

Solid-Phase Photochemical Decarboxylative Hydroalkylation of Peptides

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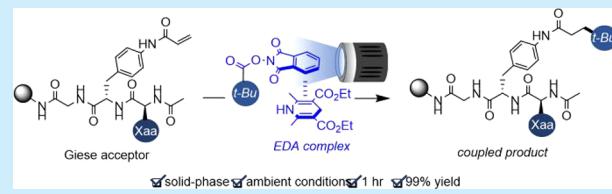
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ABSTRACT: The compatibility of photochemistry with solid-phase peptide synthesis is demonstrated via photochemical hydroalkylation to form C(sp³)–C(sp³) bonds between on-resin Giese acceptors and redox-active esters. Both iridium-based photocatalysts and Hantsch ester led to high yields, with final reaction conditions producing full conversions within 30 min under ambient conditions. The chemistry is compatible with a broad range of peptide side chains, redox-active esters, and resin. These conditions represent the first example of photochemical peptide modifications on resin.



Since the advent of solid-phase peptide synthesis (SPPS) in 1963, more than 60 peptide pharmaceuticals have been approved in the United States, Europe, and Japan, more than 150 peptides are currently undergoing clinical development, and more than 260 have been investigated in human clinical trials.¹ SPPS depends greatly on the adaptation of solution-phase reactions to solid-phase conditions. As examples, since the 1990s, C–C bond-forming transformations such as the Stille,² Heck,³ Suzuki–Miyaura,⁴ and Sonogashira⁵ reactions have been adapted to the solid phase by both industry and academia. Additionally, in 1996, the first example of solid-phase ruthenium-catalyzed metathesis was reported, and soon after, the first solid-phase peptide ring-closing metathesis (RCM) reaction was described.^{6,7} Since that time, RCM has been extensively used to form hydrocarbon-stapled peptides in biotherapeutics.⁸ Although peptide macrocycles generated via RCM can be reduced to C(sp³)–C(sp³) bonds, few direct routes to such structures exist under mild conditions.

Recently, Baran et al. reported two nickel-catalyzed decarboxylative conjugate addition procedures and demonstrated their compatibility with solid-phase conditions.^{9,10} Both procedures utilize nickel-catalyzed cross-coupling mechanisms to forge C(sp³)–C(sp³) bonds under inert conditions and required 8–16 h reaction times. Given the importance of solid-phase peptide C(sp³)–C(sp³) bond formation, an alternate route through a complementary mechanistic pathway was deemed desirable.

Although photochemistry on peptides and proteins has been a very active field,^{11,12} to the best of our knowledge, no studies on the compatibility of photochemistry with on-resin peptides have been reported. The application of photochemistry to solid-phase peptide synthesis offers several distinct advantages over extant protocols. First, photochemically induced reactions involving open-shell intermediates exhibit extraordinary toleration of diverse functional groups. Additionally, light-mediated transformations typically operate at room temper-

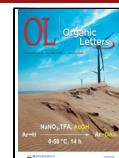
ature and permit the use of buffers or aqueous mixtures that offer biocompatible reaction conditions. The reliance on photon flux also allows the exclusion of light to quench reactions and control reaction progression precisely and conveniently.¹³ Finally, dual photocatalytic cycles have enabled multicomponent reactions, ideal for making diverse peptide macrocycles and bicycles in a single, mild synthetic step.¹⁴

With the goal of elaborating peptides by photoinduced Giese-type reactions on a solid support, several procedures were assessed. In the seminal solution-phase, photosensitized decarboxylative Giese addition reported by Okada,¹⁵ redox-active esters (RAEs) were employed as radical precursors and coupled with Giese acceptors in the presence of 1-benzyl-1,4-dihydronicotinamide as a reductant and Ru(bpy)₃Cl₂ as a photocatalyst in aqueous THF.¹⁵ Up to 69% yields were reported. However, the scope of redox-active esters was limited to three hydrocarbons. Given the structural complexity of polypeptides, reaction conditions with high functional group tolerance were imperative. Furthermore, the Okada reaction conditions utilized an aqueous reaction medium, which was expected to be challenging for nonpolar peptide substrates.

