De Novo Design, Synthesis, and Biological Activities of High-Affinity and Selective Non-Peptide Agonists of the δ-Opioid Receptor[||]

Subo Liao,[†,§] Josue Alfaro-Lopez,[†] Mark D. Shenderovich,[†,▽] Keiko Hosohata,[‡] Jun Lin,[†] Xiaoping Li,[‡] Dagmar Stropova,[‡] Peg Davis,[‡] Kevin A. Jernigan,[‡] Frank Porreca,[‡] Henry I. Yamamura,[‡] and Victor J. Hruby*[,†]

Departments of Chemistry and Pharmacology, The University of Arizona, Tucson, Arizona 85721

Received June 19, 1998

On the basis of the structure−activity relationships of δ-opioid-selective peptide ligands and on a model of the proposed bioactive conformation for a potent and selective, conformationally constrained δ-opioid peptide ligand [(2*S*,3*R*)-TMT[1]]DPDPE, a series of small organic peptide mimetic compounds targeted for the δ-opioid receptor have been designed, synthesized, and evaluated in radiolabeled ligand binding assays and in vitro bioassays. The new non-peptide ligands use piperazine as a template to present the most important pharmacophore groups, including phenol and phenyl groups and a hydrophobic moiety. This hydrophobic group was designed to mimic the hydrophobic character of the D-Pen residues in DPDPE, which has been found to be extremely important for increasing the binding affinity and selectivity of these non-peptide ligands for the δ-opioid receptor over the μ-opioid receptor. Compound **6f** (SL-3111) showed 8 nM binding affinity and over 2000-fold selectivity for the δ-opioid receptor over the μ-opioid receptor. Both enantiomers of SL-3111 were separated, and the (−)-isomer was shown to be the compound with the highest affinity for the δ-opioid receptor found in our study (IC_{50} = 4.1 nM), with a selectivity very similar to that observed for the racemic compound. The phenol hydroxyl group of SL-3111 turned out to be essential to maintain high affinity for the δ-opioid receptor, which also was observed in the case of the δ-opioid-selective peptide ligand DPDPE. Binding studies of SL-3111 and [*p*-ClPhe[4]]DPDPE on the cloned wild-type and mutated human δ-opioid receptors suggested that the new non-peptide ligand has a binding profile similar to that of DPDPE but different from that of (+)-4-[((α*R*)-α(2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide (SNC-80), another δ-opioid-selective non-peptide ligand.

Introduction

A major but difficult goal of peptidomimetic design is the translation of three-dimensional (3D) pharmacophore information from a potent bioactive peptide to a small organic non-peptide ligand with the same agonist or antagonist biological properties.[1] Some classes of non-peptide ligands may have advantages as drug candidates, such as high oral bioavailability and higher permeability across the blood−brain barrier. To successfully bridge the gap from peptide to non-peptide structures, a detailed development of a 3D peptide pharmacophore model based on the design, synthesis, and comprehensive evaluation of conformational (φ,ψ) and topographical (chi[χ]) space of a peptide ligand is required. On the basis of this information, the geometrical relationships of key phamacophore elements may become more clear.[2] In the field of opioid research, selective peptide agonists for the δ-opioid receptor have shown promising therapeutic potential as analgesics without the adverse side effects associated with morphine and other opioid drugs selective for the μ-opioid receptor.[3] Detailed structure−activity relationship (SAR) studies of opioid peptides have provided much information about the chemical, conformational, dynamic, and structural requirements for the selective agonist recognition of the δ-opioid receptors.[4] However, only a few successful attempts have been reported in the development of the δ-opioid agonist ligands of a non-peptidic nature. Several examples of non-peptide ligands have been discovered either by modification of morphine-type alkaloids or by random screening approaches,[5−7] but most of them are associated, as potential drug candidates, with various problems such as poor selectivity[6] and low efficacy in vivo[7] and display binding profiles different from those of typical δ-opioid-selective peptide agonists.[8] Therefore, development of δ-opioid receptor-selective non-peptide agonists, based on peptide structure−activity relationships, would have an important impact in understanding relationships between peptide and non-peptide ligand−receptor recognition, and signal transduction requirements, for rational drug design and for discovery of novel therapeutics.

[||] Abbreviations used for amino acids follow the rules of the IUPAC−IUB Joint Commission of Biochemical Nomenclature (*Biochem. J.* **1984**, *219*, 345−373). Other abbreviations: TMT, β-methyl-2′,6′-dimethyltyrosine; D-Pen, D-β,β-dimethylcysteine; DPDPE, c[D-Pen[2],D-Pen[5]]enkephalin; Pen, penicillamine; Aib, α-aminoisobutyric acid; ICI-174,864, $(allyl)_2$-Tyr-Aib-Aib-Phe-Leu-OH; SIOM, 7-spiroindanyloxymorphone; Boc, *tert*-butyloxycarbonyl; *p*-MeBzl, *p*-methylbenzyl; FAB-MS, fast atom bombardment mass spectrometry; TLC, thin-layer chromatography; *p*-MBHA, *p*-methylbenzhydrylamine; *m*-NBA, *m*-nitrobenzyl alcohol; DCM, dichloromethane; DIEA, diisopropylethylamine.

* To whom correspondence should be addressed.
[†] Department of Chemistry.
[‡] Department of Pharmacology.
[§] Current address: Monsanto Co., 800 N. Lindberg Blvd., St. Louis, MO 63167.
[▽] Current address: Structural Bioinformatics, Inc., 10929 Technology Place, San Diego, CA, 92127.

10.1021/jm980374r CCC: $15.00
Published on Web 11/04/1998

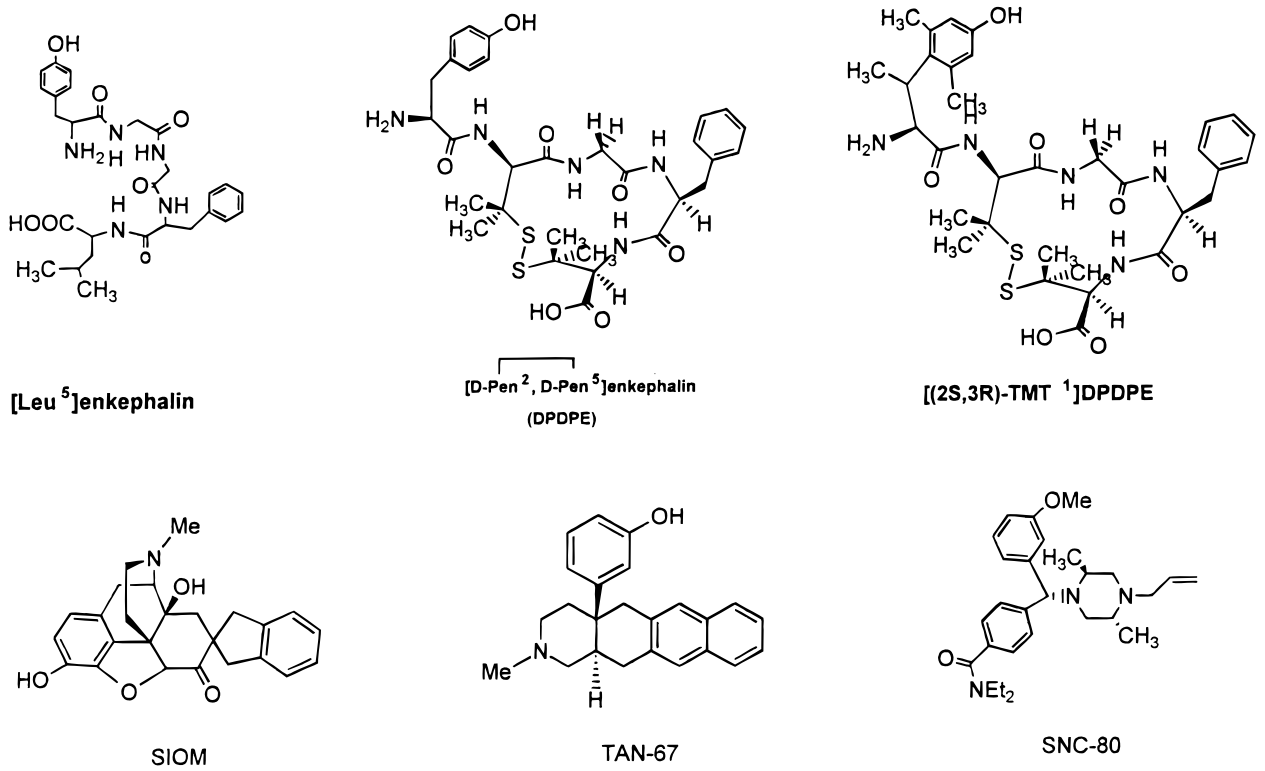


Figure 1. Structures of some typical δ-opioid-selective peptide and non-peptide ligands.

As part of our efforts in opioid ligand design, we are systematically examining approaches to cross the gap between peptide and non-peptide compounds selective for the δ-opioid receptor.[9] Recently, we have developed the conformationally and topographically constrained highly potent δ-opioid-selective peptide [(2*S*,3*R*)-TMT[1]]-DPDPE.[10] On the basis of the model of the bioactive conformation proposed for this peptide lead,[11] we have designed and synthesized a series of non-peptide ligands using the piperazine ring as a template. The lead compound in this first generation of peptidomimetics (SL-3111) shows high binding affinity, exceptionally high selectivity for δ-opioid receptors, and a binding profile similar to that of the lead peptide agonist [(2*S*,3*R*)-TMT[1]]DPDPE. In this paper, we report the design, synthesis, and pharmacological evaluation of these novel δ-opioid-selective non-peptide agonists.

