Structure−Reactivity Relationships for the Inhibition Mechanism at the Second Alkyl-Chain-Binding Site of Cholesterol Esterase and Lipase†

Gialih Lin,*,‡,§ Chuen-Tzwu Shieh,‡ Hong-Chi Ho,‡ Ju-Yueh Chouhwang,‡ Wen-Yuan Lin,‡ and Chun-Ping Lu§

Department of Chemistry and Institute of Biochemistry, National Chung-Hsing University, Taichung 402, Taiwan

Received November 23, 1998; Revised Manuscript Received May 11, 1999

ABSTRACT: Alkyl-*N*-phenyl carbamates (**2**−**8**) (see Figure 1), alkyl-*N*-phenyl thiocarbamates (**9**−**15**), 2,2′-biphenyl-2-ol-2′-*N*-substituted carbamates (**16**−**23**), and 2,2′-biphenyl-2-*N*-octadecylcarbamate-2′-*N*-substituted carbamates (**24**−**31**) are prepared and evaluated for their inhibition effects on porcine pancreatic cholesterol esterase and *Pseudomona species* lipase. All inhibitors are characterized as transient or pseudo substrate inhibitors for both enzymes. Both enzymes are not protected from inhibition and further inactivated by carbamates **2**−**8** and thiocarbamates **9**−**15** in the presence of trifluoroacetophenone. Therefore, carbamates **2**−**8** and thiocarbamates **9**−**15** are exceptions for active site binding inhibitors and are probably the second alkyl-chain binding-site-directed inhibitors for both enzymes. The inhibition data for carbamates **2**−**8** and thiocarbamates **9**−**15** are correlated with the steric constant, E_s, and the hydrophobicity constant, π; however, the inhibition data are not correlated with the Taft substituent constant, σ^*. A comparison of the inhibition data for carbamates **2**−**8** and thiocarbamates **9**−**15** toward both enzymes indicates that thiocarbamates **9**−**15** are more potent inhibitors than carbamates **2**−**8**. A comparison of the inhibition data for cholesterol esterase and *Pseudomona species* lipase by carbamates **2**−**8** or thiocarbamates **9**−**15** indicates that cholesterol esterase is more sensitive to the E_s and π values than *Pseudomona species* lipase. The negative slope values for the logarithms of inhibition data for *Pseudomona species* lipase by carbamates **2**−**8** and thiocarbamates **9**−**15** versus E_s and π indicate that the second alkyl-chain-binding site of *Pseudomona species* lipase is huge, hydrophilic, compared to that of cholesterol esterase, and prefers to interact with a bulky, hydrophilic inhibitor rather than a small, hydrophobic one. On the contrary, the second alkyl-chain-binding site of cholesterol esterase prefers to bind to a small, hydrophobic inhibitor. Both enzymes are protected from inhibition by carbamates **16**−**23** in the presence of trifluoroacetophenone. Therefore, carbamates **16**−**23** are characterized as the alkyl chain binding site, esteratic site oxyanion active site directed pseudo substrate inhibitors for both enzymes. Both enzyme inhibition data for carbamates **16**−**22** are well-correlated with σ^* alone. The negative ρ^* values for these correlations indicate that the serine residue of both enzymes and carbamates **16**−**22** forms the tetrahedral species with more positive charges than inhibitors and the enzymes and follow the formation of the carbamyl enzymes with more positive charges than the tetrahedral species. Carbamates **24**−**31** are also exceptions for active site binding inhibitors and probably the second alkyl chain binding site-directed inhibitors for both enzymes. However, the enzyme inhibition constants for carbamates **24**−**31** are correlated with values of σ^*, E_s, and π. The negative ρ^* values for these correlations indicate that both enzymes and carbamates **24**−**31** form the tetrahedral species with more positive charges than inhibitors and the enzymes and follow the formation of the carbamyl enzymes with more positive charges than those tetrahedral species. Therefore, carbamates **24**−**31** may bind to both the active sites and the second alkyl chain binding site and follow the evacuation of the active sites. A comparison of the ρ^* values for cholesterol esterase and *Pseudomona species* lipase by carbamates **24**−**31** indicates that cholesterol esterase is much more sensitive to the σ^* values than *Pseudomona species* lipase. The negative sensitivity values, δ, for the cholesterol esterase inhibitions by carbamates **24**−**31** indicate that the enzyme prefers to bind to a bulky carbamyl group rather than bind to a small one. The hydrophobicity of carbamates **24**−**31** does not play a major role in both enzyme inhibitions.

Recently, there has been increased interest in pancreatic cholesterol esterase (CEase, EC 3.1.1.13) due to correlation between enzymatic activity in vivo and absorption of dietary cholesterol (*1*, *2*). Physiological substrates include cholesteryl esters, retinyl esters, acylglycerols, vitamin esters, and phospholipids (*3*−*6*). CEase plays a role in digestive lipid absorption in the upper intestinal tract, though its role in cholesterol absorption in particular is controversial (*1*, *2*, *7*). A recent report indicates that CEase is directly involved in lipoprotein metabolism in that the enzyme catalyzes the conversion of large LDL to smaller, denser, more cholesteryl ester-rich lipoproteins and that the enzyme may regulate serum cholesterol levels (*8*). The enzyme may function in these roles by acting as a cholesterol transfer protein (*9*).

* To whom correspondence should be addressed. Fax: 886-4-286-2547.

† This work was supported by the National Science Council of Taiwan (G.L.).

‡ Department of Chemistry, National Chung-Hsing University.

§ Institute of Biochemistry, National Chung-Hsing University.

10.1021/bi982775e CCC: $18.00
Published on Web 07/13/1999

Serine lipases and CEase share the same catalytic machinery as serine proteases (*10, 11*) in that they have an active site serine residue which, with a histidine and an aspartic or glutamic acid, forms a catalytic triad. The conservation of this catalytic triad suggests that as well as sharing a common mechanism for substrate hydrolysis, that is, formation of a discrete acyl enzyme species via the active site serine hydroxy group, serine proteases, CEase, and lipases may well be expected to be inhibited by the same classes of mechanism-based inhibitors. To date, this has been demonstrated for diethyl-*p*-nitrophenol phosphate (*12*), hexadecylsulfonyl fluoride (*13*), fluoroketones (*14, 15*), boronic acid (*16*), chloroisocoumarin (*17*), β-lactones (*18, 19*), β-lactams (*20*), and carbamates (*21*−*26*). The CEase-catalyzed hydrolysis of lipid substrates via a serine protease mechanism has been proposed by Quinn et al. (*27, 28*). CEase has also been used in resolution of binaphthols, spirobiindanols (*29, 30*), and inositol derivatives (*31*).

The X-ray structure of *Candida rugosa* lipase (CRL), a homologue of CEase, has been reported (*32*). The X-ray structures of bovine pancreatic CEase have been reported recently (*33, 34*). The amino acid sequence of bovine CEase reveals about 30% amino acid residue identity to that of *Torpedo californica* acetylcholinesterase, *Geotrichum candidum* lipase, CRL, and *Candida cylindracea* CEase (*33*). The structure of the active site region of CEase (*33, 34*) is similar to *Torpedo californica* acetylcholinesterase (*35*), CRL (*32*), and *Geotrichum candidum* lipase (*36*). Therefore, it is reasonable to propose a common active site for CEase, CRL, and PSL based on the X-ray structures. The active site region of CEase, like other serine lipases, possesses a Ser194/His435/Asp320 active site triad that is involved in nucleophilic and general acid/base catalysis and a neighboring oxyanion hole, the H-bonding peptide NH functions of Gly107, Ala108, and Ala195, that stabilizes the incipient carbonyl C−O$^-$ of the ester function during turnover. Therefore, the active site of CEase (*33*, *34*) and PSL like CRL (*32*) and acetylcholinesterase (*37*) may consist of at least five major binding sites (Figure 2): (a) an alkyl-chain-binding site (ACS) that binds to the acyl chain of the substrate (*32*−*34*); (b) an oxyanion hole (OH) that stabilizes the tetrahedral species (*32*−*34*); (c) an esteratic site (ES), comprised of the active site serine which would attack the ester carbonyl (*32*−*34*); (d) a leaving group hydrophobic binding site, the peripheral site, and/or the second alkyl chain or group binding site (SACS) that binds to the cholesterol part of cholesterol ester (Figure 2A) (*33, 34*) or the second fatty acid chain of triacylglyceride (Figure 2B) (*32*), which is relatively larger than ACS; (e) a leaving group hydrophilic binding site that binds to the hydrophilic part of the leaving group and is located at the opposite direction of ACS (*32*−*34*); (f) the third alkyl chain binding site that may bind to the third fatty acid chain of triacylglyceride (Figure 2B), which is also located at the opposite direction of ACS, has exposures to the solvent, and has room to adopt many different conformations (*32*).

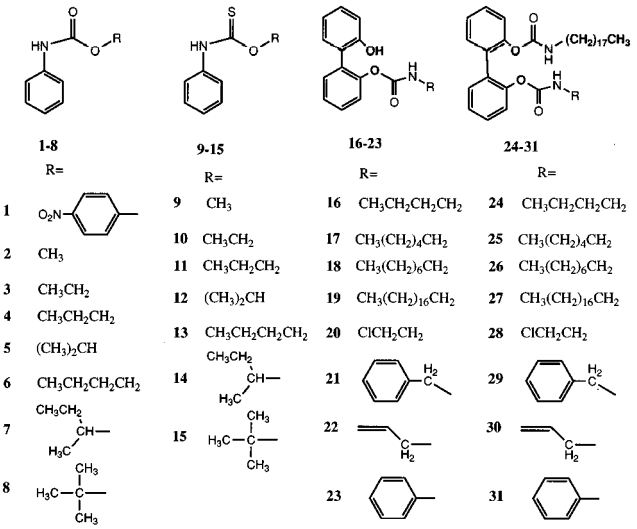


FIGURE 1: Structures of inhibitors **1**−**31**.

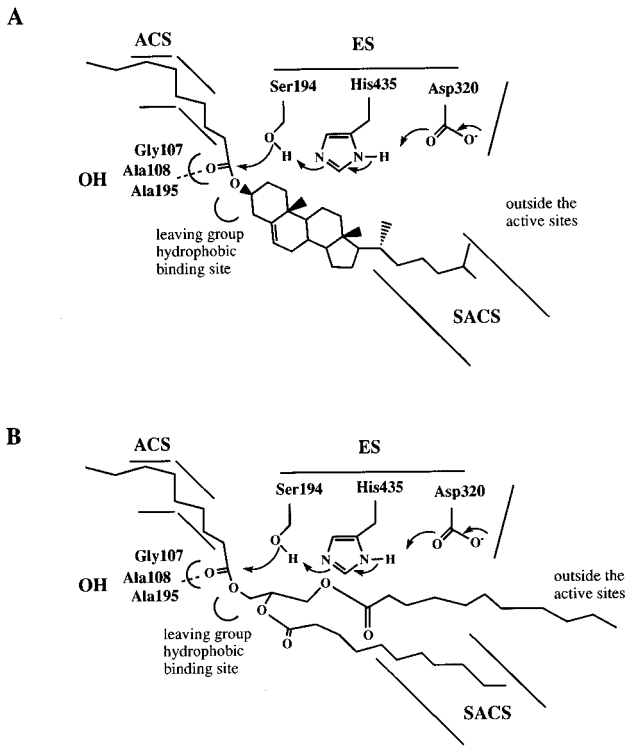


FIGURE 2: (A) Interactions between cholesteryl ester and the active sites of CEase (or PSL). The interaction for OH (Gly107, Ala108, and Ala195) is proposed by Grochulski et al. (*32*). (B) Interactions between triacylglyceride and the active sites of CEase (or PSL).

