

Photosensitizers

Near-IR Absorbing BODIPY Derivatives as Glutathione-Activated Photosensitizers for Selective Photodynamic Action

Ilke Simsek Turan,^[a] Fatma Pir Cakmak,^[b] Deniz Cansen Yildirim,^[c] Rengul Cetin-Atalay,^[c] and Engin U. Akkaya^{*[a, b]}

Abstract: Enhanced spatiotemporal selectivity in photonic sensitization of dissolved molecular oxygen is an important target for improving the potential and the practical applications of photodynamic therapy. Considering the high intracellular glutathione concentrations within cancer cells, a series of BODIPY-based sensitizers that can generate cytotoxic singlet oxygen only after glutathione-mediated cleavage of the electron-sink module were designed and synthesized. Cell culture studies not only validate our design, but also suggest an additional role for the relatively hydrophobic quencher module in the internalization of the photosensitizer.

tosensitivity is still an issue, often leading to painful edema for patients undergoing PDT treatment.^[4] Thus, more selective PDT sensitizers are needed to remove any chance of off-target sensitization. In principle, this can be done in a number of ways.^[5] Previously, we reported a photosensitizer, the activity of which is dependent on the pH and ion concentrations. Other groups have reported quenched photosensitizers, by covalent attachments to carbon nanotubes, carotenoids, and the commercial quencher BHQ₃. Self-quenching of the photosensitizer is another route for activity control.

The removal of quencher module can be accomplished enzymatically (by caspase 3, cathepsin B, β -galactosidase, β -lactamase or trypsin, among others). Unfortunately, in most cases,

More than one hundred years after the initial observation of photodynamic action,^[1] generation of cytotoxic singlet oxygen by photosensitization of molecular oxygen through the intermediacy of a dye continues to attract considerable attention.^[2] This is mostly due to its medical applications, which is the photodynamic therapy (PDT) of various cancers and non-cancerous indications.^[3] One attractive feature of photodynamic therapy is in its built-in selectivity, which results from the fact that the exciting light can be targeted to the tumor region, thus minimizing accidental excitation of the sensitizer dye at undesired locations. In practice, however, pho-

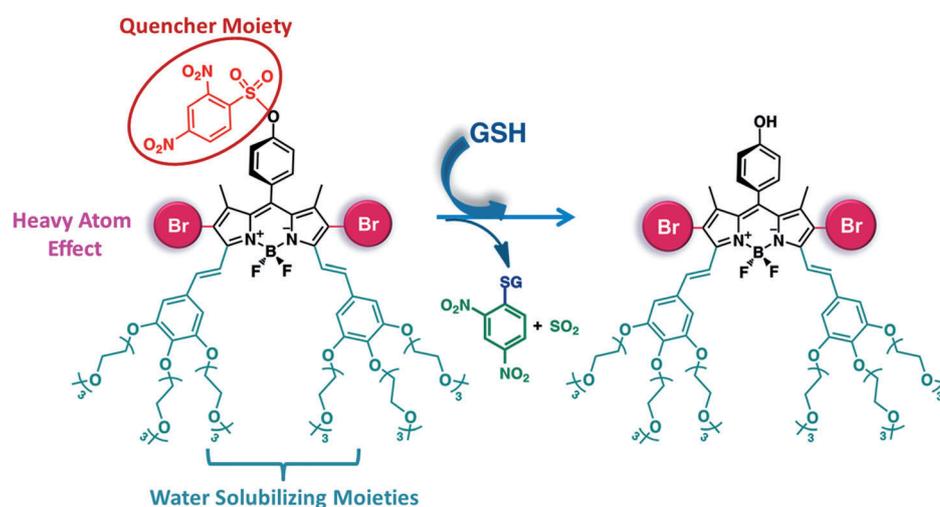


Figure 1. Mode of operation for the GSH-mediated activation of caged photosensitizers.

the change in photosensitizer activity was less than tenfold. Our goal was to design a quenched photosensitizer, which is only capable of singlet oxygen generation after a glutathione (GSH)-mediated reaction that results in the removal of the quencher moiety (Figure 1). Such compounds could aptly be called "caged photosensitizers". It should also be noted here that cancer cells reportedly have much higher concentration of GSH (up to 1000-fold) compared with normal cells.^[6]

