ACCOUNTS
of chemical research

α-Aminoxy Acids: New Possibilities from Foldamers to Anion Receptors and Channels

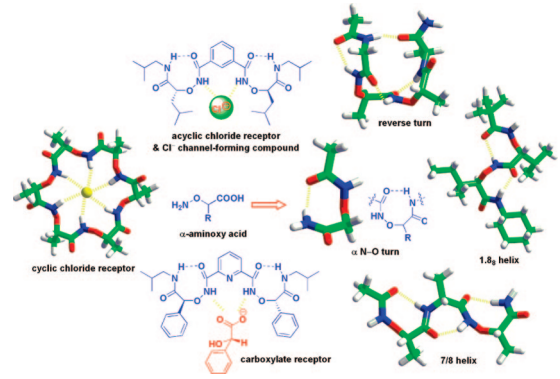
XIANG LI,[†] YUN-DONG WU,[*,§] AND DAN YANG[*,†]

[†]*Department of Chemistry, The University of Hong Kong, Pokfulam Road, Hong Kong, China,* [§]*Department of Chemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China*

RECEIVED ON JUNE 20, 2008

CONSPECTUS

Naturally occurring peptides serve important functions as enzyme inhibitors, hormones, neurotransmitters, and immunomodulators in many physiological processes including metabolism, digestion, pain sensitivity, and the immune response. However, due to their conformational flexibility and poor bioavailability, such peptides are not generally viewed as useful therapeutic agents in clinical applications. In an effort to solve these problems, chemists have developed peptidomimetic foldamers, unnatural oligomeric molecules that fold into rigid and well-defined secondary structures mimicking the structures and biological functions of these natural peptides. We have designed peptidomimetic foldamers that give predictable, backbone-controlled secondary structures irrespective of the nature of the side chains.



This Account presents our efforts to develop a novel class of peptidomimetic foldamers comprising α-aminoxy acids and explore their applications in the simulation of ion recognition and transport processes in living systems. Peptides constructed from α-aminoxy acids fold according to the following rules: (1) A strong intramolecular eight-membered-ring hydrogen bond forms between adjacent α-aminoxy acid residues (the α N−O turn). The chirality of the α-carbon, not the nature of the side chains, determines the conformation of this chiral N−O turn. (2) While homochiral oligomers of α-aminoxy acids form an extended helical structure (1.8_8 helix), heterochiral ones adopt a bent reverse turn structure. (3) In peptides of alternating α-amino acids and α-aminoxy acids, the seven-membered-ring intramolecular hydrogen bond, that is, the γ-turn, is initiated by a succeeding α N−O turn. Thus, this type of peptide adopts a novel 7/8 helical structure.

In investigating the potential applications of α-aminoxy acids, we have found that the amide NH units of α-aminoxy acids are more acidic than are regular amide NH groups, which makes them better hydrogen bond donors when interacting with anions. This property makes α-aminoxy acids ideal building blocks for the construction of anion receptors. Indeed, we have constructued both cyclic and acyclic anion receptors that have strong affinities and good (enantio-)selectivities toward chloride (Cl^-) and chiral carboxylate ions. Taking advantage of these systems' preference for Cl^- ions, we have also employed α-aminoxy acid units to construct a synthetic Cl^- channel that can mediate the passage of Cl^- ions across cell membranes. Continued studies of these peptidomimetic systems built from α-aminoxy acids should lead to a broad range of applications in chemistry, biology, medicine, and materials science.

Introduction

In Nature, most of the biologically relevant functions carried out by proteins and peptides (such as molecular recognition, catalysis, and electron transfer) are related to their unique three-dimensional structures that are characteristically stable and well-ordered. With the accumulation of knowledge on the relationship between protein/peptide structure and function, the design of peptide-based molecules that bind to therapeutically important biological targets with high affinity and selectivity has become an arena of intense research in drug discovery.[1] However, there are two major concerns about the intrinsic properties

Published on the Web 09/12/2008 www.pubs.acs.org/acr
10.1021/ar8001393 CCC: $40.75

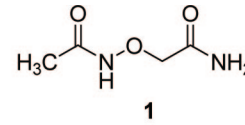
of native peptides, which limit their utility in clinical applications, namely, (1) conformational flexibility, which undermines the affinity and specificity of a peptide binding to its target, and (2) degradation by many naturally specific or nonspecific peptidases under physiological conditions. These have galvanized researchers' interest in developing peptidomimetics with more rigid conformation and enhanced biostabilities.

A current guiding principle is that a peptidomimetic molecule with a preconstrained secondary structure (which mimics the biologically active conformation of a flexible native peptide upon binding to its macromolecular target) will have higher affinity and improved selectivity profiles, because the preorganized molecule is predicted to pay a lower entropic cost upon complexation.[2] Therefore, one general strategy for designing peptidomimetics is to generate unnatural oligomeric molecules that fold into rigid and well-defined conformations. This subject has been the focus in the rapidly evolving field of peptidomimetic foldamers.[3−5] Recent research into peptidomimetic foldamers has already given birth to a diverse array of functionally interesting molecules capable of binding specifically to various targets including proteins,[6−11] RNA,[12] carbohydrates,[13] and lipids,[14−19] often with affinities close to or even higher than those of natural peptides or proteins.

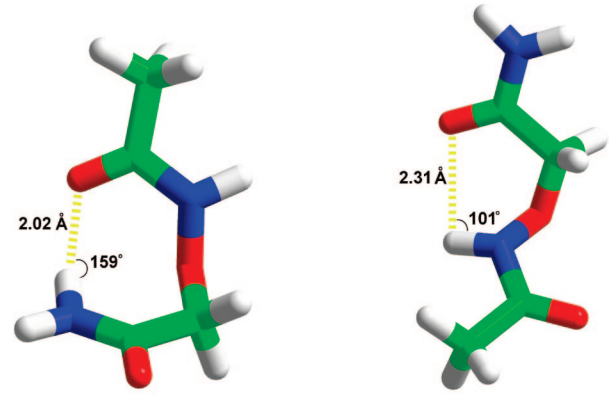
On the other hand, the side chains of a peptidomimetic molecule are known to contribute significantly to specificity and affinity when it binds to a macromolecule. This is in part because some of these side chains are involved in the direct surface interactions between the peptide and the macromolecule. More importantly, the side chains control and affect the secondary structures of the backbone, which in reverse determines the spatial orientations of the side chains themselves for the fitness with a macromolecular surface. For synthetic chemists, it remains an intriguing challenge as to how to design peptidomimetic foldamers that possess side-chain-independent backbones capable of projecting any desired side chains in defined orientations. To this end, we have initiated a program to explore a novel class of peptidomimetic foldamers comprising α-aminoxy acids that display backbone-controlled secondary structures.[20] This Account will summarize a set of folding rules for peptides based on α-aminoxy acids and propose their novel applications as building blocks for the construction of anion receptors and channels.

Monomers of α-Aminoxy Acids

α-Aminoxy acids are analogs of *β*-amino acids in which the *β*-carbon atom in the *β*-amino acid backbone is replaced with an oxygen atom. In 1996, our group first reported a novel turn structure in the peptides containing α-aminoxyacetic acids.[21] To illustrate, theoretical calculations on α-aminoxy



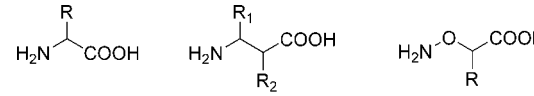
1



1a **1b**

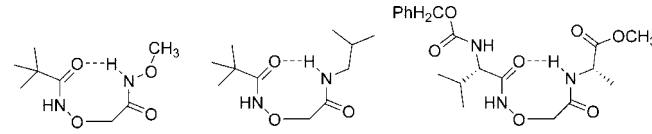
G_{rel} 0.0 (0.0) [0.0] 1.8 (3.5) [3.0]

FIGURE 1. Calculated structures of α-aminoxy amide **1**. The values of G_{rel} (kcal/mol) were calculated by using the HF/6-31G*, (MP2/6-31G*), and [HF/6-31G* $CHCl_3$ solvation] models.



