Cysteine Derivatives as Inhibitors for Carboxypeptidase A: Synthesis and Structure−Activity Relationships

Jung Dae Park and Dong H. Kim*

Department of Chemistry, Division of Molecular and Life Science, Pohang University of Science and Technology, San 31, Hyojadong, Namku, Pohang 790-784, Korea

Received June 18, 2001

A series of cysteine (Cys) derivatives having an alkyl or arylalkyl moiety on the α-amino group of the amino acid have been synthesized as a novel type of inhibitor for carboxypeptidase A. These compounds are readily prepared starting with Cys in an optically active form. The structure−activity relationship study revealed that the inhibitors prepared from D-Cys are much more potent than the corresponding inhibitors obtained from L-Cys, and the most potent inhibitor in the series, (*S*)-**1j** with a K_i value of 55 ± 4 nM, is obtained by introducing a phenethyl moiety on the amino group of D-Cys. In comparison, the most active inhibitor in the series of 2-substituted 3-mercaptopropanoic acid is found to be **20**, in which the phenyl ring is linked to the mercaptocarboxylic acid at the α-position with a methylene unit. A proposal that accounts for the different structural requirement for the maximum activity between the two series of inhibitors is provided.

Introduction

Carboxypeptidase A (CPA) is a zinc-containing proteolytic enzyme that catalyzes selectively the removal of the carboxy-terminal amino acid residue having a hydrophobic side chain.[1,2] It is one of the most extensively studied zinc proteases and represents a large family of pathologically important zinc-containing proteolytic enzymes such as matrix metalloproteases.[3] The X-ray crystal structure of CPA has been refined to 1.54 Å.[4] Because CPA is a well-characterized prototypical zinc enzyme, it has served as a model for inhibitor design protocols that can be applied to other zinc proteases. For example, captopril,[5] an inhibitor of angiotensin converting enzyme (ACE), was obtained by the application of the design strategy that originated from the synthesis of 2-benzysuccinic acid as a potent inhibitor of CPA.[6] ACE, also a zinc-containing protease, catalyzes the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor. The inhibitors of ACE have found widespread uses not only in the treatment of hypertension but also for the clinical management of congestive heart failure.[7,8] Accordingly, the inhibitor design strategies developed using CPA as a model enzyme bear considerable importance to medicinal chemistry.

We have been involved in the development of design strategies for CPA inhibitors that can be applied to other zinc proteases.[9] We describe in this paper the design and synthesis of a series of cysteine (Cys) derivatives and their structure−activity relationships (SAR) as inhibitors of CPA. Unlike most of the other CPA inhibitors, these compounds are readily synthesized in an optically active form starting with enantiomerically pure Cys.

* To whom correspondence should be addressed. Tel: +82-54-279-2101. Fax: +82-54-279-5877. E-mail: dhkim@postech.ac.kr.

Results and Discussion

Design Rationale. The topology of the CPA active site and roles of the catalytic residues for the enzymic reaction have been well-characterized. The catalytic zinc ion is bound tightly to the enzyme by coordination of His-69, Glu-72, and His-196. A molecule of water that is coordinated to the metal ion as the fourth ligand functions as the nucleophile that attacks the scissile peptide bond of the enzyme-bound substrate. Glu-270 and Arg-145 have been identified as residues that play important roles in catalysis and substrate recognition, respectively. The former functions as a general base, enhancing the nucleophilicity of the zinc-bound water molecule, and the latter forms hydrogen bonds with the C-terminal carboxylate of substrate.[2,10] In addition, there exists a hydrophobic pocket at the active site, the primary function of which is to recognize the substrate by accommodating the aromatic side chain in the P_1' residue of the substrate.[10] On the basis of the foregoing structural and functional feature of the active site of CPA, numerous inhibitors for the enzyme have been designed mostly by incorporating a zinc coordinating functionality into the substrate-like structural frame that can be recognized by the enzyme.[11] Among several zinc-coordinating groups known, the sulfhydryl group has been used most frequently by the virtue of its strong affinity for zinc ion.[11]

We have envisioned that Cys, which carries a sulfhydryl group at the β-position, may serve as a viable structural frame that is useful for the preparation of a novel type of CPA inhibitor with high potency. In the binding of Cys to CPA, the carboxylate and sulfhydryl groups in Cys are expected to interact with the guanidinium moiety of Arg-145 and the active site zinc ion, respectively. It was thought that the CPA inhibitory potency of Cys would be improved by the incorporation of a hydrophobic group that can be accommodated in

10.1021/jm010272s CCC: $22.00
Published on Web 01/17/2002

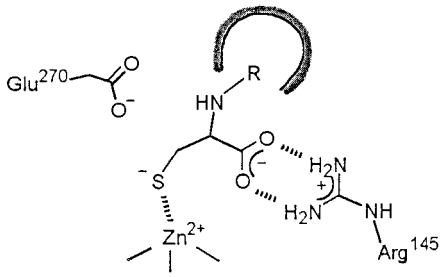
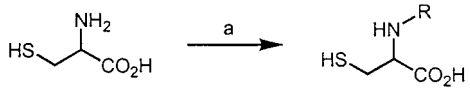


Figure 1. Rationale used for preparing *N*-substituted Cys derivatives as potential inhibitors for CPA.

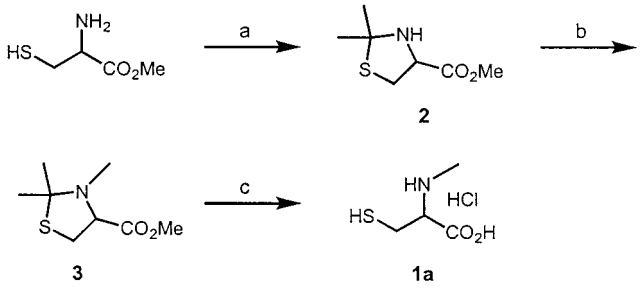
Scheme 1[a]



1

[a] Reagents, conditions, and yield: (a) Aldehydes or ketones, $NaBH_3CN$, MeOH, room temperature, 18−36 h, 30−50%.

Scheme 2[a]



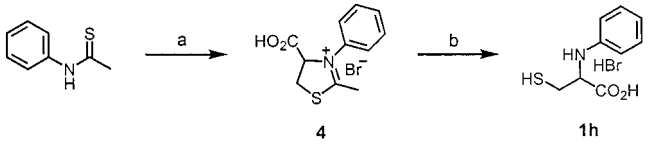
[a] Reagents, conditions, and yield: (a) Acetone, reflux, 8 h, 96%; (b) MeI, K_2CO_3, DMF, room temperature, 4 h, 45%; (c) 3 N HCl, reflux, 8 h, 88%.

the S_1' pocket of CPA. Figure 1 depicts schematically the probable binding mode of the Cys-based inhibitors that we have designed. While our study was in progress, Chong and Auld[12] reported that D-Cys is a potent inhibitor for CPA with a K_i value of 2.3 μM and subsequently the X-ray crystal structure of the CPA complex formed with D-Cys was determined to establish that the sulfhydryl group in the amino acid is coordinated to the active site zinc ion.[13]

Synthesis. Derivatives of Cys, **1b−g and 1i−n**, which carry alkyl or arylalkyl moieties at the α-amino group, were prepared from unprotected Cys simply by the reductive amination with the appropriate aldehyde or ketone using sodium cyanoborohydride in methanol in moderate yields (Scheme 1). The individual stereoisomers of **1d**, **1f**, **1i**, and **1j** were prepared starting with optically active Cys.

An attempt at *N*-methylation to obtain *N*-methylcysteine by reductive amination with formaldehyde gave a mixture of mono- and dimethylated Cys, which could not be purified. Accordingly, an alternative synthetic path was devised as follows: The condensation of the methyl ester of Cys with acetone afforded thiazolidine **2**, which was then treated with methyl iodide to give **3**. The hydrolysis of **3** under acidic conditions provided the desired compound **1a** in 38% overall yield (Scheme 2). *N*-Phenylcysteine (**1h**) was synthesized by a literature method[14] that involves the formation of **4** from *N*-phenylthioacetamide and α-bromoacrylic acid and subsequent hydrolysis under acidic conditions (Scheme 3).

