Design and Synthesis of an Orally Active Macrocyclic Neutral Endopeptidase 24.11 Inhibitor

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A potent macrocyclic inhibitor of neutral endopeptidase (NEP) 24.11 was designed using a computer model of the active site of thermolysin. This 10-membered ring lactam represents a general mimic for any hydrophobic dipeptide in which the two amino acid side chains bind to an enzyme in a contiguous orientation. The parent 10-membered ring lactam was synthesized and exhibited excellent potency as an NEP 24.11 inhibitor (IC_{50} = 3 nM). In order to improve oral bioavailability, various functionality was attached to the macrocycle. These modifications lead to CGS 25155, an orally active NEP 24.11 inhibitor that slows down the degradation of the cardiac hormone atrial natriuretic factor, producing a lowering of blood pressure in the DOCA-salt rat model of hypertension.

Introduction

Atrial natriuretic factor (ANF) is a cardiac hormone that is released into the circulation in response to changes in intra-cardiac pressure, and it plays an important role in the regulation of electrolyte levels and the suppression of renin and aldosterone secretion.[1] In animal models, as well as clinical studies, infusion of ANF produces rapid natriuresis, diuresis, and lowering of blood pressure.[2] However, because ANF is a 28-amino acid peptide, it is poorly absorbed and rapidly metabolized,[3] thus limiting its utility as a drug for cardiovascular disorders. With the discovery that neutral endopeptidase (NEP) 24.11 in the kidney is the major enzyme responsible for the hydrolysis and inactivation of ANF,[4] a number of groups have been exploring the possibility of potentiating the action of ANF by slowing down its degradation.[5] In this report, we summarize our own efforts to discover orally active inhibitors of NEP 24.11 as a new therapy for hypertension.

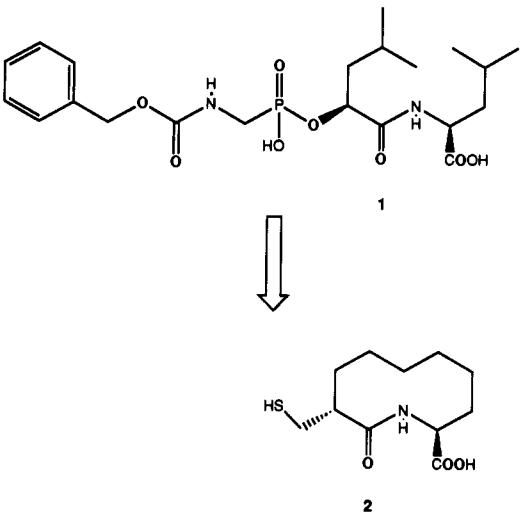

Figure 1. Macrocyclic inhibitor target.

Results and Discussion

Molecular Modeling. Although the 3-dimensional structure of NEP has not been solved, there is a similarity in terms of primary sequence and substrate specificity to the bacterial enzyme, thermolysin,[6] for which there is a good deal of structural data. In fact, several X-ray crystal structures of inhibitor–thermolysin complexes have been published.[7] For this reason, our chemistry strategy was initially guided by studying potential drug candidates in the active site of thermolysin using molecular modeling. Our expectation was that a good "fit" in thermolysin would be predictive of good affinity for NEP.

Important insight was gained by examining Bartlett's transition-state inhibitor 1 (Figure 1) in thermolysin,[7c] which revealed some of the crucial enzyme–inhibitor interactions needed for tight binding. Particularly noteworthy was the contiguous orientation of the two hydrophobic enzyme pockets that accommodated the two leucine side chains. This suggested to us that a novel inhibitor could be assembled in which these side chains were joined. Inclusion of a zinc-binding group, an internal amide bond, and a terminal carboxylic acid optimized the design to yield macrocycle 2. Using molecular modeling,[8] macrocycle 2 was docked into the active site of thermolysin, and a qualitatively good fit was readily apparent. As seen in Figure 2, the hydrophobic pockets of the enzyme make excellent van der Waals contact with the inhibitor, and key hydrogen bonds are aligned quite well. In addition, the conformation of the macrocycle necessary for binding to the enzyme is accessible (~4.5 kcal above the lowest-energy conformation). Since macrocycle 2 met our requirements for a potent inhibitor, its synthesis was undertaken.

Chemistry. The general strategy employed in the synthesis of the 10-membered ring lactam consisted of consecutive ring expansion reactions of readily available cyclooctanone (Scheme I).

Cyclooctane was first ring-expanded, following a literature procedure,[9] with ethyl diazoacetate and boron trifluoride etherate to provide the 9-membered ring β-keto ester 4 after distillation. Compound 4 was then ring-expanded a second time by treatment with sodium azide and methanesulfonic acid[10] to provide the 10-membered ring latam 5. Thus, in two simple operations, the macrocyclic template is produced lacking only the mercaptomethyl functionality.

In order to place substituents α to the amide group, lactam 5 was brominated,[11] providing exclusively the cis

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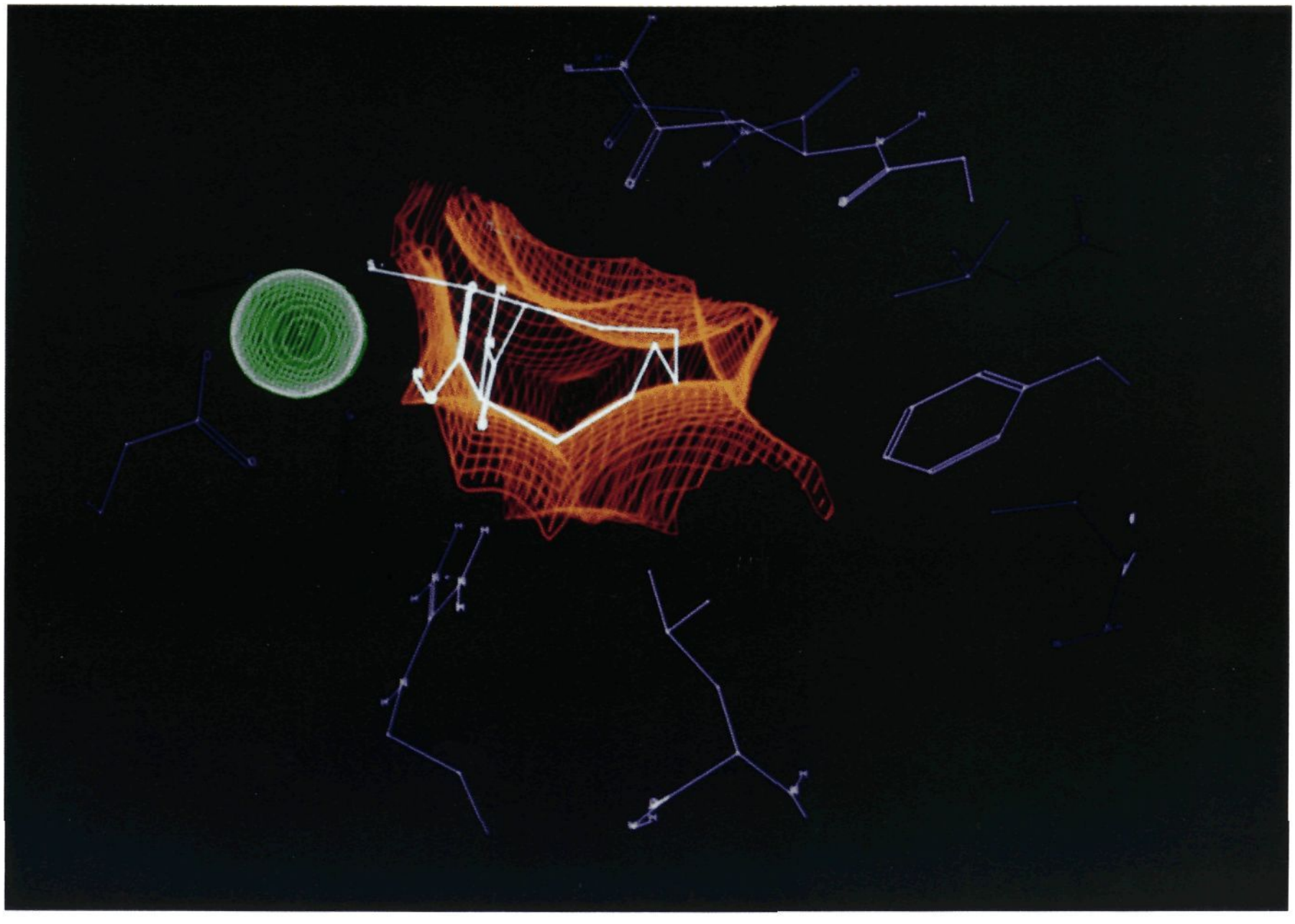


Figure 2. Macrocycle **2** (white) docked into the active site of thermolysin (purple); the zinc atom is in green, and the accessible surface of the active site is shown as an orange mesh. Note that **2** fits perfectly onto the accessible surface, except where a hydrogen bond is made between the amide of the macrocycle and an arginine side chain of thermolysin.

