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Edward E. Smissman–Bristol-Myers Squibb Award Address

The Role of Concepts in Structure–Activity Relationship Studies of Opioid Ligands†

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This award is particularly meaningful to me because I had worked with Professor Edward E. Smissman from 1958 to 1961. I spent my first 2 years at the University of Wisconsin, and I then accompanied Dr. Smissman to the University of Kansas, where I completed my Ph.D. dissertation in the spring of 1961.

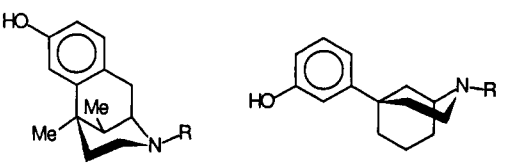
I am also indebted to Professor Louis Malspeis, my M.S. advisor at Columbia University, who directed me to the University of Wisconsin to continue my graduate studies. His advice was invaluable because it provided me with the opportunity to join Professor Smissman's research group. Professor Smissman was an outstanding role model and he had a profound influence on my scientific career. I credit him for developing my appreciation of concepts for analyzing the relationship between molecular structure and biological activity, and in the design of biologically active compounds. In this presentation I will discuss some key concepts that I have employed in my research over the past 30 years. The unifying theme in all of these concepts is that of multiple recognition sites and receptor selectivity.

The Multiple Binding Modality Concept of Ligand–Opioid Receptor Interactions

Receptor redundancy is the hallmark of biological systems, and it is the rule rather than the exception that each receptor class consists of several receptor types and subtypes. This finding is in marked contrast to the view prevalent 30 years ago when a receptor class was generally thought of as homogeneous for each major pharmacologic class of ligands. It was in this climate, as a graduate student, that I became interested in opioid ligands through my presentation of a seminar on this subject. In reviewing the literature,[1] I was both impressed and puzzled by the diverse structural and stereochemical requirements of opioid ligands. I wondered how such molecules could possibly fit a single receptor site in a lock-and-key mode. Moreover, the literature did not appear to be consistent with the then widely accepted three-point attachment model[2] for the interaction between the opioid receptor and analgesic ligands.

For example, molecules with quite different geometry possess comparable opioid agonist potency. This is illustrated by benzomorphans and phenylmorphans, whose aromatic groups are fixed in axial and equatorial conformations, respectively (Table I). Moreover, an identical change of N-substituent in both series does not afford a

Table I. Antinociceptive Potencies of Benzomorphans and Phenylmorphans[a]



R	benzomorphans ED_{50}[b]	phenylmorphans ED_{50}[b]
CH_3	2.6	2.0
$(CH_2)_2C_6H_5$	0.1	3.99

[a] Data obtained from ref 1. [b] The sc dose (mg/kg) of the racemates in mice using the hot-plate assay.

† This in part is taken from the text of the Edward E. Smissman–Bristol-Myers-Squibb Award Address delivered on August 28, 1991, at The Fourth Chemical Congress of North America, August 25–30, New York, NY.

(1) My first introduction to opioids was through a review by Eddy, N. B. Chemical Structure and Action of Morphine-Like Analgesics and Related Substances. *Chem. Ind.* **1959**, 1462–1469.
(2) Beckett, A. H.; Casy, A. F. Synthetic Analgesics: Stereochemical Considerations. *J. Pharm. Pharmacol.* **1954**, *6*, 986–999.

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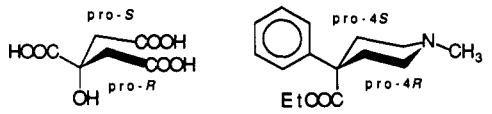


Citric acid Meperidine

Figure 1. Comparison of the enantiotopic edges of citric acid and meperidine.

parallel change of potency. Thus, the relative potency of the benzomorphan is enhanced 35-fold upon replacement of *N*-methyl with *N*-phenethyl, whereas the identical replacement in the phenylmorphan series results in an insignificant potency change.

There are, in fact, many series of opioid ligands that do not possess identical rank-order potencies with modification of the N-substituent. These include, to name only a few, opiates, 4-phenylpiperidines, and basic anilides. I suggested that the different structure–activity relationships are a consequence of dissimilar binding contributions of identical N-substituents to the receptor interaction in the different series.[3,4] In the series where the binding contributions of the N-substituent are identical (e.g., N-substituted normeperidine vs the corresponding reverse esters), an identical rank-order potency change upon changing the N-substituent is observed. This is characterized by a linear free energy relationship when the potencies of ligands in one series are plotted against those of a second series.[3]

Stereochemical data consistent with the concept of different contributions of identical substituents to opioid activity were obtained from our findings that the chiral center in the more potent enantiomers of the basic anilide analgesics is opposite to that of (−)-methadone.[5] We suggested this to be due to different receptor environments that interact with the chiral centers of ligands in each of these series.[4,5]

If one is to make some sense out of a structure–activity relationship, it is implied that all of the active ligands should interact in a similar fashion with a homogeneous population of receptors. The fact that there is no coherent relationship between structure and potency among the opioid ligands suggested that, from a conceptual point of view, it would be more realistic to consider the divergent structure–activity relationships to be a consequence of different modes of interaction with a single opioid receptor or with multiple opioid receptors.[4,5]

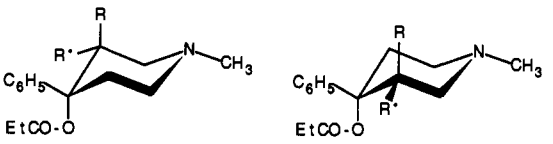
It was obviously easier to formulate this concept than to demonstrate the presence of multiple receptors and multiple modes of interaction, pharmacologically or biochemically, as over 10 years elapsed before multiple opioid receptors were demonstrated through pharmacological and binding studies. Presently, there are at least three major types of opioid receptors (μ, κ, δ) that are involved in the modulation of a variety of physiological effects via interaction with opioid peptides.[6] Because opioid receptors have not yet been cloned and characterized, it is presently difficult to evaluate the possibility of different modes of interaction of ligands with a single opioid receptor type.

The Ogston Concept

The nonequivalence of paired enantiotopic groups in substrates was first proposed by Ogston[7] in order to illustrate how only one of these groups in citric acid can be enzymatically transformed in the Krebs cycle. This concept, which is now well known and discussed in most biochemistry textbooks, was of interest in our studies because meperidine also contains enantiotopic groups (Figure 1). If meperidine or its congeners interact with the chiral environment of an opioid receptor, the enantiotopic edges (*pro*-4*R* and *pro*-4*S*) of its piperidine ring also might be distinguishable. We thought that such knowledge might provide some insight into how the mode of interaction of meperidine with opioid receptors differs from that of morphine.

There had been no reports on the application of this concept to the interaction of ligands with receptors because no biochemical transformation takes place in such cases. Hence, radiolabeling experiments were not feasible. For this reason, the enantiotopic edges of piperidine in the 4-phenylpiperidine analgesics were "labeled" with methyl groups and each enantiomer of the diastereomers were tested for antinociceptive activity. The rationale for labeling the piperidine ring with a methyl group was based on the idea that a 3-methyl group on only one of the enantiotopic edges might interfere with receptor binding. It was therefore expected that the more potent enantiomeric diastereomers should be substituted on the same enantiotopic edge of the piperidine ring.

Initial studies with enantiomeric diastereomers of prodine (**1a**, **2a**) revealed that the more potent enantiomers have the C-3 methyl group attached to the *pro*-4*S* enantiotopic edge.[8] While these results indicated a clear distinction between the enantiotopic edges of the piperidine ring by the opioid receptor, it did not reveal whether this differentiation is a consequence of steric hindrance between the 3-methyl group and the receptor or an indirect effect due to intramolecular steric factors. This was investigated with enantiomers **3** and **4** that contain a com-



	R	R'
a	H	CH_3
b	CH_3	H

(3) Portoghese, P. S. Linear Free Energy Relationship Among Analgesic N-Substituted Phenylpiperidine Derivatives: Method of Detecting Similar Modes of Molecular Binding to Common Receptors. *J. Pharm. Sci.* **1965**, *54*, 1077–1079.

(4) Portoghese, P. S. A New Concept on the Mode of Interaction of Narcotic Analgesics with Receptors. *J. Med. Chem.* **1965**, *8*, 609–616.

(5) Portoghese, P. S. Stereochemical Factors and Receptor Interactions Associated with Narcotic Analgesics. *J. Pharm. Sci.* **1966**, *55*, 865–887.

