JOC The Journal of Organic Chemistry

Article

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A Dimethyl Ketal-Protected Benzoin-Based Linker Suitable for Photolytic Release of Unprotected Peptides

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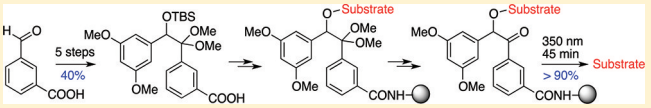
Supporting Information

ABSTRACT: Photolabile 3′,5′-dimethoxybenzoin-based linkers are advantageous for a variety of solid-phase synthetic procedures and manipulations of biomolecules because UV irradiation in aqueous media provides fast and essentially quantitative release of tethered molecules, while generating unreactive side products. Practical applications of previously reported linkers are compromised to some extent by the 1,3-dithiane protection of the benzoin carbonyl group and the lengthy synthesis. We have extended the group of photocleavable 3′,5′-dimethoxybenzoin-based linkers by designing and synthesizing a linker in which the carbonyl group is protected as a dimethyl ketal. This protection is compatible with commonly used esterification and amide bond formation techniques, including the Fmoc/tBu strategy for solid phase peptide synthesis, is stable under mild acidic conditions, and can be quantitatively removed in <5 min by 3% TFA in dichloromethane. Irradiation of beads carrying peptides attached to the linker at 350 nm in aqueous or partially aqueous media affords >90% release after 30 min. The linker was synthesized from commercially available starting materials in five steps with an overall yield of 40% and without any column chromatography purification. Additionally, we developed a route to a dithiane-protected linker that requires only two steps and proceeds in 65% yield, a significant improvement over previous synthetic routes.

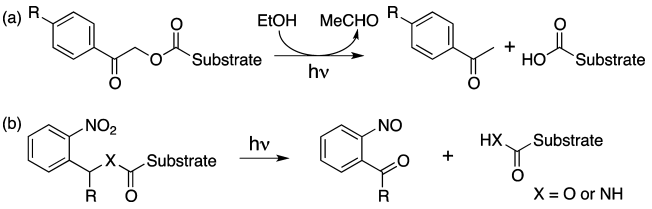


INTRODUCTION

Photolabile linkers are being used in an increasing number of chemical and biological applications. Solid-phase organic synthesis allows synthesis or transformation of substrates while bound to a solid phase through a linker that can later be degraded, allowing release of the product. If release is achieved photochemically, the product does not require purification from reagents necessary for chemical cleavage. Biological applications of photolabile linkers have increased dramatically within the past few years. Examples include enrichment of specific proteins and metabolites,[1] photoactivatable probes for protein labeling,[2] "caged" compounds for light-induced release of biological activity,[3] delivery of activators/inhibitors to organelle surfaces,[4] and construction of polymeric vesicles for imaging and drug delivery.[5]

Our applications required synthesis and then efficient release of short fully deprotected peptides and peptide libraries from a solid support into neutral aqueous medium. The most widely used photolabile linkers, which contain either a phenacyl (2-oxo-2-phenylethyl) (Scheme 1a) or a 2-nitrobenzyl (Scheme 1b) moiety, are not suitable for this purpose. Phenacyl derivatives are not stable under the nucleophilic conditions[6] of the Fmoc/tBu strategy and are prone to formation of piperazine-2,5-diones.[7] Photolysis requires a hydrogen donor, typically ethanol, which is converted to acetaldehyde that can cause side reactions. Furthermore, the efficiency of photocleavage is lower in the presence of water.[8] We attempted to use two commercially available 2-nitrobenzyl linkers—3-amino-3-(2-nitrophenyl)propionic acid[9] and 4-(4-(1-aminoethyl)-2-methoxy-5-nitrophenoxy) butanoic acid (the Holmes linker)[10,11]—but observed <40% photorelease for substrates containing free amino groups.

Scheme 1. Photocleavage of Phenacyl Esters (a) and 2-Nitrobenzyl Esters and Amides (b)

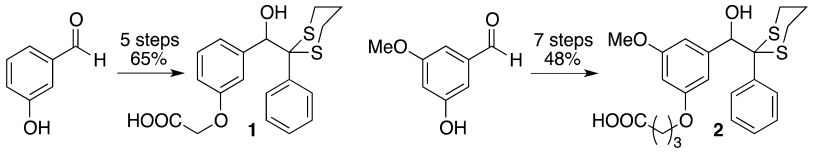


We turned our attention to the dithiane-protected benzoin-based photolinkers **1**[12] and **2**[13,14] (Scheme 2). Unfortunately, there is no reliable method for dithiane removal that is compatible with the entire set of amino acid functional groups. Rock and Chan used [bis(trifluoroacetoxy)iodo]benzene (BTI)[15] to remove the dithiane prior to release of a short peptide,[12] but this reagent promotes Hofmann rearrangement of primary amides[16] and oxidizes phenols,[17] thiols,[18] and secondary amines.[19] Further, **1** has only one meta-alkoxy substituent on the nonconjugated phenyl ring; usually two such substituents enable faster and cleaner photocleavage.[20,21]

Received: August 19, 2011
Published: September 27, 2011

   dx.doi.org/10.1021/jo2017263 | J. Org. Chem. 2011, 76, 9409–9416

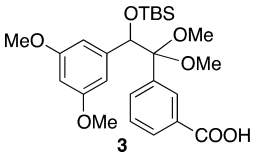
Scheme 2. Previously Reported Dithiane-Protected Benzoin Linkers



Synthesis of **2** requires 3-hydroxy-5-methoxybenzaldehyde, which is not widely commercially available.[22]

Here we report a new 3′,5′-dimethoxybenzoin-based linker **3** (Scheme 3) in which the carbonyl group is protected as a

Scheme 3. Dimethyl Ketal-Protected Benzoin-Based Photolinker 3



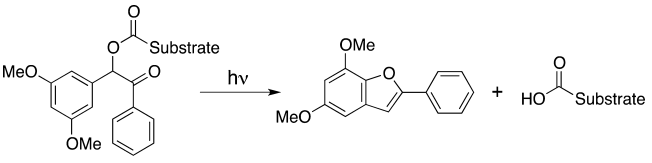
dimethyl ketal. The protection is stable under mild acidic conditions. After simultaneous deprotection of the benzoin carbonyl and amino acid side chains, unprotected peptides can be rapidly released by photolysis. The linker can be synthesized in five steps with an overall yield of 40%. *All of the intermediates are crystalline solids, and no intermediate requires purification by column chromatography.*

