Synthesis and Photolysis Properties of a Photolabile Linker Based on 3′-Methoxybenzoin†

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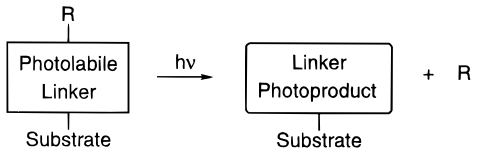
In recent years the applications of photolabile compounds, or compounds that unmask a functional group upon absorption of light, have become more diverse. Since such compounds can provide an effective means of orthogonal protection, they have been used to facilitate the synthesis of complex, polyfunctional organic molecules.[1−3] These materials also have been used to "cage" compounds by protection of an essential functional group, so that a chemical reaction may be initiated by a pulse of light. In this manner, mixing difficulties can be circumvented in kinetic measurements.[4,5] Applications of this method include the photolysis of caged ATP for studies of muscle fiber contraction, where diffusion of ATP into the muscle fiber is slow;[6] of caged fluorescent probes that only emit light after photolysis;[7] and even of caged enzymes by incorporating photolabile groups on essential side chains.[8]

Several photolabile moieties are currently available. The photolysis properties of compounds such as nitrobenzyl esters,[5] phenacyl esters,[9] and benzoin esters[10,11] of carboxylates and phosphates have been well studied. On the other hand, researchers searching for a photolabile linker, or a moiety that holds two compounds together until released by exposing the sample to light (Scheme 1), have fewer options. The photolabile compounds described above have only a single functional group for attachment, rendering them unsuitable for linkage. To provide more options in this area, a photolabile linker based on 3′-methoxybenzoin has been synthesized, and its photolysis properties were studied. Applications for this compound include linkers in solid phase synthesis, methods of caging by encapsulation, and protein folding studies.

Results and Discussion

The linker described below is based on the 3′-methoxybenzoin protecting group described by Sheehan *et al.*[12]

Scheme 1



Carboxylic and phosphate esters of appropriately substituted benzoins cleanly and rapidly photolyze in high yields when exposed to light between 308 and 366 nm. A suitable linker may be derived from this compound by incorporation of a carboxylic acid moiety. Thus, in compound **4**, a carboxy group was added to the 3′-methoxy substituent, to provide the second functional group for linkage. The benzoyl ring was left unsubstituted, as the results of Sheehan *et al.* showed that such substitution lowered the overall photolysis yield.[12]

The synthesis of the carboxylic acid **4** proceeded as outlined in Scheme 2. The central step was the addition of 2-phenyl-1,3-dithiane to **1**, via the lithium anion, to form the dithiane **2**. The hydroxyl of 3-hydroxybenzaldehyde was protected as the TBDMS ether, to circumvent dianion solubility problems. The phenolic hydroxyl was conveniently and selectively alkylated by treatment with TBAF in the presence of methyl bromoacetate, to yield the methyl ester **3**. The carboxylate was then deprotected by lithium iodide in refluxing pyridine, providing the dithiane-protected benzoin linker **4** in an overall yield of 65%.

One difficulty in the synthesis of caged compounds is premature photolysis. In the described synthesis, this problem is circumvented by dithiane protection of the benzoyl carbonyl. Photolysis of the linker is therefore prevented, until the dithiane "safety-catch" is removed (data not shown). Since dithianes may be removed under a variety of mild conditions, many substrates may be caged and manipulated with no special precautions against photolysis, until the dithiane is removed just prior to photolysis. This safety-catch offers a significant advantage over other currently available cage compounds and photolabile protecting groups.

In an effort to examine the photolysis properties of the described benzoin linker, the acetate **6** was synthesized by aminolysis of the methyl ester **3** to form the amide **5**, followed by acylation with acetic anhydride and a catalytic quantity of DMAP. The dithiane was then hydrolyzed by treatment with mercuric perchlorate in aqueous acetonitrile to yield **7**.

Irradiation of **7** resulted in a clean conversion to the phenylbenzofurans **8** and **9**. Steady-state photolysis spectra of **7** show two isosbestic points throughout the course of the photolysis (Figure 1). The two isomeric photoproducts **8** and **9** were produced in a 98% yield at a ratio of 3:1 as determined by GCMS and NMR of the isolated phenylbenzofurans, along with 1 equiv of acetic acid.

