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H-PHOSPHONATE DERIVATIVES AS NOVEL PEPTIDE DEFORMYLASE INHIBITORS

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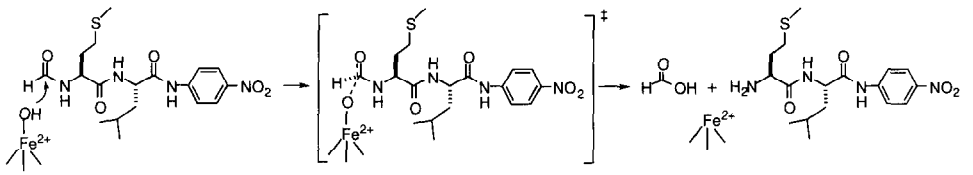
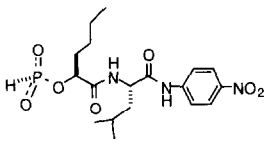
Abstract: Peptide deformylase catalyzes the removal of the N-terminal formyl group from nascent polypeptides during prokaryotic protein maturation and is essential for bacterial survival. Its absence from eukaryotic organisms makes it an attractive target for designing novel antibacterial agents. Peptidyl H-phosphonates were synthesized and shown to be competitive inhibitors of the deformylase.

In prokaryotes protein synthesis initiates with an N-formylmethionine, resulting in a formylated N-terminus in each nascent polypeptide.[1] Following translational initiation, the formyl group is removed from most polypeptides by the enzyme peptide deformylase (PDF).[2-4] While the precise function(s) of the formyl group is not well understood, genetic studies have demonstrated that PDF is essential for bacterial survival.[5] This conserved enzyme in eubacteria is, however, not present in eukaryotes. Therefore, PDF provides an attractive target for designing novel antibacterial agents.[5,6]



Scheme 1



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PDF is a new class of amide hydrolyase, which utilizes a Fe^{2+} ion as the catalytic metal.[6] Structural studies of the Zn^{2+} and Ni^{2+} substituted deformylases, which retain partial and nearly full catalytic activities, respectively, reveal that the metal ion is tetrahedrally coordinated with two histidines from the conserved HEXXH motif, a cysteine from the conserved EGCLS motif, and a water/hydroxide ion.[7-9] The proposed mechanism involves a nucleophilic attack on the formyl group by a metal bound hydroxide ion (or water molecule) to generate a tetrahedral intermediate, which is stabilized by the metal ion and the side chains of PDF active site residues (Scheme 1).[7] We envisioned that a H-phosphonate ester (**1**) could potentially resemble the tetrahedral intermediate or the transition states for the formation and/or breakdown of the intermediate and, therefore, act as a deformylase inhibitor. Stable transition-state analogs involving the corresponding phosphonates have previously been shown to be potent inhibitors of Zn^{2+} metallopeptidases such as carboxypeptidase A and leucine aminopeptidase.[10,11]

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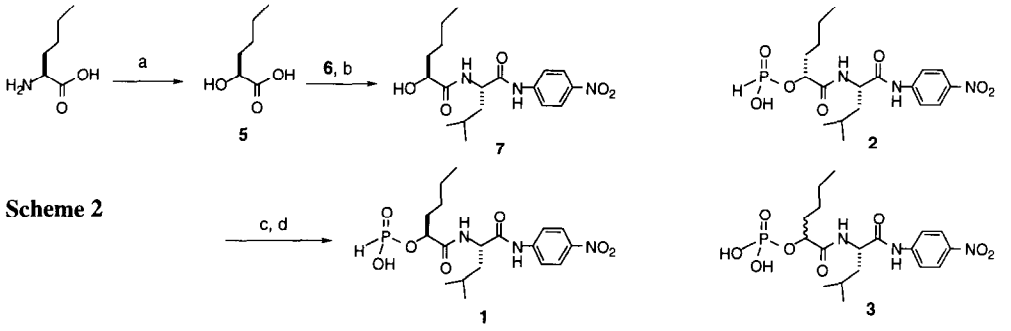
A methionine analog, 2-hydroxycaproic acid, is chosen as the P_1' residue because methionine is the N-terminal residue in all natural substrates of PDF and is strongly preferred by the enzyme.[2,12,13] An ester linkage is used because the corresponding H-phosphonamide was found to be unstable under the neutral aqueous condition. The use of leucyl-*p*-nitroanilide as the P_2'/P_3' residues of **1** is based on our previous observation that formyl-Met-Leu-*p*-nitroanilide (Scheme 1) is a high-affinity substrate of PDF (K_M = 20 μM).[14] Synthesis of **1** is shown in Scheme 2. The amino acid L-norleucine was stereoselectively converted into (*S*)-2-hydroxycaproic acid (**5**).[15,16] Condensation of **5** with L-leucyl-*p*-nitroanilide (**6**) using *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) afforded the intermediate **7**, which was subsequently phosphonylated by treating with phosphorus trichloride/triazole and triethylammonium bicarbonate (TEAB) to give **1** in 65% overall yield (three steps).[17–19] The other stereoisomer (**2**) which contains (*R*)-2-hydroxycaproic acid as the P_1' residue was similarly prepared from D-norleucine. As a comparison, **7** was also treated with $POCl_3$/pyridine followed by aqueous workup to give the corresponding phosphate monoester **3** (Scheme 2) as a racemic mixture.



Scheme 2

Reagents: (a) 1 N H_2SO_4, $NaNO_2$, 60%; (b) HBTU, HOBT, Et_3N, 80%; (c) 1,2,4-triazole, PCl_3, NMP; (d) TEAB (pH 8.5), 85% (2 steps).

