

# New “Off-On” Responsible Fluoroionophores for Alkaline Earth Metal Ions Based on Benzo-15-crown-5 Bearing 9-Phenanthreneacetamide

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The photochemical behaviors of a series of 9-phenanthreneacetamide (PA) derivatives were investigated using UV, fluorescence, and <sup>1</sup>H-NMR spectroscopy in acetonitrile solutions. To apply the electron transfer (ET) action of PA as a detecting moiety of chemosensors, 4'-(9-phenanthreneacetamido)-benzo-15-crown-5 (**1**) and 3'-methyl-4'-(9-phenanthreneacetamido)-benzo-15-crown-5 (**2**) were synthesized. After metal ion complex formation, the fluorescence intensity increased with increased concentration of the guest alkaline earth metal ions. The photochemical responses of the fluorescence intensity, as defined by the fluorescence intensity ratio ( $I_{\max}/I_0$ ) between free and complex of **1** for Ca<sup>2+</sup>, was determined to be 4.76. However, the addition of guest ions in a solution of **2** greatly enhanced the fluorescence intensity of **2**. The  $I_{\max}/I_0$  of **2** was 16.6 for Ca<sup>2+</sup>. The efficiency of ET of PA can be tuned by adding an electron-donating group onto a suitable position; fluorescent **2** was able to read out metal ions as an “Off-On” fluorescence signal.

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## Introduction

Fluoroionophores act as a nanoscale chemical interface between humans and molecules in various analytical fields. Most fluoroionophores consist of a fluorescent group with an accompanying receptor that recognizes a target substance (e.g. a metal cation) through a host-guest interaction with a coordinating moiety (e.g. crown ether).<sup>1</sup> To monitor the interactive event between the host and the guest molecule, the fluorescence signal of the fluoroionophore must be changed greatly along with the interactive event.<sup>2</sup> Consequently, the occurrence of a fluorescence spectra change upon the recognized state is an important factor to evaluate the controllability of fluoroionophores in this research.<sup>3,4</sup> Many fluoroionophores based on control of the fluorescence quenching process, excimer or exciplex emission involving a conformational change have been developed for applications in various fields of biological, medical, and environmental analyses.<sup>1,5</sup> Fluoroionophores based on crown ether, azacrown ether, cryptand,<sup>1,6</sup> cyclodextrin<sup>7-9</sup> and calixarene<sup>10,11</sup> have been reported. These fluoroionophores were operated by energy transfer<sup>12</sup> and photoinduced electron transfer (PET)<sup>13-19</sup> between a donor and acceptor linked bond.

When a PET process is prevented by complexation, the fluorescence is highly enhanced (fluorescence “On” state). We provided effective control of fluorescence “Off-On” states in previous systems.<sup>20-25</sup> In earlier papers, we described several fluoroionophores based on linear polyethers having fluorescence molecules at their terminals.<sup>20-23,26-28</sup> These ionophores gave

fluorescence behavior from weak to strong emission upon metal ion complexation. Recently, we also reported a new mode of PET in fluorophore connecting *N*-phenylamide derivatives and its applications for chemosensors based on crown ether.<sup>24,25</sup> It became clear that these photo-excited molecules showed electron transfer (ET) from the *N*-phenyl moiety to fluorophores, resulting in the quenching of fluorescence emission, which were controllable by the breakdown of  $\pi$ -conjugation for PET by bending an amide bond, which results from metal ion complexation showing a fluorescence “Off-On” signal. In our previous study, spectral behaviors of fluoroionophores based on these ET actions were classified as follows. Group 1 anthracene, pyrene: this type ET *via* amide bond occurred when the fluorescent moiety and the donor were connected directly. Group 2 naphthalene, fluorene: this type showed ET over the CH<sub>2</sub> group between the fluorescent ring and the carbonyl group. One difference between group 1 and group 2 is a difference of the UV absorption maximum wavelengths of each fluorescent moiety (e.g., anthracene, 350 nm; naphthalene, 282 nm). Consequently, these ET characteristics will be classified according to whether the excitation wavelength is longer than 300 nm or not. To study our interpretation for ET *via* an amide bond, we chose phenanthrene as the next target molecule.

