

Inhibition of Human Leukocyte Elastase (HLE) by N-Substituted Peptidyl Trifluoromethyl Ketones¹

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A series of tripeptides possessing trifluoromethyl or aryl ketone residues at P₁ 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 P₂-position have been demonstrated to be potent in vitro inhibitors of HLE, with IC₅₀ values in the submicromolar range. Sterically demanding substituents on the P₂-nitrogen have no detrimental effect on in vitro potency. The inhibition process presumably acts via hemiketal formation with the active site Ser¹⁹⁶ of HLE, and is facilitated by the strongly electron withdrawing trifluoromethyl functionality. Deletion of the amino acid at the P₃-subsite region affords inactive compounds. Valine is the preferred residue at the P₁-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 (3RS)-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-methyl-2-oxopentyl)]amide (**20i**; BI-RA-260) (IC₅₀ = 0.084 μ M). 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 ED₅₀ of 4.8 μ g. The inhibitor **20i**, 20 μ g 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 μ g 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 μ g 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 Met³⁵⁸),^{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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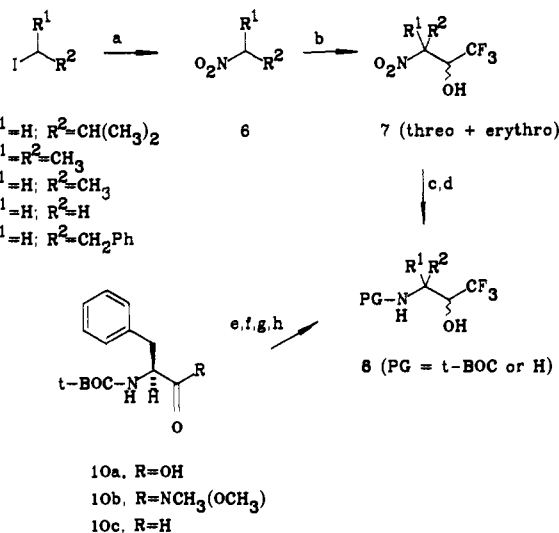
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PMN elastase activity can be potentially useful in the treatment of pulmonary emphysema and related diseases.

In recent years much attention has focused on the inhibition of elastase¹¹ 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-

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

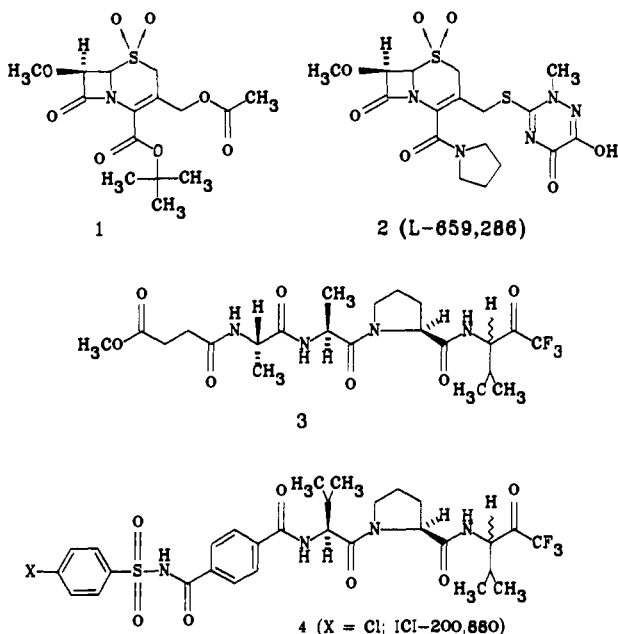


^a Reagents: (a) AgNO₃; (b) CF₃CH(OC₂H₅)OH, K₂CO₃; (c) separation of isomers; (d) LiAlH₄ to give 8 (PG = H); (e) 10a, HNC-H₃(OCH₃), CDI to give 10b; (f) 10b, LiAlH₄ to give 10c; (g) 10c, CF₃Si(CH₃)₃ (9) to give 8 (PG = t-BOC); (h) 8 (PG = t-BOC), HCl, p-dioxane to give 8 (PG = H).

β -keto amides,²⁶ latent isocyanates,²⁷ sulfonate salts,²⁸ chloroisocoumarins,^{29,30} ynenol lactones,³¹ benzoxazinones,³² 2-pyrones,³³ hydantoin,³⁴ and cephalosporins.³⁵⁻³⁷ Some

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of these inhibitors also show in vivo activity. For example, a series of cephalosporins,³⁵⁻³⁷ such as 1³⁵ and 2,³⁶ which are potent heterocyclic inhibitors of HLE, prevent lung damage in hamsters treated intratracheally with HLE.



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 model²³ 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 Ser¹⁹⁵ 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 Ser¹⁹⁵ of HLE, this process is reversible. In the case of porcine pancreatic elastase (PPE), it has been unequivocally demonstrated⁴⁸ through X-ray analysis that, in the adducts formed between the enzyme and peptidyl trifluoromethyl ketone inhibitors, the O^γ atom of the catalytic Ser¹⁹⁵ 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 analogues⁵⁰) is that they tend

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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 proline⁵¹ at the P₂-subsite.⁵² Since L-proline can effectively be replaced by achiral N-substituted glycines to afford potent angiotensin converting enzyme (ACE) inhibitors⁵³⁻⁵⁵ both in vitro and in vivo, we thought it might be of interest to see if the S₂-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 P₃-P₂ 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 P₂.

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 P₁-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 AgNO₂ in Et₂O to afford nitroalkanes 6, which were converted to nitrofluoro alcohols 7 as a mixture of *dl*-threo and *dl*-erythro isomers when R₁ ≠ R₂, by reaction with trifluoroacetaldehyde ethyl hemiacetal, CF₃CH(OC₂H₅)OH, and K₂CO₃ (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 (R₁ = H, R₂ = CH(CH₃)₂) could easily be separated from the

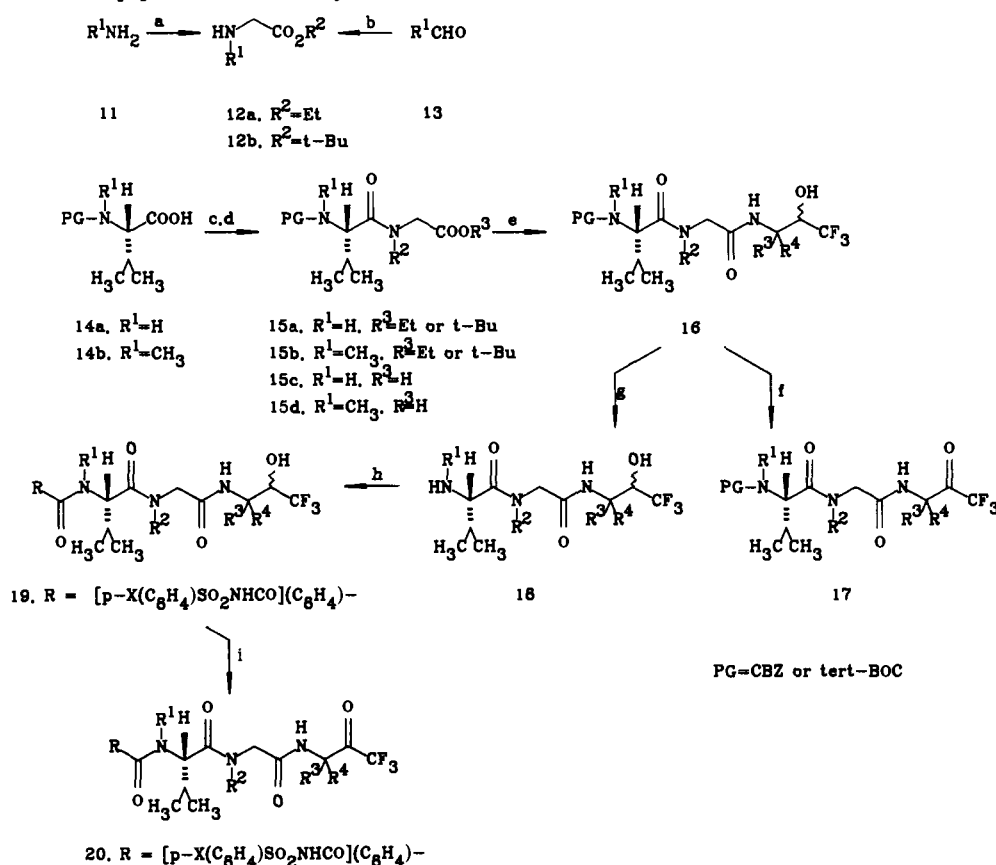
corresponding minor (*dl*)-erythro isomer [(2*S*,3*S*)+(2*R*,3*R*)] by crystallization. The 7a erythro isomer remained in the filtrate as an oil. Amino alcohols 8 were prepared by either one of two available methods. In the first method, nitroalkanes 7 were reduced effectively to the amino alcohols 8 (PG = H) by employing LiAlH₄, 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), CF₃Si(CH₃)₃. This method was applied to protected amino acid aldehydes such as *t*-BOC-L-phenylalanyl (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 BrCH₂COOR₂ (R₂ = Et or *t*-Bu) in a polar solvent such as EtOH or CH₃CN. 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 NaC-NBH₃. 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 CH₂Cl₂ 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 CH₂Cl₂/CH₃OH (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-Martin⁶⁰ 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 P₁. 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 (P₂, P₁, P₁', P₂', etc.) of a peptide substrate and the corresponding subsites (S₂, S₁, S₁', S₂', etc.) of a protease is that of Schechter and Berger: Schechter, I.; Berger, A. On the Size of the Active Site in Proteases. I. Papain. *Biochem. Biophys. Res. Commun.* 1967, 27, 157-162.
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- (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 [(Acythio)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-CF₃). A Trifluoromethyl Equivalent. *J. Am. Chem. Soc.* 1989, 111, 393-395.
- (57) Ruppert, I.; Schlich, K.; Volbach, W. The First CF₃-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)

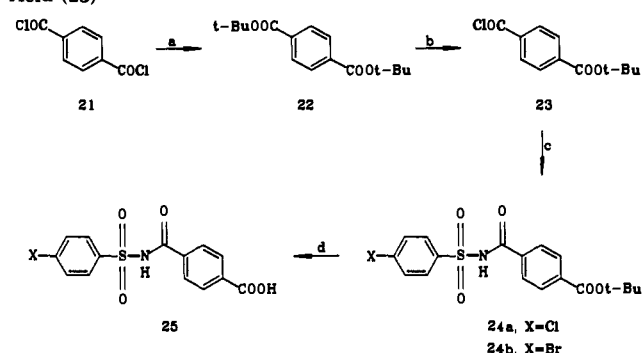


Reagents: (a) BrCH₂CO₂R₂, Et₃N; (b) H₂NCH₂CO₂R₂, NaCNBH₃; (c) 12a or 12b, CDI, THF to give 15a or 15b; (d) 15a (R₃ = Et) or 15b (R₃ = Et), KOH, EtOH to give 15c or 15d; or 15a (R₃ = *t*-BOC) or 15b (R₃ = *t*-Bu), HCl, *p*-dioxane to give 15c or 15d; (e) (PG = H), 15c or 15d, CDI, THF; (f) Dess-Martin periodinane, CF₃COOH, CH₂Cl₂; (g) 16 (PG = CBZ), 10% Pd/C, EtOH, H₂, 45 psi; or 16 (PG = *t*-BOC), HCl, *p*-dioxane; (h) 25, HOBT, WSCDI, THF; (i) Dess-Martin periodinane, CF₃COOH, THF, CH₂Cl₂.

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 P₃-P₂ reduced analogues 37b and 38c was done according to Scheme VI. *t*-BOC-L-valine was coupled with *N,O*-dimethylhydroxylamine⁶¹ in the presence of CDI to give 35b. Amide 35b was reduced with LiAlH₄ to give *t*-BOC-L-valinal (35c).⁶² The reductive alkylation of the α-amino ester 12a (R₁ = 2-indanyl) with aldehyde 35c employing NaCNBH₃ gave *t*-BOC-L-valyl-ψ(CH₂)-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine ethyl ester

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



Reagents: (a) *t*-BuOH, pyridine, THF; (b) KOH, *t*-BuOH; (c) *p*-Cl(C₆H₄)SO₂NH₂, DMAP, WSCDI, CH₂Cl₂; (d) CF₃COOH.

(36a).⁶³ Reduced dipeptide alcohol 36a was transformed to trifluoromethyl ketones 37b and 38c by methods similar to those described above (Scheme II).

