Phosphinic Acid Pseudopeptides Analogous to Glutamyl-γ-glutamate: Synthesis and Coupling to Pteroyl Azides Leads to Potent Inhibitors of Folylpoly-γ-glutamate Synthetase†

Nadya Valiaeva, David Bartley, Tsutomu Konno, and James K. Coward*

Departments of Medicinal Chemistry and Chemistry, University of Michigan, Ann Arbor, Michigan 48109-1055

jkcoward@umich.edu

Received March 14, 2001

Several routes to a complex phosphinate phosphapeptide analogous to the γ-glutamyl peptide Glu-γ-Glu have been investigated. Formation of γ-phosphono glutamate derivatives via addition of a phosphorus-based radical to protected vinylglycine was found to be of limited value because of the elevated temperatures required. Alkylation and conjugate addition reactions of trivalent phosphorus (P^{III}) species were investigated. In situ generation of bis-trimethylsilyl esters of phosphinous acids proved to be an effective route to phosphinates of modest structural complexity. However, this chemistry could not be extended to the incorporation of an amino acid moiety at the N-terminal side of the desired phosphinate. A successful synthesis of the target phosphinate phosphapeptide was effected using P^{III} chemistry and dehydrohalogenation to yield an α,β-unsaturated phosphinic acid ester, following which conjugate addition of diethylacetamido malonate and acid-mediated hydrolysis afforded the desired phosphinate phosphapeptide. Coupling of the unprotected phosphinate phosphapeptide with two acyl azides derived from folic acid and methotrexate led to the corresponding pteroylphosphapeptides of interest as possible mimics of tetrahedral intermediates in the reaction catalyzed by folylpolyglutamate synthetase.

Introduction

The design of mechanism-based enzyme inhibitors requires an understanding of enzyme catalysis at a level of sophistication that is often not available in the literature. However, in the case of ATP-dependent ligases/synthetases and various classes of proteases/hydrolases, details of catalytic mechanisms are available as a result of decades of research in laboratories around the world.[1] This mechanistic framework has allowed for the design and synthesis of many extremely potent inhibitors of these and a limited number of other classes of enzymes.[2,3] The use of phosphinic acid-containing pseudopeptides as potential inhibitors of ligases and proteases presents a significant challenge to the synthetic chemist owing to the structural diversity present in peptides. Although this synthetic effort has resulted in a variety of potent inhibitors of several ligases and proteases,[4] the phosphapeptides, $-AA_n[\psi\{P(O)(OH)X\}]-AA_m-$,[5] including not only the phosphinate (X = CH_2) but also phosphonamidate (X = NH−)[6] and phosphonate (X = O)[7] moieties, generally involve amino acids such as alanine, phenylalanine, and valine that lack side-chain functionality. Our interest in the enzymes folylpoly-γ-glutamyl synthetase (FPGS, EC 6.3.2.17) and γ-glutamyl hydrolase (GH, EC 3.4.19.9)[8] has led us to pursue the synthesis of pseudopeptides as inhibitors of these enzymes. FPGS has a strict requirement for γ-glutamyl peptide substrates (−Glu-γ-Glu−), and therefore, any pseudopeptide inhibitor must include the side-chain functionality of glutamic acid. We have described the synthesis of phosphonamidate- and phosphonate-containing pseudopeptides analogous to Glu-γ-Glu (Glu-γ-[ψ-{P(O)(OH)X}]Glu)[9] and have shown that a pteridine-based phosphonate-containing pseudopeptide is a potent inhibitor of FPGS.[10] In earlier work, it proved difficult to effect the synthesis of a corresponding phosphinate-containing pseudopeptide.[9] We report herein a synthesis of the phosphinate analogue of Glu-γ-Glu (Glu-γ-[ψ{P(O)-(OH)CH_2}]Glu) and its incorporation into folate and antifolate (methotrexate, MTX) platforms as potential inhibitors of FPGS.

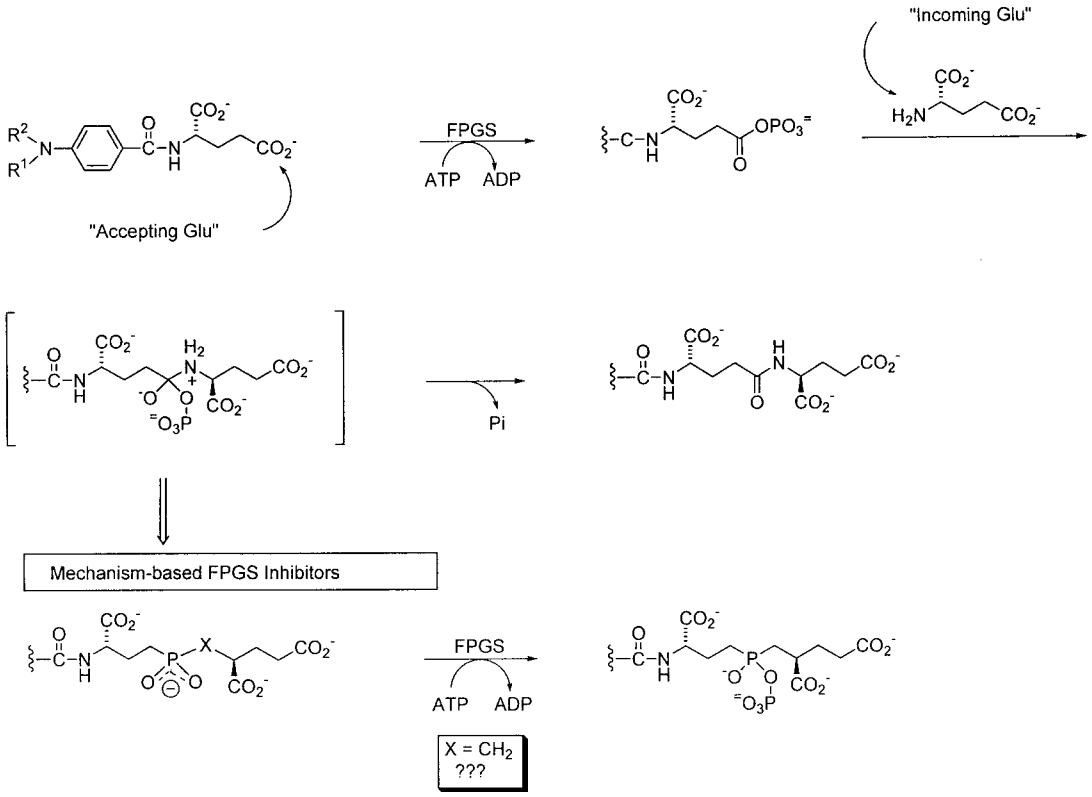
Folic acid is an important vitamin in human nutrition, and its reduced, poly-γ-glutamyl derivatives ("conjugates") are important cofactors in one-carbon biochemistry in nearly all living cells.[8,11] The need for high folate levels in the human diet during pregnancy has recently received widespread attention due to the postulated role of reduced folates in the prevention of neural tube defects. In addition, there is evidence that links hyperhomocysteinemia and associated cardiovascular disease with several postulated metabolic defects involving folate or homocysteine.[12] FPGS catalyzes the ATP-dependent liga-

† Dedicated to the memory of Prof. Arthur G. Schultz, an outstanding chemist, colleague, and friend.

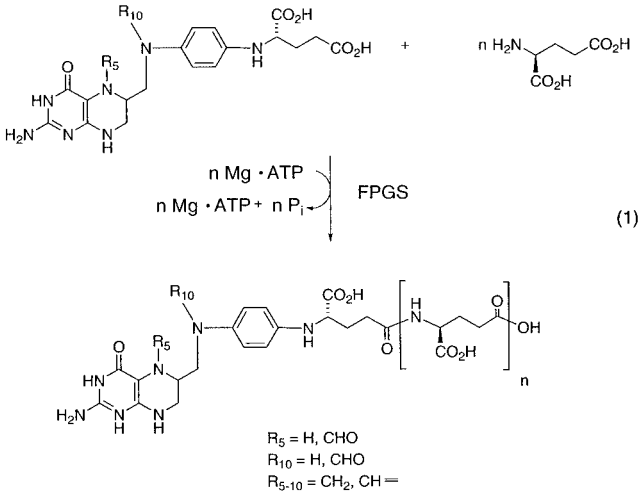
(1) Fersht, A. *Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding*; W. H. Freeman & Co.: New York, 1999.
(2) Mader, M. M.; Bartlett, P. A. *Chem. Rev.* **1997**, *97*, 1281.
(3) Radzicka, A.; Wolfenden, R. *Methods Enzymol.* **1995**, *249*, 284.
(4) Collinsova, M.; Jiracek, J. *Curr. Med. Chem.* **2000**, *7*, 629.
(5) Use of ψ as symbol for peptide surrogates as described by Spatola: IUPAC−IUB Joint Commission on Biochemical Nomenclature. *Eur. J. Biochem.* **1984**, *138*, 9.
(6) Morgan, B. P.; Holland, D. R.; Matthews, B. W.; Bartlett, P. A. *J. Am. Chem. Soc.* **1994**, *116*, 3251.
(7) Khan, A. R.; Parrish, J. C.; Fraser, M. E.; Smith, W. W.; Bartlett, P. A.; James, M. N. G. *Biochemistry* **1998**, *37*, 16839.
(8) McGuire, J. J.; Coward, J. K. In *Folates and Pterins*; Blakley, R. L., Benkovic, S. J., Eds.; Wiley: New York, 1984; Vol. 1; p 135.
(9) Malachowski, W. P.; Coward, J. K. *J. Org. Chem.* **1994**, *59*, 7625.
(10) Tsukamoto, T.; Haile, W. H.; McGuire, J. J.; Coward, J. K. *Arch. Biochem. Biophys.* **1998**, *355*, 109.
(11) Shane, B. *Vitam. Horm.* **1989**, *45*, 263.
(12) Pietrzik, K.; Bronstrup, A. *Ann. Nutrition Metab.* **1997**, *41*, 331.

