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Solid-Phase Photochemical Peptide Homologation Cyclization

Michael B. Elbaum, Mahmoud A. Elkhalifa, Gary A. Molander,* and David M. Chenoweth*

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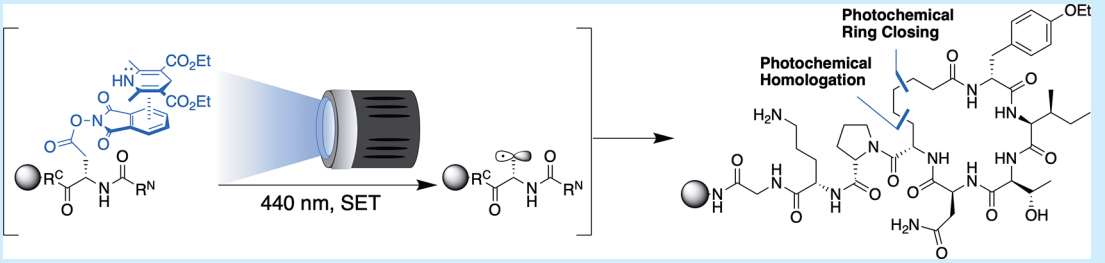

ABSTRACT: Forging new C(sp^3)−C(sp^3) bonds to central positions within a peptide backbone is critical for the development of new therapeutics and chemical probes. Currently, there are no methods for decarboxylating Asp and Glu side chains solid-phase photochemically or using such radicals to form peptide macrocycles. Herein, electron-donor-acceptor complexes between Hantzsch ester and on-resin peptide *N*-hydroxyphthalimide radical precursors are used to access these radicals, demonstrated with two-carbon homologations and homologation cyclizations of Atosiban and RGDf.

Peptides make up a critical class of therapeutics, as they occupy a chemical space regime between small molecules and large biologics and can modulate a vast array of biological processes, including protein−protein interactions. With access to a substantial pool of chiral building blocks, large peptide libraries have been developed, allowing synthetically easy access to diverse structures.[1,2] However, the flexibility of peptides decreases their binding affinity, selectivity, permeability, and metabolic stability, minimizing their efficacy.[2,3] To explore this chemical space more effectively, new strategies must be developed to stabilize polypeptide conformations. The most common strategy for reinforcing the natural conformational bias of a given peptide sequence is covalent macrocyclization.

The most prevalent methods of creating peptide macrocycles are disulfide bond formation, lactamization, and lactonization.[4] Therapeutics such as Atosiban, Oxytocin, and Vasostrict are all disulfide-cyclized peptides with an N-terminal linkage to a central position on the peptide chain. Replacing disulfide and other polar macrocycle linkages with C(sp^3)−C(sp^3) bonds serves to enhance the permeability as well as the proteolytic and chemical stability of therapeutics.[5,6] Currently, solid-phase ruthenium-catalyzed ring-closing metathesis (RCM) followed by olefin reduction is the only strategy for synthesizing secondary C(sp^3)−C(sp^3)-cyclized peptides at internal amino acid positions.[5] Furthermore, no methods for homologating Asp and Glu side chains have been reported.

Previous reports of photochemical peptide cyclizations have required activated radical precursors, preventing the use of Asp and Glu as chiral radical sources within a peptide.[7−9] The most common photochemical cyclization strategy is thus to perform a Giese-type reaction using radicals generated by oxidative decarboxylation of a free C-terminal Gly, limiting the overall structure of the macrocycles generated.[10,11] We envisioned a direct route to a series of C(sp^3)−C(sp^3)-linked macrocycles from a common intermediate utilizing natural amino acids via tandem photochemical couplings on unactivated primary radicals. To accomplish this, electron-donor-acceptor (EDA) complexes were employed to access alaninyl and homoalaninyl radicals (Figure 1A). Such radicals, embedded within peptides, could then be homologated intermolecularly with allyl acrylate or cyclized onto an acrylamide (Figure 1B,C).