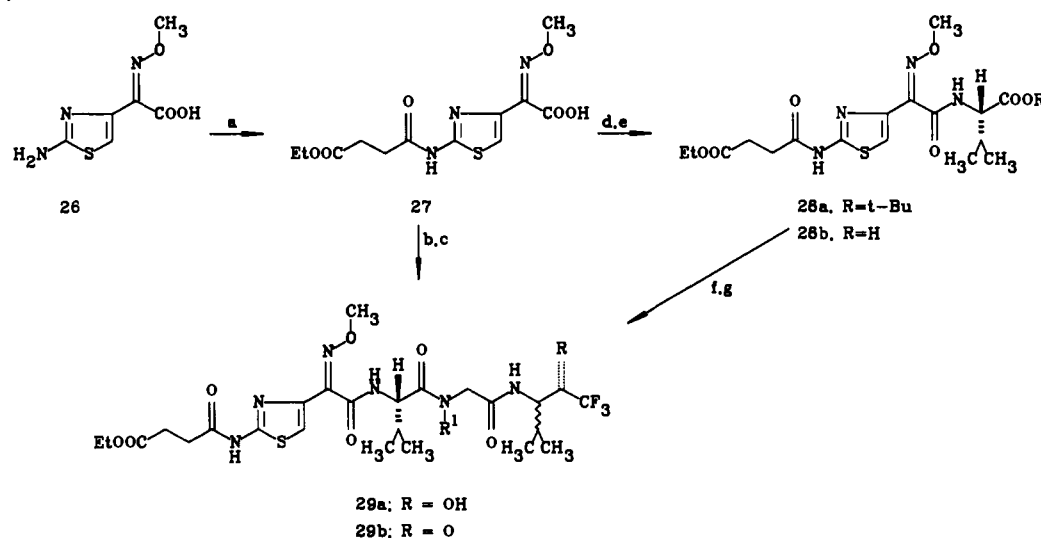
The preparations of tripeptides 41b and 41e containing aryl ketones at P₁ were performed as illustrated in Scheme VII. The required protected aryl ketones 39 were efficiently obtained by treating protected α-amino acid *N,O*-dimethylamides 35b⁶² with the appropriate aryl Grignard

(61) This material was first elegantly used by Weinreb to convert, via LiAlH₄ 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.

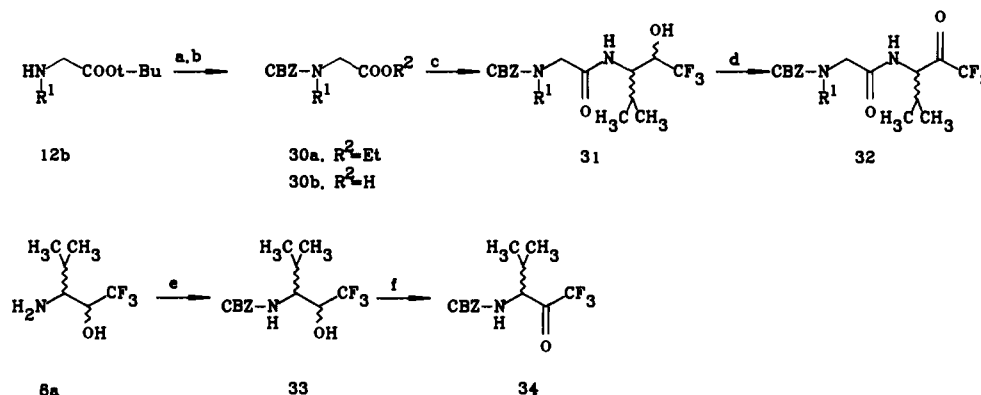
(62) Fehrentz, J. A.; Castro, B. An Effective Synthesis of Optically Active α-(*t*-Butoxycarbonylamino)-aldehydes from α-Amino Acids. *Synthesis* 1983, 676-678.

(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) ClCOCH₂CH₂CO₂Et, Et₃N, CH₂Cl₂; (b) 18, CDI, THF to give 29a; (c) 29a, Dess-Martin periodinane, CF₃COOH, CH₂Cl₂ to give 29b; (d) L-Val *t*-Bu ester, CDI, THF to give 28a; (e) 28a, CF₃COOH to give 28b; (f) 28b, 8a (PG = H), CDI, THF to give 29a; (g) 29a, Dess-Martin periodinane, CF₃COOH, CH₂Cl₂ to give 29b.

Scheme V. Synthesis of Truncated Inhibitors (P₃-P₁)

^a Reagents: (a) CBZ-Cl, Et₃N, THF to give 30a; (b) 30a, KOH, EtOH to give 30b; (c) 30b, 8a, CDI, THF; (d) Dess-Martin periodinane, CF₃COOH, CH₂Cl₂; (e) CBZ-Cl, Et₃N, THF; (f) Dess-Martin Periodinane, CF₃COOH, CH₂Cl₂.

reagent. Ketones 39 were reduced with NaBH₄ 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.

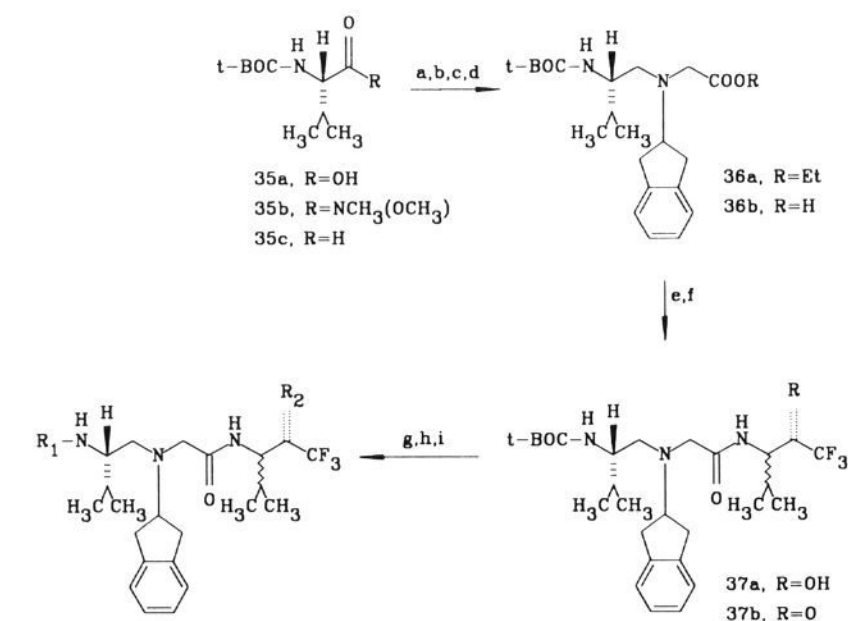
Results and Discussion

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 P₂-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 P₂-proline, this new series of inhibitors is constructed exclusively from the achiral amino acid glycine. This series of compounds has demonstrated⁶⁴ potential as therapeutic agents for em-

physema and other diseases related to the degradation of connective tissue.

The most active compounds listed in Table I are those tripeptides that span the P₅-P₁ subsites [e.g. 20b,d,f,g,i,j,p,q,s,t,v,w,x,aa and 29b (IC₅₀ = 0.03-0.217 μM)] and contain a trifluoromethyl ketone residue of valine at P₁. The most active tripeptide inhibitors correspond to those which are *N*-terminated with the functionality *p*-(*p*-ClC₆H₄SO₂NHCO)C₆H₄ (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 (IC₅₀ = 0.365 μM) is compared with 20i (IC₅₀ = 0.084 μM), or when 20r (IC₅₀ = 0.507 μM) is compared with 20s (IC₅₀ = 0.057 μM)]. Presumably, this sulfonamide functionality effectively increases binding to HLE through favorable interactions with residues in the S₅-S₄-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 P₁ 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)sulfonylamino-carbonyl]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. *FAS-EB J.* 1990, 4(4), Abstract 5212.

Scheme VI. Synthesis of P₃-P₂ Reduced Analogues

38a, R₁=H; R₂=OH
 38b, R₁=p-[p-Cl(C₆H₄)SO₂NHCO](C₆H₄)CO; R₂=OH
 38c, R₁=p-[p-Cl(C₆H₄)SO₂NHCO](C₆H₄)CO; R₂=O

^a Reagents: (a) HNCH₃(OCH₃), CDI, THF to give 35b; (b) 35b, LiAlH₄, THF to give 35c; (c) 35c, 12a (R₁ = 2-indanyl), NaCNBH₃, 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, CF₃COOH, CH₂Cl₂ to give 37b; (g) 37b, HCl/p-dioxane to give 38a; (h) 38a, 25a, WSCDI to give 38b; (i) 38b, Dess-Martin periodinane, CF₃COOH, CH₂Cl₂ to give 38c.

oxycarbonyl)propanoyl]amino]-4-thiazolyl](methoxyimino)acetyl derivative **29b** was prepared as an inhibitor with binding regions extending into the S₆-S₁ sites. The 2-(2-amino-4-thiazolyl)-2-oximinoacetic acid moiety has been widely used⁶⁵ in the design of cephalosporins. This functionality, however, did not increase the in vitro potency [e.g. **29b** (IC₅₀ = 0.452 μM)]. From Table I, it can be seen that the P₂-residue of the inhibitors has a very high degree of tolerance with regard to the substituent group. For instance, as the substituent on the P₂-glycine is increased in steric size and lipophilicity, ranging from H (**20b**), CH₃ (**20d**), cyclopentyl (**20f**), *exo*-norbornyl (**20g**), 2-indanyl (**20i**), and cycloheptyl (**20p**) to cyclooctyl (**20q**), no dramatic change in in vitro potency is observed (IC₅₀ = 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** (IC₅₀ = 0.067 μM), is as easily accommodated as is glycine itself (e.g. **20b**, IC₅₀ = 0.073 μM). The results listed in Table I also indicate that *N*-(heterocycloalkyl)glycine, as well as *N*-(arylalkyl)glycine residues at P₂, 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 (IC₅₀ = 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

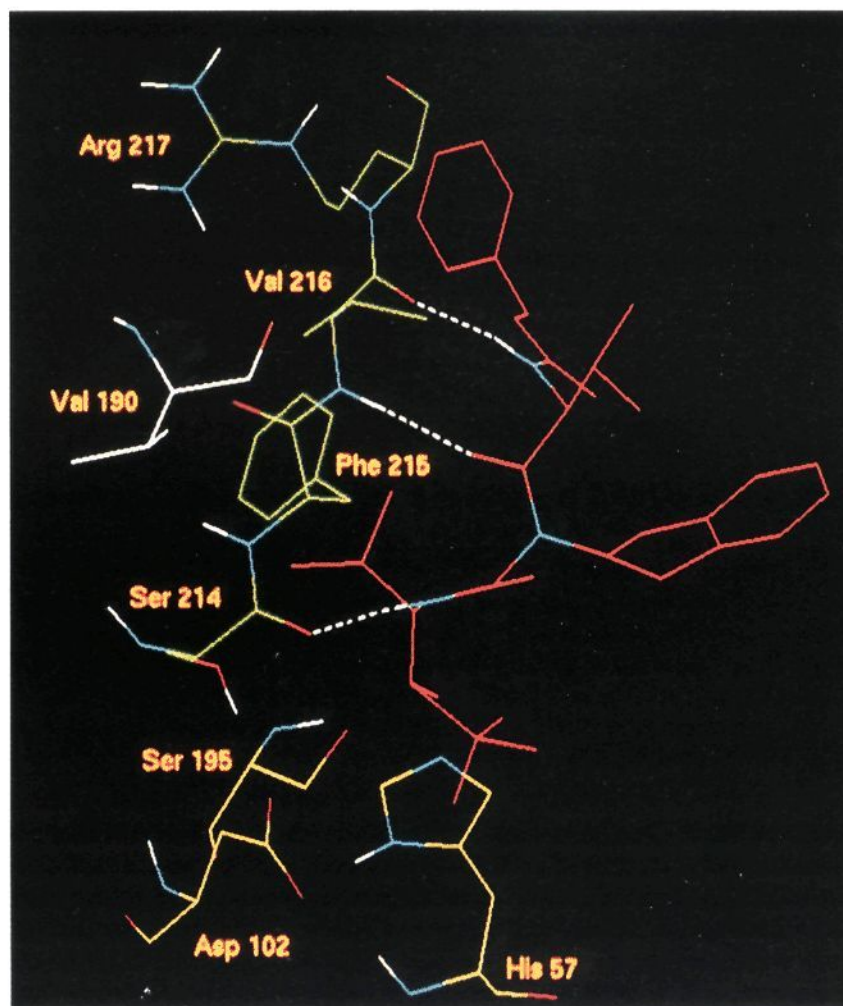


Figure 1. Minimized structure of HLE complexed with the inhibitor **20h** detailing the binding and catalytic sites. The binding residues (Ser²¹⁴, Phe²¹⁵, Val²¹⁶, and Arg²¹⁷) and the catalytic triad (Ser¹⁹⁵, His⁵⁷, and Asp¹⁰²) of HLE are given in green and yellow, respectively. The inhibitor is shown in red. For minimization calculations the constraint (1.78 Å) of covalently linking Ser¹⁹⁵ 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 (Val²¹⁶ CO to NH of P₃-Val, 1.84 Å; Val²¹⁶ NH to CO of P₃-Val, 1.97 Å; Ser²¹⁴ CO to NH of P₁-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 Ser¹⁹⁵ 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 P₂-glycines of the enzyme-inhibitor complexes must stick out into solution away from the core of HLE. Figure 1 shows the catalytic triad (Ser¹⁹⁵, Asp¹⁰², and His⁵⁷) in yellow and the binding site residues (Arg²¹⁷, Val²¹⁶, Phe²¹⁵, and Ser²¹⁴) of HLE in green. The CBZ inhibitor **20h**, complexed with

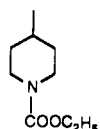
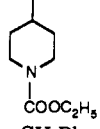
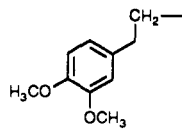
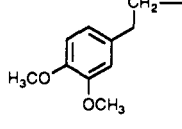
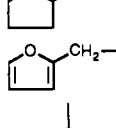
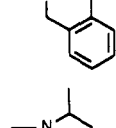
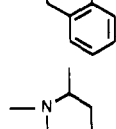
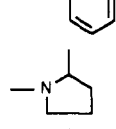
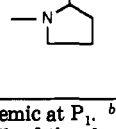
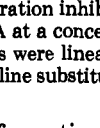
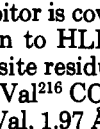
(65) *Beta-Lactam Antibiotics for Clinical Use*; Queener, S. F., Webber, J. A., Queener, S. W., Eds.; Clinical Pharmacology; Marcel Dekker: New York, 1986; Vol. 4.

