Inhibition of Human Leukocyte Elastase (HLE) by N-Substituted Peptidyl Trifluoromethyl Ketones[1]

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A series of tripeptides possessing trifluoromethyl or aryl ketone residues at P_1 were prepared and evaluated both in vitro and in vivo as potential inhibitors of human leukocyte elastase (HLE). Tripeptides containing non naturally occurring N-substituted glycine residues at the P_2-position have been demonstrated to be potent in vitro inhibitors of HLE, with IC_{50} values in the submicromolar range. Sterically demanding substituents on the P_2-nitrogen have no detrimental effect on in vitro potency. The inhibition process presumably acts via hemiketal formation with the active site Ser^{195} of HLE, and is facilitated by the strongly electron withdrawing trifluoromethyl functionality. Deletion of the amino acid at the P_3-subsite region affords inactive compounds. Valine is the preferred residue at the P_1-position, whereas the corresponding glycine, alanine, α,α-dimethylglycine, or phenylalanine analogues are all inactive. The compounds described herein all confer a high degree of in vitro specificity when tested against representative cysteine, aspartyl, metallo, and other serine proteases. One of the most potent in vitro inhibitors is (3*RS*)-*N*-[4-[[[(4-chlorophenyl)sulfonyl]amino]carbonyl]phenyl]oxomethyl]-L-valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-trifluoro-4-methyl-2-oxopentyl)]amide (**20i**; BI-RA-260) (IC_{50} = 0.084 μM). Compound **20i** was also tested in hamsters in an elastase-induced pulmonary hemorrhage (EPH) model. In this model, intratracheal (it.) administration of **20i**, 5 min prior to HLE challenge, effectively inhibited hemorrhage in a dose-dependent manner with an ED_{50} of 4.8 μg. The inhibitor **20i**, 20 μg administered it. 24, 48, and 72 h prior to HLE challenge, exhibits significant inhibition against hemorrhage at all time points (97%, 64% and 49%, respectively). In a 21-day chronic model of emphysema in hamsters, 200 μg of HLE administered it. caused an elastase-induced emphysema in the lungs which can be quantitated histologically utilizing image analysis. In this assay, **20i** significantly inhibited pulmonary lesions associated with septal destruction and increased alveolar spaces, when dosed at 20 μg it. 5 min prior to challenge with HLE.

The fibrous protein elastin, which comprises an appreciable percentage of all protein content in some tissues, such as the arteries, some ligaments, and the lungs, can be hydrolyzed or otherwise destroyed by a select group of enzymes classified as elastases. Elastases are derived from many tissues in man, including the pancreas, neutrophils, macrophages, monocytes, platelets, smooth muscle cells, and fibroblasts. 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 human polymorphonuclear leukocytes (PMN). The complete amino acid sequence of HLE has been determined.[2] 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,[3] rheumatoid arthritis,[4] adult respiratory distress syndrome (ARDS),[5] glomerulonephritis,[6] and cystic fibrosis.[7,8]

Increased proteolysis, especially elastolysis, may occur in the lung parenchyma as a result of an imbalance between HLE and its major endogenous inhibitor α_1-proteinase inhibitor (α_1-PI), because of either an acquired or an inherited deficiency of the protease inhibitor. Cigarette smoke, which has been shown to inactivate α_1-PI in vitro (through oxidation of Met^{358}),[9,10] is believed to cause a localized, functional deficiency of the protease inhibitor in the lungs of smokers. This, in turn, is thought to be a primary factor in the pathogenesis of centrilobular emphysema associated with cigarette smoking. As replacements to α_1-PI, synthetic, low molecular weight HLE inhibitors that can be delivered to the site of unregulated

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(2) 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.
(3) Janoff, A. Elastase and Emphysema. Current Assessment of the Protease-Antiprotease Hypothesis. *Am. Rev. Respir. Dis.* **1985**, *132*, 417-433.
(4) Ekerot, L.; Ohlsson, K. Interactions of Granulocyte Proteases with Inhibitors in Rheumatoid-Arthritis. *Adv. Exp. Med. Biol.* **1984**, *167*, 335-344.
(5) Merritt, T. A.; Cochrane, C. G.; Holcomb, K.; Bohl, B.; Hallman, M.; Strayer, D.; Edwards, D.; Gluck, L. Elastase and α_1PI Proteinase Inhibitor Activity in Tracheal Aspirates During Respiratory Distress Syndrome. *J. Clin. Invest.* **1983**, *72*, 656-666.
(6) 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*, 355-359.
(7) 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.
(8) 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.
(9) Johnson, D.; Travis, J. Structural Evidence for Methionine at the Reactive Site of Human α-1-Proteinase Inhibitor. *J. Biol. Chem.* **1978**, *253*, 7142-7144.
(10) Beatty, K.; Matteson, N.; Travis, J. Kinetic and Chemical Evidence for the Inability of Oxidized Alpha 1-Proteinase Inhibitor to Protect Lung Elastin from Elastolytic Degradation. *Hoppe Segler's Z. Physiol. Chem.* **1984**, *365*, 731-736.

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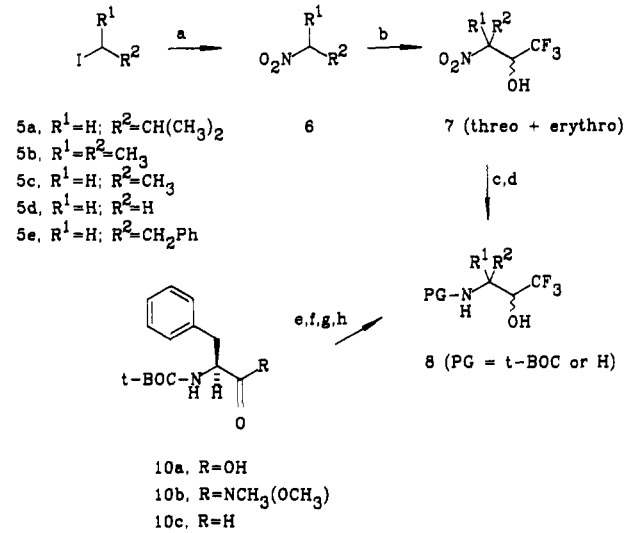
PMN elastase activity can be potentially useful in the treatment of pulmonary emphysema and related diseases.

In recent years much attention has focused on the inhibition of elastase[11] as a means of controlling emphysema. The inhibitors reported include, among others, peptide chloromethyl ketones,[12,13] azapeptides,[14-17] peptidyl carbamates,[18-20] peptidyl boronic acids,[21,22] peptidyl aldehydes,[23,24] peptidyl α-keto esters,[25] peptidyl α,α-difluoro-β-keto amides,[26] latent isocyanates,[27] sulfonate salts,[28] chloroisocoumarins,[29,30] ynenol lactones,[31] benzoxazinones,[32] 2-pyrones,[33] hydantoins,[34] and cephalosporins.[35-37] Some

Scheme I. Synthesis of 3-Substituted-3-amino-1,1,1-trifluoro 2-Alcohols (8)[a]



[a] Reagents: (a) $AgNO_2$; (b) $CF_3CH(OC_2H_5)OH$, K_2CO_3; (c) separation of isomers; (d) $LiAlH_4$ to give **8** (PG = H); (e) **10a**, $HNCH_3(OCH_3)$, CDI to give **10b**; (f) **10b**, $LiAlH_4$ to give **10c**; (g) **10c**, $CF_3Si(CH_3)_3$ (**9**) to give **8** (PG = *t*-BOC); (h) **8** (PG = *t*-BOC), HCl, *p*-dioxane to give **8** (PG = H).

(11) For recent reviews, see: (a) Trainor, D. A. Synthetic Inhibitors of Human Neutrophil Elastase. *Trends Pharmacol. Sci.* **1987**, *8*, 303–307. (b) Groutas, W. C. Inhibitors of Leukocyte Elastase and Leukocyte Cathepsin G. Agents for the Treatment of Emphysema and Related Ailments. *Med. Res. Rev.* **1987**, *7*, 227–241. (c) Powers, J. C. Serine Protease of Leukocyte and Mast Cell Origin: Substrate Specificity and Inhibition of Elastase, Chymases, and Tryptases. *Adv. Inflamm. Res.* **1986**, *11*, 145–157. (d) Stein, R. L.; Trainor, D. A.; Wildonger, R. A. Neutrophil Elastase. *Annu. Rep. Med. Chem.* **1985**, *20*, 237–246. (e) Abeles, R. H. Enzyme Inhibitors: Ground-State/Transition-State Analogs. *Drug. Dev. Res.* **1987**, *10*, 221–234.

(12) Powers, J. C.; Gupton, B. F.; Harley, A. D.; Nislino, N.; Whitley, R. J. Specificity of Porcine Elastase, Human Leukocyte Elastase and Cathepsin G. Inhibition with Peptide Chloromethyl Ketones. *Biochem. Biophys. Acta* **1977**, *485*, 156–166.

(13) McRae, B.; Nakajima, K.; Travis, J.; Powers, J. C. Studies on Reactivity of Human Leukocyte Elastase, Cathepsin G, and Porcine Pancreatic Elastase toward Peptides Including Sequences Related to the Reactive Site of α_1-Protease Inhibitor (α_1-Antitrypsin). *Biochemistry* **1980**, *19*, 3973–3978.

(14) Dorn, C. P.; Zimmerman, M.; Yang, S. S.; Yurewicz, E. C.; Ashe, B. M.; Frankshun, R.; Jones, H. Proteinase Inhibitors. 1. Inhibitors of Elastase. *J. Med. Chem.* **1977**, *20*, 1464–1468.

(15) Powers, J. C.; Carroll, D. L. Reaction of Acyl Carbazates with Proteolytic Enzymes. *Biochem. Biophys. Res. Commun.* **1975**, *67*, 639–644.

(16) Dutta, A. S.; Giles, M. B.; Gormley, J. J.; Williams, J. C.; Kusner, E. J. Inhibitors of Human Leucocyte Elastase. Peptides Incorporating an α-Azanorvaline Residue or a Thiomethylene Linkage in Place of a Peptide Bond. *J. Chem. Soc., Perkin Trans. 1* **1987**, 111–120.

(17) Dutta, A. S.; Giles, M. B.; Williams, J. C. Inhibitors of Porcine Pancreatic Elastase. Peptides Incorporating α-Aza-amino Acid Residues in the P_1 Position. *J. Chem. Soc., Perkin Trans. 1* **1986**, 1655–1664.

(18) Scofield, R. E.; Werner, R. P.; Wold, F. p-Nitrophenyl Carbamates as Active-Site-Specific Reagents for Serine Proteases. *Biochemistry* **1977**, *16*, 2492–2496.

(19) Digenis, G. A.; Agha, B. J.; Tsuji, K.; Kato, M.; Shinogi, M. Peptidyl Carbamates Incorporating Amino Acid Isosteres as Novel Elastase Inhibitors. *J. Med. Chem.* **1986**, *29*, 1468–1476.

(20) Tsuji, K.; Agha, B. J.; Shinogi, M.; Digenis, G. A. Peptidyl Carbamate Esters: A New Class of Specific Elastase Inhibitors. *Biochem. Biophys. Res. Commun.* **1984**, *122*, 571–576.

(21) Shenvi, A. B.; Kettner, C. Inhibition of the Serine Proteases Leukocyte Elastase, Pancreatic Elastase, Cathepsin G, and Chymotrypsin by Peptide Boronic Acids. *J. Biol. Chem.* **1984**, *259*, 15106–15114.

(22) Soskel, N. T.; Suetaro, W.; Hardie, R.; Shenui, A. B.; Punt, J. A.; Kettner, C. Effects of Dosage and Timing of Administration of a Peptide Boronic Acid Inhibitor on Lung Mechanics and Morphometrics in Elastase-Induced Emphysema in Hamsters. *Am. Rev. Respir. Dis.* **1986**, *133*, 635–638.

(23) Hassal, C. H.; Johnson, W. H.; Kennedy, A. J.; Roberts, N. A. A New Class of Inhibitors of Human Leucocyte Elastase. *FEBS Lett.* **1985**, *183*, 201–204.

(24) Roberts, N. A.; Surgenor, A. E. Comparison of Peptide Aldehydes with α_1-Antitrypsin as Elastase Inhibitors for Use in Emphysema. *Biochem. Biophys. Res. Commun.* **1986**, *139*, 896–902.

(25) Hori, H.; Yasutake, A.; Minematsu, Y.; Powers, J. C. Inhibition of Human Leukocyte Elastase, Porcine Pancreatic Elastase and Cathepsin G by Peptide Ketones. In *Peptides, Structure and Function. Proceedings of the Ninth American Peptide Symposium*; Deber, C. M.; Hruby, V. J.; Kopple, D. K., Ed.; Pierce Chemical Co.: Rockford, IL, 1985; p 819–822.

(26) Takahashi, L. H.; Radhakrishman, R.; Rosenfield, R. E., Jr.; Meyer, E. F., Jr.; Trainor, D. A. Crystal Structure of the Covalent Complex Formed by a Peptidyl α,α-Difluoro-β-keto Amide with Porcine Pancreatic Elastase at 1.78-Å Resolution. *J. Am. Chem. Soc.* **1989**, *111*, 3368–3374.

(27) Groutas, W. C.; Abrams, W. R.; Theodorakis, M. C.; Kasper, A. M.; Rude, S. A.; Badger, R. C.; Ocain, T. D.; Miller, K. E.; Moi, M. K.; Brubaker, M. J.; Davis, K. S.; Zandler, M. E. Amino Acid Derived Latent Isocyanates: Irreversible Inactivation of Porcine Pancreatic Elastase and Human Leukocyte Elastase. *J. Med. Chem.* **1985**, *28*, 204–209.

(28) Groutas, W. C.; Brubaker, M. J.; Zandler, M. E.; Stanga, M. A.; Huang, T. L.; Castrisos, J. C.; Crawley, J. P. Sulfonate Salts of Amino Acids: Novel Inhibitors of the Serine Proteinases. *Biochem. Biophys. Res. Commun.* **1985**, *128*, 90–93.

(29) Harper, J. W.; Hemmi, K.; Powers, J. C. Reaction of Serine Proteases with Substituted Isocoumarins: Discovery of 3,4-Dichloroisocoumarin, a New General Mechanism Based Serine Protease Inhibitor. *Biochemistry* **1985**, *24*, 1831–1841.

(30) Harper, J. W.; Powers, J. C. Reaction of Serine Proteases with Substituted 3-Alkoxy-4-chloroisocoumarins and 3-Alkoxy-7-amino-4-chloroisocoumarins: New Reactive Mechanism-Based Inhibitors. *Biochemistry* **1985**, *24*, 7200–7213.

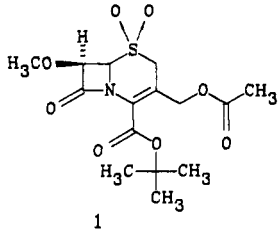
(31) Copp, L. J.; Krantz, A.; Spencer, R. W. Kinetics and Mechanism of Human Leukocyte Elastase Inactivation by Ynenol Lactones. *Biochemistry* **1987**, *26*, 169–178.

(32) Teshima, T.; Griffin, J. C.; Powers, J. C. A New Class of Heterocyclic Serine Protease Inhibitors. Inhibition of Human Leukocyte Elastase, Porcine Pancreatic Elastase, Cathepsin G, and Bovine Chymotrypsin A_α with Substituted Benzoxazinones, Quinazolines, and Anthranilates. *J. Biol. Chem.* **1982**, *257*, 5085–5091.

(33) Groutas, W. C.; Stanga, M. A.; Brubaker, M. J.; Huang, T. L.; Moi, M. K.; Carroll, R. T. Substituted 2-Pyrones, 2-Pyridones, and Other Congeners of Elasnin as Potential Agents for the Treatment of Chronic Obstructive Lung Diseases. *J. Med. Chem.* **1985**, *28*, 1106–1109.

(34) Groutas, W. C.; Stanga, M. A.; Castrisos, J. C.; Schatz, E. J. Hydantoin Derivatives. A New Class of Inhibitors of Human Leukocyte Elastase. *J. Enzyme Inhib.* **1990**, *3*, 237–243.

of these inhibitors also show in vivo activity. For example, a series of cephalosporins,[35-37] such as 1[35] and 2,[36] which are potent heterocyclic inhibitors of HLE, prevent lung damage in hamsters treated intratracheally with HLE.



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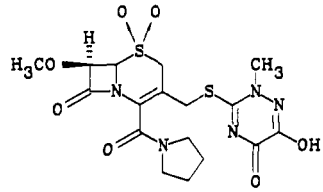
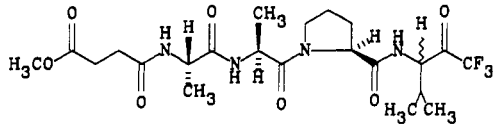


2 (L-659,286)



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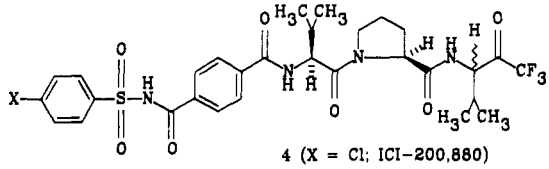


4 (X = Cl; ICI-200,880)

Recently, it has also been reported that peptidyl trifluoromethyl ketones,[38-47] such as 3 and 4,[47] are potent in vitro inhibitors of HLE. Furthermore, in an in vivo model[23] of elastase-induced emphysema, 4[47a] administered it. after elastase challenge has been demonstrated to halt the progression of HLE-induced emphysema-like lesions. Presumably, the mechanism of action of these inhibitors acts via transition-state inhibition. The enhanced electrophilicity of the fluorinated ketone carbonyl of compounds such as 3 facilitates the enzyme-catalyzed addition of active site Ser^{195} to the ketone carbonyl to form a metastable hemiketal, which resembles the tetrahedral intermediate in the reaction pathway for enzyme–substrate hydrolysis. Although the trifluoromethyl ketone inhibitors are assumed to form a covalent bond with the active site Ser^{195} of HLE, this process is reversible. In the case of porcine pancreatic elastase (PPE), it has been unequivocally demonstrated[48] through X-ray analysis that, in the adducts formed between the enzyme and peptidyl trifluoromethyl ketone inhibitors, the O^{γ} atom of the catalytic Ser^{195} residue covalently attached (1.5 Å) to the ketone carbonyl of the inhibitor via a hemiketal.[49] Due to the high electrophilicity of the fluorine atoms, however, trifluoromethyl ketone inhibitors may react with water to generate transition-state analogues in situ.

One of the principal problems associated with peptidyl inhibitors (and also β-lactam analogues[50]) is that they tend

(35) (a) 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. (b) Doherty, J. B.; Ashe, B. M.; Barker, P. L.; Blacklock, T. J.; Butcher, J. W.; Chandler, G. O.; Dahlgren, M. E.; Davies, P.; Dorn, C. P., Jr.; Finke, P. E.; Firestone, R. A.; Hagmann, W. K.; Halgren, T.; Knight, W. B.; Maycock, A. L.; Navia, M. A.; O'Grady, L.; Pisano, J. M.; Shah, S. K.; Thompson, K. R.; Weston, H.; Zimmerman, M. Inhibition of Human Leukocyte Elastase. 1. Inhibition by C-7 Substituted Cephalosporin *tert*-Butyl Esters. *J. Med. Chem.* **1990**, *33*, 2513–2521. (c) Finke, P. E.; Ashe, B. M.; Knight, W. B.; Maycock, A. L.; Navia, M. A.; Shah, S. K.; Thompson, K. R.; Underwood, D. J.; Weston, H.; Zimmerman, M.; Doherty, J. B. Inhibition of Human Leukocyte Elastase. 2. Inhibition by Substituted Cephalosporin Esters and Amides. *J. Med. Chem.* **1990**, *33*, 2522–2528. (d) Shah, S. K.; Brause, K. A.; Chandler, G. O.; Finke, P. E.; Ashe, B. M.; Weston, H.; Knight, W. B.; Maycock, A. L.; Doherty, J. B. Inhibition of Human Leukocyte Elastase. 3. Inhibition by Substituted Cephalosporin Esters and Amides. *J. Med. Chem.* **1990**, *33*, 2529–2535.

(36) Bonney, R. J.; Ashe, B.; Maycock, A.; Dellea, P.; Hand, K.; Osinga, D.; Fletcher, D.; Mumford, R.; Davies, P.; Frankenfield, D.; Nolan, T.; Schaeffer, L.; Hagmann, W.; Finke, P.; Shah, S.; Dorn, C.; Doherty, J. Pharmacological Profile of the Substituted Beta-Lactam L-659,286: A Member of a New Class of Human PMN Elastase Inhibitors. *J. Cellular Biochem.* **1989**, *39*, 47–53.

(37) Maycock, A. L.; Bonney, R. J.; Davies, P.; Doherty, J. B.; Lin, T.-Y.; Navia, M. A. Beta-Lactam Inhibitors of Human Leukocyte Elastase. In *Molecular Basis of the Action of Drugs and Toxic Substances*; Singer, T. P., Castagnoli, N., Wang, C. C., Eds.; Walter de Grutyler & Co.: New York, 1988, 138–148.

(38) 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 Peptidyl α-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.

(39) 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.

(40) Gelb, M. H.; Svaren, J. P.; Abeles, R. H. Fluoro Ketone Inhibitors of Hydrolytic Enzymes. *Biochemistry* **1985**, *24*, 1813–1817.

(41) Imperiali, B.; Abeles, R. H. Inhibition of Serine Proteases by Peptidyl Fluoromethyl Ketones. *Biochemistry* **1986**, *25*, 3760–3767.

(42) Imperiali, B.; Abeles, R. H. A Versatile Synthesis of Peptidyl Fluoromethyl Ketones. *Tetrahedron Lett.* **1986**, *27*, 135–138.

(43) Kolb, M.; Barth, J.; Neises, B. Synthesis of Fluorinated α-Amino Ketones. Part I: α-Benzamidoalkyl Mono- Di- and Trifluoromethyl Ketones. *Tetrahedron Lett.* **1986**, *27*, 1579–1582.

(44) Kolb, M.; Neises, B. Synthesis of Fluorinated α-Amino Ketones. Part II: α-Acylaminoalkyl α′,α′-Difluoroalkyl Ketones. *Tetrahedron Lett.* **1986**, *27*, 4437–4440.

(45) Fearon, K.; Spaltenstein, A.; Hopkins, P. B.; Gelb, M. H. Fluoro Ketone Containing Peptides as Inhibitors of Human Renin. *J. Med. Chem.* **1987**, *30*, 1617–1622.

(46) 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.

(47) (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), Abstract 291.

(48) 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.

