Conformational Analysis of Peptides and Pseudopeptides Incorporating an *endo*-(2*S*,3*R*)-Norborn-5-ene Residue as a Turn Inducer

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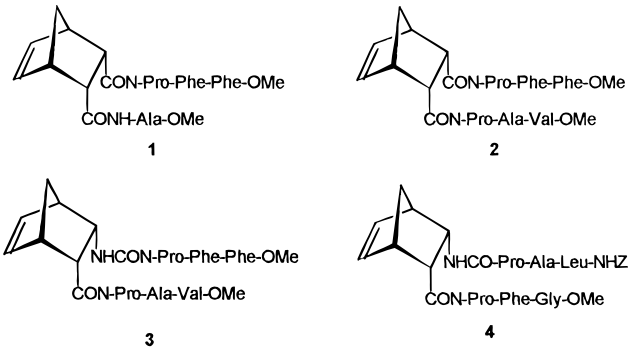
The synthesis of pseudopeptides **1**−**3** and peptide **4** were reported in the accompanying article. X-ray analysis of pseudopeptide **1** showed it to adopt a solid state conformation in which the Pro-Phe-Phe chain formed two consecutive β-turns, stabilized by hydrogen bonding between the Phe NH's and the norbornene carbonyls. However, NMR, IR, and CD studies showed that in $CDCl_3$, CH_2Cl_2, and CH_3CN solution, pseudopeptide **1** does not adopt a preferred conformation. A longer pseudopeptide **2** was found to exist in two different conformations in $CDCl_3$ solution. The major conformer adopts a structure in which both tripeptide chains form a single β-turn which is stabilized by the formation of a hydrogen bond between the *C*-terminal amino acid NH and one of the norbornene carbonyls. In the minor conformer, however, the Pro-Phe-Phe chain forms two β-turns, analogous to the X-ray structure of pseudopeptide **1**. The introduction of a urea unit into one of the peptide chains, as in pseudopeptide **3**, offsets the atom positions so as to allow interchain hydrogen bonding, and the ^3J(α-CH−NH) coupling constants and NOE's suggest that in $CDCl_3$ pseudopeptide **3** adopts a parallel β-sheet conformation. The parallel β-sheet is stabilized by the formation of two intramolecular hydrogen bonds involving the NH's of the Ala and Val residues. Finally, peptide **4**, which incorporates a conformationally constrained β-amino acid, was determined by NMR techniques to form an antiparallel β-sheet (also referred to as a β-ladder or β-hairpin). A series of model peptides lacking the norbornene unit were also prepared, and in each case NMR and IR techniques showed that the model peptides did not form well defined conformations containing intramolecular hydrogen bonds.

Introduction

In the preceding paper, we described the synthesis of peptides and pseudopeptides incorporating an *endo*-(2*S*, 3*R*)-norborn-5-ene residue. The synthetic methodology is highly flexible and allowed the synthesis of pseudopeptides such as **1** and **2** consisting of two peptide chains running in parallel directions and connected by the *endo*-(2*S*,3*R*)-norborn-5-ene unit, as well as related pseudopeptides such as **3** in which the two peptide chains again run in parallel directions but are offset due to the presence of a urea linkage in one of the peptide chains. It was also possible to prepare peptides **4** which incorporate a single conformationally constrained β-amino acid into a peptide chain.[1]

Peptides **1**−**3** were designed to investigate the ability of the *endo*-(2*S*,3*R*)-norborn-5-ene unit to induce the formation of a parallel β-sheet conformation between the two peptide chains, while peptide **4** was prepared to show that the same residue could encourage the formation of an antiparallel β-sheet by acting as a β-turn mimic.[2] β-Turns and β-sheets are important secondary structure elements of proteins,[3] which are known to be responsible for some of the biological properties exhibited by proteins. In this manuscript, we report the conformational analysis of peptides **1**−**4** which was carried out by a combination of X-ray crystallography on compound **1**, and ^{1}H NMR analysis of all four compounds. While the conformations of linear peptides containing only α-amino acids have



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(1) Throughout this paper, all α-amino acids have the (*S*)-configuration.

(2) For examples of β-turn and β-sheet mimics see: Biagini, S. C. G.; North, M. *Amino Acids, Peptides and Proteins*: *Specialist Periodical Reports Vol. 27*; (Davies, J. S., Ed.; The Royal Society of Chemistry: London, 1996; Chapter 3. Peptide Secondary Structure Mimetics: Tetrahedron Symposia in Print Number 50; Kahn, M., Ed. *Tetrahedron* **1993**, *49*, 3433-3689. Horwell, D. C.; Naylor, D.; Willems, H. M. G. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 31. Kim, K.; Germanas, J. P. *J. Org. Chem.* **1997**, *62*, 2853. Kim, K.; Germanas, J. P. *J. Org. Chem.* **1997**, *62*, 2847. Kobayashi, K.; Granja, J. R.; Ghaddidri, M. R. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 95. Tsang, K. Y. T.; Graciani, N.; Kelly, J. W. *J. Am. Chem. Soc.* **1994**, *116*, 3988. Tsang, K. Y.; Diaz, H.; Smith, A. B., III; Guzman, M. C.; Sprengeler, P. A.; Keenan, T. P.; Holcomb, R. C.; Wood, J. L.; Carroll, P. J.; Hirschmann, R. *J. Am. Chem. Soc.* **1994**, *116*, 9947. Giannis, A.; Kolter, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244. Liang, G.-B.; Rito, C. J.; Gellman, S. H. *J. Am. Chem. Soc.* **1992**, *114*, 4440. Ernest, I.; J. Kalvoda, J.; Rihs, G.; Mutter, M. *Tetrahedron Lett.* **1990**, *31*, 4011. Kemp, D. S.; Bowen, B. R.; Muendal, C. C. *J. Org. Chem.* **1990**, *55*, 4650. Kemp, D. S.; Stites, W. E. *Tetrahedron Lett.* **1988**, *29*, 5057. Kemp, D. S.; Bowen, B. R. *Tetrahedron Lett.* **1988**, *29*, 5077. Kemp, D. S.; Bowen, B. R. *Tetrahedron Lett.* **1988**, *29*, 5081 and references cited in these papers.

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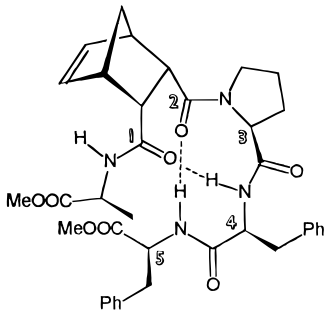

Figure 1. Representation of the solid-state structure of pseudopeptide **1**.

been extensively investigated, until very recently little was known about the conformational preferences of linear peptides containing one or more β-amino acids.[4]

Conformational Analysis of Compounds 1−4. As was discussed in the preceding manuscript, pseudopeptide **1** formed crystals suitable for X-ray crystallography. A diagram of the X-ray structure is given in the preceding paper, and an illustration of the peptide based on the X-ray structure is given in Figure 1. As Figure 1 indicates, the solid state conformation of compound **1** is stabilized by two intramolecular hydrogen bonds between the NH's of the Phe residues and the carbonyls of the norbornene units. Each of these hydrogen bonds closes a 10-membered ring, which is characteristic of a β-turn.[5] In Figure 1, the amino acid residues have been numbered 1−5 from the *N*- to the *C*-terminus. Residues 3−5 comprise ordinary α-amino acids, however residue 2 is the CH−CH−CO of the norbornene ring, so the NH of an amino acid residue has been replaced by CH in the norbornene analogue. Similarly, residue 1 is the CO−NH−CH of the alanine unit, whereas in a true peptide the sequence would be CO−CH−NH. Thus, compared to a real peptide, the NH and CH of the alanine unit have been juxtaposed.

