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# Complete Absolute Configuration of Integramide A, a Natural, 16-mer Peptide Inhibitor of HIV-1 Integrase, Elucidated by Total Synthesis

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The enzyme HIV-1 integrase catalyzes the integration of proviral DNA into the host genome through a process that is unique to the virus and absent in the host. Therefore, inhibition of this specific catalytic activity is an attractive strategy for antiretroviral drug design. A large number of HIV-1 integrase inhibitors from different chemical families,[1] including peptides,[2] have been described. Nevertheless, the emergence of resistant viral strains requires continued effort towards the discovery of novel inhibitor leads.

A few years ago, some of the co-authors of the present work reported the primary structure of the naturally-occurring integramide A, a 16-amino-acid-long, effective peptide inhibitor of HIV-1 integrase.[3] They were also able to show that the α-carbons of the (2*S*,4*R*)-4-hydroxyproline (Hyp, Figure 1), Ile and Leu residues are all L (or *S*), whereas three of the five isovaline (Iva) or $C^{\alpha}$-methyl, $C^{\alpha}$-ethyl glycine residues are L and two are D. More specifically, Iva1, Iva4, and Iva11 were identified as D, L, and L, respectively. However, despite careful analysis of a partial acid hydrolysate of the peptide inhibitor, the chirality sequence of the two C-terminal Iva14 and Iva15 residues (one L and one D) could not be assigned. An additional motivation for our interest to study integramide A stems from the observation that its primary sequence is closely related to those of peptaibols[4]/peptaibiotics,[5a–c] a class of natural, membrane active, peptides characterized by the presence of a large amount of the overwhelmingly $3_{10}$/α-helicogenic,[6] $C^{\alpha}$-tetrasubstituted α-amino acids such as α-aminoisobutyric acid (Aib) or $C^{\alpha,\alpha}$-dimethylglycine[7] and Iva.[8]

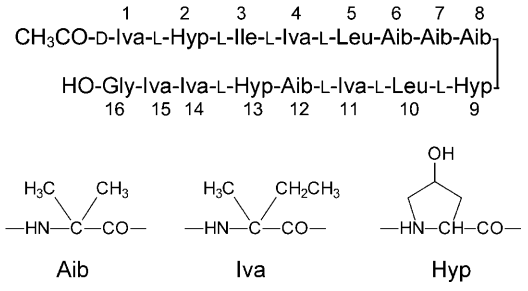


**Figure 1.** Amino acid sequence of integramide A and chemical structures of Aib, Iva, and Hyp.

To solve the unsettled stereochemistry of the Iva14-Iva15 dipeptide of integramide A, and to assess completely its primary structure-bioactivity relationship, we decided to perform the total chemical independent syntheses of both L-D and D-L 16-mer diastereomers and compare their physicochemical, analytical, and biological properties with those of the natural compound. As up to nine sterically hindered, poorly reactive, $C^{\alpha}$-tetrasubstituted α-amino acid residues, including di- and tripeptide stretches, precluded an optimal preparation of the two 16-mer peptides by the solid-phase approach, we were forced to synthesize them by a more time-consuming combination of step-by-step and segment condensation methods in solution.[9a,b] (For details of the strategy of synthesis and reagents used see Scheme S1 in the Supporting Information). The yields of each individual coupling step were from moderate to good (50–85%), and approximately 15–20 mg of each final diastereomer was produced. For the large scale production of the enantiomerically pure α-amino acids L- and D-Iva, we exploited an economically convenient and extensively applicable chemoenzymatic synthesis developed by DSM Pharmaceutical Products a few years ago.[10] Characterization of the two 16-mer peptides and their synthetic intermediates using HPLC, NMR and mass spectrometry, and chiral chromatography (with Chirasil-L-Val) analysis on the total acid hydrolyzates confirmed the chemical and optical purities of all compounds, with the single exception of a small amount (<4%) of epimerization observed only at the Leu5 residue for the L-Iva14-D-Iva15 diastereomer.

