Two alternative conformational states of α,α-dialkylglycyl-L-prolyl 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 **4** and Boc-L-Iva-L-Pro-NHBzl **5** were determined by X-ray diffraction. The diastereoisomeric peptides adopt intramolecular hydrogen-bonded β-turn conformations closely similar to each other (**4**: φ_{Iva} −51°, ψ_{Iva} −38°, φ_{Pro} −70° and ψ_{Pro} −17° and **5**: φ_{Iva} −53°, ψ_{Iva} −35°, φ_{Pro} −72° and ψ_{Pro} −14°). The Pro ring of each peptide is in C^{γ}-*exo* conformation. These conformations are essentially the same as those in the reported crystal structures of the Aib-L-Pro sequence possessing an NH group directly attached to the carbonyl of the L-Pro, indicating that replacement of either one of the two methyl groups of the Aib moiety with an ethyl group does not cause any significant change in the β-turn conformation of the Aib-L-Pro sequence in the crystalline state.

CD spectral analysis of the terminal chromophoric group-carrying peptides Dnp-Gly-X-L-Pro-Gly-*p*NA (X = Aib **6** and D/L-Iva **7**/**8**) has shown that these three tetrapeptides in $CHCl_3$ and THF solutions also adopt a β-turn-type conformation. CD spectra of glycolic acid residue-containing analogues in place of the fourth Gly residue revealed a lack of β-turn tendency in these analogues, indicating the importance of intramolecular hydrogen bonding for the β-turn conformation of the central dipeptide moieties. 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 α-aminoisobutyryl (α,α-dimethylglycyl, Aib) residue is found commonly in membrane channel-forming antibiotics such as alamethicin I and suzukacillin A.[1] 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 sterically 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,[2] and (ii) readily occurring acidolytic cleavage of the Aib-L-Pro linkage.[3] 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 β-turn conformation (φ_{Aib} −46 to −55°, ψ_{Aib} −31 to −51°, φ_{Pro} −55 to −74°, ψ_{Pro} −4 to −36°) and a rather extended conformation (φ_{Aib} +50 to +58°, ψ_{Aib} +37 to +47°, φ_{Pro} −65 to −77°, ψ_{Pro} +148 to +162°).[4] The dipeptide sequence adopts the β-turn conformation if the Pro residue is followed by an NH group, while the conformation lacking a β-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 δ-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 **1** lacking a β-turn,[4] the isovaline (α-ethyl-α-methylglycine or α-ethylalanine, Iva) containing dipeptides, Boc-D-Iva-L-Pro-OBzl **2** and Boc-L-Iva-L-Pro-OBzl **3**, were synthesized. The two diastereoisomeric dipeptides were

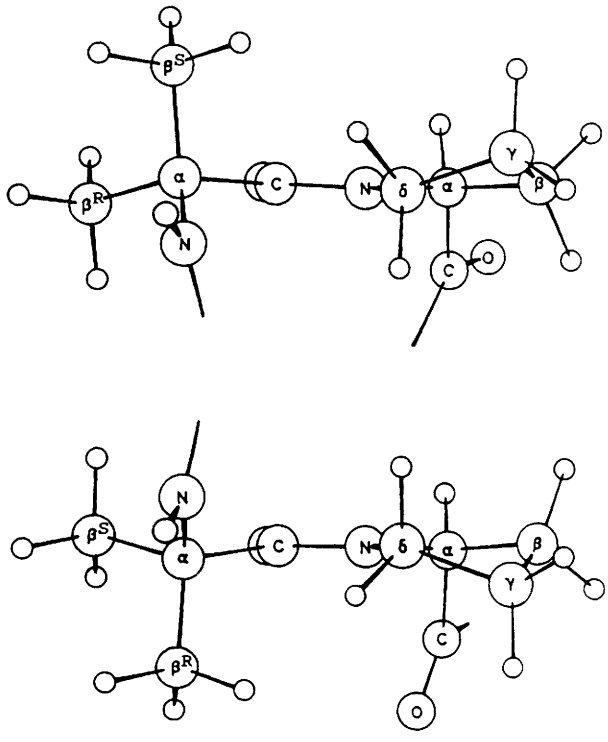

Fig. 1 Schematic drawing of two conformations of the Aib-L-Pro sequence, β-turn conformation (upper) and no-β-turn conformation (lower). In each conformation one of the methyl groups in Aib is in tight contact with the δ-methylene in the Pro residue. Replacement of the *pro-R* (β^R) and *pro-S* (β^S) methyls in the Aib residue with an ethyl affords D- and L-Iva residues, respectively.

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.[5] In the present study the amide analogues of compounds **2** and **3**, namely Boc-D-Iva-L-Pro-NHBzl **4** and Boc-L-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 chromophoric derivatives possessing 2,4-dinitrophenyl (Dnp) and *p*-nitroanilide (*p*NA) groups as N- and C-terminal chromophoric groups, respectively.[6] The magnitude of the Cotton effects in the circular dichroism (CD) spectra of the tetrapeptide derivatives Dnp-Gly-dipeptide-Gly-*p*NA was successfully employed as a measure of the turn tendency of the central dipeptide moiety consisting of D- or L-Ala and L-Pro residues.[7] For the purpose of studying the contribution of intramolecular hydrogen bonding to the stabilization of β-turn conformations in these peptides, glycolic 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-*p*NA, was smaller than that of the corresponding Gly peptides, indicating the smaller turn tendency of the depsipeptides lacking hydrogen-bonding stabilization of turn conformation.[8] In this paper, application of the chiroptical method to the conformational study of Aib-L-Pro, D-Iva-L-Pro and L-Iva-L-Pro sequences was also described by the use of chromophoric peptides Dnp-Gly-X-L-Pro-Gly-*p*NA (X = Aib/D-Iva/L-Iva **6/7/8**) and the corresponding ester analogues Dnp-Gly-X-L-Pro-Gca-*p*NA (X = Aib/D-Iva/L-Iva **9/10/11**).

