Full Paper

Applied Organometallic Chemistry

Received: 21 June 2012 Revised: 27 July 2012 Accepted: 27 July 2012

(wileyonlinelibrary.com) DOI 10.1002/aoc.2914

Non-aggregated boron–fluorine derivatives with photodynamic activity

Liang Yao, Feijun Dan*, Qiong Cao, Miaofu Mao and Shuzhang Xiao*

Novel non-aggregated boron–fluorine derivatives with large Stokes shift were facilely synthesized and characterized. These dyes show moderate absorptive coefficients and intense fluorescence emission, and excellent photo-stability in solvents and also in solid state. According to X-ray single-crystal analysis, non-covalent intermolecular interactions provide a rigid structure, which inhibits aggregate formation. These non-aggregated dyes can generate singlet oxygen under light irradiation, which makes them good candidates for photodynamic therapy. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: BOPIM; solid emission; photodynamic therapy; singlet oxygen

Introduction

Photodynamic therapy (PDT) has been pursued for several decades, primarily for treatment of cancer. During the therapy, the photosensitizers absorb light and then convert to triplet excited state through intersystem crossing (ISC) upon irradiation. The triplet state photosensitizers then transfer energy to molecular oxygen nearby to form singlet oxygen. Singlet oxygen is a highly reactive species used to attack cancer cells, causing destruction through direct cellular damage. Compared to other therapies, PDT has a number of advantages, such as non-invasive nature, high selectivity, safe treatment without initiating drug resistance, fast healing process and minimal side effects. A large number of photosensitizers have been reported for PDT study, and some of them are commercially available, such as porphyrins,[1] cyanines[2] and squarine dyes.[3] However, their practical application as photosensitizer is limited, since an ideal photosensitizer should meet all these requisites, including chemical and photo-stability, excellent ability to generate singlet oxygen under light irradiation, resistance to aggregation in aqueous environment, especially under high concentration, facile availability etc.

Recently, it was found that highly stable boron–fluorine dyes (4-bora-4,4-difluoro-pyrromethene, BODIPY) exhibit excellent photodynamic activity compared to commercial Rose Bengal.[4] In order to realize high-resolution control of generation of reactive oxygen species, environment-sensitive BODIPY for singlet oxygen generation was synthesized and studied.[5] However, most BODIPY derivatives tend to aggregate under concentrated solution, which leads to inefficient formation and potential quenching of the triplet state and singlet oxygen. Therefore the photosensitizers were only tested in dilute solutions, which restrict the generation of total amount of reactive oxygen species. Actually, non-aggregated BODIPYs and their derivatives have been successfully designed and synthesized, but no photodynamic activities have been reported for these dyes.[6] Recently we reported a new class of non-aggregated boron–fluorine derivatives, which emit intense fluorescence in solid state with large Stokes shift.[7] These dyes form a rigid structure through intermolecular non-covalent interactions to resist planar π–π stacking, thus alleviating aggregate formation. Also, efficient intramolecular charge transfer (ICT) helps to increase the Stokes shift. It occurred to us that these non-aggregated dyes should exhibit efficient intersystem crossing and generate singlet oxygen with high quantum yield. Heavy atoms incorporated into the structure have been reported to improve spin–orbit coupling to facilitate intersystem crossing. In this report, different halogen atoms (Br, I) were introduced into the structure to investigate the photodynamic activity of these non-aggregated boron-fluorine derivatives.

Results and Discussion

Synthesis and Crystal Structure

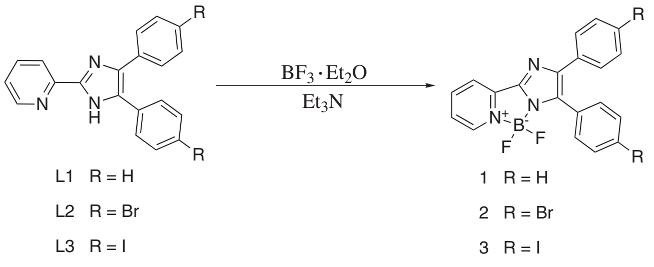
BOPIM dyes were prepared by straightforward method as shown in Scheme 1. All these dyes have been thoroughly characterized by ^{1}H NMR, ^{13}C NMR and MS.