Results and Discussion

Design. Detailed structure−activity relationship studies on endogenous δ-opioid-selective linear peptide ligands, enkephalins H-Tyr-Gly-Gly-Phe-Met(Leu)-OH (Figure 1), have demonstrated that the phenol ring and the amino group in the tyrosine residue and the phenyl ring in the phenylalanine residue are the essential pharmacophores for peptide ligands to recognize δ-opioid receptors and produce physiological effects.[4] The substitution of both Gly[2] and Met[5] (or Leu[5]) residues in enkephalin with D-Pen (β,β-dimethylcysteine), to form a disulfide bridge, produced the potent agonist c[D-Pen[2],D-Pen[5]]enkephalin (DPDPE) which shows nanomolar binding affinity at the δ-opioid receptor and over 350-fold selectivity for the δ- over μ-opioid receptor.[12] In addition, DPDPE has no inhibitory effect on gut motility, is stable to proteolytic degradation in blood, brain, and gut, and is also able to penetrate through the blood−brain barrier.[13] Most importantly, it was found to produce a potent, efficacious, and prolonged analgesia in several in vivo tests and to have low addiction potential, making it an alternative candidate for the treatment of pain without the toxicity and side effects of current drugs. Comprehensive 2D NMR,[4b] computational studies,[14] and a recently determined X-ray crystal structure of DPDPE[15] have led to the conclusion that the 14-membered ring maintains a very similar conformation in solution and in the crystal form. However, these studies also showed that the side-chain groups of the important Phe[4] and Tyr[1] phamacophores were highly flexible. Therefore, further constraints were applied to the side chains of these residues in DPDPE, using novel topographically constrained amino acids. In one of these studies, the substitution of Phe[4] with the four isomers of β-methylphenylalanine, followed by NMR and molecular modeling studies of these derivatives, led to the conclusion that the gauche (−) conformation was required for interaction of the Phe[4] side chain with the δ-opioid receptor.[16] Further substitution of Tyr[1] in DPDPE with the four isomers of β-methyl-2′,6′-dimethyltyrosine (TMT), along with detailed computational and NMR studies, revealed that the tyrosine derivative in the most potent and selective analogue, [(2*S*,3*R*)-TMT[1]]DPDPE, preferred a trans side-chain conformation.[10] Molecular modeling studies of [(2*S*,3*R*)-TMT[1]]DPDPE and two potent non-peptide δ-opioid receptor agonists, such as SIOM[6b] and 2-methyl-4aα-(3-hydroxyphenyl)-1,2,3,4,4a,5,12,-12aα-octahydroquinolino[2,3,3-*g*]isoquinoline (TAN-67),[7] resulted in a 3D pharmacophore model for this receptor. From this model a characteristic distance of 7.0 ± 1.5 Å between the phenol and phenyl rings was observed.[11] Computer-assisted template search indicated that two cis 1,4-disubstituted benzyl-like aromatic rings can be

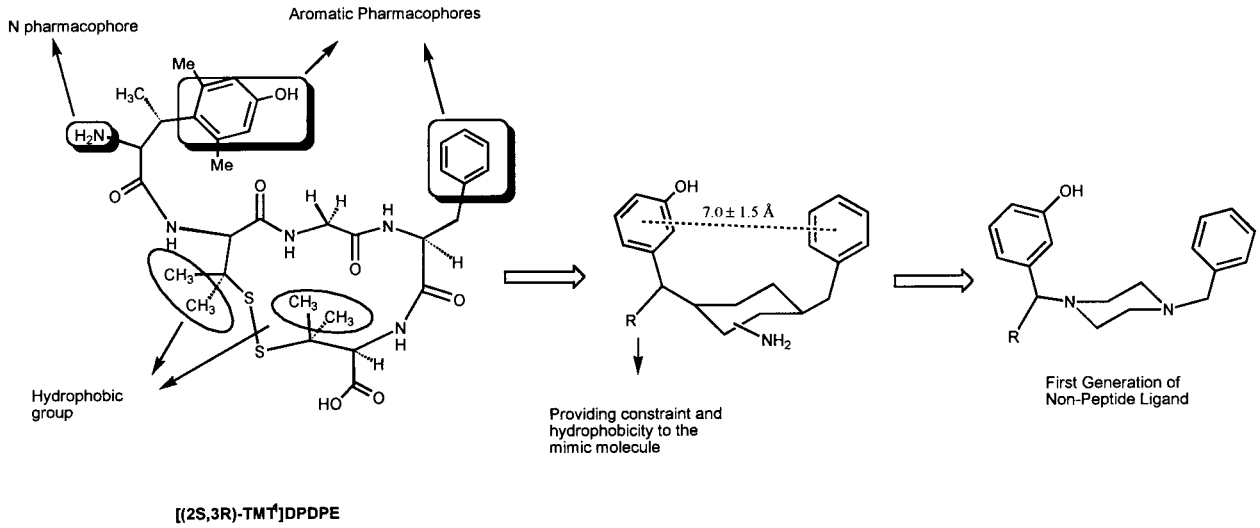
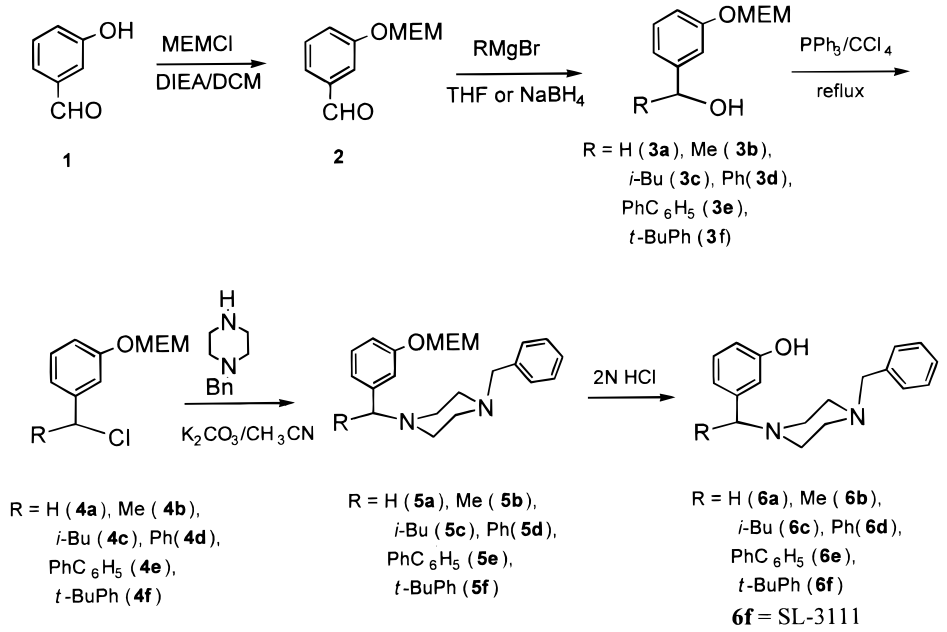


Figure 2. Design of the first generation of non-peptide mimetic ligands from the δ-opioid-selective peptide lead [(2*S*,3*R*)-TMT[1]]-DPDPE.

Scheme 1. Synthetic Route for the Preparation of Non-Peptide Mimetics **6a**−**f**



presented at such distances on a cyclohexane-like scaffold and that the hydroxy group of the phenol ring should be in a *meta* position to overlap with the corresponding pharmacophore in the bioactive conformation of [(2*S*,3*R*)-TMT[1]]DPDPE.

Additionally, an amino group may be introduced on the cyclohexane scaffold to overlap the corresponding amino group of the peptide ligand as shown in Figure 2.[11] It also has been shown that the increased hydrophobicity of the tyrosine derivatives and the β-dimethyl groups in both D-Pen residues may contribute to the enhanced selectivity and binding affinity of the peptide ligand.[11,17,18] Thus, we introduced additional substituents R, of variable size and hydrophobicity, attached to the benzyl carbon of the phenol side-chain group as shown in Figure 2, to test how this would affect the selectivity and binding affinity of these designed peptidomimetics. To facilitate the synthetic process, we used 1,4-piperazine as a 6-membered ring scaffold for the first generation of peptidomimetics.

In this first series of non-peptide ligands, a primary amino group was omitted to simplify the synthetic work. Furthermore, the nitrogen in the piperazine ring has not been ideally positioned in 3D space to mimic the terminal amino group in the peptide lead analogue (Figure 2). Thus, we postulated that with three appropriately oriented binding pharmacophore groups, high binding affinity could still be expected according to the theory proposed by Farmer.[19] Therefore, the first generation of peptidomimetic compounds was intentionally designed to explore the functional roles of the two aromatic rings and the hydrophobic group R in the ligand−receptor interaction.