In 2015, Overman et al. reported a solution-phase decarboxylative Giese-type addition involving a reductive single-electron transfer (SET) of redox-active esters via photoredox catalysis.¹⁶ The contribution by Overman and co-workers involved the photochemical coupling of various Giese acceptors and tertiary radicals generated from corresponding redox-active esters at room temperature and in

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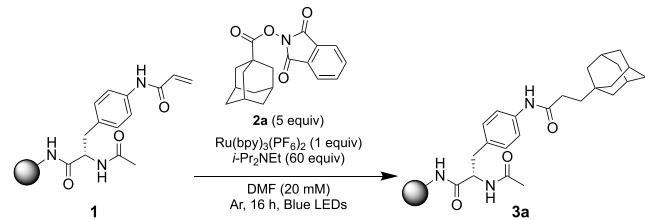
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dichloromethane as the solvent.¹⁶ The reaction employed Hantzsch ester [diethyl 1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (HE)], Ru(bpy)₃(BF₄)₂, and *i*-Pr₂NEt and featured a broad substrate scope for both coupling partners. A visible light-induced approach to the synthesis and chemical modification of solid-phase peptides was thus initiated on the basis of Overman's mild photochemical hydroalkylation.

We began our studies using ChemMatrix Rink Amide resin, which features a PEG-based polymer, rather than polystyrene, to avoid potentially reactive aromatics. Aromatic Giese acceptor **1** was employed for ease of analysis by LC-MS and ¹H NMR. Acrylamide was chosen as the electron-deficient olefin over acrylate for its ease of incorporation into peptides. Installation of the solid-phase Giese acceptor began with synthesis of Fmoc-(4-acrylamide)-Phe-OH, which was then incorporated onto resin. The Fmoc group was deprotected using non-nucleophilic DBU, and subsequently, the substrate was acylated on the N-terminus. Alternatively designed as a late-stage peptide modification, Fmoc-(4-Trt-amino)-Phe-OH was incorporated onto the resin. After N-terminal deprotection and acylation, the Trt group was removed, and the aniline was reacted with acrylic acid and (1-cyano-2-ethoxy-2-oxoethylideneaminoxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU). The purity of crude material in this protocol was 86% as judged by analytical HPLC (page S15 of the Supporting Information).

Attempts to optimize the coupling of on-resin olefin (**1**) with 1-adamantyl *N*-(acyloxy)phthalimide (**2a**) rapidly generated fruitful conditions with Ru(bpy)₃(PF₆)₂ as the photocatalyst and *i*-Pr₂NEt as the reductant in DMF with blue LED irradiation at room temperature. These conditions produced the coupled product **3a** with a 30% conversion (Table 1, entry 1). Examination of photocatalysts identified [Ir{dF(CF₃)-ppy}₂(bpy)]PF₆ (*E*^{1/2} M^{*/M⁻ = +1.32 V)¹⁷ as a suitable catalyst for the solid-phase photocoupling (Table 1, entries 2–}

Table 1. Optimization of Solid-Phase Photocatalytic Hydroalkylation Conditions



entry	deviation from initial conditions	3 (P/IS ^a)	3 (conv, % ^b)
1	none	0.26	30
2	[Ir{dF(CF ₃)-ppy} ₂ (bpy)]PF ₆	0.38	100
3	[Ir{dF(CF ₃)-ppy} ₂ (dtbbpy)]PF ₆	0.31	88
4	4CzIPN ^c	0.14	59
5	Cl-4CzIPN ^d	0.10	31
6	entry 2 with polystyrene resin	0.38	100
7	THF	0.24	89
8	no light	0.00	0
9	no <i>i</i> -Pr ₂ NEt	0.03	7
10	no photocatalyst	0.09	14
11	5 equiv of Hantzsch ester	0.40	100

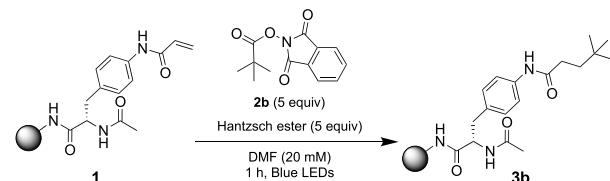
^aProduct/internal standard ratio as determined by LC-MS.

^bConversion to **3a** as determined by LC-MS. ^c1,2,3,5-Tetrakis-(carbazol-9-yl)-4,6-dicyanobenzene. ^d2,4,5,6-Tetrakis(3,6-dichloro-9H-carbazol-9-yl)isophthalonitrile.

