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From Peptides to Non-Peptide Alpha-Helix Inducers and Mimetics

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Dedicated with respect to the memory of Dr. Darshan Ranganathan

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Helix mimetics are good models for investigating the theories of protein folding and also act as good affinity ligands for therapeutic applications due to their biostability and better blood-brain barrier crossing ability. The helix mimetics display the same spatial and temporal orientation with respect to the amino acid side-chains of alpha helix part of the protein, which are involved in protein–protein interactions. The designed nonpeptidic molecules with substituents at the appropriate positions on the scaffold can mimic the amino acid side-chains of a fully folded alpha helix and can competitively bind with the target protein, thus acting as possible therapeutic agents. This was illustrated with examples like p53/MDM2, CD81/CV E2, Bcl-x_L/Bak, gp-41, and others, with therapeutic relevance.

1. Introduction

Alpha helix was the first-described secondary structure discovered by Linus Pauling in 1951, and this discovery constitutes one of the major breakthroughs in the contemporary science.[1] It is a crucial structural motif in many regulatory proteins and is involved in various vital cellular events such as protein-DNA interactions (helix-turn helix),[2] in addition to its role as dimerization motif (in zipper proteins)[3] and its ubiquitous presence in membrane-bound receptors (G-protein-coupled receptors).[4] But even after more than half a century since its discovery, the helix folding and stabilization still remains an unsolved problem.[5]

About 30% of the amino acids in proteins exist as part of the alpha-helical unit. An ideal alpha helix consists of H-bonds between the i^{th} CO and $(i+4)^{th}$ NH, with 3.6 residues per turn and a pitch of 5.5 Å. A typical right-handed alpha helix falls in the $\phi = -64 \pm 7°$ and $\psi = -41 \pm 7°$ regions of the Ramachandran map (Figure 1).[6] If the peptide chain winds up tightly, an alternate helical structure called 3_{10} helix is formed as a result of the hydrogen bond between i^{th} CO and $(i+3)^{rd}$ NH with $\phi = -49$ and $\psi = -26$.[7] If the peptide chain winds up less tightly by H-bond formation between i^{th} CO and $(i+5)^{th}$ NH, the result is a π helix ($\phi = -55$; $\psi = -70$), which is rare (Figure 1).[8] The contiguous arrays of H-bonds in the alpha helix give rise to a rigid rod-like structure. The right-handed helices are favored over left-handed ones in natural system due to the stereochemical nature of the amino acid monomers.[9]

Alpha helix plays an integral role in maintaining the structure and shape of many structural proteins.[10] Keratin and collagen are structural proteins that adopt alpha-helical and polyproline type II helical structures,[11] and they owe their characteristic properties to their supramolecular organization of helical units (Figure 1). In keratin, the helix has 3.5 residues per turn and it winds around to form a coiled-coil structure, which is responsible for its strong tensile strength.[12] Collagen is composed of three polypeptide

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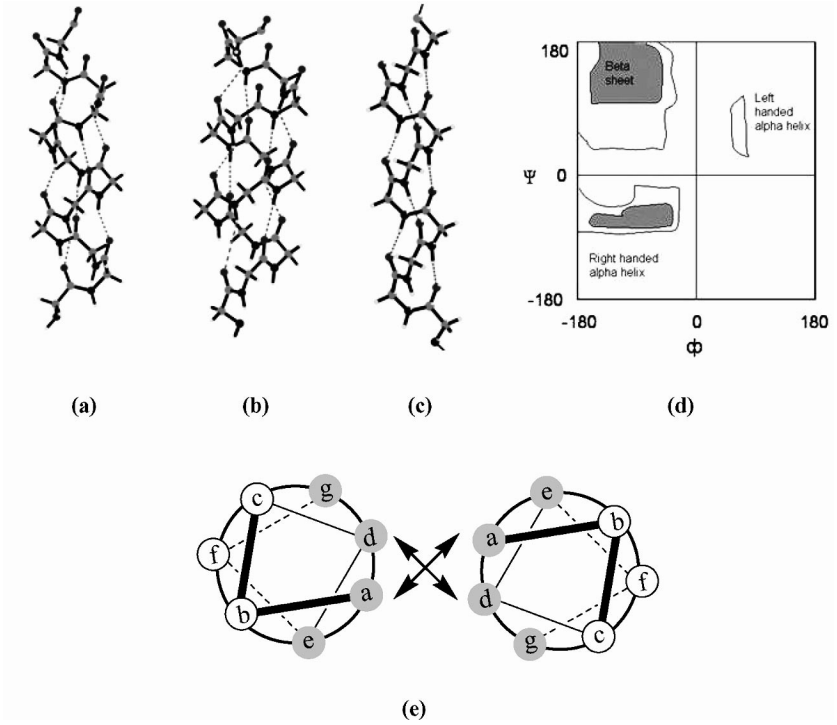


Figure 1. Ball-and-stick representation of (a) alpha helix (b) π-helix (c) 3_{10} helix (d) Ramachandran diagram (e) helical-wheel diagram of a typical coiled-coil.

chains and consists of repeating triplets of sequence Gly-X-Y, of more than 1000 residues. Here X is Pro and Y is hydroxyproline. X-ray crystal structure analysis showed that the three parallel, left-handed helical polypeptide chains in collagen wind around each other to form a right-handed triple-helical super structure.[13] Collagen thus owes its tensile strength to this well packed super-helical structure.

The structural and functional role of alpha helix is primarily due to its overall topology, which in turn, is dictated by its primary structure. The intricacies of this structure-sequence (protein folding) continue to intrigue scientists.[14] This regular helical secondary structure, with repetitive H-bonds and the side-chains lying outside the helical axis, its right-handed nature and biological relevance have triggered profound interest in studying protein folding and design.[15]

1.1 The Need to Have an Alpha Helix Mimetic

Peptide design is a fertile topic for bioorganic and synthetic chemists who wish to obtain a general theme for the folding pattern of biomacromolecules.[16] A general method for studying a stable and isolated alpha helix would constitute a useful tool for the investigation of the theories about the formation and stability of alpha helices.[17] Designing stable helical peptides with less than 15 residues is very difficult because of the lack of stability of the helical conformation over random coil conformation.[18] Attempts to design minimal and simple model systems for an alpha helix are essential not only to study and understand the intrinsic folding preferences but also to design new structural variants of helical conformation, which will enhance our ability to address problems in biology and medicine.[19] The synthesis of short and stable helical molecules employed a wide variety of methods such as the use of helix-capping templates, incorporation of unnatural amino acids, use of side-chain linkers and variety of non-peptide-based scaffold, metal assisted stabilization of helix, and additives that induce or stabilize the alpha-helical conformation. Different strategies that aim to induce the alpha-helical conformation are an essential part of bioorganic and medicinal chemistry research.[20] The central theme of this field of research is to design molecules based on peptidic, nonpeptidic or hybrid type backbone that can mimic the overall helical topology of an alpha helix, with its substituents mimicking the amino acid side-chains present in an ideal alpha helix.

Alpha helix has a functional role in many bioactive peptides, including peptide hormones. Kaizer and Kezdy have shown that the amphiphilic α-helix conformation of peptides is often more important than their specific amino acid sequences in determining the function.[21]

1.2 General Classification of Alpha Helix Mimetics

The mimetics in general can be classified in to three classes namely Type I, II and III.[22] Type I mimetics mimic the local topography of the structural motif; for example, peptide backbone mimetics such as an amide bond isoster. The analogy is applicable at the atomic level. Type II mimetics are the functional mimetics, which are generally small nonpeptide molecules that bind to the corresponding receptors. These functional mimetics need not mimic the structure of the original motifs. Type III mimetics are, in

general, nonpeptide scaffolds that serve as topographical mimetics, with their substituents mimicking the side-chains of important amino acid residues.

In this review, a classification on the basis of chemical nature of the alpha helix mimetic has been adopted and discussed.

1.3 Thermodynamic Basis of Helix Formation

Anfinsen showed that the information needed to achieve the ordered form of protein is encoded in its primary sequence.[23] Deciphering the molecular mechanism responsible for the self-assembly resulting in the folded structure is an important question in biology. The alpha helix, being the first secondary structure discovered and the most prevalent structural motif, has been extensively investigated for its thermodynamic properties of helix formation. The folding ⇌ unfolding, process involves the interplay of a large number of non-covalent interactions and is as yet an unsolved puzzle.[24] In the current view, the protein folding is funneled towards a free energy minimum conformation along a continuum of all possible trajectories.[25] The small difference (5–15 kcal/mol) in free energy between the folded and the unfolded states drives the equilibrium towards the formation of the folded structure. The contribution to this free energy is the result of balance between enthalpic and entropic factors.

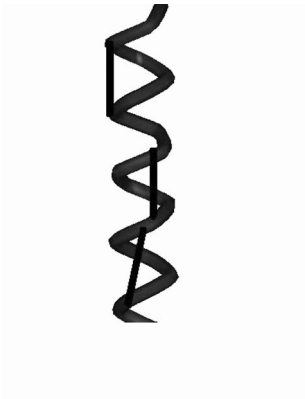
In one of the accepted views, helix formation is considered to involve two steps; nucleation and propagation. Nucleation is the process of forming the initial hydrogen bond that facilitates the folding.[26] Although nucleation can occur randomly at multiple locations along the peptide backbone, but is energetically unfavorable because it involves substantial decrease in entropy. Turns have been proposed as a nucleation point and promote the formation of neighboring interactions.[27] Once the helix has been nucleated, the entropy of fixing the side-chains is offset by the energetically favorable hydrogen bonds. The complex nature of thermodynamics involved in helix folding necessitates the development of simple chemical model systems. These designed model systems will be invaluable tools to unravel various intrinsic and extrinsic forces responsible for the folding.

2. Peptide-Based Alpha-Helix Mimetics

2.1 Alpha-Helix Stabilization by Natural and Nonnatural Amino Acids

Different amino acid residues exhibit varied preferences for different secondary structures.[28] This finding has kick-started a considerable amount of efforts directed toward the experimental and theoretical determination of the preference of individual amino acids for helix formation and stabilization.[29] Peptide design at the level of monomers (amino acids) to induce a helical structure has been investigated by Kallenbach and colleagues.[30] Specific interactions between neighboring side-chains (ion pairing or hydrophobic) might modulate the intrinsic propensities of various amino acids to stabilize or destabilize the helical structure. Replacement of alanine residues with higher linear homologs (larger alkyl substitution), for example, the substitution of a ($-CH_3$) to ($-CH_2-CH_3$) or ($-CH_2-CH_2-CH_3$) groups, increased the helical character. The *tert*-butyl group considerably destabilizes the helical conformation.[31]

Placing oppositely charged amino acid residues at appropriate positions across a helix is another alternative that can be used to nucleate and stabilize the alpha-helical conformation. For example, placing glutamic acid (Glu) at the i^{th} and lysine at $(i+4)^{th}$ positions enhances the helicity of the peptides **1** and **2** due to ion pairs or salt bridges.[32] Figure 2 illustrates the stabilization of the helix by oppositely charged side-chains.



1. Ac-A**E**AAA**KE**AAA**KE**AAA**K**A-NH_2

2. Ac-A**K**AAA**EK**AAA**EK**AAA**E**A-NH_2

Figure 2. Cartoon representation of a typical alpha helix stabilized by Glu and Lys residues at (*i, i+4*) positions.

The α,α-disubstituted amino acids (ααAAs) are extensively employed in the design of 3_{10} helical structures.[33] The ααAAs favor helical conformation because of the restricted ϕ and ψ values. The α-aminoisobutyric acid (Aib) unit was used in the *de novo* design of peptide 3_{10} helices.[34] Interestingly, there are designed ααAA-based peptides that can change conformation from the α- to the 3_{10} helix as a function of solvent polarity.[35]

Amino acids containing a C=C bond in the α, β position is called dehydroamino acids. Because of the planarity, due to the double bond, the peptide chain adopts a peculiar conformational preference. The introduction of these residues into the peptides induced 3_{10} helical structures.[36] The folding pattern depends on the specific sequence and position of dehydro residues.[37]

2.2 Helix Capping Strategy

The design of templates that can induce specific conformation, when incorporated at specific positions, has immense significance in bioorganic chemistry. Synthetic templating moieties are alternatives for generating stable helical structures with defined side-chain orientations.

