Design and Synthesis of Novel Dimeric Morphinan Ligands for κ and μ Opioid Receptors

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A novel series of morphinans were synthesized, and their binding affinity at and functional selectivity for μ, δ, and κ opioid receptors were evaluated. These dimeric ligands can be viewed as dimeric morphinans, which were formed by coupling two identical morphinan pharmacophores (cyclorphan (**1**) or MCL 101 (**2**)) with varying connecting spacers. Ligands **6** and **7** with alkyl spacers on the nitrogen position and ligands **8** and **9** in which the two morphinan pharmacophores were coupled by ether moieties at the 3-hydroxyl positions showed significant decrease in affinity at all three opioid receptors. An improvement in the affinity was achieved by introducing an ester moiety as the spacer in the dimeric morphinans. It was observed that the affinity of these ligands was sensitive to the character and length of the spacer. Compound **13** (MCL-139) with a 4-carbon ester spacer, compound **17** (MCL-144) containing a 10-carbon spacer, and compound **19** (MCL-145) with the conformationally constrained fumaryl spacer were the most potent ligands in this series, displaying excellent affinities at μ and κ receptors (K_i = 0.09−0.2 nM at μ and K_i = 0.078−0.049 nM at κ), which were comparable to the parent compound **2**. Ligand **12**, a compound containing only one morphinan pharmacophore and a long-chain ester group, had affinity at both μ and κ receptors almost identical to that of the parent ligand **2**. In the [^{35}S]GTPγS binding assay, ligands **13**, **17**, and **19** and their parent morphinans **1** and **2** stimulated [^{35}S]GTPγS binding mediated by the μ and κ receptors. Compounds **13** and **17** were full κ agonists and partial μ agonists, while compound **19** was a partial agonist at both μ and κ receptors. These novel ligands, as well as their interesting pharmacological properties, will serve as the basis for our continuing investigation of the dimeric ligands as potential probes for the pharmacotherapy of cocaine abuse and may also open new avenues for the characterization of opioid receptors.

Introduction

The opioid system modulates several physiological and behavioral processes such as pain perception, the stress response, the immune response, and neuroendocrine function.[1] Pharmacological and molecular cloning studies[2] have identified three major types of opioid receptors (μ, δ, and κ) that belong to the rhodopsin subfamily in the superfamily of over 100 G-protein-coupled receptors (GPCRs). They are highly homologous (∼60% amino acid identity) and recognize structurally diverse ligands including peptides, opiates, and a variety of synthetic non-peptides.[3]

Recent behavioral studies suggested that opioid κ agonists with varying activity at the μ receptor effectively reduced cocaine self-administration in nonhuman primates and with fewer undesirable side effects than the highly selective κ agonists.[4] These results encouraged us to continue developing novel ligands targeting both κ and μ receptors.[5,6] This report describes a series of novel dimeric morphinans that have mixed κ agonist and μ agonist/antagonist pharmacophores coupled by varying connecting spacers with different lengths. Compounds that contain two pharmacophores or one single pharmacophore and a nonpharmacophore recognition unit linked through a connecting spacer have been termed "bivalent ligands".[7] So far, only a limited number of such bivalent ligands have been synthesized.[8−13] For example, bivalent ligands containing oxymorphine or naltrexamine pharmacophores with specific spacer lengths have been reported to have enhanced opioid agonist or antagonist potencies and selectivities.[8−13]

The morphinan core structures that we employed for this study included cyclorphan (**1**)[14,15] and its *N*-cyclobutylmethyl analogue MCL-101 (**2**).[5a,15] Both **1** and **2** were found to have high affinity at κ and μ receptors and longer durations of action than ethylketocyclazocine (**5**) (Figure 1). They acted as full κ agonists but partial μ agonists.[4,5] Ligands composed of two such mixed κ agonist and μ agonist/antagonist pharmacophores may exhibit increased binding affinity and different pharmacological properties. The purpose of this study was to develop potent mixed κ and μ opioid ligands by optimizing the nature and length of the spacers. Such dimeric morphinans may serve as candidates for our continuing development of mixed κ and μ agonists/antagonists and represent intriguing potential targets for drug development. We speculated that such dimeric

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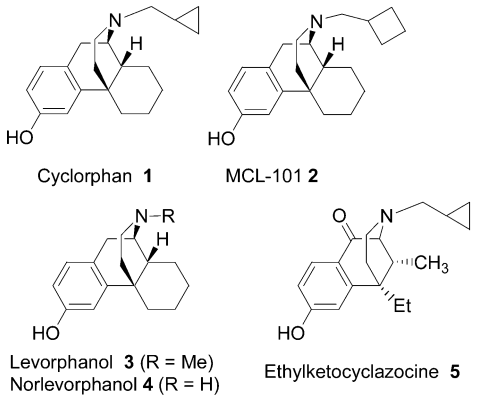
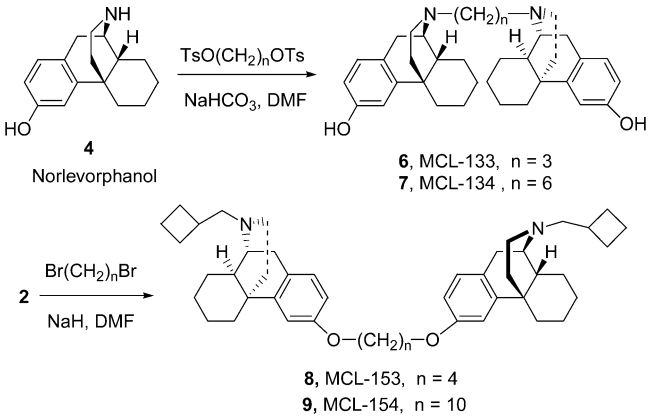


Figure 1. Selected morphinan core structures.

Scheme 1



ligands may also be recognized as the basis for the further development of novel bivalent ligands for the characterization of κ or μ opioid receptors.

Chemistry

Several series of dimeric ligands were synthesized from the monomeric cyclorphan (**1**) and MCL 101 (**2**). In the first series, the pharmacophore used in the elaboration of such novel morphinan ligands was norlevorphanol (**4**) because it has 17-nitrogen as a point of attachment for the spacer chain. The spacers employed in this series were composed of a varying number of methylene units. In the initial study, ligands **6** and **7** containing three and six methylene units at the 17-position were synthesized. Thus, the commercially available (−)-3-hydroxy-*N*-methylmorphinan (levorphanol, **3**) was N-demethylated to give the norlevorphanol (**4**) according to the procedure reported by Olfoson.[16] Reaction of **4** with propane-1,3-diyl or hexane-1,6-diyl ditosylate (prepared from propane-1,3-diol or hexane-1,6-diol) in DMF at 110 °C produced ligands **6** and **7** in 52% and 45% yields, respectively (Scheme 1).

Compounds **8** and **9** with an ether spacer at the 3-hydroxy position of morphinan **2** were prepared by coupling 2 equiv of **2** with 1,4-dibromobutane or 1,10-dibromodecane in the presence of sodium hydride in DMF (Scheme 1). A third series of morphinan derivatives (**10**, **11**, **13**−**22**) linked by ester functions at the 3-hydroxy position were also synthesized (Scheme 2). Generally, compounds **10**, **11**, and **13**−**21** were prepared from morphinan **1** or **2** with an appropriate diacid chloride in the presence of triethylamine. Compound **22** was obtained by condensation of morphinan **2** with *cis*-1,2-cyclopropyldicarboxylic acid, which was hydrolyzed from 3-oxabicyclo[3.1.0]hexane-2,4-dione, in the presence of DCC and DMAP.[17] Monomeric analogue **12** containing a capped spacer was also synthesized as a control to factor out any possible contribution of the spacer moiety.

