Peptides

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# Decarboxylative Peptide Macrocyclization through Photoredox Catalysis

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***Abstract:*** *A method for the decarboxylative macrocyclization of peptides bearing N-terminal Michael acceptors has been developed. This synthetic method enables the efficient synthesis of cyclic peptides containing γ-amino acids and is tolerant of functionalities present in both natural and non-proteinogenic amino acids. Linear precursors ranging from 3 to 15 amino acids cyclize effectively under this photoredox method. To demonstrate the preparative utility of this method in the context of bioactive molecules, we synthesized COR-005, a somatostatin analogue that is currently in clinical trials.*

Cyclic peptides have recently received significant attention from a broad range of scientists in both academic and pharmaceutical settings.[1] At the heart of this focus is the remarkable finding that this class of peptide structure delivers unprecedented and selective therapeutic benefit for a large range of disease areas that include oncology, algiatry, and neurology. While many naturally occurring macrocycles have found medicinal applications over the last century, the use of non-natural cyclic peptides has become prominent due mainly to the advent of synthetic biology techniques that allow large numbers of these macrocyclic rings to be rapidly assembled and tested on micro scale.[2]

The success of cyclic peptides as a privileged pharmacophore can be attributed to 1) conformational ring constraints and 2) enhanced pharmacokinetic (PK) properties in comparison to their acyclic counterparts.[3] With respect to the former, conformational rigidity can lead to increased biological activity and target selectivity by diminishing the entropic barrier to reaching the requisite binding conformation. In terms of PK considerations, reduced flexibility and the absence of terminal amine and carboxylate functionalities lead to greater metabolic stability.[4] As a consequence, large cyclic peptides are often better suited than small molecules to selectively disrupt important biological binding events such as protein–protein interactions.[5]

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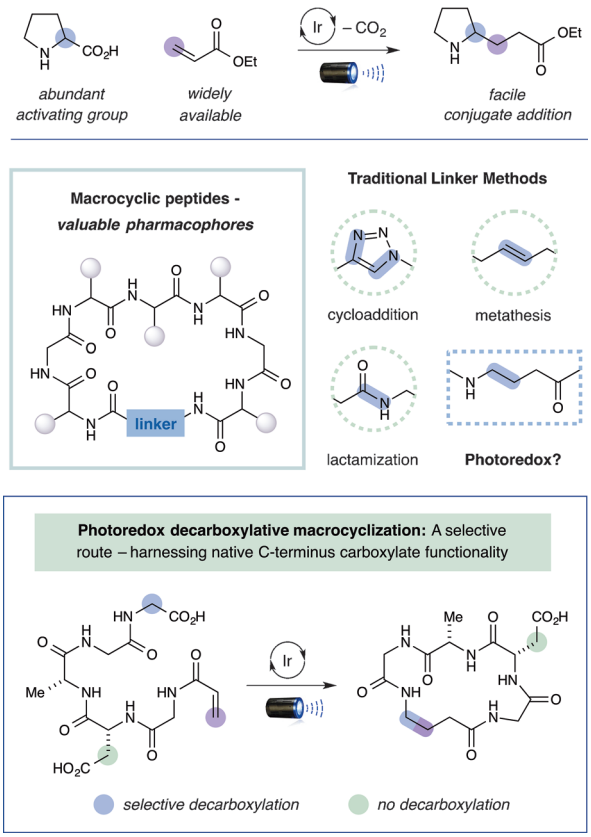
Molecules belonging to the cyclic peptide structural class can be challenging to prepare by traditional synthetic methods.[6] In contrast to all-carbon-backbone macrocycles, the ground-state *trans* geometry of multiple acyclic amide bonds results in a relatively high entropic barrier for head-to-tail engagement, while for small peptide sequences, ring strain often prevents efficient cyclization. Chief among the difficulties inherent in the synthesis of peptidic macrocycles is bimolecular couplings instead of the desired intramolecular pathway, which leads to linear oligomeric products. Selectivity for unimolecular head-to-tail cyclization can be achieved by performing reactions at low concentrations of substrate, the use of turn-inducing elements, or pseudo-dilution phenomena.[7] While traditional syntheses of macrocyclic peptides rely almost exclusively on coupling-reagent-mediated lactamization as the critical ring-forming step, in recent years a number of reports[8] have demonstrated the use of ring-closing metathesis, alkyne–azide cycloaddition, photochemical thiol–ene chemistry, and other elegant approaches to achieve macrocyclization.[9]