Crystals of BOPIM **1** and **2** suitable for X-ray measurement were obtained through slow evaporation of organic solvents. Compound **1** produces a rigid structure through intermolecular supramolecular interactions,[7b] and this phenomenon was also found for BOPIM **2**. As shown in Fig. 1, the boron atom is in the same plane containing atoms N1, N2, C5 and C6; the chromophore core is near-planar with the twisted angle between pyridine and imidazole ring being 0.80°, which shows good planarity of the main chromophore. With adjacent molecular donors, atom F1 interacts with atoms on pyridine ring to form halogen bonds, such as B-F1&cdbond;H23 (2.636 Å), and B-F1&cdbond;C1 (3.143 Å). However, F2 associates with H11 on the terminal phenyl ring with a distance 2.457 Å. The terminal Br1 atom also contributes to the supramolecular interaction by association with H36 on the phenyl ring with a distance 3.045 Å.

---

* *Correspondence to: Feijun Dan, Shuzhang Xiao, Hubei Key Laboratory of Natural Products Research and Development, College of Chemistry and Life Science, China Three Gorges University, Hubei Yichang 443002, People's Republic of China. E-mail: shuzhangxiao@gmail.com*

Hubei Key Laboratory of Natural Products Research and Development, College of Chemistry and Life Science, China Three Gorges University, Hubei Yichang 443002, People's Republic of China



Scheme 1. Synthesis of BOPIMs.

Atom Br2 interacts with adjacent Br atom (3.618 Å), and H36 on the phenyl ring (3.000 Å). With these non-covalent interactions, molecules were linked together to form non-parallel interlayered structure, which helps to prevent intermolecular planar π–π stacking. As a result, there is only slight overlap between intermolecular chromophore cores due to the rigid supramolecular network, and all of the molecules are induced to array parallel with the identical conformation and orientation in the crystal (Fig. 1). Compared to BOPIM **1**, structure **2** was even more resistant to aggregation due to the supramolecular interactions of Br atoms.

Photophysical properties

The photophysical properties of BOPIM dyes were tested in mid-polar and polar solvents, such as THF, THF/H_2O (1:1) and DMF. Most dyes, including typical BODIPY, would aggregate to present broad absorptive and fluorescent bands in polar solvents.[8] However, no aggregate formation was observed at all for these three BOPIM dyes, since they still exhibit narrow absorption and emission bands (Fig. 2). This indicates that these dyes are resistant to aggregation in aqueous conditions and even in solid state, due to the inhibition of planar π–π stacking as

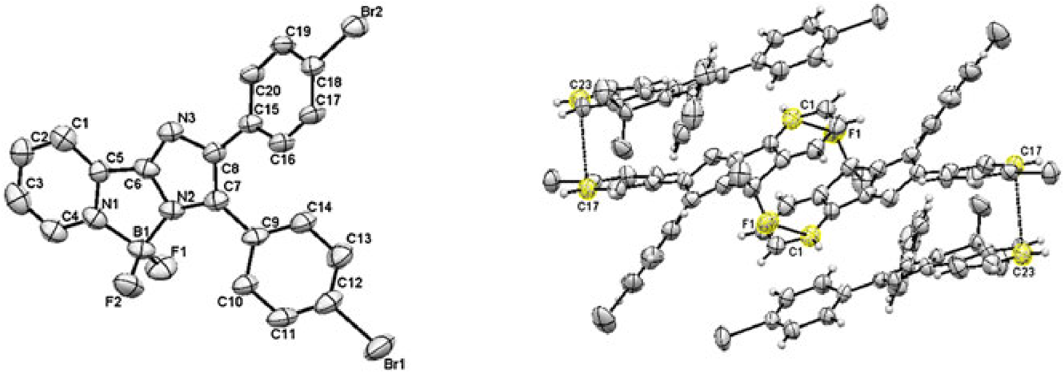


Figure 1. Single-crystal structure and packing mode of BOPIM **2**.

