Design and Synthesis of Cyclic Inhibitors of Matrix Metalloproteinases and TNF-α Production

Chu-Biao Xue,* Xiaohua He, John Roderick, William F. DeGrado,[2] Robert J. Cherney, Karl D. Hardman, David J. Nelson, Robert A. Copeland, Bruce D. Jaffee,[1] and Carl P. Decicco*,[3]

The DuPont Merck Pharmaceutical Company, Experimental Station, Wilmington, Delaware 19880-0500

Received December 19, 1997

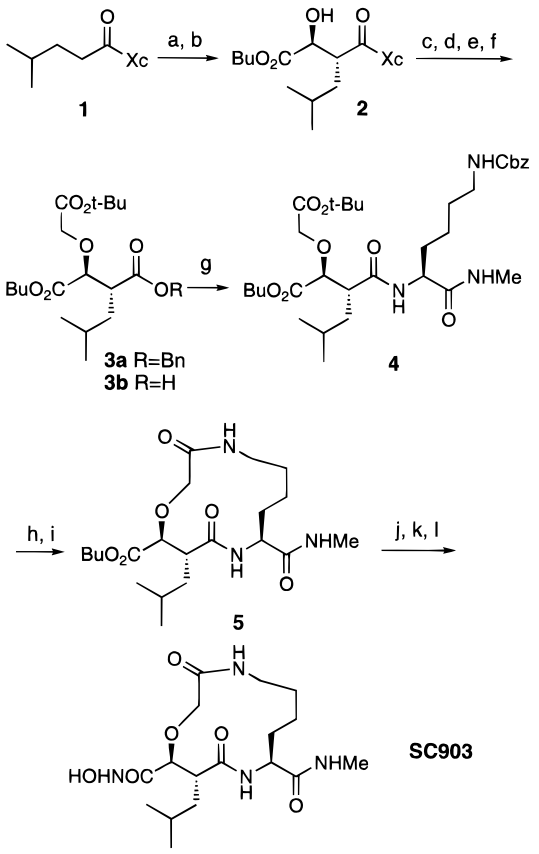
The matrix metalloproteinases (MMPs) are a family of tightly regulated enzymes involved in the catabolic aspect of remodeling and maintenance of normal tissue.[4] In pathophysiological conditions in which matrix degradation is linked to progression, incidence and severity of disease have been associated with high levels of MMP activity. Diseases such as arthritis, cancer, and periodontal and ocular disease all include as part of their progression MMP expression and activity significantly above basal levels.[5] The design and synthesis of inhibitors of key enzymes of the MMP family has become an area of vigorous investigation in drug discovery, in essence, targeting the restoration of balance to these pathodegradative processes. A collection of these agents have now progressed to the stage of clinical evaluations in cancer and arthritis in humans.[6]

We, and others, have shown that a subset of MMP inhibitors is also capable of inhibiting the production of TNF-α in cells and in animals.[7] A possible explanation for this activity has been ascribed to the inhibition of the recently discovered TNF-α converting enzyme (TACE),[8] which has been shown to cleave cell-associated 26 kd proTNF to its soluble 17 kd form. TACE has sequence homology to the reprolysins or snake venom metalloproteinases which are included in the Metzincin family of enzymes.[9] The central role of TNF-α in inflammatory disorders such as rheumatoid arthritis[10] suggests inhibition of TNF production to be a very important therapeutic target for small molecule drug design. In this communication, we disclose a new series of cyclic inhibitors of MMPs that also inhibit TNF-α release from human cells, possibly through the inhibition of TACE or a related enzyme that possesses TNF processing activity.

Succinate-based hydroxamic acids have been shown by a number of groups to be potent inhibitors of MMPs.[5] From a conformational analysis of these inhibitors using molecular modeling, and the study of inhibitor−enzyme complexes, we have observed that the preferential binding mode is an extended conformation.

