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Synthesis of *p*-Aminophenyl Aryl H-Phosphinic Acids and Esters via Cross-Coupling Reactions: Elaboration to Phosphinic Acid Pseudopeptide Analogues of Pteroyl Glutamic Acid and Related Antifolates

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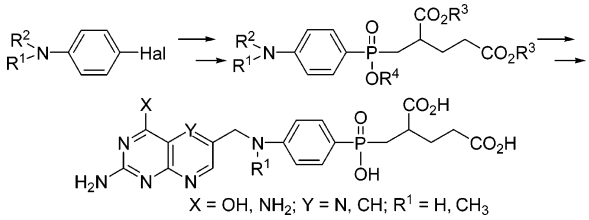

The synthesis of suitably protected *p*-aminophenyl H-phosphinic acids and esters from the corresponding para-substituted aryl halides has been accomplished via the Pd-catalyzed cross-coupling reaction of anilinium hypophosphite, either in the absence or presence of a tetraalkyl orthosilicate, to provide the free H-phosphinic acid or the corresponding ester, respectively. Subsequent conjugate addition of either a P^{III} species or phosphorus anion, generated in situ from either the free H-phosphinic acid or ester, to a 2-methylene glutaric acid ester provided the aryl phosphinic acid analogue of *p*-aminobenzoyl glutamic acid. Alkylation of these suitably protected *p*-aminophenyl phosphinic acid esters with a 6-(bromomethyl)-pteridine or the corresponding (bromomethyl)pyridopyrmidine, followed by hydrolytic removal of protecting groups, provided the target aryl phosphinic acid analogues of folic acid and related antifolates. Alternatively, for the synthesis of the folate or 5-deazafolate analogues on a slightly larger scale, reductive amination with either N^2-acetyl or N^2-pivaloyl-6-formylpterin or the corresponding formylpyridopyrmidine and the same suitably protected *p*-aminophenyl phosphinic acid esters, followed by removal of protecting groups, is preferred. In the course of this research, it was observed that the nucleophilicity of both the aniline nitrogen and various P^{III} species derived from *p*-aminophenyl phosphinic acid derivatives is significantly reduced compared to that of the unsubstituted counterpart.

Introduction

In contrast to widespread use of antibiotics and other drugs for the treatment of bacterial diseases, generally via chemotherapy, the development of chemotherapy for the treatment of parasitic diseases[1,2] has been limited, due to the close similarities between parasite and host metabolism. However, it has been known for many years that a significant difference exists between human and microorganisms, including the malaria parasites, in the folic acid biosynthesis pathway.[3−6] In mammalian cells, there is no pathway available to affect the de novo synthesis of reduced folates. Therefore, the vitamin folic acid and its reduced derivatives must be obtained from dietary intake. Unlike mammalian cells, many microorganisms such as *Plasmodium falciparum*, the important parasite causing serious malaria, have the ability to synthesize de novo the partially reduced folate, 7,8-dihydrofolic acid, which is then converted

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(1) Despommier, D. D.; Gwadz, R. W.; Hotez, P. J. *Parasitic Diseases*, 3rd ed.; Springer-Verlag New York, Inc.: New York, 1995.

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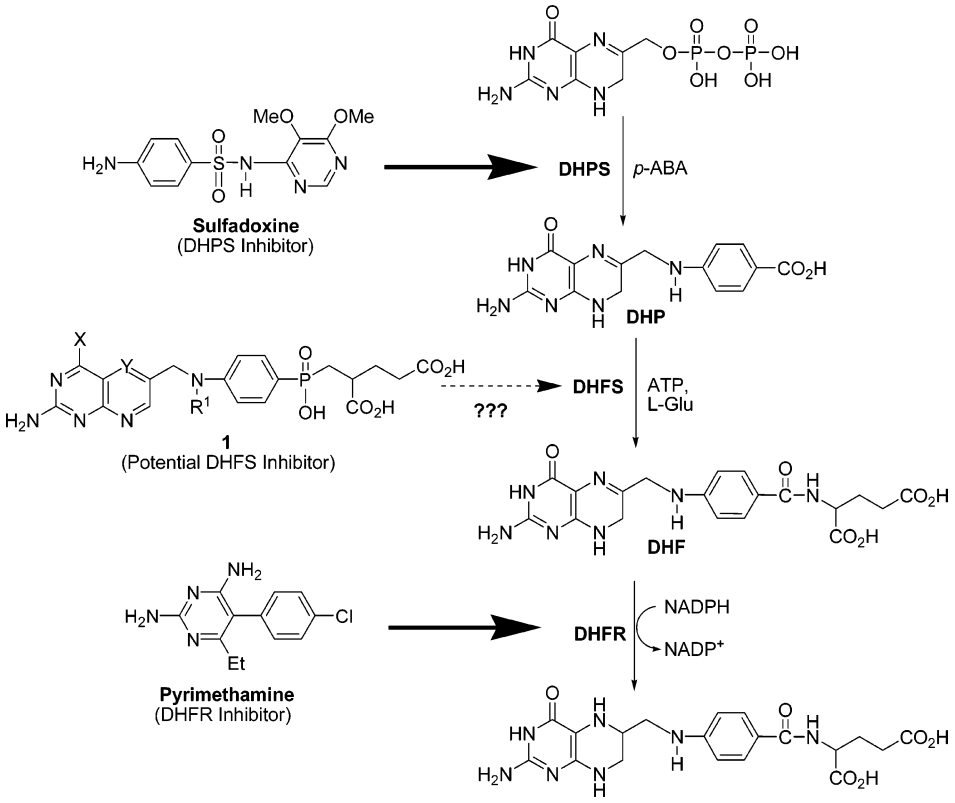

FIGURE 1. Partial folate biosynthesis pathway in microorganisms as targets for drug discovery.

to a variety of reduced folylmono- and poly-γ-glutamates via normal host and/or parasite metabolism. Intermediates that play important roles in folate biosynthesis, including 7,8-dihydropteroate (DHP), 7,8-dihydrofolate (DHF), 5,6,7,8-tetrahydrofolate (THF), and 5,10-methylene-5,6,7,8-tetrahydrofolate (5,10-CH_2-THF), have been shown to be present in the malaria parasite.[6] On the basis of this significant difference between mammalian cells and the malaria parasite, inhibition of the folate biosynthesis pathway in the parasite has been extensively investigated with the goal of developing drugs which could kill the parasite while leaving the human host unaffected.

The folate biosynthesis pathway has provided a remarkably rich source of targets for developing new drugs.[7−9] Folate antagonism has been widely developed for cancer treatment and the use of antifolates has occupied an important position for over 50 years. Folate antagonism has also proved to be a good strategy for development of antibacterial drugs, including those that target the malaria parasite. For example, sulfadoxine (4-amino-*N*-(5,6-dimethoxypyrimidin-4-yl)benzenesulfonamide), an inhibitor of dihydropteroate synthetase (DHPS, EC 2.5.1.15), and pyrimethamine (5-(4-chlorophenyl)-6-ethylpyrimidine-2,4-diamine), an inhibitor of dihydrofolate reductase (DHFR, EC 1.5.1.3), can be used in combination for the successful treatment of certain drug-resistant strains of the malaria parasite (Figure 1).[6,10] Other DHFR and DHPS inhibitor combinations have also been developed as effective antimalarials.[6,7]

Less extensively studied as a potential target for new antimalarial drugs is 7,8-dihydrofolate synthetase (DHFS, EC 6.3.2.12), which catalyzes the ATP-dependent conversion of DHP to DHF by addition of a single L-glutamate moiety.[11] We have recently reported the synthesis of potent inhibitors of folylpoly-γ-glutamate synthetase (FPGS, EC 6.3.2.17) based on pseudopeptides containing complex alkyl phosphinic acids.[12−14] Herein we describe the design of heretofore unknown pseudopeptides containing aryl phosphinic acids as potential DHFS inhibitors. Interestingly, in both *E. coli*[15] and *P. falciparum*,[16] DHFS activity is contained in a bifunctional protein that also harbors folylpoly-γ-glutamate synthetase (FPGS, EC 6.3.2.17) activity. For this reason, it has been considered unlikely that it would be possible to specifically inhibit only the DHFS locus of this bifunctional protein. However, as reported recently, an unexpected dihydropteroate binding site, distinct from the folate site previously identified in FPGS,[17] is revealed in the crystal structure of folC, the bifunctional enzyme from *E. coli*.[15] This discovery suggests the design of potential specific inhibitors of the DHFS locus of the bifunctional enzyme, including that from *P. falciparum*.

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(10) What is Malaria?; World Health Organization: ***2006***; http://www.rollbackmalaria.org/malariaFAQ.html.
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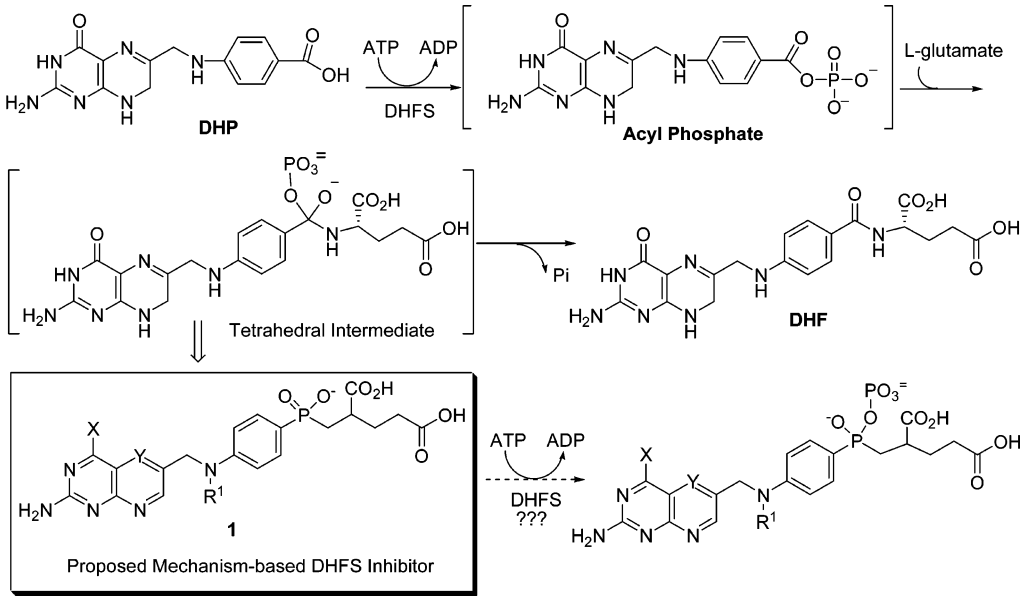
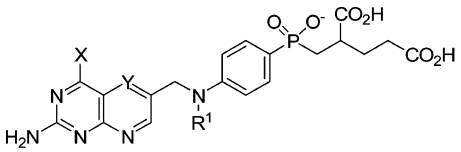
(16) Salcedo, E.; Cortese, J. F.; Plowe, C. V.; Sims, P. F. G.; Hyde, J. E. *Mol. Biochem. Parasitol.* **2001**, *112*, 239−252.
(17) Sun, X. L.; Cross, J. A.; Bognar, A. L.; Baker, E. N.; Smith, C. A. *J. Mol. Biol.* **2001**, *310*, 1067−1078.

