Peptide Macrocycles Hot Paper

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CyClick Chemistry for the Synthesis of Cyclic Peptides

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Abstract: *Here, we report a novel "CyClick" strategy for the macrocyclization of peptides that works in an exclusively intramolecular fashion thereby precluding the formation of dimers and oligomers via intermolecular reactions. The CyClick chemistry is highly chemoselective for the N-terminus of the peptide with a C-terminal aldehyde. In this protocol, the peptide conformation internally directs activation of the backbone amide bond and thereby facilitates formation of a stable 4-imidazolidinone-fused cyclic peptide with high diastereoselectivity (>99%). This method is tolerant to a variety of peptide aldehydes and has been applied for the synthesis of 12- to 23-membered rings with varying amino acid compositions in one pot under mild reaction conditions. The reaction generated peptide macrocycles featuring a 4-imidazolidinone in their scaffolds, which acts as an endocyclic control element that promotes intramolecular hydrogen bonding and leads to macrocycles with conformationally rigid turn structures.*

Introduction

Cyclic peptides have recently received considerable attention in the pharmaceutical industry because of their high stability, cell permeability, and enhanced potency as compared to their linear counterparts.[1–5] Currently, more than 40 cyclic peptides are used as pharmaceuticals, and most of them are obtained from nature.[6–8] The major driving force for the growing interest in cyclic peptides is due to their ability to interrupt protein–protein interactions (PPIs) in a highly specific manner.[9–11] Despite their importance, laboratory synthesis of cyclic peptides can be challenging. Among the most challenging cyclizations are those attempted on linear peptides containing less than seven amino acid residues.[12–16] The chain/ring conformational equilibrium[17] is the central obstacle in the synthesis of cyclic peptides from acyclic precursors. This process is characterized by an unfavorable entropy change when moving from a linear precursor to a cyclic product. The major problems associated with current cyclization strategies are C-terminal epimerization, cyclooligomerization, and formation of linear dimers and trimers (Figure 1a).[18–22] Consequently, there is a great need to develop new synthetic methodologies that can circumvent the aforementioned limitations and provide an efficient strategy for easy access to a variety of cyclic peptides. One approach to achieve this goal is to develop a strategy that could work in an exclusively intramolecular fashion. Currently, there are no such methods available to achieve this goal.

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Supporting information (including materials, instruments, reaction procedures, stability studies, and characterization of products by HRMS, HPLC, ForceGen, and NMR (PDF)) and the ORCID identification number(s) for the author(s) of this article can be found under:
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Results and Discussion

New Cyclization Strategy

To develop a chemoselective reaction that works in an intramolecular fashion only, we sought to use peptide aldehydes for macrocyclization. As a key design element, we hypothesized that formation of a cyclic imine between an N-terminal peptide and a C-terminal aldehyde might promote conformational preorganization by bringing the amide at the second position in close proximity to the cyclic imine. This would lead to nucleophilic attack by the second amidic nitrogen on the imine to generate a stable 4-imidazolidinone-fused cyclic peptide (Figure 1b). The linear imine intermediate formed by intermolecular reaction between two peptides is unable to activate the amide bond and thus would not lead to formation of stable dimers (Figure 1b). We termed this approach as "CyClick" because the reaction is highly chemoselective for formation of cyclic peptides without dimerization or oligomerization. This approach represents a rare example of conformationally induced amide bond activation that might offer a general strategy for the efficient synthesis of 4-imidazolidinone-fused cyclic peptides (Figure 1). 4-Imidazolidinone is an important structural motif found in many pharmaceuticals and biologically active compounds,[23,24] such as *N*,*N*′-methyleno-didemnin A,[25] which is cytotoxic against human colon tumor cells; spiroimidazolidinone, which exhibits anticonvulsant activity;[26] Ro 64-6198, an agonist for the nociceptin/orphanin FQ opioid peptide (NOP) receptor;[27] and ML298, a selective inhibitor of phospholipase D (PLD)[28] (Figure 1c).

Among a number of advantages, we recognized that the CyClick strategy would: 1) be triggered by the N-terminus, without the need for coupling reagents and metals; 2) be