For a stringent comparison between integramide A and the two synthetic diastereomers and an unambiguous stereochemical assignment of the natural product, we relied heavily on HPLC and NMR techniques. After a variety of attempts to chromatographically distinguish the two diastereomers by exploiting different stationary phases and elution conditions, we eventually succeeded using an analytical reversed-phase Kromasil $C_{18}$ column. Figure 2 shows the HPLC profiles obtained by co-elutions of the natural integramide A and the synthetic

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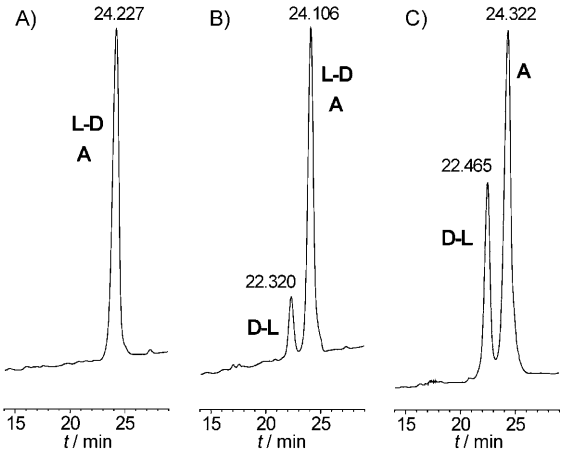


**Figure 2.** HPLC profiles for artificial mixtures of the natural integramide A (**A**), and the two synthetic diastereomers L-Iva14-D-Iva15 (L-D) and D-Iva14-L-Iva15 (D-L). A) Mixture of L-D and **A**. B) Mixture of L-D, D-L, and **A**. C) Mixture of D-L and **A**. Conditions: Kromasil $C_{18}$ column (250×4.6 mm i.d.; particle size: 5 μm; pore size: 100 Å); eluant systems: 1) 0.05% TFA/90% $H_2O$/$CH_3CN$ and 2) 0.05% TFA/10% $H_2O$/$CH_3CN$, gradient 65 to 90% 2) in 30 min.; flow rate: 1 mL min$^{-1}$; room temperature; absorbance detector at 226 nm.

L-Iva14-D-Iva15 and D-Iva14-L-Iva15 diastereomers. From this analysis we conclude that the D-Iva14-L-Iva15 16-mer exhibits a chromatographic behavior significantly different from that of integramide A (their retention times diverge by about 1.8 min; Figure 2c), whereas the integramide A and L-Iva14-D-Iva15 16-mer peaks perfectly co-elute (Figure 2a). This finding is corroborated by the HPLC profile of the ternary mixture (panel II).

Conclusive information on the complete chiral sequence of integramide A was obtained from combined 1D and 2D NMR (600 MHz) experiments in [$D_2$]-2,2,2-trifluoroethanol (TFE). In the 1D spectrum, the proton NH resonances are reasonably dispersed and minimally overlapped (Figure 3). The positions of the signals of Aib12, Iva14, Iva15, and Gly16 are the most informative. The spectra of integramide A and the L-Iva14-D-Iva15 diastereomer match very closely. (However, at this point we are still unable to explain the observation that the Gly16 triplet is slightly better resolved in the spectrum of the natural compound, whereas in the synthetic material it overlaps more with the Aib7 singlet). In contrast, the spectrum of the D-Iva14-L-Iva15 diastereomer is remarkably different. More specifically, upon going from the D-Iva14-L-Iva15 diastereomer to integramide A, the NH peaks of the Aib12, Iva14 and Gly16 residues move significantly upfield (particularly, the last two peaks), whereas that of Iva15 is shifted markedly downfield.

For the chemical shift assignment of all proton and carbon resonances, a combination of HMBC, HMQC, NOESY, and TOCSY[11] experiments were used. Figure 4 shows a comparison between the aliphatic regions of the $^{\beta}$C-selective HMQC spectra of the natural integramide A and the two synthetic 16-mer diastereomeric peptides; this comparison sheds further light on the chiral sequence of the 14-15 dipeptide segment. The peaks of the $^{13}C_{\beta}$ atoms of the β-methyl groups of the two C-terminal (14 and 15) Iva[12] residues shift considerably, almost exchanging their positions in the spectrum, on going from integramide A (and the L-Iva14-D-Iva15 synthetic 16-mer) to the D-Iva14-L-Iva15 16-mer peptide. This finding is of particular interest, and we consider it to be definitive proof that the stereochemistry of natural integramide A can be assigned as L-Iva14-D-Iva15.

