# Peptides of aminoxy acids as foldamers

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This Feature Article summarizes our efforts in developing a new family of foldamers from α-, β- and γ-aminoxy acids. From a series of conformational studies, we demonstrate that peptides consisting of aminoxy acids adopt several well-defined secondary structures, such as α N–O turns (which feature an eight-membered-ring hydrogen bond), β N–O turns (a nine-membered-ring hydrogen bond), γ N–O turns (a ten-membered-ring hydrogen bond), $1.8_8$ helices (consecutive homochiral α N–O turns), 7/8 helices (alternating α N–O turns and γ-turns), $1.7_9$ helices (consecutive β N–O turns), reverse turns (consecutive heterochiral α N–O turns) and sheet-like structures.

## Introduction

The relationship between the structures and functions of proteins is remarkable. In biological systems, most of the interesting functions performed by proteins, such as molecular recognition, electron transfer and catalysis, are related to unique secondary and tertiary structures. Studies of the structure–activity relationships (SAR) on small molecules interaction with proteins have been instrumental to drug development.

In recent years, studies of protein structures and their functions have progressed rapidly so that the mechanisms of many biological processes are now known. Chemists are presently in a position to purposely design unnatural polymers that fold into well-defined secondary structures that may perform desired functions. Gellman[1] used the term ''foldamers'' to describe polymers that have a strong tendency to adopt specific, compact conformations. Several types of foldamers have been developed in the past few years, including peptidomimetic foldamers, single-stranded abiotic foldamers, nucleotidomimetic foldamers and multistranded abiotic foldamers.[2]

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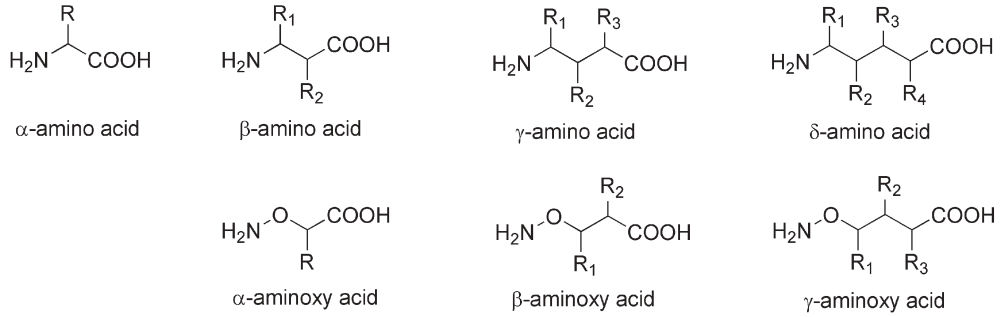
Naturally occurring peptides consisting of fewer than 10 amino acid residues usually do not possess well-defined secondary structures; in addition, they are susceptible to protease degradation. Peptidomimetic foldamers have been developed to achieve better biostablities.[1–5] As the most important models in peptidomimetic foldamer chemistry, β-peptides have been investigated and applied widely in biomolecular design.[5] Unlike α-peptides, short β-peptides—even those with as few as six residues—can fold into well-defined secondary structures, such as helices, sheets and turns. Because β-peptides have excellent stability toward proteases,[6–8] they are widely used as backbone-modified amino acids in drug design.

α-Aminoxy acids are analogs of β-amino acid in which the β-carbon atom is replaced by an oxygen atom. Because of repulsion between the lone pairs of electrons of the nitrogen and oxygen atoms, the backbone of an α-aminoxy acid is more rigid than that of β-amino acid.[9] The aminoxy amide bond is resistant to enzymatic degradation; therefore, α-aminoxy acids have been explored as peptidomimetics in several studies.[10] We have investigated the novel secondary structures, including turns and helices, that are adopted by α-aminoxy acids. In an endeavor to expand the family of aminoxy acids, we have also found that β- and γ-aminoxy acids, which are analogs of γ- and δ-amino acids, respectively, can form several well-defined secondary structures. In the following sections, we provide brief descriptions of these types of aminoxy acids, their peptides, and their potential behavior as new class of foldamers.

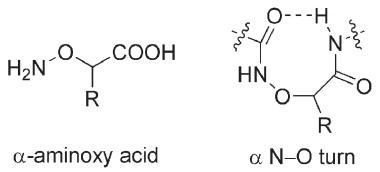
## New building blocks: monomers of aminoxy acids

### The α N–O turn

The specific biological functions of a biomolecule are, in general, closely related to its stable secondary structure; thus, understanding the secondary structures of designed peptidomimetics is also of great theoretical and pharmaceutical significance, and such studies of α-aminoxy acids are certainly no exception. We have found that when an α-aminoxy acid is

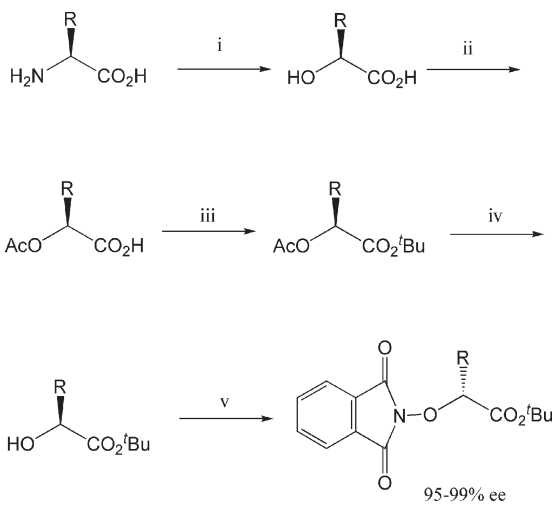



incorporated into a peptide backbone, it induces a strong eight-membered-ring intramolecular hydrogen bond between adjacent residues (the so-called α N–O turn).




Chiral α-aminoxy acids can be readily prepared from α-amino acids in several steps. Testa *et al.*[11] first reported a systematic method for synthesizing chiral α-aminoxy acids, but the optical purities of the α-aminoxy acids were not reported in literature.[10b] In this method, the conversion of α-amino acids to α-bromoacids proceeded with mainly retention at the α-carbon atom, whereas nucleophilic displacement of bromide generally followed an $S_N2$ mechanism with an inversion of configuration to give α-aminoxy acids. We used this method to synthesize several D-α-aminoxy acids from natural L-amino acids with overall yields of α-aminoxy esters about 36–55% and the optical purities in the range of 92–94% determined by HPLC analysis.[12]

We have reported a general method[12,13] for the synthesis of doubly protected chiral α-aminoxy acid monomers (Scheme 1).




**Scheme 1** *Reagents and conditions*: i, $NaNO_2$, $H_2SO_4$, $H_2O$, 80–95%; ii, AcCl, reflux, 95%; iii, DCC, *t*-BuOH, DMAP, $CH_2Cl_2$, 75–90%; iv, $K_2CO_3$, MeOH, $H_2O$, 85–98%; v, *N*-hydroxyphthalimide, DIAD, $PPh_3$, THF, 69–83%. R = $CH_2CH(CH_3)_2$, $CH_3$, $CH_2Ph$, $CH(CH_3)_2$, $CH(CH_3)CH_2CH_3$.

The key step was a Mitsunobu reaction between *N*-hydroxyphthalimide and α-hydroxy *tert*-butyl ester, which introduced the N–O segment of α-aminoxy acids and afforded the protected α-aminoxy acids with an inversion of configuration at the α-carbon. The overall yields for syntheses of α-aminoxy acids were in the range of 36–56% and no purification was needed for most of the steps. The optical purities of chiral α-aminoxy acids were found to be 95–99% by HPLC analysis, which indicates that very little or no racemization occurred in the synthesis of chiral α-aminoxy acids. Furthermore, the *N*-terminal and *C*-terminal protecting groups can be easily removed under basic and acidic conditions, respectively. Shin *et al.*[14] have synthesized a series of optically active phthaloyl aminoxy acids with nonpolar and polar side chains as building blocks for the preparation of diverse α-aminoxy peptides from α-amino acids and α-hydroxy acids. The *C*-terminal protecting group, in contrast to *tert*-butyl in our method, is replaced by benzyl.

The detailed conformational features of oligomers containing α-aminoxy acids were originally investigated within our group.[15] Similar to the β-amino acid oligomers, in theory there are several possible intramolecular hydrogen bonds formed within an α-aminoxy acid oligomer. Theoretical calculations on diamide **1** suggest that its most favorable conformation is the rigid eight-membered-ring hydrogen-bonded structure **1a** (Fig. 1). The hydrogen bond in **1a** is strong, as indicated by the short O···H distance and the near linearity of the O···H–N bond angle. This calculated conformation was confirmed through X-ray crystallography to occur in the solid-state structure of diamide **2a**, which displays a right-handed N–O turn having an $\angle NOC_\alpha C_o$ dihedral angle of +78.4° (Fig. 2).[13]

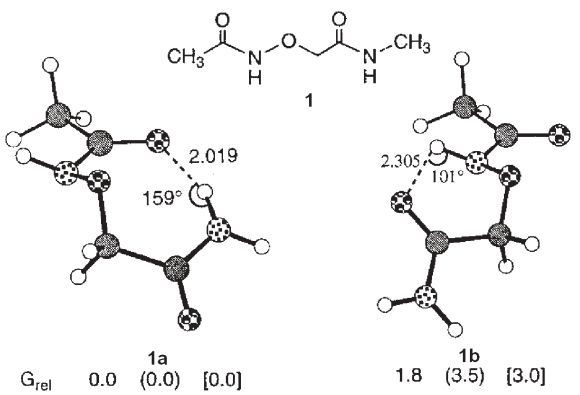



**Fig. 1** Structures of α-aminoxy amide **1**. The values of $G_{rel}$ (kcal $mol^{-1}$) were calculated using the HF/6-31G* (MP2/6-31G*) and [HF/6-31G* $CHCl_3$ solvation] models. Reprinted with permission from reference 15. Copyright 1996 American Chemical Society.

