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Perspective

Concepts and Progress in the Development of Peptide Mimetics

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Medicinal chemistry research has been dramatically transformed by biotechnology. Previously, synthetic chemistry and natural products screening dominated drug research, but now molecular biology has become a driving force behind screening and the establishment of macromolecular targets. Most of the research in biotech companies has been directed to peptide and protein therapeutics in spite of problems associated with their low bioavailability, rapid metabolism, and lack of oral activity. Because of these limitations, research groups continue to rely upon chemical synthesis of nonpeptide substances for drug discovery, recognizing that small molecules are likely to remain the most viable avenue for the identification and optimization of potential drugs. The challenge is to devise strategies that take advantage of the strengths of biotechnology to define and characterize molecular targets and of medicinal chemistry to develop compounds. A promising approach integrating these disciplines is the field of peptide mimetics, a conceptual approach which considers peptides and proteins not as potential therapeutics but rather as leads for the discovery of other classes of compounds.

The terms "peptide mimetics" and "peptidomimetics" have been utilized interchangeably to describe compounds discovered through a variety of research strategies.[1] Indeed, even compounds identified by random screening and subsequently optimized through structural modification have been termed peptidomimetics if the initial lead was found in an assay in which the natural ligand is a peptide or protein.[2] The field of enzyme inhibitors uses peptide mimetics terminology for replacements of segments of peptide-based substrates and inhibitors. In this Perspective, the emphasis will be on mimetics of peptide and protein ligands for receptors, although the methodology is applicable to enzyme inhibition as well. The broad use of the term "peptide mimetics" is unavoidable, but advocates of rational design do not favor its use to describe compounds found by screening. Nevertheless, leads found by screening contribute to the understanding of pharmacophores that recognize peptide binding sites. Similarly, natural product opiate alkaloids are frequently cited as examples of peptidomimetics[2,3] because they validate many of the concepts invoked in rational design. In fact, they also make a case for how structurally different nonpeptides may be from their peptide parents (lacking flexibility, amide bonds, and obvious pharmacophore similarity) and how their modification can lead to highly selective ligands for subtypes of receptors in both peptide and nonpeptide compounds.[4]

Another class of peptide mimetic research that lies between the screening approach and *de novo* mimetics design is centered on the replacement of individual peptide bonds by a nonpeptidic group.[5] These peptide surrogates are complemented by the synthesis of peptides incorporating unnatural amino acids, conformational constraints, and larger subunits, for example, dipeptide mimetics. These compounds bridge the gap between simple peptide analogs and the completely nonpeptide structures. In this respect, they represent a step along the path toward the rationally designed nonpeptide. In many cases, peptide surrogates are likely to be an essential stage in the development of a pharmacophoric hypothesis for conformationally flexible peptides.[6] In addition, new technologies for the rapid screening of peptide libraries[7] will generate many highly flexible lead structures with modest binding affinities. Optimization and eventual translation of a peptide lead to a small molecule drug will probably require isosteric replacements, cyclic peptide derivatives,

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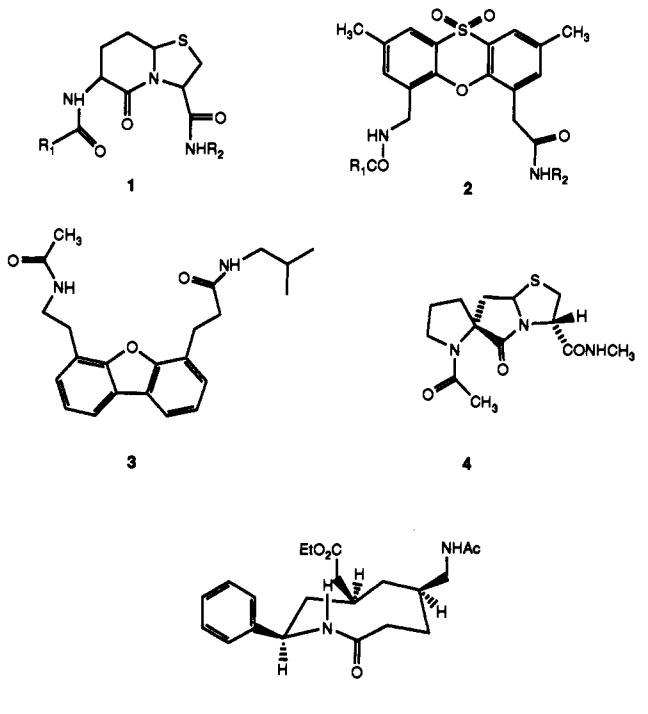


Figure 1. β-Turn mimetics (1,[11] 2,[12] 3,[13] 4,[14] 5[15]).

bond surrogates, and conformational constraints as logical steps in the process.

The rational design of nonpeptide compounds thus is not feasible without the information obtained from the study of the structure–activity relationships and conformational properties of peptide structures. When such information is available, and the indication is that the peptide backbone is not a critical element in receptor binding,[8] the design of a nonpeptide becomes feasible. The transition to a mimetic can then be made rational by adopting some general principles, adapted from the treatise of Farmer.[9]

Design Criteria for Peptide Mimetics

1. Replace as much of the peptide backbone as possible by a nonpeptide framework. If bond surrogates have been shown to retain activity, or peptide bonds are not exposed in the presumed bioactive conformation, then a structural template may be designed to eliminate amide bonds.

2. Maintain peptide side chain pharmacophoric groups as in the peptide. Rather than completely dispensing with a relationship between peptide and nonpeptide, initially designed mimetics may retain groups as found on the peptide, as these are most likely to be recognized by a receptor. For example, if a lysine residue is known to be required for activity in the peptide, then the first-generation mimetic should have a primary amino group at the end of a methylene chain. As the design progresses through other generations, amine variants, different chain lengths, conformational constraints, and substitutions on the nitrogen may be fruitful ways to enhance binding affinity.

3. Retain some conformational flexibility in first-generation mimetics. The probability that a pharmacophore hypothesis will be definitive for flexible peptide side chains is low, making it necessary that the first designs leave some of the side chain pharmacophoric groups unconstrained so that they may adopt conformations

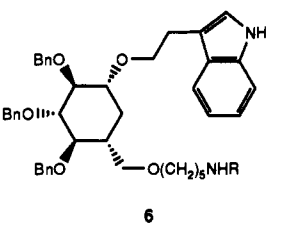


Figure 2. Somatostatin mimetic 6 (R = H) based on a glucose template.[18]

analogous to those of the peptide. Even in protein ligand structures where backbone conformations are known from X-ray crystallography, the side chains involved in interaction with a receptor may move considerably from their orientation on the crystal surface as they dock into recognition sites on the receptor. If a lead activity is seen for a flexible mimetic, adding conformational constraints to side chain groups is then a rational approach to enhance potency and selectivity.

4. Select appropriate targets based on availability of a pharmacophore hypothesis, or develop the information as a first step. Attempting to carry out a peptide mimetic project on a system for which there is no idea of structure–activity relationships and no three-dimensional hypothesis of the bioactive conformation is irrational. Such a program is likely to generate many inactive compounds and little useful guidance for further work. For systems where the biological rationale or medical need is sufficient to justify a peptide mimetics approach, efforts to provide a pharmacophore hypothesis as a first step are justified and critical to the outcome of the peptide mimetics programs.

