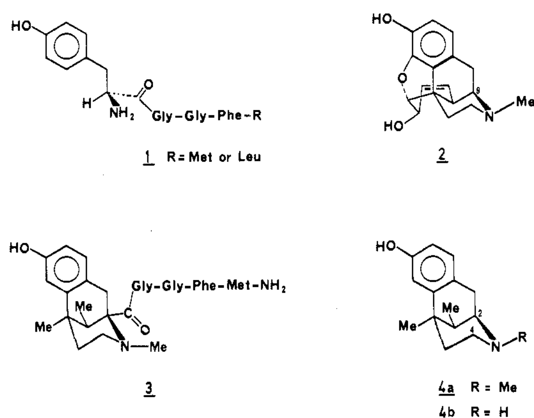
Synthesis and Biological Evaluation of a Metazocine-Containing Enkephalinamide. Evidence for Nonidentical Roles of the Tyramine Moiety in Opiates and Opioid Peptides

K. Ramakrishnan and P. S. Portoghese*

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455.
Received July 16, 1982

In an effort to test the hypothesis that the tyramine moiety present in opiates and in opioid peptides plays an identical functional role at opioid receptors, a hybrid enkephalinamide (3) that contains (–)-metazocine (4a) in place of Tyr[1] was synthesized. It was found that 3 and its congeners are inactive or feebly active in the electrically stimulated guinea pig ileum and mouse vas deferens preparations. The results of these studies suggest that the tyramine moiety in opiates and related structures does not play the same functional role as that in the opioid peptides. It is suggested that the different functional roles of the tyramine moiety in opiates and opioid peptides is a consequence of different modes of interaction with common receptors.

The discovery[1] of endogenous opioid peptides, 1, has

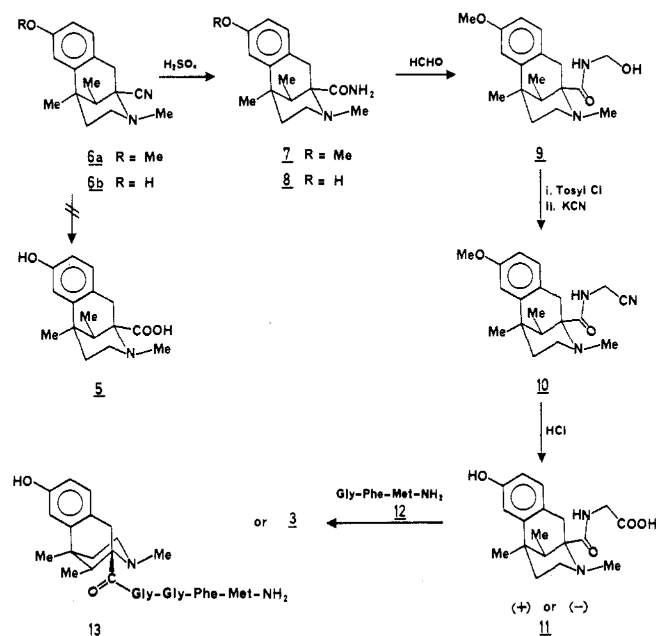


provided medicinal chemists with a fresh view of the relationship between molecular structure and analgesic activity. Soon after the structural elucidation of these peptides, it was postulated that the tyramine moiety, which is common to the enkephalins and the opiates, plays the same role in the recognition process at opioid receptors.[2-4] Further, it was proposed that the aromatic groups in an oripavine derivative simulate the aromatic residues of Tyr[1] and Phe[4] in the enkephalins.[2] Indeed, superposition of morphine (2) and related structures onto the opioid peptides reveal that the C-9 center of morphine is equivalent to the α carbon of the tyrosyl residue in the peptides when the tyramine moiety in both molecules is placed in an identical conformational arrangement.

In this report we describe the synthesis and biological evaluation of a pentapeptide (3), which is a hybrid of (–)-metazocine[5] (4a) and [Met[5]]enkephalinamide[6] (1, R = Met-NH_2), in an effort to test this hypothesis. Congeners of 3 are also described. The results of the present study suggest that the tyramine moiety of morphine-related structures and enkephalin do not play identical roles in the interaction with opioid receptors.

Chemistry. In our initial synthetic approach to the key target pentapeptide 3, we attempted to prepare amino acid 5 which we envisaged would be coupled to the appropriate tetrapeptide using conventional procedures. However,

Scheme I



subjection of the nitrile precursor[7] 6a to different concentration of H_2SO_4 afforded only the carboxamide 7 (Scheme I). Treatment of 7 with aqueous HCl hydrolyzed the methoxy group to the corresponding phenol 8. Also, nitrous acid treatment was ineffective in hydrolyzing the carboxamide group. Selective demethylation of the methoxy group of 6a was effected with BBr_3 to give 6b.

The extreme resistance of the carboxamide group to hydrolysis is probably related to the steric hindrance imposed by flanking methyl groups and to its bridgehead position. This suggested that even if 5 was obtainable, the similar steric hindrance of the carboxy group would be an obstacle to peptide bond formation. Therefore, a route for deriving the pentapeptide from carboxamide 7 was sought.