SCHEME 1



The proposed reaction mechanism of the DHFS-catalyzed reaction is depicted in Scheme 1. In this mechanism, the "accepting substrate", DHP, is converted to an acyl phosphate intermediate via an ATP-dependent process.[18] Subsequent reaction leads to a tetrahedral intermediate resulting from attack of the activated carbonyl by the amine moiety of the "incoming substrate", L-glutamate. The tetrahedral intermediate then collapses to form a new amide bond concomitant with the expulsion of P_i. On the basis of this mechanism, the synthesis of stable aryl phosphinic acid mimics, **1**, of the proposed tetrahedral intermediate as a new class of DHFS inhibitors has been investigated.

A series of aryl phosphinic acids, **1a**−**e**, has been designed to incorporate both the "tetrahedral mimic" and a heterocyclic portion analogous to the pterin moiety of the natural substrate, 7,8-dihydrofolate. The stable aryl phosphinic mimic could



1a, X = OH, Y = N, R^1 = H *(folate)*
1b, X = NH_2, Y = N, R^1 = Me *(methotrexate)*
1c, X = OH, Y = CH, R^1 = H *(5-deazafolate)*
1d, X = OH, Y = N, R^1 = Me *(10-methyl folate)*
1e, X = NH_2, Y = N, R^1 = H *(aminopterin)*

possibly be phosphorylated in the presence of ATP by a DHFS-catalyzed reaction, leading to a phosphorylated phosphinate,[19−24] thus leading to an even closer mimic of the transient intermediate formed during catalysis (Scheme 1). In the course of this investigation, development of methods for the efficient synthesis of phosphinic acid esters **7**, which carry both aryl C−P and alkyl C−P bonds, was pursued. The *para* disposition of the phosphorus substituent and the amino group results in attenuation of the reactivity of both nucleophiles: i.e., the aryl phosphinic acid (or P^{III} species derived therefrom) and the aniline nitrogen of **7** or its precursors. The consequences of this altered reactivity as it affects the synthesis of the desired target compounds and key intermediates have been investigated in detail.

Results and Discussion

The proposed DHFS inhibitor **1** is composed of three fragments including the heterocycle, a *p*-aminophenylphosphinic acid portion similar to *p*-aminobenzoic acid (pABA), and a glutarate portion similar to glutamate (Scheme 1). Under physiological conditions, the phosphinic acid would be deprotonated and, as such, mimic the tetrahedral intermediate postulated to form during DHFS-catalyzed synthesis of DHF. Retrosynthetic analysis (Scheme 2) suggests two approaches to coupling the *p*-aminophenylphosphinic acid ester moieties, **7**, to the heterocycle. Thus, an S_N2 alkylation of **7** by **8**[25−27] or a reductive amination of **9** by **7**[28−30] is proposed for the C−N bond formation. The key intermediate **7** is an unusual phosphinic acid ester that includes both an aryl C−P bond and an alkyl P−C bond. As shown in Scheme 2, a retrosynthetic analysis indicates

(18) Banerjee, R. V.; Shane, B.; McGuire, J. J.; Coward, J. K. *Biochemistry* **1988**, *27*, 9062−9070.
(19) Abell, L. M.; Villafranca, J. J. *Biochemistry* **1991**, *30*, 6135−6141.
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(22) Chen, S. J.; Lin, C. H.; Walsh, C. T.; Coward, J. K. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 505−510.
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(24) Pai, C. H.; Chiang, B. Y.; Ko, T. P.; Chou, C. C.; Chong, C. M.; Yen, F. J.; Chen, S. J.; Coward, J. K.; Wang, A. H. J.; Lin, C. H. *EMBO J.* **2006**, *25*, 5970−5982.
(25) Piper, J. R.; Montgomery, J. A. *J. Org. Chem.* **1977**, *42*, 208−211.
(26) Piper, J. R.; McCaleb, G. S.; Montgomery, J. A. *J. Heterocycl. Chem.* **1987**, *24*, 279−282.
(27) Feng, Y.; Coward, J. K. *J. Med. Chem.* **2006**, *49*, 770−788.
(28) Maunder, P.; Finglas, P. M.; Mallet, A. I.; Mellon, F. A.; Razzaque, M. A.; Ridge, B.; Vahteristo, L.; Witthoft, C. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1311−1323.
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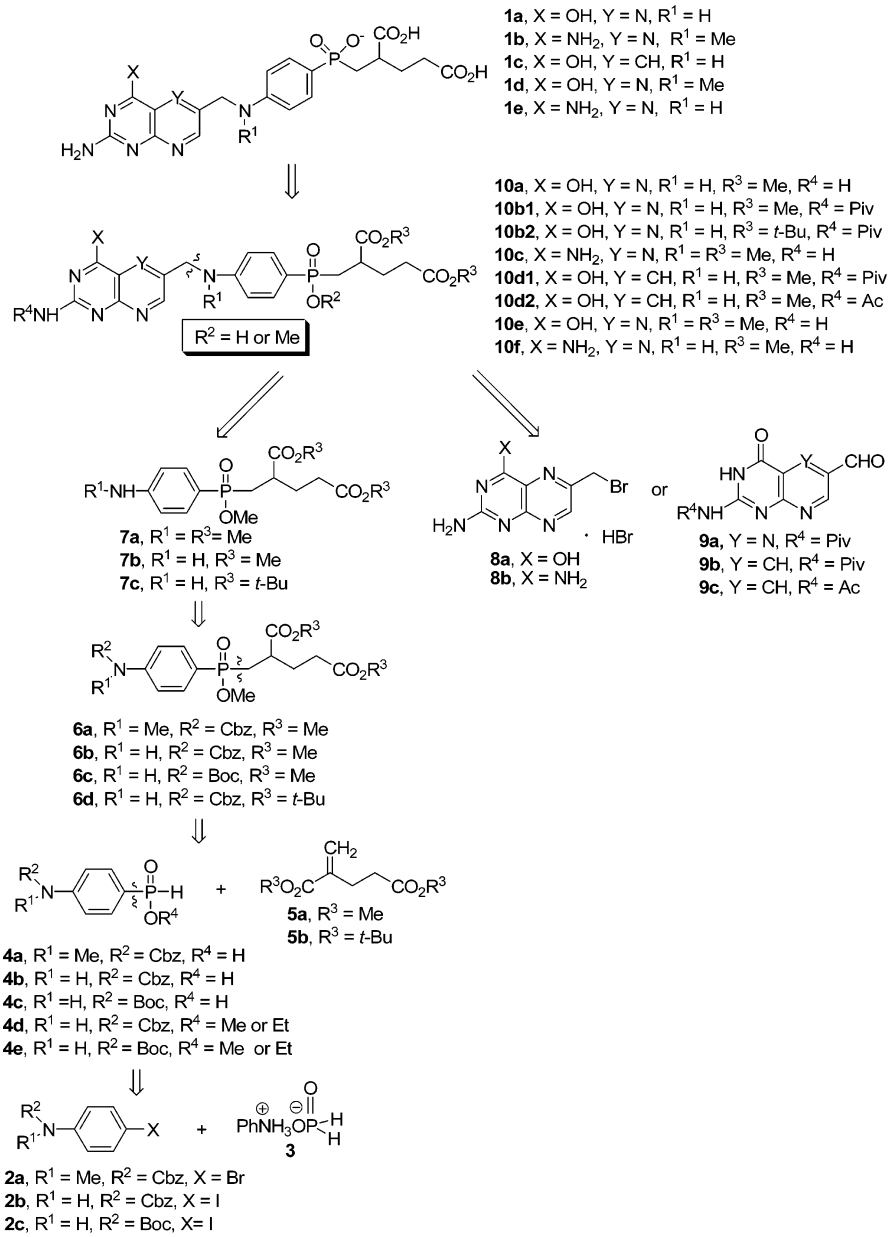
(30) Taylor, E. C.; Hamby, J. M.; Shih, C.; Grindey, G. B.; Rinzel, S. M.; Beardsley, G. P.; Moran, R. G. *J. Med. Chem.* **1989**, *32*, 1517−1522.

