Dicarboxylic Acid Dipeptide Neutral Endopeptidase Inhibitors

Gary M. Ksander,* Raj D. Ghai, Reynalda deJesus, Clive G. Diefenbacher, Andrew Yuan, Carol Berry, Yumi Sakane, and Angelo Trapani

Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation, 556 Morris Avenue, Summit, New Jersey 07901

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The synthesis of three series of dicarboxylic acid dipeptide neutral endopeptidase 24.11 (NEP) inhibitors is described. In particular, the amino butyramide **21a** exhibited potent NEP inhibitory activity (IC_{50} = 5.0 nM) *in vitro* and *in vivo*. Blood levels of **21a** were determined using an *ex vivo* method by measuring plasma inhibitory activity in conscious rats, mongrel dogs, and cynomolgus monkeys. Free drug concentrations were 10–1500 times greater than the inhibitory constant for NEP over the course of a 6 h experiment. A good correlation of free drug concentrations was obtained when comparing values determined by the *ex vivo* analysis to those calculated from direct HPLC measurements. Plasma atrial natriuretic factor (exogenous) levels were elevated in rats and dogs after oral administration of **19a**. Urinary volume and urinary sodium excretion were also potentiated in anesthetized dogs treated with **21a**.

Atrial natriuretic factor (ANF)[1] is a potent diuretic, natriuretic, and vasorelaxant hormone. These properties have led many investigators to speculate that this peptide might be effective for treating hypertension, congestive heart failure, and renal diseases.[2] To circumvent the inherent problems with the development of a peptide as a therapeutic agent, several approaches can be taken including the identification of nonpeptidic agonists or agents that affect the clearance of the peptide. It is generally accepted that there are two mechanisms responsible for the clearance of ANF. These are receptor-mediated internalization and degradation by so-called C-receptors[3] and enzymatic hydrolysis.[4] Numerous groups have independently demonstrated that kidney membrane preparations degrade ANF enzymatically. Furthermore inactivation and the loss of biological activity *in vivo*,[5] at least in part, occur via cleavage of the Cys[7]–Phe[8] peptide bond by neutral endopeptidase 24.11[6] (NEP; EC 3.4.24.11). Although the relative importance of NEP in the metabolism of endogenous ANF remains to be determined conclusively, NEP inhibitors have been shown to elicit ANF-like responses in animal models.[7a-g] Despite these encouraging experimental results, recent clinical trials have shown, at best, moderate pharmacologic activity. In these clinical studies[7h-p] several NEP inhibitors including sinorphan, SCH34826, and candoxatril have produced no or modest antihypertensive effects. Somewhat superior, albeit moderate, effects of these agents have been observed in patients with congestive heart failure. Since these poor clinical outcomes may arise from inferior pharmacokinetics or potency, we have sought to identify novel NEP inhibitors with superior pharmacologic properties.

Chemistry

The preparation of racemic unsymmetrical glutaric acids, Table 1, is outlined in Scheme 1. *p*-Phenylbenzaldehyde was converted in successive steps to the oxetane **4** by condensation with dimethyl malonate/NaOMe, hydrogenation, and LAH reduction to the diol followed by monotosylation and cyclization with *n*-butyllithium. The oxetane can be converted to a 2,4-alkyl-substituted γ-hydroxy ester (**5**) after boron trifluoride etherate-catalyzed condensation with various *tert*-butyl ester enolates at −78 to −90 °C. A variety of aliphatic, aryl-substituted, and heterosubstituted ester enolates[8] as well as phosphates readily react with 2-methylene biphenyl oxetane **4** to give diastereomeric mixtures separable by flash chromatography. However, separation at compound **7** was less tedious. The γ-hydroxy ester **5** was oxidized with pyridinium dichromate to give the 2,4-disubstituted mixed glutaric acid ester **6**. Activation of the carboxylic acid and coupling with a protected amino acid ester followed by sodium hydroxide and/or TFA hydrolysis gave the diacids **8**.

The symmetrical glutaramides **10**, Table 2, were prepared[9a] by heating β-alanine or *cis*-4-aminocyclohexanecarboxylic acid in a mixture of methylene chloride/pyridine with the appropriate *trans*-2,4-disubstituted glutaric acid anhydrides, Scheme 2.

Preparation of the chiral amino butyramides **20** (Table 3) is outlined in Scheme 3. *N*-Boc-D-tyrosine methyl ester is converted to the triflate **11** which undergoes Suzuki coupling reaction with phenylboric acid to give **12** in good yield. Conversion of the acid to the hydroxymate followed by lithium aluminum hydride reduction gave the aldehyde **15**. Wittig condensation of aldehyde **15** with (carbethoxyethylidene)triphenylphosphorane gave the olefin **16** as one geometric isomer. In the analogous series, aldehyde **15** was condensed with (carbethoxymethylene)triphenylphosphine leading to compound **21i**. Palladium-catalyzed hydrogenation of **16** gave a 6:1 diastereomeric mixture **17**. The Boc protecting group was removed with HCl and condensed with succinic anhydride affording the mixed acid ester **19**. The diastereomers are readily separated by flash chromatography after treatment of **19** with *N,N*-dimethylforamide di-*tert*-butyl acetal to give the mixed *tert*-butyl/ethyl ester **20**. Removal of the *tert*-butyl group with TFA gave the chiral prodrugs **19** which are readily converted to the chiral diacids **21** with base treatment. Compounds **21e,f** were obtained fol-

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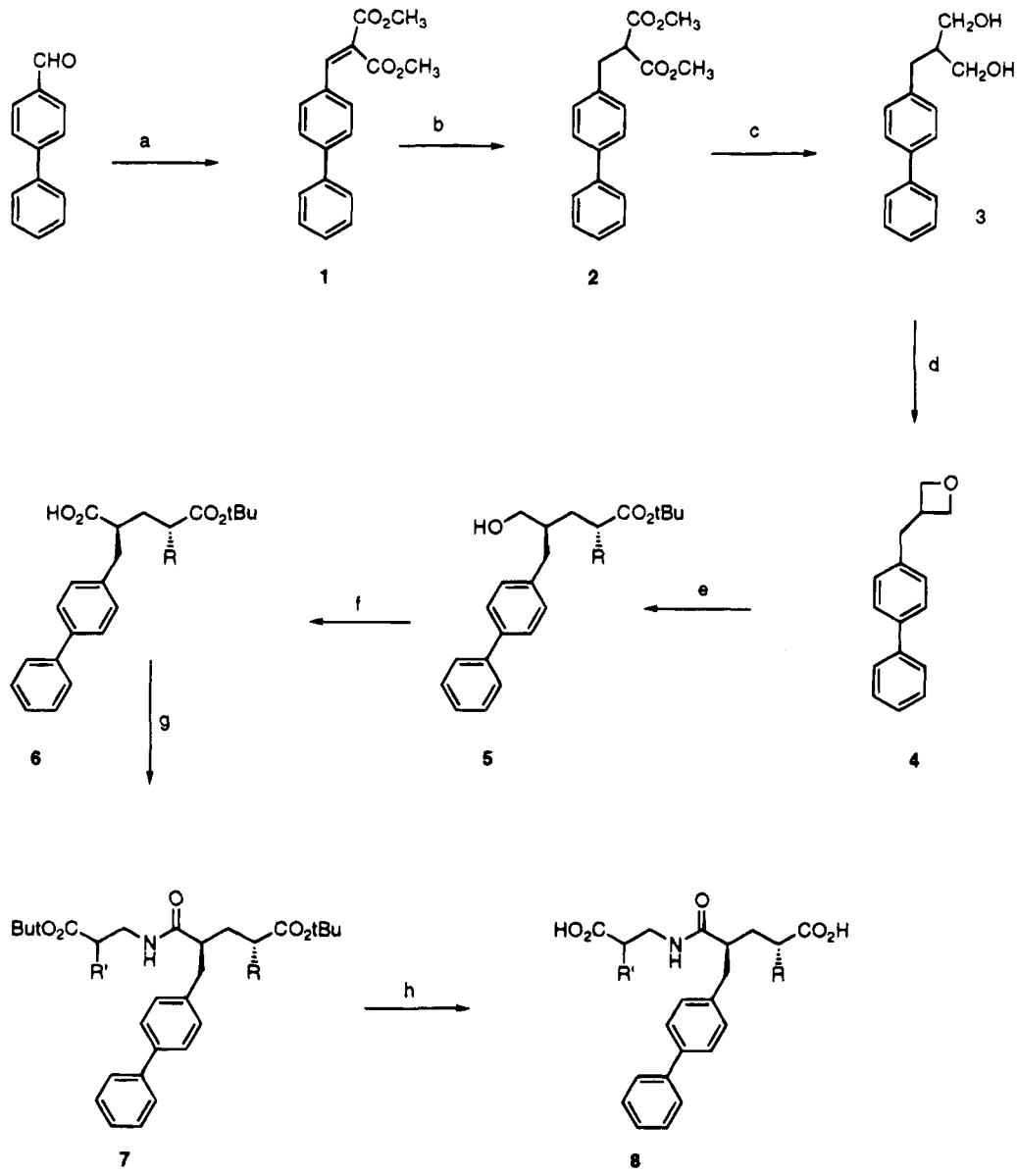
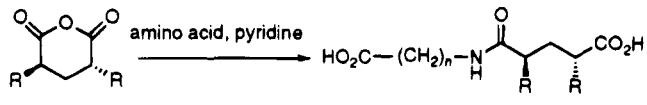
Scheme 1[a]



[a] (a) Dimethyl malonate, piperidine; (b) H_2, Pd/C; (c) $LiBH_4$; (d) TsCl, nBuLi; (e) ester enolate, $BF3OEt_2$; (f) pyridinium dichromate; (g) β-alanine *tert*-butyl ester, HOBT, EDCl; (h) TFA.

