

Univalent and Bivalent Ligands of Butorphan: Characteristics of the Linking Chain Determine the Affinity and Potency of Such Opioid Ligands[†]

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Bivalent morphinan compounds containing ester linkers were synthesized and their binding affinities at the μ , δ , and κ opioid receptors determined. Addition of methyl groups adjacent to the hydrolytically labile ester linkage increased stability while only partially affecting binding affinity. The resulting bivalent ligands with optimized spacer length and structure show potent binding profiles with the most potent compound (**4b**), having K_i values of 0.47 nM for both the μ and κ opioid receptors, and **4a**, having K_i values of 0.95 and 0.62 nM for the μ and κ receptors, respectively. Both **4a** and **4b** were partial agonists at the κ and μ receptors in the [³⁵S]GTP γ S binding assay.

Introduction

Previous reports from our laboratory have shown that bivalent morphinan (butorphan) ligands, where two identical (“homobivalent”) or two distinct (“heterobivalent”) morphinan ligands are connected by a molecular spacer, can lead to compounds with improved binding affinities and selectivities.^{1–3} The binding profile of the bivalent ligand is strongly dependent on the molecular structure of the opioid pharmacophore and on the length of the connecting spacer, with highest affinities obtained with 10 carbon atoms separating the morphinan structure.^{1,2} In our previous studies, various methods to connect the two opioid pharmacophores were investigated, the most active compounds at the opioid receptors were connected via a 10-carbon linking ester chain (Table 1, **10** (MCL-144) $K_i = 0.090$ nM at μ ; $K_i = 0.49$ nM at κ).² It has been suggested that such bivalent ligands containing ester groups produced their affinities by the hydrolysis to the active pharmacophore butorphan during the assay.

We thus wished to further investigate the factors that lead to high affinity in this series of bivalent morphinan ligands. To decrease the rate of hydrolysis, we modified the linker by introducing methyl groups in the α -position (and α' -position, respectively) of the linking dicarboxylic acid, this based in part on the observation that steric hindrance decreases the rate of hydrolysis of esters.⁴ A similar observation has been observed for a series of phenyl esters that underwent aminolysis, in which the rate decreased dramatically with α methyl substitution.⁵ A more recent study investigated methods for increasing the oral bioavailability of otherwise potent orally unavailable drugs by synthesizing prodrugs with ester functionalities.⁶ These investigators found that increased half-lives of esters branched with methyl groups in the acid moiety could be

observed both at pH = 1 ($t_{1/2}$ CH₃/iso-propyl/*tert*-butyl = 1/2/10) and pH = 10 ($t_{1/2}$ CH₃/iso-propyl/*tert*-butyl = 1/4.6/16).⁶

The possible decrease in the hydrolytic reactivity of esters of bivalent ligands containing branched spacers with esterases is important for such bivalent morphinans. By reducing the reactivity toward esterases, the effect of the ligand in contrast to the hydrolysis product butorphan could be assessed. In one study, the enzymatic rates of hydrolysis of a series of phenyl esters were determined using α -chymotrypsin and carboxylesterase. It was found that phenyl acetate is hydrolyzed much faster than phenyl trimethylacetate.⁷ Studies showing increased half-lives of highly substituted phenyl esters of phenolic drugs with different degrees of substitution in the acid moiety at physiological pH (7.4), in human plasma, and in pig liver homogenate may enable potential usage as prodrugs.⁸

We have chosen to modify the high affinity opioid agonist butorphan⁹ (MCL-101, Figure 1), which was prepared from commercially available (–)-3-hydroxy-*N*-methyl-morphinan (levorphanol). Butorphan possesses mixed κ agonist and μ agonist/antagonist binding properties with low affinity to the δ subtype,¹⁰ potentially useful for application in cocaine abuse therapy.^{11,12}

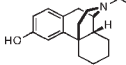
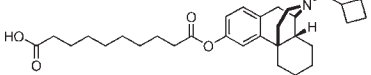
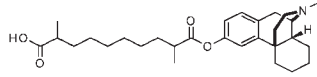
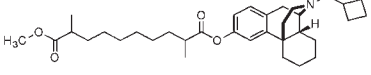
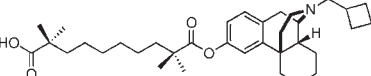
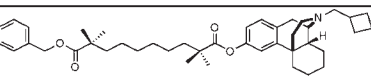
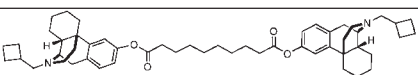
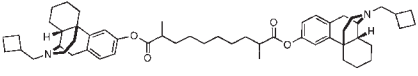
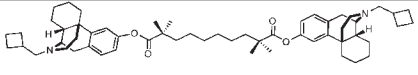
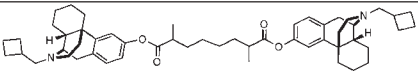
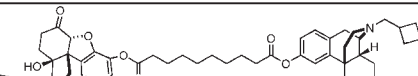
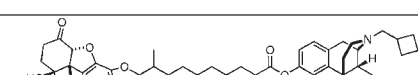
To investigate the in vitro sensitivity toward hydrolysis, the stability of the most potent bivalent ligand in buffer and in the presence of an esterase was determined. Furthermore, the affinity of compounds consisting of one butorphan unit plus the spacer (which would also be the first hydrolysis product) was determined to obtain information on how the affinity of these compounds might contribute to the affinity in the binding assay and especially to see how the spacer contributes to the binding at the receptor.

Chemistry. For the synthesis of α, α' -dimethylated dicarboxylic acid (**3a, 3c**), several synthetic approaches were investigated, e.g., hydrocarboxylation of α, ω -dienes in the presence of copper and palladium salts.¹³ The most straightforward method to obtain these dicarboxylic acids was to

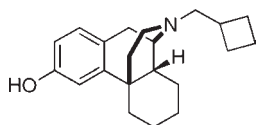
[†]Contribution to celebrate the 100th anniversary of the Division of Medicinal Chemistry of the American Chemical Society.

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Table 1. K_i Values for the Inhibition of μ , δ , and κ Opioid Binding to CHO Membranes by Uni- and Bivalent Ligands