Lewis *et al.* reported that the quenching of intramolecular ET of *N*-alkyl-9-phenanthrenecarboxamide derivatives depends on the molecular structures, such as the *Z* and *E* conformer.<sup>29</sup> In addition, 9-phenanthrenecarboxamide involving *N*-(phenylmethylaminoalkyl) exist as a mixture of *Z* and *E* conformers in solution, and a pure *Z* isomer in which the *N*-(aminoalkyl) substituent is folded over the face of the amide group and the phenanthrene and aniline rings adopt an edge-to-face geometry in the solid state, as shown by X-ray

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crystallography.<sup>30</sup> Rima *et al.* reported that inclusion phenomena of  $\beta$ -cyclodextrin with 9-alkylphenanthrene derivatives was affected by the alkyl chain length using fluorescence spectroscopy.<sup>31</sup> Phenanthrene has also been taken on the dyes of organic electroluminescence devices,<sup>32</sup> and target species for a rapid screening using electrolytic methods in environmental samples.<sup>2</sup> However, the employment of phenanthrene as a fluoroionophore based on PET is rare. Phenanthrene is structurally similar to anthracene, although its photochemical properties resemble those of naphthalene.<sup>24,29</sup> Consequently, phenanthrene will show a naphthalene-like photochemical behavior, and thus it is classified into group 2. An introduction of CH<sub>2</sub> group between phenanthrene and the amido moiety will be needed to control its ET. Herein, we report on 4'-(9-phenanthreneacetamido)-benzo-15-crown-5 (**1**) and 3'-methyl-4'-(9-phenanthreneacetamido)-benzo-15-crown-5 (**2**) as a new PET system, which was tested for its capability of fluorescence "Off-On", using fluorescence, UV, and <sup>1</sup>H-NMR spectrometry.

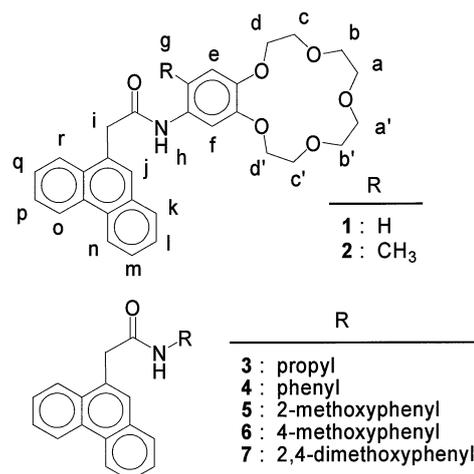
## Experimental

The synthetic pathway of 9-phenanthreneacetamide derivatives **1** and **2** is shown in Scheme S1 (Supporting Information). Actually, 9-phenanthrenecarboxylic acid (5.0 g, 0.025 mol), which was prepared according to the literature,<sup>33</sup> was suspended in THF. To this suspension, 1 g (1 eq. for 9-phenanthrenecarboxylic acid) of LiAlH<sub>4</sub> dissolved in 200 mL of THF was added dropwise at room temperature and stirred for 3 h. To this solution, distilled water was added dropwise. A crude product of 9-phenanthrenemethanol was obtained. This compound was dissolved in 50 mL of 1,4-dioxane and refluxed for 2 h with the addition to an excess amount of SOCl<sub>2</sub>. The solution was cooled to room temperature. The crude 9-chloromethylphenanthrene was precipitated through the addition of distilled water. The precipitate was collected and dried at room temperature. The precipitate and sodium cyanide (2.45 g) were dissolved in 50 mL of acetonitrile, and was heated at 70°C overnight. The reaction mixture was filtered, and 100 mL of water was added to the filtrate. The resulting precipitate 9-phenanthreneacetonitrile was filtered. The residue was suspended in 150 mL of acetic acid, and was carefully refluxed with addition to 50 mL of concentrated hydrochloric acid. Then, the reflux was continued for 5 h. The solution was cooled and precipitated by addition of water. The crude 9-phenanthreneacetic acid was dissolved in a 20% NaOH aqueous solution, and the precipitate was filtered off. Subsequently, this filtrate was added to 20 mL of concentrated hydrochloric acid. The resulting precipitate was collected by filtration (yields: 89% 5.2 g).