α-amino acid β-amino acid α-aminoxy acid

diamide **1** suggest that it adopts a rigid eight-membered-ring hydrogen-bonded structure (so-called α N−O turn) in its most favorable conformation **1a** (Figure 1). The strong hydrogen bond in **1a** is indicated by the short O···H distance (2.02 Å) and the near-linear geometry of the O···H−N bond angle (159°). Our conformational studies of α-aminoxy peptides **2**−**4** in dichloromethane using FT-IR and ^{1}H NMR spectroscopies confirmed the calculated intramolecularly hydrogen-bonding pattern in **1a**.[21]



2 **3** **4**

For peptides of α-amino acids, the propensity of turn formation is contingent on the nature, position, and relative configuration of amino acid residues. Therefore, it is interesting to know whether the α N−O turns are still favored when side chains are introduced into α-aminoxyacetic acid. We conducted *ab initio* molecular orbital calculations on L-α-aminoxypropionic acid diamide **5**.[22] Four lowest-energy conformers **5a**−**d** were found to contain eight-membered-ring hydrogen bonds (Figure 2). The hydrogen-bonding pattern of **5a** and **5b** allows a left-handed turn, while that of **5c** and **5d** results in

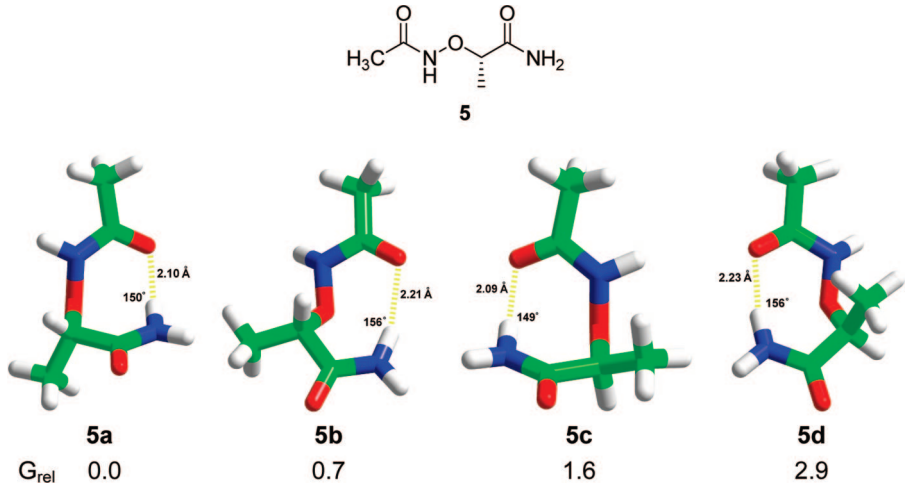
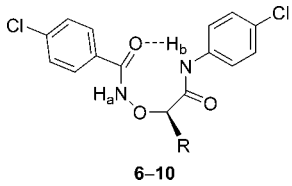


FIGURE 2. HF/6-31G**-optimized structures of diamide **5** and their relative free energies in CH_2Cl_2 solution.

a right-handed turn. The *ab initio* calculation results suggest that chiral L-α-aminoxy acids would probably prefer a left-handed chiral N−O turn (**5a** or **5b**). Furthermore, the α-methyl group was anti to the N−O bond in **5a** but gauche in **5b**, thus making **5a** the most stable conformation.

To probe the effect of side chains on the N−O turn structure, we characterized the conformations of chiral D-configuration α-aminoxy diamides **6**−**10** with different side chains through a combination of experimental techniques.[23,24]



6–10

6	R = $CH_2CH(CH_3)_2$
7	R = CH_3
8	R = CH_2Ph
9	R = $CH(CH_3)_2$
10	R = $CH(CH_3)CH_2CH_3$

^{1}H NMR dilution[25] and DMSO-d_6[26] titration studies in $CDCl_3$ revealed that in diamides **6**−**10**, NH_b forms a strong intramolecular hydrogen bond whereas NH_a is solvent-accessible and not intramolecularly hydrogen-bonded. We also applied 2D NMR methods to investigate the conformations of diamide **6** in the solution phase. The two-dimensional rotating-frame Overhauser effect (2D-ROESY) spectrum of **6** suggests that a strong nuclear Overhauser effect (NOE) exists between the NH_a and $C_\alpha H$ groups, whereas only a weak NOE exists between the NH_b and $C_\alpha H$ groups, indicating that this compound adopts a folded structure that agrees well with the most stable calculated conformer **5a**, in which the distance between NH_a and $C_\alpha H$ was 2.7 Å, compared with a corresponding distance of 3.4 Å between $C_\alpha H$ and NH_b.

We also used CD spectroscopy[27] to study the solution conformations of diamides **6**−**10**. The CD spectra of diamides **6**−**10**, which feature different side chains, exhibited strong positive exciton coupling with nearly identical maxima and minima in dichloromethane. Furthermore, diamide **6** also showed similar CD curves in different solvents like cyclohexane, dichloromethane, dioxane, acetonitrile, and methanol. This strongly indicates 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 and regardless of the solvents.

The α N−O turn structure can also be observed in the solid state. Figure 3 shows the X-ray structure of diamide **6**. An intramolecular eight-membered-ring hydrogen bond NH···O=C was found in the X-ray structure by the short H···O distance (2.12 Å). The dihedral angle ∠NOC$_\alpha$C(=O) was +78.5° in the X-ray structure, in excellent agreement with the calculation result of +78.4°, which suggests that the diamide **6** adopts a right-handed turn conformation. The isobutyl group is almost anti to the N−O bond, which agrees well with the calculation and 2D NMR studies.

Based on experimental evidence, it can be inferred that the formation of the α N−O turn is independent of side chain variation. According to the calculation result, the orientation of the α N−O turn is determined exclusively 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 would be switched from a right-handed to a left-handed structure. Figure 3 shows the solid-state structures of diamides **6** and **11**, in which their backbones are mirror image of each other. The side-chain independence of these α N−O turn structures indicates that these systems are subject to backbone-based control. Collectively, these results allow us to construct well-defined secondary structures using a variety of side chains.

Oligomers of α-Aminoxy Acids

Helix. The formation of a rigid α N−O turn structure in α-aminoxy diamides has inspired us to investigate the conforma-

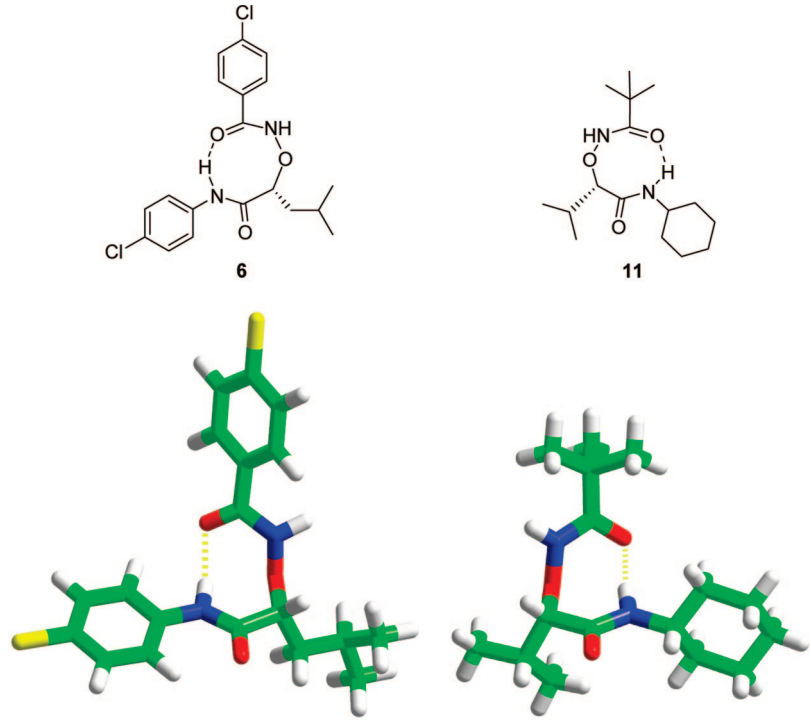
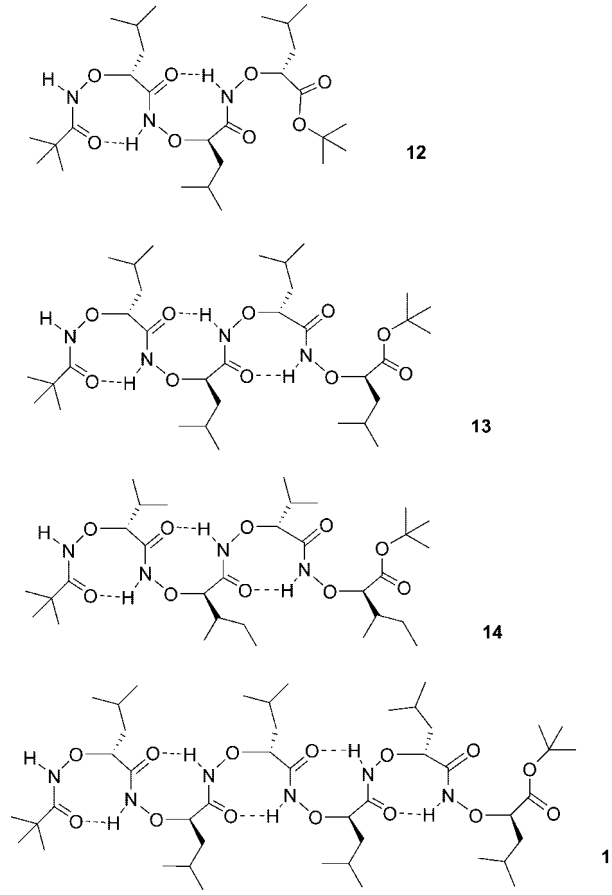


