Biochem. J. (1990) 268, 249–262 (Printed in Great Britain)

REVIEW ARTICLE

Emerging approaches in the molecular design of receptor-selective peptide ligands: conformational, topographical and dynamic considerations

Victor J. HRUBY,* Fahad AL-OBEIDI and Wieslaw KAZMIERSKI
Department of Chemistry, University of Arizona, Tucson, AZ 85721, U.S.A.

INTRODUCTION

A central goal in peptide and protein research is the development of rational approaches to the design of peptide and protein ligands with specific physical, chemical, and biological properties. In the case of peptide ligands which generally act by interactions with receptors or acceptor molecules (hormones, neurotransmitters, growth promoters and inhibitors, immunomodulators, etc.), the problem is complicated by several inherent difficulties. (1) The peptides generally are relatively small, conformationally flexible, structures and the question arises as to which of the conformations are biologically relevant. (2) The fundamental issue of the relationship(s) between peptide primary structure and conformation (the 'structure code') and topography is still unresolved. (3) Many biologically active peptides have multiple sites of interaction and hence the question of specificity and its relation to structure and conformation is a central concern. (4) The interaction of peptide with its receptor or acceptor (the 'host–guest' relationship) apparently changes the conformation of the ligand *and* the receptor; hence peptide dynamics become important. (5) The primary structure and conformation of most membrane-bound peptide receptors is not known and efforts to clone these receptors have progressed slowly. (6) Antagonist development requires that the structural, conformational and dynamic features which distinguish the 'binding message' from the 'transduction message' be dissociated [1]. (7) The three-dimensional structure of the peptide ligand, either free, or bound to the receptor/acceptor, generally is not known.

Despite these difficulties, considerable progress has been made in the development of a rational approach to the design of peptide ligands with highly potent and specific biological and conformational properties [2–13]. In this review, we summarize some of the more useful approaches which have emerged from these studies, and assess the future developments in this area.

CONFORMATIONAL CONSTRAINT, STRUCTURE–BIOLOGICAL ACTIVITIES RELATIONSHIPS AND RATIONAL DESIGN

The primary approach to the design of peptide ligands has involved the use of conformational constraints. Though this approach has provided an important rationale for peptide ligand development and will be the central concern of this review, there are a number of other issues that require comment. First, it must be emphasized that since the goal is to develop peptide ligands of importance in a biological context, it is essential that provisions for obtaining excellent biological data be an integral part of any effort. Without the availability of state-of-the-art binding and bioassay data, no chemical/physical approach, however sophisticated or powerful, will provide the necessary insights for design of ligands with high potency and specificity for the desired biological effect. In this regard, it is realized that many state-of-the-art assay systems can assess changes in the physical/chemical properties of the ligand much more sensitively than the most sophisticated biophysical tools. Thus receptors are exquisitely sensitive tools for evaluating the changes in conformation, topography and dynamics important for ligand–receptor interactions. Furthermore, though the goal is to develop a rational approach to the design of ligands with specific conformational and topographical properties important to biological activity, our knowledge of how receptor–ligand interactions are manifested in biological changes are often very incomplete. For example, though it is clear that competitive antagonists bind differently from agonists to a receptor [1,14] (and hence show different structure–activity relationships), no approach is available to predict *a priori* which 'new' ligand–receptor interactions will lead to antagonists and which will lead to agonists of greater or less potency. Hence it is necessary to perform classical structure–function studies in a systematic way to provide information about the specific amino acid residues and functional groups in a peptide that are important to biological activity. Some highly systematic approaches have been proven to be generally useful.

1. Systematic reduction of the peptide sequence one amino acid residue at a time from the *N*-terminus and from the *C*-terminus to determine the minimum active sequence necessary for bioactivity (often only four to eight residues), and to determine whether the so-called 'address' sequence and 'biologically active' sequence [9,15] are dissociated, partially overlap, or appear to be the same. This is particularly important for the development of antagonists and receptor-selective analogues.

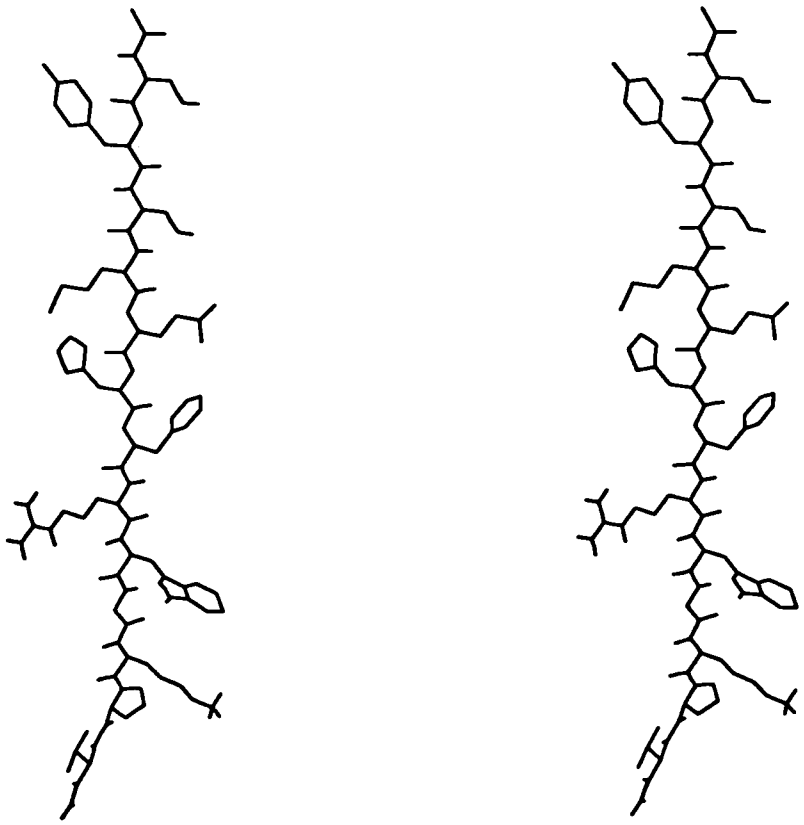
2. Systematic replacement of L-amino acids by D-amino acids. In addition to the insights this provides into the stereostructural requirements of a specific residue for peptide–receptor interaction, this approach can often provide insights into the importance of certain secondary structures (β-turns, α-helix, etc.) to the bioactivity of the peptide. It also can provide added stability to enzymic breakdown of the peptide.

3. Systematic replacements of side chain moieties by a methyl

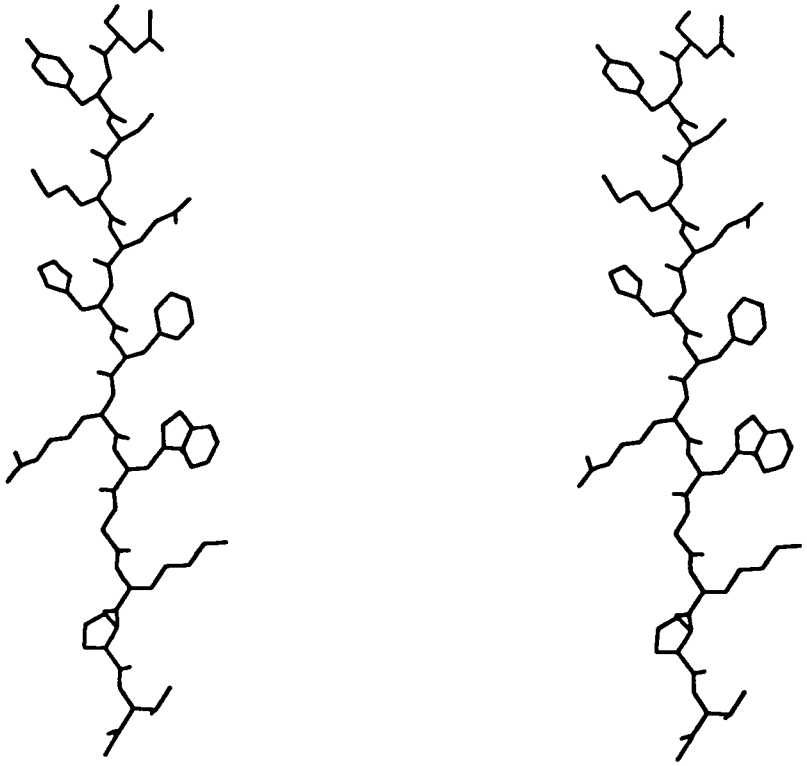
Abbreviations used: abbreviations for amino acid residues follow those recommended by the IUPAC. All amino acids, except glycine, are of the L variety unless otherwise noted. Other abbreviations include: Pen, penicillamine (β,β-dimethylcysteine); Nle, norleucine (α-aminohexanoic acid); Orn, ornithine; ACE, angiotensin converting enzyme; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; Aib, α-aminoisobutyric acid; CCK, cholecystokinin; β-Mpa, β-mercaptopropionic acid; Δ^{Z}Phe, (*Z*)-2,3-dehydrophenylalanine; $\Delta^{3,4}$Pro, 3,4-dehydroproline; 2-Tha, 2-thiazolidine-1-carboxylic acid; Acc, 1-aminocyclopropane-1-carboxylic acid; VPhe, 1-amino-2-phenylcyclopropane-1-carboxylic acid; Apmp, α-amino-β-mercapto-β-cyclopentanepropionic acid.

* To whom reprint requests should be addressed.

(a)



(b)