Pharmacological Results and Discussion

Affinity and Selectivity of the Synthesized Ligands. All the novel dimeric morphinan ligands were examined for their affinity at and selectivity for μ, δ, and κ opioid receptors with Chinese hamster ovary (CHO) cell membranes stably expressing the human opioid receptors. The data were summarized in Table 1. For comparison purposes, opioid binding affinity data for enadoline, cyclorphan (**1**), MCL-101 (**2**), and levorphanol (**3**) were also included.

Traditionally, the 3-phenolic hydroxyl group was recognized as a hydrogen bond donor and was essential for the metabolism of traditional opiates.[1] Therefore, we examined first morphinans **6** and **7** where the two morphinan pharmacophores were connected by alkyl spacers on the basic nitrogen atoms. Compared to levorphanol (**3**), their affinities were decreased 10- to 20-fold at κ, 88- to 140-fold at δ, and 228- to 247-fold at μ receptors. However, a selectivity (7- to 25-fold) for μ and κ over δ receptors was retained. Recent studies from our and Wentiand's laboratories indicated that substitution of the phenolic hydroxyl group in morphinans[18] and benzomorphans[19] with other functional groups (e.g., amino or carboxamido) may retain good opioid receptor activity. Similar results were also observed in the clocinnamox series where the 3-deoxy analogue exhibited high μ opioid receptor affinity compared to clocinnamox, 14β-cinnamoyl epoxymorphinan.[20] Thus, we investigated the effect of attachment of a spacer at the 3-OH position in the morphinans. Compounds **8** and **9**, containing an ether linkage, displayed a dramatic decrease in affinity (120- to 1500-fold) at all three opioid receptors compared to the parent morphinan **2**. We then evaluated ligands **10** and **11** containing an ester moiety between the 3-positions of the two morphinan core structures. The affinities of ligands **10** and **11** at all three opioid receptors were remarkably improved compared to the affinities of compounds **6**−**9**. Morphinan **10**, which had two methylene units between the two ester moieties, was 2-fold less potent at all three opioid receptors, while a longer ester linker (containing eight methylene units) in ligand **11** resulted in a 10- to 15-fold decrease of affinity at all three opioid receptors, compared to the parent compound **1**.

Since ligands esterified at the 3-position exhibited better affinity at opioid receptors than the ligands with a 3-position ether spacer or with an alkyl linkage at the basic nitrogen atom, we investigated the role of the character and length of the ester spacer in such ligands. Initially, with **2** as the morphinan core structure, compounds **13**−**18** with varying ester spacer lengths were evaluated. All these ligands displayed high affinity at both μ and κ receptors and a 35- to 120-fold selectivity for μ/κ over δ receptors. It is noteworthy that the affinity at opioid receptors was sensitive to the length of the ester spacer and a good relationship between the affinity and the number of methylene units in the ester spacer

Scheme 2

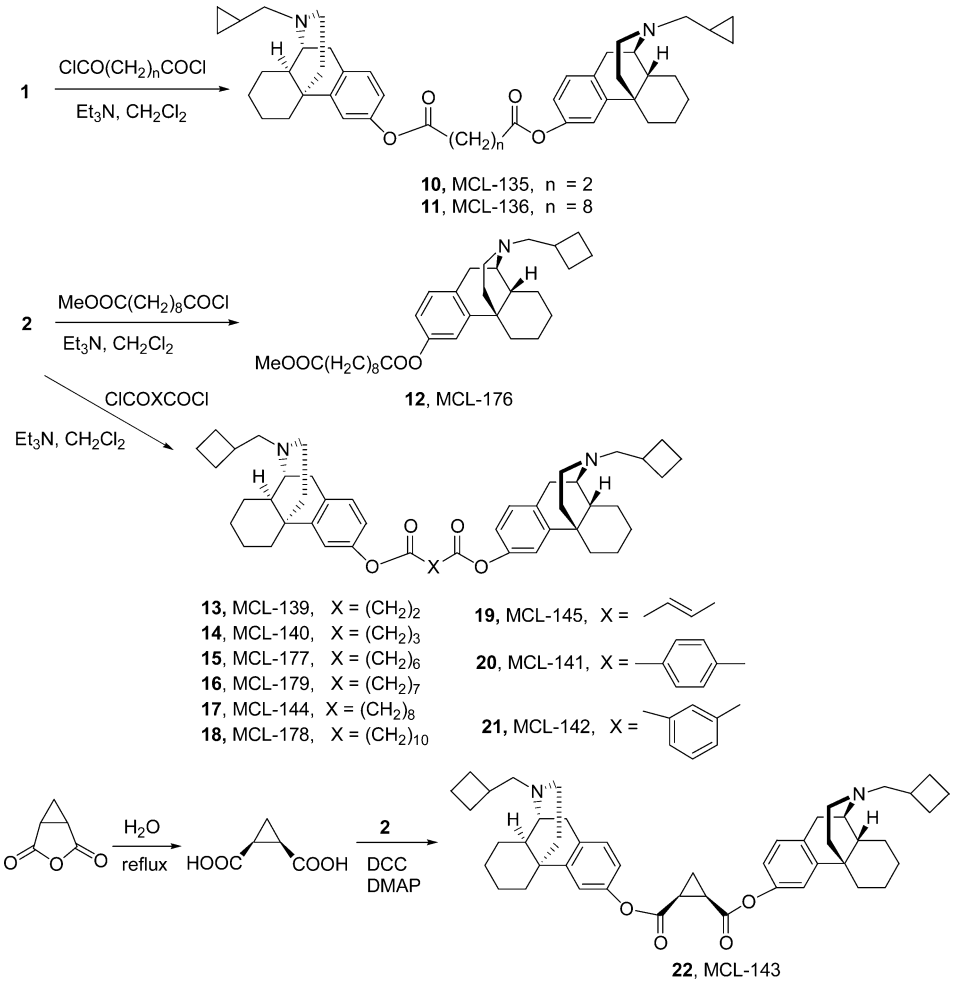


Table 1. K_i Inhibition Values of μ, δ, and κ Opioid Binding to CHO Membranes by MCL Compounds

