General Installation of (4H)-Imidazolone *cis*-Amide Bioisosteres Along the Peptide Backbone

Brendan J. Wall, Emily A. O'Brien, Krishna K. Sharma, Aaron Donovan, and Brett VanVeller*

Department of Chemistry, Iowa State University, Ames, IA 50011, USA

Received December 8, 2023; E-mail: bvv@iastate.edu

Abstract: Imidazolones represent an important class of heterocycles present in a wide-range of pharmaceuticals, metabolites, naturally-occurring bioactive natural products, and serve as the active chromophore in green fluorescent protein (GFP). Recently imidazolones have received attention for their ability to act as an amide bond bioisotere to improve pharmacokinetic properties. Herein we present a tandem amidine installation and subsequent cyclization with an adjacent ester to yield (4H)-imidazolone products. By using amino acid building blocks, we can access the first examples of α-chiral imidazolones which have been previously inaccessible. Additionally, our method is amenable to on-resin installation and can be integrated seamlessly into existing solid-phase peptide synthesis (SPPS) protocols. Finally, we show that imidazolones can act as *cis*-amide bond surrogates to assist in pre-organizing linear peptides for head-to-tail macrocyclization. This work represents the first general approach to backbone and side-chain insertion of imidazolone bioisosteres at various positions in linear and cyclic peptides.
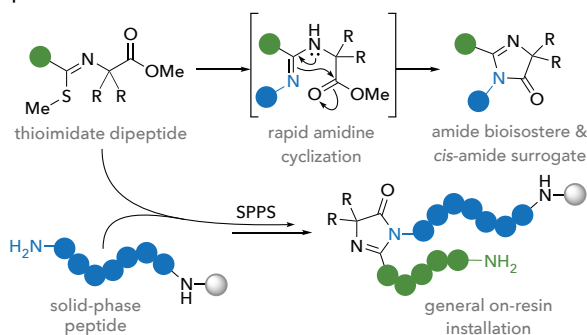
Introduction

Incorporation of heterocyclic motifs in the peptide backbone represents a critical tool to address issues in peptide drug metabolism and cell permeability.[1–4] Nature has evolved its own biological machinery to include heterocycles and expand the complexity of peptides beyond the standard suite of canonical amino acids.[5–8] The late biochemist Christopher T. Walsh credited these heterocycles as "a recurring motif in Nature's medicinal chemistry toolbox".[9] In nature, backbone heterocycles are installed via cyclization from an adjacent side-chain onto the backbone amide linkage,[8] leading to oxazole-type (from Ser or Thr), and thiazole-type (from Cys) heterocycles (Figure 1A left).[5–8] Synthetic chemists have mimicked this approach using sophisticated on-resin chemical activation of the amide bond or by incorporating non-natural side-chains into linear peptides to access aromatic backbone heterocycles such as oxazoles,[10] thiazoles,[10] imidazoles,[10] pyrazoles,[11] oxadiazoles,[12] 4-imidazolidinones,[13] 2-imidazolidines,[14] 1,2,4-

triazoles,[15] 1,2,3-triazoles,[16,17] and iminohydantoins.[18] The substitution pattern imparted by *all* of these heterocycles generates a 1,3-*trans*-amide-like conformation along the peptide backbone (Figure 1A left) which mimics the native *trans*-amide conformation.
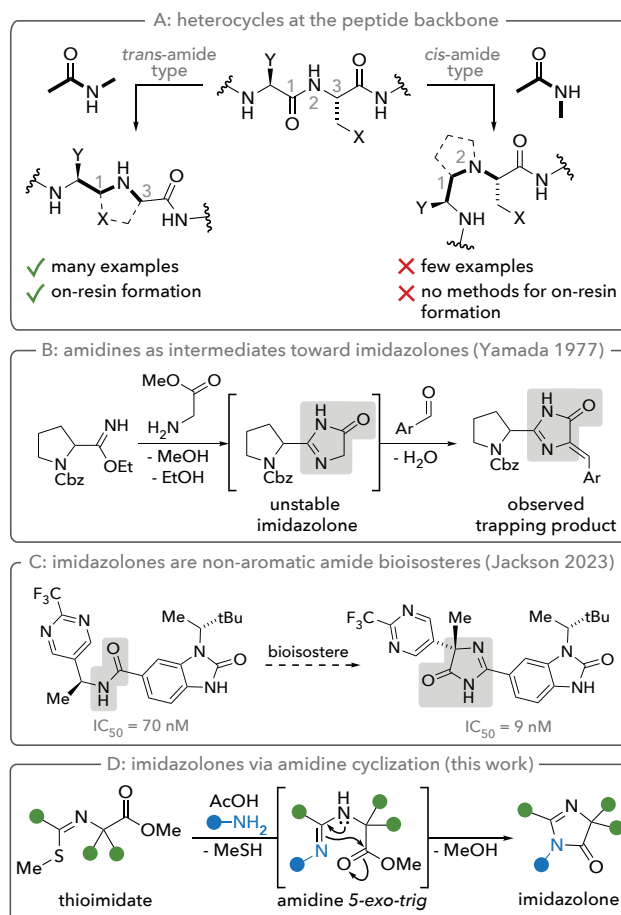

Figure 1. (A) Heterocycles can geometrically restrict the peptide backbone to either *cis*-amide-like or *trans*-amide-like motifs. (B) Amidines can act as an intermediate to form (4H)-imidazolones. (C) (4H)-Imidazolones can act as non-aromatic amide bond isosteres. (D) Thioimidate dipeptides enable facile entry to imidazolone cyclization products by use of an amidine intermediate.

One can imagine a second option for backbone heterocyclic installation, derived solely from the amide linkage. These heterocycles would yield a 1,2-*cis*-amide-like motif along the peptide backbone (Figure 1A right), geometrically constraining the amide bond to a non-native *cis*-amide conformation. *cis*-Amide bond surrogates are of exceeding interest to synthetic and medicinal chemists for their ability to initiate turn motifs in peptides,[19,20] reduce proteolysis,[11] and facilitate peptide macrocyclization.[21,22] Un-

fortunately, the amide bond of peptides only exists in the *cis*-conformation 0.1-0.2% of the time (at room temperature).[23] Properly substituted heterocycles, however, can geometrically restrict the peptide to bias the *cis*-amide conformation. Synthetic chemists have utilized pre-synthesized dipeptides with triazoles,[24,25] thiazoles,[26] pyrazoles,[11] and tetrazoles,[27–29] in place of the conventional amide bond, to install *cis*-amide surrogates with 1,2-connectivity along the peptide backbone. However, unlike *trans*-amide-like heterocycles, methods which form *cis*-amide-like heterocycles *directly on resin* during solid-phase peptide synthesis (SPPS) are seemingly absent from the literature.

