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Synthesis and Photopysical Properties of a Polioxo Ethylene Chain Alkaline Earth Metal Fluorescent Sensor with 2-Aminoanthracene As Terminal Group

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Supporting Information

ABSTRACT: A new symmetric polioxo ethylene chain fluorescent probe containing 2-aminoanthracene bichromophoric as the terminal group for the alkaline earth metal cation, 2,2'-[oxybis(3-oxapentamethyleneoxy)]bis[N-(2-anthryl)benzamide)] (1), has been synthesized. The photophysical properties of 1 have been studied by means of absorption, fluorescence spectroscopy, and ¹H NMR. The difference in emission spectra response to concentration of model compound 2-acetamidoanthracene and 1 in acetonitrile implies that intermolecular excited dimers is likely to occur. Fluorescence decay profiles of 2-acetamidoanthracene can be described by a biexponential fit, while three lifetimes, two of which are similar as those of 2-acetamido-anthracene, are found for 1. The third lifetime might be attributed to intramolecular excited dimers. Complex formation with alkaline earth metal ions are investigated in acetonitrile as solvent via fluorimetric titrations. Fluorescence intensity trend of the complex with Mg²⁺ differed from those of other alkaline earth metal ions. The compound forms 1:2 (ligand/Mg²⁺) complex with Mg^{2+} while formed 1:1 complexes with Ca^{2+} , Sr^{2+} , and



 Ba^{2+} , producing large hypochromic shifts in the emission spectra and significant cation-induced fluorescence amplifications. On the contrary, the addition of Ca^{2+} , Sr^{2+} , or Ba^{2+} lead to a decrease in the fluorescence emission first, then an increase and blue shift in emission could be found at the end.

1. INTRODUCTION

Fluorescence spectroscopy is an attractive analytical technique to measure intracellular concentrations of biologically important ions and molecules. The design and development of fluorescent chemosensors for the detection of analytically important species is still an expanding area of research.^{1–3}

Crown ether derivatives incorporating a fluorescent moiety are attractive tools for optical sensing of metal ions. Crown ethers are well-known to bind alkali and alkaline-earth metal ions, as well as other cations.^{4–6} Polioxo ethylene chain offers many advantages in separation and determination of ions.⁷ The basic molecular frame of ordinary polioxo ethylene chain molecules consists of a chain of several oxygen atoms connected by $-CH_2-$ groups. When proper fluorophore groups are introduced onto these $-CH_2-$, they will serve as potential luminescent reagents. The usual fluorophore is a planar aromatic molecule and tends to stack each other. One can expect that fluorophores will show an excellent probe and an attachment for stabilization of the pseudocyclic structure of the complex.

The early study on polioxo ethylene chains derivatives, was carried out by Vögtle et al.^{8,9} They demonstrated that the

oligo-oxyethylene compound, appending two quinoline units at its terminals, can strongly bind K^+ . Recently, the research group of Nakamura synthesized novel fluorescent reagents that have two fluorescent chromophores (anthracene, pyrene, fluorene, etc.) symmetrically or asymmetrically placed at both terminals of 1,13-diamino-4,7,10-trioxatridecane, and their complexations with alkali and alkaline earth cations were examined.^{10,11} Through the complexation with Ca^{2+} , the shape and the peak of the fluorescence spectra of two anthracene fluorescent chromophores significantly changed from a relatively structured band with three peaks around 400 nm to a structureless band at 490 nm. The peak around 400 nm of uncomplexed species and the broad peak at 490 nm of the complexed species could be attributed to fluorescence from an anthracene monomer and dimer, respectively. This phenomenon indicated that the structure of the ligand changed on complexation, and two anthracenes approached each other and then stacked, whereas compounds

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where two fluorescent chromophores are pyrene or fluorene did not show spectral changes upon addition of any metal salts. The same research group also developed another polioxo ethylene chain with two pyrenes linked at both ends of the chain show strong intramolecular excimer formation. Addition of alkaline earth metal ions leads to an increase in monomer emission at the expense of the excimer band. The helical structure of the 1:1 complexes is supported by ¹H NMR spectra.¹² In addition to this, other polioxo ethylene chains signaling of the binding event was achieved by a twisted intramolecular charge transfer (TICT)^{13,14} process and a PET¹⁵ process between two terminal chromophore units. Due to the difference in ionic radii between Mg^{2+} and Ca^{2+} , they can vary the torsion angle in the complex structure. Conformational change could be converted into physical signs, such as absorption and fluorescence change; this will be more sensitive than metal ion sensors. But some TICT processes show that the emission of complexation with Mg²⁺ was weak and gave no remarkable spectral changes compared to those of other cations.¹³ So, recently, the Nakamura group synthesized two chemosensors by substitution of the anthrance group with *n*-propyl or ethyl benzene moieties; complex results show that the peak shape and maximum wavelength of the emission of the complex with Mg^{2+} differed from those of Ca^{2+} and other alkaline earth metal ions.¹⁴ These indicate polioxo ethylene chain derivatives incorporating ionophoric functional groups located at the terminals of molecules exhibit excellent properties as carriers for metal ions.

In this work we synthesized the fluorescent chemosensor **1** based on polioxo ethylene chain with 2-aminoanthracene bichromophoric as the terminal group. The amino group was directly linked to the anthrance fluorophore. The solvent-dependent photophysical characteristics of probe **1** were investigated by steady-state absorption and emission spectroscopy, as well as time-resolved fluorimetry. Furthermore, protonation and complex formation of **1** with alkaline earth metal (Mg²⁺, Ca²⁺, Sr²⁺, Ba²⁺) ions was spectroscopically studied. Finally, the measured ground-state dissociation constants K_d for the formed complexes, the absorption and emission spectral wavelengths in the absence and presence of ions, the fluorescence quantum yields of the free and bound species of **1** are presented and discussed in this study.

