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Solution- and Solid-Phase Macrocyclization of Peptides by the Ugi–Smiles Multicomponent Reaction: Synthesis of *N*-Aryl-Bridged Cyclic Lipopeptides

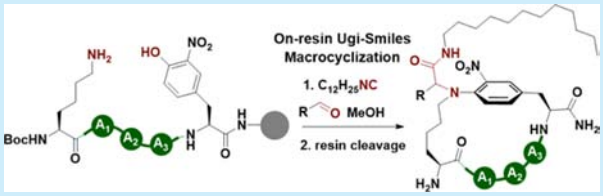
Micjel C. Morejón,[†,‡] Annegret Laub,[†] Bernhard Westermann,[†] Daniel G. Rivera,*[,†,‡] and Ludger A. Wessjohann*[,†]

[†]Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, Weinberg 3, 06120 Halle/Saale, Germany

[‡]Center for Natural Products Research, Faculty of Chemistry, University of Havana, Zapata y G, 10400 Havana, Cuba

Supporting Information

ABSTRACT: A new multicomponent methodology for the solution- and solid-phase macrocyclization of peptides is described. The approach comprises the utilization of the Ugi–Smiles reaction for the cyclization of 3-nitrotyrosine-containing peptides either by the N-terminus or the lysine side-chain amino groups. Both the on-resin and solution cyclizations took place with good to excellent efficiency in the presence of an aldehyde and a lipidic isocyanide, while the use of paraformaldehyde required an aminocatalysis-mediated imine formation prior to the on-resin Ugi–Smiles ring closure. The introduction of a turn motif in the peptide sequence facilitated the cyclization step, shortened the reaction time, and delivered crude products with >90% purity. This powerful method provided a variety of structurally novel *N*-aryl-bridged cyclic lipopeptides occurring as single atropisomers.



Peptide cyclization stands among the most effective ways of introducing conformational constraints in peptide sequences used either as protein ligands or mimetics of protein epitopes.[1] Such a covalent modification is also known to improve membrane permeability[2] and metabolic resistance of peptides,[3] while it can enhance the binding affinity to biological targets compared with their acyclic analogues.[4] Currently, macrocyclizations based on peptide coupling,[1,5] olefin metathesis,[6] and click reactions[7] are the most common methods to seek cyclic peptides with improved activity or pharmacological properties.

Isocyanide-based multicomponent reactions[8] (I-MCRs) have lately risen as powerful tools for the synthesis of cyclic peptides and peptidomimetics.[9,10] The Ugi four-component reaction (Ugi-4CR)[9] and a highly diastereoselective variant based on an amphoteric aziridine–aldehyde component[10] have been the methods employed to cyclize linear peptides either by their termini or side chains. Besides being highly efficient and diversity-oriented processes, MCRs provide a strategic benefit not inherent in traditional methods, i.e., the simultaneous incorporation of additional exocyclic moieties during the macrocyclic ring closure. In cyclic peptides, such *exo* fragments may be crucial for biological activity and may vary from functional appendages[11] for conjugation or fluorescent labeling to peptide bond *N*-alkylations and exocyclic amides capable of either controlling the conformation[12] or modulating the membrane permeability.[13] Encouraged by the possibilities provided by I-MCRs, we pursued the development of new multicomponent macrocyclization approaches capable of increasing the diversity of cyclic peptide scaffolds.

This letter describes a novel peptide cyclization method based on the Ugi–Smiles[14] multicomponent reaction. Besides being the first report describing this I-MCR for macrocyclization procedures, this article provides two innovations: (1) the employment of a lipidic isocyanide to incorporate an exocyclic lipidic tail while closing the macrocyclic ring and (2) the implementation of both solution- and solid-phase multicomponent cyclization protocols enabling the efficient synthesis of structurally unique *N*-aryl-bridged cyclic lipopeptides. Aryl-bridged cyclic peptides are among the most relevant bioactive macrocycles, including members such as K-13 and vancomycin.[15]

The Ugi–Smiles reaction[14] is a remarkable variant of the classic Ugi-4CR[8] in which the carboxylic acid component is replaced by an electron-poor phenol such as a 2- or 4-nitrophenol upon condensation with a primary amine, an aldehyde, and an isocyanide, giving rise to tertiary nitroanilines. The substrate scope of the acidic component of this reaction is remarkable, spanning beyond electron-poor phenols to hydroxyl heterocycles and conjugated enols.[16] However, we are not aware of applications in the covalent modification of peptides.