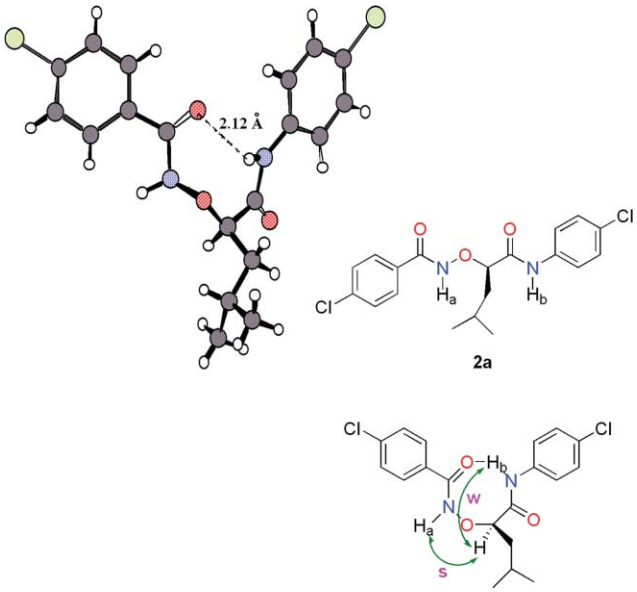
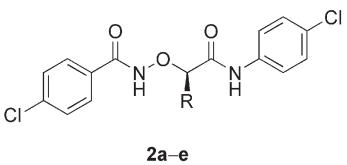



**Fig. 2** Solid-state structure of **2a** and a summary of the NOEs observed for **2a** (5 mM in $CDCl_3$); s, strong NOE; w, weak NOE.

The conformations of α-aminoxy diamides **2a–e** in nonpolar solvents were firmly established using a combination of experimental techniques.[12,13]



**2a–e**

**a** R = $CH_2CH(CH_3)_2$
**b** R = $CH_3$
**c** R = $CH_2Ph$
**d** R = $CH(CH_3)_2$
**e** R = $CH(CH_3)CH_2CH_3$

FTIR Spectra of the diamides were recorded at very low concentrations so that intermolecular hydrogen bonds were unlikely to form. These IR spectra displayed absorptions for both free (non-hydrogen-bonded) and hydrogen-bonded aminoxy amide N–H stretches, indicating that these compounds exist predominantly in the intramolecular hydrogen-bonded conformations. In our $^1$H NMR spectroscopic studies of the α-aminoxy diamides, we used two methods to characterize the intramolecular hydrogen bonds. The first was $^1$H NMR spectroscopic dilution, to determine the concentration dependence of the chemical shifts of the amide protons.[16] The second was titration with DMSO-$d_6$, *i.e.*, the gradual addition of a strong hydrogen-bond acceptor to a dilute solution of the α-aminoxy diamide in non-hydrogen-bonding solvent (*e.g.*, $CDCl_3$).[17] Our $^1$H NMR spectroscopic chemical shift data for **2a–e** suggest that the N-terminal protons of the diamides are solvent-accessible, whereas the C-terminal ones are hydrogen bonded intramolecularly. The two-dimensional rotating-frame Overhauser effect (2D-ROESY) spectrum of **2a** in Fig. 2 suggests that a strong nuclear Overhauser effect (NOE) exists between the $NH_a$ and $C_\alpha H$ groups, but only a weak NOE between the $NH_b$ and $C_\alpha H$ groups, indicating that this compound adopts a folded structure that agrees well with its crystal structure (Fig. 2).

We have used the circular dichroism (CD) exciton coupling method[18] to determine the handedness of the chiral α N–O turns. The CD spectra of diamides **2a–e** (D-configuration), which feature different side chains, displayed strong positive exciton coupling with nearly the same maxima and minima (Fig. 3), indicating that, in the D-configuration, our designed diamides all adopt right-handed α N–O turn structures, irrespective of the nature of their side chains.

The formation of the α N–O turn was independent of side chain variation; the orientation of the α N–O turn was determined solely by the configuration of the α-carbon atom. Thus, by changing the chirality of the α-carbon atom from the D configuration to the L configuration, the α N–O turn can be switched from a right-handed to a left-handed structure. Fig. 4 shows the solid-state structures of a pair of α N–O turns, in which their backbones are mirror image of each other. The side-chain-independence of these α N–O turn structures

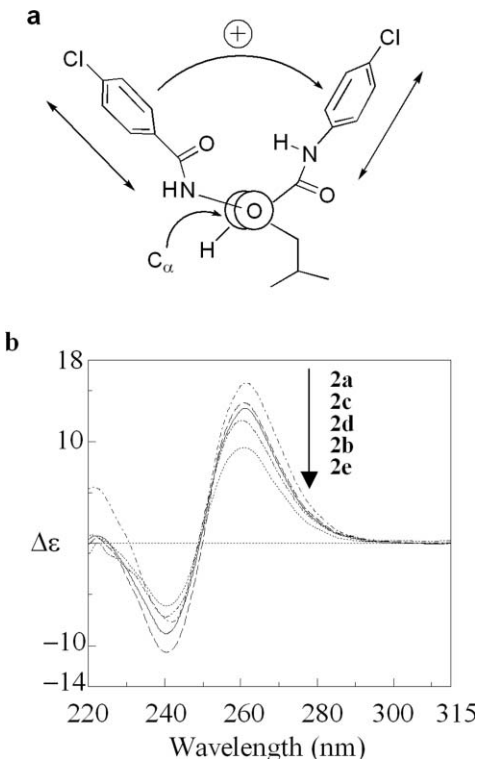



**Fig. 3** (a) Positive chirality corresponds to the positive dihedral angle $\angle NOC_\alpha C_O$ in the right-handed turn. (b) CD spectra of diamides **2a–e** (0.75 mM) in $CH_2Cl_2$.

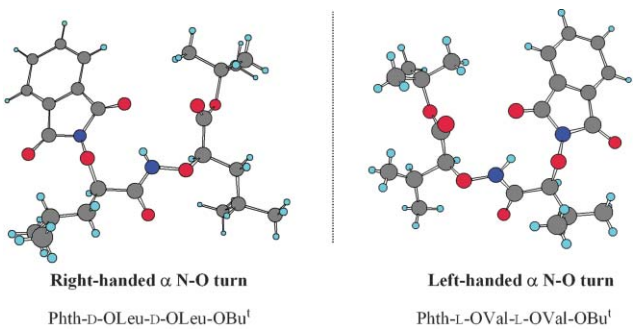
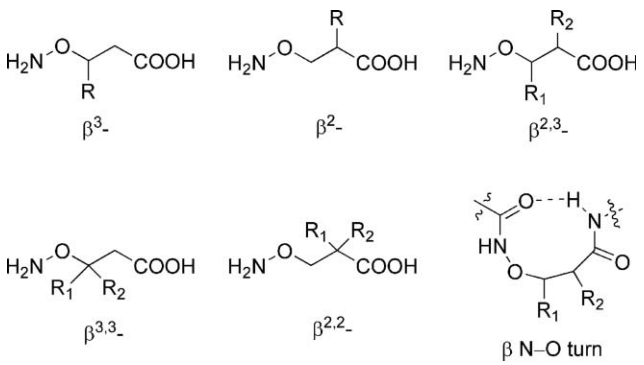



**Fig. 4** Solid-state structures of the right-handed α N–O turn and the left-handed α N–O turn.

indicates that these systems are subjected to backbone-based control. These results allow us to construct well-defined secondary structures using a variety of side chains.

### The β N–O turn

In our search for novel foldamers, we investigated the behavior of β-aminoxy acids, *i.e.*, structures in which an oxygen atom has replaced the γ-carbon atom of γ-amino acids. Relative to the α-aminoxy acids, the β-aminoxy acids possess an extra carbon atom in their backbone and, thus, provide a greater variety of substitution patterns for their peptides and many opportunities to modulate their hydrogen bonding. Similar to β-amino acids,[6,19] β-aminoxy acids can be divided into several subclasses according to their backbone substitution patterns. From our studies of diamides of $\beta^{2,2}$-, $\beta^{3}$- and $\beta^{2,3}$-aminoxy acids, we found that, despite their greater flexibility when compared with those of α-aminoxy acids, most of these diamides adopt a rigid β N–O turn, featuring a nine-membered-ring hydrogen bond between adjacent residues, when incorporated into peptide backbones.




$\beta^{2,2}$-Aminoxy acid[20] was the first member in the family of β-aminoxy acids that we studied. The crystal structure of diamide **3** revealed a nine-membered-ring hydrogen bond between the C=$O_i$ and $NH_{i+2}$ groups; the structure was stabilized further by another six-membered-ring hydrogen bond between the $NH_{i+2}$ and $NO_{i+1}$ groups. The N–O bond was positioned *anti* to the $C_\alpha$–$C_\beta$ bond with an ∠NOCβ$C_\alpha$ dihedral angle of 172° (Fig. 5(a)). In the solid state, four diamide **3** molecules formed a cyclic ring with four intermolecular hydrogen bonds between them. The C-terminal free carbonyl and N-terminal free amide NH units of one molecule of **3** were hydrogen bonded to two adjacent molecules. This molecules' ability to form hydrogen bonds is fully utilized (Fig. 5(b)).