Subsequently, 4'-(9-phenanthreneacetamido)-benzo-15-crown-5 (**1**) was synthesized from 9-phenanthreneacetic acid (1.2 g 0.005 mol) and 4'-aminobenzo-15-crown-5 according to a previous paper.<sup>34</sup> An analogous compound **2** and model compounds **3** - **7** were also synthesized from 0.005 mol of respective amino compounds: 1.64 g of 3'-methyl-4'-aminobenzo-15-crown-5<sup>35-37</sup> (**2**), the 0.30 g of propylamine (**3**), the 0.47 g of aniline (**4**), the 0.66 g of 2-anisidine (**5**), the 0.66 g of 4-anisidine (**6**), and the 0.77 g of 2,4-dimethoxyaniline (**7**).

### 4'-(9-Phenanthreneacetamido)-benzo-15-crown-5 (**1**)

Yield 71%, white solid. m.p. 236°C. <sup>1</sup>H-NMR (acetonitrile-*d*<sub>3</sub>;  $\delta$  from TMS) 3.60 (C-CH<sub>2</sub>-O-, m, 8H), 3.73 (C-CH<sub>2</sub>-O-, m, 4H), 3.99 (C-CH<sub>2</sub>-O-, m, 4H), 4.15 (CO-CH<sub>2</sub>-phenanthrene-, s, 2H), 6.79 (aromatic, d, 1H), 6.96 (aromatic, dd, 1H), 7.21 (aromatic,



Scheme 1 Structures of **1**, **2**, and model compounds **3** - **7**. Alphabets on hydrogen correspond to the assignments for <sup>1</sup>H-NMR measurement.

s, 1H), 7.65 - 7.70 (aromatic, m, 4H), 7.80 (aromatic, s, 1H), 7.92 (aromatic, d 2H), 8.13 (aromatic, d 2H), 8.28 (CO-NH-, s 1H), 8.74 (aromatic, d 1H), 8.82 (aromatic, d 1H). Found: C, 71.76; H, 6.23; N, 2.67. Calcd. for C<sub>30</sub>H<sub>31</sub>NO<sub>6</sub>: C, 71.84; H, 6.23; N, 2.79.

### 3'-Methyl-4'-(9-phenanthreneacetamido)-benzo-15-crown-5 (**2**)

Yield 67%, white solid. m.p. 227 - 229°C. <sup>1</sup>H-NMR (acetonitrile-*d*<sub>3</sub>;  $\delta$  from TMS) 3.61 (C-CH<sub>2</sub>-O-, m, 8H), 3.74 (C-CH<sub>2</sub>-O-, m, 4H), 4.00 (C-CH<sub>2</sub>-O-, m, 4H), 4.18 (CO-CH<sub>2</sub>-aromatic-, s, 2H), 6.69 (aromatic, s, 1H), 7.00 (aromatic, s, 1H), 7.62 - 7.75 (aromatic, m, 5H), 7.83 (aromatic, s, 1H), 7.93 (aromatic, d 1H), 8.17 (aromatic, d 1H), 8.75 (aromatic, d, 1H), 8.82 (aromatic, d 1H). Found: C, 72.07; H, 6.48; N, 2.69. Calcd. for C<sub>31</sub>H<sub>33</sub>NO<sub>6</sub>: C, 72.21; H, 6.45; N, 2.72.

### N-Propyl-9-phenanthreneacetamide (**3**)

Yield 64%, light-yellow solid. m.p. 188 - 190°C. <sup>1</sup>H-NMR (chloroform-*d*<sub>1</sub>;  $\delta$  from TMS), 0.67 (-CH<sub>3</sub>, t, 3H), 1.30 (-CH<sub>2</sub>-, m, 2H), 3.10 (-N-CH<sub>2</sub>-, m, 2H), 4.07 (-phenanthrene-CH<sub>2</sub>-CO-, s, 2H), 5.40 (-CONH-, s, 1H), 7.65 (aromatic, m, 5H), 7.87 (aromatic, d, 1H), 8.03 (aromatic, d, 1H), 8.69 (aromatic, d, 1H), 8.74 (aromatic, d, 1H). Found: C, 82.11; H, 6.90; N, 5.05. Calcd. for C<sub>19</sub>H<sub>19</sub>NO: C, 82.28; H, 6.90; N, 5.05.