Scheme 2



9 10

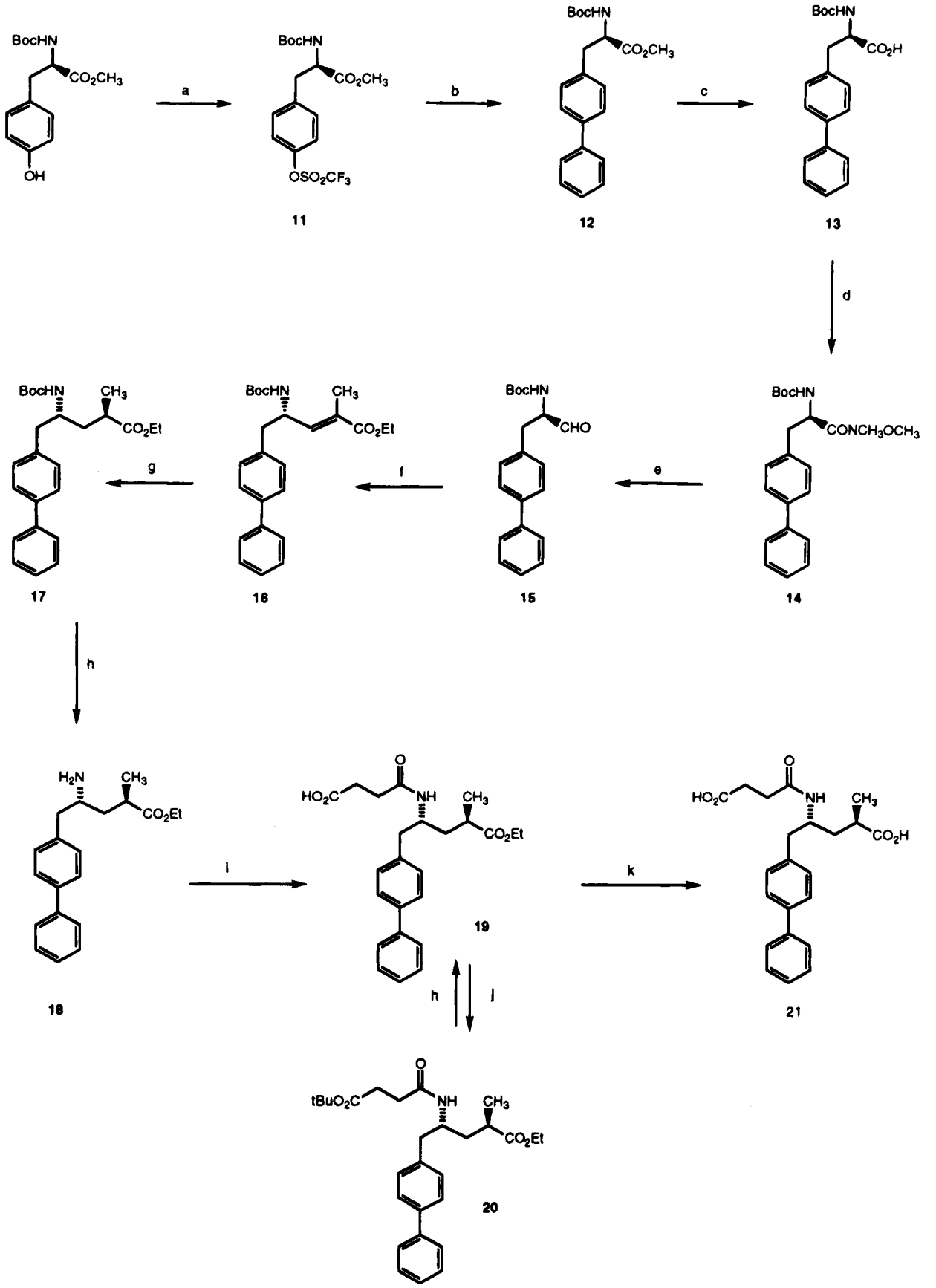
lowing the same sequence of reactions except glutamic and adipic anhydride were substituted for succinic anhydride.

In Vitro Structure–Activity Discussion

A series of glutaramides[9a] were published several years ago describing very potent compounds as assessed by an *in vitro* assay using Leu enkephalin as substrate. Changing to our present assay using the synthetic substrate GAAP, we discovered that the previously potent compounds **10a** and **21j** were no longer active in the nanomolar concentrations, 8 and 2 nM, respectively, but displayed activity in micromolar concentrations. The weak *in vitro* activity was consistent with the poor pharmacology observed with these compounds; however, many factors could lead to this observation. Therefore it was felt that this class of compounds should be reinvestigated.

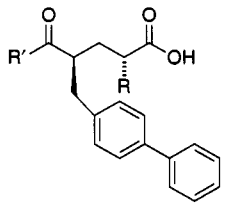
Table 1 lists the series of racemic unsymmetrical glutaramides. The activity of other series of carboxyalkyl dipeptides/glutaric acids inhibitors[7d,9] has been reported previously. The P_1' substituent, biphenylmethyl, remained constant while the P_2' substituent (R') was interchanged between β-alanine and isoserine. The P_1 substituent was modified with alkyl, aralkyl, alkoxy, and arlyoxy groups. Potency improved by a modest 3–5-fold by changing β-alanine to isoserine (**8a–d**). When β-alanine and biphenylmethyl were kept constant, very little change in inhibitory activity was observed between alkyl, aralkyl, alkoxy, and aryloxy P_1 modifications. However, in the absence of a P_1 substituent (**8l**), the activity was decreased approximately 4-fold. The stereochemistry is predictably important. Comparing the two pairs of phenoxy (**8e,f**) and methoxy

Scheme 3[a]



[a] (a) Triflic anhydride; (b) phenylboronic acid, tetrakis(triphenylphosphine)palladium(0); (c) NaOH; (d) $HNCH_3OCH_3$, EDCl, HOBT; (e) $LiAlH_4$; (f) (carbethoxyethylidene)triphenylphosphorane; (g) H_2/Pd/C; (h) HCl; (i) succinic anhydride; (j) *N,N*-dimethylformamide di-*tert*-butyl acetal; (k) NaOH.

Table 1. *In Vitro* NEP Inhibition of Unsymmetrical Glutaramides



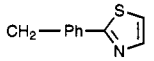
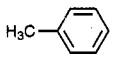
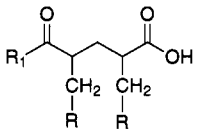
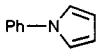
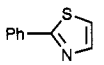
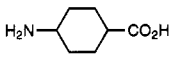
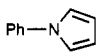
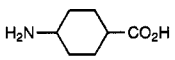
compd	R′	R	IC_{50} (nM)
8a	β-Ala	$CH_{2\text{-}Ph(3,4\text{-}OMe)}$	54
8b	isoserine	CH_2-Ph(3,4-OMe)	11
8c	β-Ala	nBu	66
8d	isoserine	nBu	21
8e	β-Ala	OPh	44
8f	β-Ala	OPh (erythreo)	>1000
8g	β-Ala	OCH_3	42
8h	β-Ala	OCH_3 (erythreo)	>1000
8i	β-Ala		19
8j	β-Ala	$CH_2CH_2OCH_3$	46
8k	β-Ala		54
8l	β-Ala	H	155
8m	β-Ala	CH_3	41

Table 2. *In Vitro* NEP Inhibition of Symmetrical Glutaramides

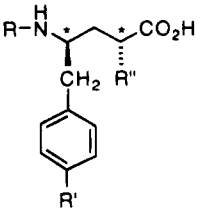


compd	R_1	R	IC_{50} (nM)
10a[9a]	β-Ala	Ph	1200
10b	β-Ala	Ph-Ph	49
10c	β-Ala		36
10d	β-Ala		>1000 (43% at 1μM)
10e	β-Ala		489
10f	β-Ala	Ph-O-Ph	203
10g			515
10h		Ph	52% at 10 μM

(**8g,h**) diastereomers, greater than a 100-fold difference in activity was observed.

Table 2 lists a series of racemic symmetrical glutaramides. The biaryl derivatives **10b,c** (IC_{50} = 49, 36 nM, respectively) were the most potent. This effect is governed entirely by the aryl−aryl P_1' substituent. The chiral β-alanine dibenzyl derivative **10a** is a relatively weak inhibitor in the GAAP assay with an IC_{50} of 1.2 μM. Substituting the monophenyl derivative in the para position with an isopropyl group (**10e**) or a phenoxy substituent (**10f**) improves activity as compared to the parent unsubstituted phenyl derivative **10a**. However, a 2−3-fold decrease in activity is observed when comparing these derivatives to the biphenylmethyl compound **10b**. The phenylthiazole **10d**, theoretically sterically compatible with the P_1' pocket on the basis of the inhibitory activity of **10b,c**, was considerably weaker than other aryl−aryl derivatives. Comparison of **8i**, Table 1 (19 nM), with **10d** (>1 μM) implies that one of the heteroatoms, possibly the nitrogen, is adversely affecting the enzyme interaction in the P_1' pocket.

Table 3. *In Vitro* NEP Inhibition of Amino Butyramides



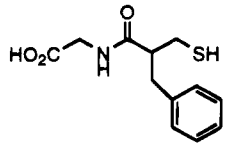
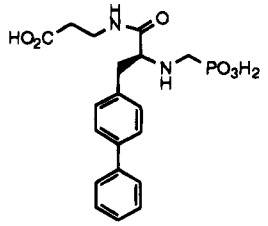
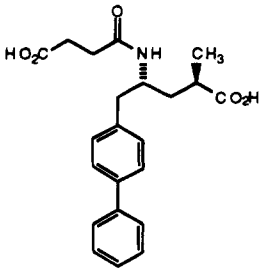
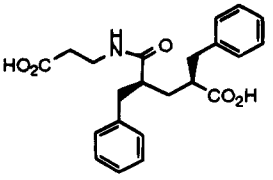
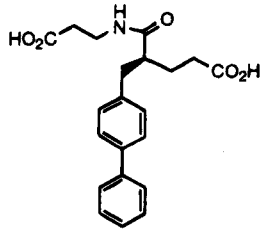
compd	R	R′	R″	IC_{50} (nM)
21a (*R,S* isomer)	$CO(CH_2)_2CO_2H$	Ph	CH_3	5
21b (*S,R* isomer)	$CO(CH_2)_2CO_2H$	Ph	CH_3	190
21c (*R,R* isomer)	$CO(CH_2)_2CO_2H$	Ph	CH_3	700
21d (*S,S* isomer)	$CO(CH_2)_2CO_2H$	Ph	CH_3	27
21e	$CO(CH_2)_3CO_2H$	Ph	CH_3	90
21f	$CO(CH_2)_4CO_2H$	Ph	CH_3	324
21g	$COCH_2CO_2H$	Ph	CH_3	92
21h	$CO(CH_2)_3CO_2H$	Ph	OCH_3	49
21i	$CO(CH_2)_2CO_2H$	Ph	H	99
21j[9a]	$CO(CH_2)_2CO_2H$	H	CH_{2Ph}	4000

The inhibitory activities of the aminobutyramides are compiled in Table 3. On the basis of the data generated in the two previous series (Tables 1 and 2), we assumed aliphatic or aralkyl modifications at the P_1 site would not significantly alter the inhibitory activity. In addition, the P_1' biphenylmethyl substituent should be nearly optimum for this series. Therefore, a limited number of compounds were prepared (Table 3) with P_1 substituents being methyl and methoxy and P_1' substituents as biphenylmethyl, while the P_2' functionality was varied from malonyl to succinyl to butyryl to glutaryl to adipyl acids.

The most active compound was **21a**, IC_{50} = 5 nM. All four diastereomers, *R,S*-**21a**, *S,R*-**21b**, *R,R*-**21c**, and *S,S*-**21d**, were prepared, and IC_{50} values of 5, 190, 700, and 27 nM were determined, respectively. The succinic acid in the P_2' site appears to be optimal since extension of the carboxylic acid chain by one (**21e**) and two (**21f**) methylene units decreased activity 18- and 65-fold. In addition decreasing the chain length by one methylene (**21g**) also showed an 18-fold decrease in activity. Although in series 1 there was no difference in activity between the P_1 methyl and methoxy substituents, a 10-fold change in activity was observed in the aminobutyramide series (**21a,h**). As expected the P_1 desmethyl derivative **21i** was considerably less potent than **21a**.