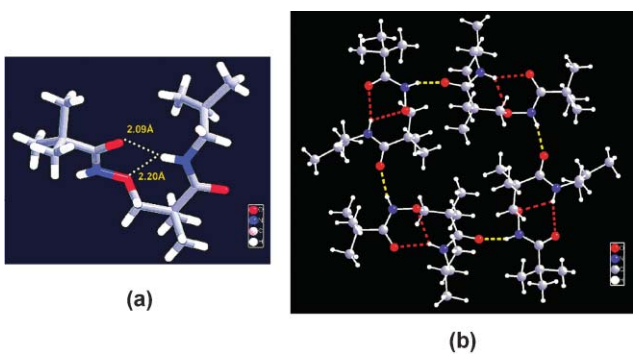
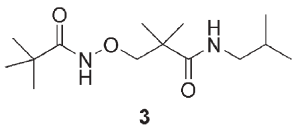



**Fig. 5** (a) Solid-state structure and (b) solid-state packing pattern of diamide **3**. Part (a) reprinted with permission from reference 20. Copyright 2002 American Chemical Society.



**3**

We used 2D NMR spectroscopy studies to confirm that the β N–O turn structure of diamide **3** exists in $CDCl_3$ also. The NOE pattern (medium NOEs between the $NH_i$ and $C_\beta H_i$ protons, but strong NOEs between the $NH_{i+1}$ and $C_\beta H_i$ protons) observed in the 2D NOESY spectrum of **3** matched that predicted from the X-ray crystallographic structure.

Diamides of $\beta^{3}$-aminoxy acids can adopt two different types of β N–O turns depending on the sizes of their side chains.[21] The expected β N–O turns involving a nine-membered-ring hydrogen bond between the C=$O_i$ and $NH_{i+2}$ units, which are stabilized further by another six-membered-ring hydrogen bond between the $NH_{i+2}$ and $NO_{i+1}$ units, are observed in the crystal structures of both diamides **4** and **5** (Fig. 6). There are, however, significant differences between these β N–O turns. In diamide **4** (Fig. 6(a)), the N–O bond of the β N–O turn is positioned *anti* to the $C_\alpha$–$C_\beta$ bond, having a dihedral angle $\theta$ of 167.2°; the H···H distance between the $NH_a$ and $C_\beta H$ units is 3.11 Å, which is longer than that between the $NH_b$ and $C_\beta H$ units (2.34 Å). The torsional angles of **4** are comparable to those of the $\beta^{2,2}$-aminoxy peptide **3** (Fig. 5), but are distinctly different from those of **5**, in which the N–O bond is *gauche* to the $C_\alpha$–$C_\beta$ bond with a dihedral angle $\theta$ of 70.4°. The H···H distance between the $NH_a$ and $C_\beta H$ units in the solid-state structure of **5** (*ca.* 2.7 Å) is shorter than that between the $NH_b$ and $C_\beta H$ units (*ca.* 3.9 Å; Fig. 6(b)). 2D NOESY studies of diamides **4** and **5** revealed conformations in nonpolar solvents that are consistent with those observed in the solid state. The NOE pattern observed for **4** is distinctly different from that of **5** (Fig. 6). In particular, the NOE between protons $H_b$ and $H_\beta$ of **4** was stronger than that between $H_a$ and $H_\beta$ (Fig. 6(c)). In contrast, for **5** we observed a slightly stronger NOE between $H_a$ and $H_\beta$ than that between $H_b$ and $H_\beta$ (Fig. 6(d)). We were interested in further understanding the specific residue-based changes that bring about this conformational switch. Theoretical calculations on a series of model diamides of $\beta^{3}$-aminoxy acids rationalized our experimental results very well. In general, a bulky substituent destabilizes the *anti* conformation because of greater crowding of the substituents.

Gellman *et al.* used cyclic ring-constrained β-amino acids, which are conformationally rigidified residues exhibiting constrained rotation about their $C_\alpha$–$C_\beta$ bonds, to construct β-peptides having well-defined conformations.[22–34] They found that the ring size of the side chain of a β-peptide has a significant effect on its secondary structures. The β-peptides of cyclohexane-containing amino acids prefer to form 14-helix structures, while those of cyclopentane-containing amino acids favor 12-helix formation; these conformations result from the

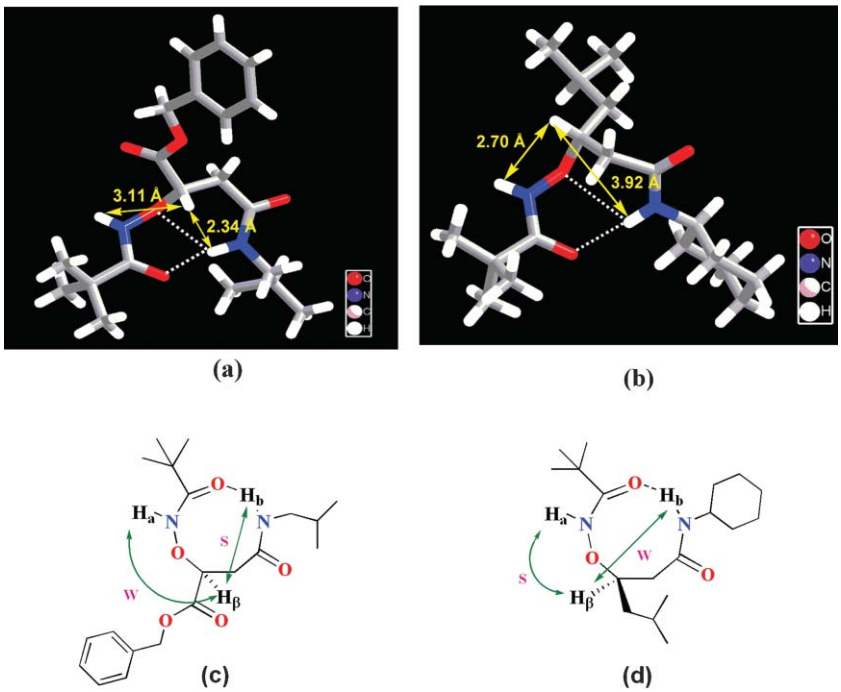



**Fig. 6** Solid-state structures of diamides (a) **4** and (b) **5** and a summary of the NOEs observed in (c) **4** and (d) **5** in $CDCl_3$ or $CD_2Cl_2$; s, strong NOE; w, weak NOE. Reprinted with permission from reference 21(*a*). Copyright 2004 American Chemical Society.

different torsional preferences of the $C_\alpha$–$C_\beta$ bonds in the rings of the individual residues.[23,35] To investigate whether this "ring-size effect" applies in our β-aminoxy acids, we explored the conformational features of the $\beta^{2,3}$-cyclic aminoxy acids, a subclass of β-aminoxy acids in which the α- and β-carbon atoms comprise part of an aliphatic ring (either a cyclopentane or cyclohexane unit).[36] Fig. 7 indicates that the most stable conformations of **6** and **7** that we obtained through theoretical calculations were the β N–O turn structures. To maintain the intramolecular hydrogen bonds of the β N–O turns, the torsional angle $\theta$ must be adjusted to compensate for changes in the torsional angle $\phi$ caused by the different ring sizes. The 2D NOESY spectra exhibit very similar NOE patterns for diamides **8** and **9**: weak NOEs exist between the $NH_i$ and $C_\beta H_i$ protons, but strong NOEs exist between the $NH_{i+1}$ and $C_\beta H_i$ protons (Fig. 8). Moreover, these NOE patterns agree well with the corresponding distances between protons in the β N–O turns of the calculated structures of **6** and **7**. This correlation suggests that, although the ring size of the side chain varies from five to six atoms, diamides **8** and **9** adopt similar β N–O turn structures in solution, in contrast to Gellman's finding for β-peptides. Furthermore, the solid-state structure of **9**, obtained through X-ray crystallography, revealed a β N–O turn structure in which the N–O bond is *anti* to the $C_\alpha$–$C_\beta$ bond with an $\angle NOC_\alpha C_\beta$ dihedral angle of 172° (Fig. 9). This structure is similar to the β N–O turns observed in the diamides of $\beta^{2,2}$-aminoxy acid.

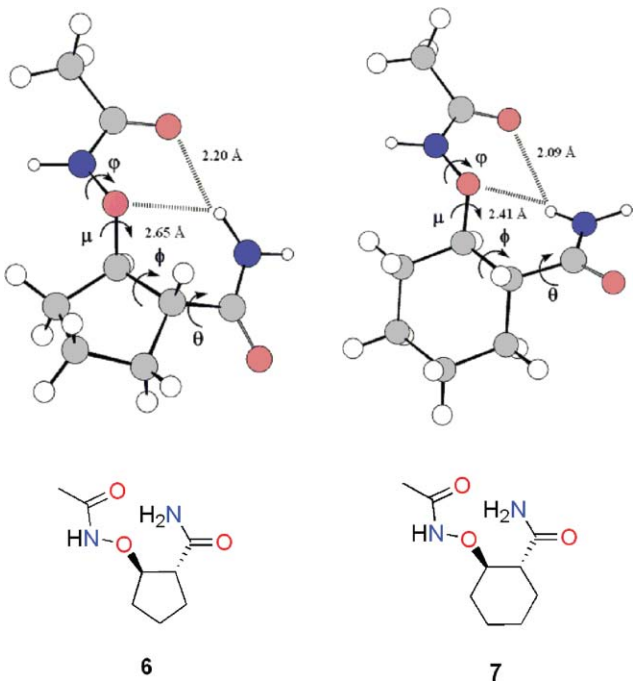



**Fig. 7** Lowest energy conformations in the calculated structures of **6** and **7**. Reproduced from reference 36 with permission of Wiley.