Scheme 3[a]



4 1h

[a] Reagents, conditions, and yield: (a) CH_2=C(Br)CO_2H, toluene, 90 °C, 1 h, 92%; (b) 48% HBr, reflux, 2 h, 82%.

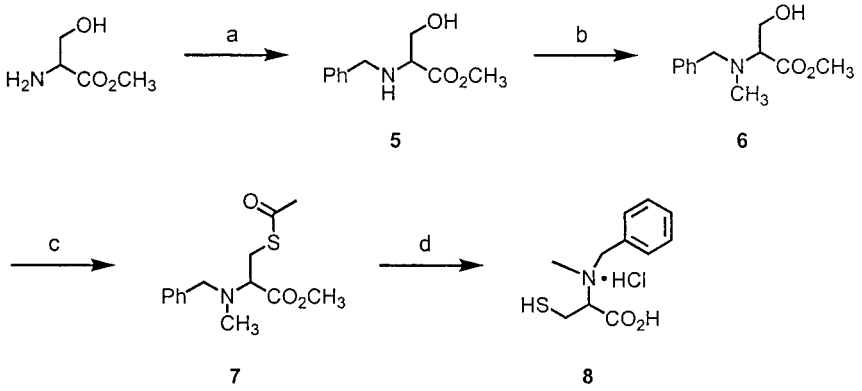
The synthesis of *N*-benzyl-*N*-methylcysteine **8** was started with the methyl ester of serine, which upon treatment with benzyl bromide in the presence of potassium carbonate, gave exclusively the *N*-monobenzylated product, **5**. The latter was allowed to react with methyl iodide to form **6**, which was then converted into thioester **7** by reacting with thioacetic acid under Mitsunobu conditions. The hydrolysis of **7** in diluted hydrochloric acid afforded **8** (Scheme 4).

To probe the effect of the amino group on the binding of the *N*-substituted Cys inhibitors to CPA, we have also synthesized **13**, **14**, and **19**, in which the amino groups are replaced with a methylene unit. Scheme 5 depicts the synthesis of 2-(mercaptomethyl)-4-methylpentanoic acid (**13**). The reaction of 3-hydroxypropionitrile in the presence of 2 equiv of LDA with isobutyl bromide in hexamethylphosphoramide (HMPA) afforded **9**. The latter was then treated with aqueous potassium hydroxide solution under reflux conditions, whereby the nitrile group was converted into carboxylate, which was then transformed into methoxycarbonyl to give **11**. The hydroxyl group in **11** was converted into an acetylmercapto group by allowing it to react with thioacetic acid under Mitsunobu conditions. The hydrolysis of **12** in 48% HBr solution under reflux conditions afforded **13** (Scheme 5). In an analogous fashion, **14** was prepared.

2-(Mercaptomethyl)-6-phenylhexanoic acid (**19**) was prepared according to the synthetic route reported by Ondetti et al.[15] (Scheme 6). Diethyl malonate was allowed to react with 1-bromo-4-phenylbutane to obtain **15**, which was subsequently subjected to saponification to give **16**. The latter was converted under Mannich-type conditions into α,β-unsaturated carboxylic acid **17** and then into **18** by treatment with thioacetic acid. The acetyl group in **18** was removed by treatment with methoxyethylamine to afford **19** (Scheme 6).

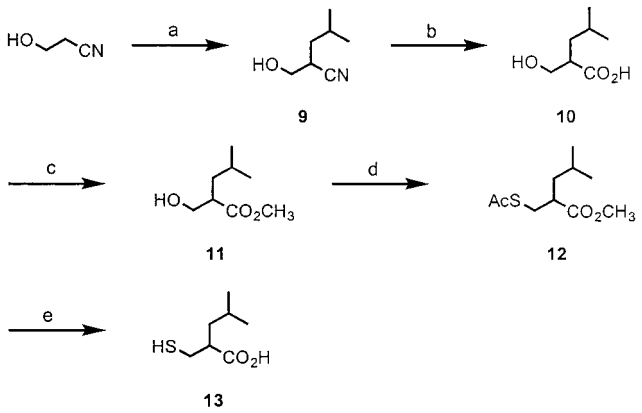
SAR. The compounds thus synthesized were evaluated as competitive inhibitors for CPA by a standard method using hippuryl-L-phenylalanine (Hipp-L-Phe) as a substrate at pH 7.5,[15] and the inhibitory constants (K_i values) were estimated from the respective Dixon plot.[16] Table 1 lists K_i values of *N*-alkylcysteine for the inhibition of CPA. It can be seen from Table 1 that the introduction of an alkyl group at the amino nitrogen of *rac*-Cys does not affect the K_i value much, suggesting that small alkyl groups such as ethyl or propyl may be accommodated in the S_1' pocket, but their effects on the binding of the inhibitors to CPA are marginal probably due to limited van der Waals contacts of the small alkyl groups with the surface of the pocket. Bulky alkyl groups such as cyclohexyl and isobutyl affect adversely, which may possibly be due to the difficulty of the large moiety getting in the S_1' pocket. It has been reported that the opening of the substrate recognition pocket is relatively narrower than the pocket, and thus, large groups experience difficulty in entering the pocket.[17]

Scheme 4[a]



[a] Reagents, conditions, and yield: (a) Benzyl bromide, K_2CO_3, DMF, room temperature, 8 h, 51%; (b) MeI, K_2CO_3, DMF, room temperature, 2 h, 84%; (c) PPh_3, DEAD, CH_3COSH, THF, 0 °C, 4 h, 36%; (d) 6 N HCl, reflux, 2 h, 90%.

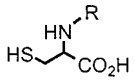
Scheme 5[a]



[a] Reagents, conditions, and yield: (a) LDA, isopropyl bromide, HMPA−THF, 0 °C, 6 h, 36%; (b) aqueous KOH (4 equiv), reflux, 8 h, quantitative; (c) $SOCl_2$, MeOH, reflux, 4 h, quantitative; (d) PPh_3, DEAD, CH_3COSH, THF, 0 °C, 2 h, 70%; (e) 48% HBr, reflux, 2 h, 82%.

As expected, the introduction of an aromatic ring at the amino group of Cys augmented the binding affinity of the inhibitors to CPA (Table 2). Several features are apparent from the analysis of the data in Table 2. First, the binding affinity increases as the number of methylene units of the linker that bridges between the phenyl ring and the amino group in the inhibitors increases, maximizing with an ethylene chain. The highest binding affinity was observed with **1j** that carries a phenethyl group at the amino nitrogen of D-Cys. Figure 2 shows the relationships between the length of the linker and

Table 1. Inhibitory Potencies (K_i Values) of *N*-Alkylcysteines for CPA Inhibition

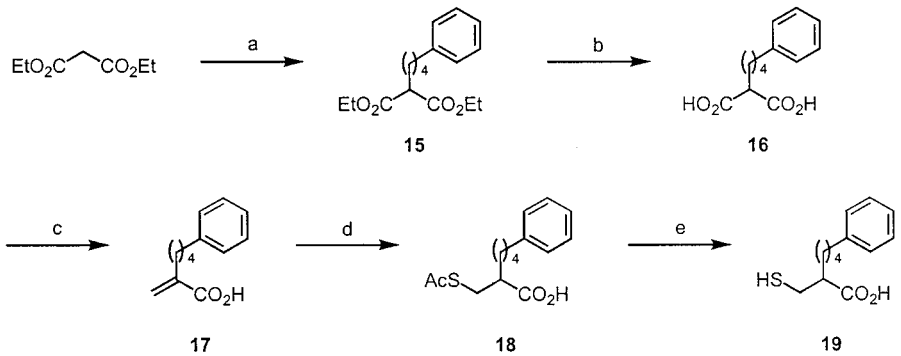


compd no.	R	K_i (μM)[a]
L-Cys	hydrogen	350 ± 5[b]
D-Cys	hydrogen	2.3 ± 1.6[b]
1a	methyl	2.4 ± 0.17
1b	ethyl	1.4 ± 0.19
1c	propyl	5.6 ± 0.5
1d	isopropyl	1.1 ± 0.16
(*R*)-**1d**	isopropyl	2.9 ± 0.23
(*S*)-**1d**	isopropyl	0.56 ± 0.02
1e	isobutyl	7.5 ± 0.42
1f	cyclohexyl	9.6 ± 0.87
(*R*)-**1f**	cyclohexyl	7.9 ± 0.63
(*S*)-**1f**	cyclohexyl	2.9 ± 1.6
1g	cyclohexylmethyl	9.4 ± 0.2

[a] K_i values are expressed as mean ± SEM ($n = 3$). [b] Ref 12.

