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Cyclols Revisited: Facile Synthesis of Medium-Sized Cyclic Peptides

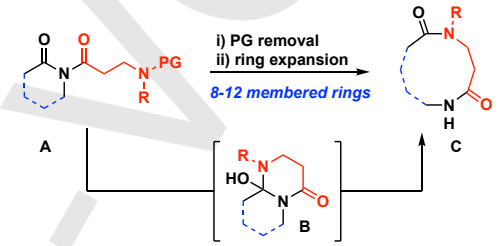
Rodrigo Mendoza-Sanchez‡[a], Victoria B. Corless‡[a], Q. Nhu N. Nguyen[b], Milan Bergeron-Brlek[a], John Frost[a], Shinya Adachi[a], Dean J. Tantillo*[b], Andrei K. Yudin*[a]

Abstract: Medium-sized rings, particularly the corresponding cyclic peptides, are challenging synthetic targets. In the present study, we report an approach to medium-sized cyclic peptides through targeted formation and collapse of cyclol intermediates. This methodology operates on β-amino imides derived from 2,5-diketopiperazines and offers a straightforward transition from frequently examined scaffolds in drug discovery to a rarely visited class of medium-sized rings.

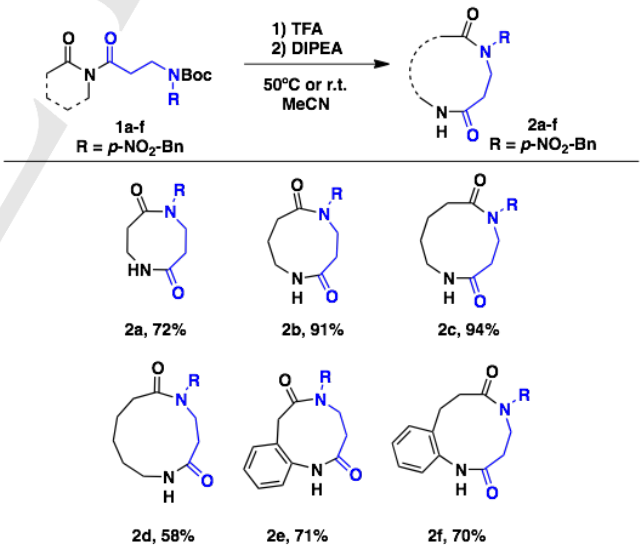
In 1936, Dorothy Wrinch proposed the first structural model for protein folding.[1–3] Repeating polypeptide motifs could hypothetically be folded into rigid protein scaffolds through what was termed a cyclol. The proposal, while intriguing, was eventually discredited by Linus Pauling, who pointed out the implausible thermodynamics of this idea.[4] Nonetheless, examples of substantially smaller cyclol-containing molecules were eventually uncovered.[5–10] Despite their thermodynamic instability, cyclols have been implicated in key biological processes such as chromophore maturation in green fluorescent protein (GFP).[11][12] In this paper, we consider factors that influence cyclol stability in settings that are likely to display transannular interactions between amides. In combination with computational analysis, our study highlights "cyclol management" as an enabling avenue to access synthetically challenging medium-sized rings.

Medium-sized rings are prevalent in many natural products and possess a range of biological activities.[13–18] They represent the “middle ground” between macrocycles and small molecules. However, despite their abundance and diverse functions, medium-sized rings are not commonly examined in drug discovery programs or found in the structures of pharmaceutical agents.[19] This absence can be attributed to unique challenges associated with the cyclization of medium-sized linear precursors which include unfavorable transannular interactions and entropic factors.[20–25] Ring expansion methodologies have been shown to circumvent some of the issues inherent in traditional cyclizations and have led to otherwise challenging substrates.[26–32] An important subclass of medium-sized rings are cyclic peptides, the synthesis of which is particularly difficult due to the preference of the amide linkage to exist in its *trans*-form.[33] As such, there is a need to develop straightforward and robust methodologies to access medium-sized ring cyclic peptides.