(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) ₃ CO	H	H	H	CH(CH ₃) ₂	71-75	D-G	C ₁₈ H ₃₀ F ₃ N ₃ O ₅	0.153
20b	<i>p</i> -(<i>p</i> -ClC ₆ H ₄ SO ₂ NHCO)C ₆ H ₄	H	H	H	CH(CH ₃) ₂	193-196	I-K	C ₂₇ H ₃₀ ClF ₃ N ₄ O ₇ S	0.073
20c	PhCH ₂ O	H	CH ₃	H	CH(CH ₃) ₂	resin	D-G	C ₂₂ H ₃₀ F ₃ N ₃ O ₅	0.153
20d	<i>p</i> -(<i>p</i> -ClC ₆ H ₄ SO ₂ NHCO)C ₆ H ₄	H	CH ₃	H	CH(CH ₃) ₂	resin	H, J, K	C ₂₈ H ₃₂ ClF ₃ N ₄ O ₇ S	0.052
20e	PhCH ₂ O	H	<i>c</i> -C ₆ H ₉	H	CH(CH ₃) ₂	69-74	D-G	C ₂₆ H ₃₆ F ₃ N ₃ O ₅	0.156
20f	<i>p</i> -(<i>p</i> -ClC ₆ H ₄ SO ₂ NHCO)C ₆ H ₄	H	<i>c</i> -C ₆ H ₉	H	CH(CH ₃) ₂	136-140	H, J, K	C ₃₂ H ₃₈ ClF ₃ N ₄ O ₇ S	0.092
20g	<i>p</i> -(<i>p</i> -ClC ₆ H ₄ SO ₂ NHCO)C ₆ H ₄	H		H	CH(CH ₃) ₂	234-239	D-F, H, J	C ₃₄ H ₄₀ ClF ₃ N ₄ O ₇ S	0.061
20h	PhCH ₂ O	H		H	CH(CH ₃) ₂	49-54	C, E-G	C ₃₀ H ₃₈ F ₃ N ₃ O ₅	0.365
20i	<i>p</i> -(<i>p</i> -ClC ₆ H ₄ SO ₂ NHCO)C ₆ H ₄	H		H	CH(CH ₃) ₂	218-226	H, J, K	C ₃₈ H ₃₈ ClF ₃ N ₄ O ₇ S	0.084
20j	<i>p</i> -(<i>p</i> -BrC ₆ H ₄ SO ₂ NHCO)C ₆ H ₄	H		H	CH(CH ₃) ₂	170-185	D-F, H, J, K	C ₃₈ H ₃₈ BrF ₃ N ₄ O ₇ S	0.149
20k	PhCH ₂ O	H		H	H	resin	D-G	C ₂₇ H ₃₀ F ₃ N ₃ O ₅	>5
20l	<i>p</i> -(<i>p</i> -ClC ₆ H ₄ SO ₂ NHCO)C ₆ H ₄	H		H	CH ₃	115-123	D-F, H, J, K	C ₃₄ H ₃₄ ClF ₃ N ₄ O ₇ S	0.817
20m	<i>p</i> -(<i>p</i> -ClC ₆ H ₄ SO ₂ NHCO)C ₆ H ₄	H		CH ₃	CH ₃	143-147	D-F, H, J, K	C ₃₆ H ₃₈ ClF ₃ N ₄ O ₇ S	>5
20n	PhCH ₂ O	CH ₃		H	CH(CH ₃) ₂	resin	O, E-G	C ₃₁ H ₃₈ F ₃ N ₃ O ₅	1.562
20o	<i>p</i> -(<i>p</i> -ClC ₆ H ₄ SO ₂ NHCO)C ₆ H ₄	H		H	CH ₂ Ph	resin	O, E, F, H, J, K	C ₄₀ H ₃₈ ClF ₃ N ₄ O ₇ S	>5
37b	(CH ₃) ₃ CO	H		H	CH(CH ₃) ₂	resin	L, E-G	C ₂₇ H ₄₀ F ₃ N ₃ O ₄	>5
38c	<i>p</i> -(<i>p</i> -ClC ₆ H ₄ SO ₂ NHCO)C ₆ H ₄	H	(reduced carbonyl between P ₃ and P ₂) 	H	CH(CH ₃) ₂	resin	L, E, F, H, J, K	C ₃₆ H ₄₀ ClF ₃ N ₄ O ₆ S	5.0
29b		H	(reduced carbonyl between P ₃ and P ₂) 	H	CH(CH ₃) ₂	94-101	D-F, H, J, K	C ₃₄ H ₄₅ F ₃ N ₆ O ₆ S	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	<i>p</i> -(<i>p</i> -ClC ₆ H ₄ SO ₂ NHCO)C ₆ H ₄	H	<i>c</i> -C ₇ H ₁₃	H	CH(CH ₃) ₂	98–102	H, J, K	C ₃₄ H ₄₂ ClF ₃ N ₄ O ₇ S	0.175
20q	<i>p</i> -(<i>p</i> -ClC ₆ H ₄ SO ₂ NHCO)C ₆ H ₄	H	<i>c</i> -C ₈ H ₁₅	H	CH(CH ₃) ₂	183–189	D–F, I–K	C ₃₆ H ₄₄ ClF ₃ N ₄ O ₇ S	0.067
20r	PhCH ₂ O	H		H	CH(CH ₃) ₂	87–91	D–G	C ₂₉ H ₄₁ F ₃ N ₄ O ₇	0.507
20s	<i>p</i> -(<i>p</i> -ClC ₆ H ₄ SO ₂ NHCO)C ₆ H ₄	H		H	CH(CH ₃) ₂	151–153	H, J, K	C ₃₅ H ₄₃ ClF ₃ N ₅ O ₉ S	0.057
20t	<i>p</i> -(<i>p</i> -ClC ₆ H ₄ SO ₂ NHCO)C ₆ H ₄	H	–CH ₂ Ph	H	CH(CH ₃) ₂	140–142	D–F, H, J, K	C ₃₄ H ₃₆ ClF ₃ N ₄ O ₇ S	0.217
20u	PhCH ₂ O	H		H	CH(CH ₃) ₂	54–59	D–G	C ₃₁ H ₄₀ F ₃ N ₃ O ₇	0.693
20v	<i>p</i> -(<i>p</i> -ClC ₆ H ₄ SO ₂ NHCO)C ₆ H ₄	H		H	CH(CH ₃) ₂	115–118	H, J, K	C ₃₇ H ₄₂ ClF ₃ N ₄ O ₉ S	0.084
20w	<i>p</i> -(<i>p</i> -ClC ₆ H ₄ SO ₂ NHCO)C ₆ H ₄	H		H	CH(CH ₃) ₂	187–190	D–F, H, J, K	C ₃₂ H ₃₈ ClF ₃ N ₄ O ₇ S	0.030
20x	<i>p</i> -(<i>p</i> -ClC ₆ H ₄ SO ₂ NHCO)C ₆ H ₄	H		H	CH(CH ₃) ₂	187–190	D–F, H, J, K	C ₃₂ H ₃₄ ClF ₃ N ₄ O ₈ S	0.138
20y ^f	PhCH ₂ O	H		H	CH(CH ₃) ₂	53–61	Q, E–G	C ₂₉ H ₃₄ F ₃ N ₃ O ₆	0.233
20z ^g	PhCH ₂ O	H		H	CH(CH ₃) ₂	54–61	Q, E–G	C ₂₉ H ₃₄ F ₃ N ₃ O ₆	0.073
20aa ^f	<i>p</i> -(<i>p</i> -ClC ₆ H ₄ SO ₂ NHCO)C ₆ H ₄	H		H	CH(CH ₃) ₂	167–172	H, J, K	C ₃₅ H ₃₈ ClF ₃ N ₄ O ₇ S	0.110
20ab ^h	(CH ₃) ₃ CO	H		H	CH(CH ₃) ₂	57–62	D–G	C ₂₁ H ₃₄ F ₃ N ₃ O ₆	0.172
20ac ^{h,i}	(CH ₃) ₃ CO	H		H	CH(CH ₃) ₂	62–65	D–G	C ₂₁ H ₃₄ F ₃ N ₃ O ₆	0.065
1	4 (X = Br) ^k								0.107 ^j 0.038 ^k

^a Except where indicated all compounds are racemic at P₁. ^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, ¹³C 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 P₂-N-substituted glycine. ^g Diastereomer of 20y. ^h L-Proline substitution for P₂-N-substituted glycine. ⁱ Diastereomer of 20ab. ^j lit.³⁵ IC₅₀ = 1.33 μM. ^k lit.^{47a} K_i = 5 × 10⁻¹⁰ M.

HLE, is shown in red. In this binding conformation of 20h, the P₁-trifluoromethyl ketone of the inhibitor is covalently linked to Ser¹⁹⁵ via hemiketal formation to HLE. Hydrogen bonds between the HLE binding site residues and those of the inhibitor 20h are as follows: Val²¹⁶ CO to NH of P₃-Val, 1.84 Å; Val²¹⁶ NH to CO of P₃-Val, 1.97 Å; Ser²¹⁴ CO to NH of P₁-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 P₃-P₁ 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 P₂-nitrogen should have very little effect on binding and

(67) Bogard, W. C., Jr.; Kato, I.; Laskowski, M., Jr. A Ser¹⁶²/Gly¹⁶² Polymorphism in Japanese Quail Ovomucoid. *J. Biol. Chem.* 1980, 255, 6569–6574.

Table II. Truncated Inhibitors (P₅-P₁)

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, ¹³C NMR, and MS spectra consistent with the assigned structures. ^b Except where indicated all compounds are racemic at P₁. ^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-pNA 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 ₂ CO		H	CH(CH ₃) ₂	resin	F, G, M, N	C ₃₆ H ₄₀ F ₃ N ₃ O ₅	>5
41e	<i>p</i> -(<i>p</i> -ClC ₆ H ₄ SO ₂ NHCO)C ₆ H ₄		H	CH(CH ₃) ₂	resin	H, J, K	C ₄₂ H ₄₂ ClF ₃ N ₄ O ₇ S	5.1

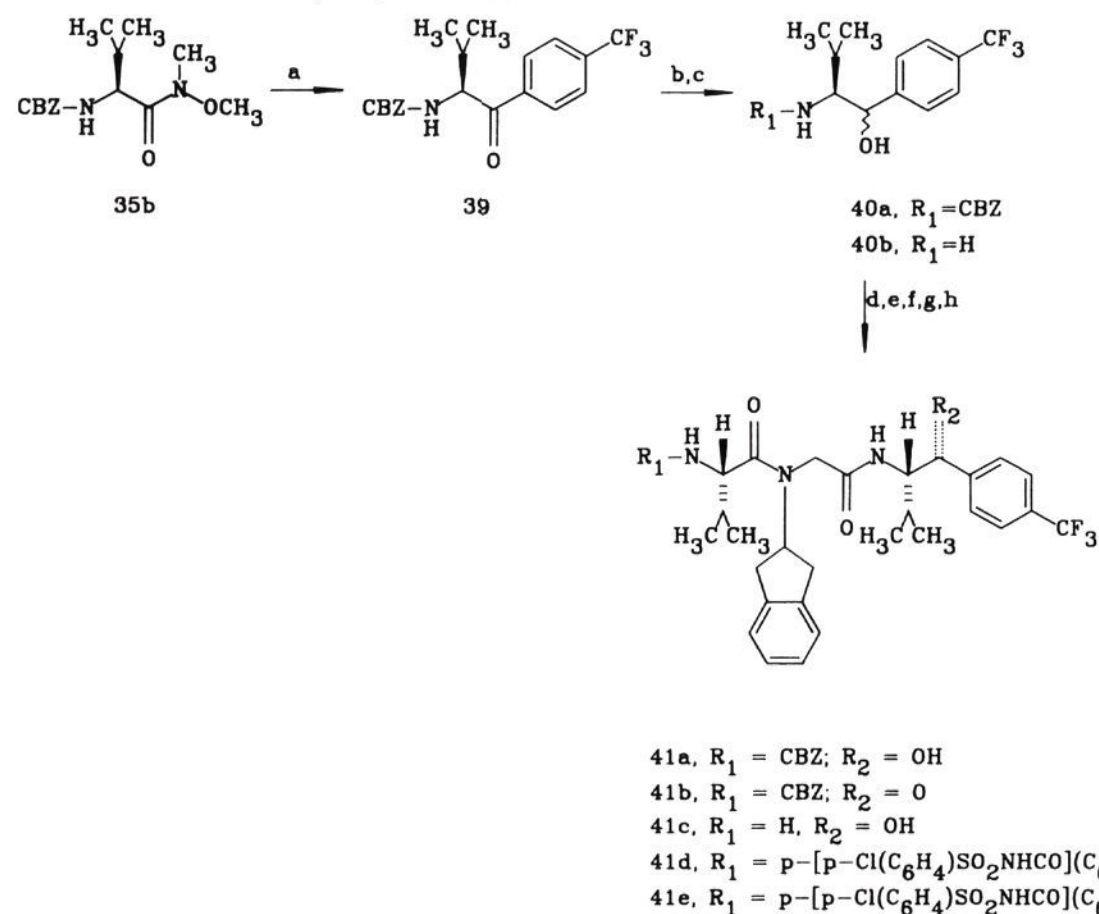
^a Compounds are of the *S* configuration at P₁. ^b Uncorrected. ^c See the Experimental Section. ^d All compounds gave satisfactory C, H, and N microanalyses and were within 0.4% of the theoretical values. ^e Concentration inhibiting 50% of the activity of HLE at pH 7.5 in 0.1 M Tris buffer containing 0.5 M NaCl with the substrate MeO-Suc-Ala-Ala-Pro-Val-pNA 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 P₂-nitrogen do not have any dramatic effect on in vitro potency.

