

A Butterfly-Shaped Pyrene Derivative of Cholesterol and Its Uses as a Fluorescent Probe

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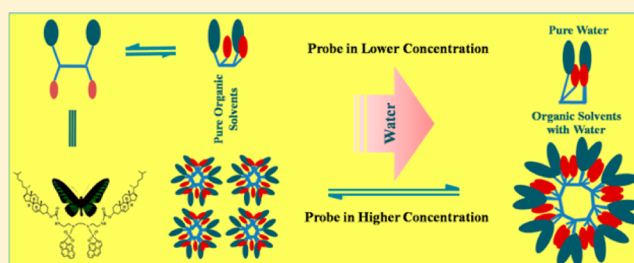
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S Supporting Information

ABSTRACT: A butterfly-shaped pyrene derivative of cholesterol, namely, *N,N'*-(ethane-1,2-diyl)-bis(*N*-(2-(chol-amino)-ethyl)pyrene-1-sulfonamide) (ECPS), has been designed and synthesized. Solvent effect studies revealed that in good solvents such as *n*-hexane, benzene, and 1,4-dioxane, the profile of the fluorescence emission of the compound is characterized by pyrene monomer emission, but in poor solvent such as water, the emission is dominated by pyrene excimer emission. Quantitatively speaking, the ratio of the excimer emission to monomer emission changes from 50 to 0

when ECPS is dissolved in water and *n*-hexane, respectively. In contrast, for a commonly used polarity probe pyrene, the ratio of I_3/I_1 varies only from ~ 0.6 to ~ 1.7 , where I_3 and I_1 stand for the intensities of the fluorescence emission at peak 3 and peak 1, respectively. This value suggests that a more powerful discriminating ability of the new compound in polarity sensing. Furthermore, unlike the main components of the compound, pyrene and cholesterol, its main chain is composed of multiple hydrophilic structures, and it is this structure that makes the emission of the compound in organic solvents sensitive to the presence of water. Accordingly, the applicability of the compound in determination of the trace amount of water in some organic solvents was evaluated. As expected, the detection limit of the compound toward water in acetonitrile reaches 7 ppm, a result never reached before. Furthermore, the fluorescence emission of the compound is also sensitive to viscosity variation. Therefore, it is assumed that ECPS may be used both as a polarity probe and a viscosity probe. On the bases of a series of steady-state and time-resolved fluorescence, as well as dynamic light scattering studies, a structural model was proposed to rationalize the fluorescence behavior of the compound in different solvents and its polarity and viscosity probing performances.



1. INTRODUCTION

Superpure organic solvents are widely used in the production of electronic and photo-electronic products and micro/nano-devices.^{1,2} One of the key procedures in the purification of organic solvents is dehydration, for which fast and accurately monitoring water content in the solvents is of great importance. Karl Fisher titration is the most widely adopted method for quantitative measurement of water in the solvents.^{3,4} Although this approach possesses several advantages and is fully automated nowadays, some disadvantages such as the use of toxic and malodorous reagents, interference from other coexisting species, consumption of substantial amount of sample solvents in the case of very low water concentration (<10 ppm), and limited sensitivity narrow its real-life applications. It is because of these reasons that development of new approaches for the measurement has never stopped during the past few decades. Continuous exploration upon IR spectroscopy,^{5,6} potentiometry,⁷ solid-phase extraction,⁸ Raman spectroscopy,⁹ holographic method,¹⁰ flow-injection,¹¹ absorbance-based film sensors,^{12,13} and the recently reported approach of cathodic stripping voltammetry at a gold

electrode¹⁴ has existed. These methods, however, are not perfect, and they also suffer from their own limitations: requirement of sophisticated equipment and limited application toward specific solvents. Of all analytical methods reported, optical methods, in particular fluorescence methods, have drawn great attention owing to their high sensitivity and reasonable selectivity.^{15–19} During the past 10–20 years, people have tried to create reusable fluorescent film sensors for water detection in organic solvents. However, these efforts have not been very successful. For example, Chang et al. reported a fluorescent film sensor for water detection, which had been fabricated by sandwiching a solvatochromic osmium complex, $[\text{Os}(\text{dppz})(\text{dppe})_2](\text{PF}_6)_2$, onto an ion-exchange resin between a thin sol–gel layer and a glass substrate.²⁰ It was reported that this sensor is insensitive to signal drift resulting from leaching and photobleaching of indicator dye, variations of light source intensity, and stability of the

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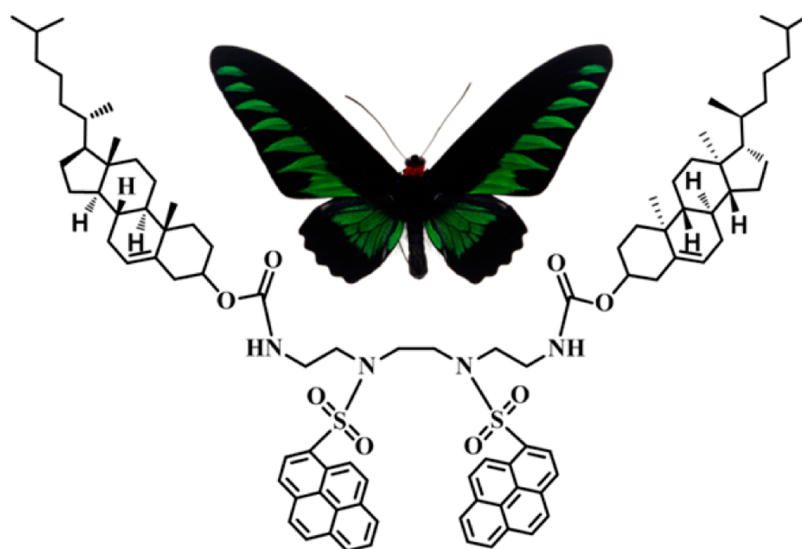


Figure 1. Molecular structure of the compound (ECPS) as synthesized.

photodetector because the measurement was conducted by measuring variation in the lifetime of the film. While simple and interesting, this sensor is subjected to low sensitivity. For example, when ethyl acetate was used as solvent, the detection limit was only 0.02% (v/v) of water. Similarly, Glenn and co-workers introduced an optical fiber sensor for the measurement of water content in a variety of organic solvents. The sensor was based on the luminescence lifetime quenching of ruthenium-(II)bisphenanthroline-di-pyridophenazine (Rudppz), which was mechanically attached to the distal tip of an optical fiber by immobilizing in a Nafion membrane.²¹ This sensor is useful for measuring the water content of organic solvents below 4% (v/v), and the detection limit was determined to be 0.06, 0.07, and 0.006% (v/v) in dimethyl sulfoxide (DMSO), ethanol, and acetonitrile, respectively. However, the sensor is only stable for four days and needs calibration before each use. Recently, Niu's group reported two fluorescent film sensors for water determination in organic solvents.^{22,23} The sensors as reported are pH-insensitive, show short response time, satisfactory reproducibility, and reversibility. However, as other film sensors, the preparation process is time-consuming. Furthermore, their conservation and activation require special skills.

