VOLUME 116, NUMBER 17
AUGUST 24, 1994

JOURNAL
OF THE
AMERICAN
CHEMICAL
SOCIETY

Conscripting the Active-Site Zinc Ion in Carboxypeptidase A in Inactivation Chemistry by a New Type of Irreversible Enzyme Inactivator†

Yasuhiro Tanaka, Ioannis Grapsas, Srikanth Dakoji, Young June Cho, and Shahriar Mobashery*

Contribution from the Department of Chemistry, Wayne State University, Detroit, Michigan 48202

Received September 17, 1993®

Abstract: A new strategy for irreversible inactivation of the metalloenzyme carboxypeptidase A (CPA) involving a proposed activation of a carbon–iodide bond in an inactivator by the active-site zinc ion toward nucleophilic substitution is described. 2-Benzyl-3-iodopropanoic acid (compound **1**) was designed to bind the active site of CPA. An energy-minimized complex of **1** in the active site of CPA reveals that the iodo moiety comes within the coordination sphere of the zinc ion. Such metal coordination was expected to facilitate the departure of the halide in an S_N2-type reaction by the side-chain functions of either Glu-270 or Tyr-248. Compound **1** was shown to inactivate CPA in a time-dependent manner, a process which was active-site directed and irreversible; a rate enhancement of approximately 10^8- to 10^9-fold is estimated for the inactivation chemistry by **1** over model metal-activated S_N2 type reactions. 2-Benzyl-4-iodobutanoic acid (compound **6**), an analog of **1** with an extended structure by one methylene unit, was shown to serve solely as a poor competitive inhibitor for CPA (K_i = 0.41 ± 0.07 mM) but not as an irreversible inactivator; a discussion of the kinetic behavior by the two compounds is provided. The results reported herein hold the promise of a novel chemistry for selective inactivation of metalloenzymes.

Transition-metal activation in Friedel–Crafts alkylation (*e.g.*, the $RX–AlX_3$ species)[1] and solvolysis of alkyl halides in the presence of silver(I)[2] constitute two classical examples of alkyl halide activation reactions. However, the nature of coordination of alkyl halides as weak Lewis bases to transition metals has been investigated only recently.[3–6] Such interactions have been documented for both intermolecular and intramolecular cases. Recently, a few well-studied examples for transition-metal-mediated enhancement of reactions at unactivated alkyl halides toward nucleophilic substitution have been reported.[4,5] The rate enhancements for nucleophilic displacement for some of these reactions have been measured at 10^5- to 10^6-fold at room temperature.[5] If such a transition-metal-mediated rate enhancement for nucleophilic substitution of an *unactivated* alkyl halide

†This paper is inscribed to Professor Koji Nakanishi on the occasion of his seventieth birthday.

• Abstract published in *Advance ACS Abstracts*, August 1, 1994.

(1) Olah, G. *Friedel-Crafts and Related Reactions*; Interscience: New York, 1963; Vols. 1 and 2. Roberts, R. M.; Khalaf, A. A. *Friedel-Crafts Alkylation Chemistry*; Marcel Dekker: New York, 1984.

(2) Kevill, D. N. In *The Chemistry of Halides, Pseudo-Halides and Azides*, Part 2, Suppl. D; Patai, S., Rappoport, Z., Eds.; Wiley-Interscience: New York, 1983; pp 933–984.

(3) Yang, P. F.; Yang, G. K. *J. Am. Chem. Soc.* **1992**, *114*, 6937. Gomes-Carneiro, T. M.; Jackson, R. D.; Downing, J. H.; Orpen, A. G.; Pringle, P. G. *J. Chem. Soc., Chem. Commun.* **1991**, 317. Kulawiec, R. J.; Faller, J. W.; Crabtree, R. H. *Organometallics* **1990**, *9*, 745. Colsman, M. R.; Newbound, T. D.; Marshall, L. J.; Noirot, M. D.; Miller, M. M.; Wulfsberg, G. P.; Frye, J. S.; Anderson, O. P.; Strauss, S. H. *J. Am. Chem. Soc.* **1990**, *112*, 2349. Brown, M.; Waters, J. M. *J. Am. Chem. Soc.* **1990**, *112*, 2442. Winter, C. H.; Arif, A. M.; Gladysz, J. A. *Organometallics* **1989**, *8*, 219. Barceló, F.; Lahuerta, R.; Ubeda, M. A.; Foces-Foces, C.; Cano, F. H.; Martínez-Ripoll, M. *Organometallics* **1988**, *7*, 584. Kulawiec, R. J.; Holt, E. M.; Lavin, M.; Crabtree, R. H. *Inorg. Chem.* **1987**, *26*, 2559. Catala, R. M.; Cruz-Garritz, D.; Hills, A.; Hughes, D. L.; Richards, R. L.; Sosa, P.; Torrens, H. *J. Chem. Soc., Chem. Commun.* **1987**, 261. Liotta, F. J.; Lahuerta, R.; Van Duyne, G.; Carpenter, B. K. *Organometallics* **1987**, *6*, 1010.

0002-7863/94/1516-7475$04.50/0

could be promoted in the active sites of metalloenzymes, this chemistry would have the potential as a general method for selective targeting of metalloenzymes for inactivation. Among metalloenzymes, zinc proteases carry out a wide range of important physiological, pathological, and regulatory processes in living cells;[7] hence, selective inhibitors for these enzymes are widely sought.[8] We report herein a new strategy for inactivation of carboxypeptidase A (CPA), a prototypic metallopeptidase, which is suggested to exploit the active-site zinc ion in its irreversible inactivation of the enzyme.

Experimental Section

Kinetic measurements were carried out on a Perkin-Elmer Lambda 3B or Hewlett-Packard 452 diode array instrument. Carboxypeptidase A (EC 3.4.17.1, Type 1 from Bovine Pancreas) was purchased from the Sigma Chemical Co. Acetylated carboxypeptidase A was prepared according to the procedure of Simpson *et al.*[9] High-performance liquid chromatographic analysis was carried out on a Perkin-Elmer Series 410-BIO system. The effluent was monitored by a Perkin-Elmer variable-wavelength LC-95 detector. ^{1}H- and ^{13}C-NMR spectra were recorded on a Nicolet QE-300 or Varian U-500 spectrometer. Melting points were determined by using Thomas Hoover Capillary melting point apparatus. Infrared and mass spectra were recorded on Nicolet 20 DX and Kratos MS 80 RFA spectrometers, respectively. Enzyme activity was measured by monitoring hydrolysis of a chromogenic ester, *O*-(*trans*-*p*-chlorocinnamoyl)-L-3-phenylacetate, at 320 nm.[10] A typical assay (1.0 mL) consists of 500 μM of the substrate ester and 20 nM enzyme in 10 mM MOPS, 0.5 M NaCl, pH 7.0.