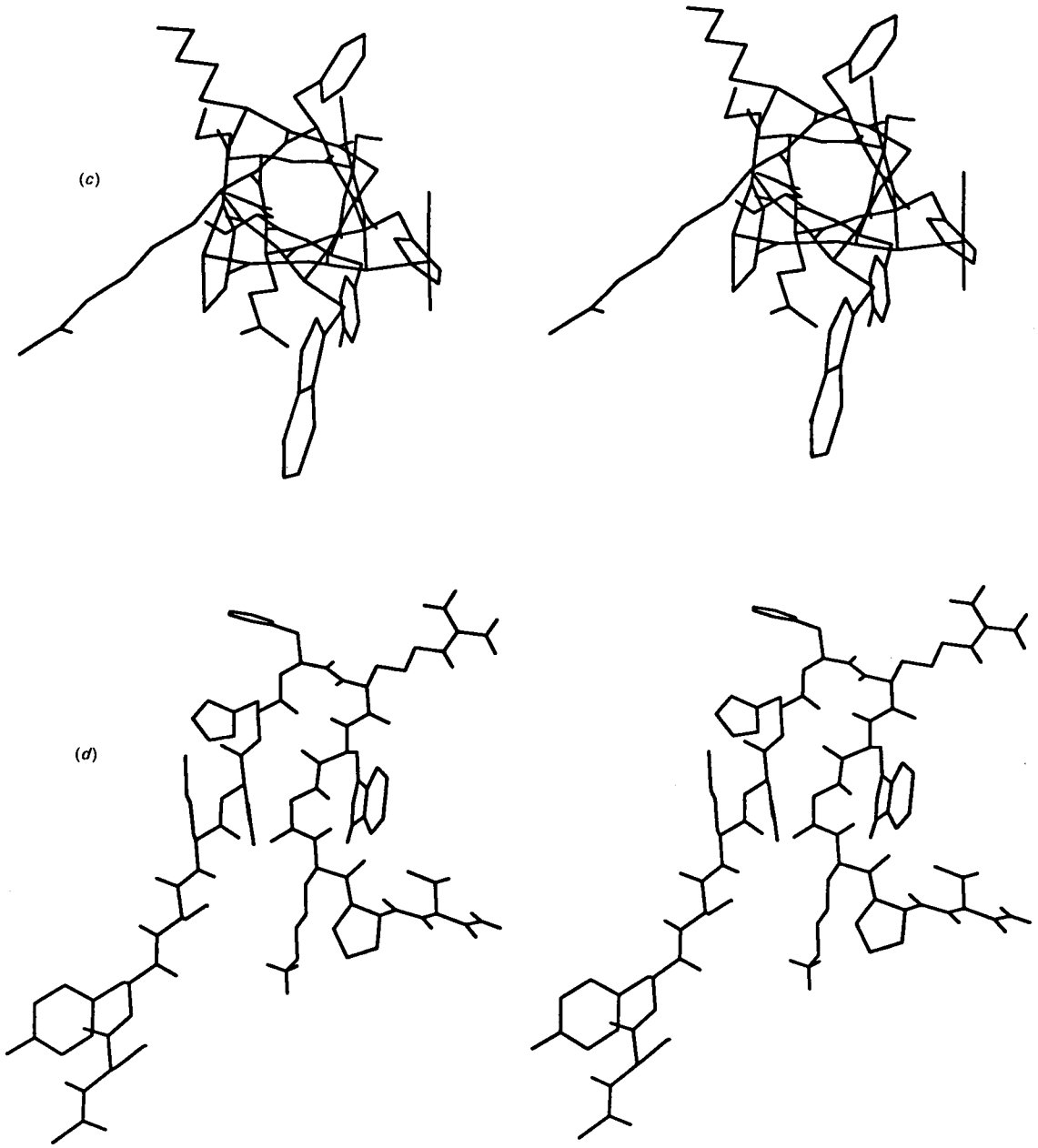


Fig. 1. Stereoviews of four common secondary structures often found in peptides and proteins using α-MSH (Ac-Ser[1]-Tyr-Ser-Met-Glu[5]-His-Phe-Arg-Trp-Gly[10]-Lys-Pro-Val-NH_2) as the peptide

The peptides are shown in the following secondary structures: (*a*) an extended conformation; (*b*) an antiparallel β-sheet structure; (*c*) an α-helix structure; (*d*) a type II β-turn at residues -His[6]-Phe[7]-Arg[8]-Trp[9]-.

group or by pseudoisosteric groups with different electronic properties to examine the importance of different side chain groups and their stereoelectronic properties for interaction with the receptor.

4. For cyclic peptides, systematic reduction or increase in ring size to determine the optimum ring size for biological activity.

5. Systematic replacement of peptide bonds with 'amide bond replacements'. This approach can provide insights into the importance of specific amide bonds for ligand–receptor (acceptor) interactions, and is especially useful in the design of protease inhibitors. This approach will not be discussed further, but an excellent review is available [16].

Finally, it is important to emphasize some of the critical biological and biochemical issues that will arise in the course of such investigations. Most peptide ligands serve as biological switches, i.e. by interacting with cellular receptors they switch on or off biochemical processes which modify cell function. As such, their information content must be related to the mechanism(s) of information transfer in the cell where the peptide–receptor (acceptor) interaction has occurred. In the case of peptide hormones and neurotransmitters, the peptide ligand has specific information related to binding (to the receptor/acceptor complex), to transduction (generally the activation of one or more biochemical processes, e.g. adenylate cyclase, channel opening or closing, etc.), and to reversal (return) to the initial state (generally this is *not* simple reversal of the binding step). Hence multiple structural, conformational and dynamic properties need to be considered in the design, adding a level of complexity. In addition,

Table 1. General methods for local non-covalent constraints in peptides

	Method	Feature
1.	Backbone N^{α}-alkylation	ϕ, ψ and χ_1 constrained; *cis–trans* peptide bond isomerism possible
2.	Backbone C^{α}-alkylation	ϕ, ψ constrained, often to α-helix, 3_{10} helix or extended structures
3.	Substitution	
	D-Amino acid	Reverse turn favoured, turn direction reversed, e.g. βII to βII′
	Proline	Compatible with different types of β-turns when at either $i+1$ or $i+2$ positions
4.	Bulky side chain groups (adamantyl, *t*-butyl, etc.)	Steric properties affect local backbone conformations and simultaneously lipophilicity is modified
5.	Amide bond surrogates	Possibility of incorporation of both *trans* (through double bond) and *cis* (through tetrazole) peptide bond mimics
6.	Cyclic amino acids	For secondary amino acids affects the ω angle, often biased to a single conformation (*cis* or *trans*); ϕ and ψ angles also are affected, often favouring various β-turn and γ-turn angles; χ_1 angles can be biased to $-60°$, or $\pm 180°$, or $+60°$ in favourable cases
7.	Dehydro amino acids	Can define side chain topography in either *Z* or *E* conformation, but chirality of C_{α} atom is lost; may lead to backbone conformational changes
8.	β or $\beta\beta$ substitutions	For β-substituted cystines, the disulphide bonds may possess either a positive or a negative helicity depending on the β-substitution pattern; in addition, transannular interactions can increase, especially in cyclic analogues; the diastereotopic environment is affected
9.	χ_1 bias for β-alkylated, β-hydroxylated and cyclic amino acids	Choice of $-60°$, $\pm 180°$ and $+60°$ possible for χ_1 angle; topographical relationships modified

Table 2. Examples of specific conformational requirements for peptide ligands interacting with their receptors

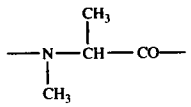
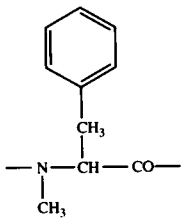
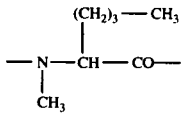
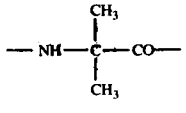
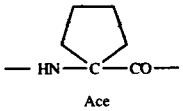
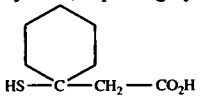
	Example	Biological activity	Reference
1	[*N*-Me-Ala]Bradykinin (substitution in place of proline) 	Sharp reduction in inhibitory potency of bradykinin-potentiating peptides in comparison to the natural peptides	[17]
2	[*N*-Me-D-Phe^1]CTOP 	Decreased potency; χ_1 effect	[18]
3	[*N*-Me-Nle^8]CCK$_5$ 	Increased selectivity for CCK-B receptor; *cis–trans* isomerism	[19]
4	[Leu]Enkephalin analogues containing Aib or Ace at positions 2 and 3  Aib  Ace	No biological data but there was conformational preference for a β-turn	[20]
5	Des-amino-β-mercapto-$\beta\beta$-pentamethylenepropionic acid in position 1 of oxytocin, replacing cysteine 	Enhanced oxytocin antagonism	[29]

Table 2 (*cont.*)

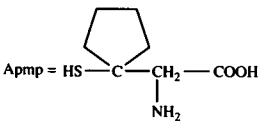
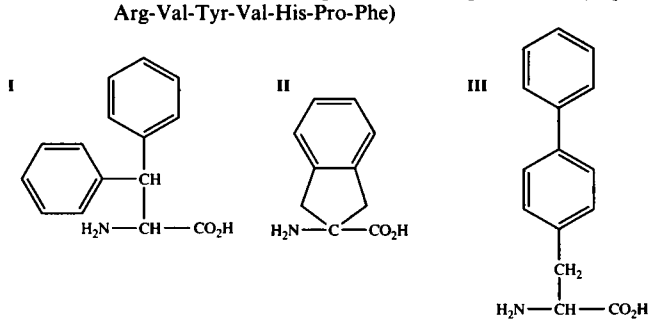
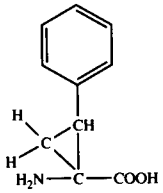
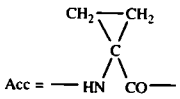
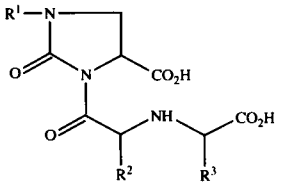
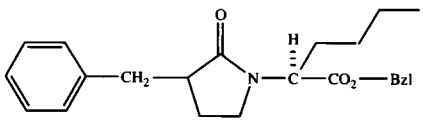
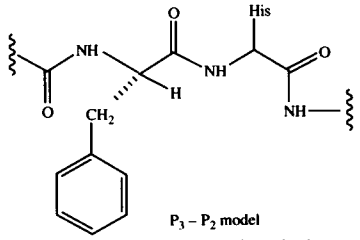
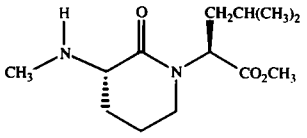
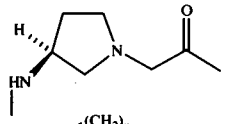
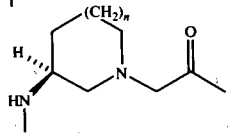
	Example	Biological activity	Reference
6	Oxytocin (OT) Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2; arginine vasopressin (AVP) Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2; [D-Pen^1]AVP; [$(CH_2)_5$-β-Mpa^1]AVP; [Et_2-β-Mpa^1]AVP; [$(C_2H_5)_2$-β-Mpa^1]OT	Enhancement or reduction in potency depending on the type of substitution at β-carbon	[24,25]
7	Tyr-D-Pen-Gly-Phe-D-Pen-OH, [D-Pen^2,D-Pen^5]enkephalin	Highly δ opioid receptor-selective ligand	[26,27]
8	[Xaa^2,Yaa^5]Enkephalins [Xaa = D-Pen, D-Cys, L(D)-Apmp; Yaa = L(D)-Cys, L(D)-Pen, L(D)-Apmp] 	Enhanced or reduced biological activity and binding depending on the residue substitution and side-chain at β-carbon of each residues involved in S-S cyclization	[30]
9	Bu^t alkylation of Ser and/or Thr side chain in enkephalins: Tyr-D-Ser(OBu^t)-Gly-Phe-Leu-Thr(OBu^t), Tyr-D-Ser-Gly-Phe-Leu-Thr(*O*Bu^t), Tyr-D-Ser(OBu^t)-Gly-Phe-Leu-Thr	Increase of potency and δ opioid receptor selectivity relative to [Leu]enkephalin	[40]
10	**I**, **II**, and **III** were incorporated into angiotensin II (Asp-Arg-Val-Tyr-Val-His-Pro-Phe) 	Enhanced the affinity to rat uterus (K_i 0.74–6.08 nM) and also enhanced inhibitory actions	[41]
11	*trans* -C=C- bond replacing -CO-NH-peptide bond at either the Trp-Leu, Leu-Asp or Asp-Phe site of CCK_4 and Gly-Trp of Boc-CCK_5	Enhancement of affinity for CCK receptor (2–3-fold), or reduction in binding activity depending on position of modification	[44]
12	Incorporation of conformationally restricted Phe in position 4 of enkephalin 	Enhanced opioid receptor selectivity and specificity	[46]
13	[1-Aminocyclopropane-1-carboxylic acid^7]Oxytocin 	Enhanced activity by about 5-fold relative to the potent analogue	[47]
14	[Acc^3]Aspartame, oxytocin, enkephalin, thyroliberin or CCK_4	Enhancement or no change in potency of oxytocin, enkephalin, and CCK_4. Also used in tentoxin as substrates or competitive inhibitors of the enzyme synthetase	[48]
15	Incorporation of 'cyclopropane Asp' (∇Asp) in Aspartame	There was a loss in activity with the bitter taste of the resulting analogue	[49]
16	Tyr-D-Ala-Gly-∇Phe-Leu (2*R*,3*S*), Tyr-D-Ala-Gly-∇Phe-Leu (2*S*,3*R*)	Enhanced selectivity and potency depending on stereochemistry of the side-chain substituent of Phe	[50]
17	[Δ^ZPhe^2]TRF (TRF sequence is pGlu-His-Pro-NH_2)	Enhancement in potency relative to the native hormone	[51]
18	Thyroliberin (TRH): [L-Δ^ZPro^3]TRH, [D-Δ^ZPro^3]TRH	No change in potency relative to the native hormone	[52]
19	Bradykinin (Bkn) (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg): [Δ^ZPhe^5]Bkn, [Δ^ZPhe^8]Bkn, [Δ^ZPhe^5,Δ^ZPhe^8]Bkn	Enhancement in potency in various bioassays	[53]