Compared to sensors in the solid state, homogeneous fluorescent sensors for water detection are more popular and more widely studied due to their convenience in using. For example, Li and co-workers developed a fluorescent active anionic cluster $\text{Pb}_4\text{B}_{11}^{3-}$, and utilized it for the determination of a microamount of water in aprotic solvents.²⁴ Suzuki et al. developed three fluorescent active acridinyl derivatives for sensitive detection of water in organic solvents.²⁵ Recently, Ooyama and co-workers obtained a number of photoinduced electron transfer (PET)-based fluorescent sensors for proton and water detection.^{26–29} However, due to the complexity and diversity of organic mediums, no method or probe is versatile to use, not to mention satisfactory in the requirement of sensitivity and selectivity during the measurement, and thereby development of the sensors for water detection with superior performances still remains as a challenge.

In the development of fluorescent water sensors, one of the key designs is to select a suitable fluorophore, which should possess superior photophysical properties including, at least,

high quantum yield, and big change in its fluorescence emission, profile or intensity, or both, upon interacting with water. In addition, another key issue might be the introduction of a specific structure, which can selectively recognize and even enrich water from the medium under study. Cholesterol (Chol) is a commonly found versatile building block for creating molecular materials, such as low-molecular mass gelators (LMMGs)-based molecular gels.^{30–32} Pyrene (Py) is famous due to the sensitivity of its emission to the change of the polarity of its microenvironment. For this reason, Py has been widely used as a polarity probe, the so-called pyrene scale.³³ Considering that the polarity of water is much higher than that of commonly found organic solvents, selection of a polarity-sensitive fluorophore as a reporter of the fluorescent water sensor to be developed should be a wise choice. However, pure Py cannot be used for such studies due to lack of water binding structures in organic solvent, and thereby another design should be considered. It is known that Py has a strong tendency to form excimer, which is also polarity dependent.³⁴ Accordingly, introduction of two or more Py moieties into a probing molecule with water binding structures may produce novel fluorophores with superior sensitivity to the presence of water in organic solvents. To enhance the solubility of the designed fluorescent probe in organic medium, and its aggregation in measurement, Chol was specifically introduced. It was expected that the combination of the two moieties would provide opportunities for creating compounds with adjustable self-assembling and fluorescent properties.

Based upon the afore-described idea, polyamine was chosen as the water binding structure, upon which Py, the so-called reporting unit, and Chol, the auxiliary unit, were affixed in a specific manner. In this way, a butterfly shaped Py derivative of Chol was developed (c.f. Figure 1), and utilized for probing trace amount of water in some organic solvents and viscosity changes in a model system. This paper reports the details.

2. EXPERIMENTAL SECTION

2.1. Reagents and Materials. Pyrenesulfonyl chloride (PSC) was synthesized by adopting a literature method.³⁵ Triethylenetetramine (TETA, Alfa, >98.0%), tert-butyl phenyl carbonate (Sigma-Aldrich, >98.0%), and cholesteryl chlor-

oformate (Sigma, >98.0%) were used directly without further purification. All organic solvents were of analytical grade and used as received or dried to eliminate any water residue before the experiment. Except where specified otherwise, all other reagents were of analytical grade and used without further purification or treatment. Doubly distilled water was used throughout.

2.2. Measurements. Steady-state fluorescence measurements were performed using a time-correlated single photon counting fluorescence spectrometer (Edinburgh Instruments FLS920). Low temperatures were achieved with the use of nitrogen-flow cryostat (Oxford OptistatDN). Temperature was controlled with a precision of 0.1 K with an Oxford ITC503 temperature controller. The emission spectra were recorded with an Edinburgh FLS920 spectrometer, using an excitation wavelength of 350 nm. The ^1H NMR spectra of the samples were obtained on a Bruker Avance NMR spectrometer. Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectra were recorded in a Microflex mass analyzer (Bruker, USA) by using α -cyano-4-hydroxycinnamic acid (CCA) as the MALDI matrixes. Dynamic Light Scattering (DLS) measurements for aggregate diameters were determined by a BI-90Plus laser particle size analyzer (Brukerhaven Instrument, USA) equipped with a 15 mW solid-state laser (659 nm).

3. RESULTS AND DISCUSSION

3.1. Aggregation of ECPS in Different Solvents. The fluorescence emission spectra of the compound in a variety of solvents including water, methanol, acetonitrile, ethanol, acetone, dichloromethane, tetrahydrofuran (THF), 1,4-dioxane, benzene, and *n*-hexane, of which the polarities are different from each other, were recorded at a concentration of 1×10^{-6} mol/L (inset of Figure 2). With reference to the emissions, it

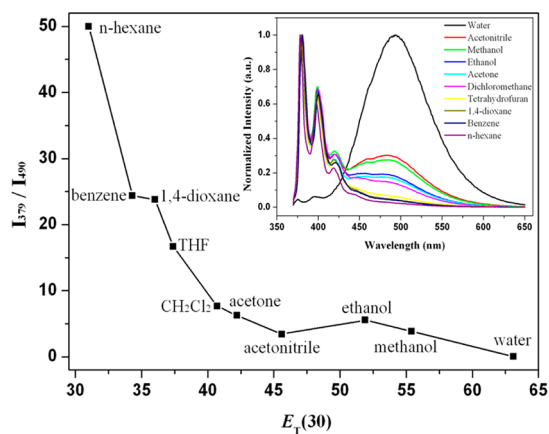


Figure 2. Plot of I_{399}/I_{490} of the fluorophore recorded in different solvents against $E_T(30)$ of the solvents. Inset is the normalized fluorescence emission spectra of ECPS in various solvents (1×10^{-6} mol/L, $\lambda_{\text{ex}} = 350$ nm).

reveals that the profile of the fluorophore is highly dependent upon the polarities of the solvents. Further inspection of the emissions reveals that excimer emission only appears at solvents of higher polarities such as water, methanol, acetonitrile, ethanol, acetone, and dichloromethane, whereas, for those of lower polarities, such as THF, 1,4-dioxane, benzene and *n*-hexane, the excimer emission disappears as that shown in the figure. This result may be understood by considering the fact

that the solvents of lower polarities are “good” solvents for the compound, but in contrast those of higher polarities are poor ones. This is because in a good solvent, both the pyrene moiety in an ECPS molecule and ECPS itself prefer to exist in monomer state, and corresponding emission should be characterized by Py monomer emission, but in a poor solvent the molecules of ECPS or Py moieties within the molecule tend to form aggregates, and the emission, of course, will be characterized by Py excimer emission.

