5-Fluoroisoproterenol (2b) Oxalate. Hydrogenolysis of 125 mg (0.31 mmol) of **8b** in 25 mL of methanol in the presence of 23 mg (0.38 mmol) of oxalic acid dihydrate as in the preparation of **1a** oxalate gave, after recrystallization from acetonitrile/water, 30 mg (0.13 mmol) of **2b** oxalate.

6-Fluoroisoproterenol (2c) Oxalate. 4,5-Bis(benzyloxy)-2-fluorophenethanolamine (**3c**; 300 mg, 0.81 mmol) was reductively alkylated as above to give 178 mg (0.43 mmol) of **8c**: NMR ($CDCl_3$) δ 1.06 (d, J = 6.5 Hz). Catalytic hydrogenolysis of 178 mg (0.43 mmol) of **8c** in the presence of oxalic acid dihydrate (58 mg, 0.46 mmol) gave, after recrystallization from ethanol, 27 mg (0.1 mmol) of **2c** oxalate.

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Synthesis, X-Ray Crystallographic Determination, and Opioid Activity of *erythro*-5-Methylmethadone Enantiomers. Evidence Which Suggests That μ and δ Opioid Receptors Possess Different Stereochemical Requirements[1]

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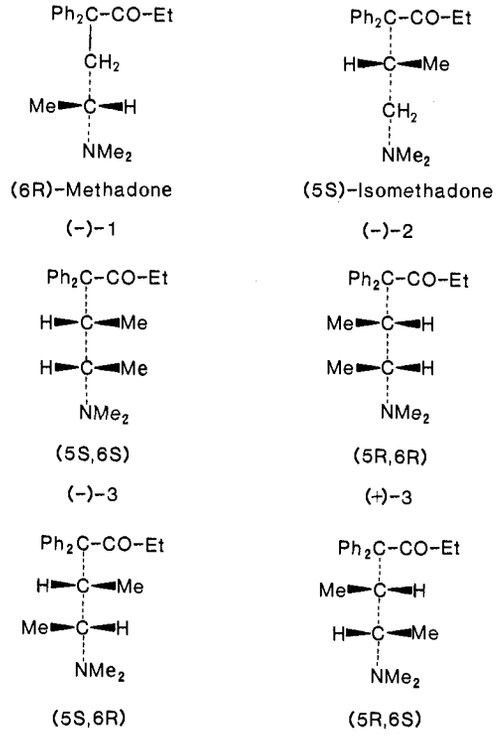
Enantiomers of *erythro*-5-methylmethadone (**3**) were synthesized from optical antipodes of *erythro*-3-(dimethylamino)-2-butanol. X-ray crystallographic analysis of (−)-**3** perchlorate revealed that it possesses the 5*S*,6*S* absolute configuration. It was found that (−)-**3** is substantially more potent than its enantiomer (+)-**3** as an opioid agonist in vivo and in vitro. In vitro tests (guinea pig ileal longitudinal muscle and mouse vas deferens preparations) suggest that (−)-**3** mediates its effect chiefly through μ opioid receptors. On the other hand, (+)-**3** and the more potent enantiomers of methadone, (−)-**1**, and isomethadone, (−)-**2**, appear to have less μ-receptor selectivity and interact with a greater fraction of δ receptors than does (−)-**3**. The fact that the solid-state conformation of (−)-**3** differs from that of (−)-**1** and (−)-**2**, which show great similarity in conformational features, suggests that μ and δ receptors have different conformational requirements. The possibility of different modes of interaction with a single opioid receptor population also is discussed.

In an effort to investigate the stereochemical relationship between the more potent enantiomers of methadone [(−)-**1**]



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and isomethadone [(−)-**2**], we have previously synthesized two diastereomeric racemates of 5-methylmethadone (**3** and **4**) and evaluated them for antinociceptive activity in mice.[2] The threo racemate **4** contains an enantiomer (5*S*,6*R*) which can be considered a hybrid of (−)-**1** and (−)-**2** by virtue of possessing the same absolute configuration at common chiral centers. It was found that the threo racemate **4** is totally devoid of antinociceptive activity, while the erythro racemate **3** is approximately 5-fold more potent than methadone, (±)-**1**.

