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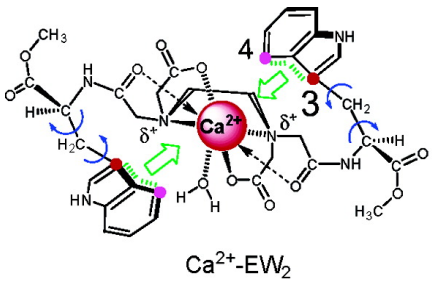

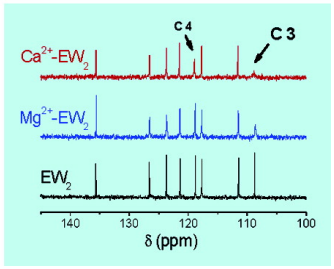

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A Tryptophan-Containing Open-Chain Framework for Tuning a High Selectivity for Ca^{2+} and ^{13}C NMR Observation of a Ca^{2+}−Indole Interaction in Aqueous Solution

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Abstract: Two molecular architectures featuring the cation-responsive tryptophan indole were designed and investigated for the development of a novel fluorescent chemosensor for Ca^{2+}. We observed that the Trp-based open-framework chemosensor EW_2 exhibits remarkable selectivity for Ca^{2+} over Mg^{2+}, Ba^{2+}, K^+, Na^+, and Li^+ in water between pH 4.6 and 7.0 on the basis of Ca^{2+}-induced high fluorescence enhancement of the Trp residue. A combined ^{13}C NMR and CD spectroscopic study has demonstrated a dynamic reorientation of the indole ring due to the cation−indole interaction accompanying the Ca^{2+}-induced dramatic fluorescence enhancement. The results suggest that the highly sensitive, metal-ion-dependent Trp indolyl C(3) chemical shifts may serve as a promising indicator for monitoring metal ion−indole noncovalent interaction in solution.

Introduction

The intriguing and prominent metal-ion-recognition property of the indole ring of tryptophan (Trp, W) has recently been subjected to much investigation due to the newly recognized importance of noncovalent interactions of metal ions with aromatic rings in chemistry and biology.[1−3] In addition, various unique metal−indole bonds, dependent upon both the bonding environment of the indole and the nature of the metal, have been identified.[4] Fluorescence spectroscopy is a powerful technique in visualizing molecular recognition events, and the inherently unique fluorescent property of the indole chromophore is sensitive to changes in the local environment.[5] Therefore, the exploration of the potential of the indole moiety in the design of fluorescent chemosensors for metal ions should provide promising results. A notable example is provided by the identification of a Trp-containing protein as a sensor for Ca^{2+}.[6]

Chemosensors not only provide analytically favorable tools for demanding biological, environmental, and medical assays but also hold great promise for molecular recognition studies.[7] Ca^{2+}, which can have a coordination number of 6−8 with a flexible geometry, plays a myriad of essential structural and functional roles in regulating diverse key cellular processes.[8,9] Despite the spectacular advances in the design of fluorescent chemosensors for physiologically important metal ions,[7,10] new molecular architectures capable of distinguishing between the biologically ubiquitous Ca^{2+} and Mg^{2+} still pose intellectual challenges to the current mainstream of sensor development.[11]

In the contemporary practice of fluorescent chemosensor configuration, while essential components commonly include macrocycle ionophores[7] armed with fluorescent signaling elements of organic chromophores that alter fluorescence properties

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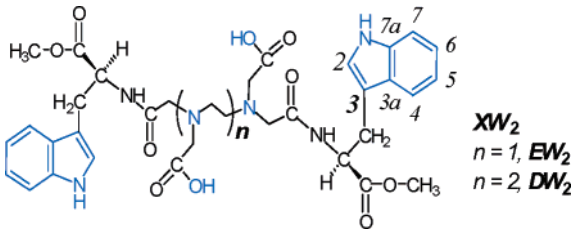
Scheme 1. Two Cation-Responsive Chemosensors Investigated



upon ion binding, ample opportunities have been provided recently by using a variety of open-chain frameworks.[11,12]

