

## Peptidic $\alpha$ -Ketocarboxylic Acids and Sulfonamides as Inhibitors of Protein Tyrosine Phosphatases

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**Abstract:** One common approach for designing protein tyrosine phosphatase (PTPase) inhibitors is to incorporate a nonhydrolyzable phosphotyrosine (pTyr) mimic into a peptide substrate for PTPases. This report describes the synthesis of three such nonhydrolyzable pTyr mimics that contain  $\alpha$ -ketoacid,  $\alpha$ -hydroxyacid, and methylenesulfonamide functional groups in place of the phosphate. These pTyr mimics were incorporated into the peptide sequence Ac-Asp-Ala-Asp-Glu-X-Leu-NH<sub>2</sub>, where X is the pTyr mimic, and analyzed for activity against the *Yersinia* PTPase and PTP1B.

There is considerable interest in the development of inhibitors for protein tyrosine phosphatases (PTPases) because PTPases are associated with a variety of human diseases.<sup>1,2</sup> For example, overexpression of the human PTPases PTP1B, LAR, and PTP $\alpha$  leads to abnormal insulin resistance that is often associated with type II diabetes.<sup>3</sup> As a consequence, several studies have shown that inhibitors of PTP1B are potential therapeutic agents for the treatment of both diabetes and obesity.<sup>4,5</sup> In a second example, the *Yersinia pestis* bacterium, the causative agent of the bubonic plague, employs the PTPase YopH that is essential for its virulence.<sup>6</sup> Thus, inhibitors of YopH are potential antibacterial agents that can be targeted for this organism.

The active site of PTPases selectively binds phosphotyrosine (pTyr),<sup>7</sup> but the amino acids that flank a pTyr residue are also crucial for recognition of substrates by PTPases.<sup>8,9</sup> One peptide that is a good substrate for a variety of PTPases is the sequence derived from the

epidermal growth factor receptor Ac-DADEpYL-NH<sub>2</sub> (EGFR<sub>988-993</sub>).<sup>9</sup> A number of investigators have shown that the Ac-DADE-X-L-NH<sub>2</sub> peptide, where X denotes a non-hydrolyzable pTyr analogue, is a useful template for designing PTPase inhibitors.<sup>10</sup> One of the most effective pTyr mimics that has been inserted into this peptide is difluoromethylphosphonophenylalanine (F<sub>2</sub>Pmp).<sup>11</sup> The difluoromethylenephosphonic acid (DFMP) group has also been used as the basis for a number of other nonpeptidic PTPase inhibitors.<sup>12</sup> Despite the potency of the DFMP group, the efficacy of phosphonates is hampered by their inability to penetrate into cells. This low permeability is likely to be due to their high charge at physiological pH.<sup>13</sup>

To improve bioavailability, efforts have been directed toward the development of less-charged, non-phosphorus-containing inhibitors. These efforts have resulted in pTyr mimics that include L-*O*-(2-malonyl)tyrosine (OMT),<sup>14</sup> its fluorinated counterpart 4'-*O*-[2-(2-fluoromalonyl)]-L-tyrosine (FOMT),<sup>15</sup> and 2-(oxalylamino)benzoic acids.<sup>16</sup> Recently, we reported that  $\alpha$ -ketoacids such as phenylglyoxylic acid (**1**) can serve as a new class of pTyr mimic.<sup>17,18</sup> While several pTyr mimics that contain a single carboxylic acid group have been found to be moderately good ligands of src homology (SH2) domains, few have shown good affinity for PTPases.<sup>19</sup>

During our investigations of new phosphate analogues that can be used for PTPase inhibition, we also became interested in sulfonamides. Sulfonamides are a common functional group in medicinal chemistry, and they have served in a variety of applications such as carbonic anhydrase inhibitors<sup>20</sup> and bacteriostatic agents.<sup>21</sup> Compounds such as benzylsulfonamide **2** may possess a similar geometry to that of aryl phosphates and aryl-methylenephosphonates. As a result, investigation of sulfonamides may lead to noncharged PTPase inhibitors that have improved bioavailability. Here, we report the synthesis of pTyr analogues that incorporate an  $\alpha$ -ketoacid or sulfonamide group that are then used in the synthesis of Ac-DADE-X-L-NH<sub>2</sub>-based inhibitors