Peptides with significant helical character show considerable fraying at the termini of the helix because the internal hydrogen bonding and the side-chain interactions are insufficient to overcome the penalty of conformational restriction.[38] At the ends of a helix, the first four NH donors and the last four CO acceptors remain unsatisfied. Amino acid residue at the helix terminus with polar side-chain can stabilize the alpha helix by hydrogen bond involving side-chain and peptide backbone (Figure 3).[39] Most often, hydrophobic interaction is also associated with hydrogen-bonding stabilization and is commonly called "helix capping".[40] The terminal helix capping inhibits the progressive fraying of the end of helix and also prevents alpha helix propagation.[41] Capping of the peptide by the side-chain of an amino acid within the helix is common at the helix N-terminus, where as capping by a peptide-backbone donor is common at the C-terminus.[42]

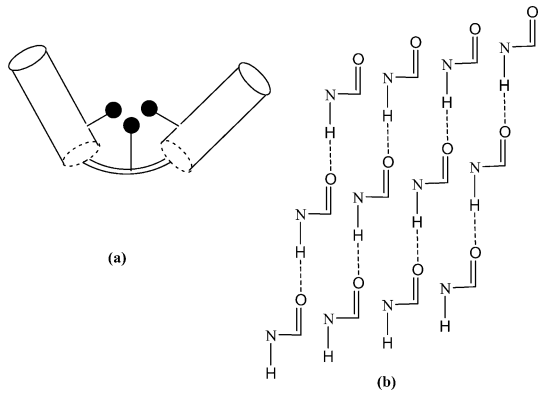
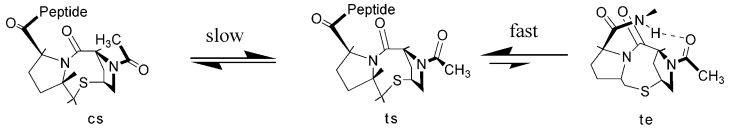


Figure 3. (a) Helix capping by hydrophobic interactions; (b) representation of helix termini, wherein four NHs and four CO groups remain free.

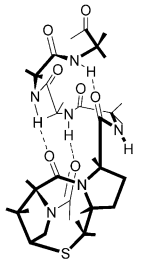
The helix stabilizing effect of particular amino acids and solvent systems have been well documented, but the fundamental questions regarding helix folding and stabilization remain unsolved and controversial. The use of rigid molecules as templates by fixing the amino acid in an orientation suitable for helix initiation is one of the ways to circumvent the entropically unfavourable nucleation step. A major hurdle in the template designing is the need to align hydrogen-bond donor/acceptor in the right orientation for hydrogen bonding. These alignments generate unfavorable dipole moments and thus destabilize the required conformation of the template.

The helix nucleating template designed by Kemp and coworkers is a conformationally rigid molecule capable of enforcing a single conformation on a linked peptide which is conformationally inhomogeneous. The conformational template **3** can be incorporated at the N-terminus which can nucleate and propagate the alpha-helical character. Such templated helices are frayed unidirectionally, unlike natural helices which are frayed bidirectionally. These unique features of the templated helices are useful to study the helix nucleation and thus templated helices act as good model systems.[43] The conformational template **3** provides three amide carbonyls that can assume helical pitch and spacing. The template **3** assumes helical angles due to the constraints imposed by proline and the -SCH_2 group that bridges the proline (Figure 4). This constrained derivative **3** can be linked by an amide bond to the N-terminus of a normal peptide to form the peptide conjugate (Figure 4). Template **3** undergoes two types of internal molecular motion as shown by NMR spectroscopy.[44] Rotation about the CO–N bond of the CH_3–CO–N group occurs slowly on the NMR time scale, generating "t" (with a potentially nucleating amide carbonyl) and "c" (with a non-nucleating amide carbonyl) states. In either population, rapid equilibration occurs between "s" (sulfur down) and "e" (sulfur up) conformations (Figure 4). The "te" conformation permits efficient helix nucleation and the "ce" state is intrinsically less stable. The $^3J_{HN}$ values of these conjugates of **3** are in the range of 5–6 Hz, reminiscent of helical conformation.



c (with a potentially non-nucleating amide carbonyl)
t (with a potentially nucleating amide carbonyl)
s (with 'sulfur up' conformation)
e (with 'sulfur down' conformation)

(a)



(b)

Figure 4. (a) Kemp's template in equilibrium with various conformations; (b) Kemp's template appended at N-terminus of a peptide.[44]

Strong ROE between $d_{NN}(i, i+1)$ and weak $d_{\alpha N}(i, i+1)$ are also characteristics of developing helical character.

The lack of rigidity decreased the efficiency of the template **3** as an alpha helix initiator. To circumvent this limitation, Kemp and co-workers have studied more rigid triproline templates **4**.[45] The triproline-based macrocycle adopts "ctt" (*cis trans trans*) and "cct" (*cis cis trans*) rotamers in aqueous solution and nucleates the alpha-helical conformation. The three amide carbonyls of the bis proline template **3** generate a hybrid 3_{10}-alpha helix at the initial hydrogen bonding site. The four amide carbonyl groups of the triproline template **4** enforce an alpha-helical conformation. Based on the molecular modeling results, an S–CH_2 bridge was introduced into template **4** to restrict the conformation ideal for helix nucleation. The designed template **5** efficiently nucleates helical conformation.

Gani et al. have reported a triproline-based macrocyclic helix cap **6**, which is similar in structure to Kemp's triproline scaffold.[46] Kemp's triproline template adopts a "ctt" conformation rather than the more helix-inducing "ttt" conformation. Gani et al. have, however, modified Kemp's template by transforming the methylene to a chiral carbon with R stereochemistry, thereby forcing the macrocycle **6** to adopt a "ttt" state that is more favorable for helix nucleation (Figure 5). The success of this strategy has been proved by a favorable cyclization of the corresponding acyclic precursor. Detailed experimental, crystallographic, and computational analyses have shown that the template favored the "ttt" state by disfavoring all other rotameric forms. This macrocyclic unit, when incorporated into the nonhelix-forming peptides propagates a helical structure.

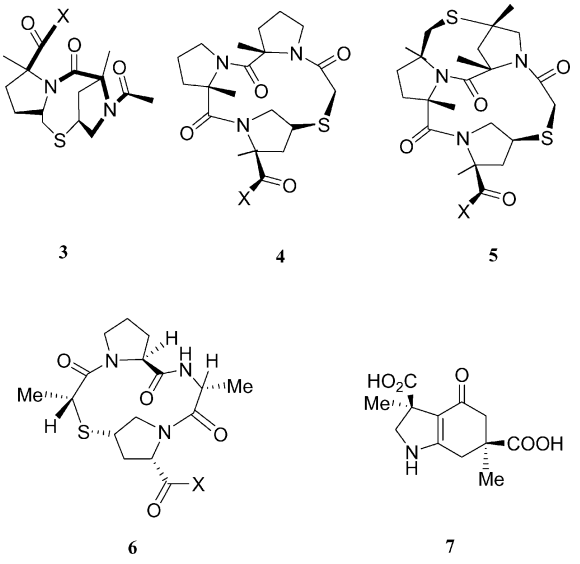


Figure 5. Various templates designed by Kemp et al.[43–45] and other groups (a) bisproline (b) triproline (c) bridged-triproline scaffold (d) Gani's bisproline template[46] (e) Bartlett's helix nucleating agelet.[47]

Bartlett et al. have reported the use of the 4-oxo-hexahydroindole-3,6-dicarboxylic acid **7** and its derivative as helix templates, when appended to the N-terminus of a peptide chain (Figure 5).[47] The positions of the carboxylate at C-6, vinylogous amide, and the carbonyl group at C-3 have been designed to mimic three successive peptide carbonyl groups in the alpha-helical conformation, and the negative charge was expected to complement the positive helix dipole. The methyl derivative at C-3 and C-6, has been particularly chosen to keep the COOH at the pseudoaxial orientation and to disfavor the formation of undesired torsional conformers. Interestingly, the amide-linked conjugate favors the 3_{10} helical conformation and the ester linked conjugate favors the alpha-helical conformation.

2.3 Helix Stabilization by Cross Linking

The induction of helicity by covalently cross linking the side-chains of otherwise unfolded peptide is an attractive alternative for generating short and stable alpha-helical structures.[48] In one approach, Taylor et al. designed peptides, with a Lys occupying the i^{th} position and an aspartic acid (Asp) or Glu at the $(i+4)^{th}$ position.[49] The single or multiple lactam bridges formed by the side-chains of (Lys^i, Asp^{i+4}) or (Lys^i, Glu^{i+4}) generate single or multiple lactam-bridged cyclic peptides (Figure 6). Conformational studies involving circular dichroism (CD) and NMR supported the enhanced helical character of the lactam-bridged cyclic peptide **8** compared to its acyclic counterpart.

In a slightly different way, Taylor et al. employed a variety of bridging spacers for stabilizing the alpha-helical conformation.[50] A peptide, with a ΔG value close to zero for the coil-helix equilibrium, was chosen for their study, because it enabled the monitoring of the influence of bridging spacers on helix induction. The 2,3-diaminopropionic acid (Dap) at i^{th} and Asp at $(i+7)^{th}$ positions were connected by condensing with 4-(aminomethyl)phenylacetic acid (AMPA) as the spacer unit (Figure 6). The cyclic peptide **9** thus obtained by linking Dap and Asp showed a pronounced alpha-helical character.[51] The authors showed that one Dap-(AMPA)-Asp bridge is effective for helix stabilization as the two Lys (i), Asp (i+4) bridges.

Phelan and co-workers used an amide-based tether to link i and i+7 residues for inducing alpha helicity in small peptides.[52] Glutamine residues were placed at i and i+7 positions of any arbitrary sequence and was tethered with alkanediyl chain between the side chain nitrogen atoms. This approach allowed complete sequence variability in all residues except the i and i+7 residues. Conformational analysis showed that the constarined peptide **10** exhibited ca. 100% helical character in aqueous medium at 25 °C.

Fairlie and colleagues synthesized a number of cyclic pentapeptides linking the i^{th} and $(i+4)^{th}$ residues with different spacers.[53] The study found that the 20-membered macrocycle **11** formed through K(i)–D(i+4) linkage is ideal and results in maximum helicity, whereas the corresponding acyclic peptide exists in a random coil conformation. On the basis of the conformational study on various control compounds, the ring size and the orientation of i, i+4 side-chains have been found to be crucial in stabilizing the helical conformation. These pentapeptides are the smallest peptides known to exist in helical conformation in water.