The fluorophore of this molecule is the 2-aminoanthracene group. On the other hand, because most studies on 2-aminoanthracene and 2-acetamido-anthracene are limited, reference experiments on 2-aminoanthracene and 2-acetamido-anthracene were performed here also.

2. EXPERIMENTAL SECTION

2.1. Instruments and Reagents. ¹H and ¹³C NMR spectra were taken on a Bruker DRX-400 spectrometer with TMS as an internal standard and CDCl₃ as solvent. Mass spectra were recorded on a Bruker Daltonics esquire6000 mass spectrometer. In the case of ¹H NMR measurements of metal complexes, concentrations of these reagents 1×10^{-2} mol/dm³ were taken in acetonitrile- d_3 . Metal ions were added in excess amount to ensure complete formation of the complex. ¹H NMR spectra were measured by a AVANCE DRX- 200 at 20 °C. The pH was measured using a FE20K pH meter (Mettler Toledo).

Chemical shift multiplicities are reported as s = singlet, d = doublet, and m = multiplet. ¹³C spectra were referenced to the CDCl₃ (77.67 ppm) signal. Mass spectra were recorded in E.I. mode. Melting points were taken on an X-4 precise micro melting point cryoscope (Beijing Fukai Instrument Co.) and are

Scheme 1. Synthesis of Compound 1



uncorrected. All the materials for synthesis were purchased from commercial suppliers and used without further purification. Deionized water was used as solvents. Perchlorate salts of magnesium, calcium, cesium, and barium were of spectroscopic grade and used as received.

2.2. Syntheses. 2-Acetamido-anthracene. 2-Acetamidoanthracene was synthesized following a literature procedure.¹⁶ ¹H NMR (DMSO- d_6 , 400 MHz): δ = 2.13 (s, 3H, -CH₃), 7.47 (m, 3H), 8.01 (d, 3H), 8.45 (m, 3H), 10.20 (s, 1H, amide NH); MS (ESI) m/z found 236.2 (M + 1, 100%).

2,2'-[Oxybis(3-oxapentamethyleneoxy)]-bis[N-(2-anthryl)benzamide)] (**1**). The synthetic pathway of compound **1** is shown in Scheme 1. Compound **2** was synthesized following a literature procedure.¹⁷

A total of 20 mL of SOCl₂ was added dropwise to the 0.43 g of dicarboxylic acid (1 mmol) in an ice bath. The mixture was stirred about 2 h at -5 °C. Excess SOCl₂ was evaporated. Then the corresponding acid chloride was obtained. A total of 0.41 g of 2-aminoanthracene (2.1 mmol) dissolved in 80 mL of acetonitrile, 2 mL of triethylamine, and a solution of the above acid chloride in 30 mL of acetonitrile was added dropwise to this 2-aminoanthracene in an ice bath. The mixture was stirred for 1 day at room temprature. The solvent was evaporated in reduced pressure, and the residue was dissolved in 30 mL of chloroform, then washed with 20 mL of water three times, and dried over MgSO₄. The crude product was purifide by silica gel column chromatography (eluent: chloroform-ethyl acetatepetroleum). Yield: 20.3%; mp 161–163 °C. ¹H NMR (CDCl₃, 400 MHz): δ = 3.38 (-C-CH₂-O, q, 4H), 3.44 (-C-CH₂-O, q, 4H), 3.65 (-C-CH₂-O, t, 4H), 3.95 (-C-CH₂-O, t, 4H), 6.67 (aromatic, d, 2H), 7.06 (aromatic, t, 2H), 7.23– 7.40 (aromatic, m, 6H), 7.47 (aromatic, d, 1H), 7.49 (aromatic, d, 1H), 7.81-7.91 (aromatic, m, 6H), 8.21 (aromatic, s, 2H), 8.27 (aromatic, t, 2H), 8.29 (aromatic, s, 2H), 8.67 (aromatic, s, 2H), 10.21 (NH, s, 2H). ¹³C NMR (CDCl₃, 100 MHz): δ 67.5, 68.7, 70.0, 70.3, 112.5, 115.7, 121.7, 122.0, 124.8, 125.4, 125.6, 125.8, 127.8, 128.1, 128.6, 129.1, 130.9, 132.0, 132.1, 132.3, 133.0, 135.5, 156.3, 163.3. MS (ESI) *m*/*z* found 785.4 (M + 1, 100%).



Figure 1. Normalized absorbance and emission spectra ($\lambda_{ex} = 340$ nm) of (a) 2-aminoanthracene and (b) 2-acetamido-anthracene in various solvents.

2.3. Steady-State UV-Vis Absorption and Fluorescence Spectroscopy. UV-vis absorption spectra were recorded on a Varian UV-Cary100 spectrophotometer, and for the corrected steady-state excitation and emission spectra, a Hitachi F-4500 spectrofluorometer was employed. Freshly prepared samples in 1 cm quartz cells were used to perform all UV-vis absorption and emission measurements. For the determination of the fluorescence quantum yields, $\phi_{\rm fr}$ of 1, only dilute solutions with an absorbance below 0.1 at the excitation wavelength ($\lambda_{ex} = 340 \text{ nm}$) were used. For the measurement of fluorescence emission spectra as the function of concentration of 1, some of the absorbance higher than 0.1. Anthrancene in cyclohexane ($\phi_{\rm f} = 0.30$) was used as fluorescence standard.¹⁸ The $\phi_{\rm f}$ values reported in this work are the averages of multiple (generally three) fully independent measurements. The majority of the ϕ_f determinations were done using undegassed samples. In all cases, correction for the solvent refractive index was applied. All spectra were recorded at 20 °C using undegassed samples. The titration experiments with alkaline earth metals were carried out by adding small quantities of a stock solution of alkaline earth metal perchlorate salts in MeCN to a much larger volume (25 mL) of solutions of 1.

