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### The chiral sequence of a natural peptide inhibitor of HIV-1 integrase elucidated

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Integramide A, an efficient inhibitor of the coupled reaction of HIV-1 integrase, is a 16-mer linear peptide characterized by nine C<sup> $\alpha$ </sup>-methylated  $\alpha$ -amino acids (five Iva, isovaline, and four Aib,  $\alpha$ -aminoisobutyric acid, residues) that was isolated from fungal extracts of *Dendrodochium sp.* The amino acid sequence was fully elucidated by the Merck groups a few years ago<sup>1</sup> (Fig. 1). On the other hand, the chiral sequence was only partially determined. In particular, the precise stereochemistry of the Iva14-Iva15 dipeptide (known to contain one D- and one L-residue) near the C-terminus was not reported.

To solve this unsettled stereochemical issue and to assess integramide A primary structure-bioactivity relationship we performed by solution methods the total chemical independent syntheses of both L-D and D-L 16-mer diastereomers and compared their properties with those of the natural inhibitor. For an unambiguous, complete stereochemical assignment of integramide A we relied heavily on HPLC (Fig. 2) and NMR (Fig. 3) techniques.



Figure 1. Amino acid sequence of integramide A and chemical structures of Aib, Iva, and Hyp.



**Figure 2.** *HPLC profiles for artificial mixtures of the natural integramide A* (**A**)*, and the two synthetic diastereomers L-Iva14-D-Iva15* (**L-D**) *and D-Iva14-L-Iva15* (**D-L**)*: mixture of L-D and A* (**I**)*; mixture of L-D, D-L, and A* (**II**)*; mixture of D-L and A* (**III**).



**Figure 3.** Comparison among the aliphatic regions of the  ${}^{\beta}C$ -selective HMQC spectra of the natural integramide A (A), and the two synthetic diastereomers L-Iva14-D-Iva15 (L-D) and D-Iva14-L-Iva15 (D-L) in TFE,d<sub>2</sub> solution at 300 K. The peaks of the Iva14 and Iva15 residues are highlighted.

Our results clearly indicate that the chirality sequence of the Iva14-Iva15 dipeptide of the natural product is L-D. The stereochemical inversion in the two integramide A diastereomers, evaluated as inhibitors of HIV-1 integrase in the coupled reaction of proviral DNA into the host cell DNA, is in general not detrimental, but it is even slightly beneficial against the strand transfer reaction.

#### References

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