JOC Note

Two-Photon Sensor for Metal Ions Derived from Azacrown Ether

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Abstract: A two-photon sensor for the metal ions derived from azacrown ether as the receptor is reported. The sensor emits strong two-photon fluorescence when excited by 800 nm laser photons. Moreover, the binding constants measured by the one- and two-photon fluorescence are similar. This result may be useful for the design of efficient two-photon fluorescence probes for biological substrates.

Organic materials exhibiting large two-photon absorption (TPA) behavior have drawn much interest because they can be excited upon irradiation of low energy photons with sufficiently high intensity.[1−5] Many applications of such materials have become reality, some of which include optical power limiting,[6−8] two-photon upconversion lasing,[9,10] two-photon fluorescence excitation microscopy,[11−15] three-dimensional optical data storage,[16−18] and photodynamic therapy.[19]

An important addition to such applications would be the development of two-photon sensors for biological applications. Currently, much research is being conducted to develop one-photon fluorescence (OPF) probes because they are useful tools for clarifying the functions in biological systems.[20] One of the major drawbacks of such sensors is that the excitation wavelengths are in the range of 350−560 nm, which may cause damage to the substrates.[21] The problem could be avoided if one develops two-photon sensors, which allow visualization of ions, small molecules, or enzyme activity in living cells by employing two-photon-induced fluorescence (TPF) microscopy that uses NIR photons as the excitation source.[11−15] More importantly, the two-photon process would make it possible to determine the three-dimensional distribution of the guest molecules in the living cells in high resolution. Recently, there has been much progress in the two-photon imaging of biological substrates by using two-photon confocal laser scanning microscopy (2PCLSM).[22]

A useful TPF sensor must possess a fluorophore with large TPA cross section (δ) and a receptor for the guest molecules or ions. The wavelength of the maximum two-photon absorption ($\lambda_{\max}^{(2)}$) for such fluorophore should be close to 800 nm, because most of the TPF microscopy experiments use an 800 nm laser beam. Recently, a variety of bis(styryl)benzene derivatives has been shown to exhibit large a two-photon cross section near 800 nm.[2] As the first step toward developing a two-photon sensor

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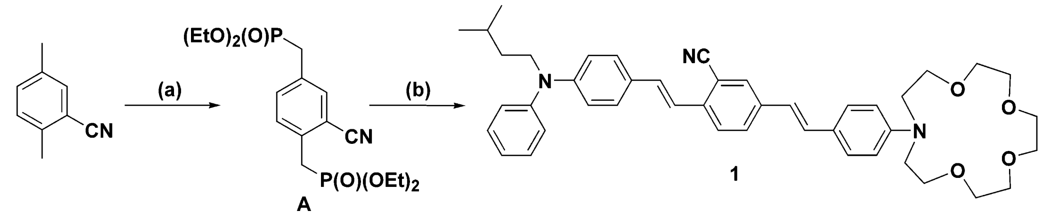
SCHEME 1[a]



[a] Reagent and conditions: (a) (i) NBS/CCl$_4$, 80 °C, 2 h (61%); (ii) P(OEt)$_3$/toluene, 120 °C, 5 h (98%); (b) (i) LDA (1 equiv)/THF, *p*-(*N*-isoamyl-*N*-phenyl)aminobenzaldehyde, 6 h (62%); (ii) LDA (1 equiv)/THF, *N*-(*p*-formylphenyl)aza-15-crown-5, 45 °C, 6 h (72%).

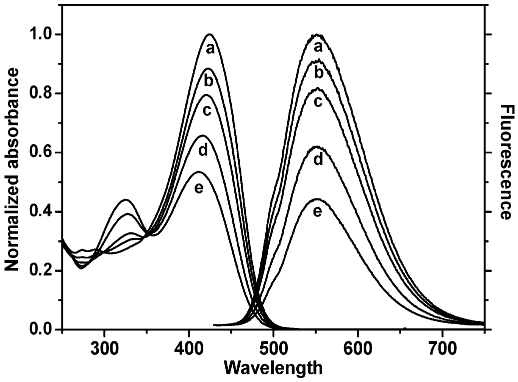


FIGURE 1. Absorption and emission spectra of **1** in MeCN (excitation at 425 nm) in the presence of (a) 0, (b) 1, (c) 3, (d) 12, or (e) 45 equiv of Ca(ClO$_4$)$_2$·4H$_2$O predissolved in MeCN (0.01 M).

