The Chemistry of Phosphapeptides: Investigations on the Synthesis of Phosphonamidate, Phosphonate, and Phosphinate Analogues of Glutamyl-γ-glutamate

William P. Malachowski and James K. Coward*

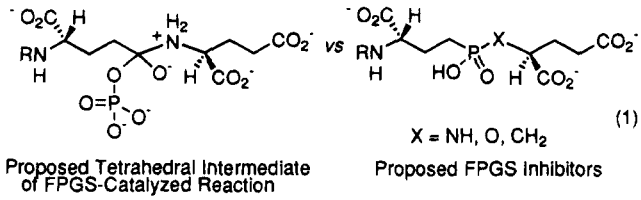
Interdepartmental Program in Medicinal Chemistry, College of Pharmacy, and Department of Chemistry, The University of Michigan, Ann Arbor, Michigan 48109

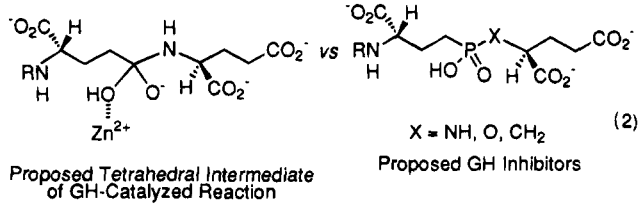
Received June 7, 1994®

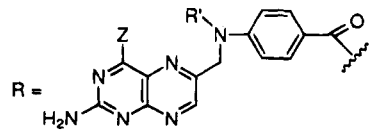
The synthesis of the phosphonamidate, **1d**, and phosphonate, **1e**, analogues of a γ-glutamyl peptide are reported. Michaelis–Arbuzov reaction with the alkyl halide, **7b**, derived from L-glutamic acid, yielded dimethyl phosphonate, **8b**. Selective aminolysis of the phosphorus diester provided monomethyl phosphonic acid, **9**. Utilizing the methodology developed in the accompanying paper, **9** was converted to the phosphonochloridate, **10**. Subsequent reaction with diethyl glutamic acid and hydrogenation afforded the complex phosphonamidate, **1d**. Mitsunobu coupling of the monomethyl phosphonic acid, **9**, with diethyl 2-hydroxyglutarate, followed by hydrogenation, provided the complex mixed phosphonate, **1e**.

The biosynthesis of poly-γ-glutamyl peptide derivatives of folic acid and related anti-folate drugs such as methotrexate (MTX) involves a nonribosomal ATP-dependent reaction catalyzed by folylpoly-γ-glutamate synthetase (FPGS, EC 6.3.2.17).[1] Our research has demonstrated that this reaction proceeds via a γ-glutamyl phosphate of a reduced folate or MTX which then reacts with an incoming molecule of L-glutamate to form a new glutamyl-γ-glutamate peptide bond. Each subsequent addition of L-glutamate proceeds through a γ-glutamyl phosphate intermediate at the C-terminus of the growing poly-γ-glutamyl peptide.[2] A tetrahedral intermediate derived from attack of the incoming L-glutamate on the γ-glutamyl phosphate of the growing peptide is assumed to be involved (eq 1, Chart 1). In the hydrolytic direction, the

Chart 1



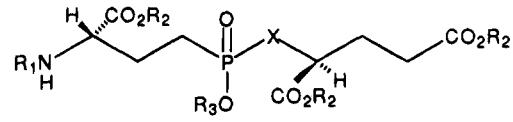




Z = OH, R' = H for folic acid analogue
Z = NH_2, R' = CH_3 for methotrexate analogue

γ-glutamyl peptides are cleaved by a specific zinc peptidase, γ-glutamyl hydrolase (GH, EC 3.4.22.12).[1] Although less is known about the mechanism of GH-catalyzed hydrolysis of γ-glutamyl peptides, it is reasonable, based on extensive research on the zinc protease, carboxypeptidase A,[3] to postulate a tetrahedral intermediate such as depicted in eq 2 (Chart 1).

We have used selected fluoroglutamic acids and fluoroglutamate-containing folates, antifols, and peptides to prevent or stimulate polyglutamate biosynthesis[4–6] or to modulate the hydrolytic breakdown of the γ-glutamyl peptides.[7] Although these fluoroglutamate-containing derivatives of folic acid and methotrexate have been extremely useful in elucidating the role of polyglutamate conjugates in one-carbon biochemistry (reduced folates) and cytotoxicity (methotrexate),[8] we wished to evaluate the possible use of phosphorus-based tetrahedral mimics to inhibit FPGS (eq 1) or GH (eq 2). In the present research, we have investigated the synthesis of blocked phosphapeptides, **1d–f**, as precursors of the proposed



1

	R_1	R_2	R_3	X
a	Pteroyl[a]	H	H	NH
b	Pteroyl	H	H	O
c	Pteroyl	H	H	CH_2
d	H	C_2H_5	CH_3	NH
e	H	C_2H_5	CH_3	O
f	H	C_2H_5	CH_3	CH_2

[a]See eq. 1 and 2.

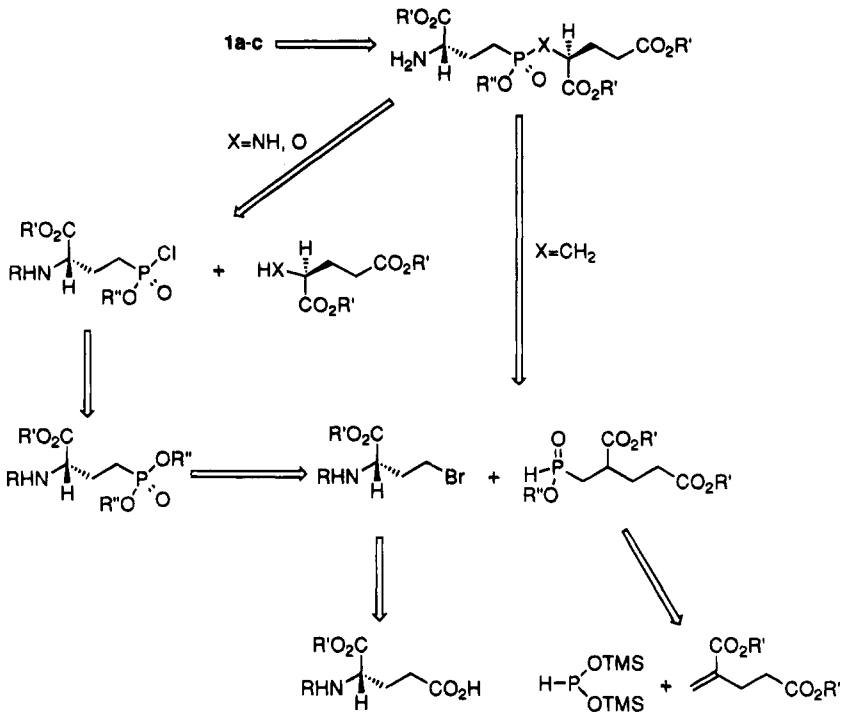
enzyme inhibitors **1a–c**. To our knowledge, this repre-

® Abstract published in *Advance ACS Abstracts*, November 15, 1994.

(1) McGuire, J. J.; Coward, J. K. In *Folates and Pterins*; Blakley, R. L., Benkovic, S. J., Eds.; John Wiley & Sons: New York, 1984; Vol. 1; p 135.
(2) Banerjee, R.; Shane, B.; McGuire, J. J.; Coward, J. K. *Biochemistry* **1988**, *27*, 9062.
(3) Christianson, D. W.; Lipscomb, W. N. *Acc. Chem. Res.* **1989**, *22*, 62.
(4) McGuire, J. J.; Coward, J. K. *J. Biol. Chem.* **1985**, *260*, 6747.
(5) McGuire, J. J.; Graber, M.; Licato, N.; Vincenz, C.; Coward, J. K.; Nimec, Z.; Galivan, J. *Cancer Res.* **1989**, *49*, 4517.

0022-3263/94/1959-7625$04.50/0

Scheme 1



sents the first synthesis of a phosphapeptide analogue of a γ-glutamyl peptide. As will be described in more detail below, most of the enzymes investigated in prior research using phosphapeptides as protease inhibitors act on proteins and peptides which contain nonfunctionalized amino acids such as leucine, alanine, phenylalanine, or glycine at or near the scissile bond. Therefore, the phosphapeptides analogous to those peptide sequences do not have the structural complexity of the phosphapeptide analogues of γ-glutamyl peptides of interest in our research.