Due to the large absorption change at 310 nm ($\Delta\epsilon$ = 35000 M^{-1} cm^{-1}), transient absorption studies of the formation of **8** and **9** were performed. Quenching studies by Sheehan *et al.* showed that photolysis of 3′,5′-dimethoxybenzoin acetate is extremely rapid, with a rate on the order of 10^{10} s^{-1}.[12] Rapid photolysis seems to have been preserved in the benzoin acetate **7**. Photolysis of **7** with a frequency tripled Nd:YAG laser at 355 nm

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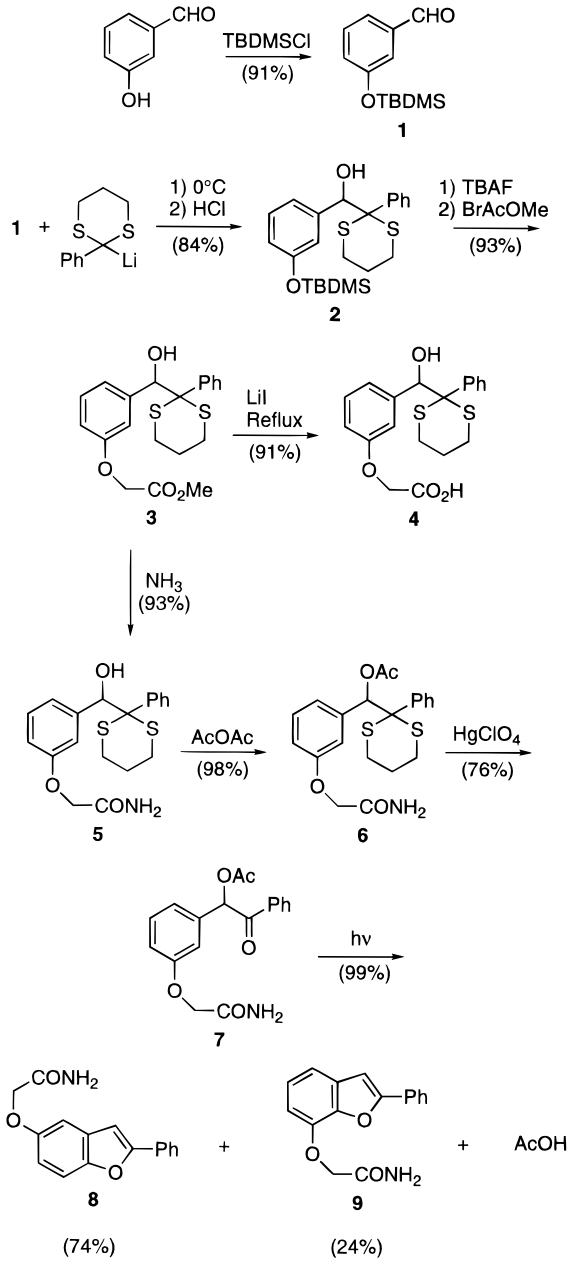
Scheme 2



resulted in a rapid absorption increase at 310 nm. The course of this increase could not be measured within the instrument response time of approximately 30 ns, which places a lower limit on the photolysis rate of 3×10^7 s^{-1} (data not shown).

As a demonstration of a potential application for this compound, the linker was incorporated within the backbone of a decapeptide (Scheme 3). Such a system would be useful for studies of protein conformation and folding, in that the amino acid composition of a target peptide could be drastically altered with a photolysis pulse. In order to facilitate the peptide synthesis, the benzylic hydroxyl of the linker **4** was protected as the Fmoc-ester **10**. This protected linker may be included in any synthetic sequence by standard Fmoc solid-phase synthesis protocols.[13] After preparation of the decapeptide, the dithiane was removed with a solution of bis(trifluoroacetoxy)iodobenzene prior to TFA cleavage, in order to avoid acidolysis of the linker. This peptide was then photolyzed to yield three products: the N-terminal peptide, and two C-terminal peptides corresponding to the two isomeric forms of the phenylbenzofuran photoproduct (Figure 2).

