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Communications to the Editor

Syntheses of *trans*-5-Oxo-hexahydro-pyrrolo[3,2-*b*]pyrroles and *trans*-5-Oxo-hexahydro-furo[3,2-*b*]pyrroles (Pyrrolidine *trans*-Lactams and *trans*-Lactones): New Pharmacophores for Elastase Inhibition

Simon J. F. Macdonald,* David J. Belton, Doreen M. Buckley, Julie E. Spooner, Michael S. Anson,† Lee A. Harrison, Keith Mills,† Richard J. Upton,‡ Michael D. Dowle, Robin A. Smith,§ Christopher R. Molloy,§ and Catherine Risley§

Enzyme Chemistry 2, Development Chemistry 3, Physical Sciences, and Enzyme Pharmacology, GlaxoWellcome Medicines Research Centre, Gunnels Wood Road, Stevenage SG1 2NY, U.K.

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This communication reports two highly unusual and significant 5,5-*trans*-fused ring systems: namely, *trans*-oxo-hexahydro-pyrrolo[3,2-*b*]pyrroles and *trans*-oxo-hexahydro-furo[3,2-*b*]pyrroles (pyrrolidine *trans*-lactams and *trans*-lactones **1** and **2**). These structures were designed as low molecular weight non-peptidic inhibitors of human neutrophil elastase[1] (HNE). Inhibition of HNE, a serine protease, is being investigated[2] as potential therapy for respiratory diseases such as acute respiratory distress syndrome, cystic fibrosis, emphysema, and chronic bronchitis.[2,3] These 5,5-*trans*-fused ring systems, derived from a natural triterpene,[4] are without precedent.[5] First, they are highly strained structures. Second, as a class, they prove of general utility as inhibitors of serine proteases (e.g., elastase and thrombin). We have identified candidates from these series for development as elastase inhibitors.[6]

Chemistry to Lactams. A route to lactams and lactones was developed from a common intermediate for efficiency. The first route started from commercially available 3-aminopropanal diethyl acetal (**3**) (Scheme 1). This was protected as its benzyl carbamate and the aldehyde **4** unmasked. Coupling with *trans*-iodoethyl acrylate (**5**)[7] using chromium(II)/nickel(II) chlorides[8] gave the crude product **6** in high purity (57−70%). This was then treated with phthalimide under Mitsunobu conditions to give the acrylate **7**.

Due to difficulties with this chemistry,[9] the preferred route to the methyl analogue of **7**, commenced from commercially available 4-aminobutanal diethyl acetal (**8**). In a three-step process **8** was doubly N-protected as its benzyl and *tert*-butyl carbamates,[10] and the aldehyde **9** was revealed (80% for three steps crude). Treatment of **9** with methylphenylsulfinyl acetate and piperidine in acetonitrile[11] gave, after chromatography, the methyl acrylate **10** (60−70%). Exposure of **10** to phthalimide under Mitsunobu conditions, followed by removal of the *tert*-butyl carbamate with trifluoroacetic acid, gave the Michael reaction precursor **11** in 71−83% yield. An intramolecular Michael reaction was then effected with 25 mol % sodium hydride in THF, to give the 2,3-*trans*-disubstituted pyrrolidine **12** (69−87%). No *cis*-related products were detected.[12] Introduction of the allyl side chain required swapping the phthaloyl N-protecting group for the *tert*-butoxycarbonyl protecting group (47−67%).[13] While this introduced two extra steps, we found that deprotonation of **13** with lithium hexamethyldisilazide (LHMDS) in 1:1 THF/DMPU, followed by addition of allyl iodide, gave the desired allyl product (64−87%) with exceptional stereoselectivity (isomer ratio > 10:1). We consider the major product is the β-isomer **14** (as drawn).[14] Saponification of **14** with potassium hydroxide in aqueous ethanol followed by treatment with 4 M hydrogen chloride in 1,4-dioxane gave the hydrochloride **15** as a non-hygroscopic solid.[15] The lactamization of **15** was best achieved with 2-chloro-1-methylpyridinium iodide[16] and Hunig's base. These conditions gave, after chromatography, the lactam **1** (64−74%).[17]

Chemistry to Lactones. In contrast to lactams, conversion of **10** to *trans*-lactones, while highly efficient,

† Development Chemistry 3.
‡ Physical Sciences.
§ Enzyme Pharmacology.

