Alpha-alkylcysteines as Inhibitors for Carboxypeptidase A. Synthesis, Evaluation, and Implication for Inhibitor Design Strategy

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(*R*,*S*)- and (*R*)-2-Benzylcysteine (**1**) and (*R*,*S*)-2-phenethylcysteine (**2**) were synthesized and evaluated as inhibitors for carboxypeptidase A (CPA) with the expectation that these compounds exhibit improved inhibitory activities over 2-benzyl-3-mercaptopropanoic acid (BMPA), a potent CPA competitive inhibitor, possibly having additional interactions of their amino group with the carboxylate of Glu-270 of the enzyme upon binding to CPA. Contrary to the expectation, however, the CPA inhibitory potencies of these compounds were found to be much reduced compared with that of BMPA, suggesting that the amino group in the inhibitors rather exerts steric hindrance in binding of these inhibitors to CPA.

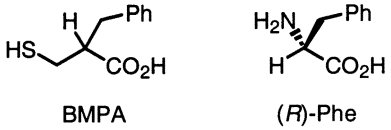
Keywords : Carboxypeptidase A, Enzyme inhibition, Hydrogen bonding, Steric hindrance.

Introduction

Carboxypeptidase A (CPA), a most extensively studied zinc-containing proteolytic enzyme, removes the C-terminal amino acid having a hydrophobic side chain, and represents a large family of physiologically and pathologically important zinc proteases such as angiotensin converting enzyme and matrix metalloproteases.[1] As a prototypical zinc proteases, CPA has served as a model target for developing design strategies of enzyme inhibitors that are effective against zinc-contaning proteases of medicinal interest.[2] The most important residues at the active site are Glu-270 and Arg-145. The former is directly involved in the catalytic hydrolysis of substrate, and the latter forms hydrogen bonds with the C-terminal carboxylate of substrate. In addition, there is present a hydrophobic pocket, the primary function of which is to recognize substrate by accommodating the aromatic side chain in the P_1' residue of substrate. The catalytically essential zinc ion is coordinated to side chain functional groups of His-69, Glu-72, and His-196. A water molecule is loosely bound to the zinc ion as the fourth ligand. The carboxylate of Glu-270 activates the zinc-bound water molecule to serve as an effective nucleophile that attacks on the scissile peptide bond of the substrate. The crystal structure of the enzyme revealed that the guanidinium moiety of Arg-145 and the carboxylate of Glu-270 are located near the surface of the enzyme molecule and the recognition pocket is invaginated deep into the core of the molecule.[3]

2-Benzyl-3-mercaptopropanoic acid (BMPA) is a potent competitive inhibitor (K_i = 0.011 μM) of CPA reported by Ondetti *et al.*[4] The design of this inhibitor was based on the observation that sulfhydryl group has a strong propensity to coordinate to the zinc ion present at the active site of angiotensin converting enzyme, a physiologically important zinc containing enzyme.[5] Thus, in binding of BMPA to CPA, the carboxylate in the inhibitor was thought to interact with the guanidinium moiety of Arg-145, the phenyl ring is fitted in the hydrophobic pocket at the active site of CPA, and the sulfhydryl group coordinates to the active site zinc ion. Figure 1 depicts schematically the binding mode of BMPA to CPA. Of a pair of enantiomers, BMPA having the (*S*)-configuration was shown to render most of the inhibitory activity.[6] (*R*)-Phe is a relatively weak inhibitor for CPA.[7] The X-ray crystal structure of CPA·(*R*)-Phe complex revealed that the amino group of the CPA-bound (*R*)-Phe is engaged in a salt bridge formation with the carboxylate of Glu-270 of CPA having the bond distance of 2.3 Å.[8] The binding mode of (*R*)-Phe to CPA is schematically shown in Figure 2.

It was thought to be of interest to evaluate compound **1** and its analog **2** as inhibitors for CPA. Compound **1** bears an amino group at the α-position of BMPA, and hence **1** was expected to bind to CPA more tightly than BMPA by virtue



Structure 1

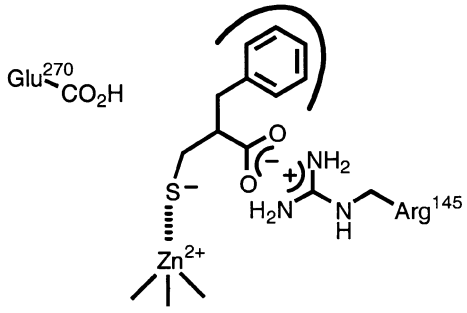


Figure 1. Schematic representation for the binding of BMPA to carboxypeptidase A. Note that the sulfhydryl group of BMPA coordinates to the zinc ion at the active site of the enzyme.

