Two alternative conformational states of \( \alpha, \alpha \)-dialkylglycyl-L-prolyl sequences governed by presence/absence of an NH group directly following the proline residue. X-ray crystal and molecular structures of \( \text{Boc-D-Iva-L-Pro-NHBzl} \) and \( \text{Boc-L-Iva-L-Pro-NHBzl} \).

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Two alternative conformational states of \(\alpha,\alpha\)-dialkylglycyl-L-proyl sequences governed by presence/absence of an NH group directly following the proline residue. X-Ray crystal and molecular structures of Boc-D-Iva-L-Pro-NHBzl and Boc-L-Iva-L-Pro-NHBzl

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The crystal structures of the isovaline-containing dipeptides, Boc-D-Iva-L-Pro-NHBzl and Boc-L-Iva-L-Pro-NHBzl were determined by X-ray diffraction. The diastereoisomeric peptides adopt intramolecular hydrogen-bonded \(\beta\)-turn conformations closely similar to each other. CD spectra of glycolic acid residue-containing analogues in place of the fourth Gly residue revealed a lack of \(\beta\)-turn tendency in these analogues, indicating the importance of intramolecular hydrogen bonding for the \(\beta\)-turn conformation of the central dipeptide moiety. The results are consistent with the reported unturned crystal structures of Aib-L-Pro and D/L-Iva-L-Pro sequence-containing peptides lacking the NH group which directly follows the Pro residue available for intramolecular hydrogen bonding.

Introduction

An \(\alpha\)-aminoisovaleryl \((\alpha,\beta\)-dimethylglycyl, Aib) residue is found commonly in membrane channel-forming antibiotics such as alamethicin I and suzukacin A. The Aib-L-Pro sequence in these antibiotic peptides is considered to contribute to the stabilization of their right-handed helical conformation. Owing to a combination of stericly crowded Aib residue and the cyclic imino acid Pro, the Aib-L-Pro sequence-containing peptides exhibit unusual chemical characteristics such as: (i) low reactivity of the free amino group of the Aib residue in N-terminal Aib-L-Pro-containing peptides, and (ii) readily occurring acidolytic cleavage of the Aib-L-Pro linkage. A survey of crystal structures of Aib-L-Pro-containing peptides revealed that only two conformations are possible for the dipeptide moiety (Fig. 1), i.e., an intramolecularly hydrogen-bonded \(\beta\)-turn conformation \(\varphi_{\text{Aib}} = 46^\circ, \psi_{\text{Aib}} = 31^\circ, \varphi_{\text{pro}} = 55^\circ, \psi_{\text{pro}} = 36^\circ\) and a relaxed conformation \(\varphi_{\text{Aib}} = 50^\circ, \psi_{\text{Aib}} = 37^\circ, \varphi_{\text{pro}} = 65^\circ, \psi_{\text{pro}} = 148^\circ\).  

