Tilted amides in amino acid and peptide derivatives

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Background: Amide bonds in peptides and proteins typically adopt planar *cis* or *trans* conformations. Conversions between *cis* and *trans* amide conformations are necessary for protein folding and for many other processes, but are difficult to achieve since they involve disruption of the planarity of the bond. As a first step to understanding *cis–trans* isomerization, we set out to synthesize and characterize peptides that mimic the tilted or twisted amide structures that are postulated to form the intermediate states in this process.

Results: We have synthesized a model amino acid and four dipeptide derivatives containing a methyl-substituted aziridine residue. Single crystals of phenacyl (2R, 3R)-benzyloxycarbonyl-3-methyl-2-aziridinecarboxylate and phenacyl (2R, 3R)-acetyl-glycyl-3-methyl-2-aziridinecarboxylate were obtained. Using X-ray diffraction analysis, we determined that the amide nitrogens of the aziridine rings have tetrahedral sp^3-like geometry with tilt angles in the range of 37–38°. The ^{13}C-NMR spectra indicate that the amide carbonyl is dramatically shifted downfield as a consequence of the tilt.

Conclusions: In peptides containing a substituted aziridine ring, the orbitals of the amide nitrogen are constrained into a tilted configuration. These peptides may mimic the transition state between *cis* and *trans* amide conformations. This technique thus provides a novel strategy for the study of isomerization and other biorecognition processes.

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Introduction

Most amide bonds in peptides and proteins adopt *cis* or *trans* conformations by a partial π-electron delocalization between carbonyl and amine units which causes the four adjacent bonded atoms of the peptide linkage to become a coplanar, rigid unit (Fig. 1). This produces a high barrier (16–22 kcal mol^{-1}) to rotation about the bonds within the linkage [1,2], which is required for *cis–trans* isomerization. Amide bond isomerization is important in many processes that require alteration to protein structure, among them the transport of polypeptides through membranes and oligomerization and folding of proteins [3–5]. For example, peptidyl-prolyl *cis–trans* isomerase catalyzes the *cis–trans* isomerization of proline peptide bonds in oligopeptides and has been shown to accelerate the refolding of several proteins *in vivo*.

The conversion between the *cis* and *trans* conformations of an amide probably involves an unstable transition state in which the planar arrangement present in the ground state is distorted by twisting or tilting. The unstable nature of this transition state has made it difficult to study. Few peptides are known to contain ground state amides that are distorted (tilted or twisted), out of plane by more than 4–6° about the bond torsion ω (C^{α}-C-N-C^{α}) [6]. To probe the complicated biophysical mechanism of amide rotation in peptides, we therefore designed linear peptides that contain stable tilted or