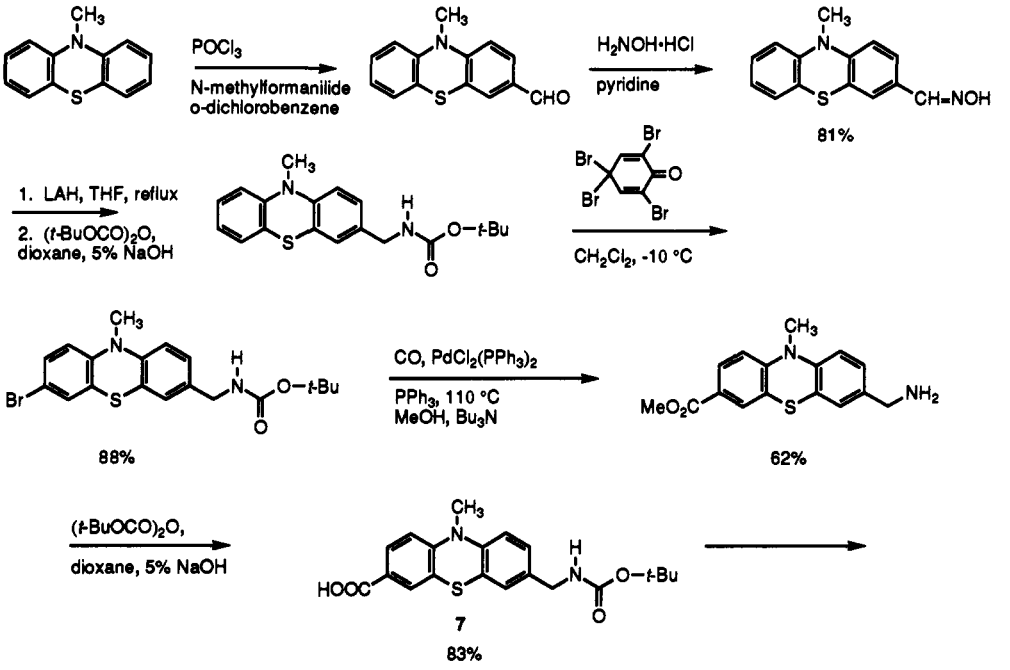
Nonpeptide leads designed by this strategy might be expected to behave more like the structures derived from screening synthetic compound collections or natural products in terms of their bioavailability, metabolic stability, and transport. With an appropriate and successful design and subsequent modification of an initial lead structure, the likelihood that these peptide mimetics will become drug candidates is high. Progress toward this goal is just beginning to emerge. In this Perspective, we will describe some of our initial efforts to design peptide mimetics to constrain secondary structural elements of peptides and will review the progress in our first cases of *de novo* design following the basic principles outlined above.

Mimicking Architectural Elements of Protein Structure

β-Turn Mimetics. Major efforts[10] have been devoted to the development of templates that mimic or stabilize several common architectural elements of peptide and protein structure, i.e., β-turns, helices, and β-sheets. The majority of the β-turn mimetics have been dipeptide mimetic replacements for the $i + 1$ and $i + 2$ residues at the corners of the turn. These have been designed to retain the intramolecular hydrogen bonding network of an appended peptide chain (e.g., Figure 1, compounds 1–4). However, these examples lack an appendage for the corner residue side chains. Since these are the residues that are most exposed to the surface of a protein, and most likely to be involved in intermolecular interactions with a receptor, their elimination limits the scope of the mimetic to that of a group that enforces a conformational constraint.

While these structures are valuable for answering fundamental questions about protein folding, we felt it

Scheme I



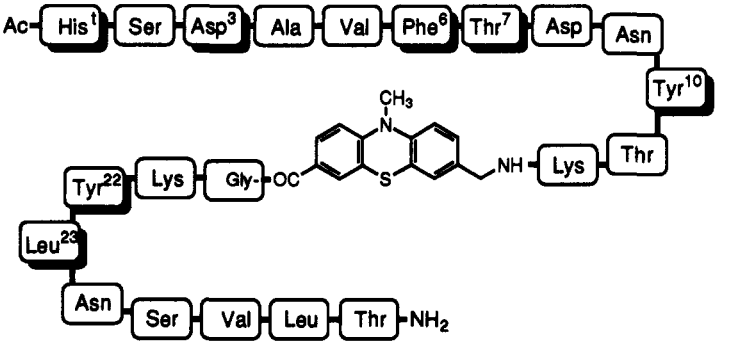


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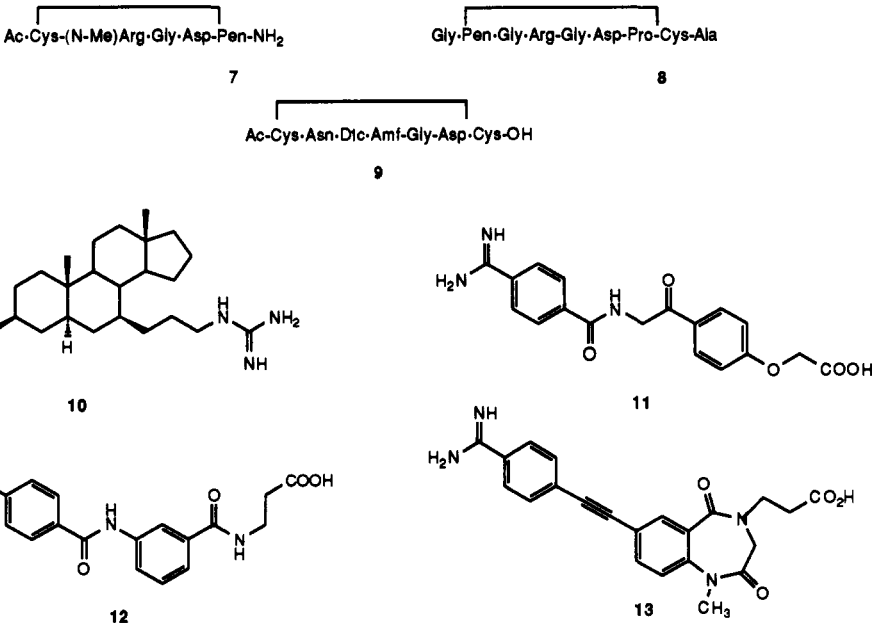
Chart I



(Pen= Penicillamine, Dtc= 5,5-dimethylthiazolidine-4-carboxylic acid, and Amf= p-aminomethylphenylalanine)

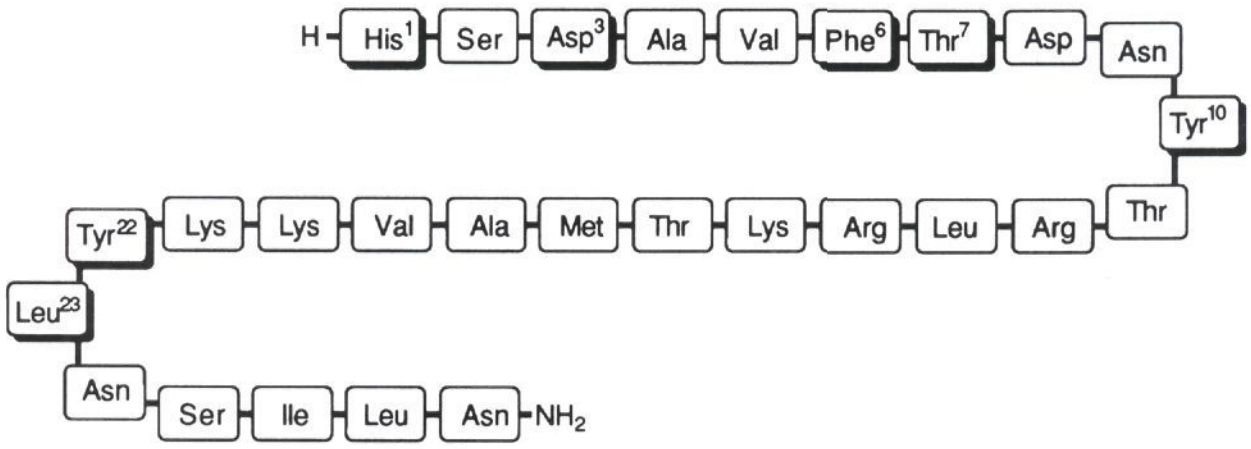


Figure 3. VIP sequence and critical residues (shadowed).