Since the active racemate (±)-**3** cannot contain the same combination of configurations found in (−)-**1**[3] and (−)-**2**[4] by virtue of its erythro stereochemistry, it was concluded[2] that each chiral center does not behave independently but rather interacts intramolecularly to afford a conformational population which facilitates the ligand–receptor recognition process.

We now report on the preparation, absolute configuration, and activity of *erythro*-5-methylmethadone enantiomers [(−)- and (+)-**3**] in an attempt to sort out the contributions of configurational and conformational factors in the ligand–receptor recognition process.

Chemistry. Attempts to resolve the erythro racemate (±)-**3** or its immediate precursor, methyl *erythro*-2,2-diphenyl-3-methyl-4-(dimethylamino)valerate, with a variety of resolving agents were unsuccessful. Ultimately, resolution of *erythro*-3-(dimethylamino)-2-butanol[5,6] (through

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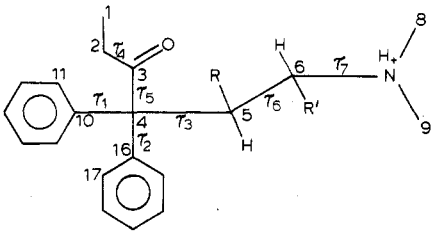
Table I. Torsion Angles of Crystallographically Observed Conformations of Methadone and Its Congeners



compd	R	R'	torsion angle,[a] deg τ_1	τ_2	τ_3	τ_4	τ_5	τ_6	τ_7	$\tau_{7'}$
(−)-1·HBr[b]	H	CH_3	−34	97	76	−29	12	−146	75	−53
(−)-2·HCl[c]	CH_3	H	−25	85	66	−10	19	−152	81	−154
(−)-3·$HClO_4$[d]	CH_3	CH_3	−40	100	64	−10	0	97	92	−156
normethadone hydrochloride[e]	H	H	−20	98	74	−15	−2	−165	72	−53
	H	H	−22	100	75	−6	−5	165	71	−53

[a] Partial atomic numbering defines torsion angles: τ_1 = C(11)-C(10)-C(4)-C(5); τ_2 = C(17)-C(16)-C(4)-C(5); τ_3 = C(3)-C(4)-C(5)-C(−6); τ_4 = C(1)-C(2)-C(3)-O(3); τ_5 = O(3)-C(3)-C(4)-C(5); τ_6 = C(4)-C(5)-C(6)-H; τ_7 = C(5)-C(6)-N-C(8); $\tau_{7'}$ = C(5)-C(6)-N-C(9). [b] A. W. Hanson and F. R. Ahmed, *Acta Crystallogr.*, 11, 724 (1958). [c] E. Shefter, *J. Med. Chem.*, 17, 1037 (1974). [d] This study. [e] E. Bye, *Acta Chem. Scand., Ser. B*, 30, 323 (1976). Two independent molecules are in a single crystal.

Table II. Relative Potencies of Methadone and Related Compounds

compd[b]	confign	relative molar potency[a] mice[c]	GPI[d]	MVD[e]	GPI/MVD[f]
(−)-1	6*R*	3.1	1.4 (2)	6.0 (4)	1.5
(−)-2	5*S*	2.3	2.2 (2)	6.3 (5)	2.3
(−)-3	5*S*,6*S*	11.3	71.4 (2)	67.7 (2)	6.9
(+)-3	5*R*,6*R*	1.8	1.7 (4)	6.6 (4)	1.7
(±)-4	5*S*,6*R*	inact at	inact[g]	inact[h]	
	5*R*,6*S*	140 μmol/kg			
morphine		1	1	1	6.5

