Enkephalinase Inhibitors. 1. 2,4-Dibenzylglutaric Acid Derivatives

Gary M. Ksander,* Clive G. Diefenbacher, Andrew M. Yuan, F. Clark, Yumi Sakane, and R. D. Ghai

Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation, 556 Morris Avenue, Summit, New Jersey 07901.
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The synthesis of two new series of dicarboxylic acid dipeptides and two sulfhydryl-containing inhibitors are described. The in vitro enkephalinase inhibition data and some in vivo analgesic data are presented for these compounds. For the dibenzylglutaric acid series structure–activity relationships and in vivo analgesic activity are discussed. The reverse amides, i.e., 4-amino-2,4-dibenzylbutyric acid derivatives, are also discussed. Two sulfhydryl-containing inhibitors showed good in vivo potency in the mouse jump-latency hot-plate test after peripheral administration at moderate low doses.

Since the two pentapeptides Met- and Leu-enkephalin were discovered, their role in the central nervous system has been studied extensively.[1] Neutral endopeptidase 24.11 (NEP),[2] which is present in cerebral synaptic membranes as well as kidney brush borders, rapidly cleaves the Gly[3]-Phe[4] amide bond of the enkephalins. Other peptides,[3] for example bradykinin, substance P, and neurotensin are also susceptible to in vitro deactivation by this endopeptidase. It has been postulated[4] that the short analgesic action of these pentapeptides when administered exogenously is due to rapid degradation by endogenous neuropeptidases, i.e., aminopeptidase and enkephalinase. If enkephalinase A is the primary degrading enzyme, inhibition of this enzyme should produce a similar but prolonged pharmacological[5] effect to that of the endogenous pentapeptides.

This paper[6] presents the synthesis, the in vitro enkephalinase A inhibitory data, and some in vivo pharmacology of a series of dibenzylglutaric acid amides, 4-aminodibenzylbutyric acid amides, and some phenolic benzylamine sulfhydryl derivatives.

Chemistry. 2,4-Dibenzylglutaric acid (**2**) was prepared by a previously described procedure.[7] Diacid **2** was converted to the cyclic anhydride **3** by heating with either acetic anhydride or acetyl chloride. The trans diastereomer was separated by crystallization from toluene. The diastereomeric mixture **3** could be largely converted to the trans anhydride **3a** due to solubility differences by warming in a toluene–triethylamine mixture. The relative stereochemistry of the trans and cis isomers **3a** and **3b** was assigned on the basis of their proton NMR spectra. Due to the pseudo-axial–equatorial positioning of the dibenzyl groups, the trans anhydride **3a** could freely change conformations; therefore the methylene protons should be equivalent and appear as a triplet. In contrast, the cis isomer **3b** should be more rigid because the pseudo-equatorial–equatorial conformation of the benzyl groups will be energetically preferred over the axial–axial conformation. Therefore, the methylene protons should be nonequivalent, as observed. The racemic mixture of diacids **2** were readily resolved to the *S,S* and *R,R* enantiomers by crystallization of their (+) and (−) α-methylbenzylamine salts, respectively. Regeneration of the chiral acids followed by acetic anhydride dehydration gave the chiral anhydrides **3c,d**. Nucleophilic opening of anhydrides **3a–d** with the appropriate amino acid (Scheme I) readily provided a series of compounds **1a–q** (Table I). The absolute configuration of the dibenzylglutaric acid side chain was confirmed by X-ray analysis of **1k** (Figure 1). Refluxing **3a** in an ethanol–toluene mixture afforded the mixed acid–ester **14**. Activated ester coupling of β-alanine benzyl ester to the mixed acid–ester **14** followed by hydrogenation gave the ethyl ester **8**. Prepared in an analogous fashion was **11** (Table I, Scheme I). Hydrogenation of *N*-CBZ-β-alanine esters in the presence of anhydride **3c** gave the mixed β-alanine ester acids **9** and **10** (Table I, Scheme I). The saturated glutaric acid derivative **4** (Table I) was prepared by Rh/C catalytic hydrogenation of **1b**. Interestingly, when absolute EtOH was used as solvent, a mono(ethyl ester) was obtained. Hydrogenation of ethyl ester **8** with Rh/C in EtOH gave a diester. Basic

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(2) (a) Malfroy, B.; Swerts, J. P.; Guyon, A.; Roques, B. P.; Swartz, J. C. *Nature* **1978**, *276*, 523. (b) DeLaBaume, S.; Patey, G.; Schwartz, J. C. *Neuroscience* **1981**, *6*, 315. (c) Llorens-Cortes, C.; Gros, C.; Schwartz, J. C. *Eur. J. Pharmacol.* **1985**, *119*, 183.
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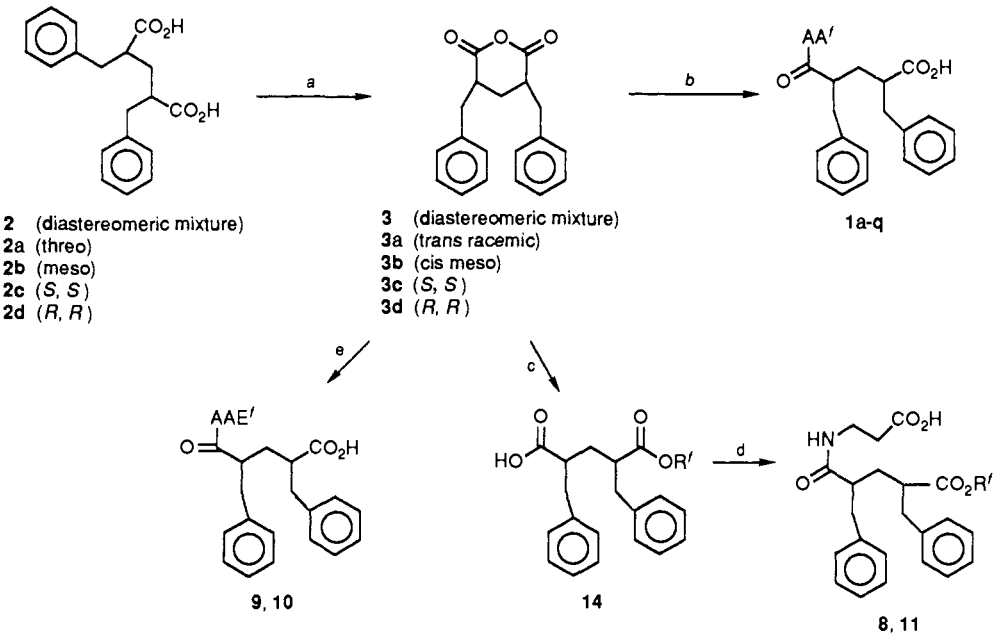
Scheme I



[a] Ac_2O or CH_3COCl, toluene. [b] Amino acid (AA), CH_2Cl_2, pyridine. [c] ROH. [d] DCC, CH_2Cl_2, β-alanine benzyl ester, H_2/Pd–C. [e] *N*-CBZ-amino acid ester (AAE), H_2/Pd–C. [f] See Table I for definition of amino acid (AA), amino acid ester (AAE), and R.

hydrolysis of both the monoester and the diester gave **4**. Although the structure of the mono(ethyl ester) was not proven by independent synthesis, these results might imply that the β-alanine carboxyl group was esterified. This problem was circumvented by using a 1:1 EtOH–H_2O mixture as solvent in the reduction step. The diphenethyl derivative **5** was prepared analogously to **1a**.

Hofmann rearrangement of 2,4-dibenzylglutaric acid amide (**1u**) gave the corresponding 4-amino-2,4-dibenzylbutyric acid (**6**). Seemingly the procedure was compatible with the chiral substrate giving the *S,S* derivative **6a**. The degree of racemization, if any, was not determined. Appropriate acylation (Scheme II) gave the inverse amides **7a–c** (Table II).

The mixed anhydride (triethylamine–ethyl chloroformate) of 2-benzyl-3-thioacetylpropionic acid was acylated with 3,4-dihydroxybenzylamine and 3-methoxy-4-hydroxybenzylamine. Hydrolysis of the *S*-acetyl group gave the sulfhydryl derivatives **12** and **13** (Table IV).