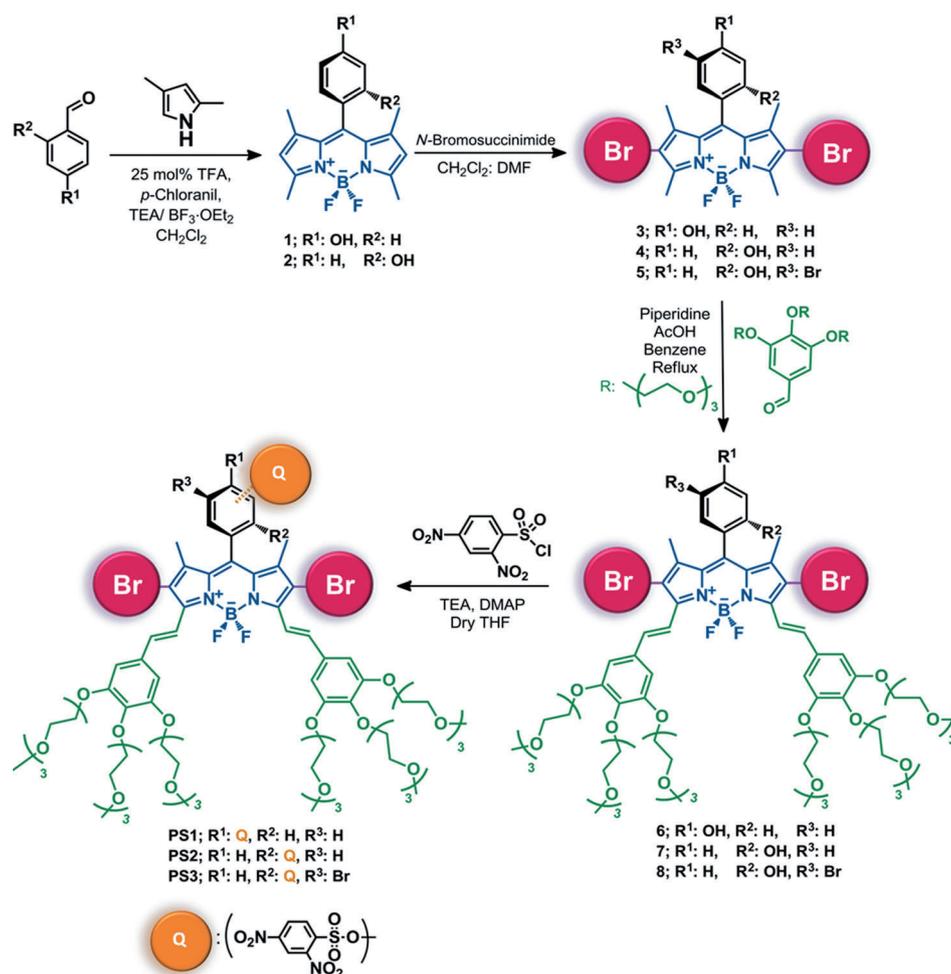
To realize this goal, we set out to synthesize the target compounds depicted in Scheme 1 as caged sensitizers, to be released on a triggering GSH reaction. The design includes a 2,4-dinitrobenzenesulfonate group, which was previously shown

[a] I. S. Turan, Prof. Dr. E. U. Akkaya
UNAM-Institute of Material Science and Nanotechnology
Bilkent University, Ankara, 06800 (Turkey)
E-mail: eua@fen.bilkent.edu.tr

[b] F. P. Cakmak, Prof. Dr. E. U. Akkaya
Department of Chemistry, Bilkent University, Ankara, 06800 (Turkey)

[c] D. C. Yildirim, R. Cetin-Atalay
Department of Molecular Biology and Genetics
Bilkent University, Ankara, 06800 (Turkey)

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Scheme 1. Synthesis of the caged photosensitizers (PS1–3) and the control compounds (6–8). TFA = trifluoroacetic acid, TEA = triethylamine, DMAP = 4-dimethylaminopyridine.

to be susceptible to thiol mediated cleavage.^[7] The design can be abbreviated as PS-Q, Q being the electron sink 2,4-dinitrobenzenesulfonyl moiety, which quenches the excited state of the photosensitizer (PS) by providing an alternative, non-radiative relaxation path.