In order to test the assertion that reduction of the P₃-P₂ amide bond [yielding the methylene (ψ(CH₂)) 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 Val²¹⁶ NH and the P₃-carbonyl of the inhibitor, which is present in **20i** (IC₅₀ = 0.084 μM), but is not possible in the inactive methylene derivative **38c** (IC₅₀ > 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*-CF₃(C₆H₄)Cl, Mg; (b) NaBH₄, CH₃OH to give 40a; (c) 40a, 10% Pd/C, EtOH, H₂, 45 psi to give 40b; (d) 15c (R₂ = 2-indanyl), 40b, HOBT, WSCDI, THF to give 41a; (e) 41a, Dess-Martin periodinane, CF₃COOH to give 41b; (f) 41a, 10% Pd/C, EtOH, H₂, 45 psi to give 41c; (g) 41c, 25a, THF, HOBT, WSCDI to give 41d; (h) 41d, Dess-Martin periodinane, CF₃COOH, CH₂Cl₂.

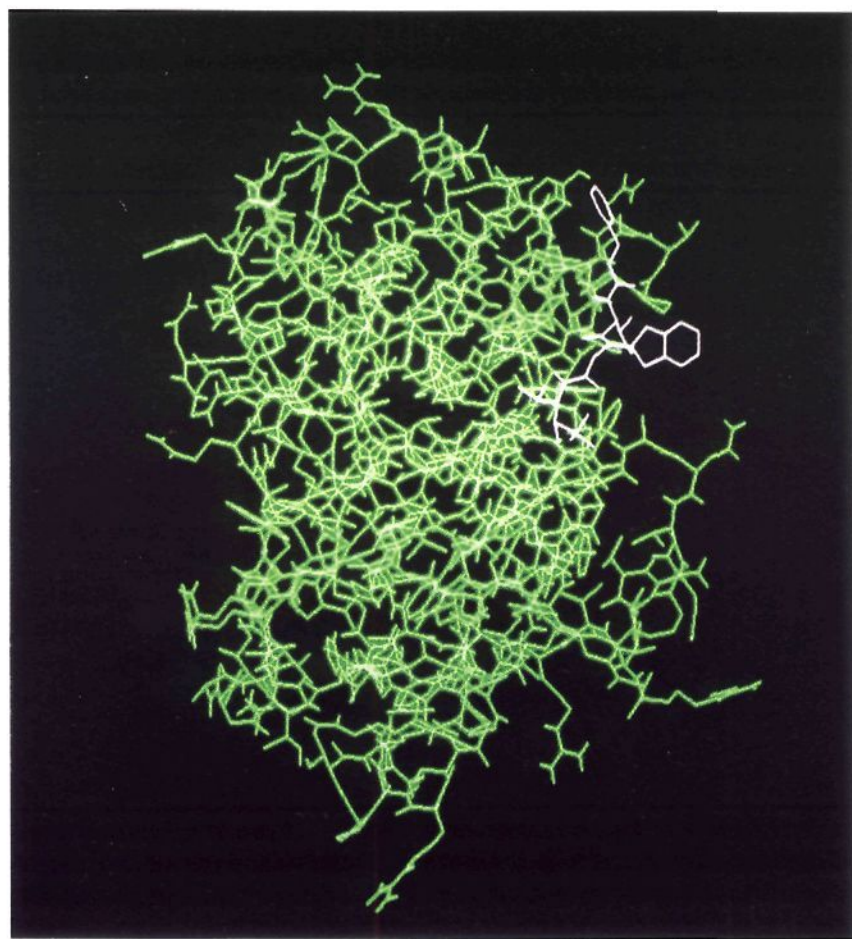


Figure 2. Minimized structure of HLE-inhibitor 20h complex. The inhibitor 20h is pink.

hemorrhage in an elastase-induced pulmonary hemorrhage (EPH) model in hamsters (see Table IV). Whereas the P₃-P₂ 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 P₃-P₂ amide bond, has an ED₅₀ 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 P₁ is valine [e.g. inhibitor 20i (BI-RA-260) (IC₅₀ = 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 later 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 ED₅₀ = 3.8 μg per animal. ^e ED₅₀ = 1.3 μg per animal.

in potency when the valine residue at the P₁-position is replaced by alanine [201 (IC₅₀ = 0.817 μM)]. Likewise, replacement of the P₁-valine in 20i to the corresponding phenylalanine (20o), α,α-dimethylglycine (20m), or glycine (20k) all afford inactive compounds (IC₅₀ >> 10 μM). This high specificity of HLE for valine at P₁ has previously been observed and is referred to as the primary specificity site of HLE. The S₁-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 S₁-region, which may result in unfavorable

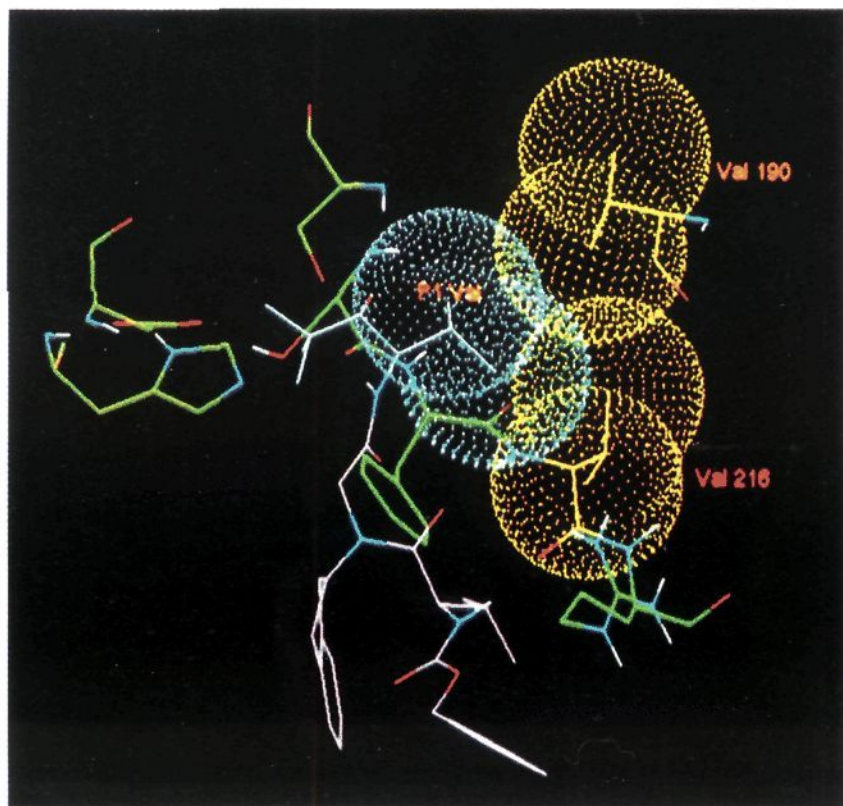


Figure 3. Minimized structure of the HLE-inhibitor **20h** (P_1 -Val) complex illustrating orbital overlap between the P_1 -side chain (blue) with the side chains of Val¹⁹⁰ and Val²¹⁶ (yellow). The van der Waals surfaces of the side chains of Val¹⁹⁰ and Val²¹⁶ of HLE and that of the P_1 -Val residue of the inhibitor are represented as dot surfaces.

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 Val¹⁹⁰ and Val²¹⁶ 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 Val¹⁹⁰ and Val²¹⁶ 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 Ser¹⁹⁵.

In the homologous series displayed in Table II, **20i** ($IC_{50} = 0.084 \mu M$), 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 ($IC_{50} = 0.365 \mu M$). 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 ($IC_{50} \gg 10 \mu M$).

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 Ser¹⁹⁵ in

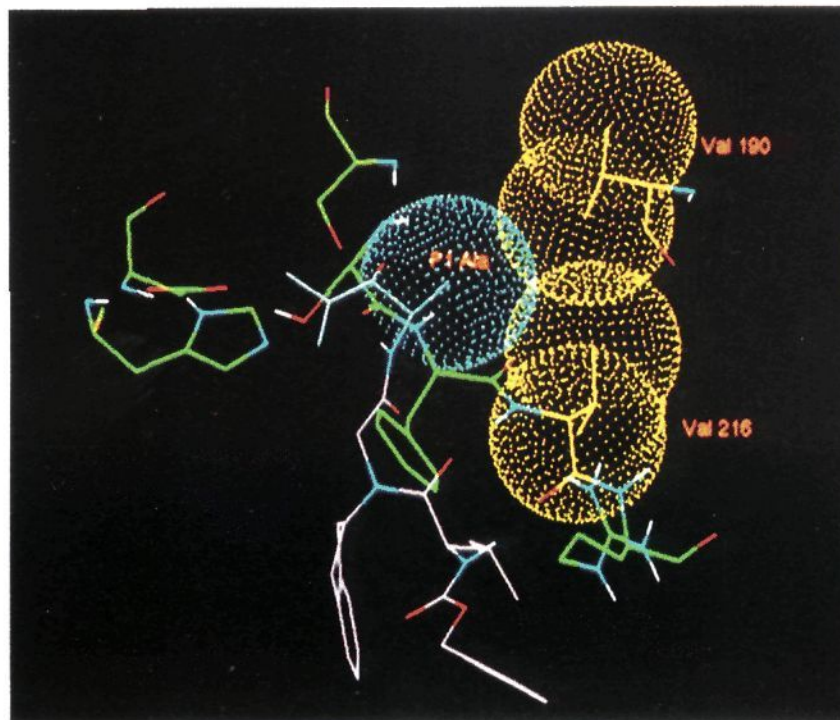


Figure 4. Minimized structure of the CBZ derivative of **20l** (P_1 -Ala) complexed with HLE illustrating orbital overlap between the P_1 -side chain (blue) with the side chains of Val¹⁹⁰ and Val²¹⁶ (yellow).

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 Ser¹⁹⁵.

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 ($IC_{50} \gg 10 \mu M$).

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 hamster²³ 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** ($IC_{50} = 0.084 \mu M$), 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 $\mu g/mL$ it. The dose-response curves for the inhibitors **20i** ($ED_{50} = 4.8 \mu g$ it. per animal) and **4** ($ED_{50} = 1.3 \mu g$ it. per animal) are presented in Figure 5. Interestingly, the *in vitro* potency of cephalosporin **1**³⁵ ($IC_{50} = 0.02 \mu M$, lit.³⁵ value 1.33 μM) does not translate into *in vivo* activity (0% inhibition of hemorrhage when tested at 100 $\mu g/mL$ 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

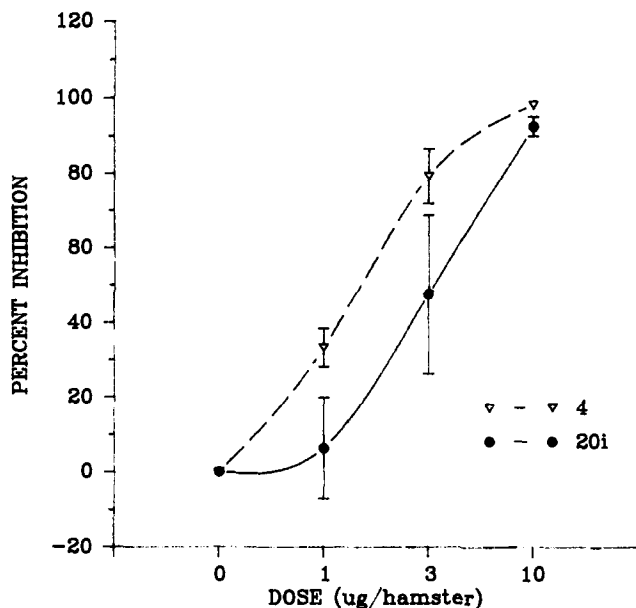


Figure 5. Inhibition of elastase-induced pulmonary hemorrhage (EPH) in hamsters by 20i and 4. Compounds were administered intratracheally (it.) in a 0.1-mL volume (DMSO, 1:100 in saline) followed 5 min later by 50 μ g (it.) of HLE in 0.1 mL of saline. Average percent reduction of red blood cells (RBC)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

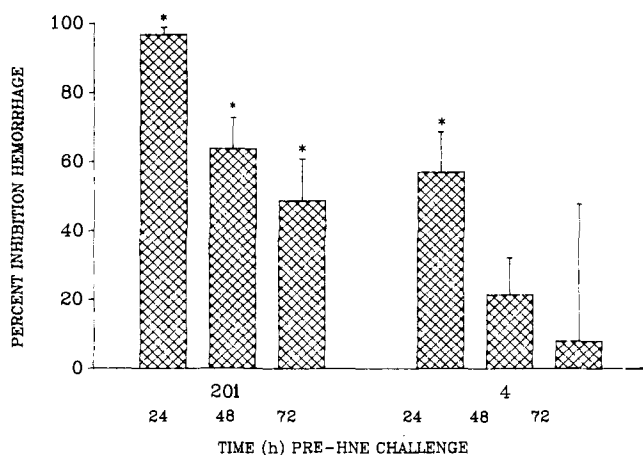


Figure 6. Effects of 20i and 4 on EPH: duration of action. Compounds were administered at 20 μ g it. to hamsters at the indicated time intervals prior to challenge with 50 μ g of HLE it.

directly compared in our laboratories, the previously reported⁴⁷ 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 μ g of 20i prior to its administration to hamsters inhibited hemorrhage by 96.7%.

