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INHIBITION OF STROMELYSIN-1 (MMP-3) BY PEPTIDYL PHOSPHINIC ACIDS

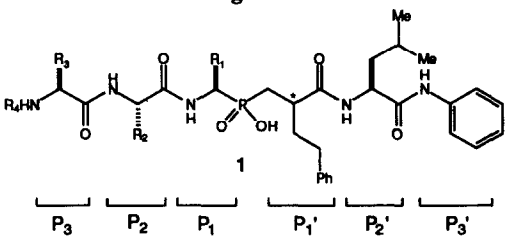
Joung L. Goulet,*† Joanne F. Kinneary,† Philippe L. Durette,† Ross L. Stein,‡• Richard K. Harrison,‡ Maria Izquierdo-Martin,‡ David W. Kuo,‡ Tsau-Yen Lin,‡° William K. Hagmann†

Departments of Medicinal Chemical Research† and Enzymology‡
Merck Research Laboratories
P.O. Box 2000, Rahway, New Jersey 07065-0900

Abstract: A series of phosphinic acid-containing peptide inhibitors of human stromelysin-1 (MMP-3) were prepared. The P_1 through P_3 subsites were varied in a systematic manner on analogs possessing an invariant P_1'-P_3' segment. The *in vitro* activity of these compounds as inhibitors of stromelysin and collagenase is discussed.

Stromelysin-1 (MMP-3) is a member of the family of zinc-containing, calcium dependent, matrix metalloproteinases and is thought to play a major role in the destruction of connective tissue components of articular cartilage.[1] Stromelysin is an attractive target for potential therapeutic intervention of rheumatoid and osteoarthritis. Our goal has been to develop potent and selective inhibitors of this enzyme. Based on other successful inhibitor designs for Zn^{2+} binding enzymes, e.g. angiotensin converting enzyme (ACE) and collagenase,[2] peptidyl phosphinic acids were chosen as one of our approaches to inhibit stromelysin. A general structure of the phosphinic acid (**1**) is shown in Figure 1. Our objective in this series of analogs was to investigate the structure-activity relationship of the P_1-P_3 sites as a means of improving potency and enzyme selectivity.

Figure 1



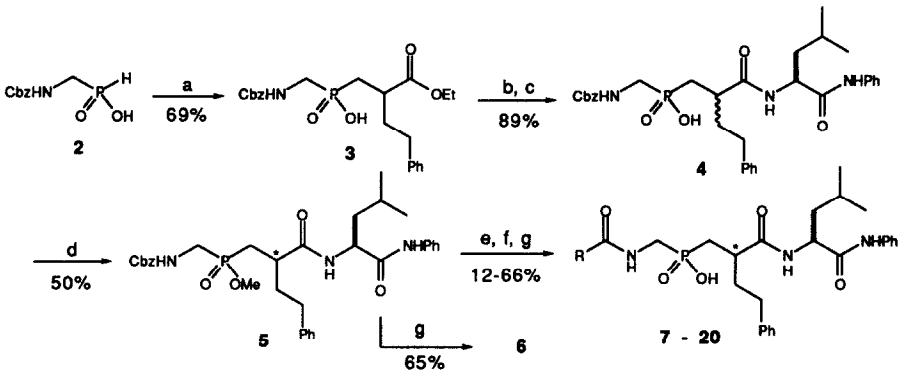
Chemistry

Based on our studies of the P_1'-P_3' residues in a related series of inhibitors,[3] these residues were kept constant as shown in Figure 1. This allowed us to focus on the systematic modification of P_1-P_3 sites. For the synthesis of an analog devoid of substitution at P_1, the aminomethylphosphonous acid (**2**) was prepared by modification of a known procedure.[4] As illustrated in Scheme 1, Michael addition of **2** to ethyl 2-(2-phenylethyl)acrylate was achieved *via* a silyl phosphonite intermediate[5] to give adduct **3**. Hydrolysis of the ethyl ester was followed by coupling with L-leucine anilide to give **4** as a mixture of diastereomers. Conversion of acid **4** to the methyl phosphinate derivative allowed for the separation of diastereomers at the carbon stereogenic center by silica gel flash chromatography. Hydrolysis and biological evaluation of each diastereomer revealed that the more polar (TLC, silica gel) isomer of **5** contained the proper configuration for the inhibition of stromelysin.

• Present address: Mycogenics, Inc. 61 Moulton Road, Cambridge, MA 02139
° Present address: Cal-Test Diagnostics/E 9365 Wickhan Way, Orlando, FL 32836

Hydrogenolysis of **5** followed by coupling with various amino acids under the standard conditions gave the corresponding homologated methyl phosphinates. Hydrolysis of these compounds provided analogs **7** to **20**.