Inhibition of CRL with sulfonyl chloride/enzyme ratios of 10:1 and greater results in a second site of covalent modification based on the X-ray structure (*32*). In addition to the inhibitor bound to Ser209, a second inhibitor molecule has covalently modified His449. These complexes are no longer transition-state analogues as the modified His side chain is rotated away from its position in the intact triad. The sulfonyl group of the second inhibitor is attached to Nϵ2 of the histidine ring. The two inhibitor molecules bind in a

[1] Abbreviations: ACS, alkyl chain binding site; CEase, pancreatic cholesterol esterase; CRL, *Candida rugosa* lipase; δ, the susceptibility (intensity factors) of the reaction to steric factors; δ/ppm, chemical shifts in parts per million; ES, esteratic site; E_s, the Taft steric constant; HRMS, high resolution mass spectra; k_{app}, the first-order rate constants; k_c, the carbamylation constants; k_i, bimolecular rate constant; OH, oxyanion hole; π, the hydrophobicity constant; PNPB, *p*-nitrophenyl butyrate; PSL, *Pseudomona species* lipase; ρ (ρ*) the reaction constant (the intensity factor of the inductive effect); σ, the Hammett substituent constant; σ*, the Taft substituent constant; SACS, the second alkyl chain or group binding site; TFA, trifluoroacetophenone; ψ, the intensity factors of the hydrophobicity.

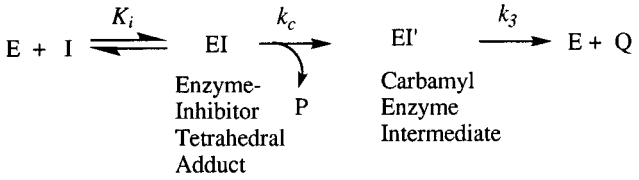
head to head arrangement and are bridged by two water molecules that hydrogen bond to one oxygen atom from each inhibitor. Only the first five carbons of this second alkyl chain are well-ordered. These carbon atoms fold between and make extensive van der Waals contacts with the phenyl rings of Phe344 and Phe448. That partial blocking of the active site of porcine pancreatic lipase by a carbamate inhibitor causes the enhancement of enantioselectivity for the resolution of 1-indanol (*38*), strongly supporting two binding sites for the alkyl groups in PSL and CEase. Thus, the overall binding site shape for all three alkyl chains of triacylglyceride in the enzyme looks like a tuning fork, which either the *sn*-1,3 or *sn*-2 fatty acyl chain binds to ACS, another fatty acyl chain binds to SACS, and the third fatty acid acyl chain extends into the solvent, binds to the third alkyl chain binding site, and has room to adopt many different conformations (Figure 2B) (*32*).

In this paper, alkyl-*N*-phenyl carbamates (**2**−**8**), alkyl-*N*-phenyl thiocarbamates (**9**−**15**), 2,2′-biphenyl-2-ol-2′-*N*-substituted carbamates (**16**−**23**), and 2,2′-biphenyl-2-*N*-octadecylcarbamate-2′-*N*-substituted carbamates (**24**−**31**) (Figure 1) are synthesized as the SACS- and/or ACS-ES-OH directed inhibitors for CEase and PSL. Aryl carbamates, such as substituted-phenyl-*N*-*n*-butyl carbamates and 4-nitrophenyl-*N*-substituted carbamates, have been characterized as transient or pseudo substrate inhibitors for CEase and have showed structure−reactivity relationships in CEase inhibition reactions (*22, 23, 25*). Carbamates **2**−**8** and thiocarbamates **9**−**15** may not bind to the ACS-ES-OH active sites of both CEase and PSL due to the fact that the leaving groups of these inhibitors, the alkoxide ions, are much poorer than the substituted phenoxide ions. Therefore, carbamates **2**−**8** and thiocarbamates **9**−**15** may bind to the enzyme other than the ACS-ES-OH active sites. Thiocarbamates **9**−**15** may be more potent inhibitors than carbamates **2**−**8** primarily because carbamates are more resonance stabilized than thiocarbamates and the activation energy for thiocarbamates is smaller than that for carbamates. Overall, carbamates **2**−**8** and thiocarbamates **9**−**15** may bind to the enzyme at the site(s) other than the ACS-ES-OH active sites. These inhibitors, therefore, may bind to the SACS of the enzyme and do not block the entry of TFA. Carbamates **16**−**23** may be very potent ACS-ES-OH-directed pseudo substrate (or transient) inhibitors of the enzyme due to the possible extra interactions between the hydroxy group of the inhibitor molecules and the enzyme (*24, 26*). On the other hand, carbamates **24**−**31** may bind to the enzyme at both ACS-ES-OH active sites and SACS by their two carbamyl groups. Overall, correlation of the structures of compounds **2**−**31** with the enzyme reactivity may lead to some information about the inhibition mechanism at SACS.

In the presence of substrate, the mechanism for inactivation of carbamate inhibitors of CEase and PSL has been proposed in Scheme 1 (*21-26, 28*). This mechanism is *pro forma* that for substrate turnover, and, therefore, carbamates are best described as pseudo substrate or transient inhibitors whose potencies derive in part from the slowness of the decarbamylation phase of the reaction (*22, 28*).

Since the inhibition follows first-order kinetics over the observed time period, the rate of hydrolysis of EI′ must be significantly slower than the rate of formation of EI′($k_c >> k_3$) (*39*). Therefore, values of K_i and k_c can be calculated from eq 1 (*21*−*26, 28, 40*):

Scheme 1. Carbamyl Enzyme Mechanism of CEase and PSL Inhibition[a]



[a] E, enzyme, I, carbamate inhibitor; P, Q, alcohol and carbamic acid products; EI, noncovalent complex or enzyme-inhibitor tetrahedral adduct (species); EI′, carbamyl enzyme intermediate.

$$k_{app} = \frac{k_c[I]}{K_i\left(1 + \frac{[S]}{K_m}\right) + [I]} \quad (1)$$

In eq 1, the k_{app} values are the first-order rate constants that can be obtained according to Hosie's method (*21*−*26, 28*). The bimolecular rate constant, $k_i = k_c/K_i$, is related to overall inhibitory potency.

Aryl carbamate inhibitors are characterized as the active site-directed pseudo substrate or transient inhibitors of CEase (*21*−*26, 28*) (Scheme 1) since they meet some of the criteria proposed by Abeles and Maycock (*41*). First, the inhibition is time-dependent and follows first-order kinetics. Second, with increasing concentration of inhibitor, the enzyme displays saturation kinetics. Third, the enzyme is protected from inhibition by carbamate inhibitors in the presence of the competitive inhibitor, TFA (*15, 26*). If the enzyme is protected from inhibition by carbamate inhibitors in the presence of TFA, then these carbamate inhibitors are the ACS-ES-OH active site-directed pseudo substrate inhibitors of the enzyme. On the other hand, if the enzyme is not protected from inhibition but further inhibited by carbamate inhibitors in the presence of TFA, then these carbamate inhibitors are still pseudo substrate inhibitors (Scheme 1); however, they are no longer the ACS-ES-OH active site-directed inhibitors. These inhibitors, based on exceptions for active site binding, may, therefore, bind to the SACS instead.

The effects of structure on reactivity can be divided into four major types (*43*−*48*): inductive effect, resonance effect, steric effect, and hydrophobicity. In most cases, two or all four of these are operating, and it is usually not easy to tell how much of the rate enhancement or decrease is caused by each of the four effects. Quantitative treatments of the effects of structure on reactivity have first been reported by Hammett, known as the Hammett equation eq 2 (*43*−*46*).

$$\log k = h + \rho\sigma \quad (2)$$

In eq 2, the h value is the log k_0 value and the parameters ρ and σ are the reaction constant (the intensity factor of the inductive effect) and the Hammett substituent constant, respectively. Taft et al. (eq 3) further take the steric effect into account and have reported the Taft−Ingold equation (eq 3) (*43*−*46*) where the parameters ρ^*, σ^*, E_s, and δ are the reaction constant, substituent constant, the steric constant, and the susceptibility of the reaction to steric factors, respectively. The constants σ^* and E_s have their usual meanings as in physical organic chemistry (*44*−*46*).

$$\log k = h + \rho^*\sigma^* + \delta E_s \quad (3)$$

Järv et al. have further reported the multiparameter correlation equation (eq 4) to characterize acetylcholinesterase reactions (*42*).

$$\log k = h + \rho^*\sigma^* + \delta E_s + \psi\pi \quad (4)$$

In eq 4, the parameters σ^*, E_s, and π are the Taft substituent constant, the Taft steric constant, and hydrophobicity, respectively, and the parameters ρ^*, ψ, and δ are the intensity factors of the inductive effect, hydrophobicity, and steric effect, respectively. The hydrophobicity constants π have been introduced by Hansch et al. (*47, 48*). Structure−activity relationships in CEase reactions have been shown as the Hammett or Taft−Ingold correlation (*22, 23, 25*). Structure−activity relationships in CEase reactions have been also shown as the multiparameter correlation of eq 4 (*25*).

MATERIALS AND METHODS

Materials. Porcine pancreatic CEase, PSL, and PNPB were obtained from Sigma; TFA and other chemicals were obtained from Aldrich. Silica gel used in liquid chromatography (Licorpre Silica 60, 200−400 mesh), medium pressure liquid chromatography column (LiChroprep Si 60), and thin layer chromatography plates (Kieselgel 60 F_{254}) were obtained from Merck. An UV lamp as well as an UV detector (Linear UV-106 or ISCO UA-6) was used in detection. Hexane/ethyl acetate solvent gradient was used in liquid chromatography and medium pressure liquid chromatography. Other chemicals and biochemicals were of the highest quality available commercially.

Instrumental Methods. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75.4 MHz (Varian-VXR 300 spectrometer), respectively. The ^{1}H and ^{13}C NMR chemical shifts were referred to internal Me_4Si. UV spectra were recorded on an UV−visible spectrophotometer (Hewlett Packard 8452A or Beckman DU-650) with a cell holder circulated with a water bath (HOTECH 631-D). HRMS were recorded at 70 eV on a Joel JMS-SX/SX-102A mass spectrophotometer. Elemental analyses were preformed on a Heraeus instrument.