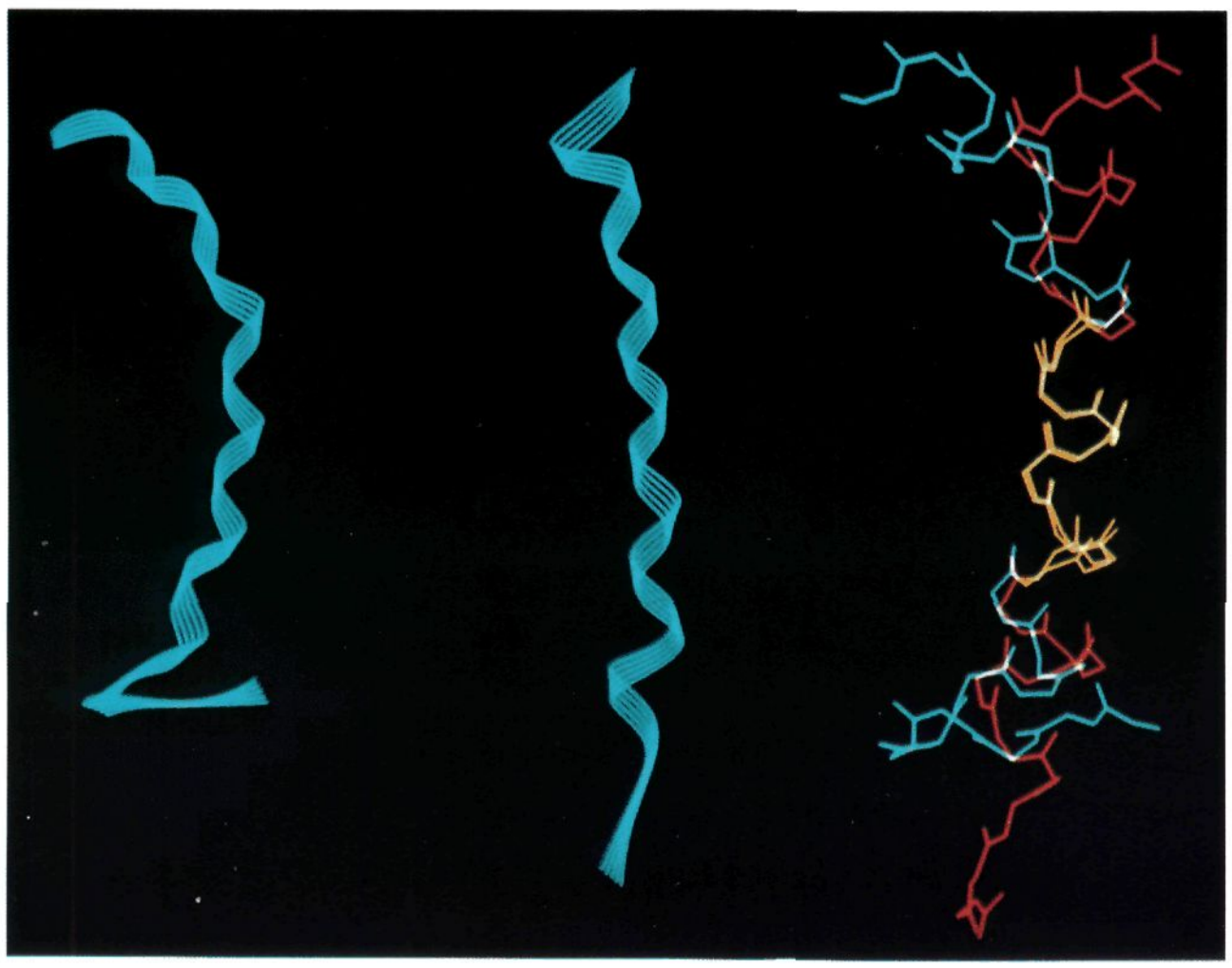


Figure 4. VIP structure by NMR in solution (left, 25% MeOH in H_2O; center, 50% MeOH in H_2O; right, superposition showing closely aligned central core).

was important to devise a more highly functionalized system that could theoretically carry side chain groups in positions that corresponded to those of the corner residues of the turn.[15] Two possibilities emerge for such compounds. In one case, the functionalized turn could be incorporated into a peptide sequence to generate the required turn and simultaneously present the corner residues to a potential receptor. Another hypothetical case would allow the corner residues and turn geometry to participate in a binding interaction in the absence of the rest of the protein molecule. In a case in which the turn is located at a critical recognition site, such compounds have the greatest potential to antagonize a biological response. The 9-membered ring lactam system **5** was designed with the idea of replacing the intrachain hydrogen bond between residues i and $i + 3$ with a methylene group and replacing all of the amide bonds except for the exposed corner with carbon–carbon bonds.

In addition to our synthetic and structural studies with model compounds related to **5**, other groups have extended this approach to specific target peptide turns and have described initial characterization of these systems.[16,17] Unfortunately, progress toward structures of this type that could be prepared by short sequences[17b,18] has been slow. Another system that has shown promise in mimicking a β-turn in a biologically active compound is the somatostatin mimetic **6** (Figure 2) based on a glucose template described by Hirschmann.[19] This compound, designed on the basis of the cyclic hexapeptide developed by the Merck group,[20] is an early example of a mimetics approach following "Farmer's rules." The fact that **6** is relatively weak in comparison to the cyclic peptide as a somatostatin mimetic[21] probably reflects the greater conformational mobility of the side chains appended to the glucose template.

RGD Mimetic Inhibitors of GpIIbIIIa–Fibrinogen Receptor Binding. The basic chemical studies and model systems for β-turn mimetics have prepared the way for the development of specific compounds with defined biological targets. Some of the most promising examples to date are the diverse structures that have emerged from work on inhibitors of GpIIbIIIa binding to fibrinogen receptors based on the ubiquitous, inhibitory RGD(S) (Arg–Gly–Asp–(Ser)) peptide sequences. This sequence has been constrained into cyclic peptides[22] (e.g., **7–9**), and a number of nonpeptide mimetics have been described (e.g., **10–13**). Benzene rings,[23,24] steroids,[25] and even

