Matrix Metalloproteinase Inhibitors Containing a (Carboxyalkyl)amino Zinc Ligand: Modification of the P1 and P2′ Residues

Frank K. Brown,‖ Peter J. Brown,*,†,# D. Mark Bickett,‡ C. Lynn Chambers,§ H. Geoff Davies,§ David N. Deaton,† David Drewry,† Michael Foley,† Andrew B. McElroy,*,†,▽ Michael Gregson,§ Gerard M. McGeehan,‡ Peter L. Myers,† David Norton,⊥ James M. Salovich,† Frank J. Schoenen,† and Peter Ward§

Glaxo Incorporated Research Institute, Five Moore Drive, Research Triangle Park, North Carolina 27709, and Glaxo Group Research Limited, Greenford Road, Greenford, Middlesex UB6 0HE, England

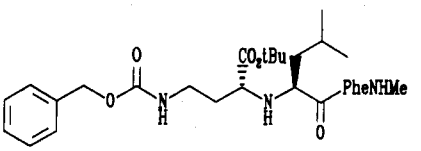
Received July 8, 1993®

Systematic modification of the presumed P1 side chain in a series of (carboxyalkyl)amino-based inhibitors of matrix metalloproteinases enabled identification of the 2-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)ethyl group as a preferred substituent imparting potent inhibition of the enzymes collagenase and gelatinase. It was subsequently found that the P2′-P3′ residues in this series could be replaced by small non-peptide residues, while maintaining inhibitory potency. The imide group in this series of compounds can undergo autocatalytic hydrolysis under neutral conditions.

Introduction

The matrix metalloproteinases (MMPs) are a family of zinc endoproteinases involved in connective tissue remodeling. Members of this family include the collagenases, stromelysins, and gelatinases.[1] These enzymes are secreted as inactive zymogens, and their expression is tightly controlled in most cell types. Aberrant regulation of these enzymes has been implicated in several pathologies including rheumatoid arthritis and metastasis. Both interstitial collagenase (MMP-1) and stromelysin (MMP-3) have been found at elevated levels in the joints of many patients suffering from active rheumatoid arthritis.[2-4] The unregulated expression of these enzymes eventually results in the destruction of the cartilage and the supporting connective tissue. MMP inhibitors have been claimed for the treatment of multiple sclerosis.[5] Several other matrix metalloproteinases have also been found at elevated levels in tumor cell lines and are implicated as important components of metastatic events. These include type IV collagenase (MMP-2),[6] gelatinase (MMP-9),[7] and the recently discovered stromelysin III (MMP-11).[8] Each of these enzymes may be important for the initial liberation of tumor cells or for the establishment of new tumor sites. Inhibition of MMP enzymatic activity represents a point for therapeutic intervention in each of these pathologies described above.

The activities of mammalian matrix metalloproteinases are modulated by proteinaceous tissue inhibitors of metalloproteinase (TIMPs).[1,9] Synthetic, small molecule inhibitors of matrix metalloproteinases have been reviewed,[10] and most classes contain a zinc-coordinating ligand, such as a thiol,[11] hydroxamic acid,[12] carboxylate,[13] phosphinate,[14] phosphonate,[15] or phosphonamidate group.[16] In these inhibitors, the zinc-coordinating ligand is attached to a peptidic residue containing a hydrophobic side chain which is recognized by the proteinase active site. It has been shown that proforms of certain MMPs are inhibited by internal coordination of the active site zinc atom by a cysteine side chain in the prosegment which is cleaved upon activation.[17] Insight into the mechanism of zinc metalloproteinases and their inhibition has been provided by studies on thermolysin[18] and astacin.[19] Irreversible-mechanism-based MMP inhibitors,[20] peptidic-ketomethylene-isostere-based collagenase inhibitors,[21] and non-peptide inhibitors of MMP-8 and bacterial collagenases, based upon tetracyclines, anthraquinones, and aranciamycin,[22] have also been reported. In our search for MMP inhibitor drugs suitable for oral therapy, we were attracted to a class of inhibitors containing a (carboxyalkyl)amino zinc ligand since this class of compounds has formed the basis of effective drugs as inhibitors of angiotensin-converting enzyme (ACE), which is also a zinc metalloproteinase.[23] (Carboxyalkyl)amino inhibitors such as 1 have been reported in the patent literature,[13] and we report here our investigations into the chemistry and structure-activity relationships of this class of compound and progress toward related MMP inhibitors of reduced peptide character.



1

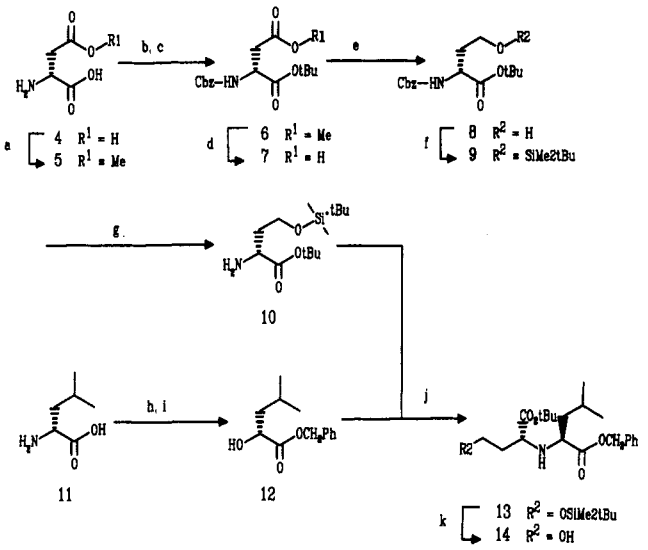
Chemistry

Compound 13 was considered a key intermediate which could be elaborated to give compounds modified at either the P1 or P2′ position, and its synthesis is described in Scheme 1. D-Aspartic acid (4) was monoesterified at the γ carbon with methanol and thionyl chloride. The product 5 was protected as the benzyl carbamate, and the free acid was converted to the *tert*-butyl ester to give compound 6. Reduction of the γ carboxyl group was carried out by saponification of the methyl ester, formation of the mixed anhydride, and reaction with $NaBH_4$ to give the alcohol 8. The alcohol was protected as the *tert*-butyldimethylsilyl ether, and the amine protecting group was removed by hydrogenation to give 10. Alcohol 12 was synthesized from

† Department of Medicinal Chemistry, Glaxo Research Institute.
‡ Department of Biochemistry, Glaxo Research Institute.
§ Department of Medicinal Chemistry, Glaxo Group Research Ltd.
⊥ Department of Cellular Science, Glaxo Group Research Ltd.
‖ Department of Molecular Sciences, Glaxo Research Institute.
▽ Current address: Discovery Chemistry, Pfizer Central Research, Sandwich, Kent CT13 9NJ, U.K.
Author to whom reprint requests should be sent.
® Abstract published in *Advance ACS Abstracts*, February 1, 1994.

0022-2623/94/1837-0674$04.50/0

Scheme 1[a]



[a] (a) $SOCl_2$, MeOH; (b) $PhCH_2OCOCl$, Na_2CO_3; (c) H_2SO_4, isobutylene; (d) aqueous NaOH; (e) i. EtOCOCl, *N*-methylmorpholine, ii. $NaBH_4$; (f) *t*-$BuMe_2SiCl$; (g) H_2, Pd/C; (h) $NaNO_2$, aqueous H_2SO_4; (i) NaOH, $PhCH_2Br$; (j) $(CF_3SO_2)_2O$, 1,8-bis(dimethylamino)naphthalene; (k) AcOH, H_2O, 45 °C.

D-leucine via a diazotization reaction in sulfuric acid/water followed by protection of the carboxyl group as the benzyl ester. This alcohol was converted to the corresponding triflate which was reacted in situ with amine **10**, in the presence of proton sponge to give the key intermediate **13**. Removal of the *tert*-butyldimethylsilyl protecting group in **13** was achieved under mild conditions using acetic acid: water at 45 °C to avoid lactonization of the product **14**.

Elaboration of the pseudodipeptide **13** to give compounds with varied P1 groups (shown in Table 1) is depicted in Scheme 2. The benzyl ester of **13** was removed by hydrogenation over 10% Pd/C, and a standard carbodiimide coupling with either phenylalanine *N*-methylamide or the (morpholinoethyl)amide of leucine gave **16** and **17**, respectively. The silyl ether was then removed by heating in 9:1 acetic acid:water at 45 °C for 3–15 h. Higher reaction temperatures led to significant amounts of lactone formation (loss of the *tert*-butyl ester). The various imide P1 substituents were then attached via Mitsunobu reaction, and the target molecules **20** and **47–57** were obtained by TFA hydrolysis of the *tert*-butyl esters.

Two non-imide P1 groups were synthesized as outlined in Scheme 3. Alcohol **18** was converted to amine **22** via a two-step procedure involving Mitsunobu reaction with bis(carbobenzyloxy) ammonia followed by removal of the benzyl groups by hydrogenation. A more standard Mitsunobu-type reaction with diphenyl phosphorazidate to form an azide intermediate did not proceed satisfactorily. Amine **22** was acylated with 2-naphthoyl chloride to give **23** and the *tert*-butyl ester removed to give the target compound **24**. Lactam **26** was synthesized in low yield by reduction of one of the imide carbonyls of **47** using zinc in hot acetic acid.

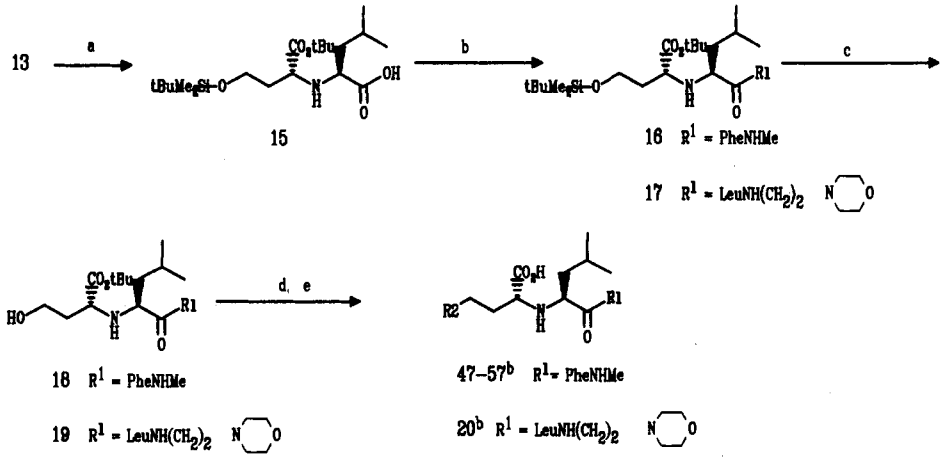
The synthesis of compounds modified at the P2′ position is outlined in Scheme 4. The naphthalimide P1 moiety was incorporated via a Mitsunobu reaction with **14** to give **27**. Hydrogenation afforded the acid **28**. Coupling of this acid with various amines (see Tables 2 and 3) via standard carbodiimide-coupling reactions followed by hydrolysis of the *tert*-butyl esters using TFA gave the target compounds **58–81**.

Three methoxy-susbtituted naphthalimides were used as P1 groups, and their syntheses are described in Scheme 5. The 5- and 6-methoxy-substituted compounds were prepared using ortho-lithiation chemistry followed by Diels–Alder reactions of isobenzofurans as described by Rodrigo.[24] *m*- and *p*-anisaldehyde were converted to the known dimethyl acetals **29** and **30**, respectively. Ortholithiation using *t*-BuLi in Et_2O at 0 °C and quenching with DMF afforded the aldehyde products **31** and **32**. Reduction of the aldehyde with $NaBH_4$ gave the isobenzofuran precursors **33** and **34**. Heating compound **33** in dimethyl acetylenedicarboxylate with a catalytic amount of glacial acetic acid gave the oxygen-bridged adduct **35**. Reduction of the double bond followed by aromatization with *p*-toluenesulfonic acid in refluxing toluene gave the naphthalene derivative **37**. Saponification of the ester moieties followed by thermal fusion of the diacid with urea afforded the desired imide **39**.

Compound **40** was prepared by the method of Mirsadeghi and Rickborn.[25] Conversion to the imide **42** proceeded under the standard neat melt conditions. The known quinoline-2,3-dicarboxylic acid (**41**)[26] was converted to the imide **43** in the same manner.

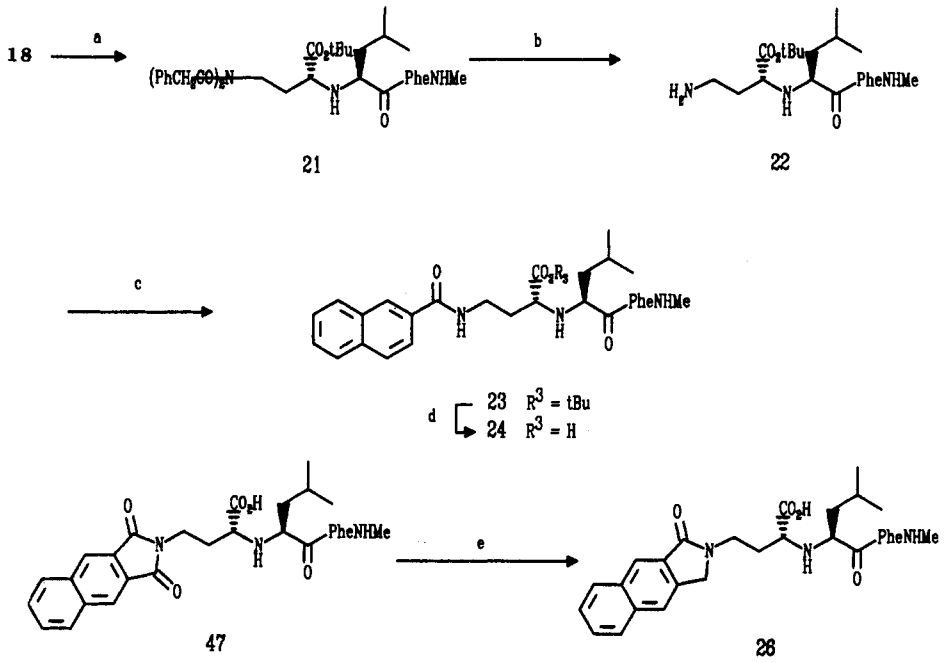
Reaction of **34** with maleic anhydride in refluxing CH_2Cl_2 containing a catalytic amount of acetic acid afforded

Scheme 2[a,b]



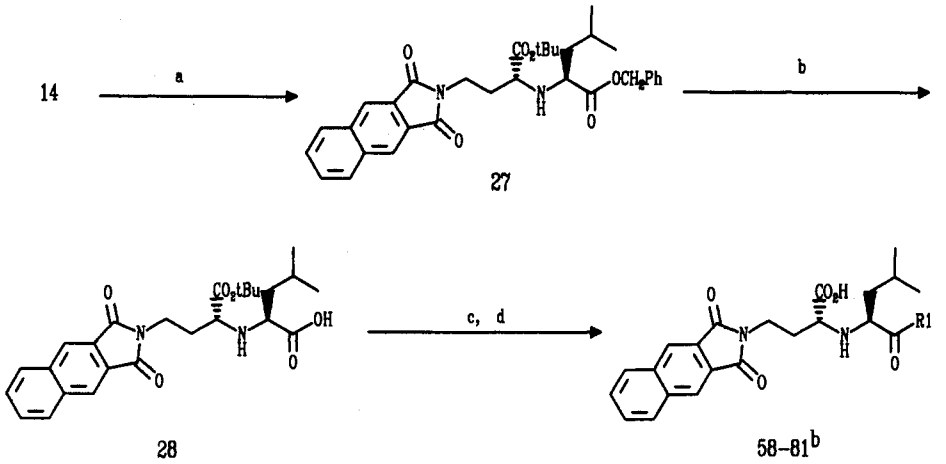
[a] (a) H_2, Pd/C, EtOH; (b) amine, DCC, HOBT; (c) AcOH:H_2O (9:1), 45 °C, 4 h; (d) Ph_3P, DEAD, phthalimide; (e) TFA:H_2O (9:1), 20 °C, 20 h. [b] R2 as defined in Table 1.

Scheme 3[a]



[a] (a) $(PhCH_2OCO)_2NH$, Ph_3P, DEAD; (b) H_2, Pd/C; (c) 2-naphthyl-COCl, Et_3N, THF; (d) TFA:H_2O (9:1), 20 °C, 20 h; (e) Zn, AcOH, 118 °C, 2 h.

Scheme 4[a,b]



[a] (a) 1,3-Dihydro-1,3-dioxo-2*H*-benz[*f*]isoindole, Ph_3P, DEAD; (b) H_2, Pd/C, EtOH; (c) amine, DCC, HOBT; (d) TFA:H_2O (9:1), 20 °C, 20 h. [b]R1 as defined in Tables 2 and 3.