The crystal structure of BB-16/MMP-3 (Figure 1) shows that the P1′ isobutyl and P1 methyl groups align toward an antiperiplanar arrangement (dihedral angle = 61°). The P2′ O-Me-phenyl group is directed away from the active site into solvent. Indeed the P2′ group of this inhibitor seems to be outside of the active site and therefore not involved in a strict P2′−S2′ interaction. This conformation clearly has P1 and P2′ directed away from the active site into solvent. We chose to exploit this observation by examining P1−P2′ linkages, which were designed to hold the succinyl-peptide inhibitor in a conformation determined by modeling, and supported by crystallographic evidence, to be correct for optimal enzyme−inhibitor interaction. Various cyclic molecules were considered, and computer modeling of the best cycles designed were determined to hold all of the inhibitor−enzyme hydrogen bonds and van der Waals contacts in analogous positions to the acyclic molecules. We disclose here two novel classes of cyclic MMP inhibitors, new synthetic methods for their preparation, and the first example of incorporating an anti-succinate peptidomimetic moiety into a macrocycle.[11,12] In vitro enzyme data for representatives of the collagenases, stromelysins, and gelatinases (MMP-1, -3, and -9); TNF inhibition data determined from a cellular assay, and X-ray crystal structures of these novel inhibitors complexed with MMP-3 are also presented.

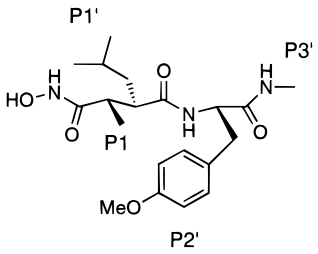
Scheme 1[a]



[a] Conditions: (a) LDA (Xc = (*S*)-4-phenylmethyloxazolidinone); (b) *n*-BuO_2CCHO, 36%; (c) LiOH, H_2O_2, 74% (d) BnBr, DBU, 79%; (e) NaH, $BrCH_2CO_2t$-Bu, 71%; (f) H_2, Pd−C, 99%; (g) *N*-Cbz-L-lysine-NHMe, BOP, 75%; (h) H_2, Pd−C, 4 N HCl/dioxane, 99%; (i) BOP, 50%; (j) 1N LiOH, 73%; (k) $BnONH_2$·HCl, BOP, 21%; (l) H_2, Pd−C, 62%.

Synthesis. Cyclic molecule SC903 containing an ether−diamide linkage was prepared as depicted in Scheme 1. 2-Hydroxy-3-isobutylsuccinate **2** was prepared through chiral oxazolidinone-controlled aldol condensation of **1** with butyl glyoxylate to give a 3:1 ratio of diastereomers of **2**.[13] The optimum sequence

