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Synthesis and folding preferences of γ-amino acid oligopeptides: stereochemical control in the formation of a reverse turn and a helix

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Abstract

The stereoselective synthesis of α-cinnamyl γ-amino acids and the corresponding oligopeptides is described. Detailed 1D and 2D NMR studies in pyridine-d_5 show that the (α*R*)-cinnamyl γ-amino acid tetrapeptide adopts a reverse turn structure, while the (α*S*)-cinnamyl γ-amino acid tetrapeptide adopts a right-handed helical structure.

The design and synthesis of unnatural oligopeptides that are able to form well-defined secondary structures has received significant attention in the past few years.[1] Recent investigations by Seebach,[2] Gellman[3] and in our laboratory[4] have shown that β-amino acid oligopeptides can adopt helix, sheet or reverse turn conformations in solution[2,4] or solid state,[3d] as evidenced by NMR, CD, X-ray or modeling studies. Further studies by us[5] as well as by Seebach[6] have revealed that γ-amino acid oligopeptides can also adopt helical conformations in solution. Gervay et al.[7] have demonstrated helix formation in a δ-peptide constructed from neuraminic acid. Oligomers of dihydroxy tetrahydrofuran amino acids also adopt a helical structure.[8] A notable contribution from Gellman reports helix formation by a foldamer in water.[9] In previous studies, we demonstrated that the folding patterns of our γ-amino acid oligopeptides could be controlled by changing the stereochemistry of an α-methyl substituent to give helical or non-helical structures.[5] In order to further investigate this phenomenon, we synthesized both (α*R*)- and (α*S*)-cinnamyl γ-amino acid oligopeptides (Fig. 1). Although X-ray quality crystals could not be obtained, detailed NMR studies revealed the existence of reverse turn and helical conformations in solution for tetrapeptides **4** and **8** respectively, depending on the configuration at the α position of each γ-amino acid residue (Fig. 1).

The (α*R*)-cinnamyl γ-amino acid tetrapeptide **4** was synthesized via the convergent route shown in Scheme 1. The *N*-Boc protected monomer **9** was prepared as previously described by homologation of L-alanine and subsequent stereoselective alkylation.[10] The stereoselective synthesis of (α*S*)-cinnamylated

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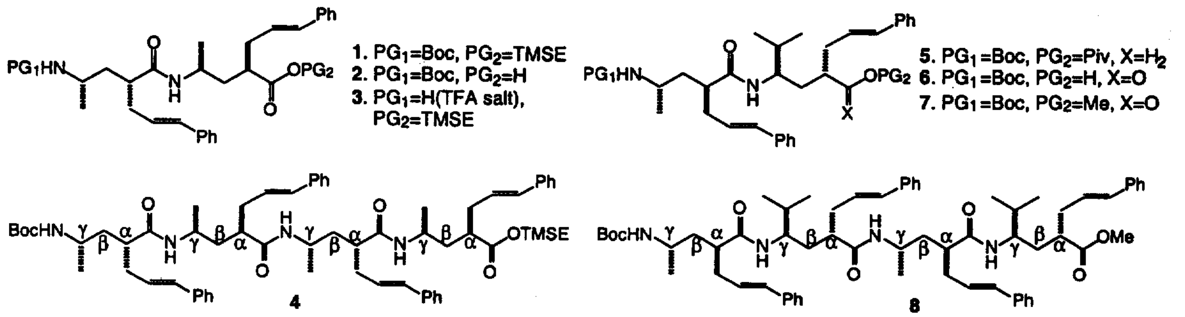
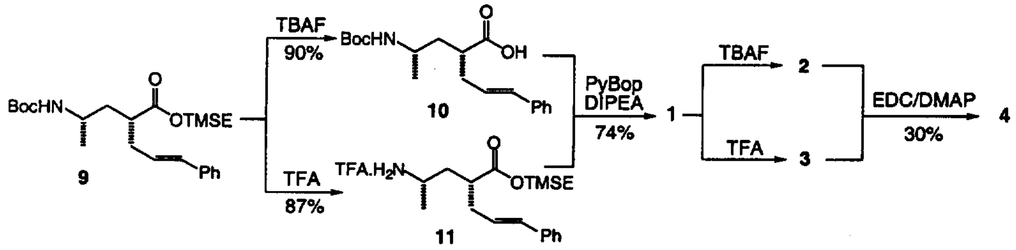
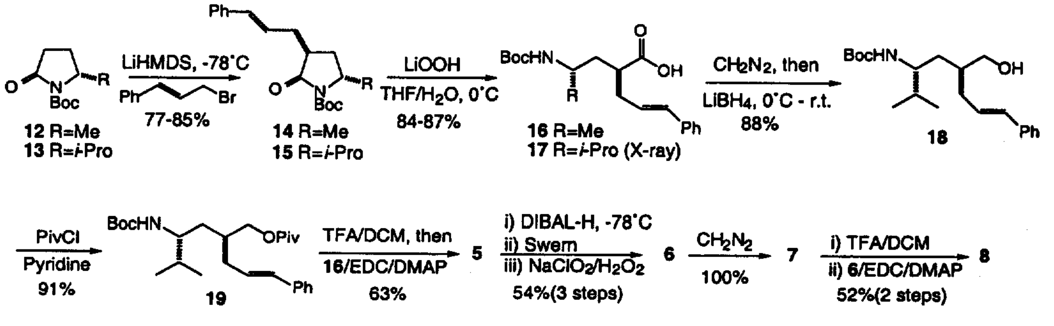


Figure 1.

