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SYNTHESIS OF A 5-AZAINDOLE PHOSPHONIC ACID AS A COMPUTATIONALLY DESIGNED INHIBITOR OF THE LOW MOLECULAR WEIGHT PHOSPHATASE HCPTP**

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**This paper is dedicated to Professor Steven M. Weinreb, one of the true master craftsmen of heterocyclic chemistry, on the occasion of his 65th birthday.

Abstract – A computationally designed inhibitor of human low molecular weight protein tyrosine phosphatase, HCPTP, was synthesized. The target compound is based on a 5-azaindole heterocyclic core bearing a phosphonic acid side chain. Its synthesis was accomplished in ten steps.

INTRODUCTION

Cycles of protein phosphorylation and dephosphorylation are ubiquitous in eukaryotic cells, serving as the means by which outside signals affect cellular behavior.[1] Protein tyrosine phosphatases (PTPs), a major family of proteins containing a conserved CX5R active site motif, are implicated in cell division, metabolism, and differentiation.[2] One subclass of PTPs, low molecular weight protein tyrosine phosphatases (LMW-PTPs), are 18 kDa enzymes which, although lacking sequence homology to other PTPs outside of the conserved active site, exhibit a remarkable structural similarity to this family of proteins.[3] LMW-PTPs are involved in cytoskeletal remodeling and in growth factor receptor down regulation in cell signaling pathways due to growth factor stimulation.[4]

In general, higher levels of tyrosine phosphorylation have been observed upon oncogenic transformation. This observation has led to the proposed model that protein tyrosine kinases stimulate transformation, while PTPs negatively regulate transformation. Yet, contrary to this simple model, Kikawa, *et al.*, have found that overexpression of LMW-PTP is sufficient to transform non-transformed epithelial cells.[5] Acting as an oncoprotein, LMW-PTP both stimulates overexpression and dephosphorylation of the receptor protein tyrosine kinase EphA2, resulting in the observed transformation.[6] Therefore, the potential for regulation of oncogenic transformation through inhibition of LMW-PTPs makes this group of enzymes an attractive drug target. For example, recent work suggests that the activity of an anticancer drug, Aplidin, is at least partly due to direct inhibition of LMW-PTP.[7]

The human LMW-PTP exists as two well-characterized isoforms, HCPTP-A and HCPTP-B. Interestingly, the purine nucleotide, adenine, inhibits the A isoform while activating the B isoform.[8] Following co-crystallization of a yeast LMW-PTP with adenine, computationally based design of an isoform-specific inhibitor of human LMW-PTP became possible. Using the modeling program AutoDock, computational studies of bonding interactions on the X-ray structure of HCPTP-A, and a homology model of HCPTP-B, provided a starting point for the design of first-generation inhibitors.[9] We have previously reported the synthesis and assay of the simpler of these inhibitors. However, of greatest interest to us among these candidate compounds was one that would mimic the key interactions represented by **1** in the co-crystal structure of adenine with the yeast LMW-PTP enzyme. A phosphonate-containing 5-azaindole derivative (**2**) showed the potential to retain the most important interactions of His52/adenine-N1 binding and Asp132/adenine-N9 binding (Figure 1). This compound would also mimic the spatial placement of phosphate relative to the adenine core through use of an ethylene linker stable to hydrolysis and capable of displacing the ordered water molecule found in the crystal structure. However, due its greater complexity, the synthesis of **2** was not performed at the time of the earlier, simpler candidates.[9] Herein, we report the successful synthesis of 5-azaindole (**2**) and the results of assays with LMW-PTP.