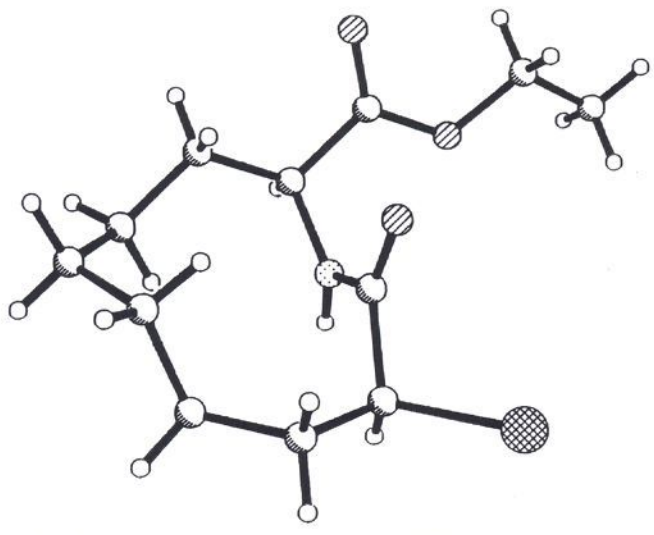


Figure 3. X-ray crystal structure of bromide **6**.

isomer **6**; the stereochemistry of bromide **6** was determined by X-ray crystallography (Figure 3). The formation of only one isomer in this reaction is probably due to selective *peripheral* attack of bromine on the 10-membered ring.[12] The cis stereochemistry turned out to be important, since all attempted S_N2 type displacements of the bromide (e.g. NaN_3 or NaOAc, 140 °C) yielded only recovered starting material. Examination of the X-ray structure suggested that back-side attack on the bromide was blocked by the carbon tether across the ring. For this reason, S_N1 type displacements were tried, and remarkably, treatment of **6** with silver acetate, followed by ethanolysis, yielded very cleanly the hydroxy macrocycle **7** as a single isomer. An

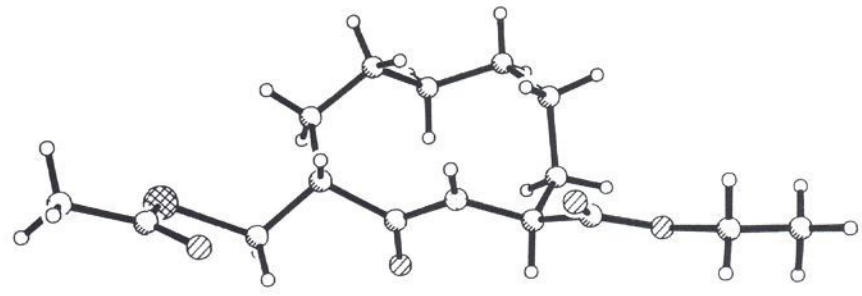
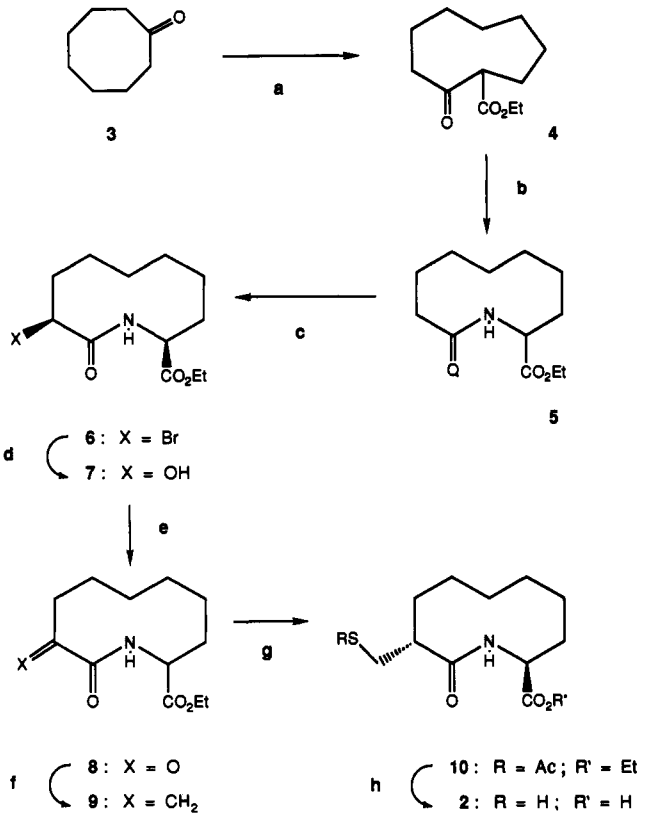


Figure 4. X-ray crystal structure of thioacetate **10**.

X-ray crystal structure of compound **7** showed that the cis isomer had again been formed, suggesting that the approach by acetate to the macrocycle occurred from the same peripheral face as bromine. Alcohol **7** was oxidized to ketone **8**, providing a handle for putting in the *exo*-methylene group. That transformation turned out to be problematical. For instance, a standard Wittig reaction only gave small amounts of impure olefin **9**. However, a two-step Peterson olefination yielded very clean olefin **9**. Stirring this intermediate overnight in thiolacetic acid gave two diastereomeric thioacetates in a 5:1 ratio, and the major isomer **10** was separated by flash chromatography. The *relative* stereochemistry of the major isomer was determined by X-ray crystallography to be trans (Figure 4). Hydrolysis of compound **10** yielded the parent macrocyclic thiol–acid **2**; since the trans isomer of **2** was about 6 times more potent in the enzyme assay than the corresponding cis isomer, all subsequent analogs were synthesized from the major trans-substituted macrocycle.

Scheme I. Synthesis of the Parent Macrocycle[a]



[a] (a) $N_2CH_2CO_2Et$, Et_3OBF_4; (b) NaN_3, $MeSO_3H$; (c) Br_2, PCl_5, I_2; (d) (1) AgOAc; (2) K_2CO_3; (e) PCC; (f) (1) $TMSCH_2MgBr$; (2) BF_3·OEt_2; (g) AcSH; (h) NaOH.

The macrocyclic amides listed in Table I (**12a–i**) were all made by standard EDC/HOBt couplings followed by removal of the protecting groups as outlined in Scheme II.

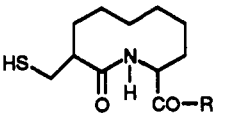
In order to synthesize the individual enantiomers of lead macrocycle **2**, an Evan's chiral oxazolidinone was attached to the carboxylic acid as outlined in Scheme III, and the two chiral diastereomers **13** were separated by flash chromatography. Initially, each isomer had to be carried forward to determine which would lead to the more potent inhibitor; this turned out to be (+)-**13**. Interestingly, the chiral auxiliary could be removed *selectively* by lithium hydroxide, providing the chiral thioacetate (+)-**11**, and allowing easy access to chiral macrocylic amide derivatives, such as hydroxyproline derivative (−)-**12i**. Chiral thiol–acid (+)-**2** was obtained by a second hydrolysis using sodium hydroxide. Chiral thiol–acid (−)-**2** was prepared similarly from (−)-**13**. Prodrug (+)-**14** was made by the standard coupling procedure, and an X-ray crystal structure was solved (Figure 5), establishing the *absolute* stereochemistry of the ring asymmetric centers (as that drawn in the scheme) by correlation to the hydroxyproline asymmetric center.

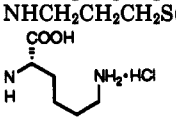
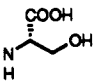
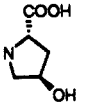
Analysis of in Vitro NEP 24.11 Inhibition. The compounds listed in Table I were evaluated for NEP 24.11 inhibition using a literature procedure.[4a] As predicted, macrocycle **2** is a very potent NEP 24.11 inhibitor, confirming that the 10-membered ring lactam is a good mimic of a dipeptide comprised of two hydrophobic residues that are contiguous in their binding mode.

Analogs **12a–e** lack a terminal carboxylic acid group and are less potent inhibitors. Moreover, the most hydrophobic analog, compound **12b**, is significantly less active than the others. These results are consistent with the notion that the P3′ area of the NEP active site is hydrophilic, possibly extending out into the surrounding surface of water, as is the case in thermolysin.

Amino acids were attached to the macrocycle in order to make analogs (**12f–i**) that retain a terminal carboxylic acid but at the same time contain a side chain that allows for modification of the compound's physical properties.