10.1021/jo010283t CCC: $20.00
Published on Web 06/27/2001

Scheme 1



tion of multiple glutamate residues to a reduced folate substrate as shown in eq 1. We have shown that each



ligation step of the oligomerization reaction proceeds via a γ-glutamyl phosphate intermediate.[13] This mechanistic background suggests the use of phosphapeptides as mechanism-based inhibitors of FPGS (Scheme 1). In the sequence of reactions shown, the "accepting glutamate" is converted to an acyl phosphate. The tetrahedral intermediate resulting from attack by the amine moiety of the "incoming glutamate" on the acyl phosphate then collapses to form a new γ-glutamyl peptide bond concomitant with the expulsion of P_i. This process is repeated until the full-length poly-γ-glutamate is synthesized. A phosphapeptide should act as an excellent mimic of the tetrahedral intermediate, a so-called "tetrahedral mimic" (Scheme 1), and thereby inhibit the FPGS-catalyzed reaction. As noted above, we have previously reported the synthesis and biochemical data for the phosphonate-containing phosphapeptide (Scheme 1, X = O), and it is a potent, reversible competitive (vs MTX) inhibitor of FPGS (K_i = 46 nM, vs K_m = 62 μM for corresponding Glu-γ-Glu substrate).[10] There is ample precedent in the literature that phosphinate-containing phosphapeptides [ψ{P(O)(OH)CH_2}] can undergo an enzyme-catalyzed, ATP-dependent reaction leading to a phosphorylated phosphinate-containing pseudopeptide, [ψ{P(O)(OPO_3^{2-})CH_2}], an even more potent inhibitor of ATP-dependent ligation (Scheme 1). The most well-studied examples of this type of inhibition involve Gln synthetase[14] and D-Ala-D-Ala ligase (Figure 1).[15,16] More recently, evidence for the enzyme-catalyzed phosphorylation of phosphinate-containing pseudopeptides has been

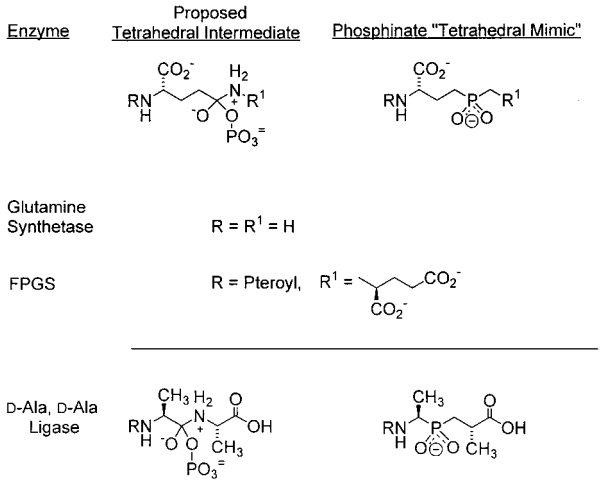
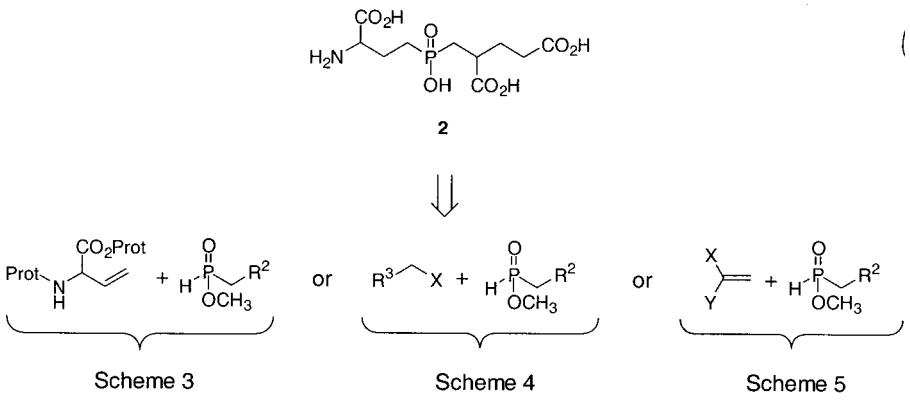


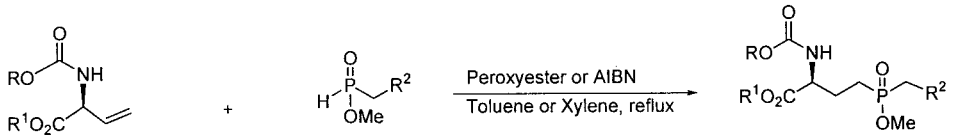
Figure 1. Phosphinate mimics of tetrahderal intermediates proposed for three ATP-dipendent liganses.

(13) Banerjee, R. V.; Shane, B.; McGuire, J. J.; Coward, J. K. *Biochemistry* **1988**, *27*, 9062.

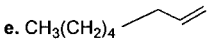
Scheme 2



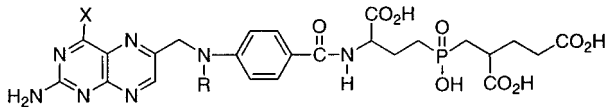
Scheme 3



3 4 5

		Reference
a. R = *t*-Bu, R^1 = Me	**a.** $R^2 = CH_2CO_2Bn$	This work
b. R = Bn, R^1 = Me	**b.** $R^2 = CHCO_2Me$ (CH$_2$CH$_3$)	Katoh et al., 1996
c. R = Bn, R^1 = Me	**c.** $R^2 = (CH_2)_6CH_3$	This work
d. R = Bn, R^1 = Me	**d.** R^2 = H	Zeiss, 1992
e. $CH_3(CH_2)_4$ 	**e.** $R^2 = CHCO_2Bn$ ($CH_2CH_2CO_2Bn$)	This work

provided for glutathione synthetase,[17] glutathionylspermidine synthetase,[18,19] and D-alanyl-D-lactate ligase.[20] The analogy with FPGS is obvious and led us to investigate the synthesis of pteroyl-based phosphinate-containing pseudopeptides **1**, as mechanism-based inhibitors of FPGS.



1

a, X = OH, R = H

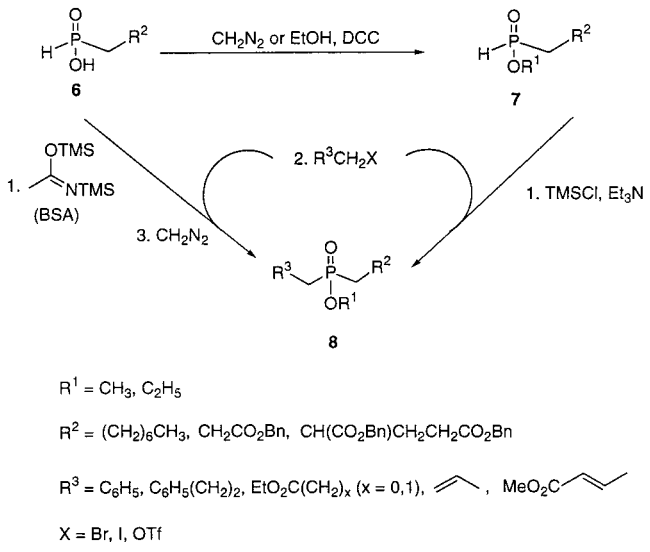
b, X = NH_2, R = CH_3

Results and Discussion

In devising a strategy for the synthesis of **1**, the most obvious disconnection for a convergent synthesis is the amide linkage between the pteroyl moiety and the phosphapeptide. We have previously applied this strategy to a wide variety of folate (e.g., **1a**) and MTX (e.g., **1b**) derivatives acylated with amino acids and peptides.[21] Therefore, the initial synthetic target was a phosphinate-containing pseudopeptide, Glu-γ-[y{P(O)(OH)CH_2}]Glu, **2**. A retrosynthetic analysis suggested three possible approaches to the synthesis of **2** (Scheme 2). Initially, the radical-based coupling of Zeiss[22] and Baylis[23] was investigated (Scheme 3). N- and C-protected vinyl glycine derivatives **3**[24,25] and excess **4** were subjected to conditions reported to generate a phosphorus-based radical (i.e., peroxyesters or AIBN) at elevated temperatures. Under these conditions, carboalkoxy-substituted phosphinic acid esters **4a**,**b**,**e** were found to be unstable. Oda's group used this methodology to synthesize an inhibitor of glutathionylcysteine synthetase, but the reported yield