(6) Jaffe, J. H.; Martin, W. R. Opioid Analgesics and Antagonists. In *The Pharmacological Basis of Therapeutics*, 8th ed.; Gilman, A. G., Rall, T. W., Nies, A. S., Taylor, P., Eds.; Pergamon Press: New York, 1990; pp 485–521.

(7) Ogston, A. G. Interpretation of Experiments on Metabolic Proteases Using Isotopic Tracer Elements. *Nature (London)* **1948**, *162*, 963.

(8) Larson, D. L.; Portoghese, P. S. Stereochemical Studies on Medicinal Agents. 12. The Distinction of Enantiotopic Groups in the Interaction of 1-Methyl-4-phenyl-4-propionoxypiperidine with Analgetic Receptors. *J. Med. Chem.* **1973**, *16*, 195–198.

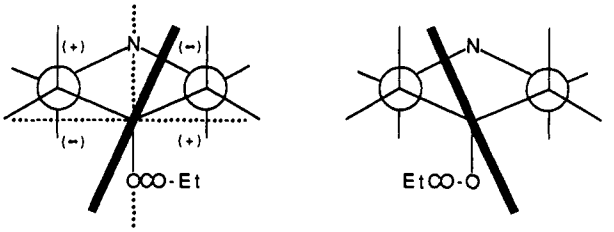
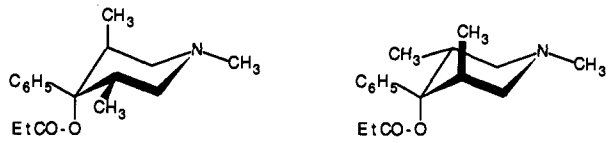


Figure 2. Projection formulas of the relationship between the phenyl group and the piperidine ring in the more potent (A) and less potent (B) enantiomers. Note that the phenyl group in A is located in the (−) quadrants.

bination of axial and equatorial methyl groups that flank the 4-phenyl substituent.[9]



The more potent enantiomer **3** is equipotent with **1a**, a finding that could not have been anticipated on the basis of configuration alone. X-ray crystallographic studies provided a clue to explaining why **3** is more potent than its enantiomer **4**, as it revealed that the main distinguishing feature of the more potent enantiomers of the prodine diastereomers **1a**, **1b**, and **3** is that the phenyl group resides in a negative quadrant (Figure 2). The data suggested that this is determined by methyl substitution on the *pro*-4*S* enantiotopic edge of the piperidine ring.

As expected, α-diastereomers with C(3) alkyl groups larger than methyl (ethyl, propyl, allyl) exhibited a similar relationship between the enantiomer potency and sign of the torsion angle of the phenyl group.[10] However, this relationship does not hold for the β-diastereomers where the 3-alkyl group is axial, as these diasteromers are of low potency. We have proposed that the larger C(3) axial substituents (allyl, propyl) cannot fit the same hydrophobic pocket that accommodates the axial 3-methyl group of β-prodine (**1b**).

All of the 4-phenylpiperidines discussed thus far contain an energetically preferred equatorial phenyl group. A potent opioid agonist whose phenyl group is known to reside preferentially in the axial conformation is α-promedol.[13,14] The chirality of enantiomers of this ligand and

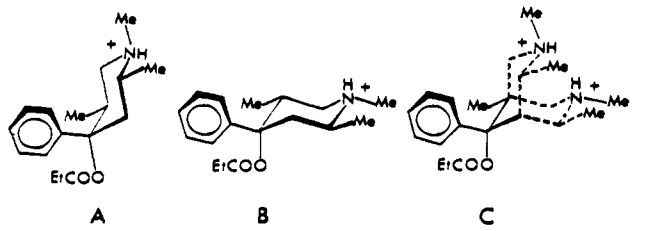


Figure 3. The C(3)–N–C(6) moiety and phenyl group of the more potent enantiomers of α-promedol (**5**) (A) and trimeperidine (**7**) (B) superposed (C). Portions not superposed are denoted by dashed lines.

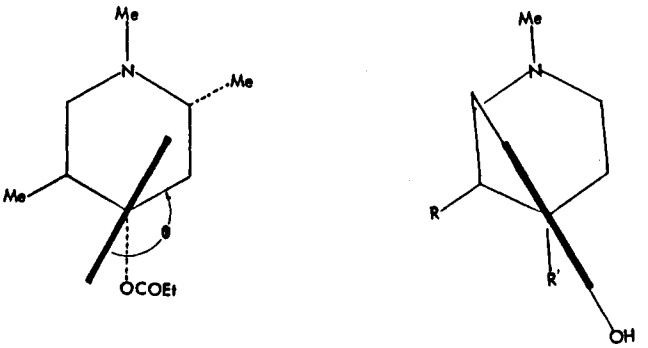
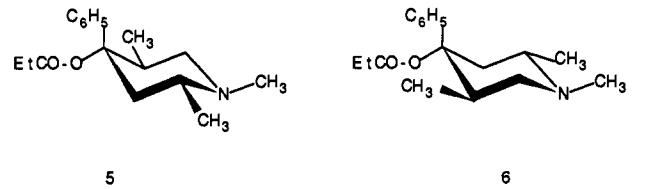


Figure 4. Projection formulas for the more potent enantiomer of α-promedol (**5**) (left) and that of the partial structure of morphine (right). Note that the torsion angle between the aromatic group and piperidine ring are of opposite signs.

those of its equatorial phenyl diastereomer, trimeperidine, were elucidated[10] to determine whether or not there is an identical recognition locus on the opioid receptor for a moiety common to both axial and equatorial 4-phenylpiperidines.[15,16] Also, because both α-promedol and morphine have axial aromatic groups, it would permit comparison of the torsional relationship of this group with opioid activity.

The 2*R*,4*S*,5*S* isomer of α-promedol (**5**) is 17 times more potent than morphine, whereas its enantiomer **6** is inactive.[15] Comparison of the absolute stereochemistry of **5** with that of its more potent equatorial diastereomer **7** reveals that they both possess the 4*S* configuration. In



other words, the more potent enantiomers of both the

(9) Portoghese, P. S.; Gomaa, Z. S. D.; Larson, D. L. Stereochemical Studies on Medicinal Agents. 13. Correlation of the Solid-State Conformations of 1,3,5-Trimethyl- and 1,3-Dimethyl-4-phenyl-4-propionoxypiperidine Enantiomers and their Absolute Steroselectivity at Analgetic Receptors. *J. Med. Chem.* 1973, *16*, 199–203.

(10) Portoghese, P. S. Stereoisomeric Ligands as Opioid Receptor Probes. *Acc. Chem. Res.* 1978, *11*, 21–29.

(11) Bell, K. H.; Portoghese, P. S. Stereochemical Studies on Medicinal Agents. 15. Absolute Configurations and Analgetic Potencies of Enantiomeric Diastereomers of 3-Allyl-1-methyl-4-phenyl-4-propionoxypiperidine. *J. Med. Chem.* 1973, *16*, 589–591.

(12) Bell, K. H.; Portoghese, P. S. Stereochemical Studies on Medicinal Agents. 17. Synthesis, Absolute Configuration and Analgetic Protency of Enantiomeric Diastereomers of 3-Ethyl and 3-Propyl Derivatives of 1-Methyl-4-phenyl-4-propionoxypiperidine. *J. Med. Chem.* 1974, *17*, 129–131.

(13) Nazarov, I. N.; Prostakov, N. S.; Mikheeva, N. N. Heterocyclic Compounds. 63. Synthetic Analgesics. XXVI. Stereoisomers of 2,5-Dimethyl-4-piperidols and 1-Acyl-2,5-dimethyl-4-phenyl-4-peridols. *J. Gen. Chem. U.S.S.R.* 1958, *28*, 2771–2780.

(14) DeCamp, W. H.; Ahmed, F. R. Structural Studies of Synthetic Analgetics. I. Crystal and Molecular Structure of the Monoclinic Form of (±)-β-promedol Alcohol. *Acta Crystallogr., Sect. B* 1972, *28*, 1796–1800.

(15) Fries, D. S.; Portoghese, P. S. Stereochemical Studies on Medicinal Agents. 20. Absolute Configuration and Analgetic Potency of α-Promedol Enantiomers. The Role of the C-4 Chiral Center in Conferring Stereoselectivity in Axial- and Equatorial-Phenyl Prodine Congeners. *J. Med. Chem.* 1976, *19*, 1155–1158.