Multidentate aminocarboxylate ligands are capable of chelating metal ions in aqueous solution, but such chelation is otherwise unattainable by hydrophobic crown ethers; hence, conjugation of amino acids with multidentate aminocarboxylate ligands provides a new vehicle for investigating the metal-ion-recognition properties of aromatic amino acid side chains in aqueous solution.[2c] Herein we report the utility of this type of configuration in a chemosensor design in which the Trp indole that serves as both a spectroscopic reporter and a metal-ion-recognition entity is incorporated into the architecture. Two molecular architectures featuring the cation-responsive Trp indole, namely EDTA-bis(L-Trp methyl ester) (EW_2) and DTPA-bis(L-Trp methyl ester) (DW_2) (Scheme 1), were investigated.[13] We established that the sensors integrated metal ion recognition and metal ion−indole interaction into one supramolecular association process and responded to binding events with a cation-dependent fluorescence spectroscopic change. It is shown by ^{13}C NMR spectroscopy that the Ca^{2+}-selective response of the sensors is due to a dynamic reorientation of the indole ring caused by a Ca^{2+}−indole interaction.

Results and Discussion

Fluorescence titrations with chloride salts of alkali metal ions (Li^+, Na^+, K^+) and alkali earth metal ions (Mg^{2+}, Ca^{2+}, Ba^{2+}) were performed in aqueous buffer. Addition of Ca^{2+} led to a dramatically pronounced enhancement in the fluorescence emission of EW_2, with a maximum at 355 nm (Figure 1).

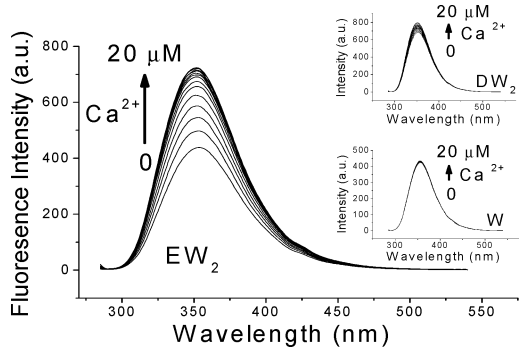


Figure 1. Ca^{2+}-induced fluorescence enhancement of 20 μM EW_2, pH 7.0, 25 °C. Insets: 20 μM DW_2 and 40 μM $NH_2TrpOCH_3$ (W), respectively.

In marked contrast, Li^+, Na^+, K^+, Mg^{2+}, and Ba^{2+} caused merely marginal changes to the fluorescence emission of the sensors,[14] while transition metal ions such as Zn^{2+} led to significant fluorescence quenching.[2c] Whereas the fluorescence intensity of the sensors was only slightly increased upon the addition of Mg^{2+}, Ba^{2+}, and Li^+, the fluorescence response is highly selective for Ca^{2+}.

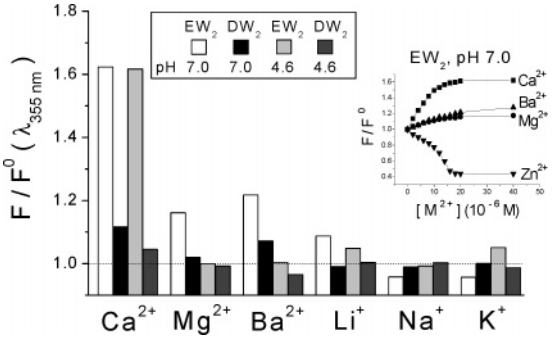


Figure 2. Fluorescence response of XW_2 to 1 equiv of metal ions in buffered water. Inset: Binding curves for Ca^{2+}, Ba^{2+}, Mg^{2+}, and Zn^{2+}.

At pH 7.0, the fluorescence intensity increase is nearly 60% upon the addition of 1 equiv of Ca^{2+} to EW_2, whereas the change is about 10% for DW_2 (Figure 2). Although the fluorescence intensities of both apo- and Ca^{2+}-bound XW_2 are sensitive to changes in pH, the selective response of EW_2 to Ca^{2+} is still significant at pH 4.6. Plots of the amplitudes of the intrinsic Trp fluorescence enhancement vs Ca^{2+} added allow the apparent association constants (K_a's) to be determined: 1.2×10^4 (Ca^{2+}-EW_2) and 7.4×10^2 M^{-1} (Ca^{2+}-DW_2) at pH 4.6, respectively.[15] As expected, with an increasing number of aminocarboxylates (in XW_2), which are much stronger ligands than the indole ring for metal ions, the sensitivity of the indole fluorophore toward Ca^{2+} significantly decreases, resulting in a more than 1 order of magnitude decrease in the fluorescence-determined apparent binding affinity. The 1:1 binding ratio was verified by ESI-MS and MALDI-TOF-MS analysis (Supporting Information).

