The Chemistry of Phosphapeptides: Formation of Functionalized Phosphonochloridates under Mild Conditions and Their Reaction with Alcohols and Amines

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A variety of methods have been explored for the convergent synthesis of phosphonamidates and phosphonates via phosphonochloridates. In the absence of complex functionality, phosphorus pentachloride is an effective reagent for the conversion of diethyl and diisopropyl phosphonates to phosphonochloridates. Subsequent reaction with amines and alcohols provides good yields of the phosphonamidates and mixed phosphonates, respectively. However, in the presence of more complex functionality, a milder method utilizing oxalyl chloride to generate the phosphonochloridate from a monomethyl phosphonic acid is required. Aminolysis of the dimethyl phosphonates generates the requisite monomethyl phosphonic acids which are cleanly converted to phosphonochloridates and reacted with amines and primary alcohols.

Introduction

The design and synthesis of extremely potent and specific inhibitors of enzyme-catalyzed reactions based on mechanistic enzymology has been actively investigated during the last decade.[1] Much of the progress in this field has been associated with the development of synthetic methods which allow for construction of these functionally complex organic molecules. Thus, elucidation of the structure of the natural product pepstatin, a potent protease inhibitor,[2] led to the synthesis of the key amino acid statine, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid,[3] and its incorporation into a myriad of peptides as potential inhibitors of a variety of proteases.[4] Other "dipeptide isostere" linkers have been synthesized, all of which are hypothesized to represent analogues of the unstable tetrahedral intermediate involved in the hydrolysis of esters, amides, and peptides.[5] The success of this approach is demonstrated by the large number of nanomolar inhibitors of aspartic proteases currently available.[6] An alternative approach is to exploit the tendency of an enzyme active site nucleophile to add to a carbonyl carbon in certain aldehydic or ketonic peptide analogues or for the enzyme to capture the hydrated form of certain heterocyles. In both cases, the change in carbon hybridization from sp^2 to sp^3 is thought to lead to an enzyme-bound mimic of the tetrahedral intermediate of normal enzyme catalysis; i.e., enzyme-catalyzed addition of water to the substrate. For example, this approach has been successfully employed in the case of fluoro ketone-containing peptides[7] and simple purines or pyrimidines.[8]

Yet another approach involves the use of stable tetrahedral phosphorous species as mimics of the unstable tetrahedral carbon intermediates noted above. This approach again has its origins in the elucidation of the structure of phosphoramidon, a potent natural product inhibitor of thermolysin, a zinc protease.[9] Following this approach, potent synthetic inhibitors of several zinc proteases such as carboxypeptidase A,[10] thermolysin,[11] and angiotensin converting enzyme[12] have been developed. The biosynthesis of amides or peptides, usually catalyzed by ATP-dependent synthetases, also proceeds via tetrahedral intermediates and therefore should be susceptible to inhibition by compounds containing tetrahedral mimics. This has been confirmed with glutamine synthetase[13,14] using a phosphinate natural product, phosphinothricin, and with D-alanyl-D-alanine ligase[15] using a synthetic phosphinate dipeptide analogue.[16]

Phosphonates, phosphonamidates, and phosphinates (Figure 1) have been the target of recent investigations in both catalytic antibody research[17,18] and protease or ligase enzyme research.[19–21] This is because they serve as stable analogues of the transient high-energy tetrahedral intermediate common to all. It was our goal to

[⊗] Abstract published in *Advance ACS Abstracts*, November 15, 1994.

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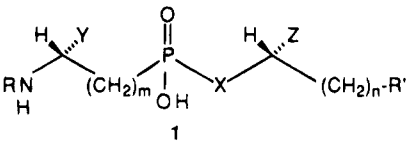
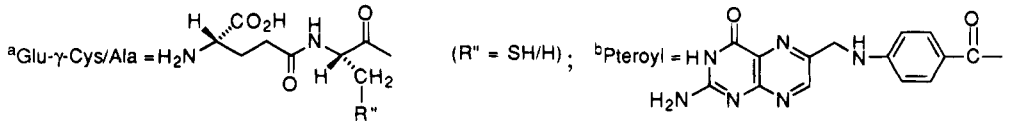
Chart 1

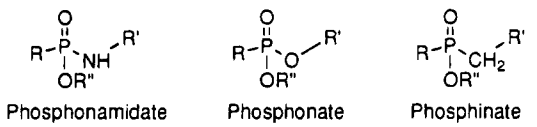


1

	R	Y	m	X	Z	n	R'
a	Glu-γ-Cys/Ala[a]	H	0	NH	H	2	$NH(CH_2)_4NH_2$
b	Glu-γ-Cys/Ala	H	0	O	H	2	$NH(CH_2)_4NH_2$
c	Pteroyl[b]	CO_2H	2	NH	CO_2H	2	CO_2H
d	Pteroyl	CO_2H	2	O	CO_2H	2	CO_2H
e	Pteroyl	CO_2H	2	CH_2	CO_2H	2	CO_2H



[a]Glu-γ-Cys/Ala = H_2N (R" = SH/H); [b]Pteroyl =



Phosphonamidate Phosphonate Phosphinate

R, R', R" = alkyl, aryl

Figure 1. Phosphorus nomenclature.

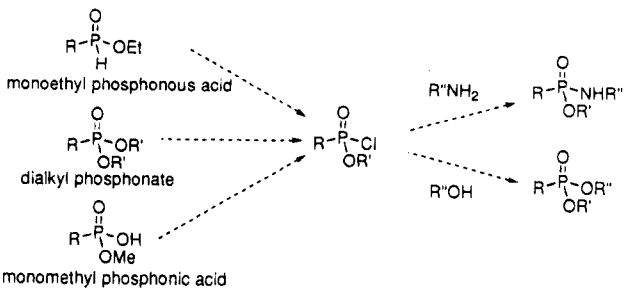
use molecules of this type as "transition state analogues"[22,23] in enzyme inhibitor design. In the research described in this and the accompanying paper,[24] we address the synthesis of complex phosphapeptides of the general structure **1** (Chart 1). Our synthetic strategy involved a convergent synthesis of the phosphonamidate (**1a**, **1c**) and phosphonate (**1b**, **1d**) transition state analogues through a common phosphonochloridate intermediate. In this paper, we describe the solutions to problems encountered during the synthesis of several phosphonates and phosphonamidates from appropriate phosphonochloridates and/or monomethylphosphonates.

A specific target for phosphorus-based tetrahedral mimics is the pair of enzymes responsible for the biosynthesis of the parasitic redox cofactor, trypanothione.[25] Specifically, we envision the use of phosphapeptides as potent and specific inhibitors of the two ATP-dependent enzymes, glutathionylspermidine (GSP) synthetase and trypanothione (TSH) synthetase, involved in the sequential addition of glutathione (GSH) to the two terminal primary amino groups of the ubiquitous polyamine, spermidine.[26] Although no data are available which demonstrate the intermediacy of an acyl phosphate in these enzyme-catalyzed reactions, we assume this to be the case based on extensive literature precedent with other ATP-dependent peptide biosynthesis. Thus, for GSP synthetase, the proposed tetrahedral intermediate in eq 1 (Chart 2) leads to the target phosphapeptides (**1a**,**b**, R" = H) including the major structural components of spermidine and GSH linked through the stable tetrahedral phosphorus moiety.