Chemistry. The synthetic route for the preparation of the non-peptide ligands **6a**−**f** is shown in Scheme 1. The hydroxyl group of **1** was protected as the methoxyethoxymethyl ether (MEM).[20] Except for alcohol **3a** (R = H) which was prepared through direct reduction of aldehyde **2** with sodium borohydride, all other alcohols **3b**−**f** were synthesized by reaction of the aldehyde **2**

Table 1. Binding Affinity of δ-Selective Opioid Peptide Ligands

compound	binding data, IC_{50} (nM) ± SEM		selectivity (μ/δ)
	[^{3}H]DAMGO (μ)	[^{3}H]*p*-Cl-DPDPE (δ)	
[(2*S*,3*R*)TMT[1]]DPDPE[3]	4300 ± 820	5.0 ± 0.1	860
6a (R = H)	8100 ± 790	6400 ± 3200	1.3
6b (R = Me)	780 ± 72	610 ± 310	1.3
6c (R = *i*-Bu)	2100 ± 600	420 ± 38	5.0
6d (R = Ph)	500 ± 52	34 ± 17	15
6e (R = C_6H_5Ph)	~27000	31 ± 4.9	~870
6f (R = *t*-BuPh) (SL-3111)	17000 ± 3000	8.4 ± 1.6	2020
(+)-SL-3111	11000 ± 1950	42 ± 3.0	260
(−)-SL-3111	7700 ± 350	4.1 ± 1.0	1900

with the corresponding Grignard reagents. Alcohols **3a**−**f** were converted into the chlorides **4** under neutral conditions in the presence of triphenylphosphine.[21] Compounds **4a**−**f** were reacted with 1-benzylpiperazine in the presence of potassium carbonate under overnight reflux to produce compounds **5a**−**f**. The hydroxyl protecting group (MEM) was then removed with 2 N HCl in a 1:1 methanol/dioxane mixture, by overnight reaction, to give the desired final products **6a**−**f** as white solid hydrochloride salts.

Structure−Activity Relationships. All the synthesized non-peptide ligands were evaluated using standard binding assays against radiolabeled δ- and μ-receptor-selective opioid ligands. The binding assay results are given in Table 1. As expected, different trends related to the size and hydrophobicity of the R groups present in these non-peptide mimetics were observed for the μ- and δ-opioid receptor binding affinities. In the case of the μ-opioid receptor an especially weak binding affinity was observed for compounds without a substituent **6a** (R = H) or with a bulky substituent (R = *i*-Bu, *p*-C_6H_5Ph, *t*-BuPh, **6c**,**e**,**f**). On the other hand, a substituent of intermediate size (methyl group **6b** or a planar phenyl group **6d**) produced compounds with higher binding affinities. These results suggest that a hydrophobic but less bulky R group may present a better fit to the lipophilic pocket of the μ-opioid receptor. For the δ-opioid receptor, it was observed that an increase in binding affinity and selectivity was proportional to the hydrophobicity and size of the substituent R in compounds **6a**−**f** (Table 1). However, for compound **6d** (R = phenyl) the substitution with a more bulky and hydrophobic R group (R = C_6H_5Ph) to produce **6e** did not improve the binding affinity of the non-peptide ligand for the δ-opioid receptor but significantly reduced the binding affinity for the μ-opioid receptor as described above. Substitution of the *p*-phenyl group in **6e** with a bulkier *p*-*tert*-butyl group led to the analogue **6f** (SL-3111), which showed a 4-fold increase on binding affinity for the δ-opioid receptor (IC_{50} = 8.4 nM) relative to **6e** and is comparable to [(2*S*,3*R*)-TMT[1]]DPDPE.[10] In addition, **6f** showed very weak affinity for the μ-receptor. This makes 1-(4-*tert*-butyl-3′-hydroxybenzhydryl)-4-benzylpiperazine (**6f**, SL-3111) one of the most selective non-peptide mimetic ligands reported so far for binding to the δ-opioid receptor in preference to the μ-opioid receptor with a 2000-fold μ/δ selectivity. Indeed, this exceeds the selectivity of the peptide lead [(2*S*,3*R*)-TMT[1]]DPDPE.

Apparently, as was postulated in the design, a bulky hydrophobic R group in SL-3111 may mimic an important lipophilic binding site in the δ-opioid peptide ligands, just as the β-dimethyl groups of the D-Pen residues in DPDPE are essential for selective binding to the δ-receptor. The two optically pure enantiomers of SL-3111 were synthesized and tested in the radiolabeled ligand binding assays (Table 1). The enantiomer (−)-SL-3111 has a very potent binding affinity for the δ-opioid receptor (IC_{50} = 4.1 nM), which is about 10-fold more potent than (+)-SL-3111, with essentially the same selectivity for the δ-opioid receptor (1900-fold) as the racemic SL-3111 (2000-fold), due to the increased binding affinity for the μ-opioid receptor. It is obvious that the chirality at this center has an important effect on the opioid ligand−receptor interactions.

Binding to the Wild-Type (Wt) and the Mutated (W284L) Human δ-Opioid Receptors. To determine if the designed non-peptide ligand SL-3111 indeed mimics the binding characteristics of the peptide ligand toward the δ-receptor, the binding profiles of SL-3111, [*p*-ClPhe[4]]DPDPE, and the non-peptide ligand SNC-80[5] were examined with the cloned human δ-opioid receptor (wild-type, Wt) and the mutated human δ-opioid receptor W248L.[22] As shown in Figure 3, the binding curves of [*p*-ClPhe[4]]DPDPE with Wt and W248L receptors were essentially identical, showing IC_{50} values of 1.53 and 1.55 nM for Wt and W248L receptors, respectively (Table 2). The binding curves of SL-3111 with the Wt and W248L receptors had only a slight difference, showing IC_{50} values of 5.5 and 16.8 nM with Wt and W248L, respectively; the 3-fold difference was not found to be significant. However, the binding curves of SNC-80 with these receptors showed important differences, with IC_{50} values of 2.8 nM at the Wt δ-opioid receptor and 49.1 nM at the W248L δ-opioid receptor. These results are consistent with the recent pharmacological studies on the binding of δ-opioid-selective peptide and non-peptide ligands to the wild-type and W284L mutant human δ-opioid receptors.[22]

The W284L mutation led to a receptor with profoundly reduced affinity to the non-peptide opioid ligand SNC-121, but one which did not alter binding affinities of typical δ-opioid peptide agonists such as [*p*-ClPhe[4]]-DPDPE and deltorphin I. The high degree of ligand specificity shown by the W284L mutation supports a conclusion that the amino acid in this position of the receptor is involved in a direct interaction with the SNC series of compounds that does not occur for peptide and peptidomimetic δ-opioid-selective ligands. Although chemically related to SNC-80, SL-3111 has a binding profile more closely related to that of the classical peptide [*p*-ClPhe[4]]DPDPE than SNC-80 discovered from screening methods.

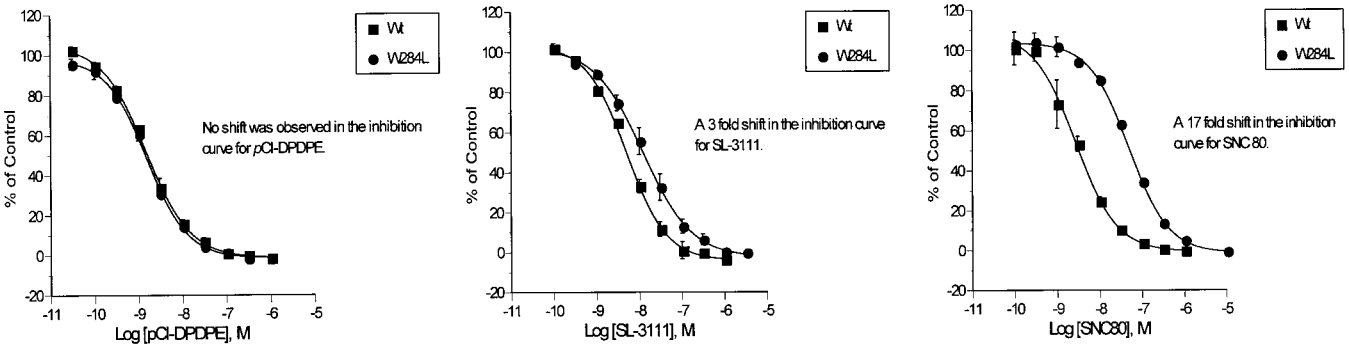


Figure 3. Inhibition of specific [^{3}H]*p*-Cl-DPDPE binding by selective opioid agonists: *p*-Cl-DPDPE, SL-3111, and SNC-80. The membrane cells were transfected with the cDNAs encoding the Wt hDOR (■) and W248L mutant hMOR (●). The specific [^{3}H]-*p*-Cl-DPDPE binding to membrane preparations was inhibited by increasing concentration of *p*-Cl-DPDPE, SL-3111, and SNC-80.

Table 2. Effect of the Mutation of Cloned Human δ-Opioid Receptor on the Binding Affinity (IC_{50}, nM) of δ-Selective Opioid Ligands[a]

ligand	Wt	W284L	W284L/Wt
p-Cl-DPDPE	1.53 ± 0.26	1.55 ± 0.11	1.0
SL-3111	5.51 ± 0.94	16.8 ± 6.13	3.1
SNC-80	2.85 ± 0.26	49.1 ± 4.33	17.2

[a] Wt, cloned human δ-opioid receptor; W284L, mutated human δ-opioid receptor in which the Trp residue at position 284 was mutated to Leu.