The core photosensitizer was based on the BODIPY chromophore, bromo or iodo-substituted derivatives of which have been shown on many occasions to be good candidates for photodynamic photosensitizers.^[8] The synthesis work was straightforward; hydroxyphenyl-BODIPY derivatives were synthesized, sulfonated, and chromatographically purified. It is important to functionalize the dyes with heavy atoms to ensure an enhanced spin-orbit coupling, and thus faster rates of intersystem crossing (ISC), which in turn translates into more efficient singlet oxygen generation. BODIPY dyes without such modifications are known to be very poor photosensitizers unless they are arranged as orthogonal dimers.^[8b,9] Oligoethyleneglycol moieties were added to improve the water solubility. Non-sulfonated BODIPY dyes 6–8 (*meso*-hydroxyphenyl-BODIPY derivatives) were utilized as control compounds for studying the change in activity in response to the removal of the dinitrobenzenesulfonate groups. All new compounds were

characterized analytically. Once the synthesis and characterization were completed, we first studied the reaction with GSH spectroscopically.

Under physiological conditions, all three sulfonates can be cleaved at reasonable rates, a process that can be followed by spectral shifts in the electronic absorption spectra. In the cleavage reaction of PS1, the major band in the visible region shows a 35 nm blueshift (Figure 2A). Other photosensitizers also show such changes in the absorption. The cleavage of the quencher moiety can also be followed by the changes in the emission spectra. In 30 min, more than 80% of the PS1 can be uncaged (see the Supporting Information). For the halogenated derivatives, intersystem crossing competes very effectively with the fluorescence emission process, resulting in low emission quantum yields.

Nevertheless, the quenching due to ISC is less than the essentially total quenching generated by the 2,4-dinitrobenzenesulfonyl moiety; thus, the emission enhanced at 683 nm is reported as another indication of the progress of the cleavage reaction (Figure 2B). As expected, the singlet oxygen generation capacity is nil when the quencher module Q is intact. But, when it is removed by the reaction with GSH, the residual BODIPY moieties are very effective in singlet oxygen generation (Figure 3). The change in the singlet oxygen generation capacity looks like an “off-on” process, demonstrating the effectiveness of the 2,4-dinitrophenyl moiety in quenching, and hence the “uncaging” process. So, it is clear that GSH at physiologically relevant concentrations is capable of transforming an ineffective chromophore into a very effective sensitizer that generates singlet oxygen when excited within the therapeutic window (in this case at 660 nm).

Next, we wanted to demonstrate the effectiveness of intracellular GSH in activating our “caged” photosensitizers. To that end, a number of human epithelial cancer cells in culture (Huh7, MCF7, and HCT116) were tested with the photosensitizers, and as a control, photosensitizers with no quencher group (in other words, the active photosensitizers obtained when the Q group is removed) were also included.

Based on our results with chemical trap studies of singlet oxygen generation, we expected higher photocytotoxic activity on cancer cells with the control series. The cell culture studies

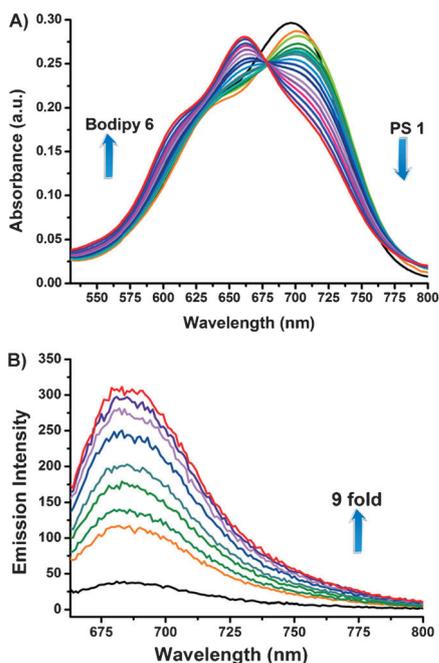


Figure 2. (A) Absorption spectra of **PS1** (20.0 μM), and (B) emission spectra of **PS1** (4.0 μM) upon addition of 50 equivalents of GSH in DMSO/1X PBS buffer (50:50, v/v, pH 7.4), incubated for 120 min. Excitation (λ_{ex}) is at 655 nm. Corresponding data for **PS2** and **PS3** are available in the Supporting Information.