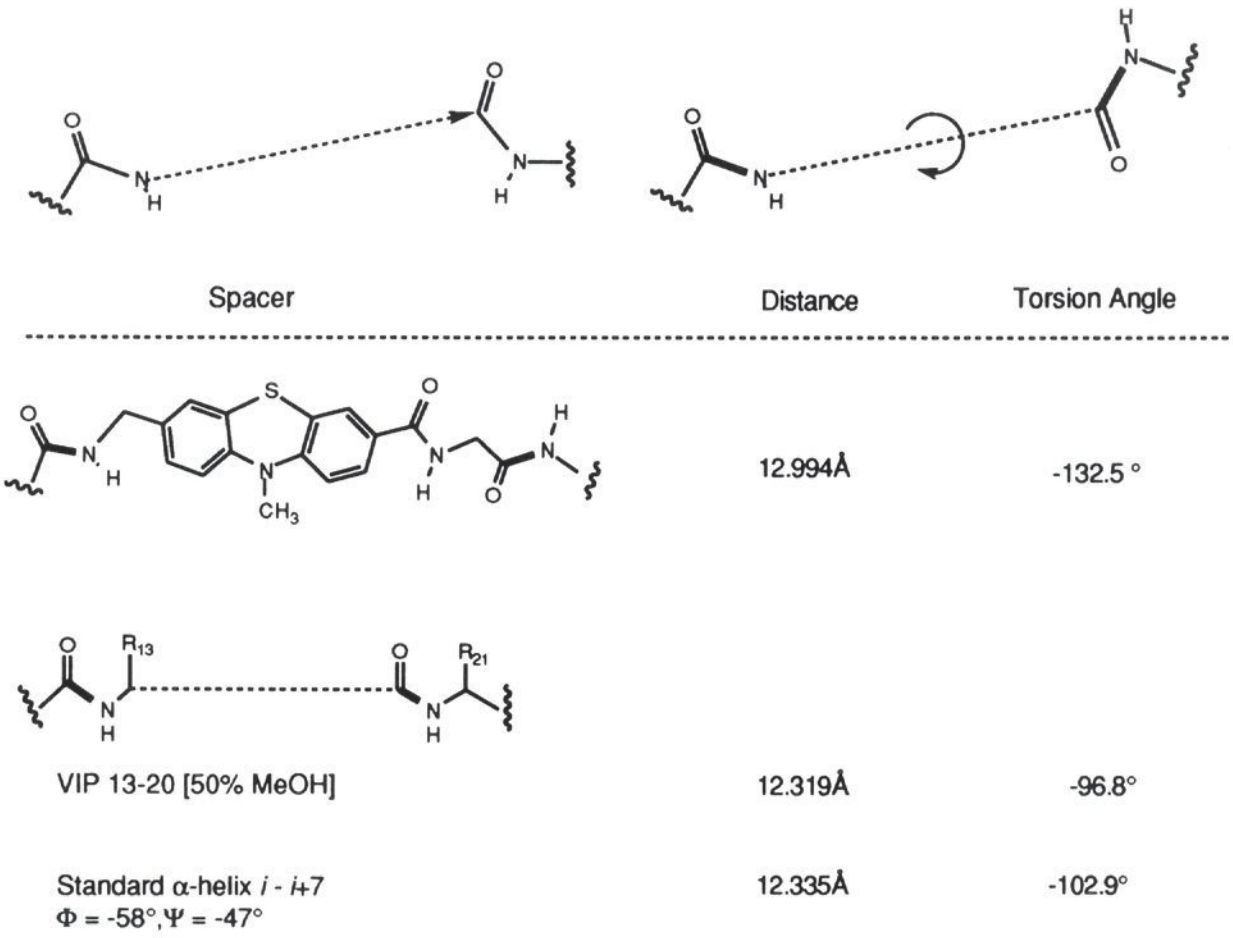


Figure 5. Parameters for an α-helix mimetic in VIP.

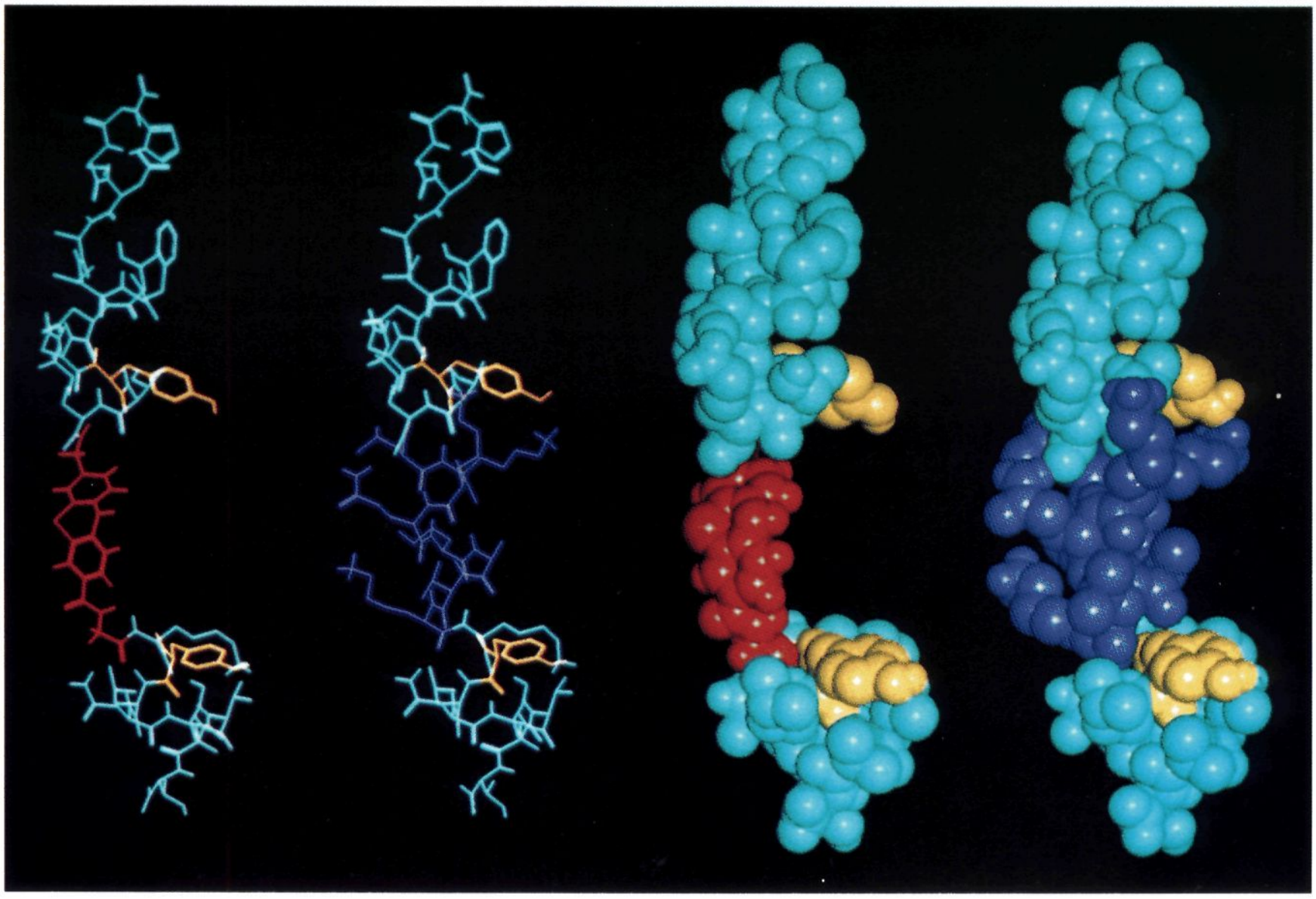


Figure 6. VIP hybrid models (left, hybrid **14** and VIP; right, space-filling models of VIP hybrid **14**).

benzodiazepines[26] have been employed as templates and represent excellent examples of the application of "Farmer's rules."

α-Helix Mimetics—Replacement of a Bridging Helix in Vasoactive Intestinal Peptide. Among the best characterized structural features of proteins is the α-helix. Unlike a β-turn, the α-helix incorporates a larger number of amino acid residues (e.g., 3.4/turn) to achieve its shape. If recognition of a particular helix involved a large number of residues, or a series of adjacent side chains, mimicry would be impractical because of the highly complex templates that would be required to present side chains correctly. However, there are two cases in which mimicry of an α-helical structure can be envisioned. (1) The residues responsible for biological recognition lie along one face of the helix,[27] so that a small bridging template

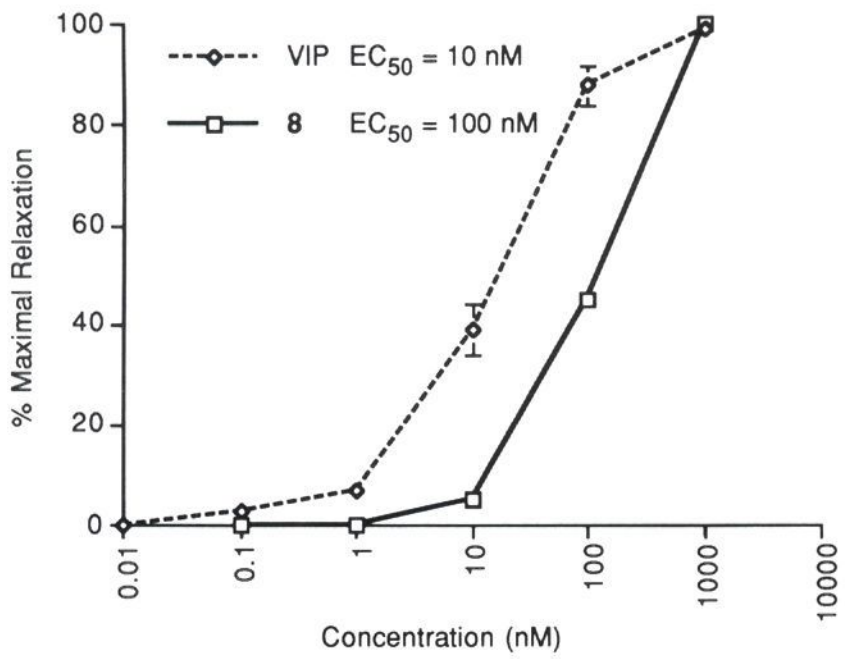


Figure 7. Relaxation of airway smooth muscle from Guinea pig trachea by VIP and VIP-helix mimetic 8.

can be designed analogously to the β-turn templates. (2) The helix performs a function as a spacer with no individual amino acid side chains specifically required for activity within a segment of the helix. In such a case, a spacer template of modest complexity could be envisioned.