RESULTS AND DISCUSSION

We began our work by exploring the utility of commercially available N-Fmoc-protected 2-nitrobenzyl linkers—3-amino-3-(2-nitrophenyl)propionic acid[9] and 4-(4-(1-aminoethyl)-2-methoxy-5-nitrophenoxy) butanoic acid[10,11] (the Holmes linker)—for photolytic release of unprotected peptides. The linkers were quantitatively coupled to TentaGel S NH_2 resin using the standard PyAOP/HOAt/DIEA method. Fmoc protection was removed by treatment with 20% piperidine in DMF for 20 min at room temperature. The simple model compounds N-Fmoc-glycine or N-Fmoc-6-aminohexanoic acid were quantitatively attached to the amino groups of the linkers and deprotected using the same protocols. The structures of the products were confirmed on-resin using high-resolution magic angle spinning (HRMAS) NMR spectroscopy. Subsequently glycinamide or 6-aminohexanamide was released from the resin (25 mg, containing 0.2−0.3 mmol of substrate per g) by irradiation at 350 nm with stirring under argon. For both linkers, the yield of released amide after irradiation in water, methanol, or acetonitrile was <40%. After 20 min of irradiation, the resins turned a deep brownish-red color, and the reactions did not proceed further. We are not the first group to encounter complications with the use of the Holmes linker for release of compounds containing amino groups. Detrimental side reactions that occur during photochemical cleavage of unprotected peptides have been reported.[23] Additionally, it has long been known that deep red azo and azoxy compounds arising from the nitroso photoproducts[24,25] of linker decomposition act as internal light filters, thus hindering complete photorelease of substrates from *o*-nitrobenzyl moieties. While it was reported that photocleavage of protected peptide fragments from an *o*-nitrobenzyl linker proceeds in high yield when degassed solvents and <300 mg of the resin containing immobilized peptide are used,[26] the yields of compounds containing free amino groups (glycinamide or 6-aminohexanamide) after photocleavage from these linkers were below 40% in our hands, even on a 25 mg scale. The low yields likely resulted from a combination of factors, including formation of internal light filters and reactions of the free amino groups on the substrate with nitrosoketone intermediates formed during photocleavage. Amines, such as hydrazine, hydroxylamine, or semicarbazide,[24] or thiols, such as DTT,[27] can be added during irradiation to scavenge nitrosoketone intermediates. However, addition of scavengers negates advantages of photocleavage for many biological applications.

Because of the difficulties we encountered with photocleavage of 2-nitrobenzyl linkers, we turned our attention to benzoin-based linkers. Facile release of esterified carboxylic acids by photocyclization of benzoin esters was reported in 1964 by Sheehan and Wilson.[28] They[20] and others[21] found that, while electron-donating substituents on the benzoyl ring interfered with photodeprotection, meta-alkoxy substitution on the nonconjugated phenyl ring allows rapid and essentially quantitative photorelease of the carboxylic acid and produces relatively inert 2-phenyl-5,7-dimethoxybenzofuran (Scheme 4).

Scheme 4. Products Formed by Photocleavage of 3,5-Dimethoxybenzoin Esters

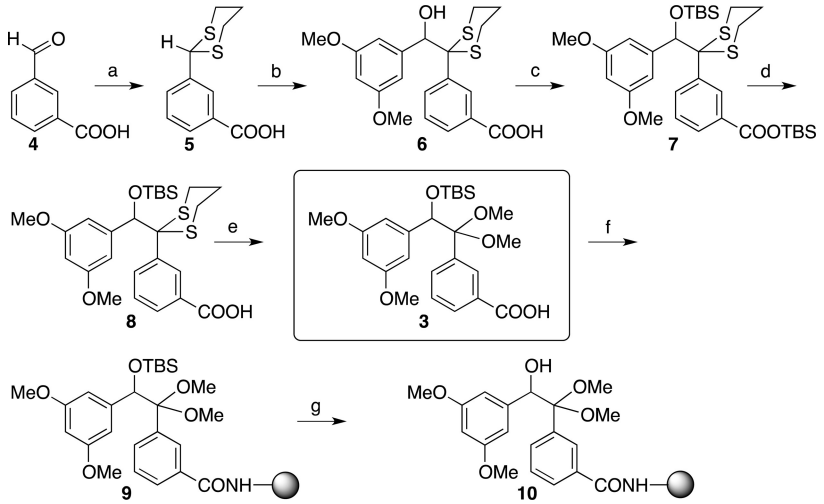


Efficient photolytic formation of the benzofuran was also observed for 3,5-dimethoxybenzoin (DMB) derivatives bearing other relatively good leaving groups, such as phosphate,[29] phosphoester,[21] carbonate,[30] or carbamate.[31] Photolysis can be carried out in a variety of solvents (benzene, THF, dioxan, acetonitrile, ethanol, water). Only two studies have reported products other than inert benzofuran. Photolysis of water-soluble 3′,5′-bis(carboxymethoxy)benzoin (BCMB) acetate in aqueous media produced around 70% of BCMB,[32] and photolysis of DMB fluoride produced 20% of parent 3′5′-dimethoxybenzoin on irradiation in acetonitrile−water (1:1 by volume). In trifluoroethanol, DMB fluoride produced 35% of a product in which the fluoride had been substituted by trifluoroethoxide.[33] Although the reaction mechanism of the photodecomposition of benzoin derivatives is still debated,[33,34] for our purposes it was germane that photolysis of 3′5′-dimethoxybenzoin esters is rapid, results in nearly quantitative release of carboxylic acids in aqueous media, and produces nonreactive byproducts.

The stability of benzoin-based linkers is enhanced by protection of the benzoin carbonyl to provide a "safety catch" that prevents premature cleavage. Our goal was to design a linker that possesses a carbonyl protecting group compatibile

dx.doi.org/10.1021/jo2017263 | J. Org. Chem. 2011, 76, 9409−9416

Scheme 5. Synthesis, Attachment to Beads, and Deprotection of Photolabile Linker 3[a]



[a]Key: (a) 1,3-propanedithiol, BF_3*Et_2O, AcOH, 75 °C, 1.5 h, 93%; (b) BuLi, −60 °C, 1 h 50 min, and then 3,5-dimethoxybenzaldehyde, −78 °C, 40 min, 70%; (c) TBSOTf, 2,6-lutidine, CH_2Cl_2, rt, 3 h, 88%; (d) 80% AcOH, 50 to 75 °C over 45 min, then 75 °C, 15 min, 86%; (e) BTI, MeOH, rt, 30 min, 81%; (f) PyAOP, HOAt, DIEA, DMF, rt, 45 min; (g) 0.17 M TBAF in THF.

with the common Fmoc/tBu strategy of SPPS but that can be removed without detrimental effects on common peptide functional groups. Additionally, we aimed to design a less laborious synthesis from commercially available starting materials.