Kinetic Analyses. Inactivation experiments were carried out with compounds **1**, **2**, **3**, and **10** and were attempted with compounds **6**, **9**, and **11**. In a typical inactivation experiment, a portion of the stock solution of the inactivator in 1,4-dioxane was added to the enzyme (0.1 μM), giving a final concentration of 10% dioxane in 100 mM buffer (pH 8.0, MOPS; pH 8.5–9.5, tris; pH 10.0, CAPS), supplemented with 0.5 M NaCl. Aliquots (10 μL) of the mixture were diluted individually into assay mixtures (990 μL) at various time intervals, and the remaining enzyme activity was monitored immediately. The data analyses were performed according to standard procedures.[11] In a typical "protection" experiment, the enzyme (0.1 μM) was incubated with (*R,S*)-benzylsuccinic acid (1 μM) for 10 min at room temperature. Subsequently, compound **1** (or **2**) was added to the mixture to give a final concentration of 5 mM. The enzyme activity was monitored over time, as described above. The reversible inhibitor constant (K_I) was determined by the Dixon method according to the protocol described previously.[11,12]

2-Benzyl-3-iodo-propanol (5). Methanesulfonyl chloride (2.76 g, 24.1 mmol) was added to a solution of diol **4** (4.0 g, 24.1 mmol) and triethylamine (2.44 g, 24.1 mmol) in 100 mL THF, and the mixture was stirred at room temperature for 1 h. The solvent was removed *in vacuo*, and the residue was allowed to react with KI (14.4 g, 86.4 mmol) in 75 mL of DMF at 80 °C for 3 h. The mixture was evaporated to dryness, and the residue was taken up in CH_2Cl_2. The solution was washed with 10% $Na_2S_2O_3$ (3 × 50 mL), dried over anhydrous Na_2SO_4, and concentrated to give an oil. Purification of the product mixture by chromatography on silica gel (a gradient of 0–10% ethyl acetate in hexane) gave the desired product as an oil (2.25 g, 41% yield). R_f 0.70 (hexane/AcOEt, 1:1); IR (CCl_4) cm^{-1} 3393; EI-MS *m/z* 276 (M^+, 13%); ^{1}H-NMR 300 MHz ($CDCl_3$) δ 1.52 (1H, s, OH), 1.75 (1H, m, CH), 2.62 (1H, dd, J = 7.8 and 13.8 Hz, benzylic), 2.70 (1H, dd, J = 6.6 and 13.5 Hz, benzylic), 3.21 (1H, dd, J = 5.1 and 9.9 Hz, CH_aI), 3.38 (1H, dd, J = 4.5 and 9.9 Hz, CH_bI), 3.57 (1H, dd, J = 6.8 and 10.9 Hz, CH_2OH), 3.70 (1H, dd, J = 5.1 and 10.9 Hz, CH_2OH), 7.2–7.4 (5H, m, phenyl); ^{13}C-NMR 75 MHz ($CDCl_3$) δ 11.5 (CH_2I), 37.0 (CH_2Ph), 43.4 (CH), 65.0 (CH_2OH), 126.4, 128.6, 129.1, 139.0 (phenyl).

(*R,S*)-2-Benzyl-3-iodo-propanoic Acid (1). A solution of compound **5** (2.25 g, 9.96 mmol) in 200 mL of acetone and 12 mL of the Jones reagent (2.67 M)[13] was stirred at room temperature for 4 h. A 1-mL portion of 2-propanol was added, and the precipitate was filtered. The filtrate was concentrated, and the mixture was purified by chromatography on silica gel (CH_2Cl_2) to afford the product as an oil (2.30 g, 80% yield). Crystallization from CH_2Cl_2/hexane gave white crystals (1.95 g). Mp 54–55 °C; R_f 0.55 (hexane/AcOEt/AcOH, 2:5:0.05); IR (KBr) cm^{-1} 1692; EI-MS *m/z* 289.9809 (M^+, 40%; calc 289.9806); ^{1}H-NMR 300 MHz ($CDCl_3$) δ 2.90 (1H, dd, J = 5.7 and 13.2 Hz, benzylic), 3.01 (1H, m, CH), 3.13 (1H, dd, J = 7.5 and 13.2 Hz, benzylic), 3.26 (1H, dd, J = 5.1 and 10.0 Hz, CH_2I), 3.32 (1H, dd, J = 6.8 and 10.0 Hz, CH_2I), 7.22 (5H, m, phenyl); ^{13}C-NMR 75 MHz ($CDCl_3$) δ 4.0 (CH_2I), 38.0 (CH_2Ph), 49.2 (CH), 127.1, 128.9, 129.1, 137.5 (phenyl), 178.2 (carboxyl).

(*R,S*)-2-Benzyl-γ-butyrolactone (8). A solution of lithium diisopropylamide (12.78 mmol) in 10 mL of anhydrous THF saturated with argon—prepared by the reaction of *n*-butyllithium (5.92 mL of a 2.16 M solution in hexanes; 12.78 mmol) with freshly distilled diisopropylamine (1.79 mL; 12.78 mmol) at 0 °C—was added dropwise to a solution of γ-butyrolactone (1.0 g, 11.62 mmol) in 70.0 mL of a 3.5:1 mixture of THF/HMPA solution at –78 °C, under an atmosphere of argon. The mixture was stirred for 1 h while allowing the temperature to rise to –45 °C. At this point, benzyl bromide (1.52 mL, 11.62 mmol) was added, and the resultant mixture was aged an additional 5 h at –45 °C. The reaction was quenched by the addition of a saturated solution of ammonium chloride (30 mL) at –45 °C. The aqueous solution was washed with CH_2Cl_2 (3 × 25 mL), and the combined organic layer was dried over anhydrous $MgSO_4$ and was evaporated *in vacuo* to dryness to give the crude product. The product was purified on silica gel (hexane/ethyl acetate, 3:2) to give 1.06 g of the title compound as a pale-yellow oil (52% yield). R_f 0.50 (hexane/AcOEt, 3:2); IR (neat) cm^{-1} 1764; EI-MS *m/z* 176.0838 (M^+, 61%; calc 176.0837); ^{1}H-NMR 500 MHz ($CDCl_3$) δ 1.98 (dddd, 1H, $J_{\beta_{ax},\beta_{eq}}$ = 12.5 Hz, $J_{\beta_{ax},\gamma_{ax}}$ = 9.3 Hz, $J_{\beta_{ax},\gamma_{eq}}$ = 8.5 Hz, $J_{\beta_{ax},\alpha}$ = 9.5 Hz, $H_{\beta_{ax}}$), 2.24 (dddd, 1H, $J_{\beta_{eq},\beta_{ax}}$ = 12.5 Hz, $J_{\beta_{eq},\gamma_{ax}}$ = 6.8 Hz, $J_{\beta_{eq},\gamma_{eq}}$ = 3.0 Hz, $J_{\beta_{eq},\alpha}$ = 8.5 Hz, $H_{\beta_{eq}}$), 2.75 (dd, 1H, $J_{CH,\alpha}$ = 9.0 Hz, J_{gem} = 13.5 Hz, PhC*H*), 2.84 (dddd, 1H, $J_{\alpha,\beta_{ax}}$ = 9.5 Hz, $J_{\alpha,\beta_{eq}}$ = 8.5 Hz, $J_{\alpha,CH}$ = 9.0 Hz, $J_{\alpha,CH}$ = 4.0 Hz, H_α), 3.24 (dd, 1H, J_{gem} = 13.5 Hz, $J_{CH,\alpha}$ = 4.0 Hz, PhC*H*), 4.13 (dt, 1H, $J_{\gamma_{ax},\gamma_{eq}}$ = 8.8 Hz, $J_{\gamma_{ax},\beta_{ax}}$ = 9.3 Hz, $J_{\gamma_{ax},\beta_{eq}}$ = 6.8 Hz, $H_{\gamma_{ax}}$), 4.22 (dt, 1H, $J_{\gamma_{eq},\gamma_{ax}}$ = 8.8 Hz, $J_{\gamma_{eq},\beta_{ax}}$ = 8.5 Hz, $J_{\gamma_{eq},\beta_{eq}}$ = 3.0 Hz, $H_{\gamma_{eq}}$), 7.26 (m, 5H, phenyl); ^{13}C-NMR 125 MHz ($CDCl_3$) δ 28.1 (C_β), 36.1 (C_α), 41.1 (PhCH_2), 66.6 (C_γ), 126.8, 128.8, 128.9 and 138.5 (phenyl), 178.7 (carbonyl).