*Synthesis of Inhibitors **2**−**31***. Carbamates **2**−**8** and thiocarbamates **9**−**15** were prepared from the condensation of phenyl isocyanate or phenyl thioisocyanate with 10 equiv of the corresponding alcohol in the presence of 1 equiv of potassium *t*-butoxide in dimethyl sulfate at 65 °C for 24 h (82−91% yield). Carbamates **16**−**23** were prepared from the condensation of 2,2′-biphenol with 1.2 equiv of the corresponding isocyanate in the presence of a catalytic amount of pyridine in dichloromethane at 25 °C for 48 h (80−85% yield). Carbamates **24**−**31** were prepared from the condensation of carbamates **16**−**23** with 1.2 equiv of *n*-octadecyl isocyanate in the presence of a catalytic amount of pyridine in dichloromethane at 25 °C for 48 h (68−81% yield). Compounds **2**−**31** were purified by liquid chromatography or medium pressure liquid chromatography and characterized by ^{1}H and ^{13}C NMR spectra, HRMS, and elemental analyses (see Appendix).

Enzyme Kinetics and Data Reduction. All kinetic data were obtained by using an UV−visible spectrophotometer (Hewlett Packard 8452A or Beckman DU-650) that was interfaced to a personal computer. Kaleida Graph (version 2.0) and Origin (version 4.0) were used for all least-squares curve fittings. The CEase inhibition was assayed by the Hosie method (*21, 28*). The temperature was maintained at 25.0 ± 0.1 °C by a refrigerated circulating water bath. All reactions were performed in sodium phosphate buffer (pH 7.0) containing NaCl (0.1 M), acetonitrile (2% by volume), and varying concentrations of substrate PNPB (50 μM for CEase, 500 μM for PSL) and inhibitors. The K_m values for CEase- and PSL-catalyzed hydrolyses of PNPB were calculated to be 140 ± 10 μM and 1.5 ± 0.1 mM, respectively, by fitting initial velocities versus substrate concentration to the Michaelis−Menten equation. Requisite volumes of stock solution of substrate and inhibitors in acetonitrile were injected into reaction buffers via Gilson Pipetmen. CEase was dissolved in sodium phosphate buffer (0.1 M, pH 7.0). Reactions were initiated by injecting enzyme and monitored at 410 nm on the UV-visible spectrometer. First-order rate constants (the k_{app} values) for inhibition of CEase were determined as described by Hosie et al. (*21, 22, 26, 28*). Values of K_i and k_c can be obtained by fitting the data of k_{app} and [I] to eq 1 by nonlinear least squares. Duplicate sets of data were collected for each inhibitor concentration.

Return of Activity and Protection by TFA. For the return of activity study, CEase or PSL was incubated with a carbamate in the absence and presence of TFA (2 μM), a known competitive inhibitor of CEase (*15, 26*) before the inhibition reaction. All the other procedures followed those of Hosie et al. (*21, 26*).

RESULTS

Inhibitors **2**−**31** are synthesized by general procedures (54−91% yield) and are characterized by ^{1}H and ^{13}C NMR spectra, HRMS, and elemental analyses (see Appendix).

Inhibitors **2**−**31** are characterized as transient or pseudo substrate inhibitors (Scheme 1) for CEase (*21*−*26, 28*) and PSL because all inhibitions are time-dependent and follow first-order kinetics. CEase and PSL are not protected from inhibition but further inactivated by carbamates **2**−**8**, thiocarbamates **9**−**15**, and carbamates **24**−**31** in the presence of TFA. Therefore, carbamates **2**−**8**, thiocarbamates **9**−**15**, and carbamates **24**−**31** are characterized as the non-ACS-ES-OH active sites-directed and probably the SACS-directed pseudo substrate inhibitors for both enzymes.

Tables 1−4 summarize the inhibition data for CEase- and PSL-catalyzed hydrolysis of PNPB in the presence of carbamates **2**−**8** and thiocarbamates **9**−**15**. Carbamates **2**−**8** and thiocarbamates **9**−**15** may be characterized as the SACS-directed pseudo substrate inhibitors for both enzymes. The inhibition data by carbamates **2**−**8** and thiocarbamates **9**−**15** are correlated with E_s and π; however, the inhibition data are not correlated with σ^*. Comparisons of the inhibition data by carbamates **2**−**8** and thiocarbamates **9**−**15** for both enzymes indicate that thiocarbamates **9**−**15** were more potent inhibitors than carbamates **2**−**8** and that the positive slope values for the logarithms of inhibition data for CEase toward the inhibitor molecules versus E_s and π but the negative slope values for PSL toward the inhibitor molecules are also observed.

Tables 5-8 summarize the inhibition data for CEase- and PSL-catalyzed hydrolysis of PNPB in the presence of carbamates **16**−**31**. CEase and PSL are protected from

Table 1: Kinetic Data and Correlation Results for the Inhibitions of CEase by Carbamates **1**−**8**[a]

compound	R	σ^*	E_s	π	K_i (μM)	k_c (10^{-3} s^{-1})	k_i (M^{-1} s^{-1})
1	4-NO_2-Ph[b]				8.9 ± 0.1	2.6 ± 0.1	290 ± 10
2	Me	0	0	0.5	1000 ± 200	0.10 ± 0.01	0.10 ± 0.02
3	Et	−0.1	−0.07	1	700 ± 200	0.10 ± 0.02	0.13 ± 0.07
4	*n*-Pr	−0.12	−0.36	1.5	6 ± 3	0.39 ± 0.03	70 ± 30
5	*i*-Pr	−0.19	−0.47	1.3	30 ± 10	0.25 ± 0.05	8 ± 3
6	*n*-Bu	−0.13	−0.39	2	1.3 ± 0.2	0.57 ± 0.05	440 ± 80
7	*i*-Bu	−0.125	−0.93	1.8	60 ± 10	0.22 ± 0.02	3.7 ± 0.7
8	*t*-Bu	−0.3	−1.54	1.98	260 ± 50	0.17 ± 0.02	0.7 ± 0.1

	-log $K_{i,}$	log k_c	log $k_{i,}$
δ^*[c]	2.0 ± 0.6	0.5 ± 0.2	2.5 ± 0.8
ψ^*[c]	2.9 ± 0.6	1.0 ± 0.2	3.9 ± 0.8
h[c]	1.1 ± 0.7	−4.9 ± 0.2	−3.8 ± 0.9
R[c]	0.924	0.934	0.930

[a] All carbamates except carbamate **1** are characterized as the non ACS-ES-OH-directed pseudo substrate inhibitors of the enzyme. [b] The ACS-ES-OH active sites-directed pseudo substrate inhibitor of the enzyme. [c] Correlations of values of -log K_i, log k_c, and log k_i with values of E_s and π by eq 4 (*42*) for all data except that of carbamate **1**. Poor correlations with the σ^* values are found.

Table 2: Kinetic Data and Correlation Results for the Inhibitions of PSL by Carbamates **1**−**8**[a]

compound	R	K_i (μM)	k_c (10^{-3} s^{-1})	k_i (M^{-1} s^{-1})
1	4-NO_2–Ph[b]	69 ± 5	3.6 ± 0.1	52 ± 4
2	Me	11 ± 3	0.50 ± 0.04	50 ± 10
3	Et	19 ± 4	0.4 ± 0.1	21 ± 7
4	*n*-Pr	18 ± 3	0.4 ± 0.1	22 ± 7
5	*i*-Pr	29 ± 2	0.4 ± 0.1	14 ± 4
6	*n*-Bu	14 ± 4	0.20 ± 0.02	14 ± 4
7	*i*-Bu	29 ± 1	0.4 ± 0.1	14 ± 4
8	*t*-Bu	5 ± 1	0.40 ± 0.01	80 ± 20

	−log $K_{i,}$	log k_c	log $k_{i,}$
δ^*[c]	−0.5 ± 0.1	−0.22 ± 0.07	−0.69 ± 0.07
ψ^*[c]	−0.2 ± 0.1	−0.30 ± 0.07	−0.54 ± 0.07
h[c]	5.0 ± 0.1	-3.11 ± 0.08	1.92 ± 0.08
R[c]	0.900	0.905	0.980

[a] All carbamates except carbamate **1** are characterized as the non ACS-ES-OH-directed pseudo substrate inhibitors of the enzyme. [b] The ACS-ES-OH active sites-directed pseudo substrate inhibitor of the enzyme. [c] Correlations of values of -log K_i, log k_c, and log k_i with values of E_s and π by eq 4 (*42*) for all data except that of carbamate **1**. Poor correlations with the σ^* values are found.

inhibition by carbamates **16**−**23** in the presence of a competitive inhibitor TFA (*15, 26*). The enzyme inhibition data of carbamates **16**−**22** are well-correlated with σ^* alone. The negative ρ^* values for these correlations are observed. Therefore, carbamates **16**−**22** are characterized as the ACS-ES-OH active sites-directed pseudo substrate inhibitors for both enzymes. Carbamates **24**−**31** are characterized as the non-ACS-ES-OH active sites-directed pseudo substrate inhibitors for both enzymes; however, both enzyme inhibition data for carbamates **24**−**31** are correlated with σ^*, E_s, and π. The negative ρ^* values for these correlations are observed. The negative δ values for the CEase inhibition by carbamates **24**−**31** are observed. The ψ values for the CEase inhibition by carbamates **24**−**31** are close to zero. The ρ^* and δ values for PSL inhibitions by carbamates **24**−**31** are also close to zero.

Table 3: Kinetic Data and Correlation Results for the Inhibitions of CEase by Thiocarbamates **9**−**15**[a]

compound	R	K_i (μM)	k_c (10^{-3} s^{-1})	k_i (10^3 M^{-1} s^{-1})
9	Me	0.8 ± 0.1	0.11 ± 0.01	0.14 ± 0.02
10	Et	0.7 ± 0.1	0.16 ± 0.01	0.23 ± 0.02
11	*n*-Pr	0.17 ± 0.07	0.456 ± 0.002	2.7 ± 0.1
12	*i*-Pr	0.21 ± 0.05	0.270 ± 0.009	1.3 ± 0.3
13	*n*-Bu	0.013 ± 0.001	1.1 ± 0.2	80 ± 20
14	*i*-Bu	0.26 ± 0.05	0.29 ± 0.02	1.1 ± 0.2
15	*t*-Bu	0.3 ± 0.1	0.22 ± 0.02	0.7 ± 0.2

	−log $K_{i,}$	log k_c	log $k_{i,}$
δ^*[b]	1.0 ± 0.3	0.6 ± 0.1	1.6 ± 0.4
ψ^*[b]	1.5 ± 0.3	0.8 ± 0.1	2.2 ± 0.4
h[b]	5.1 ± 0.4	−4.4 ± 0.1	0.7 ± 0.5
R[b]	0.923	0.970	0.946

[a] All thiocarbamates are characterized as the nonACS-ES-OH-directed pseudo substrate inhibitors of the enzyme. [b] See footnote *c* in Table 1.