Here we report the discovery of a new method to form a backbone heterocycle, (4*H*)-imidazolones, using conditions related to amidine installation into the backbone of linear peptides.[30] Yamada and co-workers hypothesized that peptide amidine intermediates could enable access to imidazolones, however these imidazolones were too unstable to be isolated and instead were observed as the benzylidene trapping product (Figure 1B).[31] Likewise, Houghten and co-workers found that resin-derived amidines could cyclize to imidazolone products during acidic HF cleavage conditions.[32] Recently, imidazolones have received attention from the medicinal chemistry community for their ability to act as a rare example of a non-aromatic bioisostere of the amide bond (Figure 1C).[33] During the course of our discovery of optimal conditions for amidine formation on peptides,[30] we noticed that when an amidine was installed one residue from a *C*-terminal ester, the amidine would rapidly attack, via *5-exo-trig* cyclization, onto the adjacent ester yielding a (4*H*)–imidazolone (Figure 1C). Thus, amidines, and now imidazolones, can be easily accessed by acid-catalyzed activation of thioimidates, a known protecting groups for thioamides during SPPS.[30,34,35]

(4*H*)-Imidazolones are high-value heterocycles appreciated for their anti-hypertensive,[36] anti-cancer,[37] anti-psychotic,[38] anti-viral,[39] and cytotoxic[40] effects; they are also known to the agrochemical field as potent broad spectrum herbicides[41] (Figure 2, **1**-**6**). Additionally, (4*H*)-imidazolones are the active fluorescent product of the green fluorescent protein (GFP) hexapeptide which has been applied in protein biology for the labeling and monitoring of protein expression and motility in cells.[42–44] New methods to form imidazolones under gentle conditions will enable access to amino-acid derived imidazolone natural products that have yet to be synthesized.[45]

Finally, the connectivity imparted by (4*H*)-imidazolones leads to the aforementioned locked *cis*-amide motif along the peptide chain, representing a new class of *cis*-amide bond surrogates with unexplored structural implications. Currently, *cis*-amide surrogates require significant pre-synthesis of protected specialty azido and alkynyl amino acid substrates prior to heterocycle formation.[25] Only then can the dipeptide be deprotected, and then finally coupled on to resin during SPPS.[11,25,29] Pyrazole and tetrazole surrogates suffer from regiochemical side-products from heterocycle formation which complicate purification.[11,29] *N*-terminal deprotection also poses a significant challenge to *cis*-amide surrogates as they pre-organize cyclization to a diketopiperazine product, which has been observed as a quantitative side product in the case of teterazole surrogates.[27] For these reasons, only a few *cis*-amide surrogates have been elaborated past dipeptides.[20,25,28]
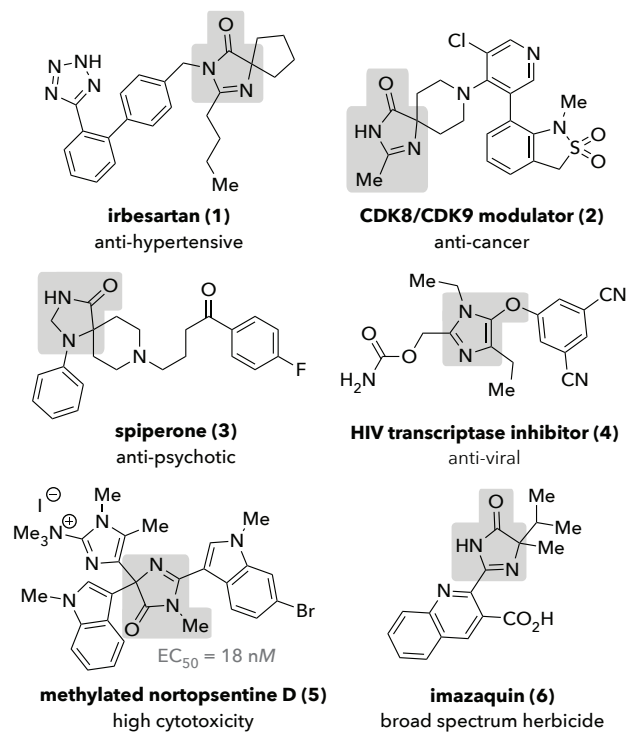
The new method reported here provides access to highly-

Figure 2. Selected examples of (4*H*)-imidazolones present in pharmaceuticals,[36–39] herbicides,[41] and natural products[40,45]

functionalized (4*H*)-imidazolones. The imidazolones can be easily accessed from commercially-available amino acid building blocks, incorporated into peptides using known methods, and cyclized to install imidazolones at both the *N*- and *C*-termini of the peptide, as well as in the middle of peptides and on the side-chain (enabling branched structures).

Imidazolones have never before been incorporated into peptides and as such have unexplored effects on the conformational landscape of peptides, as well as unknown biological properties. As mentioned previously, imidazolones have recently been shown to be an amide bond bioisostere with favorable pharmacological properties, creating a compelling need for further exploration.[33] Unfortunately, methods to install non-aromatic bioisosteres, like imidazolones, on peptides are undeveloped. This work addresses this gap in knowledge by developing a general method to access imidazolones in both small-molecules as well as linear and cyclic peptides for future applications as bioisosteres. Notably, we do not observe any diketopiperazine side-products which have been observed quantitatively from the installation of other *cis*-amide bond surrogates.[27] Finally, we show that our imidazolone performs better than other *cis*-amide surrogates in the pre-organization of a head-to-tail macrocyclization of natural product Mahafacyclin B.

Results and Discussion

Optimization of Imidazolone Formation. During our exploration of optimal conditions for the formation of amidines from thioimidates, we noticed that reactions with primary amines lead to (4*H*)-imidazolone products (Table 1, **8**) via *5-exo-trig* cyclization if an ester was present on the adjacent residue. Given the facile access to amidines on-resin, we sought to develop optimal conditions to form imidazolone products in hopes that we could install imidazolone moieties during the course of SPPS. The Cbz-

protected thioimidate dipeptide (**7**) was selected as a model substrate. The α,α-substitution of 2-aminoisobutyric acid (Aib) was chosen to mimic the majority of natural products and industrially-relevant imidazolones (Figure 2) which contain α,α-substitution. Mono-substition leads to rapid racemization of the stereocenter at this position due to the thermodynamically-stable hydroxyimidazole tautomerization.[45]

Initial testing with our previous conditions for the formation of amidines from thioimidates yielded moderate yields (Table 1, entry 1). Unfortunately 10 equivalents of the amine nucleophile complicates purification and restricts the scale of this chemistry. Therefore in an effort to reduce the equivalents of amine required for this transformation, we employed Design of Experiments (DoE) methodology to locate optimal reaction conditions. DoE (compared to standard one-variable at a time optimization) enables us to understand not only the effects of our variables (amine and acid stoichiometries) on yield, but also how these variables affect each other.[46] From this DoE optimization we observed two major trends: (1) amidine formation is driven primarily by the amount of amine nucleophile, which can be enhanced by concentration of the solution *or* stoichiometry, and (2) acid equivalency has a minor affect on yield (Table 1, entries 5-7), but is required for the transformation (See Supplementary Information).

Table 1. Optimized Conditions for Imidazolone Cyclization

entry	equiv. amine	equiv. AcOH	solvent (v/v)	temp. (°C)	time (h)	yield[a] (%)
1	10	1	DMF:TFE	23	24	73
2	5	2	DMF:TFE	23	24	93
3	5	0.5	DMF:TFE	23	24	50
4	3	1.25	DMF:TFE	23	24	64
5	1	2	DMF:TFE	23	24	31
6	1	0.5	DMF:TFE	23	24	25
7	1	5	DMF:TFE	23	24	27
8	3	1.25	DMF	23	24	7
9	3	1.25	DCM:TFE	23	24	71
10	3	1.25	THF:TFE	23	24	98
11	3	1.25	MeCN:TFE	23	24	>99
12	1	0.5	MeCN:TFE	23	24	60
13	1	0.5	MeCN:TFE	70	8	90

[a]Yield determined by crude NMR analysis with dimethyl terepthalate internal standard.
Rows 2-6 (gray) were part of the full-factorial Design of Experiments (DOE) methodology (See Supplementary Information for more details).

