J Fluoresc (2006) 16:749–753
DOI 10.1007/s10895-006-0135-3

ORIGINAL PAPER

Unexpected Fluorescence Enhancement of Naphthylethyl Lauryl Ether in Coaggregation with Variant Aggregators

Da-Yong Sang · Juan Tian · Guo-Zhen Ji

Received: 24 May 2006 / Accepted: 3 October 2006 / Published online: 22 October 2006

Abstract Unexpected fluorescence enhancement has been observed during the coaggregation process between naphthylethyl lauryl ether (the fluorescence probe) and vitamin E acetate in aqueous organic binary solutions. Variant aggregators were used to study the influence of structure features on the enhancement. The experiments indicate that a long hydrophobic hydrocarbon chain is of necessity for coaggregation, and a flat group is crucial for the fluorescence enhancement. No enhancement was observed at the absence of coaggregation. The enhancement was attributed to the hindrance of rotations and prolonged life time of the probe by steric hindrance of flat groups in coaggregate. Structure features of aggregates of variant aggregators have also been discussed.

Keywords Coaggregation · Fluorescence enhancement · Hydrophobic hydrocarbon chain · Flat moiety

Introduction

Aggregation [1] of electrically neutral organic molecules which possess at least one long chain with more than eight carbons in aggregating media driven by hydrophobic interaction [1, 2] has aroused widespread attention [2, 3]. Fluorescence analysis is one of the most frequently used methods for studying the aggregation behaviours of organic molecules [4–6]. As the concentration increased, the probe molecules aggregated in the aggregating media due to the hydrophobic interaction [1, 2], and the excimer emission appeared. The aggregation behaviours of organic molecules are evaluated by plotting the ratio of excimer to monomer emission intensities (F_e/F_m) vs. concentration of fluorescence probes in aqueous solution [4–6]. For example, an increase in F_e/F_m means the growth of aggregates [1a], and a decrease in F_e/F_m implies the possibility of aggregate destabilization [6] or dilution of fluorophore concentration by aggregators, and finally a constant and small F_e (relative to F_m) generally indicates that fluorescent probe is in monomeric state at the absence of aggregate [1].

Although the analysis remains valid for majority of cases, the fluorescence efficiency of fluorescence probes is susceptible to environment and other factors, for example, enhanced fluorescence quenching by coaggregation between different types of aggregators has been reported [7]. On the other hand, fluorescence can also be enhanced by the photo-induced electron transfer (PIET) mechanism [8] and by complex inclusion of fluorescent probes into micelles [9] or cyclodextrins [10]. It has confirmed that aggregation and host-guest interaction are competing processes [3b]. However, fluorescence enhancement caused by coaggregation of long chain organic aggregators and probes has not been described yet. In this article, we have made an attempt to understand the effects of coaggregation on the enhancements of fluorescence, and the roles of structure features of variant aggregators in the enhancement were discussed.

Experimental

Materials

Naphthylethyl lauryl ether (**1**) [11] and benzyl phytyl ether (**4**) [12] were synthesized according to literature proedures.

D.-Y. Sang · J. Tian · G.-Z. Ji (✉)
Shanghai Institute of Organic Chemistry,
Chinese Academy of Sciences,
Shanghai 200032, China
e-mail: jigz@mail.sioc.ac.cn