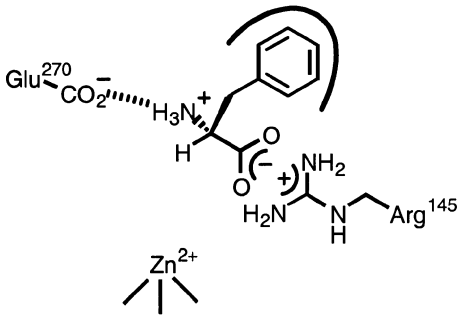


Figure 2. Schematic representation for the binding mode of (*R*)-Phe to carboxypeptidase A as determined by the X-ray structural analysis. Note that the amino group of (*R*)-Phe forms a salt bridge with the carboxylate of Glu-270 at the active site of the enzyme.

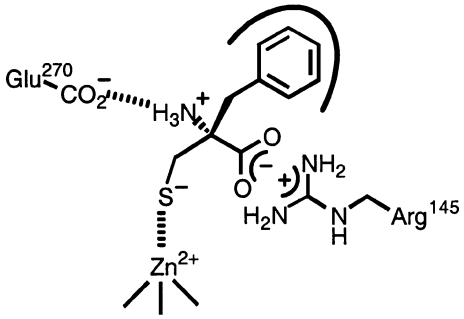
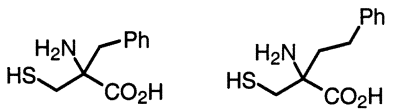


Figure 3. Postulated binding mode of (*R*)-**1** to carboxypeptidase A. The sulfhydryl and amino groups in (*R*)-**1** are thought to interact with the zinc ion and Glu-270 carboxylate, respectively, so that the binding affinity of (*R*)-**1** would be enhanced over BMPA.



1 **2**

Structures 2

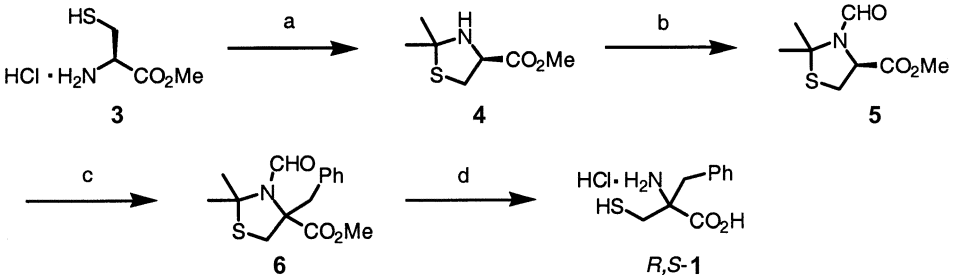
of the additional amino group interactions with the carboxylate of Glu-270 in CPA as illustrated schematically in Figure 3. This report describes synthesis of the potential inhibitors, **1** and **2** and kinetic analysis of their CPA inhibition.

Results and Discussion

Chemistry. Racemic **1** was synthesized by the route outlined in Scheme 1: (*R*)-cysteine methyl ester was treated with acetone under reflux conditions to afford thiazolidine **4** which was *N*-formylated using sodium formate in the presence of acetic anhydride and formic acid to give **5**. Treatment of a solution of **5** in tetrahydrofuran at 90 °C with lithium diisopropylamide generated the corresponding enolate which was allowed to react with benzyl bromide to yield **6**. The latter was subjected to acidic hydrolysis to obtain (*R,S*)-**1**. (*R*)-**1** was prepared by the method reported by Pattenden *et al.*[9]