A β-turn involving residues i to $i+3$ is classified according to the dihedral angles of the backbone atoms within residues $i+1$ and $i+2$.[5,6] Table 1 lists the dihedral angles of the residues involved in the β-turns of compound **1**. These values do not match those expected for any of the standard subclasses of β-turns. In view of the conformational constraints imposed by the unnatural norbornene unit, it is not surprising that the β-turn involving the norbornene ring should not fit the expected dihedral angles for a standard β-turn. The five-membered ring of the proline residue constrains the Pro-ϕ dihedral angle to −77°, and this is known to favor the formation of type II or type III′ turns when proline is in the $i+1$ position, and type II′, type III, or type VI[7] (with a *cis*-amide bond) turns with proline in the $i+2$ position.[6] Indeed, proline is often found in the $i+1$ position of peptides containing a β-turn structure.[8] In the present case, the conformational constraints within the norbornene unit seem to overcome the conformational preference of the proline residue.

Table 1. Dihedral Angles (deg) within the β-Turns of Compound 1

	residues 1−4	residues 2−5
ϕ_{i+1}	−7	−77
ψ_{i+1}	−73	−1.5
ϕ_{i+2}	−77	−66
ψ_{i+2}	−1.5	−18

The conformation of pseudopeptide **1** was also investigated in $CDCl_3$ solution by NMR techniques.[9] Each of the NH and α-CH protons could be assigned by a COSY spectrum, and the temperature coefficients[9,10] of the NH's were −3.0 ppb/°C and −5.0 ppb/°C for the two phenylalanine residues and −9.0 ppb/°C for the alanine residue, with a linear relationship between chemical shift and temperature. These temperature coefficients are all higher than the normally accepted upper limit (−2.6 ppb/°C) for a hydrogen bonded NH in chloroform,[10,11] indicating that no intramolecular hydrogen bonds are formed in chloroform solution. Further support for the absence of intramolecular hydrogen bonds came from IR spectroscopy. At a concentration of 30 mM, the IR spectrum of compound **1** exhibited a strong band at 3316 cm^{-1} attributable to a hydrogen bonded NH stretch, and a weak band at 3420 cm^{-1} attributable to a non-hydrogen bonded NH stretch.[11] However, as the concentration was reduced (to 3 mM), the relative intensity of the non-hydrogen bonded NH stretch increased, showing that the hydrogen bonding was intermolecular rather than intramolecular. Finally, the CD spectrum of compound **1** in CH_2Cl_2 or CH_3CN was also typical of a peptide that adopts no preferred conformation.[12]

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Table 2. ^{1}H NMR Assignments for the Major Conformation of Pseudopeptide 2

residue	proton	δ (multiplicity)	J (Hz)	ROE's[b]
Ala	CH_3	1.12 (d)	7.2	Val NH; Val CH_3
	α-CH	4.27 (pent.)	6.9	Val NH
	NH	7.15−7.2 (obscured)		Pro-1 α-CH
Val	CH_3	0.80 (d)	6.9	Val α-CH; Val OCH_3
	CH_3	0.82 (d)	6.9	Ala CH_3; Val α-CH
	β-CH	2.0−2.1 (m)		Val NH
	OCH_3	3.67[a] (s)		Val CH_3
	α-CH	4.42 (dd)	8.8, 5.0	Val CH_3
	NH	6.56 (d)	8.8	Ala α-CH; Ala CH_3; Val β-CH
Phe-1	β-CH_2	2.95−2.97 (m)		Phe-1 ArCH; Phe-1 NH; Phe-2 α-CH; Pro-2 α-CH
	β-CH_2	3.17 (dd)	13.7, 6.1	Phe-1 ArCH
	α-CH	4.54−4.60 (m)		
	NH	7.87 (d)	7.3	Phe-1 β-CH_2
	ArCH	7.1−7.35 (m)		Phe-1 β-CH_2
Phe-2	β-CH_2	2.97−2.99 (m)		norbornene H_h; Phe-2 ArCH
	β-CH_2	3.38−3.42 (m)		Phe-2 ArCH
	OCH_3	3.64[a] (s)		Phe-2 β-CH_2
	α-CH	4.26−4.32 (m)		Phe-2 ArCH
	NH	8.19 (d)	8.0	
	ArCH	7.1−7.35 (m)		Pro-1 α-CH; Phe-2 α-CH; Phe-2 β-CH_2
Pro-1	β-CH_2	1.86−1.94 (m)		
	γ-CH_2	1.94−2.02 (m)		
	γ-CH_2	2.12−2.22 (m)		Pro-1 α-CH
	δ-CH_2	3.59−3.64 (m)		norbornene H_f
	δ-CH_2	3.82 (td)	8.3, 3.7	norbornene H_f; H_e
	α-CH	4.19 (dd)	8.2, 2.6	Ala NH; Phe-2 ArCH; Pro-1 γ-CH
Pro-2	γ-CH_2	1.6−1.7 (m)		Pro-2 α-CH
	γ-CH_2	1.76−1.82 (m)		Pro-2 α-CH
	β-CH_2	1.88−1.95 (m)		
	β-CH_2	2.06−2.12 (m)		
	δ-CH_2	3.04−3.10 (m)		
	δ-CH_2	3.40−3.44 (m)		norbornene H_c
	α-CH	4.54−4.62 (m)		Phe-1 β-CH_2
norbornene	H_a	1.34 (d)	8.7	H_d; H_e
	H_b	1.47 (dt)	8.7, 1.7	H_g; H_d
	H_d	3.04−3.10 (m)		H_h; H_b; H_a
	H_c	3.20 (s)		H_g; Pro-2 δ-CH_2
	H_f	3.29 (s)		Pro-1 δ-CH_2; H_h
	H_e	3.52 (dd)	9.5, 3.4	
	H_g	5.86 (dd)	5.4, 3.0	H_c; H_b
	H_h	6.58 (dd)	5.4, 3.0	H_f; H_d; Phe-2 β-CH_2

[a] Indicates that the assignments may be interchanged. [b] Obtained from a ROESY spectrum, ROE's between coupled hydrogens are not reported. Norbornene hydrogens are assigned H_a−H_h as shown in Figure 2.

In contrast to pseudopeptide **1**, the corresponding pseudoheptapeptide **2** was not crystalline and was found to exist in $CDCl_3$ solution as a 6:1 mixture of two conformations. Hence, the conformational analysis of compound **2** was carried out entirely by solution state techniques (NMR, IR, and CD). A combination of DQF−COSY and TOCSY spectra[9] allowed each resonance for the major conformer in the 600 MHz ^{1}H NMR spectrum of compound **2** to be assigned as shown in Table 2. The two Pro and two Phe residues along with the norbornene hydrogens were distinguished by a ROESY experiment[9] on the basis of the ROE's they exhibited to neighboring protons. In particular, the α-hydrogen of Pro-1 (Figure 2) showed an ROE to the adjacent Ala-NH, and the Pro-1 δ-hydrogens showed ROE's to H_e and H_f of the norbornene unit. Similarly, the Pro-2 α-hydrogen showed an ROE to one of the Phe β-hydrogens, allowing this Phe unit to be assigned as Phe-1. One of the Pro-1 δ-hydrogens showed an ROE to H_c of the norbornene ring, providing confirmation of the norbornene assignments. For amide bonds involving the nitrogen of a proline residue, it is known that the cis and trans isomers are of similar energy and either can be observed.[7,13] The ROE's observed between the Pro δ-hydrogens and H_c, H_e, and H_f of the norbornene unit are indicative of *trans*-proline amide bonds. A *cis*-amide bond involving the proline units would have been expected to result in ROE's between the Pro α-hydrogens and the norbornene ring, which were not observed.

