Published in final edited form as:

Angew Chem Int Ed Engl. 2017 January 16; 56(3): 728–732. doi:10.1002/anie.201608207.

Decarboxylative Peptide Macrocyclization via Photoredox Catalysis

Stefan J. McCarver,

Merck Center for Catalysis at Princeton University, Washington Road, Princeton, NJ 08544, (USA)

Jennifer X. Qiao,

Bristol-Myers Squibb, Route 206 & Province Line Road, Princeton, NJ, 08543 (USA)

Joseph Carpenter,

Bristol-Myers Squibb, Route 206 & Province Line Road, Princeton, NJ, 08543 (USA)

Robert M. Borzilleri,

Bristol-Myers Squibb, Route 206 & Province Line Road, Princeton, NJ, 08543 (USA)

Michael A. Poss,

Bristol-Myers Squibb, Route 206 & Province Line Road, Princeton, NJ, 08543 (USA)

Martin D. Eastgate,

Bristol-Myers Squibb, Route 206 & Province Line Road, Princeton, NJ, 08543 (USA)

Michael Miller, and

Bristol-Myers Squibb, Route 206 & Province Line Road, Princeton, NJ, 08543 (USA)

Prof. Dr. David W. C. MacMillan*

Merck Center for Catalysis at Princeton University, Washington Road, Princeton, NJ 08544, (USA)

Abstract

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

Graphical Abstract

* dmacmill@princeton.edu, Homepage: <http://www.princeton.edu/chemistry/macmillan/>.

A method for the decarboxylative macrocyclization of peptides bearing *N*-terminal Michael acceptors has been developed. This synthetic protocol enables the efficient synthesis of γ -amino acid-containing cyclic peptides and is tolerant of functionality present in natural amino acids. Linear precursors ranging from 3 to 15 amino acids cyclize effectively under this photoredox protocol.

Keywords

decarboxylation; macrocycles; Michael addition; peptides; photoredox catalysis

Cyclic peptides have recently received significant attention from a broad range of scientists in both academic and pharmaceutical settings. 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, algia, and neurology.^[1] 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 technologies 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 i) conformational ring constraints and ii) 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 in reaching the requisite binding conformation. In terms of PK considerations, reduced flexibility along with 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]

Molecules belonging to the cyclic peptide structural class can be challenging to prepare by traditional synthetic methods.^[6] In

(Eq. 1)

contrast to all-carbon-backbone macrocycles, the ground state (*E*)-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 to the synthesis of peptidic macrocycles is bimolecular couplings in lieu of the desired intramolecular pathway, leading 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, and other elegant approaches to achieve macrocyclization.^[9]

Our laboratory has recently introduced a number of photoredox mediated protocols in which α -sp³ carboxylic acids are used as activating groups to generate open-shell radical species (after decarboxylation) that 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 substantial range of carboxylic acid nucleophiles (Eq

1).^[11] Given the aptitude 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 via 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 i) would be triggered using innate functionality, namely the *C*-terminus carboxylate, without the need for acid prefunctionalization, ii) would be selective for oxidation of the *C*-terminus carboxylate group over other acid-containing residues (e.g., aspartate or glutamate side chains), iii) would require the replacement of only one amino acid unit with an unsaturated acid during synthesis, and iv) would 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 ideals and present an intramolecular radical 1,4-addition platform 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 1, 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 via 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** ($E_{1/2}^{red}\bullet CH(CH_3)CO_2CH_3 = -0.66$ 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 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 with low 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, 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 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 of competent peptide substrates. As shown in Table 2,

pentamers that incorporate a structurally diverse set of amino acids are generally successful 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 photoredox conditions (**8–12**, 34%–77% yield).^[18] In the context of drug discovery, peptides containing *N*-methylated residues are particularly interesting due to increased membrane permeability and hydrophobicity.^[19] Indeed, sequences containing the non-canonical amino acids *N*-methyl alanine and propargylglycine also undergo photoredox macrocyclization with excellent efficiency (**12** and **13**, 82% and 83% 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, due 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

Photoredox Macrocyclization to Form Bioactive Cyclic Peptide (Eq. 2)