To provide a reliable synthetic tool to the repertoire of peptide cyclization methods, we endeavored to achieve the macrocyclization of 3-nitrotyrosine-containing peptides by reaction with both N-terminal and side-chain amino groups. As depicted in Scheme 1, a variety of oligopeptides bearing the 3-nitrotyrosine residue at the C-terminus were subjected to Ugi–Smiles macrocyclizations in the presence of paraformalde-

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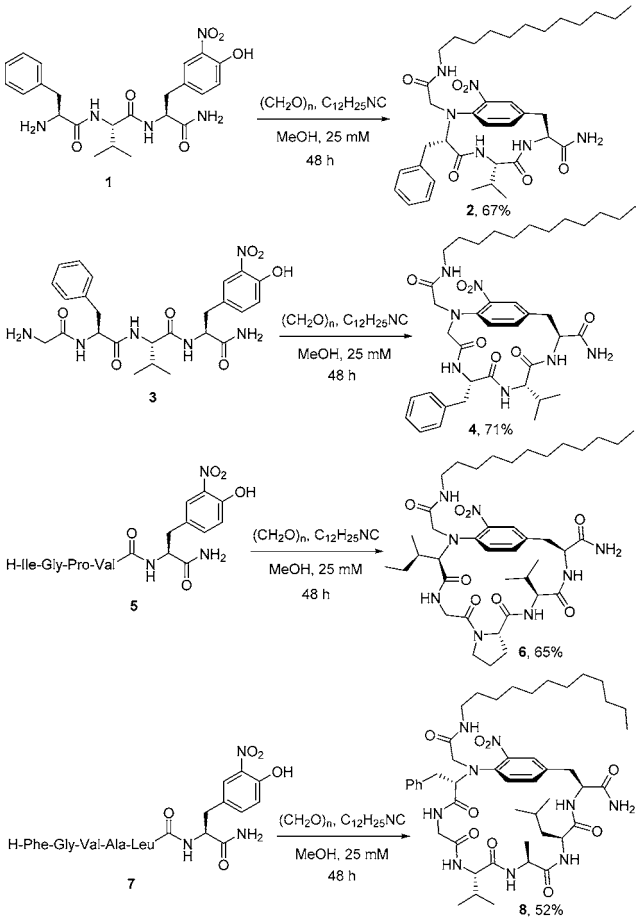
Scheme 1. Solution-Phase Synthesis of *N*-Aryl-Bridged Cyclic Lipopeptides by Ugi−Smiles Macrocyclizations



hyde and *n*-dodecyl isocyanide[17] to provide *N*-aryl-bridged cyclic lipopeptides. Peptides used in the solution-phase macrocyclizations were produced by automated solid-phase synthesis as described in the Supporting Information.

Parallel studies were carried out with peptides **1** and **5** in which both the concentration (2, 10, or 25 mM) and reaction time (12 to 96 h) were varied to assess the best macrocyclization conditions. HPLC monitoring showed that the peptidic substrates were fully consumed at 48 h of reaction at both 10 and 25 mM; there was no difference in the macrocyclization efficiencies (derived from HPLC conversion) at these two concentrations, and no oligomeric products were detected by HPLC/ESI-MS analysis. Although 25 mM may be seen as a rather high concentration for peptide cyclization, this is in agreement with previous reports of I-MCR-based cyclizations of linear peptides.[10] The ionic mechanism of this reaction goes via a zwitterionic iminium phenolate intermediate, which may aid in bringing closer the two reactive termini upon addition to the isocyanide and the subsequent Smiles rearrangement. Also, differently from the head-to-tail cyclization of short peptides, where access to *cis* peptide bonds may be energetically costly and thus detrimental to the cyclization yield, the current method involves a head-to-side chain cyclization that proved efficient even for short peptide **1**. Thus, all four cyclic peptides, containing three to six amino acids, were obtained in good isolated yields.