An \(\alpha\)-aminoisovaleryl \((\alpha,\beta\)-dimethylglycyl, Aib) residue is found commonly in membrane channel-forming antibiotics such as alamethicin I and suzukacin A. The Aib-L-Pro sequence in these antibiotic peptides is considered to contribute to the stabilization of their right-handed helical conformation. Owing to a combination of stericly crowded Aib residue and the cyclic imino acid Pro, the Aib-L-Pro sequence-containing peptides exhibit unusual chemical characteristics such as: (i) low reactivity of the free amino group of the Aib residue in N-terminal Aib-L-Pro-containing peptides, and (ii) readily occurring acidolytic cleavage of the Aib-L-Pro linkage. A survey of crystal structures of Aib-L-Pro-containing peptides revealed that only two conformations are possible for the dipeptide moiety (Fig. 1), i.e., an intramolecularly hydrogen-bonded \(\beta\)-turn conformation \(\varphi_{\text{Aib}} = 46^\circ, \psi_{\text{Aib}} = 31^\circ, \varphi_{\text{pro}} = 55^\circ, \psi_{\text{pro}} = 36^\circ\) and a relaxed conformation \(\varphi_{\text{Aib}} = 50^\circ, \psi_{\text{Aib}} = 37^\circ, \varphi_{\text{pro}} = 65^\circ, \psi_{\text{pro}} = 148^\circ\). The dipeptide sequence adopts the \(\beta\)-turn conformation if the Pro residue is followed by an NH group, while the conformation lacking a \(\beta\)-turn is favoured if the residue that immediately follows the dipeptide possesses no NH group available for hydrogen bonding. The two conformations are antipodal to each other except the carbonyl group of the L-Pro residue as can be seen in Fig. 1. In these conformations the \(\delta\)-methylene of the L-Pro residue is in tight contact with one of the methyls of the Aib residue. In order to examine the effect of replacing each of the prochiral methyl groups of the Aib residue upon the conformation of protected dipeptide Boc-Aib-L-Pro-OBzl lacking a \(\beta\)-turn, the isovaline \((\alpha\)-ethyl-\(\beta\)-methylglycine, or \(\alpha\)-ethylalalanine, Iva), containing dipeptides, Boc-D-Iva-L-Pro-OBzl and Boc-L-Iva-L-Pro-OBzl, were synthesized. The two diastereoisomeric dipeptides were...
found to possess closely similar crystal structures to that of compound 1, indicating that, irrespective of the chirality of the Iva residue, the Iva-Pro sequence adopts essentially the same unturned conformation as that of the Aib-Pro sequence in the crystalline state if the dipeptides are directly bonded to an oxygen atom lacking hydrogen-bonding ability. In the present study the amide analogues of compounds 2 and 3, namely Boc-D-Iva-L-Pro-NHBzl 4 and Boc-I-Iva-L-Pro-NHBzl 5 have been synthesized and their crystal structures have been determined in order for us to study the importance of the NH group in the β-turn conformation of these peptide sequences.

The turn tendency of peptide sequences in solution can be studied chiroptically by the use of chromatogenic derivatives possessing 2,4-dinitrophenyl (Dnp) and p-nitroanilide (pNA) groups as N- and C-terminal chromatographic groups, respectively. The magnitude of the Cotton effects in the circular dichroism (CD) spectra of the tetrapeptides Dnp-Gly-dipeptide-Gly-pNA was successfully employed as a measure of the turn tendency of the central dipeptide moiety consisting of t- or l-Ala and l-Pro residues. For the purpose of studying the contribution of intramolecular hydrogen bonding to the stabilization of β-turn conformations in these peptides, glycic acid residues (Gca) containing analogues in place of the fourth Gly residue were synthesized and their CD spectra were recorded. The magnitude of Cotton effects of the depsipeptides possessing an oxygen atom instead of an NH group, namely Dnp-Gly-dipeptide-Gca-pNA, was smaller than that of the corresponding Gly peptides, indicating the smaller turn tendency of the depsipeptides lacking hydrogen-bonding stabilization of turn conformation. In this paper, application of the chiroptical method to the conformational study of Aib-L-Pro sequences was also described by the use of chromatographic peptides Dnp-Gly-X-L-Pro-Gly-pNA (X = Aib/D-Iva/L-Iva 6/7/8) and the corresponding ester analogues Dnp-Gly-X-L-Pro-Gca-pNA (X = Aib/D-Iva/L-Iva 9/10/11).

Results and discussion

Crystal structure analysis

The crystals of Boc-I-D-Iva-L-Pro-NHBzl 4 and Boc-I-Iva-L-Pro-NHBzl 5 are isomorphous. Perspective views of the molecules of 4 and 5 are shown in Fig. 2. Their bond lengths and valence angles are shown in Figs. 3 and 4, respectively. As can be easily seen from Fig. 2, both diastereomers adopt closely similar conformations regardless of the chirality of the Iva residue. The torsion angles are summarized in Table 1, which indicates that the ϕ- and ψ-values of Iva and Pro residues correspond to the right-handed helical conformation. An intramolecular hydrogen bond is formed between the NH of the benzylamide moiety and the carbonyl oxygen of the Boc group, which constitutes a fragment of 310-helix, the N...O distance being 2.36 Å in compound 4 and 2.28 Å in compound 5. This β-turn structure is essentially the same as that observed for Aib-L-Pro sequences-containing peptides in which the L-Pro residue is directly attached to an NH group, e.g. Cbz-Aib-l-Pro-NHMe, whose torsion angles are also given in Table 1. The pyrrolidine ring of Pro in compounds 4 and 5 adopts a Cα-exo puckered conformation possessing negative χ1 and χ3 and positive χ2 and χ4 values. Molecular packing in the crystals of

