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α-Helix mimetics: Outwards and upwards

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ABSTRACT

α-Helices are common secondary structural elements forming key parts of the large, generally featureless interfacial regions of many therapeutically-relevant protein–protein interactions (PPIs). The rational design of helix mimetics is an appealing small-molecule strategy for the mediation of aberrant PPIs, however the first generation of scaffolds presented a relatively small number of residues on a single recognition surface. Increasingly, helices involved in PPIs are found to have more complex binding modes, utilizing two or three recognition surfaces, or binding with extended points of contact. To address these unmet needs the design and synthesis of new generations of multi-sided, extended, and supersecondary structures are underway.

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Proteomics has emerged as an important tool in unraveling the intricate, dynamic nature of many cellular processes.[1] The prevalence of protein–protein interactions (PPIs) in signal transduction, transcription, and apoptosis, make them attractive therapeutic targets for numerous diseases (e.g., HIV, cancer, and neurodegenerative disorders).[2–8] The development of chemical probes is important to gain further insights into these critical biological systems but despite their potential clinical importance, few small molecule inhibitors of PPIs have been developed. Many established enzymatic targets have small, well-defined catalytic domains which govern the majority of the enthalpic contribution to substrate binding.[9] Conversely, PPIs are mediated by the cumulative binding energy of many amino acid residues, over an extended surface (as large as 4500 Å^2).[10–12] Much of this surface is solvent exposed when unbound, with binding domains arranged in a noncontiguous manner, often necessitating the development of large molecular weight inhibitors.[13] Strategies to disrupt PPIs frequently focus on key 'hot spot' residues which contribute heavily to binding.[14]

An α-helix is the most common protein secondary structural element, defined by a tight helical turn (3.4 residues per turn), thereby creating three distinct binding surfaces, with the *i*, *i* + *4* and *i* + *7*, residues aligned on the same face (Fig. 1). Of the PPIs found in the Protein Data Bank (PDB), 62% have an α-helix at the interface, illustrating the importance of this structural element in protein-protein recognition.[15,16] They are also found at more complex recognition domains such as DNA binding motifs and membrane-bound proteins.[17,18]

The increase in structural data over the last 20 years (Fig. 2) has facilitated research into the mediation of therapeutically relevant PPIs, with many groups targeting key helical elements. Whilst the ratio of two- and three-sided helix-mediated PPIs relative to single-sided analogues has not changed, there has been a growing awareness of their presence at important interfaces.

Many groups have developed conformationally restricted peptides employing covalent tethers including lactams,[19,20] disulfide bridges,[21] triazoles,[22] and hydrocarbon linkers.[23–26] Grubbs showed that olefin metathesis can be used to promote helicity,[27] with Verdine later showing that stapled peptides inhibit the

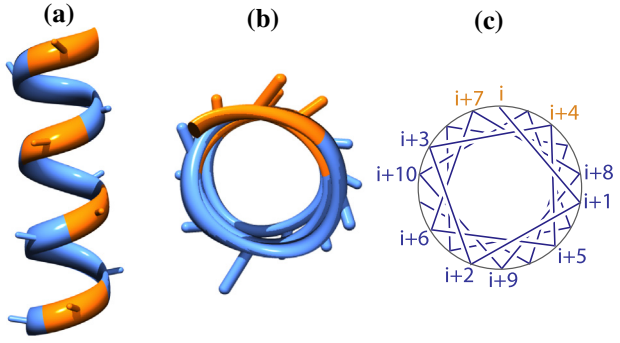

Figure 1. Distribution of residues on a canonical α-helix; (a) side-view with *i*, *i* + *4*, *i* + *7*, *i* + *11* residues displayed on the same face (orange); (b) top-down view, (c) α-helical wheel.

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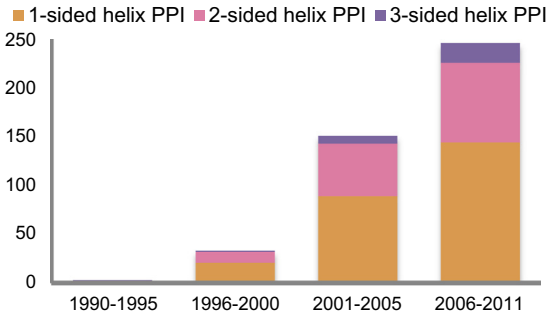


Figure 2. PDB structures with helices at the PPI interface over time. Subcategories illustrate which proportion contained helices with one, two or three key binding domains.[15,16]

Bcl-2/Bid interaction, resulting in decreased growth of human leukemia xenografts *in vivo*.[28] Strategies developed by Cabezas and Arora involve replacement of the internal α-helix hydrogen bonding network with a covalent linkage.[29–32] A number of different approaches have since been developed to introduce constraints in an effort to promote helicity (Fig. 3).[33,34] Arora et al. demonstrated inhibition of the HIF-1α/p300 PPI with an olefin-stapled hydrogen bond surrogate (HBS) where key residues are found at the *i*, *i* + *2*, *i* + *5*, *i* + *6* and *i* + *10* positions.[35] This strategy has the benefit of retaining the *i*, *i* + *4* and *i* + *7* residues and avoiding the use of synthetically challenging α,α-di-substituted amino acids. In another strategy, tethers have been used to staple long chains and stabilize extended helical structures. An impressive example is a 36 amino acid anti-viral sequence stabilized by two hydrocarbon bridges formed through ring-closing metathesis (RCM), increasing potency of a peptide inhibitor, enfuvirtide, four-fold to 2.1 nM and at the same time improving its pharmacokinetics.[36]