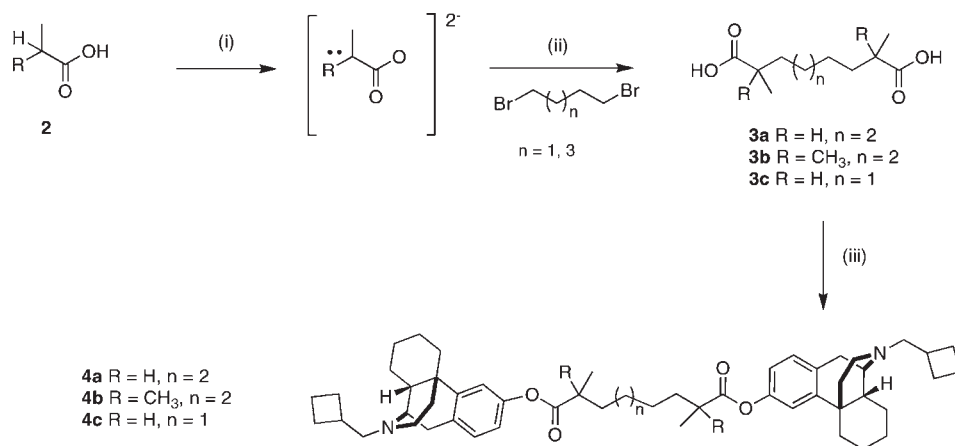
Compound/Structure	K_i (nM) \pm SE			Selectivity
	μ	δ	κ	
Butorphan ^a 	0.23 \pm 0.01	5.9 \pm 0.55	0.079 \pm 0.003	3/75/1
9 ^b 	0.71 \pm 0.02	18 \pm 0.6	0.29 \pm 0.02	2.4/62/1
7a 	0.46 \pm 0.024	20 \pm 0.074	0.42 \pm 0.0081	1/48/1
6a 	1.7 \pm 0.11	42 \pm 2.8	0.94 \pm 0.02	1.8/45/1
7b 	47 \pm 3.1	390 \pm 11	18 \pm 0.48	3/22/1
6c 	8.6 \pm 0.63	210 \pm 30	4.7 \pm 0.68	1.8/45/1
10 ^c 	0.090 \pm 0.004	4.2 \pm 0.4	0.049 \pm 0.0001	2/90/1
4a 	0.95 \pm 0.057	37 \pm 1.8	0.62 \pm 0.071	1.5/60/1
4b 	0.47 \pm 0.027	15 \pm 0.86	0.47 \pm 0.046	1/32/1
4c 	2.9 \pm 0.091	50 \pm 2.6	2.2 \pm 0.096	1.3/23/1
11 ^a 	0.43 \pm 0.001	39 \pm 2	0.13 \pm 0.03	3.3/300/1
8 	3.7 \pm 0.36	130 \pm 5.4	3.4 \pm 0.22	1/38/1

^a Reference 9. ^b Reference 1. ^c Reference 2.

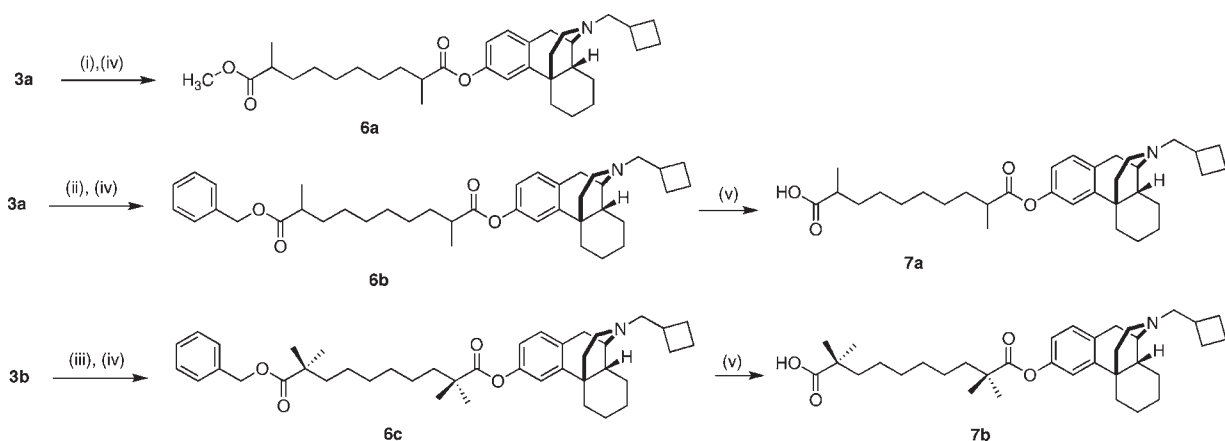
**Figure 1.** Structure of the opioid ligand butorphan (MCL-101).

deprotonate the α -carbon of 2 mol of propionic acid (α, α' -dimethyldicarboxylic acids **3a, 3c**) or isobutyric acid ($\alpha, \alpha, \alpha', \alpha'$ -tetramethyldicarboxylic acid **3b**¹⁴) using lithium diisopropylamide, followed by alkylation with dibromoalkanes (Scheme 1).

The dicarboxylic acids **3a, 3b, 3c** (1 mol) were activated to the respective dicarboxylic acid chlorides, which were then coupled to butorphan (2 mols) to yield the homobivalent compounds **4a, 4b, 4c**.² To obtain compounds containing only linker plus butorphan (**7a, 7b**) (Scheme 2), reacting equimolar amounts was not an effective method because small amounts of bivalent ligands were always formed. Protecting one carboxylic group of the diacids with a benzyl group to yield the monobenzyesters **5b, 5c**, reaction with butorphan to yield **6b, 6c**, followed by deprotection using catalytic hydrogenolysis, gave the corresponding acids (**7a, 7b**).

Scheme 1. Synthesis of Homobivalent Butorphan Ligands with Spacers Branched in α -Position^a

^a Reagents and conditions: (i) 2 equiv acid, 4 equiv LiN(*i*-Pr)₂, THF/heptane/ethylbenzene, -25 to 50 °C; (ii) cool to -30 °C, addition of dibromide, warm to 40 °C for 1 h; (iii) 4 equiv (COCl)₂/CH₂Cl₂/cat. DMF, concentration in vacuo, then 2 equiv butorphan/CH₂Cl₂/Et₃N, rt 24 h.

Scheme 2. Synthesis of Univalent Butorphan Ligands **6a–c** and **7a–b**^a

^a Reagents and conditions: (i) MeOH, DCC, DMAP, CHCl₃, rt, 24 h; (ii) C₆H₅CH₂OH, DCC, cat. DMAP, CHCl₃, rt, 24 h; (iii) C₆H₅CH₂Br, Bu₄NF, THF, rt, 24 h; (iv) 2 equiv (COCl)₂/CH₂Cl₂/cat. DMF, concentration in vacuo, then butorphan/CH₂Cl₂/Et₃N, rt 24 h; (v) 10% Pd/C, H₂, 1 atm, MeOH/EtOH, rt.

Monobenylation of α, α' -dimethyldicarboxylic acids could be easily achieved using DCC/DMAP^a and benzyl alcohol.¹⁵ Monobenylation of $\alpha, \alpha, \alpha', \alpha'$ -tetramethyldicarboxylic acid to yield **5c** could not be achieved this way due to steric hindrance. Also activation of the acid with *N, N'*-carbonyldiimidazole (CDI) did not lead to product formation and activation, as the acid chloride gave yields < 50%. Quantitative monoesterification was achieved using benzylbromide in the presence of tetrabutylammonium fluoride in THF.¹⁶ The methyl ester of the α, α' -dimethyldicarboxylic acid with butorphan (**6a**) was also prepared and together with the corresponding benzylester (**6c**) was included in the pharmacological evaluation. Because the highest affinities for unhindered bivalent butorphan esters were observed for a C10 and a C4 spacer, respective hindered C4 bivalent ligands would need 2,3-dimethyl or tetramethyl succinic acid as spacers.¹ Interestingly, neither coupling of 2,3-dimethyl nor tetramethylsuccinic acid chlorides with butorphan leads to any product formation.