After synthesis of peptides on a solid phase using the Fmoc/tBu strategy, deprotection of amino acid side chains is usually achieved by treatment with concentrated TFA. We explored the utility of protecting the benzoin carbonyl as an acid-labile ketal to allow simultaneous deprotection of the benzoin carbonyl and amino acid side chains prior to photocleavage of the peptide from the support. The stability of ketals to aqueous and nonaqueous bases and to nucleophiles allows for compatibility with the Fmoc/tBu SPPS strategy. However, we had two major concerns. First, the reactivity of carbonyl groups toward ketalization is low for aromatic ketones. Indeed, our preliminary attempts to convert the commercially available 2-hydroxy-2-phenylacetophenone to the dimethyl ketal by the common procedure ($(MeO)_3CH$, anhydrous MeOH, TsOH, reflux)[35] were unsuccessful. Second, the ketal protection might cleave prematurely under mildly acidic (pH 4) conditions that might be required during both synthesis of the linker and downstream applications. Therefore, we chose to construct the benzoin framework using a 1,3-dithiane-masked carbonyl and then to transform the *S*,*S*-acetal directly into the dimethyl ketal by the ingenious Stork and Zhao protocol.[15] Here we describe a new benzoin-based linker **3**, which can be efficiently synthesized in five steps with an overall yield of 40% (Scheme 5). The linker possesses a ketal protecting group that remains intact on heating of **3** to 95 °C in 80% aqueous acetic acid but can be quantitatively removed in 3 min at room temperature by 3% TFA in dichloromethane.

We chose to use a carboxylic acid as an anchoring group to hydroxyl- or amine-functionalized resins and began the synthesis from commercially available 3-formylbenzoic acid (**4**). The transformation of **4** to the corresponding dithiane **5** is straightforward and nearly quantitative. Conversion of **5** to **6** requires some care. The reaction begins with addition of 1 equiv of BuLi to generate the lithium salt of **5**; a second equivalent of BuLi is added to remove the acidic proton from the dithiane ring. At 0 °C, BuLi slowly attacks the lithium carboxylate, so it is important to cool the reaction to −60 °C, use no more than 2.05 equiv of BuLi, and add the BuLi slowly over at least 15 min while stirring efficiently. It is also important to use no more than 1.06 equiv of 3,5-dimethoxybenzaldehyde in the reaction to avoid formation of 3,5-dimethoxybenzoic acid (which is extremely difficult to separate from **6**), probably via a base-induced disproportionation−Cannizzaro reaction. Note that this strategy results in synthesis of the dithiane-protected linker **6** in two steps in 65% overall yield. This linker can be used in place of linkers **1** and **2** for analogous applications, but its preparation is markedly more efficient.[12−14]

Dithiane **6** could have been converted directly to the corresponding dimethyl ketal. However, leaving the OH group unprotected would have prevented applications requiring an ester bond-based attachment of the linker to a solid support (or some other moiety). Under esterification conditions, the free hydroxyl of the linker will compete with the hydroxyl group of the solid support, leading to polymerization of the linker. This concern was based upon the report that dithiane-protected 3-hydroxybenzoin immobilized on a resin was readily esterified with Fmoc derivatives of Phe, Val, and Pro using the diisopropylcarbodiimide protocol in 5 h (with yields of 100%, 65%, and 57%, respectively).[13] Furthermore, the presence of a free OH group could complicate loading of the linker onto amino group-bearing moieties via common methods for formation of amide bond. For example, sterically hindered carboxylic acids were attached to the free hydroxyl group of a resin-immobilized dithiane-protected 3-hydroxybenzoin in significant (<30%) yields[13] under standard HATU or PyBOP activation-based protocols. Moreover, the PyAOP activation procedure was used for acylation of hydroxymethyl resins with Fmoc/Trt derivatives of Cys and His.[36] To avoid any problems with polymerization of the linker during loading onto a solid phase under a broad range of conditions, we chose to protect the free hydroxyl group before converting the dithiane to a dimethyl ketal.

We could not find conditions for transformation of **6** into **7** using inexpensive *tert*-butyldimethylsilyl (TBS) chloride.

dx.doi.org/10.1021/jo2017263 | *J. Org. Chem.* 2011, 76, 9409−9416

Table 1. Yields of Dipeptides after Photorelease from Beads[a]

dipeptide	irradn time, min	yield (%) in the indicated solvent			
		CD_3CN	CD_3CN:D_2O, 6:4	CD_3OD	CD_3OD:D_2O, 6:4
His-Ile	15	37	78	62	78
His-Ile	30	41	83	73	92
His-Ile	45	44	93	75	98
Val-Tyr	45	40	90	70	95

[a]Yields were determined by ^{1}H NMR in the presence of an added internal standard (*p*-toluic acid or sodium acrylate). Calculations are based on the capacity of amino groups on the TentaGel (0.30 mmol/g).

However, reaction with TBSOTf (easily prepared from TBS chloride and triflic acid)[37] was clean and gave no side products. The silyl ester **7** was readily purified by crystallization. Complete hydrolysis of **7** to the corresponding acid **8** required heating to 75 °C in 80% aqueous acetic acid for 1 h. As expected, the *S*,*S*-acetal in **8** was smoothly converted to the corresponding dimethyl ketal **3** in high yield (81%) by treatment with BTI in anhydrous methanol by the method of Stork and Zhao.[15] Luckily, the ketal protection was entirely stable to hydrolysis on prolonged heating of **3** to 95 °C in 80% aqueous acetic acid.