Comparison of the P_1' benzyl substituent **21j** with other derivatives in Table 3 demonstrates the effects of a P_1' biphenylmethyl group. The biphenyl effect was also evident in the previous examples, shown in Table 2. This effect has been reported in the amino carboxylic acid[9b] and amino phosphonic acid[10] series. However, replacement of a benzyl group with the biphenylmethyl does not always result in a potency increase. In the thiol series,[11a] thiorphan (P_1' = benzyl, IC_{50} = 4 nM) is similar in potency to the biphenylmethyl compound (P_1' = biphenylmethyl, IC_{50} = 3 nM). In addition, a 50-fold decrease in activity has been reported for a thiol sulfonic acid series[11b] with the same P_1' biphenyl modification.

Table 4. Inhibitory Constants of NEP Inhibitors Determined from Different Substrates

Structures	IC_{50} (nM) GAAP	IC_{50} (nM) Leu ENK	IC_{50} (nM) ANF
	4.8	1.5	8.3
	1.6	0.81	2.0
	5.0	0.74	5.3
	1200	9	NT
	155	0.7	NT

Selectivity of these compounds for NEP over other Zn metalloproteases, e.g., stromelysin, endothelin converting enzyme, is not known; however, these compounds were inactive (<50% inhibition) in ACE at a concentration of 10 μM.

***In Vitro* Assays.** Leu enkephalin, glutaryl-Ala-Ala-Phe-β-naphthylamide, and ANF are used as substrates (K_{cat}/K_m = 56, 37, and 18, respectively) to identify inhibitors of NEP. We have compared the potencies of three different classes of NEP inhibitors, thiols, amino phosphonic acid, and carboxylic acid, in these three assays, Table 4. The IC_{50} values determined in these assays for thiorphan (a thiol), an amino phosphonate,[10] and **21a** (a dicarboxylic acid) were similar, although the Leu-ENK assay gave somewhat lower values, and predictive of functional potency *in vivo*. However, the correlation of inhibitory activity between assays was not always constant. Specifically within the dicarboxylic acid series of compounds, for example, **10a** and **81** gave differences in potencies of greater than 2 orders of magnitude when tested against GAAP and Leu-ENK as substrates. A possible explanation could be differences in the kinetics of individual compounds in each assay. Needless to say, the IC_{50} values for the dicarboxylic acid series of compounds vary significantly depending upon the assay used in determining these values and may not be predictive of *in vivo* functional responses.

Pharmacokinetic Profile

A pharmacokinetic profile was determined for each compound with adequate *in vitro* potency. Since pharmacokinetic measurements using HPLC techniques are labor intensive. The plasma concentrations of NEP inhibitors unbound to plasma proteins were determined by *ex vivo* analysis. This method measures the inhibitory activity in plasma and assumes that all activity is produced by the administered substance or from the active substance released via a suitable prodrug. This method does not measure total plasma levels of active substance but only the concentration of free inhibitor (i.e., not bound to plasma proteins). The total plasma concentration of compound can be calculated after the plasma protein binding is determined.

The pharmacokinetic profile of **19a,** the ethyl ester prodrug of **21a**, was determined using the *ex vivo* method by measuring plasma inhibitory activity of **21a** in conscious rats, anesthetized mongrel dogs, and conscious cynomolgus monkeys (Figure 1). In conscious rats, administration of **19a** at 30 mg/kg po produced free plasma levels of the active NEP inhibitor **21a** ranging from 0.64 to 0.05 μM over the course of the 6 h experiment. These values are 10−100 times greater than the inhibitory constant of **21a** (IC_{50} = 5 nM) for NEP. Intraduodenal administration of **19a** at 30 mg/kg in anesthetized mongrel dogs produced plasma levels of **21a** which ranged from 2.8 to 0.08 μM at 90 min and 6 h postdosing. In cynomolgus monkeys, dosing at 30 mg/kg po gave concentration values of 8.51 and 0.21 μM at 1 and 6 h, respectively. These concentrations of free inhibitor **21a** were 1500 and 38 times higher than its IC_{50} value for NEP. The apparent elimination half-life of **21a** was 4.6 ± 0.4 h in cynomolgus monkeys.

The accuracy of the *ex vivo* method was next examined using the monkey blood samples. Total drug levels of the active inhibitor **21a** were determined by HPLC.[15] The values obtained at 30, 60, 120, 180, 240, 300, 360, and 1440 min were 41, 98, 50, 21, 9.0, 4.3, 3.2, and 0.2 μM, respectively. The diacid **21a** was determined to be 94% plasma protein bound, and the free plasma concentrations of **21a** calculated from the HPLC data were in good agreement with those determined by *ex vivo* analysis (Figure 2).

ANF Potentiation Assay. Plasma ANF concentrations were determined in animals infused with exogenous ANF before and after administration of NEP inhibitors. Figure 3 shows the effects of **19a** and (±)-candoxatril[16] administered at 10 mg/kg po on plasma ANF levels in conscious rats. Plasma ANF levels are expressed as a percent of those measured in vehicle-treated animals which received the infusion of exogenous ANF. ANF levels were increased significantly at all time points (30−240 min) after the administration of **19a.** In contrast, the same dose of (±)-candoxatril produced a marked increase in plasma ANF levels initially, but this effect progressively diminished and was not significant 3 h after dosing. **19a** produced dose-

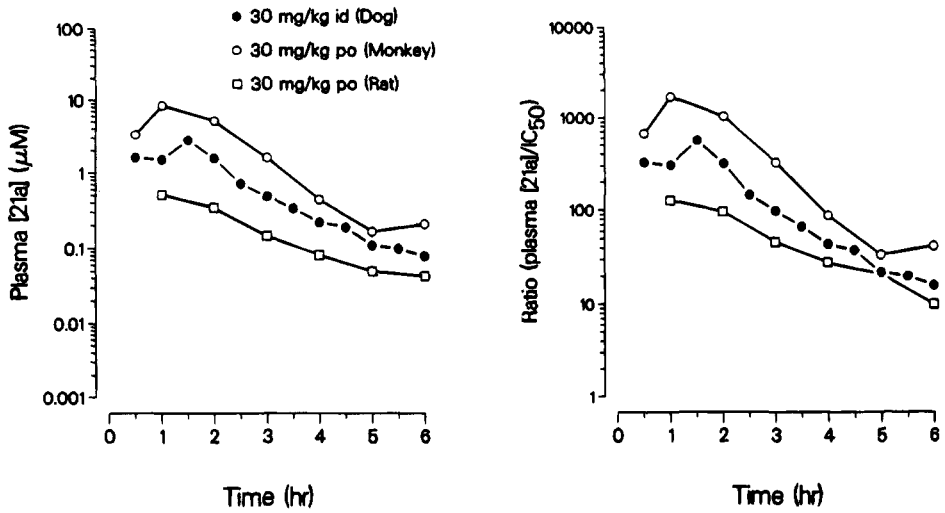


Figure 1. The pharmacokinetics of **21a** after oral administration of **19a** (30 mg/kg po) in conscious DOCA-salt rats, anesthetized dogs, and conscious monkeys (left panel). Plasma levels of **21a** shown in the figure were determined by the *ex vivo* method which measures NEP inhibitor activity and does not directly detect **21a**. The ratio of plasma **21a** concentration/IC_{50} over the course of the experiment is also shown (right panel).

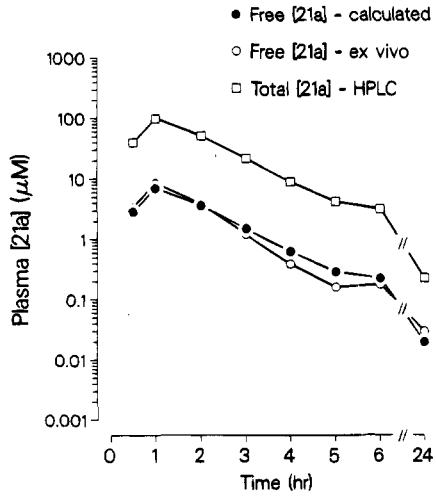


Figure 2. Comparison of free plasma concentrations of **21a** determined by the indirect *ex vivo* method and the value calculated from the total concentration obtained from HPLC measurements. The data were obtained from four cynomolgus monkeys.

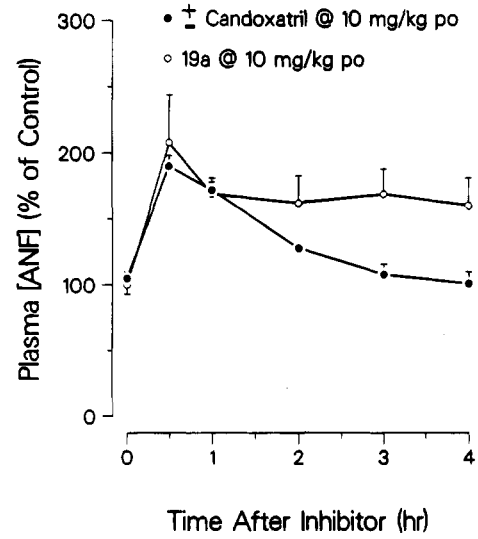


Figure 3. Effect of **19a** and (±)-candoxatril administered orally at 10 mg/kg on plasma ANF concentrations in conscious rats infused with exogenous ANF. Values are the mean ± SEM for six and three rats treated with **19a** and (±)-candoxatril, respectively.

dependent increases in plasma ANF concentrations after intraduodenal administration in anesthetized dogs (Figure 4). At 30 mg/kg id, **19a** increased ANF levels by a maximum of 107%; this effect diminished to 55% by 5 h after dosing. Substantial, albeit smaller, effects of **19a** on plasma ANF levels were observed at lower doses.

ANF-Induced Diuresis and Natriuresis. In anesthetized rats intraduodenally administered **19a** (30 mg/kg) significantly increased ANF-induced natriuresis without affecting diuresis. Prior to the administration of ANF, there were no significant differences in mean arterial pressure, urine flow, or urinary sodium excretion when rats treated with **19a** were compared to controls. In vehicle-treated rats, ANF increased urinary sodium excretion from 0.72 ± 0.25 to 3.26 ± 0.63 μequiv/kg/min. This effect was potentiated in animals which received **19a** ((0.63 ± 0.22)−(8.84 ± 2.13) μequiv/kg/min).

The effects of **21a** (10 mg/kg iv) on ANF-induced diuresis and natriuresis in mongrel dogs is shown in Figure 5. In vehicle-treated dogs, ANF increased urinary sodium excretion from 17.3 ± 3.6 to 199.5 ± 18.4 μequiv/kg/min. This effect was potentiated significantly in animals which received **21a** ((20.8 ± 4.2)−(289.2 ± 28.8) μequiv/kg/min. Urinary volume was also potentiated in animals which received an iv administration of **21a** (control, (0.09 ± .01)−(1.07 ± 0.09) mL; **21a** potentiation, (0.10 ± 0.01)−(1.59 ± 0.21) mL).