5). A similar conversion was observed using the Rink amide polystyrene resin (Table 1, entry 6). Decreasing the reaction time to <16 h proved to be detrimental to the reaction yield (page S7 of the Supporting Information). The use of THF as a solvent, which was advantageous for the coupling of *tert*-alkyl *N*-phthalimidoyl oxalates,¹⁸ decreased the conversion to **3a** to 89% (Table 1, entry 7). In the absence of light, no product was observed (Table 1, entry 8). Excluding the photocatalyst or *i*-Pr₂NEt resulted in product formation with substantially diminished yields (Table 1, entries 9 and 10), demonstrating that the superstoichiometric amine reductant was ineffective at fragmenting redox-active esters. Comparable results in the absence of the photocatalyst were observed by Okada and co-workers in the initial decarboxylative Michael addition report.¹⁵ The addition of Hantzsch ester as a reductant generated superior yields (Table 1, entry 11) particularly for primary redox-active esters (page S8 of the Supporting Information). A similar yield and conversion were produced in the absence of the photocatalyst (page S8 of the Supporting Information).

After incorporation of the Hantzsch ester, a reaction time screen revealed full conversions within 30 min (page S9 of the Supporting Information). Control studies produced similar NMR yields under air and without the amine base (Table 2,

Table 2. Development of Solid-Phase, Metal-Free Photochemical Hydroalkylation Conditions



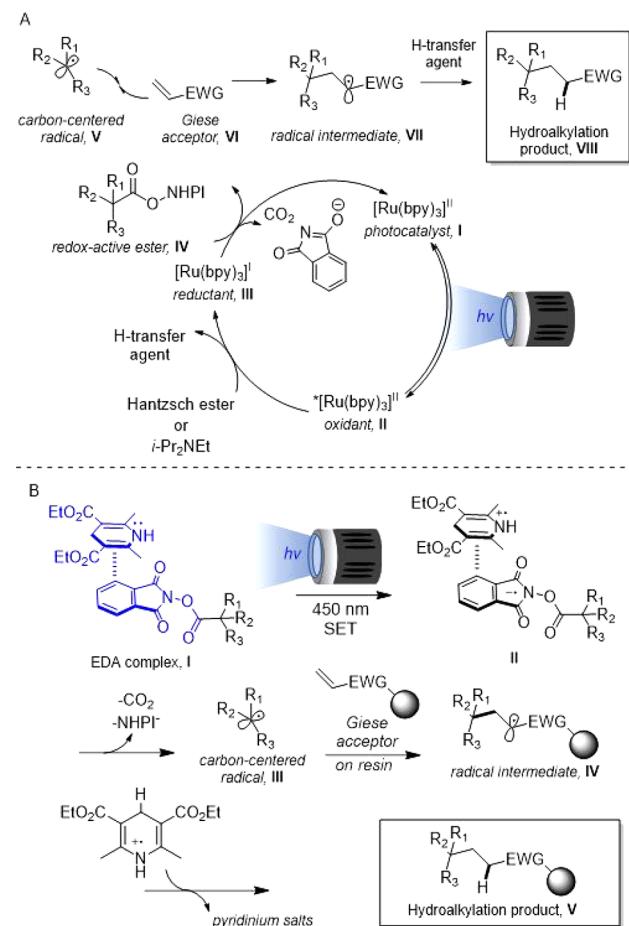
entry	deviation from initial conditions	3b (conv ^a)	yield of 3b (%) ^a
1	none	100	90
2	under argon	100	88
3	under argon with DIPEA	100	90
4	Rink amide polystyrene resin	100	90
5	no Hantzsch ester	0	0
6	no light	0	0
7	>5 equiv of HE and RAE	no improvement	no improvement
8	<5 equiv of HE and RAE	inconsistent conv	inconsistent yield

^aDetermined by crude NMR.

entries 1–3). Conversion of 100% was also observed using Rink amide polystyrene resin (Table 2, entry 4). Comparable catalyst-free conditions were reported in the original report by Okada, as well as by Overman and Shang in the solution phase.^{15,16,19} No product was formed in the absence of light or Hantzsch ester (Table 2, entries 4 and 5). Increasing the number of equivalents of Hantzsch ester and redox-active ester resulted in no improvements, while decreasing the number of equivalents of these reactants led to inconsistent results (page S10 of the Supporting Information).