Quantitative loading of **3** onto TentaGel NH_2 beads to give **9** was achieved using the standard PyAOP/HOAt/DIEA method in anhydrous DMF (2× excess of **3**). Because the Kaiser test[38] demonstrated the absence of unreacted amino groups, treatment of the solid phase with acetic anhydride to block residual amino groups was not required. Removal of the TBS protecting group with TBAF in THF to give **10** occurred in 93% yield after 3 h at room temperature and was quantitative by 6−7 h. The reaction was monitored by HRMAS ^{1}H NMR. The TBS signals at δ 0.92, 0.18, and −0.18 ppm disappeared. Concomitantly, the resonance of the proton at the carbon carrying the OTBS group in **9** and OH in **10** shifted from δ 4.99 to 5.08 ppm.

Esterification of ketal **10** proved to be more facile than esterification of more sterically hindered 1,3-dithiane protected benzoins such as **1** and **2** as well as others.[12−14] In the case of **10**, even the most sterically hindered amino acid, isoleucine, was quantitatively[39] added to the beads after 6 h at room temperature using the carbodiimide protocol (DCC/DMAP), the most common approach in the SPPS literature for the esterification of solid-phase alcohol groups.[40] For less-crowded molecules such as Fmoc-Tyr(O*t*Bu), Fmoc-6-aminohexanoic acid, and propionic acid, esterification was complete in 2 h. In contrast, only 65% loading of the sterically crowded Fmoc-Val onto the solid-supported dithiane linker **2** was achieved after extended reaction.[13] After removal of the Fmoc protecting group (20% piperidine in DMF, 15 min, rt), a second Fmoc-protected amino acid was added (His to the Ile resin and Val to the Tyr resin) using the PyAOP/HOAt/DIEA procedure.

As a next step we investigated whether the ester linkage between **10** and a substrate is stable under conditions generally employed for removal of protecting groups from amino acid side chains. After treatment with a freshly prepared mixture of TFA/H_2O/phenol/thioanisole (85 mL/5 mL/5 g/5 g) for 6 h at room temperature, we did not observe any loss of His-Ile or Val-Tyr from the beads due to hydrolysis of the ester bond. As expected, the dimethyl ketal on the linker was completely removed under these conditions.

We also explored conditions that would allow deprotection of the ketal of the linker while leaving protecting groups on the amino acid side chains intact and found that removal of the ketal is quantitative in 5 min at room temperature using 3% v/v TFA in anhydrous dichloromethane. A higher concentration of TFA (3%) than expected (1%) was necessary, most likely due to buffering of the solution by the large number of ether oxygens in the poly(ethylene glycol) chains grafted on the polystyrene matrix of TentaGel.[41]

Photocleavage of peptides (either protected or unprotected) from the resin was achieved by irradiation with a 100 W "street light type" medium-pressure mercury lamp (GE H100-A4/T), as described in the Experimental Section. Table 1 shows that photorelease was complete in 45 min, with ∼80% of the product being produced during the first 15 min of irradiation. The efficiency of photocleavage of dipeptides His-Ile and Val-Tyr depends on the nature of the solvent; yields were nearly quantitative in the presence of water in acetonitrile or methanol but considerably lower in anhydrous acetonitrile or methanol.

^{1}H NMR analysis revealed that each dipeptide was obtained as a mixture of two diastereomers. When DCC/DMAP coupling was used, the C-terminal amino acid underwent 6−8% racemization when the first amino acid was Tyr (2 h reaction) and up to 15% racemization when the first amino acid was Ile (6 h reaction). Racemization of the C-terminal amino acid due to use of DMAP as the reaction catalyst is a common problem in SPPS.[42] Less racemization is observed when acylation of hydroxymethyl-based resins with Fmoc-protected amino acids is carried out using TDO (4-hydroxy-3-oxo-2,5-diphenyl-2,3-dihydrothiophene 1,1-dioxide) esters,[43] mixed anhydrides formed from the amino acid and 2,6-dichlorobenzoyl chloride in pyridine,[44] or acyl fluorides in a mixture of pyridine and CH_2Cl_2.[45] In our case, the TDO ester obtained from Fmoc-Tyr(tBu)-OH was not reactive with **10**, and use of the corresponding acid fluoride resulted in only 20% esterification in 24 h. Reaction of **10** with a 5-fold molar excess of both Fmoc-Tyr(tBu)-OH and 2,6-dichlorobenzoyl chloride and a 20-fold molar excess of pyridine occurred in quantitative yield in 24 h but, unfortunately, resulted in 14% racemization.

The partial loss of stereochemical integrity during the esterification step, which is likely due to steric crowding around hydroxyl reaction centers in benzoin-based photolinkers, has not been previously recognized, although it would be expected to occur with linkers **1** and **2**. Previous workers did not report this problem, because they either used Fmoc-Gly as the C-terminal amino acid for peptide synthesis[12] or limited their trials to attachment following by release of a single amino acid and therefore could not have detected racemization using the techniques that were employed for characterization of the product.[13,14] Thus, this is an issue that likely needs to be resolved for the entire class of benzoin-based linkers.

dx.doi.org/10.1021/jo2017263 | *J. Org. Chem.* 2011, 76, 9409−9416

Nevertheless, our linker provides a significant improvement due to the ease of the esterification of dimethyl ketal protected **10** in comparison with the dithiane-bearing **1** and **2**. The observed racemization will not be a problem for many molecular biology applications because a spacer is often positioned between the photolabile linker and an immobilized peptide to prevent steric hindrance of binding of macromolecules to the resin.

At present, the choice of photolable linkers for the most efficient Fmoc/tBu strategy in SPPS is limited to *o*-nitrobenzyl-based linkers. As discussed above, these linkers are restricted to only relatively small amounts of peptide resin and might result in low cleavage yields, and consequently, they are rarely used for SPPS. Rather, acid-labile linkers are preferred. Deprotection of amino acid side chains and release of the final peptide from the solid support are accomplished simultaneously by acid hydrolysis with concentrated TFA containing large (up to 5%) quantities of phenol, thioanisole, and other scavengers of tBu carbonium ions. The crude peptide is precipitated in diethyl or methyl *tert*-butyl ether and contains over 50 wt % of salts, small water-soluble molecules, protecting group residues, deletion peptides, and cleavage-deteriorated peptides. Purification of the peptide to >95% is complicated by the presence of this complex mixture of salts and byproducts. A photocleavable linker should allow deprotection of side chains on the resin and then extensive washing of the resin-bound peptide before the final cleavage, delivering the product contaminated only with the inevitable deletion/truncation peptides and peptides damaged by deprotection.