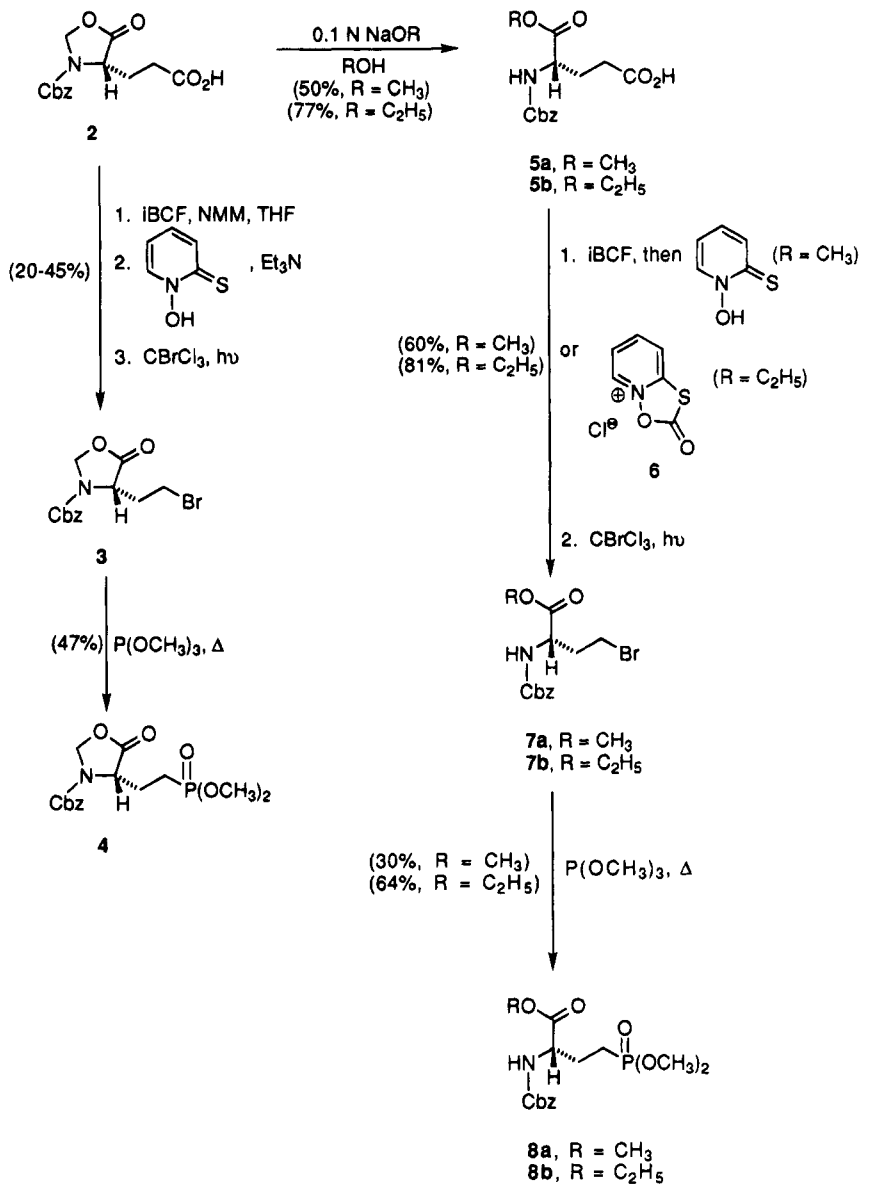
Results and Discussion

Having established that phosphonochloridates derived from fairly simple phosphonic acid monomethyl esters are reliable and accessible reagents for use in the synthesis of phosphonates and phosphonamidates,[9] we designed a synthesis of more complex phosphapeptides of interest in our research (Scheme 1). The phosphonamidate (X = NH) and the phosphonate (X = O) dipeptides, **1a** and **1b**, might be synthesized in a convergent manner from the coupling of the phosphonochloridate with either an appropriately protected glutamate or the analogous secondary alcohol, 2-hydroxyglutarate. Our retrosynthetic analysis indicated that the desired dimethyl phosphonates might be obtained via Michaelis–Arbuzov reaction between trimethyl phosphite and a suitably protected alkyl halide, the latter being derived from L-glutamic acid. The phosphinate (X = CH_2) dipeptide, **1c**, might be synthesized from a Michaelis–Arbuzov type reaction[10] between the same alkyl halide and an appropriately substituted phosphinate. Compounds similar to the desired substituted phosphinate have been synthesized recently via Michael addition of a reactive bis(trimethylsilyl)phosphonite to α,β-unsaturated esters.[11]

The phosphonochloridate which was to be derived ultimately from L-glutamic acid was considered a major challenge due to the density of heteroatoms and the disposition of these heteroatoms in the molecule. Additional challenges were found due to the disposition of the heteroatoms in the intermediate alkyl halides. A variety of intramolecular reactions can be envisioned, any of which could lead to problems. Irrespective of the path chosen, the potential problems with many of the intermediates in this synthesis, due to the spatial relation of these functionalities, were numerous. A large portion of the phosphonochloridate literature to date has resulted from research on the construction of similar amino acid analogues for enzyme inhibitor studies. However, most of this work has substituted the phosphorus moiety for the α-carboxylic acid and has synthesized analogues of relatively simple amino acids, such as glycine,[12–14] alanine,[15,16] leucine[17] or phenylalanine.[15,16] In some instances,[18] the amine functionality is eliminated so the phosphonochloridate is simplified even more. In our desired phosphonochloridate, it was necessary to differentiate between the phosphorus ester and the carboxylic ester in order that manipulations of the phos-

(6) McGuire, J. J.; Haile, W. H.; Bey, P.; Coward, J. K. *J. Biol. Chem.* **1990**, *265*, 14073.
(7) Licato, N. J.; Coward, J. K.; Nimec, Z.; Galivan, J.; Bolanowska, W. E.; McGuire, J. J. *J. Med. Chem.* **1990**, *33*, 1022.
(8) Coward, J. K.; McGuire, J. J.; Galivan, J. In *Selective Fluorination in Organic and Bioorganic Chemistry*; Welch, J. T., Ed.; American Chemical Society: Washington, D.C., 1991; p 196.
(9) Malachowski, W. P.; Coward, J. K. *J. Org. Chem.* preceding paper in this issue.
(10) Thottathil, J. K.; Przybyla, C. A.; Moniot, J. L. *Tetrahedron Lett.* **1984**, *25*, 4737.
(11) Boyd, E. A.; Corless, M.; James, K.; Regan, A. C. *Tetrahedron Lett.* **1990**, *31*, 2933.
(12) Elliott, R. L.; Marks, N.; Berg, M. J.; Portoghese, P. S. *J. Med. Chem.* **1985**, *28*, 1208.
(13) Bartlett, P. A.; Marlowe, C. K. *Biochemistry* **1983**, *22*, 4618.
(14) Jacobsen, N. E.; Bartlett, P. A. *J. Am. Chem. Soc.* **1981**, *103*, 654.
(15) Hanson, J. E.; Kaplan, A. P.; Bartlett, P. A. *Biochemistry* **1989**, *28*, 6294.
(16) Bartlett, P. A.; Lamden, L. A. *Bioorg. Chem.* **1986**, *14*, 356.
(17) Bartlett, P. A.; Hanson, J. E.; Giannousis, P. P. *J. Org. Chem.* **1990**, *55*, 6268.

Scheme 2



phorus ester could take place without cross-reactivity at the carboxylic ester. Although originally considered routine, this proved to be a difficult task. In a rare case of a phosphonochloridate which rivals the target of this research (Scheme 1) in terms of complexity, Robl et al.[19] chose a selective deprotection–reprotection to manipulate the phosphorus ester. We wished to accomplish this task in a more direct manner.

In order to differentiate between the α and γ carboxyl groups in the synthesis of the requisite alkyl halide for use in the Michaelis–Arbuzov reaction, we converted *N*-Cbz-L-glutamic acid to the oxazolidinone **2** (Scheme 2).[20,21] Based on work by Barton[22] in which his modification of the Hunsdieker reaction was applied to **2**, we attempted to effect the conversion of **2** to **3** by this method. Although an initial small scale reaction led to **3** in 73% yield, numerous repetitions of this reaction gave irreproducibly poor-moderate yields (20–45%) regardless of the scale of the reaction. Barton initially reported a 73% yield for this transformation in one communication,[22] but a later, more comprehensive publication[23] indicated a 47% yield for this same reaction.

In order to investigate this approach further, bromide **3** was dissolved in trimethyl phosphite and heated at reflux temperature in a Michaelis–Arbuzov reaction to afford a 47% yield of the dimethyl phosphonate, **4**. In parallel with the studies described in the accompanying paper[9] on the generation of phosphonochloridates from dialkyl phosphonates, the direct conversion of **4** to the corresponding phosphonochloridate with PCl_5 was investigated . Similar to the results with the simple dimethyl phosphonates, this procedure effectively formed the phosphonochloridate as determined by ^{31}P NMR analysis of the reaction solution. Unfortunately, it appeared that the phosphonochloridate decomposed when the reaction solution was concentrated as was also observed in similar

(18) Bertenshaw, S. R.; Rogers, R. S.; Stern, M. K.; Norman, B. H.; Moore, W. M.; Jerome, G. M.; Branson, L. M.; McDonald, J. F.; McMahon, E. G.; Palomo, M. A. *J. Med. Chem.* **1993**, *36*, 173.

(19) Robl, J. A.; Duncan, L. A.; Pluscec, J.; Karanewsky, D. S.; Gordon, E. M.; Ciosek, C. P., Jr.; Rich, L. C.; Dehmel, V. C.; Slusarchyk, D. A.; Harrity, T. W.; Obrien, K. A. *J. Med. Chem.* **1991**, *34*, 2804.

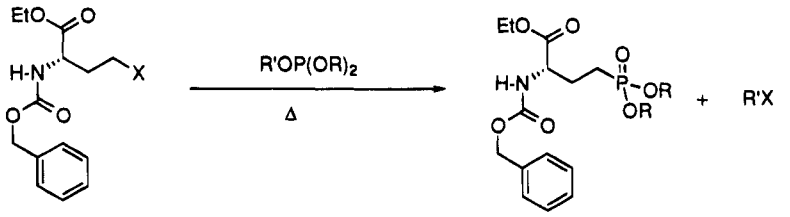
(20) Itoh, M. *Chem. Pharm. Bull.* **1969**, *17*, 1679.

(21) Scholtz, J. M.; Bartlett, P. A. *Synthesis* **1989**, 542.

(22) Barton, D. H. R.; Herve, Y.; Potier, P.; Thierry, J. *J. Chem. Soc., Chem. Commun.* **1984**, 1298.

(23) Barton, D. H. R.; Herve, Y.; Potier, P.; Thierry, J. *Tetrahedron* **1988**, *44*, 5479.