An example of this second case emerged from Bolin's studies[28] of vasoactive intestinal peptide (VIP)(Figure 3). VIP is a 28 amino acid peptide of interest as a potential therapeutic agent for the treatment of asthma. In asthmatics, VIP is present at very low levels in the lung compared to normals. VIP is synthesized in the lung, where it acts as a bronchodilator, by relaxing airway smooth muscle and by increasing mucus secretion. Extensive research has led to the discovery of highly potent, metabolically stable VIP peptide analogs of potential interest as therapeutics.

As an approach to elucidate the important residues of VIP required for biological activity, Bolin performed an "alanine scan" of the VIP sequence, synthesizing VIP analogs with Ala substitution at each individual residue. The study revealed that a significant segment of the structure between the important residues Tyr^{10} and Tyr^{22} was insensitive to the substitution by Ala. At the same time, NMR studies by Fry et al.[29] indicated that the structure of VIP in 25% and 50% aqueous methanol solution was highly helical, particularly so in the middle of the sequence where the Ala substitutions had no effect on potency (Figure 4). These two results suggested strongly that the region between Tyr^{10} and Tyr^{22} was performing as a helical spacer to hold the important residues at an appropriate distance, but without having a direct side-chain interaction with the VIP receptor.

Our approach to mimetics of a β-turn where we developed a specific replacement for the turn structure could be adapted to the VIP problem by designing an amino acid that could replace a portion of the structural α-helix segment by a nonpeptide helix mimetic. This spacer molecule would obviously have to span the required distance between the residues at either end of the excised helix. In addition, the peptide bonds emerging from either end of the spacer should be consistent with the path of the peptide helix. After some initial measurements, the segment between residues 13 and 20 was selected for replacement, giving distances and angles to be spanned by the spacer as shown in Figure 5.

Types of structures that could have the appropriate distance between the excision points and an extended, relatively rigid shape that could fit within the diameter of the helix axis were considered. After modeling some linear compounds, we realized that bent tricyclic ring systems, such as phenothiazine or dibenzazepine, would have a similar distance between the connecting points on the helix. Importantly, the bend in the middle of the ring system positioned the vectors of the amide bonds at the proper angles to follow the continuation of the peptide helix. The template selected for synthesis was the phenothiazine amino acid **7**. This structure was synthesized as outlined in Scheme I and was incorporated into a VIP sequence by solid-phase synthesis giving **14**.

Models of the VIP hybrid structure incorporating the phenothiazine spacer are illustrated in Figure 6. The spacer fits the helical segment well and does not distort the remaining peptide backbone. In addition, the tricyclic ring system lies within the volume of the helical backbone and side chains, presenting the important Tyr residues in their native orientation. What the model does not address, however, is the disruption of the hydrogen bonding lattice

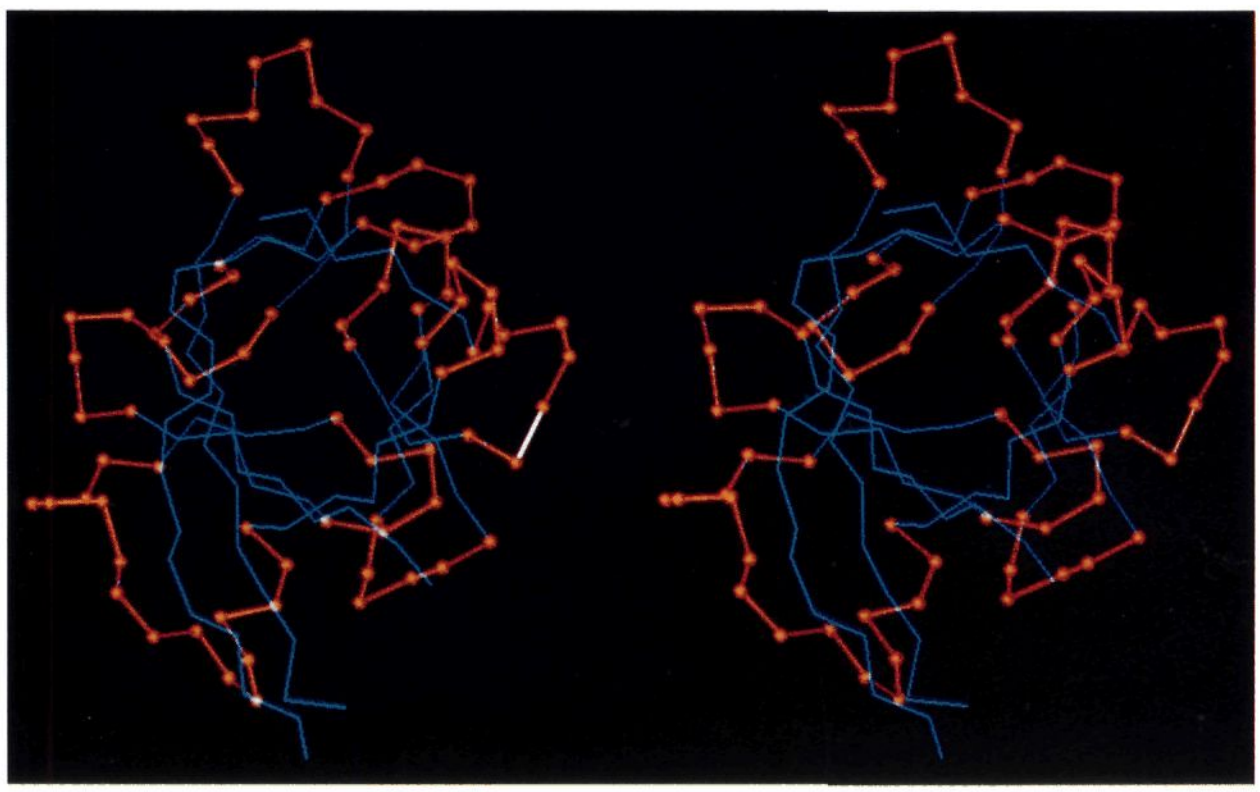


Figure 8. α-Carbon backbone of interleukin-1α showing exposed surface loops.