Previous research²³ has shown that purified preparations of elastase from neutrophils and sputum (from patients with cystic fibrosis) can lead to emphysema⁶⁸ when instilled into the lungs of dogs and hamsters. Thus, in a 21-day

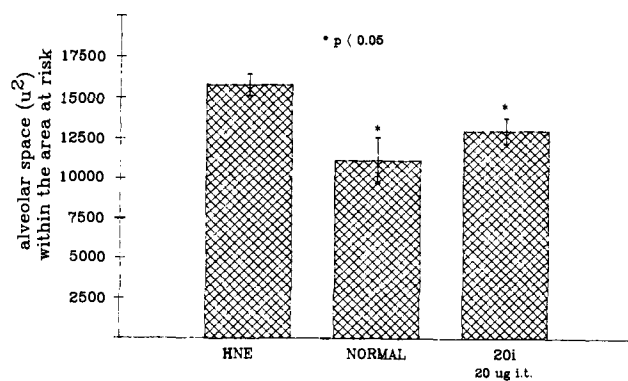


Figure 7. Effects of 20i on 21-day elastase-induced emphysema in hamsters. Five animals per group were used. HNE = Compound vehicle plus 200 μ g of HLE administered it. Normal = no treatment. Compound 20i was administered at 20 μ g it., 5 min prior to a 50 μ g it. challenge with HLE.

chronic model of emphysema in hamsters, 20 μ g 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).

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 ($IC_{50} = 0.084 \mu$ M), showed significant activity in this assay ($ED_{50} = 4.8 \mu$ g) for 72 h. As a comparison, a previously reported⁴⁷ 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 μ g 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 $\pm 0.4\%$ 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 μ A 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; $\text{R}_1 = 2$ -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 Et₂O (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 Et₂O 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, ⁺NH₂), 7.1–7.3 (m, 4 H), 4.3–4.2 (q, 2 H, CH₂), 4.05 (bs, 3 H, CH₂ + CH), 3.3–3.2 (dd, 4 H, 2 × CH₂ of indanyl), 1.2–1.3 (t, 3 H, CH₃). Anal. (C₁₃H₁₇NO₂·HCl) C, H, N, Cl.

Method B. *N*-Cyclopentylglycine Ethyl Ester Hydrochloride (12a; $\text{R}_1 = \text{c-C}_5\text{H}_9$). 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 Et₃N (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 Et₃N·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 C₆H₁₄/EtOAc (4:1) as the eluant. The collected product was dissolved in Et₂O 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 (CDCl₃) δ 9.8 (s, 2 H, ⁺NH₂), 4.3–4.2 (q, 2 H, CH₂), 3.85 (s, 2 H, α -CH₂ of Gly), 3.7–3.6 (m, 1 H, CH), 2.0–1.8 (m, 6 H, 3 × CH₂), 1.65–1.5 (m, 2 H, CH₂), 1.3–1.2 (t, 3 H, CH₃); ¹³C NMR (CDCl₃) δ 165.6 (CO), 61.8 (CH₂), 59.4 (CH), 46.1 (α -CH₂ of Gly), 28.9 (2 × CH₂), 23.5 (2 × CH₂), 13.6 (CH₃). Anal. (C₉H₁₇NO₂·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 (CDCl₃) δ 4.2 (d, 2 H, CH₂), 2.6–2.4 (m, 1 H, CH), 1.1–1.0 (d, 6 H, 2 × CH₃); ¹³C NMR (CDCl₃) δ 82.7 (CH₂NO₂), 27.9 (CH), 19.4 (2 × CH₃).

[(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), CF₃CH(OC₂H₅)OH (90%, 60.4 g, 0.377 mol), and K₂CO₃ (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 (CDCl₃) 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 × CH₃); ¹³C NMR (DMSO-*d*₆) 124.1 (q, CF₃, *J* = 284.54 Hz), 92.0 (CHNO₂), 67.4 (q, CH(OH)CF₃, *J* = 30.9 Hz), 22.7 (CH),

18.5, 16.9 (CH₃). Anal. (C₈H₁₀F₃NO₃) 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 LiAlH₄ as previously reported⁷⁰ 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/NH₃) *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 × CH₃); ¹³C NMR (DMSO-*d*₆) δ 124.65 (q, CF₃), 69.5–63.3 (m, CH(OH)-CF₃), 55.4, 53.8 (CHNH₂), 28.7, 26.2 (CH), 17.6, 17.1 (CH₃). Anal. (C₈H₁₂F₃NO·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 (CDCl₃) 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, NH₂), 1.2–1.0 (m, 6 H, 2 × CH₃).

[(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 CH₃OH 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 CH₂Cl₂ and then with 3% CH₃OH in CH₂Cl₂ gave the pure *dl*-erythro isomer 8a (PG = H) as a colorless solid: mp 63–65 °C; ¹H NMR (CDCl₃) 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 × CH₃). Anal. (C₈H₁₂F₃NO) C, H, N.

Method C. *N*-(Carbobenzyloxy)-L-valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine Ethyl Ester (15a; $\text{R}_2 = 2$ -indanyl, $\text{R}_2 = \text{Et}$). To a solution of CBZ-L-valine (5.0 g, 0.02 mol) in CH₂Cl₂ (70 mL) were added DMAP (2.44 g, 0.02 mol), 12a ($\text{R}_1 = 2$ -indanyl, $\text{R}_2 = \text{Et}$) (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 CH₂Cl₂ 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 Na₂CO₃, and saturated aqueous NaCl. The EtOAc was dried (MgSO₄), 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 (CH₂Cl₂) to give the title compound 15a as a colorless oil, *R*_f 0.7 [silica gel; CH₂Cl₂/CH₃OH (97:3)], which was used directly without further purification: MS (CI/CH₄) *m/z* (relative intensity) 453 (MH⁺, 96), 345 (100), 310 (34), 220 (84), 117 (21), 116 (23); ¹H NMR (CDCl₃) 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, CH₃), 1.1–0.9 (dd, 6 H, 2 × CH₃ of Val); ¹³C NMR (CDCl₃) 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 (PhCH₂), 60.8 (CH₂), 56.9 (α -CH of Val), 56.5 (CH of indanyl), 44.0 (α -CH₂ of Gly), 37.6 (CH₂ of indanyl), 37.0 (CH₂ of indanyl), 31.7 (β -CH of Val), 19.4 (CH₃ of Val), 17.1 (CH₃ of Val), 13.6 (CH₃).

Method D. *N*-(Carbobenzyloxy)-L-valyl-*N*-(3,4-dimethoxyphenethyl)glycine Ethyl Ester (15a; $\text{R}_2 = 3,4$ -dimethoxyphenethyl, $\text{R}_3 = \text{Et}$). By a procedure similar to method C, but employing CDI as the coupling reagent, CBZ-L-valine (6.3 g, 0.025 mol) in CH₂Cl₂ (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; CH₂Cl₂/CH₃OH (97:3)], which was used directly without further purification: MS (FAB) *m/z* (relative intensity) 501.5 (100); ¹³C NMR (CDCl₃) 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 (PhCH₂), 60.9 (CH₂), 55.7 (OCH₃), 55.4 (α -CH of Val), 50.7 (CH₂), 48.7 (α -CH₂ of Gly), 34.8 (CH₂), 30.9 (β -CH of Val), 18.9 (CH₃ of Val), 17.2 (CH₃ of Val), 13.8 (CH₃).

Method E. *N*-(Carbobenzyloxy)-L-valyl-*N*-(2,3-dihydro-1*H*-inden-2-yl)glycine (15c; $\text{R}_2 = 2$ -indanyl). The corresponding CBZ ethyl ester derivative 15a ($\text{R}_2 = 2$ -indanyl, $\text{R}_2 = \text{Et}$) (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 H₂O. 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 (MgSO₄), filtered, and concentrated under reduced pressure to afford the pure title compound 15c as a colorless semisolid (9.1 g): MS (CI/NH₃) *m/z* (relative intensity) 425 (MH⁺, 53), 317 (98), 258 (15), 192 (34); ¹H NMR (CDCl₃) 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); ¹³C NMR (CDCl₃) 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 (PhCH₂), 56.6 (CH of indanyl), 55.9 (α-CH of Val), 44.0 (α-CH₂ of Gly), 36.9 (2 × CH₂ of indanyl), 31.5 (β-CH of Val), 19.2 (CH₃ of Val), 17.3 (CH₃ of Val). Anal. (C₂₄H₂₃N₂O₅) 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; R₁ = H; R₂ = 2-indanyl, R₃ = CH(CH₃)₂, R₄ = H). To a solution of 15c (R₂ = 2-indanyl) (4.25 g, 0.01 mol) in CH₂Cl₂ (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 Et₃N (1.01 g, 0.01 mol) in CH₂Cl₂ (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 Na₂CO₃, and saturated aqueous NaCl. The organic phase was dried (MgSO₄), filtered, and concentrated to yield an oil which was purified over silica gel [CH₂Cl₂/CH₃OH (97:3)]. The title compound 16 was obtained (4.5 g, 78%) as a colorless solid: mp 64–67 °C; MS (CI/CH₄) *m/z* (relative intensity) 578 (MH⁺, 88), 470 (46), 407 (100), 345 (89), 299 (28), 172 (13); ¹H NMR (CDCl₃) 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-CH₃ of Val); ¹³C NMR (CDCl₃) 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)CF₃), 67.1 (PhCH₂), 57.9–56.4 (CH of indanyl), 55.8 (α-CH of Val), 46.9 (α-CH₂ of Gly), 36.9 (2 × CH₂ of indanyl), 31.3, 28.4 (β-CH of Val), 19.3 (CH₃ of Val), 18.7 (CH₃ of Val), 18.2 (CH₃ of Val), 17.5 (CH₃ of Val). Anal. (C₃₀H₃₃F₃N₃O₅) 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 = OCH₂Ph). To a solution of 16 (R₁ = H, R₂ = 2-indanyl, R₃ = CH(CH₃)₂, R₄ = H) (4.3 g, 7.45 mmol) in THF (120 mL) was added the Dess–Martin periodinane reagent⁶⁰ (9.5 g, 22.3 mmol) in CH₂Cl₂ (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 NaHCO₃ and Na₂S₂O₃. The organic layer was separated and washed repeatedly with dilute aqueous solutions of NaHCO₃ and Na₂S₂O₃. After a final wash with brine, the organic extract was dried (MgSO₄), filtered, and concentrated to afford a solid which was further purified by chromatography over silica gel using a gradient system of first 100% CH₂Cl₂ followed by CH₂Cl₂/CH₃OH (97:3). The title compound 20h was obtained as a colorless solid (3.1 g, 72%): mp 49–54 °C; MS (CI/CH₄) *m/z* (relative intensity) 576 (MH⁺, 100), 468 (29), 407 (95), 343 (46); ¹H NMR (CDCl₃) 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); ¹³C NMR (CDCl₃) δ 201.9 (COCF₃), 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 (PhCH₂), 59.0, 57.9, 56.3 (CH of indanyl), 46.7 (α-CH₂ of Gly), 37.1 (2 × CH₂ of indanyl), 31.5 (β-CH of Val), 29.0 (β-CH of Val), 19.5 (CH₃ of Val), 18.5 (CH₃ of Val), 17.5 (CH₃ of Val), 16.6 (CH₃ of Val). Anal. (C₃₀H₃₃F₃N₃O₅) 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; R₁ = H, R₂ = 2-indanyl, R₃ = CH(CH₃)₂, R₄ = H). To a solution of 16 (R₁ = H, R₂ = 2-indanyl, R₂ = CH(CH₃)₂, R₄ = 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/CH₄) *m/z* (relative intensity) 444 (MH⁺, 29), 426 (14), 345 (100), 273 (27); ¹H NMR (CDCl₃) 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); ¹³C NMR (CDCl₃) δ 176.0 (CO), 175.3 (CO), 140.0, 127.0, 124.5, 71.0–69.0 (m, CH(OH)CF₃), 56.9 (CH of indanyl), 54.4, 53.9 (α-CH of Val), 46.6 (α-CH₂ of Gly), 37.1 (2 × CH₂ of indanyl), 30.1 (β-CH of Val), 29.5 (β-CH of Val), 19.2 (CH₃ of Val), 18.7 (CH₃ of Val), 17.7 (CH₃ of Val), 16.7 (CH₃ 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; R₁ = H, R₂ = *c*-C₈H₁₅, R₃ = CH(CH₃)₂, R₄ = H). *t*-BOC compound 16 (R₁ = H, R₂ = *c*-C₈H₁₅, R₃ = CH(CH₃)₂, R₄ = H) (3.2 g, 6 mmol) was dissolved in Et₂O (30 mL) and then chilled to 5 °C by means of an ice/H₂O 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. (C₂₇H₃₉F₃N₃O₅·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 CH₃OH 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 × CH₃).