The natural and synthetic integramides were evaluated as inhibitors of HIV-1 integrase in the coupled and strand transfer reactions of proviral DNA into host cell DNA.[3] Natural integramide A, remeasured in parallel with the synthetic samples, inhibits coupled and strand transfer reactions with $IC_{50}$ values of 10 and 30 μM, respectively. The $IC_{50}$ values of the synthetic L-Iva14-D-Iva15 peptide are 7 and 34 μM, while those of its D-Iva14-L-Iva15 diastereomer are 8 and 55 μM for the two bioassays, respectively, which clearly shows that both synthetic peptides exhibit very tight activities that are quite comparable to those of the natural compound. (Data were collected in duplicate and average data are reported). These results show that the stereochemical inversion in the Iva14-Iva15 natural se-

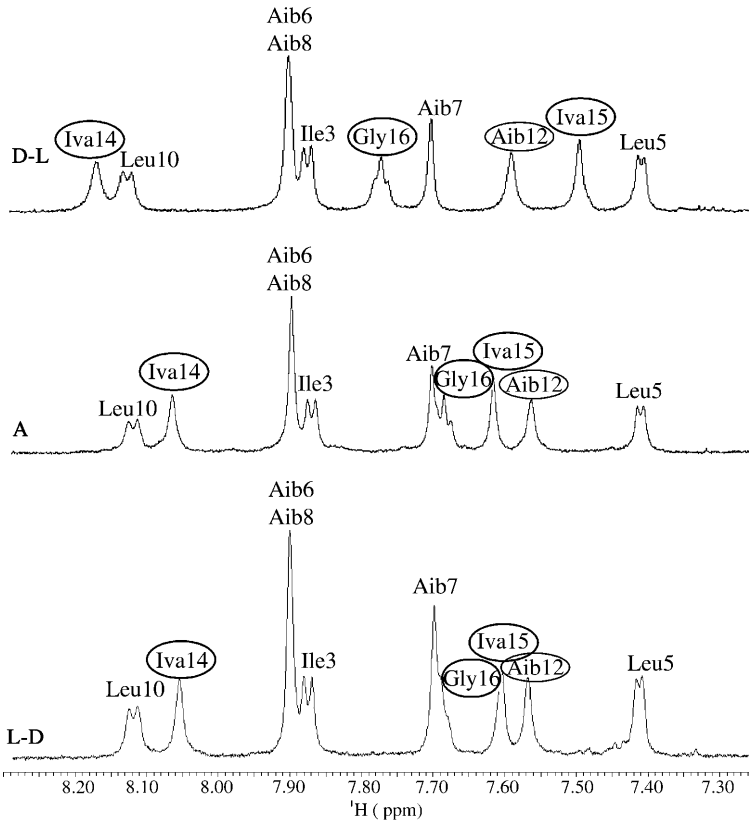


**Figure 3.** Comparison of the mono-dimensional $^1$H NMR spectra of natural integramide A (**A**), and the two synthetic diastereomers L-Iva14-D-Iva15 (L-D) and D-Iva14-L-Iva15 (D-L) in [D2]TFE solution at 300 K (recorded using a Bruker Avance 600 MHz spectrometer). Only a selected part of the NH proton resonance region is shown. The resonances of the Aib12, Iva14, Iva15, and Gly16 residues are highlighted. The NH chemical shift values for Iva1, Iva4, and Iva11 are observed below 7.15 ppm.

  