FIGURE 3. X-ray crystal structures of **6** with right-handed α N−O turn and **11** with left-handed α N−O turn.

tional features of oligomers of α-aminoxy acids. We hypothesized that in the homochiral oligomers of α-aminoxy acids, the eight-membered-ring intramolecular hydrogen bonds should occur between the adjacent residues and the consecutive homochiral N−O turns should lead to a helical structure. Accordingly, we synthesized compounds **12**−**15** and subjected them to conformational studies as performed previously for α-aminoxy diamides.[23,24] Both the FT-IR and ^{1}H NMR spectroscopies suggest that in compounds **12**−**15**, intramolecular hydrogen bonds form between the amide NH groups at the $i + 2$ position and the carbonyl oxygen atoms at the i position. Furthermore, we found that the CD curves of the oligomers **12**−**15** in 2,2,2-trifluoroethanol were almost superimposable, with a maximum at 195 nm, a minimum at 225 nm, and a zero crossing in the range of 215−222 nm, indicating that their secondary structures are very similar (Figure 4). The similar CD absorptions of tetramers **13** and **14**, which possess different side chains, suggest that the helix formation is controlled by the nature of the backbone but independent of the nature of the side chains.

We also found helical structures in the crystal structures of oligomers of α-aminoxy acids as short as dimers **16** and **17** (Figure 5).[28,29] Although they have different side chains and terminal groups, the backbones of dimers **16** and **17** adopt almost identical conformations, indicating the negligible effect of the side chains on the formation of helix. Notably, the crystal structures of **16** and **17** illuminate several features of the



novel helix: (1) the helix contains consecutive homochiral eight-membered-ring intramolecular hydrogen bonds, which

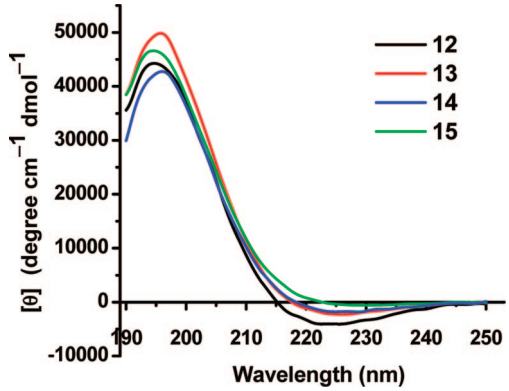


FIGURE 4. CD spectra of diamides **12**−**15** (0.4 mM) in 2,2,2-trifluoroethanol, normalized with respect to the number of potential N−O turns.

line up along the helical axis. (2) The amide carbonyl group at position $i + 2$ is twisted +31° from the one at the i position, suggesting a twisted 1.8_8 helix. Compared with other helices of conventional spiral shape (such as α-helix,[30] 3_{10} helix,[31] and newly discovered 2.5_{12}[32] and 3_{14}[33] helices in β-peptides), the new helix of small intramolecularly hydrogen-bonded rings is fairly flat when viewed in profile. (3) The backbone of the D-α-aminoxy acid oligomers forms a right-handed helix with a positive dihedral angle $\angle NOC_\alpha C_o$ in each N−O turn. (4) The side chains are located alternately on the opposite sides of the helix, resembling the side-chain orientation found in parallel β-sheets of α-peptides; (5) the distances between NH_i and $C_\alpha H_i$ in **16** and **17** are much shorter than those between NH_i and $C_\alpha H_{i-1}$, which concurs well with the NOE pattern observed in NOESY spectra of these compounds in $CDCl_3$. This suggests that the solid-state conformation of oligomers is almost identical to their solution conformation.

Reverse Turn. Our studies have established that D-α-aminoxy acids induce a right-handed α N−O turn while the L-enantiomers induce a left-handed N−O turn. As discussed before, the homochiral oligomers of α-aminoxy acids can adopt a helical structure. It is certainly worthwhile to characterize the conformations of heterochiral oligomers of α-aminoxy acids. Theoretical calculations reveal that for dipeptide **18** with two consecutive α-aminoxy acids of the same configuration, the backbone folds into an extended helical structure with two homochiral α N−O turns, while dipeptide **19** with two heterochiral α-aminoxy acids bends backward to produce a loop conformation (Figure 6).[34]

The X-ray crystal structure of triamide **20** confirms the predicted loop conformation with two heterochiral α N−O turns (Figure 7).[34] The short NH···O═C distances and the ideal N−H···O angles of the two intramolecular hydrogen bonds in **20** (2.14 Å/156° for the first hydrogen bond from the N-terminus and 2.04 Å/157° for the second one) are comparable to those observed in two consecutive homochiral α N−O turns. The looplike structure was further identified as a predominant solution conformation adopted by triamide **20** in $CDCl_3$ by using 2D NOESY spectroscopy. This loop segment was also used to constrain tetrapeptides **21** and **22** to form a reverse turn structure. Conformational studies indicate that in a nonpolar solvent, tetrapeptides **21** and **22** fold into reverse-turn conformations featuring a head-to-tail 16-membered-ring intramolecular hydrogen bond as shown in quantum mechanics calculations of model pentamide **23** (Figure 8).[34]

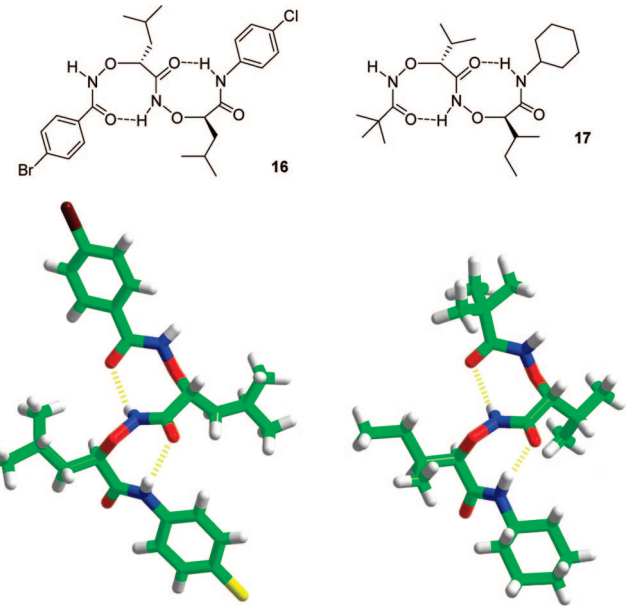


FIGURE 5. X-ray crystal structures of **16** and **17**.