We found that binary solvent mixtures containing 2,2,2-trifluroethanol (TFE) accelerate amidine installation which we attribute to solvent-enhanced hydrogen-bonding properties rapidly shuttling protons in solution (Table 1, Entry 8).[30] Solvent mixtures of THF:TFE and MeCN:TFE (1:1 %v/v) were found to be the most optimal for this transformation, however DCM:TFE and DMF:TFE furnished imidazolone product in moderate yield (Table 1, Entries 7 &

9-11). Heating the reaction (Table 1, Entry 13) enabled us to form imidazolone products with stoichiometric amine quantities which is critical for the application of this chemistry in the context of SPPS where reaction rates are greatly reduced.

With the optimized conditions from Table 1, we explored the types of amine nucleophiles that were compatible with this reaction. We found that ammonium acetate (**10a**) and sterically-unencumbered primary amines (**10b-10c**) formed imidazolone products in very-high yields. Anilines (**10d-10f**) produced the corresponding imidazolones in moderate yield, attributed to the decreased nucleophilicity of these amines due to delocalization of the nucleophilic lone-pair into aromatic π-system. Imidazolone formation also tolerated an array of pharmaceutically-valuable heterocyclic functionalities including an α-nucleophile (**10g**), pyridines (**10h** and **10j**), thiophene (**10i**) and indole (**10k**).
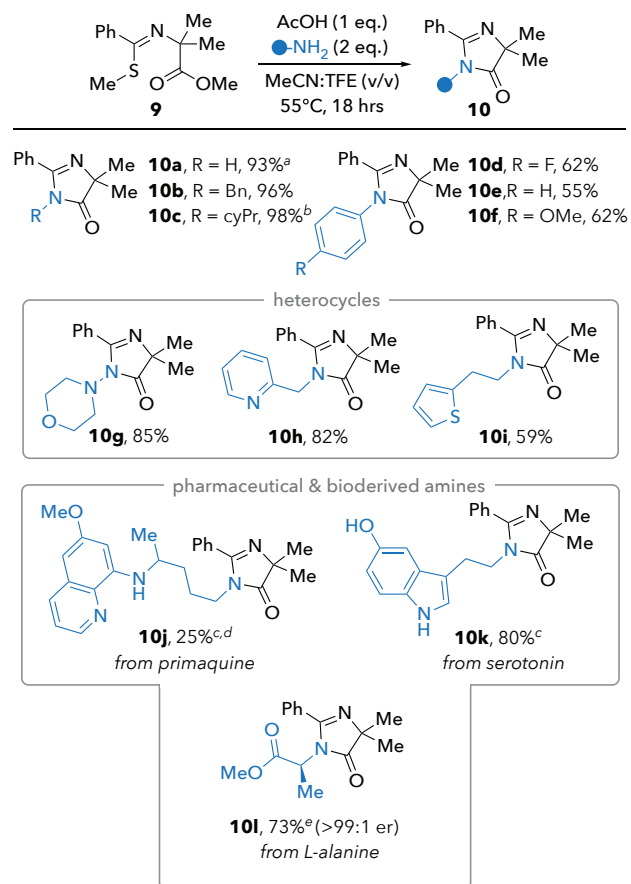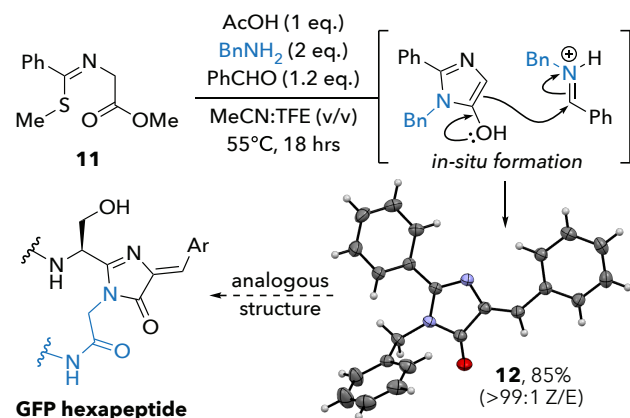

Figure 3. Scope of amine nucleophiles tolerated in imidazolone formation. Isolated yields shown. Concentration of all reactions was 0.25 *M* in **7**. [a]No AcOH added. [b]40 °C. [c]Ammonium salt neutralized with TEA (2 eq.) additive. [d]DMF:TFE (v/v) as solvent. [e]3 eq. of amine used. Ammonium salt neutralized with TEA (3 eq.) additive.

Pharmaceutical and bioderived amines (**10j-10l**) with multiple functional groups were also well-tolerated. Diminished yield for primaquine was attributed to the poor solublity observed for primaquine bisphosphate in organic solvents. Strong acid ammonium salts (i.e. HCl, H_3PO_4) were neutralized *in-situ* via addition of stoichiometric amount of triethylamine (TEA). We found that the strong acid salts alone were not able to catalyze the formation of imidazolones without additon of a weak acid. Amino acid nucleophiles were compatible with this chemistry and the reaction con-

ditions did not racemize the stereogenic center of **10l** despite the use of heat and acid (see Supplementary Information). We found that sterically-hindered amines such as tert-butyl amine and adamantyl amine only produced trace imidazolone products which were not isolated (See Supplementary Information).

Originally reported as a trapping product, the benzylidene imidazolone adduct is recognized as the primary fluorophore in GFP.[31,42] We therefore envisioned that *in-situ* imidazolone cylization of **11**, a glycine methyl ester thioimidate derivative, would enable us to form the benzylidene structure through an acid-catalyzed aldol reaction. A one-pot tandem cyclization-condensation with benzaldehyde afforded the benzylidene product **12** in high yield with excellent selectivity (Scheme 1).

Scheme 1. One-pot imidazolone cyclization-aldol condensation to access benzylidene imidazolones which mimic GFP hexapeptide.

Stereoretentive Conditions for α-Chiral Imidazolones. With an understanding of the scope of amine nucleophiles, we sought to explore the tolerance of our reaction on peptidic substrates. Notably, methods to generate chiral-enriched variants of imidazolones are largely underdeveloped.[45] While our initial attempt to synthesize unsubstituted imidazolone **8a** (Figure 4) provided good yield, a subsequent crystal structure displayed a non-centrosymmetric space group (see Supplementary Information), indicative of a mixture of both *R*- and *S*- imidazolones in the crystal (Figure 4). The formation of thioimidates and amidines on peptidic substrates has been well-studied and the conditions employed do not racemize the α-position of peptides.[30,34] Previously, Drabina and co-workers had reported trifluoroacetic acid (TFA) was capable of racemizing Boc-*L*-proline-imidazolone during the course of Boc-deprotection.[47] Thus, we hypothesized that the reaction conditions led to the racemization of the α-stereochemistry after formation of the imidazolone, presumably through protonation of the imidazolone nitrogen, leading to an increase acidity of the α-CH. We therefore sought conditions to minimize racemization of the final product.

