Probes for Narcotic Receptor Mediated Phenomena. 9.[1] Synthesis of (±)-(3α,6aα,11aβ)-1,3,4,5,6,11a-Hexahydro-2-methyl-2*H*-3,6a-methanobenzofuro[2,3-*c*]azocin-10-ol, an Oxide-Bridged 5-(*m*-Hydroxyphenyl)morphan†

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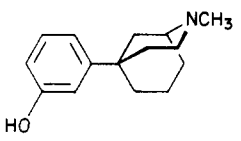
The synthesis of racemic (3α,6aα,11aβ)-1,3,4,5,6,11a-hexahydro-2-methyl-2*H*-3,6a-methanobenzofuro[2,3-*c*]azocin-10-ol (**2d**) is described. The route used acid-catalyzed ring closure of enamine **5** to yield the unsaturated phenylmorphan **6**. Conversion of **6** to oxide-bridged **2d** was accomplished in a multistep fashion that utilized the introduction of a bromine atom, followed by O-demethylation of the phenolic methyl ethers and base-catalyzed intramolecular phenoxide displacement of the bromine. Compound (±)-**2d** represents an oxide-bridged derivative of the potent 5-(*m*-hydroxyphenyl)morphan class of opioid analgesics **1**. Unlike the 5-(*m*-hydroxyphenyl)morphans that have a freely rotating phenyl group, **2d** has the phenyl ring conformationally restricted at an angle of 49° relative to atoms 1, 3, 11a, and 12 of **2d**. The low binding of (±)-**2d** to rat brain homogenate receptor preparations [IC_{50} = 1000 nM] may indicate that the phenyl angle of 49° is not suitable for binding to opioid receptors.

As one aspect of our program to study the structure and function of the opioid receptor system,[1] we are pursuing a synthetic study of the 2-methyl-5-(*m*-hydroxyphenyl)-morphan nucleus (**1**) to define the conformational requirements for optimum binding of this class of compounds to opioid receptors. Since the phenylmorphan skeleton is rigid, conformational flexibility resides predominantly in the rotation of the phenyl ring about a single bond. As such, an initial goal of this study is to determine the phenyl ring torsion orientation that results in greatest receptor affinity. As outlined more fully in two previous reports on this work,[2,3] our approach has been to freeze the conformation of the phenyl ring by means of an oxide bridge. Six unique isomeric oxide-bridged phenylmorphan-like compounds are possible with the phenyl ring held close to a 60° angle relative to the previous isomer in the series (**2a-f**).



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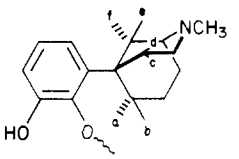
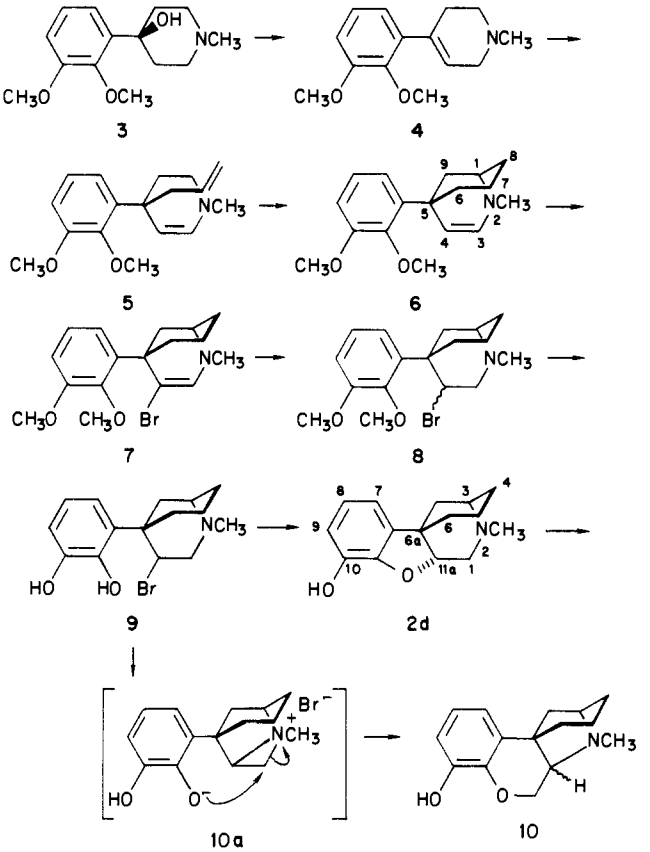