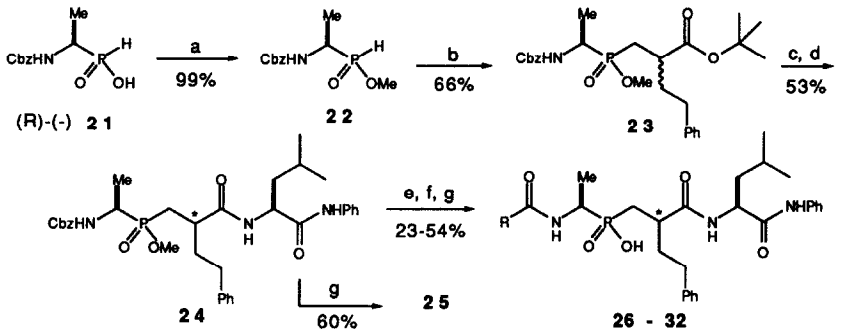
Scheme 1



a) TMSCl, Et_3N, ethyl 2-(2-phenylethyl)acrylate; b) NaOH, EtOH, H_2O; c) LeuNHPh, carbonyldiimidazole (CDI), THF; d) i) CH_2N_2/Et_2O, CH_2Cl_2, 0°C, ii) separate diastereomers; e) H_2, $Pd(OH)_2/C$, AcCl, MeOH; f) RCO_2H, HOBt, NMM, EDC, CH_2Cl_2, 0°C → rt; g) TMSBr, CH_2Cl_2 or 2N NaOH, MeOH or TFA, CH_2Cl_2

For substitution at the P_1 site, the aminoethylphosphonous acid (**21**) was synthesized and resolved according to literature procedures (Scheme 2).[6] Surprisingly, in this instance, the silyl phosphonite-mediated Michael addition[5] of **21** to ethyl 2-(2-phenylethyl)acrylate proved unsatisfactory. The phosphinic acid **21** was instead converted to methyl phosphinate **22**, which was deprotonated with sodium methoxide and treated with *tert*-butyl 2-(2-phenylethyl)acrylate to give the desired adduct **23**.[7] Cleavage of the *tert*-butyl ester was followed by coupling with L-leucine anilide and treatment with diazomethane. Separation of diastereomers at the carbon stereogenic center was accomplished by flash chromatography and, as above, it was determined that the more polar isomer **24** was the active isomer. Analogs **26** to **32** were prepared following a similar procedure as described for **7** to **20**.

Scheme 2



a) CH_2N_2/Et_2O, CH_2Cl_2, 0°C; b) NaOMe, MeOH, *t*-butyl 2-(2-phenylethyl)acrylate, 0°C → rt; c) TFA, CH_2Cl_2, 0°C → rt; d) i) LeuNHPh, CDI, THF, ii) CH_2N_2, iii) separate diastereomers; e) H_2, $Pd(OH)_2/C$, AcCl, MeOH; f) RCO_2H, HOBt, NMM, EDC, THF, 0°C → rt; g) TMSBr, CH_2Cl_2 or 2N NaOH, MeOH

Results and Discussion

The *in vitro* activities of the phosphinic acid analogs shown in the Table demonstrate that we were able to prepare inhibitors that are selective for human stromelysin versus collagenase. For the inhibition of stromelysin, methyl substitution at the P_1 site (**25**) was found to be similar in potency to the unsubstituted analog (**6**). This is consistent with a stromelysin substrate specificity study reported by Niedzwiecki and co-workers.[8a] Therefore, most of the analogs designed to probe the P_2 site SAR were prepared from compound **6**. The activity of these analogs exhibited little dependence on the nature of the P_2 substituent. Compounds with hydrophobic,

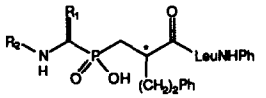


Table Inhibition of Stromelysin and Collagenase by Phosphinic Acid-Containing Peptides

Compound	R_1	R_2	SLN[a] (K_i, μM)	CGASE[a] (K_i, μM or %inh. at 10μM)
6	H	Cbz	0.28	15.9
7	H	PhtGly	0.41	14 @ 10
8	H	Bz	1.9	0 @ 10
9	H	CbzAla	0.28	0 @ 10
10	H	CbzNorLeu	1.3	0 @ 10
11	H	CbzPhe	0.71	4 @ 10
12	H	CbzGly	1.4	4 @ 10
13	H	CbzTyr	0.56	0 @ 10
14	H	CbzHis	0.21	9 @ 10
15	H	CbzAsn	0.6	14 @ 10
16	H	CbzLys	4.6	18 @ 10
17	H	CbzGlu	0.37	12 @ 10
18	H	$PhCH_2CO$	0.94	0 @ 10
25	Me	Cbz	0.33	7.5
26	Me	PhtGly	2.4	0 @ 10
27	Me	Bz	1.9	7 @ 10
28	Me	AcAla	0.34	0 @ 10
29	Me	AcPro	57% I @ 5μM	0 @ 10
19	H	AcProAla	0.015	60 @ 10
20	H	BzProAla	0.024	39 @ 10
30	Me	AcProAla	0.0061	2.9
31	Me	BzProAla	0.02	5.1
32	Me	AcAlaAla	0.042	32 @ 10

[a] see reference 3 for enzyme inhibition assay conditions.

polar, or charged groups at this site were found to be similar in potency, indicating that a broad tolerance of substitution exists in this region. However, incorporation of proline at the P_2 site (**29**) was detrimental to activity. These results are again consistent with substrate specificity studies determined for stromelysin.[8]

We then extended the peptide chain into the P_3 position. Several stromelysin substrates contain proline at P_3.[8a] The effect of proline at this position in our inhibitors was demonstrated with analogs **19**-**20** and **30**-**31**, which were found to be potent inhibitors of stromelysin and to exhibit good selectivity over collagenase. For example, compound **30** was approximately 500-fold more potent against stromelysin than collagenase.

In conclusion, a series of phosphinic acid-containing peptides was prepared and evaluated for the inhibition of stromelysin and collagenase. We have shown that potent and selective inhibition of stromelysin with these peptidyl phosphinic acids requires extension of substitution into the P_3 position.

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