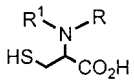
the CPA inhibitory activity. Second, to explore the stereochemistry associated with the inhibitions, representative inhibitors (**1d**, **if**, **1i**, and **1j**) were prepared in an optically active form and it was found that the inhibitory activities of these compounds reside mostly on compounds having the (*S*) configuration, that is, inhibitors derived from D-Cys (Tables 1 and 2). Apparently, inhibitors belonging to the D-series bear molecular configurations more complementary to the active site geometry of CPA. Third, it is noteworthy that the K_i value of compound **1m** with a 1-naphthylmethyl group at the amino nitrogen is over 620-fold more potent as

Scheme 6[a]



[a] Reagents, conditions, and yield: (a) 1-Bromo-4-phenylbutane, NaOEt, EtOH, room temperature, 8 h, 82%; (b) 4 N KOH, reflux, 4 h, 88%; (c) Et_2NH·HCl, HCHO, reflux, 12 h, 62 %; (d) CH_3COSH, reflux, 4 h, 88%; (e) $MeOCH_2CH_2NH_2$, room temperature, 1 h, 90%.

Table 2. Inhibitory Potencies (K_i Values) of *N*-Arylalkylcysteines for CPA Inhibition



compd no.	R	R^1	K_i (μM)[a]
1h	phenyl	H	5.5 ± 0.71
1i	benzyl	H	0.27 ± 0.01
(*R*)-**1i**	benzyl	H	33.0 ± 1.0
(*S*)-**1i**	benzyl	H	0.19 ± 0.01
8	benzyl	Me	12.0 ± 0.86
1j	phenethyl	H	0.065 ± 0.0014
(*R*)-**1j**	phenethyl	H	0.61 ± 0.058
(*S*)-**1j**	phenethyl	H	0.055 ± 0.004
1k	phenylpropyl	H	1.3 ± 0.12
1l	(*p*-methoxy)benzyl	H	0.85 ± 0.074
1m	1-naphthylmethyl	H	0.81 ± 0.14
1n	2-naphthylmethyl	H	>500

[a] K_i values are expressed as mean ± SEM (n = 3).

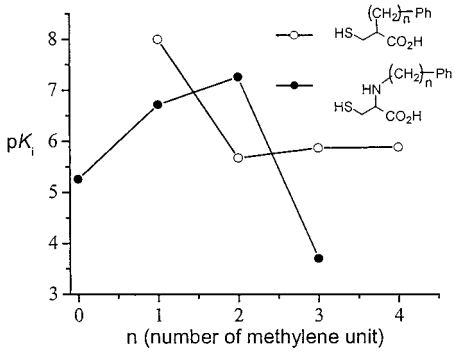
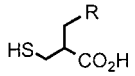


Figure 2. Relationships between the length of the linker that bridges a phenyl ring and 3-mercaptopropanoic acid and the pK_i (−log K_i) in the inhibition of CPA.

compared with its isomer in which the naphthyl moiety is linked at the 2-position. This result may be rationalized in terms of the narrow mouth of the S_1' pocket: the naphthyl ring in **1n** experiences a difficulty in entering the pocket.[17] Last, the substitution of the amino hydrogen in **1i** with a methyl group decreased the binding affinity by 44-fold, suggesting that the methyl group may experience repulsive steric interactions with neighboring amino acid residues at the active site of the enzyme.

It is instructive to compare the CPA binding affinity of the Cys-based inhibitors with those of inhibitors in which the amino group of Cys is replaced with a methylene unit. While the most potent compound in the series of Cys-based inhibitors is **1j** as described above, in the series of 2-substituted 3-mercaptopropanoic acid, the compound in which the benzene ring is linked to the mercaptopropanoic acid by a methylene unit, i.e., **20**,[15] is most active (Table 3 and Figure 2). The seemingly perplexing SAR may be reconciled as follows: The X-ray crystal structure of the CPA complex formed with D-Cys revealed that in addition to the strong interaction of the sulfhydryl group of the inhibitor with the zinc ion at the active site, the positively charged ammonium ion of the zwitterionic form of Cys interacts with the negatively charged carboxylate of Glu-270.[13] It has also been shown that upon binding of D-Cys, the side chains of Glu-270, Arg-145, and Arg-127 move toward the ammonium ion of the bound D-Cys.[13] It is not unreasonable to assume that in the CPA·D-Cys complex the Cys

Table 3. Inhibitory Constants (K_i Values) for CPA Inhibition



compd no.	R	K_i (μM)[a]
13	isopropyl	0.16 ± 0.01
14	cyclohexyl	2.35[b]
20	phenyl	0.010[c]
21	benzyl	2.1[b]
22	phenethyl	1.35[b]
19	phenylpropyl	1.3 ± 0.12

[a] K_i values are expressed as mean ± SEM (n = 3). [b] Ref 19. [c] Ref 15.

molecule may rest in such a way that its positively charged ammonium ion has maximum interactions with the Glu-270 carboxylate anion. Such interactions would be oriented closer to Glu-270 than the nonCys-based sulfhydryl-type CPA inhibitors. The 20-fold increase in the K_i value by the replacement of the amino group in **1j** with a methylene (to obtain **19**) may reflect that the amino group may indeed participate in electrostatic interactions with the carboxylate of Glu-270. As a result, the benzene ring in **1h** may not be fully accommodated in the S_1' pocket. In order for the aromatic ring in the Cys-based inhibitor to fit in the pocket fully and to have maximum hydrophobic interactions, the inhibitor would require a longer linker than an amino unit. Apparently, a chain consisting of three atoms, including the amine nitrogen, appears to be optimal, as shown by **1j**. If the chain is longer than the optimal length, the benzene ring, upon inserting into the S_1' pocket, may experience repulsive interactions with the surface of the pocket, causing the inhibitor molecule to be displaced somewhat from the position of maximum interactions with the enzyme. The reduction of the binding affinity of **1k** by 20-fold, as compared with that of **1j**, may thus be rationalized.