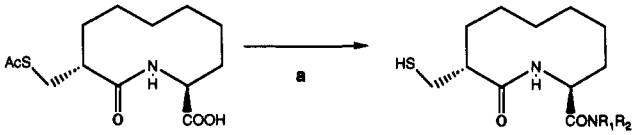
Table I. Inhibition of NEP 24.11 by Macrocycles



compd[a]	R	IC_{50}, (nM)	molecular formula[b]
(±)-**2**	OH	4	$C_{11}H_{19}NO_3S$
(+)-**2**	OH	3	$C_{11}H_{19}NO_3S$
(−)-**2**	OH	>1000	$C_{11}H_{19}NO_3S$
12a	NH(3-pyridyl)·HCl	59	$C_{16}H_{24}ClN_3O_2S$
12b	NH(phenyl)	551	$C_{17}H_{24}N_2O_2S$
12c	$NHCH_2CH_2OCH_2CH_2OH$	84	$C_{15}H_{28}N_2O_4$
12d	$NHCH_2CH_2CH_2OH$	209	$C_{14}H_{26}N_2O_3S$
12e	$NHCH_2CH_2CH_2S(O)_2CH_3$	63	$C_{15}H_{28}N_2O_4S_2$[c]
12f		14	$C_{17}H_{32}ClN_3O_4S$·$0.5CH_2Cl_2$
12g		11	$C_{14}H_{24}N_2O_5S$·$0.33CH_2Cl_2$
12h		2	$C_{15}H_{26}N_2O_4S$·$0.67H_2O$
(−)-**12i**		0.8	$C_{16}H_{26}N_2O_5S$

[a] Compounds in this table are racemic (except for (+)-**2**, (−)-**2**, and (−)-**12i**), with trans ring stereochemistry. [b] Analyses for C, H, N were within ±0.4%. [c] H: calcd, 7.69; found 7.14; N: calcd, 7.69; found, 7.16.

Scheme II. Synthesis of Amide Analogs[a]



[a] (a) (1) EDC, HOBt, various amines; (2) removal of protecting groups.

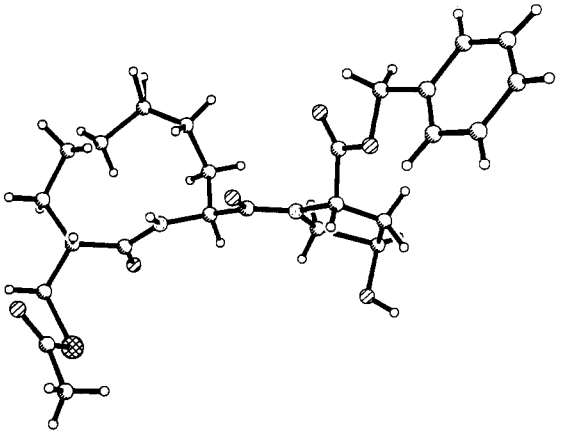
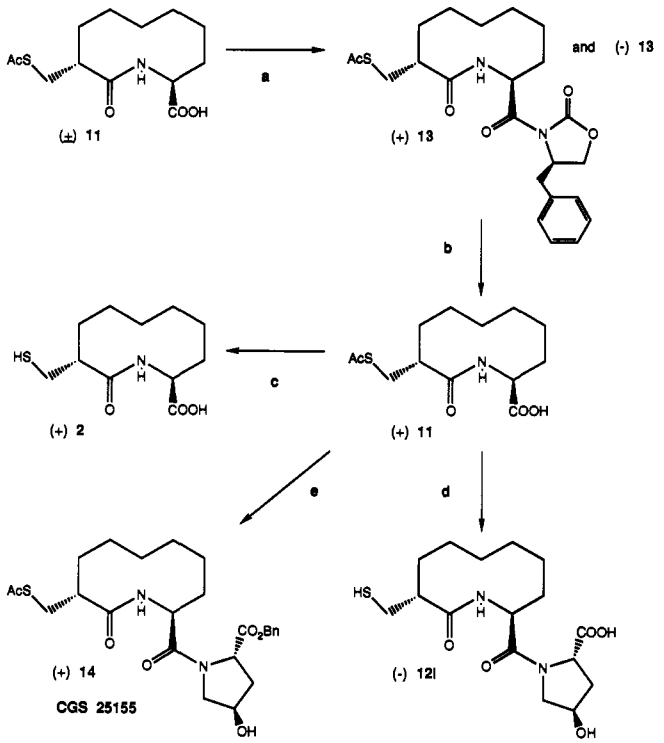


Figure 5. X-ray crystal structure of CGS 25155.

Scheme III. Synthesis of Chiral Macrocycles[a]



[a] (a) Pivaloyl chloride, (*R*)-4-benzyl-2-oxazolidinone anion; (b) LiOH; (c) NaOH; (d) (1) EDC, HOBt, 4-hydroxyproline ethyl ester; (2) NaOH; (e) EDC, HOBt, 4-hydroxyproline benzyl ester.

The conformationally restricted proline and hydroxyproline derivatives exhibit very good activity, and in fact, compound **12i** became the focus of our efforts to optimize the macrocycle series for in vivo efficacy. In addition, compound **12i** is very selective for NEP 24.11; no ACE inhibition is measured at 100 μM.

In Vivo Pharmacology. From the start of this work, an assumption was made that a long-acting, orally active thiol-based NEP inhibitor could be identified only if the thiol functionality was masked as a prodrug. This turned out to be the case, as none of the analogs listed in Table I showed any effect when given orally (30 mg/kg) in the biological assays used in this project to evaluate a compound for ANF-related antihypertensive activity. On the other hand, CGS 25155, a thioacetate–benzyl ester prodrug, demonstrated excellent in vivo activity as described in detail below. Other esters of CGS 25155 such as ethyl or 5-indanyl, combined with the thioacetate, did not show any efficacy in vivo.

The first indication that CGS 25155 had promise as a drug was inferred from its good pharmacokinetics profile, shown in Figure 6. As seen in the graph, persistent and steady plasma levels of the active inhibitor, compound (−)-**12i**, were measured for at least 6 h in rats when the prodrug was given orally at 30 mg/kg. Remarkably, even at the 6-h time point, drug levels exceed 200 times the IC_{50} of the inhibitor. It should be pointed out that drug levels in this experiment are indirectly quantitated, based on an ex vivo enzyme assay of rat plasma at each time point.

CGS 25155 was then studied in a more relevant assay that demonstrates the ability of an NEP inhibitor to affect plasma ANF levels. In this experiment, exogenous ANF is continuously infused in rats at a constant rate, while at the same time compound is administered orally in a single dose. As seen in Figure 7, treatment with CGS 25155 resulted in elevated plasma ANF levels for several hours,

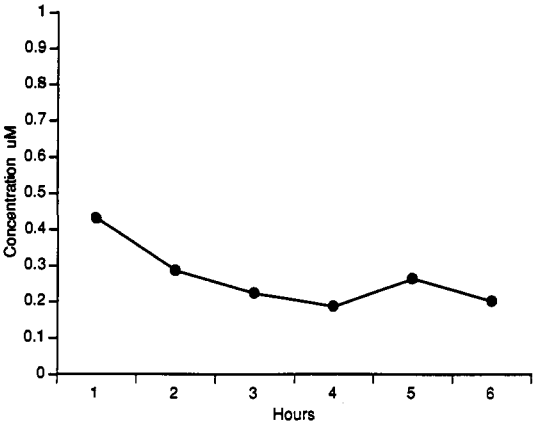


Figure 6. Plasma levels of active inhibitor in conscious rats determined by exvivo NEP assay when CGS 25155 is dosed at 30 mg/kg po. Values at each time point are the average of four animals.

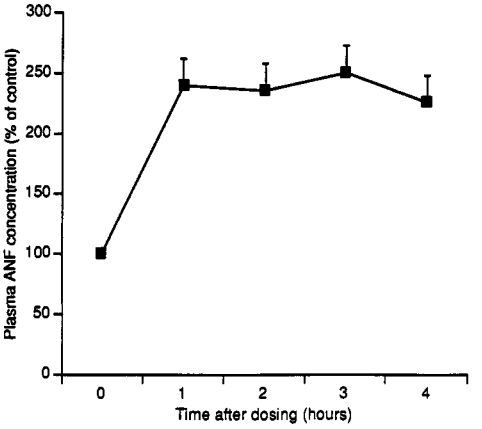


Figure 7. Potentiation of infused ANF in conscious rats due to CGS 25155 dosed at 10 mg/kg po. Plasma ANF values were determined by specific radioimmunoassay and are the average of three animals at each time point.

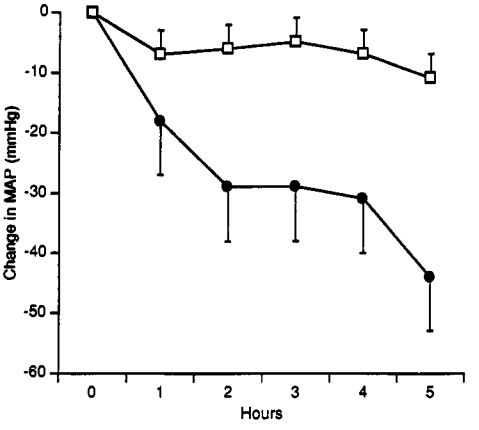


Figure 8. The decrease in mean arterial pressure (MAP) due to CGS 25155 in conscious DOCA-salt hypertensive rats. Compound (solid circles) is dosed at 30 mg/kg po; 3% cornstarch is the control (open squares). Each point is the average of seven animals.

indicating that the inhibitor could prevent the degradation of the hormone in vivo and potentiate its duration of action.

