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Design, Synthesis, and Evaluation of a Pyrrolinone-Based Matrix Metalloprotease Inhibitor

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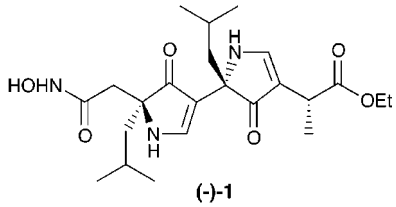
ABSTRACT



(-)-1

A pyrrolinone-based hydroxamate matrix metalloprotease inhibitor, (–)-1, has been designed and synthesized. Enzymatic assay revealed that (–)-1 inhibited three of the ten matrix metalloprotease enzymes examined and as such represents a new, potentially important lead structure.

Research in our laboratory has focused on the development of novel nonpeptidyl scaffolds that mimic β-turns, β-sheets/strands, and helices, the three principle types of secondary structure found in peptides and proteins.[1] In the area of β-sheets/strands we designed the 3,5-linked (nitrogen displaced) pyrrolinone scaffold that directly substitutes on a per residue basis for α-amino acids (except proline and glycine).[2−7] Importantly, this structural motif derived from the D-amino acids maintains both the spacial orientation of the amino acid side chains and the capacity to form intermolecular hydrogen bonds with the receptor or enzyme.[3]

The advantage of nonpeptidyl peptidomimetics, in general, is their ability to resist degradation by proteases and to possess additional favorable pharmacokinetic properties as a result of reduced solvation.[4] These critical features were demonstrated in our research on HIV-1 protease, wherein we designed a pyrrolinone-based inhibitor more potent in

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vitro than Crixivan, against a clinically significant mutant strain, which proved to be orally bioavailable in dogs (13%).[2c] In addition, we designed and synthesized a competent pyrrolinone inhibitor of renin,[2a,5] a high affinity peptide−pyrrolinone hybrid ligand for the class II major histocompatibility complex (MHC) protein HLA-DR1,[6] and most recently a polypyrrolinone β-turn mimic.[7] The latter exploited the D,L-alternating (i.e., heterochiral) polypyrrolinone structural motif. Given these successes, we sought to extend further the scope of the pyrrolinone scaffold. We report here the design, synthesis, and enzymatic evaluation of a pyrrolinone-based matrix metalloprotease inhibitor.

Matrix metalloproteases (MMPs) comprise a family of more than 20 zinc-containing enzymes that are involved in the degradation of extracellular connective tissue.[8] They have been implicated in a number of inflammatory and degenerative diseases including arthritis, multiple sclerosis, Guillian Barre′ syndrome, stroke, and cancer.[9] Thus, this enzyme class represents an attractive target for the design and synthesis of selective, small molecule inhibitors that can modulate the severity of the underlying disease.[10]

The prospective MMP inhibitor **1** (Figure 1) was designed on the basis of the Roche peptidyl inhibitor Ro-31-4724 (**2**), reported to have an IC_{50} of 9 nM against human fibroblast collagenase (MMP-1).[11] The X-ray structure of peptidyl inhibitor **2** cocrystallized with MMP-1 (2.2 Å resolution)[12] clearly indicated that the hydroxamate in the peptidyl inhibitor effectively coordinates to the active-site zinc(II), and that the S1′−S3′ pockets of the enzyme are occupied with isobutyl side chains at P1′ and P2′ and a methyl at P3′. Energy minimization of **1** employing Macromodel 5.0[13] led to a low energy model; the overlay of this model with the Roche inhibitor (**2**) held in the enzyme bound conformation (Figure 1) revealed remarkable similarities, as anticipated from our prior work.[2−7] The backbone, hydroxamate, side chains, and carbonyls all overlaid very well, while some differences were observed at the C-terminus. Not surprisingly, the amide nitrogens of **2** did not overlay with the displaced nitrogens in bispyrrolinone **1**, but the trajectories of the attached hydrogens were quite similar, providing the potential for intermolecular hydrogen bonding with the enzyme. This low energy conformation was then docked into the active site of the MMP-1 enzyme (Figure 2). As anticipated, we observed coordination of the hydroxmate to the active site zinc(II), hydrogen bonding of both the pyrrolinone carbonyls and NH's with the enzyme backbone, and S1′−S3′ occupation with the respective side chains as observed with peptidyl inhibitor **2**.