(49) Similarly the crystal structure of a covalent enzyme–inhibitor complex of PPE with a peptidyl α,α-difluoro-β-keto amide inhibitor has been reported, see: Takahashi, L. H.; Radhakrishnan, R.; Rosenfield, R. E., Jr.; Meyer, E. F., Jr.; Trainor, D. A. Crystal Structure of the Covalent Complex Formed by a Peptidyl α,α-Difluoro-β-keto Amide with Porcine Pancreatic Elastase at 1.78-Å Resolution. *J. Am. Chem. Soc.* **1989**, *111*, 3368–3374.

to have very short durations of action when administered either po or iv. The vast majority of peptide-based inhibitors of HLE that have been reported to date contain proline[51] at the P_2-subsite.[52] Since L-proline can effectively be replaced by achiral N-substituted glycines to afford potent angiotensin converting enzyme (ACE) inhibitors[53-55] both in vitro and in vivo, we thought it might be of interest to see if the S_2-region of HLE might be capable of accommodating bulky and lipophilic achiral N-substituted glycine residues in replacement of L-proline. Moreover, the presence of sterically demanding N-substituted glycine residues in peptidyl inhibitors may lead to an enhancement in the duration of action in vivo by limiting the extent of proteolytic hydrolysis of the P_3-P_2 amide bond. With these hypotheses in mind, a series of potent and specific HLE inhibitory compounds were designed and synthesized in which N-substituted glycine residues were incorporated into P_2.

Chemistry

The compounds presented in Tables I–III were conveniently prepared as shown in Schemes I–VII. The new tripeptide trifluoromethyl ketones were synthesized essentially using previously reported procedures for similar peptidyl trifluoromethyl ketones. The required trifluoromethyl ketones located at the P_1-subsite of the inhibitors were prepared as illustrated in Scheme I by means similar to those previously reported for analogous ketones.[40-44] The appropriately substituted iodoalkyl compounds **5** were reacted with $AgNO_2$ in Et_2O to afford nitroalkanes **6**, which were converted to nitrofluoro alcohols **7** as a mixture of *dl*-threo and *dl*-erythro isomers when $R_1 \neq R_2$, by reaction with trifluoroacetaldehyde ethyl hemiacetal, $CF_3CH(OC_2H_5)OH$, and K_2CO_3 (neat). Typically, the mixture of *dl*-threo and *dl*-erythro isomers was used directly without separation, except when the respective isomers were conveniently separated by flash column chromatography over silica gel or by crystallization. For example, the (*dl*)-threo isomer [(2*R*,3*S*)+(2*S*,3*R*)]-**7a** (R_1 = H, R_2 = $CH(CH_3)_2$) could easily be separated from the corresponding minor (*dl*)-erythro isomer [(2*S*,3*S*)+(2*R*,3*R*)] by crystallization. The **7a** erythro isomer remained in the filtrate as an oil. Amino alcohols **8** were prepared by either one of two available methods. In the first method, nitroalkanes **7** were reduced effectively to the amino alcohols **8** (PG = H) by employing $LiAlH_4$, DIBAL, or Raney Ni. In the second method, amino alcohol **8e** (PG = *t*-BOC) was prepared using a variation of a reported method in which trifluoromethylation of carbonyl compounds was effected by (trifluoromethyl)trimethylsilane[56,57] (**9**), $CF_3Si(CH_3)_3$. This method was applied to protected amino acid aldehydes such as *t*-BOC-L-phenylalanal (**10c**) to give directly the protected trifluoromethyl alcohols **8** (PG = *t*-BOC), which by standard methods of deprotection (HCl/*p*-dioxane) were converted to the amino alcohols **8** (PG = H).

The N-substituted glycine esters **12** were prepared by treatment of known primary amines **11** with $BrCH_2COOR_2$ (R_2 = Et or *t*-Bu) in a polar solvent such as EtOH or CH_3CN. Alternatively, the substituted glycine esters **12** could be prepared by reductive alkylation of aldehydes or ketones with α-amino acid esters in the presence of $NaCNBH_3$. The required dipeptide esters such as **15a** or **15b** were prepared via a carbonyldiimidazole (CDI) mediated condensation between L-valine (**14a** or **14b**) and α-amino acid esters **12a** or **12b** in CH_2Cl_2 or THF to afford the desired products **15a** or **15b** in high yields. The dipeptide esters were hydrolyzed to the corresponding dipeptide acids **15c** or **15d** by KOH in EtOH in the case of ethyl esters or by treatment with HCl/*p*-dioxane in the case of *tert*-butyl esters. The acids **15c** and **15d** were purified over silica gel eluting sequentially with CH_2Cl_2/CH_3OH (97:3) and (95:5). Typically, trifluoromethyl alcohols **16** were prepared by condensing acids **15c** and **15d** with amino alcohols **8** (PG = H) through the employment of CDI as the amide-generating reagent. Alternatively, tripeptides **16** were obtained by the mixed anhydride route (isopropyl chloroformate). CBZ- or *t*-BOC-protected tripeptides **16** were oxidized to the corresponding trifluoromethyl ketones **17** either by Swern[58,59] oxidation or by Dess–Martin[60] periodinane oxidation. Tripeptide ketones **17** were obtained as a mixture of diastereomers and were not further separated due to the facile and rapid enolization of the trifluoromethyl ketone functionality located at P_1. The trifluoromethyl ketones are much more prone to hydration than methyl ketones and the hydrates are stable.

For the preparation of longer trifluoromethyl ketone analogues, amino terminal trifluoromethyl alcohols **18** were required. Tripeptides **16** were deprotected catalytically over 10% Pd/C in the case in which PG = CBZ or by

(50) It has recently been reported that monocyclic β-lactams may also be inactivators of HLE; see: (a) Firestone, R. A.; Barker, P. L.; Pisano, J. M.; Ashe, B. M.; Dahlgren, M. E. Monocyclic β-Lactams of Human Leukocyte Elastase. *Tetrahedron* **1990**, *46*, 2255–2262. (b) Skiles, J. W.; McNeil, D. Spiro Indolinone Beta-Lactams, Inhibitors of Poliovirus and Rhinovirus 3C-Proteinases. *Tetrahedron Lett.* **1990**, *31*, 7277–7280.

(51) In addition to refs 12–21, 23, 38–44, see: 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.

(52) The nomenclature used for describing the individual amino acid residues (P_2, P_1, P_1', P_2', etc.) of a peptide substrate and the corresponding subsites (S_2, S_1, S_1', S_2', 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.

(53) Schwab, A.; Macerata, R.; Rogers, W.; Barton, J.; Skiles, J.; Khundwala, A. Inhibition of Angiotensin-Converting Enzyme by Dipeptide Analogs. *Res. Commun. Chem. Pathol. Pharmacol.* **1984**, *45*, 339–345.

(54) Suh, J. T.; Skiles, J. W.; Williams, B. E.; Youssefyeh, R. D.; Jones, H.; Loev, B.; Neiss, E. S.; Schwab, A.; Mann, W. S.; Khandwala, A.; Wolf, P. S.; Weinryb, I. Angiotensin-Converting Enzyme Inhibitors. New Orally Active Antihypertensive (Mercaptoalkanoyl)- and [(Acylthio)alkanoyl]glycine Derivatives. *J. Med. Chem.* **1985**, *28*, 57–66.

(55) Suh, J.; Regan, J. R.; Skiles, J. W.; Barton, J.; Piwinski, J. J.; Weinryb, I.; Schwab, A.; Samuels, A. I.; Mann, W. S.; Smith, R. D.; Wolf, P. S.; Khandwala, A. Angiotensin-Converting Enzyme Inhibitors: N-Substituted Glycine Derivatives. *Eur. J. Med. Chem.* **1985**, *20*, 563–570.

(56) Prakash, G. K. S.; Krishnamurti, R.; Olah, G. A. Fluoride-Induced Trifluoromethylation of Carbonyl Compounds with Trifluoromethyltrimethylsilane (TMS-CF_3). A Trifluoromethide Equivalent. *J. Am. Chem. Soc.* **1989**, *111*, 393–395.

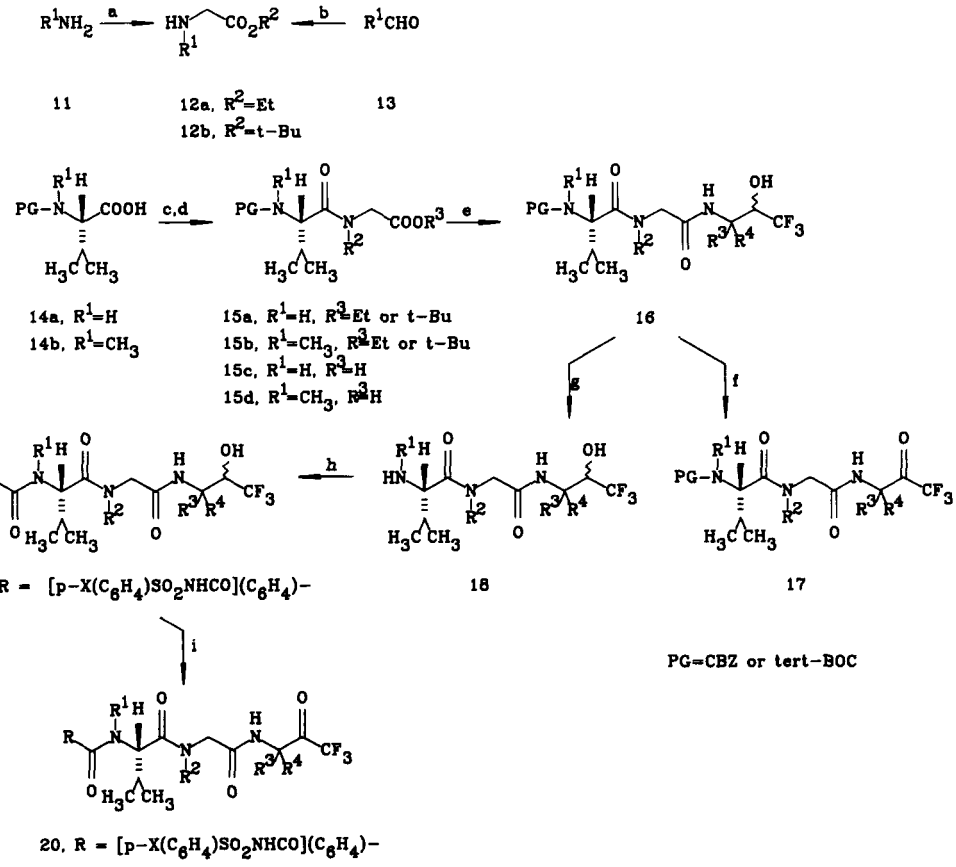
(57) Ruppert, I.; Schlich, K.; Volbach, W. The First CF_3-Substituted Organo(chloro)silane. *Tetrahedron Lett.* **1984**, *25*, 2195–2198.

(58) Omura, K.; Swern, D. Oxidation of Alcohols by "Activated" Dimethyl Sulfoxide. A Preparative Steric and Mechanistic Study. *Tetrahedron* **1978**, *34*, 1651–1660.

(59) Mancuso, A. J.; Huang, S. L.; Swern, D. Oxidation of Long-Chain and Related Alcohols to Carbonyls by Dimethyl Sulfoxide "Activated" by Oxalyl Chloride. *J. Org. Chem.* **1978**, *43*, 2480–2482.

(60) 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, p 3.

Scheme II. Synthesis of Tripeptide Trifluoromethyl Ketones (**20**)

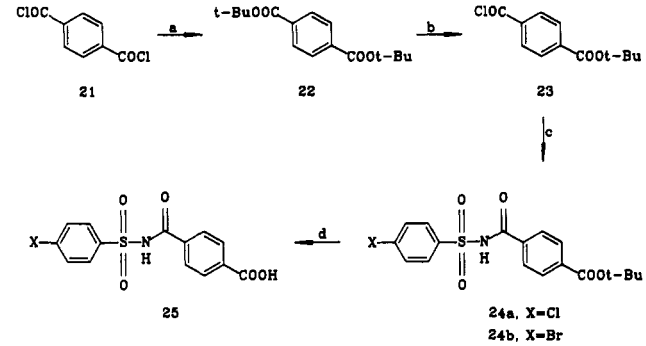


[a] Reagents: (a) $BrCH_2CO_2R_2$, Et_3N; (b) $H_2NCH_2CO_2R_2$, $NaCNBH_3$; (c) **12a** or **12b**, CDI, THF to give **15a** or **15b**; (d) **15a** (R_3 = Et) or **15b** (R_3 = Et), KOH, EtOH to give **15c** or **15d**; or **15a** (R_3 = *t*-BOC) or **15b** (R_3 = *t*-Bu), HCl, *p*-dioxane to give **15c** or **15d**; (e) (PG = H), **15c** or **15d**, CDI, THF; (f) Dess–Martin periodinane, CF_3COOH, CH_2Cl_2; (g) **16** (PG = CBZ), 10% Pd/C, EtOH, H_2, 45 psi; or **16** (PG = *t*-BOC), HCl, *p*-dioxane; (h) **25**, HOBT, WSCDI, THF; (i) Dess–Martin periodinane, CF_3COOH, THF, CH_2Cl_2.

treatment with *p*-dioxane which had previously been saturated with dry hydrogen chloride in cases where PG = *t*-BOC. Tripeptides **18** were condensed with acid **25** (see Scheme III) via a water-soluble carbonyldiimide (WSCDI) reagent, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide, to give alcohols **19** which were then oxidized to ketones **20** by a Dess–Martin periodinane oxidation. The syntheses of acids **25** and **27**, which are required for the syntheses of **20** and **29**, respectively, are illustrated in Schemes III and IV. The synthesis of *syn*-[2-[[3-(ethoxycarbonyl)-propanoyl]amino]-4-thiazolyl](methoxyimino)acetyl tripeptides with extended binding regions to HLE is illustrated in Scheme IV. The truncated inhibitors **32a** and **34** were prepared according to Scheme V by standard methods of protection and deprotection.

The synthesis of the P_3-P_2 reduced analogues **37b** and **38c** was done according to Scheme VI. *t*-BOC-L-valine was coupled with *N,O*-dimethylhydroxylamine[61] in the presence of CDI to give **35b**. Amide **35b** was reduced with $LiAlH_4$ to give *t*-BOC-L-valinal (**35c**).[62] The reductive alkylation of the α-amino ester **12a** (R_1 = 2-indanyl) with aldehyde **35c** employing $NaCNBH_3$ gave *t*-BOC-L-valyl-$\psi(CH_2)$-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine ethyl ester (**36a**).[63] Reduced dipeptide alcohol **36a** was transformed to trifluoromethyl ketones **37b** and **38c** by methods similar to those described above (Scheme II).

Scheme III. Synthesis of 4-[[[(4-Halophenyl)sulfonyl]amino]carbonyl]benzenecarboxylic Acid (**23**)



[a] Reagents: (a) *t*-BuOH, pyridine, THF; (b) KOH, *t*-BuOH; (c) *p*-$Cl(C_6H_4)SO_2NH_2$, DMAP, WSCDI, CH_2Cl_2; (d) CF_3COOH.

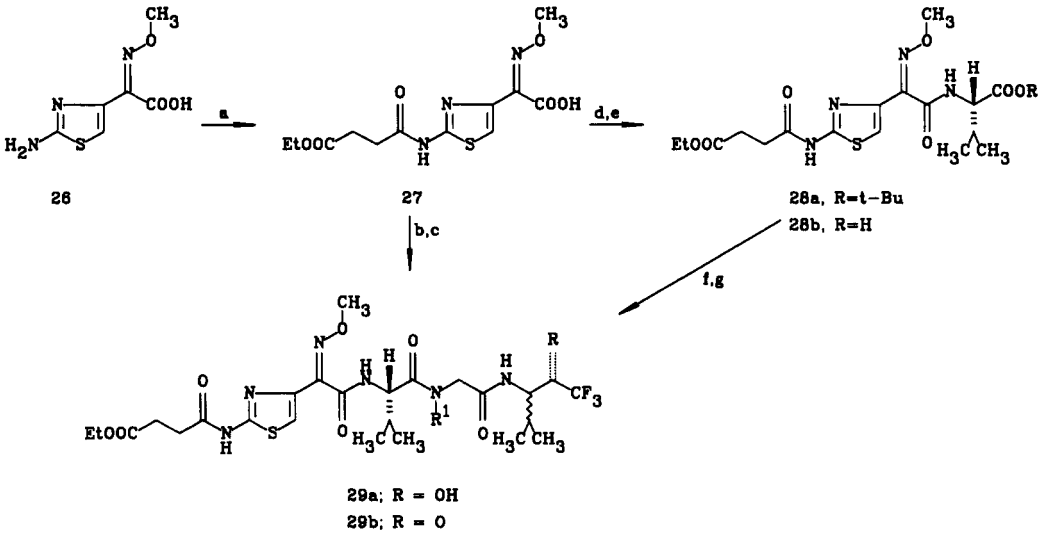
The preparations of tripeptides **41b** and **41e** containing aryl ketones at P_1 were performed as illustrated in Scheme VII. The required protected aryl ketones **39** were efficiently obtained by treating protected α-amino acid *N,O*-dimethylamides **35b**[62] with the appropriate aryl Grignard

(61) This material was first elegantly used by Weinreb to convert, via $LiAlH_4$ reduction, carboxylic acids to aldehydes, see: Nahm, S.; Weinreb, S. M. *N*-Methoxy-*N*-Methylamides as Effective Acylating Agents. *Tetrahedron Lett.* **1981**, *22*, 3815–3818.

(62) Fehrentz, J. A.; Castro, B. An Effective Synthesis of Optically Active α-(*t*-Butoxycarbonylamino)-aldehydes from α-Amino Acids. *Synthesis* **1983**, 676–678.

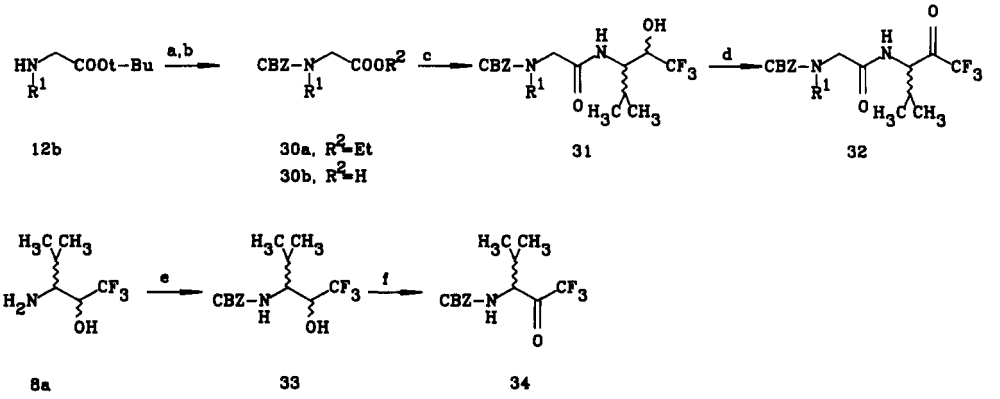
(63) The nomenclature for defining a reduced peptide bond is that of the IUPAC–IUB Joint Commission on Biochemical Nomenclature, Nomenclature and Symbolism for Amino Acids and Peptides. *J. Biol. Chem.* **1985**, *260*, 14–42.

Scheme IV. Synthesis of [2-Amino-4-thiazolyl](methoxyimino)acetyl Tripeptides



[a] Reagents: (a) $ClCOCH_2CH_2CO_2Et$, Et_3N, CH_2Cl_2; (b) 18, CDI, THF to give **29a**; (c) **29a**, Dess–Martin periodinane, CF_3COOH, CH_2Cl_2 to give **29b**; (d) L-Val *t*-Bu ester, CDI, THF to give **28a**; (e) **28a**, CF_3COOH to give **28b**; (f) **28b**, **8a** (PG = H), CDI, THF to give **29a**; (g) **29a**, Dess–Martin periodinane, CF_3COOH, CH_2Cl_2 to give **29b**.

Scheme V. Synthesis of Truncated Inhibitors (P_3-P_1)



[a] Reagents: (a) CBZ-Cl, Et_3N, THF to give **30a**; (b) **30a**, KOH, EtOH to give **30b**; (c) **30b**, **8a**, CDI, THF; (d) Dess–Martin periodinane, CF_3COOH, CH_2Cl_2; (e) CBZ-Cl, Et_3N, THF; (f) Dess–Martin Periodinane, CF_3COOH, CH_2Cl_2.

reagent. Ketones **39** were reduced with $NaBH_4$ to give *N*-CBZ amino alcohols **40a** as a mixture of *SS* and *SR* diastereomers. Using methods analogous to those described above in Scheme II, the alcohols were converted to tripeptide trifluoromethyl ketones **41b** and **41e**.

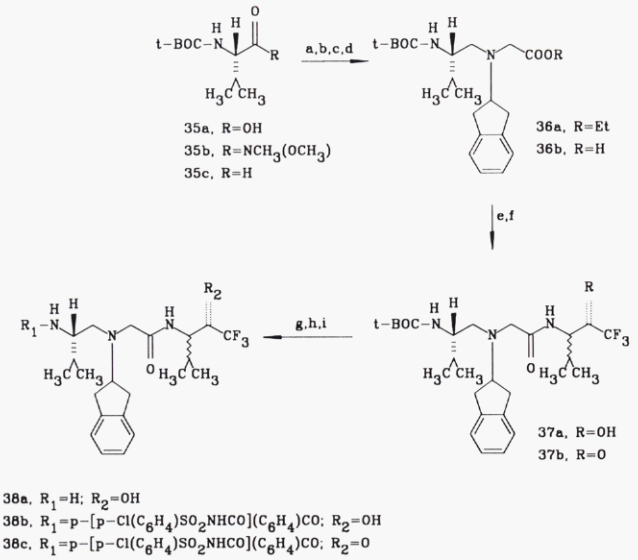
Results and Discussion

The compounds presented in Tables I–III represent an important, novel class of tripeptide trifluoromethyl and aryl ketones which contain non naturally occurring N-substituted glycine residues at the P_2-position and act as potent and specific competitive inhibitors of HLE, both in vitro and in vivo. Unlike the known peptidyl inhibitors of HLE which embody preferentially a P_2-proline, this new series of inhibitors is constructed exclusively from the achiral amino acid glycine. This series of compounds has demonstrated[64] potential as therapeutic agents for emphysema and other diseases related to the degradation of connective tissue.

The most active compounds listed in Table I are those tripeptides that span the P_5-P_1 subsites [e.g. **20b,d,f,g,-i,j,p,q,s,t,v,w,x,aa** and **29b** (IC_{50} = 0.03–0.217 μM)] and contain a trifluoromethyl ketone residue of valine at P_1. The most active tripeptide inhibitors correspond to those which are N-terminated with the functionality *p*-(*p*-$ClC_6H_4SO_2NHCO)C_6H_4$ (Table I). In accord with a previous report, this functionality confers high in vivo activity to peptidyl ketones,[47b] as well as an enhancement in in vitro potency by a factor of approximately 10 relative to the corresponding CBZ or *t*-BOC derivatives [e.g., when homologous series such as **20h** (IC_{50} = 0.365 μM) is compared with **20i** (IC_{50} = 0.084 μM), or when **20r** (IC_{50} = 0.507 μM) is compared with **20s** (IC_{50} = 0.057 μM)]. 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- or methoxysuccinyl-terminated tripeptides. Since it is known that HLE prefers extended substrates and that remote residues several amino acids away from P_1 may effect specificity and hence binding, the *syn*-[2-[[3-(eth-

(64) 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-1*H*-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), Abstract 5212.

