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## Abstract

Self-immolative aryl phthalate esters were conjugated with cleavable masking groups sensitive to light and hydrogen peroxide. The phthalate linker releases the fluorescent dye 7-hydroxycoumarin upon exposure to light or H<sub>2</sub>O<sub>2</sub>, respectively, leading to an increase in fluorescence. The light-sensitive aryl phthalate ester is demonstrated as a pro-fluorophore in cultured S2 cells

## Disciplines

Chemistry | Inorganic Chemistry | Materials Chemistry | Organic Chemistry | Other Chemistry | Polymer Chemistry

## Comments

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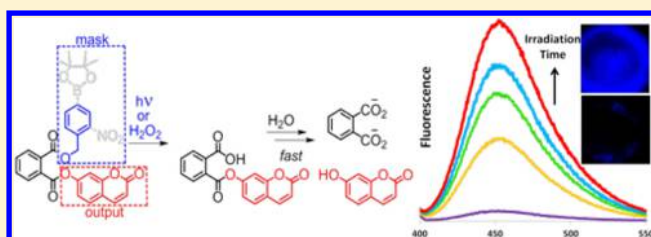
# Self-Immolative Phthalate Esters Sensitive to Hydrogen Peroxide and Light

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**S** Supporting Information

**ABSTRACT:** Self-immolative aryl phthalate esters were conjugated with cleavable masking groups sensitive to light and hydrogen peroxide. The phthalate linker releases the fluorescent dye 7-hydroxycoumarin upon exposure to light or  $\text{H}_2\text{O}_2$ , respectively, leading to an increase in fluorescence. The light-sensitive aryl phthalate ester is demonstrated as a pro-fluorophore in cultured S2 cells.

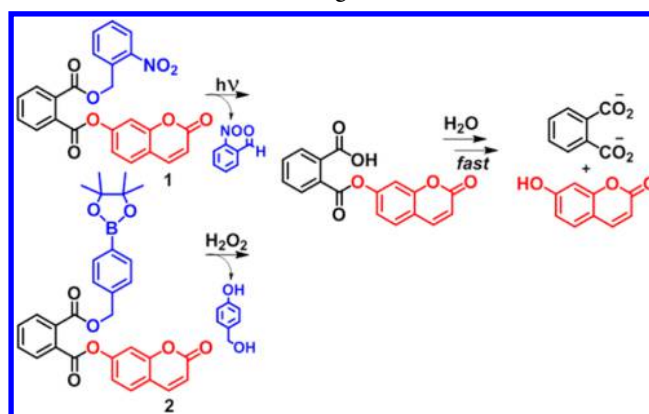


Self-immolative linkers have proven to be useful for connecting a cleavable masking molecule to an output cargo molecule.<sup>1–5</sup> Upon exposure to an input stimulus that cleaves the mask, self-immolative linkers release their cargo. Self-immolative linkers have found applications in enzyme-activated prodrugs,<sup>6–14</sup> chemical sensors,<sup>2,15</sup> traceless linkers,<sup>16–19</sup> biological probes,<sup>20–23</sup> and degradable polymers.<sup>24–26</sup> Released chemical cargos are often biomolecules, drugs, or reporters such as fluorescent dyes. Ideally, self-immolative linkers should be simple in design, stable, compatible with water, and transformed into a benign byproduct upon release of the output cargo. Additionally, they should be easy to conjugate, readily adaptable to a variety of inputs and outputs, and quickly release the output cargo. A drawback to some self-immolative linkers is that cargo release rates can be slow (minutes to hours),<sup>27</sup> leading to loss of temporal resolution.

