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# Phosphinate, sulfonate, and sulfonamidate dipeptides as potential inhibitors of *Escherichia coli* aminopeptidase N

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**Abstract**—In an effort to prepare novel inhibitors of bacterial aminopeptidase N (PepN), the phosphinate, propenylphosphinate, decylphosphinate, sulfonate, and sulfonamidate analogs of Ala–Ala were synthesized and tested as inhibitors. Phosphinate **1** was shown to inhibit PepN with a $K_i$ of 10 μM, and propenylphosphinate **2** and decylphosphinate **3** inhibited PepN with a $K_i$ of ca. 1 μM. Sulfonate and sulfonamidate analogs did not inhibit PepN.


Aminopeptidases are ubiquitous hydrolases that are involved with protein maturation, activation, and degradation.[1,2] These enzymes can hydrolyze the N-terminal amino acid(s) from a peptide by using active site serine or cysteine residues or by using metal ion catalysis. The most common metal ion found at the active site of aminopeptidases is Zn(II); however, there are known aminopeptidases that utilize Fe(II), Mn(II), or Co(II).[1] The aminopeptidases have also been grouped according to their substrate specificities, physical and mechanistic properties, and amino acid sequences.[1,3]

One particularly interesting enzyme is aminopeptidase N (PepN). In mammals, PepN is membrane-associated and has been identified as a receptor for several viruses and in the activation mechanism of collagenolysis that allows for tumor cell invasion.[4–6] Since PepN apparently is involved in a number of biomedically important processes, a large number of inhibitors of mammalian PepN have been reported. For example, several natural product dipeptides, bestatin, phebestin, probestin, and amastatin, have been shown to be potential inhibitors of PepN, leucine aminopeptidase, methionine aminopeptidase, and aminopeptidase A.[7–9] Bartlett and coworkers synthesized and tested phosphinate analogs of bestatin as inhibitors of leucine aminopeptidase.[10]

In bacteria, PepN is cytosolic,[1] and the enzymes from a number of *Lactococcus* and *Lactobacillus* strains have been studied in detail because of the roles of PepN in the making of cheese and other dairy products.[1,11] In *Escherichia coli*, PepN has been identified as the sole alanyl aminopeptidase and is the major aminopeptidase involved in ATP-independent processing during cytosolic

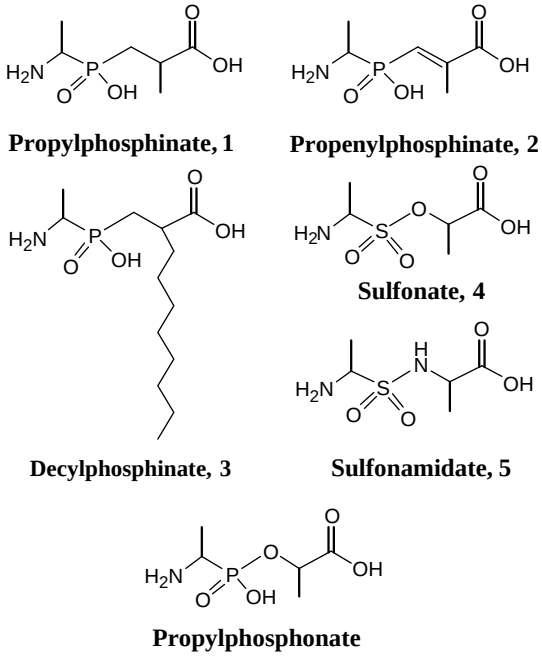


**Figure 1.** Structures of compounds described in this study.

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protein degradation.[12] Given these roles, PepN and other bacterial proteases have been recently identified as potential antibiotic targets.[13]

In this work, we have tested a number of phosphinate, sulfonate, and sulfonamidate analogs of alanyl–alanine as inhibitors of PepN from *E. coli* (Fig. 1).

Previous studies have demonstrated that similar analogs are transition state analog inhibitors of peptidases,[14–17] and these inhibitors are typically tight binding and specific.

