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Letters

Structure-Based Design of a Macrocyclic Inhibitor for Peptide Deformylase

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Abstract: A macrocyclic, peptidomimetic inhibitor of peptide deformylase was designed by covalently cross-linking the P1′ and P3′ side chains. The macrocycle, which contains an *N*-formylhydroxylamine side chain as the metal-chelating group, was synthesized from a diene precursor via olefin metathesis using Grubbs's catalyst. The cyclic inhibitor showed potent inhibitory activity toward *Escherichia coli* deformylase ($K_I = 0.67$ nM) and antibacterial activity against both Gram-positive and Gram-negative bacteria (MIC = 0.7−12 μg/mL).

The emergence of antibiotic-resistant bacteria has created an urgent demand for new antibacterial agents with novel mechanisms of action. Peptide deformylase (PDF), an essential enzyme involved in bacterial protein biosynthesis and maturation, is one of the few novel targets that is currently being pursued for antibacterial drug design.[1−3] In bacteria, protein synthesis starts with an *N*-formylmethionine, and as a result, all newly synthesized polypeptides carry a formylated N-terminus.[4] PDF catalyzes the subsequent removal of the formyl group from the majority of those polypeptides, many of which undergo further N-terminal processing by methionine aminopeptidase to produce mature proteins. As an essential activity for survival,[5−7] PDF is present in all eubacteria. On the other hand, protein synthesis in the eukaryotic cytoplasm does not involve N-terminal formylation and PDF apparently has no catalytic function in the mammalian mitochondrion.[8] Thus, PDF inhibitors are expected to act as a new class of broad-spectrum antibacterial agents.

PDF is a unique metallopeptidase, which utilizes a ferrous ion (Fe^{2+}) to catalyze the amide bond hydrolysis.[9,10] Due to sensitivity of the Fe^{2+} center to environmental oxygen and other reactive oxygen species, native PDF is extremely unstable and difficult to work with.[11] However, substitution of Ni^{2+} or Co^{2+} for the Fe^{2+} ion renders the enzyme highly stable while retaining almost full catalytic activity and substrate specificity of the native enzyme. Consequently, most of the recent biochemical, structural, and inhibition studies were carried out with the metal-substituted forms.

Numerous PDF inhibitors have been[1] reported in recent years; essentially all of them are metal chelators. On the basis of the chelator structure, they can be classified into three different types: the thiols,[12−14] the hydroxamates,[15−19] and the *N*-formylhydroxylamines or reverse hydroxamates.[20,21] Many of the hydroxamate and reverse hydroxamate inhibitors exhibit excellent antibacterial activities in vitro and in animal studies. One of the reverse hydroxamates from British Biotech (BB-86398) is currently in phase I clinical trials. However, since most of these inhibitors still have significant peptide characteristics, there are some concerns about their selectivity (e.g., inhibition of matrix metalloproteases) and in vivo stability (e.g., proteolysis of the peptide bonds). A popular approach to improving both stability against proteolysis and selectivity of peptidomimetic inhibitors is to form cyclic peptides or depsipeptides.[22,23] We report here the design, synthesis, and preliminary evaluation of the first macrocyclic inhibitor of PDF.

Structural studies of several PDF−inhibitor complexes[20,24,25] have revealed that the inhibitors are bound in an extended conformation, and the P1′ and P3′ side chains are similarly oriented. While the P2′ side chain is extended toward solvent, the P1′ and P3′ side chains are engaged in intimate interactions with the enzyme. The P1′ side chain (usually an *n*-butyl group) fits into a

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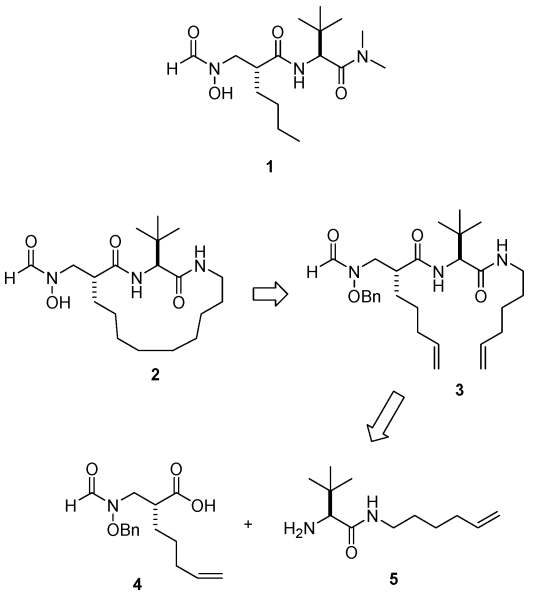
Scheme 1