Our group has recently reported aryl phthalate esters as self-immolative linkers.<sup>3</sup> In this previous work, we demonstrated that a fluoride-sensitive masking group could release cargo phenols and phenolic-based fluorescent dyes. Phthalate self-immolative linkers exploit the rapid hydrolysis of esters with adjacent catalytic carboxylate moieties, a classic case of neighboring group participation<sup>28–30</sup> (phenyl hydrogen phthalate releases phenol in <5 s in neutral water<sup>3</sup>). These intramolecularly catalyzed reactions involve neighboring group attack by the free carboxylate upon the adjacent ester to generate a transient anhydride that undergoes spontaneous hydrolysis to yield the diacid. Here, we demonstrate that phthalate esters masked with light- and peroxide-sensitive groups can release a coumarin dye upon exposure to light or peroxide (Scheme 1). Peroxide is an important biological signaling molecule, whereas light-releasable fluorescent dyes (pro-fluorophores) have found application in monitoring dynamic events in real time<sup>31–36</sup> as well as recording images with subdiffraction resolution at the nanometer level.<sup>37–40</sup>

Both **1** and **2** were synthesized by the addition of the trigger molecule to phthalic anhydride followed by the addition of 7-

**Scheme 1. General Unmasking Scheme**



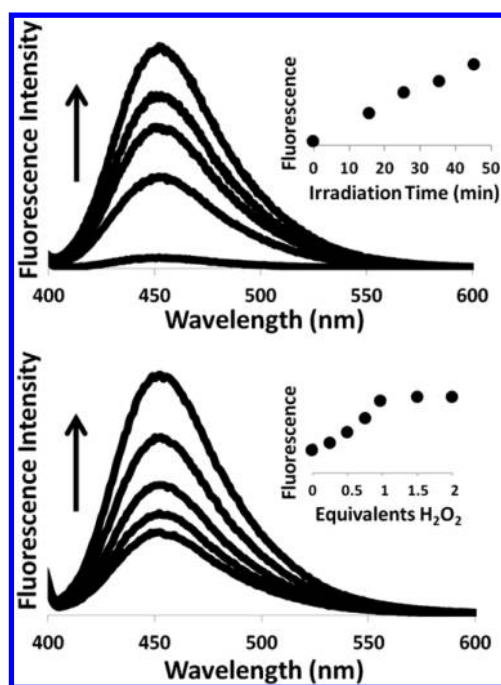
hydroxycoumarin by either a DCC/DMAP or EDC/DMAP coupling.

Compounds **1** and **2** were synthesized and exposed to UV light and hydrogen peroxide, respectively. The reactions were monitored using fluorescence and <sup>1</sup>H NMR spectroscopy. The titration of **1** resulted in an 18-fold increase in fluorescence intensity and **2** showed an 8-fold increase as a result of releasing the free fluorescent dyes.

The titrations of **1** and **2** were followed by fluorescence spectroscopy (Figure 1). To aid with solubility, experiments with **1** were carried out by first dissolving the compound in DMF and exposing the resulting solution to 350 nm light, A small aliquot (7  $\mu\text{L}$ ) of the solution was then injected into buffered water (3.0 mL, pH 7.0, 1 mM phosphate buffer) and fluorescence was followed as a function of time. Experiments for **2** were carried out by first dissolving the compound in DMF and titrating with hydrogen peroxide. This procedure was

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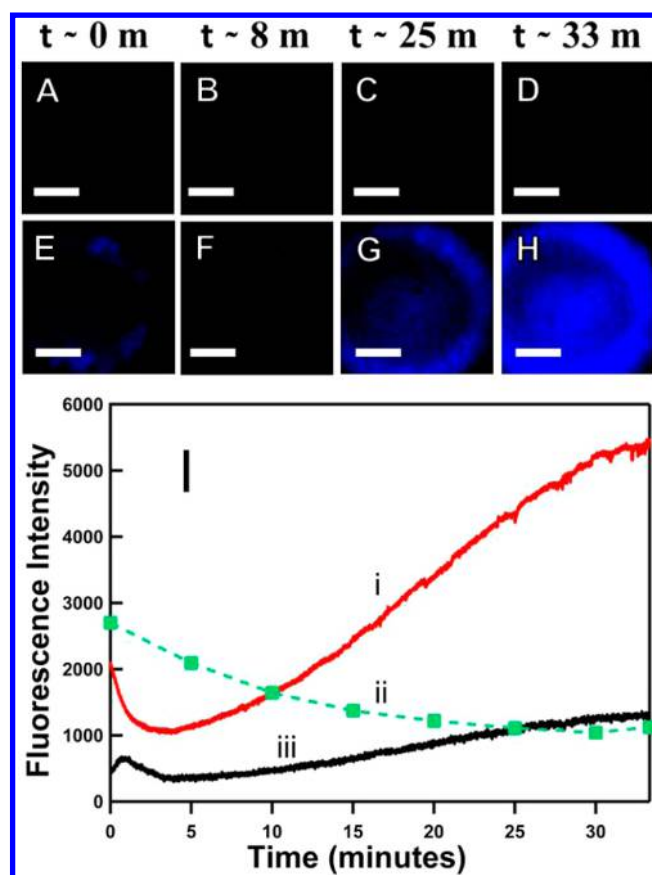
**Figure 1.** Fluorescence of compound **1** ( $6.27 \times 10^{-6}$  M) as a function of irradiation time (top); fluorescence of **2** ( $6.27 \times 10^{-6}$  M) as a function of peroxide (bottom) in pH 7.0 buffer. Plot inserts depict fluorescence at the emission maxima (453 nm) vs time of irradiation or equivalents of hydrogen peroxide.

