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Design and synthesis of AApeptides: A new class of peptide mimics

Yaogang Hu [a], Xiaolong Li [b], Said M. Sebti [c], Jiandong Chen [b], Jianfeng Cai [a,*]

[a] Department of Chemistry, University of South Florida, 4202 E. Fowler Ave, CHE 205, Tampa, FL 33620, USA
[b] Molecular Oncology Department, Moffitt Cancer Center, 12902 Magnolia Drive, Tampa, FL 33612, USA
[c] Drug Discovery Department, Moffitt Cancer Center, 12902 Magnolia Drive, Tampa, FL 33612, USA

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ABSTRACT

A new family of peptide mimics termed 'AApeptides', which are oligomers of N-acylated-*N*-aminoethyl amino acids, was proposed. The design and efficient synthesis of AApeptides are described. As proof-of-the-concept, we show that AApeptides can inhibit p53/MDM2 protein–protein interaction with significant activity (IC_{50} = 38 μM) and specificity. Preliminary data also demonstrates that AApeptides are resistant to enzymatic hydrolysis. With the ease of synthesis and diversification, potent bioactivity, and resistance to proteolysis, the development of sequence-specific AApeptides may expand the potential biomedical applications of peptidomimetics.

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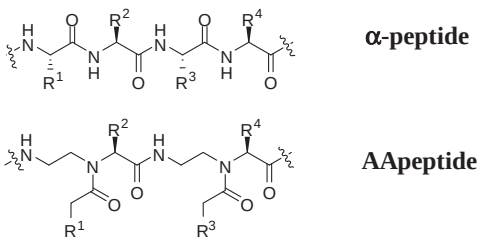
The creation and development of non-natural peptide mimics, or so called 'peptidomimetics', has become an area of high interest in bioorganic and chemical biology.[1] Examples of these sequence-specific oligomers include peptoids,[2] β-peptides,[3–5] γ- and δ-peptides,[6–8] oligoureas,[9,10] azapeptides,[11,12] α-aminoxy-peptides,[13] sugar-based peptides,[14,15] α/β-peptides,[16,17] polyamides,[18] and phenylene ethynylenes.[19] These peptidomimetics are designed to mimic peptide primary structure through the use of unnatural backbones. They are often stable against proteolysis, and are believed to have reduced immunogenicity and improved bioavailability compared to peptides.[20] They have displayed interesting structures and functions, and have begun to find some important biomedical applications.[21,22] Nonetheless, the applications of peptidomimetics are still very limited, partially hampered by the availability of frameworks.[22] A wide range of new peptide mimics with new structures and functions are urgently needed to be explored.[17,22] Such new peptide mimics are increasingly important for the generation of new focused library for drug discovery, design of potential therapeutics by disrupting protein–protein interactions or inhibiting enzyme activities, and design of novel antimicrobial peptidomimetics, etc.

In the attempt to search peptidomimetics with novel frameworks, herein we propose a family of chemically diverse peptide mimics based on a modified *N*-(2-aminoethyl)-amino acid backbone from chiral PNAs.[23,24] To the best of our knowledge, such sequence-specific peptide mimics have not been reported to mimic protein/peptide functions. They are termed 'AApeptides' because they are comprised of N-acylated-*N*-aminoethyl amino acids (Scheme 1).

Compared to natural α-peptides, the repeating unit of the AApeptide backbone is comparable to two adjacent residues of α-peptide because it contains two side chains, one of which is an α-amino acid side chain, while the other comes from a carboxylic acid residue on the adjacent tertiary amide nitrogen. As a result, AApeptides are projecting identical number of side functional groups as conventional peptides with same length of backbones. Similar to natural peptides, all the nitrogen atoms on the AApeptide backbone have formed either secondary or tertiary amide bonds. Such AApeptides are designed in a way so that they can be efficiently synthesized and easily derivatized, while potentially keep the structural and functional properties of conventional peptides. It is important to note that because AApeptide and peptide backbones are different, their hydrogen bonding properties and conformational



Scheme 1. Structures of an α-peptide and the corresponding AApeptide.

* Corresponding author. Tel.: +1 813 974 9506.
E-mail address: jianfengcai@usf.edu (J. Cai).

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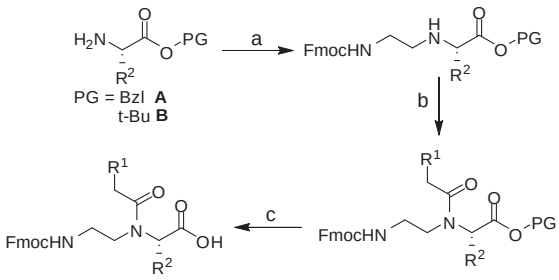


Scheme 2. Typical synthesis of an AApeptide building block. (a) Fmoc-amino ethyl aldehyde, $NaBH_3CN$, CH_3OH, overnight. (b) R_1CH_2COOH, DhBtOH/DIC, overnight. (c) Pd/C, H_2, EtOAc for **A**; 50% TFA/CH_2Cl_2 for **B**.

flexibility (AApeptides are expected to have much more conformations due to the higher flexibility of backbones and the existence of cis/trans conformations of *N*,*N*-disubstituted amide bonds) are not identical. Direct translation of sequences from peptides into AApeptides may not exhibit the same bioactivity since their conformations are directly related to their functions.