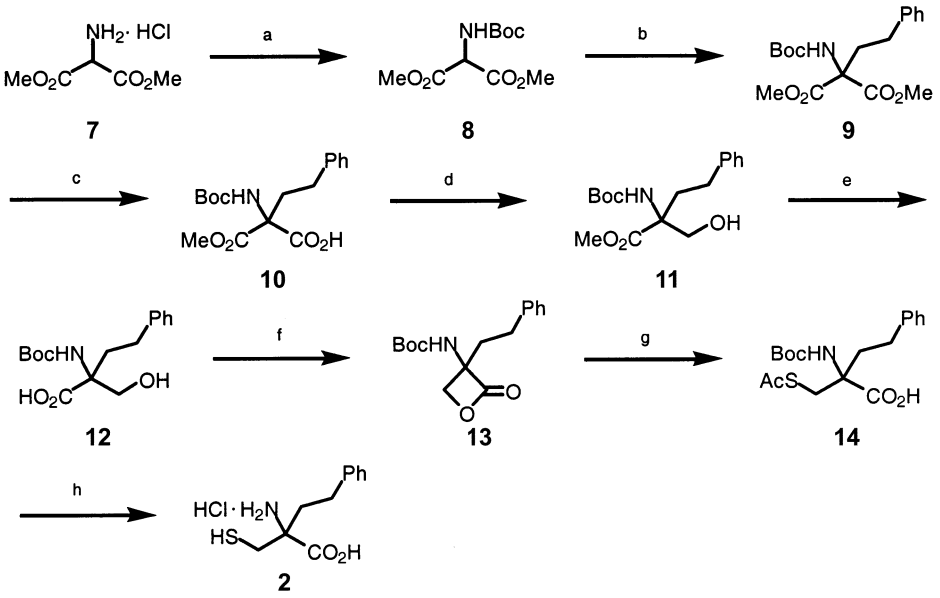
Since attempts at the introduction of a phenethyl group by the method of Pattenden met with failure, an alternative rout to the synthesis of **2** had to be devised. The reason why the enolate fails to react with phenethyl iodide is not apparent to us. Recently, Vederas *et al*. reported that *N*-protected 3-aminooxazetinoes undergo facile ring opening reactions with the C-O bond cleavage when treated with a soft nucleophile, affording *N*-protected *β*-substituted alanines.[10] Our successful synthesis of **2** makes use of the finding of Vederas *et al.* as outlined schematically in Scheme 2. Dimethyl 2-*tert*-butoxycarbonylaminomalonate (**8**) that was prepared from dimethyl 2-aminomalonate by treatment with di-*tert*-butyl dicarbonate was treated with sodium hydride in DMF to generate the corresponding enolate, and the latter was allowed to react with (2-iodoethyl)benzene at 100 °C to afford **9** in 80% yield. One of the ester moieties in **9** was converted into the corresponding carboxylate under the conditions reported by Niwayama[11] to give **10** which was then reduced with sodium borohydride to the corresponding hydroxymethyl group after being converted into an activated ester by treatment with isobutyl chloroformate. Compound **11** thus obtained was treated with aqueous lithium hydroxide solution, whereby the methyl ester moiety was hydrolyzed to obtain **12**. Lactonization of **12** to give **13** was effected in 55% yield by the Mitsunobu method modified by Vederas *et al.*[10] Treatment of **13** with potassium thioacetate in DMF cleaved the *β*-lactone ring to give **14** which was hydrolyzed under acidic conditions to yield the desired **2**.

Kinetic Studies. The compounds thus synthesized were assayed for their inhibitory activities of CPA by a standard method at pH 7.5 using hippuryl-L-phenylalanine (Hip-L-Phe) as the substrate. Their inhibitory constants (K_is) were estimated from the respective Dixon plot[12] that was constructed with the primary kinetic data obtained from the assay and are collected in Table 1. Figure 4 exemplifies the



3 **4** **5** **6** ***R,S*-1**

Scheme 1. (a) Et_3N, Acetone, reflux, 24 h, 97%; (b) acetic anhydride, sodium formate, formic acid, rt, 10 h, 75%; (c) LDA, $C_6H_5CH_2Br$, THF, -90 °C, 30%; (d) 6 N HCl, reflux, 48 h, 90%.



Scheme 2. (a) Boc_2O, Et_3N, CH_2Cl_2; (b) NaH, (2-iodoethyl)benzene, DMF, 100 °C, 2 h, 80%; (c) 1 N NaOH, H_2O/THF (5/1), rt, 4 h, 92%; (d) i) isobutyl chloroformate, Et_3N, THF, 0 °C, ii) $NaBH_4$, 0 °C, 1 h, 71% (two steps); (e) LiOH, MeOH, rt, 1 day, 93%; (f) Ph_3P, DMAD, THF, -78 °C, 55%; (g) AcSK, DMF, rt, 30 min, 90%; (h) 6 N HCl, reflux, 2 h, 98%.