^{13}C NMR (75.5 MHz) spectra of a solution of 70 mM EW_2 in 1:1 D_2O/DMSO-d_6 (v/v) revealed that the presence of 1 equiv of Ca^{2+} causes a severely broadened Trp indolyl C(3) (within ±~0.5 ppm) and a moderately broadened C(4), owing to broad distributions of isotropic chemical shifts of equivalent carbons (Figure 3).[16,17] Meanwhile, the preserved chemical structure of the indole ring is unequivocally characterized by the absence of net chemical shift change for all the aromatic carbons and further substantiated by ^1H NMR (Supporting Information).[16] Though a similar effect was observed to a moderate extent in either Ca^{2+}-DW_2 or Mg^{2+}-EW_2, the ^{13}C NMR spectra allow Ca^{2+} to be clearly distinguished from Mg^{2+}.[16] In contrast, a

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(13) EW_2 and DW_2 were prepared by coupling L-Trp methyl ester with ethylenediaminetetraacetic dianhydride and diethylenetriaminepentaacetic dianhydride, respectively (see Supporting Information).
(14) Charge densities of the metal ions increase in the following order: K^+ (0.05) < Na^+ (0.10) < Ba^{2+} (0.13) < Ca^{2+} (0.24) < Li^+ (0.33) ≪ Mg^{2+} (0.75). See ref 11c and Shannon, R. D. *Acta Crystallogr.* **1976**, *A32*, 751.
(15) Apparent association constants (K_a's, M^{-1}): Zn^{2+}(pH 4.6), 3.5×10^3 (EW_2) and 1.1×10^3(DW_2). The bindings of the rest of metal ions were too weak to estimate the accurate values at pH 4.6. EW_2(pH 7.0), 7.9×10^4 (Ca^{2+}), 1.8×10^4 (Mg^{2+}), 1.3×10^4 (Ba^{2+}), 3.1×10^3 (Li^+), and 2.0×10^4 (Zn^{2+}); DW_2 (pH 7.0), 1.2×10^4 (Ca^{2+}), 3.3×10^3 (Ba^{2+}), and 1.8×10^4 (Zn^{2+}).
(16) (a) ^{13}C NMR assignment was made on the basis of APT experiments and literature data (refs 4, 5, 18). (b) No aliphatic C(3) at ~70.0 ppm for an indolyl C(3)−M(II) covalent coordination mode was detected. (c) ^{13}C NMR spectra revealed that both carboxylate and amide carbonyl carbons are in the coordination sphere of Ca^{2+}. (d) The altered ^{13}C NMR signal of Trp C(3) was absent from acidifying EW_2 with DCl, consistent with the fluorescence and CD studies (see Supporting Information).
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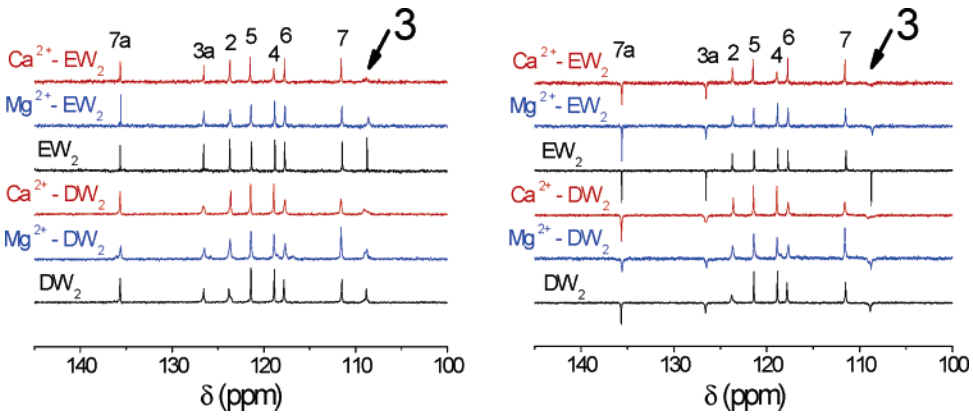
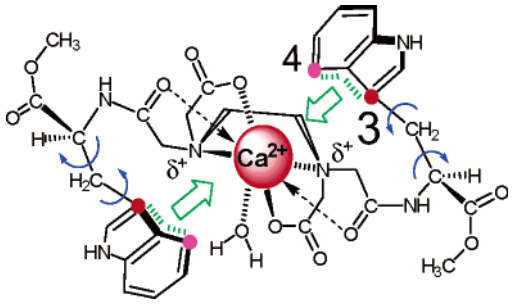