Results

We have studied the synthesis of phosphonochloridates from three different types of starting materials: monoethyl phosphonous acids, dialkyl phosphonates, and monomethyl phosphonic acids (Scheme 1). Initially, the most attractive method to phosphonochloridates was from monoethyl phosphonous acids due to the use of a rather mild and selective oxidative chlorinating agent, CCl_4 (Scheme 2).[27] This was important because we needed a method which would be compatible with the diverse functionality present in our complex target molecules. In our hands, significant difficulties were encountered with the hydrolytic lability of the phosphinate ethyl esters (**2**) and all attempts failed to yield any coupling product (**5**) in greater than 15% yield. The reaction was initially tried with ethyl 3,3-dicarbethoxy-3-acetamidopropylphosphonous acid[28] (**2a**), a masked α-amino acid synthon which, after coupling, would have served as a precursor for the dipeptide transition state analogue; i.e., **1c**,**d**. Hydrolysis of **2a** to the known phosphonous acid analogue of glutamic acid, **6**,[28] in quantitative yield provided further evidence for the structure of **2a**. When the phosphonochloridate generation reaction (Scheme 2, **2** → **4**) was monitored by ^{31}P NMR, formation of the intermediate trimethylsilylated phosphonite ethyl ester (δ = 160−175 ppm), was observed. This was true with **2a** (^{31}P NMR δ = 38.8 ppm)

Scheme 1



monoethyl phosphonous acid; dialkyl phosphonate; monomethyl phosphonic acid; R"NH_2; R"OH

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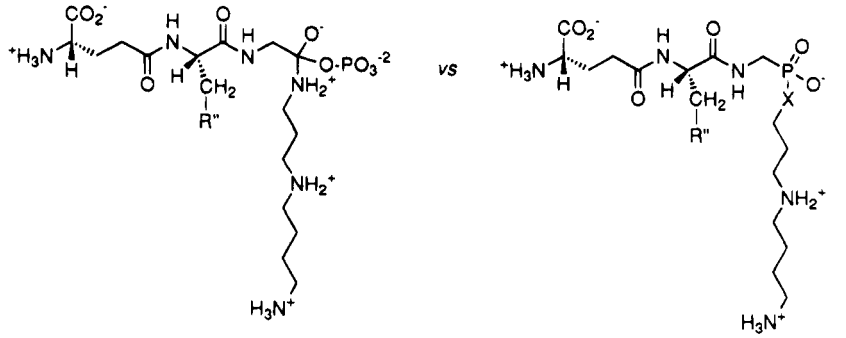
Chart 2



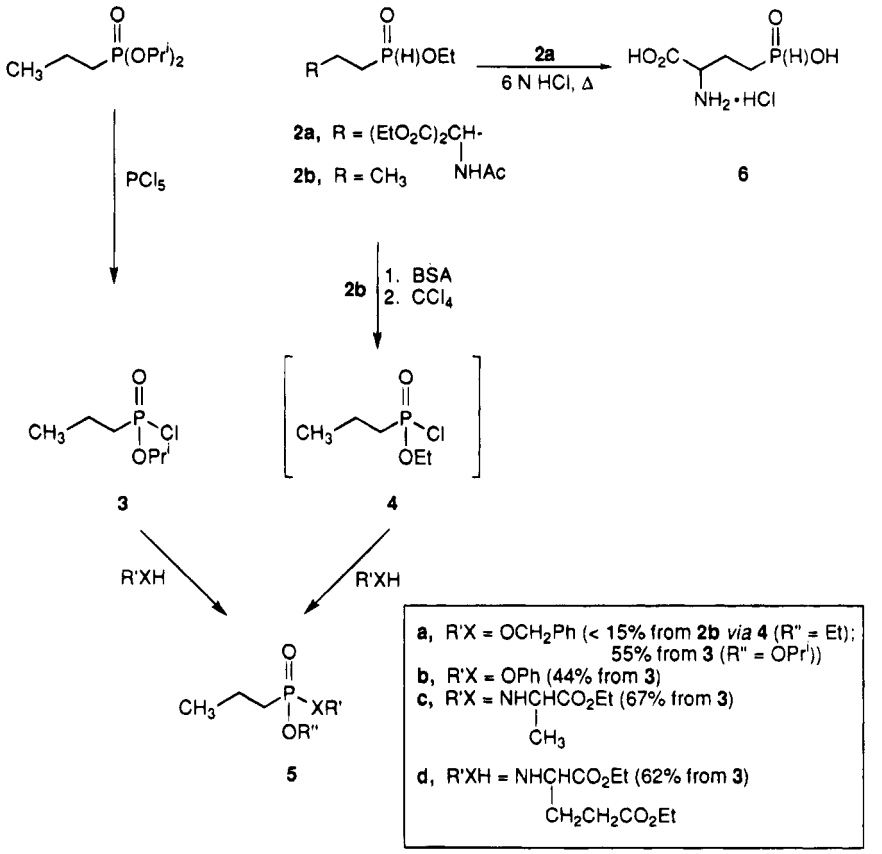
Proposed Tetrahedral Intermediate of the GSP Synthetase-Catalyzed Reaction

vs

X = NH, O (**1a,b**)
Proposed GSP Synthetase Inhibitors

(1)

Scheme 2



and later with the model compounds, monoethyl methylphosphonite (^{31}P NMR δ = 36.1 ppm) and monoethyl propylphosphonite (**2b**, ^{31}P NMR δ = 36.9 ppm). In the case of **2b**, the trimethylsilylated phosphonite ethyl ester intermediate was isolated via distillation. However, the conversion of this species to the phosphonochloridate, **4** (δ = 40–50 ppm), was rarely complete and multiple ^{31}P signals were often observed. Consequently, the yields of the subsequent coupling reaction were poor at best[29] and led us to consider alternative conditions for generating the phosphonochloridate intermediate.

The conversion of dialkyl phosphonates to monoalkyl phosphonochloridates with phosphorus pentachloride (PCl_5) was the second approach considered. PCl_5 has been used with substrates containing carboxylic acid esters[30] or phthalimides[31–33] and, therefore, has demonstrated some selectivity. The first successful phosphonochloridate generation and coupling reaction used PCl_5 in CCl_4 at reflux temperature to convert diisopropyl propylphosphonate to isopropyl propylphosphonochloridate, **3** (Scheme 2, Table 1). The desired product was purified from the crude reaction mixture by distillation to afford a 57% yield of the phosphonochloridate. The distillate was reacted immediately in four successful coupling reactions with L-alanine methyl ester, L-glutamate diethyl ester, phenol, and benzyl alcohol to give **5a–d**, respectively, as shown in Scheme 2. This was

(29) When the trimethylsilylated phosphonite and the phosphonochloridate intermediates derived from **2b** were both isolated by distillation, the coupling reaction with benzyl alcohol went in only 13% yield.

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encouraging, but we were concerned that the isopropyl phosphorus ester would be difficult to remove selectively in a total synthesis, so we tested the applicability of this technique with other dialkyl phosphonates.

Table 1. Physical Properties of Phosphonochloridates

compd	method	yield (%)	^{31}P NMR, δ	ref
3	PCl_5	57[a]	43.4	37
8a	PCl_5	88[a]	44.1	34
8b	PCl_5	nd[b]	28.4	32
14	$SOCl_2$	nd[b]	30.5	na
14	$(COCl)_2$	nd[b]	30.5	na

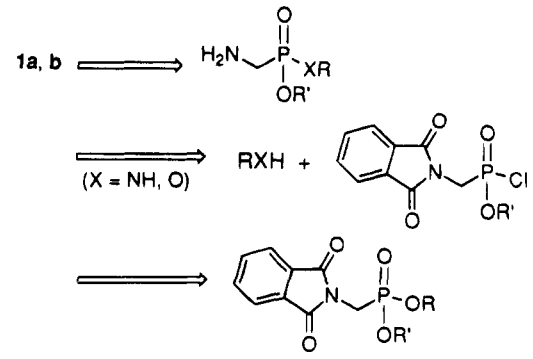
[a] Yield given is for distilled product: **3**, bp 58–62 °C/0.75 mm (lit.[37] bp 61–64 °C/3–4 mm); **8a**, bp 62–65 °C/6 mm (lit.[53] bp 73–74 °C/10 mm). [b] Yield of pure product not determined; presence of phosphonochloridate was ascertained by NMR (^{1}H, ^{13}C, ^{31}P) of crude product which then was used immediately in coupling reactions.

