Journal of
Medicinal
Chemistry

Volume 46, Number 20 September 25, 2003

Award Address

Peptide Science: Exploring the Use of Chemical Principles and Interdisciplinary Collaboration for Understanding Life Processes†

Victor J. Hruby‡

Department of Chemistry, University of Arizona, 1306 E. University, Tucson, Arizona 85721

Received June 24, 2003

Introduction

It is a great honor to be the 2002 recipient of the American Chemical Society Ralph F. Hirschmann Award. Ralph F. Hirschmann is one of the great pioneers of peptide research[1] and has been a wonderful colleague and friend for many years. This Award truly recognizes the efforts and ideas of many students and collaborators whom I have had the privilege of working with during my 37 years in peptide and protein science. Their creativity, hard work, challenging ideas and criticisms, and friendship has been an inspiration and motivation. Perhaps most important were my early mentors who set me on a path I did not plan but for which I am eternally grateful: A. William Johnson at University of North Dakota with whom I received my M.S. degree, who motivated me to become a chemist; Alfred T. Blomquist at Cornell University with whom I received my Ph.D., who guided me to the rigors of chemistry and who allowed me to do something new; Vincent du Vigneaud of Cornell Medical College and Cornell University, who introduced me to the excitement of peptide and protein chemistry and biology and who set me on a path of collaborative interdisciplinary research that I have pursued with fun and excitement; and finally Carl S. Marvel, who nurtured and protected my young career when my colleagues were wondering what I was doing in biophysics, biology, and the medical sciences.

A major goal of our research from the beginning was to develop an understanding of the physical−chemical basis for information transduction in biological systems, with particular emphasis on peptide hormones and neurotransmitters and their receptors. To investigate this research area, we took the approach that this would require the development of necessary tools and ideas from chemistry, physics, and biology and hence would require extensive collaboration with other scientists, especially biological scientists who were at the forefront of their fields. We have been very fortunate to have worked with a number of such outstanding colleagues. Our overall goal was to use chemistry to solve biological problems that involved rigorous chemistry and to address the problems from the biologist's perspective, that is, to address their problems and goals as well as our own. This is only possible with true collaboration, where everyone has "ownership" of the problem. Furthermore, a highly multidisciplinary approach is needed involving state-of-the-art chemistry, biophysics, and biology. Thus, from the beginning, we have sought to incorporate into our research, in my research group and by collaboration, whatever tools and research methods that were needed to broadly address the chemical, physical, and biological aspects of biological activity. A summary of the major chemical, physical, and biological tools we have found essential to this research is given in Table 1. In my education and training, I was fortunate to have a strong background in physical chemistry, synthetic chemistry, and theoretical chemistry, and I was additionally fortunate that Professor de Vigneaud allowed me to learn biochemistry by teaching it to graduate students and medical students, to give lectures on the chemical and physical basics of pharmacological experiments, and to participate in bioassays. He did not believe that such

† The Ralph E. Hirschmann Award Lecture in Peptide Chemistry 2002 from the American Chemical Society.

‡ Phone: (520) 621-6332. Fax: (520) 621-8407. E-mail: hruby@u.arizona.edu.

10.1021/jm0303103 CCC: $25.00
Published on Web 09/04/2003

Table 1. Chemical, Physical, and Biological Tools Essential for Peptide Science

I.	Synthesis	
	A.	Total synthesis of peptides and peptide mimetics
		1. SPPS
		2. Solution synthesis
		3. Macrocyclic synthesis
		4. Asymmetric synthesis methodology
		5. Complex orthogonal synthesis
		6. Ligation methods
	B.	Combinatorial/parallel synthesis
	C.	Design and asymmetric synthesis of novel amino acids and peptide mimetics
II.	Robust analytical and biophysical methods	
	A.	Separation science—HPLC, LPC, chiral, partition, etc.
	B.	High-resolution MS and MS/MS, CI, MALDI, etc.
	C.	Biophysical—NMR, IR, CD, UV, Raman, X-ray, PWR, fluorescence, etc. spectroscopies
III.	Conformational analysis	
	A.	X-ray crystallography
	B.	NMR
	C.	CD, IR, Raman
	D.	Computational chemistry; molecular modeling
IV.	Assays	
	1.	Binding—kinetics and thermodynamics
	2.	Bioassays—in vitro; in vivo
	3.	Animal models—disease states vs normal states

an education was "too broad" or "to general" (which unfortunately is still the attitude of many scientists) but encouraged me to appreciate the chemical aspects of all of these areas. I am eternally grateful to him and to Professors Blomquist and Johnson who encouraged me to explore the full breadth of a scientific problem, while at the same time focusing on solving the immediate scientific issues at hand. Reference to a few published studies at this time in the kind of scientific atmosphere allowed me to learn how to do science with hard work, an open mind, and when necessary, with close collaboration with others.[2−7]

Early Studies. Developing a Physical−Chemical Approach to the Chemical Biology of Peptides

In 1968, I began my independent career at the University of Arizona and set up a research laboratory that could do both synthetic peptide chemistry and organic chemistry, study the conformational properties of peptides, and examine their biological activities by collaboration with outstanding biologists. Though it was considered gauche at the time (everyone denies it today), I immediately set my lab up to do solid-phase peptide synthesis (SPPS), and with considerable help from Bruce Merrifield, we built our own automated SPPS instrument[8] (the coauthors established Vega Biotechnologies a few years later). As soon as it became available, we set up high-pressure liquid chromatography (HPLC) instrumentation to purify our peptides. These tools became of critical importance for our conformational and other biophysical studies and for our biological studies as well. Soon we had developed our synthetic and purification methods to an extent that allowed us to obtain large quantities (a few hundred milligrams) of pure peptides much more quickly than before.

Our initial biophysical studies concentrated on oxytocin and vasopressin analogues and their cyclic and linear fragments. When it became clear that the 100 MHz instrument at the University of Arizona was not adequate, we were fortunate to obtain a collaboration with Frank Bovey and Ann Brewster at Bell Labs, which gave us access to one of the few 220 MHz instruments available at that time (1969), and a further collaboration with Professor Jay Glasel at the University of Connecticut, one of the top scientists on deuterium NMR, further expanded our research. The former collaboration allowed us to examine many of the conformations and dynamic properties of our bioactive peptides using deuterated analogues to make unambiguous assignments.[9,10] The latter allowed us to be one of the first groups to directly investigate peptide hormone−macromolecular protein interactions (oxytocin−neurophysin) at the molecular and dynamic levels.[11] As part of these investigations, we also established insights into structure−activity relationships and synthetic methodologies for preparing novel ^{2}H- and ^{13}C-labeled amino acids and peptides, semisynthesis methods for modifying larger peptide hormones (glucagon), and solid-phase synthesis methods for the preparation of larger bioactive peptides such as glucagon (29 amino acids) and α-MSH (13 amino acids). Space does not allow a discussion of these studies, but a few highlights that served to stimulate our subsequent research follow: (1) with Professor Mac Hadley, our determination that α-MSH release from the pituitary was not under the control of an oxytocin fragment[12] but biogenic amines;[13] (2) the first demonstration of cis−trans isomerism about an χ-proline bond in a fragment of a bioactive peptide;[14] (3) use of specifically ^{13}C-labeled hormones, oxytocin and vasopressin, to investigate specific interactions of the hormones with their neurosecretory carrier proteins, the neurophysins,[15,16] and the direct demonstration of the microdynamics of a tyrosine ring of oxytocin when bound to neurophysin using ^{13}C NMR;[17] (4) design and discovery of the first antagonist of a large peptide hormone, glucagon,[18,19] and the demonstration with David Johnson, M.D., that such an antagonist could lower glucose levels in a diabetic animal;[20] (5) with Professor A. T. Tu, use of laser Raman in conjunction with CD spectra to determine chirality of disulfide bonds in oxytocin agonists and antagonists;[21] and (6) demonstration, using NMR, that the oxytocin−antagonist analogue [l-penicillaninine]oxytocin had a conformation different from that of the agonist oxytocin because of specific conformational constraints. This led directly to the de novo design of other antagonists with similar properties.[23,24]

Conformational Constraint, the Ramachandran Plot, and Design of Bioactive Peptides: Development of a Robust Strategy for Ligand-Based Peptide Design

During the 1970s, with the advent of high-field NMR and the lack of success of crystallizing most bioactive peptide hormones and neurotransmitters, it became clear that most of these compounds had considerable conformational flexibility in aqueous or DMSO solutions. In addition, it now had become clear that these compounds interacted with specific receptors on plasma membranes (established by development of methods for isolation of these plasma membranes and use of radioligand binding studies and second messenger assays), but no methods were available for isolating these receptors and crystallizing them. Thus, we recognized that development of a robust method of peptide hormone

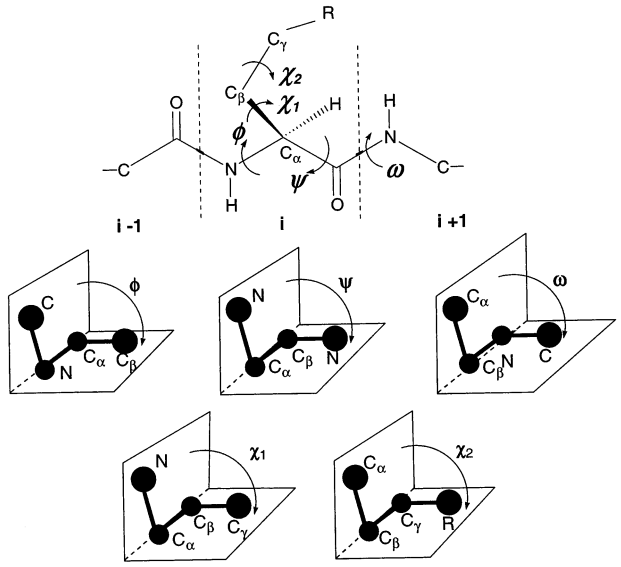


Figure 1. Definitions of ψ, φ, ω, χ^1, χ^2.

and neurotransmitter design would require the development of specific methods of conformational constraint. Our own laboratory studies of the conformational effects of β,β-dimethylcysteine (penicillaninine) in cyclic disulfide peptides and of the possibility of cis−trans isomerism about X-Pro bonds demonstrated the potential significance for use of conformational constraint in peptide ligand design of hormones and neurotransmitters. At the same time, it was becoming evident that the targets for all of these ligands were membrane proteins and even more interestingly that these ligands and their receptors (now commonly known to be G-protein-coupled receptors (GPCRs)) controlled and/or modulated many crucial biological processes and animal (including human) behaviors such as feeding behavior, pain, pigmentation, fear−flight, addiction, sexual behavior, stress response, cardiovascular function, learning and other cognitive behaviors, etc. Indeed today more than 50% of current drugs display their bioactivity by interactions with integral membrane proteins.

Thus, we (and others) developed a robust strategy to develop bioactive peptide ligands into useful biological tools (agonists, antagonists, and inverse agonists) and drugs and at the same time to determine their bioactive conformations. Three reviews early in the 1980s[24−26] focused attention on the potential of conformation constraint in bioactive peptide design.

Thus, in addition to our work on oxytocin and vasopressin design, we applied concepts of conformational constraint to all of the peptide hormone and neurotransmitter ligands we were investigating. Limitation of space will greatly limit our discussion, and thus, we will focus on aspects of our work that have provided critical impetus to subsequent studies. A major focus of our thinking about peptide conformation and design was the pioneering work of Ramachandran and co-workers,[27,28] which appeared as I entered the field and had a major impact. Ramachandran and co-workers investigated the conformational space accessible to the peptide backbone, which is defined by three torsional angles φ, ψ, and ω (see Figure 1 for definitions). Using simple computation methods, they demonstrated that only limited conformation space was available to most α-amino acids (except glycine) and that the accessible low-energy conformations were the α-helix, β-sheets, extended structures, and β-turns. Later, more sophisticated force field calculations and quantum mechanical calculations have been applied, but the basic conclusions of Ramachandran remain, and huge numbers of subsequent X-ray crystal structures have demonstrated the importance and significance of Ramachandran's insights. The lesson for us was very simple. We should try to design conformational constraints that would bias our peptides to one of these low-energy conformations and explore the significance of this conformation to the peptide's biological activity. Particularly noteworthy about peptide hormone and neurotransmitter ligands for GPCRs was the early realization that β-turn conformations might be particularly useful in many of the cases we were investigating. Table 2 provides an outline of the major approaches that were developed. Investigators who are interested in utilizing this approach in $\varphi-\psi$ space may wish to obtain information by examining reviews/overviews that have appeared in the literature.[25,26,29−32] Investigations of constraints in χ space are somewhat more limited because inherently the torsional angles about the $C_\alpha-C_\beta$, $C_\beta-C_\gamma$, etc. carbon atoms of the side chain groups (Figure 1) are more flexible than the φ, ψ, and ω torsional angles in peptides (see Figure 2 for a $\chi^1-\chi^2$ plot for L-tyrosine). Constraining α-amino acids in χ space requires careful considerations of the interplay between χ space and $\varphi-\psi$ space as well as of the inherent steric and stereoelectronic effects of modifications of the side chain groups of α-amino acids used to constrain them.[32−34] We have explored χ space by the careful use of steric and stereoelectronic effects. The use of covalent attachment of backbone nitrogen via a methylene bridge to an aromatic group in aromatic amino acids is one of the first kinds of constraints we explored. 1,2,3,4-Tetrahydroisoquinoline 3-carboxylic acids (Tic) are good examples of such a constraint because they can only exist with $\chi^1 = -60°$ or $+60°$ for an α-amino acid[35] (Figure 3). A bias to $\chi^1 = -60°$ or $+60°$ can be designed,[36,37] and by use of careful model studies, we were able to demonstrate in collaboration with Toniolo et al.[38] that these structures were completely compatible with being in either α-helix or β-turn structures. We found these compounds to be very useful and important in our de novo redesign of somatostatin from a compound that primarily interacts with somatostatin receptors to one that interacts primarily with μ opioid receptors as an antagonist.[35,39,40] Two of the designed compounds D-Phe-c[Cys-Tyr-D-Trp-Orn,Thr-Pen]-Thr-NH_2 (CTOP) and D-Phe-c[Cys-Tyr-D-Trp-Arg-Thr-Pen]-Thr-NH_2 (CTAP) are among the most potent and especially selective μ opioid receptor antagonists known and because of their complete stability against proteolytic degredation have been widely used in biological studies to help better understand the role of μ opioid receptors in a variety of biological systems[41,42] and as a ligand for binding studies.[43]

Another approach we have taken for χ constraint is to use β-alkyl-substituted amino acids, and we have developed the asymmetric synthesis of a wide variety of β-methyl- and β-phenyl-substituted analogues of aromatic amino acids.[44−58] Generally this requires the

Table 2. Some Major Approaches for Conformation Constraints of Peptides

I.	Use of D-amino acids, prolines, and related amino acid residues −Stabilize turn structures, proline helices, and others
II.	Use of α-substituted amino acids −Stabilize helical structures, turn structures, extended structures, and others, depending upon substituents
III.	Use of side chain to side chain cyclizations for cyclic disulfides, lactams, lactones, sulfides, aromatic, etc. −Stabilize β-turns, α-helices, etc., depending on macrocyclic ring size
IV.	Use of side chain N- and C-terminal residues −Stabilize various secondary structures depending on ring size
V.	Backbone to backbone cyclization −Stabilize a variety of secondary structures
VI.	Metal complexes to peptide backbone or to side chain moieties
VII.	Ring-closing metathesis

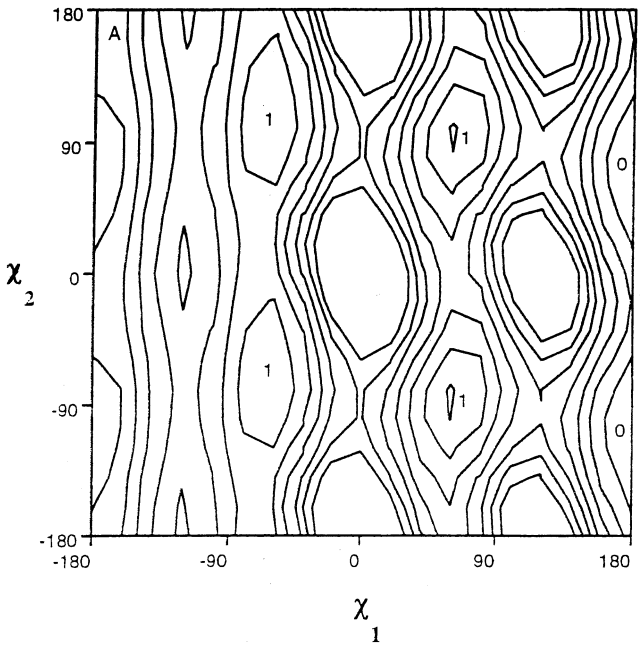


Figure 2. χ^1−χ^2 space for L-tyrosine.

