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Soluble Tag-Assisted Peptide Head-to-Tail Cyclization: Total Synthesis of Mahafacyclin B

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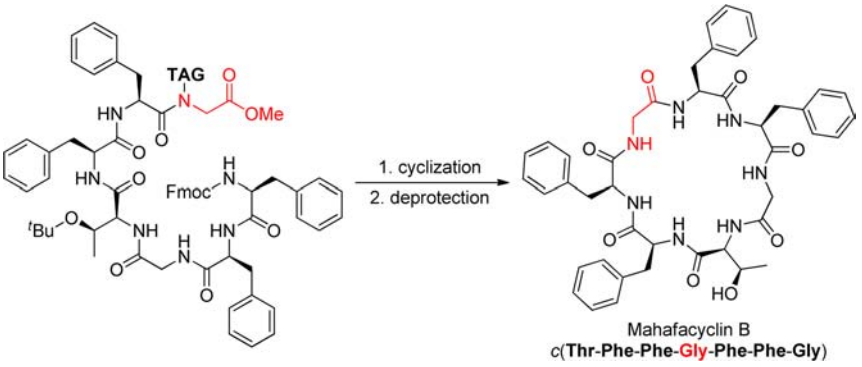
ABSTRACT



A soluble tag-assisted liquid-phase method was successfully applied to peptide head-to-tail cyclization, leading to the total synthesis of antimalarial cyclic heptapeptide, mahafacyclin B (1). The cyclization was carried out in the liquid phase with the tag remaining, which allowed rapid reaction workup and product isolation.

Since the discovery of gramicidin S,[1] cyclic peptides have offered promising medicinal candidates that preorganize their amino acid sequences into rigid conformations that are more resistant to enzymatic degradation than their linear variants.[2] Thousands of cyclic peptides from a variety of natural sources have been isolated, and they and their analogues have been synthesized, leading to potential therapeutic agents. As represented by octreotide, cyclic peptides account for an increasing portion of the pharmaceutical market.[3] A particular advantage of peptides is a fully established method of chemical synthesis based on solid-phase techniques, which also have combined well with automated and combinatorial strategies, enabling extensive and rapid screening for improved specificity and/or bioavailability. Among the most challenging syntheses is the preparation of rigid small-to-medium sized cyclic peptides because such ring closures are often slow processes and are prone to side reactions, typically, cyclodimerizations.[4]

When it comes to head-to-tail cyclized peptides, their preparation must be creative, because current solid-phase and tag-assisted liquid-phase[5] synthetic approaches have

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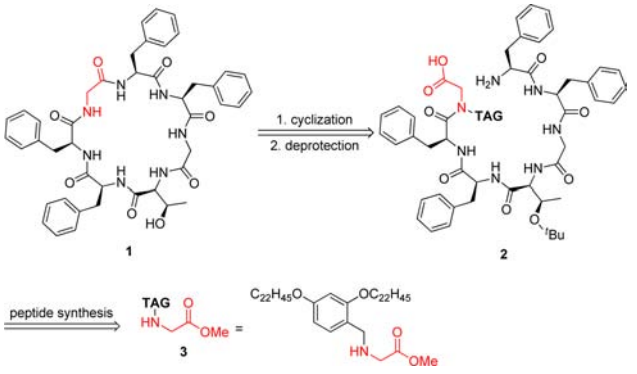
been dominated by the use of C-terminal protecting supports. This means that head-to-tail cyclizations must be carried out after removal of such supports, complicating reaction workup and product isolation. In this context, sophisticated backbone amide linker strategies have been well-established based on a solid-phase technique to realize on-resin peptide head-to-tail cyclization.[6] Such linker strategies should find further application in combination with a tag-assisted liquid-phase technique that generally can yield purer peptides on a larger scale. We have been developing soluble tag-assisted liquid-phase methods using hydrophobic benzyl alcohols as support groups for versatile production of bioactive peptides.[7] However, since our supports are also C-terminal protecting, head-to-tail cyclization is still a matter of demand. Described herein is the application of soluble tag-assisted methods toward head-to-tail cyclization using antimalarial cyclic heptapeptide, mahafacyclin B (**1**),[8] as a model.