(*R,S*)-2-Benzyl-4-iodobutanoic Acid (6). This procedure was adapted from Olah *et al.*[14] A solution of compound **8** (1.0 g, 5.7 mmol) and iodotrimethylsilane (3.23 mL, 22.7 mmol) in 25 mL of anhydrous acetonitrile was refluxed under an atmosphere of argon for 24 h in the

(4) Winter, C. H.; Arif, A. M.; Gladysz, J. A. *J. Am. Chem. Soc.* **1987**, *109*, 7560. Fernández, J.; Gladysz, J. A. *Organometallics* **1989**, *8*, 207. Kulawiec, R. J.; Crabtree, R. H. *Organometallics* **1988**, *7*, 1891.

(5) (a) Burk, M. J.; Segmuller, B.; Crabtree, R. H. *Organometallics* **1987**, *6*, 2241. (b) Winter, C. H., Veal, W. R.; Garner, C. M.; Arif, A. M.; Gladysz, J. A. *J. Am. Chem. Soc.* **1989**, *111*, 4766.

(6) For a recent review consult: Kulawiec, R. J.; Crabtree, R. H. *Coord. Chem. Rev.* **1990**, *99*, 89 and the references cited therein.

(7) Neurath, H. *J. Cell Biochem.* **1986**, *32*, 35. Matsas, R.; Stephenson, S. L.; Hryozko, J.; Kenny, A. J.; Turner, A. J. *Biochem. J.* **1985**, *231*, 445. Turner, A. J.; Matsas, R.; Kenny, A. J. *Biochem. Pharmacol.* **1985**, *34*, 1347. Brown, S. I.; Hook, C. W.; Tragakis, M. P. *Invest. Opthalmol.* **1972**, *11*, 149. Krane, S. M. *Ann. Rheum. Dis.* **1981**, *40*, 433. Liotta, L. A.; Tryggvason, K.; Garbisa, S.; Robey, P. G.; Abe, S. *Nature* **1980**, *284*, 67. Paganetti, L. A.; Caroni, P.; Schwab, M. E. *J. Cell Biol.* **1988**, *107*, 2281. Kim, H. S.; Campbell, B. J. *Biochem. Biophys. Res. Commun.* **1982**, *108*, 1638. Ballin, M.; Gomez, D. E.; Sinha, C. C.; Thorgeirsson, U. P. *Biochem. Biophys. Res. Commun.* **1988**, *154*, 832.

(8) For a review of zinc protease inhibitors see: Powers, J. C.; Harper, J. W. In *Protease Inhibitors*; Barrett, A. J., Salvesen, G., Eds.; Elsevier: New York, 1986, pp 219–298. A few examples of mechanism-based irreversible inactivators for zinc proteases are reported: (a) Ner, S. K.; Suckling, C. J.; Bell, A. R.; Wrigglesworth, R. *J. Chem. Soc., Chem. Commun.* **1987**, 480. Mobashery, S.; Ghosh, S. S.; Tamura, S. Y.; Kaiser, E. T. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 578. Ghosh, S. S.; Wu, Y. Q.; Mobashery, S. *J. Biol. Chem.* **1991**, *266*, 8759. Wu, Y.-Q.; Mobashery, S. *J. Med. Chem.* **1991**, *34*, 1914. Ghosh, S. S.; Said-Nejad, O.; Roestmadji, J.; Mobashery, S. *J. Med. Chem.* **1992**, *35*, 4175. Levy, O. E.; Taibi, P.; Mobashery, S.; Ghosh, S. S. *J. Med. Chem.* **1993**, *36*, 2408. (b) Kim, D. H.; Kim, K. B. *J. Am. Chem. Soc.* **1991**, *113*, 3200. (c) Yun, M.; Park, C.; Kim, S.; Nam, D.; Kim, S. C.; Kim, D. H. *J. Am. Chem. Soc.* **1992**, *114*, 2281.

(9) Simpson, R. T.; Riordan, J. F.; Valee, B. L. *Biochemistry* **1963**, *2*, 616.

(10) Suh, J.; Kaiser, E. T. *J. Am. Chem. Soc.* **1976**, *98*, 1940.

(11) Ghosh, S. S.; Wu, Y. Q.; Mobashery, S. *J. Biol. Chem.* **1991**, *266*, 8759.

(12) Imtiaz, U.; Billings, E.; Knox, J. R.; Manavathu, E. K.; Lerner, S. A.; Mobashery, S. *J. Am. Chem. Soc.* **1993**, *115*, 4435.

(13) Bowden, K.; Heilbron, I. M.; Jones, E. R. H.; Weedon, B. C. L. *J. Chem. Soc.* **1946**, 39.