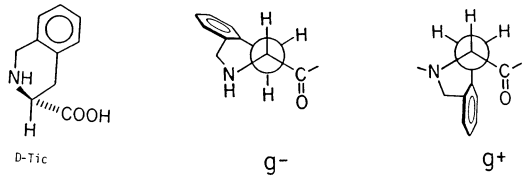


Figure 3. Conformations of D-Tic.

asymmetric synthesis of four isomers, and in most cases all four isomers were prepared. This allowed us to explore in detail the topographical requirements of the side chain groups as key pharmacophore moieties in a number of peptide hormones and neurotransmitters.[34,59−65] Among the most interesting of the novel amino acids we have prepared are the four isomers (*S*,*S*; *S*,*R*; *R*,*S*; *R*,*R*) of β-methyl-2′,6′-dimethyltyrosine (TMT) and β-methyl-2′,6′-dimethylphenylalanine TMP (Figure 4). As pointed out before (Figure 2), the energy map for most common α-amino acids, such as L-tyrosine, in χ^1−χ^2 space is quite flat, with energy differences between gauche(−) [g(−)], gauche(+) [g(+)], and trans [t] (−60°, +60°, and ±180°, respectively, for an L-amino acids) generally less than 1 kcal/mol and with the energy barrier between them generally less than 5 kcal/mol. Hence, each conformation is readily accessible at physiological temperatures (40 °C). However, for a compound

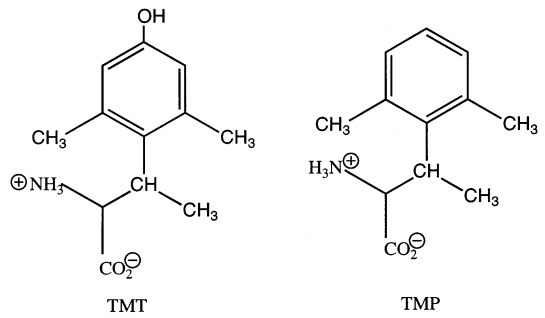


Figure 4. Structures of topographically constrained β-substituted α-amino acids β-methyl-2′,6′-dimethyltyrosine (TMT) and β-methyl-2′,6′-dimethylphenylalanine (TMP).

such as TMP (Figure 5) not only are the energy barriers between the different conformations much greater (often 5−15 kcal/mol or more)[66] but there are substantial energy differences between the gauche(−), gauche(+), and trans conformations.[34] The availability of these compounds as chirally pure derivatives allowed us to address an issue that had interested us for many years. Do biological systems (receptors, acceptors, enzymes, etc.) that utilize molecular recognition of a peptide ligand (hormone, neurotransmitter, substrate, etc.) for their biological activity utilize a *specific* χ conformation for their biological activity? How important is such a local (or specific) interaction for molecular recognition (binding) and for biological activity (agonist vs antagonist, etc.)?

Thus, we examined the effect of replacing the tyrosine-1 residue in our constrained bioactive cyclic enkephalin analogue H-Tyr-c[D-Pen-Gly-Phe-D-Pen]-OH (c[D-Pen^2,D-Pen^5]enkephalin, DPDPE),[67] which is a potent and selective ligand for the δ opioid receptor. DPDPE is an agonist with potent analgesic activities in vivo[68] and is completely stable to serum, brain homogenates, and pure enzymes to enzymatic breakdown.[69] Examination of its conformation properties in solution by NMR[70] and in the solid crystalline form by X-ray-crystallography[71] showed that the conformation of the 14-membered disulfide ring is the same in solution and in the crystal but that the preferred side chain conformations of the key pharmacophore aromatic side chain groups of Tyr^1 and Phe^4 were different in the different states. Studies with β-MePhe^4-substituted DPDPE analogues indicated that the gauche(−) conformation for χ^1 was the most favorable for the bioactivity of DPDPE. To examine the exocyclic Tyr^1 residue, we turned to β-methyl-2′,6′-dimethyltyrosine^1 (TMT^1)-substituted DPDPE analogues (all four diastereoiso-

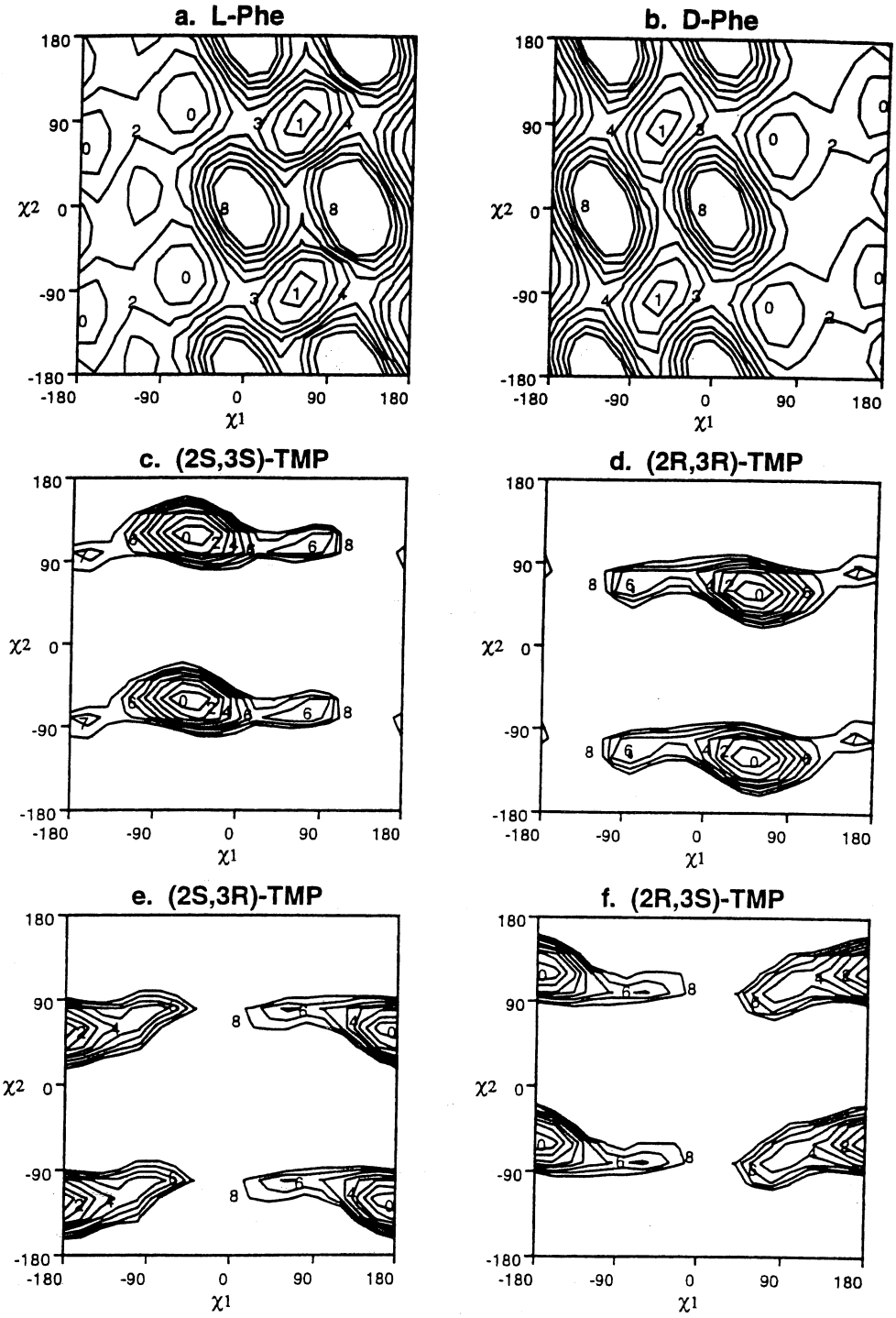


Figure 5. χ^1–χ^2 energy map for β-methyl-2′,6′-dimethylphenylalanine (TMP).

Table 3. Binding Affinities and Biological Activities of TMT[1][DPDPE] Analogues[a]

	binding data, IC_{50} (nM)		bioassay data, EC_{50} (nM)	
compd	μ^b	δ^c	GPI (μ)	MVD (δ)
H-Tyr-c[D-Pen-Gly-Phe-D-Pen]-OH(DPDPE)	610	1.6	7300	4.1
[(2*S*,3*S*)-TMT[1]]DPDPE	720	210	290	170
[(2*S*,3*R*)-TMT[1]]DPDPE	4300	5.0	0% at 60 μM, antagonist	1.8
[(2*R*,3*R*)-TMT[1]]DPDPE	77000	3500	50000	2200
[(2*R*,3*S*)-TMT[1]]DPDPE	0% at 10 μM	9% at 10 μM	75% at 82 μM	28% at 10 μM

[a] Data from ref 72. [b] Versus [^{3}H]CTOP. [c] Versus [^{3}H][*p*-ClPhe^4]DPDPE.

mers). As shown in Table 3,[72] using both binding affinity and in vitro bioassays (MVD, δ receptor; GPI, μ receptor), the (2*S*,3*R*) analogue in which χ^1 is trans was the most potent *and* selective DPDPE analogue, and the binding affinity differences for the different diastereoisomers are very consistent with the energy differences

for the various preferred side chain conformations of the four isomers of TMT. Indeed, extensive NMR studies[72] showed that the only changes in conformation between DPDPE and the TMT[1]-substituted DPDPE analogues were in the preferred side chain conformations of the TMT[1]-substituted analogues. Interestingly, in functional assays (MVD and GPI), the (2*S*,3*R*) analogue ($\chi^1 = -60°$) is a weak antagonist at the μ-opioid receptor, demonstrating that the preferred side chain conformation for agonist activity at δ and μ receptors for the Tyr[1] side chain group are very different. These results are completely consistent with the hypothesis that for peptide hormones and neurotransmitters that interact with G-protein-coupled receptors such as the opioid receptors, the topographical properties of side chain groups of key pharmacophores dominate the structure−activity relationships for these critically important modulators of bioactivity. The ancillary hypothesis is that the backbone of such peptide analogues thus serves primarily as a conformational template (privileged structure) for bioactivity. In this regard, backbone conformations of peptides and proteins (α-helix, β-sheets, β- and γ-turns, and extended conformations) serve as the most critical privileged structures in nature because they define the template structures of all peptide and proteins of biological importance.

Having a three-dimensional, topographical structure for DPDPE led us to utilize computational chemistry for the de novo design of a non-peptide mimetic of DPDPE that would be both as potent and as selective as DPDPE for the δ opioid receptors.[73,74] Space does not allow a discussion of the approach we used, which can be found in the literature.[73] Suffice it to say that in structure−activity relationships, the peptide mimetic behaved like the peptide on which its design was based and not like non-peptide δ opioids, which are known to bind to δ opioid receptors and to show bioactivities different from the bioactivities of δ opioid peptides.[74] However, these de novo designed non-peptide mimetics show significant toxicity, much like other non-peptide opioids. Thus, we are continuing to develop synthetic strategies that will lead to nontoxic peptidomimetics in related structural classes.[75,76]

As discussed above, we have hypothesized that "simple" changes in topography of key pharmacophore elements in peptide hormones and neurotransmitters can profoundly affect their bioactivities without any significant changes in backbone conformation. To examine further this hypothesis, we designed a highly constrained bicyclic oxytocin antagonist analogue in χ space[77] based on our previously designed bicyclic oxytocin analogue D-Pen-Tyr-Ile-Glu-Asn-Cys-Pro-Lys-Gly-NH_2, which was a potent, prolonged acting oxytocin antagonist at the uterine oxytocin receptor.[78,79] Detailed conformational analysis of this bicyclic oxytocin analogues using NMR, computational studies, molecular modeling, and other biophysical studies had shown that oxytocin agonists and antagonists have a different structure−biological activity relationship and different receptor-binding conformations.[80,81] Left unanswered, however, was the importance of the χ space topography of the tyrosine residue for molecular recognition at the oxytocin receptor. It previously was known that the chirality[82] and hydrophobicity[83] of the aromatic amino acid at position 2 were important for high-affinity antagonist activity. Hence, we utilized a designed novel amino acid, β-methyl-2′,6′-dimethyl-4′-methoxytyrosine (*p*-OMe-TMT all four isomers: (2*S*,3*S*), (2*S*,3*R*), (2*R*,3*S*), and (2*R*,3*R*)), which incorporated the desired hydrophobicity and chiralities, and in addition placed χ^1 and χ^2 constraints, which favored specific topographical properties for each isomer,[34,77] and incorporated each isomer into the 2-position of the bicyclic oxytocin analogue. The results of the binding studies are given in Table 4. It was very exciting to find that two of the diastereoisomer analogues were very potent binders, the (2*S*,3*S*)- and (2*R*,3*R*)-containing isomers (**2** and **5**, Table 4), whereas the other two diastereoisomers, **3** and **4** (Table 4), were virtually inactive. Indeed, compound **2** was 16 times more potent than the parent compound **1**, and the other potent binder, **5**, was nearly equipotent to **1**. Examination of the low-energy conformations of all four isomers of the topographically constrained bicyclic oxytocins **2**−**5** (Figure 6) clearly showed that a very particular topography of the tyrosine-2 side chain was critical for high affinity at the oxytocin receptor and for antagonist activity. Since the energy differences between the different χ-1 conformations (g(-), g(+), and trans) are 3−5 kcal/mol or more,[34] it is clear that a particular topography of the tyrosine side chain group is critical for high potency. Only the g(−) conformation for the (2*S*,3*S*) residue and the g(+) conformation for the (2*R*,3R) residue give potent antagonists (they both exist in the same topographical space (Figure 6)), and clearly the other two isomers, (2*S*,3*R*) and (2*R*,3*S*) structures **3** and **4**, which have different topographies at the 2-position, do not recognize the oxytocin receptor probably because of unfavorable steric effects on interaction with the oxytocin receptor. Hence, topographical differences at only one key amino acid side chain in a critical pharmacophore residue are sufficient to affect molecular recognition at receptors by a 1000-fold or more, even though all other conformational and topographical properties of the pharmacophore needed for potent molecular recognition are present in favorable conformational space.