[a] Relative to morphine. [b] Compounds 1-4 were tested as the HCl salts and morphine as the sulfate. [c] Represents the morphine ED_{50} (12.4 μmol/kg)/compound ED_{50} using the hot-plate procedure (four dose levels with five mice per dose level). [d] Relative to morphine (IC_{50} = 6.8 ± 0.84 × 10^{-8} M) on the guinea pig ileum; parentheses indicate number of replicate experiments. [e] Relative to morphine (IC_{50} = 4.4 ± 1.1 × 10^{-7} M) on the mouse vas deferens. [f] Molar potency ($1/IC_{50}$) of compound on the guinea pig ileum divided by its potency in the mouse vas deferens. [g] Potentiates contraction at ≥ 5.4 × 10^{-8} M. [h] Potentiates contraction at ≥ 1.2 × 10^{-6} M.

its *p*-toluoyltartrate salt) and the transformation of these enantiomers by a reported[2] reaction sequence yielded the desired enantiomers, (−)-**3** and (+)-**3**.

X-ray Crystallography. The absolute configuration of the more potent enantiomer of *erythro*-5-methylmethadone, (−)-**3**, was determined by X-ray crystallographic analysis of its perchorate salt. This enantiomer, (−)-**3**, was found to have 5*S*,6*S* chirality and the conformation illustrated in Figure 1. The conformation of (−)-**3** is quantitatively defined by the seven torsion angles indicated in Table I. The values of these torsion angles are compared with those observed in the X-ray crystal structures of the protonated forms of (−)-methadone [(−)-**1**], (−)-isomethadone [(−)-**2**], and normethadone (Table I).

In view of the presumed conformational flexibility of these molecules, the observed conformations are remarkably similar. The only significant conformational differences are in τ_6 and τ_7, which reflect the change in orientation of the dimethylamino groups relative to the rest of the molecule. This is illustrated in Figure 2, which compares the solid-state conformations of (−)-*erythro*-5-methylmethadone [(−)-**3**], methadone [(−)-**1**], and isomethadone [(−)-**2**]. The (−)-*erythro*-5-methylmethadone structure is distinguished from the others by its positive synclincal value of τ_6. In this connection, the amino group is in a more extended conformation in (−)-**1** and (−)-**2**.

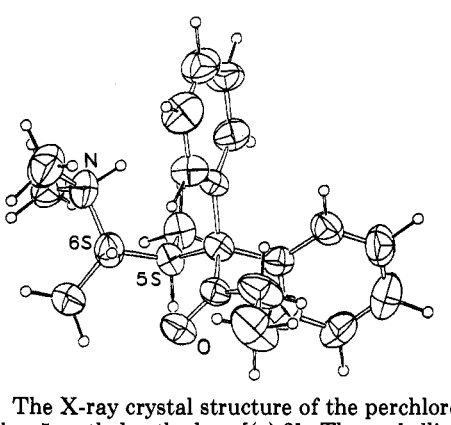


Figure 1. The X-ray crystal structure of the perchloroate salt of (−)-*erythro*-5-methylmethadone [(−)-**3**]. Thermal ellipsoids for nonhydrogen atoms are scaled to 60 probability and H atoms are represented as spheres equivalent to B = 1 Å.

Pharmacology. The hydrochloride salts (Table II) were tested in mice 15 min after subcutaneous administration using a modified[7] hot-plate procedure.[8] In vitro testing for opioid activity was conducted on the electrically stimulated guniea pig ileal longitudinal muscle[9] (GPI) and

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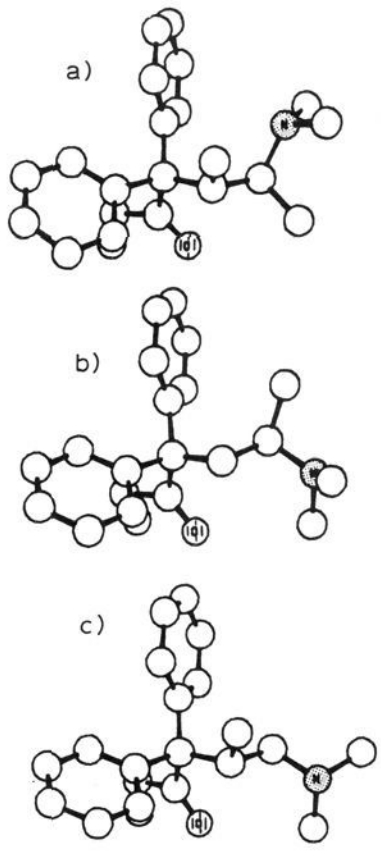

