

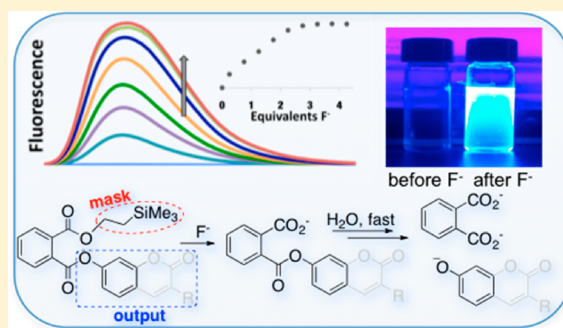
## Self-Immolative Aryl Phthalate Esters

Kaitlyn M. Mahoney, Pratik P. Goswami, and Arthur H. Winter\*

Department of Chemistry, Iowa State University, 2101d Hach Hall, Ames, Iowa 50014, United States

**S** Supporting Information

**ABSTRACT:** We report that aryl phthalate esters are robust self-immolative linkers. This linker is easy to conjugate and releases output phenols upon cleaving a fluoride-sensitive mask to yield a benign phthalic acid byproduct, making these linkers potentially useful as fluoride sensors and promising for use in biological and materials applications.



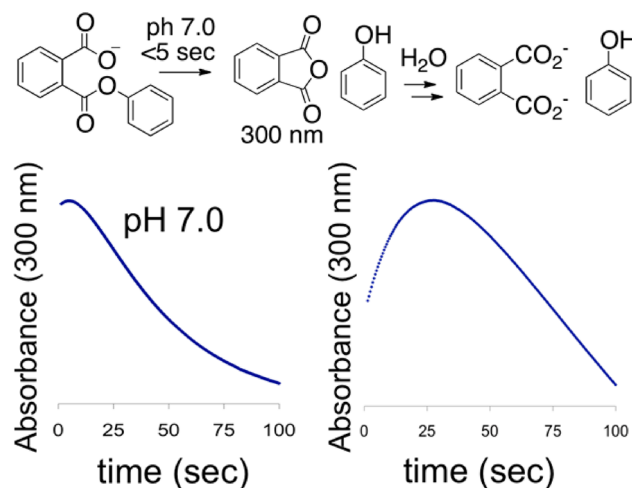
### INTRODUCTION

Self-immolative linkers have become indispensable molecules for connecting a cleavable mask to an output cargo molecule.<sup>1–3</sup> Upon an input reaction that cleaves the mask, self-immolative linkers release their output cargo. Despite their unsavory moniker, self-immolative linkers have proven to be extremely useful in enzyme-activated prodrugs,<sup>4–12</sup> chemical sensors,<sup>2,13–16</sup> traceless linkers,<sup>17–20</sup> biological probes,<sup>21–24</sup> and degradable polymers.<sup>1,25–33</sup> Released chemical cargoes are often biomolecules, drugs, or reporters such as fluorescent dyes. Linker structure can aid prodrugs by improving stability, solubility, biodistribution, pharmacokinetics, bioavailability and activation.

The ideal self-immolative linker does not impose: It is simple, stable, compatible with water, and transforms into a benign byproduct upon releasing the output cargo. Furthermore, such linkers should be easy to conjugate, readily adaptable to a variety of inputs and outputs, and quickly release the output cargo upon the input reaction. In particular, some common self-immolative linkers suffer from slow release of their output cargo. New linkers that incorporate these desirable features would be highly useful.

The hydrolysis of phenyl hydrogen phthalate is a classic case of neighboring group participation, the mechanism of which has seen extensive investigation.<sup>34–37</sup> Phenyl hydrogen phthalate is a shelf-stable compound when stored away from moisture, but this compound hydrolyzes rapidly in water (Scheme 1). It has been determined that the fast ester hydrolysis of this compound is a case of intramolecular catalysis wherein the neighboring carboxylate group displaces the phenol to generate a water-unstable anhydride that in turn spontaneously hydrolyzes to phthalic acid. In neutral water, release of phenol is too fast to obtain accurate rate constants using standard UV–vis studies ( $\tau < 5$  s), but the rate of release is slowed in more acidic water ( $\tau = 23$  s, pH 5.7). The known

**Scheme 1. Fast Hydrolysis of the Classic Phenyl Hydrogen Phthalate Hydrolysis in Water Followed by Monitoring Growth and Decay of Phthalic Anhydride (Kinetic Fits Can Be Found in Supporting Information)**



favorable kinetics of this hydrolysis led us to test aryl phthalate esters for use as self-immolative linkers.