SCHEME 2

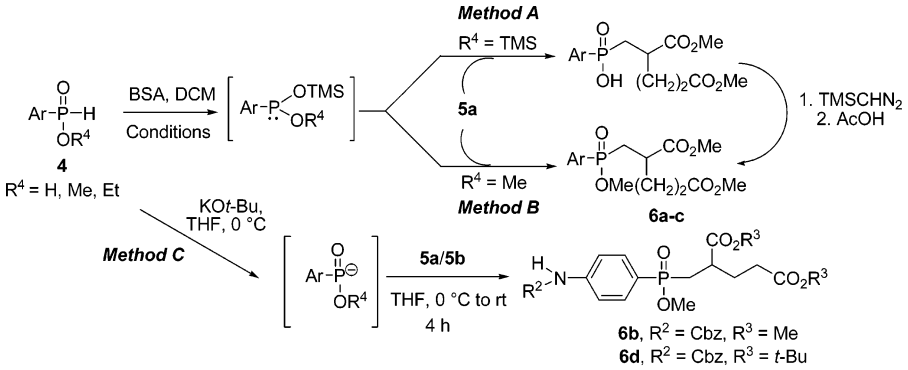


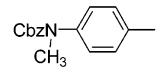
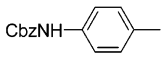
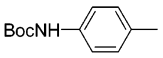
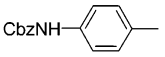
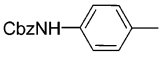
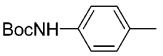
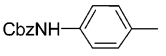
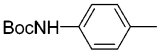
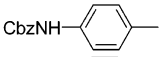
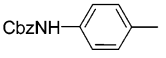
that formation of an alkyl C−P bond leading to the key intermediate **7** could be achieved by Michael addition between phosphinic acid **4**, either as the free acid **4a**−**c** or the corresponding phosphinic acid ester **4d**,**e**, and 2-methyleneglutarate **5**.[13,27] The aryl C−P bond could be established via a palladium-catalyzed cross-coupling reaction between **2** and **3** to yield **4a**−**c**,[31,32] or alternatively, the aryl C−P bond could be installed via a similar palladium-catalyzed cross-coupling reaction between **2** and **3** in the presence of $(EtO)_4Si$ or $(MeO)_4Si$ to form the phosphinic acid esters **4d** or **4e** in a single operation.[33,34]

In pursuit of this synthetic strategy, H-phosphinic acid **4a** was obtained via a palladium-catalyzed coupling reaction between benzyl 4-bromophenyl(methyl)carbamate (**2a**) and anilinium hypophosphite (**3**) in the presence of $Pd(PPh_3)_4$. ^{31}P NMR indicated that the crude H-phosphinic acid **4a** was afforded in good yield with sufficient purity for use in the next reaction. Michael addition of the corresponding $ArP(OTMS)_2$ P^{III} species, formed in situ by reaction of **4a** with *N*,*O*-(bistrimethylsilyl)acetamide (BSA), to dimethyl 2-methyleneglutarate (**5a)** followed by methylation of the resulting phosphinic acid ($TMSCHN_2$) and purification by silica gel chromatography led to **6a** in 45% yield over 3 steps (Table 1, Method A). Although **4b** and **4c** could be obtained in high yield (^{31}P NMR) by using the same Pd-catalyzed cross-coupling reaction, subsequent Michael addition to **5a** to provide **6b** or **6c** was unsuccessful in the case of **4b** and proceeded in only very low yield (6%) in the case of **4c**. It should be noted that generation of the requisite P^{III} intermediate could be observed in the BSA-mediated reaction of **4a** (^{31}P NMR, δ 140 ppm) but not in the identical reaction of **4b**.

(31) Montchamp, J.-L.; Dumond, Y. R. *J. Am. Chem. Soc.* **2001**, *123*, 510−511.
(32) Schwan, A. L. *Chem. Soc. Rev.* **2004**, *33*, 218−224.
(33) Deprele, S.; Montchamp, J. L. *J. Organomet. Chem.* **2002**, *643*, 154−163.
(34) (a) Huang, Z.; Bravo-Altamirano, K.; Montchamp, J.-L. *C. R. Chim.* **2004**, *7*, 763−768. (b) Bravo-Altamirano, K.; Huang, Z.; Montchamp, J.-L. *Tetrahedron* **2005**, *61*, 6315−6329.

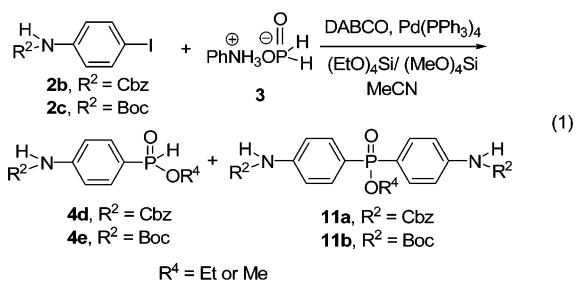
TABLE 1. Conversion of Substituted Aryl H-Phosphinic Acids or Esters (4) to the Corresponding Aryl-Alkyl Phosphinic Acid Esters (6)



Entry	Method	Ar	R^4	Conditions	Yield 6, %
1	A		H	rt, 24 h	77[a]
2	A		H	Δ, 3 d[b]	58[a]
3	A		H (**4a**)	rt, 24 h	45[c] (**6a**)
4	A		H (**4b**)	rt, 48 h	No reaction[a,d]
5	A		H (**4c**)	rt, 24 h	6[b,e]
6	B		Me (**4d**)[f]	Δ, 7 d	71[g] (**6b**)
7	B		Me (**4d**)	80 °C, 4 h[h]	50[g] (**6b**)
8	B		Me (**4e**)[f]	Δ, 7 d	53[g] (**6c**)
9	B		Me (**4d**)	Δ, 1 d[b]	25[g] (**6d**)
10	C		Me (**4e**)[i]	0 °C --> rt, 4 h	57 (**6c**)
11	C		Me (**4d**)	0 °C --> rt, 4 h	51 (**6b**)
12	C		Me (**4d**)	0 °C --> rt, 4 h	57 (**6d**)

[a] Overall yield from **4** (two steps). [b] Reaction of P^{III} species with **5b** in DCM (entry 2) or THF (entry 9). [c] Overall yield from **2** (three steps). [d] Also observed no reaction with methyl acrylate. [e] Poor reaction with methyl acrylate, yield 7%. [f] Reaction of the ethyl esters of **4d** and **4e** (R^4 = Et) under identical conditions led to **6b** and **6c** (−P(O)(OEt)−) in 53% and 47% yield, respectively. [g] Yields are for single-step reaction from **4**. [h] Microwave irradialtion, 250 W. [i] R^4 = Me, NaOMe was used as the base.

An alternative approach to **6b** or **6c** involved conjugate addition of the H-phosphinic acid alkyl ester, **4d** or **4e**, to 2-methyleneglutarate **5a**. A similar palladium-catalyzed cross-coupling of **2b** or **2c** and **3**, but in the presence of $(EtO)_4Si$ or $(MeO)_4Si$, afforded ester **4d** or **4e**, respectively, in a single step with moderate yield after purification by column chromatography (eq 1). Initially, the use of elevated temperature (80 °C)

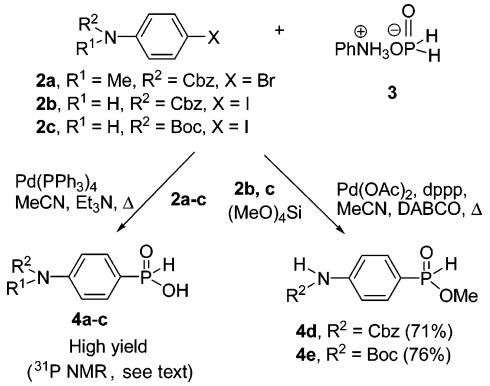


(1)

led to a low yield (26%) of **4d** and a large amount of a diaryl phosphinic acid ester **11a** (^{31}P NMR, δ 32−34 ppm). Ultimately, a decrease in temperature (50 °C) and high dilution (0.05−0.11 M of **2b** or **2c**) led to an improved yield of the desired monoaryl phosphinic acid esters, **4d** (64%) and **4e** (55%) (^{31}P NMR, δ 25−28 ppm). Unfortunately, generation of **11** was difficult to exclude completely under these conditions. However, when $Pd(OAc)_2$ and 1,3-bis(diphenylphosphino)propane (dppp) were utilized, compounds **4d** and **4e** were furnished exclusively in good yield. The bidentate phosphine ligand, dppp, could promote and facilitate concerted reductive elimination by occupying only two coordination sites cis to each other, forcing the carbon group and phosphorus group to be also cis to each other.[35] In addition, the use of $Pd(PPh_3)_4$ as catalyst results in the formation of a small amount of $Ph_3P{=}O$, which is difficult

(35) Diederich, F.; Stang, P. J. In *Metal-catalyzed Cross-coupling Reactions*; Wiley-VCH: Weinheim , Germany, 1998; pp 26−27.

SCHEME 3

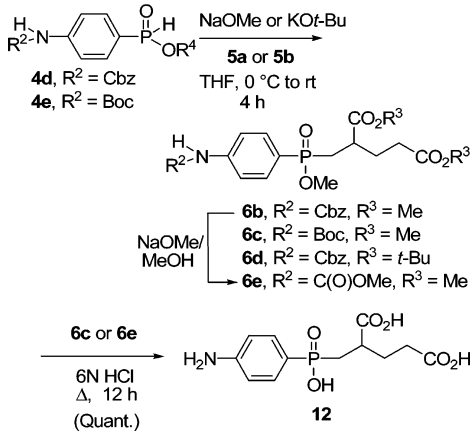


to remove completely during purification of **4d** or **4e**. The results obtained for the Pd-catalyzed cross-coupling reactions in the synthesis of aryl H-phosphinic acids or esters **4a**−**e** are summarized in Scheme 3.

There is considerable literature precedent, including from this laboratory,[13,27] for the synthesis of phosphinic acids via addition of P^{III} species, generated in situ from alkyl H-phosphinic acids, to electrophilic olefins. However, to our knowledge, very few examples involved a P^{III} species derived from an aryl H-phosphinic acid[36−38] or ester. None involved a P^{III} species derived from a substituted aryl H-phosphinic acid or ester. Thus, two routes involving a Michael reaction between **4d**,**e** and **5a**,**b** were pursued (Table 1, Method B). First, BSA-mediated formation of mono-TMS esters (^{31}P NMR, δ 146 ppm) and subsequent addition of the P^{III} species generated in situ was investigated. Under varying reaction conditions, long reaction times were required to obtain reasonable yields of the desired product. Although the use of microwave conditions to increase reaction rates[39] led to a marked reduction in reaction time, it was not effective in leading to enhanced yields (Supporting Information, Table S1).