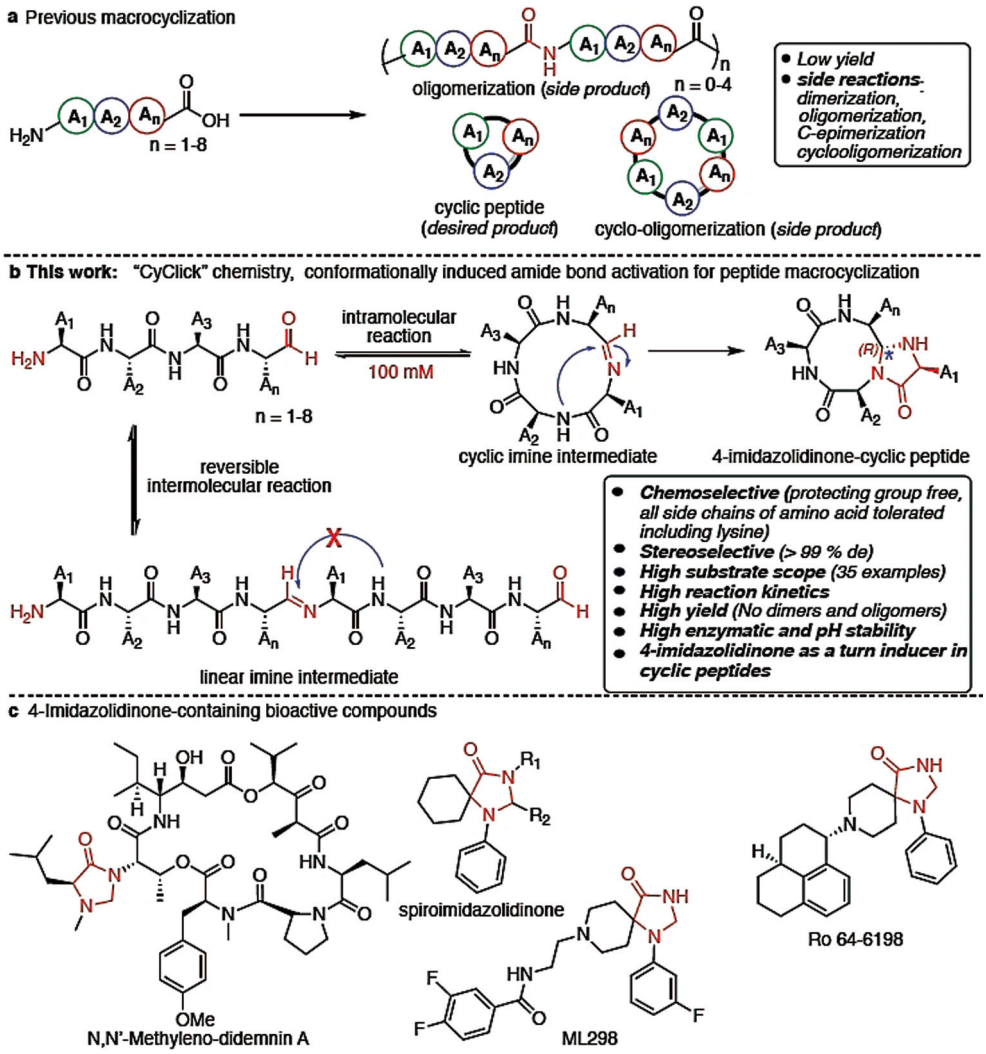

Figure 1. CyClick, an approach based on the conformationally induced activation of the amide backbone for peptide macrocyclization. a) Limitations of current peptide macrocyclization strategies. b) Synthesis of cyclic peptides via CyClick chemistry. c) 4-Imidazolidinone-containing bioactive compounds.

chemoselective for reaction between the N-terminus of a peptide and an aldehyde rather than any other amino acid residues including lysine; 3) utilize the cyclic imine–peptide conformation as an internal directing group, thus requiring no external ligand or removable directing group. In addition, this macrocyclization would lead to the generation of a new chiral center with high stereoselectivity and introduce a nonpeptidic moiety, 4-imidazolidinone, into the macrocycle. This is a feature that is known to generally improve the intrinsic pharmacokinetic profile while maintaining biological activity.[23–28]

Herein, we report the successful execution of these ideas and present a strategy that exploits the conformationally induced activation of the amide backbone for the efficient synthesis of cyclic peptides that is applicable across a wide range of peptide ring sizes with various amino acid residues. Most importantly, this reaction generates only cyclic peptides by intramolecular reaction without formation of side products due to linear and cyclic oligomerization. Since this method leads to only intramolecular reactions, a high rate of macrocyclization can be achieved when the reactions are carried out at high concentrations. Furthermore, NMR investigation revealed that the 4-imidazolidinone moiety induces a turn structure in cyclic peptides and increases their enzymatic stability; thus, this method is highly attractive for generating cyclic peptides for probing biological systems.

CyClick Reaction for Peptide Cyclization

We started our initial investigation on a peptide with the sequence of AVGPFE(CHO)Y **1a**, where the side chain of Glu was modified to an aldehyde group (Figure 2a and Supplementary Figure 1). Detailed optimization studies revealed that the macrocyclization between the N-terminus of a peptide and an aldehyde proceeds most efficiently in an aqueous medium (H_2O/DMF (1:1)) at room temperature with addition of 4-(Dimethylamino)pyridine (DMAP, 7 equiv). This resulted in the formation of a 4-imidazolidinone cyclic peptide **2a** with 99% conversion (Supplementary Table 1). We believe that DMAP facilitates macrocyclization by proton abstraction from the amidic nitrogen of the second amino acid, thereby activating the amide backbone for nucleophilic attack.[29] Importantly, coupling reagents, metal catalysts, and harsh conditions (high temperature) were not required in this procedure. The 4-imidazolidinone cyclic peptide **2a** was characterized by high-resolution mass spectrometry (HRMS) and NMR spectroscopy (Figure 2a and Supplementary Figure 2). The diagnostic aminal carbon chemical shift at 71.2 ppm for 4-imidizolidinone is much further downfield than any Cα carbon (Figure 2a and Supplementary Figure 2). ACD labs' (version 2015)[30] prediction for this chemical shift was 73.4 ppm. Moreover, heteronuclear multiple bond correlation (HMBC) NMR experiments confirmed the 4-imidazolidinone structure (Figure 2a and Supplementary Figure 2). It is noteworthy that linear and cyclic dimerization or oligomerization products were not observed by either HPLC or MS analysis. Furthermore, replacement of the second amino acid residue with proline in linear peptide aldehydes A**P**GAFE-