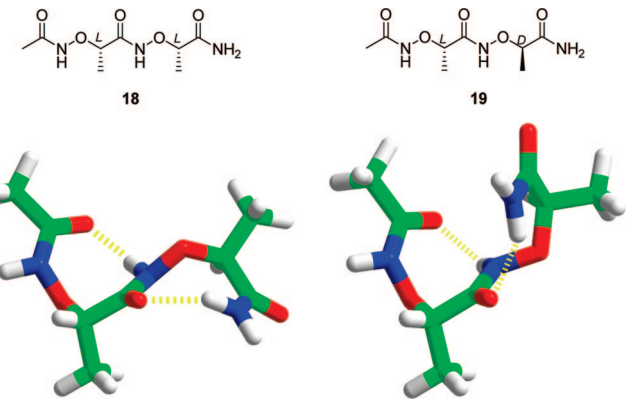


FIGURE 6. Calculated most-stable conformations of **18** and **19**.

Peptides of Alternating α-Amino and α-Aminoxy Acids

The α/β-hybrid peptides have been extensively studied in recent years,[35−41] and studies by Gellman's group have amply emphasized their biological implications[5,38] and self-assembly into quarternary helix bundles.[39,40] In exploring the

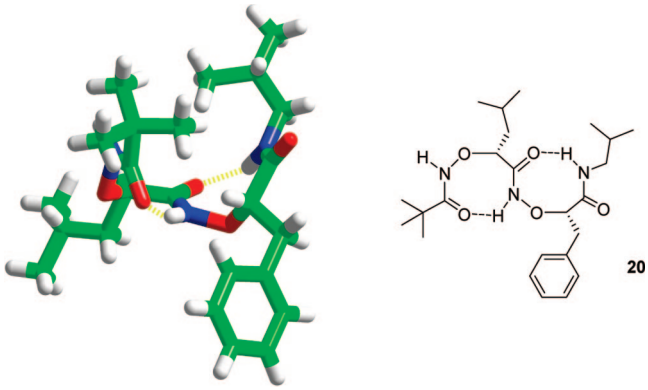
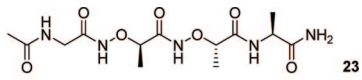


FIGURE 7. X-ray crystal structure of **20**.



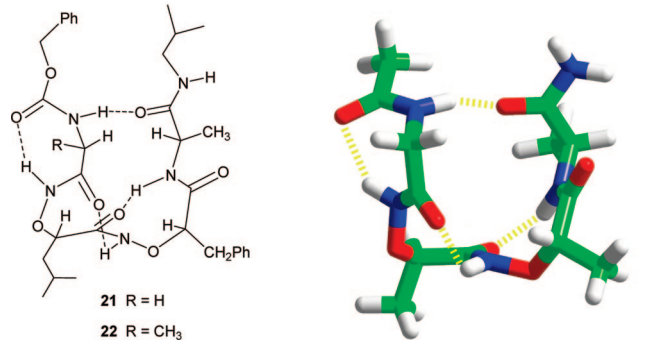
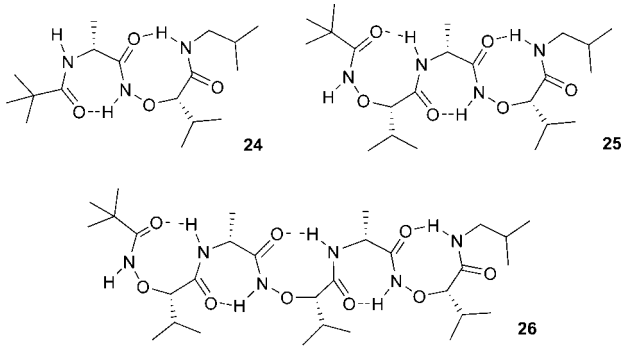


FIGURE 8. Proposed conformations of **21** and **22** in $CDCl_3$ in NMR spectroscopy and HF/6-31G**-optimized lowest-energy conformation of model pentamide **23**.

conformations of hybrid peptides containing α-amino acids and α-aminoxy acids, we have found that in peptides of alternating D-α-amino acids and L-α-aminoxy acids, such as compounds **24**–**26**, the seven-membered-ring intramolecular



hydrogen bond (i.e., γ-turn) is initiated by a succeeding α N–O turn.[42] Although γ-turns are observed less frequently than β-turns in proteins, they play vital roles in molecular recognition in biological systems. In addition, the alternating α N–O turns and γ-turns constitute a novel 7/8 helical structure in this type of peptide, which has also been proven the most stable conformation in the theoretical calculations on model peptide **27** (Figure 9).[42] It signifies a new strategy to induce γ-turn at specific sites of short peptides by incorporating an α-aminoxy acid immediately after a particular α-amino acid of interest.

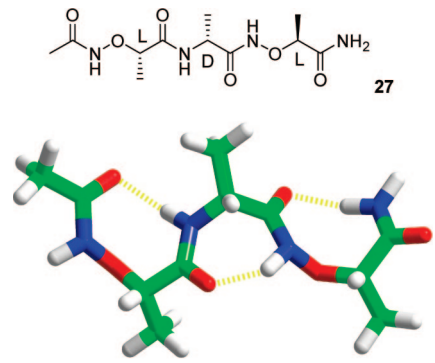


FIGURE 9. Calculated most-stable conformation of tripeptide **27** in CH_2Cl_2.

Construction of Anion Receptors and Channels with α-Aminoxy Acids as Building Blocks

Host–guest chemistry of ionic species represents a central field in supramolecular chemistry. However, anion coordination has received less attention compared with the coordination chemistry of cations. Since mechanistic understanding about the roles played by anions has profound implications in both biology and medicine,[43] simulations of the recognition, binding, and transport processes of physiologically important anions have deservedly attracted growing interest that drives the development of artificial anion receptors[43–49] and channels.[50–52]

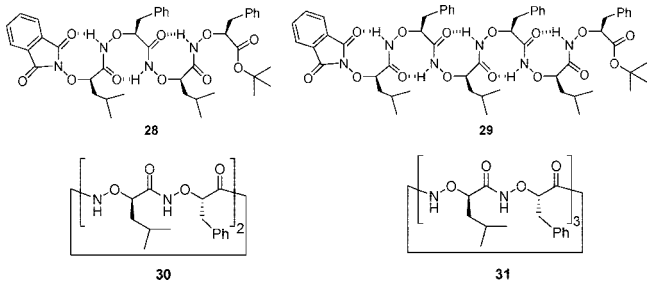
Notably, chloride (Cl^-) ions are the most abundant anions found in organisms. The Cl^- channels, unique anion channels that mediate the transfer of Cl^- ions across cell membranes, play crucial roles in controlling membrane excitability, transepithelial transport, cell volume, and intracellular pH.[53,54] In fact, dysfunctional Cl^- channels are responsible for many severe human diseases, including cystic fibrosis, inherited kidney stone diseases, myotonia, and epilepsy.[54,55] These contexts have provided the inspirations for us to create synthetic systems that mimic biological functions of natural Cl^- channels. In Nature, the high specificity of Cl^- channels stems from recognition sites in which the anion is completely desolvated and bound exclusively through hydrogen bonds.[56,57] Design of artificial Cl^- receptors to simulate such a chloride recognition process has therefore been our first consideration in developing our synthetic Cl^- channels.

In investigating the potential applications of α-aminoxy acids, we noticed that the amide NH units of α-aminoxy acids

are more acidic than are regular amide NH groups, which makes them more efficient hydrogen bond donors during interactions with anions. This property renders aminoxy acids excellent building blocks for the construction of anion receptors.

Cyclic Anion Receptors. Since a relatively rigid preorganized conformation is a general requirement of host molecules in supramolecular chemistry, cyclic peptides, in comparison with linear ones, are considered more appropriate as anion receptors. In our previous studies of α-aminoxy peptides, we found that while the homochiral oligomers of α-aminoxy acids adopt a linear helical conformation by forming consecutive homochiral N−O turns, the heterochiral ones favor a curved conformation comprising heterochiral N−O turns (Figure 6). Thus, the preorganized arch-like conformation adopted by linear heterochiral oligomers of α-aminoxy acids makes their C-terminus and the N-terminus quite close to each other. As a result, the corresponding cyclic peptides can be constructed with relative ease.