Scheme VI. Synthesis of P_3-P_2 Reduced Analogues



[a] Reagents: (a) $HNCH_3(OCH_3)$, CDI, THF to give **35b**; (b) **35b**, $LiAlH_4$, THF to give **35c**; (c) **35c**, **12a** (R_1 = 2-indanyl), $NaCNBH_3$, EtOH to give **36a**; (d) **36a**, NaOH, EtOH to give **36b**; (e) **36b**, **8a** (PG = H), CDI to give **37a**; (f) **37a**, Dess–Martin periodinane, CF_3COOH, CH_2Cl_2 to give **37b**; (g) **37b**, HCl/*p*-dioxane to give **38a**; (h) **38a**, **25a**, WSCDI to give **38b**; (i) **38b**, Dess–Martin periodinane, CF_3COOH, CH_2Cl_2 to give **38c**.

oxycarbonyl)propanoyl]amino]-4-thiazolyl](methoxyimino)acetyl derivative **29b** was prepared as an inhibitor with binding regions extending into the S_6-S_1 sites. The 2-(2-amino-4-thiazolyl)-2-oximinoacetic acid moiety has been widely used[65] in the design of cephalosporins. This functionality, however, did not increase the in vitro potency [e.g. **29b** (IC_{50} = 0.452 μM)]. From Table I, it can be seen that the P_2-residue of the inhibitors has a very high degree of tolerance with regard to the substituent group. For instance, as the substituent on the P_2-glycine is increased in steric size and lipophilicity, ranging from H (**20b**), CH_3 (**20d**), cyclopentyl (**20f**), *exo*-norbornyl (**20g**), 2-indanyl (**20i**), and cycloheptyl (**20p**) to cyclooctyl (**20q**), no dramatic change in in vitro potency is observed (IC_{50} = 0.052–0.175 μM). The results presented in Table I tend to indicate that a sterically demanding residue such as *N*-(cyclooctyl)glycine, as occurs in **20q** (IC_{50} = 0.067 μM), is as easily accommodated as is glycine itself (e.g. **20b**, IC_{50} = 0.073 μM). The results listed in Table I also indicate that *N*-(heterocycloalkyl)glycine, as well as *N*-(arylalkyl)glycine residues at P_2, are also accommodated quite easily by HLE. For example, the piperidinyl (**20s**), benzyl (**20t**), 3,4-dimethoxyphenethyl (**20v**), tetrahydrofurfuryl (**20w**), and furfuryl (**20x**) N-substituted glycine tripeptides are all potent inhibitors of HLE (IC_{50} = 0.057, 0.217, 0.084, 0.030, 0.138 μM, respectively). These results are somewhat surprising in that sterically demanding substituents such as those described above can be accommodated so easily by HLE.

Subsequent to our synthesis of inhibitors, the X-ray structure of HLE became available. In order to gain some molecular insight into the binding properties of our inhibitors, and to provide a better understanding of the structure–activity relationships, molecular modeling studies were performed utilizing the X-ray coordinates of

(65) *Beta-Lactam Antibiotics for Clinical Use*; Queener, S. F., Webber, J. A., Queener, S. W., Eds.; Clinical Pharmacology: Marcel Dekker: New York, 1986; Vol. 4.

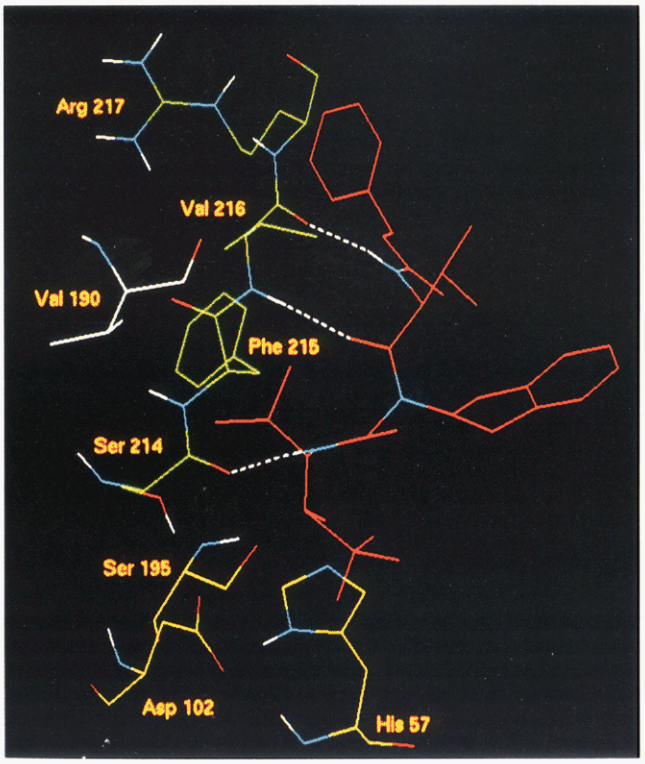
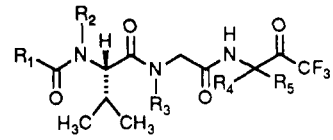


Figure 1. Minimized structure of HLE complexed with the inhibitor **20h** detailing the binding and catalytic sites. The binding residues (Ser^{214}, Phe^{215}, Val^{216}, and Arg^{217}) and the catalytic triad (Ser^{195}, His^{57}, and Asp^{102}) of HLE are given in green and yellow, respectively. The inhibitor is shown in red. For minimization calculations the constraint (1.78 Å) of covalently linking Ser^{195} with the (trifluoromethyl)carbonyl of the inhibitor **20h** via hemiketal formation was utilized. Hydrogen bonds formed between HLE residues and those of the inhibitor **20h** are indicated by dotted lines (Val^{216} CO to NH of P_3-Val, 1.84 Å; Val^{216} NH to CO of P_3-Val, 1.97 Å; Ser^{214} CO to NH of P_1-Val, 1.89 Å).

HLE.[66] If it is assumed that the carbonyl of the trifluoromethyl ketones of the active inhibitors reacts covalently but reversibly with the active site Ser^{195} 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_2-glycines of the enzyme–inhibitor complexes must stick out into solution away from the core of HLE. Figure 1 shows the catalytic triad (Ser^{195}, Asp^{102}, and His^{57}) in yellow and the binding site residues (Arg^{217}, Val^{216}, Phe^{215}, and Ser^{214}) of HLE in green. The CBZ inhibitor **20h**, complexed with

(66) 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 structure analysis as well as a description of the substrate specificity of HLE, see: (a) 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. (b) 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. (c) 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 Ovomucoid Inhibitor. *EMBO* **1986**, *5*, 2453–2458.

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



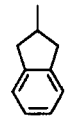
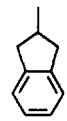
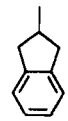
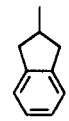
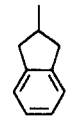
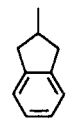
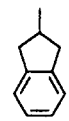
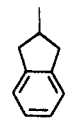
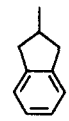
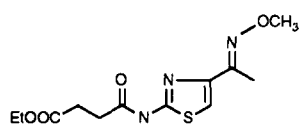
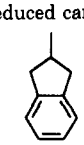
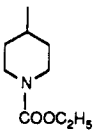
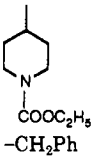
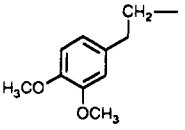
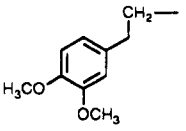
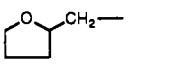
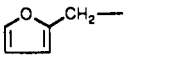
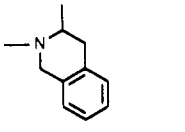
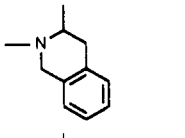
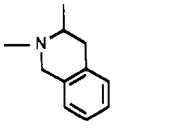
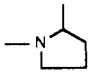
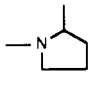
compd[a]	R_1	R_2	R_3	R_4	R_5	mp,[b] °C	procedure[c]	formula[d]	IC_{50},[e] μM
20a	$(CH_3)_3CO$	H	H	H	$CH(CH_3)_2$	71–75	D–G	$C_{18}H_{30}F_3N_3O_5$	0.153
20b	p-(p-$ClC_6H_4SO_2NHCO$)C_6H_4	H	H	H	$CH(CH_3)_2$	193–196	I–K	$C_{27}H_{30}ClF_3N_4O_7S$	0.073
20c	$PhCH_2O$	H	CH_3	H	$CH(CH_3)_2$	resin	D–G	$C_{22}H_{30}F_3N_3O_5$	0.153
20d	p-(p-$ClC_6H_4SO_2NHCO$)C_6H_4	H	CH_3	H	$CH(CH_3)_2$	resin	H, J, K	$C_{28}H_{32}ClF_3N_4O_7S$	0.052
20e	$PhCH_2O$	H	c-C_5H_9	H	$CH(CH_3)_2$	69–74	D–G	$C_{26}H_{36}F_3N_3O_5$	0.156
20f	p-(p-$ClC_6H_4SO_2NHCO$)C_6H_4	H	c-C_5H_9	H	$CH(CH_3)_2$	136–140	H, J, K	$C_{32}H_{38}ClF_3N_4O_7S$	0.092
20g	p-(p-$ClC_6H_4SO_2NHCO$)C_6H_4	H		H	$CH(CH_3)_2$	234–239	D–F, H, J	$C_{34}H_{40}ClF_3N_4O_7S$	0.061
20h	$PhCH_2O$	H		H	$CH(CH_3)_2$	49–54	C, E–G	$C_{30}H_{36}F_3N_3O_5$	0.365
20i	p-(p-$ClC_6H_4SO_2NHCO$)C_6H_4	H		H	$CH(CH_3)_2$	218–226	H, J, K	$C_{36}H_{38}ClF_3N_4O_7S$	0.084
20j	p-(p-$BrC_6H_4SO_2NHCO$)C_6H_4	H		H	$CH(CH_3)_2$	170–185	D–F, H, J, K	$C_{36}H_{38}BrF_3N_4O_7S$	0.149
20k	$PhCH_2O$	H		H	H	resin	D–G	$C_{27}H_{30}F_3N_3O_5$	>5
20l	p-(p-$ClC_6H_4SO_2NHCO$)C_6H_4	H		H	CH_3	115–123	D–F, H, J, K	$C_{34}H_{34}ClF_3N_4O_7S$	0.817
20m	p-(p-$ClC_6H_4SO_2NHCO$)C_6H_4	H		CH_3	CH_3	143–147	D–F, H, J, K	$C_{35}H_{36}ClF_3N_4O_7S$	>5
20n	$PhCH_2O$	CH_3		H	$CH(CH_3)_2$	resin	O, E–G	$C_{31}H_{38}F_3N_3O_5$	1.562
20o	p-(p-$ClC_6H_4SO_2NHCO$)C_6H_4	H		H	CH_2Ph	resin	O, E, F, H, J, K	$C_{40}H_{38}ClF_3N_4O_7S$	>5
37b	$(CH_3)_3CO$	H		H	$CH(CH_3)_2$	resin	L, E–G	$C_{27}H_{40}F_3N_3O_4$	>5
	(reduced carbonyl between P_3 and P_2)								
38c	p-(p-$ClC_6H_4SO_2NHCO$)C_6H_4	H		H	$CH(CH_3)_2$	resin	L, E, F, H, J, K	$C_{36}H_{40}ClF_3N_4O_6S$	5.0
	(reduced carbonyl between P_3 and P_2)								
29b		H		H	$CH(CH_3)_2$	94–101	D–F, H, J, K	$C_{34}H_{43}F_3N_6O_8S$	0.452

Table I (Continued)

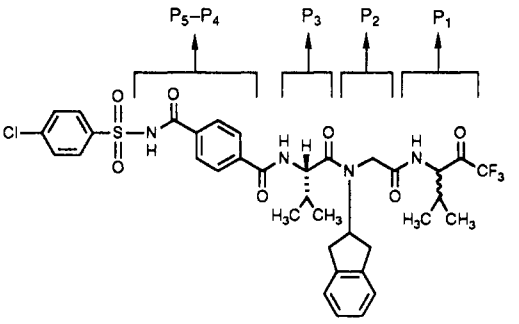
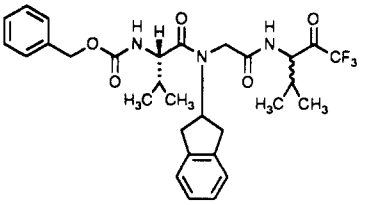
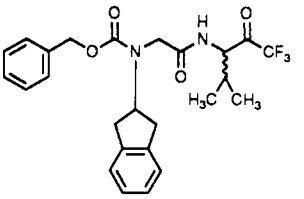
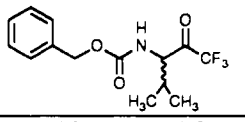
compd[a]	R_1	R_2	R_3	R_4	R_5	mp,[b] °C	procedure[c]	formula[d]	IC_{50},[e] μM
20p	*p*-(*p*-$ClC_6H_4SO_2NHCO)C_6H_4$	H	c-C_7H_{13}	H	$CH(CH_3)_2$	98–102	H, J, K	$C_{34}H_{42}ClF_3N_4O_7S$	0.175
20q	*p*-(*p*-$ClC_6H_4SO_2NHCO)C_6H_4$	H	c-C_8H_{15}	H	$CH(CH_3)_2$	183–189	D–F, I–K	$C_{35}H_{44}ClF_3N_4O_7S$	0.067
20r	$PhCH_2O$	H		H	$CH(CH_3)_2$	87–91	D–G	$C_{29}H_{41}F_3N_4O_7$	0.507
20s	*p*-(*p*-$ClC_6H_4SO_2NHCO)C_6H_4$	H		H	$CH(CH_3)_2$	151–153	H, J, K	$C_{35}H_{43}ClF_3N_5O_9S$	0.057
20t	*p*-(*p*-$ClC_6H_4SO_2NHCO)C_6H_4$	H	$-CH_2Ph$	H	$CH(CH_3)_2$	140–142	D–F, H, J, K	$C_{34}H_{36}ClF_3N_4O_7S$	0.217
20u	$PhCH_2O$	H		H	$CH(CH_3)_2$	54–59	D–G	$C_{31}H_{40}F_3N_3O_7$	0.693
20v	*p*-(*p*-$ClC_6H_4SO_2NHCO)C_6H_4$	H		H	$CH(CH_3)_2$	115–118	H, J, K	$C_{37}H_{42}ClF_3N_4O_9S$	0.084
20w	*p*-(*p*-$ClC_6H_4SO_2NHCO)C_6H_4$	H		H	$CH(CH_3)_2$	187–190	D–F, H, J, K	$C_{32}H_{38}ClF_3N_4O_7S$	0.030
20x	*p*-(*p*-$ClC_6H_4SO_2NHCO)C_6H_4$	H		H	$CH(CH_3)_2$	187–190	D–F, H, J, K	$C_{32}H_{34}ClF_3N_4O_8S$	0.138
20y[f]	$PhCH_2O$	H		H	$CH(CH_3)_2$	53–61	Q, E–G	$C_{29}H_{34}F_3N_3O_5$	0.233
20z[f,g]	$PhCH_2O$	H		H	$CH(CH_3)_2$	54–61	Q, E–G	$C_{29}H_{34}F_3N_3O_5$	0.073
20aa[f]	*p*-(*p*-$ClC_6H_4SO_2NHCO)C_6H_4$	H		H	$CH(CH_3)_2$	167–172	H, J, K	$C_{35}H_{36}ClF_3N_4O_7S$	0.110
20ab[h]	$(CH_3)_3CO$	H		H	$CH(CH_3)_2$	57–62	D–G	$C_{21}H_{34}F_3N_3O_5$	0.172
20ac[h,i]	$(CH_3)_3CO$	H		H	$CH(CH_3)_2$	62–65	D–G	$C_{21}H_{34}F_3N_3O_5$	0.065
1									0.107[j]
	4 (X = Br)[k]								0.038[k]

[a] Except where indicated all compounds are racemic at P_1. [b] Uncorrected. [c] See the Experimental Section. [d] All compounds exhibited satisfactory C, H, and N microanalyses and were within 0.4% of the theoretical values. All compounds exhibited IR, ^1H NMR, ^{13}C NMR, and MS spectra consistent with the assigned structures. [e] 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-*p*NA 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. [f] 1,2,3,4-Tetrahydroisoquinoline-4-carboxylic acid substituted for P_2-N-substituted glycine. [g] Diastereomer of **20y**. [h] L-Proline substitution for P_2-N-substituted glycine. [i] Diastereomer of **20ab**. [j] lit.[35] IC_{50} = 1.33 μM. [k] lit.[47a] $K_i = 5 \times 10^{-10}$ M.

HLE, is shown in red. In this binding conformation of **20h**, the P_1-trifluoromethyl ketone of the inhibitor is covalently linked to Ser^{195} via hemiketal formation to HLE. Hydrogen bonds between the HLE binding site residues and those of the inhibitor **20h** are as follows: Val^{216} CO to NH of P_3-Val, 1.84 Å; Val^{216} NH to CO of P_3-Val, 1.97 Å; Ser^{214} CO to NH of P_1-Val, 1.89 Å. The three amino acid residues of the inhibitor are in similar positions relative to the HLE binding site and bind in a similar conformation as the P_3-P_1 residues of the HLE turkey ovomucoid inhibitor (OMTKY3).[67] From Figure 1, it can be seen that the lipophilic 2-indanyl substituent of **20h** sticks out into an area away from the binding and catalytic sites and into the solvent. This is better illustrated, however, in Figure 2, which shows the energy-minimized structure of the enzyme–inhibitor complex between HLE and inhibitor **20h**. From this figure, it is very clear that substituents on the P_2-nitrogen should have very little effect on binding and

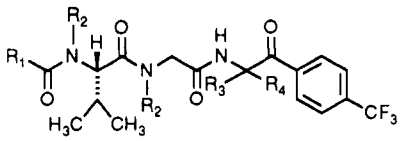
(67) Bogard, W. C., Jr.; Kato, I.; Laskowski, M., Jr. A Ser^{162}/Gly^{162} Polymorphism in Japanese Quail Ovomucoid. *J. Biol. Chem.* **1980**, *255*, 6569–6574.

Table II. Truncated Inhibitors (P_5-P_1)

compd[a]	structure[b]	IC_{50},[c] μM
20i		0.084
20h		0.365
32		≫5
34		≫5

[a] All compounds exhibited satisfactory C, H, and N microanalyses and are within 0.4% of theoretical values. All compounds exhibited IR, ^1H NMR, ^{13}C NMR, and MS spectra consistent with the assigned structures. [b] Except where indicated all compounds are racemic at P_1. [c] 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-*p*NA at a concentration of 0.5 mM. Compounds and elastase were incubated for 20 min prior to starting the reaction by addition of substrate. Initial rates were linear in each case.

Table III. In Vitro HLE Inhibitory Activities of Selected N-Substituted Tripeptide Aryl Ketones



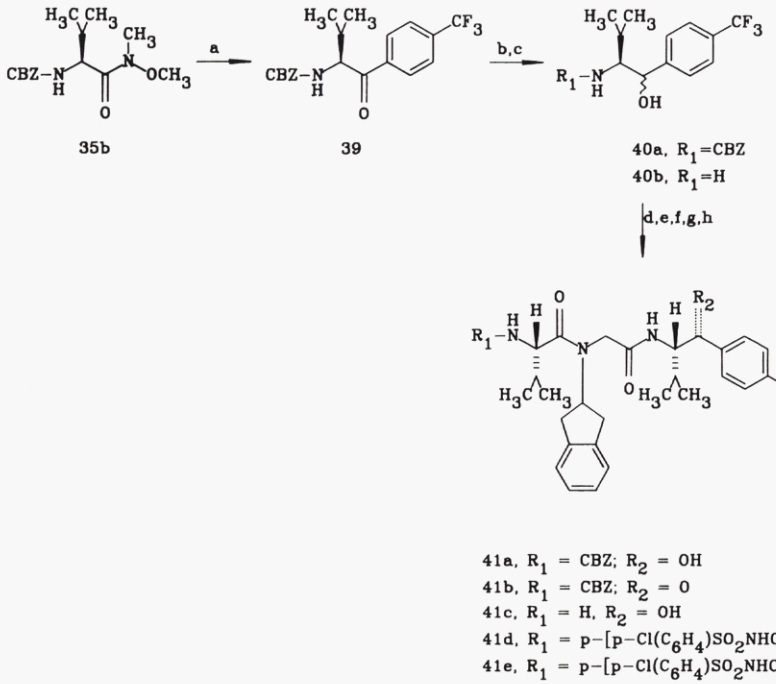
compd[a]	R_1	R_2	R_3	R_4	mp,[b] °C	procedure[c]	formula[d]	IC_{50},[e] μM
41b	$PhCH_2CO$		H	$CH(CH_3)_2$	resin	F, G, M, N	$C_{36}H_{40}F_3N_3O_5$	>5
41e	*p*-(*p*-$ClC_6H_4SO_2NHCO)C_6H_4$		H	$CH(CH_3)_2$	resin	H, J, K	$C_{42}H_{42}ClF_3N_4O_7S$	5.1

[a] Compounds are of the *S* configuration at P_1. [b] Uncorrected. [c] See the Experimental Section. [d] All compounds gave satisfactory C, H, and N microanalyses and were within 0.4% of the theoretical values. [e] 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-*p*Na at a concentration of 0.5 mM. Compounds and elastase were incubated for 20 min prior to starting the reaction by addition of substrate. Initial rates were linear in each case.

catalysis. This tends to support the results of Table I, which show that sterically demanding substituents on the P_2-nitrogen do not have any dramatic effect on in vitro potency.

In order to test the assertion that reduction of the P_3-P_2 amide bond [yielding the methylene ($\psi(CH_2)$) isostere] may potentiate in vivo activity by limiting the extent of proteolysis, derivatives **37b** and **38c** were prepared. As seen in Table I, this modification leads to a decrease in in vitro activity, presumably due to the elimination of the hydrogen bond between Val^{216} NH and the P_3-carbonyl of the inhibitor, which is present in **20i** (IC_{50} = 0.084 μM), but is not possible in the inactive methylene derivative **38c** (IC_{50} > 5 μM). The effect of producing a basic center may also alter inhibition properties. The in vitro inactivity of **37b** and **38c** is also reflected in their inability to prevent

Scheme VII. Synthesis of Trifluoromethyl Aryl Ketones



[a] Reagents: (a) p-$CF_3(C_6H_4)Cl$, Mg; (b) $NaBH_4$, CH_3OH to give **40a**; (c) **40a**, 10% Pd/C, EtOH, H_2, 45 psi to give **40b**; (d) **15c** (R_2 = 2-indanyl), **40b**, HOBT, WSCDI, THF to give **41a**; (e) **41a**, Dess–Martin periodinane, CF_3COOH to give **41b**; (f) **41a**, 10% Pd/C, EtOH, H_2, 45 psi to give **41c**; (g) **41c**, **25a**, THF, HOBT, WSCDI to give **41d**; (h) **41d**, Dess–Martin periodinane, CF_3COOH, CH_2Cl_2.