Our laboratory has recently introduced a number of photoredox-mediated methods in which α-$sp^3$ carboxylic acids are used as activating groups to generate open-shell radical species (after decarboxylation), which can thereafter be employed in a number of C–C bond forming processes.[10] In one such example, a decarboxylative conjugate addition reaction was developed that is successful with a broad range of carboxylic acid nucleophiles (Scheme 1).[11] Given the ease with which α-amino carboxylates undergo single-electron transfer (SET) decarboxylation to generate nucleophilic $C_{sp}3$ radicals, we questioned whether the C-termini of linear peptide chains might be selectively functionalized through photoredox-mediated $CO_2$ extrusion. As a key design element, we hypothesized that the resulting α-amino radicals might readily participate in intramolecular conjugate addition with pendant acrylamides, thereby enabling a rare example of C–C bond formation to close a cyclic peptide ring. Among a number of advantages, we recognized that this macrocyclization event would 1) be triggered using innate functionality, namely the C-terminal carboxylate, without the need for acid prefunctionalization, 2) be selective for oxidation of the C-terminal carboxylate group over other acid-containing residues (e.g., aspartate or glutamate side chains), 3) require the replacement of only one amino acid unit with an unsaturated acid during synthesis, and 4) introduce a non-peptidic section into the macrocycle, a feature that is known to generally improve the intrinsic pharmacokinetic profile while maintaining biological activity.[12] Herein, we report the successful execution of these ideas and present an intra-

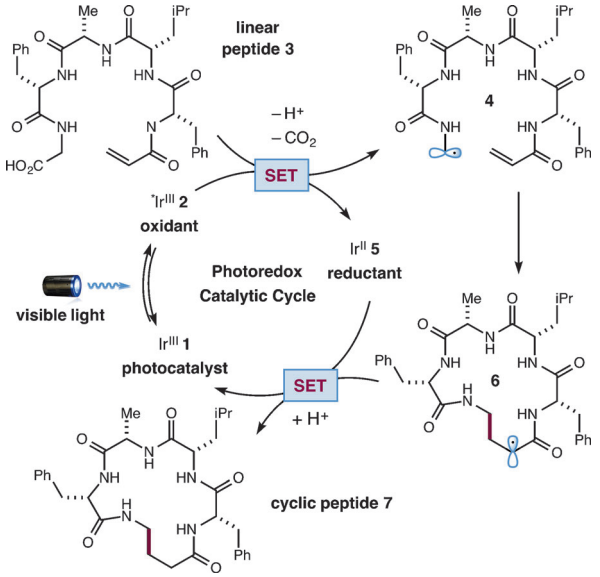
  




***Scheme 1.*** Photoredox-mediated decarboxylative conjugate addition.

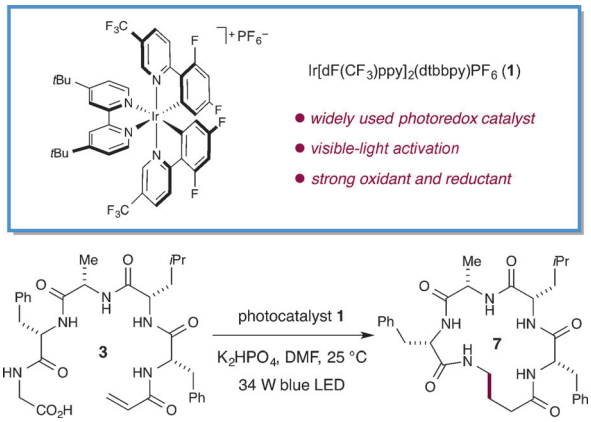
molecular radical 1,4-addition platform that is applicable across a wide range of peptide ring sizes and amino acid residues. Importantly, by leveraging the C-terminal carboxylate group, peptide macrocyclization is photoredox-enabled using the inherent oxidation potentials of naturally occurring α-amino acids. As shown in Scheme 2, our proposed mechanism begins with visible-light irradiation of photoredox catalyst $Ir[dF(CF_3)ppy]_2(dtbbpy)^+$ (**1**) to access the excited state $^*Ir[dF(CF_3)ppy]_2(dtbbpy)^+$ (**2**), a strong oxidant ($E_{1/2}^{red}[^*Ir^{III}/Ir^{II}] = +1.21$ V vs. SCE in MeCN).[13] Subsequent selective SET oxidation of the carboxylate salt of **3** ($E_{p/2}^{red}$ (Boc-Gly-$CO_2K$) = +1.2 V vs. SCE in MeCN)[14] would generate a carboxyl radical, which upon $CO_2$ extrusion would produce α-amino radical **4** and the reduced photocatalyst **5**. Intramolecular addition of nucleophilic α-amino radical **4** to the pendant Michael acceptor would then forge the desired macrocycle through a key C–C bond formation while furnishing electrophilic α-acyl radical **6**. Closure of the photoredox catalytic cycle would then involve SET reduction of the electron-deficient radical **6** ($E_{1/2}^{red}$ $^{\cdot}CH(CH_3)CO_2CH_3 = -0.66$ V vs. SCE; $E_{1/2}^{red}[Ir^{III}/Ir^{II}] = -1.37$ V vs. SCE)[15] by **5** to generate a macrocyclic enolate, which upon protonation would deliver the desired cyclic peptide **7**.[16]