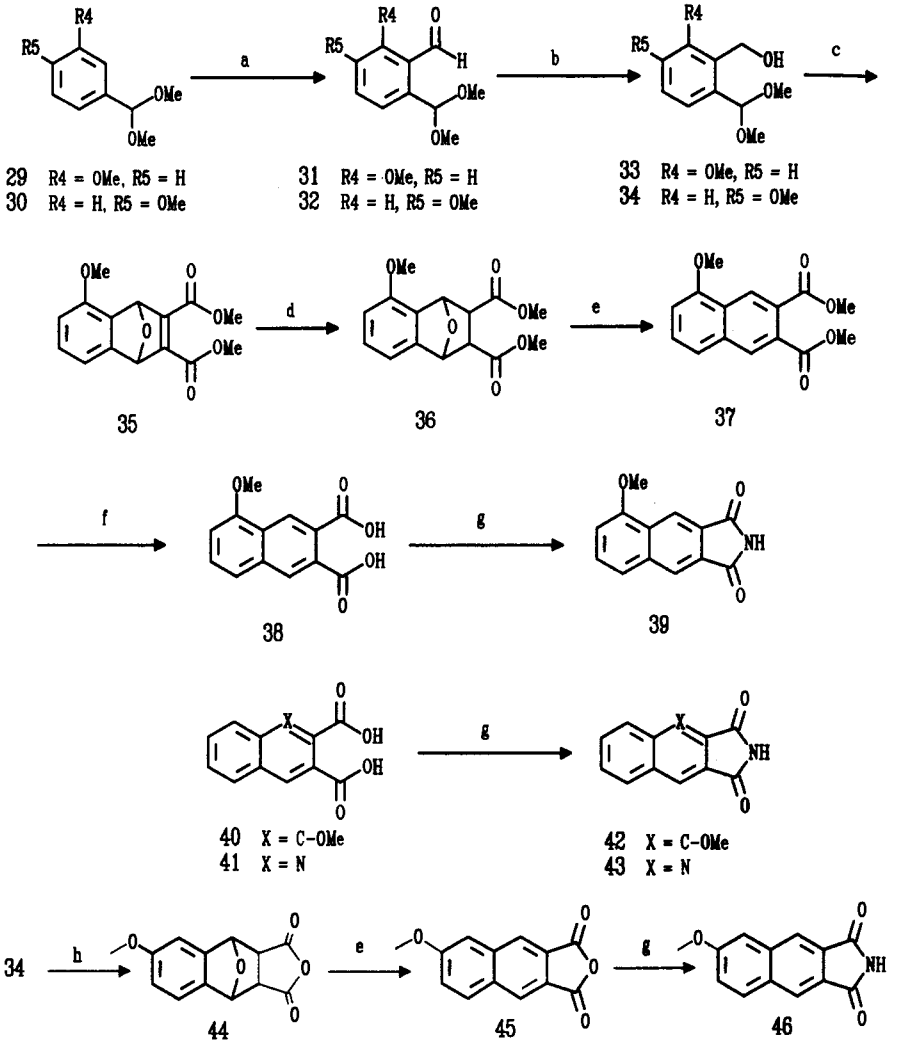
the expected Diels–Alder adduct **44**. Aromatization proceeded readily to give **45**, and melting the anhydride with urea at 170 °C gave the imide **46**.

Biological Results and Structure–Activity Discussion

Modifications of the P1 position are detailed in Table 1. Initial modifications focused on potentially isosteric replacement of the [(benzyloxy)carbonyl]amino group in **1**. It was discovered that replacement of this group with the 2,3-naphthalimide group in **47** gave a marked improvement in inhibitory potency in all three matrix metalloproteinases studied. It appears that both carbonyl groups of the imide are important for inhibitory potency since the partially reduced analog **26** has markedly reduced activity when compared to **47**. The activity of **26** is comparable to that of the napthylamide analog **24**, indicating that conformational constraint is not a major determinant of the potency of the phthalimide analog **47**.

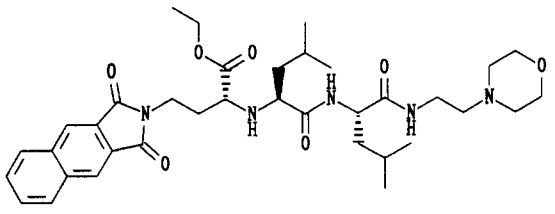
It was found that **47** slowly decomposed upon storage in solution to a single more hydrophilic product due to partial hydrolysis of the phthalimide group. Isolation of this decomposition product by HPLC and molecular weight determination by mass spectrometry indicated the addition of one water molecule to afford the carboxynaphthylamide which was inactive as an MMP inhibitor. A standardized set of conditions was employed, using [illegible] buffer at pH 6.8 to compare the stability of different analogs, and it was found that the corresponding amides **24** and **26**, which lack the imide functional group, were completely stable under the same conditions, whereas **47** decomposed with a 29-h half-life. This evidence strongly supported the hypothesis that the imide group was slowly undergoing partial hydrolysis in aqueous solution. The stability of **47** was pH dependent, becoming more unstable at higher pH and increasingly stable at more acidic pH, indicating a base-catalyzed hydrolysis. This hydrolysis reaction is probably autocatalytic in nature

Scheme 5[a]

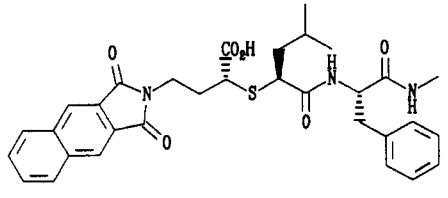


[a] (a) *t*-BuLi, DMF; (b) $NaBH_4$, MeOH; (c) dimethyl acetylenedicarboxylate, AcOH, 135 °C; (d) H_2, Pd/C, EtOH; (e) *p*-TsOH, PhMe, reflux; (f) NaOH, MeOH, H_2O; (g) H_2NCONH_2, 170 °C; (h) maleic anhydride, AcOH, 117 °C.

requiring the presence of both the carboxylate and amino groups in the central (carboxyalkyl)amino functionality of the molecule for hydrolysis to occur. Esterification of the carboxyl group in **20** (half-life 37 h, Table 2) gave the ester **2** which was found to be completely stable under the standard hydrolysis conditions. Likewise, analog **3**, in which the amino group is replaced by a sulfur atom, is also completely stable under the standard hydrolysis conditions.



2

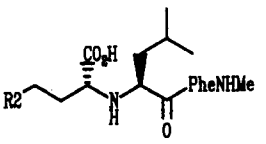


3

We postulated that the rate of this undesired base-catalyzed hydrolysis might be reduced by increasing the electron density on the naphthalimide. We were pleased to find that substitution of an electron-donating methoxy group to give **48** did markedly increase resistance to hydrolysis, giving a half-life of 87 h under the standard conditions, compared to 29 h for the unsubstituted naphthalimide analog **47**. This modification was also tolerated by collagenase and gelatinase but markedly reduced stromelysin inhibition. In contrast to this, methoxy substitution more remote to the imide in **49** increased the rate of hydrolysis. Stability was also markedly reduced in the more electron-deficient 4-aza and bis-imide analogs **51** and **53**. The high inhibitory potency of **53** against collagenase and gelatinase is notable since the phenyl ring of **47** is replaced by the markedly different imide group. It was found that the phthalimide group in **52** was slightly less resistant to hydrolysis than the naphthalimide in **47** with a half-life of only 10 h under the standard conditions. Substitution of the electron-donating methylenedioxy and propyloxy groups in **54** and **55** only slightly increased the stability in this series but provided compounds with enhanced potency compared to the parent phthalimide analog **52**.

Analogs of **47** with varied amino acid substitution in the P2′ position are detailed in Table 2. The tyrosine analog

Table 1. Modification of the P1 Substituent



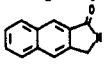
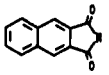
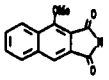
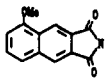
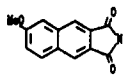
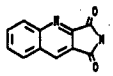
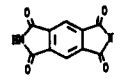
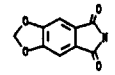
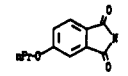
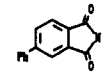
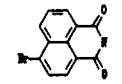
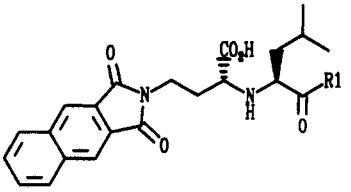
	R2	IC_{50} (nM) human collagenase (MMP-1)	human gelatinase (MMP-9)	human stromelysin (MMP-3)	pH 6.8, half-life (h)
1	$PhCH_2OCONH$-	103	580	3570	
24	2-naphthyl-CONH-	488	49	2340	>100
26		211	63	7877	>100
47		20	5.0	91	29
48		54	7.4	1370	87
49		36	15	1400	7.2
50		36			
51		22	7.5	518	1.7
52		81	59	5970	10.0
53		38	4.8	2169	1.7
54		12	5.0		15.5
55		15	2.1	427	10.9
56		19	4.5	216	7.8
57		190	28	341	

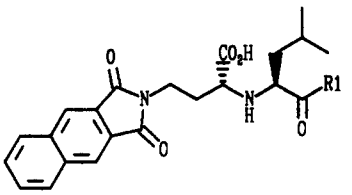
Table 2. Modification of the P2′ Amino Acid Side Chain



	R1	IC_{50} (nM) human collagenase (MMP-1)	human gelatinase (MMP-9)	human stromelysin (MMP-3)	pH 6.8, half-life (h)
47	-Phe-NHMe	20	5.0	91	29
20	-Leu-NH$(CH_2)_2$-morpholine	44	13	121	37
58	-Tyr-NHMe	29	3.8	72	
59	-phenylGly-NHMe	19	14	631	
60	-Leu-NHMe	49	11	1303	27
61	-Ala-NHMe	86	69	5633	
62	-Gly-NHMe	370	124	>10000	
63	-Arg-NHMe	152	27	481	

58 had similar potency to the parent compound against all three MMPs tested; analogs which replaced or moved the phenyl ring in this side chain markedly reduced stromelysin activity but generally reduced activity against

Table 3. Diverse Non-Peptide Modification of the P2′ Position



	R1	IC_{50} (nM) human collagenase (MMP-1)	human gelatinase (MMP-9)	human stromelysin (MMP-3)
47	-Phe-NHMe	20	5.0	91
64	$-NH(CH_2)_2Ph$	160	35	>10000
65	$-NHCH_2Ph$	87	32	1523
66	-NHPh	1170	101	12500
67	-NHMe	490	706	>10000
68	$-NHCH_2$(3-pyridyl)	139	36	309
69	## IIIb ##	270	30	2976
70	$-NHCH_2Ph$-*p*-CO_2H	56	74	178
71	$-NHCH_2Ph$-*m*-F	41	15	1485
72	$-NHCH_2Ph$-*p*-SO_2NH_2	70	6.7	295
73	$-NHCH(CH_3)Ph$ (*S*)	51	12	965
74	$-NHCH(CH_3)Ph$ (*R*)	74	76	4705
75 (isomer 1)	## IIIc ##	44	18	960
76 (isomer 2)		164	70	4084
77	## IIId ##	108	75	2473
78	## IIIe ##	84	88	2194
79	$-N(CH_3)CH_2Ph$	37	57	1159
80	## IIIf ##	54	70	1895
81	$-N(CH_3)_2$	86	82	>10000

collagenase and gelatinase to a lesser extent. The very polar arginine side chain in **63** was tolerated at this position on all three MMPs investigated, although 5–10-fold less potent than the parent phenylalanine analog **47**.

Concern that enzymic hydrolysis of the amide bonds surrounding the P2′ residue might limit the in vivo effect of these compounds led us to investigate analogs of **47** with reduced peptide character in this region of the molecule. Analogs of **47** with non-peptide replacements of the P2′ phenylalanine residue are detailed in Table 3. Removal of the carboxamide group in **47**, as in analog **64**, abolished interaction with stromelysin and reduced collagenase and gelatinase inhibition by 7–8-fold. Shortening the dimethylene linker to the phenyl ring by 1 methylene unit, as in the *N*-benzylamide **65**, marginally improved potency compared to **64**, but further shortening to the aniline amide **66** reduced potency further.

A variety of analogs containing modifications of the *N*-benzylamide were prepared (**68–79**), and it was found that the phenyl ring could be replaced with heterocyclic groups, as in **68** and **69**, without much change in inhibitory potencies. **70**, with a para carboxylate substitution on the phenyl ring, had enhanced stromelysin activity, and the *p*-sulfonamide analog **72** was a particularly potent gelatinase inhibitor, comparable in potency to **47**. Both diastereoisomers **73** and **74**, with a methyl substitution in the benzylic position, had similar potency as inhibitors of collagenase, but one diastereoisomer, **73**, showed ca. 5–6-fold higher affinity for gelatinase and stromelysin. A variety of analogs were prepared with cyclic constraints reducing the conformational freedom of the benzylic methylene group. Cyclization from the benzylic methylene onto the amide nitrogen, as in **75**, was found to enhance inhibitory potencies against collagenase and gelatinase in one of the diastereoisomers tested. Cyclization from the benzylic methylene onto the phenyl ring, as in **77**, and from the amide nitrogen onto the phenyl ring, as in **78**, was tolerated but did not enhance potencies. It was suspected that the presence of a tertiary amide in some of these analogs might be contributing to their potency, perhaps by exerting a conformational influence on the P1′ region of the molecule. The *N*-methyl-*N*-benzylamide **79** was prepared and found to be a more potent collagenase inhibitor than the *N*-benzylamide **65**, though slightly less potent as a gelatinase inhibitor. The dimethylamide analog **81** was also found to be 6–8-fold more potent than the methylamide **67** as an inhibitor of collagenase and gelatinase, though inactive against stromelysin. The *N*-methylpiperazine amide analog **80** imparted useful water solubility in this tertiary amide series while maintaining comparable potency.

Conclusions

We have shown that a variety of phthalimide and naphthalimide groups at the P1 position of (carboxyalkyl)-amino MMP inhibitors can greatly enhance their potency. The imide functional group in these compounds is susceptible to slow base-catalyzed hydrolysis in solution, but this can be inhibited by appropriate substitution and masking either the carboxylate or amino functional groups in the central region of the compounds. We have also shown that the P2′ region in this series of MMP inhibitors

is highly tolerant to substitution and can be effectively replaced by a variety of non-peptide groups.

Experimental Section

Abbreviations. Abbreviations follow the recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature for amino acids and peptides: *Eur. J. Biochem.* **1984**, *158*, 9–31. Additional abbreviations are defined in the text or as follows: DCC, dicyclohexylcarbodiimide; DEAD, diethyl azodicarboxylate; DMF, dimethylformamide; FAB, fast atom bombardment; HOBT, 1-hydroxybenzotriazole; HPLC, high-pressure liquid chromatography; MMP, matrix metalloproteinase; TFA, trifluoroacetic acid; THF, tetrahydrofuran; and TIMP, tissue inhibitor of metalloproteinase.

General Methods. All compounds for biological evaluation were characterized by mass spectroscopy using fast atom bombardment ionization on a Joel AX-505 spectrometer. Fragment ions were used to confirm the structure. Compound homogeneity was determined by analytical reverse-phase HPLC using a Dynamax-60A column using eluents A (water, 0.1% TFA) and B (acetonitrile, 0.1% TFA), with gradient elution from 85% A:15% B to 20% A:80% B over 30 min with a flow rate of 1.5 mL/min. Compound purification was effected by preparative reverse-phase HPLC using a 2-in. diameter Dynamax-60A column using the same eluents as described for analytical HPLC with an appropriately varied gradient at a flow rate of 45 mL/min. Compounds were characterized by NMR using a Varian Unity 300 spectrometer using TMS or solvent as an internal standard. Melting points were recorded on a Thomas-Hoover capillary melting point apparatus and are uncorrected.

4-Methyl D-Aspartate Hydrochloride (5). Methanol (210 mL) was slowly stirred and cooled to −10 °C, and thionyl chloride (31 mL, 425 mmol) was added dropwise over 45 min. D-Aspartic acid (40 g, 300 mmol) was added over 5 min and the reaction mixture stirred for 3 h while warming to 21 °C. Diethyl ether (600 mL) was added slowly and the mixture cooled to −10 °C. The resulting solid was filtered, washed with diethyl ether (200 mL), and dried in vacuo to afford the hydrochloride salt **5** (33.4 g, 56%) as a white solid: mp 186–188 °C; $[\alpha]^{20}_D$ −18.5° (c = 2.1; 1 N HCl) (for enantiomer, lit.[27] $[\alpha]^{20}_D$ +20.6° (c = 2.0; 1 N HCl). Anal. ($C_5H_9NO_4$·HCl) C, H, N.

***N*-[(Phenylmethoxy)carbonyl]-D-aspartic Acid, 1-(1,1-Dimethylethyl) 4-Methyl Ester (6).** 4-Methyl D-aspartate hydrochloride (**5**) (32 g, 162 mmol) was dissolved in a mixture of water (250 mL) and dioxane (250 mL), and sodium carbonate (92.4 g, 890 mmol) was added slowly with stirring. Benzyl chloroformate (25 mL, 175 mmol) was added and the mixture stirred for 15 h at 23 °C. EtOAc (250 mL) was added, and the mixture was acidified to pH 2 with concentrated HCl. The organic phase was separated, and the aqueous phase was extracted with EtOAc (2 × 150 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), and evaporated. The residue was dissolved in CH_2Cl_2 (800 mL) and treated with concentrated H_2SO_4 (2.5 mL). Isobutylene (200 mL) was condensed into the stirred mixture, and the solution was left to stand for 15 h. The mixture was neutralized with sodium bicarbonate, and the organic phase was washed with water and brine, dried (Na_2SO_4), and evaporated. The resulting oil was purified by silica chromatography using cyclohexane:EtOAc (4:1) as eluent to give **6** (31.8 g, 58%) as a clear oil: $[\alpha]^{20}_D$ +14.04° (c = 1.14; MeOH). Anal. ($C_{17}H_{23}NO_6$) C, H, N.