2.4. Time-Resolved Fluorescence Spectroscopy. Fluorescence lifetimes were measured by a FLS920 at room temperature. The samples were dissolved in CH_3CN and the concentrations were adjusted to have optical densities at the excitation wavelength (340 nm) <0.1. Solutions were purged with nitrogen for 15 min prior to analysis. The monitored wavelengths were 380, 400, 410, and 430 nm.

Fluorescence decay histograms were obtained on an Edinburgh instrument FLS920 spectrometer equipped with a hydrogen flash lamp, using the time-correlated single photon counting technique in 4096 channels. Histograms of the instrument response functions (using a LUDOX scatterer) and sample decays were recorded until they typically reached 3×10^3 counts in the peak channel. Obtained histograms were fitted as sums of the exponential, using Gaussian-weighted nonlinear leastsquares fitting based on Marquardt-Levenberg minimization implemented in the software package of the instrument. The fitting parameters (decay times and preexponential factors) were determined by minimizing the *reduced* chi-square χ_g^{-2} . An additional graphical method was used to judge the quality of the fit that included plotsof surfaces ("carpets") of the weighted residuals versus channel number. All curve fittings presented here had χ^2 values below 1.1.

2.5. Determination of K_d via Direct Fluorometric Titration. The ground-state dissociation constants K_d of the complexes between 1 and various ions were determined in CH₃CN solution at 20 °C by *direct* fluorometric titration as a function of the ion concentration [X] using the fluorescence excitation or emission spectra. Nonlinear fitting of eq 1¹⁹ to the steady-state fluorescence data *F* recorded as a function of [X] yields values of $K_{dr} F_{minr} F_{max}$ and *n*.

$$F = \frac{F_{\max}[X]^n + F_{\min}K_d}{K_d + [X]^n} \tag{1}$$

In eq 1, *F* stands for the fluorescence signal at [X], whereas F_{min} and F_{max} denote the fluorescence signals at minimal and maximal [X], respectively, and *n* is the number of ions bound per probe molecule (i.e., stoichiometry of binding). Because the fits of eq 1 to the fluorescence data *F* with *n*, K_{dv} , F_{min} , and F_{max} as freely adjustable parameters always gave values of *n* close to 1 or 2 (indicative of a 1:1 or 1:2 complex); *n* was kept fixed in the final curve fittings, from which the estimated K_d values are reported here.

3. RESULTS AND DISCUSSION

3.1. Steady-State Spectroscopic Behavior of 2-Aminoanthrancene and 2-Acetamido-anthracene. Figure 1a shows the UV/vis absorption and fluorescence emission spectra of 2-aminoanthrancene in several solvents. The absorption show typical anthracene absorption spectra that have three sharp bands at around 322, 344, and 354 nm. Furthermore, another strong and broad absorption band could be found at about 400 nm in all the solvents used. The absorption band maximum steadily shifts to longer wavelengths in going from nonpolar to polar solvents (from cyclohexane at 396 nm to acetonitrile at 407 nm).

Moreover, fluorescence emission also shows a strong solvent dependence. The common feature for 2-aminoanthrancene is the emission have three narrow bands centered near 382, 402, and 425 nm in MeCN originating from the excited state of the anthrancene (382, 402, and 425 nm in MeOH). Another red-shifted broad band, apparent here. The broad emission band maximum steadily shifts to longer wavelengths in going from nonpolar to polar solvents (from cyclohexane 438 nm to acetonitrile 487 nm). The emission spectra in solvents are of similar shape as those in the literature,²⁰ whereas the total fluorescence quantum yields are 0.28 in acetonitrile versus 0.44 in cyclohexane.

The long wavelength absorption and emission bands can be attributed to different phenomena. The amino group being a

compound	solvent	λ_{abs} (nm)	$\lambda(\text{LE})_{\text{em}}(\text{nm})$	$\lambda(ICT)_{em} (nm)$	$\phi_{ m f}{}^a$
2-aminoanthrancene	cyclohexane	336, 396	362, 380, 402	438	0.44
	toluene	336, 403	366, 385, 406	458	0.33
	MeCN	336, 407	382, 405, 428	487	0.28
2-acetamido-anthracene	cyclohexane	372, 395 ^b	380, 401, 423, 447		0.10
	toluene	354, 375, 395	408, 430, 450		0.19
	MeCN	346, 358, 375, 396	414, 431		0.31
	DMSO	358, 377, 398	380, 416, 437		0.23
^a Tatal furges and a sugestion	mield (notenan as anthe	maana in gualahawana Φ = 0	$20)^{b}$ 2 A cotomido anthracos	a is mandamatalwa salwhila in a	welshavana

Table	e 1.	Photop	hysical	Prope	erties o	f 2.	-Aminoantl	hrancene	and	2-A	cetamid	lo-ant	hracene	in	Several	So	lven	ts
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^{*a*} Total fluorescence quantum yield (reference: anthrancene in cyclohexane, $\Phi_f = 0.30$). ^{*b*} 2-Acetamido-anthracene is moderately soluble in cyclohexane; it is difficult to find all the absorption band because the absorband is very low in cylohexane.



Figure 2. Normalized emission spectra of 1 (λ_{ex} = 340 nm) in several solvents (a) and in acetonitrile (b) at different concentrations.

good electron donor and the anthrancene being a reasonable electron acceptor, the possibility of intramolecular charge transfer (ICT) may be considered.

