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Solid-Phase Synthesis of γ-AApeptides Using a Submonomeric Approach

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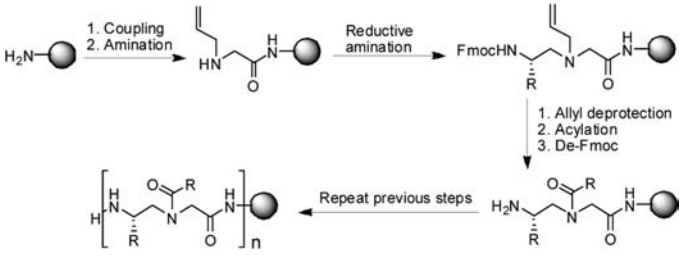
ABSTRACT



The solid-phase synthesis of γ-AApeptides using a novel submonomeric approach that utilizes an allyl protection is reported. The strategy successfully circumvents the necessity of preparing γ-AApeptide building blocks in order to prepare γ-AApeptide sequences. This method will maximize the potential of developing chemically diverse γ-AApeptide libraries and thereby facilitate the biological applications of γ-AApeptides in the future.

Unnatural peptidomimetics have been investigated for more than a decade and are of increasing importance in chemical biology and drug discovery.[1,2] Besides being resistant to protease degradation and straightforward derivatization, many classes of peptidomimetics, such as β-peptides,[3−5] peptoids,[6] α/β-peptides,[7,8] oligoureas,[9,10] azapeptides,[11−13] and oligocarbamates,[14] have shown versatile biological applications by mimicking structures and functions of bioactive peptides. One of the most important applications is to generate short peptide-like oligomeric ligands that specifically target proteins of interest, so as to facilitate the discovery of potential drug candidates[14] or identification of protein-binding molecules.[15,16] Such research efforts, with the development of proteomics, lead to an unprecedented need for the rapid generation of a chemically diverse combinatorial library.[17] A very elegant and successful example is the development of peptoid combinatorial libraries by Kodadek's group to identify short peptoid ligands that

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bind to a range of proteins with excellent specificity and affinity.[15,16,18−22] Nonetheless, there are urgent needs to develop novel combinatorial libraries with new scaffolds and functional groups in order to discover new classes of ligands with enhanced specificity, affinity, and other biological properties. This is because, with the presence of new side chains and 3D structures, ligands with distinct functional properites are expected to be identified.[23]

We have recently developed a new class of peptidomimetics termed "γ-AApeptides", as they comprise N-acylated-N-aminoethyl amino acid building blocks (Figure 1), and chiral side chains are linked to the γ-carbon in the building blocks.[24] The other half of the side chains are introduced onto the γ-AApeptide scaffold through acylation of the center N in each building block using a wide variety of commercially available carboxylic acids, which endow γ-AApeptides with a limitless potential for the generation of chemically diverse libraries. In contrast to α-peptides, each γ-AApeptide unit is comparable to a dipeptide, and γ-AApeptides and α-peptides of the same lengths project the same number of side chains. As such, there is a strong potential to identify γ-AApeptides that can mimic the structures and functions of α-peptides.

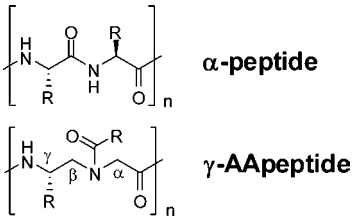

Figure 1. Representative structure of a native α-peptide and a γ-AApeptide.

Indeed, similar to other classes of peptidomimetics, γ-AApeptides have been shown to be highly resistant to protease degradation.[24] More importantly, they are able to disrupt protein−protein interactions[24] and mimic the Tat peptide by binding to HIV-1 RNA[25] and facilitating membrane translocation[26] with comparable affinity and efficiency. More recently, we have also demonstrated that γ-AApeptides are potential antibiotic agents to combat drug resistance by mimicking the mechanism of action of natural antimicrobial peptides.[27−29] Thus, it is envisioned that there is great potential to identify γ-AApeptide-based ligands from a combinatorial library to bind to proteins of interest with high specificity and affinity.