Short peptide truncates are frequently unstructured, prompting researchers to explore the helical propensities of more metabolically stable β-peptides.[37,38] Variations of this strategy have been employed to promote additional helicity, with many groups exploiting conformationally constrained variants,[39–42] and mixed sequences of α/β-amino acids.[43–46] Seebach and Gellman

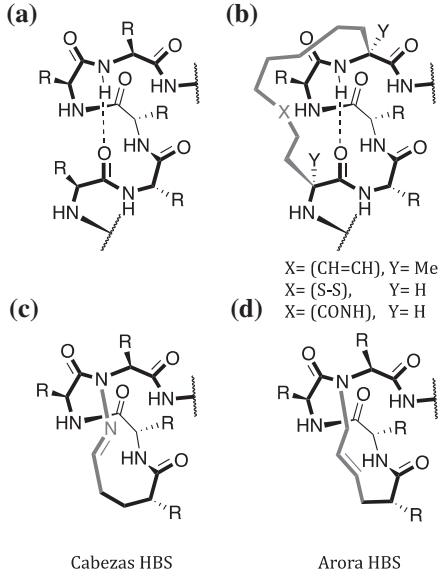


Figure 3. α-Helix tethering strategies; (a) canonical α-helix with 13-membered H-bonding, (b) stapled α-helix through residues on the *i* and *i* + *4* positions (22-ring macrocycle), (c) Cabezas' hydrazone HBS (13-ring macrocycle), (d) Arora's olefin HBS (13-ring macrocycle). HBS = hydrogen bond surrogate.

pioneered the use of β-peptides to mimic α-helical secondary structure,[47–49] with Schepartz later demonstrating inhibition of the p53/MDM2 PPI.[50] An example by Gellman showed an extended helix which accurately mimicked the native sequence, and successfully inhibited the Bcl-2/Bim PPI.[51]

A fundamentally different strategy to mediate PPIs is to mimic elements of protein secondary structure with entirely non-peptidic constructs. The rational design of helix mimics aims to project functional groups from a scaffold in order to reproduce the orientation of the native side-chains, whilst avoiding the expression, purification or chemical synthesis of peptidic elements. Such peptidomimetics present an opportunity to design compounds which have the same binding mode as the native protein, yet are metabolically stable, can permeate membranes and are readily absorbed.[11] The structural diversity provided by chemical synthesis allows a much greater array of groups, with varied steric and electronic properties, to be projected than those provided by the side chains of proteogenic amino acids. Majmudar et al. identified phenol carboxylic acid natural products, sekikaic- and lobaric acid, that inhibit the PPI between p300 and both MLL (Mixed-Lineage Leukemia) and pKID (phosphorylated kinase-inducible domain of CREB). These molecules bind at the same site as the helical region of peptides, hinting that Nature has evolved its own helix mimics.[52]

Early work focused on the mimicry of hydrophobic residues on a single helical face, yet important targets often have amphiphilic helices with hot-spot regions projected from multiple faces. Important helical sequences with hot-spot residues in the *i*, *i* + *4* and *i* + *7* positions were frequently targeted since they are found on a common face (Fig. 1).[14] Several classes of helix mimetic have been developed, many inspired by the terphenyl scaffold introduced by Orner et al. in 2001.[53] The p53/MDM2 and Bcl-xl/Bak interactions are two well-studied targets, with α-helices projecting crucial residues on a single face.[54,55] Both interactions inhibit cellular apoptosis: MDM2 binds and inactivates p53, the principal architect of cell-death, thereby arresting apoptosis.[56] Similarly, Bak is a pro-apoptotic protein whose function is disrupted on binding of Bcl-xl (Fig. 4a).[57] Both interactions are thought to be promising cancer targets since neoplastic cells often disable intracellular apoptotic mechanisms. Yin et al. designed terphenyl helix mimetics that inhibited the p53/HDM2 interaction in vitro with a K_i of 182 nM where a native peptide truncate had a K_i of 3.51 μM.[54]

Arora has highlighted the importance of binding to multiple surfaces of a single helix in many interactions.[15] The current collection of single-sided helix mimetics are ill-equipped to simultaneously mimic hot-spot residues displayed on more than one face, presenting a need for the development of more complex scaffolds (Fig. 4).[58] Moreover, the restricted length of mimics of two α-helix turns is often insufficient to inhibit interactions of extended helices. The Bcl-2/Bid interaction is an example with key residues orientated on two faces of the α-helix.[59] Key hydrophobic residues Ile82, Ile86 and Leu90 lie on the same face, with Asp95 projected on a different face to form important hydrogen bonds to Bcl-2 arginine and asparagine residues (Fig. 4b). Enzymes E1 and E2, involved in the replication of papillomavirus, are another important cancer target displaying a two-faced α-helix binding mode.[60]

Examples with hot-spot residues found on all three faces of an α-helix include the interaction of calmodulin with CaM kinase I.[61] Peptide truncates show that a particularly dense array of residues bound in the N-terminal domain of calmodulin is crucial, with the *i* (Val), *i* + *1* (Arg), *i* + *2* (His), *i* + *3* (Met) and *i* + *4* (Arg) positions of the peptide contributing strongly to binding (Fig. 4c).