Terephthalic Acid Mono-*tert*-butyl Ester (23).⁷⁰ This material was prepared from 22 according to the previously described method⁷⁰ to give 23 as a colorless solid (96%): mp 100–102 °C; ¹H NMR (CDCl₃) δ 12.0–11.6 (bs, 1 H, COOH), 8.2–8.0 (m, 4 H), 1.64 (s, 9 H, CH₃).

***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/NH₃) *m/z* (relative intensity) 413 (M⁺ + NH₄, 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 × CH₃). Anal. (C₁₈H₁₈ClNO₅S) C, H, N, Cl, S.

4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]benzoic acid (25a).⁷⁰ Compound 24a was converted to the acid 25a by treatment with trifluoroacetic acid as previously described⁷⁰ to give, after recrystallization from EtOH/H₂O (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); ¹³C 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. (C₁₄H₁₀ClNO₅S) C, H, N, Cl, S.

4-[[[(4-Bromophenyl)sulfonyl]amino]carbonyl]benzoic 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. (C₁₄H₁₀BrNO₅S) C, H, Br, N, S.

Method J. [(2*R*,3*S*)+(2*S*,3*R*)]-N-[[[[(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; R₁ = H, R₂ = 2-indanyl, R₃ = CH(CH₃)₂, R₄ = H). To a solution of 18 (R₁ = H, R₂ = 2-indanyl, R₃ = CH(CH₃)₂, R₄ = 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 Na₂CO₃, and saturated aqueous NaCl. The organic phase was dried (MgSO₄), filtered, and concentrated to give a residue which was purified by chromatography over silica gel [CH₂Cl₂/CH₃OH (97:3)]. The desired title compound 19 was obtained as a colorless solid (1.8 g, 80%): mp 140–144 °C; MS (CI/NH₃) *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 (CD₃OD) 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); ¹³C NMR (CD₃OD) 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, CH(OH)CF₃), 58.6, 56.6, 54.0, 46.4 (α -CH₂ of Gly), 38.3 (2 \times CH₂ of indanyl), 32.0 (β -CH of Val), 29.1 (β -CH of Val), 20.0 (CH₃ of Val), 19.7 (CH₃ of Val), 19.2 (CH₃ of Val), 18.7 (CH₃ of Val). Anal. (C₃₆H₄₀ClF₃N₄O₇S) C, H, F, N, S.

Method K. (3RS)-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-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 reagent⁶⁰ (2.66 g, 6.3 mmol) in CH₂Cl₂ (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 NaHCO₃ and Na₂S₂O₃. After a final wash with brine the organic extract was dried (MgSO₄), filtered, and concentrated to afford a solid which was further purified by chromatography over silica gel [CH₂Cl₂/CH₃OH (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/NH₃) m/z (relative intensity) 763 (MH⁺, 2), 746 (2), 421 (6), 377 (14), 343 (100), 325 (31), 247 (8); ¹H NMR (CD₃OD) 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); ¹³C NMR (CD₃OD) 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 -CH₂ of Gly), 38.3 (2 \times CH₂ of indanyl), 32.2 (β -CH of Val), 29.0 (β -CH of Val), 20.9 (CH₃ of Val), 20.0 (CH₃ of Val), 19.2 (CH₃ of Val), 18.8 (CH₃ of Val). Anal. (C₃₆H₃₈ClF₃N₄O₇S) C, H, F, N, S.

N-(Carbobenzyloxy)-N-(2,3-dihydro-1H-inden-2-yl)-glycine Ethyl Ester (30a; R₁ = 2-indanyl). *N*-(2,3-Dihydro-1H-inden-2-yl)glycine ethyl ester (12a; R₁ = 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/H₂O 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 Et₃N·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 (CDCl₃) δ 7.3 (s, 5 H, Ph), 7.1 (s, 4 H, 2 \times CH₂ of indanyl), 5.15 (s, 2 H, CH₂), 5.0 (m, 1 H, CH of indanyl), 4.2–4.1 (m, 2 H), 3.8 (s, 2 H, α -CH₂ of Gly), 3.3–2.9 (m, 4 H, 2 \times CH₂ of indanyl), 1.1 (t, 3 H, CH₃).

N-(Carbobenzyloxy)-N-(2,3-dihydro-1H-inden-2-yl)-glycine (30b; R₁ = 2-indanyl). Crude ester 30a (R₁ = 2-indanyl) (4.0 g, 11.2 mmol) was hydrolyzed to acid 30b (R₁ = 2-indanyl) as described in method E (96%): mp 95–97 °C; ¹H NMR (CDCl₃) 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 -CH₂ of Gly), 3.3–2.9 (m, 4 H, 2 \times CH₂ of indanyl). Anal. (C₁₉H₁₉NO₄) C, H, N.

[(2R,3S)+(2S,3R)]-N-(Carbobenzyloxy)-N-(2,3-dihydro-1H-inden-2-yl)glycine N-[3-(1,1,1-Trifluoro-4-methyl-2-hydroxypentyl)amide (31; R₁ = 2-indanyl). According to method F, 30b (R₁ = 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/CH₄) m/z (relative intensity) 479 (MH⁺, 100), 461 (24), 435 (84), 371 (14), 345 (13), 308 (7), 116 (81); ¹H NMR (CDCl₃) 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 -CH₂ of Gly), 3.6 (m, 1 H), 3.3–2.9 (m, 4 H, 2 \times CH₂ of indanyl), 2.1 (bm, 1 H, α -CH of Val), 0.95–0.8 (dd, 6 H, 2 \times CH₃); ¹³C NMR (CDCl₃) 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, CH(OH)CF₃, J = 29.43 Hz), 65.5 (s, CH₂), 55.6 (CH of indanyl), 45.8 (α -CH₂ of Gly), 35.2 (2 \times CH₂ of indanyl), 28.9 (β -CH of Val),

18.0 (CH₃ of Val), 17.15 (CH₃ of Val). Anal. (C₂₅H₂₉F₃N₂O₄) C, H, F, N.

(3RS)-N-(Carbobenzyloxy)-N-(2,3-dihydro-1H-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 (R₁ = 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/CH₄) m/z (relative intensity) 477 (MH⁺, 47), 461 (2), 433 (100), 305 (1), 241 (30), 146 (7), 117 (18), 116 (9); ¹H NMR (CDCl₃) 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 -CH₂ of Gly), 3.4–3.0 (2 m, 4 H, 2 \times CH₂ of indanyl), 2.4 (m, 1 H, β -CH of Val), 1.05 (d, 3 H, CH₃ of Val), 0.8 (d, 3 H, CH₃ of Val); ¹³C NMR (CDCl₃) δ 191 (COCF₃), 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 (CH₂), 58.8, 57.4, 48.1 (α -CH₂ of Gly), 37.1 (CH₂ of indanyl), 36.9 (CH₂ of indanyl), 29.3 (β -CH of Val), 19.6 (CH₃ of Val), 16.4 (CH₃ of Val). Anal. (C₂₅H₂₇F₃N₂O₄) C, H, F, N.

[(2R,3S)+(2S,3R)]-N-(Carbobenzyloxy)-2-hydroxy-4-methyl-1,1,1-trifluoro-3-pentylamine (33). By a method similar to that described above for 30a (R₁ = 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 (CDCl₃) 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); ¹³C NMR (CDCl₃) δ 157 (CO), 136, 128.6, 128.4, 128.3, 127.6, 127.0, 124.5 (q, CF₃, J = 283 Hz), 70.1 (q, CH(OH)CF₃, J = 30.13 Hz), 67.1 (PhCH₂), 55.5, 30.3, 19.3, 18.6.

(3RS)-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/CH₄) m/z (relative intensity) 304 (MH⁺, 100), 260 (24), 238 (34), 214 (37), 198 (40), 181 (49), 170 (44), 153 (51). Anal. (C₁₄H₁₆F₃NO₃) C, H, N.

Method L. N-(tert-Butoxycarbonyl)-L-valyl- ψ (CH₂)-N-(2,3-dihydro-1H-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-1H-inden-2-yl)glycine ethyl ester (12a; R₂ = 2-indanyl) (2.5 g, 0.01 mol) in absolute EtOH (50 mL) was added NaCNBH₃ (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 H₂O and Et₂O. The layers were separated, and the Et₂O phase was washed with dilute aqueous Na₂CO₃, dried (MgSO₄), 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% CH₂Cl₂ in petroleum ether afforded the desired pure product 36a (660 mg, 16%). The major part of the product was eluted with 20% CH₂Cl₂ in petroleum ether; ¹H NMR (CDCl₃) δ 7.15 (m, 4 H), 4.8 (bs, 1 H, NH), 4.15 (q, 2 H, CH₂CH₃), 3.8 (q, 1 H, CH of indanyl), 3.6 (m, 1 H, α -CH of Val), 3.4 (s, 2 H, α -CH₂ of Gly), 3.1–2.8 (m, 4 H, 2 \times CH₂ of indanyl), 2.7 (t, 1 H, CHCH₂N), 2.6 (t, 2 H, CHCH₂N), 2.0 (m, 1 H, α -CH of Val), 1.5 (s, 9 H, *t*-Bu), 1.25 (t, 3 H, CH₃), 0.9 (dd, 6 H, 2 \times CH₃). Anal. (C₂₈H₃₆N₂O₄) C, H, N.

N-(tert-Butoxycarbonyl)-L-valyl- ψ (CH₂)-N-(2,3-dihydro-1H-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 H₂O and the pH was adjusted to approximately 6 by the dropwise addition of 1 N aqueous HCl. The mixture was extracted several times into CH₂Cl₂. The combined CH₂Cl₂ extract was dried (MgSO₄), filtered, and concentrated to afford pure 36b (730 mg, 88%); ¹H NMR (CDCl₃) 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 -CH₂ of Gly), 3.2–2.7 (m, 6 H, CH₂N, 2 \times CH₂ of indanyl, α -CH₂ of Gly), 1.8 (m, 1 H, β -CH of Val), 1.5 (s, 9 H, *t*-Bu), 0.9 (m, 6 H, 2 \times CH₃ of Val). Anal. (C₂₁H₃₂N₂O₄) C, H, N.

[(2R,3S)+(2S,3R)]-N-(tert-Butoxycarbonyl)-L-valyl- ψ (CH₂)-N-(2,3-dihydro-1H-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/NH₃) *m/z* (relative intensity) 530 (MH⁺, 100); ¹H NMR (CDCl₃) 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, CHOH), 3.9 (m, 1 H, CH of indanyl), 3.7 (m, 1 H, α-CH of Val), 3.4 (m, 2 H, α-CH₂ of Gly), 3.2–2.7 (m, 6 H, NCH₂, 2 × CH₂ 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 × CH₃). Anal. (C₂₇H₄₂F₃N₃O₄) C, H, F, N.

(3RS)-N-(tert-Butoxycarbonyl)-L-valyl-ψ(CH₂)-N-(2,3-dihydro-1H-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/NH₃) *m/z* (relative intensity) 528 (MH⁺, 100); ¹H NMR (CDCl₃) δ 8.7–8.4 (bs, 1 H, NH), 7.2 (s, 4 H), 4.0–2.7 (m, 8 H, NCH₂, α-CH₂ of Gly, 2 × CH₂ of indanyl), 2.5 (m, 1 H, CHCOF₃), 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 × CH₃). Anal. (C₂₇H₄₀F₃N₃O₄·H₂O) C, H, N.

[(2R,3S)+(2S,3R)]-L-Valyl-ψ(CH₂)-N-(2,3-dihydro-1H-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/NH₃) *m/z* (relative intensity) 430 (MH⁺, 100); ¹H NMR (DMSO-*d*₆) δ 7.18 (bs, 4 H), 4.1 (m, 1 H, CHOH), 4.0 (bs, 4 H, +NH₃, +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 × CH₃ of Val).

[(2R,3S)+(2S,3R)]-N-[[4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]phenyl]oxomethyl]-L-valyl-ψ(CH₂)-N-(2,3-dihydro-1H-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 × CH₃). Anal. (C₃₆H₄₂ClF₃N₄O₆·S·H₂O) C, H, N.

(3RS)-N-[[4-[[[(4-Chlorophenyl)sulfonyl]amino]carbonyl]phenyl]oxomethyl]-L-valyl-ψ(CH₂)-N-(2,3-dihydro-1H-inden-2-yl)glycine N-[3-(1,1,1-Trifluoro-4-methyl-2-oxopentyl)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⁺ + H₂O, 41), 748 (M⁺, 40); ¹NMR (CF₃COOD) 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 × CH₃ of Val). Anal. (C₃₆H₄₀ClF₃N₄O₆·S·2.5H₂O·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 Et₂O. The Et₂O solution was washed with dilute aqueous HCl and twice with brine. The combined aqueous phase was back-extracted with Et₂O. The combined Et₂O extract was dried (MgSO₄), 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 Et₂O. The solid was filtered to give 39 as colorless crystals (3.37 g, 52%): mp 74–75 °C; ¹H NMR (CDCl₃) 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, PhCH₂),

2.0 (m, 1 H, β-CH of Val), 1.0 (d, 3 H, CH₃ of Val), 0.87 (d, 3 H, CH₃ of Val). Anal. (C₂₀H₂₀F₃NO₃) C, H, N.