Table 4. Binding Affinities of Bicyclic Oxytocin Analogues Containing the Four Isomers of 4′-Methoxyl-β-2′,6′-Trimethyltyrosine

peptide structure	binding affinities, IC_{50} (nM)
1 [D-Pen[1],Glu[4],Lys[8]]OT	130
2 [D-Pen[1],(2*S*,3*S*)-*p*-MeOTMT[2],Glu[4],Lys[8]]OT	8.0
3 [D-Pen[1],(2*S*,3*R*)-*p*-MeOTMT[2],Glu[4],Lys[8]]OT	36000
4 [D-Pen[1],(2*R*,3*S*)-*p*-MeOTMT[2],Glu[4],Lys[8]]OT	19000
5 [D-Pen[1],(2*R*,3*R*)-*p*-MeOTMT[2],Glu[4],Lys[8]]OT	160

α-Melanocyte Stimulating Hormone (α-MSH) and Melanocortin Receptors. A Remarkable Story of the Central Importance of Topographical Structure in Bioactivities Including Fundamental Human Behavioral Activities. α-MSH(Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2) is a potent peptide hormone and neurotransmitter that has several critical biological activities (pigmentation, feeding behavior, sexual behavior, pain, immune response,

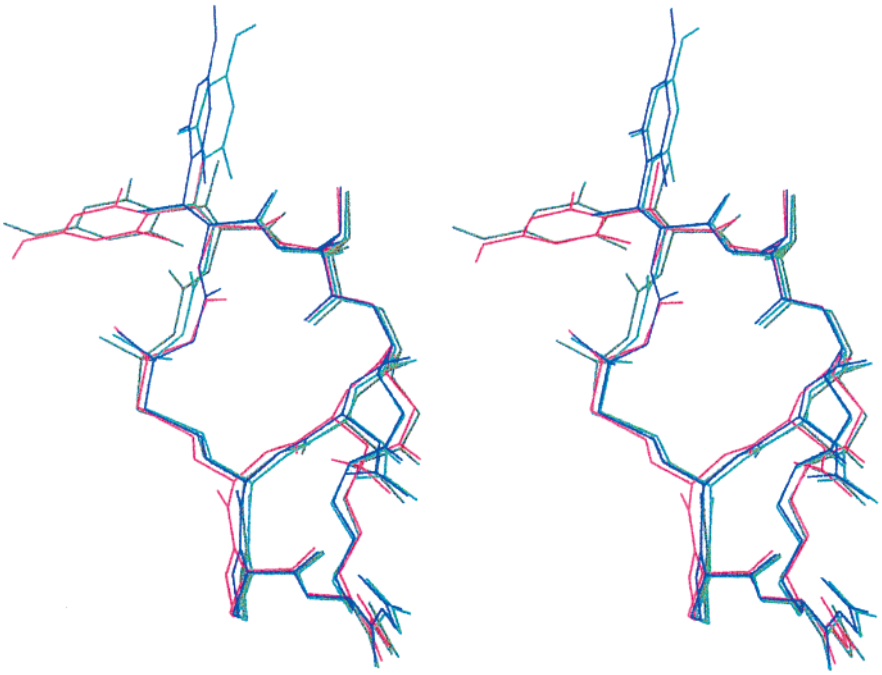


Figure 6. Stereoview of the low-energy conformations of the four [*p*-MeO-TMT^2]BC-OT analogues: [(2*S*,3*S*)-*p*-MeO-TMT^2]BC-OT (dark green); [(2*R*,3*R*)-*p*-MeO-TMT^2]BC-OT (red); [(2*R*,3*S*)-*p*-MeO-TMT^2]BC-OT (blue); [(2*S*,3*R*)-*p*-MeO-TMT^2]BC-OT (green). From Liao, S.; et al. *J. Am. Chem. Soc.* **1998**, *120*, 7393−7394. Reprinted with permission from *Journal of the American Chemical Society* (page 7394, Figure 3). Copyright 1998 American Chemical Society.

etc.) that are central to survival.[84] This peptide is a processed product of the proopiomelanocortin (POMC) gene that produces several other peptide hormones and neurotransmitters (e.g., ACTH, β-endorphin, β-lipotropin, etc.) that are central to survival in most animals.[85] Initially the primary biological activity attributed to α-MSH was its affect on pigmentation of the skin and hair of virtually all animals including humans. Extensive structure−activity studies[86] in conjunction with extensive biological studies provided a comprehensive understanding of these primary properties of the hormone and its melanocortin receptor, now referred to as the melanocortin-1 receptor (MC1R). We initially entered the field in collaboration with Professor Mac Hadley, investigating the mechanism of release of α-MSH from the pituitary, and demonstrated that the ideas related to control of release were not correct[12,13,87−89] and eventually showed that dopamine was the major neurotransmitter controlling α-MSH release.[13,90] These studies led directly to examining the in vitro and in vivo biological activities of melanotropins. It soon became clear the α-MSH and the analogues known at that time[91] were not adequate for developing good bioassay methods and especially for careful in vivo studies because of their rapid degredation by proteases. We therefore sought to develop more potent and stable analogues of α-MSH, and on the basis of previous studies of oxidation and racemization of α-MSH, we designed the ligand [Nle^4,D-Phe^7]-α-MSH[92] (NDP-α-MSH, MT-I, Ac-Ser-Tyr-Ser-Nle-Glu-His-D-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2). This compound not only was more potent than α-MSH but also showed highly prolonged (hours to weeks) biological activities both in vitro and in vivo[93] in a calcium-dependent process and was quite stable against proteolytic breakdown by proteases and serum.[94,95] These unique properties allowed the development of extensive biological studies of the role of α-MSH in pigmentation and melanoma cancer[96−100] and to the development of an adenylate cyclase assay to evaluate transduction processes in melanotropins.[101,102] Eventually, in collaboration with our colleagues at the University of Arizona, we were able to do all the preclinical studies that allowed the clinical evaluation of NDP-α-MSH as a stimulator of skin darkening in humans and for use to protect against UV radiation damage to skin.[103−106] It also provided an impetus for extensive modeling which led to the suggestion of the presence of a β-turn in the vicinity of the D-Phe^7-Arg^8 residues. This hypothesis was tested[107] by the use of conformational constraints of the linear peptide α-MSH to the cyclic peptide c[Cys^4,Cys^{10}]-α-MSH [107] in which the Met^4 and Gly^{10} residues were replaced by cystine in a process we referred to as pseudoisosteric cyclization. On the basis of this design, a superpotent analogue of α-MSH was obtained in the classical frog skin (*R. pipiens*) bioassays,[107] though in vivo activity and biostability were not as good as NDP-α-MSH. However, we were able to demonstrate using this compound that α-MSH was involved as a neurotransmitter (done in collaboration with Tom O'Donohue et al.).[108]

Our finding that NDP-α-MSH and truncated analogues of NDP-α-MSH had prolonged biological activity led us into new directions: (1) Was the unique biological activity of NDP-α-MSH an effect of conformation? (2) What is the minimum pharmacophore for α-MSH? For the last question, we did a large number of careful experiments with many truncated analogues of α-MSH and numerous in vitro and in vivo biological activity studies with our biological collaborators.[109−112] These extensive studies led to the conclusion that the minimum active sequence for full biological activity using only L-amino acids was -His-Phe-Arg-Trp-[113,114] and that when a D-Phe^7 was introduced, even tripeptides could

Table 5. Biological Activities of Cyclic Lactam Analogues of α-Melanotropins Using the Frog Skin (*R. pipiens*) and Lizard Skin (*A. carolinensis*) Assays[a]

compd	biological activities,[b] EC_{50} (nM)	
	frog skin	lizard skin
α-MSH	0.50 (−)	1.0 (−)
Ac-Nle^4-c[Glu^5,D-Phe^7,Lys^{10},Gly^{11}]α-MSH(4−13)-NH_2	0.50 (+)	0.17 (+)
Ac-Nle^4-c[Asp^5,D-Phe^7,Lys^{10},Gly^{11}]α-MSH(4−13)-NH_2	0.50 (+)	0.010 (+)
Ac-Nle^4-c[Glu^5,D-Phe^7,Lys^{10}]α-MSH(4−10)-NH_2	1.0 (+)	0.11 (+)
Ac-Nle^4-c[Asp^5,D-Phe^7,Lys^{10}]α-MSH(4−10)-NH_2 (MT-II)	0.60 (+)	0.011 (+)
Ac-Nle^4-c[Asp^5,D-Phe^7,Orn^{10}]α-MSH(4−10)-NH_2	0.50 (−)	0.05 (−)
Ac-Nle^4-c[Asp^5,D-Phe^7,Dab^{10}]α-MSH(4−10)-NH_2	0.50 (−)	0.20 (−)
Ac-Nle^4-c[Asp^5,D-Phe^7,Dpr^{10}]α-MSH(4−10)-NH_2	50 (−)	0.20 (−)
Ac-Nle^4-c[Asp^5,D-Phe^7,(2*S*,3*S*)-*β*-MeTrp^9,Lys^{10}]α-MSH(4−10)-NH_2	0.44(−)	1.0 (+)
Ac-Nle^4-c[Asp^5,D-Phe^7,(2*S*,3*R*)-*β*-MeTrp^9,Lys^{10}]α-MSH(4−10)-NH_2	29.(−)	6.7 (−)
Ac-Nle^4-c[Asp^5,D-Phe^7,(2*R*,3*S*)-*β*-MeTrp^9,Lys^{10}]α-MSH(4−10)-NH_2	0.06(+)	1.43 (+)
Ac-Nle^4-c[Asp^5,D-Phe^7,(2*R*,3*R*)-*β*-MeTrp^9,Lys^{10}]α-MSH(4−10)-NH_2	0.33(+)	1.0 (+)

[a] Adapted from refs 117 and 119. [b] (−) means no prolonged biological activity; (+) means prolonged biological activity. See text.

have potent biological activity.[115] Thus, melanotropin pharmacophore three-dimensional organization of biological function appears to be a function of a linear sequence of peptide information. This immediately raised the question about which conformations correlated to this linear structural information.

As mentioned above, our design of c[Cys^4,Cys^{10}]-α-MSH was based on a hypothesis that the potent bioactivity of NDP-α-MSH was due to a *β*-turn structure for the tetrapeptide sequence -His-D-Phe-Arg-Trp. Once appropriate force fields became available, we performed extensive conformational and dynamic calculations regarding the preferred conformation for selected melanotropins.[116] These studies led to the design of cyclic lactam analogues of α-MSH such as Ac-Nle-c[Asp-His-D-Phe-Arg-Trp-Lys]-NH_2 (MT-II) in which the critical pharmacophore sequence -His-D-Phe-Arg-Trp- was conformationally constrained in a macrocyclic 23-membered lactam ring.[116] Various sized lactam rings were investigated (Table 5),[117] and it was found that though ring size (except for the 20-membered ring) did not greatly affect potency in the classical *R. pipiens* frog skin assay, in the lizard (*A. carolinensis*) assay, which we previously had shown gave structure−activity relationships similar to those of the mammalian MC1R (whereas the *R. pipiens* assay did not), the biological potency was very dependent on ring size. Also very interesting was the observation that prolonged biological activity of the cyclic lactam analogues was dependent on ring size (Table 5).

Prolonged Biological Activity. These and other studies led us to propose that prolonged biological activity was directly related to three-dimensional topographical properties of the peptide. As pointed out above, we had found that biological activities of peptide hormones and neurotransmitters are very dependent on the three-dimensional topographical properties of the peptide in *χ* space. This led Carrie Haskell-Luevano and Lakmal Boteju in my laboratory to design and synthesize four diastereoisomeric analogues of Ac-Nle^4-c[Asp^5,D-Phe^7,Lys^{10}]-α-MSH(4−10)-NH_2 (MT-II)[118−121] in which the Trp^9 residue was replaced with all four isomers of the χ^1−χ^2 constrained amino acid *β*-methyltryptophan [(2*S*,3*S*),(2*S*,3*R*),(3*R*,3*S*), and (2*S*,3*S*)].[118] Examination of the biological activities of these compounds showed that they varied widely in potency (Table 5), and interestingly, they also varied widely in their prolonged biological activities (Figure 7). Comprehensive NMR

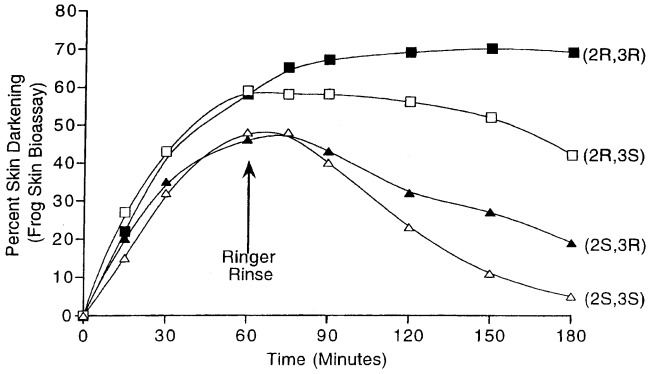


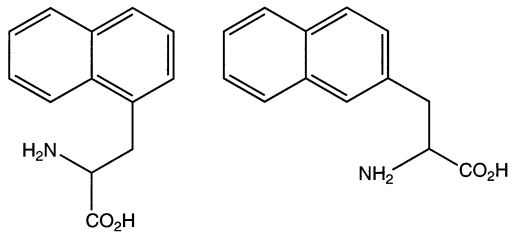
Figure 7. Prolonged biological activity of α-MSH in the frog skin (*R. pipiens*): [(2*S*,3*S*)-*β*-MeTrp^9]MT-II (△); [(2*R*,3*R*)-*β*-MeTrp^9]MT-II (■); [(2*S*,3*R*)-*β*-MeTrp^9]MT-II (▲); [(2*R*,3*S*)-*β*-MeTrp^9]MT-II (□).

strudies[119] showed that the backbone conformations of the four diastereoisomeric analogues were essentially the same and that the only conformational difference was in the topographic arrangement of side chain pharmacophore groups in *χ* space.[119] Additional careful studies of the dissociation rate constants for MT-II showed that the prolonged activity was due to the unusually slow dissociation rates from the MC1R.[121] We later showed, using a fluorescently labeled multiligand construct of MT-II, that these prolonged acting analogues of α-MSH could bind to the cell-surface receptors, undergo association (patching), be transported into the cell to the nucleus, and then be recycled to the plasma membrane again as an intact ligand−receptor complex.[122,123] These interesting constructs also could be used to examine a wide variety of cellular functions of melanotropins.[124−126] These studies, and those discussed earlier, clearly demonstrate that the three-dimensional structure of biologically active peptides, for interactions with their cognate receptors, are dependent not only on the backbone conformation (*φ*, *ψ*, *ω*) but equally, or perhaps more importantly, on the topographic relationships in *χ* space (χ^1, χ^2, etc.) of key pharmacophore moieties. From the standpoint of de novo peptide design, and especially peptide mimetic design, it clearly is very important to carefully evaluate structure−activity relationships in *χ* space. In general, these considerations have not been widely incorporated into approaches to the design of peptides and peptide mimetics, but this will change.

Effects of Genomics and Proteomics on Ligand Design. New Melancortin Genes and New Melanotropin Ligands. Until the early 1990s, the major focus of research on the products of the POMC gene were α-MSH and ACTH, which respectively influence pigmentation (α-MSH) and response to stress (ACTH, adrenal function). In the early 1990s, as a result of extensive research in several laboratories, the mammalian melanocortin-1 (MC-1) and melanocortin-2 (MC-2) receptors were sequenced and cloned.[84,127] This has proven to be very critical for subsequent studies of the biological functions of these two receptors. For example, very recently it was shown by Mogil et al.,[128] utilizing older[129] and recently discovered[130] melanocortin-1 receptor (MC1R) antagonists from our laboratory, that mammalian pathways, including human pathways, for the modulation of pain were different and distinct for males and females and that in females the MC1 receptor (MC1R) was involved in pain modulation.

From the standpoint of drug design, the most interesting findings from cloning were the discovery of three new melanocortin receptors, the MC3R, MC4R, and MC5R receptors, numbered in the order in which they were discovered.[84,127] The MC3R and MC4R were found to be located primarily in the brain, though subsequent studies also have found them in the spinal column and in various peripheral tissues. The MC5R was found throughout various tissues in the body including the brain. Numerous previous studies had suggested that α-MSH might be important in a number of biological functions that involved the central nervous system,[127,131] in the neuroendocrine system, and in the immune system. To investigate the putative biological activities ascribed to these new receptors, there is a need to obtain potent and highly selective agonist and especially antagonist analogues for these new (and old) melanocortin receptors. Fortunately, as part of our earlier studies, we had available a large collection of ligands. By use of these ligands, e.g., NDP-α-MSH, MT-II, etc., it was determined that the new receptors were primarily the targets for α-MSH, that the central core sequence His-Phe-Arg-Trp still was of critical importance as the key pharmacophore for the MC3R, MC4R, and MC5R,[132−135] and that γ-MSH had some selectivity for the MC3R. Within this context, our goal was to find selective agonists and especially antagonists for the new receptors.