Burgess has reasoned that since there is homology in the way many secondary structural elements display residues, it is possible to design 'universal peptidomimetics', able to reproduce the

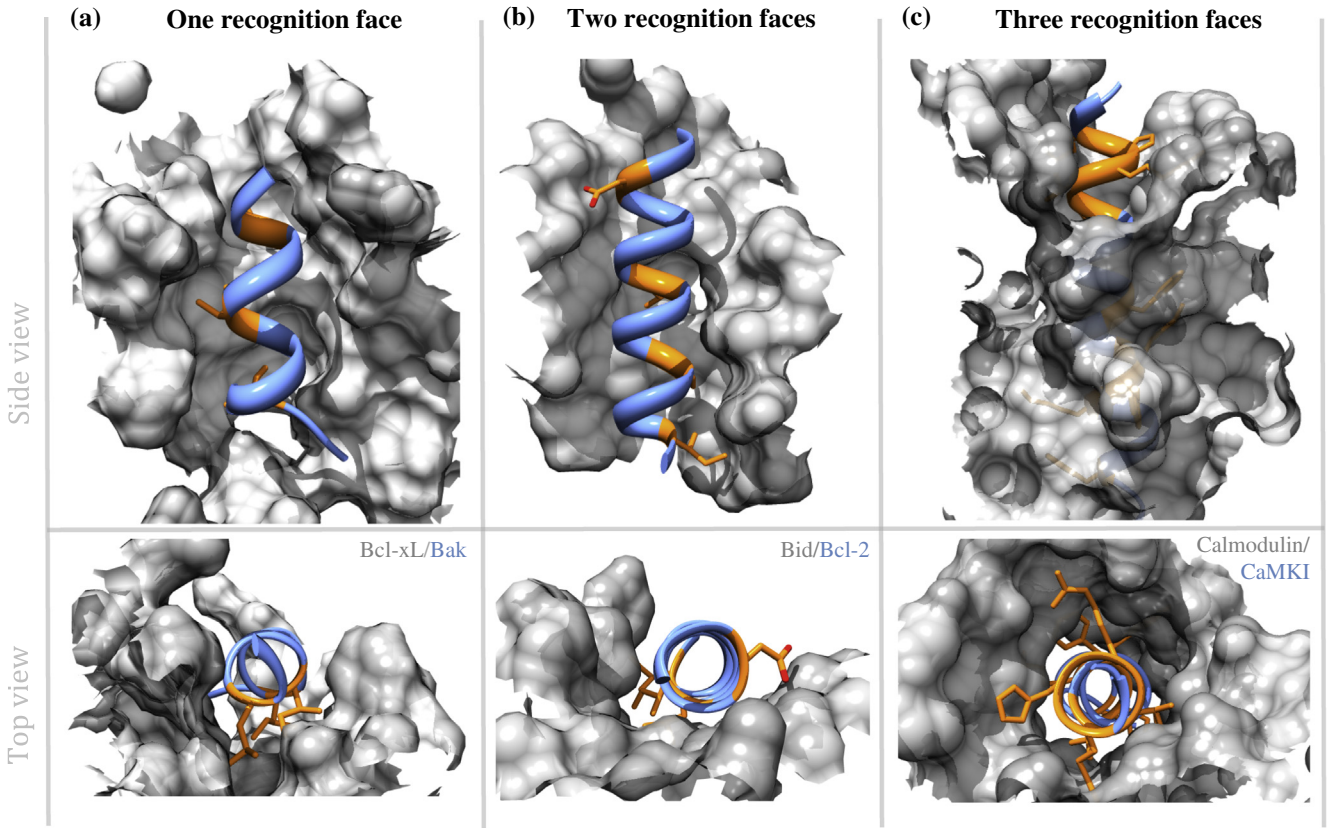
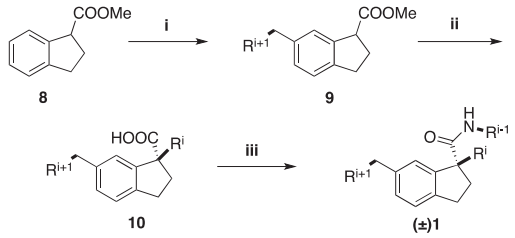


Figure 4. Examples of different α-helix binding modes; (a) Bcl-xL/Bak PPI (PDB: 1BXL), (b) Bcl-2/Bid PPI (PDB: 2VOI), (c) Calmodulin/CaMKI PPI (PDB: 1MXE).



Scheme 1. Reagents and conditions: (i) (a) $AlCl_3$, $MeNO_2$, (b) $R^{i+1}COCl$, reflux, (c) H_2, (50 psi), 10% Pd on C, CF_3CO_2H, EtOH (88%); (ii) (a) LiHMDS, THF, −78 °C, (b) XR^i, (c) NaOH, MeOH; (iii) (a) $(COCl)_2$, CH_2Cl_2, (b) $R^{i-1}NH_2$, NEt_3. R^{i+1} = Ph, XR^i = BnBr or gramine, MeI, R^{i-1} = $CH_2C(CH_3)_3$ or CH_2CH_2Ph.

recognition properties of a range of motifs.[62–65] An induced fit mode of binding is invoked in which conformationally flexible molecules are able to mimic residues on multiple faces.[66] The inherent plasticity of these scaffolds suggests that this approach would be particularly useful when targeting PPIs where the hot-spot residues are known, but the bound conformation is not. The strategy led to a peptidomimic of the *i*, *i* + *2*, *i* + *4* side chains of a peptide that inhibited dimerization of the HIV-1 protease with a K_i of 0.38 μM.[67] Some of the more conformationally flexible Hamilton scaffolds may allow mimicry of residues on multiple faces of a helix in a similar manner to the Burgess peptidomimetics, however preorganising molecules in bioactive conformations would seem to be more entropically favorable for ligand–protein binding.