Conclusion

In summary, a series of Cys derivatives (15 racemic and 8 optically active compounds) with an alkyl or arylalkyl moiety on the α-amino group of Cys have been synthesized as novel types of inhibitors for CPA. The SAR study revealed that in cases of optically active inhibitors, those prepared from D-Cys are much more potent than the corresponding inhibitors obtained from L-Cys, and the most potent inhibitor in this study is (*S*)-**1j**, obtained by introducing a phenethyl moiety on the amino group of D-Cys. The K_i value of 55 ± 4 nM exhibited by (*S*)-**1j** corresponds to a 42-fold decrease as compared with that of D-Cys, and this increase in binding affinity (2.2 kcal mol^{-1}) may be attributed to the hydrophobic interactions between the phenyl ring of the inhibitor and the S_1' pocket of CPA and the ionic interactions between the amino group in (*S*)-**1j** and the carboxylate of Glu-270. It appears therefore that in addition to the three most important binding groups (carboxylate, sulfhydryl, and aromatic) that participate in binding interactions with the enzyme, the amino group in these inhibitors also engages in binding in the form of electrostatic interactions with the Glu-270 carboxylate. The CPA inhibitor design approach that uses Cys as the starting point may be applied to other

zinc-containing proteases of medicinal interest such as ACE and matrix metalloproteases. We are currently exploring these applications.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and were uncorrected. ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra were recorded with a Bruker AM 300 (300 MHz) instrument using tetramethylsilane as the internal standard. IR spectra were recorded on a Bruker Equinox 55 Fourier transform infrared spectrometer. Mass spectra were obtained by Korea Basic Science Insititute. Silica gel 60 (230−400 mesh) was used for flash chromatography, and thin-layer chromatography was carried out on silica-coated glass sheets (Merck silica gel 60 F-254). Elemental analyses were performed at the Center for Biofunctional Molecules, Pohang University of Science and Technology, Pohang, Korea, and results were within ±0.4% of the theoretical value.

All solutions for kinetic study were prepared using doubly distilled and deionized water. Stock assay solutions were filtered (GHP Acrodic syringe filter, pore size 0.2 μm) before use. CPA was purchased from Sigma Chemical Co. (Allan form, twice crystallized from bovine pancreas, aqueous suspension in toluene) and used without further purification. CPA stock solutions were prepared by dissolving the enzyme in 0.05 M Tris/0.5 M NaCl, pH 7.5, buffer solution. Hipp-L-Phe purchased from Sigma Chemical Co. was used as substrate for CPA, and the decrease in the absorbance at 254 nm was followed at 25 °C. An HP 8453 UV/vis spectrometer was used in enzyme inhibition studies.

General Procedure for the Synthesis of *N*-Alkylcysteine. To a suspension of Cys and $NaBH_3CN$ (1 equiv) in methanol (10 mL) was added the appropriate aldehyde (1.5 equiv) or ketone (1.1 equiv) at room temperature and stirred for 18−36 h. The white precipitate formed was collected and washed with MeOH to give the product.

***rac*-*N*-Ethylcysteine (1b).** Compound **1b** was prepared from *rac*-Cys and acetaldehyde. Yield, 32%; mp 216−217 °C (dec). IR (KBr): 1596, 2587 cm^{-1}. ^{1}H NMR (D_2O, 300 MHz): δ 1.39 (t, 3H) 3.15 (m, 2H) 3.18 (d, 2H) 3.96 (t, 1H). ^{13}C NMR (D_2O, 300 MHz): δ 10.9, 23.9, 42.5, 62.7, 172.0. Anal. ($C_5H_{11}NO_2S$) C, H, N.

***rac*-*N*-Propylcysteine (1c).** Compound **1c** was prepared from *rac*-Cys and propionaldehyde. Yield, 37%; mp 229−230 °C (dec). IR (KBr): 1553, 2596 cm^{-1}. ^{1}H NMR (D_2O, 300 MHz): δ 0.99 (t, 3H) 1.75 (m, 2H) 3.06 (m, 2H) 3.10 (ddd, 2H) 3.90 (t, 1H). ^{13}C NMR (D_2O, 300 MHz): δ 5.5, 10.6, 19.5, 23.9, 48.8, 63.0, 172.0. Anal. ($C_6H_{13}NO_2S\cdot 2/3H_2O$) C, H, N.

***rac*-*N*-Isopropylcysteine (1d).** Compound **1d** was prepared from *rac*-Cys and acetone. Yield, 51%; mp 228−230 °C (dec). IR (KBr): 1585, 2576 cm^{-1}. ^{1}H NMR (D_2O, 300 MHz): δ 1.34 (q, 6H), 3.16 (ddd, 2H), 3.54 (m, 2H), 4.35 (t, 1H). ^{13}C NMR (D_2O, 300 MHz): δ 18.5, 18.6, 23.6, 51.0, 59.1, 170.3. Anal. ($C_6H_{13}NO_2S\cdot HCl\cdot 1/4H_2O$) C, H, N.

(*S*)-*N*-Isopropylcysteine ((*S*)-1d). Compound (*S*)-**1d** was prepared from (*S*)-Cys and acetone. $[\alpha]^{25}_D$ −32.6° (*c* 0.5, H_2O). mp and spectral data are identical with those of *rac*-**1d**.

(*R*)-*N*-Isopropylcysteine ((*R*)-1d). Compound (*R*)-**1d** was prepared from (*R*)-Cys and acetone. $[\alpha]^{25}_D$ +35.4° (*c* 0.25, H_2O). mp and spectral data are identical with those of *rac*-**1d**.

***rac*-*N*-Isobutylcysteine (1e).** Compound **1e** was prepared from *rac*-Cys and isobutyraldehyde. Yield, 52%; mp 208−209 °C (dec). IR (KBr): 1595, 2582 cm^{-1}. ^{1}H NMR (D_2O, 300 MHz): δ 1.03 (d, 6H), 2.07 (m, 1H), 2.95 (m, 2H), 3.10 (m, 2H), 3.89 (t, 1H). ^{13}C NMR (D_2O, 300 MHz): δ 19.4, 19.5, 23.8, 25.9, 54.3, 63.5, 171.8. Anal. ($C_7H_{15}NO_2S$) C, H, N.

***rac*-*N*-Cyclohexylcysteine (1f).** Compound **1f** was prepared from *rac*-Cys and cyclohexanone. Yield, 38%; mp 233−235 °C (dec). IR (KBr): 1596, 2585 cm^{-1}. ^{1}H NMR (D_2O, 300 MHz): δ 1.21 (m, 5H), 1.48 (d, 1H), 1.65 (d, 2H), 1.92 (t, 2H), 3.01 (m, 3H), 4.31 (t, 1H). ^{13}C NMR (D_2O, 300 MHz): δ 23.4, 24.1, 24.3, 24.6, 29.1, 54.6, 57.5, 58.3, 169.9. Anal. ($C_9H_{17}NO_2S\cdot 1/4H_2O$) C, H, N.

(*S*)-*N*-Cyclohexylcysteine ((*S*)-1f). Compound (*S*)-**1f** was prepared from (*S*)-Cys and cyclohexanone. $[\alpha]^{25}_D$ +26.2° (*c* 0.5, H_2O). mp and spectral data are identical with those of *rac*-**1f**.

(*R*)-*N*-Cyclohexylcysteine ((*R*)-1f). Compound (*R*)-**1f** was prepared from (*R*)-Cys and cyclohexanone. $[\alpha]^{25}_D$ −28.4° (*c* 0.25, H_2O). mp and spectral data are identical with those of *rac*-**1f**.

***rac*-*N*-(Cyclohexylmethyl)cysteine (1g).** Compound **1g** was prepared from *rac*-Cys and cyclohexylcarboxaldehyde. Yield, 26%; mp 216−218 °C (dec). IR (KBr): 1580, 2562 cm^{-1}. ^{1}H NMR (D_2O, 300 MHz): δ 0.79 (m, 5H), 1.33 (m, 6H), 2.59 (d, 2H), 2.80 (t, 2H), 3.94 (t, 1H). ^{13}C NMR (D_2O, 300 MHz): δ 22.6, 25.0, 25.5, 30.0, 34.5, 52.6, 61.1, 168.2. Anal. ($C_{10}H_{19}NO_2S\cdot 1/4H_2O$) C, H, N.

***rac*-*N*-Benzylcysteine (1i).** Compound **1i** was prepared from *rac*-Cys and benzaldehyde. Yield, 30%; mp 212−214 °C (dec). IR (KBr): 1595, 2562 cm^{-1}. ^{1}H NMR (D_2O, 300 MHz): δ 3.08 (ddd, 2H), 4.21 (t, 1H), 4.23 (s, 2H), 7.37 (s, 5H). ^{13}C NMR (D_2O, 300 MHz): δ 23.2, 50.4, 60.4, 129.6, 130.1, 130.3, 130.5, 169.5. Anal. ($C_{10}H_{13}NO_2S\cdot HCl$) C, H, N.

