# Inhibition of Human Leukocyte Elastase by N-Substituted Peptides Containing $\alpha.\alpha$ -Difluorostatone Residues at P<sub>1</sub>

Jerry W. Skiles,\*,†, Clara Miao,† Ronald Sorcek,† Stephen Jacober,† Philip W. Mui,†,⊥ Grace Chow,‡ Steve M. Weldon.<sup>§</sup> Genus Possanza.<sup>§</sup> Mark Skoog,<sup>‡,#</sup> James Keirns,<sup>‡</sup> Gordon Letts,<sup>§</sup> and Alan S. Rosenthal<sup>†</sup>

Departments of Medicinal Chemistry, Biochemistry, and Pharmacology, Boehringer Ingelheim Pharmaceuticals, Inc., 90 East Ridge, P.O. Box 368, Ridgefield, Connecticut 06877

Received May 6, 1992

A series of tripeptides which contain  $\alpha_{,\alpha}$ -difluorostatone residues at  $P_1$ - $P_1'$  and span the  $S_3$ - $S_1'$ subsites have been shown to be potent inhibitors of human leukocyte elastase (HLE). The tripeptides described contain the nonproteinogenic achiral residue N-(2.3-dihydro-1H-inden-2-y) glycine at the  $P_2$ -position. This residue has previously been shown in the case of HLE to be a good bioisosteric replacement for L-proline. Of the peptides prepared, those which contain the  $\alpha, \alpha$ -difluoromethylene keton derivative of L-valine (difluorostatone) are the preferred residue at the  $P_1$ -primary specificity position. Substitution at P<sub>1</sub> by the corresponding  $\alpha, \alpha$ -diffuoromethylene ketones of L-leucine and L-phenylalanine gives inactive compounds. Of the tripeptides described the most potent in vitro compound is ethyl N-[N-CBZ-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycyl]-4(S)-amino-2,2difluoro-3-oxo-5-methylhexanoate (17b) (IC<sub>50</sub> = 0.635  $\mu$ M). It is presumed that the inhibitor 17b interacts with the  $S_3$ - $S_1$  binding regions of HLE. Additionally extended binding inhibitors were prepared which interact with the  $S_3$ - $S_3$  binding subsites of HLE. In order to effect interaction with the  $S_1'-S_3'$  subsites of HLE, the leaving group side of cleaved peptides, spacers based upon Gly-Gly, and those linked via the  $N^{\epsilon}$  of L-lysine were utilized. One of the most potent extended compounds (P<sub>3</sub>-P<sub>3</sub>') in vitro is methyl N<sup>6</sup>-[4(S)-[[N-[N-CBZ-L-valyl-N-(2,3-dihydro-1H-inden-2yl)glycyl]amino]-2,2-difluoro-3-oxo-5-methylhexanoyl]-2(S)-(acetylamino)-6-aminohexanoate (24b)  $(IC_{50} = 0.057 \ \mu M)$ . The described in vitro active inhibitors were also evaluated in hamsters in an elastase-induced pulmonary hemorrhage (EPH) model. In this model, intratracheal (it.) administration of 22c, 5 min prior to HLE challenge (10  $\mu$ g, it.) effectively inhibited hemorrhage (94.6%) in a dose-dependent manner. The described  $\alpha, \alpha$ -difluoromethylene ketone inhibitors are assumed to act as transition-state analogs. The inhibition process presumably acts via hemiketal formation with the active site Ser<sup>195</sup> of HLE, and is facilitated by the strongly electron withdrawing effect of the  $\alpha, \alpha$ -difluoromethylene functionality.

Elastases are a family of proteinases that hydrolytically degrade elastin, a structural protein that is a flexible, highly cross-linked protein which is insoluble in neutral aqueous media and is generally resistant to proteolysis. Imbalances in the levels or regulation of tissue or cellular proteases are thought to manifest themselves in various disease states. Human leukocyte elastase (HLE; EC 3.4.21.37) is a glycosylated, strongly basic serine protease with a molecular weight of approximately 30 kDa, and is found in the azurophilic granules of polymorphonuclear leukocvtes (PMN). The complete amino acid sequence of HLE has been determined.<sup>1</sup> This enzyme is released from PMN upon inflammatory stimuli and has been implicated as a pathogenic agent in a number of disease states such as pulmonary emphysema,<sup>2</sup> rheumatoid arthritis,<sup>3</sup> adult respiratory distress syndrome (ARDS),<sup>4</sup> glomerulonephritis,<sup>5</sup> periodontitis, arteriosclerosis, septicemia and shock, and cvstic fibrosis.6

In order to prevent self-inflicted tissue damage due to enzymes being accidentally released, numerous endogenous inhibitors directed against neutrophil proteinases exist. In the case of HLE, the major endogenous inhibitor is  $\alpha_1$ -proteinase inhibitor ( $\alpha_1$ -PI). Extracellular HLE is normally inhibited by  $\alpha_1$ -PI; however, this delicate balance between proteinase and inhibitor may be disturbed by a genetic defect leading to a decrease in  $\alpha_1$ -PI or to the oxidative destruction of  $\alpha_1$ -PI. Cigarette smoke, which has been shown to inactivate  $\alpha_1$ -PI in vitro (through oxidation of Met<sup>358</sup>),<sup>7</sup> is believed to cause a localized, functional deficiency of the protease inhibitor. This, in

<sup>\*</sup> Author to whom correspondence should be addressed.

Department of Medicinal Chemistry.

<sup>&</sup>lt;sup>‡</sup> Department of Biochemistry.

Department of Pharmacology.
 Present address: CIBA-GEIGY Corp., Pharmaceuticals Division, 556 Morris Ave., Summit, NJ 07901.

<sup>&</sup>lt;sup>⊥</sup> Present address: Schering-Plough Research, Biotechnology Department, 60 Orange St., Bloomfield, NJ 07003.

<sup>\*</sup> Present address: Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4000, Princeton, NJ 08543.

<sup>(1)</sup> Sinha, S.; Watorek, W.; Karr, S.; Giles, J.; Bode, W.; Travis, S. Primary Structure of Human Neutrophil Elastase. Proc. Natl. Acad. Sci. U.S.A. 1987, 84, 2228-2232.

<sup>(2)</sup> Janoff, A. Elastase and Emphysema. Current Assessment of the Protective-Antiprotease Hypothesis. Am. Rev. Respir. Dis. 1985, 132, 417 - 433.

<sup>(3)</sup> Ekerot, L.; Ohlsson, K. Interactions of Granulocyte Proteases with Inhibitors in Rheumatoid-Arthritis. Adv. Exp. Med. Biol. 1984, 167, 335-344.

<sup>(4)</sup> Merritt, T. A.; Cochrane, C. G.; Holcomb, K.; Bohl, B.; Hallman, M.; Strayer, D.; Edwards, D.; Gluck, L. Elastase and  $\alpha_i$ -PI Proteinase Inhibitor Activity in Tracheal Aspirates During Respiratory Distress

Syndrome. J. Clin. Invest. 1983, 72, 656–666.
 (5) Sanders, E.; Davies, M.; Coles, A. On the Pathogenesis of Glomerulonephritis: A Clinico-Pathological Study Indicating that Neutrophils Attack and Degrade Glomerular Basement Membrane. Renal Physiol. 1980, 3, 335-359

<sup>(6) (</sup>a) Jackson, A. H.; Hill, S. L.; Afford, S. C.; Stockley, R. A. Sputum Soluble Phase Proteins and Elastase Activity in Patients with Cystic Fibrosis. J. Respir. Dis. 1984, 65, 114-124. (b) Suter, S.; Schaad, L.; Roux, L.; Nydegger, V. E.; Waldvogel, F. A. Granulocyte Neutral Proteases and Pseudomonas Elastase as Possible Causes of Airway Damage in Patients with Cystic Fibrosis. J. Infect. Dis. 1984, 149, 523-531.

<sup>(7)</sup> Johnson, D.; Travis, J. Structural Evidence for Methionine at the Reactive Site of Human  $\alpha$ -1-Proteinase Inhibitor. J. Biol. Chem. 1978, 253, 7142-7144.

turn, is thought to be a primary factor in the pathogenesis of centrilobular emphysema associated with cigarette smoking. Low molecular weight protease inhibitors as therapeutic agents may thus be beneficial as a replacement or supplement to  $\alpha_1$ -PI. Of the numerous classes of inhibitors reported, the two most exciting classes are cephalosporins<sup>8</sup> and peptidyl trifluoromethyl ketones<sup>9-12</sup> as is exemplified by 1 and 2 respectively.



2; R = CBZ 3; R =  $p_{-}[p_{-}Cl(C_{6}H_{4})SO_{2}NHCO](C_{6}H_{4})CO$ 

We,<sup>9-11</sup> as well as others,<sup>12</sup> have recently shown that peptidyl trifluoromethyl ketones (e.g., 2 and 3) are potent and specific in vitro inhibitors of HLE. Furthermore, in an in vivo model of elastase-induced emphysema, 3 administered intratracheally (it.) after elastase challenge has been demonstrated to halt the progression of HLEinduced emphysemalike lesions (ED<sub>50</sub> =  $3.8 \,\mu$ g/hamster).<sup>13</sup>

Human Leukocyte Elastase. Nature 1986, 322, 192-194.
(9) Skiles, J. W.; Fuchs, V.; Chow, G.; Skoog, M. Inhibition of Human Leukocyte Elastase by N-Substituted Tripeptide Trifluoromethyl Ketones. Res. Commun. Chem. Pathol. Pharmacol. 1990, 68, 365-374.

(10) Skiles, J. W.; Fuchs, V.; Miao, C.; Sorcek, R.; Grozinger, K. G.; Mauldin, S. C.; Vitous, J.; Mui, P. W.; Jacober, S.; Chow, G.; Matteo, M.; Skoog, M.; Weldon, S. M.; Possanza, G.; Keirns, J.; Letts, G.; Rosenthal, A. S. Inhibition of Human Leukocyte Elastase (HLE) by N-Substituted Peptidyl Trifluoromethyl Ketones. J. Med. Chem. 1992, 35, 641–662.

(11) Skiles, J. W.; Fuchs, V.; Leonard, S. F. Imidazo[1,2-a]Piperazines as Mechanistic Inhibitors of Serine Proteinases. *Bioorg. Med. Chem. Lett.* 1991, 1, 69-72. Peptidyl trifluoromethyl ketone inhibitors such as 2 and 3 are presumed to act as structural analogs of the tetrahedral intermediate formed during hydrolysis of a peptide bond. Peptidyl trifluoromethyl ketones such as 2 and 3 may deactivate HLE via transition-state inhibition by two potential means. Both di- and trifluoromethyl ketones exist in water almost entirely in the hydrated form, i.e. as the hemiketal. This tetrahedral hemiketal formation is facilitated by an enzyme-catalyzed reaction (e.g., upon binding to HLE). This hydration of an amino acid ketone is facilitated by placement of electron-withdrawing fluorine atoms adjacent to the carbonyl to give either di- or trifluoromethyl ketones. Alternatively the enhanced electrophilicity of fluorinated ketones over unfluorinated ketones may also facilitate an enzyme-catalyzed addition of the active site Ser<sup>195</sup> to the ketone carbonyl to form a metastable hemiketal, which resembles the tetrahedral intermediate in the reaction pathway for enzyme-substrate hydrolysis. Although di- and trifluoromethyl ketone inhibitors are assumed to form a covalent bond via hemiketal formation with active site Ser<sup>195</sup> of HLE, this process is reversible. In the case of porcine pancreatic elastase (PPE), it has been unequivocally demonstrated<sup>14</sup> through X-ray analysis that, in the adducts formed between the enzyme and peptidyl trifluoromethyl ketone inhibitors, the  $O^{\gamma}$  atom of the catalytic Ser<sup>195</sup> residue is covalently attached (1.5 Å) to the ketone carbonyl of the inhibitor via a hemiketal.

Most of the peptide-based inhibitors described to date occupy maximally the  $S_1$ - $S_4$  binding subsites<sup>15</sup> of the proteolytic enzyme and do not profit from the numerous possible interactions with binding subsites on the "leavinggroup" side of the cleaved peptides (i.e., the  $P_1'-P_3'$ subsites). Small synthetic inhibitors of HLE that interact in this extended manner have not been reported. In the present work we describe our work in successfully extending the trifluoromethyl ketone inhibitors 2 and 3 into the S'-region of HLE by replacing one of the fluorines of 2 and 3 by an ethoxycarbonyl functionality (e.g., COOEt) and in using this functionality as a handle to further extend into the  $S_3$ '-subsite region. This functionality has been successfully used by us to gain further enzyme-ligand interactions by the interaction with remote residues. Crystallographic studies on serine proteases bound to natural protein inhibitors indicate that the limit of the binding cleft is at the  $S_3$ '-subsite. In order to potentially facilitate a P<sub>3</sub>'-S<sub>3</sub>' inhibitor-enzyme interaction, molecular modeling in conjunction with X-ray crystallography was utilized to evaluate appropriate spacing groups. Furthermore, prior work has also provided evidence that S'interactions are important in naturally occurring inhibitors of serine protease such as turkey ovomucoid (OMTKY3).<sup>16</sup> The bulk of chemical structural literature on HLE refers to inhibitors or ligands lacking S'-functionality; thus, much

<sup>(8)</sup> Doherty, J. B.; Ashe, B. M.; Argenbright, L. W.; Barker, P. L.; Bonney, R. J.; Chandler, G. O.; Dahlgren, M. E.; Dorn, C. P., Jr.; Finke, P. E.; Firestone, R. A.; Fletcher, D.; Hagmann, W. K.; Mumford, R.; O'Grady, L.; Maycock, A. L.; Pisano, J. M.; Shah, S. K.; Thompson, K. R.;Zimmerman, M. Cephalosporin Antibiotics Can be Modified To Inhibit Human Leukocyte Elastase. *Nature* 1986, 322, 192–194.

<sup>(12) (</sup>a) Gelb, M. H.; Svaren, J. P.; Abeles, R. H. Fluoro Ketone Inhibitors of Hydrolytic Enzymes. Biochemistry 1985, 24, 1813-1817.
(b) Imperiali, B.; Abeles, R. H. Inhibition of Serine Proteases by Peptidyl Fluoromethyl Ketones. Biochemistry 1986, 25, 3760-3767. (c) Imperiali, B.; Abeles, R. H. A. Versatile Synthesis of Peptidyl Fluoromethyl Ketones. Tetrahedron Lett. 1986, 27, 135-138. (d) Dunlap, R. P.; Stone, P. J.; Abeles, R. H. Reversible Slow, Tight-Binding Inhibition of Human Leukocyte Elastase. Biophys. Res. Commun. 1987, 145, 509-513. (e) Stein, R. L.; Strimpler, A. M.; Edwards, P. D.; Lewis, J. J.; Mauger, R. C.; Schwartz, J. A.; Stein, M. M.; Trainor, D. A.; Wildonger, R. A.; Zottola, M. A. Mechanism of Slow-Binding Inhibition of Human Leukocyte Elastase by Trifluoromethyl Ketones. Biochemistry 1987, 26, 2682-2689. (f) Peet, N. P.; Burkhart, J. P.; Angelastro, M. R.; Giroux, E. L.; Mehdi, S.; Bey, P.; Kolb, M.; Neises, B.; Schirlin, D. Synthesis of Peptidyl Fluoromethyl Ketones and Peptidylα-Keto Esters as Inhibitors of Porcine Pancreatic Elastase, Human Neutrophil Elastase, and Rat and Human Neutrophil Cathepsin G. J. Med. Chem. 1990, 33, 394-407.

<sup>(13)</sup> Weldon, S. M.; Letts, L. G.; Keirns, J.; Chow, G.; Skoog, M.; Skiles, J.; Fuchs, V.; Possanza, G. J. BIRA-0260XX, [3(RS)-[[4-(4-Chlorophenyl)-sulfonylaminocarbonyl] phenyl-1-Oxomethyl]-L-Valyl-N-(2,3-Dihydro-1H-Inden-2-yl)glycyl-N-[3-(1,1,1-Trifluoro-4-Methyl-2-Oxopentyl)]amide: A Specific, Long Lasting Inhibitor of Human Neutrophil Elastase. *FASEB J.* 1990, 4(4), Abstr. 5212.
(14) Takahashi, L. H.; Rosenfield, R. E., Jr.; Meyer, E. F., Jr.; Trainor,

<sup>(14)</sup> Takahashi, L. H.; Rosenfield, R. E., Jr.; Meyer, E. F., Jr.; Trainor, D. A.; Stein, M. X-Ray Diffraction Analysis of the Inhibition of Porcine Pancreatic Elastase by a Peptidyl Trifluoromethylketone. J. Mol. Biol. 1988, 201, 423–428.

<sup>(15)</sup> The nomenclature used for describing the individual amino acid residues  $(P_2, P_1, P_1', P_2' \text{ etc.})$  of a peptide substrate and the corresponding subsites  $(S_2, S_1, S_1', S_2', \text{ etc.})$  of a protease is that of Schecter and Berger: Schecter, I.; Berger, A. On the Size of the Active Site in Proteases. I. Papain. Biochem. Biophys. Res. Commun. 1967, 27, 157-162.