The design and synthesis of double-sided mimetics is inherently more challenging than single-sided scaffolds due to the density of functionalization. Howson imitated the *i* and *i* + *1* geometry of a dipeptide fragment with a 1,6-disubstituted indane.[68] This was expanded to the racemic 1,1,6-trisubstituted indane **1** (Scheme 1), the (*S*)-enantiomer of which maps closely onto the *i* − *1*, *i* and *i* + *1* residues of an α-helix. These short helix mimics showed low

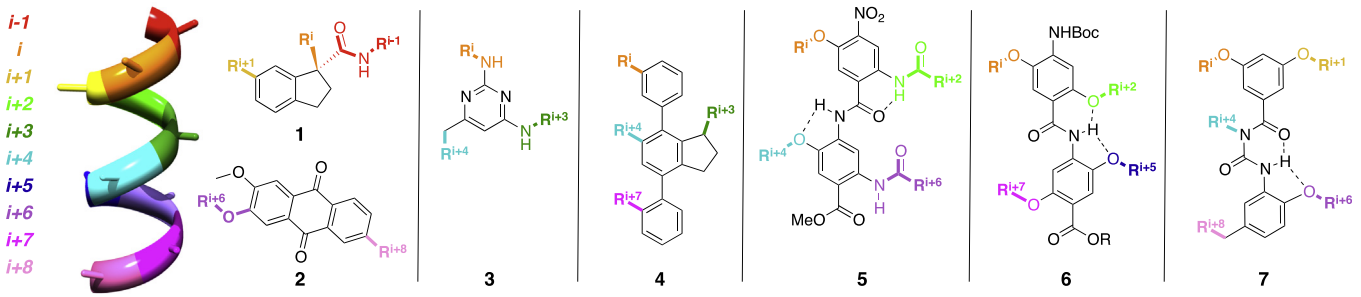


Figure 5. Schematic representation of an α-helix alongside double-sided mimics: indane **1**, anthracene **2**, pyrimidine **3**, diphenylindane **4**, oligobenzamides **5** and **6**, and benzoylurea **7**.

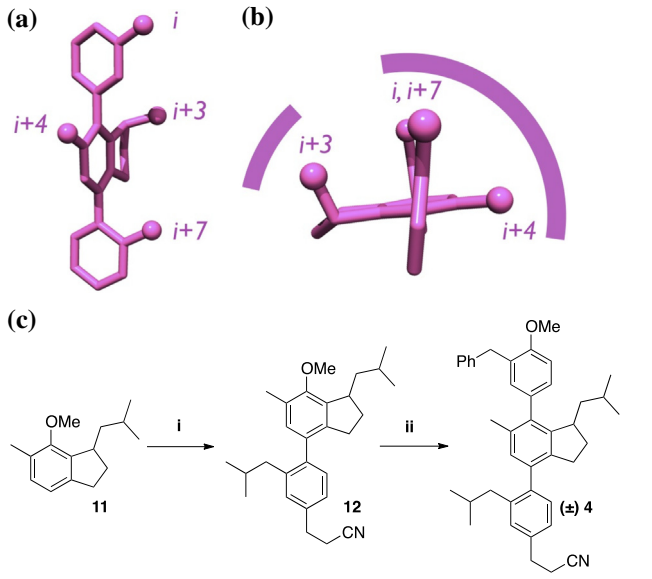


Figure 6. (a) Energy minimized conformation of a diphenylindane scaffold, side view.[74] (b) Top view: projection of side-chain groups looking down the helix mimetic. (c) Reagents and conditions: **11** six steps from *o*-cresol. (i) (a) NBS, AcOH, 85 °C, (b) ArOTf, $Pd(PPh_3)_4$, Cs_2CO_3, H_2O, DMF, 120 °C (77% over two steps); (ii) (a) BBr_3, CH_2Cl_2, (b) Tf_2O, Et_3N, CH_2Cl_2, (c) $ArSnBu_3$, CuBr, LiCl, DMF, 160 °C (overall 58%).

Table 1
*c*Log*P* values for double-sided scaffolds and the polarity of side chains that may be mimicked

Scaffold	*c*Log*P*	Side-chain mimicry	Reference
1	2.12	Hydrophobic	68,69
2	3.67	Hydrophobic	71
3	2.03	Hydrophobic	72
4	8.35	Hydrophobic	73
5	1.10	Polar and hydrophobic	78
6	3.13	Polar and hydrophobic	83
7	4.04	Polar and hydrophobic	91

*c*Log*P* values calculated with methyl substituents as side-chain mimics (ChemBioDraw 13.0, Cambridgesoft).

micromolar IC_{50}'s against tachykinin receptors, with many showing selective inhibition for NK_1R and NK_3R over NK_2R, which control dopamine release in different areas of the brain.[69,70]

Zhang et al. demonstrated that a naphthalene group could effectively span the width of an α-helix, allowing residues on opposite sides to be mimicked. An anthracene-based scaffold **2**, substituted at the 2- and 7-positions, mimicked residues in the *i* and *i* + *2* positions (Fig. 5).[71] A 24 μM inhibitor of the Bim/Bcl-2 PPI mimicked the Asp67 and Ile65 residues of the Bim helix (Fig. 4b).