The use of PCl_5 was equally effective with diethyl ethylphosphonate, **7a** (Table 2). Heating this dialkyl phosphonate at reflux with PCl_5 in CCl_4 for 20 h, followed by distillation provided 88% yield of ethyl ethylphosphonochloridate, **8a**.[34] An excess of this pure phosphonochloridate was easily coupled with **9a**,[35] a primary alcohol containing the requisite protected amine functionalities for the synthesis of **1b**, to give phosphonate, **10a**, in 94% yield. Heteroatom compatibility was initially explored with the phthalimide group, a protected amine functionality. Diethyl phthalimidomethylphosphonate, **7b**, was heated at reflux temperature overnight in benzene with PCl_5 and was completely converted to the monoethyl (phthalimidomethyl)phosphonochloridate, **8b.** Coupling of **8b** with alcohol, **9a**, was sensitive to reagent stoichiometry, with a 40% excess of **8b** leading to 95% yield of the desired phosphonate, **10b**. Successful coupling of **8b** with N^1-phthaloyl-1,4-diaminobutane (**9b**) and L-alanine methyl ester (**9c**) was also effected in unoptimized yields of 53% and 66% respectively (Table 2).

These initial results were encouraging, especially in terms of the polyamine-containing phosphapeptides, **1a** and **1b**. Thus, the alcohol **9a** contains the spermidine-like structure required in our planned synthesis of phosphonate **1b** (Scheme 3). However, selective hydrolysis of the ethyl ester of phosphonates derived from **10b** proved to be difficult. Therefore, we investigated the use of a dimethyl phosphonate, since deprotection of a methyl ester would be more facile than an ethyl ester in the phosphonate or phosphonamidate precursors of **1**. When dimethyl (phthalimidomethyl)phosphonate (**11**, Scheme 4) was heated at 65 °C for 24 h with phosphorus pentachloride, ^{31}P NMR analysis showed almost complete formation of the phosphonochloridate, **14**. However, attempts at coupling this reactive compound with several primary alcohols failed[36] and the corresponding monomethyl(phthalimidomethyl)phosphonic acid was isolated in quantitative yield, indicating the phosphonochloridate had simply hydrolyzed.

Since we were concerned that the phosphonate dimethyl esters, being susceptible to demethylation (S_N2), may be sensitive to residual PCl_5 and/or its reaction byproducts, more volatile chlorination reagents such as phosgene,[37,38] oxalyl chloride[39] and thionyl chloride,[30,40]

Scheme 3



were explored as the reagents for the conversion of **11** to **14** (Scheme 4). However, none of these reagents was found to be effective according to ^{31}P NMR analysis of the reaction mixture. Attempts to use the phosphonochloridate derived from dibenzyl phthalimidomethylphosphonate[31] were thwarted by a slow reaction of the dibenzyl ester with PCl_5. Starting material was not completely consumed after 42 h. ^{31}P NMR analysis of the reaction mixture provided evidence for the desired phosphonochloridate (δ = 29.1) but there were several other signals in the ^{31}P NMR spectra. The crude product derived from this reaction failed to react with alcohol **9a**.

Although the PCl_5 method cleanly generated selected phosphonochloridates and allowed for subsequent coupling with amines and alcohols, there were several difficulties in its general application. It could be used only with diethyl and diisopropyl phosphonates; methyl and benzyl esters were unsuitable. This limited its use in a total synthesis because, as noted previously, the ethyl and diisopropyl esters are difficult to remove selectively. Additionally, its functional group compatibility was limited to the phthaloyl group (Table 2, **7b** → **8b**). Phosphonates with more diverse functionality, such as N-Cbz amino acid esters to be discussed in the accompanying paper,[24] were not compatible with these harsh conditions for generating the phosphonochloridate. In an attempt to overcome these limitations, we pursued more mild syntheses of methyl esters of phosphonochloridates from monomethyl phosphonic acids (Scheme 1).

Using simple monomethyl alkylphosphonous acids, we initially explored the conversion of these compounds to phosphonochloridates with $SOCl_2$, based on the work of Bartlett et al.[41–44] The first attempt at this transformation used monomethyl butylphosphonic acid, **13b** (Scheme 4), synthesized by DCC-mediated coupling[45] of one equivalent of MeOH with butylphosphonic acid (**12b**). When the reaction of **13b** with $SOCl_2$ was monitored by ^{31}P NMR, the reaction proceeded slowly and after 5.5 h only about 10% conversion to the corresponding phosphonochloridate was seen. Similarly, monomethyl propylphosphonic acid, **13c**, synthesized in a manner analogous to **13b**, was treated with $SOCl_2$. Approximately 50%

(34) Razumov, A. I.; Mukjacheva, O. A.; Markovich, E. A. *Zh. Obshch. Khim.* **1957**, *27*, 2450.
(35) Lakanen, J. R. Ph.D. Thesis, The University of Michigan, Ann Arbor, MI, Jan 1994.
(36) Coupling of **14**, derived from **11** by reaction with PCl_5, with a primary amine has been effected, albeit in low (28%) yield: Gibbs, R. A.; Benkovic, S. J. Personal communication.
(37) de Roos, A. M.; Toet, H. J. *Recl. Trav. Chim. Pays-Bas* **1959**, *78*, 59.
(38) Coe, D. G.; Perry, B. J.; Brown, R. K. *J. Chem. Soc.* **1957**, 3604.
(39) Pelchowicz, Z. *J. Chem. Soc.* **1961**, 238.
(40) Maier, L. *Phosphorus, Sulfur Silicon* **1990**, *47*, 465.
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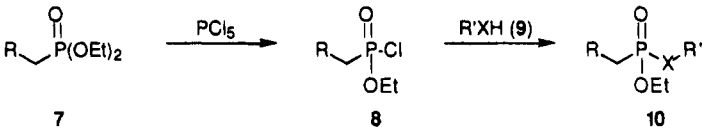
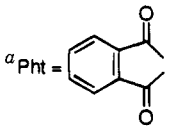
Table 2. Coupling of Polyfunctional Alcohols and Amines with Simple Phosphonochloridates

