Investigations on New Strategies for the Facile Synthesis of Polyfunctionalized Phosphinates: Phosphinopeptide Analogues of Glutathionylspermidine

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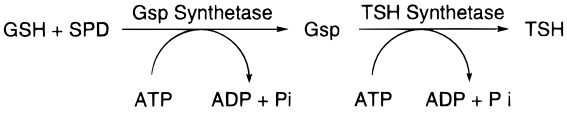
Three possible methods for the facile synthesis of functionalized phosphinates, including the core of complex phosphinopeptide analogues of glutathionylspermidine, were explored. Among these methods, the three-component condensation reaction involving benzyl carbamate, an aldehyde, and a functionalized or nonfunctionalized phosphonite can afford a variety of protected α-aminophosphinates. However, polyamine-containing α-(aminomethylene)phosphinates can be obtained only by the acid-catalyzed Pudovik−Abramov-type reaction of a polyamine-containing phosphonite with a tritylamine-derived Schiff base. Thus, despite its hydrolytic lability, a Tfa-protected polyamine-containing phosphonite reacts smoothly with tritylmethanimine and affords the corresponding phosphinate, a key intermediate for the synthesis of complex phosphinopeptide inhibitors of glutathionylspermidine synthetase. Elaboration of this intermediate via selective deprotection and coupling to a protected dipeptide provided an alanine-containing phosphinate analogue of glutathionylspermidine. The limitations and scope of the three explored methods are discussed.

Introduction

Recently, a conjugate of glutathione (GSH) and spermidine (SPD), namely N^1,N^8-bisglutathionylspermidine (trypanothione, TSH), has been found in parasites of the genera *Trypanosoma* and *Leishmania* and functions in a similar way as does GSH in mammalian cells.[1] Because TSH is found uniquely in parasites, and since GSH reductase in the host mammalian cells does not act on TSH as a substrate, the biosynthesis of TSH is an ideal target for trypanocidal drug design.[2,3] The de novo biosynthetic pathway of TSH has been elucidated,[3] and the last two steps of this pathway, i.e., the formation of TSH from GSH (eq 1), are especially attractive for drug design. Since GSH is essential to host mammalian cells



(1)

for oxidative defense, we desire to inhibit TSH biosynthesis while keeping GSH biosynthesis unaffected. Isolated from *Escherichia coli*[4,5] or *Crithidia fasciculata*,[6,7] an ATP-dependent ligase called glutathionylspermidine (Gsp) synthetase catalyzes the first of the two final steps of TSH biosynthesis.

The Gsp synthetase-catalyzed reaction probably involves formation of a tetrahedral intermediate (**1**) resulting from the attack of spermidine on the putative acyl phosphate (Figure 1). We have recently published the synthesis of phosphonate and phosphonamidate analogues of Gsp[8] and have described their inhibition of the target enzyme, a bifunctional Gsp synthetase/amidase.[8,9] Similarly, the corresponding phosphinates (**2**) would be attractive synthetic targets as potential specific inhibitors of Gsp synthetase. Compounds of this type appear to have an advantage over the corresponding phosphonate and phosphonamidate, both in terms of chemical stability and biochemical properties. Thus, **2** could be phosphorylated by the enzyme in the presence of ATP; i.e., **2** → **3**, as in the case of several other structurally similar phosphinates.[10−15] Phosphorylated phosphinates derived from **2** should act as potent and specific inhibitors of Gsp synthetase by closely mimicking the proposed tetrahedral intermediate **1**. Substrate specificity of the enzyme Gsp synthetase has been investigated, and it was found that while the glutamate and glycine residues of GSH are essential for recognition by Gsp synthetase, the cysteine residue can be replaced by other amino acids; e.g., alanine.[6,9,16] On the basis of these observations, we designed the corresponding phosphinate analogues (**2a**−

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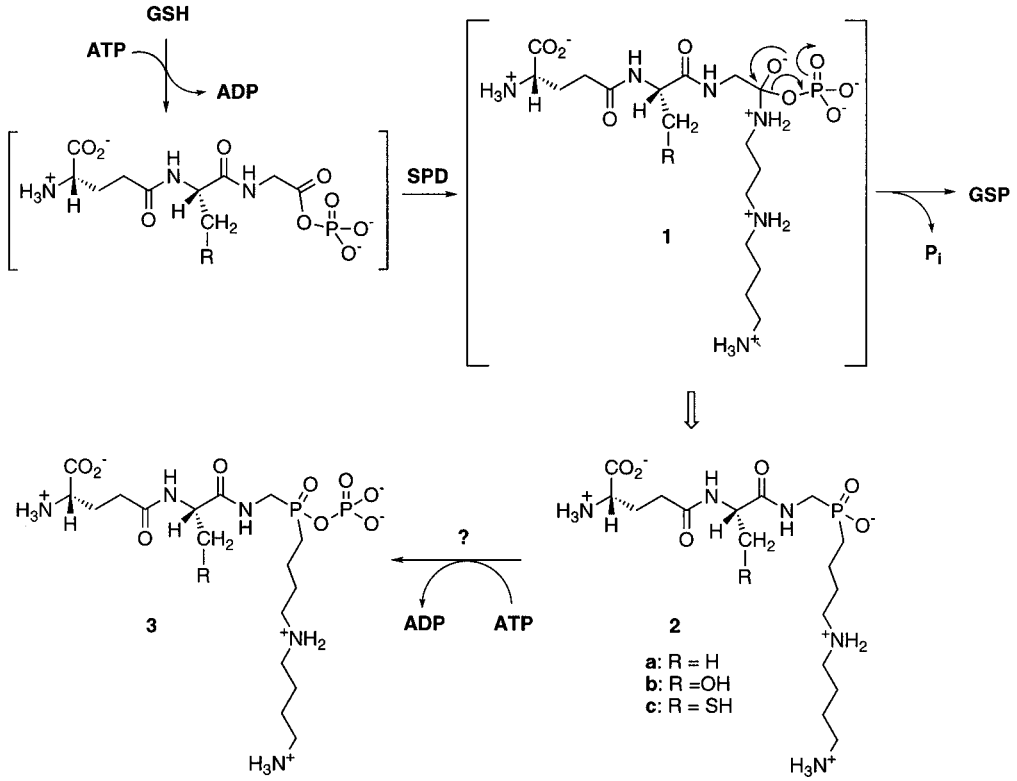

Figure 1. Postulated tetrahedral intermediate and proposed inhibitors for GSPS-catalyzed reaction.

c) as potential inhibitors of Gsp synthetase. In the present studies, we have investigated methods for the facile synthesis of core phosphinic acid building blocks leading to the desired targets **2** and report the synthesis of the first such inhibitor, an alanine-containing analogue, **2a**.