(16) DeCamp, W. H.; Ahmed, F. R. Structural Studies of Synthetic Analgetics. I. Crystal and Molecular Structure of (±)-γ-Promedol Alcohol. *Acta Crystallogr., Sect. B* 1972, *28*, 1791–1796.

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axial- and equatorial-phenyl congeners contain a 3-alkyl group on the *pro*-4*S* edge of the piperidine ring. Moreover, X-ray data show that the torsional relationship between the aromatic group and the piperidine ring in the active α-promedol enantiomer **5** is of the same sign as the more potent enantiomers (**1**, **4**, **7**) with the equatorial phenyl.[10]

This remarkable correlation led us to propose a similar recognition locus on the opioid receptor for the C(3)–C(4)–C(5) moiety and its C(4) substituents in both the equatorial- and axial-phenyl analogues. The dominant role played by this recognition locus makes it likely that the C(2)–N–C(6) moiety of axial- and equatorial-phenyl conformers are bound in different positions on the recognition site. This is illustrated in Figure 3, which shows different orientations of C(2)–N–C(6) when C(3)–C(4)–C(5) of the more potent enantiomers **5** and **7** are superposed.

The dissimilar modes of interaction between equatorial and axial phenyl is consistent with the different rank order potencies between the phenylmorphans (equatorial) and benzomorphans (axial) when their N-substituents are varied in an identical fashion (Table I). This is readily understandable if the N-substituents are projected into different receptor environments due to their dissimilar orientation.

It is noteworthy that the sign of the torsion angle of the aromatic group in the active α-promedol enantiomer **5** is opposite to that of morphine (Figure 4). This could mean that the receptor topography in proximity with morphine and **5** are different. However, there is a more compelling reason to believe that the aromatic group of α-promedol and other 4-phenylpiperidines do not interact with some recognition locus as morphine, and this is related to the presence of the phenolic group in morphine. The finding[17,18] that phenolic prodines are inactive as opioid agonists suggests that the aromatic group of nonphenolic 4-phenylpiperidines related to the prodine analgesics does not bind to the same recognition locus as the phenolic group in opiates.

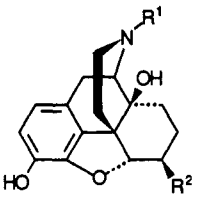
Finally, whether the different recognition loci for phenolic and nonphenolic groups represent subsites on the same receptor site or subsites on discrete recognition sites is not known. The fact that 4-phenylpiperidines related to meperidine- and prodine-type ligands are considerably more lipophilic than morphine raises the possibility that there may be more than one recognition site on the μ receptor system: one that interfaces with a hydrophilic environment, and a second that interfaces with a hydrophobic compartment (e.g., lipid bilayer). Such sites may be allosterically linked so that only one site at a time can be occupied. The implication of this concept is that changing the polarity of a ligand will alter the pathway that leads to its binding site. When viewed from this perspective, it is not difficult to visualize how ligands with widely divergent structures and with partition coefficients that differ by several orders of magnitude can bind to different recognition sites on the same receptor system. One possible example is represented by the well-known μ-selective ligands morphine (hydrophilic) and fentanyl (hydrophobic).

Affinity Labels and the Concept of Recognition Amplification

Soon after the publication of this concept of multiple opioid receptors we started to investigate affinity labels in an effort to distinguish between different receptor types. The conceptual rationale for this approach was in part based upon the studies of Baker,[19] a pioneer in the design of active site-directed irreversible inhibitors of enzymes.

With affinity labels that contain an electrophilic group, high selectivity for an opioid receptor type is dependent on (a) the affinity and selectivity of the ligand, (b) the location of the electrophilic center in the ligand, and (c) the reactivity and chemical selectivity of the electrophilic group. In considering each of these parameters, it is apparent that two consecutive recognition steps are involved in covalent binding of the receptor by an affinity label (Figure 5). The first recognition step involves a reversible association of the ligand with the receptor; the second recognition step, which leads to covalent bond formation, requires proper alignment of the electrophilic group with a receptor-based nucleophile. The second step should amplify the recognition of the affinity label when the electrophilic group is chemically selective and within covalent binding distance of a compatible receptor-based nucleophile if it is assumed that each type of opioid receptor contains a different array of nucleophiles. This I refer to as *recognition amplification*.[27]

One of the uncertainties with opioid receptor affinity labels was that we did not know what to expect with regard to in vivo pharmacological activity. The tacit assumption was that an affinity label derived from an opioid agonist would inactivate the receptor and act as an irreversible antagonist. We were not correct in that assumption because the nitrogen mustard derivative of oxymorphone, chloroxymorphamine (**8**) (COA), behaved as an irreversible



	R^1	R^2
8	CH_3	$N(CH_2CH_2Cl)_2$
9	$CH_2CH(CH_2)_2$	$N(CH_2CH_2Cl)_2$
10	$CH_2CH(CH_2)_2$	$NH\text{-}CO\text{-}C(H)=C(H)\text{-}COOCH_3$

opioid agonist in the guinea pig ileum preparation (GPI).[20]

(17) Zimmerman, D. M.; Nickander, R.; Hornig, J. S.; Wong, D. T. New Structural Concepts for Narcotic Antagonists Defined in a 4-Phenylpiperidine Series. *Nature (London)* **1978**, *275*, 332–334.

(18) Portoghese, P. S.; Alreja, B. D.; Larson, D. L. Allylprodine Analogues as Receptor Probes. Evidence that Phenolic and Nonphenolic Ligands Interact with Different Subsites on Identical Receptors. *J. Med. Chem.* **1981**, *24*, 782–787.

(19) Baker, B. R. *Design of Active-Site-Directed Irreversible Enzyme Inhibitors.* Wiley: New York, 1967.

(20) Caruso, T. P.; Takemori, A. E.; Larson, D. L.; Portoghese, P. S. Chloroxymorphamine, An Opioid Receptor Site-Directed Alkylating Agent Having Narcotic Agonist Activity. *Science* **1979**, *204*, 316–318.

(21) Paton, W. D. M. A Theory of Drug Action Based on the Rate of Drug-Receptor Combination. *Proc. R. Soc. (London)* **1961**, *154B*, 21–69.

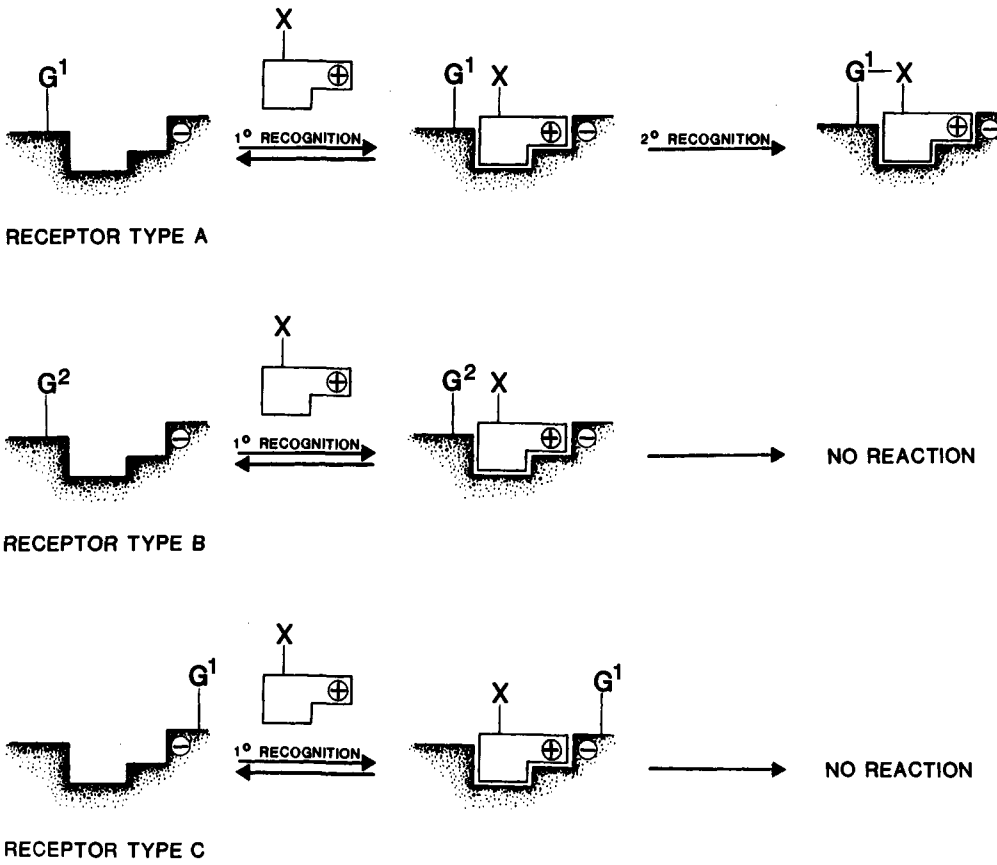


Figure 5. A schematic representation of the concept of recognition amplification in the covalent binding of receptor type A by an affinity label that contains a selective electrophilic group X. Although receptor types A–C have similar topographic features that lead to reversible binding (1° recognition), they differ with respect to the reactivity of the receptor-based nucleophiles (G^1 and G^2) and their locations. Only with receptor type A is the nucleophile G^1 reactive with respect to X and within covalent binding distance (2° recognition).