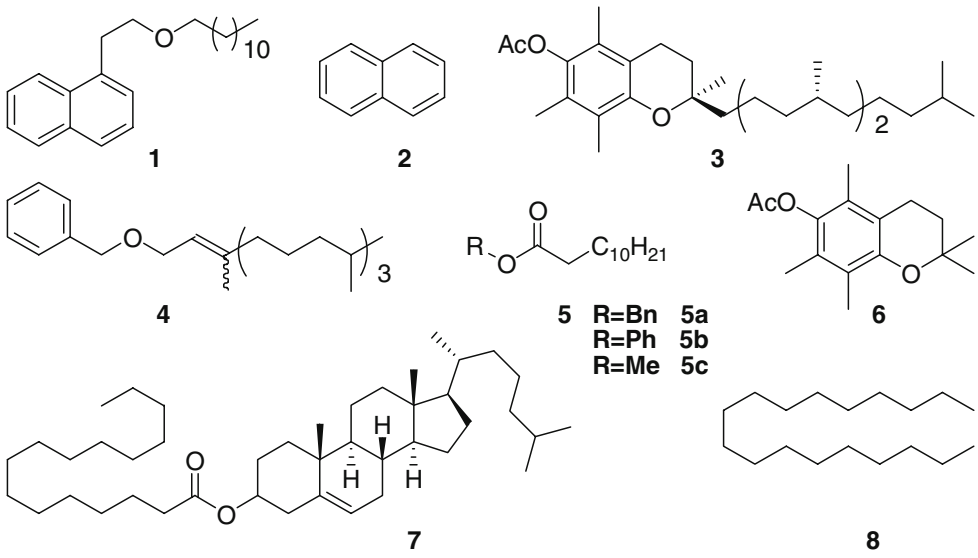

Fig. 1 Chemical structures of probes and aggregators investigated

Naphthalene (**2**), vitamin E acetate (**3**), cholesteryl octanoate (**7**), and octadecane (**8**) were commercially available and were used as received without further purification. Methanol was of spectrum grade. Water was double distilled (Fig. 1).

Fluorescence

Unless otherwise emphasized, all fluorescence spectra were recorded on a Perkin Elmer LS55 luminescence spectrometer at $T = 308$ K. A given volume of prepared solution dissolved in methanol was injected into a 2.5 mL of solution and the mixture was kept for 3 min before irradiation of the fluorophore moieties. The excitation wavelength for naphthyl fluorophore was 275 nm with a 15 nm excitation slit width and a 5 nm emission slit width.

Results and discussion

Aggregation induced fluorescence enhancement

Aggregation of **1** has been previously evaluated by steady state fluorescence method with the critical aggregate concentration (CAgC) of 2.0×10^{-6} M at $T = 308$ K in aqueous methanol mixture solution with a volume fraction of 0.45 for methanol ($\varphi = 0.45$) [13]. The probe was in the aggregated state when the concentration was above the CAgC ([**1**] $= 5.0 \times 0^{-6}$ M), and two fluorescence maxima near 340 and 380 nm were observed corresponding to the monomer and the excimer emission, respectively. Upon addition of **3**, both of the emissions were enhanced remarkably, as shown in Fig. 2. However, the fluorescence remained unaffected when naphthalene (**2**) was selected as the reference fluorescence probe. Naphthalene does not aggregate because of the absence of a long hydrophobic hydrocarbon chain [1]. The difference of influence between **1** (in aggregated state, [**1**] $= 5.0 \times 10^{-6}$ M) and **2** (in monomeric state,) by addition of aggregator **3** is depicted in Fig. 3. The extent of fluorescence enhancement could be described by fluorescence enhancement factor (FE, FE $= F/F_0$) [14] as a function of the concentration of aggregators. FE grew linearly with concentration of **3** and reached a value of 3 at [**3**] $= 5.0 \times 0^{-6}$ M for the monomer emission of **1**. The fluorescence intensity of **2** was unaffected and the FE remained constant.

Three plausible reasons might contribute to the phenomena: (1) **3** acted as a deaggregator (destabilizer), and the aggregates of **1** were deaggregated (destabilized) by **3**; (2)