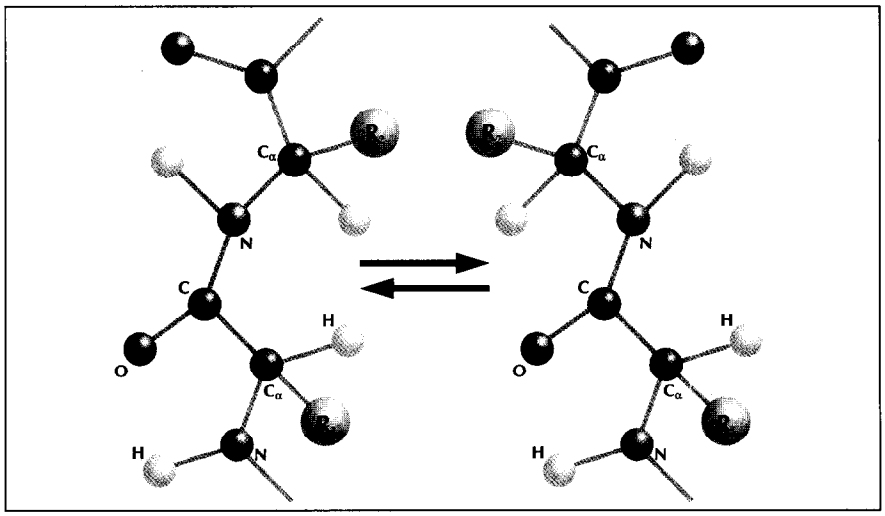

Fig. 1. *Cis–trans* isomerization in a peptide bond. Left, a *cis* amide bond; right, a *trans* amide bond. The plane of the peptide linkage is shown in yellow. The amide torsion angle ω (C^{α}–C–N–C^{α}) is 0° for the *cis* form and 180° for the *trans* form. Carbon atoms are black, nitrogen atoms are blue, oxygen atoms are red and hydrogen atoms are white. R_1 and R_2 are amino acid side chains.

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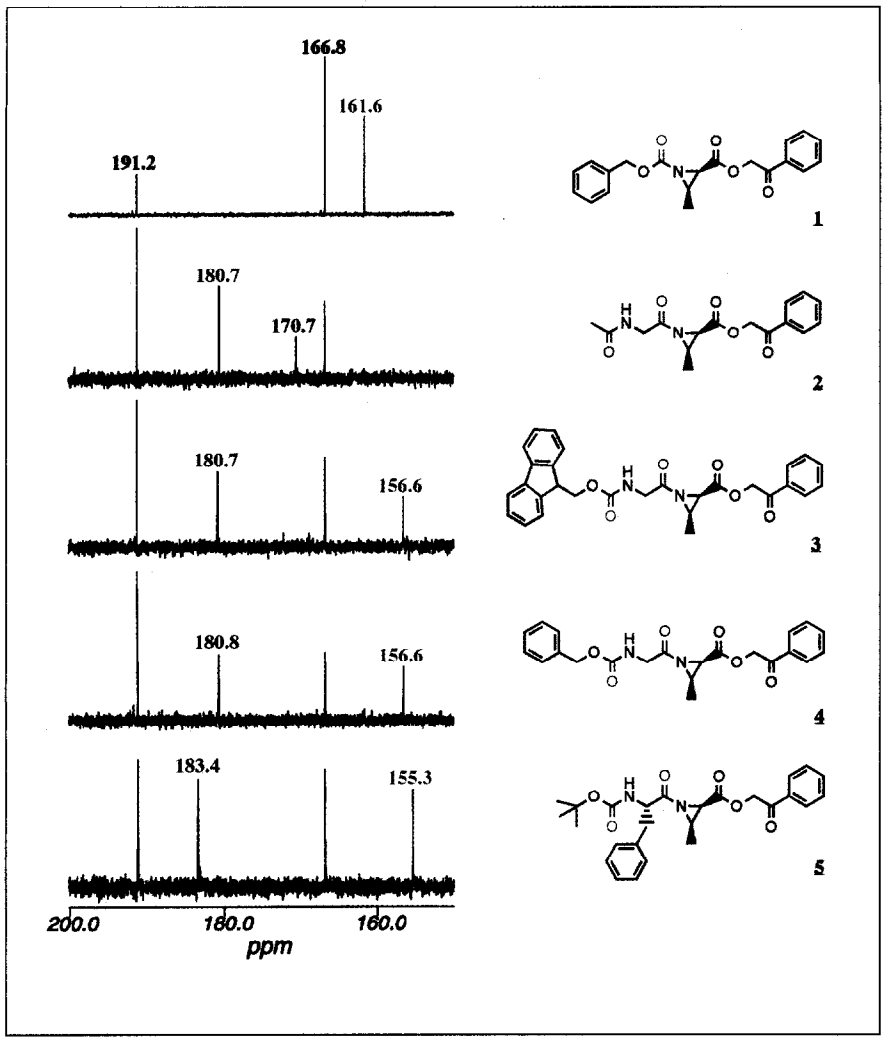