| Torsion angles (°) of compounds 4 and 5 compared with those of Cbz-Aib-L-Pro-NHMe |
|------------------|---|---|---|
| C(1)-N(2)-C(2)-C(2) | (ϕα) | -51 | -53 | -51 |
| (ϕα) | -38 | -35 | -40 |
| C(2)-N(3)-C(3)-C(3) | (ωα) | -175 | -178 | -174 |
| C(3)-N(3)-C(3)-C(3) | (ϕα) | -70 | -72 | -65 |
| N(3)-C(3)-C(3)-N(4) | (ϕα) | -17 | -14 | -25 |
| C(3)-C(3)-C(3)-C(3) | (χ1) | -14 | -19 | -18 |
| C(3)-C(3)-C(3)-N(3) | (χ2) | 28 | 33 | 29 |
| C(3)-C(3)-C(3)-C(3) | (χ3) | -29 | -33 | -27 |
| C(3)-N(3)-C(3)-C(3) | (χ4) | 19 | 21 | 16 |

* Estimated standard deviations are 0.7–1°.  
* Estimated standard deviations are 0.4–0.7°.  
* Ref. 10.  
* Value of the corresponding angle of the Aib residue.
Fig. 3  Bond lengths (Å) in Boc-D-Iva-L-Pro-NHBz1 (upper) and Boc-L-Iva-L-Pro-NHBz1 (lower). Estimated standard deviations are 0.007-0.02 Å for compound 4 and 0.005-0.02 Å for compound 5.

Fig. 4  Valence angles (°) in Boc-D-Iva-L-Pro-NHBz1 (upper) and Boc-L-Iva-L-Pro-NHBz1 (lower). Estimated standard deviations are 0.5-1° for compound 4 and 0.3-1° for compound 5.

diastereoisomers 4 and 5 is shown in Fig. 5, which also indicates the close similarity in the crystal structures of these diastereoisomeric dipeptides. In each crystal the NH groups of Iva participate in hydrogen bonding with the carbonyl groups of Pro in the neighbouring molecules, constituting an intermolecular hydrogen-bonding network, where the corresponding N⋯O distance is 2.98 Å in compound 4 and is 2.95 Å in compound 5.

The crystal structures of the benzylamides 4 and 5 are different from those of the corresponding benzyl esters Boc-D/L-Iva-L-Pro-OBz1 2/3. While the dipeptide benzyl esters adopt the same no-β-turn conformation as that of Aib-Pro peptides lacking an NH group attached to the carbonyl group of the Pro residue, the dipeptide amides 4 and 5 adopt the β-turn conformation commonly observed for peptides possessing the Aib-Pro-NH moiety. The result indicates that replacement of either methyl group of the Aib residue with an ethyl group does not cause conformational change in the β-turn structure of the Aib-Pro sequence. In the β-turn conformation of the Aib-Pro peptides, pro-S methyl is in tight contact with δ-methylene of the Pro residue. In the t-Iva-containing benzylamide 5, however, the additional methyl group in the ethyl chain is...
**Chiroptical study of solution conformations**

While solid-state conformations of the Aib-L-Pro and D/L-Iva-Pro sequences have been clarified as described above, conformational characteristics of these dipeptide sequences in solution may not be as simple to acquire as those in the crystal since a conformational equilibrium usually exists in solution. However, it can be assumed that the major conformation of these peptides is essentially the same as that observed in the crystal.

Conformational analysis of these peptides by using ¹H NMR spectroscopy, however, was found to be less fruitful than for the usual peptides since the coupling constants $J(\text{NH}-^1H)$, which reflect main chain conformation, cannot be observed for Iva and Pro residues lacking $\alpha$-H and NH, respectively.