Several precedents suggested the feasibility of this approach. Baran et al. demonstrated that an *N*-hydroxyphthalimide (NHPI) radical precursor could be formed on resin and used to generate a stabilized α-prolinyl radical via nickel-catalyzed decarboxylative hydroalkylation.[12] Furthermore, they carried out solution-phase studies demonstrating the α-prolinyl radical cyclization onto an *i*-1 Lys(acrylamide). Additionally, there are examples of Asp or Glu amino acids being used as photochemical radical precursors to make α-selenoamino acids.[13] In that work, NHPI-functionalized Asp and Glu amino acids were used to generate alanyl and homoalanyl radicals using photoexcited $[Ru(bpy)_3]Cl_2$, the radicals generated being captured by diselenides. Recently, we reported

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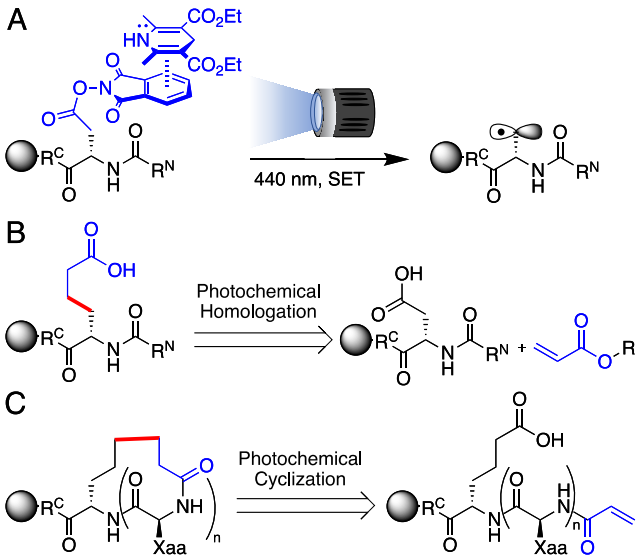


Figure 1. (A) Photochemical generation of an alaninyl radical by an EDA complex and single-electron transfer (SET) between Hantzsch ester and NHPI peptide. (B) Retrosynthetic analysis of homologation. (C) Retrosynthetic analysis of cyclization.

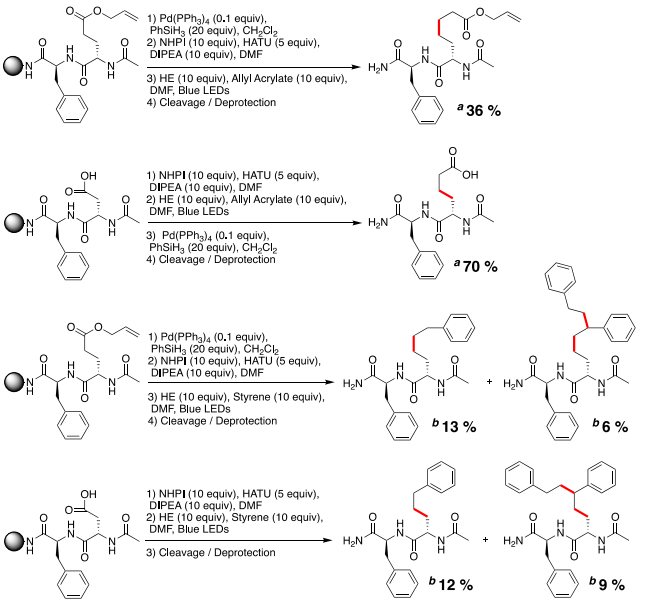
the first example of solid-phase photochemical modification of peptides, in which we developed conditions for the on-resin intermolecular hydroalkylation of peptide-bound enamides.[14] Shortly thereafter, solid-phase deaminative photochemical Giese additions using Katritzky salts were also demonstrated on peptides.[15]

The photochemical radical pathway has several advantages over RCM and nickel-catalyzed decarboxylative hydroalkylation, including being able (1) to use Asp and Glu as inexpensive chiral radical precursors, (2) to modify a common intermediate for late-stage transformations, (3) to use light-mediated transformations that are mild and often compatible with aqueous buffers and typically operate within minutes under ambient conditions, (4) to avoid organometallic catalysts by using photoinduced electron transfer activation from EDA complexes, (5) to incorporate multicomponent reactions via dual photocatalytic cycles, ideal for the synthesis of diverse peptide macrocycles,[16] and (6) to provide flexibility for the use of both RCM and radical chemistry in parallel to synthesize peptides with multiple cyclizations.

Atosiban, an oxytocin antagonist, is a hormone clinically used as a tocolytic therapeutic to prevent premature birth.[17] Atosiban is typically administered intravenously and has a poor metabolic half-life (16−18 min).[18] The short half-life has been partly attributed to the disulfide linkage within the macrocycle. Utilizing RCM, Vederas et al. demonstrated that replacement of the disulfide linkage with all $C(sp^3)-C(sp^3)$ bonds leads to a 2−3-fold increase in the half-life, with nearly identical activity.[19] Herein, the synthesis of Atosiban is used to demonstrate the viability of using solid-phase photochemical peptide homologation and cyclization to construct peptide macrocycles.