These unexpected results led us to conclude that the Paton[22] model of pharmacologic action is not tenable. You may recall that this model stated that agonists dissociate more rapidly from a receptor than antagonists, and it was hypothesized that the rate of association determines the efficiency of receptor activation. Clearly, a covalently bound agonist does not fit this model.

Since we were more interested in antagonist affinity labels because they could be employed to evaluate the selectivity of opioid agonists, our finding that COA (8) is an irreversible agonist caused us to alter our design approach, as it seemed reasonable that similar modification of naltrexone should afford an irreversible opioid antagonist. This compound, known as β-chlornaltrexamine (9) (β-CNA), is a highly potent opioid antagonist that is active both in vivo and in vitro.[22] It is irreversible in vitro (GPI and receptor binding) and produces ultralong-lasting antagomism in mice. Because β-CNA contains a highly reactive electrophilic group, it is capable of blocking all known opioid receptor types irreversibly.

After we had achieved our first objective of obtaining an affinity label with opioid antagonist activity, our goal was to obtain a *selective* ligand. In an effort to accomplish this we decided to enhance the second recognition step (Figure 5) by installing a chemically selective electrophilic group into the molecule. We therefore examined numerous groups containing the α,β-unsaturated carbonyl moiety because of its selectivity for a thiol group that was implicated as a receptor-based nucleophile. These studies led to the synthesis of β-funaltrexamine (10) (β-FNA), a highly selective μ opioid receptor antagonist.[23]

β-Funaltrexamine does not antagonize non-μ opioid agonists and its antagonist effect lasts 3–5 days in vivo after a single dose. The fact that it produces a κ agonist effect but binds noncovalently to κ receptors reflects a difference in the distribution of receptor-based nucleophiles and exemplifies the concept of recognition amplification discussed earlier. Additional evidence for recognition amplification is the finding that its C(6) epimer, α-FNA, and its maleamic acid methyl ester analogue do not irreversibly block μ receptors.[24-26] These studies emphasize that the orientation of the electrophilic group is

(22) Portoghese, P. S.; Larson, D. L.; Jiang, J. B.; Takemori, A. E.; Caruso, T. P. 6β-[N,N-Bis(2-Chloroethyl)amino]-17-cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan (Chlornaltrexamine), a Potent Opioid Receptor Alkylating Agent with Ultralong Narcotic Antagonist Activity. *J. Med. Chem.* **1978**, *21*, 598–599.

(23) Portoghese, P. S.; Larson, D. L.; Sayre, L. M.; Fries, D. S.; Takemori, A. E. A Novel Receptor Site Directed Alkylating Agent with Irreversible Narcotic Antagonist and Reversible Agonist Activities. *J. Med. Chem.* **1980**, *23*, 233–234.

(24) Sayre, L. M.; Larson, D. L.; Fries, D. S.; Takemori, A. E.; Portoghese, P. S. Importance of C-6 Chirality in Conferring Irreversible Opioid Antagonism to Naltrexone-Derived Affinity Labels. *J. Med. Chem.* **1983**, *26*, 1229–1235.

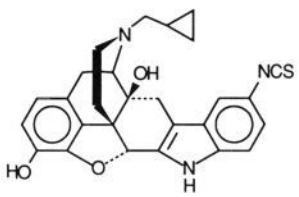
(25) Griffin, J. F.; Larson, D. L.; Portoghese, P. S. Crystal Structure of α- and β-Funaltrexamine: The Conformational Requirement of the Fumaramate Moiety in the Irreversible Blockage of μ Opioid Receptors. *J. Med. Chem.* **1986**, *29*, 778–783.

(26) Sayre, L. M.; Larson, D. L.; Takemori, A. E.; Portoghese, P. S. Design and Synthesis of Naltrexone-Derived Affinity Labels with Nonequilibrium Opioid Agonist and Antagonist Activities. Evidence for the Existence of Different μ Receptor Subtypes in Different Tissues. *J. Med. Chem.* **1984**, *27*, 1325–1335.

critical for μ receptor alkylation.

Both β-CNA and β-FNA are widely employed as pharmacological tools in opioid research and are available commercially.[27] For example, the fact that β-CNA is a universal affinity label for opioid receptors makes it useful in alkylating unprotected opioid receptors in order to obtain an enriched subpopulation of opioid receptors. β-FNA is used in pharmacological experiments to titrate μ receptors without affecting other opioid receptor populations. Also, it is presently employed in μ opioid receptor isolation and sequencing experiments.

More recently, we have developed a highly selective δ opioid receptor antagonist, naltrindole-5′-isothiocyanate (**11**) (5′–NTII).[28] The design of 5′-NTII was based upon



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the reversible δ opioid antagonist, naltrindole (**20**), which I will discuss later in this presentation. 5′-NTII has been used for one of the first identifications of δ opioid receptor subtypes.[29]

Bivalent Ligands and the Concept of Bridging Neighboring Recognition Sites

Another approach to developing highly selective opioid antagonists involved the linkage of two recognition units through a spacer.[30] The recognition units need not be the same. We call such compounds bivalent ligands. I point out that bivalent ligands are not new. The bis onium compounds that interact with nicotinic cholinergic receptors were developed over 50 years ago.[31] Our rationale for employing this approach was based on the premise that enhanced potency and selectivity may be conferred by bridging two neighboring recognition sites. These neighboring sites may be on two neighboring receptors or they may be two subsites on a single receptor system.[32]

The potency increase of a double pharmacophore bivalent ligand over a monovalent ligand should be substantially greater than a factor of two if the confinement of the free pharmacophore of a univalently bound bivalent ligand is held within the locus of the neighboring vacant recognition site, as this would be equivalent to a very high

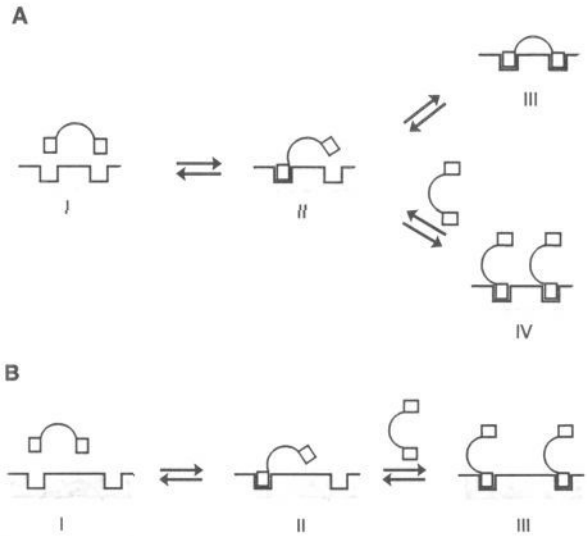
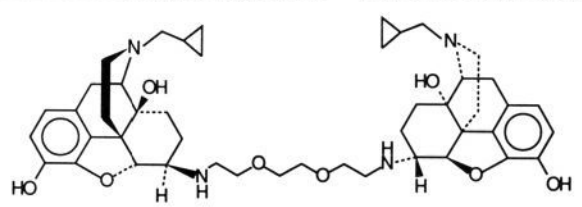


Figure 6. (A) The bridging (state III) of neighboring recognition sites by a bivalent ligand. Note that state III is favored over univalent binding (state IV) under conditions favorable for bridging. (B) For receptor types whose sites are not in proper juxtaposition, only univalent binding (state III) is possible.

concentration of pharmacophore (Figure 6A, state II). Consequently, bivalent binding (state III) should be favored over univalent binding (state IV) if the spacer permits briding of neighboring sites. The simultaneous occupation of two recognition sites (state III) should therefore lead to selectivity if such binding is favored by a single subpopulation of receptors. The dimensions of the spacer would be expected to play an important role in modulating selectivity, as factors such as the length, geometry, and conformational mobility of the spacer should influence the orientation of the unbound pharmacophore in the univalently bound state (Figure 6, II). How spacer length may modulate selectivity via the bridging principle is illustrated conceptually by comparing Figure 6A with 6B. Receptor type A is bridged by the bivalent ligand more readily than receptor type B, because the spacer does not permit both pharmacophores of a single molecule to bind neighboring sites simultaneously in receptor type B.