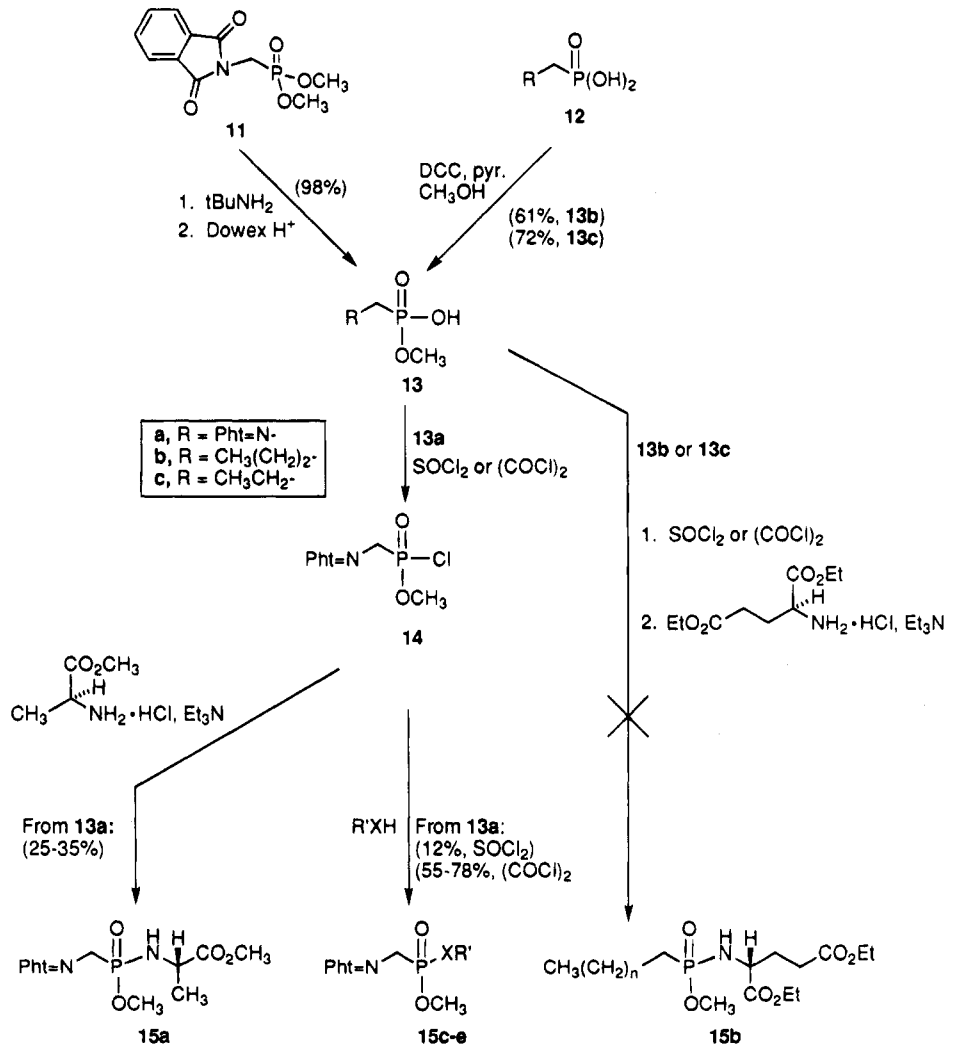


R	[8]:[R'XH]	R'XH	yield (%)
CH_3 (**7a**)	6.4	$HO(CH_2)_3N(Ts)(CH_2)_4N_3$ (**9a**)	94 (**10a**)
Pht=N[a] (**7b**)	2.0	$HO(CH_2)_3N(Ts)(CH_2)_4N_3$ (**9a**)	69 (**10b**)
Pht=N (**7b**)	1.4	$HO(CH_2)_3N(Ts)(CH_2)_4N_3$ (**9a**)	95 (**10b**)
Pht=N (**7b**)	2.0	$H_2N(CH_2)_4N$=Pht (**9b**)	53 (**10c**)
Pht=N (**7b**)	1.0	$H_2NCH(CH_3)CO_2CH_3$ (**9c**)	66 (**10d**)

[a] Pht =



Scheme 4



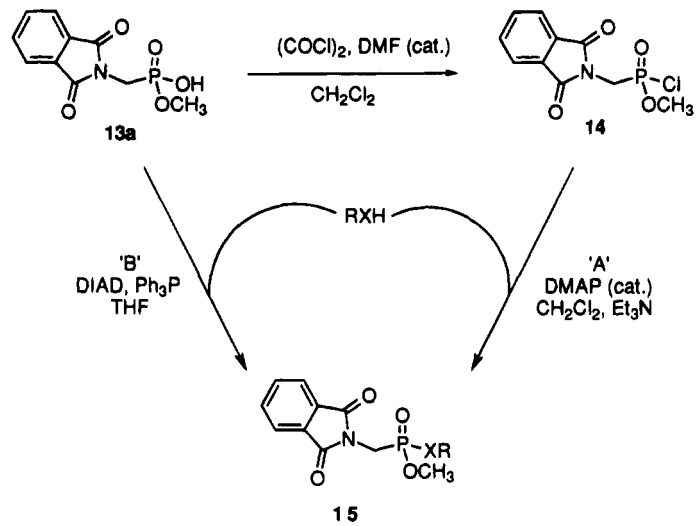
(See Scheme 5)

conversion to the corresponding phosphonochloridate was seen by ^{31}P NMR analysis, but reaction with L-glutamate diethyl ester failed to yield any of the phosphonamidate product. A third attempt at phosphonochloridate formation was performed on monomethyl (phthalimidomethyl)-phosphonic acid (**13a**, Scheme 4), obtained from dimethyl phthalimidomethylphosphonate, **11**, by selective demethylation with t-butyl amine.[46] Two different chlorinating agents, $SOCl_2$ and $(COCl)_2$,[45,47] were studied with **13a** and both proved successful, affording 25 and 35% yield, respectively, of the coupled product (**15a**) with L-alanine methyl ester. This provided two milder alternative methods to phosphonochloridates leading to the desired phosphonamidates, albeit in low overall yields. Optimization of this two-step process was investigated with

(46) Gray, M. D. M.; Smith, D. J. H. *Tetrahedron Lett.* **1980**, *21*, 859.

(47) Biller, S. A.; Forster, C. *Tetrahedron* **1990**, *46*, 6645.

Scheme 5



RXH	Yield, Method 'A'[a]	Yield, Method 'B'	Product
$CH_3(CH_2)_3OH$	55%	56%	**15c**
$N_3(CH_2)_4N(Ts)(CH_2)_3OH$ (**9a**)	72%	62%	**15d**
$N_3(CH_2)_4N(Ts)(CH_2)_3NH_2$ (**9d**)	78%	n.d.	**15e**

[a]For method 'A', yields given are for two steps from **13a** to **15**

monomethyl phthalimidomethylphosphonic acid.[48] Conditions were optimized by monitoring the formation of phosphonochloridate, **14**, by ^{31}P NMR and then by coupling this phosphonochloridate with **9a** under identical conditions and comparing yields of phosphonate, **15d** (Schemes 4 and 5). The phosphonochloridate generation step was modified by the use of $TMSNEt_2$ along with $(COCl)_2$[47] and $SOCl_2$,[41-44] but neither proved as effective as direct treatment with $(COCl)_2$. Two basic alterations were made in the coupling conditions; i.e., performing the reaction at 5 °C and using pyridine as the reaction medium,[45] but these procedures both provided a lower yield of the coupling product. Reaction of amine **9d**[49] with **14** afforded the phosphonamidate **15e** in good yield (Scheme 5).

Although primary alcohols were efficiently coupled under the optimized conditions, similar success was not realized with secondary alcohols. For example, when isopropyl alcohol or diethyl 2-hydroxyglutarate were used as nucleophiles, the coupling reaction did not occur. It appears that steric hindrance in the nucleophile can significantly hamper this reaction. However, during the course of this research, a paper was published which provided an alternate method for the synthesis of phosphonates from secondary alcohols via a variant of the Mitsunobu reaction.[50] As will be discussed in the accompanying paper,[24] we have employed this method for the successful coupling of secondary alcohols with phosphonic acid monomethyl esters. At this point, however, we wished to compare our optimized conditions for coupling via a phosphonochloridate (Scheme 5, method A) with the Mitsunobu approach (Scheme 5, method B). As shown in the data of Scheme 5, the two methods offer comparable yields in the case of primary alcohols. The Mitsunobu variant offers a clear advantage, however, in the case of secondary alcohols.[24]

Discussion

The research described in this and the accompanying paper[24] indicates that the generation and coupling of phosphonochloridates is somewhat idiosyncratic and that success is assured in complex systems only when mild conditions are employed. The reactions explored in our experiments are summarized in Scheme 6. In the case of simple aliphatic compounds such as diisopropyl propylphosphonate or diethyl ethylphosphonate, PCl_5 served as an excellent reagent for the direct generation of the phosphonochloridate (Scheme 2, Table 1). Even with molecules that contain some functionality, such as diethyl phthalimidomethylphosphonate (Table 1), the PCl_5 method was very effective. In our hands, however, the PCl_5 method was not successful with less stable phosphonate esters such as methyl or benzyl, despite the fact that the phosphonochloridate was cleanly generated in some cases; e.g., **14**. Nor was this technique effective with more complex molecules, as will be discussed in the accompanying paper.[24] Another drawback of this method involves its use in the context of a total synthesis. When the removal of the relatively more stable ethyl esters is desired after the coupling reaction, the literature[31,32,51]

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

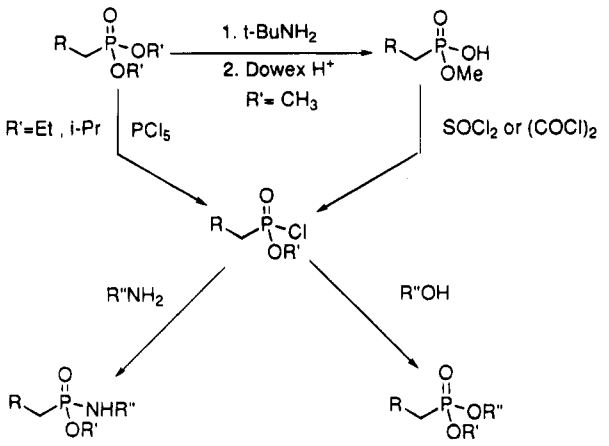
(49) Synthesis of **9d** from **9a**[35] was straightforward: RCH_2OH (**9a**) → RCH_2OMs (**16**) → RCH_2N=Pht (**17**) → RCH_2NH_2 (**9d**). Experimental details are given in the supplementary material.