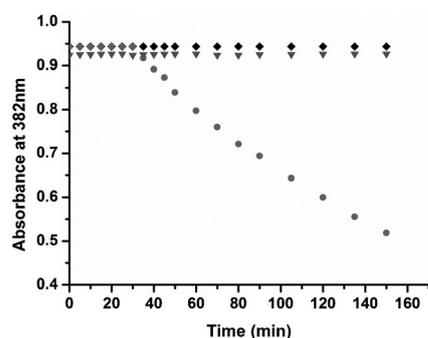


Figure 3. Absorbance values of the trap molecule (2,2'-(anthracene-9,10-diyl)bis(methylene)dimalonic acid) in DMSO/1X PBS (50:50, v/v, pH: 7.4). BODIPY-6, or **PS1** were introduced at 4 μM concentration, except for the control run. Irradiation at 660 nm was initiated at $t=30$ min (■ trap molecule alone, ● BODIPY-6, ▼ **PS1**). The light source was an LED array at 0.2 mW fluence rate.

were all done in triplicate with most of our caged photosensitizers were found to be effective. Some had relatively high dark toxicity, which was checked by additional experiments to isolate the contribution of photocytotoxicity.

The BODIPY dyes were tested both in the dark and under irradiation with red LED light. The results are presented in Table 1. In one particular colon cancer cell line, HCT116, we obtained a remarkable IC_{50} value of 20.0 nM under irradiation for **PS1**. The IC_{50} value without light is much higher, suggesting that much lower concentrations will be effective under the photodynamic regime.

Table 1. IC_{50} values of sensitizers with the HCT116 cell line. ^[a]		
Sensitizers	Red LED irradiation for 4 h, IC_{50} [μM]	No light, IC_{50} [μM]
BODIPY-6	0.35 ± 0.10	0.35 ± 0.16
BODIPY-7	0.64 ± 0.11	0.42 ± 0.27
BODIPY-8	0.43 ± 0.12	0.75 ± 0.04
BODIPY-11	0.04 ± 0.02	0.20 ± 0.03
PS1	0.02 ± 0.003	4.38 ± 0.03
PS2	0.02 ± 0.004	0.29 ± 0.11
PS3	< 0.06	0.36 ± 0.10
NC ^[b]	no inhibition	no inhibition
PS1 ^[c]	no inhibition	no inhibition

[a] IC_{50} values of sensitizers with the HCT116 cell line after 72 h of incubation with indicated sensitizers. [b] NC: Negative control compound, 1,3,5,7-tetramethyl-BODIPY. [c] The effect of **PS1** on MRC-5 human fetal lung fibroblast cells. The experiments were performed in triplicate.

It is both surprising and noteworthy that when control compounds (**6–8**) were introduced, we observed that their photocytotoxicity is significantly lower. This may be due to reduced cell permeability and lipid solubility. To investigate this matter further, we synthesized a putative positive control, compound **11**, where $R^1 = OCH_3$ and $R^2 = R^3 = H$. Control compound **11** is effective as a photosensitizer as predicted as it is unionizable and expected to be cell-permeable. Cellular uptake is an essential parameter for controlling the photocytotoxicity and even changes in the substitution pattern have an effect on intracellular availability.^[10]

However, it is clear that since the photosensitizers are in the caged form (**PS-Q**), they are not capable of producing singlet oxygen, but once the photosensitizers are inside the cells, intracellular GSH apparently cleaves the quencher module, and thus releases the active photosensitizer. Using fluorescence microscopy, (Figure 4) fluorescent-labeled Annexin-V and Hoechst-33258 co-staining show that cells clearly undergo apoptosis on irradiation in the presence of 20 nM of caged sensitizer **PS1**. This is demonstrated by the dense incorporation of the nuclear stain Hoechst-33258, and FITC-Annexin-V la-