Instead of NMR analysis, a chiroptical study using Dnp and pNA derivatives, which was known to be a useful method for estimating the turn tendency of peptides in solution,$^6$ was employed to allow us to study the solution conformation of these $\alpha,\alpha$-dialkylglycyl-L-prolyl sequences. The chromophoric tetrapeptide, Dnp-Gly-Aib-L-Pro-Gly-pNA 6, in CHCl₃ solution exhibited a characteristic CD spectrum of exciton coupling-type interaction of dinitroaniline ($
\lambda_{\text{max}} \sim 350 \text{ nm}$, $\varepsilon \sim 17,000$; $\lambda_{\text{sh}} \sim 405 \text{ nm}$, $\varepsilon \sim 7000$) and $p$-nitroaniline chromophores ($
\lambda_{\text{max}} \sim 320 \text{ nm}$, $\varepsilon \sim 15,000$) as shown in Fig. 6. The large Cotton effects indicated the presence of the terminal Dnp and pNA groups in compound 6 being consistent with the $\beta$-turn conformation of the central Aib-L-Pro sequence observed in the crystalline state. The corresponding $\alpha$- and $\beta$-Iva-containing analogues of compound 6, Dnp-Gly-D/L-Iva-L-Pro-Gly-pNA 7/8 exhibited similar CD spectra to that of compound 6 as also shown in Fig. 6. Thus, these characteristic curves can be attributed to the $\beta$-turn conformation of the central dipeptide moiety which is stabilized by hydrogen bonding between the carbonyl of the first Gly and the NH of the fourth Gly residue and probably also by stacking of the terminal Dnp and pNA chromophores. $^{12}$

The magnitude of the Cotton effects ($|\theta_{\text{max}}|^2 - |\theta_{\text{min}}|^2$) of compounds 7 (28,800) and 8 (23,000), however, is larger and smaller, respectively, than that for compound 6 (26,600), indicating the effect of the additional $\gamma$-methyl group in structures 7 and 8. Assuming the conformational equilibrium between the major ($\beta$-turn) and the minor (extended) conformers in these peptides, replacement of the pro-S methyl group of Aib, which is in tight contact with the $\delta$-methylene of Pro in structure 6, with a larger ethyl group is expected to cause a decrease in the stability of the $\beta$-turn conformer resulting in the observed smaller Cotton effects in compound 8. On the other hand, in the case of the D-Iva analogue 7 possessing an ethyl group, which is extruded outward in the $\beta$-turn conformation but is in a crowded position in the no-$\beta$-turn conformation, a slight increase in population of the major ($\beta$-turn) conformation can be reasonably expected. In other words, the replacement of the Aib residue with a D- or L-Iva residue, which gave no appreciable effect on the crystal structures, was found to cause some change in the conformational equilibrium by affecting the relative stability of the intramolecularly hydrogen-bonded $\beta$-turn conformation in CHCl₃ solution. It therefore seems reasonable that some membrane-channel-forming antibiotic peptides possess an Iva-L-Pro or Iva-L-hydroxyprolyl sequence in addition to an Aib-L-Pro sequence, $^7$ where the configuration of the Iva residue is not helix destabilizing $\ell$ but helix-stabilizing $D$.

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1. The $^1H$ NMR spectra measured in (CD₃)₂SO ([D₆]DMSO) solutions, nuclear Overhauser enhancement spectroscopic (NOESY) cross-peaks were observed between the methyl of Iva ($\delta 1.16$) and the $\delta$-proton in the $\alpha$-orientation of Pro ($\delta 3.67$) in structure 3 and between the methyl of Iva ($\delta 1.23$) and the $\delta$-proton in the $\beta$-orientation of Pro ($\delta 3.70$) in structure 4, which was consistent with the crystal structures (3, Cα4, Cα5 3.44 Å; 4, Cα3, Cα4 3.52 Å).

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Fig. 5 Molecular packing as viewed along the c axis in the crystals of Boc-L-Iva-L-Pro-NHBz 4 (upper) and Boc-L-Iva-L-Pro-NHBz 5 (lower). The broken lines indicate hydrogen bonds.
The CD spectra of the chromophoric depsipeptides Dnp-Gly-X-L-Pro-Gly-pNA (X = Aib, 6; X = d-Iva, 7; X = t-Iva, 8) measured in CHCl₃ showed different spectra compared with those of the corresponding tetrapeptides 6-8 in MeOH and DMSO. The CD spectra of Iva-containing peptides 6 measured in various solvents (CHCl₃, MeOH, DMF, DMSO) exhibited a similar solvent dependence as those in CHCl₃ and the crystalline state.

Since the stability of hydrogen bonds in solution is markedly solvent dependent, the CD spectra of the chromophoric peptides were measured using solvents other than CHCl₃. The spectra of compounds 6 and 9 measured in tetrahydrofuran (THF), MeOH, N,N-dimethylformamide (DMF), and DMSO as well as those in CHCl₃ are reproduced in Figs. 6-8. Their much smaller and completely different spectra compared with those of the corresponding tetrapeptides 6-8 can be attributed to the negligible population of the β-turn conformation of the central dipeptide moiety. The results clearly demonstrate that intramolecular hydrogen bonding plays a crucial role in the stabilization of the β-turn conformation of Aib-I-Pro and Aib-L-Pro sequences in CHCl₃ solution as well as in the crystalline state.