	K_i (nM) ± SE			selectivity	
compd	[^{3}H]DAMGO (μ)	[^{3}H]naltrindole (δ)	[^{3}H]U69,593 (κ)	κ/μ	κ/δ
enadoline	22 ± 3.2	>1 μM	0.27 ± 0.073	80	>1000
1 (cyclorphan)	0.062 ± 0.003	1.9 ± 0.072	0.034 ± 0.002	2	60
2 (MCL-101)	0.23 ± 0.01	5.9 ± 0.55	0.079 ± 0.003	3	70
3 (levorphanol)	0.21 ± 0.017	4.2 ± 0.45	2.3 ± 0.26	0.09	2
6 (MCL-133)	48 ± 2.9	600 ± 43	24 ± 0.69	2	25
7 (MCL-134)	52 ± 1.8	370 ± 23	50 ± 2.8	1	7
8 (MCL-153)	29 ± 1.2	730 ± 29	18 ± 1.3	2	40
9 (MCL-154)	66 ± 4.3	2500 ± 91	120 ± 8.2	0.5	20
10 (MCL-135)	0.14 ± 0.01	3.8 ± 0.14	0.10 ± 0.006	1	40
11 (MCL-136)	0.93 ± 0.09	32 ± 2.9	0.41 ± 0.004	2	80
12 (MCL-176)	0.23 ± 0.22	44 ± 6.8	0.070 ± 0.03	3	630
13 (MCL-139)	0.16 ± 0.01	9.4 ± 0.44	0.076 ± 0.002	2	120
14 (MCL-140)	3.3 ± 0.36	80 ± 7.9	1.2 ± 0.05	3	70
15 (MCL-177)	0.89 ± 0.13	24 ± 4.3	0.65 ± 0.52	1	37
16 (MCL-179)	0.97 ± 0.033	21 ± 0.45	0.59 ± 0.153	2	35
17 (MCL-144)	0.090 ± 0.004	4.2 ± 0.44	0.049 ± 0.001	2	90
18 (MCL-178)	1.8 ± 0.24	82 ± 5.5	1.1 ± 0.1	2	75
19 (MCL-145)	0.20 ± 0.032	9.4 ± 0.54	0.078 ± 0.01	3	120
20 (MCL-141)	4.7 ± 0.64	400 ± 46	4.2 ± 0.81	1	100
21 (MCL-142)	3.3 ± 0.26	920 ± 95	3.4 ± 0.08	1	270
22 (MCL-143)	0.28 ± 0.003	16 ± 1.0	0.17 ± 0.02	2	90

was observed (Figure 2). Compound **13** with two methylene units and **17** containing eight methylene units in the ester spacer were the most potent ligands in this subseries. The number of methylene units between two and eight (compounds **14**−**16)** or longer than eight (compound **18**) did not give better results (Table 1). These observations suggested that such dimeric ligands with an appropriate spacer (e.g., ester) and spacer length may have higher affinity (∼2-fold) and selectivity than the corresponding monomeric congener.

To further elucidate the effect of the ester spacer on the affinity, we probed another subseries of morphinans, **19**−**22**, with fumaryl, tere- or isophthalyl, and cyclopropyldicarboxyl moieties as the spacers, respectively.

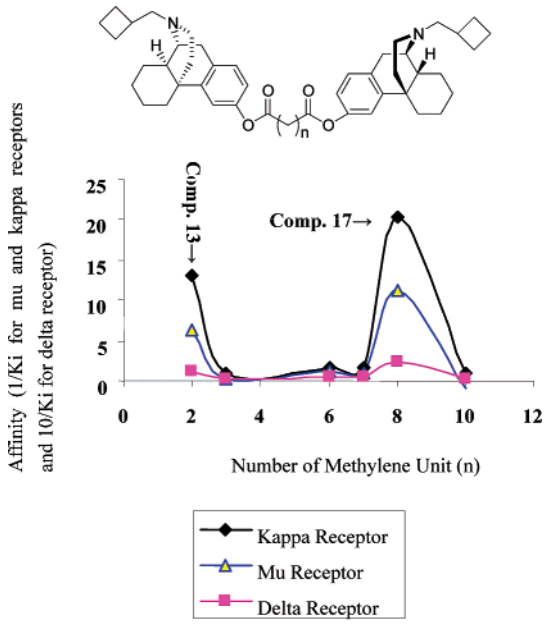


Figure 2. Relationship between ligand ester spacer length and relative potency.

It is also worth noting that compound **19** with a conformationally constrained fumaryl group (trans conformation) has almost identical affinity and selectivity as the monomeric compound **2**. Introduction of the phthalyl moiety in **20** and **21** produced a 15- to 150-fold decrease in affinity (compared to the parent compound **2**). However, ligand **22**, with a conformationally constrained *cis*-cyclopropyldicarboxyl ester linkage, was slightly less potent at μ and κ receptors than **2** and equally potent at κ receptor as the trans compound **19** (Table 1). Unexpectedly, ligand **12** with a long diester chain containing only one morphinan function retained the identical affinity at both μ and κ receptors, compared to **2**. However, a 7-fold decrease in affinity was observed at the δ receptor, which resulted in a pronounced enhancement in the selectivity for κ over δ receptors (630-fold). Thus, of the compounds we evaluated, three (**13**, **17**, and **19**) were identified with high affinity at μ and κ receptors and 45- to 120-fold selectivity for μ and κ over δ receptors.

Efficacy Assay of Selected Ligands. To characterize the relative efficacy of these morphinan ligands, compounds **10**−**13** and **15**−**19**, together with cyclorphan (**1**) and MCL 101 (**2**), were selected for the [^{35}S]GTPγS assay. Table 2 showed the agonist and antagonist properties of these ligands in stimulating [^{35}S]GTPγS binding mediated by the κ opioid receptor. Ligands **11**, **12**, **15**, **16**, **18**, and **19** produced similar maximal stimulation of [^{35}S]GTPγS binding (E_{max}) comparable to that of the monomers **1** and **2**, but the stimulation was less than that of κ selective agonist U50,488. The rank order of the EC_{50} values substantially correlated with the K_i values obtained for the compounds in the binding assays with [^{3}H]U69,593. Among the most potent ligands (**13**, **17**, and **19**), **13** and **17** had lower efficacy and ligands **17** and **19** had the lowest EC_{50} values that were not statistically different from that of the parent compound **2**. Similar to the parent compound **2**, most of these ligands except compound **19** exhibited no inhibitory effect on the stimulation of the κ selective agonist U50,488, which suggested that most of these ligands were full κ selective agonists. Ligand **19** produced good maximal stimulation of [^{35}S]GTPγS binding and also had a good maximal inhibition (I_{max}) of the U50,488 stimulated [^{35}S]GTPγS binding, although the IC_{50} was very high (2400 nM). These data indicated that ligand **19** was a partial κ agonist.

Although having different properties at κ receptor, ligands **13**, **17**, and **19** and the monomer **2** produced similar maximal stimulation of [^{35}S]GTPγS binding mediated by μ receptor. Ligands **12**, **15**, and **16** had slightly higher efficacy, but the stimulation was considerably lower than that of full μ opioid agonist DAMGO (Table 3). Ligands **10** and **18** displayed a pronounced lower efficacy than the others, which indicated that they had very weak μ agonist properties. Compounds **17** and **19** had the lowest EC_{50} values that were not statistically different from that of the parent compound **2**. Compared to the morphinans **1** and **2** that produced moderate inhibition of the full μ opioid agonist DAMGO, most of the dimeric ligands had much higher inhibitiory activity, especially ligands **11** and **19**, which produced maximal inhibitory activity (I_{max} = 100%) indicating that they were more efficacious as μ antagonists than the others. Ligand **10** and its monomeric compound **1** had similar low IC_{50} values (1.7 nM), which indicated that they were potent μ opioid antagonists.