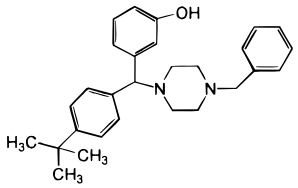
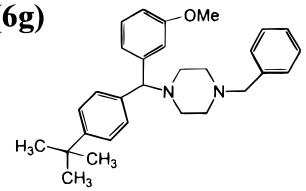
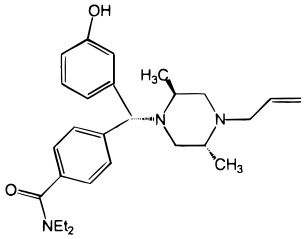
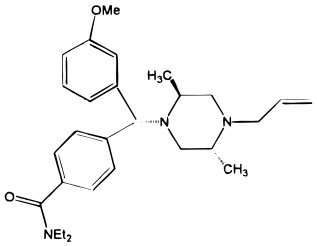
Effects of the Methylation of the Phenol Group on the Binding Affinity of δ-Opioid-Selective Peptide and Non-Peptide Ligands. Although the hydroxy functional group of the tyrosine residue in enkephalins has been identified to be a crucial pharmacophore for binding to the δ-opioid receptor, its importance in DPDPE itself has not been examined. Since methylation of (+)-4-[((α*R*)-α(2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-hydroxybenzyl]-*N*,*N*-diethylbenzamide [(+)-BW373U86] produces an extremely selective δ-opioid analogue (SNC-80), with over 2000-fold selectivity for the δ-opioid receptor while maintaining nearly the same binding affinity as (+)-BW373U86,[5] the hydroxyl group has not been thought to be an essential pharmacophore for these δ-opioid-selective ligands. Thus, to identify the importance of the hydroxyl groups in traditional δ-opioid-selective peptide ligands, a new peptide analogue **(**[*p*-MeOTyr^1]DPDPE) was synthesized. The bioassay results of this peptide analogue were compared with those from the O-methylated analogue of SL-3111 (**6g**). As shown in Table 3, methylation of the hydroxyl group of the Tyr^1 residue has a considerable effect on the binding affinity of DPDPE, causing a 150-fold drop in the binding affinity of [*p*-MeOTyr^1]DPDPE for the δ-opioid receptor. The same effect was observed for **6g**. These results clearly demonstrate that the hydroxyl group in Tyr^1 residue of the potent δ-opioid-selective peptide DPDPE and in the phenol group of SL-3111 are crucial binding pharmacophores, which probably interact with the same binding sites of the δ-opioid receptor. However, this is not the case for the SNC series of non-peptide ligands for which methylation did not affect the binding affinity to the δ-opioid receptor as discussed above. These results suggest once again that the binding profile of SL-3111 is more closely related to that of the peptide ligand DPDPE than the SNC series of non-peptide ligands, consistent with our design proposal.

In Vitro Bioassays. Compounds **6d**,**f** (SL-3111) show nanomolar binding affinities for the δ-opioid receptor in the rat brain and consequently were evaluated in the isolated mouse vas deferens (MVD) and guinea pig ileum (GPI) bioassays (Table 4). Compared to the binding affinity, **6d** shows a 5- and 2.5-fold decrease of potency in the MVD (δ) and GPI (μ) assays, respectively. On the other hand, compound **6f** (SL-3111) shows a 10-fold decrease of potency in the MVD (δ) assay and a 2-fold decrease in the GPI (μ) assay, still maintaining a high (460-fold) selectivity. The relatively low biological potency may be related to the possible improper position of the nitrogen atoms in these non-peptide analogues, which are unable to interact with the same site at the δ-receptor as the terminal amino group of the peptide lead does. In these assays, SL-3111 was used as the racemic mixture. Therefore, we sought to evaluate the biological activities of the enantiomers. However, when the optically pure isomers were tested, a 2.4- and 4.2-fold decrease in potency in MVD (δ) assay was observed for the (+)- and (−)-SL-3111 enantiomer, respectively (Table 4). As shown in Table 4, the peptide lead [(2*S*,3*R*)-TMT^1]DPDPE possesses a high potency in the δ-receptor bioassay and antagonism for the μ-receptor,[10] which makes it highly selective for the δ-opioid receptor. The difference in the binding affinity and biological potency between [(2*S*,3*R*)-TMT^1]DPDPE and the non-peptide mimetic SL-3111 raises interesting questions about the relationships between binding affinity and biological efficacy,[23] on the chemical−structural basis for binding affinity, and if additional features are required for the expected biological response. Understanding these fundamental issues are necessary for successful rational drug design. Thus, we believe that SL-3111 and the related peptidomimetic ligands presented in this paper show potential use as tools to explore the stereochemical requirements for δ-opioid-selective non-peptide ligands, providing new leads to develop novel δ-selective non-peptide mimetics.

Conclusions

A new δ-opioid-selective non-peptide agonist lead (SL-3111) has been discovered based on the structure−activity relationships of a δ-opioid peptide agonist ligand. This non-peptide ligand showed a binding affinity of 8 nM and over 2000-fold selectivity for the δ-opioid receptor over the μ-opioid receptor. The chiral

Table 3. Effect of Methoxylation of the Phenol Groups on the Binding Affinities (IC_{50}, nM) of δ-Selective Opioid Ligands

ligand	[^{3}H]DAMGO (μ)	[^{3}H]*p*-Cl-DPDPE (δ)	selectivity (μ/δ)
DPDPE[a]	609 ± 70	1.6 ± 0.2	380
[MeOTyr^1]DPDPE	11000 ± 2700	230 ± 24	48
(6f)  **SL-3111**	17000 ± 3000	8.4 ± 1.6	2020
(6g) 	>8000	1800	>4
 **(+)-BW373U86**[b]	9.7 ± 0.37	0.31 ± 0.02	31
 **SNC-80**[b]	2500 ± 200	1.06 ± 0.14	2300

[a] See refs 16, 17, and 27. [b] See ref 5.

Table 4. Biological Potencies of the Non-Peptide Mimetics

compound	bioassay data, EC_{50} (nM) ± SEM		selectivity (μ/δ)
	GPI (μ)	MVD (δ)	
[(2*S*,3*R*)TMT^1]DPDPE[c]	0% at 60 μM (antagonist, IC_{50} = 5 μM)	1.8 ± 0.3	>33000
6d	1250 ± 240	174 ± 15.5	7.1
		CTAP[a] insensitive, ICI-174,864[b] sensitive	
SL-3111 (**6f**)	39000 ± 2600	85 ± 10	460
		CTAP[a] insensitive, ICI-174,864[b] sensitive	
(+)-SL-3111	3000 ± 1500	210 ± 50	18.5
(−)-SL-3111	7600 ± 6500	360 ± 57	21.0

[a] A peptide μ-opioid receptor antagonist. [b] A δ-selective enkephalin-derived peptide antagonist. **6d** was shifted 1.3-fold by 1 μM CTAP and 6.0-fold by 1 μM ICI-174,864. The dose−response curve of SL-3111 (**6f**) was shifted to the right 2.6-fold by 1 μM CTAP and 21.8-fold by 1 μM ICI-174,864. [c] See ref 10.

HPLC resolution of the racemic SL-3111 provided the (+)- and (−)-enantiomers which were 5-fold less potent and 2-fold more potent, respectively, in our binding assay. It was observed that the hydroxyl group of the phenol pharmacophore in SL-3111 was essential to maintain high potency for the δ-opioid receptor, as was also found for the potent δ-opioid-selective peptide ligand DPDPE. Further binding studies of SL-3111 and the δ-opioid-selective peptide ligand [*p*-ClPhe^4]DPDPE on the cloned wild-type human and mutated δ-opioid receptors suggested that both present similar binding profiles which differ from those of another δ-opioid-selective non-peptide ligand (SNC-80). This makes SL-3111 a true peptidomimetic which provides a new lead structure for further design of new generations of δ-opioid non-peptide ligands.

Experimental Section

Preparation of Non-Peptide Ligands. All reagents, unless otherwise noted, were purchased from Aldrich Chemical

Co. and were used without further purification. All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded with a Bruker AM 250 spectrometer, using tetramethylsilane (TMS) or D_2O (4.66 ppm downfield from TMS) as an internal standard. Optical rotations were measured on an Autopol III polarimeter using a 1.0-dm cell. Flash column chromatography was performed using E. Merck silica gel (230 mesh). Purification of the peptide was achieved by preparative reverse-phase high-performance liquid chromatography (HPLC) on a Perkin-Elmer instrument model 410-Bio, C18-bonded silica column (VYDAC 218TP1010, 12 × 275 mm), eluting with a linear gradient of acetonitrile in 0.1% aqueous TFA (10−90%) over 30 min at a flow rate of 5 mL/min. High-resolution mass spectra (HRMS) were obtained at the University of Arizona Chemistry Department Mass Spectroscopy Facility, with a JEOL mass spectrometer model HX-110. All analytical (C, H, N) and spectroscopic (^{1}H NMR, ^{13}C NMR, MS) data are in agreement with the proposed structures.

3-(2-Methoxyethoxymethyl)benzaldehyde (2). Into a stirred solution of 3-hydroxybenzaldehyde (3.7 g, 30 mmol) in 60 mL of DCM, was added DIEA (7.6 mL, 43.7 mmol). To the resulting brown solution was added MEMCl (5.0 mL, 43.7 mmol) in 17 mL of DCM dropwise. A white fog was observed in the addition process. The resulting solution was stirred at room temperature for 3 h and then quenched by addition of 60 mL of 0.5 N HCl. The organic phase was separated and the aqueous phase extracted with DCM (3 × 60 mL). The combined organic phases were washed with 5% Na_2CO_3 (3 × 60 mL) and water (3 × 60 mL) and then dried over anhydrous $MgSO_4$. Evaporation of the dried solution yielded 5.4 g of an orange oil (86%): ^{1}H NMR ($CDCl_3$) δ 9.90 (s, 1H), 7.57−7.45 (m, 4H), 5.33 (s, 2H), 3.86−3.82 (m, 2H), 3.58−3.54 (m, 2H), 3.38 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 191.8, 157.7, 137.7, 130.0, 123.5, 122.6 93.3, 71.4, 67.7, 58.9; HRMS-FAB calcd for $C_{11}H_{15}O_4$ $[M + H]^+ = 211.0970$, found 211.0973.