The butorphan ester **7a**, when coupled with the opioid antagonist naloxone via the in situ generated acid chloride as in the schemes above, led to the heterobivalent ligand **8**.¹⁷

Binding Affinity. The affinity and selectivity of the compounds synthesized were evaluated for their binding affinities at all three opioid receptor types (μ , δ , and κ) using a previously described procedure (Table 1).¹

The introduction of methyl groups on the carbon atom adjacent to the carbonyl atom of the ester had an effect on the binding affinities and selectivity. Both compounds with one methyl group at the α -position (the dimethyl-compound **4a** and the tetramethyl compound **4b**) were approximately 10 times less active than the unsubstituted C10 bivalent ligand (**10**) with a binding affinity of 0.049 nM at the κ receptor.² The dimethyl ligand **4a** was marginally less selective for the κ receptor versus the μ receptor than **10**. The tetramethyl ligand **4b** was equipotent at both κ and μ receptors. Binding affinities at the μ and δ receptors showed no coherent tendencies. At the μ receptor, the binding affinity decreased for the dimethyl compound (**4a**) but further increased slightly for the tetramethyl compound (**4b**) with a binding affinity of 0.47 nM. Dramatic effects were observed with regard to binding affinity and selectivity at the δ opioid

^a Abbreviations: DCC, dicyclohexyl carbodiimide; DMAP, 4-dimethylaminopyridine; DMF, dimethylformamide; HPLC, high performance liquid chromatography; TFA, trifluoroacetic anhydride; THF, tetrahydrofuran.

Table 2. [³⁵S]GTPγS Binding by Uni- and Bivalent Ligands Mediated by the μ Opioid Receptor^a

μ agonist Effects	EC ₅₀ (nM) ± SEM	E _{max} (% stimulation) ± SEM	functional activity
butorphan	1.6 ± 0.15	50 ± 2.5	partial agonist
9	3.0 ± 0.64	110 ± 8.1	agonist
7a	13 ± 1.4	25 ± 3.9	partial agonist
10	1.3 ± 0.15	51 ± 6.5	partial agonist
4a	7.1 ± 3.1	26 ± 1.9	partial agonist
4b	11 ± 1.2	40 ± 6.1	partial agonist
11	2.0 ± 0.54	34 ± 1.2	partial agonist

μ antagonist effects	IC ₅₀ (nM) ± SEM	I _{max} (% inhibition) ± SEM
butorphan	20 ± 2.7	50 ± 2.6
9	no inhibition	no inhibition
7a	NA	48 ± 6.4 at 10 μM
10	16 ± 3.0	44 ± 5.1
4a	140 ± 17	50 ± 6.1
4b	81 ± 16	50 ± 0.59
11	25 ± 1.6	73 ± 1.2

^aTo determine agonist effects, 12 different concentrations of the compounds were incubated with CHO cells that expressed the μ opioid receptor. Antagonist activity was determined by measuring the inhibition of [³⁵S]GTPγS binding by the compounds. For antagonist studies, [³⁵S]GTPγS binding was stimulated with 200 nM DAMGO. The stimulation observed with DAMGO was set at 100% control binding.

receptor. Introduction of a α-methyl group (**4a**) led to a 4-fold decrease in binding affinity, whereas introduction of a α-dimethyl group (**4b**) only slightly decreased binding affinity relative to the unsubstituted bivalent ligand **10**. The dimethyl ligand **4a** lost some selectivity for μ and κ receptors versus the δ receptor, but for the tetramethyl bivalent ligand **4b**, 32-fold selectivity at κ (and μ) over δ was obtained. As in the homobivalent series, introduction of a α-methyl group also reduces the affinity of heterobivalent ligands (**11** vs **8**) by a factor of 9 at the μ opioid receptor and 26 at the κ opioid receptor (Table 1).

We have shown that the second pharmacophore is not always necessary to achieve binding to the opioid receptors.¹ This was confirmed in this study as the univalent compounds **6a** and **7a** have good binding affinities to the μ and κ opioid receptors when compared with the corresponding bivalent ligands **4a** and **4b**.

The presence of an acidic carboxylic acid in the linker chain does not appear to have any dramatic effect on the binding affinities, as methylation of **7a** to produce **6a** only decreased binding affinity by a factor of 4 at μ and a factor of 2 at the κ and δ receptors. The poor binding of **7b** could be only partially restored by the addition of a benzyl group at the terminal carboxylic acid. The benzyl ester, **6c**, of **7b** had 5 times stronger binding affinity at the μ opioid receptor than **7b**.

Efficacy of Selected Ligands. The agonist and antagonist properties of these ligands in stimulating [³⁵S]GTPγS binding mediated by the μ opioid receptor are shown in Table 2.

Ligand **9** produced maximal stimulation of [³⁵S]GTPγS binding mediated by the μ receptor while it produced no inhibition (I_{max}) of the DAMGO stimulated [³⁵S]GTPγS binding comparable to that of butorphan. These data indicate that **9** is a full μ agonist. Bivalent ligands **4a** and **4b** were partial agonists. Ligands **7a**, **4a**, **4b**, and **11** produced less maximal stimulation of [³⁵S]GTPγS binding (E_{max}), comparable to that of butorphan. The EC₅₀ values of the ligands are slightly lower than butorphan, suggesting that these ligands are less potent than butorphan.

The agonist and antagonist properties of these ligands in stimulating [³⁵S]GTPγS binding mediated by the κ receptor are shown in Table 3.

Ligands **4a**, **4b**, **7a**, **9**, and **11** produced similar minimal stimulation of [³⁵S]GTPγS binding mediated by the κ receptor, while they produced no inhibition of [³⁵S]GTPγS binding mediated by the κ receptor. These data indicate that these ligands are all κ agonists. The univalent ligand **9** was a full agonist at both the μ and κ opioid receptors.

Hydrolytic Stability. The hydrolysis results (Table 4) indicate that introduction of α-dimethyl groups does indeed increase the stability of the bivalent ligands toward in vitro hydrolysis by at least a factor of 2. The presence of the α-methyl groups appears to have no effect on the enzymatic rate of hydrolysis, as the half-life of all bivalent ligands in the presence of porcine liver esterase is approximately the same. Typical binding experiments involve incubating the ligands at 25 °C in a CHO membrane homogenate for 60 min. The hydrolysis results, conducted at physiological-like conditions of 37 °C, show that the compounds investigated would be stable during the time course of the binding experiments. It is generally accepted that the rate of a reaction doubles with an increase in temperature of 10 °C. As such, it can be predicted that the rate of hydrolysis of the bivalent esters at 25 °C will be approximately one-half of that at 37 °C. It is clear that the activity measured during the binding affinity experiments is due to the bivalent ligands and not to butorphan produced by hydrolysis. While the introduction of steric hindrance can reduce the amount of hydrolysis, chain length also appears to be a factor, as shown by **4c** being surprisingly more stable than its C10 congener **4a**.