Despite the efforts of ourselves and others, very few antagonist melanotropin ligands had been found. We earlier found weak antagonists for the MC1R,[129,136] but efforts to follow up on these leads were largely unsuccessful because modifications of these leads led to agonist activity. Thus, we were unable to formulate any general hypothesis about structure−*antagonist* activity relationships at the melanocortin receptors. An important breakthrough in our laboratory came with the use of topographical constraints in our studies of melanotropin analogues. In this case, we investigated the substitution of D-Phe^7 with naphthylalanine derivatives. There are two naphthylalanines (1′ and the 2′ isomers, Figure 8), and when they were substituted into MT-II to give the analogues Ac-Nle,^{4}c[Asp^5,D-Nal(2′)^7-Lys^{10}]-α-MSH-(4−10)-NH_2 (SHU-9119) and Ac−Nle^4-c[Asp^5,D-Nal(1′)^7-Lys^{10}]α-MSH, two quite different biological profiles were seen (Table 6).[133] In the case of the D-Nal-(2′)-containing analogue, a compound with potent antagonist activity at the MC4R and MC3R receptors was obtained, which had some agonist activity at the MC5R and modest selectivity for the MC4R. On the other hand, the D-Nal(1′)^7-containing analogue was an agonist at all of the melanocortin receptors tested. Subsequent comprehensive studies in our laboratory have shown that in general (but not always) substitution of a D-Nal(2′) in the 7-position of α-MSH analogues produces an analogue with antagonist activity at the MC4R and MC3R but not at the MC5R or mammalian MC1R.[137−145] Interestingly, SHU-9119 is a potent antagonist in the classical frog skin bioassay,[133] and this small cyclic heptapeptide is 10 times more potent at the MC4R than the natural endogenous antagonist protein ligand for the MC4R, agouti.



1 2

Figure 8. Structures of 1′-naphthylalanine (**1**) and 2′-naphthylalanine (**2**).

The discovery of SHU-9119 as a potent mammalian MC4R and MC3R antagonist has been an important tool for the development of an understanding of the roles of α-MSH in a wide variety of animal behaviors. Space does not allow a comprehensive discussion of this emerging field in drug design and development and in the physiological significance of melanotropins in animal and human behavior. However, a few focused comments will be made related to our collaborations with biologists in this area. A very early application of the use of the potent MC4R agonist MT-II in conjunction with the potent MC4R receptor antagonist SHU-9119 was its use in demonstrating that melanotropin was a critical ligand for the control of feeding behavior.[146] In collaboration with Roger Cone and his colleagues, it was found that MT-II was a potent suppressor of feeding behavior and that the antagonist SHU 9119 could block the feeding behavioral effects of MT-II *and* of endogenous neurotransmitter (presumably α-MSH or a modified version). Subsequent studies in many laboratories have demonstrated that melanotropin peptides that bind to MC4 and MC3 receptors have profound effects on feeding behavior and energy homeostasis in animals, with agonists suppressing feeding behavior and antagonist stimulating feeding behavior. The use of agonists for treatment of obesity and of antagonists for treatment of anorexia and many related applications are under investigation in many drug and biotechnology companies, and the mechanisms of action are being studied in many laboratories as more selective agonist and antagonist ligands become available. The situation has been complicated by the lack of highly selective (>500-fold selectivity) agonists and antagonists, for the melanocortin receptors. We are beginning to find success.

Table 6. Biological Activities of Macrocyclic Lactam Truncated Analogues of α-Melanotropin at Human Melanocortin Receptors[a]

compd	EC_{50} (nM)		
	hMC1R	hMC3R	hMC4R
α-MSH	0.091	0.67	0.21
[Nle^4,D-Phe^7]α-MSH	0.023	0.13	0.017
Ac-Nle^4-c[Asp^5,D-Phe^7,Lys^{10}]α-MSH(4−10)-NH_2 (MT-II)	−	0.27	0.057
Ac-Nle^4-c[Asp^5,D-Nal(2′)^7,Lys^{10}]α-MSH(4−10)-NH_2 (SHU-9119)	0.036	pA_2, 8.3	pA_2, 9.3
Ac-Nle^4-c[Asp^5,D-Phe(pI)^7,Lys^{10}]α-MSH(4−10)-NH_2	0.055	pA_2, 8.3	pA_2, 9.7

[a] Adapted from ref 133.

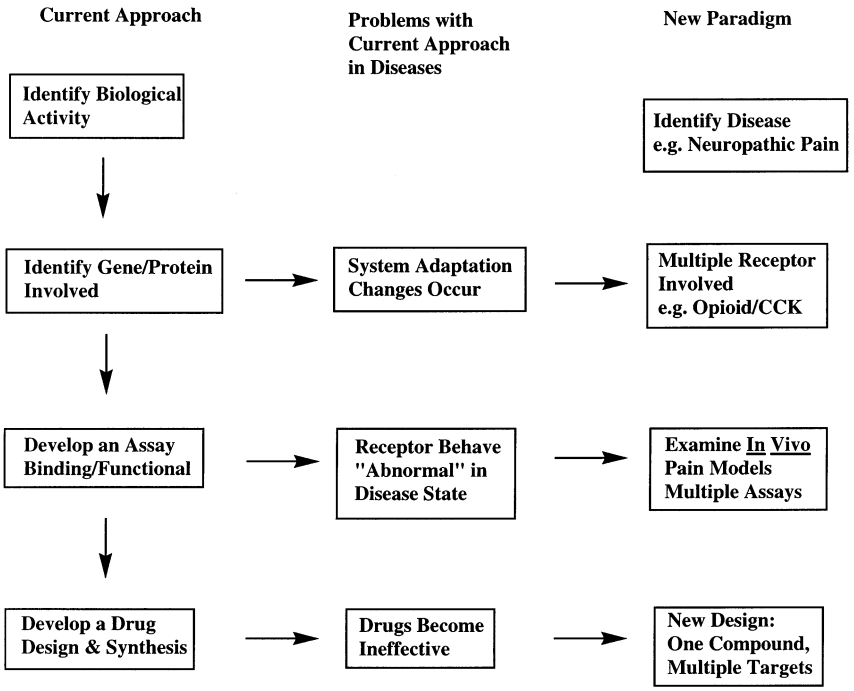


Figure 9. Drug design approaches.

An added complication in the development of drugs has been the broad spectrum of biological activities that involve melanotropin peptides, presumably via the various melanocortin receptors. Some examples from collaborations using our ligands MT-II and SHU-9119 and other more selective ligands will illustrate these exciting new areas of research. In the course of our investigations of the skin-darkening response of NDP-α-MSH[147−149] (also see above) and MT-II, we discovered that MT-II could cause an erectile response.[150] Since we already had approval for human clinical investigations related to pigmentation, we sought approval to evaluate human volunteers with psychogenic erectile dysfunction. Studies were approved for peripheral administration, and in collaboration with Dr. Hunter Wessells, placebo controlled double blind experiments were accomplished in clinical trials with exceptionally promising results.[150,151] Subsequent studies have shown that MT-II effects sexual desire and motivation in men[152,153] and involves receptors in both the brain and the spinal column.[154] In other studies with biological collaborators using our specific melanotropin agonists and antagonist, it was shown that (1) cardiovascular control by α- and γ-MSH involves two distinct pathways,[155,156] (2) that melanocortins have potent antipyretic effects,[157,158] (3) that melanocortin receptor antagonists such as SHU-9119 can prevent reflex natriuresis,[159] (4) that central melanocortins have a role in endotoxin-induced anorexia,[160] and (5) that melanocortins have differential roles mediating leptin's central effects on feeding behavior and reproduction. In view of the central role of melanotropin peptides and melanocortin receptors in all of the above biological effects, and its additional effects on learning behavior, immune response, inflammation, and other critical behavioral and function bioactivities needed for life and survival, it is clear that there is still much to learn. The development of more selective ligands for the melanocortin receptors will lead to new insights into the importance of these receptors and ligands for the maintenance of good mental and physical health and in the treatment of a wide variety of diseases.

New Dimensions in Drug Design. The enormous progress being made in determining the complete genomes of many single cell and multiple cellular life, including man, provides new opportunities for the treatment of diseases and for designing drugs that actually address the changes inherent in the disease state rather than just the symptoms associated with a particular gene product (e.g., receptor) that may be involved. As shown in Figure 9, current drug design models often (generally) do not consider systems changes associated with disease and hence do not properly take into account that the receptors in disease states do not "behave" as in the normal healthy state (adaptation). Hence, a new paradigm briefly outlined in Figure 9 is required. We have initiated studies to face this new reality directly and to design molecules (ligands, poten-

Table 7. Competitive Binding Assays of Analogue Design with Binding to δ and μ Opioids and to CCK-A and CCK-B Receptors

		opioid K_i (nM)		CCK K_i (nM)	
	compd	δ	μ	CCK-A	CCK-B
	H-Asp-Tyr-D-Phe-Gly-Trp-*N*-MeNle-Asp-Phe-NH_2 (SNF-9002)	250	5200	3330	2.1
1	H-Tyr-D-Phe-Glu-Trp-*N*-MeNle-Asp-Phe-NH_2	6.8	136	10000	2.1
2	H-Tyr-D-Nle-Gly-Trp-*N*-MeNle-Asp-Phe-NH_2	1.6	25	3900	0.6
3	H-Tyr-Gly-Gly-Tip-*N*-MeNle-Asp-Phe-NH_2	2000	610	870	1.3
4	H-Tyr-D-Phe-Gly-D-Trp-*N*-MeNle-Asp-Phe-NH_2	0.5	5.7	1080	1.6
5	H-Tyr-D-Ala-Gly-D-Trp-*N*-MeNle-Asp-Phe-NH_2	1.9	20	32	1.3

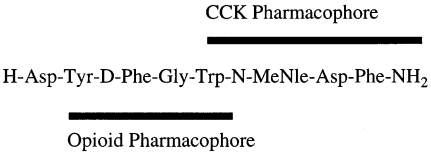


Figure 10. Design of ligands that act as agonists at opioid receptors and antagonists at CCK receptors based on SNF-9007.

tial drugs) that are designed specifically for the disease state. We have taken neuropathic pain, for which there are no currently effective drugs, as an example.[161,162] Though we are in the early stages of development, we have made significant progress, enough to warrant a few paragraphs of discussion.

In various neuropathic pain states, it has been observed that enkephalins (opioids) and cholocystokinin (CCK) and their receptors are coexpressed in the brain and spinal column. Furthermore, it has been observed that CCK can act as an anti-opioid; that is, it can cause pain. From the standpoint of peptide drug design, a reasonable hypothesis would be to design a single ligand that could act as an agonist at μ and δ opioid receptors in the brain and spinal column and as an antagonist at CCKB and CCKA receptors in the spinal column. We therefore sought to design a single molecule with all of the above biological properties. From the standpoint of design, we considered our proposed conformational pharmacophores for opioid ligands[70,71,73,163,164] and the CCK ligands.[165,166] Interestingly, we had proposed[166,167] that δ opioid and CCK bioactive conformations have similar conformational and topographical structural receptor requirements. Independently, in the course of developing highly potent and CCKB selective ligands, we had discovered a CCK-related compound H-Asp-Tyr-D-Phe-Gly-Trp-NMeNle-Asp-Phe-NH_2 (SNF-9007), which was a highly potent and selective CCKB ligand[168] but which also had weak δ opioid receptor binding affinity and in vitro and in vivo opioid biological activity. This led us to design molecules with overlapping opioid and CCK pharmacophore based on this lead structure (Figure 10). Our first goal was to convert the lead compound to a more potent opioid agonist with both δ and μ opioid receptor agonist activity. A few of the analogues we explored by removing the N-terminal Asp residue to give an N-terminal Tyr residue as is found in enkephalins, deltorphins, and other potent endogenous opioid ligands and by introducing Gly and other D-amino acids into what is now the 2-position of the des-Asp analogues are shown in Table 7. As can be seen, highly potent agonist analogues were obtained. It should be noted that to develop such analogues, at least eight in vitro bioassays are needed to evaluate the binding and biological activities of the analogues designed and synthesized (four binding assays, four second-messenger assays). In this case, two additional classic functional in vitro assays, the GPI (μ) and MVD (δ) assays, also were used. We show only the binding assays here, but the other assays, though not shown, have been done and give results consistent with this discussion.

As can be seen in Table 7, a great improvement in the binding affinity and biological activity (not shown) is seen for the ligands (**1**, **2**, and **3**, Table 6) at the opioid receptor, though interestingly the Gly-2 analogue (**3**, Table 7) was quite inactive. Our next goal was to increase the CCK-A binding affinity (without losing the CCK-A affinity) and at the same time transform the CCK-A agonist activity to antagonist activity. This was done by substituting a D-Trp residue for the L-Trp residue (**4** and **5**, Table 7). Clearly compound **5** satisfies many of the criteria we set out in our initial design because **5** has good potency at all four receptors (Table 7) and has agonist activity at the δ and μ opioid receptors and antagonist activity at CCK receptors (data not shown). To stabilize the compound against biodegradation, we have designed and synthesized both cyclic disulfide and cyclic lactam analogues of the compounds in Table 7, and in preliminary studies, some of these compounds have potent and prolonged biological activity in neuropathic pain models.

We believe this approach is applicable to many other diseases associated with the central nervous system (CNS) and the peripheral endocrine system, as well as to cardiovascular diseases, diabetes, and cancer, and have research underway to further examine this hypothesis. Clearly there is a great need for new paradigms in drug discovery for disease states, and we expect that this approach to drug design will be of great importance in the future.

Future Perspectives

These are revolutionary times in the application of chemical principles to understanding life and living systems and to treating diseases in new ways. Though the human and other genome projects have generated and are continuing to generate huge amounts of data (information), it is clear that the scientific community has not yet developed an effective means for turning all of this information into general and useful knowledge so that a greater understanding of the physical and chemical principles that underlie all biological processes, including disease, can be obtained. In addition, current approaches in high-throughput screening and synthesis are providing tremendous amounts of data, but again, very modest new knowledge and understanding have been attained given the tremendous investment. It seems that there is a need for some contrary thinking ("outside the box") about our current paradigms.

(1) Though much is made of the apparent "complexities" in living systems, in fact, nature is enormously

biased, and the real issue is the identification of the bias. From this perspective, it is not surprising that most "libraries" of non-peptide compounds being created by chemists give few, if any, good "hits" when confronted by a new biological protein or nucleic acid target. There is a critical need to develop a new view of "diversity" that is compatible with the enormous bias of living systems.

(2) Since nature has chosen peptides and proteins to do the bulk of the work, that is, work involving chemistry and structures needed for living systems, in conjunction with lipids, nucleic acids, sugars, and the rest of the periodic chart, it seems reasonable to ask why and how. We already know, as Ramachandran initially taught us, that the true "privileged" structures in nature are the α-helix, β-sheet, β-turn, and extended structures of proteins and peptides. These structures are convenient for interactions with nucleic acids, lipids, sugars, and other proteins and are wonderfully stable for supporting catalysis, information transduction, modulation of other structures, and convenient dynamic properties for scaffolding and other biological processes in time domains from nanoseconds to hours or days or years. A thorough understanding of the "simple" chemical and physical principles that make this possible and practical would be most helpful.

(3) The small differences in the genome and probably the proteome between human beings and our nearest evolutionary cousins such as apes and mice raise interesting questions about how new behavioral properties (e.g., cognition, language, music, culture, ethics, and other "human" qualities) arose. As demonstrated above, very minor changes in structure (a simple bond isomer, a torsion angle preference, etc.) for a key residue in a key ligand can lead to major changes in behavioral responses in an animal, including humans. The implications of this for drug design, especially as related to behavioral modifications, raise profound issues regarding our approach to such scientific studies, our responsibility to society, and the increasingly difficult task we will face in evaluating the potential adverse effects of our discoveries.

(4) As for new design principles and new chemistries related to peptide and peptidomimetic design and as for evaluation of their utility in biological systems, we are only at the beginning. Much more robust asymmetric synthetic methods are needed for the asymmetric synthesis of novel amino acids and amino acid mimetics that allow design in both conformational space and χ (topographical) space. Toward this end, in our most recent work using Ni(+2) chemistry, we have developed a simple scalable set of reactions that can lead to most of the possible chiral compounds that might be designed as derivatives or mimics of Glu, Gln, Asn, Asp, Pro, Cys, etc. with novel α, β, and γ substituents.[169−177] In a similar manner, we are seeking also to develop mimics for the key secondary structures of peptides and proteins, i.e., α-helix, β-sheet, β-turn, and extended structures. Our most recent efforts are directed toward bicyclic heterocyclic systems that constrain putative β-turns both in $\varphi-\psi$ space and in χ space.[177−182] The challenge of exploring all the available topographical space with 16, 32, or even more isomers is substantial, but certainly can be aided by combinatorial chemistry such as the one-bead, one-compound method.[183]. Equally challenging will be the design of non-peptide mimetics that can mimic the dynamic properties of proteins including interconversions from helix to turn structures, β-sheets to helical structures, etc.