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

(51) Yamauchi, K.; Ohtsuki, S.; Kinoshita, M. *J. Org. Chem.* **1984**, *49*, 1158.

clearly indicates this is a difficult procedure and we have confirmed this in our own research.[48]

Scheme 6



The more mild chlorinating agent, $(COCl)_2$, which generated the phosphonochloridate from monomethyl phosphonic acids, effectively resolved these problems. When optimized, this method (Schemes 4 and 5) provided a very good yield of a complex phosphonate **15d** (72%) and phosphonamidate **15e** (78%). The application of this method to the synthesis of **15** consistently provided yields in the 65–70% range. This is a slight improvement over similar phosphonate syntheses in the literature[10,41-43,45] which have reported yields in the 40–60% range. In all but one of these reported phosphonate syntheses, $SOCl_2$ was the chlorinating agent used to generate the phosphonochloridate. In our hands, $(COCl)_2$ was superior to $SOCl_2$ in the generation and coupling of the phosphonochloridate, **14** (Scheme 4). The results with amine nucleophiles to provide phosphonamidates appear to indicate the same trend, as witnessed in the reaction of L-alanine methyl ester with **14**. When $(COCl)_2$ was used to generate the phosphonochloridate the yield was better than when $SOCl_2$ was used (Scheme 4). These direct comparisons further support the utility of the $(COCl)_2$ method for the generation of phosphonochloridates and subsequent coupling to amines and alcohols.

Experimental Section

General Procedures. All reactions involving reagents sensitive to moisture were conducted under an atmosphere of argon with oven-dried glassware. 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; 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. Column chromatography was performed with Silica gel 60 (230–400 mesh) and according to the protocol of Still.[52] 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. When appropriate, carbon–phosphorous coupling is reported with the chemical shift data (multiplicity, coupling constants in Hertz). ^{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 analyses were obtained from Atlantic Microlab Inc. at Norcross, Ga. or at the Elemental Analysis Labs, Department of Chemistry, University of Michigan.

The phosphonochloridates, **3**,[37] **8a**,[34,53] and **8b**[32] were prepared from the corresponding dialkyl esters by reaction with PCl_5 as previously described in the literature. Alcohol **9a** was synthesized by John Lakanen, Department of Chemistry, University of Michigan.[35]

General Procedure for Reaction of Amine or Alcohol Nucleophiles with Isopropyl Propylphosphonochloridate. Isopropyl propylphosphonochloridate, **3**, (1.0 equiv) was dissolved in CH_3CN. The amine·HCl or alcohol (1.5 equiv) was added, followed immediately by the addition of Et_3N (2.5 equiv for amine·HCl, 1.5 equiv for alcohol). The reaction was left stirring for 1.5 h at rt and then lowered to 0 °C and quenched with MeOH. Purification by silica gel flash chromatography (eluant: EtOAc) afforded the desired product as an oil.

Benzyl Isopropyl Propylphosphonate (5a). The phosphonochloridate **3** (1.21 g, 6.50 mmol) was coupled with BnOH (1.08 g, 9.99 mmol) in CH_3CN (2.5 mL) with Et_3N (0.962 g, 9.51 mmol) to afford 0.914 g (55% yield) of **5a**. The reaction with BnOH was stirred for 42 h at rt before being quenched and the column chromatography eluant was EtOAc/hexane (2:1). TLC R_f = 0.12 (1:1 EtOAc/hexane). ^1H NMR ($CDCl_3$) δ 7.40–7.30 (m, 5), 5.10–4.95 (m, 2), 4.74–4.62 (m, 1), 1.76–1.55 (m, 4), 1.28 (dd, 6, J = 6, 12 Hz), 0.99 (t, 3, J = 6 Hz). ^{13}C NMR ($CDCl_3$) δ 136.4, 128.3, 128.0, 127.5, 69.9 (d, J = 6 Hz), 66.4 (d, J = 6 Hz), 28.3 (d, J = 140 Hz), 23.8, 15.9 (d, J = 5 Hz), 15.0 (d, J = 18 Hz). ^{31}P NMR ($CDCl_3$) δ 29.4. MS (EI, 70, rel. intensity) *m/e* 256 (6.2, M^+), 214 (28.5), 123 (10.6), 107 (43.8), 96 (27.4), 92 (12.0), 91 (100). HRMS (EI, 70) calcd for $C_{13}H_{21}O_3P$ (M^+) 256.1228, found 256.1230.

Phenyl Isopropyl Propylphosphonate (5b). The phosphonochloridate **3** (0.719 g, 3.90 mmol) was coupled with phenol (0.550 g, 5.84 mmol) in CH_3CN (4 mL) with Et_3N (0.540 g, 5.34 mmol) to afford 0.415 g (44% yield) of **5b**. Column chromatography eluant was hexane/EtOAc (4:1). TLC R_f = 0.39 (EtOAc). ^1H NMR ($CDCl_3$) δ 7.38–7.11 (m, 5), 4.87–4.75 (m, 1), 1.93–1.68 (m, 4), 1.30 (dd, 6, J = 9, 27 Hz), 1.05 (t, 3, J = 9 Hz). ^{13}C NMR ($CDCl_3$) δ 150.5 (d, J = 8 Hz), 129.5, 124.5, 120.4 (d, J = 4 Hz), 71.0 (d, J = 7 Hz), 28.2 (d, J = 140 Hz), 23.8 (d, J = 27 Hz), 16.0 (d, J = 5 Hz), 15.1 (d, J = 18 Hz). ^{31}P NMR ($CDCl_3$) δ 26.0. MS (EI, 70, rel. intensity) 242 (28.4), 227 (16.6), 200 (16.3), 185 (15.1), 172 (18.1), 118 (27.5), 94 (100). HRMS (EI, 70) calcd for $C_{12}H_{19}O_3P^+$ (M^+) 242.1072, found 242.1080.

***N*-(Methyl-L-alanyl) Isopropyl Propylphosphonamidate (5c).** The phosphonochloridate **3** (1.38 g, 7.50 mmol) was coupled with L-alanine hydrochloride, methyl ester (1.56 g, 11.2 mmol) in CH_3CN (8 mL) with Et_3N (1.94 g, 19.2 mmol) to afford 1.25 g (67% yield) of **5c**. TLC R_f = 0.33 (EtOAc). ^1H NMR δ 4.75–4.62 (m, 1), 4.10–3.99 (m, 1), 3.74 (s, 3), 3.10–3.00 (bs, 1), 1.72–1.54 (m, 4), 1.40 (t, 3, J = 6 Hz), 1.35–1.22 (m, 6), 1.00 (t, 3, J = 6 Hz). ^{13}C NMR ($CDCl_3$) δ 174.7, 68.2 (d, J = 7 Hz), 51.9, 49.2 (d, J = 7 Hz), 30.7 (d, J = 140 Hz), 23.9 (d, J = 10 Hz), 21.3 (d, J = 4 Hz), 16.0, 15.1 (d, J = 18 Hz). ^{31}P NMR ($CDCl_3$) δ 32.9, 32.5 (diastereomers). MS (EI, 70, rel. intensity) *m/e* 251 (4.1, M^+), 208 (6.3), 192 (41.8), 166 (13.7), 150 (100), 44 (74.1). HRMS (EI, 70) calcd for $C_{10}H_{23}NO_4P^+$ (MH^+) 252.1365, found 252.1349.

***N*-(Diethyl-L-glutamyl) Isopropyl Propylphosphonamidate (5d).** The phosphonochloridate **3** (0.795 g, 4.31 mmol) was coupled with L-glutamic acid hydrochloride, diethyl ester

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

(53) Sasse, K. In *Methoden der Organischen Chemie (Houben-Weyl)*; Muller, E., Ed.; Georg Thieme Verlag: Stuttgart, 1963; Vol. XII; pp 416.

