Expert Opinion

1. Introduction
2. Reverse-turn recognition and mimicry
3. Helix recognition and mimicry
4. Expert opinion

informa
healthcare

General

Privileged scaffolds targeting reverse-turn and helix recognition

Ye Che & Garland R Marshall†
Washington University, Center for Computational Biology and Department of Biochemistry and Molecular Biophysics, St. Louis, MO 63110, USA

Background: Protein–protein interactions dominate molecular recognition in biologic systems. One major challenge for drug discovery arises from the very large surfaces that are characteristic of many protein–protein interactions. *Objectives*: To identify 'drug-like' small molecule leads capable of modulating protein–protein interactions based on common protein-recognition motifs, such as α-helices, β-strands, reverse-turns and polyproline motifs for example. *Overview*: Many proteins/peptides are unstructured under physiologic conditions and only fold into ordered structures on binding to their cellular targets. Therefore, preorganization of an inhibitor into its protein-bound conformation reduces the entropy of binding and enhances the relative affinity of the inhibitor. Accordingly, this review describes a general strategy to address the challenge based on the 'privileged structure hypothesis' [Che, PhD thesis, Washington University, 2003] that chemical templates capable of mimicking surfaces of protein-recognition motifs are potential privileged scaffolds as small-molecule inhibitors of protein–protein interactions. The authors highlight recent advances in the design of privileged scaffolds targeting reverse-turn and helical recognition. *Conclusions*: Privileged scaffolds targeting common protein-recognition motifs are useful to help elucidate the receptor-bound conformation and to provide non-peptidic, bioavailable substructures suitable for optimization to modulate protein–protein interactions.

Keywords: drug discovery, helix, interaction, privileged structure, protein–protein reverse turn

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1. Introduction

Protein–protein interactions are central to many key biologic pathways and, thus, are attractive targets for drug discovery [1-6]. However, developing small molecules that modulate protein–protein interactions is generally considered difficult. The challenge with protein–protein interaction sites, is that the interaction surface involved is between 750 – 1500 Å^2, vastly exceeding the potential binding area of a low molecular weight compound. At first glance, trying to modulate an interaction of this type with a typical 'rule of five'-compliant small molecule [7] appears incredibly difficult to many people at first glance. Thus, protein–protein interactions have become known as 'hard targets' and have often been dismissed in the past as 'undruggable'. The key question in this field was whether any systematic approaches for inhibiting protein–protein interactions could be developed.

Recent studies of protein interactions involved in cell regulation and signaling have identified a large number in which one component involves a flexible or unstructured region of the polypeptide chain under physiologic condition that

folds into ordered structures only on binding to their cellular targets [8-16]. In addition, database analysis indicated that there was a high abundance of intrinsic disorder in signaling proteins, as well as in proteins associated with cancer, neurodegenerative diseases and cardiovascular diseases [17,18]. Coupled folding and binding often gives to a protein complex with high specificity and relatively low affinity, which is appropriate for signal transduction proteins that must not only associate specifically to initiate the signaling process, but must also be capable of dissociation when signaling is complete. Nature optimizes rates and system dynamics rather that affinities *per se*. Another advantage of a system that uses components that fold on binding is that the conformational flexibility facilitates the post-translational modifications of proteins [19,20]. Conformational flexibility allows a protein to bind to both its physiologic target and to modifying enzymes. It has been shown that regions undergoing disorder-to-order transitions during interaction with binding partners are very common in signaling proteins and the concept of molecular recognition features was proposed to account for these regions [21]. The thermodynamic consequence is that there is an entropic cost associated with the disorder-to-order transition that accompanies the binding of an intrinsically unstructured protein to its target. It is estimated (see Mammen *et al.* [22] for a thorough discussion of torsional entropy) that elimination of a single rotational degree of freedom of a peptide by preorganization to stabilize the receptor-bound conformation enhances affinity by ~ 1.2 – 1.6 kcal/mole assuming complete (unlikely at physiologic temperatures) loss of rotational degrees of freedom [23]. Thus, preorganization of an inhibitor into its protein-bound conformation should reduce the entropy of binding and potentially enhance the binding affinity by orders of magnitude. Therefore, it has been proposed that intrinsically disordered proteins represents a novel type of drug targets and protein–protein interactions involving one disordered partner are, perhaps, more drugable sites of interaction that can be used to fill drug discovery pipelines [1,6,24].

In fact, the recognition of peptide hormones by their receptors can be viewed as a special case of protein–protein interactions involving one unstructured partner. It has been a topic of interest ever since du Vigneaud and co-workers [25] first explored the chemical basis of specificity of the non-apeptide hormones oxytocin and vasopressin. While peptides have wide therapeutic application, they are often limited because of undesirable absorption, distribution, metabolism and excretion properties, undesired side effects due to undesirable interactions of conformationally flexible peptides with non-targeted receptors [26]. This has led to the concept of peptidomimetics, compounds which have different, and often conformationally constrained, chemical structures that still maintain the ability to interact with a specific peptide receptor [27]. Often, peptidomimetics arise from chemically significant modifications of existing peptides or by the use of rigid non-peptidic scaffolds with only limited flexibility, in order to imitate the three-dimensional structure of a peptide in its receptor-bound conformation as closely as possible. This reduction in the decrease of freedom may eventually lead to receptor binding with high affinity because of entropic reasons, provided that the receptor binding is not compromised in the modified peptide. One example was the design of a series of cyclic, conformationally restricted analogs of somatostatin, an inhibitor of hormone receptors. One of the potent analogs, a cyclic octapeptide, exhibited high affinity (the potency is 7800 times somatostatin) and selectivity for μ-opiate receptor [28]. Octreotide, a cyclic peptide analog of somatostatin, has been approved for the treatment of acromegaly and of patients with metastasizing carcinoid and vasoactive tumors [29].