Table 2. Agonist and Antagonist Properties of Compounds in Stimulating [^{35}S]GTPγS Binding Mediated by the κ Opioid Receptor[a]

compd	pharmacological properties	E_{max} (% maximal stimulation)	EC_{50} (nM)	I_{max} (% maximal inhibition)	IC_{50} (nM)
(−)-U50,488	agonist	110 ± 2.0	46 ± 16	- - -	
1 (cyclorphan)	agonist	90 ± 10	0.19 ± 0.04	- - -	
2 (MCL-101)	agonist	80 ± 6.8	1.3 ± 0.44	- - -	
10 (MCL-135)	agonist	70 ± 15	3.3 ± 0.63	- - -	
11 (MCL-136)	agonist	90 ± 7.4	4.9 ± 0.43	- - -	
12 (MCL-176)	agonist	90 ± 9.4	5.7 ± 0.62	no effect	
13 (MCL-139)	agonist	70 ± 5.7	4.5 ± 1.7	no effect	
15 (MCL-177)	agonist	80 ± 2.8	11 ± 1.1	no effect	
16 (MCL-179)	agonist	80 ± 7.5	15 ± 4.4	no effect	
17 (MCL-144)	agonist	60 ± 0.68	0.85 ± 0.053	no effect	
18 (MCL-178)	agonist	90 ± 1.6	8.1 ± 0.37	no effect	
19 (MCL-145)	partial agonist	90 ± 6.0	2.3 ± 0.34	100	2400 ± 75

[a] Membranes from CHO cells that stably expressed only the κ opioid receptor were incubated with varying concentrations of the compounds. The stimulation of [^{35}S]GTPγS binding was measured as described in the Experimental Section. To determine the antagonist properties of a compound, membranes were incubated with 100 nM of the κ agonist U50,488 in the presence of varying concentrations of the compound. The I_{max} value is the maximal percent inhibition obtained with the compound. The IC_{50} value is the concentration of compound needed to produce half maximal inhibition. Dashed lines indicate that the compound was not tested for antagonist properties because of its high E_{max} value.

Table 3. Agonist and Antagonist Properties of Compounds in Stimulating [^{35}S]GTPγS Binding Mediated by the μ Opioid Receptor[a]

compd	pharmacological properties	E_{max} (% maximal stimulation)	EC_{50} (nM)	I_{max} (% maximal inhibition)	IC_{50} (nM)
DAMGO	agonist	120 ± 12	110 ± 9.0	- - -	- - -
1 (cyclorphan)	partial agonist	40 ± 2.9	0.80 ± 0.06	50 ± 1.2	1.7 ± 0.40
2 (MCL-101)	partial agonist	50 ± 2.5	1.6 ± 0.15	50 ± 2.6	20 ± 2.7
10 (MCL-135)	antagonist	10 ± 0.60	- - -	60 ± 3.9	1.8 ± 0.14
11 (MCL-136)	partial agonist	30 ± 3.0	- - -	100	90 ± 11
12 (MCL-176)	partial agonist	60 ± 2.2	8.0 ± 0.57	50 ± 1.9	130 ± 6.0
13 (MCL-139)	partial agonist	50 ± 4.4	7.4 ± 1.8	50 ± 0.70	170 ± 32
15 (MCL-177)	partial agonist	60 ± 4.7	10 ± 0.96	100	90 ± 11
16 (MCL-179)	partial agonist	57 ± 2.4	11 ± 0.62	54 ± 4.2	360 ± 55
17 (MCL-144)	partial agonist	50 ± 6.6	1.3 ± 0.15	60 ± 5.2	16 ± 3.0
18 (MCL-178)	partial agonist	14 ± 4.0	- - -	60 ± 1.7	100 ± 2.7
19 (MCL-145)	partial agonist	50 ± 2.5	2.9 ± 0.50	100	610 ± 71

[a] Membranes from CHO cells that stably expressed only the μ opioid receptor were incubated with varying concentrations of the compounds. The stimulation of [^{35}S]GTPγS binding was measured as described in the Experimental Section. EC_{50} values were the concentration of compound needed to produce 50% of the E_{max} value. When the E_{max} value was 30% or lower, it was not possible to calculate an EC_{50} value. To determine the antagonist properties of a compound, membranes were incubated with 200 nM of the μ agonist DAMGO in the presence of varying concentrations of the compound. The I_{max} value is the maximal percent inhibition obtained with the compound. The IC_{50} value is the concentration of compound needed to produce half maximal inhibition. Dashed lines indicate that the compound was not tested.

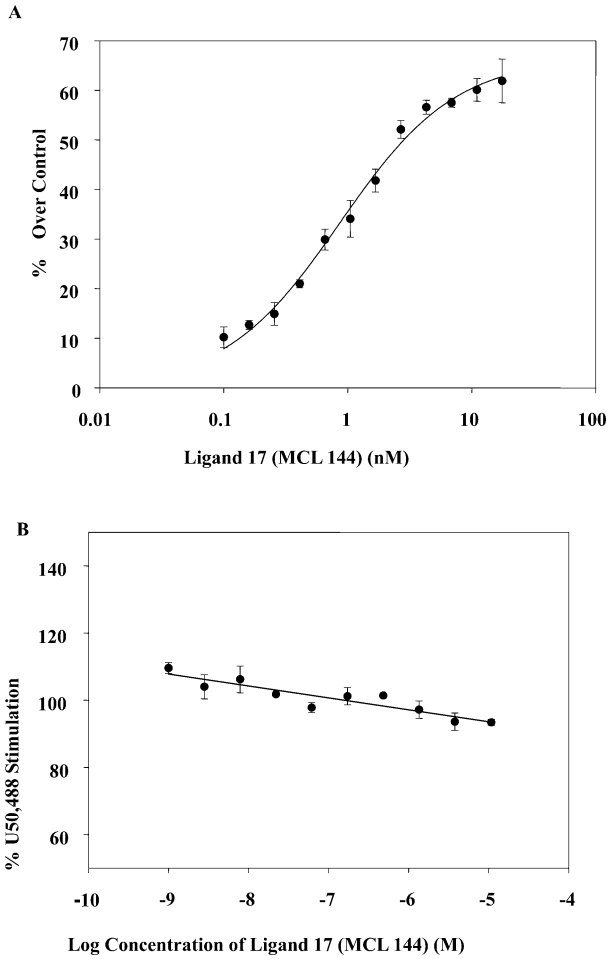


Figure 3. Partial agonist properties of **17** (MCL 144) as determined in the [^{35}S]GTPγS binding assay. To determine the agonist properties (A) of **17**, membranes from CHO cells that stably expressed the κ opioid receptor were incubated with varying concentrations of **17**. [^{35}S]GTPγS binding was measured as described in the Experimental Section. To determine the antagonist properties (B), the membranes were incubated with 100 nM of the κ agonist U50,488 in the presence of varying concentrations of **17**.