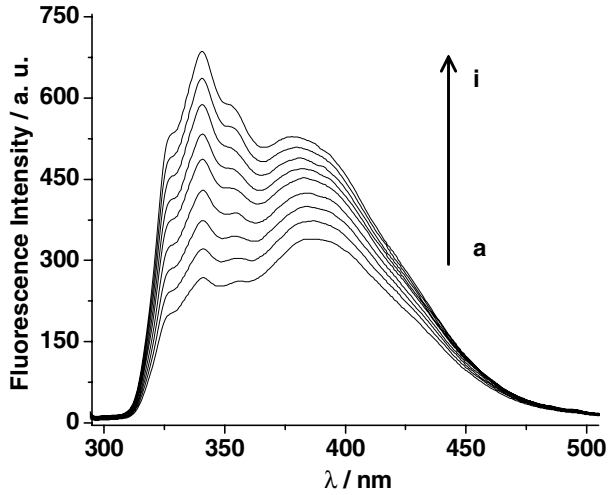

Fig. 2 Influence of **3** on the emission spectra of **1** (at aggregated state, CAgC $= 2.0 \times 10^{-6}$M). [**1**] $= 5.0 \times 10^{-6}$M. [**3**] ($\times 10^{-6}$M): a, 1.0; b, 2.0; c, 3.0; d, 4.0; e, 5.0; f, 6.0; g, 7.0; h, 8.0; i, 9.0

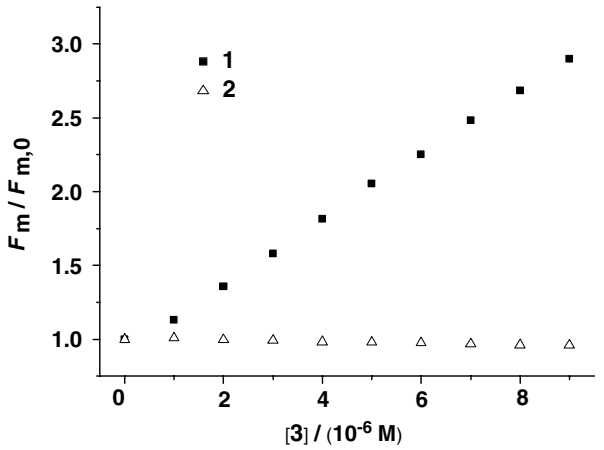

Fig. 3 Influence of **3** on the monomer intensities of **1** (in aggregated state, CAgC = 2.0 × 10^{-6} M) and **2** (in monomeric state). [**1**] = 5.0 × 10^{-6} M

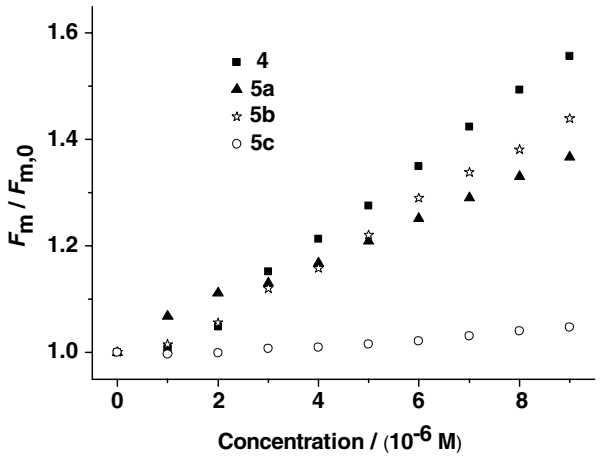

Fig. 4 Quenching effect of **4**, **5a**, **5b** and **5c** (analogues of **3**) on monomer intensities of **1** (in aggregated state, CAgC = 2.0 × 10^{-6} M, [**1**] = 5.0 × 10^{-6} M)

coaggregation as a key step towards deaggregation (by **3**) might have happened; and (3) fluorescence spectrum of **1** was overlapped by fluorescence of **3**. If deaggregation has occurred, intensity of the monomer (F_m) would be greatly enhanced at the cost of excimer intensity (F_e). This is obviously contradictory to the observed data and shall be negated. And if the enhancement was totally due to spectra overlapping, then similar result should be reproducible for **2**. But **2** does not aggregate in conventional conditions [1], as shown in Fig. 3, the fluorescence of **2** was insensitive to the addition of **3**. Thus the third possibility might be overlooked. Finally, if coaggregation might have occurred, an increase in F_e should be observable in concurrent with a decrease in F_m or vice versa, due to dilution of the local concentration of the fluorophores by aggregators [15].