***N*-[(Phenylmethoxy)carbonyl]-D-aspartic Acid, 1,1-Dimethylethyl Ester (7).** A solution of compound **6** (31.3 g, 93 mmol) in methanol (120 mL) was treated with 1 N NaOH (102 mL) and the resulting yellow solution stirred at 21 °C for 2 h. The reaction mixture was concentrated in vacuo to about 30 mL and partitioned between water (250 mL) and diethyl ether (250 mL). The aqueous layer was washed with diethyl ether (250 mL) and layered with more diethyl ether (250 mL). The mixture was stirred vigorously and acidified to pH 2 with concentrated HCl. The organic layer was separated and the aqueous layer extracted with diethyl ether (250 mL). The combined extracts were washed with brine, dried (Na_2SO_4), and evaporated to afford **7** (29.4 g, 97%) as a yellow oil: $[\alpha]^{20}_D$ +11.6° (c = 0.86; MeOH). Anal. ($C_{16}H_{21}NO_6$·0.2H_2O) C, H, N, H_2O.

***N*-[(Phenylmethoxy)carbonyl]-D-homoserine, 1,1-Dimethylethyl Ester (8).** A solution of compound **7** (29.23 g, 90.4 mmol) in dry tetrahydrofuran (120 mL) at −10 °C was treated with *N*-methylmorpholine (10 mL, 91 mmol). The mixture was stirred for 3 min before adding ethyl chloroformate (8.7 mL, 91 mmol) dropwise. The mixture was warmed to 23 °C over 15 min and filtered. The filtrate was added dropwise over 30 min to a vigorously stirred mixture of sodium borohydride (7.7 g, 200 mmol) in water (60 mL) at 3 °C. The cooling bath was removed, and the mixture was stirred at 21 °C for 3 h and then cooled to 0 °C and acidified to pH 2 with concentrated HCl. The mixture was extracted with EtOAc (3 × 200 mL), and the combined organic extracts were washed with brine, dried (Na_2SO_4), and evaporated to furnish a clear oil. Purification by silica chromatography using CH_2Cl_2:acetone (9:1) as eluent afforded **8** (21.6 g, 77%) as a clear oil: $[\alpha]^{20}_D$ +30.4° (c = 1.81; MeOH). Anal. ($C_{16}H_{23}NO_5$) C, H, N.

***N*-[(Phenylmethoxy)carbonyl]-*O*-[(1,1-dimethylethyl)-dimethylsilyl]-D-homoserine, 1,1-Dimethylethyl Ester (9).** A solution of compound **8** (26.0 g, 84 mmol) in dry DMF (300 mL) was treated with imidazole (5.72 g, 84 mmol) and then *tert*-butyldimethylsilyl chloride (12.7 g, 84.3 mmol) and the mixture stirred at 21 °C for 18 h. The mixture was poured into a mixture of EtOAc (500 mL) and 2 N HCl (500 mL), and the organic phase was separated and washed with 2 N HCl (2 × 300 mL). The combined aqueous phases were extracted with EtOAc (2 × 250 mL), and the combined organic layers were washed with brine, dried (Na_2SO_4), and evaporated to give a clear oil. Purification by silica chromatography using cyclohexane:EtOAc (4:1) as eluent furnished **9** (20.93 g, 59%) as a clear oil: $[\alpha]^{20}_D$ +28.2° (c = 0.92; MeOH). Anal. ($C_{22}H_{37}NO_5$) C, H, N.

***O*-[(1,1-Dimethylethyl)dimethylsilyl]-D-homoserine, 1,1-Dimethylethyl Ester (10).** A solution of compound **9** (26.2 g, 61.9 mmol) in ethanol (200 mL) was shaken with 10% Pd/C (2.7 g) in a hydrogen atmosphere (50 psi) at 21 °C for 3 h. The catalyst was removed by filtration through Celite, and the solvent was evaporated to afford **10** (17.3 g, 97%) as a clear oil: $[\alpha]^{20}_D$ −10.74° (c = 1.21; MeOH).

2-(*R*)-Hydroxy-4-methylpentanoic Acid, Phenylmethyl Ester (12). A solution of D-leucine (26.2 g, 200 mmol) in 1 N H_2SO_4 (300 mL) was cooled to 0 °C and treated dropwise with a solution of $NaNO_2$ (25.5 g, 370 mmol) in water (50 mL) over 0.5 h. The mixture was stirred for 3 h at 0 °C, allowed to warm up to 23 °C for 2 h, and extracted with diethyl ether (3 × 100 mL). The combined organic layers were washed with brine (100 mL), dried ($MgSO_4$), and evaporated to afford a white solid (21.0 g). This was dissolved in methanol (200 mL) and treated with a solution of NaOH (6.5 g) in water (35 mL). After 5 min, the organic solvent was evaporated and the resulting aqueous solution was lyophilized overnight. The resulting solid was slurried in DMF (400 mL) and treated with benzyl bromide (20 mL, 168 mmol). The mixture was stirred at 23 °C for 18 h and the solvent removed by evaporation. The residue was treated with 2 N HCl (200 mL) and extracted with EtOAc (3 × 150 mL). The combined organic layers were washed with aqueous sodium bicarbonate and water, dried ($MgSO_4$), and evaporated to afford **12** (32.6 g, 73%) as a light golden oil: $[\alpha]^{20}_D$ +15.2° (c = 1.3; $CHCl_3$). Anal. ($C_{13}H_{18}O_3$) C, H.

***N*-[(*R*)-1-[(1,1-Dimethylethoxy)carbonyl]-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]propyl]-L-leucine, Phenylmethyl Ester (13).** A solution of compound **12** (7.4 g, 33.3 mmol) in dry CH_2Cl_2 (100 mL) was added to a cooled (0 °C) solution of trifluoromethanesulfonic anhydride (9.40 g, 33.3 mmol) in dry CH_2Cl_2 (100 mL). 1,8-Bis(dimethylamino)naphthalene (7.14 g, 33.3 mmol) was added and the resulting orange mixture stirred at 0 °C for 30 min. A solution of compound **10** (9.65 g, 33.3 mmol) in dry dioxane (90 mL) was added dropwise along with 1,8-bis(dimethylamino)naphthalene (7.14 g, 33.3 mmol) and the mixture stirred at 21 °C for 15 h. The mixture was filtered, and the filtrate was diluted with EtOAc (300 mL), washed with water (2 × 200 mL) and brine (250 mL), dried (Na_2SO_4), and evaporated to give a brown oil. Purification by silica chromatography using cyclohexane:diethyl ether (9:1) as eluent afforded **13** (11.92 g, 73%) as a yellow oil: $[\alpha]^{20}_D$ −8.1° (c = 0.99; $CHCl_3$). Anal. ($C_{27}H_{47}NO_5Si$) C, H, N.

***N*-[(*R*)-1-[(1,1-Dimethylethoxy)carbonyl]-3-hydroxy-**

propyl]-L-leucine, Phenylmethyl Ester (14). A solution of compound 13 (5 g, 10 mmol) in acetic acid (100 mL) and water (15 mL) was heated at 45 °C for 4 h. The solvents were evaporated, and the residue was dissolved in EtOAc (100 mL) and washed with aqueous bicarbonate solution. The organic layer was dried ($MgSO_4$) and evaporated. The residue was purified by silica chromatography using EtOAc:hexane (1:9) as eluent to furnish 14 (2.5 g, 66%) as an amber oil: ^{1}H NMR ($CDCl_3$) δ 7.35 (5H, m), 5.12 (2H, AB system), 3.79 (2H, t, J = 5.2 Hz), 3.35 (2H, m), 2.00–1.50 (7H, m), 1.44 (9H, s), 0.89 (6H, d, J = 6.6 Hz).

***N*-[(*R*)-1-[(1,1-Dimethylethoxy)carbonyl]-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]propyl]-L-leucine (15).** A solution of compound 13 (1.11 g, 2.25 mmol) in ethanol (25 mL) was treated with 10% palladium on carbon (120 mg) and the mixture shaken in a hydrogen atmosphere (40 psi) for 2 h. The catalyst was removed by filtration through Celite, and evaporation of the solvent afforded 15 (950 mg, 100%) as a yellow oil. This intermediate was found to be unstable upon storage and was used without further purification within 24 h of preparation.

[*N*-[1-(*R*)-[(1,1-Dimethylethoxy)carbonyl]-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide (16). A solution of compound 15 (478 mg, 1.18 mmol) in dry DMF (5 mL) was cooled to 5 °C and treated with 1-hydroxybenzotriazole hydrate (184 mg, 1.2 mmol) and dicyclohexylcarbodiimide (248 mg, 1.20 mmol). The mixture was stirred for 3 min, and *N*-methyl-L-phenylalaninamide (214 mg, 1.20 mmol) was added. The cooling bath was removed and the mixture stirred at 21 °C for 15 h. The mixture was filtered through Celite and the solvent evaporated to afford a yellow oil. Purification by silica chromatography using CH_2Cl_2:acetone (4:1) as eluent afforded 16 (289 mg, 43%) as a cream foam. Anal. ($C_{30}H_{53}N_3O_5Si$) C, H, N.

[*N*-[1-(*R*)-[(1,1-Dimethylethoxy)carbonyl]-3-[(1,1-dimethylethyl)dimethylsilyl]oxy]propyl]-L-leucyl]-*N*-(morpholinoethyl)-L-leucinamide (17). A solution of *N*-[(1,1-dimethylethoxy)carbonyl]-L-leucine hydrate (10.4 g, 41.77 mmol), 4-(aminoethyl)morpholine (5.5 g, 42.25 mmol), and 1-hydroxybenzotriazole hydrate (3 g, 19.61 mmol) in dry CH_2Cl_2 (100 mL) was treated with dicyclohexylcarbodiimide (9.45 g, 45.87 mmol) and the mixture stirred at 23 °C for 18 h. The mixture was filtered through Celite, and the filtrate was washed with aqueous sodium bicarbonate solution, dried (Na_2SO_4), and evaporated to afford an amber gum. This was dissolved in acetonitrile (100 mL) and treated with 4-toluenesulfonic acid hydrate (24.1 g, 126.84 mmol) and the mixture heated under reflux for 3 h. The cooled solution was diluted with ether (300 mL) and the resulting solid collected by filtration to give *N*-(morpholinoethyl)-L-leucinamide, bis(4-methylbenzenesulfonate) (18.6 g, 76%), a sample of which was crystallized from acetonitrile–ether: mp 168–9 °C; ^{1}H NMR (DMSO-d_6) δ 8.50 (1H, m), 7.90 (2H, m), 7.22 (4H, d, J = 8.3 Hz), 6.87 (4H, d, J = 7.8 Hz), 3.80–2.80 (15H, m), 2.03 (6H, s), 1.30 (3H, m), 0.61 (6H, m). A solution of this intermediate (1.42 g, 2.45 mmol), compound 15 (900 mg, 2.23 mmol), 1-hydroxybenzotriazole hydrate (200 mg, 1.3 mmol), and dicyclohexylcarbodiimide (500 mg, 2.43 mmol) in dry methylene chloride (30 mL) was treated with triethylamine (1 mL) and the mixture stirred for 15 h at 23 °C. The mixture was filtered through Celite, and the filtrate was washed with aqueous sodium bicarbonate solution. The organic layer was dried (Na_2SO_4) and evaporated. The residue was purified by silica chromatography using EtOAc and then EtOAc:MeOH (49:1) as eluent to give 17 (900 mg, 64%) as a gum: ^{1}H NMR ($CDCl_3$) δ 7.96 (d, J = 9 Hz), 7.21 (m), 4.40 (m), 4.12 (m), 3.80–3.60 (m), 3.45 (m), 3.34 (m), 3.02 (m), 2.45 (m), 2.00–1.20 (br m), 1.44 (s), 0.89 (m), 0.06 (s).

[*N*-[1-(*R*)-[(1,1-Dimethylethoxy)carbonyl]-3-hydroxypropyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide (18). Compound 16 (200 mg, 3.5 mmol) was dissolved in a mixture of acetic acid (9 mL) and water (1 mL) and stirred at 45 °C for 15 h. The solvents were evaporated, and the residue was dissolved in EtOAc (20 mL) and washed with aqueous sodium bicarbonate. The organic layer was dried ($MgSO_4$) and evaporated. The residue was purified by silica chromatography using CH_2Cl_2:acetone (2:1) as eluent to give 18 (143 mg, 91%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 7.52 (1H, d, J = 8 Hz), 7.20 (5H, m), 6.80 (1H, br m), 4.68 (1H, q, J = 7 Hz), 3.73 (2H, m), 3.36 (1H, m), 3.00 (1H, m), 3.0–3.3 (2H, m), 2.75 (3H, d, J = 5 Hz), 1.42 (9H, s), 1.0–1.9 (5H, m), 0.86 (6H, m).

[*N*-[1-(*R*)-[(1,1-Dimethylethoxy)carbonyl]-3-hydroxypropyl]-L-leucyl]-*N*-(morpholinoethyl)-L-leucinamide (19). A solution of compound 17 (900 mg, 1.43 mmol) in acetic acid (45 mL) and water (5 mL) was heated at 45 °C for 3 h. The solvents were evaporated, and the residue was dissolved in ethyl acetate and washed with aqueous sodium bicarbonate solution. The organic layer was dried ($MgSO_4$) and evaporated. Purification of the residue by silica chromatography using EtOAc and then EtOAc:MeOH (19:1) as eluent afforded 19 as a white foam (500 mg, 68%): ^{1}H NMR ($CDCl_3$) δ 7.30 (1H, d), 6.84 (1H, m), 4.38 (1H, m), 3.8–3.6 (6H, m), 3.4–3.2 (3H, m), 3.15 (1H, m), 2.70 (1H, br), 2.50–2.30 (6H, m), 2.00–1.40 (9H, m), 1.42 (9H, m), 0.90 (12H, m).

[*N*-1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucyl]-*N*-(morpholinoethyl)-L-leucinamide, Ditrifluoroacetate (20). A solution of compound 19 (500 mg, 0.973 mmol), triphenylphosphine (280 mg, 1.07 mmol), and 1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindole (210 mg, 1.07 mmol) in dry tetrahydrofuran (20 mL) was cooled to 0 °C and treated with diethyl azodicarboxylate (170 μL, 1.08 mmol). The mixture was allowed to warm up to 23 °C over 15 h. The solvent was evaporated and the residue purified by silica chromatography to afford [*N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucyl]-*N*-(morpholinoethyl)-L-leucinamide (400 mg, 59%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 8.33 (2H, s), 8.07 (2H, dd), 7.72 (2H, dd), 7.55 (1H, d), 6.94 (1H, m), 4.40 (1H, m), 3.85 (2H, m), 3.65 (4H, m), 3.40–3.15 (4H, m), 2.40 (6H, m), 2.15 (2H, m), 1.90–1.60 (7H, m), 1.43 (9H, s), 0.95 (12H, m). A solution of this intermediate (90 mg, 0.13 mmol) in TFA (5 mL) was stirred at 23 °C for 15 h. The solvent was evaporated and the residue purified by reverse-phase HPLC to afford 20 (60 mg, 53%) as a white solid: mp 115–7 °C; ^{1}H NMR (D_2O) δ 7.55 (m), 7.35 (m), 4.05 (m), 3.90–3.70 (m), 3.60–3.20 (m), 3.10–2.80 (m), 1.95 (m), 1.60–1.20 (m), 0.70 (m), 0.55 (m); high-resolution FAB MS m/z found 638.3532 (MH^+), $C_{34}H_{48}N_5O_7$ requires 638.3554. Anal. ($C_{38}H_{49}F_6N_5O_{11}\cdot H_2O$) C, H, N, F, H_2O.

[*N*-[1-(*R*)-[(1,1-Dimethylethoxy)carbonyl]-3-[bis[(phenylmethoxy)carbonyl]amino]propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide (21). A solution of compound 18 (0.815 g, 2.86 mmol), bis[(phenylmethoxy)carbonyl] ammonia (1.07 g, 3.76 mmol),[28] and triphenylphosphine (0.750 g, 2.86 mmol) in dry tetrahydrofuran (10 mL) was treated with diethyl azodicarboxylate (0.45 mL, 2.86 mmol) at −5 °C. After the mixture was stirred for 16 h at 23 °C, the solvent was evaporated and the residue purified by chromatography on silica using EtOAc:hexane (9:11) as eluent to afford 21 (710 mg, 35%) as a white solid: mp 95–99 °C; positive ion electrospray MS m/z 717 (MH^+); ^{1}H NMR (CD_3OD) δ 7.65 (2H, m), 7.34 (8H, m), 7.20 (5H, m), 5.24 (4H, s), 4.57 (1H, m), 3.74 (2H, m), 3.05 (4H, m), 2.70 (3H, s), 1.84 (2H, m), 1.54 (1H, m), 1.39 (9H, s), 1.23 (2H, m), 0.82 (6H, dd).