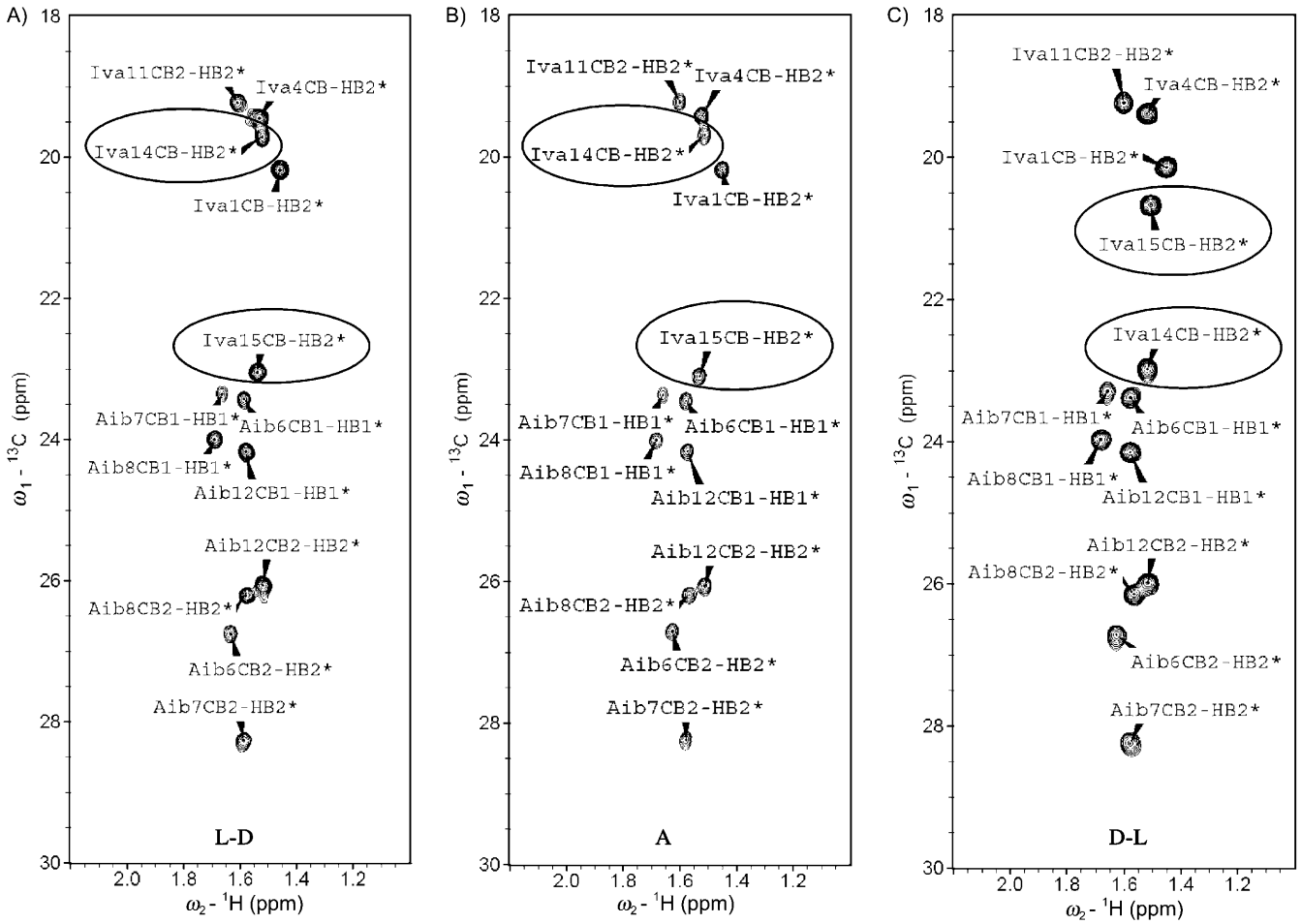


**Figure 4.** Comparison of the aliphatic regions of the $^{\beta}$C-selective HMQC spectra of B) natural integramide A (**A**), and the two synthetic diastereomers A) L-Iva14-D-Iva15 (L-D) and C) D-Iva14-L-Iva15 (D-**L**) in [D2]TFE solution at 300 K. The peaks of the Iva14 and Iva15 residues are highlighted.

quence is in general not detrimental, and might be slightly beneficial for activity against the strand transfer reaction.

In summary, using a multistep solution-phase strategy, which includes the preparation and combination of four segments, we synthesized two 16-mer diastereomeric linear peptides with the amino acid sequence of the non-ribosomal inhibitor of HIV-1 integrase, integramide A. Through HPLC and NMR comparison with an original sample of the purified fungal extract, we were able to solve the open issue of the Iva14-Iva15 stereochemistry in favor of the L–D chirality sequence. The segment-condensation synthetic strategy described here will allow in the future a relatively fast preparation of additional, appropriately designed, integramide A analogues, including a simplified version which incorporates achiral Aib residues. These residues are apparently unnecessary for bioactivity, but certainly more expensive than their chiral Iva counterparts. A detailed conformational investigation of integramides A and B,[3] the two 16-mer synthetic peptides and their shorter-sequence intermediates is currently in progress by use of combined FTIR absorption, CD, NMR and X-ray diffraction techniques.

**Keywords:** HIV inhibitor · HPLC · NMR spectroscopy · peptides · stereochemistry elucidation

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