S0022-2623(97)00849-2 CCC: $15.00
Published on Web 05/01/1998



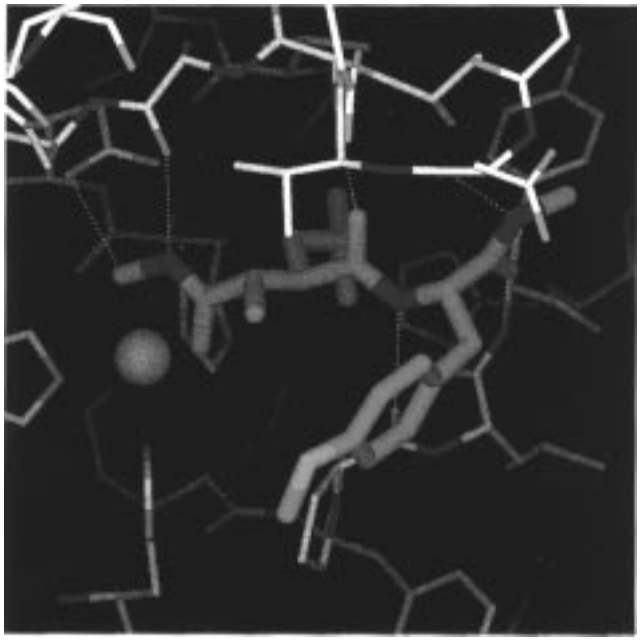


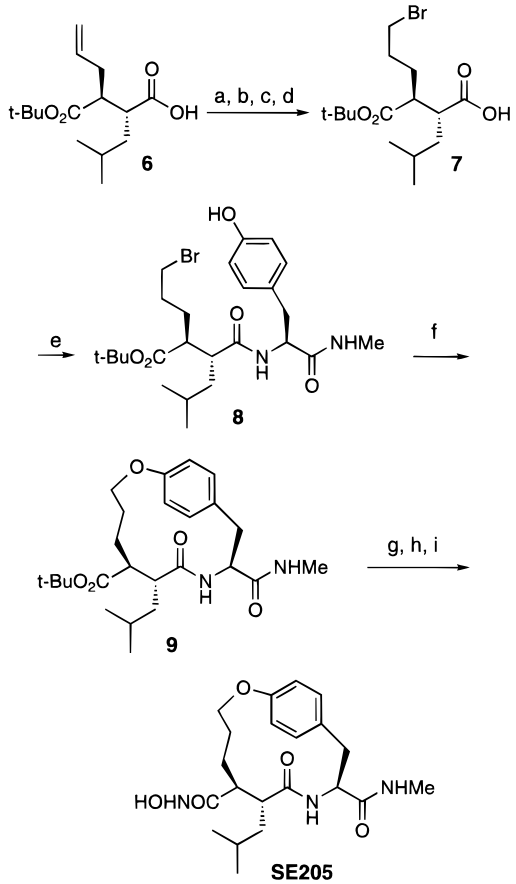
Figure 1. (a, left) BB-16 in an extended conformation. (b, right) X-ray structure of BB-16 in the active site of MMP-3.

for preparing **3b** entailed hydrolysis of the chiral auxiliary and benzyl esterification of the intermediate carboxylate, followed by alkylation of the secondary alcohol with *tert*-butyl bromoacetate to give **3a**. Hydrogenolysis of the benzyl ester gave the free carboxylic acid **3b** which was then coupled to *N*-Cbz-Lys-NHMe to give **4**. Sequential deprotection of the Cbz and *tert*-butyl ester groups followed by intramolecular cyclization using BOP gave the 13-membered lactam **5**. Conversion to the cyclic test molecule was completed by hydrolysis of the butyl ester and conversion to the benzyl protected hydroxamic acid, followed by hydrogenolysis in the final step to provide the hydroxamic acid SC903.

Starting from anti-succinate **6**,[14] cyclophane SE205 was prepared as shown in Scheme 2. Benzyl ester formation and 9-BBN oxidation of the olefin to the primary alcohol, followed by bromination with CBr_4/Ph_3P and subsequent hydrogenation of the benzyl ester, gave chiral bromo acid **7**. The benzyl ester protecting group was used in the sequence to facilitate chromatographic purification of the intermediate oxidation product. Coupling of the carboxylic acid **7** with Tyr-NHMe gave the phenoxy bromide cyclization precursor **8**. Cs_2CO_3 in DMF was used to effect a high-yield cyclization to give **9**. Removal of the *tert*-butyl ester with TFA, followed by coupling of the free carboxylic acid with *O*-benzylhydroxylamine and subsequent purification (again the benzyl group was used for ease of chromatographic purification of the penultimate product), followed by catalytic hydrogenation, provided cyclophane test molecule SE205.

Biological Results and Discussion. Macrocycles SC903 and SE205 were tested and found to be potent inhibitors of MMP-1, -3, and -9 (see Table 1).[15] Crystal structures for both SC903 and SE205 complexed with MMP-3 were also solved (Figures 2 and 3, respectively).[16] The figures depict the active site region of the enzyme extending toward the carboxy terminus (prime side) from the catalytic Zn (orange sphere). Analysis of these structures reveals a similar binding motif for both cyclic inhibitors which is comparable to the extended conformation of the linear inhibitor BB-16 shown in Figure 1, confirming our design hypothesis. Study of the overlap of the cyclophane SE205 with BB-16 (not shown) indicates that the macrocycle is functioning to maintain the succinyl-peptide backbone in an energeti-

Scheme 2[a]



[a] Conditions: (a) BnBr, DBU, 65%; (b) 9-BBN, 64%; (c) CBr_4, Ph_3P, 65%; (d) H_2, Pd−C, 99%; (e) Tyr-NHMe·HCl, TBTU, 75%; (f) Cs_2CO_3, 90%; (g) TFA, 95%; (h) $BnONH_2$, HATU, 60%; (i) H_2, $Pd/BaSO_4$, 99%.