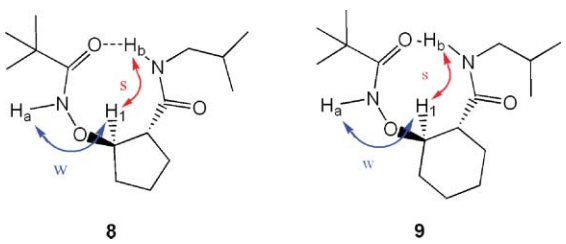



**Fig. 8** Summary of NOEs observed for compounds **8** and **9** in $CDCl_3$ (s, strong NOE; w, weak NOE).

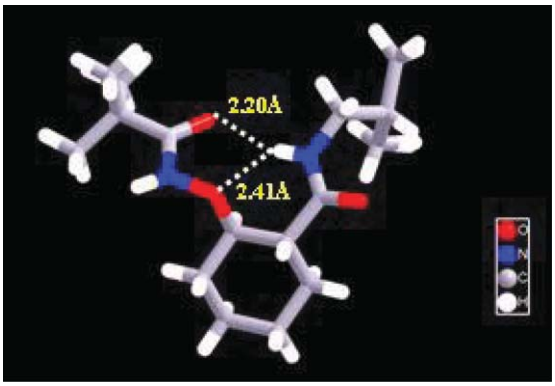



**Fig. 9** Solid-state structure of diamide **9**.

### The γ N–O turn

To expand the family of aminoxy acid residues and to test the ability of other aminoxy acids to form local intramolecular hydrogen bonds, we have also begun to explore the conformational properties of γ-aminoxy acids,[37] which belong to the δ-peptide family. β-Turns of tetrapeptide fragments are common structural features of proteins and, thus, it is not surprising that much activity in the δ-peptide family has been aimed at creating β-turn mimics.[2,38–41] To restrict the more flexible backbone of γ-aminoxy acids, initially we examined the effect of incorporating a γ-phenyl substituent. X-Ray crystallographic structural analysis of diamide **10** revealed a β-turn mimic: a γ N–O turn, featuring an intramolecular 10-membered-ring hydrogen bond between the C=$O_i$ and $NH_{i+2}$ units (Fig. 10(a)). The hydrogen bond distance (O···H) is 2.07 Å and the $C_\gamma$–O bond is positioned *gauche* to the $C_\alpha$–$C_\beta$ bond with a $\angle C_\alpha C_\beta C_\gamma O$ dihedral angle of 69°. In the 2D NOESY spectrum of **10** in $CDCl_3$, the NOE signal between regular amide N–H and γ-H protons indicates a folded backbone consistent with that observed in the solid-state structure of **10** (Fig. 10(b)). $^1$H NMR spectroscopic data also supports the existence of an intramolecular hydrogen bond between the N–H and C=O groups on the two ends of the molecule, suggesting a preference for this secondary structure in solution.

## New foldamers: oligomers of aminoxy acids

As we have discussed above, the monomers of α-, β- and γ-aminoxy acids exhibit several kinds of rigid conformations (the α, β and γ N–O turns, respectively). We were interested in utilizing these novel building blocks to construct peptides having more-diverse secondary structures. Given our focus on oligomers of aminoxy acids, in the following discussion we focus on the structural features of oligomers of aminoxy acids.

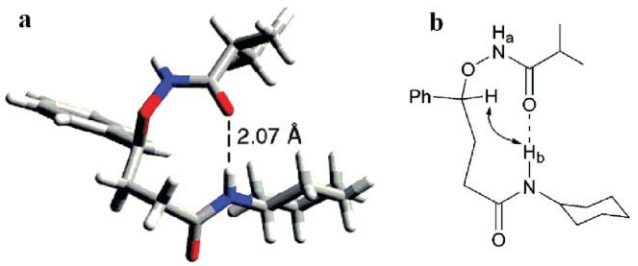
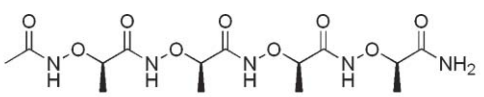



**Fig. 10** (a) Solid-state structure of diamide **10**. (b) Summary of NOEs observed for diamide **10** in $CDCl_3$. Reprinted with permission from reference 37. Copyright 2004 American Chemical Society.

### Helices

α-Aminoxy acids can induce α N–O turn in diamides. The chiral α N–O turn structure is determined by the chirality of the α-carbon atom and is independent of the nature of the side chain. We reasoned that for homochiral oligomers of α-aminoxy acids, eight-membered-ring intramolecular hydrogen bonds should exist between adjacent residues and that these consecutive homochiral α N–O turns should lead to helical structures. Quantum mechanics calculation of the oligomers of homo-D-α-aminoxy acids were performed by Wu *et al*.[42] The calculated structures of tetramer **11** reveal that its lowest-energy conformation both in the gas phase and in solution contain four consecutive right-handed α N–O turns, *i.e.*, it has a helical structure (Fig. 11). The calculated structures reveal several unique features. (1) The backbones of the oligomers of homo-D-α-aminoxy acids form right-handed helical structures, with consecutive eight-membered-ring hydrogen bonds (α N–O turns). The intramolecular hydrogen bonds are lined up along the helical axis with O···H–N angles of *ca*. 157°. (2) The side chains of the oligomers alternate on opposite sides of the helix with a distance of 6.5 Å between the groups at the *i* and *i* + 2 positions, a pattern that is reminiscent of the twisted parallel β-sheets found in proteins. (3) The amide carbonyl group at the *i* + 2 position is twisted by +50° from the one at the *i* position; this arrangement suggests a $1.8_8$ helix or a twisted $2_8$ helix with two residues per helical turn.

We performed conformational studies on the oligomers of homo-D-α-aminoxy acids in nonpolar solvents using NMR, IR and CD spectroscopic methods.[12,13] The IR and $^1$H NMR




**11**

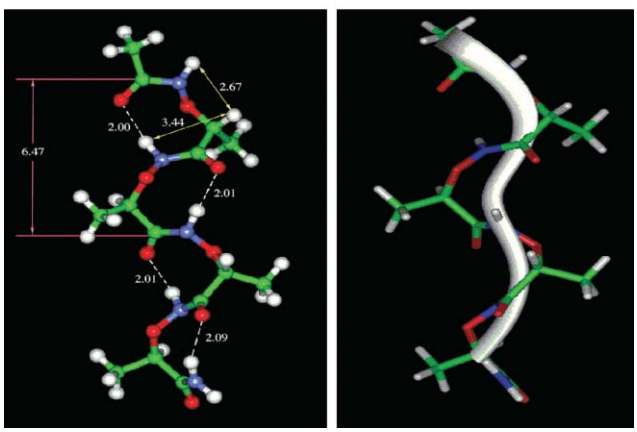
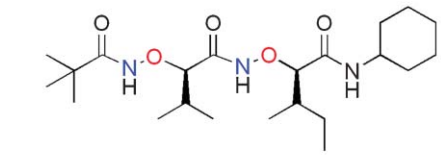



**Fig. 11** Calculated structure of **11**. Reprinted with permission from reference 13. Copyright 1999 American Chemical Society.



12

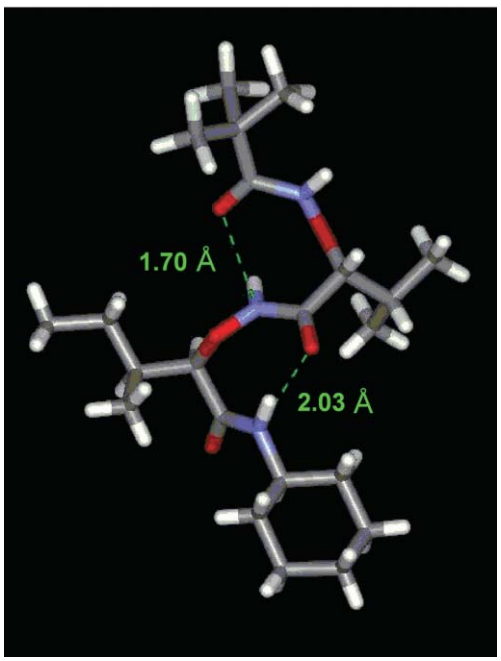


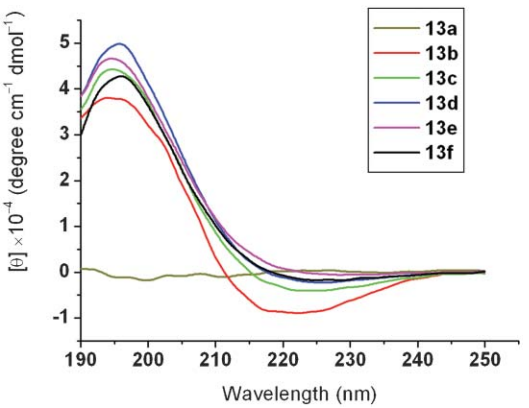

**Fig. 12** Solid-state structure of diamide **12**.

spectra suggest that in each oligomer, except for the N-terminal one, the amide NH group at the $i + 2$ position is hydrogen bonded intramolecularly to the carbonyl oxygen atom at the $i$ position. In agreement with the results of the theoretical calculation, we observed strong NOEs between the $NH_i$ and $C_\alpha H_i$ protons, but weak NOEs between the $NH_{i+1}$ and $C_\alpha H_i$ protons; this pattern is similar to that of the diamides (Fig. 2). The helical conformation was further confirmed through the X-ray crystallographic analysis of triamide **12** (Fig. 12).[43] Helix formation in biopolymers is generally length-dependent, with a significant degree of helix formation occurring only after a critical chain length has been reached.[5] In organic solvents, natural α-peptides require *ca.* 10–12 residues to form stable helices and at least six residues are needed for β-peptides. In contrast, we observed helical structures in oligomers of α-aminoxy acids as short as dimers; such structures represent the shortest helices ever found. This discovery opens up an opportunity to design relatively short peptides, which have functionalized side chains, to simulate natural α-peptides interaction with proteins.