2a-f

The first isomer synthesized,[2] **2a**, had essentially no opioid receptor affinity[4] (IC_{50} = 1766 nM), while the second isomer[3] **2f** had an 18-fold greater binding affinity[4] (IC_{50} = 96 nM). Encouraged by the latter result, we set out to prepare further members of the series **2a–f**. In this paper, we report the synthesis and X-ray structure determination of **2d**. This synthesis represents a new entry into the hexahydro-2*H*-3,6a-methanobenzofuro[2,3-*c*]azocine ring system.

Synthesis. The synthetic approach to **2d**, shown in Scheme I, contains two crucial features: the synthesis of the phenylmorphan skeleton **6** and subsequent closure of the oxide bridge to form the desired 3,6a-methanobenzofuro[2,3-*c*]azocine structure (**6** → **2d**). The acid-catalyzed

Scheme I



ring closure of **5** to form **6** was anticipated on the basis of the work of Evans in a similar system.[5] A valuable feature of this procedure is the accompanying introduction of a

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(1) For the previous paper in this series, see: Burke, T. R., Jr.; Rice, K. C.; Jacobson, A. E.; Simonds, W. F.; Klee, W. A. *J. Labelled Compd. Radiopharm.* **1984**, *21*, 693.
(2) Burke, T. R., Jr.; Jacobson, A. E.; Rice, K. C.; Silverton, J. V. *J. Org. Chem.* **1984**, *49*, 1051 (paper 4 in this series).
(3) Burke, T. R., Jr.; Jacobson, A. E.; Rice, K. C.; Silverton, J. V. *J. Org. Chem.* **1984**, *49*, 2508 (paper 6 in this series).
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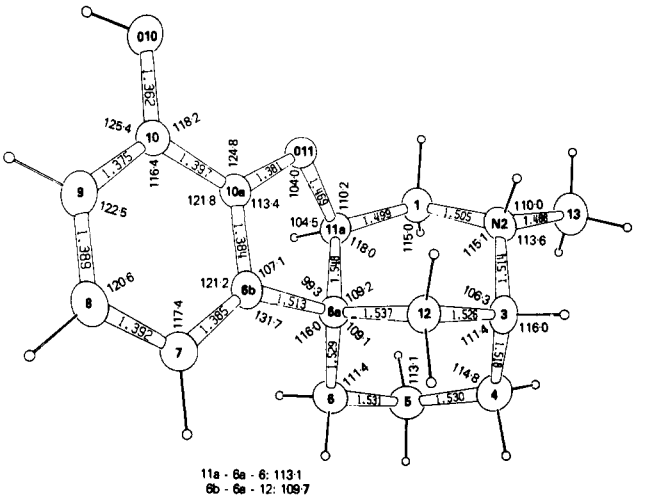

Figure 1. ORTEP[11] drawing of (±)-**2d**·HCl showing crystal conformation, bond lengths, and angles.

double bond that provides an enamine moiety suitable for the functionalization needed to close the oxide bridge. The necessary enamine **5** was synthesized in three steps from piperidol **3** by the method of Evans.[5]

In a departure from previous preparations of unsubstituted 4-phenylpiperidols,[6] piperidol **3** was obtained in 58% yield by reacting lithiated veratrol[7] with 1-methyl-4-piperidone. The dehydration of **3** was accomplished by heating with H_2SO_4 to yield the tetrahydropyridine **4** in 47% yield.[8] Finally, metalation of **4** with *n*-butyllithium at −15 °C, followed by quenching at −78 °C with allyl bromide, resulted in regioselective alkylation to give a 76% yield of enamine **5**. The ring closure of **5** to give the unsaturated phenylmorphan[5] **6** was accomplished by treatment of **5** with H_3PO_4/HCO_2H (20 °C, 4 days),[5] to yield **6** in 23% yield.