This research indicates that the nucleophilicity of the phosphorus P^{III} species derived from **4** is attenuated, apparently due to the *p*-acyloxyamino (carbamate) substituent. Thus, BSA-mediated Michael addition of *N*-Cbz- (**4d**) or *N*-Boc- (**4e**) *p*-amino phenyl H-phosphinic methyl esters, respectively, to 2-methyleneglutarate esters **5a** or **5b** required elevated temperatures and extended reaction times to obtain moderate to good yields of the desired phosphinic acids, **6** (Table 1, entries 6, 8, 9; see also Table S1, Supporting Information). In contrast, reaction of a simple, unsubstituted phenylphosphinic acid at room temperature for 24 h proceeded in good yield (Table 1, entries 1 and 2). It should be recalled that the non-esterified *N*-(Me)Cbz H-phosphinic acid, **4a**, reacted at room temperature to provide the *N*-methyl derivative, **6a**, in 45% yield (vide supra). A major benefit of the TMS group in the conjugate addition reaction involves stabilization of developing positive charge on phosphorus by silicon β to phosphorus.[40] Therefore, it is not surprising that the P−OMe esters **4d** and **4e** involving

SCHEME 4



a ArP(OMe)(OTMS) P^{III} intermediate are less reactive and require longer reaction times at elevated temperatures to provide acceptable yields of **6** than is the case with the corresponding free H-phosphinic acid **4a** involving a ArP(OTMS)$_2$ P^{III} intermediate. However, of the latter group of H-phosphinic acids, only the *N*-(Me)Cbz H-phosphinic acid **4a** led to acceptable yields. *N*(Cbz)$_2$ H-phosphinic acid resulted in very low yield, which was detected by LC-MS, and *N*(Boc)$_2$ H-phosphinic acid gave 22% yield.

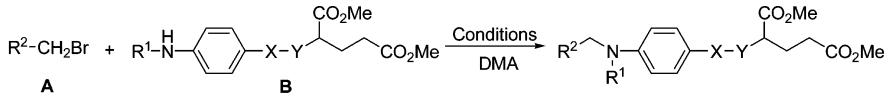
A third alternative to the synthesis of aryl-alkyl phosphinic acids **6** involved the Michaelis−Becker (Pudovik) reaction, which was examined with either NaOMe[41] or KO*t*-Bu as the base (Scheme 4; Table 1, Method C). Use of NaOMe as the base in the reaction of **4e** gave the expected product **6c** (57%). However, the identical reaction of **4d** yielded the desired product **6b** (25%) in addition to a methyl carbamate byproduct **6e** (33%). A similar methanolysis was reported recently by Shieh et al.,[42] in which a heteroaromatic Cbz carbamate was converted to the corresponding methyl carbamate. An *N*-Cbz protected aliphatic amine elsewhere on the molecule was unaffected. In the current research, when KO*t*-Bu was used as a less nucleophilic, bulky base, **6b** and **6d** were obtained in modest yields. Acid hydrolysis of **6c** and **6e** yielded **12**, the free aryl phosphinic acid analogue of *p*-aminobenzoylglutamic acid.

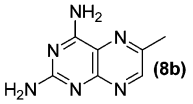
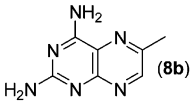
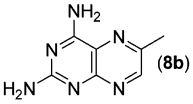
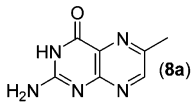
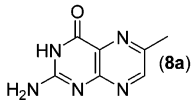
In summary, three methods to effect the synthesis of protected aryl phosphinic acid esters, **6**, from *p*-aminophenyl H-phosphinic acids **4a**−**c**, or esters **4d**,**e** were investigated. These involved reaction of the free acids or the corresponding esters via BSA-mediated formation of nucleophilic P^{III} intermediates (Methods A and B, respectively) or by a base-catalyzed Michaelis−Becker reaction of the H-phosphinic acid esters (Method C). The results of representative experiments are given in Table 1. These methods provide access to the key intermediates **6**, which were then further elaborated to **1**, the desired aryl phosphinic acid analogues of folic acid.

Compounds **7a**−**c**, destined for S_N2 alkylation by 6-(bromomethyl)pteridines **8a**,**b** and reductive amination with 6-formylpteridines **9a**−**c**, were obtained from **6a**,**b**,**d** by hydrogenolysis of the Cbz protecting group. Optimization of the S_N2 alkylation reaction conditions was investigated by varying the ratio of electrophile to nucleophile, elevating the temperature, prolonging the reaction time, or using microwave irradiation (Table 2). It

(36) Enders, D.; Saint-Dizier, A.; Lannou, M.-I.; Lenzen, A. *Eur. J. Org. Chem.* **2006**, 29−49.
(37) Boyd, E. A.; Boyd, M. E. K.; Loh, V. M. *Tetrahedron Lett.* **1996**, *37*, 1651−1654.
(38) Wehbe, J.; Rolland, V.; Fruchier, A.; Roumestant, M. L.; Martinez, J. *Tetrahedron*: *Asymmetry* **2004**, *15*, 851−858.
(39) Kappe, C. O. *Angew. Chem., Int. Ed.* **2004**, *43*, 6250−6284.
(40) Lambert, J. B.; Zhao, Y.; Emblidge, R. W.; Salvador, L. A.; Liu, X. Y.; So, J. H.; Chelius, E. C. *Acc. Chem. Res.* **1999**, *32*, 183−190.
(41) Schoen, W. R.; Parsons, W. H. *Tetrahedron Lett.* **1988**, *29*, 5201−5204.
(42) Shieh, W. C.; Xue, S.; McKenna, J.; Prasad, K.; Repic, O.; Blacklock, T. *Tetrahedron Lett.* **2006**, *47*, 5645−5648.

TABLE 2. S_N2 Alkylation of *p*-Aminophenyl Phosphinic Acid Esters: Optimization Studies

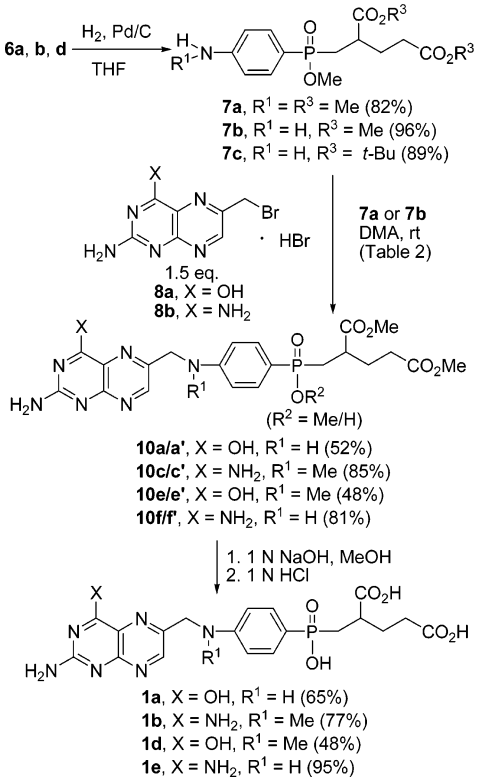


entry	electrophile, **A**, R^2 =	nucleophile, **B**	**A**:**B**	conditions	yield[a], %
1	 (**8b**)	R^1 = Me, X–Y = —C(=O)NH— [b]	1:1	1 d, rt 3 d, rt	50 80
2	 (**8b**)	R^1 = Me, X–Y = —P(=O)(OMe)—CH_2— (**7a**)	1:1	4 d, rt 12 d, rt 17 h, 35 °C 30 min, 60 °C[c] 500 min, 35 °C[c]	60 80 52[d] 37[d] 40[d]
			1.5:1	12 d, rt	100
3	 (**8b**)	R^1 = H, X–Y = —P(=O)(OMe)—CH_2— (**7b**)	1:1	3 d, rt 28 d, rt	50[e] 84
			1.5:1	11 d, rt	95
4	Ph	R^1 = H, X–Y = —P(=O)(OMe)—CH_2— (**7b**)	1:1	3 d, rt 7 d, rt	25 80
5	 (**8a**)	R^1 = Me, X–Y = —P(=O)(OMe)—CH_2— (**7a**)	1:1	14 d, rt 24 d, rt	24 41
			1.5:1	14 d, rt	90
6	 (**8a**)	R^1 = H, X–Y = —P(=O)(OMe)—CH_2— (**7b**)	1:1	3 d, rt 21 d, rt	trace 60
			1.5:1	14 d, rt	92

[a] Yield based on HPLC analysis, product could be purified by semi-prep HPLC or column chromatography (entries 1 and 4). [b] Used di-*tert*-butyl ester. [c] Microwave irradiation. [d] Byproducts detected by analytical HPLC. [e] Subsequent heating at 55 °C for ca. 20 h led to additional products (HPLC analysis) and a decreased yield (40%) of the desired product.

is well-known that the pK_a of the ammonium conjugate acid of *p*-aminobenzoic acid is unusually low (pK_a = 2.30).[43] Similarly, the nucleophilicity of the amine moiety of **7**, the phosphinic acid ester analogue of dimethyl *p*-aminobenzoylglutamate, is apparently decreased significantly in comparison to a diester of *p*-aminobenzoylglutamic acid (Table 2, entry 1). The attenuated nucleophilicity of **7b** is further supported by the slow rate of its alkylation by benzyl bromide (Table 2, entry 4). Elevation of the reaction temperature by either conventional heating or microwave irradiation resulted in the formation of several unidentified byproducts (Table 2, entries 2 and 3). The formation of these byproducts was not observed in the previously reported synthesis of prodrug esters of pseudopeptide FPGS inhibitors under similar conditions (60 °C, 30 h).[27] These observations provide further evidence for attenuation of the nucleophilicity of both the phosphorus P^{III} species derived from **4** and the aryl amine in **7**, due to the para disposition of the phosphorus substituent and the amino group. Due to the decreased solubility of **8a**, alkylation of **7a** or **7b** by **8a** is slower than the alkylation of **7a** or **7b** by **8b** (Table 2, entries 5 and 6). In all cases, the reaction rates were significantly improved by using 1.5 equiv of the electrophile on a larger scale (ca. 50 mg), presumably due to the increased concentration of all reactants. By using these optimized conditions, reaction of **7a** and **7b** with **8a** or **8b** afforded **10a**,**c**,**e**,**f**, fully protected aryl phosphinic acid analogues of folic acid (Scheme 5). In addition to the desired phosphinic acid methyl esters (P-OMe), **10a**,**c**,**e**,**f**, the free phosphinic acids (P-OH), **10a′**,**c′**,**e′**,**f′**, were also isolated from