The absorption spectra (not shown) of 2-acetamido-anthracene showed four narrow bands at around 346, 358, 375, and 396 nm in acetonitrile. In other solvents, the absorption spectra are of similar shape as in acetonitrile. No broad and red shift band could be found for compound 2-acetamido-anthracene in all the solvents used.

The emission spectra in toluene shows three bands, with the maximum at \sim 408, \sim 430, and \sim 450 nm, respectively (Figure 1b). The fluorescence emission spectra of 2-acetamido-anthracene in toluene are quite similar as that of calix[4]pyrrole-aminoanthracene in CH₂Cl₂.²¹ An emission maximum at \sim 431 nm and another band at the short wavelength side (\sim 414 nm) could be observed upon

excitation at 340 nm in acetonitrile solution. The emission spectra are of similar shape as those of 2-acetylaminoanthracene in aqueous buffer solution²² and foldamers incorporated with six decyl chains and two anthracene units at the ends in *n*-octanol.²³ As one can see, the amino group acylation brings about a dramatic hypochromic shift of the spectrum when compare with ICT band of 2-aminoanthrancene. All of these compounds (calix[4]pyrrole-aminoanthracene, 2-acetylaminoanthracene, and compounds in refs 21–23) and 2-acetamido-anthracene have the same *N*-(2-anthryl)amide group and have similar emission spectra. So the main chromophore should be *N*-(2-anthryl)amide group. These absorption and emission band are also similar as those of 9-cyanoanthracene (anthracene-9carbonitrile) in methanol or 9,10-diphenylanthracene (DPA) in methanol or cyclohexane.²⁴

The anthracene molecule is well-known to form the relatively stable excimer or exciplex.²⁵ When the excimer or exciplex was form, its fluorescence intensity will be decreased with increase concentration. Figure S1a shown the fluorescence spectra of 2-acetamidoanthracene as the fuction of concentration in acetonitrile. Its fluorescence intensity increasing with incerase concetration, then decrease intensity with further incerase concetration. We can find that fluorescence intensity at 292 μ M was lower than that at 128 μ M. Normalized their fluorescence spectra at longer band 431 nm also shown that the shorter band at 414 nm slightly decrease with increasing its concentration. The two dash curves in Figure S1b are obtained from the normalized spectra (at 431 nm) at 292 and 128 mM subtract the spectra at concentration 3.2 μ M. Because 2-acetamido-anthracene only contain one anthrance chromophore, intramolecular excimers cannot be formed. So the different ratios of two maximum bands (431 and 414 nm) of 2-acetamido-anthracene in acetonitrile as the function of concentration should be the reason that formed of the intermolecular excimer. With an increase in the concentration, there have more dimers that can form; the intensity of the longer maximum band will increase where the shorter bands of the monomer will decrease. From the two dash curves of Figure 1b, one can find that, with increasing the concentration, the contribution from the excimers will become more and more.

Table 1 summarizes the photophysical data of 2-aminoanthrancene and 2-acetamido-anthracene in several solvents.

3.2. Absorption and Emission Spectra of 1. UV/vis absorption and fluorescence emission spectra of 1 in several solvents are depicted in Figure S2 and Figure 2a, respectivity. The absorption and fluorescence emission spectra of 1 are similar to those of 2-acetamido-anthracene in all the solvents used. Table 2 summarizes the photophysical data of 1 in several solvents.

The anthracene molecule is well-known to form the relatively stable excimer or exciplex. The excimer band of the anthracence normally appears in the long wavelength area of about 490 or 505 nm.²⁶ In addition, similar emission spectra as 1 also appear in the case of other compounds only containing a single anthracene unit.^{22,23} Because intermolecular excimer can be formed for 2-acetamido-anthracene, so what about 1? We measure the fluorescence emission spectra of 1 in acetonitrile at different concentration and shown the results in Figure S3 and Figure 2b. An emission maximum at \sim 433 nm, whereas another band at \sim 415 nm, could be observed at a high concentration of **1**, this spectra is the same as those in DMSO and toluene. The maximum of the fluorescence emission band shifts hypochromic from 433 to 415 nm with an decrease in intensity when gradual decreasing the concentration of 1 and is accompanied by appear an new band at 380 nm. Figure 2b shows the normalized fluorescence emission of 1 from Figure S3. The spectra in different concentration of 1 cannot overlap. A closer inspection shows the presence of a tail at the red edge of the emission band (433 nm) with increasing in the quantity of 1, which becomes more outspoken (about 500 nm) in higher concentrations. This change in emission spectra of 1 not only shows the decrease in emission intensity at 433 nm, but shifts slightly to shorter wavelengths with decreasing in the quantity of ligand in addition to the appearance of isoemissiom points at 398 nm. These spectral changes with the clean isosbestic point at 398 nm indicate that the present bisanthracene systems exist in equilibrium, at least between two states. The clearly different emission spectra response to concentration here implies that intermolecular excited dimers $(1^* \cdot 1)$ obtained from the excited state of the 1* reaction with the group state of 1 is likely to occur in acetonitrile.

In DMSO, the ration of two bands, 440/420 nm, changed with concentration, which is also similar as in MeCN. So, intermolecular excited dimers can be formed in DMSO, also.

In addition, the molecule **1** has two of the same anthrance chromophores that are linked by a flexible chain. Intramolecular excimers may also be formed in the excited state. The intramolecular anthracene dimer linked with ethyleneoxy units (bis(9,10-anthracenediyl)coronand) has been reported to form a metal-sensitive intramolecular excimer.²⁷ One cannot exclude that intramolecular excimers can also be formed for **1**.