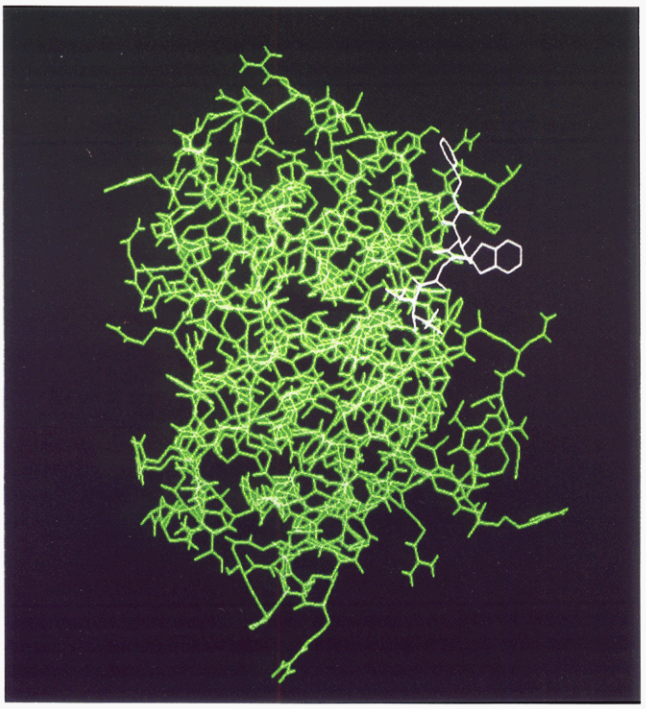


Figure 2. Minimized structure of HLE–inhibitor **20h** complex. The inhibitor **20h** is pink.

hemorrhage in an elastase-induced pulmonary hemorrhage (EPH) model in hamsters (see Table IV). Whereas the P_3-P_2 methylene isostere **38c** is inactive in the EPH model (10 μg administered it. causing a maximum inhibition of hemorrhage of only 2.7%), the closely related derivative **20i**, which contains a P_3-P_2 amide bond, has an ED_{50} of 4.8 μg it. per animal (10 μg administered it. causing an inhibition of hemorrhage of 90.5%).

The results presented in Table I also show that the most preferred residue at P_1 is valine [e.g. inhibitor **20i** (BIRA-260) (IC_{50} = 0.084 μM)]. There is a 10-fold decrease

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

compd[a]	dose, μg/mL (it.)	N[b]	max hemorrhage[c]
20b	20	4	85.1
20f	20	2	99.2
20g	20	3	93.8
20h	20	2	24.2
20i[d]	3	4	58.1
	10	4	90.5
	30	4	99.3
20o	20	3	43.8
20p	20	4	84.3
20q	10	4	96.7
20s	20	4	98.4
20x	10	4	96.2
20aa	20	3	88.4
38c	10	4	2.7
41e	10	4	7.7
1	100	3	0
4[e]	1	3	33.3
	3	4	79.4
	10	4	98.5

[a] Compounds were administered intratracheally (it.) in a 0.1-mL volume (DMSO, 1:100 in saline) followed 5 min latter by 50 μg (it.) of HLE in 0.1 mL of saline. [b] Number of animals. [c] Average percent reduction of red blood cells (RBC) per mL of cell suspension over vehicle control. [d] ED_{50} = 3.8 μg per animal. [e] ED_{50} = 1.3 μg per animal.

in potency when the valine residue at the P_1-position is replaced by alanine [**20l** (IC_{50} = 0.817 μM)]. Likewise, replacement of the P_1-valine in **20i** to the corresponding phenylalanine (**20o**), α,α-dimethylglycine (**20m**), or glycine (**20k**) all afford inactive compounds ($IC_{50} \gg 10$ μM). This high specificity of HLE for valine at P_1 has previously been observed and is referred to as the primary specificity site of HLE. The S_1-area of HLE is hydrophobic in nature and is well adapted to accommodate medium-sized aliphatic side chains such as valine and leucine. Accommodation of large side chains such as phenylalanine require an expansion of the S_1-region, which may result in unfavorable

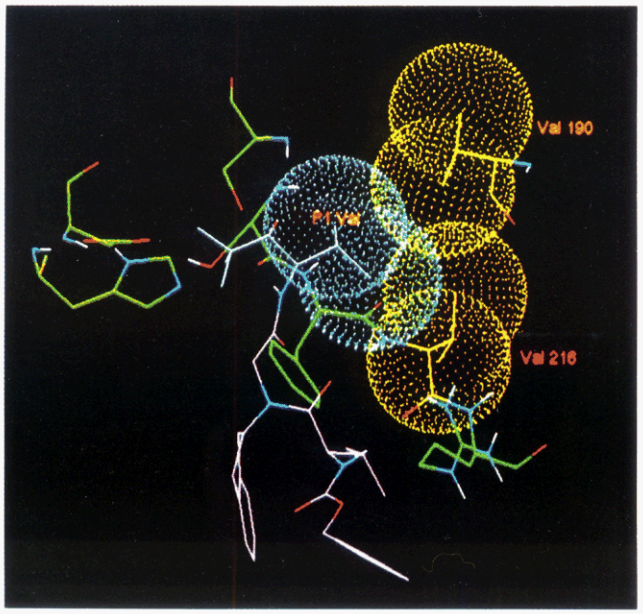


Figure 3. Minimized structure of the HLE–inhibitor **20h** (P_1-Val) complex illustrating orbital overlap between the P_1-side chain (blue) with the side chains of Val^{190} and Val^{216} (yellow). The van der Waals surfaces of the side chains of Val^{190} and Val^{216} of HLE and that of the P_1-Val residue of the inhibitor are represented as dot surfaces.

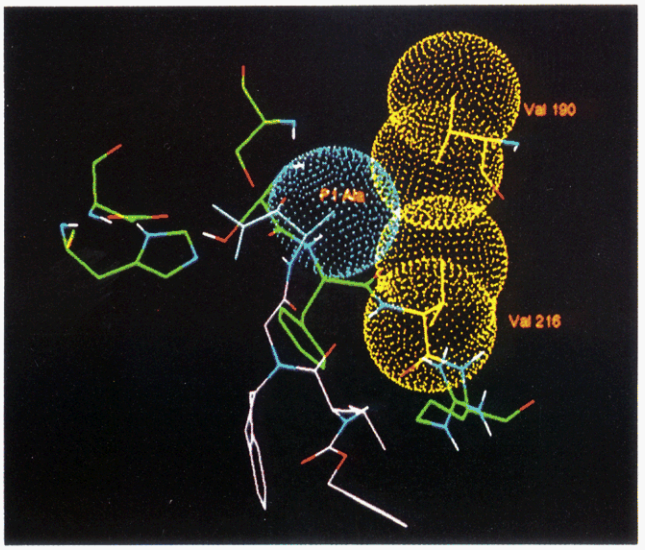


Figure 4. Minimized structure of the CBZ derivative of **20l** (P_1-Ala) complexed with HLE illustrating orbital overlap between the P_1-side chain (blue) with the side chains of Val^{190} and Val^{216} (yellow).

steric interactions. It is tempting to speculate as to why analogues incorporating smaller residues at P_1, for example Ala, leads to a reduction in in vitro potency. As illustrated in Figure 3, it can be seen from molecular modeling studies that, in the enzyme–inhibitor complex, the side chain of the Val residue at P_1 of **20h** is involved in favorable hydrophobic contact with those of Val^{190} and Val^{216} of the enzyme. Replacement of Val at P_1 by Ala to give **20l** leads to a smaller side chain which does not extend deep enough into the hydrophobic pocket in order to elicit such favorable interactions (Figure 4). It can be further surmised from molecular modeling studies that, while the side chain of Ile can also be accommodated easily into the S_1 pocket, fitting of the lengthier and hence more extended side chain of Leu (with methyl branching at the C_δ-position as compared to C_β in Ile) into the pocket leads to significant unfavorable steric interactions between the terminal methyls of the Leu side chain and those of Val^{190} and Val^{216} of the enzyme. The inactivity of *gem*-dimethyl species **20m** may be due to a combination of factors, side chains too short to elicit effective lipophilic binding with the residues of the S_1-pocket and/or steric compression around the trifluoromethyl carbonyl moiety, consequently making it inaccessible to the attack of Ser^{195}.

In the homologous series displayed in Table II, **20i** (IC_{50} = 0.084 μM), which spans P_5-P_1, exhibits the best in vitro activity. However, when the P_5-P_4 substituent that exists in **20i** is removed to give the CBZ-truncated inhibitor **20h**, which spans instead only P_3-P_1, the in vitro activity decreases by approximately 10-fold (IC_{50} = 0.365 μM). When the inhibitor **20h** is further truncated to give dipeptide **32** (P_2-P_1) and ketone **34** (P_1), any remaining inhibitory activity is completely lost in both cases ($IC_{50} \gg 10$ μM).

In order to see if the valine trifluoromethyl ketone residue that occurs at P_1 in the inhibitor **20i** might be replaced by electron-withdrawing aryl ketones, tripeptide **41e** was prepared. The *p*-trifluoromethylphenyl residue was selected with the hope that the electron-withdrawing CF_3 group might, through the inductive effect, facilitate the formation of a hemiketal with the active site Ser^{195} in a manner analogous to that in the case of a trifluoromethyl ketone. However, as can be seen in Table III, this was not the case, as **41e** is completely devoid of activity. This lack of activity may be due to the increased steric requirements of the relatively large aryl substituent in the S_1-binding pocket; alternatively, the presence of a bulky group may also make the neighboring carbonyl moiety inaccessible to the attack of the active site Ser^{195}.

The inhibitors presented in Table I are also highly selective toward inhibition of HLE. The inhibitors have been tested against representative examples of all four classes of proteinases (e.g., serine, cysteine, aspartic, and metallo) and have been found to inhibit only HLE. Enzymes such as cathepsins D, B, and G, urokinase, TPA, thrombin, C_1-esterase, renin, plasmin, HIV-protease, thrombin, and trypsin are not inhibited ($IC_{50} \gg 10$ μM).

The results pertaining to the inhibition of elastase-induced pulmonary hemorrhage (EPH) in hamsters by selected representative agents are presented in Table IV. HLE induces acute hemorrhage in the hamster[23] 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, **20i** (IC_{50} = 0.084 μM), 5 min prior to HLE challenge, effectively inhibited hemorrhage in a dose-dependent manner with an ED_{50} of 4.8 μg. It can also be seen that the in vitro potencies closely parallel the in vivo activities as measured in the EPH model. Furthermore, CBZ substituents at P_4 afford inactive in vivo compounds. For example, the corresponding sulfonamide analogue **20i** of the in vivo inactive CBZ derivative **20h** is quite active in vivo, exhibiting 90.5% inhibition against hemorrhage at 10 μg/mL it. The dose–response curves for the inhibitors **20i** (ED_{50} = 4.8 μg it. per animal) and **4** (ED_{50} = 1.3 μg it. per animal) are presented in Figure 5. Interestingly, the in vitro potency of cephalosporin 1[35] (IC_{50} = 0.02 μM, lit.[35] value 1.33 μM) does not translate into in vivo activity (0% inhibition of hemorrhage when tested at 100 μg/mL in our laboratories).

Inhibitor **20i**, when administered (20 μg it.) to hamsters at 24, 48, and 72 h prior to HLE challenge, exhibited significant inhibition against hemorrhage at all time points, 97%, 64%, and 49%, respectively[47b] (Figure 6). When

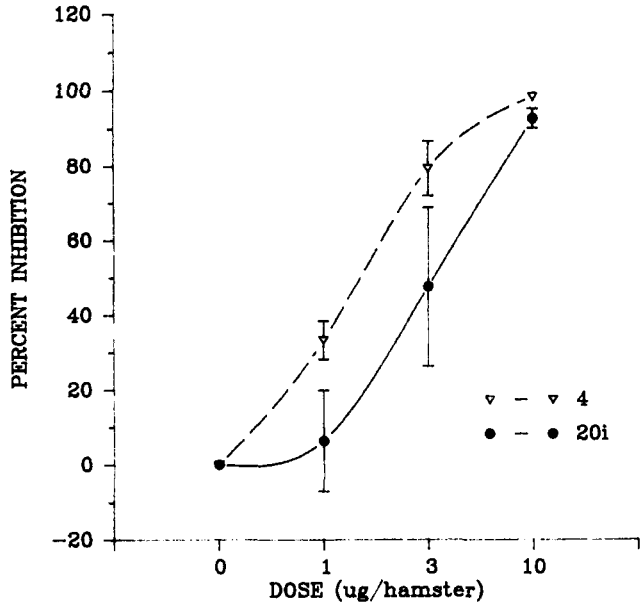


Figure 5. Inhibition of elastase-induced pulmonary hemorrhage (EPH) in hamsters by **20i** and **4**. Compounds were administered intratracheally (it.) in a 0.1-mL volume (DMSO, 1:100 in saline) followed 5 min later by 50 μg (it.) of HLE in 0.1 mL of saline. Average percent reduction of red blood cells (RBC) per milliliter of cell suspension over vehicle control. Data represents mean ± SD, $N = 4$. $ED_{50} = 3.8$ μg it. per animal for **20i**. $ED_{50} = 1.3$ μg it. per animal for **4**.

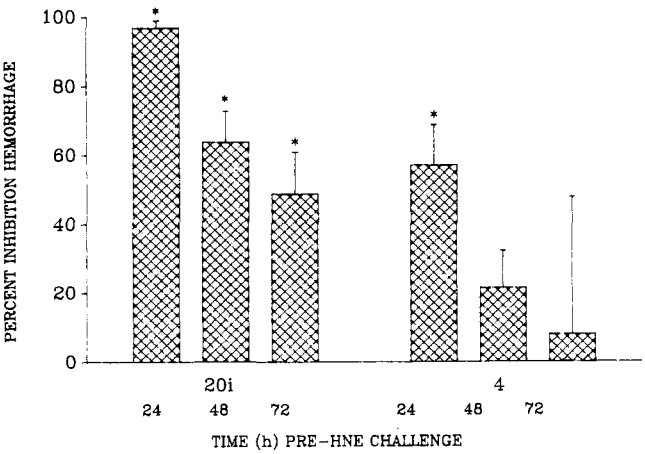


Figure 6. Effects of **20i** and **4** on EPH: duration of action. Compounds were administered at 20 μg it. to hamsters at the indicated time intervals prior to challenge with 50 μg of HLE it.

directly compared in our laboratories, the previously reported[47] P_2-proline derivative **4** exhibits significant inhibition (59%) against hemorrhage only at the 24-h time point. At the 48- and 72-h time points, the inhibitory effect of **4** is rather insignificant (21% and 7%, respectively). It is also of interest to note that the maximum inhibition observed for **4** was only 59%, whereas for **20i** it was 97% when administered at equivalent doses. Also, ex vivo pretreatment of HLE with 20 μg of **20i** prior to it. administration to hamsters inhibited hemorrhage by 96.7%.

Previous research[23] has shown that purified preparations of elastase from neutrophils and sputum (from patients with cystic fibrosis) can lead to emphysema[68] when instilled into the lungs of dogs and hamsters. Thus, in a 21-day chronic model of emphysema in hamsters, 20 μg of HLE administered it. causes an elastase-induced emphysematous state which can be quantitated histologically utilizing image analysis. Under these conditions, **20i** significantly inhibited pulmonary lesions associated with septal destruction and increased alveolar spaces (Figure 7).

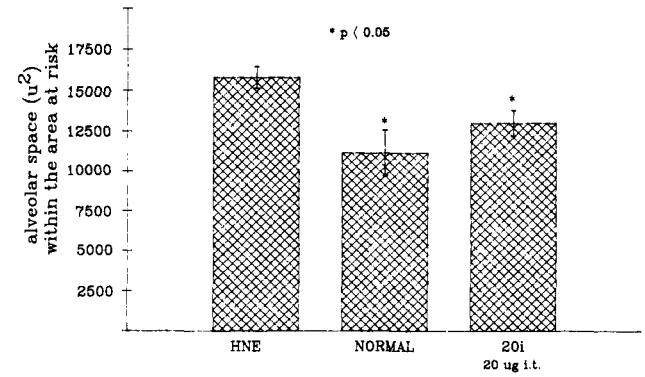


Figure 7. Effects of **20i** on 21-day elastase-induced emphysema in hamsters. Five animals per group were used. HNE = Compound vehicle plus 200 μg of HLE administered it. Normal = no treatment. Compound **20i** was administered at 20 μg it., 5 min prior to a 50 μg it. challenge with HLE.

In conclusion, the tripeptides presented in Table I containing achiral N-substituted glycine residues at P_2 in replacement of L-proline and having a trifluoromethyl ketone of valine at P_1 are effective in vitro HLE inhibitors with IC_{50} values in the submicromolar range. Sterically demanding substituents on the P_2-nitrogen have no detrimental effect on in vitro potency. In an in vivo situation, the inhibitors reported in the present paper have also been found to inhibit hemorrhage in a model of elastase-induced pulmonary hemorrhage in hamsters when administered it. One of the most active compounds of the series, **20i** (IC_{50} = 0.084 μM), showed significant activity in this assay (ED_{50} = 4.8 μg) for 72 h. As a comparison, a previously reported[47] L-proline derivative, **4**, showed significant activity in our laboratories for only 24 h. In a 21-day chronic model of emphysema in hamsters, **20i** significantly inhibited pulmonary lesions associated with septal destruction and increased alveolar spaces, when dosed at 20 μg it., 5 min prior to challenge with HLE.

Experimental Section

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 60F-254 plates of 0.25-mm thickness and were visualized with UV, I_2, or ninhydrin spray reagent. Preparative high-performance LC separations were determined on a Waters Prep LC/System 500 instrument. Chemical microanalyses for carbon, hydrogen, and nitrogen were conducted by Midwest Laboratories (Indianapolis, IN) and are within ±0.4% of theoretical values. Solid samples were purified by recrystallization and dried in vacuo at appropriate temperatures. IR spectra were determined on a Perkin-Elmer 781 spectrophotometer. Solid samples were taken in KBr pellets. Liquid samples were taken neat on NaCl salt plates. ^1H and ^{13}C NMR were determined with Varian EM-390 (^1H = 90 MHz), Bruker AM 500 (^1H = 500.13 MHz, ^{13}C = 125.77 MHz), Bruker AC 270 (^1H = 270.13 MHz, ^{13}C = 67.92 MHz), Bruker WM 250 (^1H = 250.13 MHz), or Bruker WP 100 (^{13}C = 25.18 MHz) spectrometers using $(CH_3)_4Si$ as an internal standard. Chemical shifts for ^1H NMR signals are reported in ppm downfield from TMS (δ). 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 μA emission current.

(68) For a review of animal models of emphysema, see: Snider, G. L.; Lucey, E. C.; Stone, P. J. Animal Models of Emphysema. *Am. Rev. Respir. Dis.* **1986**, *133*, 149–169.

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_3 (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 °C, the electron energy 200 eV, and the emission current 0.1 mA. The mass range, 50–650 Da, was scanned in 1.95 s. Optical rotations were determined at λ 589 (sodium D line) in CH_3OH with a Perkin-Elmer 241 polarimeter.

Method A. *N*-(2,3-Dihydro-1*H*-inden-2-yl)glycine Ethyl Ester Hydrochloride (12a; R_1 = 2-Indanyl). 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 $NaCNBH_3$ (25.8 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_2O. The product was extracted several times into EtOAc. The organic extract was washed consecutively with saturated aqueous solutions of $NaHCO_3$ and NaCl before being dried over $MgSO_4$ and filtered. After concentration under reduced pressure the oily residue was taken up in Et_2O (300 mL) and then cooled 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_2O to afford the title compound **12a** (21 g) as a colorless solid: mp 166–168 °C; ^1H NMR (DMSO-d_6) δ 10.1 (s, 2 H, $^+NH_2$), 7.1–7.3 (m, 4 H), 4.3–4.2 (q, 2 H, CH_2), 4.05 (bs, 3 H, CH_2 + CH), 3.3–3.2 (dd, 4 H, 2 × CH_2 of indanyl), 1.2–1.3 (t, 3 H, CH_3). Anal. ($C_{13}H_{17}NO_2$·HCl) C, H, N, Cl.

Method B. *N*-Cyclopentylglycine Ethyl Ester Hydrochloride (12a; R_1 = c-C_5H_9). Ethyl bromoacetate (167 g, 1.0 mol) in THF (200 mL) was added dropwise to a chilled (0–5 °C) solution of cyclopentylamine (85.2 g, 1.0 mol) and Et_3N (101.2 g, 1.0 mol) in THF (750 mL). After the addition was complete the mixture was warmed to room temperature and then stirred for 16 h at ambient temperature. The precipitated Et_3N·HCl was filtered and washed with a small amount of THF. The filtrate was concentrated under reduced pressure to yield an oil which was purified by chromatography over silica gel using C_6H_{14}/EtOAc (4:1) as the eluant. The collected product was dissolved in Et_2O and chilled by means of an ice/water bath. Dry hydrogen chloride was bubbled into the solution whereby the HCl salt of the product precipitated. Filtration afforded the title compound **12a** as a colorless solid (138 g, 66.5%): mp 174–176 °C; ^1H NMR ($CDCl_3$) δ 9.8 (s, 2 H, $^+NH_2$), 4.3–4.2 (q, 2 H, CH_2), 3.85 (s, 2 H, α-CH_2 of Gly), 3.7–3.6 (m, 1 H, CH), 2.0–1.8 (m, 6 H, 3 × CH_2), 1.65–1.5 (m, 2 H, CH_2), 1.3–1.2 (t, 3 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 165.6 (CO), 61.8 (CH_2), 59.4 (CH), 46.1 (α-CH_2 of Gly), 28.9 (2 × CH_2), 23.5 (2 × CH_2), 13.6 (CH_3). Anal. ($C_9H_{17}NO_2$·HCl) C, H, N, Cl.

2-Methyl-1-nitropropane (6a).[69] This material was prepared by the previously reported method.[69] The crude product was vacuum distilled (bp 55–60 °C, 50 mmHg; lit.[69] bp 71 °C, 65 mm), to give **6a** (75.5%) as a colorless oil which was used directly: ^1H NMR ($CDCl_3$) δ 4.2 (d, 2 H, CH_2), 2.6–2.4 (m, 1 H, CH), 1.1–1.0 (d, 6 H, 2 × CH_3); ^{13}C NMR ($CDCl_3$) δ 82.7 (CH_2NO_2), 27.9 (CH), 19.4 (2 × CH_3).

[(2*R*,3*S*)+(2*S*,3*R*)]-4-Methyl-3-nitro-1,1,1-trifluoro-2-pentanol (7a).[70] A mixture of **6a** (38.9 g, 0.377 mol), $CF_3CH(OC_2H_5)OH$ (90%, 60.4 g, 0.377 mol), and K_2CO_3 (2.15 g, 0.0156 mol) was stirred at 60 °C for 3 h followed by 3 days at room temperature as previously described.[70] After the usual workup an oily residue was obtained which was placed in a freezer overnight whereby *dl-threo*-4-methyl-3-nitro-1,1,1-trifluoro-2-pentanol (**7a**) crystallized. The solid was filtered and washed with cold petroleum ether (bp 37–50 °C) to yield the *dl*-threo isomer **7a** as a colorless solid: mp 80–82 °C; ^1H NMR ($CDCl_3$) δ 4.5 (t, 1 H), 4.6 (bm, 1 H), 3.8 (d, 1 H, OH), 2.45–2.55 (m, 1 H), 1.0–1.2 (dd, 6 H, 2 × CH_3); ^{13}C NMR (DMSO-d_6) 124.1 (q, CF_3, J = 284.54 Hz), 92.0 ($CHNO_2$), 67.4 (q, $CH(OH)CF_3$, J = 30.9 Hz), 22.7 (CH), 18.5, 16.9 (CH_3). Anal. ($C_6H_{10}F_3NO_3$) C, H, N. From the filtrate the *dl*-erythro isomer was obtained as a pale yellow oil.