### N-Phenyl-9-phenanthreneacetamide (**4**)

Yield 73%, white solid. m.p. 235 - 236°C. <sup>1</sup>H-NMR (chloroform-*d*<sub>1</sub>;  $\delta$  from TMS), 4.24 (phenanthrene-CH<sub>2</sub>-CO, s, 1H), 7.04 (aromatic, m, 2H), 7.25 (aromatic, m, 5H), 7.69 (aromatic, m, 4H), 7.80 (-CONH-, s, 1H), 7.91 (aromatic, d, 1H), 8.10 (aromatic, d, 1H), 8.71 (aromatic, d, 1H), 8.77 (aromatic, d, 1H). Found: C, 84.90; H, 5.67; N, 4.53. Calcd. for C<sub>22</sub>H<sub>17</sub>NO: C, 84.86; H, 5.50; N, 4.50.

### N-(2-Methoxyphenyl)-9-phenanthreneacetamide (**5**)

Yield 67%, light-yellow solid. m.p. 166.5 - 169°C. <sup>1</sup>H-NMR (chloroform-*d*<sub>1</sub>;  $\delta$  from TMS), 3.36 (-OCH<sub>3</sub>, s, 3H), 4.25 (-phenanthrene-CH<sub>2</sub>-CO-, s, 2H), 6.66 (aromatic, d, 1H), 6.92 (aromatic, m, 2H), 7.68 (aromatic, m, 5H), 7.80 (-CONH-, s, 1H), 8.12 (aromatic, d, 1H), 8.29 (aromatic, d, 1H), 8.71 (aromatic, d, 1H), 8.76 (aromatic, d, 1H). Found: C, 81.06; H, 5.75; N, 4.15. Calcd. for C<sub>23</sub>H<sub>19</sub>NO: C, 80.92; H, 5.61; N, 4.10.

*N*-(4-Methoxyphenyl)-9-phenanthreneacetamide (**6**)

Yield 70%, white solid. m.p. 252 – 255°C. <sup>1</sup>H-NMR (chloroform-*d*<sub>1</sub>; δ from TMS), 3.72 (–OCH<sub>3</sub>, s, 3H), 4.22 (phenanthrene-CH<sub>2</sub>-CO, s, 2H), 6.74 (aromatic, d, 2H), 6.97 (aromatic, s, 1H), 7.17 (aromatic, d, 2H) 7.70 (aromatic, m, 4H), 7.79 (–CONH–, s, 1H), 7.91 (aromatic, d, 1H), 8.10 (aromatic, d, 1H), 8.71 (aromatic, d, 1H), 8.77 (aromatic, d, 1H). Found: C, 80.97; H, 5.74; N, 4.15. Calcd. for C<sub>23</sub>H<sub>19</sub>NO<sub>2</sub>: C, 80.92; H, 5.61; N, 4.10.

*N*-(2,4-Dimethoxyphenyl)-9-phenanthreneacetamide (**7**)

Yield 63%, white solid. m.p. 204 – 207°C. <sup>1</sup>H-NMR (chloroform-*d*<sub>1</sub>; δ from TMS), 3.34 (–OCH<sub>3</sub>, s, 3H), 3.71 (–OCH<sub>3</sub>, s, 3H), 4.21 (CO-CH<sub>2</sub>-phenanthrene–, s, 2H), 6.25 (aromatic, d, 1H), 6.39 (aromatic, dd, 1H), 7.64 (aromatic, m, 5H), 7.77 (–CO-NH–, s, 1H), 7.88 (aromatic, d, 1H), 8.11 (aromatic, d, 2H), 8.68 (aromatic, d, 2H), 8.74 (aromatic, d, 2H). Found: C, 77.51; H, 5.86; N, 3.71. Calcd. for C<sub>24</sub>H<sub>21</sub>NO<sub>3</sub>: C, 77.61; H, 5.70; N, 3.77.