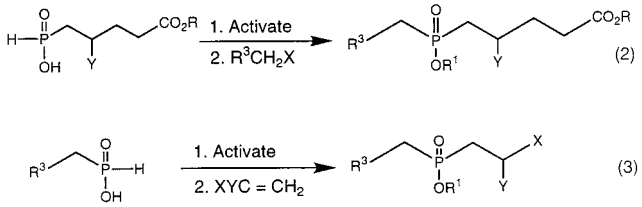
(14) Abell, L. M.; Villafranca, J. J. *Biochemistry* **1991**, *30*, 6135.
(15) McDermott, A. E.; Creuzet, F.; Griffin, R. G.; Zawadzke, L. E.; Ye, Q. Z.; Walsh, C. T. *Biochemistry* **1990**, *29*, 5767.
(16) Fan, C.; Moews, C.; Walsh, C. T.; Knox, J. R. *Science* **1994**, *266*, 439.
(17) Hiratake, J.; Kato, H.; Oda, J. *J. Am. Chem. Soc.* **1994**, *116*, 12059.
(18) Chen, S.; Lin, C.-H.; Walsh, C. T.; Coward, J. K. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 505.
(19) Lin, C.-H.; Chen, S.; Kwon, D. S.; Coward, J. K.; Walsh, C. T. *Chem. Biol.* **1997**, *4*, 859.
(20) Kuzin, A. P.; Sun, T.; Jorczak-Baillass, J.; Healy, V. L.; Walsh, C. T.; Knox, J. R. *Struct. Folding Design* **2000**, *8*, 463.
(21) Tsukamoto, T.; Coward, J. K.; McGuire, J. J. In *Biomedical Frontiers of Fluorine Chemistry*; Ojima, I., McCarthy, J. R., Welch,J. T., Eds.; American Chemical Society: Washington, DC, 1996; p 118.
(22) Zeiss, H.-J. *Tetrahedron* **1992**, *48*, 8263.
(23) Baylis, K. E. *Tetrahedron. Lett.* **1995**, *36*, 9389.
(24) Hanessian, S.; Sahoo, S. P. *Tetrahedron Lett.* **1984**, *25*, 1425.
(25) Carrasco, M.; Jones, R. J.; Kamel, S.; Rapoport, H.; Truong, T. *Organic Syntheses*; Wiley: New York, 1998; Collect. Vol. IX, p 63.

Scheme 4



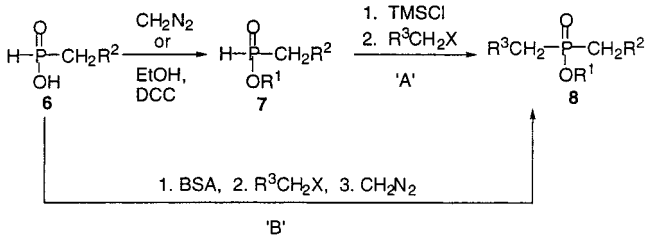
of **5b** was only 22%.[26] The higher yields reported by Zeiss and Baylis involved the use of less complex phosphinic acid esters **4** (e.g., R_2 = H (**4d**, Zeiss), which are apparently considerably more stable at the elevated temperatures required for the reaction shown in Scheme 3 than are **4a**,**b**,**e**. Considering the fact that the reaction of **3a** and **4a** failed to yield **5a**, the reaction of an alkyl phosphinic acid ester **4c** was investigated. Although ^{31}P NMR analysis of the reaction mixture indicated formation of the desired product **5c**, unreacted **4c** proved difficult to separate from the desired product. Thus, our results indicate that synthesis of **2** via phosphorus-based radicals is untenable due to the instability of functionalized phosphinic acid esters **4** at the elevated temperatures employed.

In terms of the target phosphinates **2**, the ability to form two P−C bonds involving glutamyl-like moieties is required. Equations 2 and 3 show two different approaches to the formation of this array. The substituted



phosphinic acids are available either via the reaction of $(TMSO)_2PH$ or NaH_2PO_2 (± AIBN) with the appropriate olefin.[27−29] Given the instability of functionalized phosphorus-based radicals in the experiments just described, our attention turned to the transient generation of P^{III} species for use in alkylation (Scheme 4) and conjugate addition (Scheme 5) reactions under mild conditions. Two methods for the in situ generation of P^{III} species were

Table 1. S_B2 Displacement by P^{III} Species



entry	R^1	R^2	R^3	X	method[a]	yield, %
1	C_2H_5	CH_2CO_2Bn	CH_2=CH	Br	A	0
2	C_2H_5	CH_2CO_2Bn	C_6H_5	Br	A	34
3	C_2H_5	CH_2CO_2Bn	$C_6H_5(CH_2)_2$	OTf	A	0
4	C_2H_5	$CHCO_2Et$ | $CH_2CH_2CO_2Et$	EtO_2C	Br	A	28[b]
5	CH_3	$(CH_2)_6CH_3$	CH_2=CH	Br	B	73
6	CH_3	$(CH_2)_6CH_3$	C_6H_5	Br	B	74
7	CH_3	$(CH_2)_6CH_3$	MeO_2C-CH=CH	Br	B	40
8	CH_3	$CH_3(CH_2)_6CH_3$	EtO_2C	Br	B	90
9	CH_3	$(CH_2)_6CH_3$	EtO_2CCH_2	Br	B	0
10	CH_3	$(CH_2)_6CH_3$	CH_3CH_2	I	B	0
11	CH_3	CH_2CO_2Bn	CH_2=CH	Br	B	82
12	CH_3	$CHCO_2Bn$ | $CH_2CH_2CO_2Bn$	CH_2=CH	Br	B	73

[a] Method A: (i) TMSCl, Et_3N, rt, 1 h; (ii) R^3CH_2X, rt, overnight. Method B: (i) BSA, 0 °C, 15 min; (ii) R^3CH_2X, 0 °C → rt, overnight; (iii) CH_2N_2, 0 °C, 30 min. [b] Reference 9.

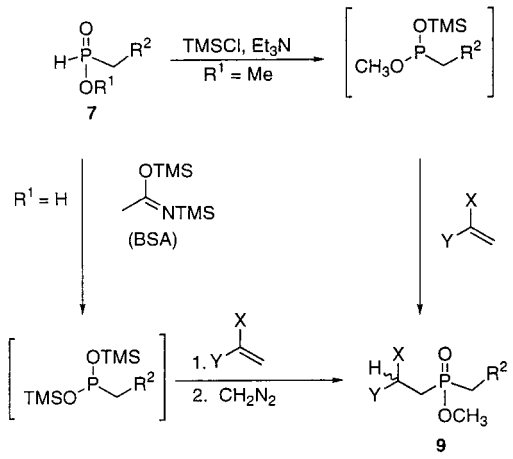
investigated. Subsequent reaction with either alkyl halides or electron-deficient olefins allowed for the evaluation of these two different methods. As shown in Tables 1 and 2, the use of preformed phosphinic acid esters (R^1 = Me, Et) followed by reaction with TMSCl to generate a mixed phosphonite (method A) proved to be less successful than the generation of bis-TMS phosphonite (method B).

Among the various alkyl halides evaluated via method A, only benzyl bromide and ethyl bromoacetate[9] afforded any of the desired product **8**, and that at only a low yield of 34% and 28%, respectively (Table 1, entries 2 and 4). In contrast, use of method B, involving in situ formation of the bis-TMS phosphonite, provided good to excellent yields (Table 1, entries 5, 6, and 8). Of interest in terms of our ultimate synthetic target, the successful alkylations by allyl bromide (Table 1, entries 11 and 12) proceeded in high yield to afford a functionalized phosphinate containing a glutamyl-like moiety at a position analogous to the C-terminus of the target phosphapeptide **2**. These results are in agreement with those of Reiter and Jones,[30] who attributed this enhanced reactivity to stabilization of the developing positive charge on phosphorus by silicon at the β-position.[31] It should be noted that method B was not uniformly successful; unactivated alkyl halides were unable to effect the desired formation of **8** (Table 1, entries 9 and 10) under this set of reaction conditions.

The use of conjugate addition to access phosphinates **9** appeared to be less sensitive to the nature of the P^{III} species and more sensitive to the electron density at the olefin (Table 2). Thus, addition of a phosphonite to phenyl vinyl sulfone (Table 2, entry 1) and benzyl acrylate (Table

(26) Katoh, M.; Hiratake, J.; Kato, H.; Oda, J. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1437.
(27) Jackson, P. F.; Cole, D. C.; Slusher, B. S.; Stetz, S. L.; Ross, L. E.; Donzanti, B. A.; Trainor, D. A. *J. Med. Chem.* **1996**, *39*, 619.
(28) Nan, F.; Bzdega, T.; Pshenichkin, S.; Wroblewski, J. T.; Wroblewska, B.; Neale, J. H.; Kozikowski, A. P. *J. Med. Chem.* **2000**, *43*, 772.
(29) Karanewsky, D. S.; Badia, M. C.; Cushman, D. W.; DeForrest, J. M.; Dejneka, T.; Loots, M. J.; Perrie, M. G.; Petrillo, E. W., Jr.; Powell, J. R. *J. Med. Chem.* **1988**, *31*, 204.
(30) Reiter, L. A.; Jones, B. J. *J. Org. Chem.* **1997**, *62*, 2808.
(31) El Gihani, M. T.; Heaney, H. *Synthesis* **1998**, 357.