Treatment of the emission spectra shown in Figure 2 reveals that the ratios of the intensities of the monomer emissions to those of the excimer emissions are well correlated to the values of the classical polarity parameter, $E_T(30)$,³⁶ of the solvents under study. The results are shown in Figure 2. As it can be expected, other factors such as hydrogen bond formation may also affect the ratios of the emissions. To reveal whether the excimer emission is originated from intramolecule, as there are two Py moieties in an ECPS molecule or intermolecule interactions, concentration-dependent experiment was performed.

Concentration Effect. From the structure of ECPS, it should not be difficult to anticipate that the profile of its fluorescence emission in a solvent of reasonable solubility should be concentration dependent because aggregation of the Py moieties must result in a broad and structure-less emission that is the excimer emission. To examine this, a number of ECPS solutions in methanol with different concentrations were prepared, and their fluorescence emission spectra were recorded, of which some typical results are shown in Figure 3. It is clearly seen that with progressive increase in the

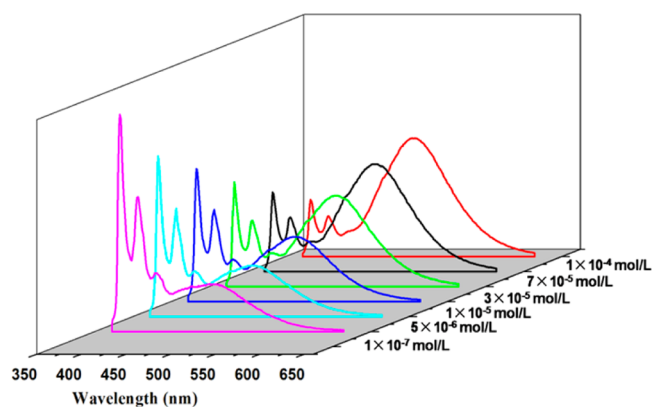


Figure 3. Fluorescence emission spectra of ECPS recorded in methanol at different concentrations.

concentration, the excimer emission around 495 nm increased dramatically while the monomer emission at lower wavelengths reduced. The emission is almost dominated by the excimer emission when the concentration of the compound reaches 1×10^{-4} mol/L as that shown in the figure. The increase in the excimer emission can be considered as a result of increase in the numbers of the molecules of the compound in aggregated state, of which the emission should be characterized by excimer emission, a typical intermolecular interaction. However, it is to be noted that excimer emission is still obvious even at concentration as low as 1×10^{-7} mol/L, which is less than one-third of the solubility of Py in water (6.7×10^{-7} mol/L).³⁷ Considering the fact that methanol is a better solvent than water toward the compounds, including both Py and ECPS, and the introduction of Chol structure favors the dissolution of

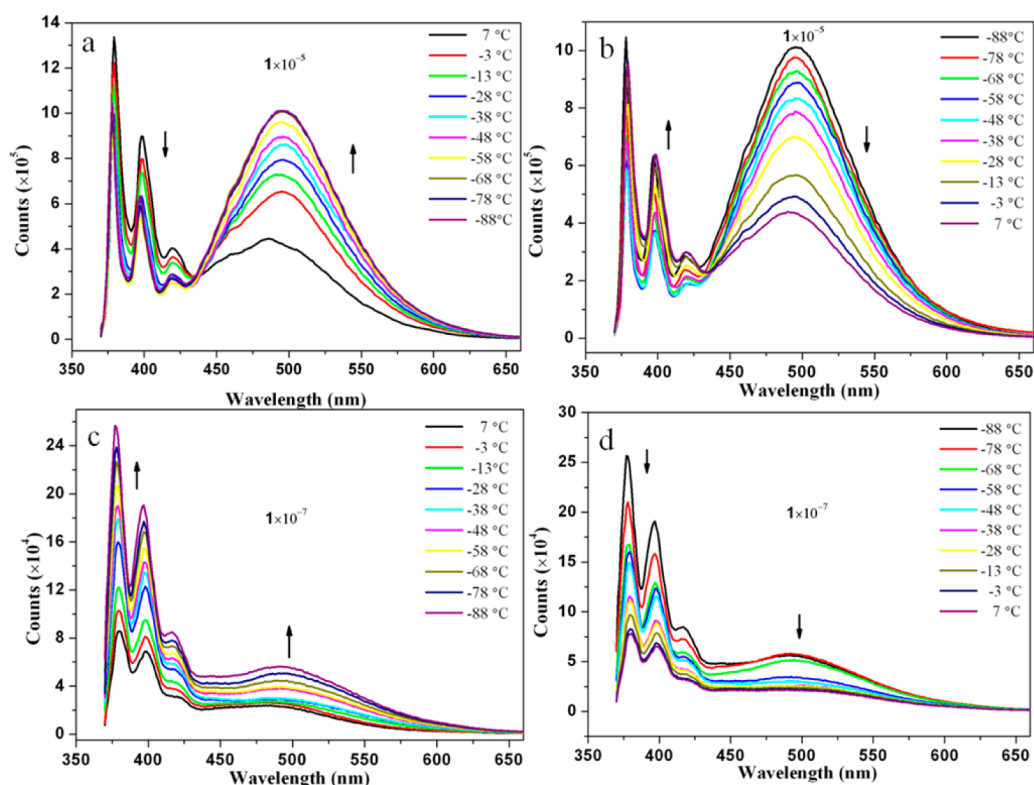


Figure 4. Fluorescence emission spectra of ECPS in methanol recorded at different temperatures and concentrations: a and c display the results with cooling from 7 °C to −88 °C, and b and d display those with heating from −88 to 7 °C, at concentrations of 1×10^{-5} mol/L and 1×10^{-7} mol/L, respectively.

