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Macrocyclization of Linear Peptides Enabled by Amphoteric Molecules

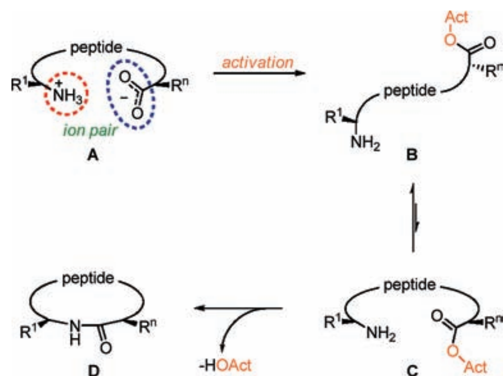
Ryan Hili, Vishal Rai, and Andrei K. Yudin*

Davenport Research Laboratories, Department of Chemistry, University of Toronto, 80 St. George Street, Toronto, Ontario, Canada M5S 3H6

Received November 9, 2009; E-mail: ayudin@chem.utoronto.ca

There has been enormous interest in both naturally occurring and synthetic cyclic peptides as scaffolds that preorganize an amino acid sequence into a rigid conformation.[1] Among the vast number of known cyclic peptides, rigid small-to-medium sized rings have been of particular interest. The branches of science where cyclic peptides have found application include nanomaterials,[2] imaging agents,[3] and therapeutics.[4] Various cyclolactamization and non-peptidic cyclization methods[5] have been developed. The macrocyclization of linear precursors is afflicted by several thermodynamic and kinetic challenges that arise from the conformational preferences of linear peptides. Short linear peptides can easily adopt a circular conformation, which is driven by ion pairing between the N- and C-termini (Scheme 1, **A**).[6] Despite the unfavorable entropy, these circular conformations are thermodynamically favored due to the enthalpy garnered through electrostatic and other polar interactions. As shown in Scheme 1, conventional activation reagents tend to remove the zwitterionic character of the peptide, rendering it incapable of forming ion pairs. Consequently, without enthalpic contribution from electrostatics and other polar interactions, the activated peptide adopts a random linear conformation (Scheme 1, **B**). In order for macrocyclization to occur, the activated peptide must adopt a precyclization conformation (**C**) prior to forming the desired cyclic molecule (**D**). High dilution, on the order of 10^{-4} or greater, is essential to limiting the formation of byproduct arising from cyclodimerization,[7] cyclotrimerization, and polymerization.[8] Unfortunately, dilution brings about long reaction times, which in turn provoke background processes such as epimerization. Among the most challenging cyclizations are those attempted on linear peptides containing less than seven residues.[9,10]

Scheme 1. A Common Peptide Macrocyclization Strategy



We became interested in applying amphoteric molecules[11] toward peptide macrocyclization. As a medium, we chose trifluoroethanol (TFE), known for its capacity to stabilize peptide secondary structure and promote polar interactions.[12] To generate zwitterionic cyclization precursors, we resorted to the Ugi four-component condensation, a well-known reaction involving carboxylic acids, amines, aldehydes, and isocyanides.[13] Mechanistically, the Ugi reaction goes through a series of reversible transformations that tend toward the thermodynamic driving force of amide bond formation. When an α-amino acid, aldehyde, and isocyanide are used as starting materials, the reaction traverses a zwitterionic iminium ion intermediate, which upon attack by the isocyanide produces an electrophilic mixed anhydride. Subsequent reaction with methanol gives a linear peptide ester as a mixture of diastereoisomers.[14] This reaction has been attempted in the synthesis of cyclic peptides from linear peptides and conventional (monofunctional) aldehydes. However, low yields are accompanied by the lack of diastereoselectivity and dominant cyclodimerization products.[15,16]

When we reacted L-phenyl alanine, *tert*-butyl isocyanide, and aziridine aldehyde, a cyclic piperazinone was obtained in 92% yield in approximately 1 h (Table 1, entry 1). The relative stereochemistry of **1** was established using X-ray analysis. A range of amino acids were subjected to this reaction, and in all cases the corresponding piperazinone products were obtained as single diastereoisomers without formation of linear peptides (Table 1).