Figure 2. A comparison of the crystallographically observed conformations of salts of (a) (−)-*erythro*-5-methylmethadone [(−)-**3**], (b) (−)-methadone [(−)-**1**], and (c) (−)-isomethadone [(−)-**2**], illustrating the similarity in all features except the relative orientation of the dimethylamino group.

mouse vas deferens[10] (MVD) preparations. The hot-plate data are expressed as the morphine ED_{50} (standard) divided by the test compound ED_{50}. The in vitro data are expressed as ratios of the morphine IC_{50} (control) divided by the IC_{50} of the test compound in the same preparation. The greater potency of morphine in the GPI than in the MVD by a factor of 6.5 is in the range reported by others.[11]

The rank-order potencies of the in vivo and in vitro data are in harmony with one another, suggesting that events affecting access to receptors are not overriding factors which govern potency in this series. Interestingly, the threo racemate (±)-**4**, which is known[2] to be inactive as an agonist or antagonist in mice, also exhibits no activity in vitro and, in fact, potentiates the contraction of both the GPI and MVD.

Stereostructure–Activity Relationship. A feature of this relationship that is most apparent is that the more potent *erythro*-5-methylmethadone entantiomer [(−)-**3**] possesses the 5*S* configuration. This is in harmony with the opioid receptor 5*S* stereoselectivity of isomethadone [(−)-**2**] and all other structurally related ligands with an equivalent chiral center.[12-14]

On the other hand, the 6*S* stereochemistry of (−)-**3** is opposite that of the more potent methadone enantiomer (−)-**1**. In this connection, there are many examples of inverted enantioselectivity at the C-6 or equivalent position in related series.[12-14] The biological data therefore are consistent with the apparent greater importance[14] of the C(5) chiral center over C(6) in the recognition process at opioid receptors.

While the opioid potency of (±)-**3** resides principally with the (−)-5*S*,6*S* enantiomer both in vitro and in vivo, the enantiomeric potency ratio depends on the test system employed. Thus, the potency ratio, (−)-**3**/(+)-**3**, is 6 in mice, 40 on the GPI, and 10 on the MVD (Table II). Since the GPI preparation is known to contain predominantly μ opioid receptors and the MVD is rich in δ opioid receptors,[15] it seems likely that the different enantiomeric potency ratios reflect stereochemical differences among these opioid receptor subtypes. These data suggest that greater enantioselectivity is associated with μ receptors. The fact that the enantiomeric potency ratio in mice is closer to that determined on the MVD preparation suggests δ-like stereoselectivity for the recognition sites which mediate analgesia in mice.

As indicated by the GPI/MVD ratios, all the optical isomers (Table II) are more effective on the GPI than on the MVD. In this regard, it appears that (−)-**3** has a substantially greater GPI/MVD ratio (6.9) than that of the other optical isomers [(−)-**1**, (−)-**2**, and (+)-**3**], whose ratios are close to 2. The high GPI/MVD ratio associated with (−)-**3** suggests that it interacts more effectively with μ than with δ opioid receptors. This ratio is close to that of morphine (6.5), which is considered to be the prototypical μ agonist. The lower GPI/MVD ratios of the other compounds [(−)-**1**, (−)-**2**, and (+)-**3**] are consistent with the mediation of the agonist response through a greater percentage of δ opioid receptors.

Evidence relating divergent conformational preferences between (±)-**3** and (±)-**4** to their marked potency difference has been reported.[2] Such conformational differences probably arise as a consequence of nonbonded repulsive interaction between the vicinal *C*-methyl groups of these diastereomers. The present study provides evidence that conformational factors are in part responsible for the enhanced potency of (−)-**3**.