(14) Olah, G. A.; Gupta, B. G. B.; Malhotra, R.; Narang, N. C. *J. Org. Chem.* **1980**, *45*, 1638.

dark. After the mixture was cooled to room temperature, 10 mL of water was added and the mixture was evaporated to dryness *in vacuo*. The resulting dark-brown oil was taken up in ethyl acetate (75 mL) and was washed with 10% sodium thiosulfate (2 × 20 mL). The organic layer was dried over anhydrous $MgSO_4$ and was then purified on silica gel (benzene/ethyl acetate, 3:1) to afford 1.4 g of the product as a pale-yellow solid (81% yield). Mp 52–55 °C; R_f 0.40 (benzene/AcOEt/AcOH, 6:2:0.5); IR (neat) cm^{-1} 1689; EI-MS *m/z* 176 (M – HI, 33.2%); ^{1}H-NMR 500 MHz ($CDCl_3$) δ 1.99 (m,1H, ICH_2–CH_2), 2.19 (m, 1H, ICH_2CH_2), 2.80 (dd, 1H, *J* = 7.0 and 13.5 Hz, $PhCH_2$), 2.87 (m, 1H, methine), 3.03 (dd, 1H, *J* = 7.0 and 13.5 Hz, $PhCH_2$), 3.14 (dt, 1H, *J* = 7.8 and 10.0 Hz, CH_2I), 3.25 (m, 1H, CH_2I), 7.25 (m, 5H, phenyl); ^{13}C-NMR 125 MHz ($CDCl_3$) δ 2.59 (CH_2I), 34.99 (CH_2CH_2I), 37.57 (methine), 47.68 ($PhCH_2$), 126.78, 128.72, 128.9, and 138.01 (phenyl), 181.20 (carboxyl).

(*R,S*)-2-Benzyl-2-deuterio-1,3-propanediol. A solution of dimethyl 2-benzylmalonate (2.8 g, 12.6 mmol) in ethyl ether (10 mL) was added to a suspension of NaH (0.5 g of a 60% dispersion in oil, washed previously with hexane, approximately 12.5 mmol) in 50 mL of anhydrous ethyl ether; the mixture was stirred until all the solid (NaH) was dissolved (approximately 0.5 h). The resultant solution of malonate anion was added dropwise to a solution of DCl in D_2O (generated by mixing 1 mL of $SOCl_2$ into 3 mL of D_2O) at room temperature over 15 min. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated to give an oil (2.6 g). A solution of the resultant deuterated diester in ethyl ether (10 mL) was added dropwise to a suspension of lithium aluminium hydride (1.2 g, 31.6 mmol) in 20 mL of ethyl acetate in an ice/water bath. After completion of the addition, the mixture was refluxed for 15 h. Subsequently, 1.5 mL of water was added, followed by a sufficient amount of 1.0 N HCl which dissolved all of the solid. The solution was washed with ethyl acetate (3 × 100 mL); then the combind organic layer was dried over anhydrous Na_2SO_4. The solution was concentrated, and the mixture was purified by chromatography on silica gel (a gradient of 0–20% ethyl acetate in hexane); 1.55 g of the product was isolated as white crystals (80% yield). R_f 0.5 (CH_2Cl_2/CH_3OH, 10:1); mp 68–69 °C; IR (KBr) 3220 cm^{-1}; EI-MS *m/z* 149.0946 (M – H_2O; calc 149.09508); ^{1}H-NMR 300 MHz ($CDCl_3$) δ 2.60 (2H, s, CH_2-Ph), 2.81 (2H, t, *J* = 4.4 Hz, OH), 3.65 (2H, dd, *J* = 4.4 and 10.5 Hz, CH_2OH), 3.77 (2H, dd, *J* = 4.4 and 10.8 Hz, CH_2OH), 7.2–7.4 (5H, m); ^{13}C-NMR 300 MHz ($CDCl_3$) δ 34.2 (CH_2Ph), 65.3 (CH_2OH), 126.1, 128.5, 129.0, and 140.0 (phenyl).

(*R,S*)-2-Benzyl-2-deuterio-3-iodopropanol. The synthesis was carried out as described for **5**, using 2-benzyl-2-deuterio-1,3-propanediol as the starting material. IR (neat) 3393 cm^{-1}; EI-MS *m/z* 277.0076 (M^+; calc 277.0075); ^{1}H-NMR 300 MHz ($CDCl_3$) δ 1.61 (1H, t, *J* = 4.8 Hz, OH), 2.61 (1H, d, *J* = 13.5 Hz, benzylic), 2.70 (1H, d, *J* = 13.2 Hz, benzylic), 3.21 (1H, d, *J* = 9.9 Hz, CH_2I), 3.36 (1H, d, *J* = 9.9 Hz, CH_2I), 3.57 (1H, dd, *J* = 4.8 and 10.8 Hz, CH_aOH), 3.69 (1H, dd, *J* = 4.4 and 11.0 Hz, CH_bOH), 7.2–7.4 (5H, m); ^{13}C-NMR 75 MHz ($CDCl_3$) δ 11.4 (CH_2I), 36.9 (CH_2Ph), 64.9 (CH_2OH), 126.4, 128.6, 129.1, and 139.1 (phenyl).

(*R,S*)-2-Benzyl-2-deuterio-3-iodopropanoic Acid (10). Oxidation of 2-benzyl-2-deuterio-3-iodopropanol was carried out as described for the synthesis of **1**. Mp 54.5–55.5 °C; IR (KBr) 1701 cm^{-1}; EI-MS *m/z* 290.9873 (M^+; calc 290.98684); ^{1}H-NMR 300 MHz ($CDCl_3$) δ 2.92 (1H, d, *J* = 13.8 Hz, benzylic), 3.13 (1H, d, *J* = 13.8 Hz, benzylic), 3.27 (1H, d, *J* = 10.2 Hz, CH_2I), 3.30 (1H, d, *J* = 9.9 Hz, CH_2I), 7.2–7.4 (5H, m, phenyl), 9.6 (1H, br s, COOH); ^{13}C-NMR 75 MHz ($CDCl_3$) δ 3.6 (CH_2I), 37.8 (CH_2Ph), 127.0, 128.8, 129.0, and 137.3 (phenyl), 178.8 (carboxyl).

(*R,S*)-1-((*p*-Tolylsulfonyl)oxy)-2-benzyl-*n*-propanol. *p*-Toluenesulfonyl chloride (4.0 g, 21.0 mmol) was added to a solution of 2-benzyl-1,3-propanediol (3.47 g, 20.9 mmol) and triethylamine (2.1 g, 20.8 mmol) in 100 mL of CH_2Cl_2. The mixture was stirred at room temperature for 20 h, followed by the addition of 100 mL of water. The organic layer was separated, and the aqueous solution was washed twice with CH_2Cl_2. The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated to give a crude product mixture. Purification by chromatography on silica gel (a gradient of 0–20% ethyl acetate in hexane) gave the desired product as an oil (2.25 g, 34% yield). R_f 0.6 (CH_2Cl_2/MeOH, 10:1); IR (neat) cm^{-1} 3554 (OH), 1344, 1169 (S = O). CI-MS *m/z* 321 (M^+ + H); ^{1}H-NMR 300 MHz ($CDCl_3$) δ 1.82 (1H, t, *J* = 5.4 Hz, OH), 2.11 (1H, m, CH), 2.46 (3H, s, CH_3), 2.61 (1H, dd, *J* = 13.8 and 7.5 Hz, CH_2Ph), 2.65 (1H, dd, *J* = 7.5 and 13.8 Hz, CH_2Ph), 3.57 (1H, ddd, *J* = 6.0, 6.0, and 11.1 Hz, CH_2OH), 3.64 (1H, ddd, *J* = 5.1, 5.1, and 11.1 Hz, CH_2OH), 4.01 (1H, dd, *J* = 5.4 and 9.9 Hz, CH_2-OTos), 4.10 (1H, dd, *J* = 4.5 and 9.9 Hz, CH_2OTos), 7.07–7.10 (2H, m, phenyl), 7.18–7.28 (3H, m, phenyl), 7.33–7.36 (2H, m, phenyl), 7.7–7.80 (2H, m, phenyl); ^{13}C-NMR 75 MHz ($CDCl_3$) δ 21.6 (CH_3), 33.5 (CH_2Ph), 42.5 (CH), 61.3 (CH_2OH), 69.6 (CH_2OTos), 126.3, 127.9, 128.5, 129.0, 129.9, 132.8, 138.7, 144.9 (aromatic).