The synthesis of AApeptide sequences is very simple and highly efficient by assembling AApeptide building blocks (Scheme 2) on solid phase, which is similar to standard solid phase synthesis of conventional peptides.

AApeptide building blocks can be prepared readily using low cost commercially available agents. In this basic process, Fmoc-amino ethyl aldehyde reacts with amino acid esters to form secondary amines, which are subsequently acylated with carboxylic acids. Deprotection of the coupling products gives the desired AApeptide building blocks. Starting materials are readily available, and the potential of rapidly forming AApeptide derivatives with a wide variety of side chains is almost limitless because there are thousands of carboxylic acids available for acylation.

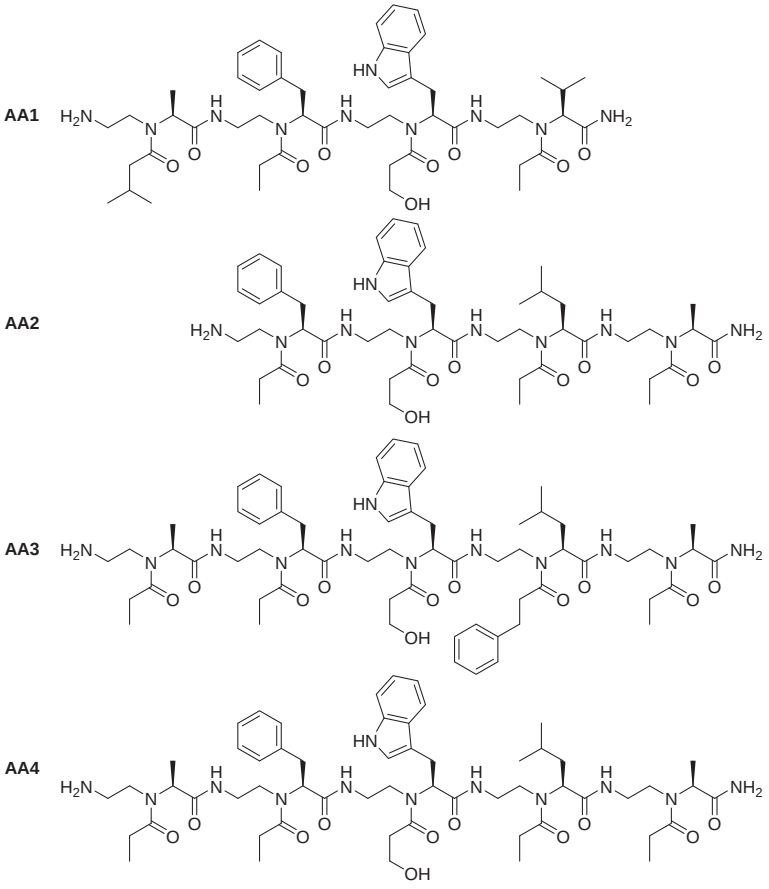
To demonstrate the facile synthesis and potential bioactivity of AApeptides, as a proof-of-principle, we designed four AApeptide sequences to target p53/MDM2 protein–protein interaction (Scheme 3) by preliminary computer modeling (Fig. S1, Supplementary data), and synthesized them on the solid phase.

These sequences were purified by HPLC with over 95% purity. For convenience, in each AApeptide residue in a sequence, the amide side chain is given the same designation corresponding to the cognate amino acid, with the prime ′, and the name of the chiral side chain is kept the same as the corresponding α-amino acid. These sequences were then tested for the inhibition of p53/MDM2 protein–protein interaction by the ELISA assay[25] (Table 1).