## Measurement of fluorescence and UV spectra

Fluorescence spectra were measured using a spectrometer (RF-5300PC; Shimadzu Corp.) in a spectral-grade acetonitrile solution at 25°C. The excitation wavelength was set to 297 nm, unless described otherwise. Various alkaline earth metal cations were added to a solution of phenanthreneacetamido derivatives as perchlorate salts. The initial concentration of phenanthreneacetamido derivatives was 5 × 10<sup>−6</sup> mol/L. All fluorescence spectra were normalized by the absorbance at 297 nm among all compounds while comparing the fluorescence intensity. Because the fluorescence lifetimes of free and metal complexes were less than 10 ns, the sample solutions were used without degassing. The UV spectra were measured (UV-2400PC) under the same conditions as those used for fluorescence measurements.

Measurement of <sup>1</sup>H-NMR

The <sup>1</sup>H-NMR spectra were measured at 30°C (JNM-EX400; JEOL). The phenanthreneacetamido derivatives concentrations were 5 × 10<sup>−3</sup> mol/L in acetonitrile-*d*<sub>3</sub>. For measurements of alkaline earth metal complexes, excess amounts of metal cations as perchlorates were added to these solutions.

## Results and Discussion

Fluorescence properties of model compounds **3** – **7**

For comparing the electron transfer (ET) effects, a fluorescence study using model compounds **3** – **7** in Scheme 1 was conducted; it can be summarized as follows: 1) phenanthrene is an electron acceptor and the *N*-phenyl moiety worked as a donor when phenanthrene was excited; 2) an effective ET could be induced by higher electron donating groups.<sup>27,29</sup> Fluorescence spectra of **3** – **7** were measured in a 1 × 10<sup>−5</sup> mol/L acetonitrile solution at 25°C. The results are presented in Fig. 1. The fluorescence spectrum of model compound **3**, which is used as a reference, shows the original phenanthrene emission, because no ET was expected between the electron donor and the acceptor. The order of the fluorescence intensity in all model compounds was **3** > **4** > **5** > **6** >> **7**, which shows the efficiency of the ET process by corresponding to the electron-donating substituent. In a previous report, the fluorescence intensity of *N*-(2-methoxyphenyl) or (4-methoxyphenyl)-naphthaleneacetamide drastically decreased due to the resulting ET. However, the

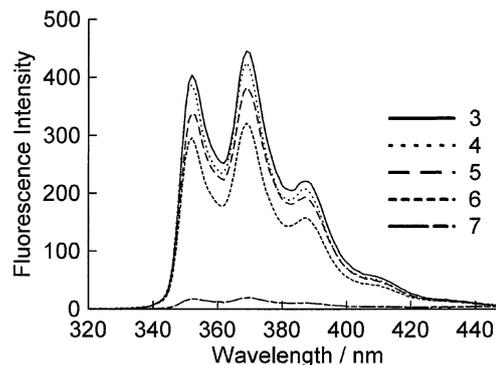


Fig. 1 Fluorescence spectra of model compounds **3** – **7**. Those intensities were corrected by their absorbances at 297 nm in acetonitrile at 25°C. The excitation wavelength was 297 nm. The concentrations of all model compounds were 1 × 10<sup>−5</sup> mol/L.

fluorescence spectra of **4** – **6** were similar to those of reference **3**. The present results suggest that an electron-donating ability of the phenyl group with one methoxy group to phenanthrene was insufficient to ET. The fluorescence intensity of **7**, which has two methoxy substituent at *ortho*- and *para*-positions in the benzene moiety, decreased to 95% for **5** and 94% for **6**, respectively. From that reason, to quench the fluorescence emission, this ET system consisting of 9-phenanthreneacetamide (PA) needs the addition of an electron-releasing substituent on a benzene ring in the electron donor. In consideration of this photochemical property, new fluoroinophores **1** and **2** involving a fluorescence “Off-On” ability were synthesized, and the photochemical properties were measured in the presence of various alkaline earth metal ions.