Finally, CGS 25155 was studied in the DOCA-salt rat model of hypertension. As seen in Figure 8, this compound produced a maximum drop in blood pressure of 44 ± 13 mmHg at 5 h. Thus, due to significant pharmacological effects in all of these in vivo assays, CGS 25155 was selected for toxicological studies and clinical development.

Conclusion

Examination of the way known compounds bind to thermolysin leads to a general understanding of exactly what kinds of interactions were important in an inhibitor–metalloprotease complex. On the basis of the assumption that the active site of thermolysin is a reasonable model for the active site of NEP 24.11, a novel macrocycle was designed by molecular modeling with the expectation that it would be a potent NEP inhibitor. Once this template had been identified, analogues were synthesized until one prodrug was optimized for significant antihypertensive activity.

Experimental Section

General. Molecular modeling studies using MacroModel[13] were run on a VAX 8820 computer with an Evans and Sutherland PS 390 color graphics terminal. Solvents and reagents were used as received. Flash chromatography on silica gel was performed with a forced flow of the indicated solvent system on EM Science silica gel 60 (230–400 mesh). ^{1}H NMR spectra were recorded on a Bruker AC-250 (250 MHz) or a Varian XL-300 (300 MHz) in the indicated solvent. Mass spectra were determined on a Hewlett-Packard 5985B quadrupole mass spectrometer using desorption chemical ionization with methane. Melting points were determined on a Hoover capillary melting point apparatus. Optical rotations were determined on a Jasco DIP-370 polarimeter.

2-(Ethoxycarbonyl)cyclononanone (4). Cyclooctanone (25 g, 198 mmol) is dissolved in methylene chloride (500 mL), and the solution is cooled to 0 °C. Triethyloxonium tetrafluoroborate (121.6 g, 640 mmol) is added. Ethyl diazoacete (41.61 g, 365 mmol) is then added dropwise over 25 min, and the reaction is stirred at 0 °C for 4 h. This reaction is quenched by pouring into a solution of sodium bicarbonate (160 g) in water (1.6 L) and stirring overnight. The reaction mixture is then extracted several times with methylene chloride, the combined organic layers are dried (Na_2SO_4), and the solvent is evaporated. The product is purified by vacuum distillation (0.2–1.0 mmHg), and the fraction boiling between 100 and 125 °C is collected to give 42.0 g of the title compound (~100%): NMR (300 MHz, $CDCl_3$) δ 4.1 (m, 2 H), 3.6 (dd, J = 6 Hz, 5 Hz, 1 H), 2.6 (m, 2 H), 2.4 (m, 1 H), 2.1 (m, 2 H), 1.9 (m, 2 H), 1.7 (m, 1 H), 1.5 (m, 6 H), 1.2 (t, J = 7 Hz, 3 H).

Ethyl 2-Oxo-1-azacyclodecane-10-carboxylate (5). Keto ester 4 (13.72 g, 64.7 mmol) is dissolved in chloroform (200 mL) and cooled to 0 °C. Methanesulfonic acid (62.4 g, 650 mmol) is added, followed by sodium azide (12.68 g, 195 mmol). The reaction mixture is stirred at room temperature for 30 min and then heated to reflux for 4 h. The reaction mixture is poured onto ice, made basic with concentrated ammonium hydroxide (pH = 9), and extracted several times with methylene chloride. The combined organic layers are dried ($MgSO_4$), and the solvent is evaporated. The product is purified by silica gel chromatography (40% ethyl acetate/hexane) to give 5.80 g of the title compound (40%): mp 101–103 °C; NMR (300 MHz, $CDCl_3$) δ 6.3 (m, 1 H), 4.7 (m, 1 H), 4.2 (m, 2 H), 2.3 (m, 3 H), 1.7 (m, 6 H), 1.4 (m, 5 H), 1.3 (t, J = 7 Hz, 3 H); MS (DCI, CH_4) m/e 228 (M + 1). Anal. ($C_{12}H_{21}NO_3$) C, H, N.

Ethyl 3-Bromo-2-oxo-1-azacyclodecane-10-carboxylate (6). Lactam 5 (4.0 g, 17.6 mmol) is dissolved in methylene chloride (50 mL) and cooled to 0 °C. Phosphorus pentachloride (3.85 g, 18.5 mmol) and iodine (.051 g, 0.2 mmol) are added. The reaction mixture is stirred for 15 min at 0 °C. Then, bromine (3.10 g, 19.4 mmol) is added, and the reaction is stirred at 0 °C for 4 h. The reaction is quenched by adding saturated sodium sulfate (until the color of excess bromine disappears) and extracted several times with methylene chloride. The combined organic layers are dried (Na_2SO_4), and the solvent is evaporated. The product is purified by silica gel chromatography (15% ethyl acetate/hexane) to give 3.09 g of the title compound (57%): mp 132–134 °C; NMR (300 MHz, $CDCl_3$) δ 7.2 (broad s, 1 H), 4.6 (m, 1 H), 4.35 (dd, J = 7 Hz, 5 Hz, 1 H), 4.2 (m, 2 H), 2.25 (m, 3 H), 1.75 (m, 1 H), 1.5 (m, 8 H), 1.35 (t, J = 7 Hz, 3 H); MS (DCI, CH_4) m/e 306, 308 (M + 1). Anal. ($C_{12}H_{20}BrNO_3$) C, H, N.

Ethyl 3-Hydroxy-2-oxo-1-azacyclodecane-10-carboxylate (7). Bromide 6 (2.96 g, 9.67 mmol) is dissolved in dimethylformamide (30 mL). Silver acetate (6.35 g, 38 mmol) is added, and the reaction is heated to 100 °C for 3 h. After cooling back to room temperature, the reaction mixture is diluted with ethyl acetate (100 mL) and filtered through a pad of silica gel, and the solvent is evaporated to give the crude acetate (9.82 g).

This acetate (9.82 g) is then dissolved in ethanol (150 mL). Potassium carbonate (1.35 g, 9.67 mmol) is added, and the reaction mixture is stirred at room temperature for 4 hours. The reaction mixture is diluted with ethyl acetate (200 mL) and filtered through a pad of silica gel, and the solvent is evaporated to give 2.3 g of the title compound (~100% from 6): mp 93–94 °C; NMR (250 MHz, $CDCl_3$) δ 7.6 (d, J = 6 Hz, 1 H), 4.6 (m, 1 H), 4.3 (dd, J = 3 Hz, 3 Hz, 1 H), 4.2 (m, 2 H), 3.5 (broad s, 1 H), 2.15 (m, 1 H), 1.85 (m, 3 H), 1.4 (m, 8 H), 1.25 (t, J = 7 Hz, 3 H); MS (DCI, CH_4) m/e 244 (M + 1). Anal. ($C_{12}H_{21}NO_4$) C, H; N: calcd, 5.76; found, 5.19.

Ethyl 2,3-Dioxo-1-azacyclodecane-10-carboxylate (8). Alcohol 7 (1.63 g, 6.7 mmol) is dissolved in methylene chloride (70 mL), and pyridinium chlorochromate (2.89, 13.4 mmol) is added. The reaction mixture is stirred at room temperature overnight, diluted with ethyl acetate, and filtered through a pad of florisil. The product is purified by silica gel chromatography (30% ethyl acetate/hexane) to give 1.21 g of the title compound (75%): mp 71–73 °C; NMR (250 MHz, $CDCl_3$) δ 6.0 (d, J = 8 Hz, 1 H), 4.5 (m, 1 H), 4.2 (q, J = 6 Hz, 2 H), 3.2 (dd, J = 7 Hz, 11 Hz, 1 H), 2.2 (m, 1 H), 1.8 (m, 2 H), 1.5 (m, 8 H), 1.3 (t, J = 6 Hz, 3 H); MS (DCI, CH_4) m/e 242 (M + 1). Anal. ($C_{12}H_{19}NO_4$) C, H, N.

Ethyl 3-Methylidene-2-oxo-1-azacyclodecane-10-carboxylate (9). Ketone 8 (5.02 g, 20.81 mmol) is dissolved in ether (50 mL), and the mixture is cooled to 0 °C. [(Trimethylsilyl)methyl]-magnesium chloride (42.0 mL of a 1.0 M solution in ether, 42.0 mmol) is added dropwise, and the reaction mixture is stirred at 0 °C for 20 min and then heated to reflux overnight. The reaction is quenched with saturated ammonium chloride and extracted several times with ethyl acetate. The combined organic layers are dried (Na_2SO_4), and the solvent is evaporated. The product is purified by silica gel chromatography (10% ethyl acetate/hexane) to give separately the two diastereomers of ethyl-3-[(trimethylsilyl)methyl]-3-hydroxy-2-oxo-1-azacyclodecane-10-carboxylate: 2.16 g of the major isomer (31%) [mp 129–130 °C; NMR (250 MHz, $CDCl_3$) δ 7.7 (d, J = 5 Hz, 1 H), 4.5 (m, 1 H), 4.2 (m, 2 H), 2.1 (m, 1 H), 1.7 (m, 2 H), 1.5 (d, J = 16 Hz, 1 H), 1.4 (m, 10 H), 1.3 (t, J = 6 Hz, 3 H), 1.0 (d, J = 16 Hz, 1 H), 0.0 (s, 9 H); MS (DCI, CH_4) m/e 330 (M + 1), 242] and 1.11 g of the minor isomer (16%) [mp 175–177 °C; NMR (250 MHz, $CDCl_3$) δ 6.2 (d, J = 5 Hz, 1 H), 4.65 (m, 1 H), 4.2 (m, 2 H), 2.45 (m, 1 H), 1.8 (m, 2 H), 1.4 (m, 10 H), 1.3 (t, J = 6 Hz, 3 H), 1.15 (d, J = 16 Hz, 1 H), 1.0 (d, J = 16 Hz, 1 H), 0.0 (s, 9 H); MS (DCI, CH_4) m/e 330 (M + 1)].