The first truly selective κ opioid receptor antagonist, TENA (**12**), was developed from the double pharmacophore bivalent ligand approach.[33] TENA consists of two



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naltrexone-derived pharmacophores connected to a spacer obtained from triethylene glycol. The spacers that were employed in subsequent studies are composed of glycyl units. This permitted varying the spacer length by the number of glycyl units and it provided facile elaboration of such spacers through standard peptide chemistry. Also, glycyl units were preferred over an alkyl chain in order to avoid incremental increases in the hydrophobic properties

(27) Takemori, A. E.; Portoghese, P. S. Affinity Labels for Opioid Receptors. *Annu. Rev. Pharmacol.* **1985**, *25*, 193–223.

(28) Portoghese, P. S.; Sultana, M.; Takemori, A. E. Naltrindole 5′-Isothiocyanate: A Nonequilibrium, Highly Selective δ Opioid Receptor Antagonist. *J. Med. Chem.* **1990**, *33*, 1547–1548.

(29) Jiang, Q.; Takemori, A. E.; Sultana, M.; Portoghese, P. S.; Bowen, W. D.; Mosberg, H. I.; Porreca, F. Differential Antagonism of Opioid Delta Antinociception by [D-Ala^2,Leu^5,Cys^6]Enkephalin and Naltrindole 5′-Isothiocyanate: Evidence for Delta Receptor Subtypes. *J. Pharmacol. Exp. Ther.* **1991**, *257*, 1059–1075.

(30) Erez, M.; Takemori, A. E.; Portoghese, P. S. Narcotic Antagonist Potency of Bivalent Ligands Which Contain β-Naltrexamine. Evidence for Bridging Between Proximal Recognition Sites. *J. Med. Chem.* **1982**, *25*, 847–849.

(31) Cocolas, G. H. Cholinergic Drugs and Related Agents. In *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry*, 9th ed.; Delgado, J. N., Remers, W. A., Eds.; J. B. Lippincott: Philadelphia, 1991; pp 443–505.

(32) Portoghese, P. S. Bivalent Ligands and the Message–Address Concept in the Design of Selective Opioid Antagonists. *Trends Pharmacol. Sci.* **1989**, *10*, 230–235.

(33) Portoghese, P. S.; Takemori, A. E. TENA, A Selective Kappa Opioid Receptor Antagonist. *Life Sci.* **1985**, *36*, 801–805.

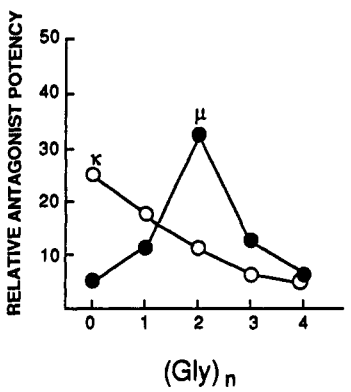
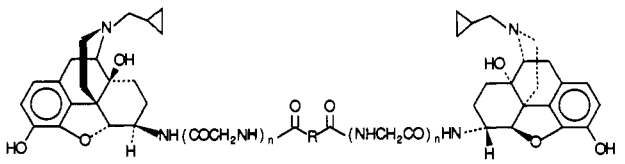


Figure 7. Relationship of μ and κ opioid receptor antagonist potency (guinea pig ileum preparation) and the number of Gly units in each half of the spacer in series **13**.

of the bivalent ligand upon lengthening the spacer. Symmetry was introduced into the spacers by a central succinyl or fumaryl group (series **13** and **14**, respectively). Both groups were employed in order to compare the relationship between the conformational flexibility of the spacer and antagonist potency.[34,35]



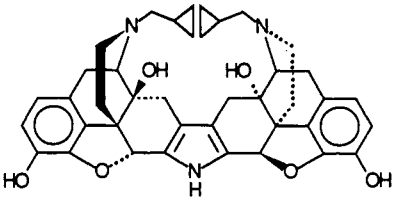
13, R = CH_2CH_2

14, R = $\underset{H}{\overset{H}{C{=}C}}$

The structure–activity profile of series **13** is presented in Figure 7. These studies were carried out on the guinea pig ileum preparation (GPI) which contains μ and κ receptors. The graph illustrates the relative effectiveness of members of the series to antagonize either morphine (μ-selective agonist) or ethylketazocine (κ-selective agonist) as a function of the number of glycyl units (n) in the spacer. Significantly, the structure–activity relationship profile of the succinyl series **13** for antagonism of morphine is substantially different from that of ethylketazocine (Figure 7); peak antagonism of morphine is observed at $n = 2$, whereas maximum antagonism of ethylketazocine is seen in the bivalent ligand having the shortest spacer length ($n = 0$). The structure–activity data for series **13** are qualitatively consistent with the data obtained with TENA (**12**) and its higher homologue, in that the bivalent ligands with the shortest spacers are the more potent κ antagonists.

The large increase of antagonist potency at μ receptors is consistent with the bridging of either two neighboring receptor sites or two neighboring subsites on a single μ opioid receptor. In an effort to distinguish between these two possibilities, the bivalent ligand containing a combination of (−) and (+) enantiomeric elements (Figure 8) was synthesized. This meso isomer possesses the same spacer ($n = 2$) that afforded peak antagonism at μ receptors. The (+)-enantiomer was incorporated into this molecular because it has been established that (+)-naltrexone is inactive as an opioid antagonist.[36] It was found that the meso isomer and the monovalent ligand possess nearly equal antagonist potencies, but $\sim 1/30$ less than that of the (−)-(−) isomer, thereby confirming that the neighboring site has an enantio-preference characteristic of an opioid receptor site.[37]

The observation that the shortest spacer in the series afforded the most potent κ antagonist (Figure 7) led to the synthesis of bivalent ligands that have a pyrrole moiety as a very short rigid spacer.[38] The most potent and selective member of this series, norbinaltorphamine (**15**) (norBNI), possesses exceptionally high κ opioid receptor



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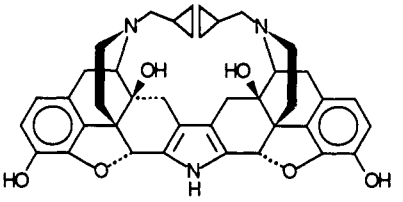
antagonist potency and unprecedented κ antagonist selectivity.[39] This high in vitro antagonist selectivity of norBNI is paralleled by its high binding selectivity for κ opioid sites and its κ antagonist selectivity in mice.[40]

As it was uncertain whether norBNI (**15**) derives its κ selectivity by interacting with two neighboring κ opioid receptor sites or with two subsites on a single κ receptor, the meso isomer **16** was synthesized from a combination



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of the antagonist pharmacophore derived from (−)-naltrexone and its inactive (+)-enantiomer. The perspective formulas (Figure 9) corresponding to **15** and **16** illustrate the different geometry of these molecules. In smooth muscle preparations, **16** is ~5 times more potent than norBNI (**15**) and it is κ-selective. This is consistent with the idea that only one of the two antagonist pharmacophores of norBNI is required for κ opioid antagonist activity and selectivity.

Our study suggested that the κ opioid receptor site is comprised of two key subsites. One subsite recognizes the

(34) Portoghese, P. S.; Larson, D. L.; Yim, C. B.; Sayre, L. M.; Ronsisvalle, G.; Tam, S. W.; Takemori, A. E. Opioid Agonist and Antagonist Bivalent Ligands. The Relationship of Spacer Length and Selectivity at Multiple Opioid Receptors. *J. Med. Chem.* **1986**, *29*, 1855–1861.

(35) Portoghese, P. S.; Ronsisvalle, G.; Larson, D. L.; Takemori, A. E. Synthesis and Opioid Antagonist Potencies of Naltrexamine Bivalent Ligands with Conformationally Restricted Spacers. *J. Med. Chem.* **1986**, *29*, 1650–1653.