Using a fluoride-sensitive 2-(trimethylsilyl)ethyl ether group to mask the catalytic carboxyl group, in combination with three phenolic cargos (phenol **1** plus the fluorescent dyes 7-hydroxycoumarin **2** and 3-(2-benzothiazolyl)-7-hydroxycoumarin **3**), we find that aryl phthalate esters can indeed be exploited as self-immolative linkers (Figure 1). We show that these linkers can be conjugated easily starting from phthalic anhydride, a cheap industrial starting material in the

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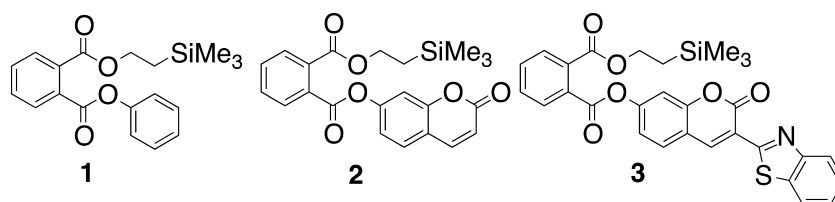
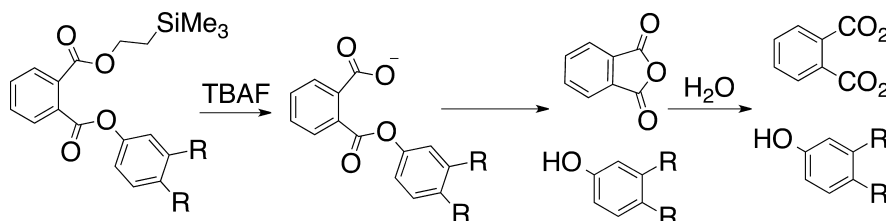


Figure 1. Aryl phthalate esters described in this study.

### Scheme 2. Putative Mechanism of Decomposition of 1–3 with F<sup>-</sup> Ion



manufacture of plastics, and “self-immolate” to ultimately yield phthalic acid as a biologically benign byproduct upon release of the phenolic output.

## RESULTS AND DISCUSSION

Compounds 1–3 were synthesized and titrated with fluoride ion (Scheme 2). The titration of 1 was followed by <sup>1</sup>H NMR spectroscopy, and titrations of 2 and 3 were followed with fluorescence spectroscopy. The cleavage of the 2-(trimethylsilyl)ethyl mask for 2 and 3 by potassium fluoride (KF) in neat water occurs successfully but is very slow, requiring excess fluoride ion to achieve reasonable deprotection over several hours (1 is not very soluble in pure H<sub>2</sub>O; see the Supporting Information for details). Thus, the titration experiments were performed by initially dissolving 1–3 in organic solvent (CH<sub>3</sub>CN or DMSO) with varying equivalents of tetrabutylammonium fluoride (TBAF) to cleave the mask (which is much more rapid in organic solvent), followed by injection of a small aliquot of this solution into buffered water for the fluorescence analysis for 2 and 3. The titration of compound 2 is remarkable because we observe a 730-fold increase in fluorescence upon complete fluoride deprotection as a consequence of the release of the highly fluorescent 7-hydroxycoumarin dye. Thus, compound 2 acts as a fluoride sensor. Curiously, compound 3 shows a decrease in fluorescence during the titration even though the highly fluorescent free coumarin dye is released. This decrease in fluorescence is due to the starting ester 3 being highly fluorescent, whereas ester 2 is essentially nonfluorescent.