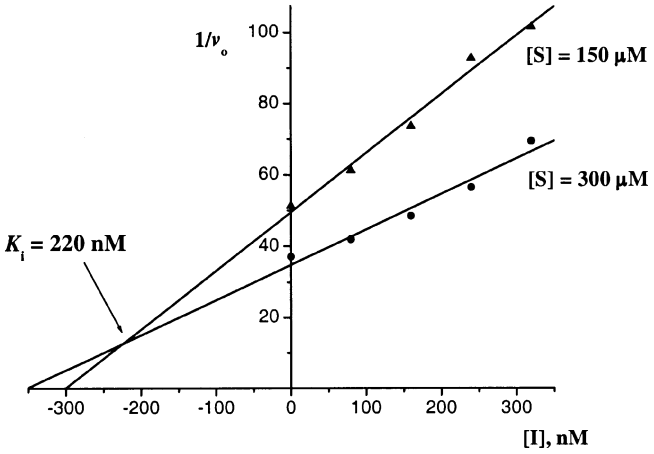


Figure 4. The Dixon plot for the inhibition of carboxypeptidase A with (*R*,*S*)-**1**.

Dixon plot.

Contrary to the expectation, (*R*,*S*)-**1** was a less potent inhibitor for CPA than BMPA by 20-fold. Although (*R*)-**1** is more potent than its racemic form, it is also less potent than the optically active BMPA that belongs to the same configurational series as (*R*)-**1**, *i.e.*, (*S*)-BMPA by 13-fold. It occurred to us that the binding interactions of the amino group in the inhibitors with the Glu-270 carboxylate may cause the inhibitor molecules to move closer towards the carboxylate, and as a result the benzene ring in **1** may not be perfectly fitted in the S_1' pocket of CPA as the benzene ring in BMPA does. If this would occur, the binding affinity of the inhibitors may be reduced. That is, the reduced inhibitory activity shown by (*R*,*S*)- and (*R*)-**1** may be resulted by the imperfect anchoring of the benzene ring of these inhibitors. In order to probe this hypothesis, we have evaluated

Table 1. K_i values for CPA inhibition

Inhibitor	K_i (μM)
(*R*,*S*)-BMPA	0.011[a]
(*S*)-BMPA	0.0078[b]
(*R*)-Phe	2000[c]
(*R*,*S*)-**1**	0.22 ± 0.07
(*R*)-**1**	0.1 ± 0.03
(*R*,*S*)-**2**	0.17 ± 0.02

[a]ref. 4. [b]ref. 6. [c]ref. 7.

inhibitor **2** in which the benzene ring is separated from the α-position to the carboxylate in BMPA by an ethylene unit. The K_i value obtained for (*R*,*S*)-**2** was, however, found to be not significantly different from that for (*R*,*S*)-**1** as can be seen in Table 1. These kinetic results suggest strongly that the amino group in **1** as well as **2** may not be engaged in the salt bridge that is seen in the complex of CPA·(*R*)-Phe.[8] It appears that the amino group in these inhibitors rather exert steric hindrance in their binding to CPA. Streochemically, the amino group in (*R*)-**1** corresponds to the proton at the α-position of the P_1' residue of substrate, but the active site of the enzyme that has been evolved to selectively accommodate substrate can difficultly accept a large group at the position.[13] These inhibitors may not anchor in the active site of CPA freely and the sulfhydryl group cannot form a coordinative bond of maximum strength with the active site zinc ion.

In conclusion, it can be surmised that the amino group in **1** and **2** renders deterrent effect in binding of BMPA to CPA by virtue of the unfavorable steric effect of the amino group in binding of the inhibitors to CPA, rather than improving the inhibitory activity with formation of the salt bridge with the carboxylate of Glu-270 expected on the basis of the X-ray

crystal structure of CPA·(*R*)-Phe complex. The present study demonstrates that design of CPA inhibitors that can bind the target enzyme through contacts at more than three points is hard to achieve.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and were uncorrected. IR spectra were recorded on a Bruker Equinox 55 FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were obtained with a Bruker AM 300 (300 MHz) NMR spectrometer using tetramethylsilane as the internal standard. Silica gel 60 (230-400 mesh) was used for flash chromatography and thin layer chromatography (TLC) was carried out on silica coated glass sheets (Merck silica gel 60 F-254). Elemental analyses were performed at Pohang University of Science and Technology, Pohang, Korea.