A general method for the synthesis of α-aminophosphonous acids was reported by Baylis in 1984.[17] Reaction of the protected α-aminophosphonous acids or esters with a variety of Michael acceptors can afford diverse phosphinates and has been used in the synthesis of potent inhibitors of several ligase and protease enzymes as noted above.[15,18−20] However, this approach is not suitable for synthesis of the phosphinates of interest in our current research. First, the structures of intermediates in the synthetic approach to our targets do not contain a Michael acceptor. Second, the yield of a key intermediate α-(aminomethyl)phosphonous acid, required in this approach, is extremely low (6%).[17] Although an improved procedure for the synthesis of this precursor was reported recently,[21] its use in the synthesis of the highly functionalized phosphinates of interest in this research would involve a series of protection−reprotection steps. We wished to synthesize a variety of protected α-aminophosphinates in a more direct manner. As outlined in Figure 2, three alternative routes were proposed for the synthesis of the core phosphinates. These include the three-component condensation, Michaelis−Becker, and Pudovik−Abramov reactions.

Results and Discussion

The three-component condensation (TCC) reaction involving an amide functionality, an aldehyde or a ketone, and a trivalent phosphorus compound was first reported by Birum[22] in 1974 and was subsequently extended by Oleksyszyn et al.[23−26] for the synthesis of α-aminophosphonic acids or α-aminophosphinic acids. Because the free aminophenylphosphonic acids are unsuitable for peptide coupling,[27,28] facile and direct methods for the synthesis of protected α-aminophosphonic acids and phosphonopeptides were developed by Yuan and co-workers.[29−32] This type of reaction was also extended for the asymmetric synthesis of α-aminophosphonic acids.[33,34] However, there is no precedent for the direct use of phosphonous acids or alkylphosphonites, especially functionalized alkylphosphonites, in this type

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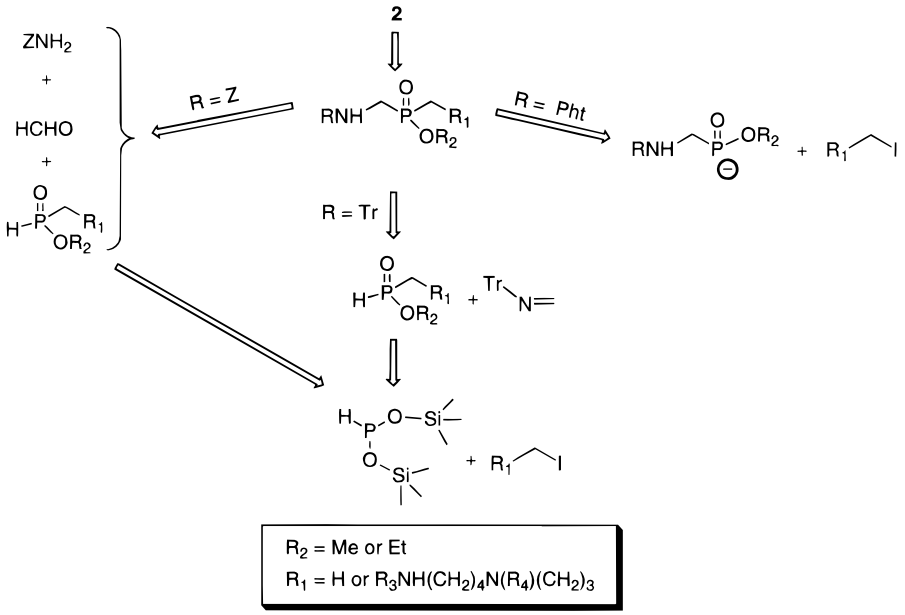
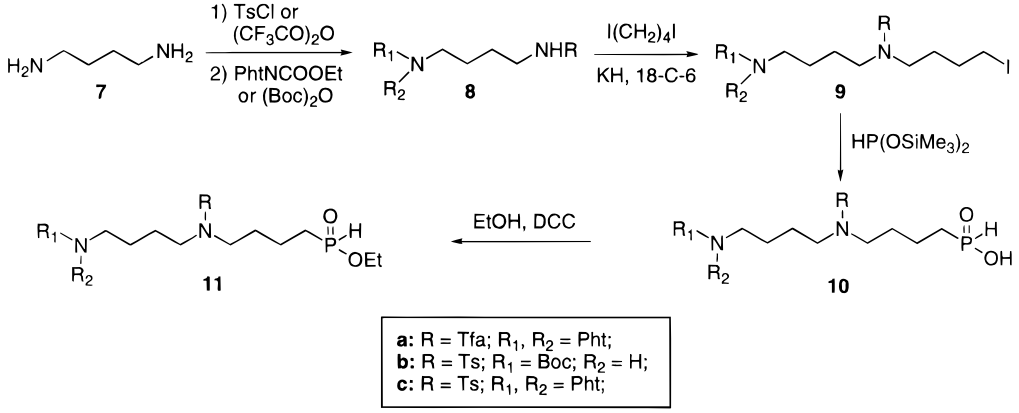
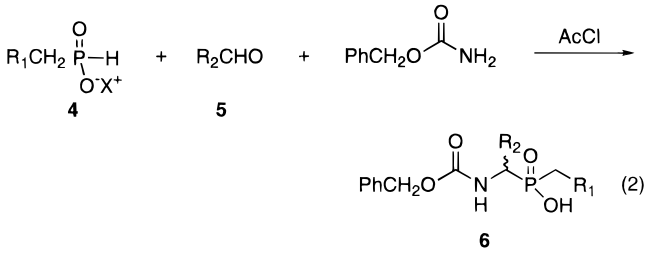


Figure 2. Retrosynthetic pathway for phosphinates.