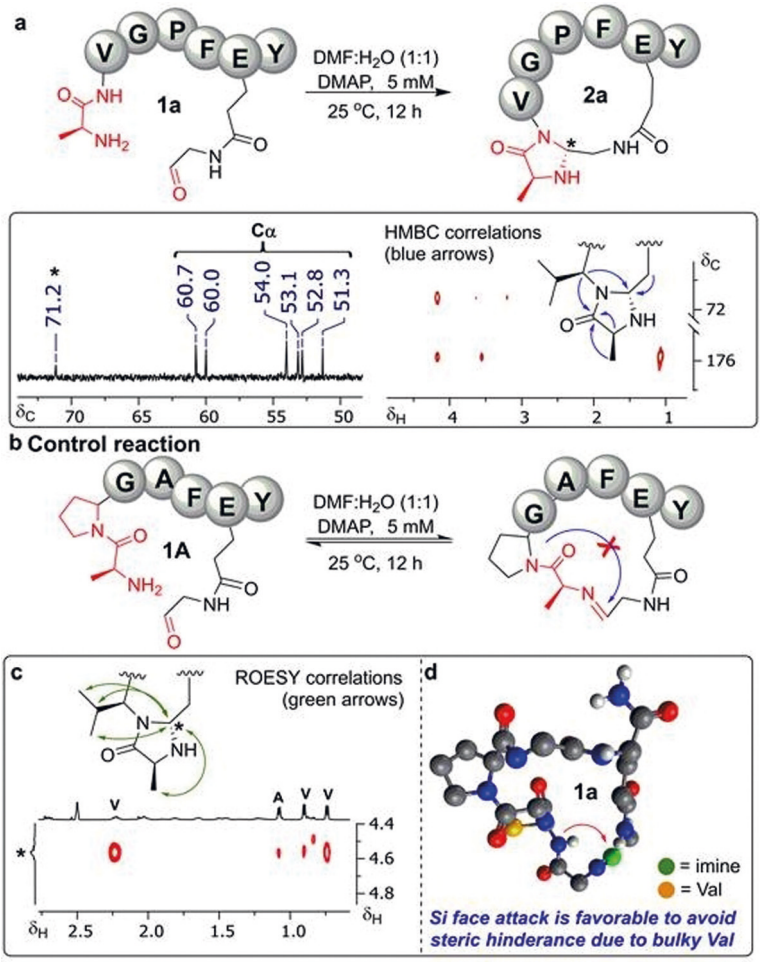

Figure 2. Structural characterization of cyclic peptide **2a** by NMR spectroscopy. a) ^{13}C NMR spectrum with the diagnostic aminal chemical shift highlighted (*) and HMBC correlations confirming the 4-imidazolidinone ring structure of **2a**. b) Control reaction using APGAFE(CHO)Y **1A**, which contains Pro in the second position, validating that no macrocyclization occurs. c) Key ROEs of **2a** to assign the (*R*)-configuration of the new chiral center. Full-scale spectra are included in the Supporting Information. d) Proposed mechanistic pathway for observed the stereoselectivity in the cyclic peptide.

(CHO)Y **1A** and A**P**CA(CHO) completely abolished the cyclization, indicating that the amide proton at the second amino acid is essential for the formation of the 4-imidazolidinone moiety (Figure 2b and Supplementary Figure 3).

Most importantly, we attempted the intermolecular reaction between an aldehyde, such as pentanal, and the N-terminus of a linear peptide FVA under various reaction conditions, including longer reaction times, a great excess of aldehyde, and high amounts of DMAP. Intermolecular coupling leading to the formation of 4-imidazolidinone was not observed between the aldehyde and N-terminus of the peptide (Supplementary Figure 4). Although the intermolecular reaction led to the formation of a reversible linear imine, the synthesis of a stable 4-imidazolidinone moiety was not detected. We confirmed the formation of linear imine by reduction with $NaCNBH_3$. Next, we attempted the intermolecular reaction between a highly reactive keto peptide aldehyde CHOVF and the N-terminus of a linear peptide ASVF under CyClick reaction conditions; however, we did not observe the formation of any 4-imidazolidinone-containing product under these conditions (Supplementary Figure 4).

We hypothesize that this selectivity is based on the proximity of the amidic nitrogen to the cyclic imine. Together, these results confirm that the CyClick reaction takes place in an intramolecular fashion only. This is the first report for such a macrocyclization where the intermolecular reaction is not possible.