The crystallographically observed conformations of (−)-**3**, (−)-**1**, and (−)-**2** are compared in Figure 2. Crystallographically observed conformations are generally very near local if not global minimum energy conformations.[16] In those cases where two or more conformers are in equilibrium in solution, crystal structure determination will usually identify one of them.[17] The reported[2] NMR coupling constant, $J_{5,6}$ = 6 Hz, of (±)-**3** in D_2O and the fact that a change in solvent polarity induces a change in $J_{5,6}$ indicate that it consists of a mixture of conformers of comparable energies. It is therefore likely that the crystallographically observed conformation (Figure 2a) is one of several conformers in solution.

Although it is not the sole component in solution, the demonstration of this unusual conformation in the solid state is almost certainly a result of the simultaneous presence of the methyl substituents on C(5) and C(6) and may have a direct relationship to the compound's enhanced activity. The fact that both (−)-**1** and (−)-**2** readily achieve an antiperiplanar-type arrangement of $^{+}NHMe_2$ and Ph_2CCOEt in the solid state and solution[2] may also suggest that some alternate conformation is responsible for the enhanced activity of (−)-**3**. At the very least the data provide support for conformational differences between these molecules.

Since the present studies suggest that μ and δ receptors possess different stereochemical requirements, such con-

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formational differences may contribute to the greater μ opioid receptor selectivity of (−)-**3**. Thus, one possible explanation for the greater μ receptor activity of (−)-**3** might be related to a more proximal conformational relationship between the basic nitrogen and one of the phenyl groups, as illustrated in Figure 2. In view of the differences in the structural requirements for agonist activity at μ and δ receptors,[18] a difference in receptor stereoselectivity is not unexpected.

Alternately, but not mutually exclusive, the mode of interaction of (−)-**3** may differ from that of (−)-**1** or (−)-**2** with the same recognition site. This might arise as a consequence of a different orientation of the $^{+}HNMe_2$ group with the anionic site on the receptor. This has been proposed for diastereomic 4-phenylpiperidine analgetics[19] and for α-methadol.[20] The opioid activity of enkephalin analogues that contain a basic amino acid residue linked through a peptide bond with the Tyr nitrogen also is consistent with this concept.[21]

Experimental Section

Melting points were determined in open capillary tubes with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, and are within ±0.4% of theoretical values. IR (Perkin-Elmer 281) and NMR (Varian HFT 80) spectra are consistent with the structures. Optical rotations were taken using a 1-cm cell on a Perkin-Elmer 141 polarimeter. (−)-Methadone and (−)-isomethadone were gifts of Mallinckrodt, Inc., St. Louis, MO.

Resolution of (±)-*erythro*-3-(Dimethylamino)-2-butanol. To a hot solution of 40 g (1.04 mol) di-*p*-toluoyl-*d*-tartaric acid in methanol (120 mL) was added 12 g (1.03 mol) of (±)-*erythro*-3-(dimethylamino)-2-butanol.[5-7] After the solution cooled to 25 °C, anhydrous ether was added to induce crystallization. After 1 day at room temperature, the salt was filtered: mp 159–160 °C; $[\alpha]^{20}_D$ −116° (*c* 2, CH_3OH). Four additional recrystallizations from methanol–2-propanol afforded a salt (6 g, 23%) with constant rotation and melting point: mp 167.5–168 °C; $[\alpha]^{25}_D$ −113.5° (*c* 2.0, CH_3OH). The salt was dissolved in water, and the solution was made basic with 10% aqueous sodium hydroxide. Extraction with ether (4 × 50 mL) and drying (magnesium sulfate) yielded the free base, $[\alpha]^{20}_D$ +54.3° (*c* 3.14, $CHCl_3$), which was converted to the hydrochloride salt: mp 141–2 °C; $[\alpha]^{20}_D$ −24.0° (*c* 2.92, CH_3OH).