Table 1. Scope of Piperazinone Synthesis (L-Amino Acid Derived Product Is Shown)

R^1-aziridine aldehyde (dimer) + H_2N–CH(R^2)–COOH → (^{t}Bu–N≡C; TFE, rt, 0.2 M, >20:1 dr) piperazinone (N-^{t}Bu amide)

entry[a]	R^1	amino acid	time (h)	product[b]
1	$CH_2OTBDMS$	L-Phe	1	**1** (92)
2	$CH_2OTBDMS$	D-Phe	1.5	**2** (90)
3	$CH_2OTBDMS$	L-Ala	1.5	**3** (82)
4	$CH_2OTBDMS$	L-Pro	1	**4** (98)
5	$CH_2{}^iPr$	D-Arg	1	**5** (83)
6[c]	$CH_2{}^iPr$	L-Lys HCl[g]	1	**6** (76)
7	$CH_2OTBDMS$	L-Gly	1	**7** (80)
8[e]	$CH_2{}^iPr$	L-Asp	3	**8** (76)
9[f]	$CH_2OTBDMS$	L-Cys	2	**9** (82)
10[d]	$CH_2OTBDMS$	L-His	1	**10** (88)
11	$CH_2OTBDMS$	L-Ser	3	**11** (77)

[a] Unless specified otherwise, reactions were performed at room temperature using 0.2 mmol of isocyanide and amino acid, and 0.1 mmol of amino aldehyde dimer in TFE (0.2 M). [b] % isolated yield. [c] HFIP/H_2O (20:1) was used as solvent (0.2 M). [d] HFIP was used as solvent (0.2 M). [e] Cyclization occurs at the more acidic α-carboxylic acid. [f] TFE was degassed prior to use. [g] Lysine side chain amine is protonated.

We next examined how peptide chain length affects the reaction outcome in terms of selectivity (Table 2). With our protocol, the challenging medium-sized rings are readily prepared; the reaction times are less than 10 h, proceeding with high yields and diastereoselectivities. The workup procedure is simple, involving product precipitation from diethyl ether and hexanes. Most of the