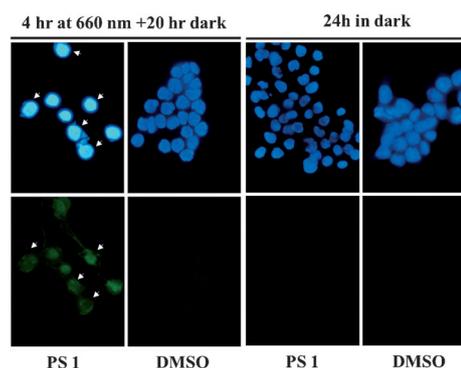


Figure 4. Fluorescence microscope images of Annexin-V-fluoro stained HCT116 cells in the presence of 40 nM sensitizer **1**. Cells were either subjected to red LED irradiation at 660 nm for 4 h, followed by 20 h incubation in the dark, or 24 h of incubation in the dark. Hoechst-33258 stains nuclear DNA in all conditions. Arrows point to the apoptotic cells with fragmented chromatin (bright blue) and Annexin V positive membrane (green) corroborating apoptosis in the presence of light and **PS1**. Images were captured at 100× magnification.

belonging of the exposed phosphatidylserines on the outer cytoplasmic membrane. Without red light irradiation, cells show no such changes, keeping their usual appearance.

The response to the varying concentrations of the best performing sensitizers is also shown in the form of a bar graph (Figure 5).

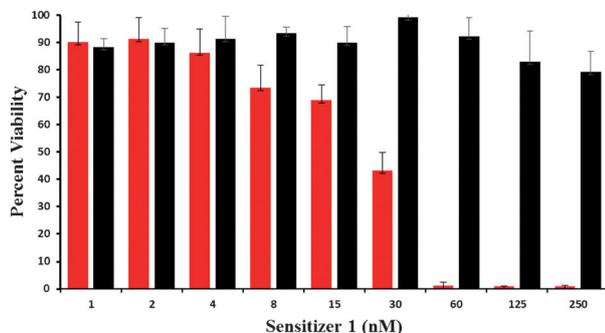


Figure 5. Percent viability of HCT116 cells was determined by NCI-SRB assay. Various concentrations of sensitizer **PS1** (1–250 nM) were used to determine cell death. Red bars show cell viability after 4 h irradiation with red LED, followed by 20 h incubation in the dark, black bars indicate cell viability following 24 h incubation in the dark.

Additional support for **PS1** inducing apoptosis in the HCT116 cell line was obtained by a fluorescence-activated cell sorting (FACS) analysis. The analysis shows that when the cells are treated with **PS1** and red light, the percentage of cells with fractional (sub-G1) DNA content increase significantly compared with the control (Figure S20 in the Supporting Information).

Finally, we are very pleased that **PS1** showed no apparent photocytotoxicity (or dark toxicity) on the MRC-5 (human fetal lung fibroblast cells) cell line, which is a normal cell line.

In conclusion, improved selectivity in any therapeutic agent is highly desirable. In this work, we took advantage of the activation (uncaging) of a photosensitizer by a cancer-related cellular parameter, glutathione concentration. Before the uncaging reaction, the PS-Q conjugate has low to negligible toxic activity on the selected cell cultures. GSH-mediated intracellular uncaging results in a highly active photodynamic agent. We are confident that as the stumbling blocks hindering the broader applicability of photodynamic therapy are removed, the methodology will be more effective competitor of the current established treatment protocols. We shall continue to do our part in providing chemical/photophysical avenues towards that end.

Experimental Section

Cell culture

HCT116 human colon carcinoma cells (ATCC) were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen GIBCO), with 10% fetal bovine serum (FBS) (Invitrogen GIBCO), glutamine (2 mM L), nonessential amino acids (0.1 mM), penicillin

(100 units mL⁻¹), and streptomycin (100 g mL⁻¹). MRC-5 human fetal lung fibroblast cells (ATCC) were maintained in Minimum Essential Medium (MEM) (Invitrogen GIBCO), with 10% FBS (Invitrogen GIBCO), glutamine (2 mM L), penicillin (100 units mL⁻¹), and streptomycin (100 g mL⁻¹) at 37 °C in a humidified incubator under 5% CO₂.