In summary, *in vitro* data are presented for three series of NEP inhibitors. The pharmacokinetic profile of **19a/21a** was determined in three species. The prodrug **19a** was also shown to increase exogenous levels of ANF and enhance ANF's natriuretic and diuretic activity. Although these experiments do not prove **21a** will enhance endogenous ANF levels and natriuretic and diuretic activity, it does demonstrate the potential to elicit these activities.

Experimental Section

General Procedures. ^1H NMR spectra were recorded on a Varian XL 400 MHz, Varian VR 300 MHz, and/or Bruker

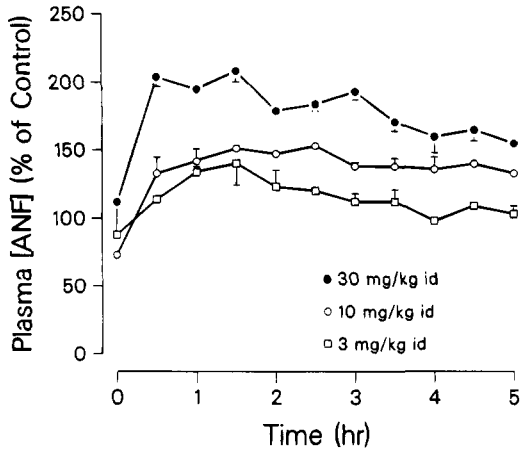


Figure 4. Effect of **19a** on plasma ANF concentrations in anesthetized dogs following intraduodenal administration at 3 (n = 2), 10 (n = 3), and 30 (n = 2) mg/kg. Values are the mean ± SEM.

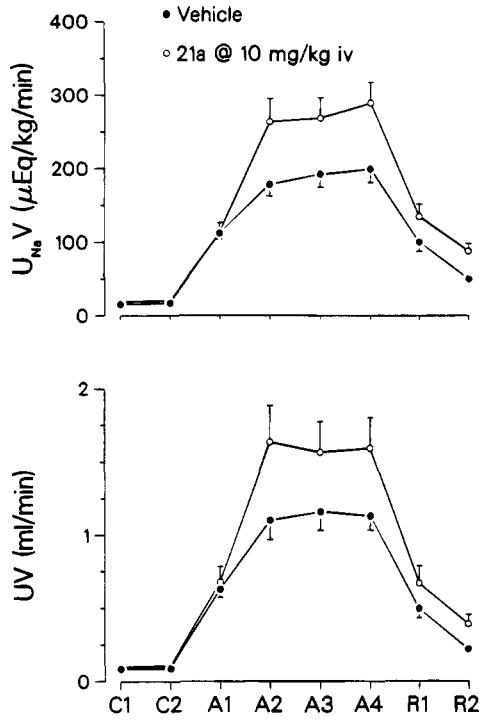


Figure 5. Effect of **21a** administered at 10 mg/kg iv on ANF-induced diuresis and natriuresis in anesthetized mongrel dogs. Values are the mean ± SEM for 11 and 15 dogs treated with **21a** and vehicle, respectively. The experiment consisted of two control periods (C_1, C_2), a period of intrarenal arterial ANF infusion alone (A_1), and three points of ANF infusion with or without **21a** (A_1–A_3) followed by two periods of recovery (termination of ANF infusion; R_1, R_2). Urine volume (UV) and urinary sodium excretion were determined for each of these collection periods.

AC 250 MHz spectrometer with tetramethylsilane as internal standard. Infrared spectra were recorded on a Nicolet 5SXFT spectrometer. 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.

Dimethyl 2-([1,1′-Biphenyl]-4-ylmethylene)-1,3-propanedioate (1). A solution of dimethyl malonate (34.7 g, 0.26 mol), 4-biphenylcarboxaldehyde (30 g, 0.16 mol), benzoic acid (2.0 g, 16 mmol), and piperidine (2.1 g, 25 mmol) was refluxed in 100 mL of toluene. The water was removed azeotropically with a Dean–Stark trap for 1 h. The reaction mixture was cooled, diluted with ether/ethyl acetate, washed with 3 × 1 N HCl, 3 × $NaHCO_3$, and brine, dried ($MgSO_4$), filtered, and concentrated to give 60 g of **1** as a yellow oil. The crude product which contained excess dimethyl malonate was used as is in the next step.

Dimethyl 2-([1,1′-Biphenyl]-4-ylmethyl)-1,3-propanedioate (2). Crude **1** was dissolved in 200 mL of hot THF/CH_3OH and cooled, and 2 g of 5% Pd/C was added. The mixture was hydrogenated at room temperature and pressure for 20 h. The mixture was filtered through a pad of Celite, concentrated, and distilled using a Kugelrohr apparatus. The dimethyl malonate was removed between 100–150 °C, while the product was collected at 180–230 °C. The product was redistilled which solidified upon standing to a sticky solid. The solid was heated; hexanes were added and cooled to 0 °C. The product was collected and dried to give 49.5 g of **2**, mp 72–73 °C: ^{1}H NMR ($CDCl_3$) δ 7.56 (d, 2H), 7.50 (d, 2H), 7.40 (t, 2H), 7.30 (t, 1H), 7.27 (d, 2H), 3.70 (t, 1H), 3.69 (s, 6H), 3.24 (d, 2H).

2-([1,1′-Biphenyl]-4-ylmethyl)-1,3-propanediol (3). To a solution of **2** (29 g, 97 mmol) in 70 mL of THF was added 68 mL of 2.0 M lithium borohydride. TLC indicated the presence of **3** after refluxing for 5 h. Therefore, an additional 20 mL of lithium borohydride was added and refluxing continued another 2 h. The mixture was cooled, poured into ice–water, extracted three times with ether/EtOAc, dried ($MgSO_4$), filtered, concentrated, and slurried with ether. The solid was collected affording 18.9 g of **3** melting at 97–99 °C: ^{1}H NMR ($CDCl_3$) δ 7.6–7.2 (m, 9H), 3.8 (m, 2H), 3.7 (m, 2H), 2.68 (d, 2H), 2.4 (s, 2H), 2.1 (m, 1H).

3-([1,1′-Biphenyl]-4-ylmethyl)oxetane (4). To a 0 °C solution of **3** (19.6 g, 81.0 mmol) in 600 mL of THF was added 32.4 mL of 2.5 M nBuLi. The thick suspension was kept at 0 °C and stirred for 30 min. To this suspension was added tosyl chloride (15.4 g, 81.0 mmol) in 75 mL of THF. The mixture was stirred for 1 h followed by the addition of a second equivalent of nBuLi (32.4 mL, 81.0 mmol). The mixture was heated to 60 °C for 4 h, cooled, poured into ice–water, and extracted three times with ether. The organic extract was washed with brine, dried ($MgSO_4$), concentrated, and chromatographed on silica gel eluting with ether:hexane (1:4) to give 10.1 g of **4** melting at 79–80 °C: ^{1}H NMR ($CDCl_3$) δ 7.57 (d, 2H), 7.52 (d, 2H), 7.42 (1, 2H), 7.34 (t, 1H), 7.2 (d, 2H), 4.82 (dd, 2H), 4.5 (1, 2H), 3.35 (septet, 1H), 3.06 (d, 2H).

1,1-Dimethylethyl (α*R*,γ*S*)-α-Butyl-γ-(hydroxymethyl)-[1,1′-biphenyl]-4-pentanoate (5). A solution of *tert*-butyl hexanoate (3.69 g, 21.4 mmol) in 20 mL of dry THF was added to a −78 °C solution of LDA (21.4 mmol) in 20 mL of THF and stirred for 1 h. The enolate was cooled to −110 °C to which the oxetane **4** (0.96 g, 4.3 mmol) in 20 mL of THF was added. To this mixture was added boron trifluoride etherate (2.91 g, 21 mmol) in 20 mL of THF. The reaction temperature was raised to −40 °C and the mixture stirred for an additional 90 min. The reaction was quenched with 20 mL of 3:1 (THF:HOAc) and the mixture diluted with Et_2O and washed with 2 × H_2O, 2 × 1 N HCl, 2 × $NaHCO_3$, and brine. The organic layer was dried ($MgSO_4$), filtered, concentrated, and chromatographed eluting with ether:hexane (1:4) to give 1.4 g of **5** as a mixture of diastereomers. The diastereomers can be separated by chromatography. However, the separation was less tedious at a later stage (compound **7**).

1,1-Dimethylethyl (α*R*,γ*S*)-α-Butyl-γ-carboxy[1,1′-biphenyl]-4-pentanoate (6). To a solution of pyridinium dichromate (9.0 g, 23.9 mmol) in 125 mL of DMF was added **5** (1.9 g, 4.79 mmol) in 25 mL of DMF, and the mixture was stirred for 28 h. The reaction mixture was poured into 500 mL of water and extracted with ether. The organic layer was washed with 4 × H_20, 2 × $NaHCO_3$, and brine, dried ($MgSO_4$), filtered, and concentrated to give 1.8 g of **6** as a brown oil.

1,1-Dimethylethyl (α*R*,γ*S*)-α-Butyl-γ-[[[3-(phenylmethoxy)-3-oxopropyl]amino]carbonyl][1,1′-biphenyl]-4-pentanoate (7a). To a solution of **6** (0.76 g, 1.85 mmol), β-alanine *tert*-butyl ester hydrochloride (0.673 g, 3.72 mmol), HOBT (0.50 g, 3.72 mmol), and triethylamine (0.47 g, 4.64 mmol) in 8 mL of methylene chloride was added EDCI (0.88 g, 4.6 mmol). The mixture was stirred for 17 h, diluted with 200 mL of ether/ethyl acetate, washed with 1 N HCl, H_2O, $NaHCO_3$, and brine, dried ($MgSO_4$), and concentrated. The mixture was chromato-

graphed on silica gel eluting with hexane:ethyl acetate (9:1) to give 190 mg of the desired diastereomer **7a** as a colorless oil, 300 mg of mixed fractions (mostly the desired diastereomer), and 445 mg of the undesired diastereomer **7b**.

Compound 7a: ^{1}H NMR ($CDCl_3$) δ 7.56 (d, 2H), 7.46 (t, 2H), 7.38 (d, 2H), 7.3 (t, 1H), 7.17 (d, 2H), 6.01 (t, 1H), 3.48 (m, 2H), 2.95 (dd, 1H), 2.7 (dd, 1H), 2.4–2.1 (m, 4H), 1.9–1.6 (m, 2H), 1.4 (s, 9H), 1.3 (s, 9H), 1.2 (m, 6H), 0.86 (t, 3H).