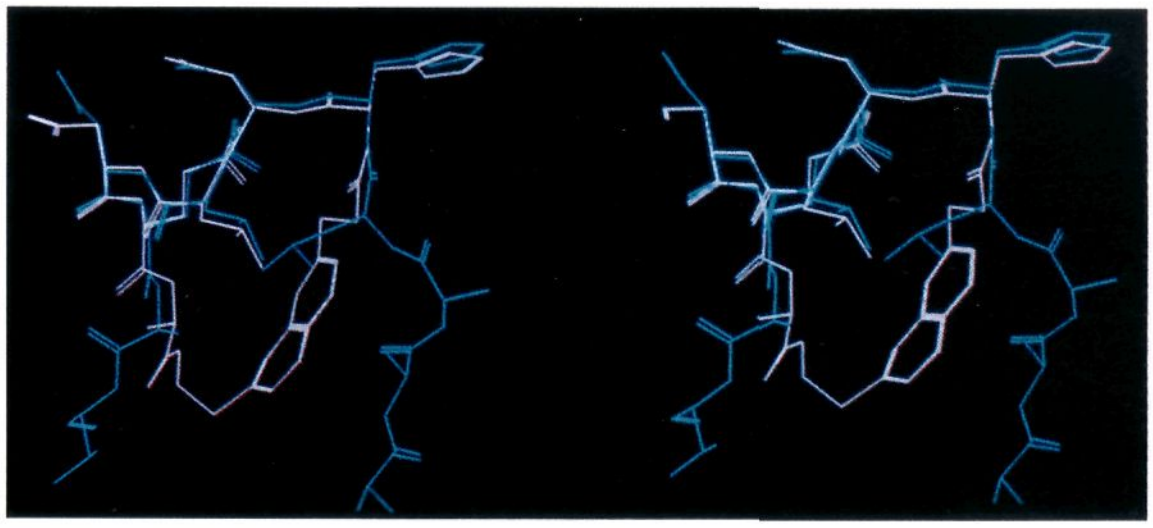
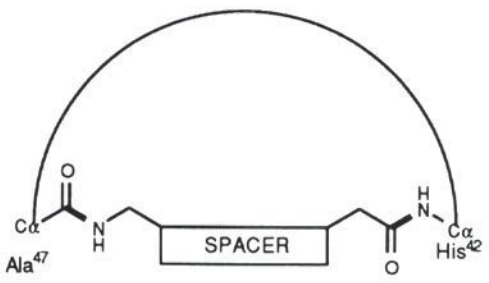


Figure 9. Left: Ω-loop in IL-1α sequence 41–48. Right: Ω-loop mimetic **16** incorporating naphthalene spacer.



Distance between Leu ^{41} CO and Val ^{48} NH ..... 10.944 Å

Torsion Angle between the highlighted bonds ..... 125.9°

Figure 10. Structural parameters for Ω-loop spacers.

that is present in the normal helix and missing in the hybrid molecule. The tricyclic ring system does not have hydrogen bond donors or acceptors to initiate a helical turn following the spacer and thus might mimic the biologically irrelevant portion well while failing to preserve the conformation needed for activity at those regions outside of the spacer.

The results of testing the VIP hybrid **14** in a model of airway smooth muscle relaxation are shown in Figure 7. In this test, guinea pig tracheal rings are treated with test compounds, and their relaxation is compared to the maximal relaxation elicited by the β-agonist, isoproterenol. The hybrid **14** was remarkably potent, exhibiting full agonist activity at about 10% of the potency of native VIP. Unfortunately, the low solubility of **14** precluded an NMR analysis of the structure to determine the extent of helicity of the hybrid. Nevertheless, the activity of **14** as a full agonist supports the data from the Ala scan that the core of the helix in VIP may be characterized as a structural scaffolding. Properly designed mimetics, like the phenothiazine system, have the potential to replace the polypeptide spacer helix and to position the N- and C-terminal pharmacophores. Further research on these systems will be needed to prepare more potent variants as well as soluble hybrids whose structures can be studied directly.

Ω-Loop Mimetic in Interleukin-1α. The approach devised for the design of the α-helix mimetic for VIP was to replace a segment of the peptide with a spacer that would maintain the distance and torsion angles of the native helix. A similar strategy can be applied to mimic an Ω-loop structure[30] if a spacer is designed to constrain the ends of a loop sequence. We have applied this idea[31] to mimic a prominent loop of the immunomodulatory cytokine, IL-1α, whose crystal structure was determined by Graves and Hatada.[32] In IL-1α, the 12-stranded beta barrel is interconnected by a series of loops and turns (Figure 8). Among these is a large, prominent Ω-loop between residues 41 and 48 of the sequence.

The ends of this loop are too far apart (ca. 11 Å) to be constrained by formation of a cyclic peptide or by disulfide bridges. However, since the side chains of residues Leu[41] and Val[48] project into the center of the loop, they could be replaced by a nonpeptide spacer with the proper distances and torsional constraints (Figures 9 and 10).

The system which was chosen for this purpose is the 7-(2-aminoethyl)naphthalene-2-propionic acid, **15**. This amino acid was capable of fitting into the space occupied by the side chains of Leu[41] and Val,[48] and modeled low-energy conformations of the loop with the spacer included were consistent with the loop conformation from the X-ray structure. Thus, the hybrid cyclic peptide **16** could be expected to mimic the role of the remainder of the IL-1α protein in presenting the loop in a conformation that could potentially be recognized by a receptor.

The synthesis of amino acid **15** and the cyclic peptide hybrid **16** have been described.[31] The structure of **16** was studied by 2D-NMR (COSY and NOESY) experiments in

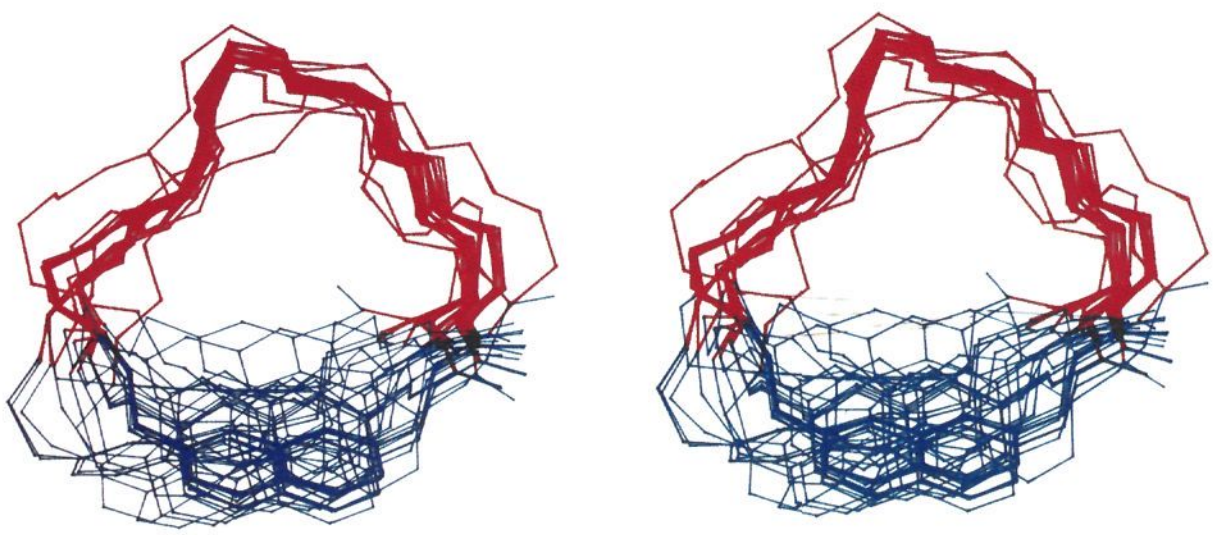


Figure 11. Overlay of 30 lowest-energy conformations of Ω-loop hybrid **10** consistent with NMR data.

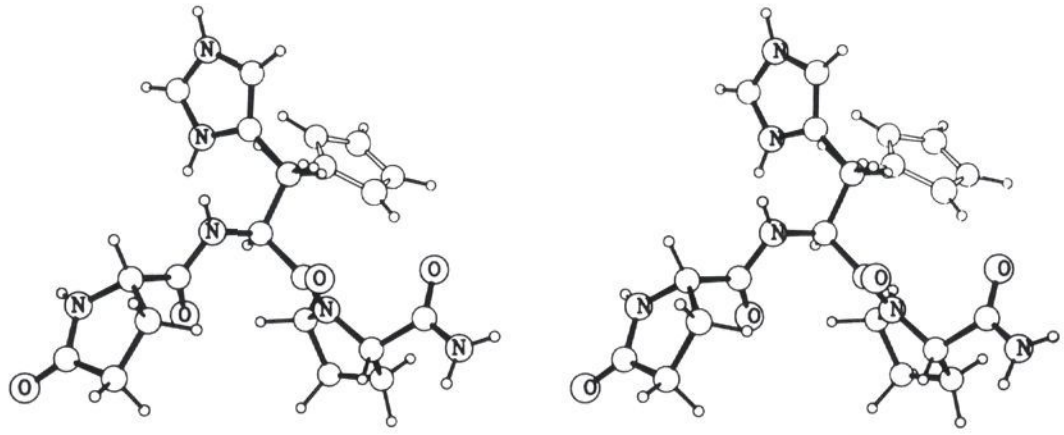


Figure 12. X-ray (open bonds) and proposed bioactive conformations (solid bonds) of TRH.