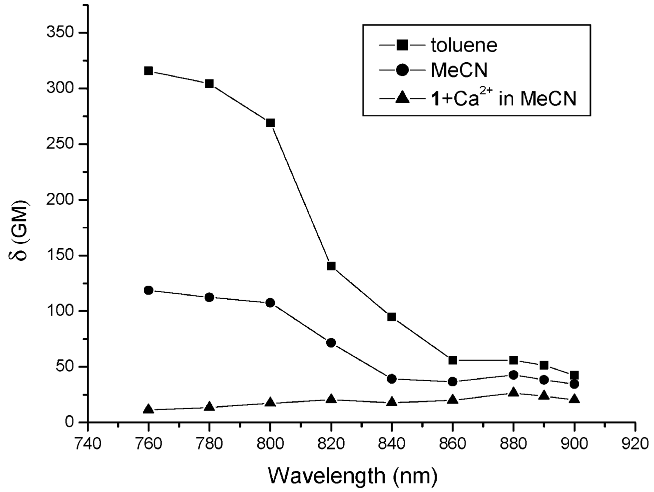


FIGURE 2. Two-photon excitation spectra of **1** in toluene or MeCN and in the presence of 45 equiv of Ca^{2+}.

for metal ions, we have synthesized **1** with 1-cyano-2,5-bis(styryl)benzene as the fluorophore and aza-15-crown-5 as the receptor. We now report that **1** is an effective TPF sensor for the metal ions.

Synthesis of the sensor **1** is shown in Scheme 1. Bromination of 2-cyano-1,4-dimethylbenzene followed by phosphorylation produced **A** in high yield. Compound **1** was obtained in modest yield by successive condensation of **A** with *p*-(*N*-isoamyl-*N*-phenyl)aminobenzaldehyde and *N*-(*p*-formylphenyl)aza-15-crown-5.

Compound **1** is strongly fluorescent in MeCN ($\Phi = 0.47$). Addition of Ca^{2+} to the solution of **1** in MeCN induces a blue shift in the absorption spectra and a gradual decrease in the absorption and fluorescence intensity with a slight decrease in the fluorescent quantum yield (Figure 1).

This may be due to the fact that complexation by a metal ion reduces the donor character of the nitrogen atom in the crown ether to induce the spectra changes. The result of a Job's plot and the appearance of an isosbestic point at 355 nm indicate the formation of 1:1 complex (Figure S1). The binding constant, expressed as log *K*, estimated with the extent of fluorescence quenching decreases in the order Ca^{2+} (4.26 ± 0.06) > Ba^{2+} (3.65 ± 0.02) > Mg^{2+} (3.32 ± 0.03) > Na^{+} (3.24 ± 0.16) > K^{+} (2.40 ± 0.50) (Figures S2 and S4). Similar results were previously reported.[23]

The two-photon cross section (δ) of **1** was determined by using the two-photon-induced fluorescence measurement technique.[2a,5a] Figure 2 shows that the two-photon cross section of **1** in toluene is 320 GM at 740 nm. The value of δ for **1** is slightly larger than 260 GM ($\lambda^{(2)}_{\max} = 740$ nm) reported for 1-cyano-2,5-bis(diethylaminostyryl)benzene.[2c] This may be due to the presence of the diphenylamino group as the donor and/or the contribution of the excited-state excitation in the nanosecond experiment. Moreover, δ also decreases by the change in the solvent from toluene to MeCN. The origin of this solvent effect is not clear at present. Nevertheless, the intensity of the two-photon fluorescence was strong enough for the measurement. When excess Ca^{2+} was added, δ decreased even further, probably because the electron-donating ability of the azacrown moiety is attenuated upon complexation (Figure 2). Hence it is possible to measure the metal ion concentration from the decrease in the TPF upon addition of metal ions.