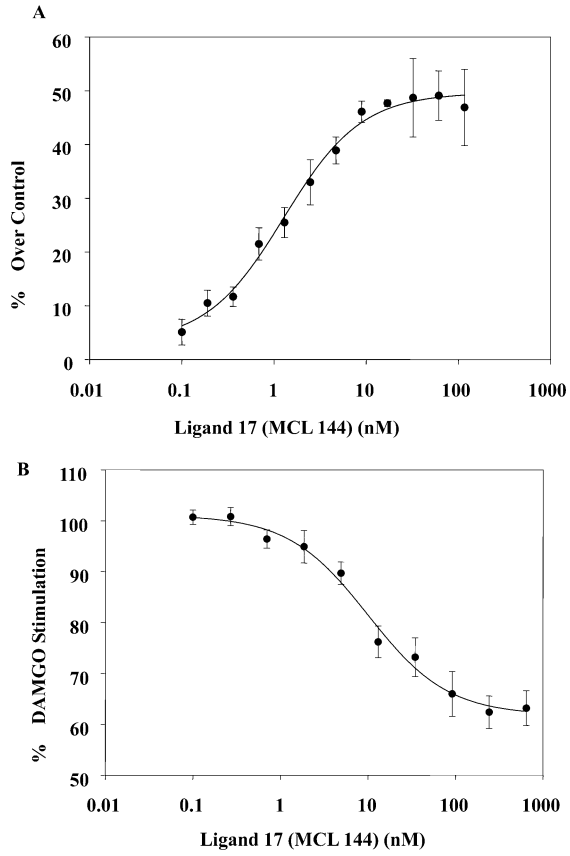


Figure 4. Partial agonist properties of **17** (MCL 144) as determined in the [^{35}S]GTPγS binding assay. To determine the agonist properties (A) of **17**, membranes from CHO cells that stably expressed the μ opioid receptor were incubated with varying concentrations of **17**. [^{35}S]GTPγS binding was measured as described in the Experimental Section. To determine the antagonist properties (B), the membranes were incubated with 200 nM DAMGO in the presence of varying concentrations of **17**.

The preliminary assay for agonist and antagonist properties of these ligands in stimulating [^{35}S]GTPγS binding mediated by the κ and μ opioid receptors illustrated that most of these ligands were κ agonists and μ partial agonists except for compound **10**, which was a κ agonist and μ antagonist, and compound **19**, which was a partial agonist at both κ and μ receptors. Compound **12**, compared to its parent **2**, gave similar pharmacological properties at both μ and κ receptors. The different pharmacological properties of the highly

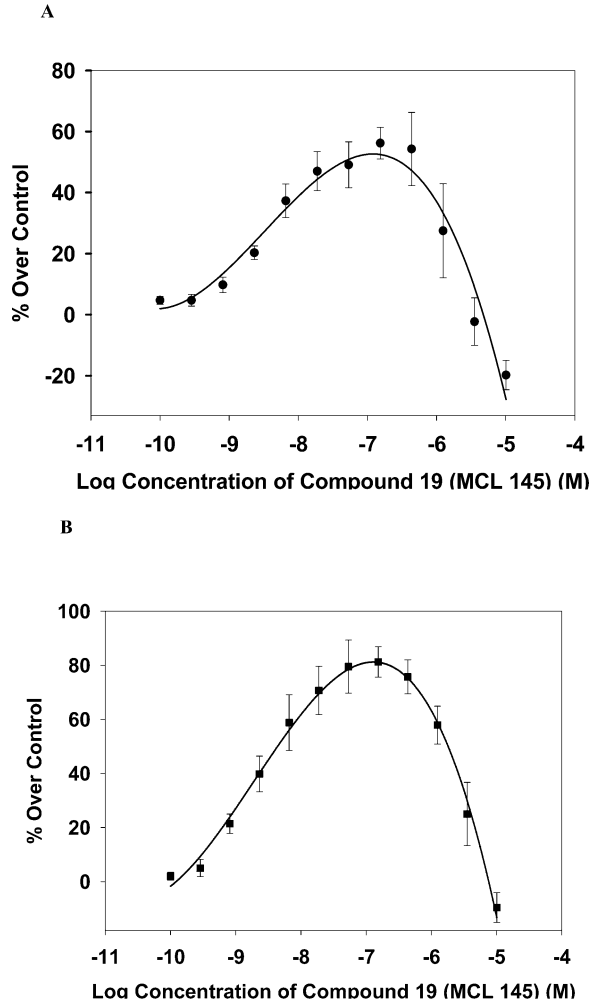


Figure 5. Determining the pharmacological properties of **19** (MCL 145) at the κ and μ opioid receptors as measured with the [^{35}S]GTPγS binding assay. Membranes from CHO cells that stably expressed the μ opioid receptor (A) or κ opioid receptor (B) were incubated with increasing concentrations of **19**.

potent ligands **17** and **19** were further demonstrated in Figures 3−5.

At concentrations from 0.1 to 100 nM, ligand **17** produced an increasing stimulation with a maximal stimulating [^{35}S]GTPγS binding of 60% and 50% (E_{max}) at κ and μ receptors, respectively (Figures 3A and 4A). When membranes were incubated with the κ agonist U50,488 in the presence of varying concentrations of **17**, there was no significant inhibition of U50,488-induced [^{35}S]GTPγS binding (Figure 3B). However, when membranes were incubated with the μ agonist DAMGO in the presence of varying concentrations of **17**, it significantly antagonized DAMGO-induced stimulation (Figure 4B). Altogether, these findings demonstrated that ligand **17** exerts agonist properties at κ and μ receptors and also acts as a μ antagonist.

Pharmacological properties of ligand **19** were determined by incubating membranes from CHO cells with increasing concentrations of **19** (Figure 5). Interestingly, it showed an inverted "U" concentration effect curve. At lower concentration (less than 100 nM), it produced a dose-dependent increase in stimulation at both μ and κ receptors. A strong antagonism of its self-induced stimulation was observed when the concentration of **19** was greater than 100 nM. These findings illustrated that ligand **19** acted as an agonist at both μ and κ receptors at low concentrations, while at higher concentrations, it inhibited its agonist effects and ultimately acted as an inverse agonist as shown by its inhibition of basal [^{35}S]GTPγS binding to both receptors. The reason behind this ability of **19** was not clear.

Conclusion

This study was conducted to evaluate the important structural features of morphinan ligands with mixed μ and κ receptor selectivity. By the choice of cyclorphan (**1**) and MCL 101 (**2**) as the critical pharmacophores, a novel series of dimeric ligands were synthesized by coupling two identical morphinans with spacers with varying lengths. Compounds **6** and **7** with alkyl spacers at the nitrogen position and compounds **8** and **9** in which the two morphinan pharmacophores were coupled by ether moieties at the 3-hydroxyl position showed significantly decreased affinity at all three opioid receptors. An appreciable improvement in affinity was observed by introducing an ester moiety as the spacer. After optimization of the morphinan core structure, the spacer character, and the spacer length, three compounds (**13**, **17**, and **19**) were identified as the most potent ligands within this entire series. Compound **17** displayed a 2-fold higher affinity at μ and κ receptors as the monomeric congener **2**. Since the interaction between the 3-phenolic hydroxyl group and opioid receptor proteins (μ, δ, and κ) was historically recognized as crucial for high affinity, the high potency of these ligands with a 3-position ester spacer may be indicative of the existence of some opioid receptor complex whose pharmacological profiles are distinct from classic μ, δ, and κ receptors. The unexpected high affinity of **12** (the monomeric morphinan) at μ and κ receptors indicated that the spacer may also represent a recognition site and only one pharmacophore was required for such ligands. This observation is in agreement with our and others' recent findings that the phenolic hydroxyl group could be replaced by other functional groups without significant decrease in the opioid receptor activity.[18−20] Studies to further elucidate the function of the spacer itself or to explain the pharmacological difference between monomeric and dimeric ligands are now ongoing.