To further illustrate the enhancement of fluorescence of the probe, structure features of **3** were brought into consideration. Should the hydrophobic hydrocarbon chain be responsible for the enhancement, or should the fluorophore (chromanol group) be the reason? Other molecules (**4**, **5a**, **5b** and **5c**) with variant aggregating tendencies were examined. Aggregator **4** was derived from phytol and used to study the influence of branch methyl groups on the enhancement. Aggregators **5a** and **5b** were analogues of **4** without branch methyl groups, and **5c** was an analogue of **4** with neither branch methyl groups nor an aromatic group. F_m increased linearly when these compounds (except **5c**) were added to the solution of **1** (in aggregated state), as shown in Fig. 4. When **5c** was added, the FE ($F_m/F_{m,0}$) remained constant. The aggregators of **5a** and **5b** enhanced the fluorescence of **1** in a similar way to **4**. This observation excluded the branch methyl groups from possible reasons for the enhancement as they did not exist in the hydrophobic chains of **5a** and **5b**. And an aromatic group attached to a long hydrocarbon chain seemed to be crucial for the enhancement of fluorescence. No enhancement was observed in the case of **5c** at the absence of an aromatic group.

No fluorescence enhancement is observed at the absence of aggregation

Since all the abovementioned cases of fluorescence enhancement were correlated with aggregation, it was of interest to study what would be at the absence of aggregation. Generally, neither flat molecules without a side chain nor chained molecules shorter than eight hydrocarbon moieties would aggregate [1–12]. There would be no coaggregation if molecules that can not aggregate are added into a solution of a fluorescence probe (in aggregated state) or if molecules with aggregating power are put into a solution of a fluorescence probe without aggregating power [1]. Naphthalene (**2**), for example, could not aggregate [1] and was selected as the reference fluorescence probe in contrast to **1**. As an analogue of **3** without a hydrophobic tail, aggregator **6** could not aggregate, too. Indeed, all these mismatched groups led to quenched fluorescence of probes. Figure 5 showed the fluorescence quenching of **1** by **6** and of **2** by **3** and **4**. The quenching processes obeyed the Stern–Volmer law, indicating that a hydrophobic chain is crucial for the enhancement.

With a long tail, **3** remarkably enhanced the fluorescence of **1**. And without the long tail, **6** decreased the fluorescence of **1**. Although both **3** and **4** could enhance the fluorescence of **1** which coaggregate with the probe **1** readily, there are no effects on the fluorescence of **2**. The major difference between **1** and **2** is that **1** has a long hydorcarbon chain attached to the naphthyl moiety which facilitates aggregation while flat molecules without a tail like **2** do not aggregate. These results strongly demonstrate that the above observed fluorescence enhancement was closely related to coaggregation between aggregators and probes.

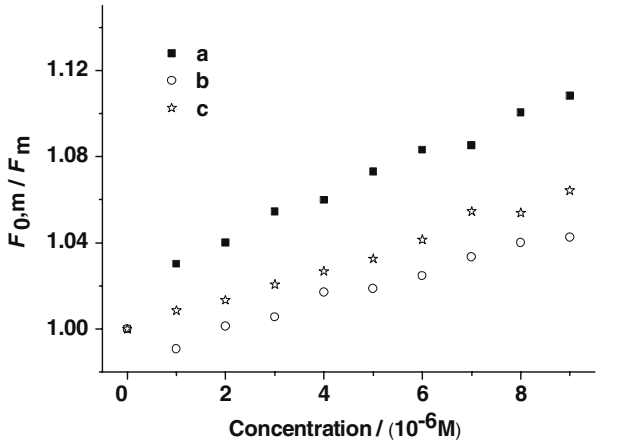

Fig. 5 Fluorescence quenching effect at the absence of coaggregation. Fluorescence of **1** (in aggregated state, CAgC = 2.0×10^{-6} M) was quenched by **6** (a); and fluorescence of **2** (in monomeric state) was quenched by **3** (b) and **4** (c) in accordance to the Stern-Volmer law. [**1**] = 5.0×10^{-6} M, [**2**] = 5.0×10^{-6} M, excitation slit = 15 nm, emission slit = 5 nm, excitation wavelength = 275 nm