A Lorentz fit was applied to the fluorescence spectrum of 1 in acetonitrile and three peaks were picked at 412, 436, and 490 nm, respectively. In a fit of the spectra, the position, half-width, and skewness of these two components were kept fixed. An example of a fit is given in Figure S3. The band at 412 nm can be

	Table 2.	Photophy	vsical Pro	perties of 1	l in	Several	Solvents
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solvent	λ_{abs} (nm)	$\lambda_{\rm em} ({\rm nm})$	$\phi_{ m f}{}^a$				
toluene	350, 377, 396	380, 416, 436	0.05				
MeCN	355, 372, 393	380, 415, 436 ^{<i>a</i>}	0.02				
DMSO	350, 377, 396	380, 420, 440	0.03				
$^{\prime}$ High concentration of 1 (absorbance higher than 0.1).							

considered the monomer emission of N-(2-anthryl)amide and 490 nm is therefore due to an excimer formed via an association of two 2-aminoanthracene units situated at the ends of the polyoxyethylene chain. This eximer is not so obvious in the emission spectra of 1.

3.3. Complex Formation between 1 and Alkaline-Earth Metal lons in Acetonitrile Solution. Complex formation was investigated for 1 with alkaline-earth metal (Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+}) in acetonitrile. The results are summarized in Table 3.

Upon addition of Mg^{2+} in the μM concentration range to a mM solution of 1 in acetonitrile, a slight decrease in intensity and hypochromic shift of the UV/vis absorption could be found.

The fluorescence emission spectra of 1 as a function of $[Mg^{2+}]$ are also shown in Figure 3. In salt-free acetonitrile, the emission spectrum of 1 displays one weak, broad band with a maximum



Figure 3. (a) Fluorescence emission ($\lambda_{ex} = 340 \text{ nm}$) spectra of 1 in acetonitrile solution as a function of [Mg²⁺]. (b) Best fit of eq 1 with n = 2 to the *direct* emission fluorimetric titration data of 1 obtained from the spectra of (a): $\lambda_{ex} = 340 \text{ nm}$, $\lambda_{em} = 381 \text{ nm}$.

Table 3. Spectral and Photophysical Characteristics of 1 in the Absence and Presence of Alkaline-Earth Metal Ions in Acetonitrile⁴

complex	cation size $(\text{\AA})^{28}$	ctoichiometry ligand/metal	$\lambda_{\rm em}$ (nm)	log K	Φ_{f}
1			380, 415		0.020
$1 - Mg^{2+}$	1.44	1:2	366, 380, 401	8.11	0.088
$1 - Ca^{2+}$	2.12	1:1	366, 380, 401	3.58	0.020
$1 - Sr^{2+}$	2.52	1:1	366, 380, 401	4.60	0.060
$1-Ba^{2+}$	2.94	1:1	366, 380, 401	4.17	0.059

^{*a*}K_d is the average value determined from all direct analyses of fluorometric and spectrophotometric titrations.



Figure 4. ESIMS for the Mg adduct of $1 [(1 + 2Mg + H^+): 834.5]$.

at \sim 415 nm and a shoulder band at \sim 380 nm. Upon increasing $[Mg^{2+}]$, the emission band blue-shifted with a maximum at 366, 380, and 401 nm originating from anthrancene excited state increases its intensity. From the analyses of all the spectrophotometric and fluorometric titrations of 1 with Mg^{2+} ions, it is clear that a 1:2 ligand/cation stoichiometry is found. The $\log K_d$ $(K_{d1} \times K_{d2})$ value for the 1–Mg²⁺ complex, determined by direct fluorometric titration (eq 1) of emission spectral data F, equals 8.11. The response of the absorption and fluorescence spectra of 1 in acetonitrile upon addition of Mg^{2+} is nearly the same as that found for 1 in the presence of H^+ (Figure S6), only $\Phi_{\rm f}$ of 1–H⁺ is somewhat lower than $\Phi_{\rm f}$ of 1–Mg²⁺. Because direct fluorometric titrations emission spectra show that 1:1 stoichiometry between 1 and H⁺, whereas there is a 1:2 ligand/ cation stoichiometry between 1 and Mg²⁺. One cannot exclude that with another H^+ to $1-H^+$ the emission intensity will increase further until it is the same as that of $1-2Mg^{2+}$. With an increase in the concentration of Mg^{2+} , the free excited state of 1* will decrease due to the formation of the complex of Mg₂²⁺ \cdot 1. The intermolecular excited dimers $(1^* \cdot 1)$ will become less and

less due to a decrease in the excited state of 1*; then the group state of monomer of 1 will become more and more. So we can observe the anthrancen-like emission spectra upon addition of Mg^{2+} to 1. In addition to inhibiting the formation of intermolecular excited dimers (1*·1), coordination of the Mg^{2+} polyoxyethylene and carbonyl oxygen atoms suppresses the required bending movement of the polyoxyethylene chain for the association of two fluorophores, so the intramolecular excimer can not be formed, leading to a huge cation-induced fluorescence enhancement with spectral shift.

The formation of $Mg_2^{2+} \cdot 1$ adduct was further confirmed by mass spectrometry. The electrospray ionization mass spectrum of the Mg adducts for 1 showed a molecular mass of 834.5, which correspond to the formula of $[1 + 2Mg + H^+]$ (Figure 4).

Upon complexation by Ca^{2+} in acetonitrile, the absorption spectrum of 1 is blue-shifted combine with a decrease in the absorption (Figure S7).