Although Overman proposed a photocatalytic reduction of the redox-active esters (Scheme 1A),^{16,20} recent investigations have revealed that the reduction takes place largely via a light-induced charge transfer within an electron donor–acceptor (EDA) complex formed between the Hantzsch ester and the redox-active ester (Scheme 1B).²¹ Photoactivation of the EDA complex induces an inner sphere electron transfer from the

Scheme 1. Mechanistic View of the Light-Mediated Decarboxylative Hydroalkylation^a



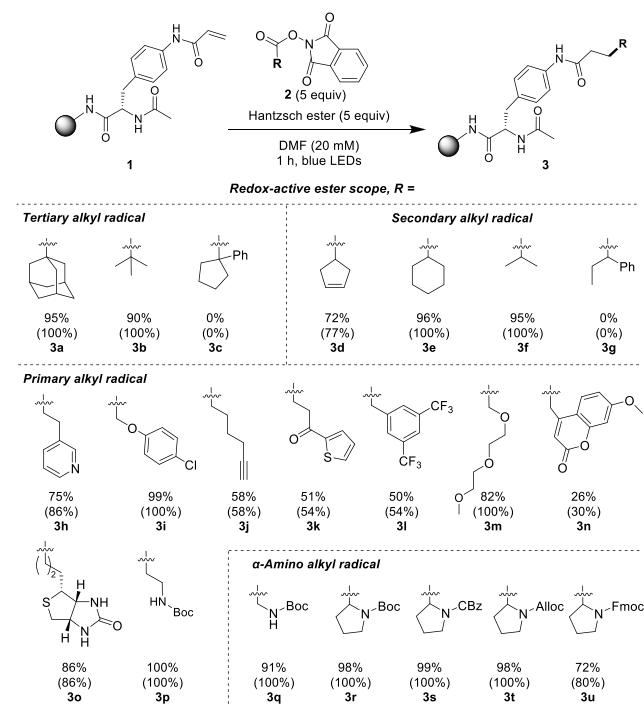
^a(A) Envisioned mechanism for the photocatalytic decarboxylative hydroalkylation. (B) Mechanistic view of the catalyst-free photochemical decarboxylative hydroalkylation.

Hantzsch ester to the redox-active ester, leading to carbon-centered radical III via homolytic fragmentation and decarboxylation. The radical then adds to the electron-deficient olefin and generates intermediate IV, which abstracts a hydrogen atom from the oxidized Hantzsch ester to generate pyridinium salts, furnishing the desired hydroalkylation product. Because of the speed, cost, and operational simplicity of the catalyst-free conditions, they were utilized to explore the scope of the process.

The performance of the hydroalkylation using a series of simple redox-active esters was first investigated (Scheme 2). The on-resin Giese acceptor coupled efficiently with primary, secondary, and tertiary alkyl RAE partners. Tertiary (3b) and secondary (3f) carbon-centered radicals performed nearly identically, while their primary analogues produced diminished yields, except for α -benzyloxy radical (3i) and Boc- β -alanine (3p). Cyclic redox-active esters, including adamantyl (3a), 3-cyclopentenyl (3d), and cyclohexyl (3e), all converted to the desired product with excellent yields. NHPI esters featuring nitrogen-based (3h and 3p), oxygen-based (3n), and sulfur-based (3k and 3o) heterocyclic moieties were also compatible with the hydroalkylation protocol.

Cycloadditions between terminal alkynes and azides (“click chemistry”) are widely utilized for bioorthogonal trans-

Scheme 2. Scope of Solid-Phase Photochemical Hydroalkylation^a



^aYields determined by quantitative ¹H NMR; conversions determined by quantitative ¹H NMR and reported in parentheses.

formations in which considerations of reaction efficiency, chemical inertness, cost, and compatibility with aqueous conditions are paramount. An alkynyl-substituted RAE was synthesized and found to deliver a 58% yield of 3j. The moderate yield was expected because of the difficulty of generating primary radicals as well as their reduced nucleophilicities. Furthermore, reports of terminal alkynes undergoing decarboxylative radical addition to form alkenes suggested a loss of reactant may be partially responsible, as well.^{22,23} The interaction between biotin and streptavidin is one of the strongest in nature and widely utilized in biology and biochemistry ($K_d = 10^{-15}$ M).²⁴ A biotinyl-substituted RAE was synthesized and found to react with the solid-phase substrate in 86% yield, which is high given the yields of other primary RAEs (3o).

We subsequently incorporated an example of a PEGylation (3m) featuring an α -alkoxy group that conveniently aided radical generation. Mini-PEGs are frequently used to tune the lipophilicity of biomolecules. A coumarin moiety was also reasonably well-tolerated by the reaction protocol (3n). Coumarins are found in a broad range of medicinally relevant natural products, synthetic pharmaceuticals, and fluorescent labels.^{25,26} The low yield of coumarin 3n is likely a result of the low nucleophilicity of the highly stabilized radical generated (*vide infra*). Recognition of protein conformers by fluorine NMR necessitates the integration of fluorinated moieties whose chemical shifts are responsive to slight variations in the local dielectric and magnetic shielding environment. To that end, an NHPI ester incorporating a fluorine (¹⁹F) NMR tag^{27,28} was evaluated, with the 3,5-bis(trifluoromethyl)phenyl moiety 3l selected as a model fluorine probe that was successfully coupled with a reasonable yield. A single ¹⁹F NMR