In the [^{35}S]GTPγS binding assays, ligands **13**, **17**, and **19** and their parent compounds **1** and **2** stimulated [^{35}S]-GTPγS binding mediated by the μ and κ receptors. They produced E_{max} values lower than that of the κ-selective agonist arylacetamide (U50,488), suggesting that these morphinan ligands, like most classic monomeric morphinans, were less efficacious than U50,488 at the κ receptor as measured by the stimulating [^{35}S]GTPγS binding. The most potent ligands **13**, **17**, and **19** behaved differently at μ and κ receptors. Both **13** and **17** were full κ agonists and partial μ agonists. **19** exhibited a unique pharmacological profile; it showed an inverted "U" concentration effect curve. The different pharmacological profile of **19** makes it unlikely that MCL 101 (**2**), a possible metabolite of this dimeric ligand, was responsible for the pharmacological properties observed. The monomeric ligand **12**, compared to its parent **2**, not only retained high affinity but also displayed similar

pharmacological properties at both μ and κ receptors. To establish if such dimeric ligands act as bivalent ligands, it is necessary to prepare additional compounds in this series as well as to undertake further pharmacological investigations. Such studies will be necessary to further rule out the metabolic conversion of these dimeric ligands to the corresponding monomers with a free hydroxyl group, and to address the role of the spacer moiety in compound **12**.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary tube apparatus and are reported uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC300 spectrometer using tetramethylsilane as an internal reference. Element analyses, performed by Atlantic Microlabs, Atlanta, GA, were within ±0.4% of theoretical values. Analytical thin-layer chromatography (TLC) was carried out on 0.2 mm Kieselgel 60F 254 silica gel plastic sheets (EM Science, Newark). Flash chromatography was used for the routine purification of reaction products. The column output was monitored by TLC.

1,3-Bis(3-hydroxymorphinan-17-yl)propane (6, MCL-133). A mixture of norlevorphanol **4** (499 mg, 1.9 mmol), propane-1,3-diyl ditosylate (294 mg, 0.76 mmol), and $NaHCO_3$ (320 mg, 3.8 mmol) in anhydrous DMF (8 mL) was stirred at 110 °C overnight. The solvent was removed in vacuo. The residue was partitioned between ethyl acetate and water. The aqueous phase was extracted with ethyl acetate. The combined extracts were washed with water and brine and then dried over Na_2SO_4. After removal of the solvent, a dark residual oil remained, which was purified by column chromatography on silica gel (EtOAc/MeOH.Et_3N, 5:1:0.1) to give the product **6** as white solid (207 mg, 52%): mp 162−164 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.89 (d, J = 8.1 Hz, 1H), 6.73 (d, J = 2.7 Hz, 1H), 6.61 (dd, J = 2.7, 8.4 Hz, 1H), 2.91−0.89 (complex, 20H). Anal. ($C_{35}H_{46}N_2O_2\cdot H_2O$) C, H, N.

1,6-Bis(3-hydroxymorphinan-17-yl)hexane (7, MCL-134). To a solution of 1,6-hexanediol (2.36 g, 20 mmol) in anhydrous dichloromethane (100 mL) was added triethylamine (8.5 mL, 60 mmol) and tosyl chloride (9.55 g, 50 mmol) at 0 °C. The resulting mixture was stirred at room temperature overnight. After dilution with $CHCl_3$, the layers were separated and the organic phase was washed with brine, dried with Na_2SO_4, and concentrated to give a pale-yellow oil. The crude product was purified by column chromatography on silica gel (EtOAc/MeOH/Et_3N, 50:1:0.1) to afford the corresponding hexane-1,6-diyl ditosylate as a white crystal: mp 72−73 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.78 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.4 Hz, 2H), 3.98 (t, J = 6.6 Hz, 2H), 2.45 (s, 3H), 1.63−1.57 (m, 2H), 1.29−1.24 (m, 2H). Condensation of the ditosylate (225 mg, 0.53 mmol) with **4** (244 mg, 1 mmol) by using the same procedure as in the case of **6** gave product **7** as a white solid (128 mg, 45%): mp 156−158 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.96 (s, 1H), 6.88 (d, J = 8.4 Hz, 1H), 6.63 (d, J = 2.1 Hz, 1H), 6.50 (dd, J = 2.4, 8.4 Hz, 1H), 2.84−0.98 (complex, 22H). Anal. ($C_{38}H_{52}N_2O_2\cdot H_2O$) C, H, N.

1,4-Bis(*N*-cyclobutylmethylmorphinan-3-oxy)butane (8, MCL-153). To a solution of **2** (311 mg, 1 mmol) in anhydrous DMF was added NaH (48 mg, 1.2 mmol) at 0 °C. The mixture was stirred at room temperature for 1 h. Dibromobutane (130 mg, 0.6 mmol) was added dropwise, and the resulting mixture was stirred at room temperature for 24 h. After removal of the solvent, the mixture was diluted with CH_2Cl_2. The organic phase was separated, washed with brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was chromatographed on silica gel with hexanes/ethyl acetate (1:1) as the eluent to give product **8** as pale-yellow oil (250 mg, 72.5%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.00 (d, J = 8.4 Hz, 1H), 6.78 (d, J = 2.4 Hz, 1H), 6.68 (dd, J = 2.4, 8.4 Hz, 1H), 3.98 (t, J = 6.6 Hz, 2H), 2.97−1.25 (complex, 27H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.3, 138.9, 127.2, 125.3, 108.5, 108.2, 63.6, 58.5, 52.9, 42.8, 42.1, 38.9, 34.7, 33.6, 31.9, 30.5, 26.5, 25.0, 24.8, 23.8, 23.5, 21.0, 19.2. Anal. ($C_{46}H_{64}N_2O_2$) C, H, N.

1,4-Bis(*N*-cyclobutylmethylmorphinan-3-oxy)decane (9, MCL-154) was prepared as a pale-yellow oil in 40% yield from **2** (311 mg, 1 mmol) and dibromodecane (180 mg, 0.6 mmol) using a similar procedure as in the case of **8**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.01 (d, J = 8.4 Hz, 1H), 6.78 (d, J = 2.4 Hz, 1H), 6.68 (dd, J = 8.4, 2.4 Hz, 1H), 3.91 (t, J = 6.6 Hz, 2H), 3.01−1.21 (complex, 27H). Anal. ($C_{50}H_{72}N_2O_2$) C, H, N.

General Procedure for the Preparation of Ligands 10−21. To a solution of morphinan **1** or **2** (1 mmol) and Et_3N (0.5 mL) in $CHCl_3$ (10 mL) was added dropwise an appropriate diacid chloride (0.6 mmol) at 0 °C. The mixture was stirred at room temperature for 48 h and then diluted with dichloromethane. The organic layer was separated, washed with saturated Na_2CO_3 and brine, dried with Na_2SO_4, and then concentrated to give a dark oil. The crude product was purified by column chromatography on silica gel (EtOAc/Et_3N, 100:1) to afford the corresponding bivalent ligands.

Bis(*N*-cyclopropylmethylmorphinan-3-yl) succinate 10 (MCL-135): pale-yellow foam (70%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.07 (d, J = 8.1 Hz, 1H), 6.95 (d, J = 2.1 Hz, 1H), 6.85 (dd, J = 2.4, 8.4 Hz, 1H), 3.10−0.11 (complex, 26H). Anal. ($C_{45}H_{58}N_2O_4\cdot 0.5H_2O$) C, H, N.