followed by injection of a small aliquot of the DMF/H<sub>2</sub>O<sub>2</sub> solutions into buffered water for the fluorescence analysis.

Compound **1** was stable in water/DMF mixtures in the absence of light for at least 1 day at room temperature (see the Supporting Information for details). Compound **2** did show some instability, as seen by a small increase in fluorescence after a 16-h period in a water/DMF mixture in the absence of hydrogen peroxide (see the Supporting Information for spectra). Additionally, it is noteworthy that this structure **2** is quite unstable under the seemingly mild conditions required to synthesize it (e.g., DCC/DMAP ester coupling), possibly the result of the boronate ester under the reaction conditions catalyzing a spontaneous ester hydrolysis (**2** is stable as a solid or dissolved in a solution lacking hydrogen peroxide, however). Additionally, NMR product studies of **1** after exposure to light indicate that the organic products are the expected free 7-hydroxycoumarin as well as phthalic acid. The toxicity of phthalic acid has been studied due to its industrial use in the synthesis of phthalate plastics and esters; it has not been found to be very toxic in mice (LD<sub>50</sub> (mouse) is 2.53 g/kg).<sup>41,42</sup>

Because **1** showed the largest increase in fluorescence intensity and the greatest stability during the titration studies, we chose to use it for cellular experiments. Compound **1** was incubated with *Drosophila* S2 cells, and dye release was monitored using fluorescence microscopy. See Figure 2.

The *Drosophila* S2 cells were loaded with **1** and subjected to continuous irradiation with 335 nm light. Fluorescence images were collected every 500 ms for a total of 33 min (Figure 2). Fluorescence emission was observed at 450 nm. Fluorescence intensity for Figure 2 (i–iii) was taken from the periphery of the cell where the concentration of **1** was highest. As shown in Figure 2E–H, at the beginning of the experiment there was minimal fluorescence; however, after exposure to light a gradual increase in fluorescence is seen for up to 33 min. The initial



**Figure 2.** Fluorescence images of a cell with no compound **1** (A–D) and cell incubated with compound **1** ( $50 \mu\text{M}$ ) (E–H) as a function of irradiation time. (I) Average fluorescence intensity as a function of time for (i) four cells incubated with compound **1** and exposed to continuous irradiation for 35 min, (ii) four cells incubated with compound **1** and only exposed to irradiation briefly every 5 min to obtain an image, and (iii) a cell without compound **1** and exposed to continuous irradiation for 35 min. Scale bar represents  $5 \mu\text{m}$  in all images.

fluorescence seen at time zero can be attributed to cellular auto fluorescence, which undergoes initial bleaching prior to significant release of the free coumarin dye. Control studies were performed to make sure the fluorescence was due to the controlled release of 7-hydroxycoumarin by irradiation. Parts A–D of Figure 2 show that there is a minimal change in fluorescence of cells when they are irradiated without being loaded with **1**. Another control study (Figure 2 (ii)) was performed with cells incubated with **1** but not exposed to irradiation. There was an initial fluorescence signal due to cellular auto fluorescence; however, a decrease in fluorescence signal is seen, eventually leveling off to an intensity similar to that of the unloaded cells, indicating that the **1** does not release the dye in the absence of irradiation.

