Synthesis and Biological Activity of a Novel Methylamine-Bridged Enkephalin Analogue (MABE): A New Route to Cyclic Peptides and Peptidomimetics

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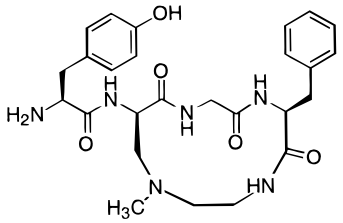
The synthesis and biological activity of a methylamine-bridged enkephalin analogue (MABE) is presented. The key step in the synthesis of the target compound involves the ring opening of Cbz-D-serine β-lactone with Boc-Phe-$NHCH_2CH_2NHCH_3$. Further synthetic elaboration of the resulting building block yielded compound **1** (MABE, Tyr-c[($N_\beta CH_3$)-D-A_2pr-Gly-Phe-$NHCH_2$-CH_2-], where A_2pr is a 2,3-diaminopropionic acid residue). Utilizing a combination of NMR and molecular modeling, the structure−biological activity relationships for compound **1** were studied. Using an in vitro isolated receptor assay, MABE was found to have affinities for isolated μ, δ, and κ opioid receptors of 1.6, 2.1, and 340 nM, respectively. By an in vivo thermal escape assay, MABE was found to have an ED_{50} of 0.027 μg in the rat when administered intrathecally. This effect was reversed by naloxone. By comparison, DAMGO, morphine, and DPDPE were found to yield ED_{50} values of 0.14, 2.4, and 54 μg, respectively, in the same assay.

Introduction

Enkephalins are a class of endogenous peptide opioids first isolated and identified by Hughes in 1975.[1] Since this time, a variety of naturally occurring analogues has been discovered, all of which contain the sequence Tyr-X_1-Y_2-Phe, where X_1 and Y_2 are amino acid residues. Many studies have noted the importance of enkephalins in a variety of physiological functions ranging from the modulation of pain to the regulation of the immune system.[2] To date, three major opioid receptor subtypes have been identified: μ, δ, and κ, and each has been associated with various physiological roles. Consequently, the synthesis of potent and receptor-specific enkephalin analogues is an area of active interest.[3]

Since the synthesis of the first cyclic enkephalin analogue (H-Tyr-c[D-A_2bu-Gly-Phe-Leu]) by Schiller,[4] many analogues have been synthesized to yield derivatives with enhanced selectivity, increased potency, and extended biological stability when compared to their parent enkephalins.[5] Many cyclic analogues have been synthesized that rely on the use of disulfide or lanthionine (monosulfide)[6] bridges. In general, a variety of structures can be utilized as bridges in the synthesis of cyclic peptides. Commonly used functionalities include amide bonds that result from head-to-tail or main chain-to-side chain cyclizations, disulfide bonds, urethanes, and diaryl ethers. Other functionalities based on phosphorus, silicon, and sulfur heteroatoms have also been used.[7,8] We have recently embarked on developing the use of the nitrogen heteroatom as a bridge in the design of cyclic peptides. More specifically, we synthesized the cyclic enkephalin analogue **1** (MABE, Tyr-c[($N_\beta CH_3$)-D-A_2pr-Gly-Phe-$NHCH_2CH_2$-]; Chart 1) with a methylamine bridge. The resulting target MABE exhibits a high affinity for the μ and δ receptors and a modest affinity for the κ receptor. In addition, MABE was found to be a potent opioid agonist as measured in vivo.

Chart 1. Structure of Tyr-c[($N_\beta CH_3$)-D-A_2pr-Gly-Phe-$NHCH_2CH_2$-] (MABE)



1 (MABE)

Results and Discussion

The key step in the synthesis of compound **1** (MABE; see Scheme 1) relies on a tertiary amine-containing tripeptide unit, **4**, that becomes the cyclic peptide bridge. In our previous synthesis of cyclic lanthionine enkephalin analogues, the monosulfide bridging unit was derived from the ring opening of [*N*-benzyloxycarbonyl (Cbz)]-serine β-lactone (the Vederas lactone)[9] with Boc-Cys-OMe.[10] Here, a similar strategy was employed utilizing the secondary amine **2**. This amine was obtained from the ring opening of Boc-Phe-*N*-carboxyanhydride with *N*-methylethylenediamine. The resulting secondary amine nucleophile was used to ring-open Cbz-D-serine β-lactone in DMF with Cs_2CO_3 as a base to neutralize the proton derived from *N*-methylethylenediamine reactant. Unlike the previous synthesis of lanthionine dipeptide bridging units, the carboxylate derivative **3** was not isolated but was coupled directly to H-Gly-O*t*-Bu using BOP as a coupling reagent.[11] This "one-pot" procedure avoided the difficult isolation of the