Sulforhodamine B assay

Cells were plated in 96-well plates (2000 cell/well in 150 μL) and grown for 24 h at 37 °C prior to treatment with different concentrations of sensitizers and negative control (0.25–0.0005 μM for sensitizers 1, 2, and 3; 5.0–0.06 μM for BODIPYs dissolved in DMSO). After 72 h of incubation, the medium was aspirated, washed once with 1X PBS (Gibco, Invitrogen), followed by addition of 50 μL of a cold (4 °C) solution of 10% (v/v) trichloroacetic acid (MERCK) for fixation. Then the plates were washed five times with dd-H₂O and were left to air-dry. A solution of sulforhodamine (50 μL, 0.4%, m/v; Sigma–Aldrich) in 1% acetic acid solution was then added to each well and left at room temperature for 10 min. The sulforhodamine B (SRB) solution was removed and the plates were washed five times with 1% acetic acid and left for air-drying. Protein-bound sulforhodamine B was solubilized in a Tris-base solution (200 μL, 10 mM) and the plates were shaken for 10 min on a plate shaker before the measurement of absorbance. The absorbance was read in a 96-well plate reader at 515 nm. Cells incubated in DMSO alone were used as controls for percent inhibition and IC₅₀ calculations either in irradiated plates (for 4 h) or the plates kept in dark. Percent inhibition (%) values were calculated with the given formula: 1–[average (OD of treated wells)/average (OD of DMSO treated cells)] × 100.

Detection of apoptosis

Cells were seeded onto coverslips in 6-well plates. After 24 h in culture, cells were treated with sensitizer 1 (40 nM/well). One group was irradiated with red LED at 625 nm for 4 h and kept 20 h in the dark. Another group was incubated in the dark for 24 h. Apoptosis was determined with Annexin-V-Fluos (Roche) staining together with Hoechst-33258 (Sigma–Aldrich) counterstaining that shows the nuclear condensation. Cells were washed twice with ice-cold 1X PBS. Hoechst-33258 staining was performed with 1 μg mL⁻¹ (final concentration) in each well followed by incubation for 10 min in the dark. Cells were destained with 1X PBS for 5 min. Then, Annexin-V-Fluos staining was carried out according to the manufacturer's recommendations (Roche). Slides were then analyzed under the fluorescence microscope (Nikon Eclipse 50).

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- [1] a) O. Raab, *Z. Biol.* **1900**, *39*, 524–546; b) A. Jesionek, H. von Tappeiner, *MMW Muench. Med. Wochenschr.* **1903**, *47*, 2042–2044; c) H. von Tappeiner, A. Jodlbauer, *Dtsch. Arch. Klin. Med.* **1904**, *80*, 427–487.
- [2] a) A. Kamkaew, S. H. Lim, H. B. Lee, L. V. Kiew, L. Y. Chung, K. Burgess, *Chem. Soc. Rev.* **2013**, *42*, 77–88; b) A. B. Ormond, H. S. Freeman, *Materials* **2013**, *6*, 817–840; c) O. J. Stacey, S. J. A. Pope, *RSC Adv.* **2013**, *3*, 25550–25564; d) S. G. Awuah, Y. You, *RSC Adv.* **2012**, *2*, 11169–11183.