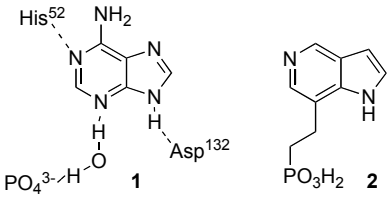


Figure 1. Key interactions of adenine with LMW-PTP in the X-ray co-crystal structure (**1**) and structure of a proposed inhibitor (**2**)

RESULTS AND DISCUSSION

Azaindoles in general have been widely recognized as pharmacologically significant scaffolds for drug development efforts.[10] Most commonly studied have been 6-aza- and 7-azaindoles, whereas the 5-

azaindole system has received less attention.[11] Of the previously developed methods, we chose to build upon the work of Yakhontov, *et al.*,[12,13] which would provide 6-chloro-7-cyano-1-(4-methoxybenzyl)-5-azaindoline (**3**) as a possible precursor of our target (**2**, Figure 2).

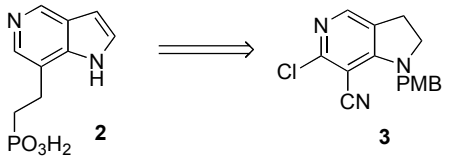
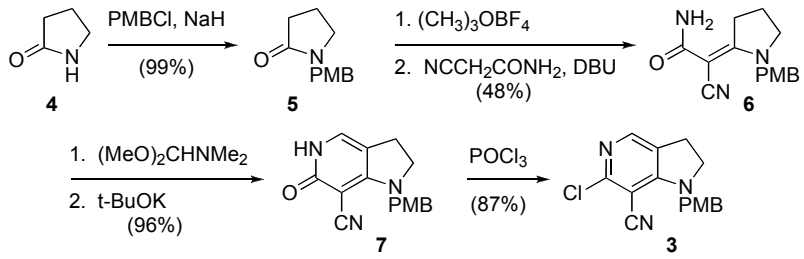


Figure 2. 5-Azaindoline (**3**) as a potential precursor of proposed inhibitor (**2**)

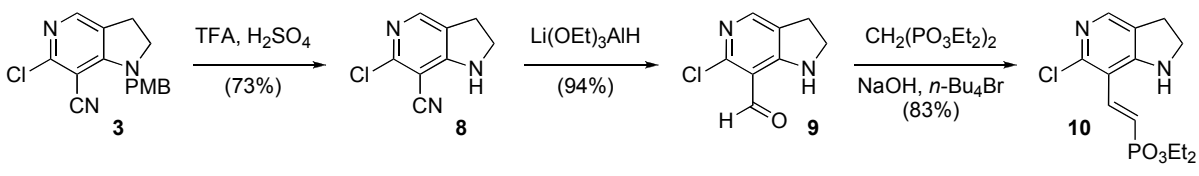
We modified the Yakhontov pathway to provide key intermediate (**3**) as depicted in Scheme 1. The synthesis began with the *p*-methoxybenzyl protection of 2-pyrrolidinone (**4**). Treatment of the protected pyrrolidinone (**5**) with trimethyloxonium tetrafluoroborate followed by the addition of 2-cyanoacetamide afforded 2-cyano-2-[1-(4-methoxybenzyl)pyrrolidin-2-ylidene]acetamide (**6**). Formation of the 5-azaindoline precursor (**7**) was accomplished by the addition of *N,N*-dimethylformamide dimethyl acetal to **6** followed by reaction with potassium *t*-butoxide. Treatment of **7** with phosphorus oxychloride gave the desired chlorinated 5-azaindoline (**3**).[13]

Scheme 1



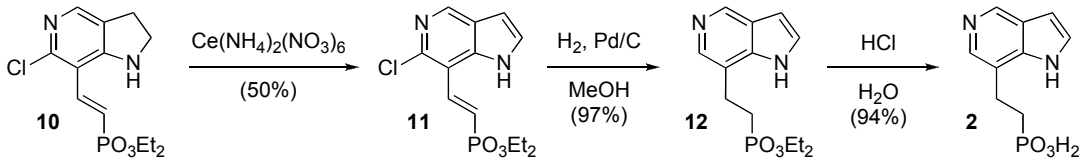
With the 5-azaindoline core in place, efforts were next directed at incorporation of the desired phosphonate side chain (Scheme 2). Deprotection of **3** with trifluoroacetic acid and sulfuric acid gave indoline (**8**) which was followed by reduction of the nitrile group with lithium triethoxyaluminohydride to yield the aldehyde (**9**).[14] This reduction step proved troublesome with several other reducing agents. Useful results were obtained only after thorough optimization of reagent selection and reaction conditions. In a key step, a Horner-Wadsworth-Emmons reaction of **9** with tetraethyl methylenediphosphonate afforded the unsaturated phosphonate (**10**).