Table I. In Vitro HLE Inhibitory Activities of N-Substituted Tripeptide Difluoromethylene Ketones



compd <sup>a</sup>	R <sub>1</sub>	R <sub>2</sub>	$\mathbf{R}_{3}$	$method^b$	formula <sup>c</sup>	$\mathrm{IC}_{50}$ , <sup>d</sup> $\mu \mathrm{M}$
22a 17a 22b 17b 19 22c 23b	$\begin{array}{l} p-[p-Cl(C_6H_4)SO_2NHCO](C_6H_4)\\ PhCH_2O\\ p-[p-Cl(C_6H_4)SO_2NHCO](C_6H_4)\\ PhCH_2O\\ PhCH_2O\\ p-[p-Cl(C_6H_4)SO_2NHCO](C_6H_4)\\ PhCH_2O\\ PhCH_2O\end{array}$	PhCH <sub>2</sub> CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub> CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	OEt  OEt  OEt  OEt  OEt  OH  OEt  OEt  OH  OEt  OEt  OH  OEt  O	A-G, M, N A-H A-G, M, N AH A-I A-G, M, N A-E, L	$\begin{array}{c} C_{43}H_{43}CIF_2N_4O_9S\\ C_{34}H_{43}F_2N_3O_7\\ C_{40}H_{43}CIF_2N_4O_9S\\ C_{33}H_{41}F_2N_3O_7\\ C_{31}H_{39}F_2N_3O_7\\ C_{39}H_{43}CIF_2N_4O_9S\\ C_{37}H_{47}F_2N_5O_9\end{array}$	≫5 11.1 4.9 0.635 ≫5 0.404 0.069
24b	PhCH₂O	CH(CH <sub>3</sub> ) <sub>2</sub>		A-E, J, K	$C_{40}H_{53}F_2N_5O_9$	0.057
1e 2 <sup>f</sup> 3s						0.107 0.365 0.084

<sup>a</sup> All compounds are of the (S)-configuration at P<sub>3</sub> and P<sub>1</sub>. <sup>b</sup> See Experimental Section. <sup>c</sup> All compounds exhibited satisfactory C, H, and N microanalyses and were within 0.4% of theoretical values. All compounds exhibited <sup>1</sup>H NMR and MS spectra consistent with the assigned structures. <sup>d</sup> Concentration inhibiting 50% of the activity of HLE at pH 7.5 in 0.1 M Tris buffer containing 0.5 M NaCl with the substrate MeO-Suc-Ala-Ala-Pro-Val-pNA at a concentration of 0.5 mM. Compounds and HLE were incubated for 20 min prior to starting the reaction by addition of substrate. Initial rates were linear in each case. <sup>e</sup> Literature<sup>8</sup> IC<sub>50</sub> = 1.33  $\mu$ M. <sup>f</sup> Literature<sup>10</sup> IC<sub>50</sub> = 0.365  $\mu$ M. <sup>g</sup> Literature<sup>10</sup> IC<sub>50</sub> = 0.084  $\mu$ M.

less is known about receptor-ligand interactions in the P'"leaving group" side.

Our working hypothesis was that additional interactions between the S'-subsites of the enzyme and the P'-fragments of the peptidyl difluoro ketone inhibitors may contribute to overall tight binding. It was also believed that targeted syntheses in the prime region could provide improved S'-P' interactions which may in turn provide enhanced specificity and potency.

# Chemistry

Compounds of Table I were conveniently prepared as is illustrated in Schemes I–III. The suitably protected statine<sup>17,18</sup> derivatives 7 were prepared as is shown in Scheme I. The *t*-BOC amino acids 4 were coupled with Scheme I<sup>a</sup>



<sup>a</sup> Reagents: (a) CDI, HN(OCH<sub>3</sub>)(CH<sub>3</sub>), CH<sub>2</sub>Cl<sub>2</sub>; (b) LiAlH<sub>4</sub>, THF; (c) Zn, BrCF<sub>2</sub>CO<sub>2</sub>Et, THF to give 7; (d) 7, CF<sub>3</sub>COOH to give 8; (e) 7, H<sub>2</sub>NCH<sub>2</sub>CONHCH<sub>2</sub>CO<sub>2</sub>Et; (*i*-Pr)<sub>2</sub>NEt, EtOH to give 9; (f) 9, CF<sub>3</sub>COOH to give 10; (g) 7,  $N^{a}$ -acetyl-L-Lys-OCH<sub>3</sub>, (*i*-Pr)<sub>2</sub>NEt, EtOH to give 11; (h) 11, CF<sub>3</sub>COOH to give 12.

N,O-dimethylhydroxylamine<sup>19</sup> employing carbonyldiimidazole (CDI) to give the protected amides 5. The amides 5 were reduced with LiAlH<sub>4</sub> to give the protected amino

<sup>(16)</sup> The X-ray coordinates of HLE complexed with both the chloromethyl ketone inhibitor MeO-Suc-Ala-Ala-Pro-Val chloromethyl ketone and that with the third domain of the natural inhibitor of the turkey ovomucoid inhibitor (OMTKY3) were obtained from the Max Planck Institute (Bode, W., Martinsried, Germany). For discussions on the X-ray analysis as well as a description of the substrate specificity of HLE, see (a) Bode, W.; Meyer, E., Jr.; Powers, J. C. Human Leukocyte and Porcine Pancreatic Elastase: X-ray Crystal Structures, Mechanism, Substrate Specificity, and Mechanism-Based Inhibitors. *Biochemistry* 1989, 28, 1951–1963. (b) Bode, W.; Wei, A.-Z.; Huber, R.; Meyer, E.; Travis, J.; Neumann, S. X-Ray Crystal Structure of the Complex of Human Leukocyte Elastase (PMN Elastase) and the Third Domain of the Turkey Ovonucoid Inhibitor. *EMBO* 1986, 5, 2453–2458. (c) An-Zhi, W.; Mayr, I.; Bode, W. The Refined 2.3 Å Crystal structure of Human Leukocyte Elastase in a Complex with a Valine Chloromethyl Ketone Inhibitor. *FEBS Lett.* 1988, 234, 367–373.

<sup>(17)</sup> Thaisrivongs, S.; Pals, D. T.; Kati, W. M.; Turner, S. R.; Thomasco, L. M. Difluorostatine- and Difluorostatone-Containing Peptides as Potent and Specific Renin Inhibitors. J. Med. Chem. 1985, 28, 1553–1555.

<sup>(18)</sup> Thaisrivongs, S.; Pals, D. T.; Kati, W. M.; Turner, S. R.; Thomasco, L. M.; Watt, W. Design and Synthesis of Potent and Specific Renin Inhibitors Containing Difluorostatine, Difluorostatone, and Related Analogues. J. Med. Chem. 1986, 29, 2080-2087.



<sup>a</sup> Reagents: (a)  $H_2NCH_2CO_2Et$ ,  $NaBH_3CN$ , EtOH; (b) CBZ-L-valine, CDI,  $CH_2Cl_2$  to give 15a; (c) 15a, KOH, EtOH to give 15b; (d) 8, 15b, WSCDI, HOBT, THF; (e) Dess-Martin periodinane,  $CF_3COOH$ ,  $CH_2Cl_2$ ; (f) KOH, EtOH to give 18; (g) 18, Dess-Martin periodinane,  $CF_3COOH$ ,  $CH_2Cl_2$ ; (c) using 19; (h) 10% Pd/C,  $H_2$  (45 psi), EtOH, to give 20; (i) 20, p-[p-Cl( $C_6H_4$ )SO<sub>2</sub>NHCO]( $C_6H_4$ )COOH, WSCDI, HOBT, THF to give 21; (i) 21, Dess-Martin periodinane,  $CF_3COOH$ ,  $CH_2Cl_2$  to give 22.

acid aldehydes  $6.^{20}$  A Reformatsky reaction of the *t*-BOC aldehydes 6 with ethyl bromodifluoroacetate in the presence of activated zinc dust afforded the diastereomeric alcohols 7, which were used directly without further purification. Deprotection of the *t*-BOC alcohols 7 with CF<sub>3</sub>COOH gave the amino alcohols 8 as their trifluoroacetate salts.

In order to extend the potential inhibitors into the  $S_{1'}$ - $S_{3'}$  subsites, the statine derivatives 10 (R = CH(CH<sub>3</sub>)<sub>2</sub>) and 12 (R = CH(CH<sub>3</sub>)<sub>2</sub>) were prepared as is illustrated in Scheme I. Treatment of the *t*-BOC- $\alpha,\alpha$ -difluorostatine ethyl ester 7 with ethyl glycylglycinate in refluxing EtOH afforded the protected amides 9, which ultimately corresponds to a P<sub>1</sub>-P<sub>3</sub>' spacer link in the inhibitor 23b. Treatment of 9 with CF<sub>3</sub>COOH effected the removal of the t-BOC group to give the amine 10. In a similar manner, treatment of the protected  $\alpha, \alpha$ -difluorostatine analog 7 with N<sup> $\alpha$ </sup>-acetyl-L-lysine ethyl ester in refluxing EtOH gave, after deprotection with CF<sub>3</sub>COOH, the amino alcohol 12 as a mixture of diasteriomers which were used directly without further purification. The amide 12 ultimately corresponds to the P<sub>1</sub>-P<sub>3</sub>' tether in the extended inhibitor 24b.

The N-substituted tripeptide inhibitors 17 and 22 were prepared by standard peptide coupling and deprotection procedures as is illustrated in Scheme II. In our study nonproteinogenic achiral N-substituted glycine residues were utilized at P<sub>2</sub>, in particular N-(2,3-dihydro-1H-inden-2-yl)glycine (14). We have previously shown<sup>9,10</sup> that in the case of trifluoromethyl ketone inhibitors this residue may replace L-proline to give potent and specific inhibitors of HLE that possess long duration of action in an in vivo

<sup>(19)</sup> Nahm, S.; Weinreb, S. M. N-Methoxy-N-Methylamides as Effective Acylating Agents. Tetrahedron Lett. 1981, 22, 3815-3818.
(20) Fehrentz, J. A.; Castro, B. An Effective Synthesis of Optically

<sup>(20)</sup> Fehrentz, J. A.; Castro, B. An Effective Synthesis of Optically Active  $\alpha$ -(t-Butoxycarbonylamino)-aldehydes from  $\alpha$ -Amino Acids. Synthesis 1983, 676–678.

## Inhibition of HLE by N-Substituted Peptides

model of elastase induced pulmomary hemorrhage. Ethyl N-(2,3-dihydro-1H-inden-2-yl)glycinate (14) was prepared by treatment of a mixture of 2-indanone (13) and glycine ethyl ester hydrochloride in EtOH with NaCNBH<sub>3</sub>. The required dipeptide ester 15a was prepared via a CDImediated condensation between N-CBZ-L-valine and 14 in  $CH_2Cl_2$  or THF. The dipeptide ester 15a was hydrolyzed to the corresponding dipeptide acid 15b with KOH in EtOH. The acid 15b was then condensed with the  $\alpha, \alpha$ difluorostatine ethyl ester derivatives 8 via a water-soluble carbonyl diimide (WSCDI) reagent, 1-[3-(dimethylamino)propy]-3-ethylcarbodiimide, in THF as the amide-forming reagent to give the tripeptides 16 as a mixture of diastereomers which were used directly without separation. The N-CBZ-protected tripeptides 16 were oxidized to the corresponding  $\alpha$ , $\alpha$ -difluoromethylene ketones 17 by Dess-Martin periodinane.<sup>21</sup> Treatment of the tripeptide esters 16 with 1 equiv of KOH in EtOH gave the alcohol acids 18, which were then oxidized via Dess-Martin periodinane to the  $\alpha, \alpha$ -difluoromethylene keto acid 19.

In order to extend the tripeptides 16 into the  $S_5$ - $S_4$  subsite region the amino terminal alcohols 20 were required. The *N*-CBZ tripeptides 16 were deprotected catalytically over 10% Pd/C (H<sub>2</sub>, 45 psi) in EtOH to give the amines 20. The tripeptides 20 were condensed with the acid<sup>10,22,23</sup> *p*-[*p*-Cl(C<sub>6</sub>H<sub>4</sub>)SO<sub>2</sub>NHCO](C<sub>6</sub>H<sub>4</sub>)CO<sub>2</sub>H using WSCDI/HOBT to give the alcohols 21, which were then oxidized by Dess-Martin periodinane<sup>21</sup> to afford the  $\alpha$ , $\alpha$ -difluoromethylene ketones 22.

The extended binding inhibitors 23b and 24b were prepared as shown in Scheme III. Condensation of the *N*-CBZ tripeptide acid 15b with the amine 10 in the presence of WSCDI/HOBT gave 23a as a mixture of diastereomers which were used directly without further separation. Oxidation of the diastereomeric alcohols 23a with Dess-Martin periodinane gave the ketone 23b. In a similar manner the ketone 24b was efficiently obtained by condensing the acid 15b with the amine 12 in the presence of WSCDI/HOBT to give, after Dess-Martin periodinane oxidation, 24b.

#### **Results and Discussion**

The compounds presented in Table I represent a novel peptidyl series of potent and specific human leukocyte elastase (HLE) inhibitors which contain derivatives of  $\alpha$ , $\alpha$ fluorostatone at P<sub>1</sub>-P<sub>1</sub>'. The peptides described contain L-valine at P<sub>3</sub> and the nonproteinogenic achiral residue N-(2,3-dihydro-1*H*-inden-2-yl)glycine at the P<sub>2</sub>-position. This N-substituted glycine residue has previously been shown<sup>9,10</sup> in the case of trifluoromethyl ketone inhibitors of HLE to be a good bioisosteric replacement for L-proline.

#### Scheme III<sup>a</sup>



<sup>a</sup> Reagents: (a) 15b, 10, WSCDI, HOBT to give 23a; (b) Dess-Martin periodinane, CF<sub>3</sub>COOH, CH<sub>2</sub>Cl<sub>2</sub> to give 23b; (c) 15b, 12, WSCDI, HOBT to give 24a; (d) Dess-Martin periodinane, CF<sub>3</sub>COOH, CH<sub>2</sub>Cl<sub>2</sub> to give 24b.

As is seen from the Table I, the  $\alpha$ . $\alpha$ -diffuoromethylene ketone (difluorostatone) derivative of L-valine, e.g. 17b and 22c (IC<sub>50</sub> = 0.635 and 0.404  $\mu$ M, respectively), is the preferred residue at the P1-primary specificity position of the inhibitors described herein. Substitution at  $P_1$ - $P_1$  by the corresponding  $\alpha, \alpha$ -difluoromethylene ketones of Lleucine to give 17a and 22b (IC<sub>50</sub> = 11.1 and 4.9  $\mu$ M, respectively) or by that of L-phenylalanine to give 22a  $(IC_{50} = \gg 5 \mu M)$  all afford less active compounds when compared to the  $P_1$ - $P_1'$  L-value derivative 22c (IC<sub>50</sub> = 0.404  $\mu$ M). The inhibitors 17a and 17b span the P<sub>3</sub>-P<sub>1</sub>' subsites whereas the inhibitor 22c spans the  $P_5$ - $P_1$ ' subsites and all of these inhibitors contain derivatives of  $\alpha, \alpha$ difluorostatone at  $P_1$ - $P_1'$ . The tripeptide 22c is N-terminated with the functionality  $p-[p-Cl(C_6H_4)SO_2NHCO]$ - $(C_6H_4)CO$ . In accord with our previous report,<sup>9,10</sup> and others,<sup>22</sup> this sulfonamide functionality confers high in vivo activity as well as an enhancement in in vitro potency to peptidyl trifluoromethyl ketones relative to the corresponding CBZ or t-BOC derivatives. This is also true in the present report (e.g.,  $\alpha, \alpha$ -difluoromethylene ketones), as is seen when the homologous series such as 17b (IC<sub>50</sub> = 0.635  $\mu$ M) is compared with 22c (IC<sub>50</sub> = 0.404  $\mu$ M). Presumably, this sulfonamide functionality effectively increases binding to HLE through favorable interactions with residues in the  $S_5$ - $S_4$ -subsites. Furthermore, in an in vivo situation, the acidic nature of the sulfonamide may prevent the rapid clearance of the inhibitors from the lungs when compared to CBZ N-terminated tripeptides.

From Table I it is seen that besides requiring a derivative of L-valine at P<sub>1</sub> it is crucial that the tripeptides described contain an ester functionality at the carboxy terminal end of the peptide for good in vitro potency. For example, the ethyl ester 17b has an IC<sub>50</sub> of 0.635  $\mu$ M whereas the corresponding carboxylic acid compound 19 is inactive (IC<sub>50</sub> =  $\gg 5 \mu$ M). This may not be surprising since it is well-known that HLE prefers small uncharged residues at

<sup>(21)</sup> Dess, D. B.; Martin, J. C. Readily Accessible 12-I-5 Oxidant for the Conversion of Primary and Secondary Alcohols to Aldehydes and Ketones. J. Org. Chem. 1983, 48, 4155-4156. Caution: Care should be taken in the handling of this reagent. The explosive nature of periodinane and its precursor 2-iodoxybenzoic acid has recently been described: Plumb, J. B.; Harper, D. J. Chem. Eng. News 1990, July 16, 3. (22) (a) Krell, R. D.; Stein, R. L.; Strimpler, A. M.; Trainor, D.; Edwards,

<sup>(22) (</sup>a) Krell, R. D.; Stein, R. L.; Strimpler, A. M.; Trainor, D.; Edwards,
P.; Wolanin, D.; Wildonger, R.; Schwartz, J.; Hesp, B.; Giles, R. E.;
Williams, J. C. Biochemical Characterization of ICI 200,880: A Novel,
Potent and Selective Inhibitor of Human Neutrophil Elastase. *FASEB*J. 1988, 2 (4), Abstract 290. (b) Williams, J. C.; Stein, R. L.; Knee, C.;
Egan, J.; Falcone, R.; Trainor, D.; Edwards, P.; Wolanin, D.; Wildonger,
R.; Schwartz, J.; Hesp, B.; Giles, R. E.; Krell, R. D. Pharmacologic
Characterization of ICI 200,880: A Novel Potent and Selective Inhibitor
of Human Neutrophil Elastase. *FASEB J.* 1988, 2 (4), Abstr. 291.
(23) Bergeson, S.; Schwartz, J. A.; Stein, M. M.; Wildonger, R. A.;

<sup>(23)</sup> Bergeson, Š.; Schwartz, J. A.; Stein, M. M.; Wildonger, R. A.; Edwards, P. D.; Shaw, A.; Trainor, D. A.; Wolanin, D. J. EP Patent Appl. 0 189 305, 1986.