A linear dependence of the chemical shift of the NH protons on temperature was observed, with temperature coefficients as follows: Phe-1 −5.4 ppb/°C; Phe-2 −2.6 ppb/°C; and Val −1.9 ppb/°C; the Ala NH was obscured by the aromatic protons. The low-temperature coefficients (≤−2.6 ppb/°C) for the Phe-2 and Val NH's suggest that these hydrogens are involved in intramolecular hydrogen bonds.[9−11] Both the absolute values and the large difference between the values for Phe-1 and Phe-2 is strongly indicative of a difference in their hydrogen bonding status. Further support for the presence of intramolecular hydrogen bonds came from the lack of any concentration dependence (30 mM to 3.5 mM) of the IR spectrum of pseudoheptapeptide **2** in $CHCl_3$. The IR spectrum showed a strong band at 3293 cm^{-1} attributable to a hydrogen bonded NH stretch, and only

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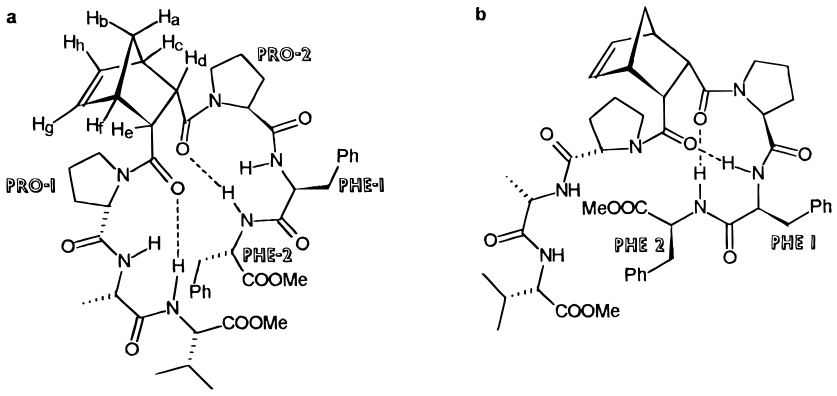

Figure 2. The conformation of **a**, the major conformer, and **b**, the minor conformer, of pseudopeptide **2**.

a weak band at 3420 cm^{-1} for a non-hydrogen bonded NH stretch.[11]

The fact that the NH's of both terminal amino acids are involved in hydrogen bonds suggests the formation of β-turns stabilized by hydrogen bonds between these NH's and the norbornene carbonyls as shown in Figure 2. The ^3J (α-CH to NH) values of 6.9−8.8 Hz (Table 2) are also consistent with the formation of β-turns involving all of the amino acid residues,[9,14] as is the observation of long-range ROE's in the ROESY spectrum between one of the Phe-2 β-hydrogens and H_h of the norbornene ring, and between the aromatic hydrogens of Phe-2 and Pro-1 α-CH. A similar close contact (2.6 Å) between a Phe-2 β-hydrogen and a vinyl hydrogen of the norbornene ring is seen in the X-ray structure of compound **1**. The alternative explanation for the formation of intramolecular hydrogen bonds, the formation of a parallel β-sheet type conformation, can be discounted due to the lack of interchain ROE's between opposite residues (Pro-1 to Pro-2; Phe-1 to Ala; Phe-2 to Val). This conformation would also be expected to involve only one of the NH's of the terminal amino acids in a hydrogen bond. CD spectra of compound **2** (in CH_2Cl_2 or CH_3CN) also indicated the presence of an ordered conformation, but due to the presence of the norbornene unit could not be used to determine which conformation(s) were present.[12]

Many of the ^{1}H NMR resonances of the minor conformer were obscured by resonances of the major conformer. However, the minor conformer NH protons were visible, as were the Pro α-CH protons and H_a of the norbornene. From these the TOCSY spectrum could be used to determine the chemical shifts of the protons in the minor conformer, these being given in Table 3. For the minor conformer, a ROESY cross-peak was observed between the α-CH of one of the phenylalanine residues and one of the methyl esters. This allowed the phenylalanine and methyl ester signals to be assigned using the numbering shown in Figure 2. It was not possible, however, to distinguish between the two proline residues.

The temperature coefficients of the NH protons were as follows: Phe-2 −1.7 ppb/°C; Phe-1 −2.3 ppb/°C; Val −5.1 ppb/°C and Ala −8.2 ppb/°C, which indicated that for the minor conformer, the NH protons of both phenylalanine residues are hydrogen bonded, while the NH protons of the alanine and valine residues are not hydrogen bonded.[9,10] Again, a linear relationship between chemical shift and temperature was observed, and there is a large difference between the values for the Phe-1 and Val NH's, which is as indicative of a change in hydrogen bonding status as the absolute values of the temperature coefficients. This is the hydrogen bonding pattern found in the X-ray structure of compound **1** and suggests that the minor conformation of pseudoheptapeptide **2** corresponds to the solid state conformation of pseudopentapeptide **1**. Consistent with this is the observation of a number of ROESY cross-peaks between the β-protons of one of the phenylalanine residues and the NH protons of the other phenylalanine residue as detailed in Table 3. It is also significant that while the ^3J (α-CH to NH) for the Phe residues were >7.5 Hz, the corresponding coupling constants for the Ala and Val residues were <7.0 Hz, values which are consistent with the formation of a random coil involving the Pro-Ala-Val chain but β-turns being formed in the Pro-Phe-Phe chain.[9,14]

In pseudopeptide **2**, the carbonyl and NH groups are not correctly positioned to allow interchain hydrogen bonding. Rather, each carbonyl in the Pro-Phe-Phe chain is opposite a carbonyl in the Pro-Ala-Val chain and similarly with the NH groups. Pseudopeptide **3** was designed to alleviate this problem by introducing a urea linkage into the Pro-Phe-Phe, thus offsetting the two peptide chains so that the carbonyls of one chain were opposite the NH groups of the other chain. In this way it was hoped to encourage interchain hydrogen bonding and the formation of a parallel β-sheet conformation.

In $CDCl_3$, pseudopeptide **3** exists as a 10:1 ratio of two conformers, and the ^{1}H NMR data for the major conformer is given in Table 4. The ROESY cross-peaks between the δ-CH_2 of Pro-1 and H_e and H_f of the norbornene ring, along with the cross-peak between the α-CH of Pro-1 and the Ala NH, allowed this proline to be assigned as the proline in the Pro-Ala-Val sequence. Similarly, ROESY correlations were observed between the δ-CH_2 of Pro-2 and the norbornene NH as well as between the α-CH of Pro-2 and aromatic protons, allowing Pro-2 to be assigned as part of the Pro-Phe-Phe sequence. The two phenylalanine residues were distinguished by the ROESY cross-peak between the α-CH of Phe-1 and the Ala-NH. A molecular model showed that this was only possible if Phe-1 was the phenylalanine unit attached to Pro-2. This cross-peak is the only interchain ROE seen in the ROESY or NOESY spectra of compound **3**.