The hydrolysis rate results agree with a previous study where the mono phenyl ester of 2-methylsuccinic acid was 43 times more stable at pH 7.4 than mono phenyl succinate.⁸ It has also been shown that the introduction of α gem-dimethyl groups onto the phenyl ester of 4-pentenoic acid increases its hydrolytic stability in acidic solutions by a factor of 2 and in alkaline solution by a factor of 215.¹⁸

In summary, our data suggests that the introduction of α-methyl groups increases the hydrolytic stability of the bivalent ligands and has the effect of decreasing the binding affinities to opioid receptors for the di- as well as for the tetra-substituted compound. The difference in binding affinities could be due to the inherent properties of the bivalent ligands themselves and not due to hydrolytic products that may develop during the binding assay.

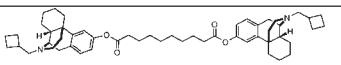
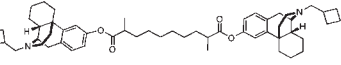
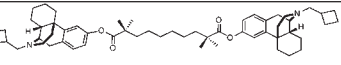
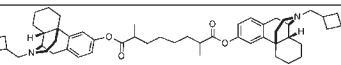
Table 3. [³⁵S]GTPγS Binding by Uni- and Bivalent Ligands Mediated by the κ Opioid Receptor^a

κ agonist effects	EC ₅₀ (nM) ± SEM	E _{max} (% stimulation) ± SEM	functional activity
butorphan	1.3 ± 0.44	80 ± 6.8	agonist
9	2.8 ± 0.43	75 ± 1.1	agonist
7a	4.9 ± 0.82	58 ± 7.4	agonist
10	0.85 ± 0.053	65 ± 0.68	agonist
4a	7.3 ± 1.6	54 ± 3.5	agonist
4b	6.4 ± 0.87	60 ± 8.0	agonist
11	1.6 ± 0.23	86 ± 7.3	agonist

κ antagonist effects	IC ₅₀ (nM) ± SEM	I _{max} (% inhibition) ± SEM
butorphan	NT	NT
9	no inhibition	no inhibition
7a	no inhibition	no inhibition
10	no inhibition	no inhibition
4a	no inhibition	no inhibition
4b	no inhibition	no inhibition
11	no inhibition	no inhibition

^aTo determine agonist effects, 12 different concentrations of the compounds were incubated with CHO cells that expressed the κ opioid receptor. Antagonist activity was determined by measuring the inhibition of [³⁵S]GTPγS binding by the compounds. For antagonist studies, [³⁵S]GTPγS binding was stimulated with 100 nM U50,488. The stimulation observed with U50,488 was set at 100% control binding.

Table 4. Hydrolysis of Bivalent Ligands

Compound	t _{1/2} (minutes) 37 °C	
	Buffer pH 7.4	Liver esterase
10 	46	110
4a 	83	131
4b 	101	100
4c 	221	114

Experimental Section

General Synthetic Methods. ¹H and ¹³C NMR spectra were recorded at 300 MHz using CDCl₃ or DMSO-*d*₆ on a Varian Mercury 300 spectrometer. Chemical shifts are given as δ value (ppm) downfield from tetramethylsilane as an internal reference. Melting points were determined on a Thomas–Hoover capillary tube apparatus and are reported uncorrected. LC-MS and HRMS was performed on an Agilent 6210 time-of-flight spectrometer using an ESI source, with the LC being performed on an Agilent 1200 series system with an autosampler and the mobile phases A: H₂O with 0.1% formic acid; B: acetonitrile with 0.1% formic acid. All target compounds showed one peak only in the LC prior to MS. Elemental 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 μm Kieselgel 60F-254 silica gel plastic sheets (EM Science, Newark, NJ). Flash chromatography was used for the routine purification of reaction products. Eluent systems are described for the individual compounds.

General Procedure for the Preparation of α,α'-Dimethyl- and α,α,α',α'-Tetramethyldicarboxylic Acids (3a,b,c). The α,α'-dimethyldicarboxylic acids were prepared in a similar fashion as described for α,α,α',α'-tetramethyldicarboxylic acids.¹⁴ In an ice/acetone bath, 42 mL of a 2.0 M solution of lithium diisopropylamide (84 mmol) in heptane/THF/ethylbenzene was cooled to −25 °C. Very slowly (caution: vigorous reaction!) 35 mmol of propionic acid (for preparation of **3a** and **3c**) or isobutyric acid

(for preparation of **3b**) were added so that the temperature did not rise over −20 °C. The mixture was vigorously stirred and after addition of acid the mixture was slowly warmed to 50 °C. After ~30 min, a yellow precipitate had formed. After warming for 2 h at 50 °C, the mixture was cooled to −30 °C and 1,6-dibromohexane (for preparation of **3a** and **3b**) or 1,4-dibromobutane (for preparation of **3c**) were added dropwise. After addition, the temperature was raised to ~40 °C, while the suspension lost its brown color and became yellow. Stirring at 40 °C was continued for 1 h, after which concentrated hydrochloric acid was added under ice-cooling until pH = 1. The mixture was extracted three times with dichloromethane. The combined organic phases were washed three times with 10% hydrochloric acid (20 mL) and twice with 100 mL of water. The organic phase was dried over Na₂SO₄ and the solvent evaporated. High vacuum was used to remove residues of ethylbenzene. The brown oily residue was recrystallized from ethyl acetate to yield colorless crystals.

2,9-Dimethyldecanedioic Acid (3a). Yield 23%; mp: 104 °C. ¹H (DMSO-*d*₆): 12.00 (bs, 2H, COOH), 2.28 (sex, 2H, *J* = 7 Hz, CH), 1.52 (qui, 2H, CH₂CH), 1.35 (qui, 2H, *J* = 7 Hz, CH₂CH), 1.28 (bs, 8H, CH₂), 1.03 (d, 6H, *J* = 7 Hz, CH₃) ppm. ¹³C (DEPT, DMSO-*d*₆): 178.19 (COOH), 39.37 (CH), 33.93 (CH₂), 29.54, 27.29, 17.66 (CH₃) ppm.

2,2,8,8-Tetramethyldecanedioic acid (3b). Yield 40%; mp: 117 °C (lit.:⁸ 118 °C); 1.52 (m, 4H), 1.28 (m, 8H), 1.18 (bs, 12H) ppm. ¹³C (CDCl₃): 185.0 (COOH), 42.4, 40.7, 30.0, 25.2, 24.9 ppm.

2,7-Dimethyloctanedioic Acid (3c). Yield 23%; mp: 121 °C. ¹H (DMSO-*d*₆): 12.02 (bs, 2H, COOH), 2.28 (sex, 2H, *J* = 7 Hz, CH), 1.52 (m, 2H, *J* = 7 Hz), 1.21–1.33 (m, 8H), 1.03 (d, 6H, *J* = 7 Hz, CH₃) ppm. ¹³C (DMSO-*d*₆): 178.15 (COOH), 39.31 (CH), 33.80 (CH₂), 27.28, 17.63 (CH₃) ppm.

General Procedure for the Preparation of 10-Methoxy- (5a) and 10-(Benzyloxy)-2,9-dimethyl-10-oxodecanoic Acid (5b). To 2 mL of anhydrous chloroform were added 1 mmol of methanol (dried over Mg, for preparation of **5a**) or benzyl alcohol (for preparation of **5b**), followed by 230 mg (1 mmol) of 2,9-dimethyldecanedioic acid, and then a catalytic amount of DMAP was added, followed by 240 mg (1.2 mmol) of DCC. The mixture was stirred at rt under nitrogen overnight. To the suspension were added 1 mL of water and additional chloroform and the mixture was vigorously stirred for an additional hour to hydrolyze excess DCC. The precipitated urea was filtered off, the solution dried over Na₂SO₄, and the solvent evaporated. The oil obtained was used for the next reaction step without further purification.