(*S*)-*N*-Benzylcysteine ((*S*)-1i). Compound (*S*)-**1i** was prepared from (*S*)-Cys and benzaldehyde. $[\alpha]^{25}_D$ +20.4° (*c* 0.5, 3 N HCl). mp and spectral data are identical with those of *rac*-**1i**.

(*R*)-*N*-Benzylcysteine ((*R*)-1i). Compound (*R*)-**1i** was prepared from (*R*)-Cys and benzaldehyde. $[\alpha]^{25}_D$ −18.0° (*c* 0.4, 3 N HCl). mp and spectral data are identical with those of *rac*-**1i**.

***rac*-*N*-Phenethylcysteine (1j).** Compound **1j** was prepared from *rac*-Cys and phenylacetaldehyde. Yield, 30%; mp 220−221 °C (dec). IR (KBr): 1600, 2567 cm^{-1}. ^{1}H NMR (D_2O, 300 MHz): δ 2.96 (t, 2H), 3.03 (m, 2H), 3.27 (t, 2H), 4.22 (t, 1H), 7.24 (m, 5H). ^{13}C NMR (D_2O, 300 MHz): δ 22.9, 31.8, 47.8, 61.0, 127.7, 129.1, 129.4, 136.5, 169.4. Anal. ($C_{11}H_{15}NO_2S\cdot 1/4H_2O$) C, H, N.

(*S*)-*N*-Phenethylcysteine ((*S*)-1j). Compound (*S*)-**1j** was prepared from (*S*)-Cys and phenylacetaldehyde. $[\alpha]^{25}_D$ +23.2° (*c* 0.25, 3 N HCl). mp and spectral data are identical with those of *rac*-**1j**.

(*R*)-*N*-Phenethylcysteine ((*R*)-1j). Compound (*R*)-**1j** was prepared from (*R*)-Cys and phenylacetaldehyde. $[\alpha]^{25}_D$ −22.7° (*c* 0.25, 3 N HCl). mp and spectral data are identical with those of *rac*-**1j**.

***rac*-*N*-Phenylpropylcysteine (1k).** Compound **1k** was prepared from *rac*-Cys and 3-phenylpropionaldehyde. Yield, 43%; mp 205−206 °C (dec). IR (KBr): 1582, 2561 cm^{-1}. ^{1}H NMR (D_2O, 300 MHz): δ 1.69 (m, 2H), 2.35 (t, 2H), 2.76 (t, 2H), 2.78 (ddd, 2H), 3.93 (t, 1H), 6.92 (m, 5H). ^{13}C NMR (D_2O, 300 MHz): δ 22.7, 27.1, 31.9, 46.2, 60.7, 126.6, 128.6, 128.9, 140.7, 169.3. Anal. ($C_{12}H_{17}NO_2S\cdot 1/4H_2O$) C, H, N.

***rac*-*N*-(*p*-Methoxy)benzylcysteine (1l).** Compound **1l** was prepared from *rac*-Cys and *p*-anisaldehyde. Yield, 47%; mp 209−211 °C (dec). IR (KBr): 1596, 2568 cm^{-1}. ^{1}H NMR (D_2O, 300 MHz): δ 2.87 (ddd, 2H), 3.51 (s, 3H), 4.00 (s, 2H), 4.01 (t, 1H), 6.71 (d, 2H), 7.13 (d, 2H). ^{13}C NMR (D_2O, 300 MHz): δ 23.1, 49.9, 55.6, 59.9, 114.8, 122.4, 132.1, 160.1, 169.3. Anal. ($C_{11}H_{15}NO_2S\cdot 1/4H_2O$) C, H, N.

***rac*-*N*-(1-Naphthylmethyl)cysteine (1m).** Compound **1m** was prepared from *rac*-Cys and 1-naphthaldehyde. Yield, 40%; mp 204−205 °C (dec). IR (KBr): 1598, 2568 cm^{-1}. ^{1}H NMR (D_2O, 300 MHz): δ 2.77 (d, 2H), 3.98 (t, 1H), 4.27 (s, 2H), 7.18 (m, 4H), 7.58 (m, 3H). ^{13}C NMR (D_2O, 300 MHz): δ 23.3, 47.6, 60.9, 122.7, 125.7, 125.9, 126.8, 127.7, 129.2, 130.5, 131.0, 131.2, 133.7, 169.3. Anal. ($C_{14}H_{15}NO_2S\cdot 1/4H_2O$) C, H, N.

***rac*-*N*-(2-Naphthylmethyl)cysteine (1n).** Compound **1n** was prepared from *rac*-Cys and 2-naphthaldehyde. Yield, 45%; mp 177−179 °C (dec). IR (KBr): 1622, 2578 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.78 (m, 2H), 3.33 (t, 1H), 4.00 (q, 2H), 7.50 (m, 3H), 7.87 (m, 4H). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 27.0, 51.4, 62.6, 126.3, 127.0, 127.7, 128.4, 128.5, 128.7, 133.2, 137.7, 173.2. Anal. ($C_{14}H_{15}NO_2S\cdot 1/4H_2O$) C, H, N.

***rac*-*N*-2,2-Dimethyl-4-thiazolidinecarboxylate Methyl Ester (2).** A mixture of *rac*-Cys methyl ester hydrochloride (3.4 g, 20 mmol) and acetone (50 mL) was refluxed for 8 h and

then evaporated under reduced pressure. The residue was diluted with ethyl acetate, washed successively with water, 5% aqueous sodium bicarbonate solution (50 mL × 3), and brine (50 mL × 3), and then dried over anhydrous $MgSO_4$. The solution was concentrated, and the crude product was purified by column chromatography (EtOAc/*n*-hexane = 1/8) to give **2** as a pale yellow oil (3.4 g, 96%). IR ($CHCl_3$): 1744 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.52 (d, 3H), 1.70 (d, 3H), 2.51 (s, 1H), 3.24 (m, 2H), 3.79 (s, 3H), 4.08 (s, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 30.7, 30.9, 33.3, 53.4, 64.4, 65.1, 172.4.

***rac*-*N*-Methyl-2,2-dimethyl-4-thiazolidinecarboxylate Methyl Ester (3).** A mixture of **2** (1.2 g, 6.9 mmol), potassium carbonate (0.95 g, 6.9 mmol), and methyl iodide (0.5 mL, 8.3 mmol) in dimethylformamide (DMF, 30 mL) was stirred at room temperature for 4 h. The residue was diluted with ethyl acetate, washed successively with water, 5% sodium thiosulfate (50 mL × 3), and brine (50 mL × 3), and dried over anhydrous $MgSO_4$. The solution was evaporated in vacuo, and the crude product was purified by column chromatography (EtOAc/*n*-hexane = 1/4) to give **3** as a pale yellow oil (0.58 g, 45%). IR ($CHCl_3$): 1747 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.37 (s, 3H), 1.46 (s, 3H), 2.24 (s, 3H), 3.10 (m, 2H), 3.70 (s, 3H), 3.72 (t, 3H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 27.3, 29.4, 32.3, 34.6, 52.8, 70.0, 75.1, 172.5.

***rac*-*N*-Methylcysteine Hydrochloride (1a).** A mixture of **3** (0.50 g, 2.6 mmol) and 6 N HCl (10 mL) was refluxed for 8 h and then evaporated under reduced pressure. The residue was recrystallized from ether and ethanol to give a white powder (0.40 g, 88%): mp 127−129 °C (literature 127 °C).[18] ^1H NMR (D_2O, 300 MHz): δ 2.59 (s, 3H), 3.07 (m, 2H), 4.15 (t, 1H). ^{13}C NMR (D_2O, 300 MHz): δ 22.8, 31.7, 62.3, 170.0.