- [3] a) W. M. Gallagher, L. T. Allen, C. O'Shea, T. Kenna, M. Hall, A. Gorman, J. Killoran, D. F. O'Shea, *Br. J. Cancer* **2005**, *92*, 1702–1710; b) S. G. Rockson, P. Kramer, M. Razavi, A. Szuba, S. Filardo, P. Fitzgerald, J. P. Cooke, S. Yousuf, A. R. DeVault, M. F. Renschler, D. C. Adelman, *Circulation* **2000**, *102*, 2322–2324; c) D. E. J. G. J. Dolmans, D. Fukurama, R. K. Jain, *Nat. Rev. Cancer* **2003**, *3*, 380–387; d) R. Waksman, P. E. McEwan, T. I. M. Moore, R. Pakala, F. D. Kolodgie, D. G. Hellinga, R. C. Seabron, S. J. Rychnovsky, J. Vasek, R. W. Scott, R. Virmani, *J. Am. Coll. Cardiol.* **2008**, *52*, 1024–1032; e) Y. Choi, R. Weissleder, C.-H. Tung, *Cancer Res.* **2006**, *66*, 7225–7229; f) T. J. Dougherty, C. J. Gomer, B. W. Henderson, G. Jori, D. Kessel, M. Korbelik, J. Moan, Q. Peng, *J. Natl. Cancer Inst.* **1998**, *90*, 889–905; g) T. J. Dougherty, J. E. Kaufman, A. Goldfarb, K. R. Weishaupt, D. Boyle, A. Mittleman, *Cancer Res.* **1978**, *38*, 2628–2635.
- [4] a) J. Gołab, D. Olszewska, P. Mróz, K. Kozar, R. Kamiński, A. Jallili, M. Jakóbiak, *Clin. Cancer Res.* **2002**, *8*, 1265–1270; b) H. Hirschberg, F. A. Uzal, D. Chighvinadze, M. J. Zhang, Q. Peng, S. J. Madsen, *Lasers Surg. Med.* **2008**, *40*, 535–542; c) M. S. Mathews, D. Chighvinadze, H. M. Gach, F. A. Uzal, S. J. Madsen, H. Hirschberg, *Lasers Surg. Med.* **2011**, *43*, 892–900.
- [5] a) S. Ozlem, E. U. Akkaya, *J. Am. Chem. Soc.* **2009**, *131*, 48–49; b) S. Erbas, A. Gorgulu, M. Kocakusakogullari, E. U. Akkaya, *Chem. Commun.* **2009**, 4956–4958; c) S. O. McDonnell, M. J. Hall, L. T. Allen, A. Byrne, W. M. Gallagher, D. F. O'Shea, *J. Am. Chem. Soc.* **2005**, *127*, 16360–16361; d) J. Chen, K. Stefflova, M. J. Niedre, B. C. Wilson, B. Chance, J. D. Glickson, G. Zheng, *J. Am. Chem. Soc.* **2004**, *126*, 11450–11451; e) G. Zheng, J. Chen, K. Stefflova, M. Jarvi, H. Li, B. C. Wilson, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 8989–8994; f) T. Yogo, Y. Urano, A. Mizusumus, H. Sunahara, T. Inoue, K. Hirose, M. Lino, K. Kikuchi, T. Nagano, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 28–32.
- [6] a) Z.-B. Zheng, G. Zhu, H. Tak, E. Joseph, J. L. Eiseman, D. J. Creighton, *Bioconjugate Chem.* **2005**, *16*, 598–607; b) H. S. Jung, X. Chen, K. Seung, J. Yoon, *Chem. Soc. Rev.* **2013**, *42*, 6019–6031; c) C.-C. Yeh, M.-F. Hou, S.-H. Wu, S.-M. Tsai, S.-K. Lin, L. A. Hou, H. Ma, L. Y. Tsai, *Cell Biochem. Funct.* **2006**, *24*, 555–559; d) S. C. Barranco, R. R. Perry, M. E. Durm, M. Quarishi, A. L. Werner, S. G. Gregorczyk, P. Kolm, *Dis. Colon Rectum* **2000**, *43*, 1133–1140.