1 Boc-Aib-L-Pro-OBzl
2 Boc-D-Iva-L-Pro-OBzl
3 Boc-L-Iva-L-Pro-OBzl
4 Boc-D-Iva-L-Pro-NHBzl
5 Boc-D-Iva-L-Pro-NHBzl
6 Dnp-Gly-Aib-L-Pro-Gly-*p*NA
7 Dnp-Gly-D-Iva-L-Pro-Gly-*p*NA
8 Dnp-Gly-L-Iva-L-Pro-Gly-*p*NA
9 Dnp-Gly-Aib-L-Pro-Gca-*p*NA
10 Dnp-Gly-D-Iva-L-Pro-Gca-*p*NA
11 Dnp-Gly-D-Iva-L-Pro-Gca-*p*NA

Results and discussion

Crystal structure analysis

The crystals of Boc-D-Iva-L-Pro-NHBzl **4** and Boc-L-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 diastereoisomers 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 3_{10}-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 sequence-containing peptides in which the L-Pro residue is directly attached to an NH group,[4] *e.g.* Cbz-Aib-L-Pro-NHMe,[9] whose torsion angles are also given in Table 1. The pyrrolidine ring

Table 1 Torsion angles (°) of compounds **4** and **5** compared with those of Cbz-Aib-L-Pro-NHMe

Angle		**4**[a]	**5**[b]	Aib-L-Pro[c]
C(1)–N(2)–C$^{\alpha}$(2)–C(2)	(φ_{Iva})	−51	−53	−51[d]
N(2)–C$^{\alpha}$(2)–C(2)–N(3)	(ψ_{Iva})	−38	−35	−40[d]
C$^{\alpha}$(2)–C(2)–N(3)–C$^{\alpha}$(3)	(ω_{Iva})	−175	−178	−174[d]
C(2)–N(3)–C$^{\alpha}$(3)–C(3)	(φ_{Pro})	−70	−72	−65
N(3)–C$^{\alpha}$(3)–C(3)–N(4)	(ψ_{Pro})	−17	−14	−25
N(3)–C$^{\alpha}$(3)–C$^{\beta}$(3)–C$^{\gamma}$(3)	(χ^1_{Pro})	−14	−19	−18
C$^{\alpha}$(3)–C$^{\beta}$(3)–C$^{\gamma}$(3)–C$^{\delta}$(3)	(χ^2_{Pro})	28	33	29
C$^{\beta}$(3)–C$^{\gamma}$(3)–C$^{\delta}$(3)–N(3)	(χ^3_{Pro})	−29	−33	−27
C$^{\alpha}$(3)–N(3)–C$^{\delta}$(3)–C$^{\gamma}$(3)	(χ^4_{Pro})	19	21	16

[a] Estimated standard deviations are 0.7–1°. [b] Estimated standard deviations are 0.4–0.7°. [c] Ref. 10. [d] Value of the corresponding angle of the Aib residue.

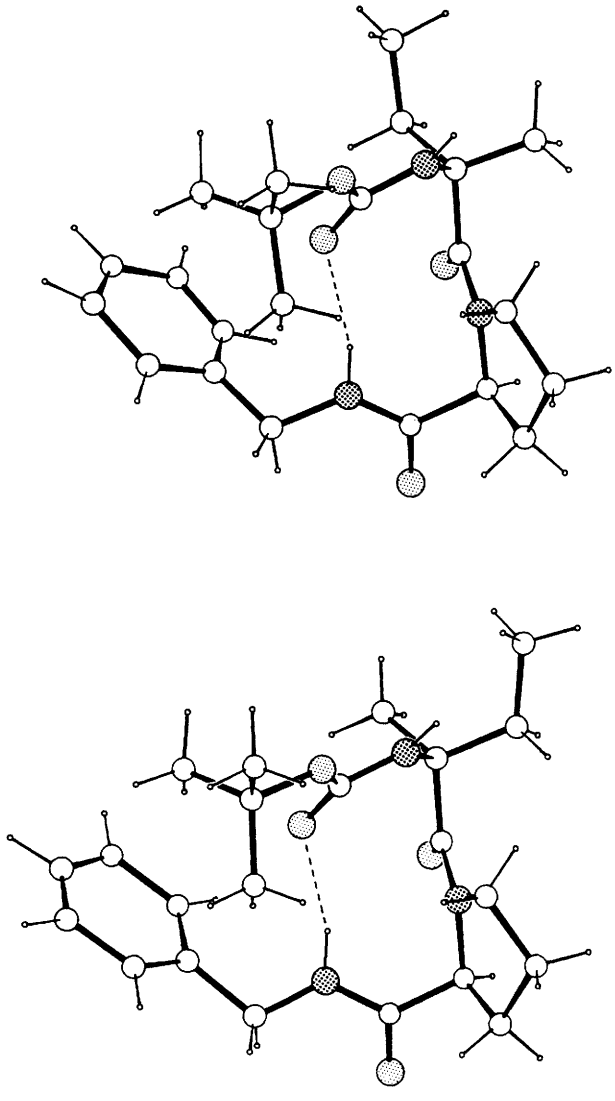

Fig. 2 Perspective view of the molecular structures of Boc-D-Iva-L-Pro-NHBzl **4** (upper) and Boc-L-Iva-L-Pro-NHBzl **5** (lower)

of Pro in compounds **4** and **5** adopts a C$^{\gamma}$-*exo* puckered conformation possessing negative χ^1 and χ^3 and positive χ^2 and χ^4 values.[10] Molecular packing in the crystals of