(1.35 g, 5.63 mmol) in CH_3CN (8.5 mL) with Et_3N (0.868 g, 8.58 mmol) to afford 0.937 g (62% yield) of **5d**. TLC R_f = 0.36 (EtOAc). ^1H NMR ($CDCl_3$) δ 4.67–4.53 (m, 1), 4.13 (q, 2, J = 4,7 Hz), 4.07 (q, 2, J = 4,7 Hz), 3.98–3.85 (m, 1), 3.67–3.52 (m, 1), 2.44–2.31 (m, 2), 2.10–1.82 (dm, 2), 1.63–1.48 (m, 4), 1.26–1.15 (m, 12), 0.95 (t, 3, J = 7 Hz). ^{13}C NMR ($CDCl_3$) δ 173.1, 172.2, 68.1 (t, J = 7 Hz), 60.9, 60.0, 52.7 (d, J = 15 Hz), 31.0, 29.6, 23.9, 23.6, 15.7, 14.9 (d, J = 18 Hz), 13.7. ^{31}P NMR ($CDCl_3$) δ 33.4, 32.9 (diastereomers). MS (EI, 70, rel. intensity) *m/e* 351 (1.4, M^+), 278 (39.3), 264 (14.1), 236 (21.5), 190 (17.2), 162 (22.3), 130 (19.3), 84 (100). HRMS (EI, 70) calcd for $C_{15}H_{30}NO_6P^+$ (M^+) 351.1811, found 351.1809.

General Procedure for Reaction of Amine or Alcohol Nucleophiles with Ethyl Alkylphosphonochloridates. The amine or alcohol (1.0 equiv) was dissolved in $CHCl_3$ and cooled to 0 °C. Et_3N (3.0 equiv for amine hydrochloride salt or 2.0 equiv for alcohol) was added, followed immediately by the dropwise addition of the ethyl alkylphosphonochloridate (**8a** or **8b**) (1.4 equiv) in THF at 0 °C. The reaction was allowed to warm to rt and stirred overnight. The reaction solution was filtered and the filtrate concentrated in vacuo to remove THF. The resulting concentrate was diluted with $CHCl_3$, washed with 5% $NaHCO_3$ and H_2O, dried with Na_2SO_4, and concentrated in vacuo. Further purification was achieved by silica gel flash column chromatography (eluant: EtOAc).

3-[*N*-(*p*-Toluenesulfonyl)-*N*-(4′-azidobutyl)amino]propyl Ethyl Ethylphosphonate (10a). The alcohol **9a** (0.039 g, 0.119 mmol) was coupled with ethyl ethylphosphonochloridate (**8a**) (0.120 g, 0.766 mmol) in $CHCl_3$ (2 mL) with Et_3N (0.042 g, 0.42 mmol). Purification by silica gel flash column chromatography (eluant $CHCl_3$/MeOH (95:5)) afforded 0.050 g (94% yield) of **10a**. TLC R_f = 0.55 (95:5 $CHCl_3$/MeOH); ^1H NMR ($CDCl_3$) δ 7.70–7.30 (dd, 4), 4.15–3.95 (m, 4), 3.31 (t, 2, J = 7 Hz), 3.21 (t, 2, J = 7 Hz), 3.14 (t, 2, J = 7 Hz), 2.43 (s, 3), 1.99–1.89 (m, 2), 1.76 (dq, 2, J = 7, 14, 22 Hz), 1.77–1.52 (m, 4), 1.33 (t, 3, J = 7 Hz), 1.17 (dt, 3, J = 7, 18 Hz). ^{31}P NMR ($CDCl_3$) δ 31.6.

3-[*N*-(*p*-Toluenesulfonyl)-*N*-(4′-azidobutyl)amino]propyl Ethyl Phthalimidomethylphosphonate (10b). The phosphonochloridate **8b** (1.93 g, 6.72 mmol) was dissolved in THF (30 mL) and reacted with 1-oxy-3-N-(*p*-toluenesulfonyl)-3-*N*-(4′-azidobutyl)propylamine (**9a**) (1.58 g, 4.84 mmol) dissolved in $CHCl_3$ (25 mL) with Et_3N (1.04 g, 10.3 mmol). Following extractive workup, **10b** was obtained as a light yellow oil (2.66 g, 95% yield). TLC R_f = 0.35 (EtOAc); ^1H NMR ($CDCl_3$) δ 7.87–7.73 (dm, 4), 7.49 (dd, 4), 4.24–4.08 (m, 4), 4.10 (d, 2, J = 11 Hz), 3.29 (t, 2, J = 7 Hz), 3.21 (t, 2, J = 7 Hz), 3.13 (t, 2, J = 7 Hz), 2.43 (s, 3), 2.00–1.90 (m, 2), 1.67–1.56 (m, 4), 1.33 (t, 3, J = 7 Hz). ^{13}C NMR ($CDCl_3$) δ 166.8, 143.2, 136.3, 134.1, 131.8, 129.6, 127.0, 123.4, 63.6 (d, J = 5 Hz), 63.0 (d, J = 5 Hz), 50.8, 48.3, 45.2, 33.0 (d, J = 156 Hz), 30.1, 30.0, 25.7 (d, J = 18 Hz), 21.3, 16.2. ^{31}P NMR ($CDCl_3$) δ 17.2. MS (CI w/NH_3, rel. intensity) *m/e* 595 (9.7, $[M + NH_4]^+$), 578 (12.8, MH^+), 377 (21.7), 327 (33.7), 315 (24.3), 298 (78.9), 287 (84.9), 270 (63.2), 174 (100), 153 (45), 139 (59.3). HRMS (CI w/NH_3) calcd for $C_{25}H_{33}N_5O_7PS^+$ (MH^+) 578.1838, found 578.1818. Anal. Calcd for $C_{25}H_{32}N_5O_7PS \cdot 0.75H_2O$: C, 50.79; H, 5.71; N, 11.85. Found: C, 50.85; H, 5.48; N, 11.89.

***N*-(4-*N*-Phthaloyl-1,4-diaminobutyl) Ethyl (Phthalimidomethyl)phosphonamidate (10c).** The phosphonochloridate **8b** (0.350 g, 1.22 mmol) was dissolved in THF (4 mL) and reacted with 4-phthalimidobutylamine hydrochloride (**9b**) (0.155 g, 0.608 mmol) dissolved in $CHCl_3$ (3.5 mL) with Et_3N (0.280 g, 2.77 mmol). Following extractive workup, **10c** was obtained as a white crystalline product (0.152 g, 53% yield), mp = 147–149 °C. TLC R_f = 0.14 (EtOAc); ^1H NMR ($CDCl_3$) δ 7.86–7.70 (dm, 8), 4.17–4.00 (m, 4), 3.69 (t, 2, J = 7 Hz), 3.08–3.00 (m, 2), 2.93–2.83 (m, 1), 1.74–1.67 (m, 2), 1.58–1.53 (m, 2), 1.28 (t, 3, J = 7 Hz). ^{13}C NMR ($CDCl_3$) δ 168.3, 167.3, 134.1, 133.9, 132.1, 131.9, 123.5, 123.2, 61.3, 40.1, 37.5, 35.0 (d, J = 144 Hz), 29.2, 25.7, 16.3. ^{31}P NMR ($CDCl_3$) δ 21.0. MS (rel. intensity, EI, 70) *m/e* 469 (0.4, M^+), 424 (0.3), 321 (7.9), 252 (45.3), 217 (100), 201 (22.1), 160 (52.4), 84 (40.3), 49 (51.9). HRMS (EI, 70) calcd for $C_{23}H_{24}N_3O_2P$ (M^+) 469.1403, found 469.1398. Anal. Calcd for $C_{23}H_{24}N_3O_2P \cdot H_2O$: C, 56.66 H, 5.38; N, 8.62. Found: C, 56.99; H, 5.03; N, 8.64.