We began our investigation into the proposed decarboxylative cyclization by exposing the *N*-acryloyl peptide Phe-Leu-Ala-Phe-Gly (**3**), photocatalyst **1**, and $K_2HPO_4$ in DMF to a 34 W blue LED lamp at room temperature (Table 1). To our delight, intramolecular cyclic peptide formation was observed under these preliminary conditions, albeit in low




***Scheme 2.*** Proposed mechanism for the decarboxylative peptide macrocyclization.

***Table 1:*** Initial results and optimization.




| Entry | Conditions | Concentration | **1** [mol %] | Yield [%][a] |
|---|---|---|---|---|
| 1 | as shown | 10 mM | 8 | 33 |
| 2 | as shown | 5 mM | 8 | 63 |
| 3 | as shown | 2.5 mM | 8 | 72 |
| 4 | as shown | 2.5 mM | 12 | 86 |
| 5 | no base | 2.5 mM | 12 | 31 |
| 6 | no photocatalyst | 2.5 mM | 0 | 0 |
| 7 | no light | 2.5 mM | 12 | 0 |

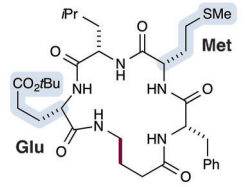
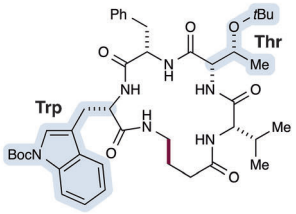
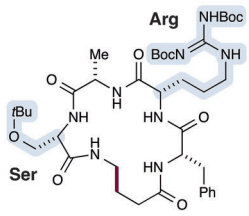
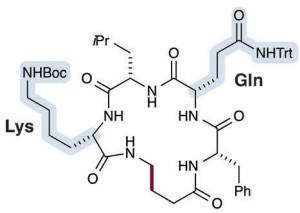
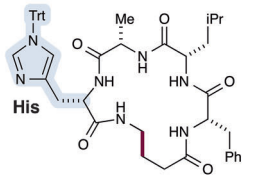
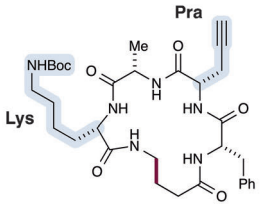
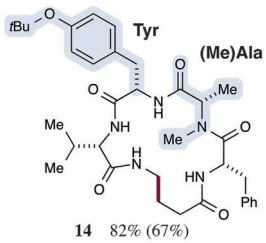
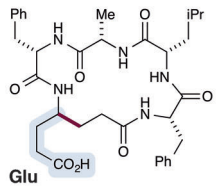
[a] Yields determined by HPLC (see the Supporting Information). DMF = *N*,*N*-dimethylformamide

yield (entry 1, 33% yield). As expected, lowering the concentration of the peptide substrate helped to circumvent oligomerization pathways while improving efficiency (entries 2 and 3). Similar increases in yield were observed with higher photocatalyst loadings, which is consistent with the necessary reduction of the α-acyl radical species in lieu of oligomerization (entry 4). It should be noted that the removal of base led to greatly diminished efficiency, and control

experiments revealed that both the photocatalyst and light were critical for product formation (entries 5–7).