[(2*R*,3*S*)+(2*S*,3*R*)]-3-Amino-4-methyl-1,1,1-trifluoro-2-pentanol Hydrochloride (8a).[70] The *dl-threo*-nitro compound **7a**, corresponding to the 2*R*,3*S*+2*S*,3*R* diastereomer (21.8 g, 0.108 mol), was reduced with $LiAlH_4$ as previously reported[70] to give amine **8a**. The *dl-threo*-amino hydrochloride **8a** (PG = H) was prepared in the usual way to afford a colorless solid (11 g, 49%): mp 123–125 °C (lit.[70] mp 118–120 °C); MS (CI/NH_3) m/z (relative intensity) 172 (MH^+, 100); ^1H NMR (DMSO-d_6) δ 8.5–8.0 (2 × bs, 3 H), 7.6–7.2 (2 bd, 1 H, OH), 4.6–4.2 (2 bm, 1 H, CH), 3.2–3.1 (bs, 1 H, CH), 2.2–2.0 (bm, 1 H, CH), 1.1–0.9 (m, 6 H, 2 × CH_3); ^{13}C NMR (DMSO-d_6) δ 124.65 (q, CF_3), 69.5–63.3 (m, $CH(OH)CF_3$), 55.4, 53.8 ($CHNH_2$), 28.7, 26.2 (CH), 17.6, 17.1 (CH_3). Anal. ($C_6H_{12}F_3NO$·HCl) C, H, N. The free base **8a** (PG = H) could be regenerated from the hydrochloride under standard conditions to give a colorless solid: mp 80–82 °C; ^1H NMR ($CDCl_3$) δ 5.3–4.8 (bs, 1 H, OH), 3.9–3.7 (q, 1 H, CH), 3.1–2.9 (dd, 1 H, CH), 2.0–1.8 (m, 1 H, CH), 1.5–1.2 (bs, 2 H, NH_2), 1.2–1.0 (m, 6 H, 2 × CH_3).

[(2*S*,3*S*)+(2*R*,3*R*)]-3-Amino-4-methyl-1,1,1-trifluoro-2-pentanol (8a). The *dl-erythro*-nitro isomer **7a**, corresponding to the 2*S*,3*S* + 2*R*,3*R* diastereomer, was reduced by Raney nickel in CH_3OH at 45 psi for 1.5 h. The catalyst was filtered and the filtrate was concentrated in vacuo to give the crude amine. Flash chromatography over silica gel first with CH_2Cl_2 and then with 3% CH_3OH in CH_2Cl_2 gave the pure *dl*-erythro isomer **8a** (PG = H) as a colorless solid: mp 63–65 °C; ^1H NMR ($CDCl_3$) δ 4.2–4.0 (m, 1 H, CH), 2.8–2.6 (m, 1 H, CH), 2.1–1.9 (m, 1 H, CH), 1.3–1.0 (m, 6 H, 2 × CH_3). Anal. ($C_6H_{12}F_3NO$) C, H, N.

Method C. *N*-(Carbobenzyloxy)-L-valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine Ethyl Ester (15a; R_2 = 2-indanyl, R_3 = Et). To a solution of CBZ-L-valine (5.0 g, 0.02 mol) in CH_2Cl_2 (70 mL) were added DMAP (2.44 g, 0.02 mol), **12a** (R_1 = 2-indanyl, R_2 = Et) (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_2Cl_2 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_2CO_3, 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 product was purified by chromatography over silica gel (CH_2Cl_2) to give the title compound **15a** as a colorless oil, R_f 0.7 [silica gel; CH_2Cl_2/CH_3OH (97:3)], which was used directly without further purification: MS (CI/CH_4) m/z (relative intensity) 453 (MH^+, 96), 345 (100), 310 (34), 220 (84), 117 (21), 116 (23); ^1H NMR ($CDCl_3$) 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_3), 1.1–0.9 (dd, 6 H, 2 × CH_3 of Val); ^{13}C NMR ($CDCl_3$) δ 171.9 (CO), 168.9 (CO), 156.2 (CO), 140.0, 139.9, 136.5, 128.3, 127.8, 127.0, 126.9, 124.4, 124.3, 66.7 ($PhCH_2$), 60.8 (CH_2), 56.9 (α-CH of Val), 56.5 (CH of indanyl), 44.0 (α-CH_2 of Gly), 37.6 (CH_2 of indanyl), 37.0 (CH_2 of indanyl), 31.7 (β-CH of Val), 19.4 (CH_3 of Val), 17.1 (CH_3 of Val), 13.6 (CH_3).

Method D. *N*-(Carbobenzyloxy)-L-valyl-*N*-(3,4-dimethoxyphenethyl)glycine Ethyl Ester (15a; R_2 = 3,4-dimethoxyphenethyl, R_3 = Et). By a procedure similar to method C, but employing CDI as the coupling reagent, CBZ-L-valine (6.3 g, 0.025 mol) in CH_2Cl_2 (60 mL) was condensed with *N*-(3,4-dimethoxyphenethyl)glycine ethyl ester (2.7 g, 0.0268 mol) to give the title compound **15a** as a pale yellow oil (4.3 g), R_f 0.6 [silica gel; CH_2Cl_2/CH_3OH (97:3)], which was used directly without further purification: MS (FAB) m/z (relative intensity) 501.5 (100); ^{13}C NMR ($CDCl_3$) δ 172.4 (CO), 168.6 (CO), 156.1 (CO), 136.3, 130.1, 128.0, 127.8, 127.7, 127.5, 126.7, 120.5, 112.1, 111.6, 66.5 ($PhCH_2$), 60.9 (CH_2), 55.7 (OCH_3), 55.4 (α-CH of Val), 50.7 (CH_2), 48.7 (α-CH_2 of Gly), 34.8 (CH_2), 30.9 (β-CH of Val), 18.9 (CH_3 of Val), 17.2 (CH_3 of Val), 13.8 (CH_3).

Method E. *N*-(Carbobenzyloxy)-L-valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine (15c; R_2 = 2-indanyl). The corresponding CBZ ethyl ester derivative **15a** (R_2 = 2-indanyl, R_2 = Et) (13.8 g, 0.0305 mol) was dissolved in EtOH (200 mL) and then treated with 1 N aqueous KOH (30 mL) in portions of 5 mL. The mixture was stirred at room temperature for 16 h. The EtOH was concentrated under reduced pressure and the residue was

(69) Kornblum, N.; Taub, B.; Ungnade, H. E. The Reaction of Silver Nitrite with Primary Alkyl Halides. *J. Am. Chem. Soc.* **1954**, *76*, 3209–3211.

(70) Bergeson, S.; Schwartz, J. A.; Stein, M. M.; Wildonger, R. A.; Edwards, P. D.; Shaw, A.; Trainor, D. A.; Wolaunin, D. J. EP Patent Appl. 0 189 305, 1986.

treated with H_2O. 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 ($MgSO_4$), filtered, and concentrated under reduced pressure to afford the pure title compound **15c** as a colorless semisolid (9.1 g): MS (CI/NH_3) m/z (relative intensity) 425 (MH^+, 53), 317 (98), 258 (15), 192 (34); ^1H NMR ($CDCl_3$) δ 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), 4.1 (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); ^{13}C NMR ($CDCl_3$) δ 172.7 (CO), 171.9 (CO), 156.3 (CO), 139.9, 136.3, 128.3, 127.9, 127.8, 127.0, 126.6, 124.3, 66.9 (PhCH_2), 56.6 (CH of indanyl), 55.9 (α-CH of Val), 44.0 (α-CH_2 of Gly), 36.9 (2 × CH_2 of indanyl), 31.5 (β-CH of Val), 19.2 (CH_3 of Val), 17.3 (CH_3 of Val). Anal. ($C_{24}H_{28}N_2O_5$) C, H, N.

Method F. [(2*R*,3*S*)+(2*S*,3*R*)]-*N*-(Carbobenzyloxy)-L-valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-trifluoro-4-methyl-2-hydroxypentyl)]amide (16; R_1 = H; R_2 = 2-indanyl, R_3 = $CH(CH_3)_2$, R_4 = H). To a solution of **15c** (R_2 = 2-indanyl) (4.25 g, 0.01 mol) in CH_2Cl_2 (60 mL) was added CDI (1.62 g, 0.01 mol). After 2 h of stirring at room temperature a suspension of *dl-threo*-**8a** (PG = H) hydrochloride (2.1 g, 0.01 mol) and Et_3N (1.01 g, 0.01 mol) in CH_2Cl_2 (30 mL) was added. The mixture was stirred for 16 h and afterward it was concentrated under reduced pressure. The remaining residue was treated with EtOAc and washed sequentially with 1 N aqueous HCl, 5% aqueous Na_2CO_3, and saturated aqueous NaCl. The organic phase was dried ($MgSO_4$), filtered, and concentrated to yield an oil which was purified over silica gel [CH_2Cl_2/CH_3OH (97:3)]. The title compound **16** was obtained (4.5 g, 78%) as a colorless solid: mp 64–67 °C; MS (CI/CH_4) m/z (relative intensity) 578 (MH^+, 88), 470 (46), 407 (100), 345 (89), 299 (28), 172 (13); ^1H NMR ($CDCl_3$) δ 7.3 (s, 5 H), 7.15 (s, 4 H), 5.6–5.5 (d, 1 H), 5.15–5.0 (m, 3 H), 4.6–4.5 (m, 1 H), 4.2–3.8 (m, 6 H), 3.3–2.9 (m, 4 H), 2.2–1.9 (m, 2 H), 1.05–0.8 (m, 12 H, 4 × δ-CH_3 of Val); ^{13}C NMR ($CDCl_3$) δ 173.6 (CO), 170.5 (CO), 156.6 (CO), 139.7, 136.3, 128.4, 128.1, 127.9, 127.1, 124.1, 71.5–68.9 (q, $CH(OH)CF_3$), 67.1 (PhCH_2), 57.9–56.4 (CH of indanyl), 55.8 (α-CH of Val), 46.9 (α-CH_2 of Gly), 36.9 (2 × CH_2 of indanyl), 31.3, 28.4 (β-CH of Val), 19.3 (CH_3 of Val), 18.7 (CH_3 of Val), 18.2 (CH_3 of Val), 17.5 (CH_3 of Val). Anal. ($C_{30}H_{38}F_3N_3O_5$) C, H, N.

Method G. (3*RS*)-*N*-(Carbobenzyloxy)-L-valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-trifluoro-4-methyl-2-oxopentyl)]amide (20h, *R* = OCH_2Ph). To a solution of **16** (R_1 = H, R_2 = 2-indanyl, R_3 = $CH(CH_3)_2$, R_4 = H) (4.3 g, 7.45 mmol) in THF (120 mL) was added the Dess–Martin periodinane reagent[60] (9.5 g, 22.3 mmol) in CH_2Cl_2 (110 mL). Trifluoroacetic acid (2.55 g, 22.3 mmol) was slowly added and the reaction was stirred at room temperature for 16 h. The reaction was concentrated under reduced pressure and the residue was treated with a mixture of EtOAc and saturated aqueous solutions of $NaHCO_3$ and $Na_2S_2O_3$. The organic layer was separated and washed repeatedly with dilute aqueous solutions of $NaHCO_3$ and $Na_2S_2O_3$. After a final wash with brine, the organic extract was dried ($MgSO_4$), filtered, and concentrated to afford a solid which was further purified by chromatography over silica gel using a gradient system of first 100% CH_2Cl_2 followed by CH_2Cl_2/CH_3OH (97:3). The title compound **20h** was obtained as a colorless solid (3.1 g, 72%): mp 49–54 °C; MS (CI/CH_4) m/z (relative intensity) 576 (MH^+, 100), 468 (29), 407 (95), 343 (46); ^1H NMR ($CDCl_3$) δ 7.4 (s, 5 H), 7.25 (s, 4 H), 7.1 (d, 1 H), 5.55 (m, 1 H), 5.25–5.0 (m, 4 H), 4.8–4.7 (m, 1 H), 4.3 (d, 1 H), 3.9 (d, 1 H), 3.5–3.1 (m, 4 H), 2.4 (m, 1 H), 2.25 (m, 1 H), 1.2–0.9 (m, 12 H); ^{13}C NMR ($CDCl_3$) δ 201.9 ($COCF_3$), 173.6 (CO), 169.4 (CO), 156.4 (CO), 139.7, 136.3, 128.4, 128.1, 127.9, 127.0, 124.5, 67.0 (PhCH_2), 59.0, 57.9, 56.3 (CH of indanyl), 46.7 (α-CH_2 of Gly), 37.1 (2 × CH_2 of indanyl), 31.5 (β-CH of Val), 29.0 (β-CH of Val), 19.5 (CH_3 of Val), 18.5 (CH_3 of Val), 17.5 (CH_3 of Val), 16.6 (CH_3 of Val). Anal. ($C_{30}H_{36}F_3N_3O_5$) C, H, N.

Method H. [(2*R*,3*S*)+(2*S*,3*R*)]-L-Valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-Trifluoro-4-methyl-2-hydroxypentyl)]amide (18; R_1 = H, R_2 = 2-indanyl, R_3 = $CH(CH_3)_2$, R_4 = H). To a solution of **16** (R_1 = H, R_2 = 2-indanyl, R_3 = $CH(CH_3)_2$, R_4 = H) (1.7 g, 2.94 mmol) in EtOH (65 mL) was added 10% Pd/C (100 mg) and then the resulting mixture was hydrogenated at 45 psi by means of a Parr shaker for 3 h. The reaction was filtered through Celite and the filtrate was concentrated under reduced pressure to afford the title compound **18** as a semisolid which was used directly without further purification: MS (CI/CH_4) m/z (relative intensity) 444 (MH^+, 29), 426 (14), 345 (100), 273 (27); ^1H NMR ($CDCl_4$) δ 7.2–7.05 (m, 4 H), 5.7–5.5 (m, 1 H), 5.1–4.8 (m, 1 H), 4.3–3.6 (m, 6 H), 2.2–1.8 (m, 2 H), 1.1–0.8 (m, 12 H); ^{13}C NMR ($CDCl_3$) δ 176.0 (CO), 175.3 (CO), 140.0, 127.0, 124.5, 71.0–69.0 (m, $CH(OH)CF_3$), 56.9 (CH of indanyl), 54.4, 53.9 (α-CH of Val), 46.6 (α-CH_2 of Gly), 37.1 (2 × CH_2 of indanyl), 30.1 (β-CH of Val), 29.5 (β-CH of Val), 19.2 (CH_3 of Val), 18.7 (CH_3 of Val), 17.7 (CH_3 of Val), 16.7 (CH_3 of Val).

Method I. [(2*R*,3*S*)+(2*S*,3*R*)]-L-Valyl-*N*-cyclooctylglycine *N*-[3-(1,1,1-Trifluoro-4-methyl-2-hydroxypentyl)]amide (18; R_1 = H, R_2 = c-C_8H_{15}, R_3 = $CH(CH_3)_2$, R_4 = H). *t*-BOC compound **16** (R_1 = H, R_2 = c-C_8H_{15}, R_3 = $CH(CH_3)_2$, R_4 = H) (3.2 g, 6 mmol) was dissolved in Et_2O (30 mL) and then chilled to 5 °C by means of an ice/H_2O bath. Diethyl ether (8 mL) which had previously been saturated with dry HCl was added. The mixture was stirred at 5 °C for 15 min and then for 6 h at room temperature. The reaction was concentrated under reduced pressure to afford the pure title amino hydrochloride **18** as a colorless solid (2.0 g), mp 146–156 °C. Anal. ($C_{21}H_{36}F_3N_3O_3$·HCl) C, H, N.

Terephthalic Acid Di-*tert*-butyl Ester (22).[70] This material was prepared from terephthaloyl chloride according to the previously reported procedure.[70] The crude product was recrystallized from CH_3OH to give **22** (78%) as a colorless crystalline solid: mp 116–118 °C; ^1H NMR (DMSO-d_6) δ 8.15–8.0 (m, 4 H), 1.55 (s, 18 H, 6 × CH_3).

Terephthalic Acid Mono-*tert*-butyl Ester (23).[70] This material was prepared from **22** according to the previously described method[70] to give **23** as a colorless solid (96%): mp 100–102 °C; ^1H NMR ($CDCl_3$) δ 12.0–11.6 (bs, 1 H, COOH), 8.2–8.0 (m, 4 H), 1.64 (s, 9 H, CH_3).

***tert*-Butyl 4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]benzoate (24a).**[70] According to the previously described procedure,[70] **24a** was obtained as a colorless solid (42.3%): mp > 300 °C; MS (CI/NH_3) m/z (relative intensity) 413 (M^+ + NH_4, 50), 239 (100); ^1H NMR (DMSO-d_6) δ 8.5–7.4 (bs, 1 H, NH), 8.0–7.8 (2 d, 6 H), 7.5 (d, 2 H), 1.55 (s, 9 H, 3 × CH_3). Anal. ($C_{18}H_{18}ClNO_5S$) C, H, N, Cl, S.

4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]benzenecarboxylic Acid (25a).[70] Compound **24a** was converted to the acid **25a** by treatment with trifluoroacetic acid as previously described[70] to give, after recrystallization from EtOH/H_2O (1:1), a colorless solid (63%): mp 285–287 °C; ^1H NMR (DMSO-d_6) δ 14.1–12.1 (bs, 2 H, NH, COOH), 8.1–7.9 (m, 6 H), 7.75 (d, 2 H); ^{13}C NMR (DMSO-d_6) δ 166.2 (CO), 164.2 (CO), 138.5, 138.1, 134.9, 134.6, 129.4, 129.1, 128.4. Anal. ($C_{14}H_{10}ClNO_5S$) C, H, N, Cl, S.

4-[[[(4-Bromophenyl)sulfonyl]amino]carbonyl]benzenecarboxylic Acid (25b).[70] This material was obtained in 68.4% yield in a manner analogous to the preparation of the corresponding chloro derivative **25a** described above, mp 272–273 °C (lit.[70] mp 193–194 °C). Anal. ($C_{14}H_{10}BrNO_5S$) C, H, Br, N, S.

Method J. [(2*R*,3*S*)+(2*S*,3*R*)]-*N*-[[4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]phenyl]oxomethyl]-L-valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-Trifluoro-4-methyl-2-hydroxypentyl)]amide (19; R_1 = H, R_2 = 2-indanyl, R_3 = $CH(CH_3)_2$, R_4 = H). To a solution of **18** (R_1 = H, R_2 = 2-indanyl, R_3 = $CH(CH_3)_2$, R_4 = H) (1.3 g, 2.93 mmol) and **25a** (0.9 g, 2.64 mmol) in THF (40 mL) cooled to 0–5 °C was added HOBT (0.36 g, 2.66 mmol) followed by WSCDI (0.56 g, 2.92 mmol). The resulting mixture was stirred at 0–5 °C for 30 min and then for 4 h at room temperature. The reaction was concentrated under reduced pressure and the residue was treated with EtOAc. The EtOAc was washed sequentially with 1 N aqueous HCl, 5% aqueous Na_2CO_3, and saturated aqueous NaCl. The organic phase was dried ($MgSO_4$), filtered, and concentrated to give a residue which was purified by chromatography over silica gel [CH_2Cl_2/CH_3OH (97:3)]. The desired title compound **19** was obtained as a colorless solid (1.8 g, 80%): mp 140–144 °C; MS (CI/NH_3) m/z (relative intensity) 765 (MH^+, 10), 747 (56), 591 (20), 573 (53), 421 (74), 377 (58), 345 (100), 327 (44), 247 (33); ^1H

NMR (CD_3OD) δ 8.1 (bs, 2 H), 8.0 (d, 2 H), 7.9–7.8 (m, 2 H), 7.45 (d, 2 H), 7.2–7.1 (m, 4 H), 5.3 (m, 1 H), 5.0 (m, 1 H), 4.0–3.7 (m, 6 H), 3.4–2.9 (m, 6 H), 2.3–2.1 (bm, 2 H), 1.2–0.8 (m, 12 H); ^{13}C NMR (CD_3OD) δ 174.0 (CO), 173.2 (CO), 170.6 (CO), 169.2 (CO), 143.3, 141.5, 141.1, 138.6, 138.0, 137.7, 130.0, 129.5, 129.2, 128.6, 127.8, 127.2, 71.1–68.5 (q, *C*H(OH)CF_3), 58.6, 56.6, 54.0, 46.4 (α-CH_2 of Gly), 38.3 (2 × CH_2 of indanyl), 32.0 (β-CH of Val), 29.1 (β-CH of Val), 20.0 (CH_3 of Val), 19.7 (CH_3 of Val), 19.2 (CH_3 of Val), 18.7 (CH_3 of Val). Anal. ($C_{36}H_{40}ClF_3N_4O_7S$) C, H, F, N, S.

Method K. (3*RS*)-*N*-[[4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]phenyl]oxomethyl]-L-valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-Trifluoro-4-methyl-2-oxopentyl)]amide (20i). To a solution of corresponding **19**, see compound directly above (1.6 g, 2.1 mmol), in THF (25 mL) was added the Dess–Martin periodinane reagent[60] (2.66 g, 6.3 mmol) in CH_2Cl_2 (45 mL). Trifluoroacetic acid (0.72 g, 6.3 mmol) was slowly added and then the reaction mixture was stirred at room temperature for 16 h. The reaction was concentrated under reduced pressure and the remaining residue was treated with a mixture of EtOAc and saturated aqueous solutions of $NaHCO_3$ and $Na_2S_2O_3$. After a final wash with brine the organic extract was dried ($MgSO_4$), filtered, and concentrated to afford a solid which was further purified by chromatography over silica gel [CH_2Cl_2/CH_3OH (first 97:3 and then 90:10)]. The desired title compound **20i** was obtained as a colorless solid (0.9 g): mp 218–226 °C; MS (CI/NH_3) *m*/*z* (relative intensity) 763 (MH^+, 2), 746 (2), 421 (6), 377 (14), 343 (100), 325 (31), 247 (8); ^1H NMR (CD_3OD) δ 8.1 (m, 2 H), 7.9 (d, 2 H), 7.8 (m, 2 H), 7.45 (d, 2 H), 7.1 (m, 4 H), 5.2 (bm, 1 H), 5.0 (d, 1 H), 4.4–3.8 (m, 4 H), 3.4–2.9 (m, 6 H), 2.4–2.1 (bm, 2 H), 1.1–0.7 (m, 12 H); ^{13}C NMR (CD_3OD) δ 174.1 (CO), 173.7 (CO), 170.7 (CO), 169.3 (CO), 143.6, 141.4, 138.7, 137.7, 130.2, 129.6, 129.0, 127.9, 125.3, 58.8, 56.6, 49.8, 46.4 (α-CH_2 of Gly), 38.3 (2 × CH_2 of indanyl), 32.2 (β-CH of Val), 29.0 (β-CH of Val), 20.9 (CH_3 of Val), 20.0 (CH_3 of Val), 19.2, (CH_3 of Val), 18.8 (CH_3 of Val). Anal. ($C_{36}H_{38}ClF_3N_4O_7S$) C, H, F, N, S.