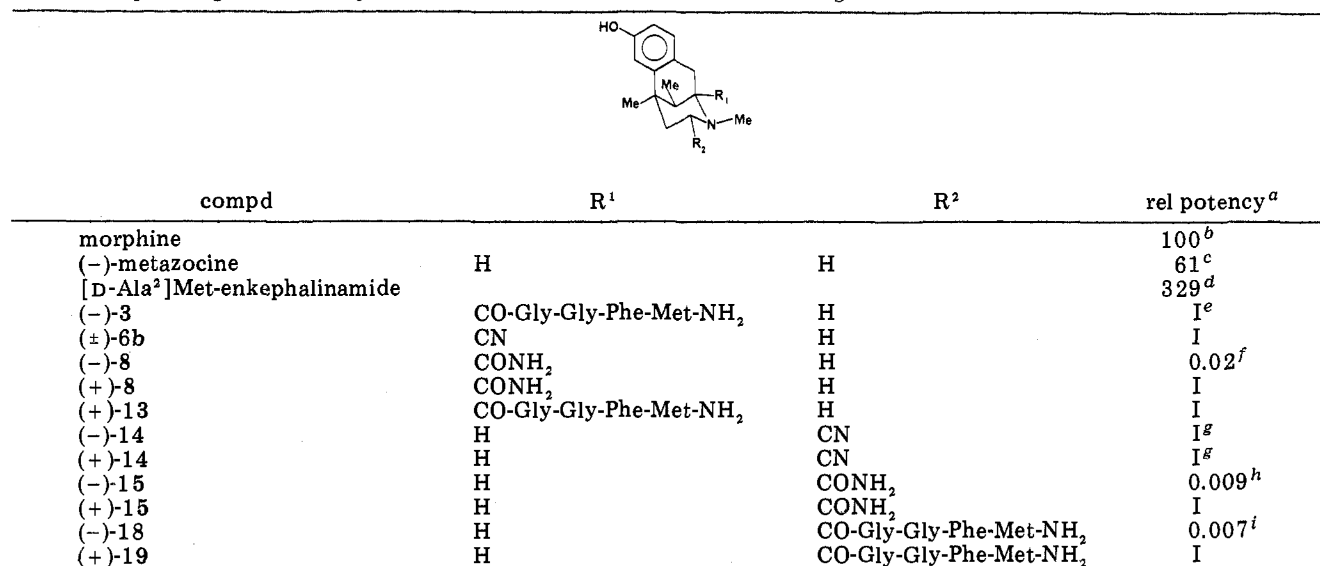
This approach involved the elaboration of the methylol adduct 9 by treatment of 7 with formaldehyde. Tosylation of 9, followed by in situ treatment with KCN, afforded the nitrile 10. The desired dipeptide intermediate 11 was obtained by refluxing 10 in 6 N HCl.

The above sequence was carried out using racemic material initially, but in subsequent experiments enantiomers were prepared. The enantiomers were obtained by optical resolution of (±)-7 via its acid tartrate salt. The derived

(1) Terenius, L. *Annu. Rev. Pharmacol. Toxicol.* **1978**, *18*, 189.
(2) Bradbury, A.; Smith, D.; Snell, C. *Nature (London)* **1976**, *260*, 165.
(3) Horn, A. S.; Rodgers, J. R. *Nature (London)* **1976**, *260*, 795.
(4) Gorin, F. A.; Marshall, G. R. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5179.
(5) Fry, E. M.; May, E. L. *J. Org. Chem.* **1959**, *24*, 116.
(6) Morley, J. S. *Annu. Rev. Pharmacol. Toxicol.* **1980**, *20*, 81 (1980).
(7) Ramakrishnan, K.; Portoghese, P. S. *J. Org. Chem.* **1980**, *45*, 4240.

0022-2623/82/1825-1423$01.25/0 © 1982 American Chemical Society

Table I. Opioid Agonist Activity of Metazocine Derivatives on the Guinea Pig Ileum



compd	R^1	R^2	rel potency[a]
morphine			100[b]
(−)-metazocine	H	H	61[c]
[D-Ala[2]]Met-enkephalinamide			329[d]
(−)-3	CO-Gly-Gly-Phe-Met-NH_2	H	I[e]
(±)-6b	CN	H	I
(−)-8	$CONH_2$	H	0.02[f]
(+)-8	$CONH_2$	H	I
(+)-13	CO-Gly-Gly-Phe-Met-NH_2	H	I
(−)-14	H	CN	I[g]
(+)-14	H	CN	I[g]
(−)-15	H	$CONH_2$	0.009[h]
(+)-15	H	$CONH_2$	I
(−)-18	H	CO-Gly-Gly-Phe-Met-NH_2	0.007[i]
(+)-19	H	CO-Gly-Gly-Phe-Met-NH_2	I

[a] [IC_{50} (morphine)/IC_{50} (compd)] × 100 = relative potency. Inactive compounds were tested (n = 2) at 1×10^{-6} M. [b] $IC_{50} = 5.73 \pm 2.02 \times 10^{-8}$ M (n = 6). [c] $IC_{50} = 9.4 \times 10^{-8}$ M (n = 2). [d] $IC_{50} = 1.74 \pm 1.10 \times 10^{-8}$ M (n = 3). [e] Weak antagonism with agonist morphine pA_2 of <6 (naloxone pA_2 = 8.53 ± 0.07). [f] $IC_{50} = 2.82 \pm 5.47 \times 10^{-6}$ M (n = 4). [g] Concentration-dependent increase in contractile amplitude with a mean IC_{50} of 5.10×10^{-7} M (n = 2). [h] $IC_{50} = 6.09 \pm 3.86 \times 10^{-6}$ M (n = 3). [i] $IC_{50} = 7.97 \pm 1.58 \times 10^{-6}$ M (n = 4).

enantiomers [(−)-7, (+)-7] then were converted to (−)- and (+)-11. Inasmuch as (−)-7 also was synthesized from normetazocine (**4b**) of known absolute configuration,[8] the chirality of these intermediates are therefore known.

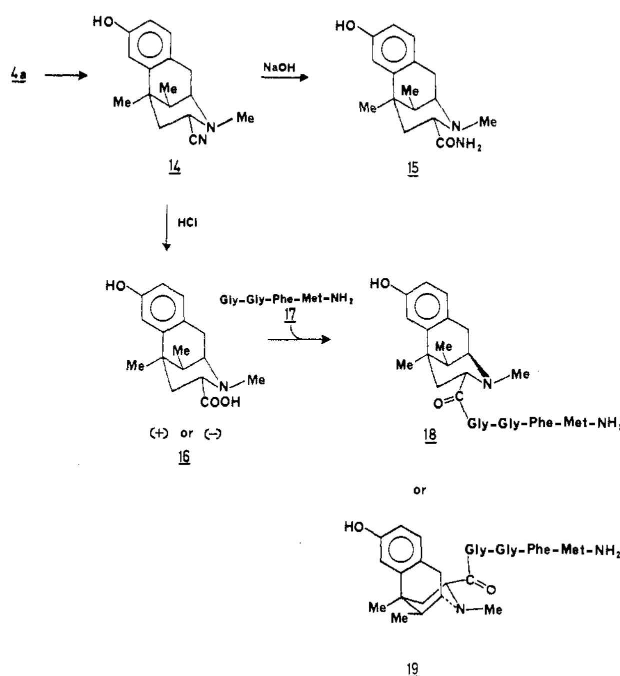
Each of the enantiomers of **11** was employed in the coupling with tripeptide **12**. Thus, (−)- and (+)-**11** afforded the corresponding diastereomeric pentapeptides whose configurations are depicted by structures **3** and **13**, respectively.