Indeed, cyclization of the linear oligomers **28** and **29** can produce cyclic tetrapeptide **30** and hexapeptide **31**, respectively.[58] The conformations of **30** and **31** are novel in peptide chemistry. Solution-phase conformational studies by FT-IR, ^{1}H NMR, and 2D NMR spectroscopies demonstrate that in agreement with the calculated structures of model cyclic tetrapeptide **32** and hexapeptide **33**, respectively, cyclic peptides **30** and **31** adopt highly symmetrical bracelet-like conformations, wherein all of the amide NH units and carbonyl groups are involved in the eight-membered-ring intramolecular hydrogen bonds (Figure 10).



Because **30** and **31** have preorganized symmetrical conformations with cylindrical cavities inside, they meet the general requirements of host molecules. The van der Waals diameter of the cavity in model cyclic hexapeptide **33** is 3.22 Å, which would probably be suitable for binding some small ions, whereas the internal cavity in model cyclic tetrapeptide **32** is too small to be useful (1.28 Å in van der Waals diameter). Indeed, cyclic hexapeptide **31** shows affinities for halide anions with very high selectivity for Cl^- ions (association constant K_a = 11 880 M^{-1} in CD_2Cl_2).[58] The HF/6-31G*-optimized lowest-energy conformation of complex **33**·Cl^- is shown in Figure 11. The Cl^- ion at the center of **33** is hydrogen bonded to six NH protons simultaneously. The calculated H···Cl distance and N−H···Cl angle are 2.40 Å and 154°, respectively.

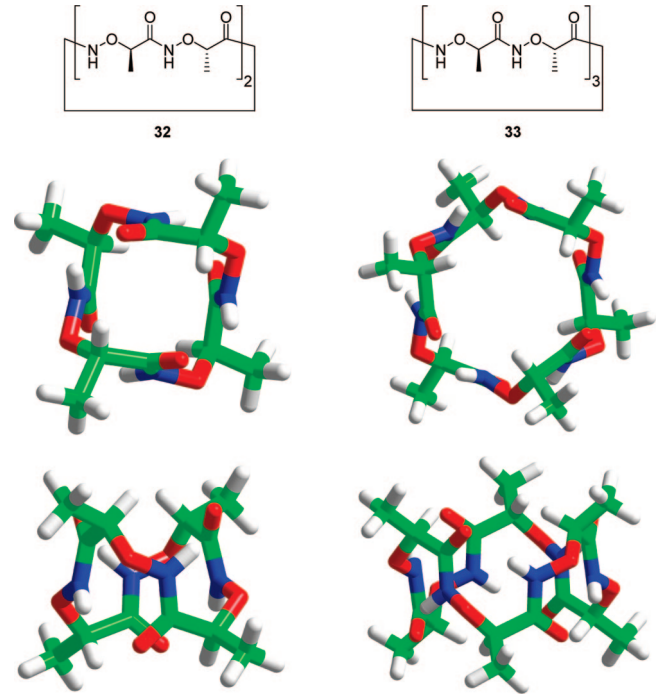


FIGURE 10. Top views and side views of HF/6-31G*-optimized lowest-energy conformations of model cyclic tetrapeptide **32** and cyclic hexapeptide **33**.

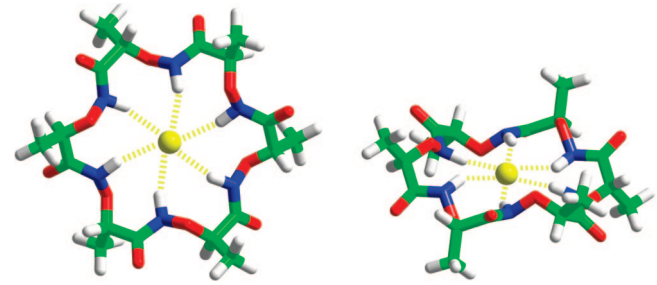
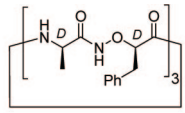


FIGURE 11. Top view and side view of HF/6-31G*-optimized lowest-energy conformation of **33**·Cl^- complex.

To extend the scope of our inquiry beyond peptides composed of only aminoxy acids, we synthesized cyclic hexapeptide **34** comprising alternating D-α-aminoxy acids and D-α-amino acids to further explore the potential of α-aminoxy acids in constructing anion receptors.[59] Conformational studies by both experimental and theoretical methods reveal that **34** adopts a C_3-symmetric conformation with alternate seven-membered ring (γ-turn) and eight-membered ring (N−O turn) hydrogen bonds (Figure 12).[59] Quantitative assessments of the anion-binding affinities of **34** in CD_2Cl_2 suggest that **34** is not only effective in forming a 1:1 complex with anions but also



28

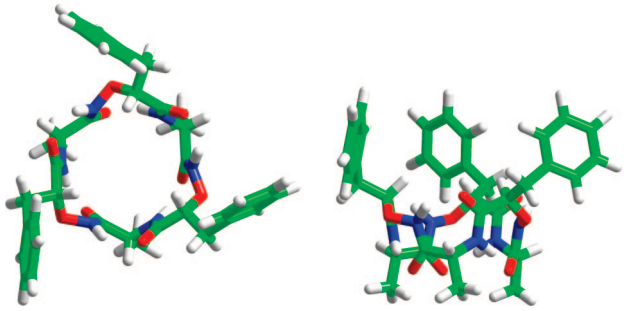


FIGURE 12. Top view and side view of HF/6-31G*-optimized lowest-energy conformation of **34**.

TABLE 1. Association Constants for the Binding of **31**, **34**, and **35** with Anions[a]

anions	**31**[b]	**34**[b]	**35**[c]
Cl^-	11 800	15 000	>100 000
Br^-		910	18 000
I^-		51	1 500
NO_3^-		440	1 100
$H_2PO_4^-$			1 400

[a] Anions were added as their tetrabutylammonium salts. In all cases, 1:1 receptor/anion stoichiometry was observed. Errors were estimated to be no more than ±10%. [b] Determined in CD_2Cl_2. [c] Determined in $CDCl_3$.

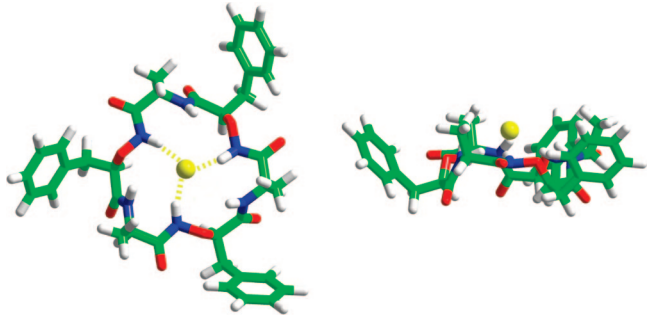


FIGURE 13. Top view and side view of HF/6-31G*-optimized lowest-energy conformation of the cyclic hexapeptide **34**·Cl^- complex.

highly selective for chloride ions (association constant K_a = 15 000 M^{-1} in CD_2Cl_2). Compared with cyclic hexapeptide **31** comprising D,L-α-aminoxy acids, the cyclic hexapeptide **34**, which has fewer aminoxy amide NH units, shows enhanced binding toward various anions while maintaining high selectivity toward the chloride ion (Table 1).

As shown in Figure 13, upon binding with a Cl^- ion, all six of the hydrogen bonds initially present in **34** are disrupted. The backbone of **34** is rearranged into a flat conformation with all of the amide NH hydrogen atoms pointing inward. The Cl^- ion sits above the plane of the peptide backbone and forms strong hydrogen bonds with the three O−NH hydrogen atoms, as indicated by a Cl···H distance of 2.21 Å. While the three regular amide NH groups do not show strong binding with the Cl^- ion (Cl···H distance is 3.00 Å), these hydrogen atoms are positioned close to the backbone O atom of the adjacent N−O unit (O···H distance is 2.13 Å), possibly forming a weak five-membered-ring hydrogen bond.