Table 1. Optimization of Michaelis–Arbuzov Reaction



entry	compd	X	R'	R	phosphite bp	product	yield (%)	R'X, bp[a]
1		I	Me	Me	111–112	**8b**	52	MeI, bp = 41–43 °C
2		I	Me	Me	*b*	**8b**	0	MeI
3	**7b**	Br	Me	Me	111–112	**8b**	64	MeBr, bp = 4 °C
4	**7b**	Br	TMS	Me	149–150	**8b**	0	TMSBr
5	**7b**	Br	Et	Et	156		74	EtBr, bp = 37–40 °C
6	**7b**	Br	i-Pr	i-Pr	180–182		68	i-PrBr

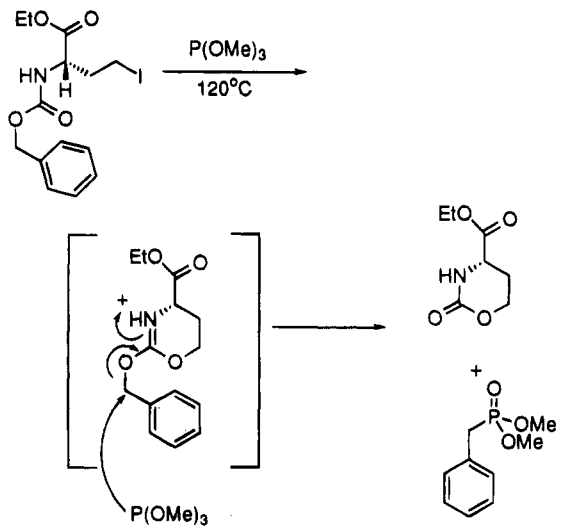
[a] bp is reported for reactions in which RX was removed with a heated condensor. [b] Three equivalents of $(CH_3O)_3P$ were used. Reaction solvent was toluene, bp 110 °C.

reactions with simple dimethyl phosphonates.[9] Therefore, subsequent reaction with L-glutamic acid diethyl ester did not provide any of the desired phosphonamidate products. The low and irreproducible yields on the Hunsdieker reaction in this sequence significantly hampered access to bromide, **3**, so we explored the Hunsdieker reaction with alternative substrates.

Glutamic acids with different α-carboxylic acid protective groups have been reported to yield the desired alkyl bromides (64–82%) in the modified Hunsdieker reaction.[22,23] For this reason, the oxazolidine, **2**, was transformed into the α-methyl ester, **5a** (Scheme 2), with 0.1M NaOMe/MeOH according to the procedure of Hanessian.[24] This transformation was achieved in a moderate 50% yield of **5a**, together with a significant amount of *N*-Cbz-glutamic acid, even when the reaction was run under meticulously dry conditions. We speculate that competition between intramolecular attack of the γ-carboxylate anion and intermolecular attack of the methoxide anion is a significant problem. After acidification of the reaction solution during the extraction, the glutamic anhydride is cleaved to afford the side product, N-Cbz-glutamic acid.

The α-methyl ester, **5a**, was subjected to the Hunsdieker reaction conditions and afforded an improved 60% yield of the bromide, **7a**. When the α-methyl ester was reacted with trimethyl phosphite in the Michaelis–Arbuzov reaction, it afforded only a 30% yield of the dimethyl phosphonate, **8a**. Despite the low yield in this step, we investigated reactions to obtain the desired phosphonochloridate from **8a**. Model studies following several failed PCl_5 reactions with **4**, had shown that a milder method to generate phosphonochloridates of complex molecules was achieved via reaction of monomethyl phosphonic acids with $(COCl)_2$.[9] Therefore, we attempted to selectively deprotect the phosphorus methyl ester in the presence of the carboxylic methyl ester to obtain the phosphonic acid t-butylamine salt. Despite the precedent[25] for this type of transformation, the reaction of **8a** did not show the desired selectivity in the cleavage of the phosphorus methyl ester over the carboxylic methyl ester.[26]

The lack of selectivity observed in the reaction of t-butylamine with both **4** and **8a**, led us to pursue a more stable protecting group at the α-carboxylic acid. Conversion of the oxazolidine, **2**, to the α-ethyl ester, **5b**, with 0.1M NaOEt/EtOH, a procedure analogous to that employed in the methyl ester synthesis, went in good yield (77%). Treatment of **5b** with **6**,[27] followed by irradiation, afforded an 81% yield of the bromide, **7b**. This reaction still had some difficulties due to the purity and stability of **6**, but reproducible yields in the high 70% range were achieved.[28] Bromide **7b** was converted into the corresponding iodide followed by reaction with trimethyl phosphite to afford initially a 52% yield of the dimethyl phosphonate, **8b**. However, when this reaction was performed on a larger scale, two side products were also formed (eq 3). This side reaction is hypothesized to be a

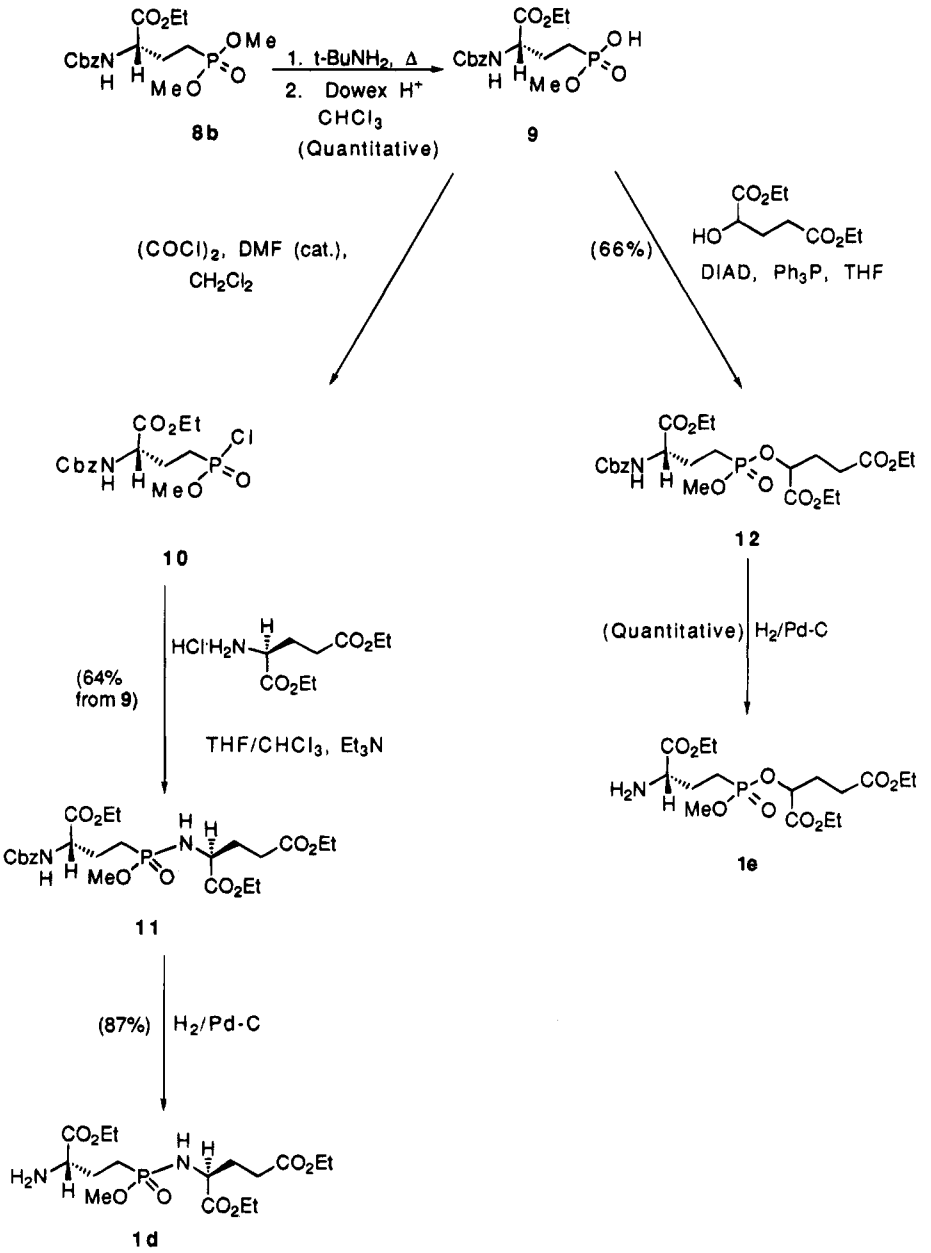


(3)

result of intramolecular attack of the carbonyl oxygen of the Cbz protecting group on the alkyl iodide to form the intermediate shown.[29] Attack of trimethyl phosphite on the benzylic position of the intermediate afforded the side

(24) Hanessian, S.; Sahoo, S. P. *Tetrahedron Lett.* **1984**, *25*, 1425.
(25) Gray, M. D. M.; Smith, D. J. H. *Tetrahedron Lett.* **1980**, *21*, 859.
(26) Similar problems with the oxazolidinone-based phosphonic acid dimethyl ester, **4** were observed. Bromide **3** was obtained in an improved synthesis (62% yield) from **2** and 1-oxa-2-oxothiaindolizinium chloride, **6**, an activated version of 2-mercaptopyridine *N*-oxide.[27] Conversion of **3** to the corresponding iodide, followed by reaction of with trimethyl phosphite provided **4**. Reaction of **4** with t-butylamine failed to give the desired phosphonic acid monomethyl ester.
(27) Barton, D. H. R.; Crich, D.; Motherwell, W. B. *Tetrahedron* **1985**, *41*, 3901.
(28) Acid-mediated (HBr/HOAc) solvolysis of D,L-homoserine lactone followed by esterification and N-protection provides D,L- **7** (R = $CH_2C_6H_5$).[49] In the present research, attempts to obtain **7b** directly from *N*-Cbz-L-homoserine failed.
(29) Coward, J. K.; Lok, R. *J. Org. Chem.* **1973**, *38*, 2546.