Figure 3. Regions of the ^{13}C NMR CPD (left) and APT (right) spectra of 70 mM XW_2 in the presence and absence of 1 equiv of Ca^{2+} and Mg^{2+}, respectively, in D_2O/DMSO-d_6 (1:1, v/v), pH 7.0.

Scheme 2. Proposed Ca^{2+}−Indole Interaction Model in Ca^{2+}-EW_2



marked quenching of the EW_2 fluorescence by Zn^{2+} is coupled to relatively sharp splitting of not only the Trp indolyl C(3) and C(4), but also the C(7) and C(7a) resonances, indicative of a mechanistically distinct interaction of the indole with Zn^{2+} (Supporting Information).

Since the Trp indolyl C(3) chemical shift is exquisitely sensitive to changes of the side-chain torsion angles,[18] the experimentally observed ±~0.5 ppm chemical-shift range for Trp indolyl C(3) therefore reflects a substantial degree of orientational mobility of the indole unit.[17] Thus, upon a preorganization of the indole unit via chelating Ca^{2+} by aminocarboxylates, a subsequently attained equilibrium fluctuation of the Trp side chain can conceivably lead to conformations in which the bound Ca^{2+} and the proximal indole moiety come into dynamic contact, as shown in Scheme 2, thereby leading to the virtually unfixed torsion angles of the Trp side chain and eventually the splitting of the Trp indolyl C(3) resonance.[18] A closely associated possibility remains: due to the susceptibility of the indolyl C(3) to eletrophilic attack,[4] direct electrostatic interactions between the ligated Ca^{2+} and the antenna indolyl C(3) may contribute to the broadened resonances of C(3) and C(4). Evidently, both scenarios are in support of the involvement of a noncovalent interaction between the indole and a ligated Ca^{2+}.[16] In addition, the resulting regioselective broadening of the aromatic protons (^1H NMR) accompanying the binding of Ca^{2+} to EW_2 is indicative of a fairly well-defined packing of the indole ring. The metal-ion-dependent conformational changes associated with the bindings of Ca^{2+} and Zn^{2+} to EW_2 were ascertained by CD titration experiments (Supporting Information).

(18) The Trp indolyl C(3) chemical shifts show no evident dependence on the backbone torsion angles but are strongly dependent on the side-chain torsion angles. See: Sun, H.; Oldfield, E. *J. Am. Chem. Soc.* **2004**, *126*, 4726.

Resonance broadenings or chemical shift nonequivalences in ^{13}C NMR spectroscopy are frequently observed in biomolecular associations, due to the broad distributions of isotropic chemical shifts of equivalent carbon atoms in biomolecular binding equilibria.[17] In general, ^{13}C NMR spectroscopy displays a more than 180 ppm chemical shift range for normal organic compounds, and it is one of the most sensitive spectroscopic methods for precisely visualizing chemical structural changes of organic compounds from either organic reactions or molecular interactions, or both. The experimentally observed broad distribution of the Trp indolyl C(3) isotropic chemical shifts (~0.5 ppm) not only directly reflects that the Trp indole side-chain torsion angles are rapidly changing within a certain limit upon Ca^{2+} binding, which clearly indicates the involvement of the bound Ca^{2+}−indole interaction, but also, interestingly, reveals that the metal ion−indole attraction is favored over the potential metal ion−ester carbonyl oxygen binding (Scheme 2). This result was further supported by the experimentally identified resonance broadenings of only two types of carboxyl carbons in the ^{13}C NMR spectrum of the complex Ca^{2+}-EW_2 (there are overall three types of carboxyl carbons in the sensor molecule EW_2), as the resonance of the carboxyl carbon in the methyl ester group ($COOCH_3$) was virtually unaffected by the addition of Ca^{2+} (Supporting Information).