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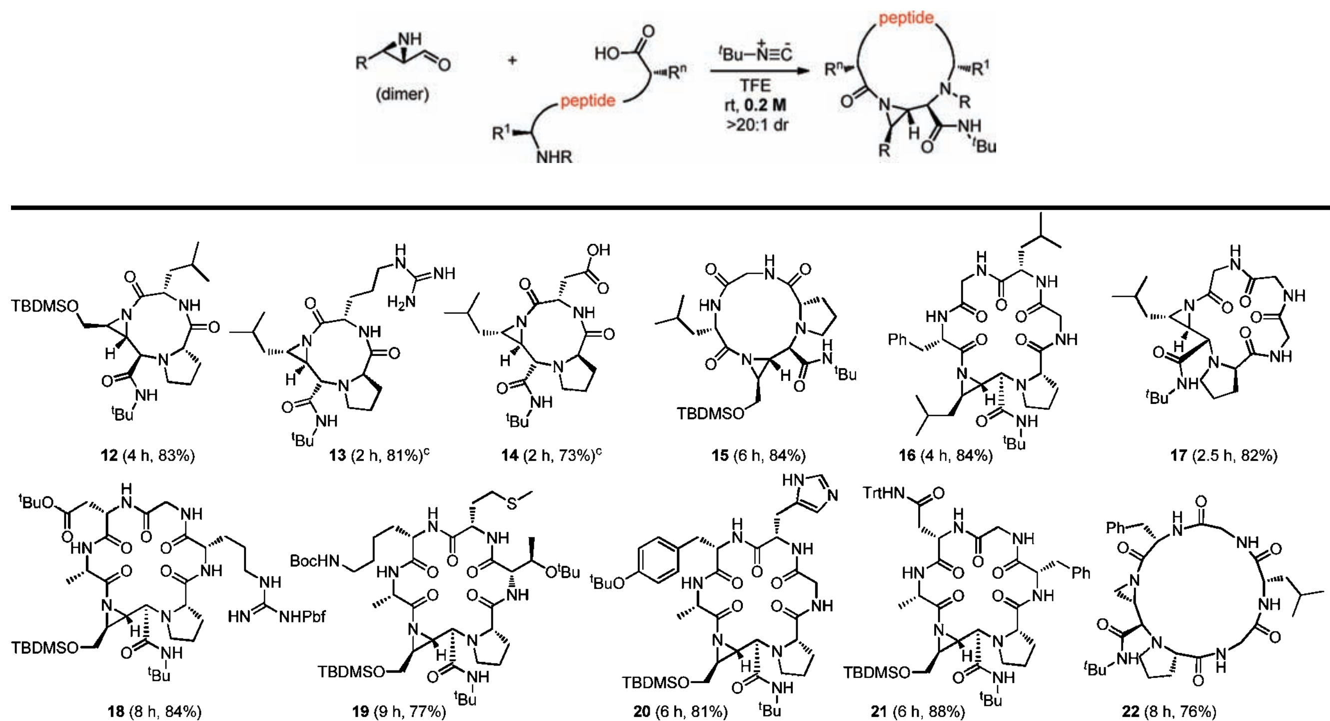
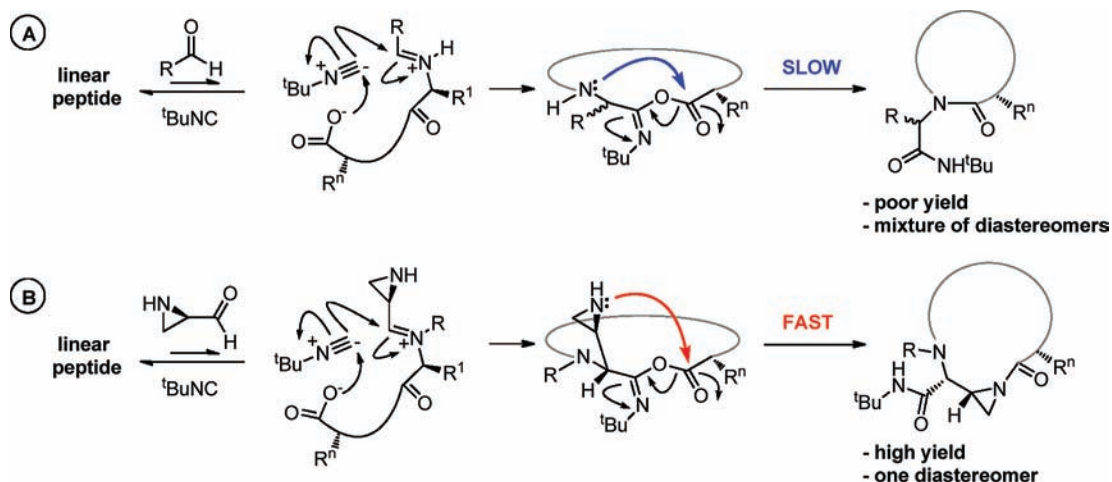
Table 2. Scope of Linear Peptide Macrocyclization[a–c]



[a] Unless specified otherwise, reactions were performed at room temperature using 0.2 mmol of isocyanide and amino acid, and 0.1 mmol of amino aldehyde dimer in TFE (0.2 M). [b] Isolated yield. [c] Diastereoselectivity of >20:1 was confirmed by ^1H NMR analysis of each crude reaction mixture. The relative stereochemistry was established by correlating the methine region of each ^1H NMR spectrum with that of piperazinone **1** (see Supporting Information for X-ray crystal structure of **1**). [d] HFIP was used as solvent (0.2 M).

Scheme 2. (A) Macrocyclization with a Monofunctional Aldehyde; (B) Macrocyclization Mediated by an Amphoteric Amino Aldehyde



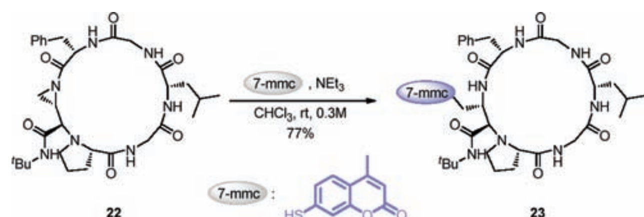
cyclic peptides require no further purification by HPLC. In addition, racemization was not detected throughout the course of the reaction or during product isolation. The lack of epimerization is further evidenced by high stereoselectivity; aziridine aldehydes with *S* stereocenters next to the carbonyl group undergo macrocyclization with the peptides that contain an L-amino acid residue at the N-terminus. The "mismatched" reaction with the D-amino acid terminated peptide is unproductive, leading to the formation of stable aminals (see Supporting Information).