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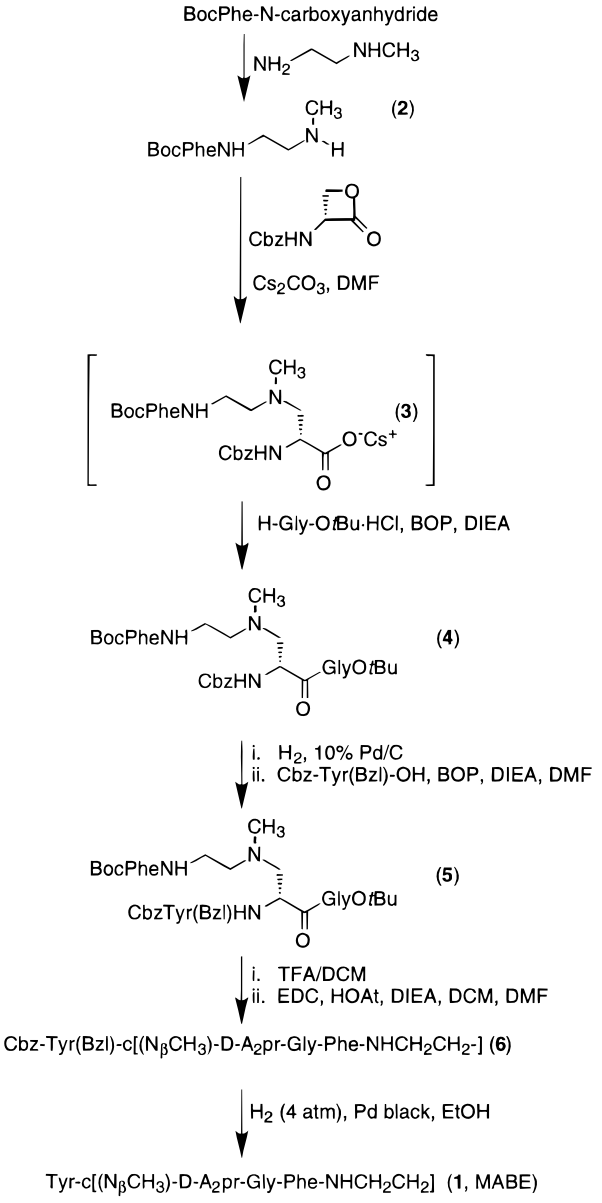
Scheme 1. Synthesis of Tyr-c[($N_\beta CH_3$)-D-A_2pr-Gly-Phe-$NHCH_2CH_2$-] (MABE)



highly polar intermediate **3**. The resulting tripeptide **4** was then readily purified using silica gel chromatography in an overall yield of 72% from **2**.

The resulting building block **4** was incorporated into the target MABE as follows: Catalytic hydrogenolysis of compound **4** yielded a free amine that was coupled to Cbz-Tyr(Bzl)-OH to yield the branched intermediate **5**. The acid-labile protecting groups of compound **5** were removed using TFA/DCM (1:1), and the resulting crude product was converted to its HCl salt. Cyclization under dilute conditions using EDC, DIEA, and HOAt yielded compound **6**. This cyclic precursor to the final product was deprotected using palladium black under 4 atm of H_2 to yield MABE.