Initial efforts focused on optimizing the formation of the NHPI redox active ester (RAE) using solid-phase techniques. To do so requires activation of Asp or Glu side chains, making them prone to intramolecular cyclization to form aspartimide or glutarimide. Studies were begun on a model dipeptide Ac-Glu-Phe-Rink amide polystyrene resin. Phenylalanine was incorporated to increase the retention time on the HPLC column and to provide a chromophore for UV−vis detection. Glutamic acid was chosen as the radical precursor to minimize formation of the less favorable six-membered cyclization compared to aspartic acid's five-membered cyclization. Previous work to install the NHPI RAE solid phase utilized 1,3-diisopropylcarbodiimide (DIC) as the coupling reagent and required reaction for 2 h at 37 °C. We observed the desired product by LCMS along with a substantial peak consistent with the peptide backbone-cyclized side product (page S5 of the Supporting Information). Using hexafluorophosphate azabenzotriazole tetramethyl uronium (HATU) for 45 min under ambient conditions decreased the level of the cyclized side product by 91% and led to an overall cleaner reaction (page S6 of the Supporting Information). Freshly synthesized RAE dipeptides were treated with allyl acrylate (10 equiv) and Hantzsch ester (HE, 10 equiv) at 0.2 M and irradiated with blue LEDs for 2 h (Scheme 1). All starting

Scheme 1. On-Resin Homologations of Asp- and Glu-Containing Dipeptides



[a]Quantitative HPLC yields reported. [b]Isolated yields reported.

material was consumed, and crude HPLC yields were obtained for both Glu (36%) and Asp (41%) homologation reactions. Interestingly, side products of the reaction were hydrodecarboxylation of Asp and Glu, giving alanine and homoalanine, respectively. For the Ac-Asp-Phe-resin substrate, increasing the reaction concentration to 0.3 and 0.5 M increased the yield to 70% and 56%, respectively. Substitution of NMP for DMF decreased the yield from 70% to 48%. Peptide Ac-Glu-Phe was reacted on a 50 μmol scale without allyl acrylate, cleanly affording homoalanine with an HPLC isolated yield of 22% (page S16 of the Supporting Information). In our previous work on solid-phase Giese addition, we reported the compatibility of Trp, His, Cys, and Met with the overall transformation involving on-resin enamides and 10 equiv of RAE.[14] In the work presented here, we performed the homologation on Ac-Xaa-Gly-Glu-resin peptides examining the same functional groups. All peptides exhibited comparable isolated yields of 6−16% (S35−S37 of the Supporting Information). Met was not stable in the

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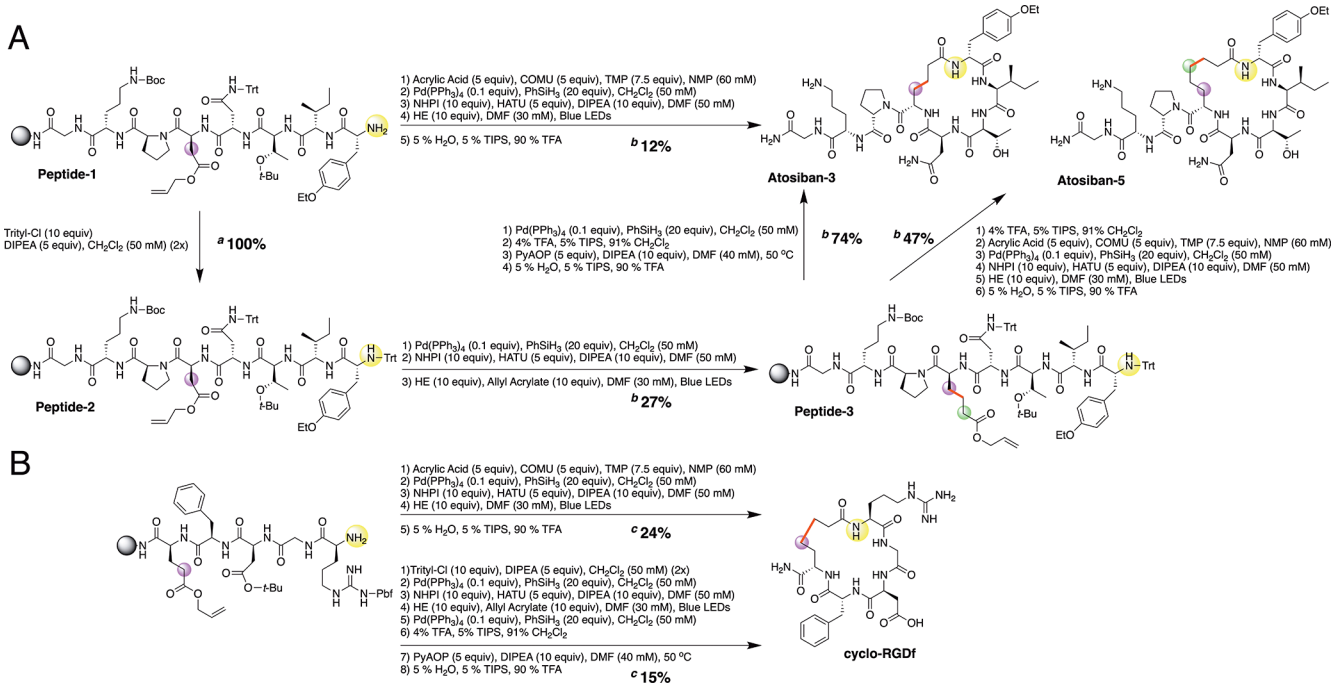
Scheme 2. Synthetic Scheme for the Photochemical Homologation and Cyclizations of (A) Atosiban and (B) Cyclo-RGDf Peptides[d]