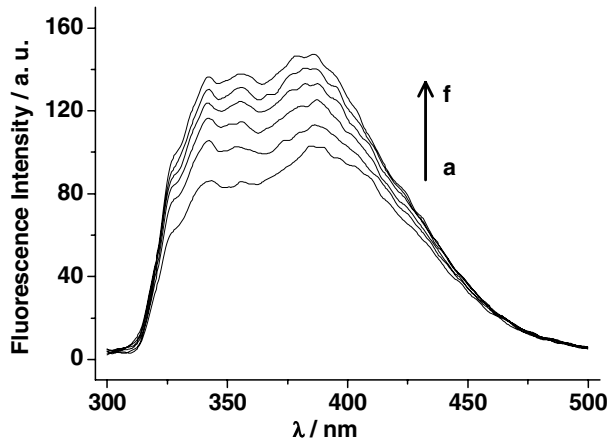

Fig. 6 Influence of **7** on fluorescence emission of **1** (in aggregated state, CAgC < 0.5×10^{-6} M) in aqueous dimethylsulfoxide solution (φ = 0.45). [**1**] = 5.0×10^{-6} M), T = 298 K, excitation slit = 15 nm, emission slit = 3 nm, excitation wavelength = 275 nm. [**7**] ($\times 10^{-6}$ M): a, 0.0; b, 2.0; c, 4.0; d, 6.0; e, 8.0; f, 10.0

Some aggregators without aryl groups also could enhance fluorescence of the probe

All the aggregators (**3**, **4**, **5a**, and **5b**) showing strong influence on fluorescence of **1** have an aryl moiety attached to a long hydrocarbon chain despite the existence of branch methyl groups. Two aggregators without aryl groups, cholesteryl palmitate (**7**) and octadecane (**8**), were selected to study the relation between an aryl group and the enhancement. For consideration of solubility, the study was carried out in aqueous dimethylsulfoxide (φ = 0.45) and aqueous dioxane (φ = 0.40) mixture solutions at T = 298 K respectively. As shown in Fig. 6, addition of **7** in aqueous dimethylsulfoxide solution resulted in remarkable enhancement in both monomer intensity and excimer intensity of the probe. However, an experiment carried out in dioxane water mixture solution showed that an increase in excimer intensity at the cost of monomer emission has occurred, indicating the occurrence of aggregation between cholesteryl palmitate **7** and **1** (in monomeric state, [**1**] = 5.0×10^{-6} M, CAgC = 5.3×0^{-6} M) in aqueous dioxane solution (φ = 0.40), as shown in Fig. 7. We have investigated the structural features of aggregates formed by **7** [12] and found that **7** could reduce the microscopic polarity of aggregates formed by dodecyl 1-pyrenylmethyl ether. Since aggregation between **7** and **1** in aqueous dioxane mixture solution has been observed, the polarity of the aggregate might not be a reason for the fluorescence enhancement. In effect, decreasing the volume fraction of dioxane to 0.20 led to remarked fluorescence enhancement. Also aggregate has been generally accepted as a paralleled assembly of long chain aggregators [1a, 12], which implied that structure features of aggregates are dependent upon the aggregating powers of solvents [1a], and a stronger aggregating media leads to aggregates in better order than the structures of aggregates formed by the same aggregators in lower aggregating media. In fact, Jiang has related the degree of aggregation of a particular aggregator to the solvent aggregating power of a particular aquiorgano solvent media [1, 3b].