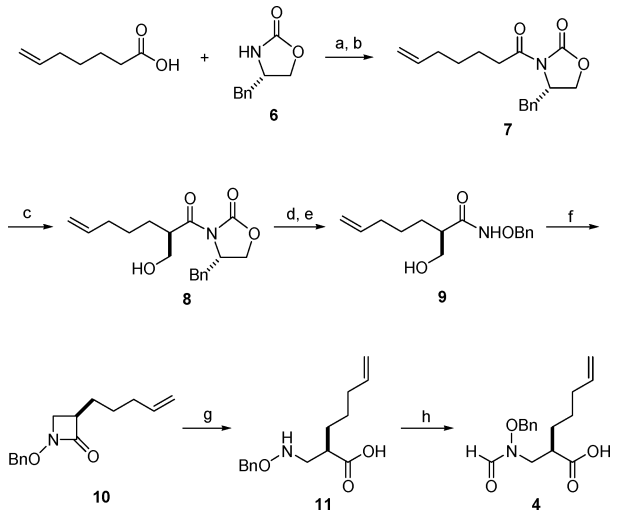


deep hydrophobic pocket in the PDF active site. The P3′ side chain makes hydrophobic contacts with a shallow pocket near the active site as well as one face of the P1′ side chain. It appears that covalent cross-linking of the P1′ and P3′ side chains would be accommodated by the PDF active site. Moreover, the rigidity introduced by cyclization may lock the inhibitor into the PDF-binding conformation and thus improve binding affinity as well as selectivity by preventing binding to other enzymes. On the basis of the structure of reverse hydroxamate **1** (BB-3497) (Scheme 1),[20] a potent PDF inhibitor and antibacterial agent, we designed cyclic compound **2**, in which a nonyl group serves as the cross-linked P1′ and P3′ side chains (Scheme 1). Molecular modeling indicated that the nonyl group should have the sufficient length to link the P1′ Cα carbon and the P3′ amino group, while maintaining the extended conformation of the peptide backbone. We chose *tert*-leucine as the P2′ residue because other PDF inhibitors containing this P2′ residue have shown excellent antibacterial activity and bioavailability.[20] Retrosynthetic analysis (Scheme 1) shows that the macrocycle can be conveniently prepared via olefin metathesis from diene **3**, which in turn can be prepared from acid **4** and amine **5**. This synthetic strategy would allow for the convergent synthesis of macrocycles of different ring sizes by using the common acid **4** and varying the length of the alkenylamino group in **5**.

Synthesis of acid **4** started from the commercially available 6-heptenoic acid (Scheme 2). A hydroxymethyl group was introduced at the C-2 position using 4*S*-benzyloxazolidin-2-one as the chiral auxiliary group.[26] Removal of the auxiliary group by hydrolysis followed by condensation with *O*-benzylhydroxylamine gave the hydroxamate **9**, which was converted into β-lactam **10** through an intramolecular Mitsunobu reaction.[27] Hydrolysis of the β-lactam furnished acid **11**, which was subsequently formylated at its benzyloxyamine moiety to give the acid **4**.

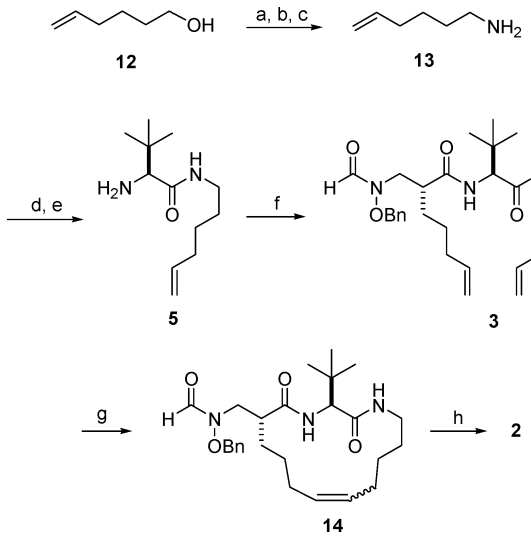
Synthesis of compound **2** is shown in Scheme 3. Amine **13**, which was obtained from the corresponding alcohol **12**, was condensed with *N*-Boc-*tert*-leucine.