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Table 3. ^{1}H NMR Assignments for the Minor Conformation of Pseudopeptide 2

residue	proton	δ (multiplicity)	J (Hz)	ROE's[b]
Ala	CH_3	1.42 (d)	7.0	Ala NH
	α-CH	4.48−4.52 (m)		
	NH	6.79 (d)	7.2	Ala-CH_3; Val α-CH
Val	CH_3	0.89 (d)	6.0	
	CH_3	0.90 (d)	6.8	
	β-CH	2.2−2.25 (obscured)		
	OCH_3	3.66 (s)		
	α-CH	4.55−4.6 (obscured)		Ala NH
	NH	6.48 (d)	8.8	
Phe-1	β-CH_2	2.90 (dd)	14.2, 12.1	Phe-2 α-CH
	β-CH_2	3.2−3.3 (m)		
	α-CH	4.6−4.65 (obscured)		
	NH	8.13 (d)	9.3	Phe-2 NH; Phe-1 β-CH_2
Phe-2	β-CH_2	3.05−3.1 (obscured)		
	β-CH_2	3.2−3.3 (m)		
	OCH_3	3.74 (s)		
	α-CH	4.78 (td)	8.4, 6.5	Phe-2 OCH_3; Phe-1 β-CH_2
	NH	7.55 (d)	8.6	Phe-1-NH
Pro-1[a]	β-CH_2	1.65−1.8 (obscured)		
	γ-CH_2	1.65−1.8 (obscured)		
	γ-CH_2	1.8−1.85 (obscured)		
	β-CH_2	2.0−2.2 (obscured)		
	δ-CH_2	3.44−3.49 (m)		
	δ-CH_2	3.5−3.51 (obscured)		
	α-CH	4.38 (dd)	9.0, 3.8	
Pro-2[a]	γ-CH_2	2.14−2.20 (obscured)		
	β-CH_2	2.28−2.36 (m)		
	δ-CH_2	3.22−3.26 (obscured)		
	δ-CH_2	3.50−3.51 (obscured)		
	α-CH	4.62−4.64 (m)		
norbornene	H_a	1.29 (d)	8.5	H_d
	H_b	1.43 (obscured)		
	H_d	3.04−3.08 (obscured)		H_a
	H_f	3.17−3.22 (obscured)		
	H_e	3.17−3.22 (obscured)		
	H_c	3.27−3.3 (obscured)		
	H_g	5.70 (dd)	5.5, 3.0	
	H_h	6.75 (dd)	5.6, 3.1	

[a] Assignments may be interchanged. [b] Obtained from a ROESY spectrum, ROE's between coupled hydrogens are not reported. Norbornene hydrogens are assigned H_a−H_h as shown in Figure 2.

The temperature coefficients of the NH protons were as follows: Ala −1.8 ppb/°C; Val −1.9 ppb/°C; Phe-2 −4.6 ppb/°C; norbornene −6.3 ppb/°C; with the NH of Phe-1 obscured by the aromatic signals, and the variation of chemical shift with temperature was linear. These results indicated that the Ala and Val NH's were involved in intramolecular hydrogen bonds, while the Phe-2 and norbornene NH's were not hydrogen bonded.[9,10] Once again, a significant difference was observed between the temperature coefficients of the hydrogen bonded and non-hydrogen bonded NH's. The ROESY cross-peaks seen between the Pro δ-protons and the norbornene ring and NH protons show that both proline residues adopt a *trans*-conformation as shown in Figure 3. All of the ^3J (α-CH to NH) values (Table 4) were consistent with the formation of β-sheet structures in both tripeptide chains,[9,14] as was the large number of ROESY and NOESY cross-peaks (Table 4) between the NH, α-CH, and β-CH's of adjacent residues in both peptide chains.[9] In addition, the ROESY cross-peak between the Ala-NH and the α-CH of a phenylalanine (Phe-1) provides evidence that the two peptide chains are close together. All of this information suggests the formation of a parallel β-sheet conformation as shown in Figure 3, with interchain hydrogen bonds involving the Ala NH and Pro-2 CO, and Val NH and Phe-1 CO. Construction of a molecular model showed that this was the only hydrogen bonding pattern which was possible given the observed temperature coefficients. Attempts to form hydrogen bonds between both NH's of the Ala-Val unit and other carbonyls resulted in severe steric interaction between the two chains.

The NH region of the IR spectrum of compound **3** (in $CHCl_3$) was concentration dependent, indicating that at high concentrations (30 mM) aggregation occurs. The presence of a urea NH complicates the interpretation of the IR spectrum of this compound; however, no significant changes in the spectra occurred at concentrations below 15 mM, and a hydrogen bonded NH stretch (3300 cm^{-1}) was still present at this concentration. The NMR spectra of pseudopeptide **3** were obtained at a concentration of ca. 10 mM, in the region where no aggregation occurs. Finally, CD spectra of compound **3** (in CH_2Cl_2 and CH_3CN) showed that the pseudopeptide adopted an ordered conformation,[12] though the presence of the norbornene and urea units prevented a more detailed analysis.

Peptide **4** had been designed to adopt an antiparallel[15] rather than a parallel β-sheet, with the conformationally constrained *endo*-(2*S*,3*R*)-2-amino-3-carboxy-norborn-5-ene unit acting as a β-turn mimetic. This type of structure is also referred to as a β-hairpin or β-ladder.[16]

(15) For other examples of antiparallel β-sheet formation in peptides see: Kawano, K.; Yoneya, T.; Miyata, T.; Yoshikawa, K.; Tokunga, F.; Terada, Y.; Iwanaga, S. *J. Biol. Chem.* **1990**, *265*, 15365. Blanco, F. J.; Jimenez, M. A.; Herranz, J.; Rico, M.; Santoro, J.; Nieto, J. L. *J. Am. Chem. Soc.* **1993**, *115*, 5887. Sieber, V.; Moe, G. R. *Biochemistry* **1996**, *35*, 181.

Table 4. ^{1}H NMR Assignments for the Major Conformation of Pseudopeptide 3

residue	proton	δ (multiplicity)	J (Hz)	ROE's and NOE's[b]
Ala	CH_3	1.39 (d)	7.1	Ala NH; *Val NH*
	α-CH	4.41 (pent.)	7.1	Val NH
	NH	7.15 (d)	7.3	Ala CH_3; Pro-1 α-CH; Phe-1 α-CH
Val	CH_3	0.90 (d)	7.0	Val NH; *Val α-CH*
	CH_3	0.93 (d)	6.8	Val α-CH
	β-CH	2.1−2.2 (m)		
	OCH_3	3.66[a] (s)		
	α-CH	4.50 (dd)	8.7, 4.9	Val CH_3
	NH	6.61 (d)	8.7	Val CH_3; Ala α-CH; *Ala CH_3*
Phe 1	*β*-CH_2	2.97 (dd)	14.1, 7.9	
	β-CH_2	3.13 (dd)	14.0, 5.9	
	α-CH	4.65 (td)	8.3, 5.9	Phe-2 α-CH; Phe-2 NH; ArCH; Ala NH
	NH	7.2−7.3 (obscured)		
	ArCH	7.1−7.3 (m)		
Phe 2	*β*-CH_2	3.03 (dd)	14.0, 7.0	
	β-CH_2	3.13 (dd)	14.0, 5.9	
	OCH_3	3.74[a] (s)		
	α-CH	4.79 (q)	7.0	Phe-1 α-CH; ArCH
	NH	6.99 (d)	7.9	Phe-1 α-CH
	ArCH	7.1−7.3 (m)		
Pro 1	*β*-CH_2	1.79−1.85 (m)		
	γ-CH_2	1.95−2.0 (m)		
	γ-CH_2	2.03−2.12 (m)		
	β-CH_2	2.26−2.31 (m)		
	δ-CH_2	3.6−3.72 (m)		H_e; H_f
	α-CH	4.37 (dd)	8.3, 2.5	Ala NH
Pro 2	*γ*-CH_2	1.6−1.7 (m)		
	β-CH_2	1.75−1.80 (m)		
	γ-CH_2	1.80−1.85 (m)		Pro-2 α-CH
	β-CH_2	2.13−2.20 (m)		
	δ-CH_2	2.95−3.00 (m)		
	δ-CH_2	3.03−3.08 (m)		norbornene NH
	α-CH	4.24 (dd)	8.4, 1.7	Pro-2 *γ*-CH_2; ArCH
norbornene	H_a	1.46 (d)	7.1	H_d; H_e; *H_g*
	H_b	1.51 (dt)	9.0, 1.9	H_e; *H_h*
	H_c	3.01 (s)		H_f; *H_g*; norbornene NH
	H_f	3.10 (s)		H_c; H_h; *H_g*; Pro-1 *δ*-CH_2
	H_e	3.27 (dd)	9.0, 3.2	H_a; *H_g*; Pro-1 *δ*-CH_2
	H_d	4.73 (td)	9.0, 3.7	H_a
	NH	5.80 (d)	8.9	H_c; H_g; H_h; Pro-2 *δ*-CH_2
	H_h	6.14 (dd)	5.6, 3.0	H_b; norbornene NH
	H_g	6.34 (dd)	5.7, 3.0	*H_a*; *H_c*; *H_e*; *H_f*; norbornene NH