Methyl (4*R*)-2,2-dimethyl-1,3-thiazolidine-4-carboxylate (4). A solution of (*R*)-cysteine methyl ester · hydrochloride (**4**, 8.58 g, 50 mmol) and triethylamine (7.68 mL, 55 mmol) in acetone (80 mL) was refluxed for 24 h. The reaction mixture was filtered and the filtrate was concentrated *in vacuo* to afford the product (8.50 g, 97%) as a colorless oil. IR (neat) 1742 cm^{-1}; ^{1}H NMR 300 MHz ($CDCl_3$) δ 1.33 (s, 3H), 1.51 (s, 3H), 2.84 (dd, 1H), 3.24 (dd, 1H) 3.90 (dd, 1H); ^{13}C NMR 300 MHz ($CDCl_3$) δ 30.68, 32.88, 40.44, 52.70, 64.59, 76.00, 172.18.

Methyl (4*R*)-2,2-dimethyl-3-formyl-1,3-thiazolidine-4-carboxylate (5). Acetic anhydride (11.3 mL, 120 mmol) was added to an ice-chilled formic acid solution (50 mL) containing thiazolidine **4** (7.01 g, 40 mmol) and sodium formate (3.22 g, 48 mmol). The reaction mixture was stirred for 10 h at room temperature. The solvent was removed in vacuo and the residue was then carefully neutralized with saturated aqueous sodium bicarbonate solution and extracted with ether. The extract was dried over magnesium sulfate and evaporated *in vacuo* to give the product (6.11 g, 75%) as a colorless oil. IR (neat) 1751, 1666 cm^{-1}; ^{1}H NMR 300 MHz ($CDCl_3$) δ 1.75 (s, 3H), 1.78 (s, 3H), 3.26 (m, 2H), 3.71 (s, 3H) 5.00 (dd, 1H), 8.29 (s, 1H); ^{13}C NMR 300 MHz ($CDCl_3$) δ 31.35, 31.45, 32.37, 53.22, 62.74, 70.55, 160.33, 170.04.

Methyl (4*R*)-2,2-dimethyl-3-formyl-4-benzyl-1,3-thiazolidine-4-carboxylate (6). A solution of butyl lithium (1.6 M in hexane, 13.8 mL, 22 mmol) was added dropwise to a stirred solution of diisopropylamine (4.2 mL, 30 mmol) in dry THF (80 mL) at -78 °C under nitrogen atmosphere. 1,3-Dimethyl-3,4,5,6-tetrehydro-2(1H)-pyrimidinone (10 mL) was added in one portion, and the mixture was then stirred at -78 °C for 1 h. To the resulting mixture was added dropwise a solution of thiazolidine **5** (4.07 g, 20 mmol) in THF with stirring at -90 °C, and the stirring was continued for 45 min at -90 °C. Benzyl bromide (2.9 mL, 24 mmol) was added dropwise. The reaction mixture was stirred for 2 h at -90 °C, warmed to room temperature, treated with brine, then extracted with ether. The organic layer was dried over magnesium sulfate and concentrated *in vacuo*. The crude product was purified by column chromatography (EtOAc/hexane = 1/3) to afford the product (1.76 g, 30%) as a colorless oil. IR (neat) 1740, 1668 cm^{-1}; ^{1}H NMR 300 MHz ($CDCl_3$) δ 1.13 (s, 3H), 1.77 (s, 3H), 3.05-3.38 (4H, m), 3.74 (s, 3H), 7.12-7.31 (m, 5H), 8.31 (s, 1H); ^{13}C NMR 300 MHz ($CDCl_3$) δ 31.78, 31.83, 35.66, 38.50, 53.41, 72.01, 75.05, 160.60, 172.18.