Bis(*N*-cyclopropylmethylmorphinan-3-yl) sebacoylate 11 (MCL-136): pale-yellow foam (76.2%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.07 (d, J = 8.4 Hz, 1H), 6.93 (s, 1H), 6.83 (d, J = 8.1 Hz, 1H), 3.09−0.11 (complex, 31H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.5, 149.5, 142.3, 135.4, 128.6, 118.7, 118.3, 60.2, 55.9, 45.8, 45.2, 42.0, 38.1, 36.8, 34.6, 29.2, 27.0, 26.7, 25.0, 24.5, 22.3, 9.7, 4.2, 3.8. Anal. ($C_{50}H_{68}N_2O_4\cdot {}^1/_2H_2O$) C, H, N.

Methyl(*N*-cyclobutylmethylmorphinan-3-yl) sebacate 12 (MCL-176) was prepared as a pale-yellow oil (230 mg, 88%) from **2** (155 mg, 0.5 mmol), Et_3N (0.2 mL), and methyl 10-chloro-10-oxodecanoate (175.5 mg, 0.75 mmol) by using a similar procedure as in the preparation of **11**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.09 (d, J = 8.1 Hz, 1H), 6.91 (d, J = 2.7 Hz, 1H), 6.84 (dd, J = 8.4, 2.7, 1H), 3.66−1.32 (complex, 46H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.4, 172.6, 149.6, 142.0, 134.9, 128.7, 118.8, 118.4, 61.1, 55.8, 51.6, 45.7, 44.4, 41.4, 37.7, 36.6, 34.6, 34.5, 34.2, 28.1, 28.0, 26.8, 26.6, 25.1, 25.0, 24.6, 22.2, 19.0. Anal. ($C_{33}H_{49}NO_4\cdot H_2O$) C, H, N.

Bis(*N*-cyclobutylmethylmorphinan-3-yl) succinate 13 (MCL-139): pale-yellow foam (23%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.06 (d, J = 8.4 Hz, 1H), 6.91 (s, 1H), 6.83 (dd, J = 1.8, 8.1 Hz, 1H), 3.01−1.28 (complex, 28H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.0, 149.2, 142.3, 135.6, 128.7, 123.7, 118.6, 118.2, 61.8, 56.1, 46.0, 45.1, 42.0, 38.1, 36.8, 35.2, 29.7, 28.2, 27.1, 26.8, 24.7, 22.4, 19.2. Anal. ($C_{47}H_{62}N_2O_4\cdot 0.5H_2O$) C, H, N.

Bis(*N*-cyclobutylmethylmorphinan-3-yl) glutamate 14 (MCL-140): pale-pink foam (41%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.09 (d, J = 8.4 Hz, 1H), 6.94 (d, J = 2.7 Hz, 1H), 6.84 (dd, J = 2.7, 8.4 Hz, 1H), 3.01−1.25 (complex, 29H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.8, 149.3, 142.4, 135.6, 128.7, 118.6, 118.3, 61.7, 56.0, 45.9, 45.1, 42.0, 38.0, 36.7, 35.1, 33.5, 28.0, 27.0, 26.7, 24.6, 22.3, 20.2, 19.0. Anal. ($C_{48}H_{64}N_2O_4\cdot 1.5H_2O$) C, H, N.

Bis(*N*-cyclobutylmethylmorphinan-3-yl) suberate 15 (MCL-177): pale-yellow oil (70.9%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.09 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 2.1 Hz, 1H), 6.84 (dd, J = 8.4, 2.1 Hz, 1H), 3.01−1.25 (complex, 32H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.5, 149.4, 142.3, 135.5, 128.7, 118.6, 118.3, 61.7, 56.0, 45.9, 45.1, 42.0, 38.0, 36.8, 35.2, 34.5, 28.9, 28.0, 27.0, 26.7, 24.9, 24.6, 22.3, 19.0. Anal. ($C_{51}H_{70}N_2O_4$) C, H, N.

Bis(*N*-cyclobutylmethylmorphinan-3-yl) azelaoate 16 (MCL-179): pale-yellow foam (42%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.08 (d, J = 8.4 Hz, 1H), 6.92 (d, J = 2.1 Hz, 1H), 6.83 (dd, J = 8.4, 2.1 Hz, 1H), 3.01−1.19 (complex, 33H). Anal. ($C_{52}H_{72}N_2O_4\cdot 0.5H_2O$) C, H, N.

Bis(*N*-cyclobutylmethylmorphinan-3-yl) sebacoylate 17 (MCL-144): pale-yellow foam (47%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.07 (d, J = 8.4 Hz, 1H), 6.89 (s, 1H), 6.81 (dd, J = 8.4, 2.1 Hz, 1H), 2.97 (d, J = 18.3 Hz, 1H), 2.79 (s, 1H), 2.61−1.25 (complex, 32H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.4, 149.3, 142.2, 135.4, 128.6, 118.6, 118.3, 61.8, 56.1, 46.0, 45.2, 42.1,

38.1, 36.9, 35.3, 34.7, 29.4, 29.3, 28.2, 27.1, 26.9, 25.2, 24.8, 22.5, 19.2. Anal. ($C_{53}H_{74}N_2O_4 \cdot 0.4Et_3N$) C, H, N.

Bis(*N*-cyclobutylmethylmorphinan-3-yl) dodecanedioate 18 (MCL-178): pale-yellow oil (60.3%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.09 (d, J = 8.4 Hz, 1H), 6.91 (d, J = 2.1 Hz, 1H), 6.83 (dd, J = 7.8, 2.1 Hz, 1H), 3.01−0.86 (complex, 36H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.4, 149.2, 142.0, 135.2, 128.4, 118.4, 118.1, 61.5, 55.8, 45.6, 44.9, 41.7, 37.7, 36.5, 34.9, 34.4, 29.3, 29.2, 29.1, 27.8, 26.7, 26.5, 24.9, 24.3, 22.1, 18.8. Anal. ($C_{55}H_{78}N_2O_4$) C, H, N.

Bis(*N*-cyclobutylmethylmorphinan-3-yl) fumarate 19 (MCL-145): pale-yellow foam (45%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.18 (s, 1H), 7.12 (d, J = 8.4, 1H), 6.98 (d, J = 2.1 Hz, 1H), 6.91 (dd, J = 2.1, 8.1 Hz, 1H), 3.04−1.25 (complex, 26H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.5, 148.9, 142.5, 136.1, 134.6, 128.8, 118.3, 118.0, 61.8, 56.1, 46.0, 45.1, 42.0, 38.1, 36.8, 35.2, 28.2, 27.1, 26.8, 24.8, 22.4, 19.2. Anal. ($C_{47}H_{60}N_2O_4 \cdot 0.5Et_3N \cdot H_2O$) C, H, N.

Bis(*N*-eyclobutylmethylmnorphinan-3-yl) terephthate 20 (MCL-141): pale-yellow foam (52%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.32 (s, 2H), 7.17 (d, J = 8.1 Hz, 1H), 7.09 (d, J = 2.1 Hz, 1H), 7.00 (dd, J = 2.4, 8.1 Hz, 1H), 3.03 (d, J = 18.6 Hz, 1H), 2.83 (s, 1H), 2.83−1.25 (complex, 26H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.7, 149.4, 142.6, 136.3, 136.0, 134.2, 130.4, 128.9, 118.8, 118.3, 61.7, 56.1, 45.9, 45.1, 42.0, 38.1, 36.8, 35.1, 28.0, 27.0, 26.7, 24.7, 22.4, 19.0. Anal. ($C_{51}H_{62}N_2O_4 \cdot 0.5H_2O$) C, H, N.