With **6** in hand, completion of the synthesis by oxide-bridge closure at C(4) of **6** was achieved in four steps. The transformation required introduction of a bromine at C(4) of **6**, which subsequently underwent intramolecular displacement by phenoxide anion to give oxide-bridged product **2d**. Thus, reaction of enamine **6** with *N*-bromoacetamide in dry THF, followed by treatment with aqueous $NaHCO_3$, gave the vinylic bromide **7** in 62% yield. It was next desired to reduce the $\Delta^{3,4}$ double bond of **7** to give the secondary bromide **8**. Catalytic hydrogenation of **7** gave a complex mixture; however, treatment of a solution of **7** in aqueous methanolic HCl with $NaCNBH_3$ gave **8** as a single, epimerically pure product in 72% yield. The NMR coupling constants for the hydrogen geminal to the bromide (C(4)-H; δ 5.31, J = 7, 13 Hz) were ambiguous when Dreiding models were used to determine which epimer was present.

Prior to oxide ring closure it was necessary to deprotect the aromatic oxygen by demethylation. This was effected in $CHCl_3$ with BBr_3 at 20 °C,[9] yielding **9** quantitatively. Compound **9** was now set for the final oxide ring closure to give **2d**. This was first attempted by heating **9** in pyridine (90 °C, $5^1/_2$ h). The major product of this reaction as determined from single-crystal X-ray analysis[10] was the isomeric compound **10**. This unexpected product could result by initial intramolecular displacement of bromine by nitrogen to give an aziridinium intermediate **10a**, which could subsequently undergo intramolecular ring opening by phenoxide to yield **10**, as depicted in Scheme I. As a second approach, a suspension of **9**·HBr was treated with 3 equiv of potassium *tert*-butoxide in dry THF to give a clean conversion of **9** to **2d** (85%).

X-ray Analysis. In general, bond lengths and angles, as shown in Figure 1, are as might be expected from the molecular structure. The bond lengths at N(2) show the usual slight lengthening appropriate to an ionized N atom. Molecular strain is mainly indicated by the far from tetrahedral angles at C(6a). The unusually large angle C(7)–C(6b)–C(6a) (131.7°) is a consequence of the fact that the other two angles at the planar C atom are constrained to ca. 120° and 109° by ring formation.

There are no unusually short intermolecular distances, and the controlling feature of the packing appears to be electrostatic interaction and hydrogen bonding along the screw axis. The appropriate distances and angles are Cl···N(2), 3.020 Å; Cl···H(2), 2.16 Å; Cl···H–N(2), 173°; Cl···O(10), 3.043 Å; Cl···H(10), 2.25 Å; and Cl···H(10)–O(10), 173°.

Biological Results and Conclusion

The synthesis of **2d** represents the extension of a facile synthesis of the phenylmorphan nucleus[5] to a new class of oxide-bridged phenylmorphans. Analysis of **2d**·HCl by single-crystal X-ray diffractometry confirmed the structure and allowed calculation of the angle between the phenyl ring and the piperidine ring as 49°. (The plane of the piperidine ring was calculated as a least-squares plane through atoms 1, 3, 11a, and 12 of **2d**.) Opioid receptor binding of **2d** was measured with rat brain homogenate by using a modification of the procedure of Itzhak et al.[12] as previously reported.[4] Opioid receptor affinity was very low (IC_{50} = 1000 nM), indicating that perhaps the phenyl ring is held at an unsuitable orientation. In addition, receptor binding of side product **10** was also very low. At 10 μM less than 10% displacement of [^{3}H]dihydromorphine in rat brain homogenate was observed.[4] Further work is in progress on the synthesis of the remaining isomers in this series.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are corrected. NMR spectra were recorded with a Varian 220 MHz spectrometer with $(CH_3)_4Si$ as the internal reference in the specified solvent. IR spectra were recorded on a Beckman IR 4230 spectrometer. Chemical-ionization mass spectra (CIMS) were obtained on a Finnigan 1015D spectrometer with a Model 6000 data collection system, and electron-ionization mass spectra (EIMS) were obtained with a Hitachi Perkin-Elmer RMU-6E spectrometer (70 eV). Column chromatography was performed with use of 230–400-mesh EM silica gel. Mass spectra and elemental analyses were obtained from the Section on Analytical

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(10) Crystals were monoclinic, space group $P2_1/n$, cell dimensions a = 10.654 (2) Å, 9.944 (1) Å, 14.089 (2) Å, β = 111.02 (2)°, Z = 4. Full three-dimensional X-ray data were collected. The best E map from MULTAN[13] indicated the structure proposed here. The structure was refined to a R factor of 10%, but the investigation was not continued further since the results were not relevant to this study.
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Services and Instrumentation, NIADDK.