Fig. 2. The ^{13}C-NMR spectra and the chemical structures of compounds (**1**)–(**5**). The chemical shift of all carbonyls in the spectra are denoted by using the color-coding to each of the molecules studied. Thus, the typical urethane carbonyls are shown in green and fall between 155.3 and 156.6 ppm. The urethane of compound (**1**) is shifted downfield to 161.6 ppm as a result of its tilted structure. All esters fall between 166 and 167 ppm and are noted in black. The amide bond of the acetylglycyl group in compound (**2**) is shown in blue and falls at 170.7 ppm while the amide carbonyls attached to the aziridines of compounds (**2**)–(**5**) are shifted downfield to the range of 180.7–183.4 ppm (shown in red). The ketone group of the phenacyl structures (shown in black) is observed at 191.2 ppm for all compounds.

twisted amides to obtain accurate information on their structures [7, 8]. Here we describe the first X-ray diffraction analysis of substituted aziridine-containing amino acid and dipeptide derivatives. Others have also reported similar synthetic methodology for the preparation of aziridine-containing peptides [9,10]. The investigation described here extends the synthetic methodology to structural studies in solution and in the solid state.

The structures we have generated contain a highly tilted amine in their ground state, both in the solid state and in solution, that may resemble the transition state of *cis–trans* isomerization for amide bonds in natural peptides, and should thus contribute to a better understanding of dynamic processes in proteins, including isomerization catalyzed by enzymes [3,4,11,12].

Results and discussion

A model amino acid (compound (**1**) in Fig. 2) and four dipeptide derivatives (compounds (**2**)–(**5**) in Fig. 2) containing conformationally constrained methyl-substituted aziridines were synthesized based on published methodology [13]. The methyl group on the aziridine ring was included as a side-chain mimic. The presence of the aziridine ring was expected to induce specific conformational

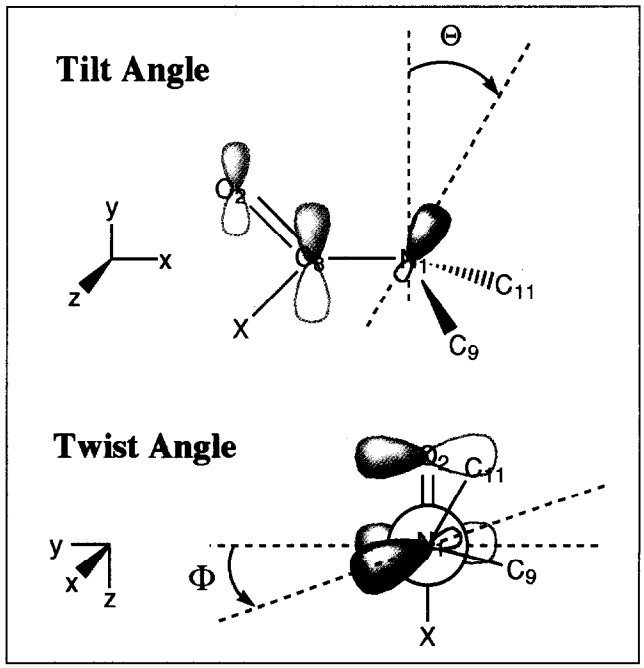

Fig. 3. The tilt and twist angles of an amide bond as defined by Somayaji and Brown [15]. X is O_1 in Fig. 4 and C_7 in Fig. 5.