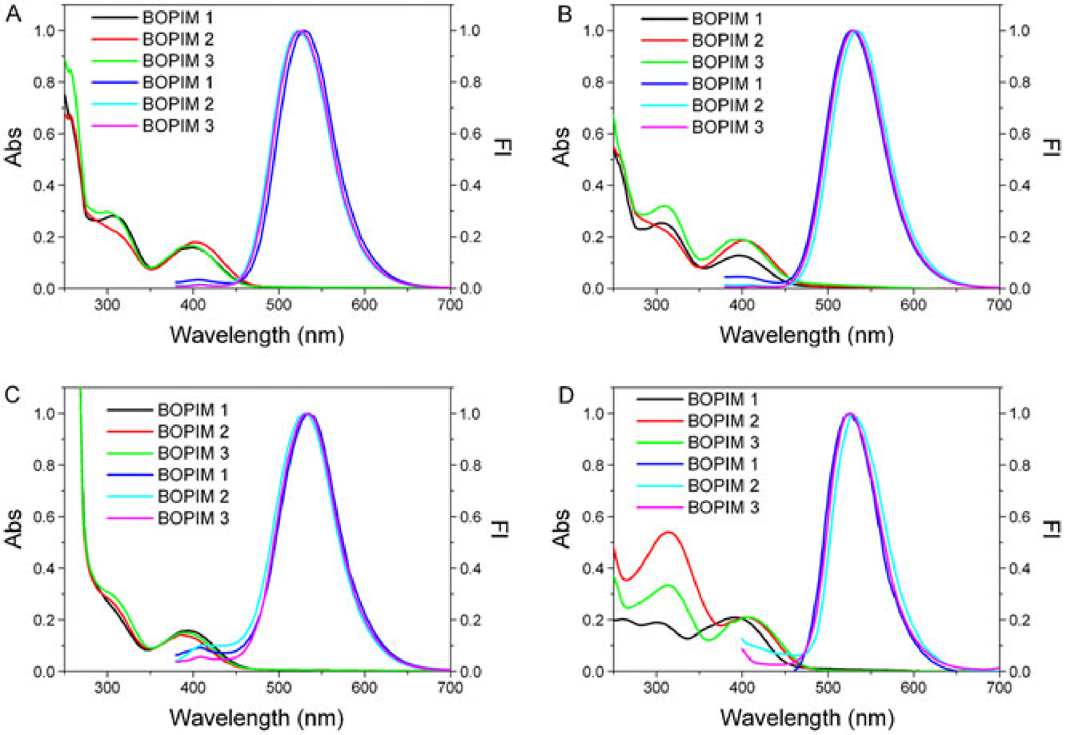


Figure 2. Absorption and fluorescence spectra of BOPIM dyes in THF (A), THF/H_2O, 1:1 (B), DMF (C) and in solid state (D).

revealed by X-ray single-crystal measurement. According to Fig. 2, the substitution of H atoms by halogen atoms induces a slight bathochromic shift. For BOPIM **1** in THF/H_2O (1:1), the absorption band was centered at 397 nm, and the fluorescent emission was observed at 528 nm. BOPIM **2** and **3** absorb at 403 and 401 nm, and fluoresce at 534 and 529 nm, respectively. The bathochromic shift should be due to enlargement of the π system, as a result of p–π conjugation.

Besides their sharp emission bands, the non-aggregated mode makes them exhibit comparatively high fluorescence quantum yields in aqueous condition, and even in solid state. Fluorescence quantum yields under all conditions were over 0.15. Especially for BOPIM **2** with Br atoms, its fluorescence quantum yields in polar solvent (DMF) and in solid state were even higher than those in non-polar solvents (Table 1), which is interesting and unusual for boron–fluorine dyes. In solid state, these BOPIMs also exhibit intense fluorescent emission. The absolute fluorescent quantum yields of BOPIM **1**, **2**, **3** in solid film were calculated by the integrating sphere method, and estimated to be 0.18, 0.24 and 0.16, respectively. This indicates that the presence of Br atoms can enhance the quantum yield. One factor is the enlargement of the π system resulting from p–π conjugation. The non-covalent interactions of Br atoms could be another key factor, since it can help to further alleviate aggregate formation as revealed by X-ray analysis. However, heavy atoms are known quenchers and thus the quantum yield of **3** is lower. The non-aggregated modes maintain their fluorescence in solid state, but on the other hand increases their lifetime of the excited triplet states, which makes them potential candidates as photosensitizers for photodynamic therapy.