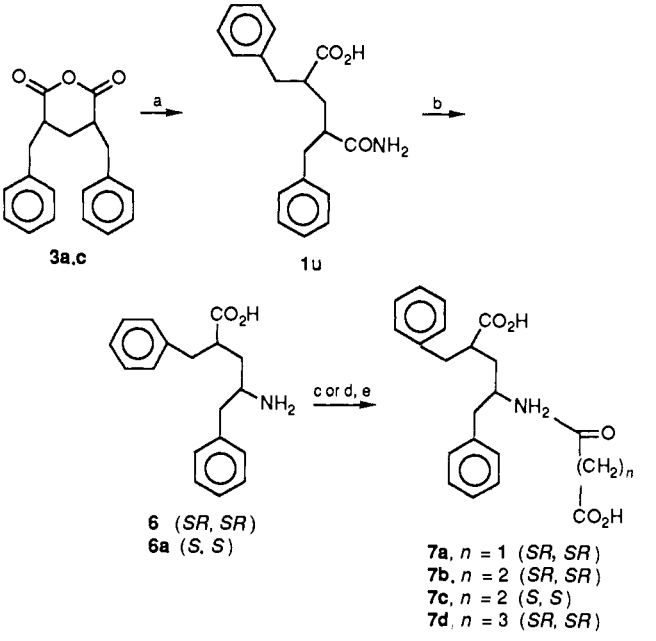
Structure–Activity Relationships

The in vitro data for the substituted dibenzylglutaric acids are summarized in Table I. Varying the chain length of the amino acid substituent from glycine **1a** to β-alanine **1c** to 4-aminobutyric acid **1f** gave similar potent inhibitory activity, 4, 5, and 1.4 nM, respectively. Unlike angiotensin converting enzyme (ACE) inhibitors, considerable flexibility must exist within the enzyme–inhibitor complex in this region.

As with ACE inhibitors the carboxylate site does appear to be of primary importance since replacement of the carboxylate with hydroxyl **1t** or nitrile **1s** decreases activity at least 100-fold. However, the phenolic derivative **1r** has good inhibitory activity.

Two structurally more rigid β-alanine and gaba derivatives, the *o*-anthranilic acid **1m** and the *o*-aminophenylacetic acid **1n**, were prepared. Due to the 100-fold decrease in potency of **1m**, the necessary conformation of the β-alanine carboxyl group for enzyme interaction is probably not cis to the amide nitrogen, although potential steric interactions cannot be ignored. The less rigid *o*-aminophenylacetic acid derivative **1n** has good enkephalinase

Scheme II



[a] NH_3, CH_2Cl_2. [b] NaOH, Br_2, H_2O. [c] Succinic or glutaric anhydride, CH_2Cl_2. [d] Ethyl malonyl chloride, pyridine, CH_2Cl_2. [e] NaOH, MeOH, H_2O.

inhibitory activity. Decreased restriction about the acetic acid carbonyl function allows free rotation around this bond, thus enabling the molecule to bind more effectively to the enzyme. In the anthranilic acid series the meta isomer **1o** was more active than the ortho (**1m**) or para (**1p**) derivatives. A more definitive explanation of spatial orientation is presently under investigation.

The α-amino acid derivatives leucine **1g** and phenylalanine **1h** had similar activities (30–40 nM). Extending the aryl substituent by one or two atoms as in the derivatives of homophenylalanine **1j**, *O*-benzylserine **1l** and *S*-benzylcysteine **1k**, improved the activity almost 10-fold. For the sulfydrol-containing inhibitors (thiorphan derivatives),[5o] this difference was not observed. The inhibitory

activity of the leucine and O-benzylserine derivatives were nearly equivalent. However, the tryptophan derivative **1i** was 15-fold less active than **1j**. Possibly the indole ring and one of the benzyl groups on the glutaric acid chain sterically interact, thus changing the conformation of the molecule, and an unfavorable enzyme interaction results.

The absolute configuration of the benzyl substituents in the S_1 and S_1' region was critical for potent activity. A 10^3-fold difference in activity was observed between the *S,S* isomer **1c** and the *R,R* isomer **1d**. The *SR,RS* gaba diastereomeric mixture **1e**[8] was 40-fold less active than **1f**, the *S,S* isomer. In contrast it has been reported[9] that both enantiomers of thiorphan, a sulfhydryl-containing inhibitor, have similar low nanomolar in vitro potency.

As compared with **1b**, extension of the dibenzylglutaric acid to the diphenethylglutaric acid derivative **5** decreased the activity almost 50-fold. A similar decrease in activity was observed with the bis(cyclohexylmethyl)glutaric acid derivative **4**. These results would imply a specific $\pi-\pi$ interaction between inhibitor and enzyme or a sterically restricted lipophilic pocket might exist in the S_1' region.

For the inverse amide series **7a–d** (Table II), a 15-fold decrease in activity was observed between **7a** and **1a**. A 3-fold decrease in activity was also observed between the glutaric acid **7d** and the gaba derivative **1f**. Similarly for thiorphan, inversion of the amide bond (retrothiorphan)[10] resulted in a 5-fold decrease of enkephalinase inhibitory activity. However, amide inversion of the β-alanine derivative **1c** to the succinyl derivative **7c** reversed the trend, and a slight improvement in activity was observed.

Biological Results

The analgesic test results in the 55 °C mouse hot-plate jump-latency test for thiorphan (**1c**) and **8–11** are shown in Table III. Only **11** was effective in prolonging the jump latencies when administered subcutaneously at 30 mg/kg. However, intraventricular (icv) administration of **1c**, **8**, and thiorphan elicited potent Naloxone reversible, dose-dependent increase of the jump-latency time. Diacid **1c** at 30 μg (icv) surpassed the 240 second cut-off time in the hot-plate jump-latency test. A result usually not obtainable at these doses except for dual aminopeptidase–enkephalinase inhibitors.[5p] Compound **1c** did not inhibit ACE or aminopeptidase. Diacid **1c** and the ethyl ester prodrug **8** were approximately 5 times more potent than thiorphan in doubling the jump latencies. This data would imply that the ethyl ester is readily cleaved to **1c** in the central nervous system (CNS) (assuming that analgesia is due to enkephalinase inhibition) since no in vitro enkephalinase inhibition was detected even at 1 μM concentration.

D-Ala^2-Met-enkephalin (DAEM), an aminopeptidase-resistant substrate for enkephalinase, is active in the tail-withdrawal assay. When it is coadministered with thiorphan[5e] a dose-related potentiation of the analgesic activity has been reported. Compounds **1c**, **9**, and **10** potentiated the Naloxone reversible analgesic response of DAEM, whereas **8** was inactive (Table III). The increased potency of thiorphan in this test system may be due to better blood–brain barrier permeability. However, it is somewhat surprising that peripheral administration of **1c** or thiorphan did not elicit a more potent effect in the mouse hot-plate jump-latency test. Since ethyl ester **8** is very potent in the mouse hot-plate test when administered icv, as mentioned earlier, deesterification probably occurs in the CNS. However, the inactivity of **8** in the DAEM potentiation test implies minor blood–brain penetration and limited hydrolysis to the active diacid **1c** in the periphery. One might also speculate that it is diacid **1c** that crosses the blood–brain barrier and not the monoester.

It was hoped that by increasing lipophilicity from a diacid (**1c**) to a monoacid dihydroxybenzylamine derivative **1o**, improved CNS penetration would result. Good in vitro potency was found although **1o** did not display any improved analgesic activity after systemic administration. As a comparison to **1r** and thiorphan, the hydroxybenzylamine sulfhydryl derivatives **12** and **13** were tested (Table IV). The in vitro potency was similar to that of **1o** and 3 times weaker than that of thiorphan. Surprisingly **12** and **13** were quite potent after subcutaneous administration in the mouse hot-plate jump-latency test as well as the acetic acid stretching screen (Table IV). These two compounds are considerably more active in the mouse hot-plate test after peripheral administration than any of the other tested compounds in this report. Compounds **12** and **13** displaced tritium-labeled Naloxone 15 and 30%, respectively, at 10 μM. Therefore, these compounds are assumed to be virtually inactive at the opiate receptors. In addition, these compounds did not inhibit aminopeptidase. Although opiate receptor interaction and aminopeptidase inhibition are unlikely, the analgesic activity is probably not unique to enkephalinase but due to other combined unidentifiable interactions.