The target compound was tested for its potency in vitro and for its efficacy in vivo (Table 1). Using an in vitro radioligand displacement assay, MABE was found to have an affinity for isolated μ, δ, and κ opioid receptors of 1.6, 2.1, and 340 nM, respectively. Using

Table 1. In Vitro and in Vivo Bioactivity Data for MABE, DAMGO, Morphine, and DPDPE[a]

	K_i (nM)			
compd	μ	δ	κ	ED_{50} (μg)
MABE	1.6 (1.0−2.4) $n = 5$	2.1 (1.3−3.5) $n = 3$	340 (110−1020) $n = 2$	0.027 (0.014−0.053) $n = 30$
DAMGO	14 (7.4−27) $n = 8$	>10000	>10000	0.14 (0.094−0.20) $n = 30$
morphine	17 (6.8−43) $n = 4$	150 (63−360) $n = 4$	260 (100−690) $n = 3$	2.4 (1.8−3.1) $n = 30$
DPDPE	>10000	2.2 (1.0−3.9) $n = 5$	>10000	54 (38−77) $n = 24$

[a] The K_i and ED_{50} values are presented wth the 95% confidence intervals and the number (n) of determinations.

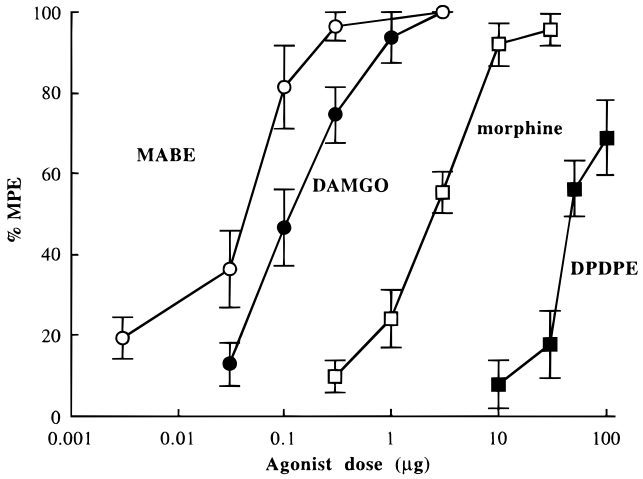


Figure 1. Dose dependency of MABE, DAMGO, morphine, and DPDPE on the thermal escape test.

an in vivo thermal escape assay, MABE was found to have an ED_{50} of 0.027 μg. This effect was reversed by naloxone. By comparison, DAMGO, morphine, and DPDPE were found to have ED_{50} values of 0.14, 2.4, and 54 μg, respectively, in the same assay (Figure 1). Thus, MABE was found to be a potent and broad-spectrum opioid agonist.

Utilizing a combination of ^{1}H NMR and molecular modeling, MABE has been shown to exhibit both folded and extended spatial arrangements of the aromatic rings of phenylalanine and tyrosine which are critical in defining selectivity between the μ and δ opioid receptors (see Supporting Information).[12−14] Furthermore, conformational analysis of MABE indicated that its methlyamine bridge was highly flexible and conformational exchange was observed on the NMR time scale. Interestingly, MABE exhibits weak κ receptor activity unlike its lanthionine enkephalin congener of the sequence c[D-Cys^2,D-Cys^5].[15] Consequently, the κ activity of MABE may originate from the methylamine bridge.

Conclusions

MABE represents a new approach in the design of cyclic peptides. Advantages of using an amine as a bridging atom include the potential to construct a diverse number of bridges based on commercially available or readily synthesized amines. Furthermore, amine

bridges also contain a site for protonation or metal chelation. Finally, the use of the trivalent amine offers a "handle" by which to functionalize the bridge without disturbing the pharmacophores. Such modifications have the potential to modulate biological activity or biodistribution.

The potency of MABE in vivo makes it a promising new lead compound in our search for enkephalin analogues with specific activities. Future efforts will be focused on maintaining the high efficacy of this derivative while improving its selectivity for the various opioid receptors. The amine bridge offers several unique possibilities to modify the activity of this class of compounds. Because the methylamine bridge of MABE was found to be highly flexible, bicyclic enkephalin analogues derived from cyclic amine bridges might offer a way to constrain the pharmacophores conformationally to improve receptor selectivity. Additionally, modification of the bridgehead of the amine with charged or hydrophobic functional groups might alter selectivity for the various membrane-bound opioid receptors. Such syntheses are now underway in our laboratories.