The postulation was further strengthened by the fact that aggregation of **8** and **1** resulted in no fluorescence enhancement (data not shown) even in strong aggregating media (φ = 0.45 aqueous methanol). Interestingly, there was no enhancement in the case of **5c**, too. These two aggregators were in lack of a bulky flat group (an aryl group or a cholesteryl group, for example). Long chain molecules with a flat moiety such as porphin [16] might tend to generate stack-like aggre-

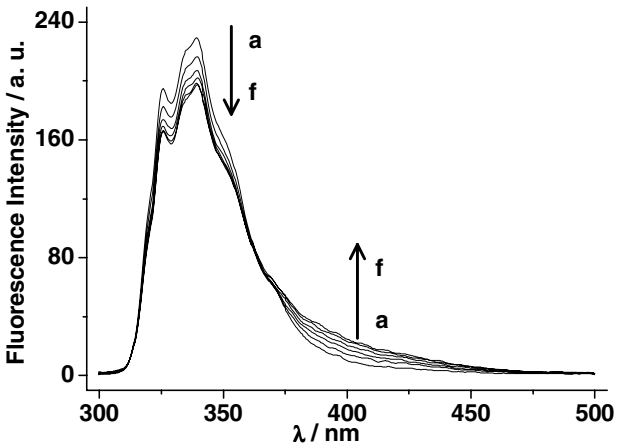

Fig. 7 Influence of **7** on fluorescence emission of **1** (in monomeric state, CAgC = 5.3×10^{-6} M) in aqueous dioxane solution (φ = 0.40). [**1**] = 5.0×10^{-6} M, T = 298 K, excitation slit = 15 nm, emission slit = 5 nm, excitation wavelength = 275 nm. [**7**] ($\times 10^{-6}$ M): a, 0.0; b, 2.0; c, 4.0; d, 6.0; e, 8.0; f, 10.0

gates, which might restrict the rotation [10b] of fluorophores accommodated in aggregates and prolong its lifetime and as a result, enhance its fluorescence efficacy. Jiang reported that cholesteryl caprylate prolonged the lifetime of monomer of hexadecyl 2-naphthoate from $\tau_0 = 10.3$ ns to $\tau = 13.6$ ns and cholesteryl laurate to $\tau = 13.5$ ns [17]. Long chain molecules without a flat moiety, on the other hand, coils randomly around the probe and the aggregates they formed might be different from those with a flat group [12].

Conclusion

In summary, we have demonstrated a phenomenon of fluorescence enhancement induced by coaggregation driven by hydrophobic interaction [1, 2] in aquiorgano solutions. No fluorescence enhancement was observed at the absence of coaggregation, but coaggregation alone does not necessarily lead to fluorescence enhancement. As to linear aggregators such as **5c** and **8**, only coaggregation with the probe (**1**) was observed at the absence of fluorescence enhancement. Aggregator **7** coaggregated with **1** in less aggregating media, but enhanced the fluorescence in strong aggregating media. The enhancement was attributed to the hindrance of rotations [10b] and prolonged life time [17] of the probe by steric hindrance of flat groups in coaggregate [12]. Long chain molecules without a flat moiety could not provide the hindrance and as a result no fluorescence enhancement was recorded. The present study also indicated that coaggregates of aggregators with flat moieties (with the fluorescence probe) were different from those formed by linear molecules.

Acknowledgments This work was supported by the National Natural Science Foundation of China (No. 20272078) and the Shanghai Municipal Science and Technology Commission (No. 03JC14083).