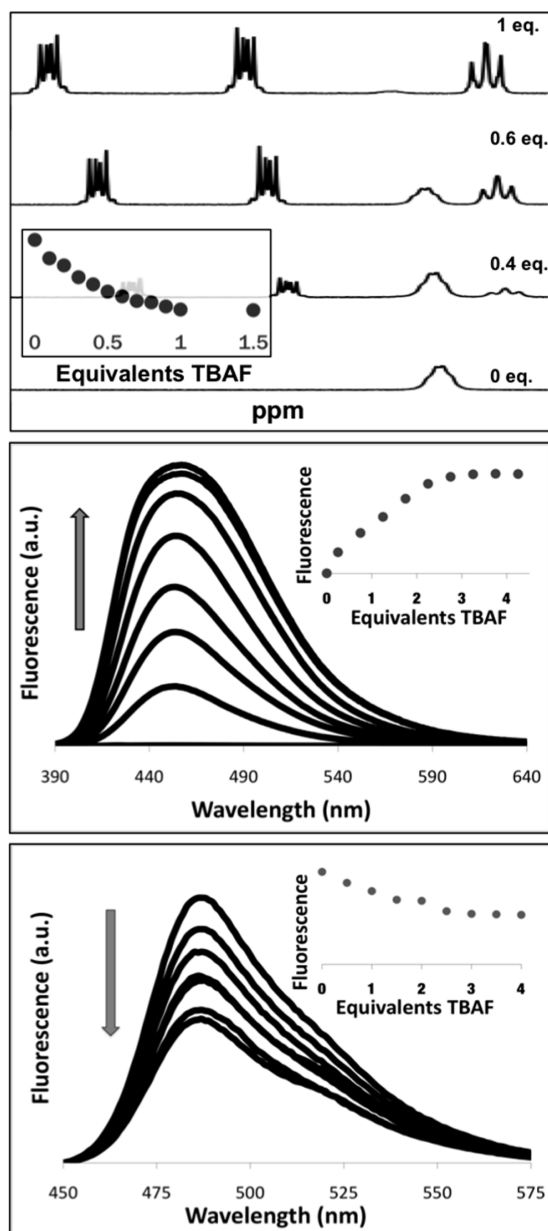
Compounds 1–3 are stable in water/CH<sub>3</sub>CN/DMSO in the absence of fluoride, with no decomposition observed after 1 day at room temperature. Additionally, NMR product analysis after fluoride deprotection indicates that the organic end products are the free phenolic compound as well as phthalic acid as the byproduct of the self-immolative linker. Of note, 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 toxic (LD<sub>50</sub> (mouse) is 2.53 g/kg).<sup>38,39</sup> These products lead us to postulate the mechanism of release shown in Scheme 2. Surprisingly, our titrations indicate that compounds 2 and 3 require three equivalents of fluoride to achieve complete deprotection, while 1 requires the expected 1 equiv of fluoride (Figure 2). This “excess” F<sup>-</sup> required for 2 and 3 is interesting

since the presumed mechanism for TMSE deprotection involves a single fluoride ion adding to the silicon to eliminate ethane gas and trimethylsilyl fluoride. To ensure that this observation was not an artifact of fluorescence, we added 1 equiv of fluoride to 2 and observed only ca. 33% decomposition of 2 by <sup>1</sup>H NMR. Additionally, <sup>19</sup>F NMR following deprotection suggest a different product for 1 than for 2 and 3 (see the Supporting Information). Possibly, deprotection of 2 and 3 proceed through a hypervalent silicon mechanism, although further work would be needed to verify this mechanistic possibility.

## CONCLUSION

In conclusion, we have shown that aryl phthalate esters are robust self-immolative linkers in water using a fluoride sensitive mask as a test case and phenolic outputs. The phthalate scaffold also appears to be highly promising for latent fluorophores, given the ~10<sup>3</sup> fluorescence enhancement upon releasing 7-hydroxycoumarin. Ester 2 may be useful as an interesting fluoride sensor. The advantages of this linker include a simple synthesis from inexpensive starting materials and a biologically benign byproduct. Given the favorable release kinetics of phenol from phenyl hydrogen phthalate (Scheme 1), the release kinetics of the output phenol may prove to be favorable, although we cannot directly measure the release directly from 1–3 due to the slow rate of fluoride deprotection of the silyl mask. The possibility of tuning the rate of release by chemical substitutions to the phthalate ring system, as well as the scope of this linker for different masking groups and output cargos, is currently under investigation in our laboratory. Through the use of different masks for the catalytic carboxylate such as those cleaved by light or enzymes, these phthalate esters may find use in biological and materials applications. For example, our preliminary studies indicate that substitution of the 2-(trimethylsilyl)ethyl group in compound 2 with the 2-nitrobenzyl photocage leads to release of the free coumarin dye upon photolysis in neat water.

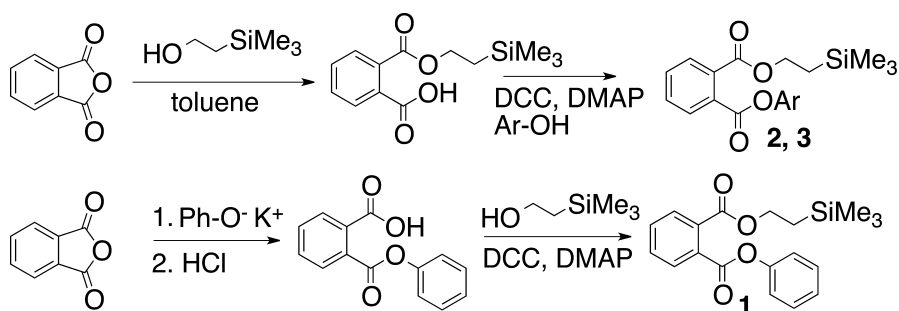
**Synthetic Schemes.** Compounds 1–3 were prepared from phthalic anhydride (see Scheme 3). Addition of TMSE to phthalic anhydride yields the TMSE-protected acid ester, which was further converted to aryl esters 2 and 3 using the Stieglich DCC/DMAP coupling procedure. For 1, esterification of