Scheme 2



Continuation of the synthesis from this point provided the target, saturated 5-azaindole (**2**) (Scheme 3). Oxidation of the indoline (**10**) with ceric ammonium nitrate gave the chloroindole (**11**). Reduction of the conjugated phosphonate side chain and removal of the chloro group by catalytic hydrogenation and hydrogenolysis, respectively, were accomplished in a single operation. Finally, the resulting phosphonate ester (**12**) was converted to the 5-azaindole (**2**) by hydrolytic removal of the ethyl groups using concentrated hydrochloric acid.

Scheme 3



To determine the ability of the 5-azaindole (**2**) to inhibit HCPTP, *in vitro p*-nitrophenyl phosphate (pNPP) assays[15] were conducted with purified enzyme to which varying concentrations of **2** were added in order to calculate the inhibition constant, K_i. The inhibition constant for the reaction was evaluated based on the assumption of competitive inhibition of the reaction, and found to be 1.79 mM for HCPTP-A and 2.76 mM for HCPTP-B. Although these values are higher than predicted computationally, they are lower than most of those found for the previously synthesized compounds[9] and demonstrate the ability of the 5-azaindole scaffold (**2**) to inhibit the activity of HCPTP.

In conclusion, modifications of the synthetic pathway to 5-azaindoline (**3**) developed by Yakhontov, *et al.*,[12,13] have provided straightforward and relatively high-yielding access to one of our key, originally designed inhibitors (**2**) of LMW-PTP. Although the predicted and experimental activities are not in close agreement, our results have provided us with a good basis for the design of second-generation inhibitors for which syntheses are now underway in our laboratories. Of broader potential utility, we have developed a route for synthesis of usefully functionalized 5-azaindoles for which many other applications may be anticipated, including those involving synthetic nucleotide mimics and modified nucleic acids.

EXPERIMENTAL

1-(4-Methoxybenzyl)-2-pyrrolidinone (5).[16] To hexane-washed sodium hydride (2.35 g, 61 mmol, 60% dispersion in mineral oil) in tetrahydrofuran (25 mL, anhyd), at 0 ºC with stirring under nitrogen, was added a solution of 2-pyrrolidinone (3.84 mL, 51 mmol) in tetrahydrofuran (25 mL, anhyd) via a cannula. The mixture was left for 10 min at 0 ºC, and a solution of 4-methoxybenzyl chloride (6.9 mL, 51 mmol) in tetrahydrofuran (25 mL) was added dropwise. Solid tetrabutylphosphonium bromide (1.73 g, 5.1 mmol) was then added, and the solution was stirred for 24 h at 25 °C. The solution was quenched with saturated ammonium chloride, and the solvent was removed under vacuum. The residue was partitioned

between diethyl ether and water and extracted further with diethyl ether. The organic extracts were washed with brine, dried over magnesium sulfate, and filtered, and the solvent was removed under vacuum to afford the title compound as a colorless oil. The compound was further purified by column chromatography (9:1 ethyl acetate:hexanes, silica gel) to give a clear colorless oil (99% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (d, *J* = 8.7 Hz, 2H), 6.87 (dt, *J* = 8.4, 2.1 Hz, 2H), 4.40 (s, 2H), 3.81 (s, 3H), 3.26 (t, *J* = 6.6 Hz, 2H), 2.45 (t, *J* = 8.1 Hz, 2H), 1.99 (quin, *J* = 7.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.8, 159.4, 129.6, 129.0, 114.3, 55.4, 46.6, 46.2, 31.1, 17.9; IR 2935, 2837, 1682, 1610, 1514, 1247, 1176, 1032, 848, 819 cm^{-1}; HRMS (FAB$^+$, *m/z*) calcd for $C_{12}H_{16}NO_2$ (M+H$^+$) 206.1181, found 206.1173.