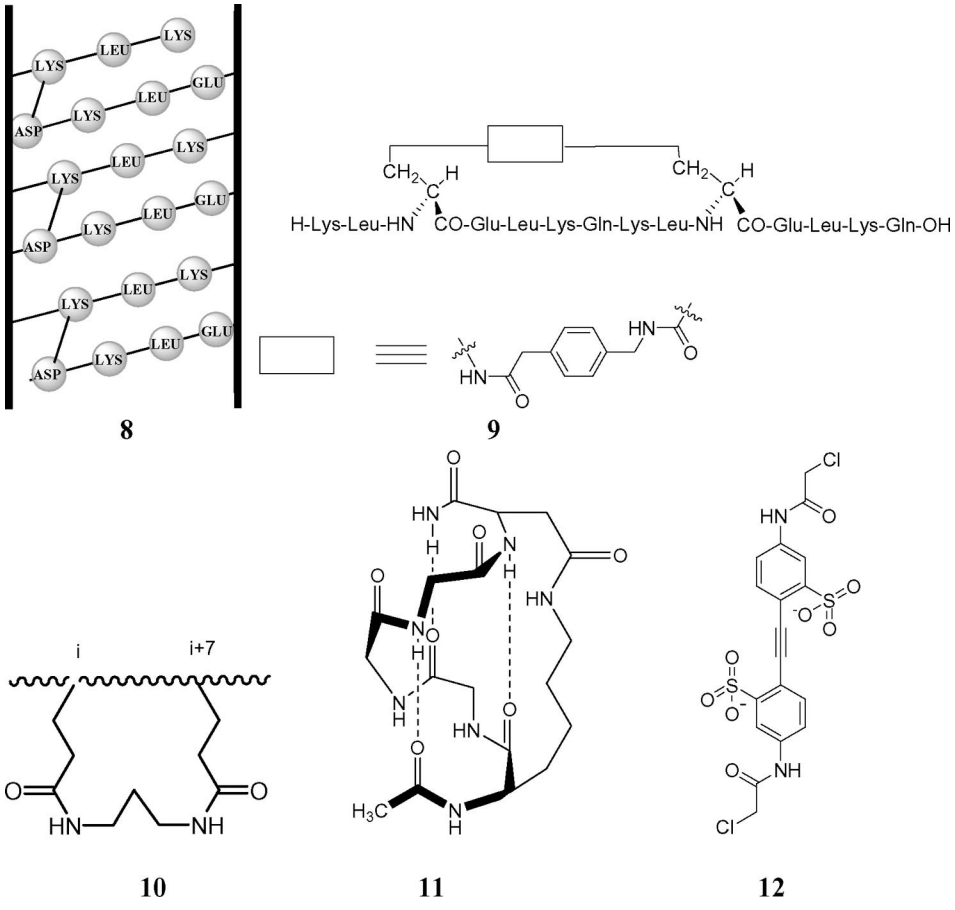


Figure 6. (a) Helical net diagram representing peptides with linking side chains at (*i, i+4*) position; (b) smallest helical peptide designed by Fairlie and co-workers;[53] (c) Woolley's rigid spacer for helix stabilization;[54] (d) helical stabilization by bridging residues at (*i, i+7*) positions; (e) lactam-based cyclic helical peptides.

Woolley et al. recently reported a long rigid crosslinker 3,3′-ethyne-1,2-diylbis{6-[(chloroacetyl)amino]benzenesulfonic acid} (**12**), for use in peptides and proteins.[54] A helical peptide with Cys at the i^{th} and $(i+11)^{th}$ positions can be efficiently cross-linked with the linker **12** (Figure 6). The cross-linked peptide showed a higher helical character than the uncrosslinked peptide as observed from the detailed CD spectral studies. The linker **12** was incorporated into a small β-sheet protein (FynSH3) and was shown to increase the melting temperature (Tm) by ca. 10 °C. This finding indicates that the linker enhances the conformational stability of the protein.

Schultz et al. reported a short alpha-helical peptide stabilized by an intramolecular disulfide bond bridging the i^{th} and $(i+7)^{th}$ residues.[55] The D and L forms of the amino acid **13** are incorporated at i^{th} and $(i+7)^{th}$ positions of the peptide (Figure 7). The intramolecular disulfide bond formation led to an increased helicity as was evident from the θ_{222} value in the CD spectrum. The disulfide peptide was reduced to give the dithiol peptide, its helicity being lower than that of the disulfide. This study thus demonstrates the use of intramolecular disulfide bond in stabilizing the helical conformation of short peptides.

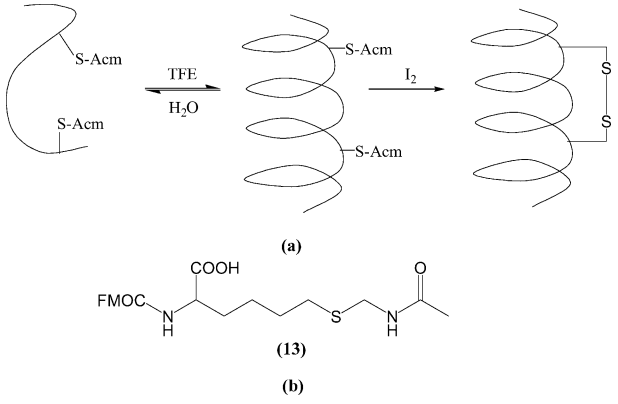


Figure 7. (a) Helix stabilization by disulfide linkage; (b) The structure of amino acid used to form the disulfide linkage.

2.4 Helix Stabilization via Olefin Metathesis

Grubbs et al. used the ring closing metathesis (RCM) reaction to synthesize a series of macrocyclic structures.[56] The hydrophobic peptide Boc-Val-Ala-Leu-Aib-Val-Ala-Leu-OMe, with alanine placed at the i^{th} and $(i+4)^{th}$ posi-

tions was chosen as the model system. Alanine units were replaced with serine and modified to carry the alkene unit. Metathesis reaction, followed by hydrogenation, generated the desired macrocyclic structures (Figure 8). The yield from the metathesis reaction was good, indicating that the olefinic units were in close proximity. These macrocyclic structures did not show any appreciable difference in their helical character. Verdine et al. showed that the versatility of such metathesis reactions by incorporating amino acids **14a** and **14b** with olefinic side-chains on different positions in the helix *i*, (*i*+4) and *i*, (*i*+7).[57] Such peptides, with different configuration of the synthetic amino acids and varying side-chain lengths, showed different reactivity towards metathesis due to preorganization. These cyclized peptides were analyzed by CD spectroscopy, and the results showed that the peptides with synthetic amino acids at the i^{th} position with an *R*-configuration, and the $(i+7)^{th}$ position with an *S* configuration having 10 methylene groups in the ring, showed enhanced helical character. Overall, these studies show that the incorporation of the crosslinking unit renders the peptide resistant to trypsin digestion by a factor of 41.

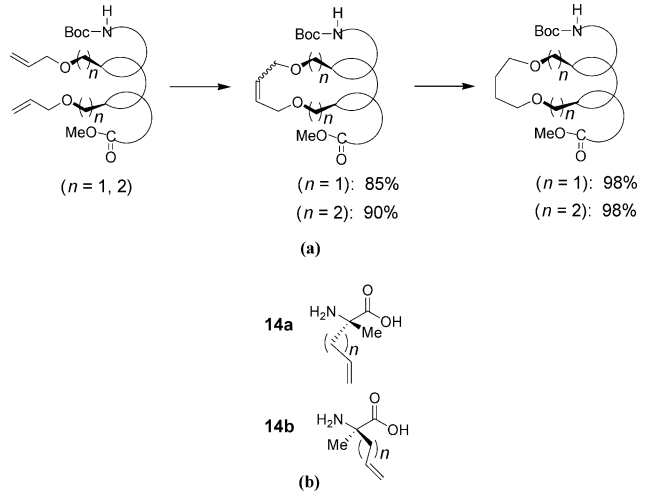


Figure 8. (a) Helix stabilization by metathesis reaction; (b) designed amino acids by Verdine et al. for the metathesis reaction to stabilize alpha helix.[57]

2.5 Covalent Hydrogen Bond Mimetics

The alpha helix is defined by a pattern of sequential (*i*+4→*i*) H-bonds. Generally, hydrogen bonds alone are weak and inadequate for stabilizing short peptides in to a well defined conformation. Hence, designing peptides that adopt stable conformations similar to those of their cognate sequences in proteins is an important challenge for bioorganic chemists.

Replacement of putative hydrogen bonds in alpha helix with a covalent mimic and thus constraining the peptide to adopt an alpha-helical conformation was envisioned and developed by Satterthwait et al.[58] The hydrazone link (N–N=CH–CH_2–CH_2) was designed to replace the putative hydrogen bond [NH···O=C(R)NH] that forms between the main-chain amide proton of an upstream amino acid and the main chain carbonyl oxygen of a downstream amino acid (*i*+4→*i*) (Figure 9). The hydrogen bond in the peptide was replaced by the N=CH and the associated peptide linkage was replaced with an ethylene unit. Replacement of (*i*+4→*i*) hydrogen bond with a hydrazone link yielded a 13-membered hydrogen-bonded helical turn of the alpha helix. Space filling CPK models suggest that the peptides with hydazone linkage mimic the alpha helix (Figure 9). Although, neither the H-bond nor the angles were precisely mimicked by the double bond (N=CH), the flexibility of

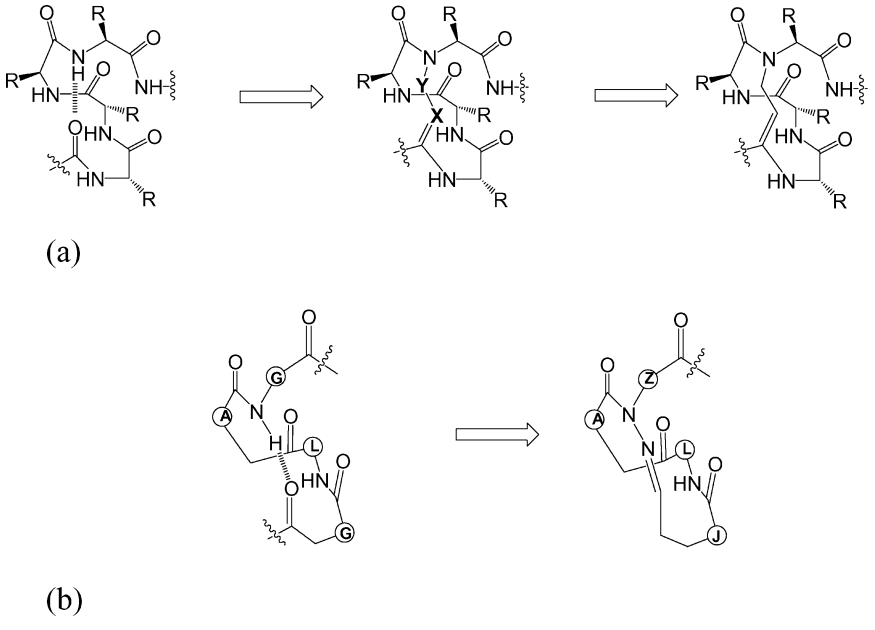


Figure 9. HBS approach for stabilizing alpha helix; (a) peptide stabilized by using HBS approach; (b) Satterthwait et al. used hydrazone as the hydrogen bond mimic L = Leu, A = Ala, J = CH_2, Z = CH_2.[58]

the hydrazone link can induce the conformation favoring the helix. The replacement of (i+4→i) hydrogen bond with the hydrazone linkage at the N-terminus of acetyl-GLA-GAEAAKA-NH_2 to give [JLAZ]AEAAKA-NH_2 converted the peptide it to a full-length alpha helix in water at ambient temperature, as indicated by detailed NMR studies. Further, substituting L-alanine with proline [JLPZ]-AEAAKA-NH_2 enhanced the helicity of the peptide.

Inspired by the work of Satterthwait, Arora and co-workers used a hydrogen-bond surrogate (HBS) approach, wherein a covalent bond of the type C=X–Y–N is used to mimic the C=O···H–N hydrogen bond, where X and Y are parts of the i and (i+4) residues, respectively (Figure 9).[59] In this approach, the hydrogen bond is replaced with a carbon–carbon bond derived from a RCM reaction. The advantage of HBS approach using RCM is that it maintains all the side-chains untouched, which are therefore available for molecular recognition and does not place any steric encumbrances on the helix surface.

2.6 β-Turn as α-Helix Mimetic

Robinson and co-workers designed and synthesized a series of β-turn cyclic peptides that mimic the α-helix. The hairpin helix mimetic **15** consists of an eight-residue loop preorganized into a β-hairpin by using a D-Pro-L-Pro dipeptide template.[60] The sequence D-Pro-L-Pro acts as a template to stabilize the β-hairpin conformation. The hairpin cyclic peptides incorporate Phe1, Trp3 and Leu4, so that they mimic the critical residues Phe19, Trp23 and Leu26 of p53, as is evident from molecular modeling studies (Figure 10). These β-turn cyclic peptides bind strongly (in the μm range) to human double minute 2 (HDM2) protein as observed from BIAcore and NMR studies.