 $P_1$  and the vicinity. We have recently reported<sup>9,10,11</sup> upon peptidyl trifluoromethyl ketones as potent and specific inhibitors of HLE. The primary specificity pocket of HLE  $(S_1)$  is hydrophobic, consisting of the residues Val<sup>190</sup> and Val<sup>216</sup> of HLE, which may explain the enzyme's preference for hydrophobic, nonpolar, uncharged residues. The loss of potency by the carboxylic acid **19** may simply be the result of placing a very hydrophilic residue (COOH) near or into the hydrophobic  $S_1$ - $S_1$ ' region, which, by the presence of a negatively charged species, may result in a decrease of hydrophobic interactions between the inhibitor and the enzyme. On the other hand the neutrally charged ethyl ester **17b** is expected to have a favorable hydrophobic interaction with Val<sup>190</sup> and Val<sup>216</sup> of HLE.

One of the most potent trifluoromethyl inhibitors reported<sup>9,10</sup> is 3 (BI-RA-260) (IC<sub>50</sub> = 0.084  $\mu$ M). The in vitro potency of the trifluoromethyl ketone inhibitor 3 is 1 order of magnitude greater than the corresponding  $\alpha, \alpha$ difluorostatone derivative 22c (IC<sub>50</sub> =  $0.404 \ \mu$ M). This difference in potency may be due to the overall difference in the electronegative inductive effect of a trifluoromethyl residue when compared to that of a difluoromethylene residue. Presumably the mechanism of inhibition of HLE by trifluoromethyl ketone compounds, such as 2 and 3, and of  $\alpha$ ,  $\alpha$ -diffuoromethylene ketone compounds, such as 17b and 22c, is through transition-state inhibition. The enhanced electrophilicity of the fluorinated ketone carbonyl of compounds such as 2 and 3 or 17b and 22c facilitates the enzyme-catalyzed addition of active site Ser<sup>195</sup> to the ketone carbonyl to form a metastable hemiketal, which resembles the tetrahedral intermediate in the reaction pathway for enzyme-substrate hydrolysis. Although the  $\alpha, \alpha$ -difluoromethyl ketone inhibitors are assumed to form a covalent bond with the active site Ser<sup>195</sup> of HLE, this process is reversible. In the case of porcine pancreatic elastase (PPE), it has been unequivocally demonstrated<sup>14</sup> through X-ray analysis that, in the adducts formed between the enzyme and the peptidyl trifluoromethyl ketone inhibitors, the  $O^{\gamma}$  atom of the catalytic Ser<sup>195</sup> residue covalently attached (1.5 Å) to the ketone carbonyl of the inhibitor via hemiketal formation. A similar type mechanistic inhibition is presumed to be the case with the  $\alpha, \alpha$ -difluoromethylene ketone inhibitors described herein. Because of the high electrophilicity of the fluorine atoms, an alternative mechanism may be due to the generation of transition-state inhibitors in situ resulting from the addition of H<sub>2</sub>O to the  $\alpha$ , $\alpha$ -difluoromethylene ketone.

The X-ray structure of HLE complexed with both synthetic and natural inhibitors has recently become available.<sup>16</sup> In order to gain some molecular insight into the binding properties of extended inhibitors  $(P_1'-P_3')$ , and to provide a better understanding of the structureactivity relationships, molecular modeling studies were performed utilizing the X-ray coordinates of the complex of the third domain of the natural inhibitor of the turkey ovomucoid (OMTKY3) with HLE. We have previously shown<sup>10</sup> that if it is assumed on the basis of mechanistic grounds that the carbonyl of the trifluoromethyl ketone of the active inhibitors reacts covalently but reversibly with the active site Ser<sup>195</sup> of HLE, then it is seen from molecular docking studies that, in order to best fit and have desirable hydrogen-bonding interactions, the bulky substituents on the P<sub>2</sub>-glycines of the enzyme-inhibitor complexes must stick out into solution away from the core



Figure 1. Minimized structure of HLE complexed with the  $\alpha,\alpha$ difluoromethylene inhibitor 17b detailing the binding and catalytic sites of HLE. The binding residues (Ser<sup>214</sup>, Phe<sup>215</sup>, Val<sup>216</sup>, and Arg<sup>217</sup>) and the catalytic triad (Ser<sup>195</sup>, His<sup>67</sup>, and Asp<sup>102</sup>) of HLE are given in blue and white, respectively. The inhibitor is shown in yellow. For minimization calculations the constraint (1.78 Å) of covalently linking Ser<sup>195</sup> with the  $\alpha,\alpha$ -difluoromethylene carbonyl of the inhibitor 17b via hemiketal formation was utilized. Hydrogen bonds formed between HLE residues and those of the inhibitor 17b are indicated by dotted lines (Val<sup>216</sup> CO to NH of P<sub>3</sub>-Val, 1.83 Å; Val<sup>216</sup> NH to CO of P<sub>3</sub>-Val, 1.90 Å; Ser<sup>214</sup> CO to NH of P<sub>1</sub>-Val, 1.82 Å).

of HLE. Molecular modeling indicates that a similar situation exists in the case of  $\alpha, \alpha$ -difluoromethylene ketone inhibitors. Figure 1 shows the catalytic triad (Ser<sup>195</sup>, Asp<sup>102</sup>, and His<sup>57</sup>) of HLE in white and the binding site residues (Val<sup>216</sup>, Phe<sup>215</sup>, and Ser<sup>214</sup>) of HLE in blue. The  $\alpha, \alpha$ -difluoromethylene inhibitor 17b is in yellow. In the binding conformations depicted in Figure 1, the P<sub>1</sub>difluoromethylene ketone carbonyl of the inhibitor 17b is covalently linked (1.78 Å) to the Ser<sup>195</sup> of HLE via hemiketal formation. Hydrogen bonds between the HLE binding site residues and those of the inhibitor 17b are as follows: Val<sup>216</sup> CO to NH of P<sub>3</sub>-Val, 1.83 Å; Val<sup>216</sup> NH to CO of P<sub>3</sub>-Val, 1.90 Å; Ser<sup>214</sup> CO to NH of P<sub>1</sub>-Val, 1.82 Å. The angles of the generated hydrogen bonds between the inhibitor 17b and HLE are as follows: Val<sup>216</sup> CO to N of P3-Val, 164.57°; Val<sup>216</sup> O to NH of P3-Val, 169.15°; Val<sup>216</sup> NH to O of P<sub>3</sub>-Val, 166.85°; Val<sup>216</sup> H to OC of P<sub>3</sub>-Val, 167.43°; Ser<sup>214</sup> CO to N of P1-Val, 154.80°; Ser<sup>214</sup> O to HN of P1-Val, 149.40°. The ethoxy carbonyl of 17b makes no contribution to the overall binding other than to fit into the hydrophobic  $S_1$ '-pocket and thus helps to stabilize the complex.

In order to gain some insight into the possibility of extending the inhibitor 17b further into the prime region and thus take further advantage of P'-S'-interactions, molecular modeling studies were conducted with the natural inhibitor OMTKY3 and the trifluoromethyl ketone inhibitor 2. Figure 2 depicts, on the basis of the X-ray structure, how the third domain of the turkey ovomucoid inhibitor aligns itself with the catalytic site of HLE. The catalytic site of HLE (Ser<sup>195</sup>, Asp<sup>102</sup>, and His<sup>57</sup>) is shown in white and the partial structure of OMTKY3 in yellow (residues  $Pro^{14}$ -Ala<sup>15</sup>-Cys<sup>16</sup>-Thr<sup>17</sup>-Leu<sup>18</sup>-Glu<sup>19</sup>-Tyr<sup>20</sup>). In this figure the P<sub>1</sub>'-Glu<sup>19i</sup> is within the oxyanion hole of

Inhibition of HLE by N-Substituted Peptides



Figure 2. X-ray structure corresponding to a complex between HLE and the third domain of the turkey ovomucoid inhibitor (OMTKY3). The OMTKY residues  $Pro^{14}$ -Ala<sup>15</sup>-Cys<sup>16</sup>-Thr<sup>17</sup>-Leu<sup>18</sup>-Glu<sup>19</sup>-Tyr<sup>20</sup> are shown in yellow. The catalytic site of HLE (Asp<sup>102</sup>, His<sup>57</sup>, and Ser<sup>195</sup>) is shown in white and the Phe<sup>41</sup> binding residue in blue. Donor-acceptor distances between HLE residues and those of OMTKY3 are indicated by dotted lines (P<sub>3</sub>'-N to O of Phe<sup>41</sup>, 3.03 Å). Donor-acceptor distances between residues of the catalytic site are also indicated by dotted lines (O of Asp<sup>102</sup> to imidazole N of His<sup>57</sup>, 2.56 Å; N imidazole of His<sup>57</sup> to O<sup> $\gamma$ </sup> of Ser<sup>195</sup>, 2.48 Å).



Figure 3. Overlay of 2 in yellow with  $P_5$ - $P_1'$  residues of turkey ovomucoid (OMTKY3) in white. The residues  $Pro^{14}$ -Ala<sup>15</sup>-Cys<sup>16</sup>-Thr<sup>17</sup>-Leu<sup>18</sup>-Glu<sup>19</sup>-Tyr<sup>20</sup> of OMTKY3 are illustrated.

HLE created by the residues Val<sup>190</sup>, Val<sup>216</sup>, and Asp<sup>226</sup>. From the X-ray structure it is clearly seen that Tyr<sup>20i</sup> of OMTKY3 makes a very effective hydrogen bond with the carbonyl of Phe<sup>41</sup> (Phe<sup>41</sup> CO to NH of P<sub>2</sub>'-Tyr<sup>20i</sup>). The donor-acceptor distance is 3.03 Å (P<sub>3</sub>'-N to oxygen of Phe<sup>41</sup>) and the angle between Phe<sup>41</sup> CO and the nitrogen of  $P_2$ '-Tyr<sup>20i</sup> is 142.73°. It was thought that the hydrogen bonding that is observed in the natural inhibitor between Phe<sup>41</sup> and Tyr<sup>20i</sup> and which is incapable of forming in the trifluoromethyl ketone series of inhibitors (e.g., 2) might be taken advantage of in the case of the  $\alpha, \alpha$ -difluoromethylene series through chain extension. The first step in the design process of preparing inhibitors extended into the S2'-S3' region is to utilize molecular modeling to overlay the least energy conformation of the trifluoromethyl ketone 2 with that of OMTKY3 in order to obtain a proper alignment with statone-containing inhibitors which contain one atom less than a true dipeptide mimic. This overlay and alignment is seen in Figure 3. In this figure the following alignment is made: P3-Val, P2-(N-indanyl)-Gly, and P3-Val of 2 with P3-Cys16, P2-Thr17, and P1-Leu17 of OMTKY3, respectively. The distance from the carbon of the P<sub>1</sub>-carbonyl of OMTKY3 (i.e., Leu<sup>18</sup>) to the oxygen



 $P_2' - N$  (Tyr<sup>20i</sup>) to oxygen of Phe<sup>41</sup> is 3.03 Å.

**Figure 4.** Schematic representation of the hydrogen-bonding interactions, as taken from the X-ray structure, of turkey ovomucoid (OMTKY3) with Phe<sup>41</sup> of HLE  $[P_2'-N (Tyr^{20i})$  to the O of Phe<sup>41</sup> is 3.03 Å)]. The distance from the C of the P<sub>1</sub>-CO to the O of Phe<sup>41</sup> is 5.36 Å.



Figure 5. A schematic representation of potential hydrogenbonding interactions of the inhibitor 17b with remote residues, i.e.  $Asn^{61}$ ,  $Ala^{60}$ , and  $Phe^{41}$ . The following distances are observed: from the carbon of the P<sub>1</sub>-CO to the O of Ala<sup>60</sup>, 10.79 Å; from the C of the P<sub>1</sub>-CO to the side-chain N of  $Asn^{60}$ , 14.15 Å; from the carbon of the P<sub>1</sub>-CO to the O of Phe<sup>41</sup>, 5.78 Å.

of Phe<sup>41</sup> of HLE is 5.36 Å as taken from the X-ray structure. This distance measurement is shown schematically in Figure 4. This is the distance which we used to approximate the length of an appropriate spacing group that could take advantage of the accessibility of Phe<sup>41</sup> and which could act as a hydrogen donor to effectively hydrogen bond with the carbonyl of  $Phe^{41}$ . As is seen in Figure 5 the distance from the carbon of the P1-CO of the inhibitor 17b to the oxygen of the carbonyl of Phe<sup>41</sup> is 5.78 Å compared to 5.36 Å for OMTKY3. The spacing group which we employed to elongate 17b into the  $P_3$ ' region was Gly-Gly. By incorporating this fragment the inhibitor 23b was obtained (Figure 6A). The distance from the carbon of the P<sub>1</sub>-CO of 23b to the P<sub>3</sub>'-NH of 23b is 5.44 Å, which is in the range described above to potentially hydrogen bond with the carbonyl of Phe<sup>41</sup> (see Figure 6A). The  $P_3$ '-NH of 23b acts as a hydrogen donor while the carbonyl of Phe<sup>41</sup> acts as a hydrogen acceptor. The distance of this hydrogen bond is 1.99 Å. Figure 7 depicts the minimized structure of the complexation of HLE with the inhibitor 23b (Gly-Gly spacing group) detailing the binding and catalytic sites of HLE. For minimization calculations the



Figure 6. Schematic representations of (a) the distance from the C of the  $P_1$ -CO of 23b to the  $P_2$ '-N of 23b, 5.44 Å, and (b) the distance from the C of the  $P_1$ -CO of 24b to the  $P_3$ -O of 24b, 10.62 Å, and from the C of the  $P_1$ -CO of 24b to the  $P_3$ '-N of 24b, 8.75 Å.



Figure 7. Minimized structure of the complexation of HLE with the inhibitor 23b (Gly-Gly) in yellow detailing the binding and catalytic sites. The binding residues (Phe<sup>41</sup>, Ser<sup>214</sup>, Phe<sup>215</sup>, and Val<sup>216</sup>) and the catalytic triad (Ser<sup>195</sup>, His<sup>57</sup>, Asp<sup>102</sup>) are given in blue and white, respectively. For minimization calculations the constraint (1.78 Å) of covalently linking Ser<sup>195</sup> with the  $\alpha,\alpha$ -difluoromethylene carbonyl of the inhibitor 23b. Hydrogen bonds formed between HLE residues and those of the inhibitor 23b are indicated by dotted lines (Phe<sup>41</sup> CO to NH of P<sub>3</sub>'-Gly, 1.99 Å; Val<sup>216</sup> CO to NH of P<sub>3</sub>-Val, 1.82 Å; Val<sup>216</sup> NH to CO of P<sub>3</sub>-Val, 1.93 Å; Ser<sup>214</sup> CO to NH of P<sub>1</sub>-Val, 1.84 Å).

constraint (1.78 Å) of covalently linking Ser<sup>195</sup> with the  $\alpha, \alpha$ -difluoromethylene carbonyl of the inhibitor **23b** was made. The hydrogen-bonding distances formed between HLE residues and those of the inhibitor **23b** are as follows: Phe<sup>41</sup> CO to NH of P<sub>3</sub>'-Gly, 1.99 Å; Val<sup>216</sup> CO to NH of P<sub>3</sub>-Val, 1.82 Å; Val<sup>216</sup> NH to CO of P<sub>3</sub>-Val, 1.93 Å; Ser<sup>214</sup> NH to NH of P<sub>1</sub>-Val, 1.84 Å. The corresponding hydrogen-bonding angles between the inhibitor **23b** and HLE are as follows: Phe<sup>41</sup> CO to N of P<sub>3</sub>'-Gly, 139.78°; Phe<sup>41</sup> O to NH of P<sub>3</sub>'-Gly, 151.80°; Val<sup>216</sup> CO to H of P<sub>3</sub>-Val, 165.26°; Val<sup>216</sup> O to HN of P<sub>3</sub>-Val, 171.66°; Val<sup>216</sup> NH to O of P<sub>3</sub>-Val, 166.99°; Val<sup>216</sup> H to OC of P<sub>3</sub>-Val, 163.65°; Ser<sup>214</sup> CO to H of P<sub>1</sub>-Val, 158.06°; Ser<sup>214</sup> O to HN of P<sub>1</sub>-Val, 146.77°. These results correspond to the inhibitor





**Figure 8.** Minimized structure of the complexation of HLE with the inhibitor **24b** in yellow detailing the binding and catalytic sites. The binding residues (Ala<sup>60</sup>, Asn<sup>61</sup>, Ser<sup>214</sup>, Phe<sup>215</sup>, and Val<sup>216</sup>) and the catalytic triad (Ser<sup>195</sup>, His<sup>57</sup>, and Asp<sup>102</sup>) are given in blue and white, respectively. For minimization calculations the constraint (1.78 Å) of covalently linking Ser<sup>195</sup> with the ( $\alpha,\alpha$ -difluoromethylene)carbonyl of the inhibitor **24b** via hemiketal formation was utilized. Hydrogen bonds formed between HLE residues and those of the inhibitor **24b** are indicated by dotted lines (Ala<sup>60</sup> CO to P<sub>3</sub>'-NH, 1.89 Å; Asn<sup>61</sup> NH to CO of P<sub>3</sub>'-CO, 3.22 Å; Ser<sup>214</sup> CO to NH of P<sub>1</sub>-Val, 1.88 Å; Val<sup>216</sup> CO to NH of P<sub>3</sub>-Val, 1.84 Å; Val<sup>216</sup> NH to CO of P<sub>3</sub>-Val, 1.87 Å).