Table 4: Kinetic Data and Correlation Results for the Inhibitions of PSL by Thiocarbamates **9**-**15**[a]

compound	R	K_i (μM)	k_c (10^{-3} s^{-1})	k_i (M^{-1} s^{-1})
9	Me	25 ± 2	1.00 ± 0.03	40 ± 3
10	Et	15 ± 1	1.00 ± 0.03	67 ± 5
11	*n*-Pr	12 ± 1	1.8 ± 0.1	150 ± 20
12	*i*-Pr	20 ± 3	1.8 ± 0.1	90 ± 10
13	*n*-Bu	23 ± 1	1.6 ± 0.2	70 ± 9
14	*i*-Bu	5.0 ± 0.1	1.8 ± 0.1	360 ± 20
15	*t*-Bu	1.2 ± 0.1	2.0 ± 0.1	1,700 ± 200

	−log $K_{i,}$	log k_c	log $k_{i,}$
δ[b]	−1.0 ± 0.2	−0.1 ± 0.1	−1.1 ± 0.2
ψ^*[b]	−0.3 ± 0.2	0.14 ± 0.09	−0.1 ± 0.2
h[b]	4.8 ± 0.2	−3.1 ± 0.1	1.7 ± 0.2
R[b]	0.954	0.856	0.974

[a] All thiocarbamates are characterized as the nonACS-ES-OH-directed pseudo substrate inhibitors of the enzyme. See footnote *c* in Table 1.

DISCUSSION

Because CEase, PSL, and CRL are homologs to each other and have similar active site amino acid residues (*32*−*35*), we, therefore, assume all three enzymes have the same active site (Figure 2) and, therefore, we use the same model to explain all these catalyses. Carbamate **1** is characterized as the ACS-ES-OH active sites-directed pseudo substrate inhibitors for CEase (*23, 25*) and PSL. However, carbamates **2**−**8** and thiocarbamates **9**−**15** are characterized as the non-ACS-ES-OH active sites-directed pseudo substrate inhibitors for both enzymes . Both CEase and PSL are not protected from inhibition and further inhibited by carbamates **2**−**8** and thiocarbamates **9**−**15** in the presence of a competitive inhibitor, TFA (*15, 26*). Therefore, these inhibitors may block the site other than the ACS-ES-OH active sites. Thus, **carbamates 2**−**8 and thiocarbamates 9**−**15 may bind to the SACS of the enzyme**, and TFA can still enter the ACS-ES-OH active sites of the enzyme (Figure 3A).

The inhibition data for carbamates **2**−**8** and thiocarbamates **9**−**15** are correlated with E_s and π; however, the inhibition data are not correlated with σ^* (Tables 1−4). In other words, these reactions are sensitive to the steric effect and hydro-

Table 5: Kinetic Data and Correlation Results for the Inhibitions of CEase by Carbamates **16**−**23**[a]

compound	R	σ^*	E_s	K_i (μM)	k_c (10^{-3} s^{-1})	k_i (10^3 M^{-1} s^{-1})
16	*n*-Bu	−0.13	−0.39	0.21 ± 0.02	10 ± 1	48 ± 7
17	*n*-Hex	−0.15	−0.40	0.22 ± 0.02	10 ± 1	45 ± 4
18	*n*-Oct	−0.13	−0.33	0.12 ± 0.02	9.7 ± 1	80 ± 20
19	*n*-$C_{18}H_{37}$	−0.13	−0.33	0.15 ± 0.02	10 ± 1	70 ± 10
20	C_2H_4Cl	0.39	−0.43	2.4 ± 0.2	1.6 ± 0.1	0.667 ± 0.007
21	CH_2Ph	0.22	−0.38	0.7 ± 0.1	0.95 ± 0.09	1.3 ± 0.2
22	Allyl	0.1	−0.39	0.20 ± 0.02	2.4 ± 0.2	12 ± 2
23	Ph	0.6	−2.55	0.041 ± 0.004	0.40 ± 0.05	10 ± 2

	−log K_i,	log k_c	log k_i,
ρ^{*b}	−2.0 ± 0.4	−1.9 ± 0.4	−3.9 ± 0.4
h^b	6.55 ± 0.08	−2.30 ± 0.07	4.25 ± 0.07
R^b	0.911	0.925	0.978

[a] Carbamates **16**−**23** were characterized as the ACS-ES-OH active sites-directed pseudo substrate inhibitors of the enzyme. [b] Correlations of values of −log K_i, log k_c, and log k_i with the σ^* values by the Hammett equation (eq 2) (*43*−*46*) for all data except those of carbamate **23**.

Table 6: Kinetic Data and Correlation Results for the Inhibitions of PSL by Carbamates **16**−**23**[a]

compound	R	K_i (nM)	k_c (10^{-3} s^{-1})	k_i (10^3M^{-1} s^{-1})
16	*n*-Bu	50 ± 6	5.1 ± 0.5	100 ± 20
17	*n*-Hex	50 ± 5	5.5 ± 0.6	110 ± 20
18	*n*-Oct	37 ± 3	5.7 ± 0.6	150 ± 20
19	*n*-$C_{18}H_{37}$	32 ± 3	5.6 ± 0.6	180 ± 20
20	C_2H_4Cl	420 ± 40	0.42 ± 0.04	1.0 ± 0.1
21	CH_2Ph	100 ± 10	0.95 ± 0.09	10 ± 1
22	Allyl	87 ± 9	1.5 ± 0.2	17 ± 3
23	Ph	40 ± 4	0.31 ± 0.03	8 ± 1

	−log K_i,	log k_c	log k_i,
ρ^{*b}	−1.6 ± 0.3	−2.15 ± 0.08	−3.8 ± 0.3
h^b	7.18 ± 0.05	−2.56 ± 0.02	4.62 ± 0.06
R^b	0.937	0.997	0.985

[a] Carbamates **16**−**23** were characterized as the ACS-ES-OH active sites-directed pseudo substrate inhibitors of the enzyme. [b] Correlations of values of −log K_i, log k_c, and log k_i with the σ^* values by the Hammett equation (eq 2) (*43*−*46*) for all data except those of carbamate **23**.

phobicity but insensitive to the inductive effect of the inhibitors. Thus, **the SACS of the enzyme is not correlated with the σ^* values of the inhibitor molecules.**

A comparison of the CEase and PSL inhibitions by carbamates **2**−**8** or thiocarbamates **9**−**15** indicates that the E_s and π values for CEase are more sensitive and more positive than those for PSL (Tables 1−4). This may be due to the fact that the bulkiness of the inhibitor substituents inactivates CEase but activates PSL and that the hydrophobicity of the inhibitor substituents activates CEase but inactivates PSL. Thus, the SACS of CEase is small but hydrophobic toward the inhibitor molecules; however, the SACS of PSL is relatively too bulky to sense the steric effect of the inhibitor substituents, and the site is relatively hydrophilic toward the inhibitor molecules.

When comparing the CEase inhibitions by carbamates **2**−**8** (Table 1) and thiocarbamates **9**-**15** (Table 3), we find that values of δ and ψ for CEase are more sensitive toward carbamates **2**−**8** than those toward thiocarbamates **9**−**15**. This is probably due to the fact that the sulfur (compared to oxygen) atom makes the enzyme−thiocarbamate tetrahedral

Table 7: Kinetic Data and Correlation Results for the Inhibitions of CEase by Carbamates **24**-**31**[a]

compound	R	π	K_i (nM)	k_c (10^{-3} s^{-1})	k_i (10^3 M^{-1} s^{-1})
24	*n*-Bu	2	70 ± 10	1.4 ± 0.1	18 ± 3
25	*n*-Hex	3	70 ± 10	1.39 ± 0.05	18 ± 3
26	*n*-Oct	4	110 ± 10	0.87 ± 0.05	7.9 ± 0.8
27	*n*-$C_{18}H_{37}$	9	100 ± 10	0.80 ± 0.05	8.0 ± 0.8
28	C_2H_4Cl	1.39	370 ± 30	0.30 ± 0.04	0.8 ± 0.1
29	CH_2Ph	2.26	250 ± 50	0.34 ± 0.05	14 ± 3
30	Allyl	1.23	170 ± 20	0.77 ± 0.09	4.4 ± 0.7
31	Ph	2.13	20 ± 1	0.40 ± 0.06	20 ± 3

	−log K_i,	log k_c	log k_i,
ρ^{*b}	−1.37 ± 0.09	−1.4 ± 0.2	−2.7 ± 0.2
δ^b	−0.74 ± 0.03	−0.23 ± 0.06	−0.98 ± 0.08
ψ^b	−0.010 ± 0.007	−0.03 ± 0.01	−0.04 ± 0.02
h^b	6.65 ± .03	−3.09 ± 0.06	3.57 ± 0.08
R^b	0.997	0.974	0.988

[a] Carbamates **24**−**31** are characterized as the non ACS-ES-OH-directed pseudo substrate inhibitors of the enzyme. [b] See footnote c in Table 1.

Table 8: Kinetic Data and Correlation Results for the Inhibitions of PSL by Carbamates **24**−**31**[a]

compound	R	K_i (nM)	k_c (10^{-3}s^{-1})	k_i (10^3 M^{-1} s^{-1})
24	*n*-Bu	40 ± 4	2.9 ± 0.2	73 ± 9
25	*n*-Oct	41 ± 3	3.0 ± 0.2	73 ± 7
27	*n*-$C_{18}H_{37}$	40 ± 4	3.0 ± 0.2	73 ± 7
28	C_2H_4Cl	70 ± 6	4.3 ± 0.1	61 ± 5
29	CH_{2Ph}	51 ± 5	3.5 ± 0.3	69 ± 9
30	Allyl	47 ± 4	3.7 ± 0.5	80 ± 10
31	Ph	11 ± 1	4.1 ± 0.3	370 ± 40

	−log K_i,	log k_c	log k_i,
ρ^{*b}	−0.35 ± 0.05	0.33 ± 0.06	−0.02 ± 0.08
δ^b	0.07 ± 0.02	0.04 ± 0.02	0.11 ± 0.03
h^b	7.39 ± 0.01	−2.49 ± 0.01	4.90 ± 0.02
R^b	0.994	0.966	0.967

[a] See footnote *a* in Table 7. [b] Correlations of −log K_i, log k_c, and log k_i with values of σ^* and E_s by Taft-Ingold equation (eq 3) (*43*−*46*) for all data.

species too crowded to sense the difference in the E_s and π values.

With a good leaving group, such as 4-nitrophenoxide ($\sigma^* > 0$), carbamate **1** is bound to the ACS-ES-OH of the enzyme. However, carbamates **2**−**8** do not bind to the ACS-ES-OH active sites of the enzyme probably because the inhibitor molecules do not have a good leaving group (alkoxide instead). Therefore, the leaving group ability plays a major role in the selection of the reaction site. Thus, the leaving group binding sites of the enzyme are probably positively charged at the one end where the SACS of the enzyme neighbors are (Figure 4A) and negative charged at the other end where the ACS-ES-OH active sites of the enzyme contact (Figure 4B).

