An Achiral Dipeptide Mimetic That Promotes β-Hairpin Formation

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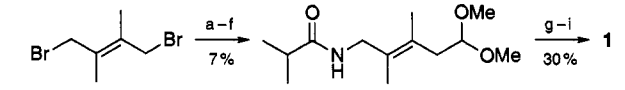
β-Hairpins are elements of polypeptide secondary structure comprised of two adjacent strands of antiparallel β-sheet and a connecting loop. The shortest common loop is two residues, in which case the loop and the two adjacent residues constitute a β-turn.[1] Strategies for stabilizing β-hairpin conformations in short peptides and peptidomimetic molecules should be useful for the development of medicinal agents and larger polypeptides with well-defined folding propensities.[2−4] We describe the design, synthesis, and evaluation of a dipeptide mimetic unit that promotes β-hairpin formation.

We have recently shown that simply enforcing a β-turn conformation across a dipeptide loop is not sufficient to induce β-hairpin folding.[5] Indeed, the most common β-turn conformations available to peptides comprised of L-amino acids appear to be ill-suited for β-hairpin formation within an all-L sequence, according to statistical surveys of β-turns in crystalline proteins[6] and model studies in solution.[5] In contrast, β-hairpin formation is promoted by so-called "mirror image" β-turns, a rare class in which the ϕ and ψ backbone torsion angles along the two-residue loop are opposite to the torsion angles normally favored by β-turns formed from L-residues.[6]

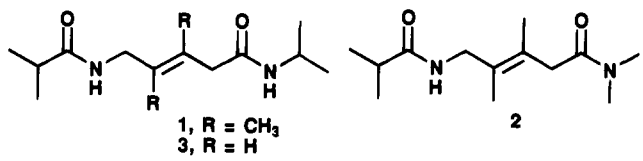
In designing a nonpeptidic unit to promote β-hairpin formation, we sought a dipeptide mimic devoid of stereogenic centers, so that the energetic distinction between mirror image turns would not exist. For an authentic dipeptide, the absence of stereogenic centers leads to an extremely flexible backbone (Gly-Gly). It was therefore necessary to identify a source of conformational rigidification that would promote β-turn-like folding without introducing chirality. We focused on *trans*-5-amino-3,4-dimethylpent-3-enoate because we expected $A^{1,2}$- and $A^{1,3}$-strain to disfavor extended conformations.[7] Molecule **1** is a derivative of this dipeptide mimetic unit bearing amide capping groups; the synthesis of **1** is outlined in Scheme 1.[8]

We compared **1**−**3** by IR spectroscopy (1 mM each in CH_2Cl_2) in order to assess the extent to which avoidance of allylic strain influences the turn-forming propensity of the tetrasubstituted *trans*-alkene peptidomimetic unit. Figure 1a shows N−H stretch region IR data obtained for **1**; the substantial band at 3329 cm^{-1} indicates intramolecular C=O···H−N hydrogen bonding.[9] We excluded the possibility of intermolecular hydrogen bonding under these conditions by monitoring the

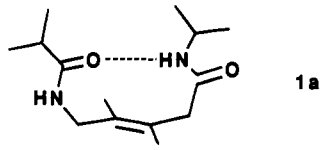
Scheme 1[a]



[a] Key: (a) 1 equiv of 2-lithio-1,3-dithiane; (b) NaN_3, H_2O, *n*-Bu_4NBr, heat; (c) Ph_3P, wet THF; (d) isobutyryl chloride, Et_3N; (e) $PhI(O_2CCF_3)_2$, CH_3OH; (f) alkene isomer separation; (g) 1 N HCl, THF; (h) $NaClO_2$, pH 4; (i) DCC, HOSu; $(CH_3)_2CHNH_2$.