Stereoselectivity of the CyClick Reaction

Another unique feature of this reaction is that it generates a new chiral center at the site of macrocyclization with high diastereoselectivity (de > 99%), which is in contrast to conventional methods of macrocyclization leading to C-terminal epimerization.[17–21] The absolute configuration of a new chiral center in the 4-imidazolidinone cyclic peptide *cyc*(AVGPFEY) **2a** is (*R*) and was determined by ROESY NMR spectroscopy (Figure 2c, and for detailed analysis see Supplementary Figure 5). To determine the source of the high stereoselectivity of the CyClick reaction, NMR analysis of another cyclic peptide *cyc*(aVGPFEY) **2a'** containing D-Ala at the N-terminus was conducted. The results showed the formation of a new chiral center with (*R*)-configuration (de > 99%) (Supplementary Figure 6), thus allowing the conclusion that the configuration of the N-terminal amino acid is not responsible for directing the configuration of the new chiral center. Next, we synthesized a cyclic peptide *cyc*(AiGPFEY) with a D-Ile at the second position, and the spectroscopic data established that the configuration of the new chiral center in the cyclic peptide is (*S*) (Supplementary Figure 7). This validated that high stereoselectivity and the configuration of the new chiral center in cyclic peptides was conferred by the configuration of the second amino acid, which most likely directs nucleophilic attack of the amidic nitrogen on the cyclic imine intermediate from the *Si* face. Attack from the *Re* face would be hindered due to the bulky Val residue (Figure 2d).

Macrocyclization vs. Oligomerization

One of the major limitations with current methods for peptide cyclization is their tendency to undergo intermolecular reactions to generate linear dimers, linear trimers, cyclodimers, and cyclotrimers.[17–21] Conventional macrocyclization reactions are carried out at high dilution, on the order of 10^{-4} M or greater, to limit the formation of side products such as dimers or oligomers.[31–33] Unfortunately, high dilution leads to long reaction times, which in turn can provoke unwanted background processes such as epimerization. Some

strategies have been reported for the synthesis of cyclic peptides in solution at high concentrations; however, they are limited by their ability to undergo intermolecular reactions, require protected amino acids, such as Lys or Glu/Asp, to avoid side reactions, and lead to the formation of a mixture of diastereoisomers.[34,35]

The unique feature of our approach is that it works in intramolecular fashion only. Thus we carried out the macrocyclization of a linear peptide AVGPFE-(CHO)Y **1a** using high concentrations (25 mM, 25 times higher than usually employed) and compared it with a conventional method of macrocyclization (reductive amination, Figure 3 and Supplementary Figure 8).[36] Insights into the impact of CyClick chemistry on the efficiency of macrocyclization at high concentrations were revealed by LC-MS analysis (Figure 3).

In the conventional reductive amination approach[35] for the cyclization of linear peptide **1a** at high concentration (25 mM), significant quantities of unwanted linear dimers and cyclodimers were produced (Figure 3, bottom chromatogram). In contrast, the cyclization of the linear peptide **1a** by CyClick chemistry at high concentration (25 mM) generated the desired cyclic peptide **2a** with high conversion (98%) (Figure 3, top chromatogram). The major corresponding by-products were not seen even in trace quantities (Figure 3, top chromatogram). Thus, a significant improvement in macrocyclization is realized by using the CyClick approach. We also carried out the macrocyclization of linear peptide AVGPFE(CHO)Y **1a** at 100 mM concentration with 21 equiv of DMAP and stirring for 8 h at room temperature. The reaction generated only the desired cyclic peptide **2a** with high conversion (89%) under the reaction conditions without the formation of any side products due to dimerization and oligomerization (HPLC trace of the reaction, Supplementary Figure 8).

To gain a deeper understanding of reaction rates and the products formed, time-course studies on linear peptide AVGPFE(CHO)Y **1a** were undertaken. For this investigation, quantitative monitoring was carried out by injecting samples for HPLC analysis at regular time intervals. The peptide AVGPFE(CHO)Y **1a** (0.67 mM) was subjected to the CyClick reaction and conversion was monitored over 4 h. From the data, it is clear that the initial rate of the formation of a cyclic peptide **2a** is considerably fast with >80% conversion achieved in 4 h (Figure 4, and Supplementary Figure 9). Taken together, these studies establish that the CyClick reaction employs mild conditions, proceeds quickly, gives higher yields and does not generate any side products, such as linear and cyclic dimers or oligomers even at high concentrations.