(36) Iijima, I.; Minamikawa, J.-I.; Jacobson, A. E.; Brossi, A.; Rice, K. C.; Klee, W. Studies in the (+)-Morphinan Series. 5. Synthesis and Biological Properties of (+)-Naloxone. *J. Med. Chem.* **1978**, *21*, 398–400.

(37) Portoghese, P. S.; Larson, D. L.; Yim, C. B.; Sayre, L. M.; Ronsisvalle, G.; Lipkowski, A. W.; Takemori, A. E.; Rice, K. C.; Tam, S. W. Stereostructure–Activity Relationship of Opioid Agonist and Antagonist Bivalent Ligands. Evidence for Bridging between Vicinal Opioid Receptors. *J. Med. Chem.* **1985**, *28*, 1140–1141.

(38) Portoghese, P. S.; Lipkowski, A. W.; Takemori, A. E. Bimorphinans as Highly Selective, Potent, κ Opioid Receptor Antagonists. *J. Med. Chem.* **1987**, *30*, 238–239.

(39) Portoghese, P. S.; Lipkowski, A. W.; Takemori, A. E. Binaltorphimine and Norbinaltorphimine, Potent and Selective κ-Opioid Receptor Antagonists. *Life Sci.* **1987**, *40*, 1287–1292.

(40) Takemori, A. E.; Ho, B. Y.; Naeseth, J. S.; Portoghese, P. S. Norbinaltorphimine, a Highly Selective Kappa-Opioid Antagonist in Analgesic and Receptor Binding Assays. *J. Pharmacol. Exp. Ther.* **1988**, *246*, 255–258.

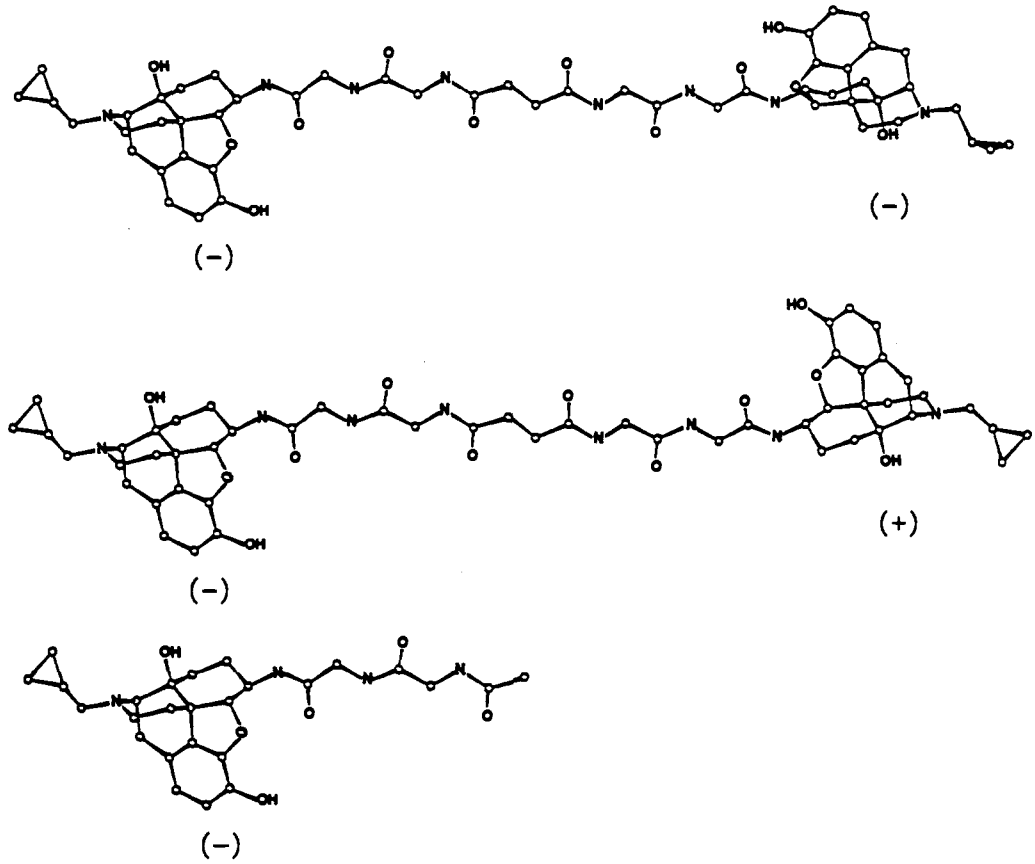


Figure 8. The structural relationship between the bivalent ligand with peak μ antagonist potency 13 (n = 2), its meso isomer (center), and the monovalent ligand (bottom).

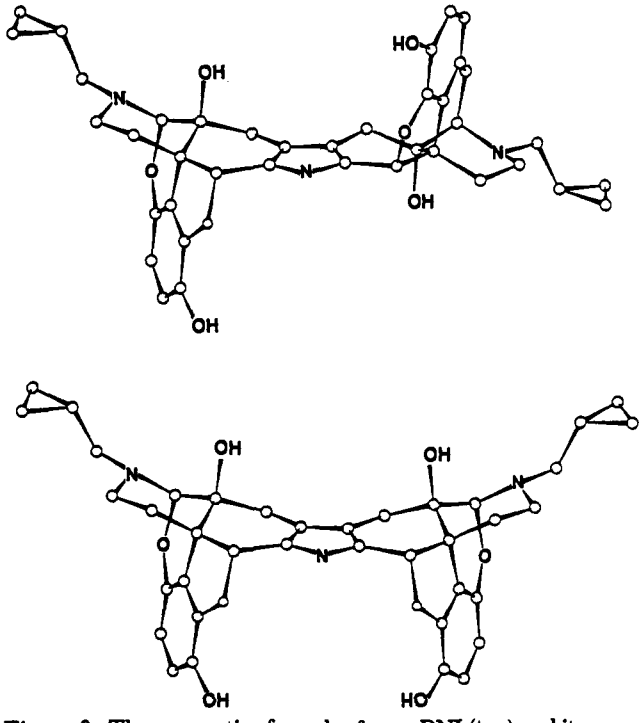
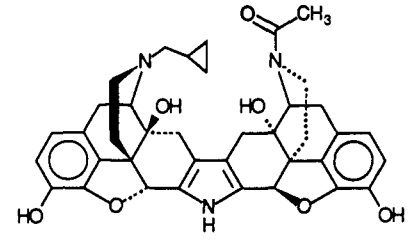


Figure 9. The perspective formulas for norBNI (top) and its meso isomer (bottom). Note that the basic nitrogens in the right halves of the molecules have similar orientations with respect to the antagonist pharmacophore to the left.

tyramine moiety of a single antagonist pharmacophore in norBNI, while the second subsite interacts with an element of the second pharmacophore. On the basis that the basic nitrogens of norBNI and meso-norBNI are in similar positions relative to one another (see Figure 9), we proposed that one of the basic groups in each of these ligands mimics the Arg[7] residue of dynorphin at the κ receptor recognition site. Our conclusion was based on a report[41] that Arg[7] is essential for κ opioid agonist activity. Moreover, the low potency and lack of selectivity of the monobasic norBNI analogue 17 is consistent with this model.[42]



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Since norBNI contains recognition elements that function as an opioid antagonist pharmacophore and as a κ receptor "discriminator" unit (second basic nitrogen) that enhances the affinity for κ sites, it is of interest to divide norBNI into functional elements. From this perspective, the rigid spacer that connects the tyramine moiety in the first half of norBNI with the basic nitrogen (κ receptor discriminator) in the second half consists of the C rings of each of the morphinans and one piperidine carbon. The most relevant segment of the dynorphin is depicted below

(41) Chavkin, C.; Goldstein, A. Specific Receptor for the Opioid Peptide Dynorphin: Structure–Activity Relationships. *Proc. Natl. Acad. Sci. U.S.A.* 1981, *78*, 6543–6547.

(42) Lin, C.-E.; Takemori, A. E.; Portoghese, P. S. The Analysis of the Second Pharmacophore Fragment of norBNI in Kappa Opioid Antagonist Potency and Selectivity; 4th Chemical Congress of North America, New York, August 25–30, 1991; MEDI 157.