As described above, the resonance broadening of the Trp indolyl C(3) was observed to only a moderate extent in the ^{13}C NMR spectrum of either Ca^{2+}-DW_2 or Mg^{2+}-EW_2 (Figure 3), in which the metal-ion-induced fluorescence enhancement was much less significant (Figure 2). These findings clearly suggest that the metal-ion-induced Trp indolyl C(3) resonance broadening is strongly correlated with the metal-ion-induced changes in the fluorescence intensity. That is, the cation-selective response of the sensors, as well as the metal-ion-induced three-dimensional structural transformation as evidenced by the CD absorption enhancements at 278 nm (see Supporting Information), is characteristic of the indole moiety in a relatively ordered three-dimensional structure.[2c,19] On the basis of these data, the altered ^{13}C NMR chemical shifts of the Trp indolyl C(3) may provide direct evidence for the Ca^{2+}−indole attraction (Scheme

(19) (a) Vitale, D. J.; Goldbeck, R. A.; Kim-Shapiro, D. B.; Esquerra, R. M.; Parkhurst, L. J.; Kliger, D. S. *Biochemistry* **2000,** *39*, 7145. (b) Whereas a pH−fluorescence titration showed that acidification of the sensor solutions decreased the fluorescence emission intensity of the sensors, a pH−CD titration demonstrated that the acidification of the sensor solutions was not accompanied by any CD absorption enhancement at 278 nm (see Supporting Information).

2), and hence the ^{13}C NMR spectroscopic analysis is shown to be very helpful in unambiguously delineating this metal-ion-receptor binding model.

In summary, we have presented the use of a Trp-based fluorescent framework in fine-tuning a markedly high selectivity for Ca^{2+} over Mg^{2+}, Ba^{2+}, K^+, Na^+, and Li^+ in aqueous solution. A combined NMR and CD spectroscopic study has demonstrated that the Ca^{2+}-induced fluorescence enhancement correlates with a dynamic reorientation of the indole ring due to the cation−indole attraction, suggesting that the highly sensitive, metal-ion-dependent Trp indolyl C(3) chemical shifts may serve as a promising indicator for monitoring metal ion−indole noncovalent interaction involving the Trp indole in biomolecules in solution. Given the structural simplicity of this Ca^{2+}-specific framework, its straightforward design may offer great potential for metal ion sensing and may open new perspectives in exploring the significant role of metal ion−aromatic ring interactions in supramolecular chemistry and biology.

Experimental Section

L-Tryptophan methyl ester was obtained from Novabiochem Corp. and used as received. EDTA dianhydride, DTPA dianhydride, and DMSO-d_6 were purchased from Aldrich and used as received. Deuterium oxide was obtained from Acros. All other solvents and reagents, including dichloromethane, triethylamine, methanol, and diethyl ether, were of analytical grade and were dried and purified before use. EDTA-bis(L-tryptophan methyl ester) (EW_2) and DTPA-bis(L-tryptophan methyl ester) (DW_2) were synthesized following our recently described procedure,[2c] and their identities were established by NMR and mass spectroscopy. The ^1H and ^{13}C NMR spectra were recorded on a Bruker AV-300 NMR spectrometer. Mass spectra were recorded at the Beijing Mass Spectrometry Center at the Institute of Chemistry, the Chinese Academy of Science. The matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectroscopy instrument used was a BIFLEX III (Bruker Inc.) with a α-cyano-4-hydroxycinnamic acid (CCA) matrix. The electrospray ionization mass spectroscopy instrument used was a Finnigen LCQ-Advantage 10 grade ion-trap LC-MS, and the flow rate of the LC pump was 4 μL/min.