To explore the usefulness of linker **3**, we took on the challenge of synthesizing a very difficult arginine-rich peptide sequence (Lys-Tyr-Arg-Arg-Arg-Pro-Arg-Arg-Ser-Gln-Arg-Lys-Arg-Gly). The synthesis was carried out on a 0.04 mmol scale. A common problem with ester-based linkers is formation of piperazine-2,5-diones (diketopiperazines), resulting in release of the substrate during coupling of the third amino acid and re-formation of the bead-linked linker **10**. However, HRMAS ^{1}H NMR of the beads carrying Lys-Arg-Gly after the third coupling step shoved no signal at 5.08 ppm characteristic of the C*H*OH proton of **10**. Therefore, we conclude that formation of piperazine-2,5-diones is not a concern.

According to the Proteomics Resource Center of the Rockefeller University, a 0.04 mmol scale synthesis is expected to produce 39−53 mg of a crude 14-mer peptide, and "HPLC purification to >95% purity will reduce the yield by at least 50%". Using our linker, after completion of the synthesis and irradiation of the beads for 40 min in 5 mL of 30% aqueous methanol, we obtained 62 mg of desalted peptide that was >75% pure according to HPLC (Figure 1). Thus, the yield of crude peptide we obtained was higher than expected, even though the standard SPPS procedure results in significant contamination by salts and residual protecting groups. The ^{1}H NMR spectrum showed no visible traces of small molecules or residual protecting groups (see Supporting Information). The predicted monoisotopic mass for the peptide is 1837.1208; the observed mass of the major peak is 1837.0793.

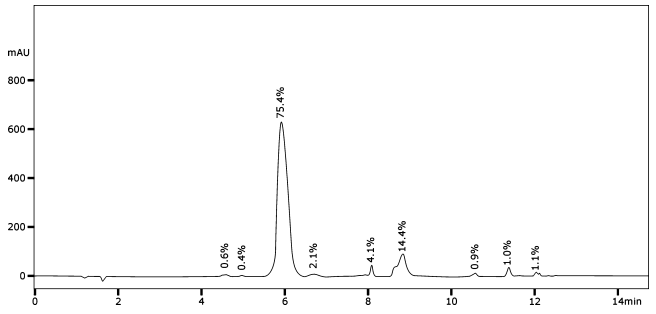


Figure 1. HPLC trace of crude Lys-Tyr-Arg-Arg-Arg-Pro-Arg-Arg-Ser-Gln-Arg-Lys-Arg-Gly. The detector was set at 217 nm.

CONCLUSION

We have succeeded in synthesizing a photocleavable benzoin linker **3** that has significant advantages over currently available benzoin-based linkers due to the dimethyl ketal protection of the benzoin carbonyl and the straightforward synthesis in 40% overall yield from readily available starting materials via five steps, all of which afford stable crystalline products. Linker **3** shares the previously recognized valuable photochemical properties of 3′,5-dimethoxybenzoin-based protective groups that allow rapid and essentially quantitative photorelease while producing nonreactive and colorless 2-phenyl-5,7-dimethoxybenzofuran. Partial loss of stereochemical integrity of acids possessing an α-stereogenic center occurs on esterification of our linker and, most likely, other benzoin-based linkers under common conditions. However, our linker provides considerable advantages for a multitude of molecular biology applications in which a spacer between the resin and the immobilized ligand or peptide is commonly used and therefore racemization is not a problem. Further, our linker may provide an improvement over 2-nitrobenzyl linkers in photoactivatable probes for *in vivo* applications because photocleavage does not produce reactive intermediates that can damage amino acid side chains.

EXPERIMENTAL SECTION

General. Melting points were determined on a hot-stage apparatus and are uncorrected. On-resin NMR experiments were performed on a 400 MHz spectrometer using with a 4 mm ghxNano probe. Magic angle sample spinning (MAS) was performed at a spinning speed of 2.8−3.0 kHz. Prior to spinning, samples on polystyrene beads were swelled in $CDCl_3$ to allow sufficient line narrowing for high-resolution NMR. High-resolution mass spectra were obtained on an ABI QSTAR in ECI (−) mode. Air-sensitive reactions were performed in flame-dried glassware under argon. Reagents and solvents were used as purchased unless otherwise indicated.

*3-(1,3-Dithian-2-yl)benzoic Acid (**5**).* 3-Formylbenzoic acid (**4**, 3.00 g, 20.0 mmol, 1.0 equiv) was dissolved in acetic acid (30 mL) at 75 °C in an oil bath. Neat 1,3-propanedithiol (2.40 mL, 2.58 g, 23.9 mmol, 1.2 equiv) was added, followed by boron trifluoride etherate (1.00 mL, 1.15 g, 8.1 mmol, 0.41 equiv). The reaction mixture was stirred in the bath for 1.5 h and then evaporated to dryness at reduced pressure. The residue was dissolved by refluxing in methanol (30 mL). The boiling solution was treated with 12 mL of water, which caused the solution to become cloudy, and allowed to cool to room temperature. Formation of white crystals of **5** was complete in 1 h at room temperature. The crystals were filtered, washed with water, dried, and washed with hexanes to remove residual dithiol. The white solid 5 (4.70 g, 19.6 mmol, 98%) was pure by ^{1}H and ^{13}C NMR. Recrystallization from methanol/water (30 mL/12 mL) was performed to remove any traces of boron derivatives, yielding 5 (4.47 g, 18.6 mmol, 93%) as snow-white crystals; mp = 164−165 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.23 (s, 1H), 8.06 (d, J = 7.8 Hz, 1H), 7.75 (d, J = 7.7 Hz, 1H), 7.47 (app. t, J = 7.75 Hz, 1H), 5.23 (s, 1H), 3.15−3.00 (m, 2H), 3.00−2.85 (m, 2H), 2.25−2.13 (m, 1H), 2.05−1.86 (m, 1H). ^{13}C NMR (300 MHz, $CDCl_3$): δ 172.0, 139.9, 133.5, 130.5, 130.0, 129.3, 51.1, 51.0, 32.2, 25.2. Predicted exact mass for $C_{11}H_{11}O_2S_2$ 239.0206; observed mass 239.0193.