The mother liquors from the above procedure were evaporated to dryness, and the free base (8.0 g, 0.063 mol) was dissolved in methanol (40 mL). To this solution was added di-*p*-toluoyl-*l*-tartaric acid (25 g, 0.065 mol) dissolved in ethanol (200 mL). 2-Propanol (30 mL) was then added to initiate crystallization. After 24 h at 4 °C, the crude product was collected and recrystallized four times from ethanol to give 6 g (23%) of the salt: mp 166–167 °C; $[\alpha]^{20}_D$ +114.5° (*c* 2.14, CH_3OH). The salt was dissolved in water, and the solution was made basic with 10% aqueous sodium hydroxide. Extraction with ether (4 × 50 mL) and drying (magnesium sulfate) afforded the free base, $[\alpha]^{20}_D$ −52° (*c* 3.1, $CHCl_3$). The free base was converted to the hydrochloride salt: mp 138–140 °C; $[\alpha]^{20}_D$ +24.6° (*c* 2.91, CH_3OH). The spectral data of (+)- and (−)-*erythro*-3-(dimethylamino)-2-butanol were identical with those of the racemate.

Methyl *erythro*-2,2-Diphenyl-3-methyl-4-(dimethylamino)valerate. Into a three-neck flask modified for downward addition and fitted with a mechanical stirrer, nitrogen inlet, septum, and drying tube, was placed methyllithium in ether (75.81 mL, 1.4 M). The ether was removed by evaporation with a stream of nitrogen. To the residue was added 25.56 g (0.105 mol) of triphenylmethane in anhydrous THF (122 mL). After the solution was stirred at 25 °C for 3 h, methyl diphenylacetate (23.77 g, 0.105 mol) in anhydrous THF (82 mL) was added slowly through the septum with a syringe. Into a second dry three-neck flask fitted to the bottom of the above apparatus, to which was attached an injection septum, nitrogen inlet, and a drying tube, was placed (+)-*erythro*-3-(dimethylamino)-2-butanol (2.67 g, 0.024 mol) in anhydrous THF (41 mL). Butyllithium in hexane (8.54 mL, 2.5 M) was injected rapidly in small fractions into the cooled (−5 °C) solution. After stirring for 5 min, *p*-toluenesulfonyl chloride (4.20 g, 0.024 mol) in anhydrous THF (41 mL) was injected in small fractions. After the suspension was stirred for 1 h, allowing it to rise to room temperature, the contents of the upper flask were then added to the lower flask, and the reaction mixture was stirred for 4 days at room temperature. The reaction mixture was poured into water (500 mL), stirred for 5 min, and then extracted with ether (5 × 100 mL) and dried (magnesium sulfate). Removal of solvent left an oil, which was dissolved in 10% HCl and extracted with ether (3 × 75 mL). The aqueous phase was made basic with cold 10% NaOH and extracted with ether (5 × 100 mL). The organic phase was dried (magnesium sulfate) and evaporated in vacuo to yield 5 g of oil, which was purified by chromatography using a silica gel column (8 × 1 in.) with petroleum ether (60–110 °C) and ethyl ether as eluents. The product (2.0 g, 26%) was obtained as a colorless oil, $[\alpha]^{20}_D$ −45.92° (*c* 1.69, $CHCl_3$). Employing (−)-*erythro*-3-(dimethylamino)-2-butanol in an identical procedure yielded methyl (+)-*erythro*-2,2-diphenyl-3-methyl-4-(dimethylamino)valerate, $[\alpha]^{20}_D$ +43.6° (*c* 1.69, $CHCl_3$). The IR, NMR, and MS of both antipodes were identical with those of its racemate.[2]