The regioisomeric pentapeptides **18** and **19** also were synthesized in order to evaluate the effect of placing the tetrapeptide chain in a different position with respect to the tyramine moiety. The synthesis involved cyanation of optically active metazocine (**4a**) using a reported procedure (Scheme II).[9] Each of the optical antipodes of the resulting nitrile **14** were then hydrolyzed to the optically active amides **15** or amino acids **16**. Coupling (−)-**16** and (+)-**16** with tetrapeptide **17** afforded the corresponding diastereomeric pentapeptides **18** and **19**.

Pharmacology. The compounds were tested in the electrically stimulated myenteric plexus of the guinea pig ileum[10] (GPI) (Table I) and mouse vas deferens[11] (MVD). Compounds that exhibited opioid activity were tested for naloxone reversibility, and all compounds were examined for antagonism against morphine and [D-Ala[2]]Met-enkephalinamide.

Morphine and the parent benzomorphan, (−)-metazocine (**4a**, R = Me), possessed comparable activity on the GPI, and both were less potent than [D-Ala[2]]Met-enkephalinamide. Introduction of substitution at the 2- or 4-position greatly decreased or abolished agonist activity. The key enkephalin analogue **3** was inactive as an agonist or antagonist at 1×10^{-6} M. The only congeners that exhibited agonist activity in the micromolar range were the carboxamides [(−)-**8** and (−)-**15**] and the regioisomeric pentapeptide **18**. The agonist activity observed with these compounds was naloxone reversible. Interestingly, both enantiomeric cyano compounds, (+)- and (−)-**14**, produced an increase in the amplitude of contraction of the GPI in the micromolar range, which was not antagonized by naloxone. In the MVD preparation, none of the compounds showed agonist or antagonist activity at concentrations of 10^{-6} M or above. The IC_{50} of [D-Ala[2]]Met-enkephalinamide on the MVD was 7.6×10^{-9} M (n = 2).

Scheme II



Discussion

The fact that the key enkephalin analogue **3** is inactive at 10^{-6} M in both the GPI or MVD preparations suggests that the tyramine moiety in (−)-metazocine (**4a**) and in the Tyr[1] residue of the opioid peptides **2** does not play a functionally equivalent role in the interaction with opioid

(8) Casy, A. F.; Parulkar, A. P. *J. Med. Chem.* **1969**, *12*, 178.
(9) Groutas, W. C.; Essawi, M.; Portoghese, P. S. *Synth. Commun.* **1980**, *10*, 495.
(10) Rang, H. P. *Br. J. Pharmacol.* **1965**, *22*, 356.
(11) Henderson, G.; Hughs, J.; Kosterlitz, H. W.; *Br. J. Pharmacol.* **1972**, *46*, 764.

receptors. Since the tyramine moiety is conformationally restricted with **3**, but highly flexible in **1**, it is conceivable that the inactivity of **3** is related to the rigidity of this pharmacophore. This suggests that the pharmacophoric conformation of the tyramine moiety in **1** differs from that found in morphine (**2**) and related structures. Also, it is possible that the tyramine moiety in **1** and **2** interacts with different subsites on the same opioid receptor. Either or both of the above possibilities might contribute to the inactivity of **3**. The different structure–activity relationships between the enkephalins and morphine-related structures upon nitrogen substitution are consistent with this notion.

Since a large drop in agonist potency was observed when (−)-metazocine was modified with a carboxamide group [(−)-**8**], it is likely that this functionality is largely responsible for the loss of activity in the corresponding pentapeptide **3**. This abolition of activity is probably of electronic rather than of steric origin, since retention of opioid activity has been reported[12] in 2-methylmetazocine. This, too, is consistent with the idea that the opiates and related structures interact with opioid receptors in a way that is different from the opioid peptides **1**.

It is noteworthy that the decrease or loss of potency was seen in both the GPI and MVD preparations, which are rich in μ and δ opioid receptors, respectively. It therefore appears that both these receptor subtypes[13] show decreased recognition for the hybrid peptide **3** and its 2-substituted congeners when compared to (−)-metazocine[14] and Met-enkephalinamide.

In conclusion, the hypothesis[2–4] that the tyramine moiety in opiates and in opioid peptides plays an equivalent functional role in the receptor recognition process is not supported by the present study. Alternative possibilities for the apparent divergent recognition of opiates and opioid peptides by common receptors include the interaction of different conformations of the tyramine moiety in these molecules with an identical subsite or with different subsites. In view of the considerable stereochemical and structure–activity evidence[15–18] for different modes of interaction at opioid receptors, it is not unexpected that a similar situation might occur with molecules such as the opiates and opioid peptides, whose chemical constitution differs substantially.

Experimental Section

Melting points were determined with a Mel-Temp capillary apparatus and are uncorrected. Infrared spectra were taken with Perkin-Elmer Model 281 instrument. NMR spectra were obtained (Me_4Si or DSS as internal standards) from Varian A-60, T-60, and FT-80 instruments. An AE 1 MS-30 or Finnigan 4000 spectrometer was employed for mass spectra. Electron-ionization mass spectra were conducted at 10–70 eV, and chemical-ionization spectra were carried out with ammonia as reagent gas. Optical rotations were taken using a 1 cm cell on a Perkin-Elmer 141 polarimeter. TLC plates, silica gel GF, 0.25 mm thick, were obtained from Analtech Inc., Newark, DE. HPLC analysis was done on a Beckman Model 110-A system with a reverse-phase Beckman Ultrasphere-ODS column. Elemental analyses were performed by MHW Laboratories, Phoenix, AZ. Amino acid analyses were performed by Sequemat Inc., Watertown, MA. All chemicals and solvents were of reagent grade. Peptides and amino acids were obtained from Sigma Chemical Co., St. Louis, MO. Normetazocine was a generous gift from Sterling Winthrop Research Institute, Rensselaer, NY.