Experimental Section

General Procedures. Proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a QE-300 NMR spectrometer using the residual peaks in the deuterated solvents as internal standards. Fast atom bombardment (FAB) positive ion mass spectra were obtained on a VG ZAB-VSE double focusing high-resolution mass spectrometer equipped with a cesium ion gun. 3-Nitrobenzyl alcohol was used as the matrix for FAB mass spectrometry. Electrospray ionization (ESI) mass spectra were obtained on an API III Perkin-Elmer SCIEX triple quadrupole mass spectrometer. Analytical reverse-phase high-performance liquid chromatography (RP-HPLC) was conducted with a C_{18} 90 Å silica column (5 μm, 4.6 × 250 mm, Vydac). Preparative RP-HPLC was conducted with a C_{18} 90 Å silica column (10 μm, 22 × 250 mm, Vydac). All reagents were of the highest grade available and were purchased from the Aldrich Chemical Co. unless indicated otherwise. Dichloromethane was distilled from CaH_2 under N_2. Boc-Phe-*N*-carboxyanhydride was prepared according to the method of Fuller et al.[16] Cbz-Tyr(Bzl)-OH was purchased from Bachem California (Torrance, CA).

Boc-Phe-NHCH$_2$CH$_2$NHCH$_3$ (2). To a solution of *N*-methylethylenediamine (2.00 g, 6.87 mmol) in dry CH_2Cl_2 (15 mL) was added Boc-Phe-NCA. The resulting solution was stirred for 1.5 h at room temperature under an atmosphere of N_2. The solvent was removed under reduced pressure to obtain a yellow oil. The residue was dissolved in EtOAc (50 mL), washed with brine (2 × 35 mL), dried with Na_2SO_4, and filtered to remove inorganic solids. The filtrate was evaporated under reduced pressure, and the resulting residue was purified on a short column of silica gel using a gradient of MeOH/CH_2Cl_2 (1−10%) to obtain 1.10 g of a light-yellow solid (50%): ^{1}H NMR ($CDCl_3$) δ 1.43 (9H, s), 2.36 (3H, s), 2.63 (2H, m), 3.08 (2H, b), 3.30 (2H, m), 4.34 (1H, quad), 5.41 (1H, d), 6.54 (1H, b), 7.2−7.4 (5H, m); ^{13}C NMR ($CDCl_3$) δ 27.8, 35.2, 38.1, 38.5, 49.8, 55.5, 126.1, 127.8, 128.8, 136.7, 155.1, 171.6; MS (FAB+) 266, 322, 344 (M + Na^+); HRMS (FAB+) [M + H]$^+$ calcd for $C_{17}H_{28}N_3O_3$ 322.2131, found 322.2139.

Cbz-D-A$_2$pr(Boc-Phe-NHCH$_2$CH$_2$N$_\beta$CH$_3$)-Gly-O*t*-But (4). A suspension of compound **2** (321 mg, 1.0 mmol), Cbz-D-serine β-lactone[8] (250 mg, 1.13 mmol) and Cs_2CO_3 (180 mg, 0.6 mmol) was dissolved in DMF (3 mL) and rapidly stirred for 24 h under a blanket of N_2 at room temperature. Thin-layer chromatography (10% MeOH/$CHCl_3$) indicated that compound **2** had been completely consumed. To this flask were then added Gly-O*t*-Bu·HCl (251 mg, 1.5 mmol) and BOP (531 mg, 1.2 mmol). The reaction mixture was allowed to stir at room temperature for 52 h. The DMF solvent was evaporated under high vacuum, and the crude material was taken up in 10% K_2CO_3 (15 mL, pH > 10) and extracted with EtOAc (5 × 25 mL). The organic layers were combined, washed with brine (15 mL), and dried with Na_2SO_4. The solution was filtered, and the resulting filtrate was evaporated under reduced pressure to obtain an oily yellow residue. The residue was chromatographed on silica gel using a gradient of MeOH/$CHCl_3$ (1−6%) to obtain 480 mg of a yellow solid (73% yield from compound **2**): ^{1}H NMR ($CDCl_3$) δ 1.36 (9H, s), 1.46 (9H, s), 2.26 (3H, s), 2.4−2.8 (4H, m), 2.97 (2H, m), 3.30 (2H, m), 3.83 (2H, m), 3.97 (2H, m), 4.27 (1H, s), 5.10 (2H, s), 5.21 (1H, b), 5.82 (1H, b), 6.67 (1H, b), 7.1−7.34 (10H, m), 7.90 (1H, b); MS (FAB+) 656, 788 (M + Cs^+); HRMS (FAB+) [M + Cs]$^+$ calcd for $C_{34}H_{49}CsN_5O_8$ 788.2635, found 788.2660.