In summary, new dibenzylglutaric acid, dibenzyl gaba, and some sulfhydryl derivatives were found to be active in vitro as enkephalinase inhibitors. Potent analgesic activity was obtained for some compounds in vivo after subcutaneous and/or intraventricular administration.

Experimental Section

Proton NMR spectra were determined on a Varian XL 400 and/or a Bruker AM-300. Infrared spectra were recorded on a Nicolet 5SXFT. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. X-ray analysis was determined on a Nicolet SHELXTL PLUS (Micro Vax II).

2,4-Dibenzylglutaric Acid Anhydrides 3a–d. A solution of **2**[7] (52 g, 0.17 mol) and acetyl chloride (250 mL) in 150 mL of toluene was refluxed for 2 h. The mixture was concentrated, toluene was added (twice), and the mixture was reconcentrated. The paste was dissolved in hot toluene and let stand overnight. The trans anhydride **3a** was collected, rinsed with toluene, and dried to give 24 g of shiny plates melting at 150–152 °C: ^{1}H NMR ($CDCl_3$) δ 7.2 (m, 6 H), 6.95 (m, 4 H), 3.21 (dd, J = 6, 3 Hz, 2 H), 2.85 (m, 2 H), 2.69 (dd, J = 4, 10 Hz, 2 H), 1.65 (t, J = 7 Hz, 2 H).

The mother liquor was concentrated and seeded with **3b** (obtained from allowing the mother liquor to sit at room temperature

(8) The meso-cis anhydride **3b** contains approximately 5% of the trans anhydride **3a**. Therefore some active *S,S* isomer may be present in **1e**.

(9) (a) Mendelsohn, L.; Johnson, B.; Scott, W.; Frederickson, R. *J. Pharmacol. Exp. Ther.* **1985**, *234*, 2. (b) Scott, W.; Mendelsohn, L.; Cohen, M.; Evans, D.; Frederickson, R. *Life Sci.* **1985**, *36*, 1307. (c) Giros, B.; Gros, C.; Schwartz, J. C.; Danvey, D.; Plaquevent, J. C.; Duhamel, L.; Duhamel, P.; Vlaiculescu, A.; Costentin, J.; Lecomte, J. M. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 2. (d) Fournie-Zaluski, M. C.; Coulaud, A.; Boubouotou, R.; Chaillet, P.; Devin, J.; Waksman, G.; Costentin, J.; Roques, B. P. *J. Med. Chem.* **1985**, *28*(9), 1158.

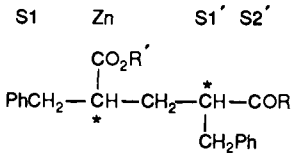
(10) Roques, B. P.; Lucas-Soroca, F.; Chaillet, P.; Costentin, J.; Fournie-Zaluski, M. C. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 3178.

(11) This procedure is adapted from the method of Altstein et al.: *Life Sci.* **1981**, *28*, 185.

(12) This procedure is adapted from the method of Maurer, R. *J. Biochem. Biophys. Methods* **1980**, *2*, 183.

(13) This procedure is adapted from the method of Taber, R. *Adv. Biochem. Psychopharmacol. NY* **1974**, Vol. 8.

Table I. Substituted Dibenzylglutaric Acid Amides



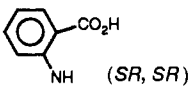
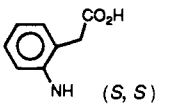
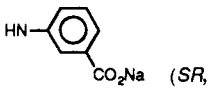
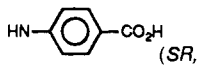
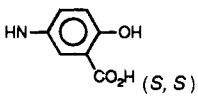
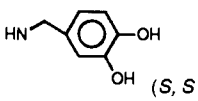
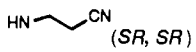
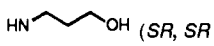
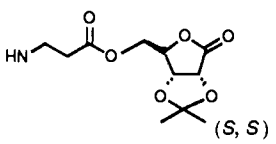
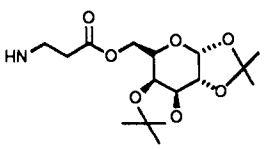
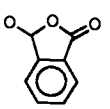
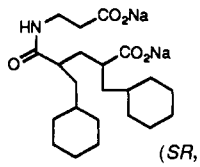
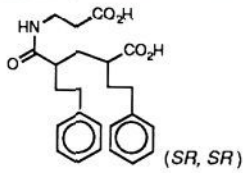
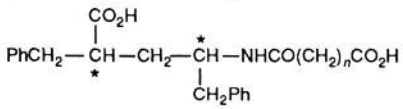
compd	R(*stereochemistry)	R′	enkephalinase IC_{50},[c] nM
1a	Gly (*S,S*)	H	4[b]
1b	β-Ala (*SR,SR*)	H	18[a]
1c	β-Ala (*S,S*)	H	8[a], 5[b]
1d	β-Ala (*R,R*)	H	>1000[a]
1e	gaba (*SR,RS*)	H	57[b]
1f	gaba-ONa (*S,S*)	Na	1.4[b]
1g	Leu (*S,S*)	H	40[a]
1h	Phe-ONa (*S,S*)	Na	29[a]
1i	Trp-ONa (*S,S*)	Na	74[a]
1j	homoPhe (*S,S*)	H	5[a]
1k	S-Benzyl Cys (*S,S*)	H	4[a]
1l	O-Benzyl Ser (*S,S*)	H	6[a]
1m	 (*SR, SR*)	H	1000[a]
1n	 (*S, S*)	H	25[a]
1o	 (*SR, SR*)	Na	90[a]
1p	 (*SR, SR*)	H	800[a]
1q	 (*S, S*)	H	38[a]
1r	 (*S, S*)	Na	20[a]
1s	 (*SR, SR*)	H	>1000[a]
1t	 (*SR, SR*)	Na	1000[a]
1u	NH_2 (*SR,SR*)	H	NT
8	β-Ala (*SR,SR*)	CH_2CH_3	>1000[a]
9	 (*S, S*)	H	NT
10	 (*S, S*)	Na	NT
11	β-Ala (*S,S*)		NT
4	 (*SR, SR*)		1000[a]

Table I (Continued)

compd	R(*stereochemistry)	R′	enkephalinase IC_{50},[c] nM
5	 (SR, SR)		1000[a]
phosphoramidon			0.6,[a] 0.4[b]
thiorphan			3,[a] 5[b]

[a] Method 1. [b] Method 2. [c] The differences between methods 1 and 2 are noted in the Experimental Section. The inhibitory activity from either method appear to be virtually identical.

Table II. 2,4-Dibenzyl-4-aminobutyric Acid Amides



compd	n	stereochemistry (*)	enkephalinase IC_{50}, nM
7a	1	(*SR*,*SR*)	62[b]
7b	2	(*SR*,*SR*)	9[a]
7c	2	(*S*,*S*)	2[b]
7d	3	(*SR*,*SR*)	12[b]

[a] Method 1. [b] Method 2.

for 1 week). The needles were collected and rinsed with cyclohexane–toluene (3:1) to give 10 g of **3b** melting at 57–60 °C: ^{1}H NMR ($CDCl_3$) δ 7.2 (m, 6 H), 7.1 (m, 4 H), 3.25 (m, 2 H), 2.7 (m, 4 H), 1.7 (m, 1 H), 1.45 (m, 1 H).

The remainder of the mother liquor was concentrated and slurried with cyclohexane to give another 17 g of mostly cis anhydride. The cis anhydride (16 g) was isomerized to the trans anhydride by heating at 50 °C with 15% Et_3N in toluene for 15 min. The solution was left at 22 °C overnight. The solid was collected and recrystallized from hot toluene to give another 12.3 g of **3a**.