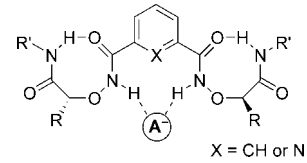
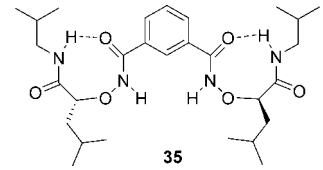


FIGURE 14. The designed acyclic anion receptors and assumed anion-binding pattern (A^-, anions).

Acyclic Anion Receptors. Since low overall yields in the synthesis of cyclic peptides have limited the subsequent studies on their chloride transport properties, we have also developed acyclic anion receptors based on a C_2-symmetric isophthalamide scaffold, featuring two α-aminoxy acid units. Based on comparisons of anion binding abilities between cyclic hexapeptides **31** and **34**, we believe that removing the constraint of original intramolecular hydrogen bonds involving the aminoxy amide NH units can increase the receptors' binding affinities for anions. Therefore, unlike the case of their counterparts in cyclic peptides, the aminoxy amide NH units are not involved in any intramolecular hydrogen bonds in the new designed acyclic receptors, which make them capable of binding to anions directly (Figure 14).

Analyses of anion-binding properties of **35** with respect to different anions suggest that **35** is not only an effective 1:1



35

anion-binding agent in solution but also a selective one that shows a remarkable preference for Cl^- relative to other anions (Br^-, I^-, NO_3^-, and $H_2PO_4^-$) (Table 1).[60] Compared with cyclic peptides **31** and **34**, the less rigid receptor **35** displays impressive strong binding abilities for many anions while maintaining significant selectivity for chloride ions.

In view of the biological and pharmaceutical significance of chiral carboxylates, we have been interested in discovering anion receptors for enantioselective recognition of chiral molecules containing carboxylate groups. We designed C_2-symmetric receptor **36** to gauge the effect of the receptor on enantioselective recognition for chiral carboxylates.[61] We found that **36** bound two enantiomers of mandelate with different binding affinities (association constants for (*R*)-mande-

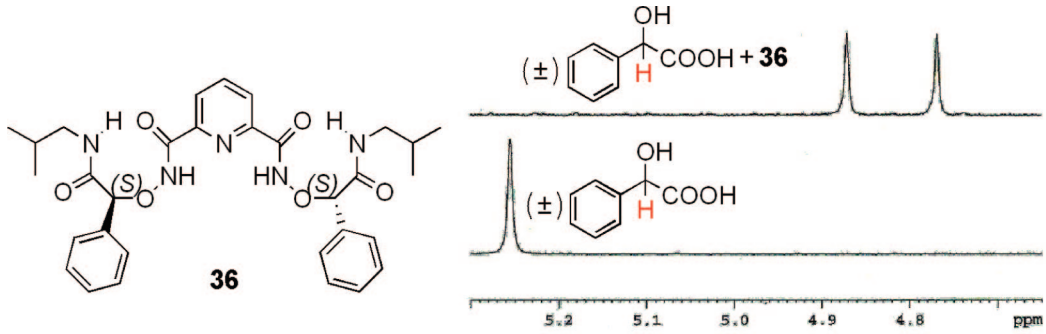


FIGURE 15. The $C_\alpha H$ region of overlaid ^1H NMR spectra of racemic mandelic acid and the 1:1 mixtures of racemic tetrabutylammonium mandelate with receptor **36**.

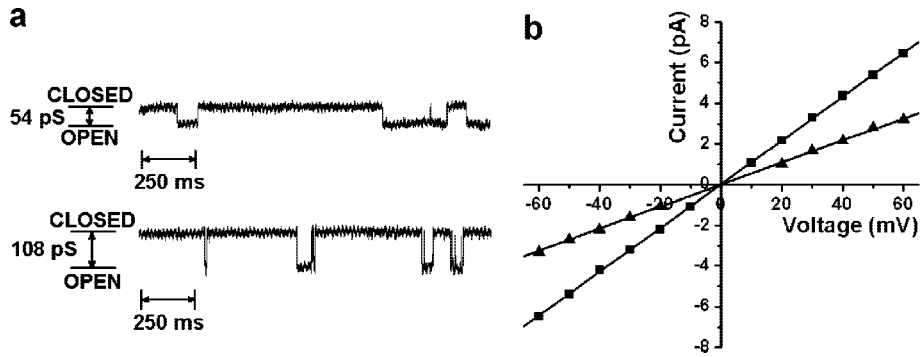


FIGURE 16. (a) Single-channel recording traces obtained at −60 mV with **35** and (b) current−voltage relationships for the channels formed by **35**. The conductances of the channels were 54 (▲) and 108 pS (■), respectively. Reprinted from ref 60. Copyright 2007 American Chemical Society.

late $K_a = 4300\ M^{-1}$ and (*S*)-mandelate $K_a = 8100\ M^{-1}$ in $CDCl_3$). As shown in Figure 15, the addition of 1 equiv of **36** to a racemic mixture of mandelates induced a large enough chemical shift nonequivalence ($\Delta\Delta\delta$) of 0.10 ppm for the methine proton of each enantiomer, resulting in an excellent baseline resolution for accurate integrations of the proton signals. Thus we utilized receptor **36** as a chiral shift reagent for the determination of enantiomeric purities of a broad variety of chiral carboxylic acids by ^1H NMR.

Synthetic Chloride Channel. Impressed by the effectiveness of chloride-selective anion receptor **35**, we moved on to test the ability of receptor **35** in facilitating Cl^- transport across lipid bilayer membranes in liposomes.[60] We prepared liposomes encapsulating sodium nitrate ($NaNO_3$) and suspended them in a sodium chloride (NaCl) bulk solution. We studied the Cl^- influx into liposomes by monitoring the fluorescence intensity of an entrapped Cl^--sensitive indicator, 6-methoxy-*N*-(3-sulfopropyl)quinolinium (SPQ). Addition of receptor **35** to such liposome suspensions induced a rapid drop in SPQ fluorescence level, indicating that **35** did, in fact, transport Cl^- into the liposomes. Single-channel recording by patch-clamp techniques is the most critical test for distinguishing ion channels from other ion transport mechanisms such as ion carriers. The characteristic single-channel currents that we observed for **35** in giant liposomes indicated that **35** can form functional ion channels (Figure 16). Furthermore, results of the SPQ fluorescent assay in Madin−Darby canine kidney (MDCK) cells reveal that **35** partitions into plasma membranes of living cells and therein mediates Cl^- transport by increasing anion permeability. Thus, compound **35** represents the smallest synthetic molecule that can mediate Cl^- transport efficiently in the plasma membranes of living cells.

Conclusions and Outlook

Since our first report in 1996,[21] we have been extensively exploring the rigid secondary structures induced by α-aminoxy acids. As analogs of β-amino acids, α-aminoxy acids have received less attention in the arena of peptidomimetic foldamers. However, by virtue of their rigid, side-chain-independent backbone conformations, α-aminoxy acids have proven their versatility as building blocks in the construction of peptidomimetic foldamers. We have discovered a broad variety of well-defined secondary structures within the family of α-aminoxy acids and established a clear set of folding rules for oligomers of α-aminoxy acids. On the other hand, by taking advantage of the aminoxy amide NH groups as excellent hydrogen bond donors, we have also used α-aminoxy acids as building blocks to construct anion receptors and chan-

nels to mimic anion recognition and transport processes in living systems. We expect applications of α-aminoxy acids in creating functional foldamers capable of binding specifically to various biological targets and transmembrane ion channels that can mediate the flow of specific ion species across cell membranes will continue to drive this work forward. We believe that focused research into these versatile molecular systems will, in time, lead to a broad range of new applications in chemistry, biology, medicine, and materials science.

We thank our co-workers Fei-Fu Ng, Jin Qu, Bing Li, Yu-Hui Zhang, Wei Li, Dan-Wei Zhang, Yu Hao, Ze-Min Dong, Fei Chen, Ke-Sheng Song, K.-W.-K. Chan, De-Ping Wang, Yi Ren, Shi-Wei Luo, Yao Sha, Prof. Xiao-Qiang Yao, and Bing Shen for their enthusiasm and hard work, which made this research program successful. We thank the University of Hong Kong, Hong Kong Research Grants Council (Grants HKU 7117/97P, HKU 7098/01P, HKU 7367/03M, HKU7654/06M, and HKU 2/06C), Fudan University, the National Natural Science Foundation of China (Project No. 20202001), Bristol-Myers Squibb Foundation (Unrestricted Grants in Synthetic Organic Chemistry to D.Y.), Croucher Foundation (Croucher Senior Research Fellowship to Y.D.W. and D.Y.), and Morningside Foundation for providing financial support.