N-Boc protected γ-amino acid units **16** and **17**, however, was achieved by a different route as shown in Scheme 2.



Scheme 1.



Scheme 2.

Pyrrolidinones **12** and **13** were synthesized from α-amino acids via *N*-Cbz protected α,β-unsaturated γ-amino esters,[11] or Meldrum's acid derivatives.[12] Cinnamylation of **12** and **13** in the presence of LiHMDS at −78°C gave **14** and **15** in good isolated yields and with high diastereoselectivities (*anti*:*syn*=18:1 and 40:1, respectively). Hydrolysis of **14** and **15** under mild conditons[13] afforded **16** and **17** without detectable epimerization at the newly generated stereocenters, as evidenced by ^{1}H NMR and X-ray crystal structure analysis.

The synthesis of dipeptide **6** was challenging since lactamization of both the Boc-protected γ-amino acid **16** and the Boc-deprotected γ-amino ester derived from **17** occurred under several conditions of peptide coupling. Although lactamization of **16** could be minimized when EDC/DMAP was used, it was necessary to adopt a slightly longer route in the case of **17** by going through the pivalate ester **19**. Thus, peptide coupling followed by deprotection and oxidation gave the dipeptide **6** albeit in moderate yield. The target tetrapeptide **8** was then obtained by a convergent synthesis.

Table 1

Key inter-residue NOEs for peptides **4** and **8** in pyridine-d_5 at room temperature (1 mM)

Peptide	Proton	Residue	Proton	Residue	NOE	Proton	Residue	Proton	Residue	NOE
4	NH	2	Hα	1	strong	Hα	3	Hγ	2	strong
	NH	3	Hα	2	medium	NH	4	Hβ(pro S)	3	weak
	NH	3	Hβ(pro S)	2	strong	NH	4	Hα	3	strong
8	NH	2	Hα	1	strong	NH	4	Hα	3	strong
	NH	3	Hα	2	strong	NH	4	Hγ	3	strong
	NH	3	Hγ	1	medium	NH	4	Hγ	2	medium

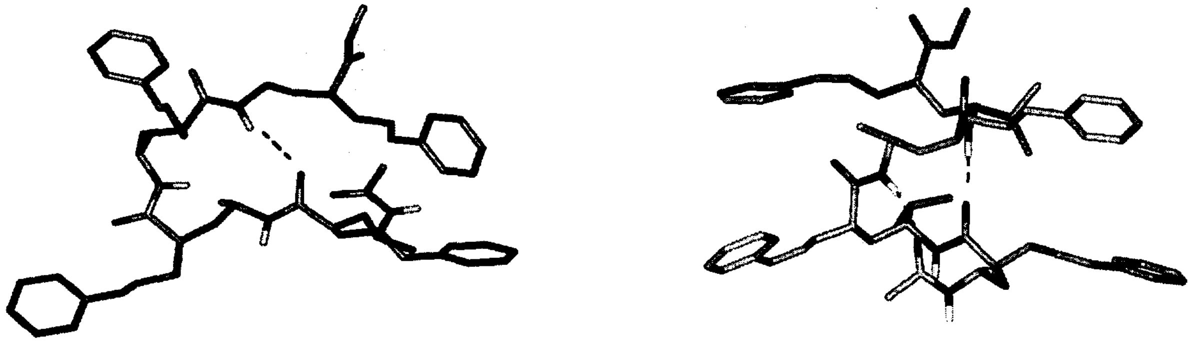


Figure 2. Computer molecular model of tetrapeptides **4** (left) and **8** (right) from COSY and NOE constraints[19]

2D ^{1}H NMR experiments (COSY, TOCSY, ROESY) were performed on peptides **4** and **8** in pyridine-d_5 at 1 mM since intra- rather than inter-molecular processes are favored at these concentrations.[3a] Complete proton resonance assignments in each residue of the peptides were easily achieved by means of a combination of both TOCSY and COSY data. Sequence specific assignments for each peptide were obtained by analyzing short-range NOEs between Hα(i) and NH(i+1).