This and many other challenges will make the coming years a continually exciting time to be a chemist, and I am looking forward with great joy to what we might learn in the coming years.

Acknowledgment. This work was made possible by the contributions and help of my mentors, students, colleagues, collaborators, and friends. I am eternally grateful for the opportunity to work with them and to interact with so many talented and enthusiastic people. Their ideas, criticisms, hard work, creativity, and humanity have been a constant inspiration. The support over the years primarily by the U.S. Public Health Service, National Institutes of Health, and in my earlier career by the National Science Foundation, and occasionally other sponsors is gratefully acknowledged.

Biography

Victor J. Hruby received his Ph.D. from Cornell University (A. T. Blomquist) and did a Postdoctoral with Vincent du Vigneaud. In 1968, he joined the Department of Chemistry, University of Arizona, where he is currently a Regents Professor of Chemistry. He was a recipient of Guggenheim and Senior Humboldt Fellowships, Pierce Award (now Merrifield Award), Javits Award, MERIT Award, and several other awards and honors. His major research interests include de novo design and synthesis of biologically active peptides and peptide mimetics, asymmetric synthesis, computation chemistry, combinatorial chemsity, conformation−biological activity relationships, design of ligands that affect pain, addictions, feeding behavior, sexual behavior, cancer, etc., the chemical−physical basis for behavior, and structure−function of GPCRs. He is a strong proponent of interdisciplinary research and has collaborated extensively on the biological and medical implications of his research.

References

(1) Hirschmann, R. Medicinal Chemistry in the Golden Age of Biology. Lessons from Peptide and Steroid Research. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1278−1301.
(2) Johnson, A. W.; Hruby, V. J.; Williams, J. L. Chemistry of Ylids, Diphenylsulfonium Alkylides. A Stereoselective Synthesis of Epoxides. *J. Am. Chem. Soc.* **1964**, *86*, 918−922.
(3) Blomquist, A. T.; Hruby, V. J. The Preparation and Properties of 1,2-Bis(triphenylphosphoranyl)benzocyclobutene. *J. Am. Chem. Soc.* **1967**, *89*, 4996−5007.
(4) Blomquist, A. T.; Rich, D. H.; Hruby, V. J.; Nangeroni, L.; Glose, P.; du Vigneaud, V. Deuterated Oxytocins: The Synthesis and Biological Properties of Three Crystalline Analogs of Deaminooxytocin Deuterated in the 1-β-Mercaptopropionic Acid Residue. *Proc. Natl. Acad. Sci. U.S.A.* **1968**, *61*, 688−692.
(5) Hruby, V. J.; Yamashiro, D.; du Vigneaud, V. The Structure of Acetone-oxytocin with Studies on the Reaction of Acetone with Various Peptides. *J. Am. Chem. Soc.* **1968**, *90*, 7106−7110.
(6) Hruby, V. J.; du Vigneaud, V. The Detection of a Schiff Base Intermediate in the Formation of Acetone−Oxytocin. *J. Am. Chem. Soc.* **1969**, *91*, 3624−3628.
(7) Hruby, V. J.; du Vigneaud, V.; Chan, W. Y. [2,4-Diisoleucine]-oxytocin. An Analog of Oxytocin with Natriuretic and Diuretic Activities. *J. Med. Chem.* **1970**, *13*, 185−187.
(8) Hruby, V. J.; Barstow, L. E.; Linhart, T. A New Machine for Automated Solid Phase Peptide Synthesis. *Anal. Chem.* **1972**, *44*, 343−350.
(9) Brewster, A. I. R.; Hruby, V. J.; Spatola, A. F.; Bovey, F. A. Carbon-13 NMR Spectroscopy of Oxytocin, Related Oligopeptides, and Selected Analogs. *Biochemistry* **1973**, *12*, 1643−1649.
(10) (a) Brewster, A. I. R.; Hruby, V. J. 300 MHz Nuclear Magnetic Resonance Study of Oxytocin in Aqueous Solution: Conformational Implications. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 3806−3809; (b) Hruby, V. J.; Deb, K. K.; Spatola, A. F.; Upson, D. A.; Yamamoto, D. M. ^{13}C Nuclear Magnetic Resonance Studies of

the Peptide Hormones Oxytocin, Arginine Vasopressin, Isotocin, Mesotocin, Glumitocin, Aspartocin, Related Analogues, and Diastereoisomers: Use of Specifically Deuterated Hormone Derivatives for Assignments and Effects of Structural Changes on ^{13}C NMR Chemical Shifts of Peptides. *J. Am. Chem. Soc.* **1979**, *101*, 202−212.

(11) Glasel, J. A.; McKelvy, J. F.; Hruby, V. J.; Spatola, A. F. Deuteron Magnetic Resonance Studies of Neurohypophyseal Hormones. *Ann. N. Y. Acad. Sci.* **1973**, *222*, 778−788.

(12) Hruby, V. J.; Smith, C. W., Sr.; Bower, A.; Hadley, M. E. MSH Release Inhibition by Ring Structures of Neurohypophyseal Hormones. *Science* **1972**, *176*, 1331−1332.

(13) Bower, A., Sr.; Hadley, M. E.; Hruby, V. J. Biogenic Amines and the Control of Melanophore Stimulating Hormone (MSH) Release. *Science* **1974**, *184*, 70−72.

(14) Hruby, V. J.; Brewster, A. I.; Glasel, J. A. NMR Study on the Conformation of Derivatives of the Side Chain of Oxytocin: Examples of cis−trans Isomerism. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 450−453.

(15) Blumenstein, M.; Hruby, V. J. Carbon-13 Nuclear Magnetic Resonance Studies of the Interaction of Specifically Labeled (90%-^{13}C) Oxytocin and Arginine Vasopressin with Neurophysins. *Biochem. Biophys. Res. Commun.* **1976**, *68*, 1052−1058.

(16) Blumenstein, M.; Hruby, V. J. The Interactions of Oxytocin with Bovine Neurophysins I and II. Use of ^{13}C Nuclear Magnetic Resonance and Hormones Specifically Enriched with ^{13}C in the Glycinamide-9 and Half-Cystine-1 Positions. *Biochemistry* **1977**, *16*, 5169−5177.

(17) Blumenstein, M.; Hruby, V. J.; Viswanatha, V. The Tyrosine Ring of Oxytocin Undergoes Hindered Rotation When the Hormone Is Bound to Neurophysin. *Biochem. Biophys. Res. Commun.* **1980**, *94*, 431−437.

(18) Bregman, M. D.; Hruby, V. J. Synthesis and Isolation of a Glucagon Antagonist. *FEBS Lett.* **1979**, *101*, 191−194.

(19) Bregman, M. D.; Trivedi, D. B.; Hruby, V. J. Glucagon Amino Groups: Evaluation of Modifications Leading to Antagonism and Agonism. *J. Biol. Chem.* **1980**, *255*, 11725−11733.

(20) Johnson, D. G.; Goebel, C. U.; Hruby, V. J.; Bregman, M. D.; Trivedi, D. B. Decrease in Hyperglycemia of Diabetic Rats by a Glucagon Receptor Antagonist. *Science* **1982**, *215*, 1115−1116.

(21) Hruby, V. J.; Deb, K. K.; Fox, J.; Bjarnason, J.; Tu, A. T. Conformational Studies of Peptide Hormones Using Laser Raman and Circular Dichroism: A Comparative Study of Oxytocin Agonists and Antagonists. *J. Biol. Chem.* **1978**, *253*, 6060−6067.

(22) Meraldi, J.-P.; Hruby, V. J.; Brewster, A. I. R. Relative Conformational Rigidity in Oxytocin and [1-Penicillamine]-oxytocin: A Proposal for the Relationship of Conformational Flexibility to Peptide Hormone Agonism and Antagonism. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 1373−1377.

(23) Hruby, V. J.; Deb, K. K.; Yamamoto, D. M.; Hadley, M. E.; Chan, W. Y. [1-Penicillamine,2-leucine]oxytocin: Synthesis, Pharmacological and Conformational Studies of a Potent Peptide Hormone Inhibitor. *J. Med. Chem.* **1979**, *22*, 7−12.

(24) Hruby, V. J. Relation of Conformation to Biological Activity in Oxytocin, Vasopressin, and Their Analogues. In *Topics in Molecular Pharmacology*; Elsevier/North-Holland Biomedical Press: Amsterdam, The Netherlands, 1981; Vol. 1, pp 99−126.

(25) Kessler, H. Conformation and Biological Activity of Cyclic Peptides. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 512−521.

(26) Hruby, V. J. Conformational Restrictions of Biologically Active Peptides via Amino Acid Side Chain Groups. *Life Sci.* **1982**, *31*, 189−199.

(27) Ramachandran, G. N.; Ramakrishnan, C.; Sasisekharan, V. Stereochemistry of Polypeptide Chain Configurations. *J. Mol. Biol.* **1963**, *7*, 95−96.

(28) Ramachandran, G. N.; Sasisekharan, V. Conformation of Polypeptides and Proteins. *Adv. Protein Chem.* **1968**, *23*, 283−438.

(29) Hruby, V. J.; Al-Obeidi, F.; Kazmierski, W. M. Emerging Approaches in the Molecular Design of Receptor Selective Peptide Ligands: Conformational, Topographical and Dynamic Considerations. *Biochem. J.* **1990**, *268*, 249−262.

(30) Rizo, J.; Gierasch, L. M. Constrained Peptides: Models of Bioactive Peptides and Protein Substructures. *Annu. Rev. Biochem.* **1992**, *61*, 387−418.

(31) Marshall, G. R. A Hierarchical Approach to Peptidomimetic Design. *Tetrahedron* **1993**, *49*, 3547−3555.

(32) Hruby, V. J. Conformational and Topographical Considerations in the Design of Biologically Active Peptides. *Biopolymers* **1993**, *33*, 1073−1082.

(33) Hruby, V. J.; Nikiforovich, G. V. The Ramachandran Plot and Beyond: Conformational and Topographical Considerations in the Design of Peptides and Proteins. In *Molecular Conformation and Biological Interactions*; Balaram, P., Ramaseshan, S., Eds.; Indian Academy of Science: Bangalore, India, 1991; pp 429−445.

(34) Hruby, V. J.; Li, G.; Haskell-Luevano, C.; Shenderovich, M. D. Design of Peptides, Proteins, and Peptidomimetics in Chi Space. *Biopolymers* **1997**, *43*, 219−266.

(35) Kazmierski, W. M.; Hruby, V. J. A New Approach To Receptor Ligand Design: Synthesis and Conformation of a New Class of Potent and Highly Selective μ Opioid Antagonists Utilizing Tetrahydroisoquinoline Carboxylic Acid. *Tetrahedron* **1988**, *44*, 697−710.

(36) Kazmierski, W. M.; Yamamura, H. I.; Hruby, V. J. Topographic Design of Peptide Neurotransmitters and Hormones on Stable Backbone Templates: Relation of Conformation and Dynamics to Bioactivity. *J. Am. Chem. Soc.* **1991**, *113*, 2275−2283.

(37) Kazmierski, W. M.; Urbanczyk-Lipkowski, Z.; Hruby, V. J. New Amino Acids for the Topographical Control of Peptide Conformation: Synthesis of All Isomers of α,β-Dimethylphenylalanine and α,β-Dimethyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid of High Optical Purity. *J. Org. Chem.* **1994**, *59*, 1789−1795.

(38) Valle, G.; Kazmierski, W. M.; Crisma, M.; Bonora, G. M.; Toniolo, C.; Hruby, V. J. Constrained Phenylalanine Analogues. Preferred Conformation of the 1,2,3,4-Tetrahydroisoquinoline-3-Carboxylic Acid (Tic) Residue. *Int. J. Pept. Protein Res.* **1992**, *40*, 222−232.

(39) Pelton, J. T.; Gulya, K.; Hruby, V. J.; Duckles, S. P.; Yamamura, H. I. Conformationally Restricted Analogs of Somatostatin with High μ-Opiate Receptor Specificity. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 236−239.

(40) Kazmierski, W. M.; Wire, W. S.; Lui, G. K.; Knapp, R. J.; Shook, J. E.; Burks, T. F.; Yamamura, H. I.; Hruby, V. J. Design and Synthesis of Somatostatin Analogues with Topographical Properties That Lead to Highly Potent and Specific μ Opioid Receptor Antagonists with Greatly Reduced Binding at Somatostatin Receptors. *J. Med. Chem.* **1988**, *31*, 2170−2177.

(41) Ayres, E. A.; Villar, R.; Kramer, T. H.; Kazmierski, W. M.; Hruby, V. J.; Burks, T. F. Highly Selective Mu Opioid Antagonist Peptides Block Spinal Mu Opioid Inhibition of Gastrointestinal Transit. *Proc. West. Pharmacol. Soc.* **1988**, *31*, 41−43.

(42) Shook, J. E.; Lemcke, P. K.; Gehrig, C. A.; Hruby, V. J.; Burks, T. F. Antidiarrheal Properties of Supraspinal Mu and Delta, and Peripheral Mu, Delta and Kappa Opioid Receptors: Inhibition of Diarrhea without Constipation. *J. Pharmacol. Exp. Ther.* **1989**, *249*, 83−90.

(43) Hawkins, K. N.; Knapp, R. J.; Lui, G. K.; Gulya, K.; Kazmierski, W. M.; Wan, Y.-P.; Pelton, J. T.; Hruby, V. J.; Yamamura, H. I. [^3H]-[H-D-Phe-Cys-Tyr-D-Tyr-Orn-Thr-Pen-Thr-NH_2], a Potent and Highly Selective Peptide for Mu Opioid Receptors in Rat Brain. *J. Pharmacol. Exp. Ther.* **1989**, *248*, 73−81.

(44) Dharanipragada, R.; Nicolás, E.; Tóth, G.; Hruby, V. J. Asymmetric Synthesis of Unusual Amino Acids: Synthesis of Optically Pure Isomers of β-Methylphenylalanine. *Tetrahedron Lett.* **1989**, *30* (49), 6841−6844.

(45) Nicolás, E.; Dharanipragada, R.; Tóth, G.; Hruby, V. J. Asymmetric Synthesis of Unusual Amino Acids: Synthesis of Optically Pure Isomers of β-Methyltyrosine. *Tetrahedron Lett.* **1989**, *30* (49), 6845−6848.

(46) Dharanipragada, R.; Van Hulle, K.; Bannister, A.; Bear, S.; Kennedy, L.; Hruby, V. J. Asymmetric Synthesis of Unusual Amino Acids: An Efficient Synthesis of Optically Pure Isomers of β-Methylphenylalanine, *Tetrahedron* **1992**, *48*, 4733−4748.

(47) Boteju, L. W.; Wegner, K.; Hruby, V. J. Asymmetric Synthesis of Unusual Amino Acids: Synthesis of the Optically Pure Isomers of Indole-Protected β-Methyltryptophan Suitable for Peptide Synthesis. *Tetrahedron Lett.* **1992**, *33*, 7491−7494.

(48) Nicolás, E.; Russell, K. C.; Hruby, V. J. Asymmetric 1,4-Addition of Organocuprates to Chiral α,β-Unsaturated *N*-Acyl-4-phenyl-2-oxazolidinones: A New Approach to the Synthesis of Chiral β-Branched Carboxylic Acids. *J. Org. Chem.* **1993**, *58*, 766−770.

(49) Li, G.; Jarosinski, M. A.; Hruby, V. J. Diastereospecific Tandem Michael-Like Addition/Electrophilic Bromination: A One Pot Tandem Asymmetric Synthesis of Precursors of Unusual Amino Acids. *Tetrahedron Lett.* **1993**, *34*, 2561−2564.