3-(2-Methoxyethoxymethyl)benzylic Alcohol (3a). Into a stirred solution of **2** (1 g, 8.2 mmol) in 30 mL of ethanol was added sodium borohydride powder until the bubbling stopped. Then the reaction was quenched with 15 mL of 2 N HCl. The product was extracted with ether (3 × 30 mL). The combined organic extracts were washed with $NaHCO_3$ (40 mL) and brine (40 mL) and dried over anhydrous $MgSO_4$. Evaporation yielded a light-yellow oil, which was purified by flash chromatography (EtOAc/hexane, 3:7), to give a colorless oil (95%): ^{1}H NMR ($CDCl_3$) δ 7.30−6.95 (m, 4H), 5.27 (s, 2H), 4.65 (s, 2H), 3.84−3.80 (m, 2H), 3.57−3.54 (m, 2H), 3.37 (s, 3H), 1.94 (bs, 1H); ^{13}C NMR ($CDCl_3$) δ 157.4, 142.8, 129.6, 120.3, 115.4 114.7, 93.3, 71.5, 67.6, 65.1, 59.0.

Synthesis of 1-(3-Hydroxybenzyl)-4-benzylpiperazine (6a) from 3a. Preparation of 3-(2-Methoxyethoxymethyl)benzyl Chloride (4a). A mixture of **3a** (2.38 mmol) and triphenylphosphine (3.43 mmol) in 6.0 mL of carbon tetrachloride was refluxed under Ar for 3 h. A white precipitate was formed. After the mixture cooled to room temperature, 10 mL of anhydrous ether was added to the mixture. The solid was filtered off and washed with ether (3 × 10 mL). The combined filtrate was evaporated to dryness and purified by flash chromatography (AtOAc/hexane, 3:7): colorless oil (20%); ^{1}H NMR ($CDCl_3$) δ 7.30−6.99 (m, 4H), 5.27 (s, 2H), 4.55 (s, 2H), 3.85−3.81 (m, 2H), 3.58−3.54 (m, 2H), 3.38 (s, 3H).

Preparation of 1-[3-(2-Methoxyethoxymethyl)benzyl]-4-benzylpiperazine (5a). A mixture of 1-benzylpiperazine (0.8 g, 4.8 mmol), potassium carbonate (0.4 g, 2.9 mmol), and **4a** (1.3 mmol) in 6.0 mL of acetonitrile was refluxed under Ar for 2 h. After cooling to room temperature, the solid was filtered out and washed with acetonitrile (3 × 10 mL). The filtrates were combined and concentrated to yield a yellow oil, which was purified by flash chromatography (EtOAc/hexane/Et_3N, 30:70:1): colorless oil (80%); ^{1}H NMR ($CDCl_3$) δ 7.31−6.93 (m, 9H), 5.26 (s, 2H), 3.84−3.80 (m, 2H), 3.57−3.53 (m, 2H), 3.50 (s, 2H), 3.48 (s, 2H), 3.37 (s, 3H), 2.47 (bs, 8H).

Preparation of 1-(3-Hydroxybenzyl)-4-benzylpiperazine (6a). General Method for Cleavage of MEM Protecting Group (Compounds 6a−g). HCl 2 N (2 mL/0.1 g) was added into a solution of **5a** dissolved in a 1:1 MeOH/dioxane mixture. The solution was stirred at room temperature for 24 h, and the volatiles were removed *in vacuo* at room temperature. To the residue was added cold ether to precipitate the product. If no precipitate was formed, the residue was redissolved in ethyl acetate and hexane was added to precipitate the product. The white precipitate was filtered off, washed with ether, and dried in vacuo: hydrochloride salt, white solid (95%); ^{1}H NMR (CD_3OD) δ 7.32−6.78 (m, 9H), 4.27 (s, 2H), 4.20 (s, 2H), 3.43 (bs, 8H). ^{13}C NMR (D_2O) δ 157.2, 132.7, 132.0, 131.7, 130.5, 130.1, 128.5, 124.1, 118.9, 118.5, 61.5, 61.2, 49.1, 36.1; FAB-HRMS calcd for $C_{18}H_{23}N_2O$ $[M + H]^+ = 283.1810$, found 283.1808. Anal. Calcd for $C_{18}H_{22}N_2O\cdot 2HCl$: C, 61.06; H, 6.84; N, 7.91. Found: C, 60.01; H, 7.03; N, 7.42.

Synthesis of 1-(3-Hydroxy-α-methylbenzyl)-4-benzylpiperazine (6b) from 2. Preparation of 1-[3-(2-Methoxyethoxymethyl)phenyl]ethanol (3b). Into a stirred solution of methylmagnesium bromide (3.0 M, 4.5 mL, 5 equiv) in 10 mL of THF at 0 °C was added dropwise a solution of **2** in 5 mL of THF. The resulting gray suspension was allowed to warm to room temperature and monitored by TLC. Then, the reaction was quenched by addition of 0.5 N HCl (30 mL). The product was extracted with ether (3 × 40 mL), and the combined organic fractions were washed with brine (60 mL), water (60 mL) and and dried over anhydrous $MgSO_4$. Evaporation of the solution gave the product which was further purified by flash chromatography (EtOAc/hexanes, 2:8): colorless oil (95%); ^{1}H NMR ($CDCl_3$) δ 7.30−6.998 (m, 4H), 5.27 (s, 2H), 4.87 (q, 1H, J = 6.5 Hz), 3.85−3.81 (m, 2H), 3.58−3.55 (m, 2H), 3.38 (s, 3H), 1.97 (bs, 1H), 1.48 (d, 3H, J = 6.8 Hz).

Preparation of 1-Chloro-1-[3-(2-methoxyethoxymethyl)phenyl]ethane (4b). Following the same procedure outlined for **4a** (vide supra) but starting with **3b** led to **4b** as a colorless oil (60%): ^{1}H NMR ($CDCl_3$) δ 7.16−6.87 (m, 4H), 5.17 (s, 2H), 4.93 (q, 1H), 3.74−3.70 (m, 2H), 3.47−3.43 (m, 2H), 3.27 (s, 3H), 1.72 (d, 3H, J = 6.8 Hz).

Preparation of 1-[3-(2-Methoxyethoxymethyl)-α-methylbenzyl]-4-benzylpiperazine (5b). Following the same method as used to prepare **5a** but starting from **4b** led to **5b** as a colorless oil (62%): ^{1}H NMR ($CDCl_3$) δ 7.30−6.90 (m, 9H), 5.26 (s, 2H), 3.85−3.81 (m, 2H), 3.49 (s, 2H), 3.37 (s, 3H), 3.35 (q, 1H, J = 6.7 Hz), 2.44 (bs, 8H), 1.34 (d, 3H, J = 6.7 Hz).

Synthesis of 1-(3-Hydroxy-α-methylbenzyl)-4-benzylpiperazine (6b). Following the same method as used to prepare **6a** but starting with **5b** led to **6b** as the hydrochloride salt: white solid (95%); ^{1}H NMR (CD_3OD) δ 7.34−6.81 (m, 9H), 4.34 (q, 1H J = 6.9 Hz), 4.28 (s, 2H), 3.45 (s, 4H), 3.30 (bs, 4H), 1.58 (d, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$) δ 156.0, 142.7, 136.5, 129.7, 129.0, 128.3, 127.3, 119.6, 115.0, 114.6, 64.3, 63.0, 53.0, 49.3, 19.0; FAB-HRMS calcd for $C_{19}H_{25}N_2O$ $[M + H]^+ = 297.1967$, found 297.1972. Anal. Calcd for $C_{19}H_{24}N_2O$: C, 76.98; H, 8.19; N, 9.46. Found: C, 77.07; H, 8.00; N, 9.20.

Synthesis of 1-(3-Hydroxy-α-isobutylbenzyl)-4-benzylpiperazine (6c). Preparation of 1-[3-(2-Methoxyethoxymethyl)phenyl]-3-methylbutanol (3c). Following the same general procedure outlined for **3b** but using isobutylmagnesium bromide led to **3c** as a colorless oil (45%): ^{1}H NMR ($CDCl_3$) δ 7.27−6.93 (m, 4H), 5.27 (s, 2H), 4.69 (m, 1H), 3.83−3.79 (m, 2H), 3.56−3.52 (m, 2H), 3.37 (s, 3H), 1.94−1.60 (m, 2H), 1.49−1.45 (m, 1H), 0.94 (d, 6H, J = 6.3 Hz).

Preparation of 1-Chloro-1-[3-(2-methoxyethoxymethyl)phenyl]-3-methylbutane (4c). Following the same method as used to prepare **4a** (vide supra) but starting with **3c** led to **4c** as a colorless oil (80%): ^{1}H NMR ($CDCl_3$) δ 7.29−6.97 (m, 4H), 5.27 (s, 2H), 4.88 (dd, 1H, J = 6.0 Hz), 3.85−3.81 (m, 2H), 3.57−3.53 (m, 2H), 3.37 (s, 3H), 2.12−1.95 (m, 1H), 1.84−1.76 (m, 2H), 0.95 (dd, 6H, J = 6.4 Hz).