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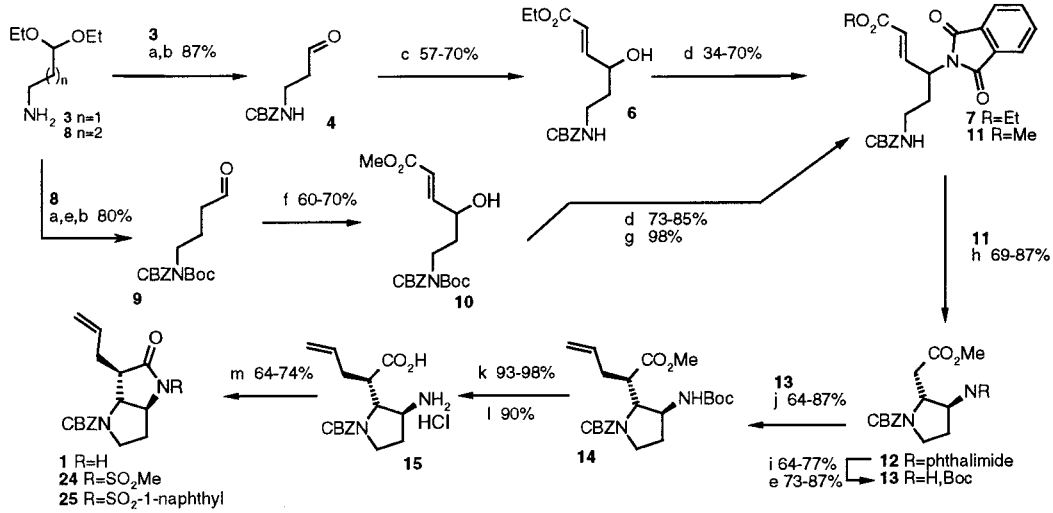
Scheme 1[a]



[a] (all compounds racemic) (a) CBZCl, 1 M Na_2CO_3, CH_2Cl_2, rt; (b) TsOH·py, H_2O, Me_2CO, 45 °C; (c) $CrCl_2$, $NiCl_2$ (catalytic), *trans*-ICHCHCO$_2$Et **5**, DMF, rt; (d) phthalimide, PPh_3, EtO_2CNNCO_2Et, THF, rt; (e) Boc_2O, Et_3N, DMAP, MeCN, rt; (f) PhS(O)CH_2CO_2Me, piperidine, MeCN, rt; (g) CF_3CO_2H, CH_2Cl_2, rt; (h) NaH (catalytic), THF, rt; (i) NH_2NH_2, EtOH, reflux; (j) LiN(SiMe_3)$_2$, CH_2CHCH_2I, DMPU, THF, −78 °C; (k) KOH, H_2O, EtOH, reflux; (l) 4 M HCl in dioxane, rt; (m) 2-chloro-1-methylpyridinium iodide, iPr_2NEt, CH_2Cl_2, rt.

Table 1. Enzyme IC_{50} Values of **1**, **2**, **22**−**25**, and L-694,458 at Varying Preincubation Times Against HNE, Thrombin, Chymotrypsin, and Cathepsin G[a]

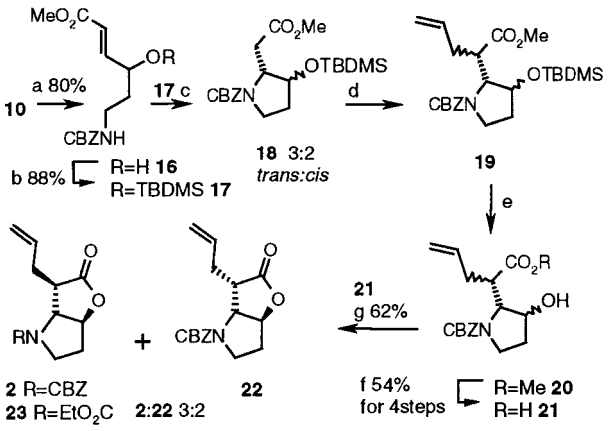
	HNE (μM)				
compd	0 min (error)[b]	40 min (error)	thrombin (μM) 15 min (error)	chymotrypsin (μM) 15 min (error)	cathepsin G (μM) 15 min[c]
1	>500	>500	>100	>100	
2	0.336 (8%)	0.047 (13%)	48.99 (8%)	15.9 (5.6%)	>100
22	12.8 (18%)	0.074 (15%)	44.58 (5.5%)	4.86 (8.5%)	67
23		0.069[c,d]	>200	4.0[c]	3.6
24	0.370 (43%)	0.056 (10%)	53.17 (8%)	40.6 (15%)	53
25	>100	0.088 (13%)	>100	11.5 (14%)	
L-694,458[e]	0.023 (15%)	0.036 (10%)	78.76 (11.6%)	0.98 (4.6%)	