Scheme 5



R^1 = H, CH_3

R^2 = $(CH_2)_6CH_3$, CH_2CO_2Bn

X, Y = BnO_2C, H; BnO_2C, $BnO_2C(CH_2)_2$

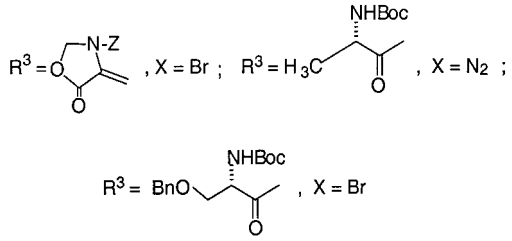
X, Y = $C_6H_5SO_2$, H; $C_6H_5SO_2$, CH_3; *p*$TolSO_2$, CH_3; $C_6H_5SO_2$, EtO_2CCH_2

2, entry 4) proceeded in good to excellent yield.[32] However, addition of electron-donating groups to the olefin led to complete ablation of the reaction (Table 2, entries 2, 3, and 5). Once again, the use of BSA to generate an intermediate bis-TMS phosphonite led to the desired conjugate addition to the less reactive olefins (Table 2, entry 6). The latter reaction also leads to a phosphinate containing a glutamyl-like element at the C-terminal portion of phosphapeptide **2** (Scheme 2).

Although these investigations provided the framework for introducing the C-terminal glutamyl-like residue (eqs 2 and 3, Y = CO_2R), several attempts to introduce the N-terminal amino acid moiety to phosphinates **8** and **9** failed. This included the use of appropriately substituted alkyl halides and diazoketones (eq 2) as amino acid surrogates.[33] The synthesis of phosphinic acid analogues of glutamic acid has been reported by Kurdyumova et al.[34] Extending this approach to the synthesis of **2** required the synthesis of a complex α,*β*-unsaturated phosphinic acid (e.g., **12**, Scheme 6) that would be susceptible to conjugate addition by the anion of diethylacetamido malonate. Investigations into this synthetic route led to the successful synthesis of **2** as outlined in Scheme 6. Thus, conversion of **10** to the bis-TMS ester followed by alkylation with 1,2-dibromoethane led to **11**

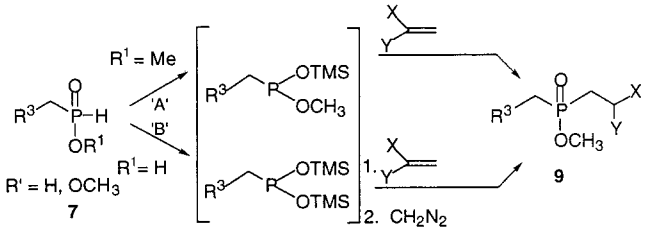
(32) Thottathil, J. K.; Ryono, D. E.; Przybyla, C. A.; Moniot, J. L.; Neubeck, R. *Tetrahedron Lett.* **1984**, *25*, 4741.

(33) These included halomethyl- and diazomethyl ketone derivatives (R^3CH_2X, Table 1) of dehydroalanine, alanine, and serine as shown below:



(34) Kurdyumova, N. R.; Ragulin, V. V.; Tsvetkov, E. N. *Mendeleev Commun.* **1997**, *1997*, 69.

Table 2. Conjugate Addition by P^{III} Species



entry	R^3	X	Y	method[a]	yield, %
1	$(CH_2)_6CH$	$C_6H_5SO_2$	H	A	97
2	$(CH_2)_6CH_3$	$C_6H_5SO_2$	CH_3	A	0
3	$(CH_2)_6CH_3$	*p*-$CH_3C_6H_4$-SO_2	CH_3	B	0
4	$(CH_2)_6CH_3$	$BnCO_2$	H	A	73
5	$(CH_2)_6CH_3$	$BnCO_2$	$BnCO_2(CH_2)_2$	A	0
6	$(CH_2)_6CH_3$	$BnCO_2$	$BnCO_2(CH_2)_2$	B	62

[a] Method A: (i) TMSCl, Et_3N, 0 °C → rt, 1 h; (ii) XYC=CH_2, 0 °C → rt, 6 h. Method B: (i) BSA, 0 °C, 15 min; (ii) XYC=CH_2, 0 °C → rt, overnight; (iii) CH_2N_2, 0 °C, 30 min.

together with the α,*β*-unsaturated phosphinic acid **12a**. Esterification of the mixture with $HC(OEt)_3$ and concomitant dehydrohalogenation led to **12**, which could be purified by silica gel chromatography. Conjugate addition of diethyl acetoamidomalonate followed by removal of the benzyl esters and acidic hydrolysis led to the desired complex phosphinic acid **2**.

Previous publications from this laboratory have described the synthesis of numerous pteroyl and 4-amino-10-methylpteroyl derivatives of glutamates,[13] fluoroglutamates,[21] and *γ*-glutamyl peptides.[35,36] In these syntheses, considerable time and material was consumed in a series of protection and deprotection steps to join the heterocycle and peptide moieties. Two approaches have been used in our laboratory and those of others with interests in folate biochemistry or antifolate pharmacology. One involves the linear assembly of the peptide, *p*-aminobenzoyl, and pteridine portions[37] while the other involves a more convergent synthesis based on the coupling of an activated pteroic acid derivative with a protected *γ*-glutamyl peptide.[38,39] Recently, Fuchs and colleagues[40] described an efficient synthesis of pteroyl azide (**13a**) from folic acid and its reaction with free glutamic acid to yield folic acid. More recent applications of this reaction have suggested that **13a** reacts with a wide variety of amines to form pteroyl derivatives (e.g., pteroyl bridged to taxol)[41] for use in drug delivery research. In addition, Modest and colleagues reported the synthesis of the corresponding 2-amino-10-methylpteroyl azide (**13b**) many years ago for use in the synthesis of methotrexate analogues.[42] We have extended the Fuchs approach to the synthesis of the target pteroyl-Glu-*γ*-[*ψ*-{P(O)(OH)CH_2}]Glu (**1a**) and the corresponding meth-

(35) Licato, N. J.; Coward, J. K.; Nimec, Z.; Galivan, J.; Bolanowska, W. E.; McGuire, J. J. *J. Med. Chem.* **1990**, *33*, 1022.

(36) Coward, J. K.; Parameswaran, K. N.; Cashmore, A. R.; Bertino, J. R. *Biochemistry* **1974**, *13*, 3899.

(37) Piper, J. R.; Montgomery, J. A. *J. Org. Chem.* **1977**, *42*, 208.

(38) Meienhofer, J.; Jacobs, P. M.; Godwin, H. A.; Rosenberg, I. H. *J. Org. Chem.* **1970**, *35*, 4137.

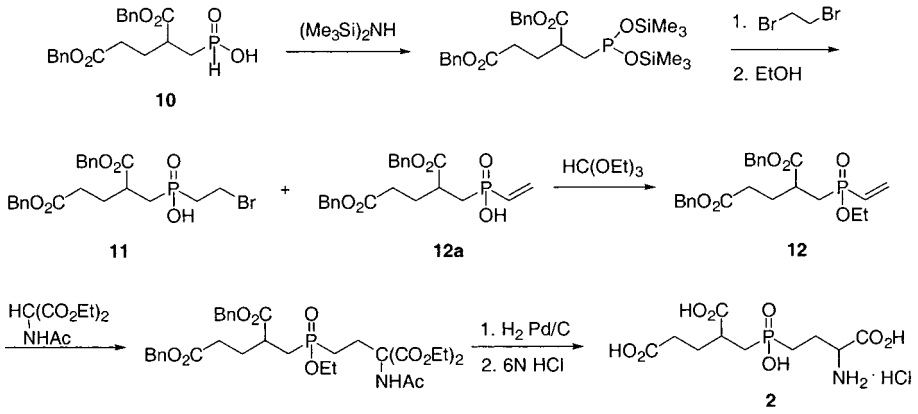
(39) Rosowsky, A.; Freisheim, J. H.; Bader, H.; Forsch, R. A.; Susten, S. S.; Cucchi, C. A.; Frei, E., III. *J. Med. Chem.* **1985**, *28*, 660.

(40) Luo, J.; Smith, M. D.; Lantrip, D. A.; S., W.; Fuchs, P. L. *J. Am. Chem. Soc.* **1997**, *119*, 10004.

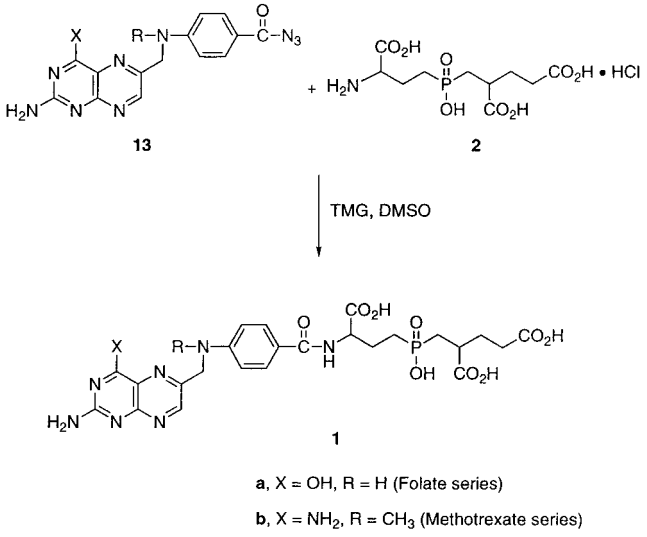
(41) Lee, J. W.; Fuchs, P. L. *Org. Lett.* **1999**, *1*, 179.