Preparation of 1-[3-(2-Methoxyethoxymethyl)-α-isobutylbenzyl]-4-benzylpiperazine (5c). Following the same procedure as described for **5a** but starting with **4c** led to **5c** as a colorless oil (22%): ^{1}H NMR ($CDCl_3$) δ 7.27−6.81 (m, 9H), 5.26 (s, 2H), 3.84−3.81 (m, 2H), 3.57−3.55 (m, 2H), 3.45 (s,

2H), 3.40−3.36 (m, 4H), 2.43 (bs, 8H), 1.68 (dd, 2H, J = 6.7 Hz), 1.35 (m, 1H), 0.84 (t, 6H, J = 6.3 Hz).

Synthesis of 1-(3-Hydroxy-α-isobutylbenzyl)-4-benzylpiperazine (6c). Following the same method as used to prepare **6a** but starting with **5c** led to **6c** as the hydrochloride salt: white solid (95%); ^{1}H NMR (CD_3OD) δ 7.35−6.80 (m, 9H), 4.24 (m, 3H), 3.40 (s, 4H), 3.20 (bs, 4H), 2.00 (m, 1H), 1.03 (m, 1H), 0.69 (d, 3H, J = 9.8 Hz), 0.66 (d, 3H, J = 9.6 Hz); FAB-HRMS calcd for $C_{22}H_{31}N_2O$ $[M + H]^+$ = 339.2436, found 339.2425. Anal. Calcd for $C_{22}H_{30}N_2O$·2HCl: C, 64.45; H, 7.80; N, 6.83. Found: C, 68.83; H, 8.80; N, 6.50.

Synthesis of 1-(3-Hydroxybenzhydryl)-4-benzylpiperazine (6d) from 2. Preparation of 3-(2-Methoxyethoxymethyl)benzhydrol (3d). Following the same general procedure outlined for **3b** but using phenylmagnesium bromide led to **3d** as a colorless oil (95%): ^{1}H NMR ($CDCl_3$) δ 7.35−6.85 (m, 9H), 5.75 (s, 1H), 5.24 (s, 2H), 3.78−3.72 (m, 2H), 3.41−3.48 (m, 2H), 3.35 (s, 3H), 2.30 (bs, 1H).

Preparation of 3-(2-Methoxyethoxymethyl)benzhydryl Chloride (4d). Following the same method as used to prepare **4a** (vide supra) but starting with **3d** led to **4d** as a colorless oil (65%): ^{1}H NMR ($CDCl_3$) δ 7.43−7.06 (m, 9H), 6.08 (s, 1H), 5.25 (s, 2H), 3.83−3.78 (m, 2H), 3.54−3.52 (m 2H), 3.36 (s, 3H).

Preparation of 1-[3-(2-Methoxyethoxymethyl)benzhydryl]-4-benzylpiperazine (5d). Following the same procedure as described for **5a** but starting with **4d** led to **5d** as a colorless oil (77%): ^{1}H NMR ($CDCl_3$) δ 7.40−6.84 (m, 14H), 5.23 (s, 2H), 4.18 (s, 1H), 3.80−3.77 (m, 2H), 3.54−3.50 (m, 4H), 3.35 (s, 3H), 2.44 (bs, 8H).

Synthesis of 1-(3-Hydroxybenzhydryl)-4-benzylpiperazine (6d). Following the same method as used to prepare **6a** but starting with **5d** led to **6d** as the hydrochloride salt: white solid (95%); ^{1}H NMR (CD_3OD) δ 7.37−6.58 (m, 14H), 4.17 (s, 1H), 3.53 (s, 2H), 2.48 (bs, 8H), 2.00 (m, 1H), 1.03 (m, 1H), 0.69 (d, 3H, J = 9.8 Hz), 0.66 (d, 3H, J = 9.6 Hz); ^{13}C NMR (DMSO-d_6) δ 157.9, 137.2, 136.1, 131.5, 130.4, 129.6, 129.2, 129.0, 128.8, 128.3, 118.5, 115.8, 115.3, 57.3, 45.4, 32.9; FAB-HRMS calcd for $C_{24}H_{27}N_2O$ $[M + H]^+$ = 359.2123, found 359.2128. Anal. Calcd for $C_{24}H_{26}N_2O$·2HCl: C, 67.03; H, 6.51; N, 6.51. Found: C, 66.15; H, 6.71; N, 6.30.

Synthesis of 1-(3-Hydroxy-4′-phenylbenzhydryl)-4-benzylpiperazine (6e) from 2. Preparation of 3-(2-Methoxyethoxymethyl)-4′-phenylbenzhydrol (3e). Following the same general procedure outlined for **3b** but using 4-phenylphenylmagnesium bromide led to **3e** as a colorless oil (85%): ^{1}H NMR ($CDCl_3$) δ 7.36−6.98 (m, 8H), 5.77 (d, 1H, J = 3.5 Hz), 5.24 (s, 2H), 3.82−3.78 (m,2H), 3.55−3.51 (m, 2H), 3.35 (s, 3H), 2.29 (s, 1H, J = 3.5 Hz), 1.29 (s, 9H).

Preparation of 3-(2-Methoxyethoxymethyl)-4′-phenylbenzhydryl Chloride (4e). Following the same method as used to prepare **4a** (vide supra) but starting with **3e** led to **4e** as a colorless oil (34%): ^{1}H NMR ($CDCl_3$) δ 7.345−7.00 (m, 8H), 6.06 (s, 1H), 5.25 (s, 2H), 3.82−3.70 (m, 2H), 3.64−3.50 (m, 2H), 3.35 (s, 3H), 1.29 (s, 9H).

Preparation of 1-[3-(2-Methoxyethoxymethyl)-4′-phenylbenzhydryl]-4-benzylpiperazine (5e). Following the same procedure as described for **5a** but starting with **4e** led to **5e** as a colorless oil (70%): ^{1}H NMR ($CDCl_3$) δ 7.28−7.12 (m, 13H), 5.23 (s, 2H), 4.15 (s, 1H), 3.81−3.77 (m, 2H), 3.35−3.49 (m, 2H), 3.48 (s, 2H), 3.35 (s, 3H), 2.45 (bs, 8H), 1.25 (s, 9H).

Synthesis of 1-(3-Hydroxy-4-phenylbenzhydryl)-4-benzylpiperazine (6e). Following the same method as used to prepare **6a** but starting with **5e** led to **6e** as the hydrochloride salt: white solid (80%); ^{1}H NMR (CD_3OD) δ 7.76−7.13 (m, 18H), 4.17 (s, 1H), 5.43 (s, 1H), 4.36 (s, 2H), 3.60 (bs, 4H), 3.45 (s, 4H); ^{13}C NMR (DMSO-d_6) δ 158.0, 140.5, 139.2, 137.3, 135.1, 131.5, 130.5, 129.7, 129.0, 128.8, 127.8, 127.5, 126.7, 118.5, 115.9, 115.3, 38.2; FAB-HRMS calcd for $C_{30}H_{31}N_2O$ $[M + H]^+$ = 435.2436, found 435.2427. Anal. Calcd for $C_{30}H_{30}N_2O$·2HCl: C, 71.20; H, 6.38; N, 5.53. Found: C, 69.66; H, 6.57; N, 5.31.

Synthesis of 1-(4-*tert*-Butyl-3-hydroxybenzhydryl)-4-benzylpiperazine (6f) (SL-3111) from 2. Preparation of 4-*tert*-Butyl-3-(2-methoxyethoxymethyl)benzhydrol (3f). Following the same general procedure outlined for **3b** but using 4-*tert*-butylphenylmagnesium bromide led to **3f** as a colorless oil (80%): ^{1}H NMR ($CDCl_3$) δ 7.36−6.76 (m, 8H), 5.77 (d, 1H), 3.78 (s, 3H), 2.26 (bs, 1H), 1.29 (s, 9H).

Preparation of 4-*tert*-Butyl-3-(2-methoxyethoxymethyl)benzhydryl Chloride (4f). Following the same method as used to prepare **4a** (vide supra) but starting with **3f** led to **4f** as a colorless oil (70%): ^{1}H NMR ($CDCl_3$) δ 7.34−6.80 (m, 8H), 6.08 (s, 1H), 3.75 (s, 3H), 1.29 (s, 9H).

Preparation of 1-[4-*tert*-Butyl-3′-(2-methoxyethoxymethyl)benzhydryl]-4-benzylpiperazine (5f). Following the same procedure as described for **5a** but starting with **4f** led to **5f** as a colorless oil (70%): ^{1}H NMR ($CDCl_3$) δ 7.34−7.68 (m, 13H), 4.13 (s, 1H), 3.77 (s, 3H), 3.50 (s, 2H), 2.45 (bs, 8H), 1.28 (s, 9H).