Table 1. Comparison of in Vitro Data for SE205, SC903, and BB-16

	K_i, nM			IC_{50}, nM
	MMP-1	MMP-3	MMP-9	TNF-WBA
SE205	1.2	32.7	1.8	1200
SC903	2.8	24.1	2.6	6500
BB-16	0.87	1.93	0.13	1800

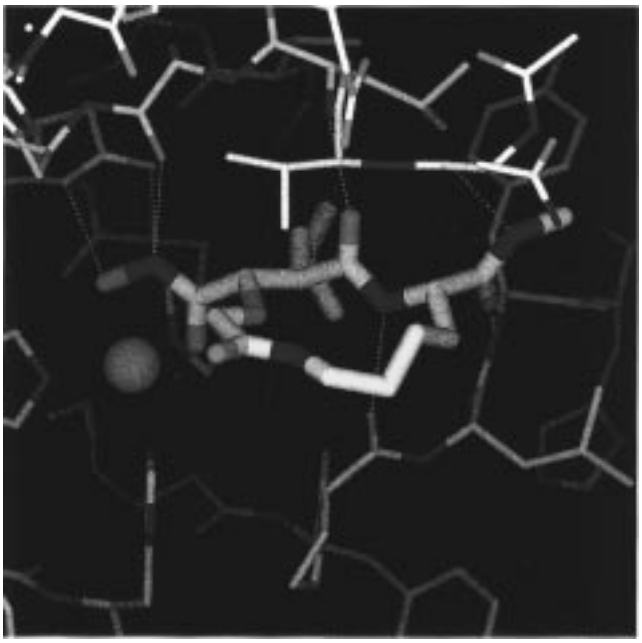


Figure 2. X-ray structure of SC903 in the active site of MMP-3.

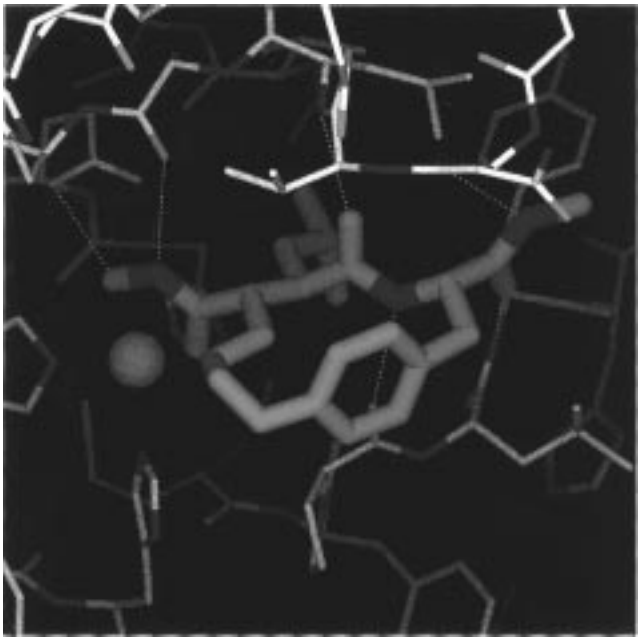


Figure 3. X-ray structure of SE205 in the active site of MMP-3.

cally favorable conformation suitable for active site inhibition of MMP-3. Experimentally, the cyclic molecules were determined to be 1 order of magnitude less potent than BB-16, suggesting that further refinement and optimization of the cyclic framework may be possible, which could potentially lead to even more potent inhibitors of these enzymes.

The cyclics were also determined to inhibit TNF release from LPS-stimulated human whole blood,[17] possibly through inhibition of TNF-α converting enzyme or related metalloproteinase TNF processing enzyme.[18] These data indicate that the cyclic molecules SE205 and SC903 represent new leads in the pursuit of metalloproteinase inhibitors that are antiinflammatory through inhibition of the key inflammatory mediator TNF. SE205 in particular was found to have excellent water solubility (13 mg/mL) in comparison to BB-16 (0.3 mg/mL), making it an attractive prototypical molecule for further studies in vivo.