Scheme 3



products, a cyclic carbamate and dimethyl benzyl phosphonate, both of which were isolated and characterized by ^{1}H NMR.

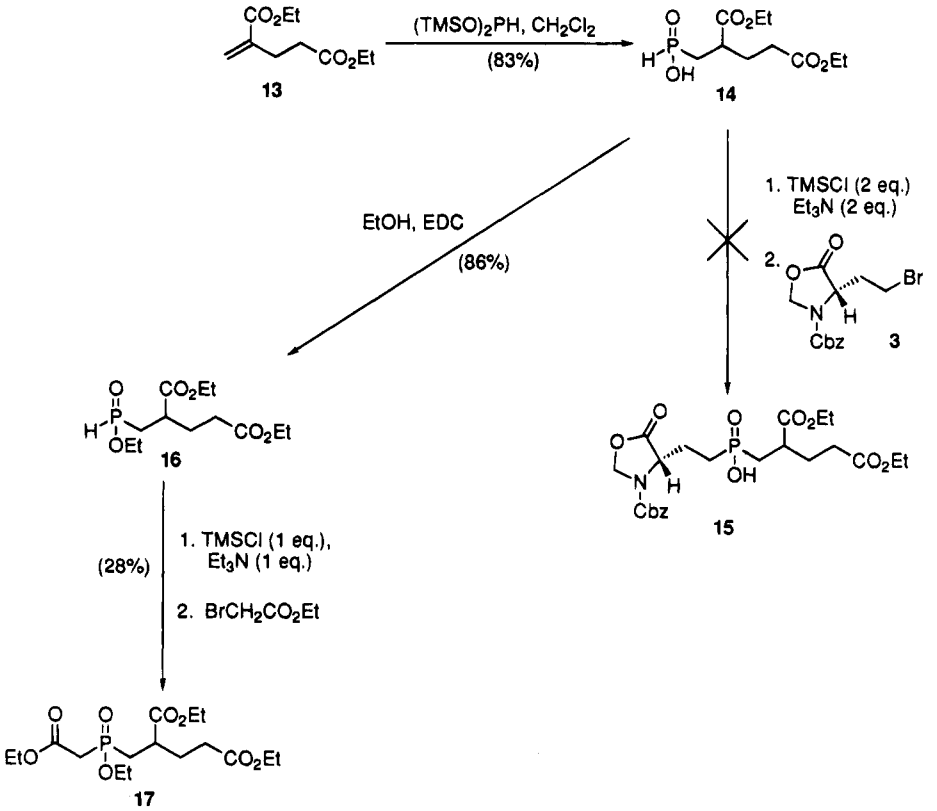
A systematic study of the Michaelis–Arbuzov reaction was undertaken in an attempt to improve the yield of **8b** and to minimize the side reaction depicted in eq 3. These experiments are summarized in Table 1. The well-known tendency of the alkyl halide product, R′X, to react with the trialkyl phosphite reagent was minimized by removal of R′X via a warmed (hot water) condensor.[30] However, even under these conditions, considerable dimethyl methylphosphonate was observed when R′X = CH_3I. In contrast, CH_3Br is much more volatile than CH_3I and very little dimethyl methylphosphonate was observed in the reaction, providing a 64% yield of **8b**. Several alternatives to trimethyl phosphite were explored (Table 1, entries 4–6). The intermediate TMS ester resulting from the use of $TMSOP(OCH_3)_2$ was formed extremely rapidly[31] but the TMSBr (R′X) formed in the reaction led to several undesired decomposition products and none of the desired phosphonate. Finally, the use of triethyl phosphite and triisopropyl phosphite was investigated to provide assurance that reaction of **7b** with phosphites known to be less problematic than trimethyl phosphite in the Michaelis–Arbuzov reaction could proceed in good yield. However, the resulting diethyl and diisopropyl phosphonate ester analogues of **8b** are unsuitable for our total synthesis objective.

Having achieved an efficient synthesis of the dimethyl phosphonate **8b** (Scheme 2, Table 1), this phosphonate was subjected to aminolysis by t-butyl amine. The desired phosphonic acid monomethyl ester t-butylamine salt was formed in quantitative yield (Scheme 3). Thus, the carboxylic acid ethyl ester of **8b** was refractory to the nucleophilic demethylation conditions and permitted differentiation between the carboxylic acid ester and the phosphonate ester. The t-butylamine salt was converted

(30) Kosolapoff, G. M. *J. Am. Chem. Soc.* **1944**, *66*, 109.

(31) Engel, R. *The Synthesis of Carbon–Phosphorous Bonds*; CRC Press: Boca Raton, FL, 1988; Chapter 2.

Scheme 4



to the free monomethyl phosphonic acid, **9**, also in quantitative yield. The monomethyl phosphonate, **9**, was then treated with $(COCl)_2$ and catalytic DMF in CH_2Cl_2 initially at 0 °C, then at rt with stirring for 1 h. Analysis of a reaction aliquot by ^{31}P NMR demonstrated complete conversion of **9** to **10**. Concentration in vacuo eliminated the volatile reagents and left the phosphonochloridate, **10**, as a yellow oil residue. This residue was dissolved immediately in THF and added to L-glutamic acid diethyl ester in $CHCl_3$ at 0 °C. Following chromatographic purification of the crude product, a 22% yield of the phosphonamidate, **11**, was obtained.

With the alkyl bromides, **3** and **7** synthesized in adequate yields, we considered approaches to the phosphinate dipeptide, **1c**, and investigated the synthesis of the phosphonite, **14** (Scheme 4). In the retrosythetic analysis (Scheme 1), we would obtain **14** from the Michael addition of bis(trimethylsilyl)phosphonite to a diester of 2-methyleneglutarate. Initially, we attempted to synthesize diethyl 2-methyleneglutarate (**13**) from the Wittig reaction of diethyl 2-ketoglutarate with methylenetriphenylphosphorane. When this failed, we resorted to a novel procedure[32] in which ethyl acrylate is treated with a catalytic amount of hexamethylphosphorous triamide (HMPT). Although a significant amount of polymerization occurred by this procedure, sufficient **13** was obtained to study its conversion to **14**.

The bis(trimethylsilyl)phosphonite, $(TMSO)_2PH$, was obtained by treatment of triethylammonium phosphinate with trimethylsilyl chloride and triethylamine.[11,33-36] Despite the pyrophoric nature of $(TMSO)_2PH$, it could be isolated and purified by distillation. Treatment of **13** with $(TMSO)_2PH$ afforded an 83% yield of the phosphinic acid, **14**. Thottathil et al. reported the mild Michaelis–Arbuzov reaction of 4-phenylbutylphosphinic acid with a number of alkyl halides[10] and Michael acceptors.[37] In situ bis-silylation of the phosphinic acid affords a nucleophilic tervalent derivative of the phosphinic acid, which can react with these electrophiles. When this procedure was applied to **14**, reaction with **3** did not afford any of the coupled product, **15**. Similarly, **14** failed to react in three different model Michaelis–Arbuzov reactions with bromopropane, iodopropane and ethyl bromoacetate.

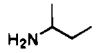
Since the monoethyl ester of 4-phenylbutylphosphinic acid demonstrated better reactivity than the free acid,[10] we treated **14** with EtOH and ethyl(dimethylamino)-propylcarbodiimide (EDC) to obtain the ethyl ester, **16**. Unfortunately, **16** was found to be only marginally more reactive than **14**. Monosilylation of **16**, followed by Michaelis–Arbuzov reaction with bromopropane and allyl bromide both failed, but ethyl bromoacetate reacted to provide 28% yield of the phosphinate, **17**. The poor reactivity of both **14** and **16** in this mild Michaelis–Arbuzov reaction led us to abandon this approach to the synthesis of the phosphinate dipeptide, **1c**. On closer scrutiny, the literature, either overtly[38] or by omission,[10] indicates that unactivated halides fail to react in this procedure.

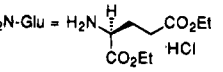
Since the phosphonochloridate, **10**, was formed completely (^{31}P NMR analysis) using the $(COCl)_2$ method, modifications of the coupling conditions for the synthesis

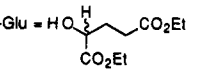
(32) Amri, H.; Rambaud, M.; Villieras, J. *Tetrahedron Lett.* **1989**, *30*, 7381.
(33) Stawinski, J.; Thelin, M.; Westman, E.; Zain, R. *J. Org. Chem.* **1990**, *55*, 3503.
(34) Grobelny, D. *Synthesis* **1987**, 942.
(35) Majewski, P. *Synthesis* **1987**, 555.
(36) Voronkov, M. G.; Marmur, L. Z.; Dolgov, O. N.; Pestunovich, V. A.; Pokrovskii, E. I.; Popel, Y. I. *Zh. Obshch. Khim.* **1971**, *41*, 2005.
(37) Thottathil, J. K.; Ryono, D. E.; Przybyla, C. A.; Moniot, J. L.; Neubeck, R. *Tetrahedron Lett.* **1984**, *25*, 4741.
(38) Majewski, P. *Phosphorus, Sulfur Silicon Relat. Elem.* **1989**, *45*, 151.

of **11** and **12** were explored (Table 2). It was noted that, in the first successful coupling reaction of **10** described previously, a THF/$CHCl_3$ mixture was used which led to the precipitation of Et_3N·HCl from the reaction solution. When the reaction was performed in CH_2Cl_2 and CH_3CN, the reaction mixture remained homogeneous but the coupled products **11** and **12** were isolated in under 5% yield (Table 2, entries 1–4). Precipitation of Et_3N·HCl could be affecting the reaction dynamics[39] so THF was used as the sole solvent (entry 5), affording a 29% yield of **11** after chromatography. The reaction of diethyl 2-hydroxyglutarate with the phosphonochloridate, **10**, under identical conditions (entry 6) yielded only 6.7% of the phosphonate product, **12**. The use of a catalytic amount of DMAP with this alcohol (entry 10) did not enhance the yield.