***N*-[1-(*R*)-[(1,1-Dimethylethoxy)carbonyl]-3-aminopropyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide (22).** A solution of compound 21 (580 mg, 0.81 mmol) in methanol (40 mL) containing 10% Pd/C (60 mg) was stirred in a hydrogen atmosphere. After the mixture was stirred for 3 h, additional 10% Pd/C (60 mg) was added and stirring was continued for 16 h. The catalyst was removed by filtration through Celite and the solvent evaporated to give 22 as a white semisolid (277 mg, 76%): ^{1}H NMR (CD_3OD) δ 7.63 (2H, m), 7.23 (3H, m), 4.59 (1H, m), 3.24–2.98 (5H, m), 2.70 (5H, m), 1.79–1.56 (2H, m), 1.45 (9H, s), 1.26 (2H, m), 0.86 (6H, dd).

[*N*-[1-(*R*)-Carboxy-3-(2-naphthoylamino)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide, Trifluoroacetate (24). To a stirred solution of compound 22 (70 mg, 0.16 mmol) in THF (2 mL) at 0 °C was added triethylamine (0.03 mL, 0.18 mmol) followed by 2-naphthoyl chloride (32 mg, 0.17 mmol). The ice bath was removed, and the light yellow mixture was stirred for 3 h. The reaction mixture was concentrated in vacuo and the residue diluted with acetonitrile, filtered, and reconcentrated to give an oil (84 mg). This residue was dissolved in TFA (5 mL) and stirred at room temperature for 3 h and then concentrated in vacuo, and the residue was purified by reverse-phase HPLC to give 24 (34.1 mg, 40%) as a white lyophilized trifluoroacetate

salt: ^{1}H NMR ($CDCl_3$) δ 8.2 (1H, s), 7.9–7.8 (4H, m), 7.5 (2H, m), 7.2–7.0 (5H, m), 4.6 (2H, dd), 3.9 (1H, m), 3.4 (3H, m), 3.0–2.8 (2H, m), 2.5 (3H, s), 2.0–1.9 (2H, m), 1.7–1.5 (3H, m), 0.9 (6H, dd); high-resolution FAB MS *m/z* found 547.2936 (MH^+), $C_{31}H_{39}N_4O_5$ requires 547.2920.

[*N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1-oxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide, Trifluoroacetate (26). To a solution of the trifluoroacetate salt **47** (88 mg, 0.127 mmol) in glacial acetic acid (1.3 mL, 22.9 mmol) was added zinc dust (42 mg, 0.635 mmol). The resulting suspension was heated at reflux under nitrogen for 2 h. Further zinc dust (42 mg, 0.635 mmol) was added and heating continued for 2 h. The reaction mixture was cooled, diluted with methanol, filtered, and concentrated to give a yellow oil which was purified by reverse-phase HPLC to give **26** (11 mg, 12%) as a lyophilized trifluoroacetate salt: ^{1}H NMR δ 8.22 (1H, s), 8.00–7.85 (3H, m), 7.60–7.40 (2H, m), 7.20–7.00 (5H, m), 4.70–4.60 (1H, m), 4.55 (2H, s), 3.85–3.80 (1H, m), 3.75–3.50 (2H, m), 3.50–3.40 (1H, m), 3.10–2.75 (2H, ABX), 2.60 (3H, s), 2.20–2.05 (1H, m), 2.00–1.85 (1H, m), 1.80–1.60 (3H, m), 0.93 (3H, d), 0.88 (3H, d); high-resolution FAB MS *m/z* found 559.2944 (MH^+), $C_{32}H_{39}N_4O_5$ requires 559.2920.

***N*-[(*R*)-1-[(1,1-Dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucine, Phenylmethyl Ester (27).** A solution of compound **14** (2.5 g, 6.4 mmol), triphenylphosphine (1.85 g, 7.03 mmol), and 1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindole (1.4 g, 7.10 mmol) in dry tetrahydrofuran (100 mL) was cooled to 0 °C and treated with diethyl azodicarboxylate (1.1 mL, 7.06 mmol). The resulting mixture was allowed to warm up to 23 °C over 24 h and the solvent removed by evaporation. The residue was purified by silica chromatography using CH_2Cl_2 as eluent to afford a light yellow solid. Trituration with methanol (20 mL) and filtration gave **27** as a white solid (1.9 g, 53%) which was crystallized from methanol: mp 136–137 °C; ^{1}H NMR ($CDCl_3$) δ 8.32 (2H, s), 8.04 (2H, m), 7.69 (2H, m), 7.33 (5H, m), 5.10 (2H, AB system, J_{ab} = 12.4 Hz), 3.87 (2H, m), 3.38 (1H, t, J = 7.0 Hz), 3.22 (1H, dd, J = 5.1 Hz, J = 6.3 Hz), 2.25 (1H, br s), 2.10–1.70 (5H, m), 1.47 (9H, s), 0.90 (6H, d, J = 6.6 Hz). Anal. ($C_{33}H_{38}N_2O_6$) C, H, N.

***N*-[(*R*)-1-[(1,1-Dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucine (28).** A solution of compound **27** (1.9 g, 3.4 mmol) in EtOAc (50 mL) was shaken with 10% Pd/C (500 mg) in a hydrogen atmosphere (50 psi) for 24 h. The catalyst was removed by filtration through Celite and the solvent evaporated. The residue was purified by silica chromatography using 50% ethyl acetate–hexane as eluent to afford **28** as a cream solid (1.5 g, 94%) which was crystallized from EtOAc–hexane: mp 168–169 °C; ^{1}H NMR ($CDCl_3$) δ 8.34 (2H, s), 8.05 (2H, m), 7.70 (2H, m), 3.87 (2H, m), 3.31 (2H, m), 2.20–1.60 (5H, m), 1.46 (9H, s), 0.95 (6H, d, J = 6.6 Hz). Anal. ($C_{26}H_{32}N_2O_6$) C, H, N.

2-Methoxy-6-(dimethoxymethyl)benzaldehyde (31). A solution of the dimethyl acetal of *m*-anisaldehyde **29** (490 mg, 2.7 mmol) in dry diethyl ether (10 mL) at 0 °C was treated with *tert*-butyllithium (1.75 mL, 1.7 M in hexanes, 2.97 mmol). The solution was stirred for 1 h at 0 °C followed by addition of anhydrous dimethylformamide (0.25 mL). The solution was allowed to warm to 23 °C over 15 h and the reaction quenched by the addition of water. The solution was extracted with ethyl acetate (2 × 50 mL). The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by chromatography on silica using hexane:EtOAc (9:1) as eluent to afford **31** as a pale yellow oil (255 mg, 45%): ^{1}H NMR ($CDCl_3$) δ 10.60 (1H, s), 7.53 (1H, t, J = 8.1 Hz), 7.38 (1H, t, J = 7.2 Hz), 6.99 (1H, d, J = 7.5 Hz), 6.05 (1H, s), 3.91 (3H, s), 3.42 (6H, s).

5-Methoxy-2-(dimethoxymethyl)benzaldehyde (32). A solution of the dimethyl acetal of *p*-anisaldehyde **30** (5.3 g, 29.1 mmol) in dry diethyl ether (90 mL) at 0 °C was treated with *tert*-butyllithium (20.5 mL, 1.7 M in hexanes, 34.9 mmol). The solution was stirred for 1.5 h at 0 °C followed by addition of anhydrous dimethylformamide (2.9 mL). The solution was allowed to warm to 23 °C over 1 h and the reaction quenched by the addition of water. The solution was extracted with ethyl acetate (2 × 200 mL). The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by chromatography on silica using 90% hexane–ethyl acetate as eluent to afford **32** as a pale yellow oil (2.63 g, 43%): ^{1}H NMR ($CDCl_3$) δ 10.45 (1H, s), 7.29 (1H, d, J = 8.7 Hz), 6.97 (1H, dd, J = 2.4, 8.5 Hz), 6.88 (1H, d, J = 2.1 Hz), 6.02 (1H, d, J = 2.2 Hz), 3.83 (3H, s), 3.44 (3H, s), 3.42 (3H, s).

6-Methoxy-2-(dimethoxymethyl)benzenemethanol (33). A solution of compound **31** (5.7 g, 27.4 mmol) in methanol (50 mL) was treated with a solution of sodium borohydride (2.1 g, 52.9 mmol) in methanol (40 mL). The reaction mixture was stirred for 4 h and the reaction quenched by the addition of water. The mixture was extracted with chloroform (3 × 100 mL). The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by chromatography on silica using hexane:EtOAc (8:2) as eluent to afford **33** as an oil (2.98 g, 51%): ^{1}H NMR ($CDCl_3$) δ 7.27 (1H, m), 7.14 (1H, d, J = 7.5 Hz), 6.92 (1H, d, J = 7.8 Hz), 5.51 (1H, s), 4.81 (2H, br d), 3.87 (3H, s), 3.37 (6H, s).

5-Methoxy-2-(dimethoxymethyl)benzenemethanol (34). A solution of **32** (2.6 g, 12.4 mmol) in MeOH (45 mL) was treated with sodium borohydride (2 g, 52.9 mmol). The reaction mixture was stirred for 2 h and the reaction quenched by the addition of water. The mixture was extracted with chloroform (3 × 100 mL). The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was purified by chromatography on silica using hexane:EtOAc (8:2) as eluent to afford **34** as an oil (1.36 g, 52%): ^{1}H NMR ($CDCl_3$) δ 7.28 (1H, d, J = 8.1 Hz), 6.87 (1H, dd, J = 2.4, 8.4 Hz), 6.77 (1H, d, J = 1.8 Hz), 6.13 (1H, d, J = 2.2 Hz), 5.17 (1H, m), 4.99 (1H, m), 3.82 (3H, s), 3.41 (6H, s).

1,4-Dihydro-5-methoxy-1,4-epoxynaphthalene-2,3-dicarboxylic Acid, Dimethyl Ester (35). A solution of compound **33** (1.17 g, 5.5 mmol) in dimethyl acetylenedicarboxylate (8 mL) was treated with glacial acetic acid (0.5 mL). The reaction mixture was heated to 135 °C for 45 min, and then, the excess reactants were distilled off. The residue was chromatographed on silica using hexane:EtOAc (7:3) as eluent to afford **35** (820 mg, 51%) as an oil: ^{1}H NMR ($CDCl_3$) δ 7.07 (2H, m), 6.67 (1H, dd, J = 1.5, 7.5 Hz), 6.20 (1H, d, J = 0.9 Hz), 5.93 (1H, d, J = 1.2 Hz), 3.87 (3H, s), 3.81 (3H, s), 3.79 (3H, s).

1,2,3,4-Tetrahydro-5-methoxy-1,4-epoxynaphthalene-2,3-dicarboxylic Acid, Dimethyl Ester (36). A solution of compound **35** (464 mg, 1.6 mmol) in ethyl acetate (6 mL) was treated with 10% Pd/C (35 mg) and stirred under a hydrogen atmosphere for 15 h. The catalyst was removed by filtration, and the solvent was evaporated to afford **36** (455 mg, 97%) as a clear oil: ^{1}H NMR ($CDCl_3$) δ 7.19 (1H, m), 7.02 (1H, d, J = 7.2 Hz), 6.75 (1H, d, J = 8.4 Hz), 5.71 (1H, m), 5.48 (1H, m), 3.84 (3H, s), 3.62 (2H, m), 3.50 (3H, s), 3.49 (3H, s).

5-Methoxy-2,3-naphthalenedicarboxylic Acid, Dimethyl Ester (37). A solution of **36** (455 mg, 1.56 mmol) in toluene (10 mL) was treated with *p*-toluenesulfonic acid (20 mg, 0.1 mmol) and heated to reflux for 4 h. The solvent was evaporated, and the residue was purified by chromatography on silica using 80% hexane–ethyl acetate as eluent to afford **37** (370 mg, 86%) as a clear oil: ^{1}H NMR ($CDCl_3$) δ 8.69 (1H, s), 8.15 (1H, s), 7.49 (2H, m), 6.91 (1H, dd, J = 0.96, 7.4 Hz), 4.00 (3H, s), 3.95 (6H, s).

5-Methoxy-2,3-naphthalenedicarboxylic Acid (38). A solution of compound **37** (379 mg, 1.38 mmol) in methanol (3 mL) and water (10 mL) was treated with sodium hydroxide (166 mg, 4.15 mmol) at 65 °C for 3 h. The solution was allowed to cool, and the solvent was evaporated. The aqueous solution was diluted with water and acidified with concentrated hydrochloric acid. The pale yellow precipitate was collected by filtration, washed with water, and dried to yield **38** (300 mg, 88%): mp 210–212 °C; ^{1}H NMR (DMSO-d_6) δ 8.48 (1H, s), 8.20 (1H, s), 7.62 (2H, m), 7.13 (1H, dd, J = 2.0, 6.8 Hz), 4.00 (3H, s).

5-Methoxy-1*H*-benz[*f*]isoindole-1,3(2*H*)-dione (39). A mixture of compound **38** (270 mg, 1.1 mmol) and urea (132 mg, 2.2 mmol) was heated at 170 °C for 2 h. The mixture was cooled and triturated with water (30 mL) and the resulting solid collected by filtration and dried to give **39** (237 mg, 95%) as a tan solid: mp >250 °C; ^{1}H NMR (DMSO-d_6) δ 11.51 (1H, s), 8.47 (1H, s), 8.40 (1H, s), 7.79 (1H, d, J = 8.1 Hz), 7.69 (1H, t, J = 7.8 Hz), 7.24 (1H, d, J = 7.6 Hz), 4.03 (3H, s).

4-Methoxy-1*H*-benz[*f*]isoindole-1,3(2*H*)-dione (42) was prepared as described for compound **39** from 4-methoxynaphtho-[2,3-c]furan-1,3-dione[25] (229 mg, 1 mmol) and urea (120 mg, 2

mmol); yield 215 mg, 95%: mp 216–220 °C; ^{1}H NMR (DMSO-d_6) δ 10.6 (1H, s), 7.52 (1H, d), 7.34 (1H, s), 6.92 (2H, m), 3.43 (3H, s).

1,3-Dihydro-2*H*-pyrrolo[3,4-*b*]quinoline-1,3-dione (43) was prepared as described for **39** from quinoline-2,3-dicarboxylic acid[26] (866 mg, 4 mmol) and urea (480 mg, 8 mmol); yield 540 mg, 68%, as a white solid: mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 8.67 (1H, s), 8.03 (2H, m), 7.74 (1H, br t), 7.57 (1H, br t).

3a,4,9,9a-Tetrahydro-6-methoxy-4,9-epoxynaphtho[2,3-*c*]furan-1,3-dione (44). A solution of compound **34** (844 mg, 4 mmol) and maleic anhydride (1.18 g, 12 mmol) in methylene chloride (10 mL) was treated with acetic acid (0.2 mL, 3.5 mmol) and acetic anhydride (0.1 mL, 1 mmol) and heated to reflux for 16 h. The reaction mixture was allowed to cool and treated with saturated aqueous sodium bicarbonate. The aqueous layer was extracted with $CHCl_3$ (175 mL), and the combined organic layers were dried (Na_2SO_4) and evaporated. The residue was purified by chromatography on silica using hexane:EtOAc (7:3) as eluent to afford **44** (800 mg, 81%) as a clear oil: ^{1}H NMR ($CDCl_3$) δ 7.22 (1H, m), 6.90 (1H, m), 6.76 (1H, m), 5.75 (2H, m), 3.98 (1.4H, m), 3.76 (3H, m), 3.23 (0.6H, s).

6-Methoxynaphtho[2,3-*c*]furan-1,3-dione (45). A solution of compound **45** (241 mg, 0.98 mmol) in toluene (5 mL) was treated with *p*-toluenesulfonic acid (20 mg, 0.1 mmol) and heated to reflux for 4.5 h. The solvent was evaporated and the residue dissolved in ethyl acetate. The solution was washed with saturated aqueous sodium bicarbonate and brine, dried ($MgSO_4$), and evaporated to give a brown solid. The solid was triturated with cold EtOAc, and the desired material was obtained by filtration. The solvent was evaporated from the filtrate, and the brown solid residue was again triturated with cold EtOAc. The pale tan solid was filtered off and combined with the previous crop to give 45 (211 mg, 95%): mp 238–240 °C; ^{1}H NMR (DMSO-d_6) δ 8.43 (1H, s), 8.33 (1H, s), 8.00 (1H, d), 7.52 (1H, s), 7.25 (1H, d), 3.69 (3H, s).

6-Methoxy-1*H*-benz[*f*]isoindole-1,3(2*H*)-dione (46). Compound **45** (159 mg, 0.7 mmol) and urea (84 mg, 1.4 mmol) were combined and heated at 170 °C for 45 min. The mixture was cooled and triturated with water (15 mL) and the resulting solid collected by filtration and dried to give **46** (134 mg, 84%): mp >250 °C; ^{1}H NMR (DMSO-d_6) δ 8.36 (1H, s), 8.30 (1H, s), 8.15 (1H, d, J = 9 Hz), 7.69 (1H, m), 7.39 (1H, dd, J = 2.7, 9 Hz), 3.92 (3H, s).