Fig. 13 indicates that the CD curve of monomer **13a** is featureless because it did not possess a defined secondary structure. Dipeptide **13b** exhibited a slightly weak CD absorption because it only contains one N–O turn. The CD curves of the oligomers **13c–e** were almost superimposable—a maximum at 195 nm, a minimum at 225 nm, and a zero crossing in the range of 212–222 nm—indicating the absence of a significant positive cooperative effect. It seems that once the length satisfies the smallest helix, as in the case of tripeptide **13c**, elongation of the peptide does not provide extra stability to the helical conformation. The similar CD absorptions of tetramers **13d** and **13f**, which possess different side chains, suggest that the helix formation is independent of the nature of the side chain; *i.e.*, helix formation from peptides of α-aminoxy acids is controlled by the nature of the backbone.

γ-Turns, which are reversed-turn secondary structures found in proteins, are formed by a 3 → 1 hydrogen bond between the C=O group of amino acid residue $i$ and the NH group of amino acid residue $i + 2$.[44–46] Although γ-turns are observed less frequently than β-turns in proteins,[46] they play important roles in biological recognitions.[47–50] It is challenging to investigate the roles of γ-turns in protein–peptide recognition, however, because they seldom exist in short, linear peptides. In an exploration of the conformations of hybrid peptides containing α-amino acids and α-aminoxy acids, we demonstrated that in peptides of alternating D-α-amino acids and L-α-aminoxy acids, the seven-membered-ring intramolecular hydrogen bond, *i.e.*, γ-turn, is initiated by a following α N–O turn. Thus, a novel 7/8 helical structure is induced in this type of peptide.[51]

Conformational studies, performed using NMR, FT-IR and CD spectroscopic techniques, suggested that oligomers **14b**, **14d** and **14e** form N–O turns and γ-turns simultaneously in solution—even in a protic solvent, methanol. Fig. 14(a) displays the CD spectra of compounds **14a–e** in



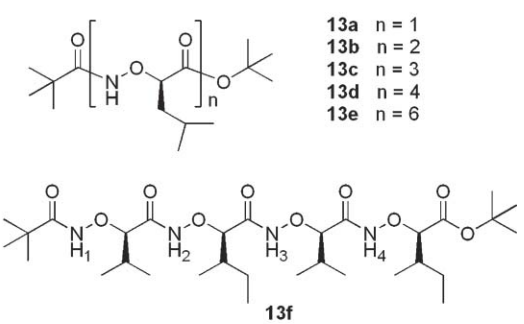




**Fig. 13** CD spectra of compounds **13a–f** in 2,2,2-trifluoroethanol.

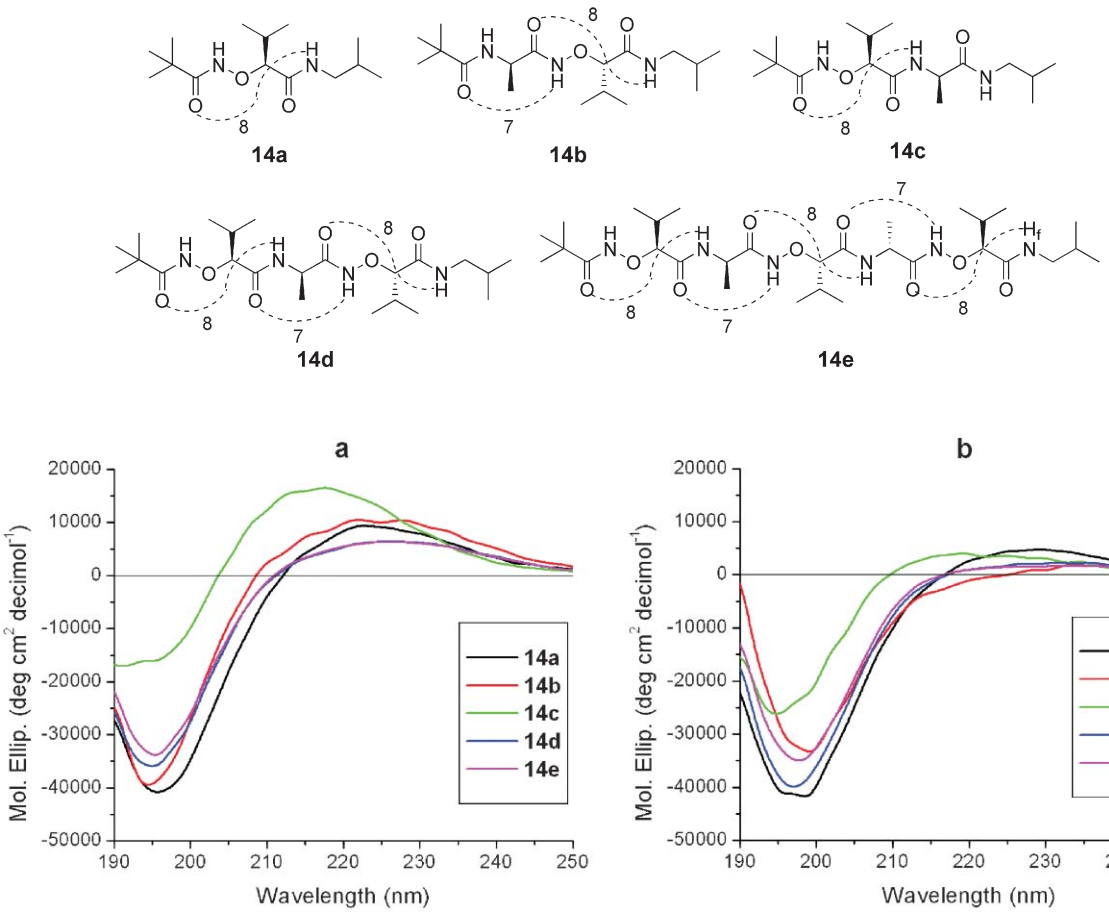
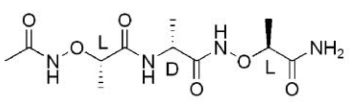



**Fig. 14** CD spectra of compounds **14a–e** recorded at 25 °C in (a) 2,2,2-trifluoroethanol and (b) methanol.

trifluoroethanol after normalizing for the concentration and the number of aminoxy acid residues. Peptide **14c** exhibited a CD curve that is unlike those of the other peptides, possibly because the γ-turn between the C-terminal NH unit and the C=O group of the aminoxy acid residue of **14c** was not formed, as revealed by $^1$H NMR spectroscopy. The CD curves of oligomers **14d** and **14e** were almost superimposable—a maximum at 227 nm, a minimum at 195 nm, and a zero crossing at 211 nm—suggesting that oligomers **14d** and **14e** adopt the same type of secondary structure: a novel mixed 7/8 helix. The CD curves of peptides **14a–e** in methanol (Fig. 14(b)) display similar patterns and intensities as those recorded in 2,2,2-trifluoroethanol, suggesting that the mixed 7/8 helix remains unchanged in methanol.

The structure of this novel 7/8 helical conformation was further confirmed from theoretical calculations. For example, the most stable conformation of tripeptide **15** features an alternating α N–O turn and γ-turn structure (Fig. 15). In the alanine residue, the α-methyl group occupies an equatorial position, which is characteristic of an inverse γ-turn. The $C_\alpha$–$C_\beta$ bonds of the aminoxy acid residues are aligned *anti* to the N–O bonds.

Helical structures are also observed in oligomers of β-aminoxy acids. Fig. 16(a) presents the well-defined helical conformation found in the solid-state structure of dipeptide **16**, which comprises $\beta^{2,2}$-aminoxy acids.[20] The helix was composed from two consecutive nine-membered-ring intramolecular hydrogen bonds, *i.e.*, two β N–O turns. The lengths of the hydrogen bonds between the $NH_{i+2}$ and $O{=}C_i$ units were 1.93 Å for the first β N–O turn and 2.29 Å for the second. The shorter NH···O=C distance in the former turn reflects the higher acidity of an aminoxy amide NH group relative to that of a common amide NH unit. Similar to the structure of diamide **3**, each of the β N–O turns of **16** featured an N–O bond aligned *anti* to the $C_\alpha$–$C_\beta$ bond; their ∠$NOC_\beta C_\alpha$




**15**

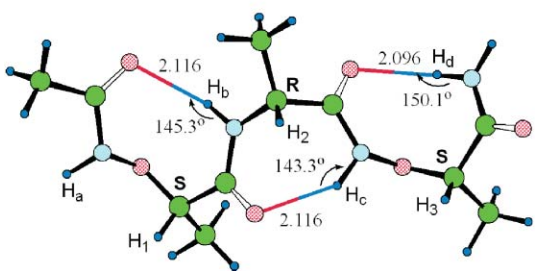



**Fig. 15** Calculated most-stable conformation of tripeptide **15** in $CH_2Cl_2$. Reprinted with permission from reference 51. Copyright 2003 American Chemical Society.