***N*-[Methyl-L-alanyl] Ethyl Phthalimidophosphonamidate (10d).** The phosphonochloridate **8b** (0.967 g, 3.36 mmol) was dissolved in THF (7 mL) and reacted with L-alanine hydrochloride, methyl ester (**9c**) (0.469 g, 3.36 mmol) dissolved in $CHCl_3$ (15 mL) with Et_3N (4.2 mL). Following extractive workup, **10d** was obtained as a white solid (0.79 g, 66% yield), mp = 118–125 °C. TLC R_f = 0.21 (EtOAc); ^1H NMR ($CDCl_3$) δ 7.90–7.70 (dm, 4), 4.27–4.00 (m, 4), 3.71 (d, 3, J = 4 Hz), 3.70–3.40 (m, 1), 3.08 (q, 1, J = 6, 12 Hz), 1.41 (t, 3, J = 6 Hz), 1.35–1.24 (m, 3). ^{31}P NMR ($CDCl_3$) δ 20.3, 19.7 (diastereomers).

Methyl (Phthalimidomethyl)phosphonic Acid (13a). Dimethyl (phthalimidomethy)phosphonate (**11**) (3.0 g, 0.011 moles) was suspended in t-$BuNH_2$ (29 mL, 0.28 moles) and the mixture was refluxed for 24 h. The reaction was concentrated to provide 3.6 g (quantitative yield) of the white t-butylamine salt of monomethyl phthalimidomethylphosphonate, mp = 197–205 °C. TLC R_f = 0.24 (4:1 $CHCl_3$/MeOH); ^1H NMR (D_2O) δ 7.73–7.60 (dm, 4), 3.72 (d, 2, J = 14 Hz), 3.44 (d, 3, J = 11 Hz), 1.18 (s, 9). ^{13}C NMR (D_2O) δ 169.5, 134.7, 131.4, 123.5, 52.1 (d, J = 14 Hz), 33.8 (d, J = 150 Hz), 27.7, 26.7. ^{31}P NMR (D_2O) δ 17.0. IR (KBr) 1044, 1062, 1188, 1200, 1711, 2400–3200 cm^{-1}. MS (EI, 70, rel. intensity) *m/e* 255 (12.0, M^+-Bu^tNH_2), 160 (100), 104 (18.1), 77 (20.2), 76 (20.7), 58 (40.0). HRMS (EI, 70) calcd for $C_{10}H_{10}NO_5P$ (M^+-Bu^tNH_2) 255.0297, found 255.0314. Anal. Calcd for $C_{14}H_{21}N_2O_5P \cdot 0.5\ H_2O$: C, 49.85; H, 6.57; N, 8.31. Found: C, 49.51; H, 6.83; N, 7.93.

The free acid was generated from the t-butyl amine salt (0.513 g, 1.56 mmol) by treatment with cation exchange resin (Dowex 50W-X8 (H^+ form) 200–400 mesh, 2.2 g (dry)) in MeOH. Filtration removed the resin and the filtrate was concentrated to afford 0.393 g (98% yield) of methyl (phthalimidomethyl)phosphonic acid, **13a**, as a white salt. mp = 183–190 °C. ^1H NMR (D_2O) δ 7.75–7.60 (dm, 4), 3.74 (d, 2, J = 11 Hz), 3.43 (d, 3, J = 10 Hz). ^{13}C NMR (D_2O) δ 169.5, 134.8, 131.4, 123.5, 52.2, 33.7 (d, J = 150 Hz). ^{31}P NMR (D_2O) δ 17.2. IR 1037, 1200, 1250, 1720 cm^{-1}. MS (EI, 70, rel. intensity) *m/e* 255 (14.0, M^+), 161 (49.8), 160 (100), 104 (17.1), 91 (15.8), 77 (20.1), 76 (20.1). HRMS (EI, 70) calcd for $C_{10}H_{10}NO_5P$ (M^+) 255.0297, found 255.0289.

Methyl (Phthalimidomethyl)phosphonochloridate (14). A. $SOCl_2$ Method. Thionyl chloride (0.124 g, 1.04 mmol) was added to methyl phthalimidomethylphosphonic acid (**13a**) (0.113 g, 0.443 mmol) suspended in $CHCl_3$ (8 mL) at rt. This mixture was stirred for 4 h, until the reaction solution became homogeneous. The reaction was concentrated in vacuo, diluted with $CHCl_3$, and reconcentrated to remove all volatile contaminants. The phosphonochloridate was immediately reacted with amines or alcohols as described below. ^{31}P NMR ($CDCl_3$) δ 30.5.

B. $(COCl)_2$ Method. Methyl phthalimidomethylphosphonic acid (**13a**) (0.630 g, 2.47 mmol) was suspended in CH_2Cl_2 (20 mL) and the mixture was cooled to 0 °C. DMF (0.007 g, 0.1 mmol) was added, followed by dropwise addition of oxalyl chloride (0.359 g, 2.83 mmol). The reaction was stirred at 0 °C for 20 min. and then allowed to warm to rt and stirred for 1.5 h as the reaction solution became homogeneous. The reaction was concentrated, diluted with toluene (2 mL), and then reconcentrated in vacuo to provide the product as a white salt. The phosphonochloridate was immediately reacted with an amine or an alcohol. ^1H NMR ($CDCl_3$) δ 7.95–7.75 (dm, 4), 4.47 (d, 2, J = 7 Hz), 3.94 (d, 3, J = 13 Hz). ^{13}C NMR ($CDCl_3$) δ 166.3, 134.5, 131.6, 123.8, 53.8 (d, J = 8 Hz), 39.6 (d, J = 144 Hz). ^{31}P NMR ($CDCl_3$) δ 30.5.

General Procedure for Reaction of Amine or Alcohol Nucleophiles with Methyl (Phthalimidomethyl)phosphonochloridate. The amine or alcohol (1.0 equiv) was dissolved in $CHCl_3$ or CH_2Cl_2 and cooled to 0 °C. Triethylamine (3.0 equiv for an amine hydrochloride salt, 2.0 equiv for a free amine or an alcohol) and DMAP were added to the reaction, followed immediately by the dropwise addition of the phosphonochloridate **14** (1.2 equiv), dissolved in $CHCl_3$ or CH_2Cl_2. The reaction was allowed to warm to rt and stirred overnight. The reaction solution was then concentrated in vacuo, diluted with EtOAc, filtered to remove Et_3N·HCl, washed with saturated $NaHCO_3$ and H_2O, dried with Na_2SO_4 and concentrated to

afford the desired product. Silica gel flash column chromatography of this material provided pure product.

***N*-[Methyl-L-alanyl] Methyl Phthalimidophosphonamidate (15a).** A. The phosphonochloridate **14** (0.216 g, 0.789 mmol), prepared by the $SOCl_2$ method, was dissolved in $CHCl_3$ (3 mL) and added dropwise to L-alanine hydrochloride, methyl ester (0.110 g, 0.789 mmol) in $CHCl_3$ (3 mL) with Et_3N (0.399 g, 3.94 mmol) at 0 °C (Note: DMAP was not used) to give **15a**, a yellowish white solid (0.068 g, 25% yield). This product was spectroscopically identical to the sample prepared by the $(COCl)_2$ method described below.

B. The phosphonochloridate **14** (0.220 g, 0.803 mmol), prepared by the $(COCl)_2$ method, was reacted in $CHCl_3$ (6 mL) with L-alanine hydrochloride, methyl ester (**9c**) (0.117 g, 0.839 mmol) and Et_3N (0.319 g, 3.15 mmol) at 0 °C (Note: DMAP was not used) to afford **15a**, a yellowish white solid (0.097 g, 35% yield), mp = 127–142 °C . TLC R_f = 0.17 (EtOAc); ^1H NMR ($CDCl_3$) δ 7.90–7.72 (dm, 4), 4.23–4.01 (m, 3), 3.88–3.69 (m, 6), 3.65–3.40 (m, 1), 1.43 (t, 3, J = 6 Hz). ^{13}C NMR ($CDCl_3$) δ 174.1, 167.0, 134.0, 131.6, 123.3, 52.1, 51.4 (dd, J = 6, 25 Hz), 49.1, 35.1 (dd, J = 33, 142 Hz), 21.1 (d, J = 32 Hz). ^{31}P NMR ($CDCl_3$) δ 21.6, 21.0 (diastereomers). MS (CI w/NH_3) 341 (MH^+, 100), 307 (4.7), 281 (13.8), 255 (17.9), 238 (24.3), 104 (58.4). HRMS (CI w/NH_3) calcd for $C_{14}H_{18}N_2O_6P$ (MH^+) 341.0902, found 341.0894. Anal. Calcd for $C_{14}H_{17}N_2O_6P$: C, 49.40; H, 5.04; N, 8.23. Found: C, 49.65; H, 5.26; N, 8.31.