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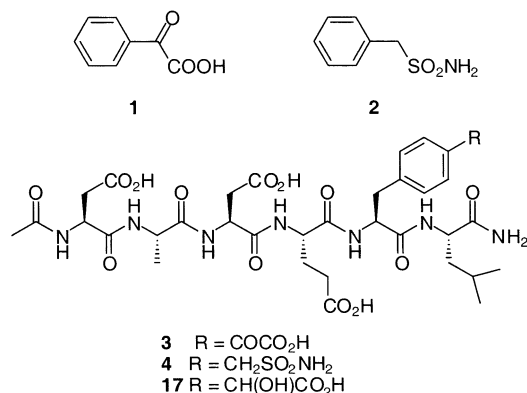
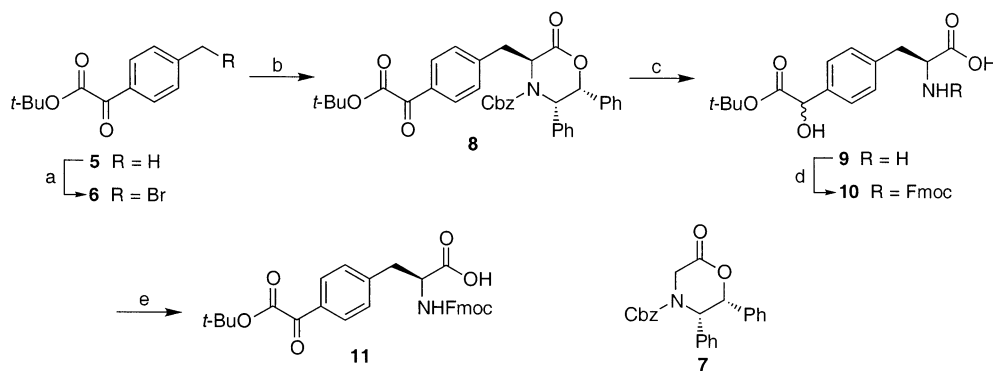
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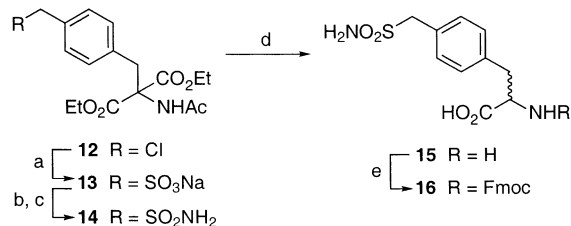
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SCHEME 1<sup>a</sup>

(compounds **3** and **4**). The activity of these peptide-based inhibitors is evaluated against two PTPases, the *Yersinia* PTPase and PTP1B.

Compound **11** (Scheme 1) is a protected form of the  $\alpha$ -ketoacid-containing pTyr analogue that is suitable for use in Fmoc-based solid-phase peptide synthesis. We began the synthesis of compound **11** by bromination of  $\alpha$ -ketoester **5** with *N*-bromosuccinimide and benzoyl peroxide to give benzyl bromide **6**. Compound **5** was prepared according to the procedure of Nimitz and Mosher.<sup>22</sup> To synthesize the amino acid in stereochemically pure form, we employed the diphenyloxazinone chiral auxiliary **7** that has been developed by Williams and Im.<sup>23</sup> Treatment of compound **7** with LiHMDS gave the corresponding glycine-based enolate. Alkylation of this enolate with benzyl bromide **6** afforded oxazinone **8** with the desired stereochemistry. Subsequent hydrogenation of **8** with palladium hydroxide on carbon removed the chiral auxiliary to generate free amino acid **9**. During this reaction, the ketone was also reduced to give the  $\alpha$ -hydroxy ester as a mixture of two diastereomers. After the amine was protected with an Fmoc group, the ketone was regenerated by oxidation with MnO<sub>2</sub> to give compound **11**.