4-Carboxy-2-methyl-3-phenyl-Δ^2-thiazolinium Bromide (4). A mixture of thioacetamide (1.0 g, 6.6 mmol) and α-bromoacrylic acid (1.1 g, 7.3 mmol) in dry toluene (20 mL) was heated at 90 °C for 1 h. The reaction mixture was cooled to room temperature, and then, the precipitate was collected on a filter and washed with acetone (30 mL × 3), and the filter cake was recrystallized from MeOH/EtOAc/hexane (1:1:2) to give a white solid (1.8 g, 90%): mp 216−218 °C (literature[14] 215−216 °C). IR (KBr): 1735 cm^{-1} (literature[14] 1722 cm^{-1}). ^1H NMR (TFA, 300 MHz): δ 2.45 (s, 3H), 4.21 (ddd, 2H), 5.75 (dd, 1H), 7.36 (s, 5H).

***rac*-*N*-Phenylcysteine Hydrobromide (1h).** A mixture of **4** (1.0 g, 3.3 mmol) and 1.5 N HBr solution (20 mL) was refluxed for 3 h and then evaporated under reduced pressure. The residue was dissolved in 48% HBr solution (10 mL) and evaporated in vacuo to give a brown solid. The solid was recrystallized from *n*-PrOH and benzene to give the product as a white solid (0.83 g, 90%): mp 173−175 °C (literature[14] 173−174 °C). IR (KBr): 1739, 2543 cm^{-1}. ^1H NMR (TFA, 300 MHz): δ 3.22 (dd, 2H), 6.87 (t, 1H), 7.51 (m, 5H).

***rac*-*N*-Benzylserine Methyl Ester (5).** A mixture of *rac*-serine methylester (4.7 g, 30 mmol), potassium carbonate (8.3 g, 60 mmol), and benzyl bromide (4.0 mL, 33 mmol) in DMF (100 mL) was stirred at room temperature for 8 h. The residue was diluted with ethyl acetate, washed successively with water, 5% aqueous sodium thiosulfate solution (50 mL × 3), and brine (50 mL × 3), and then dried over anhydrous $MgSO_4$. The solution was concentrated, and the crude product was purified by column chromatography (EtOAc/*n*-hexane = 1/2) to give the product as a pale yellow oil (3.2 g, 51%). IR ($CHCl_3$): 1736, 3321 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 3.43 (t, 1H), 3.69 (m, 2H), 3.75 (dd, 2H), 7.33 (m, 5H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 52.5, 52.6, 62.3, 62.9, 127.7, 128.7, 128.9, 139.6, 173.9.

***rac*-*N*-Benzyl-*N*-methylserine Methyl Ester (6).** A mixture of **5** (3.2 g, 15.3 mmol), potassium carbonate (4.2 g, 30.6 mmol), and methyl iodide (1.9 mL, 30.6 mmol) in DMF (100 mL) was stirred at room temperature for 2 h. The residue was diluted with ethyl acetate, washed successively with water, 5% aqueous sodium thiosulfate solution (50 mL × 3), and brine (50 mL × 3), and then dried over anhydrous $MgSO_4$. The solution was concentrated, and the crude product was purified by column chromatography (EtOAc/*n*-hexane = 1/8) to give the product as a pale yellow oil (2.5 g, 84%). IR ($CHCl_3$): 1732, 3446 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 2.34 (s, 3H), 3.55 (q, 1H), 3.76 (m, 2H), 3.78 (s, 3H), 3.82 (dd, 2H), 7.31 (m, 5H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 37.8, 51.8, 59.3, 59.6, 66.2, 127.8, 128.9, 129.3, 138.9, 171.6.

***rac*-*N*-Benzyl-*N*-methyl-*S*-acetylcysteine Methyl Ester (7).** To a solution of triphenylphosphine (1.0 g, 4 mmol) in tetrahydrofuran (THF, 15 mL) was slowly added diethyl azodicarboxylate (0.63 mL, 4 mmol) at 0 °C under a nitrogen atmosphere, and the resulting mixture was stirred for 30 min at 0 °C. To the reaction mixture was added slowly a mixture of **6** (0.44 g, 2 mmol) and thioacetic acid (0.3 mL, 4 mmol) by a cannular and stirred for 4 h at room temperature. The reaction mixture was diluted with ethyl acetate and washed with a saturated $NaHCO_3$ solution (10 mL × 3). After the organic layer was collected and acidified with 3 N HCl, the aqueous layer was washed with ethyl acetate (10 mL × 3), then basified with 2 N NaOH, and extracted with ethyl acetate (10 mL × 3). The combined extracts were dried over anhydrous $MgSO_4$ and concentrated in vacuo, and the crude product was purified by column chromatography (EtOAc/*n*-hexane = 1/4) to give **7** as an oil (0.2 g, 36%). IR ($CHCl_3$): 2244, 3446 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.92 (d, 6H), 1.45 (m, 2H), 1.79 (m, 1H), 2.78 (m, 1H), 3.69 (d, 2H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 21.9, 23.3, 26.5, 33.7, 37.5, 63.3, 121.6.

***rac*-*N*-Benzyl-*N*-methylcysteine (8).** A mixture of **7** (0.07 g, 0.25 mmol) and concentrated HCl (5 mL) was refluxed for 2 h and then evaporated under reduced pressure. The residue was recrystallized from ether and ethanol to give a white powder (0.66 g, 90%): mp 68−70 °C. IR (KBr): 1739, 2585 cm^{-1}. ^1H NMR (D_2O, 300 MHz): δ 2.71 (s, 3H), 3.05 (m, 2H), 4.06 (t, 1H), 4.67 (s, 2H), 7.33 (m, 5H). ^{13}C NMR (D_2O, 300 MHz): δ 21.3, 37.8, 59.5, 67.5, 128.7, 129.7, 130.8, 131.7, 169.5. Anal. ($C_{11}H_{15}NO_2S \cdot 1/2H_2O$) C, H, N.

***rac*-2-(Hydroxymethyl)-4-methylvaleronitrile (9).** To a solution of diisopropylamine (5.6 mL, 40 mmol) in THF (100 mL) under a nitrogen atmosphere at 0 °C was added a *n*-BuLi (2.5 M in hexane, 16 mL, 40 mmol) by a microsyringe. The mixture was stirred for 30 min at that temperature and cooled to −78 °C. To the chilled and stirring solution of LDA was added slowly a solution of 3-hydroxypropanitrile (1.4 mL, 20 mmol) in 30 mL of THF, and the stirring was continued for an additional 1 h at −78 °C. Isobutyl bromide (2.2 mL, 20 mmol) was added to the reaction mixture at −78 °C, and the resulting mixture was warmed to 0 °C slowly and stirred at the temperature for 12 h. The reaction was quenched by the addition of 6 N HCl solution (30 mL). The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (10 mL × 3). The combined organic layers were washed with 5% $Na_2S_2O_3$ (10 mL × 3) and brine (10 mL × 3), dried under anhydrous $MgSO_4$, and then evaporated in vacuo to dryness. The crude product was purified by column chromatography (EtOAc/*n*-hexane = 1/1) to give **9** as an oil (0.91 g, 36%). IR ($CHCl_3$): 2244, 3446 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.92 (d, 6H), 1.45 (m, 2H), 1.79 (m, 1H), 2.78 (m, 1H), 3.69 (d, 2H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 21.9, 23.3, 26.5, 33.7, 37.5, 63.3, 121.6.

***rac*-2-(Hydroxymethyl)-4-methylpentanoic Acid (10).** A mixture of **9** (1.3 g, 10 mmol) and 4 N KOH (10 mL, 40 mmol) was refluxed for 8 h. The reaction mixture was cooled to room temperature and was acidified with 3 N HCl. The product was extracted with ethyl acetate (10 mL × 3). The combined extracts were dried over anhydrous $MgSO_4$ and evaporated on the reduced pressure to give **10** as an oil (1.39 g, 95%). IR ($CHCl_3$): 1714, 3346 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.90 (m, 6H), 1.31 (m, 1H), 1.62 (m, 2H), 2.69 (m, 1H), 3.74 (d, 2H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 22.7, 22.9, 26.2, 37.6, 46.0, 63.7, 181.2.