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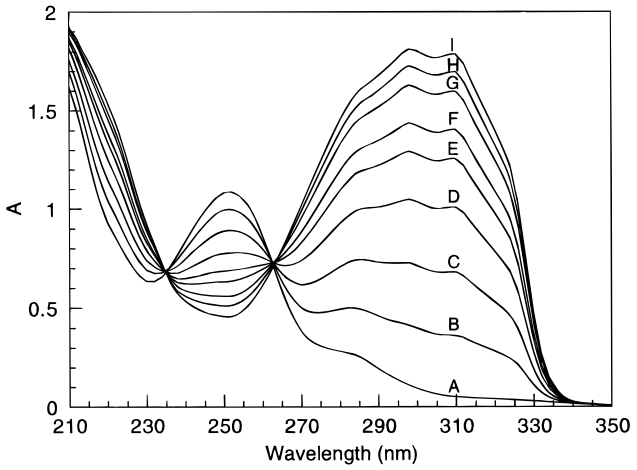
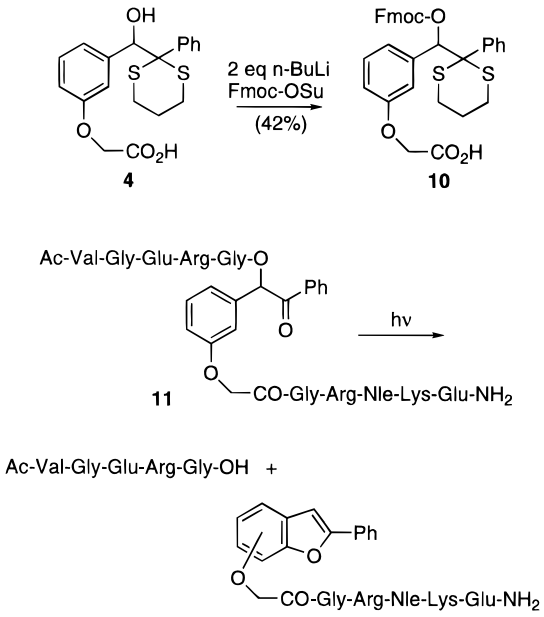


Figure 1. Steady-state photolysis of benzoin acetate **7**. A 47.7 μM solution of **7** in 1:1 methanol/Tris·HCl (0.05 M, pH 7.40) was irradiated with an Oriel 66011 Hg vapor lamp; irradiation time in seconds: A, 0; B, 2; C, 5; D, 10; E, 15; F, 20; G, 30; H, 40; I, 90.

Scheme 3



Many of the properties of the substituted benzoin described above combine to make it an ideal photolabile linker for a variety of applications. Photolysis is extremely rapid, producing a high yield of isolated photoproducts. The linker may be photolyzed by 308−366 nm light, which allows photolysis to proceed without competing absorption by organic moieties that absorb further to the blue. On the other hand, the linker itself is sufficiently transparent from 308−366 nm to prevent inner filter effects from interfering with the course of the photolysis. The phenylbenzofuran photoproducts are expected to be inert,[11] and their production may be easily monitored at 310 nm. Finally, the linker **4** is photochemically inert until removal of the dithiane. Combined, these attributes should make **4** a valuable photolabile linker.

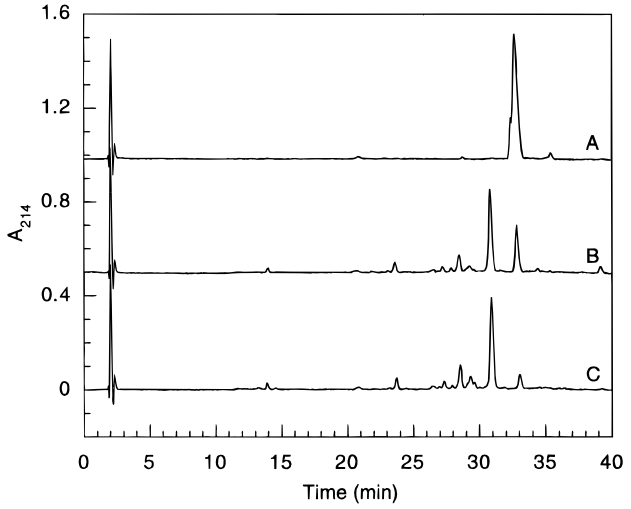


Figure 2. Steady-state photolysis of peptide **11**, as monitored by reversed-phase (C18) HPLC. The two C-terminal phenylbenzofuran-containing peptides elute at 28.5 and 31.0 min, while the N-terminal peptide elutes in the void volume. Some photodegradation of the benzofuran is also apparent at retention times below 30 min. Gradient: 0−35% acetonitrile in water, 0.1% TFA, detection: 214 nm. Irradiation time in minutes: A, 0; B, 5; C, 10.