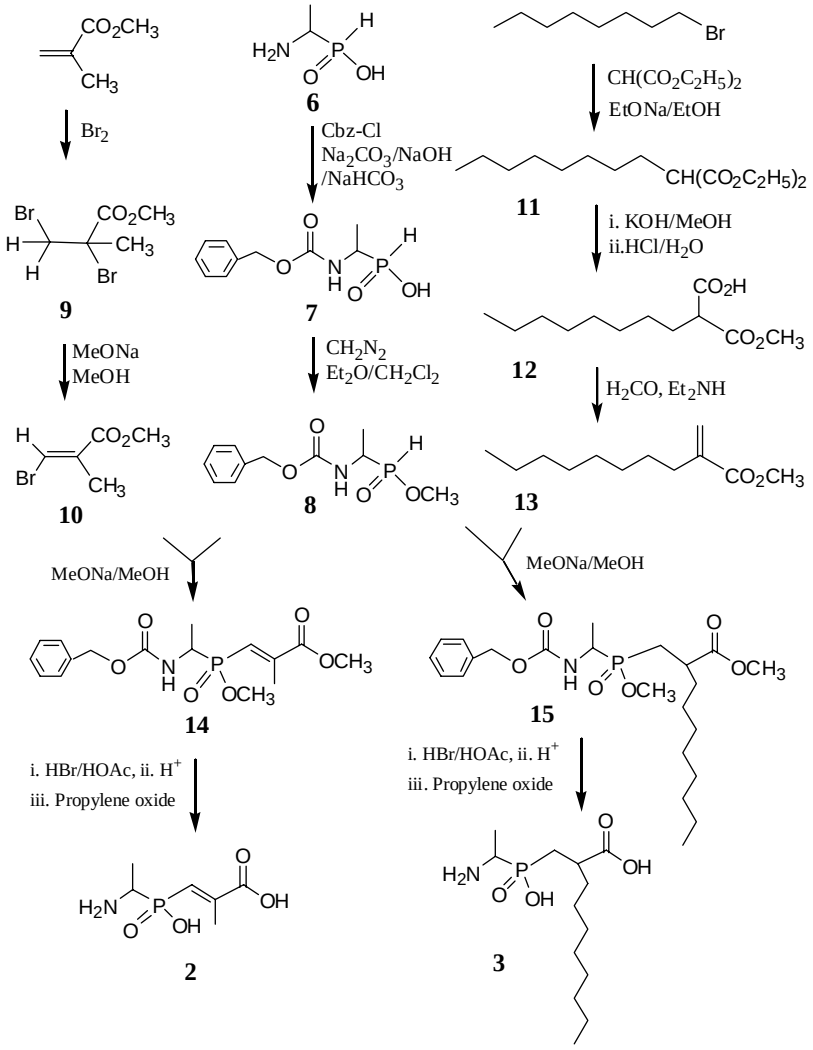
**Table 1.** Inhibition constants for compounds **1**–**5**[a]

| Compound | $K_i$ (μM) | Mode of inhibition |
|---|---|---|
| **1** | 10 ± 2 | Competitive |
| **2** | 1.0 ± 0.2 | Competitive |
| **3** | 1.1 ± 0.1 | Competitive |
| **4** | >300 | |
| **5** | >300 | |

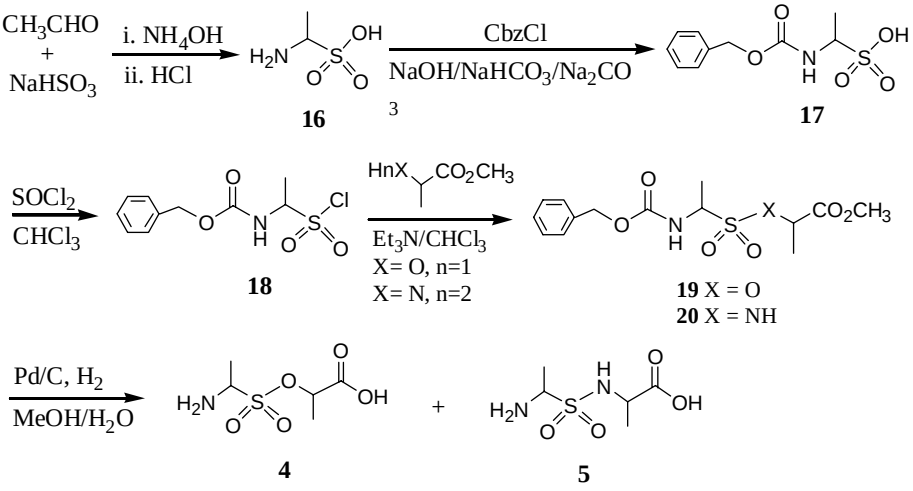
[a] $K_i$ values and modes of inhibition were determined as previously described and using L-ala-*p*-nitroanilide as substrate and PepN from *E. coli* as the enzyme in 50 mM Hepes, pH 7.0, at 25 °C.[20]