The cytotoxicity of **1** in the cells was determined by incubating the cells ( $1 \times 10^6$  cells/mL) with different dilutions (100, 50, 25, 12.5, 6.25, and  $3.125 \mu\text{M}$ ) of **1** in phosphate buffer saline (PBS, pH = 7.1) for 1 h. At a compound concentration of  $50 \mu\text{M}$ , 83% of the cells remained viable after 1 h, and this concentration was used in all fluorescence imaging cell studies.

In conclusion, we have shown that aryl phthalate self-immolative linkers are easily conjugated with the light-sensitive 2-nitrobenzyl alcohol group and the hydrogen peroxide sensitive group 4-(hydroxymethyl)phenylboronic acid pinacol

ester. Compound **2** could be potentially useful as a hydrogen peroxide sensor. Compound **1** is of interest because it is able to deliver cargo in a temporally and spatially controlled manner using irradiation. Release of caged 7-hydroxycoumarin occurs upon irradiation within S2 cells. We note finally that the known fast rates of hydrolysis of phthalate self-immolative linkers may make these structures good candidates for domino self-immolative linkers,<sup>43–47</sup> wherein a single input reaction results in the spontaneous release of numerous cargo molecules. Current domino self-immolative linkers tend to have slow kinetics of release.<sup>27</sup> A recent method to synthesize aryl mellitic acid esters<sup>48</sup> may enable these structures to be used within fast-releasing domino self-immolative systems.

## EXPERIMENTAL SECTION

**Synthesis of 2-(Nitrobenzyl) Hydrogen Phthalate.** Phthalic acid (0.100 g, 0.675 mmol) and 2-nitrobenzyl alcohol (0.103 g, 0.675 mmol) were refluxed in toluene under argon overnight. The crude product was collected by evaporation under reduced pressure. The resulting mixture was dissolved in ethyl acetate, and the product was extracted with aqueous sodium bicarbonate followed by acidification with 1 M aqueous hydrogen chloride. Final collection of a white solid was performed by vacuum filtration. The product was dried under vacuum and used without any further purification (0.203 g, 36%): <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 13.35 (s, 1H), 8.15 (dd, *J* = 8.1, 1.2 Hz, 1H), 7.85–7.60 (m, 7H), 5.63 (s, 2H); <sup>13</sup>C (DMSO-*d*<sub>6</sub>, 100 MHz) δ 168.4, 167.6, 147.8, 134.6, 132.1, 131.9, 131.9, 131.5, 129.9, 129.8, 129.4, 128.8, 125.3, 64.1; mp 142–145 °C; HRMS (ESI) *m/z* [M + Na]<sup>+</sup> for C<sub>15</sub>H<sub>11</sub>NNaO<sub>6</sub> requires 324.0479, found 324.0480.

**Synthesis of 7-Hydroxycoumarinyl 2-(Nitrobenzyl) Hydrogen Phthalate (1).** 2-(Nitrobenzyl) hydrogen phthalate (0.200 g, 0.664 mmol), *N*-(3-(dimethylamino)propyl)-*N'*-ethylcarbodiimide hydrochloride (0.126 g, 0.657 mmol), and 4-(*N,N*-dimethylamino)pyridine (0.089 g, 0.728 mmol) were dissolved in dry DCM (10 mL), followed by continuous stirring of the solution. 7-Hydroxycoumarin potassium salt (0.132 g, 0.660 mmol) and 18-crown-6 (0.174 g, 0.660 mmol) were added next to the reaction mixture, and the reaction was stirred under an argon atmosphere for 12 h. The product was washed with an aqueous saturated sodium bicarbonate solution. The solvent was then removed under reduced pressure to yield the crude product as a white solid. Flash chromatography (Hex/EtOAc, 30:70 → chloroform/MeOH 95:5) gave the pure final product (0.123 g, 42%): <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ 8.16–8.08 (m, 2H), 8.06–7.91 (m, 2H), 7.90–7.72 (m, 5H), 7.67–7.56 (m, 1H), 7.34 (d, *J* = 2.2 Hz, 1H), 7.23 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.53 (d, *J* = 9.6 Hz, 1H), 5.74–5.69 (m, 2H), 1.24 (s, 1H), 0.84 (t, *J* = 6.8 Hz, 1H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 100 MHz) δ 166.3, 165.7, 160.0, 154.5, 153.0, 148.0, 144.2, 134.5, 132.8, 131.0, 130.3, 130.0, 129.9, 129.8, 125.3, 118.6, 117.4, 116.2, 110.2, 79.6, 79.4, 79.1, 64.4; mp >260 °C; HRMS (ESI) calcd for C<sub>24</sub>H<sub>15</sub>NO<sub>8</sub> [M + H]<sup>+</sup> requires 446.0870, found 446.0872.