Rodriguez et al. expanded the concept of double-sided mimicry in developing an inhibitor of the p106 helical peptide truncate/nuclear hormone receptor (NR) interaction.[72] X-ray crystallography showed that protein fragments with an LXXLL motif in a two-turn helical orientation bound in a hydrophobic groove in the NR. A pyrimidine scaffold **3** (Fig 5) mimicking residues in the *i*, *i* + *3* and *i* + *4* positions had a K_i of 29 μM. Substituting pyrimidine for trithiane or cyclohexyl ablated binding, while a triazine decreased inhibition by an order of magnitude. Larger hydrophobic side-chains resulted in increased activity, illustrating the importance of subtle structural modification and the advantage of non-proteogenic approaches in which a large library of synthetic building blocks may be used. The strategy was further expanded with the design of the diphenylindane *i*, *i* + *3*, *i* + *4* and *i* + *7* mimic **4** (Fig. 6).[73,74] The synthetic route provides the final product as a racemate but with sufficiently low energy aryl–aryl rotational barriers for interconversion of the diastereomers at room temperature, thus allowing an induced fit mode of binding for one of the enantiomers. To increase the utility of this scaffold, asymmetric approaches for the synthesis of single enantiomer indanes are required.

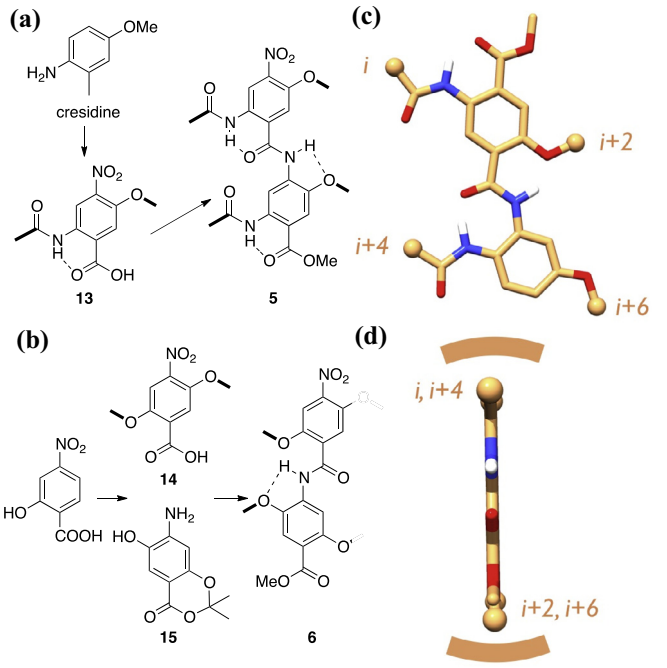


Figure 7. Syntheses of double-sided scaffolds: (a) Hamilton's oligobenzamide; (b) Marimganti's oligobenzamide. Energy minimized conformation of the oligobenzamide: (c) side view; (d) top view: projection of side-chain groups looking down the helix mimetic.[74]

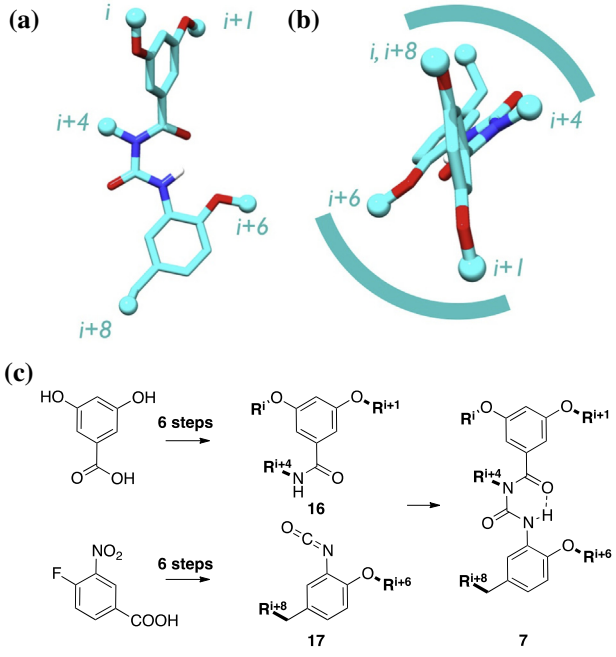


Figure 8. (a) Energy minimized conformation of double-sided benzoylurea scaffold, side view.[74] (b) Top view: projection of side-chain groups looking down the helix mimetic. (c) Synthesis of double-sided benzoylurea scaffold. The monomeric units were easily assembled from commercially available starting materials.