Fluorescence spectra of **1** and **2**

Figure 2 portrays the fluorescence spectra of **1**, and its Ca<sup>2+</sup> complex in acetonitrile. Fluorescence emissions from the phenanthrene moiety in the absence of Ca<sup>2+</sup> were weak compared to those of **3**. In contrast, dramatically enhanced emissions were observed in the presence of Ca<sup>2+</sup>. The shapes of these spectra indicate that they are phenanthrene monomer emissions, whose maximum wavelength is *ca.* 370 nm. A similar fluorescence enhancement behavior was also observed in the other alkaline earth metal ions. These “Off-On” behaviors were expressed quantitatively as a fluorescence intensity ratio,  $I_{\max}/I_0$ , where  $I_{\max}$  and  $I_0$  are the fluorescence intensities in the presence ( $I_{\max}$ ) and absence ( $I_0$ ) of excess amounts of alkaline earth metal ions. These data are presented in Table 1. A more improved  $I_{\max}/I_0$  value for **2** (16.6) than that of **1** (4.8) for Ca<sup>2+</sup> was obtained, suggesting that the electron-donating methyl group in benzocrown moiety can control the fluorescence “Off-On” behavior.

UV-vis and excitation spectra of **1** and **2**

Figure 3 shows UV spectra of **2** with and without Ca<sup>2+</sup>. Whether Ca<sup>2+</sup> was present or not, the absorbance and shape of the spectrum between 254 and 297 nm corresponding to the phenanthrene moiety is hardly changed. The UV spectral change of **1** (see also Fig. S1) with Ca<sup>2+</sup> resembled that of **2**. These results show that **1** and **2** did not have a charge-transfer interaction based on a large conformational change by complexation with Ca<sup>2+</sup> in the ground state, and a quenching process took place *via* electron transfer.

Figure S2 shows fluorescence excitation spectra of **2** and its

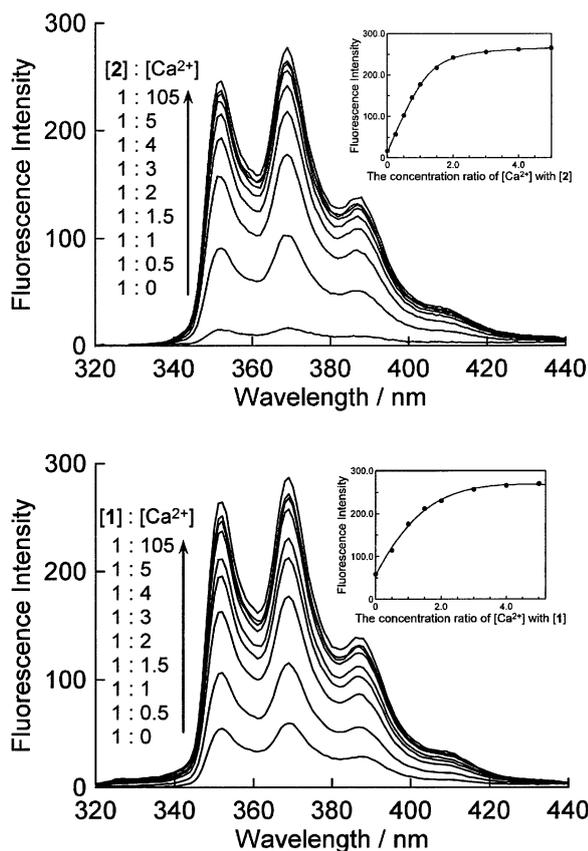


Fig. 2 Fluorescence spectra of **1** and its  $\text{Ca}^{2+}$  complex (lower), and fluorescence spectra of **2** and its  $\text{Ca}^{2+}$  complex (upper); they were corrected by the absorbance at 297 nm, in acetonitrile at 25°C. Excitation wavelength, 297 nm. [**1**] and [**2**] =  $5 \times 10^{-6}$  mol/L. The stoichiometric ratios with  $\text{Ca}^{2+}$  are listed respectively in columns. Insets: dependence of fluorescence intensity at 369 nm on the concentration of  $\text{Ca}^{2+}$ , and theoretical curve of stoichiometric 1:1 complex.

$\text{Ca}^{2+}$  complex. After the addition of  $\text{Ca}^{2+}$ , the response of the phenanthrene ring at 297 nm is notably increased without a spectral shift; it is similar to the UV-vis spectra of **2**. These results support the interpretation obtained from UV measurements.