(*S*,*S*)-2,4-Dibenzylglutaric anhydride (**3c**) was prepared as described for **3a** except acetic anhydride was used in place of acetyl chloride. The chiral anhydride was obtained as colorless flakes (98%) melting at 173–175 °C; $[\alpha]_D$ = −18.3° (*c* 1, $CHCl_3$). The preparation of the starting material **2c** is described below.

(*R*,*R*)-2,4-Dibenzylglutaric anhydride (**3d**) was prepared as described above: mp 172–175 °C; $[\alpha]_D$ = +19.0° (*c* 0.85, $CHCl_3$). The starting material **2d** (mp 149–152 °C) was prepared as described for **2c** accept the diacid was resolved with (*S*)-(−)-α-methylbenzylamine.

(*S*,*S*)-2,4-Dibenzylglutaric Acid (2c). To a solution of **2a** (prepared by refluxing the trans anhydride **3a** in dioxane–H_2O) (39.5 g, 0.126 mol) in 1.1 L of hot 2-propanol was added (*R*)-(+)-α-methylbenzylamine (15.3 g, 0.126 mol) in 700 mL of 2-propanol. The mixture crystallized overnight. The solid was collected and recrystallized from 2-propanol to give 20.2 g of a colorless solid melting at 205–208 °C. The salt (17.8 g) was dissolved in a warm solution of 39 mL of 1 N HCl and 200 mL of MeOH and then concentrated and extracted with 2:1 EtOAc–Et_2O. The organic layer was washed with 1 N HCl, H_2O, NaCl (saturated), dried ($MgSO_4$), and concentrated to give 12.5 g of **2c** as a colorless solid melting at 150–152 °C; $[\alpha]_D$ = +8.6° (*c* 1.1, MeOH).

Typical Procedure for the Formation of Dibenzylglutaric Acid Amides from (*S*,*S*)-2,4-Dibenzylglutaric Anhydride (3c). *N*-[(*S*,*S*)-2,4-Dibenzyl-4-carboxylbutyryl]-β-alanine (1c). To a suspension of **3c** (1.0 g, 3.4 mmol) in 20 mL of 1:1 pyridine–CH_2Cl_2 under an atmosphere of N_2 was added powdered β-alanine (10.2 mmol). The mixture was stirred at room temperature for 16 h. The solvent was removed under reduced pressure. The residue was diluted with EtOAc, washed with 2 N HCl, H_2O, and NaCl, dried ($MgSO_4$), filtered, and concentrated to give an oil. Ether was added, and the solid was collected and washed with a small amount of ether to give 1.2 g (92%) of **1c** as a colorless solid melting at 142–144 °C: $[\alpha]_D$ +25.6° (*c* 2, MeOH); IR (Nujol) 1697, 1640, 1601, 1532 cm^{-1}, ^{1}H NMR (Me_2SO-d_6) δ 12.1 (br s, 2 H), 7.8 (br t, *J* = 5 Hz, 1 H), 7.2 (m, 10 H), 3.1 (m, 2 H), 2.75 (m, 3 H), 2.55 (m, 3 H), 2.2 (d, d, *J* = 7, 2 Hz, 2 H), 1.6 (m, 2 H). Anal. ($C_{22}H_{25}NO_5$) C, H, N.

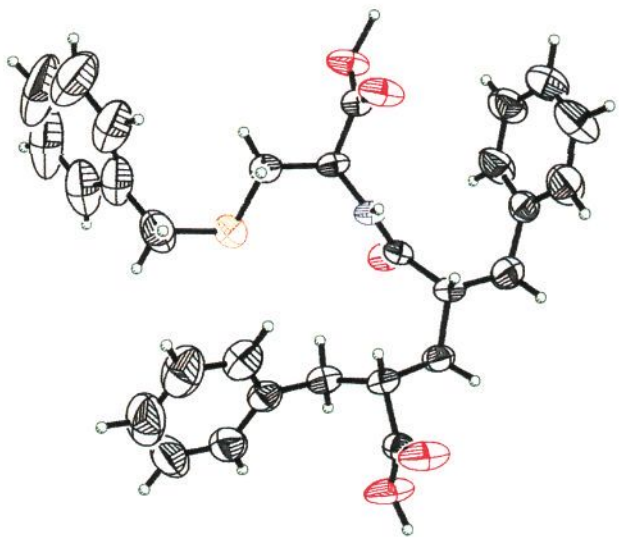


Figure 1. X-ray crystal structure (with thermal ellipsoids), showing absolute configuration of compound **1k**.

N-[(*S*,*S*)-2,4-Dibenzyl-4-carboxybutyryl]glycine (**1a**) was prepared as described above. The diacid was purified via esterification ($SOCl_2$–MeOH), chromatography (SiO_2, 4:1 CH_2Cl_2–EtOAc), and saponification (1 N NaOH, MeOH) to give **1a** (31%) as a colorless solid melting at 61–63 °C: $[\alpha]_D$ +22° (*c* 1, MeOH); IR (Nujol) 1720, 1632, 1535 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) 12.2 (br s, 2 H), 8.2 (t, *J* = 6 Hz, 1 H) 7.2 (m, 10 H), 3.65 (m, 2 H), 2.7 (m, 6 H), 1.6 (m, 2 H). Anal. ($C_{21}H_{23}NO_5$) C, H, N.

N-[(*SR*,*SR*)-2,4-Dibenzyl-4-carboxybutyryl]-β-alanine (**1b**) was prepared as described above (90%) by substituting **3a** for **3c** to give colorless solid: mp 122–124 °C.

N-[(*R*,*R*)-2,4-Dibenzyl-4-carboxybutyryl]-β-alanine (**1d**) was prepared as described above (88%) by substituting **3d** for **3c** to give a colorless solid: mp 143–145 °C, $[\alpha]_D$ −25.0° (*c* 2, MeOH).

N-[(*RS*,*SR*)-2,4-Dibenzyl-4-carboxybutyryl]-4-aminobutyric acid (**1e**) was prepared as described above by substituting **3b** for **3c**. After recrystallization from EtOAc–Et_2O, **1e** was obtained (72%) as a colorless solid melting at 124–126 °C; IR (KBr) 1700, 1632, 1550 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 12.1 (br s, 2 H), 7.95 (t, *J* = 7 Hz, 1 H), 7.1 (m, 10 H), 2.2–2.5 (m, 8 H), 2.02 (t, *J* = 7 Hz, 2 H), 1.9 (m, 1 H), 1.7 (m, 3 H). Anal. ($C_{23}H_{27}NO_5$) C, H, N.

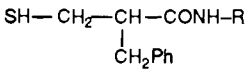
N-[(*S*,*S*)-2,4-Dibenzyl-4-carboxybutyryl]-4-aminobutyric acid disodium salt (**1f**) (the disodium salts were prepared by mixing 1 equiv of 1 N NaOH with the diacid and concentrating) was prepared and described above (80%) to give a colorless solid: mp 108–113 °C dec; $[\alpha]_D$ +11.7° (*c* 1, MeOH); IR (KBr) 1650, 1570, 1410 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 8.3 (br s, 1 H), 7.2 (br s, 10 H),

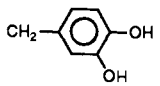
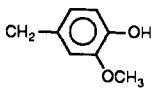
Table III. Antinociceptive Effects in the Mouse Hot-Plate and DAEM Potentiation Tests

compound	mouse hot plate MED[a] (sc), mg/kg	ED_{200}[b] (icv), μg	DAEM potentiation control (s) (n = 10)	DAEM potentiation reactn time, s (n = 10)	DAEM potentiation dose (sc), mg/kg
thiorphan	100[c]	75	3.3	7.5[d]	3
1c	>30	15	4.2	8.6[e]	30
8	>100	16	-	inactive	30
9	>30	NT[h]	2.9	9.8[f]	30
10	>30	NT	3.5	8.1[g]	30
11	30	NT		NT	

[a] Minimum effective dose. [b] Doubling of the jump latency. [c] Reference 5f: (100 mg/kg) MED. [d] $p \leq 0.0001$. [e] $p \leq 0.006$. [f] $p \leq 0.001$. [g] ≤ 0.05. [h] NT = not tested.