(50) Nicolas, E.; Russell, K. C.; Knollenberg, J.; Hruby, V. J. Efficient Method for the Total Asymmetric Synthesis of the Isomers of β-methyltyrosine. *J. Org. Chem.* **1993**, *58*, 7565−7571.

(51) Boteju, L. W.; Wegner, K.; Qian, X.; Hruby, V. J. Asymmetric Synthesis of Unusual Amino Acids: Synthesis of Optically Pure Isomers of *N*-Indole-(2-mesitylenesulfonyl)-β-methyltryptophan. *Tetrahedron* **1994**, *50*, 2391−2404.

(52) Lung, F. D.; Li, G.; Lou, B.-S.; Hruby, V. J. A New Strategy for the Synthesis of Four Individual Isomers of β-Methylphenylalanine. *Synth. Commun.* **1995**, *25*, 57−61.

(53) Qian, X.; Russell, K. C.; Boteju, L. W.; Hruby, V. J. Stereoselective Total Synthesis of Topographically Constrained Designer Amino Acids: 2′,6′-Dimethyl-β-methyltyrosines. *Tetrahedron* **1995**, *51*, 1033−1054.

(54) Xiang, L.; Wu, H.; Hruby, V. J. Stereoselective Synthesis of All Individual Isomers of β-Methyl-2′, 6′-dimethylphenylalanine. *Tetrahedron: Asymmetry* **1995**, *6*, 83−86.

(55) Liao, S.; Hruby, V. J. Asymmetric Synthesis of Optically Pure β-Isopropylphenylalanine: A New β-Branched Unusual Amino Acid. *Tetrahedron Lett.* **1996**, *37*, 1563−1566.

(56) Liao, S.; Han, Y.; Qui, W.; Bruck, M.; Hruby, V. J. Syntheses of Highly Constrained β-Aryl Isohexanoic Acid Derivatives via Asymmetric Michael Addition. *Tetrahedron Lett.* **1996**, *37*, 7917−7920.

(57) Yuan, W.; Hruby, V. J. Asymmetric Synthesis of Unusual Amino Acids: Synthesis of Four Isomers of β-Methyl-3-(2′-naphthyl)-alanine. *Tetrahedron Lett.* **1997**, *38*, 3853−3856.

(58) Han, Y.; Liao, S.; Qiu, W.; Cai, C.; Hruby, V. J. Total Asymmetric Syntheses of Highly Constrained Amino Acids β-Isopropyl-2′,6′-Dimethyl-tyrosines. *Tetrahedron Lett.* **1997**, *38*, 5135−5138.

(59) Hruby, V. J.; Tóth, G.; Prakash, O.; Davis, P.; Burks, T. F. Cyclic Enkephalins Which Are Optically Pure Isomers of [β-Me-*p*-NO_2-Phe[4]]-DPDPE Possess Extraordinary δ-Opioid Receptor Selectivities. In *Peptides 1988*; Jung, G., Bayer, E., Eds.; W. de Gruyter & Co.: Berlin, Germany, 1989; pp 616−618.

(60) Hruby, V. J.; Kazmierski, W. M.; Tóth, G.; Kao, L.-F.; Gehrig, C. A.; Burks, T. F.; Yamamura, H. I. Design of Specific Topographical Features in Polypeptide Neurotransmitters Leads to Unique Biological Properties. In *Highlights in Modern Biochemistry*; Kotyk, A., Skoda, J., Paces, V., Kostka, V., Eds.; VSP International Science Publishers: Zeist, The Netherlands, 1989; pp 43−52.

(61) Hruby, V. J.; Tóth, G.; Gehrig, C. A.; Kao, L.-F.; Knapp, R.; Lui, G. K.; Yamamura, H. I.; Kramer, T. H.; Davis, P.; Burks, T. F. Topographically Designed Analogues of [D-Pen[2],D-Pen[5]]enkephalin. *J. Med. Chem.* **1991**, *34*, 1823−1830.

(62) Nikiforovich, G. V.; Prakash, O.; Gehrig, C. A.; Hruby, V. J. Solution Conformations of the Peptide Backbone for DPDPE and Its β-Me-Phe[4]-Substituted Analogues. *Int. J. Pept. Protein Res.* **1993**, *41*, 347−361.

(63) Boteju, L. W.; Zalewska, T.; Yamamura, H. I.; Hruby, V. J. Tryptophan-Norleucine 1,5-Disubstituted Tetrazoles as Cis Peptide Bond Mimics: Investigation of the Bioactive Conformation of a Potent and Selective Peptide for the Cholecystokinin-B Receptor. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2011−2016.

(64) Qian, X.; Kövér, K. E.; Shenderovich, M. D.; Lou, B.-S.; Misicka, A.; Zalewska, T.; Horvath, R.; Davis, P.; Bilsky, E. J.; Porreca, F.; Yamamura, H. I.; Hruby, V. J. Newly Discovered Stereochemical Requirements in Side Chain Conformation of δ Opioid Agonists for Recognizing Opioid δ Receptors. *J. Med. Chem.* **1994**, *37*, 1746−1757.

(65) Boteju, L. W.; Nikiforovich, G. V.; Haskell-Luevano, C.; Fang, S.-N.; Zalewska, T.; Stropova, D.; Yamamura, H. I.; Hruby, V. J. The Use of Topographical Constraints in Receptor Mapping: Investigation of the Topographical Requirements of the Tryptophan 30 Residue for Receptor Binding of Asp-Tyr-D-Phe-Gly-Trp-(N-Me)Nle-Asp-Phe-NH_2 (SNF 9007), a Cholecystokin (26−33) Analogue That Binds to Both CCK-B and δ-Opioid Receptors. *J. Med. Chem.* **1996**, *39*, 4120−4124.

(66) Jiao, D.; Russell, K. C.; Hruby, V. J. Locally Constrained Tyrosine Analogues with Restricted Side Chain Dynamics. *Tetrahedron* **1993**, *49*, 3511−3520.

(67) Mosberg, H. I.; Hurst, R.; Hruby, V. J.; Gee, K.; Yamamura, H. I.; Galligan, J. J.; Burks, T. F. Bis-penicillamine Enkephalins Possess Highly Improved Specificity toward Delta Opioid Receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 5871−5874.

(68) Porreca, F.; Mosberg, H. I.; Hurst, R.; Hruby, V. J.; Burks, T. F. A Comparison of the Analgesic and Gastrointestinal Transit Effects of [d-Pen[2],L-Cys[5]]Enkephalin after Intracerebroventricular and Intrathecal Administration in Mice. *Life Sci.* **1983**, *33* (Suppl. I), 457−460.

(69) Weber, S. J.; Greene, D. L.; Sharma, S. D.; Yamamura, H. I.; Kramer, T. H.; Burks, T. F.; Hruby, V. J.; Hersh, L. B.; Davis, T. P. Distribution and Analgesia of [D-Pen[2],D-Pen[5]]enkephalin and Two Halogenated Analogues after Intravenous Administration. *J. Pharmacol. Exp. Ther.* **1991**, *259*, 1109−1117.

(70) Hruby, V. J.; Kao, L.-F.; Pettitt, B. M.; Karplus, M. The Conformational Properties of the δ Opioid Peptide [D-Pen[2],D-Pen[5]]-enkephalin in Aqueous Solution Determined by NMR and Energy Minimization Calculations. *J. Am. Chem. Soc.* **1988**, *110*, 3351−3359.

(71) Flippen-Anderson, J. L.; Hruby, V. J.; Collins, N.; George, C.; Cudney, B. X-ray Structure of [D-Pen[2],D-Pen[5]]Enkephalin, a Highly Potent, δ Opioid Receptor Selective Compound: Comparisons with Proposed Solution Conformations. *J. Am. Chem. Soc.* **1994**, *116*, 7523−7531.

(72) Qian, X.; Shenderovich, M. D.; Kövér, K. E.; Davis, P.; Horváth, R.; Zalewska, T.; Yamamura, H. I.; Porreca, F.; Hruby, V. J. Probing the Stereochemical Requirements for Receptor Recognition of δ Opioid Agonists through Topographic Modifications in Position 1. *J. Am. Chem. Soc.* **1996**, *118*, 7280−7290.

(73) Shenderovich, M. D.; Liao, S.; Qian, X.; Hruby, V. J. A Three-Dimensional Model of the δ Opioid Pharmacophore: Comparative Molecular Modeling of Peptide and Non-Peptide Ligands. *Biopolymers* **2000**, *53*, 565−580.

(74) Liao, S.; Alfaro-Lopez, J.; Shenderovich, M. D.; Hosohata, K.; Lin, J.; Li, X.; Stropova, D.; Davis, P.; Jernigan, K. A.; Porreca, F.; Yamamura, H. I.; Hruby, V. J. De Novo Design, Synthesis, and Biological Activities of High-Affinity and Selective Non-Peptide Agonists of the δ-Opioid Receptor. *J. Med. Chem.* **1998**, *41*, 4767−4776.

(75) Alfaro-Lopez, J.; Okayama, T.; Hosohata, K.; Davis, P.; Porreca, F.; Yamamura, H. I.; Hruby, V. J. The Fine Tuning of High Affinity and Selective Non-Peptide Agonists of the δ-Opioid Receptor via Solution and Solid-Phase Synthesis. In *Peptides for the New Millennium*; Fields, G. B., Tam, J. P., Barany, G., Eds.; Kluwer Academic: Dordrecht, The Netherlands, 2000; pp 38−39.

(76) Alfaro-Lopez, J.; Okayama, T.; Hosohata, K.; Davis, P.; Porreca, F.; Yamamura, H. I.; Hruby, V. J. Exploring the Structure−Activity Relationships of [1-(4-*tert*-Butyl-3′-hydroxy)benzhydryl-4-benzylpiperazine] (SL-3111), a High-Affinity and Selective δ-Opioid Receptor Nonpeptide Agonist Ligand. *J. Med. Chem.* **1999**, *42*, 5359−5368.

(77) Liao, S.; Shenderovich, M. D.; Zhang, Z.; Maletinska, L.; Slaninova, J.; Hruby, V. J. Substitution of the Side-Chain Constrained Amino Acids β-Methyl-2′,6′-dimethyl-4′-methoxytyrosine in Position 2 of a Bicyclic Oxytocin Analogue Provides Unique Insights into the Bioactive Topography of Oxytocin Antagonists. *J. Am. Chem. Soc.* **1998**, *120*, 7393−7394.

(78) Hill, P. S.; Smith, D. D.; Slaninová, J.; Hruby, V. J. Bicyclization of a Weak Oxytocin Agonist Produces a Highly Potent Oxytocin Antagonist. *J. Am. Chem. Soc.* **1990**, *112*, 3110−3113.

(79) Smith, D. D.; Slaninová, J.; Hruby, V. J. Structure−Activity Studies of a Novel Bicyclic Oxytocin Antagonist. *J. Med. Chem.* **1992**, *35*, 1558−1563.

(80) Shenderovich, M. D.; Wilke, S.; Kövér, K. E.; Collins, N.; Hruby, V. J.; Liwo, A.; Ciarkowski, J. Solution Conformation of a Potent Bicyclic Antagonist of Oxytocin. *Pol. J. Chem.* **1994**, *68*, 921−927.

(81) Shenderovich, M. D.; Kövér, K. E.; Wilke, S.; Collins, N.; Hruby, V. J. Solution Conformations of Potent Bicyclic Antagonists of Oxytocin by Nuclear Magnetic Resonance Spectroscopy and Molecular Dynamics Simulations. *J. Am. Chem. Soc.* **1997**, *119*, 5833−5846.

(82) Lebl, M.; Barth, T.; Servitova, L.; Slaninova, J.; Jöst, K. Amino-Acids and Peptides 190. Oxytocin Analogs with Inhibiting Properties, Containing in Position 2 a Hydrophobic Amino-Acid of D-Configuration. *Collect. Czech. Chem. Commun.* **1985**, *50*, 132−145.

(83) Zhuze, A. L.; Kasafire, E.; Jöst, K.; Rudinger, J. Amino Acids and Peptides 45. Analogues of Oxytocin with *O*-Ethyltyrosine *p*-Methylphenylalanine and *p*-Ethylphenylalanine Replacing Tyrosine. *Collect. Czech. Chem. Commun.* **1964**, *29*, 2648−2662.

(84) Cone, R. D., Ed. *The Melanocortin Receptors*; Humana Press: Totowa, NJ, 2000; pp 551 and references therein.

(85) Cutaneous Neuroimmunomodulation. The Proopiomelanocortin System. In *Annals of the New York Academy of Science*; Luger, T. A., Paus, R., Lipton, J. M., Slominski, A. T., Eds.; New York Academy of Sciences: New York, 1999; Vol. 885, p 479 and references therein.

(86) Hruby, V. J.; Wilkes, B. C.; Cody, W. L.; Sawyer, T. K.; Hadley, M. E. Melanotropins: Structural, Conformational, and Biological Considerations in the Development of Superpotent and Superprolonged Analogs. *Pept. Protein Rev.* **1984**, *3*, 1−64.

(87) Bower, A., Sr.; Hadley, M. E.; Hruby, V. J. Comparative MSH Release-Inhibiting Activities of Tocinoic Acid (The Ring of Oxytocin) and the L-Pro-L-Leu-Gly-NH_2 (The Side Chain of Oxytocin). *Biochem. Biophys. Res. Commun.* **1971**, *45*, 1185−1191.

(88) Hruby, V. J.; Bower, A., Sr.; Hadley, M. E. The MSH-Release Inhibiting Factor Activities of Tocinoic Acid (The Ring of Oxytocin), L-Pro-L-Leu-Gly-NH_2 (The Side Chain of Oxytocin) and Related Compounds. In *Structure−Activity Relationships of Protein and Polypeptide Hormones*; Margoulies, M., Greenwood, F. C., Eds.; Excerpta Medica: Amsterdam, The Netherlands, 1972; pp 525−527.

(89) Hadley, M. E.; Bower, A., Sr.; Hruby, V. J. Regulation of Melanophore Stimulating Hormone (MSH) Release. *Yale J. Biol. Med.* **1973**, *46*, 602−616.

(90) Hadley, M. E.; Hruby, V. J.; Bower, A., Sr. Cellular Mechanisms Controlling Melanophore Stimulating Hormone (MSH) Release. *Gen. Comp. Endocrinol.* **1975**, *26*, 24−35.

(91) Schwyzer, R. ACTH A Short Introductory Review. *Ann. N. Y. Acad. Sci.* **1977**, *297*, 3−26.

(92) Sawyer, T. K.; Sanfilippo, P. J.; Hruby, V. J.; Engel, M. H.; Heward, C. B.; Burnett, J. B.; Hadley, M. E. [Nle[4],D-Phe[7]]-α-Melanocyte Stimulating Hormone: A Highly Potent α-Melanotropin with Ultralong Biological Activity. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 5754−5758.

(93) Hadley, M. E.; Anderson, B.; Heward, C. B.; Sawyer, T. K.; Hruby, V. J. Calcium-Dependent Prolonged Effects on Melanophores of [4-Norleucine,7-D-Phenylalanine]-α-Melanotropin. *Science* **1981**, *213*, 1025−1027.

(94) Akiyama, K.; Yamamura, H. I.; Wilkes, B. C.; Cody, W. L.; Hruby, V. J.; de Lauro Castrucci, A.-M.; Hadley, M. E. Relative Stability of α-Melanotropin and Related Analogues to Rat Brain Homogenates. *Peptides* **1984**, *5*, 1191−1195.

(95) de Lauro Castrucci, A.-M.; Hadley, M. E.; Sawyer, T. K.; Hruby, V. J. Enzymological Studies of Melanotropins. *Comp. Biochem. Physiol.* **1984**, *78B*, 519−524.

(96) de Lauro Castrucci, A.-M.; Hadley, M. E.; Yorulmazoglu, E. I.; Wilkes, B. C.; Sawyer, T. K.; Hruby, V. J. Synthesis and Studies of Superpotent Melanotropins Resistant to Enzyme Degradation. *Pigm. Cell* **1985**, 145−151.