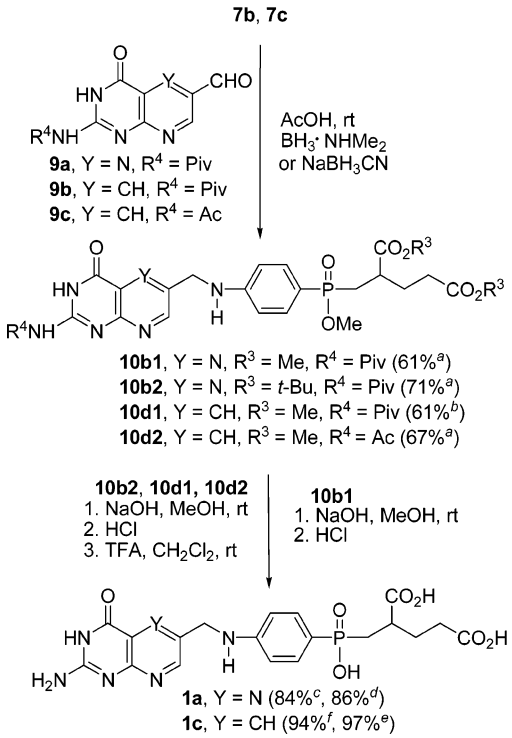
SCHEME 5



reaction mixture. Demethylation of these methyl esters is attributed to the presence of bromide ion formed in the alkylation reaction, as previously noted in our syntheses of pseudopeptide

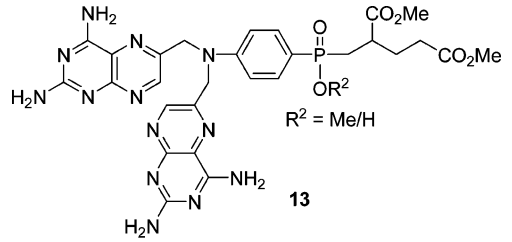
(43) Jencks, W. P. In *Handbook of biochemistry*: *selected data for molecular biology*; Sober, H. A., Ed.; Chemical Rubber Co: Cleveland, OH, 1968; pp J144−J149.

SCHEME 6



[a] Method A: Two-step reaction, imine formation, reduction by BH_3·$NHMe_2$. [b]Method B: One-step reaction, imine formation, in situ reduction by $NaBH_3CN$. [c]Hydrolyzed from **10b1**. [d]Hydrolyzed from **10b2**. [e]Hydrolyzed from **10d1**. [f]Hydrolyzed from **10d2**.

prodrug esters.[27] In the alkylation of **7b** by **8b**, a small amount (8%) of an *N*-10-dialklylated compound **13** was also isolated.



The target compounds **1a**,**b**,**d**,**e** were obtained after hydrolysis of the carboxylic acid esters under basic conditions. Hydrolysis of the phosphinic acid methyl esters (P-OMe) of **10a**,**c**,**e**,**f** proceeded very slowly and was complete only after several days at room temperature. In contrast, the corresponding phosphinic acid **10f′** was hydrolyzed overnight in excellent yield. It should be noted that the *N*-10 methyl derivatives **10c** and **10e** were hydrolyzed more rapidly than the *N*-H derivatives **10a** and **10f**. Although the S_N2 alkylation was successful in forming the C−N bond between heterocycles and phosphinic acid esters, several experimental limitations were also discovered in the course of this investigation, such as low solubility and high polarity of the mixture of free phosphinic acids and the corresponding methyl esters. Purification via crystallization or column chromatography was not generally applicable to these compounds. As a result, purification of the desired target compounds, **1**, or its precursors **10a**/**a′**,**c**/**c′**,**e**/**e′**,**f**/**f′** required the use of preparative HPLC, with associated loss of product during purification.

As an alternative approach, reductive amination was pursued (Scheme 6). The advantage of reductive amination over S_N2 alkylation is the use of N^2-pivaloylated or acetylated heterocycles in the former reaction, which allows for purification of the product by column chromatography. Reductive amination via a two-step pathway, involving imine formation followed by reduction of intermediate imine with BH_3·$NHMe_2$ as reductant, was investigated. As previously reported by Taylor's group for the synthesis of N^2-acetyl 5-deazafolate, the imine intermediate formed by reaction of **9c** and *p*-AB-Glu$(OMe)_2$ is insoluble in acetic acid, and precipitates from reaction solution within 10 min, thus driving the reaction to completion in 6 h.[29] However, under the same reaction conditions, no precipitate was observed in the reaction of **9c** and **7b**, and additional **9c** (1.1 equiv) was required to drive the reaction to completion after a total reaction time of 27 h as determined by HPLC and MS. In contrast, a rapid reductive amination was observed in the reaction of **9a** with **7b** (1.1 equiv) or **7c** (1.1 equiv), in agreement with reports in the literature for the preparation of folic acid.[28] Imine formation was complete within 15 min, and subsequent reduction required only an additional 10 min. Extended exposure to BH_3·$NHMe_2$ led to a tetrahydropteroyl derivative as detected by LC-MS.

Alternatively, reductive amination of **9a** or **9b** can be effected via a one-pot procedure, which involves imine formation and in situ reduction of intermediate imine by using $NaBH_3CN$ as reductant. In the case of reductive amination of pterin **9a** by either **7b** or **7c**, reduction of the pyrazine ring led to the 5,6,7,8-tetrahydro derivative of **10b1** (77%) or **10b2** (57%). However, reductive amination of the 5-deaza compound **9b** (1.5 equiv) by **7b** mediated by $NaBH_3CN$ provided compound **10d1** in modest yield. The advantage of the reductive amination method (Scheme 6) over S_N2 alkylation (Scheme 5) is that the fully protected products **10b1**,**2** and **10d1**,**2** can be purified by column chromatography and the reductive amination reaction is less affected by the attenuation of amine nucleophilicity in **7**. In addition, this method is more amenable to scale up due to the rapid reaction and ease of product purification.

Conditions for hydrolysis of the fully protected precursors, **10**, obtained by reductive amination of the N^2-protected heterocyclic aldehydes, **9**, to the target aryl phosphinic acids, **1**, varied depending on the nature of the protecting groups. Precursor **10b1** was treated with aqueous base (1 N NaOH, room temperature, 5−7 days) leading to free acid **1a** in good yield. The closely related precursor **10b2** was first treated with aqueous base (1 N NaOH, room temperature), which resulted in complete removal of the pivaloyl protecting group, accompanied by partial removal of the *tert*-butyl ester as indicated by LC-MS analysis. Similarly, **10d1** or **10d2** was treated with aqueous base (1 N NaOH, room temperature), resulting in complete removal of the pivaloyl or acetyl protecting group as well as all carboxylic acid methyl esters. The basic reaction solutions (**10b2**, **10d1**, or **10d2**) were then neutralized (HCl) and the solutions lyophilized. Treatment of the resulting residue with TFA in CH_2Cl_2 provided the free acids, **1a** or **1c**, in good yield after crystallization. The more facile cleavage of methyl β-carboxyphosphinates under both alkaline and acidic conditions is attributed to intramolecular catalysis involving the formation of a five-membered cyclic mixed anhydride intermediate.[44] Under acidic conditions, the free β-carboxyphosphinic acid was obtained rapidly. Under basic condition, a similar mechanistic pathway proceeded at a much slower rate. On the basis of these

(44) Georgiadis, D.; Dive, V.; Yiotakis, A. *J. Org. Chem.* **2001**, *66*, 6604−6610.

observations, an improved procedure involved hydrolysis of the carboxylic acid ester under basic condition (1 N NaOH:MeOH = 2:1) followed by lyophilization of the neutralized reaction mixture, and treatment of the resulting residue with TFA in CH_2Cl_2 (TFA:CH_2Cl_2 = 1:5). This provides the free phosphinic acid in high purity following a shorter reaction time.

In summary, several *p*-aminophenyl H-phosphinic acids and esters (**4**) have been synthesized from the corresponding substituted aryl halides via a Pd-catalyzed cross-coupling reaction. Subsequent conversion of the aryl H-phosphinic acids or esters to phosphinic acid ester analogues of *p*-aminobenzoyl glutamate (**6**) was pursued by using three methods (Table 1). Elaboration to the target compounds, **1**, was investigated via two synthetic routes: (1) alkylation by a bromomethyl heterocycle (Scheme 5) or (2) reductive amination of a heterocyclic aldehyde (Scheme 6). In the course of this research, it was observed that the nucleophilicity of both substituents in *p*-aminophenylphosphinic acid derivatives is markedly reduced. Thus, the aniline nitrogen and various P^{III} species derived from the aryl phosphinic acid each react at significantly lower rates than the unsubstituted counterpart.

Preliminary biological evaluation of these compounds indicates that the fully oxidized compounds, **1a**−**e**, are at best only modest inhibitors of glutamate ligation catalyzed by the DHFS-FPGS bifunctional protein, folC, from *E. coli* (A. Bognar, University of Toronto, personal communication). However, this is not surprising since the natural substrate for this reaction is a 7,8-dihydropterin derivative. Synthesis and biochemical evaluation of the more labile 7,8-dihyrdo derivative of **1a** is currently in progress. Results of these studies will be reported in a separate publication.

Experimental Section

General Procedures. See the Supporting Information. Representative procedures are given in this Experimental Section. Details on the synthesis of additional compounds using the procedures described below are given in the Supporting Information.