From the authors' perspective, the best place to look for small molecules that interfere with protein–protein interactions are peptidomimetics; chemical scaffolds that mimic the most common protein recognition motifs. By suitable decorating such chemical scaffolds, they are able to provide ligands for multiple, unrelated classes of protein targets with high affinity. Therefore, these chemical scaffolds can be viewed as privileged structures [30] that provide the medicinal chemist with common, non-peptidic, orally available substructures as suitable starting points in combinatorial synthesis. Common protein recognition motifs comprise repetitive structures, such as α-helix or β-sheet and non-repetitive structures, such as a reverse-turn or loop. This review highlights recent advances in the design of privileged scaffolds targeting reverse-turn and helical recognition.

2. Reverse-turn recognition and mimicry

A reverse-turn is a structural motif that invariably lies on the surface of proteins that often participates in protein–protein interactions [31]. Receptor recognition, substrate specificity and catalytic function generally reside in these loop regions, which often connect residues of adjacent α-helices and β-strands, contributing to the structural stability of proteins. Reverse-turns comprise a diverse group of structures with a well-defined three-dimensional orientation of amino acid side chains. β-Turns constitute the most important subgroup and are formed by four consecutive amino acids. Examples of turns as recognition motifs can be readily found in peptide antigen–antibody complexes [32]. Structure–activity relationship studies of many peptide hormones interacting with G-protein-coupled receptors (GPCRs) have indicated that the hormones are probably in reverse-turn conformations when bound to their receptors [33,34].

2.1 Non-peptidyl reverse-turn mimetics

It is desirable to have a repertoire of scaffolds that reliably transform the information present in reverse-turn motifs, seen in proteins, into non-peptidyl compounds of low

molecular weight. The desired reverse-turn conformation should be imitated as closely as possible and the synthetic route for the non-peptidyl mimetic should permit the introduction of appropriate side chains onto the mimetic scaffold. Thus, the mode of action of a biologically active peptides on the protein target can be imitated by the small molecule (agonist) or can be blocked (antagonist). Today, such compounds – that combine bioavailability and stability superior to that of bioactive peptides with increased receptor selectivity – are the subject of major interest by pharmaceutical companies.

Examples of privileged structures used to mimic reverse-turn motifs include, for instance, the benzodiazepine (**Figure 1** (**1**)) scaffolds [30,35,36]. The benzodiazepine ring is a core element of a natural product, asperlicin, which was discovered during a screening of fungal metabolites and was found to be a cholecystokinin A (involved in the control of appetite) antagonist [37]. Asperlicin was combined with a D-Trp structural motif, culminating in the synthesis of a selective orally administered peptidomimetic antagonist of the peptide hormone cholecystokinin [38]. The benzodiazepine derivatives continue to generate leads against multiple protein receptors [39-43]. The benzodiazepine scaffold, which is probably the best known privileged platform, has also produced farnesyl transferase inhibitors, reverse transcriptase inhibitors and ligands for the HIV-1 Tat protein [44], in addition to leads for GPCRs and ion channels. This use in targeting peptide receptors is rationalized by the ability of benzodiazepines to mimic the entire set of classical β-turns in its ability to orient four side chains (Ripka *et al.* [24], Hata *et al.* [25]).

Monosaccharides provide an excellent platform to tailor molecular diversity by appending desired substituents at selected positions around the sugar scaffold (**Figure 1** (**2**)). It was Hirschmann *et al.* [45,46], who conduced the pioneering work and demonstrated the use of β-D-glucose as a scaffold in the synthesis of somatotropin release-inhibiting factor peptidomimetics targeting somatostatin receptors. Three residues, Phe-Trp-Lys, contain the necessary functional information, but it is the relative positioning of these side chains that determine the affinity and selectivity for one or more of the five subtypes of somatostatin receptors. Substituents mimicking these amino acid side chains were positioned on a β-D-glucose scaffold in a way that ensure the distances between the pharmacophoric groups were similar to those of somatostatin. Hirschmann *et al.* [47] later showed that compounds with modulated receptor subtype affinity are obtained by altering stereochemical centers in the scaffold. D-Glucose, L-glucose and L-mannose structural isomers were synthesized and displayed different subtype selectivity for somatostatin receptors. Kessler and co-workers [48] also employed the carbohydrate scaffold to develop ligands for the integrin family. Starting from identifying a bioactive cyclic peptide and NMR determination of bioactive peptide conformations, molecular modeling was used to design a small set of mimetics based on β-D-mannose. This led to the identification of $\alpha_4\beta_1$-selective integrin antagonists. Carbohydrate-like scaffolds are being used increasing in drug design: scaffolds, such as tetrahydrofuran rings from D-mannitol [49], artificial amino pyranose rings [50] and the chemically more challenging natural glycosides, such as β-mannoside, have been explored (see recent reviews [51-55]).