Having determined reaction conditions leading to efficient macrocyclization, we turned our attention to establishing the scope with respect to the peptide substrate. As shown in Table 2, pentamers that incorporate a structurally diverse

***Table 2:*** Scope with respect to the amino acids of the peptide substrate.

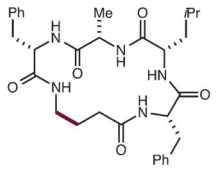
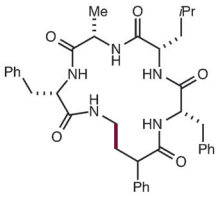
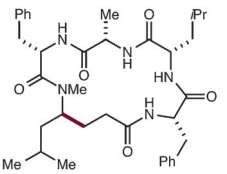
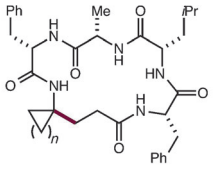
| product, % yield[a] | product, % yield[a] |
|---|---|
|  |  |
| **8** 73%[b] (33%) | **9** 46% (25%) |
|  |  |
| **10** 45%[b] (44%) | **11** 77% (70%) |
|  |  |
| **12** 67% (27%) | **13** 83% (60%) *crystal structure* |
|  |  |
| **14** 82% (67%) | **15** 50%, 2:1 dr (25%) |

[a] Yield determined by HPLC of crude reaction (2 trials; see the Supporting Information). Yield of isolated product obtained by preparative HPLC in parentheses. [b] 10 mol% 2,4,6-triisopropylthiophenol added.

set of amino acids can be successfully cyclized using this decarboxylative method. HMBC correlations and X-ray diffraction for examples **7** and **13,** respectively, unambiguously confirmed their cyclic nature.[17] Importantly, substrates containing many functional side chains can be cyclized readily under the photoredox conditions (**8**–**12**, 45–77% yield).[18] In the context of drug discovery, peptides containing *N*-methylated residues are particularly interesting owing to their increased membrane permeability and hydrophobicity.[19] Indeed, sequences containing the non-canonical amino acids *N*-methyl alanine and propargylglycine also undergo the photoredox macrocyclization with excellent efficiency (**13** and **14**, 83% and 82% yield, respectively). As a critical design element for these studies, we hypothesized that high selectivity should be observed for decarboxylation of the α-amino acid C-terminal residue in preference to any side-chain carboxylic acids, owing to the lower $pK_a$ and oxidation potential. As such, we were delighted to observe that a substrate containing a Glu residue undergoes uniformly selective decarboxylation at the α-amino C-terminal residue in preference to the γ-amino carboxylate side chain (**15**, 50% yield, 2:1 d.r.).

This method is also amenable to terminal amino acid and Michael-acceptor substitution, as shown in Table 3. More-

***Table 3:*** Scope with respect to the C-terminal and acryloyl functionality.

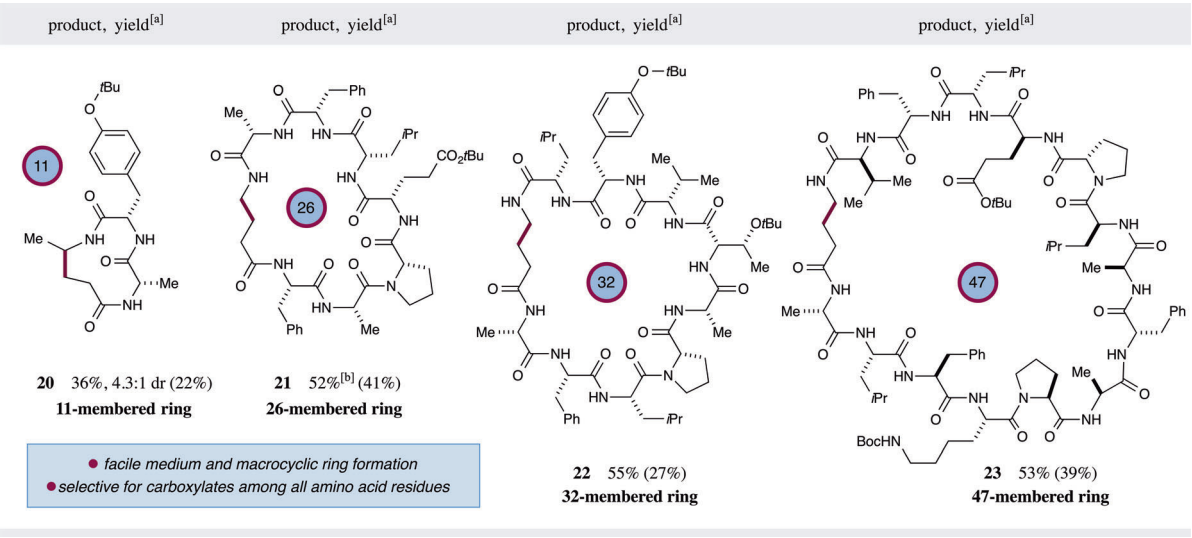
| product, yield[a] | product, yield[a] |
|---|---|
|  |  |
| **7** 86% (71%) | **16** 85%, 10:1 dr (41%) |
|  |  |
| **17** 51%, 1.6:1 dr (28%) | **18** $n=1$ 51%[b] (21%)<br>**19** $n=3$ 56%[b] (51%) |

