

α -Helix Stabilized Peptides via an all Hydrocarbon-staple Conferring an Improved Inhibitory Activity against 3'-Processing of HIV-1 Integrase

Ya-Qiu Long^{1*}, Shao-Xu Huang¹, Hui-Yuan Li¹ and Nouri Neamati²

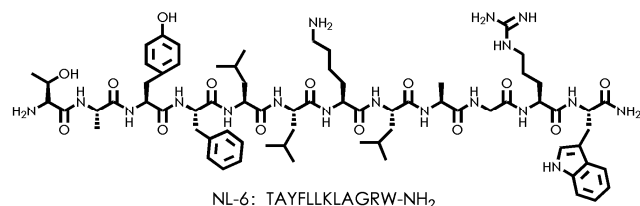
¹State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, Shanghai, China; ²Department of Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, California, United States

E-mail: yqlong@mail.shcnc.ac.cn

Introduction

Integration of viral cDNA into the host genome is a vital step in the HIV replication cycle.¹ This process is mediated by HIV-1 integrase (IN), a 32 kDa viral enzyme. IN has no mammalian counterpart, and uses a single active site to accommodate two different configurations of DNA substrates, which may constrain the ability of HIV to develop drug resistance to integrase inhibitors, so IN₃ has become an attractive target for antiviral drug design.^{2,3}

By employing a novel "sequence walking" strategy across the entire sequence of HIV-1 integrase, we identified an active peptide segment NL-6 (TAYFLLKLAGRW, IC₅₀ = 21 μ M for the 3'-processing) (Scheme 1)⁴.



Scheme 1. The structure of peptide NL-6

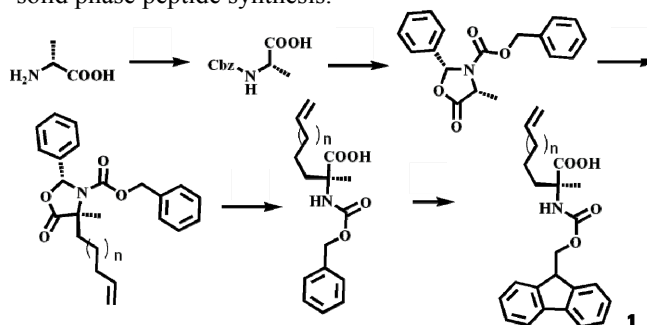
Since NL-6 is derived from the α 1 helix region of HIV-1 integrase and there is strong interaction between α -1 and α -5' helix regions of two integrase monomers during dimerization which is required for the catalytic function of the integrase, we suppose that stabilizing the helix conformation should enhance the interaction of NL-6 with the integrase thus improve the inhibitory activity. So we designed α -helix stapled peptides derived from NL-6 to enhance the inhibitory activity against HIV-1 integrase.

Results and Discussion

α -helix conformation of a linear peptide can be stabilized by introducing disubstituted olefinic amino acids in the sequence between $i/i + 4$ and $i/i + 7$ positions, and then bridging the side chain of the peptide by ruthenium-catalyzed ring-closing metathesis (RCM) reaction.⁵

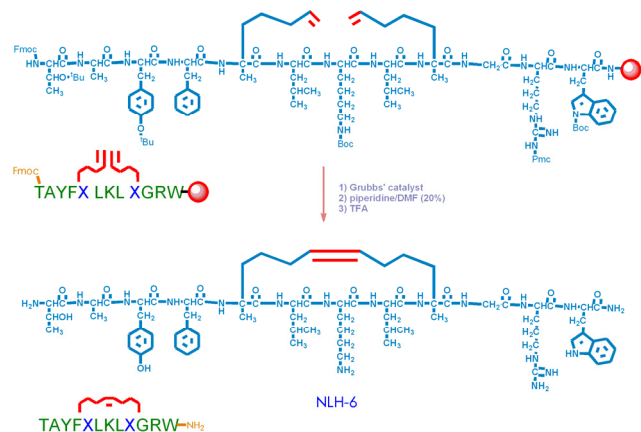
First, an efficient synthesis was established to synthesize the α -methyl- α -alkene amino acid suitably protected for solid phase peptide synthesis. As depicted in Scheme 2, we adopted Seebach's oxazolidinone methodology with optimized conditions to achieve the synthesis of

(S)-N-Fmoc-2-(4'-pentenyl)alanine (1) suitable for the solid phase peptide synthesis.



Scheme 2. The synthesis of (S)-N-Fmoc-2-(4'-pentenyl)alanine by using optimized Seebach's oxazolidinone methodology




The appropriate incorporation of α,α -disubstituted amino acid into position i and $i+4$ of the active peptide NL-6 followed by the cyclization via RCM reaction on solid phase (Scheme 3) resulted in an increase of an α -helicity and metabolic stability, thus gaining a corresponding improvement of the HIV-1 integrase inhibitory activity.



Scheme 3. The synthesis of all-hydrocarbon bridged peptide NLH-6 by using Fmoc chemistry and RCM reaction

Bioassay indicated that the HIV-1 integrase inhibitory activity of the active peptide is correlated with the content of α -helix, and the incorporation of the α,α -disubstituted amino acid in position i and $i+4$ in the middle really stabilize the α -helix structure (Table 1).

Table 1. The α -helix content and their IC₅₀ against 3'-processing activity of HIV-1 integrase of the hydrocarbon-stapled peptides

No	Peptide Sequences	Content of α -Helix (%) in PBS/CH ₃ CN (V/V=1:1)	IC ₅₀ (μ M) (3'-processing activity)
1	TAYFLLKLAGRW	21	21
2	 XAYFXLKLAGRW	20	53
3	 TAYFXLKLXGRW	34	9
4	 TAYFLLXLAGXW	38	> 114

Our work is the first attempt to apply the concept of hydrocarbon-stapled α -helix peptides in the design of HIV-1 integrase inhibitors. The positive results of an enhanced α -helicity and increased HIV-1 integrase inhibitory activity proved the effectiveness of the novel strategy, and provided new templates for structural optimization of potent HIV-1 integrase inhibitors.

Acknowledgments

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