Synthesis of 1-(4-*tert*-Butyl-3′-hydroxybenzhydryl)-4-benzylpiperazine (6f) (SL-3111). Following the same method as used to prepare **6a** but starting with **5f** led to **6f** as the hydrochloride salt: white solid (83%); ^{1}H NMR (CD_3OD) δ 7.73−7.21 (m, 13H), 4.49 (s, 1H), 3.73 (s, 2H), 3.63 (bs, 4H), 3.55 (s, 4H), 1.20 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 157.9, 152.9, 131.2, 130.5, 129.5, 129.4, 128.0, 127.2, 126.7, 119.8, 117.3, 114.5, 112.9, 48.4, 48.3, 34.6, 31.0; FAB-HRMS calcd for $C_{28}H_{35}N_2O$ $[M + H]^+$ = 415.2749, found 415.2742. Anal. Calcd for $C_{28}H_{34}N_2O$·2HCl: C, 69.06; H, 7.46; N, 5.75. Found: C, 66.40; H, 7.56; N, 5.31.

Synthesis of 1-(4-*tert*-Butyl-3′-methoxybenzhydryl)-4-benzylpiperazine (6g). Following the same procedure described above for the preparation of analogues **6b**−**f** but using 3-methoxybenzaldehyde instead of **2** led to **6g** as the hydrochloride salt: white solid (85%); ^{1}H NMR ($CDCl_3$) δ 7.54−6.83 (m, 13H), 4.70 (s, 1H), 4.25 (s, 2H), 3.80 (s, 3H), 3.62 (m, 4H), 3.33 (bs, 4H); ^{13}C NMR ($CDCl_3$) δ 160.5, 152.5, 137.0, 132.2, 130.9, 130.5, 71.8, 62.08, 60.4, 55.3, 48.6, 34.6; FAB-MS calcd for $C_{29}H_{36}N_2O$ $[M + H]^+$ = 429.2906, found 429.2902. Anal. Calcd for $C_{29}H_{36}N_2O$·2CF_3CO_2H: C, 60.41; H, 5.85; N, 4.27. Found: C, 60.22; H, 5.81; N, 4.08.

The two optically pure enantiomers of SL-3111 (**6f**) were obtained by HPLC separation performed on a Shimadzu liquid chromatograph model SCL-10A, using a chiral column Chiralpack-AD (DAICEL) (25 cm × 0.5 cm). An isocratic eluent mixture of hexane/2-propanol (97:3) was used as mobile phase with the detector set at 254 nm. The concentration of each injection was 1.5 mg/mL to obtain an adequate resolution. (+)-SL-3111: white solid; mp 109−110 °C; t_R = 7.2 min; $[\alpha]_D^{22}$ = +11.5 (c 1, $CHCl_3$). (−)-SL-3111: white solid; mp 109−110 °C; t_R = 8.1 min; $[\alpha]_D^{22}$ = −10.6 (c 1, $CHCl_3$).

Preparation of [*p*-MeOTyr^1]DPDPE. This peptide was synthesized on a solid support using the N$^\alpha$-Boc strategy. N$^\alpha$-Boc-D-Pen(S-*p*-MeBzl)-resin (1 g, 0.4 mmol/g) was used as starting material. The attachment of the N$^\alpha$-Boc-D-Pen(S-*p*-MeBzl) to the chloromethylated Merrifield resin (Advanced Chemtech, KY) was performed by Gisin's method.[24] The following protected amino acids were assembled on a solid support in a stepwise fashion to the growing peptide chain: N$^\alpha$-Boc-Phe, N$^\alpha$-Boc-Gly, N$^\alpha$-Boc-D-Pen(S-*p*-MeBzl), and N$^\alpha$-Boc-Tyr(OMe)-OH (Bachem, CA). The cyclization of the linear peptide was achieved by procedures reported in the literature[25] and purified by HPLC as described previously: t_R = 16.5 min; HRMS-FAB (*m*-NBA matrix) calcd for $C_{31}H_{42}N_5O_7S_2$ $[M + H]^+$ = 660.2526, found 660.2529. The purity of the synthesized peptide was also analyzed by TLC in three different solvent systems and visualized by staining with 0.2% ninhydrin in ethanol and heating:[26] (A) R_f = 0.70 (*n*-BuOH/AcOH/H_2O, 4:1:5); (B) R_f = 0.75 (*n*-BuOH/pyridine/AcOH/H_2O, 3.75:2.5:0.75:3); (C) R_f = 0.95 (AcOEt/pyridine/AcOH/H_2O, 6:1.5:2.1:1.1).

Radiolabeled Ligand Binding Assays. Membranes were prepared according to the following procedure: adult male Sprague−Dawley rats (200−300 g) obtained from Harlan Sprague−Dawley, Inc. (Indianapolis, IN) were sacrificed and their brains immediately removed and placed on ice. The

whole brain was homogenized in 20 volumes of 50 mM Tris-HCl stock buffer (pH 7.4) with a glass−Teflon homogenizer. The homogenate was centrifuged (48000*g* for 15 min), resuspended, and preincubated (25 °C for 30 min) to remove endogenous opioids. The homogenate was centrifuged and resuspended again (0.5% final concentration). The cloned human δ-opioid receptor for study of the binding profiles of SL-3111 (**6f**), [Phe(*p*-Cl)[4]]DPDPE, and SNC-80 was prepared based on literature method.[22] Binding affinities of the compounds were measured against [^{3}H][*p*-Cl-Phe[4]]DPDPE ([^{3}H]-Tyr-c[D-Pen-Gly-(*p*-Cl)Phe-D-Pen-OH]) (41.0 Ci/mmol)[27] and [^{3}H]DAMGO ([^{3}H]Tyr-D-Ala-Gly-*N*-methyl-Phe-Gly-ol) (48.9 Ci/mmol)[28] (New England Nuclear) by a rapid filtration technique. A solution of 0.75 nM [^{3}H][*p*-Cl-Phe[4]]DPDPE or 1.0 nM [^{3}H]-DAMGO in a total volume of 1 mL of 50 mM Tris-HCl buffer (pH 7.4) containing bovine serum albumin (1.0 mg/mL), bacitracin (50 μg/mL), bestatin (30 μg/mL), captopril (10 μM), and phenylmethanesulfonyl fluoride (0.1 mM) was used. Assays were done in duplicate with 10 μM naltrexone hydrochloride to define nonspecific tissue binding. The binding reaction was terminated by rapid filtration through presoaked (0.5% poly(ethylenimine) solution) GF/B Whatman glass fiber strips with a Brandel cell harvester followed immediately by three rapid washes with 4-mL aliquots of ice-cold saline solution. The filters were removed and soaked in 10 mL of scintillation fluid at 4 °C for at least 6 h before bound radioactivity was measured. The data were analyzed by nonlinear least-squares regression analysis.

In Vitro Bioassays. Electrically induced smooth muscle contractions from mouse vas deferens (MVD) and guinea pig ileum (GPI) longitudinal muscle-myenteric plexus were used for bioassays.[29] Tissues came from male ICR mice weighing 25−30 g and from male Hartley guinea pigs weighing 150−400 g. The tissues were tied to gold chains with suture silk, suspended in 20-mL baths containing 37 °C oxygenated (95% O_2, 5% CO_2) Krebs bicarbonate solution (magnesium-free for the MVD), and allowed to equilibrate for 15 min. The tissues were then stretched to optimal length previously determined to be 1 g tension (0.5 g for MVD) and allowed to equilibrate for 15 min. The tissues were stimulated transmurally between platinum plate electrodes at 0.1 Hz for 0.4-ms pulses (2.0-ms pulses for MVD) and supramaximal voltage. Drugs were added to the baths in 20−60-μL volumes. The agonists remained in tissue baths for 3 min and were removed by rinsing several times with fresh Krebs solution. Tissues were given 8 min to reequilibrate and regain predrug contraction height. Antagonists were added to the bath 2 min prior to the addition of the agonists. Percent inhibition was calculated by dividing height for 1 min preceding the addition of the agonist by the contraction height 3 min after exposure to the agonist. The IC_{50} values represent the mean of not less than four tissues. Relative potency estimates were determined by fitting the mean data to the Hill equation by using a computerized nonlinear least-squares method. In some cases, the weak μ agonist action of these analogues did not permit completion of full dose−response curves in the GPI.

Acknowledgment. The authors wish to acknowledge the financial support of grants from NIDA (U.S.P.H.S. DA08657 and DA06284), the Dean's Fellowship from the Graduate College of The University of Arizona for S.L., and CONACyT-Mexico for J.A.L. The results are those of the authors and do not necessarily represent the official views of the U.S. Public Health Service.

References

(1) (a) Hirschmann, R. F. Medicinal Chemistry in the Golden Age of Biology. Lessons from Stereoid and Peptide Research. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1278−1301. (b) Moore, G. J. Design Peptide Mimetics. *Trends Pharmacol. Sci.* **1994**, *15*, 124−129. (c) Giannis, A.; Kolter, T. Peptidomimetics for Receptor Ligands Discovery, Development and Medical Perspectives. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244−1267. (d) Sawyer, T. K. Peptidomimetic Design and Chemical Approaches to Peptide Metabolism. In *Peptide-Based Drug Design: Controlling Transport and Metabolism*; Taylor, M. D., Amidon, G. L., Eds.; American Chemical Society: Washington, DC, 1995; pp 387−421. (e) Hruby, V. J. Prospects For Peptidomimetic Drug Today. *Drug Discovery Today* **1997**, *2*, 165−167.

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

(3) Porreca, F.; Bilsky, E. J.; Lai, J. Pharmacological Characterization of Opioid δ- and κ-Receptors. In *The Pharmacology of Opioid Peptides*; Tseng, T. F., Ed.; Harwood Academic Publishers: New York, 1995; pp 219−238.