Experimental Section

General. THF was refluxed over sodium and benzophenone and was distilled prior to use. 3-Hydroxybenzaldehyde (Fluka) was dissolved in diethyl ether, filtered through a plug of neutral alumina, and evaporated. The 1.0 M tetrabutylammonium fluoride (TBAF) solution in THF was dried over 3A molecular seives. All other starting materials were from Aldrich and used without further purification. IR spectra were acquired from a thin film of the sample on a polyethylene substrate. ^{1}H and ^{13}C NMR spectra were collected at 500 and 125 MHz, respectively.

Synthesis of 3-(*tert*-Butyldimethylsilyloxy)benzaldehyde (1). To a solution of 3-hydroxybenzaldehyde (12.21 g, 100 mmol) in 600 mL THF was added *tert*-butyldimethylsilyl chloride (TBDMSCl, 18.84 g, 125 mmol). The solution was cooled to 0 °C and triethylamine (12.65 g, 17.4 mL, 125 mmol) was added dropwise. The reaction mixture was brought to room temperature and stirred 5 h. The mixture was filtered and the THF removed under reduced pressure. The oil was repeatedly dissolved in 200 mL portions of THF and evaporated, until no more triethylamine hydrochloride precipitated. The oil was then dissolved in 150 mL diethyl ether, filtered through a plug of neutral alumina and activated charcoal to remove the salt and the yellow color, and evaporated. The colorless, mobile oil was dried *in vacuo* overnight. Yield: 21.43 g (91%). IR: 1703, 1583, 1482, 1278, 1145, 840 cm^{-1}. ^{1}H NMR ($CDCl_3$, TMS) δ 9.927 (s, 1 H), 7.447 (d, J = 7.50 Hz, 1 H), 7.379−7.335 (m, 2 H), 7.096−7.074 (m, 1 H), 0.994 (s, 9 H), 0.215 (s, 6 H). ^{13}C NMR ($CDCl_3$, TMS) δ 191.60, 156.34, 138.03, 130.03, 126.34, 123.46, 119.70, 25.59, 18.12, −4.52. Anal. Calcd for $C_{13}H_{20}O_2Si$: C, 66.05; H, 8.53. Found: C, 66.13; H, 8.53.

Synthesis of (±)-1-Hydroxy-1-[3-(*tert*-butyldimethylsilyloxy)phenyl]-2-phenyl-2-(1,3-dithian-2-yl)ethane (2). A solution of 2-phenyl-1,3-dithiane (15.71 g, 80 mmol) in 125 mL of THF was prepared. The solution was treated at 0 °C under a nitrogen atmosphere with 40 mL of *n*-butyllithium (2.0 M in cyclohexane, 80 mmol). After 30 min, **1** (18.91 g, 80 mmol) was added. The solution was stirred for 1 h at 0 °C and then poured into 100 mL of 1 N HCl and extracted with methylene chloride (4 × 50 mL). The organic phase was washed with brine, dried with Mg_2SO_4, filtered through a plug of activated charcoal and silica gel, and evaporated under reduced pressure. The resulting oil was crystallized from ethanol/water to form a white powder. Yield: 28.98 g (84%). Mp 75−76 °C. IR: 3449 (br), 1601, 1484, 1275, 1152, 834 cm^{-1}. ^{1}H NMR ($CDCl_3$, TMS) δ 7.70 (d, J = 7.50 Hz, 2 H), 7.308−7.235 (m, 3 H), 6.937 (t, J = 7.79 Hz, 1 H), 6.682−6.660 (m, 1 H), 6.427−6.404 (m, 2 H), 4.926 (d, J = 3.73 Hz, 1 H), 2.936 (d, J = 3.76 Hz, 1 H), 2.739−2.610 (m, 4 H), 1.942−1.879 (m, 2 H), 0.935 (s, 9 H), 0.111 (s, 6 H). ^{13}C NMR ($CDCl_3$, TMS) δ 154.43, 138.89, 137.47, 130.42, 128.00, 127.69, 127.36, 121.23, 119.89, 119.54, 80.74, 66.36, 27.22, 26.93, 25.65, 24.69, 18.03, −4.40. Anal. Calcd $C_{23}H_{32}O_2S_2Si$: C, 63.84; H, 7.45. Found: C, 63.83; H, 7.26.