Since the stability of hydrogen bonds in solution is markedly dependent on the nature of the solvent, the CD spectra of the chromophoric tetrapeptides were measured using solvents other than CHCl₃. The spectra of compounds 6 and 9 measured in tetrahydrofuran (THF), MeOH, N,N-dimethylformamide (DMF), and DMSO as well as those in CHCl₃ are reproduced in Figs. 6-8. The CD spectrum of the chromophoric tetrapeptide 6 in THF solution was quite similar to that in CHCl₃ solution, although the magnitude of the Cotton effects was about two-thirds of that in CHCl₃ as shown in Fig. 6. A further decrease in the [θ]₅₀₀ value of 6 was observed in MeOH, DMF, and especially in DMSO solution, and the spectra in MeOH and DMSO did not show typical exciton coupling pattern any more. The CD spectra of Iva-containing peptides 7 and 8 also exhibited quite similar solvent dependence to that of compound 6. Thus, it has been shown that in these peptides the intramolecularly hydrogen-bonded β-turn conformation of the central dipeptide is destabilized to a large extent by the surrounding solvent molecules which can participate in intramolecular hydrogen bonding with the solute peptide molecules partly breaking the intramolecular hydrogen bonding.

The CD spectrum of the chromophoric depsipeptides 9 in which the carbonyl group of the first Gly residue cannot form intramolecular hydrogen bonding to stabilize the β-turn conformation, was also solvent-dependent as can be seen in Fig. 9. It seems difficult, however, to find a rule describing the relationships between the spectra and the nature of the solvents. As for the central Aib-L-Pro moiety, it is quite reasonable to assume the equilibrium between the two conformations observed in the crystalline state which undergoes an extreme shift to favour the no-β-turn conformation in the Gca-containing depsipeptides. The diversity in these CD spectra, therefore, could be attributed to the conformational freedom of the Gly and Gca residues, which is not investigated in the present study.

In summary, a CD spectral study of Aib-L-Pro and D/L-Iva-L-Pro sequences using terminal chromophore-carrying derivatives has shown that, in solution, the presence of an NH group which is directly bonded to the Pro residue is crucial for the stability of their β-turn conformation. In a comparison of Aib- and Iva-containing peptides, a change in the relative stability of the β-turn conformation by the replacement of the Aib with an Iva residue was found to be dependent on the chirality of the Iva residue, which could not be observed in the crystalline form. The solvent environment has a great influence on the stability of the β-turn conformation, which is shown to be destabilized by the solvent with strong hydrogen-bonding ability. Consequently, the dialkylglycyl-L-Pro sequences, which adopt only the two conformational states in equilibrium depending on the surrounding environment, can be considered as good candidates for building blocks of functional molecules or systems, such as artificial enzymes, receptors, etc.
Experimental

Mps were obtained on a hot-plate apparatus and are uncorrected. Column chromatography was performed with silica gel (Merck, #7734) using CHCl₃-MeOH as eluent. 1H NMR spectra were measured on a JEOL JNM-GSX-400 or Varian XL-GEM200 spectrometer at 27 °C, for solutions in [D₆]DMSO. CD spectra were recorded on a JASCO J-600 spectrophotometer at room temperature at a concentration ~4 × 10⁻⁴ mol dm⁻³ and cell length 1 cm, except for DMSO solution which was measured at ~2 × 10⁻⁴ mol dm⁻³ in a 0.2 cm cell.

Synthesis of dipeptides 4 and 5 for crystallography

Boc-D-Iva-L-Pro-NHBzl 4. Catalytic hydrolysis of Boc-D-Iva-L-Pro-OBzl [2] (201 mg, 0.53 mmol) over Pd-black (20 mg) in MeOH (10 cm³) under atmospheric H₂ for 18 h afforded, after usual work-up, Boc-D-Iva-L-Pro-OBzl (147 mg, 94%) as a yellow powder, mp 145-147 °C. The Boc-tetrapeptide-pNA (95 mg, 0.18 mmol) was dissolved in TFA (1 cm³) and the solution was stirred for 20 min at room temperature. After evaporation of TFA, the residue was dissolved in CHCl₃ (2 cm³) and the solution was neutralized by Et₃N, to which were added, under ice-cooling, Boc-Gly-Aib-OMe (108 mg, 0.41 mmol), DCC (137 mg, 0.62 mmol) and HOBt (28 mg, 0.21 mmol). After being stirred overnight at room temperature, the mixture underwent the usual work-up to yield Boc-Gly-Aib-L-Pro-Gly-pNA as a powder (135 mg, 61%), mp 135.5-147 °C.