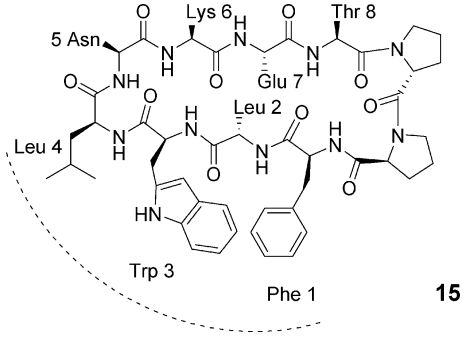


Figure 10. β-turn peptide mimicking the alpha-helical p53 peptide. The marked residue Leu4, Trp3, Phe1 correspond to Leu(26), Trp (23) and Phe (19) of p53 unit.

2.7 Metal Clipping

Ghadiri et al. used an elegant approach to stabilize alpha-helical peptides.[61] Peptides with histidine and cysteine residues at the i^{th} and (i+4)th positions could interact with transition-metal ions to form a bidentate metal complex and thus fix the peptide in an alpha-helical conformation (Figure 11). Two peptides were synthesized, one with Cys and His at the i^{th} and (i+4)th positions, respectively **16**, and another with His residues at both the i^{th} and (i+4)th positions **17**. Histidine was placed near the C-terminal to enhance the charge-dipole interactions. Peptide **16** was 54% helical and was ca. 90% helical in the presence of cadmium ions. Peptide **17**, with a pair of His residues, showed ca. 90% helicity in the presence of Cu^{+2}. The metal-ion selectivity was determined by the affinity of the metal ions toward the ligands and the coordination geometry.

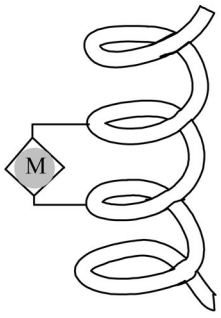
Fairlie and colleagues designed peptides with modified histidine residues (methylated histidine) at the i^{th} and (i+4)th positions.[62] These peptides showed random coil structure as observed from the NMR and CD spectral studies. On treatment with [Pd(en)(NO_3)$_2$] in the appropriate ratio, these peptides generated Pd complexes like structure **18**. Detailed NMR studies of theses complexes in solvents such as water and DMF gave convincing evidences of their helical structures (Figure 11). The coupling constants $<$ 6 Hz and dδ/dT value of $<$ 3 ppb/K, and the characteristic NOE peaks d_{NN} i, i+1, $d_{\alpha N}$ i, i+1 are compelling evidences in support of the helical conformation in solution. The CD spectral studies of these complexes showed a characteristic spectrum, with absorption at 202 and 222 nm, which are characteristic of alpha helix. This strategy is thus useful in generating short peptides in an alpha-helical conformation, which is otherwise improbable.

3. Nonpeptide-Based Helix Mimetics

One of the main goals of protein design is the development of minimalistic molecules to emulate the structure and functions of complex protein folds.[63] The ubiquitous nature of the alpha-helical structure in protein–protein interaction networks stimulated more interest in nonpeptide helix mimicry. The nonpeptide-based helical systems can be designed to disrupt the protein-protein interactions (PPIs) and thus act as an important starting point for the development of therapeutic agents.[64] Various organic structures such as biphenyl, polycyclic ethers, indanes and imidazole-based systems, have been exploited for the designing of the helix mimetics (Figure 12) because these mimetics are small in size, maintain solubility, and are devoid of some of the shortcomings of conventional peptides.

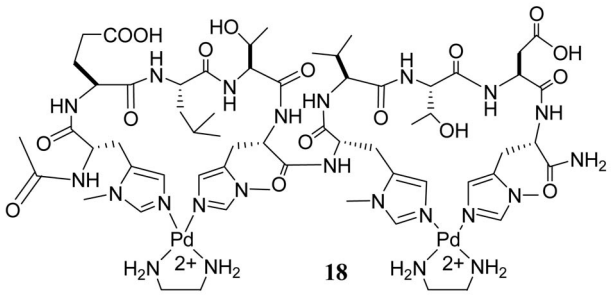
3.1 Marine Natural Products as Helix Mimetic

Polycyclic ether marine toxins such as brevetoxins and ciguatoxins exert their function by binding to an alpha subunit of voltage-sensitive sodium channels.[65] The mode of action of these ladder-like toxins is assumed to be the topological similarity of these *trans*-fused cyclic ethers to the alpha-helical peptide conformation. The length and the relatively straight conformation of the cyclic ether skeleton are critical for the binding and modulation of voltage sensitive sodium channel. The interaction between the polycyclic ethers and the receptor site is believed to be due to the hydrophobic interaction in addition to the hydrogen



Ac-Ala-Glu-Ala-Ala-Ala-Lys-Glu-Ala-Ala-Ala-Lys-**X1**-Ala-Ala-Ala-**X2**-Ala-NH_2

16: **X1** = Cys; **X2** = His
17: **X1** = **X2** = His **(a)**



(b)

Figure 11. (a) Stabilization of alpha-helical conformation by metal ions; (b) helix stabilization by Pd complex formation.

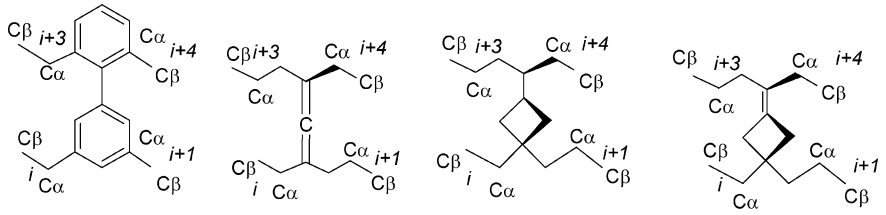


Figure 12. Molecules with helical twist (a) biphenyl (b) allene (c) cycloalkane (d) alkylidinecycloalkane.

bonding, with the ether oxygen (Figure 13). In addition to this, axially oriented CH groups adjacent to the oxygen atom in the skeleton might also play an integral role by way of CH-π interactions with aromatic amino acid residues. The distance between oxygen atom in the cyclic ether in brevetoxin is identical to the distance between the side-chains of the i^{th}, $(i+4)^{th}$ positions of the alpha helix. This led to the design and synthesis of polyether ring systems like **19** as helix mimetics.[66] The designed alpha helix-mimetics with two equatorial hydroxy groups separated by a distance of 4.8 Å is analogous to the ridges formed by the *i*, *i*+4 side-chains of alpha helix in the *i*, *i*+4 relationship.

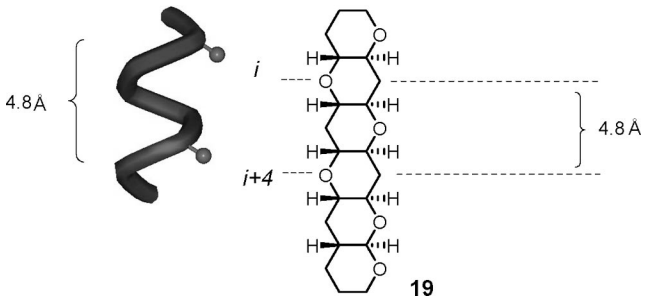
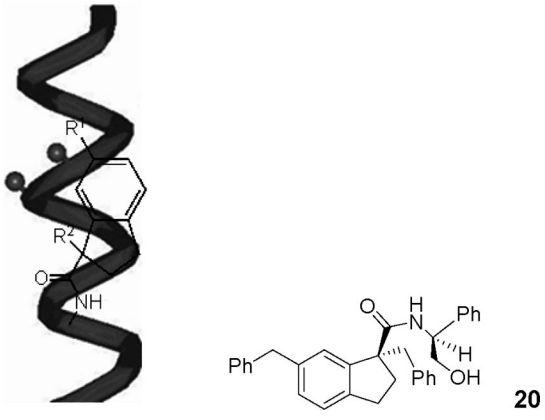


Figure 13. Cyclic polyether compound as alpha helix mimetics.

3.2 The Indane Framework as Alpha-Helix Mimetic

The amino acid side-chains of alpha helices appear to be important in the molecular recognition between binding partners. The non-peptide templates, onto which the substituents are attached such that they mimic the amino acid side-chains in an alpha helix, are important probes to investigate the bioactive conformation of the peptide ligands. Rees et al. selected disubstituted indane skeleton as a template with the aid of molecular modeling.[67] An examination of molecular models of the 1, 6 disubstituents of an indane template indicated that they compliment the Cα and Cβ of adjacent residues in an alpha helix (Figure 14). Many of these model compounds are found to bind to tachykinin receptor with micromolar affinity. X-ray crystal structure of the indane-carboxamide **20** showed that it adopts an alpha-helical conformation.[68]



20

Figure 14. Indane-based alpha-helix mimetic. The substituents correspond to (*i, i*+1) side-chain in an ideal alpha helix.

3.3 Biaryl, Terphenyl, and Arylamides as Peptide Alpha-Helix Mimetics

Organic molecules such as biphenyl, allenes, alkylidene cycloalkanes and spiranes are structures with helical twist (Figure 15). The superimposition of the above substituted molecular skeletons onto polyalanine helix showed that the systems other than biphenyl do not comply with all other requirements of alpha-helical conformation, for example, disposition of the amino acid side-chain and twist angle.[69] Moreover; the biphenyl unit was attractive because 2.1% of all reference drug molecules contain this scaffold. The biphenyl unit can bind to a wide variety of protein pockets, owing to its flexibility, size, and shape. The small molecule alpha helix mimetic should display helical shape, steric orientation of amino acid side-chain, and the N-cap to C-cap helix dipole moment. The first bis or oligo phenylic unit as alpha helix mimetics was reported recently by Hamilton et al. The 3,2′,2′′-trisubstituted terphenyl derivative mimics the *i*, *i*+3 and *i*+7 of a 12-mer alpha-helical peptide.[70]

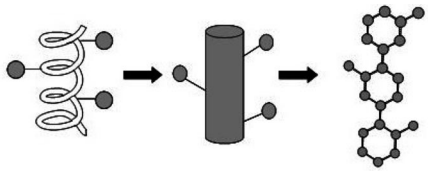


Figure 15. Appropriately substituted biphenyl scaffold mimics an ideal alpha helix.

3.3.1 Bcl-x_L-Antagonist-Based Alpha-Helix Mimicry

The design and synthesis of low molecular weight ligand that disrupt PPIs is a challenge for medicinal chemists. Hamilton and colleagues designed and synthesized variety of terphenyl-based molecules containing alkyl or aryl substituents on the three ortho positions to mimic the hydrophobic substituents on the exterior side of the helical unit of Bcl-antagonist killer (Bak) or Bcl-associated death promoter (Bad) proteins.[71] The design is based on the fact that an appropriately substituted terphenyl scaffold can compliment the *i*, *i*+4 and *i*+7 side-chains of an α-helix (Figure 16). The binding affinity of the terphenyl system to Bcl-x_L was assessed by fluorescence spectroscopy, the terphenyl derivative **21** with two carboxylic acids, isobutyl, 1-naphthalenemethylene, and isobutyl sequence showed maximum binding affinity (nm range) to Bcl-x_L, compared to other compounds tested, suggesting the importance of hydrophobic interactions. The carboxyl units on either sides of the terphenyl unit are for mimicking the ionic interactions. Interestingly, scrambling the positions of the substituents resulted in the loss of binding affinity due to the non-complimentarity of the shape (Figure 16). Molecular modeling and NMR spectral studies supported this view.