However, the previous approach of solid-phase synthesis of γ-AApeptides (Figure 2)[24−29] is not suitable for the development of combinatorial libraries. In this method, a γ-AApeptide sequence is prepared by assembling γ-AApeptide building blocks on solid phase. Each building block requires a three-step synthesis (reductive amination, acylation, and deprotection) starting from the corresponding Fmoc-amino aldehyde. For instance, to prepare a random library of short γ-AApeptides containing three building blocks (6 side chains, comparable to 6-mer peptides), with the availability of 10 Fmoc-amino aldehydes (R = 10) and 10 carboxylic acids (R = 10), 100 different building blocks have to be generated, which is almost impossible to achieve.

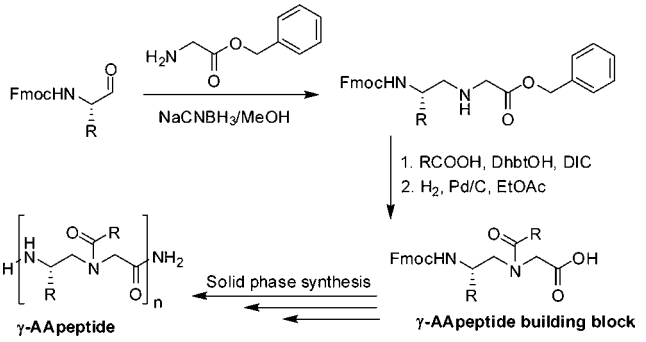

Figure 2. Previous method for the synthesis of γ-AApeptides.

Submonomer approach has been used by many groups to synthesize different classes of oliogmeric peptidomimetics.[30−33] To rapidly develop γ-AApeptide libraries, so as to maximize their biological potential, herein we report the development of a novel submonomeric approach for the solid-phase synthesis of short γ-AApeptides by utilizing an allyl protection. This method circumvents the necessity of γ-AApeptide building block preparation, thereby it is expected to greatly facilitate the application of γ-AApeptides in biomedical sciences in the future.

The new route for the solid-phase synthesis of γ-AApeptides using the submonomeric approach is shown in Figure 3. The first two steps have been used in the microwave-assisted

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preparation of peptoids[17] and have proven to be highly efficient. In brief, **1** is obtained through the microwave-assisted coupling of bromoacetic acid with the amino group on the Rink amide resin using DIC as the activation agent.[17] With the assistance of a 1000 W commercial microwave, the reaction is accomplished in 4 min (8 × 30 s). Then excess allyl amine is added as the nucleophilic agent to form a secondary amine on the solid phase to give **2**, which again is assisted by microwave and finished in 4 min (8 × 30 s). We reason the introduction of the allyl protecting group is critical since it completely avoids the constant overalkylation occurring in the reductive amination of Fmoc-amino aldehyde with the primary amino group on the solid phase.[34,35] Although over alkylation can be potentially alleviated by draining out excess aldehyde remaining in the solution during the imine formation step, it does not solve the problem;[34] on the contrary, incomplete imine formation is seen when the draining method is used since the formation of the imine is not efficient.[34] As such, successful preparation of sequences employing repetitive reductive amination reactions on the solid phase is rare due to such complexity of overalkylation and incomplete reaction.

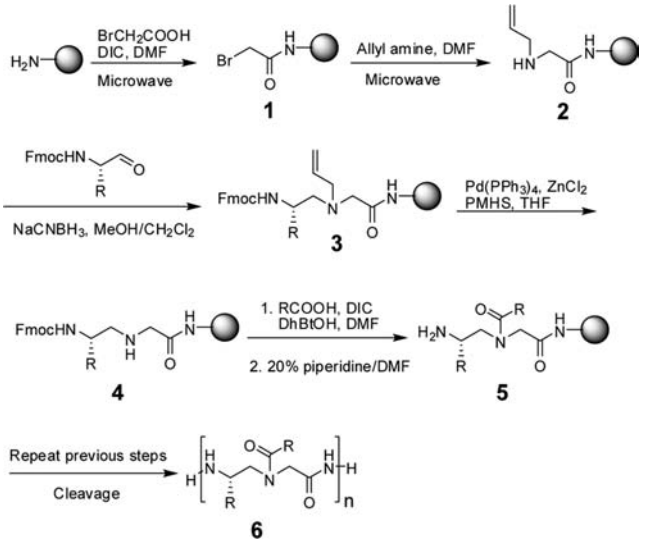

Figure 3. New route for the synthesis of γ-AApeptides by submonomeric approach. DIC = Diisopropylcarbodiimide, PMHS = polymethylhydrosiloxane, DhBtOH = 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine.