Table 2 (*cont.*)

	Example	Biological activity	Reference
20	Tyr-Gly-Δ^ZAla-Phe-Leu, Tyr-Gly-Gly-Δ^ZPhe-Leu, Tyr-Δ^ZAla-Gly-Phe-Leu, Tyr-Gly-Gly-Phe-Δ^ZLeu, Tyr-D-Ala-Δ^ZAla-Phe-Leu, Tyr-D-Ala-Gly-Δ^ZPhe-Leu	Enhanced or reduced activity depending on the position of substitution	[54]
21	[$\Delta^{3,4}$Pro^7]AVP, [2-Tha^3]LVP	No change or reduction in potency relative to the parent compound	[55]
22	Bicyclization via S-S of atriopeptin-(103–125) amide were prepared by synthesizing novel bicyclic peptides in which a second disulphide bridge linking residues 108 and 117 was introduced	The conformationally restricted analogues were more active than parent compounds (EC_{50} 0.05–3 μM)	[56]
23	Disulphide cyclization of myoglobin-(26–54)-fragment	Enhanced antigenicity relative to the parent myoglobin	[57]
24	Side-chain cyclization by S-S of *C*-terminal of Substance P via [Cys^5-Cys^{11}]SP-(5–11), [Cys^5, Cys^6]SP-(5–11), [Cys^6, Cys^{11}]SP-(6–11) and -(5–11), etc.	All cyclized analogues showed lower potency relative to the linear analogues, which indicate that a full agonistic activity may require an extended conformation	[64,65]
25	Cyclic lactams of CCK_8: Boc-Asp-Tyr(SO_3H)-Nle-D-Lys-Trp-Nle-Asp-Phe-NH_2	Reduction in activity by about 1000-fold in comparison to linear parent compound	[71]
26	Ac-Nle-Asp-His-D-Phe-Arg-Trp-Lys-NH_2	A superpotent and prolonged activity melanotropin	[74,75]
27	1. [β-Mpa^1,Glu^4,Cys^6,Lys^8]OT 2. [β-Mpa^1,Glu^4,Cys^6,Lys^8]OT	A cyclic oxytocin agonist converted to a bicyclic potent antagonist	[77]
28		Enhancement in activity (IC_{50} 1.1×10^{-8}–1.5×10^{-9} M and ID_{50} 0.24 mg/kg) in comparison to the native compound (ACE inhibitors).	[80]
29	 γ-Lactam model  P_3 – P_2 model	Enhancement in renin inhibition (IC_{50} 6–5 nM) upon substitution in human angiotensinogen	[81]
30	 β-II lactam analogue	Some loss in immunosuppressive activity due to steric hindrance effect on receptor–drug binding	[82]
31	Substitution in (A^{11}–B^6-Phe^7-D-Trp^8-Lys^9-Thr^{10}) 1. A–B =  2. A–B = 	Decrease in potency relative to the parent hormone. There was no difference whether $n = 1$ or 2	[83]
32	[Glu^{21}]Glucagon, [Lys^{17,18},Glu^{21}]glucagon, [Phe^{13}, Lys^{17,18}, Glu^{21}]glucagon, [Phe^{10,13},Lys^{17,18},Glu-^{21}]glucagon	Enhanced potency. Substitutions promote α-helix formation	[87–89]
33	D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2, D-Tic-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2, D-Phe-Cys-Tic-D-Trp-Orn-Thr-Pen-Thr-NH_2, *N*-Me-D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2, Gly-D-Tic-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2	Enhancement or decrease in binding and selectivity to opioid receptors depending only on side chain conformations of amino acids (backbone conformations stay the same in all analogues)	[18,101]

of course, if the peptide ligand is to be utilized *in vivo*, questions of biodistribution, passage through membrane barriers, biodegradation, etc. also become important issues.

Local conformational constraints

A variety of approaches are available to modify the local conformation to a specific or highly restricted conformation. Some of the most important *general* approaches are listed in Table 1. A rational utilization of these approaches is best undertaken within the context of a structure that already provides a measure of conformational stability (e.g. a β-turn, an α-helix, etc.; see Fig. 1 for examples of various secondary structures which often are desired). In these cases, very specific insights into the conformational preferences of a particular site of interaction between the receptor and the ligand can be obtained. In Table 2 we outline some examples from the literature that have utilized these approaches in a specific manner to provide insights into specific conformational requirements for peptide ligands interacting with their receptors.

For example, use of *N*-methylalanine in place of proline in bradykinin analogues causes a sharp reduction in potency (**1**, Table 2) and *N*-Me-D-Phe substitution at a terminal position in a μ opioid receptor-specific peptide *N*-Me-D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (**2**, Table 2) led to a 200-fold decrease in potency due to a change of the χ_1 angle of the D-Phe[1] residue ($\chi_1 = 180°$ instead of $-60°$). On the other hand, substitution of a *N*-Me-Nle for a Nle in position 29 of a cholecystokinin-(28–32) analogue dramatically increased its specificity for the CCK-β (brain) receptor, and n.m.r. studies suggested that this may involve a *cis* peptide bond [19].

C_α-Alkylation, especially C_α-methylation, has been widely used to explore the conformational requirements for bioactivity because it generally constrains residues to specific α-helical, 3_{10} helical or β-turn conformations [6,8] (Fig. 1). For example, it biases enkephalin analogues (**4**, Table 2) to a β-turn. When both Gly[2] and Gly[3] were replaced by Aib residues and the *N*-terminal group was bis-allyl substituted a highly specific δ opioid antagonist was obtained [21], though the conformational basis for this has not been determined. The use of Aib in peptide design has been reviewed [6,8].

β,β-Disubstituted amino acids were among the first amino acid derivatives used to examine the effects of conformational constraint on peptide conformation and biological activity. In particular, penicillamine (β,β-dimethylcysteine) was used to examine the structural, conformational and dynamic properties of oxytocin analogues which distinguish agonist from antagonist biological activity [22,23]. Its use in this application was particularly valuable because it provided insights into both local conformational constraints (disulphide bond angles and helicity), as well as overall conformational, dynamic and topographical properties (due to the geminal dimethyl effect in a medium-sized ring). Its potential in design has been amply illustrated subsequently in the development of potent vasopressin V_1 and V_2 antagonists [24,25], in the development of cyclic, highly δ-receptor selective, opioid agonists [26,27], and in the development of highly μ-receptor selective opioid antagonists [28]. β,β-Dialkyl-substituted analogues with larger R groups in the β-position have provided further insights into the relationship of structure to biological activity in oxytocin, vasopressin and enkephalin analogues [24,25,29,30] (**5**, **6**, **7** and **8**, Table 2, illustrate some examples).

The use of D-amino acids to stabilize or promote reverse turn conformations also has been widely applied in peptide design including the development of LHRH agonists and antagonists [31,32], substance P antagonists [33], oxytocin antagonists [34,35], melanotropin superagonists [36], enkephalin agonists [37], somatostatin analogues [11,38] and others. D-Amino acid residues also can help stabilize a peptide from enzymic degradation. D-Amino acid substitution will continue to be an important design consideration in many peptides, both for its conformational and stereochemical effects.

Amino acids with bulky side chain groups are primarily used to explore the steric and stereoelectronic requirements of a particular residue in a peptide for interaction with a particular receptor. For example, in development of vasopressin antagonists for the kidney (V_2) receptor initially it was necessary to utilize a β,β-cyclopentamethylenecysteine derivative in position 1 as well as other changes to obtain antagonist activity [39]. β,β-Dimethyl- or β,β-diethyl-substituted Cys did not work. Another interesting example is development of linear δ-opioid receptor agonists such as Tyr-D-Thr(OBu[t])-Gly-Phe-Leu-Thr(OBu[t]) ('DTLET', **9**, Table 2) which give high δ versus μ receptor selectivity only when the O[t]Bu groups are present [40]. While the conformational flexibility of this linear peptide makes a conformational analysis difficult, molecular modelling in conjunction with comparison with conformationally constrained highly δ-selective enkephalins such as [D-Pen[2], D-Pen[5]]enkephalin [27] can provide important insights into the steric requirements for δ versus μ opioid receptors. Many other kinds of amino acid derivatives are possible, such as β,β-dialkyl or diarylamino acids such as in **10**, Table 2 [41]. The possibilities are endless (cf. [42]).

Though several examples of replacing peptide bonds with *trans* double bonds has given some success in enzyme inhibitors, in biologically active peptides, the analogues produced have generally lost potency (cf. [43,44]) (**11**, Table 2). Efforts to prepare *cis* peptide bonds have been largely unsuccessful, and instead it has been suggested that a 1,5-disubstituted tetrazole ring can serve as a surrogate for a *cis* amide bond [45].

The use of cyclic amino acid analogues to constrain amino acid residues to particular conformational states has been under rapid development in recent years, and numerous examples now exist which illustrate its potential. The use of $\alpha\alpha'$- and $\beta\beta'$-substituted cyclic amino acids such as 1-aminocyclopentanecarboxylic acid (cycloleucine) and β,β-cyclopentamethylene-β-mercaptopropionic acid have been discussed in other contexts. α,β-Cyclopropane derivatives of amino acids deserve special mention. They can exist as four isomers and thus can be used to explore topographical relationships of particular side chain groups with respect to other side chain groups in the peptide and with respect to receptor requirements. These amino acids appear to be compatible with α-helical and certain β-turn conformations (Fig. 1), and also may be applicable to other conformational motifs. A number of examples are presented in Table 2 (**12–16**); increased potency and/or selective receptor selectivities often are observed. The use of such amino acids in rational design should increase dramatically in the future as an increasing number of constrained conformation templates become available as models for the 'biologically active' conformation, since such amino acids can be used to ask very specific steric, stereoelectronic, conformational, and topographical questions regarding a particular ligand–receptor/acceptor interaction, and for receptor mapping. In a similar manner, they should be most useful in examining similar questions in protein folding, stabilization of protein conformation, protein–ligand docking, etc. It will be important for synthetic chemists to develop high yield asymmetric syntheses of these compounds.