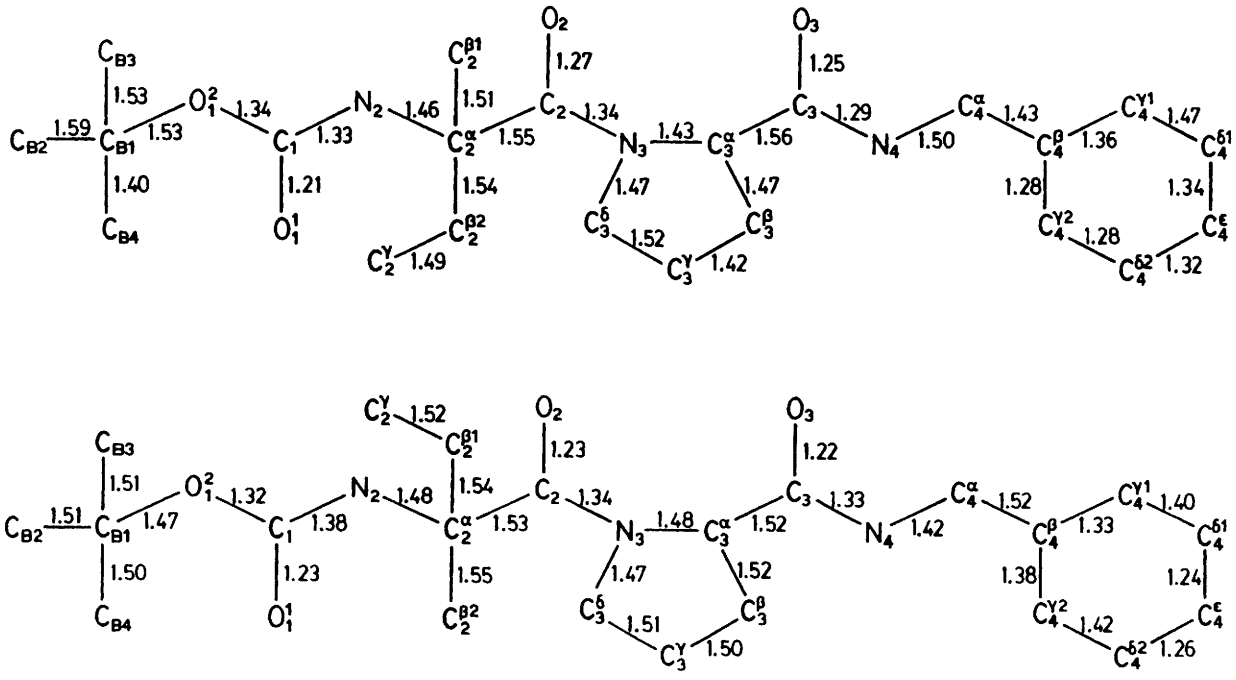

Fig. 3 Bond lengths (Å) in Boc-D-Iva-L-Pro-NHBzl **4** (upper) and Boc-L-Iva-L-Pro-NHBzl **5** (lower). Estimated standard deviations are 0.007–0.02 Å for compound **4** and 0.005–0.02 Å for compound **5**.

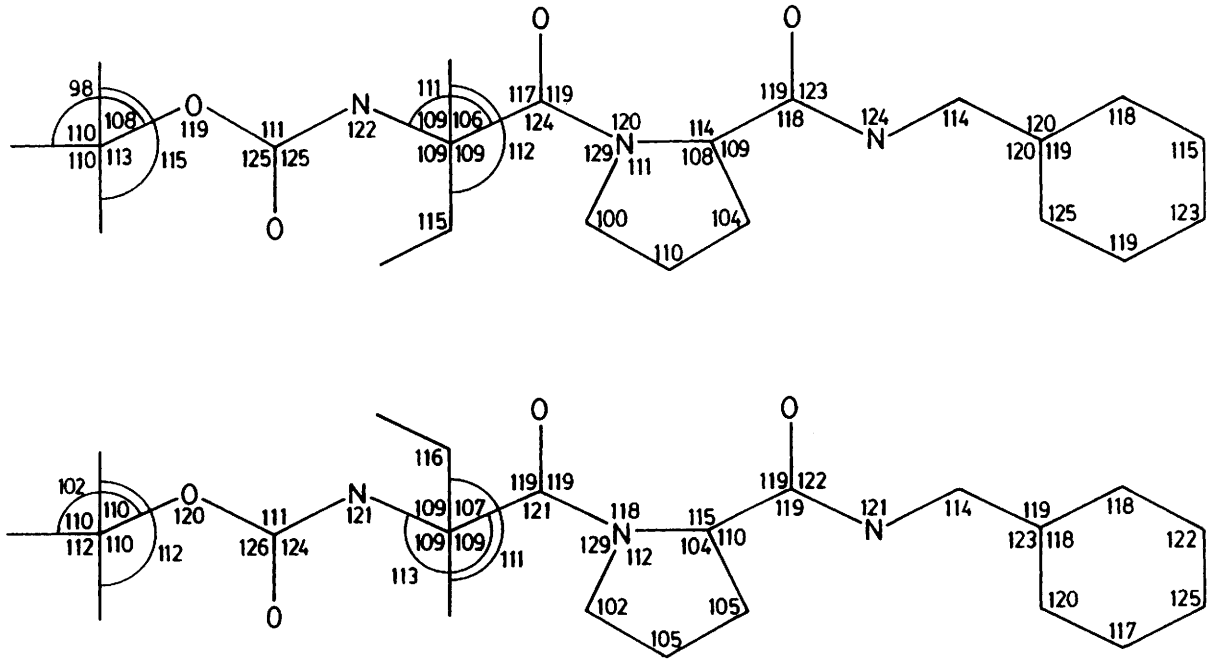

Fig. 4 Valence angles (°) in Boc-D-Iva-L-Pro-NHBzl **4** (upper) and Boc-L-Iva-L-Pro-NHBzl **5** (lower). Estimated standard deviations are 0.5–1° for compound **4** and 0.3–1° for compound **5**.

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-OBzl **2**/**3**.[5] 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 L-Iva-containing benzylamide **5**, however, the additional methyl group in the ethyl chain is

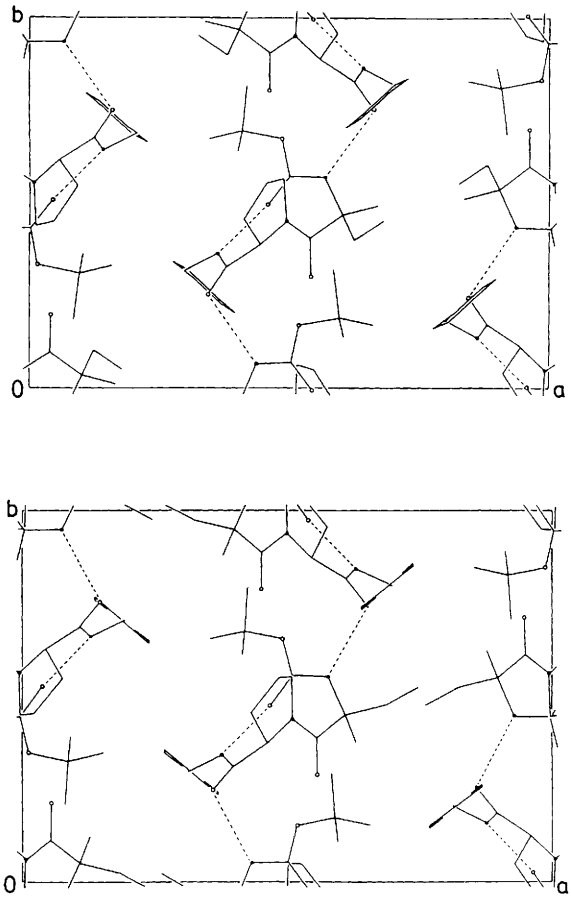

Fig. 5 Molecular packing as viewed along the *c* axis in the crystals of Boc-D-Iva-L-Pro-NHBzl **4** (upper) and Boc-L-Iva-L-Pro-NHBzl **5** (lower). The broken lines indicate hydrogen bonds.