***N*-(Carbobenzyloxy)-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine Ethyl Ester (30a; R_1 = 2-indanyl).** *N*-(2,3-Dihydro-1*H*-inden-2-yl)glycine ethyl ester (**12a**; R_1 = 2-indanyl) (3.2 g, 13.7 mmol) was dissolved in dry THF (120 mL). Triethylamine (2.77 g, 27.4 mmol) was added and the solution was chilled (0–5 °C) by means of an ice/H_2O bath. Benzyl chloroformate (2.33 g, 13.7 mmol) in THF (15 mL) was added dropwise while the temperature was maintained between 0 and 5 °C. After the addition was complete the mixture was allowed to reach room temperature and was then stirred for a further 16 h. Precipitated Et_3N·HCl was filtered off and the filtrate was concentrated in vacuo to afford **30a** as a colorless oil (4.0 g, 83%) which was used directly without further purification; ^1H NMR ($CDCl_3$) δ 7.3 (s, 5 H, Ph), 7.1 (s, 4 H, 2 × CH_2 of indanyl), 5.15 (s, 2 H, CH_2), 5.0 (m, 1 H, CH of indanyl), 4.2–4.1 (m, 2 H), 3.8 (s, 2 H, α-CH_2 of Gly), 3.3–2.9 (m, 4 H, 2 × CH_2 of indanyl), 1.1 (t, 3 H, CH_3).

***N*-(Carbobenzyloxy)-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine (30b; R_1 = 2-indanyl).** Crude ester **30a** (R_1 = 2-indanyl) (4.0 g, 11.2 mmol) was hydrolyzed to acid **30b** (R_1 = 2-indanyl) as described in method E (96%): mp 95–97 °C; ^1H NMR ($CDCl_3$) δ 10.1 (bs, 1 H), 7.3 (d, 5 H, Ph), 7.1 (s, 4 H, indanyl), 5.1 (s, 2 H), 5.0 (m, 1 H, CH of indanyl), 3.9 (s, 2 H, α-CH_2 of Gly), 3.3–2.9 (m, 4 H, 2 × CH_2 of indanyl). Anal. ($C_{19}H_{19}NO_4$) C, H, N.

[(2*R*,3*S*)+(2*S*,3*R*)]-*N*-(Carbobenzyloxy)-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-Trifluoro-4-methyl-2-hydroxypentyl)]amide (31; R_1 = 2-indanyl). According to method F, **30b** (R_1 = 2-indanyl) (3.3 g, 0.01 mol) was condensed with the hydrochloride of *dl-threo*-**8a** (PG = H) (2.2 g, 10.5 mmol) to give the title compound **31** as a colorless solid (3.85 g, 79.4%): mp 160–163 °C; MS (CI/CH_4) *m*/*z* (relative intensity) 479 (MH^+, 100), 461 (24), 435 (84), 371 (14), 345 (13), 308 (7), 116 (81); ^1H NMR ($CDCl_3$) δ 7.3 (s, 5 H, Ph), 7.1 (s, 4 H, indanyl), 7.0–6.3 (bs, 1 H, NH, exchangeable), 5.2 (s, 2 H), 5.0 (m, 1 H, CH of indanyl), 4.6 (bs, 1 H, OH, exchangeable), 4.1 (bm, 1 H, α-CH of Val), 3.85 (s, 2 H, α-CH_2 of Gly), 3.6 (m, 1 H), 3.3–2.9 (m, 4 H, 2 × CH_2 of indanyl), 2.1 (bm, 1 H, α-CH of Val), 0.95–0.8 (dd, 6 H, 2 × CH_3); ^{13}C NMR ($CDCl_3$) δ 167.4 (CO), 154.4 (CO), 139.4, 135.2, 126.9, 126.3, 126.1, 125.9, 125.1, 122.9, 121.7, 66.6 (q, *C*H(OH)CF_3, *J* = 29.43 Hz), 65.5 (s, CH_2), 55.6 (CH of indanyl), 45.8 (α-CH_2 of Gly), 35.2 (2 × CH_2 of indanyl), 28.9 (β-CH of Val), 18.0 (CH_3 of Val), 17.15 (CH_3 of Val). Anal. ($C_{25}H_{29}F_3N_2O_4$) C, H, F, N.

(3*RS*)-*N*-(Carbobenzyloxy)-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-Trifluoro-4-methyl-2-oxopentyl)]amide (32). By means similar to that described in method G **31** (R_1 = 2-indanyl) (2.7 g, 5.64 mmol) was oxidized to afford the title compound **32** as a colorless solid (1.9 g, 71%): mp 116–117 °C; MS (CI/CH_4) *m*/*z* (relative intensity) 477 (MH^+, 47), 461 (2), 433 (100), 305 (1), 241 (30), 146 (7), 117 (18), 116 (9); ^1H NMR ($CDCl_3$) δ 7.4 (s, 5 H, Ph), 7.2 (s, 4 H, indanyl), 5.3 (s, 2 H), 5.25–5.0 (2 m, 2 H, CH of indanyl, α-CH of Val), 4.1–3.9 (dd, 2 H, α-CH_2 of Gly), 3.4–3.0 (2 m, 4 H, 2 × CH_2 of indanyl), 2.4 (m, 1 H, β-CH of Val), 1.05 (d, 3 H, CH_3 of Val), 0.8 (d, 3 H, CH_3 of Val); ^{13}C NMR ($CDCl_3$) δ 191 (*C*OCF_3), 169.8 (CO), 156.8 (CO), 140.4, 140.3, 136.1, 128.6, 128.3, 128.0, 126.9, 124.6, 124.5, 68.1 (CH_2), 58.8, 57.4, 48.1 (α-CH_2 of Gly), 37.1 (CH_2 of indanyl), 36.9 (CH_2 of indanyl), 29.3 (β-CH of Val), 19.6 (CH_3 of Val), 16.4 (CH_3 of Val). Anal. ($C_{25}H_{27}F_3N_2O_4$) C, H, F, N.

[(2*R*,3*S*)+(2*S*,3*R*)]-*N*-(Carbobenzyloxy)-2-hydroxy-4-methyl-1,1,1-trifluoro-3-pentylamine (33). By a method similar to that described above for **30a** (R_1 = 2-indanyl) the *dl-threo*-amino hydrochloride **8a** (PG = H) was converted to the title compound **33** (95%). Product **33** was obtained as a colorless oil (4.3 g) which was used directly without further purification: ^1H NMR ($CDCl_3$) δ 7.3 (bs, 5 H, Ph), 5.5 (d, 1 H), 5.1 (s, 2 H), 4.9–4.4 (s, 1 H, OH), 4.1–4.0 (m, 1 H), 3.6–3.5 (m, 1 H), 2.05–1.9 (m, 1 H), 1.9 (m, 6 H); ^{13}C NMR ($CDCl_3$) δ 157 (CO), 136, 128.6, 128.4, 128.3, 127.6, 127.0, 124.5 (q, CF_3, *J* = 283 Hz), 70.1 (q, *C*H(OH)CF_3, *J* = 30.13 Hz), 67.1 (PhCH_2), 55.5, 30.3, 19.3, 18.6.

(3*RS*)-*N*-(Carbobenzyloxy)-4-methyl-2-oxo-1,1,1-trifluoro-3-pentylamine (34). By method G, **33** (0.79 g, 2.59 mmol) was oxidized to afford ketone **34** (0.70 g, 90%) as a pale yellow oil; MS (CI/CH_4) *m*/*z* (relative intensity) 304 (MH^+, 100), 260 (24), 238 (34), 214 (37), 198 (40), 181 (49), 170 (44), 153 (51). Anal. ($C_{14}H_{16}F_3NO_3$) C, H, N.

Method L. *N*-(*tert*-Butoxycarbonyl)-L-valyl-ψ(CH_2)-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine Ethyl Ester (36a). To a mixture of *t*-BOC-L-valinal (**35c**)[62] (2.0 g, 0.01 mol) and the hydrochloride of *N*-(2,3-dihydro-1*H*-inden-2-yl)glycine ethyl ester (**12a**; R_2 = 2-indanyl) (2.5 g, 0.01 mol) in absolute EtOH (50 mL) was added $NaCNBH_3$ (1.57 g, 0.025 mol) in portions. The resulting mixture was stirred for 16 h at room temperature. The EtOH was removed under reduced pressure and the residue was treated with H_2O and Et_2O. The layers were separated, and the Et_2O phase was washed with dilute aqueous Na_2CO_3, dried ($MgSO_4$), filtered, and concentrated to give crude **36a** (4.5 g). Column chromatography over silica gel eluting first with petroleum ether and then with incremental increases of 10–20% CH_2Cl_2 in petroleum ether afforded the desired pure product **36a** (660 mg, 16%). The major part of the product was eluted with 20% CH_2Cl_2 in petroleum ether; ^1H NMR ($CDCl_3$) δ 7.15 (m, 4 H), 4.8 (bs, 1 H, NH), 4.15 (q, 2 H, CH_2CH_3), 3.8 (q, 1 H, CH of indanyl), 3.6 (m, 1 H, α-CH of Val), 3.4 (s, 2 H, α-CH_2 of Gly), 3.1–2.8 (m, 4 H, 2 × CH_2 of indanyl), 2.7 (t, 1 H, CHCH_2N), 2.6 (t, 2 H, CHCH_2N), 2.0 (m, 1 H, α-CH of Val), 1.5 (s, 9 H, *t*-Bu), 1.25 (t, 3 H, CH_3), 0.9 (dd, 6 H, 2 × CH_3). Anal. ($C_{23}H_{36}N_2O_4$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-L-valyl-ψ(CH_2)-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine (36b).** To a solution of ethyl ester **36a** (890 mg, 2.20 mmol) in 95% EtOH (25 mL) was added 1 N aqueous NaOH (2.5 mL, 2.50 mmol). The resulting mixture was warmed to 60 °C for 1 h. The reaction was cooled to room temperature and then 1 N aqueous HCl (2.5 mL) was added dropwise to neutralize the solution. The resulting mixture was concentrated under reduced pressure while the temperature was maintained below 45 °C. The residue was dissolved in H_2O and the pH was adjusted to approximately 6 by the dropwise addition of 1 N aqueous HCl. The mixture was extracted several times into CH_2Cl_2. The combined CH_2Cl_2 extract was dried ($MgSO_4$), filtered, and concentrated to afford pure **36b** (730 mg, 88%); ^1H NMR ($CDCl_3$) δ 7.15 (s, 4 H), 4.9 (bs, 1 H, NH), 3.9 (m, 1 H, CH of indanyl), 3.7 (m, 1 H, α-CH of Val), 3.4 (m, 2 H, α-CH_2 of Gly), 3.2–2.7 (m, 6 H, CH_2N, 2 × CH_2 of indanyl, α-CH_2 of Gly), 1.8 (m, 1 H, β-CH of Val), 1.5 (s, 9 H, *t*-Bu), 0.9 (m, 6 H, 2 × CH_3 of Val). Anal. ($C_{21}H_{32}N_2O_4$) C, H, N.

[(2*R*,3*S*)+(2*S*,3*R*)]-*N*-(*tert*-Butoxycarbonyl)-L-valyl-ψ(CH_2)-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-

Trifluoro-4-methyl-2-hydroxypentyl)]amide (37a). By following a procedure similar to method F, the title compound **37a** was obtained as a colorless resin (81%): MS (CI/NH_3) m/z (relative intensity) 530 (MH^+, 100); ^1H NMR ($CDCl_3$) δ 8.7–8.4 (bs, 1 H, NH), 7.2 (s, 4 H), 5.8 (t, 1 H, OH), 4.9–4.7 (bs, 1 H, NH), 4.3 (m, 1 H, C*H*OH), 3.9 (m, 1 H, CH of indanyl), 3.7 (m, 1 H, α-CH of Val), 3.4 (m, 2 H, α-CH_2 of Gly), 3.2–2.7 (m, 6 H, NCH_2, 2 × CH_2 of indanyl), 2.3–2.1 (m, 1 H, β-CH of Val), 1.6 (m, 1 H, β-CH of Val), 1.5 (s, 9 H, *t*-Bu), 1.2–0.9 (m, 12 H, 4 × CH_3). Anal. ($C_{27}H_{42}F_3N_3O_4$) C, H, F, N.

(3*RS*)-*N*-(*tert*-Butoxycarbonyl)-L-valyl-ψ(CH_2)-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-Trifluoro-4-methyl-2-oxopentyl)]amide (37b). By following a procedure similar to method G, the title compound **37b** was obtained in 64% yield as a colorless resinous solid: MS (CI/NH_3) m/z (relative intensity) 528 (MH^+, 100); ^1H NMR ($CDCl_3$) δ 8.7–8.4 (bs, 1 H, NH), 7.2 (s, 4 H), 4.0–2.7 (m, 8 H, NCH_2, α-CH_2 of Gly, 2 × CH_2 of indanyl), 2.5 (m, 1 H, C*H*$COCF_3$), 1.9 (m, 2 H, 2 × β-CH of Val), 1.5 (s, 9 H, *t*-Bu), 1.2–0.9 (m, 12 H, 4 × CH_3). Anal. ($C_{27}H_{40}F_3N_3O_4 \cdot H_2O$) C, H, N.

[(2*R*,3*S*)+(2*S*,3*R*)]-L-Valyl-ψ(CH_2)-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-Trifluoro-4-methyl-2-hydroxypentyl)]amide (38a). By following a procedure similar to method I, *t*-BOC tripeptide **37a** (520 mg, 0.98 mmol) was converted to the title amino compound **38a** (440 mg, 90%). Product **38a** was obtained as a colorless resinous solid which was used directly without further purification. The ^1H NMR indicated the loss of a *t*-BOC group as desired: MS (CI/NH_3) m/z (relative intensity) 430 (MH^+, 100); ^1H NMR (DMSO-d_6) δ 7.18 (bs, 4 H), 4.1 (m, 1 H, C*H*OH), 4.0 (bs, 4 H, $^+NH_3$, ^+NH), 3.8 (m, 1 H, CH of indanyl), 3.6–2.6 (m, 10 H), 1.8 (m, 2 H, β-CH of Val), 0.8 (m, 12 H, 4 × CH_3 of Val).

[(2*R*,3*S*)+(2*S*,3*R*)]-*N*-[[4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]phenyl]oxomethyl]-L-valyl-ψ(CH_2)-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-Trifluoro-4-methyl-2-hydroxypentyl)]amide (38b). This material was prepared in 81% yield as a colorless resinous solid in a manner similar to that described in method J: MS (FAB/thioglycerol) m/z (relative intensity) 751 (M^+, 100); ^1H NMR (DMSO-d_6) δ 8.0–8.2 (m, 1 H, NH), 8.0–7.8 (m, 6 H), 7.4 (d, 2 H), 7.1 (m, 4 H, indanyl), 6.6 (m, 1 H, NH), 4.1–2.5 (m, 13 H), 1.8 (m, 2 H, 2 × β-CH of Val), 0.9 (m, 12 H, 4 × CH_3). Anal. ($C_{36}H_{42}ClF_3N_4O_6S \cdot H_2O$) C, H, N.

(3*RS*)-*N*-[[4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]phenyl]oxomethyl]-L-valyl-ψ(CH_2)-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-Trifluoro-4-methyl-2-oxypentyl)]amide (38c). By following a procedure similar to that described in method K, the title compound **38c** was obtained in 55% yield as a colorless resinous solid: MS (FAB/thioglycerol) m/z (relative intensity) 766 ($M^+ + H_2O$, 41), 748 (M^+, 40); ^{1}NMR (CF_3COOD) δ 8.3 (m, 6 H), 7.8 (d, 2 H), 7.4 (m, 4 H), 5.1–2.6 (m, α-protons), 2.5–2.1 (m, 2 H, 2 × β-CH of Val), 1.4–1.0 (m, 12 H, 4 × CH_3 of Val). Anal. ($C_{36}H_{40}ClF_3N_4O_6S \cdot 2.5H_2O \cdot Na$) C, H, Cl, N, Na, S.

Method M. (*S*)-[2-[(Benzyloxycarbonyl)amino]-3-methylbutanoyl]-*p*-(trifluoromethyl)benzene (39). To a suspension of Mg turnings (1.24 g, 0.051 mol) in dry THF (50 mL) was added 10 mL of a solution of 4-bromobenzotrifluoride (12.6 g, 0.056 mol) in dry THF (50 mL) under argon with slight heating (45–50 °C). After the Grignard reaction initiated the remainder of the solution was added dropwise and the heating was removed as the reaction mixture turned brown. At the end of the addition, the reaction mixture was further refluxed for 3.5 h. After cooling, the freshly formed Grignard reagent was then added quickly to a stirring solution of CBZ-L-valine methoxymethylamide (**35b**)[61] (5.0 g, 0.017 mol) in dry THF (100 mL) at room temperature. The resulting mixture was stirred at room temperature for 15 min before it was diluted with Et_2O. The Et_2O solution was washed with dilute aqueous HCl and twice with brine. The combined aqueous phase was back-extracted with Et_2O. The combined Et_2O extract was dried ($MgSO_4$), filtered, and concentrated to give a brown oil (8.12 g) which crystallized upon standing. The solid was triturated with a mixture of petroleum ether and Et_2O. The solid was filtered to give **39** as colorless crystals (3.37 g, 52%): mp 74–75 °C; ^1H NMR ($CDCl_3$) δ 8.0 (dd, 4 H), 7.4 (s, 5 H), 5.5 (bs, 1 H, NH), 5.3 (m, 1 H, α-CH of Val), 5.2 (dd, 2 H, $PhCH_2$), 2.0 (m, 1 H, β-CH of Val), 1.0 (d, 3 H, CH_3 of Val), 0.87 (d, 3 H, CH_3 of Val). Anal. ($C_{20}H_{20}F_3NO_3$) C, H, N.

Method N. [(2*S*,1*RS*)]-2-[(Benzyloxycarbonyl)amino]-3-methyl-1-[*p*-(trifluoromethyl)phenyl]-1-butanol (40a). Phenyl ketone **39** (2.86 g, 7.54 mmol) was dissolved in CH_3OH (25 mL) with stirring at room temperature. To this solution was added $NaBH_4$ (0.57 g, 15.1 mmol) in portions. After the usual workup the pure title compound **40a** (2.26 g, 79%) was obtained as a colorless solid: mp 97.5–100 °C; ^1H NMR ($CDCl_3$) δ 7.5 (dd, 4 H), 7.3 (s, 5 H, Ph), 5.1 (dd, 2 H, $PhCH_2$), 4.9 (t, 1 H, C*H*OH), 4.6 (d, 1 H, NH), 3.87 (q, 1 H, α-CH of Val), 2.95 (s, 1 H, OH), 1.75 (m, 1 H, β-CH of Val), 1.0 (d, 3 H, CH_3 of Val), 0.9 (d, 3 H, CH_3 of Val). Anal. ($C_{20}H_{22}F_3NO_3$) C, H, N.

(2*S*,1*RS*)-2-Amino-3-methyl-1-[*p*-(trifluoromethyl)phenyl]-1-butanol (40b). The title compound was prepared by means similar to method H. Purification by flash chromatography over silica gel was done by using a gradient system of EtOH in CH_2Cl_2. Most of the desired title product **40b** was collected from the 20% EtOH in CH_2Cl_2 fractions as a mixture of *SS* and *SR* diastereomers, which was used directly without further separation (83.5%). The NMR was consistent with the desired product and indicated the loss of a CBZ group; ^1H NMR ($CDCl_3$) δ 7.5 (dd, 4 H), 4.5 (q, 1 H, C*H*OH), 2.65 (t, 1 H, α-CH of Val), 2.2 (bs, 2 H, NH + OH), 1.6 (m, 1 H, β-CH of Val), 0.9 (dd, 6 H, 2 × CH_3 of Val).

(2*S*,1*RS*)-*N*-(Carbobenzyloxy)-L-valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[2-[3-Methyl-1-[4-(trifluoromethyl)phenyl]-1-hydroxybutyl]]amide (41a). The title compound **41a** was prepared by means similar to that described in method F. Purification by flash chromatography over silica gel using a gradient system of 15%, 20%, and 40% $(CH_3)_2CO$ in petroleum ether afforded pure **41a** (88%) as a colorless amorphous solid; ^1H NMR ($CDCl_3$) δ 7.5 (m, 4 H), 7.3 (s, 4 H), 7.1 (s, 4 H), 6.6 (m, 1 H, NH), 5.5 (m, 1 H, C*H*OH), 5.1 (m, 2 H, $PhCH_2$), 4.9–4.7 (m, 2 H, CH of indanyl), 4.6 (m, 1 H, α-CH of Val), 4.1 (m, 1 H, α-CH_2 of Gly), 3.7 (m, 1 H, α-CH_2 of Gly), 3.4–3.1 (m, 4 H, 2 × CH_2 of indanyl), 2.8 (bs, 1 H, OH), 1.8 (m, 2 H, 2 × α-CH of Val), 1.0–0.8 (m, 6 H, 2 × CH_3 of Val). Anal. ($C_{36}H_{42}F_3N_3O_3$) C, H, F, N.

(2*S*)-*N*-(Carbobenzyloxy)-L-valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[2-[3-Methyl-1-[4-(trifluoromethyl)phenyl]-1-oxobutyl]]amide (41b). The title compound **41b** was prepared by means similar to that described in method G. Purification was achieved by preparative TLC employing the solvent system $(CH_3)_2CO$/petroleum ether (1:4). In this manner the title compound **41b** was obtained as a colorless amorphous solid (605 mg, 81%): MS (CI/NH_3) m/z (relative intensity) 652 (MH^+, 100); ^1H NMR ($CDCl_3$) δ 8.0 (d, 2 H), 7.7 (d, 2 H), 7.3 (s, 5 H), 7.15 (m, 4 H), 6.95 (d, 1 H, NH), 5.6 (d, 1 H, NH), 5.5 (m, 1 H, α-CH of Val), 5.2–5.0 (m, 3 H, $PhCH_2$, CH of indanyl), 4.7 (m, 1 H, α-CH of Val), 4.0 (dd, 2 H, α-CH_2 of Gly), 3.2–2.9 (m, 4 H, 2 × CH_2 of indanyl), 2.1 (m, 2 H, β-CH of Val), 1.1 (m, 12 H, 4 × CH_3 of Val). Anal. ($C_{36}H_{40}F_3N_3O_3$) C, H, F, N.

(2*S*,1*RS*)-L-Valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[2-[3-Methyl-1-[4-(trifluoromethyl)phenyl]-1-hydroxybutyl]]amide (41c). The title compound **41c** was prepared by means similar to that described in method H to afford a colorless amorphous solid which was used directly without further purification. The NMR of this material was consistent with the desired product and showed the loss of the CBZ protecting group; ^1H NMR ($CDCl_3$) δ 7.5 (bs, 4 H), 7.18 (bs, 4 H), 6.7 (m, 1 H, NH), 5.6 (m, 1 H, OH), 4.8–2.6 (m, 6 H), 3.0 (bs, 2 H, NH_2), 1.8 (m, 2 H, 2 × β-CH of Val), 0.8 (m, 12 H, 4 × CH_3 of Val).