**Figure 2.** Fluoride titrations by NMR for **1** (top) and by fluorescence detection for **2** (middle) and **3** (bottom) in pH 7.0 buffer. Plot inserts depict fluorescence (or NMR integration) at the emission maxima vs equivalents of tetrabutylammonium fluoride.

phenyl hydrogen phthalate was accomplished in a similar way using DCC/DMAP conditions.

### Scheme 3. Synthesis of 1–3



### EXPERIMENTAL SECTION

Phenyl hydrogen phthalate,<sup>37</sup> 2-(trimethylsilyl)ethyl hydrogen phthalate,<sup>40</sup> and 3-(2-benzothiazolyl)-7-hydroxycoumarin<sup>41</sup> were prepared by published procedures. All NMR matched the known spectra.

**Synthesis of Phenyl 2-(Trimethylsilyl)ethyl Phthalate (1).** Phenyl hydrogen phthalate (1.50 g, 6.21 mmol), 2-trimethylsilylethanol (1 mL, 6.98 mmol), and 4-*N,N*-dimethylaminopyridine (0.085 g, 0.69 mmol) were dissolved in dry DMF (4 mL), followed by continuous stirring of the solution. *N,N*-Dicyclohexylcarbodiimide (1.54 g, 7.45 mmol), dissolved in dry DMF (2 mL), 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 yellow oil. Flash chromatography (Hex/EtOAc, 90:10) gave the pure final product (0.595 g, 28%) as a colorless oil: <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz) δ 7.90 (m, 1H), 7.83 (m, 1H), 7.70 (m, 2H), 7.46 (m, 2H), 7.29 (m, 3H), 4.44–4.40 (m, 2H), 1.09 (m, 2H), 0.04 (s, 9H); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 100 MHz) δ 169.1, 167.9, 152.5, 133.9, 133.1, 132.9, 132.7, 130.7, 130.4, 130.2, 127.3, 122.7, 65.4, 18.3, –1.4; high-res MS (ESI) calcd for formula C<sub>19</sub>H<sub>23</sub>O<sub>4</sub>Si (M + H)<sup>+</sup> requires 343.1360, found 343.1360; for formula C<sub>19</sub>H<sub>23</sub>O<sub>4</sub>SiNa (M + Na)<sup>+</sup> requires 365.1180, found 365.1187.

**Synthesis of 7-Hydroxycoumarinyl 2-(Trimethylsilyl)ethyl Phthalate (2).** 2-(trimethylsilyl)ethyl hydrogen phthalate (1.29 g, 4.83 mmol), 7-hydroxycoumarin (1.21 g, 4.83 mmol), and 4-dimethylaminopyridine (0.65 g, 5.3 mmol) were dissolved in a mixture of anhydrous methylene chloride (15 mL) and anhydrous DMF (9 mL). *N,N*-Dicyclohexylcarbodiimide was quickly added to the reaction mixture, which was stirred under argon overnight. Dicyclohexyl urea was filtered off, and the filtrate was diluted in 10 mL of methylene chloride. The solution was washed with brine and then dried over anhydrous MgSO<sub>4</sub>. The crude product was collected by evaporation under reduced pressure and then purified by flash chromatography on silica gel (Hex/EtOAc, 70:30) to yield **2** (0.65 g, 33%) as a white solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.87 (m, 2H), 7.73 (d, 1H, *J* = 8 Hz), 7.64 (m, 2H), 7.57 (d, 1H, *J* = 8 Hz), 7.34 (s, 1H), 7.30 (s, 1H), 6.43 (d, 1H, *J* = 8 Hz), 4.43 (t, 2H, *J* = 8 Hz), 1.11 (t, 2H, *J* = 8 Hz), 0.06 (s, 9H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 167.2, 166.2, 160.7, 155.1, 153.7, 143.2, 132.0, 131.8, 131.7, 129.6, 129.4, 128.9, 118.7, 117.2, 116.5, 110.8, 64.7, 17.7, –1.1; high-res MS (ESI) calcd for formula C<sub>22</sub>H<sub>22</sub>O<sub>6</sub>Si (M + H)<sup>+</sup> requires 411.1258, found 411.1261, C<sub>22</sub>H<sub>22</sub>O<sub>6</sub>SiNa (M + Na)<sup>+</sup> requires 433.1078, found 433.1083.