The application of α,β-, β,γ- etc. unsaturated amino acids should be mentioned. It must be emphasized that since hybridization of the α and β carbons are changed from sp^3 to sp^2, the stereoelectronic properties of the local environment change considerably relative to normal α-amino acids. On the other hand, the fixed *Z*- or *E*-configuration appears to be compatible

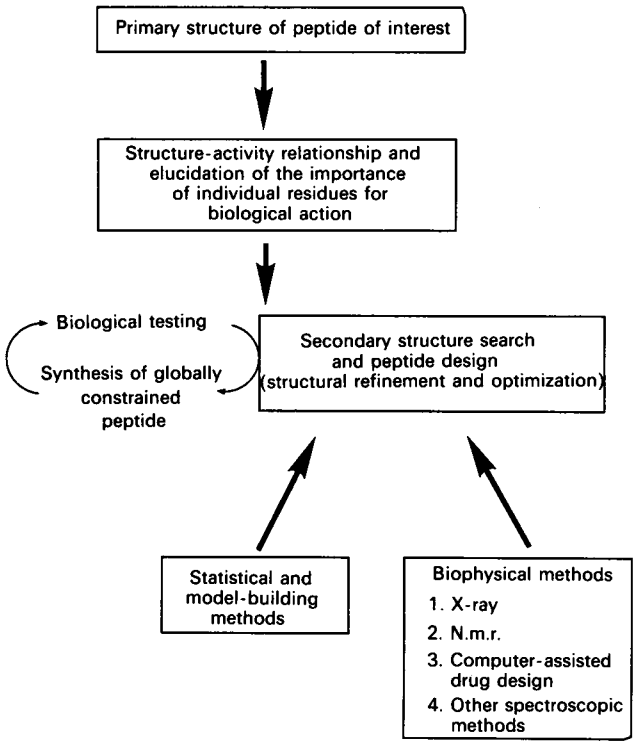


Fig. 2. An outline of the general strategy used for global constraint in the design of peptides

with most peptide secondary structure (α-helix, β-turn, β-sheet, extended, etc.). This permits investigation of very specific three-dimensional structural properties with respect to bioactivity, especially in constrained analogues. An additional difficulty, however, is the conservation of the peptide backbone conformation in a template since (among others) the C–C_α–N angle is now 120° (sp^2) instead of 109.5° (sp^3). In Table 2 a number of examples of the application of these amino acids to peptide structure-function studies (**17–21**) are provided. The use of double bonds further out on the side chain is largely unexplored.

Global conformational constraints

The use of local constraints in the design of specific conformational and topographical features in a peptide is most rationally applied within the context of a conformationally defined or semistable 'template' for the biologically active conformation.

Arriving at a conformationally defined template generally requires the development in each case of a systematic strategy of design leading to global constraints (Fig. 2). Such an approach includes but is not limited to the following considerations. (1) Examination of general structural requirements for bioactivity using the classical approach. For example variation of specific amino acid residues by elimination of the side chain groups, examination of stereochemical requirements, or variations in different physicochemical properties [e.g. basic (Lys, Arg), acidic (Asp, Glu), aromatic (Phe, Trp), hydrophilic (Ser, Thr), etc.] can provide powerful insight and occasionally critical leads. In addition, replacement of physiologically unstable amino acid residues by their isosteric more stable analogues (e.g. Nle for Met) also can be useful. These approaches have been useful for example, in the design of critically important analogues of α-MSH [36,63], enkephalins [27,69] and somatostatins [11,101]. (2) Examination of potential conformational preferences using techniques such as Chou–Fasman calculations, model building, simple c.d. and/or n.m.r. studies, etc. to suggest possible secondary structural features important to bioactivity, and for designing analogues that may enhance these properties (e.g. [63,86], etc.). (3) Biophysical studies of analogues developed from (1) and (2) by using various techniques [n.m.r., c.d., computer-assisted drug design (CADD), etc.] (see [104] for discussions of these methods) to obtain insights for design of globally constrained peptide analogues (e.g. see [74,78]). Recent advances in theoretical methods and computer-assisted design are now available for direct use in the analysis and design of conformationally constrained analogues (e.g. see [74] and references therein). (4) Receptor binding and/or bioassay results often are the most sensitive probes of conformational and dynamic requirements for a peptide ligand, often exceeding most physicochemical methods. The critical interplay of design, synthesis, biological activity and biophysical studies will often provide the critical insights needed for rational design. An outline of the general strategies leading to the design of peptide analogues with global constraints is given in Fig. 2.

Using this general approach, it has been possible in several instances to suggest models for the biologically active conformation of peptide ligands for receptors and acceptors. In general, these 'bioactive conformations' were proposed as a result of global conformational constraints (e.g. cyclization; Table 1) that also led to high receptor affinity (potency) and/or selectivity. The results further suggested a similarity between the receptor-bound conformation and that in the free state, allowing direct examination of the latter by n.m.r., etc. In this section we will examine such approaches which have promise for the rational design of biologically active peptide ligands. In general, the purpose of these constraints is to promote or stabilize more comprehensive structural features as α-helices, β-sheets, reverse turns, pseudocyclic conformations, loops, etc. (Fig. 1) which are important or essential for the biological activity of the compound. In Table 3 we list some of the general approaches that have been taken, and will now illustrate these with selected examples.

A few principles should be emphasized prior to the discussion. First, since the peptide ligands are interacting with receptors, and side chain groups often need to be significantly modified to establish a cyclic structure, in many cases it is critical that the side chain group modified *not* be one critical to receptor recognition or transduction. However, if is necessary to modify such a side chain group, it should be done in such a way that it is isosteric and isoelectronic to the native structure. Thus generally it is not useful simply to make a covalent bridge at convenient residues, but rather to choose and/or design sites that are compatible with receptor requirements. This may require synthesis of special amino acids or amino acid derivatives with specific stereostructural properties. In this regard, one must also consider whether the groups to be joined covalently should be in close spatial proximity in the desired conformation. This is particularly emphasized since even if distal amino acid residues appear close to one another in a modelled analogue, as suggested, for example,

Table 3. General methods for global constraints in peptides

1. Cyclic disulphides; stabilization of secondary structure
2. Cyclic, covalent side chain-to-side chain; lactams, lactones, ethers, thioethers, etc.
3. Cyclic, covalent side chain-to-backbone; lactams, lactones, heterocyclics, etc.
4. Stabilized amphiphilic helices; non-covalent stabilization
5. Specific transannular interactions; non-covalent stabilization
6. Peptido-mimetics
7. Topographical considerations

by C_α-to-C_α distances, the side chain groups (aromatic groups, OH groups, etc.) may be quite distant from one another and will not readily interact with one another as a result of their preferred side chain conformations. Thus, careful modelling of conformational and dynamic properties of such side chain groups to determine whether their proximity is feasible without too high an energy penalty can be most useful.

Cyclic disulphides. In eucaryotic cells, Nature has used disulphide bonds as its major covalent bond for conformational control and stabilization. In most cases, it appears that proteins are 'programmed' to fold in a manner that is compatible with the disulphide motif found in that protein. Indeed, as shown in Table 2 (**22**), an additional S–S bridge can increase activity. In a similar manner (**23**, Table 2), introduction of a specific disulphide in a myoglobin fragment enhanced its antigenicity relative to the parent myoglobin. While disulphide-containing peptide rings have reduced conformational properties relative to their linear counterparts, many cyclic disulphide-containing peptides still can be flexible. Thus, for peptides already containing a disulphide ring, two different approaches have been taken to constrain the structure further. The first is to reduce the size of the ring. Subtle modifications include removing one carbon or one sulphur atom from the ring or replacing the disulphide bridge with a C–S or S–C bridge (thioether) or a C–C bridge (carba). All of these have been examined in oxytocin (Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2) and vasopressin (Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2) (for reviews see [58,59]) and dramatically different results were obtained. In the case of oxytocin some analogues were superagonists, others slightly weaker agonists than oxytocin. In the case of vasopressin, most analogues were less potent. In all cases the relative effect greatly depended on which neurohypophyseal hormone receptor was examined. Interestingly, *all* efforts to reduce or increase the size of oxytocin's 20-membered ring by a single residue (three atoms) or a single atom (be it C or S) led to a drastic decrease (> 100-fold) in potency.

A quite different and useful experience occurred with somatostatin, Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys. This 38-membered ring compound has had its disulphide ring structure reduced to a 20-membered ring in the analogue D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol [60] which is superactive in inhibiting growth hormone release, and to an 18-membered cyclic hexapeptide ring structure, [Pro-Phe-D-Trp-Lys-Thr-Phe] [61] which is equipotent to somatostatin in inhibition of growth hormone, glucagon and insulin release. These results were obtained by a systematic reduction in the ring size of somatostatin to arrive at the 'biologically active' conformation (for a review see [11]). Interestingly, Hruby and co-workers, using a different approach [28,62,101], have utilized the somatostatin β-turn template -Phe-D-Trp-Lys-Thr- as a conformationally stable moiety to design cyclic peptides that have high potency and selectivity for μ opioid receptors, but little or no activity at somatostatin receptors. In this case, penicillamine replacement for cysteine was used to enhance receptor selectivity and potency at the μ opioid receptor and decrease potency at somatostatin receptor. For example, the analogue D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 [62] interacted with the μ opioid receptor in the nM range, but with the somatostatin receptor in the 20000 nM range. Further development of these μ-specific peptides using topographical constraints is described later in this paper.

Disulphide ring closure can be used to explore whether certain folded conformations are compatible with the biologically active conformation of linear peptides. In the case of α-melanotropin (Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2), a postulated turn centred at the Phe^7 position as the biologically active conformation was examined by preparing the pseudo-isosteric cyclic analogue Ac-[Cys^4,Cys^{10}]α-MSH in which the Gly^4 and Met^{10} residue were replaced by cystine to give a superpotent analogue [63]. Similarly, replacement of the Gly^2 and Met^5 residues with the disulphide bridge of D-Pen^2 and D-Pen^5 gave the cyclic constrained analogue [D-Pen^2,D-Pen^5]-enkephalin, a potent and highly receptor selective δ-opioid receptor agonist [27]. Alternatively, disulphide cyclization has been used to suggest that for substance P the receptor preferred a more extended conformation, since a variety of cyclic disulphide-containing analogues led to greatly reduced potencies [64] (**24**, Table 2). Of course, negative results can never be taken as final, since it is always possible that the appropriate constraint has not been examined. In any case, the examples cited above and others [3] demonstrate that careful use of disulphide and thioether cyclization is a powerful tool for examining the conformational requirements for peptides at biological receptors.