Numerous additional non-peptidyl systems have been designed to mimic different types of reverse-turns. Of particular interest has been the replacement of a dipeptide motif in a given bioactive peptide with a constrained or rigidified counterpart (**Figure 1** (**3**)). Freidinger *et al.* [56] have prepared an analog of luteinizing hormone-releasing hormone containing a γ-lactam as a conformational constraint. The analog was more active as a luteinizing hormone-releasing hormone agonist than the parent hormone and provided evidence for a bioactive conformation containing a β-turn. The attachment of one or more rings to the basic Freidinger lactam structure was also possible. Fused lactam [57-61], spirolactam bicyclic [62] and tricyclic [63] systems were all examples that partially constrained the four backbone torsion angles of residues $i + 1$ and $i + 2$ and enhance reverse-turn propensity. By its very nature, such a motif could also encompass heteroatom analogs, in which carbon is replaced by sulfur, oxygen or nitrogen, at different synthetically attainable sites. The presence of functional groups as pendant substituents on the lactam ring system or its heteroatom congeners also provides opportunities for additional diversification.

2.2 Conformationally constrained peptides for reverse-turn mimicry

Conformational and topographical restrictions are particularly suited as manipulation for reverse-turn mimicry towards an increase of receptor selectivity, metabolic stability and the development of highly potent agonists or antagonists. One straightforward approach for peptide modification is to introduce a covalent linkage between residues i and $i + 3$, such as head-to-tail cyclization, which retaining the reverse-turn conformation. Cyclic peptides form a large class of naturally occurring or synthetic compounds with a variety of biologic activities, such as hormones, antibiotics, ion-transport regulators, toxins for example. They have been reported to bind multiple, unrelated classes of receptors with high affinity. Thus, cyclic peptides are considered to be privileged structures capable of providing useful ligands for more than one receptor, due to their high content of reverse-turn motifs. Another approach is to incorporate heterochiral dipeptides as residues $i + 1$ and $i + 2$. Nearly all biologic polymers are homochiral: all amino acids coded and incorporated by protein synthesis are left-handed; whereas all sugars in DNA/RNA and in metabolic pathways, are right-handed. It is the homochirality of naturally occurring amino acids that allows proteins to adopt