With the protection of the allyl group, **3** can be obtained free of side reactions, as the reductive amination step can be repeated in order to achieve quantitative conversion. The allyl protecting group is selectively removed by using PMHS-$ZnCl_2$/$Pd(PPh_3)_4$ in THF for 4 h (twice) to provide **4**. Although this method has only been used to convert allyl-protected secondary amines to primary amines,[36] we found that deprotection of tertiary amines **3** under the same condition is also extremely efficient. Followed by double coupling of carboxylic acids and deprotection of Fmoc, the first building block **5** is accomplished, which ends the first synthetic cycle on the solid phase. The desired γ-AApeptides therefore can be generated by repeating the synthetic cycles.

To demonstrate the efficiency of this approach, Fmoc-Phe-CHO was first used for the reductive amination of **2a**, and CH_3COOH was used to acylate **4a**. Both **4a** and **5a** were cleaved by 95% TFA/H_2O and analyzed by HPLC. As shown in Figure 4, every step in the synthetic cycle, including reductive amination, allyl deprotection, and acylation, is highly efficient, as crude **4a** and **5a** showed more than 95% purity.

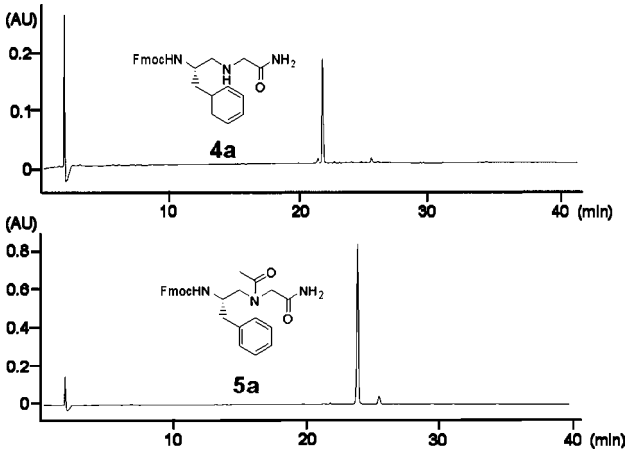

Figure 4. HPLC traces of crude **4a** and **5a** that were monitored at 215 nm.

To further prove the practical application of this approach in the future development of a γ-AApeptide combinatorial library, three random γ-AApeptide sequences **6a**, **6b**, and **6c** (Figure 5a) were synthesized from a pool of Fmoc-amino aldehydes[34,37] and carboxylic acids (Figure 5b) that contain a variety of charged and hydrophobic groups. As shown in Figure 5a, **6a** and **6b** are γ-AApeptides containing three building blocks, which are comparable to 6-mer peptides in length, whereas **6c** is a γ-AApeptide having five building blocks and thereby a 10-mer peptide mimic. We believe these γ-AApeptide sequences are sufficiently long enough to compose combinatorial libraries in the future for the identification of potential drug candidates or protein-binding ligands. If the satisfactory yield of these γ-AApeptides can be achieved, this submonomeric approach will definitely be able to be used to generate γ-AApeptide libraries much more rapidly than the current building block strategy.

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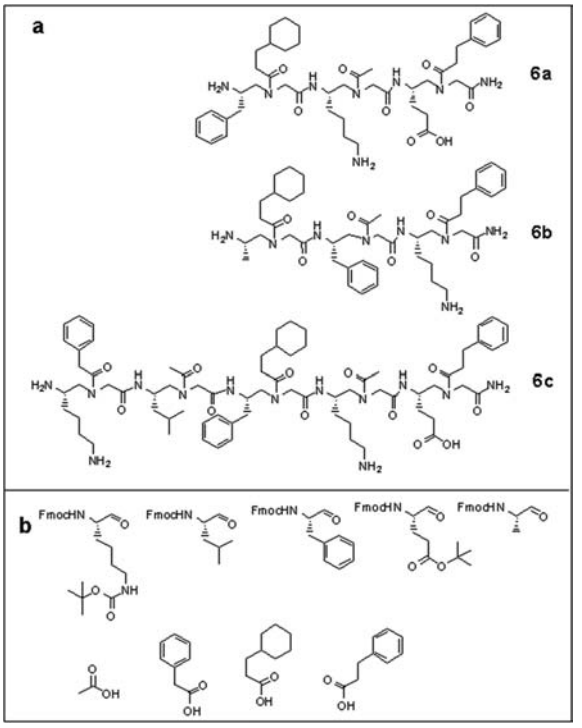