Synthesis of (±)-1-Hydroxy-1-[3-((carbomethoxy)methoxy)phenyl]-2-phenyl-2-(1,3-dithian-2-yl)ethane (3). A solution of **2** (28.12 g, 65 mmol) and methyl bromoacetate (12.43 g, 81.25 mmol) in 150 mL of dry THF was prepared under a nitrogen atmosphere. The solution was treated with 1 M TBAF in THF (68.25 mL, 68.25 mmol) dropwise. The solution was allowed to react overnight and then was poured into ethyl acetate (200 mL) and washed with water (5 × 50 mL). The organic phase was dried with Mg_2SO_4 and evaporated. The residue was dissolved in 200 mL of diethyl ether, filtered through a small quantity of neutral alumina and activated charcoal, and dried *in vacuo*. The product was crystallized from ethyl acetate/hexanes, to afford a white powder. Yield: 23.61 g (93%). Mp 122−122.5 °C. IR: 3471 (br), 1760, 1595, 1441, 1211, 714 cm^{-1}. ^{1}H NMR ($CDCl_3$, TMS) δ 7.679 (dd, J = 8.16, 1.55 Hz, 2 H), 7.265−7.325 (m, 3 H), 7.045 (t, J = 7.92 Hz, 1 H), 6.804−6.782 (m, 1 H), 6.553 (d, J = 7.61 Hz, 1 H), 6.302 (s, 1 H), 4.960 (d, J = 3.51 Hz, 1 H), 4.367 (s, 2 H), 3.778 (s, 3 H), 3.023 (d, J = 3.52 Hz), 2.757−2.620 (m, 4 H), 1.951−1.891 (m, 2 H). ^{13}C NMR ($CDCl_3$, TMS) δ 169.17, 156.61, 138.88, 137.40, 130.46, 128.08, 127.98, 127.49, 121.76, 115.35, 113.68, 80.73, 66.32, 52.11, 27.30, 26.99, 24.74. Anal. Calcd for $C_{20}H_{22}O_4S_2$: C, 61.51; H, 5.68. Found: C, 61.34; H, 5.75.

Synthesis of (±)-1-Hydroxy-1-[3-(carboxymethoxy)phenyl]-2-phenyl-2-(1,3-dithian-2-yl)ethane (4). A solution of anhydrous lithium iodide (2.68 g, 20 mmol, Aldrich) in 25 mL of dry pyridine was brought to reflux under a nitrogen atmosphere and treated with **3** (1.95 g, 5 mmol). The reaction was refluxed for 6 h and then allowed to cool to room temperature under a stream of nitrogen. The solution was poured into 1 N HCl (300 mL) and extracted with ethyl acetate (3 × 50 mL). The combined ethyl acetate layers were extracted with 5% sodium bicarbonate (4 × 50 mL). The aqueous phase was acidified to pH 2 and extracted with ethyl acetate (3 × 50 mL). The organic phase was dried with Mg_2SO_4, filtered through activated charcoal, evaporated, and triturated with hexanes to yield a white solid. Yield: 1.71 g (91%). Mp 99−101 °C. IR: 3448 (br), 1735, 1595, 1462, 1232, 719 cm^{-1}. ^{1}H NMR ($CDCl_3$, TMS) δ 7.656 (dd, J = 8.00, 1.71 Hz, 2 H), 7.299−7.254 (m, 3 H), 7.036 (t, J = 7.98 Hz, 1 H), 6.794−6.773 (m, 1 H), 6.573 (d, J = 7.55 Hz, 1 H), 6.251 (s, 1 H), 4.955 (s, 1 H), 4.357 (s, 2 H), 2.728−2.620 (m, 4 H), 1.914−1.868 (m, 2 H). ^{13}C NMR ($CDCl_3$, TMS) δ 173.47, 156.32, 139.01, 137.40, 130.50, 128.12, 128.07, 127.56, 122.02, 115.43, 113.66, 80.56, 66.13, 64.73, 27.26, 26.96, 24.67. Anal. Calcd for $C_{19}H_{20}O_4S_2$: C, 60.62; H, 5.35. Found: C, 60.36; H, 5.21.