10-Methoxy-2,2,9,9-tetramethyl-10-oxodecanoic Acid (5a). Yield 70%; colorless oil. ¹H (CDCl₃): 3.67 (s, 3H), 2.44 (m, 1H), 1.61–1.71 (m, 3H), 1.15–1.42 (m, 10H), 1.13 (d, *J* = 7 Hz, 6H) ppm. ¹³C (CDCl₃): 177.4, 156.2, 51.5, 39.4, 33.8, 33.7, 33.6, 29.3, 27.1, 25.5, 24.9, 17.1 ppm.

10-(Benzyloxy)-2,9-dimethyl-10-oxodecanoic Acid (5b). Yield 79%; colorless oil. ¹H (CDCl₃): 7.37 (bs, 5H, arom.), 5.11 (s, 2H, Bn-CH₂), 2.46 (qui, 2H, *J* = 7 Hz), 1.50–1.81 (m, 4H), 1.14–1.38 (m, 14H), ppm. ¹³C (CDCl₃): 176.6 (CO), 136.04, 128.5, 128.1, 128.0, 65.9 (Bn-CH₂), 39.5, 33.7, 29.3, 27.1, 27.0, 17.0 (CH₃) ppm.

Synthesis of 10-(Benzyloxy)-2,2,9,9-tetramethyl-10-oxodecanoic Acid (5c). To a solution of 0.57 mmol of 2,2,9,9-tetramethyldecanedioic acid in THF were added dropwise 0.57 mL of 1.0 M tetrabutylammonium fluoride in THF under stirring at rt, followed by addition of 0.57 mmol of benzylbromide in 1 mL of THF over 3 h under vigorous stirring. The solution was allowed to stand overnight at rt, after which 3 mL of 2 N HCl were added and the mixture extracted 4× with EtOAc. The combined organic phases are subsequently washed 4× with a small amount of 1 N HCl to remove TBA ions. The organic phase was dried over sodium sulfate to yield a colorless oil (143 mg, 72%), which was sufficiently pure for the following reaction step. ¹H (CDCl₃): 7.34 (bs, 5H, arom.), 5.10 (s, 2H, BnCH₂), 1.42–1.58 (m, 4H), 1.15–1.30 (m, 20H) ppm. ¹³C (CDCl₃): 184.4 (COOH), 178.2 (COOBn), 136.7, 128.7, 128.2, 128.1, 128.0, 66.2 (BnCH₂), 42.6, 42.3, 41.0, 40.7, 30.1, 29.9, 25.4, 25.3, 25.2, 25.1, 25.0, 24.8 ppm.

General Procedure for the Synthesis of Butorphan Univalent Diesters (6a,b,c). In 2 mL of anhydrous methylene chloride, 0.75 mmol of the respective dicarboxylic monoester (**5a,b,c**) were dissolved and two drops of dimethylformamide were added, followed by the addition of 1.5 mmol of oxalylchloride. Gas evolution could be observed, and the solution was stirred for 4 h at rt under nitrogen. Then solvent and excess oxalyl chloride were removed under reduced pressure and high vacuum, respectively. The residual acid chloride of the monoester was dissolved in 4 mL of anhydrous methylene chloride, and 0.5 mmol of butorphan were added. Dropwise addition of 2 mmol of triethylamine led to dissolution of butorphan, and the solution was refluxed overnight. After cooling, additional methylene chloride was added and the mixture washed with sat. NaHCO₃ solution and brine. The organic phase was dried over Na₂SO₄ and solvent removed under reduced pressure. The residual oil was column chromatographed using hexane/ethyl acetate/triethylamine 10/10/1 as eluent system.

Methyl 17-((-)-N-Cyclobutylmethyl)morphinan-3-yl 2,9-Dimethyldecanedioate (6a). Colorless oil (21%). ¹H (CDCl₃): 7.11 (d, *J* = 8 Hz, 1H), 6.92 (d, *J* = 2 Hz, 1H), 6.85 (dd, *J* = 8, 2 Hz, 1H), 3.67 (s, 3H), 2.98 (m, 2H), 2.60–2.67 (m, 6H), 2.21–2.45 (m, 2H), 2.06–2.11 (m, 3H), 1.16–1.92 (m, 29H), 1.14 (d, *J* = 7 Hz,

3H) ppm. ¹³C (CDCl₃): 177.3, 175.4, 149.3, 142.0, 135.1, 128.5, 118.4, 118.0, 61.5, 55.8, 51.4, 45.7, 44.8, 41.7, 39.6, 39.4, 37.7, 36.5, 34.9, 33.7, 33.6, 29.4, 29.3, 27.8, 27.1, 26.7, 26.5, 24.4, 22.1, 19.8, 17.1, 17.0, 16.9 ppm. MS (TOF ESI+): *m/z* = 538 ([M + H]⁺). HRMS Calcd for C₃₄H₅₂NO₄: 538.3891. Found 538.4082. Anal. Calcd for C₃₄H₅₁NO₄·¹/₃H₂O: C, 74.28; H, 9.59; N, 2.55. Found: C, 74.14; H, 9.45; N, 2.87.

Benzyl 17-((-)-N-Cyclobutylmethyl)morphinan-3-yl 2,9-Dimethyldecanedioate (6b). Colorless oil (30%). ¹H (CDCl₃): 7.12 (d, 1H, *J* = 8.4 Hz), 6.92 (d, 1H, *J* = 2.4 Hz), 6.87 (dd, 1H, *J* = 8.4, 2.4 Hz), 2.98 (d, 2H, *J* = 18 Hz), 2.50–2.78 (m, 8H), 1.01–2.18 (m, 36H) ppm. ¹³C (CDCl₃): 176.9, 175.7, 149.7, 142.6, 136.5, 128.8, 128.7, 128.3, 128.29, 118.7, 118.3, 66.2, 61.6, 56.1, 46.0, 39.9, 39.4, 37.9, 36.7, 34.0, 29.6, 28.1, 27.4, 27.0, 26.7, 24.7, 22.3, 19.1, 17.3 ppm.