[a] Peak assignments may be exchanged. [b] ROE's/NOE's between coupled hydrogens are not reported. Italicized correlations are observed only in the NOESY spectrum; other correlations are observed in the ROESY spectrum or both spectra. Norbornene hydrogens are assigned H_a−H_h as shown in Figure 2.

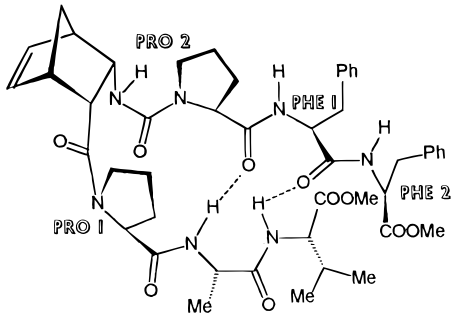


Figure 3. The conformation of the major conformer of compound **3**.

The peptide was analyzed by NMR techniques in the way described for pseudopeptides **2** and **3**. In this case, the analysis was simplified due to the presence of a single Phe residue, and the Pro residues were distinguished by the ROE's they showed to the adjacent residues and norbornene hydrogens (Table 5). In particular, the α-CH of Pro-1 showed a ROESY cross-peak to the Phe NH. The Pro-2 α-CH, however, showed a ROESY correlation to the norbornene NH, and one of the Pro-2 *β*-CH_2 hydrogens showed a ROESY correlation to the Ala α-CH.

The temperature coefficients of the NH protons were as follows: Phe 2.3 ppb/°C; norbornene 0.7 ppb/°C; Gly −0.3 ppb/°C; Ala −4.5 ppb/°C; Leu −8.0 ppb/°C; a linear variation of chemical shift with temperature was observed for each NH. The large difference in temperature coefficient between the Gly and Ala NH's as well as the absolute values of the temperature coefficients are characteristic of a difference in hydrogen bond status.[9,10] These results indicated that the Phe, Gly, and norbornene NH's were involved in intramolecular hydrogen bonds, while the Ala and Leu NH's were not hydrogen bonded. Consistent with this analysis, the IR spectrum of peptide **4** (in $CHCl_3$) showed two NH stretching bands of equal intensity at 3326 cm^{-1} and 3411 cm^{-1} corresponding to hydrogen bonded and non-hydrogen bonded NH stretches, respectively. The IR spectrum was concentration independent over the concentration range 30 mM to 7 mM.[11]

A large number of long-range ROESY and NOESY correlations were seen for compound **4**, and these are shown in Figure 4. In particular, the Ala *β*-CH_3 shows an ROE to both H_g and H_h of the norbornene ring, indicating that the Pro-Ala-Leu chain is folded back

(16) Sibanda, B. L.; Thornton, J. M. *J. Mol. Biol.* **1993**, *229*, 428.

Table 5. ^{1}H NMR Assignments for the Major Conformation of Peptide 4

residue	proton	δ (multiplicity)	*J* (Hz)	NOE's and ROE's[a]
Ala	CH_3	1.35 (d)	6.8	Ala NH; Pro-2 δ-CH_2; *Pro−2 β- CH_2*; norbornene NH; H_g; H_h
	α-CH	4.55 (pent.)	6.8	Pro-2 δ-CH_2
	NH	7.40 (d)	6.2	Ala CH_3; Leu α-CH; Leu NH
Leu	CH_3	0.91 (d)	6.3	Leu β-CH_2; Leu α-CH; Gly α-CH_2
	CH_3	0.93 (d)	6.9	Gly α-CH_2
	β-CH_2	1.46−1.52 (m)		Leu NH; Leu δ-CH_3
	β-CH_2	1.60−1.65 (m)		Leu δ-CH_3
	γ-CH	1.70−1.80 (m)		Leu NH; Leu α-CH
	α-CH	4.20−4.25 (m)		Leu γ-CH; Leu δ-CH_3; Ala NH
	OCH_2	5.02 (d)	12.4	
	OCH_2	5.15 (d)	12.4	
	NH	6.37 (d)	8.2	Leu β-CH_2; Leu γ-CH; Leu δ-CH_3; Ala NH
Phe	β-CH_2	3.03 (dd)	13.9, 6.3	Pro-2 δ-CH_2; Gly NH
	β-CH_2	3.35 (dd)	14.0, 5.7	Pro-2 δ-CH_2; Gly NH
	α-CH	4.68−4.71 (m)		Gly NH; Pro-2 δ-CH_2
	NH	6.32 (d)	8.5	Pro-1 α-CH; Gly NH
	ArCH	7.1−7.3 (m)		
Gly	α-CH_2	3.74 (dd)	17.6, 6.0	
	OCH_3	3.71 (s)		
	α-CH_2	4.13 (dd)	17.6, 6.0	Leu δ-CH_3
	NH	7.23 (d)	7.5	Phe α-CH; Phe β-CH_2; Phe NH
Pro-1	β-CH_2	1.7−1.8 (m)		
	γ-CH_2	1.7−1.8 (m)		
	β-CH_2	1.92−1.94 (m)		Pro-1 δ-CH_2
	δ-CH_2	3.28−3.33 (m)		
	δ-CH_2	3.55−3.60 (m)		Pro-1 α-CH
	α-CH	4.53 (dd)	12.2, 7.4	Pro-1 δ-CH_2; Phe-NH; *H_b*
Pro-2	γ-CH_2	1.92−1.94 (m)		Pro-2 α-CH
	β-CH_2	1.98−2.04 (m)		Pro-2 δ-CH_2; *Ala α-CH*
	β-CH_2	2.06−2.11 (m)		
	δ-CH_2	3.41−3.45 (m)		Ala α-CH; Ala β-CH_3; Phe α-CH; Phe β-CH_2
	δ-CH_2	3.55−3.60 (m)		Ala β-CH_3; Phe β-CH_2
	α-CH	4.38 (dd)	8.0, 4.2	Pro-2 γ-CH_2; norbornene NH
norbornene	H_a	1.29 (d)	8.9	H_d; H_e
	H_b	1.44 (d)	8.9	H_g; H_h; *Pro−1 α-CH*
	H_f	2.69 (s)		
	H_c	2.93 (s)		
	H_e	2.98 (d)	8.4	H_a
	H_d	4.87 (td)	9.7, 3.8	H_a
	NH	6.06 (d)	10.0	H_c; H_g; H_h; Pro-2 α-CH; Ala β-CH_3
	H_h	5.86 (t)	1.7	H_b; norbornene NH; Ala β-CH_3
	H_g	6.25 (s)		Ala β-CH_3

[a] NOE's/ROE's between coupled hydrogens are not reported. Italicized correlations are observed only in the NOESY spectrum, other correlations are observed in the ROESY spectrum or both spectra. Norbornene hydrogens are assigned H_a−H_h as shown in Figure 2.