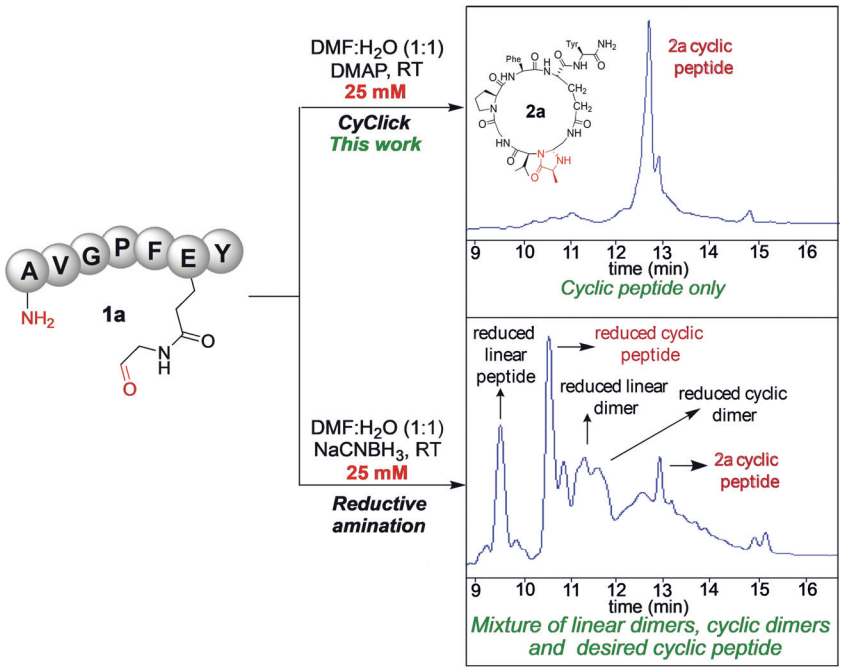

Figure 3. Macrocyclization vs. oligomerization. Direct comparison of CyClick reaction and reductive amination approach for the synthesis of cyclic peptide at high concentrations (25 mM). Chromatograms of the crude reaction mixtures.

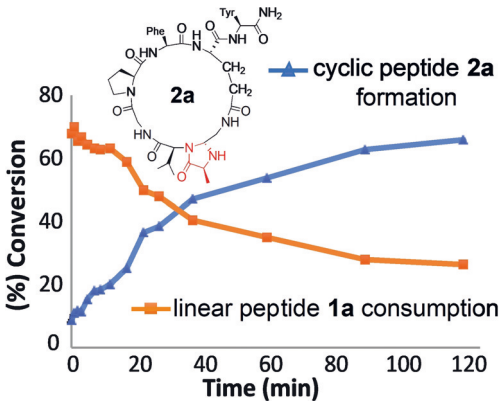

Figure 4. Rate studies for the synthesis of cyclic peptide **2a** by CyClick chemistry. Peptides were quantified by HPLC.

Scope of CyClick Chemistry

Having established the optimal conditions, we sought to demonstrate the generality of CyClick chemistry with different amino acids at the N-terminus. As outlined in Figure 5, substrates bearing aromatic and aliphatic amino acids at the N-terminus including Trp, Tyr, and β-branched Val (**1b–1d**) were fully tolerated in this protocol and the corresponding cyclic peptides (**2b–2d**) were generated with good conversions (37–84%, Figure 5a and Supplementary Figure 10). Reactions with N-terminal amino acids bearing reactive side chains such as Gln, Asn, Asp, and Lys (**1e–1h**) did not interrupt the cyclization process, and afforded desired cyclic peptides (**2e–2h**) with good conversions(64–94%, Figure 5a, **2e** and **2f** NMR-Supplementary Figure 2, and HRMS-Supplementary Figure 10). Surprisingly, linear peptide **1i** with