Bis(*N*-cyclobutylmethylmorphinan-3-yl) isophthalate 21 (MCL-142): pale-yellow foam (60%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.02 (t, J = 1.8 Hz, 1H), 8.45 (dd, J = 1.8, 8.1 Hz, 2H), 7.67 (t, J = 8.1 Hz, 1H), 7.16 (d, J = 8.4 Hz, 2H), 7.09 (d, J = 2.1 Hz, 2H), 7.00 (dd, J = 2.1, 8.4 Hz, 2H), 3.03 (d, J = 18.6 Hz, 2H), 2.83 (s, 2H), 2.66−1.26 (complex, 24H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.7, 149.4, 142.6, 136.0, 135.0, 131.9, 130.7, 129.2, 128.9, 118.7, 118.4, 61.7, 56.0, 45.9, 45.1, 42.0, 38.1, 36.8, 35.1, 28.0, 27.0, 26.7, 24.7, 22.3, 19.0. Anal. ($C_{51}H_{62}N_2O_4 \cdot 0.5H_2O$) C, H, N.

***cis*-1,2-(*N*-Cyclobutylmethylmorphinan-3-yl)cyclopropyl Dicarboxylate 22 (MCL-143).** 3-Oxabicyclo[3.1.0]hexane-2,4-dione (500 mg, 4.46 mmol) was refluxed in water (2 mL) for 1 h. The solvent was evaporated in vacuo to give cis-1,2-cyclopropyldicarboxylic acid as a white solid (550 mg, 95%): mp 135−138 °C [lit.[17] 139 °C]. The prepared 1,2-cyclopropyldicarboxyl acid (65 mg, 0.5 mmol), morphinan **2** (311 mg, 1 mmol), and a catalytic amount of 4-dimethylaminopyridine were dissolved in a solution of dichloromethane (5 mL) and cooled to 0 °C. A solution of *N*,*N*′-dicyclohexylcarbodiimide (206 mg, 1 mmol) in dichloromethane (2 mL) was added slowly. After being stirred for 48 h at room temperature, the reaction mixture was filtered and evaporated to dryness. Purification of the crude product by column chromatography gave product **22** as pale-yellow foam (210 mg, 57.5%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.03 (d, J = 8.4 Hz, 1H), 6.91 (t, J = 2.1 Hz, 1H), 6.82 (m, 1H), 2.96 (d, J = 18.6 Hz, 1H), 2.80 (dd, J = 3.0, 5.4 Hz, 1H), 2.59−1.0 (complex, 26H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 168.8, 168.7, 149.4, 149.3, 142.3, 135.6, 128,7, 118.6, 118.3, 118.2, 61.7, 56.0, 45.9, 45.1, 41.9, 37.9, 36.7, 35.1, 28.0, 26.9, 26.7, 24.6, 22.4, 22.2, 22.1, 19.0, 12.6. Anal. ($C_{48}H_{62}N_2O_4 \cdot H_2O$) C, H, N.

Opioid Binding to the Human μ, δ, and κ Opioid Receptors. Chinese hamster ovary (CHO) cells stably transfected with the human κ opioid receptor (hKOR-CHO), δ-opioid receptor (hDOR-CHO), and the μ-opioid receptor (hMOR-CHO) were obtained from Drs. Larry Toll (SRI International, Palo Alto, CA) and George Uhl (NIDA Intramural Program, Bethesda, MD), respectively. The cells were grown in 100 mm dishes in Dulbecco's modified Eagle's media (DMEM) supplemented with 10% fetal bovine serum (FBS) and penicillin−streptomycin (10 000 units/mL) at 37 °C in a 5% CO_2 atmosphere. The affinity and selectivity of the compounds for the multiple opioid receptors were determined by incubating the membranes with radiolabeled ligands and 12 different concentrations of the compounds at 25 °C in a final volume of 1 mL of 50 mM Tris-HCl, pH 7.5. Incubation times of 60 min were used for the μ-selective peptide [^{3}H]DAMGO and the κ-selective ligand [^{3}H]U69,593. A 3 h incubation was used with the δ-selective antagonist [^{3}H]naltrindole. To determine the IC_{50} values for the inhibition of binding by the compounds, the final concentrations of [^{3}H]DAMGO, [^{3}H]naltrindole, and [^{3}H]U69,593 were 0.25, 0.2, and 1 nM, respectively. Nonspecific binding was measured by inclusion of 10 μM naloxone. Binding was terminated by filtering the samples through Schleicher & Schuell no. 32 glass fiber filters (Keene, NH) using a Brandel 48-well cell harvester. Filters were soaked for at least 60 min in 0.25% polyethylenimine for [^{3}H]naltrindole and [^{3}H]U69,593 binding experiments. After filtration, filters were washed three times with 3 mL of cold 50 mM Tris-HCl, pH 7.5, and were counted in 2 mL of Ecoscint A scintillation fluid. The K_i values of unlabelled compounds were calculated from the equation $K_i = IC_{50}/(1 + S)$, where S = (concentration of radioligand)/(K_D of radioligand).[21]

[^{35}S]GTPγS Binding Studies To Measure Coupling to G Proteins. Membranes from CHO cells stably expressing either the human κ or μ opioid receptor were used in the experiments. Cells were scraped from tissue culture plates and then centrifuged at 1000*g* for 10 min at 4 °C. The cells were resuspended in phosphate-buffered saline, pH 7.4, containing 0.04% EDTA. After centrifugation at 1000*g* for 10 min at 4 °C, the cell pellet was resuspended in membrane buffer, which consisted of 50 mM Tris-HCl, 3 mM $MgCl_2$, and 1 mM EGTA, pH 7.4. The membranes were homogenized by with a Dounce homogenizer, followed by centrifugation at 40000*g* for 20 min at 4 °C. The membrane pellet was resuspended in membrane buffer, and the centrifugation step was repeated. The membranes were then resuspended in assay buffer, which consisted of 50 mM Tris-HCl, 3 mM $MgCl_2$, 100 mM NaCl, and 0.2 mM EGTA, pH 7.4. The protein concentration was determined by the Bradford assay[22] using bovine serum albumin as the standard. The membranes were frozen at −80 °C until use.

CHO cell membranes expressing either the human κ opioid receptor (15 μg of protein per tube) or μ opioid receptor (7.5 μg of protein per tube) were incubated with 12 different concentrations of the agonist in assay buffer for 60 min at 30 °C in a final volume of 0.5 mL. The reaction mixture contained 3 μM GDP and 80 pmol of [^{35}S]GTPγS. Basal activity was determined in the presence of 3 μM GDP and in the absence of an agonist, and nonspecific binding was determined in the presence of 10 μM unlabeled GTPγS. Then, the membranes were filtered onto glass fiber filters by vacuum filtration, followed by three washes with 3 mL of ice-cold 50 mM Tris-HCl, pH 7.5. Samples were counted in 2 mL of Ecoscint A scintillation fluid. Data represent the percent of agonist-stimulation [^{35}S]GTPγS binding over the basal activity, defined as [(specific binding/basal binding) × 100] − 100. All experiments were repeated at least three times and were performed in triplicate. To determine antagonist activity of a compound at the μ opioid receptors, CHO membranes expressing the μ opioid receptor were incubated with the compound in the presence of 200 nM of the μ agonist DAMGO. To determine antagonist activity of a compound at the κ opioid receptors, CHO membranes expressing the κ opioid receptor were incubated with the compound in the presence of 100 nM of the κ agonist U50,488.

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