Herein, we report an efficient protocol for the synthesis of challenging medium-sized cyclic peptides from β-amino imides.[34–39] The enabling feature of this study is control over the collapse of the cyclol intermediate **B** through which a ring expansion pathway takes place (Scheme 1).[40–47]



Scheme 1. Ring expansion via cyclol intermediate to access synthetically challenging medium-sized rings and cyclic tripeptides.



Scheme 2. Synthesis of medium-sized rings using from amino imides derived from lactams.

As proof of concept, we began by assessing the likelihood of forming the desired medium-sized rings using Boc-protected β-amino imide **1c** as a model substrate. While numerous methodologies for the synthesis of acyclic imides have been reported in the literature, we sought a protocol that was compatible with our Boc-protected β-amino acid fragments (Scheme 2). We therefore opted to use the direct acylation of amides using pentafluorophenol active esters as coupling partners[48–50] and obtained β-amino imides **1a-f** in moderate to good yields (See *Supporting Information*).

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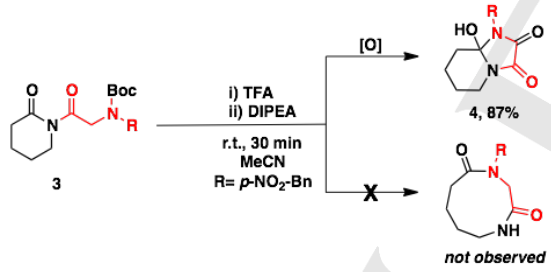
Computational analysis (See *Supporting Information*) of the ring expansion of **1c** predicted that the resulting ring expanded form **2c** would be favored through the collapse of the cyclol intermediate as outlined in Scheme 1B. Removal of the Boc protecting group was carried out by stirring **1c** in TFA for one hour. Neutralization of the isolated TFA salt was achieved using 5.0 equivalents of DIPEA and yielded the desired 10-membered ring **2c** after 28 h stirring at room temperature. Upon heating to 50 ºC, we observed complete conversion to **2c** in under 4h with no adverse effect on isolated yield.

The generality of the ring expansion was investigated using several β-amino imides derived from simple lactams. Gratifyingly, an array of 8–12 membered rings were obtained in moderate to good isolated yields (Scheme 2). Compounds **2a-d** were obtained using our optimized conditions. However, for compounds **2e-f**, allowing the reactions to proceed at room temperature resulted in clean and complete conversion to the desired medium-sized rings in just under 2h. Neither the ring size nor the aromatic substituents adversely affected the reaction outcome, which showed promise in extending this methodology to more complex systems. Having secured a reliable route to medium-sized rings from simple lactams, we investigated the possibility of using an α-amino imide fragment.[51–53] Using glycine-derived α-amino imide **3** as our model substrate we calculated the probability of forming a 9-membered ring however, computational analysis predicted that the cyclol form of this system would be thermodynamically favored over the ring expanded product (see *Supporting Information*). Synthesis of the α-amino imide **3** and removal of the Boc-protected amine proceeded smoothly, but after subjecting the unprotected amine to the standard ring expansion conditions we isolated the oxidized cyclol **4** as a single product (Scheme 3).



Scheme 3. Formation of oxidized cyclol **4** from glycine-derived amino imide **3**.

Next, we applied our ring expansion methodology to the synthesis of cyclic tripeptides from 2,5-diketopiperazines, which are common by-products of peptide coupling reactions.[54] Computational analysis using the unprotected imide of **6a** indicated favorable formation of the 10-membered cyclic tripeptide (Figure 1). We therefore synthesized imides **6a-j** derived from functionalized 2,5-diketopiperazines (see *Supporting Information*) and applied our optimized ring expansion conditions. The reactions proceeded with clean conversion to the desired cyclic tripeptides **7a-j** (Scheme 4).