**Synthesis of 4-(Hydroxymethyl)benzeneboronic Acid Pinacol Hydrogen Phthalate.** Phthalic anhydride (0.049 g, 0.294 mmol) and 4-(hydroxymethyl)benzeneboronic acid pinacol (0.077 g, 0.329 mmol) were refluxed in toluene overnight. The crude product was collected by evaporation under reduced pressure. The resulting mixture was dissolved in ethyl acetate, and the product was extracted with aqueous sodium bicarbonate followed by acidification with hydrogen chloride. Vacuum filtration was used to collect the white solid. The product was dried under vacuum and used without any further purification (0.045 g, 40%): <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz) δ 7.85 (s, 1H), 7.72 (m, 6H), 7.52 (d, 1H, *J* = 0.8 Hz), 1.36 (s, 12H); <sup>13</sup>C (DMSO-*d*<sub>6</sub>, 100 MHz) δ 168.4, 167.9, 139.4, 137.0, 135.0, 134.7, 132.6, 131.9, 129.4, 128.8, 127.8, 127.4, 84.2, 67.1, 25.1; mp 121–123 °C; HRMS (ESI) calcd for C<sub>21</sub>H<sub>23</sub>BO<sub>6</sub> (M – H)<sup>+</sup> requires 380.1551, found 380.1550 (mass calculated using boron isotope <sup>10</sup>B).

**Synthesis of 7-Hydroxycoumarinyl 2-(4-Hydroxymethyl)benzeneboronic Phthalate (2).** 4-(Hydroxymethyl)benzeneboronic acid pinacol hydrogen phthalate (0.100 g, 0.262 mmol), *N,N*-

dicyclohexylcarbodiimide (0.068 g, 0.314 mmol), 4-(*N,N*-dimethylamino)pyridine (0.011 g, 0.087 mmol), and 18-crown-6 ether (0.069 g, 0.262 mmol) were dissolved in dry DMF (3 mL) followed by continuous stirring of the solution. 7-Hydroxycoumarin potassium salt (0.052 g, 0.262 mmol) was next added to the reaction mixture, and the reaction was stirred under an argon atmosphere overnight. The dicyclohexylurea byproduct was filtered off as a white solid. The solvent was then removed under reduced pressure to yield the crude product as a white solid. Flash chromatography (Hex/EtOAc, 50:50) gave the pure final product (5.6 mg, 4.1%): <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.10 (d, *J* = 9.6 Hz, 1H), 8.02–7.90 (m, 2H), 7.87–7.75 (m, 3H), 7.62 (dd, *J* = 7.0, 1.3 Hz, 2H), 7.43 (d, *J* = 7.6 Hz, 2H), 7.32 (d, *J* = 2.1 Hz, 1H), 7.18 (ddd, *J* = 8.5, 2.2, 0.8 Hz, 1H), 6.52 (dd, *J* = 9.6, 0.8 Hz, 1H), 5.40 (s, 2H), 1.27 (d, *J* = 0.8 Hz, 12H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 100 MHz) δ 166.6, 165.8, 160.3, 154.7, 153.3, 142.8, 138.2, 135.1, 131.8, 131.6, 131.5, 129.5, 129.2, 128.6, 127.5, 118.4, 116.8, 116.2, 110.4, 80.9, 67.6, 24.9; mp; HRMS (ESI) calcd for C<sub>30</sub>H<sub>27</sub>BO<sub>8</sub> (M + H)<sup>+</sup> requires 526.1904, found 526.1908 (mass calculated using boron isotope <sup>10</sup>B).