23b having one further hydrogen bond than 17b. This extra hydrogen bond results in a factor of 10 in the relative in vitro potencies of the elongated inhibitor 23b (IC<sub>50</sub> = 0.069  $\mu$ M) versus the shorter 17b (IC<sub>50</sub> = 0.635  $\mu$ M).

The X-ray structure of the OMTKY3-HLE complex was further examined to see if there are any accessible residues other than Phe<sup>41</sup> within 15 Å of the carbon of the  $P_1$ -CO of 17b that might be taken advantage of through effective hydrogen bonding. This examination indicated that the oxygen of Ala<sup>60</sup> and the side-chain nitrogen of Asn<sup>61</sup> fit the above criteria, the distances being 10.79 and 14.15 Å, respectively. These distance measurements are shown schematically in Figure 5. The spacing group which we employed to elongate 17b into the P3'-region and thus take advantage of the remote residues Ala<sup>60</sup> and Asn<sup>61</sup> was  $N^{\alpha}$ -acetyllysine ethyl ester, as is seen in Figure 6B. The distance from the carbon of the P<sub>1</sub>-CO of 24b to the P<sub>3</sub>'-NH of 24b is 8.75 Å. The distance from the P<sub>1</sub>-CO of 24bto the P<sub>3</sub>'-ester carbonyl of 24b is 10.63 Å. Figure 8 depicts the minimized structure of the complexation of the inhibitor 24b (side-chain of L-lysine being used as the spacing group) detailing the binding and catalytic sites. As in the example above, for minimization purposes the constraint (1.78 Å) of covalently linking Ser<sup>195</sup> with the  $\alpha, \alpha$ -difluoromethylene carbonyl of the inhibitor 24b was made. The hydrogen-bonding distances formed between HLE residues and those of the inhibitor 24b are as follows: Ala<sup>60</sup> CO to P<sub>3</sub>'-NH, 1.89 Å; Asn<sup>61</sup> NH to CO of P<sub>3</sub>'-CO, 3.22 Å; Ser<sup>214</sup> CO to NH of P<sub>1</sub>-Val, 1.88 Å; Val<sup>216</sup> CO to NH of P3-Val, 1.84 Å; Val<sup>216</sup> NH to CO of P3-Val, 1.87 Å. The hydrogen-bonding angles between the inhibitor 24b and HLE are as follows: Ala<sup>60</sup> CO to H of P3'-Lys, 135.55°; Ala<sup>60</sup> O to HN of P3'-Lys, 156.74°, Asn<sup>61</sup> NH to O of  $P_{3}$ '-Lys, 143.83°; Asn<sup>61</sup> H to OC of  $P_{3}$ '-Lys, 154.29°; Ser<sup>214</sup> CO to H of  $P_{1}$ -Val, 149.11°; Ser<sup>214</sup> O to HN of P1-Val, 153.68°; Val<sup>216</sup> CO to H of P3-Val, 165.52°; Val<sup>216</sup>

Table II. Inhibition of Elastase-Induced Pulmonary Hemorrhage (EPH) in Hamsters by Selected Agents

compdª	dose, µg/mL (it.)	$N^b$	max % reduction in hemorrhage <sup>c</sup>
17 <b>a</b>	NT		
1 <b>7b</b>	20	4	51.9
19	20	4	55.8
22a	10	4	12.2
22b	20	4	55.2
22c	10	4	94.6
23b	20	4	33.3
24b	20	4	7.6
3 <sup>d</sup>	3	4	58.1
	10	4	90.5

<sup>a</sup> Compounds were administered intratracheally (it.) in a 0.1-mL volume (DMSO, 1:100 in saline) followed 5 min later by 50  $\mu$ g (it.) of HLE in 0.1 mL of saline. <sup>b</sup> Number of animals. <sup>c</sup> Average percent reduction of red blood cells (RBC) per milliliter of cell suspension over vehicle control. <sup>d</sup> Literature<sup>10</sup>  $ED_{50} = 3.8 \mu g/animal$ .

O to HN of P<sub>3</sub>-Val, 167.79°. Although 24b contains one hydrogen bond more than 23b (IC<sub>50</sub> = 0.069  $\mu$ M), there is only a slight enhancement of in vitro potency of 24b (IC<sub>50</sub> = 0.057  $\mu$ M). This is probably due to the fact that the hydrogen bond between the  $P_3$ '-CO of 24b and the side-chain nitrogen of Asn<sup>61</sup> is weak (3.22 Å) and is not of an optimal value.

The results pertaining to the inhibition of elastaseinduced pulmonary hemorrhage (EPH) in hamsters by selected representative agents are presented in Table II. HLE induces acute hemorrhage in the hamster<sup>24</sup> lung when administered intratracheally (it.). Hemorrhage can be quantitated 18 h later by measuring red blood cell concentration in bronchial alveolar lavage fluid. In this model, it. administration of one of the most potent in vitro inhibitors, 22c (IC<sub>50</sub> = 0.404  $\mu$ M), 5 min prior to HLE challenge, effectively inhibited hemorrhage in a dosedependent manner, a dose of 10  $\mu$ g/mL (it.) resulting in a 94.6% inhibition of hemorrhage. We have previously shown<sup>13</sup> in the case of trifluoromethyl ketone containing inhibitors that tripeptides having a CBZ substituent at the amino terminal end, although they may be potent in vitro, translate into inactive compounds in vivo. For example, the corresponding sulfonamide analog 22c of the in vivo inactive CBZ derivative 17b is quite active in vivo, exhibiting 94.6% inhibition against hemorrhage at  $10 \,\mu g/$ mL it.

In conclusion, the peptides presented in Table I which contain achiral nonproteinogenic N-substituted glycine residues at  $P_2$  in replacement of L-proline and possess a  $\alpha, \alpha$ -difluoromethylene ketone derivative of L-valine at P<sub>1</sub>- $P_1'$  are effective in vitro HLE inhibitors with IC<sub>50</sub> values in the submicromolar range. As previously demonstrated, sterically demanding substituents on the P2-nitrogen have no detrimental effect on in vitro potency. When substitution is made at  $P_1$  by the  $\alpha, \alpha$ -difluoromethylene ketones of L-leucine or L-phenylalanine, activity is lost. The described  $\alpha$ ,  $\alpha$ -diffuoromethylene tripeptide inhibitors, i.e. 17b, interact with the  $S_3$ - $S_1'$  binding regions of HLE. Additionally the extended inhibitors 23b and 24b were designed through molecular modeling to interact with S<sub>3</sub>- $S_{3}$  binding subsites of HLE. The described in vitro active inhibitors were also evaluated in hamsters in an elastaseinduced pulmonary hemorrhage (EPH) model. In this

model, it. administration of 22c, 5 min prior to HLE challenge (10  $\mu$ g, it.), effectively inhibited hemorrhage (94.6%) in a dose-dependent manner.

Journal of Medicinal Chemistry, 1992, Vol. 35, No. 26 4803

## **Experimental Section**

General. All melting points were determined with a Büchi SMP-20 melting point apparatus and are uncorrected. TLC analyses were performed with E. Merck silica gel 60 F-254 plates of 0.25 mm thickness and were visualized with UV, I<sub>2</sub>, or ninhydrin spray reagent. Chemical microanalyses for carbon, hydrogen, and nitrogen were conducted by Midwest Laboratories (Indianapolis, IN) and are within  $\pm 0.4\%$  of theoretical values. <sup>1</sup>H NMR were determined with Bruker AM 500 (<sup>1</sup>H = 500.13 MHz), Bruker AC 270 ( ${}^{1}\text{H} = 270.13 \text{ MHz}$ ), and Bruker WM 250 ( ${}^{1}\text{H} = 250.13 \text{ MHz}$ ) MHz) spectrometers using (CH<sub>3</sub>)<sub>4</sub>Si as an internal standard. Chemical shifts for <sup>1</sup>H NMR signals are reported in ppm downfield from TMS ( $\delta$ ). Fast atom bombardment (FAB) mass spectra were obtained using a Kratos MS 80RFAQ mass spectrometer (Manchester, U.K.) equipped with a Phrasor Scientific (Duarte, CA) Capillatron Fast Atom Gun. Instrument resolution was 1200  $(m/\Delta m)$ , the accelerating voltage was 3 kV. A 1:1 mixture of glycerol and thioglycerol was used as the FAB matrix. The FAB gun was operated with Xenon at 8 kV and  $35 \,\mu$ A emission current. Chemical ionization (CI) mass spectra were obtained using a Finnigan 4023 GC/MS/DS (San Jose, CA) instrument modified for high-pressure operation. Methane (1.5 Torr) or NH<sub>3</sub> (2.0 Torr) was used as reagent gas. Samples were introduced via direct probe, heated ballistically from 50 to 350 °C. The source temperature was 300  $^{\circ}\mathrm{C},$  the electron energy was 200 eV, and the emission current was 0.1 mA. The mass range, 50-650 Da, was scanned in 1.95 s.

N-(2,3-Dihydro-1H-inden-2-yl)glycine Ethyl Ester Hydrochloride (14).<sup>10</sup> Glycine ethyl ester hydrochloride (34.5 g. 0.247 mol) and 2-indanone (25.1 g, 0.19 mol) were dissolved in absolute EtOH (700 mL) and then NaCNBH<sub>3</sub> (25.2 g, 0.41 mol) was added portionwise. The reaction was stirred at room temperature for 16 h. The EtOH was removed under reduced pressure and the residue was treated with H<sub>2</sub>O. The product was extracted several times into EtOAc. The organic extract was washed sequentially with saturated aqueous solutions of NaHCO3 and NaCl before being dried (MgSO4) and filtered. After concentration in vacuo the oily residue was taken up in Et<sub>2</sub>O (300 mL) and then cooled to 10 °C by means of an ice/water bath. Diethyl ether which had previously been saturated with anhydrous hydrogen chloride was slowly added. The precipitated hydrochloride was filtered and washed with chilled Et<sub>2</sub>O to afford the title compound 14 (21 g) as a colorless solid: mp 166-168 °C; <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  10.1 (s, 2 H, <sup>+</sup>NH<sub>3</sub>), 7.3–7.1 (m, 4 H), 4.3-4.2 (q, 2 H, CH<sub>3</sub>), 4.05 (bs, 3 H, CH<sub>2</sub>, CH<sub>3</sub>), 3.3-3.2 (dd, 4 H,  $2 \times CH_2$  of indanyl), 1.3-1.2 (t, 3 H, CH<sub>3</sub>). Anal. (C<sub>13</sub>H<sub>17</sub>NO<sub>2</sub>·HCl) C, H, N.

Method A. N-(Carbobenzyloxy)-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycine Ethyl Ester (15a).<sup>10</sup> To a solution of CBZ-L-valine (5.0 g, 0.02 mol) in CH<sub>2</sub>Cl<sub>2</sub> (70 mL) were added DMAP (2.44 g, 0.02 mol), 14 (5.1 g, 0.02 mol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (WSCDI) (3.83 g, 0.02 mol). The resulting mixture was stirred at room temperature for 16 h. The CH<sub>2</sub>Cl<sub>2</sub> was concentrated under reduced pressure and the residue was treated with EtOAc and 1 N aqueous HCl. The organic layer was separated and washed consecutively with 1 N aqueous HCl, 5% aqueous Na<sub>2</sub>-CO<sub>3</sub>, and saturated aqueous NaCl. The EtOAc was dried  $(MgSO_4)$ , filtered, and concentrated in vacuo to give the crude title compound 15a as a pale yellow oil. The compound was purified by chromatography over silica gel (CH<sub>2</sub>Cl<sub>2</sub>) to give the title compound 15a as a colorless oil: MS (CI/CH<sub>4</sub>) m/z (relative intensity) 453 (MH<sup>+</sup>, 96), 345 (100), 310 (34), 220 (84), 117 (21), 116 (23); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.35 (s, 5 H), 7.15 (s, 4 H), 5.6 (d, 1 H), 5.15-5.0 (m, 3 H), 4.8-4.7 (m, 1 H), 4.2-4.1 (m, 3 H), 3.7-3.6 (d, 1 H), 3.4–2.9 (m, 4 H), 2.15–2.0 (m, 1 H), 1.25 (t, 3 H, CH<sub>3</sub>), 1.10-0.9 (dd, 6 H, 2 × CH<sub>3</sub> of Val). Anal. ( $C_{26}H_{32}N_2O_5$ ) C, H, N.

Method B. N-(Carbobenzyloxy)-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycine (15b).<sup>10</sup> The corresponding CBZ ethyl ester derivative 15a (13.8 g, 0.0305 mol) was dissolved in EtOH

<sup>(24)</sup> Hassal, C. H.; Johnson, W. H.; Kennedy, A. J.; Roberts, N. A. A New Class of Inhibitors of Human Leukocyte Elastase. FEBS Lett. 1985, 183, 201-204.

(200 mL) and then treated with 1 N aqueous KOH (30 mL) in 5-mL portions. The mixture was stirred at room temperature for 16 h. The EtOH was concentrated under reduced pressure and the residue was treated with H<sub>2</sub>O. The aqueous mixture was washed three times with EtOAc, and the layers were separated. The aqueous layer was acidified to pH 3 by the dropwise addition of 1 N aqueous HCl. The product was extracted into EtOAc, and the layers were separated. The organic phase was washed with brine, dried (MgSO<sub>4</sub>), filtered, and concentrated under reduced pressure to afford the pure title compound 15b as a colorless semisolid (9.1g): MS (CI/NH<sub>3</sub>) m/z (relative intensity) 425 (MH<sup>+</sup>, 53), 317 (98), 258 (15), 192 (34); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  10.4 (bs, 1 H, COOH), 7.35 (m, 5 H), 7.2 (s, 4 H), 6.0 (m, 1 H), 5.15–5.0 (m, 3 H), 4.75 (m, 1 H), 3.7–3.6 (dd, 1 H), 3.35–2.85 (m, 4 H), 2.05 (d, 2 H), 1.1–0.9 (dd, 6 H). Anal. (C<sub>24</sub>H<sub>28</sub>N<sub>2</sub>O<sub>5</sub>) C, H, N.

Method C. (tert-Butoxycarbonyl)-L-valine Methoxymethylamide (5c).<sup>20</sup> Under argon at 0 °C, N-t-BOC-L-valine (4c) (40.0 g, 0.184 mol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> with stirring. To this solution was added 1,1'-carbonyldiimidazole (CDI) (39.70 g, 0.245 mol). After stirring for 0.5 h, a mixture of N,O-dimethylhydroxylamine hydrochloride (23.88 g, 0.245 mol) and Et<sub>3</sub>N (24.77 g, 0.245 mol) was added at 0 °C. After 1 h of stirring at 0°C, the reaction mixture was left overnight at room temperature. The reaction mixture was left overnight at room temperature. The reaction mixture was washed with 1 N HCl (2×), saturated aqueous Na<sub>2</sub>CO<sub>3</sub> (2×), and brine. The CH<sub>2</sub>Cl<sub>2</sub> phase was dried (MgSO<sub>4</sub>), filtered, and concentrated to give the amide 5c (47.54 g, 99%): <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  5.1 (bs, 1 H, NH), 4.7 (m, 1 H, CHCO), 3.8 (s, 3 H, OCH<sub>3</sub>), 3.3 (s, 3 H, NCH<sub>3</sub>), 2.0 (m, 1 H, CH(CH<sub>3</sub>)<sub>2</sub>), 1.5 (s, 9 H, t-Bu), 1.05 (d, 3 H, CH<sub>3</sub>), 0.9 (d, 3 H, CH<sub>3</sub>). Anal. (Cl<sub>12</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>) C, H, N.