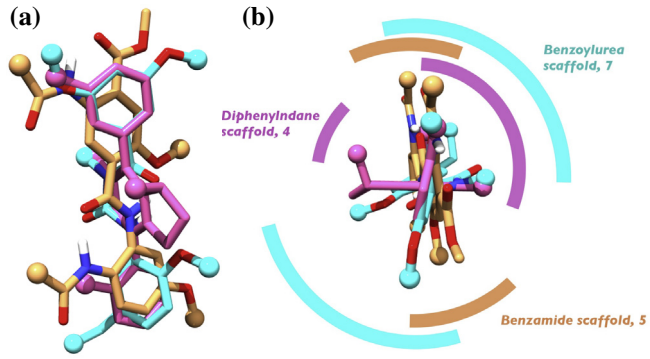


Figure 9. Superimposition of lowest energy conformations of diphenylindane (pink), benzamide (brown) and benzoylurea (cyan) scaffolds showing differing side chain projections: (a) side view; (b) top view.[74]

Many of the early mimics mediate binding predominantly through hydrophobic interactions and the exclusion of water—also a major thermodynamic driving force in the association of proteins.[75,76] Interactions of this type are not necessarily non-specific: for example a 1-naphthyl-substituted terphenyl displayed selective inhibition of the HDM2/p53 PPI over the Bcl-2/Bak PPI, while a 2-naphthyl analogue reversed the selectivity.[54] The hydrophobicity of these molecules is an important factor in the good cell permeability of this series,[77] but limits aqueous solubility,[58] providing an impetus for the development of new scaffolds. For example scaffolds **1**–**3** have moderate *c*log*P* values (Table 1), however only hydrophobic groups can be appended, further increasing their hydrophobicity. Later scaffolds, for example, **5**–**7** incorporate heteroatoms that act to increase aqueous solubility and provide additional synthetic versatility for the incorporation of a greater range of side-chains.

An example of a heteroatom-containing scaffold is the double-sided oligobenzamide **5** based on an earlier single-sided variant (Fig. 7a).[78,79] The single-sided oligobenzamide has been employed successfully to inhibit the Bak/Bcl-xL, p53/hMDM2 interactions, and to inhibit islet amyloid peptide aggregation.[80–82] The oligobenzamide scaffold allows for facile incorporation of a variety of different side-chain mimics and with increased aqueous solubility. This new mimic uses an amide linker to install the *i* + *2* and *i* + *6* residues, which also defines the geometry of the molecule through the formation of two further hydrogen-bonded six-membered rings. The densely functionalized monomer **13** (Fig. 7a) was synthesized in three steps (50% overall yield) from *meta*-cresidine. Sequential coupling of different monomeric units from a common starting material adds to the synthetic flexibility.

The Ahn group developed a conformationally restricted bisbenzamide scaffold **6** (Fig. 7b).[83] Five-membered hydrogen bonds between the backbone amide proton and *ortho*-oxy group places the *i* + *2*/*i* + *5* and *i*/*i* + *7* groups on opposite faces. A common precursor was used to synthesize both monomeric units **14** and **15**. Sequential alkylation of the hydroxyl groups allows for different residue mimics to be installed in a linear manner, providing a rapid and flexible synthesis. The Wilson group detailed the synthesis of several benzamide helix mimics with N- and O-alkylation allowing side-chain attachment.[84,85] The synthesis of oligobenzamides was further facilitated by loading of the monomer onto Wang resin allowing for solid-phase assembly of large libraries.[86] A single sided benzamide from the same group showed an impressive 40 nM potency against the androgen receptor/PELP1 co-regulator PPI, a pathway which is often upregulated in tumors.[87] Cell-based assays and in vivo mouse studies highlight the increased aqueous solubility and cell permeability of these second generation compounds.

The planarity of benzamides limits side-chain projection to opposite faces, restricting the use of such scaffolds to PPIs with