Benzyl 17-((-)-N-Cyclobutylmethyl)morphinan-3-yl 2,2,9,9-Tetramethyldecanedioate (6c). Colorless oil (9%). ¹H (CDCl₃): 7.33 (bs, 5H), 7.10 (d, *J* = 8 Hz, 1H), 6.88 (d, *J* = 2.4 Hz, 1H), 6.80 (dd, *J* = 8, 2.4 Hz, 1H), 5.11 (s, 2H), 2.89 (d, *J* = 42 Hz, 1H), 2.70 (m, 1H), 2.63–2.41 (m, 4H), 2.26 (m, 1 H), 2.09–2.01 (m, 2H), 1.91–1.63 (m, 7H), 1.61–1.50 (m, 4H), 1.40–1.15 (m, 29H) ppm. ¹³C (CDCl₃): 178.1, 177.0, 149.8, 142.2, 136.7, 128.7, 128.2, 128.1, 118.7, 118.3, 66.2, 61.7, 56.1, 45.9, 45.2, 44.0, 42.8, 42.6, 42.0, 41.0, 40.5, 38.0, 36.8, 35.2, 30.3, 30.2, 28.1, 27.0, 26.8, 25.4 ppm. MS (TOF ESI+): *m/z* = 642 ([M + H]⁺). HRMS Calcd for C₄₂H₆₀NO₄: 642.4517. Found 642.4546. Anal. Calcd for C₄₂H₅₉NO₄·¹/₃EtOAc: C, 77.53; H, 9.26; N, 2.09. Found: C, 77.55; H, 9.48; N, 1.89.

General Procedure for the Deprotection of Butorphan Univalent Benzyl Esters (7a,b). The respective benzyl ester (0.12 mmol; **6b,c**) was dissolved in 3 mL of a 1:1 mixture of methanol and ethanol, and 10 wt % of Pd/C was added. The atmosphere over the mixture was evacuated and flushed with hydrogen twice, after which the mixture was vigorously stirred at rt under hydrogen overnight. On the next day, a further 10 wt % of Pd/C was added and the procedure repeated until no starting material could be seen by TLC control (hexane/ethyl acetate/triethylamine 10/10/1). The catalyst was filtered off, washed with methylene chloride, and the solvents evaporated to yield the pure products.

2,9-Dimethyldecanedioic Acid 10-((-)-N-Cyclobutylmethylmorphinan-3-yl) Ester (7a). Colorless oil (30%). ¹H (CDCl₃): 7.16 (d, 1H, *J* = 8.5 Hz), 6.96 (s, 1H, *J* = 2.4 Hz), 6.95 (dd, 1H, *J* = 8.5, 2.4 Hz), 2.85–3.18 (m, 5H), 2.21–2.87 (m, 8H), 1.07–2.47 (m, 33H) ppm. ¹³C (CDCl₃): 181.8, 175.6, 142.0, 140.1, 134.4, 129.1, 120.1, 118.8, 59.0, 56.5, 40.5, 39.8, 38.7, 36.8, 35.6, 34.2, 33.9, 31.4, 29.8, 29.6, 28.2, 28.0, 27.6, 27.4, 27.2, 26.1, 25.9, 21.8, 18.9, 17.7, 17.2 ppm. MS (TOF ESI+): *m/z* (% rel. int.) = 524 ([M + H]⁺, 100). Anal. Calcd for C₃₃H₅₀NO₄Cl (hydrochloride)·H₂O: C, 68.55; H, 9.06; N, 2.42. Found: C, 68.62; H, 9.01; N, 2.62.

2,2,9,9-Tetramethyldecanedioic Acid 10-((-)-N-Cyclobutylmethylmorphinan-3-yl) Ester (7b). Colorless oil (23%). ¹H (CDCl₃): 7.15 (d, *J* = 8 Hz, 1H), 6.95 (d, *J* = 2 Hz, 1H), 6.89 (dd, 1H, *J* = 8, 2 Hz), 3.43 (bs, 1 H), 2.93–3.10 (m, 5H), 2.05–2.34 (m, 4H), 1.81 (m, 3H), 1.66 (m, 3H), 1.10–1.52 (m, 34H) ppm. ¹³C (CDCl₃): 182.9, 176.6, 150.4, 128.8, 127.8, 119.7, 118.4, 58.8, 56.0, 42.6, 42.2, 42.1, 40.8, 40.7, 36.6, 35.4, 31.3, 30.1, 30.0, 27.9, 27.7, 26.0, 25.7, 25.3, 25.2, 25.0, 24.5, 21.6, 18.6 ppm. MS (TOF ESI+): *m/z* (% rel. int.) = 552 ([M + H]⁺, 100). HRMS (TOF ESI+) Calcd for C₃₅H₅₄NO₄: 552.4053. Found: 552.4044. Anal. Calcd for C₃₅H₅₄NO₄HCl·H₂O: C, 69.34; H, 9.31; N, 2.31. Found: C, 69.37; H, 9.20; N, 2.53.

General Procedure for the Preparation of Homobivalent Ligands of Butorphan (4a,b,c). To a solution of 0.3 mmol of the respective dicarboxylic acid (**3a,b,c**) in anhydrous methylene chloride (part of the diacid keeps suspended) were added two drops of dimethylformamide, followed by the addition of 1.2 mmol of oxalylchloride. Gas evolution was observed and the solution is stirred for 4 h at rt under nitrogen. Then solvent and excess

oxalyl chloride were removed under reduced pressure and high vacuum, respectively. The residual diacid dichloride was dissolved in a small amount of anhydrous methylene chloride and 0.9 mmol of butorphan were added and a clear solution was formed. Dropwise addition of 1.2 mmol of triethylamine were added and the solution was stirred at rt overnight. Additional methylene chloride was added and the mixture was washed with sat. NaHCO_3 solution and brine. The organic phase was dried over Na_2SO_4 and solvent removed under reduced pressure. The residual oil was column chromatographed using hexane/ethyl acetate/triethylamine 10/10/1 as eluent system.

Bis((-)-N-cyclobutylmethylmorphinan-3-yl) 2,9-Dimethyldecanedioate (4a). Colorless oil (71%). ^1H (CDCl_3): 7.10 (d, 2H, $J=8.4$ Hz), 6.91 (d, 2H, $J=2.4$ Hz), 6.87 (dd, 2H, $J=8.4, 2.4$ Hz), 3.01 (d, 2H, $J=19$ Hz), 2.80 (m, 2H), 2.22–2.73 (m, 16H), 1.05–2.17 (m, 50H) ppm. ^{13}C (CDCl_3): 175.7, 149.5, 142.3, 135.5, 128.7, 118.7, 118.3, 61.8, 56.0, 45.9, 45.2, 42.0, 39.9, 38.0, 36.8, 35.2, 34.0, 29.7, 28.1, 28.1, 27.5, 27.0, 26.8, 24.6, 22.4, 19.1, 17.3 ppm. MS (TOF ESI+): m/z (% rel. int.) = 816 ($[\text{M}]^+$, 11), 409 ($[\text{M} + 2\text{H}]^{2+}$, 100). Anal. Calcd for $\text{C}_{54}\text{H}_{78}\text{N}_2\text{O}_4\text{Cl}_2$ (dihydrochloride)· H_2O : C, 71.42; H, 8.88; N, 3.02. Found: C, 71.37; H, 8.98; N, 3.00.