Conclusion. We have described the design and synthesis of two novel cyclic inhibitors of MMPs, SC903, and SE205. These molecules were cocrystallized with MMP-3, and the high-resolution structures were solved. The structures clearly show the cyclic inhibitors bound in the predicted orientation. These molecules were also determined to inhibit the release of TNF from human cells stimulated with LPS. The implication for further iterations in design are numerous, and we are currently studying several variations of these prototypes in our labs. The physical properties, absorption, metabolism, and pharmacokinetics, as well as comprehensive SAR and potential therapeutic value for these series, are the subject of subsequent disclosures from our labs.

Supporting Information Available: Experimental details for the synthesis of SE205 (Scheme 2), spectroscopic data on SC903, and X-ray crystallographic data (5 pages). Ordering information is given on any current masthead page.

References

(1) Department of Inflammatory Diseases Research, DuPont Merck.
(2) Current address: Department of Biochemistry and Biophysics, The University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6059.
(3) To whom correspondence should be addressed.
(4) Woessner, J. F. *FASEB J.* **1991**, *5*, 2145−2154.
(5) (a) Levy, D. E.; Ezrin, A. M. Matrix metaloproteinase inhibitor drugs. *Emerging Drugs* **1997**, *2*, 205−230. (b) Ye, Q.-Z.; Hupe, D.; Johnson, L. Catalytic domains of matrix metalloproteinases: a molecular biology approach to drug discovery. *Curr. Med. Chem.* **1996**, *3*, 407−418. (c) Beckett, R. P. Recent advances in the field of matrix metalloproteinase inhibitors. *Exp. Opin. Ther. Pat.* **1996**, *6*, 1305−1315. (d) Becket, R. P.; Davidson, A. H.; Drummond, A. H.; Huxley, P.; Whittaker, M. Recent advances in matrix metalloproteinase inhibitor research. *Drug Discovery Today* **1996**, *1*, 16−26. (e) Hagmann, W. K.; Lark, M. W.; Becker, J. W. Inhibition of matrix metalloproteinases. *Annu. Rep. Med. Chem.* **1996**, *31*, 231−240. (f) Porter, J. R.; Millican, T. A.; Morphy, J. R. Recent developments in matrix metalloproteinase inhibitors *Exp. Opin. Ther. Pat.* **1995**, *5*, 1287−1296. (g) Dean, D. D.; Martel-Pelletier, Pelletier, J.-P.; Howell, D. S.; Woessner, J. F. Evidence for metalloproteinase and metalloproteinase inhibitor imbalance in human osteoarthritic cartilage. *J. Clin. Invest.* **1989**, *84*, 678−685.
(6) (a) Ray, J. M.; Stetler-Stevenson, W. G. Matrix metalloproteinases and malignant disease: recent developments. *Exp. Opin. Invest. Drugs* **1996**, *5*, 323−335. (b) Lewis, E. J.; Bishop, J.; Bottomley, K. M. K.; Bradshaw, D.; Brewster, M.; Broadhurst, M. J.; Brown, P. A.; Budd, J. M.; Elliott, L.; Greenham, A. K.; Johnson, W. H.; Nixon, J. S.; Rose, F.; Sutton, B.; Wilson, K. Ro 32-3555, an orally active collagenase inhibitor, prevents cartilage breakdown in vitro and in vivo. *Br. J. Pharmacol.* **1997**, *121*, 540−541.
(7) (a) Decicco, C. P.; Jaffee, B. D.; Copeland, R.; Jones, B.; DiMeo, T.; Gardner, T.; Collins, R.; Czerniak, P.; Nelson, D.; Magolda, R. TNF−C inhibitors in models of inflammation. *Eur. Cytokine Netw.* **1996**, *7*, 290. (b) Mohler, K. M.; Sleath, P. R.; Fitzner, J. N.; Cerretti, D. P.; Alderson, M.; Kerwar, S. S.; Torrance, D. S.; Otten-Evans, C.; Greenstreet, T.; Weerawarna, K.; Kronheim, S. R.; Petersen, M.; Gerhart, M.; Kozlosky, C. J.; March, C. J.; Black, R. A. Protection against a lethal dose of endotoxin by an inhibitor of tumor necrosis factor processing. *Nature* **1994**, *370*, 218−220. (c) Gearing, A. J. H.; Beckett, P.; Christodoulou, M.; Churchill, M.; Clements, J.; Davidson, A. H.; Drummond, A. H.;