($2\alpha,6\alpha,11\alpha$)-1,2,3,4,5,6-Hexahydro-8-hydroxy-3,6,11-trimethyl-2,6-methano-3-benzazocine-2-nitrile (6b). The O-demethylation procedure of Rice[19] was employed. A solution of the racemic nitrile **6a** (100 mg, 0.37 mmol) in chloroform (5 mL) was cooled to −20 °C (dry ice–CCl_4 bath) and a solution of boron tribromide (500 mg, 2 mmol) in chloroform (3 mL) was added under nitrogen over a 15-min period. The mixture was stirred at −20 °C for 1 h and at 25 °C for 4 h. Saturated sodium bicarbonate solution (3 mL) was added, and the mixture was stirred for 5 min. It was further basified with 10% NaOH to pH 12, and the unreacted **6a** that was insoluble in NaOH was extracted with chloroform (2 × 5 mL). The aqueous phase containing **6a** was readjusted to pH 9.0 and was extracted with chloroform. The chloroform layer was separated, washed with water, and dried (Na_2SO_4). Removal of chloroform gave **6b**: yield 55 mg (60%); mp 162–164 °C; R_f 0.48 (EtOAc/$CHCl_3$/ammonia, 2:1:0.01); EIMS, m/e 256 (M^+). Anal. ($C_{16}H_{20}N_2O{\cdot}HCl{\cdot}0.5H_2O$) C, H, N.

($2\alpha,6\alpha,11\alpha$)-1,2,3,4,5,6-Hexahydro-8-methoxy-3,6,11-trimethyl-2,6-methano-3-benzazocine-2-carboxamide (7). The aminonitrile **6a** (1.00 g, 3.7 mmol) was dissolved in 31% (v/v) sulfuric acid (10 mL), and the solution was refluxed for 30 h. It was cooled, poured into water (100 mL), and extracted with methylene chloride after basification with 10% sodium hydroxide. Drying (Na_2SO_4) the extract and evaporating the solvent gave the amide **7**, which was crystallized from ethyl acetate: yield 1.04 g (97%); mp 207–210 °C; R_f 0.6 (EtOAc/CH_3OH, 4:1); EIMS, m/e 288 (M^+). Anal. ($C_{17}H_{24}N_2O_2$) C, H, N.

Resolution of Normetazocine. (a) (−)-Normetazocine [(−)-4b]. The resolution was accomplished by the modification of a reported[20] procedure. A solution of (+)-tartaric acid (15.01 g, 0.10 mol) in water (600 mL) was added to a solution of (±)-normetazocine (**4b**; 43.40 g, 0.20 mol) in ethanol (300 mL). The mixture was heated on a steam bath to obtain a clear solution. While the solution was cooled, one of the neutral tartrate salts was obtained as solid. This was crystallized twice from alcohol–water (1:2) to yield 17.10 g (56%) of the pure salt: mp 302–304 °C; $[\alpha]^{25}{}_D$ −28.0° (*c* 0.5, ethanol/water, 1:1). Anal. ($C_{32}H_{44}N_2O_8$) C, H, N. The salt (17.10 g) was dissolved in water (200 mL) with heating, and the base was precipitated with aqueous sodium bicarbonate. The precipitate was filtered, washed with water, and dried. Crystallization from 80% alcohol gave the base (−)-**4b**: yield 10.0 g (51%); mp 261–262 °C; $[\alpha]^{25}{}_D$ −69.2° (*c* 0.5, absolute EtOH). [lit.[20] mp 260–262 °C; $[\alpha]^{25}{}_D$ −69.3° (*c* 1, absolute EtOH)]. Anal. ($C_{14}H_{19}NO$) C, H, N.

(b) (+)-Normetazocine [(+)-4b]. The mother liquid from the preceding resolution procedure was neutralized with sodium bicarbonate to obtain partially resolved (+)-normetazocine (21.15 g). To a solution of this base in ethanol (150 mL) was added a solution of (−)-tartaric acid (7.30 g, 0.05 mol) in water (300 mL). The mixture was heated to obtain a clear solution, which upon cooling afforded the neutral tartrate salt. This was crystallized twice from alcohol/water (1:2) to yield the pure salt (7.34 g, 32%): mp 302–304 °C; $[\alpha]^{25}{}_D$ +27.6° (*c* 0.5, EtOH/water, 1:1). Basification of salt with sodium bicarbonate, followed by filtration, gave (+)-**4b**, which was crystallized from 80% EtOH: yield 4.04 g (15%); mp 261–262 °C; $[\alpha]^{25}{}_D$ +69.0° (*c* 0.5, absolute EtOH). [lit.[20] mp 260–262 °C; $[\alpha]^{25}{}_D$ +70.1° (*c* 1, absolute EtOH)].

Resolution of ($2\alpha,6\alpha,11\alpha$)-1,2,3,4,5,6-Hexahydro-8-methoxy-3,6,11-trimethyl-2,6-methano-3-benzazocine-2-carboxamide (7). (a) (−)-7. The racemic carboxamide **7** (1.152 g 4.00 mmol) and (+)-tartaric acid (0.600 g, 400 mmol) were dissolved in refluxing acetone (200 mL). The solution was concentrated to about 75 mL and allowed to stand for 24 h at room temperature. The salt (0.89 g) that separated was filtered and recrystallized twice from acetone to afford the acid tartrate salt (0.750 g): mp

(12) Sallay, S. I. *Chem. Abstr.* **1975**, *83*, 179354*g* and 193576*n*.
(13) Iwamoto, E. T.; Martin, W. R. *Med. Res. Rev.* **1981**, *1*, 411.
(14) Studies with μ-receptor-depleted GPI preparation using the μ-specific receptor alkylator β-FNA (Portoghese, P. S.; Larson, D. L.; Sayre, L. M.; Fries, D. S.; Takemori, A. E. *J. Med. Chem.* **1980**, *23*, 233) indicate that (−)-metazocine is highly selective for μ receptors (unpublished data).
(15) Portoghese, P. S. *J. Med. Chem.* **1965**, *8*, 609.
(16) Portoghese, P. S. *J. Pharm. Sci.* **1966**, *55*, 865.
(17) Portoghese, P. S. *Acc. Chem. Res.* **1978**, *11*, 21.
(18) Portoghese, P. S.; Alreja, B. D.; Larson, D. L. *J. Med. Chem.* **1981**, *24*, 782.
(19) Rice, K. C. *J. Med. Chem.* **1977**, *20*, 164.
(20) Tullar, B. F.; Harris, L. S.; Perry, R. L.; Pierson, A. K.; Soria, A. E. *J. Med. Chem.* **1967**, *10*, 383.