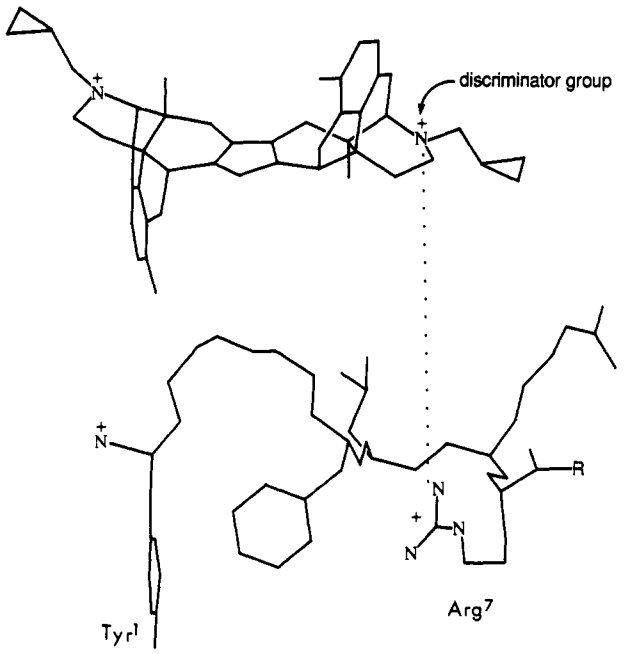
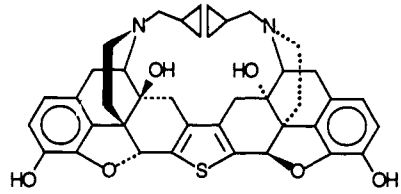


Figure 10. An illustration of the possible relationship between the basic nitrogen (κ receptor discriminator) of **15** (norBNI) and the guanidinium group of Arg^7 in dynorphin (arbitrary conformation).

norBNI in Figure 10 in order to illustrate the possible correspondence of the key basic moieties in each of these molecules. From the standpoint of recognition, the κ receptor discriminator of norBNI possibly mimics Arg^7 of dynorphin, perhaps through ion pairing with an anionic group at a unique κ receptor subsite.

In order to define the position of the discriminator subsite relative to the pharmacophore recognition site, the pyrrole moiety of norBNI was replaced with either thiophene **18** or pyran **19**.[43] The relative affinities for κ



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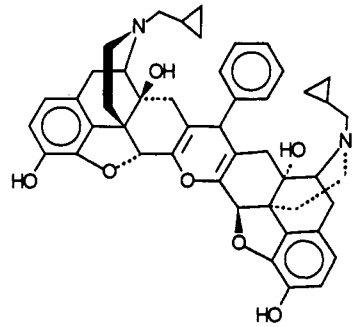

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sites are norBNI > **18** > **19**. Both **18** and **19** are κ-selective; the thiophene analogue, **18**, exhibits a binding selectivity profile that resembles that of norBNI, while the pyran analogue shows considerably lower κ selectivity. The differences in binding selectivities and in affinities may reflect the different orientations of the κ subsite discriminator due to the geometric constraint imposed by the spacers. Since the geometry of norBNI and its thiophene analogue, **18**, bear the closest resemblance, it is reasonable that they possess similar selectivity ratios. One interpretation of the data is that a hypothetical anionic group on a putative subsite of the κ recognition site may be bridged more easily when the heterocyclic portion if the spacer is pyrrole or an isosteric moiety.

The Message–Address Concept in the Design of Selective δ Opioid Receptor Antagonists

The "message–address" concept was proposed by Schwyzer who employed it to analyze the structure–activity relationship of ACTH and related peptide hormones.[44] Accordingly, peptide hormones in the "sychnologic" class contain a "message" sequence and an "address" sequence of amino acid residues, each being sequential and close to one another in the peptide chain. The message component is responsible for signal transduction, while the address provides additional binding affinity and is not essential for the transduction process. It is apparent that this concept bears a formal resemblance to the idea of discrete pharmacophore and discriminator moieties of norBNI in its relationship to dynorphin.

The endogenous opioid peptides appear to conform to this model in that they contain an N-terminal tetrapeptide sequence, Tyr-Gly-Gly-Phe, that is an important requirement for opioid activity. It has been proposed that this sequence carries the "message" responsible for mediating the opioid effect, and that the C-terminal segment of these peptides that differ in amino acid sequence functions as an "address" to bind to a unique subsite that is complementary to each type of opioid receptor.[41] It is important to point out that this conceptual model should not be viewed too literally, as it is possible that some portion of the message and address elements may be confluent with respect to function as illustrated schematically in Figure 11.

The message–address concept was evaluated with respect to opioid receptor selectivity by the attachment of "address" sequences to the μ-opioid agonist oxymorphone (Figure 12).[45] The "δ address", which was considered to be Phe-Leu, is found in the δ-selective opioid peptide leucine enkephalin; the "κ address" (Phe-Leu-Arg-Arg-Ile-OMe) constitutes a portion of the κ-selective peptide dynorphin.[41] Although the δ and κ binding selectivities resulting from these modifications are relatively low, they represented a dramatic change from that of the unsubstituted semicarbazone (A = NH_2, Figure 12) which is μ-selective. These results suggested that the Tyr^1 residue of the opioid peptides comprises the message component and the sequence starting with Phe^4 constitutes the address; in this context, Gly^2-Gly^3 serves as a spacer. This is consistent with the well-known structure–activity relationships of nonpeptide opioid ligands (e.g., morphine or oxymorphone) that contain only one aromatic ring which presumably mimics the Tyr^1 residue. The C ring of the morphinan structure and semicarbazone moiety in these hybrid molecules may serve as a mimic for the Gly^2-Gly^3 spacer. Alternately, it is conceivable that the Phe^4 residue

(43) Portoghese, P. S.; Garzon-Aburbeh, A.; Nagase, H.; Lin, C.-E.; Takemori, A. E. Role of Spacer in Conferring κ Opioid Receptor Selectivity to Bivalent Ligands Related to Norbinaltorphimine. *J. Med. Chem.* **1991**, *34*, 1292–1296.

(44) Schwyzer, R. ACTH: A Short Introductory Review. *Ann. N.Y. Acad. Sci.* **1977**, *247*, 3–26.

(45) Lipkowski, A. W.; Tam, S. W.; Portoghese, P. S. Peptides as Receptor Selectivity Modulators of Opiate Pharmacophores. *J. Med. Chem.* **1986**, *29*, 1222–1225.

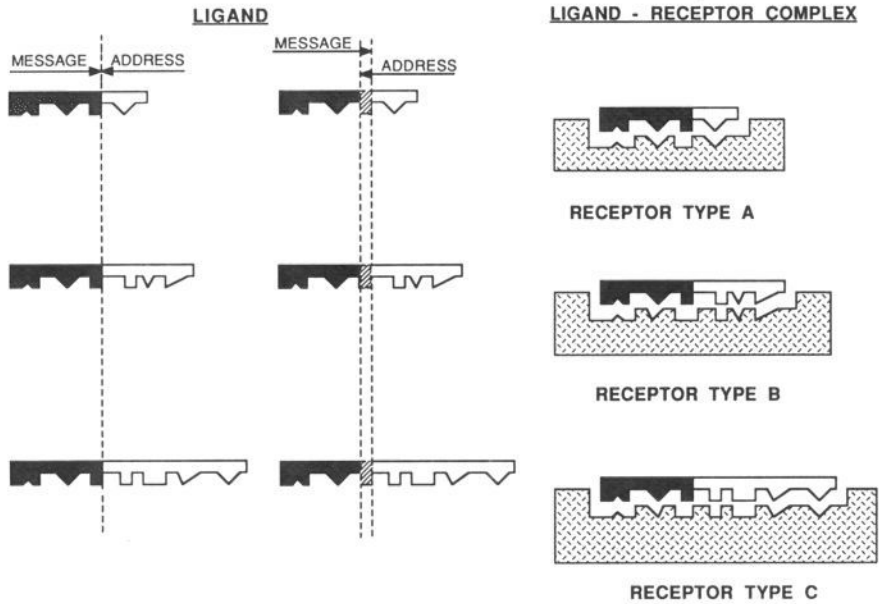
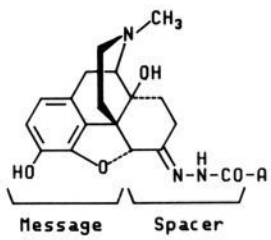


Figure 11. A cartoon of the message–address concept as applied to opioid peptides. The message and address elements of the ligands illustrated on left have completely separate functions, while those ligands to the right have partially overlapping functions.