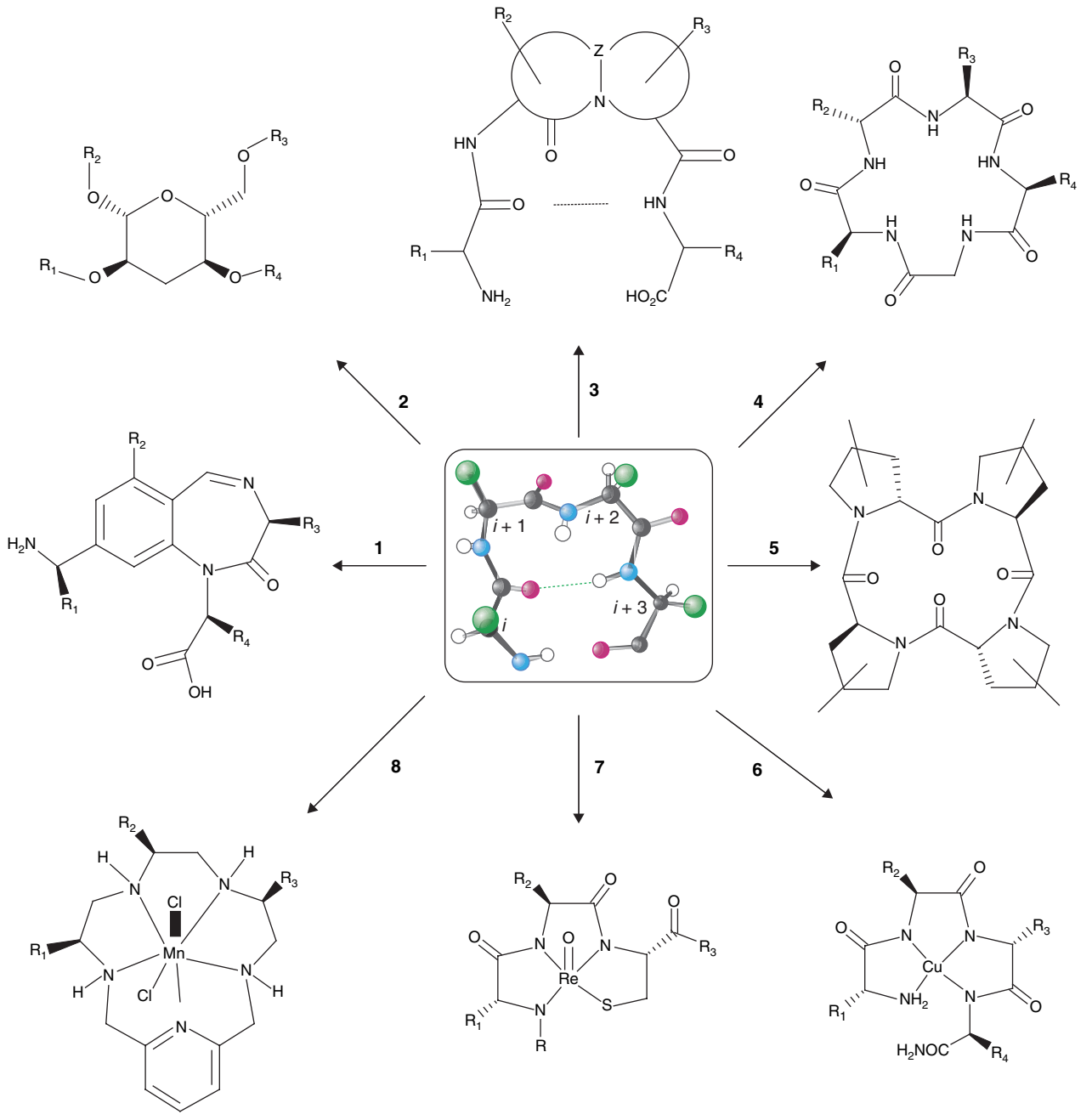

Figure 1. Privileged scaffolds for reverse-turn recognition: benzodiazepines (1), sugars (2), lactams (3), cyclopentapeptides with heterochiral dipeptide segments (4), cyclotetraprolines with chimeric amino acids (5), metal complexes of linear peptides (6), metal ion-induced distinctive array of structures (7) and metal complexes of chiral azacrowns (8).

regular conformations, such as the α-helix and the β-sheet. The incorporation of heterochiral (D,L-alternating) dipeptides into a peptide chain abruptly changes the direction of the peptide. For example, Marshall and co-workers [64,65] suggested that D-Pro-L-Pro, L-Pro-D-Pro, D-Pro-L-Pip, L-Pro-D-Pip, D-Pro-L-NMe-AA and L-Pro-D-NMe-AA (where AA: amino acid other than Gly; Pip: pipecolic amino acid; NMe: *N*-methylation) offer relatively rigid scaffolds on which to orient side chains for interactions with receptors that recognize reverse-turn structures. Similarly, Gellman and co-workers [66,67] described that the β-amino acid heterochiral dinipecotic acid segments, R-Nip-S-Nip and S-Nip-R-Nip (where Nip: nipecotic acid), could also promote reverse-turn formation. Smith *et al.* [68] also demonstrated that heterochiral pyrrolinones preferentially adopt a turn structure.

Kessler *et al.* [69] first established the concept of 'spatial screening' (**Figure 1** (**4**)), whereby small libraries of cyclic heterochiral penta- and hexapeptides as conformational scaffolds for probing receptor recognition, where a recognition motif (such as Arg-Gly-Ser or Leu-Asp-Thr tripeptide segments for integrin receptors) were systematically shifted around cyclic peptide–backbone structures containing different chiralities to sample different three-dimensional presentations of pharmacophoric side chain groups, ultimately yielding compounds with nanomolar affinities and high selectivity [70-73]. The Kessler group in collaboration with Merck KgaA has used the results from the 'spatial screening' with constrained cyclic peptides to guide the development of selective nanomolar non-peptide molecule inhibitors for $\alpha_V\beta_3$, $\alpha_V\beta_5$ and $\alpha_V\beta_6$ integrins [73]. One peptidic $\alpha_V\beta_3$ inhibitor, c[RazaGDf(NMe)V], was reported in Phase II clinical studies and formed the basis the for design of nanomolar non-peptidic clinical candidates [74]. A similar overall philosophy was employed by Fujii *et al.* [75] to discover potent antagonists of C-X-C motif receptor 4, the GPCR co-receptor that interacts with the complex of gp120 and CD4, that blocked HIV infectivity. Porcelli *et al.* [76] also used this approach to discover a novel substance P antagonist. However, earlier theoretical and experimental studies [77] have demonstrated a considerable degree of conformational averaging in NMR studies of cyclopentapeptides advocated as receptor probes. This has stimulated Che and Marshall [78] to examine cyclotetrapeptides (CTPs), the minimalist reverse-turn mimetic, based on heterochiral dipeptides of chimeric amino acids to be used as conformational templates, for instance, c[D-Pro-L-Pro-D-Pro-L-Pro] (**Figure 1** (**5**)), as synthetic routes to chimeric prolines containing 2-, 3-, 4- or 5-position substituents on proline are abundant. The presence of four functionalized and stereochemically controlled centers on each proline ring offers chemists ample opportunity to custom design molecules to fit a pharmacophoric model; libraries of such CTPs comprised of chimeric prolines would lead to rapid identification of geometrical requirements from compounds found active in library screening. Theoretical studies [78] indicated that most reverse-turn motifs seen in proteins could be mimicked effectively with a subset of CTP scaffolds.