(*R,S*)-2-Benzyl-3-fluoropropanol. A mixture of 1-((*p*-tolylsulfonyl)-oxy)-2-benzyl-*n*-propanol (2.25 g, 7.0 mmol) and KF (0.87 g, 15 mmol) in diethyleneglycol (5 mL) was stirred at 135 °C for 16 h. The reaction mixure was subjected to column chromatography on silica gel (a gradient of 0–10% ethyl acetate in hexane) to give the title product as an oil (600 mg, 51% yield). R_f 0.7 (hexane/ethyl acetate, 1:1); IR (neat) 3370 cm^{-1}; EI-MS *m/z* 168.0948 (M^+; calc 168.0950); ^{1}H-NMR 300 MHz ($CDCl_3$) δ 1.44 (1H, t, *J* = 5.3 Hz, OH), 2.1–2.2 (1H, m, CH), 2.71 (2H, d, *J* = 7.5 Hz, CH_2Ph), 3.68 (1H, dd, *J* = 6.0 and 11.4 Hz, CH_2OH), 3.74 (1H, dd, *J* = 5.3 and 11.1 Hz, CH_2OH), 4.45 (1H, ddd, J_{HF} = 47.4 Hz, *J* = 5.3, 9.2 Hz, CH_2F), 4.52 (1H, ddd, J_{HF} = 47.4 Hz, *J* = 4.4, 9.2 Hz, CH_2F), 7.2–7.4 (5H, m, phenyl); ^{13}C-NMR 75 MHz ($CDCl_3$) δ 33.3 (J_{CF} = 5.6 Hz, CH_2Ph), 43.7 (J_{CF} = 9.9 Hz, CH), 62.1 (J_{CF} = 4.8 Hz, CH_2OH), 83.5 (J_{CF} = 166.3 Hz, CH_2F), 126.3, 128.5, 129.1, and 139.3 (phenyl).

(*R,S*)-2-Benzyl-3-fluoropropanoic Acid (11). A solution of 2-benzyl-3-fluoropropanol (600 mg, 3.57 mmol) in 100 mL of acetone and the Jones reagent (6.0 mL, 2.67 M) was stirred at room temperature for 4 h. Subsequently, 1 mL of 2-propanol was added and the resultant precipitate was filtered. The filtrate was concentrated, and the residue was purified by chromatography on silica gel (a gradient of 0–100% CH_2Cl_2 in hexane) to give the product as an oil (600 mg, 92% yield). R_f 0.65 (hexane/AcOEt, 1:1); IR (neat) 1712 (C = O) cm^{-1}; EI-MS *m/z* 182.0740 (M^+; calc 182.0743); ^{1}H-NMR 300 MHz ($CDCl_3$) δ 2.9–3.2 (3H, m, benzylic methylene and methine), 4.58 (2H, m, J_{HF} = 46.5 Hz, CH_2F), 7.2–7.6 (5H, m, phenyl), 10.4 (1H, br s, COOH); ^{13}C-NMR 75 MHz ($CDCl_3$) δ 33.0 (J_{CF} = 5.2 Hz, CH_2Ph), 47.6 (J_{CF} = 20.6 Hz, CH), 82.0 (J_{CF} = 170.0 Hz, CH_2F), 126.9, 128.7, 129.0, and 137.5 (phenyl), 178.4 (carboxyl).

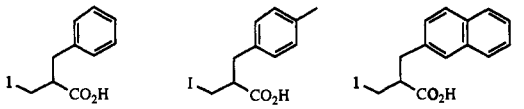
2-Benzyl-2-propenoic Acid (9). A mixture of methyl 2-benzyl-2-propenoate (1.0 g, 5.7 mmol), prepared according to the literature,[15] and NaOH (0.4 g, 10 mmol) in 5 mL of water and 20 mL of methanol was stirred at room temperature for 20 h. The mixture was diluted with 250 mL of water and was acidified with concentrated HCl to pH 2.0. Subsequently, the solution was washed with ethyl ether (3×). The combined ether solution was dried over Na_2SO_4 and was concentrated *in vacuo* to give a residual oil. The sample was purified by chromatography on silica gel (CH_2Cl_2) to give the desired product as an oil (600 mg, 65% yield). Crystallization from CH_2Cl_2/hexane gave the title compound as needles (450 mg). Mp 69.5–70.5 °C (lit.[16] 66–67 °C); IR (KBr) 1682 cm^{-1}; EI-MS *m/z* 162.0677 (M^+; calc 162.06807); ^{1}H-NMR 300 MHz ($CDCl_3$) δ 3.64 (2H, s, CH_2Ph), 5.60 (1H, s, CH_2), 6.40 (1H, s, CH_2), 7.20–7.32 (5H, m, phenyl), 11.3 (1H, br s, carboxyl); ^{13}C-NMR 75 MHz ($CDCl_3$) δ 37.5 (CH_2Ph), 126.4, 128.5, 129.0, 128.7, 138.4, 139.5 (phenyl and olefin), 172.3 (C=O).

Results and Discussion

The inhibitors reported herein were designed on the basis of our knowledge of the binding of known inhibitors to the active site of CPA, of which several examples have been reported.[17] A feature common to the known inhibitors is the presence of the terminal carboxyl group and the hydrophobic benzyl function. Therefore, we retained these features in our initially conceived molecule. We elaborated the structure of the molecule in the distal direction to the carboxylate by computer modeling to realize the length of the carbon backbone which was necessary to bring a halogen moiety to the coordination sphere of the active-site zinc ion. A single methylene unit was deemed necessary. Therefore, compound **1** was conceived as the prototype of a molecule that has the minimal structural features needed for active-site binding, which furthermore would allow for the

(15) Madruga, E. L.; San Román, J.; Lavia, M. A.; Fernandez-Monreal, M. C. *Macromolecules* **1984**, *17*, 989.
(16) Wilt, J. W.; Pawlikowski, W. W.; Wieczorek, J. J. *J. Org. Chem.* **1972**, *37*, 820.
(17) Christianson, D. W.; Lipscomb, W. N. *Acc. Chem. Res.* **1989**, *22*, 62.