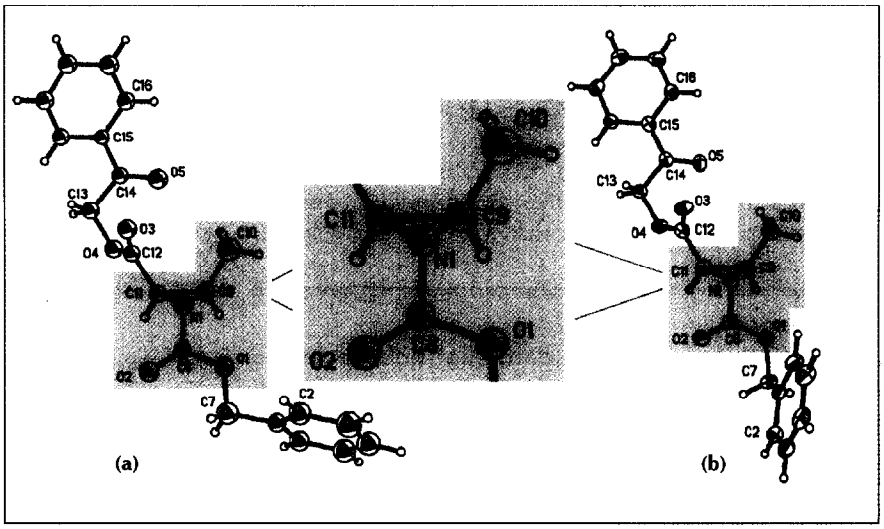

Fig. 4. X-ray crystal structures of phenyl (2R, 3R)-benzyloxycarbonyl-3-methyl-2-aziridinecarboxylate (**1**) with a tilt angle of (**a**) 37.5º and (**b**) 36.8º. Substituted aziridine groups are highlighted in green and expanded in the inset for clarity.

preferences in peptide backbones. To investigate the actual conformations of these peptides, single crystals of compound (**1**) and (**2**) were grown from methanol and ethyl acetate, respectively, for X-ray diffraction studies. In addition, compounds (**1**)–(**5**) were examined by NMR spectroscopy to determine chemical shifts and the geometries of the molecules in solution.

The two lowest energy arrangements for typical amide bonds of peptides are *trans* ($\omega \approx 180°$) or *cis* ($\omega \approx 0°$) [1,2,6]. The *cis* amide bond is frequently observed for residues having tertiary nitrogen atoms such as proline or N-methylated amino acids. Tilted or twisted amide bonds have not been observed in peptides and proteins although much effort has been expended to mimic these structures in other biomolecules [14]. The tilt and twist angles for distorted amide bonds have been defined as shown in Fig. 3 [15]. A normal amide bond is coplanar in the x–z plane. The constrained aziridine structure forces the pyramidization and rehybridization of the nitrogen from sp^2 toward sp^3-like geometry [16]. The N-pyramidization generates a net tilt angle (Θ) of the lone pair away from the y axis in the x axis direction. Along with this tilt, a rotation of the C_9–N_1–C_{11} plane about the x axis can occur to produce a twist angle (Φ).

In the X-ray diffraction study of the amino acid compound (**1**), two crystal structures were found which have very similar conformations except for the orientation of the aromatic ring of the benzyloxycarbonyl group (Fig. 4a,b). One structure adopts a tilt angle of 37.5° and a small twist angle of 3.0° in the urethane group (Fig. 4a), while the other has a tilt angle of 36.8° and a twist angle of 7.5° (Fig 4b). Both twist angles are well within the normal limits of amide bonds in peptides and proteins. The differences in the crystal structures are attributed to crystal packing requirements. Moreover, relocalization of the electron pair of the nitrogen reduces the amide resonance, leading to an increased bond length of C_8–N_1 from the 1.35 Å of a typical urethane group to 1.38 Å. In the crystal structure of the linear dipeptide (compound (**2**), Fig. 4), the amide bond to the aziridine ring generates a tilt angle of 38.4° and a twist angle of 14.9°. The amide bond C_8–N_1 is longer (1.40 Å) than the standard peptide bond (1.32 Å) because of the reduced amide resonance [5]. It is worth noting that, in the same structure, the other amide bond C_6–N_2 in the acetyl-glycyl part of the molecule exists with the standard bond length and in a standard *trans* conformation ($\omega = 175°$).