Covalent side chain-to-side chain cyclizations. The use of side chain groups on amino acid residues to form covalent attachments to other side chain groups on the peptide has, in principle, an almost infinite number of possibilities. The side chain functional groups on peptides are numerous and one can easily envisage the formation of esters (lactones), amides (lactams), thioethers, ethers, a variety of substituted aromatics and pseudo-aromatics, ureas, ketones, etc. by a variety of side chain-to-side chain covalent interactions. Indeed a variety of these have been used already. The coupling of aromatic rings to give biphenyls or substituted biphenyls is of great current interest since a number of macrocyclic antibiotics such as vancomycin [65,66] have such structures. The chemistry being developed for the synthesis of these strained macrolids should be directly applicable to peptides. The synthesis of a monocyclic derivative of the antitumour agent bovardin was reported [67], but the tyrosine-to-tyrosine ring formation was not achieved. An enkephalin analogue with a diazobridge has been reported and it has some activity [68], but further analogues apparently were not prepared. Numerous possibilities exist to create interesting conformations and topographical surfaces, especially where side chain aromatic residues are important for biological activity.

Cyclic lactams utilizing a side chain amino and a side chain carboxylate group have become more widely used recently. An important early success was that of Schiller and co-workers who prepared cyclic lactams between the 2 and 4 (or 5) positions of enkephalin to give analogues with considerable potency and selectivity for μ opioid receptors. For example Tyr-D-Orn-Phe-Asp-NH_2 is a 13-membered ring compound with high potency and selectivity for the μ opioid receptor [69]. Similar studies with the dynorphin analogues were less successful [70]. The cyclic lactam analogue of CCK-8, Boc-Asp-Tyr(SO_3H)-Nle-D-Lys-Trp-Nle-Asp-Phe-NH_2 (**25**, Table 2) showed a large decrease in potency at the CCK-8 peripheral receptor [71]. On the other hand when a bis-lactam analogue of CCK-8 was prepared by replacement of the Met residues in CCK-8 at positions 28 and 31 with Lys residues, and amide bonds were formed by reaction of the Lys ϵ-amino acid groups with the carboxylate groups of succinic acid, a highly selective analogue for the CCK-B (brain) receptor was obtained [72]. Interestingly, these selectivities were closely matched by CCK-8 analogues in which the Met residues were replaced by *N*-Me-Nle residues where *cis–trans* isomerism is now possible [19]. It will be interesting to compare the conformational similarities of these two different kinds of CCK analogues which have very similar potencies and selectivities for CCK-B receptors. The use of computer modelling techniques in

conjunction with quenched molecular dynamics simulations as a rational approach to the design of peptide ligands was recently explored for melanotropins [73–75]. Using quenched dynamic simulations on α-MSH, [Nle^4,D-Phe^7]α-MSH and Ac-[Cys^4,Cys^{10}]α-MSH allowed an exploration of conformational space including interconversion of conformations through rotational barriers. It was observed that α-MSH and its analogues tended to form folded conformations about the tetrapeptide sequence His^6-Phe^7-Arg^8-Trp^9 with segregation of lipophilic and hydrophilic side chains to opposite faces. It was further observed that although the Lys^{11} and Glu^5 side chain groups were on the same side of the folded pseudocyclic structures they did not form a salt bridge readily. However if the Lys side chain group was moved to the pro-*S* face of the α-carbon of Gly^{10}, salt bridges formed readily. These observations prompted the synthesis of linear [76] and cyclic lactam [74,75] analogues of α-MSH, α-MSH-(4–13) and α-MSH-(4–10). Compounds with very high potency were obtained particularly in the cyclic case, with Ac-[Nle^4,Asp^5,D-Phe^7,Lys^{10}]α-MSH-(4–10)-NH_2 (Table 2, **26**) being respectively 90 times and 100 times more potent than α-MSH in the lizard skin and mammalian melanoma tyrosine assay, respectively. An example of the effect of conformational space restriction on biological activity using cyclic lactam formation is the conversion of a very weak monocyclic oxytocin agonist analogue, [β-Mpa^1,Glu^5,Cys^6,Lys^8]oxytocin to a highly potent bicyclic antagonist (pA_2 = 8.2) [β-Mpa^1,Glu^5,Cys^6,Lys^8]oxytocin (**27**, Table 2) by cyclic lactam formation [77]. This represents a > 1000-fold increase in binding for the bicyclic analogue, and its simultaneous conversion to an antagonist. Such an analogue should provide new insights into the relationships of topography and conformational rigidity on agonist and antagonist activity in oxytocin analogues. Another example of exploring conformational–biological activity relationship at hormone receptors has been the studies of Struthers *et al.* [78] on GnRH analogues such as cyclo[Δ^{3,4}Pro-D-Phe(4-Cl)-D-Trp-Ser-Tyr-D-Trp-*N*-Me-Leu-Arg-Pro-β-Ala]GnRH which were prepared to test a model for the biological conformation of GnRH.

There are many other possibilities for side chain-to-side chain cyclization awaiting those with the ideas and synthetic approaches which will make it possible. A wide variety of bond types including organometallics should be possible. In addition to bonding, bridging is also possible, as in the bis thioethers of Mosberg & Omnaas [79].

Cyclic side chain-to-backbone modifications. Cyclization of peptides from a side chain functional group to the peptide backbone offers numerous possibilities for rational design of peptides with specified conformational properties. In a sense cyclic amino acids such as proline, α-aminocyclopropylcarboxylate, α-aminocyclohexylcarboxylate, and other such amino acids are members of this class. These have been discussed in other contexts, though some interesting modified analogues should be mentioned (**28–31**, Table 2). Many of these kinds of modifications favour the formation of, or stabilize, specific conformational features (α-helices, β-turns, etc.), and thus can be readily utilized for rational design. Others have less well-defined properties which need to be determined by experimental means (e.g. n.m.r. and X-ray analysis) and/or by calculations. Many possibilities can be imagined and a number have been tried. A comprehensive review has been prepared recently [8].

Stabilized amphiphilic helical structure. It has been proposed that the biological activity of many larger polypeptide hormones may be due to the presence of amphiphilic (amphipathic) helices. There has been a considerable amount of work in this area which has been reviewed [7]. A basic hypothesis of Kaiser and co-workers was that the major structural features for biological activity were the presence of a stabilized amphipathic helix without need for specific primary structural or topographical features. Thus specific side chain structures or conformations were not considered in the design except that they be compatible with an amphipathic helix. This works for a number of biologically active polypeptides such as β-endorphin [84], where specific structure features can be greatly modified without large losses in potency. In other cases, e.g. glucagon [85], it was found that replacement of the address sequence with a stabilized but structurally non-specific sequence led to a drastic loss in potency. On the other hand, Hruby *et al.* [86–88] found that enhancement of α-helical potential (based on Chou–Fasman probability parameters) but with 'conservative' amino acid replacements greatly increase binding to the glucagon receptor (**32**, Table 2). In a similar extensive investigation of the anticoagulant peptide hirudin, it was suggested that an amphipathic α-helical structure was critical for bioactivity [89] and the conformations as further stabilized by formation of a [D-Cys^{58},Cys^{61}]hirudin-(54–65) fragment analogues (14-membered ring), which had 14% of the potency of the full sequence [90]. Also most interesting has been the work of Baldwin, Stewart and co-workers who have utilized specific conservative structural modifications to greatly increase the helical content of ribonuclease S peptide such that in aqueous solution it is essentially α-helical [91,92]. These and other studies now make it possible to design α-helical structures of reasonable short sequence (10–20 residues) with considerable fidelity.

Specific non-covalent transannular interactions. The use of specific non-covalent transannular interactions to stabilize the three-dimensional structure of peptides has long been a goal of peptide and protein chemistry. Intramolecular hydrogen bonding stabilizes α-helices, β-turns, γ-turns and other turn structures. Much experimental data is now available that allows one to design turns in peptides with success. This has been thoroughly reviewed [93]. One important caveat is that though reverse turns can be designed readily, it is sometimes difficult to design them with the appropriate side chain groups for specific peptide–receptor (acceptor) interaction. This aspect is often neglected in modelling and design, but it is critical that it be a major consideration. Salt bridges and side chain (Ser, Thr, etc.) to backbone hydrogen bonding also can be used to stabilize secondary structure [94]. Other forms of non-covalent interaction, including aromatic–aromatic side chain interactions [95], appear to stabilize tertiary structure in peptides and proteins but their systemic use in the design of peptide conformation has not been developed. In this regard, the proposal of Rose [96] that 'loops' may constitute yet another important secondary structure feature in proteins has met with wide acceptance. Here again, very little has been done to utilize this in peptide design, owing in large part no doubt to our lack of ability to utilize in a predictive way collective non-covalent interactions (e.g. van der Waal attractions) to stabilize three-dimensional structures of peptides. This should be an important goal of future work in peptide and protein design.

Peptide mimetics. The idea of replacing all or part of a peptide structure with a non-peptide that can mimic some structural or conformational property of the peptide has been widely considered. Amide bond replacements are the most simple 'mimics' to consider [16], and though they have been useful in the design of peptidase enzyme inhibitors, often they have not been successful in modifying peptide hormones and neurotransmitters to obtain more potent and selective analogues, presumably because most of these modifications do not truly mimic a peptide bond in

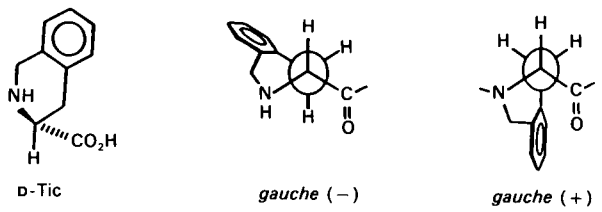


Fig. 3. Structure of D-1,2,3,4-tetrahydroisoquinoline-2-carboxylic acid (D-Tic) and its two low-energy chair-like conformations

its ground state. Nonetheless, they have many potential uses [16]. Another area under rapid development is the design of mimics of peptide and protein secondary structure (β-turns, α-helices, β-sheets, γ-turns, etc.). Though a number of successes have been reported in obtaining mimetics which can force or stabilize peptide secondary structure (cf. [97–99]), little success has been reported in incorporating mimetics at the active site of a peptide hormone or neurotransmitter, probably because to date most mimetics do not possess appropriately positioned side chain groups. It is anticipated that this will change in the near future since peptide mimetics have many potential applications. A good example of the potential in this area is the recent report of Flynn *et al.* [100] in which a tricyclic, conformationally constrained lactam was obtained which is among the most potent ACE inhibitors. In this case special attention was paid to side chain conformations. Other interesting examples have appeared recently [105–107].

TOPOGRAPHICAL DESIGN – POSSIBILITIES AND PROSPECTS

Topographical design provides a largely uncharted, but in our view highly important, approach to peptide and protein design which offers great potential in the design of highly potent and receptor/acceptor selective peptides and proteins. Furthermore, it can provide critical new insights in our efforts to understand such diverse but closely related problems as protein folding, peptide–receptor interactions (the docking problem), enzyme–substrate specificity, etc. A few initial efforts have been made that have been surprisingly successful. They will be briefly discussed here with additional comments about the future.