coordination of the iodo moiety to the active-site metal.[18] The energy of the complex of the *R*-enantiomer of **1** in the CPA active site was minimized.[19] The features of the binding interactions of the carboxylate and the benzyl group remained similar to those for cocrystal structures of other inhibitors in the CPA active site. The Tyr-248 side chain was retained in the "down" position.[17] This complex reveals that the iodo moiety remained near the coordination sphere of the zinc ion (2.3 Å) after energy minimization. The binding of alkyl halides to transition metals has been shown to be a rapid process in model systems.[20] Furthermore, such coordination in the active site of CPA would be favored on entropic grounds; that is to say that once the binding of the carboxylate and the benzyl group of the inhibitor in the active site takes place, coordination of the iodo group may be imposed on the metal, despite the weak nature of such an interaction. This may not be an unreasonable expectation in light of the fact that a very simple molecule such as 3-phenylpropionate has been shown to be a competitive inhibitor for CPA.[21] The hydrophobic pocket that accommodates the benzyl group appears rather large, so we decided to prepare analogues of **1** which may benefit from the full potential for hydrophobic interaction in this cavity. Toward that goal, compounds **2** and **3** were also synthesized.



1 2 3

We envisioned that the metal coordination which is proposed for the iodo moiety for a compound such as **1** should increase the polarity of the carbon–iodide bond, whereby an enhanced S_N2 reaction with an active-site nucleophilic amino acid residue would be possible as a consequence of the increased electrophilicity of the carbon bonded to the iodo group and improved leaving ability of the halide.[22] We note that the oxygen atoms of the side chains of both Glu-270 and Tyr-248 come close to the C_3-methylene of compound **1** in the energy-minimized enzyme–inhibitor complex—3.2 and 3.5 Å, respectively (data not shown).

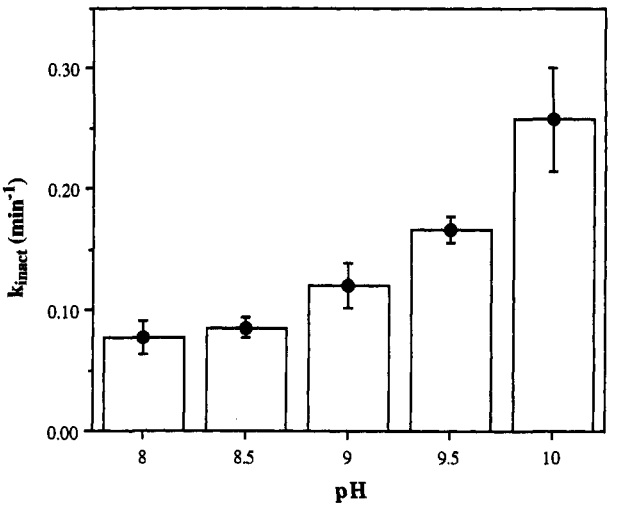


Figure 1. Dependence of the value of k_{inact} on pH (buffers: pH 8.0, MOPS; pH 8.5–9.5, tris; pH 10.0, CAPS. Each buffer was supplemented with 0.5 M NaCl).

Compound **1** was synthesized in two steps starting from diol **4**. Monomesylation of **4**, followed by the reaction with KI in DMF afforded **5** in 41% yield. Jones oxidation of **5** gave the racemic mixture of **1** in 80% yield. Compounds **2** and **3** were synthesized essentially by similar methodology (supplementary material). Compounds **1**, **2**, and **3** all inactivated CPA in a saturable and time-dependent manner, which followed pseudo-first-order kinetics. A double-reciprocal analysis of the observed inactivation rates versus the inhibitor concentration at pH 8.0 furnished the values for k_{inact} of 0.078 ± 0.014 min^{-1} and a K_I of 2.0 ± 0.4 mM for **1** and k_{inact} of 0.027 ± 0.005 min^{-1} and a K_I of 3.2 ± 0.7 mM for **2**. Whereas incubation of compound **3** with CPA resulted clearly in a time-dependent loss of activity, the rate of the inactivation process was sufficiently slower than those for **1** and **2** that this compound was not studied further. The following experiments were carried out with compound **1**. Compound **1** bound to the CPA active site (competitive inhibition) at pH ≤ 7.5; however, no inactivation of the enzyme was observed. Enzyme inactivation was noted at pH > 7.5, and the value of k_{inact} increased as a function of increasing pH (Figure 1) (a similar result was noted with **2**); the effect of pH on K_I was not significant. The results of Figure 1 suggest that a residue that titrates at pH > 8 may be involved in inactivation, indicating that Tyr-248 is a likely amino-acid residue for covalent modification. The dissociation constant (K_i) for the reversible enzyme–inhibitor complex was evaluated for the racemate at 130 μM by the method of Dixon.[23,24] The following experiments shed light on the nature of the inactivation process: (i) A complete protection of CPA inactivation by **1** was noted when CPA was preincubated with (*R*,*S*)-benzylsuccinate (1 μM),[21] a known competitive inhibitor for CPA; therefore, inactivation chemistry takes place in the enzyme active site. (ii) Neither extensive dialysis over 2 days (>10 buffer changes)[25] nor rapid gel filtration (Sephadex-15) of

(18) We hasten to add that a structurally related compound to **1**, 2-benzyl-3-mercaptopropanoate, was reported as an inhibitor of CPA, for which the mercapto group was suggested to coordinate to the active-site zinc ion upon binding to the active site (Ondetti, M. A.; Condon, M. E.; Reid, J.; Sabo, E. F.; Cheung, H. S.; Cushman, D. W. *Biochemistry* **1979**, *18*, 1427). A similarly designed mercapto-containing inhibitor for thermolysin, (2-benzyl-3-mercaptopropanoyl)-L-alanylglycinamide, has been shown conclusively to achieve thiol coordination to the active-site zinc ion—presumably as a thiolate—in the crystal structure of the enzyme–inhibitor complex (Monzingo, A. F.; Matthews, B. W. *Biochemistry* **1982**, *21*, 3390). These molecules do not exhibit irreversible inhibition of their respective target enzymes, since the deprotonated mercaptan may not serve as a reasonable leaving group, despite coordination to the metal.