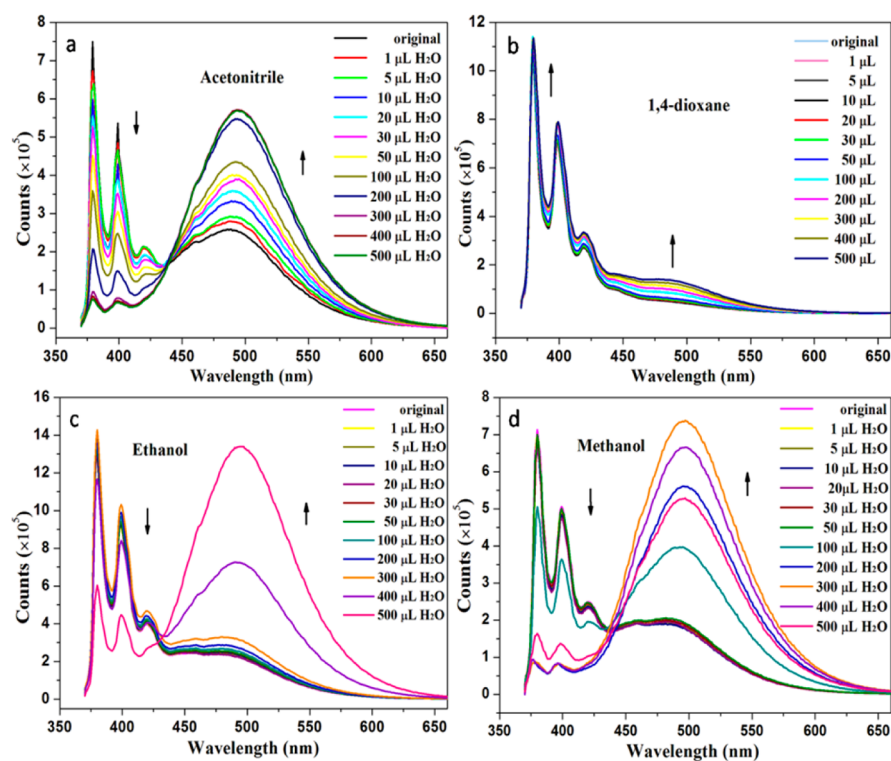


Figure 5. Fluorescence emission spectra of ECPS recorded in 3 mL of different organic solvents and in the presence of different amounts of water: (a) acetonitrile, (b) 1,4-dioxane, (c) ethanol, and (d) methanol.

Py into the organic solvent, it should be safe to say that ECPS stays mainly in the monomer state in the solvent, and thereby the emission at this concentration should be originated from

free ECPS rather than its aggregates. Further inspection of the emission spectrum of the system of lowest ECPS concentration reveals that the fluorescence emission at this case is

characterized by both Py monomer emission and its excimer emission, a fact in support of the presence of intramolecular interaction between the two Py moieties within an ECPS molecule.

Temperature Effect. Reasonably, the efficiency of an excimer emission of a system is also temperature dependence. For the excimer of different origins, such as that from intramolecular interaction and the other from intermolecular interaction, the dependence might be different, and thereby, temperature effect on the excimer formation of different concentrations was examined. Figure 4 shows the fluorescence emission spectra of ECPS in methanol at two different concentrations, of which one is 1×10^{-7} mol/L, and the other is 1×10^{-5} mol/L. It was expected that for the system of lower ECPS concentration, the excimer should form mainly from intramolecular interaction, and in contrast, for the one of higher ECPS concentration, the excimer mainly forms from intermolecular interaction. Reference to Figure 4a, of which the fluorophore concentration is high, reveals that along with temperature decrease from 7 °C to −88 °C, the excimer emission around 495 nm enhanced dramatically while the monomer emission between 360 and 430 nm reduced. However, for the system of lower fluorophore concentration, the situation is different (c.f. Figure 4c). With examination of the figure, it is seen that both the excimer and the monomer emission increased slowly along with temperature decrease. As expected, increase in temperature results in reversed phenomena as those shown in Figure 4b and Figure 4d, respectively. The phenomena observed for the system of higher fluorophore concentration may be explained by intermolecular interaction because decrease in temperature favors aggregation of the fluorophore molecules, which is a priority for excimer emission. As for the system of lower fluorophore concentration, aggregation of the molecules is impossible, thus resulting in a different situation. Decrease in temperature reduces the mobility of the molecules of the fluorophore and decreases the probability of nonradiation decay from its excited state, implying a greater probability of radiation decay and of course a higher fluorescence quantum yield and brighter emission. The explanations are strongly supported by the results from time-resolved emission spectroscopy (TRES) studies, which will be presented later on.

Spectral Changes Induced by the Presence of Water.

It can also be anticipated that introduction of water into a less-polar solvent must result in change in the profile of the fluorescence emission of ECPS in less-polar solvent. To examine this effect, the fluorescence emission of ECPS in acetonitrile, ethanol, methanol and 1,4-dioxane was recorded at various water contents. Except for 1,4-dioxane, as shown in Figure 5, with increasing water content, the profiles of the fluorescence emission spectra changed dramatically. For acetonitrile, introduction of water, even a trace amount of it, enhances excimer emission while it reduces monomer emission (c.f. Figure 5a). A similar phenomenon was observed for ethanol and methanol. However, significant change can be observed only when the content of water exceeds a certain value, for example, above 300 μ L/3 mL (water to ethanol; c.f. Figure 5c). It is surprising to find that water has little effect on the fluorescence behavior of the compound in 1,4-dioxane (c.f. Figure 5b). Addition of 17% of water only results in less than 5% increase in the monomer emission, and a little bit more increase in the excimer emission. Further inspection of the spectra shown in the figure shows that the profile of the

spectrum at pure 1,4-dioxane is dominated by Py monomer emission, while the corresponding excimer emission is negligible. This is a rather interesting result because at a concentration of 5×10^{-6} mol/L, significant excimer emission should be expected as those observed in other systems (c.f. Figure 5a, c, and d). Negligible water effect and no obvious excimer emission reveals that 1,4-dioxane may be one of the best solvents for the compound obtained in the present work. This observation can only be explained by considering that this solvent may possess a specific interaction with the probe, which can be attributed to the binding of the solvent molecules to the probe molecules via hydrogen bonding, which of course inhibits the aggregation of the probe molecules both in pure solvents and in solvents containing water. Figure 6 shows the plots of

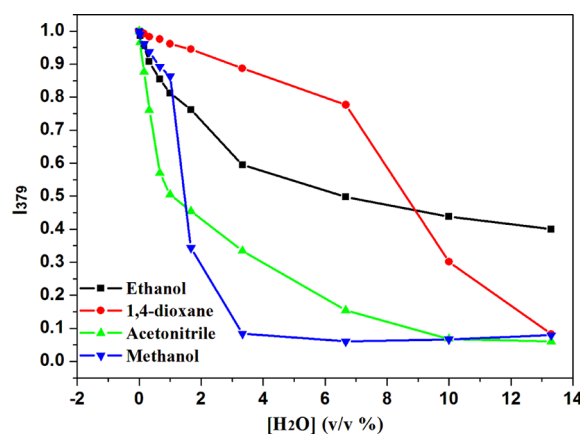


Figure 6. Plot of I_{379} as a function of water volume in 3 mL of different organic solvents. Note: the intensities at pure organic solvents have been normalized to 1.