Hamilton et al. designed and synthesized trispyridylamide scaffold as an alpha helix mimetic.[72] The preorganization to an α-helical conformation was achieved by introducing bifurcated hydrogen bonding of the amide NHs with the pyridyl nitrogens and the ether oxygen atoms (Figure 16). This stabilization through hydrogen bond consequently destabilized other alternative conformations. Energy minimization studies of the scaffold **22** showed the polyamide backbone to be planar and the alkoxy side-chains are approximately 45° out of the plane of the carboxamide backbone to optimize the hydrogen-bonding interactions. Overlaying the polyalanine alpha helix with the pyridinecarboxamide derivative revealed a close resemblance. The *i*, (*i*+4) and (*i*+7) alanine methyl corresponded with the pyridinecarboxamide side-chains. Bak and Bcl-x_L are the BH3 domains of the proapoptotic and the antiapoptotic proteins respectively. The NMR structure analysis showed that the interactions between Bak domain and Bcl-x_L are helix–helix interactions and hydrophobic in nature. The oligo-pyridinecarboxamide was used as a competitive binder to Bak and was found to bind with Bcl-x_L protein in low micromolar (μM) affinity.

Recently Ahn et al. reported trisbenzamide scaffold **23** as an alpha-helix mimetic.[73] The substituents R^1, R^2, and R^3 represent the *i*, *i*+4, and *i*+7 residues of an ideal alpha helix as was evident from computer modeling studies.

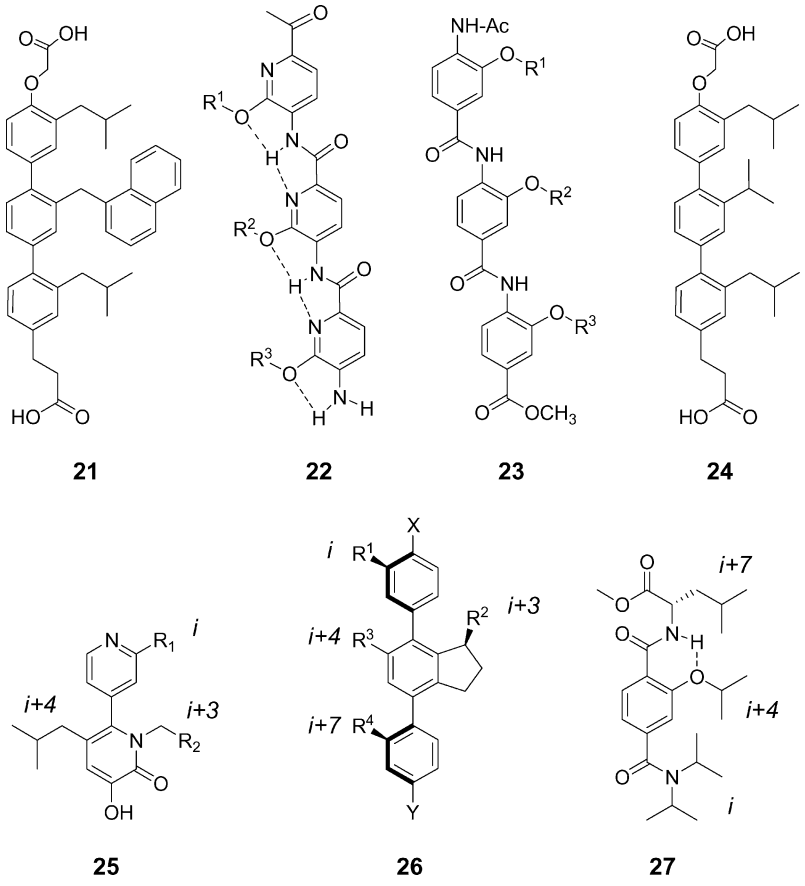


Figure 16. Various helical scaffolds (a) terphenyl (b) trispyridylamide (c) 1,6-disubstituted 4,7-diphenylindane scaffold (d) terephthamide (e) pyridylpyridone (f) terphenyl (g) trisbenzamide.

3.3.2 As an Inhibitior of gp41 Assembly

The surface glycoproteins of the human immunodeficiency virus type-1 (HIV-1) play a critical role in infection. The envelope glycoprotein of HIV-1 consists of two noncovalently associated subunits gp120 and gp41. The gp41 is a transmembrane protein containing a hydrophobic glycine-rich fusion peptide used in membrane fusion.[74] It contains two helical regions with heptad repeats at both the N- and C-terminii. The X-ray crystallographic studies showed that the N-helical region forms a parallel trimeric coiled-coil. The N- and C- regions assembled into a six-helix bundle during the fusion of the virus with the host cell (Figure 17).[75] Hamilton et al. designed and synthesized terphenyl derivative **24** that mimics the alpha-helical portion of the N- or C-helical region.[76] The substituents at the ortho positions of terphenyl template correspond to the side-chains *i*, *i*+4, and *i*+7 residues of the C-helix. Compound **24** disrupted the formation of a six-helix-bundle assembly and hence inhibited the gp-41-mediated fusion, with an IC_{50} of ca. 15.7 $\mu g\,mL^{-1}$. The inhibition was the result of the binding of the compound **24** with the N-terminal helix, such that the formation of the six-helix-bundle by association with C-terminal helix was impossible. The terminal carboxylate groups on the terphenyl unit were used to mimic the anionic character of the C-helical region of the peptide.

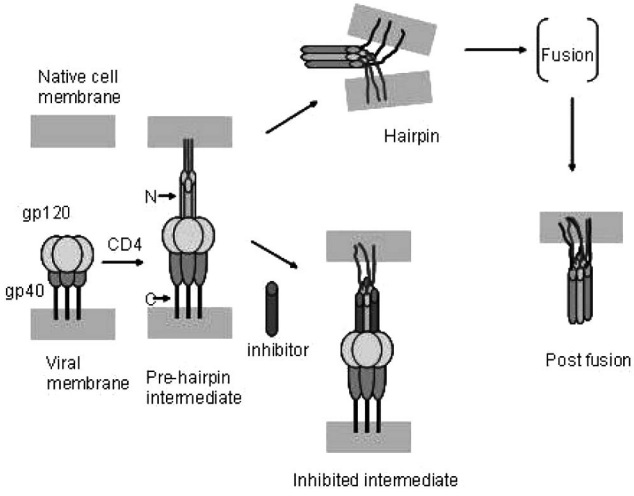


Figure 17. The mechanism of of HIV-virus entry into the host cell. The inhibitor acts by inhibiting the six helix bundle formation.

3.3.3 As an Inhibitor of p53-HDM2 Interaction

In human cancers, 50% show either mutation or an inactive state of the p53 protein. The HDM2 promotes the degradation of p53 and an overexpression of HDM2 has been observed in human cancer.[77] Therefore, disruption of p53/HDM2 interaction by using small molecule mimic is an im-

portant goal in anticancer drug development. X-ray crystal structure analysis of HDM2/p53 complex showed that three hydrophobic residues (F19, W23 and L26) in p53 helix are essential for binding. Hamilton et al. showed that a terphenyl derivative with appropriate substituents on the three *ortho* positions (Figure 16) can mimic the *i*, *i*+4 and *i*+7 residues of p53.[78] Terphenyl derivatives with isobutyl, 2-naphthyl, isobutyl substituents showed the strongest binding (K_i = 0.182 ± 0.020 μM). It is important to note that the terphenyl compound **21** with the 1-naphthylmethylene substituent binds stronger to Bcl-x_L than HDM2.

3.3.4 As an Inhibitor of Estrogen Receptors

The estrogen receptor (ER) has been associated with breast cancer, osteoporosis and cardiovascular diseases. Binding of estrogen to the ligand-binding domain (LBD) of ER ultimately leads to the recruitment of coactivator proteins. Coactivator proteins are involved in the assembly of transcriptional machinery and hence are essential for the expression of ER regulated genes.[79] Inhibition of the interaction between the estrogen-activated ER and coactivator protein is an important therapeutic target. The coactivator proteins possess multiple copies of the LXXLL motif also called nuclear receptor box, which is involved in binding to ER. Crystal structure analysis showed that the LXXLL peptide adopts an α-helical conformation, with Leu at i^{th} and $(i+4)^{th}$ positions projected to a hydrophobic pocket of ER. Hamilton et al. synthesized pyridylpyridone derivative **25** to mimic the *i*, *i*+3, and *i*+4 residues of the α-helix.[80] Pyridylpyridone compounds with appropriate substitutions on the aryl ring are able to inhibit the ER-coactivator interaction with low micromolar activity.

3.3.5 4,7 Diphenyl-1,6-Disubstituted Indanes as Helix Mimetic

Hamilton et al. designed and synthesized nonpeptidic scaffolds based on 1,6-disubstituted 4,7-diphenylindanes **26**, mimicking the *i*, *i*+3, *i*+4, and *i*+7 residues of an α-helix.[81] Theoretical calculations for the substituted diphenylindanes showed that the aryl–aryl torsional angle of 62° and 67° causes the substituents to mimic the disposition of amino acid side-chains at *i*, *i*+3, *i*+4, and *i*+7 of an α-helix.

3.3.6 Terephthalamide as Alpha Helix Mimetics

The more tedious synthesis of terphenyl compounds as alpha helix mimetic prompted Hamilton and co-workers to search for simpler scaffolds. The scaffold should mimic the *i*, *i*+4 and *i*+7 side-chains of the alpha helix.[82] This was accomplished by terephthalamide derivative **27** as the scaffold. The two flanking phenyl rings in terphenyl helix mimetics were replaced with amide bonds that retain the planar geometry due to the restricted rotation, and a further conformational lock was imposed by an intramolecular hydrogen bond between the amide NHs and the alkoxy oxygen atom to influence the position of amino acid side-chain. Terephthalamide-based molecules disrupted the Bcl-x_L/Bak interaction in vitro.

3.4 Pyrimidine-Based Helix Mimetics

Nuclear receptors (NR) are ligand-modulated transcription factors and are associated with various diseases. The coactivator proteins interact with the LBD of NRs through an amphiphilic alpha helix containing the LXXLL motif. Peptides having LXXLL sequence are known to block gene transcription induced by estrogen agonists through ER.[83] A careful analysis of the coactivator protein shows that the positions of the three leucine residues in the LXXLL motif are on the edges of an equilateral triangle. Katzenellenbogen et al. designed Erα coactivator binding inhibitors **28** and **29** (Figure 18) based on a central core with substituents attached to it, so that it mimicked the leucine residues of the LXXLL motif.[84] Various central cores such as pyrimidine, trithianes, and cyclohexane, with leucine-like substituents on them were synthesized to inhibit the coactivator protein from binding to NR. A fluorescence anisotropy-based assay gave the best binding for pyrimidine derivative **30**, with a K_i value of approximately 30 μM. The greater torsional flexibilty of the pyrimidine compounds has been attributed as the cause for the high binding affinity.

The second design criterion was based on the fact that the four hydrophobic residues of the NR box approximated the shape of a parallelogram. The naphthalene core unit was designed to mimic the leucine residues and the substituents were attached such that they mimicked the leucine and isoleucine residues. Binding studies showed that these molecules are weak binders. This is attributed to the flat nature of the aromatic unit, which is not a real structural mimic of the leucine residues.

3.5 Imidazole-Based Helix Mimetics

The hepatitis C virus (HCV) is a lethal virus infecting about 170 million people world wide. The cell surface protein, CD81, was identified to bind with the envelop protein (E2) of HCV. Recent work supports the fact that the amino acid residues I182, N184 and F186 on the helix-D region of CD81 are important for binding to HCV-E2. It was shown that the CD81 helix D peptides inhibit the binding of HCV-E2 to CD81. Todd et al. showed that bisimidazole derivative with appropriate substituents mimic the solvent-exposed face of the CD81 helix D region.[85] Compounds **31** and **32** show bioactivity by blocking the binding of HCV-E2 to CD81. Compound **31** show stronger binding and almost completely block the HCV-E2 to CD81 binding.