Scheme 1



of reaction. We envisioned that a variety of functionalized alkylphosphonites may also participate in this type of reaction and afford the functionalized α-aminophosphinates suitable for our target synthesis. As described briefly in a previous communication,[35] this condensation can be realized under very mild conditions (AcCl, rt) and affords a small molecular library of α-aminophosphinates (eq 2). These phosphinates are not only useful for our



current research but are also potential building blocks for a variety of phosphinic peptides for use in inhibition studies of many ligase or protease enzymes.

Having established a convenient method for the synthesis of protected α-aminophosphinic acids using the TCC reaction, we sought to use this method for the synthesis of the polyamine-containing phosphinic acids required in our target synthesis. Starting from the natural polyamine, putrescine (**7**), the polyamine-containing phosphonous acid **10** and its ethyl ester **11** bearing various protective groups were synthesized as shown in Scheme 1. The Michaelis−Arbuzov reaction of **9**[36] with bis(trimethylsilyl) hydrogen phosphonite (BTH) was effected by a slight modification of a recently published procedure.[40] At elevated temperature (105 °C, toluene), **9** reacted smoothly with BTH to give the desired

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(36) Substituted 1,4-diaminobutanes **9** were synthesized by the monoacylation (**9a**) or monotosylation (**9b**,**c**) of 1,4-diaminobutane with trifluoroacetic anhydride or tosyl chloride.[37] The remaining free amino group was then protected by reaction with di-*tert*-butyl dicarbonate or *N*-(ethoxycarbonyl)phthalimide to give the orthogonally protected 1,4-diaminobutane, **8**. Alkylation of **8** with 1,4-diodobutane was effected in the presence of NaH.[38,39]

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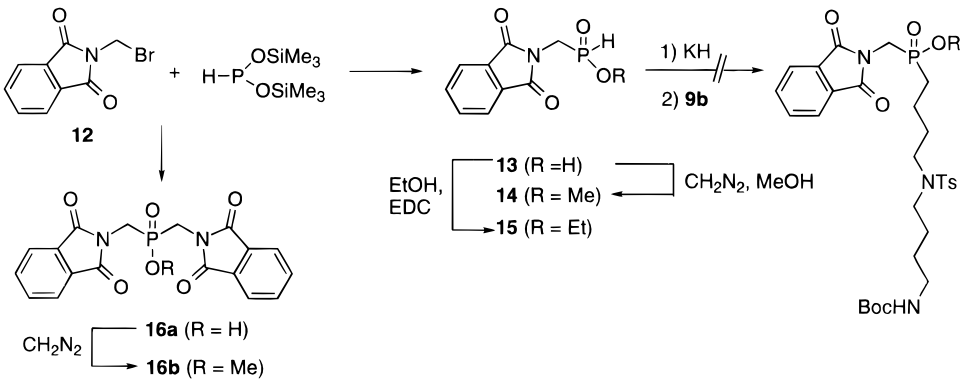
Scheme 2



phosphonous acid **10** in satisfactory yields. Conversion of **10** to the ethyl esters **11** was achieved in the usual manner by using DCC[41] as the coupling reagent. Of the two ethyl phosphonites (**11a** and **11c**) synthesized, the Tfa derivative **11a** was found to be extremely susceptible to hydrolysis; i.e., **11a** was converted completely to **10** when standing exposed to air at rt for several days. In contrast, the tosyl-protected phosphonite **11c** was very stable; no detectable hydrolysis was found upon standing at rt exposed to air for over 6 months. With the polyamine-containing phosphonous acid **10** and ethyl phosphonite **11** in hand, use of the TCC reaction for the synthesis of polyamine-containing phosphinates was investigated. Unfortunately, neither **10** nor **11** would react with ZNH_2 and formaldehyde to give the polyamine-containing α-aminomethanephosphinic acid, a key intermediate in our target synthesis.

Our attention then turned to the Michaelis−Becker reaction strategy (Scheme 2). Reaction of (bromomethyl)phthalimide **12** with BTH did not proceed as reported for this type of alkylation reaction.[40] Instead of the desired phosphonous acid **13**, the corresponding symmetric phosphinate **16a** was obtained when equimolar amounts of **12** and BTH were mixed in CH_2Cl_2 at 0 °C to rt. This appears to be the result of a double Arbuzov reaction of the bromide with BTH.[42,43] A similar observation was also mentioned by Grobelny.[44] We found that the proportion of the products **13** and **16a** can be controlled by manipulating the relative amount of starting material **12** and BTH used. When a 1:4 ratio of **12**:BTH was employed, the desired product **13** was obtained as the major product but the separation of **13** from the bis-alkylation product, **16a**, proved to be problematic. The mixture was therefore treated directly with diazomethane to give the corresponding methyl esters **14** and **16b**, which were then separated easily by silica gel chromatography. Unfortunately, treatment of **14** with **9b** under various Michaelis−Becker conditions; e.g., KH or NaH[45,46] and sodium bis(trimethylsilyl)amide,[47] failed to form any trace of the desired phosphinate. Instead, the starting material, phosphonite **14**, was found to decompose under these conditions. Since it is known that methyl esters of phosphonous acids are very susceptible to hydrolysis,[48] **13** was converted to the more stable ethyl ester by treatment with EtOH/EDC.[49] The resultant ethyl ester **15** also failed to react with **9b** under Michaelis−Becker conditions. Similar problems have been encountered using the Michaelis−Becker reaction for the synthesis of complex phosphonates.[50]

The Pudovik−Abramov reaction has been used widely for the synthesis of phosphonates from dialkyl or trialkyl phosphites and carbonyl compounds.[45] Because of the mild conditions employed for the removal of a trityl group, Soroka first synthesized trityl-protected α-aminophosphonates from dialkyl phosphites and tritylamine-derived Schiff bases by slight modification of the existing literature procedure.[51] The use of a functionalized phosphonite such as **11a** or **11c** in Soroka's procedure should afford the core phosphinate for our target synthesis. Before the synthesis of the desired polyfunctionalized phosphinates was initiated, studies were done to optimize the reaction conditions using a model reaction. Thus, a simple phosphonite, ethyl phenylphosphonite (**18**), was subjected to the reaction with the imine, **17**, under the reported conditions (toluene, 100 °C, 4 h).[51] However, no trace of addition product could be detected after prolonged time (24 h). Many other conditions previously employed for this type of reaction such as KF/18-C-6/CH_3CN,[52] NaOMe/MeOH,[15,20] and a silylphosphorus reagent[53] were also evaluated but none gave satisfactory results. Finally, when catalytic amounts of a Lewis acid such as boron trifluoride were added, the reaction was successfully effected in hot toluene and gave the desired phosphinate product **19** in 82% yield. Satisfactory yields could also be obtained from the analogous reaction involving functionalized phosphonites such as **15**, **11a**, and **11c** to provide **20**−**22**, respectively (eq 3). It should be noted that boron trifluoride in catalytic amounts is