Targeting p53/MDM2 protein–protein interaction was chosen to demonstrate our AApeptide approach for peptide mimicry because the interaction has been a proving ground for the new non-natural peptide mimic strategy.[26–28] Phe19, Trp23 and Leu26 from p53 helical domain are largely responsible for the binding

Table 1
ELISA results of AApeptides for the disruption of p53/MDM2

AApeptides	IC_{50} (μM)
AA1. Val′-Ala-Ala′-Phe-Ser′-Trp-Ala′-Val	>1000
AA2. Ala′-Phe-Ser′-Trp-Ala′-Leu-Ala′-Ala	120 ± 10
AA3. Ala′-Ala-Ala′-Phe-Ser′-Trp-Phe′-Leu-Ala′-Ala	120 ± 16
AA4. Ala′-Ala-Ala′-Phe-Ser′-Trp-Ala′-Leu-Ala′-Ala	38 ± 8
p53-derived peptide (Ac-QETFSDLWKLLP)	8.7[26]



Scheme 3. AApeptide sequences synthesized for p53/MDM2 disruption.

of p53 to MDM2. Potent oligomeric peptidomimetics have been reported, including scaffolds based on peptoids,[29,30] β-peptides,[5,31,32] *N*-acylpolyamine,[33] β-sheet cyclic peptides,[34] etc. These oligomers were able to project functional groups to occupy the positions of those three critial residues of p53. Here AApeptides were designed to bear either all or some of the three functional groups (Phe, Trp and Leu), which were assumed to compete with Phe19, Trp23 and Leu26 of p53 and disrupt p53/MDM2 interaction. The other functional groups were randomly chosen and introduced from carboxylic acids into AApeptides through the formation of tertiary amide bonds. Indeed, the ELISA results show that AApeptide **AA4** has an IC_{50} of 38 μM, which is only fourfold less active than the wild-type p53-derived peptides,[26] and comparable to several previously reported peptoids and β-peptides.[29,35,36] As shown in Figure S1, the side chains of Phe, Trp and Leu of **AA4** overlap very well with those residues (Phe19, Trp23 and Leu26) in p53, which are responsible for recognizing MDM2 in its binding cleft. Such inhibition, on the other hand, may indicate that the AApeptides are likely to adopt extended conformations when binding to MDM2 as shown in the computer modeling results (Fig. S1). The information will be very valuable for rational design of bioactive and functional AApeptides in the future. Based on the ELISA results, more potent AApeptide inhibitors should be obtained by introducing halogen atoms,[26,29,32] restraining backbone to stabilize secondary structure, and carrying out further computer-aided design.

The AApeptides also exhibit excellent selectivity. AApeptide **AA1** is a poor inhibitor of p53/MDM2 interaction, while **AA2** and **AA3** are weaker inhibitors compared to **AA4**. Structure–activity relationship (SAR) is consistent to previous reported studies.[26] Phe, Typ and Leu functionalities are necessary for strong binding, which are absent in **AA1** but present in all other sequences. Comparing **AA2** to the same length AApeptide **AA1**, the change of Leu into Val decreases the activity at least 10-fold. Second, it seems longer sequences have better activities, as seen for **AA4** and **AA2**, possibly due to the higher stability of the backbone conformations. Side chains that are not involved in the recognition of MDM2 hydrophobic binding cleft also play a very important role for the overall interactions, since **AA3** and **AA4** differ for only one residue. In **AA3**, the Phe′ side chain may clash with the residues of MDM2 near the edge of the binding domain, which probably increases its binding energy to MDM2. Detailed SAR study for sequences with a variety of lengths and distribution of functional groups along the AApeptide backbone is currently ongoing, which could further shed light on the rational design of AApeptide library for drug discovery.

A significant disadvantage of peptides is their susceptibility to proteolysis. To assess the sensitivity of AApeptides to enzymatic hydrolysis, we incubated a representative sequence **AA3** with chymotrypsin, trypsin, and pronase (0.1 mg/ml) respectively in 100 mM pH 7.8 ammonium bicarbonate buffer for 24 h. The reaction mixtures were analyzed by HPLC by comparing their retention time and integration to those of the starting material. The results (see Supplementary data) show that AApeptide **AA3** is highly resistant to proteolysis. After 24 h incubation, **AA3** was not cleaved by the enzymes.

In conclusion, we have designed a new family of peptide mimics-AApeptides and described a simple approach for their efficient synthesis based on N-acylated-*N*-Fmoc-amino ethyl amino acid building blocks. The potential of AApeptide diversification by introducing a wide variety of side groups is substantial. The preliminary results demonstrated their superior stability against proteolysis, significant bioactivity and excellent selectivity toward p53/MDM2 protein–protein interaction. The development of such sequence-specific AApeptides may expand the applications of peptidomimetics in the areas of biomedical and material sciences, such as modulation of protein–protein interactions, and generation of focused library for drug discovery, etc. We are currently carrying out systematic studies to probe structural requirements for AApeptides to adopt predicted conformations using 2D-NMR, Circular Dichroism (CD) and X-ray crystallography. Optimization of AApeptide sequences through rational design to achieve more potent bioactivity towards p53/MDM2 and other proteins/nucleic acids/carbohydrates interactions are also under investigation.

Acknowledgments

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Supplementary data

Supplementary data (experimental details include AApeptide building block and sequence synthesis, purification, characterization, ELISA, CD, and assay for enzymatic hydrolysis) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.005.

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