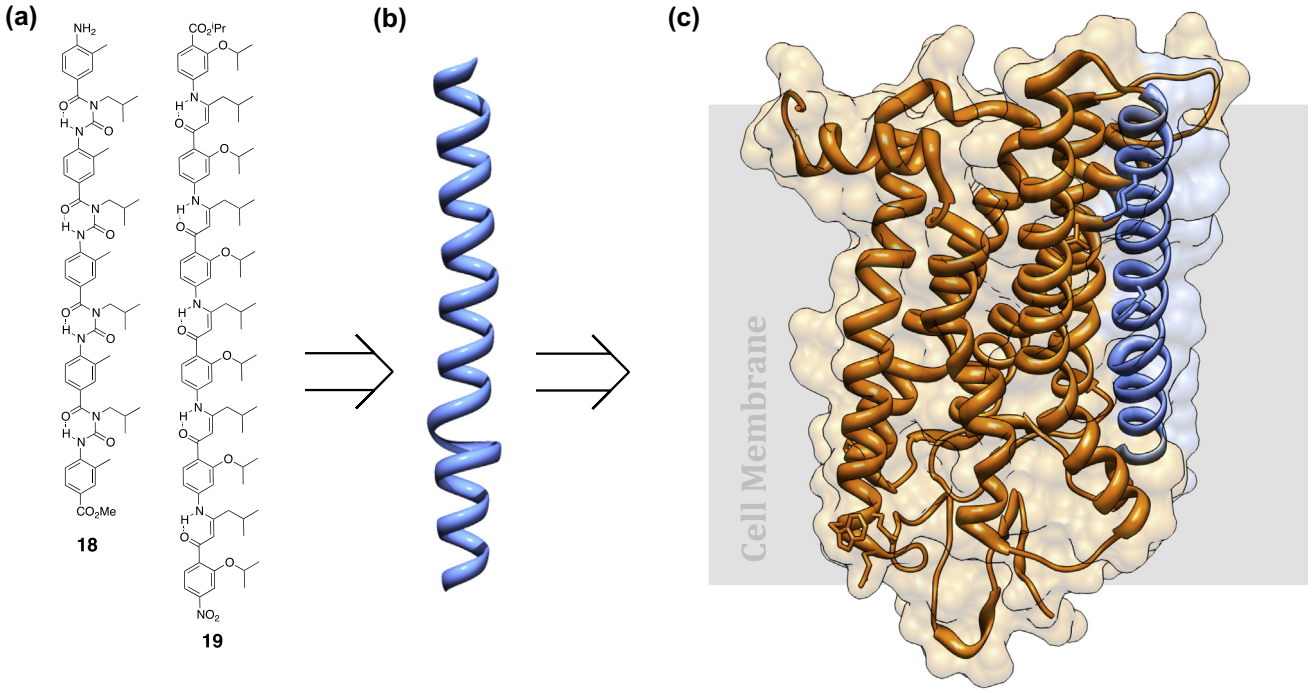


Figure 10. (a) Extended benzoylurea **18** and enaminone **19** scaffolds, (b) nine turn α-helix from a membrane spanning opsin GPCR, (c) PDB: 3CAP. GPCR = G protein-coupled receptor.

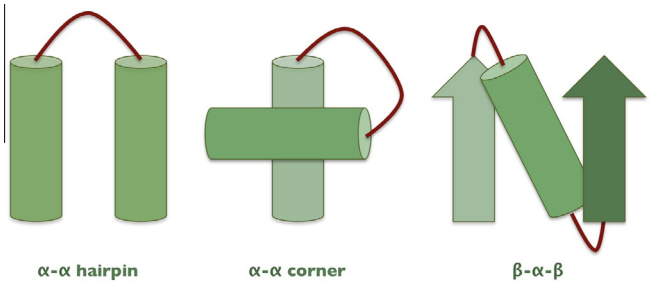


Figure 11. Common super secondary structural motifs containing α-helices (cylinders), β-strands (arrows) and loop sequences (red).

hot-spot residues at 180° to one another (Fig. 7c and d). The Spivey group overcame this limitation by designing a scaffold with an azabicyclo[2.2.2]octane moiety coupled to an aryl fragment, mimicking the *i*, *i* + *1*, *i* + *2*, *i* + *4* and *i* + *5* residues.[88]

The benzoylurea scaffold can similarly mimic a more complex presentation of residues, with a convergent synthesis allowing incorporation of hydrophobic and hydrophilic groups.[89,90] The Hamilton group synthesized double-sided mimic **7** from a benzamide **16** and an isocyanate **17** (Fig. 8c).[91] The helical backbone is reminiscent of earlier benzamide helices created by the group,[82,91] but allows for a more comprehensive distribution of side chains (Fig. 8b). Existing scaffolds reproduce groups on a number of different faces of the helix (Fig. 9), suggesting that while a three-sided helical peptidomimetic remains elusive, several frameworks exist for targeting PPIs with complex binding modes.

Extended helices are found in important therapeutic targets; for example transmembrane GPCR (G protein-coupled receptor) helices which span cell membranes and assemble due to both hydrophobic and hydrophilic side-chain interactions.[92] Membrane-spanning amphiphilic α-helices form artificial ion-channels, mimicking the acetylcholine receptor.[93] Synthetic variants may be able to replicate this behavior or inhibit endogenous