(42) Chaykovsky, M.; Brown, B. L.; Modest, E. J. *J. Med. Chem.* **1975**, *18*, 909.

Scheme 6



Scheme 7



otrexate derivative (**1b**) (Scheme 7). Preliminary biochemical evaluation of **1b** as an inhibitor of human FPGS has shown it to be a potent inhibitor with IC_{50} = ca. 20 nM. Detailed kinetic studies including investigation of possible FPGS- and ATP-dependent formation of **1**−PO_3^{2-} (Scheme 1) are currently in progress. Considering the known stereochemical preference of FPGS substrates is 2*S* at both the accepting and incoming glutamates[8] and that **1b** is a mixture of four isomers (two diastereomeric racemates), it is likely that the IC_{50} for the *S*,*S* isomer corresponding to the naturally occurring (2*S*)Glu-γ-(2*S*)-Glu peptide is ca. 5 nM. It should be noted that several recent syntheses of complex phosphinates also yield racemic products.[43−45] The development of methodology for the stereoselective synthesis of complex phosphinate phosphapeptides such as (2*S*, 2′*S*)-**2** is under investigation in our laboratory.

In conclusion, we have investigated several routes to a phosphinate phosphapeptide (**2**) analogous to the γ-glutamyl peptide, Glu-γ-Glu. The use of phosphorus radical chemistry to form a second P−C bond via addition across the double bond of vinyl glycine (and other olefins) was first studied. This approach failed due to the instability of highly functionalized phosphinic acid esters at the high temperatures required. Trivalent phosphorus (P^{III}) species were then investigated, and it was found that bis-TMS phosphonites were most effective in a series of alkylation and conjugate addition reactions to afford a wide range of phosphinates. Unfortunately, this chemistry could not be extended to the incorporation of an amino acid moiety at the N-terminal side of **2**. A successful synthesis of **2** was effected using P^{III} chemistry followed by dehydrohalogenation to yield an α,β-unsaturated phosphinic acid ester. Conjugate addition of diethylacetamido malonate followed by acid-mediated hydrolysis afforded the phosphapeptide **2**. Coupling of **2** to two pteroyl azides, **13a** and **13b**, led to the corresponding pteroylphosphapeptides **1a** and **1b**. The latter compound is an extremely potent inhibitor of human FPGS.

Experimental Section

General Procedures. For general experimental techniques, see recent publications from this laboratory on this type of chemistry.[9,46,47] All reactions involving moisture-sensitive reagents were performed in oven-dried glassware under an atmosphere of nitrogen or argon. All solvents used in moisture-sensitive reactions were dried as follows. Tetrahydrofuran was freshly distilled form sodium/benzophenone. Methanol was freshly distilled from 4 Å molecular sieves prior to use. Toluene was freshly distilled over sodium metal, and 1,2-dibromoethane was freshly distilled over P_2O_5. All flash chromatography was carried out using silica gel (0.04−0.06 mm, 230−400 mesh) as the stationary phase. Folic acid, potassium thiocyanate, and *tert*-butylnitrite were purchased from Acros Chemicals. Ammonium hypophosphite (H_2POONH_4) was purchased from Fluka. All other reagents were purchased from Sigma-Aldrich and were used without further purification. *n*-Octylphosphinic acid **6** ($R^2 = (CH_2)_6CH_3$) and 2-[(hydroxyphosphinyl)methyl]pentane-1,5-dioic acid dibenzyl ester **6** (R^2 = $CH(CO_2Bn)CH_2CH_2CO_2Bn$) were prepared using methods previously described.[29,48,49] The dibenzyl and dimethyl esters of 2-methyleneglutarate were prepared by literature procedures.[27,50] With minor modifications, pteroylhydrazide[40] and the 4-amino-4-deoxy-10-methyl analogue of pteroic acid[51]

(43) Zeng, B. Q.; Wong, K. K.; Pompliano, D. L.; Reddy, S.; Tanner, M. E. *J. Org. Chem.* **1998**, *63*, 10081.
(44) Miller, D. J.; Hammond, S. M.; Anderluzzi, D.; Bugg, T. D. H. *J. Chem. Soc., Perkin Trans. 1* **1998**, 131.
(45) Georgiadis, D.; Matziari, M.; Vassiliou, S.; Dive, V.; Yiotakis, A. *Tetrahedron* **1999**, *55*, 14635.
(46) Malachowski, W. P.; Coward, J. K. *J. Org. Chem.* **1994**, *59*, 7616.
(47) Chen, S.; Coward, J. K. *J. Org. Chem.* **1998**, *63*, 502.
(48) Boyd, E. A.; Regan, A. C.; James, K. *Tetrahedron Lett.* **1992**, *33*, 813.
(49) In more recent research. a generous gift of compound **10** (Scheme 6) was provided by Dr. Takashi Tsukamoto, Guilford Pharmaceuticals, Baltimore, MD.
(50) Moiseenkov, A. M.; Cheskis, B. A.; Shpiro, N. A.; Stashina, G. A.; Zhulin, V. M. *Bull. Acad. Sci. USSR, Div. Chem. Sci* **1990**, 517.
(51) Kralovec, J.; Spencer, G.; Blair, A. H.; Mammen, M.; Singh, M.; Ghose, T. *J. Med. Chem.* **1989**, *32*, 2426.

were synthesized as described previously. Experimental details, including complete spectral characterization, for the synthesis of these two precursors of azides **13a** and **13b** are contained in the Supporting Information. Melting points were taken on a Thomas-Hoover Mel-Temp apparatus and are uncorrected. All NMR spectra were recorded on Bruker AVANCE DRX300 or DRX500 spectrometers. ^{1}H NMR spectra were recorded at 300 or 500 MHz and are reported as follows: chemical shifts in ppm downfield from internal tetramethylsilane (multiplicity, integrated intensity, coupling constant in Hz). ^{13}C NMR spectra were obtained at 75 or 126 MHz and referenced to tetramethylsilane. ^{31}P NMR spectra were recorded at 121 MHz with 1% aqueous H_3PO_4 as an external reference. ^{19}F NMR spectra were recorded at 282 MHz with TFA as an external standard. Unless otherwise noted, all ^{13}C, ^{19}F, and ^{31}P NMR spectra are proton decoupled.

***n*-Octylphosphinic Acid Methyl Ester (7, $R^1 = CH_3$, $R^2 = (CH_2)_6CH_3$).** To a solution of the phosphinic acid **6** ($R^2 = (CH_2)_6CH_3$) (0.456 g, 2.56 mmol) in Et_2O (10 mL) was added at 0 °C an ethereal solution of CH_2N_2 in excess of the required stoichiometry. The reaction solution was stirred for 30 min, after which time HOAc was added to quench the excess CH_2N_2. After concentration of the quenched reaction mixture in vacuo, the crude reaction product was purified by silica gel chromatography (EtOAc) to give the desired methyl ester (0.46 g, 93% yield): ^{1}H NMR ($CDCl_3$) δ 0.90 (3H, t, J = 4.03 Hz), 1.30−1.80 (14H, m), 3.80 (3H, d, J = 7.03 Hz), 7.07 (1H, dt, J = 1.10, 317 Hz); ^{31}P NMR ($CDCl_3$) δ 43.4.

3-(Methoxyphosphinyl)propanoic Acid Benzyl Ester (7, $R^1 = CH_3$, $R^2 = CH_2CO_2Bn$).[27] Hypophosphorous acid (4.59 g, 34.8 mmol, 50% aqueous solution) and triethylamine (2.45 g, 34.8 mmol) were mixed and dried by azeotropic removal of toluene in vacuo. The residue was dissolved in dry CH_2Cl_2 (40 mL) and cooled to 0 °C. Triethylamine (8.25 mL, 60.8 mmol) and chlorotrimethylsilane (7.45 mL, 60.8 mmol) were added followed after 5 min by benzyl acrylate (0.81 g, 5.0 mmol) in CH_2Cl_2. The mixture was stirred overnight at room temperature and then filtered. The filtrate was washed with 1 N hydrochloric acid and water, dried over anhydrous $MgSO_4$, and concentrated to afford the phosphinic acid, **6**. This compound was dissolved in ether (10 mL) and cooled to 0 °C. To this solution was added diazomethane in ether solution (10 mmol) slowly. The reaction mixture was allowed to warm to room temperature, stirred for 3 h, and then concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc) to produce 0.68 g of the methyl ester, **7** (56% overall yield): ^{1}H NMR ($CDCl_3$) δ 2.0−2.3 (m, 2 H), 2.6−2.9 (m, 2 H), 3.79 (3 H, d, J = 11.8 Hz), 5.16 (s, 2 H), 7.19 (dt, 1 H, J = 1.82, 548 Hz), 7.2−7.4 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 24.8, 26.5 (d, J = 2.94 Hz), 53.3 (d, J = 6.94 Hz), 67.4, 128.8, 128.9, 129.1, 135.8, 172.2 (d, J = 12.4 Hz); ^{31}P NMR ($CDCl_3$) δ 40.4.