Table IV. Antinociceptive Effects in the Mouse Hot-Plate and Acetic Acid Stretching Tests



compd	R	mouse hot plate:[c,d] ED_{200} (sc), mg/kg	acetic acid stretching:[c,d] ED_{50} (sc), mg/kg	enkephalinase IC_{50}, nM
12		15	5	12[a]
13		6	5.5	9[b]
thiorphan	CH_2CO_2H	>100	38	3,[a] 5[b]
1r		>100	NT	20[a]

[a] Method 1. [b] Method 2. [c] Vehicle is 5% Emulphor, 5% ethanol, 90% saline. [d] n = 20, $p < 0.05$.

3.3–1.2 (m, 14 H). Anal. ($C_{23}H_{25}Na_2O_5 \cdot 2H_2O$) C, H, N.

N-[(*S*,*S*)-2,4-Dibenzyl-4-carboxybutyryl]-(*S*)-leucine (**1g**) was prepared as described above (75%) to give a colorless solid: mp 129–131 °C; $[\alpha]_D$ 0° (*c* 1, MeOH); IR (KBr) 1742, 1690, 1506, 1565 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 12.3 (br s, 2 H), 8.13 (d, *J* = 9 Hz, 1 H), 7.2 (m, 10 H), 4.2 (m, 1 H), 2.90 (dd, *J* = 7, 6 Hz, 1 H), 2.7 (m, 5 H), 1.6 (m, 5 H), 0.82 (dd, *J* = 11, 6 Hz, 6 H). Anal. ($C_{25}H_{31}NO_5$) C, H, N.

N-[(*S*,*S*)-2,4-Dibenzyl-4-carboxybutyryl]-(*S*)-phenylalanine disodium salt (**1h**) was prepared as described above (57%) to give a colorless solid: mp >300 °C dec; $[\alpha]_D$ +20.7° (*c* 1, MeOH); IR (KBr) 1618, 1599, 1561, 1523, 1415 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.6 (d, *J* = 10 Hz, 1 H), 7.2 (m, 15 H), 3.90 (q, *J* = 9 Hz, 1 H), 2.2–3.1 (m, 8 H), 1.4 (m, 2 H). Anal. ($C_{28}H_{27}NNa_2O_5$) C, H, N.

N-[(*S*,*S*)-2,4-Dibenzyl-4-carboxybutyryl]-(*S*)-tryptophan disodium salt (**1i**) was prepared as described above (69%) to give a colorless solid: mp >300 °C dec; $[\alpha]_D$ +11.1° (*c* 1, MeOH); IR (KBr) 1621, 1583, 1522, 1410 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 10.7 (s, 1 H), 9.0 (d, *J* = 7 Hz, 1 H), 7.55 (d, *J* = 8 Hz, 1 H), 6.8–7.3 (m, 14 H), 4.15 (t, *J* = 5 Hz, 1 H), 2.2–3.2 (m, 8 H), 1.4 (m, 2 H). Anal. ($C_{30}H_{28}N_2Na_2O_5$) C, H, N.

N-[(*S*,*S*)-2,4-Dibenzyl-4-carboxybutyryl]-(*S*)-homophenylalanine (**1j**) was prepared as described above (74%) to give a colorless solid: mp 165–167 °C; $[\alpha]_D$ +13.7° (*c* 1, MeOH); IR (Nujol) 1710, 1645, 1525, cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 12.3 (br s, 2 H), 8.3 (d, *J* = 8 Hz, 1 H), 7.2 (m, 15 H), 4.1 (m, 1 H), 2.90 (dd, *J* = 8, 6 Hz, 1 H), 2.4–2.7 (m, 7 H), 1.4–2.0 (m, 4 H). Anal. ($C_{29}H_{31}NO_5$) C, H, N.

N-[(*S*,*S*)-2,4-Dibenzyl-4-carboxybutyryl]-(*S*)-*S*-benzylcysteine (**1k**) was prepared as described above (77%) to give a colorless solid: mp 134–136 °C; $[\alpha]_D$ −17.1° (*c* 1, MeOH); IR (KBr) 1710, 1655, 1536; ^1H NMR (Me_2SO-d_6) δ 12.4 (br, 2 H), 8.3 (d, *J* = 8 Hz, 1 H), 7.2 (m, 15 H), 4.4 (m, 1 H), 3.7 (s, 1 H), 2.4–3.0 (m, 8 H), 1.6 (m, 2 H). Anal. ($C_{29}H_{31}NO_5S$) C, H, N.

N-[(*S*,*S*,)-2,4-Dibenzyl-4-carboxybutyryl]-(*S*)-*O*-benzylserine (**1l**) was prepared as described above (90%) to give a colorless solid: mp 149–151 °C; [α] +25.2° (*c* 1, MeOH); IR (KBr) 1740, 1715, 1637; ^1H NMR (Me_2SO-d_6) δ 12.4 (br, 2 H), 8.25 (d, *J* = 8 Hz, 1 H), 7.2 (m, 15 H), 4.45 (s, 2 H), 4.5 (m, 1 H), 3.7 (m, 2 H), 2.4–3.0 (m, 6 H), 1.6 (m, 2 H). Anal. ($C_{29}H_{31}NO_6$) C, H, N.

N-[(*SR*,*SR*)-2,4-Dibenzyl-4-carboxybutyryl]-2-aminobenzoic acid (**1m**) was prepared as described above (44%) to give a colorless solid: mp 180–183 °C; IR (KBr) 1740, 1690, 1668, 1602, 1583, 1530 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 12.1 (s, 2 H), 8.43 (dd, *J* = 7, 1 Hz, 1 H), 7.90 (dd, *J* = 6, 1.5 Hz, 1 H), 7.55 (dt, *J* = 2, 7 Hz, 1 H), 7.2 (m, 11 H), 2.5–2.9 (m, 6 H), 1.8 (m, 2 H). Anal. ($C_{26}H_{25}NO_5$) C, H, N.

N-[(*S*,*S*)-2,4-Dibenzyl-4-carboxybutyryl]-2-aminophenylacetic acid (**1n**) was prepared as described above (32%) to give a colorless solid: mp 108–115 °C; $[\alpha]_D$ +25.0° (*c* 1, MeOH); IR (KBr) 1698, 1652, 1590, 1525 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 12.5 (br, 2 H), 9.4 (br s, 1 H), 7.2 (m, 14 H), 3.35 (AB q, *J* = 21, 16 Hz, 2 H), 2.8 (m, 6 H), 1.7 (m, 2 H). Anal. ($C_{27}H_{27}NO_5$) C, H, N.

N-[(*S*,*S*)-2,4-Dibenzyl-4-carboxybutyryl]-3,4-dihydroxybenzylamine sodium salt (**1r**) was prepared as described above (84%) to give a yellow solid: mp 150 °C dec; $[\alpha]_D$ +19.0° (*c* 1, MeOH); IR (KBr) 1735, 1650, 1564 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.8 (br, 1 H), 6.5–7.3 (m, 15 H), 4.0 (m, 2 H), 2.3–3.0 (m, 6 H), 1.5 (m, 2 H). Anal. ($C_{25}H_{26}NNaO_5$) C, H, N.

N-[(*SR*,*SR*)-2,4-Dibenzyl-4-carboxybutyryl]-3-aminopropanenitrile (**1s**) was prepared as described above (89%) to give a colorless solid: mp 123–125 °C; IR (KBr) 2260, 1730, 1640, 1548 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 12.1 (br s, 1 H), 8.3 (t, *J* = 6 Hz, 1 H), 7.2 (m, 10 H), 3.3 (m, 2 H), 2.4–2.8 (m, 8 H), 1.6 (m, 2 H). Anal. ($C_{22}H_{24}N_2O_3$) C, H, N.

