Peptides:
Breaking Away

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Synthesis and Conformational Analysis of a Natural Peptide Inhibitor of HIV-1 Integrase

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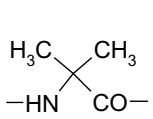
Introduction

Integramide A, an effective inhibitor of the coupled reaction of HIV-1 integrase, is a 16-mer linear peptide characterized by nine C^{α}-methylated α-amino acids [five Iva, isovaline, and four Aib, α-aminoisobutyric acid, residues (Figure 1)] that was isolated from fungal extracts of *Dendrodochium sp*. The amino acid sequence was fully elucidated by the Merck group a few years ago [1]. On the other hand, the chiral sequence was only partially determined. In particular, the precise stereochemistry of the Iva^{14}-Iva^{15} dipeptide (known to contain one D- and one L-residue) near the C-terminus was not reported.

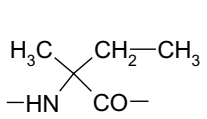
We solved this unsettled issue by performing *via* solution methods the total chemical independent syntheses of both L-D and D-L 16-mer diastereomers and compared their chromatographic and spectroscopic properties with those of the natural inhibitor [2].

1 5 10 14

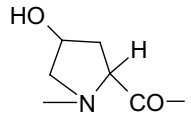
CH_3CO-D-Iva-L-Hyp-L-Ile-L-Iva-L-Leu-Aib-Aib-Aib-L-Hyp-L-Leu-L-Iva-Aib-L-Hyp-Iva-Iva-Gly-OH



Aib



Iva



Hyp

Fig. 1. Amino acid sequence of integramide A and chemical structures of Aib, Iva, and Hyp. Here, Hyp is (2S, 4R)-4-hydroxyproline.

Results and Discussion

The occurrence in the sequence of integramide A of as many as nine poorly reactive residues (Aib and Iva), including di- and tripeptide stretches, precludes an efficient peptide synthesis by the solid-phase approach. Therefore, we decided to synthesize by solution methods the L-Iva^{14}-D-Iva^{15} and D-Iva^{14}-L-Iva^{15} 16-mer diastereoisomeric peptides. To speed up the preparation of the two peptides and allow, in the future, a relatively fast synthesis of additional analogues, we followed a segment-condensation strategy [2].

Taking into account (i) the racemization risks associated with segment couplings, (ii) the acid lability of the Aib-Pro(Hyp) amide bond, and (iii) the remarkable tendency of the H-Aib-Pro- N-terminal dipeptide sequence to cyclize to the Aib-Pro diketopiperazine, we designed and prepared 4 segments: **A** (CH_3CO-D-Iva-OH), **B** (residues 2-8), **C** (residues 9-12), and **D** (residues 13-16). **B**, **C** and **D** were N- and C-protected with Z and O*t*Bu, respectively, whereas the secondary alcoholic function of the three L-Hyp residues was left unprotected. After selective removal of the protecting groups, the four segments were covalently linked (from **D** to **A**) by means of the highly effective EDC/HOAt activation procedure. The yields of each individual coupling step were from moderate to good (50-85%).

The two 16-mer peptides and their synthetic intermediates were characterized by HPLC, NMR, mass spectrometry, and chiral chromatography (with Chirasil-L-Val) analysis on the total acid hydrolyzates.

For the unambiguous assignment of the Iva^{14}-Iva^{15} stereochemistry of the natural integramide A, we relied on HPLC and NMR techniques. In particular, we observed that the HPLC retention times and the NMR chemical shifts of a natural sample (obtained from a purified fungal extract) were perfectly matching those of the synthetic L-Iva^{14}-D-Iva^{15} diastereoisomer [2].

We also performed an in-depth conformational analysis of the two final synthetic compounds and selected intermediates, of different main-chain length, in the crystal state (by X-ray diffraction) and in solvents of different polarities (using CD, FT-IR absorption, and 2D NMR techniques). By a combination of HMBC, HMQC, NOESY, and TOCSY experiments we were able to assign all proton and carbon NMR resonances. These data, together with molecular dynamics calculations, were also extremely useful to elucidate the preferred 3D-structure (Figure 2) for integramide A: a helical conformation characterized by the alignment on one face of all three L-Hyp residues.

Overall, our 3D-structural results have provided useful information to shed light on the mechanism of inhibition of HIV-1-integrase, an important target for anti-HIV therapy.

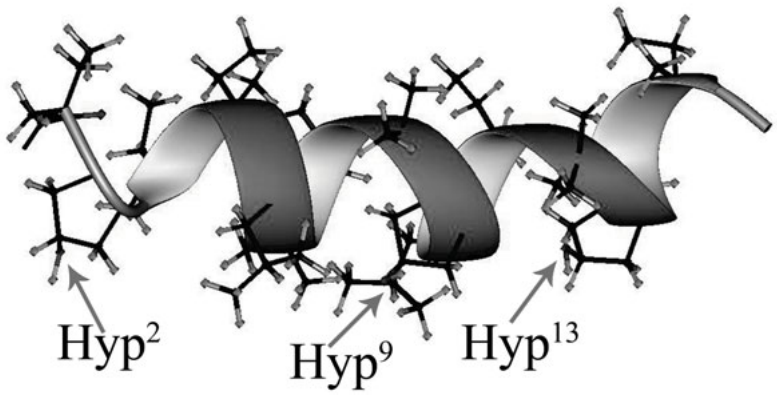


Fig. 2. Representation of the 3D-structure with the lowest energy obtained for integramide A from 2D-NMR experiments and molecular dynamics calculations. The three L-Hyp residues are located on the same face of the amphiphilic helical structure.

References

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2. De Zotti, M., Formaggio, F., Kaptein, B., Broxterman, Q.B., Felock, P.J., Hazuda, D.J., Singh, S.B., Brückner, H., Toniolo, C. *ChemBioChem* **10**, 87-90 (2009).