We suspect that consistent yields in this chemistry are obtained due to the mechanism that governs cyclization. It is at this point that a departure from the Ugi reaction with monofunctional aldehydes becomes apparent. When monofunctional aldehydes are used in the reaction with isocyanides and peptides, low diastereoselectivities are observed. More importantly, the undesired cyclodimerization occurs during the cyclization of linear peptides containing less than six residues; the cyclization of tripeptides yields only the cyclodimers.[16] This low selectivity is due to a slow *transannular* attack of the amine onto the mixed anhydride (Scheme 2, **A**), thus allowing the intermolecular process to be kinetically competitive. By adding an amphoteric amino aldehyde to the cyclization reaction mixture containing a secondary amine-terminated peptide, the slow transannular attack is replaced with a fast attack by the nucleophilic aziridine, which is positioned exocyclic to the mixed anhydride (Scheme 2, **B**). This provides an unencumbered trajectory of attack. Since TFE is a non-nucleophilic solvent, premature solvolysis of the mixed anhydride is not observed. The reaction mechanism ensures that the C-terminus is

activated only upon formation of the intermediate cyclic mixed anhydride, which is then attacked by the exocyclic aziridine. This not only secures selectivity for the intramolecular macrocyclization but also avoids prolonged C-terminus activation and potential epimerization. Accordingly, high dilution, a critical condition normally required to achieve high yields in conventional cyclic peptide synthesis, is not necessary.[9] Furthermore, no oligomeric or polymeric byproducts have been detected in our experiments. We ran a side-by-side comparison of our reaction with the traditional lactamization of a linear tetrapeptide, widely used in the synthesis of cyclic peptides (see Supporting Information). The amino aldehyde mediated macrocyclization delivered rapid, selective, and efficient formation of the cyclic peptide. In contrast, only a trace amount of the cyclic peptide was detected among myriad structures formed in the course of the lactamization. The undesired cyclodimerization[8] has dominated this process, which is a testament to the sluggish kinetics of the conventional intramolecular process.

The incorporation of an activated aziridine ring into the framework of a cyclic peptide provides a useful point for conjugation to various side chains via nucleophilic ring opening, a well developed methodology that has been demonstrated using nucleophilic biomolecules ranging from carbohydrates to biotin and farnesyl derivatives.[17] We chose to demonstrate the cyclic peptide conjugation strategy through the nucleophilic ring opening of an aziridine moiety with the widely used fluorescent tag 7-mercapto-4-methyl-coumarin (7-mmc) (Scheme 3). The cyclic peptide **22** was generated by subjecting the sterically unencumbered serine-derived aziridine aldehyde to our macrocyclization protocol. Other nucleophiles such as aliphatic thiols, thioacids, and imides worked equally well during ring opening (see Supporting Information).

Scheme 3. Late Stage, Site-Specific Attachment of a Fluorescent Tag onto a Cyclic Peptide



In conclusion, amphoteric amino aldehydes have led to the development of a novel peptide macrocyclization process. The presence of the nucleophilic center at the α-position of the amphoteric amino aldehyde is responsible for high yields and stereoselectivities. The molecules of cyclic peptides can be made in a one-step process from amino acids or linear peptides, isocyanides, and amphoteric amino aldehydes. The analytically pure product of the reaction can be isolated by precipitation from the reaction mixture. There are no observable byproducts in this chemistry. Most importantly, the resulting molecules possess useful structural features that allow specific modification at defined positions. Thereby, control elements such as fluorescent tags, solubilizing groups, and conformation-tuning substituents are amenable to incorporation into cyclic peptide frameworks at a late stage of synthesis. Given the prevalence of cyclic peptides in chemistry and biology, this operationally simple method should find utility in many areas.

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Note Added after ASAP Publication. Scheme 2 contained an error in the version published ASAP on February 15, 2010; the corrected version posted to the web on March 3, 2010.

Supporting Information Available: Experimental procedures, supplementary data, and chemical characterizations of cyclization products. This material is available free of charge via the Internet at http://pubs.acs.org.

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