Initial experiments involved amino imides **6a-c**, which contain various combinations of D- and L-proline and alanine. Gratifyingly, the stereochemistry of the amino acids did not hinder the outcome of the ring expansions. A number of phenylalanine containing amino imides were then synthesized. Starting from *N*-methyl phenylalanine, the ring expanded product **7d** was obtained in good yield. *N*-allyl-phenylalanine was used to generate **6e-h**, which produced the desired ring expanded products **7e-h** in comparable yields to the proline derived amino imides with the exception of **7g**, which was obtained in moderate yield. Reaction times ranged from 12 to 36 hours.

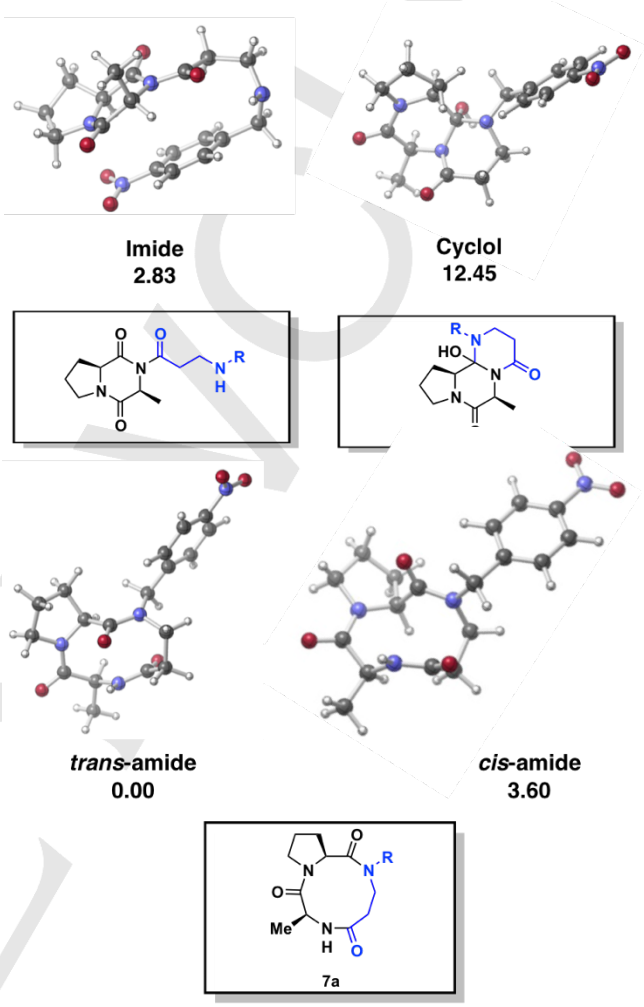


Figure 1. R = *p*-NO_2-Bn. Lowest energy conformers for the unprotected β-amino imide of **6a**, the corresponding cyclol and 10-membered ring expansion product **7a**. Free energies (gas phase) shown in kcal mol^{-1} were computed using density functional theory at M06-2X/6-31G(d) (See *Supporting Information*). All energies are normalized relative to *trans*-amide **7a**.