After proving the success of the Ugi−Smiles macrocyclization in solution, we turned to the development of the on-resin protocol, as this shows great promise in parallel combinatorial synthesis and high-throughput screening. Scheme 2A shows the first solid-phase protocol implemented, which comprises the initial incorporation of 3-nitrotyrosine to the Tentagel S RAM resin followed by a typical Fmoc/*t*Bu strategy for the peptide elongation. Finally, Boc-Lys(Fmoc)-OH is installed at the N-terminus followed by removal of the Fmoc group at the Lys side chain, thus leaving the two reactive functional groups free for the side chain-to-side chain macrocyclization. A key step in Ugi-type reactions is the imine formation, which is typically accomplished prior to addition of the isocyanide component to avoid the competing Passerini reaction. However, on-resin imine formation with paraformaldehyde turned out to be inefficient even at the reactive amino group of a Lys side chain. To solve this, we implemented an aminocatalysis-mediated transimination protocol recently developed by our group to enable on-resin Ugi-4CR on peptide N-termini.[18] The procedure involves the addition to the resin-bound peptide of a piperidinium ion arising from reaction of paraformaldehyde and piperidine. After 20 min of shaking, complete transimination was achieved at the Lys side chain according to the ninhydrin test.[18]

A relatively low resin loading (0.23 mmol/g) was used to provide pseudodilution conditions in order to minimize cyclodimerization processes. As shown in Scheme 2A, peptides from three to eight amino acids were efficiently cyclized under these conditions, providing macrocyclic peptides in good yields with 79% to 90% purity after cleavage from the resin, as determined by HPLC analysis after 48 h. To check for an optimum reaction time, the synthesis of cyclic peptide **10** was taken as model, and mini-cleavages were performed at 24, 48, 72, and 96 h with HPLC/ESI-MS analysis (see the Supporting Information). This proved that the cyclization efficiency was already high at 48 h. Analytical samples of all of the cyclic peptides were purified by semipreparative RP-HPLC for NMR and HRMS characterization. We believe that on-resin head-to-side chain macrocyclizations can be as efficient as those in solution since the transimination step has proven to be very effective at the N-terminus as well.[18]

This approach creates cyclic peptides featuring an *N*-aryl bridge between the Tyr(NO_2) side chain and either the N-terminus or the Lys side chain. This class of covalent linkage has no equivalent in any known natural or synthetic cyclopeptide fragment, but it is related to *ansa*-cyclopeptides.[15] Because of the tertiary nature of the aniline and the presence of an *o*-nitro substituent on the phenyl ring, it is likely that these compounds show atropisomerism due to the high rotational energy barrier at the N−C(sp^2) single bond. As previously described,[19] sterically congested anilines with bulky *o*-aryl substituents provide stereoisomers that can be isolated by chromatographic purification and with different NMR spectra (upon the existence of other chirality elements). HPLC and NMR analyses of compounds in Schemes 1 and 2A suggest the presence of a single atropisomer, although fast interconversion cannot be ruled out at this point. Depending on how high the rotational barrier is, dynamic NMR and HPLC experiments at low or elevated temperatures could provide useful insight into the interconversion process around the N−C(sp^2) bond of these cyclic peptides.[19] Whereas such a study was previously done for Ugi-4CR-derived compounds,[20] it is beyond the goal of this report.