Dömling et al. designed and synthesized trisubstituted imidazole compounds as alpha-helix mimetics based on molecular-modeling-derived structural considerations (Figure 19).[86] The imidazole-based alpha-helix mimetics can disrupt PPI and induce cellular apoptosis as the trisubstituted imidazoles exhibited micromolar potency for cell growth inhibition in a HL-60 cell line. The advantage is that these compounds are amenable by a one step synthetic protocol compared to Hamilton's terphenyl compounds, which required elaborate synthesis. The trisubstituted imid-

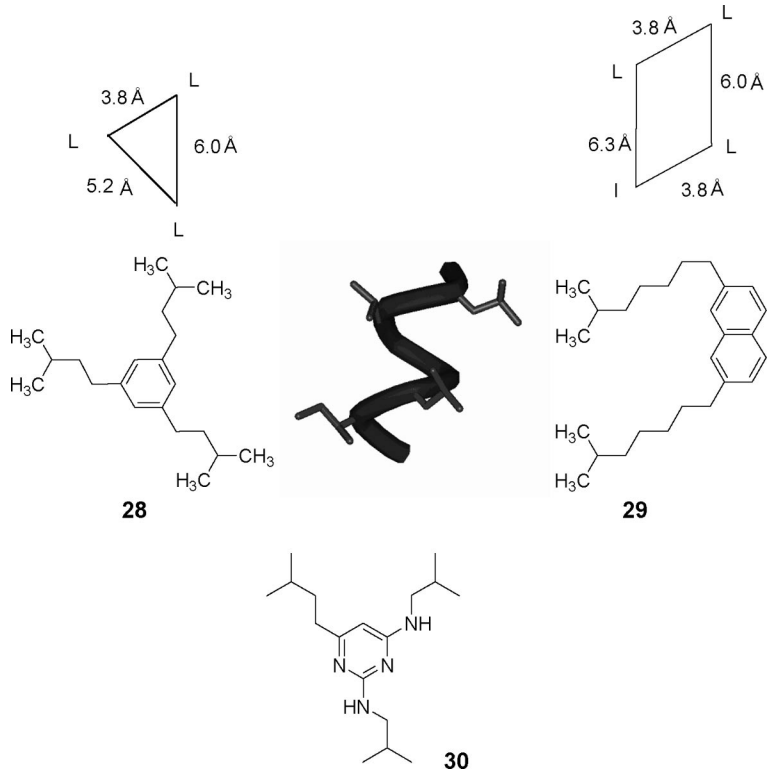


Figure 18. The helix subunit in the ER was mimicked by different template structures.

azoles are synthesized by van Leusen multi-component reaction involving phenyl-substituted tosylmethyl isocyanide, benzaldehyde and an amine.

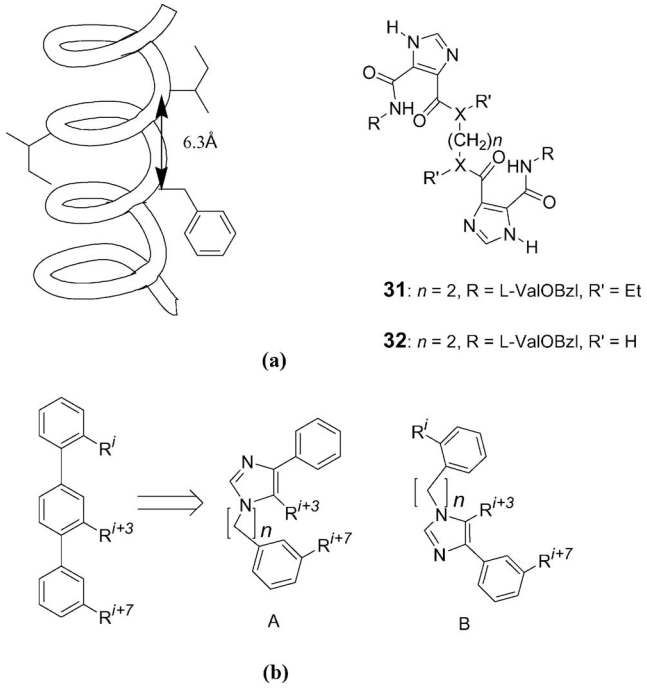


Figure 19. (a) An imidazole-based alpha-helix mimetic (b) comparison of terphenyl scaffold with imidazole-based helix mimetics.

3.6 Benzodiazipines as Helix Mimetic

Benzodiazipinedione (BDP) is a chemotype that binds to HDM2 with high affinity, representing a functional agonism of p53 (Figure 20). The three critical residues Phe19, Trp23 and Leu26 of p53 bind to the predominantly hydrophobic cleft of HDM2. X-ray crystal structure analysis of a p53-based 9mer peptide **33** complexed with HDM2 clearly testified these interactions. X-ray crystallographic studies showed that the three phenyl rings of HDM2 bound BDP occupying the position of p53 derived helical peptide.[87] The 1,4-benzodiazipine-2,5-dione **34** scaffold with appropriate substituents mimics the side-chains presented by p53 helix. The BDP analogs decrease the cell proliferation in cultured tumor cells and thus are promising candidates for use as potential cancer drugs.

3.7 Chalcones as Helix Mimetics

Chalcone derivatives have been shown to inhibit MDM2 binding to the p53 peptide (Figure 20). Chalcones **35** and **36** act as helix mimetics because the MDM2/p53 interaction is a well known helix–helix interaction. The binding of chalcone near the Trp-binding pocket of the p53 binding cleft of human MDM2 was demonstrated by NMR spectroscopy.[88] The NMR studies showed changes in the chemical shifts and line widths of the backbone amide resonances of ^{15}N-enriched MDM2, as a function of chalcone concentration, indicating their binding interactions.

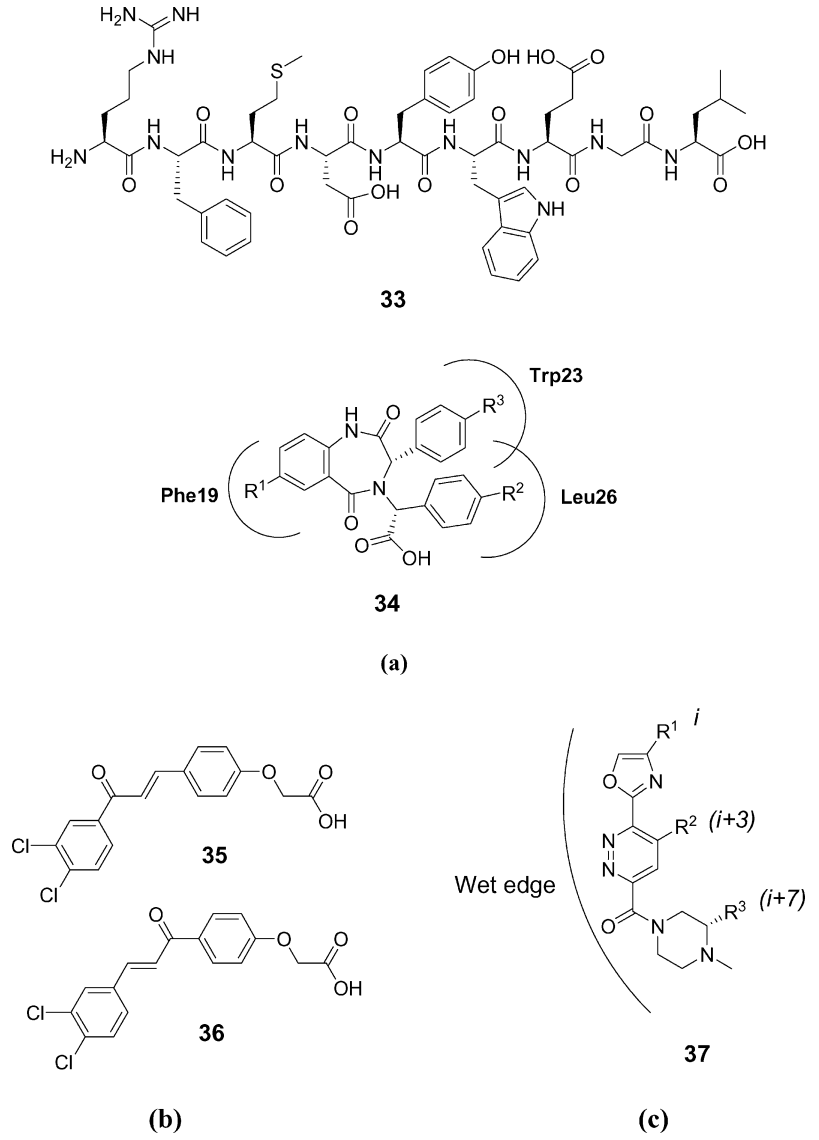


Figure 20. (a) p53 peptide (b) BDP scaffold with substituents R^1, R^2, and R^3 mimicking Phe, Leu and Trp residues of p53 peptide (b) chalcones (c) pyridazine-based helix mimetic.

3.8 Pyridazine-Based Helix Mimetics

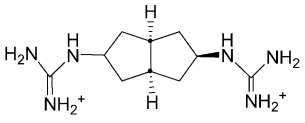
Rebek Jr. and co-workers synthesized various pyridazine-based amphiphilic α-helix mimetics.[89] The design was inspired from Hamilton's terphenyl α-helix mimetic design. These heterocyclic helix mimetics contain a central pyridazine ring, an oxazole, and a piperazine ring (Figure 20). The presentation of substituents on the scaffold is similar to the *i*, *i*+3, and *i*+7 side-chains of a natural α-helix. The molecule **37** clearly has a hydrophobic and a hydrophilic part, thus resembling an amphiphilic helix, and it inhibited the Bcl-x_L/Bak interaction, a well known helix–helix interaction. The binding affinity of this heterocyclic helix mimetic is lower than that of the Hamilton's terphenyl mimetics.

4. Molecular-Recognition-Directed Folding

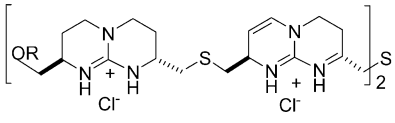
4.1 Guanidine-Based Helix-Inducing Additives

Hamilton et al. exploited the ability of alkyl-guanidinium groups to bind strongly to carboxylates in polar solvents.[90] The receptor **38** with rigid scaffold orients two guanidinium units at 4–5 Å in an approximately parallel arrangement. The 16mer peptide **40** possessing modest helical character with two Asp residues was synthesized. Addition of guanidinium receptor molecule **38** to the peptide solution enhanced the helical character, as was evident from the CD spectrum. NMR and CD spectral studies and comparison with the respective spectra of control compounds with one guanidinium group clearly indicated the formation of 1:1

complex, wherein the side-chains of Asp residues bind to guanidine groups of **38**, thus constricting the peptide to assume an alpha-helical conformation. In a similar manner, a 16-mer peptide **41** with four Asp residues was found to bind tetraguanindinium-based receptor **39** and enhanced the helicity. The spacing between the guanidinium groups and the positions of the aspartate residues are crucial for molecular recognition (Figure 21).



38

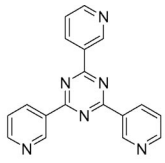


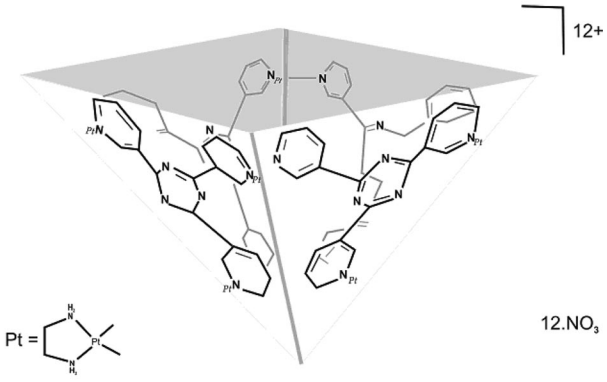
39

40 Ac-A-A-Q-D-A-A-D-A-A-A-A-A-Q-A-A-Y-$CONH_2$

41 Ac-A-A-A-D-Q-L-D-A-L-D-A-Q-D-A-A-Y-$CONH_2$

Figure 21. Guanidine-based receptors for inducing helical conformation in peptide containing Asp units.