***rac*-2-(Hydroxymethyl)-4-methylpentanoic Acid Methyl Ester (11).** To a solution of **10** (0.90 g, 6.2 mmol) in MeOH (20 mL) was added slowly thionyl chloride (2.3 mL, 31.0 mmol) at 0 °C, and the resulting mixture was refluxed for 4 h. The reaction mixture was evaporated in vacuo, diluted with ethyl acetate, washed with 5% aqueous $NaHCO_3$ solution (10 mL ×

3) and brine (10 mL×3), and then dried over anhydrous $MgSO_4$. The solution was concentrated, and the crude product was purified by column chromatography (EtOAc/*n*-hexane = 1/10) to give the product as an oil (0.99 g, quantitative). IR ($CHCl_3$): 1696, 1738 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.92 (d, 6H), 1.36 (m, 1H), 1.61 (m, 2H), 2.34 (s, 3H), 2.68 (m, 1H), 3.05 (m, 2H), 3.70 (s, 3H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 22.6, 23.0, 26.4, 31.0, 31.2, 41.7, 44.3, 52.2, 175.5, 195.7.

***rac*-2-(Acetylthiomethyl)-4-methylpetanoic Acid Methyl Ester (12).** To a solution of triphenylphosphine (1.0 g, 4 mmol) in THF (15 mL) was added slowly diethyl azodicarboxylate (0.63 mL, 4 mmol) at 0 °C under a nitrogen atmosphere and stirred for 30 min at 0 °C. To the resulting mixture was added slowly a mixture of **11** (0.44 g, 2 mmol) and thioacetic acid (0.3 mL, 4 mmol) by a cannular and stirred for 4 h at room temperature. The reaction mixture was diluted with ethyl acetate and washed with saturated $NaHCO_3$ solution (10 mL × 3), then dried over anhydrous $MgSO_4$, and concentrated in vacuo, and the crude product was purified by column chromatography (EtOAc/*n*-hexane = 1/4) to give **12** as an oil (0.2 g, 36%). IR ($CHCl_3$): 2244, 3446 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.92 (d, 6H), 1.45 (m, 2H), 1.79 (m, 1H), 2.78 (m, 1H), 3.69 (d, 2H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 21.9, 23.3, 26.5, 33.7, 37.5, 63.3, 121.6.

***rac*-2-(Mercaptomethyl)-4-methylpentanoic Acid (13).** A mixture of **12** (0.11 g, 0.5 mmol) and 48% HBr (5 mL) was refluxed for 2 h. To the reaction mixture was added deoxygenated water (10 mL) and was extracted with ethyl acetate (10 mL × 3). The organic layer was dried over $MgSO_4$ and evaporated in vacuo to give **13** as an oil (0.08 g, 92%). IR ($CHCl_3$): 1708, 2574 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.93 (d, 6H), 1.44 (m, 2H), 1.63 (m, 2H), 2.11 (m, 3H), 2.71 (m, 2H), 2.95 (m, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 22.6, 23.1, 26.3, 26.5, 40.9, 47.8, 181.5. High-resolution mass spectroscopy (HRMS) (fast atom bombardment (FAB+)) $(M + H)^+$: calcd for $C_{11}H_{15}NO_2S$, 162.0715; found, 162.0714.

***rac*-2-(Mercaptomethyl)-3-cyclohexylpropanoic Acid (14).** Compound **14** was prepared as described in the literature.[19]

***rac*-2-(Phenylbutyl)propanedioic Acid Diethyl Ester (15).** To absolute EtOH (100 mL) at 0 °C was added sodium metal (1.03 g, 44.8 mmol). To the solution were added diethyl malonate (6.8 mL, 44.8 mmol) and 1-bromo-4-phenylbutane (6.4 g, 30 mmol). After the mixture was stirred at room temperature for 8 h, the solution was concentrated, acidified with 3 N HCl, and extracted with ether. The combined extracts were dried over anhydrous $MgSO_4$ and evaporated to give a crude product, which was purified by column chromatography (EtOAc/*n*-hexane = 1/4) to give **15** as a pale yellow oil (7.2 g, 82%). IR ($CHCl_3$): 1733 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.24 (t, 6H), 1.36 (m, 2H), 1.64 (m, 2H), 1.88 (m, 2H), 2.60 (t, 2H), 3.30 (t, 1H), 4.17 (q, 4H), 7.16 (m, 5H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 14.5, 27.3, 29.0, 31.4, 36.0, 52.4, 61.7, 126.1, 128.7, 128.8, 142.7, 169.9.

***rac*-(2-Phenylbutyl)propanedioic Acid (16).** A mixture of **15** (2.9 g, 10.0 mmol) and 4 N KOH solution (8.7 mL) was refluxed for 2 h. The solution was diluted with water, washed with ether (50 mL × 3), acidified with 3 N HCl, and extracted with ether (50 mL × 3). The organic phase was dried over anhydrous $MgSO_4$ and concentrated under the reduced pressure to give **16** as a white solid (2.08 g, 88%): mp 93−94 °C. IR ($CHCl_3$): 1716 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.44 (m, 2H), 1.65 (m, 2H), 1.98 (m, 2H), 2.62 (m, 2H), 3.43 (t, 1H), 7.23 (m, 5H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 27.2, 28.9, 31.3, 35.9, 52.0, 126.2, 128.7, 128.8, 142.5, 175.5.

***rac*-α-Methylene-6-phenylhexanoic Acid (17).** To a mixture of **16** (1.8 g, 7.6 mmol) and diethylamine hydrochloride (1.0 g, 9.1 mmol) was added 37% aqueous formaldehyde solution (1.1 mL, 15.1 mmol) at room temperature, and the resulting mixture was refluxed for 12 h. The solution was washed with ether (50 mL × 3), acidified with 3 N HCl, and extracted with ether (50 mL × 3). The organic phase was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give **17** as an oil (1.0 g, 62%). IR ($CHCl_3$): 1627, 1695 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.59 (m, 2H), 1.68 (m, 2H), 2.36 (m, 2H), 2.68 (m, 2H), 5.65 (s, 1H), 6.31 (s, 1H), 7.27 (m, 5H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 28.4, 31.4, 31.7, 36.1, 126.1, 127.5, 128.7, 128.8, 140.5, 142.9, 173.3.

***rac*-α-(Acetylthiomethyl)-6-phenylhexanoic Acid (18).** A mixture of **17** (0.38 g, 1.9 mmol) and thioacetic acid (0.2 mL, 5.7 mmol) in benzene (10 mL) was refluxed for 12 h. The solution was concentrated under reduced pressure, diluted with ethyl acetate, and washed with water (50 mL × 3). The organic phase was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give **18** as a pale yellow oil (0.47 g, 88%). IR ($CHCl_3$): 1698 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.43 (m, 2H), 1.66 (m, 2H), 1.71 (m, 2H), 2.32 (s, 3H), 2.61 (m, 2H), 2.62 (m, 1H), 3.07 (m, 2H), 7.21 (m. 5H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 27.0, 30.5, 31.0, 31.6, 32.0, 36.0, 46.0, 126.1, 128.7, 128.8, 142.7, 180.7, 195.9.