(4-(*N*-Benzyloxycarbonyl-*N*-methylamino)phenyl)phosphinic Acid (4a). Palladium-Catalyzed Cross-Coupling Reaction with $Pd(PPh_3)_4$: General Procedure. To an oven-dried round-bottom flask containing **2a** (482 mg, 1.51 mmol), anilinium hypophosphite (**3**) (642 mg, 4.03 mmol), and $Pd(PPh_3)_4$ (60 mg, 52 μmol) were added Et_3N (1 mL) and CH_3CN (12 mL) via syringes under a N_2 atmosphere. The reaction mixture was heated under reflux for 24 h. The reaction was monitored by ^{31}P NMR, and the integration ratio of the starting material of **3** and product **4a** indicated when the reaction was complete. The reaction mixture was then cooled to room temperature. Water (5 mL) and a solution of $KHSO_4$ (1 M) saturated with NaCl (5 mL) were added to the reaction mixture, followed by addition of ethyl acetate (30 mL). The organic layer was separated. The aqueous layer was back-extracted with ethyl acetate (3 × 25 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The concentrated solution was passed through a Celite filter to remove the remaining catalyst. The solvent was removed in vacuo to afford **4a** as a brown oil with sufficient purity (450 mg, 98% (^{31}P NMR yield)), which was used without further purification: ^1H NMR ($CDCl_3$) δ 12.39 (1 H, br s), 7.76−7.64 (2 H, m), 7.59 (1 H, d, J_{HP} = 561 Hz), 7.57−7.35 (7 H, m), 5.20 (2 H, s), 3.34 (3 H, s); ^{31}P NMR ($CDCl_3$) δ 19.3; MS (ESI) *m*/*z* 304.0 ([M − H]$^-$, 100).

(4-(*N*-Benzyloxycarbonylamino)phenyl)phosphinic Methyl Ester (4d). Palladium-Catalyzed Cross-Coupling Reaction with $Pd(OAc)_2$ and dppp: General Procedure. To an oven-dried round-bottomed flask containing **2b** (520 mg, 1.47 mmol), anilinium hypophosphite (**3**) (703 mg, 4.42 mmol), DABCO (496 mg, 4.42 mmol), tetramethyl orthosilicate (0.66 mL, 4.43 mmol), 1,3-bis-(diphenylphosphino)propane (dppp) (60 mg, 0.145 mmol), and $Pd(OAc)_2$ (30 mg, 0.134 mmol) was added CH_3CN (20 mL) via a cannula under an Ar atmosphere. The reaction mixture was stirred under reflux (85 °C). After an additional 2 h, the reaction mixture was allowed to cool to room temperature. The reaction was monitored with ^{31}P NMR, and the integration ratio of the starting material **3** and product **4d** indicated when the reaction was complete. A solution of $KHSO_4$ (1 M) saturated by NaCl (20 mL) was added to the reaction mixture, followed by ethyl acetate (50 mL). The organic layer was separated. The aqueous layer was back-extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by silica gel column chromatography, using CH_2Cl_2:ethyl acetate = 1:1 as the eluant, to afford **4d** (320 mg, 1.05 mmol, 71%) as a white solid: mp 108−110 °C; ^1H NMR ($CDCl_3$) δ 7.74−7.65 (2 H, m), 7.64−7.52 (2 H, m), 7.51 (1 H, d, J_{HP} = 567 Hz), 7.47−7.30 (6 H, m), 5.21 (2 H, s), 3.75 (3 H, d, J_{HP} = 12.0 Hz); ^{13}C NMR ($CDCl_3$) δ 152.9, 142.7 (d, $^4J_{CP}$ = 2.9 Hz), 135.6, 132.3 (d, $^2J_{CP}$ = 12.8 Hz), 128.6, 128.5, 128.4, 123.0 (d, $^1J_{CP}$ = 136.7 Hz), 118.1 (d, $^3J_{CP}$ = 14.3 Hz), 67.3, 52.0 (d, $^2J_{POC}$ = 6.6 Hz); ^{31}P NMR ($CDCl_3$) δ 28.0; MS (ESI) *m*/*z* 328.1 ([M + Na]$^+$, 100); ESI-HRMS (*m*/*z*) calcd for $C_{15}H_{16}NO_4PNa$ [M + Na]$^+$ 328.0715, found 328.0711.

2-[((4-(*N*-Benzyloxycarbonyl-*N*-methylamino)phenyl)(methoxy)-phosphinoyl)methyl]pentane-1,5-dioic Acid Dimethyl Ester (6a, Table 1, entry 3). BSA-Mediated Michael Addition Reaction of $ArP(OTMS)_2$ to α,β-Unsaturated Esters and Subsequent Methylation of the Resulting Phosphinic Acid: General Procedure (Table 1, Method A). To an oven-dried round-bottomed flask containing **4a** (83 mg, 0.272 mmol) and dimethyl 2-methyleneglutarate **5a** (94 mg, 0.544 mmol) was added anhydrous CH_2Cl_2 (1 mL), followed by *N*,*O*-(bistrimethylsilyl)acetamide (BSA) (0.34 mL, 1.36 mmol) under an Ar atmosphere. The reaction mixture was stirred at room temperature for 24 h. The starting material was consumed as indicated by ^{31}P NMR. The reaction was quenched by the addition of a 1 N solution of HCl (5 mL) and stirring was continued for an additional 30 min. The layers were separated by adding ethyl acetate (30 mL) and water (30 mL). The aqueous layer was back-extracted with ethyl acetate (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo to afford the crude product as a precursor of **6a** as a light brown oil (^{31}P NMR δ 44.3), which was used for next step reaction without further purification. To a flask containing of a solution of crude product (130 mg) in MeOH (8 mL) was added a large excess of a solution of TMS−diazomethane (2 M) in diethyl ether until a persistent yellow color was observed at 0 °C. In addition, gas evolution was observed during the addition. After an additional 2 h at room temperature, TLC indicated the starting material was consumed. The reaction mixture was quenched by adding acetic acid. The solvent and the excess of acetic acid were removed in vacuo. The crude product was purified by silica gel chromatography, using ethyl acetate as the eluant, to afford **6a** (60 mg, 0.122 mmol, 45% (3 steps)) as a colorless oil: ^1H NMR ($CDCl_3$) δ 7.76−7.71 (2 H, m), 7.44−7.29 (7 H, m), 5.20 (2 H, s), 3.69−3.38 (12 H, m), 2.87−2.80 (1 H, m), 2.50−2.29 (3 H, m), 2.09−1.91 (3 H, m); ^{13}C NMR ($CDCl_3$) δ 174.2 (dd, $^3J_{CP}$ = 22.9, 7.0 Hz), 172.7 (d, $^5J_{CP}$ = 5.4 Hz), 154.9, 147.0 (t, $^4J_{CP}$ = 3.6 Hz), 136.0, 132.4 (dd, $^2J_{CP}$ = 16.3, 10.8 Hz), 128.5, 128.1, 127.9, 126.3 (dd, $^1J_{CP}$ = 127.0, 42.8 Hz), 124.8 (dd, $^3J_{CP}$ = 12.4, 6.7 Hz), 67.7, 51.8 (d, $^2J_{POC}$ = 17.4 Hz), 51.6 (d, $^7J_{POC}$ = 1.3 Hz), 51.1 (dd, $^5J_{POC}$ = 6.4, 2.5 Hz), 38.4, 37.1, 31.7 (dd, $^1J_{CP}$ = 101.6, 40.8 Hz), 31.1, 28.5 (d, $^2J_{CP}$ = 11.9 Hz); ^{31}P NMR ($CDCl_3$) δ 41.7, 41.4; MS (ESI) *m*/*z* 514.1 ([M + Na]$^+$, 100).

2-[((4-(*N*-Benzyloxycarbonylamino)phenyl)(methoxy)phosphinoyl)methyl]pentane-1,5-dioic Acid Dimethyl Ester (6b, Table 1, entry 6). BSA-Mediated Michael Addition Reaction of ArP(OMe/OEt)OTMS to α,β-Unsaturated Esters: General Procedure (Table 1, Method B). To an oven-dried round-bottomed

flask containing **4d** (224 mg, 0.734 mmol) and dimethyl 2-methyleneglutarate (**5a**) (253 mg, 1.47 mmol) was added anhydrous CH_2Cl_2 (5 mL), followed by BSA (1.82 mL, 7.34 mmol) under an Ar atmosphere. The reaction mixture was heated under reflux for 7 days. The reaction was quenched by the addition of water (10 mL) and stirred for 30 min. The layers were separated by adding ethyl acetate (50 mL) and brine (10 mL). The aqueous layer was back-extracted with ethyl acetate (3 × 40 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by silica gel chromatography, using ethyl acetate as the eluant, to afford **6b** (250 mg, 0.524 mmol, 71%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.75 (1 H, br s), 7.69−7.63 (2 H, m), 7.62−7.57 (2 H, m), 7.38−7.31 (5 H, m), 5.20 (2 H, s), 3.65−3.48 (9 H, m), 2.84−2.76 (1 H, m), 2.46−2.25 (3 H, m), 2.01−1.90 (3 H, m); ^{13}C NMR ($CDCl_3$) δ 174.3 (dd, $^3J_{CP}$ = 18.9, 7.2 Hz), 172.8 (d, $^5J_{CP}$ = 5.2 Hz), 153.1, 142.3, 135.7, 133.0 (dd, $^2J_{CP}$ = 13.7, 10.9 Hz), 128.6, 128.4, 128.3, 123.1 (dd, $^1J_{CP}$ = 130.3, 41.7 Hz), 118.2 (dd, $^3J_{CP}$ = 12.8, 8.7 Hz), 67.2, 51.9 (d, $^2J_{POC}$ = 10.6 Hz), 51.6, 51.1 (d, $^5J_{POC}$ = 5.4 Hz), 38.5 (d, $^4J_{CP}$ = 4.8 Hz), 31.7 (dd, $^1J_{CP}$ = 101.9, 32.9 Hz), 31.2, 28.5 (d, $^2J_{CP}$ = 11.5 Hz); ^{31}P NMR ($CDCl_3$) δ 44.4, 44.0; MS (ESI) *m*/*z* 500.1 ([M + Na]$^+$, 100); ESI-HRMS (*m*/*z*) calcd for $C_{23}H_{28}NO_8PNa$ [M + Na]$^+$ 500.1450, found 500.1457.