[*N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide, Trifluoroacetate (47). Method A: A solution of **18** (190 mg, 0.42 mmol) in dry tetrahydrofuran (5 mL) at 0 °C was treated with triphenylphosphine (121 mg, 0.46 mmol), 1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindole (91 mg, 0.46 mmol), and diethyl azodicarboxylate (72 μL, 0.46 mmol). The mixture was allowed to warm up to 23 °C over 15 h. The solvent was evaporated, and the residue was purified by preparative reverse-phase HPLC to give [*N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide, Trifluoroacetate (**25**) as a white lyophilized solid (133 mg, 50%): ^{1}H NMR ($CDCl_3$) δ 8.35 (2H, s), 8.09 (2H, m), 7.86 (1H, d, J = 8 Hz), 7.72 (2H, m), 7.28 (5H, s), 7.18 (1H, m), 4.74 (1H, m), 3.80 (2H, m), 3.36 (2H, m), 3.20 (1H, dd, J = 13, 8 Hz), 2.98 (1H, m), 2.69 (3H, d, J = 6 Hz), 2.10 (2H, m), 1.0–1.6 (3H, m), 1.36 (9H, s), 0.86 (6H, d, J = 6 Hz); chemical ionization MS m/z found 629 (MH^+). **Method B:** A solution of this intermediate (118 mg, 0.19 mmol) in trifluoroacetic acid (3 mL) was stirred at 23 °C for 6 h. The solvent was removed by evaporation and the residue purified by reverse-phase HPLC to afford **47** as a white lyophilized solid (95 mg, 73%): mp 199–200 °C; ^{1}H NMR (DMSO-d_6) δ 8.94 (1H, d, J = 8 Hz), 8.04 (1H, q, J = 5 Hz), 8.54 (2H, s), 8.26 (2H, m), 7.80 (2H, m), 7.20 (5H, m), 4.60 (1H, q, J = 6 Hz) 3.6–3.8 (3H, m), 3.43 (1H, t, J = 5 Hz), 2.95 (1H, dd, J = 12 Hz), 2.79 (1H, dd, J = 12, 8 Hz), 2.50 (3H, obscured by DMSO), 2.08 (2H, m), 1.4–1.6 (3H, m), 0.87 (3H, d, J = 6 Hz), 0.83 (3H, d, J = 6 Hz); high-resolution FAB MS m/z found 573.2701 (MH^+), $C_{32}H_{37}N_4O_6$ requires 573.2722.

[*N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-4-methoxy-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide, Trifluoroacetate (48). Reaction of **42** (73 mg, 0.32 mmol) with **18** (130 mg, 0.29 mmol) using method A (above) gave [*N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-4-methoxy-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide, trifluoroacetate (70 mg, 31%): ^{1}H NMR (CD_3OD) δ 8.31 (1H, m), 7.99 (1H, s), 7.63 (2H, m), 7.09 (6H, m), 4.63 (1H, m), 4.32 (3H, s), 3.60–3.80 (2H, m), 3.40–3.60 (2H, m), 2.77–3.00 (2H, m), 2.53 (3H, s), 1.88 (2H, m), 1.51–1.71 (3H, m), 1.43 (9H, s), 0.89 (3H, d, J = 5.9 Hz), 0.83 (3H, d, J = 6.1 Hz). Hydrolysis of this intermediate (70 mg, 0.09 mmol) using method B (above) gave **48** (30 mg, 47%) as a white lyophilized powder: ^{1}H NMR (CD_3OD) δ 8.33 (1H, m), 7.97 (1H, s), 7.63 (2H, m), 7.10 (6H, m), 4.57 (1H, m), 4.31 (3H, s), 3.77 (1H, m), 3.66 (3H, m), 2.80–3.00 (2H, m), 2.51 (3H, s), 1.95 (2H, m), 1.60 (3H, m), 0.88 (3H, d, J = 5.6 Hz), 0.83 (3H, d, J = 5.9 Hz); high-resolution FAB MS m/z found 603.2817 (MH^+), $C_{33}H_{39}N_4O_7$ requires 603.2821.

[*N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-5-methoxy-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide, Trifluoroacetate (49). Reaction of **39** (75 mg, 0.33 mmol) with **18** (136 mg, 0.3 mmol) using method A (above) gave [*N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-5-methoxy-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide, trifluoroacetate (156 mg, 67%): ^{1}H NMR (CD_3OD) δ 8.58 (1H, s), 8.22 (1H, s), 7.57 (2H, m), 7.09 (6H, m), 4.60 (1H, m), 3.96 (3H, s), 3.67 (3H, m), 3.40 (1H, m), 2.81–2.97 (2H, m), 2.52 (3H, s), 1.9 (2H, m), 1.52 (3H, m), 1.42 (9H, s), 0.88 (3H, d, J = 5.7 Hz), 0.82 (3H, d, J = 5.7 Hz). Hydrolysis of this intermediate (136 mg, 0.18 mmol) using method B (above) gave **49** (80 mg, 62%) as a white lyophilized powder: ^{1}H NMR (CD_3OD) δ 8.52 (1H, s), 8.16 (1H, s), 7.53 (2H, m), 7.08 (6H, m), 4.55 (1H, m), 3.93 (3H, s), 3.69 (3H, m), 3.57 (1H, m), 2.79–2.99 (2H, m), 2.49 (3H, s), 1.97 (2H, m), 1.59 (3H, m), 0.86 (3H, d, J = 5.6 Hz), 0.81 (3H, d, J = 5.9 Hz); high-resolution FAB MS m/z found 603.2808 (MH^+), $C_{33}H_{39}N_4O_7$ requires 603.2821.

[*N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-6-methoxy-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide, Trifluoroacetate (50). Reaction of **46** (40 mg, 0.18 mmol) with **18** (66 mg, 0.15 mmol) using method A (above) gave [*N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-6-methoxy-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide, trifluoroacetate (30 mg, 26%): ^{1}H NMR (CD_3OD) δ 8.19 (1H, d, J = 3.2 Hz), 7.92 (1H, d, J = 9 Hz), 7.43 (1H, d, J = 2.1 Hz), 7.24 (1H, dd, J = 2.4, 9 Hz), 7.15 (6H, m), 4.63 (1H, m), 3.86 (3H, s), 3.60 (3H, m), 3.39 (1H, m), 2.8–3.01 (2H, m), 2.52 (3H, s), 1.92 (2H, m), 1.55 (3H, m), 1.42 (9H, s), 0.82 (6H, m). Hydrolysis of this intermediate (28 mg, 0.036 mmol) using method B (above) gave **50** (19 mg, 73%) as a white lyophilized powder: ^{1}H NMR (CD_3OD) δ 8.16 (1H, d, J = 3.9 Hz), 7.91 (1H, d, J = 9 Hz), 7.41 (1H, d, J = 2.7 Hz), 7.24 (1H, dd, J = 2.4, 9 Hz), 7.10 (6H, m), 4.56 (1H, m), 3.86 (3H, s), 3.70 (2H, m), 3.56 (1H, m), 3.40 (1H, m), 2.81–3.02 (2H, m), 2.51 (3H, s), 1.97 (2H, m), 1.62 (3H, m), 0.87 (3H, d, J = 5.9 Hz), 0.82 (3H, d, J = 5.9 Hz); high-resolution FAB MS m/z found 603.2808 (MH^+), $C_{33}H_{39}N_4O_7$ requires 603.2817.

[*N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-pyrrolo-[3,4-*b*]quinolin-2-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide, Trifluoroacetate (51). Reaction of **43** (57 mg, 0.29 mmol) with **18** (115 mg, 0.26 mmol) using method A (above) gave [*N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-pyrrolo[3,4-*b*]quinolin-2-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide, trifluoroacetate (100 mg, 51%): ^{1}H NMR ($CDCl_3$) δ 8.67 (1H, s), 8.45 (1H, d), 8.11 (1H, d), 7.98 (1H, t), 7.60 (5H, m), 4.72 (m), 3.85 (m), 3.50–3.10 (m), 2.71 (3H, d), 2.15 (m), 1.50 (m), 1.40 (9H, s), 1.25 (m), 0.84 (6H, m). Hydrolysis of this intermediate (100 mg, 0.13 mmol) using method B (above) gave **51** (10 mg, 11%) as a white lyophilized powder: ^{1}H NMR (CD_3OD) δ 8.75 (1H, s), 8.13 (2H, m), 7.89 (1H, m), 7.72 (1H, m), 7.13 (5H, m), 4.54 (m), 3.87 (m), 3.65 (m), 3.10–2.80 (m), 2.10 (m), 1.56 (m), 0.85 (6H, m); high-resolution FAB MS m/z found 574.2664 (MH^+), $C_{31}H_{36}N_5O_6$ requires 574.2660.

[*N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide, Trifluoroacetate (52). Method C: Diethyl azodicarboxylate (0.025 mL, 0.16 mmol) was added to a stirred solution of **18** (66 mg, 0.15 mmol), triphenylphosphine (42 mg, 0.16 mmol), and 1,3-dihydro-1,3-dioxo-2*H*-isoindole (23 mg, 0.16 mmol) in dry tetrahydrofuran

at 5 °C, and the resulting mixture was stirred at 21 °C for 16 h before concentrating in vacuo. The residue was purified by silica chromatography using CH_2Cl_2:acetone (9:1) as eluent to give [*N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide (58 mg, 56%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 7.0–7.9 (11H, m), 4.72 (1H, m), 3.72 (2H, m), 3.34 (2H, m), 3.15 (1H, dd, *J* = 13, 9 Hz), 2.96 (1H, dd, *J* = 9, 4 Hz), 2.70 (3H, d, *J* = 5 Hz), 2.04 (2H, m), 1.38 (9H, s), 1.0–1.6 (3H, m), 0.84 (6H, d, *J* = 6 Hz); time of flight MS *m/z* found 580.4 (MH^+). **Method D**: A solution of this intermediate (58 mg, 0.08 mmol) in trifluoroacetic acid (1 mL) was stirred for 10 min under nitrogen before concentrating in vacuo. The residue was purified by preparative reverse-phase HPLC to give **52** (28 mg, 55%) as a white lyophilized solid: ^{1}H NMR (DMSO-d_6) δ 8.75 (1H, br), 8.00 (1H, q, *J* = 5 Hz), 7.88 (4H, m), ca. 7.20 (5H, m), 4.57 (1H, q, *J* = 7 Hz), 3.2–3.9 (4H, m), 2.96 (1H, dd, *J* = 13, 6 Hz), 2.71 (1H, dd, *J* = 13, 6 Hz), 2.54 (3H, d, *J* = 5 Hz), 2.00 (2H, m), 1.47 (3H, m), 0.86 (3H, d, *J* = 7 Hz), 0.82 (3H, d, *J* = 7 Hz); high-resolution FAB MS *m/z* found 523.2540 (MH^+), $C_{28}H_{35}N_4O_6$ requires 523.2557.

[*N*-[1-(*R*)-Carboxy-3-(1,3,5,7-tetraoxo-2*H*,6*H*-benzo[1,2-*c*:4,5-*c*']dipyrrol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide, Trifluoroacetate (53). Reaction of 1,3,5,7-tetraoxo-2*H*,6*H*-benzo[1,2-c:4,5-c']dipyrrole (315 mg, 1.46 mmol) with **18** (503 mg, 1.33 mmol) using method A (above) gave [*N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3,5,7-tetraoxo-2*H*,6*H*-benzo[1,2-c:4,5-c']dipyrrol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide, trifluoroacetate (81 mg, 8%): ^{1}H NMR ($CDCl_3$) δ 8.5 (1H, m), 8.4 (2H, s), 8.3 (1H, m), 7.4–7.2 (6H, m), 6.8 (1H, m), 4.7 (1H, m), 4.3 (1H, q), 3.8 (1H, m), 3.3 (1H, dd), 3.2–3.0 (2H, m), 2.8 (3H, d), 2.1 (2H, m), 1.6 (3H, m), 1.4 (9H, s), 0.9 (6H, d). Hydrolysis of this intermediate (76 mg, 0.09 mmol) using method B (above) gave **53** (41 mg, 57%) as a white lyophilized powder: ^{1}H NMR (CD_3OD) δ 8.1 (2H, s), 7.0 (5H, s), 4.6 (1H, t), 3.6–3.8 (4H, br m), 2.7–3.0 (2H, m), 2.5 (3H, s), 1.8–2.0 (2H, br m), 1.4–1.7 (3H, br m), 0.8–1.0 (6H, d); high-resolution FAB MS *m/z* found 592.2372 (MH^+), $C_{30}H_{35}N_5O_8$ requires 592.2397.

[*N*-[1-(*R*)-Carboxy-3-(5,7-dihydro-5,7-dioxo-6*H*-1,3-dioxolo[4,5-*f*]isoindol-6-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide (54). Reaction of 5*H*-1,3-dioxolo[4,5-*f*]isoindole-5,7(6*H*)-dione (80 mg, 0.42 mmol)[29] with **18** (188 mg, 0.42 mmol) using method C (above) gave [*N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(5,7-dihydro-5,7-dioxo-6*H*-1,3-dioxolo[4,5-*f*]isoindol-6-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide, trifluoroacetate (48 mg, 16%): ^{1}H NMR (CD_3OD) δ 7.17 (7H, m), 6.09 (2H, s), 4.69 (1H, m), 3.67 (1H, m), 3.57 (1H, m), 3.40 (2H, m), 2.95 (2H, m), 2.55 (3H, s), 1.90 (2H, m), 1.50–1.77 (3H, m), 1.44 (9H, s), 0.88 (6H, dd). Hydrolysis of this intermediate (40 mg, 0.054 mmol) using method D (above) gave **54** (7 mg, 19%) as a white lyophilized powder: mp 100–110 °C; ^{1}H NMR (CD_3OD) δ 7.26 (7H, m), 6.19 (2H, m), 4.68 (1H, m), 3.86 (1H, m), 3.66 (3H, m), 2.96 (2H, m), 2.64 (3H, s), 2.03 (2H, m), 1.62–1.80 (3H, m), 0.95 (6H, dd); high-resolution FAB MS *m/z* found 567.2439 (MH^+), $C_{29}H_{35}N_4O_8$ requires 567.2455.

[*N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-5-(propyloxy)-2*H*-isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide (55). Reaction of 1,3-dihydro-1,3-dioxo-5-(propyloxy)-2*H*-isoindole (92 mg, 0.448 mmol)[30] with **18** (200 mg, 0.448 mmol) using method C (above) gave [*N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-5-(propyloxy)-2*H*-isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide (190 mg, 74%): ^{1}H NMR ($CDCl_3$) δ 8.26 (1H, br d, *J* = 8.5 Hz), 7.71 (1H, d, *J* = 8.3 Hz), 7.29 (1H, d, *J* = 2 Hz), 7.24–7.12 (5H, m), 6.00 (2H, br), 4.80 (1H, m), 4.02 (2H, t, *J* = 6.6 Hz), 3.80–3.60 (3H, m), 3.48 (1H, t, *J* = 5.5 Hz), 3.24 (1H, A part of ABX system, dd, J_{ab} = 1.04 Hz, J_{ax} = 5.7 Hz), 3.02 (1H, B part of ABX system, dd, J_{ab} = 14.0 Hz, J_{bx} = 9.2 Hz), 2.74 (3H, d, *J* = 4.4 Hz), 2.20 (2H, m), 1.86 (2H, m), 1.70–1.45 (3H, m), 1.38 (9H, s), 1.05 (3H, t, *J* = 7.3 Hz), 0.85 (6H, t, *J* = 6.0 Hz). Hydrolysis of this intermediate (170 mg, 0.293 mmol) using method D (above) gave **55** (55 mg, 27%) as a white lyophilized powder: mp 91–101 °C; ^{1}H NMR (CD_3OD) δ 7.88 (1H, br m), 7.67 (1H, d, *J* = 8.3 Hz), 7.25 (1H, d, *J* = 2.2 Hz), 7.20–7.05 (6H, m), 4.56 (1H, dd, *J* = 6.6 Hz, *J'* = 8.8 Hz), 3.97 (2H, t, *J* = 6.3 Hz), 3.70–3.56 (3H, m), 3.52 (1H, dd, *J* = 5.1 Hz, *J'* = 6.6 Hz), 3.20 (1H, quin, *J* = 1.6 Hz), 3.01 (1H, A part of ABX system, J_{ab} = 13.8 Hz, J_{ax} = 6.7 Hz), 2.86 (1H, B part of ABX system, J_{ab} = 13.7 Hz, J_{bx} = 9.0 Hz), 2.55 (3H, s), 1.95 (2H, m), 1.76 (2H, hex, *J* = 6.9 Hz), 1.65–1.45 (3H, m), 0.96 (3H, t, *J* = 7.4 Hz), 0.87 (3H, d, *J* = 5.8 Hz), 0.83 (3H, d, *J* = 5.8 Hz); high-resolution FAB MS *m/z* found 581.2979 (MH^+), $C_{31}H_{41}N_4O_7$ requires 581.2975.