1-Methyl-4-hydroxy-4-(2,3-dimethoxyphenyl)piperidine (3). A solution of veratrole (69.0 g, 0.5 mol) in anhydrous ether (450 mL) was cooled in an ice bath and stirred under argon while 1.6 M *n*-butyllithium (250 mL, 0.4 mol) was added. The reaction mixture was then stirred under argon at 20 °C for 18 h. The resulting white suspension was cooled on ice water while a solution of 1-methyl-4-piperidone (45.2 g, 0.4 mol) in ether (75 mL) was slowly added over 5 min. The solid dissolved, giving a cloudy yellow solution which was washed with aqueous $NaHCO_3$ (3 × 100 mL), filtered to remove some white crystalline solid, and evaporated, yielding a yellow oil (99.0 g). The oil was distilled (110 °C bath, 56 °C head, 0.2 mm) to remove unreacted amine along with a volatile side product (34.0 g). The reddish undistilled oil (58.0 g, 58%) remaining was product **3**, sufficiently pure for further work.

A sample was crystallized from acetone as the oxalate salt and recrystallized from MeOH–acetone for analysis: mp 196–198 °C (loss of solvent above 180 °C); CIMS (NH_3), *m/e* 252 (M^+H). Anal. ($C_{14}H_{21}NO_3 \cdot C_2H_2O_4 \cdot {}^1/_2C_3H_6O \cdot {}^1/_2H_2O$) C, H, N.

1-Methyl-4-(2,3-dimethoxyphenyl)-1,2,3,6-tetrahydropyridine (4). Addition of **3** (105.0 g, 0.42 mol) and H_2SO_4 (60 mL) to H_2O (100 mL) gave a red solution, which was stirred at 115 °C. After 1 h the mixture was cooled on ice water and made alkaline (pH 10) by addition of concentrated aqueous NaOH. Extraction with $CHCl_3$ (2 × 200 mL), washing with H_2O (2 × 300 mL), and evaporation gave a clear brown oil (93.0 g). Flash chromatography (500 g of silica gel, CH_2Cl_2–MeOH–NH_4OH, 9:1.5:0.1) gave product **4** as a brown oil (73.0 g). The brown color was removed by distillation (111 °C head, 165 °C bath, 3 mm), giving pure **4** [**CAUTION**][8] as a viscous yellow oil (46.0 g, 47%). A sample of **4**·HCl was prepared by treatment of a methanolic solution of **4** with 37% HCl followed by evaporation to dryness and crystallization from MeOH: mp 235–236 °C; CIMS (NH_3), *m/e* 234 (M^+H). Anal. ($C_{14}H_{19}NO_2 \cdot HCl$) C, H, N.

(±)-1-Methyl-4-(2,3-dimethoxyphenyl)-4-(3-propenyl)-1,4,5,6-tetrahydropyridine (5). A solution of **4** (46.0 g, 197 mmol) in dry THF (30 mL) was stirred under argon at −78 °C. A solution of *n*-butyllithium, 1.6 M in hexane (210 mL, 336 mmol), was added to the reaction, producing a deep red color. The mixture was stirred at −15 °C for 75 min and then cooled to −78 °C. Allyl bromide (41.0 g, 336 mmol) was added, producing a yellow solution, which was then brought to 20 °C and mixed with H_2O (20 mL). The reaction mixture was evaporated, and the residue was partitioned between $CHCl_3$ (2 × 200 mL) and H_2O (200 mL), evaporated to an oil, and distilled (172 °C head, 235 °C bath, 5 mm), giving the desired product **5** as a light yellow oil (41.0 g, 76%): CIMS (NH_3), *m/e* 290 ($M^+ + NH_4$).