Addition of Ca^{2+} to a solution of 1 in acetonitrile shows a decrease in the fluorescence emission intensity at ~415 nm in addition to the red tail at around 500 nm that slowly disappears



Figure 5. (a) Fluorescence emission ($\lambda_{ex} = 340 \text{ nm}$) spectra of 1 in acetonitrile solution as a function of $[Ca^{2+}]$. (b) Best fit of eq 1 with n = 1 to the direct emission fluorimetric titration data of 1 obtained from the spectra of (a): $\lambda_{ex} = 340 \text{ nm}$, $\lambda_{em} = 381 \text{ nm}$.

first (Figure 5a); then the fluorescence emission band hypochromic shifts from 415 nm in ion-free acetonitrile to 366, 380, and 401 nm and increases its intensity with increasing the calcium ion concentration. This mean that the presence of calcium at low concentration will decrease emission of the monomer and dimer first. From the analyses of fluorometric titrations of 1 with Ca²⁺ ions, it is clear that a 1:1 ligand/cation stoichiometry is found. The log K_d value for the Ca²⁺ complex with 1 determined by direct fluorimetric titration (eq 1) of emission spectral data Fequals 3.58. The emission spectral changes of 1 in the presence of Sr²⁺ or Ba²⁺ (Figures S8 and S9) are similar to those of Ca²⁺.

A closer inspection shows the fluorescence emission spectra change of 1 in the presence of Ca^{2+} , Sr^{2+} , or Ba^{2+} at low concentrations are completely the same as the fluorescence emission spectra of 1 in acetonitrile at different concentrations and accompanied by the appearance of an isoemissiom point at 398 nm. This means that the cation (Ca^{2+} , Sr^{2+} , or Ba^{2+}) binding model is not the same as Mg^{2+} ; this should have a relationship with the structure of the ligand 1. But after the alkaline-earth metal completely complexes with 1, the rigid structure of the complex may hinder intramolecular excimer formation. At the same time, complexes formed can also suppress the form of intermolecular excited dimers $(1^* \cdot 1)$ form, which will lead to an increase in the monomer emission of anthrance.

The stability constants measured for the Ca²⁺, Sr²⁺, or Ba²⁺ cation complexes of 1 are in the order of Ca²⁺ < Sr²⁺ \approx Ba²⁺, which can be explained by the cation size and the size of the pseudocrown cavity. Binding affinity of macrocyclic crown ethers mainly depends on the ionic size of the cation, whereas in pseudocyclic systems, combined effects of the size of the pseudocrown ether cavity, number of oxygen atoms, the charge density, and the coordination number of the cations play a considerable role. The exceptional behavior of the Mg²⁺ complex showing 1:2 ligand/cation stoichiometry value can be ascribed to the fact that the Mg²⁺ ion is too small to only fit well one cation into the cavity (see Table 3).

3.4. Time Resolved Fluorescence Spectroscopy and Their Complexes with Alkaline-Earth. To directly probe the excited state dynamics of 2-acetamido-anthracene and 1, fluorescence decay traces in different solvents were collected as a function of emission wavelength λ_{em} by the single-photon timing technique. Fluorescence decay measurements reveal a more complex situation for 1. Each fluorescence decay trace was analyzed individually as a sum of mono, bi, or three exponential functions in terms of decay times τ_i and associated pre-exponential factors a_i (i = 1-3). Details are given in Tables 4 and S1.

The fluorescence decay of 2-acetamido-anthracene is fitted to the bi- exponential profile with the lifetime of 10 and 3.0 ns in acetonitrile in all of the fluorescence region. In DMSO, the fluorescence decays also show two main components with time constants of about 18.0 and 1.5 ns (Table S1). Contributions of the faster decay times τ_1 decrease with increasing the concentration of 2-acetamido-anthracene in acetonitrile, so fast decay might be attributed to the lifetime of nonaggregated molecules, while slow decay may be attributed to the lifetime of intermolecular excited dimers.

In contrast, the decay profiles of 1 are more complicated than those of 2-acetamido-anthracene. In acetonitrile, the emission is triple-exponential (0.2, 3.8, and 10.5 ns). The contributions of the two slower constant decay times τ_2 (3.8 ns) and τ_3 (10.5 ns) components are about 15 and 40%, respectively. The fluorescence decay is also a triple-exponential in DMSO. In DMSO, the longest decay time has the largest contribution. The longer component τ_2 decreases from 19.0 ns at $\lambda_{\rm em} = 410$ nm to 9.5 ns at $\lambda_{\rm em} = 430$ nm. These may be due to the reason that oxygen is easily quenched in this long lifetime. Solutions were purged with nitrogen to elimate the effect on oxygen; when measured for a long time, oxygen will quench the lifetime of the solution.

The fluorescence lifetimes of anthracene excimers strongly depend on the environment, solvent polarity, and the substituents. Lifetimes of 52–91 ns have recently been reported for the excimer of a 9,10-dimethylene substituted anthracene chromophore in a cyclodextrin-derived bianthracene system in water²⁹ and an even longer lifetime than 200 ns for the π -stacked dimer.³⁰ In DMSO and MeCN, the decay profile is analyzed by using three exponential functions, where two of the parameters (10.0 and 3.0 ns for MeCN, 18.0 and 2.0 ns for DMSO) are similar to that obtained from the emission analysis of 2-acetamido-anthracene. In addtion, contributions of the decay times 2-3 ns (about 10-15%) are almost the same as 2-acetamido-anthracene. In DMSO, it is clearly indicated that the third slowest one (140-160 ns) is mainly from the excimer emission. The difference in the structure of 1 compared with 2-acetamido-anthracene is 1 has two of the same anthrance chromophores, so the third lifetime in DMSO maybe could be Table 4. Time-Resolved Emission Data of 1 and Complexes, Fitted Individually as a Sum of up to Three Exponential Functions with Time Constants τ_i (ns) and Amplitudes α_i (i = 1-3)