2.3 Use of metals for reverse-turn mimicry

Efforts have extended conventional cyclization by disulfide, amide or carbon–carbon bonds through the use of metals and the introduction of specific metal-binding sites in the peptide itself. The use of a metal template as a strategy for controlling the conformation of a short peptide to mimic a reverse-turn motif was clearly enunciated and demonstrated by Tian and Bartlett [79]. Peptide complexes of the Cu(II) ion (**Figure 1** (**6**)) were used to adopt the appropriate conformation to mimic the Trp-Arg-Tyr segment of tendamistat, a protein inhibitor of α-amylase. The metal complexes oriented the triad around a β-turn in a fashion similar to tendamistat, for which these residues are central to binding interactions with α-amylase. These mimetics were based on the structure of the complex of Cu(II) with pentaglycine where the N-terminal amino group and the next three amide nitrogens showed square-planar coordination to the metal. Three tetrapeptides containing Trp, Arg and Tyr residues showed ~ 100-fold increases in inhibition in the presence of Cu(II). One complicating factor in this study was the dissociation of copper from the complex with its inherent amylase–inhibitor activity. It is most desirable that any metal complex has stability in the relevant biologic milieu to reduce ambiguity in its mechanism of action and to reduce possible toxicity.

Shi and Sharma [80] have developed a combinatorial approach entitled metal-ion induced distinctive array of structures in which the amide nitrogens of the N-terminal two amide acids of a peptide preceding a cysteine residue react with a rhenium reagent leading to formation of a stable rhenium complex (**Figure 1** (**7**)). This leads to stable complexes with similar geometry to the Cu(II) complexes of Tian and Bartlett. A selective inhibitor of human neutrophil elastase [80] and a highly selective agonist of the melanocortin-1 receptor [81] were discovered with the metal-ion induced distinctive array of structures approach.

Marshall and co-workers [82-85] explored the use of metal complexes of chiral azacrowns (MACs) derived from amino acid synthons as a strategy for controlling the conformation and fixing chiral side chains in orientations comparable with those of reverse turns (**Figure 1** (**8**)). Reduction of the amide bonds to secondary amines of a cyclic peptide precursor leads to a flexible azacrown and the flexibility can be limited by complexation with a metal to fix the side chain orientations into a manageable set [86]. Proof of concept of MACs providing a novel approach to peptidomimetics came from two examples, where the receptor-bound conformations had been previously determined by X-ray crystallography of peptide–receptor complexes [83]. One MAC was designed to mimic the proposed receptor-bound conformation of the Arg-Gly-Asp motif to the cyclic pentapeptide, c[RGDfMeV], complexed with the $\alpha_V\beta_3$ integrin receptor. And the other MAC was designed to mimic the α-amylase-bound conformation of a Trp-Arg-Tyr β-turn motif from tendamistat. The metal center is buried in the middle of a MAC complex, acting like glue to keep the pharmacophoric groups correctly oriented in their desired directions. One must design a complex that affords the proper geometrical orientations, but it is essential that the metal be bound tightly so that no redox-active metals are allowed to dissociate from the complex *in vivo* to complicate bioassays with potentially toxic side effects. Riley and co-workers [87-93] have demonstrated that MACs possessed catalytic superoxide dismutase activity in a wide range of MAC analogs when complexed with manganese. These metal complexes showed reasonable thermodynamic

stabilities and excellent kinetic stability with the metal complexes completely intact under physiologic conditions and no metal dissociation for many hours even in the presence of ethylene-diamine-tetra-acetic acid. Clinical candidates for a variety of inflammation conditions, as well as ischemia–reperfusion injury, refractory hypotension and HIV-1 infection emerged from this class of metal complexes [90,94-96]. The fact that one MAC, M40403, has successfully completed Phase I and II clinical trials demonstrated that this class of metal complexes is relatively safe and possesses suitable pharmacokinetic properties (e.g., log *P*) for use as pharmacologic probes and potential therapeutic agents.

Several other groups have also used amino acid side chains (e.g., cysteine, histidine, lysine, aspartic acid) or chemically modified backbone to participate in specific metal ligation. A few examples serve to further illustrate this approach. Tamamura *et al.* [97-99] have shown that three peptides with significantly different cyclic constraints, including a Zn(II) complex, bind to C-X-C motif receptor 4. T22, a precursor of T134, has four Cys residues making two disulfide bonds and a β-hairpin conformation in solution. T22 (Zn), a derivative of T22 in which the four sulfurs of the Cys residues are bonded to Zn(II), has 4-fold the activity of T22. T134 has a characteristic turn motif (D-amino acid-Pro) and a disulfide bridge constraint to impose a β-hairpin structure in solution. The Marshall group [100-103] developed synthetic routes to modify the amide backbone to a hydroxymate, or phosphinic acid (Ye *et al.* Biopolymers, in press), group to provide multiple metal-binding sites. Similarly, Akiyama *et al.* [104] had previously replaced the amide bond with a hydroxymate in enkephalin to generate a metal-binding site. These peptides mimic the naturally occurring hydroxymate-containing siderophores involved in iron transport. Combinations of these approaches and complexation of the resulting compounds with different metals should provide useful probes of conformational preorganization with novel constraints for reverse-turn recognition.

3. Helix recognition and mimicry

The helix is a common secondary structural motif in proteins, a crucial recognition motif in many protein–protein and protein–nucleic acid interactions. Helices are found in proteins predominantly as α-helices, but occasionally as 3_{10}-helices. 3_{10}-Helices have also been implicated as recognition motifs in a number of protein–protein complexes [105,106]. In isolated helices, transition between the α- and 3_{10}-helical forms is facile with an estimated energy barrier of 3 – 4 kcal/mole [107]. This is primarily due to the fact that helix geometry of the peptide backbone allows a single amino group to makes two weaker bifurcated H-bonds in the transition state between the α- and 3_{10}-helices. The lowness of this barrier suggests that small peptide helices can be easily induced to bind in either helical conformation by interaction with their receptors. So far, helical peptidomimetics were designed primarily to imitate α-helical recognition functions [108].