N-[(*SR*,*SR*)-2,4-Dibenzyl-4-carboxybutyryl]-3-aminopropanol sodium salt (**1t**) was prepared as described above (74%) to give a colorless solid melting at 90–100 °C: IR (KBr) 1660, 1648, 1580 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 9.16 (t, *J* = 8 Hz, 1 H), 7.2 (m, 10 H), 3.35 (t, *J* = 8 Hz, 2 H), 3.05 (m, 1 H), 2.97 (m, 1 H), 2.85 (m, 2 H), 3.62 (m, 1 H), 2.4 (m, 3 H), 1.4 (m, 4 H). Anal. ($C_{21}H_{26}NNaO_4$) C, H, N.

***N*-[(*SR*,*SR*)-2,4-Bis(cyclohexylmethyl)-4-carboxybutyryl]-β-alanine Disodium Salt (4).** To a solution of **1b** (0.5 g, 1.3 mmol) in 25 mL of EtOH–H_2O (1:1) was added 0.5 g of 5% Rh/C. The suspension was shaken under an atmosphere of hydrogen at 50 psi for 36 h. The mixture was filtered through Celite and concentrated to give a colorless oil. The residue was taken up in 5 mL of MeOH and 2.7 g of 1.0 N NaOH (2 equiv) was added. The mixture was concentrated and the solid collected, washed with cold MeOH, and dried 16 h at 40 °C under high vacuum to give 320 mg (56%) of a pink powder melting at 200–210 °C dec: IR (KBr) 1645, 1585, 1560, 1452 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.1 (br s, 1 H), 3.2 (m, 2 H), 2.3 (m, 1 H), 2.1 (m,

3 H), 0.6–1.7 (m, 29 H). Anal. ($C_{22}H_{35}NNa_2O_5 \cdot H_2O$) C, H, N.

N-[(*SR,SR*)-2,4-Diphenethyl-4-carboxybutyryl]-β-alanine disodium salt (5) was prepared (76%) as described for **1b** by substituting *trans*-2,4-diphenethylglutaric anhydride for **3a** to give a colorless solid: mp 205–215 °C dec; IR (Nujol) 1660, 1640, 1635, 1570, 1495 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.2 (t, J = 8 Hz, 1 H), 7.2 (m, 10 H), 3.3 (m, 2 H), 1.3–2.5 (m, 14 H). Anal. ($C_{24}H_{27}NNa_2O_5 \cdot H_2O$) C, H, N.

(*SR,SR*)-2,4-Dibenzyl-4-aminobutyric Acid (6). To a solution of (*SR,SR*)-2,4-dibenzylglutaric acid amide (25.9 g, 83 mmol) [the amide was prepared by stirring the anhydride **3c** in CH_2Cl_2 saturated with NH_3 gas at room temperature for 30 min; $[\alpha]_D$ +21.4° (*c* 1, MeOH); mp 144–147 °C] and 2 N NaOH (124 mL, 0.25 mol) at 0 °C was added a 0 °C solution of bromine (4.26 mL, 83 mmol) in 124 mL of 2 N NaOH. After the addition was complete the solution was warmed to room temperature and then heated to 70 °C for 2 h, cooled, and acidified with 330 mL of 1 N HCl. The solid was collected, washed with H_2O and acetone, and dried to give 15.3 g (65%) of **6**: mp 158–161 °C; ^1H NMR (MeOD) δ 7.1 (m, 10 H), 3.50 (m, 1 H), 3.18 (dd, J = 13, 7 Hz, 1 H), 2.87 (dd, J = 13, 7 Hz, 1 H), 2.75 (dd, J = 13, 7 Hz, 1 H), 2.72 (m, 1 H), 2.59 (dd, J = 13, 5 Hz, 1 H), 1.7 (m, 2 H). The *S,S* isomer **6a** was prepared as described above, $[\alpha]_D$ −10° (*c* 1, 1 N TFA/MeOH).

***N*-(2-Carboxy-1-oxoethyl)-(*SR,SR*)-2,4-dibenzyl-4-aminobutyric Acid (7a).** To a solution of **6** (1.4 g, 4.9 mmol) in 10 mL of pyridine was added ethyl malonyl chloride (483 mg, 3.2 mmol) in 10 mL of CH_2Cl_2. The mixture was stirred for 3 days, diluted with EtOAc–Et_2O, and washed with 2 N HCl, H_2O, and NaCl (saturated), dried ($MgSO_4$), and concentrated. The residue was dissolved in 15 mL of MeOH and 1 N NaOH (10.6 g) and stirred for 2 days. The mixture was shaken with 200 mL of Et_2O (3,5-dibenzyl-2-pyrrolidinone, mp 85–87 °C, was isolated from the ether layer) and 100 mL of 0.5 N NaOH. The basic layer was acidified with 2 N HCl and extracted with EtOAc, washed with H_2O, dried ($MgSO_4$), and concentrated. The residue was triturated with toluene, and the solid was collected to give 200 mg (11%) of **7a**: mp 92–94 °C; IR (KBr) 1749, 1689, 1613, 1546 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 12.2 (br s, 2 H), 7.91 (d, J = 8 Hz, 1 H), 3.94 (m, 1 H), 3.05 (s, 2 H), 2.8–2.6 (m, 5 H), 1.7 (m, 1 H), 1.4 (m, 1 H). Anal. ($C_{21}H_{23}NO_5$) C, H, N.

***N*-(3-Carboxy-1-oxopropyl)-(*SR,SR*)-2,4-dibenzyl-4-aminobutyric Acid (7b).** A suspension of **6** (0.5 g, 1.8 mmol) and succinic anhydride (0.16 g, 1.6 mmol) in 15 mL of 1:1 CH_2Cl_2–pyridine was stirred at room temperature for 2 days. The solvent was removed under reduced pressure. The residue was diluted with EtOAc and washed with 1 N HCl, H_2O, and NaCl (saturated), dried ($MgSO_4$), and concentrated to give an oil. The oil was covered with ether and scratched and the product crystallized to give 0.4 g (57%) of **7b**: mp 157–159 °C; IR (KBr) 1715, 1675, 1610, 1569 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 12.0 (s, 2 H), 7.7 (d, J = 8 Hz, 1 H), 7.2 (m, 10 H), 3.92 (m, 1 H), 2.6–2.8 (m, 5 H), 2.35 (m, 2 H), 2.20 (m, 2 H), 1.7 (m, 1 H), 1.4 (m, 1 H). Anal. ($C_{22}H_{25}NO_5$) C, H, N.

N-(3-Carboxy-1-oxopropyl)-(*S,S*)-2,4-dibenzyl-4-aminobutyric acid (**7c**) was prepared (48%) as described above by substituting the *S,S* diastereomer **6a** for **6** to give a colorless solid: mp 147–149 °C; $[\alpha]_D$ +8.4° (*C* 1.2, MeOH).

N-(4-Carboxy-1-oxobutyl)-(*SR,SR*)-2,4-dibenzyl-4-aminobutyric acid (**7d**) was prepared as described above by substituting glutaric anhydride for succinic anhydride to give a colorless solid: mp 127–130 °C; IR (KBr) 1710, 1695, 1635, 1547 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 12.0 (br s, 2 H), 7.6 (d, J = 8 Hz, 1 H), 7.2 (m, 10 H), 3.96 (m, 1 H), 2.78 (m, 1 H), 2.62 (m, 4 H), 2.5 (t, J = 8 Hz, 2 H), 2.0 (dt, J = 7, 4 Hz, 2 H), 1.65 (m, 1 H), 1.62 (t, J = 7 Hz), 1.42 (m, 1 H). Anal. ($C_{23}H_{27}NO_5$) C, H, N.

***N*-[(*SR,SR*)-2,4-Dibenzyl-4-carbethoxybutyryl]-β-alanine (8).** A solution of **3a** (5.0 g, 17.0 mmol) in 45 mL of ethanol–toluene (2:1) was refluxed for 16 h. Concentration of the solution gave 2,4-dibenzylglutaric acid ethyl monoester **14** (6 g) as an oil: ^1H NMR ($CDCl_3$) δ 12.5 (br s, 1 H), 7.2 (m, 10 H), 4.05 (q, 2 H), 2.6–3.1 (m, 6 H), 1.8 (m, 2 H), 1.1 (t, 3 H).