(97) Sawyer, T. K.; Hruby, V. J.; Hadley, M. E.; Engel, M. H. α-Melanocyte Stimulating Hormone: Chemical Nature and Mechanism of Action. *Am. Zool.* **1983**, *23*, 529−540.

(98) Vinson, G. P.; Whitehouse, B. J.; Bateman, A.; Hruby, V. J.; Sawyer, T. K.; Darman, P. S. α-MSH Analogues and Adrenal Zona Glomerulosa Function. *Life Sci.* **1984**, *35*, 603−610.

(99) Hadley, M. E.; Mieyr, J. H.; Martin, B. E.; Upton, J. L.; de Lauro Castrucci, A.-M.; Hruby, V. J.; Sawyer, T. K.; Powers, E. A.; Ranga Rao, K. [Nle^4,D-Phe^7]-α-MSH: A Superpotent Melanotropin with Prolonged Action on Vertebrate Chromatophores. *Comp. Biochem. Physiol.* **1985**, *81A*, 1−6.

(100) Abdel Malek, Z. A.; Kreutzfeld, K. L.; Marwan, M. M.; Hadley, M. E.; Hruby, V. J.; Wilkes, B. C. Prolonged Stimulation of S91 Melanoma Tyrosinase by [Nle^4,D-Phe^7]-Substituted α-Melanotropins. *Cancer Res.* **1985**, *45*, 4735−4740.

(101) de Lauro Castrucci, A.-M.; Hadley, M. E.; Hruby, V. J. Melanotropin Bioassays: In Vitro and in Vivo Comparisons. *Gen. Comp. Endocrinol.* **1984**, *55*, 104−111.

(102) Marwan, M. M.; Abdel Malek, Z. A.; Kreutzfeld, K. L.; Hadley, M. E.; Wilkes, B. C.; Hruby, V. J.; de Lauro Castrucci, A.-M. Stimulation of S91 Melanoma Tyrosinase Activity by Superpotent α-Melanotropins. *Mol. Cell. Endocrinol.* **1985**, *41*, 171−177.

(103) Hadley, M. E.; Wood, S. H.; Jessen, G. L.; Lemus-Wilson, A. L.; Dawson, B. V.; Levine, N.; Dorr, R. T.; Hruby, V. J. Topical Application of a Melanotropic Peptide Induces Systemic Follicular Melanogenesis. *Life Sci.* **1987**, *40*, 1889−1895.

(104) Hadley, M. E.; Hruby, V. J.; Levine, N.; Dorr, R. T.; Sharma, S. D.; Sheftel, S. N.; Eytan, T.; Weinrach, J. C.; Ertl, G. A.; Toth, K. A Melanotropic Peptide Induces Pigmentation (Tanning) of Human Skin. In *Peptides: Chemistry and Biology*; Smith, J., Rivier, J., Eds.; ESCOM Publishers: Leiden, The Netherlands, 1992; pp 429−430.

(105) Hadley, M. E.; Hruby, V. J.; Sharma, S. D.; Dorr, R. T.; Levine, N. Melanotropic Peptides for Therapeutic and Cosmetic Tanning of Human Skin. *Ann. N. Y. Acad. Sci.* **1993**, *680*, 424−439.

(106) Ugwu, S. O.; Blanchard, J.; Dorr, R. T.; Levine, N.; Brooks, C.; Hadley, M. E.; Aickin, M.; Hruby, V. J. Skin Pigmentation and Pharmacokinetics of Melanotan-I in Humans. *Biopharm. Drug Dispos.* **1997**, *18*, 259−269.

(107) Sawyer, T. K.; Hruby, V. J.; Darman, P. S.; Hadley, M. E. [4-Half-Cystine,10-Half-Cystine]-α-Melanocyte Stimulating Hormone: A Cyclic α-Melanotropin Exhibiting Superagonist Biological Activity. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 1751−1755.

(108) Hirsch, M. D.; O'Donohue, T. L.; Wilson, R.; Sawyer, T. K.; Hruby, V. J.; Hadley, M. E.; Cody, W. L.; Knittel, J. J.; Crawley, J. N. Structural and Conformational Modifications of α-MSH/ACTH$_{4-10}$ Provide Melanotropin Analogues with Highly Potent Behavioral Activities. *Peptides* **1984**, *5*, 1197−1201.

(109) Hadley, M. E.; Mieyr, J. H.; Martin, B. E.; Upton, J. L.; de Lauro Castrucci, A.-M.; Hruby, V. J.; Sawyer, T. K.; Powers, E. A.; Ranga Rao, K. [Nle^4,D-Phe^7]-α-MSH: A Superpotent Melanotropin with Prolonged Action on Vertebrate Chromatophores. *Comp. Biochem. Physiol.* **1985**, *81A*, 1−6.

(110) Abdel Malek, Z. A.; Kreutzfeld, K. L.; Marwan, M. M.; Hadley, M. E.; Hruby, V. J.; Wilkes, B. C. Prolonged Stimulation of S91 Melanoma Tyrosinase by [Nle^4,D-Phe^7]-Substituted α-Melanotropins. *Cancer Res.* **1985**, *45*, 4735−4740.

(111) Abdel Malek, Z. A.; Hadley, M. E.; Bregman, M. D.; Meyskens, F. L., Jr.; Hruby, V. J. Actions of Melanotropins on Mouse Melanoma Cell Growth in Vitro. *J. Natl. Cancer Inst.* **1986**, *76*, 857−863.

(112) Levine, N.; Lemus-Wilson, A.; Wood, S. H.; Abdel-Malek, Z. A.; Al-Obeidi, F.; Hruby, V. J.; Hadley, M. E. Stimulation of Follicular Melanogenesis in the Mouse by Topical and Injected Melanotropins. *J. Invest. Dermatol.* **1987**, *89*, 269−273.

(113) Hruby, V. J.; Wilkes, B. C.; Hadley, M. E.; Al-Obeidi, F.; Sawyer, T. K.; Staples, D. J.; de Vaux, A. E.; Dym, O.; de Lauro Castrucci, A.-M.; Hintz, M. F.; Riehm, J. R.; Rao, R. R. α-Melanotropin: The Minimum Active Sequence in the Frog Skin Bioassay. *J. Med. Chem.* **1987**, *30*, 2126−2130.

(114) de Lauro Castrucci, A.-M.; Hadley, M. E.; Sawyer, T. K.; Wilkes, B. C.; Al-Obeidi, F.; Staples, D. J.; de Vaux, A. E.; Dym, O.; Hintz, M. F.; Riehm, J. P.; Rao, R. R.; Hruby, V. J. α-Melanotropin: The Minimal Active Sequence in the Lizard Skin Bioassay. *Gen. Comp. Endocrinol.* **1989**, *73*, 157−163.

(115) (a) Sawyer, T. K.; de Lauro Castrucci, A.-M.; Marwan, M.; Staples, D. J.; Affholter, J. A.; DeVaux, A. E.; Hruby, V. J.; Hadley, M. E. Structure−Activity Relationships of [Nle^4,D-Phe^7]α-MSH: Discovery of a Tripeptidyl Agonist Exhibiting Sustained Bioactivity. *Ann. N. Y. Acad. Sci.* **1993**, *680*, 597−599. (b) Haskell-Luevano, C.; Sawyer, T. K.; Hendrata, S.; North, C.; Panahinia, L.; Stum, M.; Staples, D. J.; de Lauro Castrucci, A.-M.; Hadley, M. E.; Hruby, V. J. Truncation Studies of α-Melanotropin Peptides Identify Tripeptide Analogues Exhibiting Prolonged Agonist Bioactivity. *Peptides* **1996**, *17*, 995−1002.

(116) Al-Obeidi, F.; Hadley, M. E.; Pettitt, B. M.; Hruby, V. J. Design of a New Class of Superpotent Cyclic α-Melanotropins Based on Quenched Dynamic Simulations. *J. Am. Chem. Soc.* **1989**, *111*, 3413−3416.

(117) Al-Obeidi, F.; de Lauro Castrucci, A.-M.; Hadley, M. E.; Hruby, V. J. Potent and Prolonged Acting Cyclic Lactam Analogues of α-Melanotropin: Design Based on Molecular Dynamics. *J. Med. Chem.* **1989**, *32*, 2555−2561.

(118) Haskell-Luevano, C.; Boteju, L. W.; Miwa, H.; Dickinson, C.; Gantz, I.; Yamada, T.; Hadley, M. E.; Hruby, V. J. Topographical Modification of Melanotropin Peptide Analogues with *β*-Methyltryptophan Isomers at Position 9 Leads to Differential Potencies and Prolonged Biological Activities. *J. Med. Chem.* **1995**, *38*, 4720−4729.

(119) Haskell-Luevano, C.; Toth, K.; Boteju, L.; Job, C.; de Lauro Castrucci, A.-M.; Hadley, M. E.; Hruby, V. J. *β*-Methylation of the Phe^7 and Trp^9 Melanotropin Side Chain Pharmacophores Affects Ligand−Receptor Interactions and Prolonged Biological Activity. *J. Med. Chem.* **1997**, *40*, 2740−2749.

(120) Boteju, L. W.; Wegner, K.; Qian, X.; Hruby, V. J. Asymmetric Synthesis of Unusual Amino Acids: Synthesis of Optically Pure Isomers of *N*-Indole-(2-mesitylenesulfonyl)-*β*-methyltryptophan. *Tetrahedron* **1994**, *50*, 2391−2404.

(121) Haskell-Luevano, C.; Miwa, H.; Dickinson, C.; Hadley, M. E.; Hruby, V. J.; Yamada, T.; Gantz, I. Characterization of the Unusual Dissociation Properties of Melanotropin Peptides from the Melanotropin Receptor, hMC1R. *J. Med. Chem.* **1996**, *39*, 432−435.

(122) Sharma, S. D.; Granberry, M. E.; Jiang, J.; Leong, S. D. L.; Hadley, M. E.; Hruby, V. J. Multivalent Melanotropic Peptide and Fluorescent Macromolecular Conjugates: New Reagents for Characterization of Melanotropin Receptors. *Bioconjugate Chem.* **1994**, *5*, 591−601.

(123) Sharma, S. D.; Jiang, J.; Hadley, M. E.; Bentley, D. L.; Hruby, V. J. Melanotropic Peptide-Conjugated Beads for Microscopic Visualization and Characterization of Melanoma Melanotropin Receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 13715−13720.

(124) Jiang, J.; Sharma, S. D.; Fink, J. L.; Hadley, M. E.; Hruby, V. J. Melanotropic Peptide Receptors: Membrane Markers of Human Melanoma Cells. *Exp. Dermatol.* **1996**, *5*, 325−333.

(125) Jiang, J.; Sharma, S. D.; Hruby, V. J.; Fink, J. L.; Hadley, M. E. Human Epidermal Melanocyte and Keratinocyte Melanotropin Receptors: Visualization by Melanotropic Peptide Conjugated Macrospheres (Polyamide Beads). *Exp. Dermatol.* **1997**, *6*, 6−12.

(126) Jiang, J.; Sharma, S. D.; Hruby, V. J.; Bentley, D. L.; Fink, J. L.; Hadley, M. E. Human Epidermal Melanocyte and Keratinocyte Melanocortin Receptors: Visualization by Melanotropic Peptide Conjugated Microspheres (Latex Beads). *Pigm. Cell Res.* **1996**, *9*, 240−247.

(127) The Melanotropic Receptors. In *Annals of the New York Academy of Sciences*; Vaudry, H., Eberle, A. N., Eds.; New York Academy of Sciences: New York, 1993; Vol. 680, pp 1−687.

(128) Mogil, J. S.; Wilson, S. G.; Chesler, E. J.; Rankin, A. L.; Lariviere, W. R.; Groce, M. K.; Wallace, M. R.; Kaplan, L.; Staud, R.; Ness, T. J.; Glover, T. L.; Grisel, J. E.; Fillingim, R. B. The Melanocortin-1 Receptor Gene Mediates Female-Specific Mechanisms of Analgesia in Mice and Humans. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 4867−4872.

(129) Al-Obeidi, F.; Hruby, V. J.; Hadley, M. E.; Sawyer, T. K.; de Lauro Castrucci, A.-M. Design, Synthesis and Biological Activities of a Potent and Selective α-Melanotropin Antagonist. *Int. J. Pept. Protein Res.* **1990**, *35*, 228−234.

(130) Han, G.; Quillan, J. M.; Carlson, K.; Sadée, W.; Hruby, V. J. Design of Novel Chimeric Melanotropin−Deltorphin Analogues: Discovery of the First Potent Human Melanocortin 1 Receptor Antagonist. *J. Med. Chem.* **2003**, *46*, 810−819.

(131) O'Donohue, T. L.; Dorsa, D. M. The Opiomelanotropin Neuronal and Endocrine Systems. *Peptides* **1982**, *3*, 353−395.

(132) Haskell-Luevano, C.; Miwa, H.; Dickinson, C.; Hruby, V. J.; Yamada, T.; Gantz, I. Binding and cAMP Studies of Melanotropin Peptides with the Cloned Human Peripheral Melanocortin Receptor, hMC1R. *Biochem. Biophys. Res. Commun.* **1994**, *204*, 1137−1142.

(133) Hruby, V. J.; Lu, D.; Sharma, S. D.; de Lauro Castrucci, A.; Kesterson, R. A.; Al-Obeidi, F. A.; Hadley, M. E.; Cone, R. D. Cyclic Lactam α-Melanotropin Analogues of Ac-Nle^4-c[Asp^5,D-Phe^7,Lys^{10}]α-MSH(4−10)-NH_2 with Bulky Aromatic Amino Acids at Position 7 Show High Antagonist Potency and Selectivity at Specific Melanocortin Receptors. *J. Med. Chem.* **1995**, *38*, 3454−3461.

(134) Haskell-Luevano, C.; Hendrata, S.; North, C.; Sawyer, T. K.; Hadley, M. E.; Hruby, V. J.; Dickinson, C.; Gantz, I. Discovery of Prototype Peptidomimetic Agonists at the Human Melanocortin Receptors MC1R and MC4R. *J. Med. Chem.* **1997**, *40*, 2133−2139.

(135) Schiöth, H. B.; Muceniece, R.; Mutulis, F.; Prusis, P.; Lindeberg, G.; Sharma, S. D.; Hruby, V. J.; Wikberg, J. E. S. Selectivity of Cyclic [D-Nal^7]- and [D-Phe^7]-Substituted MSH Analogues for the Melanocortin Receptor Subtypes. *Peptides* **1997**, *18*, 1009−1013.

(136) Sawyer, T. K.; Staples, D. J.; de Lauro Castrucci, A.-M.; Hadley, M. E.; Al-Obeidi, F.; Cody, W. L.; Hruby, V. J. α-Melanocyte Stimulating Hormone Message and Inhibitory Sequences: Comparative Structure−Activity Studies on Melanocytes. *Peptides* **1990**, *11*, 351−358.

(137) Hruby, V. J.; Sharma, S. D.; Lim, S.; Yuan, W.; Haskell-Luevano, C.; Han, G.; Hadley, M. E.; Cone, R. D.; Gantz, I. Design of Potent and Specific Melanotropin Agonists and Antagonists: Investigating Ligands for New Receptors. In *Peptides 1996*; Ramage, R., Epton, R., Eds.; Mayflower Science Ltd.: Kingswinford, England, 1998; pp 485−486.

(138) Hruby, V. J.; Han, G.; Yuan, W.; Lim, S.; Haskell-Luevano, C.; Cone, R. D.; Kunos, G.; Hadley, M. E. Design of Novel Melanotropin Antagonists and Agonists for the Recently Discovered MC3, MC4 and MC5 Receptors and Their Use To Determine New Biological Roles. In *Peptides: Frontiers of Peptide Science*; Tam, J. P., Kaumaya, P. T. P., Eds.; Kluwer Academic: Dordrecht, The Netherlands, 1999; pp 723−725.

(139) Hadley, M. E.; Hruby, V. J.; Jiang, J.; Sharma, S. D.; Fink, J. L.; Haskell-Luevano, C.; Bentley, D. L.; Al-Obeidi, F.; Sawyer, T. K. Melanocortin Receptors: Identification and Characterization by Melanotropic Peptide Agonists and Antagonists. *Pigm. Cell Res.* **1996**, *9*, 213−234.