#### <sup>1</sup>H-NMR spectra of **2**

The fluorescence “Off-On” ability of **2** was enhanced by introduction of a methyl group to **1**. The <sup>1</sup>H-NMR spectra of **2** in the absence and presence of various alkaline earth metal ions were measured to analyze the conformational effect concerning the fluorescence behavior of **2** in acetonitrile-*d*<sub>3</sub>. Respective peaks were assigned by <sup>1</sup>H-<sup>1</sup>H COSY and NOESY 2D spectra; their chemical shifts are presented in Table 1 (alphabets on respective peaks correspond to hydrogens in Scheme 1). Spectral changes upon  $\text{Ca}^{2+}$  are presented in Fig. S3 as the representative result. Remarkable chemical shift changes ( $\Delta\delta$  ppm) of **2** upon complexation with  $\text{Ca}^{2+}$  were observed as follows: 3.61 – 3.90 ( $\Delta\delta = 0.29$ , a, b in Fig. S3), 3.74 – 3.97 ( $\Delta\delta = 0.23$ , c), and 4.00 – 4.30 ( $\Delta\delta = 0.30$ , d) for crown ether moiety, 6.69 – 6.98 ( $\Delta\delta = 0.29$ , e) and 7.01 – 7.32 ( $\Delta\delta = 0.31$ , f) for benzene moiety, and *ca.* 7.7 – 8.1 (*ca.*  $\Delta\delta = 0.4$ , h) for amide proton. These changes were attributed to a coordinating moiety interaction with positive ions, suggesting that the  $\text{Ca}^{2+}$  complex was formed cooperatively with the benzocrown moiety and a carbonyl group. This complexation can restrict molecular motion around the amide group and a benzene ring. No protons

Table 1 Main chemical shifts ( $\delta$  ppm) for **2** and their changes ( $\Delta\delta$  ppm) upon complexation with various alkaline earth metal ions<sup>a</sup>

	a, a'	b, b'	c, c'	d	d'	e	f	g <sup>b</sup>	h <sup>b</sup>	i
None	3.61	3.61	3.74	4.00	3.97	6.69	7.01	1.9	7.7	4.18
$\text{Mg}^{2+}$	0.35	0.35	0.39 <sup>b</sup>	0.39 <sup>b</sup>	0.42 <sup>b</sup>	0.11 <sup>b</sup>	0.0 <sup>b</sup>	0.1	— <sup>c</sup>	0.14 <sup>b</sup>
$\text{Ca}^{2+}$	0.29	0.29	0.23	0.30	0.33	0.29	0.31	0.1	0.4	0.12
$\text{Sr}^{2+}$	0.24	0.24	0.18	0.27 <sup>b</sup>	0.30 <sup>b</sup>	0.25	0.27	0.0	0.3	0.09
$\text{Ba}^{2+}$	0.21	0.21	0.08 <sup>b</sup>	0.22 <sup>b</sup>	0.25 <sup>b</sup>	0.22	0.24	0.0	0.2	0.04 <sup>b</sup>

a. Alphabets on respective peaks correspond to hydrogens in Scheme 1. Positive values show lower field shifts. The values in “none” denote chemical shifts ( $\delta$  ppm from TMS) of protons of **2** in acetonitrile-*d*<sub>3</sub> at 30°C.

b. This value includes uncertainty because of overlapping with the peak with acetonitrile, water, or other peaks.

c. This value was not assigned because of peak overlapping or broadening.

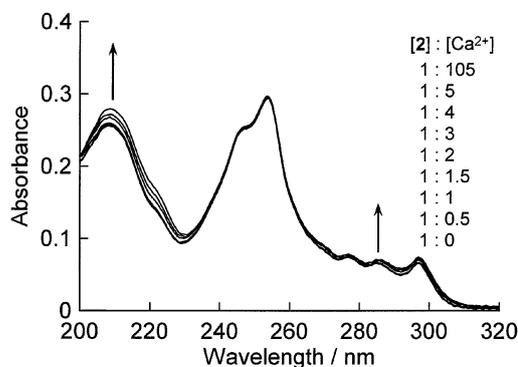


Fig. 3 UV spectra **2**, and their  $\text{Ca}^{2+}$  complexes in acetonitrile at 25°C. [**2**] =  $5 \times 10^{-6}$  mol/L. Stoichiometric ratios of metal ion to those ligand are listed in this column.