Galloway, W. A.; Gilbert, R.; Gordon, J. L.; Leber, T. M.; Mangan, M.; Miller, K.; Nayee, P.; Owens, K.; Patel, S.; Thomas, W.; Wells, G.; Wood, L. M.; Woolley, K. Processing of tumor necrosis factor-α precursor by metalloproteinases. *Nature* **1994**, *370*, 555−557. (d) McGeehan, G. M.; Becherer, J. D.; Bast Jr, R. C.; Boyer, C. M.; Champion, B.; Connolly, K. M.; Conway, J. G.; Furdon, P.; Karp, S.; Kidao, S.; McElroy, A. B.; Nichols, J.; Pryzwansky, K. M.; Schoenen, F.; Sekut, L.; Truesdale, A.; Verghese, M.; Warner, J.; Ways, J. P. Regulation of tumor necrosis factor-α processing by a metalloproteinase inhibitor. *Nature* **1994**, *370*, 558−561.

(8) (a) Black, R. A.; Rauch, C.; March, C. J.; Cerretti, D. P. Tumor necrosis factor-α converting enzyme. Patent Appl. WO 9641624, 1997. (b) Black, R. A.; Rauch, C. T.; Kozlosky, C. J.; Peschon, J. J.; Slack, J. L.; Wolfson, M. F.; Castner, B. J.; Stocking, K. L.; Reddy, P.; Srinivasan, S.; Nelson, N.; Boiani, N.; Schooley, K. A.; Gerhart, M.; Davis, R.; Fitzner, J. N.; Johnson, R. S.; Paxton, R. J.; March, C. J.; Cerretti, D. P. A metalloproteinase disintegrin that releases tumor-necrosis factor-α from cells. *Nature* **1997**, *385*, 729−733. (c) Moss, M. L.; Jin, S.-L. C.; Milla, M. E.; Bickett, D. M.; Burkhart, W.; Carter, H. L.; Chen, W.-J.; Clay, W. C.; Didsbury, J. R.; Hassler, D.; Hoffman, C. R.; Kost, T. A.; Lambert, M. H.; Leesnitzer, M. A.; McCauley, P.; McGeehan, G.; Mitchell, J.; Moyer, M.; Pahel, G.; Rocque, W.; Overton, L. K.; Schoenen, F.; Seaton, T.; Su, J.-L.; Warner, J.; Willard, D.; Becherer, J. D. Cloning of a disintegrin metalloproteinase that processes precursor tumor-necrosis factor-α. *Nature* **1997**, *385*, 733−736.

(9) Hooper, N. Families of zinc metalloproteinases. *FEBS Lett.* **1994**, *354*, 1−6.

(10) Heath, P. K. Use of anti-TNF-α therapies as potential treatments for rheumatoid arthritis. *Rheumatoid Arthritis* **1997**, *1*, 235.

(11) Initial presentations on these inhibitor: (a) Decicco, C. P.; Nelson, D. J.; Kennedy, K.; Hardman, K.; Copeland, R.; Covington, M.; Magolda, R.; Arner, E.; Duan, J.; Cherney, R.; Xue, C.-B. Novel macrocyclic inhibitors of matrix metalloproteinases and TNF-α converting enzyme. ACS National Meeting, September 7−11, 1997, Las Vegas, NV. (b) Nelson, D. J.; Xue, C.-B.; Hardman, K.; Copeland, R.; Covington, M.; Magolda, R.; Arner, E.; Decicco, C. P. Novel cyclophane inhibitors of matrix metalloproteinases and TNF-α converting enzyme. ACS National Meeting, September 7−11, 1997, Las Vegas, NV. (c) Xue, C.-B.; He, X.; Roderick, J.; Hardman, K.; Copeland, R.; Jaffee, B.; Arner, E.; Magolda, R.; DeGrado, W. F.; Decicco, C. P. Rational design of matrix metalloproteinase inhibitors: a novel series of macrocyclic compounds featuring a linkage between the P1 and P2′ residues. ACS National Meeting, September 7−11, 1997, Las Vegas, NV. The procedure for making these and related macrocylce can also be found in the following: Xue, C.-B.; Cherney, R. J.; Decicco, C. P.; Degrado, W. F.; He, X.; Hodge, C. N.; Jacobson, I. C.; Magolda, R. L.; Arner, E. Novel Macrocylic Compounds as Metalloproteinase Inhibitors. Patent Appl. WO9718207, 1997.