Table 2. Optimization of Phosphonochloridate Coupling Reaction

entry	nucleophile (RXH)	equiv of RXH	solvent	DMAP (cat.)	product	yield (%)
1	H_2N-Glu[a]	0.8	CH_2Cl_2	yes	**11**	<5
2	HO-Glu[b]	1.0	CH_2Cl_2	yes	**12**	<5
3	H_2N-Glu	0.8	CH_3CN	yes	**11**	0
4	HO-Glu	1.1	CH_3CN	yes	**12**	0
5	H_2N-Glu	0.9	THF	no	**11**	29
6	HO-Glu	0.9	THF	no	**12**	6.7
7		1.6	THF	yes		0
8		1.4	THF	no		70
9	H_2N-Glu	1.1	THF	no	**11**	64
10	HO-Glu	1.1	THF	yes	**12**	6.4
11	H_2N-Glu	5.0	THF	no	**11**	18

[a] H_2N-Glu = 

[b] HO-Glu = 

In order to assess the reactivity of phosphonochloridate **10** with simple secondary amines and alcohols, we performed model coupling reactions with sec-butylamine and isopropyl alcohol. The reaction of **10** with isopropyl alcohol (Table 2, entry 7) led to the same poor results as seen with the secondary alcohol of interest, diethyl 2-hydroxyglutarate. However, the reaction of **10** with sec-butylamine (entry 8) afforded 70% of the expected phosphonamidate product. In both of these reactions, the nucleophiles were present in excess over the phosphonochloridate, **10**, and the possibility was considered that the enhanced yield in the sec-butylamine reaction was due to a simple change in reagent stoichiometry. When the equivalents of the phosphonochloridate, **10**, and the nucleophile, L-glutamic acid diethyl ester, were modified so that the nucleophile was in 10% excess, a tremendous enhancement was seen (Table 2, entry 9). After extractive purification, a 64% yield of the phosphonamidate, **11**, was achieved. This material began to crystallize after several days at rt and was not submitted to chromatography; some decompostion was observed previously during silica gel chromatography with **11**. A large excess (5-fold) of amine, however, failed to improve the yield (entry 11).

The conditions which afforded a 64% yield of the phosphonamidate, **11**, from **10** were unsuccessful with the secondary alcohol nucleophile, diethyl 2-hydroxyglutarate (entry 10). Therefore, a different approach was used to obtain the phosphonate, based on recent work[40] with diisopropyl azodicarboxylate (DIAD) and triphenyl phosphine (Ph_3P). This method was also explored in the accompanying paper as an alternative method to synthesize phosphonates from primary alcohols[9]. Treatment of **9** and diethyl 2-hydroxyglutarate with Ph_3P and DIAD at rt resulted in the formation of **12** in 66% yield.

Removal of the Cbz protecting group from the phosphonamidate **11** and phosphonate **12** was effected by standard hydrogenolysis conditions to afford acid-protected phosphapeptides **1d** and **1e**, in 87% and quantitative yields, respectively. Our retrosynthesis outlined in Scheme 1 suggests that compounds such as **1d** and **1e** should lead to the desired enzyme inhibitors, **1a** and **1b** via a simple coupling to appropriate pteroic acid derivatives[7] followed by removal of the acid protecting groups. Coupling of **1d** and **1e** with 4-amino-10-methylpteroic acid in the presence of DEPC has been effected.[41] Removal of the acid protecting groups followed by purification of the final target compounds will allow for their evaluation as inhibitors of FPGS and/or GH. The results of this ongoing research will be reported in future publications.

Experimental Section

General Procedures. All reactions involving reagents sensitive to moisture were conducted under an atmosphere of argon with oven-dried glassware. Sodium ethoxide was generated according to Zaugg's procedure.[42] 2-Mercaptopyridine *N*-oxide was recrystallized from ethanol prior to use and stored in amber bottles. Trialkyl phosphites were distilled prior to use. t-Butylamine was stored under nitrogen over molecular sieves. All solvents used in moisture-sensitive reactions were dried as follows: benzene and pyridine were distilled from CaH_2 and stored over 4 Å molecular sieves; absolute ethanol was used as purchased and kept under nitrogen; tetrahydrofuran (THF) was freshly distilled from a sodium/benzophenone mixture; triethylamine was distilled from KOH and stored over molecular sieves. Chloroform was washed successively with concentrated sulfuric acid and brine, dried over $CaCl_2$, then heated at reflux over P_2O_5 and distilled from P_2O_5. Dichloromethane was dried over CaH_2 and freshly distilled. All other purchased materials were used without further purification. Irradiations were performed with a standard 150 W flood lamp. Column chromatography was performed with silica gel 60 (230–400 mesh) and according to the protocol of Still.[43] Thin layer chromatography was performed with aluminum-backed silica gel 60-F254 plates unless otherwise noted. Melting points were obtained on a Thomas-Hoover Mel-Temp apparatus and are uncorrected. ^1H NMR spectra were recorded at 300 and 360 MHz and are reported in the following manner: chemical shift in ppm downfield from internal tetramethyl silane (multiplicity, integrated intensity, coupling constants in Hertz, assignment). ^{13}C NMR spectra were obtained at 90 and 50 MHz and referenced to tetramethylsilane. ^{31}P NMR spectra were recorded at 145 MHz with 85% H_3PO_4 as an external reference and with broad-band ^1H decoupling. Infrared spectra were recorded on a Nicolet 5-DX spectrometer. Mass spectra and high resolution mass spectra were performed on a Finnigan 4500 GC/MS-EICI system or on a VG Analytical system, Model 70–250S. Elemental

(39) Morgan, B. P.; Scholtz, J. M.; Ballinger, M. D.; Zipkin, I. D.; Bartlett, P. A. *J. Am. Chem. Soc.* **1991**, *113*, 297.

(40) Campbell, D. A. *J. Org. Chem.* **1992**, *57*, 6331.

(41) Malachowski, W. P. Ph.D. Thesis, The University of Michigan, Ann Arbor, MI, May 1993.

(42) Fieser, L. F.; Fieser, M. *Reagents for Organic Synthesis*; John Wiley & Sons, Inc.: New York, 1967; Vol. 1, pp 1065.

(43) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

analyses were obtained from Atlantic Microlab Inc. at Norcross, Ga. or at the Elemental Analysis Labs, Department of Chemistry, University of Michigan.

4-Amino-4-deoxy-N^{10}-methyl-pteroate was synthesized by a literature procedure.[2] δ-3-Benzyloxycarbonyl-5-oxo-4-oxazolidine-3-propanoic acid (**2**) was synthesized as previously described.[20,21] Diethyl DL-2-hydroxyglutarate was synthesized by reduction ($NaBH_4$) of the corresponding keto ester. This material was used without further purification because it was susceptible to lactonization during either silica gel chromatography or distillation.[44] Bis(trimethylsilyl)hydrogen phosphonite,[35,36] diethyl 2-methyleneglutarate,[45] and diethyl 2-ketoglutarate[46] were synthesized as described in the literature with minor modifications.[41]

***N*-Cbz-L-glutamic Acid, α-Ethyl Ester (5b).** (*S*)-3-(Benzyloxycarbonyl)-5-oxo-4-oxazolidinone-3′-propanoic acid (**2**) (11.42 g, 39 mmol) was dissolved in EtOH (800 mL) and cooled to 0 °C. Sodium ethoxide (25 mL of a 3.3M solution, 82 mmol) was added over 15 min. The reaction mixture was stirred at 0 °C for 1.5 h, after which it was quenched by diluting with H_2O and EtOAc and acidified with 3M HCl. The reaction was concentrated in vacuo without heating and then washed with EtOAc. The combined organic extracts were washed with brine, dried with $MgSO_4$, and concentrated to a white viscous oil. Silica gel chromatography (eluants: 1% EtOH/$CHCl_3$, 3% EtOH/$CHCl_3$, 5% EtOH/$CHCl_3$) of the oil provided 9.32 g (77% yield) of **5b**, a slightly white viscous oil which crystallizes on standing (mp = 32–33 °C). TLC R_f = 0.38 $CHCl_3$/EtOH (9:1). ^1H NMR ($CDCl_3$) δ 9.9–9.0 (bs, 1), 7.33 (s, 5), 5.67 (d, 1, J = 4 Hz), 5.09 (s, 2), 4.40 (q, 1, J = 5, 8 Hz), 4.18 (q, 2, J = 7 Hz), 2.55–2.35 (m, 2), 2.24–1.90 (dm, 2), 1.25 (t, 3, J = 7 Hz). ^{13}C NMR ($CDCl_3$) δ 177.7, 171.9, 156.0, 136.0, 128.4, 128.1, 128.0, 67.0, 61.7, 53.2, 29.8, 27.3, 14.0. IR (film) 1530, 1680–1760, 2400–3600 cm^{-1}. MS (EI, 70, rel. intensity) *m/e* 309 (3.5, M^+), 279 (19.4), 236 (14.9), 192 (19.5), 167 (31.0), 149 (65.4), 91 (100). HRMS (EI, 70) calcd for $C_{15}H_{19}NO_6$ (M^+) 309.1212, found 309.1199. $[\alpha]_D^{22}$ = −23.6° (c = 0.5 abs. MeOH), (lit. $[\alpha]_D^{19}$ = −21.4° (c = 6.8 abs. MeOH),[47] $[\alpha]_D^{21}$ = −21.4°.(c = 7.7 in abs. EtOH).[48] Anal. Calcd for $C_{15}H_{19}NO_6$: C, 58.24; H, 6.20; N, 4.53. Found: C, 58.24; H, 6.23; N, 4.50.