For discussion purposes, topography refers to the 'relative, co-operative three dimensional arrangements of the side chain groups in a polypeptide'. Accordingly it is possible to have a different topography with a particular secondary structure (e.g. β-turn), or alternatively, to have a similar topography with a different secondary structure. There is a need to examine this problem systematically and determine its implications for the host–guest problem in peptide structure–activity relationships. We propose that this can be done by appropriately constraining, biasing or fixing the side chain conformers to specific conformers generally found for amino acid residues, *gauche*(−) ($\chi_1 = -60°$), *trans* ($\chi_1 = \pm 180°$) or *gauche*(+) ($\chi_1 = +60°$). In this way it should be possible to modify the surface architecture, especially of already constrained analogues, to provide specific topographical relationships.

One approach to do this is to enforce specific side chain conformations on aromatic (or aliphatic) amino acid residues, for example, by connecting its α-amino group to the 2′-aromatic position with a methylene group. Thus, 1,2,3,4-tetrahydroisoquinoline-2-carboxylic acid (D-Tic, Fig. 3) can be viewed as a topographically constrained analogue of Phe. Note that χ_1 is now restricted to either *gauche*(−) or *gauche*(+) (the *trans* rotamer is excluded). It was found (Table 2, **33**) [18,101] that when the side chain conformation of the *N*-terminal aromatic residue in position 1 of [D-Tic[1]]CTP (D-Tic-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2) is *gauche*(−), analogues with maximum potency and receptor selectivity are obtained. N.m.r.-based conformational analysis suggested a topography in which the essential pharmacophores are on one face of the molecule (Fig. 4). A *gauche*(+) conformation for this residue leads to large drops in potency and selectivity, though the backbone conformation remained unchanged, as in Gly-D-Tic-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (Fig. 5). Finally, examination was made of the 3 position with an L-Tic residue in CTOP [102,105]. In this latter case a topography is observed in which the L-Tic[3] side chain is rotated to an opposite face as a result of its *gauche*(+) side chain conformation (Fig. 6), but the backbone conformation remains the same. This compound had greatly reduced potency at the μ opioid receptors. This and other

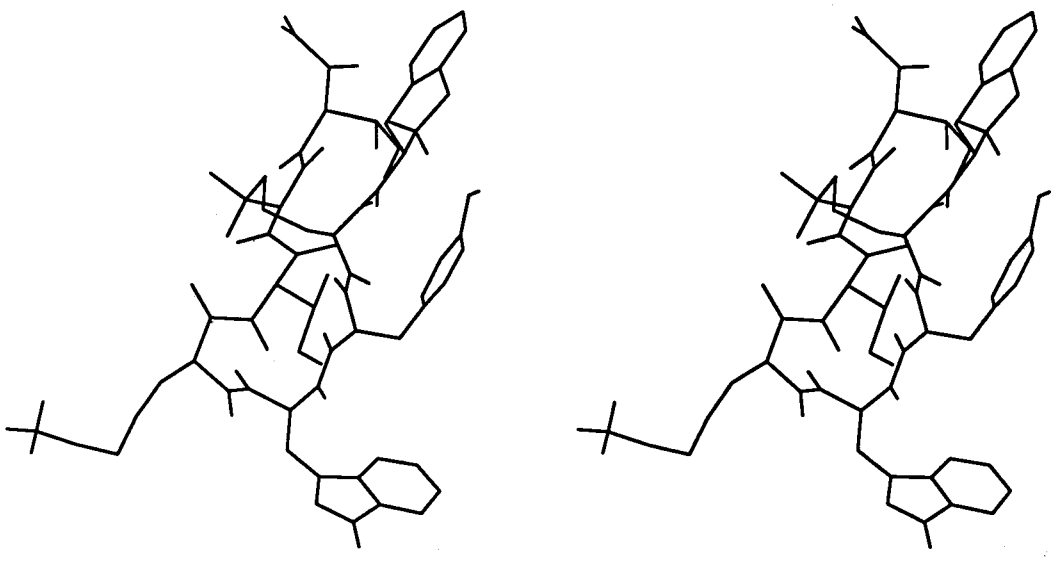


Fig. 4. Conformation of [D-Tic[1]]CTP (D-Tic-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2): stereo view

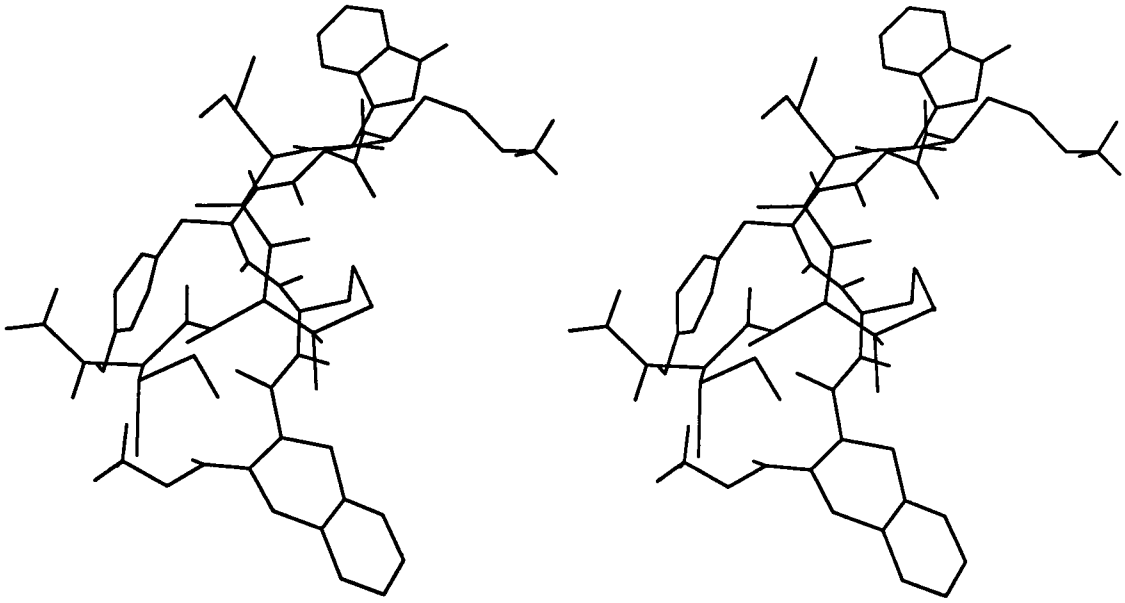


Fig. 5. Conformation of [Gly^0,D-Tic^3]CTOP (Gly-D-Tic-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2): stereo view

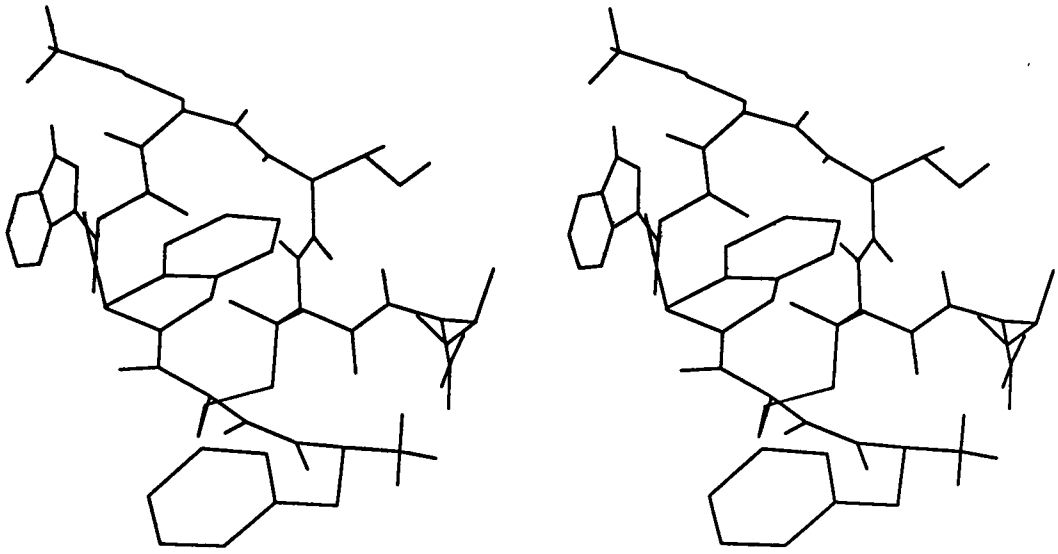


Fig. 6. Conformation of [Tic^3]CTOP (D-Phe-Cys-Tic-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2: stereo view

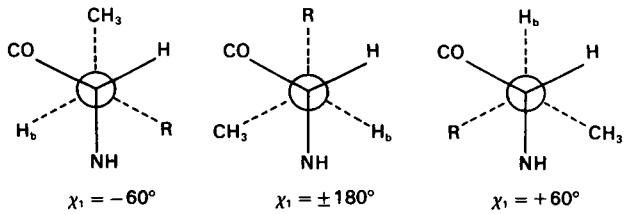


Fig. 7. The low-energy side chain conformations for (2*S*,3*S*)-β-substituted α-amino acids about the C_α–C_β bond [*gauche*(−) = −60°, *trans* = ±180°, *gauche*(+) = +60°]

related amino acids (cf. [8]) should find wide use in exploring topographical requirements.

α,β-Unsaturated amino acids (see above) are interesting in this regard in that the Δ^E conformer is similar to the *trans* conformation. However the Δ^Z conformation places the aromatic ring (for dehydrophenylalanine) approximately half way between *gauche*(−) and *gauche*(+). Also it should be noted that the α-carbon and β-carbon are now sp^2, which will modify other angles as previously discussed. Hence the general applicability of these as mimics of endogenous amino acids for topographical constraint is unclear.

Another way to approach this problem is to utilize diastereoscopic positions for substitution. For example, in β-methylphenylalanine its side chain conformation can be biased by virtue of nonbonding interactions between vicinal substituents. Thus, in the *S,S* isomer (Fig. 7), steric considerations suggest that a *gauche*(−) side chain conformation is preferred to *trans* or *gauche*(+). Similar arguments can be made for the other three isomers, and in view of the asymmetric centre at the α-carbon, each derivative would have its own unique topographical relationship in a peptide (especially a constrained peptide with a particular conformation). Indeed Hruby *et al.* [103] have shown, examining all four diastereoisomers of [D-

Pen[2],β-Me-p-NO_2Phe[4], D-Pen[5]]enkephalin, that though each has the same backbone conformation, they have different topographies and widely different potencies and specificities at δ and μ opioid receptors. Thus, topographical changes alone can greatly affect receptor potency and selectivity.

Similar considerations can be applied to other diastereotopic positions in amino acid residues in peptides using a wide variety of functions groups to probe steric and stereoelectronic effects.