BIOGRAPHICAL INFORMATION

Xiang Li was born in Kunming, China, in 1981. He received his B.Sc. in Chemistry at Fudan University in 2003. He obtained a Ph.D. in 2008 from The University of Hong Kong under the guidance of Professor Dan Yang. He has been working on the construction of anion receptors and synthetic ion channels by using α-aminoxy acids as building blocks.

Yun-Dong Wu received his B.Sc. from Lanzhou University in 1981 and Ph.D. from University of Pittsburgh in 1986. He had a long association with K. N. Houk, both as a graduate student and as a research associate (shortly with Paul. v. R. Schleyer). In 1992, he joined the Hong Kong University of Science & Technology (HKUST) and is now a Chair Professor of Chemistry. His research group is interested in understanding the mechanisms of catalytic reactions, molecular designs with peptides, modeling of protein folding, and protein/protein interactions.

Dan Yang received her B.Sc. in Chemistry from Fudan University in 1985, M.A. from Columbia with Professor Ronald Breslow in 1988, and a Ph.D. from Princeton under the guidance of Professor Daniel Kahne in 1991. She then spent two years as a postdoctoral fellow with Professor Stuart Schreiber at Harvard. In 1993, she joined The University of Hong Kong, where she is currently the Morningside professor in chemical biology and chair professor of chemistry. Her research interests include asymmetric catalysis and total synthesis, biological applications of novel foldamers, and fluorescent sensors for probing cellular processes.

FOOTNOTES

*To whom correspondence should be addressed. E-mail address: chydwu@ust.hk; yangdan@hku.hk.

REFERENCES

1 Babine, R. E.; Bender, S. L. Molecular recognition of protein−ligand complexes: applications to drug design. *Chem. Rev.* **1997**, *97*, 1359–1472.
2 Nakanishi, H.; Kahn, M. Design of Peptidomimetics. In *The Practice of Medicinal Chemistry*, 2nd ed.; Wermuth, C. G.,Ed.; Academic Press: London, U.K., 2003; pp 477−500, and references therein.
3 Gellman, S. H. Foldamers: A manifesto. *Acc. Chem. Res.* **1998**, *31*, 173–180.
4 Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. A field guide to foldamers. *Chem. Rev.* **2001**, *101*, 3893–4011.
5 Goodman, C. M.; Choi, S.; Shandler, S.; DeGrado, W. F. Foldamers as versatile frameworks for the design and evolution of function. *Nat. Chem. Biol.* **2007**, *3*, 252–262.
6 Gademann, K.; Ernst, M.; Hoyer, D.; Seebach, D. Synthesis and biological evaluation of a cyclo-β-tetrapeptide as a somatostatin analogue. *Angew. Chem., Int. Ed.* **1999**, *38*, 1223–1226.
7 Koglin, N.; Zorn, C.; Beumer, R.; Cabrele, C.; Bubert, C.; Sewald, N.; Reiser, O.; Beck-Sickinger, A. G. Analogues of neuropeptide Y containing β-aminocyclopropane carboxylic acids are the shortest linear peptides selective for the Y1-receptor. *Angew. Chem., Int. Ed.* **2003**, *42*, 202–205.
8 Sadowsky, J. D.; Schmitt, M. A.; Lee, H.-S.; Umezawa, N.; Wang, S.; Tomita, Y.; Gellman, S. H. Chimeric (α/β + α)-peptide ligands for the BH3-recognition cleft of Bcl-XL: Critical role of the molecular scaffold in protein surface recognition. *J. Am. Chem. Soc.* **2005**, *127*, 11966–11968.
9 English, E. P.; Chumanov, R. S.; Gellman, S. H.; Compton, T. Rational development of β-peptide inhibitors of human cytomegalovirus entry. *J. Biol. Chem.* **2006**, *281*, 2661–2667.
10 Kritzer, J. A.; Lear, J. D.; Hodsdon, M. E.; Schepartz, A. Helical β-peptide inhibitors of the p53-hDM2 interaction. *J. Am. Chem. Soc.* **2004**, *126*, 9468–9469.
11 Stephens, O. M.; Kim, S.; Welch, B. D.; Hodsdon, M. E.; Kay, M. S.; Schepartz, A. Inhibiting HIV fusion with a β-peptide foldamer. *J. Am. Chem. Soc.* **2005**, *127*, 13126–13127.
12 Gelman, M. A.; Richter, S.; Cao, H.; Umezawa, N.; Gellman, S. H.; Rana, T. M. Selective binding of TAR RNA by a Tat-derived β-peptide. *Org. Lett.* **2003**, *5*, 3563–3565.
13 Choi, S.; Clements, D. J.; Pophristic, V.; Ivanov, I.; Vemparala, S.; Bennett, J. S.; Klein, M. L.; Winkler, J. D.; DeGrado, W. F. The design and evaluation of heparin-binding foldamers. *Angew. Chem., Int. Ed.* **2005**, *44*, 6685–6689.
14 Werder, M.; Hauser, H.; Abele, S.; Seebach, D. β-Peptides as inhibitors of small-intestinal cholesterol and fat absorbtion. *Helv. Chim. Acta* **1999**, *82*, 1774–1783.
15 Hamuro, Y.; Schneider, J. P.; DeGrado, W. F. De novo design of antibacterial β-peptides. *J. Am. Chem. Soc.* **1999**, *121*, 12200–12201.
16 Porter, E. A.; Wang, X.; Lee, H. S.; Weisblum, B.; Gellman, S. H. Antibiotics: Non-haemolytic β-amino-acid oligomers. *Nature* **2000**, *404*, 565.
17 Liu, D.; DeGrado, W. F. De novo design, synthesis, and characterization of antimicrobial β-peptides. *J. Am. Chem. Soc.* **2001**, *123*, 7553–7559.
18 Epand, R. F.; Raguse, T. L.; Gellman, S. H.; Epand, R. M. Antimicrobial 14-helical β-peptides: Potent bilayer disrupting agents. *Biochemistry* **2004**, *43*, 9527–9535.
19 Seurynck, S. L.; Patch, J. A.; Barron, A. E. Simple, helical peptoid analogs of lung surfactant protein B. *Chem. Biol.* **2005**, *12*, 77–88.
20 Li, X.; Yang, D. Peptides of aminoxy acids as foldamers. *Chem. Commun.* **2006**, 3367–3379.
21 Yang, D.; Ng, F.-F.; Li, Z.-J.; Wu, Y.-D.; Chan, K. W. K.; Wang, D.-P. An unusual turn structure in peptides containing α-aminoxy acids. *J. Am. Chem. Soc.* **1996**, *118*, 9794–9795.
22 Wu, Y.-D.; Wang, D.-P.; Chan, K. W. K.; Yang, D. Theoretical study of peptides formed by aminoxy acids. *J. Am. Chem. Soc.* **1999**, *121*, 11189–11196.
23 Yang, D.; Qu, J.; Li, B.; Ng, F.-F.; Wang, X.-C.; Cheung, K.-K.; Wang, D.-P.; Wu, Y.-D. Novel turns and helices in peptides of chiral α-aminoxy acids. *J. Am. Chem. Soc.* **1999**, *121*, 589–590.
24 Yang, D.; Li, B.; Ng, F. F.; Yan, Y.-L.; Qu, J.; Wu, Y.-D. Synthesis and characterization of chiral N−O turns induced by α-aminoxy acids. *J. Org. Chem.* **2001**, *66*, 7303–7312.
25 Gellman, S. H.; Dado, G. P.; Liang, G.-B.; Adams, B. R. Conformation-directing effects of a single intramolecular amide-amide hydrogen bond: Variable-temperature NMR and IR studies on a homologous diamide series. *J. Am. Chem. Soc.* **1991**, *113*, 1164–1173.