Cbz-Tyr(Bzl)-D-A$_2$pr(Boc-Phe-NHCH$_2$CH$_2$N$_\beta$CH$_3$)-Gly-O*t*-But (5). Compound **4** (380 mg, 0.6 mmol) and 10% Pd/C (90 mg) in EtOH (20 mL) were stirred under an atmosphere of H_2 for 19 h. The catalyst was filtered off using vacuum filtration, and the resulting filtrate was evaporated under reduced pressure to obtain a clear oil (71% yield). This crude material were taken on without further purification. The oil was taken up in DMF (1.5 mL), and to this solution were added Cbz-Tyr(Bzl)-OH (216 mg, 0.618 mmol), DIEA (0.11 mL, 0.62 mmol), and BOP (274 mg, 0.62 mmol). The reaction mixture was stirred for 2 days and the solvent removed under reduced pressure. The crude material was partitioned between EtOAc (25 mL) and 10% K_2CO_3 (25 mL, pH > 10). The layers were separated, and the aqueous layer was extracted with EtOAc (4 × 25 mL). The organic layers were combined, washed with brine (15 mL), and dried with Na_2SO_4. The solution was filtered, and the resulting filtrate was evaporated under reduced pressure. Purification using a gradient of MeOH/CH_2Cl_2 (0−3%) yielded 242 mg of a clear oil (46% yield from compound **4**): ^{1}H NMR ($CDCl_3$) δ 1.36 (9H, s), 1.47 (9H, s), 2.19 (3H, s), 2.41 (2H, m), 2.58 (2H, m), 2.8−3.2 (4H, m), 3.32 (2H, m), 3.88 (2H, m), 4.25 (1H, b), 4.42 (2H, m), 5.02 (2H, s), 5.05 (2H, s), 5.47 (1H, b), 5.71 (1H, m), 6.88 (2H, d), 7.1−7.4 (18H, m), 7.91 (1H, b); MS (FAB+) 910, 1041 (M + Cs^+); HRMS (FAB+) [M + Cs]$^+$ calcd for $C_{50}H_{64}CsN_6O_{10}$ 1041.3738, found 1041.3738.

Cbz-Tyr(Bzl)-c[(N$_\beta$CH$_3$)-D-A$_2$pr-Gly-Phe-NHCH$_2$CH$_2$-] (6). Compound **5** (216 mg, 0.24 mmol) in CH_2Cl_2 (10 mL) and TFA (6 mL) was stirred for 1 h. The solvent was evaporated under reduced pressure, the residue was taken up in THF, and concentrated HCL was added (~5 drops) whereupon the HCl salt precipitated out. The solvent was removed under reduced pressure, and toluene (20 mL) was added to azeotrope off traces of residual acid and water. The resulting foam was dried overnight under high vacuum. To this residue were added HOAt (95 mg, 0.70 mmol), DIEA (0.1 mL, 0.57 mmol), DMF (3 mL), and CH_2Cl_2 (120 mL), and the reaction mixture was cooled to 4 °C. EDC (181 mg, 0.95 mmol) was added, and the reaction was stirred at 4 °C overnight and then at room temperature for 1 week. The solution was then washed with 10% K_2CO_3 (10 mL, pH > 10), the organic layer was separated, and the solvent was evaporated under reduced pressure. Purification using a gradient of MeOH/CH_2Cl_2 (0−3%) yielded 86 mg of a solid (49% yield from compound **5**): ^{1}H NMR ($CDCl_3$) δ 2.12 (3H, s), 2.97 (4H, m), 3.26 (2H, m), 3.49 (2H, m), 3.71 (2H, m), 4.12 (2H, m), 4.3 (2H, b), 5.27 (3H, m), 5.35 (2H, s), 5.38 (1H, b), 7.09 (2H, d), 7.14 (1H, b), 7.17−7.28 (17H, m), 8.49 (1H, b); MS (ESI) 735, 757 (M + Na^+).