(Eq. 2)

to the γ -amino carboxylate side chain (**15**, 50% yield, 2:1 dr). This protocol is also amenable to terminal amino acid and Michael acceptor substitution, as shown in Table 3. Moreover, 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 dr). The use of *N*-methyl leucine at the precursor *C*-terminus also resulted in an efficient macrocyclization, albeit without control at the newly formed stereocenter (**17**, 51% yield, 1.6:1 dr). 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 using this photoredox protocol (**21–23**, 52–55% yield). It has long been established that medium peptidic ring synthesis is challenging due to the ground state (*E*)-conformation of the amide bond and detrimental transannular

interactions. As such, we were 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 technology for preparing cyclic structures of therapeutic value, we sought to construct COR-005, a somatostatin analog currently in Phase II clinical trials.^[20] Somatostatin receptor agonists have shown high 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 while not interfering with receptor-binding ability. Additionally, **25** is resistant to biodegradation in comparison to somatostatin. As shown in Eq 2, photoredox-mediated cyclization of linear peptide **24**, followed by acid-mediated deprotection, leads to COR-005 (**25**) in 56% yield.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors are grateful for financial support provided by the NIH General Medical Sciences (Grant NIHGM5 (R01 GM078201-05) and gifts from Bristol-Myers Squibb, Merck and Abbvie. Purification of compounds was supported by Sergey Malnikov at BMS. X-ray diffraction was performed by Dr. Phil Jeffrey at Princeton University.

References

1. a) Fairlie DP, Abbenante G, March DR. *Curr. Med. Chem.* 1995; 2:654. b) Wang S, Blois A, El Rayes T, Liu JF, Hirsch MS, Gravidal K, Palakurthi S, Bielenberg DR, Akslen LA, Drapkin R, Mittal V, Watnick RS. *Sci. Transl. Med.* 2016; 8:329. c) Janecka A, Gentilucci L. *Fut. Med. Chem.* 2014; 6:2093. d) Thell K, Hellinger R, Sahin E, Michenthaler P, Gold-Binder M, Haider T, Kuttke M, Liutkevicius Z, Göransson U, Gründemann C, Schabbauer G, Gruber CW. *PNAS.* 2016; 113:3960. [PubMed: 27035952]
2. a) Driggers EM, Hale SP, Lee J, Terrett NK. *Nat. Rev. Drug Discov.* 2008; 7:608. [PubMed: 18591981] b) Craik DJ, Fairlie DP, Liras S, Price D. *Chem. Biol. Drug Des.* 2013; 81:136. [PubMed: 23253135] c) Yudin AK. *Chem. Sci.* 2015; 6:30. d) Horton DA, Bourne GT, Smythe ML. *J. Comput. Aided Mol. Des.* 2002; 16:415. [PubMed: 12489688]
3. a) Roxin A, Zheng G. *Future Med. Chem.* 2012; 4:1601. [PubMed: 22917248] b) Adessi C, Soto C. *Curr. Med. Chem.* 2002; 9:963. [PubMed: 11966456]
4. a) Bogdanowich-Knipp SJ, Jois DSS, Siahhaan TJ. *J. Peptide Res.* 1999; 53:523. [PubMed: 10424347] b) Piserchio A, Salinas GD, Li T, Marshall J, Spaller MR, Mierke DF. *Chemistry & Biology.* 2004; 11:469. [PubMed: 15123241]
5. a) Cardote TAF, Ciulli A. *ChemMedChem.* 2016; 11:787. [PubMed: 26563831] b) Zhang Z, Lin Z, Zhou Z, Shen HC, Yan SF, Mayweg AV, Xu Z, Qin N, Wong JC, Zhang Z, Rong Y, Fry DC, Hu T. *ACS Med. Chem. Lett.* 2014; 5:993. [PubMed: 25221655]
6. Davies JS. *J. Peptide Sci.* 2003; 9:471. [PubMed: 12952390]
7. Martí-Centelles V, Pandey MD, Burguete MI, Luis SV. *Chem. Rev.* 2015; 115:8736. [PubMed: 26248133]
8. a) White CJ, Yudin AK. *Nat. Chem.* 2011; 3:509. [PubMed: 21697871] b) Griesbeck AG, Heinrich T, Oelgemöller M, Molis A, Heidtmann A. *Helv. Chim. Acta.* 2002; 85:4561. c) Zhang L, Tam JP. *J. Am. Chem. Soc.* 1997; 119:2363. d) Yu L, Lai Y, Wade JV, Coutts SM. *Tetrahedron Lett.* 1998; 39:6633.
9. a) Miller SJ, Blackwell HE, Grubbs RH. *J. Am. Chem. Soc.* 1996; 118:9606. b) Punna S, Kuzelka J, Wang Q, Finn MG. *Angew. Chem. Int. Ed.* 2005; 44:2215. c) Aimetti AA, Shoemaker RK, Lin C-C,