As expected, the TPF intensity decreased gradually upon addition of metal ions (Figures S2 and S3). Moreover, the binding constants calculated with the TPF are similar to those measured by OPF, i.e., Ca^{2+} (4.34 ± 0.08) > Ba^{2+} (3.73 ± 0.04) > Mg^{2+} (3.57 ± 0.05) > Na^{+} (3.20 ± 0.30) > K^{+} (2.10 ± 0.70). The larger standard deviation for the two-photon process compared to that of the one-photon process underlines the experimental difficulties associated with measuring the weaker spectral intensities (Figures S2 and S3). On the other hand, the extent of TPF quenching by the metal ions is slightly larger than that of the one-photon process (Figure 3). Also, the two-photon process shows slightly larger selectivity for Ca^{2+}. Nevertheless, the trends observed in both experiments are more or less the same. These results seem to indicate

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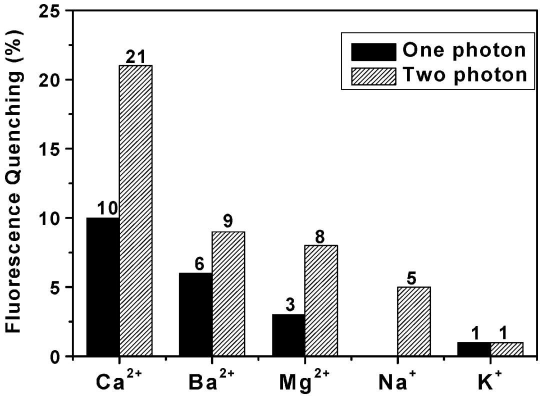

FIGURE 3. A comparison of the percent quenching of one- and two-photon excited fluorescence of **1** in MeCN in the presence of 1 equiv of metal ions. The excitation wavelengths for one- and two-photon processes are 425 and 800 nm, respectively.

that similar mechanisms are operating in both one- and two-photon sensors. Furthermore, this result suggests an interesting possibility that efficient TPF probes for biological substrates could be designed by using the same strategy developed for the synthesis of one-photon sensors by using an appropriate TPA chromophore.

In conclusion, we have synthesized a two-photon sensor for the metal ions by using bis-styrylbenzene and aza-crown ether as the fluorophore and receptor, respectively. The sensor emits strong two-photon fluorescence when excited by 800 nm laser photons. Moreover, the fluorescence is quenched upon complexation with metal ions, and the binding constants measured by the one- and two-photon fluorescence are similar. This result provides a useful design strategy for the synthesis of the two-photon sensors for biological applications.

Experimental Section

Synthesis. 2-Cyano-1,4-bis(bromomethyl)benzene. To a solution containing 2-cyano-1,4-dimethylbenzene (10.0 g, 76.2 mmol) in CCl_4 (300 mL) were added NBS (30.0 g, 168 mmol) and benzoyl peroxide (0.40 g, 1.7 mmol). The mixture was vigorously stirred under reflux for 2 h. After the usual workup, the product was purified using flash column chromatography on silica gel using hexane/ethyl acetate = 10:1 as the eluent. Yield: 13 g (61%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.69 (d, 1H, J = 3.0 Hz), 7.60 (dd, 1H, J = 3.0, 9.0 Hz), 7.53 (d, 1H, J = 9.0 Hz), 4.62 (s, 2H), 4.46 (s, 2H).

2-Cyano-1,4-bis[(diethylphosphoryl]methyl]benzene (A). A solution of 2-cyano-1,4-α,α′-dibromomethylbenzene (3.70 g, 12.8 mmol) and $P(OEt)_3$ (10.6 g, 64.0 mmol) in toluene were heated to 120 °C for 5 h. Excess $P(OEt)_3$ was removed in vacuo, and the product was extracted with ethyl acetate. After drying of the solution with $MgSO_4$, the solvent was evaporated to obtain slightly yellow oil. Yield: 5.0 g (98%). ^{1}HNMR (300 MHz, $CDCl_3$): δ 7.58 (s, 1H), 7.50 (s, 2H), 4.08 (m, 8H), 3.34 (d, 2H, J = 9.0 Hz), 3.10 (d, 2H, J = 9.0 Hz), 1.27 (m, 12H).