CONCLUDING REMARKS

In this brief overview we have tried to provide some insight into recent developments in the rational design of peptides utilizing conformational, topographical, stereoelectronic and dynamic considerations. Though these approaches are recent, considerable progress has been made, and some very impressive peptide hormone and neurotransmitter analogues have been designed which offer enormous potential as future drugs. Hopefully, we also have illustrated the considerable power which conformational thinking, in combination with chemical design and synthesis, can bring to this field. It has not been possible to outline all of the numerous approaches which utilize this thinking. However, it should be quite clear that this approach has enormous potential for further development. Given the great importance of peptides and proteins in living systems, we have no doubt that exciting progress will continue.

Work was supported by grants from the U.S. Public Health Service DK 17420, DK 21085, NS 19972 and DA 04248 and from the National Science Foundation. The help of Natasha Johnson in typing and editing this paper is gratefully acknowledged.

REFERENCES

1. Hruby, V. J. (1981) in Topics in Molecular Pharmacology (Burgen, A. S. V. & Roberts, G. C. K., eds.), pp. 99–126, Elsevier/North Holland, Amsterdam
2. Marshall, G. R., Gorin, F. A. & Moore, M. L. (1978) Annu. Rep. Med. Chem. **13**, 227–238
3. Hruby, V. J. (1982) Life Sci. **31**, 189–199
4. Hruby, V. J., Krstenansky, J. L. & Cody, W. L. (1984) Annu. Rep. Med. Chem. **19**, 303–312
5. Schiller, P. W. & DiMaio, J. (1983) in Peptides: Structure and Function (Hruby, V. J. & Rich, D. H., eds.), pp. 269–278, Pierce Chem. Co., Rockford, IL
6. De Grado, W. F. (1988) Adv. Protein Chem. **39**, 51–124
7. Kaiser, E. T. & Kezdy, F. J. (1984) Science **223**, 249–255
8. Toniolo, C. (1990) Int. J. Peptide Protein Res. **35**, 287–300
9. Hruby, V. J. & Hadley, M. E. (1986) in Design and Synthesis of Organic Molecules Based on Molecular Recognition (Van Binst, G., ed.), pp. 269–289, Springer/Verlag, Heidelberg
10. Hruby, V. J. (1984) in Conformationally Directed Drug Design (Vida, J. A. & Gordon, M., eds.), pp. 9–29, ACS Symposium Series 251, Washington, DC
11. Freidinger, R. M. & Veber, D. F. (1984) in Conformationally Directed Drug Design (Vida, J. A. & Gordon, M. eds.), pp. 169–189, ACS Symposium Series 251, Washington, DC
12. Hruby, V. J. (1985) Trends Pharmacol. Sci. **6**, 259–262
13. Hruby, V. J. (1981) in Perspectives in Peptide Chemistry (Eberle, A., Geiger, R. & Wieland, T., eds.), pp. 207–221, Karger, Basel
14. Hruby, V. J. & Mosberg, H. I. (1982) in Hormone Antagonists (Agarwal, M. K., ed.), pp. 433–474, Walter de Gruyter & Co., Berlin
15. Schwyzer, R. (1977) Ann. N.Y. Acad Sci. **297**, 3–26
16. Spatola, A. F. (1983) in Chemistry and Biochemistry of Amino Acids, Peptides and Proteins (Weinstein, B., ed.), vol. 7, pp. 267–357, Marcel Dekker, New York
17. Filatova, M. P., Kri, N. A., Komarova, O. M., Orfkchovich, V. N., Reiss, S., Liepinya, I. T. & Nikiforovich, G. V. (1986) Bioorg. Khim. **12**, 59–70
18. Kazmierski, W., Wire, W. S., Lui, G. K., Knapp, R. J., Shook, J. E., Burks, T. F., Yamamura, H. I. & Hruby, V. J. (1988) J. Med. Chem. **31**, 2170–2177
19. Hruby, V. J., Fang, S., Knapp, R., Kazmierski, W., Lui, G. K. & Yamamura, H. I. (1990) in Peptides: Chemistry, Structure and Biology (Rivier, J. E. & Marshall, G. R., eds.), pp. 53–55, ESCOM Publishers, Leiden
20. Kishore, K. & Balaram, P. (1984) Ind. J. Chem. **231**, 1137–1144
21. Thornber, C. W., Shaw, J. S., Miller, L., Hayward, C. F., Morley, J. S., Timms, D. & Wilkinson, A. (1986) NIDA Res. Monogr. (Prog. Opioid Res.) **75**, 177–186
22. Meraldi, J.-P., Yamamoto, D., Hruby, V. J. & Brewster, A. I. R. (1975) in Peptides: Chemistry, Structure and Biology (Walter, R. & Meienhofer, J., eds.), pp. 803–814, Ann Arbor Science Publishers, Ann Arbor, MI
23. Meraldi, J.-P., Hruby, V. J. & Brewster, A. I. R. (1977) Proc. Natl. Acad. Sci. U.S.A. **74**, 1373–1377
24. Bankowski, K., Manning, M., Haldar, J. & Sawyer, W. H. (1978) J. Med. Chem. **21**, 850–853; Manning, M., Lammek, B., Kruszynski, M., Seto, J. & Sawyer, W. H. (1982) J. Med. Chem. **25**, 408–414
25. Dyckes, D. F., Nestor, J. J., Jr., Ferger, M. F. & du Vigneaud, V. (1974) J. Med. Chem. **17**, 250–252
26. Mosberg, H. I., Hurst, R., Hruby, V. J., Galligan, J. J., Burks, T. F., Gee, K. & Yamamura, H. I. (1983) Biochem. Biophys. Res. Commun. **106**, 506–512
27. Mosberg, H. I., Hurst, R., Hruby, V. J., Gee, K., Yamamura, H. I., Galligan, J. J. & Burks, T. F. (1983) Proc. Natl. Acad. Sci. U.S.A. **80**, 5871–5874
28. Pelton, J. T., Gulya, K., Hruby, V. J., Duckles, S. P. & Yamamura, H. I. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 236–239
29. Kruszynski, M., Lammek, B., Manning, M., Seto, J., Haldar, J. & Sawyer, W. H. (1980) J. Med. Chem. **23**, 364–368
30. Bryan, W. M., Callahan, J. F., Codd, E. E., Lemieux, C., Moore, M. L., Schiller, P. W., Walker, R. F. & Huffman, W. F. (1989) J. Med. Chem. **32**, 302–304
31. Coy, D. H., Horvath, A., Nekola, M. V., Coy, F. J., Erchogyi, J. & Schally, A. V. (1982) Endocrinology (Baltimore) **110**, 1445–1447
32. Freidinger, R. M., Colton, C. D., Randall, W. C., Pitzenberger, S. M., Veber, D. F., Saperstein, R., Brady, E. J. & Arison, B. H. (1985) in Peptides: Structure and Function (Deber, C. M., Hruby, V. J. & Kopple, K. D., eds.), pp. 549–552, Pierce Chemical Co., Rockford, IL
33. Piercy, M. F., Schroeder, A., Einspahr, F. J., Folkers, K., Xu, J.-C. & Hörig, J. (1981) in Peptides: Synthesis-Structure-Function (Rich, D. H. & Gross, E., eds.), pp. 589–592, Pierce Chemical Co., Rockford, IL
34. Melin, P., Trojnar, J., Vilhardt, H. & Akerlund, M. (1983) in Peptides: Structure and Function (Hruby, V. J. & Rich, D. H., eds.), pp. 361–364, Pierce Chemical Co., Rockford, IL
35. Lebl, M., Barth, T., Servitova, L., Slaninova, J. & Jost, K. (1985) Collec. Czech. Chem. Commun. **50**, 132–145
36. Sawyer, T. K., Sanfilippo, P. J., Hruby, V. J., Engel, M. H., Heward, C. B., Burnett, C. B. & Hadley, M. E. (1980) Proc. Natl. Acad. Sci. U.S.A. **77**, 5754–5758
37. Morgan, B. A., Bower, J. D., Guest, K. P., Handa, B. K., Metcalf, G. & Smith, C. F. C. (1977) in Peptides (Goodman, M. & Meienhofer, J., eds.), pp. 111–113, Wiley, New York, and references therein
38. Torchiana, M. L., Cook, P. G., Weise, S. R., Saperstein, R. & Veber, D. F. (1978) Arch. Int. Pharmacol. Ther. **235**, 170–176
39. Sawyer, W. H., Pang, P. K., Seto, J., McEnroe, M., Lammeck, B. & Manning, M. (1981) Science **212**, 49–51
40. Zajac, Z.-M., Gacel, G., Petit, F., Dodey, P., Rossignol, P. & Roques, B. P. (1983) Biochem. Biophys. Res. Commun. **111**, 390–397
41. Hsieh, K., Lattan, T. R. & Speth, R. C. (1989) J. Med. Chem. **32**, 899–903
42. Barrett, G. C. (ed.) (1985) Chemistry and Biochemistry of Amino Acids, pp. 1–684, Chapman and Hall, London
43. Tourwe, D., De Cock, E., Van Marsenille, M., Van der Auwera, L., Van Binst, G., Viville, R., Degeloen, J. & Scarso, A. (1989) Peptides 1988 (Jung, G. & Bayer, E., eds.), pp. 562–564, de Gruyter, Berlin
44. Nadzan, A. M., Shue, Y.-K., Carrera, G. M., Jr., Kerwin, J. F., Jr., Kopecka, H., Tufano, M. D. & Chun, W. L. (1988) in Peptide Chemistry 1987 (Shiba, T. & Sakakibara, S., eds.), pp. 549–552, Protein Research Foundation, Osaka; Shue, Y.-K., Correra, G. M., Jr. & Nadzen, A. M. (1987) Tetrahedron Lett. 3225–3228
45. Zabrocki, Z., Smith, G. D., Danbar, J. B., Jr., Iiyama, H. & Marshall, G. R. (1988) J. Am. Chem. Soc. **110**, 5875–5880
46. Shimohigashi, Y., Costa, T., Herz, A. & Stammer, C. H. (1988) in Peptide Chemistry 1987 (Shiba, T. & Sakakibara, S., eds.), pp. 562–572, Protein Research Foundation, Osaka