[a]Conversion determined by acylation and crude HPLC. [b]Crude HPLC yields are reported and obtained by standard curves based on quantitative ^{1}H NMR. [c]Isolated yields reported. [d]Concentrations and equivalents are based on resin loading.

presence of allyl acrylate. We also sought to capture the radical with styrene to make phenylalanine homologues. Although styrene is less radicophilic with electron-rich radicals than acrylates, we still obtained appreciable yields of approximately 1 mg on a 25 μmol scale using both Asp and Glu side chains. Interestingly, a dicarbo-functionalized peptide was found to be an additional product isolated with comparable yields. This provides evidence that the newly generated α-radical may be further captured for other dicarbo-functionalization reactions.[16,20,21]

Having optimized conditions for both on-resin RAE formation and homologation, we began the synthesis of Atosiban on a 100 μmol resin scale, reporting overall yields based on label loading. For the sake of accuracy, we relied on crude HPLC yields and standard curves derived from NMR. After peptide elongation, 5 μmol of resin was cleaved, worked up, and found to contain 4.5 μmol of **Peptide-1** by quantitative HPLC (page S17 of the Supporting Information) (Scheme 2A). For formation of **Atosiban-3**, 20 μmol of resin was acylated with acrylic acid and then treated with Pd(0) to remove the allyl group. The aspartic acid side chain was converted to the NHPI ester and reacted with Hantzsch ester under 440 nm light for 2 h, leading to a 12% overall yield as determined by HPLC and an isolated yield of 5%. Upon cyclization, a large upfield shift was observed in the ^{1}H NMR spectra for Asp (linker) (0.38 ppm), Thr (0.49 ppm), and Ile (0.31 ppm) amides, all amino acids within the cycle. Half the resin of **Peptide-1** (50 μmol) was protected on the N-terminus, using trityl chloride to avoid potential issues with Fmoc incompatibility in photochemistry. A small amount of resin (~3 mg) was subjected to acylation conditions, where the trityl protection was found to proceed to 100% conversion (page S18 of the Supporting Information). The allyl group was deprotected, and Asp was homologated, leading to **Peptide-3** in 27% yield (page S19 of the Supporting Information). The hydrodecarboxylated peptide (alanine) was again found to be the major side product in the reaction as shown clearly by MS and an additional methyl doublet in the ^{1}HNMR spectra (page S52 of the Supporting Information). Cyclization of **Peptide-3** on a 5 μmol scale using PyAOP led to a clean 18% overall yield of **Atosiban-3** (page S21 of the Supporting Information). Alternatively, 25 μmol of **Peptide-3** was acylated on the N-terminus and then subjected to Pd(0), revealing the free carboxylic acid. The peptide was then activated, converted to the NHPI ester, and cyclized to **Atosiban-5** in 12% overall yield by HPLC and an isolated yield of 5%. Cyclization led to a large upfield shift in the ^{1}H NMR spectra for the linker (0.14 ppm), Thr (0.46 ppm), Ile (0.35 ppm), and Asn (0.44 ppm) amides, all amino acids within the cycle. ^{1}H NMR assignments based on TOCSY spectra are consistent with literature precedents (page S47 of the Supporting Information). The high cyclization yield of 47% for **Atosiban-5** is similar to the findings of both MacMillan[10] and Xu[11] on photochemical C−N cyclizations of peptides.