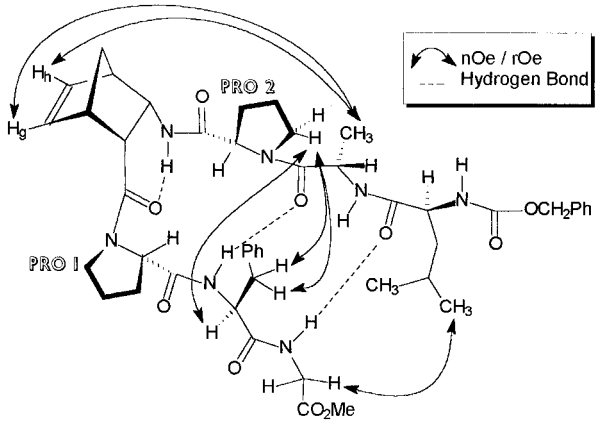


Figure 4. Long-range NOE's and ROE's seen in the NMR spectrum of peptide **4**.

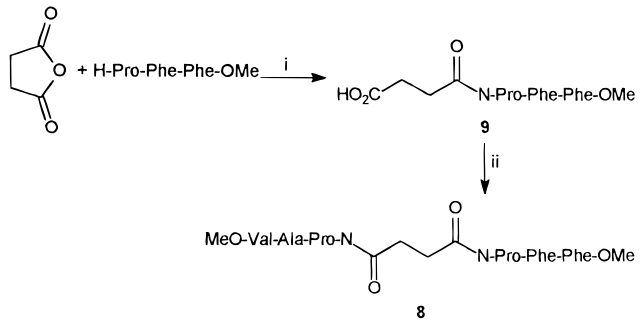
underneath the norbornene ring. The Phe α-CH and β-CH_2's all show ROESY correlations to the Pro-2 δ-CH_2, showing that amino acids on opposite sides of the norbornene ring are close together, and the Leu δ-CH_3's show an ROE to the Gly α-CH_2, again showing that even the *N*- and *C*-terminal amino acids are close together. In addition to these long-range ROE's, a large number of ROE's and NOE's were observed between adjacent residues (Table 5) and are characteristic of β-sheet formation.[9] Thus both the temperature coefficients and the ROE's are indicative of the formation of an antiparallel β-sheet conformation. Finally, all of the ^3J (α-CH to NH) values (except Ala) were ≥7.5 Hz, which is again consistent with β-sheet formation.[9,14]

The CD spectra of peptide **4** (in CH_2Cl_2 or CH_3CN) indicated that the peptide adopted a preferred conformation and were almost exactly those expected of a β-sheet conformation, showing a maximum at 190 nm and a minimum at 225 nm.[12] However, a shoulder was also present at 210 nm, which may be due to the presence of a β-amino acid or a conformationally constrained norbornene unit.

Synthesis and Analysis of Model Compounds. Aromatic amino acids such as phenylalanine are often found in β-sheet forming regions of proteins,[3,17] and since each of compounds **1**−**4** contained at least one phenylalanine residue, four model compounds were prepared to investigate whether the formation of β-turn and β-sheet type conformations in compounds **1**−**4** was due to the influence of the aromatic amino acids. Both *N*-Ac-

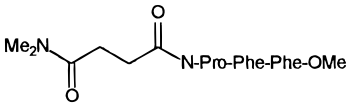
(17) Chou, P. Y.; Fasman, G. D. *Annu. Rev. Biochem.* **1978**, *47*, 251. Wilmot, C. M.; Thornton, J. M. *J. Mol. Biol.* **1988**, *203*, 221.

Scheme 1[a]



[a] Reagents: (i) Et_3N; (ii) HN-Pro-Ala-Val-OMe/EDC/HOBt/Et_3N.

Pro-Phe-Phe-OMe **5** and *N*-Ac-Pro-Ala-Val-OMe **6** were prepared by acetylation of the corresponding tripeptide methyl esters.[18] Analogue **7** which consists of a tripeptide attached to an unconstrained succinamide unit was



prepared by reaction between H-Pro-Phe-Phe-OMe and *N*,*N*-dimethylsuccinamide, and analogue **8**, which incorporates both peptide chains and the unconstrained succinamide unit, was prepared as shown in Scheme 1.

For compounds **5**−**7**, the temperature coefficients of the NH's (in $CDCl_3$ solution) were all between −3.3 and −4.3 ppb/°C, indicating that none of the NH's were involved in intramolecular hydrogen bonds.[9,10] Similarly, the IR spectra of compounds **5**−**7** at low concentrations (<3.5 mM in $CHCl_3$) showed a strong absorption at 3400−3420 cm^{-1} corresponding to a non-hydrogen bonded NH stretch and either a very weak absorption at 3320−3340 cm^{-1} corresponding to a hydrogen bonded NH stretch or no absorption in this region.[11] Hence it is apparent that model peptides **5**−**7** adopt no preferred conformation in chloroform solution.

The NMR spectrum of compound **8** was extremely complex, showing evidence of at least four conformations. Furthermore, the variation of chemical shift of the NH signals with temperature was not linear, indicating that conformational changes were occurring as the temperature changed.[9,10] The IR spectrum of compound **8** as a 2 mM solution in $CHCl_3$, however, showed only a non-hydrogen bonded NH stretch at 3419 cm^{-1}, though at higher concentrations a hydrogen bonded NH stretch (3326 cm^{-1}) was observed and became dominant at 30 mM. The most likely explanation of these results is that compound **8** exists as a mixture of conformations which differ in the geometry of the tertiary amide bonds involving the proline residues,[7,13] but which do not adopt any preferred secondary structure.

Conclusions

We have demonstrated that the 2,3-dicarboxy-*endo*-(2*S*,3*R*)-norborn-5-ene and 2-amino-3-carboxy-*endo*-(2*S*,3*R*)-norborn-5-ene residues which are readily prepared from *endo*-norborn-5-ene 2,3-dicarboxylic anhydride are suitable templates for the formation of β-turn and β-sheet structures. Depending upon the nature of the template, and the way in which the peptides are attached to the template, it is possible to use these units to induce the formation of β-turns, parallel β-sheets, or antiparallel β-sheets. The conformational constraint of the norbornene unit is necessary for the formation of these conformations, since neither the peptides themselves nor the acyclic analogue **8** adopted well defined conformations. The above conclusions are based on the solid state conformation of compound **1** and the solution state conformations of compounds **1**−**4** in nonpolar solvents such as chloroform and acetonitrile. The NMR spectra of compounds **1**−**4** were also recorded in DMSO-d_6, but no evidence for intramolecular hydrogen bonding or interchain NOE's were observed in this case. Presumably the DMSO is such a good hydrogen bond acceptor that it breaks the intramolecular hydrogen bonds, as a result of which compounds **1**−**4** adopt disordered conformations in DMSO. Our work on the applications of these templates in the preparation of biologically active peptides with defined conformations is continuing and will be reported in due course.