2-[(Methoxyphosphinyl)methyl]pentane-1,5-dioic Acid Dibenzyl Ester (7, $R^1 = CH_3$, $R^2 = CH(CO_2Bn)CH_2CH_2CO_2Bn$). A mixture of H_2POONH_4 (0.125 g, 1.5 mmol) and hexamethyldisilazane (0.32 mL, 1.5 mmol) was heated at 100−110 °C for 1 h. After the mixture was cooled to 0 °C, dry CH_2Cl_2 and dibenzyl 2-methyleneglutarate (0.189 g, 0.58 mmol) were added, and the resulting mixture was stirred at room temperature for 24 h, after which time it was quenched with 1 N HCl and extracted three times with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo. The resulting crude phosphinic acid **6** ($R^2 = CH(CO_2Bn)CH_2CH_2CO_2Bn$, 0.10 g, 0.26 mmol) was dissolved in 4 mL of MeOH, and a solution of ethereal CH_2N_2 was added. The resulting reaction mixture was stirred overnight at room temperature and then concentrated in vacuo. Partial purification by silica gel chromatography (EtOAc) afforded the desired ester **7** as a diastereomeric mixture together with an inseparable impurity (0.152 g): ^{1}H NMR ($CDCl_3$) δ 1.8−1.95 (1H, m), 1.96−2.10 (2H, m), 2.13−2.28 (1H, m), 2.30−2.45 (2H, m), 2.85−3.00 (1H, m), 3.69 (3H, t, J = 11.5 Hz), 5.0−5.2 (4H, m), 6.52−6.55 (0.5H, m) 7.27−7.40 (10H, m), 7.60−7.80 (0.5H, m); ^{13}C NMR ($CDCl_3$) δ 28.07, 28.10, 28.17, 28.20, 29.92, 30.11, 30.67, 30.85, 31.20, 31.21, 37.93, 37.95, 38.03, 38.06, 52.79, 52.84, 52.91, 52.96, 66.41, 66.96, 67.03, 128.21, 128.26, 128.32, 128.39, 128.44, 128.52, 128.57, 135.28, 135.31, 135.64, 172.10, 173.30, 173.37, 173.42; ^{31}P NMR 38.7, 37.7, 35.6, 34.6. This crude material was used directly for the synthesis of **8** ($R^1 = CH_3$, $R^2 = CH_2(CO_2Bn)CHCH_2\ CH_2C\ CO_2Bn$, $R^3 = CH_2 = CH$; Table 1, entry 12).

3-[Ethoxy(phenylmethyl)phosphinyl]propanoic Acid Benzyl Ester (8, $R^1 = C_2H_5$, $R^2 = CH_2CO_2Bn$, $R^3 = C_6H_5$). Method A (Table 1, Entry 2). To a solution of benzyl 3-(ethoxyphosphinyl)propionate (**7**, $R^1 = C_2H_5$, $R^2 = CH_2CO_2$-Bn, 0.10 g, 0.391 mmol) in CH_2Cl_2 (5 mL) was added a mixture of TMSCl (0.17 g, 1.57 mmol) and triethylamine (0.16 g, 1.57 mmol) at room temperature. After being stirred for 1 h, benzyl bromide (10 equiv) was added to the above mixture at room temperature and stirred for 1 day at that temperature. The reaction was quenched with aqueous $NaHCO_3$, and the mixture was extracted with CH_2Cl_2 three times. The combined organic layers were dried over anhydrous $MgSO_4$ and filtered and the filtrate evaporated. The residue was purified by silica gel column chromatography (EtOAc) to afford the desired compound (46 mg, 34%): ^{1}H NMR ($CDCl_3$) δ 1. 26 (3 H, t, J = 7.02 Hz), 1.9−2.1 (2 H, m), 2.4−2.6 (2 H, m), 3.17 (2 H, d, J = 16.9 Hz) 3.8−4.1 (2 H, m) 5.13 (2 H, s), 6.1−7.4 (10 H, m); ^{13}C NMR ($CDCl_3$) δ 17.0 (d, J = 5.62 Hz) 22.5, 23.8, 27.1 (d, J = 3.28 Hz), 36.6, 37.7, 61.3 (d, J = 6.45 Hz) 67.2, 127.4, 128.7, 128.8, 129.0, 129.2, 129.3, 130.0, 130.1, 136.0, 172.3; ^{31}P NMR ($CDCl_3$) δ 52.2.

3-[Methoxy(2-propenyl)phosphinyl]propanoic Acid Benzyl Ester (8, $R^1 = CH_3$, $R^2 = CH_2CO_2Bn$, $R^3 = CH_2{=}CH$). Method B. (Table 1, Entry 11). To a solution of phosphinic acid **6** ($R_2 = (CH_2)_2CO_2Bn$, 1.79 g, 7.87 mmol) and allyl bromide (2.85 g, 23.7 mmol) in CH_2Cl_2 (20 mL) at room temperature was added *N*,*O*-(bis-trimethylsilyl)acetamide (BSA) (9.61 g, 47.2 mmol). The reaction mixture was stirred at room temperature overnight, after which time it was quenched with 1 N HCl, stirred an additional 10 min, and then extracted three times with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo. The resulting crude phosphinic acid **7** was used directly in the next step without further purification.

To an ethereal solution of the crude phosphinic acid **7** described above was added an ethereal solution of excess diazomethane at 0 °C and then allowed to warm to room temperature. The light yellow solution became colorless after several hours, the reaction was quenched with HOAc, and the solvent was removed in vacuo. The resulting residue was purified by silica gel chromatography (EtOAc) to give 1.83 g (82%, two steps) of the desired *P*-methyl ester **8**: ^{1}H NMR ($CDCl_3$) δ 2.0−2.2 (2 H, m), 2.5−2.7 (2 H, m), 3.72 (3 H, d, J = 10.7 Hz), 5.15 (2 H, s), 5.20−5.30 (2 H, m), 5.60−5.80 (1 H, m), 7.20−7.40 (5 H, m); ^{13}C NMR ($CDCl_3$) δ 21.5 (d, J = 94.4 Hz), 27.0 (d, J = 2.98 Hz), 34.5 (d, J = 87.8 Hz), 51.8 (d, J = 6.79 Hz), 67.2, 121.0 (d, J = 12.7 Hz), 127.7 (d, J = 8.98 Hz), 128.7, 128.8, 129.0, 136.0, 172.5; ^{31}P NMR ($CDCl_3$) δ 54.3.

3-(Methoxy(octyl)phosphinyl)propanoic Acid Benzyl Ester (9, $R^3 = (CH_2)_6CH_3$, X = CO_2Bn, Y = H). Method A (Table 2, Entry 4). To a mixture of TMSCl (0.23 g)/Et_3N (0.22 g) in CH_2Cl_2 (5 mL) was added **7** ($R^1 = CH_3$, $R^3 = (CH_2)_6CH_3$, 0.10 g, 0.52 mmol) at 0 °C. The mixture was stirred at room temperature for 1 h and cooled to 0 °C again. To the above solution was added benzyl acrylate (0.17 g). The whole was stirred at room temperature for 6 h. The reaction was quenched with 1 N HCl, and the solution was extracted with CH_2Cl_2 three times. The combined organic layers were dried over anhydrous $MgSO_4$ and filtered, and the filtrate was evaporated. The residue was purified by silica gel column chromatography (EtOAc) to give the desired phosphinic acid *P*-methyl ester **9** (0.13 g, 73%): ^{1}H NMR ($CDCl_3$) δ 0.90 (3 H, t, J = 3.95 Hz), 1.20−1.40 (10 H, m), 1.50−1.60 (2 H, m), 1.60−1.70 (2 H, m), 2.08 (2 H, dt, J = 4.86, 7.76 Hz), 2.50−2.70 (2 H, m), 3.69 (3 H, d, J = 6.15 Hz), 5.16 (2 H, s), 7.30−7.50 (5 H, m); MS (CI/NH_3) *m*/*z* 355.3 (MH^+, 100); HRMS calcd for $C_{19}H_{31}O_4P$ 355.2038 (MH^+), found 355.2035.

2-[(Methoxy(*n*-octyl)phosphinyl)methyl]pentane-1,5-dioic Acid Dibenzyl Ester (9, $R^3 = (CH_2)_6CH_3$, X = CO_2Bn, Y = $(CH_2)_2CO_2Bn$). Method B (Table 2, Entry 6). To a

solution of *n*-octylphosphinic acid (**6**, $R^2 = (CH_2)_6CH_3$, 0.10 g, 0.562 mmol) and dibenzyl 2-methylene glutarate (0.549 g, 1.69 mmol) in 5 mL of CH_2Cl_2 was added 0.686 g (3.37 mmol) of *N*,*O*-(bis-trimethylsilyl)acetamide (BSA) at room temperature. The reaction mixture was stirred overnight at room temperature, after which time it was quenched with 1 N HCl and extracted three times with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and then concentrated in vacuo. The resulting crude phosphinic acid **8** (R^1 = H) was used in the next reaction without further purification. Formation of the *P*-methyl ester was accomplished with ethereal CH_2N_2 as described above to provide 0.185 g (62%, two steps) of the desired phosphinic acid *P*-methyl ester **8**: ^{1}H NMR ($CDCl_3$) δ 0.90 (3 H, t, J = 6.20 Hz), 1.20−2.40 (23 H, m), 2.70−2.90 (1 H, m), 3.60 (3 H, d, J = 11.8 Hz), 5.10 (2 H, s), 5.11 (2 H, s), 5.14 (2 H, s), 5.15 (2 H, s), 7.20−7.40 (5 H, m); ^{13}C NMR ($CDCl_3$) δ 14.51, 21.96, 22.02, 22.07, 22.13, 22.98, 27.79, 28.20, 29.00, 29.10, 29.24, 29.30, 29.38, 29.41, 29.50, 30.40, 30.69, 30.80, 31.03, 31.24, 31.62, 31.70, 32.00, 32.13, 38.81, 38.86, 39.04, 39.08, 51.18, 51.27, 51.37, 66.68, 67.15, 67.21, 128.57, 128.70, 128.74, 128.79, 128.90, 135.92, 135.95, 136.16, 172.52, 174.25, 174.34; ^{31}P NMR ($CDCl_3$) δ 56.5, 56.2; MS (CI, NH_3) *m*/*z* 517.3 (MH^+, 58% bp); HRMS calcd for $C_{29}H_{41}O_6P$, 517.2719 (MH^+), found 517.2720.