To examine the homologation cyclization efficiency on a different five-amino acid peptide substrate, we performed a C−N cyclization on integrin ligand cyclo-RGDf peptide (Scheme 2B).[22] Performing the photochemical intermolecular homologation followed by PyAOP cyclization led to an isolated yield of 15%, similar to that found for **Atosiban-3**, suggesting that structure does not significantly impact the transformation. Performing the photochemical cyclization led to a greater isolated yield of 24%, comparable to that of **Atosiban-5**. Given the similar reactivities of Glu and Asp and its homologue, the position of the radical within the peptide appears to matter less than the ring strain. For macrocycles with less ring strain, the

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yield of the intramolecular cyclization will likely be higher than that of the intermolecular homologation. For macrocycles with greater ring strain, the intermolecular homologation followed by PyAOP cyclization would be preferred.

In conclusion, we have demonstrated the solid-phase use of Asp and Glu side chains to generate alaninyl and homoalaninyl radicals by photochemical EDA decarboxylation. The method was used to homologate peptide precursors to Atosiban and cyclo-RGDf, which was subsequently cyclized to the target macrocycle. Reported are the first conditions to offer a route to a series of $C(sp^3)-C(sp^3)$-linked macrocycles, solid phase, as a late-stage modification from a common intermediate. The chemistry is operationally simple, using natural amino acids under ambient conditions and expedient reaction times. The research described herein foretells the potential for other solid-phase peptide radical transformations beyond decarboxylative hydroalkylation, providing new strategies for accessing this unique regime of chemical space.[23]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.2c02012.

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

■ AUTHOR INFORMATION

Corresponding Authors

Gary A. Molander − *Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6323, United States;* orcid.org/0000-0002-9114-5584; Email: gmolandr@sas.upenn.edu

David M. Chenoweth − *Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6323, United States;* orcid.org/0000-0002-0819-4669; Email: dcheno@sas.upenn.edu

Authors

Michael B. Elbaum − *Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6323, United States*

Mahmoud A. Elkhalifa − *Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6323, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.orglett.2c02012

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Sohrabi, C.; Foster, A.; Tavassoli, A. Methods for Generating and Screening Libraries of Genetically Encoded Cyclic Peptides in Drug Discovery. *Nat. Rev. Chem.* **2020**, *4* (2), 90−101.

(2) Lian, W.; Upadhyaya, P.; Rhodes, C. A.; Liu, Y.; Pei, D. Screening Bicyclic Peptide Libraries for Protein-Protein Interaction Inhibitors: Discovery of a Tumor Necrosis Factor-α Antagonist. *J. Am. Chem. Soc.* **2013**, *135* (32), 11990−11995.

(3) Vinogradov, A. A.; Yin, Y.; Suga, H. Macrocyclic Peptides as Drug Candidates: Recent Progress and Remaining Challenges. *J. Am. Chem. Soc.* **2019**, *141* (10), 4167−4181.

(4) White, C. J.; Yudin, A. K. Contemporary Strategies for Peptide Macrocyclization. *Nat. Chem.* **2011**, *3* (7), 509−524.

(5) Walensky, L. D.; Bird, G. H. Hydrocarbon-Stapled Peptides: Principles, Practice, and Progress. *J. Med. Chem.* **2014**, *57* (15), 6275−6288.

(6) Tian, Y.; Jiang, Y.; Li, J.; Wang, D.; Zhao, H.; Li, Z. Effect of Stapling Architecture on Physiochemical Properties and Cell Permeability of Stapled α-Helical Peptides: A Comparative Study. *ChemBioChem.* **2017**, *18* (21), 2087−2093.

(7) Bottecchia, C.; Noël, T. Photocatalytic Modification of Amino Acids, Peptides, and Proteins. *Chem. - A Eur. J.* **2019**, *25* (1), 26−42.

(8) Raynal, L.; Rose, N. C.; Donald, J. R.; Spicer, C. D. Photochemical Methods for Peptide Macrocyclisation. *Chem. - A Eur. J.* **2021**, *27* (1), 69−88.