206–207 °C; $[\alpha]^{25}_{D}$ −48.0° (*c* 1, absolute EtOH). Anal. ($C_{17}H_{24}N_2O_2 \cdot C_4H_6O_6$) C, H, N.

The aqueous solution of the salt was basified with 10% sodium hydroxide and the precipitated free base was extracted with methylene chloride. Drying (Na_2SO_4) the extract followed by removal of the solvent gave (−)-**7** which was crystallized from ethyl acetate (0.345 g, 70%): mp 231–232 °C; $[\alpha]^{25}_{D}$ −73.6° (*c* 0.5, absolute EtOH). The spectral properties (IR, NMR, and MS) of (−)-**7** obtained by this resolution are identical with those of the racemate and (−)-**7** obtained by synthesis from (−)-**4a**.

(**b**) (+)-**7**. The mother liquor from the preceding resolution procedure was seeded with a few crystals of the acid tartrate salt of (−)-**7** to remove an additional quantity of this enantiomer from solution. The supernatant was filtered, and the solvent was evaporated from the filtrate to afford the acid tartrate salt of (+)-**7** (0.610 g). This was dissolved in water, the solution was basified with 10% sodium hydroxide, and the precipitate was extracted with methylene chloride. Drying (Na_2SO_4) the extract, followed by removal of the solvent, gave (+)-**7**, which was crystallized from ethyl acetate (0.371 g, 78%): mp 231–232 °C; $[\alpha]^{25}_{D}$ +70.8° (*c* 0.5, absolute EtOH). The spectral properties of (+)-**7** are identical with that of its enantiomer and racemate.

(2α,6α,11α)-1,2,3,4,5,6-Hexahydro-8-hydroxy-3,6,11-trimethyl-2,6-methano-3-benzazocine-2-carboxamide (8). The amide **7** (0.345 g, 1.04 mmol) was dissolved in 6 N HCl (10 mL) and refluxed for 24 h. The solution was adjusted to pH 12 with 40% sodium hydroxide and was extracted with methylene chloride to remove the unreacted **7**. The aqueous phase was readjusted to pH 9.0 and extracted with methylene chloride. Washing the extract with water and then drying (Na_2SO_4), followed by evaporation of the solvent, gave (±)-**8**: yield 0.279 g (48%); mp 127–129 °C. (−)-**8**, $[\alpha]^{25}_{D}$ −66.8° (*c* 0.25, absolute EtOH), and (+)-**8**, $[\alpha]^{25}_{D}$ +68.0° (*c* 0.25, absolute EtOH), mp 127–129 °C (both antipodes), R_f 0.47 (EtOAc/CH_3OH, 10:1), were obtained from (−)- and (+)-**7**, respectively: EIMS, *m/e* 274 (M^+). Anal. ($C_{16}H_{22}N_2O_2$) C, H, N.

(2α,6α,11α)-1,2,3,4,5,6-Hexahydro-*N*-(hydroxymethyl)-8-methoxy-3,6,11-trimethyl-2,6-methano-3-benzazocine-2-carboxamide (9). A solution of (±)-**7** (0.604 g, 2.1 mmol) in THF (30 mL) was stirred with 40% formalin (30 mL) and potassium carbonate (300 mg) for 48 h. The THF was removed in vacuo, and water (50 mL) was added to the reaction mixture. The precipitated product was filtered and washed several times with aqueous sodium bicarbonate to remove formalin and paraformaldehyde. The residue was redissolved in methylene chloride, and the solution was extracted with sodium bicarbonate twice to ensure complete removal of paraformaldehyde. Drying (Na_2SO_4) the extract, followed by evaporation of the methylene chloride, gave (±)-**9**, which was crystallized from ethyl acetate in a yield of 0.66 g (99%). (−)-**9**, $[\alpha]^{25}_{D}$ −50.4° (*c* 0.5, absolute EtOH), and (+)-**9**, $[\alpha]^{25}_{D}$ +48.6° (*c* 0.5, EtOH), were obtained from (−)- and (+)-**7**, respectively: mp 166–167 °C (racemate and antipodes); R_f 0.6 (EtOAc/CH_3OH, 4:1); EIMS, *m/e* 318 (M^+). Anal. ($C_{18}H_{26}O_3N_2$) C, H, N.

(2α,6α,11α)-1,2,3,4,5,6-Hexahydro-*N*-(cyanomethyl)-8-methoxy-3,6,11-trimethyl-2,6-methano-3-benzazocine-2-carboxamide (10). The methylol adduct (±)-**9** (0.52 g, 1.62 mmol) was heated under N_2 at 95 °C for 15 h with tosyl chloride (420 mg, 2.2 mmol) in dry Me_2SO (24 mL). Potassium cyanide (1.0 g) was then added, and the mixture was heated for another 24 h. The solvent was removed in vacuo, and the residue was extracted with methylene chloride after basification with sodium bicarbonate. Washing the extract with water and then drying (Na_2SO_4), followed by removal of the solvent, gave an oil (0.52 g), which was chromatographed on silica gel (20 g) with ethyl acetate/chloroform (1:2) to afford pure (±)-**10** as an oil: yield 0.4 g (75%). (−)-**10**, $[\alpha]^{25}_{D}$ −32°, and (+)-**10**, $[\alpha]^{25}_{D}$ +31.8° (*c* 0.4, absolute EtOH), were derived from (−)- and (+)-**7**, respectively: R_f 0.5 ($CHCl_3$/EtOAc, 1:2); EIMS, *m/e* 327 (M^+). Anal. ($C_{19}H_{25}O_2N_3$) C, H, N.