3.1 Nonpeptidyl α-helix mimetics

As the critical surface for α-helical recognition often involves the side chains of residues *i*, *i* + 3 and/or *i* + 4 and *i* + 7, along one face of the α-helix, one can design appropriate scaffolds with limited conformations to orient attached functional groups that closely resemble the surface of α-helices. There are 3.6 residues per turn of an α-helix, with a rise of 1.5 Å per residue. The characteristic axial rise between these four key residues is 4.5 or 6.0 Å, respectively. Looking down the helical axis, residues are projected at -60° and 40° for $i \rightarrow i + 3$ and $i \rightarrow i + 4$ interactions, respectively. Hamilton and co-workerss [109-113] described a terphenyl scaffold (**Figure 2** (**9**)) that can reasonably imitate side chain orientations seen in α-helices in which the 3,2′,2″-substituents on the phenyl rings present functionalities in a spatial relationship that mimic the *i*, *i* + 3 or *i* + 4 and *i* + 7 residues on an α-helix. Comparing the terphenyl scaffold and the ideal α-helical structure, when the terphenyl is in a staggered conformation, the three substituents project from the terphenyl core with similar angular relationships and 5 – 30% shorter distances in the characteristic rise corresponding to $i \rightarrow i + 3$ and $i \rightarrow i + 4$ interactions in a native α-helix. Proof of concept for helix mimetics in protein–protein recognition came from successfully disrupting the interaction between calmodulin and an α-helical domain of smooth muscle light-chain kinase [109]; inhibiting the assembly of HIV-1 gp41 and, thereby, reducing levels of viral entry into host cells [110]; preventing the interaction between the proapoptotic protein Bak and the antiapoptotic protein Bcl-xL [111,112]; and blocking the complex formation of the tumor-suppressor p53 with the oncoprotein human double minute (HDM2) [113]. Based on theoretical arguments, Jacoby [114] proposed that 2,6,3′,5′-substituted biphenyl derivatives are protein α-helix mimetics superimposing the side chains of the residues *i*, *i* + 1, *i* + 3 and *i* + 4, better than other templates with a chiral axis, such as allene, alkylidene cycloalkane and spirane. Similarly, scaffolds based on terephthalamide [115], piperazinyl-pyrimidone [116], benzoylurea [117] and pyridazine heterocycle [118] have also been described as nonpeptidyl α-helix mimetics.

However, the terphenyl scaffold is not rigid; for example, it adopts both right- and left-handed twists. There are 16 energetically almost equal conformers, only two of which can mimic either of the desired α-helical side chain orientations. Thus, the terphenyl scaffold is not optimally preorganized in terms of α-helical mimicry, due to its conformational heterogeneity. Based on molecular modeling, Che *et al.* [108] described novel α-helix mimetics that are more effective than the terphenyl at constraining the