Despite the high conversion achieved in the on-resin Ugi−Smiles macrocyclization at 48 h, we pursued shortening the reaction time by the introduction of structural motifs capable of facilitating the ring-closing step. For this, we devised a solid-phase synthetic route comprising the incorporation of the

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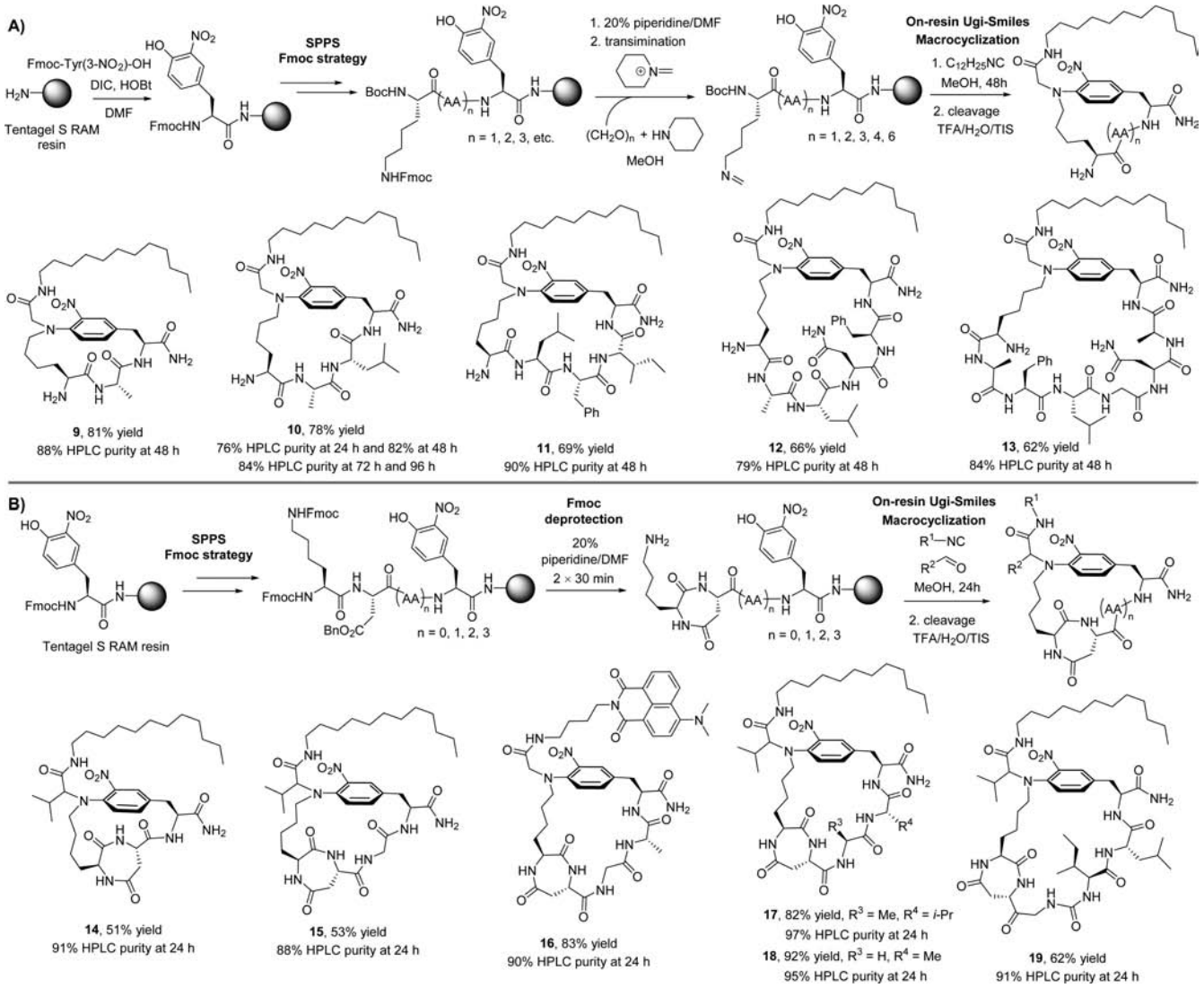
Scheme 2. Solid-Phase Synthesis of Side-Chain *N*-Aryl Cross-Linked Peptides by On-Resin Ugi–Smiles Macrocyclization[a]