(19) The *R*-enantiomer is expected to bind CPA with higher affinity on the basis of the literature precedent. Crystal coordinates for CPA (6CPA, Protein Data Bank, Brookhaven National Laboratory) were used in the three-dimentional modeling and energy minimization. The structure of the *R*-enantiomer of inactivator **1**, which is expected to bind the CPA much more tightly than the *S*-enantiomer, was docked into the active site. A total of 148 crystallographic water molecules were retained, and the active-site-bound inactivator was then capped by the addition of 54 Monte Carlo water molecules. The additional water molecules also covered the areas of the enzyme that were previously sheltered by the original inhibitor in the crystal coordinates, which was removed prior to docking of **1**. The hydrogen atoms were added in the calculated positions, and atomic charges were computed by the method of Gasteiger-Hückel (Gasteiger, J.; Marsili, M. *Tetrahedron* **1980**, *36*, 3219. Gasteiger, J.; Marsili, M. *Org. Magn. Reson.* **1981**, *15*, 353). The energy minimization was performed by the geometry optimization algorithm MAXMIN2, using the Tripos force field by the Sybyl molecular modeling software in a Silicon Graphics R4000 Indigo computer. The Powell method (Powell, M. J. D. *Math. Programming* **1977**, *12*, 241) was used to determine the descending direction in the minimization. Minimization was performed in three stages: (1) the inhibitor and water molecules were allowed to move; (2) the inhibitor, water molecules, and the protein backbone were allowed to move; and (3) finally, the entire enzyme–inhibitor complex was allowed to minimize without any constraints. The minimization in a radius of 15 Å from the active site in each stage was continued until the change in energy was less than 0.001 kcal/(mol Å) between iterations. A dielectric constant of 1.0 was used for the calculations.

(20) O'Driscoll, E.; Simon, J. D. *J. Am. Chem. Soc.* **1990**, *112*, 6580.

(21) Byers, L. D.; Wolfenden, R. *Biochemistry* **1973**, *12*, 2070.

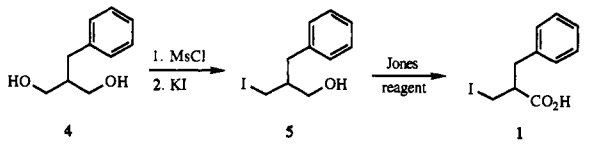
(22) Suh, J. H. *Acc. Chem. Res.* **1992**, *25*, 273.

(23) Dixon, M. *Biochem. J.* **1953**, *55*, 170. The experimental protocols were essentially as established earlier in experiments with β-lactamase.[12]

(24) The dissociation constant, K_i, should not be confused with K_I, which is a collection of a number of microscopic rate constants, similar to K_m for enzyme substrates. The results presented herein indicate that these two parameters are obviously not equal to one another for compound **1**. The value of K_i, and not K_I, gives an indication of the affinity of the enzyme for compound **1**.

(25) Under these conditions even enzymes inhibited by slow- and tight-binding inhibitors have been shown to recover activity (Littlewood, G. M.; Hooper, N. M.; Turner, A. J. *Biochem. J.* **1989**, *257*, 361).

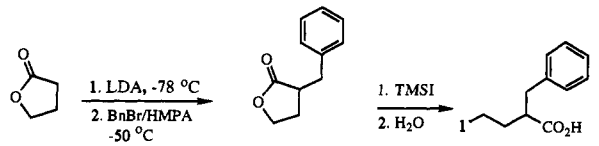
the inactivated CPA resulted in any regeneration of enzymic activity, indicating the existence of a covalent bond between the inhibitor and the enzyme. (iii) HPLC analysis of the inactivation mixture revealed no product formation after several hours of incubation of **1** with CPA. For example, formation of 2-benzyl-3-hydroxylpropanoate as a consequence of nucleophilic attack by water at the metal-activated iodide was not seen; hence **1** is not turned over by CPA.



4 5 1

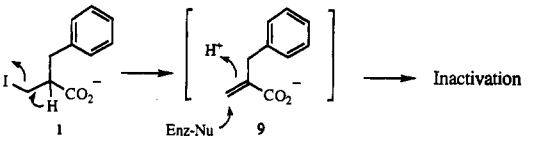
Simpson *et al.* have reported the preparation of acetylated CPA, which has been suggested to be incorporated with the acetyl moiety at the Tyr-248 side chain.[9] The activity of the modified enzyme is attenuated by approximately 90%, compared to the wild type. We made an interesting observation that acetylated CPA was still inactivated by compound **1** at pH 8.0, although the inactivation process was slower than with the wild-type enzyme (k_{inact} = 0.057 ± 0.012 min^{-1} and a K_I = 4.6 ± 1.1 mM). An investigation of the pH dependence of inactivation could not be carried out successfully, since deacetylation of the acetylated enzyme proceeded more rapidly than the inactivation chemistry. Inactivation experiments of acetyl-CPA at pH > 8.5 with **1** showed that enzyme activity increased first—due to deacetylation of acetyl-CPA—followed by the subsequent loss of activity. A plausible explanation for the inactivation is that since Tyr-248 may no longer serve as the nucleophile in the inactivation chemistry of the acetylated CPA, a second nucleophilic residue—possibly Glu-270—may conceivably be modified by **1** with acetylated CPA. We note that if Tyr-248 is modified, an ether linkage results, whereas modification of Glu-270 would give an ester; the putative ester linkage to the Glu-270 side chain should be labile to alkaline conditions, so a distinction of the type of linkage may be made on this basis. All attempts at measuring any recovery of activity by prolonged incubation of the inactivated CPA at pH 10 failed. Whereas this observation may suggest that Tyr-248 may indeed be the modified residue, we hasten to add that should Glu-270 be modified, the bulk of the enzyme structure would shelter the ester linkage from the medium and may prevent its hydrolysis. The exact nature of the modified residue in the inactivation chemistry remains unclear at the present, although future peptide mapping of the inactivated CPA would clarify this issue. A comparison of these findings with those disclosed by Kim and Kim[8b] and Yun *et al.*[8c] for inactivation of CPA by an oxirane containing inhibitor is relevant here. These workers suggested that the oxygen of the oxirane may coordinate to the active-site zinc ion, facilitating the departure of the oxyanion upon nucleophilic attack of an active-site residue at the epoxide moiety. These workers showed by determination of the crystal structure of the inactivated protein that Glu-270 was the modified active-site residue.[8c]

We also synthesized compound **6**, which has a longer backbone by one methylene unit compared to **1**. γ-Butyrolactone (**7**) was deprotonated by LDA at −78 °C, followed by alkylation with benzyl bromide to give **8** in 52% yield. The reaction of iodotrimethylsilane (TMSI) with **6**, and the subsequent hydrolysis of the resultant trimethylsilyl ester, furnished **6** in 81% yield. We



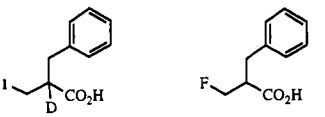
7 8 6

find it significant that, in contrast to **1**, compound **6** does not function as an irreversible inactivator for CPA but serves merely as a poor competitive inhibitor for the enzyme (K_i = 0.41 ± 0.07 mM for the racemate); as noted earlier, compound **1** was designed to have the ability to coordinate to the active-site zinc ion, a function that compound **6** cannot fulfill. It occurred to us that the fact that compound **1** inactivated CPA, whereas **6** does not, could be explained by another conceivable mechanism. An enzyme-mediated deprotonation at the α-position to the carboxylate in **1** would result in β-elimination of the iodide, giving rise to intermediate **9** in the active site. Intermediate **9** may