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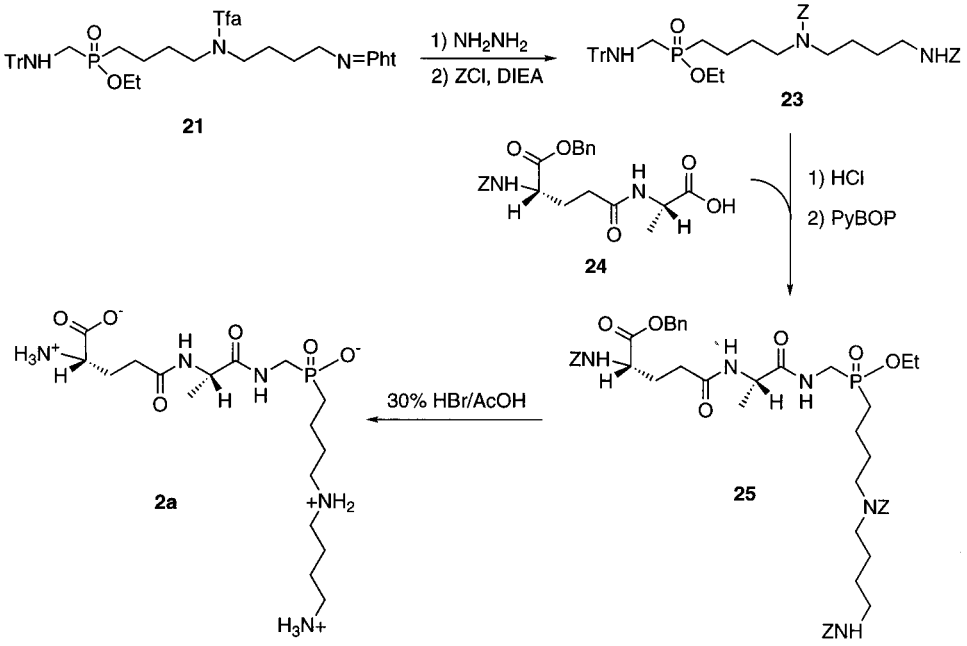
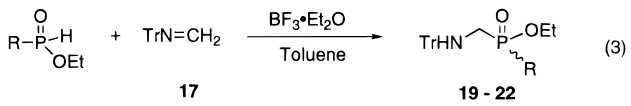
Scheme 3[a]



[a] Z = carbobenzyloxy.

essential for this reaction; using either stoichiometric or excess boron trifluoride results in drastically decreased yields.



(3)

R	Product	%Yield
Ph (**18**)	**19**	82
$PhtNCH_2$ (**15**)	**20**	86
$PhtN(CH_2)_4N(Tfa)(CH_2)_4$ (**11a**)	**21**	81
$PhtN(CH_2)_4N(Ts)(CH_2)_4$ (**11c**)	**22**	70

Unlike the aforementioned TCC reaction that can be applied to a variety of both aliphatic and aromatic aldehydes, this last method is not very successful with an aromatic aldehyde; e.g., trying to extend this reaction to a Schiff base derived from tritylamine and benzaldehyde was unsatisfactory. However, in the case of more complex phosphonites such as the polyamine-containing phosphonites **11a** and **11c**, this modified Pudovik–Abramov method is required. Combination of these two methodologies is therefore capable of providing many structural variants of protected α-aminophosphinates for different purposes. Thus, compound **6a** can be easily obtained by the TCC method and is the core phosphinate for synthesis of a nonpolyamine-containing phosphinate for enzyme inhibition studies.[54] Compounds **21** and **22**, obtained by the Pudovik–Abramov method, contain the core phosphinate required for the successful synthesis of the complex polyamine-containing phosphinate inhibitors **2a**–**c**.

As outlined in Scheme 3, culmination of this building block strategy for the construction of potent inhibitors of glutathionylspermidine synthetase is illustrated by the synthesis of **2a**, a potent, slow-binding inhibitor (K_i = 3.2 μM, K_i* = 7.8 nM) of Gsp synthetase.[54]

Experimental Section

General Methods. Standard analytical and solvent purification methods have been described previously.[8] ^{19}F NMR spectra were obtained on a Bruker 300 spectrometer at 282 MHz; chemical shift data are reported in reference to TFA. *J* values are reported in Hz. *N*-(Trifluoroacetyl)-1,4-diaminobutane[37] and the *N*-tritylimine **17**[51] were prepared according to known procedures. "Usual workup" refers to dissolving the concentrated reaction mixture in EtOAc followed by washing with 5% citric acid, 5% $NaHCO_3$ solution, and brine, followed by drying (Na_2SO_4) and removal of EtOAc.

Methyl [[*N*-(Benzyloxycarbonyl)amino]methyl]phosphinic acid (6a, R_1 = R_2 = H). A mixture of benzyl carbamate (160 mg, 1.05 mmol) and formaldehyde (90 mg of 37% aqueous solution, 1.1 mmol) in AcCl (5 mL) was stirred at 0 °C for 30 min. Adamantanamine methylphosphonite **4a** (240 mg, 1.0 mmol) was then added. The resultant suspension was then stirred at rt for 6 h. Volatile components were then removed under reduced pressure, and the residue was partitioned between 5% $NaHCO_3$ (45 mL) and Et_2O (30 mL). The aqueous layer was separated and acidified with 6 N HCl to pH 2 and then extracted with EtOAc (4 × 30 mL). The combined EtOAc extracts were dried (Na_2SO_4), and solvent was removed to afford 0.17 g (67%) of the crude product **6a** as a powder, crystallized from EtOAc/hexane: mp 123–125 °C; ^{1}H NMR ($CDCl_3$) δ 1.38 (d, 3H, J = 14.1), 3.47 (d, 2H, J = 6.3), 5.03 (s, 2H), 5.54 (br, 1H), 7.25 (s, 5H), 11.65 (br, 1H); ^{13}C NMR δ 156.6, 136.3, 128.8, 128.5, 128.4, 67.5, 40.8 (d, J = 104), 12.8 (d, J = 95.6); ^{31}P NMR δ 50.6; MS (FAB) *m*/*z* 244 (MH^+, 100), 177 (3), 154 (3), 136 (4); HRMS (FAB) calcd for $C_{10}H_{14}NO_4PH$ (MH^+) 244.0739, found 244.0728. Anal. Calcd for $C_{10}H_{14}NO_4P$: C, 49.39; H, 5.80; N, 5.76. Found: C, 49.75; H, 5.75; N, 5.80.