Bis((-)-N-cyclobutylmethylmorphinan-3-yl) 2,2,9,9-Tetramethyldecanedioate (4b). Colorless oil (70%). ^1H (CDCl_3): 7.10 (d, 2H, $J=8.4$ Hz), 6.91 (d, 2H, $J=2.4$ Hz), 6.87 (dd, 2H, $J=8.4, 2.4$ Hz), 3.01 (d, 2H, $J=19$ Hz), 2.90 (m, 2H), 2.22–2.73 (m, 16H), 1.05–2.17 (m, 54H) ppm. ^{13}C (CDCl_3): 177.0, 149.7, 142.2, 135.3, 128.7, 118.7, 118.3, 61.7, 56.0, 45.9, 45.2, 42.8, 42.0, 41.0, 37.0, 36.8, 35.2, 30.3, 28.1, 27.0, 26.8, 25.4, 25.4, 25.3, 24.6, 22.4, 19.1 ppm. MS (TOF ESI+): m/z (% rel. int.) = 846 ($[\text{M} + \text{H}]^+$, 11), 423.5 ($[\text{M} + 2\text{H}]^{2+}$, 100). HRMS (TOF ESI+) Calcd for $\text{C}_{56}\text{H}_{80}\text{N}_2\text{O}_4$: 845.6196. Found: 845.6228. Anal. Calcd for $\text{C}_{56}\text{H}_{82}\text{N}_2\text{O}_4\text{Cl}_2$ (dihydrochloride)· $1.3\text{H}_2\text{O}$: C, 71.43; H, 9.06; N, 2.98. Found: C, 71.38; H, 8.95; N, 3.00.

Bis((-)-N-cyclobutylmethylmorphinan-3-yl) 2,7-Dimethyldecanedioate (4c). Colorless oil (47%). ^1H (CDCl_3): 7.10 (d, 2H, $J=8.4$ Hz), 6.91 (d, 2H, $J=2.4$ Hz), 6.85 (dd, 2H, $J=8.4, 2.4$ Hz), 2.98 (d, 2H, $J=19$ Hz), 2.87 (m, 2H), 2.22–2.74 (m, 14H), 1.01–2.10 (m, 48H) ppm. ^{13}C (CDCl_3): 175.6, 149.5, 142.3, 135.4, 128.7, 118.7, 118.3, 61.7, 56.1, 45.9, 45.1, 41.9, 39.8, 38.0, 36.8, 35.1, 33.8, 28.1, 27.4, 27.0, 26.8, 24.6, 22.4, 19.1, 17.2 ppm. MS (TOF ESI+): m/z (% rel. int.) = 789 ($[\text{M} + \text{H}]^+$, 13), 395 ($[\text{M} + 2\text{H}]^{2+}$, 100). Anal. Calcd for $\text{C}_{52}\text{H}_{72}\text{N}_2\text{O}_4 \cdot \frac{1}{3}\text{EtOAc}$: C, 78.21; H, 9.23; N, 3.54. Found: C, 78.34; H, 9.34; N, 3.45.

(5 α)-17-Allyl-14-hydroxy-6-oxo-4,5-epoxymorphinan-3-yl-17-((-)-N-cyclobutylmethyl)morphinan-3-yl) 2,9-Dimethyldecanedioate (8). The compound was synthesized from equimolar amounts of 2,9-dimethyldecanedioic acid 10-((-)-N-cyclobutylmethylmorphinan-3-yl) ester (7a) and naloxone free base according to the general procedure for the synthesis of butorphan univalent diesters (6a,b,c). The compound was purified using hexane/EtOAc/ NEt_3 10/10/1 as eluent.

Colorless oil (11%). ^1H (CDCl_3): 7.08 (d, 2H, $J=8.4$ Hz), 6.87 (d, 2H, $J=2.1$ Hz), 6.82 (dd, 2H, $J=8.4, 2.1$ Hz), 6.68 (d, 1H, $J=8.4$ Hz), 5.76–5.89 (m, 1H), 5.18–5.25 (m, 2H), 4.67 (s, 1H), 2.98–3.18 (m, 6H), 1.03–2.82 (m, 51 H) ppm. ^{13}C (CDCl_3): 206.5, 174.5, 173.5, 148.3, 146.8, 141.0, 134.2, 134.0, 131.7, 129.0, 127.7, 127.5, 121.8, 118.3, 117.5, 117.2, 117.1, 89.5, 69.1, 61.0, 60.5, 56.6, 54.8, 49.6, 44.7, 43.9, 42.2, 40.7, 38.6, 38.3, 36.7, 35.5, 35.0, 33.9, 32.8, 30.1, 29.6, 28.4, 28.3, 26.9, 26.2, 26.0, 25.8, 25.5, 23.4, 22.0, 21.1, 17.8, 16.0 ppm. MS (TOF ESI+): m/z (% rel. int.) = 833 ($[\text{M} + \text{H}]^+$, 27), 418 ($[\text{M} + 2\text{H}]^{2+}$, 100). Anal. Calcd for $\text{C}_{52}\text{H}_{68}\text{N}_2\text{O}_7 \cdot 1.5\text{H}_2\text{O}$: C, 72.61; H, 8.32; N, 3.26. Found: C, 72.79; H, 8.21; N, 3.41.

HPLC Analysis. HPLC analysis was performed on a Varian Prostar HPLC modular system operated by Star Chromatography Workstation software, version 5. Chromatographic separations were performed on a Supelco Discovery C18 column (4.6 mm \times 25 cm, 5 μm) operated at ambient temperature. The samples were injected using a Rheodyne 7725 manual sample

injector equipped with a 20 μL injection loop. The mobile phase of 0.1% TFA in acetonitrile and 0.1% TFA in water was operated at a gradient of 60–100% acetonitrile over 10 min at 1.0 mL/min. Detection was at 265 nm.

Determination of Hydrolysis of Bivalent Ligands. The trifluoroacetate salts of the bivalent ligands were dissolved in acetonitrile and diluted in pH 7.4 20 mM phosphate buffer to give a final concentration of 100 $\mu\text{g}/\mu\text{L}$ of ligand at a final acetonitrile concentration of 20%. The samples were incubated in duplicate in a 37 $^\circ\text{C}$ water bath and at appropriate times removed and injected directly for analysis. The half-life for each ligand was calculated assuming pseudo-first-order kinetics from the rate constant measured from a plot of $\ln(\% \text{ester from } t=0)$ versus time (min). The esterase mediated hydrolysis was conducted as above using approximately 0.15 units of porcine liver esterase (esterase reported as a specific activity of 24 units/mg where 1 unit will hydrolyze 1.0 μmole of ethyl butyrate to butyric acid and ethanol per min at pH 8.0 at 25 $^\circ\text{C}$).