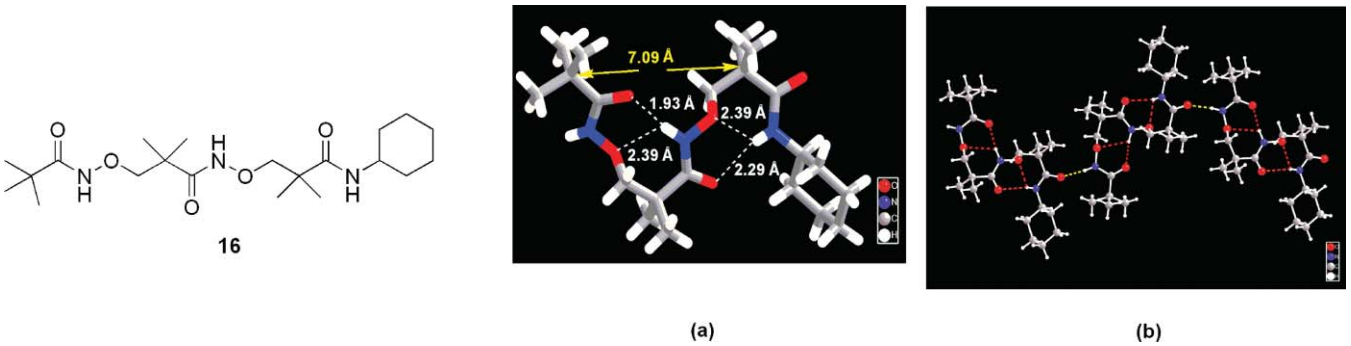
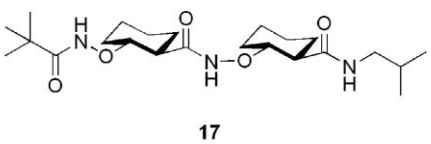



**Fig. 16** (a) Solid-state structure and (b) solid-state packing pattern of tripeptide **16**. Part (a) reprinted with permission from reference 20. Copyright 2002 American Chemical Society.

dihedral angle are similar (170 and 174°). The amide carbonyl group at position $i + 2$ was twisted by +65.9° from that at position $i$, suggesting a novel $1.7_9$ helix. Similar to the $1.8_8$ helices found in peptides of D-α-aminoxy acids, the side chains were projected laterally from the axis of the helix, but, in contrast, the distance between the α-carbon atoms at the $i$ and $i + 2$ positions of the $1.7_9$ helix was longer (7.1 Å) than that found in the $1.8_8$ helix (6.3 Å). In the solid state, when compared with $\beta^{2,2}$-aminoxy diamide **3** (Fig. 5(b)), one molecule of compound **16** formed two intermolecular hydrogen bonds with two adjacent molecules in a linear fashion (Fig. 16(b)). A similar $1.8_9$ helical structure, composed from two consecutive β N–O turns, was also observed in the solid state for a tripeptide **17**, which comprises $\beta^{2,3}$-cyclic aminoxy acids (Fig. 17).[36] In the first N–O turn, the length of the O···H hydrogen bond between the $C{=}O_i$ and $NH_{i+2}$ units is 1.93 Å, which again reflects the relatively higher acidity of aminoxy amide protons. In the second N–O turn, the O···H hydrogen bond distance between the $C{=}O_{i+1}$ and $NH_{i+3}$ units (2.07 Å) is shorter than that present in **9** (2.20 Å), indicating that hydrogen bonding is enhanced upon increasing the number of N–O turns; *i.e.*, a cooperative effect exists.

Because there are two types of β N–O turns induced by $\beta^3$-aminoxy acids, we used dipeptides **18** and **19**,[21] which have



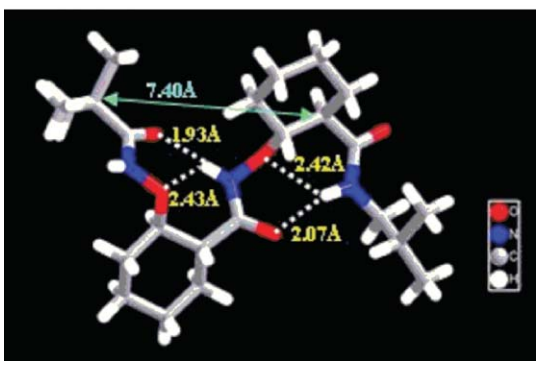




**Fig. 17** Solid-state structure of tripeptide **17**.

small (methyl) and large (isobutyl) side chains, respectively, to probe the effect that the side chain has on the helical conformation of a $\beta^3$-aminoxy peptide. $^1$H NMR, FT-IR and CD spectroscopy studies of **18** and **19** in nonpolar solvents all suggest that the helical structures possess two nine-membered-ring hydrogen bonds, *i.e.*, two consecutive β N–O turns. Interestingly, we observed two different NOE patterns for dipeptides **18** and **19** (Fig. 18) that are analogous to those observed in the β N–O turns of diamides **4** and **5** (Fig. 6). Therefore, two types of β N–O helices constructed from two types of β N–O turns are dominant in **18** and **19**. The N–O bond is aligned *anti* to the $C_\alpha$–$C_\beta$ bond in **18**, which features methyl side chains, but it is *gauche* in **19**, which possesses isobutyl side chains.

## Reverse turns

β-Turns, in which peptide backbones reverse their directions, are common secondary structures of peptides.[52–55] We are interested in constructing reverse turns using aminoxy acids as building blocks. We have established that a D-α-aminoxy acid induces a right-handed α N–O turn and its L-enantiomer leads to a left-handed α N–O turn.[13,15] While oligomers of homochiral α-aminoxy acids adopt an extended helical structure ($1.8_8$ helix),[13] we were interested in exploring the conformational features of oligomers of heterochiral α-aminoxy acids. Our theoretical calculations revealed that a loop-like conformation was the most stable structure of dipeptide **20**, which consists of two heterochiral α-aminoxy acids, while a helical structure was dominant for dipeptide **21**, which contains homochiral α-aminoxy acids (Fig. 19).[56] The calculations were confirmed by the solid-state structure of dipeptide **22**, in which two heterochiral α N–O turns were characterized

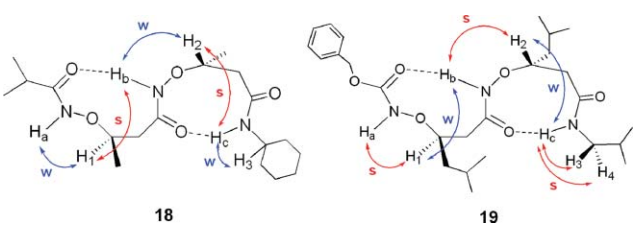



**Fig. 18** Summary of NOEs observed in dipeptides **18** and **19** in $CDCl_3$ or $CD_2Cl_2$ (s, strong NOE; w, weak NOE).

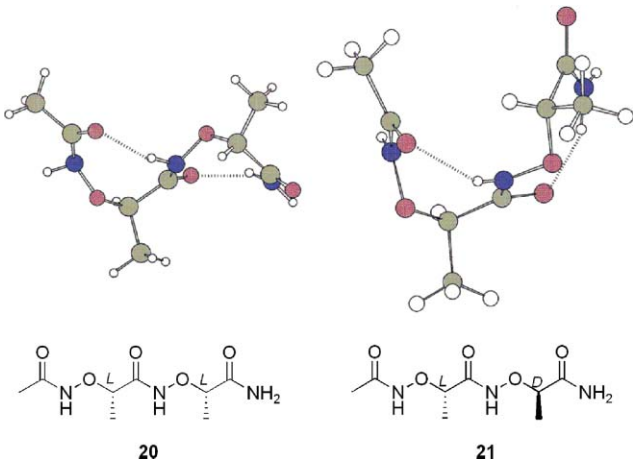



**Fig. 19** Calculated most-stable conformations of **20** and **21**.

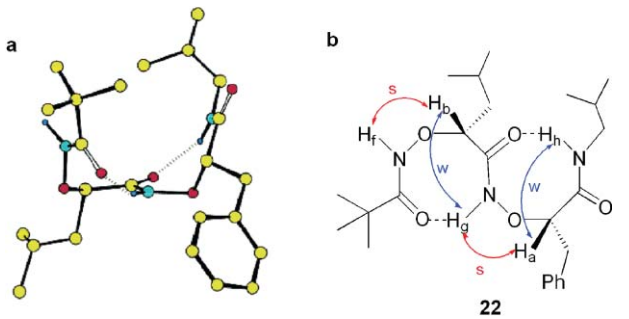
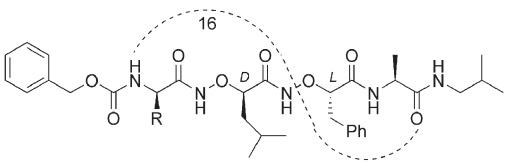



**Fig. 20** (a) Solid-state structure of dipeptide **22**. (b) Summary of NOEs observed in **22** in $CDCl_3$ (s, strong NOE; w, weak NOE). Reprinted in part with permission from reference 56. Copyright 2003 American Chemical Society.

by short NH···O=C distances and ideal N–H···O angles (Fig. 20). The conformation of **22** in $CDCl_3$ was determined from 2D NOESY experiments. The NOE pattern of **22** was similar to that observed in the spectrum of two consecutive homochiral α N–O turns; *i.e.*, **22** has a conformational preference for two consecutive α N–O turns in solution.[12,13] We used this loop segment to constrain the tetrapeptides **23** and **24** in the form of a reverse-turn structure. $^1$H NMR spectroscopic dilution and DMSO-$d_6$ addition experiments and 2D-NOESY data indicated that the tetrapeptides **23** and **24** folded into reverse-turn conformations that featured head-to-tail 16-membered-ring intramolecular hydrogen bonds.[56]




Shin *et al.* have also reported β-turn mimics prepared using α-aminoxy acids.[57] They designed and characterized the α-aminoxy tripeptide **25**, which consists of an oxanipecotic acid dimer and an α-aminoxy acid. Conformational studies in solution, performed using FT-IR and NMR spectroscopy, suggested that this compound adopts a loop conformation

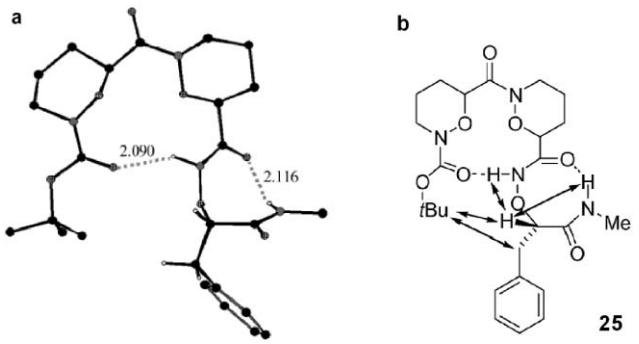
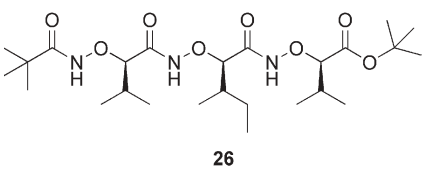



**Fig. 21** (a) Energy-minimized structure of **25**. (b) Summary of NOEs observed in **25** in $CDCl_3$. Reprinted with permission from reference 57. Copyright 2003 American Chemical Society.

with consecutive α N–O turn and β turn-like structures. Theoretical calculations of the structure of tripeptide **25** supported these conformational features and agreed well with the NOE data (Fig. 21).