Synthesis of (±)-1-Hydroxy-1-[3-(carbamylmethoxy)-phenyl]-2-phenyl-2-(1,3-dithian-2-yl)ethane (5). A solution of **4** (391 mg, 1 mmol) was prepared in 50 mL of methanol with gentle warming. The solution was cooled to 0 °C, and gaseous ammonia was bubbled through for 30 min. The flask was wrapped in a towel, securely stoppered, and allowed to come to room temperature. After 2 h, the solvent was removed under reduced pressure to yield a white solid. Yield: 348 mg (93%). Mp 140−141°C. IR: 3460, 3346 (br), 1680, 1586, 1442, 1252, 1058, 714 cm^{-1}. ^{1}H NMR ($CDCl_3$, TMS) δ 7.686 (dd, J = 7.82, 1.70 Hz, 2 H), 7.336−7.291 (m, 3 H), 7.088 (t, J = 7.93 Hz, 1 H), 6.774 (dd, J = 8.09, 2.55 Hz, 1 H), 6.626 (d, J = 7.67 Hz, 1 H), 6.463 (s, br, 1 H), 6.335 (s, 1 H), 5.582 (s, br, 1 H), 4.971 (d, J = 3.16 Hz, 1 H), 4.245 (s, 2 H), 3.092 (d J = 3.24 Hz, 1 H), 2.777−2.635 (m, 4 H), 1.962−1.905 (m, 2 H). ^{13}C NMR ($CDCl_3$, TMS) δ 170.699, 156.059, 139.293, 137.529, 130.437, 128.245, 127.696, 122.261, 114.772, 114.270, 80.706, 67.077, 66.462, 27.342, 27.002, 24.729. Anal. Calcd for $C_{19}H_{21}NO_3S_2$: C, 60.77; H, 5.64; N, 3.73. Found: C, 60.93; H, 5.79; N, 3.76.

Synthesis of (±)-1-Acetoxy-1-[3-(carbamylmethoxy)-phenyl]-2-phenyl-2-(1,3-dithian-2-yl)ethane (6). To a solution of **5** (192 mg, 0.5 mmol) in 10 mL of THF were added DMAP (2 mg), triethylamine (70 μL, 0.5 mmol), and acetic anhydride (94 μL, 1.0 mmol). The solution was stirred at room temperature for 4 h and then partitioned between ethyl acetate (50 mL) and 5% sodium bicarbonate (50 mL). The organic phase was washed

with water (3 × 50 mL), dried with Mg_2SO_4, and evaporated to yield a colorless oil. Yield 205 mg (98%). IR: 3479, 3331 (br), 1747, 1694, 1589, 1443, 1224, 1033, 910, 718 cm^{-1}. ^1H NMR ($CDCl_3$, TMS) δ 7.740 (dd, J = 8.15, 1.55 Hz, 2 H), 7.348−7.281 (m, 3 H), 7.111 (t, J =7.96 Hz, 1 H), 6.801 (dd, J = 8.18, 2.53 Hz, 1 H), 6.686 (d, J = 7.59 Hz, 1 H), 6.521 (s, 2 H), 6.312 (s, 1 H), 6.136 (s, 1 H), 4.238 (s, 2 H), 2.751−2.597 (m, 4 H), 2.104 (s, 3 H), 1.933−1.857 (s, 1 H). ^{13}C NMR ($CDCl_3$, TMS) δ 171.080, 169.318, 156.012, 136.974, 130.771, 128.402, 128.045, 127.738, 122.586, 115.072, 114.635, 79.890, 66.982, 63.133, 27.270, 27.109, 24.568, 20.848. Anal. Calcd for $C_{21}H_{23}NO_4S_2$: C, 60.41; H, 5.55; N, 3.35. Found: C, 59.92; H, 5.76; N, 3.14.