(±)-2-Methyl-5-(2,3-dimethoxyphenyl)-2-azabicyclo[3.3.1]non-3-ene Oxalate (6·oxalate). A solution of **5** (41.0 g, 0.15 mol) in 85% H_3PO_4 (40 mL) and 88% formic acid (40 mL) was left at 20 °C for 4 days. The resulting dark brown mixture was diluted with H_2O (100 mL) and cooled in ice while aqueous NaOH (100 g in 300 mL H_2O) was added. Extraction with $CHCl_3$ (2 × 250 mL), washing the $CHCl_3$ extract with aqueous $NaHCO_3$ (300 mL), and evaporation gave an oil (46.0 g). A solution of this oil in acetone (200 mL) was treated with oxalic acid (15.3 g) in acetone (100 mL) to yield **6** as a white oxalate salt (12.6 g, 23%): mp 148–151 °C; CIMS (CH_4), *m/e* 274 (M^+H). Anal. ($C_{17}H_{23}NO_2 \cdot C_2H_2O_4$) C, H, N.

(±)-2-Methyl-4-bromo-5-(2,5-dimethoxyphenyl)-2-azabicyclo[3.3.1]non-3-ene (7). Neutralization of the oxalate salt of **6** (2.54 g, 7.0 mmol) (partitioned between saturated aqueous $NaHCO_3$ and $CHCl_3$) gave the free base as a light orange oil (1.82 g, 6.67 mmol). To a solution of the free base in dry THF (20 mL) at −78 °C was added *N*-bromoacetamide (1.01 g, 7.3 mmol) in dry THF (4 mL). The mixture was stirred at 20 °C for 20 min and then evaporated to an orange, crystalline solid. Partitioning between aqueous saturated $NaHCO_3$ (10 mL) and $CHCl_3$ (2 × 20 mL) and evaporation of solvent gave a crystalline orange solid (3.51 g), which was purified by flash chromatography (CH_2Cl_2–MeOH, 99:1) to yield **7** as a white crystalline solid (1.45 g, 62%): mp 152–155 °C; CIMS (CH_4), *m/e* 352, 354 (M^+H). Anal. ($C_{17}H_{22}BrNO_2$) C, H, N.

(±)-2-Methyl-4-bromo-5-(2,3-dimethoxyphenyl)-2-azabicyclo[3.3.1]nonane Hydrobromide (8·HBr). Addition of 37% HCl (1.0 mL) to a suspension of **7** (1.00 g, 2.8 mmol) in MeOH (20 mL) gave a light brown solution to which was added $NaCNBH_3$ (215 mg, 3.4 mmol). The resulting milky mixture was stirred 20 min at 20 °C and then diluted with saturated aqueous $NaHCO_3$ (30 mL), extracted (3 × 60 mL $CHCl_3$), and evaporated to a light brown oil. The oil was dissolved in MeOH, acidified with 37% HBr, evaporated to a foam, and triturated with 2-propanol to yield **8**·HBr as white crystals (870 mg, 72%): mp 190–192 °C; CIMS (CH_4), *m/e* 354, 356 (M^+H); NMR ($CDCl_3$, free base) δ 3.17 (dd, 1 H, C(3)-H, *J* = 7, 13 Hz), 3.38 (t, 1 H, C(3)-H, *J* = 13 Hz), 5.31 (dd, 1 H, C(4)-H, *J* = 7, 13 Hz). Anal. ($C_{17}H_{24}BrNO_2 \cdot HBr$) C, H, N.

(±)-2-Methyl-4-bromo-5-(2,3-dihydroxyphenyl)-2-azabicyclo[3.3.1]nonane Hydrobromide (9·HBr). A solution of crude **8**·HBr (1.64 g, 4.6 mmol) in $CHCl_3$ (50 mL) was stirred at 20 °C while BBr_3 (10 mL, 15.1 mmol) was added, giving an emulsion. After 45 min the reaction was terminated by cautious addition of MeOH (20 mL). Solvent was evaporated, leaving a foam, which was redissolved in MeOH (20 mL) and reevaporated to yield **9**·HBr as a foam (1.87 g, 100%). Treatment with 2-propanol gave crystalline **9**·HBr: mp 219–223 °C; CIMS (NH_3), *m/e* 326, 328 (M^+H). Anal. ($C_{15}H_{20}BrNO_2 \cdot HBr$) C, H, N.