***N*-(Trifluoroacetyl)-*N*′-phthaloyl-1,4-diaminobutane (8a).** A solution of *N*-Tfa-1,4-diaminobutane (3.4 g, 15.4 mmol), *N*-(ethoxycarbonyl)phthalimide (4.06 g, 18.5 mmol), and Et_3N (5.3 mL) in dry THF (120 mL) was refluxed for 12

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h. The volatile components were then removed under reduced pressure, and the residue was dissolved in EtOAc (150 mL), washed with 5% citric acid and brine, and dried over sodium sulfate. Removal of the solvents afforded a solid that was crystallized from a mixture of $CHCl_3$ and hexane; 4.5 g (93%) of the product was obtained as a white solid: R_f 0.35 (hexane/EtOAc, 2:1); mp 146−147 °C; ^{1}H NMR ($CDCl_3$) δ 1.61−69 (m, 2H), 1.70−1.80 (m, 2H), 3.39−3.47 (m, 2H), 3.73 (t, 2H, J = 7), 6.81 (b, 1H), 7.70−7.79 (m, 2H), 7.81−7.88 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 168.7, 157.7, 134.3, 132.1, 123.5, 39.7, 37.3, 26.2, 26.1; ^{19}F NMR ($CDCl_3$) δ 0.06. Anal. Calcd for $C_{14}H_{13}F_3N_2O_3 \cdot 0.4H_2O$: C, 52.30; H, 4.33; N, 8.71. Found: C, 52.45; H, 4.33; N, 8.85.

***N*-(*tert*-Butoxycarbonyl)-*N′*-tosyl-1,4-diaminobutane (8b).** A stirred solution of *N*-tosyl-1,4-diaminobutane (5.8 g, 25.4 mmol), obtained as described below for the synthesis of **8c**, and di-*tert*-butyl dicarbonate (5.55 g, 25.4 mmol) in dry CH_2Cl_2 (50 mL) was heated to reflux for 4 h. After removal of the volatile components, the product was crystallized from hexane to afford 8.1 g (93%) of **8b** as a white solid: mp 84−86 °C (hexane); IR (KBr) ν 3285, 1688 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 1.48−1.54 (m, 4H), 2.43 (s, 3H), 2.86−2.95 (m, 2H), 3.00−3.12 (m, 2H), 4.55 (br, 1H), 4.83 (br, 1H), 7.31 (d, 2H, J = 8), 7.74 (d, 2H, J = 8); ^{13}C NMR ($CDCl_3$) δ 143.5, 137.0, 129.9, 127.2, 79.9, 42.8, 40.0, 28.6, 27.3, 26.8, 21.7.

***N*-Tosyl-*N′*-phthaloyl-1, 4-diaminobutane (8c).** A solution of TsCl (3.16 g, 16 mmol) in dry THF (15 mL) was added dropwise to a stirred solution of putrescine **7** (2 mL, 20 mmol) and NMM (2.23 mL, 20 mmol) in THF (20 mL) over a period of 2 h. The volatile components were removed under reduced pressure, and the residue was partitioned between 5% $NaHCO_3$ and CH_2Cl_2 (40 mL each). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 30 mL). Combined CH_2Cl_2 solutions were dried (Na_2SO_4) and concentrated to afford an oil that was extracted with 1 N HCl (2 × 20 mL), and precipitated material was filtered off. The filtrate was extracted with Et_2O (2 × 40 mL) and was then basified (pH 11) by the addition of ammonium hydroxide (29%) and extracted with CH_2Cl_2 (4 × 40 mL). The CH_2Cl_2 extracts were combined and dried over sodium sulfate. Removal of the solvent afforded 2.3 g (61%) of *N*-tosyl-1,4-diaminobutane as an amorphous powder: mp 65−68 °C; IR ($CDCl_3$) ν 3363, 3290, 3055, 1322, 1155 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.38−1.50 (m, 2H), 1.50−1.60 (m, 2H), 2.62−2.69 (m, 2H), 2.88−2.95 (m, 2H), 7.29 (d, 2H, J = 8), 7.74 (d, 2H, J = 8); ^{13}C NMR ($CDCl_3$) δ 143.2, 138.4, 129.8, 127.2, 43.3, 41.6, 30.8, 27.7, 21.7.

A solution of *N*-tosyl-1,4-diaminobutane (5.55 g, 24 mmol), obtained as described above, *N*-(ethoxylcarbonyl)phthalimide (5.55 g, 25 mmol), and Et_3N (4.9 mL) in dry THF (150 mL) was heated at reflux temperature for 4 h. The volatile components were then removed under reduced pressure, and the residue was dissolved in EtOAc (200 mL), washed with 5% citric acid and brine, and dried over sodium sulfate. Removal of the solvents afforded a solid that was crystallized from a mixture of EtOAc and hexane; 7.0 g (78%) of the product **8c** was obtained as a white powder: mp 128−129 °C; IR (KBr) ν 3289, 1770, 1712 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.48−1.56 (m, 2H), 1.65−1.74 (m, 2H), 2.41 (s, 3H), 2.95−3.02 (m, 2H), 3.65 (t, 2H, J = 7), 4.52 (t, 1H, J = 6), 7.29 (d, 2H, J = 10), 7.68−7.76 (m, 4H), 7.82−7.88 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 168.5, 143.4, 138.0, 134.1, 132.0, 129.8, 127.1, 123.2, 42.6, 37.3, 26.8, 25.8, 21.6.