1-Oxa-2-oxo-3-thiaindolizinium Chloride (6).[27] 2-Mercaptopyridine N-oxide (8.25 g, 65 mmol) was dissolved in benzene. Phosgene (20% solution in toluene, 7.2 g, 140 mmol) was added and the reaction solution was stirred at rt. After 15 min, the precipitate which had formed was filtered, washed with benzene and dried in vacuo (0.3 mm Hg) for 6 h at 50 °C. Caution: This material reverts to mercaptopyridine N-oxide on extended heating. This provided 10.6 g (86% yield) of the pyridinium salt, **6**, mp = 185–187 °C (lit.[27] mp = 108–110 °C). IR (KBr) 1784 cm^{-1} (C = O stretch) (lit.[27] IR (nujol) 1770 cm^{-1}).

Ethyl (*S*)-2-(*N*-(Benzyloxycarbonyl)amino)-4-bromobutanoate (7b). *N*-Cbz-L-glutamic acid, α-ethyl ester (**5b**) 3.72 g, 12 mmol) was dissolved in CH_2Cl_2 (20 mL). Triethylamine (1.42 g, 14 mmol) was added, and the reaction flask was shielded from light with aluminum foil. Shortly thereafter, 1-oxa-2-oxo-3-thiaindolizinium chloride (**6**, 2.70 g, 14 mmol) was added in one portion and the reaction was stirred for 10 min at rt. The reaction was diluted with bromotrichloromethane (50 mL, 630 mmol) and then irradiated for 15 min. The reaction mixture was filtered and the filtrate concentrated to a light brown oil. Flash column chromatography (eluants: hexane, 1:1 CH_2Cl_2/hexane, CH_2Cl_2) of this oil afforded 3.35 g (81% yield) of **7b**, a dark yellow oil. TLC R_f = 0.49 (4:1 EtOAc: hexane). ^1H NMR ($CDCl_3$) δ 7.46 (s, 5), 5.40 (d, 1, J = 6 Hz), 5.15 (s, 2), 4.50 (q, 1, J = 4, 7 Hz), 4.23 (q, 2, J = 7 Hz), 3.44 (t, 2, J = 9 Hz), 2.50–2.15 (dm, 2), 1.29 (t, 3, J = 7 Hz). ^{13}C NMR ($CDCl_3$) δ 171.4, 155.9, 136.0, 128.6, 128.3, 128.1, 67.2, 61.9, 52.9, 35.8, 28.0, 14.1. IR (film) 690, 740, 1216, 1532, 1727, 3337 cm^{-1}. MS (EI, 70, rel. intensity) *m/e* 345 (3.8, M^+), 343 (3.7), 302 (0.5), 272 (12.7), 270 (12.4), 228 (17.3), 226 (17.4), 108 (31.7), 91 (100). HRMS (EI, 70) calcd for $C_{14}H_{18}{}^{79}BrNO_4$ (M^+) 343.0419, found 343.0424. $[\alpha]_D^{21}$ = −33° (c = 6.8 in abs. MeOH).

Dimethyl ((*S*)-3-(*N*-(Benzyloxycarbonyl)amino)-4-carbethoxybutyl)phosphonate (8b). Ethyl (*S*)-2-(*N*-(benzyloxycarbonyl)amino)-4-bromobutanoate (**7b**, 0.188 g, 0.546 mmol) was dissolved in trimethyl phosphite (5 mL, 42 mmol) and heated at reflux. The reflux condensor was flushed continuously with water at 50 °C[30] and an argon stream was maintained to remove the side product, methyl bromide, (bp = 4 °C). After five days at reflux temp., the reaction was concentrated in vacuo and then submitted to Kugelrohr distillation to remove unreacted trimethyl phosphite and volatile side products. Flash column chromatography (eluants: 1:1 $CHCl_3$/EtOAc, 9:9:2 $CHCl_3$/EtOAc/MeOH) of the concentrated material afforded 0.130 g (64% yield) of the product, a yellow oil. TLC R_f = 0.36 (9:9:2 $CHCl_3$/EtOAc/MeOH). ^1H NMR ($CDCl_3$) δ 7.35 (s, 5), 5.58 (d, 1, J = 7 Hz), 5.11 (s, 2), 4.45–4.35 (m, 1), 4.20 (q, 2, J = 7 Hz), 3.72 (dd, 6, J = 4, 11 Hz), 2.25–1.65 (m, 4), 1.27 (t, 3, J = 7 Hz). ^{13}C NMR ($CDCl_3$) δ 171.4, 155.9, 136.1, 128.5, 128.2, 128.1, 67.0, 61.8, 53.9 (d, J = 18 Hz), 52.4 (d, J = 6 Hz), 25.8 (d, J = 3 Hz), 20.7 (d, J = 140 Hz), 14.1. ^{31}P NMR ($CDCl_3$) δ 30.9. IR (neat) 817, 1046, 1218,1256, 1542, 1725, 3252 cm^{-1}. MS (EI, 70, rel. intensity) *m/e* 373 (0.7, M^+), 328 (0.18), 300 (10.4), 256 (13.8), 166 (8.5), 146 (31.2), 91 (100), 79 (11.1), 65 (10.7). HRMS (EI, 70) calcd for $C_{16}H_{24}NO_7P$ (M^+) 373.1290, found 373.1283. $[\alpha]_D^{22}$ = −15.1 (c = 6.8 in abs. MeOH). Anal. Calcd for $C_{16}H_{24}NO_7P{\cdot}0.5H_2O$: C, 50.25; H, 6.59; N, 3.66. Found: C, 50.50; H, 6.42; N, 3.61.

Methyl ((*S*)-3-(*N*-(Benzyloxycarbonyl)amino)-4-carbethoxybutyl)phosphonic Acid (9). Dimethyl ((*S*)-3-(*N*-(benzyloxycarbonyl)amino)-4-(ethoxycarbonyl)butylphosphonate (**8b**) 0.221 g, 0.592 mmol) was dissolved in t-butylamine (8 mL, 76 mmol) and heated at reflux temp. for 4 days. The reaction was concentrated to provide the product as a white salt, 0.256 g (quantitative yield). TLC R_f = 0.47 (4:1 $CHCl_3$/MeOH). ^1H NMR ($CDCl_3$) δ 8.6–8.2 (bs, 3), 7.32 (s, 5), 6.86 (d, 1, J = 7 Hz), 5.07 (q, 2, J = 11, 22 Hz), 4.45–4.30 (m, 1), 4.13 (q, 2, J = 7, 14 Hz), 3.44 (d, 3, J = 11 Hz), 2.1–1.9 (m, 2), 1.8–1.5 (dm, 2), 1.27 (s, 9), 1.20 (t, 3, J = 7 Hz). ^{13}C NMR ($CDCl_3$) δ 172.3, 156.2, 136.3, 128.4, 128.2, 128.0, 66.7, 61.2, 54.6 (d, J = 15 Hz), 51.6, 50.6, 27.8, 26.6, 22.0 (d, J = 125 Hz), 14.1. ^{31}P NMR ($CDCl_3$) δ 21.9. IR (KBr) 1047, 1171, 1210, 1266, 1552, 1720, 2400–3600 cm^{-1}. MS (CI w/NH_3, rel. intensity) *m/e* 360 (5.7, (MH − Bu^tNH_2)$^+$), 211 (6.0), 204 (8.5), 136 (98.6), 91 (86.8), 88 (22.4), 75 (100). HRMS (CI w/NH_3) calcd for $C_{15}H_{22}NO_7PH^+$ (MH − Bu^tNH_2)$^+$) 360.1212, found 360.1206. $[\alpha]_D^{23}$ = −5.2 (c = 6.8 in abs. MeOH).

Methyl ((*S*)-3-(*N*-(benzyloxycarbonyl)amino)-4-(ethoxycarbonyl)butyl)phosphonate, t-butyl amine salt (0.290 g, 0.670 mmol) was dissolved in $CHCl_3$ and treated with cation exchange resin (Dowex 50W-X8 (H^+ form) 200–400 mesh, 2.6 g (dry)). The Dowex resin was removed by filtration and the filtrate was concentrated in vacuo to afford **9** as an oil, 0.241 g (quantitative yield). ^1H NMR ($CDCl_3$) δ 7.35 (s, 5), 6.1–5.8 (bs, 1), 5.6–5.4 (bs, 1), 5.10 (s, 2), 4.45–4.35 (m, 1), 4.20 (q, 2, J = 7 Hz), 3.69 (d, 3, J = 11 Hz), 2.3–1.8 (dm, 2), 1.9–1.7 (m, 2), 1.27 (t, 3, J = 7 Hz). ^{13}C NMR ($CDCl_3$) δ 171.5, 155.9, 136.1, 128.5, 128.2, 128.1, 67.1, 61.8, 53.8, 51.6, 25.7, 21.5 (d, J = 100 Hz), 14.1. ^{31}P NMR ($CDCl_3$) δ 32.6. MS (CI w/NH_3, rel. intensity) *m/e* 360 (13.29, (MH^+)), 253 (18.8), 204 (15.6), 191 (12.1), 176 (20.5), 136 (100), 128 (20.7), 124 (11.6), 114 (12.0), 108 (19.5), 106 (18.5). HRMS (CI w/NH_3) calcd for

(44) An attempt was made to obtain chirally pure diethyl L-2-hydroxyglutarate via diazotization of L-glutamic acid diethyl ester, a process that reportedly[50,51] proceeds with retention of configuration. However, in our hands, a 1:1 mixture of the 2-hydroxyglutarate and an elimination product, diethyl *trans*-1,5-pent-2-enedioate, was obtained. Lowering the temperature did not reduce the amount of the elimination product.