[a] Full experimental details for inhibition of these enzymes are included in the Supporting Information. For HNE $t = 0$ min, the compound was preincubated at 30 °C with the substrate MeO-Succ-Ala-Ala-Pro-Val-*p*-nitroanilide and the reaction started with HNE. For $t = 40$ min, the compound was preincubated with elastase for 40 min and the reaction started with the substrate above. The progress of the reactions was followed by monitoring spectrophotometrically evolution of *p*-nitroanilide. [b] Unless otherwise stated, errors are quoted as standard deviations. [c] These values are a mean of three experiments. All values are within 30% of the mean. [d] This IC_{50} was obtained after a 15-min preincubation time with HNE. [e] See ref 26.

showed little stereoselectivity (Scheme 2). Removal of the *tert*-butyl carbamate from the acrylate **10** gave the alcohol **16** which was protected as its TBDMS ether **17**. Intramolecular cyclization with 10 mol % sodium hydride gave 3:2 *trans*:*cis*-pyrrolidines **18**.[18] The crude material was allylated with LHMDS and allyl bromide to give the allyl esters **19** (β:α 1:1). The TBDMS ethers **19** were converted into the alcohol esters **20** with tetrabutylammonium fluoride, and the esters **20** saponified to the acid **21** (54% crude yield from **17**).[19] Lactonization under Yamaguchi conditions[20] followed by chromatography gave the β-allyl *trans*-lactone **2** and the α-allyl analogue **22** in 62% yield (β:α 3:2).[17] All crude products from **17** to **2** (and **22**) were of sufficient purity for the subsequent stage.

Medicinal Chemistry of Lactams and Lactones. *trans*-Lactones **2**, **22**, and **23** and *trans*-lactams **24** and **25** (Table 1) are potent serine protease inhibitors.[21] We attribute this property to a combination of molecular fit with the enzyme and their intrinsic ring strain.[22] In particular they are highly potent inhibitors of HNE *in vitro* with the β-allyl lactone more active than α-allyl lactone. The enantiomers of the *trans*-lactones **2** and **22** were separated by preparative chiral HPLC.[23] The

Scheme 2[a]

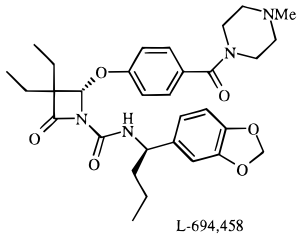


[a] (all compounds racemic) (a) CF_3CO_2H, CH_2Cl_2, 4 °C; (b) $tBuMe_2SiCl$, imidazole, DMF, rt; (c) NaH (catalytic), THF, 4 °C; (d) LiN(SiMe_3)$_2$, CH_2CHCH_2Br, THF, −78 °C; (e) Bu_4NF, THF, rt; (f) LiOH, H_2O, THF, 60 °C; (g) 2,4,6-trichlorobenzoyl chloride, Et_3N, DMAP, PhMe, reflux.

activities against HNE for the enantiomers of **2** are 0.024 and 0.215 μM, and for the enantiomers of **22** they are 0.676 and 1.603 μM.[24] While the activity of the

lactones **2**, **22**, and **23** is ascribed in part to their intrinsic ring strain (see later), the lactams require additional activation. Thus the sulfonamide analogues **24** and **25** show at least a 1000-fold increase in activity over the unsubstituted lactam **1**. All these compounds show at least a 10-fold selectivity over other serine proteases, presumably reflective of the diversity of the S-1 binding sites in these enzymes.[25] However, the intrinsic activities of these analogues against thrombin, chymotrypsin and cathepsin G are significant and enticing. Replacement of the allyl group with substituents derived from the P-1 residues of substrates normally cleaved by these enzymes may lead to increased potency against those enzymes.