2-[(Ethoxy(vinyl)phosphinyl)methyl]pentane-1,5-dioic Acid Dibenzyl Ester (12). 2-(Hydroxyphosphinoylmethyl)-pentane-1,5-dioic acid dibenzyl ester (**10**) (2.7 g, 6.9 mmol) was dissolved in dry 1,2-dibromoethane (2.4 mL, 27.6 mmol). Hexamethyldisilazane (3 mL, 13.8 mmol) was added to the solution, which was stirred at 120 °C for 20 h. The reaction mixture was cooled to room temperature, after which time EtOH (15 mL) was added, and the resulting solution was stirred at reflux temperature for 0.5 h. Following removal of the solvent in vacuo, the residue was dissolved into EtOAc (60 mL) and washed with water (3 × 15 mL). The organic layer was dried over $MgSO_4$ and evaporated to give **11** (2.4 g) containing ca. 50% **12a** resulting from dehydrohalogenation of **11** in the presence of hexamethyldisilazane: ^{31}P NMR ($CDCl_3$) δ 31.37, 38.97.

This residue was treated with $HC(OEt)_3$ (4.0 mL, 19.2 mmol) for 1.5 h at reflux temperature with simultaneous removal of resulting EtOH, following which excess $HC(OEt)_3$ was removed in vacuo. The residue was subjected to silica gel chromatography (EtOAc/acetone 3:1) to give **12** (1.71 g, 57% over two steps) as a colorless oil: TLC R_f = 0.48 (EtOAc/acetone 3:1); ^{1}H NMR ($CDCl_3$) 1.23−1.29 (m, 3H), 1.80−2.29 (dm, 2H, J = 120 Hz), 2.01−2.03 (m, 2H), 2.34 (t, 2H, J = 8 Hz), 2.80−2.95 (m, 1H), 3.80−4.09 (dm, 2H, J = 22 Hz), 5.09−5.14 (m, 4H), 5.96−6.35 (m, 3H), 7.29−7.38 (m, 10H); ^{13}C NMR ($CDCl_3$) 16.78, 16.86, 28.99, 30.67, 30.84, 31.72, 31.77, 32.00, 32.18, 38.96, 60.96, 61.05, 66.77, 67.18, 128.62, 128.66, 128.75, 128.79, 128.96, 129.89, 130.17, 136.20, 136.88, 172.64, 174.15, 174.19, 174.24, 174.28; ^{31}P NMR ($CDCl_3$) 39.0, 39.5; MS (CI, NH_3) *m*/*z* 445.2 (MH^+, 100). HRMS (DCI with ammonia) calcd for $C_{24}H_{29}O_6P$ 445.1780 $[M + H]^+$, found 445.1780. Anal. Calcd for $C_{24}H_{29}O_6P$: C, 64.86; H, 6.58. Found: C, 64.89; H, 6.51.

2-[((3-Amino-3-carboxypropyl)(hydroxy)phosphinyl)-methyl]pentane-1,5-dioic Acid (2). A solution of α,β-unsaturated phosphinic acid ester **12** (0.79 g, 1.8 mmol) in dry EtOH (1 mL) was added to a solution of diethyl acetoamidomalonate (0.35 g, 1.8 mmol) in dry EtOH (2 mL) containing metallic Na (0.04 g, 1.8 mmol). The reaction mixture was stirred at 80 °C overnight. Ethanol was evaporated. The residue was dissolved in $CHCl_3$ (30 mL) and washed with 3% HCl (5 mL) and water (2 × 5 mL). $CHCl_3$ was evaporated. Unreacted diethyl acetoamidomalonate was removed by silica gel plug filtration ($CHCl_3$). The resulting 2-(ethoxycarbonyl)-2-(acetamido)-4-[(2,4-dibenzoxycarbonylbutyl)(ethoxy)-phosphinyl]butanoic acid ethyl ester (0.78 g) was dissolved in EtOH (5 mL). Pd/C (0.021 g, 0.02 mmol) was added to the solution, which was shaken overnight under an atmosphere of H_2. Pd/C was removed by filtration, after which EtOH was evaporated.

HCl (6 N, 5 mL) was added to the resulting residue (0.49 g). The mixture was heated for 20 h at reflux temperature as solution was effected. The resulting solution was cooled to room temperature. Light organic byproducts were removed by extraction with ether. The aqueous layer was evaporated to give **2** as a white solid (0.39 g, 88%); mp 121−125 °C dec; TLC on cellulose R_f = 0.5 (BuOH/AcOH/H_2O 4:1:1) ^{1}H NMR (D_2O, pH 0.5−1) δ 1.71−1.85 (m, 5H), 2.00−2.16 (m, 3H), 2.30 (t, 2H, J = 7 Hz), 2.57−2.72 (m, 1H), 4.07 (t, 1H, J = 6 Hz); ^{13}C NMR (D_2O, pH 0.5−1) δ 22.5, 24.0, 25.2, 28.3, 28.5, 29.5, 30.7, 31.2, 38.6, 52.8, 53.1, 171.1, 177.4, 178.2, 178.3; ^{31}P NMR (D_2O, pH 0.5−1) δ 52.7; MS (FAB) *m*/*z* 312.2 (MH^+, 41.4); HRMS (FAB) calcd for $C_{10}H_{18}NO_8P$ 312.0800 $[M + H]^+$, found 312.0840. Anal. Calcd for $C_{10}H_{18}NO_8P$·HCl: C, 34.54; H, 5.51; N, 4.03. Found: C, 34.84; H, 5.77; N, 3.90.

Pteroyl Azide, 13a.[40] Ice-cold TFA (32 mL) was added to a 100 mL round-bottom flask containing pteroyl hydrazide (4.1 g, 12.5 mmol) and cat. KSCN (0.061 g, 0.63 mmol), and the mixture was stirred until all solids dissolved. The reaction mixture was cooled to −10 °C, and tBuONO (1.49 mL, 12.5 mmol) was added dropwise. The reaction was monitored by analytical HPLC. The reaction was stirred at −10 °C for 6.5 h, at which time HPLC indicated that none of the starting material was still present in the mixture. The reaction mixture was allowed to warm to room temperature, and 60 mL of 2-propanol was slowly added to afford an orange precipitate that was collected by centrifugation (30 min × 10 000 rpm). The pellet was washed with 60 mL of H_2O, 60 mL of acetonitrile, and 60 mL of diethyl ether, centrifuging after each wash (30 min × 10 000 rpm). The pellet was dried under high vacuum (0.25 mmHg) for 24 h to give 3.52 g of **13a** as an orange solid (83%): mp 185−190 °C dec (lit.[40] mp ∼180 °C); IR (KBr) 2134 cm^{-1} (N_3); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.69 (s, 1H), 7.69 (d, 2H, J = 8.7 Hz), 7.42 (broad s, D_2O exchangeable, 1H), 6.70 (d, 2H, J = 8.7 Hz), 4.56 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.3, 160.4, 154.0, 153.7, 153.4, 148.8, 148.3, 131.4, 128.1, 116.9, 111.7, 45.5; UV/vis (HPLC diode array, phosphate buffer, pH 7.0/acetonitrile) λ_{max} 235 nm, 275 nm, 338 nm; (0.01 M NaOH) 256, 280 sh, 365 nm; (0.01 M HCl) 333 nm; MS (FAB) *m*/*z* 338 (MH^+); analytical HPLC t_R = 24.3 min (flow rate: 0.7 mL/min; eluent A: water and phosphate buffer, pH 7.0; eluent B: acetonitrile; gradient: 0 min, 2% B, 25 min, 50% B; column: Chrompack Kromasil 100 C18, 250 mm × 4.6 mm).