(2α,6α,11α)-[(1,2,3,4,5,6-Hexahydro-8-hydroxy-3,6,11-trimethyl-2,6-methano-3-benzazocin-2-yl)carbonyl]glycine (11). The cyanomethylcarboxamide (±)-**10** (0.400 g, 1.2 mmol) was refluxed in 6 N HCl (25 mL) for 48 h. The hydrochloric acid was removed in vacuo and the residue was dissolved in water (1 mL) and applied to a column of cation-exchange resin AG, 50 W-X8 (4.0 g). After the ammonium chloride was removed by washing with water, the dipeptide (±)-**11** was eluted with 2 N ammonia. The water and ammonia were evaporated from the eluate, and the residue was dissolved in methanol. Filtration and removal of solvent gave pure (±)-**11**: yield 0.304 g (75%); mp 197–200 °C. (−)-**11**, $[\alpha]^{25}_{D}$ −48.4° (*c* 0.5, absolute EtOH), and (+)-**11**, $[\alpha]^{25}_{D}$ +49.8° (*c* 0.5, absolute EtOH), were obtained from (−)- and (+)-**10**, respectively: mp 187–190 °C (both the antipodes); R_f 0.3 ($CHCl_3$/CH_3OH/ammonia, 20:7:0.3); EIMS, *m/e* 332 (M^+). Anal. ($C_{18}H_{24}N_2O_4 \cdot CH_3OH$) C, H, N.

Glycylphenylalanylmethioninamide Hydrochloride (12). Glycylphenylalanine (222 mg, 1.0 mmol) and di-*tert*-butyl dicarbonate (240 mg, 1.1 mmol) were dissolved in THF (12 mL), and the mixture was stirred for 2.25 h with 1 N NaOH (1.0 mL) at 25 °C. The reaction mixture was diluted water (50 mL), basified with sodium bicarbonate, and extracted with methylene chloride to remove the unreacted reagent. The aqueous phase was acidified with cold 10% citric acid and was extracted with ethyl acetate. Washing the ethyl acetate extract with saturated sodium chloride solution and then drying ($NaSO_4$), followed by removal of the solvent, gave the *N*-(*tert*-butyloxycarbonyl)glycylphenylalanine: 300 mg (94%); mp 138–140 °C; $[\alpha]^{25}_{D}$ +41.4° (*c* 0.5, absolute EtOH); R_f 0.23 (EtOAc/CH_3OH, 4:1). The *N*-(*tert*-butyloxycarbonyl)glycylphenylalanine was dissolved in THF (10 mL) containing *N*-methylmorpholine (94 mg, 0.93 mmol). Isobutyl chloroformate (128 mg, 0.93 mmol) in THF (10 mL) was added to the dipeptide solution kept at −20 °C, and the mixture was stirred for 20 min. A solution of methioninamide hydrochloride (172 mg, 0.93 mmol) and triethylamine (94 mg, 0.93 mmol) in a 2:1 mixture of THF and water (10 mL) was added in one portion, and the reaction mixture was allowed to warm to room temperature. After stirring for 2.5 h, the THF was removed in vacuo and the residue was dissolved in ethyl acetate. This extract was washed with cold 10% citric acid (2 × 10 mL) to remove *N*-methylmorpholine and unreacted methioninamide and then with sodium bicarbonate to remove unreacted *N*-(*tert*-butyloxycarbonyl)glycylphenylalanine. Washing the extract with water and then drying (Na_2SO_4), followed by removal of the ethyl acetate, gave a solid, which was dissolved in absolute ethanol (20 mL) and stirred at 25 °C for 0.5 h with 15% ethanolic HCl (2 mL). Removal of the HCl and solvent in vacuo gave **12** as a solid, which was crystallized from methanol/acetone (1:10): 239 mg (90%); mp 232–233 °C; $[\alpha]^{25}_{D}$ −18.5° (*c* 1, DMF) [lit.[21] mp 238–240 °C; $[\alpha]^{25}_{D}$ −20.0° (*c* 1, DMF)]; R_f 0.4 (EtOAc/CH_3OH/ammonia, 20:7:0.3). Anal. ($C_{16}H_{24}N_4O_3S \cdot HCl \cdot 0.5H_2O$) C, H, N.

(−)-(2*R*,6*R*,11*R*)-[(1,2,3,4,5,6-Hexahydro-8-hydroxy-3,6,11-trimethyl-2,6-methano-3-benzazocin-2-yl)carbonyl]glycylglycylphenylalanylmethioninamide (3). The dipeptide (−)-**11** (50 mg, 0.15 mmol) and glycylphenylalanylmethioninamide hydrochloride (**12**; 60 mg, 0.15 mmol) were dissolved in dry DMF (2 mL). 1-Hydroxybenzotriazole (20 mg, 0.17 mmol), DCC (35 mg, 0.17 mmol), and triethylamine (16 mg, 0.15 mmol) in DMF (2 mL) were added. The solution was stirred at room temperature for 48 h. The DMF was removed in vacuo, and the residue was triturated with dilute HCl (3 × 1 mL). The extract was filtered, and the pentapeptide was precipitated with saturated sodium bicarbonate. The precipitate was separated, washed with water, and dissolved in absolute ethanol. Filtration and removal of ethanol gave pure **3**: yield 50 mg (49%); mp 200 °C dec; $[\alpha]^{25}_{D}$ −25° (*c* 0.5, absolute EtOH); R_f 0.51 (EtOAc/CH_3OH/ammonia, 20:7:0.3); NMR (CD_3OD) δ 7.25 (s, 5 H, aromatic), 6.75 (m, 3 H, aromatic), 2.1 (s, 3 H, aromatic), 2.1 (s, 3 H, S-CH_3), 1.35 (s, 3 H, C_6 CH_3), 0.75 (d, 3 H, C_{11} CH_3); CIMS, *m/e* 387 (negative ion, benzazocin-2-ylcarbonylglycylglycyl moiety). Anal. ($C_{34}H_{46}N_6O_6S \cdot H_2O$) C, H, N, S. Amino acid analysis: Gly, 1.05; Phe, 1.00; Met, 1.00.