Experimental Section

General experimental details are given in the preceding paper. NMR spectra used for conformational analysis were measured in $CDCl_3$ using a Varian 600 INOVA spectrometer operating at 599.9 MHz for protons and 149.9 MHz for ^{13}C nuclei. The following details for compound **4** are typical of the parameters used. DQFCOSY spectra were obtained using hypercomplex acquisition, preexcitation delay = 1.4 s, and an FID acquisition time of 0.17 s. Other parameters were SW = 6000 Hz, 2K data points, and 200 increments each with 16 transients per FID. The data were processed using shifted sine-bell squared functions in both dimensions with zero filling of the F_1-data to 1K. TOCSY spectra were obtained using hypercomplex acquisition, preexcitation delay = 1.5 s, mixing time = 80 ms, and FID acquisition time = 0.17 s. Other parameters were SW = 6000 Hz, 2K data points, and 256 increments each with 4 transients per FID. NOESY and ROESY spectra were obtained using hypercomplex acquisition, preexcitation delay = 1.4 s; mixing time = 150 ms, and FID acquisition time = 0.34 s. Other parameters were SW = 6000 Hz, 4K data points, and 256 increments each with 16 (NOESY) or 24 (ROESY) transients per FID. The data were processed using shifted sine-bell squared functions in both dimensions with zero filling of the F_1-data to 1K. 2-D Proton-detected one-bond $^1H-^{13}C$ correlation (HMQC) spectra were obtained using hypercomplex acquisition, preexcitation delay = 1.4 s; D2 = 3.7 ms (0.5 $^1J_{CH}$), null period = 300 ms (to minimize signals from protons bonded to ^{12}C nuclei), and FID acquisition time = 0.34 s. The experiment was preceded by 128 dummy scans to establish thermal equilibrium, and ^{13}C broad band decoupling was employed during acquisition of the proton signals. Other parameters were SW(^1H) = 6000 Hz, 4K data points, SW(^{13}C) = 30000 Hz, and 128 increments with 8 scans per FID. The data were processed using shifted sine-bell squared functions in both dimensions with zero filling of the F_1-data to 512K before transformation.

N-Ac-Pro-Phe-Phe-OMe (5). Triethylamine (0.39 mL, 2.79 mmol) was added to a cooled (0 °C) suspension of CF_3CO_2H·HN-Pro-Phe-Phe-OMe[19] (0.30 g, 0.54 mmol) and acetyl chloride (0.2 mL, 2.79 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred at room temperature for 14 h and subsequently washed with 0.5 M HCl (3 mL), saturated aqueous Na_2CO_3 (3 mL), and H_2O (3 mL) and dried ($MgSO_4$).

(18) Allen, M. C.; Brandish, D. E.; Fullerton, J. D.; Wade, R. *J. Chem. Soc.*, Perkin Trans. 1 **1986**, 989; Krois, D.; Lehner, H. *J. Chem. Soc., Perkin Trans. 2* **1990**, 1745.

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The solvent was evaporated in vacuo and the residue subjected to flash chromatography using EtOAc as eluent affording (R_f = 0.08, EtOAc) 0.21 g (81%) of a white solid, mp 34−38 °C. $[\alpha]^{22}_D$ −68.3 (c = 1, $CHCl_3$). IR ($CHCl_3$): 3415, 3314, 3018, 1743, 1672. ^{1}H NMR: 1.7−2.4 (m, 4), 1.94 (s, 3), 2.9−3.3 (m, 6), 3.72 (s, 3), 4.44 (dd, 1, J = 7.6, 2.9), 4.6−4.7 (m, 1), 4.83 (q, 1, J = 6.7), 6.69 (d, 1, J = 7.6), 7.05−7.3 (m, 11). ^{13}C NMR: 22.3, 24.8, 27.3, 37.0, 37.7, 48.0, 52.3, 53.3, 53.8, 59.6, 126.6, 126.9, 128.3, 128.5, 129.2, 136.0, 136.9, 170.5, 171.1, 171.4, 171.6. CI-MS m/e (relative intensity): 483 (M^+ + 18, 3), 466 (M^+ + 1, 12). HRMS (CI, NH_3) m/e: 466.2342 (MH^+ $C_{26}H_{32}N_3O_5$ requires 466.2341).

N-Ac-Pro-Ala-Val-OMe (6). Triethylamine (0.26 mL, 1.86 mmol) was added to a cooled (0 °C) suspension of glacial acetic acid (0.21 mL, 0.37 mmol), water soluble carbodiimide, EDC (0.093 g, 0.48 mmol), HOBt (0.065 g, 0.48 mmol), and CF_3CO_2H·HN-Pro-Ala-Val-OMe[18] (0.20 g, 0.48 mmol) in CH_2Cl_2 (8 mL). The reaction mixture was stirred for 16 h and subsequently washed with 0.5 M HCl (8 mL), saturated aqueous Na_2CO_3 (8 mL), and H_2O (8 mL) and dried ($MgSO_4$). The solvent was evaporated in vacuo, affording 0.11 g (67%) of a clear oil. $[\alpha]^{22}_D$ −26.2 (c = 1, $CHCl_3$). IR ($CHCl_3$): 3307, 1741, 1633. ^{1}H NMR: 0.83 (d, 3, J = 5.1), 0.85 (d, 3, J = 5.1), 1.29 (d, 3, J = 7.0), 1.8−2.5 (m, 4), 2.04 (s, 3), 3.3−3.6 (m, 2), 3.65 (s, 3), 4.3−4.5 (m, 3), 6.86 (d, 1, J = 9.0), 7.36 (d, 1, J = 6.9). ^{13}C NMR: 17.3, 17.7, 18.9, 22.5, 25.0, 28.1, 31.0, 48.3, 49.0, 52.1, 57.2, 59.7, 170.8, 171.6, 172.1, 172.2. CI-MS m/e (relative intensity): 359 (M^+ + 18, 9), 342 (M^+ + 1, 100). HRMS (CI, NH_3) m/e: 342.2029 (MH^+ $C_{16}H_{27}N_3O_5$ requires 342.2029).

Pseudotripeptide 7. Triethylamine (0.83 mL, 5.99 mmol) was added to a cooled (0 °C) suspension of succinic anhydride (0.2 g, 2.0 mmol) and Me_2NH·HCl (0.17 g, 2.2 mmol) in CH_2Cl_2 (5 mL). After stirring for 16 h at room temperature, DCC (0.53 g, 2.6 mmol) and *N*-hydroxysuccinimide (0.30 g, 2.60 mmol) were added to the reaction mixture. Stirring was continued for a further 12 h, after which the dicyclohexylurea byproduct was removed by filtration. The filtrate was washed with H_2O (3 × 5 mL), dried ($MgSO_4$), and evaporated to dryness in vacuo, yielding the crude active ester (0.25 g, 1.03 mmol) which was used without further purification. The active ester was then redissolved in CH_2Cl_2 (5 mL), and CF_3CO_2H·HN-Pro-Phe-Phe-OMe[19] (0.72 g, 1.34 mmol) and Et_3N (0.43 mL, 3.10 mmol) were added at 0 °C. The solution was stirred at room temperature for 9 h and then washed sequentially with 1 M HCl (5 mL), saturated aqueous Na_2CO_3 (5 mL), and H_2O (5 mL). The organic phase was dried ($MgSO_4$), filtered, and evaporated to dryness in vacuo, affording a yellow oil. Flash chromatography (10% MeOH/ 90% EtOAc) gave (R_f = 0.18, 10% MeOH/ 90% EtOAc) 0.42 g (38%) of a white powder, mp 35−39 °C. $[\alpha]^{23}_D$ −45.4 (c = 1, $CHCl_3$). IR ($CHCl_3$): 3316, 3015, 1744, 1629. ^{1}H NMR: 1.8−2.1 (m, 4), 2.15−2.8 (m, 4), 2.9−3.15 (m, 4), 2.93 (s, 3), 2.98 (s, 3), 3.4−3.8 (m, 2), 3.60 (s, 3), 4.5−5.0 (m, 1), 4.6−4.8 (m, 2), 7.2−7.35 (m, 11), 7.57 (d, 1, J = 9.1). ^{13}C NMR (only peaks corresponding to the major conformer are reported): 23.7, 28.8, 29.3, 29.5, 36.1, 38.0, 53.9, 54.6, 60.4, 126.4, 126.7, 128.2, 128.3, 128.5, 129.0, 136.7, 138.4, 171.3, 171.4, 171.6, 172.4, 173.1. CI-MS m/e (relative intensity): 551 (M^+ + 1, 100). HRMS (CI, NH_3) m/e: 551.2869 (MH^+, $C_{30}H_{39}N_4O_6$ requires 551.2869). Anal. Calcd for $C_{30}H_{38}N_4O_6$ (H_2O): C, 63.35; H, 7.09; N, 9.86. Found C, 63.59; H, 6.95; N, 9.73.