Characterization of EDTA-bis(L-tryptophan methyl ester) (EW_2). ^1H NMR (300 MHz, D_2O): δ 2.492−2.616 (4H, m, NCH_2CH_2N), 3.102−3.265 (12H, m, all other CH_2), 3.706 (6H, s, OCH_3), 4.727 (2H, dd, chiral −CH−), 7.105 (2H, t, indolyl H5), 7.188 (4H, m, indolyl H6, H2), 7.444 (2H, d, indolyl H7), 7.579 (2H, d, indolyl H4). ^{13}C NMR (75.5 MHz, 1:1 D_2O/DMSO-d_6; v/v): δ 172.57, 172.33, 168.74, 135.66, 126.58, 123.72, 121.36, 118.80, 117.72, 111.47, 108.75, 56.66, 56.04, 53.23, 52.21, 51.07, 26.42. ESI^+-MS: for $C_{34}H_{40}N_6O_{10}$ calcd, 692.3; found, 693.4 ([EW_2 + H^+]). MALDI-TOF-MS: found, 693.7.

Characterization of DTPA-bis(L-tryptophan methyl ester) (DW_2). ^1H NMR (300 MHz, D_2O): δ 2.455−2.580 (8H, m, NCH_2CH_2N), 3.106−3.365 (14H, m, all other CH_2), 3.700 (6H, s, OCH_3), 4.700 (2H, dd, chiral −CH−), 7.095−7.196 (6H, m, indolyl H5, H6, H2), 7.406 (2H, d, indolyl H7), 7.555 (2H, d, indolyl H4). ^{13}C NMR (75.5 MHz, 1:1 D_2O/DMSO-d_6; v/v): δ 177.23, 176.24, 172.63, 172.30, 135.66, 126.56, 123.82, 121.41, 118.87, 117.82, 111.51, 108.84, 57.91, 57.67, 54.02, 52.94, 52.29, 51.56, 49.31, 26.15. ESI^+-MS: for $C_{38}H_{47}N_7O_{12}$ calcd, 793.3; found, 794.4 ([DW_2 + H^+]). MALDI-TOF-MS: found, 794.3.

Mass Spectroscopic Characterization of 1:1 Complexes of Ca^{2+} Ions with EW_2 and DW_2, Respectively. ESI^+-MS for Ca^{2+}-EW_2, $C_{34}H_{40}N_6O_{10}Ca$ calcd, 732.2; found, 731.3 ([EW_2 + Ca^{2+} − H^+]). MALDI-TOF-MS: found, 731.2. ESI^+-MS for Ca^{2+}-DW_2, $C_{38}H_{47}N_7O_{12}$-Ca calcd, 833.3; found, 832.3 ([DW_2 + Ca^{2+} − H^+]). MALDI-TOF-MS: found, 832.0.

Fluorescence titration experiments were conducted on a computer-controlled Varian Cary eclipse fluorescence spectrophotometer (Varian Instruments, Palo Alto, CA) attached to a circulating water bath for thermal regulation. A quartz cell with a 1 cm path length in both the excitation and emission directions was used in all the experiments, which were conducted at 25.0 °C. With the excitation wavelength set at 278.0 nm, fluorescence emission spectra (with maximum at 355 nm) were acquired from 550 to 250 nm in 1 nm increments with an averaging time of 0.1 s. The excitation and emission slit widths were set at 5 nm. The scan rate was 600 nm/min. The PMT detector voltage was 600 V. A pH of 7.0 was maintained by using phosphate buffer for titration experiments with Ca^{2+}, Mg^{2+}, Ba^{2+}, Zn^{2+}, and Li^+, and by using Tris-HCl buffer for titrations with Na^+ and K^+. A pH of 4.6 was maintained by using sodium acetate buffer for titration experiments with Ca^{2+}, Mg^{2+}, Ba^{2+}, Zn^{2+}, and Li^+, and by using ammonium acetate buffer for titrations with Na^+ and K^+.

Circular dichroism (CD) absorbance spectra were obtained with a JASCO J-715 spectropolarimeter (JASCO International Co., Ltd., Tokyo, Japan) interfaced to a personal computer. CD titrations were carried out for solutions of 100 μM XW_2 with Ca^{2+} and Zn^{2+}, respectively, at both pH 4.60 (sodium acetate buffer) and pH 7.0 (phosphate buffer), 25.0 °C.

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Supporting Information Available: Synthesis details and fluorescence, NMR, CD, ESI-MS, and MALDI-MS spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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