[a] Yields determined by HPLC of crude reaction (2 trials; see the Supporting Information). Yield of isolated product obtained by preparative HPLC in parentheses. [b] DMSO as solvent.

over, incorporating a radical-stabilizing phenyl group at the α-position of the α,β-unsaturated carbonyl results in excellent reaction efficiency and diastereocontrol (**16**, 85% yield, 10:1 d.r.). The use of *N*-methyl leucine at the precursor C-terminus also resulted in efficient macrocyclization, albeit without control at the newly formed stereocenter (**17**, 51% yield, 1.6:1 d.r.). Notably, spirocenter-containing macrocycles can be readily generated (**18** and **19**, 51% and 56% yield, respectively).

Finally, we sought to assess the range of different ring sizes that could be generated (Table 4). Notably, peptide sequences containing 8, 10, and 15 amino acids (arbitrarily selected residue numbers) undergo efficient cyclization when using this photoredox method (**21**–**23**, 52–55% yield). It has long been established that the synthesis of medium-sized peptidic rings is challenging owing to the ground-state *trans* confor-

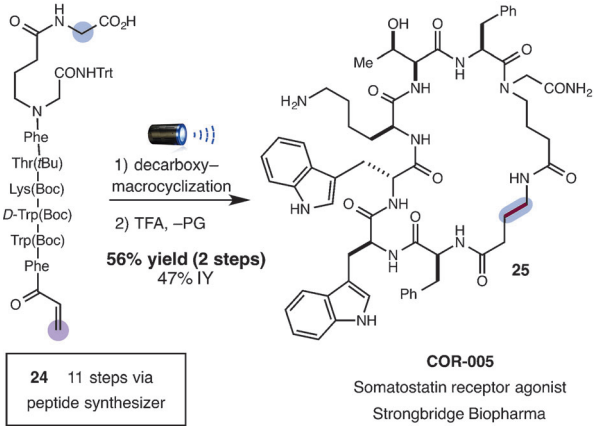
***Table 4:*** Scope with respect to the peptide macrocycle ring size.




[a] Yields determined by HPLC (2 trials; see the Supporting Information). Yield of isolated product obtained by preparative HPLC in parentheses. [b] 10 mol% of 2,4,6-triisopropylthiophenol.

mation of the amide bond and detrimental transannular interactions. We were thus delighted to find that a tripeptide substrate underwent intramolecular bond formation with useful levels of efficiency (**20**, 36% yield).

Last, to highlight the utility of this method for preparing cyclic structures of therapeutic value, we sought to construct COR-005, a somatostatin analogue that is currently in Phase II clinical trials.[20] Somatostatin-receptor agonists have shown great potential for the treatment of gastrointestinal indications, non-insulin-dependent diabetes, and acromegaly.[21] The γ-amino acids serve to optimize the conformational rigidity and stability of the compound without interfering with receptor-binding ability. Additionally, **25** is resistant to biodegradation in comparison to somatostatin. As shown in Scheme 3, photoredox-mediated cyclization of linear peptide **24**, followed by acid-mediated deprotection, leads to COR-005 (**25**) in 56% yield.




***Scheme 3.*** Photoredox macrocylization to form the bioactive cyclic peptide COR-005.

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**Keywords:** decarboxylation · macrocycles · Michael addition · peptides · photoredox catalysis

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# Communications

**Peptides**

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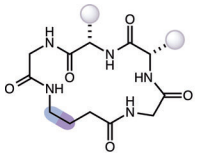
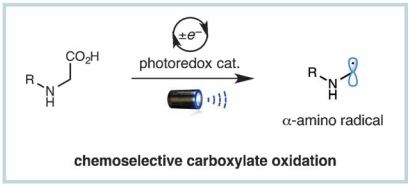
Decarboxylative Peptide Macrocyclization through Photoredox Catalysis




**Oh, take me back to the start**: A method for the decarboxylative macrocyclization of peptides bearing N-terminal Michael acceptors was developed. This photoredox method enables the efficient synthesis of cyclic peptides containing γ-amino acids, is tolerant of functionalities present in natural amino acids. Linear precursors ranging from 3 to 15 amino acids can be effectively cyclized by using this method.