42

43 Ac-Trp-Ala-Glu-Ala-Ala-Ala-Glu-Ala-Trp-NH_2

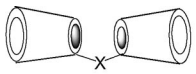
Figure 22. Platinum bowl for binding to peptide to induce alpha-helical conformation.

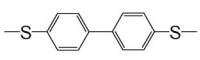
4.2 Platinum-Based Coordination Bowl

The dimeric capsule of the bowl-shaped coordination compound **42** with a large hydrophobic interior encapsulates a nine-residue peptide, WAEAAAEAW, and induces the alpha-helical conformation. NMR titration experiments showed that, in solution, the capsule exists as 1:1 and 2:1 (Bowl:peptide) complexes (Figure 22). Even in presence of excess of bowl, these 1:1 and 2:1 complexes are in equilibrium.[91] The addition of $NaNO_3$ resulted in the predominance of the 2:1 complex and this is rationalized as due to enhanced ionic strength that increased the hydrophobic interaction between the bowl **42** and peptide **43**. The secondary structure of peptide **43** within the dimeric capsule was found to be alpha-helical from NOESY and CD studies. The driving forces for the formation of the dimeric capsule are thought to be the hydrophobic and charge-transfer interactions between the bowl and Trp residues in the peptide.

4.3 Designed Cyclodextrin Analogs

Breslow and colleagues designed dimeric cyclodextrin analogs with varied linker regions that bind to peptides with hydrophobic amino acid residues at (*i*, *i*+11) or (*i*, *i*+14) positions.[92] β-cyclodextrin with 1,1′-biphenyl-4,4′-dithiol (**44**) could induce folding of a peptide bearing *p*-*tert*-butylphenylalanine in the *i* and *i*+11 positions (Figure 23). In aqueous medium, peptide Ac-F(*p*-*t*Bu)EAAAKEAAAKF-(*p*-*t*Bu)-NH_2 (**45**) adopts a β-sheet structure as was evident from the characteristic 218 nm negative absorption in the CD spectrum. Addition of the receptor **44** with a biphenyl linker showed a characteristic change in the CD spectrum from β-sheet to α-helical conformation. Circular dichroism and calorimetric studies yielded an apparent K_a on the order of 10^4–10^5 M^{-1}. Various cyclodextrin monomers and dimers with different linkers were synthesized as control compounds to establish the mechanism of folding. The study conclusively proved molecular-recognition-directed folding of peptides and is one of the first approaches to utilize the hydrophobic effect of peptides to induce the desired folding in designed oligopeptides.



44 X = 

45 Ac-F(*p*-*t*Bu)EAAAKEAAAKF(*p*-*t*Bu)-NH_2

Figure 23. Designed cyclodextrin-based receptor for binding to peptides with hydrophobic side-chains.

Conclusions

Peptide-based helix mimetics serve as chemical models to understand protein folding, whereas nonpeptide-based helix mimetics will serve as effective therapeutic agents for many diseases by disrupting important PPIs. Molecular-recogni-

tion-based peptide folding models may shed light into various protein folding activities of chaperones. The diverse array of chemical systems studied in an attempt to mimic the essential features of helix will continue till a reasonable solution to the problem of protein folding and associated protein–protein interactions is obtained. The contributions of many scientists to mimic the helix backbone by various structurally diverse skeletons will definitely fuel many more discoveries in this direction with potential for therapeutic drugs. The fundamental questions around the protein folding problem such as sequence dependence of the folding process and the conformational search by protein molecule (Levinthal paradox) are still to be addressed.

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[1] L. Pauling, R. B. Corey, *Proc. Natl. Acad. Sci. USA* **1951**, *37*, 241–250.
[2] R. G. Brennan, B. W. Matthews, *J. Biol. Chem.* **1989**, *264*, 1903–1906 and references there in.
[3] W. H. Landschulz, P. F. Johnson, S. L. McKnight, *Science* **1988**, *240*, 1759–1764.
[4] K. Palczewski, T. Kumasaka, T. Hori, C. A. Behnke, H. Motoshima, B. A. Fox, I. L. Trong, D. C. Teller, T. Okada, R. E. Stenkamp, M. Yamamoto, M. Miyano, *Science* **2000**, *289*, 739–745.
[5] V. Daggett, A. R. Fersht, *Trends Biochem. Sci.* **2003**, *28*, 18–25.
[6] a) G. N. Ramachandran, V. Sasisekharan, *Adv. Protein Chem.* **1968**, *23*, 283–437; b) G. N. Ramachandran, C. Ramakrishnan, V. Sasisekharan, *J. Mol. Biol.* **1963**, *7*, 95–99.
[7] J. Donohue, *Proc. Natl. Acad. Sci. USA* **1953**, *39*, 470–478.
[8] B. W. Low, R. B. Baybutt, *J. Am. Chem. Soc.* **1952**, *74*, 5806–5807.
[9] A. Sali, E. Shaknovich, M. Karplus, *Nature* **1994**, *369*, 248–251.
[10] R. Aurora, T. P. Creamer, R. Srinivasan, G. D. Rose, *J. Biol. Chem.* **1997**, *272*, 1413–1416.
[11] G. N. Ramachandran, G. Kartha, *Nature* **1955**, *176*, 593–595.
[12] a) L. Pauling, R. B. Corey, *Proc. Natl. Acad. Sci. USA* **1951**, *37*, 256–261; b) L. Pauling, R. B. Corey, *Proc. Natl. Acad. Sci. USA* **1951**, *37*, 261–271.
[13] a) L. Pauling, R. B. Corey, *Proc. Natl. Acad. Sci. USA* **1951**, *37*, 272–281; b) G. N. Ramachandran, G. Kartha, *Nature* **1954**, *174*, 269–270.
[14] a) M. W. Peczuh, A. D. Hamilton, *Chem. Rev.* **2000**, *100*, 2479–2494; b) T. Ooi, M. Oobatake, *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 2859–2863; c) C. M. Dobson, A. Šali, M. Karplus, *Angew. Chem. Int. Ed.* **1998**, *37*, 868–893.
[15] S. F. Betz, J. W. Bryson, W. F. DeGrado, *Curr. Opin. Struct. Biol.* **1995**, *5*, 457–463.
[16] a) A. Horovitz, J. M. Matthews, A. R. Fersht, *J. Mol. Biol.* **1992**, *227*, 560–568; b) K. R. Shoemaker, P. S. Kim, E. J. York, J. M. Stewart, R. L. Baldwin, *Nature* **1987**, *326*, 563–567.
[17] Y. Chen, F. Ding, H. Nie, A. W. Serohijos, S. Sharma, K. C. Wilcox, S. Yin, N. V. Dokholyan, *Arch. Biochem. Biophys.* **2008**, *469*, 4–19.
[18] B. H. Zimm, J. K. Bragg, *J. Chem. Phys.* **1959**, *31*, 526–535.
[19] Y. Fezoui, D. H. Hartley, D. M. Walsh, D. J. Selkoe, J. J. Osterhout, D. B. Teplow, *Nat. Struct. Biol.* **2000**, *7*, 1095–1099.
[20] J. M. Davis, L. K. Tsou, A. D. Hamilton, *Chem. Soc. Rev.* **2007**, *36*, 326–334.
[21] E. T. Kaiser, F. J. Kezdy, *Science* **1984**, *223*, 249–255.
[22] A. S. Ripka, D. H. Rich, *Curr. Opin. Chem. Biol.* **1998**, *2*, 441–452.
[23] C. B. Anfinsen, *Science* **1973**, *181*, 223–230.
[24] G. D. Rose, P. J. Fleming, J. R. Banavar, A. Maritan, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 16623–16633.
[25] E. I. Shakhnovich, *Curr. Opin. Struct. Biol.* **1997**, *7*, 29–40.
[26] R. L. Baldwin, G. D. Rose, *TIBS* **1999**, *24*, 26–33.
[27] a) L. Monticelli, D. P. Tieleman, G. Colombo, *J. Phys. Chem. B* **2005**, *109*, 20064–20067; b) E. L. McCallister, E. Alm, D. Baker, *Nat. Struct. Biol.* **2000**, *7*, 669–673; c) S. Nauli, B. Kuhlman, D. Baker, *Nat. Struct. Biol.* **2001**, *8*, 602–605; d) A. M. C. Marcelino, L. M. Gierasch, *Biopolymers* **2008**, *89*, 380–391; e) D. E. Kim, C. Fisher, D. Baker, *J. Mol. Biol.* **2000**, *298*, 971–984.
[28] a) E. R. Blout, P. Doty, J. T. Yang, *J. Am. Chem. Soc.* **1957**, *79*, 749–750; b) P. Doty, A. Wada, T. Yang, E. R. Blout, *J. Polym. Sci.* **1957**, *23*, 851–861.
[29] P. Y. Chou, G. D. Fasman, *Biochemistry* **1974**, *13*, 222–245.
[30] a) P. C. Lyu, M. I. Liff, L. A. Marky, N. R. Kallenbach, *Science* **1990**, *250*, 669–673; b) P. C. Lyu, L. A. Marky, N. R. Kallenbach, *J. Am. Chem. Soc.* **1989**, *111*, 2733–2734.
[31] P. C. Lyu, J. C. Sherman, A. Chen, N. R. Kallenbach, *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 5317–5320.
[32] a) J. M. Scholtz, H. Qian, V. H. Robbins, R. L. Baldwin, *Biochemistry* **1993**, *32*, 9668–9676; b) J. A. Vila, D. R. Ripoll, H. A. Scheraga, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 13075–13079; c) S. Marqusee, R. L. Baldwin, *Proc. Natl. Acad. Sci. USA* **1987**, *84*, 8898–8902.
[33] C. Toniolo, G. M. Bonora, A. Bavoso, E. Benedetti, B. D. Blasio, V. Pavone, C. Pedone, *Biopolymers* **1983**, *22*, 205–215.
[34] I. L. Karle, P. Balaram, *Biochemistry* **1990**, *29*, 6747–6756.
[35] a) P. Pengo, L. Pasquato, S. Moro, A. Brigo, F. Fogolari, Q. B. Broxterman, B. Kaptein, P. Scrimin, *Angew. Chem. Int. Ed.* **2003**, *42*, 3388–3392; b) M. Bellanda, S. Mammi, S. Geremia, N. Demitri, L. Randaccio, Q. B. Broxterman, B. Kaptein, P. Pengo, L. Pasquato, P. Scrimin, *Chem. Eur. J.* **2007**, *13*, 407–416.
[36] a) P. Mathur, S. Ramakumar, V. S. Chauhan, *Biopolymers* **2004**, *76*, 150–161; b) Y. Inai, T. Hirabayashi, *Biopolymers* **2001**, *59*, 356–369.
[37] R. Jain, V. S. Chauhan, *Biopolymers* **1996**, *40*, 105–119.
[38] U. R. Doshi, V. Muñoz, *J. Phys. Chem. B* **2004**, *108*, 8497–8506.
[39] H. X. Zhou, P. C. Lyu, D. E. Wemmer, N. R. Kallenbach, *J. Am. Chem. Soc.* **1994**, *116*, 1139–1140.
[40] A. J. Doig, R. L. Baldwin, *Protein Sci.* **1995**, *4*, 1325–1336.
[41] M. Sagermann, [1].-G. Martensson, W. A. Baase, B. W. Matthews, *Protein Sci.* **2002**, *11*, 516–521.
[42] R. Aurora, G. D. Rose, *Protein Sci.* **1998**, *7*, 21–38.
[43] a) D. S. Kemp, T. P. Curran, *Tetrahedron Lett.* **1988**, *29*, 4931–4934; b) D. S. Kemp, T. J. Allen, S. L. Oslick, J. G. Boyd, *J. Am. Chem. Soc.* **1996**, *118*, 4240–4248; c) D. S. Kemp, S. L. Oslick, T. J. Allen, *J. Am. Chem. Soc.* **1996**, *118*, 4249–4255.
[44] a) D. S. Kemp, T. P. Curran, W. M. Davis, J. G. Boyd, C. Muendel, *J. Org. Chem.* **1991**, *56*, 6672–6682; b) D. S. Kemp, T. P. Curran, J. G. Boyd, T. J. Allen, *J. Org. Chem.* **1991**, *56*, 6683–6697.
[45] a) D. S. Kemp, J. H. Rothman, T. C. Curran, D. E. Blanchard, *Tetrahedron Lett.* **1995**, *36*, 3809–3812; b) D. S. Kemp, J. H. Rothman, *Tetrahedron Lett.* **1995**, *36*, 3813–3816; c) D. S. Kemp, J. H. Rothman, *Tetrahedron Lett.* **1995**, *36*, 4019–4022; d) D. S. Kemp, J. H. Rothman, *Tetrahedron Lett.* **1995**, *36*, 4023–4026.
[46] a) A. Lewis, M. D. Ryan, D. Gani, *J. Chem. Soc. Perkin Trans. 1* **1998**, 3767–3775; b) A. Lewis, J. Wilkie, T. J. Rutherford, D.