2-Cyano-2-[1-(4-methoxybenzyl)pyrrolidin-2-ylidene]acetamide (6). To a solution of **5** (10 g, 49 mmol) in acetonitrile (40 mL, anhyd) was added solid trimethyloxonium tetrafluoroborate (7.2 g, 49 mmol) slowly at 0 ºC while being stirred under nitrogen. The mixture was stirred at 25 °C for 2 h, and a solution of 2-cyanoacetamide (4.9 g, 58 mmol) in acetonitrile (200 mL, anhyd) was added. 1,8-Diazabicyclo[5.4.0]undecane (DBU, 8.8 mL, 58 mmol) was then added dropwise, and the mixture was stirred for 2 h at 25 °C. The reaction was quenched with water (100 mL), and acetonitrile was removed by distillation under vacuum. The solution was partitioned between water and dichloromethane and extracted with dichloromethane. The combined organic layers were washed with brine, dried over magnesium sulfate, and filtered, and the solvent was removed until **6** crystallized from the solution as a yellow solid. The crude product was purified by recrystallization from methanol to yield a colorless solid (6.4g, 48%). mp 166-168 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 (d, *J* = 8.4 Hz, 2H), 6.92 (dd, *J* = 6.3, 1.8 Hz, 2H), 4.99 (s, 2H), 3.82 (s, 3H), 3.49 (t, *J* = 7.5 Hz, 2H), 3.48 (t, *J* = 7.5 Hz, 2H), 3.43 (t, *J* = 8.1 Hz, 2H), 1.95 (quin, *J* = 7.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.7, 170.7, 168.2, 159.7, 129.4, 127.2, 121.7, 114.6, 55.6, 55.0, 51.3, 36.3, 20.1; IR 3419, 3333, 3000, 2957, 2170, 1670, 1634, 1030, 812 cm^{-1}; HRMS (FAB$^+$, *m/z*) calcd for $C_{15}H_{18}N_3O_2$ (M+H$^+$) 272.1399, found 272.1419.

7-Cyano-1-(4-methoxybenzyl-)-5-azaindolin-6(*5H*)-one (7). To a solution of **6** (5 g, 18 mmol) in *t*-butanol (75 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (5.15 g, 43 mmol) dropwise, and the solution was heated at reflux for 2 h, while being stirred. The reaction was cooled to 25 °C, solid potassium *tert*-butoxide (2.4 g, 21.5 mmol) was added, and the mixture was heated at reflux for another 2 h. The solvent was removed under vacuum, and the residue was partitioned between water and dichloromethane. The dichloromethane solution was washed with brine, dried over magnesium sulfate, and filtered, and the solvent was removed by distillation under vacuum to afford **7** as a fine yellow powder. The powder was triturated with ethyl acetate to form a colorless powder (4.9g, 96%). mp 222 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.22 (br s, 1H), 7.25 (dd, *J* = 7.0, 1.8 Hz, 2H), 7.00 (s, 1H), 6.91 (dd, *J* = 6.4, 2.1 Hz, 2H), 4.89 (s, 2H), 3.81 (s, 3H), 3.66 (t, *J* = 8.7 Hz, 2H), 2.84 (td, *J* = 7.8, 1.2 Hz, 2H); ^{13}C

NMR (125 MHz, $CDCl_3$) δ 166.0, 160.6, 159.7, 129.8, 129.6, 127.3, 116.9, 114.6, 114.1, 74.7, 55.6, 53.3, 49.9, 22.6; IR 2920, 2204, 1621, 1514, 1493, 1469, 1440, 1246, 1176, 1024 cm^{-1}; HRMS (FAB^+, *m/z*) calcd for $C_{16}H_{16}N_3O_2$ ($M+H^+$) 282.1243, found 282.1243.