Method D. (tert-Butoxycarbonyl)-L-valinal (6c).<sup>20</sup> To a solution of the amide 5c (49.46 g, 0.19 mol) in freshly distilled THF (500 mL) under argon at 0 °C was added LiAlH<sub>4</sub> (7.21 g, 0.19 mol) in portions over a 0.5-h period. After stirring 15 min in the cold, an aqueous solution of KHSO<sub>4</sub> (25.87 g, 0.19 mol) was slowly added. The reaction mixture was filtered through Celite and the filter cake was washed consecutively with 2 N HCl (2×), saturated aqueous Na<sub>2</sub>CO<sub>3</sub> (2×), and brine. After drying (MgSO<sub>4</sub>), filtering, and concentrating, the aldehyde 6c (32.3 g, 92%) was obtained as an oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  9.6 (s, 1 H, CHO), 5.1 (bs, 1 H, NH), 4.2 (m, 1 H, CHCO), 2.1 (m, 1 H, CH-(CH<sub>3</sub>)<sub>2</sub>), 1.45 (s, 9 H, t-Bu), 1.05 (d, 3 H, CH<sub>3</sub>), 0.95 (d, 3 H, CH<sub>3</sub>).

Method E. Ethyl4(S)-[(tert-Butoxycarbonyl)amino]-2,2difluoro-3(RS)-hydroxy-5-methylhexanoate (7c).<sup>17,18</sup> Ethyl bromodifluoroacetate (1.5 mL) was added to a refluxing suspension of freshly activated Zn (13.62 g, 0.2 mol) in anhydrous THF (100 mL). After 1 min, a solution of t-BOC-L-valinal (6c) (16.77 g, 0.083 mol) and ethyl bromodifluoroacetate (40.59 g, 0.2 mol) in THF (75 mL) was added at a rate such that a gentle reflux was maintained. At the end of the addition the reaction mixture was refluxed for another 30 min. The reaction mixture was allowed to cool to room temperature and then KHSO<sub>4</sub> (28.3 g, 0.21 mol) in H<sub>2</sub>O (150 mL) was added. The reaction mixture was stirred for 30 min and was then filtered through Celite to remove the solid suspension. The filter cake was washed well with Et<sub>2</sub>O. To the filtrate was added brine (150 mL). The filtrate was extracted with  $Et_2O$  (3×). The combined  $Et_2O$  extract was dried (MgSO<sub>4</sub>), filtered, and concentrated in vacuo to afford crude 7c ( $\mathbf{R} = CH(CH_3)_2$ ) (34.3 g) as an oil. The product was further purified by chromatography over silica gel (2% EtOH in CH<sub>2</sub>- $Cl_2$ ) to give pure 7c (R = CH(CH\_3)\_2) (20.53 g, 76%) as a mixture of diastereomers which was used directly without further separation: <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  5.1 (b, 1 H, NH), 4.3 (m, 3 H, CH<sub>2</sub>CH<sub>3</sub>, CHOH), 4.1–3.3 (m, 2 H, α-CH, OH), 2.1 (m, 1 H, CH-(CH<sub>3</sub>)<sub>2</sub>), 1.5 (s, 9 H, 3 × CH<sub>3</sub>), 1.3 (t, 3 H, CH<sub>2</sub>CH<sub>3</sub>), 1.0 (d, 6 H,  $2 \times CH_3$ ). Anal. (C<sub>14</sub>H<sub>25</sub>F<sub>2</sub>NO<sub>5</sub>) C, H, N.

Method F. 4(S)-Amino-2,2-difluoro-3(RS)-hydroxy-5methylhexanoate (8c). The t-BOC compound 7c (R = CH-(CH<sub>3</sub>)<sub>2</sub>) (5.0 g, 15.4 mol) was dissolved in a mixture (20 mL) of 30% TFA in CH<sub>2</sub>Cl<sub>2</sub>. The resulting mixture was stirred at room temperature for 90 min. The TFA and CH<sub>2</sub>Cl<sub>2</sub> were concentrated in vacuo (<45 °C). The residue was azeotroped with toluene (5×) at 45 °C by means of a rotary evaporator. After drying overnight in vacuo, the trifluoroacetate salt of 8c (R = CH(CH<sub>3</sub>)<sub>2</sub>) (5.15 g, 98%) was obtained as a tan oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.6 (b, 3 H, <sup>+</sup>NH<sub>3</sub>), 4.3 (q, 3 H, CH<sub>2</sub>CH<sub>3</sub>, CHOH), 3.9–3.3 (m, 2 H, α-CH, OH), 2.2 (m, 1 H, CH(CH<sub>3</sub>)<sub>2</sub>), 1.4 (t, 3 H, CH<sub>2</sub>CH<sub>3</sub>), 1.1 (d, 6 H, 2 × CH<sub>3</sub>). Anal. (C<sub>9</sub>H<sub>17</sub>F<sub>2</sub>NO<sub>3</sub>·CF<sub>3</sub>COOH) C, H, N.

Method G. Ethyl 4(S)-[[N-(Carbobenzyloxy)-L-valyl-N-(2,3-dihydro-1*H*-inden-2-yl)glycyl]amino]-2,2-difluoro-3-(RS)-hydroxy-5-methylhexanoate (16;  $R = CH(CH_3)_2$ ). Under argon, HOBT (2.39 g, 17.7 mmol) in dry THF (10 mL) was added to a solution of N-CBZ-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycine (15b) (7.68 g, 17.7 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) at 0 °C. To this solution was added WSCDI (3.39 g, 17.7 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL). The reaction mixture was stirred at 0 °C for 30 min and then the trifluoroacetate salt 8c ( $\mathbf{R} = CH(CH_3)_2$ ) (5.0 g, 14.7 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added. The reaction mixture was stirred at 0 °C for 30 min and then for 16 h at room temperature. The reaction mixture was diluted with  $Et_2O$ . The  $Et_2O$  was washed sequentially with 2 N HCl  $(2\times)$ , saturated aqueous Na<sub>2</sub>- $CO_3$  (2×), and brine. The organic phase was dried (MgSO<sub>4</sub>), filtered, and concentrated to afford crude 16 ( $R = CH(CH_3)_2$ ) (10.36 g) as a viscous oily residue. Flash chromatography over silica gel with increasing solvent polarity (15, 20, 25, and 40%) $(CH_3)_2CO$  in petroleum ether) afforded pure 16 (R = CH(CH\_3)\_2) (3.02 g, 41%) as a colorless resinous solid: MS (CI/NH<sub>3</sub>) m/z(relative intensity) 632 (MH+, 100); <sup>1</sup>H NMR & 7.35 (s, 5 H, aromatics), 7.24 (s, 4 H, indanyl aromatics), 6.95 (d, 1 H, NH), 5.5 (m, 1 H, NH), 5.1 (d, 2 H, PhCH<sub>2</sub>), 5.0 (m, 1 H, indanyl CH), 4.6 (t, 1 H, α-CH), 4.3 (q, 2 H, CH<sub>2</sub>CH<sub>3</sub>), 4.2–3.6 (m, 5 H, CH<sub>2</sub> of Gly,  $\alpha$ -CH, CHOH), 3.2 (m, 4 H, indanyl CH<sub>2</sub>), 2.0 (m, 2 H,  $2 \times CH(CH_3)_2$ ), 1.3 (t, 2 H,  $CH_2CH_3$ ), 1.0 (m, 12 H,  $4 \times CH_3$ ). Anal.  $(C_{33}H_{43}F_2N_3O_7)$  C, H, F, N.

Method H. Ethyl 4(S)-[[N-(Carbobenzyloxy)-L-valy]-N-(2,3-dihydro-1H-inden-2-yl)glycyl]amino]-2,2-difluoro-3-oxo-5-methylhexanoate  $(17; \mathbf{R} = \mathbf{CH}(\mathbf{CH}_3)_2)$ . To the alcohol 16 (R = CH(CH<sub>3</sub>)<sub>2</sub>) (500 mg, 0.79 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added Dess-Martin periodinane<sup>21</sup> (1.3 g, 3.05 mmol) followed by TFA (100  $\mu$ L). The reaction mixture was stirred for 3 h at room temperature and then aqueous NaHSO3 was added. The reaction mixture was stirred for 15 min before the aqueous phase was extracted with EtOAc (2×). The combined EtOAc extract was washed with brine, dried (MgSO<sub>4</sub>), filtered, and concentrated to afford crude 17 ( $R = CH(CH_3)_2$ ) as a pale yellow oil. Preparative TLC (25% (CH<sub>3</sub>)<sub>2</sub>CO in petroleum ether) provided the pure product 17 (R = CH(CH<sub>3</sub>)<sub>2</sub>) (320 mg) as a colorless oil: MS (CI/  $NH_3$ ) m/z (relative intensity) 630 (MH<sup>+</sup>, 100); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ7.35 (s, 5 H, aromatics), 7.2 (s, 4 H, aromatics), 7.0 (d, 1 H, NH), 5.58 (d, 1 H, NH), 5.1 (d, 2 H, PhCH<sub>2</sub>), 5.0 (m, 2 H, α-CH, indanyl CH), 4.7 (t, 1 H, α-CH), 4.35 (q, 2 H, CH<sub>2</sub>CH<sub>3</sub>), 4.0-3.85 (m, 2 H, CH<sub>2</sub> of Gly), 3.3-3.0 (m, 4 H,  $2 \times CH_2$  of indanyl), 2.38 (m, 1 H, CH(CH<sub>3</sub>)<sub>2</sub>), 2.05 (m, 1 H, CH(CH<sub>3</sub>)<sub>2</sub>), 1.3 (t, 3 H, CH<sub>2</sub>CH<sub>3</sub>), 1.0-0.8 (m, 12 H,  $4 \times CH_3$ ). Anal. (C<sub>33</sub>H<sub>41</sub>F<sub>2</sub>N<sub>3</sub>O<sub>7</sub>·H<sub>2</sub>O) C, H, F, N.

Method I. 4(S)-[[N-(Carbobenzyloxy)-L-valy]-N-(2.3-dihydro-1*H*-inden-2-yl)glycyl]amino]-2,2-difluoro-3(*RS*)-hydroxy-5-methylhexanoic Acid (18:  $\mathbf{R}_1 = \mathbf{CH}(\mathbf{CH}_3)_2$ ). To a solution of the ester 16 ( $R = CH(CH_3)_2$ ) (250 mg, 0.413 mmol) in EtOH (10 mL) was added at 50 °C 1 N KOH (0.41 mL). The resulting mixture was stirred at ambient temperature for 2 h and then diluted with  $H_2O$ . The reaction mixture was extracted (3×) with  $Et_2O$ /petroleum ether (1:1). The aqueous phase was acidified with 2 N HCl to pH 3 and then extracted with  $Et_2O$  (3×). The combined Et<sub>2</sub>O extract was washed with brine, dried (MgSO<sub>4</sub>), filtered, and concentrated to afford the pure acid 18 ( $R_1 = CH$ -(CH<sub>3</sub>)<sub>2</sub>) (230 mg, 96%) as a colorless resinous solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>) § 7.3 (bs, 5 H, aromatics), 7.15 (bs, 4 H, aromatics), 6.0 (m, 1 H, NH), 5.1 (m, 3 H, PhCH<sub>2</sub>, indanyl CH), 4.8-3.9 (m, 5 H,  $\alpha$ -CH of Val, CHOH, CH<sub>2</sub> of Gly), 3.2 (m, 4 H, 2 × CH<sub>2</sub> of indanyl), 2.0 (m, 2 H, 2 × CH(CH<sub>3</sub>)<sub>2</sub>), 0.9 (m, 12 H, 4 × CH<sub>3</sub>). Anal.  $(C_{31}H_{39}F_2N_3O_7)$  C, H, F, N.

4(S)-[[N-(Carbobenzyloxy)-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycyl]amino]-2,2-difluoro-3-oxo-5-methylhexanoic Acid (19;  $\mathbf{R}_1 = CH(CH_3)_2$ ). A procedure similar to method H was followed on a 230-mg (0.38 mmol) scale. After workup, crude 19 ( $\mathbf{R}_2 = CH(CH_3)_2$ ) (210 mg) was obtained. Purification by preparative TLC over silica gel (0.5% HOAc and 3% EtOH in CH<sub>2</sub>Cl<sub>2</sub>) provided pure 19 (90 mg, 40%) as a colorless resinous solid: MS (FAB) (gly/thio) m/z (relative intensity) 640 (MH<sup>+</sup> + K<sup>+</sup>, 20), 602 (MH<sup>+</sup>, 80); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.4 (m, 5 H, aromatics), 7.1 (m, 4 H, aromatics). Anal. (C<sub>31</sub>H<sub>36</sub>F<sub>2</sub>N<sub>3</sub>O<sub>7</sub>-K) C, H, F, N, K.

## Inhibition of HLE by N-Substituted Peptides

Method J. Methyl  $N^{5}$ -[4(S)-[(tert-Butoxycarbonyl)amino]-2,2-difluoro-3(RS)-hydroxy-5-methylhexanoyl]-6amino-2(S)-(aminoacetyl)hexanoate (11; R = CH(CH<sub>3</sub>)<sub>2</sub>). A mixture of the ester 7c (R = CH(CH<sub>3</sub>)<sub>2</sub>) (500 mg, 1.54 mmol),  $N^{\alpha}$ -acetyl-L-lysine methyl ester (1.83 g, 7.68 mmol), and (*i*-Pr)<sub>2</sub>-NEt (990 mg, 7.65 mmol) in CH<sub>3</sub>OH (10 mL) was refluxed for 48 h. The reaction was concentrated in vacuo and to the residue was added EtOAc. The EtOAc was washed with brine (3×), dried (MgSO<sub>4</sub>), filtered, and concentrated to give the pure title amide 11 (R = CH(CH<sub>3</sub>)<sub>2</sub>) (660 mg, 89%) as a pale yellow oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.4 (m, 1 H, NH), 7.0 (m, 1 H, NH), 5.2 (m, 2 H, indanyl CH, NH), 4.7-4.2 (m, 3 H,  $\alpha$ -CH of lysine, CHCHOH, OH), 2.2 (m, 4 H,  $\alpha$ -CH of Val, NHCOCH<sub>3</sub>), 1.6-1.0 (m, 21 H, *t*-Bu, 3 × CH<sub>2</sub>, 2 × CH<sub>3</sub>). Anal. (C<sub>21</sub>H<sub>37</sub>F<sub>2</sub>N<sub>3</sub>O<sub>7</sub>) C, H, N.

Method K. Methyl N<sup>6</sup>-[4(S)-[[N-(Carbobenzyloxy)-Lvalyl-N-(2,3-dihydro-1H-inden-2-yl)glycyl]amino]-2,2-difluoro-3(RS)-hydroxy-5-methylhexanoyl]-6-amino-2(S)-(aminoacetyl)hexanoate (24a). To the t-BOC amine 11 (R =CH(CH<sub>3</sub>)<sub>2</sub>) (500 mg, 1.04 mmol) was added at room temperature a mixture (2 mL) of 30% TFA in CH<sub>2</sub>Cl<sub>2</sub>. The reaction was stirred at room temperature for 90 min. The reaction was concentrated in vacuo (<45 °C) and azeotroped (3×) with toluene to afford the trifluoroacetate amine salt 12 ( $R = CH(CH_3)_2$ ) as a pale yellow oil (590 mg), which was used directly without further purification. The TLC analysis indicated clean transformation to the amine 12. The coupling of N-CBZ-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycine (15b) (530 mg, 1.25 mmol) with the trifluoroacetate salt 12 (R =  $CH(CH_3)_2$ ) described above was done in a similar manner to that described in method G to give crude 24a (930 mg) as an oil. Flash chromatography over silica gel (2% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>) provided pure 24a (530 mg, 65%) as a colorless resinous solid: MS (CI/NH<sub>3</sub>) m/z (relative intensity) 788 (MH<sup>+</sup>, 100); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.5 (m, 1 H, NH), 8.2 (m, 1 H, NH), 7.5 (m, 1 H, NH), 7.2 (s, 5 H, aromatics), 7.15 (m, 4 H, aromatics), 6.0 (m, 1 H, NH), 5.2-5.0 (m, 3 H, PhCH<sub>2</sub>, indanyl CH), 4.5-4.1 (m, 4 H,  $\alpha$ -CH of Val,  $\alpha$ -CH of Lys, CH<sub>2</sub> of Gly), 3.8-3.6 (m, 4 H, CHOH, OCH<sub>3</sub>), 3.3 (m, 6 H, NHCOCH<sub>3</sub>, indanyl CH2, CHCHOH), 3.0 (m, 4 H, indanyl CH2, CONHCH2), 2.0 (m, 2 H,  $\beta$ -CH of Val), 1.7–1.2 (m, 6 H, 3 × CH<sub>2</sub>), 0.9 (m, 12 H, 4 × CH<sub>3</sub>). Anal. (C<sub>40</sub>H<sub>55</sub>F<sub>2</sub>N<sub>5</sub>O<sub>9</sub>·H<sub>2</sub>O) C, H, N.