[*N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-5-phenyl-2*H*-isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide (56). Reaction of 2,3-dihydro-5-phenyl-1*H*-isoindole-1,3-(2*H*)-dione[31] (116 mg, 0.52 mmol) with **18** (150 mg, 0.9 mmol) using method C (above) gave [*N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-5-phenyl-2*H*-isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide (138 mg, 59%): ^{1}H NMR ($CDCl_3$) δ 8.03 (2H, m), 7.88 (1H, d), 7.64 (2H, m), 7.44 (3H, m), 7.14 (5H, m), 4.65 (1H, m), 3.70 (2H, m), 3.57 (1H, m), 3.46 (1H, m), 2.98 (2H, m), 2.57 (3H, s), 1.95 (2H, m), 1.65 (3H, m), 1.48 (9H, s), 0.88 (6H, dd). Hydrolysis of this intermediate (100 mg, 0.16 mmol) using method D (above) gave **56** (52 mg, 57%) as a white lyophilized powder: mp 83–100 °C; ^{1}H NMR (CD_3OD) δ 7.96 (2H, m), 7.81 (1H, d), 7.55 (2H, m), 7.38 (3H, m), 7.10 (5H, m), 4.57 (1H, m), 3.72 (1H, m), 3.60 (3H, m), 2.85 (2H, m), 2.49 (3H, s), 1.95 (2H, m), 1.53 (3H, m), 0.85 (6H, dd); high-resolution FAB MS *m/z* found 599.2853 (MH^+), $C_{34}H_{39}N_4O_6$ requires 599.2870.

[*N*-[1-(*R*)-Carboxy-3-(6-bromo-1,3-dioxo-1*H*-benz[*de*]isoquinolin-2(3*H*)-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide, Trifluoroacetate (57). Reaction of 6-bromo-1*H*-benz[*de*]isoquinoline-1,3(2*H*)-dione (171 mg, 0.62 mmol) with **18** (250 mg, 0.56 mmol) using method C (above) gave [*N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(6-bromo-1,3-dioxo-1*H*-benz[*de*]isoquinolin-2(3*H*)-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylalaninamide (260 mg, 66%) as a pale yellow foam: ^{1}H NMR ($CDCl_3$) δ 8.67 (1H, d, *J* = 8 Hz), 8.63 (1H, d, *J* = 8 Hz), 8.44 (1H, d, *J* = 8 Hz), 8.07 (1H, d, *J* = 8 Hz), 7.88 (1H, dd, *J* = 7, 8 Hz), 7.1–7.3 (6H, m), 4.75 (1H, m), 4.19 (2H, m), 3.32 (1H, dd, *J* = 6, 14 Hz), 3.15 (1H, dd, *J* = 8, 14 Hz), 3.19 (1H, t, *J* = 6 Hz), 3.00 (1H, m), 2.74 (3H, d, *J* = 5 Hz), 2.06 (2H, m), 1.40 (9H, s), 1.00–1.70 (3H, m), 0.86 (6H, d, *J* = 6 Hz); IR ($CHBr_3$) 3395 (NH), 1727 (C=OO), 1659 cm^{-1} (C=ONH); chemical ionization MS *m/z* found 707/709 (MH^+). Hydrolysis of this intermediate (230 mg, 0.32 mmol) using method D (above) gave **57** as a pale yellow foam: ^{1}H NMR (DMSO-d_6) δ 8.96 (1H, d, *J* = 8 Hz), 8.57 (2H, m), 8.36 (1H, d, *J* = 8 Hz), 8.23 (1H, d, *J* = 8 Hz), 8.02 (2H, m), 7.1–7.3 (5H, m), 4.57 (1H, q, *J* = 7 Hz), 4.11 (2H, m), 3.92 (1H, t, *J* = 6 Hz), 3.53 (1H, t, *J* = 5 Hz), 2.89 (1H, dd, *J* = 6, 13 Hz), 2.75 (1H, dd, *J* = 8, 13 Hz), ca. 2.50 (3H, s, obscured by DMSO), 2.11 (2H, m), 1.40–1.60 (3H, m), 0.86 (3H, d, *J* = 6 Hz), 0.82 (3H, d, *J* = 6 Hz); IR (Nujol) 3260 (br OH and NH), 1700 (COOH), 1652 cm^{-1} (C=ONH); chemical ionization MS *m/z* found 651/653 (MH^+).

[*N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-tyrosinamide, Trifluoroacetate (58). Reaction of L-tyrosine *N*-methylamide (51 mg, 0.22 mmol) with **28** (50 mg, 0.11 mmol) using method E (below) gave [*N*-[(*R*)-1-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-tyrosinamide, trifluoroacetate (60 mg, 84%): ^{1}H NMR ($CDCl_3$) δ 8.3 (2H, s), 8.0 (2H, m), 7.6 (1H, br s), 7.5 (2H, m), 6.7 (2H, d), 6.3 (2H, d), 4.2 (2H, m), 3.4 (4H, m), 3.1 (1H, m), 2.6 (3H, d), 1.8 (4H, m), 1.4 (9H, s), 1.2 (2H, m), 0.9 (6H, d); chemical ionization MS *m/z* found 645.3 (MH^+). This intermediate (60 mg, 0.09 mmol) was hydrolyzed using method F (below) to give **58** (20 mg, 54%) as a white lyophilized solid: mp 175–180 °C; ^{1}H NMR (DMSO-d_6) δ 8.9 (1H, s), 8.3 (2H, s), 8.0 (2H, m), 7.6 (1H, br s), 7.5 (2H, m), 6.7 (2H, d), 6.3 (2H, d), 4.2 (2H, m), 3.4 (4H, m), 3.1 (1H, m), 2.6 (3H, d), 1.8 (4H, m), 1.2 (2H, m), 0.6 (6H, d); high-resolution FAB MS *m/z* found 589.2652 (MH^+), $C_{32}H_{37}N_4O_7$ requires 589.2658.

[*N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylglycinamide, Trifluoroacetate (59). Reaction of *N*-methyl-L-phenylglycinamide (15 mg, 0.075 mmol) with **28** (35 mg, 0.075 mmol) using method E (below) gave crude [*N*-[(*R*)-1-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-phenylglycinamide (62 mg). This intermediate was hydrolyzed using method F (below)

to give **59** (19 mg, 38%) as a white lyophilized solid: ^{1}H NMR (DMSO-d_6) δ 8.52 (2H, s), 8.22 (2H, m), 7.79 (2H, m), 7.16–7.42 (5H, m), 5.40 (1H, d), 3.20–3.80 (m, obscured by water), 2.56 (3H, d), 2.10 (m), 1.40–1.70 (3H, m), 0.89 (6H, m); high-resolution FAB MS m/z found 559.2552 (MH^+), $C_{31}H_{35}N_4O_6$ requires 559.2557.

[*N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]-isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-leucinamide, Trifluoroacetate (60). Method E: A solution of compound **28** (100 mg, 0.214 mmol), *N*-methyl-L-leucinamide (31 mg, 0.215 mmol), and 1-hydroxybenzotriazole hydrate (17 mg, 0.111 mmol) in CH_2Cl_2 (10 mL) was treated with dicyclohexylcarbodiimide (48 mg, 0.233 mmol) and the mixture stirred for 15 h at 23 °C. The mixture was filtered through Celite and the solvent evaporated. The residue was purified by reverse-phase HPLC to afford [*N*-[1-(*R*)-[(1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-leucinamide, trifluoroacetate (61 mg, 48%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 8.35 (s), 8.10 (m), 7.70 (m), 4.60–4.20 (m), 3.90 (m), 3.70 (m), 3.10 (m), 2.90–2.70 (m), 2.40–1.60 (m), 1.45 (s), 1.00 (m). **Method F**: A solution of this intermediate (40 mg, 0.067 mmol) in TFA (5 mL) was allowed to stand at 23 °C for 15 h. The solvent was evaporated and the residue purified by reverse-phase HPLC to furnish **60** (16 mg, 36%) as a white foam: mp 115–125 °C; ^{1}H NMR (CD_3OD) δ 8.30 (m), 8.05 (m), 7.65 (m), 4.30 (m), 3.90–3.65 (m), 2.55 (s), 2.20 (m), 1.70–1.40 (m), 0.90–0.75 (m); high-resolution FAB MS m/z found 539.2838 (MH^+), $C_{29}H_{39}N_4O_6$ requires 539.2859.

[*N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]-isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-alaninamide, Trifluoroacetate (61). Reaction of *N*-methyl-L-alaninamide (40 mg, 0.25 mmol) with **28** (50 mg, 0.11 mmol) using method E (above) gave [*N*-[(*R*)-1-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-alaninamide, trifluoroacetate (37 mg, 60%): ^{1}H NMR ($CDCl_3$) δ 8.4 (2H, s), 8.2 (2H, br s), 8.1 (2H, m), 7.8 (2H, m), 7.1 (1H, m), 4.5 (1H, m), 4.0 (2H, m), 3.7 (1H, t), 3.6 (1H, t), 3.2 (1H, br s), 2.8 (3H, d), 2.4–2.2 (2H, m), 1.7 (2H, m), 1.6 (1H, m), 1.4 (9H, s), 1.2 (3H, d), 0.9 (6H, d); chemical ionization MS m/z found 645.3 (MH^+). This intermediate was hydrolyzed using method F (above) to give **61** (16 mg, 44%) as a white lyophilized solid: mp 133–135 °C; ^{1}H NMR (CD_3OD) δ 8.3 (2H, s), 8.0 (2H, dd), 7.6 (2H, dd), 4.2 (1H, q), 3.9 (2H, m), 3.7 (1H, t), 3.6 (1H, t), 2.6 (3H, s), 2.2 (2H, dd), 1.7 (2H, m), 1.5 (1H, m), 1.3 (3H, d), 0.9 (6H, d); high-resolution FAB MS m/z found 497.2411 (MH^+), $C_{26}H_{33}N_4O_6$ requires 497.2400.

[*N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]-isoindol-2-yl)propyl]-L-leucyl]-*N*-methylglycinamide, Trifluoroacetate (62). Reaction of *N*-methylglycinamide (59 mg, 0.22 mmol) with **28** (50 mg, 0.11 mmol) using method E (above) gave [*N*-[(*R*)-1-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucyl]-*N*-methylglycinamide, trifluoroacetate (27 mg, 48%): chemical ionization MS m/z found 539.0 (MH^+). This intermediate was hydrolyzed using method F (above) to give **62** (20 mg, 67%) as a white lyophilized solid: mp 175–180 °C; ^{1}H NMR (CD_3OD) δ 8.3 (2H, s), 8.0 (2H, d), 7.8 (2H, d), 3.9 (1H, m), 3.85 (1H, m), 3.8 (2H, m), 3.7 (2H, m), 2.5 (3H, s), 2.3 (2H, m), 1.8–1.7 (1H, m), 1.7–1.6 (2H, m), 0.9 (6H, m); high-resolution FAB MS m/z found 483.2223 (MH^+), $C_{25}H_{31}N_4O_6$ requires 483.2244.

[*N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]-isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-argininamide, Trifluoroacetate (63). Reaction of *N'*-nitro-*N*-methyl-L-argininamide (51 mg, 0.22 mmol) with **28** (50 mg, 0.11 mmol) using method E (above) gave [*N*-[(*R*)-1-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-*N'*-nitro-L-argininamide, trifluoroacetate (63 mg, 84%): chemical ionization MS m/z found 683.3 (MH^+). A solution of this intermediate (63 mg, 0.09 mmol) in ethanol (10 mL) was shaken with 10% Pd/C (10 mg) in a hydrogen atmosphere (50 psi) at 23 °C for 15 h. The catalyst was removed by filtration through Celite, and the solvent was evaporated to afford [*N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucyl]-*N*-methyl-L-argininamide, trifluoroacetate (28 mg, 49%) as a yellow oil: ^{1}H NMR (D_2O) δ 8.1 (2H, s), 7.9 (2H, m), 7.5 (2H, m), 4.4 (1H, t), 3.9 (2H, t), 3.6 (2H, m), 3.4 (1H, t), 3.3 (1H, t), 2.8 (2H, m), 2.3 (3H, s), 1.9 (2H, m), 1.4 (2H, m), 1.3 (2H, m), 1.2 (1H, m), 1.1 (9H, s), 0.6 (6H, d); chemical ionization MS m/z found 638.3 (MH^+). Hydrolysis of this intermediate (20 mg, 0.03 mmol) using method F (above) gave **63** (5 mg, 20%) as a white lyophilized solid: ^{1}H NMR (CD_3OD) δ 8.3 (2H, s), 8.3 (2H, s), 8.0 (2H, m), 7.6 (2H, m), 7.5 (2H, m), 4.3 (1H, m), 3.8 (2H, m), 3.5 (2H, m), 3.4 (1H, t), 3.2 (1H, m), 3.0 (2H, m), 2.6 (3H, s), 2.2 (2H, m), 1.9–1.5 (5H, m), 0.9 (6H, d); high-resolution FAB MS m/z found 582.3039 (MH^+), $C_{29}H_{40}N_7O_6$ requires 582.3040.

***N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-(phenylethyl)-L-leucinamide, Trifluoroacetate (64). Method G**: Dicyclohexylcarbodiimide (52.8 mg, 0.26 mmol) was added to a stirred solution of compound **28** (100 mg, 0.21 mmol), phenylethylamine (0.032 mL, 0.26 mmol), and hydroxybenzotriazole hydrate (39.2 mg, 0.26 mmol) in DMF (2.5 mL) cooled at 0 °C. Stirring was continued at room temperature for 24 h before the solvents were removed by evaporation and the resultant residue was diluted with EtOAc. This mixture was washed with a saturated aqueous sodium bicarbonate solution, and the organic layer was separated, dried ($MgSO_4$), and filtered. The solvent was removed by evaporation to afford crude *N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-(phenylethyl)-L-leucinamide (120 mg, 115%). This intermediate was dissolved in TFA:H_2O (9:1) and allowed to stand for 20 h at room temperature. The reaction mixture was concentrated in vacuo and the residue purified by reverse-phase HPLC to give **64** (53 mg, 40%) as a colorless solid: ^{1}H NMR (CD_3OD) δ 8.40 (2H, s), 8.10–8.20 (2H, m), 7.70–7.80 (2H, m), 7.10–7.30 (5H, m), 3.80–4.00 (2H, m), 3.50–2.70 (3H, m), 3.40–3.50 (1H, m), 2.80–2.90 (2H, m), 2.10–2.30 (2H, m), 1.80–1.10 (5H, m), 0.80 (6H, d, J = 6 Hz); high-resolution FAB MS m/z found 516.2502 (MH^+), $C_{30}H_{34}N_3O_5$ requires 516.2421.

***N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-(phenylmethyl)-L-leucinamide, Trifluoroacetate (65).** Reaction of benzylamine (0.028 mL, 0.26 mmol) with **28** (100 mg, 0.21 mmol) using method G (above) gave *N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-(phenylmethyl)-L-leucinamide (60 mg, 50%) as a tan oil. Hydrolysis of this intermediate using method F (above) gave **65** (28 mg, 42%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 8.10 (2H, br s), 7.90–8.00 (2H, m), 7.60–7.70 (2H, m), 7.20–7.30 (5H, m), 7.10–7.20 (1H, m), 4.40 (2H, d), 4.10–4.20 (1H, m), 3.80–4.00 (2H, m), 3.60–3.70 (1H, m), 3.40–3.50 (1H, m), 2.30–2.40 (2H, m), 1.00–1.40 (3H, m), 0.90–1.00 (6H, m); high-resolution FAB MS m/z found 502.2334 (MH^+), $C_{29}H_{32}N_3O_5$ requires 502.2342.

***N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-phenyl-L-leucinamide, Trifluoroacetate (66).** Reaction of aniline (0.03 mL, 0.33 mmol) with **28** (100 mg, 0.21 mmol) using method G above and purification of the product by reverse-phase HPLC gave *N*-[1-(*R*)-[(1,1-dimethylethoxy)-carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)-propyl]-*N'*-phenyl-L-leucinamide, trifluoroacetate (69 mg, 59%) as a white solid. Hydrolysis of this intermediate using method F (above) gave **66** (56 mg, 85%) as a white lyophilized solid: ^{1}H NMR (CD_3OD) δ 8.20 (2H, s), 7.98–8.03 (2H, m), 7.61–7.67 (2H, m), 7.36 (2H, d, J = 9 Hz), 7.06 (2H, t, J = 8, 8 Hz), 6.88–6.94 (1H, m), 3.83–3.95 (4H, m), 2.17–2.26 (2H, m), 1.57–1.83 (3H, m), 0.90 (3H, d, J = 6 Hz), 0.87 (3H, d, J = 6 Hz); high-resolution FAB MS m/z found 488.2186 (MH^+), $C_{28}H_{30}N_3O_5$ requires 488.2185.