***rac*-2-(Mercaptomethyl)-6-phenylhexanoic Acid (19).** A mixture of **18** (0.10 g, 0.4 mmol) and 2-methoxyethylamine (5 mL) was stirred at room temperature for 1 h. The resulting mixture was acidified with 3 N HCl (10 mL) and extracted with ethyl acetate (10 mL × 3). The organic layer was dried over $MgSO_4$ and evaporated in vacuo to give **18** as an oil (0.09 g, 90%). IR ($CHCl_3$): 1705, 2565 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.41 (m, 2H), 1.61 (m, 4H), 2.60 (t, 2H), 2.82 (m, 2H), 2.98 (m, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 27.0, 31.6, 31.7, 36.0, 40.5, 45.6, 126.2, 128.7, 128.8, 142.7, 181.2. HRMS (FAB+) $(M + H)^+$: calcd for $C_{11}H_{16}NO_2S$, 239.1106; found, 239.1108.

Determination of K_i Value. Typically, the enzyme stock solution was added to a solution containing Hipp-L-Phe (final concentrations, 150 and 300 μM) and inhibitor (five different final concentrations in the range of 0.023−1.0 μM) in 0.05 M Tris/0.5 M NaCl, pH 7.5, buffer (1 mL cuvette), and the change in absorbance at 254 nm was measured immediately. The final concentration of CPA was 68.5 nM. Initial velocities were then calculated from the linear initial slopes of the change in absorbance where the amount of substrate consumed was less than 10%. The K_i values were then estimated from the semireciprocal plot of the initial velocity vs the concentration of the inhibitors according to the method of Dixon.[16] The correlation coefficients for the Dixon plots were above 0.992.

Acknowledgment. This work was supported by the Brain Korea 21 Project.

References

(1) Lipscomb, N. W.; Sträter, N. Recent Advances in Zinc Enzymology. *Chem. Rev.* **1996**, *96*, 2375−2433.
(2) Christianson, D. W.; Lipscomb, N. W. Carboxypeptidase A. *Acc. Chem. Res.* **1989**, *22*, 62−69.
(3) Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. Design and Therapeutic Application of Matrix Metalloproteinase Inhibitors. *Chem. Rev.* **1999**, *99*, 2735−2776.
(4) Rees, D. C.; Lewis, M.; Lipscomb, W. N. Refined Crystal Structure of Carboxypeptidase A at 1.54 Å Resolution. *J. Mol. Biol.* **1983**, *168*, 367−387.
(5) (a) Ondetti, M. A.; Rubin, B.; Cushman, D. W. Design of Specific Inhibitors of Angiotensin-Converting Enzyme: New Class of Orally Active Antihypertensive Agents. *Science* **1977**, *196*, 441−444. (b) Cushman, D. W.; Cheung, H. S.; Sabo, E. F.; Ondetti, M. A. Design of Potent Competitive Inhibitors of Angiotensin-Converting Enzyme. Carboxyalkanoyl and Mercaptoalkanoyl Amino Acids. *Biochemistry* **1977**, *16*, 5484−5491.
(6) Byers, L. D.; Wolfenden, R. Binding of the By-Product Analogue Benzylsuccinic Acid by Carboxypeptidase A. *Biochemistry* **1973**, *12*, 2070−2078.
(7) Horovitz, Z. P., Ed. Angiotensin Converting Enzyme Inhibitors. *Mechanisms of Action and Clinical Implications*; Urbans & Schwarzenberg: Baltimore−Munich, 1981.
(8) Ehlers, M. R. W.; Riordan, J. F. Angiotensin-converting Enzyme: New Concepts Concerning Its Biological Role. *Biochemistry* **1989**, *28*, 5312−5318.
(9) (a) Kim, D. H.; Kim, K. B. Design of a Novel Type of Zinc-Containing Protease Inhibitor. *J. Am. Chem. Soc.* **1991**, *113*, 3200−3202. (b) Kim, D. H.; Chung, S. J. Inactivation of Carboxypeptidase A by 2-Benzyl-3,4-epithiobutanoic Acid. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1667−1672. (c) Lee, K. J.; Kim, D. H. Inactivation of a Prototypic Zinc-Containing Protease with (*S*)-2-Benzyl-2-(oxo-2-isoxazolidinyl)acetic Acid. *Bioorg. Med. Chem.*

Lett. **1996**, *6*, 2431−2436. (d) Kim, K. J.; Joo, K.-J.; Lee, M.; Kim, D. H. A New Type of Carboxypeptidase A Inhibitors Designed Using an Imidazole as a Zinc Coordinating Ligand. *Bioorg. Med. Chem*. **1997**, *5*, 1989−1998. (e) Kim, D. H.; Lee, K. J. *O*-(Hydroxyacetyl)-L-*β*-phenylactic Acid as a New Type of Mechanism-Based Inactivator for Carboxypeptidase A. *Bioorg. Med. Chem. Lett*. **1997**, *7*, 2607−2612. (f) Lee, K. J.; Kim, D. H. Synthesis and Inhibitory Study of *N*-Oxide Containing Substrate Analogue Inhibitors of Carboxypeptidase A. *Bull. Korean Chem. Soc*. **1997**, *18*, 1100−1104. (g) Kim, D. H.; Chung, S. J.; Kim, E.-J.; Tian, G. R. Irreversible Inhibition of Zinc-Containing Protease by Oxazolidinone Derivatives. Novel Inactivation Chemistry. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 859−864. (h) Kim, D. H.; Jin, Y. First Hydroxamate Inhibitors for Carboxypeptidase A. *N*-Acyl-*N*-hydroxy-*β*-phenylalanines. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 691−696. (i) Lee, M.; Jin, Y.; Kim, D. H. 2-Benzyl-2-methylsuccinic Acid as Inhibitor for Carboxypeptidase A. Synthesis and Evaluation. *Bioorg. Med. Chem*. **1999**, *7*, 1755−1760. (j) Chung, S. J.; Kim, D. H. *N*-(Hydroxyaminocarbonyl)phenylalanine: A Novel Class of Inhibitor for Carboxypeptidase A. *Bioorg. Med. Chem*. **2001**, *9*, 185−189.

(10) Kim, D. H. A Three-Dimensional Active Site Model of Carboxypeptidase A. *Bull. Korean Chem. Soc*. **1994**, *15*, 805−807.

(11) Powers, J. C.; Harper, J. W. Inhibitors of Metalloproteases. In *Proteinase Inhibitors*; Barrett, A. J., Salvesan, G., Eds.; Elsevier Science Publishers: Amsterdam, 1986; pp 219−298.

(12) Chong, C. R.; Auld, D. S. Inhibition of Carboxypeptidase A by D-Penicillamine: Mechanism and Implications for Drug Design. *Biochemistry* **2000**, *39*, 7580−7588.

(13) van Aalten, D. M.; Chong, C. R.; Joshua-Tor, L. Crystal Structure of Carboxypeptidase A Complexed with D-Cysteine at 1.75 Å−Inhibitor-Induced Conformational Changes. *Biochemistry* **2000**, *39*, 10082−10089.

(14) Lee, G. H.; Pak, C. S.; Lee, H. W. Synthesis of *N*-Phenylcysteine. *Bull. Korean Chem. Soc*. **1988**, *9*, 25−26.

(15) Ondetti, M. A.; Condon, M. E.; Reid, J.; Sabo, E. F.; Cheung, H. S.; Cuchman, D. W. Design of Potent and Specific Inhibitors of Carboxypeptidase A and B. *Biochemistry* **1979**, *18*, 1427−1430.

(16) Dixon, M. Determination of Enzyme−Inhibitor Constants. *Biochem. J*. **1953**, *55*, 170−171.

(17) Kim, D. H.; Shin, Y. S.; Kim, K. B. The Structural Feature of S_1' Subsite of Carboxypeptidase A. *Bioorg. Med. Chem. Lett*. **1991**, *1*, 317−322.

(18) Blondeau, P.; Berse, C.; Gravel, D. Dimerization of an Intermediate during the Sodium in Liquid Ammonia Reduction of L-Thiazolidine-4-carboxylic Acid. *Can. J. Chem*. **1967**, *45*, 49−52.

(19) Kim, K. B. Studies on the Nature of Hydrophobic Pocket of Carboxypeptidase A. M.S. Thesis, Pohang University of Science and Technology, 1991.

JM010272S