Generation of Singlet Oxygen

The ability of BOPIM dyes to generate singlet oxygen under light irradiation was determined in a mixture of THF and water (1:1). The appearance of singlet oxygen was detected by trapping with 1,3-diphenylisobenzofuran (DPBF) and followed by monitoring the absorptive decrease at 410 nm. The DPBF has an absorption maximum at 410 nm and forms a colorless endoperoxide product when it reacts with singlet oxygen. In each entry, samples (10 μM) and DPBF (50 μM) were irradiated with light from an iodine–tungsten lamp. It is worth noting that the absorption of BOPIM dyes overlaps with DPBF on some level. To avoid the direct irradiation of DPBF, the irradiative wavelength should not be shorter than 450 nm; thus a filter was adopted to remove wavelengths below 450 nm. The blank test proves that DPBF shows slight degradation after 10 min irradiation under this condition, while all BOPIM dyes exhibit excellent photo-stability (there is no absorption decrease at all after 1 h light irradiation for all these three dyes). Since the absorption of BOPIMs and DPBF overlaps, the consumption of DPBF can be calculated from the equation $\Delta A_{DPBF} = \Delta A_{total} - A_{BOPIM}$. The time-dependent DPBF consumption is shown in Fig. 3.

Table 1. Fluorescence quantum yields of non-aggregated BOPIM dyes

	THF	THF/H_2O (1:1)	DMF	Solid
BOPIM **1**	0.17	0.22	0.15	0.18
BOPIM **2**	0.19	0.22	0.24	0.24
BOPIM **3**	0.22	0.23	0.17	0.16

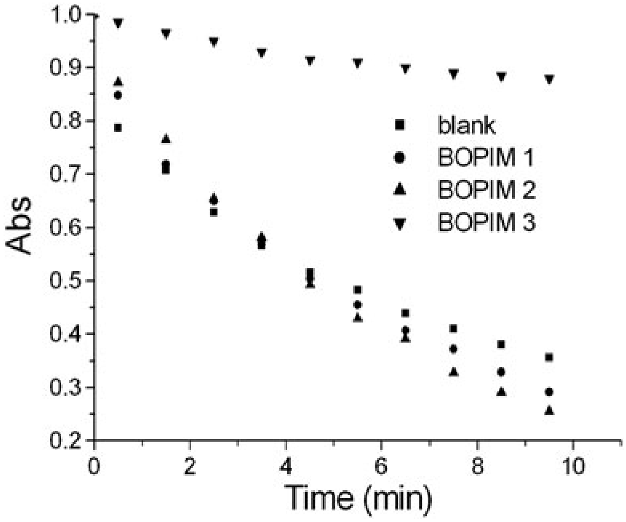


Figure 3. Variation of absorbance at 410 nm of DPBF in the presence of BOPIMs.

Although the absorption of BOPIM dyes is small above 450 nm (Fig. 2), these dyes still show photosensitizing properties under daylight irradiation. After 30 min light irradiation, DPBF degrades 80% for **1**, **2**, **3** respectively (blank DPBF degrades 10%). For BOPIM **1**, the DPBF band was consumed with a half-life of 5 min. The half-lives for BOPIM **2** and **3** were 4.5 and 4.2 min respectively, indicating that heavy atoms introduced at the end of the phenyl ring contribute little to the photosensitizing character. However, it is expected to improve the singlet oxygen generation efficiency by placing the heavy atoms directly on the chromophore core.[9] For these compounds, it is hard to evaluate the absolute quantum yield of $^1O_2$ for two reasons: one is the overlapping of the absorption of BOPIMs and DPBF; the other is that DPBF decomposes to some degree during light irradiation.

Conclusions

We have synthesized a series of novel boron–fluorine derivatives bearing halogen atoms. These dyes show solid-emissive properties due to non-covalent intermolecular interactions, which inhibit aggregate formation. They can also generate singlet oxygen under light irradiation. Future work will tune the absorptive band to longer wavelengths, especially in the near-infrared region, and introduce halogen atoms directly on to the BOPIM core.