(12) For related examples of macrocyclic inhibitors of Zn metalloproteinases, see: (a) Ksander, G. M.; de Jesus, R.; Yuan, A.; Ghai, R. D.; McMartin, C.; Bohacek, R. Meta-substituted Benzofused Macrocyclic Lactams as Zinc Metalloprotease Inhibitors. *J. Med. Chem.* **1997**, *40*, 506. (b) Orthosubstituted Benzofused Macrocyclic Lactams as Zinc Metalloproteinase Inhibitors. *J. Med. Chem.* **1997**, *40*, 495.

(13) Full experimental details on this reaction will be presented in a subsequent communication.

(14) Becket, R. P.; Crimmins, M. J.; Davis, M. H.; Spavold, Z. A short diastereoselective synthesis of 2,3-disubstituted succinates. *Synlett* **1993**, 137−138.

(15) The enzymatic activities of recombinant MMP-1, -3, and -9 were measured at 25 °C with a fluorometric assay (Copeland, R. A.; Lombardo, D.; Giannaras, J.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1947−1952). Final enzyme concentrations in the assay were between 0.05 and 10 nM depending on the enzyme and the potency of the inhibitor tested. The permisive peptide substrate, MCA-Pro-Leu-Gly-Leu-DPA-Ala-Arg-NH_2, was present at a final concentration of 10 μM in all assays. Initial velocities, in the presence or absence of inhibitor, were measured as slopes of the linear portion of the product progress curves. IC_{50} values were determined by plotting the inhibitor concentration dependence of the fractional velocity for each enzyme, and fitting the data by nonlinear least-squares methods to the standard isotherm equation (Copeland, R. A. *Enzymes: A practical Introduction to Structure, Mechanism and Data Analysis*; Wiley-VHC: New York, 1996; pp 187−223). All of the hydroxamic acids studied here were assumed to act as competitive inhibitors of the enzyme, binding to the active site Zn atom as previously demonstrated by crystallographic studies of MMP-3 complexed with related hydroxamic acids (Rockwell, A.; Melden, M.; Copeland, R. A.; Hardman, K.; Decicco, C. P. and DeGrado, W. F. *J. Am. Chem. Soc.* **1996**, *118*, 10337−10338). On the basis of the assumption of competitive inhibiton, the IC_{50} values were converted to K_i values as previously described.

(16) A summary of the crystallographic analysis of SC903 and SE205 with MMP-3 is presented in the Supporting Information.

(17) Whole blood assays were performed using a modified procedure reported by Desch et al. (Desch, C. E.; Kovach, N. L.; Present, W.; Boyles C. and Harlan, J. M. *Lymphokine Res.* **1989**, *8*, 141−146.) Heparinized human blood (225 μL) was dispensed into 1 mL sterile polypropylene tubes. Ten micoliter aliquots of test compounds dissolved in DMSO were added to the tubes. Samples were vortexed and incubated for 15 min, and then 15 μL of LPS (*Salmonella typhimurium*, 5 mg/mL in AIM-V media) was added, and the cultures were incubated for 5 h at 37 °C in a humidified atmosphere of 5% CO_2. AIM-V media (750 μL) was added to each tube. The samples were centrifuged and supernatants collected for TNF-α quantification by ELISA.

(18) Van Dyk, D. E.; Marchand, P.; Bruckner, R. C.; Fox, J. W.; Jaffee, B. D.; Gunyuzlu, P. L.; Davis, G. L.; Nurnberg, S.; Covington, M.; Decicco, C. P.; Trzaskos, J.; Magolda, R. L.; Copeland, R. A. Comparison of snake venom reprolysin and matrix metalloproteinases as models of TNF-α converting enzyme. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1219−1224.

JM970849Z