6-Chloro-7-cyano-1-(4-methoxybenzyl)-5-azaindoline (3). To **7** (390 mg, 1.39 mmol) was added phosphorus oxychloride (3 mL), and the reaction was warmed to reflux for 30 min. The reaction was allowed to cool and was then partitioned between satd. aq. $NaHCO_3$ and CH_2Cl_2. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated under reduced pressure. The residual solid was purified by column chromatography [40:1 CH_2Cl_2 (satd. NH_3): MeOH, silica gel] resulting in 360 mg (87%) of a white solid. mp 123-126 °C; ^1H NMR (300 MHz, DMSO-d_6) δ 7.74 (s, 1H), 7.23 (d, *J* = 9.0 Hz, 2H), 6.94 (d, *J* = 8.7, 2H), 4.77 (s, 2H), 3.73 (s, 3H), 3.67 (t, *J* = 9.0 Hz, 2H), 2.96 (t, *J* = 8.7 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.6, 158.6, 152.7, 143.2, 129.5, 127.3, 126.5, 115.6, 114.5, 85.8, 55.5, 52.8, 49.8, 23.6; IR 2959, 2835, 2214, 1606, 1568, 1514, 1441, 1392, 1348, 1288, 1247, 1177, 1031, 992, 917, 814, 741 cm^{-1}; HRMS (FAB^+, *m/z*) calcd for $C_{16}H_1N_3OCl$ ($M+H^+$) 300.0904, found 300.0884.

6-Chloro-7-cyano-5-azaindoline (8). To **3** (2.3 g, 7.7mmol) in trifluoroacetic acid (11 mL) was added conc. H_2SO_4 (0.82 mL, 15.3 mmol) dropwise with stirring. The bright red solution was stirred at 25 °C for 1 h, quenched with water (5 mL), and poured carefully into satd. aq. $NaHCO_3$ (excess) with vigorous stirring. The precipitate was collected by filtration and washed with ice-cold methanol and dichloromethane/petroleum ether (1/1) to afford **8** as a white solid (1.01g, 73%). mp 248 °C; ^1H NMR (300 MHz, DMSO-d_6) δ 8.28 (br s, 1H), 7.74 (s, 1H), 3.71 (t, *J* = 8.4 Hz, 2H), 2.99 (t, *J* = 9.0 Hz, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.3, 149.8, 143.8, 127.3, 115.4, 85.3, 47.9, 25.2; IR 3193, 3154, 2219, 1607, 1513, 1421, 1333, 1321, 1281, 1225, 1140, 1034, 1001, 906, 760 cm^{-1}; HRMS (FAB^+, *m/z*) calcd for $C_8H_7N_3Cl$ ($M+H^+$) 180.0329, found 180.0347.

6-Chloro-5-azaindoline-7-carbaldehyde (9). To a solution of **8** (24 mg, 0.13 mmol) dissolved in THF (3 mL, anhyd) was added 1 N $Li(EtO)_3AlH$ (0.27 mL, 0.27 mmol) in THF. The mixture was stirred at 25 °C for 1 h, and 5 N H_2SO_4 (2 mL) was added. The mixture was partitioned between satd. $NaHCO_3$ (aq) and CH_2Cl_2. The aq. layer was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated. The residual oil was purified by column chromatography [40:1 CH_2Cl_2 (satd. NH_3):MeOH, silica gel] yielding 23 mg (94%) of **9** as an off-white solid. mp 128-131 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 10.23 (s, 1H), 7.75 (s, 1H), 7.64 (br s, 1H), 3.92 (t, *J* = 8.7 Hz, 2H), 3.07 (t, *J* = 8.7 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.6, 160.2, 152.4, 143.9, 127.4, 109.5, 47.9, 24.4; IR 3338, 2920, 1648, 1601, 1580, 1509, 1297, 1207, 1027, 918 cm^{-1}; HRMS (FAB^+, *m/z*) calcd for $C_8H_8N_2OCl$ ($M+H^+$) 183.0325, found 183.0348.