- Anseth KS. *Chem. Commun.* 2010; 46:4061.d) Wang Y, Chou DH-C. *Angew. Chem. Int. Ed.* 2015; 54:10931.
10. a) Zuo Z, Ahneman DT, Chu L, Terrett JA, Doyle AG, MacMillan DWC. *Science*. 2014; 345:437. [PubMed: 24903563] b) Noble A, McCarver SJ, MacMillan DWC. *J. Am. Chem. Soc.* 2015; 137:624. [PubMed: 25521443] c) Zuo Z, Cong H, Li W, Choi J, Fu GC, MacMillan DWC. *J. Am. Chem. Soc.* 2016; 138:1832. [PubMed: 26849354] d) Noble A, MacMillan DWC. *J. Am. Chem. Soc.* 2014; 136:11602. [PubMed: 25026314] e) Zuo Z, MacMillan DWC. *J. Am. Chem. Soc.* 2014; 136:5257. [PubMed: 24712922]
11. Chu L, Ohta C, Zuo Z, MacMillan DWC. *J. Am. Chem. Soc.* 2014; 136:10886. [PubMed: 25032785]
12. a) Oddo A, Nyberg NT, Frimodt-Møller N, Thulstrup PW, Hansen PR. *Eur. J. Med. Chem.* 2015; 102:574. [PubMed: 26318064] b) Chen Y-C, Muhlrads A, Shteyer A, Vidson M, Bab I, Chorev M. *J. Med. Chem.* 2002; 45:1624. [PubMed: 11931616] c) Ohshiro Y, Nakajima E, Goto Y, Fuse S, Takahashi T, Doi T, Suga H. *ChemBioChem*. 2011; 12:1183. [PubMed: 21506233]
13. Lowry MS, Goldsmith JJ, Slinker JD, Rohl R, Pascal RA Jr, Malliaris GG, Bernhard S. *Chem. Mater.* 2005; 17:5712.
14. Reported as half peak potential. See Supporting Information.
15. Bortolamei N, Isse AA, Gennaro A. *Electrochim. Acta*. 2010; 55:8312.
16. Detailed mechanistic investigations are ongoing.
17. See Supporting Information. CCDC 1507663 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
18. For several substrates, addition of a thiol co-catalyst led to increased efficiency. See Supporting Information for details.
19. a) Chatterjee J, Rechenmacher F, Kessler H. *Angew. Chem. Int. Ed.* 2013; 52:254.b) White TR, Renzelman CM, Rand AC, Rezai T, McEwen CM, Gelev VM, Turner RA, Linington RG, Leung SSF, Kalgutkar AS, Bauman JN, Zhang Y, Liras S, Price DA, Mathiowetz AM, Jacobson MP, Lokey RS. *Nat. Chem. Biol.* 2011; 7:810. [PubMed: 21946276]
20. Hornik, V.; Afargan, M.; Gellerman, G. WO 99/65508. 1999 Dec 23.
21. Plöckinger U, Hoffmann U, Geese M, Lupp A, Buchfelder M, Flitsch J, Vajoczy W, Saeger W, Schulz S, Dohrmann C. *Eur. J. Endocrinol.* 2012; 166:223. [PubMed: 22065857]

Scheme 1.
Proposed Decarboxylative Peptide Macrocyclization

Table 1

Initial Results and Optimization

entry	conditions	concentration	1	yield ^a
1	as shown	10 mM	8 mol%	33%
2	as shown	5 mM	8 mol%	63%
3	as shown	2.5 mM	8 mol%	72%
4	as shown	2.5 mM	12 mol%	86%
5	no base	2.5 mM	12 mol%	31%
6	no photocatalyst	2.5 mM	0 mol%	0%
7	no light	2.5 mM	12 mol%	0%

^aYields determined by HPLC, see Supporting Information.

Table 2

Scope with Varied Amino Acid Residues

product, % yield ^a	product, % yield ^a
<p>8 73%^b (33%)</p>	<p>9 46% (25%)</p>
<p>10 45%^b (44%)</p>	<p>11 77% (70%)</p>
<p>12 67% (27%)</p>	<p>13 83% (60%) crystal structure</p>
<p>14 82% (67%)</p>	<p>15 50%, 2:1 dr (25%)</p>

^aYield determined by HPLC of crude reaction (2 trials), see Supporting Information. Isolated yield by preparative HPLC in parenthesis.^bAdded 10 mol% 2,4,6-triisopropylthiophenol.

Table 3

Scope of C-Terminus 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%)
		19 n = 3	56% ^b (51%)

^aYields determined by HPLC of crude reaction (2 trials), see Supporting Information. Isolated yield in parenthesis.^bDMSO as solvent.

Table 4

Scope of Peptide Macrocyclic Ring Sizes

product, yield ^a	product, yield ^a	product, yield ^a	product, yield ^a
<p>20 36%, 4.3:1 dr (22%) 11-membered ring</p>	<p>21 52%^b (41%) 26-membered ring</p>	<p>22 55% (27%) 32-membered ring</p>	<p>23 53% (39%) 47-membered ring</p>
<ul style="list-style-type: none"> ● facile medium and macrocyclic ring formation ● selective for carboxylates among all amino acid residues 			

^aYields determined by HPLC (2 trials), see Supporting Information. Isolated yield in parenthesis.

^b10 mol% of 2,4,6-triisopropylthiophenol.