***erythro*-4,4-Diphenyl-5-methyl-6-(dimethylamino)-3-heptanone.** A mixture of lithium wire (0.54 g, 0.078 mol) in anhydrous ethyl ether (60 mL) was cooled (−40 °C) and stirred under nitrogen, and freshly distilled ethyl bromide (4.21 g, 38.74 mmol) was added dropwise. More ethyl bromide was added periodically to ensure complete reaction of the lithium. After about 3 h no lithium remained. A solution of methyl (−)-*erythro*-2,2-diphenyl-3-methyl-4-(dimethylamino)valerate (1.63 g, 4.99 mmol) in anhydrous ether (60 mL) was added to the ethyllithium solution. The solution was then allowed to warm to 0 °C, where it was held for 6 h and then stored at 5 °C for 2 days. The solution was poured into vigorously stirred ice–water (1 L) and extracted with ether (4 × 150 mL). The product was taken up in 10% HCl, which was then cooled to 0 °C and neutralized with cold 15% KOH. The resulting solution was extracted with ether (5 × 100 mL), which was dried (magnesium sulfate) and evaporated to afford 1.4 g of oil, which was dissolved in chloroform and converted into its hydrochloride salt. After removal of the solvent in vacuo, the salt was purified by column chromatography (2 times) using silica gel and ethyl acetate and methanol as eluants. The salt (0.25 g, 16%) was obtained as a white solid: mp 179–182 °C; $[\alpha]^{20}_D$ −61.7° (*c* 1.56, CH_3OH). Its enantiomer (+)-**3**·HCl was prepared and purified in the same manner: mp 177 °C $[\alpha]^{20}_D$ +59.4° (*c* 1.54, CH_3OH). The IR, NMR, and MS of the antipodes were identical with those of its racemate. Anal. for (−)- and (+)-**3**·HCl ($C_{22}H_{30}ClNO\cdot H_2O$) C, H, N.

X-ray Crystallography. Crystals of the perchlorate salt of (−)-*erythro*-5-methylmethadone, grown by evaporation of an aqueous solution, are orthorhombic, space group $P2_12_12_1$, with cell constants (18 °C) a = 10.823 (3) Å, b = 23.56 (1) Å, c = 8.735 (2) Å, Z = 4, and P_c = 1.312 g-cm^{-3}. A single crystal (0.24 × 0.3 × 0.52 mm) was mounted, and 2916 independent reflections ($\sin/\lambda$ = 0.704 Å^{-1}) were collected with niobium-filtered Mo Kα radiation (0.71069 Å) on a Syntex p3 automated 4-circle diffractometer using 2θ scans. Three reference reflections which were monitored throughout the course of the data collection showed no significant

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change in intensity. A total of 1556 data were found to have $F < 3\sigma(f)$ and were therefore considered to be unobserved. The intensities were corrected for Lorentz and polarization factors but not for extinction or absorption ($\mu_{MoK\alpha} = 2.13$ cm^{-1}). Real and imaginary dispersion corrections were applied to the atomic scattering factors. The variance of each structure factor was calculated according to the method of Stout and Jensen:[22] $\sigma^2(F) = 1LpI[\sigma^2(I) + (0.06I)^2]$; $w(F) = 1/\sigma^2(F)$. Unobserved data were assigned zero weight.

The structure was solved through the use of the direct methods program, MULTAN,[23] from which the position of all nonhydrogen atoms were determined. Isotropic refinement of the structure by full-matrix least-squares, minimizing $\sum w(F_o - F_c)^2$, converged at a residual ($R = \sum||F_o|-|F_c||/\sum|F_o|$) of 13.2%. The refinement was continued, treating the vibration of all atoms anisotropically and including the hydrogen atom contributions to the structure factors by calculating their positions on the basis of idealized geometry. The refinement converged at a residual of 0.068 for the 1360 observed data ($R = 0.13$ for all data) and a weighted residual ($R_w = \sum w(F_o - F_c)^2/\sum wF_o^2$) of 0.071. S, the standard deviation of an observation of unit weight [$S = \sum w\Delta^2/(m - n)$, where m is the number of observations and n is the number of parameters], was 1.73 and suggests that the standard deviations may be underestimated by a factor of nearly 2. The absolute configuration was determined by measurement of the relative intensities of 49 Freidel pairs with large differences in calculated intensities (0.25–0.75). In 44 of 49 cases the signs of the observed differences between F_{hkl} and $F_{\overline{hkl}}$ (0.76–4.44) were in agreement with the signs of the corresponding calculated differences for the structure with the 5*S*,6*S* configuration. Positional parameters for the nonhydrogen atoms are given in Table III (see paragraph at the end of paper concerning supplementary material). The equivalent B_{iso} for the nonhydrogen atoms was calculated as defined by eq 18 of Hamilton.[24]