amide proton NMR resonances of **1** in CD_2Cl_2 over the range 0.05−50 mM; these data indicated that aggregation, signaled by downfield shifts of δNH, occurs only above 1 mM.[10] Diamide **2** (Figure 1b) shows only one N−H stretch band, at 3440 cm^{-1}, in the non-hydrogen-bonding region. This result demonstrates that the eight-membered ring C=O···H−N hydrogen bond potentially available to **1** does not form. Thus, the 3329 cm^{-1} band observed for **1** arises exclusively from a β-turn-like 10-membered ring hydrogen bond (**1a**). The bands



1a

at 3452 and 3440 cm^{-1} for **1** (Figure 1a) presumably arise from the completely non-hydrogen-bonded N-terminal NH and the non-hydrogen-bonded portion of the C-terminal NH.[9] The weak band displayed by **1** at 3410 cm^{-1} must result from the C-terminal NH, because there is no such band for **2**. This band is assigned to a small population of NH that is intramolecularly hydrogen bonded to the alkene π-system, based on a similar π - - H−N interaction detected in the *N*-methyl amide of *trans*-hex-3-enoate.[9b] Dipeptide mimetic **3** is a Gly-Gly analogue containing the commonly used disubstituted *trans*-alkene isosteric replacement for the backbone amide group.[11] IR data for **3** (Figure 1c) indicate that there is very little intramolecular hydrogen bonding in the absence of backbone preorganization by allylic strain.

Having established that our dipeptide mimetic residue does indeed promote β-turn-like folding in solution, we examined **4**, the analogue of Ac-Val-Gly-Gly-Leu-NMe_2 containing this peptidomimetic unit, to determine whether *β-hairpin* formation is promoted. We also examined **5**, the Ac-Val-Gly-Gly-Leu-NMe_2 analogue lacking the methyl substituents on the alkene moiety. Figure 2 shows the effect of concentration on amide proton NMR chemical shifts in CD_2Cl_2 for **4** and **5** over the range 0.05−20 mM at 20 °C. These data indicate that **5** aggregates avidly in this concentration range, but that **4** does not. IR data for **4** (Figure 1d) reveal that most N−H in this

(1) For leading references, see: Sibanda, B. L.; Thornton, J. M. *J. Mol. Biol.* **1993**, *229*, 428.

(2) β-Hairpin formation in short, linear peptides: (a) Ueki, T.; Bando, S.; Ashida, T.; Kakudo, M. *Acta Crystallogr.* **1971**, *B27*, 2219. (b) Kopple, K. D.; Go, A.; Pilipauskas, D. R. *J. Am. Chem. Soc.* **1975**, *97*, 6830. (c) Blanco, F. J.; Jiménez, M. A.; Herranz, J.; Rico, M.; Santoro, J.; Nieto, J. L. *J. Am. Chem. Soc.* **1993**, *115*, 5887.

(3) For discussion of a well-developed nonpeptidic β-hairpin nucleator, see: (a) Díaz, H.; Tsang, K. Y.; Choo, D.; Espina, J. R.; Kelly, J. W. *Tetrahedron* **1993**, *49*, 3533. (b) Tsang, K. Y.; Diaz, H.; Graciani, N.; Kelly, J. W. *J. Am. Chem. Soc.* **1994**, *116*, 3988.

(4) Other nonpeptide β-hairpin nucleators: (a) Kemp, D. S.; Bowen, B. R.; Muendel, C. C. *J. Org. Chem.* **1990**, *55*, 4650. (b) Balaram, H.; Uma, K.; Balaram, P. *Int. J. Pept. Protein Res.* **1990**, *35*, 495.

(5) Haque, T. S.; Little, J. C.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 4105.

(6) (a) Sibanda, B. L.; Thornton, J. M. *Nature* **1985**, *316*, 170. (b) Wilmot, C. M.; Thornton, J. M. *J. Mol. Biol.* **1988**, *203*, 221.

(7) (a) Johnson, F. *Chem. Rev.* **1968**, *68*, 375. (b) Berg, U.; Sandström, J. *Adv. Phys. Org. Chem.* **1989**, *25*, 1. (c) Hoffmann, R. W. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1124.