Compound 7b: ^{1}H NMR ($CDCl_3$) δ 7.56 (d, 2H), 7.46 (t, 2H), 7.38 (d, 2H), 7.3 (t, 1H), 7.17 (d, 2H), 5.84 (t, 1H), 3.4 (m, 2H), 2.85 (m, 2H), 2.3 (m, 2H), 2.2–1.9 (m, 2H), 1.7–1.5 (m, 2H), 1.49 (s, 9H), 1.83 (s, 9H), 1.25 (m, 6H), 0.88 (t, 3H).

($\alpha R,\gamma S$)-α-Butyl-γ-[[(2-carboxyethyl)amino]carbonyl]-[1,1′-biphenyl]-4-pentanoic Acid (8c). To a solution of **7** (150 mg, 0.28 mmol) in 2 mL of methylene chloride was added 5 mL of trifluoroacetic acid. The mixture was stirred for 4 h and concentrated, toluene was added, and the mixture was concentrated and solidified when slurried with ether. The solution was collected and dried to give 110 mg of **8c**, mp 151–152 °C: ^{1}H NMR (DMSO-d_6) δ 12.1 (br, 2H), 7.89 (t, 1H), 7.6 (d, 2H), 7.5 (d, 2H), 7.42 (t, 2H), 7.30 (t, 1H), 7.22 (d, 2H), 3.16 (m, 2H), 2.25 (m, 1H), 2.26 (m, 1H), 2.22 (t, 2H), 2.10 (m, 1H), 1.55 (t, 2H), 1.35 (m, 2H), 1.2 (m, 5H), 0.82 (t, 3H). Anal. ($C_{25}H_{31}NO_5$) C,H,N.

The following compounds were prepared similarly.

Compound 8a: mp 144–145 °C; ^{1}H NMR (DMSO-d_6) δ 12.0 (br, 2H), 7.88 (t, 1H), 7.60 (d, 2H), 7.50 (d, 2H), 7.40 (t, 2H), 7.30 (t, 1H), 7.18 (d, 2H), 6.79 (d, 1H), 6.68 (s, 1H), 6.60 (d, 2H), 3.68 (s, 3H), 3.67 (s, 3H), 3.16 (m, 2H), 2.8–2.4 (m, 6H), 2.24 (t, 2H), 1.6 (m, 2H). Anal. ($C_{25}H_{31}NO_5$) C,H,N.

Compound 8b: mp 118–120 °C; ^{1}H NMR (DMSO-d_6) δ 12.3 (br, 2H), 7.92 (t, 1H), 7.63 (d, 2H), 7.52 (d, 2H), 7.41 (t, 2H), 7.30 (t, 1H), 7.18 (d, 2H), 6.79 (d, 1H), 6.68 (s, 1H), 6.60 (d, 2H), 5.35 (br, 1H), 3.98 (m, 1H), 3.68 (s, 3H), 3.67 (s, 3H), 3.2–2.6 (m, 8H), 1.65 (m, 2H). Anal. ($C_{25}H_{31}NO_5$) C,H,N.

Compound 8d: mp 220–230 °C (mixture of diastereomers); ^{1}H NMR (CD_3OD) δ 7.55 (d, 2H), 7.5 (d, 2H), 7.4 (t, 2H), 7.38 (t, 1H), 7.25 (d, 2H), 4.1 (m, 1H), 3.5 (m, 2H), 2.9 (m, 1H), 2.7 (m, 1H), 2.6 (m, 1H), 2.8 (m, 1H), 1.75 (m, 2H), 1.6 (m, 1H), 1.4 (m, 1H), 1.3 (m, 5H), 0.9 (t, 3H). Anal. ($C_{25}H_{29}NNa_2O_6 \cdot 2H_2O$) C,H,N.

Compound 8e: mp 70–75 °C; ^{1}H NMR ($CDCl_3$) δ 7.52 (d, 2H), 7.47 (d, 2H), 7.38 (t, 2H), 7.3–7.1 (m, 5H), 6.95 (t, 1H), 6.84 (d, 2H), 6.1 (t, 1H), 5.6 (H_2O plus exchangeable protons), 4.67 (m, 1H), 3.29 (m, 2H), 2.96 (m, 1H), 2.80 (m, 1H), 2.75 (m, 1H), 2.45 (m, 1H), 2.2 (m, 3H). Anal. ($C_{27}H_{27}NO_6 \cdot 0.75H_2O$) C,H,N.

Compound 8f: mp 75–78 °C; ^{1}H NMR ($CDCl_3$) δ 8.4 (exchangeable protons), 7.50 (d, 2H), 7.49 (d, 2H), 7.35 (t, 2H), 7.3–7.1 (m, 5H), 6.90 (t, 1H), 6.80 (d, 2H), 6.4 (t, 1H), 4.65 (t, 1H), 3.84 (m, 1H), 3.70 (m, 1H), 2.9 (m, 1H), 3.75 (m, 2H), 2.4 (m, 2H), 2.25 (m, 1H), 2.1 (m, 1H). Anal. ($C_{27}H_{27}NO_6$) C,H,N.

Compound 8g: mp 142–146 °C; ^{1}H NMR (DMSO-d_6) δ 12.0 (2H), 8.0 (t, 1H), 7.62 (d, 2H), 7.54 (d, 2H), 7.40 (t, 2H), 7.32 (t, 1H), 7.25 (d, 2H), 3.47 (m, 1H), 3.3 (m, 2H), 3.17 (s, 3H), 2.8 (m, 1H), 2.7 (m, 1H), 2.55 (m, 1H), 2.31 (t, 2H), 1.89 (t, 1H), 1.47 (t, 1H). Anal. ($C_{22}H_{25}NO_6$) C,H,N.

Compound 8h: mp 120–125 °C; ^{1}H NMR (DMSO-d_6) δ 12.0 (br, 2H), 7.86 (t, 1H), 7.62 (d, 2H), 7.54 (d, 2H), 7.40 (t, 2H), 7.30 (t, 1H), 7.2 (d, 2H), 3.65 (m, 1H), 3.20 (s, 3H), 3.15 (m, 2H), 2.9–2.6 (m, 3H), 2.26 (t, 2H), 1.8 (m, 1H), 1.7 (m, 1H). Anal. ($C_{22}H_{25}NO_6$) C,H,N.

Compound 8i: mp >100 °C dec.; ^{1}H NMR (CD_3OD) δ 7.86 (m, 3H), 7.55 (m, 3H), 7.50 (d, 2H), 7.39 (t, 2H), 7.28 (d, 2H), 7.27 (m, 1H), 7.20 (d, 2H), 3.0–2.5 (m, 6H), 2.35 (m, 2H), 1.84 (m, 2H). Anal. ($C_{31}H_{30}N_2O_5S \cdot 1H_2O$) C,H,N.

Compound 8j: mp 160–162 °C; ^{1}H NMR ($CDCl_3$) δ 12.0 (br, 2H), 7.52 (d, 2H), 7.46 (d, 2H), 7.37 (t, 2H), 7.30 (t, 1H), 7.20 (d, 2H), 6.2 (t, 1H), 3.72 (m, 1H), 3.38 (t, 2H), 3.25 (s, 3H), 3.15 (m, 1H), 3.0 (m, 1H), 2.67 (m, 1H), 2.57 (m, 1H), 2.4 (m, 3H), 1.9 (m , 2H), 1.7 (m, 2H). Anal. ($C_{24}H_{29}NO_6$) C,H,N.

Compound 8k: mp 65–70 °C; ^{1}H NMR (DMSO-d_6) δ 12.1 (br, 2H), 7.91 (t, 1H), 7.62 (d, 2H), 7.54 (d, 2H), 7.40 (t, 2H), 7.31 (t, 1H), 7.17 (d, 2H), 3.14 (m, 2H), 2.80 (m, 1H), 2.6 (m, 2H), 2.25 (t, 3H), 1.6 (m, 10H), 1.5–1.0 (5H). Anal. ($C_{28}H_{35}NO_5$) C,H,N.

Compound 8l: mp 165–167 °C; ^{1}H NMR (DMSO-d_6) δ 12.0 (br, 2H), 7.92 (t, 1H), 7.60 (d, 2H), 7.50 (d, 2H), 7.40 (t, 2H), 7.31 (t, 1H), 7.20 (d, 2H), 3.20 (dd, 2H), 2.8 (m, 1H), 2.6 (m, 1H), 2.45 (m, 1H), 2.28 (t, 2H), 2.10 (t, 2H), 1.6 (m, 2H). Anal. ($C_{21}H_{23}NO_5 \cdot 0.5H_2O$) C,H,N.

Compound 8m: mp 59–65 °C; ^{1}H NMR (DMSOd$_6$) δ 12.1 (br, 2H), 7.92 (t, 1H), 7.62 (d, 2H), 7.5 (d, 2H), 7.42 (t, 2H), 7.32 (t, 1H), 7.2 (d, 2H), 3.19 (m, 2H), 2.80 (m, 1H), 2.6 (m, 2H), 2.22 (m, 3H), 1.6 (m, 2H), 1.1 (d, 3H). Anal. ($C_{22}H_{25}NO_5$) C,H,N.

($\alpha R,\gamma R$)-γ-[[(2-Carboxyethyl)amino]carbonyl]-α-([1,1-biphenyl]-4-ylmethyl)[1,1′-biphenyl]-4-pentanoic Acid (10b). A solution of β-alanine (300 mg, 3.37 mmol) and **9b** (150 mg, 0.34 mmol) in 30 mL of methylene chloride:pyridine (1:1) was stirred at room temperature for 2 days. The mixture was concentrated, redissolved in ether:ethyl acetate:toluene (1:1:1), washed with 2 N HCl, 3 × H_2O, and brine, dried ($MgSO_4$), and concentrated. The solid was collected and dried to give 140 mg of **10b**, mp 205–208 °C: ^{1}H NMR (DMSO-d_6) δ 12.1 (br, 2H), 7.88 (t, 1H), 7.61 (d, 2H), 7.53 (d, 2H), 7.40 (t, 2H), 7.31 (t, 1H), 7.21 (d, 2H), 3.2 (m, 2H), 2.7 (m, 3H), 2.6–2.4 (m, 3H), 2.25 (m, 2H), 1.7 (m, 2H). Anal. ($C_{23}H_{33}NO_5$) C,H,N.

The following compounds were prepared similarly.

Compound 10c: mp 201–203 °C; ^{1}H NMR (DMSO-d_6) δ 12.1 (br, 2H), 7.88 (t, 2H), 7.4 (two overlapping doublets, 4H), 7.26 (s, 4H), 7.18 (d, 4H), 6.22 (s, 4H), 3.2 (m, 2H), 2.7 (m, 3H), 2.5 (m, 3H), 2.23 (t, 2H), 1.65 (m, 2H). Anal. ($C_{30}H_{31}N_3O_5$) C,H,N.

Compound 10d:[14] mp 182–188 °C; ^{1}H NMR (DMSO-d_6) δ 12.1 (br, 2H), 7.94 (t, 1H), 7.86 (d, 2H), 7.83 (d, 2H), 7.80 (d, 2H), 7.74 (d, 2H), 7.35 (d, 4H), 3.2 (m, 2H), 2.66 (m, 3H), 2.55 (m, 3H), 2.27 (t, 2H), 1.6 (m, 2H); MS m/e 550 (M + 1).