***N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-methyl-L-leucinamide, Trifluoroacetate (67). Method H**: Triethylamine (0.5 mL, 3.6 mmol) was added to a stirred solution of compound **28** (300 mg, 0.64 mmol), hydroxybenzotriazole hydrate (120 mg, 0.78 mmol), dicyclohexylcarbodiimide (150 mg, 0.73 mmol), and methylamine hydrochloride (600 mg, 8.9 mmol) in CH_2Cl_2 (30 mL), and the resulting mixture was stirred for 18 h at room temperature. The mixture was filtered through Celite, and the solvent was removed by evaporation. The resulting residue was purified by silica chromatography using hexane:EtOAc:CH_2Cl_2 (2:1:1) as eluent to give *N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-phenyl-L-leucin-

amide (320 mg, 100%) as a white solid. Hydrolysis of this intermediate using method F (above) gave **67** (190 mg, 55%) as a white lyophilized solid: ^{1}H NMR (CD_3OD) δ 8.11 (2H, s), 7.86 (2H, m), 7.45 (2H, m), 3.65 (3H, m), 3.52 (1H, m), 2.00 (2H, m), 1.6–1.2 (3H, m), 0.69 (6H, m); high-resolution FAB MS *m/z* found 426.2020 (MH^+), $C_{23}H_{28}N_3O_5$ requires 426.2029.

***N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-(3-pyridylmethyl)-L-leucinamide, Trifluoroacetate (68).** Reaction of 3-(aminomethyl)pyridine (0.026 mL, 0.26 mmol) with **28** (100 mg, 0.21 mmol) using method G (above) gave crude *N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-(3-pyridylmethyl)-L-leucinamide (139 mg, 117%). Hydrolysis of this intermediate using method F (above) gave **68** (97 mg, 74%) as a white colorless solid: ^{1}H NMR (CD_3OD) δ 8.75 (1H, br s), 8.63 (1H, m), 8.40 (2H, s), 8.30 (1H, m), 8.15 (2H, m), 7.70–7.90 (3H, m), 4.6 (2H, ABq), 3.90–4.00 (2H, m), 3.80–3.90 (2H, m), 1.60–1.85 (4H, m), 0.95 (6H, apparent t, J = 6 Hz); high-resolution FAB MS *m/z* found 503.2295 (MH^+), $C_{28}H_{31}N_4O_5$ requires 503.2222.

***N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-[(2-methyl-2*H*-tetrazol-5-yl)methyl]-L-leucinamide, Trifluoroacetate (69). Method I:** Dicyclohexylcarbodiimide (53 mg, 0.26 mmol) was added to a stirred solution of compound **28** (100 mg, 0.21 mmol), hydroxybenzotriazole hydrate (39 mg, 0.26 mmol), *N*-methylmorpholine (26 mg, 0.26 mmol), and 2-methyl-5-(aminomethyl)-2*H*-tetrazole[32] (38 mg, 0.26 mmol) in DMF (2 mL) cooled at 0 °C. The mixture was allowed to warm to 23 °C and stirred for 24 h. The solvents were removed by evaporation, and the resultant residue was diluted with EtOAc. The mixture was extracted with saturated aqueous sodium bicarbonate, the organic layer was separated, dried ($MgSO_4$), and filtered, and the solvent was removed by evaporation to afford *N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-[(2-methyl-2*H*-tetrazol-5-yl)methyl]-L-leucinamide (120 mg, 100%). Hydrolysis of this intermediate using method F (above) gave **69** (50 mg, 38%) as a white colorless solid: ^{1}H NMR (CD_3OD) δ 8.25 (2H, s), 8.00–8.10 (2H, m), 7.60–7.65 (2H, m), 4.55 (2H, s), 4.20 (3H, s), 3.70–3.90 (4H, m), 2.10–2.30 (2H, m), 1.50–1.80 (3H, m), 0.86 (6H, apparent t, J = 6 Hz); high-resolution FAB MS *m/z* found 508.2307 (MH^+), $C_{25}H_{30}N_7O_5$ requires 508.2230.

***N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-[(4-carboxyphenyl)methyl]-L-leucinamide, Trifluoroacetate (70). Method J:** Compound **28** (200 mg, 0.42 mmol) was added to a stirred solution of diisopropylethylamine (0.174 mL, 1 mmol) and hydroxybenzotriazole–benzotriazolyltetramethyluronium hexafluorophosphate (1.1 mL, 0.45 M, 1:1 stock solution in DMF) in DMF (1 mL) at 5 °C. 4-(Aminomethyl)benzoic acid (75 mg, 0.5 mmol) was added, and the mixture was stirred for 24 h. The solvents were removed by evaporation to give crude *N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-[(4-carboxyphenyl)methyl]-L-leucinamide. Hydrolysis of this intermediate using method F (above) gave **70** (84 mg, 30%) as a white solid: ^{1}H NMR (CD_3OD) δ 8.38 (2H, s), 8.14 (2H, m), 7.96 (2H, d, J = 8 Hz), 7.74 (2H, m), 7.40 (2H, d, J = 8 Hz), 4.51 and 4.44 (2H, ABq, J = 15 Hz), 3.80–4.0 (4H, m), 2.20–2.40 (2H, m), 1.58–1.90 (3H, m), 0.9–1.1 (6H, m); high-resolution FAB MS *m/z* found 546.2230 (MH^+), $C_{30}H_{32}N_3O_7$ requires 546.2240.

***N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-[(3-fluorophenyl)methyl]-L-leucinamide, Trifluoroacetate (71).** Reaction of 3-fluorobenzylamine (0.030 mL, 0.26 mmol) with **28** (100 mg, 0.21 mmol) using method G (above) gave *N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-[(3-fluorophenyl)methyl]-L-leucinamide, trifluoroacetate (60 mg, 48%) after purification by reverse-phase HPLC. Hydrolysis of this intermediate using method F (above) gave **71** (33 mg, 55%) as a white solid: ^{1}H NMR (CD_3OD) δ 8.31 (2H, s), 8.05–8.11 (2H, m), 7.63–7.70 (2H, m), 7.18–7.26 (1H, m), 6.95–7.03 (2H, m), 6.83–6.92 (1H, m), 4.23 (2H, ABq, J_{ab} = 15 Hz), 3.69–3.92 (4H, m), 2.09–2.27 (2H, m), 1.48–1.78 (3H, m), 0.78–0.96 (6H, m); high-resolution FAB MS *m/z* found 520.2233 (MH^+), $C_{29}H_{31}FN_3O_5$ requires 520.2248.

***N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-[(4-sulfamoylphenyl)methyl]-L-leucinamide, Trifluoroacetate (72).** Reaction of 4-(aminoethyl)benzenesulfonamide (57 mg, 0.26 mmol) with **28** (100 mg, 0.21 mmol) using method I (above) with stirring for 72 h and purification of the product by reverse-phase HPLC gave *N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-[(4-sulfamoylphenyl)methyl]-L-leucinamide, trifluoroacetate (104 mg, 76%) as a white solid. Hydrolysis of this intermediate using method F (above) gave **72** (30 mg, 28%) as a white solid: ^{1}H NMR (CD_3OD) δ 8.32 (2H, s), 8.05–8.06 (2H, m), 7.76 (2H, d, J = 6 Hz), 7.64–7.67 (2H, m), 7.37 (2H, d, J = 6 Hz), 4.38 (2H, m), 3.73–3.86 (4H, m), 2.17–2.20 (2H, m), 1.57–1.58 (3H, m), 0.88 (3H, d, J = 6 Hz), 0.85 (3H, d, J = 6 Hz); high-resolution FAB MS *m/z* found 581.2060 (MH^+), $C_{29}H_{33}N_4O_7S$ requires 581.2070.

***N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-(1-(*S*)-phenylethyl)-L-leucinamide, Trifluoroacetate (73).** Reaction of 1-(*S*)-phenylethylamine (0.033 mL, 0.26 mmol) with **28** (100 mg, 0.21 mmol) using method G (above) and purification of the crude product by reverse-phase HPLC gave *N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-(1-(*S*)-phenylethyl)-L-leucinamide, trifluoroacetate (42 mg, 34%) as a white solid. Hydrolysis of this intermediate using method F (above) gave **73** (20 mg, 45%) as a white solid: ^{1}H NMR (CD_3OD) δ 8.28 (2H, s), 8.02–8.07 (2H, m), 7.60–7.66 (2H, m), 7.12–7.26 (4H, m), 6.97–7.03 (1H, m), 4.90 (2H, ABq, J_{ab} = 6 Hz), 3.69–3.79 (3H, m), 3.56–3.65 (1H, m), 1.96–2.11 (2H, m), 1.55–1.75 (3H, m), 1.33 (3H, d, J = 9 Hz), 0.90–0.91 (3H, d, J = 6 Hz), 0.88 (3H, d, J = 6 Hz); high-resolution FAB MS *m/z* found 516.2487 (MH^+), $C_{30}H_{34}N_3O_5$ requires 516.2498.

***N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-(1-(*R*)-phenylethyl)-L-leucinamide, Trifluoroacetate (74).** Reaction of 1-(*R*)-phenylethylamine (0.033 mL, 0.26 mmol) with **28** (100 mg, 0.21 mmol) using method G (above) and purification of the crude product by reverse-phase HPLC gave *N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-(1-(*R*)-phenylethyl)-L-leucinamide, trifluoroacetate as a white solid. Hydrolysis of this intermediate using method F (above) gave **74** (42 mg, 31%) as a white solid: ^{1}H NMR (CD_3OD) δ 8.33 (2H, s), 8.07–8.10 (2H, m), 7.65–7.69 (2H, m), 7.10–7.25 (5H, m), 4.97–4.99 (2H, m), 3.72–3.95 (4H, m), 2.16–2.27 (2H, m), 1.46–1.71 (3H, m), 1.42 (3H, d, J = 6 Hz), 0.83 (3H, d, J = 6 Hz), 0.75 (3H, d, J = 6 Hz); high-resolution FAB MS *m/z* found 516.2501 (MH^+), $C_{30}H_{34}N_3O_5$ requires 516.2498.

***N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucine, 2-(*R*)-(Pyridin-3-yl)pyrrolidine Amide, Trifluoroacetate and *N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucine, 2-(*S*)-(Pyridin-3-yl)pyrrolidine Amide, Trifluoroacetate (75 and 76).** Reaction of 3-(pyrrolidin-2-yl)pyridine (50 mg, 0.34 mmol) with **28** (103 mg, 0.22 mmol) using method J (above) gave crude *N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucine, 2-(pyridin-3-yl)pyrrolidine amide (145 mg) as a dark solid. Hydrolysis of this intermediate using method F (above) and purification by reverse-phase HPLC using a 15–80% CH_3CN in H_2O gradient over 30 min gave two fractions eluting at 11.78 and 12.03 min, which yielded two diastereoisomers as white solids upon lyophilization. **75** (25 mg, 15%, 11.78-min peak): ^{1}H NMR (CD_3OD) δ 8.77–8.78 (1H, m), 8.62–8.63 (1H, m), 8.41 (2H, s), 8.15–8.19 (2H, m), 7.82–7.86 (1H, m), 7.74–7.77 (2H, m), 5.21–5.24 (1H, m), 4.21–4.26 (1H, m), 3.69–4.04 (5H, m), 2.47–2.54 (1H, m), 1.74–2.30 (8H, m), 1.02–1.07 (6H, m); high-resolution FAB MS *m/z* found 543.2612 (MH^+), $C_{31}H_{35}N_4O_5$ requires 543.2607. **76** (27 mg, 16%, 12.03-min peak): ^{1}H NMR (CD_3OD) δ 8.65–8.70 (2H, m), 8.39 (2H, s), 8.29–8.37 (1H, m), 8.13–8.17 (2H, m), 7.73–7.88 (2H, m), 5.24 (1H, t, J = 6 Hz), 4.32 (1H, t, J = 6 Hz), 3.82–4.05 (5H, m), 2.51–2.57 (1H, m), 2.26–2.35 (2H, m), 2.13–2.22 (2H, m), 1.95–2.04 (1H, m), 1.76–1.81 (3H, m), 1.01–1.06 (6H, m); high-resolution FAB MS *m/z* found 543.2618 (MH^+), $C_{31}H_{35}N_4O_5$ requires 543.2607.

***N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-(1,2,3,4-tetrahydronaphth-1-yl)-L-leucinamide, Trifluoroacetate (77).** Reaction of 1,2,3,4-

tetrahydronaphthalen-1-ylamine (101 mg, 0.55 mmol)[33] with **28** (200 mg, 0.42 mmol) using method J (above) gave crude *N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-(1,2,3,4-tetrahydronaphth-1-yl)-L-leucinamide (300 mg) as an oil. Hydrolysis of this intermediate using method F (above) gave **77** (47 mg, 17%) as a white solid: ^{1}H NMR (CD_3OD) δ 8.43 (2H, s), 8.19 (2H, m), 7.79 (2H, m), 7.07–7.37 (4H, m), 5.07–5.2 (1H, m), 3.82–4.12 (4H, m), 2.80 (2H, m), 2.33 (2H, m), 1.67–2.10 (7H, m), 1.05 (6H, m); high-resolution FAB MS *m/z* found 542.2655 (MH^+), $C_{32}H_{36}N_3O_5$ requires 542.2655.

***N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucine, 1*H*-3,4-Dihydroisoquinoline Amide, Trifluoroacetate (78).** Reaction of 1,2,3,4-tetrahydroisoquinoline (0.063 mL, 0.5 mmol) with **28** (200 mg, 0.42 mmol) using method J (above) gave crude *N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]-isoindol-2-yl)propyl]-L-leucine, 1*H*-3,4-dihydroisoquinoline amide (274 mg) as a white solid. Hydrolysis of this intermediate using method F (above) gave **78** (51 mg, 19%) as a colorless oil: ^{1}H NMR (CD_3OD) δ 8.33 (2H, s), 8.11 (2H, m), 7.72 (2H, m), 7.13 (4H, m), 4.56–4.84 (4H, m), 3.60–4.24 (6H, m), 2.78–3.08 (2H, m), 2.16–2.42 (2H, m), 1.64–1.96 (3H, m), 1.00 (6H, m); high-resolution FAB MS *m/z* found 528.2510 (MH^+), $C_{31}H_{34}N_3O_5$ requires 528.2498.

***N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*-(phenylmethyl)-*N'*-methyl-L-leucinamide, Trifluoroacetate (79).** Reaction of benzylmethylamine (61 mg, 0.5 mmol) with **28** (200 mg, 0.42 mmol) using method J (above) gave crude 4-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)-2-(*R*)-[[3-methyl-1-(*S*)-[[(benzylmethyl)amino]carbonyl]-butyl]amino]butanoic acid, 1,1-dimethylethyl ester (280 mg) as a white solid. Hydrolysis of this intermediate using method F (above) gave **79** (104 mg, 39%) as a white solid: ^{1}H NMR (CD_3OD) (rotameric mixture) δ 8.33 (minor) and 8.35 (major) (2H, s), 8.12 (2H, m), 7.72 (2H, m), 7.15–7.38 (5H, m), 4.76–4.38 (3H, m), 3.77–4.05 (3H, m), 3.01 (minor) and 3.04 (major) (3H, s), 2.17 (minor) and 2.30 (major) (2H, m), 1.67–1.94 (3H, m), 0.86–0.97 (minor) and 1.00 (major) (6H, m); high-resolution FAB MS *m/z* found 516.2491 (MH^+), $C_{30}H_{34}N_3O_5$ requires 516.2498.

***N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucine, 4-Methylpiperazine Amide, Trifluoroacetate (80).** Reaction of 4-methylpiperazine (0.055 mL, 0.5 mmol) with **28** (200 mg, 0.42 mmol) using method J (above) gave crude *N*-[1-(*R*)-[(1,1-dimethylethoxy)carbonyl]-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-L-leucine, 4-methylpiperazine amide (244 mg) as a white solid. Hydrolysis of this intermediate using method F (above) gave **80** (175 mg, 58%) as a white solid: ^{1}H NMR (CD_3OD) δ 8.36 (2H, s), 8.13 (2H, m), 7.73 (2H, m), 4.52 (1H, m), 3.20–4.25 (11H, br m), 2.97 (3H, s), 2.30 (2H, m), 1.80 (3H, m), 1.02 (6H, m); high-resolution FAB MS *m/z* found 495.2598 (MH^+), $C_{27}H_{35}N_4O_5$ requires 495.2607.

***N*-[1-(*R*)-Carboxy-3-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]isoindol-2-yl)propyl]-*N'*,*N'*-dimethyl-L-leucinamide, Trifluoroacetate (81).** Reaction of dimethylamine (0.16 mL of a 5.6 M solution in EtOH, 0.88 mmol) with **28** (103 mg, 0.22 mmol) using method J (above) gave 4-(1,3-dihydro-1,3-dioxo-2*H*-benz[*f*]-isoindol-2-yl)-2-(*R*)-[[3-methyl-1-(*S*)-[(dimethylamino)carbonyl]-butyl]amino]butanoic acid, 1,1-dimethylethyl ester, trifluoroacetate (61 mg, 56%) as a white solid after purification by reverse-phase HPLC. Hydrolysis of this intermediate using method F (above) gave **81** (35 mg, 57%) as a white solid: ^{1}H NMR (CD_3OD) δ 8.31 (2H, s), 8.04–8.08 (2H, m), 7.63–7.68 (2H, m), 4.38 (1H, t, J = 6 Hz), 3.82–3.89 (3H, m), 3.03 (3H, s), 2.88 (3H, s), 2.15–2.23 (2H, m), 1.64–1.73 (3H, m), 0.90–0.94 (6H, m); high-resolution FAB MS *m/z* found 440.2188 (MH^+), $C_{24}H_{30}N_3O_5$ requires 440.2185.