The NOE data observed for peptide **4** (Table 1) suggest a reverse turn structure as shown in Fig. 2 (left). The large cinnamyl substituents are arranged towards the exterior of the turn while the amide proton of residue 4 is oriented towards the carbonyl group of residue 1. Deuterium exchange studies in pyridine-d_5 and 10% CD_3OD (Fig. 3) show that the gradual disappearance of the NH-4 peak is slower than that of the NH-2 and NH-3 peaks, which supports the intramolecular H-bond conformation adopted by the peptide. This reverse turn bears resemblance to a natural peptide β II′ turn in that the orientation of NH-3 and the α substituents are above the plane of the turn.[14] That such a flexible peptide can adopt a reverse turn is even more remarkable in light of the fact that classical β-turns are favored by more conformationally constrained amino acids like proline.[15]

The long-range NOE data in Table 1 indicate a right-handed 14-helical secondary structure (Fig. 2, right) for peptide **8**, which is similar to the helical structures of γ-amino acid oligopeptides we have reported previously.[5] The helix is stablized by two H-bonds (i–1)C=O···HN(i+2) (i.e. C=O of Boc to NH-3, and C=O of residue 1 to NH-4), which is further substantiated by variable temperature ^{1}H NMR experiments.[16] Fig. 4 shows the temperature dependence of chemical shifts of amide protons in hexapeptide **8**. A linear relationship for δ vs 1/T is observed for all residues. The temperature coefficients (–dδ/dT, ppb/K) of NH-3 (1.64) and NH-4 (2.94) are much lower than NH-1 (11.74) and NH-2 (9.98). The observation of such a low dδ/dT (≤6.0 ppb/K) has generally been attributed to intramolecularly hydrogen bonded amide groups in linear peptides,[17] which indicates that NH-3 and NH-4 are H-bonded and shielded from the solvent. This is consistent with a solution conformation in which the amide protons in residue 3 and residue 4 are part of the H-bonding network of a well-defined helical structure.

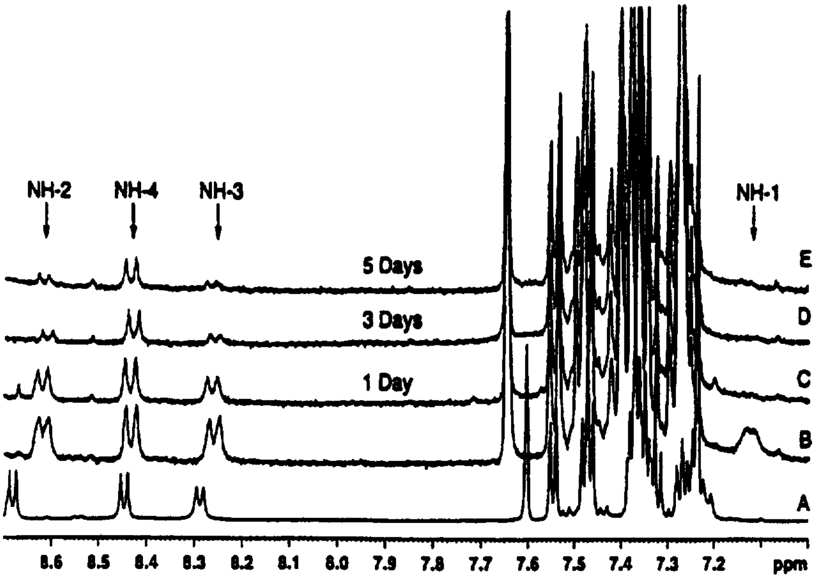


Figure 3. Deuterium exchange of the amide protons for peptide 4 in py-d$_5$/10% CD$_3$OD at 1 mM

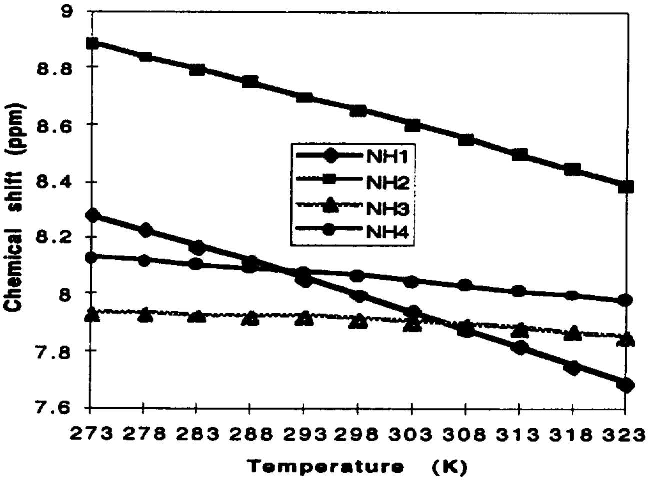


Figure 4. Temperature dependence of the chemical shifts of the amide protons of peptide 8

Compared to the unsubstituted and (α*S*)-methyl substituted analogs,[5] amide protons in peptide 8 show smaller temperature coefficients, indicating that the helical structure may be further stabilized by the cinnamyl substituents at the α position of the γ-amino acid residues. A preferred backbone conformation in the context of helical secondary structures of β- and γ-amino acid oligopeptides has recently been discussed by Seebach.[18]

In summary, we have demonstrated that the (α*R*)-cinnamyl γ-amino acid tetrapeptide 4 can adopt a reverse turn in solution, while the (α*S*)-cinnamyl analog 8 adopts a helical structure. Both of these structures are stabilized by intramolecular H-bonds as evidenced by NMR experiments. The strong dependence of secondary structure and conformation on the stereochemistry of the α-substituent in these γ-amino acid tetrapeptides will be useful in their utilization as peptidomimetics in drug design.

Acknowledgements

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