Experimental

Measurements

^{1}H NMR and ^{13}C NMR spectra were recorded on Varian or Bruker instruments, using $CDCl_3$ as solvent. Chemical shifts are reported in ppm with $CDCl_3$ as reference (7.26 ppm for ^{1}H NMR, and 77.0 ppm for ^{13}C NMR). UV–visible and fluorescent measurements were carried out on Hitachi U-3010 and F-4500 instruments, respectively. Mass spectral data were recorded on a Waters Quattro Micro API mass spectrometer and elemental analyses on a Vario EL III. The fluorescent quantum yield was calculated using rhodamine B as reference in solution, and the integrating sphere method in solid state.

X-Ray Structural Analysis of BOPIM 2

The X-ray crystal structural analysis of **2** was carried out at room temperature (293(2) K) on a Bruker Smart CCD area-detector diffractometer with graphite- monochromated Mo KR radiation ($\lambda = 0.71073$ Å). Formula weight: 502.96. Crystal system: triclinic. Space group: P-1. Unit cell dimensions: $a = 12.098(10)$ Å, $b = 13.022$ (12) Å, $c = 13.279(12)$ Å, $\alpha = 85.69(3)^\circ$, $\beta = 84.90(3)^\circ$, $\gamma = 66.276(18)$. Volume $V = 1906(3)$ Å^3. $Z = 4$. Absorption coefficient $\mu = 4.283$. $F(000)$: 984. Crystal size: $0.20 \times 0.20 \times 0.20$ mm^3. Theta range for data collection: 2.25–27.58°. Index ranges: $-15 \leq h \leq 15$, $-16 \leq k \leq 16$, $-17 < l \leq 17$. Reflections collected: 20470. Independent reflections: 8717 [R(int) = 0.1685]. Completeness to $\theta = 27.58$: 99%. Data/restraints/parameters: 8717/0/505. Goodness-of-fit on F^2: 0.881. Final R indices [$I > 2\sigma(I)$]: $R1 = 0.0741$, $wR2 = 0.1781$. R indices (all data): $R1 = 0.1120$, $wR2 = 0.1997$. Largest difference peak and hole: 1.044 and −1.345 e Å^{-3}. Structure solution and refinement were accomplished using WinGX and SHELXL-97. The data have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 870758. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre, via www.ccdc.cam.ac.uk/data request/cif.

Singlet Oxygen Formation

Dye solutions (10 μM) were prepared in a mixture of THF and water (1:1). An aliquot of DPBF (50 μM) was added and irradiated using a 500 W iodine–tungsten lamp with a yellow filter to remove light at $\lambda < 450$ nm. This avoids direct excitation of the DPBF molecule, which absorbs at 410 nm.

Synthesis

Ligands were synthesized following a previously reported procedure.[10] Typical synthesis of boron–fluorine complexes was described as follows.

The ligand (4 mmol) was dissolved in anhydrous dichloromethane (30 ml), followed by addition of triethylamine (4.46 ml, 32 mmol) under N_2. This mixture was cooled by ice, and then $BF_3.Et_2O$ (5.10 ml, 40 mmol) was added dropwise. The reaction mixture was stirred at room temperature overnight. After quenching with water, the organic phase was separated and the aqueous phase was extracted with dichloromethane. The combined organic phases were washed with water, dried over Na_2SO_4 and evaporated *in vacuo*. The crude residue was subjected to a silica gel column (petroleum ether:ethyl acetate = 1:4) to afford target product as a yellow solid. Besides the target product, the starting material can be recycled.

BOPIM ***1***

32%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.43 (d, $J = 5.4$ Hz, 1 H, Py-6-*H*), 8.16 (m, 1 H, Py-4-*H*), 8.09 (d, $J = 7.8$ Hz, 1 H, Py-3-*H*), 7.60 (m, 4 H, Ph-2-*H*), 7.46 (m, 1 H, Py-5-*H*), 7.36 (m, 6 H, Ph-3-*H* + Ph-4-*H*). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 144.68 (Py), 141.26 (Py), 134.56 (Ph), 130.66 (Ph), 128.68 (Im), 128.59 (Im), 128.34 (Py), 128.14 (Ph), 127.31 (Ph), 122.78 (Ph), 117.73 (Py). ^{19}F NMR ($CDCl_3$, 376 MHz): δ −155.4, −155.4. IR (KBr): $\nu = 1634$ (C N), 1143 (B-F), 994 (B-N), 958 (B-N) cm^{-1}. UV–visible (THF): λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}) = 404 (1.72×10^4). Ms [M + H]$^+$ 346.4, Calcd 346.1. Anal. Calcd for $C_{20}H_{14}BF_2N_3$: C, 69.60; H, 4.09; N, 12.17. Found: C, 68.94; H, 4.07; N, 12.02. HRMS calcd for $C_{20}H_{15}BF_2N_3$ [M + H]$^+$ 346.1325. Found 346.1315.