Compounds **1–3** were tested for inhibition of both the Fe^{2+} and Zn^{2+} deformylases using formyl-Met-Leu-*p*-nitroanilde as substrate in a continuous assay.[14] Stock solutions of **1–3** were prepared in water and their concentrations were determined by absorbance measurement at 405 nm after alkaline hydrolysis with 1 N sodium hydroxide (ε = 10,600 $M^{-1}cm^{-1}$). Assay reactions were carried out in 50 mM potassium phosphate (pH 7.0) and 10 mM NaCl. Initial rates obtained were plotted against substrate concentration to determine the apparent K_M values (Figure 1). Secondary plot of apparent K_M's against inhibitor concentration gives the inhibition constant, K_I. Compounds **1** and **2** act as competitive inhibitors of both Fe^{2+} and Zn^{2+} deformylases. As expected, the H-phosphonate derivative (**1**) which has the P_1' residue in L- form is most potent, with K_I values of 37 μM and 76 μM for the Fe^{2+} and Zn^{2+} enzymes, respectively. The corresponding D-isomer (**2**) is also an inhibitor of the Fe^{2+} deformylase although with a threefold lower potency relative to **1** (K_I = 125 μM). Its affinity to the Zn^{2+} enzyme is also estimated to be approximately threefold lower than that of **1**; the high K_I value, however, did not permit its accurate measurement. This relatively small difference between the K_I values for **1** and **2** is somewhat surprising. A likely explanation may be that PDF recognizes primarily residues at the P_1'–P_4' positions for affinity and specificity and does not have extensive interactions with the small formyl group.[12] Thus, the side chain of D-2-

hydroxycaproic acid could fit into the same pocket that is normally occupied by the side chain of L-methionine, by placing the H-phosphonate group in the opposite orientation. This notion is consistent with our observation that the free alcohol **7**, which does not contain the phosphonate moiety, is also an inhibitor of PDF (its limited solubility prevented any measurement of K_I value).

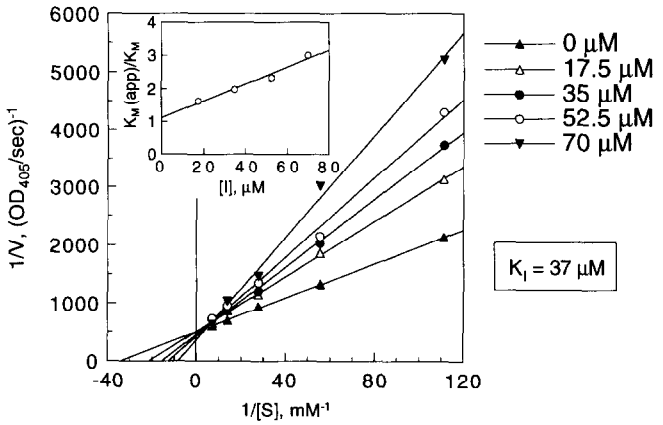


Figure 1. Lineweaver–Burk plots for the inhibition of Fe^{2+} PDF by **1**. *Inset*, secondary plot of slopes [K_M(app)/K_M] derived from the primary plots vs [I].

Interestingly, the phosphate ester **3** is a much poorer inhibitor of the deformylase than **1** or **2**, resulting in ~20% inhibition at 1 mM inhibitor.[20] This may be because of the larger size of the phosphate group (relative to H-phosphonate), which does not fit into the active site. In this regard, PDF strongly favors a formyl group vs an acetyl group by a factor of ~10^5–fold.[14] Another possibility may be the different geometry of the H-phosphonate group from that of the phosphate group; the O–P–O bond angle in the former is expected to be significantly larger than that in the latter.

In summary, we have described the synthesis and evaluation of the first rationally designed inhibitor for peptide deformylase. To our knowledge, this work also represents the first case in which H-phosphonate is used to inhibit an amide hydrolase. The availability of this substrate analog will facilitate future mechanistic and structural studies of the peptide deformylase.

Acknowledgment

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13. The side chain of norleucine is used instead of that of methionine to faciliate the synthesis. Substitution of norleucine for methionine slightly reduces the activity of PDF to a substrate.[12]
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19. Compound **1** (triethylammonium salt): ^{1}H NMR (200 MHz, D_2O): δ 8.43 (d, J = 9.2 Hz, 2H), 7.89 (d, J = 9.2 Hz, 2H), 7.02 (d, J = 643 Hz, 1H), 4.82 (m, 1H), 4.68 (m, 1H), 3.39 (q, J = 7.4 Hz, 6H), 2.00 (m, 9H), 1.47 (t, J = 7.4 Hz, 9H), 1.17 (d, J = 5.2 Hz, 3H), 1.14 (d, J = 5.2 Hz, 3H), 1.00 (t, J = 6.6 Hz, 3H). ^{31}P NMR (D_2O): δ 51.8. FABMS (M + H$^+$): *m/z* 531. **2**: δ 8.28 (d, J = 7.9 Hz, 2H), 7.74 (d, J = 7.9 Hz, 2H), 7.49 (d, J = 582 Hz), 4.66 (m, 1H), 4.59 (m, 1H), 3.20 (q, J = 7.4 Hz, 6H), 1.85 (m, 9H), 1.29 (t, J = 7.4 Hz, 9H), 0.99 (d, J = 5.2 Hz, 6H), 0.91 (t, J = 6.6 Hz, 3H). FABMS (M + H$^+$): *m/z* 531.
20. Assay was performed with 10 mM formyl-Met-Ala-Ser (ref 12) as substrate.