## ASSOCIATED CONTENT

### Supporting Information

All experimental data including stability tests, product studies, cell study procedures, and compound characterization. This material 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) Amir, R. J.; Pessah, N.; Shamis, M.; Shabat, D. *Angew. Chem., Int. Ed.* **2003**, *42*, 4494–4499.
- (2) Chandran, S. S.; Dickson, K. A.; Raines, R. T. *J. Am. Chem. Soc.* **2005**, *127*, 1652–1653.
- (3) Mahoney, K. M.; Goswami, P. P.; Winter, A. H. *J. Org. Chem.* **2012**, *78*, 702–705.
- (4) Schmid, K. M.; Jensen, L.; Phillips, S. T. *J. Org. Chem.* **2012**, *77*, 4363–4374.
- (5) Sella, E.; Shabat, D. *Chem. Commun.* **2008**, 5701–5703.
- (6) Amir, R. J.; Popkov, M.; Lerner, R. A.; Barbas, C. F.; Shabat, D. *Angew. Chem., Int. Ed.* **2005**, *44*, 4378–4381.
- (7) Dubowchik, G. M.; Firestone, R. A.; Padilla, L.; Willner, D.; Hofstead, S. J.; Mosure, K.; Knipe, J. O.; Lasch, S. J.; Trail, P. A. *Bioconjugate Chem.* **2002**, *13*, 855–869.
- (8) Gopin, A.; Pessah, N.; Shamis, M.; Rader, C.; Shabat, D. *Angew. Chem., Int. Ed.* **2003**, *42*, 327–332.
- (9) Grinda, M.; Legigan, T.; Clarhaut, J.; Peraudeau, E.; Tranoy-Opalinski, I.; Renoux, B.; Thomas, M.; Guilhot, F.; Papot, S. *Org. Biomol. Chem.* **2013**, *11*, 7129–7133.
- (10) Niculescu-Duvaz, D.; Niculescu-Duvaz, I.; Friedlos, F.; Martin, J.; Spooner, R.; Davies, L.; Marais, R.; Springer, C. J. *J. Med. Chem.* **1998**, *41*, 5297–5309.
- (11) Redy, O.; Shabat, D. *J. Controlled Release* **2012**, *164*, 276–282.
- (12) Saez, J. A.; Escuder, B.; Miravet, J. F. *Tetrahedron* **2010**, *66*, 2614–2618.
- (13) Weinstain, R.; Baran, P. S.; Shabat, D. *Bioconjugate Chem.* **2009**, *20*, 1783–1791.