*3-[2-(Hydroxy(3,5-dimethoxyphenyl)methyl)-1,3-dithian-2-yl]-benzoic Acid (**6**).* Preparation of **6** was done under argon with

dx.doi.org/10.1021/jo2017263 | J. Org. Chem. 2011, 76, 9409−9416

vigorous mechanical stirring. Acid **5** (3.00 g, 12.5 mmol, 1.00 equiv) was dissolved in anhydrous THF (250 mL). The solution was cooled to −60 °C, and BuLi (16 mL of 1.6 M solution in hexane, 25.6 mmol, 2.05 equiv) was added dropwise over 20 min. After addition of about 4.5 mL (7.2 mmol, 0.58 equiv) of BuLi, an increasing amount of white precipitate appeared in the reaction mixture. After addition of about 8.5 mL (13.6 mmol, 1.09 equiv), the liquid became brown and the precipitate began to disappear. By the end of the addition of BuLi, all of the precipitate had dissolved. The very dark brown solution was stirred at −60 °C for 1.5 h and then further cooled to −78 °C. A solution of 3,5-dimethoxybenzaldehyde (2.20 g, 13.2 mmol, 1.06 equiv) in anhydrous THF (50 mL) was added dropwise over 10 min, resulting in formation of a whitish precipitate. The reaction mixture was warmed to room temperature over 30 min, stirred for an additional 30 min, and treated with 1 M HCl (27 mL). The precipitate immediately dissolved. The solvent was removed by rotary evaporation (*be careful: foams*) to produce a yellow oil. The oil was extracted with aqueous NaOH (20 mmol in 120 mL, 30 min stirring; then twice with 5 mmol in 30 mL). The combined aqueous extracts were centrifuged at room temperature for 10 min. The pH of the aqueous extract was adjusted to 2 with HCl, resulting in the separation of acid **6** as an oil. The aqueous solution was treated with NaCl until saturation to ensure the complete recovery of **6**. The oil was extracted with diethyl ether (3 × 100 mL), and the combined extracts were dried over $MgSO_4$, filtered, and concentrated to produce a light yellow oil of crude **6**. Recrystallization from ethyl acetate gave **6** (3.55 g, 8.73 mmol, 70%) as white crystals; mp = 184−185 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.55 (app t, J = 1.7 Hz, 1H), 8.04 (app dt, J_d = 7.7 Hz, J_t = 1.4 Hz, 1H), 7.92 (ddd, J_d = 8.0 Hz, J_d = 2.1 Hz, J_d = 1.2 Hz, 1H), 7.41 (app t, J = 7.8 Hz, 1H), 6.31 (t, J = 2.3, 1H), 6.01 (d, J = 2.3, 2H), 4.98 (s, 1H), 3.58 (s, 6H), 2.85−2.51 (m, 4H), 2.07−1.83 (m, 2H). ^{13}C NMR (300 MHz, $CDCl_3$): δ 172.1, 159.8, 139.2, 138.8, 136.8, 132.9, 129.6, 129.7, 128.5, 106.3, 101.2, 81.1, 65.8, 55.4, 27.5, 27.3, 24.9. Predicted exact mass for $C_{20}H_{21}O_5S_2$ 405.0836; observed mass 405.0844.

*3-[2-(tert-Butyldimethylsilyloxy(3,5-dimethoxyphenyl)methyl)-1,3-dithian-2-yl]benzoic Acid tert-Butyldimethylsilyl Ester (**7**).* Acid **6** (2.30 g, 5.66 mmol, 1.00 equiv) was dried in vacuum over $CaCl_2$ overnight, suspended with stirring in dichloromethane (35 mL), and treated with 2,6-lutidine (3.50 mL, 3.23 g, 30.1 mmol, 5.33 equiv), which resulted in complete dissolution. The solution was treated dropwise with neat *tert*-butyldimethylsilyl triflate (4.40 mL, 5.08 g, 19.2 mmol, 3.39 equiv) and stirred at rt for 3 h until the starting material had completely disappeared based upon TLC analysis (toluene:AcOH, 9:1). The reaction mixture was poured into a mixture of water (75 mL) and ethyl acetate (150 mL). The organic layer (whose volume was kept at ca. 150 mL by the addition of extra ethyl acetate when necessary) was washed with water (3 × 75 mL) and brine (2 × 50 mL) and then dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to produce light yellow crystals of crude ester **7**. Recrystallization from the minimal amount of hexanes (rt for at least 5 h and then at 4 °C for 5 h) gave pure **7** (3.15 g, 4.96 mmol, 88%) as white crystals; mp = 153−154 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.33 (br s, 1H), 8.01 (br d, J = 7.1 Hz), 7.95 (app dt, J_d = 7.7 Hz, J_t = 1.4 Hz), 7.38 (app t, J = 7.8 Hz, 1H), 6.28 (t, J = 2.3, 1H), 5.94 (very broad app s, 2H), 5.00 (s, 1H), 3.54 (s, 6H), 2.70−2.40 (m, 4H), 1.90−1.75 (m, 2H), 0.96 (s, 9H), 0.84 (s, 9H), 0.35 (s, 6H), 0.09 (s, 3H), −0.34 (s, 3H). ^{13}C NMR (300 MHz, $CDCl_3$): δ 166.8, 159.4, 140.9, 137.5, 137.0, 134.5, 131.1, 129.1, 127.6, 107.5−106.0 (br), 101.0, 81.8, 65.3, 55.2, 27.5, 27.1, 26.0, 25.8, 25.1, 18.5, 18.0, −4.4, −4.6, −4.7, −4.9.

*3-[2-(tert-Butyldimethylsilyloxy(3,5-dimethoxyphenyl)methyl)-1,3-dithian-2-yl]benzoic Acid (**8**).* Ester **7** (4.20 g, 6.61 mmol, 1.0 equiv) was suspended in 80% aqueous acetic acid (160 mL) and heated with stirring from 50 to 75 °C over 45 min. The solution was kept at 75 °C for an additional 15 min and then cooled. The solvent was removed by rotary evaporation. The oily residue was coevaporated with toluene (3 × 50 mL) to produce an off-white foam. The foam was dissolved in a minimal amount (10−15 mL) of dichloromethane and diluted with pentane (ca. 120 mL). After standing for 2 h at room temperature followed by 2 h at 4 °C, crystallization of the product was complete. The crystals were collected by filtration, washed with pentane, and dried to give **8** (2.96 g, 5.68 mmol, 86%) as white crystals; mp =135−137 °C. (*Important note*: do not crystallize the product from hexanes, as it crystallizes as a complex with some hexane isomers.) ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.67 (br s, 1H), 8.04 (app dt, J_d = 7.7 Hz, J_t = 1.4 Hz, 1H), 7.94 (br d, J = 7.2 Hz, 1H), 7.39 (app t, J = 7.8 Hz, 1H), 6.30 (t, J = 2.3, 1H), 5.90 (very broad app s, 2H), 5.04 (s, 1H), 3.55 (s, 6H), 2.75−2.40 (m, 4H), 1.98−1.77 (m, 2H), 0.88 (9H), 0.10 (3H), −0.33 (3H). ^{13}C NMR (300 MHz, $CDCl_3$): δ 172.4, 159.3, 140.7, 137.8, 137.7, 134.3, 128.9, 128.8, 127.4, 106.4 (br), 100.7, 81.6, 66.8, 55.0, 27.4, 27.0, 25.7, 24.9, 18.3, −4.6, −5.1. Predicted exact mass for $C_{26}H_{35}O_5S_2Si$ 519.1701; observed mass 519.1636.