(±)-(2*S*,6*S*,11*S*)-[(1,2,3,4,5,6-Hexahydro-8-hydroxy-3,6,11-trimethyl-2,6-methano-3-benzazocin-2-yl)carbonyl]glycylglycylphenylalanylmethioninamide (13). The dipeptide (+)-**11** (50 mg, 0.15 mmol) was subjected to the preceding procedure for peptide coupling. The pentapeptide **13** was isolated and purified by the same procedure in 50% yield (51 mg): mp

(21) Lipkowski, A. W.; Majewski, T.; Drabarek, S. *Pol. J. Chem.* **1980**, *54*, 373.

>200 °C dec; $[\alpha]^{25}_D$ +13.4° (c 0.5, absolute EtOH); R_f 0.51 (EtOAc/CH_3OH/ammonia, 20:7:0.3); NMR (CD_3OD) δ 7.25 (s, 5 H, aromatic), 6.75 (m, 3 H, aromatic), 2.1 (s, 3 H, S-CH_3), 1.35 (s, 3 H, C_6 CH_3), 0.75 (d, 3 H, C_{11} CH_3); CIMS, m/e 387 (negative ion, benzazocin-2-ylcarbonylglycylglycyl moiety). Amino acid analysis: Gly, 1.02; Phe, 0.96; Met, 0.92.

(2α,6α,11α)-1,2,3,4,5,6-Hexahydro-8-hydroxy-3,6,11-trimethyl-2,6-methano-3-benzazocine-4-nitrile (14). This was prepared by a modification of Groutas et al.[9] A solution of metazocine (**4a**; 2.31 g, 0.1 mmol) in methanol (25 mL) was stirred with 30% hydrogen peroxide (5 mL) at 55 °C for 4 h. After cooling to 25 °C, the mixture was treated with 5% palladium on carbon (0.1 g) for 1 h to decompose the excess hydrogen peroxide. The mixture was filtered and the solvent was removed. The N-oxide residue was dissolved in ethyl acetate and dried (Na_2SO_4) to afford 2.47 g of oily residue on removal of solvent. A mixture of the oil in methylene chloride (24 mL) was cooled to 0 °C, and trifluoroacetic anhydride (8 mL) was added dropwise with stirring under nitrogen. After the solution was stirred for 1 h at 25 °C and for 30 min at 50 °C, the solvents were removed in vacuo under anhydrous conditions. The residue was dried in vacuo and cooled to 0 °C, and a solution of potassium cyanide (9.80 g) in water (20 mL) was added dropwise. The mixture was stirred for 45 min at 25 °C, acidified with dilute HCl (in hood), and basified with sodium bicarbonate. Extraction with ethyl acetate and removal of solvent gave an oil, which was chromatographed on silica gel ($CHCl_3$/EtOAc, 2:1) to afford the nitrile **14**, which was homogeneous by TLC and HPLC (ultrasphere-ODS column, 40% methanol): yield 2.2 g (86%); $[\alpha]^{25}_D$ −107.5° (c 0.2, absolute EtOH) for (−)-**14**·HCl obtained from (−)-**4a**; $[\alpha]^{25}_D$ +109.0° (c 0.2, absolute EtOH) for (+)-**14**·HCl obtained from (+)-**4a**; mp 134–136 °C for hydrochloride salts of both the antipodes; R_f 0.5 ($CHCl_3$/EtOAc, 2:1); IR (neat) 2220 (CN) cm^{-1}; NMR ($CDCl_3$) δ 6.65 (m, 3 H, Ar H), 2.55 (s, 3 H, N-CH_3), 1.3 (s, 3 H, C_6 CH_3), 0.85 (d, 3 H, C_{11} CH_3); EIMS, m/e 256.1587 (M^+; calcd for $C_{16}H_{20}N_2O$, 256.1577).

(2α,6α,11α)-1,2,3,4,5,6-Hexahydro-8-hydroxy-3,6,11-trimethyl-2,6-methano-3-benzazocine-4-carboxamide (15). Compound **14** (500 mg, 1.95 mmol) was dissolved in 1 N NaOH (10 mL) and heated on a steam bath for 1.25 h. The solution was acidified with 10% HCl, basified with sodium bicarbonate, and extracted with ethyl acetate. Washing the extract with water and then drying (Na_2SO_4), followed by filtration and removal of the solvent, gave **15**, which was crystallized from ethyl acetate: yield 410 mg (77%); mp 287–290 °C; $[\alpha]^{25}_D$ −141.5° (c 0.2, absolute EtOH) for (−)-**15** obtained from (−)-**14**; $[\alpha]^{25}_D$ +140.1° (c 0.14, absolute EtOH) for (+)-**15** obtained from (+)-**14**; mp 287–290 °C for both the antipodes; R_f 0.50 (CH_3OH/EtOAc/ammonia, 1:5:0.1); EIMS, m/e 274 (M^+). Anal. ($C_{16}H_{22}N_2O_2$) C, H, N.

(2α,6α,11α)-1,2,3,4,5,6-Hexahydro-8-hydroxy-3,6,11-trimethyl-2,6-methano-3-benzazocine-4-carboxylic Acid (16). Compound **14** (320 mg, 1.25 mmol) was refluxed in 6 N HCl (6 Ml) for 15 h. The acid was removed in vacuo, and the residue was chromatographed on a column of cation-exchange resin AG, 50W-X8 (1.0 g). The resin was washed with water until neutral, and the crude amino acid was loaded on the column as an aqueous solution (1 mL). The column then was washed with water until neutral in order to remove ammonium chloride and HCl. The amino acid was eluted with 2 N NH_4OH. Evaporation of ammonia gave **16**, which was crystallized from ethanol: yield 250 mg (65%); mp 222–223 °C; $[\alpha]^{25}_D$ −98.8° (c 1, CH_3OH) for (−)-**16** obtained from (−)-**14**; $[\alpha]^{25}_D$ +98.6° (c 0.5, CH_3OH) for (+)-**16** obtained from (+)-**14**; mp 229–232 °C (both antipodes); R_f 0.50 (EtOAc/CH_3OH, 3:2); CIMS, m/e 275 (M^+). Anal. ($C_{16}H_{21}NO_3$·EtOH) C, H, N.