Scheme 2[a]



[a] Conditions: (a) pivaloyl chloride, TEA, THF; (b) LiCl, 95% (two steps); (c) $TiCl_4$, DIPEA, CH_2Cl_2, $(HCHO)_n$, 52%; (d) H_2O_2, LiOH, THF/H_2O; (e) $BnONH_2$, HBTU, TEA, CH_3CN, 87% (two steps); (f) DIPAD, Ph_3P, THF, 88%; (g) LiOH, i-PrOH/H_2O, 93%; (h) HCO_2H, Ac_2O, CH_2Cl_2, quantitative.

Scheme 3[a]



[a] Conditions: (a) MsCl, TEA, CH_2Cl_2; (b) NaN_3, $DMF-H_2O$; (c) $LiAlH_4$, Et_2O; 57% (three steps); (d) Boc-*tert*-Leu-OH, EDC, CH_2Cl_2; (e) TFA, 86% (two steps); (f) **4**, EDC, CH_2Cl_2, 89%; (g) $(Pcy_3)_2Cl_2Ru{=}CHPh$, CH_2Cl_2, reflux, 83%; (h) Pd/C, H_2, MeOH−EtOAc, quantitative.

Treatment of the resulting amide with TFA resulted in the amine **5**. Coupling of amine **5** with acid **4** gave the diene **3**. The terminal alkenes were cross-linked using Grubbs's ruthenium catalyst[28,29] to produce the 15-membered macrocycle **14**. The configuration of the ring C=C bond in this intermediate was not determined. Catalytic hydrogenation of **14** reduced the double bond and simultaneously removed the benzyl group from the *N*-hydroxyl moiety to give the *N*-formylhydroxylamine **2**. NMR and HPLC analyses indicated a purity of ∼96%.

Compound **2** was assayed against Co(II)-substituted *E. coli* PDF using a dehydrogenase assay.[30,31] It acted as a potent inhibitor, with an apparent K_I value of 0.67 ± 0.2 nM. Thus, cyclization of the P1′ and P3′ side chains renders compound **2** ∼10-fold more potent than the acyclic parent compound **1** (IC_{50} = 7 nM).[20] To gain

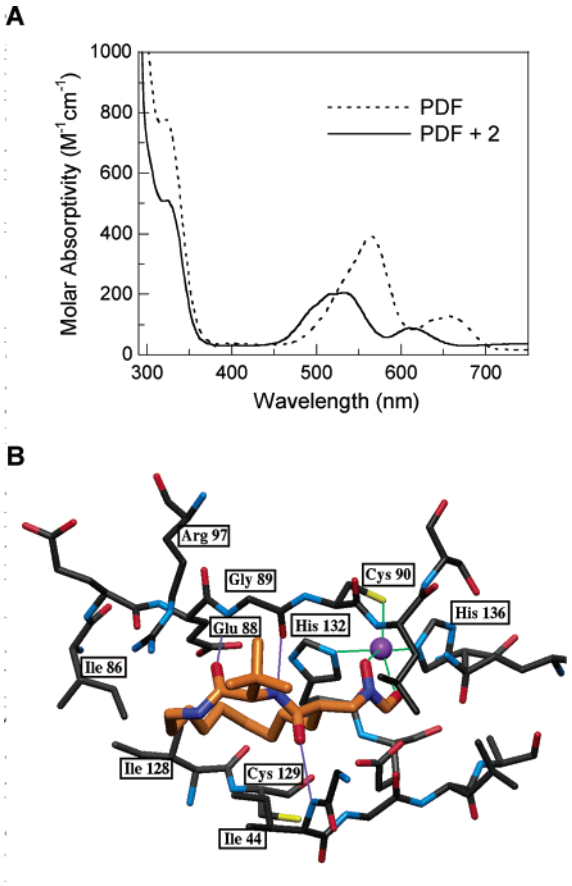


Figure 1. (A) Electronic absorption spectra of Co(II)-substituted PDF (240 μM) in the absence and presence of inhibitor **2** (500 μM). (B) Model showing the binding mode of inhibitor **2** to the *E. coli* PDF active site. Modeling was carried out by docking compound **2** into the structure of *E. coli* PDF bound with reverse hydroxamate inhibitor **1** (PDB access code 1G27). Protein residues involved in hydrophobic interactions with the inhibitor nonyl ring, as well as the metal ligands, are labeled (atom color code: protein C = gray, inhibitor C = caramel, N = blue, O = red, metal = purple; metal bonds = green; hydrogen bonds = purple).