Reaction of 9·HBr in Hot Pyridine. A solution of **9**·HBr (1.87 g, 5.7 mmol) in pyridine (25 mL) was heated at 90 °C under argon for $5^1/_2$ h. At the end of this time TLC ($CHCl_3$–MeOH–NH_4OH, 90:10:1) showed **10** as the major spot, R_f = 0.54 with a secondary spot at R_f 0.43, and a slower spot, R_f 0.07. Pyridine was evaporated and the residue partitioned between aqueous Na_2CO_3 and $CHCl_3$. Evaporation of solvent gave a brown oil, which was purified by silica gel flash chromatography ($CHCl_3$–MeOH, 9:1) to yield pure **10**, which was crystallized from MeOH as the hydrochloride salt, **10**·HCl:[10] mp 268–273 °C; CIMS (CH_4), *m/e* 246 (M^+H); NMR (**10** base, $CDCl_3$) δ 1.18–2.07 (m, 8 H), 2.45 (s, 3 H), 2.48 (dd, 1 H, *J* = 6, 11 Hz), 3.18 (t, 1 H, *J* = 6 Hz), 4.32–4.45 (m, 2H), 6.50 (dd, 1 H, *J* = 3, 7 Hz), 6.66–6.77 (m, 2 H); high-resolution MS ($C_{15}H_{19}NO_2$) calcd 245.1416, found 245.1410.

(±)-(3α,6aα,11aβ)-1,3,4,5,6,11a-Hexahydro-2-methyl-2*H*-3,6a-methanobenzo[2,3-*c*]azocin-10-ol Hydrochloride (2d·HCl). To a suspension of **9**·HBr (600 mg, 1.47 mmol) in dry THF (50 mL) was added potassium *tert*-butoxide (495 mg, 4.42 mmol). The resulting grey suspension was stirred at 20 °C under argon for 30 min and then acidified with 37% HCl (500 μL), evaporated to an off-white solid, and partitioned between dilute aqueous NH_4OH (10 mL) and $CHCl_3$ (2 × 30 mL). Evaporation of solvent gave a syrup, which was acidified with methanolic HCl and crystallized from MeOH to afford **2d**·HCl as a white salt (350 mg, 85%): mp 299–300 °C (dec); CIMS (NH_3), *m/e* 246 (M^+H); NMR (**2d** base, $CDCl_3$) δ 1.20–1.39 (m, 1 H), 1.48 (d, 1 H, *J* = 14 Hz), 1.59–1.93 (m, 6 H), 2.00–2.16 (m, 1 H), 2.45 (s, 3 H), 2.80 (br s, 1 H), 2.92 (dd, 1 H, *J* = 7, 11 Hz), 3.11 (dd, 1 H, *J* = 6, 12 Hz), 4.66 (t, 1 H, *J* = 7 Hz), 6.59 (dd, 1 H, *J* = 2, 6 Hz), 6.70–6.75 (m, 2 H). Anal. ($C_{15}H_{19}NO_2 \cdot HCl \cdot {}^1/_2CH_3OH$) C, H, N.

X-ray. Recrystallization from methanol afforded prismatic crystals, space group $P2_1/c$, dimensions *a* = 7.130 (1) Å, *b* = 15.786 (1) Å, *c* = 12.818 (1) Å, β = 103.58°, *Z* = 4, *V* = 1402.4 Å^3, D_x = 1.334 g cm^{-3}, formula $C_{15}H_{20}NO_2Cl$, M = 281.77. The X-ray intensity data were collected by standard methods by using an Enraf-Nonius CAD4 diffractometer with graphite-monochromated Cu Kα radiation (λ = 1.5418 Å; max $(\sin\theta)/\lambda$ = 0.6228 Å^{-1}). There were 2841 independent reflections (1110 with $I < \sigma(I)$). The phase problem was solved with programs of MULTAN 78,[13] and all heavier atoms were found in an *E* map. Standard refinement techniques allowed the detection of all H atoms and the structure was refined, with the programs of XRAY72[14] to an *R* factor of 0.054 (R_w = 0.052). The function minimized in the refinement was $\sum\omega\Delta^2$ with weights as in Peterson and Levy.[15] A table of the heavier atom parameters and full refinement parameters are deposited as supplemental

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material. A table of observed and calculated structure factors was submitted to the referees and may be obtained from J.V.S.