2-Cyano-4-(diethylphosphoryl)methyl-4′-[(*N*-isoamyl-*N*-phenylamino)stilbene. LDA (1.5 M in *c*-Hex, 3.3 mL) was slowly added to a solution containing **A** (2.0 g, 4.9 mmol) in THF (50 mL) at −78 °C under Ar. To this solution was added *p*-(*N*-isoamyl-*N*-phenyl)aminobenzaldehyde (1.2 g, 4.5 mmol) in THF (20 mL), and the mixture was stirred for 6 h. The reaction was quenched with water, and the product was extracted with ethyl acetate. The organic layer was dried with $MgSO_4$, and the solvent was removed in vacuo. The product was purified by column chromatography using ethyl acetate as the eluent. Yield: 1.6 g (62%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.70 (d, 1H, J = 9.0 Hz), 7.50 (m, 2H), 7.41 (d, 2H, J = 9 Hz), 7.32 (t, 2H, J = 9 Hz), 7.20 (d, 2H, J = 9 Hz), 7.12 (m, 3H), 6.68 (d, 2H, J = 9 Hz), 4.06 (m, 4H), 3.74 (t, 2H, J = 8 Hz), 3.10 (d, 2H, J = 21 Hz), 1.57 (t, 3H, J = 8 Hz),1.28 (t, 6H, J = 6 Hz), 0.93 (d, 6H, J = 6 Hz).

Compound 1. Synthesized by the same procedure as above. The residue was purified by column chromatography using ethyl acetate/hexane = 2:1 as the eluent. Yield: 0.60 g (72%). Mp: 52 °C. IR: 2223 (CN). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.69 (d, 1H, J = 9 Hz), 7.65 (s, 1H), 7.59 (d, 1H, J = 9 Hz), 7.41 (d, 2H, J = 9 Hz), 7.36 (d, 2H, J = 9 Hz), 7.34 (d, 1H, J = 18 Hz), 7.31 (s, 1H), 7.20 (d, 2H, J = 9 Hz), 7.12 (d, 2H, J = 9 Hz), 7.09 (d, 1H, J = 18 Hz), 7.02 (d, 1H, J = 18 Hz), 6.84 (d, 2H, J = 9 Hz), 6.77 (d, 1H J = 18 Hz), 6.64 (d, 2H J = 9 Hz), 3.76 (t, 2H, J = 6 Hz), 3.64 (m, 20H), 1.60 (m, 3H), 0.93 (d, 6H, J = 6 Hz). Anal. Calcd for $C_{44}H_{51}N_3O_4$: C, 77.05; H, 7.49; N, 6.13. Found: C, 77.02; H, 7.46; N, 6.15.

General Procedure for Spectroscopic Studies. The fluorescence quantum yield of **1** was determined in MeCN by using fluorescein ($\Phi = 0.90$) as the reference.[24] It decreased from 0.47 to 0.45 upon addition of excess amount of Ca^{2+}. The TPA cross sections of **1** (5.0 μM) in toluene and MeCN both in the presence and absence of Ca^{2+} were measured by the two-photon-induced fluorescence method by using fluorescein (80 μM, pH = 11) as the reference.[2a,5a] One photon absorption and one- and two-photon fluorescence titration experiments were performed by adding various amounts of metal ions to a solution containing 5.0 μM of **1** in MeCN (Figures S2−S4). The binding constant, log K, for each metal ion was calculated from the change in the spectral intensities by using the Excel program as reported.[25]

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Supporting Information Available: Job's plot, one-photon absorption and emission, and two-photon fluorescence spectra of **1** in the presence of Ba^{2+}, Mg^{2+}, Na^{+}, and K^{+}, and plots of A_0/A of one- and two-photon fluorescence vs the molar equivalent of metal ions. This material is available free of charge via the Internet at http://pubs.acs.org.

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