The silyl alcohol intermediate (0.98 g, 2.98 mmol) is dissolved in methylene chloride (50 mL), and boron triflate etherate (1.8 mL, 15 mmol) is added. The reaction mixture is stirred at room temperature for 26 h. The reaction is quenched with saturated ammonium chloride and extracted several times with methylene chloride. The combined organic layers are dried ($MgSO_4$), and the solvent is evaporated. The product is purified by silica gel chromatography (20% ethyl acetate/hexane) to give 0.52 g of the title compound (73%): mp 91–92 °C; NMR (250 MHz, $CDCl_3$) δ 6.3 (d, J = 4 Hz, 1 H), 5.8 (d, J = 2 Hz, 1 H), 5.3 (d, J = 2 Hz, 1 H), 4.7 (m, 1 H), 4.2 (m, 2 H), 2.45 (dd, J = 5 Hz, 5 Hz, 2 H), 1.5 (m, 10 H), 1.3 (t, J = 7 Hz, 3 H); MS (DCI, CH_4) m/e 240 (M + 1).

Ethyl 3-[(Acetylthio)methyl]-2-oxo-1-azacyclodecane-10-carboxylate (10). Olefin 9 (1.0 g, 4.18 mmol) is dissolved in thiolacetic acid (6 mL). The reaction mixture is stirred at room temperature overnight, and the solvent is evaporated. The product is purified by silica gel chromatography (25% ethyl acetate/hexane) to give separately the two disatereomers of the title compound: 0.80 g of the major (trans) isomer (61%) [mp 115–117 °C; NMR (250 MHz, $CDCl_3$) δ 6.3 (d, J = 6 Hz, 1 H), 4.70 (m, 1 H), 4.2 (m, 2 H), 3.0 (m, 2 H), 2.4 (m, 1 H), 2.3 (s, 3 H), 2.2 (m, 1 H), 1.85 (m, 2 H), 1.6 (m, 2 H), 1.4 (m, 7 H), 1.25

(t, J = 6 Hz, 3 H); MS (DCI, CH_4) m/e 316 (M + 1). Anal. ($C_{15}H_{25}NO_4S$) C, H, N] and 0.16 g of the minor (cis) isomer (12%) [mp 179–180 °C; NMR (250 MHz, $CDCl_3$) δ 6.2 (d, J = 6 Hz, 1 H), 4.55 (m, 1 H), 4.2 (m, 2 H), 3.1 (ddd, 2 H), 2.45 (m, 1 H), 2.3 (s, 3 H), 1.6 (m, 5 H), 1.35 (m, 7 H), 1.25 (t, J = 6.5 Hz, 3 H); MS (DCI, CH_4) m/e 316 (M + 1). Anal. ($C_{15}H_{25}NO_4S$) C, H, N].

3-(Mercaptomethyl)-2-oxo-1-azacyclodecane-10-carboxylic Acid [(±)-2]. The major (trans)thioacetate **10** (1.35 g, 4.29 mmol) is dissolved in nitrogen-degassed ethanol (30 mL). Sodium hydroxide (13.0 mL of a 1.0 M aqueous solution, 13.0 mmol) is also nitrogen-degassed and then added. After the mixture is stirred for 75 min, the reaction is quenched with 1 M hydrochloric acid (15.0 mL), and the solvent is removed. The residue is partitioned between ethyl acetate and water, and the aqueous phase is thoroughly extracted with ethyl acetate. The combined organic layers are dried ($MgSO_4$), and the solvent is evaporated to give 1.05 g of the title compound (~100%): mp 180–185 °C; NMR (250 MHz, CD_3OD) δ 8.2 (d, J = 6 Hz, 1 H), 4.55 (m, 1 H), 2.75 (dd, J = 10 Hz, 12 Hz, 12 Hz, 1 H), 2.55 (m, 1 H), 2.45 (m, 1 H), 2.45 (m, 1 H), 2.15 (m, 1 H), 1.85 (m, 2 H), 1.45 (m, 10 H); MS (DCI, CH_4) m/e 246 (M + 1). Anal. ($C_{11}H_{19}NO_3S$) C, H, N.

3-[(Acetylthio)methyl]-2-oxo-1-azacyclodecane-10-carboxylic Acid [(±)-11]. Thiol (±)-2 (0.75 g, 3.06 mmol) is dissolved in pyridine (20 mL). Acetyl chloride (0.26 g, 3.37 mmol) is added, and the reaction is stirred at room temperature overnight. The reaction is quenched by cooling to 0 °C, adding concentrated hydrochloric acid (20 mL), diluting with water, and extracting several times with ethyl acetate. The combined organic layers are dried ($MgSO_4$), and the solvent is evaporated to give 0.90 g of the title compound (~100%) as an oil: NMR (300 MHz, $CDCl_3$) δ 9.5 (broad s, 1 H), 6.6 (d, J = 5 Hz, 1 H), 4.65 (m, 1 H), 3.0 (m, 2 H), 2.4 (m, 1 H), 2.3 (s, 3 H), 2.2 (m, 1 H), 1.90 (m, 2 H), 1.4 (m, 9 H); MS (DCI, CH_4) m/e 288 (M + 1), 246.

General Procedure for Analogs 12a-e. The procedure used to synthesize these compounds is the same as that described in detail for compound (–)-**12i** below, except that racemic macrocycle (±)-11 is the starting material.

General Procedure for Analogs 12f-h. The procedure used to synthesize these compounds is described below in detail for **12**.

***N*-[[*trans*-3-(Mercaptomethyl)-2-oxo-1-azacyclodec-10-yl]carbonyl]-L-serine (12g).** The thioacetate (±)-11 (1.15 g, 4.0 mmol), 1-hydroxybenzotriazole (0.61 g, 4.0 mmol), 4-methylmorpholine (1.32 mL, 12.0 mmol), and L-serine *tert*-butyl ether *tert*-butyl ester hydrochloride (1.02 g, 4.0 mmol) are all dissolved in methylene chloride (40.0 mL), and the reaction mixture is cooled to 0 °C. To this solution is added *N*-[(dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride (1.54 g, 8.0 mmol), and the reaction mixture is allowed to warm up to room temperature and stir overnight. The solvent is then removed, and the residue is partitioned between ethyl acetate and water. After the aqueous layer is discarded, the organic layer is washed with 2.5 N hydrochloric acid, saturated sodium bicarbonate and brine and dried (Na_2SO_4), and the solvent is evaporated. The product is purified by silica gel chromatography (30% ethyl acetate/hexane) to give 1.03 g of *N*-[[*trans*-3-[(acetylmethyl)thio]-2-oxo-1-azacyclodec-10-yl]carbonyl]-L-serine *tert*-butyl ether *tert*-butyl ester (53%) as a mixture of diastereomers: NMR (300 MHz, $CDCl_3$) δ 6.5 (m, 1 H), 4.55 (m, 2 H), 3.75 (m, 1 H), 3.5 (m, 1 H), 3.0 (m, 2 H), 2.25 (m, 2 H), 2.25 (s, 3 H), 1.85 (m, 1 H), 1.65 (m, 2 H), 1.4 (s, 9 H), 1.3 (m, 9 H), 1.1 (s, 9 H); MS (DCI, CH_4) m/e 487 (M + 1), 431, 375. Anal. ($C_{24}H_{42}N_2O_6S$) C, H, N.

This intermediate (0.87 g, 1.78 mmol) is dissolved in nitrogen-degassed ethanol (18.0 mL). An aqueous solution of 1 N sodium hydroxide (3.6 mL, 3.6 mmol), also nitrogen-degassed, is added, and the reaction mixture is stirred for 90 min. The reaction is quenched with 1 M hydrochloric acid (5.0 mL), and the mixture is partitioned between ethyl acetate and water. The aqueous layer is extracted several times with ethyl acetate, the combined organic layers are dried (Na_2SO_4), and the solvent is evaporated. The product is purified by silica gel chromatography (25% ethyl acetate/hexane) to give 0.66 of *N*-[[*trans*-3-(mercaptomethyl)-2-oxo-1-azacyclodec-10-yl]carbonyl-L-serine *tert*-butyl ether *tert*-butyl ester (83%) as a mixture of diastereomers: NMR (300 MHz, $CDCl_3$) δ 6.6 (d, J = 7 Hz, 2 NH of diastereomer 1), 6.5 (d, J = 7 Hz, NH of diastereomer 2), 6.4 (d, J = 7 Hz, NH of diastereomer 2), 4.60 (m, 2 H), 3.80 (m, 1 H), 3.5 (m, 1 H), 2.9 (m, 1 H), 2.3 (m, 3 H), 1.8 (m, 1 H), 1.5 (s, 9 H), 1.35 (m, 11 H), 1.1 (s, 9 H); MS (DCI, CH_4) m/e 445 (M + 1), 389,333.