26 Copeland, G. T.; Jarvo, E. R.; Miller, S. J. Minimal acylase-like peptides. Conformational control of absolute stereospecificity. *J. Org. Chem.* **1998**, *63*, 6784–6785.

27 Woody R. W. In *Circular Dichroism: Principles and Applications*; Nakanishi, K., Berova, N., Woody, R., Eds. VCH: New York, 1994; pp 473−497.

28 Li, B. Ph. D Thesis, The University of Hong Kong, 2000.

29 Qu, J. Ph. D Thesis, The University of Hong Kong, 2001.

30 Zubay, G. In *Biochemistry*; Addison-Wesley publishing Company Inc.: Reading, MA, 1983; pp 87.

31 Toniolo, C.; Benedetti, E. The polypeptide-3_{10}-helix. *Trends Biochem. Sci.* **1991**, *16*, 350–353.

32 Appella, D. H.; Christianson, L. A.; Klein, D. A.; Powell, D. R.; Huang, X.-L.; Barchi, J. J., Jr.; Gellman, S. H. Residue-based control of helix shape in β-peptide oligomers. *Nature* **1997**, *387*, 381–384.

33 Seebach, D.; Overhand, M.; Kühnle, F. N. M.; Oberer, L.; Martinoni, B. β-Peptides: Synthesis by Arndt−Eistert homologation with concomitant peptide coupling. Structure determination by NMR and CD spectroscopy and by X-ray crystallography. Helical secondary structure of a β-hexapeptide in solution and its stability towards pepsin. *Helv. Chim. Acta* **1996**, *79*, 913–941.

34 Yang, D.; Qu, J.; Li, W.; Wang, D.-P.; Ren, Y.; Wu, Y.-D. A reverse turn structure induced by a D,L-α-aminoxy acid dimer. *J. Am. Chem. Soc.* **2003**, *125*, 14452–14457.

35 Hayen, A.; Schmitt, M. A.; Nagassa, N.; Thomson, K. A.; Gellman, S. H. Two helical conformations from a single foldamer backbone: "Split personality" in short α/β-peptides. *Angew. Chem., Int. Ed.* **2004**, *43*, 505–510.

36 De Pol, S.; Zorn, C.; Klein, C. D.; Zerbe, O.; Reiser, O. Surprisingly stable helical conformations in α/β-peptides by incorporation of cis-β-aminocyclopropane carboxylic acids. *Angew. Chem., Int. Ed.* **2004**, *43*, 511–514.

37 Sharma, G. V. M.; Nagendar, P.; Radha Krishna, P.; Jayaprakash, P.; Ramakrishna, K. V. S.; Kunwar, A. C. 9/11 Mixed helices in α/β peptides derived from C-linked carbo-β-amino acid and L-Ala repeats. *Angew. Chem., Int. Ed.* **2005**, *44*, 5878–5882.

38 Schmitt, M. A.; Weisblum, B.; Gellman, S. H. Unexpected relationships between structure and function in α,β-peptides: Antimicrobial foldamers with heterogeneous backbones. *J. Am. Chem. Soc.* **2004**, *126*, 6848–6849.

39 Seth Horne, W.; Price, J. L.; Keck, J. L.; Gellman, S. H. Helix bundle quaternary structure from α/β-peptide foldamers. *J. Am. Chem. Soc.* **2007**, *129*, 4178–4180.

40 Price, J. L.; Seth Horne, W.; Gellman, S. H. Discrete heterogeneous quaternary structure formed by α/β-peptide foldamers and α-peptides. *J. Am. Chem. Soc.* **2007**, *129*, 6376–6377.

41 Seebach, D.; Jaun, B.; Sebesta, R.; Mathad, R. I.; Flogel, O.; Limbach, M; Sellner, H.; Cottens, S. Synthesis, and helix or hairpin-turn secondary structures of "mixed" α/β-peptides consisting of residues with proteinogenic side chains and of 2-amino-2-methylpropanoic acid (Aib). *Helv. Chim. Acta* **2006**, *89*, 1801–1825.

42 Yang, D.; Li, W.; Qu, J.; Luo, S. W.; Wu, Y. D. A new strategy to induce γ-turns: Peptides composed of alternating α-aminoxy acids and α-amino acids. *J. Am. Chem. Soc.* **2003**, *125*, 13018–13019.

43 Bianchi, A., Bowman-James, K., Garcia-España, E. Eds., *Supramolecular Chemistry of Anions*, Wiley-VCH: New York, 1997. 000

44 Sessler, J. L.; Gale, P. A.; Cho, W. S. *Anion Receptor Chemistry*; Royal Society of Chemistry: Cambridge, U.K., 2006. 000

45 Gale, P. A.; Quesada, R. Anion coordination and anion-templated assembly: Highlights from 2002 to 2004. *Coord. Chem. Rev.* **2006**, *250*, 3219–3244.

46 Gale, P. A. Anion and ion-pair receptor chemistry: Highlights from 2000 and 2001. *Coord. Chem. Rev.* **2003**, *240*, 191–221.

47 Gale, P. A. Anion receptor chemistry: Highlights from 1999. *Coord. Chem. Rev.* **2001**, *213*, 79–128.

48 Gale, P. A. Anion coordination and anion-directed assembly: Highlights from 1997 and 1998. *Coord. Chem. Rev.* **2000**, *199*, 181–233.

49 Schmidtchen, F. P.; Berger, M. Artificial organic host molecules for anions. *Chem. Rev.* **1997**, *97*, 1609–1646.

50 Matile, S.; Som, A.; Sordé, N. Recent synthetic ion channels and pores. *Tetrahedron* **2004**, *60*, 6405–6435.

51 Sisson, A. L.; Shah, M. R.; Bhosale, S.; Matile, S. Synthetic ion channels and pores (2004−2005). *Chem. Soc. Rev.* **2006**, *35*, 1269–1286.

52 Davis, A. P.; Sheppard, D. N.; Smith, B. D. Development of synthetic membrane transporters for anions. *Chem. Soc. Rev.* **2007**, *36*, 348–357.

53 Jentsch, T. J.; Stein, V.; Weinreich, F.; Zdebik, A. A. Molecular structure and physiological function of chloride channels. *Physiol. Rev.* **2002**, *82*, 503–568.

54 Ashcroft, F. M. *Ion Channels and Disease*; Academic Press, San Diego, CA, 2000; p 185.

55 Jentsch, T. J.; Hübner, C. A.; Fuhrmann, J. C. Ion channels: Function unravelled by dysfunction. *Nat. Cell Biol.* **2004**, *6*, 1039–1047.

56 Dutzler, R.; Campbell, E. B.; Cadene, M.; Chait, B. T.; MacKinnon, R. X-ray structure of a ClC chloride channel at 3.0 angstrom reveals the molecular basis of anion selectivity. *Nature* **2002**, *415*, 287–294.

57 Dutzler, R.; Campbell, E. B.; MacKinnon, R. Gating the selectivity filter in ClC chloride channels. *Science* **2003**, *300*, 108–112.

58 Yang, D.; Qu, J.; Li, W.; Zhang, Y.-H.; Ren, Y.; Wang, D.-P.; Wu, Y.-D. Cyclic hexapeptide of D,L-α-aminoxy acids as a selective receptor for chloride ion. *J. Am. Chem. Soc.* **2002**, *124*, 12410–12411.

59 Yang, D.; Li, X.; Sha, Y.; Wu, Y.-D. A cyclic hexapeptide comprising alternating α-aminoxy acids and α-amino acids is a selective chloride ion receptor. *Chem.—Eur. J.* **2005**, *11*, 3005–3009.

60 Li, X.; Shen, B.; Yao, X.-Q.; Yang, D. A small synthetic molecule forms chloride channels to mediate chloride transport across cell membranes. *J. Am. Chem. Soc.* **2007**, *129*, 7264–7265.

61 Yang, D.; Li, X.; Fan, Y.-F.; Zhang, D.-W. An enantioselective carboxylate receptor derived from α-aminoxy acids functions as a chiral shift reagent. *J. Am. Chem. Soc.* **2005**, *127*, 7996–7997.