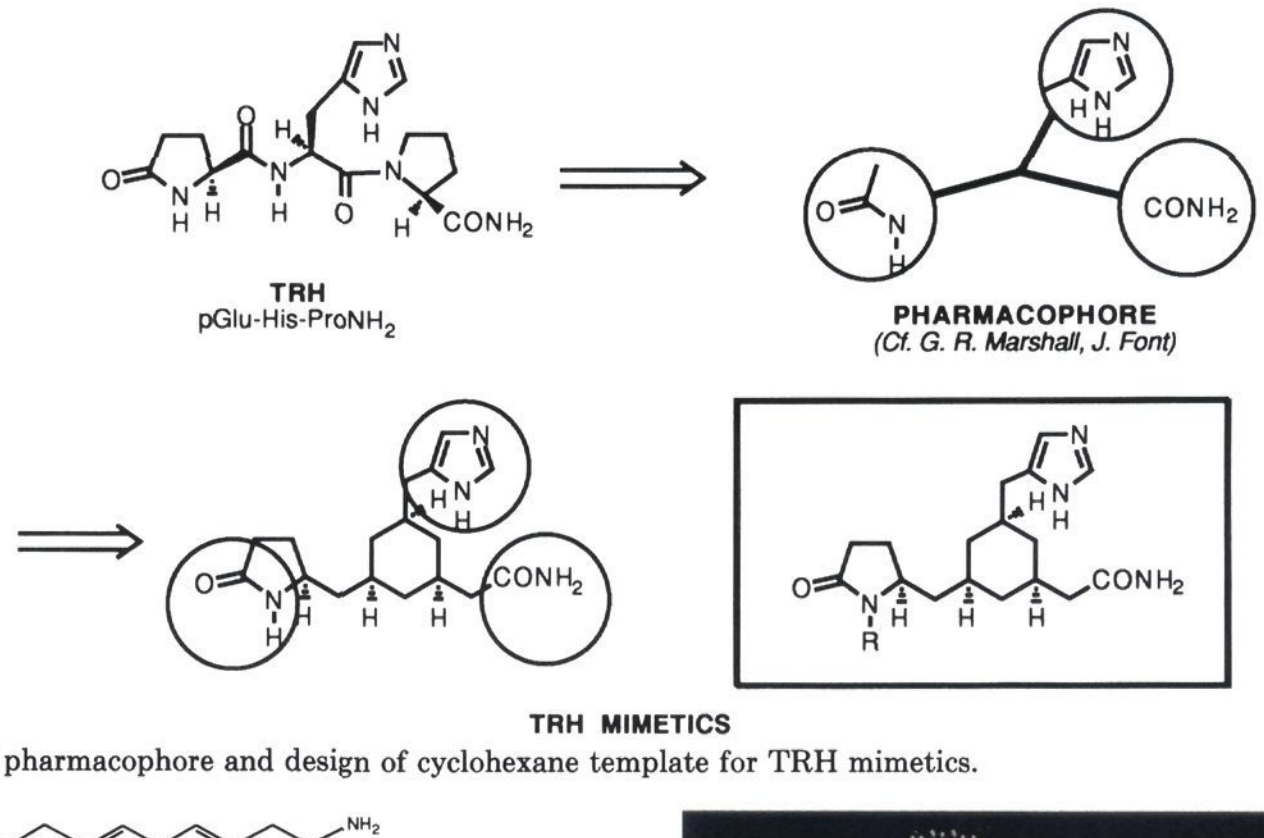
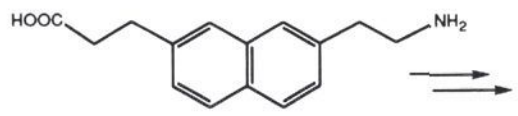


Figure 13. TRH pharmacophore and design of cyclohexane template for TRH mimetics.



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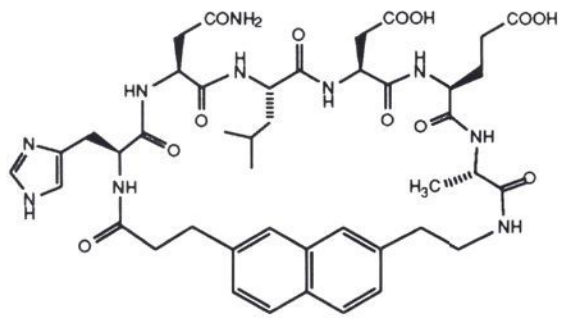

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DMSO-d_6 at room temperature. NOE's were quantified and converted into distances which were then employed as constraints for molecular dynamics simulations. For the cyclic peptide hybrid **16**, optimization yielded 30 low-energy conformations consistent with the NMR data. A superposition of the backbones of these 30 structures is illustrated in Figure 11. The figure illustrates how the naphthalene spacer is able to constrain the backbone C_α to similar loop structures for all the conformations of the peptide loop, even though the spacer itself is capable of adopting a number of low-energy conformations.

The cyclic peptide hybrid **16** was assayed for its ability to compete with ^{125}I-labeled IL-1α in binding to type I IL-1 receptors on mouse EL-4 cell membranes.[33] These assays indicated that **16** did not inhibit IL-1 binding up to a concentration of 2 mM. This observation suggests that the constrained Ω-loop mimetic is not recognized by the receptor, a conclusion that is supported by recent findings that the IL-1 binding epitope consists of 7–9 residues which are clustered in space, but none of which are found in the 41–47 sequence of the Ω-loop.[34] Current work is directed toward mimetics that could present important residues from the binding epitope on nonpeptide scaffolding.

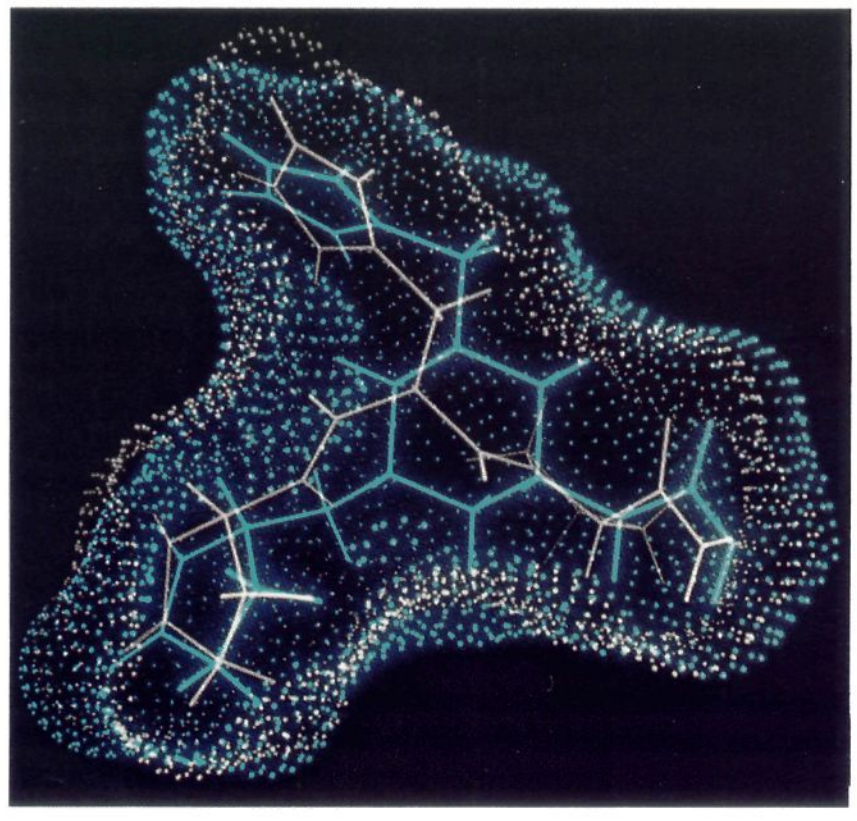

Figure 14. Superposition of TRH and cyclohexane TRH mimetics and volume overlap.