located away (χ^1 −65°) from the Pro, causing no further steric constraint in the molecule although the methylene in the ethyl chain is close to the δ-methylene of the Pro residue (C_{Iva} ··· C_{Pro} 3.48 Å). A similar crystallographic result to this was reported for C-terminal peptide sequence of emericins III and IV, *i.e.*, the right-handed 3_{10}-helical conformation was not affected by the replacement of Aib-L-Pro with a D-Iva-L-Pro sequence.[11] Therefore it has been shown that, in the β-turn conformation, as well as in the other conformation, replacement of the Aib with a D- or L-Iva residue brings about no significant change in the conformational state of an Aib-L-Pro sequence.

The present result combined with our previous study[4,5] concerning the solid-state structure of α,α-dialkylglycyl-L-prolyl sequences is summarized as follows:

(i) For Aib-L-Pro and D/L-Iva-L-Pro sequences only two conformational states are possible; *i.e.*, the intramolecularly hydrogen-bonded β-turn conformation constituting a fragment of right-handed 3_{10}-helix, and the rather extended unturned conformation which is enantiomeric to the β-turn conformation except for the orientation of the carbonyl group of the Pro residue as shown in Fig. 1 for the Aib-L-Pro sequence.

(ii) The preference of the two conformational states is governed by the presence/absence of an NH group which directly follows the dipeptide moiety; *i.e.*, if an NH attached to the Pro carbonyl group is present the dipeptide invariably adopts the β-turn conformation, while the extended conformation is taken if such an NH is not present but the carbonyl group is followed by an oxygen atom forming an ester function or by an imide nitrogen of the Pro residue forming a Pro-Pro linkage.

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 conformer of these peptides is essentially the same as that observed in the crystal.

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

Instead of NMR analysis, a chiroptical study using Dnp and *p*NA 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 α,α-dialkylglycyl-L-prolyl sequences. The chromophoric tetrapeptide, Dnp-Gly-Aib-L-Pro-Gly-pNA **6**, in $CHCl_3$ solution exhibited a characteristic CD spectrum of exciton coupling-type interaction of dinitroaniline (λ_{max} ~350 nm, ε ~17 000; λ_{sh} ~405 nm, ε ~7000) and *p*-nitroanilide chromophores (λ_{max} ~320 nm, ε ~15 000) as shown in Fig. 6. The large Cotton effects indicated the steric proximity of the terminal Dnp and *p*NA groups in compound **6** being consistent with the β-turn conformation of the central Aib-L-Pro sequence observed in the crystalline state. The corresponding D- and L-Iva-containing analogues of compound **6**, Dnp-Gly-D/L-Iva-L-Pro-Gly-*p*NA **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 β-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 *p*NA chromophores.[12] The magnitude of the Cotton effects ($|[\theta]^{max}_{350} - [\theta]^{max}_{300}|$) 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 γ-methyl group in structures **7** and **8**. Assuming the conformational equilibrium between the major (β-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 δ-methylene of Pro in structure **6**, with a larger ethyl group is expected to cause a decrease in the stability of the β-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 β-turn conformation but is in a crowded position in the no-β-turn conformation, a slight increase in population of the major (β-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 β-turn conformation in $CHCl_3$ 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,[1] where the configuration of the Iva residue is not helix-destabilizing L but helix-stabilizing D.

† In the ^{1}H NMR spectra measured in $(CD_3)_2SO$ ([$^2H_6$]DMSO) solutions, nuclear Overhauser enhancement spectroscopic (NOESY) cross-peaks were observed between the methyl of Iva (δ 1.16) and the δ-proton in the α-orientation of Pro (δ 3.67) in structure **3** and between the methyl of Iva (δ 1.23) and the δ-proton in the β-orientation of Pro (δ 3.76) in structure **4**, which was consistent with the crystal structures (**3**, C_{Iva} ··· C_{Pro} 3.44 Å; **4**, C_{Iva} ··· C_{Pro} 3.52 Å).

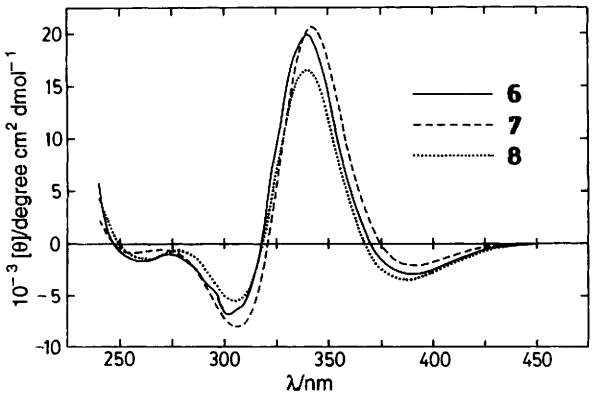

Fig. 6 CD spectra of Dnp-Gly-X-L-Pro-Gly-*p*NA (**6**, X = Aib; **7**, X = D-Iva; **8**, X = L-Iva) measured in $CHCl_3$

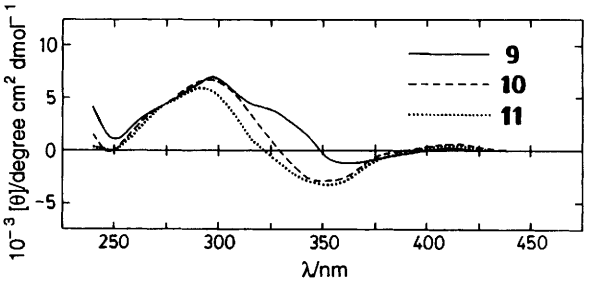

Fig. 7 CD spectra of Dnp-Gly-X-L-Pro-Gca-*p*NA (**9**, X = Aib; **10**, X = D-Iva; **11**, X = L-Iva) measured in $CHCl_3$