This intermediate (0.43 g, 0.97 mmol) is dissolved in methylene chloride (25.0 mL). Anhydrous hydrochloric acid gas from a lecture bottle is bubbled into the solution for 15 min, and the reaction mixture is stirred for 24 h. The solvent is then evaporated to give 0.30 g of the title compound (93%) as a mixture of diastereomers: NMR (300 MHz, CD_3OD) δ 8.4 (d, J = 7 Hz, NH of diastereomer 1), 8.2 (d, J = 7 Hz, NH of diastereomer 1), 8.0 (d, J = 7 Hz, NH of diastereomer 2), 7.9 (d, J = 7 Hz, NH of diastereomer 2), 4.7 (m, 1 H), 4.5 (m, 2 H), 3.9 (m, 1 H), 2.75 (m, 1 H), 2.55 (m, 1 H), 2.45 (m, 1 H), 2.15 (m, 1 H), 1.8 (m, 2 H), 1.4 (m, 10 H); MS (DCI, CH_4) m/e 333 (M + 1), 315, 228. Anal. ($C_{14}H_{24}N_2O_5S \cdot 0.33CH_2Cl_2$) C, H, N.

***(R)*-*N*-[[3(*R*)-[(Acetylthio)methyl]-2-oxo-1-azacyclodec-10(*S*)-yl]carbonyl-4-benzyl-2-oxazolidinone [(+)-13].** The carboxylic acid (±)-11 (2.90 g, 10.1 mmol) is dissolved in tetrahydrofuran (45.0 mL), triethylamine (1.48 mL, 10.2 mmol) is added, and the reaction mixture is cooled to –78 °C. Pivaloyl chloride (1.31 mL, 10.2 mmol) is added, and the reaction mixture is warmed to 10 °C, stirred for 3 h, and then cooled down again to –78 °C. Meanwhile, in a separate flask, (*R*)-4-benzyl-2-oxazolidinone (1.88 g, 10.2 mmol) is dissolved in tetrahydrofuran (35 mL) and cooled to –78 °C, butyllithium (6.63 mL of a 1.6M solution in hexane, 10.2 mmol) is added, and the reaction mixture is stirred at –78 °C for 30 min. This oxazolidinone anion is then cannulated into the first reaction flask, which is stirred at –78 °C for an additional 1 h and then allowed to warm up to room temperature and stir overnight. The reaction is quenched with saturated ammonium chloride, and all of the solvent is evaporated. The residue is partitioned between ethyl acetate and water, the organic layer is washed with 1 N hydrochloric acid and saturated sodium bicarbonate and dried ($MgSO_4$), and the solvent is evaporated. The product is purified by silica gel chromatography (15% ether/40% hexane/45% methylene chloride) to give 1.08 g of the less polar, minor chiral isomer (24%) [$[\alpha]_D$ = –102.90° (c = 7.58 mg/mL, CH_2Cl_2)] and 1.37 g of the more polar, major chiral isomer, the title compound (30%): mp 65–66 °C; NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 3 H), 7.2 (m, 2 H), 6.5 (d, J = 7 Hz, 1 H), 5.75 (m, 1 H), 4.7 (m, 1 H), 4.25 (m, 2 H), 3.2 (dd, J = 9 Hz, 2 Hz, 1 H), 3.0 (m, 2 H), 2.8 (dd, J = 9 Hz, 11 Hz, 1 H), 2.4 (m, 1 H), 2.3 (s, 3 H), 2.2 (m, 1 H), 1.85 (m, 2 H), 1.5 (m, 3 H), 1.35 (m, 6 H); MS (DCI, CH_4) m/e 447 (M + 1), 270; $[\alpha]_D$ = +10.97° (c = 7.52 mg/mL, CH_2Cl_2).

3(*R*)-[(Acetylthio)methyl]-2-oxo-1-azacyclodecane-10(*S*)-carboxylic Acid [(+)-11]. The macrocyclic oxazolidinone (+)-**13** (1.23 g, 2.75 mmol) is dissolved in tetrahydrofuran (45.0 mL) and water (15.0 mL), and the reaction mixture is cooled to 0 °C. Lithium hydroxide hydrate (0.116 g, 2.75 mmol) is added, and the reaction is stirred at 0 °C for 45 min. The solvent is then evaporated, and the residue is partitioned between saturated sodium bicarbonate and methylene chloride. The aqueous layer is extracted several times with methylene chloride to remove the chiral auxiliary, and then the aqueous layer is acidified with 2.5 N hydrochloric acid to pH = 3. The aqueous layer is extracted several times with ethyl acetate, the combined organic layers are dried ($MgSO_4$), and the solvent is evaporated to give 0.620 g of the title compound (79%): mp 144–145 °C; NMR (300 MHz, $CDCl_3$) δ 6.65 (d, J = 5 Hz, 1 H), 5.0 (broad s, 1 H), 4.70 (m, 1 H), 3.0 (d, J = 7 Hz, 2 H), 2.5 (m, 1 H), 2.3 (s, 3 H), 2.2 (m, 1 H), 1.90 (m, 2 H), 1.4 (m, 9 H); MS (DCI, CH_4) m/e 288 (M + 1), 246; $[\alpha]_D$ = +88.36° (c = 6.9 mg/mL, CH_2Cl_2).

3(*R*)-(Mercaptomethyl)-2-oxo-1-azacyclodecane-10(*S*)-carboxylic Acid [(+)-2]. The thioacetate (+)-11 (0.15 g, 0.52 mmol) is dissolved in nitrogen-degassed ethanol (3.5 mL). Nitrogen-degassed sodium hydroxide (1.5 mL of a 1 N aqueous solution, 1.5 mmol) is added, and the reaction mixture is stirred at room temperature for 90 min. The reaction is quenched by adding 1 N hydrochloric acid (1.6 mL), and the solvent is evaporated. The residue is triturated with water, and the precipitate is collected by filtration to give 0.090 g of the title compound (70%): mp 213–215 °C; NMR (300 MHz, CD_3OD) δ 8.2 (d, J = 6 Hz, 1 H), 4.55 (m, 1 H), 2.75 (m, 1 H), 2.55 (m, 1

H), 2.45 (m, 1 H), 2.15 (m, 1 H), 1.85 (m, 2 H), 1.45 (m, 10 H); $[\alpha]_D$ = +48.56° (c = 4.3 mg/mL, MeOH). Anal. ($C_{11}H_{19}NO_3S$) C, H, N.

3(*S*)-(Mercaptomethyl)-2-oxo-1-azacyclodecane-10(*R*)-carboxylic Acid [(+)-2]. In the same manner as described above for (+)-2, the less active enantiomer (-)-2 was synthesized from the less polar chiral oxazolidine (-) 13: mp 220 °C; NMR (300 MHz, CD_3OD) δ 8.2 (d, J = 6 Hz, 1 H), 4.55 (m, 1 H), 2.75 (m, 1 H), 2.55 (m, 1 H), 2.45 (m, 1 H), 2.15 (m, 1 H), 1.85 (m, 2 H), 1.45 (m, 10 H); MS (DCI, CH_4) m/e 246 (M + 1). Anal. ($C_{11}H_{19}NO_3S$) C, H, N.

***trans*-*N*-[[3(*R*)-(Mercaptomethyl)-2-oxo-1-azacyclodec-10(*S*)-yl]carbonyl]-4-hydroxy-L-proline [(-)-12i].** The thioacetate (+)-11 (0.23 g, 0.80 mmol), 1-hydroxybenzotriazole (0.11 g, 0.80 mmol), 4-methylmorpholine (0.22 mL, 2.0 mmol), and L-hydroxyproline ethyl ester hydrochloride (0.16 g, 0.80 mmol) are dissolved in methylene chloride (5.0 mL), and the reaction mixture is cooled to 0 °C. To this solution is added *N*-[(dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride (0.31 g, 1.60 mmol), and the reaction mixture is allowed to warm up to room temperature and stir overnight. The reaction mixture is diluted with more methylene chloride, and the organic layer is washed with 1 N hydrochloric acid and saturated sodium bicarbonate and dried ($MgSO_4$), and the solvent is evaporated. The product is purified by silica gel chromatography (80% ethyl acetate/hexane) to give 0.278 g of *trans*-*N*-[[3(*R*)-[(acetylthio)-methyl]-2-oxo-1-azacyclodec-10(*S*)-yl]carbonyl-4-hydroxy-L-proline ethyl ester (81%): NMR (300 MHz, $CDCl_3$) δ 7.3 (d, J = 7 Hz, 1 H), 4.75 (m, 1 H), 4.60 (dd, J = 7 Hz, 7 Hz, 1 H), 4.5 (m, 1 H), 4.2 (d, J = 4 Hz, 1 H), 4.15 (m, 2 H), 3.80 (d, J = 9 Hz, 1 H), 3.65 (dd, J = 9 Hz, 3 Hz, 1 H), 2.85 (m, 2 H), 2.4 (m, 2 H), 2.25 (s, 3 H), 1.95 (m, 2 H), 1.8 (m, 2 H), 1.55 (m, 1 H), 1.35 (m, 8 H), 1.3 (t, J = 7 Hz, 3 H); $[\alpha]_D$ = +5.73° (c = 4.1 mg/mL, MeOH).