***N*-(Trifluoroacetyl)-*N*-(4-iodo)butyl-*N′*-phthaloyl-1,4-diaminobutane (9a).** To a suspension of KH (1.42 g, 12.4 mmol) in dry THF (60 mL) was added **8a** (3.0 g, 9.55 mmol) slowly at 0 °C under a dry atmosphere of N_2. Crown ether 18-C-6 (0.25 g) was then added followed by the addition of 1,4-diiodobutane (11.9 g, 36.2 mmol) at 0 °C. The mixture was stirred overnight at rt and then was quenched by the addition of AcOH (5 mL) and MeOH until a clear solution was obtained (ca. 20 mL). This solution was concentrated under reduced pressure. After usual workup and column chromatography on silica gel (gradient elution from 100% hexane to 67% hexane in EtOAc), 2.6 g (55%) of the product was obtained as a light yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.56−1.85 (m, 8H), 3.21 (t, 2H, J = 9.5), 3.35−3.52 (m, 4H), 3.73 (t, 2H, J = 9.1), 7.70−7.79 (m, 2H), 7.80−7.91 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 168.0, 155.4 (q, J = 34.3 Hz), 134.4, 131.6, 123.0, 116.5 (q, J = 289 Hz), 46.6, 46.0, 45.3, 37.0, 36.9, 30.1, 29.8, 29.1, 27.3, 25.5, 25.2, 25.0, 23.6, 18.2, 7.9, 7.7; ^{19}F NMR ($CDCl_3$) δ 7.58, 7.54; MS (CI/CH_4) m/z 497 (MH^+, 100), 371 (23), 369 (16), 273 (59); HRMS (CI/CH_4) calcd for $C_{18}H_{20}F_3IN_2O_3H$ (MH^+) 497.0549, found 497.0555. Anal. Calcd for $C_{18}H_{20}F_3IN_2O_3$: C, 43.56; H, 4.07; N, 5.65. Found: C, 43.72; H, 4.04; N, 5.62.

***N*-Tosyl-*N*-(4-iodobutyl)-*N′*-Boc-1,4-diaminobutane (9b)** was obtained in a similar manner with a yield of 64%: IR (neat) ν 3355, 1707, 1159 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 9H), 1.49−1.65 (m, 6H), 1.75−1.84 (m, 2H), 2.43 (s, 3H), 3.07−3.15 (m, 6H), 3.20 (t, 2H, J = 7), 4.51 (br, 1H), 7.32 (d, 2H, J = 5), 7.75 (d, 2H, J = 8); ^{13}C NMR ($CDCl_3$) δ 156.1, 143.3, 136.8, 129.8, 127.2, 79.2, 48.3, 47.4, 40.1, 30.4, 29.6, 28.6, 27.4, 26.2, 21.6, 6.2; MS (FAB) m/z 525 (MH^+, 1.6), 425 (16), 243 (100), 154 (20).

***N*-Tosyl-*N*-(4-iodobutyl)-*N′*-phthaloyl-1,4-diaminobutane (9c)** was obtained in 64% yield in a similar manner: mp 93−94 °C; IR (KCl) ν 1712 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.54−1.75 (m, 6H), 1.75−1.90 (m, 2H), 2.40 (s, 3H), 3.10−3.20 (m, 6H), 3.69 (t, 2H, J = 6), 7.28 (d, 2H, J = 8), 7.62−7.78 (m, 4H), 7.82−7.88 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 168.5, 143.4, 136.8, 134.2, 132.2, 129.8, 127.2, 123.4, 48.1, 47.5, 37.4, 30.4, 29.6, 26.2, 25.9, 21.6, 6.3. Anal. Calcd for $C_{23}H_{27}IN_2O_4S$: C, 49.82; H, 4.92; N, 5.05. Found: C, 50.40; H, 5.14; N, 5.00.

[4-[*N*-(Trifluoroacetyl)-*N*-(4-phthalimidobutyl)amino]-butyl]phosphonous Acid (10a). A mixture of ammonium hypophosphite[55] (1.86 g, 22.4 mmol) and HMDS (4.74 mL, 22.4 mmol) was stirred at 110 °C under argon for 1.5 h. Toluene (70 mL) was then added via a syringe followed by the addition of **9a** (2.20 g, 4.4 mmol) in toluene (40 mL). After being stirred at 105−108 °C overnight, the mixture was cooled naturally to rt and quenched with AcOH (4 mL) and MeOH (20 mL). Insoluble materials were removed by filtration through a layer of Celite, and the filtrate was concentrated under reduced pressure. The resultant oily residue was partitioned between CH_2Cl_2 (150 mL) and concentrated hydrochloric acid (40 mL). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 40 mL). The combined organic layers were dried ($NaSO_4$), and removal of solvent afforded 1.29 g (67%) of the crude product **10a** as an oil that was used for the synthesis of **11a**. A small portion was purified by DEAE ion-exchange chromatography (0.1 M NH_4OAc/MeOH): ^{1}H NMR ($CDCl_3$) δ 1.55−1.95 (m, 10H), 3.35−3.49 (m, 4H), 3.71 (t, 2H, J = 6.4), 7.11 (d, 1H, J = 543), 7.68−7.80 (m, 2H), 7.81−7.92 (m, 2H), 9.65−9.82 (br, 1H); ^{19}F NMR ($CDCl_3$) δ 7.11, 7.06; ^{31}P NMR ($CDCl_3$) δ 37.5, 36.9; MS (FAB) m/z 435 (MH^+, 100), 401 (13), 268 (25), 121 (17). Anal. Calcd for $C_{18}H_{22}F_3N_2O_5P \cdot H_2O$: C, 47.78; H, 5.36; N, 6.19. Found: 47.79; H, 5.15; N, 6.10.