*3-[1,1-Dimethoxy-2-tert-butyldimethylsilyloxy-2-(3,5-dimethoxyphenyl)ethyl]benzoic Acid (**3**).* Dithiane **8** (1.00 g, 1.92 mmol, 1.00 equiv) was dissolved in a mixture of anhydrous methanol (20 mL) and dichloromethane (3 mL). A solution of [bis-(trifluoroacetoxy)iodo]benzene (2.10 g, 4.88 mmol, 2.54 equiv) in dichloromethane (8 mL) was added dropwise. The reaction mixture was stirred for 30 min at room temperature and then poured into cold aqueous 0.17 N NaOH (90 mL). The organic solvents were removed by rotary evaporation (foams). The remaining aqueous solution was filtered and treated with CO_2 gas until the pH decreased to 6 and then with solid NaCl until saturation. Acid **3** separated as an oil. To collect the oil, the mixture was extracted with dichloromethane. The dichloromethane solution was dried over $MgSO_4$, and the dichloromethane was removed by rotary evaporation to leave a light-yellow oil. The product was crystallized from a minimal volume of hexanes (15−20 mL) to give **3** (0.74 g, 1.56 mmol, 81%) as white crystals; mp = 129−130 °C. (*Important note:* If <2.5 equiv excess of [bis(trifluoroacetoxy)iodo]benzene is used or if this reagent is old, the final product can be contaminated with 5−10% of dithiane **8**. Recrystallization does not enrich the mixture in **3**. A route to purify **3** from **8** is by additional treatment of the mixture with [bis(trifluoroacetoxy)iodo]benzene as above.) ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06 (br s, 1H overlaps with 8.06−7.98 (m, 1H), 7.29−7.32 (m, 2H), 6.27 (t, J = 2.3), 5.98 (d, J = 2.3 Hz, 2H), 4.97 (s, 1H), 3.56 (s, 6H), 3.42 (s, 3H), 3.25 (s, 3H), 0.89 (s, 9H), 0.10 (s, 3H), −0.20 (s, 3H). ^{13}C NMR (300 MHz, $CDCl_3$): δ 172.3, 159.2, 142.4, 137.7, 134.6, 131.4, 129.4, 128.0, 126.7, 106.3, 104.2, 99.7, 75.7, 55.0, 49.6, 49.4, 25.7, 18.2, −4.5, −5.0. Predicted exact mass for $C_{25}H_{35}O_7Si$ 475.2158; observed mass 475.2109.

*Compound **9**: Linker **3** Loaded onto TentaGel NH_2 Beads.* TentaGel NH_2 beads (1.00 g, 300 μmol of NH_2, 1 equiv) were swollen in anhydrous DMF (5 mL) for 15 min, then washed three times with 5 mL of anhydrous DMF, and finally suspended in DMF (3 mL). Solid linker **3** (287 mg, 0.602 μmol, 2 equiv) was added to the beads, and the mixture was gently shaken for 1 min to allow the linker to dissolve. Solid PyOAP (312 mg, 598 μmol, 2 equiv) and solid HOAt (81 mg, 595 μmol, 2 equiv) were added, followed by *N,N*-diisopropylethylamine (210 μL, 156 mg; 1207 μmol, 4 equiv). The reaction mixture was shaken gently for 40 min. The beads were drained, washed with anhydrous DMF (5 times × 5 mL), anhydrous MeOH (5 times × 5 mL), and anhydrous dichloromethane (5 times × 5 mL), and dried.

*Compound **10**.* One half of the TentaGel beads carrying the linker (**3**) from the step above (562 mg) were swollen in THF (5 mL) for 15 min, washed three times with 5 mL of THF, and drained. The volume of the swollen drained beads was ca. 2.7 mL. THF (3.1 mL) and TBAF (1.20 mL, 1 M in THF, 1.20 mmol) were added, resulting in a final TBAF concentration of ca. 0.17 M. The reaction mixture was gently shaken for 7 h (93% deprotection occurred in 3 h). The beads were drained, washed with THF (5 times × 5 mL), water (5 times × 5 mL), anhydrous MeOH (5 times × 5 mL), and anhydrous dichloromethane (5 times × 5 mL), and dried. *Important note:* Complete removal of water and/or the increase of the final TBAF concentration over 0.2 mM slows or stops the reaction. Do not use anhydrous THF.

*Acylation of **10**. a. DCC/DMAP Protocol.* The reaction was accomplished using an 8-fold molar excess of Fmoc-N-protected amino acid and a 4-fold molar excess of DCC relative to the amount of linker on the beads. (The TentaGel NH_2 beads carry 300 μmol of

dx.doi.org/10.1021/jo2017263 | J. Org. Chem. 2011, 76, 9409−9416

NH_2/g; quantitative loading results in 275 μmol of NH-Linker-OH/g of beads due to the increased mass of the loaded beads.) The molar ratio of DMAP/DCC was 1/10. The beads were swollen in dichloromethane and drained. The Fmoc-N-protected amino acid to be added to the linker was suspended/dissolved in anhydrous dichloromethane (1 mL of CH_2Cl_2 for 0.2 mmol of the acid). If necessary, anhydrous DMF was added dropwise to dissolve the amino acid. The solution was cooled to 4 °C, and DCC was added as a dry powder that dissolved immediately. After stirring for 15 min at 4 °C, a white precipitate formed in the reaction mixture, which was removed by filtration. The filtrate was added to the beads, followed by DMAP, and the reaction was stirred overnight at room temperature. The beads were drained, washed eight times with alternating dichloromethane and methanol (5 mL/g beads), and dried. Fmoc protecting groups were removed by treatment with 20% piperidine in DMF (5 mL/g beads; two treatments: 5 and 15 min). The beads were drained, washed with anhydrous DMF (3 times, 5 mL/g beads) and dichloromethane (3 times, 5 mL/g beads), and dried.