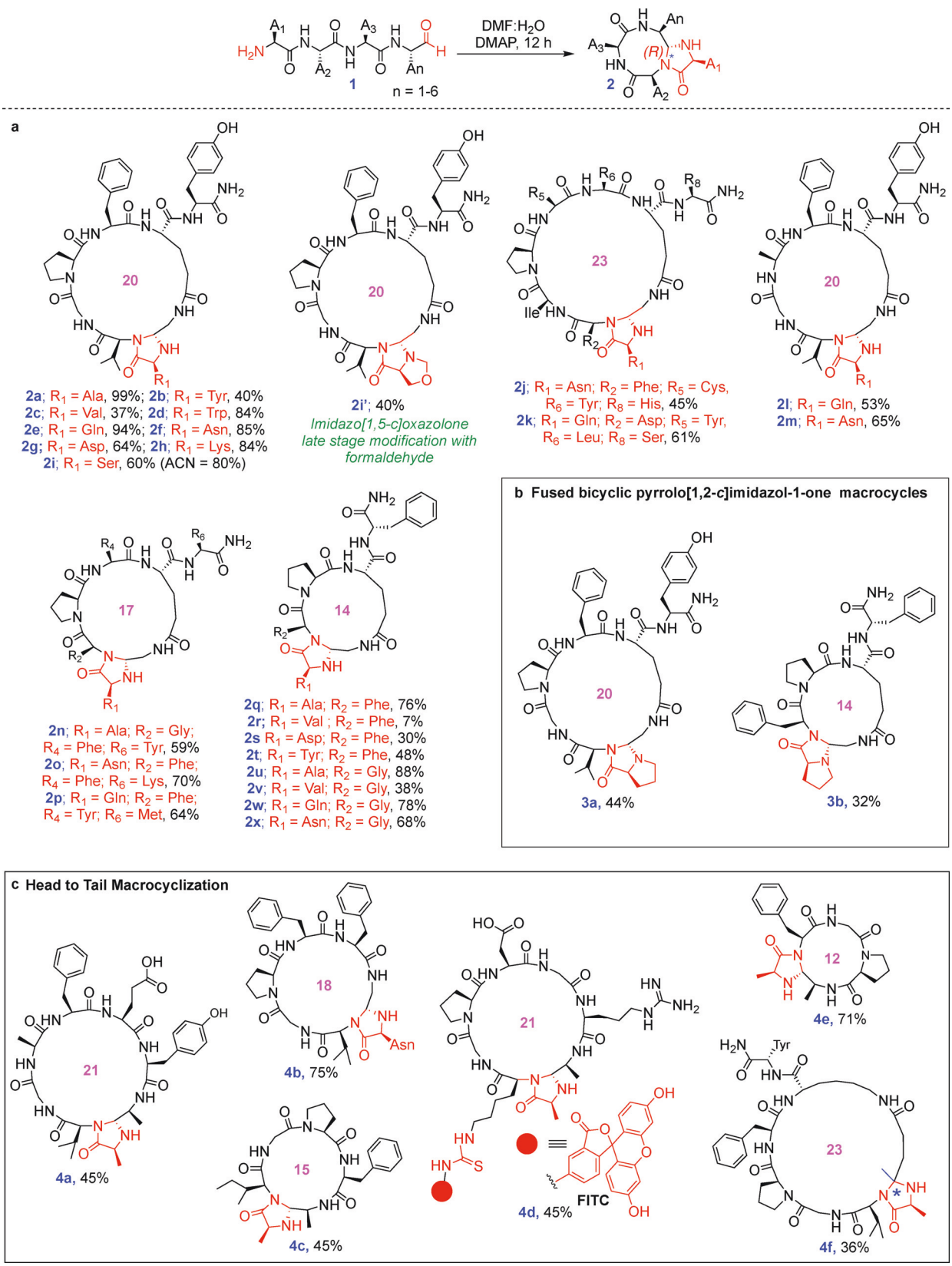

Figure 5. Substrate scope of CyClick chemistry. a) High conversions of cyclic peptides (12- to 23-membered) with various amino acid residues and lengths of peptide chains. b) Fused bicyclic pyrrolo[1,2-*c*]imidazolone macrocycles at the site of macrocyclization. c) Head-to-tail macrocyclization of cyclic peptides by CyClick chemistry; number in the middle of the rings denotes ring size. Macrocyclic peptide **4 f** with quaternary chiral center (*) was generated by reaction with peptide ketone.

serine at the N-terminus generated cyclic peptide **2i′** with a fused five-membered bicyclic imidazo[1,5-*c*]oxazol-7-one (40% conversion) along with the expected 4-imidazolidinone cyclic peptide **2i** (60% conversion) under the reaction conditions (H_2O/DMF (1:1), DMAP (7 equiv), 25 °C, Figure 5a).

The detailed NMR and HRMS analysis of the imidazo-[1,5-*c*]oxazol-7-one cyclic peptide **2i′** revealed the late-stage insertion of the formyl group in the 4-imidazolidinone moiety of the cyclic peptide **2i** and the hydroxymethyl group of the side chain of serine (Figure 5a, HRMS-Supplementary Figure 10, and **2i′** NMR-Supplementary Figure 11).[37] This is most likely due to the formaldehyde present in the undistilled DMF. We validated the source of formaldehyde by carrying out the reaction in H_2O/ACN. We did not observe the formation of fused five-membered bicyclic imidazo[1,5-*c*]oxazol-7-one cyclic peptide **2i′** in H_2O/ACN and the desired 4-imidazolidinone cyclic peptide **2i** was obtained with 80% conversion (Figure 5a).

Unprotected linear peptides **1j** and **1k** with reactive amino acids such as Asn, Asp, His, Tyr, Cys, Gln, and Ser afforded 23-membered cyclic peptides **2j**–**2k** with good conversions (45–61%, Figure 5a and Supplementary Figure 10), demonstrating the versatility of the CyClick reaction. The reaction was also utilized for the cyclization of the difficult sequences with all L-amino acids without any turn inducers such as NVGAFE(CHO)Y **1l** and QVGAFE-(CHO)Y **1m.** Linear peptides **1l** and **1m** cyclized smoothly with high amounts of DMAP (21 equiv) and generated corresponding cyclized products **2l** and **2m** (53–65%, Figure 5a and Supplementary Figure 10). Notably, we did not observe the formation of any linear or cyclic oligomers.

Most importantly, the γ-amino groups of lysine residues do not undergo CyClick reaction because of the lack of a neighboring amide group required for facile cyclization. To probe the impact of lysine residues, linear peptide aldehydes **1h** and **1o** bearing unprotected Lys residues were prepared and cyclized under CyClick chemistry conditions. The reactions generated 4-imidazolidinone cyclic products **2h** and **2o** with good conversion (**2h**, 84% and **2o**, 70%, Figure 5a). Peptides lacking a Lys group (**2a**–**2g**, **2n**, and **2p,** Figure 5a) produced nearly similar yields of the cyclic products, suggesting that unprotected Lys does not influence the overall yield of the CyClick products.

Encouraged by these results, we continued to test the versatility of this procedure with different chain lengths of peptides such as pentapeptides and hexapeptides **1n**–**1x**. All the substrates cyclized efficiently and provided the corresponding 14- to 17-membered macrocycles **2n**–**2x** with good conversions (Figure 5a and Supplementary Figure 10).