**Sheets**




In the course of our conformational studies into the oligomers of α-aminoxy acids, we found that the conformation of tripeptide **26** in the solid state was different from that existing in solution. Solution-phase $^1$H NMR and 2D ROESY studies on tripeptide **26** suggested that it adopts a helical conformation with two consecutive N–O turns. The X-ray crystallographic analysis of tripeptide **26** indicated, however, that no intramolecular hydrogen bond formed; instead, the molecule adopted a more-extended conformation, akin to that of an arched β-strand (Fig. 22).[43] The amide bonds lay in the plane of the β-strand and the backbone bulged up and down,

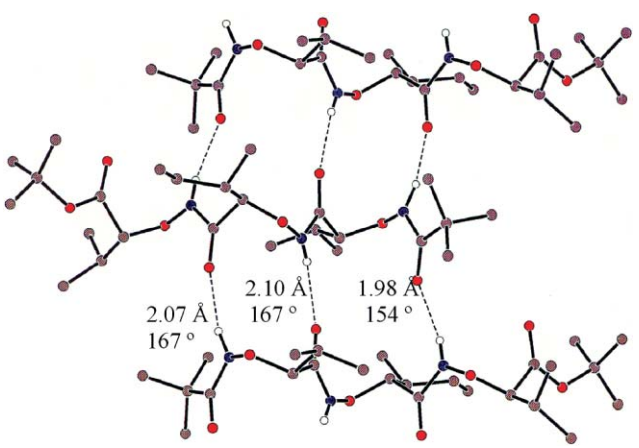



**Fig. 22** Antiparallel sheet structure in the packing of tripeptide **26** in the solid state.

directing the side chains above and below the plane of the β-strand. The distance between the side chains at the *i* and *i* + 2 positions was 6.34 Å, quite close to the distances found in parallel β-sheet structures of α-peptides. In this special β-strand, all of the amide groups were involved in intermolecular hydrogen bonding in an antiparallel fashion (Fig. 22). Each residue had a *gauche* NO–$C_\alpha$C(=O) torsion angle and the carbonyl groups were oriented in an antiparallel manner, resulting in a sheet with no net dipole. In contrast to the antiparallel β-sheets of α-peptides, the sheet conformation of **26** in the solid state featured consecutive 12- and 16-membered-ring hydrogen bonds. The fact that tripeptide **26** has a helical conformation in solution, but adopts an antiparallel sheet conformation in the solid state, serves as an example to show that the solid-state structure of a molecule does not always reflect its conformation in solution.[58]

Such sheet-like structures were also observed in the solid-state structures of peptides of $\beta^3$-aminoxy acids.[21] The conformation of dipeptide **18** in the solid state led to more-extended parallel sheet-like structures stabilized through intermolecular hydrogen bonds (Fig. 23). In these extended conformations, all of the amide groups are involved in intermolecular 16-membered-ring hydrogen bonding in a parallel fashion. According to the theoretical calculations, the extended conformers of $\beta^3$-aminoxy acids are less stable than are bent ones (β N–O turns), but the difference in energy between the two is not too great. For dipeptide **18**, which has small side chains, it is possible that van der Waals interactions between the protecting groups at both the C- and N-termini result in the sheet structures being more favorable in the solid state, *i.e.*, when the molecules are packed at exceedingly "high concentration."

### Cyclic peptides

In addition to our studies of linear peptides, we have also prepared and characterized two cyclic hexapeptides containing α-aminoxy acids.[59,60] Fig. 24 presents the calculated lowest-energy conformation of model compound **27**, which comprises DL-α-aminoxy acids. The intramolecularly hydrogen bonded cyclopeptide adopts a bracelet-like conformation with consecutive α N–O turns; the α protons of all of the residues point inward and the side chains decorate two edges of the ring (all of the D-aminoxy acid side chains are located on one side of the bracelet, with all of the L-aminoxy acid side chains on the other).[59] This conformation makes **27** quite polar on the inside of the bracelet, but nonpolar on its external surface. Instead of forming consecutive α N–O turns, a similar conformation, featuring alternating α N–O turns and γ turns, was induced in the cyclic hexapeptide **28**, which is composed of alternating D-α-aminoxy acids and D-α-amino acids (Fig. 25).[60]

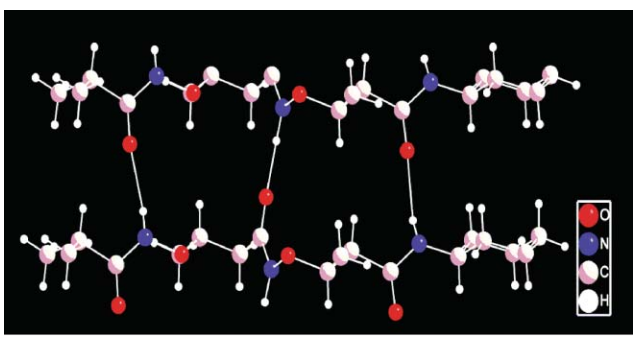
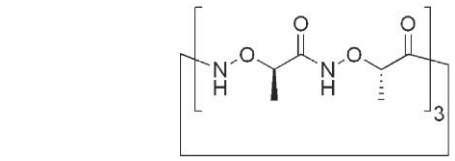



**Fig. 23** Parallel sheet structure found the packing of dipeptide **18** in the solid state. Reprinted in part with permission from reference 21(*a*). Copyright 2004 American Chemical Society.




**27**

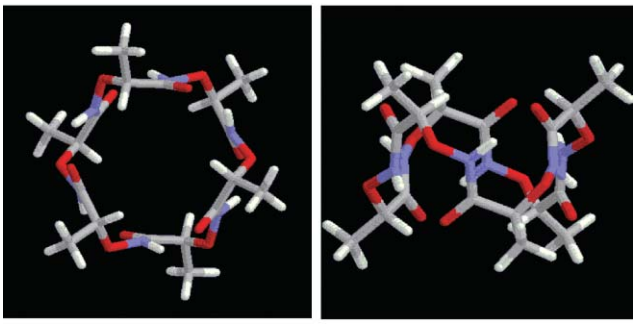
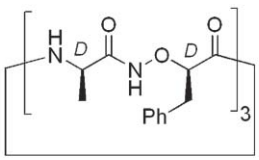



**Fig. 24** Calculated lowest-energy conformation of the cyclic hexapeptide **27**. Reprinted with permission from reference 59. Copyright 2002 American Chemical Society.

## Conclusion and outlook

Since we first published—almost ten years ago—our studies of the secondary structures induced by α-aminoxy acids, a surprisingly large number of well-defined secondary structures have been discovered within the family of aminoxy acids. Because of their rigid conformations, aminoxy acids are




**28**

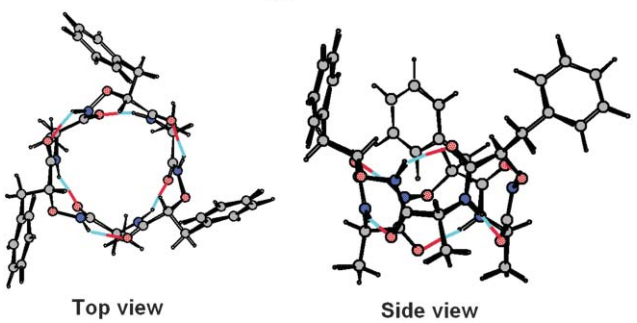



**Fig. 25** Calculated lowest-energy conformation of the cyclic hexapeptide **28**. Reproduced from reference 60 with permission of Wiley.

versatile building blocks with which to construct peptidomimetic foldamers. Peptidomimetics that adopt well-defined secondary structures in buffers or in polar solvents to mimic natural peptide–protein interactions are interesting in drug discovery. Our current studies are, therefore, concentrated on design and synthesis of aminoxy peptides with functional side chains and characterization of their secondary structures in polar solvents and buffers. Considering their biological stability towards proteases, aminoxy peptides that adopt well-defined secondary structures—with their functional side chains positioned appropriately—are expected to have the potential to simulate natural α-peptides, *i.e.*, to bind to target receptors and proteins. We believe that further research in this field will lead to potential applications for these systems in the design of pharmaceuticals, materials and molecular devices.