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Registry No. (±)-**2d**, 100448-10-0; **3**, 82359-62-4; **3**·oxalate, 100430-71-5; **4**, 82359-63-5; **4**·HCl, 100430-72-6; (±)-**5**, 100430-70-4; (±)-**6**, 100448-03-1; (±)-**6**·oxalate, 100448-11-1; (±)-**7**, 100448-04-2; (±)-**7**·HCl, 100448-05-3; **8**, 100448-06-4; **8**·HBr, 100448-07-5; **9**·HBr, 100448-08-6; **10**, 100448-09-7; **10**·HCl, 100448-12-2; veratrole, 91-16-7; 1-methyl-4-piperidone, 1445-73-4; allyl bromide, 106-95-6.

Supplementary Material Available: Tables of atomic parameters for all atoms, bond angles, and torsion angles (4 pages). Ordering information is given on any current masthead page.

^{1}H NMR Configurational Correlation for Retro-Inverso Dipeptides: Application to the Determination of the Absolute Configuration of "Enkephalinase" Inhibitors. Relationships between Stereochemistry and Enzyme Recognition

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A stereospecific synthesis of thiorphan [*N*-[2(*RS*)-(mercaptomethyl)-1-oxo-3-phenylpropyl]glycine] and *retro*-thiorphan [3-[[1(*RS*)-(mercaptomethyl)-2-phenylethyl]amino]-3-oxopropanoic acid], two highly potent inhibitors of enkephalinase, a neutral endopeptidase involved in enkephalin metabolism, is reported. Due to a rapid isomerization process, derivatives of *retro*-thiorphan, which contains a 2-substituted malonyl moiety, cannot be separated by classical methods. However, a separation of the diastereoisomeric mixtures of these *retro*-thiorphan derivatives was achieved by HPLC. The absolute configuration of each isomer was determined by using an NMR configurational correlation. The inhibitory potency of the various inhibitors indicates that, in the thiorphan series, the affinity for enkephalinase is independent of the stereochemistry of the 2-(mercaptomethyl)-1-oxo-3-phenylpropyl moiety. In contrast, in the *retro*-thiorphan series a 100-fold difference in the inhibitory activity of the two enantiomers is observed. This indicates that there are large differences in the conformational behavior of the two series of inhibitors at the active site of the enzyme.

The neutral endopeptidase (EC 3.4.24.11)[1] designated enkephalinase[2] is involved in the metabolism of the endogenous opioid peptides Met-enkephalin (Tyr-Gly-Gly-Phe-Met) and Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu). Inhibition of this enzyme has been shown to be a useful method for investigating the physiological functions of opioid peptides.[3-6] Moreover, inhibition of enkephalin metabolism represents a new approach in the search for analgesics.[3,7] The design of potent and specific inhibitors of a given peptidase requires the knowledge of both its mechanism of action and its substrate specificity. Crystallographic studies of various zinc metallopeptidases such as carboxypeptidase A[8] and thermolysin[9] have shown that they have a common mechanism of catalysis and that their specificity is related to well-defined interactions (ionic, hydrophobic, H bonds, etc.) between the side chains of the substrate and the enzyme subsites surrounding the catalytic site.

On the basis of these findings, highly potent inhibitors of metallopeptidases such as angiotensin converting enzyme, ACE (EC 3.4.15.1),[10] a metallopeptidase involved in blood pressure regulation, and enkephalinase[3,7,11,12] have been rationally designed after a careful characterization of their subsite specificity. The specificity of enkephalinase is essentially insured by the preferential interactions of the S_1' subsite with aromatic or large hydrophobic residues and of the S_2' subsite with small lipophilic moieties[13,14] (the nomenclature used for the individual subsites of the enzyme, S_1', S_2', ..., is that of Schechter and Berger[15]).

Taking these features into account, two highly potent enkephalinase inhibitors have been designed that are able to interact with the catalytic site by a thiol group and to recognize the S_1'–S_2' subsites. However, these two compounds, designated thiorphan [*N*-[2(*RS*)-(mercaptomethyl)-1-oxo-3-phenylpropyl]glycine][3] and *retro*-thiorphan [3-[[1(*RS*)-(mercaptomethyl)-2-phenylethyl]-amino]-3-oxopropanoic acid][7] were synthesized as racemic mixtures. The retro-inversion of the amide bond in thiorphan and derivatives was shown to induce a complete differentiation between enkephalinase and ACE inhibition.[7] Generally, enzymes bind preferentially to compounds that mimic the configuration of the natural substrate, and studies on enkephalinase inhibition, by various

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