Interestingly, the reaction of linear peptides with proline at the N-terminus gave fused bicyclic five-membered 1*H*-pyrrolo[1,2-*c*]imidazole-1-one cyclic peptides **3a** and **3b** (Figure 5b, HRMS-Supplementary Figure 10, and **3a** NMR-Supplementary Figure 12).[38] Similarly, head-to-tail macrocyclization of octa-, hepta-, and hexapeptides yielded the corresponding 15- to 21-membered macrocycles **4a**–**4c** with good conversions under the reaction conditions (45–75%, Figure 5c and Supplementary Figure 10). The head-to-tail macrocyclization of the difficult sequence with all L-amino acids without any turn inducers such as AVGAFEYA(CHO) proceeded smoothly and the corresponding cyclized product **4a** was generated with good conversion without the formation of any side products due to linear and cyclic oligomerization (Figure 5c and Supplementary Figure 10).

We further highlighted the utility of this protocol by synthesizing a fluorescent-labeled 4-imidazolidinone cyclic RGD peptide **4d** which has the potential to bind to breast cancer cells overexpressing αvβ3 integrin (Figure 5c and Supplementary Figure 10).[39,40] This result demonstrates the validity of our approach in synthesizing bioactive peptidomimetics. We next challenged our method by cyclizing a head-to-tail pentapeptide, which is extremely difficult to achieve by current cyclization techniques due to their high tendency to form oligomers.[12–21,31–33] To our delight, the highly strained 12-membered cyclic peptide **4e** was formed with good conversion (71%, Figure 5c, Supplementary Figure 10). To further challenge our method, we attempted the cyclization of the head-to-tail tetrapeptide FGPA(CHO) using various reaction conditions including high amounts of DMAP. We knew that a tetrapeptide aldehyde should give a nine-membered ring, which is impossible, but we also did not observe the formation of any linear and cyclodimer by CyClick chemistry (Supplementary Figure 10). We confirmed these results by reducing the reaction mixture with sodium cyanoborohydride; this resulted in the formation of reduced linear tetrapeptide and reduced linear dimer. These studies further confirmed that our method works in intramolecular fashion only (Supplementary Figure 10). Next, we examined the scope of CyClick chemistry on less reactive peptide ketones instead of peptide aldehydes. Interestingly, cyclic peptide **4f** was generated with moderate conversion from the peptide ketone with a quaternary chiral center at the site of cyclization, further expanding the substrate scope of this reaction (36%, Figure 5c, Supplementary Figure 10). In the reactions described above, we have synthesized more than 35 cyclic peptides that vary in ring size (12- to 23-membered) and amino acid composition highlighting that CyClick is a powerful, self-guided, intramolecular amide backbone activation approach for the efficient synthesis of cyclic peptides in high purity, free from the typical contaminating species normally encountered during the synthesis of cyclic peptides using conventional methodology.

Structural Impact of 4-Imidazolidinone in Cyclic Peptides

To determine the ability of the 4-imidazolidinone to induce secondary structure in cyclic peptides, we conducted NMR studies on the head-to-tail cyclic peptide *cyc*-(AVGAFEYA) **4a** in aqueous medium. 2D TOCSY, COSY, HSQC, HMBC, and ROESY spectra were acquired to assign the ^1H and ^{13}C signals of **4a** (Supplementary Figure 13). Variable-temperature (VT-NMR) studies were then performed to determine the intramolecular H-bonding pattern of cyclic peptide **4a** (Figure 6a and Supplementary Figure 14), between the Tyr amide proton and Ala carbonyl oxygen. The observed ROEs were used to construct a ROE

