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INHIBITION OF MATRIX METALLOPROTEINASES BY N-CARBOXYALKYL PEPTIDES CONTAINING EXTENDED ALKYL RESIDUES AT P_1'

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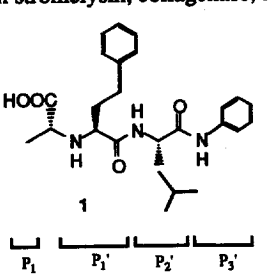
Abstract: A series of N-carboxyalkyl peptides were prepared to test their inhibitory activity against human stromelysin (MMP-3), collagenase (MMP-1), and gelatinase A (MMP-2). Linear alkyl and ω-aminoalkyl residues were employed as replacements for a phenethyl group yielding inhibitors with *in vitro* activities comparable to their corresponding aromatic analogs.

The matrix metalloproteinases (MMP's) are a family of zinc-containing, calcium dependent mammalian proteinases that are capable of degrading the extracellular matrix of connective tissues and basement membranes.[1] These enzymes have been implicated in a variety of biological processes, including degradative diseases such as rheumatoid and osteoarthritis.[2] Earlier work by our group established a structure-activity relationship (SAR) for the inhibition of MMP's by N-carboxyalkyl peptides.[3] This SAR revealed a deep, hydrophobic pocket at the S_1' subsite of stromelysin, a conclusion that has since been confirmed by NMR structure determination.[4] The most potent stromelysin inhibitor to emerge from that effort was [N-1(R)-carboxyethyl)-α-(S)-(phenylethyl)]glycine-(S)-leucine, N-phenylamide, **1**, with a $K_i = 0.47$ μM.

In 1990, Shirota and coworkers demonstrated that a long-chain, ω-aminoalkyl group could be used as a substitute for the phenethyl group in the angiotensin converting enzyme (ACE) inhibitor enalaprilat.[5] Since our lead compound **1** for stromelysin also contained the phenethyl side-chain, we explored a similar replacement in our molecule in an effort to improve its activities against MMP's and selectivity for stromelysin. To that end, we have prepared a series of analogs containing long-chain alkyl and ω-aminoalkyl groups at the P_1' position and measured their activities against human stromelysin, collagenase, and gelatinase A.



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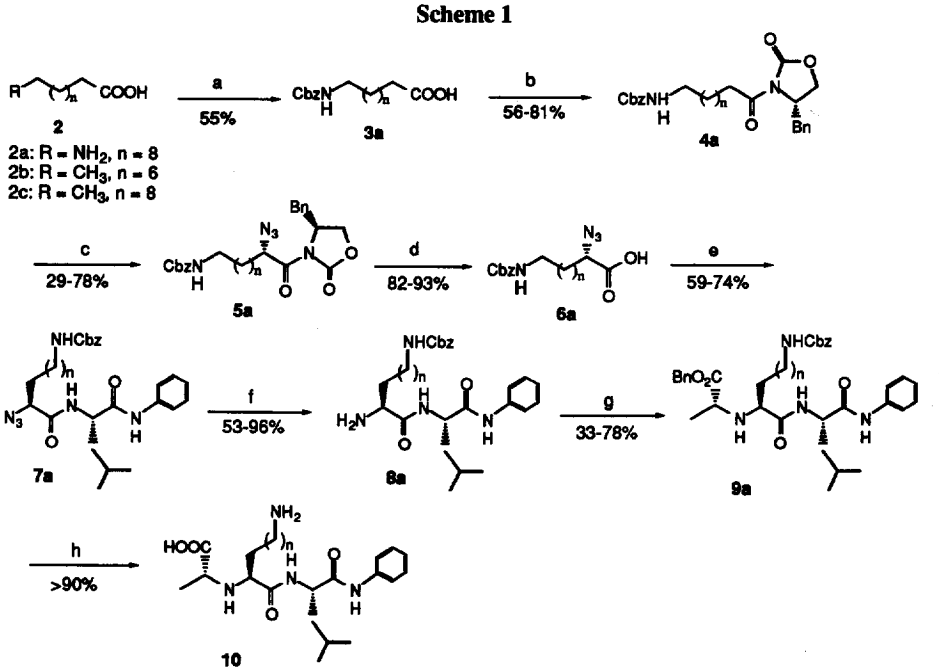
Chemistry