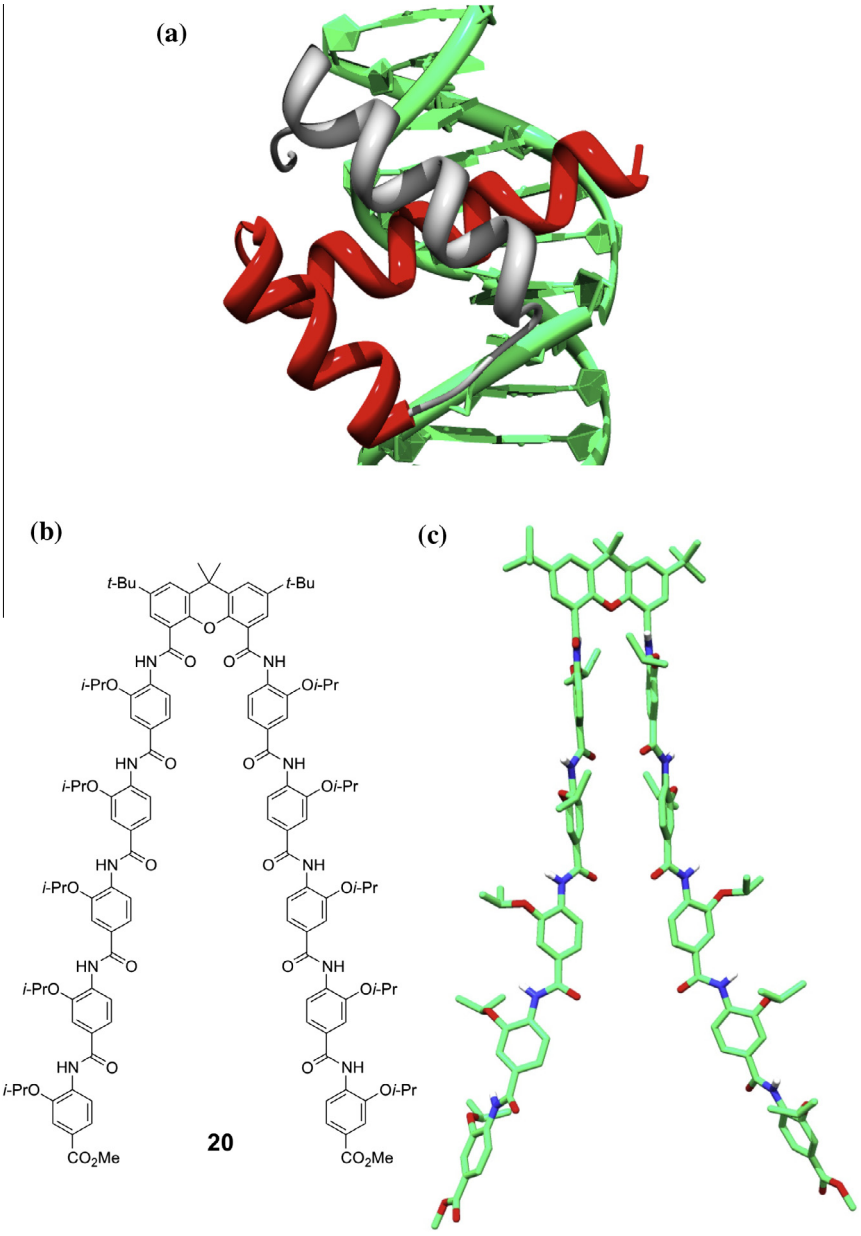


Figure 12. (a) HTH (red) of Pax class homeodomain dimer bound to DNA (green, PDB: 1FJL). (b) Structure and energy minimized conformation of bis-pentabenzamide HTH mimic **20**.[105]

ion channels. The Hamilton group elongated the single-sided benzoylurea scaffold to an oligomeric species mimicking a 37 Å α-helix **18** (Fig. 10). The easily assembled benzoylurea ring surrogate enabled facile extension of the scaffold resulting in a mimic that exhibited a preference for the extended conformation.[89] A similar result was attained by the same group with an enaminone scaffold **19** which was extended to a ten turn α-helix mimic.[94] It was also possible to incorporate polar side-chains, thus broadening the potential for therapeutic applications. Other extended scaffolds have been designed by the Rebek, Ahn and Wilson groups, with the latter exploiting solid phase synthesis to facilitate oligomerisation.[95–98]

Peptidomimetic design principles may be harnessed to replicate elements of supersecondary structure.[17] Commonly occurring combinations of secondary structural elements constitute α-hairpins, α-corners and β-α-β motifs (Fig. 11). Helical hairpins are important structures aiding protein insertion and transport across cell membranes.[99] Given the majority of folded protein is buried in the hydrophobic core, loop sequences are found at protein surfaces. To replicate these more complex structures, non-peptidic loop or hairpin sequences have been designed to template the display of α-helical mimics.[100–104] A series of linked oligobenzamide and oligopyridylamide mimetics have the potential to function in a similar way.[105] Flexible syntheses with a variety of linking groups give versatile helix-turn-helix (HTH) mimics that can be tuned for inter-helix angle and length (Fig. 12b). Linked helix mimetics may find a role in inhibiting important protein–DNA interactions, such as the helix-turn-helix (Fig. 12a) or basic-helix-loop-helix, which regulate gene transcription. The DNA binding domain of the proto-oncogene c-Myc is an example of a HTH tri-helical protein.[106] The helical turn forms a hydrophobic cavity, while simultaneously projecting polar residues towards the negatively charged phosphate DNA backbone. These classes of HTH mimic allow controlled projection of side chains in three-dimensional space and thus the reproduction of relatively large protein surfaces. Surface mimicry has been successfully attempted with both cyclic peptides and backbone grafting onto protein scaffolds.[107,108] Synthetic scaffolds have also been employed by Ghosh and Hamilton in immobilizing peptide loops on G-quadruplexes and thus imitating multi-loop protein sufaces.[109] The same group previously designed a large protein surface mimic by coupling cyclic peptides on to a calixarene scaffold, which showed strong binding to cytochrome c.[110] Surface recognition has been demonstrated with porphyrin species,[111] copper- and ruthenium-complexes,[112,113] illustrating the diversity of scaffolds available.

If the lessons learned from the development of early peptidomimetics are extended to produce synthetic antibodies, these non-peptidic constructs may allow several problems with the use of biologics in vivo to be circumvented. Despite the growing number of this type of therapeutic, prohibitive cost, poor oral bioavailability and the difficulties associated with maintaining homogeneity currently present major obstacles.

The last two decades have seen extensive efforts in the design and synthesis of α-helix mimetics as synthetic agents for the mediation of PPIs. Many of these constructs have been thoroughly characterized and shown to provide good structural mimicry, with some demonstrating functional mimicry in vitro. Researchers have targeted a relatively small number of interfaces, which have frequently relied on single face binding mediated by hydrophobic groups. Given the myriad of therapeutically relevant PPIs the challenge for researchers is to extend the scope of these mimetics to provide a diverse collection allowing intervention at a broader range of interfaces, and with improved pharmacokinetic properties.

Acknowledgments

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