(4) (a) Hruby, V. J.; Gehrig, C. A. Recent Developments in Design of Receptor Specific Opioid Peptides. *Med. Res. Rev.* **1989**, *9*, 343−401 (b) Hruby, V. J.; Kao, L. F.; Pettitt, B. M.; Karplus, M. The Conformational Properties of the Delta Opioid Peptide [*D*-Pen[2],*D*-Pen[5]]enkephalin in Aqueous Solution Determined by NMR and Energy Minimization Calculation. *J. Am. Chem. Soc.* **1988**, *110*, 3351−3359. (c) Nikiforovich, G. V.; Hruby, V. J.; Prakash, O.; Gehrig, C. A. Topographical Requirements for δ-Selective Opioid Peptides. *Biopolymers* **1991**, *31*, 941−955. (d) Zimmerman, D. M.; Leander, J. D. Selective Opioid Receptor Agonists and Antagonists: Research Tools and Potential Therapeutic Agents. *J. Med. Chem.* **1990**, *33*, 895−902. (e) Mosberg, H. I.; Omnas, J. R.; Lomize, A.; Heyl, D. L.; Norde, I.; Mousigian, C.; Davis, P.; Porreca, F. Development of a Model for the δ-opioid Receptor Pharmacophore. 2. Conformationally Restricted Phe[3] Replacements in the Cyclic δ-Receptor Selective Tetrapeptide Tyr-c[D-Cys-Phe-D-Pen]-OH (JOM-13). *J. Med. Chem.* **1994**, *37*, 4384−4391.

(5) Knapp, R. J.; Santoro, G.; De Leon, I. A.; Lee, K. B.; Edsall, S. A.; Waite, S.; Malatynska, E.; Varga, E.; Calderon, S. N.; Rice, K. N.; Rothman, R. B.; Porreca, F.; Roeske, W. R.; Yamamura, H. Y. Structure−Activity Relatioships for SNC80 and Related Compounds at Cloned Human *Delta* and *Mu* Opioid Receptors. *J. Pharmacol. Exp. Ther.* **1996**, *277*, 1284−1291.

(6) (a) Knapp, R. J.; Landsman, R.; Waite, S.; Malatynska, E.; Varga, E.; Haq, W.; Hruby, V. J.; Roeske, W. R.; Nagase, H.; Yamamura, H. Y. Properties of TAN-67, a Nonpeptidic δ-Opioid Receptor Agonist, at Cloned Human δ- and μ-Opioid Receptors. *Eur. J. Pharmacol.* **1995**, *291*, 129−134 (b) Portoghese, P. S.; Moe, S. T.; Takemori, A. E. A Selective δ_1 Opioid Receptor Agonist Derived from Oxymorphone. Evidence for Separate Recognition Sites for δ_1 Opioid Receptor Agonists and Antagonists. *J. Med. Chem.* **1993**, *36*, 2572−2574.

(7) Suzuki, T.; Tsuji, M.; Mori, T.; Misawa, M.; Endoh, T.; Nagase, H. Effects of A Highly Selective Nonpeptide δ Opioid Receptor Agonist, TAN-67, on Morphine-Induced Antinociception in Mice. *Life Sci.* **1995**, *57*, 155−168.

(8) Li, X.; Knapp, R. J.; Stropova, D.; Varga, E.; Wang, Y.; Malatynska, E.; Calderon, S. N.; Rice, K.; Rothman, R.; Porreca, F.; Hruby, V. J.; Roeske, W. R.; Yamamura, H. I. Trp[284] of the Human δ Opioid Receptor is Required for SNC121 Binding But Not Binding of *p*Cl-DPDPE, Deltorphin II or Naltrindole. *Analgesia* **1995**, *1*, 539−542.

(9) Qian, X.; Liao, S.; Stropova, D.; Yamamura, H. I.; Hruby, V. J. Novel Scaffolds for Non-Peptide Mimetics of δ Opioid Receptor Agonists Based on Peptide Leads. *Regul. Pept.* **1996**, *65*, 79−82.

(10) 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.

(11) Shenderovich, M. D.; Liao, S.; Qian, X.; Hruby, V. J. Three-Dimensional Models of δ Opioid Pharmacophore: Molecular Modeling and QSAR Study of Peptide and Non-Peptide Ligands. In *Peptides: Chemistry, Structure and Biology, Proceedings of the 15th American Peptide Symposium*; Tam, J. P., Kaumaya, P. T. P., Eds.; ESCOM Kluwer Acad. Publ.: Dordrecht, The Netherlands, 1998, in press.

(12) 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.

(13) (a) Weber, S. J.; Greene, D. L.; Hruby, V. J.; Yamamura, H. I.; Porreca, F.; Davis, T. P. Whole Body and Brain Distribution of [^{3}H]Cyclic[*D*-Pen[2],*D*-Pen[5]]Enkephalin After Intraperitoneal, Intravenous, Oral and Subcutaneous Administration. *J. Pharmacol. Exp. Ther.* **1992**, *263*, 1308−1316. (b) Williams, S. A.; Abbruscato, J. J.; Hruby, V. J.; Davis, T. P. Passage of a δ-Opioid Receptor Selective Enkephalin, [*D*-Penicillamin[2,5]]Enkephalin, Across the Blood-Brain and the Blood-Cerebrospinal Fluid Barriers. *J. Neurochem.* **1996**, *66*, 1289−1299.

(14) 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.
(15) 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, Delta Opioid Receptor Selective Compound: Comparisons with Proposed Solution Conformations. *J. Am. Chem. Soc.* **1994**, *116*, 7523−7531.
(16) Hruby, V. J.; Töth, 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.
(17) Mosberg, H. I.; Hurst, R.; Hruby, V. J.; Gee, K.; Akiyama, K.; Yamamura, H. I.; Galligan, J. J.; Burks, T. F. Cyclic Penicillamine Containing Enkephalin Analogues Display Profound Delta Receptor Selectivities. *Life Sci.* **1984**, *33*, 447−450.
(18) Nikiforovich, G. V. Towards Nonpeptide Agonists: Design of "True" Peptidomimetics. *Lett. Pept. Sci.* **1995**, *2*, 172−176.
(19) Farmer, P. S. Binding the Gap Between Bioactives Peptides and Non-Peptides: Some Perspectives in Design. In *Drug Design*; Ariens, E. J., Ed.; Academic Press: New York, 1980; Vol. X, p 127.
(20) Corey, E. J.; Gras J.-L.; Ulrich, P. A New General Method for Protection of the Hydroxyl Functions. *Tetrahedron Lett.* **1976**, 809−812.
(21) Lee, J. B.; Nolan, T. J. Sugar Esters III. New Reagent for Deoxyhalo Sugar Derivatives. *Can. J. Chem.* **1966**, *44*, 1331−1334.
(22) Varga, A. E.; Li, X.; Stropova, D.; Zalewska, T.; Landsman, R. S.; Knapp, R. J.; Malatynska, E.; Kawai, K.; Mizusura, A.; Nagase, H.; Calderon, S. N.; Rice, K. C.; Hruby, V. J.; Roeske, W. R.; Yamamura, H. I. The Third Extracellular Loop of the Human δ-Opioid Receptor Determines the Selectivity of δ-Opioid Agonist. *Mol. Pharmacol.* **1996**, *50*, 1619−1624.
(23) Quock, R. M.; Hosohata, Y.; Knapp, R. J.; Burkey, T. H.; Hosohata, K.; Zhang, X.; Rice, K. C.; Nagase, H.; Hruby, V. J.; Porreca, F.; Roseke, W. R.; Yamamura, H. I. Relative Efficacies of δ-Opioid Receptor Agonists at the Cloned Human δ-Opioid Receptor. *Eur. J. Pharmacol.* **1997**, *326*, 101−104.
(24) Gisin, B. F. The Preparation of Merrifield-Resins Through Total Esterification With Cesium Salts. *Helv. Chim. Acta* **1973**, *56*, 1476−1482.
(25) Misicka, A.; Hruby, V. J. Optimization of Disulfide Bond Formation. *Polish J. Chem.* **1994**, *68*, 893−899.
(26) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. J. Color Test for Detection of Free Terminal Amino Groups in the Solid-Phase Synthesis of Peptides. *Anal. Biochem.* **1970**, *34*, 595−598.
(27) Vaughn, L. K.; Knapp, R. J.; Toth, G.; Wan, Y.-P.; Hruby, V. J.; Yamamura, H. I. A Highly Affinity, Highly Selective Ligand for the Delta Opioid Receptor: [^{3}H]-[*D*-Pen^2,*p*Cl-Phe^4,*D*-Pen^5]-Enkephalin. *Life Sci.* **1989**, *45*, 1001−1008.
(28) Rothman, R. B.; Xu, H.; Seggel, M.; Jacobson, A. E.; Rice, K. C.; Brine, G. A.; Carroll, F. I. RTI-4614-4: An Analogue of (+)-Cis-3-methylfentanyl with a 27,000-Fold Binding Selectivity for Mu Versus Delta Opioid Binding Sites. *Life Sci.* **1991**, *48*, PL111−116.
(29) Kramer, T. H.; Davis, P.; Hruby, V. J.; Burks, T. F.; Porreca, F. In Vitro Potency, Affinity and Agonist Efficacy of Highly Selective Delta Opioid Receptor Ligands. *J. Pharmacol. Exp. Ther.* **1993**, *266*, 577−584.

JM980374R