors are expected to show enhanced response times compared to low EWC hydrogels due to their relatively high permeability to water; however, very high EWC gels, with resulting highly expanded polymer network structure, will facilitate the release of noncovalently incorporated sensors such as those employed here. Importantly, it was observed that all the hydrogels retained the **Eu1** complex entirely within the matrix for >1 month, as both absorbance and luminescence measurements of the solution in which the hydrogels were soaked showed no detectable levels of complex. The ability of all the matrixes to physically retain the **Eu1** complex is ascribed to hydrogen bonding interactions between the polymer chains and the complex. This interaction is proposed to take place through the free, ninth coordination site, which may be occupied by either a water solvent molecule (as demonstrated in solution) or a complex−polymer hydrogen bond. The irreversible retention of the complex in all the matrixes examined is attributable to the latter interaction.

Luminescence Measurements of Eu1 in Hydrogels. Hydrogel films were investigated using confocal laser-scanning microscopy (CLSM) and steady state luminescence.

CLSM allows direct, noninvasive measurement of fluorescence from the surface of a sample following excitation from an appropriate laser source. To build up a single image, data is collected pixel by pixel with the laser rastering over the sample, with measurement of overall fluorescence intensity within a defined wavelength range being translated into a false color for each pixel which correlates with that intensity. A full spectrophotometric measurement of luminescence emission from any defined area within a collected series of images can thus be obtained. This allowed characterization of both the distribution of the sensors within the hydrogel matrixes and the luminescence emission characteristics of the sensors on a macro (from square micrometer up to square millimeter) scale.

For all films the Eu(III) emission from the films was modulated by changing from an alkaline to an acidic medium. After the strips were soaked in acidic or alkaline solution for 1 h, the changes in the Eu(III) emission were recorded using CLSM as shown in the luminescence emission micrographs in Figure 10. Figure 10, which effectively maps Eu emission from the hydrogel surface, demonstrates the pH-dependent changes in the Eu(III) emission. These results show that the sensor is homogeneously dispersed within the medium and that in acidic solution the emission is switched "*on*" (Figure 10A), whereas in basic solution the Eu(III) was not observed and emission is switched "*off*" (Figure 10B). Hence, the luminescence of the films is highly sensitive to pH, as in solution.

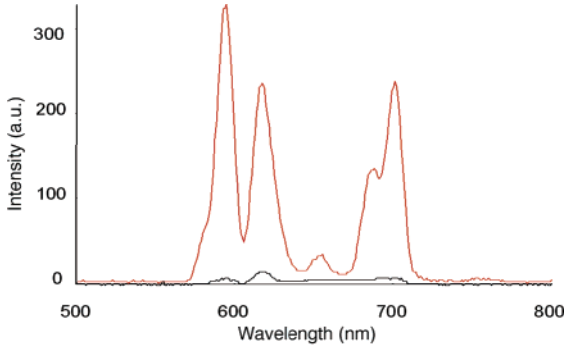

Figure 11. Eu(III) emission of the 1:3 MMA/HEMA film after soaking in pH 2 (red line) and pH 9 (black line) solutions for 24 h.

All the hydrogels showed a significant (>1 order of magnitude) difference in their Eu(III) emission intensities as a function of pH. These results mirror that seen in the aqueous solution; e.g. the emission was switched "*on*" in acid whereas in a basic environment the emission was switched "*off*". The steady state fluorescence results for the 1:3 w/w MMA/HEMA hydrogel are shown in Figure 11.

By comparison with Figure 7, the relative intensities of each luminescence band ($\Delta J = 0$, 1, 2, 3, and 4) are, within experimental error, in agreement, indicating the close similarity of the europium environment in solution and hydrogel matrixes. Due to the experimental method, the resolution of spectra obtained for hydrogels is slightly lower than in solution, with the $\Delta J = 0$ band (580 nm) observed as a shoulder on the $\Delta J = 1$ band (593 nm). These results clearly demonstrate that such "*on*−*off*" pH dependent switching is possible both in hydrogels and in solution. The maximum switching factor (luminescent enhancement), observed for the MMA/HEMA, 1:3 w/w material, is 53, which is greater than the factor of 30 observed in solution and is a clear "*on*−*off*" signal. However, the time taken to achieve the fully switched "*on*" equilibrium was significantly different in the films to that observed in solution. In solution, the response is instantaneous; the hydrogel media respond more slowly, as the aqueous medium to be analyzed must diffuse into the hydrogel matrix. Nevertheless, the materials studied all give measurable "*on*−*off*" switching on the minute time scale.

Conclusion

In this paper we have described the synthesis and the characterization of the pH sensor **Eu1**. The Eu(III) emission of the complex was shown to be highly pH sensitive, and the emission could be reversibly switched "*off-on*" as a function of pH in solution. The permanent incorporation of **Eu1** in a noncovalent manner into three hydrogels of composition 1:1 (MMA:HEMA, w/w), 1:3 (MMA:HEMA,

w/w), and 100% HEMA was achieved. Of significant importance, the emission of these water-permeable materials was on all occasions found to be highly pH sensitive and reversible, as in solution. These materials thus represent matrixes which can be switched "*on*" or "*off*", with potential application in the interference-free analysis of biological media.

We are currently improving on these design principles with the aim of achieving the sensing of other cations.

Acknowledgment. We thank Trinity College Dublin, Centre for Synthesis and Chemical Biology, the Queen's University Belfast, and The Royal Society (for a University Research Fellowship to McCoy) for financial support, Dr. John E. O'Brien for assisting with NMR, and Dr. Ryan J. Morrow for help with the preparation of hydrogels.

CM060603V