To identify if the pK_a of the acid used for the transformation impacted the racemization, we explored pyridinium *p*-toluenesulfonate (PPTS, pK_a = 5.2) and hexafluoroiso-propanol (HFIP, pK_a = 8.3) compared to acetic acid (AcOH, pK_a = 4.8). Intuitively, weaker acids such as PPTS and HFIP should protonate the imidazolone to a lesser extent and reduce racemization. Unfortunately, protonation of the thioimidate is also necessary for initial formation of the amidine ahead of cyclization (Figure 1C). Accordingly, weak acids like HFIP did not furnish imidazolone in sufficient yield

Table 2. Stereoretention of the α-position of imidazolones.

entry	eq. amine	acid	eq. acid	temp. (°C)	isolated yield (%)	er
1	1	AcOH	1	70	75	50:50
2	3	AcOH	1	50	89	77:23
3	3	PPTS	1	50	83	83:17
4	3	HFIP	1	50	34	87.5:12.5
5	3	AcOH	1	23	71	91:9
6[a]	3	AcOH	1	23	79	85.5:14.5
7	3	AcOH	0.5	23	40	94.5:5.5
8	3	PPTS	1	23	58	97:3
9	5	PPTS	1	23	71	73:27
10	10	PPTS	1	23	78	77:23

[a]Reaction concentration 0.25 *M*.

(Table 2, entry 2-4). We then attempted the reaction at lower temperatures, resulting in an enantiomeric ratio (er) of 90:10 using AcOH, however attempts to further improve the er using AcOH were unsuccessful (Table 2, entry 6 & 7). Thus, a combination of a weaker acid, PPTS, and room temperature reaction conditions provided the best compromise between isolated yield and enantiomeric ratio (Table 2, Entry 8).
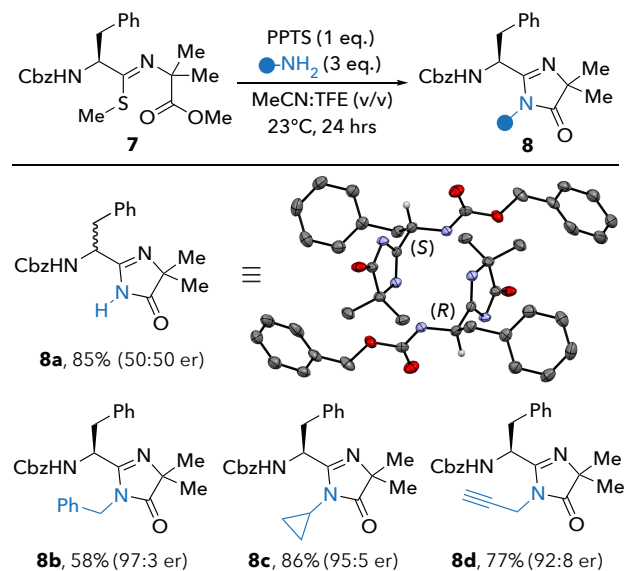

Figure 4. Stereoretentive imidazolone formation with primary amine nucleophiles. Isolated yields shown. Non-stereogenic H-atoms of **8a** were ommitted for clarity.

Unfortunately despite the development of stereoretentive conditions, attempts to generate enantiopure variants of unsubstituted imidazolone **8a** were not successful. With our conditions we prepared the first examples of enantiopure α-chiral imidazolones (**8b**–**8d**). We found that nucleophilic alkyl amines performed best for this chemistry to yield imidazolones in an overall >92:8 er. Our attempts to use aniline derivatives or hindered amines did not yield imidazolone

products due to their poor room temperature reactivity (see Supplementary Information).

Solid-Phase Imidazolone Formation. With an efficient method to form imidazolones established, we sought to incorporate these heterocycles in to peptides through conventional solid-phase peptide synthesis (SPPS) procedures.

N-Terminal imidazolone formation. We selected Apidaecin Ib (1-7, H-GNNRPVY-NH$_2$) as our model peptide to test imidazolone cyclization using the free –NH$_2$ group on the resin as our amine nucleophile. Apidaecin Ib and synthetic derivatives are currently being evaluated for their potential to treat multi-drug resistant gram-negative pathogens.[48,49] Recently, Moore and co-workers showed that N-terminal guanidinylation of Apidaecin Ib peptide enhanced the anti-microbial activity and proteolytic stability.[50] We hypothesized that imidazolones, a basic heterocyclic motif, might impart similar activity to the guanidinylated form by introducing a site for protonation. Positive charge has also been demonstrated as a useful tool to enhance the accumulation and uptake of anti-bacterial peptides and small molecules.[51–53] However, on-resin installation of imidazolones required new considerations for our reaction conditions. THF was chosen as a co-solvent instead of MeCN because it swells polystyrene-based SPPS resins similarly to DMF and DCM, but provided the highest yields in our solution-phase solvent screening (Entries 4 & 9-11, Table 1).[54] Since the amine nucleophile is immobilized on resin, we utilize an excess of thioimidate, Fmoc-Gly^{SMe}-Aib-OMe (**13**, Scheme 2) for the reaction. We found that the typical concentration employed for amide coupling on resin (0.1 M) was sufficient to effect complete installation of the imidazolones when treated with 0.1 M **13** and 0.1 M AcOH under gentle heating conditions at 55 °C (Scheme 2). Final resin cleavage and purification afforded **14**, the N-terminal imidazolone analogue of Apidaecin Ib (1-7).

Scheme 2. On-resin N-terminal imidazolone formation on Apidaecin Ib (1-7).
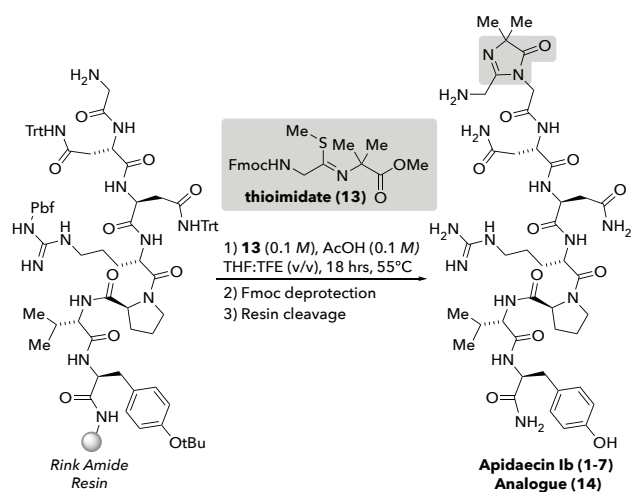
C-Terminal imidazolone formation. To form C-terminal imidazolones we hypothesized that reaction of thioimidate with the amine of an amide resin itself (e.g. Rink amide) could yield N-unsubstituted imidazolones, enabling facile access to C-terminal imidazolone analogues in which the imidazolone is the direct link to the solid support. We selected C-terminal imidazolone **15** which mimics the molecular shape of the proline found at the C-terminus of the thyrotropin-releasing hormone (TRH). The clinically-used

synthetic analogue of TRH, taltirelin, contains an unnatural dihydropyrimidine heterocycle.[55] We envisioned that the imidazolone synthesis platform, which enables rapid diversification of the imidazolone core through SPPS, could be used in the drug discovery of new TRH analogues. After iterative cycles of SPPS coupling and Fmoc-deprotection the imidazolone remained intact and no discernible by-products related to scaffold degradation were isolated upon HPLC purification of peptide **15**.