2-[((4-(*N*-Benzyloxycarbonylamino)phenyl)(methoxy)phosphinoyl)methyl]pentane-1,5-dioic Acid Dimethyl Ester (6b, Table 1, entry 7). BSA-Mediated Michael Addition Reaction of ArP(OMe/OEt)OTMS to α,β-Unsaturated Ester under Microwave Conditions: General Procedure (Table 1, Method B, Microwave). To an oven-dried microwave reaction vessel containing **4d** (32 mg, 0.105 mmol) and dimethyl 2-methyleneglutarate (**5a**) (36 mg, 0.209 mmol) was added anhydrous CH_2Cl_2 (1 mL), followed by BSA (0.26 mL, 1.05 mmol) under an Ar atmosphere. The reaction mixture was heated by microwave reactor at 80 °C for 4 h (250 W). The reaction was quenched by the addition of HCl (0.1 N, 2 mL) and water (5 mL) and stirred for 10 min. The layers were separated by adding ethyl acetate (20 mL) and brine (10 mL). The aqueous layer was back-extracted with ethyl acetate (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by silica gel chromatography, using ethyl acetate as the eluant, to afford **6b** (25 mg, 52 μmol, 50%) as a colorless oil. Spectra are identical with those obtained for **6b** by Method B.

2-[((4-(*N*-Benzyloxycarbonylamino)phenyl)(methoxy)phosphinoyl)methyl]pentane-1,5-dioic Acid Dimethyl Ester (6b, Table 1, entry 10). Michael Addition Reaction of ArP(O)(OMe)-H to α,β-Unsaturated Esters Promoted by KO*t*-Bu (Table 1, Method C: General Procedure). To an oven-dried round-bottomed flask containing **4d** (110 mg, 0.360 mmol) and KO*t*-Bu (49 mg, 0.437 mmol) was added anhydrous THF(5 mL) at 0 °C. The reaction mixture was stirred for an additional 20 min at 0 °C before a solution of dimethyl 2-methyleneglutarate **5a** (105 mg, 0.610 mmol) in THF (5 mL) was added. After an additional 2 h at room temperature, the starting material was consumed as indicated by ^{31}P NMR. The reaction was quenched by the addition of water (5 mL) and stirred for 10 min. The layers were separated by adding ethyl acetate (20 mL) and brine (10 mL). The aqueous layer was back-extracted with ethyl acetate (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by silica gel chromatography, using ethyl acetate as the eluant, to afford **6b** (87 mg, 0.182 mmol, 51%) as a colorless oil. Spectra are identical with those obtained for **6b** by Method B.

If NaOMe was used as base, in addition to the desired product, **6b**, a side-product compound **6e** was isolated (33%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 8.01 (1 H, br s), 7.70−7.61 (4 H, m), 3.76−3.50 (12 H, m), 2.88−2.72 (1 H, m), 2.46−2.25 (3 H, m), 2.00−1.90 (3 H, m); ^{13}C NMR ($CDCl_3$) δ 174.3 (dd, $^3J_{CP}$ = 17.9, 7.2 Hz), 172.8 (d, $^5J_{CP}$ = 5.5 Hz), 153.8, 142.6, 132.9 (dd, $^2J_{CP}$ = 14.1, 11.1 Hz), 122.9 (dd, $^1J_{CP}$ = 130.7, 41.9 Hz), 118.1 (dd, $^3J_{CP}$ = 12.8, 8.6 Hz), 52.4, 51.9 (d, $^2J_{POC}$ = 10.6 Hz), 51.6, 51.1 (d, $^5J_{POC}$ = 6.6 Hz), 38.5, 31.7 (dd, $^1J_{CP}$ = 101.8, 32.9 Hz), 31.2, 28.5 (d, $^2J_{CP}$ = 11.5 Hz); ^{31}P NMR ($CDCl_3$) δ 43.8, 43.7, 43.4,43.3; MS (ESI) *m*/*z* 402.1 ([M + Na]$^+$, 100).

2-[((4-Aminophenyl)(methoxy)phosphinoyl)methyl]pentane-1,5-dioic Acid (12). A solution of **6e** (135 mg, 0.336 mmol) in 5 mL of 6 N HCl was heated under reflux for 10 h. The solution was allowed to cool to room temperature. Solvent was removed in vacuo to afford **12** as a colorless oil: ^{1}H NMR (CD_3OD) δ 7.73−7.66 (2 H, m), 7.31−7.12 (2 H, m), 2.42−2.38 (1 H, m), 2.13−1.99 (3 H, m), 1.80−1.57 (3 H, m); ^{31}P NMR (D_2O) δ 36.8. Note: Compound **12** obtained from **6c** has identical spectral data as that from **6e**.

2-[((4-(*N*-Methylamino)phenyl)(methoxy)phosphinoyl)methyl]pentane-1,5-dioic Acid Dimethyl Ester (7a). Preparation of *p*-Aminophenylphosphinic Acid Methyl Esters via Hydrogenation To Remove the Cbz Protecting Group: General Procedure. To a suspension of **6a** (270 mg, 0.549 mmol) and 5% Pd/C (200 mg) in THF (20 mL) was bubbled H_2 under liquid for 10 min, then the mixture was kept under H_2 atmosphere for 24 h. Pd/C was removed by filtration through a Celite pad. The filtrate was concentrated in vacuo. The crude product was purified by a silica gel chromatography, using ethyl acetate as the eluant, to afford **7a** (160 mg, 0.448 mmol, 82%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.55−7.50 (2 H, m), 6.64−6.61 (2 H, m), 4.35 (1 H, br s), 3.72−3.51 (9 H, m), 2.88−2.81 (3.5 H, m), 2.80−2.75 (0.5 H, s), 2.44−2.28 (3 H, m), 2.03−1.86 (3 H, m); ^{13}C NMR ($CDCl_3$) δ 174.6 (dd, $^3J_{CP}$ = 23.7, 7.4 Hz), 172.9 (d, $^5J_{CP}$ = 5.3 Hz), 152.5 (t, $^4J_{CP}$ = 2.2 Hz), 133.4 (dd, $^2J_{CP}$ = 18.5, 11.3 Hz), 115.0 (dd, $^1J_{CP}$ = 136.4, 53.7 Hz), 111.6 (dd, $^3J_{CP}$ = 13.5, 7.9 Hz), 51.8 (d, $^2J_{POC}$ = 9.3 Hz), 51.6 (d, $^7J_{POC}$ = 1.9 Hz), 50.7 (d, $^5J_{POC}$ = 6.3 Hz), 38.6 (dd, $^4J_{CP}$ = 5.1, 2.7 Hz), 32.0 (dd, $^1J_{CP}$ = 102.3, 34.4 Hz), 31.3, 30.0, 28.6 (dd, $^2J_{CP}$ = 10.3, 1.4 Hz); ^{31}P NMR ($CDCl_3$) δ 44.8, 44.4; MS (ESI) *m*/*z* 358.1 ([M + H]$^+$, 100); ESI-HRMS (*m*/*z*) calcd for $C_{16}H_{25}NO_6P$ [M + H]$^+$ 358.1420, found 358.1412.

[((4-(*N*-((2-Amino-3,4-dihydro-4-oxo-6-pteridinyl)methyl)amino)phenyl)(methoxy)phosphinoyl)methyl]pentane-1,5-dioic Acid Dimethyl Ester (10a, Table 2, entry 6)). Preparation of Heterocyclic Phosphinic Acid Methyl Esters by S_N2 Alkylation: General Procedure. A mixture of **7b** (45 mg, 0.131 mmol) and **8a** (66 mg, 0.196 mmol) in DMA (2 mL) was stirred at room temperature. The reaction mixture was monitored by analytical HPLC, and consumption of the limiting reagent, **7b**, was complete after 14 d. The crude product was purified by semipreparative HPLC (see the Supporting Information for details) and solvent was removed via lyophilization to afford **10a** (P-OMe) (9 mg, 17 μmol, 13%) as a light brown solid and **10a′** (P-OH) (26 mg, 52 μmol, 39%) as a greenish yellow solid. **10a**: mp >300 °C (210 °C dec); ^{1}H NMR (DMSO) δ 8.64 (1 H, s), 7.38−7.33 (2 H, m), 7.16−7.12 (1 H, m), 7.05 (1 H, br s), 6.76−6.71 (2 H, m), 4.48 (2 H, d, *J* = 5.5 Hz), 3.56−3.35 (9 H, m), 2.57−2.51 (1 H, m), 2.26−1.92 (4 H, m), 1.85−1.68 (2 H, m); ^{31}P NMR (DMSO) δ 43.8, 43.6; MS (ESI) *m*/*z* 541.1 ([M + Na]$^+$, 100); ESI-HRMS (*m*/*z*) calcd for $C_{22}H_{27}N_6O_7PNa$ [M + Na]$^+$ 541.1577, found 541.1589; UV λ_{max} (0.1 N NaOH) 235, 267, 329, 369 nm; (0.1 N HCl) 268, 320 nm; analytical HPLC t_R = 19.3 min. **10a′**: mp >300 °C (210 °C dec); ^{1}H NMR (DMSO) δ 8.62 (1 H, s), 7.37−7.33 (2 H, m), 7.28 (2 H, br s), 6.73 (1 H, br s), 6.59−6.58 (2 H, m), 4.44 (2 H, s), 3.60−3.38 (6 H, m), 2.55−2.45 (2 H, m), 2.17 (2 H, t, *J* = 7.5 Hz), 1.89−1.78 (2 H, m), 1.73−1.63 (1 H, m), 1.63−1.53 (1 H, m); ^{13}C NMR (DMSO) δ 175.5, 173.1, 161.9, 156.6, 154.8, 149.6 (d, $^4J_{CP}$ = 114.4 Hz), 149.6, 148.1, 132.8 (dd, $^2J_{CP}$ = 168.3, 18.2 Hz), 128.3, 124.9 (d, $^1J_{CP}$ = 125.8 Hz), 111.7 (d, $^3J_{CP}$ = 156.6 Hz), 53.4, 52.3, 51.1, 49.9, 47.5, 46.5, 45.4, 39.0, 38.8, 38.6, 38.5, 32.4, 31.4, 30.4, 29.6, 28.6, 27.6; ^{31}P NMR (DMSO) δ 27.1; MS (ESI) *m*/*z* 527.1 ([M + Na]$^+$, 100); ESI-HRMS (*m*/*z*) calcd for $C_{21}H_{25}N_6O_7PNa$ [M + Na]$^+$ 527.1420, found 527.1425; UV λ_{max} (0.1 N NaOH) 259, 366 nm; (0.1 N HCl) 263, 322 nm; analytical HPLC t_R = 14.4 min.