(9) Liu, J. Q.; Shatskiy, A.; Matsuura, B. S.; Kärkäs, M. D. Recent Advances in Photoredox Catalysis Enabled Functionalization of α-Amino Acids and Peptides: Concepts, Strategies and Mechanisms. *Synth.* **2019**, *51* (14), 2759−2791.

(10) McCarver, S. J.; Qiao, J. X.; Carpenter, J.; Borzilleri, R. M.; Poss, M. A.; Eastgate, M. D.; Miller, M. M.; MacMillan, D. W. C. Decarboxylative Peptide Macrocyclization through Photoredox Catalysis. *Angew. Chemie - Int. Ed.* **2017**, *56* (3), 728−732.

(11) Wang, M.; Wang, C.; Huo, Y.; Dang, X.; Xue, H.; Liu, L.; Chai, H.; Xie, X.; Li, Z.; Lu, D.; Xu, Z. Visible-Light-Mediated Catalyst-Free Synthesis of Unnatural α-Amino Acids and Peptide Macrocycles. *Nat. Commun.* **2021**, *12* (1), 6873.

(12) Qin, T.; Malins, L. R.; Edwards, J. T.; Merchant, R. R.; Novak, A. J. E.; Zhong, J. Z.; Mills, R. B.; Yan, M.; Yuan, C.; Eastgate, M. D.; Baran, P. S. Nickel-Catalyzed Barton Decarboxylation and Giese Reactions: A Practical Take on Classic Transforms. *Angew. Chem.* **2017**, *129* (1), 266−271.

(13) Jiang, M.; Yang, H.; Fu, H. Visible-Light Photoredox Synthesis of Chiral α-Selenoamino Acids. *Org. Lett.* **2016**, *18* (9), 1968−1971.

(14) Elkhalifa, M.; Elbaum, M. B.; Chenoweth, D. M.; Molander, G. A. Solid-Phase Photochemical Decarboxylative Hydroalkylation of Peptides. *Org. Lett.* **2021**, *23* (21), 8219−8223.

(15) Openy, J.; Amrahova, G.; Chang, J.-Y.; Noisier, A.; 't Hart, P. Solid-phase Peptide Modification via Deaminative Photochemical Csp3-Csp3 Bond Formation Using Katritzky Salts. *Chem. - Eur. J.* **2022**, e202201121.

(16) Badir, S. O.; Molander, G. A. Developments in Photoredox/Nickel Dual-Catalyzed 1,2-Difunctionalizations. *Chem.* **2020**, *6* (6), 1327−1339.

(17) Melin, P.; Trojnar, J.; Johansson, B.; Vilhardt, H.; Akerlund, M. Synthetic Antagonists of the Myometrial Response to Vasopressin and Oxytocin. *J. Endocrinol.* **1986**, *111* (1), 125−131.

(18) Tsatsaris, V.; Carbonne, B.; Cabrol, D. Atosiban for Preterm Labour. *Drugs* **2004**, *64* (4), 375−382.

(19) Stymiest, J. L.; Mitchell, B. F.; Wong, S.; Vederas, J. C. Synthesis of Oxytocin Analogues with Replacement of Sulfur by Carbon Gives Potent Antagonists with Increased Stability. *J. Org. Chem.* **2005**, *70* (20), 7799−7809.

(20) Dhungana, R. K.; KC, S.; Basnet, P.; Giri, R. Transition Metal-Catalyzed Dicarbofunctionalization of Unactivated Olefins. *Chem. Rec.* **2018**, *18* (9), 1314−1340.

(21) Derosa, J.; Apolinar, O.; Kang, T.; Tran, V. T.; Engle, K. M. Recent Developments in Nickel-Catalyzed Intermolecular Dicarbofunctionalization of Alkenes. *Chem. Sci.* **2020**, *11* (17), 4287−4296.

(22) Kapp, T. G.; Rechenmacher, F.; Neubauer, S.; Maltsev, O. V.; Cavalcanti-Adam, E. A.; Zarka, R.; Reuning, U.; Notni, J.; Wester, H. J.; Mas-Moruno, C.; Spatz, J.; Geiger, B.; Kessler, H. A Comprehensive Evaluation of the Activity and Selectivity Profile of Ligands for RGD-Binding Integrins. *Sci. Rep.* **2017**, *7*, 39805.
(23) Malins, L. R. Decarboxylative Couplings as Versatile Tools for Late-Stage Peptide Modifications. *Pept. Sci.* **2018**, *110* (3), e24049.

https://doi.org/10.1021/acs.orglett.2c02012
Org. Lett. XXXX, XXX, XXX−XXX