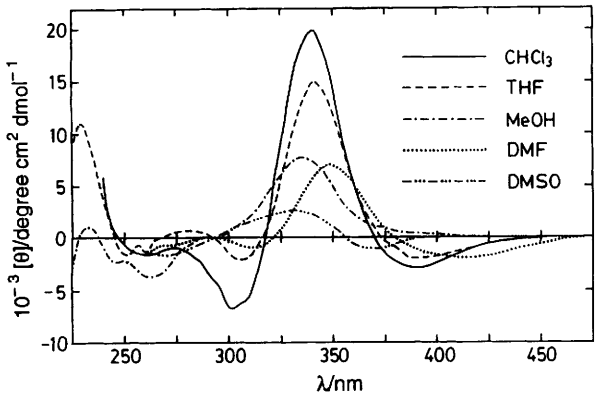

Fig. 8 CD spectra of Dnp-Gly-Aib-L-Pro-Gly-*p*NA **6** measured in various solvents

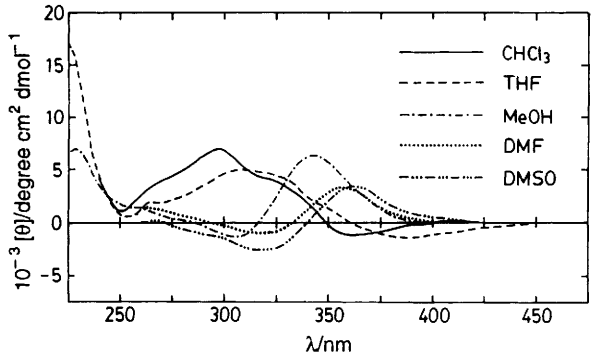

Fig. 9 CD spectra of Dnp-Gly-Aib-L-Pro-Gca-*p*NA **9** measured in various solvents

The CD spectra of the chromophoric depsipeptides Dnp-Gly-X-L-Pro-Gca-*p*NA (X = Aib/D-Iva/L-Iva **9**/**10**/**11**) are reproduced in Fig. 7. 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-L-Pro and D/L-Iva-L-Pro sequences in $CHCl_3$ 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, CD spectra of the chromophoric peptides were measured using solvents other than $CHCl_3$. The spectra of compounds **6** and **9** measured in tetrahydrofuran (THF), MeOH, *N*,*N*-dimethylformamide (DMF), and DMSO as well as those in $CHCl_3$ are reproduced in Figs. 8 and 9. The CD spectrum of the chromophoric tetrapeptide **6** in THF solution was quite similar to that in $CHCl_3$ solution, although the magnitude of the Cotton effects was about two-thirds of that in $CHCl_3$ as shown in Fig. 8. A further decrease in the $[\theta]_{max}$-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 intermolecular 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 crystallographic study. The solution conformation was found to be greatly solvent dependent and the β-turn conformation was 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 $CHCl_3$–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 [$^2H_6$]DMSO CD spectra were recorded on a JASCO J-600 spectrophotometer at room temperature at a concentration ~4 × 10^{-5} 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 hydrogenolysis of Boc-D-Iva-L-Pro-OBzl[5] **2** (201 mg, 0.5 mmol) over Pd-black (20 mg) in MeOH (10 cm^3) under atmospheric H_2 for 18 h afforded, after usual work-up, Boc-D-Iva-L-Pro-OH (147 mg, 94%) as a solid, mp 194–195 °C. To the ice-cooled solution of the Boc-dipeptide (121 mg, 0.38 mmol) in $CHCl_3$ (5 cm^3) were added benzylamine (0.04 cm^3, 0.40 mmol), dicyclohexylcarbodiimide (DCC; 309 mg, 1.5 mmol), and 1-hydroxybenzotriazole (HOBt; 14 mg, 0.10 mmol). After stirring of the mixture for 48 h at room temp., usual work-up followed by column chromatographic purification afforded compound **4** (139 mg, 91%) as prisms, mp 183.5–185 °C (from AcOEt) (Found: C, 65.6; H, 8.45; N, 10.45. $C_{22}H_{33}N_3O_4$ requires C, 65.5; H, 8.25; N, 10.4%); δ_H(400 MHz) 0.76 (t, *J* 7, Iva γ-Me), 1.23 (s, Iva β-Me), 1.29 (s, Boc CMe_3), ~1.75 (m, Iva-β and Pro-β), ~1.85 (m, Pro-γ,γ′), ~1.95 (m, Iva-β′), 2.11 (m, Pro-β′), 3.46 (m, Pro-δ), 3.77 (m, Pro-δ′), 4.21 (dd, *J* 14 and 5, BzlNC*H*), 4.32 (dd, *J* 14 and 5, BzlNC*H*′), 4.39 (m, Pro-α), 7.24 (m, BzlPh) and 8.08 (m, BzlN*H*).

Boc-L-Iva-L-Pro-NHBzl 5. Boc-L-Pro-NHBzl was prepared from Boc-L-Pro-OH and benzylamine by using DCC–HOBt as coupling agent in 65% yield; compound **5** was a solid, mp 125.5–126 °C. Boc-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 $CHCl_3$ (5 cm^3). The solution so obtained was brought to pH 7–8 (moistened universal pH paper) by treatment with Et_3N. To the solution of L-Pro-NHBzl thus obtained were added, under ice-cooling, Boc-L-Iva-OH (110 mg, 0.51 mmol), DCC (224 mg, 1.1 mmol), and HOBt (37 mg, 0.27 mmol), and the mixture was stirred overnight at room temperature. Usual work-up and column chromatography of the crude product afforded compound **5** (153 mg, 76%) as prisms, mp 197–198 °C (from AcOEt) (Found: C, 65.6; H, 8.45; N, 10.45%); δ_H(400 MHz) 0.79 (t, *J* 7.5, Iva γ-Me), 1.27 (s, Boc CMe_3), 1.32 (s, Iva β-Me), ~1.65 (m, Iva-β), ~1.7 (m, Iva-β′), 1.76 (m, Pro-β), ~1.8 (m, Pro-γ,γ′), 2.10 (m, Pro-β′), 3.44 (dt, *J* 12 and 6, Pro-δ), 3.75 (dt, *J* 12 and 6, Pro-δ′), 4.20 (dd, *J* 15 and 5, BzlNC*H*), 4.35 (dd, *J* 15 and 6, BzlNC*H*′), 4.39 (m, Pro-α), 7.24 (m, BzlPh) and 8.02 (t, *J* 6, BzlN*H*).