(8) A reviewer has pointed out that the Gly-Gly sequence is relatively uncommon among β-turns. We anticipate that it will be possible to mimic other dipeptide sequences with derivatives of the *trans*-5-amino-3,4-dimethylpent-3-enoate residue that bear substituents on the alkene methyl groups.

(9) For interpretation of N−H stretch region IR data in CH_2Cl_2, see: (a) Gellman, S. H.; Dado, G. P.; Liang, G.-B.; Adams, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 1164. (b) Liang, G.-B.; Desper, J. M.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 925.

(10) Data may be found in the supplementary material.

(11) For leading references: Wipf, P.; Fritch, P. C. *J. Org. Chem.* **1994**, *59*, 4875.

(12) We use the term "strong C=O···H−N hydrogen bonding" to distinguish interresidue hydrogen bonds, which give rise to N−H stretch bands ≤ 3360 cm^{-1}, from the weak intraresidue five-membered ring C=O···H−N interaction (C_5), which gives rise to an N-H stretch band in the range 3420−3430 cm^{-1}. For more information on the C_5 interaction, see: (a) Avignon, M.; Huong, P. V.; Lascombe, J.; Marraud, M.; Neel, J. *Biopolymers* **1969**, *8*, 69. (b) Burgess, A. W.; Scheraga, H. A. *Biopolymers* **1973**, *12*, 2177.

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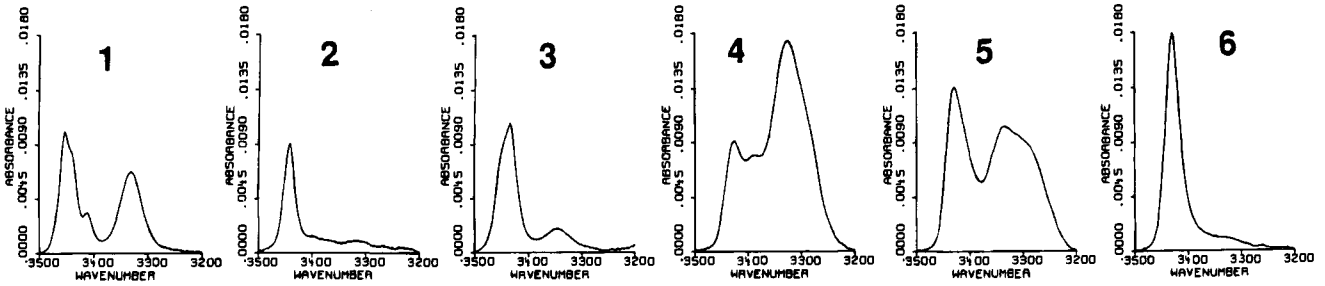
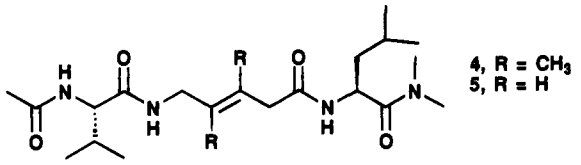
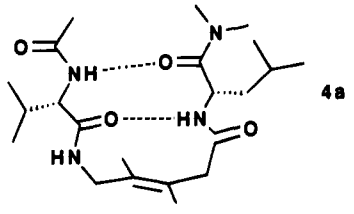


Figure 1. N−H stretch FT-IR data for 1 mM samples in CH_2Cl_2 at 20 °C, after subtraction of the spectrum of pure CH_2Cl_2: **1**, maxima at 3452, 3440 (shoulder), 3410, and 3329 cm^{-1}; **2**, maximum at 3440 cm^{-1}; **3**, maxima at 3448 (shoulder), 3434, and 3345 cm^{-1}; **4**, maxima at 3429, 3398, 3331, and 3287 (shoulder) cm^{-1}; **5**, maxima at 3429, 3336, and 3283 (shoulder) cm^{-1}; **6**, maximum at 3429 cm^{-1}.