I_{379} against water fraction in the organic solvents. Reference to the insets shown in the figure reveals that in the low water content region (<1%, v/v) and for all the systems, the intensity of the emission increased almost linearly with the increase of water content. Mathematical simulation of the plots in the region gives equation: $Y = a + bX$, where X is the volume fraction of water in the mixture solvents, and Y stands for the corresponding value of I_{379} . “ a ” and “ b ” are constants that are different based on different systems. The values of a and b are found to be 1 and −0.63, 1 and −0.2, 1 and −0.13, and 1 and −0.03 for acetonitrile, ethanol, methanol, and 1,4-dioxane, respectively. Clearly, the absolute magnitude of the slope is largest for acetonitrile, followed by ethanol, then methanol, and last 1,4-dioxane. No doubt, these calibration equations can serve as the basis for quantitative analysis of trace amounts of water in the solvents. The detection limits (DL) of this method, the details of which are provided in the Supporting Information, of water in the solvents are 0.0007, 0.06, 0.2, and 0.4 (% v/v), respectively, of which the sensitivity to water in acetonitrile is the highest when compared to those reported in the literature.

Spectral Changes Induced by Viscosity. Considering the molecular beacon-like structure of ECPS (c.f. Figure 1), it is expected that the fluorescence emission from this compound might be sensitive to the change in the microviscosity of its medium. Accordingly, the fluorescence behavior of ECPS in methanol of different viscosities, which could be adjusted by the addition of different volumes of glycerol, a widely accepted method to obtain solvents of different viscosities, was studied.³⁸

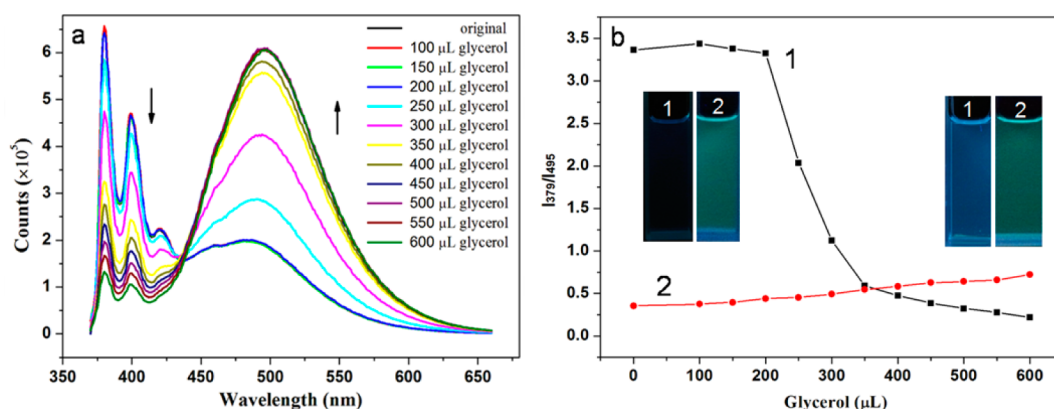


Figure 7. (a) Fluorescence emission spectra of ECPS recorded in the presence of different amounts of glycerol. (b) Plots of I_{379}/I_{490} against the volume of glycerol in 3 mL of acetonitrile, in which 1 stands for ECPS, and 2 is the corresponding molecular beacon.

Figure 7 shows the fluorescence emission spectra of ECPS in methanol (1×10^{-5} mol/L) containing different concentrations of glycerol, in which the viscosities are different from one another. It is seen that along with increasing viscosity, the excimer emission around 495 nm enhanced dramatically, while the monomer emission between 360 and 430 nm reduced. This is a rather surprising result because it is contrary to that reported using Py-based molecular beacons as probes.³⁹ It is known that for traditional Py-based molecular beacons, the ratio of excimer emission to monomer emission increases along with decreasing the viscosity of its medium. Such result is because, for this kind of molecular beacon, the excimer forms mainly via Birk's scheme, which relies on the frequency of the collision between the two Py moieties within a molecule of the beacons. However, for our system the situation is different because the solubility of this compound is much lower than those of the commonly found Py-based molecular beacons. Therefore, at the concentration employed, the molecules of ECPS may exist in both monomolecular state and aggregated state (clusters). In other words, there is a balance between the two states. At this case, the excimer emission mainly originates from intermolecular interaction of the Py moieties belonging to different molecules of the probe within the clusters. Thus, the phenomenon observed may be rationalized by considering that addition of glycerol increases the viscosity of the system, leading to diminishing of the mobility of the molecules of the probe, resulting in shift of the balance to the cluster side, suggesting more excimer formation.

Not only is the mechanism behind monitoring the viscosity change of the fluorescent probe developed in the present work different from that behind the routine molecular beacons, but their monitoring abilities are also different. As a control, a classical bis-pyrenyl molecular beacon, of which the structure is shown in the Supporting Information (c.f. Figure S1), was chosen to conduct the same viscosity measurement. The results are shown in Figure 7b and Figure S2. With references to the figure and the spectra, it is clearly seen that compared to the probe we developed, this probe is insensitive to the changes, at least within the range of the test, even though the ratio of I_{379}/I_{495} slightly increases along with increasing the viscosity of the system.

TRES Studies. To rationalize the fluorescence behavior of ECPS in solvents of different polarities and at different concentrations, TRES of the compound in methanol at different concentrations and with or without water were

measured. The results are shown in Figure 8. It is clearly seen from the figure that for the system of higher ECPS

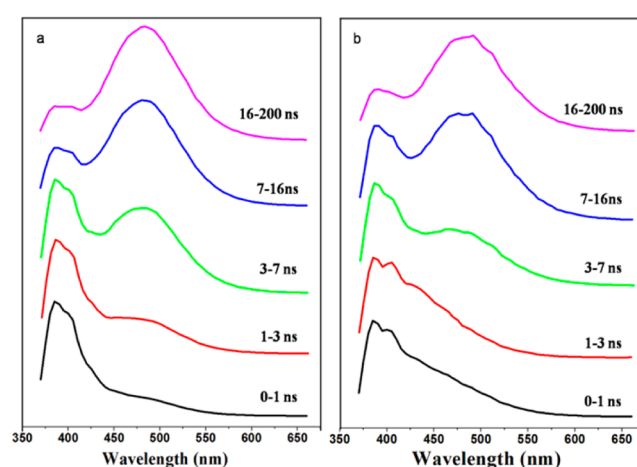


Figure 8. Time-resolved emission spectra of ECPS in methanol of a concentration of 1×10^{-4} mol/L (a) without water and (b) with 300 μ L water in 3 mL of the solution.

concentration (1×10^{-4} mol/L) and in pure methanol (c.f. Figure 8a), the profile of the earlier time gate (0–1 ns) spectrum is dominated by Py monomer emission, and the contribution from Py excimer emission is small. However, with the time-gate moving to longer times, it is seen that contribution from the excimer emission increases, and the latest time gate (16–200 ns) spectrum is actually dominated by the excimer emission. These results suggest that in this system, Py excimer forms mainly via Birk's scheme because the excimer mainly forms after the system is excited.⁴⁰ Such result strongly suggests that dense aggregation of the Py moieties both within an ECPS molecule and between the ECPS molecules of the system is impossible. However, with introduction of water into the system aforementioned, contribution from the excimer emission to the early time gate spectrum at this case increased significantly, and at the same time the contribution appears mainly at shorter wavelengths if compared to the emission from normal excimer formed via Birk's scheme, of which the maximum emission appears around 490–500 nm, indicating that the presence of water promotes the aggregation of the Py moieties, which is a prerequisite for excimer formation via preformed scheme. It is to be noted that with the exception of

the excimer formed via the preformed scheme, excimer in this system forms also via Birk's scheme as the maximum emission of the excimer at later time gate shifts to longer wavelength and eventually to the position between 490 and 500 nm (c.f. Figure 8b).