Diethyl (*E*)-2-(6-Chloro-5-azaindolin-7-yl)ethenylphosphonate (10). To a solution of **9** (125 mg, 0.69 mmol) in CH_2Cl_2 (2 mL) was added tetraethyl methylenediphosphonate (0.32 mL, 1.3 mmol), 50% aq. NaOH (0.5 mL) and a catalytic amount of tetrabutylammonium bromide. The mixture was stirred for 2 h at 25 ^{0}C and was then partitioned between CH_2Cl_2 and satd. aq. $NaHCO_3$. The aq. layer was extracted with CH_2Cl_2 (2 x 30 mL). The combined organic layers were dried over $MgSO_4$ and concentrated. The residual oil was purified by first triturating the oil with hexanes followed by column chromatography [30:1 CH_2Cl_2 (satd. NH_3): MeOH, silica gel], yielding 180 mg (83%) of **10** as a white solid. mp 160-162 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.58 (dd, *J* = 24.2, 18.0 Hz, 1H), 6.18 (t, *J* = 18.0 Hz, 1H), 5.72 (br s, 1H), 4.10 (dq, *J* = 8.0, 7.0 Hz, 4H), 3.75 (t, *J* = 8.5 Hz, 2H), 3.03 (t, *J* = 8.6 Hz, 2H), 1.32 (t, *J* = 6.9 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.4 (d, *J* = 1.6 Hz), 150.0, 142.3, 126.1, 117.7, 116.2, 109.6 (d, *J* = 24.3 Hz), 62.3 (d, *J* = 5.7 Hz), 47.9, 25.8, 16.5 (d, *J* = 6.2 Hz); IR 3337, 2981, 1614, 1570, 1498, 1472, 1394, 1306, 1232, 1020, 967, 912, 852 cm^{-1}; HRMS (FAB$^+$, *m/z*) calcd for $C_{13}H_{19}N_2O_3ClP$ (M+H$^+$) 317.0822, found 317.0810.

Diethyl (*E*)-2-(6-Chloro-5-azaindol-7-yl)ethenylphosphonate (11). To **10** (70 mg, 0.22 mmol) in dichloromethane (2 mL) was added ceric ammonium nitrate (CAN, 242 mg, 0.44 mmol) and water (0.2 mL). The biphasic solution was bright orange and bubbled vigorously. The mixture was stirred at 25 °C for 1 h and partitioned between dichloromethane and satd. aq. sodium bicarbonate. The organic layer was dried over magnesium sulfate, filtered, and concentrated. The reaction had not gone to completion, and therefore, the solid residue was redissolved in CH_2Cl_2 (5 mL). H_2O (0.5 mL) and CAN (242 mg) were added, and the mixture was stirred at 25 °C for 2 h. Workup as above gave a residual oil which was purified by column chromatography [30:1 CH_2Cl_2 (satd. NH_3): MeOH, silica gel] to give 35 mg (50%) of **11** as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.60 (br s, 1H), 8.66 (s, 1H), 7.89 (dd, *J* = 24.2, 18.2 Hz, 1H), 7.35 (t, *J* = 2.6 Hz, 1H), 6.80 (t, *J* = 18.2 Hz, 1H), 6.65-6.68 (m, 1H), 4.22 (dq, *J* = 7.5, 7.0 Hz, 4H), 1.41 (t, *J* = 6.9 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.2, 141.8 (d, *J* = 6.5 Hz), 139.5, 128.0, 126.0, 120.5, 119.0, 113.6 (d, *J* = 24.3 Hz), 102.3, 62.9 (d, *J* = 5.8 Hz), 16.6 (d, *J* = 6.5 Hz); IR 3215, 2982, 1615, 1512, 1449, 1393, 1240, 1024, 968, 854, 791, 736, 665 cm^{-1}; HRMS (FAB$^+$, *m/z*) calcd for $C_{13}H_{17}N_2O_3ClP$ (M+H$^+$) 315.0665, found 315.0670.