These amide distortions were also maintained in solution as shown by ^{13}C-NMR experiments (see Fig. 2). Depending on the structure, the characteristic chemical shifts of carbonyl groups of amides in peptides fall between 175 ppm and 167 ppm. As a consequence of the tilt and twist angles, the carbonyl group attached to the aziridine nitrogen is more ketone-like, which is revealed in the ^{13}C-NMR spectra by a downfield chemical shift. For the dipeptides (**2**) to (**5**), the chemical shift of C=O in the tilted amide is in the range of 180.7–183.4 ppm. Similarly, the chemical shift of 161.6 ppm observed in the urethane structure of amino acid (**1**) is downfield

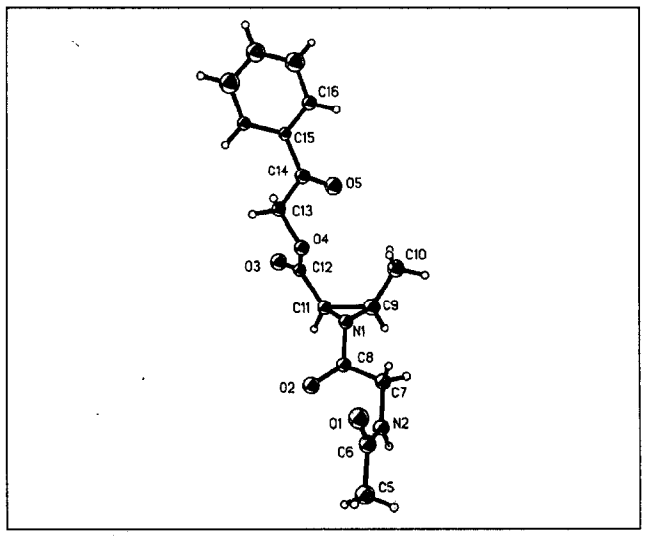

Fig. 5. X-ray crystal structure of phenacyl (2R, 3R)-acetyl-glycyl-3-methyl-2-aziridinecarboxylate (**2**) with a tilt angle of 38.4° and a twist angle of 14.9°.

from that of 155.3–156.6 ppm in the normal urethane groups shown in compounds (**3**), (**4**) and (**5**).

In a normal amide, the nitrogen adopts an sp^2 hybrid orbital to achieve conjugation with the carbonyl group. In the rigid aziridine structure, the hybridization of the nitrogen is altered to an sp^3-like structure. The conjugation is maintained, but weaker than a normal amide bond.

Significance

An amide bond is the fundamental linkage in the structures of peptides and proteins. The isomerization between *cis* and *trans* amide conformations is important in many processes that involve alteration of protein structure, such as protein synthesis, the transport of polypeptides through membranes, ligand binding, and the oligomerization and folding of proteins [3,4]. Isomerization is believed to occur via a high-energy transition state; to understand the isomerization process, we have attempted to decipher the structure of the transition state by studying peptides with distorted amides in their ground state. In this study, we characterized the highly tilted amides in linear amino acid and peptide derivatives. The structures are consistent with those of highly constrained, non-peptidic amides [7,8,17–19].

The incorporation of tilted and twisted amides in synthetic peptides provides a novel strategy in the study of *cis–trans* isomerization and other biorecognition processes [3–5]; their incorporation into a peptide backbone as conformationally constrained peptidomimetic building blocks may also prove useful in altering the properties of the peptide. Since the aziridines are thiol-reactive structures, peptides containing the simple aziridine ring have been developed as irreversible inhibitors for cysteine proteases [9,17]. The peptides containing methyl-substituted aziridine, with suitable modification, might represent a novel class of cysteine protease inhibitors and antibiotics.

Materials and methods:

Chemical synthesis

Phenacyl (2R, 3R)-trityl-3-methyl-aziridine-2-carboxylate was synthesized based on the published methodology [9]. Compounds (**1**) to (**5**) were synthesized with the typical peptide protection and coupling methods, purified by chromatography on silica gel and recrystallization, and fully characterized by ^1H and ^{13}C NMR, high-resolution mass spectroscopy and optical rotations. Detailed syntheses and the data for molecular characterization will be published elsewhere.

NMR experiments

All NMR experiments were carried out on a Bruker AMX-500 at 300 °K. Compounds (**1**) to (**5**) were dissolved in $CDCl_3$ for 1D and 2D NMR studies.

Supplementary material available

Full reports are available for X-ray crystallographic analysis of compounds (**1**) and (**2**) including crystal data, data collection, solutions and refinements of the structures.

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