compound	solvent	monitored wavelength	$ au_1$ (ns)	$ au_2$ (ns)	τ_3 (ns)
1	MeCN	410	3.82 (19%)	10.97 (50%)	0.22 (31%)
		420	3.88 (13%)	10.47 (40%)	0.09 (47%)
		430	3.22 (15%)	9.99 (40%)	0.26 (45%)
	DMSO	410	2.33 (14%)	19.31 (13%)	164.07 (73%)
		420	0.74 (16%)	11.16 (23%)	135.36 (61%)
		430	1.79 (23%)	9.52 (19%)	146.74 (58%)
$1 \cdot Mg^{2+}$	MeCN	380	1.58 (20%)	6.64 (53%)	27.42 (27%)
		400	2.46 (30%)	8.38 (17%)	27.92 (53%)
$1 \cdot Ca^{2+}$	MeCN	380	0.91 (12%)	4.63 (35%)	16.88 (16.88%)
		400	1.09 (9%)	4.95 (44%)	14.69 (47%)
$1 \cdot Sr^{2+}$	MeCN	380	1.8 (18%)	6.56 (48%)	18.31 (34%)
		400	2.62 (17%)	7.47 (60%)	18.50 (23%)
$1 \cdot Ba^{2+}$	MeCN	380	1.41 (15%)	6.89 (46%)	21.51 (39%)
		400	1.13 (10.5%)	7.02 (61.2%)	18.20 (28.3%)



Figure 6. Partial 1 H NMR spectra of 1 in CD₃CN: (a) $1-Mg^{2+}$, (b) $1-Ca^{2+}$, and (c) 1 alone.

assigned to intramolecular excimers. In acetonitrile, the fastest decay times τ_1 of about 0.2 ns maybe also attributed to the lifetime of intramolecular excimers. At all wavelengths, the shortest decay time $(\tau_1 \approx 0.2 \text{ ns})$ has the largest contribution (45%), which is the same as the longest lifetime (>50%) in DMSO. Another possible reason may be that the energy transfer from the excited anthracene monomer to the anthracene dimer is facile in aggregrates; the observation of short-lived components in MeCN is not unexpected.

The fluorescence decay of monoanthracene is well fitted to the double exponential profile, the excimer decay profile is analyzed by using three exponential functions that have been reported in literature before.²⁹ In general, the decay profiles of **1** are more complicated than 2-acetamido-anthracene due to the involvement

of at least two different emitting electronic states and the likely presence of inter- and intramolecular excimers since anthrance tend to aggregate.

Fluorescence decay measurements also reveal a more complex complexation behavior of **1**. The fluorescence decay curves of all the alkaline-earth metal ion complexes measured can be described by a triple exponential fit. The lifetimes of all longer-lived and shorter-lived emissions changed upon complexation. This result shows that the environment around the compounds changes upon complexation.

On the contrary, as in the absence of alkaline-earth metal cations in MeCN, most of the contribution are from two slows decays ($\tau_2 \approx 6.6-8.4$ ns and $\tau_3 \approx 27.5$ ns) in the presence of Mg²⁺. The longest lifetime can be attributed to the complex with



Figure 7. Schematic representation of the proposed conformational changes before and after binding with Mg²⁺ and Ca²⁺, Sr²⁺, or Ba²⁺ in acetonitrile.

alkaline-earth metal cations, where another slow decay may be caused by the photoinduced decoordination of the cation. In case of photodecoordination of the cation,^{31,32} the fluorescence lifetimes of the decoordinated complexes are similar for different cations, which is the same as our measurements ($\tau_2 \approx 7.0$ ns for $1 \cdot \text{Mg}^{2+}$ and other alkaline-earth metals, except that $\tau_2 \approx 5.0 \text{ ns}$ for $1 \cdot \text{Ca}^{2+}$). The real nature of the third species remains unclear at present. The short lifetimes from 1.0 to 2.5 ns may correspond to the transition from complexation to photodecoordination. This shows the existence of a slow exchange rate between free and complexed species. As a result, three decay components were observed. The absence of the red edge of the emission band when in the presence of alkaline-earth metal cations does not support that another slow decay can be attributed to the lifetime of the aggregated intermolecule dimer. Complexes with Mg^{2+} , Ca^{2+} , Sr^{2+} , and Ba^{2+} reveal that

fluorescence lifetimes increase with increasing values for quantum

yield. This suggests that the increase in the fluorescence lifetimes of both components seems to be the direct consequence of the complex formation in the ground state and the emission of both excited complex conformers. The fluorescence decay times of the longest decay components of the Mg²⁺ complex are longer than that of the other alkaline-earth metal complexes, pointing to higher fluorescence quantum yields of Mg^{2+} compared to that of the other alkaline-earth metal complexes.

3.5. ¹H NMR Measurenment of the Complexes. To clarify the structure of complexes in detail, a ¹H NMR study was carried out in the absence and in the presence of metal cations in acetonitrile- d_3 at 25 °C. The spectra of 1 before and after addition of Mg^{2+} and Ca^{2+} are shown in Figure 6.

The oxyethylene proton peaks of 1 became broad and shifted to low magnetic field in the presence of Mg^{2+} . The reason is the reduction of the electron density on the oxygen atoms by the coordinated cations. This also indicated that Mg²⁺ was bound to oxyethylene parts. On the other hand, a high magnetic shift change of the amide proton was observed, whereas the amide proton peak of the complex $Mg^{2+}-2,2'$ -[oxybis(3-oxapentamethyleneoxy)]bis[N-(1-pyrenylmethyl)benzamide] shifted to a low magnetic field. Protons of the anthracence ring and benzene moiety showed the high-field shifts.¹² This result can be explained by deshielding due to stacking of the benzene and pyrene rings. These observations indicated that Mg^{2+} was mainly bound by the carbonyl group and the oxygen atoms of oxyethylene moieties. Further details of the assigned change of each proton of the spectra were too complicated for complete assignments to be made.