[4-[*N*-Tosyl-*N*-(4-phthalimidobutyl)amino]butyl]-phosphonous Acid (10c). In a two-necked round-bottom flask equipped with a refluxing condenser were placed ammonium hypophosphite (0.39 g, 4.7 mmol) and HMDS (1 mL, 4.7 mmol). The stirred mixture was heated to 110 °C under dry N_2 for 2 h. Toluene (8 mL) was then added through a syringe followed by the addition of a solution of **9c** (0.52 g, 0.94 mmol) in dry toluene (8 mL). The mixture was stirred at 105 °C for 24 h. The reaction was then quenched by the addition of methanol (4 mL) and AcOH (0.5 mL) at rt. After removal of the solvents and volatile materials, the residue was dissolved in CH_2Cl_2 (60 mL) and washed with 6 N HCl (10 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by ion-exchange chromatography on a DEAE column eluting with 0.1 M ammonium acetate in MeOH to afford 0.32 g (70%) of the product as a hygroscopic solid: ^{1}H NMR ($CDCl_3$) δ 1.40−1.62 (m, 10H), 2.36 (s, 3H), 3.20−3.14 (m, 4H), 3.62 (t, 2H, J

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= 6), 7.00 (d, 1H, J = 501), 7.23 (d, 2H, J = 8), 7.62 (d, 2H, J = 8), 7.65−7.77 (m, 2H), 7.88−7.85 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 168.5, 143.3, 136.8, 134.2, 132.2, 129.9, 127.3, 123.4, 48.3, 48.1, 37.5, 31.3 (d, J = 91), 30.0 (d, J = 15), 26.1, 25.9, 21.6, 19.2; ^{31}P NMR ($CDCl_3$) δ 28.3; MS (FAB) m/z 493 (MH^+, 100), 479 (30), 160 (40), 337 (40); HRMS (FAB) calcd for $C_{23}H_{29}N_2O_6$-PSH 493.1562, found 493.1562.

***O*-Ethyl [4-[*N*-(Trifluoroacetyl)-*N*-(4-phthalimidobutyl)amino]butyl]phosphonite (11a).** To a stirred solution of **10a** (1.21 g, 2.8 mmol) in THF (25 mL) and ethanol (3.0 mL) was added DMAP (36 mg) followed by DCC (0.75 g, 3.6 mmol). The mixture was stirred overnight at rt, the resultant precipitated material was removed by filtration, and the filtrate was concentrated under reduced pressure. After usual workup and column chromatography on silica gel using EtOAc as eluant, 1.0 g (76%) of the product **11a** was obtained as a syrup: ^{1}H NMR ($CDCl_3$) δ 1.34−1.40 (m, 3H), 1.57−1.83 (m, 10 H), 3.30−3.42 (m, 4H), 3.72 (t, 2H, J = 6.5), 4.04−4.22 (m, 2H), 7.12 (dm, 1H, J = 533), 7.68−7.74 (m, 2H), 7.76−7.88 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 168.1, 156.6 (q, J = 35.4), 134.0, 133.9, 131.9, 123.1, 123.0, 116.5 (q, J = 288), 62.4, 62.2, 46.9, 46.3, 46.2, 37.0, 36.9, 29.2 (d, J = 14.1 Hz), 28.1 (d, $^1J_{CP}$ = 93.6 Hz), 27.4 (d, J = 14.6 Hz), 25.8, 25.7, 23.9, 18.0, 17.8, 16.1, 16.0; ^{19}F NMR ($CDCl_3$) δ 4.86; ^{31}P NMR ($CDCl_3$) δ 38.1, 37.4; MS (CI/CH_4) m/z 463 (MH^+, 100), 427 (7), 365 (13), 301 (10); HRMS (CI/CH_4) calcd for $C_{20}H_{26}F_3N_2O_5PH$ 463.1610, found 463.1600. Anal. Calcd for $C_{20}H_{26}F_3N_2O_5P$: C, 51.95; H, 5.68; N, 6.06. Found: C, 51.84; H, 5.66; N, 6.18.

***O*-Ethyl [4-[*N*-Tosyl-*N*-(4-phthalimidobutyl)amino]butyl]phosphonite (11c).** To a stirred solution of **10c** (0.3 g, 0.62 mmol), ethanol (29 mg), and DMAP (8 mg) in THF (6 mL) was added DCC at 0 °C. The mixture was stirred at rt overnight. Precipitate DCU was filtered off, and the filtrate was concentrated and dissolved in EtOAc (40 mL). Some more precipitated DCU was filtered off again, and the EtOAc solution was washed with 5% $NaHCO_3$ (2 × 30 mL) and brine (30 mL). After being dried over sodium sulfate, the solvent was removed. The resultant solid product was then recrystallized from $CHCl_3$/hexane to afford 0.26 g (81%) of the product as a white powder: mp 102−105 °C; ^{1}H NMR ($CDCl_3$) δ 1.35 (t, 3H, J = 7), 1.55−1.75 (m, 10H), 2.40 (s, 3H), 3.08−3.15 (m, 4H), 3.67 (t, 2H, J = 7), 4.08−4.18 (m, 2H), 7.10 (d, 1H, J = 530), 7.28 (d, 2H, J = 8), 7.66 (d, 2H, J = 8), 7.70−7.75 (m, 2H), 7.82−7.89 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 168.5, 143.4, 136.7, 134.2, 132.2, 131.4, 129.8, 128.2, 127.3, 123.7, 123.4, 122.9, 62.5 (d, J = 6), 48.2, 48.1, 37.4, 29.7, (d, J = 15), 28.3 (d, J = 94), 26.2, 25.9, 21.6, 18.2, 16.4 (d, J = 6); ^{31}P NMR ($CDCl_3$) δ 38.6. Anal. Calcd for $C_{25}H_{33}N_2O_6PS$: C, 57.68; H, 6.40; N, 5.38. Found: C, 57.86; H, 6.64; N, 5.51.