Method N. [(2S,1RS)]-2-[(Benzyloxycarbonyl)amino]-3-methyl-1-[*p*-(trifluoromethyl)phenyl]-1-butanol (40a). Phenyl ketone 39 (2.86 g, 7.54 mmol) was dissolved in CH₃OH (25 mL) with stirring at room temperature. To this solution was added NaBH₄ (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 (CDCl₃) δ 7.5 (dd, 4 H), 7.3 (s, 5 H, Ph), 5.1 (dd, 2 H, PhCH₂), 4.9 (t, 1 H, CHOH), 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, CH₃ of Val), 0.9 (d, 3 H, CH₃ of Val). Anal. (C₂₆H₂₂F₃NO₃) C, H, N.

(2S,1RS)-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 CH₂Cl₂. Most of the desired title product 40b was collected from the 20% EtOH in CH₂Cl₂ 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 (CDCl₃) δ 7.5 (dd, 4 H), 4.5 (q, 1 H, CHOH), 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 × CH₃ of Val).

(2S,1RS)-N-(Carbobenzyloxy)-L-valyl-N-(2,3-dihydro-1H-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% (CH₃)₂CO in petroleum ether afforded pure 41a (88%) as a colorless amorphous solid; ¹H NMR (CDCl₃) 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, CHOH), 5.1 (m, 2 H, PhCH₂), 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-CH₂ of Gly), 3.7 (m, 1 H, α-CH₂ of Gly), 3.4–3.1 (m, 4 H, 2 × CH₂ 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 × CH₃ of Val). Anal. (C₃₆H₄₂F₃N₃O₃) C, H, F, N.

(2S)-N-(Carbobenzyloxy)-L-valyl-N-(2,3-dihydro-1H-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 (CH₃)₂CO/petroleum ether (1:4). In this manner the title compound 41b was obtained as a colorless amorphous solid (605 mg, 81%): MS (CI/NH₃) *m/z* (relative intensity) 652 (MH⁺, 100); ¹H NMR (CDCl₃) 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, PhCH₂, CH of indanyl), 4.7 (m, 1 H, α-CH of Val), 4.0 (dd, 2 H, α-CH₂ of Gly), 3.2–2.9 (m, 4 H, 2 × CH₂ of indanyl), 2.1 (m, 2 H, β-CH of Val), 1.1 (m, 12 H, 4 × CH₃ of Val). Anal. (C₃₆H₄₀F₃N₃O₃) C, H, F, N.

(2S,1RS)-L-Valyl-N-(2,3-dihydro-1H-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 (CDCl₃) 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, NH₂), 1.8 (m, 2 H, 2 × β-CH of Val), 0.8 (m, 12 H, 4 × CH₃ of Val).

(2S)-[[4-[[[(4-Chlorophenyl)sulfonyl]aminocarbonyl]phenyl]oxomethyl]-L-valyl-N-(2,3-dihydro-1H-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 CH₂Cl₂. 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 -CH₂ of Gly), 3.7 (d, 1 H, α -CH₂ of Gly), 3.1–2.8 (m, 4 H, 2 \times CH₂ of indanyl), 2.2 (m, 2 H, β -CH of Val), 1.0–0.7 (m, 12 H, 4 \times CH₃ of Val). Anal. (C₄₂H₄₂ClF₃N₄O₇S) 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 (R₁ = 2-indanyl), the title compound 14b (PG = CBZ) was obtained in 57% yield as a colorless crystalline solid: mp 65–67 °C; ¹H NMR (CDCl₃) δ 10.2 (s, 1 H, COOH), 7.33 (s, 5 H), 5.17 (s, 2 H, CH₂), 4.6 (m, 1 H, α -CH of Val), 2.95 (s, 3 H, NCH₃), 2.27 (m, 1 H, β -CH of Val), 0.97 (t, 6 H, 2 \times CH₃). Anal. (C₁₄H₁₉NO₄) C, H, N.

Method O. N-(Carbobenzyloxy)-N-methyl-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycine Ethyl Ester (15b; R₂ = 2-indanyl, R₃ = Et). To a solution of CBZ-N-methyl-L-valine (14b; PG = CBZ) (2.0 g, 7.54 mmol) in CH₂Cl₂ (20 mL) cooled to 0 °C was added diethyl chlorophosphate⁷¹ (1.09 mL, 1.3 g, 7.54 mmol; *d* = 1.194) followed by Et₃N (1.05 mL, 7.54 mmol). After stirring at 0 °C for 15 min, a suspension of the amino acid ester hydrochloride 12a (R₁ = 2-indanyl) (1.93 g, 7.54 mmol) in CH₂Cl₂ (20 mL) containing Et₃N (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 \times), 10% aqueous NaHCO₃ (2 \times), and brine. The organic phase was dried (MgSO₄), 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% (CH₃)₂CO in petroleum ether afforded the pure title product 15b (1.5 g, 43%) as a colorless oil; ¹H NMR (CDCl₃) 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, PhCH₂), 4.2–3.85 (m, 4 H, CH₂CH₃, α -CH₂ of Gly), 3.25–2.8 (m, 4 H, 2 \times CH₂ of indanyl), 2.95 (s, 3 H, NCH₃), 2.4 (m, 1 H, β -CH of Val), 1.2 (t, 3 H, CH₃), 0.97 (t, 6 H, 2 \times CH₃ of Val). Anal. (C₂₇H₃₄N₂O₆) 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-hydroxybutyl)]amide (16; R₁ = CH₃, R₂ = 2-indanyl, R₃ = CH(CH₃)₂, R₄ = H). Ethyl ester 15b (R₂ = 2-indanyl, R₃ = Et, PG = CBZ) (2.24 g, 4.8 mmol) was hydrolyzed to the acid 15d (R₂ = 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 (CDCl₃) δ 7.4–7.2 (2 s, 9 H), 5.3–4.8 (m, 4 H, CHOH, CH of indanyl, PhCH₂), 4.2–3.7 (m, 4 H, α -CH₂ of Gly, 2 \times α -CH of Val), 3.2–2.8 (m, 4 H, 2 \times CH₂ of indanyl), 2.9 (s, 3 H, NCH₃), 2.4–1.9 (m, β -CH of Val), 0.95 (d, 12 H, 4 \times CH₃ of Val). Anal. (C₃₁H₄₀F₃N₃O₆) 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 CH₂Cl₂; MS (CI/NH₃) *m/z* (relative intensity) 590 (MH⁺, 100); ¹H NMR (CDCl₃) 7.3–7.1 (m, 9 H), 5.3–4.8 (m, 5 H, PhCH₂, CH of indanyl, NH), 4.1–3.8 (m, 2 H, α -CH₂ of Gly), 3.2–2.8 (m, 4 H, 2 \times CH₂ of indanyl), 2.9 (s, 3 H, NCH₃), 2.35 (m, 2 H, β -CH of Val), 1.1–0.8 (m, 12 H, 4 \times CH₃). Anal. (C₃₁H₃₅F₃N₃O₅H₂O) 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 (CDCl₃) δ 7.3 (s, 5 H, Ph), 5.2–4.9 (m, 2 H, α -CH of Phe, NH), 3.7 (s, 3 H, NCH₃), 3.2 (s, 3 H, NCH₃), 3.0 (dd, 2 H, β -CH₂ of Phe), 1.5 (s, 9 H, *t*-Bu).

(tert-Butoxycarbonyl)-L-phenylalanine (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 (CDCl₃) 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, PhCH₂), 1.4 (s, 9 H, *t*-Bu).

(Trifluoromethyl)trimethylsilane (9).⁶⁶ Bromotrimethylmethane (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 CF₃Br 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 CF₃Si(CH₃)₃ and THF. The ¹H NMR of the distilled material indicated that it was composed of 29.5% CF₃Si(CH₃)₃ in THF (2.07 mmol/g). This material was used without further purification; ¹³C NMR (CDCl₃) δ 131.4 (q, CF₃, *J* = 324 Hz), –5.01 (s, Si(CH₃)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, CF₃Si(CH₃)₃) (5.47 g, 38 mmol) followed by the addition of [CH₂(CH₂)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 Et₂O and then washed with brine. The aqueous phase was back-extracted with Et₂O. The combined Et₂O extract was dried (MgSO₄), 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% (CH₃)₂CO in petroleum ether gave six fractions. Fraction number two which was eluted with 10% (CH₃)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/CH₄) and weighed 4.34 g; MS (CI/CH₄) *m/e* (relative intensity) 320 (MH⁺, 28). This oily material was used directly without further purification; ¹H NMR (CDCl₃) 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; R₁ = H, R₂ = 2-indanyl, R₃ = CH₂Ph, R₄ = H). The impure *t*-BOC alcohol 8e (PG = *t*-BOC) (4.2 g) was treated with 15 mL of a solution containing 30% CF₃COOH in CH₂Cl₂ for 1 h at room temperature under an anhydrous atmosphere. The reaction mixture was concentrated in vacuo and then azeotroped with toluene (4 \times) 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-1H-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 CH₂Cl₂ followed by 5% EtOH in CH₂Cl₂ afforded the crude title product 16 in 66% purity based upon GC–MS (CI): MS (CI/NH₃) *m/z* (relative intensity) 626 (MH⁺, 100); ¹H NMR (CDCl₃) δ 7.4–7.0 (m, 14 H), 5.4–4.4 (m, 6 H, 2 \times NH, CHOH, α -CH of Val, PhCH₂), 4.2–3.8 (m, 4 H, α -CH₂ of Gly, α -CH of Phe, CH of indanyl), 3.2–2.7 (m, 6 H, 2 \times CH₂ of indanyl, β -CH₂ of Phe), 2.0 (m, 1 H, β -CH of Val), 1.0 (m, 6 H, 2 \times CH₃ 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; R₁ = H, R₂ = 2-indanyl, R₃ = PhCH₂, R₄ = H). Crude (3S,2RS)-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% (CH₃)₂CO in petroleum ether. The desired amino compound 18 eluted with 40% (CH₃)₂CO. This material (420 mg) was of sufficient purity to be used in the next reaction without further purification: MS (CI/C₄H₁₀) *m/z* (relative intensity) 492 (MH⁺, 100); ¹H NMR (CDCl₃) δ 2.1 (m, 1 H, β-CH of Val), 1.9 (bs, 1 H, OH), 0.9 (m, 6 H, 2 × CH₃ 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 (R₁ = H, R₂ = 2-indanyl, R₃ = PhCH₂, R₄ = H) (0.4 g, 0.81 mmol). Flash chromatography of the crude product over silica gel using as the eluant EtOH/HOAc/CH₂Cl₂ (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 re-dissolving in CH₂Cl₂ followed by filtration, concentration, and drying in vacuo to give pure the title compound 19 (111 mg) as a pale yellow resin: MS (CI/NH₃) *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 × CH₃ of Val). Anal. (C₄₀H₄₀ClF₃N₄O₅S) 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-oxobutyl)]amide (20c). The above title compound 20c was prepared in 33% yield by a Dess-Martin periodinane⁶⁰ oxidation analogous to that described in method K. The desired material 20c was obtained as a colorless amorphous solid: MS (CI/NH₃) *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-CH₂ of Gly), 3.2–2.4 (m, 6 H, β-CH₂ of Phe), 2.15 (m, 1 H, β-CH of Val), 0.9 (m, 6 H, 2 × CH₃). Anal. (C₄₀H₃₈ClF₃N₄O₅S) 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 NH₃ 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 Et₂O to give 8d (PG = H) (19.4 g, 92%), mp > 300 °C. The product was used directly without further purification: MS (CI/NH₃) *m/z* (relative intensity) 130 (MH⁺, 100); ¹H NMR (D₂O) δ 4.5 (m, 1 H, CH(OH)CF₃), 3.35 (m, 2 H, CH₃).