b. 2,6-Dichlorobenzoyl Chloride Protocol. The reaction was accomplished using a 5-fold molar excess of both Fmoc-N-protected amino acid and 2,6-dichlorobenzoyl chloride and a 20-fold molar excess of pyridine relative to the concentration of linker on the beads (275 μmol of NH-Linker-OH/g of beads). The beads were swollen in anhydrous DMF and then drained. An additional amount of DMF (ca. 1 mL per 0.2 g of dry resin) was added, followed by the solid Fmoc-N-protected amino acid. The mixture was shaken for 10−15 min until all the amino acid dissolved. 2,6-Dichlorobenzoyl chloride and pyridine were added, and the reaction was stirred for 24 h at room temperature. The beads were drained, washed eight times with alternating dichloromethane and methanol, and dried. Fmoc protecting groups were removed by treatment with 20% piperidine in DMF (5 mL/g beads; two treatments: 5 and 15 min). The beads were drained, washed with anhydrous DMF (3 times, 5 mL/g beads) and dichloromethane (3 times, 5 mL/g beads), and dried.

Peptide Synthesis. Amino acids for peptide synthesis were protected as follows: Fmoc-Tyr(Trt)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-His(Boc)-OH, Fmoc-Pro-OH, Fmoc-Val-OH, Fmoc-Gly-OH.

Beads carrying the first amino acid (attached as described above) were swollen in anhydrous DMF (5 mL/1 g beads) for 15 min, then washed three times with anhydrous DMF (5 mL/1 g beads each washing), and finally suspended in the minimal amount of anhydrous DMF required to achieve a homogeneous solution of the reagents. A 4-fold molar excess of solid Fmoc-protected amino acid was added to the beads, and the mixture was gently shaken for 1−2 min for the acid to dissolve. Solid PyOAP (4-fold molar excess) and solid HOAt (4-fold molar excess) were added, followed by *N,N*-diisopropylethylamine (8-fold molar excess). The reaction mixture was gently shaken for 25 min. The beads were drained and washed with anhydrous DMF (5 mL/g beads), and the coupling step was repeated a second time to ensure complete derivatization of the beads. The beads were drained, washed with anhydrous DMF (7 times, 5 mL/g beads) and anhydrous dichloromethane (5 times, 5 mL/g beads), and dried.

Between coupling steps, Fmoc protecting groups were removed by treatment with 20% piperidine in DMF (5 mL/g beads; two treatments: 5 and 15 min). The beads were drained, washed with anhydrous DMF (7 times, 5 mL/g beads), and used for the next step of peptide synthesis using the protocol described above.

After completion of peptide synthesis, side chain protecting groups and the dimethylketal protecting group on the linker were removed by treatment with TFA/water/phenol/thioanisole (85 mL/5 mL/5 g/5 g) at room temperature for 4−6 h. The beads were washed with dichloromethane (10 × 1 mL per 200 mg beads) and dried. *Important note:* Once the dimethyl ketal protecting group on the linker is removed, the beads should not be exposed to pH > 7, as side-reactions and/or decomposition might occur.

UV Irradiation. Irradiation was carried out using a 100 W "street light type" medium-pressure mercury lamp (GE H100-A4/T). The lamp had a transparent glass envelope (diameter about 35 mm) that was not removed. The lamp was placed at one focus of an elliptical mirror while a water-jacketed Pyrex reactor was placed at the second focus of the same mirror. The mirror was made by simply bending a band of thin polished stainless steel plate over two aluminum elliptical guides, which were made on an in-house computer numerical controlled (CNC) mill (mirror dimensions: small diameter 50 mm, large diameter 100 mm, height 60 mm—same as the height of the lamp active zone). Solvents were not degassed to remove O_2. Argon (Scientific grade, grade 6, oxygen content 20−50 ppm) was slowly bubbled through the suspension of beads (20−100 mg) in the solvents listed in Table 1 for cleavage of dipeptides (0.5−2.5 mL) or in 30% aqueous methanol (5 mL for a 0.04 mmol scale synthesis) for cleavage of larger peptides to provide stirring during irradiation. Yields of products released by photocleavage were determined by ^{1}H NMR in the presence of an added internal standard (*p*-toluic acid, sodium acrylate, or pyridine hydrochloride) or by weight. The concentration of amino groups on the TentaGel as provided by the manufacturer was 0.30 mmol/g. This concentration was confirmed to be accurate by UV analysis of fulvene−piperidine adducts[46] released during the acylation of the beads with the first Fmoc-protected amino acid. The yields in Table 1 of the text are calculated based on the capacity of amino groups on the TentaGel beads.

After synthesis of Lys-Tyr-Arg-Arg-Arg-Pro-Arg-Arg-Ser-Gln-Arg-Lys-Arg-Gly on a 0.04 mmol scale, the beads were irradiated for 40 min in 5 mL of 30% aqueous methanol. The solvent was drained, and the beads were washed three times with 0.05% TFA in water. The drained solvent and the aqueous washings were combined, filtered through a 0.2 μm membrane filter, and concentrated by rotary evaporation to ca. 0.3 mL. The peptide was precipitated by addition of methanol (ca. 3 mL). The precipitate was collected by centrifugation, washed with anhydrous methanol, and dried before NMR and HPLC analysis. HPLC analysis was carried out using an Eclipse XDB-C18 column (4.6 × 150 mm). Samples were injected in water, isocratic elution, 1 mL/min, 95% solvent A (0.1% aqueous TFA), 5% solvent B (0.085% TFA/acetonitrile). Compounds were detected at 217 nm. Predicted monoisotopic mass for the peptide: 1837.1208; observed mass (major peak) 1837.0793.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra for all synthesized materials, assignment of the NMR signals for **6**−**10** and Lys-Tyr-Arg-Arg-Arg-Pro-Arg-Arg-Ser-Gln-Arg-Lys-Arg-Gly, and a figure detailing the photoreactor assembly. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENTS

This work was supported by the National Science Foundation under Grant 0526747.

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