Scheme 3. Imidazolone cyclization on Rink amide resin linker and subsequent elongation toward C-terminal imidazolone TRH analogue (**15**).
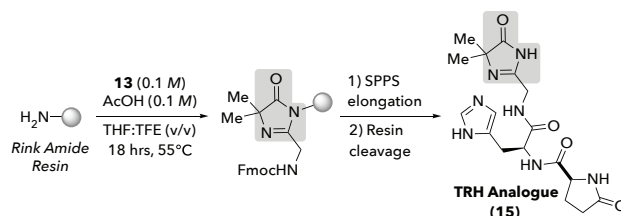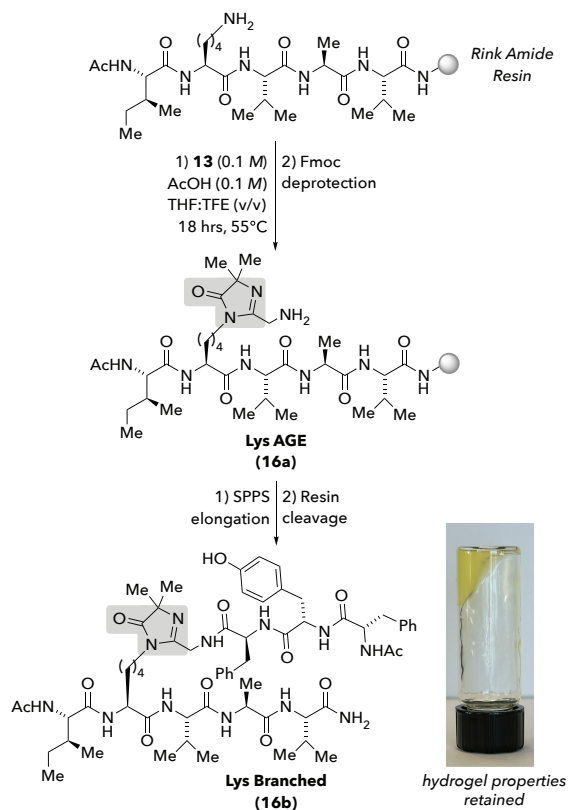

Side-chain functionalization and branched peptides. (4H)-Imidazolones are also a well-known class of biological metabolites known as advanced glycation end products (AGEs), which are non-enzymatic metabolites formed by the reaction of protein side-chains and sugars.[56] AGEs are a product of normal metabolism, however high levels of AGEs have been linked to oxidative stress and inflammation; which are indicative of metabolic disorders, namely diabetes.[57,58] AGEs react with the side-chains of cell surface receptor proteins, and alter their structure and function.[59] For this reason we chose the peptide H-IKVAV-NH$_2$ which is a functional component of the laminin α1 chain, an extracellular matrix (ECM) protein responsible for cell adhesion and appreciated for its use as a hydrogel.[60,61]

By selectively deprotecting the ϵ-NH$_2$ of the Lys residue (using a commercially-available 4-methoxytrityl protecting group), we could install a side-chain imidazolone modification to form **16a** which mimics AGE formation common to Arg and Lys side chains.[62] Branched peptides have been appreciated by the medicinal chemistry community for their enhanced proteolytic stability.[63,64] Accordingly, subsequent elongation from the side-chain imidazolone afforded a branched peptide structure **16b**, another demonstration of the stability of the imidazolone core to SPPS conditions.

(cis)-Amide bond surrogates and peptide cyclization. Finally, with effective conditions to install imidazolones on-resin, we sought to explore their utility as cis-amide bond surrogates. Head-to-tail macrocyclization of peptides is assisted by the introduction of at least one cis-amide bond which helps to pre-organize the ends of cyclic peptides.[6,65] Mahafacyclin B (cyclo-TFFGFFG), a cyclic peptide natural product appreciated for its anti-malarial properties, has been used as a benchmark structure to test head-to-tail cyclization strategies.[66] Native cyclization of the linear Mahafacyclin B yields only 30% of the cyclic peptide and requires extremely long reaction times of up to 3 days.[22,66] Jolliffe and coworkers, however, found that pseudoproline, a cis-amide surrogate derived from condensation with acetone at the Thr site, could pre-organize the peptide for cyclization. Their strategy yielded 50% of the target cyclic peptide in less than 3 hours, underscoring the importance of cis-amide linkages in assisting the cyclization of Mahafacyclin B.[22] Robinson and coworkers used a non-canonical amino acid with alkene side chains and a ring-closing metathesis cata-

Scheme 4. Imidazolone formation on Lys side-chain to yield AGE-related modification (**16a**) and subsequent elongation to the Lys branched peptide (**16b**).
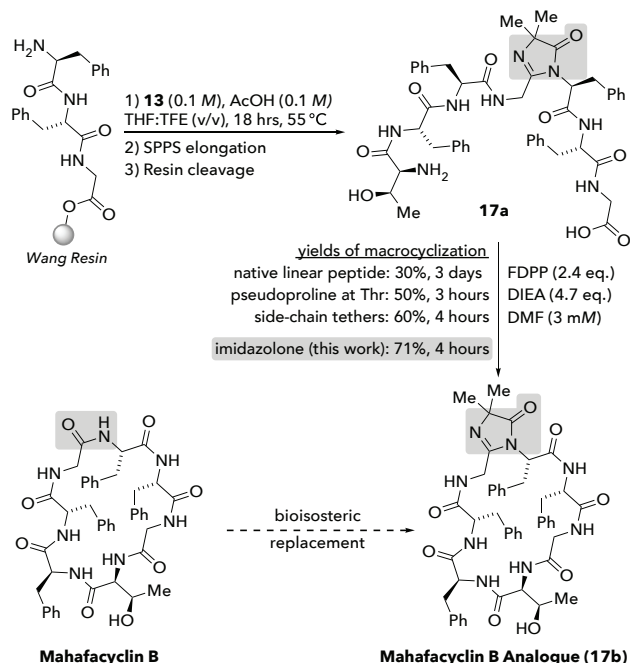

Scheme 5. Imidazolones as *cis*-amide surrogates to pre-organize head-to-tail macrocyclization of Mahafacyclin B analogue with bioisosteric replacement.

lyst to form a tethered linkage to pre-organize the peptide.[67] Their tethered structure yielded 60% of the cyclic peptide in 4 hours which could be later deprotected using ring-opening metathesis to yield a Mahafacyclin B analogue.[67] We opted to use Mahafacyclin B as a benchmark cyclization for our *cis*-amide inducing imidazolone.

To a Gly-loaded Wang resin we synthesized and installed our imidazolone at the central glycine residue. Cleavage from Wang resin afforded the linear peptide precursor (H-TFFG$^{\mathrm{Imi}}$FFG-OH, **17a**) which was allowed to react under the same macrocyclization conditions employed by Jollife and Robinson using a pentafluorophenyl diphenylphosphinate (FDPP) coupling reagent.[22,67] Gratifyingly, the isolated yield of peptide **17b** (after preparatory HPLC purification) was higher than either previously reported macrocyclization of the Mahafacyclin B sequence. Additionally, since imidazolones have recently been employed as bioisosteric replacements for the amide bond, the geometric constraints imparted by imidazolones on bioactive linear peptides may be a useful strategy to retain bioactivity in cyclic form.[33]

Conclusion

(4H)-imidazolones are important heterocycles found in natural products,[40,45] pharmaceuticals,[36–39] agrochemicals,[41] fluorescent probes,[42,43] and biological metabolites.[62] Our method enables access to novel imidazolone scaffolds which can be synthesized rapidly from commercially-available starting materials. We have shown that imidazolone cyclization is tolerated by a wide-scope of primary amine nucleophiles, with excellent functional group tolerance. Previous methods to form imidazolones required high temperatures and strong acid or base to form.[45] The mild con-

ditions employed enable us to access imidazolone products with α-stereochemistry—derived from amino acid starting materials—the first examples of stereochemical retention at this postion.