(45) Feit, B. A.; Sasson, S. *Eur. Polym. J.* **1971**, *7*, 1435.
(46) Slama, J. T.; Satsangi, R. K.; Simmons, A.; Lynch, V.; Bolger, R. E.; Suttie, J. *J. Med. Chem.* **1990**, *33*, 824.
(47) Weygand, V. F.; Hunger, K. *Z. Naturforsch. B: Chem. Sci.* **1958**, *13B*, 50.
(48) Le Quesne, W. J.; Young, G. T. *J. Chem. Soc.* **1950**, 1954.
(49) Chang, C.-D.; Coward, J. K. *J. Med. Chem.* **1976**, *19*, 684.
(50) Knapp, S.; Hale, J. J.; Bastos, M.; Molina, A.; Chen, K. Y. *J. Org. Chem.* **1992**, *57*, 6239.
(51) Van Draunen, N. A.; Arseniyadis, S.; Crimmins, M. T.; Heathcock, C. H. *J. Org. Chem.* **1991**, *56*, 2499.

$C_{15}H_{22}NO_7PH^+$ (MH^+) 360.1212, found 360.1223. Anal. Calcd for $C_{15}H_{22}NO_7P \cdot 1.5\ H_2O$: C, 46.63; H, 6.52; N, 3.63. Found: C, 46.46; H, 6.40; N, 3.28.

Methyl ((*S*)-3-(*N*-(Benzyloxycarbonyl)amino)-4-carbethoxybutyl)phosphonochloridate (10). Oxalyl chloride (0.132 g, 1.04 mmol) was added dropwise to a solution of methyl ((*S*)-3-(*N*-(benzyloxycarbonyl)amino-4-(ethoxycarbonyl)-butyl)phosphonic acid (**9**) (0.240 g, 0.668 mmol) and DMF (2.5 μL, 0.033 mmol) dissolved in CH_2Cl_2 at 0 °C. The solution was stirred at 0 °C for 20 min and then warmed to rt and stirred for 1.5 h. The reaction was concentrated, dissolved in toluene (2 mL), and then reconcentrated in vacuo to remove the volatile reagents. This left the phosphonochloridate as a yellow oil which was used immediately in a reaction with an amine or alcohol. ^1H NMR ($CDCl_3$) δ 7.37 (s, 5), 5.45–5.40 (bs, 1), 5.13 (s, 2), 4.50–4.40 (m, 1), 4.24 (q, 2, J = 6, 12 Hz), 3.88 (d, 3, J = 12 Hz), 2.4–2.0 (m, 4), 1.29 (t, 3, J = 6 Hz). ^{31}P NMR ($CDCl_3$) δ 42.1.

General Procedure for Reaction of Amine or Alcohol Nucleophiles with 10. The amine or alcohol (1.0 equiv) was dissolved in THF and cooled to 0 °C. Et_3N (3.0 equiv for an amine hydrochloride salt, 2.0 equiv for a free amine or an alcohol) was added to the reaction, followed immediately by the dropwise addition of the phosphonochloridate **10** (1.1 equiv), dissolved in THF. The reaction was allowed to warm to rt and stirred overnight. The $Et_3N \cdot HCl$ precipitate was removed by filtration and the filtrate was concentrated in vacuo. This oil was dissolved in EtOAc and washed successively with 5% $NaHCO_3$, 5% $KHSO_4$ and brine, dried with $MgSO_4$, filtered and concentrated to afford the product. Silica gel flash column chromatography (eluant: EtOAc or $CHCl_3$/i-PrOH (9:1)) of this oil provided pure product but some decomposition occurred on the column, thus leading to decreased yields.

2-[(*S*)-*N*-[Methoxy((*S*)-3′-(*N*-(benzyloxycarbonyl)amino)-4′-carbethoxybutyl)phosphinyl]aminopentane-1,5-dioic Acid, Diethyl Ester (11). The phosphonochloridate **10** (0.375 g, 0.993 mmol) was coupled with L-glutamic acid hydrochloride, diethyl ester (0.262 g, 1.09 mmol) in THF (28 mL) with Et_3N (0.381 g, 3.77 mmol). After extractive workup the opaque oil (0.344 g, 64% yield) crystallized upon sitting to afford white crystals, mp = 116–118 °C. TLC R_f = 0.22 (EtOAc). ^1H NMR ($CDCl_3$) δ 7.35 (s, 5), 5.61 (d, 1, J = 7 Hz), 5.11 (s, 2), 4.45–4.35 (m, 1), 4.25–4.05 (m, 6), 4.00–3.85 (m, 1), 3.64 (d, 3, J = 11 Hz), 3.12 (t, 1, J = 11 Hz), 2.45–2.35 (m, 2), 2.25–2.05 (m, 2), 2.00–1.85 (m, 2), 1.85–1.60 (m, 2), 1.35–1.15 (m, 9). ^{13}C NMR ($CDCl_3$) δ 173.2 , 172.6, 171.6, 156.1, 136.2, 128.5, 128.1, 128.1, 67.0, 61.6, 60.6, 54.0 (d, J = 18 Hz), 53.0, 50.8, 30.1, 29.7, 25.6, 23.6 (d, J = 125 Hz), 14.1. ^{31}P NMR ($CDCl_3$) δ 31.5. IR (KBr) 1021, 1051, 1201, 1217, 1259, 1536, 1733, 1747 cm^{-1}. MS (DCI w/NH_3, rel. intensity) *m/e* 546 (36.9, $[M + 2H]^+$), 545 (100, (MH^+)), 531 (14.4), 437 (10.8), 342 (32.4), 320 (23.6), 204 (28.9), 134 (20.7), 130 (24.8), 91 (63.7). HRMS (CI w/NH_3) calcd for $C_{24}H_{38}N_2O_{10}P^+$ (MH^+) 545.2264, found 545.2227. $[\alpha]_D^{23} = -27.4$ (c = 2.1 in abs. MeOH). Anal. Calcd for $C_{24}H_{37}N_2O_{10}P \cdot 0.5\ H_2O$: C, 52.07; H, 6.92; N, 5.06. Found: C, 52.27; H, 6.58; N, 5.07.

2-[*O*-[Methoxy((*S*)-3′-(*N*-(benzyloxycarbonyl)amino)-4′-carbethoxybutyl)phosphinyl]hydroxypentane-1,5-dioic Acid, Diethyl Ester (12). A. The phosphonochloridate **10** (0.125 g, 0.331 mmol) was coupled with diethyl 2-hydroxyglutarate (0.061 g, 0.301 mmol) in THF (10 mL) with Et_3N (0.069 g, 0.682 mmol). After extractive workup of the reaction mixture, the crude product was purified with silica gel flash chromatography (eluant: EtOAc) to afford 0.011 g (7% yield) of the phosphonate **12**. The product was spectroscopically identical to the sample prepared by the DIAD/Ph_3P method (below).

B. Diethyl 2-hydroxyglutarate (0.150 g, 0.63 mmol) and Ph_3P (0.164 g, 0.63 mmol) were added to the phosphonate monoacid (**7**, 0.15 g, 0.42 mmol) dissolved in THF (4 mL). Diisopropyl azodicarboxylate (0.139 g, 0.69 mmol) was added dropwise to this mixture at rt. After two days, the reaction solution was concentrated in vacuo and purified by flash column chromatography (eluant: 95:5 EtOAc:CH_3CN) to provide 0.150 g (66% yield) of the product as an opaque oil. TLC R_f = 0.32 (EtOAc). ^1H NMR ($CDCl_3$) δ 7.35 (s, 5), 5.65–5.55 (bs, 1), 5.11 (s, 2), 5.00–4.85 (m, 1), 4.45–4.30 (m, 1), 4.25–4.10 (m, 6), 3.78 (d, 1.3, J = 11 Hz) and 3.70 (d, 1.7, J = 11 Hz) (diastereomers), 2.50–2.35 (m, 2), 2.35–1.85 (m, 6), 1.35–1.20 (m, 9). ^{13}C NMR ($CDCl_3$) δ 172.2, 171.4, 170.0, 156.0, 136.1, 128.5, 128.2, 128.1, 73.1 and 72.8 (diastereomers), 67.0, 61.73, 61.67, 60.7, 54.0 (d, J = 18 Hz), 51.7, 29.3, 28.1, 25.6, 22.0 (dd, J = 24, 144 Hz), 14.1. ^{31}P NMR ($CDCl_3$) δ 31.1, 31.0, 29.9. MS (DCI w/NH_3, rel. intensity) *m/e* 546 (51.5, (MH^+)), 532 (6.2), 472 (8.0), 412 (7.9), 342 (5.5), 188 (14.6), 187 (100, MS), 91 (31.2). HRMS (CI w/NH_3) calcd for $C_{24}H_{37}NO_{11}P^+$ (MH^+) 546.2104, found 546.2056. $[\alpha]_D^{23} = -2.5$ (c = 2.6 in abs. MeOH). Anal. Calcd for $C_{24}H_{36}NO_{11}P$: C, 52.84; H, 6.65; N, 2.57. Found: C, 52.44; H, 6.53; N, 2.11.