A comparison of the inhibitions by carbamates **2**−**8** and thiocarbamates **9**−**15** indicates that thiocarbamates **9**−**15** are more potent inhibitors than carbamates **2**−**8** probably because (a) the SACS EI tetrahedral species (Scheme 1) for thiocarbamates **9**−**15** are too crowded compared to those for carbamates **2**−**8**, and (b) the stabilization by resonance in the carbamate bonds makes a stable, inactive species (high activation energy); however, the absence of that resonance

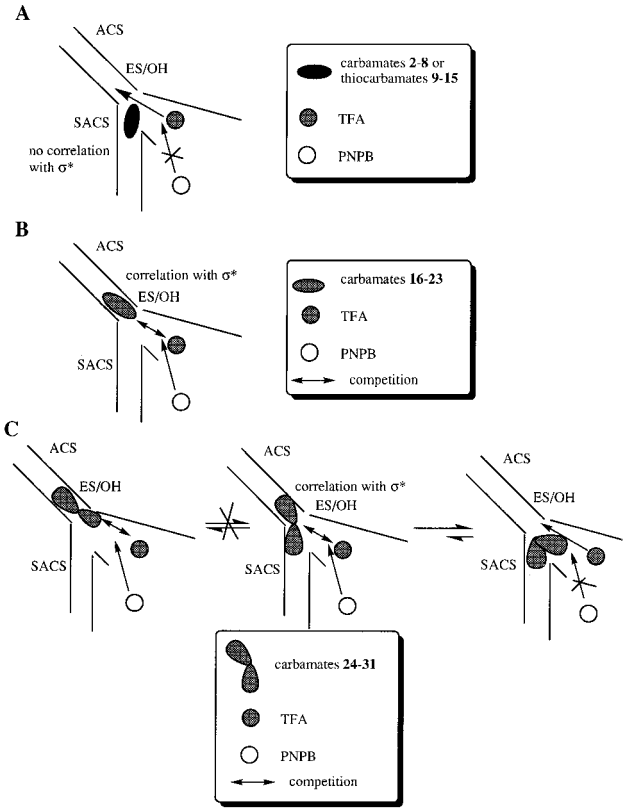


FIGURE 3: (A) Interactions between the active sites of CEase (or PSL) and carbamates **2**−**8** or thiocarbamates **9**−**15** in the presence of TFA and PNPB. The SACS of the enzyme is not correlated with the σ^* values of the inhibitor molecules. (B) Interactions between the active sites of CEase (or PSL) and carbamates **16**−**23** in the presence of TFA and PNPB. The ACS-ES-OH active sites of the enzyme are correlated with the σ^* values of the inhibitor molecules. (C) Interactions between the active sites of CEase (or PSL) and carbamates **24**−**31** in the presence of TFA and PNPB. The ACS-ES-OH active sites of the enzyme are correlated with the σ^* values of the inhibitor molecules and are evacuated in the second step.

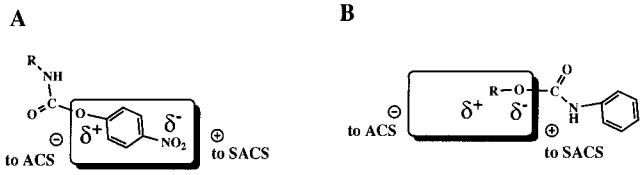


FIGURE 4: (A) Interactions between 4-nitrophenyl-*N*-alkyl carbamate and the leaving group binding site of CEase (or PSL). (B) Interactions between carbamates **2**−**8** and the leaving group binding site of CEase (or PSL).

in thiocarbamates **9**−**15** makes a relatively unstable, active species (low activation energy).

Carbamates 16−**23 are the ACS-ES-OH active sites-directed pseudo substrate inhibitors for both enzymes** (Figure 3B). For CEase, carbamates **16**−**23** are more potent inhibitors than 4-nitrophenyl-*N*-substituted carbamates (*24*, *26*) probably due to the fact that carbamates **16**−**23** interact well with the ACS-ES-OH active site of the enzyme through the H-bonding between the hydroxy group of the inhibitor molecules and the His435 residue of the enzyme (Figure 5). The enzyme inhibition data for carbamates **16**−**22** are well-correlated with σ^* alone. The negative ρ^* values for these correlations (Tables 5 and 6) indicate that the serine residue of both enzymes and carbamates **16**−**23** form the tetrahedral species with more positive charges than inhibitors and the

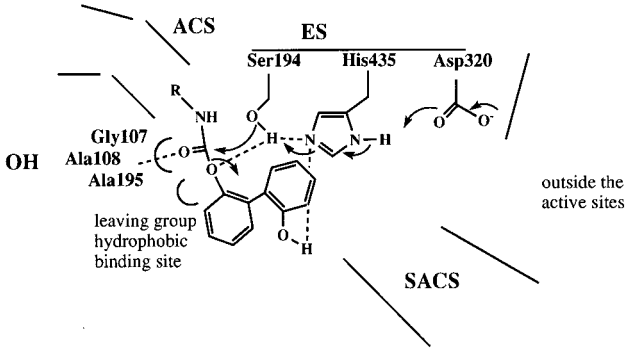


FIGURE 5: Interactions between the active sites of CEase (or PSL) and carbamates **16**−**23**. The H-binding between His435 and the hydroxy group of the inhibitor molecules is proposed.

enzymes and follow the formation of the carbamyl enzymes with more positive charges than the tetrahedral species. However, it is believed that the EI tetrahedral species are negative in charge (*22*, *23*, *28*). Therefore, the K_i step of Scheme 1 may be further divided into two steps as proposed previously (*25*). There are two possible explanations for the deviations of carbamate **23** in these correlations (Tables 5 and 6): first, that the rate-determining steps are changed; second, that the σ^* value for the phenyl group is not a constant value (0.6) and is varied with reactions.

Like carbamates **2**−**8** and thiocarbamates **9**−**15**, **carbamates 24**−**31 are also characterized as the pseudo substrate inhibitors and are probably the SACS-directed inhibitors for CEase and PSL.** The SACS of the enzyme is not correlated with the σ^* values of inhibitors **2**−**15** (Tables 1−4). However, **the CEase and PSL inhibition data for carbamates 24**−**31 are correlated with values of σ^*.** A possible explanation for this is that carbamates **24**−**31** bind to the ACS-ES-OH active sites of the enzyme before the sites of the enzyme evacuate the inhibitor molecules (Figure 3C). The negative ρ^* values for these correlations (Table 7) indicate that CEase and carbamates **24**−**31** form the tetrahedral species with more positive charges than inhibitors and the enzyme and follow the formation of the carbamyl enzymes with more positive charges than the tetrahedral species. The negative sensitivity δ values (Table 7) indicate that the SACS of CEase prefers to bind to bulky carbamates **24**−**31** rather than small ones and confirm that the size of SACS is relatively larger than that of ACS (*32*−*34*). Moreover, the fact that values of ρ^* and δ for PSL inhibitions by carbamates **24**−**31** are close to zero (Table 8) indicates that PSL is insensitive to both inductive and steric effects of the inhibitor molecules. Further, the hydrophobicity of carbamates **24**−**31** does not play a major role in both CEase and PSL inhibitions.

APPENDIX: SPECTROSCOPIC CHARACTERIZATION OF INHIBITORS 2−31

Methyl-N-phenyl carbamate (2): ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm 3.79 (s, 3H, C*H*$_3$), 6.77 (s, 1H, N*H*), 7.03−7.40 (m, 5H, phenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ/ppm 52.18 (*C*H$_3$), 118.71, 123.94, 128.94, 137.87 (phenyl *C*), 154.23 (*C*=O). HRMS calcd for $C_8H_9O_2N$: 151.0635. Found: 151.0633.

Ethyl-N-phenyl carbamate (3): ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm 1.30 (t, J = 6.9 Hz, 3H, C*H*$_3$), 4.22 (q, J =

7.2 Hz, 2H, CH_2), 6.78 (s, 1H, N*H*), 7.03−7.40 (m, 5H, phenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ/ppm 14.34 (*C*H_3), 61.01(*C*H_2), 118.67, 123.18, 128.88, 138.00 (phenyl *C*), 153.83 (*C*═O). HRMS calcd for $C_9H_{11}O_2N$: 165.0790. Found: 165.0797.

*n-Propyl-N-phenyl carbamate (**4**):* ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm 0.97 (t, J = 7.2 Hz, 3H, CH_3), 1.72 (sextet, J = 7.0 Hz, 2H, OCH_2CH_2), 4.12 (t, J = 7.0 Hz, 2H, OCH_2-CH_2), 6.74 (s, 1H, N*H*), 7.02−7.40 (m, 5H, phenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ/ppm 10.25 (*C*H_3), 22.17 (OCH_2CH_2), 66.78 (OCH_2CH_2), 118.62, 123.30, 129.02, 137.99 (phenyl *C*), 153.80 (*C*═O). HRMS calcd for $C_{10}H_{13}O_2N$: 179.0946. Found: 179.0951.

*i-Propyl-N-phenyl carbamate (**5**):* ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm 1.30 (d, J = 6.3 Hz, 6H, CH_3), 5.02 (quintet, J = 7.0 Hz, 1H, C*H*), 6.59 (s, 1H, N*H*), 7.02−7.39 (m, 5H, phenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ/ppm 21.98 (*C*H_3), 68.62 (*C*H), 118.58, 123.18, 128.98, 138.11 (phenyl *C*), 153.31 (*C*═O). HRMS calcd for $C_{10}H_{13}O_2N$: 179.0946. Found: 179.0944.

*n-Butyl-N-phenyl carbamate (**6**):* ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm 0.95 (t, J = 7.2 Hz, 3H, CH_3), 1.41 (sextet, J = 7.0 Hz, 2H, $OCH_2CH_2CH_2CH_3$), 1.65 (quintet, J = 7.0 Hz, 2H, $OCH_2CH_2CH_2CH_3$), 4.17 (t, J = 6.6 Hz, 2H, OCH_2-$CH_2CH_2CH_3$), 6.69 (s, 1H, N*H*), 7.03−7.40 (m, 5H, phenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ/ppm 13.62 (*C*H_3), 18.96 ($OCH_2CH_2CH_2CH_3$), 30.86 ($OCH_2CH_2CH_2CH_3$), 66.03 ($OCH_2CH_2CH_2CH_3$), 118.61, 123.27, 128.99, 138.00 (phenyl *C*), 153.82 (*C*═O). HRMS calcd for $C_{11}H_{15}O_2N$: 193.1102. Found: 193.1095.

*i-Butyl-N-phenyl carbamate (**7**):* ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm 0.96 (d, J = 6.9 Hz, 6H, CH_3), 1.97 (quintet, J = 7.0 Hz, 1H, C*H*), 3.95 (d, J = 6.6 Hz, 2H, CH_2), 6.73 (s, 1H, N*H*), 7.03−7.40 (m, 5H, phenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ/ppm 18.96 (*C*H_3), 27.86 (*C*H), 71.29 (*C*H_2), 118.61, 123.30, 129.02, 137.99 (phenyl *C*), 153.82 (*C*═O). HRMS calcd for $C_{11}H_{15}O_2N$: 193.1102. Found: 193.1111.