To a solution of Boc-Aib-Gly-OMe (274 mg, 1 mmol) in MeOH (20 cm³) was added benzylamine (0.5 cm³) to bring to pH 7-8 (moistened universal pH paper) by the addition of Et₃N. The mixture was stirred for 15 min. Under ice-cooling, Aib-OMe-HCl (676 mg, 4.4 mmol) was added to the mixture, which was then stirred overnight while the temperature was raised gradually to ambient. Usual work-up gave Boc-Gly-Aib-OMe (701 mg, 4 mmol) as a yellow powder (86%, mp 88-89 °C). Saponification of Boc-Gly-Aib-OMe (701 mg, 4 mmol) using DCC-HOBt as coupling agent in 65% yield; compound 4 was obtained as a yellow powder (301 mg, 70%), mp 130.5-132.5 °C.

Boc-Iva-L-Pro-NHBzl 5. Boc-Iva-L-Pro-NHBzl was prepared from Boc-Iva-L-Pro-OMe and benzylamine by using DCC-HOBt as coupling agent in 65% yield; compound 5 was a solid, mp 125.5-126 °C. Boc-Iva-L-Pro-NHBzl (185 mg, 0.60 mmol) was dissolved in trifluoroacetic acid (TFA) and stirred for 30 min. The TFA was evaporated under reduced pressure and the residue was dissolved in CHCl₃ (5 cm³). The solution so obtained was brought to pH 7-8 (moistened universal pH paper) by treatment with Et₃N. After usual work-up, the residue was stirred overnight at room temperature. After evaporation of TFA, the residue was dissolved in MeOH (10 cm³) and the solution was neutralized by the addition of Et₃N, to which were added, under ice-cooling, Boc-Gly-Aib-OMe (108 mg, 0.41 mmol), DCC (137 mg, 0.62 mmol) and HOBt (28 mg, 0.21 mmol). After being stirred overnight at room temperature, usual work-up and column chromatographic purification afforded compound 4 (139 mg, 91%) as prisms, mp 153.5-158 °C (from AcOEt) (Found: C, 65.6; H, 8.45; N, 10.45; C₁₂H₁₄N₂O₄ requires C, 65.5; H, 8.25; N, 10.4%). 1H NMR (400 MHz) 0.76 (t, J 7, Iva-γ-Me), 1.23 (s, Iva β-Me), 1.29 (s, Boc CMe₂), 1.75 (m, Iva-β and Pro-β), 1.85 (m, Pro-γ), ~1.95 (m, Iva-β), 2.11 (m, Pro-β), 3.46 (m, Pro-α), 3.77 (m, Pro-α), 4.21 (dd, J 15 and 4, BzlnCH'), 4.32 (dd, J 14 and 5, BzlnCH'), 4.39 (m, Pro-α), 7.24 (m, BzlnH) and 8.08 (m, BzlnH).
Gly\textsuperscript{\texttextacutesign}-\texttextacutesign), 3.86 (dd, J 17 and 6, Gly\textsuperscript{\texttextacutesign}-\texttextacutesign), 4.30 (m, Gly\textsuperscript{\texttextacutesign}-\texttextacutesign)-, 4.41 (dd, J 8.2 and 4.3, Pro-\texttextacutesign), 6.96 (d, J 9.6, Dnp-o\texttextacutesign), 7.85 (d, J 9.2, p\texttextacutesign-Na-o\texttextacutesign), 7.92 (m, Gly\textsuperscript{\texttextacutesign}-\texttextacutesign), 8.17 (d, J 9.2, p\texttextacutesign-Na-m\texttextacutesign-m\texttextacutesign), 8.29 (dd, J 9.6 and 2.7, Dnp-m\texttextacutesign), 8.52 (d, J 2.7, Dnp-m\texttextacutesign), 8.88 (s, Iva-NH), 9.09 (t, J 5.0, Gly\textsuperscript{\texttextacutesign}-\texttextacutesign)-NH and 10.07 (s, p\texttextacutesign-Na-NH).