Methyl N<sup>6</sup>-[4(S)-[[N-(Carbobenzyloxy)-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycyl]amino]-2,2-difluoro-3-oxo-5methylhexanoyl]-6-amino-2(S)-(aminoacetyl)hexanoate (24b). Following a procedure similar to that described in method H, the alcohol 24a (400 mg, 0.508 mmol) was oxidized to afford, after workup, the crude ketone 24b (390 mg). Preparative TLC purification over silica gel (4% CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub>) provided after double development pure 24b (274 mg, 69%) as a colorless resinous material: MS (CI/NH<sub>3</sub>) m/z (relative intensity) 786 (MH<sup>+</sup>, 100); <sup>1</sup>H NMR (CDCl<sub>3</sub>) § 9.1 (m, 1 H, NH), 8.2 (m, 2 H, 2 × NH), 7.5 (m, 1 H, NH), 7.35 (s, 5 H, aromatics), 7.15 (m, 4 H, aromatics), 5.15 (m, 1 H, indanyl CH), 5.03 (d, 2 H, PhCH<sub>2</sub>), 4.8 (m, 1 H, α-CH of Val), 4.47 (m, 1 H, NHCHOCF<sub>2</sub>), 4.1 (m, 2 H, CH<sub>2</sub> of Gly), 3.75 (m, 1 H, α-CH of Lys), 3.6 (s, 3 H, OCH<sub>3</sub>), 3.37 (s, 3 H, NHCOCH<sub>3</sub>), 3.1 (m, 6 H,  $2 \times CH_2$  of indanyl, CONHCH<sub>2</sub>), 2.23 (m, 1 H,  $\beta$ -CH of Val), 2.0 (m, 1 H,  $\beta$ -CH of Val),  $1.6-1.27 (m, 6 H, 3 \times CH_2), 0.9-0.8 (m, 12 H, 4 \times CH_3)$ . Anal.  $(C_{40}H_{53}F_2N_5O_9)$  C, H, F, N.

Method L. [4(S)-[(tert-Butoxycarbonyl)amino]-2,2-difluoro-3(RS)-hydroxy-5-methylhexanoyl]glycylglycine Ethyl Ester (9; R = CH(CH<sub>3</sub>)<sub>2</sub>). A diastereomeric mixture of the alcohol 7c (R = CH(CH<sub>3</sub>)<sub>2</sub>) (500 mg, 1.54 mmol) was dissolved in absolute EtOH (10 mL). To this solution was added glycylglycine ethyl ester (1.51 g, 7.68 mmol) and *i*-Pr<sub>2</sub>NEt (990 mg, 7.68 mmol). The resulting mixture was refluxed for 48 h. The reaction was concentrated in vacuo and the residue was treated with EtOAc. The EtOAc was washed with brine (3×), dried (MgSO<sub>4</sub>), filtered, and concentrated to afford the pure amide 9 (R = CH(CH<sub>3</sub>)<sub>2</sub>) (570 mg, 84%): <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.85 (m, 2 H, 2 × NH), 5.4 (m, 1 H, NH), 4.4-3.7 (m, 9 H,  $\alpha$ -CH of Val, CHOH, 2 × CH<sub>2</sub> of Gly, OCH<sub>2</sub>CH<sub>3</sub>, OH), 2.0 (m, 1 H, CH(CH<sub>3</sub>)<sub>2</sub>), 1.5 (s, 9 H, t-Bu), 1.35-1.0 (m, 9 H, CH<sub>2</sub>CH<sub>3</sub>, 2 × CH<sub>3</sub>). Anal. (C<sub>18</sub>H<sub>31</sub>F<sub>2</sub>N<sub>3</sub>O<sub>7</sub>) C, H, N.

[4(S)-[[N-(Carbobenzyloxy)-L-valyl-N-(2,3-dihydro-1Hinden-2-yl)glycyl]amino]-2,2-difluoro-3(RS)-hydroxy-5methylhexanoyl]glycylglycine Ethyl Ester (23a). The tBOC amine 9 (R = CH(CH<sub>3</sub>)<sub>2</sub>) (550 mg, 1.25 mmol) was added at room temperature to a mixture of 30% TFA in CH<sub>2</sub>Cl<sub>2</sub>. The reaction mixture was stirred at room temperature for 2 h. The reaction was concentrated in vacuo (<45 °C) and the residue was azeotroped with toluene  $(3\times)$  to afford the crude trifluoroacetate salt of the amine 10 ( $R = CH(CH_3)_2$ ) (640 mg) as a yellow oil. This material was used directly without further purification. The coupling of the trifluoroacetate salt of 10 ( $R = CH(CH_3)_2$ ) (640 mg, 1.5 mmol) with N-CBZ-L-valyl-N-(2.3-dihydro-1H-inden-2yl)glycine (15b) (850 mg, 2.0 mmol) was carried out according to method G to give crude 23a (1.29 g) as an oil. Purification by flash chromatography over silica gel (1, 2, and 3% EtOH in CH<sub>2</sub>-Cl<sub>2</sub>) provided pure 23a (450 mg, 48%) as a colorless resinous solid: MS (CI/C<sub>4</sub>H<sub>10</sub>) m/z (relative intensity) 746 (MH<sup>+</sup>, 30); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 8.7 (m, 1 H, NH), 8.38 (m, 1 H, NH), 7.5 (m, 1 H, NH), 7.35 (s, 5 H, aromatics), 7.15 (m, 4 H, aromatics), 6.1 (m, 1 H, NH), 5.15-5.0 (m, 3 H, PhCH<sub>2</sub>, indanyl CH), 4.45 (m, 1 H, α-CH of Val), 4.1 (m, 4 H, OCH<sub>2</sub>, CHOH, CHCHOH), 3.8-3.65 (m, 6 H, 3 × CH<sub>2</sub> of Gly), 3.1-3.0 (m, 4 H, 2 × CH<sub>2</sub> of indanyl), 2.0 (m, 1 H, β-CH of Val), 1.8 (m, 1 H, β-CH of Val), 1.2 (t, 3 H,  $CH_2CH_3$ , 0.85 (m, 12 H, 4 × CH<sub>3</sub>). Anal. ( $C_{37}H_{49}F_2N_5O_9 H_2O$ ) C, H, F, N.

[4(S)-[[N-(Carbobenzyloxy)-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycyl]amino]-2,2-difluoro-5-methyl-3oxohexanoyl]glycylglycine Ethyl Ester (23b). According to method H the alcohol 23a (300 mg, 0.402 mmol) was converted via Dess-Martin<sup>21</sup> oxidation to the difluoro ketone 23b. After workup, 23b (300 mg) was obtained as an oil. Preparative TLC over silica gel (4% EtOH in CH<sub>2</sub>Cl<sub>2</sub>) provided pure 23b (205 mg) as a colorless resin: MS (CI/C<sub>4</sub>H<sub>10</sub>) m/z (relative intensity) 744 (MH<sup>+</sup>, 100); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 9.3 (m, 1 H, NH), 8.45 (m, 1 H, NH), 8.15 (m, 1 H, NH), 7.5 (m, 1 H, NH), 7.35 (s, 5 H, aromatics), 7.15 (m, 4 H, aromatics), 5.18-5.03 (m, 3 H, PhCH<sub>2</sub>, indanyl CH), 4.8 (m, 1 H,  $\alpha$ -CH of Val), 4.45 (m, 1 H,  $\alpha$ -CH of Val), 4.1-3.8 (m, 8 H, CH<sub>2</sub>CH<sub>3</sub>, 3 × CH<sub>2</sub> of Gly), 3.0 (m, 4 H, 2  $\times$  CH<sub>2</sub> of indanyl), 2.25 (m, 1 H,  $\beta$ -CH of Val), 1.95 (m, 1 H,  $\beta$ -CH of Val), 1.18 (t, 3 H, CH<sub>2</sub>CH<sub>3</sub>), 0.9-0.77 (m, 12 H, 4 × CH<sub>3</sub>). Anal.  $(C_{37}H_{47}F_2N_5O_9\cdot H_2O)$  C, H, F, N.

(tert-Butoxycarbonyl)-L-phenylalanine Methoxymethylamide (5a; R = PhCH<sub>2</sub>).<sup>20</sup> By means similar to that described in method C the methoxymethylamide 5a was obtained (98%) as a colorless oily material: <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.3 (s, 5 H, Ph), 5.2-4.9 (m, 2 H,  $\alpha$ -CH of Phe, NH), 3.7 (s, 3 H, NOCH<sub>3</sub>), 3.2 (s, 3 H, NCH<sub>3</sub>), 3.0 (dd, 2 H,  $\beta$ -CH<sub>2</sub> of Phe), 1.5 (s, 9 H, t-Bu).

(tert-Butoxycarbonyl)-L-phenylalanal (6a;  $R = PhCH_2$ ).<sup>20</sup> By means similar to method D the aldehyde 6a was obtained as a colorless solid (28.44 g, 70%): mp 80–83 °C (lit.<sup>20</sup> mp 86 °C); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  9.6 (s, 1 H, CHO), 7.5 (s, 5 H, aromatic), 5.0 (b, 1 H, NH), 4.4 (m, 1 H,  $\alpha$ -CH), 3.1 (d, 2 H, PhCH<sub>2</sub>), 1.4 (s, 9 H, t-Bu).

Ethyl 4(S)-[(tert-Butoxycarbonyl)amino]-2,2-difluoro-3-(RS)-hydroxy-5-phenylpentanoate (7a; R = PhCH<sub>2</sub>). A suspension of freshly activated Zn (13.11 g, 0.2 mol) in freshly distilled dry THF was brought to reflux under argon. To this suspension was added BrCF<sub>2</sub>CO<sub>2</sub>Et (1.5 mL). After 1 min a mixture of t-BOC-L-phenylalanal (6a) (20.0 g, 0.08 mol) and  $BrCF_2CO_2Et$  (39.08 g, 0.19 mol) in THF (75 mL) was added at a rate such that a gentle reflux was maintained. After the addition was complete the reaction mixture was refluxed for an additional 30 min. The reaction mixture was cooled to room temperature and then KHSO<sub>4</sub> (27.37 g, 0.2 mol) in  $H_2O$  (150 mL) was added and the resulting mixture was stirred for 30 min. The reaction mixture was filtered through Celite and the filter cake was washed with EtOAc followed by brine (150 mL). The aqueous phase was extracted with EtOAc  $(2\times)$ . The combined EtOAc extract was dried over MgSO<sub>4</sub>, filtered, and concentrated to give a brown oil (34.7 g). The oil was filtered through silica gel and eluted with 2% EtOH in CH<sub>2</sub>Cl<sub>2</sub> to afford three fractions. Fractions 1 and 2 provided the desired pure product  $(7a; R = PhCH_2)$  as a colorless oil (13.53 g, 45%): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.2 (m, 6 H, 5 H, aromatics, NH), 4.88 (d, 1 H, CHOH), 4.3-4.0 (m, 4 H, CH<sub>2</sub>CH<sub>3</sub>, α-CH, OH), 3.0 (m, 2 H, PhCH<sub>2</sub>), 1.4 (s, 9 H, 3 × CH<sub>3</sub>), 1.3 (t, 3 H, CH<sub>2</sub>CH<sub>3</sub>). Anal.  $(C_{18}H_{25}F_2NO_5)$  C, H, F, N.

Ethyl 4(S)-Amino-2,2-difluoro-3(RS)-hydroxy-5-phenylpentanoate (8a;  $R = PhCH_2$ ). To a solution (15 mL) of 30% TFA in CH<sub>2</sub>Cl<sub>2</sub> was added 7a ( $R = PhCH_2$ ) (3.65 g, 9.78 mmol) and the resulting solution was stirred at 0 °C for 1 h. The reaction was concentrated (<55 °C) in vacuo to afford a syrupy residue (4.83 g) which was identified to be 8a (R = PhCH<sub>2</sub>). This material was used directly in the next reaction without further purification: <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  9.3 (bs, 3 H, NH<sub>3</sub>), 7.2 (bs, 5 H), 4.3–3.9 (m, 4 H, CH<sub>2</sub>CH<sub>3</sub>, CHOH,  $\alpha$ -CH), 3.14 (m, 3 H, PhCH<sub>2</sub>, OH), 1.3 (t, 3 H, CH<sub>3</sub>).

Ethyl4(S)-[[N-(Carbobenzyloxy)-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycyl]amino]-2,2-difluoro-3(RS)-hydroxy-5phenylpentanoate (16:  $\mathbf{R} = \mathbf{PhCH}_2$ ). To a solution of CBZ-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycine (15b) (4.98 g, 11.7 mol) in CH<sub>2</sub>Cl<sub>2</sub> (25 mL), cooled to -10 °C under argon, was added HOBT (1.5 g, 11.7 mol) in THF (10 mL) followed by the addition of WSCDI (2.25 g, 11.7 mol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL). After stirring for 30 min at -10 °C the trifluoroacetate salt 8a (R = PhCH<sub>2</sub>) (4.83 g, 9.78 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added. The reaction mixture was stirred at -10 °C for 15 min before Et<sub>3</sub>N (1.63 g, 11.7 mol) was added. The resulting mixture was stirred at 0 °C for 1 h followed by 2 h at room temperature. The reaction was washed consecutively with 10% aqueous HCl (2×), saturated aqueous NaHCO<sub>3</sub>  $(2\times)$ , and brine. The organic phase was dried (MgSO<sub>4</sub>), filtered, and concentrated to give a brown glassy residue (7.3 g). Flash chromatography over silica gel (3% EtOH in CH<sub>2</sub>Cl<sub>2</sub>) provided the product 16 ( $R = PhCH_2$ ) as a resin. Further purification of the resin (4.35 g) was achieved by flash chromatography over silica gel utilizing a gradient system of 10, 20, and 30% (CH<sub>3</sub>)<sub>2</sub>CO in petroleum ether. In this manner pure 16 (R = PhCH<sub>2</sub>) (2.9 g, 44%) was obtained from the 30% (CH<sub>3</sub>)<sub>2</sub>CO/ petroleum ether fraction as a colorless amorphous solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>) & 7.4-7.1 (m, 14 H, aromatics), 6.1 (b, 1 H, OH), 5.6 (d, 1 H, NH), 5.5 (m, 1 H, indanyl CH), 5.2-5.0 (m, 4 H, PhCH<sub>2</sub>, CHOH, NH), 4.7 (m, 1 H, α-CH), 4.2-3.8 (m, 4 H, α-CH<sub>2</sub> of Gly, CH2CH3), 3.4-2.9 (m, 6 H, indanyl CH2, PhCH2), 2.6 (m, 1 H, CHCHOH), 2.05 (m, 1 H), 1.1-0.9 (m, 9 H, CH<sub>2</sub>CH<sub>3</sub>, 2 × CH<sub>3</sub>). Anal.  $(C_{37}H_{43}F_2N_3O_7)$  C, H, N.

Method M. Ethyl 4(S)-[[L-Valyl-N-(2,3-dihydro-1H-inden-2-yl)glycyl]amino]-2,2-difluoro-3(RS)-hydroxy-5phenylpentanoate (20;  $R_2 = CH_2Ph$ ). To a solution of 16 (R =  $PhCH_2$ ) (2.90 g, 4.27 mmol) in absolute EtOH (40 mL) under argon was added 10% Pd/C (290 mg). The resulting mixture was hydrogenated in a Parr shaker at 45  $psi/H_2$  for 2 days. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> and then filtered through Celite. The filter cake was washed well with CH<sub>2</sub>Cl<sub>2</sub>/ EtOH (1:1). The filtrate was concentrated to afford a pale yellow gummy resin (2.23 g). Flash chromatography over silica gel (2.8%EtOH in CH<sub>2</sub>Cl<sub>2</sub>) provided the pure desired amino compound 20  $(R_2 = PhCH_2)$  (1.19 g, 47%) as a mixture of two diastereomers which was used directly without further separation: MS (FAB) m/z (relative intensity) 546.3 (MH<sup>+</sup>, 100), <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$ 7.3-7.1 (m, 9 H, aromatic), 6.5 (b, 1 H, NH, or OH), 5.5 (m, 1 H indanyl CH<sub>2</sub>), 5.1-4.6 (bm, 3 H, CHOH, NH, α-CH), 4.3 (m, 3 H, CH<sub>2</sub>CH<sub>3</sub>, NHCHCHOH), 4.1-3.8 (m, 3 H, CH<sub>2</sub> of Gly), 3.3-2.5 (m, 7 H, PhCH<sub>2</sub>, indanyl CH<sub>2</sub>, OH), 1.8 (m, 1 H, CHCH<sub>3</sub>), 1.4 (t, 3 H, CH<sub>2</sub>CH<sub>3</sub>), 1.0 (m, 6 H,  $2 \times$  CH<sub>3</sub>).