*t-Butyl-N-phenyl carbamate (**8**):* ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm 1.52 (s, 9H, CH_3), 6.47 (s, 1H, N*H*), 7.00−7.37 (m, 5H, phenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ/ppm 28.28 (*C*H_3), 80.48 (*C*$(CH_3)_3$), 118.51, 123.04, 129.00, 138.34 (phenyl *C*), 152.79 (*C*═O). HRMS calcd for $C_{11}H_{15}O_2N$: 193.1102. Found: 193.1109.

*Methyl-N-phenyl thiocarbamate (**9**):* ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm 4.20 (s, 3H, CH_3), 7.17−7.64 (m, 5H, phenyl-*H*), 8.48 (s, 1H, N*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ/ppm 59.93 (*C*H_3), 121.46, 125.42, 128.98, 137.09 (phenyl *C*), 187.53 (*C*═S). HRMS calcd for C_8H_9ONS: 167.0405. Found: 167.0412.

*Ethyl-N-phenyl thiocarbamate (**10**):* ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm 1.41 (t, J = 7.2 Hz, 3H, CH_3), 4.63 (q, J = 7.1 Hz, 2H, CH_2), 7.17−7.64 (m, 5H, phenyl-*H*), 8.75 (s, 1H, N*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ/ppm 14.01 (*C*H_3), 68.81(*C*H_2), 121.43, 125.30, 129.03, 137.12 (phenyl *C*), 188.65 (*C*═S). HRMS calcd for $C_9H_{11}ONS$: 181.0561. Found: 181.0568.

*n-Propyl-N-phenyl thiocarbamate (**11**):* ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm 1.00 (t, J = 7.5 Hz, 3H, CH_3), 1.81 (sextet, J = 7.3 Hz, 2H, OCH_2CH_2), 4.54 (t, J = 7.1 Hz, 2H, OCH_2-CH_2), 7.16−7.36 (m, 5H, phenyl-*H*), 8.87 (s, 1H, N*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ/ppm 10.39 (*C*H_3), 21.77 (OCH_2CH_2), 74.46 (OCH_2CH_2), 121.51, 125.31, 129.00, 137.11 (phenyl *C*), 188.75 (*C*═S). HRMS calcd for $C_{10}H_{13}$-ONS: 195.0718. Found: 195.0724.

*i-Propyl-N-phenyl thiocarbamate (**12**):* ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm 1.41 (d, J = 6.3 Hz, 6H, CH_3), 5.66 (quintet, J = 6.7 Hz, 1H, C*H*), 7.16−7.36 (m, 5H, phenyl-*H*), 8.60 (s, 1H, N*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ/ppm 21.56 (*C*H_3), 77.00 (*C*H), 121.35, 125.12, 128.94, 137.20 (phenyl *C*), 187.73 (*C*═S). HRMS calcd for $C_{10}H_{13}ONS$: 195.0718. Found: 195.0722.

n-Butyl-*N*-phenyl thiocarbamate (**13**): ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm 0.96 (t, J = 7.5 Hz, 3H, CH_3), 1.43 (sextet, J = 7.3 Hz, 2H, $OCH_2CH_2CH_2CH_3$), 1.77 (quintet, J = 7.2 Hz, 2H, $OCH_2CH_2CH_2CH_3$), 4.58 (t, J = 7.1 Hz, 2H, OCH_2-$CH_2CH_2CH_3$), 7.17−7.64 (m, 5H, phenyl-*H*), 8.65 (s, 1H, N*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ/ppm 13.62 (*C*H_3), 19.05 ($OCH_2CH_2CH_2CH_3$), 30.38 ($OCH_2CH_2CH_2CH_3$), 72.77 ($OCH_2CH_2CH_2CH_3$), 121.80, 125.31, 128.99, 137.10 (phenyl *C*), 188.75 (*C*═S). HRMS calcd for $C_{11}H_{15}ONS$: 209.0874. Found: 209.0883.

*i-Butyl-N-phenyl thiocarbamate (**14**):* ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm 0.99 (d, J = 6.9 Hz, 6H, CH_3), 2.11 (quintet, J = 7.0 Hz, 1H, C*H*), 4.36 (d, J = 6.8 Hz, 2H, CH_2), 7.15−7.37 (m, 5H, phenyl-*H*), 8.73 (s, 1H, N*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ/ppm 19.11 (*C*H_3), 27.65 (*C*H), 79.01 (*C*H_2), 121.67, 125.41, 129.01, 137.03 (phenyl *C*), 188.90 (*C*═S). HRMS calcd for $C_{11}H_{15}ONS$: 209.0874. Found: 209.0872.

*t-Butyl-N-phenyl thiocarbamate (**15**):* ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm 1.73 (s, 9H, CH_3), 6.67−7.34 (m, 5H, phenyl-*H*), 8.33 (s, 1H, N*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ/ppm 28.14 (*C*H_3), 88.37 (*C*$(CH_3)_3$), 124.93, 128.92, 129.29, 137.58 (phenyl *C*), 186.48 (*C*═S). HRMS calcd for $C_{11}H_{15}$-ONS: 209.0874. Found: 209.0881.

*2,2′-Biphenyl-2-N-butylcarbamate-2′-ol (**16**):* ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm 0.85 (t, J = 7 Hz, 3H, ω-CH_3), 1.20 (m, 2H, γ-CH_2), 1.25 (m, 2H, β-CH_2), 3.16 (m, 2H, α-CH_2), 4.78 (br, 1H, N*H*), 5.17 (br, 1H, O*H*), 6.92−7.48 (m, 8H, biphenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ/ppm 13.62 (ω-*C*), 19.53 (γ-*C*), 31.55 (β-*C*), 40.54 (α-*C*), 122.74, 125.25, 128.43, 130.44, and 130.76 (biphenyl *C*-1 and *C*-3-*C*-6), 148.46 (biphenyl *C*-2), 156.63 (*C*═O). HRMS calcd for $C_{17}H_{19}O_3N$: 285.1365. Found: 285.1376. Elemental analysis calcd for $C_{17}H_{19}O_3N$: C, 71.54; H, 6.72; N, 4.91. Found: C, 71.72; H, 6.59, N, 5.02.

*2,2′-Biphenyl-2-N-hexylcarbamate-2′-ol (**17**):* ^{1}H NMR ($(CD_3O)_2SO_2$, 300 MHz) δ/ppm 0.85 (t, J = 7 Hz, 3H, ω-CH_3), 1.22 (m, 8H, β to ω-1-CH_2), 2.91 (s, 1H, O*H*), 2.93 (m, 2H, α-CH_2), 6.74−7.43 (m, 8H, biphenyl-*H*), 9.28 (s, 1H, N*H*); ^{13}C NMR ($(CD_3O)_2SO_2$, 75.4 MHz) δ/ppm 13.94 (ω-*C*), 22.08, 28.66, 31.23, (β to ω-1-*C*), 40.32 (α-*C*), 115.56, 118.37, 123.21, 124.15, 124.40, 127.65, 128.50, 130.71, 131.60, and 131.73 (biphenyl *C*-1 and *C*-3-*C*-6), 148.51 (biphenyl *C*-2), 154.53 (*C*═O). HRMS calcd for $C_{19}H_{23}O_3N$: 313.1678. Found: 313.1685. Elemental analysis calcd for $C_{19}H_{23}O_3N$: C, 72.80; H, 7.40; N, 4.47. Found: C, 72.87; H, 7.32, N, 4.28.

*2,2′-Biphenyl-2-N-octylcarbamate-2′-ol (**18**):* ^{1}H NMR ($(CD_3O)_2SO_2$, 300 MHz) δ/ppm 0.87 (t, J = 7 Hz, 3H, ω-CH_3), 1.25 (m, 12H, β to ω-1-CH_2), 2.86 (br, 1H, O*H*), 2.94 (m, 2H, α-CH_2), 6.74−7.43 (m, 8H, biphenyl-*H*), 9.25 (s, 1H, N*H*); ^{13}C NMR ($(CD_3O)_2SO_2$, 75.4 MHz) δ/ppm

13.95 (*ω*-*C*), 22.10, 26.07, 28.66, 29.15, and 31.25, (*β* to *ω*-1-*C*), 40.33 (α-*C*), 115.60, 118.40, 123.24, 124.17, 124.42, 127.65, 128.50, 130.72, 131.62, and 131.75 (biphenyl *C*-1 and *C*-3-*C*-6), 148.52 (biphenyl *C*-2), 154.55 (*C*═O). HRMS calcd for $C_{21}H_{27}O_3N$: 341.1991. Found: 341.2001 Elemental analysis calcd for $C_{21}H_{27}O_3N$: C, 73.86; H, 7.98; N, 4.10. Found: C, 73.97; H, 7.85, N, 4.20.

2,2′-Biphenyl-2-N-octadecylcarbamate-2′-ol (***19***): ^{1}H NMR ($(CD_3O)_2SO_2$, 300 MHz) *δ*/ppm 0.88 (t, *J* = 7 Hz, 3H, *ω*-C*H*$_3$), 1.24 (m, H, *β* to *ω*-1-C*H*$_2$), 2.87 (br, 1H, O*H*), 2.93 (m, 2H, α-C*H*$_2$), 6.74−7.43 (m, 8H, biphenyl-*H*), 9.25 (s, 1H, N*H*); ^{13}C NMR ($(CD_3O)_2SO_2$, 75.4 MHz) *δ*/ppm 14.90 (*ω*-*C*), 22.66, 26.53, 29.23, 29.34, and 31.90 (*β* to *ω*-1-*C*), 41.13 (α-*C*), 115.61, 118.40, 123.23, 124.18, 124.41, 127.64, 128.50, 130.71, 131.62, and 131.74 (biphenyl *C*-1 and *C*-3−*C*-6), 148.51 (biphenyl *C*-2), 154.56 (*C*═O). HRMS calcd for $C_{31}H_{47}O_3N$: 481.3556. Found: 481.3550. Elemental analysis calcd for $C_{21}H_{27}O_3N$: C, 77.28; H, 9.84; N, 2.91. Found: C, 77.18; H, 9.76, N, 2.87.

2,2′-Biphenyl-2-N-(2′′-chloroethyl)-carbamate-2′-ol (***20***): ^{1}H NMR ($(CD_3O)_2SO_2$, 300 MHz) *δ*/ppm 3.28 (m, 2H, CH_2CH_2Cl), 3.53 (m, 2H, CH_2CH_2Cl), 6.74−7.43 (m, 8H, biphenyl-*H*), 9.28 (s, 1H, N*H*); ^{13}C NMR ($(CD_3O)_2SO_2$, 75.4 MHz) *δ*/ppm 42.38 (CH_2CH_2Cl), 43.10 (CH_2CH_2Cl), 118.50, 123.34, 124.57, 124.62, 125.10, 127.67, 128.65, 130.03, 130.75, and 131.62 (biphenyl *C*-1 and *C*-3−*C*-6), 148.12 (biphenyl *C*-2), 154.57 (*C*═O). HRMS calcd for $C_{15}H_{14}O_3NCl$: 291.0662. Found: 291.0670. Elemental analysis calcd for $C_{15}H_{14}O_3NCl$: C, 61.84; H, 4.85; N, 4.81. Found: C, 61.80; H, 4.79, N, 4.70.