The synthesis of the racemic sulfonamide-containing amino acid **16** is shown in Scheme 2. Compound **12**, which was prepared according to the procedure of

SCHEME 2<sup>a</sup>

<sup>a</sup> Reagents: (a) Na<sub>2</sub>SO<sub>3</sub>, acetone, H<sub>2</sub>O, 59%; (b) PCl<sub>5</sub>, CH<sub>2</sub>Cl<sub>2</sub>; (c) NH<sub>4</sub>OH, CH<sub>2</sub>Cl<sub>2</sub>, 53% (two steps); (d) 6 N HCl; propylene oxide, 88%; (e) Fmoc-OSu, NaHCO<sub>3</sub>, 1:1 H<sub>2</sub>O:dioxane, 85%.

Marseigne and Roques,<sup>24</sup> was allowed to react with sodium sulfite to give the corresponding sodium sulfonate **13**. Sulfonate **13** was treated with phosphorus pentachloride to give the sulfonyl chloride, which was then converted to sulfonamide **14** by reaction with ammonium hydroxide. Compound **14** was heated at reflux in 6 N HCl to effect the hydrolysis of the ester and *N*-acetyl groups, and to decarboxylate the resulting dicarboxylic acid. Workup of the reaction with propylene oxide in ethanol gave free amino acid **15**. Finally, the amine was protected with an Fmoc group to give compound **16** as a racemic mixture.

Hexapeptides **3** and **4** were synthesized on Rink amide resin<sup>25</sup> with use of manual solid-phase methods with Fmoc chemistry. Resin cleavage and side-chain deprotections were performed with a solution of 95% trifluoroacetic acid, 2.5% H<sub>2</sub>O, and 2.5% triisopropylsilane. During the cleavage and deprotection of peptide **3**, we observed that the ketoacid moiety was reduced to the corresponding  $\alpha$ -hydroxyacid to give peptide **17** as a 1:1 mixture of two diastereomers. This type of ionic reduction of ketones to alcohols mediated by trifluoroacetic acid and triisopropylsilane has been studied by a number of investigators.<sup>26</sup> When the cleavage and deprotection was performed in the absence of triisopropylsilane, the reaction yielded the desired ketoacid-containing peptide **3** in diastereomerically pure form. Since we prepared the sulfonamide-based amino acid **16** as a racemic mixture,

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**TABLE 1. Inhibition of Phosphatases by Peptide Inhibitors 3, 4, and 17**

| compd                 | IC <sub>50</sub> (μM)  |                 |
|-----------------------|------------------------|-----------------|
|                       | <i>Yersinia</i> PTPase | PTP1B           |
| <b>2</b>              | 370 ± 60               | ND <sup>c</sup> |
| <b>3</b>              | 150 ± 15               | 270 ± 30        |
| <b>4a<sup>a</sup></b> | 370 ± 50               | >2500           |
| <b>4b<sup>a</sup></b> | 2100 ± 400             | ND <sup>c</sup> |
| <b>17<sup>b</sup></b> | 180 ± 20               | ND <sup>c</sup> |

<sup>a</sup> **4a** and **4b** are two different diastereomers of peptide **4** that differ in stereochemistry of the sulfonamide-containing amino acid. <sup>b</sup> Mixture of two diastereomers that differ in the stereochemistry of the hydroxyl group of the α-hydroxyacid moiety. <sup>c</sup> Not determined.

peptide **4** was obtained as a mixture of two diastereomers. These diastereomers were separated by reverse-phase HPLC and assayed separately.

Assays were performed with *p*-nitrophenyl phosphate (*p*-NPP) as the substrate at 25 °C in pH 5.5 acetate buffer (100 mM) that contained 100 mM NaCl and 10% DMSO to increase the solubility of the inhibitors. As shown in Table 1, benzylsulfonamide (**2**) has an IC<sub>50</sub> value against the *Yersinia* PTPase of 370 μM. We then investigated the potential of the arylmethylenesulfonamide within the context of a peptide-based inhibitor. However, one diastereomer of this peptide (**4a**) has an IC<sub>50</sub> value against the *Yersinia* PTPase of 370 μM. Thus, we did not observe any improvement in activity over the simple unsubstituted benzylsulfonamide inhibitor **2**. Compound **4a** is a millimolar inhibitor of PTP1B, while its diastereomer **4b** is a millimolar inhibitor against the *Yersinia* enzyme.