Compound 10e: mp 141–144 °C; ^{1}H NMR ($CDCl_3$) δ 12.1 (br, 2H), 7.15 (m, 8H), 6.04 (t, 1H), 3.52 (m, 1H), 3.2 (m, 1H), 2.35 (m, 4H), 2.6 (m, 4H), 2.3 (m, 2H), 1.95 (t, 1H), 1.7 (t, 1H), 1.27 (d, 6H), 1.20 (d, 6H). Anal. ($C_{28}H_{37}NO_5$) C,H,N.

Compound 10f: mp 288–290 °C, disodium salt; ^{1}H NMR ($CDCl_3$) δ 7.3 (m, 6H), 7.03 (m, 6H), 6.90 (m, 3H), 6.80 (m, 3H), 6.01 (t, 1H), 3.65 (m, 1H), 3.15 (m, 1H), 2.88 (m, 2H), 2.73 (m, 1H), 2.40 (m, 3H), 2.37 (m, 1H), 2.23 (m, 1H), 1.92 (t, 1H), 1.70 (t, 1H). Anal. ($C_{34}H_{31}NNa_2O_7$) C,H,N.

Compound 10g: mp 215–220 °C, disodium salt; ^{1}H NMR (diacid; $CDCl_3$) δ 12.0 (br, 2H), 7.4–7.1 (m, 10H), 5.39 (d, 1H), 3.9 (m, 1H), 2.9 (m, 2H), 2.8–2.5 (m, 3H), 2.4 (m, 1H), 2.2 (m, 1H), 1.96 (t, 1H), 1.8–1.2 (m, 9H). Anal. ($C_{34}H_{35}N_3Na_2O_5 \cdot H_2O$) C,H,N.

Compound 10h: mp 91–94 °C; ^{1}H NMR (diacid; $CDCl_3$) δ 12.1 (br, 2H), 7.3–7.0 (m, 10H), 5.37 (d, 1H), 3.9 (m, 1H), 2.9 (m, 2H), 2.8–2.6 (m, 4H), 2.4 (m, 1H), 2.2 (m, 1H), 1.95 (t, 1H), 1.8–1.1 (m, 8H). Anal. ($C_{26}H_{31}NO_5$) C,H,N.

Preparation[9a] of Glutaric Acid Anhydrides 9. Compound 9b, (*R,R*)-3,5-Bis([1,1′-biphenyl]-4-ylmethyl)dihydro-2*H*-pyran-2,6(3*H*)-dione: mp 244–246 °C, recrystallized from toluene; ^{1}H NMR ($CDCl_3$) δ 7.4 (m, 14H), 7.03 (d, 4H), 3.30 (dd, 2H), 2.9 (m, 2H), 2.75 (dd, 2H), 1.76 (t, 2H).

Compound 9c: mp 238–240 °C, recrystallized from toluene; ^{1}H NMR ($CDCl_3$) δ 7.3–7.15 (m, 7H), 7.07 (m, 7H), 6.3 (m, 4H), 3.25 (dd, 2H), 2.86 (m, 2H), 2.72 (dd, 2H), 1.72 (t, 2H).

Compound 9e: mp 116–117 °C, recrystallized from toluene; ^{1}H NMR ($CDCl_3$) δ 7.19 (d, 4H), 6.90 (d, 4H), 3.19 (dd, 2H), 2.85 (m, 4H), 2.68 (dd, 2H), 1.7 (t, 2H), 1.22 (d, 12H).

Compound 9f: mp 175–177 °C, recrystallized from toluene; ^{1}H NMR ($CDCl_3$) δ 7.30 (t, 4H), 7.20 (t, 2H), 6.97 (d, 8H), 6.88 (d, 4H), 3.20 (dd, 2H), 2.88 (m, 2H), 2.7 (dd, 2H), 1.7 (t, 2H).

Methyl (*R*)-α-[[(1,1-Dimethylethoxy)carbonyl]amino]-4-[(methylsulfonyl)oxy]benzenepropanoate (11). To a mixture of *N*-tBoc-D-tyrosine methyl ester (9.75 g, 33mmol) in 30 mL of methylene chloride which contained pyridine (6.53 g, 82.54 mmol) at −15 °C was added trifluoromethanesulfonic anhydride (11.17 g, 39.62 mmol). The mixture was stirred for 5 min, the reaction quenched with water (1 × 30 mL), and the mixture washed with 0.5 N NaOH (2 × 30 mL) and 15% citric acid (2 × 30 mL). The organic layer was separated, dried ($MgSO_4$), filtered, and concentrated on a rotary evaporator to

yield 13.61 g (96%) of **11**: ^{1}H NMR ($CDCl_3$) δ 7.22 (s, 4H), 5.0 (d, 1H), 4.6 (m, 1H), 3.70 (s, 3H), 3.17 (dd, 1H), 3.05 (dd, 1H), 1.40 (s, 9H).

Methyl (*R*)-α-[[(1,1-Dimethylethoxy)carbonyl]amino]-[1,1′-biphenyl]-4-propanoate (12). Tetrakis(triphenylphosphine)palladium(0) (1.10 g, 0.955 mmol) was added to a suspension of phenylboronic acid (7.76 g, 63.68 mmol) and potassium carbonate (6.59 g, 47.76 mmol) in 160 mL of toluene. The reaction mixture was degassed and heated to 80 °C before adding a 20 mL toluene solution of **11** (13.61 g, 31.84 mmol). The thick suspension was stirred at 80 °C for 2 h and then filtered through Celite. Ethyl acetate was added and the organic layer washed with 0.5 N NaOH (2 × 100 mL), 15% citric acid (2 × 100 mL), and brine (2 × 100 mL), dried ($MgSO_4$), filtered, and concentrated to give a quantitative yield of **12**, mp 87–89 °C. This material was used without further purification: $[\alpha]^{25}_D$ −54.8° (*c* = 7 in $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.55 (d, 2H), 7.50 (d, 2H), 7.4 (t, 2H), 7.3 (t, 1H), 7.15 (d, 2H), 5.0 (d, 1H), 4.60 (q, 1H), 3.74 (s, 3H), 3.10 (m, 2H), 1.40 (s, 9H).

(*R*)-α-[[(1,1-Dimethylethoxy)carbonyl]amino][1,1′-biphenyl]-4-propanoic Acid (13). To a solution of **12** (11.4 g, 32.07 mmol) in 120 mL of THF was added 39 mL of 1 N NaOH in 39 mL of methanol. The mixture was stirred at ambient temperature until hydrolysis was complete (TLC). The reaction mixture was concentrated and the residue partitioned between ether and water. The aqueous layer was separated, acidified with 1 N HCl, extracted with EtOAc (3 × 100 mL), dried ($MgSO_4$), and evaporated to dryness to yield 10.41 g of **3** (95%): ^{1}H NMR ($CDCl_3$) δ 7.55 (t, 4H), 7.45 (t, 2H), 7.35 (d, 1H), 7.25 (d, 2H), 5.0 (d, 1H), 4.65 (q, 1H), 3.15 (m, 2H), 1.45 (s, 9H).

(*R*)-α-[[(1,1-Dimethylethoxy)carbonyl]amino]-*N*-methoxy-*N*-methyl[1,1′-biphenyl]-4-propanamide (14). The reaction mixture of **13** (10.41 g, 30.53 mmol), *O,N*-dimethylhydroxylamine hydrochloride (3.57 g, 26.62 mmol), triethylamine (3.70 g, 36.34 mmol), 1-ethyl-3-[(3-dimethylamino)propyl]carbodiimide hydrochloride (11.70 g, 61.06 mmol), and hydroxybenzotriazole (6.18 g, 45.80 mmol) in 100 mL of CH_2Cl_2 was stirred at ambient temperature for 16 h. The reaction mixture was concentrated and the residue taken up in EtOAc (100 mL), washed with $NaHCO_3$ (2 × 50 mL), 1 N HCl (2 × 50 mL), and brine (1 × 50 mL), dried ($MgSO_4$), and concentrated to yield 11.4 g of **4** (97%): ^{1}H NMR ($CDCl_3$) δ 7.55 (d, 2H), 7.50 (d, 2H), 7.42 (t, 2H), 7.3 (t, 1H), 7.22 (d, 2H), 5.2 (d, 1H), 4.95 (q, 1H), 3.65 (s, 3H), 3.15 (s, 3H), 3.08 (dd, 1H), 2.88 (dd, 1H), 1.35 (s. 9H).

(*R*)-α-[[(1,1-Dimethylethoxy)carbonyl]amino]-*N*-methoxy-*N*-methyl[1,1′-biphenyl]-4-propanal (15). Lithium aluminum hydride (1.13 g, 29.69 mmol) added to a 500 mL ethereal solution of **4** (11.4 g, 29.69 mmol) at 0 °C and stirrred for 20 min before the reaction was quenched with 50 mL of aqueous solution of $KHSO_4$ (10.9 g, 74.22 mmol). The reaction mixture was shaken with cold 1 N HCl (100 mL). The ethereal layer was separated, washed with brine (100 mL), dried ($MgSO_4$), and concentrated on a rotary evaporator to yield 9.65 g (100%) of **15** melting at 83–85 °C after crystallizing from a *tert*-butyl methyl ether:heptane mixture (5:2): $[\alpha]^{25}_D = -38.45°$ (*c* = 9.9 in $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 9.65 (s, 1H), 7.55 (t, 4H), 7.42 (t, 2H), 7.40 (t, 1H), 7.2 (d, 2H), 5.07 (d, 1H), 4.45 (q, 1H), 3.15 (d, 2H), 1.4 (s, 9H).

Ethyl 5-(4-[1,1-Biphenyl]yl)-4-[[(1,1-dimethylethoxy)carbonyl]amino]-2-methyl-2-pentenoate (16). (Carbethoxyethylidene)triphenylphosphorane (21.52 g, 59.38 mmol) was added to a solution of **15** (9.65 g, 29.69 mmol) in 500 mL of CH_2Cl_2 and stirred at ambient temperature for 16 h. The reaction mixture was concentrated and chromatographed on silica gel eluting with ether:hexane (3:7) to yield 9.45 g of **16** (78%). Recrystallization from a *tert*-butyl methyl ether:heptane (5:2) mixture gave a white solid, mp 125–127 °C: ^{1}H NMR ($CDCl_3$) δ 7.57 (d, 2H), 7.52 (d, 1H), 7.44 (t, 2H), 7.35 (t, 1H), 7.25 (d, 2H), 6.55 (d, 1H), 4.70 (s, 1H), 4.60 (s, 1H), 4.19 (q, 2H), 2.95 (dd, 1H), 2.82 (dd, 1H), 1.75 (s, 3H), 1.40 (s, 9H), 1.30 (t, 3H).