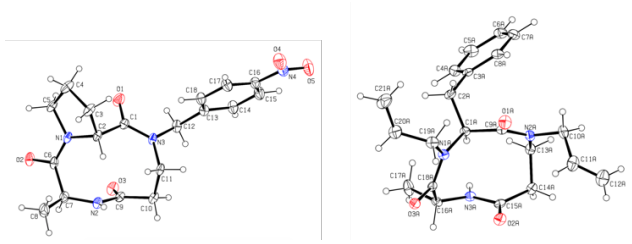
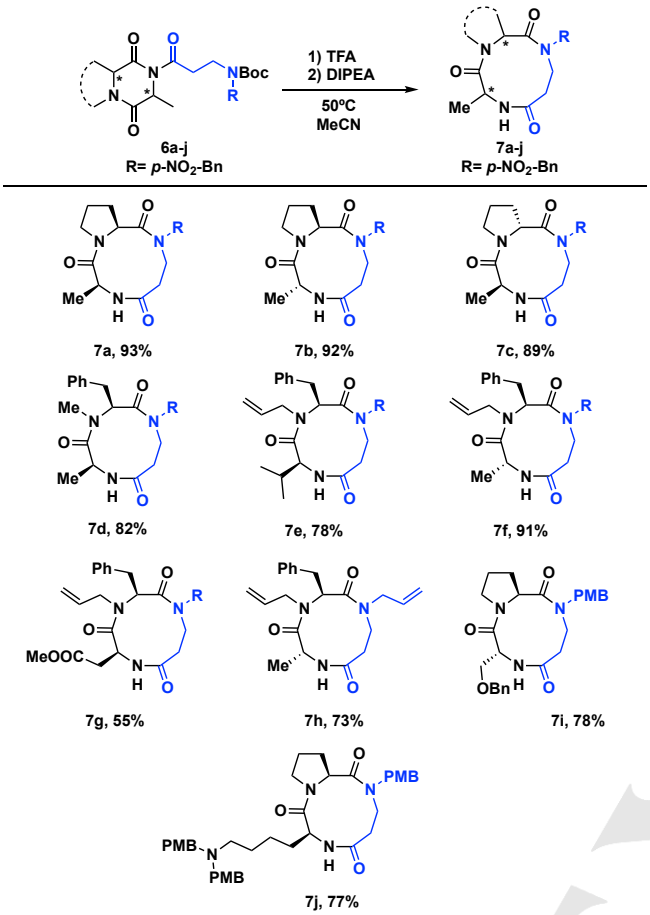


Figure 2. Solid state structure of **7a** (left) and **7h** (right) as determined by single-crystal X-ray diffraction. **7a** was recrystallized from acetonitrile in water and **7h** from deuterated methanol.



Scheme 4: Synthesis of cyclic tripeptides through the ring expansion of 2,5-diketopiperazines.

The active ester containing the para-methoxybenzyl (PMB) protecting group (see *Supporting Information*) was used to synthesize cyclic tripeptides **7i-j**. These cyclic tripeptides contain the polar amino acid side chains, serine and lysine, and were obtained in comparable yields. X-ray crystal structures were obtained for compounds **7a** and **7h** (Figure 2). In both molecules, the newly formed amide bond displays a *trans* conformation, alleviating allylic strain present in an all *cis*-amide linkage.

In summary, we have developed a robust methodology for the synthesis of challenging medium-sized rings through the collapse of cyclol intermediates derived from the intramolecular cyclization of β-amino imides. The results of our study suggest that cyclols can act as useful pivot points in the construction of medium-sized rings, but only if the conversion to the open form is exergonic by at least 6-12 kcal/mol (see *Supporting Information* for in-depth analysis), as determined through computational analysis. As observed, more readily reversible systems would promote undesired decomposition pathways of these fragile intermediates. Our findings also encourage consideration of non-peptidic systems, where "cyclol management" could facilitate rational design of new transformations. This methodology was successfully applied to the synthesis of challenging cyclic tripeptides and has addressed some long-standing challenges in this area such as high entopic barriers, unwanted oligomerization, poor yields, and limited substrate accessibility. By avoiding the well-known pitfalls of cyclization reactions, our study opens doors for the evaluation of medium sized cyclic peptides in library production for the purposes of drug discovery.

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Keywords: cyclol • medium-sized ring • cyclic peptide • ring expansion • β-amino imide

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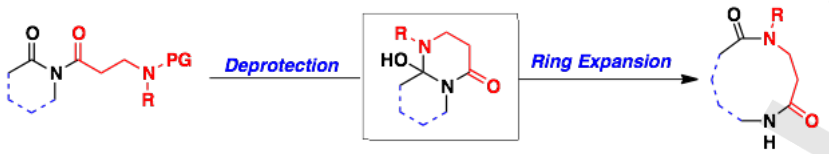

- *Access to amide rich 8-12 membered medium-sized rings and cyclic tripeptides*
- *Mild and convergent synthesis*
- *14 examples*

Rodrigo Mendoza-Sanchez‡, Victoria B. Corless‡, Q. Nhu Nguyen, Milan Bergeron-Brlek, John Frost, Shinya Adachi, Dean J. Tantillo, Andrei K. Yudin**

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