By contrast, for the system of lower ECPS concentration ($<1 \times 10^{-7}$ mol/L) and with pure methanol as solvent, the situation is different. In this case, the earlier time gate spectrum (0–1 ns) is characterized by Py monomer emission and with a slight contribution from Py excimer emission (c.f. Figure S3a). The fact that the excimer emission increases along with the time gate moving to longer times indicates again that in pure methanol Py moieties within an ECPS molecule are either mobile or loosely aggregated, in agreement with the result from TRES studies of the system of higher ECPS concentration. For the system of similar composition but containing water, contribution from excimer emission to the earlier time gate spectrum of this system is increased (c.f. Figure S3b), suggesting that the presence of water promotes the aggregation of the Py moieties within an ECPS molecule, again in support of the result from the TRES studies of the system of higher ECPS concentration and containing water.

Tentative probing mechanism. As revealed by fluorescence studies afore-presented, aggregation of the Py moieties, no matter if they are within the same ECPS molecule or between different ECPS molecules, is the origin of the change of the profile of its fluorescence emission observed in solvent, water, temperature, or viscosity dependence studies. To further confirm this tentative explanation, DLS was employed to monitor the aggregation of the compound in acetonitrile (1×10^{-5} mol/L) as a function of water content. The results are shown in Figure 9. Reference to the figure reveals that (1) for

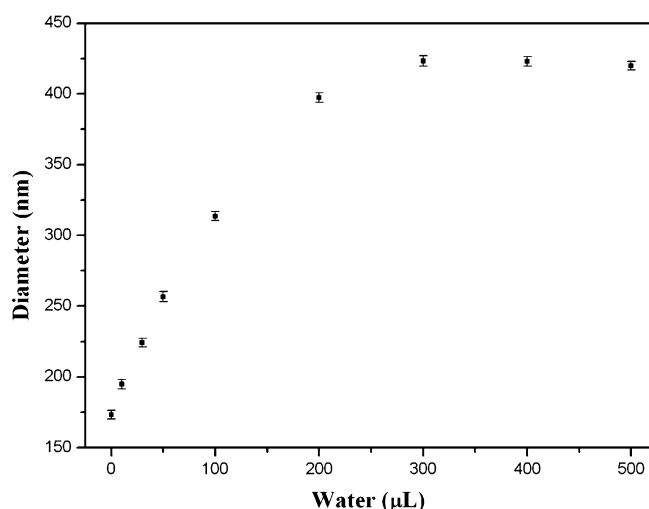


Figure 9. Plot of the diameters of the aggregates of ECSP in 3 mL of acetonitrile (1×10^{-5} mol/L) in the presence of different amounts of water; results from more than three parallel measurements.

the system studied, aggregates exist in the system with no water, (2) the average size of the aggregates increases along with increasing water content, and (3) no more increase occurred in the average size of the aggregates when the water content exceeded a certain value, which is about 1:10 (v:v, water to acetonitrile) in this case. This is a result in accordance with that obtained from fluorescence studies, as shown in Figure 5a. Clearly, below the critical concentration, the excimer

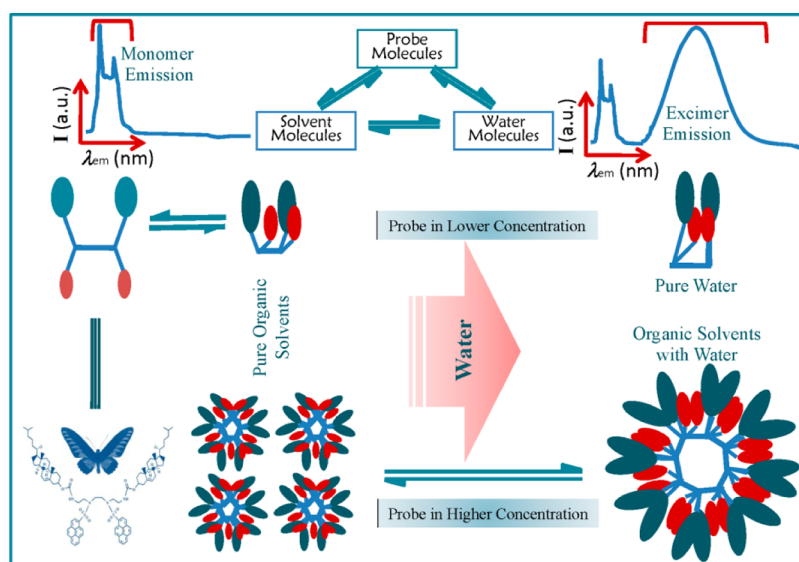
emission increases and the monomer emission decreases along with increasing water content, but both emissions remained almost unchanged when water content exceeds the value, suggesting that almost all of the ECPS molecules in the system may have joined the aggregates. Accordingly, a cartoon was drawn to show the possible mechanism for the response of the probe to mediums of different compositions or properties (c.f. Scheme 1). With reference to the cartoon, it is seen that for systems of higher probe concentrations as employed in the present study, introduction of water into a miscible and pure organic solvent promotes the probe molecules to move close to each other, which is a necessity for excimer formation, no matter if it forms via Birk's scheme or the preformed scheme. However, for organic solvents that show specific affinity to probe molecules, the situation might be different. For example, 1,4-dioxane may combine the probe strongly, resulting in inhibition of the aggregation both in the absence and presence of water, implying lower excimer formation efficiency. This is to say that our probe functions through aggregate (cluster) formation, which is very different from those reported for routine Py-based molecular beacons, which functions, as reported in the literature, through intramolecular excimer formation via Birk's scheme. The cartoon can also be used for the rationalization of the results from temperature-dependent and viscosity-dependent experiments because both decreasing temperature and increasing viscosity diminish the mobility of the probe molecules, which favors formation of the aggregates, suggesting a higher excimer emission.