Glycylglycylphenylalanylmethioninamide Hydrochloride (17). A solution of glycylglycylphenylalanine (1.33 g, 4.75 mmol) in THF (75 mL) was stirred at room temperature for 3 h with di-*tert*-butyl dicarbonate (1.07 g, 4.8 mmol), 1 N NaOH (4.75 mL), and water (24 mL). The THF was removed in vacuo, and the mixture was extracted with ethyl acetate to remove excess reagent after basifying with sodium bicarbonate. The aqueous phase was acidified with 10% citric acid and was extracted with ethyl acetate. Washing the ethyl acetate extract with water and then drying (Na_2SO_4), followed by removal of the solvent, gave the N-t-Boc tripeptide: mp 149–150 °C; $[\alpha]^{25}_D$ +25.6° (c 0.5, absolute EtOH); R_f 0.23 (EtOAc/CH_3OH, 5:1); CIMS, m/e 379 (M^+). It was dissolved in DMF (18 mL) containing N-methylmorphine (0.418 g, 4.14 mmol) and cooled to −20 °C, and isobutyl chloroformate (0.570 g, 4.2 mmol) in DMF (5 mL) was added dropwise. After this solution was stirred for 15 min, a solution of methioninamide hydrochloride (0.766 g, 4.14 mmol) and trimethylamine (0.420 g, 4.14 mmol) in DMF (10 mL) was added in one portion, and the mixture was allowed to warm up to room temperature. After the mixture was stirred, for 3 h, the DMF was removed in vacuo. The residue was dissolved in ethyl acetate, and the solution was washed with cold 10% citric acid. Washing with water and then drying (Na_2SO_4), followed by removal of the solvent, gave the N-t-Boc tetrapeptide: yield 1.20 g (57%); mp 185–187 °C; $[\alpha]^{25}_D$ −5.6° (c 0.5, absolute EtOH); R_f 0.40 (EtOAc/CH_3OH, 4:1); EIMS, m/e 509 (M^+). This was dissolved in methanol (64 mL) and stirred with 15% ethanolic HCl (2 mL) at 0 °C for 30 min. Removing the solvent and HCl in vacuo and washing the residue with ethyl acetate gave the hygroscopic tetrapeptide HCl **17**, which was homogeneous on TLC: yield 0.47 g (93%); mp 198–200 °C dec; $[\alpha]^{25}_D$ −18.4° (c 0.5, absolute EtOH); R_f 0.17 (EtOAc/CH_3OH/ammonia, 25:10:02); CIMS, m/e 409 (M^+). Amino acid analysis: Gly, 2.00; Phe, 1.05; Met, 0.95.

(−)-(2*R*,6*R*,11*R*)-[(1,2,3,4,5,6-Hexahydro-8-hydroxy-3,6,11-trimethyl-2,6-methano-3-benzazocin-4-yl)carbonyl]glycylglycylphenylalanylmethioninamide (18). A solution of (−)-**16** (60 mg, 0.22 mmol) in dry Me_2SO (2 mL) was treated with DCC (49 mg, 0.24 mmol), 1-hydroxybenzotriazole (31 mg, 0.23 mmol), the tetrapeptide HCl **17** (93 mg, 0.22 mmol), and triethylamine (21.7 mg, 0.22 mmol) in 1 mL of Me_2SO. After the solution was stirred at room temperature for 24 h, the solvent was removed in vacuo, and the residue was triturated with dilute HCl (3 × 0.5 mL). The acid extract was filtered, and the pentapeptide was precipitated with saturated sodium bicarbonate. For purification, the pentapeptide was redissolved in dilute HCl and precipitated with sodium bicarbonate. The precipitate was washed with water and dissolved in absolute ethanol. Filtration and removal of ethanol in vacuo afforded **18**: yield 58 mg (40%); mp >200 °C dec; $[\alpha]^{25}_D$ −55.2° (c 0.25, absolute EtOH); R_f 0.41 (EtOAc/CH_3OH/ammonia, 25:10:0.2); NMR (CD_3OD) δ 7.15 (s, 5 H, phenylalanyl Ar H), 2.85 (s, 3 H, N-CH_3), 2.0 (s, 3 H, S-CH_3), 1.35 (s, 3 H, C_6 CH_3), 0.9 (d, 3 H, C_{11} CH_3); CIMS, m/e 273 (benzomorphan CONH). Anal. ($C_{34}H_{46}N_6O_6S$·H_2O) C, H, N, S. Amino acid analysis: Gly, 2.07; Phe, 1.11; Met, 0.81.

(+)-(2*S*,6*S*,11*S*)-[(1,2,3,4,5,6-Hexahydro-8-hydroxy-3,6,11-trimethyl-2,6-methano-3-benzocin-4-yl)carbonyl]glycylglycylphenylalanylmethioninamide (19). The amino acid (+)-**16** was subjected to the preceding procedure for peptide coupling. The pentapeptide was purified and isolated by the same procedure in 40% yield: mp >190 °C dec; $[\alpha]^{25}_D$ +37.6° (c 0.3, absolute EtOH); R_f 0.41 (EtOAc/CH_3OH/ammonia, 25:10:0.2); NMR (CD_3OD) δ 7.15 (s, 5 H, phenylalanyl Ar H), 6.70 (m, 3 H, benzomorphan Ar H), 2.45 (s, 3 H, N-CH_3), 2.05 (s, 3 H, S-CH_3), 1.35 (s, 3 H, C_6 CH_3), 0.85 (d, 3 H, C_{11} CH_3); CIMS, m/e 273 (benzomorphan CONH). Amino acid analysis: Gly, 2.00; Phe, 1.07; Met, 0.91.

Acknowledgment. The authors thank Professor A. E. Takemori and Mrs. M. Ikeda for advice concerning biological testing. We also thank Dr. A. E. Soria of the Sterling-Winthrop Research Institute, Rensselaer, NY, for the generous supply of normetazocine. This research was supported in part by NIDA Research Grant DA 02220.