## Acknowledgements

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## Notes and references

1 S. H. Gellman, *Acc. Chem. Res.*, 1998, **31**, 173.
2 D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes and J. S. Moore, *Chem. Rev.*, 2001, **101**, 3893.
3 M. S. Cubberley and B. L. Iverson, *Curr. Opin. Chem. Biol.*, 2001, **5**, 650.
4 J. A. Patch and A. E. Barron, *Curr. Opin. Chem. Biol.*, 2002, **6**, 872.
5 (*a*) R. P. Cheng, S. H. Gellman and W. F. DeGrado, *Chem. Rev.*, 2001, **101**, 3219; (*b*) D. Seebach and J. L. Matthews, *Chem. Commun.*, 1997, 2015.
6 D. Seebach, M. Overhand, F. N. M. Kuhnle, B. Martinoni, L. Oberer, U. Hommel and H. Widmer, *Helv. Chim. Acta*, 1996, **79**, 913.
7 (*a*) T. Hintermann and D. Seebach, *Chimia*, 1997, **50**, 244; (*b*) D. Seebach, S. Abele, J. V. Schreiber, B. Martinoni, A. K. Nussbaum, H. Schild, H. Schulz, H. Hennecke, R. Woessner and F. Bitsch, *Chimia*, 1998, **52**, 734.
8 J. Frackenpohl, P. I. Arvidsson, J. V. Schreiber and D. Seebach, *ChemBioChem*, 2001, **2**, 445.
9 Y.-D. Wu, D.-P. Wang, K. W. K. Chan and D. Yang, *J. Am. Chem. Soc.*, 1999, **121**, 11189.
10 (*a*) I. Schon, L. Kisfaludy, J. Nafradi, L. Varga and V. Varro, *Hoppe-Seyler's Z. Physiol. Chem.*, 1978, **Bd. 359**, 897; (*b*) M. Briggs and J. S. Morley, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2138.
11 E. Testa, B. J. R. Nicolaus, L. Mariani and G. Pagani, *Helv. Chim. Acta*, 1963, **46**, 766.
12 D. Yang, B. Li, F. F. Ng, Y.-L. Yan, J. Qu and Y.-D. Wu, *J. Org. Chem.*, 2001, **66**, 7303.
13 D. Yang, J. Qu, B. Li, F.-F. Ng, X.-C. Wang, K.-K. Cheung, D.-P. Wang and Y.-D. Wu, *J. Am. Chem. Soc.*, 1999, **121**, 589.
14 I. Shin, M. R. Lee, J. Lee, M. Jung, W. Lee and J. Yoon, *J. Org. Chem.*, 2000, **65**, 7667.
15 D. Yang, F.-F. Ng, Z.-J. Li, Y.-D. Wu, K. W. K. Chan and D.-P. Wang, *J. Am. Chem. Soc.*, 1996, **118**, 9794.
16 T. S. Haque, J. C. Little and S. H. Gellman, *J. Am. Chem. Soc.*, 1994, **116**, 4105.
17 G. T. Copeland, E. R. Jarvo and S. J. Miller, *J. Org. Chem.*, 1998, **63**, 6784.
18 N. Harada and K. Nakanishi, in *Circular Dichroic Spectroscopy: Exciton Coupling in Organic Stereochemistry*, University Science Books, Mill Valley, CA, 1983.
19 (*a*) D. Seebach, P. E. Ciceri, M. Overhand, B. Jaun, D. Rigo, L. Oberer, U. Hommel, R. Amstutz and H. Widmer, *Helv. Chim. Acta*, 1996, **79**, 2043; (*b*) Y.-D. Wu and D.-P. Wang, *J. Am. Chem. Soc.*, 1999, **121**, 9352; (*c*) D. Seebach, S. Abele, K. Gademann, G. Guichard, T. Hintermann, B. Jaun, J. L. Matthews and J. Schreiber, *Helv. Chim. Acta*, 1998, **81**, 932; (*d*) D. Seebach, K. Gademann, J. L. Matthews, T. Hintermann, B. Jaun, L. Oberer, U. Hommel and H. Widmer, *Helv. Chim. Acta*, 1997, **80**, 2033.
20 D. Yang, Y.-H. Zhang and N.-Y. Zhu, *J. Am. Chem. Soc.*, 2002, **124**, 9966.
21 (*a*) D. Yang, Y.-H. Zhang, B. Li, D.-W. Zhang, C.-Y. J. Chan, N.-Y. Zhu, S.-W. Luo and Y.-D. Wu, *J. Am. Chem. Soc.*, 2004, **126**, 6956; (*b*) D. Yang, Y.-H. Zhang, B. Li and D.-W. Zhang, *J. Org. Chem.*, 2004, **69**, 7577.
22 D. H. Appella, L. A. Christianson, I. L. Karle, D. R. Powell and S. H. Gellman, *J. Am. Chem. Soc.*, 1996, **118**, 13071.
23 D. H. Appella, L. A. Christianson, D. A. Klein, D. R. Powell, X. Huang, J. J. Barchi, Jr. and S. H. Gellman, *Nature*, 1997, **387**, 381.
24 D. H. Appella, L. A. Christianson, D. A. Klein, M. R. Richards, D. R. Powell and S. H. Gellman, *J. Am. Chem. Soc.*, 1999, **121**, 7574.
25 D. H. Appella, L. A. Christianson, I. L. Karle, D. R. Powell and S. H. Gellman, *J. Am. Chem. Soc.*, 1999, **121**, 6206.
26 J. J. Barchi, Jr., X. Huang, D. H. Appella, L. A. Christianson, S. R. Durell and S. H. Gellman, *J. Am. Chem. Soc.*, 2000, **122**, 2711.
27 D. H. Appella, J. J. Barchi, Jr., S. R. Durell and S. H. Gellman, *J. Am. Chem. Soc.*, 1999, **121**, 2309.
28 X. Wang, J. F. Espinosa and S. H. Gellman, *J. Am. Chem. Soc.*, 2000, **122**, 4821.
29 H.-S. Lee, F. A. Syud, X. Wang and S. H. Gellman, *J. Am. Chem. Soc.*, 2001, **123**, 7721.
30 M. G. Woll, J. D. Fisk, P. R. LePlae and S. H. Gellman, *J. Am. Chem. Soc.*, 2002, **124**, 12447.
31 P. R. LePlae, J. D. Fisk, E. A. Porter, B. Weisblum and S. H. Gellman, *J. Am. Chem. Soc.*, 2002, **124**, 6820.
32 T. L. Raguse, E. A. Porter, B. Weisblum and S. H. Gellman, *J. Am. Chem. Soc.*, 2002, **124**, 12774.
33 T. L. Raguse, J. R. Lai and S. H. Gellman, *J. Am. Chem. Soc.*, 2003, **125**, 5592.
34 J.-S. Park, H.-S. Lee, J. R. Lai, B. M. Kim and S. H. Gellman, *J. Am. Chem. Soc.*, 2003, **125**, 8539.
35 Y. D. Wu and D. P. Wang, *J. Am. Chem. Soc.*, 1998, **120**, 13485.
36 D. Yang, D.-W. Zhang, Y. Hao, Y.-D. Wu, S.-W. Luo and N.-Y. Zhu, *Angew. Chem., Int. Ed.*, 2004, **43**, 6719.
37 F. Chen, N.-Y. Zhu and D. Yang, *J. Am. Chem. Soc.*, 2004, **126**, 15980.
38 E. Graf von Roedern and H. Kessler, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 687.
39 E. Graf von Roedern, E. Lohof, G. Hessler, M. Hoffmann and H. Kessler, *J. Am. Chem. Soc.*, 1996, **118**, 10156.
40 R. R. Gardner, G.-B. Liang and S. H. Gellman, *J. Am. Chem. Soc.*, 1995, **117**, 3280.
41 R. R. Gardner, G.-B. Liang and S. H. Gellman, *J. Am. Chem. Soc.*, 1999, **121**, 1806.
42 Y.-D. Wu, D.-P. Wang, K. W. K. Chan and D. Yang, *J. Am. Chem. Soc.*, 1999, **121**, 11189.
43 J. Qu, PhD Thesis, The University of Hong Kong, 2001.
44 G. Némethy and M. P. Printz, *Macromolecules*, 1972, **5**, 755.
45 C. Toniolo, *Crit. Rev. Biochem.*, 1980, **9**, 1.
46 G. D. Rose, L. M. Gierasch and J. A. Smith, *Adv. Protein Chem.*, 1985, **37**, 1.

 

47 A. Milon, T. Miyazawa and T. Higashijima, *Biochemistry*, 1990, **29**, 65.
48 M. Coles, V. Sowemimo, D. Scanlon, S. L. A. Munro and D. J. Craik, *J. Med. Chem.*, 1993, **36**, 2658.
49 S. M. Vogen, O. Prakash, L. Kirnarsky, S. D. Sanderson and S. A. Sherman, *J. Pept. Res.*, 1999, **54**, 74.
50 A. M. Andrianov and Y. A. Sokolov, *J. Biomol. Struct. Dyn.*, 2003, **20**, 603.
51 D. Yang, W. Li, J. Qu, S.-W. Luo and Y.-D. Wu, *J. Am. Chem. Soc.*, 2003, **125**, 13018.
52 C. M. Venkatachalam, *Biopolymers*, 1968, **6**, 1425.
53 P. Y. Chou and G. D. Fasman, *J. Mol. Biol.*, 1977, **115**, 135.
54 B. L. Sibanda and J. M. Thornton, *Nature*, 1985, **316**, 170.
55 C. M. Wilmot and J. M. Thornton, *J. Mol. Biol.*, 1988, **203**, 221.
56 D. Yang, J. Qu, W. Li, D.-P. Wang, Y. Ren and Y.-D. Wu, *J. Am. Chem. Soc.*, 2003, **125**, 14452.
57 B. H. Baek, M. R. Lee, K. Y. Kim, U. I. Cho, D. W. Boo and I. Shin, *Org. Lett.*, 2003, **5**, 971.
58 Y.-J. Chung, B. R. Huck, L. A. Christianson, H. E. Stanger, S. Krauthäuser, D. R. Powell and S. H. Gellman, *J. Am. Chem. Soc.*, 2000, **122**, 3995.
59 D. Yang, J. Qu, W. Li, Y.-H. Zhang, Y. Ren, D.-P. Wang and Y.-D. Wu, *J. Am. Chem. Soc.*, 2002, **124**, 12410.
60 D. Yang, X. Li, Y. Sha and Y.-D. Wu, *Chem.–Eur. J.*, 2005, **11**, 3005–3009.