Synthesis of chromophoric peptides 6–8 and depsipeptides 9–11 for chiroptical study

Dnp-Gly-Aib-L-Pro-Gly-*p*NA 6. Benzenesulfonyl chloride (0.773 cm^3, 6 mmol) was added to an ice-cooled solution of Boc-Gly-OH (701 mg, 4 mmol) in dry pyridine (8 cm^3) and 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* (785 mg, 72%), mp 95–96 °C (from AcOEt) (Found: C, 52.65; H, 8.25; N, 10.2. $C_{12}H_{22}N_2O_5$ requires C, 52.55; H, 8.1; N, 10.2%).

To a solution of Boc-Aib-Gly-OMe (274 mg, 1 mmol) in MeOH (4 cm^3) was added 1 mol dm^{-3} NaOH (2 cm^3) and the mixture was stirred for 2 h at room temperature. Acidification of the mixture followed by extraction with AcOEt afforded, after usual work-up, Boc-Aib-Gly-OH as an oil (216 mg, 83%). Boc-L-Pro-Gly-*p*NA (163 mg, 0.415 mmol) was dissolved in TFA (2 cm^3) and the solution was stirred for 30 min at room temperature. After evaporation of TFA, the residue was dissolved in CH_2Cl_2 (2 cm^3) and the solution was neutralized by Et_3N, to which were added, under ice-cooling, Boc-Gly-Aib-OH (108 mg, 0.415 mmol), DCC (137 mg, 0.623 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-*p*NA as a powder (135 mg, 61%), mp 145–147 °C.

The Boc-tetrapeptide-*p*NA (95 mg, 0.178 mmol) was dissolved in TFA (1 cm^3) and the solution was stirred for 20 min at room temperature. After evaporation of TFA, the residue was dissolved in acetone (2 cm^3), to which Dnp-F (66 mg, 0.36 mmol) was added and the solution was neutralized by the addition of Et_3N. After stirring of the mixture overnight at room temperature, usual work-up and column chromatographic purification afforded *Dnp-Gly-Aib*-L-*Pro-Gly*-p*NA* **6** as yellow crystals (59 mg, 55%), mp 168–169 °C (from MeOH) (Found: C, 48.95; H, 4.65; N, 18.45. $C_{25}H_{28}N_8O_{10}$·(1/2)H_2O requires C, 49.25; H, 4.8; N, 18.3%); δ_H(200 MHz) 1.41 (s, AibMe), 1.43 (s, Aib Me′), ~1.9 (m, Pro-β,β′,γ,γ′), ~3.6 (m, Pro-δ,δ′), ~3.8 (m, Gly^4-α), 3.85 (m, Gly^4-α′), 4.27 (br d, *J* 5, Gly^1-α,α′), 4.38 (dd, *J* 7.8 and 4.6, Pro-α), 6.96 (d, *J* 9.6, Dnp-*o*′), 7.85 (d, *J* 9.4, *p*NA-*o*,*o*′), 7.97 (br t, *J* 6.4, Gly^4-NH), 8.17 (d, *J* 9.4, *p*NA-*m*,*m*′), 8.29 (dd, *J* 9.6 and 2.8, Dnp-*m*′), 8.74 (d, *J* 2.8, Dnp-*m*), 9.00 (s, Aib-NH), 9.08 (t, *J* 5, Gly^1-NH) and 10.05 (s, *p*NA-NH).

Dnp-Gly-D-Iva-L-Pro-Gly-*p*NA 7. Reaction of Boc-L-Gly-OH (385 mg, 2.2 mmol) and D-Iva-OMe·HCl (335 mg, 2.0 mmol) using DCC–HOBt as coupling agent afforded Boc-Gly-D-Iva-OMe (471 mg, 82%), mp 88–89 °C. Saponification of Boc-Gly-D-Iva-OMe (346 mg, 1.2 mmol) as described above for Boc-Gly-Aib-OMe gave Boc-Gly-D-Iva-OH as a powder (327 mg, 88%), mp 164–165.5 °C. From Boc-Gly-D-Iva-OH (219 mg, 0.80 mmol) and Boc-Pro-Gly-*p*NA (353 mg, 0.90 mmol), in a similar manner to the preparation of the corresponding Aib-peptide described above, Boc-Gly-D-Iva-L-Pro-Gly-*p*NA was prepared as a slightly yellow solid (301 mg, 70%), mp 130.5–132.5 °C. Removal of the Boc group followed by 2,4-dinitrophenylation of Boc-Gly-D-Iva-L-Pro-Gly-*p*NA (165 mg, 0.30 mmol) as also described above afforded *Dnp-Gly*-D-*Iva*-L-*Pro-Gly*-p*NA* **7** as a yellow powder (201 mg, 95%), mp 155–157 °C (Found: C, 49.15; H, 4.85; N, 17.6. $C_{26}H_{30}N_8O_{10}$·H_2O requires C, 49.4; H, 5.1; N, 17.7%); δ_H(200 MHz) 0.74 (t, *J* 7.3, Iva γ-Me), 1.39 (s, Iva β-Me), ~1.8 (m, Pro-β), 1.83 (m, Pro-γ), 1.85 (m, Iva-β,β′), 1.86 (m, Pro-γ′), ~2.0 (m, Pro-β′), 3.58 (m, Pro-δ,δ′), 3.78 (dd, *J* 17.4 and 5.9, Gly^4-α), 3.91 (dd, *J* 17.4 and 6.4, Gly^4-α′), 4.29 (d, *J* 5.1, Gly^1-α,α′), 4.37 (m, Pro-α), 6.95 (d, *J* 9.6, Dnp-*o*′), 7.91 (d, *J* 9.2, *p*NA-*o*,*o*′), 8.16 (m, Gly^4-NH), 8.18 (d, *J* 9.2, *p*NA-*m*,*m*′), 8.30 (dd, *J* 9.6 and 2.6, Dnp-*m*′), 8.78 (d, *J* 2.6, Dnp-*m*), 8.79 (s, Iva-NH), 9.08 (t, *J* 4.9, Gly^1-NH) and 10.02 (s, *p*NA-NH).