This thioacetate ester (0.137 g, 0.32 mmol) is dissolved in nitrogen-degassed ethanol (2.5 mL). Nitrogen-degassed sodium hydroxide (1.0 mL of a 1 M aqueous solution, 1.0 mmol) is added, and the reaction mixture is stirred at room temperature for 90 min. The reaction is quenched by adding 1 N hydrochloric acid (1.1 mL), and the solvent is evaporated. The residue is partitioned between ethyl acetate and water, the aqueous layer is extracted well with ethyl acetate and dried ($MgSO_4$), and the solvent is evaporated to give 0.090 g of the title compound (78%): mp 128–130 °C; NMR (300 MHz, CD_3OD) δ 8.1 (d, J = 7 Hz, 1 H), 4.80 (m, 1 H), 4.50 (m, 2 H), 3.75 (m, 2 H), 2.70 (m, 1 H), 2.50 (m, 1 H), 2.40 (m, 1 H), 2.30 (m, 1 H), 2.0 (m, 3 H), 1.80 (m, 2 H), 1.5 (m, 4 H), 1.3 (m, 6 H); MS (DCI, CH_4) m/e 359 (M + 1); $[\alpha]_D$ = -50.30° (c = 4.5 mg/mL, MeOH). Anal. ($C_{16}H_{26}N_2O_5S$) C, H, N.

***trans*-*N*-[[3(*R*)-[(Acetylthio)methyl]-2-oxo-1-azacyclodec-10(*S*)-yl]carbonyl]-4-hydroxyl-L-proline Benzyl Ester [(+)-14, CGS 25155].** The thioacetate (+)-11 (0.30 g, 1.04 mmol), 1-hydroxybenzotriazole (0.141 g, 1.04 mmol), 4-methylmorpholine (0.287 mL, 2.60 mmol), and L-hydroxyproline benzyl ester hydrochloride (0.269 g, 1.04 mmol) are dissolved in methylene chloride (22.0 mL), and the reaction mixture is cooled to 0 °C. To this solution is added *N*-[(dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride (0.400 g, 2.08 mmol), and the reaction mixture is allowed to warm up to room temperature and stir overnight. The reaction mixture is diluted with more methylene chloride, the organic layer is washed with 1 N hydrochloric acid and saturated sodium bicarbonate and dried ($MgSO_4$), and the solvent is evaporated. The product is purified by silica gel chromatography (3% methanol/methylene chloride) to give 0.325 g of the title compound (64%): mp 145 °C; NMR (300 MHz, $CDCl_3$) δ 7.35 (broad s, 5 H), 7.0 (d, J = 7 Hz, 1 H), 5.20 (dd, J = 22 Hz, 9 Hz, 2 H), 4.75 (m, 2 H), 4.55 (m, 1 H), 3.75 (m, 2 H), 3.0 (m, 2 H), 2.8 (d, J = 3 Hz, 1 H), 2.4 (m, 2 H), 2.3 (s, 3 H), 2.0 (m, 2 H), 1.9 (m, 1 H), 1.65 (m, 2 H), 1.3 (m, 8 H); MS (DCI, CH_4) m/e 491 (M + 1), 222; $[\alpha]_D$ = +4.36° (c = 8.9 mg/mL, CH_2Cl_2). Anal. ($C_{25}H_{34}N_2O_6S$) C, H, N.

Biological Assays. NEP 24.11 activity was determined by the hydrolysis of the substrate glutaryl-Ala-Ala-Phe-2-naphthylamide using a modified procedure[4a] of Orlowski and Wilk.[14] In brief, the incubation mixture (total volume 125 μL) contained 4.2 μg of protein (rat kidney cortex membranes prepared by the method of Maeda[15]), 50 mM tris buffer, pH 7.4 at 25 °C, 500 μM substrate, various concentrations of inhibitor, and leucine aminopeptidase M (2.5 μg). The mixture was incubated for 10 min at 25 °C, and then 100 μL of fast garnet was added. Enzyme activity was measured spectrophotometrically at 540 nm.

Ex vivo pharmacokinetic studies were conducted using DOCA-salt hypertensive rats. After dosing compound po blood samples were obtained via a catheter in the femoral artery from conscious, unrestrained animals. The blood was collected in EDTA and centrifuged for 1 min in a microfuge. Protein-bound compound was separated from the plasma by centrifugation through an ultrafiltration membrane. The concentration of unbound inhibitor in the plasma ultrafiltrate was then determined (ex vivo) in the standard in vitro NEP assay. It should be emphasized that this assay measures NEP inhibitor activity, not the direct direction of the compound.

For the ANF potentiation assay, rats were infused continuously with ANF at 450 ng/kg/min iv through a catheter in the femoral vein for the duration of the experiment. After baseline plasma ANF levels were established (1 h), rats were dosed with compound, and blood samples were taken from a catheter in the femoral artery at the various time points. Plasma ANF levels were determined by a specific radioimmunoassay, using rabbit anti-rANF serum, [^{125}I]rANF, and goat anti-rabbit IgG serum coupled to paramagnetic particles.

DOCA-salt hypertensive rats were prepared by the standard method. Rats underwent a unilateral nephrectomy, and 1 week later were implanted with silastic pellets containing 100 mg/kg

Table II. Single-Crystal X-ray Crystallographic Analysis of **6**

A. Crystal Parameters	
formula	$C_{12}H_{20}NO_3Br$
formula weight	306.2
crystal size (nm)	0.1 × 0.2 × 0.5
crystal system	triclinic
space group	$P\bar{1}$
molecules/unit cell	2
unit cell dimensions: a, Å	4.9470(15)
b, Å	8.6776(16)
c, Å	16.498(5)
α, deg	95.13(2)
β, deg	93.26(3)
γ, deg	102.32(2)
density (calcd), mg/m^3	1.480
absorption coefficient, mm^{-1}	4.090
B. Refinement Parameters	
system used	Nicolet SHELXTL PLUS
solution	direct methods
refinement method	full-matrix least-squares
number of reflections	1827
observed reflections	1066
wavelength, λ, Å	1.541 84
R index	0.0743

Table III. Single-Crystal X-ray Crystallographic Analysis of **7**

A. Crystal Parameters	
formula	$C_{12}H_{21}NO_4$
formula weight	243.3
crystal size (mm)	0.3 × 0.5 × 0.8
crystal system	monoclinic
space group	$P2_1/a$
molecules/unit cell	4
unit cell dimensions: a, Å	9.974(2)
b, Å	12.7195(16)
c, Å	11.334(2)
β, Å	115.147(12)
density (calc), mm/m^3	1.242
absorption coefficient, mm^{-1}	0.726
B. Refinement Parameters	
system used	Nicolet SHELXTL PLUS
solution	direct methods
refinement method	full-matrix least-squares
number of reflections	1761
observed reflections	1518
wavelength, λ, Å	1.541 84
R index	0.0496

Table IV. Single-Crystal X-ray Crystallographic Analysis of 10

A. Crystal Parameters	
formula	$C_{15}H_{25}NO_4S$
formula weight	315.4
crystal size (mm)	0.05 × 0.07 × 0.7
crystal system	monoclinic
space group	$P2_1$
molecules/unit cell	2
unit cell dimensions: a, Å	11.335(2)
b, Å	4.9181(6)
c, Å	16.163(3)
β, deg	106.009(13)
density (calcd), mg/m^3	1.209
absorption coefficient, mm^{-1}	1.742
B. Refinement Parameters	
system used	Nicolet SHELXTL PLUS
solution	direct methods
refinement method	full-matrix least-squares
number of reflections	1352
observed reflections	1114
wavelength, λ, Å	1.541 84
R index	0.0562

Table V. Single-Crystal X-ray Crystallographic Analysis of CGS 25155

A. Crystal Parameters	
formula	$C_{25}H_{34}N_2O_6S$
formula weight	490.6
crystal size (mm)	0.1 × 0.2 × 0.2
crystal system	orthorhombic
space group	$P2_12_12_1$
molecules/unit cell	8
unit cell dimensions: a, Å	12.583(2)
b, Å	17.870(3)
c, Å	22.559(4)
density (calcd), mg/m^3	1.280
absorption coefficient, mm^{-1}	1.442
B. Refinement Parameters	
system used	Siemens SHELXTL PLUS
solution	direct methods
refinement method	full-matrix least-squares
number of reflections	3877
observed reflections	2394
wavelength, λ, Å	1.541 84
R index	0.0652

of DOCA. The rats were maintained on 1% NaCl/0.2% KCl drinking water for 3–5 weeks until sustained hypertension was established. To evaluate a drug, baseline mean arterial blood pressure was first measured through a catheter in the femoral artery. Compound was then given po, and blood pressure was monitored for the duration of the experiment.