For comparison purposes these compounds were tested alongside a potent elastase inhibitor from Merck, L-694,458.[26] At the 40-min time point against HNE, these compounds (except **1**) were of similar inhibitory activity. At the 0-min time point, they are less active suggestive of slower binding in comparison to L-694,458. Further kinetics have not been explored as the IC_{50} values have only been used for the purpose of ranking compounds for further biological testing.



L-694,458

On the basis of modeling studies of **2** and **25** in the active site of porcine pancreatic elastase (derived from known X-ray structures), we propose that the allyl group docks into the S-1 specificity pocket and the carbonyl group of the benzyl carbamate hydrogen bonds to the NH of valine-216 (chymotrypsin numbering).

As already mentioned, a feature of the *trans*-lactones and *trans*-lactams described here is their ring strain. Thus the infrared lactam and lactone carbonyl stretching frequencies of **1**, **24**, **25**, **2**, and **23** are ν 1713, 1768, 1759, 1791, and 1785 cm^{-1} respectively, and show a 10−20-cm^{-1} shift to higher frequency in comparison to similar *cis*-fused systems.[17]

Empirical observation from aqueous basic and acidic workups of *trans*-lactones and *trans*-lactams suggested that lactones, in contrast to lactams, were unstable at basic pH. We therefore examined the stability of the ethyl carbamate **23** in deuterated water at various pD's. Solutions of **23** in 1:1 buffer:acetonitrile at pD 0.8 at 20 or 39 °C showed no decomposition up to 24 h as monitored by infrared spectroscopy. However at pD 9.3, **23** showed 10% decomposition at 1 h and 60% at 24 h. By infrared analysis, the decomposition product is the corresponding hydroxy acid.[27]

The human plasma and whole blood stabilities of representative *trans*-lactones and *trans*-lactams were also examined. Thus, the half-lives of **2** and **25** in human plasma are 6 min and 2 h and in human whole blood 2 min and 4.5 h, respectively, suggesting that the lactams are metabolically more robust than the lactones.

In summary, we have described syntheses and activities of new pharmacophores for HNE inhibition. The stability of the lactams in blood gives confidence that acceptable concentrations of inhibitor may be possible after oral administration. We will report further details of this work shortly.

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Supporting Information Available: Experimental details and analytical data (20 pages). Ordering information is given on any current masthead page.

References

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

(2) (a) Merck: Finke, P. E.; Shah, S. K.; Fletcher, D. S.; Ashe, B. M.; Brause, K. A.; Chandler, G. O.; Dellea, P. S.; Hand, K. M.; Maycock, A. L.; Osinga, D. G.; Underwood, D. J.; Weston, H.; Davies, P.; Doherty, J. B. Orally Active β-Lactam Inhibitors of Human Leukocyte Elastase. 3. Stereospecific Synthesis and Structure−Activity Relationships for 3,3-Dialkylazetidin-2-ones. *J. Med. Chem.* **1995**, *38*, 2449−2462. (b) Zeneca: Veale, C. A.; Bernstein, P. R.; Bohnert, C. M.; Brown, F. J.; Bryant, C.; Damewood, J. R.; Earley, J.; Feeney, S. W.; Edwards, P. D.; Gomes, B.; Hulsizer, J. M.; Kosmider, B. J.; Krell, R. D.; Moore, G.; Salcedo, T. W.; Shaw, A.; Silberstein, D. S.; Steelman, G. B.; Stein, M.; Strimpler, A.; Thomas, R. M.; Vacek, E. P.; Williams, J. C.; Wolanin, D. J.; Woolson, S. Orally Active Trifluoromethyl Ketone Inhibitors of Human Leukocyte Elastase. *J. Med. Chem.* **1997**, *40*, 3173−3181. (c) Sterling Winthrop: Hlasta, D. J.; Ackermann, J. H.; Court, J. J.; Farrell, R. P.; Johnson, J. A.; Kofron, J. L.; Robinson, D. T.; Talomie, T. G.; Dunlap, R. P.; Franke, C. A. A Novel Class of Cyclic β-Dicarbonyl Leaving Groups and Their Use in the Design of Benzisothiazolone Human Leukocyte Elastase Inhibitors. *J. Med. Chem.* **1995**, *38*, 4687−4692. (d) Hoechst: Burkhart, J. P.; Mehdi, S.; Koehl, J. R.; Angelastro, M. R.; Bey, P.; Peet, N. P. Preparation of α-Keto Ester Enol Acetates as Potential Prodrugs of Human Neutrophil Elastase Inhibitors. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 63−64. (e) Wichita State University: Kuang, R.; Venkataraman, R.; Ruan, S.; Groutas, W. C. Use of the 1,2,5-Thiadiazolidin-3-one 1,1 dioxide and Isothiazolidin-3-one 1,1 dioxide Scaffolds in the Design of Potent Inhibitors of Serine Proteinases. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 539−544.