47. Prochazka, Z., Lebl, M., Barth, T., Hlavacek, J., Trka, A., Budensinsky, M. & Jost, K. (1984) Collect. Czech. Chem. Commun. **49**, 642–652
48. Toniolo, C., Crisma, M., Valle, G., Bonora, G. M., Barone, V., Benedetti, E., Di Blasio, B., Pavone, C., Pedone, C., Santini, A. & Lelj, F. (1987) in Peptide Chemistry 1987 (Shiba, T. & Sakakibara, S., eds.), pp. 45–48, Protein Research Foundation, Osaka, Japan; Toniolo, C., Bonora, G. M., Bavoso, A., Benedetti, E., Di Blasio, B., Pavone, V. & Pedone, C. (1983) Biopolymers **22**, 209–215; Varughese, K. I., Srinivasan, A. R. & Stammer, C. H. (1985) Int. J. Peptide Protein Res. **26**, 242–251; Crisma, M., Bonora, G. M., Toniolo, C., Bavoso, A., Benedetti, E., Di Blasio, B., Pavone, V. & Pedone, C. (1988) Int. J. Biol. Macromol. **10**, 300–304
49. Tamura, M., Park, J.-A. & Stammer, C. H. (1988) in Peptide Chemistry 1987 (Shiba, T. & Sakakibara, S., eds.), pp. 705–708, Protein Research Foundation, Osaka
50. Shimohigashi, Y., Costa, T., Pfeiffer, A., Herz, A., Kimura, H. & Stammer, C. H. (1987) FEBS Lett. **222**, 2671–2673
51. Grim, M. D., Chauhan, V., Shimohigashi, Y., Kolar, A. J. & Stammer, C. H. (1981) J. Org. Chem. **46**, 2671–2673
52. Felix, A. M., Wan, C. T., Liebman, A. A., Delaney, C. M., Mowles, T., Berrghardt, B. A., Charnecki, A. M. & Meienhofer, J. (1977) Int. J. Peptide Protein Res. **10**, 299–310
53. Fisher, G. H., Berryer, P., Ryan, J. W., Chauc, V. & Stammer, C. V. (1981) Arch. Biochem. Biophys. **211**, 269–274
54. Shimohigashi, Y. & Stammer, C. H. (1982) Int. J. Peptide Protein Res. **20**, 199–205
55. Meienhofer, J., Trzeciak, A., Havran, R. T. & Walter, R. (1970) J. Am. Chem. Soc. **92**, 7199–7202
56. Spur, K. L., Reinho, E. J., McMahon, E. G., Palomo, M. A. & Patton, D. R. (1989) J. Med. Chem. **32**, 67–72
57. Dorrow, D. S., Shi, P.-T., Carbonne, F. R., Minasian, E., Todd, P. E. E. & Leach, S. J. (1985) Mol. Immunol. **22**, 1255–1264
58. Hruby, V. J. & Smith, C. W. (1987) in The Peptides: Analysis, Synthesis, Biology (Smith, C. W., ed.), vol. 8, pp. 77–197, Academic Press, NY
59. Jost, K. (1987) in Handbook of Neurohypophyseal Hormone Analogs, Vol. 1, Part 2 (Jost, K., Lebl, M. & Brtnik, F., eds.), pp. 144–156, CRC Press, Boca Raton, FL
60. Bauer, W., Boiner, U., Doepfner, W., Haller, R., Huguenin, R., Marbach, P., Petcher, T. J. & Pless, J. (1983) in Peptides 1982 (Blaha, K. & Malon, P., eds.), pp. 583–588, de Gruyter, Berlin
61. Veber, D. F., Freidinger, R. M., Perlow, D. S., Poleveda, W. J., Holly, F. W., Strachan, R. G., Nutt, R. F., Arison, B. H., Homnick, C., Randall, W. C., Glitzer, M. S., Saperstein, R. & Hirschmann, R. (1981) Nature (London) **292**, 55–57
62. Pelton, J. T., Kazmierski, W., Gulya, K., Yamamura, H. I. & Hruby, V. J. (1986) J. Med. Chem. **29**, 2370–2375
63. Sawyer, T. K., Hruby, V. J., Darman, P. S. & Hadley, M. E. (1982) Proc. Natl. Acad. Sci. U.S.A. **79**, 1751–1755
64. Theodoropoulos, D., Poulos, C., Gatos, D., Cordopatis, P., Eschi, E., Mizrahi, J., Kegoli, D., Dalietos, D., Furst, A. & Lee, T. D. (1985) J. Med. Chem. **28**, 1536–1536; Darman, P. S., Landis, G. G., Smits, J. R., Hirning, L. D., Gulya, K., Yamamura, H. I., Burks, T. F. & Hruby, V. J. (1985) Biochem. Biophys. Res. Commun. **127**, 656–662
65. Rosenber, D. E., Beadle, J. R., Korzeniowski, S. H. & Gokel, G. W. (1980) Tetrahedron Lett. 4141–4144
66. Bates, R. B. & Janda, K. D. (1982) J. Org. Chem. **47**, 4374–4376
67. Bates, R. B., Gin, S. L., Hassen, M. A., Hruby, V. J., Janda, K. D., Kriek, G. R., Michand, J.-P. & Vine, D. B. (1984) Heterocycles **22**, 785–790
68. Siemion, I. Z., Szewezuk, Z., Herman, Z. S. & Stachura, Z. (1980) Mol. Cell. Biochem. **34**, 213–219
69. Schiller, P. W., Nguyen, T. M.-D., Maziak, L. A. & Lemieux, C. (1985) Biochem. Biophys. Res. Commun. **127**, 558–564
70. Schiller, P. W., Nguyen, T. M.-D. & Lemieux, C. (1988) Tetrahedron **44**, 733–743
71. Charpentier, B., Durieux, C., Menant, E. & Roques, B. P. (1987) J. Med. Chem. **30**, 962–968
72. Rodriguez, M., Lignon, M.-F., Galas, M.-C. & Martinez, J. (1990) in Peptides: Chemistry, Structure and Biology (Rivier, J. E. & Marshall, G. R., eds.), pp. 108–110, ESCOM Publishers, Leiden
73. Hruby, V. J., Kazmierski, W., Pettitt, B. M. & Al-Obeidi, F. A. (1988) in Molecular Biology of Brain and Endocrine Peptidergic Systems (Chretien, M. & McKerns, K. W., eds.), pp. 13–17, Plenum, New York
74. Al-Obeidi, F., Hadley, M. E., Pettitt, B. M. & Hruby, V. J. (1989) J. Am. Chem. Soc. **111**, 3413–3416
75. Al-Obeidi, F., Castrucci, A. M. L., Hadley, M. E. & Hruby, V. J. (1989) J. Med. Chem. **32**, 2555–2561
76. Al-Obeidi, F., Hruby, V. J., Castrucci, A. M. L. & Hadley, M. E. (1989) J. Med. Chem. **32**, 174–179
77. Hill, P. S., Slaninova, J. & Hruby, V. J. (1989) in Peptides: Chemistry and Biology (Marshall, G. R., ed.), pp. 468–470, ESCOM Publishers, Leiden; Hill, P. S., Smith, D. D., Slaninova, J. & Hruby, V. J. (1990) J. Am. Chem. Soc. **112**, 3110–3113
78. Struthers, R. S., Rivier, J. & Hagler, A. (1984) Ann. N.Y. Acad. Sci. **439**, 81–96
79. Mosberg, H. I. & Omnaas, J. R. (1985) J. Am. Chem. Soc. **107**, 2986–2987
80. Hayashi, K., Nunami, K., Kato, J., Yoneda, N., Kubo, M., Ochiai, T. & Ishida, R. (1989) J. Med. Chem. **32**, 289–297
81. Thaisrivong, S., Pals, D. T., Turner, S. R. & Ki, L. T. (1988) J. Med. Chem. **31**, 1369–1376
82. Aebi, J. D., Guillaume, G., Dulap, B. E. & Rich, D. H. (1988) J. Med. Chem. **31**, 1805–1815
83. Van der Elst, P., Van den Berg, E., Pepermans, H., Van der Auwera, L., Zeeuws, R., Tourwe, D. & Van Binst, G. (1987) Int. J. Peptide Protein Res. **29**, 318–330
84. Taylor, J. W. & Kaiser, E. T. (1989) Int. J. Peptide Protein Res. **34**, 75–80
85. Musso, G. F., Assoian, R. K., Kaiser, E. T., Kezdy, F. J. & Tager, H. S. (1984) Biochem. Biophys. Res. Commun. **119**, 713–719
86. Hruby, V. J., Krstenansky, J., Gysin, B., Pelton, J. T., Trivedi, D. & McKee, R. L. (1986) Biopolymers **25**, 135–155
87. Krstenansky, J. L., Trivedi, D., Johnson, D. & Hruby, V. J. (1987) J. Am. Chem. Soc. **108**, 1696–1698
88. Krstenansky, J. L., Zechel, C., Trivedi, D. & Hruby, V. J. (1988) Int. J. Peptide Protein Res. **32**, 468–478
89. Krstenansky, J. L., Owen, T. J., Yates, M. T. & Mao, S. J. T. (1987) J. Med. Chem. **30**, 1688–1691
90. Krstenansky, J. L., Mao, S. J. T., Owen, T. J. & Yates, M. T. (1989) in Peptides: Chemistry and Biology (Marshall, G. R., ed.), pp. 447–448, ESCOM Publishers, Leiden
91. Shoemaker, K. R., Kim, P. S., Brems, D. N., Marquese, S., York, E. J., Chaiken, I. M., Stewart, J. M. & Baldwin, R. L. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 2349–2353
92. Shoemaker, K. R., Kim, P. S., York, E. J., Stewart, J. M. & Baldwin, R. L. (1987) Nature (London) **326**, 563–565
93. Rose, G., Gierasch, L. M. & Smith, J. A. (1985) Adv. Protein Chem. **37**, 1–109
94. Marraud, M., Premilat, S. & Aubry, A. (1990) in Peptides: Chemistry, Structure and Biology (Rivier, J. E. & Marshall, G. R., eds.), pp. 535–538, ESCOM Publishers, Leiden
95. Burley, S. K. & Petsko, G. A. (1985) Science **229**, 23–28
96. Leszczynski, F. J. & Rose, G. D. (1986) Science **234**, 849–855
97. Kemp, D. S. & Curran, T. P. (1988) Tetrahedron Lett. 4935–4938
98. Kemp, D. S. & Carter, J. S. (1987) Tetrahedron Lett. 4645–4648
99. Kemp, D. S. & Stites, W. F. (1988) Tetrahedron Lett. 5057–5060
100. Flynn, G. A., Giroux, E. L. & Beight, D. W. (1988) in Peptide Chemistry 1987 (Shiba, T. & Sakakibara, S., eds.), pp. 631–636, Protein Research Foundation, Osaka
101. Kazmierski, W. & Hruby, V. J. (1988) Tetrahedron **44**, 697–710
102. Hruby, V. J., Toth, G., Prakash, O., Davis, P. & Burks, T. F. (1989) in Peptides 1988 (Jung, G. & Bayer, E., eds.), pp. 616–618, de Gruyter, Berlin
103. Hruby, V. J. (ed.) (1985) The Peptides: Analysis, Synthesis, Biology. Vol. 7: Conformation in Biology and Drug Design, pp. 495, Academic Press, New York
104. Kazmierski, W., Yamamura, H. I., Burks, T. F. & Hruby, V. J. (1989) in Peptides 1988 (Jung, G. & Bayer, E., eds.), pp. 643–645, de Gruyter, Berlin
105. Nicolau, K. C., Salvino, J. M., Rayner, K., Pietranico, S., Reisine, T., Freidinger, R. M. & Hirschmann, R. (1990) in Peptides: Chemistry, Structure and Biology (Rivier, J. E. & Marshall, G. R., eds.), pp. 881–884, ESCOM Publishers, Leiden
106. Olson, G. L., Voss, M. E., Hill, D. E., Kahn, M., Madison, V. S. & Cook, C. M. (1990) J. Am. Chem. Soc. **112**, 323–333
107. Olson, G. L., Cheung, H.-C., Voss, M. E., Hill, D. E., Kahn, M., Madison, V. S., Cook, C. M., Sepinwall, J. & Vincent, G. (1990) BIOTECH USA 1989, pp. 348–360