Ethyl (α*R*,γ*S*)-γ-[[(1,1-Dimethylethoxy)carbonyl]amino]-α-methyl[1,1′-biphenyl]-4-pentanoate (17). The unsaturated ester **16** (9.45 g, 23 mmol) was dissolved in 500 mL of ethanol and hydrogenated at 50 psi for 6 h in the presence of 3.0 g of 10% Pd/C. The catalyst was filtered and the filtrate concentrated on a rotary evaporator to yield 9.5 g of a 6:1 threo:erythro mixture of ester **17**: ^{1}H NMR ($CDCl_3$) δ 7.57 (d, 2H), 7.52 (d, 2H), 7.44 (t, 2H), 7.30 (t, 1H), 7.22 (d, 2H), 4.10 (q, 2H), 3.90 (s, 1H), 2.79 (d, 2H), 2.58 (m, 1H), 1.90 (m, 1H), 1.35 (s, 9H), 1.25 (t, 3H), 1.25 (d, 3H).

Ethyl (α*R*,γ*S*)-γ-Amino-α-methyl[1,1′-biphenyl]-4-pentanoate (18). Compound **17** (9.50 g, 23.10 mmol) was dissolved in 100 mL of CH_2Cl_2 and cooled to 0 °C. Hydrogen chloride gas was bubbled into the reaction mixture for 5 min, and then the mixture was allowed to warm to room temperature and stirred for 5 h. The reaction mixture was concentrated on a rotary evaporator to yield 7.61 g of **18** (95%): ^{1}H NMR (DMSO-d_6) δ 8.12 (s, 3H), 7.65 (dd, 4H), 7.46 (t, 2H), 7.35 (dd, 3H), 4.00 (q, 2H), 3.40 (m, 1H), 3.02 (dd, 1H), 2.32 (dd, 1H), 2.70 (m, 1H), 1.87 (m, 1H), 1.56 (m, 1H), 1.15 (t, 3H), 1.1 (dd, 3H).

α-Ethyl (α*R*,γ*S*)-γ-[(3-Carboxy-1-oxopropyl)amino]-α-methyl[1,1′-biphenyl]-4-pentanoate Monosodium Salt (19). Succinic anhydride (3.29 g, 32.9 mmol) was added to a solution of **18** (7.61 g, 21.93 mmol) in 60 mL of methylene chloride and 60 mL of pyridine. The reaction mixture was stirred at ambient temperature for 16 h and then concentrated on a rotary evaporator. The residue was taken up with 100 mL of EtOAc, washed with 1 N HCl (3 × 50 mL) and brine (1 × 50 mL), dried ($MgSO_4$), and evaporated to dryness to yield **19** as a mixture of diastereomers. The crude acid ester was used as is in the next reaction.

α-Ethyl (α*R*,γ*S*)-γ-[[3-(1,1-Dimethylethoxy)-1-oxopropyl]amino]-α-methyl[1,1′-biphenyl]-4-pentanoate (20). *N,N*-Dimethylformamide di-*tert*-butyl acetal (18.43 g, 90.80 mmol) was added to an 80 °C toluene (100 mL) solution of **19** (9.0 g, 21.90 mmol). The reaction mixture was kept at 80 °C for 1 h and then poured into 50 mL of cold 1 N HCl and extracted with ether (3 × 50 mL). The organic layer was dried ($MgSO_4$) and concentrated on a rotary evaporator. The diastereomers were separated through a 75 mm × 8 in. silica gel column using toluene:EtOAc (3:1) eluent to yield 4.71 g of pure 2*R*,4*S* diastereomer **20a** and 3.43 g of mixed fractions containing 70% of **20a.**

20a (2*R*,4*S*): ^{1}H NMR ($CDCl_3$) 7.57 (d, 2H), 7.52 (d, 2H), 7.44 (t, 2H), 7.32 (t, 1H), 7.24 (d, 2H), 5.63 (d, 1H), 4.22 (m, 1H), 4.10 (q, 2H), 2.82 (d, 2H), 2.52 (t, 2H), 2.35 (t, 2H), 1.92 (m, 1H), 1.48 (m, 1H), 1.43 (s, 9H), 1.25 (t, 3H), 1.15 (d, 3H).

20b (2*S*,4*S*): ^{1}H NMR ($CDCl_3$) 7.75 (d, 2H), 7.50 (d, 2H), 7.42 (t, 2H), 7.30 (t, 1H), 7.22 (d, 2H), 5.57 (d, 1H), 4.25 (m, 1H), 4.07 (m, 2H), 2.80 (m, 2H), 2.50 (q, 2H), 2.32 (q, 2H), 1.77 (m, 1H), 1.55 (m, 1H), 1.40 (s, 9H), 1.22 (t, 3H), 1.10 (d, 3H).

α-Ethyl (α*R*,γ*S*)-γ-[(3-Carboxy-1-oxopropyl)amino]-α-methyl[1,1′-biphenyl]-4-pentanoate Monosodium Salt (19a). Hydrogen chloride gas was bubbled through a solution of *tert*-butyl ester **20a** (4.71 g, 10.08 mmol) in 50 mL of methylene chloride for 5 min. The reaction mixture was warmed to room temperature for 16 h and concentrated to dryness to give the 2*R*,4*S* diastereomer **19a** as a foam: ^{1}H NMR ($CDCl_3$) δ 7.55 (d, 2H), 7.50 (d, 2H), 7.42 (t, 2H), 7.30 (t, 1H), 7.22 (d, 2H), 5.80 (d, 1H), 4.23 (m, 1H), 4.10 (q, 2H), 2.85 (d, 2H), 2.65 (t, 2H), 2.55 (m, 1H), 2.40 (t, 2H), 1.92 (m, 1H), 1.50 (m, 1H), 1.25 (t, 3H), 1.14 (d, 3H).

The sodium salt of **19a** (4.15 g, 10.08 mmol) was prepared by stirred the monoacid in 1 N NaOH (10 mL) and THF (50 mL) for 5 min at ambient temperature. The solvents were evaporated, and a CH_2Cl_2/hexane mixture was added to triturate the sodium salt of **19a** (4.09 g, 93%), mp 159–160 °C: $[\alpha]^{25}_D$ −11.41° (*c* = 10.34 in MeOH); ^{1}H NMR (DMSO-d_6) δ 8.45 (d, 1H), 7.64 (d, 2H), 7.56 (d, 2H), 7.42 (t, 2H), 7.31 (t, 1H), 7.22 (d, 2H), 3.98 (q, 2H), 3.88 (m, 1H), 2.67 (t, 2H), 2.46 (m, 1H), 2.15 (m, 4H), 1.74 (m, 1H), 1.35 (m, 1H), 1.12 (t, 3H), 1.05 (d, 3H). Anal. ($C_{24}H_{28}NNaO_5$) C,H,N.

Prepared similarly was the following compound.

Compound 19d: ^{1}H NMR ($CDCl_3$) δ 7.55 (d, 2H), 7.50 (d, 2H), 7.41 (t, 2H), 7.30 (t, 1H), 7.20 (d, 2H), 6.15 (d, 1H), 4.25 (m, 1H), 4.10 (t, 2H), 2.80 (dd, 2H), 2.75 (m, 1H), 2.62 (dd,

2H), 2.45 (t, 2H), 1.85 (m, 1H), 1.60 (m, 1H), 1.22 (t, 3H), 1.12 (d, 3H). Anal. ($C_{24}H_{28}NNaO_5$) C,H,N.

(αR,γS)-γ-[(3-Carboxy-1-oxopropyl)amino]-α-methyl-[1,1'-biphenyl]pentanoic Acid (21a). To the sodium salt of **19a** (0.73 g, 1.68 mM) in 20 mL of THF:EtOH was added 1 N NaOH (5.0 mL, 5.0 mmol). The reaction mixture was stirred overnight and then washed with ether. The aqueous layer was acidified with 1 N HCl, re-extracted with EtOAc (3 × 10 mL), dried ($MgSO_4$), and evaporated to dryness. The solid was recrystallized from ethanol to yield 435 mg of **21a** melting at 165–167 °C: $[\alpha]^{25}_D$ −28.73° (c = 10.1 in MeOH); ^{1}H NMR (DMSO-d_6) δ 12.0 (s, 2H), 7.75 (d, 1H), 7.62 (d, 2H), 7.55 (d, 2H), 7.45 (t, 2H), 7.32 (t, 1H), 7.25 (d, 2H), 4.92 (m, 1H), 2.70 (d, 2H), 2.35 (t, 3H), 2.25 (m, 2H), 1.75 (m, 1H), 1.32 (m, 1H), 1.03 (d, 3H). Anal. ($C_{22}H_{25}NO_5$) C,H,N.

Prepared similarly were the following compounds.

Compound 21b (2S,4R): mp 145–149 °C; $[\alpha]^{25}_D$ +17.40° (c = 5.58 in MeOH); ^{1}H NMR (DMSO-d_6) δ 7.72 (d, 1H), 7.65 (d, 2H), 7.57 (d, 2H), 7.45 (t, 2H), 7.35 (t, 1H), 7.25 (d, 2H), 3.92 (m, 1H), 2.70 (d, 2H), 2.37 (m, 3H), 2.25 (m, 2H), 1.76 (m, 1H), 1.35 (m, 1H), 1.05 (d, 3H). Anal. ($C_{22}H_{25}NO_5$) C,H,N.

Compound 21c (2R,4R): mp 162–165 °C; ^{1}H NMR (DMSO-d_6) δ 7.75 (d, 1H), 7.65 (d, 2H), 7.57 (d, 2H), 7.45 (t, 2H), 7.32 (t, 1H), 7.25 (d, 2H), 4.0 (m, 1H), 2.70 (d, 2H), 2.35 (m, 3H), 2.25 (m, 2H), 1.75 (m, 1H), 1.40 (m, 1H), 0.97 (d, 3H). Anal. ($C_{22}H_{25}NO_5$) C,H,N.

Compound 21d (2S,4S): mp 165–167 °C: ^{1}H NMR (DMSO-d_6) δ 12.0 (s, 2H), 7.85 (d, 1H), 7.65 (d, 2H), 7.57 (d, 2H), 7.45 (t, 2H), 7.35 (t, 1H), 7.25 (d, 2H), 4.02 (m, 1H), 2.70 (d, 2H), 2.37 (m, 3H), 2.25 (m, 2H), 1.75 (m, 1H), 1.40 (m, 1H), 0.98 (d, 3H). Anal. ($C_{22}H_{25}NO_5 \cdot 0.25H_2O$) C,H,N.

Compound 21e: mp 152–155 °C; ^{1}H NMR (DMSO-d_6) δ 12.0 (br, 2H), 7.65 (d, 1H), 7.61 (d, 2H), 7.54 (d, 2H), 7.4 (t, 2H), 7.31 (t, 1H), 7.22 (d, 2H), 3.95 (m, 1H), 2.65 (d, 2H), 2.35 (m, 1H), 2.1 (t, 2H), 2.02 (t, 2H), 1.75 (m, 1H), 1.66 (t, 2H), 1.3 (m, 1H), 1.04 (d, 3H). Anal. ($C_{23}H_{27}NO_5$) C,H,N.