Tyr-c[(N$_\beta$CH$_3$)-D-A$_2$pr-Gly-Phe-NHCH$_2$CH$_2$-] (1, MABE). Compound **6** (25 mg, 0.03 mmol) and Pd black (40 mg) in 20 mL of EtOH were shaken in a Parr hydrogenator overnight under 4 atm of H_2. The catalyst was removed using vacuum filtration and the filtrate was evaporated under reduced pressure. Purification of compound **1** using preparative RP-HPLC yielded a peak at 20.0 min using an isocratic setting of 15% (0.1% TFA/CH_3CN in 0.1% TFA/H_2O) at 8.0 mL/min: ^{1}H NMR (DMSO-d_6) δ 2.09 (4H, b, $-CH_3$, D-A$_2$pr^{2}C$_\beta$H), 2.29 (1H, b, C_bH), 2.40 (2H, b, D-A$_2$pr^{2}C$_\beta$H, C_bH), 2.83 (3H, m, Tyr^{1}H$_{\beta2}$, Phe^{4}H$_\beta$), 3.01 (1H, b, C_aH), 3.12 (3H, m, Gly^{3}H$_\alpha$, Phe^{4}H$_\beta$, C_aH),

3.97 (2H, m, Gly^{3}H$_\alpha$, Tyr^{1}H$_\alpha$), 4.12 (1H, quad, Gly^{3}H$_\alpha$), 4.31 (1H, quint, Phe^{4}H$_\alpha$), 4.48 (1H, b, D-A$_2$pr^{2}C$_\alpha$), 6.69 (2H, d, Tyr^1-H$_{3,5}$), 6.94 (1H, b, NH^5), 7.00 (2H, d, Tyr^1-H$_{2,6}$), 7.19−7.26 (5H, m, Phe^{4}H$_{Ar}$), 8.08 (3H, s, Tyr^{1}NH$_3$), 8.36 (1H, d, NH^4), 8.47 (1H, d, NH^2), 9.01 (1H, b, NH^3), 9.19 (1H, s, −OH); MS (ESI−) 509 (M − H$^-$), 531 (M + Cl$^-$), 623 (M + TFA$^-$); MS (ESI+) 511 (M + H$^+$), 533 (M + Na$^+$), 549 (M + K$^+$); HRMS (FAB+) [M + Cs]$^+$ calcd for $C_{26}H_{34}CsN_6O_5$ 643.1645, found 643.1665; RP-HPLC 14.7 min using a gradient of 10−40% (0.1% TFA/CH$_3$CN in 0.1% TFA/H$_2$O) over 20 min at 1 mL/min.

In Vitro Radioligand Inhibition Assay. 1. Preparation of Cell Membranes Expressing Opiate Receptors. This method is a modification of the method of Raynor et al.[17] CHO cells stably expressing cloned human μ, δ, and κ receptors were harvested by scraping from the culture flasks, centrifuging at 1000*g* for 10 min, resuspending in assay buffer (50 mM tris(hydroxymethyl)aminomethane HCl, pH 7.8, 1.0 mM ethylene glycol bis(β-aminoethyl ether) *N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA free acid), 5.0 mM $MgCl_2$, 10 mg/L leupeptin, 10 mg/L pepstatin A, 200 mg/L bacitracin, 0.5 mg/L aprotinin), and centrifuging again. The resulting pellet was resuspended in assay buffer homogenized with a Polytron homogenizer (Brinkmann, Westbury, NY) for 30 s at a setting of 1. The homogenate was centrifuged at 48000*g* for 10 min at 4 °C and the pellet resuspended at 1 mg of protein/mL of assay buffer and stored at −80 °C until use.

2. [^{3}H]Diprenorphine Binding to μ, δ, and κ Opiate Receptors. After dilution in assay buffer and homogenization as before, membrane proteins (50−100 mg) in 250 μL of assay buffer were added to mixtures containing test compound and radioligand (1.0 nM, 40 000−45 000 dpm) in 250 μL of assay buffer in 96-well deep-well polystyrene titer plates (Beckman) and incubated at room temperature for 60 min ([^{3}H]diprenorphine). Reactions were terminated by vacuum filtration with a Brandel MPXR-96T harvester through GF/B filters that had been pretreated with a solution of 0.5% poly(ethylenimine) and 0.1% bovine serum albumin for at least 1 h. The filter-bottom plates were washed four times with 1.0 mL of ice-cold 50 mM Tris-HCl, pH 7.8, 30 μL of Microscint-20 (Packard Instrument Co., Meriden, CT) was added to each filter, and radioactivity on the filters was determined by scintillation spectrometry in a Packard TopCount.