General Procedure for the Hydrogenation of Phosphonamidate 11 or Phosphonate 12. The phosphonamidate or phosphonate was dissolved in MeOH in a Parr hydrogenation bottle and Pd/C (15–20 wt %) was added. The bottle was charged with H_2 (45–50 psi) and left shaking overnight. The reaction material was filtered through celite and concentrated to afford the products as light yellow oils.

2-[(*S*)-N-[Methoxy((*S*)-3′-amino-4′-carbethoxybutyl)-phosphinyl]amino]pentane-1,5-dioic Acid, Diethyl Ester (1d). The phosphonamidate **11** (0.152 g, 0.279 mmol) was shaken overnight with Pd/C (0.030 g) in MeOH (10 mL) under H_2 (50 psi). After concentration, 0.100 g (87% yield) of the free amine, **1d**, was obtained. TLC R_f = 0.48 (4:1 $CHCl_3$/MeOH). ^1H NMR ($CDCl_3$) δ 4.25–4.05 (m, 6), 4.00–3.90 (m, 1), 3.75 (d, 1.5, J = 11 Hz) and 3.66 (dd, 1.5, J = 1, 11 Hz) (diastereomers), 3.60–3.40 (m, 2), 3.25–2.75 (bs, 2), 2.50–2.30 (m, 2), 2.20–1.70 (m, 6) 1.35–1.10 (m, 9). ^{13}C NMR ($CDCl_3$) δ 174.8, 173.2, 172.6, 61.4, 60.9, 60.5, 54.2 (d, J = 15 Hz), 52.9 (d, J = 16 Hz), 50.4 (d, J = 18 Hz), 30.0, 29.6 (d, J = 5 Hz), 27.3, 23.8 (dd, J = 20, 135 Hz), 14.0. ^{31}P NMR ($CDCl_3$) δ 32.9, 32.8, 32.4, 32.3, 31.8. MS (CI w/NH_3, rel. intensity) *m/e* 411 (84.5, (MH^+)), 397 (9.5), 361 (6.4), 240 (11.3), 225 (14.7), 208 (38.9), 204 (100), 175 (19.5). HRMS (DCI w/NH_3) calcd for $C_{16}H_{32}N_2O_8P^+$ (MH^+) 411.1896, found 411.1894.

2-[*O*-[Methoxy((*S*)-3′-amino-4′-carbethoxybutyl)phosphinyl]hydroxy]pentane-1,5-dioic Acid, Diethyl Ester (1e). The phosphonate **12** (0.205 g, 0.376 mmol) was shaken overnight with Pd/C (0.032 g) in MeOH (10 mL) under H_2 (47 psi). After concentration, 0.165 g (quantitative yield) of the free amine, **1e**, was obtained. TLC R_f = 0.55 (4:1 $CHCl_3$/MeOH). ^1H NMR ($CDCl_3$) δ 5.00–4.88 (m, 1), 4.28–4.10 (m, 6), 3.80 (d, 1.2, J = 12 Hz) and 3.73 (d, 1.8, J = 12 Hz) (diastereomers), 3.60–3.50 (m, 1), 2.90–2.55 (bs, 2), 2.55–2.35 (m, 2), 2.35–1.90 (m, 6), 1.36–1.24 (m, 9). ^{13}C NMR ($CDCl_3$) δ 174.3, 172.1, 169.9, 72.7 (dd, J = 6, 27 Hz), 61.5, 61.0, 60.5, 54.0 (d, J = 17 Hz), 52.1 (dd, J = 7, 81 Hz), 29.2, 28.1, 27.0 (d, J = 9 Hz), 21.9 (dd, J = 14, 143 Hz), 14.0. ^{31}P NMR ($CDCl_3$) δ 31.9, 30.8. MS (CI w/NH_3, rel. intensity) *m/e* 426 (11.7), 412 (100, (MH^+)), 398 (33.9), 237 (20.1), 208 (5.7), 201 (29.2), 187 (14.7), 136 (24.5). HRMS (DCI w/NH_3) calcd for $C_{16}H_{31}NO_9P^+$ (MH^+) 412.1736, found 412.1748.

2,5-Bis(carbethoxy)pentane-1-phosphinic Acid (14). Diethyl 2-methyleneglutarate **13** (1.27 g, 6.30 mmol) was added to a solution of bis(trimethylsilyl)hydrogen phosphonite (5.77 g, 32.0 mmol) in methylene chloride (20 mL) at 0 °C. After the addition, the reaction was stirred overnight at rt. The reaction was quenched with dilute (1M) HCl and the resulting solution was washed with CH_2Cl_2. The combined organic layers were dried with $MgSO_4$ and concentrated to afford 1.53 g (83% yield, based on diethyl 2-methyleneglutarate) of a yellow oil, **14**. TLC R_f = 0.17 (9.5:0.5:0.5 $CHCl_3$:MeOH:AcOH). ^1H NMR ($CDCl_3$) δ 12.41 (bs, 1), 7.1 (d, 1, J = 570 Hz), 4.17–4.04 (m, 4), 2.88–2.71 (m, 1), 2.35–2.25 (m, 2), 2.20–1.70 (dm, 2, J = 94 Hz), 1.99–1.88 (m, 2), 1.21 (q, 6, J = 6, 9 Hz). ^{13}C NMR ($CDCl_3$) δ 173.4 (d, J = 24 Hz), 172.2, 60.9, 60.3, 38.1, 31.0 (d, J = 378 Hz), 31.3, 28.0 (d, J = 48 Hz, 1-CH_2), 14.0, 13.9. ^{31}P NMR ($CDCl_3$) δ 34.3. MS (FAB in 3-NBA, rel. intensity) *m/e* 267 (((MH^+), 100), 221 (34.9), 175 (51.4), 147 (24.8).

2,5-Dicarbethoxypentane-1-phosphinic Acid, Ethyl Ester (16). Phosphinic acid **14** (0.277 g, 1.04 mmol) was dissolved in CH_2Cl_2 (4 mL) and treated with EtOH (1.5 mL,

28 mmol) and EDC (0.221 g, 1.14 mmol). After stirring for 19 h at rt, the reaction mixture was diluted with EtOAc, washed with saturated KH_2PO_4, $NaHCO_3$, brine, dried with Na_2SO_4 and concentrated to afford 0.262 g (86% yield) of a viscous cloudy oil. TLC $R_f = 0.14$ (EtOAc). ^1H NMR ($CDCl_3$) δ 7.13 (dm, 1, $J = 555$ Hz), 4.18–3.97 (m, 6), 2.9–2.7 (m, 1), 2.35–2.26 (m, 2), 2.25–1.75 (m, 2, $J = 105$ Hz), 2.0–1.9 (m, 2), 1.32–1.17 (m, 9). ^{13}C NMR ($CDCl_3$) δ 173.2, 171.8, 62.0, 60.6, 60.1, 38.0, 31.2, 30.2, 28.0, 15.9, 13.8, 13.7. ^{31}P NMR ($CDCl_3$) δ 34.4, 33.4, 30.7, 29.8 (diastereomers).

Diethyl 2-[[Ethoxy(2′-carbethoxyethyl)phosphinyl]-methyl]-pentanedioate (17). Phosphinic acid **16** (0.177 g, 0.601 mmol) was dissolved in $CHCl_3$ (4 mL), treated with Et_3N (0.080 g, 0.79 mmol) and cooled to 0 °C. TMSCl (0.093 g, 0.86 mmol) was added and the reaction was stirred at 0 °C. After 2.25 h, ethyl 2-bromoacetate (0.116 g, 0.661 mmol) was added and the reaction was warmed to rt and stirred overnight. The reaction was concentrated in vacuo after 18 h and the product was purified by silica gel flash chromatography (eluant: EtOAc) as a viscous, colorless oil (0.064 g, 28% yield). TLC $R_f = 0.18$ (EtOAc). ^1H NMR ($CDCl_3$) δ 4.2–4.0 (m, 8), 3.01–2.89 (m, 2), 2.89–2.77 (m, 1), 2.42–2.20 (m, 3), 2.02–1.88 (m, 3), 1.30–1.15 (m, 12). ^{13}C NMR ($CDCl_3$) δ 173.8, 172.4, 166.1, 61.5, 61.3, 61.2, 60.4, 38.4 (d, $J = 16$ Hz), 37.1 (dd, $J = 27$, 78 Hz), 31.4, 30.5 (dd, $J = 9$, 90 Hz), 28.4 (dd, $J = 11$, 27 Hz), 16.3 (d, $J = 5$ Hz), 14.1. ^{31}P NMR ($CDCl_3$) δ 42.8, 42.2 (diastereomers).

Acknowledgment. This research was supported by a grant from the National Cancer Institute (CA28097). W.P.M. was supported in part by a National Research Service Award (T32 GM07767), a fellowship from the University of Michigan Comprehensive Cancer Center, and a fellowship from the American Foundation for Pharmaceutical Education. The syntheses of diethyl 2-ketoglutarate and diethyl DL-2-hydroxyglutarate were carried out by Anne Lynn Gillian. We gratefully acknowledge the helpful discussions with Professor David Crich, University of Illinois at Chicago Circle, concerning the chemistry of the modified Hunsdieker reaction. We thank Mrs. Jane MacDonald for careful preparation of the manuscript.

Supplementary Material Available: Procedures for the synthesis of **2**, reaction product: **10** and *sec*-butylamine, diethyl 2-ketoglutarate, diethyl DL-2-hydroxyglutarate, **13**, and bis(trimethylsilyl)hydrogenphosphonite with complete spectral data. ^1H NMR spectra for **7b**, **10**, **1d**, **1e**, **14**, **16**, and **17**. IR spectrum for **6** (11 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.