A (address)	Selectivity
Phe-Leu-OH	delta
Phe-Leu-Arg-Arg-Ile-OMe	kappa

Figure 12. Opiate–peptide hybrids with an opioid agonist pharmacophore (message) and an address (peptide).

in the "address" component may serve both as a functional but nonessential part of the message and as an essential part of the address in these hybrid molecules. Also, it is conceivable that there may be overlap of these functions in the opioid peptides (see Figure 11).

Although the concept of a message and an address was proposed for peptide agonists, we believed it could serve as a useful model for the design of antagonists based on the premise that such ligands also interact with the same message and address subsites. Moreover, since the structure–activity relationship studies of norBNI-related structures and the opiate–peptide hybrids suggested that their selectivities are consistent with a message–address model, the design of nonpeptide δ opioid receptor antagonists was undertaken.

An important consideration in this design was the conformational restriction of the nonpeptide address moiety, as this would preclude possible conformational adaption in the binding to other opioid receptor types. In fact, the relatively low binding selectivity of endogenous opioid peptides[46] may be a consequence of such conformational

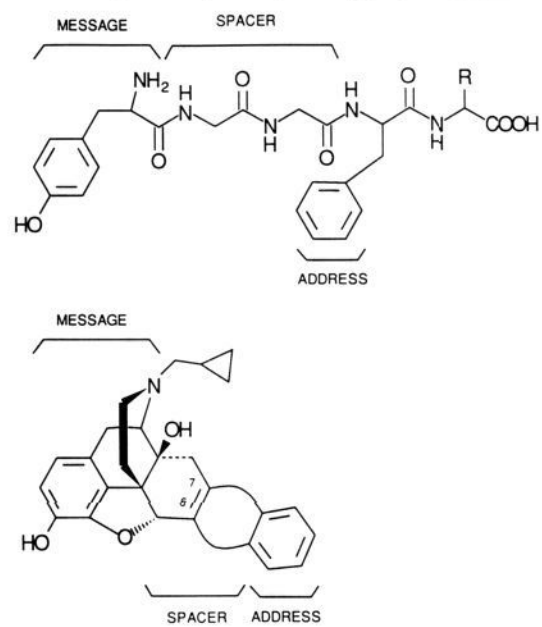


Figure 13. The relationship between message and address elements in enkephalin and an opiate as an approach to the design of non-peptide δ opioid antagonists.

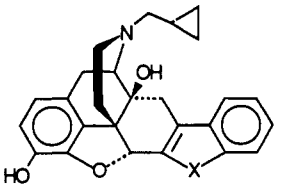
adaption due to their flexible nature.

The design strategy for nonpeptide, δ-selective antagonists employed the naltrexone pharmacophore for the message moiety and a key element in the leucine-enkephalin δ address.[32] The key element, which was hypothesized to be the phenyl group to Phe[4], was joined to the morphinan structure of naltrexone through a rigid spacer. The relationship of the functional components of the nonpeptide to leucine-enkephalin is illustrated in Figure 13.

The first target compound we synthesized contained a pyrrole spacer because it was easily accessible from naltrexone through the Fischer indole synthesis. This permitted quick access to the target compound in order to test the model. The initial member of this series, naltrindole (**20**) (NTI), is the first reported nonpeptide δ-selective opioid receptor antagonist.[47,48] The δ antagonist potency

(46) Hruby, V. J.; Gehrig, C. A. Recent Developments in the Design of Receptor Specific Opioid Peptides. *Med. Res. Rev.* **1989**, *9*, 343–401.

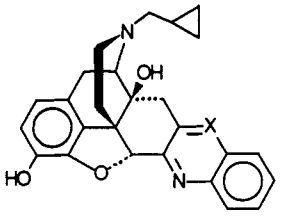
in vitro and its binding are about 3 orders of magnitude greater than the δ-selective enkephalin-derived antagonist (allyl)$_2$Tyr-Aib-Aib-Phe-Leu-OH[49] (ICI174864).



20, X = NH

21, X = O

Since the pyrrole moiety functions as a spacer, other heterocycles can play a similar role.[50] Replacement of pyrrole with furan afforded the benzofuran analogue **21** (NTB) which also is a potent, δ-selective antagonist. Analogues with quinoline or quinoxaline systems **22** are δ-selective, but substantially less potent and selective than NTI, possibly due to the fact that a 6-membered spacer orients the address mimic (benzene moiety) differently from a 5-membered ring.



22, X = CH or N

Our recent observation that NTB (**21**) differentially antagonizes the antinociceptive effect of δ opioid agonists has provided evidence for subtypes of δ receptors.[51] Cross-tolerance studies between the δ-selective peptides that were differentially antagonized were consistent with the existence of at least two types of δ opioid receptor sites.

Recent studies have demonstrated that NTI (**20**) and NTB (**21**) afford prolonged suppression of ethanol intake in P line rats (a line of rats that prefer ethanol).[52] This suppression is selective, as water intake is unaffected. These results indicate that enkephalinergic neurons are in some way involved in ethanol dependence, and they suggest a new approach for the treatment of ethanol addiction.

Also, we have observed that NTI suppresses morphine-induced tolerance and physical dependence in mice without affecting antinociception.[53] These data are of interest from the standpoint of preventing tolerance and physical dependence in patients who receive morphine on a chronic basis.

The results of both these studies suggest that there may be a common enkephalinergic pathway that modulates the reward centers in the brain. If this is the case, δ opioid antagonists may have potential clinical applications in the general treatment of substance abuse.

Summary and Conclusions

Various concepts have served as the basis for the development of models to explore the relationship between molecular structure and biological activity. In this presentation I have outlined five concepts that have been useful in our investigation of opioid receptor multiplicity and in the design of selective opioid receptor antagonists. The first of these, the multiple binding modality concept, led to our application of four other concepts in the development of opioid receptor probes. Some of these probes are now standard tools in opioid research. These include the μ-selective affinity label β-FNA (**10**), the κ opioid receptor antagonist norBNI (**15**), and the δ opioid antagonist NTI (**20**). These highly selective antagonists have advantages over the universal opioid antagonists naloxone and naltrexone because they are of value in probing the interaction of endogenous opioid peptides with opioid receptor types. Additionally, they are useful in evaluating the selectivity of new opioid agonists. Also, selective opioid antagonists have potential clinical applications in the treatment of a variety of disorders where endogenous opioids play a modulatory role. These include constipation, immune function, drug addiction, and alcoholism, to name only a few.

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(47) Portoghese, P. S.; Sultana, M.; Nagase, H.; Takemori, A. E. Application of the Message–Address Concept in the Design of Highly Potent and Selective Non-Peptide δ Opioid Receptor Antagonists. *J. Med. Chem.* **1988**, *31*, 281–282.

(48) Portoghese, P. S.; Sultana, M.; Takemori, A. E. Design of Peptidomimetic δ Opioid Antagonists Using the Message-Address Concept. *J. Med. Chem.* **1990**, *33*, 1714–1720.

(49) Cotton, R.; Giles, M. G.; Miller, L.; Shaw, J. S.; Timms, D. ICI174864: A Highly Selective Antagonist for Opioid δ-Receptors. *Eur. J. Pharmacol.* **1984**, *97*, 331–332.

(50) Portoghese, P. S.; Nagase, H.; MaloneyHuss, K. E.; Lin, C.-E.; Takemori, A. E. Role of Spacer and Address Component in Peptidomimetic δ Opioid Receptor Antagonists Related to Naltrindole. *J. Med. Chem.* **1991**, *34*, 1715–1720.

(51) Sofuoglu, M.; Portoghese, P. S.; Takemori, A. E. Differential Antagonism of Delta Opioid Agonists by Naltrindole (NTI) and its Benzofuran Analog (NTB) in Mice: Evidence for Delta Opioid Receptor Subtypes. *J. Pharmacol. Exp. Ther.* **1991**, *257*, 676–680.

(52) Froehlich, J. C.; Zweifel, M.; Li, T. K.; Portoghese, P. S. Delta Opioid Antagonists Produce Prolonged Suppression of Ethanol Intake. Society of Alcoholism Conference, St. Marcos Island, FL, June 8–13, 1991; Abstract 20.

(53) Abdelhamid, E. E.; Sultana, M.; Portoghese, P. S.; Takemori, A. E. Selective Blockage of Delta Opioid Receptors Prevents the Development of Morphine Tolerance and Dependence in Mice. *J. Pharmacol. Exp. Ther.* **1991**, *258*, 299–303.