(3) Vender, R. L. Therapeutic Potential of Neutrophil-Elastase Inhibition in Pulmonary Disease. *J. Invest. Med.* **1996**, *44*, 531−539.

(4) O'Neill, M. J.; Lewis, J. A.; Noble, H. M.; Holland, S.; Mansat, C.; Farthing, J. E.; Foster, G.; Noble, D.; Lane, S. J.; Sidebottom, P. J.; Lynn, S. M.; Hayes, M. V.; Dix, C. J. Isolation of Translactone-Containing Triterpenes with Thrombin Inhibitory Activities from the Leaves of *Lantana Camara* L. (Verbenaceae). *J. Nat. Prod.*, submitted for publication.

(5) We are unaware of precedent for the systems described. Cyclopentane *trans*-lactones are known from (a) synthesis: Fukuzawa, S.; Lida, M.; Nakanishi, A.; Fujinami, T.; Sakai, S. Intramolecular Reductive Cyclization of Unsaturated Keto or Aldo Esters by Samarium(II) Diiodide: A Ready Synthesis of Bicyclic γ-Lactones. *J. Chem. Soc., Chem. Commun.* **1987**, 920−921. (b) Natural sources[4]: Kelecom, A.; Cabral, M. M. O.; Garcia, E. S. A New Euphane Triterpene from the Brazilian Melia Azedarach. *J. Braz. Chem. Soc.* **1996**, *7*, 39−41.

(6) Dowle, M. D.; Finch, H.; Harrison, L. A.; Inglis, G. G. A.; Johnson, M. R.; Macdonald, S. J. F.; Shah, P.; Smith, R. A. WO 9736903 A1 971009.

(7) **5** is prepared from propiolic acid by (a) 57% HI, reflux (98% crude), (b) K_2CO_3, EtI, DMSO (93% crude). No purification is necessary; cf. Biougne, J.; Theron, F. β-Haloacrylic Acids. Reactions with Certain Nucleophiles. *C. R. Acad. Sci. Ser. C* **1971**, *272*, 858−861.

(8) Takai, K.; Tagashiri, T.; Kuroda, T.; Oshima, K.; Utimoto, K.; Nozaki, H. Reactions of Alkenylchromium Reagents Prepared from Alkenyl Trifluoromethanesulfonates (Triflates) with Chromium(II) Chloride under Nickel Catalysis. *J. Am. Chem. Soc.* **1986**, *108*, 6048−6050.