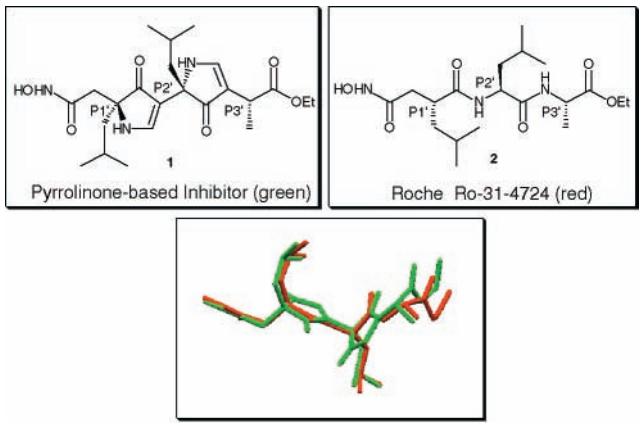

Figure 1. Overlay of active-site conformation of Roche compound Ro-31-4724 (**2**) with model of bispyrrolinone **1**.

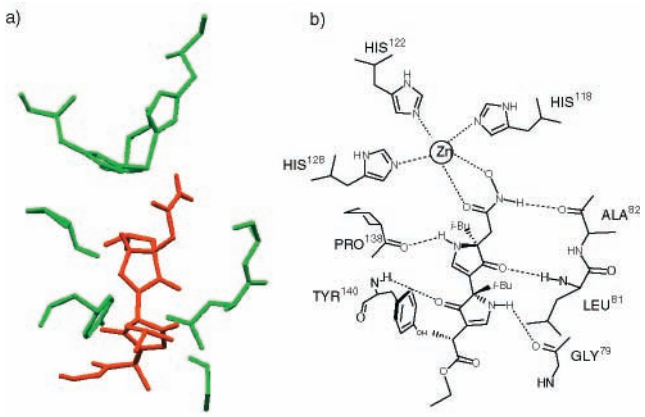

Figure 2. (a) Bispyrrolinone **1** (red) docked into the active site of human fibroblast collagenase (MMP-1) and (b) a cartoon of the active site for clarity.

From the synthetic perspective, we envisioned construction of **1** to exploit our base-mediated pyrrolinone ring synthetic protocol,[2−7] followed by functional group manipulation (Scheme 1). Bispyrrolinone **9** would thus require 2 equiv of known amino ester (−)-**7**[6] and 1 equiv of aldehyde **6**.

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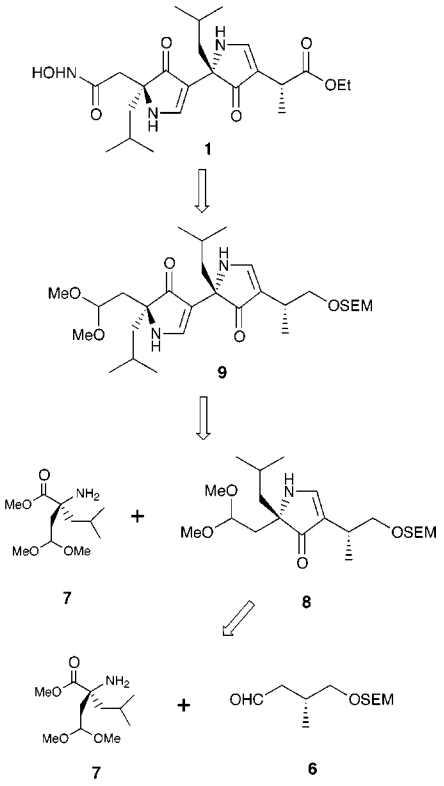
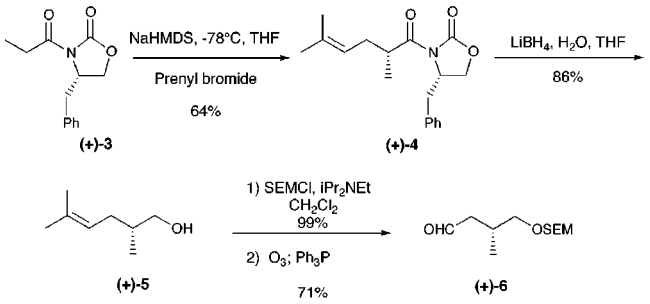
Scheme 1