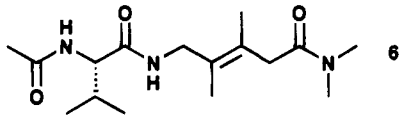


molecule is engaged in strong C=O···H—N hydrogen bonding[12] at 1 mM in CH_2Cl_2 (major band at 3326 cm^{-1}), and the concentration independence of the NMR data for **4** (Figure 2) suggests that this hydrogen bonding is strictly intramolecular. For **5**, on the other hand, IR data show that a much smaller proportion of the N−H is involved in strong C=O···H—N hydrogen bonding at 1 mM (Figure 1e), and the NMR data show that some of this hydrogen bonding is intermolecular. These observations indicate that **4** adopts a compact intramolecularly hydrogen-bonded folding pattern (or set of folding patterns) that is sufficiently robust to inhibit intermolecular association, while **5** has a weaker folding propensity and readily engages in hydrogen bond-driven aggregation.

Comparisons among IR and NMR data for **4** and truncated compound **6** indicate that a β-hairpin-like conformation of **4** (e.g., **4a**) is highly populated. Formation of the 14-membered



ring hydrogen bond between the C-terminal amide carbonyl and the N-terminal amide proton of **4** is the key criterion for β-hairpin folding. IR data for **6**, 1 mM in CH_2Cl_2 (Figure 1f), show that this molecule contains no strong intramolecular C=O···H—N hydrogen bonds (no bands < 3380 cm^{-1}). The



downfield ^1H NMR shift of the N-terminal amide proton of tetrapeptide analogue **4** (7.26 ppm) relative to the signal for the analogous proton of **6** (6.03 ppm) indicates that the N-terminal amide proton of **4** experiences considerable intramolecular hydrogen bonding (1 mM in CD_2Cl_2). Together, these

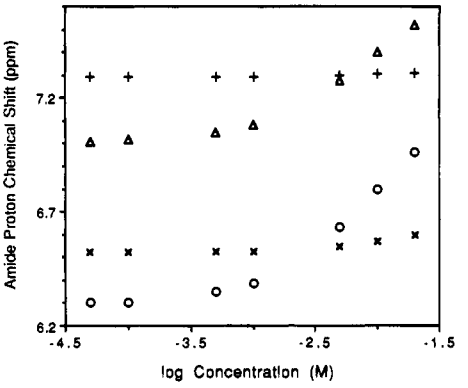


Figure 2. Amide proton NMR chemical shifts in CD_2Cl_2 at 20 °C, as a function of the logarithm of concentration: (+), Val NH of **4**; (△), Val NH of **5**; (×), *trans*-5-amino-3,4-dimethylpent-3-enoate NH of **4**; (○), *trans*-5-amino-3,4-dimethyl-pent-3-enoate NH of **5**. (The data for Leu NH of **4** and **5** may be found in the supplementary material.)

observations imply that the intramolecular hydrogen bonding of the N-terminal amide proton of **4** involves the 14-membered ring β-hairpin interaction. The N−H stretch region IR data for **4** (Figure 1d) are consistent with extensive β-hairpin formation, because it is clear that the amide protons of **4** are predominantly involved in strong C=O···H—N hydrogen bonds (major band at 3326 cm^{-1}).

We have shown that the dipeptide mimetic *trans*-5-amino-3,4-dimethylpent-3-enoate residue promotes β-hairpin formation in solution. The smaller extent of intramolecular hydrogen bonding and the greater tendency for aggregation of **5**, relative to **4**, indicate that $A^{1,2}$- and $A^{1,3}$-strain effects predispose the backbone of **4** to adopt a β-hairpin conformation. These results suggest that the tetrasubstituted *trans*-alkene replacement for a backbone amide group will be a useful alternative to the more common disubstituted *trans*-alkene units[11] for the design of peptidomimetic molecules with specified folding propensities.

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Supplementary Material Available: Synthetic details for **1** and **4** and concentration-dependent NMR data for **1** and 4 (9 pages). This material is contained in many libraries on microfiche, immediately fllows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Intemet; see any current masthead page for ordering information and Intemet access instructions.

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