(9) Two significant problems were encountered with this route: first, the chromium/nickel-catalyzed coupling generates 30 L of foul smelling waste solvent per 50−75 g of product; second, the Mitsunobu reaction requires excess reagents leading to tedious chromatographic purification.
(10) The double protection of the nitrogen was essential for the next step—the SPAC[11] reaction.
(11) (a) Tanikaga, R.; Nozaki, Y.; Tamura, T.; Kaji, A. Facile Synthesis of 4-Hydroxy-(E)-2-alkenoic Esters from Aldehydes. *Synthesis* **1983**, 134−135. (b) Burgess, K.; Cassidy, J.; Henderson, I. Optically Active Building Blocks from the SPAC Reaction: A Completely Asymmetric Synthesis of (4S-*cis*)-5-(Cyclohexylmethyl)-4-hydroxy-2-pyrrolidinone, a Statine Analogue. *J. Org. Chem*. **1991**, *56*, 2050−2058.
(12) As determined by examination of the crude ^{1}H NMR.
(13) Attempted allylation of the phthalimide **12** failed. In the conversion of **12** to **13**, there was no observable lactamization of the intermediate free base suggestive of *trans* stereochemistry in **12** and **13**. From unpublished work it is known that the corresponding *cis*-amino ester spontaneously lactamizes.
(14) We have been unable to rigorously prove that the stereochemistry of the allyl group in **14** is *β*. However we believe that in the conversion of **14** to **1**, this stereochemical center is configurationally stable. This is supported by unpublished work.
(15) The deprotection sequence of **14** to **15** may be reversed. There was no observable lactamization of the intermediate amino ester, suggestive of a *trans* relationship between the substituents on C-2 and C-3 on the pyrrolidine (see ref 12).
(16) Mukaiyama, T. New Synthetic Reactions Based on the Onium Salts of Aza-Arenes. *Angew. Chem. Chem. Int. Ed. Engl.* **1979**, *18*, 707−721.
(17) The relative stereochemistry is assigned on the basis of coupling constants and NOE experiments in the ^{1}H NMR spectrum. We have also prepared the analogous *cis*-lactam and *cis*-lactone which exhibit clear differences in their spectral properties.
(18) The *cis:trans* ratio was determined by comparison with a pure sample of the *trans* product available by another route and whose structure has been secured. Intramolecular cyclization of **16** on a 250-mg scale with NaH (0.5 equiv) in 9:1 PhMe:THF gave an 80% crude yield of a 10:1 *trans:cis* mixture of pyrrolidines. However this was not reproducible on a larger scale.
(19) The *cis*-related products (i.e., *cis* across C-2 and C-3 of the pyrrolidine) in crude **21** (carried through from **18**) are removed at this stage by an ether wash of a dilute basic solution of the crude product. The *cis* products are removed as ether-soluble−base-insoluble lactones.
(20) Inanaga, J.; Katsuki, T.; Takimoto, S.; Ouchida, S.; Inoue, K.; Nakano, A.; Okukado, N.; Yamaguchi, M. Total Synthesis of Methynolide. *Chem. Lett.* **1979**, 1021−1024.
(21) The HNE and cathepsin G enzyme assays were adapted from: Nakajima, K.; Powers, J. C.; Ashe, B. M.; Zimmerman, M. Mapping the Extended Substrate Binding Site of Cathepsin G and Human Leukocyte Elastase. Studies with Peptide Substrates Related to the α1-Protease Inhibitor Reactive Site. *J. Biol. Chem*. **1979**, *254*, 4027−4032. The thrombin assay was adapted from: Lottenberg, R.; Hall, J. A.; Fenton, J. W.; Jackson, C. M. The Action of Thrombin on Peptide *p*-nitroanilide Substrates: Hydrolysis of Tos-Gly-Pro-Arg-pNA and D-Phe-Pip-Arg-pNA by Human α and *γ* and Bovine α- and *β*-Thrombins. *Thrombin Res.* **1982**, *28*, 313−332. The chymotrypsin assay was similar to: Kerrigan, J. E.; Oleksyszyn, J.; Kam, C.-M.; Selzler, J.; Powers, J. C. Mechanism Based Isocoumarin Inhibitors for Human Leukocyte Elastase. Effects of 7-Amino Substituents and 3-Alkoxy Groups in 3-Alkoxy-7-amino-4-chloroisocoumarins on Inhibitory Potency. *J. Med. Chem*. **1995**, *38*, 544−552.
(22) A similar explanation is given for the biological activity of *β*-lactams. Page, M. I. Structure−Activity Relationships: Chemical. In *The Chemistry of β-Lactams*; Page, M. I., Ed.; Blackie: Glasgow, 1992; pp 79−100.
(23) Purified on a Chiralcel-OD column (25 mm × 4.6 mm) eluting with 85:15 hexane:2-isopropanol at 1 mL/min with UV detection at 200 nm. Under these conditions the enantiomers of **2** eluted at 21.14 and 23.94 min and **22** at 22.81 and 26.23 min.
(24) These IC_{50}'s were calculated after a 15-min preincubation.
(25) Barrett, A. J. An Introduction to The Proteinases. In *Research Monographs in Cell and Tissue Physiology, Vol. 12: Proteinase Inhibitors*; Barrett, A. J., Salvesen, G., Eds.; Elsevier: Amsterdam, 1986; p 9.
(26) Cvetovich, R. J.; Chartrain, M.; Hartner, F. W.; Roberge, C.; Amato, J.; Grabowski, E. J. J. An Asymmetric Synthesis of L-694,458. A Human Leukocyte Elastase Inhibitor, via Novel Enzyme Resolution of *β*-Lactam Esters. *J. Org. Chem*. **1996**, *61*, 6575−6580.
(27) Up to millimolar concentrations of analogous hydroxy acids did not inhibit HNE.

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