- [7] a) S. Ji, H. Guo, X. Yuan, X. Li, H. Ding, P. Gao, C. Zhao, W. Wu, W. Wu, J. Zhao, *Org. Lett.* **2010**, *12*, 2876–2879; b) J. Shao, H. Guo, S. Ji, J. Zhao, *Biosens. Bioelectron.* **2011**, *26*, 3012–3017; c) J. Ålander, K. Johansson, V. D. Heuser, H. Farebo, J. Järviden, H. Abe, A. Shibata, M. Ito, Y. Ito, R. Morgenstern, *Anal. Biochem.* **2009**, *390*, 52–56; d) A. Shibata, K. Furukawa, H. Abe, S. Tsuneda, Y. Ito, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2246–2248; e) H. Maeda, H. Matsuno, M. Ushida, K. Katayama, K. Saeki, N. Itoh, *Angew. Chem.* **2005**, *117*, 2982–2985; *Angew. Chem. Int. Ed.* **2005**, *44*, 2922–2925; f) W. Jiang, Q. Fu, H. Fan, J. Ho, W. Wang, *Angew. Chem.* **2007**, *119*, 8597–8600; *Angew. Chem. Int. Ed.* **2007**, *46*, 8445–8448; g) X. Li, S. Qian, Q. He, B. Yang, J. Li, Y. Hu, *Org. Biomol. Chem.* **2010**, *8*, 3627–3630; h) Z. Dai, L. Tian, Z. Ye, B. Song, R. Zang, J. Yuan, *Anal. Chem.* **2013**, *85*, 11658–11664; i) K. Cui, Z. Chen, Z. Wang, G. Zhang, D. Zhang, *Analyst* **2011**, *136*, 191–195; j) S.-Q. Wang, Q.-H. Wu, H.-Y. Wang, X.-X. Zheng, S.-L. Shen, Y.-R. Zhang, J.-Y. Miao, B.-X. Zhao, *Analyst* **2013**, *138*, 7169–7174; k) H. Guo, Y. Jing, X. Yuan, S. Ji, J. Zhao, X. Li, Y. Kan, *Org. Biomol. Chem.* **2011**, *9*, 3844–3853; l) H. He, P.-C. Lo, D. K. P. Ng, *Chem. Eur. J.* **2014**, *20*, 6241–6245.
- [8] a) S. Atilgan, Z. Ekmekci, A. L. Dogan, D. Guc, E. U. Akkaya, *Chem. Commun.* **2006**, 4398–4400; b) Y. Cakmak, S. Kolemen, S. Duman, Y. Dede, Y. Dolen, B. Kilic, Z. Kostereli, L. T. Yildirim, A. L. Dogan, D. Guc, E. U. Akkaya, *Angew. Chem.* **2011**, *123*, 12143–12147; *Angew. Chem. Int. Ed.* **2011**, *50*, 11937–11941; c) M. Isik, T. Ozdemir, I. S. Turan, S. Kolemen, E. U. Akkaya, *Org. Lett.* **2013**, *15*, 216–219; d) T. Yogo, Y. Urano, Y. Ishitsuka, F. Maniwa, T. Nagano, *J. Am. Chem. Soc.* **2005**, *127*, 12162–12163; e) X. Zhang, Y. Xiao, J. Qi, J. Qu, B. Kim, X. Yue, K. D. Belfield, *J. Org. Chem.* **2013**, *78*, 9153–9160; f) H. Yu, Y. Dai, H. Lib, Y. Xiao, *New J. Chem.* **2013**, *37*, 1688–1691; g) S. Kou, H. N. L. Lee, D. van Noort, K. M. K. Swamy, S. H. Kim, J. H. Soh, K.-M. Lee, S.-W. Nam, J. Yoon, S. Park, *Angew. Chem.* **2008**, *120*, 886–890; *Angew. Chem. Int. Ed.* **2008**, *47*, 872–876; h) L. Li, J. Han, B. Nguyen, K. Burgess, *J. Org. Chem.* **2008**, *73*, 1963–1970; i) G. Ulrich, R. Ziesel, A. Harriman, *Angew. Chem.* **2008**, *120*, 1202–1219; *Angew. Chem. Int. Ed.* **2008**, *47*, 1184–1201.
- [9] a) W. Pang, X.-F. Zhang, J. Zhou, C. Yu, E. Hao, L. Jiao, *Chem. Commun.* **2012**, *48*, 5437–5439; b) X.-F. Zhang, X. Yang, *J. Phys. Chem. B* **2013**, *117*, 9050–9055.
- [10] Y. You, S. L. Gibson, R. Hilf, S. R. Davies, A. R. Oseroff, I. Roy, T. Y. Ohulichansky, E. J. Bergy, M. E. Detty, *J. Med. Chem.* **2003**, *46*, 3734–3747.

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