Guinea Pig Ileal Longitudinal Muscle Preparation. The preparation employed a strip of longitudinal muscle (with myenteric plexus intact) obtained from a segment of male guinea pig (300–450 g) ileum. The segment was taken from a location 10–15 cm above the ileocecal junction and gently placed on a glass rod, and the longitudinal muscle layer was carefully stripped off with the aid of a wet cotton swab. The muscle strip was suspended under a 1-g tension between two platinum wire electrodes located on either side. The mounted strip was immersed in an organ bath (10 mL) containing a Krebs–Ringer bicarbonate solution of the following composition (in mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.52; $MgSO_4$, 1.19; KH_2PO_4, 1.19; $NaHCO_3$, 25; dextrose, 11.48. Chlorpheniramine maleate (1.2 μM) was added as an antihistaminic. A mixture of 95% O_2 and 5% CO_2 was bubbled through the bath, which was maintained at 37 °C by means of an external jacket through which warm water was circulated. Contractions of the muscle were induced by square wave pulses (Grass S44 stimulator; 0.5-ms duration, 0.1 Hz) that were initially applied as a gradient of 10 V every 2 min up to a supramaximal value of 90 V. The contractions were amplified through an isometric transducer (Statham UC3) connected to one end of the muscle and were recorded by a Gilson polygraph. The preparation was allowed to equilibrate for a minimum of 90 min under continuous stimulation before exposure to drugs.

Compounds to be tested were added as solutions (1–10 μL) of the hydrochloride salt in normal saline. The percent inhibition of the muscle twitch was recorded at each level of serial additions of compound up to a total of approximately 80%. Prior to applying compound, the response to standard solutions of morphine sulfate was recorded for each muscle preparation, followed by several washings of the muscle with fresh bath to remove drug. Two to four independent preparations were employed for each compound tested. Using the parallel line assay method of Finney,[24] an estimate of the IC_{50} for each compound and its potency relative to morphine was made from a plot of the percent inhibition of muscle twitch vs. the log concentration of the compound.

Mouse Vas Deferens Preparation. Male albino mice, weighing 25–40 g, were killed by cervical dislocation, and their vasa deferentia were dissected out, carefully stripped of surrounding tissue, and gently pressed to expel their seminal contents. The tissue was placed in a bath (10 mL) containing (in mM) NaCl, 118; KCl, 4.7; $CaCl_2$, 2.52; KH_2PO_4, 1.19; $NaHCO_3$, 25; and dextrose, 11.48. The bath was gassed with 95% O_2 and 5% CO_2 and maintained at 37 °C by means of a circulating water jacket. The muscle was suspended within the bath under 100 mg of tension and positioned so as to place each end at the center of a circular platinum wire electrode. Contractions of the muscle were induced by supramaximal rectangular pulses (70 V, 0.1 Hz, 1.0-ms duration) using a Grass S44 stimulator and were measured isometrically using a Statham UC3 transducer and Gilson recorder. The muscle was allowed to equilibrate under continuous stimulation for a minimum of 15 min before exposure to drugs.

Compounds were applied to the tissue by the addition of microliter amounts (1–10 μL) of aqueous solutions of the hydrochloride salt to the bath. The maximum decrease of the twitch was noted, and serial additions of compound solutions were made until approximately 80% inhibition was obtained. Prior to applying compounds, a control response to morphine was determined for each tissue, followed by several washings with fresh bath to reestablish the original twitch value. Only one compound was tested per tissue, and each compound was evaluated on two to five preparations. From a plot of the percent inhibition against the log bath concentration, a value for the IC_{50} and potency relative to morphine was calculated for each compound using the parallel line assay method of Finney.[25]

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Supplementary Material Available: Atomic coordinates and isotropic thermal parameters for (–)-*erythro*-5-methylmethadone perchlorate (1 page). Ordering information is given on any current masthead page.

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