References

1. (a) Jiang XK (1988) Hydrophobic-lipophilic interactions. Aggregation and self-coiling of organic molecules. Acc Chem Res 21(10):362–367; (b) De Rossi U, Moll J, Kriwanek J, Daehne S (1994) Influence of the N-alkyl chain length on the J-aggregation behavior of a cyanine dye. J Fluorescence 4(1):53–55
2. Blokzijl W, Engberts JBFN (1993) Hydrophobic effects. Opinions and facts. Angew Chem Int Ed Engl 32(11):1545–1579
3. (a) Otto S, Engberts JBFN (2003) Hydrophobic interactions and chemical reactivity. Org Biomol Chem 1(16):2809–2820; (b) Jiang XK (1994) New concepts for studying and understanding the aggregation, co-aggregation, de-aggregation and self-coiling of organic molecules. Why are cholesterols and triglycerides culprits of arteriosclerosis? Pure Appl Chem 66(8):1621–1628
4. Jiang XK, Ji GZ, Tu B, Zhang XY, Shi JL, Chen X (1995) Shape and aggregating tendency. The aggregating behavior of eight esters of eight-carbon carboxylic acids. J Am Chem Soc 117(51):12679–12682
5. Zhang XY, Zhu Y, Ji GZ, Jiang XK (1996) Assessment of critical aggregate concentration (CAgC) by UV. A new methodology. Chin Chem Lett 7(8):759–762
6. Zhang JT, Nie J, Ji GZ, Jiang XK (1994). Effective neutral deaggregators. Langmuir 10(1):2814–2816
7. Shi JL, Qiu ZH, Jiang XK (1999) Effect of aggregate formation on the fluorescence quenching of anthracene derivatives by m-N,N-diethylaminophenyl carboxylates with different chain lengths as quenchers. J Phy Org Chem 12(10):735–740
8. Badugu R (2005) Fluorescence sensor design for transition metal ions: the role of the PIET Interaction Efficiency. J Fluorescence 15(1):71–83
9. Singh HN, Hinze WL (1982) Micellar enhanced fluorometric determination of 1-N,N-dimethylaminonaphthalene-5-sulfonyl chloride and o-phthalaldehyde-2- mercaptoethanol derivatives of amino acids. Analyst 107(1278):1073–1080
10. (a) Muthu IV, Enoch V, Swaminathan M (2004) Inclusion complexation of 2-amino-7-bromofluorene by β-cyclodextrin: Spectral characteristics and the effect of pH. J Fluorescence 14(6):751–756; (b) Li S, Purdy WC (1992) Cyclodextrins and their applications in analytical chemistry. Chem Rev 92(6):457–1470
11. Shi JL, Chen X, Jiang XK (1996) Hydrophobic Acceleration of Electron Transfer Processes. J Org Chem 61(14):698–4702
12. Sang DY, Tian J, Ji GZ (2006) Aggregation of dodecyl 1-pyrenylmethyl ether and its application in structure-polarity relations of aggregates. Chin J Chem 24(9):1109–1116
13. Jiang XK, Hui YZ, Fei ZX (1987) The effect of hydrophobic-lipophilic interactions on chemical reactivity. 8. Large-ring intramolecular excimer formation brought about by hydrophobic forces. J Am Chem Soc 109(19):5862–5864
14. Magri DC, Callan JF, de Silva AP, Fox DB, McClenaghan ND, Sandanayake KRAS (2005) The anthracen-9-ylmethyloxy unit: an underperforming motif within the fluorescent PET (photoinduced electron transfer) sensing framework. J Fluorescence 15(5):769–775
15. Ji HF, Xu XH, Tung CH (2001) Chromophore end-labelled long chain perfluoropolyether as probes to study the formation ofaggregates in aqueous organic solventand their asymmetrical coaggregationwith hydrocarbons. J Photochem Photobio A 144(2–3):179–184
16. (a) Kubat P, Lang K, Zelinger Z, Kral V (2004) Aggregation and photophysical properties of water-soluble sapphyrins. Chem Phy Lett 395(1–3):82–86; (b) Prochazkova K, Zelinger Z, Lang K, Kubat P (2004) meso-Tetratolylporphyrins substituted by pyridinium groups: aggregation, photophysical properties and complexation with DNA. J Phy Org Chem 17(10):890–897
17. Jiang XK, Zhang JT, Tong ZH (1993) Chain foldability effect on coaggregation. Evidence derived from time-resolved fluorescence spectroscopy. Chin J Chem 11(2):187–189