Biological Methods. K_i values were determined using the chromogenic substrate Ac-Pro-Leu-Gly-Ψ[CO-S]-Leu-Leu-Gly-OC_2H_5 (TES)[34] (Bachem Inc., Torrance, CA) for collagenase or the fluorogenic substrates Dnp-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(Nma)-NH_2 for gelatinase[35] and Dnp-Pro-Gln-Gln-Phe-Lys-Arg-Lys(Nma)-NH_2 for stromelysin. Assays were conducted in a total volume of 0.3 mL of assay buffer (200 mM NaCl, 50 mM Tris, 5 mM $CaCl_2$, 20 μM $ZnSO_4$, 0.05% Brij 35, pH 7.6) in either black (gelatinase and stromelysin) or clear (collagenase) 96-well microtiter plates. Collagenase, gelatinase, and stromelysin concentrations were adjusted to 3 nM, 1 nM, and 10 nM, respectively. Inhibitors were diluted from 10 000 nM to 0.057 nM by serial 3-fold dilution. The fluorogenic assays with gelatinase and stromelysin were initiated with substrate addition (10 μM final concentration), and the product formation was measured at EX_{365}/EM_{450} nm after 40 min using a Baxter (FCA) fluorescence analyzer. TES assays were initiated with TES (100 mM final concentration) in the presence of 1 mM DTNB (5,5'-dithiobis(2-nitrobenzoic acid)), and the rate of product formation was monitored at 405 nm with a Molecular Devices THERMOmax plate reader. Under the above conditions, the substrate concentrations are $<<< K_m$ and the K_i can be determined directly by plotting percent inhibition versus the log of the inhibitor concentration.

Stability of the inhibitors in 20 mM sodium phosphate buffer (pH 6.8) was monitored by HPLC (Vydac, C18, 5 μm, 4.1 × 250 mm) using a 5–60% acetonitrile gradient containing 0.1% TFA. Each inhibitor was adjusted to 20 μM in the phosphate buffer and placed in an autosampler at 30 °C; 200-μL aliquots were injected every 3 h over a 24-h period. The compounds and associated degradation products were monitored at 214 nm. The half-life of each compound was determined by plotting the log (percent remaining) versus time.

Supplementary Material Available: Analytical HPLC analyses and proton NMR spectra are available for all compounds for which biological data were obtained (64 pages). Ordering information is given on any current masthead page.

References

(1) Woessner, J. F. Matrix Metalloproteinases and their Inhibitors in Connective Tissue. *FASEB J.* **1991**, *5*, 2145–2154.

(2) Gravallese, E. M.; Darling, J. M.; Ladd, A. L.; Katz, J. N.; Glimcher, L. H. In Situ Hybridization Studies of Stromelysin and Collagenase Messenger RNA Expression in Rheumatoid Synovium. *Arthritis Rheum.* **1991**, *34*, 1076–1084.

(3) McCachren, S. S. Expression of Metalloproteinases and Metalloproteinase Inhibitors in Human Arthritic Synovium. *Arthritis Rheum.* **1991**, *34*, 1085–1093.

(4) Firestein, G. S.; Paine, M. M.; Littman, B. H. Gene Expression (Collagenase, Tissue Inhibitor of Metalloproteinase, Complement, and HLA-DR) in Rheumatoid Arthritic and Osteoarthritic Synovium. *Arthritis Rheum.* **1991**, *34*, 1094–1105.

(5) Harper, G. S. Use of Collagenase Inhibitors in the Treatment of Demyelinating Diseases. European Patent 423,943, 1991.

(6) Liotta, L. A.; Trygvasson, K.; Garbisa, S.; Roby, P. G.; Abe, S. Partial Purification and Characterization of a Neutral Protease Which Cleaves Type IV Collagen. *Biochemistry* **1981**, *20*, 100–104.

(7) Wilhelm, S. M.; Collier, I. E.; Marmer, B. L.; Eisen, A. Z.; Grant, G. A.; Goldberg, G. I. SV40-Transformed Human Lung Fibroblasts Secrete a 92-kDa Type IV Collagenase Which is Identical to that Secreted by Normal Human Macrophages. *J. Biol. Chem.* **1989**, *264*, 17213–17221.

(8) Basset, P.; Bellocq, J. P.; Wolf, C.; Stott, I.; Hutin, P.; Limacher, J. M.; Podhajcer, O. L.; Chinard, M. P.; Rio, M. C.; Chambon, P. A Novel Metalloproteinase Gene Specifically Expressed in Stromal Cells of Breast Carcinomas. *Nature* **1991**, *348*, 699–704.

(9) Goldberg, G. I. Tissue Inhibitor of Metalloproteinase (TIMP-2). European Patent 404,750, 1990.

(10) (a) Johnson, W. H.; Roberts, N. A.; Borkakoki, N. Collagenase inhibitors: Their Design and Potential Therapeutic Use. *J. Enzyme Inhib.* **1987**, *2*, 1–22. (b) Henderson, B.; Docherty, A. J. P.; Beeley, N. R. A. Design of Inhibitors of Articular Cartilage Destruction. *Drugs Future* **1990**, *15*, 495–508. (c) Schwartz, M. A.; Van Wart, H. E. Synthetic Inhibitors of Bacterial and Mammalian Interstitial Collagenases. In *Progress in Medicinal Chemistry*; Ellis, G. P., Luscombe, D. K., Eds.; Elsevier Science Publishers B.V.: The Netherlands, 1992; Vol. 29. (d) Emonard, H.; Grimaud, H. Matrix Metalloproteinases. A Review. *Cell. Mol. Biol.* **1990**, *36*, 131–153. (e) Wahl, R. C.; Dunlap, R. P.; Morgan, B. P. Biochemistry and Inhibition of Collagenase and Stromelysin. In *Annual Reports in Medicinal Chemistry*; Academic Press Inc.: New York, 1989; Vol. 25. (f) Rich, D. H. Peptidase Inhibitors. In *Comprehensive Medicinal Chemistry*; Hansch, C., Sammes, P. G., Taylor, J. B., Eds.; Pergamon Press: New York, 1990.

(11) (a) Darlak, K.; Moiller, R. B.; Stack, S.; Spatola, A. F.; Gray, R. D. Thiol-based Inhibitors of Mammalian Collagenase. *J. Biol. Chem.* **1990**, *265*, 5199–5205. (b) Donald, D. K.; Hann, M. M.; Saunders, J.; Wadsworth, H. J. Thiol Based Collagenase Inhibitors. United States Patent 4,595,700, 1986. (c) Markwell, R. E.; Smith, S. A.;

Hughes, I. Derivatives of N-(2-alkyl-3-mercapto-glutaryl) alpha-amino acids and their use as Collagenase Inhibitors. European Patent 273,689, 1988. (d) Hughes, I. Novel Compounds. Australian Patent 8936675, 1990. (e) Markwell, R. E.; Hughes, I. Novel Compounds. European Patent 438,223, 1991. (f) Sundeen, J. E.; Dejneka, T. Inhibitors of Mammalian Collagenase. United States Patents 4,297,275, 4,263,293, 4,235,885; European Patent 67,429, 1981.

(12) (a) Odake, S.; Okayama, T.; Obata, M.; Morikawa, T.; Hattori, S.; Hori, H.; Yutaka, N. Vertebrate Collagenase Inhibitor. I. Tripeptidyl Hydroxamic Acids. *Chem. Pharm. Bull.* **1990**, *38*, 1007–1011; Vertebrate Collagenase Inhibitor. II. Tetrapeptidyl Hydroxamic Acids. *Chem. Pharm. Bull.* **1991**, *39*, 1489–1494. (b) Grobelny, D.; Poncz, L.; Galardy, R. E. Inhibition of Human Skin Fibroblast Collagenase, Thermolysin and Pseudomonas Aeruginosa Elastase by Peptide Hydroxamic Acids. *Biochemistry* **1992**, *31*, 7152–7154. (c) Dickens, J. P.; Donald, D. K.; Kneen, G.; McKay, W. R. Hydroxamic Acid Based Collagenase Inhibitors. European Patent 214,639, 1987. (d) Spilburg, C. A.; Moore, W. M. Method of Inhibiting Vertebrate Collagenase Activity Using Peptide Hydroxamic Acid Derivatives. United States Patent 4,720,486, 1988. (e) Beckett, R. P.; Crimmin, M. J.; Davidson, A. H. Hydroxamic Acids Derivatives, Process for their Preparation and Use Thereof. European Patent 498,665, 1992. (f) Campion, C.; Davidson, A.; Dickens, J. P.; Crimmin, M. J. Hydroxamic Acid Based Collagenase Inhibitors. World Patent 91/02716, 1991. (g) Davidson, A.; Dickens, J. P.; Crimmin, M. J. Hydroxamic Acid Based Collagenase Inhibitors. World Patent 90/05716, 1990. (h) Campion, C.; Davidson, A.; Dickens, J. P.; Crimmin, M. J. Hydroxamic Acid Based Collagenase Inhibitors. World Patent 90/05719, 1990. (i) Porter, J. R.; Morphy, J. R.; Millican, T. A.; Beeley, N. R. Peptidyl Derivatives. World Patent 92/09565, 1992. (j) Galardy, R. E.; Grobelny, D. Improved Matrix Metalloproteinase Inhibitors. World Patents 92/09556, 92/09563, 92/09282, 1992. (k) Handa, B. K.; Johnson, W. H.; Machin, P. J. European Patent 236,872. (l) Tanzawa, K.; Ogita, T.; Takahashi, S.; Kinoshita, T.; Haruyama, H.; Kagasaki, T.; Okazaki, T.; Enokita, R. Novel Compound Matristatin. World Patent 91/17982, 1991.

(13) (a) McCullagh, K.; Wadsworth, H.; Hann, M. Carboxyalkyl Peptide Derivatives. European Patent 126,974, 1984. (b) McCullagh, K.; Wadsworth, H.; Hann, M. Carboxyalkyl Peptide Derivatives. European Patent 159,396, 1985.

(14) Broadhurst, M. J.; Handa, B. K.; Johnson, W. H.; Lawton, G.; Machin, P. J. Phosphinic Acid Derivatives. European Patent 276,-436, 1988.

(15) (a) Hunter, D. J.; Markwell, R. E.; Ward, R. W. Peptides with Collagenase Inhibiting Activity. European Patent 320,118, 1989. (b) Markwell, R. E.; Rahman, S. S.; Ward, R. W. Phosphonopeptides with Collagenase Inhibiting Activity. World Patent 91/15506, 1991. (c) Markwell, R. E.; Ward, R. W.; Ratcliffe, S. J. Phosphonopeptides with Collagenase Inhibiting Activity. World Patent 91/15507, 1991.

(16) Kortylewicz, Z. P.; Galardy, R. E. Phosphonamidate Peptide Inhibitors of Human Skin Fibroblast Collagenase. *J. Med. Chem.* **1990**, *33*, 263–273.

(17) Springman, E. B.; Angleton, E. L.; Birkedal-Hansen, H.; Van Wart, H. Multiple Modes of Activation of Latent Human Fibroblast Collagenase: Evidence for the Role of a Cys-73 Active-site Zinc Complex in Latency and a "Cysteine Switch" Mechanism for Activation. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, 87, 356–368.

(18) (a) Matthews, B. W. Structural Basis of the Action of Thermolysin and Related Zinc Peptidases. *Acc. Chem. Res.* **1988**, *21*, 333–340. (b) Bartlett, P. A.; Marlowe, C. K. Phosphonamidates as Transition-state Analogue Inhibitors of Thermolysin. *Biochemistry* **1983**, *22*, 4618–4624.

(19) Bode, W.; Gomis-Rueth, F. X.; Huber, R.; Zwilling, R.; Stoecker, W. Structure of Astacin and Implications for Activation of Astacins and Zinc-ligation of Collagenases. *Nature* **1992**, *358*, 164–167.

(20) (a) Ghosh, S. S.; Mobashery, S. N-Acyl Peptide Metalloendopeptidase Inhibitors and Methods of Using the Same. World Patent 91/05555, 1991. (b) Ghosh, S. S. Dipeptide-analog-based Metalloendopeptidase Inhibitors and Methods of Using the Same. World Patent 91/05763, 1991.

(21) Wallace, D. A.; Bates, S. R. E.; Walker, B.; Kay, G.; White, J.; Guthrie, J. S.; Blumsom, N. L.; Elmore, D. T. Competitive Inhibition of Human Skin Collagenase by N-benzyloxycarbonyl-L-prolyl-L-alanyl-3-amino-oxopropyl-L-leucyl-L-alanylglycine Ethyl ester. *Biochem. J.* **1986**, *239*, 797–799.

(22) (a) Bols, M. Inhibition of Collagenase by Aranciamycin and Aranciamycin Derivatives. *J. Med. Chem.* **1992**, *35*, 2768–2771. (b) Golub, L. M.; McNamara, T. F.; D'Angelo, G.; Greenwald, R. A.; Ramamurthy, N. S. A Non-bacterial Chemically-modified Tetracycline Inhibits Mammalian Collagenase Activity. *J. Dent. Res.* **1987**, *66*, 1310–1314. (c) Tanaka, T.; Metori, K.; Mineo, S.; Matsumoto, H.; Satoh, T. Studies on Collagenase Inhibitors. II. Inhibitory Effects of Anthraquinones on Bacterial Collagenase. *J. Pharm. Soc. Jpn.* **1990**, *110*, 688–692. (d) Greenwald, R. A.; Moak, S. A.; Ramamurthy, N. S.; Golub, L. M. Tetracyclines Suppress Matrix Metalloproteinase Activity in Adjuvant Arthritis and in Combination with Flurbiprofen, Ameliorate Bone Damage. *J. Rheumatol.* **1992**, *19*, 927–938.

(23) Angiotensin Converting Enzyme Inhibitors. Kostis, J. B., DeFelice, E. A., Eds.; Alan R. Liss Inc.: New York, 1987; ISBN 0-8451-4240-2.

(24) (a) Keay, B. A.; Lee, D. K. W.; Rodrigo, R. A New Method for the Generation of Isobenzofurans: A Simple Entry to Substituted Naphthalenes. *Tetrahedron Lett.* **1980**, *21*, 3663–3666. (b) Keay, B. A.; Plaumann, H. P.; Rajapaksa, D.; Rodrigo, R. Hydroxyacetals, Phthalans, and Isobenzofurans Therefrom. *Can. J. Chem.* **1983**, *61*, 1987–1995.

(25) Mirsadeghi, S.; Rickborn, B. 1-Methoxyisobenzofuran from Base-Induced and Acid-Catalyzed Reactions of 1,3-Dihydro-1,3-dimethoxyisobenzofuran. *J. Org. Chem.* **1987**, *52*, 787–792.

(26) Los, M. Herbicidal 2-(2-imidazolin-2-yl)pyridine Derivatives. GB 2174395A, 1986.

(27) Albert, R.; Danklmaier, J.; Hoenig, H.; Kandolf, H. A Simple and Convenient Synthesis of β-Aspartates and γ-Glutamates. *Synthesis* **1987**, 635–637.

(28) Grehn, L.; Lurdes, M.; Almeida, S.; Raguarsson, U. Convenient Preparation of Alkyl Benzyl Imidodicarbonates, Useful Reagents for the Direct Synthesis of Protected Amines. *Synthesis* **1988**, 992–994.

(29) Dallacker, F.; Schmets, G. Derivatives of (methylenedioxy)benzene. 34. Dimethoxy(methylenedioxy)benzene Dicarboxylic acids. *Chem. Ber.* **1971**, *104*, 2534–2544.

(30) Kanevskaya, S. I.; Brasyunas, V. B. Derivatives of Dicarboxylic Acids. I. Alkylation of Diethyl Ester of 4-hydroxyphthalic Acids. *Zh. Obsch. Khim.* **1959**, *29*, 1930–1932.

(31) Elks, J.; Hey, D. H. Union of Aryl Nuclei. Part VI. Reactions With 1-Aryl-3,3-dimethyltriazens. *J. Chem. Soc.* **1943**, 441–445.

(32) Moderhack, D.; Goos, K. H. 5-(Diazomethyl)tetrazoles Substituted in Position 1 or 2. *Chem. Ber.* **1987**, *120*, 921–930.

(33) Ghislandi, V.; Vercesi, D. Optical Resolution and Conflguration of α-Cyclohexylbenzylamine, 1,2,3,4-Tetrahydro-1-naphthylamine, and 1,2,3,4-Tetrahydro-2-naphthylamine. *Farmaco, Ed. Sci.* **1971**, *26*, 474–486.

(34) Weingarten, H.; Martin, R.; Feder, J. Synthetic Substrates of Vertebrate Collagenase. *Biochemistry* **1985**, *24*, 6730–6734.

(35) Bickett, D. M.; Green, M. D.; Berman, J.; Dezube, M.; Howe, A. S.; Brown, P. J.; Roth, J. T.; McGeehan, G. M. A High Throughput Fluorogenic Substrate for Interstitial Collagenase (MMP-1) and Gelatinase (MMP-9). *Anal. Biochem.* **1993**, *212*, 58–64.