Additionally, we show that imidazolones can be easily incorporated onto the *N*-terminus or side-chain of an elongating peptide chain during SPPS by reaction with a thioimidate dipeptide. *C*-terminal installation can be achieved by direct reaction with amide resins, which cleave to the free *N*–*H* imidazolone. Imidazolones also tolerated peptide elongation after installation, enabling them to be installed into the center of peptides and on to side-chains. Side-chain imidazolones provided access to branched peptides and AGE-related products with potential for the study of receptors for AGEs (RAGE).[59] As a newly discovered bioisostere of the amide bond,[33] our general method for insertion and functionalization of imidazolone motifs will assist in the discovery of other small-molecule and peptide-based therapeutics.

Finally, the geometric constraints imparted by the imidazolones heteorcycle leads to *cis*-amide surrogates which are especially useful in pre-organizing linear peptides for head-to-tail macrocyclization.[6,65] We show that the imidazolone surrogate performs better than existing methods for the head-to-tail cyclization of the Mahafacyclin B sequence.[22,66,67] We anticipate that this work will assist in the synthesis of currently inaccessible imidazolone natural products,[45] and provide a new conformational tool to peptide chemists when designing cyclic peptides.

Corresponding Author

bvv@iastate.edu

ORCID

Brendan J. Wall: 0000-0002-0784-7752
Emily O'Brien: 0000-0002-6995-9282
Krishna K. Sharma: 0000-0003-4927-745X
Aaron Donovan: 0009-0009-4814-3485
Brett VanVeller: 0000-0002-3792-0308

Supporting Information Available

ASSOCIATED CONTENT:
- Data Availability Statement

The data underlying this study are available in the published article and its Supporting Information.
- Supporting Information Statement

The Supporting Information is available free of charge on the ACS Publications website.

CCDC 2312772 and CCDC 2312773 contains the supplementary crystallographic data for this paper.

References

(1) Wang, M.; Rakesh, K.; Leng, J.; Fang, W.-Y.; Ravindar, L.; Channe Gowda, D.; Qin, H.-L. Amino acids/peptides conjugated heterocycles: A tool for the recent development of novel therapeutic agents. *Bioorg. Chem.* **2018**, *76*, 113–129.

(2) Soor, H. S.; Appavoo, S. D.; Yudin, A. K. Heterocycles: Versatile control elements in bioactive macrocycles. *Bioorg. Med. Chem.* **2018**, *26*, 2774–2779, Peptide Therapeutics.

(3) Nielsen, D. S.; Shepherd, N. E.; Xu, W.; Lucke, A. J.; Stoermer, M. J.; Fairlie, D. P. Orally Absorbed Cyclic Peptides. *Chem. Rev.* **2017**, *117*, 8094–8128, PMID: 28541045.

(4) Kaldas, S. J.; Yudin, A. K. Achieving Skeletal Diversity in Peptide Macrocycles through The Use of Heterocyclic Grafts. *Chem. Eur. J.* **2018**, *24*, 7074–7082.

(5) Smolyar, I. V.; Yudin, A. K.; Nenajdenko, V. G. Heteroaryl Rings in Peptide Macrocycles. *Chem. Rev.* **2019**, *119*, 10032–10240, PMID: 31157971.

(6) Hamdan, F.; Tahoori, F.; Balalaie, S. Synthesis of novel cyclopeptides containing heterocyclic skeletons. *RSC Adv.* **2018**, *8*, 33893–33926.

(7) Walsh, C. T.; Malcolmson, S. J.; Young, T. S. Three Ring Post-translational Circuses: Insertion of Oxazoles, Thiazoles, and Pyridines into Protein-Derived Frameworks. *ACS Chem. Bio.* **2012**, *7*, 429–442, PMID: 22206579.

(8) Dong, S.-H.; Liu, A.; Mahanta, N.; Mitchell, D. A.; Nair, S. K. Mechanistic Basis for Ribosomal Peptide Backbone Modifications. *ACS Cent. Sci.* **2019**, *5*, 842–851, PMID: 31139720.

(9) Walsh, C. T.; Nolan, E. M. Morphing peptide backbones into heterocycles. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 5655–5656.

(10) Biron, E.; Chatterjee, J.; Kessler, H. Solid-Phase Synthesis of 1,3-Azole-Based Peptides and Peptidomimetics. *Org. Lett.* **2006**, *8*, 2417–2420, PMID: 16706540.

(11) De Luca, L.; Falorni, M.; Giacomelli, G.; Porcheddu, A. New pyrazole containing bicarboxylic -amino acids: mimics of the cis amide bond. *Tetrahedron Lett.* **1999**, *40*, 8701–8704.

(12) Abdildinova, A.; Yang, S.-J.; Gong, Y.-D. Solid-phase parallel synthesis of 1,3,4-oxadiazole based peptidomimetic library as a potential modulator of protein-protein interactions. *Tetrahedron* **2018**, *74*, 684–691.

(13) Rinnová, M.; Nefzi, A.; Houghten, R. A. An efficient approach for solid-phase synthesis of peptidomimetics based on 4-imidazolidinones. *Tetrahedron Lett.* **2002**, *43*, 2343–2346.

(14) Jones, R. C.; Ward, G. J. Amide bond isosteres: Imidazolines in pseudopeptide chemistry. *Tetrahedron Lett.* **1988**, *29*, 3853–3856.

(15) Boeglin, D.; Cantel, S.; Heitz, A.; Martinez, J.; Fehrentz, J.-A. Solution and Solid-Supported Synthesis of 3,4,5-Trisubstituted 1,2,4-Triazole-Based Peptidomimetics. *Org. Lett.* **2003**, *5*, 4465–4468, PMID: 14602026.

(16) Horne, W. S.; Stout, C. D.; Ghadiri, M. R. A Heterocyclic Peptide Nanotube. *J. Am. Chem. Soc.* **2003**, *125*, 9372–9376, PMID: 12889966.

(17) Angelo, N. G.; Arora, P. S. Solution- and Solid-Phase Synthesis of Triazole Oligomers That Display Protein-Like Functionality. *J. Org. Chem.* **2007**, *72*, 7963–7967, PMID: 17880241.

(18) Gavrilyuk, J. I.; Evindar, G.; Batey, R. A. Peptide—Heterocycle Hybrid Molecules: Solid-Phase Synthesis of a 400-Member Library of N-Terminal 2-Iminohydantoin Peptides. *J. Comb. Chem.* **2006**, *8*, 237–246, PMID: 16529519.