2,2′-Biphenyl-2-N-benzylcarbamate-2′-ol (***21***): ^{1}H NMR ($(CD_3O)_2SO_2$, 300 MHz) *δ*/ppm 4.19 (m, 2H, C*H*$_2$), 6.80−8.10 (m, 13H, phenyl-*H* and biphenyl-*H*), 9.38 (s, 1H, N*H*); ^{13}C NMR ($(CD_3O)_2SO_2$, 75.4 MHz) *δ*/ppm 43.74 (*C*H$_2$), 115.68, 118.62, 123.34, 124.13, 124.69, 126.73, 127.77, 130.83, 131.71, 132.50, and 139.44 (phenyl *C*, biphenyl *C*-1, and *C*-3−*C*-6), 148.47 (biphenyl *C*-2), 154.62, 154.87 (*C*═O). HRMS calcd for $C_{20}H_{17}O_3N$: 319.1208. Found: 319.1216. Elemental analysis calcd for $C_{20}H_{17}O_3N$: C, 75.21; H, 5.37; N, 4.39. Found: C, 75.29; H, 5.29, N, 4.31.

2,2′-Biphenyl-2-N-allylcarbamate-2′-ol (***22***): ^{1}H NMR ($(CD_3O)_2SO_2$, 300 MHz) *δ*/ppm 3.58 (m, 2H, $CH_2CH{=}CH_2$), 4.99 (m, 2H, $CH_2CH{=}CH_2$), 5.75 (m, 1H, $CH_2CH{=}CH_2$), 6.75−7.65 (m, 8H, biphenyl-*H*), 9.38 (s, 1H, N*H*); ^{13}C NMR ($(CD_3O)_2SO_2$, 75.4 MHz) *δ*/ppm 42.51 ($CH_2CH{=}CH_2$), 114.70 ($CH_2CH{=}CH_2$), 115.59 ($CH_2CH{=}CH_2$), 118.50, 123.21, 124.13, 124.55, 127.69, 128.58, 130.78, 131.62, 131.75, and 135.10 (biphenyl *C*-1 and *C*-3-*C*-6), 148.44 (biphenyl *C*-2), 154.51, 154.60 (*C*═O). HRMS calcd for $C_{16}H_{16}O_3N$: 270.1130. Found: 270.1136. Elemental analysis calcd for $C_{16}H_{16}O_3N$: C, 71.08; H, 5.97; N, 5.18. Found: C, 70.99; H, 5.88, N, 5.07.

2,2′-Biphenyl-2-N-phenylcarbamate-2′-ol (***23***): ^{1}H NMR ($(CD_3O)_2SO_2$, 300 MHz) *δ*/ppm 6.72−7.75 (m, 13H, phenyl-*H* and biphenyl-*H*), 9.39 (s, 1H, N*H*); ^{13}C NMR ($(CD_3O)_2SO_2$, 75.4 MHz) *δ*/ppm 118.54, 123.21, 122.60, 122.98, 123.40, 125.59, 125.78, 128.78, 128.96, 131.03, 131.14, 136.13, 137.64, 147.88, 148.29, 149.59 (biphenyl and phenyl *C*), 155.13 (*C*═O). HRMS calcd for $C_{19}H_{15}O_3N$: 303.1052. Found: 303.1059. Elemental analysis calcd for $C_{19}H_{15}O_3N$: C, 74.73; H, 4.95; N, 4.59. Found: C, 74.65; H, 4.89, N, 4.50.

2,2′-Biphenyl-2-N-butylcarbamate-2′-N-octadecylcarbamate (***24***): ^{1}H NMR ($CDCl_3$, 300 MHz) *δ*/ppm 0.88 (t, *J* = 7 Hz, 6H, *ω*-C*H*$_3$), 1.25−1.31 (m, 32H, *β* to *ω*-1-C*H*$_2$ of octadecyl), 1.39 (m, 2H, *γ*-CH_2 of butyl), 1.62 (m, *β*-CH_2 of butyl), 3.11 (m, 4H, α-C*H*$_2$), 5.38 (m, 2H, N*H*), 7.25−7.29 (m, 8H, biphenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) *δ*/ppm 13.68 and 14.07 (*ω*-*C*), 19.60, 22.60, 26.52, 29.18, 29.57, 31.65, and 31.77 (*β* to *ω*-1-*C*); 40.63 and 40.97 (α-*C*), 122.89, 125.37, 128.59, 130.62, 130.67, and 130.95 (biphenyl *C*-1 and *C*-3−*C*-6), 148.44 (biphenyl *C*-2), 154.97 (*C*═O). HRMS calcd for $C_{36}H_{56}O_4N_2$: 580.4240. Found: 580.4245. Elemental analysis calcd for $C_{36}H_{56}O_4N_2$: C, 74.43; H, 9.72; N, 4.83. Found: C, 74.35; H, 9.69, N, 4.69.

2,2′-Biphenyl-2-N-hexylcarbamate-2′-N-octadecylcarbamate (***25***): ^{1}H NMR ($CDCl_3$, 300 MHz) *δ*/ppm 0.88 and 0.89 (t, *J* = 7 Hz, 6H, *ω*-C*H*$_3$), 1.25−1.81 (m, 40H, *β* to *ω*-1-C*H*$_2$), 3.12 (m, 4H, α-C*H*$_2$), 5.36 (q, *J* = 6 Hz, 2H, N*H*), 7.25−7.30 (m, 8H, biphenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) *δ*/ppm 13.69 and 14.07 (*ω*-*C*), 19.62, 22.61, 26.52, 29.19, 29.57, 31.62, and 31.76 (*β* to *ω*-1-*C*), 40.63 and 40.99 (α-*C*), 122.89, 125.39, 128.57, 130.61, 130.66, and 130.95 (biphenyl *C*-1 and *C*-3−*C*-6), 148.44 (biphenyl *C*-2), 154.97 (*C*═O). HRMS calcd for $C_{38}H_{60}O_4N_2$: 608.4553. Found: 608.4559. Elemental analysis calcd for $C_{38}H_{60}O_4N_2$: C, 74.94; H, 9.94; N, 4.60. Found: C, 74.88; H, 9.86, N, 4.48.

2,2′-Biphenyl-2-N-octadecylcarbamate-2′-N-octylcarbamate (***26***): ^{1}H NMR ($CDCl_3$, 300 MHz) *δ*/ppm 0.88 and 0.89 (t, *J* = 7 Hz, 6H, *ω*-C*H*$_3$), 1.25−1.80 (m, 44H, *β* to *ω*-1-C*H*$_2$), 3.10 (q, $^3J_{H-C-C-H} = {}^3J_{H-C-N-H} = 7$ Hz, 4H, α-C*H*$_2$), 5.38 (q, *J* = 6 Hz, 2H, N*H*), 7.25−7.29 (m, 8H, biphenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) *δ*/ppm 13.69 and 14.07 (*ω*-*C*), 19.61, 22.62, 26.51, 29.18, 29.59, 31.64, and 31.78 (*β* to *ω*-1-*C*), 40.64 and 40.98 (α-*C*), 122.88, 125.38, 128.59, 130.60, 130.67, and 130.94 (biphenyl *C*-1 and *C*-3−*C*-6), 148.45 (biphenyl *C*-2), 154.99 (*C*═O). HRMS calcd for $C_{40}H_{64}O_4N_2$: 636.4866. Found: 636.4885. Elemental analysis calcd for $C_{40}H_{64}O_4N_2$: C, 75.41; H, 10.13; N, 4.40. Found: C, 75.51; H, 10.01, N, 4.49.

2,2′-Biphenyl-di-N-octadecyl carbamate (***27***): ^{1}H NMR ($CDCl_3$, 300 MHz) *δ*/ppm 0.89 (t, *J* = 7 Hz, 6H, *ω*-C*H*$_3$), 1.25−1.80 (m, 64H, *β* to *ω*-1-C*H*$_2$), 3.11 (q, $^3J_{H-C-C-H} = {}^3J_{H-C-N-H} = 7$ Hz, 4H, α-C*H*$_2$), 5.37 (q, *J* = 6 Hz, 2H, N*H*), 7.24−7.29 (m, 8H, biphenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) *δ*/ppm 13.69 (*ω*-*C*), 14.72, 19.60, 22.62, 26.50, 29.19, 29.59, 31.63, and 31.78 (*β* to *ω*-1-*C*), 40.63 (α-*C*), 122.88, 125.39, 128.59, 130.61, 130.67, and 130.93 (biphenyl *C*-1 and *C*-3−*C*-6), 148.44 (biphenyl *C*-2), 155.00 (*C*═O). HRMS calcd for $C_{50}H_{84}O_4N_2$: 776.6431. Found: 776.6440. Elemental analysis calcd for $C_{50}H_{84}O_4N_2$: C, 77.26; H, 10.90; N, 3.61. Found: C, 77.15; H, 10.83, N, 3.54.

2,2′-Biphenyl-2-N-(2′′-chloroethyl)-carbamate-2′-N-octadecyl carbamate (***28***): ^{1}H NMR ($CDCl_3$, 300 MHz) *δ*/ppm 0.88 (t, *J* = 7 Hz, 3H, *ω*-C*H*$_3$), 1.26 (m, 30H, *γ* to *ω*-1-C*H*$_2$), 1.42 (m, 2H, *β*-C*H*$_2$), 3.17 (m, 2H, α-C*H*$_2$), 3.42 (m, 4H, CH_2CH_2Cl), 5.27 and 5.78 (m, 2H, N*H*), 7.22−7.40 (m, 8H, biphenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) *δ*/ppm 14.00 (*ω*-*C*), 22.62, 26.52, 29.23, 29.32, 31.78, 41.01, 42.78, and 43.06 (*C*H$_2$), 122.66, 123.06, 125.50, 125.73, 128.77, 131.00, and 149.55 (biphenyl *C*), 155.10 (*C*═O). HRMS calcd for $C_{32}H_{51}O_4N_2Cl$: 562.3537. Found: 562.3542. Elemental analysis calcd for $C_{32}H_{51}O_4N_2Cl$: C, 68.28; H, 9.14; N, 4.98. Found: C, 68.19; H, 9.10, N, 4.87.