(140) de Lauro Castrucci, A.-M.; Almeida, A. L. K.; Al-Obeidi, F. A.; Hadley, M. E.; Hruby, V. J.; Staples, D. J.; Sawyer, T. K. Comparative Biological Activities of α-MSH Antagonists in Vertebrate Pigment Cells. *Gen. Comp. Endocrinol.* **1997**, *105*, 410−416.

(141) Haskell-Luevano, C.; Lim, S.; Yuan, W.; Cone, R. D.; Hruby, V. J. Structure−Activity Studies of the Melanocortin Antagonist SHU 9119 Modified at the 6, 7, 8, and 9 Positions. *Peptides* **2000**, *21*, 49−57.

(142) Cai, M.; Grieco, P.; Wiens, J.; Trivedi, D.; Hruby, V. J. Structure−Activity Relationship Studies of New Cyclic MSH Analogues Using Cloned-Human Melanocortin Receptors Lead to Greater Selectivity and Inverse Agonists. In *Peptides: The Wave of the Future*; Lebl, M., Houghten, R. A., Eds.; American Peptide Society: San Diego, CA, 2001; pp 892−893.

(143) Grieco, P.; Han, G.; Weinberg, D.; MacNeil, T.; Van der Ploeg, L. H. T.; Hruby, V. J. Design and Synthesis of Highly Potent and Selective Melanotropin Analogues of SHU9119 Modified at Position 6. *Biochem. Biophys. Res. Commun.* **2002**, *292*, 1075−1080.

(144) Balse-Srinivasan, P. M.; Grieco, P.; Cai, M.; Trivedi, D.; Hruby, V. J. Structure−Activity Relationships of Novel Cyclic α-MSH/*β*-MSH Hybrid Analogues Which Lead to Potent and Selective Ligands for the Human MC3R and Human MC5R. *J. Med. Chem.*, in press.

(145) Grieco, P.; Lavecchia, A.; Cai, M.; Trivedi, D.; Weinberg, D.; MacNeil, T.; Van der Ploeg, L. H. T.; Hruby, V. J. Structure−Activity Studies of the Melanocortin Peptides: Discovery of Potent and Selective Antagonists for the hMC3 and hMC4 Receptors. *J. Med. Chem.* **2002**, *45*, 5287−5294.

(146) Fan, W.; Boston, B. A.; Kesterson, R. A.; Hruby, V. J.; Cone, R. D. Role of Melanocortinergic Neurons in Feeding and the Agouti Obesity Syndrome. *Nature* **1997**, *385*, 165−168.

(147) Dorr, R. T.; Dvorakova, K.; Brooks, C.; Lines, R.; Levine, N.; Schram, K.; Miketova, P.; Hruby, V.; Alberts, D. S. Increased Eumelanin Expression and Tanning Is Induced by a Superpotent Melanotropin [Nle^4,D-Phe^7]α-MSH in Humans. *Protochem. Photobiol.* **2000**, *72*, 526−532.

(148) Hadley, M. E.; Hruby, V. J.; Blanchard, J.; Dorr, R. T.; Levine, N.; Dawson, B. V.; Al-Obeidi, F.; Sawyer, T. K. Discovery and Development of Novel Melanogenic Drugs, Melanotan-I and II. In *Integration of Pharmaceutical Discovery and Development: Case Studies*; Borchardt, R., Ed.; Plenum Press: New York, 1998; pp 575−595.

(149) Dorr, R. T.; Lines, R.; Levine, N.; Brooks, C.; Xiang, L.; Hruby, V. J.; Hadley, M. E. Evaluation of Melanotan II, a Superpotent Cyclic Melanotropic Peptide in a Pilot Phase-I Clinical Study. *Life Sci.* **1996**, *58*, 1777−1784.

(150) Wessells, H.; Fuciarelli, K.; Hansen, J.; Hadley, M. E.; Hruby, V. J.; Dorr, R.; Levine, N. Synthetic Melanotropic Peptide Initiates Erections in Men with Psychogenic Erectile Dysfunction: Double-Blind, Placebo Controlled Crossover Study. *J. Urol.* **1998**, *160*, 389−393.

(151) Hadley, M. E.; Hruby, V. J. Induction of Erectogenic Activity in the Human Male by Systemic Delivery of a Melanotropin Peptide. In *Peptides: Frontiers of Peptide Science*; Tam, J. P., Kaumaya, P. T. P., Eds.; Kluwer Academic: Dordrecht, The Netherlands, 1999; pp 658−659.

(152) Wessells, H.; Gralnek, D.; Dorr, R.; Hruby, V. J.; Hadley, M. E.; Levine, N. Effect of an Alpha-Melanocyte Stimulating Hormone Analog on Penile Erection and Sexual Desire in Men with Organic Erectile Dysfunction. *Urology* **2000**, *56*, 641−646.

(153) Wessells, H.; Levine, N.; Hadley, M. E.; Dorr, R.; Hruby, V. J. Melanocortin Receptor Agonists, Penile Erection, and Sexual Motivation: Human Studies with Melanotan II. *Intl. J. Impot. Res.* **2000**, *12*, S74−S79.

(154) Wessells, H.; Hruby, V.; Hackett, J.; Han, G.; Balse-Srinivasan, P.; Vanderah, T. W. MT-II Induces Penile Erection via Brain and Spinal Melanocortin Receptors. *Neuroscience* **2003**, *118*, 755−762.

(155) Li, S.-J.; Varga, K.; Archer, P.; Hruby, V. J.; Sharma, S. D.; Kesterson, R. A.; Cone, R. D.; Kunos, G. Melanocortin Antagonists Define Two Distinct Pathways of Cardiovascular Control by α- and *γ*-Melanocyte-Stimulating Hormones. *J. Neurosci.* **1996**, *16*, 5182−5188.

(156) Kunos, G.; Li, S.-J.; Varga, K.; Archer, P.; Kesterson, R. A.; Cone, R. D.; Hruby, V. J.; Sharma, S. D. Novel Neural Pathways of Cardiovascular Control by α- and *γ*-MSH. *Fundam. Clin. Pharmacol.* **1997**, *11* (Suppl. 1), 44s−48s.

(157) Huang, Q.-H.; Entwistle, M. L.; Alvaro, J. D.; Duman, R. S.; Hruby, V. J.; Tatro, J. B. Antipyretic Role of Endogenous Melanocortins Mediated by Central Melanocortin Receptors During Endotoxin-Induced Fever. *J. Neurosci.* **1997**, *17*, 3343−3351.

(158) Huang, Q.-H.; Hruby, V. J.; Tatro, J. B. Systemic α-MSH Suppresses LPS Fever via Central Melanotropin Receptors Independently of Its Suppression of Corticosterone and IL-6 Release. *Am. J. Physiol.: Regul., Integr. Comp. Physiol.* **1998**, *275*, R524−R530.

(159) Ni, X.-P.; Kesterson, R. A.; Sharma, S. D.; Hruby, V. J.; Cone, R. D.; Wiedemann, E.; Humphreys, M. H. Prevention of Reflex Natriuresis after Acute Unilateral Nephrectomy by Melanocortin Receptor Antagonists. *Am. J. Physiol.* **1998**, *274* (43), R931−R938.

(160) Huang, Q.-H.; Hruby, V. J.; Tatro, J. B. Role of Central Melanocortins in Endotoxin-Induced Anorexia. *Am. J. Physiol.* **1999**, *276*, R864−R871.

(161) Hruby, V. J.; Agnes, R. S.; Lee, Y. S.; Davis, P.; Ma, S.-W.; Lai, J.; Porreca, F. Designing Peptide Drugs/Ligands for Pathological States. A New Paradigm for Design of Bioactive Peptide Hormones and Neurotransmitters. In *Peptides: The Wave of the Future*; Lebl, M., Houghten, R. A., Eds.; American Peptide Society: San Diego, CA, 2001; pp 969−970.

(162) Hruby, V. J.; Agnes, R. S.; Davis, P.; Ma, S.-W.; Lee, Y. S.; Vanderah, T. W.; Lai, J.; Porreca, F. Design of Novel Peptide Ligands Which Have Opioid Agonist Activity and CCK Antagonist Activity for the Treatment of Pain. *Life Sci.* **2003**, *73*, 699−704.

(163) Collins, N.; Flippen-Anderson, J. L.; Haaseth, R. C.; Deschamps, J. R.; George, C.; Kövér, K.; Hruby, V. J. Conformational Determinants of Agonist Versus Antagonist Properties of [D-Pen^2,D-Pen^5]Enkephalin (DPDPE) Analogs at Opioid Receptors. Comparison of X-ray Crystallographic Structure, Solution ^{1}H NMR Data, and Molecular Dynamic Simulations of [L-Ala^2]-DPDPE and [D-Ala^3]DPDPE. *J. Am. Chem. Soc.* **1996**, *118*, 2143−2152.

(164) Hruby, V. J. Designing Peptide Receptor Agonists and Antagonists. *Nat. Rev. Drug Discovery* **2002**, *1*, 847−858.

(165) Nikiforovich, G. V.; Hruby, V. J. Models for the A- and B-Receptor-Bound Conformations of CCK-8. *Biochem. Biophys. Res. Commun.* **1993**, *194*, 9−16.

(166) Hruby, V. J.; Fang, S. N.; Kramer, T. H.; Davis, P.; Parkhurst, D.; Nikiforovich, G. V.; Boteju, L. W.; Slaninová, J.; Yamamura, H. I.; Burks, T. F. Analogues of Cholecystokinin$_{26-33}$ Selective for B-Type CCK Receptors Possess Delta Opioid Receptor Agonist Activity in Vitro and in Vivo: Evidence for Similarities in CCK-B and *δ* Opioid Receptor Requirements. In *Peptides: Chemistry, Structure and Biology*; Hodges, R. S., Smith, J. A., Eds.; ESCOM Publishers: Leiden, The Netherlands, 1994; pp 669−671.

(167) Fang, S. N.; Nikiforovich, G. V.; Knapp, R. J.; Jiao, D.; Yamamura, H. I.; Hruby, V. J. Development of Models for the Bioactive Conformations of Ligands for CCK A and B Receptors and Their Use in the Design and Synthesis of Highly Potent and Selective Analogs. In *Peptides: Chemistry and Biology*; Smith, J. A., Rivier, J. E., Eds.; ESCOM Publishers: Leiden, The Netherlands, 1992; pp 142−143.
(168) Slaninová, J.; Knapp, R. J.; Wu, J.; Fang, S. N.; Kramer, T.; Hruby, V. J.; Yamamura, H. I. Opioid Receptor Binding Properties of Analgesic Analogues of Cholecystokinin Octapeptide. *Eur. J. Pharmacol.* **1991**, *200*, 195−198.
(169) Soloshonok, V. A.; Cai, C.; Hruby, V. J.; Van Meervelt, L. Asymmetric Synthesis of *Novel* Highly Sterically Constrained (2*S*,3*S*)-3-Methyl-3-trifluoromethyl- and (2*S*,3*S*,4*R*)-3-Trifluoromethyl-4-methylpyroglutamic Acids. *Tetrahedron* **1999**, *55*, 12045−12058.
(170) Soloshonok, V. A.; Cai, C.; Hruby, V. J. Toward Design of a Practical Methodology for Stereocontrolled Synthesis of χ-Constrained Pyroglutamic Acids and Related Compounds Virtually Complete Control of Simple Diastereoselectivity in the Michael Addition Reactions of Glycine Ni(II) Complexes with *N*-(Enoyl)-oxazolidinones. *Tetrahedron Lett.* **2000**, *41*, 135−139.
(171) Soloshonok, V. A.; Cai, C.; Hruby, V. J. (*S*)- or (*R*)-3-(E-enoyl)-4-phenyl-1,-3-oxazolidin-2-ones: Ideal Michael Acceptors To Afford a Virtually Complete Control of Simple and Face Diastereoselectivity in Addition Reactions with Glycine Derivatives. *Org. Lett.* **2000**, *2*, 747−750.
(172) Tang, X.; Soloshonok, V. A.; Hruby, V. J. Convenient, Asymmetric Synthesis of Enantiomerically Pure 2′,6′-Dimethyltyrosine (DMT) via Alkylation of Chiral Equivalent of Nucleophilic Glycine. *Tetrahedron: Asymmetry* **2000**, *11*, 2917−2925.
(173) Soloshonok, V. A.; Tang, X.; Hruby, V. J.; Van Meervelt, L. Asymmetric Synthesis of α,β-Dialkyl-α-phenylalanines via Direct Alkylation of a Chiral Alanine Derivative with Racemic α-Alkylbenzyl Bromides. A Case of High Enantiomer Differentiation at Room Temperature. *Org. Lett.* **2001**, *3*, 341−343.
(174) Cai, C.; Soloshonok, V. A.; Hruby, V. J. Michael Addition Reactions between Chiral Ni(II) Complex of Glycine and 3-(*trans*-Enoyl)oxazolidin-2-ones. A Case of Electron Donor−Acceptor Attractive Interaction-Controlled Face Diastereoselectivity. *J. Org. Chem*. **2001**, *66*, 1339−1350.
(175) Soloshonok, V. A.; Tang, X.; Hruby, V. J. Large-Scale Asymmetric Synthesis of Novel Sterically Constrained 2′,6-Dimethyl- and α,2′,6′-Trimethyltyrosine and -phenylalanine Derivatives via Alkylation of Chiral Equivalents of Nucleophilic Glycine and Alanine. *Tetrahedron* **2001**, *57*, 6375−6382.
(176) Wang, W.; Xiong, C.; Yang, J.; Hruby, V. J. Practical, Asymmetric Synthesis of Aromatic-Substituted Bulky and Hydrophobic Tryptophan Derivatives. *Tetrahedron Lett.* **2001**, *42*, 7717−7719.
(177) Wang, W.; Xiong, C.; Zhang, J.; Hruby, V. J. Practical, Asymmetric Synthesis of Aromatic-Substituted Bulky and Hydrophobic Tryptophan and Phenylalanine Derivatives. *Tetrahedron* **2002**, *58*, 3101−3110.
(178) Wang, W.; Xiong, C.; Yang, J.; Hruby, V. J. Two Novel and Efficient Approaches to Synthesis of Enantiopure Dipeptide β-Turn Mimetics: Indolizidinone Amino Acids. In *Peptides: The Wave of the Future*; Lebl, M., Houghten, R. A., Eds.; American Peptide Society: San Diego, CA, 2001; pp 30−31.
(179) Gu, X.; Qiu, W.; Ying, J.; Ndungu, J. M.; Hruby, V. J. Synthesis of β-Turn Mimetics: [5,5]-Fused Bicyclic γ-Lactam Dipeptide Analogues. In *Peptides: The Wave of the Future*; Lebl, M., Houghten, R. A., Eds.; American Peptide Society: San Diego, CA, 2001; pp 602−603.
(180) Wang, W.; Yang, J.; Ying, J.; Xiong, C.; Zhang, J.; Cai, C.; Hruby, V. J. Stereoselective Synthesis of Dipeptide β-Turn Mimetics: 7-Benzyl and 8-Phenyl Substituted Azabicyclo[4.3.0]nonane Amino Acid Esters. *J. Org. Chem*. **2002**, *67*, 6353−6360.
(181) Zhang, J.; Xiong, C.; Wang, W.; Ying, J.; Hruby, V. J. Stereoselective Bromination−Suzuki Cross-Coupling of Dehydroamino Acids To Form Novel Reverse-Turn Peptidomimetics: Substituted Unsaturated and Saturated Indolizadinone Amino Acids. *Org. Lett*. **2002**, *4*, 4029−4032.
(182) Gu, X.; Tang, X.; Cowell, S.; Ying, J.; Hruby, V. J. A Novel Strategy toward [6.5]Bicyclic β-Turn Dipeptides. *Tetrahedron Lett.* **2003**, *43*, 6669−6672.
(183) Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.; Kazmierski, W. M.; Knapp, R. J. A New Type of Synthetic Peptide Library for Identifying Ligand-Binding Activity. *Nature* **1991**, *354*, 82−84.

JM0303103