The same trends in ¹H NMR studies on $1 \cdot Ca^{2+}$ were also obtained. The protons of the anthracence ring and benzene moiety show small high-field shifts more than $1 \cdot Mg^{2+}$; the complex $1 \cdot Ca^{2+}$ had a much larger value of chemical shift changes than those of $1 \cdot Mg^{2+}$.

On the basis of fluorescence spectral and ¹H NMR changes of all the compounds, an expected structural change in 1 in the presence of alkaline earth metal ions is depicted in Figure 7. Due to the ionic radii of Mg^{2+} , which is smaller than those of other earth-alkaline-metal cations (Ca²⁺ or Sr²⁺ or Ba²⁺; Table 3), the torsion angle varies in the complex structure, which will be different for them. Conformational change will be reflected in the fluorescence spectrum as a feature and a position of fluorescence maximum. Before the formation of the complex, 1 shows weak fluorescence emission of the N-(2-anthryl)amide group at \sim 380, \sim 420, and/or \sim 440 nm due to the intermolecular and intramolecular excimers that can be formed from two molecules and two anthrances of one of the same molecules, respectively. Complexes formed will decrease the form of intermolecular dimers $(1^* \cdot 1)$ due to the decrease of 1^* . On the other hand, after binding with Mg²⁺, the complex formed a rigid conformation structure; two anthrance groups were separated. When complexed with other alkaline earth metal ions, the two anthrance groups approached each other first and lead to a decrease in the monomer emission of 1. The complex can be further reduced in conformational flexibility. These complexes can hinder excimer formation and also coordinate with a carbonyl group, which results in a fluorescence emission of a local excited state of 2-aminoanthrance enhancement at \sim 360, \sim 380, and \sim 400 nm (Table 1).

What are the different properties of 1 compared with other polioxo ethylene chain probes? Linear polyether N,N'-[oxybis-(3-oxapentamethylenoxy)-2-phenyl] bis(9-anthracenecarbonamide) and its analogues have been synthesized, and fluorescence "Off-On" behaviors for alkaline earth metal ions (Ca²⁺, Sr²⁺, and Ba²⁺) were reported by Hiroshi Nakamura.¹³ These compounds showed almost no fluorescence emission (fluorescence quantum yield = 0.0003) because twisted intramolecular charge transfer (TICT) occurred between the carbonyl group of the amide bond and a plane of the anthracene ring in the absence of a metal ion. The fluorescence lifetime of these compounds and their calcium complexes was too short and could not be determined. The same group also reported¹² 2,2'-[oxybis(3-oxapentamethyleneoxy)]bis-[N-(1-pyrenylmethyl)benzamide)] and its analogues have two pyrenes at both terminals of polyoxyethylene compounds, showing strong intramolecular excimer emissions around 480 nm in the fluorescence spectra. On the complexation with alkaline earth metal cations, the increase of monomer emission around 400 nm accompanied by the disappearance of intramolecular excimer emission of free reagents was observed. These reagents formed a 1:1 complex with alkaline-earth metal cations.

Two new asymmetric fluorescent reagents were synthesized by one of the anthracene aromatic amide moieties substituted by *n*-propyl and ethyl benzene moieties.¹⁴ In the absence of metal ions, the fluorescence emissions of these compounds were quite weak, but their intensities were much greater in the presence of alkaline earth metal ions. The peak shape and maximum wavelength of the emission of the complex with Mg^{2+} differed from those of Ca^{2+} and other alkaline earth metal ions, but synthesis is not so easy for these symmetric compounds. Complex alkalineearth metal cations with 1 not only increase the emission band, but also the emission band hypochromic shift and shown maximum at 366, 380, and 401 nm, which is the same as the locally excited (LE) state of 2-aminoanthrance that could be observed. It formed a 1:2 complex with Mg^{2+} , while 1:1 with Ca^{2+} , Sr^{2+} , or Ba^{2+} , and the spectra change with Mg^{2+} is not the same as those with other alkaline earth metal ions.

4. CONCLUSION

We have synthesized a polioxo ethylene chain fluorescent dye 1 with 2-aminoanthracene as the terminal group. Model compound 2-acetamido-anthracene shows a dependence of fluorescence emission on the concentration in acetonitrile, which indicates intermolecular excited dimers can be formed. Fluorescence decay profiles of 2-acetamido-anthracene can be described by a biexponential fit, which may be attributed to nonaggregated molecules and intermolecular excited dimers. Three lifetimes are found of 1 in MeCN and DMSO, which implies that intramolecular excited dimers are likely to occur also. The dye forms 1:2 complexes with Mg^{2+} but 1:1 complexes with other earth-alkaline-metal ions (Ca²⁺, Sr²⁺, Ba²⁺). The Mg^{2+} complexation-induced effects on the emission spectra led to a large blue shift and a strong increase in the emission. The addition of other earth-alkaline-metal cations (Ca²⁺, Sr²⁺, or Ba²⁺) has a very different effect on the emission than in the presence of Mg^{2+} , which leads to a decrease in the fluorescence emission first, then an increase and blue shift in emission could be found at the end. The new dye is an example of a very sensitive fluorescent probe for Mg²⁺ metal ions, displaying large fluorescence changes in wavelength and intensity than those for other earth-alkalinemetal cations. This difference change of spectra allowed a discriminating detection between Mg²⁺ and other earth-alkaline-metal cation by means of fluorescence spectroscopy.

ASSOCIATED CONTENT

Supporting Information. Absorption and fluorescence emission spectra of 1 in acetonitrile as a function of Sr²⁺ or Ba²⁺. Protonation fluorescence emission spectra of 1 in acetonitrile. ¹H and ¹³C NMR spectra of 2-acetamido-anthracene and 1. This material is available free of charge via the Internet at http:// pubs.acs.org.

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