*2,2′-Biphenyl-2-N-benzylcarbamate-2′-N-octadecyl carbamate (**29**):* ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm 0.87 (t, J = 7 Hz, 3H, ω-CH_3), 1.26 (m, 32H, β to ω-1-CH_2), 3.17 (q, $^3J_{H-C-C-H} = {}^3J_{H-C-N-H}$ = 6 Hz, 2H, α-CH_2), 4.34−4.39 (m, 2H, CH_2Ph), 5.25 and 5.78 (m, 2H, N*H*), 7.04−7.39 (m, 13H, phenyl and biphenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ/ppm 14.10 (ω-*C*), 22.68, 26.51, 29.25, 29.34, 29.54, 29.68, 31.91, 41.00, 44.44, and 44.81 (α to ω-1-*C* of octadecyl and CH_2Ph), 122.89, 125.50, 125.62, 127.02, 127.20, 127.37, 128.49, 128.54, 128.70, 130.91, 131.02, 138.02, 139.54, and 148.97 (biphenyl and phenyl *C*), 155.10 (*C*═O). HRMS calcd for $C_{39}H_{54}O_4N_2$: 614.4083. Found: 614.4078. Elemental analysis calcd for $C_{39}H_{54}O_4N_2$: C, 76.17; H, 8.86; N, 4.56. Found: C, 76.07; H, 8.76, N, 4.59.

*2,2′-Biphenyl-2-N-allylcarbamate-2′-N-octadecyl carbamate (**30**):* ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm 0.88 (t, J = 7 Hz, 3H, ω-CH_3), 1.25 (m, 30H, γ to ω-1-CH_2), 1.40 (m, 2H, β-CH_2), 3.09 (q, $^3J_{H-C-C-H} = {}^3J_{H-C-N-H}$ = 6 Hz, 2H, α-CH_2), 3.70 (m, 2H, CH_2CH═CH_2), 5.03 and 5.08 (s, 2H, N*H*), 5.30 and 5.50 (m, 2H, CH_2CH═CH_2), 5.73 (m, 1H, CH_2CH═CH_2), 7.20−7.45 (m, 8H, biphenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ/ppm 14.10 (ω-*C*), 22.66, 26.53, 29.25, 29.33, 29.68, 31.90, 41.01, and 43.22 (α to ω-1-*C* of octadecyl and CH_2CH═CH_2), 115.62 (CH_2CH═CH_2), 130.67 (CH_2CH═CH_2), 122.83, 122.92, 125.44, 125.53, 128.66, 131.01, 133.92, and 148.44 (biphenyl), 155.00 (*C*═O). HRMS calcd for $C_{35}H_{52}O_4N_2$: 564.3927. Found: 564.3934. Elemental analysis calcd for $C_{35}H_{52}O_4N_2$: C, 74.42; H, 9.29; N, 4.96. Found: C, 76.34; H, 9.21, N, 4.89.

*2,2′-Biphenyl-2-N-octadecylcarbamate-2′-N-phenyl carbamate (**31**):* ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm 0.89 (t, J = 7 Hz, 3H, ω-CH_3), 1.26 (m, 30H, γ to ω-1-CH_2), 1.42 (m, 2H, β-CH_2), 3.17 (q, $^3J_{H-C-C-H} = {}^3J_{H-C-N-H}$ = 6 Hz, 2H, α-CH_2), 5.23 (m, 2H, N*H*), 7.04−7.29 (m, 13H, phenyl and biphenyl-*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ/ppm 14.10 (ω-*C*), 22.66, 26.53, 29.23, 29.34, 29.68, and 31.90 (β to ω-1-*C*), 41.13 (α-*C*); 118.55, 122.60, 123.00, 123.41, 125.59, 125.79, 128.79, 128.88, 128.98, 131.03, 131.15, 136.12, 137.57, 147.92, 148.33, and 149.55 (biphenyl and phenyl), 155.14 (*C*═O). HRMS calcd for $C_{38}H_{52}O_4N_2$: 600.3927. Found: 600.3939. Elemental analysis calcd for $C_{38}H_{52}O_4N_2$: C, 75.95; H, 8.73; N, 4.66. Found: C, 76.07; H, 8.62, N, 4.79.

REFERENCES

1. Bhat, S. G. and Brockman, H. L. (1982) *Biochem. Biophys. Res. Commun. 109*, 486−492.
2. Gallo, L. L., Clark, S. B., Myers, S., and Vohouny, G. V. (1984) *J. Lipid Res. 25*, 604−612.
3. Brockerhoff, H., and Jensen, R. G. (1974) in *Lipolytic enzymes*, Academic Press, New York.
4. Fredrikzon, B., Hernell, O., Bläckberg, L., and Olivecrona, T. (1978) *Pediatr. Res. 12*, 1048−1052.
5. Kritchevsky, D., and Kothari, H. V. (1978) *Adv. Lipid Res. 16*, 221−226.
6. Rudd, E. A., and Brockman, H. L. (1984) in *Lipases* (Borgström, B., and Brockman, H. L., Eds.), Elsevier, Amsterdam.
7. Watt, S. M., and Simmonds, W. J. (1981) *J. Lipid Res. 22*, 157−165.
8. Brodt-Eppley, J., White, P., Jenkins, S., and Hui, D. Y. (1995) *Biochim. Biophys. Acta 1272*, 69−72.
9. Mayers-Payne, S. C., Hui, D. Y., and Brockman, H. L. (1995) *Biochemistry 34*, 3942−3947.
10. Boland, W., Frössl, C., Lorenz, N. (1991) *Synthesis 12*, 1049−1072.
11. Svendsen, A. (1994) in *Lipases, Their Structure Biochemistry and Application* (Woolley, P. and Petersen, S. B., Eds.), pp 1−21, Cambridge University Press, Cambridge, U.K.
12. Derewenda, U., Brzozowski, A. M., Lawson, D. N., and Derewenda, Z. S. (1992) *Biochemistry 31*, 1532−1541.
13. Leuveling Tjeenk, M., Bulsink, Y. B., Slotboon, A. J., Verheij, H. M., de Haas, G. H., Demleitner, G., and Gotz, F. (1994) *Protein Eng. 7*, 579−583.
14. Bartoli, F., Lin, H. K., Ghomashchi, F., Gelb, M. H., Jain, M. K., and Apitz-Castro, R. (1994) *J. Biol. Chem. 269*, 15625−15630.
15. Sohl, J., Sutton, L. D., Burton, D. J., and Quinn, D. M. (1988) *Biochem. Biophys. Res. Commun. 151*, 554−560.
16. Sutton, L. D., Stout, J. S., Hosie, L., Spencer, P. S., and Quinn, D. M. (1986) *Biochem. Biophys. Res. Commun. 134*, 386−392.
17. Tew, D. G., Southan, C., Rice, S. Q., Lawrence, M. P., Li, H., Boyd, H. F., Moores, K., Gloger, I. S., and Macphee, C. H. (1996) *Arterioscler. Thromb. Vasc. Biol. 16*, 591−599.
18. Imanaka, T., Moriyama, Y., Ecsedi, G. G., Aoyagi, T., Amanuma-Muto, K., Ohkuma, S., and Takano, Y. (1983) *J. Biochem. 94*, 1017−1020.
19. Hadvarry, P., Lengsfield, H., and Wolfer, H. (1988) *Biochem. J. 256*, 357−361.
20. Tew, D. G., Boyd, H. F., Ashman, S., Theobald, C., and Leach, C. A. (1994) *Biochemistry 37*, 10087−10093.
21. Hosie, L., Sutton, L. D., and Quinn, D. M. (1987) *J. Biol. Chem. 262*, 260−264.
22. Feaster, S. R., Lee, K., Baker, N., Hui, D. Y., and Quinn, D. M. (1996) *Biochemistry 35*, 16723−16734.
23. Lin, G., and Lai, C.-Y. (1995) *Tetrahedron Lett. 36*, 6117−6120.
24. Lin, G., Liu, H.-C., and Tsai, Y.-C. (1996) *Bioorg. Med. Chem. Lett. 6*, 43−46.
25. Lin, G., and Lai, C.-Y. (1996) *Tetrahedron Lett. 37*, 193−196.
26. Lin, G., Tsai, Y.-C., Liu, H.-C., Liao, W.-C., and Chang, C.-H. (1998) *Biochim. Biophys. Acta 1388*, 161−174.
27. Sutton, L. D., Stout, J. S., and Quinn, D. M. (1990) *J. Am. Chem. Soc. 112*, 8398−8403.
28. Feaster, S. R., and Quinn, D. M. (1997) *Methods Enzymol. 286*, 231−252.
29. Kazlauskas, R. J. (1989) *J. Am. Chem. Soc. 111*, 4953−4959.
30. Lin, G., Liu, S.-H., Chen, S.-J., Wu, F.-C., and Sun, H.-L. (1993) *Tetrahedron Lett. 34*, 6057−6058.
31. Liu, Y.-C., and Chen, C.-S. (1989) *Tetrahedron Lett. 30*, 1617−1620.
32. Grochulski, P., Bouthillier, F., Kazlauskas, R. J., Serreqi, A. N., Schrag, J. D., Ziomek, E., and Cygler, M. (1994) *Biochemistry 33*, 3494−3500.
33. Wang, X., Wang, C.-S., Tang, J., Dyda, F., and Zhang, X. C. (1997) *Structure 5*, 1209−1218.
34. Chen, J. C.-H., Miercke, L. J. W., Krucinski, J., Starr, J. R., Saenz, G., Wang, X., Spilburg, C. A., Lange, L. G., Ellsworth, J. L., and Stroud, R. M. (1998) *Biochemistry 37*, 5107−5117.
35. Sussman, J. L., Harel, M., Frolow, F., Oefner, C., Goldman, A., Toker, L., and Silman, I. (1991) *Science 253*, 872−879.
36. Schrag, J. D., and Cygler, M. (1993) *J. Mol. Biol. 230*, 575−591.
37. Quinn, D. M. (1987) *Chem. Rev. 87*, 955−979.
38. Lin, G., Lin, W.-Y., and Shieh, C.-T. (1998) *Tetrahedron Lett. 39*, 8881−8884.
39. Aldridge, W. N., and Reiner, E. (1972) in *Enzyme Inhibitors as Substrates* (Neuberger, A., and Tatun, E. L., Eds.), pp 123−145, North-Holland Publishing Co., Amsterdam.

40. Hart, G. J., and O'Brien, R. D. (1973) *Biochemistry 12*, 2940−2945.
41. Abeles, R. H., and Maycock, A. L. (1976) *Acc. Chem. Res. 9*, 313−319.
42. Järv, Kesvatera, T., and Aaviksaar, A. (1976) *Eur. J. Biochem. 67*, 315−322.
43. March, J. (1992) *Advanced Organic Chemistry* 4th ed., John Wiley & Sons, Inc., New York.
44. Hine, J. (1975) *Structural Effects on Equilibria in Organic Chemistry*, John Wiley & Sons, Inc., New York.
45. Isaacs, N. (1995) *Physical Organic Chemistry* 2nd ed., Longman, U.K.
46. Lowry, T. H., and Richardson, K. S. (1987) *Mechanism and Theory in Organic Chemistry* 3rd ed., Harper & Row, New York.
47. Leo, A., Hansch, C., and Elkins, D. (1971) *Chem. Rev. 71*, 525−616.
48. Fujita, T., Iwasa, J., and Hansch, C. (1964) *J. Am. Chem. Soc. 86*, 5175−5180.

BI982775E