- (14) Yang, J. J.; Kularatne, S. A.; Chen, X.; Low, P. S.; Wang, E. *Mol. Pharmacol.* **2011**, *9*, 310–317.
- (15) Meyer, Y.; Richard, J.-A.; Delest, B.; Noack, P.; Renard, P.-Y.; Romieu, A. *Org. Biomol. Chem.* **2010**, *8*, 1777–1780.
- (16) Ding, S.; Gray, N. S.; Ding, Q.; Schultz, P. G. *J. Org. Chem.* **2001**, *66*, 8273–8276.
- (17) Horton, J. R.; Stamp, L. M.; Routledge, A. *Tetrahedron Lett.* **2000**, *41*, 9181–9184.
- (18) Hulme, C.; Peng, J.; Morton, G.; Salvino, J. M.; Herpin, T.; Labaudiniere, R. *Tetrahedron Lett.* **1998**, *39*, 7227–7230.
- (19) Stieber, F.; Grether, U.; Waldmann, H. *Angew. Chem., Int. Ed.* **1999**, *38*, 1073–1077.
- (20) Antczak, C.; Jaggi, J. S.; LeFave, C. V.; Curcio, M. J.; McDevitt, M. R.; Scheinberg, D. A. *Bioconjugate Chem.* **2006**, *17*, 1551–1560.
- (21) Duimstra, J. A.; Femia, F. J.; Meade, T. J. *J. Am. Chem. Soc.* **2005**, *127*, 12847–12855.
- (22) Jeffrey, S. C.; Torgov, M. Y.; Andreyka, J. B.; Boddington, L.; Cerveny, C. G.; Denny, W. A.; Gordon, K. A.; Gustin, D.; Haugen, J.; Kline, T.; Nguyen, M. T.; Senter, P. D. *J. Med. Chem.* **2005**, *48*, 1344–1358.
- (23) Leu, Y.-L.; Chen, C.-S.; Wu, Y.-J.; Chern, J.-W. *J. Med. Chem.* **2008**, *51*, 1740–1746.
- (24) Shabat, D. *J. Polym. Sci. A Polym. Chem.* **2006**, *44*, 1569–1578.
- (25) Weinstain, R.; Sagi, A.; Karton, N.; Shabat, D. *Chem.—Eur. J.* **2008**, *14*, 6857–6861.
- (26) Blencowe, C. A.; Russell, A. T.; Greco, F.; Hayes, W.; Thornthwaite, D. W. *Polym. Chem.* **2011**, *2*, 773–790.
- (27) Amir, R.; Shabat, D. In *Polymer Therapeutics I*; Satchi-Fainaro, R., Duncan, R., Eds.; Springer: Berlin, 2006; Vol. 192; pp 59–94.
- (28) Pasto, D. J.; Serve, M. P. *J. Am. Chem. Soc.* **1965**, *87*, 1515–1521.
- (29) Andres, G. O.; Granados, A. M.; de Rossi, R. H. *J. Org. Chem.* **2001**, *66*, 7653–7657.
- (30) Thanassi, J. W.; Bruice, T. C. *J. Am. Chem. Soc.* **1966**, *88*, 747–752.
- (31) Dirks, R. W.; Molenaar, C.; Tanke, H. J. *Histochem. Cell Biol.* **2001**, *115*, 3–11.
- (32) Xu, Y.; Melia, T. J.; Toomre, D. K. *Curr. Opin. Chem. Biol.* **2011**, *15*, 822–830.
- (33) Zaitsev, S.; Zaitsev, S. Y. *World Appl. Sci. J.* **2013**, *26*, 712.
- (34) Agasti, S. S.; Kohler, R. H.; Liong, M.; Peterson, V. M.; Lee, H.; Weissleder, R. *Small* **2013**, *9*, 222–227.
- (35) Adams, S. R.; Tsien, R. Y. *Annu. Rev. Physiol.* **1993**, *55*, 755–784.
- (36) Politz, J. C. *Trends Cell Biol.* **1999**, *9*, 284–287.
- (37) Fernandez-Suarez, M.; Ting, A. Y. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 929–943.
- (38) Huang, B.; Bates, M.; Zhuang, X. *Annu. Rev. Biochem.* **2009**, *78*, 993–1016.
- (39) Toomre, D.; Bewersdorf, J. *Annu. Rev. Cell Dev. Biol.* **2010**, *26*, 285–314.
- (40) van de Linde, S.; Heilemann, M.; Sauer, M. *Annu. Rev. Phys. Chem.* **2012**, *63*, 519–540.
- (41) Lin, V. S.; Dickinson, B. C.; Chang, C. J. In *Methods in Enzymology*; Enrique, C., Lester, P., Eds.; Academic Press: New York, 2013; Vol. 526, pp 19–43.
- (42) *National Cancer Institute Carcinogenesis Technical Report Series*; National Cancer Institute: Bethesda, 1979; pp 1–123.
- (43) Turan, I. S.; Akkaya, E. U. *Org. Lett.* **2014**, *16*, 1680–1683.
- (44) Shamis, M.; Shabat, D. *Chem.—Eur. J.* **2007**, *13*, 4523–4528.
- (45) Amir, R. J.; Danieli, E.; Shabat, D. *Chem.—Eur. J.* **2007**, *13*, 812–821.
- (46) Sagi, A.; Segal, E.; Satchi-Fainaro, R.; Shabat, D. *Bioorg. Med. Chem.* **2007**, *15*, 3720–3727.
- (47) Karton-Lifshin, N.; Shabat, D. *New J. Chem.* **2012**, *36*, 386–393.
- (48) Geraskina, M. R.; Juetten, M. J.; Winter, A. H. *J. Org. Chem.* **2014**, *79*, 5334–5337.