It is to be noted, however, that the results from the temperature-dependent and/or the viscosity-dependent experiments are opposite to those obtained by using routine Py-based molecular beacons as probes. This contradiction can be easily understood by considering that excimer emission observed in Py-based routine molecular beacons originates from intramolecular interaction of the Py moieties or Birk's scheme-based dynamic mechanism. However, for the Py-based probe developed here, the emission originates from intermolecular interaction of the Py moieties. In other words, Py-based routine molecular beacons probe via a single molecule, but the newly developed Py-based molecular probe functions through its aggregates or clusters.

4. CONCLUSION

In conclusion, we have designed and synthesized a butterfly-shaped Py derivative of Chol (ECPS). The fluorescence behavior of ECPS in various solvents has been studied, and it was revealed that ECPS is sensitive to the change both in the polarity and the viscosity of its microenvironment. For this reason, the compound can be used as a probe for the determination of trace amounts of water in acetonitrile, ethanol, methanol and 1,4-dioxane. The DLs of the method for water in these solvents are 0.0007, 0.06, 0.2, and 0.4 (% (v/v), respectively, of which the DL for water in acetonitrile is the lowest value reported to date. The sensing behavior of the compound has been rationalized by proposing an existence of a balance between the molecules of ECPS in monomolecular state and those in aggregated state, as it is believed that the molecules of the compound in the monomolecular state correspond mainly to the monomer emission of Py even though the probe contains two Py moieties molecularly, and those in aggregated state, no matter how big it is, to excimer emission. Furthermore, for the organic solvents studied, the water content in them is linearly dependent on the ratio of the

Scheme 1. A Cartoon Showing the Possible Mechanism for the Response of the Probe to Mediums of Different Compositions and Properties^a



^aNote: the number of the probe molecules participating in the clusters is just a representative. In fact, for a given system, initially the number increases along with increasing water content.

excimer emission to the monomer emission of the probe when the provided water content in them is sufficiently low. Accordingly, ECPS may be used as a quantitative probe for trace water monitoring in them. Of course, ECPS may also find uses in other fields relevant to water determination and viscosity monitoring.

■ ASSOCIATED CONTENT

● Supporting Information

The synthetic procedures, the characterization data, and some TRES spectra of the compound. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Bühlmann, P.; Pretsch, E.; Bakker, E. Carrier-Based Ion-Selective Electrodes and Bulk Optodes. 2. Ionophores for Potentiometric and Optical Sensors. *Chem. Rev.* **1998**, *98*, 1593–1688.
- (2) Kakabadse, G. J.; Al-Aziz, M. S.; Hamilton, I. C.; Olatoye, E. O.; Perry, R.; Tipping, A. E.; Vaudrey, S.; Al-Yawer, N. F. N. On-Line Direct Potentiometry of Residual Water in Organic Solvents Using Cells Without a Liquid Junction Under Proton Isoconcentration Conditions. *Analyst* **1988**, *113*, 1365–1368.
- (3) Oradd, C.; Cedergren, A. Coulometric Study of Recovery Rates for Karl Fischer Titration of Water in Aldehydes and Ketones Using

Rapidly Reacting Methanolic and 2-Methoxyethanolic Reagents. *Anal. Chem.* **1995**, *67*, 999–1004.

(4) Liang, Y. Y. Automation of Karl Fischer Water Titration by Flow Injection Sampling. *Anal. Chem.* **1990**, *62*, 2504–2506.

(5) Ludvik, J.; Hilgard, S.; Volke, J. Determination of Water in Acetonitrile, Propionitrile, Dimethylformamide and Tetrahydrofuran by Infrared and Near-Infrared Spectrometry. *Analyst* **1988**, *113*, 1729–1731.

(6) Luzinova, Y.; Zdyrko, B.; Luzinov, I.; Mizaikoff, B. Detecting Trace Amounts of Water in Hydrocarbon Matrices with Infrared Fiberoptic Evanescent Field Sensors. *Analyst* **2012**, *137*, 333–341.

(7) Karim, M. R. O. Direct Potentiometry of Residual Water in Acetonitrile Using the Proton Isoconcentration Technique. *Analyst* **1987**, *112*, 1369–1371.

(8) Saraullo, A.; Martos, P. A.; Pawliszyn, J. Water Analysis by Solid Phase Microextraction Based on Physical Chemical Properties of the Coating. *Anal. Chem.* **1997**, *69*, 1992–1998.

(9) Lombardi, D. R.; Mann, C. K.; Vickers, T. J. Determination of Water in Slurries by Fiber-Optic Raman Spectroscopy. *Appl. Spectrosc.* **1995**, *49*, 220–223.

(10) Blyth, J.; Millington, R. B.; Mayes, A. G.; Frears, E. R.; Lowe, C. Holographic Sensor for Water in Solvents. *Anal. Chem.* **1996**, *68*, 1089–1094.

(11) Baptista, M. S.; Tran, C. D.; Gao, G. H. Near-infrared Detection of Flow Injection Analysis by Acoustooptic Tunable Filter-Based Spectrophotometry. *Anal. Chem.* **1996**, *68*, 971–976.

(12) Hisamoto, H.; Manabe, Y.; Yanai, H.; Tohma, H.; Yamada, T.; Suzuki, K. Molecular Design, Characterization, and Application of Multiinformation Dyes for Multidimensional Optical Chemical Sensings. 2. Preparation of the Optical Sensing Membranes for the Simultaneous Measurements of pH and Water Content in Organic Media. *Anal. Chem.* **1998**, *70*, 1255–1261.

(13) Fong, A.; Hieftje, G. M. Near-Infrared Measurement of Relative and Absolute Humidity through Detection of Water Adsorbed on a Silica Gel Layer. *Anal. Chem.* **1995**, *67*, 1139–1146.

(14) Zhao, C.; Bond, A. M.; Compton, R. G.; O'Mahony, A. M.; Rogers, E. I. Modification and Implications of Changes in Electrochemical Responses Encountered When Undertaking Deoxygenation in Ionic Liquids. *Anal. Chem.* **2010**, *82*, 3856–3861.