Butyl Methyl Phthalimidomethylphosphonate (15c). The phosphonochloridate **14** (0.895 g, 3.27 mmol), prepared by the $(COCl)_2$ method, was coupled with n-butanol (0.213 g, 2.87 mmol) in CH_2Cl_2 (25 mL) with Et_3N (0.681 g, 6.73 mmol) and DMAP (0.017 g, 0.14 mmol). Silica gel column chromatography (elutant: EtOAc) provided the phosphonate product as a viscous opaque oil in 55% yield (0.491 g). TLC R_f = 0.38 (EtOAc). ^1H NMR ($CDCl_3$) δ 7.90–7.70 (dm, 4), 4.20–4.00 (m, 2), 4.12 (d, 2, J = 11 Hz), 3.84 (d, 3, J = 11 Hz), 1.70–1.60 (m, 2), 1.45–1.30 (m, 2), 0.91 (t, 3, J = 7 Hz). ^{13}C NMR ($CDCl_3$) δ 166.8, 134.1, 131.8, 123.4, 66.5 (d, J = 5 Hz), 52.9 (d, J = 5 Hz), 32.6 (d, J = 150 Hz), 32.3 (d, J = 5 Hz), 18.5, 13.4. ^{31}P NMR ($CDCl_3$) δ 18.4. IR (neat)1024, 1046, 1248, 1721 cm^{-1}. MS (CI w/NH_3, rel. intensity) *m/e* 313 (21.5, $[M + 2H]^+$), 312 (100, MH^+), 256 (12.7). HRMS (CI w/NH_3) calcd for $C_{14}H_{29}NO_5P^+$ (MH^+) 312.1001, found 312.0095. Anal. Calcd for $C_{14}H_{18}NO_5P{\cdot}0.25\ H_2O$: C, 53.24; H, 5.91; N, 4.44. Found: C, 53.41; H, 5.88; N, 4.44.

3-[*N*-(*p*-Toluenesulfonyl)-*N*-(4′-azidobutyl)amino]propyl Methyl (Phthalimidomethyl)phosphonate (15d). A. The phosphonochloridate **14** (0.079 g, 0.29 mmol), prepared by the $SOCl_2$ method, was dissolved in CH_2Cl_2 (7 mL) and added dropwise to 1-oxy-3-[*N*-(*p*-toluenesulfonyl)-3-*N*-(4′-azidobutyl)propylamine (**9a**), (0.074 g, 0.23 mmol) in CH_2Cl_2 (5 mL) with Et_3N (0.042 g, 4.64 mmol) and DMAP (0.002 g, 0.02 mmol) to afford a yellow oil. Silica gel flash column chromatography of this oil (gradient eluant: hexane, EtOAc:hexane (2:1), and EtOAc) provided pure **14d** (0.014 g, 12% yield). This material was spectroscopically identical to the sample prepared by the $(COCl)_2$ method described below.

B. The phosphonochloridate **14** (0.045 g, 0.16 mmol), prepared by the $(COCl)_2$ method, was coupled with 1-oxy-3-*N*-(*p*-toluenesulfonyl)-3-*N*-(4′-azidobutyl)propylamine (**9a**) (0.038 g, 0.12 mmol) in CH_2Cl_2 (5 mL) with Et_3N (0.042 g, 4.64 mmol) and DMAP (0.001 g, 0.008 mmol). Silica gel flash column chromatography (gradient eluant: EtOAc/hexane (2:1), EtOAc/hexane (3:1), EtOAc/hexane (4:1)) of the concentrated reaction material provided the product as a yellow oil in 72% yield (0.047 g). TLC R_f = 0.34 (EtOAc). ^1H NMR ($CDCl_3$) δ 7.95–7.75 (dm, 4), 7.75–7.30 (dd, 4), 4.30–4.10 (m, 2), 4.13 (d, 2, J = 11 Hz), 3.87 (d, 3, J = 11 Hz), 3.32 (t, 2, J = 5 Hz), 3.22 (t, 2, J = 7 Hz), 3.20–3.10 (m, 2), 2.46 (s, 3), 2.05–1.90 (m, 2), 1.70–1.55 (m, 4). ^{13}C NMR ($CDCl_3$) δ 166.9, 143.4, 136.3, 134.3, 131.8, 129.7, 127.2, 123.6, 63.9 (d, J = 6 Hz), 53.4, 50.9, 48.5, 45.3, 32.7 (d, J = 150 Hz), 30.3 (d, J = 6 Hz), 26.0, 25.8, 21.5. ^{31}P NMR ($CDCl_3$) δ 18.7. IR (neat)1025, 1027, 1031, 1158, 1721, 2096 cm^{-1}. MS (DCI w/NH_3, rel. intensity) *m/e* 581(100, $[M + NH_4]^+$), 564 (78.6, MH^+), 536 (12.8), 408 (28.7), 309 (64.0), 270 (36.1), 256 (23.9), 153 (46.1). HRMS (DCI w/NH_3) calcd for $C_{24}H_{31}N_5O_7PS^+$ (MH^+) 564.1682, found 564.1650.

***N*-[3-*N*-(*p*-Toluenesulfonyl)-3-*N*-(4′-azidobutyl)-1,3-diaminopropyl] Methyl Phthalimidomethylphosphonamidate (15e).** The phosphonochloridate **14** (0.127 g, 0.464 mmol) was coupled with 1-amino-3-*N*-(*p*-toluenesulfonyl)-3-*N*-(4′-butylazido)propylamine (**9d**) (0.126 g, 0.387 mmol) in CH_2Cl_2 (8 mL) with Et_3N (0.118 g, 1.16 mmol) and DMAP (0.002 g, 0.019 mmol). Silica gel column chromatography (elutant: EtOAc) provided the phosphonamidate product **15e** as a white solid (0.170 g, 78% yield), mp = 100–103 °C. TLC R_f = 0.12 (EtOAc). ^1H NMR ($CDCl_3$) δ 7.90–7.70 (dm, 4), 7.70–7.25 (dd, 4), 4.20–4.00 (m, 2), 3.76 (d, 3, J = 9 Hz), 3.29 (t, 2, J = 6 Hz), 3.40–3.05 (m, 9) , 2.44 (s, 3), 1.85–1.65 (m, 2), 1.65–1.50 (m, 4). ^{13}C NMR ($CDCl_3$) δ 167.3, 143.4, 136.2, 134.2, 131.9, 129.7, 127.1, 123.5, 51.7 (d, J = 6 Hz), 50.9, 48.4, 45.7, 37.3, 34.6 (d, J = 140 Hz), 31.1 (d, J = 5 Hz), 26.0, 26.0, 21.5. ^{31}P NMR ($CDCl_3$) δ 22.9. IR (KBr) 1048, 1305, 1333, 1378, 1717, 2099 cm^{-1}. MS (DCI w/NH_3, rel. intensity) *m/e* 563 (100, MH^+), 522 (31.1), 407 (20.3), 326 (27.8), 255 (44.6), 139 (21.0). HRMS (DCI w/NH_3) calcd for $C_{24}H_{32}N_6O_6SP^+$ (MH^+) 563.1842, found 563.1816. Anal. Calcd for $C_{24}H_{31}N_6O_6PS{\cdot}0.75\ H_2O$: C, 50.04; H, 5.69; N, 14.59. Found: C, 50.14; H, 5.62; N, 14.29.

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Supplementary Material Available: Procedures for the synthesis of **3**, **8a**, **8b**, and **9d** with complete spectral data. ^1H NMR spectra for **5a**, **5b**, **5c**, **5d**, and **10a** (8 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.