**Synthesis of 3-(Benzo[d]thiazol-2-yl)-7-hydroxycoumarinyl-2-(trimethylsilyl)ethyl Phthalate (3).** 2-(Trimethylsilyl)ethyl hydrogen phthalate (50 mg, 0.34 mmol), 3-(2-benzothiazolyl)-7-hydroxycoumarin (99 mg, 0.34 mmol), and 4-dimethylaminopyridine (4 mg, 0.034 mmol) were dissolved in DMF (5 mL). *N,N*-Dicyclohexylcarbodiimide (69 mg, 0.34 mmol) was quickly added to the reaction mixture, which was stirred under argon for 12 h. The white solid was filtered off, and the DMF was removed by evaporation under reduced pressure. The crude mixture was purified by preparatory thin-layer chromatography (200 μm) using a (Hex/EtOAc, 70:30) eluent followed by an additional prep TLC purification

using (hexane/EtOAc, 50:50) to yield the product **3** (37 mg, 20%) as a yellow solid:  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  9.11 (s, 1H), 8.12 (d, 1H,  $J=8$  Hz), 8.01 (d, 1H,  $J=8$  Hz), 7.89 (m, 2H), 7.81 (d, 1H,  $J=8$  Hz), 7.66 (m, 2H), 7.56 (t, 1H,  $J=8$  Hz), 7.48 (s, 1H), 7.45 (s, 1H), 7.40 (dd, 2H,  $J=4$  Hz), 4.45 (t, 2H,  $J=8$  Hz), 1.12 (t, 2H,  $J=8$  Hz), 0.07 (s, 9H);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  167.1, 166.2, 160.0, 159.9, 154.9, 152.7, 141.24, 137.2, 132.3, 132.1, 131.9, 131.8, 130.6, 129.7, 129.5, 126.9, 125.8, 123.3, 122.1, 120.1, 119.7, 117.3, 110.68, 64.8, 17.7, -1.1; high-res MS (ESI) calcd for formula  $\text{C}_{29}\text{H}_{26}\text{NO}_6\text{SSi}$  ( $\text{M} + \text{H}$ )<sup>+</sup> requires 544.1245, found 544.1248.

**$^1\text{H NMR}$  Titration of **1**.** A stock solution of **1** was prepared ( $9.05 \times 10^{-2}$  M) in  $\text{DMSO}-d_6$  and distributed equally ( $97 \mu\text{L}$ ) into 12 vials. To these vials were added varying equivalents of a second stock solution made of 1 M TBAF/THF ( $7.44 \times 10^{-2}$  M) in  $\text{DMSO}-d_6$ . After 4 h, 0.5 mL of  $\text{D}_2\text{O}$  was then added to each vial.  $^1\text{H NMR}$  spectra of each were then recorded. The titration was repeated three times, and the results were averaged. Conversion was calculated by measuring the ratio of  $\text{DMSO}-d_6$  signal integration with the integration of the  $-\text{CH}_2$  peak ( $\delta$  4.42 ppm) in **1**.

**Fluorescence Titration of **2** and **3**.** A stock solution of **2** was prepared ( $7.68 \times 10^{-5}$  M) in acetonitrile and distributed equally ( $52 \mu\text{L}$ ) into vials. These samples were titrated using varying equivalents of a 1 M TBAF in THF solution for 4 h to ensure complete deprotection of the TMSE group. The samples were then diluted with 1 mM phosphate buffered (pH = 7.0) water to 3.0 mL. Excitation was carried out at 370 nm with all excitation and emission slit widths at 2 nm. The titration was repeated three times, and the data were averaged. The same experimental procedure was used in the titration of compound **3** except the stock solution ( $2.3 \times 10^{-6}$  M) was prepared in DMF, and the excitation of these scans was carried out at 440 nm.

## ■ ASSOCIATED CONTENT

### ● Supporting Information

All experimental data including kinetic plots, stability tests, full titration plots, synthetic procedures, and compound characterizations. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## ■ AUTHOR INFORMATION

### Corresponding Author

\*E-mail: winter@iastate.edu.

### Notes

The authors declare no competing financial interest.

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