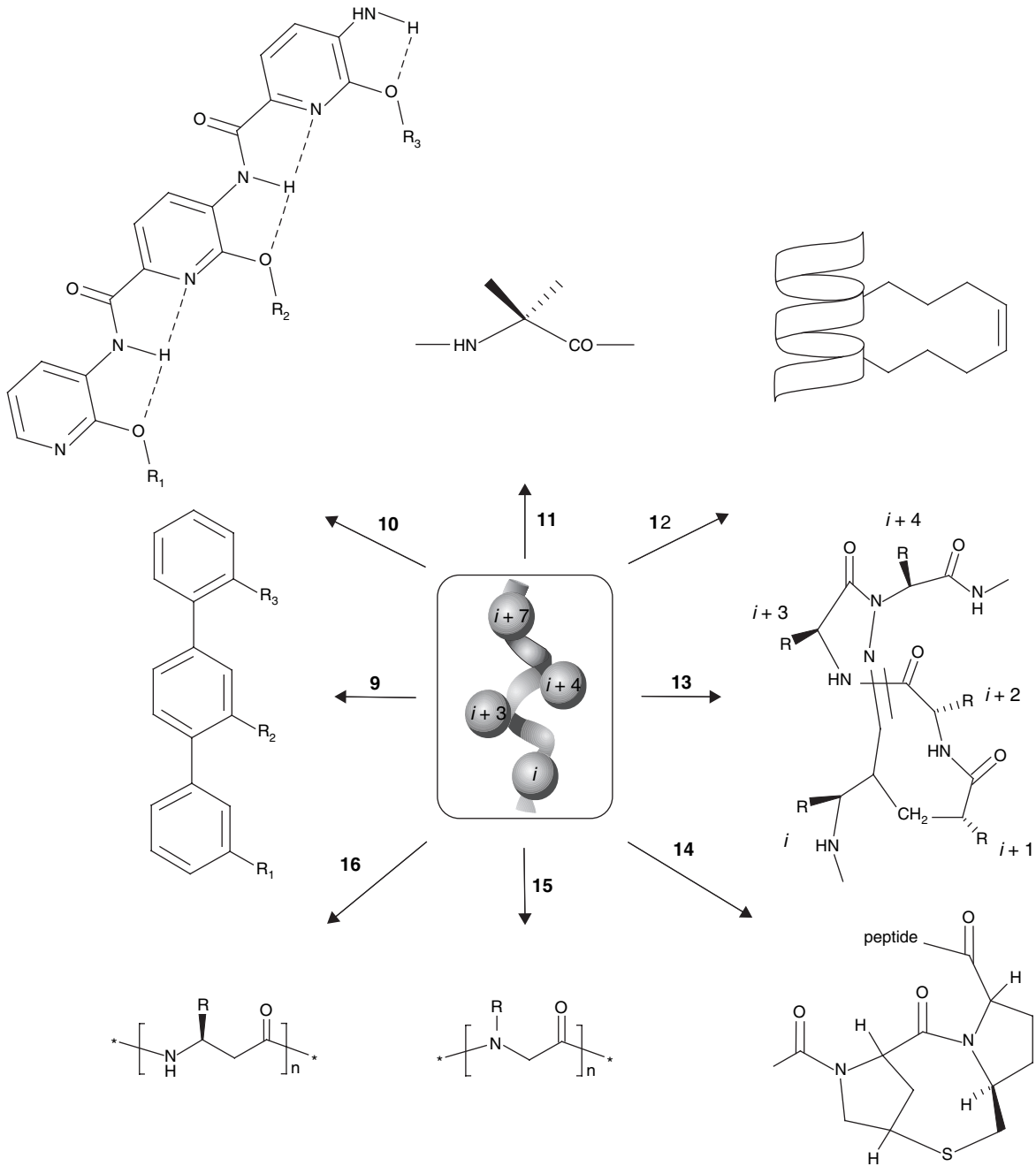

Figure 2. Privileged scaffolds for α-helical recognition: terphenyls (9), trispyridylamides (10), α,α-dialkyl amino acids (11), crosslinked interfacial peptides (12), H-bond surrogates (13), end-capping templates (14), β^3-peptides (15) and peptoids (16).

aryl–aryl torsion angles to those associated with structures suitable for mimicking the α-helical twist for side chain orientation and for superimposing those four key residues when compared with the α-β side chain vectors of the regular α-helix with improved root mean square deviation values. As an example of one alternative scaffold, the terpyridyl one is able to limit side chain orientation to a greater extent than does the terphenyls. The computational study also indicated that rotamer distributions around the Cα-Cβ bonds of these helix mimetics are similar to those of α-helices, except that the rotamer distributions show a 60° shift compared with those of α-helices when the mimetic axis is superimposed on the helix axis. This change in rotamer orientation complicates mimicry of the helix surface as it implies that one cannot simply transfer side chains from the helix to the aryl scaffold.

The low solubility of the terphenyl scaffold has prompted the Hamilton group [119] to develop another scaffold, trispyridylamide (**Figure 2** (**10**)), for α-helix mimicry. The template adopts a preferred conformation in which all three functional groups are projected on the same face of the scaffold. This preorganization is accomplished through a stabilizing bifurcated H-bonding network, as well as through the minimization of alternative conformations. The characteristic axial rise of 5.7 Å is close to that of the $i \rightarrow i + 4$ interaction in an α-helix. However, the alkoxy side chains are rotated 45° out of the plane of the carboxamide backbone. This may partially explain why trispyridylamide derivatives only had affinity in the low μmolar range for Bcl-xL, compared with a binding affinity of 114 nM for a terphenyl compound and 300 nM for the 16 residue BH3-domain peptide from the protein Bak.

3.2 Conformationally constrained α-helix motifs

A short synthetic peptide corresponding to a helical recognition motif does not typically fold stably in isolation and is usually flexible and conformationally disordered in solution. Such flexible peptides present side chains in a plethora of relative orientations increasing undesirable interactions at multiple recognition sites. This inherent flexibility also limits binding affinity when these peptides bind to their targeted receptors in a unique conformation, due to a more significant loss of entropy. Marshall and Bosshard [120] predicted in 1972 that α,α-dialkyl amino acids (**Figure 2** (**11**)), such as α-aminoisobutyric acid (Aib or α-methylalanine, MeA), would severely restrict the ϕ and ψ torsion angles of that residue to those associated with right- or left-handed helices (both α- and 3_{10}-helices). Subsequent experimental validation of that prediction is abundant [121]. An example where α,α-dialkyl amino acids were used to induce an α-helix of the peptide in water that enhanced binding involves the p53/HDM2 helix recognition: IC_{50} of 5 nM for an Aib-containing peptide and 8.7 μM for the native α-helical peptide [122].

Alternatively, the helical structure can be stabilized through the incorporation of covalent or noncovalent linkages between side chains of two residues separated in sequence, but spatially close in a helix, such as residues i and $i + 4$ of an α-helix (**Figure 2** (**12**)). Examples of chemical linkages shown to enhance helical propensity include: salt bridges [123], hydrophobic interactions [124,125], aromatic–charge [126] or aromatic–sulfur [127] interactions, disulfide bonds [128,129], lactam bridges [130-132], hydrocarbon staplings [133,134], diaminoalkanes [135], acetylenes [136] and metal ligation between natural [137,138] and unnatural amino acids [139,140]. These crosslinked interfacial peptides have been demonstrated to yield a marked enhancement of peptide helicity, stability and *in vitro* and *in vivo* biologic activity. For example, the interaction between the proapoptotic protein BID and the antiapoptotic protein Bcl-xL was disrupted by a hydrocarbon-stapled helix combined with α-methyl substituents on the two linked amino acids [141]. This conformationally constrained peptide segment, derived from the helical BH3 domain of BID, was found to protease resistant, cell-permeable and bound to Bcl-xL with a 6-fold higher affinity than the unconstrained helix. Cellular uptake was observed and apoptosis was activated within cells after treatment with the stapled helix. In addition, the stapled helix effectively inhibited the growth of human leukemia xenografts *in vivo*.