Gani, *J. Chem. Soc. Perkin Trans. 1* **1998**, 3777–3793; c) A. Lewis, T. J. Rutherford, J. Wilkie, T. Jenn, D. Gani, *J. Chem. Soc. Perkin Trans. 1* **1998**, 3795–3806.
[47] R. E. Austin, R. A. Maplestone, A. M. Sefler, K. Liu, W. N. Hruzewicz, C. W. Liu, H. S. Cho, D. E. Wemmer, P. A. Bartlett, *J. Am. Chem. Soc.* **1997**, *119*, 6461–6472.
[48] M. E. Houston Jr., C. L. Gannon, C. M. Kay, R. S. Hodges, *J. Pept. Sci.* **1995**, *1*, 274–282.
[49] a) C. Yu, J. W. Taylor, *Tetrahedron Lett.* **1996**, *37*, 1731–1734; b) G. Osapay, J. W. Taylor, *J. Am. Chem. Soc.* **1992**, *114*, 6966–6973.
[50] a) C. Bracken, J. Gulyas, J. W. Taylor, J. Baum, *J. Am. Chem. Soc.* **1994**, *116*, 6431–6432; b) C. Yu, J. W. Taylor, *Bioorg. Med. Chem.* **1999**, *7*, 161–175.
[51] J. W. Taylor, *Pept. Sci.* **2002**, *66*, 49–75.
[52] J. C. Phelan, N. J. Skelton, A. C. Braisted, R. S. McDowell, *J. Am. Chem. Soc.* **1997**, *119*, 455–460.
[53] N. E. Shepherd, H. N. Hoang, G. Abbenante, D. P. Fairlie, *J. Am. Chem. Soc.* **2005**, *127*, 2974–2983.
[54] F. Zhang, O. Sadovski, S. J. Xin, G. A. Woolley, *J. Am. Chem. Soc.* **2007**, *129*, 14154–14155.
[55] D. Y. Jackson, D. S. King, J. Chmielewski, S. Singh, P. G. Schultz, *J. Am. Chem. Soc.* **1991**, *113*, 9391–9392.
[56] H. E. Blackwell, R. H. Grubbs, *Angew. Chem. Int. Ed.* **1998**, *37*, 3281–3284.
[57] C. E. Schafmeister, J. Po, G. L. Verdine, *J. Am. Chem. Soc.* **2000**, *122*, 5891–5892.
[58] E. Cabezas, A. C. Satterthwait, *J. Am. Chem. Soc.* **1999**, *121*, 3862–3875.
[59] a) R. N. Chapman, G. Dimartino, P. S. Arora, *J. Am. Chem. Soc.* **2004**, *126*, 12252–12253; b) G. Dimartino, D. Wang, R. N. Chapman, P. S. Arora, *Org. Lett.* **2005**, *7*, 2389–2392; c) D. Wang, K. Chen, J. L. Kulp III, P. S. Arora, *J. Am. Chem. Soc.* **2006**, *128*, 9248–9256.
[60] R. Fasan, R. L. A. Dias, K. Moehle, O. Zerbe, J. W. Vrijbloed, D. Obrecht, J. A. Robinson, *Angew. Chem. Int. Ed.* **2004**, *43*, 2109–2112.
[61] a) M. R. Ghadiri, C. Choi, *J. Am. Chem. Soc.* **1990**, *112*, 1630–1632; b) M. R. Ghadiri, A. K. Fernholz, *J. Am. Chem. Soc.* **1990**, *112*, 9633–9635.
[62] a) M. J. Kelso, R. L. Beyer, H. N. Hoang, A. S. Lakdawala, J. P. Snyder, W. V. Oliver, T. A. Roberston, T. G. Applenton, D. P. Fairlie, *J. Am. Chem. Soc.* **2004**, *126*, 4828–4842; b) M. J. Kelso, H. N. Hoang, T. G. Appleton, D. P. Fairlie, *J. Am. Chem. Soc.* **2000**, *122*, 10488–10489; c) R. L. Beyer, H. N. Hoang, T. G. Appleton, D. P. Fairlie, *J. Am. Chem. Soc.* **2004**, *126*, 15096–15105.
[63] B. Lovejoy, S. Choe, D. Cascio, D. K. McRorie, W. F. DeGrado, D. Eisenberg, *Science* **1993**, *259*, 1288–1293.
[64] L. Zhao, J. Chmielewski, *Curr. Opin. Struct. Biol.* **2005**, *13*, 31–34.
[65] A. Lombet, J.-N. Bidard, M. Lazdunski, *FEBS Lett.* **1987**, *219*, 355–359.
[66] a) H. Oguri, A. Oomura, S. Tanabe, M. Hirama, *Tetrahedron Lett.* **2005**, *46*, 2179–2183; b) H. Oguri, S. Tanabe, A. Oomura, M. Umetsu, M. Hirama, *Tetrahedron Lett.* **2006**, *47*, 5801–5805.
[67] a) D. C. Horwell, W. Howson, W. P. Nolan, G. S. Ratcliffe, D. C. Rees, H. M. G. Willems, *Tetrahedron* **1995**, *51*, 203–216; b) W. P. Nolan, G. S. Ratcliffe, D. C. Rees, *Tetrahedron Lett.* **1992**, *33*, 6879–6882.
[68] D. C. Horwell, W. Howson, G. S. Ratcliffe, H. M. G. Williams, *Bioorg. Med. Chem.* **1996**, *4*, 33–42.
[69] E. Jacoby, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 891–893.
[70] B. P. Orner, J. T. Ernst, A. D. Hamilton, *J. Am. Chem. Soc.* **2001**, *123*, 5382–5383.
[71] O. Kutzki, H. S. Park, J. T. Ernst, B. P. Orner, H. Yin, A. D. Hamilton, *J. Am. Chem. Soc.* **2002**, *124*, 11838–11839.
[72] J. T. Ernst, J. Becerril, H. S. Park, H. Yin, A. D. Hamilton, *Angew. Chem. Int. Ed.* **2003**, *42*, 535–539.
[73] J.-M. Ahn, S.-Y. Han, *Tetrahedron Lett.* **2007**, *48*, 3543–3547.
[74] D. C. Chan, D. Fass, J. M. Berger, P. S. Kim, *Cell* **1997**, *89*, 263–273.
[75] D. C. Chan, P. S. Kim, *Cell* **1998**, *93*, 681–684.
[76] J. T. Ernst, O. Kutzki, A. K. Debnath, S. Jiang, H. Lu, A. D. Hamilton, *Angew. Chem. Int. Ed.* **2002**, *41*, 278–281.
[77] P. Chéne, *Mol. Cancer Res.* **2004**, *2*, 20–28.
[78] H. Yin, G-in Lee, H. S. Park, G. A. Payne, J. M. Rodriguez, S. M. Sebti, A. D. Hamilton, *Angew. Chem. Int. Ed.* **2005**, *44*, 2704–2707.
[79] Y.-H. Lee, S. A. Coonrod, W. L. Kraus, M. A. Jelinek, M. R. Stallcup, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 3611–3616.
[80] J. Becerril, A. D. Hamilton, *Angew. Chem. Int. Ed.* **2007**, *46*, 4471–4473.
[81] I. C. Kim, A. D. Hamilton, *Org. Lett.* **2006**, *8*, 1751–1754.
[82] H. Yin, G-in Lee, K. A. Sedey, J. M. Rodriguez, H.-G. Wang, S. M. Sebti, A. D. Hamilton, *J. Am. Chem. Soc.* **2005**, *127*, 5463–5468.
[83] C.-Y. Chang, J. D. Norris, H. GrØn, L. A. Paige, P. T. Hamilton, D. J. Kenan, D. Fowlkes, D. P. McDonnel, *Mol. Cell Biol.* **1999**, *19*, 8226–8239.
[84] A. L. Rodriguez, A. Tamrazi, M. L. Collins, J. A. Katzenellenbogen, *J. Med. Chem.* **2004**, *47*, 600–611.
[85] S. E. VanCompernolle, A. V. Wiznycia, J. R. Rush, M. Dhanasekaran, P. W. Baures, S. C. Todd, *Virology* **2003**, *314*, 371–380.
[86] W. Antuch, S. Menon, Q.-Z. Chen, Y. Lu, S. Sakamuri, B. Beck, V. Schauer-Vukasinovic, S. Agarwal, S. Hess, A. Dömling, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1740–1743.
[87] a) B. L. Grasberger, T. Lu, C. Schubert, D. J. Parks, T. E. Carver, H. K. Koblish, M. D. Cummings, L. V. LaFrance, K. L. Milkiewicz, R. R. Calvo, D. Maguire, J. Lattanze, C. F. Franks, S. Zhao, K. Ramachandren, G. R. Bylebyl, M. Zhang, C. L. Manthey, E. C. Petrella, M. W. Pantoliano, I. C. Deckman, J. C. Spurlino, A. C. Maroney, B. E. Tomczuk, C. J. Molloy, R. F. Bone, *J. Med. Chem.* **2005**, *48*, 909–912; b) M. D. Cummings, C. Schubert, D. J. Parks, R. R. Calvo, L. V. LaFrance, J. Lattanze, K. L. Milkiewicz, T. Lu, *Chem. Biol. Drug Des.* **2006**, *67*, 201–205.
[88] R. Stoll, C. Renner, S. Hansen, S. Palme, C. Klein, A. Belling, W. Zeslawski, M. Kamionka, T. Rehm, P. Mühlhahn, R. Schumacher, F. Hesse, B. Kaluza, W. Voelter, R. A. Engh, T. A. Holak, *Biochemistry* **2001**, *40*, 336–344.
[89] a) S. M. Biros, L. Moisan, E. Mann, A. Carella, D. Zhai, J. C. Reed, J. Rebek Jr., *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4641–4645; b) A. Volonterio, L. Moisan, J. Rebek Jr., *Org. Lett.* **2007**, *9*, 3733–3736.
[90] a) M. W. Peczuh, A. D. Hamilton, J. S-Quesada, J. de Mendoza, T. Haack, E. Giralt, *J. Am. Chem. Soc.* **1997**, *119*, 9327–9328; b) J. S. Albert, M. S. Goodman, A. D. Hamilton, *J. Am. Chem. Soc.* **1995**, *117*, 1143–1144.
[91] S. Tashiro, M. Tominaga, Y. Yamaguchi, K. Kato, M. Fujita, *Chem. Eur. J.* **2006**, *12*, 3211–3217.
[92] a) D. Wilson, L. Perlson, R. Breslow, *Bioorg. Med. Chem.* **2003**, *11*, 2649–2653; b) R. Breslow, Z. Yang, R. Ching, G. Trojandt, F. Odobel, *J. Am. Chem. Soc.* **1998**, *120*, 3536–3537.

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