Opioid Binding to Human μ , δ , and κ Opioid Receptors. To determine the affinity and selectivity of the peptides for the μ , δ , and κ opioid receptors, Chinese hamster ovary (CHO) cells that stably expressed one type of human opioid receptor were used as previously described.¹⁹ Cell membranes were incubated at 25 $^\circ\text{C}$ with the radiolabeled ligands 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 [^3H]DAMGO and the κ -selective ligand [^3H]U69,593, and a 3 h incubation was used with the δ -selective antagonist [^3H]naltrindole. The final concentrations of [^3H]DAMGO, [^3H]naltrindole, and [^3H]U69,593 were 0.25, 0.2, and 1 nM, respectively. Nonspecific binding was measured by inclusion of 10 μM naloxone for μ and κ binding and 100 μM for δ binding. The binding was terminated by filtering the samples through Schleicher & Schuell no. 32 glass fiber filters using a Brandel 48-well cell harvester. The filters were washed three times with 3 mL of cold 50 mM Tris-HCl, pH 7.5, and were counted in 2 mL of ScintiSafe 30% scintillation fluid (Fisher Scientific, Fair Lawn, NJ). For [^3H]U69,593 binding, the filters were soaked in 0.1% polyethylenimine for at least 30 min before use. IC_{50} values were calculated by least-squares fit to a logarithm-probit analysis. K_i values of unlabeled compounds were calculated from the equation $K_i = (\text{IC}_{50}) / (1 + S)$ where S = (concentration of radioligand) (K_d of radioligand).²⁰

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References

- (1) Neumeyer, J. L.; Zhang, A.; Xiong, W.; Gu, X.; Hilbert, J. E.; Knapp, B. I.; Negus, S. S.; Mello, N. K.; Bidlack, J. M. Design and Synthesis of Novel Dimeric Morphinan Ligands for κ and μ Opioid Receptors. *J. Med. Chem.* **2003**, *46*, 5162–5170.
- (2) Peng, X.; Knapp, B. I.; Bidlack, J. M.; Neumeyer, J. L. Synthesis and Preliminary In vitro Investigation of Bivalent Ligands Containing Homo- and Heterodimeric Pharmacophores at μ , δ , and κ Opioid Receptors. *J. Med. Chem.* **2006**, *49*, 256–262.
- (3) Peng, X.; Neumeyer, J. L. Kappa Receptor Bivalent Ligands. *Curr. Top. Med. Chem.* **2007**, *7*, 363–373.
- (4) Newman, M. S. Some Observations Concerning Steric Factors. *J. Am. Chem. Soc.* **1950**, *72*, 4783–4786.
- (5) Shawali, A. S. A. S.; Biechler, S. S. Aminolysis of Esters. I. Kinetics and Mechanism in Anhydrous Dioxane. *J. Am. Chem. Soc.* **1967**, *89*, 3020–3026.
- (6) Bender, D. M.; Peterson, J. A.; McCarthy, J. R.; Gunaydin, H.; Takano, Y.; Houk, K. N. Cyclopropanecarboxylic Acid Esters as Potential Prodrugs with Enhanced Hydrolytic Stability. *Org. Lett.* **2008**, *10*, 509–511.
- (7) Stoops, J. K.; Horgan, D. J.; Runnegar, M. T. C.; de Jersey, J.; Webb, E. C.; Zerner, B. Carboxylesterase (EC 3.1.1). Kinetic Studies on Carboxylesterases. *Biochemistry* **1969**, *8*, 2026–2033.

- (8) Fredholt, K.; Mørk, N.; Begtrup, M. Hemiesters of Aliphatic Dicarboxylic Acids as Cyclization-Activated Prodrug Forms for Protecting Phenols against First-Pass Metabolism. *Int. J. Pharm.* **1995**, *123*, 209–216.
- (9) Neumeyer, J. L.; Bidlack, J. M.; Zong, R.; Bakthavachalam, V.; Gao, P.; Cohen, D. J.; Negus, S. S.; Mello, N. K. Synthesis and Opioid Receptor Affinity of Morphinan and Benzomorphan Derivatives: Mixed κ Agonist and μ Agonists and Antagonists as Potential Pharmacotherapeutics for Cocaine Dependence. *J. Med. Chem.* **2000**, *43*, 114–122.
- (10) Neumeyer, J. L.; Gu, X.-H.; van Vliet, L. A.; DeNunzio, N. J.; Rusovici, D. E.; Cohen, D. J.; Negus, S. S.; Mello, N. K.; Bidlack, J. M. Mixed κ Agonists and μ Agonists/Antagonists as Potential Pharmacotherapeutics for Cocaine Abuse: Synthesis and Opioid Binding Affinity of *N*-substituted Derivatives of Morphinan. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2735–2740.
- (11) Negus, S. S.; Mello, N. K.; Portoghese, P. S.; Lin, C.-E. Effects of Kappa Opioids on Cocaine Self-Administration by Rhesus Monkeys. *J. Pharmacol. Exp. Ther.* **1997**, *282*, 44–55.
- (12) Mello, N. K.; Negus, S. S. Effects of Kappa Opioid Agonists on Cocaine- and Food-Maintained Responding by Rhesus Monkeys. *J. Pharmacol. Exp. Ther.* **1998**, *286*, 812–824.
- (13) Alper, H.; Woell, J. B.; Despeyroux, B.; Smith, D. J. H. The Regio-specific Palladium Catalyzed Hydrocarboxylation of Alkenes under Mild Conditions. *J. Chem. Soc., Chem. Commun.* **1983**, 1270–1271.
- (14) Newkome, G. R.; Baker, B.; Caruso, A.; Greenwald, M. M.; Hanson, P. G.; Mangogna, G. A.; Mathes, P. D.; Pascal, A.; Rigby, H. O.; Riser, J. M.; Schnabel, J. J.; Sonnier, J. A.; Steinkampf, M. P.; Johnson, J. L. An Improved Synthesis of $\alpha,\alpha,\alpha',\alpha'$ -Tetramethyl-dicarboxylic Acids. *Synthesis* **1975**, *8*, 517–518.
- (15) Babler, J. H.; Moy, R. K. A Facile Method for Monoesterification of α,ω -Dicarboxylic Acids: Application to the Synthesis of Traumatic Acid, a Prostaglandin Synthase. *Synth. Commun.* **1979**, *9*, 669–675.
- (16) Wu, C.-Y.; Brik, A.; Wang, S.-K.; Chen, Y.-H.; Wong, C.-H. Tetrabutylammonium Fluoride-Mediated Rapid Alkylation Reaction in Microtiter Plates for the Discovery of Enzyme Inhibitors in Situ. *ChemBioChem* **2005**, *6*, 2176–2180.
- (17) (a) Peng, X.; Knapp, B. I.; Bidlack, J. M.; Neumeyer, J. L. Pharmacological Properties of Bivalent Ligands Containing Butorphan Linked to Nalbuphine, Naltrexone and Naloxone at μ , δ , and κ Opioid Receptors. *J. Med. Chem.* **2007**, *50*, 2254–2258. (b) Mathews, J. L.; Fulton, B. S.; Negus, S. S.; Neumeyer, J. L.; Bidlack, J. M. In Vivo Characterization of (-)(-)MCL-144 and (+)(+)MCL-193: Isomeric, Bivalent Ligands with kappa/mu Agonist Properties. *Neurochem. Res.* **2008**, *33*, 2142–2150.
- (18) Almeida, M. I.; Amaral, A. T.; Amaral, L. Effects of α Substitution on the Carbonyl Stretching Frequencies of Phenyl Carboxylates. *J. Org. Chem.* **1982**, *47*, 1567–1571.
- (19) Parkhill, A. L.; Bidlack, J. M. Several δ -Opioid Receptor Ligands Display No Subtype Selectivity to the Human δ -Opioid Receptor. *Eur. J. Pharmacol.* **2002**, *451*, 257–264.
- (20) Cheng, Y. C.; Prusoff, W. H. Relationship Between the Inhibition Constant (K_i) and the Concentration of Inhibitor Which Causes 50% Inhibition (IC_{50}) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.