To a solution of **14** (2.0 g, 5.9 mmol), β-alanine benzyl ester pTSA (2.25 g, 6.4 mmol), triethylamine (0.65 g, 6.4 mmol) in 20 mL of CH_2Cl_2 was added 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (1.3 g, 6.8 mmol). The reaction was stirred at room temperature for 16 h. The solution was diluted with EtOAc and washed with 1 N HCl, H_2O, $NaHCO_3$, and NaCl (saturated). The organic layer was separated, dried ($MgSO_4$), filtered, concentrated, and chromatographed on SiO_2 with EtOAc–CH_2Cl_2 (1:4) as eluent to give 1.5 g of diester **15** as a colorless oil: ^1H NMR ($CDCl_3$) δ 7.4 (s, 5 H), 7.3 (m, 10 H), 5.85 (t, 1 H), 5.1 (s, 2 H), 4.1 (q, 2 H), 3.4 (m, 2 H), 2.8 (m, 5 H), 1.6–2.5 (m, 5 H), 1.1 (t, 3 H).

A solution of **15** (1.5 g, 3.0 mmol) in 50 mL of EtOH with 0.5 g of suspended 5% Pd/C was stirred under 1 atm of H_2 for 2 h at room temperature. The mixture was filtered through Celite. The filtrate was concentrated to give 880 mg of **8** as a crystalline solid: mp 84–86 °C; IR (KBr) 1728, 1702, 1620, 1575 cm^{-1}; ^1H NMR ($CDCl_3$) δ 12.1 (br s, 1 H), 7.1 (m, 10 H), 6.09 (t, J = 7 Hz, 1 H), 4.0 (q, J = 8 Hz, 2 H), 3.4 (m, 2 H), 2.8 (m, 5 H), 1.6–2.4 (m, 5 H), 1.1 (t, J = 8 Hz, 3 H). Anal. ($C_{24}H_{29}NO_5$) C, H, N.

***N*-[(*S,S*)-2,4-Dibenzyl-4-carboxybutyryl]-β-alanine 2,3-*O*-Isopropylidene-D-ribono-1,4-lactone Ester (9).** A solution of *N*-CBZ-β-alanine 2,3-*O*-isopropylidene-D-ribono-1,4-lactone ester (0.67 g, 1.70 mmol) [the ester was prepared by coupling *N*-CBZ-β alanine and 2,3-*O*-isopropylidene-D-ribono-1,4-lactone with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride and DMAP in CH_2Cl_2] and **3c** (0.5 g, 1.70 mmol) in 50 mL of EtOAc with 250 mg of suspended 5% Pd/C was hydrogenated at room temperature for 5 h. The mixture was filtered through Celite. The filtrate was concentrated, Et_2O was added, and the mixture was left to stand for 2 days. The solid was collected to give 0.60 g (64%) of **9**: mp 106–109 °C; $[\alpha]_D$ −0.94° (*c* 0.8, MeOH); IR (KBr) 1783, 1743, 1730, 1629, 1538 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.0 (t, J = 6 Hz, 1 H), 7.2 (m, 10 H), 4.9 (m, 3 H), 4.2 (d, J = 3 Hz, 2 H), 3.2 (m, 2 H), 2.1–2.8 (m, 8 H), 1.7 (m, 2 H), 1.35 (s, 3 H), 1.32 (s, 3 H). Anal. ($C_{30}H_{35}NO_9$) C, H, N.

N-[(*S,S*)-2,4-Dibenzyl-4-carboxybutyryl]-β-alanine 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose ester sodium salt (**10**) was prepared as described for **9** except the acid ester was purified by flash chromatography on SiO_2 eluting with 90:6:4 CH_2Cl_2–EtOH–EtOAc: mp 70–75 °C; $[\alpha]_D$ −12.4° (*c* 1, MeOH); IR (KBr) 1740, 1660, 1580 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.8 (br, 1 H), 7.2 (m, 10 H), 5.5 (d, J = 5 Hz, 1 H), 2.1–4.5 (m, 16 H), 1.6 (m, 2 H), 1.42 (s, 3 H), 1.46 (s, 3 H), 1.33 (s, 6 H). Anal. ($C_{34}H_{42}NNaO_{10} \cdot {}^3/_4H_2O$) C, H, N.

N-[(*S,S*)-2,4-Dibenzyl-4-[[(1-oxo-2-benzofuran-3-yl)oxy]-carbonyl]butyryl]-β-alanine (**11**) was prepared by a method similar to that described for **8**: mp 46–49 °C; $[\alpha]_D$ +14.0° (*c* 1, MeOH); IR (Nujol) 1790, 1768, 1713, 1673, 1518 cm^{-1}; ^1H NMR δ 7.9 (m, 4 H), 7.2 (m, 10 H), 6.5 (m, 1 H), 5.45 (s, 1 H), 3.2 (m, 2 H), 2.0–2.9 (m, 8 H), 1.6 (m, 2 H). Anal. ($C_{30}H_{29}NO_7$) C, H, N.

2-Benzyl-3-mercaptopropionic Acid 3,4-Dihydroxybenzylamide (12). To a 0 °C solution of 2-benzyl-3-thioacetylpropionic acid[14] (2.0 g, 8.4 mmol) and triethylamine (1.9 g, 8.4 mmol) in 50 mL of THF was added ethyl chloroformate (1.0 g, 9.2 mmol). The mixture was stirred at 0 °C for 30 min and then 3,4-dihydroxybenzylamine hydrobromide (1.85 g, 8.4 mmol) was added. Stirring was continued for 16 h. The residue was dissolved in EtOAc, washed 1 N HCl, H_2O, saturated $NaHCO_3$, dried (Na_2SO_4), filtered, concentrated, and flash chromatographed on SiO_2 with EtOAc–CH_2Cl_2 (1:1) as eluent to give 1.3 g (43%) of the *S*-acetyl derivative as an oil.

To a solution of the above *S*-acetyl derivative (1.1 g, 3.1 mmol) in 10 mL of MeOH was added 9.2 mL of 1 N NaOH (the methanol and sodium hydroxide solutions were deoxygenated with bubbling nitrogen for 10 min prior to mixing). The solution was stirred under an atmosphere of nitrogen for 2.5 h, 10 mL of 1 N HCl was added, and the mixture was concentrated. The residue was extracted with EtOAc, dried (Na_2SO_4), filtered, and concentrated. Methylene chloride (3 mL) was added, and the crystalline solid was collected to give 0.81 g (84%) of **12**: mp 108–111 °C; IR (Nujol) 1637, 1605, 1545 cm^{-1}; ^1H NMR (Me_2SO-d_6) 8.75 (s, 1 H)

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8.69 (s, 1 H), 8.2 (t, J = 7 Hz, 1 H), 7.2 (m, 5 H), 6.61 (d, J = 4 Hz, 1 H), 6.59 (d, J = 11 Hz, 1 H), 6.33 (dd, J = 8, 2 Hz, 1 H), 4.05 (d, J = 6 Hz, 2 H), 2.4–3.0 (m, 5 H), 2.15 (t, J = 8 Hz, 1 H). Anal. ($C_{17}H_{19}NO_3S$) C, H, N.

2-Benzyl-3-mercaptopropionic acid 3-methoxy-4-hydroxybenzylamide (**13**) was prepared as described above to give a colorless solid: mp 98–101 °C; IR (Nujol) 1640, 1603, 1518 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.8 (s, 1 H), 8.3 (t, J = 7 Hz, 1 H), 7.2 (br s, 5 H), 6.4–6.8 (m, 3 H), 4.15 (d, J = 7 Hz, 2 H), 3.7 (s, 3 H), 3.3 (s, 1 H), 2.0–2.9 (m, 5 H). Anal. ($C_{18}H_{20}NO_3S$) C, H, N.