(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; R₁ = H, R₂ = 2-indanyl, R₃ = H, R₄ = 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 CH₂Cl₂. The desired title alcohol 16 was obtained as a colorless amorphous solid: MS (CI/NH₃) *m/z* (relative intensity) 536 (MH⁺, 62) 407 (100); ¹H NMR (CDCl₃) 7.5–7.20 (m, 9 H), 7.10 (bm, 1 H, CHCF₃), 5.80–5.30 (m, 1 H, CH of indanyl), 5.20–5.00 (m, 1 H, OH), 5.10 (s, 2 H, PhCH₂), 4.90–4.50 (m, 1 H, α-CH of Val), 4.30–3.60 (m, 4 H, CH₂), 3.50–2.90 (m, 4 H, 2 × CH₂ of indanyl), 2.10–1.90 (bm, 1 H, β-CH of Val), 1.0 (dd, 6 H, 2 × CH₃ 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 periodinane⁶⁰ oxidation by means similar to that described in method G. The crude product was purified by chromatography over silica gel (1% EtOH in CH₂Cl₂) to give pure 20k as a colorless amorphous solid (11%): MS (CI/NH₃) *m/z* (relative intensity) 534 (MH⁺, 30), 407 (100); ¹H NMR (CDCl₃) δ 7.60–7.20 (m, 9 H), 5.90–5.30 (m, 1 H, CH of indanyl), 5.20 (s, 2 H, PhCH₂), 5.20 (m, 1 H, α-CH of Val), 4.90–3.70 (m, 4 H, CH₂), 3.60–2.70 (m, 4 H, 2 × CH₂ of indanyl), 2.40, 1.90 (bm, 1 H, β-CH of val), 1.10 (dd, 6 H, 2 × CH₃ of Val). Anal. (C₂₇H₃₀F₃N₃O₅) 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 K₂CO₃ (1.38 g, 0.01 mol) was added CF₃CH(OC₂H₅)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 (MgSO₄), filtered, and concentrated. The residue was dissolved in CH₃OH (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; R₁ = H, R₂ = 2-indanyl, R₃ = CH₃, R₄ = CH₃). 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 CH₂Cl₂) to give the desired title product 16 as colorless crystals (6.4%) after recrystallization from Et₂O: mp 135–140 °C; MS (CI/NH₃) *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, CHCF₃), 5.30–4.90 (m, 1 H, CH of indanyl), 5.10 (s, 2 H, PhCH₂), 4.50 (m, 1 H, α-CH of Val), 4.30–3.60 (m, 2 H, CH₂), 3.20–2.80 (m, 4 H, 2 × CH₂ of indanyl), 2.20–1.90 (bm, 1 H, β-CH of Val), 1.00 (m, 12 H, 4 × CH₃). Anal. (C₂₅H₃₆F₃N₃O₅) 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; R₁ = H, R₂ = 2-indanyl, R₃ = CH₃, R₄ = CH₃). A mixture of 16 (R₁ = H, R₂ = 2-indanyl, R₃ = CH₃, R₄ = CH₃) (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; R₁ = H, R₂ = 2-indanyl, R₃ = CH₃, R₄ = CH₃). 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 CH₂Cl₂) followed by recrystallization from CH₂Cl₂/Et₂O to afford the title compound 19 (2.3 g, 51%) as a colorless solid: mp 150–165 °C dec; MS (CI/NH₃) *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, CHCF₃), 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, CH₂), 3.30–2.80 (m, 4 H, 2 × CH₂ of indanyl), 2.40 (bm, 1 H, β-CH of Val), 1.50–0.80 (m, 12 H, 4 × CH₃ 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; R₁ = H, R₂ = 2-indanyl, R₃ = CH₃, R₄ = CH₃). 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% CH₃OH in CH₂Cl₂) to give 20m as a colorless solid (20.1%): mp 143–147 °C; MS (CI/NH₃) *m/z* (relative intensity) 749 (MH⁺, 15), 185 (100); ¹H NMR (CDCl₃ + (CD₃)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, CH₂), 3.50–2.90 (m, 4 H, 2 × CH₂ of indanyl), 2.40–2.00 (bm, 2 H, 2 × β-CH of Val), 1.70 (m, 12 H, 4 × CH₃).

[(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-hydroxybutyl)]amide (29a; R = OH, R₁ = 2-indanyl). Amine 18 (R₁ = H, R₂ = 2-indanyl, R₃ = CH(CH₃)₂, R₄ = 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 CH₂Cl₂ and then CH₂Cl₂/CH₃OH (97:3) as the eluting solvent to afford a colorless solid which was filtered and washed with a small amount of cold Et₂O to give the desired title compound 29a (1.1 g, 41%): mp 127–132 °C; MS (CI/NH₃) *m/z* (relative in-

tensity) 755 (MH⁺, 100), 725 (5), 709 (6), 679 (6), 470 (5), 345 (18), 254 (11); ¹³C NMR (CDCl₃) 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, CF₃), 115.3 (thiazole C), 69.4–67.0 (q, CH(OH)CF₃), 62.1 (OCH₃), 60.6 (CH₂), 58.4, 56.4, 54.4, 46.5 (α-CH₂ of Gly), 37.2 (CH₂ of indanyl), 35.9 (CH₂ of indanyl), 30.8 (β-CH of Val), 30.1 (β-CH of Val), 28.7 (CH₂), 19.7, 19.4, 18.8, 18.1, 17.4, 16.4, 13.9 (CH₃). Anal. (C₃₄H₄₅F₃N₆O₈S) C, H, N, S.

(2RS)-syn-N-[[2-[[3-(Ethoxycarbonyl)propanoyl]amino]-4-thiazolyl](methoxyimino)acetal]-L-valyl-N-(2,3-dihydro-1H-inden-2-yl)glycine N-[3-(1,1,1-Trifluoro-4-methyl-2-oxopentyl)]amide (29b; R = O, R₁ = 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 CH₂Cl₂ and then CH₂Cl₂/CH₃OH (97:3) as the eluting solvent. In this manner **29b** (R = O, R₁ = 2-indanyl) was obtained as a colorless solid (52%): mp 94–101 °C; MS (CI/NH₃) *m/z* (relative intensity) 753 (MH⁺, 100), 723 (1), 707 (10), 677 (5), 468 (11), 254 (11); ¹³C NMR (CDCl₃) 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 (OCH₃), 60.8 (CH₂), 58.3, 56.3, 46.0 (α-CH₂ of Gly), 37.3, 35.9 (CH₂ of indanyl), 30.5 (β-CH of Val), 29.4 (CH₂), 28.9 (β-CH of Val), 28.3 (CH₂), 19.7, 19.4, 17.6, 17.2, 14.1 (CH₃). Anal. (C₃₄H₄₃F₃N₆O₈S) C, H, N, S.

Method Q. N-(Carbobenzyloxy)-L-valine N-[3-(Ethoxycarbonyl)-1,2,3,4-tetrahydroisoquinolinyl]amide (15a; R₁ = H, R₃ = Et). CBZ-L-valine (12.6 g, 0.05 mol) and Et₃N (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 Et₃N (6.1 g, 0.06 mol) in CH₂Cl₂ (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 Na₂CO₃, and saturated aqueous NaCl. After drying over MgSO₄, filtration and concentration under reduced pressure afforded an oil (12.5 g) which was purified over silica gel [*n*-C₆H₁₄/EtOAc (4:1)]. The desired title product **15a** was obtained as a colorless viscous oil (12.9 g): MS (CI/NH₃) *m/z* (relative intensity) 456 (MH⁺ + NH₃, 8), 439 (MH⁺, 28), 357 (6), 331 (100), 259 (3), 206 (5); ¹H NMR (CDCl₃) 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); ¹³C NMR (CDCl₃) 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 (PhCH₂), 61.1 (CH₂), 56.2 (α-CH of Val), 52.1 (CH of tetrahydroisoquinoline), 45.7 (CH₂ of tetrahydroisoquinoline), 31.3 (β-CH of Val), 30.7 (CH₂ of tetrahydroisoquinoline), 19.4 (CH₃ of Val), 17.2 (CH₃ of Val), 13.9 (CH₃). Anal. (C₂₅H₃₀N₂O₅) 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 OD₄₀₅ 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:

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

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 $\times 40$ 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 p 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 Allinger⁷⁵ 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 inhibitor⁶⁷ (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 Ser¹⁹⁵ and the ketone carbonyl of the inhibitor. To simulate this situation in the modeling process, the O atom of Ser¹⁹⁵ 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; (\pm)-8b (PG = H), 137301-93-0; (\pm)-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 ($R_1 = \text{c-C}_6\text{H}_5$), 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, $R_3 = \text{Et}$, PG = Cbz), 131506-23-5; 15a ($R_2 = 3,4$ -dimethoxyphenethyl, $R_3 = \text{Et}$, PG = Cbz), 131506-25-7; 15b ($R_2 = 2$ -indanyl, $R_3 = \text{Et}$, 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 ($R_1 = \text{H}$, $R_2 = 2$ -indanyl, $R_3 = i$ -Pr, $R_4 = \text{H}$, PG = Cbz), 137429-26-6; (2*S*,3*R*)-16 ($R_1 = \text{H}$, $R_2 = 2$ -indanyl, $R_3 = i$ -Pr, $R_4 = \text{H}$, PG = Cbz), 137429-27-7; (2*R*,3*S*)-16 ($R_1 = \text{H}$, $R_2 = \text{c-C}_8\text{H}_{15}$, $R_3 = i$ -Pr, $R_4 = \text{H}$, PG = Boc), 137301-56-5; (2*S*,3*R*)-16 ($R_1 = \text{H}$, $R_2 = \text{c-C}_8\text{H}_{15}$, $R_3 = i$ -Pr, $R_4 = \text{H}$, PG = Boc), 137429-30-2; (2*R*,3*S*)-16 ($R_1 = \text{Me}$, $R_2 = 2$ -indanyl, $R_3 = i$ -Pr, $R_4 = \text{H}$, PG = Cbz), 137301-82-7; (2*S*,3*R*)-16 ($R_1 = \text{Me}$, $R_2 = 2$ -indanyl, $R_3 = i$ -Pr, $R_4 = \text{H}$, PG = Cbz), 137429-38-0; (2*R*,3*S*)-16 ($R_1 = \text{H}$, $R_2 = 2$ -indanyl, $R_3 = \text{CH}_2\text{Ph}$, $R_4 = \text{H}$, PG = Cbz), 137301-57-6; (2*S*,3*R*)-16 ($R_1 = \text{H}$, $R_2 = 2$ -indanyl, $R_3 = \text{CH}_2\text{Ph}$, $R_4 = \text{H}$, PG = Cbz), 137429-41-5; (2*R*)-16 ($R_1 = \text{H}$, $R_2 = 2$ -indanyl, $R_3 = R_4 = \text{H}$, PG = Cbz), 137301-90-7; (2*S*)-16 ($R_1 = \text{H}$, $R_2 = 2$ -indanyl, $R_3 = R_4 = \text{H}$, PG = Cbz), 137301-91-8; (2*R*)-16 ($R_1 = \text{H}$, $R_2 = 2$ -indanyl, $R_3 = R_4 = \text{Me}$, PG = Cbz), 137301-94-1; (2*S*)-16 ($R_1 = \text{H}$, $R_2 = 2$ -indanyl, $R_3 = R_4 = \text{Me}$, PG = Cbz), 137301-95-2; (2*R*,3*S*)-18 ($R_1 = \text{H}$, $R_2 = 2$ -indanyl, $R_3 = i$ -Pr, $R_4 = \text{H}$), 137429-28-8; (2*S*,3*R*)-18 ($R_1 = \text{H}$, $R_2 = 2$ -indanyl, $R_3 = i$ -Pr, $R_4 = \text{H}$), 137429-29-9; (2*R*,3*S*)-18 ($R_1 = \text{H}$, $R_2 = \text{c-C}_6\text{H}_{15}$, $R_3 = i$ -Pr, $R_4 = \text{H}$), 137301-57-6; (2*S*,3*R*)-18 ($R_1 = \text{H}$, $R_2 = \text{c-C}_6\text{H}_{15}$, $R_3 = i$ -Pr, $R_4 = \text{H}$), 137429-31-3; (2*R*,3*S*)-18 ($R_1 = \text{H}$, $R_2 = 2$ -indanyl, $R_3 = \text{CH}_2\text{Ph}$, $R_4 = \text{H}$), 137301-87-2; (2*S*,3*S*)-18 ($R_1 = \text{H}$, $R_2 = 2$ -indanyl, $R_3 = \text{CH}_2\text{Ph}$, $R_4 = \text{H}$), 137429-42-6; (2*R*)-18 ($R_1 = \text{H}$, $R_2 = 2$ -indanyl, $R_3 = R_4 = \text{Me}$), 137301-96-3; (2*S*)-18 ($R_1 = \text{H}$, $R_2 = 2$ -indanyl, $R_3 = R_4 = \text{Me}$), 137301-97-4; (2*R*,3*S*)-19 (X = Cl, $R_1 = \text{H}$, $R_2 = 2$ -indanyl, $R_3 = i$ -Pr, $R_4 = \text{H}$), 137432-15-6; (2*S*,3*R*)-19 (X = Cl, $R_1 = \text{H}$, $R_2 = 2$ -indanyl, $R_3 = i$ -Pr, $R_4 = \text{H}$), 137490-09-6; (2*R*,3*S*)-19 (X = Cl, $R_1 = \text{H}$, $R_2 = 2$ -indanyl, $R_3 = \text{CH}_2\text{Ph}$, $R_4 = \text{H}$), 137301-88-3; (2*S*,3*S*)-19 (X = Cl, $R_1 = \text{H}$, $R_2 = 2$ -indanyl, $R_3 = \text{CH}_2\text{Ph}$, $R_4 = \text{H}$), 137429-43-7; (2*R*)-19 (X = Cl, $R_1 = \text{H}$, $R_2 = 2$ -indanyl, $R_3 = R_4 = \text{Me}$), 137301-98-5; (2*S*)-19 (X = Cl, $R_1 = \text{H}$, $R_2 = 2$ -indanyl, $R_3 = R_4 = \text{Me}$), 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 2), 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,

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79-2; HLE, 9004-06-2; Cbz-Cl, 501-53-1; H-Gly-OEt·HCl, 623-33-6; BrCH₂CO₂Et, 105-36-2; (*c*-C₅H₉)NH₂, 1003-03-8; CF₃CH(OEt)OH, 433-27-2; 3,4-(MeO)₂C₆H₃CH₂CH₂-Gly-OEt, 56014-42-7; 4-BrC₆H₄CF₃, 402-43-7; BrCF₃, 75-63-8; ClSiMe₃, 75-77-4; (±)-BrCH₂CH(OH)CF₃, 137429-44-8; O₂NCHMe₂, 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.