[((4-((2-Pivaloylamino-4-hydroxy)pteroylamino)phenyl)-(methoxy)phosphinoyl)methyl]pentane-1,5-dioic Acid Dimethyl Ester (10b1). Preparation of Heterocyclic Phosphinic Acid Esters by Reductive Amination via a Two-Step Sequence: General Procedure (Method A). The solution of phosphinic methyl ester **7b** (55 mg, 0.160 mmol) and **9a** (40 mg, 0.145 mmol) in AcOH (2 mL) was stirred at room temperature for 15 min. A solution of $BH_3{\cdot}NHMe_2$ (17 mg, 0.289 mmol) in AcOH (0.5 mL) was added to reduce the imine intermediate. After an additional 10 min, the solvent was removed in vacuo to afford the crude product, which was purified by silica gel chromatography, using ethyl acetate as eluant, to remove excess **7b**, followed by a mixture of 5% of MeOH and 95% of ethyl acetate to afford **10b1** (54 mg, 90 μmol, 61%) as a yellow solid: mp 80−82 °C; ^{1}H NMR ($CDCl_3$) δ 12.43 (1 H, br s), 8.89 (1 H, s), 8.52 (1 H, br s), 7.57−7.53 (2 H, m), 6.75−6.73 (2 H, m), 5.30 (1 H, br s), 4.73 (2 H, d, J = 5.6 Hz), 3.64−3.49 (9 H, m), 2.86−2.76 (1 H, m), 2.44−2.28 (3 H, m), 2.05−1.86 (3 H, m), 1.36−1.34 (9 H, m); ^{13}C NMR ($CDCl_3$) δ 180.9, 174.5 (dd, $^3J_{CP}$ = 21.8, 7.1 Hz), 172.9 (d, $^5J_{CP}$ = 4.5 Hz), 159.5, 154.5, 151.9, 150.5, 149.4, 149.2, 133.6 (dd, $^2J_{CP}$ = 16.8, 11.4 Hz), 130.6, 116.6 (dd, $^1J_{CP}$ = 135.6, 48.4 Hz), 112.5 (dd, $^3J_{CP}$ = 13.4, 7.1 Hz), 51.8 (d, $^2J_{POC}$ = 8.5 Hz), 51.6, 50.9 (d, $^5J_{POC}$ = 6.3 Hz), 46.6, 40.5, 38.6 (dd, $^4J_{CP}$ = 7.9, 2.8 Hz), 31.8 (dd, $^1J_{CP}$ = 102.3, 37.4 Hz), 31.2, 28.5 (d, $^2J_{CP}$ = 11.4 Hz), 26.8; ^{31}P NMR ($CDCl_3$) δ 44.6, 44.3; MS (ESI) m/z 625.2 ([M + Na]$^+$, 100); ESI-HRMS (m/z) calcd for $C_{27}H_{35}N_6O_8PNa$ [M + Na]$^+$ 625.2152, found 625.2150; UV λ_{max} (0.1 N NaOH) 267, 356 nm; (0.1 N HCl) 270, 326 nm; analytical HPLC t_R = 26.8 min.

[((4-((2-Pivaloylamino-4-hydroxy)-5-deazapteroylamino)phenyl)(methoxy)phosphinoyl)methyl]pentane-1,5-dioic Acid Dimethyl Ester 10d1. Preparation of Heterocyclic Phosphinic Methyl Esters by Reductive Amination via a One-Step Sequence: General Procedure (Method B). The mixture of phosphinic methyl ester **7b** (25 mg, 73 μmol), **9b** (30 mg, 0.109 mmol), and $NaBH_3$-CN (7 mg, 0.111 mmol) in AcOH (1.5 mL) was stirred at room temperature for 48 h. The solvent was removed in vacuo to afford the crude product, which was purified by silica gel chromatography, using a mixture of 5% of MeOH and 95% of EtOAc as eluant, to afford **10d1** (27 mg, 45 μmol, 61%) as a light yellow solid: mp 80−82 °C; ^{1}H NMR ($CDCl_3$) δ 12.13 (1 H, br s), 8.88 (1 H, s), 8.52 (1 H, br s), 8.49 (1 H, s), 7.56−7.51 (2 H, m), 6.68−6.66 (2 H, m), 4.90 (1 H, br s), 4.54 (2 H, d, J = 5.7 Hz), 3.69−3.50 (9 H, m), 2.88−2.77 (1 H, m), 2.45−2.29 (3 H, m), 2.06−1.87 (3 H, m), 1.30 (9 H, s); ^{13}C NMR ($CDCl_3$) δ 180.7, 174.6 (dd, $^3J_{CP}$ = 23.3, 7.0 Hz), 173.0 (d, $^5J_{CP}$ = 4.6 Hz), 161.0, 158.2, 155.8, 150.6, 149.1, 134.8, 133.7 (dd, $^2J_{CP}$ = 18.6, 11.3 Hz), 131.7, 116.9 (dd, $^1J_{CP}$ = 134.9, 52.5 Hz), 115.2, 112.5 (dd, $^3J_{CP}$ = 13.3, 8.4 Hz), 51.9 (d, $^2J_{POC}$ = 8.3 Hz), 51.7, 50.9 (d, $^5J_{POC}$ = 6.3 Hz), 44.7, 40.5, 38.7 (dd, $^4J_{CP}$ = 5.1, 2.8 Hz), 32.0 (dd, $^1J_{CP}$ = 102.4, 39.9 Hz), 31.3, 28.7 (d, $^2J_{CP}$ = 10.7 Hz), 26.9; ^{31}P NMR ($CDCl_3$) δ 44.4, 44.0; MS (ESI) m/z 624.2 ([M + Na]$^+$, 100); UV λ_{max} (0.1 N NaOH) 246, 274, 324 nm; (0.1 N HCl) 276, 339 nm; analytical HPLC t_R = 29.0 min.

[((4-(*N*-((2-Amino-3,4-dihydro-4-oxo-6-pteridinyl)methyl)amino)phenyl)(hydroxy)phosphinoyl)methyl]pentane-1,5-dioic Acid (1a). Method A: Preparation of Heterocyclic Aryl Phosphinic Acids from the Corresponding Unprotected 2-Amino Heterocycles and Glutarate Methyl Esters via Hydrolysis under Basic Conditions: General Procedure. A degassed solution of **10a** obtained by S_N2 alkylation (Scheme 5) (30 mg, 59 μmol) and 1 N NaOH (3.0 mL) in CH_3OH (3.0 mL) was stirred at room temperature protected from the light. The reaction mixture was monitored by analytical HPLC, and consumption of **10a** was >95% after 5 days. The crude reaction mixture was concentrated in vacuo to remove CH_3OH, followed by acidification with 1 N HCl to pH 2−3 at 0 °C. After the solvent was removed via lyophilization, a solid was precipitated out by addition of a very small amount of ice-cold water to the residue, collected by centrifuge, washed with ice-cold water twice, and dried in vacuo to afford the first batch **1a** (16.3 mg). After the supernatant was dried by lyophilization, the resulting residue was dissolved in DMA. The mixture was filtered through a nylon filter before it was purified by semipreparative HPLC (see the Supporting Information for details) and solvent was removed via lyophilization to afford the second batch **1a** (2 mg) (18.3 mg (total), 38 μmol, 65%) as a yellow solid: mp >300 °C dec; ^{1}H NMR (D_2O) δ 8.63 (1 H, s), 7.37 (2 H, t, J = 8.7 Hz), 6.72 (2 H, d, J = 8.0 Hz), 4.53 (2 H, s), 2.41−2.32 (1 H, m), 2.10 (2 H, t, J = 7.4 Hz), 2.02−1.93 (1 H, m), 1.65−1.58 (3 H, m); ^{31}P NMR (D_2O) δ 32.5, 32.3; MS (ESI) m/z 477.1 ([M + H]$^+$, 100); ESI-HRMS (m/z) calcd for $C_{19}H_{22}N_6O_7P$ [M + H]$^+$ 477.1288, found 477.1293. UV λ_{max} (0.1 N NaOH) 258, 364 nm; (0.1 N HCl) 263, 321 nm; analytical HPLC t_R = 9.6 min.

Method B: Preparation of Heterocyclic Aryl Phosphinic Acids from the Corresponding Protected 2-Amino Heterocycles and Glutarate Di-*tert*-butyl Esters via Hydrolysis of the Pivaloyl Group under Basic Conditions Followed by TFA-Mediated Hydrolysis of the *tert*-Butyl Ester: General Procedure.[45] A degassed solution of **10b2** obtained by reductive amination (Scheme 6) (50 mg, 73 μmol) and 1 N NaOH (3.0 mL) in CH_3OH (1.5 mL) was stirred at room temperature protected from the light. The reaction mixture was monitored by analytical HPLC and LC-MS frequently. Complete removal of the *N*-pivaloyl group and partial removal of *tert*-butyl esters was observed after 1 day. The crude reaction mixture was concentrated in vacuo to remove CH_3OH, followed by acidification with 1 N HCl to pH 2−3 at 0 °C. After the solvent was removed via lyophilization, a suspension of the resulting solid in CH_2Cl_2 (2.5 mL) was treated overnight with TFA (0.5 mL), after which solvent was removed in vacuo. The desired product was precipitated by addition of a very small amount of ice-cold water, collected by centrifuge (SpeedVac SPD111V, 4 min), washed with ice-cold water twice, and dried in vacuo to afford **1a** (30 mg, 63 μmol, 86%) as an orange-yellow solid. Spectra are identical with those obtained for **1a** by Method A.

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Supporting Information Available: Experimental procedures and characterization of compounds **2a**,**b**, **4b**−**e**, compounds noted in Table 1 (entries 1, 2, 6, 8, 9, and 11), **7b**,**c**, **10b2**, **10c**/**c′**, **10d2**, **10e**/**e′**, **10f**/**f′**, **11a**,**b** (R^4 = Et), and **1a**−**e**, Table S1 (optimization experiments, Table 1, method B), and ^{1}H NMR, ^{13}C NMR, and ^{31}P NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(45) This method is also applicable to glutarate dimethyl esters by completely removing the dimethyl ester under basic condition, followed by removing the P-OMe by TFA.