Biological Tests. Assay for Enkephalinase Activity. Method 1. Procedure.[11] Synaptic membranes from rat striatum were incubated with ^3H-Tyr-Leu-enkephalin for 15 min at 30 °C, pH 6.5, in the presence of 10^{-6} M Bestatin (to inhibit aminopeptidase activity). The reaction was stopped by the addition of 30% acetic acid and the reaction product ^3H-Tyr-Gly-Gly is separated from unreacted ^3H-Tyr-Leu-enkephalin on a Porapak Q column followed by a Cu^{2+} chelex column. The ^3H-Tyr-Gly-Gly is counted by liquid scintillation and 15–20% hydrolysis of substrate is routinely detected.

Assay for Enkephalinase Activity. Method 2. Procedure.[12] Rabbit protease 24.11 (15 ng of protein) was incubated with a mixture of [Try-^3H]Leu-enkephalin (43.6 Ci/mmol, NEN) and unlabeled Leu-enkephalin (1 μM final concentration, 30 000 cpm) for 10 min at 37 °C in a final volume of 100 μL. The assay buffer used was 50 mM Tris-HCl, pH 6.5 at 37 °C. The reaction was terminated by adding 20 μL of 0.6% TFA. The degradative product Tyr-Gly-Gly was separated from Leu-Enk by RP-HPLC. Ninety microliters (75% of total volume) of the reaction mixture was separated with a C4 cartridge and an isocratic elution for 8 min, 85% buffer A (0.09% TFA in water) and 15% buffer B (0.09% TFA in 90% acetonitrile), 1 mL/min. Fractions (0.6 mL) were collected and counted in a Beckman Model LS 6800 scintillation counter.

Hot-Plate Test. Procedure. Fifteen minutes postinjection (icv, iv, or sc), male mice (CF_1; 18–23 g) were individually exposed to a copper hot plate, thermostatically maintained at 55 °C. The latency between the animal being placed on the hot plate and jumping up from the hot plate was recorded. Cutoff time was 240 s.

[D-Ala^2,Met^5]enkephalin (DAEM) Test. Procedure.[5g] Male CF-1 mice (20–22 g) were injected sc with either test agent or vehicle followed immediately by DAEM (1 μg) or vehicle (icv). Tail-flick latencies were measured 30 min later. Cutoff time was 10 s.

Acetic Acid Stretching Mouse. Procedure.[13] Potential analgesics were administered (sc) 15 min prior to the mice receiving an intraperitoneal injection of 3% aqueous solution of acetic acid in a volume of 10 mL/kg. Twelve male CF·1 mice (18–22 g) were used per dose of test agent or vehicle. Five minutes after the irritant, the animals were placed into individual Plexiglas observation cages and the number of characteristic stretching episodes were recorded over a 10-min period.

Amino Peptidase Inhibition. Procedure. One unit will hydrolyze 1.0 μmol of L-leucine p-nitroaniline to L-leucine and p-nitroaniline per minute at pH 7.4 at 37 °C. The incubation mixture (total volume 900 μL) contained 50 mM Tris buffer, pH 7.4, 0.8 mM substrate (final concentration) and kidney cortex membranes (75 μg of protein). The mixture was incubated for 15 min at 37 °C and 100 μL of 2 M TCA added to terminate the reaction. The reaction tubes were centrifuged for 2 min and OD determined at 410 nm. The inhibitor studies were carried out in similar fashion except that the inhibitors were preincubated with enzyme for 10 min at 37 °C.

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Registry No. **1a**, 112965-83-0; **1b**, 105148-48-9; **1c**, 105182-21-6; **1d**, 122442-15-3; **1e**, 105129-12-2; **1f**·2Na, 122410-30-4; **1f** (free acid), 105226-94-6; **1g**, 112893-89-7; **1h**·2Na, 112914-67-7; **1h** (free acid), 122442-18-6; **1i**·2Na, 112893-91-1; **1i** (free acid), 122519-16-8; **1j**, 112893-84-2; **1k**, 112893-85-3; **1l**, 122410-31-5; **1m**, 105129-17-7; **1n**, 122410-32-6; **1o**·2Na, 122410-33-7; **1o** (free acid), 122410-42-8; **1p**, 105129-20-2; **1q**, 122410-34-8; **1r**·Na, 122410-35-9; **1r** (free acid), 122410-43-9; **1s**, 105129-19-9; **1t**·Na, 122410-36-0; **1t** (free acid), 122410-44-0; (*SR,SR*)-**1u**, 122410-26-8; (*S,S*)-**1u**, 122442-13-1; **2a**, 105129-14-4; **2b**, 122442-10-8; **2c**, 105182-19-2; **2c**·(*R*)-PhCH(NH$_2$)CH$_3$, 105182-20-5; **2d**, 122442-11-9; **2d**·(*S*)-PhCH(NH$_2$)CH$_3$, 122442-12-0; **3a**, 105129-09-7; **3b**, 105129-11-1; **3c**, 105226-92-4; **3d**, 122517-32-2; **4**·2Na, 105129-26-8; **4** (free acid), 122410-45-1; **5**·2Na, 105129-22-4; **5** (free acid), 122424-10-6; **6**, 122410-27-9; **6a**, 122442-14-2; **7a**, 122410-37-1; **7b**, 122410-38-2; **7c**, 112442-16-4; **7d**, 122410-39-3; **8**, 105129-28-0; **9**, 105129-50-8; **10**·Na, 122442-17-5; **10** (free acid), 105129-53-1; **11**, 105148-64-9; **12**, 122410-40-6; **12** (*S*-acetyl derivative), 122410-25-7; **13**, 122410-41-7; **13** (*S*-acetyl derivative), 122424-09-2; **14** (R = Et), 105129-29-1; **15**, 122410-29-1; (*R*)-PhCH(NN$_2$)CH$_3$, 3886-69-9; (*S*)-PhCH(NH$_2$)CH$_3$, 2627-86-3; H-β-Ala-OH, 107-95-9; H-Gly-OH, 56-40-6; H$_2$N(CH$_2$)$_3$COOH, 56-12-2; H-Leu-OH, 61-90-5; H-Phe-OH·Na, 16480-57-2; H-Trp-OH·Na, 32450-62-7; H-homoPhe-OH, 943-73-7; H-Cys-(CH$_2$Ph)-OH, 3054-01-1; H-Ser(CH$_2$Ph)-OH, 4726-96-9; 2-H$_2$NC$_6$H$_4$COOH, 118-92-3; 2-H$_2$NC$_6$H$_4$CH$_2$COOH, 3342-78-7; 3-H$_2$NC$_6$H$_4$COOH·Na, 17264-94-7; 4-H$_2$NC$_6$H$_4$COOH, 150-13-0; H$_2$NCH$_2$CH$_2$CN, 151-18-8; H$_2$N(CH$_2$)$_3$OH, 156-87-6; EtOCOCH$_2$COCl, 36239-09-5; H-β-Ala-OCH$_2$Ph·TsOH, 27019-47-2; Cbz-β-Ala-OH, 2304-94-1; AcSCH$_2$CH(CH$_2$Ph)COOH, 91702-98-6; 5-amino-2-hydroxybenzoic acid, 89-57-6; 4-(aminomethyl)-1,2-benzenediol, 37491-68-2; succinic anhydride, 108-30-5; glutaric anhydride, 108-55-4; 2,3-*O*-isopropylidene-D-ribono-1,4-lactone, 30725-00-9; *N*-Cbz-β-alanine 2,3-*O*-isopropylidene-D-ribono-1,4-lactone ester, 122410-24-6; *N*-Cbz-β-alanine 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose ester, 122442-74-4; 3,4-dihydroxybenzylamine hydrobromide, 16290-26-9; 4-hydroxy-3-methoxybenzylamine, 1196-92-5; *trans*-(±)-3,5-dibenzyl-2-pyrrolidinone, 122410-28-0; *trans*-(±)-2,4-diphenethylglutaric anhydride, 122410-46-2; enkephalinase A, 70025-49-9.