(2*S*)-[[4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]phenyl]oxomethyl]-L-valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)-glycine *N*-[2-[3-Methyl-1-[4-(trifluoromethyl)phenyl]-1-oxobutyl]]amide (41e). Acid **25a** (2.2 g, 6.14 mmol) was condensed with **41c** according to method J to give crude **41d** (5.6 g) as a yellow resinous powder which was used directly without further purification. Crude alcohol **41d** (1.0 g, 1.19 mmol) was oxidized to ketone **41e** by means similar to that described in method G. Crude product **41e** was purified by preparative TLC employing the solvent system 0.5% EtOAc and 3% EtOH in CH_2Cl_2. In this manner pure product **41e** was obtained as a colorless amorphous solid (396 mg): MS (FAB) m/e (relative intensity) 839 (M^+, 100); ^1H NMR (DMSO-d_6) δ 8.8–8.6 (m, 1 H,

NH), 9.0 (m, 8 H), 7.7 (d, 2 H), 7.1 (s, 4 H), 5.25 (m, 1 H, α-CH of Val), 4.8 (m, 1 H, CH of indanyl), 4.1 (d, 1 H, α-CH_2 of Gly), 3.7 (d, 1 H, α-CH_2 of Gly), 3.1–2.8 (m, 4 H, 2 × CH_2 of indanyl), 2.2 (m, 2 H, β-CH of Val), 1.0–0.7 (m, 12 H, 4 × CH_3 of Val). Anal. ($C_{42}H_{42}ClF_3N_4O_7S$) C, H, Cl, F, N.

N-(Carbobenzyloxy)-N-methyl-L-valine (14b; PG = CBZ). By means similar to that described above for the preparation of **30a** (R_1 = 2-indanyl), the title compound **14b** (PG = CBZ) was obtained in 57% yield as a colorless crystalline solid: mp 65–67 °C; ^1H NMR ($CDCl_3$) δ 10.2 (s, 1 H, COOH), 7.33 (s, 5 H), 5.17 (s, 2 H, CH_2), 4.6 (m, 1 H, α-CH of Val), 2.95 (s, 3 H, NCH_3), 2.27 (m, 1 H, β-CH of Val), 0.97 (t, 6 H, 2 × CH_3). Anal. ($C_{14}H_{19}NO_4$) C, H, N.

Method O. N-(Carbobenzyloxy)-N-methyl-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycine Ethyl Ester (15b; R_2 = 2-indanyl, R_3 = Et). To a solution of CBZ-*N*-methyl-L-valine (**14b**; PG = CBZ) (2.0 g, 7.54 mmol) in CH_2Cl_2 (20 mL) cooled to 0 °C was added diethyl chlorophosphate[71] (1.09 mL, 1.3 g, 7.54 mmol; *d* = 1.194) followed by Et_3N (1.05 mL, 7.54 mmol). After stirring at 0 °C for 15 min, a suspension of the amino acid ester hydrochloride **12a** (R_1 = 2-indanyl) (1.93 g, 7.54 mmol) in CH_2Cl_2 (20 mL) containing Et_2N (1.05 mL, 7.54 mmol) was added. The resulting mixture was stirred at 0 °C for 1 h and then stirred at room temperature for 18 h. The reaction mixture was washed consecutively with dilute aqueous HCl (2×), 10% aqueous $NaHCO_3$ (2×), and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo to give the crude title product **15b** (3.08 g) as an oily material. Flash chromatography over silica gel employing a gradient system of 5%, 8%, and 10% $(CH_3)_2CO$ in petroleum ether afforded the pure title product **15b** (1.5 g, 43%) as a colorless oil; ^1H NMR ($CDCl_3$) δ 7.4 (s, 5 H), 7.2 (s, 4 H, indanyl), 5.2–4.6 (m, 4 H, CH of indanyl, α-CH of Val, PhCH_2), 4.2–3.85 (m, 4 H, CH_2CH_3, α-CH_2 of Gly), 3.25–2.8 (m, 4 H, 2 × CH_2 of indanyl), 2.95 (s, 3 H, NCH_3), 2.4 (m, 1 H, β-CH of Val), 1.2 (t, 3 H, CH_3), 0.97 (t, 6 H, 2 × CH_3 of Val). Anal. ($C_{27}H_{34}N_2O_5$) C, H, N.

[(2R,3S)+(2S,3R)]-N-(Carbobenzyloxy)-N-methyl-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycine N-[3-(1,1,1-Trifluoro-4-methyl-2-hydroxypentyl)]amide (16; R_1 = CH_3, R_2 = 2-indanyl, R_3 = $CH(CH_3)_2$, R_4 = H). Ethyl ester **15b** (R_2 = 2-indanyl, R_3 = Et, PG = CBZ) (2.24 g, 4.8 mmol) was hydrolyzed to the acid **15d** (R_2 = 2-indanyl, PG = CBZ) (2.02 g, 96%) by method E to give a colorless oil which was used directly without further purification. The NMR indicated the loss of an ethyl group. Acid **15d** (2.02 g, 4.6 mmol) was condensed with the *dl-threo*-amino compound **8a** (0.96 g, 4.6 mmol) according to method J to afford the title compound **16** (2.76 g, 100%) as a colorless amorphous solid: ^1H NMR ($CDCl_3$) δ 7.4–7.2 (2 s, 9 H), 5.3–4.8 (m, 4 H, C*H*OH, CH of indanyl, PhCH_2), 4.2–3.7 (m, 4 H, α-CH_2 of Gly, 2 × α-CH of Val), 3.2–2.8 (m, 4 H, 2 × CH_2 of indanyl), 2.9 (s, 3 H, NCH_3), 2.4–1.9 (m, β-CH of Val), 0.95 (d, 12 H, 4 × CH_3 of Val). Anal. ($C_{31}H_{40}F_3N_3O_5$) C, H, N.

(3RS)-L-(Carbobenzyloxy)-N-methyl-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycine N-[3-(1,1,1-Trifluoro-4-methyl-2-oxopentyl)]amide (20n). Alcohol **16** (0.5 g, 0.845 mmol) was oxidized to the desired product **20n** (409 mg, 82.1%) as described in method G. The pure product was obtained as a colorless resin after preparative TLC employing the solvent system 3% EtOH in CH_2Cl_2: MS (CI/NH_3) *m/z* (relative intensity) 590 (MH^+, 100); ^1H NMR ($CDCl_3$) 7.3–7.1 (m, 9 H), 5.3–4.8 (m, 5 H, PhCH_2, CH of indanyl, NH), 4.1–3.8 (m, 2 H, α-CH_2 of Gly), 3.2–2.8 (m, 4 H, 2 × CH_2 of indanyl), 2.9 (s, 3 H, NCH_3), 2.35 (m, 2 H, β-CH of Val), 1.1–0.8 (m, 12 H, 4 × CH_3). Anal. ($C_{31}H_{38}F_3N_3O_5 \cdot H_2O$) C, H, N, F.

(tert-Butoxycarbonyl)-L-phenylalanine N-Methoxy-N-methylamide (10b).[62] By means previously described,[62] **10b** was obtained (98%) as a colorless oily material (45.3 g, 98%); ^1H NMR ($CDCl_3$) δ 7.3 (s, 5 H, Ph), 5.2–4.9 (m, 2 H, α-CH of Phe, NH), 3.7 (s, 3 H, $NOCH_3$), 3.2 (s, 3 H, NCH_3), 3.0 (dd, 2 H, β-CH_2 of Phe), 1.5 (s, 9 H, *t*-Bu).

(tert-Butoxycarbonyl)-L-phenylalanal (10c).[62] By means previously described,[62] **10c** was obtained as a colorless crystalline material (28.44 g, 70%): mp 80–83 °C (lit.[62] mp 86 °C); ^1H NMR ($CDCl_3$) δ 9.6 (s, 1 H, CHO), 7.5 (s, 5 H, Ph), 5.0 (d, 1 H, NH), 4.4 (m, 1 H, α-CH of Phe), 3.1 (d, 2 H, PhCH_2), 1.4 (s, 9 H, *t*-Bu).

(Trifluoromethyl)trimethylsilane (9).[56] Bromotrifluoromethane (10 mL, 67 mmol) was condensed from a lecture bottle into a Dean–Stark trap which was cooled by means of a dry ice/acetone bath. The exit of the Dean–Stark trap was connected to a 500-mL three-necked flask having a dry ice condenser and addition flask. The three-necked flask was charged with dry THF (20 mL) and chlorotrimethylsilane (8.5 mL, 67 mmol; *d* = 0.856). In the addition funnel was placed tris(*N*,*N*-diethylamino)phosphine (16.61 g, 67 mmol). The three-necked flask was cooled in a dry ice/acetone bath. The dry ice/acetone bath was removed from the Dean–Stark trap whereby the CF_3Br condensed into the cooled reaction flask. The contents of the addition funnel was added dropwise over 1 h while the temperature was maintained at −78 °C. After addition was complete the mixture was stirred for 30 min at −78 °C and then the dry ice condenser was replaced with a 6 in. Vigraux column containing a distillation head. The dry ice bath which cooled the reaction flask was removed and the reaction was allowed to slowly warm to room temperature. The contents of the reaction flask was distilled, and fractions were taken. The desired material **9** was obtained as a colorless oil which by ^1H NMR proved to be a mixture of $CF_3Si(CH_3)_3$ and THF. The ^1H NMR of the distilled material indicated that it was composed of 29.5% $CF_3Si(CH_3)_3$ in THF (2.07 mmol/g). This material was used without further purification; ^{13}C NMR ($CDCl_3$) δ 131.4 (q, CF_3, *J* = 324 Hz), −5.01 (s, $Si(CH_3)_3$).

Method P. (3S,2RS)-3-[(tert-Butoxycarbonyl)amino]-2-hydroxy-4-phenyl-1,1,1-trifluorobutane (8e). To a solution of the aldehyde **10c** (8.0 g, 32 mmol) in THF (75 mL) cooled to 0 °C under argon was added (trifluoromethyl)trimethylsilane (**9**, $CF_3Si(CH_3)_3$) (5.47 g, 38 mmol) followed by the addition of $[CH_3(CH_2)_3]_4NF$ (0.5 mL, 0.5 mmol) in a small amount of THF. The reaction mixture was allowed to warm to room temperature and then stirred for 1 h. The reaction mixture was diluted with Et_2O and then washed with brine. The aqueous phase was back-extracted with Et_2O. The combined Et_2O extract was dried ($MgSO_4$), filtered, and evaporated to afford a yellow oil (10.23 g). Flash chromatography over silica gel employing a gradient system of 5%, 10%, and 20% $(CH_3)_2CO$ in petroleum ether gave six fractions. Fraction number two which was eluted with 10% $(CH_3)_2CO$ in petroleum ether afforded a mixture that contained 33% of the desired product **8e** (PG = *t*-BOC) as a mixture of *SS* and *SR* diastereomers based upon GC–MS (CI/CH_4) and weighed 4.34 g; MS (CI/CH_4) *m/e* (relative intensity) 320 (MH^+, 28). This oily material was used directly without further purification; ^1H NMR ($CDCl_3$) δ 7.3 (s, 5 H), 1.4 (s, 9 H, *t*-Bu).

(3S,2RS)-N-(Carbobenzyloxy)-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycine N-[3-(1,1,1-trifluoro-4-phenyl-2-hydroxybutyl)]amide (16; R_1 = H, R_2 = 2-indanyl, R_3 = CH_2Ph, R_4 = H). The impure *t*-BOC alcohol **8e** (PG = *t*-BOC) (4.2 g) was treated with 15 mL of a solution containing 30% CF_3COOH in CH_2Cl_2 for 1 h at room temperature under an anhydrous atmosphere. The reaction mixture was concentrated in vacuo and then azeotroped with toluene (4×) to give the crude trifluoroacetate salt of amine **8e** (PG = H) as an orange oil (3.60 g) which was used directly without further purification. By means similar to that described in method F, *N*-CBZ-L-Val-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine (**15c**) (4.59 g, 0.01 mol) was condensed with crude alcohol **8e** (3.60 g) from above. Flash chromatography over silica gel eluting first with 3% EtOH in CH_2Cl_2 followed by 5% EtOH in CH_2Cl_2 afforded the crude title product **16** in 66% purity based upon GC–MS (CI): MS (CI/NH_3) *m/z* (relative intensity) 626 (MH^+, 100); ^1H NMR ($CDCl_3$) δ 7.4–7.0 (m, 14 H), 5.4–4.4 (m, 6 H, 2 × NH, C*H*OH, α-CH of Val, PhCH_2), 4.2–3.8 (m, 4 H, α-CH_2 of Gly, α-CH of Phe, CH of indanyl), 3.2–2.7 (m, 6 H, 2 × CH_2 of indanyl, β-CH_2 of Phe), 2.0 (m, 1 H, β-CH of Val), 1.0 (m, 6 H, 2 × CH_3 of Val).

(3S,2RS)-N-[[4-[[[(4-Chlorophenyl)sulfonyl]amino]-carbonyl]phenyl]oxomethyl]-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycine N-[3-(1,1,1-Trifluoro-4-phenyl-2-hydroxybutyl)]amide (19; R_1 = H, R_2 = 2-indanyl, R_3 = $PhCH_2$, R_4 = H). Crude (3*S*,2*RS*)-CBZ-L-Val-*N*-(2,3-dihydro-

(71) Steinberg, G. M. Reactions of Dialkyl Phosphites. Synthesis of Dialkyl Chlorophosphates, Tetraalkyl Pyrophosphates, and Mixed Orthophosphate Esters. *J. Org. Chem.* **1950**, *15*, 637–647.

1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-trifluoro-4-phenyl-2-hydroxybutyl)]amide (16) (1.75 g, 2.8 mmol) was hydrogenated according to method H to give the crude title amine **19**. The product was purified by flash chromatography over silica gel employing a gradient system of 20%, 25%, 40%, and 100% $(CH_3)_2CO$ in petroleum ether. The desired amino compound **18** eluted with 40% $(CH_3)_2CO$. This material (420 mg) was of sufficient purity to be used in the next reaction without further purification: MS (CI/C_4H_{10}) *m/z* (relative intensity) 492 (MH^+, 100); ^1H NMR ($CDCl_3$) δ 2.1 (m, 1 H, β-CH of Val), 1.9 (bs, 1 H, OH), 0.9 (m, 6 H, 2 × CH_3 of Val). The NMR indicated the loss of a CBZ group, as desired.

By means similar to that described in method J, acid **25a** (0.35 g, 0.98 mmol) was condensed with amine **18** (R_1 = H, R_2 = 2-indanyl, R_3 = $PhCH_2$, R_4 = H) (0.4 g, 0.81 mmol). Flash chromatography of the crude product over silica gel using as the eluant EtOH/HOAc/CH_2Cl_2 (4:0.1:95.9) gave the pure title compound **19** as a colorless resinous material. An analytical sample was prepared by taking a portion (130 mg) of the product and redissolving in CH_2Cl_2 followed by filtration, concentration, and drying in vacuo to give pure the title compound **19** (111 mg) as a pale yellow resin: MS (CI/NH_3) *m/z* (relative intensity) 813 (MH^+, 2.23); ^1H NMR (DMSO-d_6) δ 7.9 (bs, 6 H), 7.6 (d, 2 H), 7.1 (m, 9 H), 2.2 (m, 1 H, α-CH of Val), 0.9 (m, 6 H, 2 × CH_3 of Val). Anal. ($C_{40}H_{40}ClF_3N_4O_7S$) C, H, Cl, F, N.

(3*S*)-[[4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]phenyl]oxomethyl]-L-valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-Trifluoro-4-phenyl-2-oxybutyl)]amide (20o). The above title compound **20o** was prepared in 33% yield by a Dess–Martin periodinane[60] oxidation analogous to that described in method K. The desired material **20o** was obtained as a colorless amorphous solid: MS (CI/NH_3) *m/z* (relative intensity) 811 (MH^+, 3.53); ^1H NMR (DMSO-d_6) δ 8.7 (m, 1 H, NH), 7.9 (m, 6 H), 7.4 (d, 2 H), 7.1 (m, 9 H), 4.8–4.1 (m, 3 H, α-CH of Val), 3.9–3.7 (m, 2 H, α-CH_2 of Gly), 3.2–2.4 (m, 6 H, β-CH_2 of Phe), 2.15 (m, 1 H, β-CH of Val), 0.9 (m, 6 H, 2 × CH_3). Anal. ($C_{40}H_{38}ClF_3N_4O_7S$) C, H, Cl, N, S.

(2*RS*)-3-Amino-2-hydroxy-1,1,1-trifluoropropane Hydrochloride (8d). A solution of 3-bromo-1,1,1-trifluoro-2-propanol (19.3 g, 0.1 mol) in absolute EtOH (100 mL) was saturated with anhydrous NH_3 at 0–5 °C. The mixture was stirred for 3 days at room temperature and then concentrated until crystals separated. The colorless crystalline material was filtered and washed with Et_2O to give **8d** (PG = H) (19.4 g, 92%), mp > 300 °C. The product was used directly without further purification: MS (CI/NH_3) *m/z* (relative intensity) 130 (MH^+, 100); ^1H NMR (D_2O) δ 4.5 (m, 1 H, C*H*(OH)CF_3), 3.35 (m, 2 H, CH_3).

(2*RS*)-*N*-(Carbobenzyloxy)-L-valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-Trifluoro-2-hydroxypropyl)]amide (16; R_1 = H, R_2 = 2-indanyl, R_3 = H, R_4 = H). The desired title compound was prepared in 68% yield by means similar to that described in method F. The product was purified by chromatography over silica gel while eluting with 2% EtOH in CH_2Cl_2. The desired title alcohol **16** was obtained as a colorless amorphous solid: MS (CI/NH_3) *m/z* (relative intensity) 536 (MH^+, 62) 407 (100); ^1H NMR ($CDCl_3$) 7.5–7.20 (m, 9 H), 7.10 (bm, 1 H, $CHCF_3$), 5.80–5.30 (m, 1 H, CH of indanyl), 5.20–5.00 (m, 1 H, OH), 5.10 (s, 2 H, $PhCH_2$), 4.90–4.50 (m, 1 H, α-CH of Val), 4.30–3.60 (m, 4 H, CH_2), 3.50–2.90 (m, 4 H, 2 × CH_2 of indanyl), 2.10–1.90 (bm, 1 H, β-CH of Val), 1.0 (dd, 6 H, 2 × CH_3 of Val).

***N*-(Carbobenzyloxy)-L-valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-Trifluoro-2-oxopropyl)]amide (20k).** The title compound **20k** was prepared by a Dess–Martin periodinane[60] oxidation by means similar to that described in method G. The crude product was purified by chromatography over silica gel (1% EtOH in CH_2Cl_2) to give pure **20k** as a colorless amorphous solid (11%): MS (CI/NH_3) *m/z* (relative intensity) 534 (MH^+, 30), 407 (100); ^1H NMR ($CDCl_3$) δ 7.60–7.20 (m, 9 H), 5.90–5.30 (m, 1 H, CH of indanyl), 5.20 (s, 2 H, $PhCH_2$), 5.20 (m, 1 H, α-CH of Val), 4.90–3.70 (m, 4 H, CH_2), 3.60–2.70 (m, 4 H, 2 × CH_2 of indanyl), 2.40, 1.90 (bm, 1 H, β-CH of val), 1.10 (dd, 6 H, 2 × CH_3 of Val). Anal. ($C_{27}H_{30}F_3N_3O_5$) C, H, F, N.

(2*RS*)-3-Amino-3-methyl-1,1,1-trifluoro-2-butanol (8b). To a stirred mixture of 2-nitropropane (24 g, 0.27 mol) and K_2CO_3 (1.38 g, 0.01 mol) was added $CF_3CH(OC_2H_5)OH$ (38 g, 0.264 mol) at room temperature. The mixture was heated to 60 °C for 3 h. The reaction was cooled to room temperature and then quenched by the addition of a saturated aqueous NaCl solution. After acidification with 2 N HCl, the organic phase was separated, dried ($MgSO_4$), filtered, and concentrated. The residue was dissolved in CH_3OH (75 mL) and then hydrogenated with Raney Ni at 40 psi for 5 h by means of a Parr shaker. The catalyst was removed by filtration and the filtrate was concentrated to dryness. The residue was dried in vacuo to give the desired product **8b** as a colorless solid (18.5 g, 43.6%) which was sufficiently pure to be used directly without further purification.

(2*RS*)-*N*-(Carbobenzyloxy)-L-valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-Trifluoro-3-methyl-2-hydroxybutyl)]amide (16; R_1 = H, R_2 = 2-indanyl, R_3 = CH_3, R_4 = CH_3). The title compound **16** was prepared in low yield (6.4%) by means analogous to method F. The product was purified by flash chromatography over silica gel (2% EtOH in CH_2Cl_2) to give the desired title product **16** as colorless crystals (6.4%) after recrystallization from Et_2O: mp 135–140 °C; MS (CI/NH_3) *m/z* (relative intensity) 564 (MH^+, 15), 407 (100); ^1H NMR (DMSO-d_6) 7.50–7.20 (m, 9 H), 7.20 (m, 1 H, $CHCF_3$), 5.30–4.90 (m, 1 H, CH of indanyl), 5.10 (s, 2 H, $PhCH_2$), 4.50 (m, 1 H, α-CH of Val), 4.30–3.60 (m, 2 H, CH_2), 3.20–2.80 (m, 4 H, 2 × CH_2 of indanyl), 2.20–1.90 (bm, 1 H, β-CH of Val), 1.00 (m, 12 H, 4 × CH_3). Anal. ($C_{29}H_{36}F_3N_3O_5$) C, H, F, N.

(2*RS*)-L-Valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-Trifluoro-3-methyl-2-hydroxybutyl)]amide (18; R_1 = H, R_2 = 2-indanyl, R_3 = CH_3, R_4 = CH_3). A mixture of **16** (R_1 = H, R_2 = 2-indanyl, R_3 = CH_3, R_4 = CH_3) (12.5 g, 0.024 mol) in EtOH (120 mL) was hydrogenated at 40 psi with 10% Pd/C (0.7 g) for 3 h by means of a Parr shaker. The catalyst was filtered off and the filtrate was concentrated to afford the title amine **18** (8.9 g, 86.3%) as a colorless resin which was used directly without further purification.

(2*RS*)-*N*-[[4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]phenyl]oxomethyl]-L-valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-Trifluoro-3-methyl-2-hydroxybutyl)]amide (19; R_1 = H, R_2 = 2-indanyl, R_3 = CH_3, R_4 = CH_3). The title compound **19** was prepared by means similar to that described in method J. The crude product was purified by chromatography over silica gel (3% EtOH in CH_2Cl_2) followed by recrystallization from CH_2Cl_2/Et_2O to afford the title compound **19** (2.3 g, 51%) as a colorless solid: mp 150–165 °C dec; MS (CI/NH_3) *m/z* (relative intensity) 751 (MH^+, 10) 117.1 (100); ^1H NMR (DMSO-d_6) δ 8.70–8.50 (m, 1 H), 8.10–7.80 (m, 6 H), 7.50 (d, 2 H), 7.30–7.10 (dd, 3 H), 6.60–6.40 (m, 1 H, $CHCF_3$), 5.40–5.20 (m, 1 H, CH of indanyl), 5.10–4.80 (m, 1 H, OH), 4.80–4.20 (m, 1 H, α-CH of Val), 4.30–3.30 (m, 2 H, CH_2), 3.30–2.80 (m, 4 H, 2 × CH_2 of indanyl), 2.40 (bm, 1 H, β-CH of Val), 1.50–0.80 (m, 12 H, 4 × CH_3 of Val).