(*R,S*)-2-Benzylcysteine · hydrochloride ((*R,S*)-1). A solution of **6** (0.21 g, 0.7 mmol) in 6 N HCl was refluxed for 48 h and cooled to room temperature. The reaction mixture was washed with ethyl acetate to remove any unreacted **6** and the aqueous layer was evaporated *in vacuo* to afford the product (0.17 g, 90%) as a hygroscopic solid. IR (KBr) 2514, 1732 cm^{-1}; ^{1}H NMR 300 MHz (D_2O) δ 2.81 (d, 1H), 3.03 (d, 1H), 3.18 (d, 1H), 3.23 (d, 1H), 7.11-7.26 (m, 5H); ^{13}C NMR 300 MHz (D_2O) δ 29.4, 41.1, 66.2, 128.7, 129.5, 130.5, 132.8, 172.1.

Dimethyl (*R,S*)-α-(*tert*-butoxycarbonylamino)malonate (8). Triethylamine (0.84 mL, 6.0 mmol) and di-*tert*-butyl dicarbonate (1.31 g, 6.0 mmol) was added dropwise to a solution of dimethyl aminomalonate · hydrochloride (**7**, 1.00 g, 5.5 mmol) in dichloromethane at 0 °C and the reaction mixture was stirred for 24 h at room temperature. The solution was washed with 0.1 N HCl, 5% aqueous $NaHCO_3$ solution, and brine. The organic layer was dried over magnesium sulfate and concentrated *in vacuo* to give the product (1.29 g, 95%) as a colorless oil. IR (neat) 3381, 2979, 1743, 1720 cm^{-1}; ^{1}H NMR 300 MHz ($CDCl_3$) δ 1.42 (s, 9H), 3.79 (s, 6H), 4.97 (d, 1H), 5.54 (d, 1H); ^{13}C NMR 300 MHz ($CDCl_3$) δ 28.6, 53.8, 57.6, 81.2, 155.1, 167.5.

Dimethyl (*R,S*)-α-(*tert*-butoxycarbonylamino)-α-phenethylmalonate (9). Compound **8** (5.0 g, 20.2 mmol) was added to the suspension of sodium hydride (60% dispersion in mineral oil, 0.81 g, 20.2 mmol) in DMF at 0 °C. When H_2 evolution was ceased, (2-iodoethyl)benzene was added to the mixture and stirred for 2 h at 100 °C. The reaction mixture was cooled to room temperature and extracted with ethyl acetate. The extract was washed with 5% sodium thiosulfate to remove DMF. The organic layer was dried over magnesium sulfate and concentrated under reduced pressure. The crude product thus obtained was purified by column chromatography (EtOAc/hexane = 1/5) to obtain the product (5.7 g, 80%) as a colorless oil. IR (neat) 3425, 2980, 1743, 1720 cm^{-1}; ^{1}H NMR 300 MHz ($CDCl_3$) δ 1.44 (s, 9H), 2.52 (m, 2H), 2.64 (m, 2H), 3.71 (s, 6H), 5.99 (s, 1H), 7.14-7.29 (m, 5H); ^{13}C NMR 300 MHz ($CDCl_3$) δ 28.7, 30.3, 34.8, 53.7, 66.7, 80.9, 126.5, 128.7, 128.9, 141.0, 154.3, 169.0.

Monomethyl (*R,S*)-α-(*tert*-Butoxycarbonylamino)-α-phenethylmalonate (10). Compound **9** (0.84 g, 2.4 mmol) was dissolved in 8 mL of THF, and 40 mL of water was added. To the resulting mixture was added 4.8 mL of 1 N NaOH at 0 °C, and stirred at room temperature for 4 h. The reaction mixture was washed with ethyl acetate, acidified with 1 N HCl, and extracted with ethyl acetate. The combined extract was dried over magnesium sulfate and evaporated

in vacuo to give a white solid which was recrystallized from EtOAc/hexane to yield **10** (0.74 g, 92%). Mp 128-129 °C; IR (KBr) 3295, 2980, 1729, 1660 cm^{-1}; ^1H NMR 300 MHz ($CDCl_3$) δ 1.42 (s, 9H), 2.61 (t, 4H), 3.78 (s, 3H), 7.16-7.28 (m, 5H), 7.89 (s, 1H); ^{13}C NMR 300 MHz ($CDCl_3$) δ 28.6, 30.4, 35.8, 53.4, 67.1, 83.3, 126.4, 128.82, 128.84, 141.4, 156.4, 169.4, 170.6. Anal. Calcd. for $C_{17}H_{23}NO_6$: C, 60.52; H, 6.87; N, 4.15. Found: C, 60.59; H, 6.92; N, 4.01.