BOPIM ***2***

28%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.39 (d, $J = 5.6$ Hz, 1 H, Py-6-*H*), 8.16 (t, $J = 8.0$ Hz, 1 H, Py-4-*H*), 8.02 (d, $J = 8.0$ Hz, 1 H, Py-3-*H*), 7.40 (m, 9 H, Py-2-*H* + Py-5-*H* + Ph-*H*). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.86 (Py), 141.42 (Py), 133.30 (Ph), 132.01 (Ph), 131.62 (Im), 130.22 (Im), 129.62 (Py), 123.17 (Ph), 122.61 (Ph), 121.53 (Ph), 117.87 (Py). ^{19}F NMR ($CDCl_3$, 376 MHz): δ −155.1, −155.2. IR (KBr): $\nu = 1636$ (C N), 1138 (B-F), 1065 (C-Br), 997 (B-N), 951 (B-N) cm^{-1}. UV–visible (THF): λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}) = 387 (1.82×10^4). Ms [M]$^+$ 500.9. Calcd 500.9. Anal. Calcd for $C_{20}H_{12}BBr_2F_2N_3$: C, 47.76; H, 2.40; N, 8.35. Found: C, 47.61; H, 2.42; N, 8.24.

BOPIM ***3***

20%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.50 (d, $J = 5.2$ Hz, 1 H, Py-6-*H*), 8.27 (t, $J = 7.6$ Hz, 1 H, Py-4-*H*), 8.13 (d, $J = 8.0$ Hz, 1 H, Py-3-*H*), 7.75 (d, $J = 8.4$ Hz, 2 H, Ph-2-*H*), 7.69 (d, $J = 8.0$ Hz, 2 H, Ph-2′-*H*), 7.58 (t, $J = 6.4$ Hz, 2 H, Py-5-*H*), 7.36 (m, 4 H, Ph-3-*H* + Ph-3′-*H*). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.92 (Py), 141.40 (Py), 137.93 (Ph), 137.55 (Ph), 133.89 (Im), 130.31 (Py), 129.93 (Im), 129.79 (Ph), 123.14 (Ph), 117.92 (Py), 94.41 (Ph), 93.15 (Ph). ^{19}F NMR ($CDCl_3$, 376 MHz): δ −155.1, −155.2. IR (KBr): $\nu = 1633$ (C N), 1138 (B-F), 1066 (C-I), 998 (B-N), 953 (B-N) cm^{-1}. UV–visible (THF): λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}) = 387 (1.84×10^4). Ms [M]$^+$ 596.8, Calcd 596.9. Anal. Calcd for $C_{20}H_{12}BF_2I_2N_3$: C, 40.24; H, 2.03; N, 7.04. Found: C, 40.11; H, 2.08; N, 6.96.

Acknowledgements

We are grateful for the financial support from the National Natural Science Foundation of China (21002059), and China Three Gorges University (2011CX089, KJ2010B035).

References

[1] a) A. Wiehe, Y. M. Shaker, J. C. Brandt, S. Mebs, M. O. Senge, *Tetrahedron* **2005**, *61*, 5535; b) M. B. Bakar, M. Oelgemoller, M. O. Seger, *Tetrahedron* **2009**, *65*, 7064; c) V. Sarrazy, G. Garcia, J. P. MBakidi, C. L. Morvan, G. Begaud-Grimaud, R. Granet, V. Sol, P. Krausz, *J. Photochem. Photobiol. B* **2011**, *103*, 201; d) J. F. Lovell, M. W. Chan, Q. Qi, J. Chen, G. Zheng, *J. Am. Chem. Soc.* **2011**, *133*, 18580. e)M. Ethirajan, Y. Chen, P. Joshi, R. K. Pandey, *Chem. Soc. Rev.* **2011**, *40*, 340.