in the phenanthrene ring indicated a considerable chemical shift change upon complexation with any alkaline earth metal ions. This result demonstrates that these protons did not receive an electron or ring-current effect upon complexation (see Fig. 4). When  $\text{Mg}^{2+}$  and  $\text{Ba}^{2+}$  were added, detailed <sup>1</sup>H-NMR spectra arrangements of the complex with these ions could not be obtained because of the complicated and poorly resolved spectra. It is assumed that these phenomena resulted from differences of the complexation behaviors based on the size of the crown ether ring and an alkaline earth metal ion radius.

#### Complex formation constants

The complex formation constant (*K*), which is useful to estimate the binding affinities of the crown ether moiety with various alkaline earth metal ions, is defined as

$$K = \frac{[\text{ML}]}{[\text{M}][\text{L}]}$$

Therein, [M], [L], and [ML] signify the concentrations of the alkaline earth metal ion, crown ether, and complex, respectively. Then, the values of log *K* were determined using a curve-fitting method of a nonlinear least-squares curve fitting (Marquardt's method<sup>38</sup>). The fluorescence intensities of **1** and **2** at 369 nm against the metal to ligand ratio are shown in Fig. 3. These plots show that **1** and **2** formed a complex with alkaline earth metal

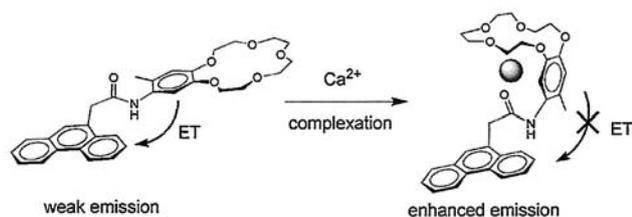


Fig. 4 Schematic representation of complexation with  $\text{Ca}^{2+}$  involving a conformational change.

Table 2 Complex formation constants ( $\log K$ )<sup>a</sup> and fluorescence responses ( $I_{\text{max}}/I_0$ ) of **1** and **2** for various metal ions in acetonitrile at 25°C

		$\text{Mg}^{2+}$	$\text{Ca}^{2+}$	$\text{Sr}^{2+}$	$\text{Ba}^{2+}$
<b>1</b>	$I_{\text{max}}/I_0$	4.36	4.76	4.27	4.66
	$\log K$	6.30	6.36	5.82	5.74
<b>2</b>	$I_{\text{max}}/I_0$	15.50	16.60	13.20	13.60
	$\log K$	6.58	6.29	5.68	5.30

a.  $K = [\text{ML}]/([\text{M}][\text{L}])$ .

ion corresponding to 1:1 stoichiometry. Consequently, the obtained complex formation constants for alkaline earth metal ions are presented in Table 2. In our previous study, all values of  $\log K$  showed a similarity to crown ether compounds.<sup>28</sup> Consequently, all guest species were suitable for fluorescence “Off-On” controlling because of a high response to the benzo-15-crown-5 moiety.

## Conclusion

Using new fluoroionophores **1** and **2**, a fluorescence “Off-On” intramolecular charge-transfer system was produced. The photochemical response ( $I_{\text{max}}/I_0$ ) of **2**, which has an electron-donating substituent on **1**, was increased compared to **1**. This indicates that the introduction of a methyl group that has an electron-donating character at the 3'-position of the benzocrown ring induced an effective electron transfer (ET) action. On the other hand, a complex formation constant,  $\log K$ , and the conformations of the complex were similar to those of previous fluoroionophores. Therefore, the fluorescence “Off-On” ability was improved without disturbing the complexation with crown ether. These results indicate that the introduction of a suitable electron-donating group to benzocrown ethers makes a new or improved fluoroionophore available in various chemical situations. This study will be expanded to include possibilities for the development of a new optical analysis method and chemical signal devices for biological materials *in vivo* and *in vitro*.

## Supporting Information

The synthetic pathway, excitation spectra, and <sup>1</sup>H-NMR spectra of **1** and **2**. This material is available free of charge on the Web at <http://www.jsac.or.jp/analsci/>.

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