(19) Caramiello, A. M.; Bellucci, M. C.; Marti-Rujas, J.; Sacchetti, A.; Volonterio, A. Turn-Mimic Hydantoin-Based Loops Constructed by a Sequential Multicomponent Reaction. *J. Org. Chem.* **2023**, *88*, 15790–15804, PMID: 37932902.

(20) Pokorski, J. K.; Miller Jenkins, L. M.; Feng, H.; Durell, S. R.; Bai, Y.; Appella, D. H. Introduction of a Triazole Amino Acid into a Peptoid Oligomer Induces Turn Formation in Aqueous Solution. *Org. Lett.* **2007**, *9*, 2381–2383, PMID: 17506576.

(21) Rečnik, L.-M.; Kandioller, W.; Mindt, T. L. 1,4-Disubstituted 1,2,3-Triazoles as Amide Bond Surrogates for the Stabilisation of Linear Peptides with Biological Activity. *Molecules* **2020**, *25*.

(22) Sayyadi, N.; Skropeta, D.; Jolliffe, K. A. N,O-Isopropylidenated Threonines as Tools for Peptide Cyclization: Application to the Synthesis of Mahafacyclin B. *Org. Lett.* **2005**, *7*, 5497–5499, PMID: 16288540.

(23) Zhang, J.; Germann, M. W. Characterization of secondary amide peptide bond isomerization: Thermodynamics and kinetics from 2D NMR spectroscopy. *Biopolymers* **2011**, *95*, 755–762.

(24) Hitotsuyanagi, Y.; Motegi, S.; Fukaya, H.; Takeya, K. A cis Amide Bond Surrogate Incorporating 1,2,4-Triazole. *J. Org. Chem.* **2002**, *67*, 3266–3271, PMID: 12003534.

(25) Tam, A.; Arnold, U.; Soellner, M. B.; Raines, R. T. Protein Prosthesis: 1,5-Disubstituted[1,2,3]triazoles as cis-Peptide Bond Surrogates. *J. Am. Chem. Soc.* **2007**, *129*, 12670–12671, PMID: 17914828.

(26) Abdildinova, A.; Gong, Y.-D. Traceless solid-phase synthesis and -turn propensity of 1,3-thiazole-based peptidomimetics. *RSC Adv.* **2021**, *11*, 1050–1056.

(27) Zabrocki, J.; Smith, G. D.; Dunbar, J. B.; Iijima, H.; Marshall, G. R. Conformational mimicry. 1. 1,5-Disubstituted tetrazole ring as a surrogate for the cis amide bond. *J. Am. Chem. Soc.* **1988**, *110*, 5875–5880.

(28) Beusen, D. D.; Zabrocki, J.; Slomczynska, U.; Head, R. D.; Kao, J. L.-F.; Marshall, G. R. Conformational mimicry: Synthesis and solution conformation of a cyclic somatostatin hexapeptide containing a tetrazole cis amide bond surrogate. *Biopolymers* **1995**, *36*, 181–200.

(29) May, B. C.; Abell, A. D. The synthesis and crystal structure of alpha-keto tetrazole-based dipeptide mimics. *Tetrahedron Lett.* **2001**, *42*, 5641–5644.

(30) O'Brien, E. A.; Sharma, K. K.; Byerly-Duke, J.; Camacho, L. A. I.; VanVeller, B. A General Strategy to Install Amidine Functional Groups Along the Peptide Backbone. *J. Am. Chem. Soc.* **2022**, *144*, 22397–22402, PMID: 36469014.

(31) Yamada, T.; Suegane, K.; Kuwata, S.; Watanabe, H. Studies of Unusual Amino Acids and Their Peptides. VII. The Syntheses and the Reactions of Iminopeptides. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 1088–1093.

(32) Yu, Y.; Ostresh, J. M.; Houghten, R. A. Solid-Phase Synthesis of 2,3,5-Trisubstituted 4H-Imidazolones. *J. Comb. Chem.* **2001**, *3*, 521–523, PMID: 11703145.

(33) Jackson, J. J. et al. Imidazolone as an Amide Bioisostere in the Development of -1,3-N-Acetylglucosaminyltransferase 2 (B3GNT2) Inhibitors. *J. Med. Chem.* **0**, *0*, null, PMID: 37988652.

(34) Camacho, L. A. I.; Lampkin, B. J.; VanVeller, B. A Bottom-Up Approach To Preserve Thioamide Residue Stereochemistry during Fmoc Solid-Phase Peptide Synthesis. *Org. Lett.* **2019**, *21*, 7015–7018, PMID: 31403302.

(35) Camacho, L. A. I.; Nguyen, Y. H.; Turner, J.; VanVeller, B. Deprotection Strategies for Thioimidates during Fmoc Solid-Phase Peptide Synthesis: A Safe Route to Thioamides. *J. Org. Chem.*

2019, *84*, 15309–15314, PMID: 31725287.

(36) Hernandez-Olmos, V.; Heering, J.; Bischoff-Kont, I.; Kaps, A.; Rajkumar, R.; Liu, T.; Fürst, R.; Steinhilber, D.; Proschak, E. Discovery of Irbesartan Derivatives as BLT2 Agonists by Virtual Screening. *ACS Med. Chem. Lett.* **2021**, *12*, 1261–1266.

(37) Mallinger, A. et al. Discovery of Potent, Selective, and Orally Bioavailable Small-Molecule Modulators of the Mediator Complex-Associated Kinases CDK8 and CDK19. *J. Med. Chem.* **2016**, *59*, 1078–1101, PMID: 26796641.

(38) Metwally, K. A.; Dukat, M.; Egan, C. T.; Smith, C.; DuPre, A.; Gauthier, C. B.; Herrick-Davis, K.; Teitler, M.; Glennon, R. A. Spiperone: Influence of Spiro Ring Substituents on 5-HT2A Serotonin Receptor Binding. *J. Med. Chem.* **1998**, *41*, 5084–5093, PMID: 9836624.

(39) Jones, L. H.; Dupont, T.; Mowbray, C. E.; Newman, S. D. A Concise and Selective Synthesis of Novel 5-Aryloxyimidazole NNRTIs. *Org. Lett.* **2006**, *8*, 1725–1727, PMID: 16597151.

(40) Mancini, I.; Guella, G.; Pietra, F.; Debitus, C.; Waikedre, J. From Inactive Nortopsentin D, a Novel Bis(indole) Alkaloid Isolated from the Axinellid Sponge Dragmacidon sp. from Deep Waters South of New Caledonia, to a Strongly Cytotoxic Derivative. *Helv. Chim. Acta* **1996**, *79*, 2075–2082.

(41) Tan, S.; Evans, R. R.; Dahmer, M. L.; Singh, B. K.; Shaner, D. L. Imidazolinone-tolerant crops: history, current status and future. *Pest Manag. Sci* **2005**, *61*, 246–257.

(42) Cody, C. W.; Prasher, D. C.; Westler, W. M.; Prendergast, F. G.; Ward, W. W. Chemical structure of the hexapeptide chromophore of the Aequorea green-fluorescent protein. *Biochemistry* **1993**, *32*, 1212–1218, PMID: 8448132.

(43) Chatterjee, S.; Ahire, K.; Karuso, P. Room-Temperature Dual Fluorescence of a Locked Green Fluorescent Protein Chromophore Analogue. *J. Am. Chem. Soc.* **2020**, *142*, 738–749, PMID: 31846319.