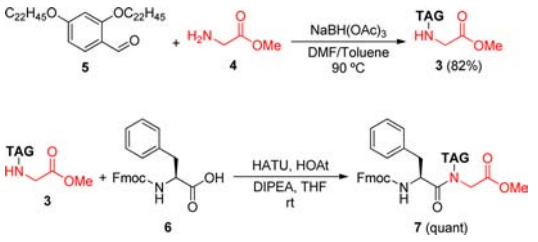
The present work began with the preparation of hydrophobically tagged Gly-OMe (**3**) (Scheme 1). H-Gly-OMe (**4**) was introduced into hydrophobic benzaldehyde (**5**) through reductive amination to afford desired tagged Gly-OMe (**3**) (Scheme 2). Several amino acid methyl esters, including H-Phe-OMe and H-Thr(^{t}Bu)-OMe, can be introduced into hydrophobic benzaldehyde (**5**) under the same reaction conditions (Table S1, Supporting Information), providing an amide nitrogen as a tagging site. To avoid commonly occurring epimerization, the glycine residue was selected as a C-terminus. Fmoc-Phe-OH (**6**) was then coupled to give the tagged dipeptide (**7**). In this case, the use of HATU in combination with HOAt was found to be effective for the coupling, while HBTU was used in combination with HOBt for peptide synthesis unless otherwise mentioned.

Basic deprotection of the hydrophobically tagged dipeptide (**7**), however, did not give the desired product (**8**), instead leading to the formation of diketopiperazine (**9**) (Scheme S1, Supporting Information). It is well-known that basic deprotection induces diketopiperazine formation when the C-terminal residue is a proline. The *N*-alkylation of peptides also has a significant impact on their conformations (Table S2, Supporting Information). When a Boc-protected variant was used instead, both the Boc-group and the tag were removed through acidic deprotection (Scheme S2, Supporting Information). This could be avoided through the coupling of Fmoc-Phe-Phe-OH to the hydrophobically tagged Gly-OMe (**3**) (Scheme 3). This step was evaluated by HPLC, and only a trace amount of epimerized variant was observed (Figure S1, Supporting Information). Basic deprotection of the hydrophobically tagged tripeptide (**10**) gave the desired product (**11**) predominantly, accompanied by the formation of only a trace amount of cyclized compound (**12**) (Scheme 4). This could be simply rationalized based on the resulting ring sizes; thus, the formation of six-membered rings was much more favorable than that of nine-membered rings.

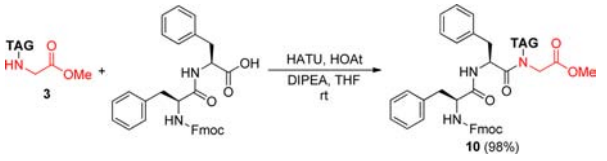
Scheme 1. Retrosynthetic Strategy for Mahafacyclin B (**1**)



Scheme 2. Preparation of the Hydrophobically Tagged Gly-OMe (**3**) and the Hydrophobically Tagged Dipeptide (**7**)



Scheme 3. Preparation of the Hydrophobically Tagged Tripeptide (**10**)



With the hydrophobically tagged tripeptide (**11**) in hand, the desired sequence (**13**) was then elaborated using general Fmoc-chemistry followed by basic deprotection, which removed both the Fmoc-group and the C-terminal methyl ester to give the desired product (**2**) in one step (Scheme 5). All reactions took place efficiently in less polar solvents followed by dilution with poor solvents to give the product

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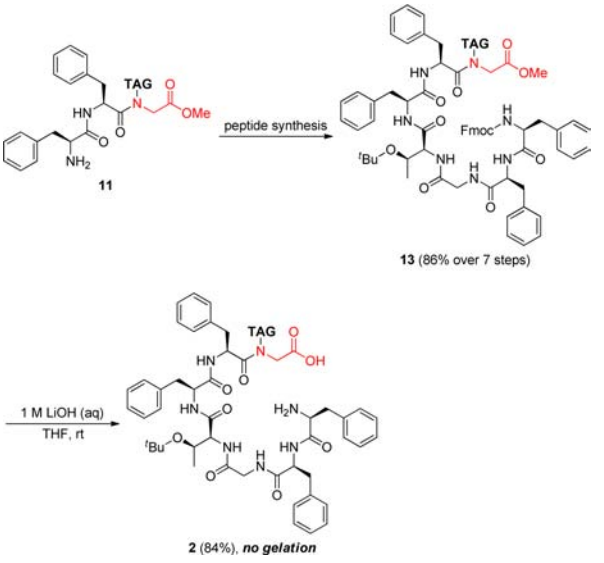
Scheme 4. Basic Deprotection of the Hydrophobically Tagged Tripeptide (**10**)