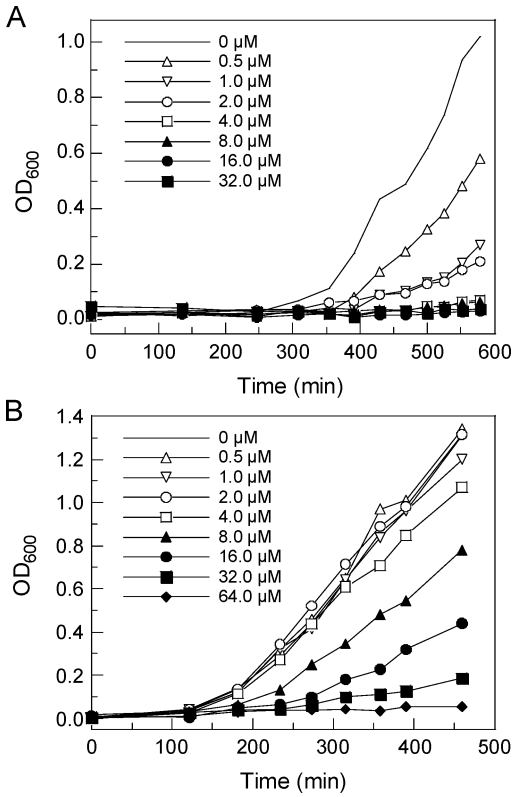


Figure 2. Inhibition of *B. subtilis* (A) and *E. coli* (B) cell growth by inhibitor **2**. An overnight culture was diluted 1000-fold into 2 mL of fresh LB medium containing the specified concentrations of inhibitor **2** and incubated at 37 °C. Cell densities were measured at the specified times on a UV−vis spectrophotometer.

insight into the mechanism of inhibition, the Co−PDF−inhibitor **2** complex was examined by UV−visible spectroscopy. Binding of the inhibitor resulted in marked blue shift (by ~40 nm) and reduction in the maximum intensity of the D−D transition bands in the absorption spectrum of the cobalt ion (Figure 1a). This result suggests that compound **2** is directly ligated to the metal ion. The maximum absorptivity of ~200 M^{-1} cm^{-1} for the PDF−inhibitor complex is consistent with a bidentate interaction between the *N*-formylhydroxylamine group and the metal and the formation of a pentacoordinated cobalt.[32] Molecular modeling showed that inhibitor **2** fits snugly in the active site of *E. coli* PDF (Figure 1b). The *N*-formylhydroxylamine is coordinated with the metal ion via both oxygen atoms. There are three hydrogen bonds formed between the protein and the inhibitor: from Ile-44 backbone amide to P1′ carbonyl, from P2′ amide to Gly-89 carbonyl, and from Gly-89 amide to P2′ carbonyl group. The nonyl group is engaged in extensive hydrophobic interactions with protein side chains including those of Ile-44, Ile-86, Glu-88, Ile-128, Cys-129, and His-132. Overall, these interactions are very similar to those observed in the PDF−inhibitor **1** complex.[20]

The in vitro antibacterial activity of the cyclic inhibitor was tested against *E. coli* and *Bacillus subtilis*, the representative Gram-negative and Gram-positive bacteria, respectively. Figure 2 shows the bacterial cell growth curves in the presence of varying concentrations of inhibitor **2**. Compound **2** exhibited potent antibacterial activity against *B. subtilis*, with a minimal inhibitory concentration (MIC) of 2−4 μM (or 0.7−1.4 μg/mL). It is only moderately active against *E. coli*, with an MIC of ~32 μM (~12 μg/mL). The lower activity against *E. coli* is likely due to its inefficient permeation of the bacterial outer membrane and/or being removed from the cells by the efflux pump.

In conclusion, based on our earlier observations that the P1′ and P3′ side chains of PDF inhibitors are closely packed in the PDF−inhibitor complex, we have developed a new class of macrocyclic PDF inhibitor by covalently linking the two side chains. The cyclic inhibitor is highly potent against PDF and has excellent to moderate antibacterial activity against both Gram-positive and Gram-negative bacteria. This result demonstrates that cyclization of the P1′ and P3′ side chains is a viable approach to developing potent PDF inhibitors. Due to their more rigid structures, cyclic inhibitors of this type may also have improved stability and selectivity. Further evaluation of inhibitor **2** with regard to its selectivity and in vivo stability as well as SAR studies of macrocycles of different ring sizes are already underway in our laboratories. These cyclic inhibitors may provide a novel class of antibiotics.

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Supporting Information Available: Experimental details for the synthesis and assay of compound **2** and the spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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