[2] a) A. R. Oseroff, D. Ohuoha, G. Ara, D. Mcauliffe, J. Foley, L. Cincotta, *Proc. Natl Acad. Sci. USA* **1986**, *83*, 9729. b) E. Delaey, F. van Laar, D. De Vos, A. Kamuhabwa, P. Jaobs, P. De Witte, *J. Photochem. Photobiol. B* **2000**, *55*, 27. c) M. P. A. Williams, M. Ethirajan, K. Ohkubo, P. Chen, P. Pera, J. Morgan, W. H. White III, M. Shibata, S. Fukuzumi, K. M. Kadish, R. K. Pandey, *Bioconjugate Chem.* **2011**, *22*, 2283. d) J. F. Xiang, Y. X. Liu, D. Sun, S. J. Zhang, Y. L. Fu, X. H. Zhang, L. Y. Wang, *Dyes Pigments* **2012**, *93*, 1481; e)Y. Koide, Y. Urano, A. Yatsushige, K. Hanaoka, T. Terai, T. Nagano, *J. Am. Chem. Soc.* **2009**, *131*, 6058.

[3] a) E. Arunkumar, P. K. Sudeep, K. V. Kamat, B. Bruce Noll, B. D. Smith, *New J. Chem.* **2007**, *31*, 677; b) J. J. Lee, A. Goncalves, B. A. Smith, R. Palumbo, A. G. White, B. D. Smith, *Aust. J. Chem.* **2011**, *64*, 604; c) J. M. Baumes, J. J. Gassensmith, J. Giblin, J. J. Lee, A. G. White, W. J. Culligan, W. Matthew Leevy, M. Kuno, B. D. Smith, *Nat. Chem.* **2010**, *2*, 1025; d) P. Salice, J. Arnbjerg, B. W. Pedersen, R. Toftegaard, L. Beverina, G. A. Pagani, P. R. Ogilby, *J. Phys. Chem. A* **2010**, *114*, 2518.

[4] T. Yogo, Y. Urano, Y. Ishitsuka, F. Maniwa, T. Nagano, *J. Am. Chem. Soc.* **2005**, *127*, 12162.

[5] T. Yogo, Y. Urano, A. Mizushima, H. Sunahara, T. Inoue, K. Hirose, M. Iino, K. Kikuchi, T. Nagano, *Proc. Natl Acad. Sci. USA* **2008**, *105*, 28.

[6] a) Y. Zhou, Y. Xiao, D. Li, M. Y. Fu, X. H. Qian, *J. Org. Chem.* **2008**, *73*, 1571; b) Y. Kubota, T. Tsuzuki, K. Funabiki, M. Ebihara, M. Matsui, *Org. Lett.* **2010**, *12*, 4010; c) G. Q. Zhang, J. W. Lu, M. Sabat, C. L. Fraser, *J. Am. Chem. Soc.* **2010**, *132*, 2160; d) Y. Zhou, Y. Xiao, S. M. Chi, X. H. Qian, *Org. Lett.* **2008**, *10*, 633; e) J. F. Araneda, W. E. Piers, B. Heyne, M. Parvez, R. McDonald, *Angew. Chem. Int. Ed.* **2011**, *50*, 12214; f) Y. Kubota, H. Hara, S. Tanaka, K. Funabiki, M. Matsui, *Org. Lett.* **2011**, *13*, 6544.

[7] a) M. F. Mao, R. H. Zhang, S. Z. Xiao, K. Zou, *J. Coord. Chem.* **2011**, *64*, 3303; b) M. F. Mao, S. Z. Xiao, J. F. Li, Y. Zou, R. H. Zhang, J. R. Pan, F. J. Dan, K. Zou, T. Yi, *Tetrahedron* **2012**, *68*, 5037.

[8] Y. Tokoro, A. Nagai, Y. Chujo, *Tetrahedron Lett.* **2010**, *51*, 3451.

[9] L. Beverina, A. Abbotto, M. Landenna, M. Cerminara, R. Tubino, F. Meinardi, S. Bradamante, G. A. Pagani, *Org. Lett.* **2005**, *7*, 4257.

[10] X. J. Wu, R. Jiang, X. P. Xu, X. M. Su, W. H. Lu, X. J. Ji, *J. Comb. Chem.* **2010**, *12*, 839.