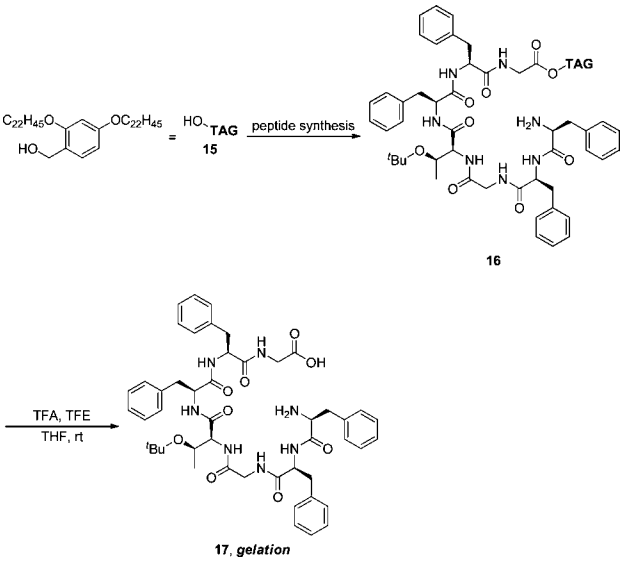
as a precipitate. Generally, a high content of hydrophobic residues tends to cause peptide aggregation in polar solvents, which might be a crucial drawback for peptide cyclizations, because folding of their linear precursors is impeded. Indeed, when conventional C-terminal hydrophobic benzyl alcohol (**15**) was used instead as a support for construction of the same sequence (**16**), acidic deprotection of the tag required to form the linear precursor (**17**) induced severe gelation (Scheme 6 and Scheme S3, Supporting Information). In sharp contrast, the linear precursor (**2**) was highly soluble in less polar solvents due to the hydrophobic tag. Finally, the head-to-tail cyclization took place effectively to give the cyclic version (**14**) with the tag remaining without any gelation (Scheme 7). This facilitated the reaction workup and product isolation significantly, and acidic deprotection gave mahafacyclin B (**1**).

Scheme 5. Preparation of the Linear Precursor (**2**)

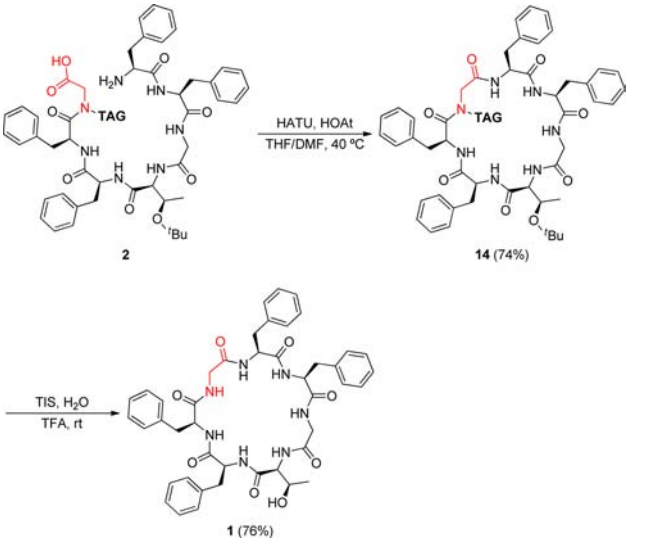


In conclusion, we successfully applied a soluble tag-assisted liquid-phase method to peptide head-to-tail cyclization, leading to the total synthesis of mahafacyclin B (**1**). The cyclization was carried out in the liquid phase with the tag remaining, which allowed rapid reaction workup and product isolation. Because this strategy employs an amide nitrogen as the tagging site instead of the C-terminus, it should find further application in the preparation of several head-to-tail cyclized peptides in a sequence-independent manner.

Scheme 6. Preparation of the Linear Precursor (**17**) Using Conventional C-Terminal Protecting Tag (**15**)



Scheme 7. Synthesis of Mahafacyclin B (**1**)



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Supporting Information Available. Additional schemes, tables, figures, general information, experimental information, spectral information, and copies of ^{1}H and ^{13}C NMR. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.