

## A Facile Synthesis of a Difluoromethylene Analog of $\beta$ -Aspartyl Phosphate as an Inhibitor of L-Aspartate- $\beta$ -semialdehyde Dehydrogenase

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**Abstract:** An efficient and high yield procedure for the synthesis of the difluoromethylene analog of  $\beta$ -aspartyl phosphate,  $\beta$ -aspartyl difluoromethylenephosphonate ( $\beta$ AFP), is described. Preincubation of  $\beta$ AFP with L-aspartate- $\beta$ -semialdehyde dehydrogenases from several infectious microorganisms leads to potent time-dependent enzyme inactivation.

**Key words:** L-aspartate- $\beta$ -semialdehyde dehydrogenase, difluoromethylenephosphonate, antibacterial,  $\beta$ -aspartylphosphate, time-dependent inhibition

The scourge of bacterial resistance to antibiotics is a problem of ever-increasing concern among the scientific and medical communities.<sup>1</sup> The identification and characterization of new targets for inhibition is necessary for the development of new antimicrobial compounds. One such target is L-aspartate- $\beta$ -semialdehyde dehydrogenase (ASADH), which catalyzes the reductive dephosphorylation of  $\beta$ -aspartyl phosphate to L-aspartate semialdehyde. This step is the first branch point in the aspartate pathway,<sup>2</sup> culminating in the synthesis of lysine, isoleucine, threonine, and methionine in plants and microorganisms. Diaminopimelic acid, a direct metabolic precursor of lysine, is a fundamental component of bacterial cell walls.<sup>3</sup> Perturbations to the *asd* gene encoding for ASADH can be lethal to a microorganism<sup>4,5</sup> and cell lines with deletions of the *asd* gene are auxotrophic for diaminopimelic acid. Since the aspartate pathway is not found in humans, ASADH is an attractive target for the development of new antimicrobial and herbicidal compounds.<sup>3</sup> Although there is a continued interest in developing effective aspartate pathway inhibitors, there has been only limited success to date.

Difluoromethylenephosphonate analogues of phosphate esters are of general interest in inhibitor design due to their hydrolytic stability and the similarity in polarity of the difluoromethylene moiety to an oxygen atom. The most potent inhibitor of ASADH reported to date is  $\beta$ -aspartyl difluoromethylenephosphonate ( $\beta$ AFP), an analog of  $\beta$ -aspartylphosphate ( $\beta$ AP) in which the oxygen atom connecting the  $\beta$ -carbonyl group and the phosphorus atom of  $\beta$ AP is replaced by a difluoromethylene group.<sup>6</sup> Our interest in the aspartate pathway<sup>7</sup> prompted us to evaluate  $\beta$ AFP as a potential ASADH inhibitor. Towards this goal,

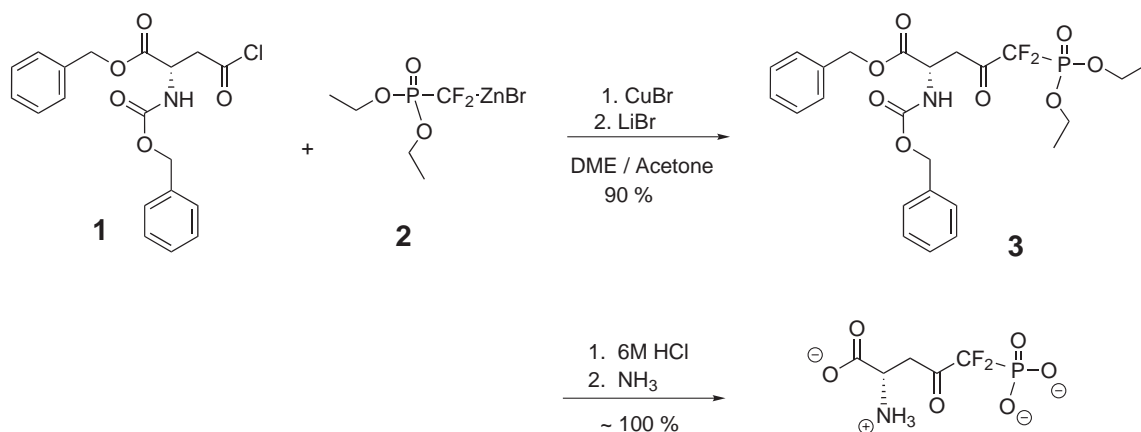
we have developed a new synthesis of  $\beta$ AFP for the purpose of testing its inhibition of ASADHs from selected infectious microorganisms.<sup>8</sup>

The reported synthesis of  $\beta$ AFP involves more than 8 steps, leading to a low overall yield of the final product.<sup>6,9</sup> Our aim was to develop a more efficient synthesis of  $\beta$ AFP by finding a coupling reaction between the carbonyl group of the L-aspartate acid chloride and the difluoromethylene moiety of the phosphonate, thus affording the protected target in one step from the protected amino acid precursor. Lithio(difluoromethyl)-phosphonates have been used to react with methyl esters for the direct preparation of ketones.<sup>10</sup> This approach has also been used in the synthesis of  $\gamma$ -glutamyl difluoromethylenephosphonate.<sup>11</sup> Unfortunately, coupling of lithio(difluoromethyl)diethylphosphonate to the protected L-aspartate methyl ester was unsuccessful.<sup>6</sup> However, phosphonodifluoromethyl ketones have been prepared by the direct coupling of difluoromethylene zinc reagents with acyl chlorides,<sup>12</sup> often with catalysis by CuBr.<sup>13,14</sup> Another catalyst, PdCl<sub>2</sub>(Ph<sub>3</sub>P)<sub>2</sub>, has also been used to facilitate coupling of organozinc reagents with acid halides to prepare ketones.<sup>15,16</sup> The coupling of **1** and **2** had not been accomplished using any of these published procedures.