Method N. Ethyl4(S)-[[[[4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]phenyl]carbonyl]-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycyl]amino]-2,2-difluoro-3(RS)-hydroxy-5phenylpentanoate (21;  $R_2 = PhCH_2$ ). The acid<sup>10,23</sup> p-[p-Cl(C6H4)SO2NHCO](C6H4)COOH (790 mg, 2.2 mmol) was added to dry THF (10 mL) and the stirring mixture was cooled to 0 °C under argon. To this mixture was added HOBT (300 mg, 2.2 mmol) in THF (10 mL) followed by WSCDI (420 mg, 2.2 mmol) in  $CH_2Cl_2$  (10 mL). After 30 min the amine 20 ( $R_2 = PhCH_2$ ) (1.0 g, 1.83 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added while the temperature was maintained at 0 °C. The reaction mixture was stirred at 0 °C for 30 min followed by 2 h at room temperature. The reaction mixture was diluted with EtOAc. The organic phase was washed consecutively with saturated aqueous Na<sub>2</sub>CO<sub>3</sub> (2×), 2 N HCl  $(2\times)$ , and brine  $(2\times)$ . The EtOAc phase was dried (MgSO<sub>4</sub>), filtered, and concentrated to give a yellow resin (1.49 g, 94%)after flash chromatography over silica gel (0.2-10% EtOH in CH<sub>2</sub>Cl<sub>2</sub>). For analytical purposes a small sample (300 mg) was further purified by preparative TLC (0.5% HOAc and 3% EtOH in  $CH_2Cl_2$ ). In this manner an analytically pure sample of 21 ( $R_2$ = PhCH<sub>2</sub>) (164 mg) was obtained as a colorless resinous solid: MS (CI/NH<sub>3</sub>) m/z (relative intensity) 867 (MH<sup>+</sup>, 11); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  8.7 (m, 1 H, NH), 7.9 (bs, 6 H, aromatics), 7.6 (d, 2 H, aromatics), 7.1 (m, 9 H, aromatics), 6.5 (m, 1 H, NH), 5.3 (m, 1 H, indanyl CH), 4.9–4.5 (m, 2 H,  $\alpha$ -CH, NH), 4.4–3.3 (m, 5 H, CH<sub>2</sub> of Gly, CH<sub>2</sub>CH<sub>3</sub>, CHOH), 3.2–2.7 (m, 7 H, indanyl CH<sub>2</sub>, PhCH<sub>2</sub>, CHOH), 2.2 (m, 1 H, CH(CH<sub>3</sub>)<sub>2</sub>), 1.2–0.8 (m, 9 H, m, CH<sub>2</sub>CH<sub>3</sub>, 2 × CH<sub>3</sub>). Anal. (C<sub>43</sub>H<sub>45</sub>ClF<sub>2</sub>N<sub>4</sub>O<sub>9</sub>S) C, H, Cl, F, N, S.

Ethyl 4(S)-[[[[4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]phenyl]carbonyl]-L-valyl-N-(2,3-dihydro-1*H*-inden-2-yl)glycyl]amino]-2,2-difluoro-3-oxo-5-phenylpentanoate (22;  $R_2 = PhCH_2$ ). The title compound was prepared by method H. Preparative TLC purification over silica gel (0.5% HOAc, 3% EtOH in CH<sub>2</sub>Cl<sub>2</sub>) afforded pure 22 ( $R_2 = PhCH_2$ ) as a colorless resin in 60% yield: MS (CI/NH<sub>3</sub>) m/z (relative intensity) 865 (MH<sup>+</sup>, 98); <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  8.8–8.6 (m, 2 H, 2 × NH), 7.9 (bs, 6H, aromatics), 7.6 (d, 2 H, aromatics), 7.1 (m, 9 H, aromatics), 5.2 (m, 1 H, indanyl CH), 4.9–4.6 (m, 2 H,  $\alpha$ -CH, NH), 4.3 (m, 2 H, CH<sub>2</sub>CH<sub>3</sub>), 4.0–3.3 (m, 2 H, CH<sub>2</sub> of Gly), 3.3–2.7 (m, 5 H, PhCH<sub>2</sub>, 2 × CH<sub>2</sub> of indanyl,  $\alpha$ -CH of Phe), 2.2 (m, 1 H, CH-(CH<sub>3</sub>)<sub>2</sub>), 1.2 (t, 3 H, CH<sub>3</sub>), 0.9 (m, 6 H, CH(CH<sub>3</sub>)<sub>2</sub>). Anal. (C<sub>43</sub>H<sub>43</sub>-CIF<sub>2</sub>N<sub>4</sub>O<sub>9</sub>S·H<sub>2</sub>O) C, H, Cl, F, N, S.

Ethyl 4(S)-[[L-Valyl-N-(2,3-dihydro-1H-inden-2-yl)glycyl]amino]-2,2-difluoro-3(RS)-hydroxy-5-methylhexanoate (20;  $\mathbf{R}_2 = \mathbf{CH}(\mathbf{CH}_3)_2$ ). To a solution of 16 ( $\mathbf{R} = \mathbf{CH}$ -(CH<sub>3</sub>)<sub>2</sub>) (2.0 g, 3.17 mmol) in absolute EtOH (40 mL) was added 10% Pd/C (400 mg). The resulting mixture was hydrogenated (45 psi) for 16 h by means of a Parr shaker. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (40 mL) and filtered through Celite. The filtrate was concentrated in vacuo to give an oily residue (1.84 g). Flash chromatography over silica gel with first 3% and then 8% EtOH in  $CH_2Cl_2$  provided pure 20 ( $R_2 = CH(CH_3)_2$ ) (1.55 g) as a diastereomeric mixture which was used directly without further separation: <sup>1</sup>H NMR (CDCl<sub>3</sub>) & 7.2 (bs, 4 H, aromatic), 6.5 (b, 1 H, NH), 5.6 (m, 1 H, CH of indanyl), 4.3 (m, 4 H, OCH<sub>2</sub>CH<sub>3</sub>, CHOH, α-CH), 4.2-3.5 (m, 4 H, CH<sub>2</sub> of Gly, CHCHOH, OH), 3.2-2.8 (m, 4 H, 2 × CH<sub>2</sub> of indanyl), 1.8 (m, 2 H, 2 × CH(CH<sub>3</sub>)<sub>2</sub>), 1.3 (t, 3 H, CH<sub>2</sub>CH<sub>3</sub>), 0.9 (m, 12 H, 4 × CH<sub>3</sub>). Anal.  $(C_{25}H_{37}F_2N_3O_5)$  C, H, N.

Ethyl 4(S)-[[[[4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]phenyl]carbonyl]-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycyl]amino]-2,2-difluoro-3(RS)-hydroxy-5-methylhexanoate (21;  $R_2 = CH(CH_3)_2$ ). The acid<sup>10,23</sup> p-[p-Cl(C<sub>6</sub>-H<sub>4</sub>)SO<sub>2</sub>NHCO](C<sub>6</sub>H<sub>4</sub>)COOH (350 mg, 0.965 mmol) was dissolved in dry THF (5 mL) and the resulting solution was chilled to 0 °C and placed under a positive argon atmosphere. To this solution was added HOBT (130 mg, 0.965 mmol) in THF (5 mL) followed by WSCDI (180 mg, 0.965 mmol). After stirring at 0 °C for 30 min the amine 20  $(R_2 = CH(CH_3)_2)$  (400 mg, 0.804 mmol) in  $CH_2Cl_2$  (5 mL) was added. The resulting mixture was stirred at 0°C for 45 min followed by 1 h at room temperature. The reaction mixture was diluted with Et<sub>2</sub>O and then washed sequentially with saturated aqueous  $NaHCO_3$  (2×), 2 N HCl (2×), and brine. The organic phase was dried (MgSO<sub>4</sub>), filtered, and concentrated to give crude 21 ( $R_2 = CH(CH_3)_2$ ) (710 mg) as a white colorless resin. Preparative TLC over silica gel (0.5% HOAc and 5% EtOH in CH<sub>2</sub>Cl<sub>2</sub>) provided pure 21 ( $R_2 = CH(CH_3)_2$ ) (520 mg) as a colorless white powder: MS FAB (gly/thio) m/z (relative intensity) 819 (MH<sup>+</sup>, 80); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 10.5 (bs, 1 H, SO<sub>2</sub>NH), 8.1 (m, 2 H, aromatics), 7.8 (m, 4 H, aromatics), 7.5 (m, 2 H, aromatics), 7.3 (m, 1 H, NH), 7.2 (m, 4 H, aromatics), 6.8 (d, 1 H, NH), 5.2 (m, 1 H, CH of indanyl), 5.1 (m, 1 H, α-CH), 4.3-3.8 (m, 5 H, OCH<sub>2</sub>CH<sub>3</sub>, CH<sub>2</sub> of Gly, CHOH), 3.4-3.1 (m, 5 H, 2 × CH<sub>2</sub> of indanyl, CHCHOH), 2.3-1.8 (m, 3 H, 2 × CH(CH<sub>3</sub>)<sub>2</sub>, OH), 1.3 (t, 3 H, CH<sub>2</sub>CH<sub>3</sub>), 1.1–0.7 (m, 12 H,  $4 \times$  CH<sub>3</sub>). Anal. (C<sub>39</sub>H<sub>45</sub>-ClF<sub>2</sub>N<sub>4</sub>O<sub>9</sub>S) C, H, Cl, F, N, S

Method O. Ethyl4(S)-[[[4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]phenyl]carbonyl]-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycyl]amino]-2,2-difluoro-3-oxo-5-methylhexanoate (22;  $R_2 = CH(CH_3)_2$ ). To the alcohol 21 ( $R_2 = CH(CH_3)_2$ ) (1.14 g, 1.39 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (25 mL) was added Dess-Martin periodinane<sup>21</sup> (2.18 g, 5.15 mmol) followed by TFA (110  $\mu$ L). The reaction mixture was stirred at room temperature for 3 h before a saturated aqueous mixture of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> and NaHCO<sub>3</sub> was added. The resulting mixture was stirred for 30 min. The aqueous mixture was extracted several times with EtOAc (3×). The combined EtOAc extract was washed with 10% aqueous HCl and then with brine. The EtOAc was dried

## Inhibition of HLE by N-Substituted Peptides

(MgSO<sub>4</sub>), filtered, and concentrated to afford crude 22 (R<sub>2</sub> = CH(CH<sub>3</sub>)<sub>2</sub>) (1.2 g) as a yellow resin. Flash chromatography over silica gel (0.5% HOAc and 2% EtOH in CH<sub>2</sub>Cl<sub>2</sub>) gave pure 22 (R<sub>2</sub> = CH(CH<sub>3</sub>)<sub>2</sub>) (520 mg) as a colorless resin: MS FAB (gly/ thio) m/z (relative intensity) 817 (MH<sup>+</sup>, 80); <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  8.75 (d, 1 H, SO<sub>2</sub>NH), 8.6 (m, 1 H, NH), 8.2 (d, 1 H, NH), 7.9 (m, 6 H, aromatics), 7.6 (d, 2 H, aromatics), 7.15 (m, 4 H, aromatics), 5.3 (m, 1 H, indanyl CH), 4.9 (m, 1 H,  $\alpha$ -CH), 4.65 (m, 1 H,  $\alpha$ -CH), 4.3 (q, 2 H, OCH<sub>2</sub>CH<sub>3</sub>), 3.6 and 4.1 (2 dd, 2 H, CH<sub>2</sub>o f Gly), 3.2–3.0 (m, 4 H, 2 × CH<sub>2</sub> of indanyl), 2.2 (m, 2 H, 2 × CH(CH<sub>3</sub>)<sub>2</sub>), 1.2 (t, 3 H, CH<sub>2</sub>CH<sub>3</sub>), 1.0–0.8 (m, 12 H, 4 × CH<sub>3</sub>). Anal. (C<sub>39</sub>H<sub>43</sub>ClF<sub>2</sub>N<sub>4</sub>O<sub>9</sub>S) C, H, Cl, F, N, S.

(tert-Butoxycarbonyl)-L-leucine Methoxymethylamide (5b; R = CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>).<sup>20</sup> By a procedure similar to method C the methoxymethylamide 5b was obtained in 86% yield as a colorless oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  5.2-4.6 (m, 2 H, CHCO, NH), 4.74 (s, 3 H, N(OCH<sub>3</sub>), 3.24 (s, 3 H, NCH<sub>3</sub>), 1.6-1.4 (m, 3 H, CH<sub>2</sub> CH(CH<sub>3</sub>)<sub>2</sub>), 1.5 (s, 9 H, t-Bu), 1.05 (d, 3 H, CH<sub>3</sub>), 0.9 (d, 3 H, CH<sub>3</sub>).

(tert-Butoxycarbonyl)-L-leucinal (6b;  $\mathbf{R} = \mathbf{CH}_2\mathbf{CH}(\mathbf{CH}_3)_2$ ).<sup>20</sup> By a procedure similar to method D the aldehyde 6b was obtained in 75% yield as a colorless viscous oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  9.64 (s, 1 H, CHO), 5.0 (m, 1 H, NH), 4.25 (m, 1 H, CH), 2.0–1.35 (m, 3 H, CH<sub>2</sub>, CH(CH<sub>3</sub>)<sub>2</sub>), 1.45 (s, 9 H, t-Bu), 1.0 (d, 6 H, 2 × CH<sub>3</sub>).

Ethyl4(S)-[(tert-Butyloxycarbonyl)amino]-2,2-difluoro-3(RS)-hydroxy-6-methylheptanoate (7b; R = CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>). A stirred suspension of freshly activated Zn (17.08 g, 0.26 mol) in dry THF (150 mL) was placed under nitrogen and then bromodifluoroacetate (1.5 g) was added. The mixture was heated to a gentle reflux. After 1 min a mixture of t-BOC-L-leucinal (6b) (22.5 g, 0.1 mol) and ethyl bromodifluoroacetate (49.41 g, 0.25 mol) in THF (100 mL) was added dropwise while a gentle reflux was maintained. At the end of the addition the reaction was further refluxed for 30 min and then cooled to room temperature. To the reaction mixture was added 1 M aqueous KHSO<sub>4</sub> (150 mL). The resulting mixture was stirred for 15 min and then filtered through Celite. The filter cake was washed with EtOAc. The organic filtrate was separated and the aqueous phase was extracted with EtOAc  $(3\times)$ . The combined organic extract was washed with brine (150 mL), dried (MgSO<sub>4</sub>), filtered, and concentrated to give crude 7b ( $R = CH_2CH(CH_3)_2$ ) (48.14 g). Flash chromatography over silica gel  $(2\% \text{ EtOH in CH}_2\text{Cl}_2)$ provided pure 7b ( $\mathbf{R} = CH_2CH(CH_3)_2$ ) (21.16 g) as an oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 4.7 (bs, 1 H, NH), 4.4 (m, 1 H, CHOH), 4.35 (q, 2 H, OCH<sub>2</sub>CH<sub>3</sub>), 4.1-3.85 (m, 2 H, CHCHOH, OH), 1.7 (m, 3 H,  $\beta$ -CH, CH(CH<sub>3</sub>)<sub>2</sub>), 1.4 (m, 9 H, t-Bu), 1.35 (t, 3 H, CH<sub>2</sub>CH<sub>3</sub>), 0.9 (m, 6 H, 2 × CH<sub>3</sub>). Anal. ( $C_{15}H_{27}F_2NO_5$ ) C, H, N.

Ethyl 4(S)-Amino-2,2-difluoro-3(RS)-hydroxy-6methylheptanoate Trifluoroacetate Salt (8b;  $\mathbf{R} = CH_2CH_{-}(CH_3)_2$ ). The *t*-BOC amine 7b ( $\mathbf{R} = CH_2CH(CH_3)_2$ ) (5.0 g, 14.7 mmol) was added to a mixture (20 mL) of 30% TFA in  $CH_2Cl_2$ and the resulting solution was stirred at room temperature for 1 h. The reaction was concentrated in vacuo (<45 °C) and then azeotroped (3×) with toluene to afford a tan oil. The residue was dried for 16 h under vacuum to afford the trifluoroacetate salt 8b ( $\mathbf{R} = CH_2CH(CH_3)_2$ ) (5.88 g) which was used directly without further purification.

Ethyl4(S)-[[N-(Carbobenzyloxy)-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycyl]amino]-2,2-difluoro-3(RS)-hydroxy-6methylheptanoate (16;  $\mathbf{R} = \mathbf{CH}_2\mathbf{CH}(\mathbf{CH}_3)_2$ ). By means similar to method G, the trifluoroacetate salt 8b ( $R = CH_2CH(CH_3)_2$ ) (4.89 g, 13.1 mmol) was condensed with N-CBZ-L-valyl-N-(2,3dihydro-1H-inden-2-yl)glycine (15b) (5.6 g, 13.1 mmol). Purification of the crude product was accomplished by flash chromatography over silica gel using a gradient system of 10, 15, 20, and 40 % (CH<sub>3</sub>)<sub>2</sub>CO in petroleum ether. Most of the desired product 16 (R = CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>) was collected in the 20% (CH<sub>3</sub>)<sub>2</sub>-CO fraction. Fraction 2 afforded a pure diastereomer (3.0 g) as a colorless oil. Fraction 3 was a mixture of diastereomers. The physical data for fraction 2 is as follows: MS FAB (gly/thio) m/z(relative intensity) 646 (MH<sup>+</sup>, 30); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.4 (s, 5 H, aromatics), 7.27 (m, 4 H, aromatics), 5.6 (m, 1 H, NH), 5.2 (m, 3 H, PhCH<sub>2</sub>, indanyl CH), 4.9 (m, 1 H, α-CH), 4.7-3.9 (m, 6 H, CHOH, OCH<sub>2</sub>CH<sub>3</sub>, CH<sub>2</sub> of Gly, CHCHOH), 3.3-3.0 (m, 4 H, CH<sub>2</sub> of indanyl), 2.1 (m, 1 H,  $\beta$ -CH of Val), 1.7 (m, 3 H,  $\beta$ -CH<sub>2</sub> of Leu, δ-CH of Leu), 1.4 (t, 3 H, CH<sub>2</sub>CH<sub>3</sub>), 1.0 (m, 12 H, 4 × CH<sub>3</sub>). Anal.  $(C_{34}H_{45}F_2N_3O_7)$  C, H, F, N.