Propylphosphinate **1** was previously reported to be an inhibitor of the aminopeptidase VanX[18,19] and was synthesized as previously described.[20] This compound was shown to be a competitive inhibitor of PepN from *E. coli* with a $K_i$ value of 10 ± 2 μM (Table 1). The comparable phosphonate analog (Fig. 1) of **1**[19] did not appreciably inhibit PepN at concentrations up to 200 μM (data not shown). We reasoned that the P–X–C bond angle (ideally 109.5° in **1** and 104° in the phosphonate analog) may play a role in the relative binding strengths of these compounds. Therefore, a compound with a large P–X–C bond angle was tested as an inhibitor. (1-Aminoethyl)(2-carboxy-1-propenyl) phosphinic acid **2** was synthesized as shown in Scheme 1 and as described in supplementary materials. The overall yield of this synthetic strategy was 27%. Propenylphosphinate **2** was a competitive inhibitor with a $K_i$ of 1.0 ± 0.2 μM (Table 1). As we hypothesized, the lengthening of the P–X–C bond angle resulted in tighter binding of the inhibitor.

In an effort to improve the binding affinity of these phosphinate dipeptide analogs to PepN, a hydrophobic substituent ($C_8H_{17}$) was attached to the β-carbon of **1** to yield decylphosphinate **3**. We reasoned that **3** would




**Scheme 1.** Synthesis of propenylphosphinate **2** and decylphosphinate **3**.




**Scheme 2.** Synthesis of sulfonate **4** and sulfonamide **5**.

be a bifunctional inhibitor in which the substituent would provide additional points of attachment to PepN. (1-Aminoethyl)(2-carboxy-1-decyl) phosphinic acid **3** was synthesized in 34% overall yield as shown in Scheme 1 and as described in supplementary materials. Decylphosphinate **3** was shown to be a competitive inhibitor of PepN with a $K_i$ of 1.1 ± 0.1 μM (Table 1). The inclusion of the hydrophobic substituent on phosphinate **1** did result in improved binding affinity. Unfortunately, efforts to synthesize a decylphosphinate analog of propenylphosphinate **2** have been unsuccessful.

Previously, phosphinate analogs of peptides have been reported to be very tight binding inhibitors of several peptidases with $K_i$ values reported as low as $10^{-15}$ M.[16] The relative weaker binding to PepN suggested that these compounds may not be good transition state analogs. Nonetheless, we attempted to improve the binding of the dipeptide analogs by preparing sulfonate and sulfonamide analogs of Ala–Ala. We reasoned that the sulfonate and sulfonamide analogs would require less desolvation than the phosphinate analogs, and the reduced desolvation energy would result in relatively tighter binding.

1-Aminoethyl-*O*-1-carboxyethyl sulfonate **4** and 1-aminoethyl-*N*-1-carboxyethyl sulfonamidate **5** were synthesized as shown in Scheme 2 and as described in Supplementary materials. Inhibition studies revealed that neither compound inhibited PepN at concentrations up to 300 μM.

These studies demonstrate that the value of the P–X–C bond angle in phosphinate analogs of Ala–Ala plays a role in the relative binding affinity of these compounds to PepN. In addition, the presence of a hydrophobic substituent on the β-carbon of the inhibitor also improves the binding affinity of the compounds. Recently, Grembecka et al.[21] reported that the phosphinate analog of PheTyr is a tight binding inhibitor ($K_i$ of 36 nM for diastereomeric mixture) of mammalian PepN. The work presented herein suggests that the binding affinity of this compound could be improved by ca. 100-fold if a double bond and a hydrophobic substituent are included in the phosphinate dipeptide. These studies also show that the use of sulfonate/sulfonamide analogs to replace phosphinates is not an effective strategy to improve the binding strength of the compounds.

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## Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.08.055

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