**Dnp-Gly-Alb-I-Pro-Gca-pNA 9.** A mixture of Ac-Gca-OH (11.5 g, 0.97 mmol) and SOC\textsubscript{3} (7 cm\textsuperscript{3}, 100 mmol) was stirred for 1 h and the volatile compounds were evaporated off. The residue was dissolved in dry THF (20 cm\textsuperscript{3}), to which were added H\texttextacutesign-p\texttextacutesign (20.1 mg, 145 mmol) and pyridine (1 cm\textsuperscript{3}). After being stirred overnight the mixture was washed successively with hot water (90°C) and then with cold benzene to afford Ac-Gca-pNA as a pale yellow solid (312 mg, 85%). Removal of the Boc group followed by 2,4-dinitrophenylation as described above for the X-ray analysis. Crystal data, orthorhombic, a = 17.629(8), b = 12.402(8), c = 10.515(4) Å, V = 2319.9 Å\textsuperscript{3}, space group P2\textsubscript{1}2\textsubscript{1}2\textsubscript{1}, Z = 4, D\textscript{c} = 1.166 g cm\textsuperscript{-3}, compound 3, C\textsubscript{26}H\textsubscript{39}N\textsubscript{3}O\textsubscript{12}*CH\textsubscript{3}OH requires: C, 50.1; H, 5.1; N, 15.1%; δ\textsubscript{d}(200 MHz) 0.77 (t, J 7.5, Iva-γ\texttextacutesign), 1.35 (s, Iva-β\texttextacutesign), 1.77 (m, Iva-β\texttextacutesign)-, 1.98 (m, Iva-β\texttextacutesign), ~2.0 (m, Pro-β\texttextacutesign)-γ, 3.51 (m, Pro-β\texttextacutesign), 3.46 (d, J 5.5, Gly-\texttextacutesign), 4.41 (m, Pro-β\texttextacutesign), 5.95 (m, Pro-β\texttextacutesign), 6.75 (m, Pro-β\texttextacutesign), 7.85 (d, J 9.2, p\texttextacutesign-Na-o\texttextacutesign), 8.22 (d, J 9.2, p\texttextacutesign-Na-m\texttextacutesign-m\texttextacutesign), 8.52 (d, J 9.6 and 2.8, Dnp-m\texttextacutesign), 8.89 (s, Iva-NH), 8.85 (d, J 2.5, Dnp-m\texttextacutesign), 9.07 (t, J 4.9, Gly-NH) and 10.49 (s, p\texttextacutesign-Na-NH).

**X-Ray crystallography**

Crystal data. Compound 4: C\textsubscript{2}H\textsubscript{3}N\textsubscript{2}O\textsubscript{3}, M = 403.5 g mol\textsuperscript{-1}, orthorhombic, a = 17.351(7), b = 12.477(7), c = 10.764(4) Å, V = 2139.9 Å\textsuperscript{3}, space group P2\textsubscript{1}2\textsubscript{1}2\textsubscript{1}, Z = 4, D\textscript{c} = 1.155 g cm\textsuperscript{-3}, compound 4, C\textsubscript{2}H\textsubscript{3}N\textsubscript{2}O\textsubscript{3}, M = 403.5 g mol\textsuperscript{-1}, orthorhombic, a = 17.629(8), b = 12.402(8), c = 10.515(4) Å, V = 2299.3 Å\textsuperscript{3}, space group P2\textsubscript{1}2\textsubscript{1}2\textsubscript{1}, Z = 4, D\textscript{c} = 1.166 g cm\textsuperscript{-3}.

Data collection and processing. Rigaku AFC-SRU four-circle diffractometer, ω-2θ scanning mode within a range of 20 = 12° graphite-monochromated Cu-K\textsubscript{a} radiation; 1493 reflections for compound 4 and 4200 for compound 5 with θ = 2θ(111).

Full X-ray crystallographic details have been deposited with the Cambridge Crystallographic Data Centre.

**Structure analysis and refinement.** Direct methods were used, using the program MULTAN78. Hydrogen atoms were located from a D-map. Refinement was with a block-diagonal weighting scheme.

For compound 4, 120 reflections for compound 5 with θ = 2θ(111).


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