Ethyl 4(S)-[[L-Valyl-N-(2,3-dihydro-1H-inden-2-yl)glycyl]amino]-2,2-difluoro-3(RS)-hydroxy-6-methylheptanoate (20;  $R_2 = CH_2CH(CH_3)_2$ ). To a solution of the N-CBZ peptide 16 (R =  $CH_2CH(CH_3)_2$ ) (2.33 g, 3.61 mmol) in absolute EtOH (40 mL) was added 10% Pd/C (250 mg). The resulting mixture was hydrogenated (45 psi) by means of a Parr apparatus for 16 h. The reaction was filtered through Celite and the filtrate was concentrated in vacuo to give crude amine 20 ( $R_2 = CH_2$ - $CH(CH_3)_2$ ) (1.89 g) as an oil. Purification of the crude product by flash chromatography over silica gel eluting first with 20%  $(CH_3)_2CO$  in petroleum ether followed by 3% EtOH in  $CH_2Cl_2$ gave the pure title compound 20 ( $R_2 = CH_2CH(CH_3)_2$ ) (970 mg) as a colorless oil: MS FAB (gly/thio) m/z (relative intensity) 512 (MH<sup>+</sup>, 50); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.2 (m, 4 H, aromatics), 6.1 (m, 1 H, NH), 5.65 (m, 1 H, indanyl CH), 4.6 (m, 1 H,  $\alpha$ -CH), 4.35 (m, 2 H, CH<sub>2</sub>CH<sub>3</sub>), 4.2 (d, 1 H, CHOH), 3.9-3.7 (m, 3 H, CH<sub>2</sub> of Gly, CHCHOH), 3.3-2.8 (m, 5 H,  $2 \times CH_2$  of indanyl, OH), 1.9 (m, 1 H,  $\beta$ -CH of Val), 1.6 (m, 2 H,  $\beta$ -CH of Leu), 1.4 (t, 3 H,  $CH_2CH_3$ , 1.3 (m, 1 H,  $\delta$ -CH of Leu), 1.1–0.90 (m, 12 H, 4 × CH<sub>3</sub>). Anal.  $(C_{26}H_{39}F_2N_3O_5)$  C, H, N.

Ethyl 4(S)-[[[[4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]phenyl]carbonyl]-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycyl]amino]-2,2-difluoro-3(RS)-hydroxy-6-methylheptanoate (21;  $\mathbf{R}_2 = \mathbf{CH}_2\mathbf{CH}(\mathbf{CH}_3)_2$ ). To a chilled (0 °C) solution of the acid<sup>10,23</sup> p-[p-Cl(C<sub>6</sub>H<sub>4</sub>)SO<sub>2</sub>NHCO](C<sub>6</sub>H<sub>4</sub>)COOH (800 mg, 2.23 mmol) in THF (10 mL) was added HOBT (300 mg, 2.23 mmol) in THF (100 mL). To this solution was added WSCDI (430 mg, 2.23 mmol) in  $CH_2Cl_2$  (10 mL). The resulting mixture was stirred at 0 °C for 30 min and then a solution of the amine 20 ( $R_2 = CH_2CH(CH_3)_2$ ) (950 mg, 1.86 mmol) in  $CH_2Cl_2$  (10 mL) was added. The resulting mixture was stirred at 0 °C for 30 min and then at room temperature for 2 h. The reaction mixture was diluted with  $Et_2O$  and washed sequentially with 2 N HCl (2×), saturated aqueous  $Na_2CO_3$  (2×), and brine. The Et<sub>2</sub>O phase was dried (MgSO<sub>4</sub>), filtered, and concentrated to give crude 21 (R<sub>2</sub> =  $CH_2CH(CH_3)_2$  (1.73) as a colorless amorphous solid. Flash chromatography over silica gel (3% EtOH in CH<sub>2</sub>Cl<sub>2</sub>) provided pure 21 ( $R_2 = CH_2CH(CH_3)_2$ ) (1.41 g, 91%) as a colorless amorphous solid: MS FAB (gly/thio) m/z (relative intensity) 855 (MH<sup>+</sup>, 40); <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$  8.7 (m, 1 H, NH), 7.95 (m, 6 H, aromatics), 7.75 (m, 1 H, NH), 7.6 (m, 2 H, aromatics), 7.15 (m, 4 H, aromatics), 6.17 (m, 1 H, NH), 5.3 (m, 1 H, indanyl CH), 4.88 (m, 1 H, α-CH of Val), 4.27 (m, 3 H, OCH<sub>2</sub>CH<sub>3</sub>, CHOH), 4.0 (m, 2 H, CH<sub>2</sub> of Gly), 3.6-2.9 (m, 6 H,  $2 \times CH_2$  of indanyl, CHCHOH, OH), 2.1 (m, 1 H, β-CH of Val), 1.5 (m, 1 H, δ-CH of Leu), 1.25 (m, 5 H, CH<sub>2</sub>CH<sub>3</sub>, β-CH<sub>2</sub> of Leu), 1.0-0.8 (m, 12 H, 4  $\times$  CH<sub>3</sub>). Anal. (C<sub>40</sub>H<sub>46</sub>ClF<sub>2</sub>N<sub>4</sub>O<sub>9</sub>S·Na) C, H, Cl, F, N, Na.

Ethyl 4(S)-[[[[4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]phenyl]carbonyl]-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycyl]amino]-2,2-difluoro-3-oxo-6-methylheptanoate (22;  $\mathbf{R}_2 = \mathbf{CH}_2\mathbf{CH}(\mathbf{CH}_3)_2$ ). According to method O the alcohol 21 ( $R_2 = CH_2CH(CH_3)_2$ ) (1.21 g, 1.45 mmol) was converted, via a Dess-Martin periodinane oxidation,<sup>21</sup> to the ketone 22 ( $\dot{R}_2$  = CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>). The crude product 22 (1.16 g) was further purified by flash chromatography over silica gel (5% EtOH in  $CH_2Cl_2$ ) to afford the pure title compound 22 (700 mg, 58%) as a colorless resin: MS FAB (gly/thio) m/z (relative intensity) 869 aromatics), 7.2 (m, 6 H, aromatics, NH), 5.3-5.1 (m, 2 H, CH<sub>2</sub> of indanyl, a-CH), 4.4 (q, 3 H, OCH<sub>2</sub>CH<sub>3</sub>, a-CH of Leu), 4.2-4.0 (dd, 2 H, CH<sub>2</sub> of Gly), 3.5 (m, 2 H, CH<sub>2</sub> of indanyl), 3.1 (m, 2 H, CH<sub>2</sub> of indanyl), 2.2 (m, 1 H,  $\beta$ -CH of Val), 1.8 (m, 1 H,  $\delta$ -CH of Leu), 1.45 (m, 2 H, β-CH<sub>2</sub> of Leu), 1.35 (t, 3 H, CH<sub>2</sub>CH<sub>3</sub>), 1.15-0.75 (m, 12 H,  $4 \times CH_3$ ). Anal. (C<sub>40</sub>H<sub>44</sub>ClF<sub>2</sub>N<sub>4</sub>O<sub>9</sub>S·K) C, H, Cl, F, N, S

Ethyl 4(S)-[[(N-Carbobenzyloxy)-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycyl]amino]-2,2-difluoro-3-oxo-6-methylhexanoate (17;  $\mathbf{R} = CH_2CH(CH_3)_2$ ). By means similar to that described in method H the alcohol 16 ( $\mathbf{R} = CH_2CH(CH_3)_2$ ) (800 mg, 1.24 mmol) was oxidized to the corresponding ketone 17. The crude ketone 17 (810 mg) was obtained as an oil. The crude product was purified by flash chromatography over silica gel employing first the solvent system  $CH_2Cl_2$ /petroleum ether (1:1) followed by the system 2% EtOH in  $CH_2Cl_2$  to give the pure title compound 17 ( $\mathbf{R} = CH_2CH(CH_3)_2$ ) (300 mg, 38%) as a colorless oil: MS FAB (gly/thio) m/z (relative intensity) 644 (MH<sup>+</sup>, 30); <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.30 (s, 5 H, aromatics), 7.17 (s, 4 H, aromatics), 6.87 (d, 1 H, NH), 5.5 (d, 1 H, NH), 5.3–5.0 (m, 5 H, PhCH<sub>2</sub>, OCH<sub>2</sub>CH<sub>3</sub>,  $\alpha$ -CH of Val), 4.65 (m, 1 H,  $\alpha$ -CH of Leu), 4.3 (m, 3 H, OCH<sub>2</sub>CH<sub>3</sub>, indanyl CH), 3.9 (m, 2 H, CH<sub>2</sub> of Gly), 3.3–3.0 (m, 4 H, 2 × CH<sub>2</sub> of indanyl), 2.05 (m, 1 H,  $\beta$ -CH of Val), 1.7 (m, 2 H,  $\beta$ -CH<sub>2</sub> of Leu), 1.4 (m, 1 H,  $\delta$ -CH of Leu), 1.3 (t, 3 H, CH<sub>2</sub>CH<sub>3</sub>), 0.9 (m, 12 H, 4 × CH<sub>3</sub>). Anal. (C<sub>34</sub>H<sub>43</sub>F<sub>2</sub>N<sub>3</sub>O<sub>7</sub>) C, H, F, N.

Human Leukocyte Elastase (HLE) Inhibition Screen in Vitro. The method of Nakajima was adapted to a microtiter format.<sup>25</sup> The in vitro assay was based upon the hydrolysis of the commercially available (Sigma Chemical Co., St. Louis, MO) substrate MeO-Suc-Ala-Ala-Pro-Val p-nitroanilide and the resulting release of p-nitroanilide (pNA) which absorbs at 405 nm. The release of pNA was followed spectrophotometrically. The equipment used in the assay were microtiter flat bottom plates having 96 wells/plate, a  $V_{max}$  kinetic microtiter plate reader equipped with a 405-nm filter (Molecular Devices), a microtiter plate mixer (Fisher Scientific), and a Cary 118 spectrophotometer. Human sputum elastase (HSE) (Elastin Products Co., Owensville, MO) was dissolved at 1 mg/mL in 0.05 M NaCl and frozen (50- $\mu$ L aliquots) at -20 °C until used. A stock solution of MeO-Suc-Ala-Ala-Pro-Val p-nitroanilide was prepared by dissolving at 15 mM in DMSO and frozen (4-mL aliquots) at -20 °C until used. The assay buffer was 0.1 M Tris buffer, pH 7.5, containing 0.5 M NaCl. Screening was performed in microtiter plates using 0.5 mM of substrate. Enzyme activity (+/- test compound) was determined as the rate of pNA release (linear regression analysis of the slope). The inhibitory activity of the test compound was calculated relative to the uninhibited enzyme control as follows:

% inhibition = 
$$100 - \frac{\text{rate (with test compound)} \times 100}{\text{rate (enzyme control)}}$$

A frozen aliquot of HSE was thawed and diluted with the assay buffer to a stock concentration of 0.02 mg/mL (30× the assay concentration). A frozen aliquot of the substrate stock solution was thawed and diluted to 0.5 mM with the assay buffer (the final concentration of DMSO was 10%). A 10- $\mu$ L sample of the test compound stock solution (or the assay buffer) and 10  $\mu$ L of the HSE stock solution were pipetted into each microtiter well in duplicate. The plates were well mixed and preincubated at room temperature for 15 min. A substrate solution (300  $\mu$ L) was then added to each well and the OD<sub>405</sub> was followed for approximately 30 min.

Elastase-Induced Pulmonary Hemorrhage (EPH) Model in Hamsters. Experiments were conducted using four male, Syrian golden hamsters per group weighing 90-130 g, obtained from Charles River Laboratories. The animals were quarantined for a minimum of 3 days before use during which time they were maintained under routine animal care procedures. Anesthesia required for the intratracheal administration of elastase was induced by ip injection of Nembutal (sodium pentobarbital, 50-100 mg/kg of body weight). Hamsters were anesthetized as described above and the trachea was surgically exposed. Test compounds were solubilized in DMSO at 20 mg/mL and diluted 1:100 in normal saline for a working concentration of  $200 \,\mu g/mL$ . The compound or vehicle (DMSO, 1:100 in saline) were administered intratracheally (it.) via a 27-gauge needle, inserted directly into the trachea in 0.1 mL volume (20  $\mu$ g of compound). Five minutes later, purified human sputum elastase (Elastin Products Co., Owensville, MO), 50  $\mu$ g in 0.1 mL saline, was administered directly into the trachea. Eighteen hours later animals were sacrificed by an overdose of Nembutal. Whole lung lavage fluid was performed using normal saline at room temperature. A 6-mL sample of lavage fluid was collected from each animal and each sample was assayed for red blood cell (RBC) concentration (manual counts) as a measure of pulmonary hemorrhage. Each 6-mL lavage sample was centrifuged at approximately 1400 rpm for 10 min. The supernatant was discarded and the cell pellet was gently resuspended in 1 mL of normal saline. A  $25-\mu$ L aliquot of the cell suspension was added to  $475 \,\mu\text{L}$  of Trypan Blue (1:20

dilution) and manual RBC counts were performed. The number of RBCs/mL of cell suspension was calculated. The percent inhibition of pulmonary hemorrhage was calculated as follows:

% inhibition =  $100 \times [no. RBC/(mL vehicle control)] -$ 

[no. RBC/(mL compound tested)]/[no. RBC/

(mL vehicle control)]

Statistical analysis<sup>26</sup> of the data was performed using analysis of variance (ANOVA) and Dunnett's multiple comparison test.

Conformational Analysis and Molecular Modeling. Initial optimized geometries of inhibitors were accomplished using the force field program MM2/MMP2 (Molecular Design Ltd. Hayward, CA) of Allinger<sup>27</sup> employing a VAX 11/750 (Boston, MA) computer. In order to model the enzyme-inhibitor interactions, we made use of two experimental X-ray structures corresponding to a complex between HLE and the third domain of the turkey ovomucoid inhibitor (OMTKY3) as well as that of HLE with the inhibitor MeO-Suc-Ala-Ala-Pro-Val-chloromethyl ketone (MPC-MK). The X-ray coordinates of both complexes were kindly provided to us by Dr. W. Bode (Max Planck Institute, Martinsried, Germany). The backbone atoms of the previously minimized (MM2/MMP2) structures of the inhibitors were superimposed onto the corresponding atoms of the inhibitors in either X-ray structure. Presumably, the mechanism of inhibition of the enzyme by such inhibitors containing trifluoromethyl ketone moieties lies in the formation of a stable hemiketal between Ser<sup>195</sup> and the ketone carbonyl of the inhibitor. To simulate this situation in the modeling process, the  $O^{\gamma}$  atom of  $Ser^{195}$  and the C atom of the ketone group of the inhibitor were constrained (1.78 Å) to their corresponding positions as observed in the X-ray structure (MPCMK) through the use of a forcing potential. Energy refinement was performed on the "active-site region", which was defined as the region consisting of all residues with any atom within a 15-Å sphere centered on the N atom of the N-substituted glycine residue. In order to avoid large atomic movements resulting in unrealistic distortion or deviation from the initial structure, the energy refinement process was performed in two stages. First, a harmonic forcing potential was applied to every atom in the energy minimization procedure so as to relax bad steric contact and to constrain the structure close to its initial conformation. Second, the forcing potential was slowly relaxed in stages to bring the system to a stable conformation, with a convergence criterion of the maximum derivative being set at 0.01. Typically, a total of about 4000-5000 iteration steps are required for such convergence to occur. Minimizations of the HLE-inhibitor complexes was done using the program CHARMm (Polygen Corp., Waltham, MA) and utilizing a Silicon Graphics 4D/50 (Mountain View, CA) computer. Molecular graphics interpretation of the results was done via QUANTA (Polygen Corp.).

Acknowledgment. We are indebted to S. Campbell, S. Leonard, and T. Saboe for providing <sup>1</sup>H NMR of all new compounds, G. Hanson and K. McKellop for providing mass spectral data, and Professor W. Bode of the Max Planck Institute (Martinsried, Germany) for providing the X-ray coordinates of the complexes of HLE with MPCMK and with OMTKY3.

<sup>(25)</sup> Nakajima, K.; Powers, J. C.; Ashe, B. M.; Zimmerman, M. Mapping the Extended Substrate Binding Site of Cathepsin G and Human Leukocyte Elastase. J. Biol. Chem. 1979, 254, 4027-4032.

<sup>(26) (</sup>a) Blaker, W. D. Computer Program for the Parametric and Nonparametric Comparison of Several Groups to a Control. Comput. Biol. Med. 1987, 17, 37-44. (b) Kramer, C. Y. Extension of Multiple Range Tests to Group Means with Unequal Numbers of Replications. Biometrics 1956, 12, 307. (c) SAS Institute Inc., SAS User's Guide, Version 5 Edition; SAS Institute Inc.; Cary, NC, 1985; pp 434-506.

<sup>Range Tests to Group Interns with Chedual runners of replactons.
Biometrics 1956, 12, 307. (c) SAS Institute Inc., SAS User's Guide,
Version 5 Edition; SAS Institute Inc.; Cary, NC, 1985; pp 434-506.
(27) (a) Burkert, U.; Allinger, N. L. Molecular Mechanics; ACS
monograph; American Chemical Society: Washington, DC, 1982. (b)
Allinger, N. L. Conformational Analysis 130. MM2. A Hydrocarbon
Force Field Utilizing V<sub>1</sub> and V<sub>2</sub> Torsional Terms. J. Am. Chem. Soc.
1977, 99, 8127-8134.</sup>