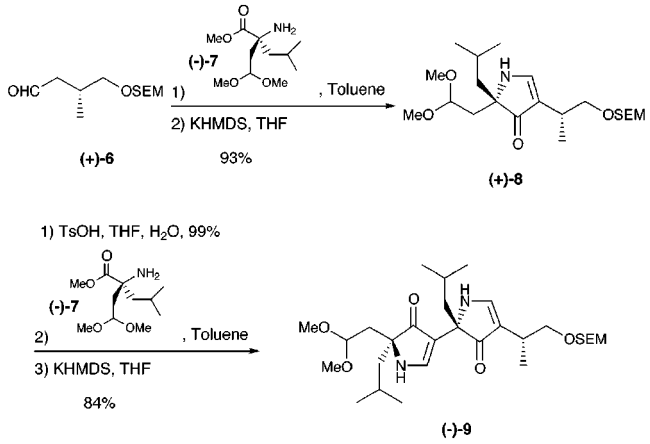
Synthesis of aldehyde **6** (Scheme 2) began with Evans alkylation of (*S*)-propionyloxazolidinone (+)-**3** with prenyl bromide to furnish oxazolidinone (+)-**4** in 64% yield (>98% ee). Reduction with lithium borohydride in wet THF,[14] followed by protection of the hydroxyl with 2-(trimethylsilyl)ethoxymethyl chloride (SEM-Cl) led to the SEM ether, which was subjected to ozonolysis to furnish aldehyde (+)-**6**; the overall yield from (+)-**3** was 39%.

Scheme 2



To construct monopyrrolinone (+)-**8**, amino ester (−)-**7**[6] was condensed with aldehyde (+)-**6** (Scheme 3); dehydration, followed by base-promoted pyrrolinone ring formation (KHMDS) then furnished (+)-**8** in 93% yield (two steps). Pleasingly, hydrolysis of the dimethylacetal with TsOH at 40 °C led to the corresponding aldehyde in nearly quantitative yield; a second pyrrolinone ring construction, again using amino ester (−)-**7**[6] led to bispyrrolinone (−)-**9**. The efficiency of our iterative pyrrolinone construction protocol was clearly demonstrated by the 77% overall yield of (−)-**9** from (+)-**6**.

Scheme 3



Hydrolysis of the dimethylacetal in (−)-**9** was next achieved with 1 N HCl at 40 °C. Unfortunately, oxidation of the derived aldehyde to the corresponding carboxylic acid by employing a variety of different conditions (e.g., Jones, sodium chlorite, PCC, etc.) proceeded only in low yield. Careful examination of the product mixture revealed that the pyrrolinone rings were not stable to the oxidation conditions. To circumvent this problem, (−)-**9** was protected as the bis Cbz derivative (+)-**10** (Scheme 4); although this operation led to a less reactive pyrrolinone ring, the acetal proved resilient to hydrolysis. We therefore decided to remove the SEM group first. Treatment of (+)-**10** with TsOH and methanol at 40 °C furnished alcohol (+)-**11**. A two-step oxidation with Dess−Martin periodinane[15] and then with sodium chlorite produced the C-terminal acid in 81% yield. Esterification followed by removal of the acetal (TsOH in wet THF at 40 °C) furnished the intermediate bispyrrolinone aldehyde; immediate oxidation with sodium chlorite led to (+)-**13**. We speculate that the steric congestion in (+)-**10**, emanating from the SEM and Cbz groups, inhibits acetal hydrolysis. Completion of the synthesis was achieved via coupling (+)-**13** with *O*-benzyl hydroxylamine (EDCI and HOBt), followed by hydrogenolysis with Pd/$BaSO_4$;[16] the overall yield of (−)-**1** for the two steps was 51%. Since the carboxylic acid functionality is also known to coordinate to the active-site zinc(II) in matrix metalloproteases,[9g] (+)-**13** was subjected to hydrogenolysis to furnish acid (−)-**14** (Scheme 5).