2-[[[3-Carboxy-3-[[4-[[(2-amino-3,4-dihydro-4-oxopteridin-6-yl)methyl]amino]benzoyl]amino]propyl]-hydroxyphosphinyl]methyl]pentane-1,5-dioic Acid Tris-(triethylamine) Salt (1a). To a stirring suspension of pteroyl azide (**13a**, 0.21 g, 0.63 mmol) and **2** (0.33 g, 0.95 mmol) in anhydrous DMSO (10 mL) was added neat tetramethylguanidine (0.48 mL, 3.8 mmol). The reaction was stirred at room temperature for 18 h. Acetonitrile (30 mL) was slowly added to the reaction with vigorous stirring. The orange-yellow precipitate was collected by centrifugation (10 000 rpm × 30 min). The pellet was washed with aqueous 1% HCl (30 mL), acetonitrile (30 mL), and diethyl ether (30 mL × 2), centrifuging after each wash (30 min × 10 000 rpm). The pellet was dried under high vacuum (0.25 mmHg) for 24 h to give 0.200 g of crude **1a** as an orange solid (52%; analytical HPLC indicates 85% **1a**, 15% pteroic acid). A portion of the crude product (100 mg) was purified by ion-exchange chromatography on a DEAE-cellulose (DE52) column (15 mL of wet resin volume poured into a 22 × 2 cm glass Bio-Rad column) equilibrated with 0.01 M triethylammomium bicarbonate, pH 8.0. The column was eluted with a linear gradient ranging from 0.01 to 1.0 M triethylammonium bicarbonate, pH 8.0, with a flow rate of 1 mL/min and a total volume of 120 mL. Fractions containing the product were lyophilized to give 130 mg of **1a** as a fluffy, yellow solid (45%): mp 200 °C dec; ^{1}H NMR (D_2O) δ 8.68 (s, 1H), 7.63 (d, 2H), 6.63 (d, 3H), 4.47 (s, 2H), 4.31 (d, 1H), 3.15 (q, 6H) 2.54 (m, 1H), 2.23 (m, 2H), 2.05 (m, 1H), 2.0−1.65 (m, 4H), 1.62 (m, 3H), 1.24 (t, 9H); ^{13}C NMR (D_2O) δ 181.94, 181.89, 180.13, 180.00, 179.01, 178.92, 169.88, 169.81, 165.11, 154.60, 153.39, 150.89, 150.84, 149.51, 149.42, 149.32, 129.41, 127.44, 127.40, 121.54, 112.44, 112.35, 59.02, 56.49, 47.01, 46.86, 45.96, 45.79, 45.77, 45.74, 45.56, 42.62, 41.62,

41.55, 33.57, 33.52, 33.27, 33.18, 32.55, 32.46, 30.13, 30.02, 29.94, 26.80, 25.48, 25.38, 10.90, 8.69, 7.59; ^{31}P (D_2O) δ 42.7, 42.5; UV λ_{max} (0.01 M NaOH) 255, 283, 364 nm; (0.01 M HCl) 246, 296 nm; MS (FAB) *m/z* 606.2 (MH^+, 22); HRMS (FAB) calcd for $C_{24}H_{29}N_7O_{10}P$ 606.1714 $[M + H]^+$, found 606.1721; analytical HPLC t_R = 11.0 min (flow rate: 0.7 mL/min; eluent A: water and phosphate buffer, pH 7.0; eluent B: acetonitrile; gradient: 0 min, 2% B, 25 min, 50% B; column: Chrompack Kromasil 100 C18, 250 mm × 4.6 mm). Due to the extreme hygroscopic nature of the tris(triethylamine) salt, **1a**, all attempts at obtaining a satisfactory elemental analysis failed.

4-Amino-4-deoxy-10-methylpteroyl Azide (13b).[39,42] A solution of 4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]-benzoic acid (0.4 g, 1.2 mmol), diphenylphosphoryl azide (0.64 mL, 3.2 mmol), and triethylamine (0.3 mL, 2.2 mmol) in dry DMSO (4 mL) was stirred at room temperature for 2 days. THF (50 mL) was added to the reaction mixture, and the resulting precipitate was removed by filtration, washed with THF, and dried in vacuo over refluxing hexane for 6 h to give 0.36 g (85%) of **14b**: mp 287−291 °C dec (lit.[42] mp 280−290 °C); IR (KBr) cm^{-1} 2135 (N_3); ^{1}H NMR (CF_3COOD) δ 3.31 (s, 3H), 7.53 (d, 2H, J = 8.6 Hz), 7.90 (d, 2H, J = 8.6 Hz), 8.56 (s, 1H); ^{13}C NMR (CF_3COOD) δ 43.1, 49.9, 114.7, 115.0, 119.1, 127.5, 129.1, 137.3, 150.8, 152.7, 153.8, 155.5, 161.5, 165.5, 178.2; UV/vis (HPLC diode array, phosphate buffer, pH 7.0/acetonitrile) λ_{max} 230, 256, 345 nm; (0.1 N NaOH) 257.5, 282.0, 370.5 nm; (0.1 N HCl) 311.0 nm; analytical HPLC t_R = 28.282 min (flow rate: 0.7 mL/min; eluent A: water and phosphate buffer pH 7; eluent B: acetonitrile; gradient: 0 min, 2% B, 25 min, 50% B; column: Chrompack Kromasil 100 C18, 250 × 4.6 mm).

2-[[[3-Carboxy-3-[[4-[[(2,4-diamino-6-pteridinyl)methyl]-methylamino]benzoyl]amino]propyl]hydroxy-phosphinyl]methyl]pentane-1,5-dioic Acid Tetra(triethylamine) Salt (1b). To a solution of **13b** (0.07 g, 0.20 mmol) and **2** (0.104 g, 0.30 mmol) in dry DMSO (2.5 mL) was added 1,1,3,3-tetramethylguanidine (0.15 mL, 1.2 mmol). The mixture was stirred at room temperature for 2 days. The resulting solution was filtered through a pad of Celite to remove a trace of solid residue, and acetonitrile (15 mL) was slowly added to the stirred filtrate. The mixture was centrifuged, and the supernatant was decanted. The procedure was repeated with acetonitrile and then ether (2×), and the solid pellet was dried in vacuo overnight to give 0.18 g of crude product **1b** (86%; ^{1}H NMR indicated ca. 65% **1b**, 35% **2**). A portion of crude product (45 mg) was purified by ion-exchange chromatography on DEAE-cellulose (DE53) column (12 mL of wet resin volume poured into a 22 × 1.5 cm glass Bo-Rad column) equilibrated with 0.01 M triethylammonium bicarbonate, pH 7.8. The column was eluted with a linear gradient ranging from 0.01 to 1.0 M triethylammonium bicarbonate, pH 7.8, with a flow rate of 1 mL/mn and a total volume of 250 mL. Fractons containing the product were lyophilized to give 29 mg of **1b** as a fluffy**,** yellow solid (56%): mp 197−203 °C dec; ^{1}H NMR (D_2O) δ 0.70−1.30 (m, 36H), 1.35−2.10 (m, 10H), 2.26−2.42 (m, 1H), 2.70−3.29 (m, 25H), 4.12−4.15 (m, 1H), 4.46 (s, 2H), 6.52 (d, 2H, J = 9 Hz), 7.46 (d, 2H, J = 10 Hz), 8.37 (s, 1H); ^{13}C NMR (D_2O) δ 7.8, 8.6, 10.9, 25.6, 26.6, 27.8, 31.2, 31.4, 33.0, 34.1, 35.6, 39.1, 42.6, 43.0, 43.4, 47.0, 55.0, 56.7, 57.0, 59.2, 61.8, 111.9, 120.1, 120.8, 122.3, 129.2, 149.6, 150.2, 151.8, 158.2, 159.4, 163.2, 169.3, 179.2, 182.4, 183.6; ^{31}P NMR (D_2O), δ 44.0, 44.2; MS (FAB) *m/z* 619.2 (MH^+, 1.6); HRMS (FAB) calcd for $C_{25}H_{31}N_8O_9P$ 619.2030 $[M + H]^+$, found 619.2000 (free acid); UV/vis (HPLC diode array, phosphate buffer, pH 7.0/acetonitrile) λ_{max} 260, 305, 375 nm; (0.1 N HCl) 311.5 (26 258) nm, λ_{max} (0.1 N NaOH) 258.0 (23 717), 301.0 (22 043), 371.0 (7652) nm; analytical HPLC t_R= 4.473 min (eluent A: 0.1 M NaOAc, pH 5.5; eluent B: acetonitrile; column: Chrompack Kromasil 100 C18, 250 × 4.6 mm). Due to the extreme hygroscopic nature of the tetra(triethylamine) salt, **1b**, all attempts at obtaining a satisfactory elemental analysis failed.

Acknowledgment. This research was supported by a grant from the National Cancer Institute (CA28097). We are most grateful to Dr. John J. McGuire and Mr. William Haile, Roswell Park Cancer Institute, Buffalo, NY, and Mr. John Tomsho of this laboratory for preliminary data on the inhibition of human FPGS by **1**. We thank Dr. Takashi Tsukamoto for several insightful discussions about this research and for carrying out some of the initial experiments with phosphorus radical chemistry (Scheme 3). In addition, we thank Dr. Tsukamoto and Guilford Pharmaceuticals for a generous gift of **10** (Scheme 6). The careful preparation and thorough editing of this manuscript by Ms. Joyce Reese is gratefully acknowledged.

Supporting Information Available: ^{1}H, ^{13}C, and ^{31}P NMR spectral data for compounds in Tables 1 and 2 not described in the Experimental Section. ^{1}H and ^{13}C spectra for compounds **7** ($R^1 = CH_3$, $R^2 = (CH_2)_6CH_3$; $R^1 = CH_3$, $R^2 = CH_2$-CO_2Bn; $R^1 = CH_3$, $R^2 = CH(CO_2Bn)$ $CH_2CH_2CO_2Bn$, **8** (Table 1, entries 2 and 11), **9** (Table 2, entries 4 and 6), **13a**,**b**, and **1a**,**b**. Chemical shift data obtained from these spectra, or in some cases from the expanded regions of these spectra, are included in the Experimental Section of this paper. Full experimental details and complete spectral characterization of pteroic acid derivatives used in the synthesis of aryl azides **13a** and **13b**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO010283T