Dnp-Gly-L-Iva-L-Pro-Gly-*p*NA 8. This compound was prepared in essentially the same manner as the preparation of the D-Iva diastereoisomer **7**, *via* Boc-Gly-L-Iva-OMe (powder; 76%; mp 88–89.5 °C), Boc-Gly-L-Iva-OH (powder; 97%; mp 168–169 °C), and Boc-Gly-L-Iva-L-Pro-Gly-*p*NA (powder; 65%; mp 114.5–117 °C). *Dnp-Gly*-L-*Iva*-L-*Pro-Gly*-p*NA* **8** was obtained as a yellow powder (58%), mp 148.5–151 °C (Found: C, 49.7; H, 5.0; N, 17.45. $C_{26}H_{30}N_8O_{10}$·H_2O requires C, 49.4; H, 5.1; N, 17.7%); δ_H(200 MHz) 0.85 (t, *J* 7.2, Iva γ-Me), 1.38 (s, Iva β-Me), ~1.8 (m, Pro-β), 1.85 (m, Pro-γ,γ′ and Iva-β,β′), ~2.0 (m, Pro-β′), ~3.6 (m, Pro-δ,δ′), 3.80 (dd, *J* 17 and 6,

Gly⁴-α), 3.86 (dd, *J* 17 and 6, Gly⁴-α'), 4.30 (m, Gly¹-α,α'), 4.41 (dd, *J* 8.2 and 4.3, Pro-α), 6.96 (d, *J* 9.6, Dnp-*o'*), 7.85 (d, *J* 9.2, *p*NA-*o,o'*), 7.92 (m, Gly⁴-NH), 8.17 (d, *J* 9.2, *p*NA-*m,m'*), 8.29 (dd, *J* 9.6 and 2.7, Dnp-*m'*), 8.72 (d, *J* 2.7, Dnp-*m*), 8.88 (s, Iva-NH), 9.09 (t, *J* 5.0, Gly¹-NH) and 10.07 (s, *p*NA-NH).

Dnp-Gly-Aib-L-Pro-Gca-*p*NA 9. A mixture of Ac-Gca-OH (11.50 g, 0.97 mmol) and $SOCl_2$ (7 cm^3, 100 mmol) was stirred for 1 h and the volatile compounds were evaporated off. The residue was dissolved in dry THF (20 cm^3), to which were added H-*p*NA (20.1 g, 145 mmol) and pyridine (1 cm^3). After being stirred overnight the mixture was washed successively with hot water (90 °C) and then with cold benzene to afford Ac-Gca-*p*NA as a pale yellow solid (19.8 g, 85%), mp 141.5–142 °C.

To a solution of Ac-Gca-*p*NA (481 mg, 2.0 mmol) in MeOH (4 cm^3)–THF (4 cm^3) was added 1 mol dm^{-3} aq. NaOH (2.2 cm^3, 2.2 mmol) and the mixture was stirred for 10 min. Usual work-up afforded Gca-*p*NA as a yellow solid (312 mg, 80%), mp 196–196.5 °C (lit.,[13] 194 °C).

Under ice-cooling, benzenesulfonyl chloride (0.435 cm^3, 3.4 mmol) was added to a solution of Boc-L-Pro-OH (538 mg, 2.5 mmol) in pyridine (3.2 cm^3) and was stirred for 15 min. Gca-*p*NA (736 mg, 3.75 mmol) was added and, after being stirred overnight, the reaction mixture was worked up as usual. Column chromatography of the crude product afforded *Boc-L-Pro-Gca-pNA* as pale yellow powder (660 mg, 67%), mp 143–144 °C (from AcOEt–hexane) (Found: C, 55.0; H, 5.95; N, 10.75. $C_{18}H_{23}N_3O_7$ requires C, 54.95; H, 5.9; N, 10.7%).

Boc-L-Pro-Gca-*p*NA (60 mg, 0.15 mmol) was treated with TFA and subjected to a coupling reaction with Boc-Gly-Aib-OH (52 mg, 0.20 mmol) using DCC–HOBt as described for the preparation of compound **6** to give Boc-Gly-Aib-L-Pro-Gca-*p*NA as a yellow oil (80 mg, ~100%). Removal of the Boc group followed by 2,4-dinitrophenylation as described above and column chromatographic purification afforded *Dnp-Gly-Aib-L-Pro-Gca-pNA* **9** as yellow powder (12 mg, 13%), mp 145–146 °C (Found: C, 48.5; H, 4.55; N, 15.8. $C_{25}H_{27}N_7O_{11}\cdot H_2O$ requires C, 48.5; H, 4.7; N, 15.8%); δ_H(200 MHz) 1.38 (s, Aib-Me), 1.40 (s, Aib-Me'), ~1.9 (m, Pro-β,β'), ~2.0 (Pro-γ,γ'), 3.45 (m, Pro-δ), 3.65 (m, Pro-δ'), 4.22 (br d, *J* 5, Gly-α,α'), 4.41 (dd, *J* 8 and 3, Pro-α), 4.70 (d, *J* 16, Gca-α), 4.72 (d, *J* 16, Gca-α'), 6.93 (d, *J* 9.6, Dnp-*o'*), 7.85 (d, *J* 9.2, *p*NA-*o,o'*), 8.22 (d, *J* 9.2, *p*NA-*m,m'*), 8.32 (dd, *J* 9.6 and 2.8, Dnp-*m'*), 8.68 (s, Aib-NH), 8.86 (d, *J* 2.8, Dnp-*m*), 9.06 (t, *J* 5, Gly-NH) and 10.49 (s, *p*NA-NH).

Dnp-Gly-D-Iva-L-Pro-Gca-*p*NA 10. Boc-Gly-D-Iva-OH (219 mg, 0.80 mmol) and L-Pro-OBzl·HCl (290 mg, 1.2 mmol) were subjected to a coupling reaction in $CHCl_3$ using DCC–HOBt as coupling reagent. Column chromatography followed by crystallization from hexane afforded Boc-Gly-D-Iva-L-Pro-OBzl (318 mg, 69%), mp 92–92.5 °C.