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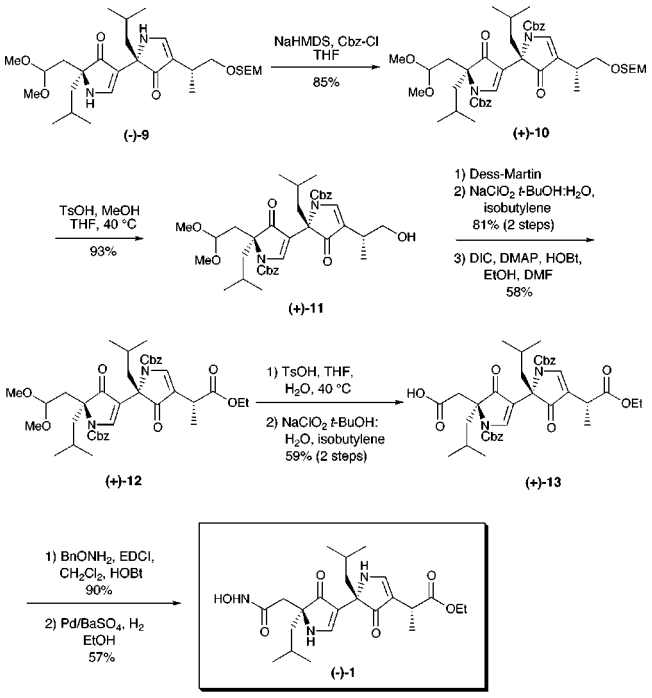
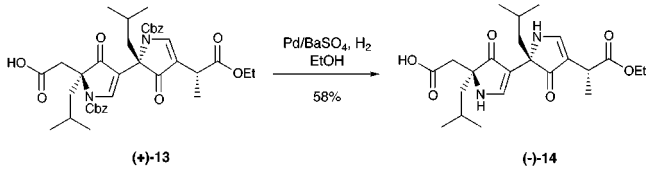
Scheme 4



Although bispyrrolinone (−)-**1** was designed for the MMP-1 isozyme, inhibitory activity was observed only for gelatinase (MMP-2), matrilysin (MMP-7), and the membrane type 2 matrix metalloprotease (MMP-15) with K_i values of 2.9, 6.4, and 6.8 μM, respectively. The bispyrrolinone carboxylic acid (−)-**14**, on the other hand, failed to inhibit the 10 proteases assayed, a result presumably of the shorter overall chain length compared to (−)-**1** and/or the known reduced affinity of the carboxylate to zinc(II) compared to the hydroxamate functionality.[9g,17]

Scheme 5



In summary, we have designed and synthesized a pyrrolinone-based inhibitor for a series of matrix metalloproteases. The synthesis reveals that unprotected pyrrolinone rings can be susceptible to oxidation but that acylation alleviates this propensity. Although only modest activity was observed, we believe that (−)-**1** represents an important new lead structure.[18] In addition, these results further demonstrate that excellent peptide mimicry can be achieved with the pyrrolinone scaffold. Studies to increase the potency of the pyrrolinone-based MMP inhibitors by increasing the size and hydrophobicity of the P1′ side chain, exploiting our recently disclosed solid-support polypyrrolinone synthetic protocol for focused library construction,[19] are currently underway in our laboratory.

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Supporting Information Available: Spectroscopic and analytical data as well as experimental procedures for all intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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