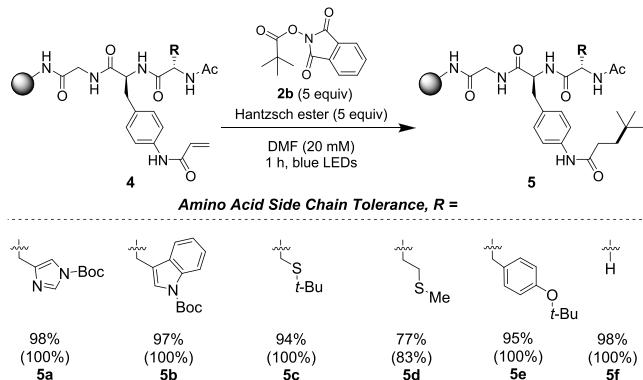
peak confirmed the presence of the fluorine (^{19}F) NMR tag (page S79 of the Supporting Information).

The amenability of resonance-stabilized radicals, which exhibit nucleophilicities lower than those of localized carbon-centered radicals, was also examined. Unfortunately, the success of substrates generating such stabilized radicals was limited. Products **3l** and **3n** derived from primary resonance-stabilized radicals were obtained in 50% and 26% yields, respectively. Neither secondary nor tertiary benzylic RAE substrates produced the product (**3c** or **3g**), perhaps because of the combination of steric and stabilizing resonance effects.

The abundance of structurally diverse, commercially available amino acids makes them a particularly appealing radical feedstock for peptide modification. They are also attractive building blocks because the amine functional group promotes radical addition and provides a site for further functionalization and peptide elongation. The scope of the coupling using α -amino alkyl radical precursors was therefore evaluated. Couplings employing Boc-, CBz-, Alloc-, and Fmoc-protected prolines all produced excellent yields (**3q**–**3u**). Fmoc protection is seldom used in photochemistry, but it is used abundantly with SPPS. Fmoc-proline was well-tolerated, albeit with a slightly diminished yield of 72%.

Toleration of the transformation to structural and functional complexity was further evaluated using a series of tripeptides (Scheme 3). The Giese acceptor was incorporated as a late-

Scheme 3. Tolerance of Various Amino Acid Side Chains^a



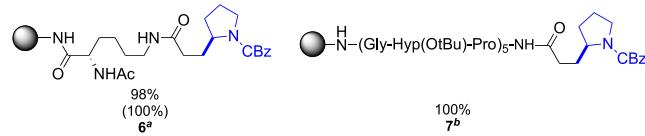
^aYields determined by quantitative ^1H NMR; conversions determined by quantitative ^1H NMR and reported in parentheses.

stage modification to prevent attack of the N-terminus on the Giese acceptor during peptide elongation. His, Trp, Cys, Met, and Tyr moieties have all participated in photochemistry under various solution-phase conditions,¹² but their behavior under similar conditions protected on the solid phase has not been tested. In the event, tripeptides incorporating these amino acid derivatives exhibited high conversions and yields.

The amenability of aliphatic Giese acceptors was then demonstrated by on-resin acylation of lysine **6** and subsequent reaction with CBz-Pro-NHPI (Scheme 4). A 98% yield was observed. Next, 15-mer collagen model peptide **7** was synthesized and acylated on the N-terminus. A reaction with CBz-Pro-NHPI led to full conversion by HPLC (page S112 of the Supporting Information).

In summary, the development of the photochemical hydroalkylation of electron-deficient olefins has been reported in the solid phase. These conditions offer mild, expeditious, and operationally simple routes for introducing $\text{C}(\text{sp}^3)$ –

Scheme 4. Tolerance of Aliphatic Giese Acceptors



^aYield determined by quantitative ^1H NMR; conversions determined by quantitative ^1H NMR and reported in parentheses. ^bConversion determined by HPLC.

$\text{C}(\text{sp}^3)$ bonds into peptides on resin. They also represent the first example of a light-mediated peptide modification in the solid phase and demonstrate the potential for further elaboration of polypeptides on a solid support.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.orglett.1c02928>.

Preparation of starting materials, optimization and control studies, and characterization data of products (NMR and LC-MS) (PDF)

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Notes

The authors declare no competing financial interest.

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■ NOTE ADDED AFTER ASAP PUBLICATION

Table 2 graphic was replaced on October 21, 2021.