X-ray Structure Determinations. The X-ray structure determinations were performed with a Siemens R3m/v diffractometer and SHELLXTL PLUS software on a MicroVax II computer; see also Tables II–V.

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References

(1) (a) Brenner, B. M.; Ballerman, B. J.; Gunning, M. E.; Zeidel, M. L. Diverse Biological Actions of Atrial Natriuretic Peptide. *Physiol. Rev.* **1990**, *70*, 665–700. (b) Needleman, P.; Blaine, E. H.; Greenwald, J. E.; Michener, M. L.; Saper, C. B. The Biochemical Pharmacology of Atrial Peptides. *Annu. Rev. Pharamcol. Toxicol.* **1989**, *29*, 23–54. (c) Lang, R. E.; Unger, T.; Ganten, D. Atrial Natriuretic Peptide: A New Factor in Blood Pressure Control. *J. Hypertension* **1987**, *5*, 255–278.

(2) (a) Raine, A. E. G.; Firth, J. G.; Ledingham, J. G. G. Renal Actions of Atrial Natriuretic Factor *Clin. Sci.* **1989**, *76*, 1–8. (b) Bussien, J. P.; Biollaz, J.; Waeber, B.; Nussberger, J.; Turini, G. A.; Brunner, H. R.; Brunner-Ferber, F.; Gomez, H. J.; Otterbein, E. S. Dose-Dependent Effect of Atrial Natriuretic Peptide on Blood Pressure, Heart Rate, and Skin Blood Flow of Normal Volunteers. *J. Cardiovasc. Pharmacol.* **1986**, *8*, 216–220.

(3) Yandle, T. G.; Richards, A. M.; Nicholls, M. G.; Cuneo, R.; Espiner, E. A.; Livesey, J. H. Metabolic Clearance Rate and Plasma Half-life of α-Human Atrial Natriuretic Peptide in Man. *Life Sci.* **1986**, *38*, 1827–1833.

(4) (a) Sonnenberg, J. L.; Sakane, Y.; Jeng, A. Y.; Koehn, J. A.; Ansell, J. A.; Wennogle, L. P.; Ghai, R. D. Identification of Protease NEP 24.11 as the Major Atrial Natriuretic Factor Degrading Enzyme in the Rat Kidney. *Peptides* **1988**, *9*, 173–180. (b) Kenny, A. J.; Stephenson, S. L. Role of Endopeptidase 24.11 in the Inactivation of Atrial Natriuretic Peptide. *FEBS Lett.* **1988**, *232*, 1–8.

(5) (a) Roques, B. P.; Beaumont, A. Neutral Endopeptidase-24.11 Inhibitors: from Analgesics to Antihypertensives? *TIPS* **1990**, *11*, 245–249. (b) Schwartz, J. C.; Gros, C.; LeComte, J.-M.; Bralet, J. Enkephalinase (EC 3.4.24.11) Inhibitors: Protection of Endogenous ANF against Inactivation and Potential Therapeutic Applications. *Life Sci.* **1990**, *47*, 1279–1297. (c) Gros, C.; Souque, A.; Schwartz, J. C.; Duchier, J.; Cournot, A.; Baumer, P.; LeComte, J.-M. Protection of Atrial Natriuretic Factor Against Degradation: Diuretic and Natriuretic Responses after *in vivo* Inhibition of Enkephalinase (EC 3.4.24.11) by Acetorphan. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 7580–7584. (d) LeComte, J.-M.; Baumer, P.; Lim, C.; Duchier, J.; Cournot, A.; Dussaule, J. C.; Ardaillou, R.; Gros, C.; Chaignon, B.; Souque, A.; Schwartz, J. C. Stereoselective Protection of Exogenous and Endogenous Atrial Natriuretic Factor by Enkephalinase Inhibitors in Mice and Humans. *Eur. J. Pharmacol.* **1990**, *179*, 65–73. (e) Danilewicz, J. C.; Barclay, P. L.; Barnish, I. T.; Brown, D.; Campbell, S. F.; James, K.; Samuels, G. M. R.; Terrett, N. K.; Wythes, M. J. UK-69,578 a Novel Inhibitor of 3.4.24.11 Which Increases Endogenous ANF Levels and is Natriuretic and Diruetic. *Biochem. Biophys. Res. Commun.* **1989**, *164*, 58–65. (f) Haslanger, M. F.; Sybertz, E. J.; Neustadt, B. R.; Smith, E. M.; Nechuta, T. L.; Berger, J. Carboxyalkyl Dipeptides with Atrial Natriuretic Factor Potentiating and Antihypetensive Activity. *J. Med. Chem.* **1989**, *32*, 737–739.

(6) (a) Benchetrit, T.; Bissery, V.; Mornon, S. P.; Devault, A.; Crine, P.; Roques, B. P. Primary Structure Homologies between Two Zinc Metalloproteases, the Neutral Endopeptidase 24.11 ('Enkephalinase') and Thermolysin, through Clustering Analysis. *Biochemistry* **1988**, *27*, 592–596. (b) Benchetrit, T.; Fournie-Zaluski, M. C.; Roques, B. P. Relationship between the Inhibitory Potencies of Thiorphan and Retrothiorphan Enantiomers on Thermolysin and Neutral Endopeptidase 24.11 and Their Interactions with the Thermolysin Active Site by Computer Modeling. *Biochem. Biophys. Res. Commun.* **1987**, *147*, 1034–1040.

(7) (a) Roderick, S. L.; Fournie-Zaluski, M. C.; Roques, B. P.; Matthews, B. W. *Biochemistry* **1989**, *28*, 1493–1497. (b) Tronrud, D. E.; Holden, H. M.; Matthews, B. W. Structure of Two Thermolysin-Inhibitor Complexes that Differ by a Single Hydrogen Bond. *Science* **1987**, *235*, 571–574. (c) Holden, H. M.; Tronrud, D. E.; Monzingo, A. F.; Weaver, L. H.; Matthews, B. W. Slow- and Fast-Binding Inhibitors of Thermolysin Display Different Modes of Binding: Crystallographic Analysis of Extended Phosphonamidate Transition-State Analogues. *Biochemistry* **1987**, *26*, 8542–8553. (d) Holden, H. M.; Matthews, B. W. The Binding of L-Valyl-L-Tryptophan to Crystalline Thermolysin Illustrates the Mode of Interaction of a Product of Peptide Hydrolysis. *J. Biol. Chem.* **1988**, *263*, 3256–3260. (e) Monzingo, A. F.; Matthews, B. W. Binding of N-Carboxymethyl Dipeptide Inhibitors to Thermolysin Determined by X-ray Crystallography: A Novel Class of Transition-State Analogues for Zinc Peptidases. Biochemistry **1984**, *23*, 5724–5729.

(8) Guida, W. C.; Bohacek, R. S.; Erion, M. D. Probing the Conformational Space Available to Inhibitors in the Thermolysin Active Site Using Monte-Carlo/Energy Minimization Techniques. *J. Comput. Chem.* **1992**, *13*, 214–228.

(9) Mock, W. L.; Hartman, M. E. Synthetic Scope of the Triethyloxonium Ion Catalyzed Homologation of Ketones with Diazoacetic Acids. *J. Org. Chem.* **1977**, *42*, 459–465.

(10) Georg, G. I.; Guan, X.; Kant, J. Asymmetric Synthesis of α-Alkylated α-Amino Acids Via Schmidt Rearrangement of α,α-Bisalkylated β-Keto Esters. *Tetrahedron Lett.* **1988**, *29*, 403–406.

(11) Nagasawa, H. T.; Elberling, J. A.; Fraser, P. S.; Mizuno, N. S. Medium Ring Homologs of Proline as Potential Amino Acid Antimetabolites. *J. Med. Chem.* **1971**, *14*, 501–508.

(12) Still, W. C.; MacPherson, L. J.; Harada, T.; Callahan, J. F.; Rheingold, A. L. Macrocycles in the Construction of Acyclic Stereochemistry. *Tetrahedron* **1984**, *40*, 2275–2282.

(13) Mohamadi, F.; Richards, N. G.; Gulda, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. Macro-Model-An Integrated Software System for Modeling Organic and Bioorganic Molecules Using Molecular Mechanics. *J. Comput. Chem.* **1990**, *90*, 440–467.

(14) Orlowski, M.; Wilk, S. Purification and Specificity of a Membrane Bound Metalloendoprotease from Bovine Pituitary. *Biochemistry* **1981**, *20*, 4924–4950.

(15) Maeda, T.; Balakrishman, K.; Mehdi, S. Q. The Simple and Rapid Method for the Preparation of Plasma Membranes. *Biochem. Biophys. Acta* **1983**, *731*, 115–120.