Diethyl 2-(5-Azaindol-7-yl)ethylphosphonate (12). To a solution of **11** (9 mg, 0.0286 mmol) dissolved in MeOH (2 mL) was added 5% palladium on carbon (6 mg, 0.00286 mmol), and the mixture was placed under H_2 at 1 atmosphere for 17 h. The mixture was filtered, concentrated, and purified by column chromatography [20:1 CH_2Cl_2 (satd. NH_3): MeOH, silica gel], yielding 7.8 mg (97%) of **12** as a clear colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.83 (br s, 1H), 8.84 (s, 1H), 8.10 (s, 1H), 7.29 (dd, *J* = 3.2, 2.2 Hz, 1H), 6.63 (dd, *J* = 2.2, 1.9 Hz, 1H), 4.06 (dq, *J* = 7.5, 7.0 Hz, 4H), 3.20 (dt, *J* = 19.2, 6.9 Hz, 2H), 2.16 (dt, *J* = 18.8, 6.9 Hz, 2H), 1.27 (t, *J* = 7.05 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 142.8, 140.0,

139.1, 126.1, 125.0, 119.6 101.9, 62.3 (d, *J* = 6.5 Hz), 29.9, 26.2, 25.1, 22.0 (d, *J* = 4.5 Hz), 16.5 (d, *J* = 6.2 Hz); IR 3152, 2925, 1610, 1589, 1443, 1349, 1234, 1162, 1103, 1053, 1027, 966, 736 cm^{-1}; HRMS (FAB^+, *m/z*) calcd for $C_{13}H_{20}N_2O_3P$ ($M+H^+$) 283.1212, found 283.1200.

2-(5-Azaindol-7-yl)ethylphosphonic acid (2). Compound (**12**) (8 mg, 0.03 mmol) was dissolved in concd. aq. HCl (4 mL) and heated at reflux for 20 h. The mixture was cooled to 25 °C and concentrated. The residual solid was twice redissolved in water and concentrated under vacuum to remove excess HCl, resulting in **2** as an oil complexed with HCl (8 mg, 94%). ^1H NMR (300 MHz, D_2O) δ 8.81 (s, 1H), 8.07 (s, 1H), 7.68 (d, *J* = 3.0 Hz, 1H), 6.94 (d, *J* = 3.0 Hz, 1H), 4.67-4.83 (m, 3H), 3.11-3.23 (m, 2H), 2.00-2.14 (m, 2H); ^{13}C NMR (125 MHz, CD_3OH) δ 143.3, 135.6, 133.2, 130.3, 126.1, 125.5, 125.4, 106.0, 35.5, 28.2, 27.3, 23.8; IR 3034, 2812, 1630, 1463, 1350, 1102, 985 cm^{-1}; HRMS (FAB^+, *m/z*) calcd for $C_9H_{12}N_2O_3P$ ($M+H^+$) 227.0586, found 227.0573.

Enzymatic inhibition assays. Enzymatic activity was monitored via a continuous 10-min reaction with the substrate analog *p*-nitrophenyl phosphate (pNPP).[15] The accumulation of *p*-nitrophenol was measured at 405 nm with an extinction coefficient of 185 $M^{-1}cm^{-1}$. Enzymatic assays were performed at 25 °C in 100 mM sodium acetate, pH 5.0, which had been adjusted to an ionic strength of 150 mM with NaCl. For these assays, the concentration of BSA was adjusted to 0.5 mM, and pNPP was adjusted to 1.0 mM. Inhibitors were evaluated at 0 mM, 0.5 mM, 1.0 mM, 2.0 mM and 5 mM concentrations. The assays were initiated by the addition of enzyme, after incubation with the 5-azaindole for 2 min. The enzyme concentrations used were 55.6 nM HCPTP-A and 111 nM HCPTP-B. The reactions were carried out in duplicate for each isoenzyme. The kinetic parameters of maximum velocity and the Michaelis constant were obtained for each reaction. The inhibition constant for the 5-azaindole (**2**) was then calculated using the formula $K_i = I/((K_{m(apparent)}/K_m)-1))$, where K_m is the Michaelis constant and $K_{m(apparent)}$ is the apparent Michaelis constant for the reactions conducted with inhibitor present.

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