Helical peptides are stabilized by extensive but weak intrachain H-bonds; design of covalent surrogates of intrachain H-bonds (**Figure 2** (**13**)) reinforces the helical structure [142,143]. Such artificial helical peptides are attractive scaffolds for molecular recognition because the backbone H-bond surrogate neither blocks solvent-exposed recognition surface nor removes important side chain functionalities. For example, one peptide analog of a human papillomavirus peptide segment was conformationally restricted to an α-helical structure using a hydrazone link and was shown to have a very strong reaction with sera from women having invasive cervical carcinoma [144]. Though the main body of a peptide helix is stabilized by intrachain H-bonds, free amino groups at the N-terminus and carboxyl groups at the C-terminus of the helix do not participate in such internal peptide H-bonding. Thus, preorganized helix-nucleating templates (**Figure 2** (**14**)) [145,146] have been developed in which the orientation of the first 4 amino groups or the last 4 carboxyl groups were fixed in a rigid structure to template helix formation and prevent fraying of either end.

3.3 Helical foldamers

Foldamers are sequence-specific oligomers, akin to peptides and oligonucleotides that fold into well-defined three-dimensional structures. They offer templates for presenting complex array of functional groups in virtually unlimited geometrical patterns and, thereby, providing attractive opportunities for the design of molecules that bind in a sequence- and structural-specific manner to protein surfaces [147]. A number of foldamers with a strong tendency to adopt helical structures has been employed to interfere with protein–protein interactions. Many of these are structural variants of peptides, but are essentially stable to most proteases. One such family of foldamers is the poly-*N*-substituted glycines or 'peptoids' (**Figure 2** (**15**)) on which the amino acid side chains are appended to amide nitrogens rather than to the α-carbons [148]. Despite the achirality of the *N*-substituted glycines backbone and its loss of amide H-bonds, peptoids containing α-chiral, sterically bulky side chains are able to adopt stable, chiral helices with *cis*-amide bonds. The periodicity of the peptoids helix is 3 residues per turn, with a pitch of 6 Å. Appella and co-workers [149] explored the structural requirements of peptoids optimized for inhibition of p53–HDM2 interactions. The other family of foldamers is β-peptides (**Figure 2** (**16**)) that differ from α-peptides by one additional

backbone carbon atom between the amino and carboxyl groups [150,151]. β-peptides composed of β^3-L-amino acids are able to form left-handed 14-helices characterized by a periodicity of 3.25 residues per turn with a pitch of 4.7 Å and H-bonds between the backbone amide proton of residue *i* and the carbonyl oxygen of residue *i* + 2. The ability to form stable helices makes β-peptides good candidates for mimicry of structures and functions of α-helical recognition motifs. Schepartz and co-workers have designed adaptable β^3-peptide scaffolds with enhanced 14-helix structure by neutralization of the helix macrodipole [152] that inhibited the p53–MDM2 interaction [153], as well as gp41-mediated HIV-1 fusion [154]. Alternative helical structures of regular and hybrid peptides consisting of homologous amino acids, such as β-, γ- and δ-amino acids, have been implicated as potential inhibitors to modulate α-helix recognition [155-158].

4. Expert opinion

One major drug discovery paradigm often begins with a known chemical starting point that has a desirable biologic activity with therapeutic relevance, such as a natural substrate or regulator; such information is not readily available if the object is to disrupt a protein–protein interaction. However, if the protein–protein interface consists of short continuous recognition motifs, such as an α-helix or a reverse turn, privileged scaffolds targeting these binding sites may serve as lead compounds for subsequent optimization. In addition, the concept of privileged scaffold targeting common protein recognition motifs is highly attractive because the rational design of new leads for many protein–protein interactions has been limited by the lack of detailed structural information for a particular targets. Privileged scaffolds can provide medicinal chemists with common, non-peptidic, bioavailable substructures as suitable starting points in parallel synthesis. Ultimately, a single, large combinatorial library of privileged structures might provide ligands for a whole series of protein targets.

Although research to discover small-molecule drugs that target protein–protein interactions is still at an early stage, accelerated activity in this area will occur as compounds move through clinical trials and the science and technology base continues to develop. The prospective of developing drugs that target biomolecules that are relatively well validated in terms of biologic function and role in disease is important in driving advances in this field.

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Affiliation

Ye Che & Garland R Marshall[†]
[†]Author for correspondence
Washington University,
Center for Computational Biology and
Department of Biochemistry and
Molecular Biophysics,
St. Louis, MO 63110, USA
Tel: +1 314 362 1567; Fax: +1 314 747 3330;
E-mail: garland@biochem.wustl.edu