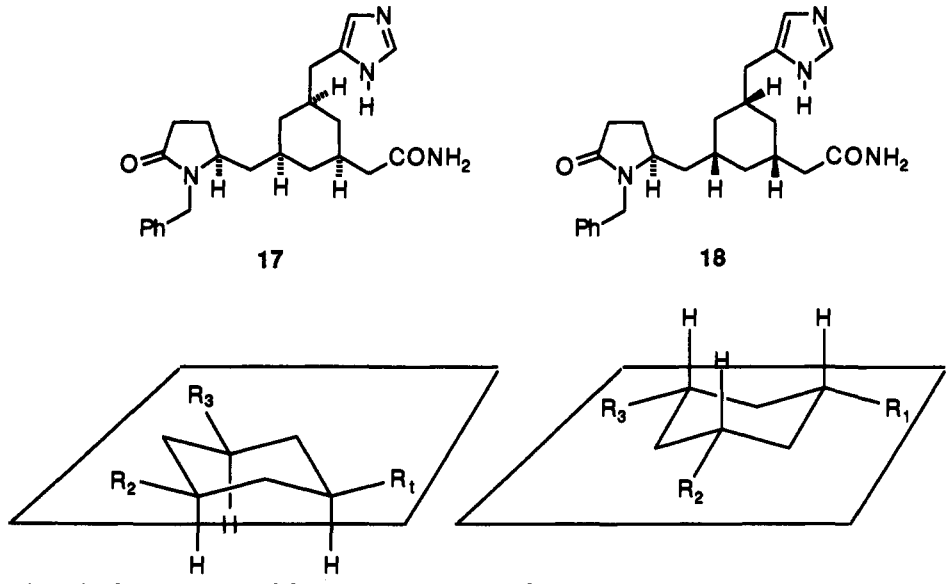

Figure 15. Orientation of side chains in cyclohexane TRH mimetic diastereoisomers.

Mimetics of a Target Peptide—TRH Mimetics Based on a Cyclohexane Framework. The concepts outlined above, and the β-turn, α-helix, and Ω-loop examples described, illustrate the validity of our approach to design mimetics of defined structural elements of peptide and protein architecture. In the case of smaller, conformationally mobile peptides, the design of a mimetic requires an appreciation of the bioactive conformation of the peptide. Except for systems in which enzyme- or receptor-bound peptides have been observed directly by X-ray or NMR, this information can be obtained only through the study of constrained peptide analogs and peptides containing rigid bond surrogates. One system for which much of this information is available is the simple tripeptide, thyrotropin-releasing hormone (TRH, pGlu-His-ProNH$_2$).

TRH is a hypothalamic peptide which functions as a neuroendocrine hormone by increasing thyrotropin-stimulating hormone (TSH) leading to an elevation of thyroid hormone levels. TRH binds to high- and low-affinity receptors labeled by ^{3}H-TRH and ^{3}H-3-MeHis^2-TRH in discrete brain regions.[35] TRH has pronounced CNS effects[36] and is able to enhance performance in cognitive behavioral models in animals[37] suggesting its potential to treat cognitive disorders, including those associated with Alzheimer's disease.

Crystal[38,39] and solution structures[40] of TRH and TRH peptide analogs are known, and models have been proposed for the pharmacophore and bioactive conformation(s) of TRH and TRH analogs. Moore and Marshall[41] proposed the lactam moiety of the pyroglutamyl residue, the histidine imidazole ring, and the carboxamide of the terminal prolineamide as pharmacophoric groups based on activities in TSH release and high affinity receptor binding.

For the purposes of our mimetics design, we chose a starting conformation in which the peptide backbone approximates the Y-shaped X-ray structure of TRH[42] except that, in our model, the imidazole ring is not locked in the hydrogen-bonded interaction with the terminal carboxamide as in the crystal but is oriented in a conformation consistent with the observations from solution NMR studies (Figure 12). This model is analogous to that proposed by Marshall and colleagues.[43,44]

In designing our mimetic, we wanted to maintain the spatial orientation of the pharmacophore using a scaffolding which would not extend beyond the boundaries of the peptide. For this purpose, we chose the 1,3,5-cis-trisubstituted cyclohexane framework to replace the peptide backbone (Figure 13). The mimetic retains the pyroglutamyl amide, prolineamide, and imidazole pharmacophoric groups hypothesized to be required for activity and permits orientations consistent with the proposed bioactive conformation (Figure 14). The cyclohexane ring system should contribute only nonspecific, hydrophobic character to the mimetic while permitting conformational mobility at the side chains. The design, synthesis, and biological activity of our first TRH mimetics have recently been reported[45] following our initial disclosures of the results.[46]

The most potent of the mimetics in the Morris water maze test,[47] a behavioral model of cognitive impairment, was found to be the *N*-benzyl compound 17 (Ro 24-9975). In this test, TRH reduced mean latencies over a dose range from 0.003 to 3 mg/kg ip but was inactive by the oral route. Compound 17 was active from 0.0003 to 3.0 mg/kg ip and from 0.0003 to 3.0 mg/kg po in this test.

Because of our route of synthesis, it was also possible to determine the effects of diastereoisomers in the series. When the ring center bearing the imidazolylmethyl group was inverted, the compounds were ca. 10-fold less active. By contrast, the compound in which all three of the ring asymmetric centers is reversed (18) was virtually equipotent to 17. This result is significant from the perspective of the ability of the cyclohexane ring to serve as a neutral scaffolding for the pendant groups, since the ring inversion at all three centers, as in 18, also orients the pharmacophoric groups in a 1,3,5-cis relationship as indicated in Figure 15.

The conclusion that the cyclohexane compounds are mimicking TRH is also supported by the activity of other compounds[48] having substituents chosen from among those known to lead to active TRH peptide analogs. Thus, analogs of 17 having phenyl, 2-imidazolyl-, and 1-methyl-4-imidazolyl groups in place of the 4-imidazolyl group gave structure–activity results which paralleled those obtained from corresponding TRH peptide analogs. Although 17 does not compete with ^{3}H-3-MeHis^2-TRH in the standard

TRH binding assay, direct binding studies with ^{3}H-Ro 24-9975 have recently identified a lower affinity binding site in rat brain, and work on biochemical characterization of this receptor and its regional localization by autoradiography is being pursued.[49] Further studies will be required to establish whether the cyclohexane mimetics are exhibiting cognitive enhancement activities by a mechanism that is associated with this receptor or through some other process.

The peptide mimetics projects described in this Perspective are representative of efforts being made in many laboratories to translate peptide and protein structures into small molecular weight, nonpeptide compounds that could mimic their biological functions. The research is truly interdisciplinary and dependent upon molecular biology and peptide chemistry to provide targets and structure-activity data. These results, coupled with structural insights from crystallography, NMR, and modeling provide the basis for design, while the implementation and practicality of the ideas is ultimately dependent on organic synthesis. With the progress being made now, it is reasonable to predict that some general principles of *de novo* peptide mimetics design will evolve from today's efforts and that important therapeutic agents will emerge as a result of this collaboration between biotechnology and medicinal chemistry. The future will present many opportunities for these two sciences to work together and indeed to become partners in the search for new treatments for disease.

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