Synthesis of (±)-*O*-Acetyl-3′-(carbamylmethoxy)benzoin (7). To a solution of **6** (110 mg, 0.26 mmol) in 5 mL 9:1 (v/v) acetonitrile/water was added mercuric perchlorate (148 mg, 0.33 mmol). The solution was stirred for 15 min, filtered through a 0.45 μm PTFE syringe filter (Gelman) into a 5% sodium bicarbonate solution (10 mL), and extracted with 50 mL of methylene chloride. The organic phase was dried and evaporated under reduced pressure to yield a colorless oil. Samples for analysis were evaporated from methanol, dissolved in warm water, and lyophilized. Yield: 65 mg (76%). IR: 3445, 1743, 1694, 1462, 1236, 1075, 720 cm^{-1}. ^1H NMR ($CDCl_3$, TMS) δ 7.936 (d, J = 7.82 Hz, 2 H), 7.529 (t, J = 7.56 Hz, 1 H), 7.412 (t, J = 7.57 Hz, 2 H), 7.319 (t, J = 7.86 Hz, 1 H), 7.139 (d, J = 7.51 Hz, 1 H), 7.051 (s, 1 H), 6.884 (dd, J = 8.21, 2.75 Hz, 1 H) 6.835 (s, 1 H), 6.558 (s, 1 H), 6.148 (s, 1 H), 4.462 (s, 2 H), 2.207 (s, 3 H). ^{13}C NMR ($CDCl_3$, TMS) δ 193.577, 170.825, 170.348, 157.593, 135.473, 134.494, 133.637, 130.516, 128.768, 128.711, 122.371, 115.205, 115.140, 77.144, 67.115, 20.715. Anal. Calcd for $C_{18}H_{17}NO_5 \cdot H_2O$: C, 62.59; H, 5.54; N, 4.05. Found: C, 62.53; H, 5.12; N, 3.90.

Steady-State Photolysis of 7. A 47.7 μM solution of **7** in 1:1 methanol/Tris·HCl (0.05 M, pH 7.40) was prepared in a 1 cm pathlength quartz cuvette. The sample was irradiated by an Oriel 66011 Hg vapor lamp operating at 450 W and filtered with a water cooled Schott glass UG11 filter. At intervals, the sample was removed, and the UV absorption spectrum from 210−400 nm was taken by a HP 8452 spectrophotometer. Complete photolysis occurred within a 90 s exposure.

For isolation of the photoproduct, a 25.6 mg sample of **7** in 50 mL of methanol was irradiated in 3 mL batches as above, until no further change was observed in the absorption spectrum of the sample. The methanol was removed under reduced pressure to yield 20.6 mg (99%) of the photoproduct. The composition of this material was 74% 2-phenyl-5-(carbamylmethoxy)benzofuran (**8**), 24% 2-phenyl-7-(carbamylmethoxy)-benzofuran (**9**), and 2% other material, as determined by GCMS. Standard samples were obtained by preparative-TLC (silica gel/diethyl ether) of the crude photolyzed sample and identified by ^1H NMR and IR.

Transient Photolysis of 7. A 9.54 μM solution of **7** in 1:1 methanol/Tris·HCl (0.05 M, pH 7.40) was prepared in a 1 cm pathlength quartz cuvette. The sample was photolyzed using the third harmonic at 355 nm from a Q-switched Spectra Physics DCR-12 Nd:YAG laser. Typical pulses were 10−20 ns (FWHM) in duration at an energy of 1.5 mJ/pulse. The sample was monitored with a 75 W xenon arc lamp filtered with a Schott glass UG11 filter placed between the arc lamp and the cuvette. The probe light exiting the cuvette was then wavelength-selected by a SA 1690B double monochromator set at 310 nm and was detected with a photomultiplier. The signal was amplified with a Keithly 427 current amplifier and digitized by a Tektronix R710 200 MHz transient digitizer interfaced to a microcomputer. Samples were acquired at a 10 Hz photolysis pulse repetition rate, and scans represented the average of 20 pulses.