[a]Yields of crude products arising from the on-resin peptide synthesis and macrocyclization.

dipeptide fragment Fmoc-Lys(Fmoc)-Asp(OBzl) at the peptide N-terminus. As shown in Scheme 2B, a 1,4-diazepane-2,5-dione ring was readily formed upon treatment of the resin-bound peptide with the mixture of 20% piperidine in DMF employed for Fmoc removal, thus capping the N-terminus and deprotecting the Lys side chain for the subsequent multicomponent cyclization. 1,4-Diazepanediones and their analogous 1,4-diazepines are known mimics of β-turns and have proven merit in peptidomimetic medicinal chemistry.[21] Our rationalization for the installation of such a moiety at the end of the peptide sequence was to provide a turn motif capable of bringing the reacting side chains closer. For this, a set of oligopeptides were produced on resin and subjected to diazepane ring closure followed by Ugi–Smiles macrocyclization.

To our delight, HPLC/ESI-MS analysis proved in most cases that the linear peptides were fully consumed after 24 h, thus enabling the reaction time required to complete the multicomponent macrocyclizations to be reduced by half. In addition, the crude cyclic peptides arising from cleavages showed high purity, in most cases over 90% and up to 97% and 95% for compounds **17** and **18**, respectively. Indeed, the high purity of the crude products resulting from on-resin cyclizations shown in Scheme 2 is a desired characteristic for solid-phase protocols intended to be used in the creation of combinatorial libraries for biological screening. An advantage of the on-resin approach is that the peptide side chains can be protected during cyclization, which enables the use of all amino acids in the peptide sequence. Even various Lys can be present if a three-dimensional orthogonal strategy is employed, i.e., the use of Alloc at a specific Lys side chain to enable orthogonal deprotection without affecting other Boc-protected Lys side chains.

To prove the wider synthetic scope, we also aimed at varying the nature of the lipidic and aldehyde components. Accordingly, isobutyraldehyde was employed as an alternative oxo component, which led to efficient on-resin imine formation and thus avoided the use of a previous transimination step required with paraformaldehyde. Cyclic peptides derived from isobutyraldehyde were obtained as mixtures of two diastereomers of variable ratio as a result of the poor stereoselectivity of this reaction. Alternatively, the synthesis of fluorescently labeled cyclic peptide **16** demonstrated the effectiveness of this methodology in the production of tagged cyclic peptides. This conveys one of the

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greatest advantages over other methodologies, as it allows for cyclopeptide labeling at the same time as the ring closure. As known from previous Ugi–Smiles reports,[14] the scope of the isocyanide component is great, and it may enable the incorporation of further exocyclic fragments.

In conclusion, we have developed a robust methodology for the cyclization of linear peptides by means of the Ugi–Smiles reaction. To our knowledge, this is the first application of this multicomponent reaction in the synthesis of cyclic peptides and in macrocyclization approaches in general. The macrocycles reported herein are structurally novel, as they integrate a cyclopeptide skeleton cross-linked (either at the side chains or head-to-side chain) by an *N*-aryl bridge having a lipidic (or fluorescent) tail arising from the isocyanide component. HPLC and NMR evidence confirmed the presence of a single diastereomer for compounds produced with paraformaldehyde (i.e., either a single atropisomer is formed or the atropisomers rapidly interconvert). Additionally, we demonstrated the feasibility of this multicomponent method in both solution- and solid-phase cyclization protocols. For the use of paraformaldehyde as the oxo component in the on-resin protocol, an aminocatalysis-mediated transimination step was required prior to the addition of the isocyanide. Finally, the installation of a 1,4-diazepanedione moiety (β-turn mimic) in the resin-bound peptide shortened the reaction time, likely by bringing closer the two reactive ends of the peptide precursor.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02001.

Experimental procedures, RP-HPLC chromatograms, and NMR and HR-ESI-MS spectra of cyclic peptides (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: dgr@fq.uh.cu.
*E-mail: wessjohann@ipb-halle.de.

Notes

The authors declare no competing financial interest.

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