1 9

potentially serve as a Michael acceptor for a side-chain function of a nucleophilic residue in the active site. Of course, such an elimination reaction is not possible with compound **6**. Furthermore, CPA-mediated deprotonation reactions are precedented for a few substrate analogues.[26] An argument against this mechanism is the fact that the pK_a for the hydrogen α to a carboxylate should be higher that 25. However, one may envision that strong interactions with the carboxylate in the active site of CPA may lower the pK_a of the α-hydrogen somewhat. Nonetheless, to investigate this mechanistic possibility, we synthesized and studied compounds **9**, **10**, and **11**. If the proposed depro-



10 11

tonation were rate-limiting in the inactivation chemistry, a primary-isotope effect should be noted in inactivation of CPA by **10**. Compound **10** inactivated CPA with the kinetic parameters k_{inact} = 0.052 ± 0.007 min^{-1} and K_I = 2.5 ± 0.4 mM. An isotope effect of 1.5 on k_{inact} falls in the range expected for a secondary-isotope effect, which is consistent with the S_N2-type reaction at the adjacent methylene moiety. In addition, compound **11** cannot be activated by the active-site metal toward an S_N2-type reaction, but could result in enzyme inactivation by the elimination mechanism. Compound **11** did not inactivate CPA in a time-dependent manner in the pH range 8.0–10.0; however, it served as a competitive inhibitor for CPA (K_i = 0.22 ± 0.07 mM). Finally, since according to the elimination mechanism compound **9** would be an intermediate in the course of enzyme inactivation, its incubation with CPA should result in irreversible inactivation of the enzyme. As with **11**, compound **9** did not result in any time-dependent loss of activity in the pH range 8.0–10.0, but merely served as a competitive inhibitor for CPA (K_i = 0.61 ± 0.51 mM).

Second-order rates for reactions of methyl iodide with various nucleophiles in both methanol[27] and water[28] have been reported. The magnitudes of these rates do not change appreciably between the two solvents for a given reaction. Since the two likely nucleophiles for reaction with our inactivators are the side-chain functions of Glu-270 or Tyr-248, the rates for the S_N2 displacement of iodide from iodomethane by the acetate and phenolate ions are most relevant; these reactions afford rates of 2.7 × 10^{-6}

(26) Spratt, T. E.; Kaiser, E. T. *J. Am. Chem. Soc.* **1984**, *106*, 6440. Sugimoto, T.; Kaiser, E. T. *J. Am. Chem. Soc.* **1978**, *100*, 7750. Sugimoto, T.; Kaiser, E. T. *J. Am. Chem. Soc.* **1979**, *101*, 3946. Nashed, N. T.; Kaiser, E. T. *J. Am. Chem. Soc.* **1981**, *103*, 3611. Nashed, N. T.; Kaiser, E. T. *J. Am. Chem. Soc.* **1986**, *108*, 2710.

(27) Pearson, R. G.; Sobel, H.; Songstad, J. *J. Am. Chem. Soc.* **1968**, *90*, 319.

(28) Lalor, G. C.; Moelwyn-Hughes, E. A. *J. Chem. Soc.* **1965**, 2201.

and 7.3 × 10^{-5} M^{-1} s^{-1} in methanol at room temperature, respectively.[27] Branching at either the α- or the β-carbon is known to decrease the rates of S_N2 reactions. The trends that have been established indicate that an isopropyl or isobutyl halide reacts approximately 1000-fold more slowly than the corresponding methyl halide on grounds of steric encumbrance for the transition-state structure for the reaction.[29]

The halide moiety in **1** is doubly branched at the β-position; hence, a relative comparison of the reaction rate of this molecule with that of isobutyl iodide is called for. The value of k_{inact}/K_I gives an indication of the second-order rate constant for inactivation of CPA by **1**, which is measured at 0.7 to 1.5 M^{-1} s^{-1} for pH 8.0–10.0. Therefore, compared to the estimated rates for the model systems cited above, it appears that the rate of the nucleophilic displacement of the iodo moiety from **1** has been enhanced by approximately 10^8- to 10^9-fold.

Kahne and Still reported the rate for the uncatalyzed hydrolysis of an amide bond at neutral pH and at room temperature to be 3 × 10^{-9} s^{-1}.[30] This value translates to a pseudo-second-order rate for amide hydrolysis of approximately 10^{-10} M^{-1} s^{-1} under such conditions.[31] Carboxypeptidase A catalyzed hydrolysis of some of its preferred peptide substrates (e.g., Bz-Gly-Gly-L-Phe or Cbz-Gly-Gly-L-Leu) with k_{cat}/K_m values ranging about 10^3–10^4 M^{-1} s^{-1}.[32] Hence, CPA catalyzes hydrolysis of its peptide substrates with a rate enhancement of 10^{13}- to 10^{14}-fold over the uncatalyzed reaction, The active-site zinc ion is believed to play a critical role for this rate enhancement.[17,22]

The analysis presented in the preceding paragraphs indicates that an approximate rate enhancement of 10^8- to 10^9-fold for displacement of the iodo function from **1** is much higher than the 10^5- to 10^6-fold rate acceleration for the transition-metal activation of halides reported for the model systems; however, it is lower than the rate enhancement of 10^{13}- to 10^{14}-fold for the CPA-catalyzed hydrolysis of peptides. We suggest that some proximity effects may play a role in enhancement of the rate of iodo displacement above and beyond the values measured for the model metal-activated S_N2 reactions. Whereas the extent of rate enhancement for inactivation chemistry does not approach the value for amide hydrolysis, we rationalize this observation on the grounds that the CPA active site has evolved for turnover of the substrates and not the chemistry reported for enzyme inactivation by **1**. Nonetheless, the significant rate enhancement for inactivation chemistry reported herein constitutes the first example of such an application for an S_N2 reaction in enzymic reactions.

Acknowledgment. The funds for the purchase of the Silicon Graphics computer used in this research were provided by a grant from the National Institutes of Health (AI 33170). We wish to thank Dr. Charles Winter for drawing our attention to ref 5b.

Supplementary Material Available: Synthetic procedures for compounds **2** and **3** and their spectroscopic characteristics (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(29) Hughes, E. D. *Q. Rev. Chem. Soc.* **1948**, *2*, 107. March, J. *Advanced Organic Chemistry*, 3rd ed.; John Wiley & Sons: New York, 1985; p 299. Caldwell, G.; Magnera, T. F.; Kebarle, P. *J. Am. Chem. Soc.* **1984**, *106*, 959.

(30) Kahne, D.; Still, W. C. *J. Am. Chem. Soc.* **1988**, *110*, 7529.

(31) That is, 3 × 10^{-9} s^{-1} divided by 55 M for water concentration.

(32) Auld, D. S.; Holmquist, B. *Biochemistry* **1974**, *13*, 4355. Auld, D. S.; Vallee, B. L. *Biochemistry* **1970**, *9*, 4352.