Methyl (*R,S*)-2-(*tert*-butoxycarbonylamino)-2-(hydroxymethyl)-4-phenylbutanoate (11). Isobutyl chloroformate (0.27 mL, 2.1 mmol) and triethylamine (0.31 mL, 2.3 mmol) were added to an ice-chilled THF solution containing **10** (0.70 g, 2.1 mmol). After stirring for 5 min at 0 °C, the reaction mixture was filtered. Sodium borohydride (95 mg, 2.5 mmol) was added carefully to the ice-chilled filtrate with stirring. The stirring was continued for 1 h, and the reaction was quenched with 0.1 N HCl and the solvent was evaporated *in vacuo*. The concentrated residue was dissolved in ethyl acetate, and the ethyl acetate solution was washed with 5% aqueous $NaHCO_3$ solution, 0.1 N HCl and water. The extract was dried over magnesium sulfate and concentrated *in vacuo*. The crude product was purified by column chromatography (EtOAc/hexane = 1/5) to obtain the product (0.48 g, 71%) as a colorless oil. IR (neat) 3425, 2980, 1716 cm^{-1}; ^1H NMR 300 MHz ($CDCl_3$) δ 1.46 (s, 9H), 2.05 (m, 1H), 2.42 (m, 2H), 2.61 (m, 1H), 3.72 (s, 3H), 3.81 (d, 1H), 4.15 (d, 1H), 5.67 (s, 1H), 7.12-7.28 (m, 5H); ^{13}C NMR 300 MHz ($CDCl_3$) δ 28.7, 30.5, 34.1, 53.2, 65.8, 66.0, 80.6, 126.5, 128.8, 141.2, 155.3, 173.4.

(*R,S*)-2-(*tert*-Butoxycarbonylamino)-2-(hydroxymethyl)-4-phenylbutanoic acid (12). 1 N LiOH (1.5 mL) was added to a solution of **11** (0.4 g, 1.2 mmol) in MeOH and the mixture was stirred for 12 h at room temperature. Solvent was removed *in vacuo*, the residue was dissolved in ethyl acetate, and washed with 0.1 N HCl. The organic layer was dried over magnesium sulfate and evaporated *in vacuo* to afforded the product (0.37 g, 98%) as a white solid. Mp 134-135 °C; IR (KBr) 3425, 2062, 1641 cm^{-1}; ^1H NMR 300 MHz ($CDCl_3$) δ 1.49 (s, 9H), 2.08 (m, 1H), 2.51 (m, 2H), 2.62 (m, 1H), 3.94 (d, 1H), 4.14 (d, 1H), 5.72 (s, 1H), 6.63 (br, 1H), 7.15-7.29 (m, 5H); ^{13}C NMR 300 MHz ($CDCl_3$) δ 28.8, 30.5, 34.2, 65.6, 66.0, 81.2, 126.5, 128.8, 128.9, 141.3, 156.0, 175.6. Anal. Calcd. for $C_{16}H_{23}NO_5$: C, 62.12; H, 7.49; N, 4.53. Found: C, 62.18; H, 7.68; N, 4.41.

(*R,S*)-3-(*tert*-Butoxycarbonylamino)-3-phenethyl β-lactone (13). To a stirred solution of triphenylphosphine (0.64 g, 2.5 mmol) in anhydrous THF was added dimethyl azodicarboxylate (40% solution in toluene, 0.9 mL, 2.5 mmol) at -78 °C, and the stirring was continued for 10 min. A solution of compound **12** (0.63 g, 2.1 mmol) in THF was added dropwise to the stirred white slurry at -78 °C. The mixture was stirred for additional 20 min at -78 °C and then for 2.5 h at 20 °C. The solvent was removed *in vacuo*, and the residue was purified by column chromatography (EtOAc/hexane = 1/5) to afford the product (0.34 g, 55%) as a white crystalline solid. Mp 108-109 °C; IR (KBr) 3434, 1831, 1739, 1646 cm^{-1}; ^1H NMR 300 MHz ($CDCl_3$) δ 1.47 (s, 9H), 2.22 (t, 2H), 2.82 (m, 2H), 4.12 (d, 1H), 4.67 (d, 1H), 4.89 (br, 1H), 7.19-7.35 (m, 5H); ^{13}C NMR 300 MHz ($CDCl_3$) δ 28.5, 30.4, 35.9, 70.4, 70.8, 78.8, 127.1, 128.7, 129.2, 140.1, 154.5, 176.2. Anal. Calcd. for $C_{16}H_{21}NO_4$: C, 65.96; H, 7.27; N, 4.81. Found: C, 65.54; H, 7.33; N, 4.85.