Catalytic hydrogenolysis of the protected tripeptide (254 mg, 0.55 mmol) in MeOH (10 cm^3) over Pd-black (11 mg) under atmospheric H_2 overnight afforded Boc-Gly-D-Iva-L-Pro-OH as a solid (204 mg, ~100%), mp 109.5–111 °C.

To an ice-cooled solution of the Boc-tripeptide (186 mg, 0.50 mmol) in THF (5 cm^3) were added Gca-*p*NA (151 mg, 0.80 mmol), DCC (405 mg, 2.0 mmol), and 4-(dimethylamino)pyridine (50 mg, 0.40 mmol) and the mixture was stirred for 48 h at room temp. Usual work-up and column chromatography afforded Boc-Gly-D-Iva-L-Pro-Gca-*p*NA as a pale yellow powder (134 mg, 49%), mp 98.5–99.5 °C (from hexane).

Treatment of the Boc-depsipeptide-*p*NA (111 mg, 0.20 mmol) with TFA followed by 2,4-dinitrophenylation and column chromatography of the crude product gave *Dnp-Gly-D-Iva-L-Pro-Gca-pNA* **10** as a yellow powder (87 mg, 71%), mp 249.5–251 °C (Found: C, 50.15; H, 4.8; N, 15.5. $C_{26}H_{29}N_7O_{11}\cdot(1/2)H_2O$ requires C, 50.0; H, 4.8; N, 15.7%); δ_H(200 MHz) 0.80 (t, *J* 7.4, Iva γ-Me), 1.37 (s, Iva β-Me), 1.74 (m, Iva-β), 1.83 (m, Iva-β'), ~1.95 (m, Pro-β,γ,γ'), ~2.08 (m, Pro-β'), 3.48 (m, Pro-δ), 3.67 (m, Pro-δ'), 4.24 (m, Gly-α,α'), 4.39 (dd, *J* 7.7 and 3.6, Pro-α), 4.73 (s, Gca-α,α'), 6.93 (d, *J* 9.6, Dnp-*o'*), 7.85 (d, *J* 9.2, *p*NA-*o,o'*), 8.23 (d, *J* 9.2, *p*NA-*m,m'*), 8.33 (dd, *J* 9.6 and 2.8, Dnp-*m'*), 8.51 (s, Iva-NH), 8.87 (d, *J* 2.7, Dnp-*m*), 9.07 (t, *J* 4.9, Gly-NH) and 10.53 (s, *p*NA-NH).

Dnp-Gly-L-Iva-L-Pro-Gca-*p*NA 11. This compound was prepared in essentially the same manner as the preparation of the D-Iva diastereoisomer **10**, *via* Boc-Gly-L-Iva-L-Pro-OBzl (powder; 43%; mp 82–83.5 °C), Boc-Gly-L-Iva-L-Pro-OH (powder; ~100%; mp 126–127 °C), and Boc-Gly-L-Iva-L-Pro-Gca-*p*NA (pale yellow powder; 85%; mp 84–86 °C). *Dnp-Gly-L-Iva-L-Pro-Gca-pNA* **11** was obtained as a yellow powder (71%), mp 249.5–251 °C (Found: C, 50.0; H, 4.8; N, 15.0. $C_{26}H_{29}N_7O_{11}\cdot CH_3OH$ requires: C, 50.1; H, 5.1; N, 15.1%); δ_H(200 MHz) 0.77 (t, *J* 7.5, Iva γ-Me), 1.35 (s, Iva β-Me), 1.77 (m, Iva-β), 1.98 (m, Iva-β'), ~2.0 (m, Pro-β,β',γ,γ'), 3.51 (m, Pro-δ), 3.67 (m, Pro-δ'), 4.25 (d, *J* 5, Gly-α,α'), 4.42 (dd, *J* 8.2 and 3.3, Pro-α), 4.70 (d, *J* 16, Gca-α), 4.71 (d, *J* 16, Gca-α'), 6.94 (d, *J* 9.5, Dnp-*o'*), 7.84 (d, *J* 9.3, *p*NA-*o,o'*), 8.22 (d, *J* 9.3, *p*NA-*m,m'*), 8.32 (dd, *J* 9.5 and 2.5, Dnp-*m'*), 8.49 (s, Iva-NH), 8.85 (d, *J* 2.5, Dnp-*m*), 9.07 (t, *J* 5, Gly-NH) and 10.49 (s, *p*NA-NH).

X-Ray crystallography

Crystal data. Compound **4**: $C_{22}H_{33}N_3O_4$, M = 403.5. Orthorhombic, a = 17.315(7), b = 12.477(7), c = 10.764(4) Å, V = 2319.9 Å³, space group $P2_12_12_1$, Z = 4, D_x = 1.155 g cm^{-3}; compound **5**, $C_{22}H_{33}N_3O_4$, M = 403.5. Orthorhombic, a = 17.629(8), b = 12.402(8), c = 10.515(4) Å, V = 2298.9 Å³, space group $P2_12_12_1$, Z = 4, D_x = 1.166 g m^{-3}.

Data collection and processing. Rigaku AFC-5RU four-circle diffractometer, ω–2θ scanning mode within a range of $2\theta < 120°$, graphite-monochromated Cu-Kα radiation; 1493 reflections for compounds **4** and 1809 reflections for compound **5** with $I > 2\sigma(I)$. 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.[14] Hydrogen atoms were located from a *D*-map. Refinement was with a block-diagonal least-squares method including the hydrogen atoms in the structure-factor calculations. The weighting scheme was $w = 1/[\sigma(F) + (0.023F)^2]$. Final R- and wR-values for compounds **4**, 0.0786 and 0.0987; and for compounds **5**, 0.0734 and 0.0860. Atomic scattering factors were taken from the International Tables for X-ray Crystallography,[15] and all computations were performed on a FACOM M382 in the Data Processing Center of Kyoto University using the program system KPPXRAY[16] for the X-ray analysis.

‡ For details see 'Instructions for Authors (1995)', *J. Chem. Soc., Perkin Trans 1*, 1995, Issue 1.

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