Here we report optimized conditions for the high-yield coupling of **1** and **2** in the presence of CuBr-2LiBr. Deprotection was effected with HCl, yielding  $\beta$ AFP in 90% overall yield.

The protected aspartic acid chloride (**1**) was obtained in quantitative yield as a pale yellow solid from *N*-(benzyloxycarbonyl)-L-aspartate- $\alpha$ -benzylester according to a reported procedure.<sup>17</sup> Compound **2** was prepared by treatment of diethyl (bromodifluoromethyl)-phosphate with acid-washed zinc powder in monoglyme.<sup>12</sup>

A solution of **2** (15.4 mmol) in monoglyme (25 mL) was added dropwise to a mixture of CuBr (2.3 g, 15.7 mmol) in acetonitrile (30 mL, -5 °C) and further stirred for 1 hour. To this mixture, **1** (5.1 g, 14.0 mmol) in acetonitrile (30 mL, -5 °C) was added dropwise by cannula and stirred for 4 hours. The solution was transferred to a flask containing LiBr (2.8 g, 31.4 mmol), stirred overnight at room temperature, then concentrated by rotary evaporation. The yellow semisolid was dissolved in ethyl acetate (400 mL) and washed successively with saturated NaHCO<sub>3</sub>, 2 M HCl, water, and brine, then dried over sodium sulfate. Flash column chromatography on silica gel



**Scheme 1** Synthesis of  $\beta$ -aspartyl difluoromethylenephosphonate

affords **3**, the coupled protected product (6.5 g, 12.6 mmol) as a pale yellow liquid in 90% yield (Scheme 1).<sup>18</sup>

Deprotection was afforded by treatment of **3** (2.0 g, 3.9 mmol) with 6 M HCl (20 mL) under reflux overnight. The acid was removed in vacuo and the residue was dissolved in ethanol (20 mL). As ethanolic ammonia (15.6 mmol) was slowly added to the solution, a white precipitate formed. The solid was filtered, washed with acetone, and dried in vacuo over  $\text{P}_2\text{O}_5$ , affording the final product,  $\beta$ AFP, in 90% overall yield.<sup>19</sup>

When ASADHs from the infectious organisms, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, and *Vibrio cholera*, are pre-incubated with  $\beta$ AFP at pH 9.0 and at concentrations as low as 10  $\mu\text{M}$ , the activity of each enzyme decreases dramatically over a period of several hours. These preliminary studies show that  $\beta$ AFP is potent, slow-binding inhibitor of ASADH.  $\beta$ AFP could act as a lead compound in the design of broad-spectrum antibacterial compounds against infectious microorganisms based upon ASADH inhibition.

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- (18) **Analytical Data of Compound 3**:  $R_f$  0.49 (hexanes–EtOAc, 2:1).  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 7.29 (m, 10 H), 5.92 (d, 1 H,  $J$  = 8.4 Hz), 5.06 (s, 2 H), 5.10 (s, 2 H), 4.73 (m, 1 H), 4.21 (q, 4 H,  $J$  = 6.8 Hz), 3.4 (dd, 2 H,  $J$  = 8.0 Hz), 1.3 (t, 6 H,  $J$  = 6.8 Hz).  $^{19}\text{F NMR}$  (188.2 MHz,  $\text{CDCl}_3$ ):  $\delta$  = –119.1 (d,  $J_{\text{F-P}}$  = 95.4 Hz).
- (19) **Analytical Data of  $\beta$ AFP**: TOF-ES MS [ $\text{C}_5\text{H}_8\text{F}_2\text{NPO}_6$ ] $\text{H}^+$ : Calcd: 248.0674. Found: 248.0156.  $^1\text{H NMR}$  (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 3.9 (m, 1 H), 3.4 (t, 2 H,  $J$  = 3.4 Hz).  $^{13}\text{C NMR}$  (100.6 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 205.2, 175.9, 120.6 (dt,  $J_{\text{C-F}}$  = 270.5 Hz,  $J_{\text{C-P}}$  = 156.4 Hz), 51.9, 33.0.  $^{19}\text{F NMR}$  (188.2 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = –119.1 (d,  $J_{\text{F-P}}$  = 74.9 Hz), –124.5 (d,  $J_{\text{F-P}}$  = 78.7 Hz).  $^{31}\text{P NMR}$  (81.0 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 2.35 (t,  $J_{\text{F-P}}$  = 75.6 Hz). Elemental analysis [ $\text{C}_5\text{H}_6\text{F}_2\text{NPO}_6(\text{NH}_4)_3\text{Cl}$ ]: Calcd: C, 17.98%; H, 5.41%; N, 16.74%. Found: C, 18.14%; H, 5.19%; N, 16.34%.