- (15) Papkovsky, D. B.; Ponomarev, G. V.; Chernov, S. F.; Ovchinnikov, A. N.; Kurochkin, I. N. Luminescence Lifetime-Based Sensor for Relative Air Humidity. *Sens. Actuators B* **1994**, *22*, 57–61.
- (16) Franke, H.; Wagner, D.; Kleckers, T.; Reuter, R.; Rohitkumar, H. V.; Blech, B. A. Measuring Humidity with Planar Polyimide Light Guides. *Appl. Opt.* **1993**, *32*, 2927–2935.
- (17) Fernández, J. M. C.; Medel, A. S. Air Moisture Sensing Materials Based on the Room Temperature Phosphorescence Quenching of Immobilized Mercurochrome. *Anal. Chim. Acta* **2000**, *407*, 61–69.
- (18) Wang, K. M.; Seiler, K.; Haug, J. P.; Lehmann, B.; West, S.; Hartman, K.; Simon, W. Hydration of Trifluoroacetophenones as the Basis for an Optical Humidity Sensor. *Anal. Chem.* **1991**, *63*, 970–974.
- (19) Glenn, S. J.; Cullum, B. M.; Nair, R. B.; Nivens, D. A.; Murphy, C. J.; Angel, S. M. Lifetime-Based Fiber-Optic Water Sensor Using a Luminescent Complex in a Lithium-Treated NafionTM Membrane. *Anal. Chim. Acta* **2001**, *448*, 1–8.
- (20) Chang, Q.; Murtaza, Z.; Lakowicz, J. R.; Rao, G. A Fluorescence Lifetime-Based Solid Sensor for Water. *Anal. Chim. Acta* **1997**, *350*, 97–104.
- (21) Bai, M. Q.; Seitz, W. R. A Fiber Optic Sensor for Water in Organic Solvents Based on Polymer Swelling. *Talanta* **1994**, *41*, 993–999.
- (22) Niu, C. G.; Guan, A. L.; Zeng, G. M.; Liu, Y. G.; Li, Z. W. Fluorescence Water Sensor Based on Covalent Immobilization of Chalcone Derivative. *Anal. Chim. Acta* **2006**, *577*, 264–270.
- (23) Niu, C. G.; Qin, P. Z.; Zeng, G. G.; Gui, X. Q.; Guan, A. L. Fluorescence Sensor for Water in Organic Solvents Prepared from Covalent Immobilization of 4-Morpholinyl-1,8-naphthalimide. *Anal. Biochem.* **2007**, *387*, 1067–1074.
- (24) Li, S. H.; Chen, F. R.; Zhou, Y. F.; Xu, J. G. Pb₄Br₁₁³⁻ Cluster as a Fluorescent Indicator for Micro Water Content in Aprotic Organic Solvents. *Analyst* **2009**, *134*, 443–446.
- (25) Citterio, D.; Minamihashi, K.; Kuniyoshi, Y.; Hisamoto, H.; Sasaki, S.; Suzuki, K. Optical Determination of Low-Level Water Concentrations in Organic Solvents Using Fluorescent Acridinyl Dyes and Dye-Immobilized Polymer Membranes. *Anal. Chem.* **2001**, *73*, 5339–5345.
- (26) Ooyama, Y.; Matsugasako, A.; Oka, K.; Nagano, T.; Sumomogi, M.; Komaguchi, K.; Imae, I.; Harima, Y. Fluorescence PET (Photo-Induced Electron Transfer) Sensors for Water Based on Anthracene–Boronic Acid Ester. *Chem. Commun.* **2011**, *47*, 4448–4450.
- (27) Ooyama, Y.; Egawa, H.; Yoshida, K. The Design of a Novel Fluorescent PET Sensor for Proton and Water: A Phenylaminonaphtho[1,2-d]oxazol-2-yl-Type Fluorophore Containing Proton Donor and Acceptor Groups. *Dyes Pigm.* **2009**, *82*, 58–64.
- (28) Ooyama, Y.; Egawa, H.; Yoshida, K. A New Class of Fluorescent Dye for Sensing Water in Organic Solvents by Photo-induced Electron Transfer - A (Phenylamino)naphtho[1,2-d]oxazol-2-yl-Type Fluorophore with Both Proton-Binding and Proton-Donating Sites. *Eur. J. Org. Chem.* **2008**, *31*, 5239–5243.
- (29) Ooyama, Y.; Sumomogi, M.; Nagano, T.; Kushimoto, K.; Komaguchi, K.; Imae, I.; Harima, Y. Detection of Water in Organic Solvents by Photo-induced Electron Transfer Method. *Org. Biomol. Chem.* **2011**, *9*, 1314–1316.
- (30) Murata, K.; Aoki, M.; Suzuki, T.; Harada, T.; Kawabata, H.; Komori, T.; Ohseto, F.; Ueda, K.; Shinkai, S. Thermal and Light Control of the Sol–Gel Phase Transition in Cholesterol-Based Organic Gels. Novel Helical Aggregation Modes As Detected by Circular Dichroism and Electron Microscopic Observation. *J. Am. Chem. Soc.* **1994**, *116*, 6664–6676.
- (31) George, M.; Weiss, R. G. Molecular Organogels. Soft Matter Comprised of Low-Molecular-Mass Organic Gelators and Organic Liquids. *Acc. Chem. Res.* **2005**, *39*, 489–497.
- (32) Lin, Y.; Weiss, R. G. A Novel Gelator of Organic Liquids and the Properties of Its Gels. *Macromolecules* **1987**, *20*, 414–417.
- (33) Kwon, G. S.; Naito, M.; Kataoka, K.; Yokoyama, M.; Sakurai, Y.; Okano, T. Block Copolymer Micelles as Vehicles for Hydrophobic Drugs. *Colloids Surf., B* **1994**, *2*, 429–434.
- (34) Khakhel, O. A.; Sakhno, T. V.; Romashko, T. P.; Granchak, V. M. Polarization of Excimer Vinylpyrene. *J. Appl. Spectrosc.* **2004**, *71*, 48–53.
- (35) Ezzell, S. A.; McCormick, C. L. Water-Soluble Copolymers. 39. Synthesis and Solution Properties of Associative Acrylamido Copolymers with Pyrenesulfonamide Fluorescence Labels. *Macromolecules* **1992**, *25*, 1881–1886.
- (36) Reichardt, C. Solvatochromic Dyes as Solvent Polarity Indicators. *Chem. Rev.* **1994**, *94*, 2319–2358.
- (37) Yang, H.; Morris, J. J.; Lopina, S. T. Polyethylene Glycol-Polyamidoamine Dendritic Micelle as Solubility Enhancer and the Effect of the Length of Polyethylene Glycol Arms on the Solubility of Pyrene in Water. *J. Colloid Interface Sci.* **2004**, *273*, 148–154.
- (38) Blake, T. D.; Shikhmurzaev, Y. D. Dynamic Wetting by Liquids of Different Viscosity. *J. Colloid Interface Sci.* **2002**, *253*, 196–202.
- (39) Zachariasse, K. A.; Kuhnle, W.; Weller, A. Intramolecular Excimer Fluorescence as a Probe of Fluidity Changes and Phase Transitions in Phosphatidylcholine Bilayers. *Chem. Phys. Lett.* **1980**, *73*, 6–11.
- (40) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum Press: New York, 2006.