Compound 21f: mp 124–127 °C; ^{1}H NMR (DMSO-d_6) δ 12.0 (br, 2H), 7.78 (d, 1H), 7.6 (d, 2H), 7.5 (d, 2H), 7.4 (t, 2H), 7.3 (t, 1H), 7.2 (d, 2H), 3.95 (m, 1H), 2.68 (d, 2H), 2.35 (m, 1H), 2.12 (t, 2H), 2.0 (t, 2H), 1.77 (m, 1H), 1.4 (m, 1H), 1.02 (d, 3H). Anal. ($C_{24}H_{29}NO_5$) C,H,N.

Compound 21g: mp 160–161 °C; ^{1}H NMR (CD_3OD) δ 7.6–7.5 (m, 4H), 7.4 (t, 2H), 7.25 (m, 3H), 4.2 (m, 1H), 3.2 (s, 2H), 2.8 (d, 2H), 2.55 (m, 1H), 1.9 (m, 1H), 1.5 (m, 1H), 1.15 (d, 3H). Anal. ($C_{21}H_{23}NO_5 \cdot H_2O$) C,H,N.

Compound 21h: mp 180–185 °C; ^{1}H NMR (CD_3OD) δ 7.45 (d, 2H), 7.4 (d, 2H), 7.32 (t, 2H), 7.23 (t, 1H), 7.15 (t, 2H), 6.02 (d, 1H), 4.39 (m, 1H), 3.74 (d, 1H), 3.32 (s, 3H), 2.8–2.2 (m, 6H), 1.8 (m, 2H); MS m/e 400 (M + 1).

Compound 21i: mp 163–166 °C; ^{1}H NMR (DMSO-d_6) δ 12.0 (br, 2H), 7.75 (d, 1H), 7.64 (d, 2H), 7.57 (d, 2H), 7.44 (t, 2H), 7.34 (t, 1H), 7.28 (d, 2H), 3.9 (m, 1H), 2.7 (d, 2H), 2.35 (m, 2H), 2.2 (m, 4H), 1.75 (m, 1H), 1.53 (m, 1H). Anal. ($C_{21}H_{23}NO_5$) C,H,N.

Preparation of Kidney Cortex Homogenate. Kidney cortex homogenates were prepared from male Sprague–Dawley rats, Tac:(SD) (Taconic Farms, Germantown, NY), as follows. The cortex from each kidney was removed and homogenized six times for 15 s in 5 volumes (wt/vol) of 50 mM Tris-HCl buffer, pH 7.4, using a polytron tissue homogenizer at a setting of 7. The homogenate was centrifuged at 1000g for 10 min at 4 °C in a Sorvall RC-5B centrifuge. The supernatant (sup I) was saved, and the particulate fraction was homogenized in 1.5 volumes of Tris buffer and centrifuged again at 1000g for 10 min (sup II). Supernatants I and II were pooled and again centrifuged at 1000g for 10 min. The supernatant was saved and further centrifuged at 30 000g for 60 min at 4 °C. The pellet obtained was resuspended in Tris buffer (2 volumes of original weight of tissue) and stored frozen in 100 μL aliquots at −70 °C. Purified protease 3.4.24.11 was prepared according to the method of Sonnenberg et al.[4a]

Enzyme Assay. The determination of NEP activity was carried out using glutaryl-Ala-Ala-Phe-2-naphthylamide (GAAP) as a substrate. NEP activity was determined in duplicate by the hydrolysis of the substrate using a modified procedure of Orlowski and Wilk.[12] The incubation mixture (total volume of 125 μl) containing 50 μL of membrane homogenate (4.0 μg of protein) and 2.5 μg of aminopeptidase M was added to 25 μL of test compound in 50 mM Tris-HCl buffer (pH 7.4). Reaction mixtures were preincubated for 10 min at 25 °C, and then 50 μL of 1.25 mM GAAP was added to achieve a final concentration of 500 μM. Reaction mixtures were further incubated for 25 min at 25 °C, and then 100 μL of fast garnet was added to terminate the reaction. For color development the reaction mixture was incubated for an additional 30 min at 25 °C. The OD was read at 540 nm. One unit (U) of NEP activity is defined as 1 nmol of 2-naphthylamine released/min at 25 °C at pH 7.4.

NEP activity was determined in duplicates by measuring the disappearance of the substrate ANF using a 3 min RP-HPLC separation. NEP (5.0 μg, 30 μL) was preincubated for 10 min with 50 μl of inhibitor in 50 mM Tris-HCL buffer (pH 7.4), and the reaction was initiated by the addition of 20 μL of 100 μl of r-ANF (final concentration 20 μM). The reaction mixture was further incubated for 10 min at 37 °C and the reaction terminated by the addition of 20 μL of 0.6% TFA. Eighty microliters of the mixture was injected into a RP-HPLC column and analyzed using a C4 cartridge in a 3 min isocratic separation at a flow rate of 2 mL/min. Twenty-five percent buffer B (0.08% TFA in 80% acetonitrile) was used. Buffer A was 0.1% TFA in water. One unit of ANF degrading activity is defined as the hydrolysis of 1 nmol of r-ANF/min at 37 °C at pH 7.4.

NEP activity was determined in duplicates by measuring the disappearance of the substrate Leu-ENK using a 6 min RP-HPLC separation. NEP (30 ng, 40 μL) was preincubated for 10 min with 20 μl of inhibitor in 50 mM Tris-HCL buffer (pH 7.4), and the reaction was initiated by the addition of 20 μL of 125 μL of Leu-ENK (final concentration 25 μM). The reaction mixture was further incubated for 10 min in a total volume of 100 μL at 37 °C and the reaction terminated by the addition of 20 μL of 0.6% TFA. Ninety microliters of the mixture was injected into a RP-HPLC column and analyzed using a C4 cartridge in a 6 min isocratic separation at a flow rate of 1 mL/min. Sixteen percent buffer B (0.09% TFA in 90% acetonitrile) was used. Buffer A was 0.09% TFA in water. One unit of Leu-ENK degrading activity is defined as the hydrolysis of 1 nmol of Leu-ENK/min at 37 °C at pH 7.4.

***Ex Vivo* Pharmacokinetics of NEP Inhibitors.** Pharmacokinetic studies were carried out using DOCA-salt rats. Once sustained hypertension was established,[13] the rats were anesthetized with methoxyfluorane and instrumented with a catheter in the femoral artery to obtain blood samples. The catheter was threaded through a swivel system which enabled the rats to move freely after regaining consciousness. Rats were allowed to recover from this surgical procedure for 24 h and then studied in the conscious, unrestrained state. Arterial blood samples (250 μL) were obtained 10, 30, 60, 120, and 240 min after drug administration at 10 mg/kg iv. Each sample was replaced with an equivalent volume of saline. The blood was collected into a tube containing 25 μL of 27.5 mM EDTA and centrifuged immediately at 13 600g for 1 min. Plasma (140 μl) was placed in a ultrafiltration unit (MW cut off 30 000), and ultrafiltrates were collected following centrifugation at 850g for 25 min at 4 °C. The collecting well of the ultrafiltration unit contained 5 μL of 0.1 N HCl to acidify the ultrafiltrate. The free concentrations of NEP inhibitors were then determined by an *ex vivo* analysis of NEP inhibitory activity present in the ultrafiltrate.

Plasma ANP Assay. Male Sprague–Dawley rats (275–350 g) were anesthetized with ketamine (150 mg/kg)/acepromazine (1.5 mg/kg) im and instrumented as described for the pharmacokinetic studies. In this assay, plasma ANP immunoreactivity (ANP_{ir}) levels were determined in the presence and absence of inhibitors. On the day of the study, all rats were infused continously with ANP(99-126) at 450 ng/kg/min iv for the entire 5 h experiment. Sixty minutes after beginning the infusion, blood samples for base line ANP_{ir} measurements were obtained (time 0), and the rats were randomly divided into groups treated with a NEP inhibitor prodrug (10 mg/kg po) or vehicle (3% cornstarch at 1 mL/kg po). Additional blood samples were taken at 30, 60, 120, 180, and 240 min after

oral administration of the compounds. Plasma concentrations of ANP_{ir} were determined by a specific radioimmunoassay. The plasma was diluted 12.5–25- and 50-fold in buffer containing 50 mM Tris (pH 6.8), 154 mM NaCl, 0.3% bovine serum albumin, and 0.01% EDTA. One hundred microliters of standards [rANP(99-126)] or samples was added to 100 μL of rabbit anti-rANP serum and incubated at 4 °C for 16 h; 10 000 cpm of [^{125}I]rANP was then added to the reaction mixture which was incubated at 4 °C for an additional 24 h. Goat anti-rabbit IgG serum coupled to paramagnetic particles was added to the mixture, and bound [^{125}I]rANP was pelleted by exposing the mixture to an attracting magnet rack. The supernatant was decanted, and the pellets were counted in a γ-counter. All determinations were performed in duplicate.

Renal Studies in Dogs. Male mongrel dogs were anesthetized with sodium pentobarbital (30 mg/kg iv, supplemented as needed) and placed on a warming blanket maintained at 37 °C. The right femoral vein was cannulated for vehicle and drug administration. The ureter of the left kidney was cannulated for the collection of urine, and a hook needle catheter was inserted into the left renal artery for intrarenal arterial infusions of ANF or saline. Following surgery, dogs were allowed to equilibrate for 60 min; at 50 min an infusion of saline (0.5 mL/min ira) was started and continued throughout the experiment. Following equilibration and during each of eight 20 min clearance periods, the volume of urine (UV) was collected and determined. Urine sodium concentrations were measured by flame photometry and urine sodium excretions ($U_{Na}V$) calculated. The first two collection periods (C_1 and C_2) served as controls to establish base line renal parameters. ANF was infused at 3.6 ng/kg/min ira during the next four periods (A_1–A_4). Vehicle (0.25 M $NaHCO_3$) or **21a** (10 mg/kg iv) was administered immediately following the A_1 period. R_1 and R_2 served as the recovery periods; the infusion of ANF was discontinued during these times.

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(14) The bis-thiazole was prepared from the corresponding *p*-bromodibenzyl ester derivative. The aryl bromide was refluxed in THF for 17 h with 10 mol % $Pd(Cl_2)(PPh_3)_2$ and 2.2 equiv of (tributylstannyl)thiazole.

(15) Plasma was acidified to pH 2–3 with 0.1 N HCl and extracted with ethyl acetate. The HPLC aqueous mobile phase A was 0.1 M $NaClO_4$. It was prepared in a 2.0 L flask by dissolving 28.1 g of $NaClO_4 \cdot H_2O$ in 1500 mL of H_2O. To this was added 2.0 mL of 85% H_3PO_4, and the mixture was diluted to volume with H_2O. The organic phase was prepared by adding 200 mL of mobile phase A to 1500 mL of CH_3CN. To this was added 2.0 mL of 85% H_3PO_4 and the solution brought to volume with CH_3CN.

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