Figure 5. (a) Random γ-AApeptide sequences **6a**, **6b**, and **6c**. (b) Fmoc-amino aldehydes and carboxylic acids used to prepare **6a**, **6b**, and **6c**.

Table 1. Product Characteristics Based on the Monomeric Approach

γ-AApeptide	**6a**	**6b**	**6c**
purity[a]	68%	60%	56%
yield[b]	31%	27%	22%

[a] Calculated based on crude HPLC. [b] Calculated based on the purified product after lyophilization.

Surprisingly, the submonomeric method led to the production of these three γ-AApeptide sequences with good yield and purity (Table 1, Figure 6, and Supporting Information Figure S1). Although there are some impurities seen in crude HPLC traces, which may come from the long-time exposure of resin to air and to water moisture in the solvents during solid-phase synthesis, the quality of these crude γ-AApeptides is consistent and considerably high. They can be easily purified (Figure 6) and provided with the excellent overall yields. However, as seen in Figure S2, to prepare same three γ-AApeptide sequences **6a**, **6b**, and **6c**, seven building blocks would have to be prepared using the previous building block strategy, which is much more tedious and time-consuming. Thus, this new submonomeric approach is a real breakthrough.

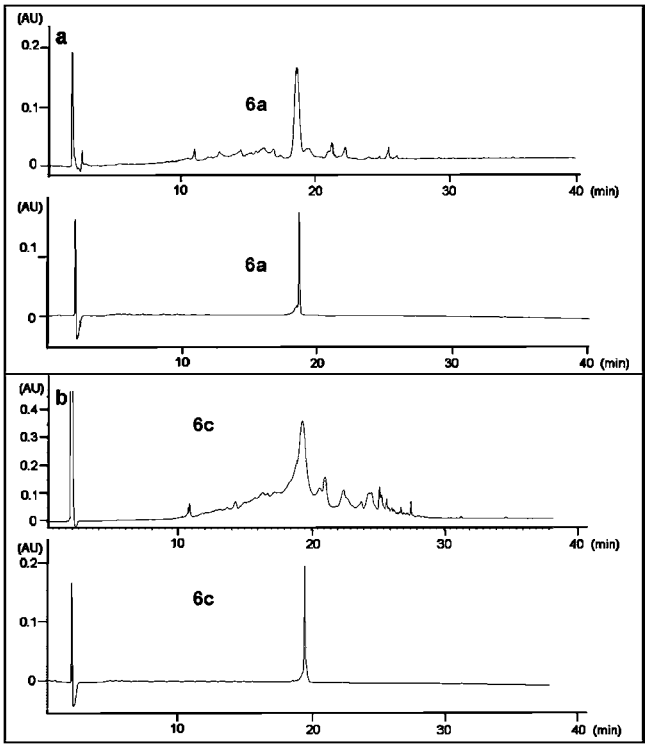

Figure 6. HPLC profiles of γ-AApeptide **6a** and **6c**. (a) Top, HPLC trace of crude **6a**; bottom, HPLC trace of purified **6a**. (b) Top, HPLC trace of crude **6c**; bottom, HPLC trace of purified **6c**.

In summary, we have reported a novel submonomeric method to prepare short γ-AApeptides. This strategy circumvents the needs to prepare γ-AApeptide building blocks and therefore greatly facilitates the rapid preparation of chemically diverse γ-AApeptide libraries. The application of this approach will unprecedentedly enhance the biological potential of γ-AApeptides. The preparation of the γ-AApeptide combinatorial library for specific protein targeting is currently under investigation.

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Supporting Information Available. Experimental details, characterization of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.