The synthesis of the inhibitors generally followed procedures as described in reference 3. The unnatural amino acids incorporated at the P_1' position were prepared by stereoselective azide transfer methods described by Evans et al.[6] Scheme 1 illustrates the synthesis of [N-1(R)-carboxyethyl)-α-(S)-(9-aminononyl)]glycine-(S)-leucine, N-phenylamide as an example of our typical procedure. Starting with commercially available 11-aminoundecanoic acid **2a**, the amino functionality was protected as its benzyl carbamate (Cbz) to furnish **3a**. Stereoselective introduction of azide to the oxazolidinone **4a**, followed by hydrolysis of the chiral auxiliary afforded the chiral α-azido acid **6a** in moderate yield. Coupling of the α-azido acid **6a** to amino acid anilides (L-leucinanilide, in this case) was accomplished using standard EDC/HOBt conditions.[7] Reduction of the azide in the presence of the Cbz group proceeded smoothly with $SnCl_2$[8] to give the dipeptide **8a**. Displacement of the triflate[9] derived from benzyl (S)-lactate with the dipeptide **8a**, followed by catalytic hydrogenation of the benzyl ester afforded the N-carboxyalkyl peptides listed in Table 1. Analogous procedures were followed for alkyl carboxylates **2b** and **2c**.

Scheme 1



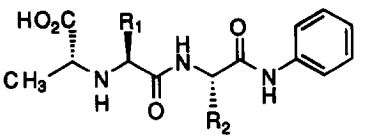
a) benzyl chloroformate, aq. $NaHCO_3$, THF, 0°-25°; b) trimethylacetyl chloride, Et_3N, THF, 0°, (S)-(-)-4-benzyl-2-oxazolidinone, n-BuLi, -78°; c) $KN(SiMe_3)_2$, Trisyl-N_3, -78°, AcOH; d) LiOH, H_2O_2, THF, H_2O; e) (L)-LeuNHPh or diCbz-(L)-ArgNHPh, HOBt, EDC, THF, 25°; f) $SnCl_2$ MeOH, 25°; g) benzyl (S)-lactate, Tf_2O, 2,6-lutidine, $Et(^iPr)_2N$, CH_2Cl_2, 0-25°; h) H_2, $Pd(OH)_2/C$, MeOH.

Results and Discussion

Compounds of the general structure shown below were evaluated *in vitro* against human fibroblast stromelysin (MMP-3), human fibroblast collagenase (MMP-1), and human gelatinase A (MMP-2). The data in Table 1 indicate that stromelysin and gelatinase A can accommodate extended hydrocarbon and ω-amino hydrocarbon chains in their S_1' pockets, while collagenase will not. Activities against stromelysin and gelatinase A were found to be comparable to those of the corresponding inhibitors containing a phenethyl group at the P_1' position.[3] Similar results have been reported with hydroxamic acid inhibitors of MMP's with long-chain alkyl groups at P_1'.[10]

In conclusion, we have demonstrated that linear alkyl chains can be substituted for a hydrophobic phenethyl group at P_1' in this class of MMP inhibitors without adversely affecting the potency against stromelysin and gelatinase A. Subsequent structural determination of stromelysin has revealed a deep, hydrophobic pocket at S_1' that extends completely through the enzyme that could easily accommodate these extended alkyl groups.[4] The X-ray crystal structure of human fibroblast collagenase indicates a much smaller S_1' specificity pocket for this enzyme compared to stromelysin.[11] The loss of activity versus fibroblast collagenase with compounds **10-14** can be explained by the inability of this enzyme to fit a long alkyl chain in its much shallower S_1' pocket.

Table 1. Inhibition of Human Stromelysin, Collagenase, and Gelatinase A by N-Carboxyalkyl Peptides with Long Chain Alkyl and Aminoalkyl Substituents at P_1'



Compound Number	R_1 (P_1')	R_2 (P_2')	Stromelysin K_i, μM (± S.E.)	Collagenase K_i, μM (± S.E.)	Gelatinase A K_i, μM (± S.E.)
1	$(CH_2)_2Ph$	i-C_4H_9	0.47 (0.08)	0.76 (0.22)	0.20 (0.04)
10	n-$C_9H_{18}NH_2$	i-C_4H_9	0.24 (0.04)	> 10	0.50 (0.04)
11	n-C_8H_{17}	i-C_4H_9	0.57 (0.05)	>10	0.34 (0.05)
12	n-C_8H_{17}	$(CH_2)_3NHC(NH)NH_2$	1.60 (0.10)	>10	0.12 (0.03)
13	n-$C_9H_{18}NH_2$	$(CH_2)_3NHC(NH)NH_2$	0.37 (0.04)	>10	0.11 (0.01)
14	n-$C_{10}H_{21}$	i-C_4H_9	0.85 (0.06)	>10	0.56 (0.11)

Stromelysin, collagenase, and gelatinase A assays were all performed at pH = 7.5 and 25° C according to the procedures detailed in Reference 3.

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