***N*-[[4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]phenyl]oxomethyl]-L-valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-Trifluoro-3-methyl-2-oxobutyl)]amide (20m; R_1 = H, R_2 = 2-indanyl, R_3 = CH_3, R_4 = CH_3).** The title compound **20m** was prepared in an analogous manner to that described in method J. The product was purified by flash chromatography over silica gel (3% CH_3OH in CH_2Cl_2) to give **20m** as a colorless solid (20.1%): mp 143–147 °C; MS (CI/NH_3) *m/z* (relative intensity) 749 (MH^+, 15), 185 (100); ^1H NMR ($CDCl_3$ + $(CD_3)_2CO$) δ 8.10 (m, 3 H), 7.90 (bd, 3 H), 7.70 (s, 1 H), 7.40 (d, 2 H), 7.10 (bs, 3 H), 5.20–4.90 (m, 1 H, α-CH of indanyl), 4.70–4.30 (m, 1 H, α-CH of Val), 4.20–3.80 (m, 2 H, CH_2), 3.50–2.90 (m, 4 H, 2 × CH_2 of indanyl), 2.40–2.00 (bm, 2 H, 2 × β-CH of Val), 1.70 (m, 12 H, 4 × CH_3).

[(2*R*,3*S*)+(2*S*,3*R*)]-*syn*-*N*-[[2-[[3-(Ethoxycarbonyl)propanoyl]amino]-4-thiazolyl](methoxyimino)acetyl]-L-valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-Trifluoro-4-methyl-2-hydroxypentyl)]amide (29a; R = OH, R_1 = 2-indanyl). Amine **18** (R_1 = H, R_2 = 2-indanyl, R_3 = $CH(CH_3)_2$, R_4 = H) (1.6 g, 3.6 mmol) was condensed with **27** (1.3 g, 3.8 mmol) according to method J. The crude product was purified by chromatography over silica gel employing a gradient system of first CH_2Cl_2 and then CH_2Cl_2/CH_3OH (97:3) as the eluting solvent to afford a colorless solid which was filtered and washed with a small amount of cold Et_2O to give the desired title compound **29a** (1.1 g, 41%): mp 127–132 °C; MS (CI/NH_3) *m/z* (relative in-

tensity) 755 (MH^+, 100), 725 (5), 709 (6), 679 (6), 470 (5), 345 (18), 254 (11); ^{13}C NMR ($CDCl_3$) δ 174.3 (CO), 173.6 (CO), 172.6 (CO), 170.3 (CO), 168.8 (CO), 158.8 (quaternary C), 148.3, 141.1 (thiazole C), 140.7, 130.4–118.9 (q, CF_3), 115.3 (thiazole C), 69.4–67.0 (q, $CH(OH)CF_3$), 62.1 (OCH_3), 60.6 (CH_2), 58.4, 56.4, 54.4, 46.5 (α-CH_2 of Gly), 37.2 (CH_2 of indanyl), 35.9 (CH_2 of indanyl), 30.8 (β-CH of Val), 30.1 (β-CH of Val), 28.7 (CH_2), 19.7, 19.4, 18.8, 18.1, 17.4, 16.4, 13.9 (CH_3). Anal. ($C_{34}H_{45}F_3N_6O_8S$) C, H, N, S.

(2*RS*)-*syn*-*N*-[[2-[[3-(Ethoxycarbonyl)propanoyl]amino]-4-thiazolyl](methoxyimino)acetal]-L-valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine *N*-[3-(1,1,1-Trifluoro-4-methyl-2-oxopentyl)]amide (29b; R = O, R_1 = 2-indanyl). The corresponding alcohol **29a** (0.23 g, 0.305 mmol) was oxidized to the title ketone **29b** according to method K. The crude product was purified over silica gel using initially CH_2Cl_2 and then CH_2Cl_2/CH_3OH (97:3) as the eluting solvent. In this manner **29b** (R = O, R_1 = 2-indanyl) was obtained as a colorless solid (52%): mp 94–101 °C; MS (CI/NH_3) *m/z* (relative intensity) 753 (MH^+, 100), 723 (1), 707 (10), 677 (5), 468 (11), 254 (11); ^{13}C NMR ($CDCl_3$) δ 174.4 (CO), 173.1 (CO), 170.1 (CO), 168.9 (CO), 163.9 (CO), 159.0 (CO), 148.4, 141.3 (thiazole C), 139.8, 127.1, 124.5, 115.5 (thiazole C), 62.3 (OCH_3), 60.8 (CH_2), 58.3, 56.3, 46.0 (α-CH_2 of Gly), 37.3, 35.9 (CH_2 of indanyl), 30.5 (β-CH of Val), 29.4 (CH_2), 28.9 (β-CH of Val), 28.3 (CH_2), 19.7, 19.4, 17.6, 17.2, 14.1 (CH_3). Anal. ($C_{34}H_{43}F_3N_6O_8S$) C, H, N, S.

Method Q. *N*-(Carbobenzyloxy)-L-valine *N*-[3-(Ethoxycarbonyl)-1,2,3,4-tetrahydroisoquinolinyl]amide (15a; R_1 = H, R_3 = Et). CBZ-L-valine (12.6 g, 0.05 mol) and Et_3N (5.06 g, 0.05 mol) were dissolved in THF (100 mL), and then the mixture was cooled to 0–5 °C. Ethyl chloroformate (5.44 g, 0.05 mol) in THF (35 mL) was added dropwise while the temperature was maintained at 0–5 °C. Following the addition, the resulting mixture was stirred for 1 h in the cold. Ethyl 1,2,3,4-tetrahydroisoquinoline-3-carboxylate hydrochloride (14.5 g, 0.06 mol) and Et_3N (6.1 g, 0.06 mol) in CH_2Cl_2 (60 mL) and THF (30 mL) were added while the temperature was maintained at 0–5 °C. The resulting mixture was stirred for 30 min at 0–5 °C and then for 16 h at room temperature. Concentration of the reaction under reduced pressure yielded a viscous semisolid which was treated with EtOAc followed by 1 N aqueous HCl. The layers were separated, and the organic phase was washed consecutively with 1 N aqueous HCl, 5% aqueous Na_2CO_3, and saturated aqueous NaCl. After drying over $MgSO_4$, filtration and concentration under reduced pressure afforded an oil (12.5 g) which was purified over silica gel [*n*-C_6H_{14}/EtOAc (4:1)]. The desired title product **15a** was obtained as a colorless viscous oil (12.9 g): MS (CI/NH_3) *m/z* (relative intensity) 456 ($MH^+ + NH_3$, 8), 439 (MH^+, 28), 357 (6), 331 (100), 259 (3), 206 (5); ^1H NMR ($CDCl_3$) δ 7.4–7.3 (s, 5 H), 7.2–7.1 (m, 4 H), 5.6 (d, 1 H), 5.4 (t, 1 H), 5.2–4.6 (m, 2 H), 4.15–4.0 (m, 2 H), 3.35–3.05 (m, 2 H), 2.2–2.0 (m, 1 H), 1.2–0.9 (m, 12 H); ^{13}C NMR ($CDCl_3$) δ 171.6 (CO), 170.5 (CO), 156.2 (CO), 136.9, 131.9, 128.4, 128.2, 127.8, 127.2, 126.9, 126.0, 66.8 ($PhCH_2$), 61.1 (CH_2), 56.2 (α-CH of Val), 52.1 (CH of tetrahydroisoquinoline), 45.7 (CH_2 of tetrahydroisoquinoline), 31.3 (β-CH of Val), 30.7 (CH_2 of tetrahydroisoquinoline), 19.4 (CH_3 of Val), 17.2 (CH_3 of Val), 13.9 (CH_3). Anal. ($C_{25}H_{30}N_2O_5$) C, H, N.

Human Leukocyte Elastase (HLE) Inhibition Screen in Vitro. The method of Nakajima[51a] was adapted to a microtiter format. The in vitro assay was based upon the hydrolysis of the commercially available (Sigma Chemical Company, 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 per 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., Pacific, MO) was dissolved in 1 mg/mL in 0.05 M NaCl and frozen (50-μL 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 slope). The inhibitory activity of the test compound was calculated relative to the uninhibited enzyme control as follows:

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

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-μL sample of the test compound stock solution (or the assay buffer) and 10 μL 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 μL) was then added to each well and the OD_{405} 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 their trachea were 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 μg/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 a 0.1-mL volume (20 μg of compound). Five minutes later, purified human sputum elastase (Elastin Products Co., Owensville, MO), 50 μg 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 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-μL aliquot of the cell suspension was added to 475 μL of Trypan Blue (1:20 dilution), and manual RBC counts were performed. The number of RBC's per mL of cell suspension was calculated. The percent inhibition of pulmonary hemorrhage was calculated as follows:

$$\% \text{ inhibition} = 100 \times [\text{no. RBC}/(\text{mL vehicle control})] - [\text{no. RBC}/(\text{mL compound tested})]/[\text{no. RBC}/(\text{mL vehicle control})]$$

Statistical analysis[72–74] of the data was performed using analysis of variance (ANOVA) and Dunnett's multiple comparison test.

Twenty-One-Day Emphysema Model. Golden Syrian hamsters weighing 150–200 g were used. Anesthesia and surgery was described in the EPH assay described above. Animals were set up into three different groups consisting of five animals per group: (a) "normal", no treatment; (b) "control", 200 μg of HNE plus the compound vehicle; (c) "test compound", 200 μg of HNE plus 20 μg of test compound. Compounds were prepared in DMSO as in the EPH assay and diluted in normal saline. Each animal in the control group or test compound group received either the vehicle or test compound respectively in a 0.1-mL volume it., 5 min prior to HNE challenge. Wounds were surgically closed and the animals were placed in normal animal care for 21 days. At

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

the end of 21 days the animals were sacrificed by the administration of a Nembutal overdose. Lungs were inflated with 10% neutral buffered formalin, being "very careful" not to over inflate. The lungs were removed intact from the animal and processed histologically for paraffin microtomy and hematoxylin/eosin staining (H/E). Cross-sections of the intact lungs were prepared from each animal, stained by H/E and observed under image analysis for sign of emphysema (pulmonary lesions of septal destruction and increased alveolar spaces). Each histology specimen (five per group, each representing a respective animal in the normal, control, and test groups) were viewed under ×40 magnification utilizing an image analyzer (40–10, Optomax, Inc.). The image analyzer was used to measure alveolar spaces within the areas at risk. Ten locations within the areas at risk, per specimen, were measured. Three measurements (in μm^2) per location, were calculated by the image analyzer and data was collected by an Hewlett-Packard Vectra computer. Thus, 30 measurements per specimen and 150 measurements per group were performed. The grouped data was analyzed and subjected to Tukeys standardized range test. Tukeys multiple comparison procedure suggests significance at the ρ 0.05 level.

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[75] 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[67] (OMTKY3) as well as that of HLE with the inhibitor MeO-Suc-Ala-Ala-Pro-Val-chloromethyl ketone (MPCMK). 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^{195} and the ketone carbonyl of the inhibitor. To simulate this situation in the modeling process, the O 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 an 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 constraint 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.).

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Registry No. **6a**, 625-74-1; *dl-threo*-**7a**, 105108-34-7; *dl-erythro*-**7a**, 137301-54-3; *dl-threo*-**8a**·HCl (PG = H), 105108-35-8; *dl-threo*-**8a** (free base, PG = H), 136182-45-1; *dl-erythro*-**8a** (PG = H), 136235-55-7; (±)-**8b** (PG = H), 137301-93-0; (±)-**8d** (PG = H), 127590-68-5; (2*R*,3*S*)-**8e** (PG = Boc), 137301-85-0; (2*S*,3*S*)-**8e** (PG = Boc), 137332-28-6; (2*R*,3*S*)-**8e**·TFA (PG = H), 137432-18-9; (2*S*,3*S*)-**8e**·TFA (PG = H), 137429-40-4; **9**, 81290-20-2; **10b**, 87694-53-9; **10c**, 72155-45-4; **12a** (R_1 = c-C_5H_9), 89479-61-8; **12a** (R_1 = 2-indanyl), 84827-59-8; **14a** (PG = Cbz), 1149-26-4; **14b** (PG = Cbz), 42417-65-2; **15a** (R_2 = 2-indanyl, R_3 = Et, PG = Cbz), 131506-23-5; **15a** (R_2 = 3,4-dimethoxyphenethyl, R_3 = Et, PG = Cbz), 131506-25-7; **15b** (R_2 = 2-indanyl, R_3 = Et, PG = Cbz), 137301-80-5; **15c** (R_2 = 2-indanyl, PG = Cbz), 131506-24-6; **15d** (R_2 = 2-indanyl, PG = Cbz), 137301-81-6; (2*R*,3*S*)-**16** (R_1 = H, R_2 = 2-indanyl, R_3 = *i*-Pr, R_4 = H, PG = Cbz), 137429-26-6; (2*S*,3*R*)-**16** (R_1 = H, R_2 = 2-indanyl, R_3 = *i*-Pr, R_4 = H, PG = Cbz), 137429-27-7; (2*R*,3*S*)-**16** (R_1 = H, R_2 = c-C_8H_{15}, R_3 = *i*-Pr, R_4 = H, PG = Boc), 137301-56-5; (2*S*,3*R*)-**16** (R_1 = H, R_2 = c-C_8H_{15}, R_3 = *i*-Pr, R_4 = H, PG = Boc), 137429-30-2; (2*R*,3*S*)-**16** (R_1 = Me, R_2 = 2-indanyl, R_3 = *i*-Pr, R_4 = H, PG = Cbz), 137301-82-7; (2*S*,3*R*)-**16** (R_1 = Me, R_2 = 2-indanyl, R_3 = *i*-Pr, R_4 = H, PG = Cbz), 137429-38-0; (2*R*,3*S*)-**16** (R_1 = H, R_2 = 2-indanyl, R_3 = CH_2Ph, R_4 = H, PG = Cbz), 137301-86-1; (2*S*,3*S*)-**16** (R_1 = H, R_2 = 2-indanyl, R_3 = CH_2Ph, R_4 = H, PG = Cbz), 137429-41-5; (2*R*)-**16** (R_1 = H, R_2 = 2-indanyl, R_3 = R_4 = H, PG = Cbz), 137301-90-7; (2*S*)-**16** (R_1 = H, R_2 = 2-indanyl, R_3 = R_4 = H, PG = Cbz), 137301-91-8; (2*R*)-**16** (R_1 = H, R_2 = 2-indanyl, R_3 = R_4 = Me, PG = Cbz), 137301-94-1; (2*S*)-**16** (R_1 = H, R_2 = 2-indanyl, R_3 = R_4 = Me, PG = Cbz), 137301-95-2; (2*R*,3*S*)-**18** (R_1 = H, R_2 = 2-indanyl, R_3 = *i*-Pr, R_4 = H), 137429-28-8; (2*S*,3*R*)-**18** (R_1 = H, R_2 = 2-indanyl, R_3 = *i*-Pr, R_4 = H), 137429-29-9; (2*R*,3*S*)-**18** (R_1 = H, R_2 = c-C_8H_{15}, R_3 = *i*-Pr, R_4 = H), 137301-57-6; (2*S*,3*R*)-**18** (R_1 = H, R_2 = c-C_8H_{15}, R_3 = *i*-Pr, R_4 = H), 137429-31-3; (2*R*,3*S*)-**18** (R_1 = H, R_2 = 2-indanyl, R_3 = CH_2Ph, R_4 = H), 137301-87-2; (2*S*,3*S*)-**18** (R_1 = H, R_2 = 2-indanyl, R_3 = CH_2Ph, R_4 = H), 137429-42-6; (2*R*)-**18** (R_1 = H, R_2 = 2-indanyl, R_3 = R_4 = Me), 137301-96-3; (2*S*)-**18** (R_1 = H, R_2 = 2-indanyl, R_3 = R_4 = Me), 137301-97-4; (2*R*,3*S*)-**19** (X = Cl, R_1 = H, R_2 = 2-indanyl, R_3 = *i*-Pr, R_4 = H), 137432-15-6; (2*S*,3*R*)-**19** (X = Cl, R_1 = H, R_2 = 2-indanyl, R_3 = *i*-Pr, R_4 = H), 137490-09-6; (2*R*,3*S*)-**19** (X = Cl, R_1 = H, R_2 = 2-indanyl, R_3 = CH_2Ph, R_4 = H), 137301-88-3; (2*S*,3*S*)-**19** (X = Cl, R_1 = H, R_2 = 2-indanyl, R_3 = CH_2Ph, R_4 = H), 137429-43-7; (2*R*)-**19** (X = Cl, R_1 = H, R_2 = 2-indanyl, R_3 = R_4 = Me), 137301-98-5; (2*S*)-**19** (X = Cl, R_1 = H, R_2 = 2-indanyl, R_3 = R_4 = Me), 137301-99-6; (3*R*)-**20a**, 137302-04-6; (3*S*)-**20a**, 137302-05-7; (3*R*)-**20b**, 137302-06-8; (3*S*)-**20b**, 129585-35-9; (3*R*)-**20c**, 137302-07-9; (3*S*)-**20c**, 137302-08-0; (3*R*)-**20d**, 137302-09-1; (3*S*)-**20d**, 129601-47-4; (3*R*)-**20e**, 137302-10-4; (3*S*)-**20e**, 137302-11-5; (3*R*)-**20f**, 137429-47-1; (3*S*)-**20f**, 129585-36-0; (3*R*)-**20g**, 137429-48-2; (3*S*)-**20g**, 137429-49-3; (3*R*)-**20h**, 137301-55-4; (3*S*)-**20h**, 129601-48-5; (3*R*)-**20i**, 137432-16-7; (3*S*)-**20i**, 129585-37-1; (3*R*)-**20j**, 137302-12-6; (3*S*)-**20j**, 137302-13-7; **20K**, 137301-92-9; (3*R*)-**20l**, 137332-51-5; (3*S*)-**20l**, 137332-52-6; **20m**, 129585-42-8; (3*R*)-**20n**, 137301-83-8; (3*S*)-**20n**, 137301-84-9; (3*S*)-**20o**, 137301-89-4; (3*R*)-**20p**, 137302-14-8; (3*S*)-**20p**, 137302-15-9; (3*R*)-**20q**, 137302-16-0; (3*S*)-**20q**, 129585-39-3; (3*R*)-**20r**, 137302-17-1; (3*S*)-**20r**, 129585-40-6; (3*R*)-**20s**, 137302-18-2; (3*S*)-**20s**, 137302-19-3; (3*R*)-**20t**, 137302-20-6; (3*S*)-**20t**, 137302-21-7; (3*R*)-**20u**, 137302-22-8; (3*S*)-**20u**, 137302-23-9; (3*R*)-**20v**, 137429-50-6; (3*S*)-**20v**, 137429-51-7; **20w**, 137302-24-0; (3*R*)-**20x**, 137302-25-1; (3*S*)-**20x**, 129622-81-7; **20y** (diastereomer 1), 137302-26-2; **20y** (diastereomer 2), 137429-52-8; **20y** (diastereomer 3), 137429-53-9; **20y** (diastereomer 4), 137429-54-0; **20aa**, 137302-27-3; **20ab** (diastereomer 1), 137302-28-4; **20ab** (diastereomer 1), 137429-55-1; **20ab** (diastereomer 3), 137429-56-2; **20ab** (diastereomer 4), 137429-57-3; **21**, 100-20-9; **22**, 28313-42-0; **23**, 20576-82-3; **24a**, 105080-62-4; **25a**, 105080-63-5; **25b**, 137301-58-7; **27**, 137302-00-2; (2*R*,3*S*)-**29a** (R_1 = 2-indanyl), 137302-01-3; (2*S*,3*R*)-**29a** (R_1 = 2-indanyl), 137429-45-9; (3*R*)-**29b**, 137302-02-4; (3*S*)-**29b**, 137429-46-0; **30a** (R_1 = 2-indanyl), 137301-59-8; **30b** (R_1 = 2-indanyl), 137301-60-1; *dl-threo*-**31** (R_1 = 2-indanyl), 137301-61-2; **32** (R_1 = 2-indanyl), 137301-62-3; *dl-threo*-**33**,

(75) For a comprehensive account of principles used to derive the MM2 force field, see: (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_1 and V_2 Torsional Terms. *J. Am. Chem. Soc.* **1977**, *99*, 8127–8134.

137301-63-4; **34**, 107576-66-9; **35b**, 87694-52-8; **35c**, 79069-51-5; **36a**, 137301-64-5; **36b**, 137301-65-6; (2*R*,3*S*)-**37a**, 137301-66-7; (2*S*,3*R*)-**37a**, 137429-32-4; (3*R*)-**37b**, 137301-67-8; (3*S*)-**37b**, 137332-44-6; (2*R*,3*S*)-**38a**, 137301-68-9; (2*S*,3*R*)-**38a**, 137429-33-5; (2*R*,3*S*)-**38b**, 137301-69-0; (2*S*,3*R*)-**38b**, 137429-34-6; (3*R*)-**38c**, 137332-50-4; (3*S*)-**38c**, 137301-70-3; **39**, 137301-71-4; (1*R*)-**40a**, 137301-72-5; (1*S*)-**40a**, 137301-73-6; (1*R*)-**40b**, 137301-74-7; (1*S*)-**40b**, 137332-27-5; (1*R*)-**41a**, 137301-75-8; (1*S*)-**41a**, 137429-35-7; **41b**, 137301-76-9; (1*R*)-**41c**, 137301-77-0; (1*S*)-**41c**, 137429-36-8; (1*R*)-**41d**, 137301-78-1; (1*S*)-**41d**, 137429-37-9; **41e**, 137301-79-2; HLE, 9004-06-2; Cbz-Cl, 501-53-1; H-Gly-OEt·HCl, 623-33-6; $BrCH_2CO_2Et$, 105-36-2; $(c\text{-}C_5H_9)NH_2$, 1003-03-8; $CF_3CH(OEt)OH$, 433-27-2; $3,4\text{-}(MeO)_2C_6H_3CH_2CH_2$-Gly-OEt, 56014-42-7; $4\text{-}BrC_6H_4CF_3$, 402-43-7; $BrCF_3$, 75-63-8; $ClSiMe_3$, 75-77-4; (±)-$BrCH_2CH(OH)CF_3$, 137429-44-8; O_2NCHMe_2, 79-46-9; 2-indanone, 615-13-4; (±)-ethyl 1,2,3,4-tetrahydroisoquinoline-3-carboxylate hydrochloride, 54656-72-3; *N*-(carbobenzyloxy)-L-valine *N*-[3-(*R*)-(ethoxycarbonyl)-1,2,3,4-tetrahydroisoquinolinyl]amide, 137302-03-5; *N*-(carbobenzyloxy)-L-valine *N*-[3(*S*)-(ethoxycarbonyl)-1,2,3,4-tetrahydroisoquinolinyl]amide, 137302-29-5.