(*R,S*)-2-*tert*-Butoxycarbonylamino-2-(acetylthiomethyl)-4-phenylbutanoic acid (14). Potassium thioacetate (0.40 g, 2.8 mmol) was added to a solution of **13** (0.34 g, 1.17 mmol) in DMF and the reaction mixture was stirred for 30 min at room temperature. The reaction mixture was diluted with ethyl acetate and washed with 5% aqueous solution of sodium thiosulfate to remove DMF. The organic layer was washed with 0.1 N HCl, dried over magnesium sulfate, and concentrated *in vacuo* to afford the product (0.39 g, 90%) as a white crystalline solid. Mp 133-134 °C; IR (KBr) 3416, 3026, 2980, 2581, 1697 cm^{-1}; ^1H NMR 300 MHz ($CDCl_3$) δ 1.47 (s, 9H), 2.18 (t, 1H), 2.29 (s, 3H), 2.56 (m, 3H), 3.60 (d, 1H), 3.82 (d, 1H), 5.64 (br, 1H), 7.15-7.29 (m, 5H), 8.84 (br, 1H); ^{13}C NMR 300 MHz ($CDCl_3$) δ 28.7, 30.8, 30.9, 34.9, 37.3, 63.7, 80.9, 126.6, 128.8, 128.9, 141.0, 151.4, 155.7, 176.0. Anal. Calcd. for $C_{18}H_{25}NO_5S$: C, 58.83; H, 6.86; N, 3.81. Found: C, 58.75; H, 7.06; N, 3.64.

(*R,S*)-2-Phenethylcysteine · hydrochloride (2). A solution of **14** (0.18 g, 0.49 mmol) in 6 N HCl was refluxed for 2 h and cooled to room temperature. The reaction mixture was washed with ethyl acetate and the aqueous layer was concentrated *in vacuo* to afford the product (0.13 g, 98%) as a hygroscopic solid. IR (KBr) 2558, 1639 cm^{-1}; ^1H NMR 300 MHz (MeOH-D_4) δ 2.17 (t, 2H), 2.54 (m, 1H), 2.77 (m, 1H), 3.00 (d, 1H), 3.15 (d, 1H), 7.14-7.29 (m, 5H); ^{13}C NMR 300 MHz (MeOH-D_4) δ 29.6, 29.8, 37.7, 64.7, 126.6, 128.3, 128.7, 140.2, 170.7; HRMS (FAB+) calcd for ($C_{11}H_{15}NO_2S$, H^+): 226.0902; found: 226.0904.

General remarks for kinetic experiments. Carboxypeptidase A was purchased from Sigma Chemical Co. (Allan form, twice crystallized from bovine pancrease, aqueous suspension in toluene) and used without further purification. Hip-L-Phe purchased from Sigma Chemical Co. was used as substrate. All solutions were prepared by dissolving in doubly distilled and deionized water and stock assay solutions were filtered before use. CPA stock solutions were prepared by dissolving CPA in 0.05 M Tris/0.5 M NaCl, pH 7.5 buffer solution and their concentrations were estimated from the absorbance at 278 nm (ε_{278} = 64,200). A Perkin-Elmer HP 8453UV-vis spectrophotomer was used for UV absorbance measurements.

Dertermination of K_i. The initial velocities were obtained from the linear plot of the substrate hydrolysis monitored by following the increase of absorbance at 254 nM. The K_i values were then estimated from the semireciprocal plot of the initial velocity versus the concentration of the inhibitors according to the substrate were used. Typically, an aliquot of the enzyme stock solution was added to a cuvette (1 mL) containing the buffer solution of the inhibitor and substrate, and the rate of absorption increase at 254 nm was recorded immediately.

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