Synthesis of (±)-1-(Fluorenylmethoxycarbonyloxy)-1-[3-(carboxymethoxy)phenyl]-2-phenyl-2-(1,3-dithian-2-yl)-ethane (10). A solution of **4** (1.57 g, 4.17 mmol) in 50 mL of THF was prepared under a nitrogen atmosphere. The solution was cooled to −78 °C, and *n*-butyllithium (2.0 M in cyclohexane, Aldrich) was added until the dianion precipitated and a persistent yellow color remained (approximately 4 mL, 8 mmol). The suspension was treated with 9-fluorenylmethyl succinimidyl carbonate (Fmoc-OSu, 3.18 g, 9.43 mmol, Bachem), and the cold bath was removed. After 1 h, the suspension was poured into 1 N HCl and extracted with ethyl acetate. The organic phase was dried and evaporated under reduced pressure. The resulting oil was purified by reversed-phase HPLC (C18, 70% to 90% acetonitrile in water, 0.1% TFA, 40 min). Yield: 1.06 g (42%). IR: 1747, 1597, 1462, 1256, 730 cm^{-1}. ^1H NMR ($CDCl_3$, TMS) δ 7.778 (d, J = 6.85 Hz, 2 H), 7.739 (dd, J = 7.40, 2.47 Hz, 2 H), 7.590 (dd, J = 12.18, 7.47 Hz, 2 H), 7.404−7.237 (m, 7 H), 7.115 (t, J = 7.94 Hz, 1 H), 6.842 (dd, J = 8.24, 2.38 Hz, 1 H), 6.710 (d, J = 7.64 Hz, 1 H), 6.346 (s, 1 H), 6.024 (s, 1 H), 4.430−4.393 (m, 3 H), 4.298−4.256 (m, 2 H), 2.778−2.603 (m, 4 H), 1.984−1.856 (m, 2 H). ^{13}C NMR ($CDCl_3$, TMS) δ 173.41, 156.42, 154.02, 143.32, 143.25, 141.25, 141.24, 136.79, 136.17, 130.78, 128.44, 128.13, 127.13, 125.26, 122.51, 119.98, 115.92, 114.17, 83.94, 70.19, 64.77, 62.93, 46.66, 27.26, 27.18, 24.48. Anal. Calcd for $C_{34}H_{30}O_6S_2 \cdot H_2O$: C, 66.21; H, 5.23. Found C, 66.44; H, 5.44.

Synthesis of Photolabile Peptide 11. The sequence Ac-Val-Gly-Glu-Arg-Gly-linker-Gly-Arg-Nle-Lys-Glu-NH_2 was prepared on an Fmoc-amide resin (Applied Biosystems, Inc.) on a 0.1 mmol scale. Couplings were performed with 1 mmol each of DCC, HOBt, and Fmoc-AA or **10** in NMP for 1 h, with the exception of Gly-5 which was coupled to the benzylic hydroxyl of the linker with 1 mmol DCC and 0.01 mmol DMAP for 24 h. The Fmoc groups were removed by 3 × 3 min treatments with 30% piperidine in NMP. Couplings were monitored by the absorbtion of the dibenzofulvene-piperidine adduct or the qualitative Kaiser test.[14] The N-terminal valine was capped with 1 mmol of acetic anhydride and 0.01 mmol of DMAP, and the dithiane was removed by treatment with 0.5 mmol of bis-(trifluoroacetoxy)iodobenzene in 9:1 acetonitrile:water for 10 min. The resin was dried and then cleaved with 1.8 mL of TFA, 0.1 mL of water, and 100 mg of phenol for 90 min. The crude peptide was isolated by precipitation in methyl butyl ether and purified by reversed-phase HPLC (C18, water/acetonitrile gradient containing 0.1% TFA).

Steady-State Photolysis of Peptide 11. The purified peptide HPLC fractions were diluted 10:1 with 25 mM sodium acetate buffer, pH 5.5, and irradiated as for the photolysis of **7**. Aliquots were periodically analyzed by reversed-phase HPLC. Maximum photolysis occurred after 10 min.

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