In previous work, we have found that aryl α-ketocarboxylic acids comprise a new class of inhibitors for protein tyrosine phosphatases.<sup>17,18</sup> For example, phenylglyoxylic acid **1** is a 2.7 mM inhibitor of the *Yersinia* PTPase.<sup>17</sup> This value can be improved upon significantly by adding electron-donating substituents to the phenyl ring, or by exchanging the phenyl ring for more electron-rich aromatic groups. In light of these studies, we synthesized peptide **3** that incorporates an aryl α-ketoacid as a mimic of phosphotyrosine. During the synthesis of peptide **3**, we found that cleavage of the peptide from the solid support with a combination of trifluoroacetic acid and triisopropylsilane produced the corresponding α-hydroxycarboxylic acid-containing peptide **17**. This failed reaction was somewhat fortuitous since it provided us with an additional compound for evaluation. Our earlier studies demonstrated that α-hydroxyacids retain some activity against PTPases.<sup>17</sup>

The α-hydroxyacid-containing peptide **17** is a reasonable inhibitor of the *Yersinia* PTPase with an IC<sub>50</sub> value of 180 μM, while the α-ketoacid-containing peptide **3** has similar activity with an IC<sub>50</sub> value of 150 μM (Table 1). Thus, peptide **3** represents a 15-fold improvement over the simple phenylglyoxylic acid (**1**) starting point. These compounds are not as potent as other peptide-based inhibitors that incorporate doubly charged phosphotyrosine mimics such as fluoro-*O*-malonyl tyrosine and difluorophosphonomethyl phenylalanine.<sup>27</sup> However, they

have better activity than other Ac-DADE-X-L-NH<sub>2</sub>-based inhibitors that contain pTyr analogues with a single carboxylic acid.<sup>19</sup> Of the pTyr analogues that are monoanionic, only the difluoromethylenesulfonates that have been developed by Taylor and co-workers show higher activity.<sup>28</sup>

Burke and co-workers have suggested a rationale for why some carboxylic acid-based peptide inhibitors may have low affinity for phosphatases.<sup>19</sup> While the peptide chain provides favorable binding interactions with residues outside of the catalytic site of PTPases, it limits the geometrical freedom and depth of insertion of the aryl phosphate analogue into the active site. The optimal positioning of aryl α-ketoacids in the active site may be somewhat different than that of aryl phosphates. Therefore, by restricting the binding geometry of the α-ketoacid segment in peptide **3** into the same geometry as is observed for aryl phosphates, the α-ketoacid moiety may not be able to make optimal contacts with the active site residues. This results in relatively weak binding compared to compounds such as difluoromethylenephosphonates that closely mimic the geometry of the natural aryl phosphate substrates. In conclusion, we have synthesized three new nonhydrolyzable pTyr mimics, and investigated their activity within the context of peptide-based inhibitors. These compounds are not as active as many dianionic pTyr analogues, but they are more active than most of the monoanionic analogues that have been reported to date.

## Experimental Section

**PTPase Assays.** The phosphatase activities of the *Yersinia* PTPase and PTP1B (purchased from Calbiochem) were assayed with use of *p*-NPP as the substrate and the reaction progress was monitored by UV spectroscopy. Initial rates were determined by monitoring the hydrolysis of *p*-NPP at 420 nm, from 10 to 120 s after mixing. Assay solutions contained 1 mM EDTA, 100 mM NaCl, 100 mM acetate at pH 5.5, and 10% DMSO. Substrate concentrations were 2.5 and 1.2 mM for the *Yersinia* PTPase and PTP1B, respectively. IC<sub>50</sub> values were calculated by using a Dixon analysis. Data analysis was performed with the commercial graphing package Grafit (Erithacus Software Ltd.). The *K<sub>m</sub>* values under these conditions were found to be 2.5 mM for the *Yersinia* PTPase and 0.62 mM for PTP1B. The *K<sub>m</sub>* value for PTP1B in the absence of DMSO and in acetate buffer at pH 5.5 is reported to be 0.75 mM.<sup>29</sup>

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**Supporting Information Available:** Experimental procedures and characterization for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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