[^{3}H]Diprenorphine was purchased from Amersham Life Science, Inc. (Arlington Heights, IL) and had a specific activity of 39−45 Ci/mmol. Preliminary experiments were performed to show that no specific binding was lost during the wash of the filters, that binding achieved equilibrium within the incubation time and remained at equilibrium for at least an additional 60 min, and that binding was linear with regard to protein concentration. Nonspecific binding, determined in the presence of 10 μM unlabeled naloxone, was less than 10% of total binding.

The data from competition experiments were fit by nonlinear regression analysis by Prism (GraphPad Software, Inc., San Diego, CA) using the 4-parameter equation for one-site competition and subsequently calculating K_i from EC_{50} by the Cheng−Prusoff equation.

In Vivo Thermal Escape Assay. 1. Rat Preparation. Animal surgeries and tests were approved by the institutional animal care committee of the University of California, San Diego. Male Sprague−Dawley rats (Harlan Industries, Indianapolis, IN), weighing 250−350 g, were housed in separate plastic cages and maintained on a 12-h cycle (on, 7:00 a.m.; off, 7:00 p.m.) with food and water given ad libitum.

Chronic intrathecal (i.t.) catheters were implanted under 2−3% halothane (50% O_2/air) anesthesia.[18] Briefly, for intrathecal injection, an 8.5-cm polyethylene catheter (PE-10) was inserted through a slit in the cisternal atlanto-occipital membrane and was passed to the rostral edge of the lumbar (L4) subarachnoid space. The external portion was tunneled subcutaneously to exit at the top of the skull. Prior to insertion, the catheter was flushed with saline and, after insertion, plugged with stainless steel wire to prevent leakage of cerebrospinal fluid.

2. Assay of Thermal Nociception. This test was described previously in detail.[19] In brief, the animal was placed on a glass surface which was maintained at 30 °C by a feedback-controlled, under-glass, forced-air heating system.[20] A projection bulb under the glass was focused on the foot pad of the animal which induced an abrupt withdrawal. A cutoff time was set at 20 s to prevent the tissue damage. The test was started approximately 1 h after animal was placed in the chamber for acclimation. After the baseline latency was measured, the drugs were injected intrathecally and the test was performed.

3. Drugs and Injections. All agonists were injected i.t. in a total volume 10 μL followed by 10 μL of saline to flush the catheter. Naloxone was injected i.t. in a total volume of 10 μL followed by 10 μL of saline, 10 min before agonists were injected. Naloxone hydrochloride (DuPont; Supporting Information Figure 2), MABE (Supporting Information Figure 3), DAMGO ([D-Ala^2,N-Me-Phe^4,Gly-ol^5]-enkephalin; RBI, MA; Supporting Information Figure 4), and morphine sulfate (Merck, Sharpe and Dohme, West Point, PA) were dissolved in physiological saline (0.9% NaCl w/v), and DPDPE ([D-Pen^2, D-Pen^5]-enkephalin; RBI, MA) was dissolved in 10% 2-hydroxypropyl-β-cyclodextrin (Research Biochemicals, Inc., Natick, MA).

4. Data Analysis. Response latency data from the hot box test are presented as the mean ± SE at 0, 15, 30, 60, 90, and 120 min after drug injection and were converted to percent maximum possible effect (% MPE) according to the formula:

$$\%\ \text{MPE} = (\text{postdrug latency} - \text{predrug latency})/(\text{cutoff latency}^* - \text{predrug latency}) \times 100\%$$

where the cutoff latency is 20 s in this study.

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Supporting Information Available: NMR characterization (^{1}H chemical shifts, coupling constants, amide proton temperature coefficients, and NOE data), conformational analysis (preferred conformational families and torsional angle data), and biological assay data (effects of naloxone on drug analgesia, comparative intrathecal dose dependency of DAMGO and MABE on the thermal escape test) for MABE (12 pages). Ordering information is given on any current masthead page.

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