(44) Petkova, I.; Dobrikov, G.; Banerji, N.; Duvanel, G.; Perez, R.; Dimitrov, V.; Nikolov, P.; Vauthey, E. Tuning the Excited-State Dynamics of GFP-Inspired Imidazolone Derivatives. *J. Phys. Chem. A* **2010**, *114*, 10–20, PMID: 20000560.

(45) Keel, K. L.; Tepe, J. J. The preparation of (4H)-imidazol-4-ones and their application in the total synthesis of natural products. *Org. Chem. Front.* **2020**, *7*, 3284–3311.

(46) Murray, P. M.; Bellany, F.; Benhamou, L.; Bučar, D.-K.; Tabor, A. B.; Sheppard, T. D. The application of design of experiments (DoE) reaction optimisation and solvent selection in the development of new synthetic chemistry. *Org. Biomol. Chem.* **2016**, *14*, 2373–2384.

(47) Panov, I.; Drabina, P.; Hanusek, J.; Sedlák, M. The synthesis and characterisation of N-(1-carbamoyl-1,1-dialkyl-methyl)-(S)-prolinamides and related pyrrolidin-2-yl-4,5-dihydro-1H-imidazol-5-ones as potential enantioselective organocatalysts. *Tetrahedron: Asymm.* **2011**, *22*, 215–221.

(48) Czihal, P. et al. Api88 Is a Novel Antibacterial Designer Peptide To Treat Systemic Infections with Multidrug-Resistant Gram-Negative Pathogens. *ACS Chem. Bio.* **2012**, *7*, 1281–1291, PMID: 22594381.

(49) Bluhm, M. E.; Knappe, D.; Hoffmann, R. Structure-activity relationship study using peptide arrays to optimize Api137 for an increased antimicrobial activity against Pseudomonas aeruginosa. *Eur. J. Med. Chem.* **2015**, *103*, 574–582.

(50) Skowron, K. J.; Baliga, C.; Johnson, T.; Kremiller, K. M.; Castroverde, A.; Dean, T. T.; Allen, A. C.; Lopez-Hernandez, A. M.; Aleksandrova, E. V.; Klepacki, D.; Mankin, A. S.; Polikanov, Y. S.; Moore, T. W. Structure–Activity Relationships of the Antimicrobial Peptide Natural Product Apidaecin. *J. Med. Chem.* **2023**, *66*, 11831–11842, PMID: 37603874.

(51) Richter, M. F.; Drown, B. S.; Riley, A. P.; Garcia, A.; Shirai, T.; Svec, R. L.; Hergenrother, P. J. Predictive compound accumulation rules yield a broad-spectrum antibiotic. *Nature* **2017**, *545*, 299–304.

(52) Haloi, N.; Vasan, A. K.; Geddes, E. J.; Prasanna, A.; Wen, P.-C.; Metcalf, W. W.; Hergenrother, P. J.; Tajkhorshid, E. Rationalizing the generation of broad spectrum antibiotics with the addition of a positive charge. *Chem. Sci.* **2021**, *12*, 15028–15044.

(53) López Cascales, J. J.; Zenak, S.; García de la Torre, J.; Lezama, O. G.; Garro, A.; Enriz, R. D. Small Cationic Peptides: Influence of Charge on Their Antimicrobial Activity. *ACS Omega* **2018**, *3*, 5390–5398, PMID: 30221230.

(54) Magtaan, J. K.; Devocelle, M.; Kelleher, F. Assessing the correlation of microscopy-based and volumetry-based measurements for resin swelling in a range of potential greener solvents for SPPS. *J. Pep. Sci.* **2020**, *26*, e3250, e3250 PSC-20-0006.R1.

(55) Urayama, A.; Yamada, S.; Kimura, R.; Zhang, J.; Watanabe, Y. Neuroprotective effect and brain receptor binding of taltirelin, a novel thyrotropin-releasing hormone (TRH) analogue, in transient forebrain ischemia of C57BL/6J mice. *Life Sci.* **2002**, *72*, 601–607, The Thrid International Symposium on the study on Brain Function.

(56) Uribarri, J.; Woodruff, S.; Goodman, S.; Cai, W.; Chen, X.; Pyzik, R.; Yong, A.; Striker, G. E.; Vlassara, H. Advanced Glycation End Products in Foods and a Practical Guide to Their Reduction in the Diet. *J. Am. Diet Assoc.* **2010**, *110*, 911–916.e12.

(57) Reddy, V. P.; Aryal, P.; Darkwah, E. K. Advanced Glycation End Products in Health and Disease. *Microorganisms* **2022**, *10*.

(58) Singh Varun Parkash, S. N. J. A. S., Bali Anjana Advanced Glycation End Products and Diabetic Complications. *Korean J. Physiol. Pharmacol.* **2014**, *18*, 1–14.

(59) Sergi, D.; Boulestin, H.; Campbell, F. M.; Williams, L. M. The Role of Dietary Advanced Glycation End Products in Metabolic Dysfunction. *Mol. Nutr. Food Res.* **2021**, *65*, 1900934.

(60) Nakamura, M.; Yamaguchi, K.; Mie, M.; Nakamura, M.; Akita, K.; Kobatake, E. Promotion of Angiogenesis by an Artificial Extracellular Matrix Protein Containing the Laminin-1-Derived IKVAV Sequence. *Bioconjugate Chem.* **2009**, *20*, 1759–1764, PMID: 19689157.

(61) Yin, Y.; Wang, W.; Shao, Q.; Li, B.; Yu, D.; Zhou, X.; Parajuli, J.; Xu, H.; Qiu, T.; Yetisen, A. K.; Jiang, N. Pentapeptide IKVAV-engineered hydrogels for neural stem cell attachment. *Biomater. Sci.* **2021**, *9*, 2887–2892.

(62) Uribarri, J.; Woodruff, S.; Goodman, S.; Cai, W.; Chen, X.; Pyzik, R.; Yong, A.; Striker, G. E.; Vlassara, H. Advanced Glycation End Products in Foods and a Practical Guide to Their Reduction in the Diet. *J. Am. Diet. Assoc.* **2010**, *110*, 911–916.e12.

(63) Falciani, C.; Lozzi, L.; Pini, A.; Corti, F.; Fabbrini, M.; Bernini, A.; Lelli, B.; Niccolai, N.; Bracci, L. Molecular Basis of Branched Peptides Resistance to Enzyme Proteolysis. *Chem. Biol. Drug Des.* **2007**, *69*, 216–221.

(64) Brunetti, J.; Falciani, C.; Bracci, L.; Pini, A. Branched peptides as bioactive molecules for drug design. *Pep. Sci.* **2018**, *110*, e24089.

(65) White, C. J.; Yudin, A. K. Contemporary strategies for peptide macrocyclization. *Nat. Chem.* **2011**, *3*, 509–524.

(66) Baraguey, C.; Blond, A.; Cavelier, F.; Pousset, J.-L.; Bodo, B.; Auvin-Guette, C. Isolation, structure and synthesis of mahafacyclin B, a cyclic heptapeptide from the latex of Jatropha mahafalensis. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2098–2103.

(67) Illesinghe, J.; Guo, C. X.; Garland, R.; Ahmed, A.; van Lierop, B.; Elaridi, J.; Jackson, W. R.; Robinson, A. J. Metathesis assisted synthesis of cyclic peptides. *Chem. Commun.* **2009**, 295–297.