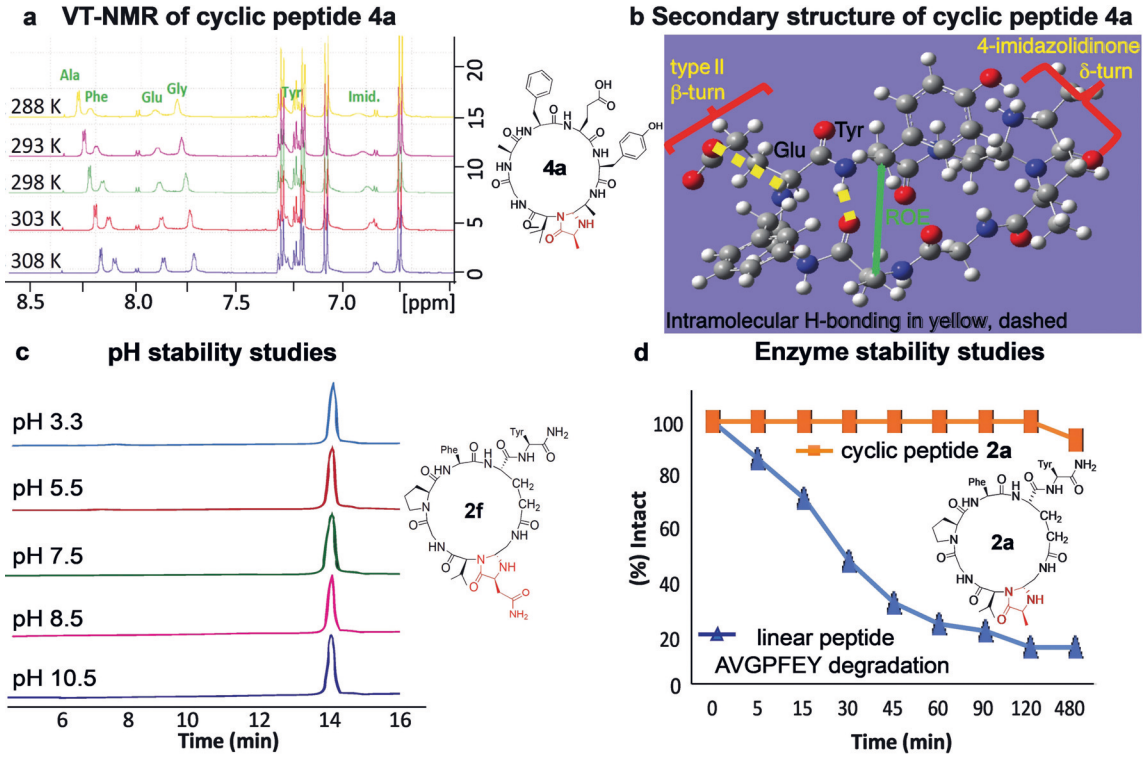

Figure 6. Impact of 4-imidazolidinone in cyclic peptides. a) VT-NMR spectra of head-to-tail 4-imidazolidinone cyclic peptide **4a** in aqueous solutions. b) Turn structure of 4-imidazolidinone cyclic peptide **4a** obtained by running ForceGen[41] with NMR constraints. c) 4-Imidazolidinone cyclic peptide **2f** exhibited enhanced resistance towards hydrolysis under different pH conditions. d) Proteolytic stability of 4-imidazolidinone cyclic peptide **2a** as compared to its linear counterpart.

"connectivity" map, which summarizes sequential (i.e. residues i to $i+1$) and long-range ROEs that are commonly observed in peptides with higher order structure. Long-range ROEs were observed for peptide **4a** between residues Ala (ith) and Tyr ($i+3$) indicating their proximity. Furthermore, the secondary structure adopted by **4a** was determined by running ForceGen[41] with NMR constraints (Figure 6b and Supplementary Figure 15). Together, these NMR studies provided the first direct experimental evidence that 4-imidazolidinone is indeed a turn inducer.

Biological Evaluation of 4-Imidazolidinone Cyclic Peptides

The stability of cyclic peptides is a major concern for pharmaceutical applications. To evaluate the stability of a 4-imidazolidinone cyclic peptide, we incubated cyclic peptide *cyc*(NVGPFEY) **2f** under different pH conditions. HPLC analysis showed that 4-imidazolidinone cyclic peptide **2f** was resistant to hydrolysis/degradation under acidic and basic conditions and remained unchanged for up to 24 h (10 mM phosphate-buffered saline (PBS) buffer, pH 3.5–10.5, Figure 6c and Supplementary Figure 16).

To evaluate the potential of 4-imidazolidinone cyclic peptides for biological applications, we examined the proteolytic stability of a cyclic peptide *cyc*(AVGPFEY) **2a** in comparison with its linear counterpart AVGPFEY. Linear peptide AVGPFEY and cyclic peptide *cyc*(AVGPFEY) **2a** were incubated with chymotrypsin, which hydrolyzes peptide bonds at the C-terminal side of aromatic residues, such as Phe. Results showed that in the presence of chymotrypsin cyclic peptide **2a** remained intact for up to 24 h with only 20% cleavage observed, whereas its linear counterpart AVGPFEY degraded quickly with a half-life of 20 min and was fully consumed in 90 min, as determined by HPLC and MS analysis (Figure 6d, Supplementary Figure 17). These results demonstrated that the 4-imidazolidinone moiety generated during cyclization significantly improved the stability of cyclic peptides against both proteolysis as well as degradation over a range of pH conditions. Together, these results demonstrate the applicability of the CyClick chemistry in generating potentially bioactive cyclic peptidomimetics as molecular tools to study biological systems.

Conclusion

In summary, we have developed the CyClick reaction, an approach based on the conformationally induced activation of the amide backbone, for the cyclization of peptides. This method is highly selective for intramolecular reaction and leads to the efficient synthesis of cyclic peptides even at high concentrations without the formation of any undesired side products due to linear and cyclic dimerization or oligomerization. The potency of the CyClick reaction is well demonstrated by the broad substrate scope encompassing a variety of peptides with different amino acid compositions including difficult sequences containing all L-amino acids without any

turn inducers, various aldehydes and ketones, and different chain lengths, including generation of highly strained 12-membered cyclic peptide(s) as shown in Figure 5. CyClick chemistry leads to the formation of a 4-imidazolidinone moiety in a cyclic peptide, which further induces a turn structure as determined by detailed NMR investigation. The 4-imidazolidinone cyclic peptides exhibit high stability over a range of pH conditions and towards enzymatic degradation, demonstrating the potential utility of this chemistry for the development of pharmaceutically active compounds and biological probes. Moreover, the 4-imidazolidinone moiety introduces a secondary amine in cyclic peptides which can be used for further diversification. This work is currently underway in our laboratory. The increasing significance of bioactive cyclic peptides containing pharmacophores should render this method attractive for synthetic and medicinal chemistry.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: chemoselectivity · CyClick chemistry · peptides · macrocycles · stereoselectivity

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