Acid 9. Triethylamine (0.36 mL, 2.57 mmol) was added to a cooled (0 °C) suspension of succinic anhydride (0.086 g, 0.86 mmol) and CF_3CO_2H·HN-Pro-Phe-Phe-OMe[19] (0.60 g, 1.10 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred at room temperature for 24 h and subsequently washed with 0.5 M HCl (5 mL) and H_2O (2 × 5 mL) and dried ($MgSO_4$), and the solvent was evaporated in vacuo to leave a yellow solid. Purification by flash chromatography using EtOAc as eluent afforded (R_f = 0.05, EtOAc) 0.3 g (67%) of a white solid, mp 57−61 °C. $[\alpha]^{22}_D$ −62.8 (c = 1, $CHCl_3$). IR ($CHCl_3$): 3500−2400, 3340, 1738, 1666. ^{1}H NMR: 1.7−2.5 (m, 4), 2.5−3.7 (m, 10), 3.64 (s, 3), 4.45 (t, 1, J = 5.6), 4.63 (q, 1, J = 6.8), 4.65−4.75 (m, 1), 7.0−7.3 (m, 12). ^{13}C NMR (only peaks corresponding to the major conformer are reported): 24.1, 28.6, 28.8, 29.3, 36.4, 37.7, 47.5, 52.1, 54.0, 54.1, 60.3, 126.6, 126.9, 128.5, 129.1, 129.2, 136.1, 137.3, 171.3, 171.5, 171.5, 172.4, 175.8. CI-MS m/e (relative intensity): 524 (M^+ + 1, 50). HRMS (CI, NH_3) m/e: 524.2400 (MH^+, $C_{29}H_{34}N_3O_7$ requires 524.2396). Anal. Calcd for $C_{28}H_{33}N_3O_7$: C, 64.23; H, 6.35; N, 8.03. Found C, 64.22; H, 6.29; N, 7.79.

Pseudoheptapeptide 8. Triethylamine (0.27 mL, 1.87 mmol) was added to a cooled (0 °C) suspension of acid **9** (0.20 g, 0.38 mmol), water soluble carbodiimide, EDC (0.09 g, 0.46 mmol), HOBt (0.062 g, 0.46 mmol), and CF_3CO_2H·HN-Pro-Ala-Val-OMe[20] (0.24 g, 0.57 mmol) in CH_2Cl_2 (8 mL). The reaction mixture was stirred at room temperature for 16 h and washed with 0.5 M HCl (8 mL), saturated aqueous Na_2CO_3 (8 mL), and H_2O (8 mL) and dried ($MgSO_4$). The solvent was evaporated in vacuo and the residue subjected to flash chromatography using 10% MeOH/90% EtOAc as eluent to give (R_f = 0.17, 10% MeOH/90% EtOAc) 0.23 g (75%) of a white powder, mp 79−81 °C. $[\alpha]^{22}_D$ −71.3 (c = 1, $CHCl_3$). IR ($CHCl_3$): 3419, 3326, 1741, 1668, 1630. ^{1}H NMR (peaks reported as multiplets refer to all conformers, other peaks refer to individual conformers): 0.74−0.9 (m, 6), 1.32 (d, 3, J = 7.1), 1.38 (d, 3, J = 7.3), 1.39 (d, 3, J = 7.1), 1.54 (d, 3, J = 7.3), 1.6−2.3 (m, 9), 2.4−2.8 (m, 4), 2.8−3.2 (m, 4), 3.3−3.8 (m, 4), 3.58 (s, 3), 3.66 (s, 3), 3.67 (s, 3), 3.68 (s, 3), 3.69 (s, 3), 3.72 (s, 3), 4.17 (d, 1, J = 4.5), 4.25 (d, 1, J = 8.1), 4.29 (d, 1, J = 3.3), 4.35 (dd, 1, J = 8.5, 5.6), 4.4−4.7 (5H, m), 4.8−4.9 (m, 1), 6.57 (d, 1, J = 8.8), 6.63 (d, 1, J = 7.6), 6.83 (d, 1, J = 6.5), 6.85 (d, 2, J = 6.9), 7.00 (d, 1, J = 7.1), 7.0−7.3 (m, 12), 7.6−7.7 (m, 3), 7.97 (d, 1, J = 7.4). ^{13}C NMR: 16.7, 17.3, 17.5, 17.6, 17.7, 17.8, 17.9, 18.1, 18.8, 18.9, 21.6, 22.0, 22.3, 22.5, 23.7, 24.3, 24.4, 24.6, 28.1, 28.7, 28.9, 29.2, 29.4, 29.5, 29.9, 30.0, 30.1, 30.4, 30.8, 31.0, 31.1, 31.3, 31.5, 31.6, 31.9, 36.1, 36.8, 37.1, 37.5, 37.8, 38.1, 46.5, 46.7, 46.9, 47.3, 47.5, 47.6, 47.8, 49.1, 49.2, 49.3, 51.5, 51.9, 52.0, 52.1, 52.2, 52.3, 53.3, 53.7, 53.9, 54.1, 54.7, 56.6, 56.8, 57.3, 57.4, 57.5, 59.8, 60.2, 60.4, 60.5, 61.0, 61.1, 61.4, 126.4, 126.6, 126.7, 126.8, 127.1, 128.2, 128.3, 128.4, 128.5, 128.7, 128.9, 129.1, 129.2, 129.3, 129.5, 135.9, 136.4, 136.6, 137.4, 137.5, 138.2, 170.4, 170.5, 170.7, 170.8, 171.2, 171.3, 171.4, 171.5, 171.6, 171.7, 171.8, 171.9, 172.0, 172.2, 172.3, 172.5, 172.6, 172.7, 173.0, 173.3, 173.7. CI-MS m/e (relative intensity): 805 (M^+ + 1, 56), 182 (100). HRMS (FAB) m/e: 804.4042 (M^+, $C_{42}H_{56}N_6O_{10}$ requires 804.4057). Anal. Calcd for $C_{42}H_{56}N_6O_{10}$ (3 H_2O): C, 58.73; H, 7.28; N, 9.78. Found C, 58.73; H, 7.19; N, 10.18.

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Supporting Information Available: Copies of ^{1}H NMR spectra (2 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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