***O*-Methyl (Phthalimidomethyl)phosphonite (14) and *O*-Methyl Bis(phthalimidomethyl)phosphinate (16b).** A mixture of ammonium hypophosphite (3.15 g, 38 mmol) and HMDS (6.16 g, 38 mmol) was stirred and heated to 120 °C under dry N_2 for 2 h. After being cooled to rt, CH_2Cl_2 (30 mL) was added, and the reaction flask was then chilled in an ice bath. A solution of bromomethyl phthalimide (2.28 g, 9.5 mmol) in dry CH_2Cl_2 (15 mL) was then added. The mixture was stirred at 0 °C for 1 h and then at rt for 12 h. The mixture was filtered through a layer of Celite into a mixture of MeOH (6 mL) and CH_2Cl_2 (100 mL), and the filtrate was washed with 6 N HCl (20 mL). The aqueous layer was separated and back-extracted with CH_2Cl_2 (2 × 40 mL). The combined CH_2Cl_2 solution was then dried (Na_2SO_4). Removal of solvents afforded 1.74 g (78% conversion) of a mixture of **13** and **16a** (41:9) as a light yellow solid. This material was used directly for the reaction with diazomethane described below. An analytical sample of (phthalimidomethyl)phosphonous acid (**13**) was obtained by precipitation from EtoAc: mp 209−211 °C; ^{1}H NMR (D_2O) δ 3.83 (d, 2H, J = 11), 7.17 (d, 1H, J = 580), 7.70−7.84 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 166.8, 134.4, 134.3, 131.2, 123.0, 38.2 (d, J = 97); ^{31}P NMR (D_2O) δ 16.4 (ammonium salt; free acid δ 24 ppm vs **16a** δ 38 ppm); MS (FAB) m/z 226 (MH^+, 100), 153 (35), 103 (34); HRMS (DCI/NH_3) calcd for $C_9H_8NO_4P$ 226.0269, found 226.0262. Anal. Calcd for $C_9H_8NO_4P$: C, 48.01; H, 3.59; N, 6.22. Found: C, 47.99; H, 3.77; N, 6.27.

To a suspension of the mixture containing **13** and **16a** (1.1 g) in MeOH (15 mL) was added a solution of diazomethane in ether until a yellow color persisted for 5 min. The resultant solution was stirred at rt for 1.5 h. Removal of the solvent afforded the crude products **14** and **16b**. Column chromatography on silica gel eluting with EtOAc/MeOH (10:1) afforded 0.96 g of **14** (99% based on **13**) and 0.16 g of **16b** (78% based on **16a**) as white solids. **14**: R_f 0.57 (EtOAc); mp 101−102 °C; ^{1}H NMR ($CDCl_3$) δ 3.84 (d, 3H, J = 11.7), 4.11 (dd, 2H, J = 2.2, 9.8), 7.4 (d, 1H, J = 583), 7.75−7.81 (m, 2H), 7.86−7.95 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 167.2, 134.5, 131.9, 123.8, 52.9 (d, J = 7), 36.2 (d, J = 99); ^{31}P NMR ($CDCl_3$) δ 28.1. **16b**: R_f 0.47 (EtOAc); mp 198−200 °C; ^{1}H NMR ($CDCl_3$) δ 3.83 (dd, 3H, J = 2, 11), 4.21−4.40 (m, 2H), 7.75−7.81 (m, 2H), 7.86−7.93 (m, 2H); ^{13}C NMR δ 167.4, 134.3, 132.2, 123.8, 52.5, 36.9 (d, J = 98); ^{31}P NMR ($CDCl_3$) δ 39.7; MS (CI/NH_3) m/z 399 (MH^+, 100), 238 (28); HRMS (CI/NH_3) calcd for $C_{19}H_{15}N_2O_6$-PH 399.0746, found 399.0741. Anal. Calcd for $C_{19}H_{15}N_2O_6P\cdot 0.3H_2O$: C, 56.52; H, 3.90; N, 6.94. Found: C, 56.80; H, 4.09; N, 6.69.

***O*-Ethyl (Phthalimidomethyl)phosphonite (15).** A solution of **13** (0.30 g, 1.6 mmol), ethanol (0.5 mL), EDC (0.28 g, 1.6 mmol), and DMAP (10 mg) in CH_2Cl_2 (10 mL) was stirred at rt for 12 h. After removal of the volatile components, the crude product was purified by column chromatography on silica gel (EtOAc) to afford 0.26 g (78%) of **15** as a powder: R_f 0.5 (EtOAc); mp 90−92 °C; ^{1}H NMR ($CDCl_3$) δ 1.35 (t, 3H, J = 7), 4.09 (dd, 2H, J = 8, 11), 4.12−4.28 (m, 2H), 7.38 (dt, 1H, J = 2, 580), 7.75−7.84 (m, 2H), 7.87−7.95 (m, 2H); ^{13}C NMR δ 166.9, 135.7, 134.2, 131.5, 123.5, 62.9, 36.3 (d, J = 100), 16.3; ^{31}P NMR ($CDCl_3$) δ 26.2; MS (DCI/NH_3) m/z 254 (MH^+, 100), 160 (5), 134 (8); HRMS (CI/NH_3) calcd for $C_{11}H_{12}NO_4PH$ 254.0582, found 254.0590. Anal. Calcd for $C_{11}H_{12}NO_4P$: C, 52.17; H, 4.79; N, 5.53. Found: C, 52.17; H, 4.88; N, 5.67.

***O*-Ethyl Phenyl [(*N*-Tritylamino)methyl]phosphinate (19).** A solution of ethyl phenylphosphonite **18** (18 mg, 0.11 mmol), **17** (29 mg, 0.11 mmol), and a drop of $BF_3\cdot Et_2O$ in dry toluene (8 mL) was stirred and heated to reflux for 8 h. Removal of the volatile components followed by silica gel column chromatography (EtOAc) afforded 39 mg (82%) of **19** as a solid: mp 114−116 °C; ^{1}H NMR ($CDCl_3$) δ 1.32 (m, 3H), 2.20 (t, 1H, J = 7.8), 2.48−2.72 (m, 2H), 3.88−4.20 (m 2H), 7.11−7.42 (m, 15H), 7.55−7.82 (m, 2H), 7.94−8.07 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 145.2, 132.9, 132.8, 132.7, 129.8, 129.0, 128.9, 128.4, 126.9, 71.9, 42.4 (d, J = 117), 17.0; ^{31}P NMR ($CDCl_3$) δ 41.4; MS (FAB) m/z 442 (MH^+, 3.4), 243 (100), 91 (11); HRMS (FAB) calcd for $C_{28}H_{28}NO_2PH$ 442.1936, found 442.1913. Anal. Calcd for $C_{28}H_{28}NO_2P\cdot 0.3H_2O$: C, 75.23; H, 6.46; N, 3.13. Found: C, 75.17; H, 6.56; N, 3.10.

***O*-Ethyl [(*N*-(phthalimidomethyl-*N′*-tritylamino)methyl]phosphinate (20)** was obtained in a similar manner in 86% yield as an oil: R_f 0.7 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.35 (t, 3H, J = 7), 2.40−2.85 (m, 3H), 4.05−4.38 (m, 4H), 7.10−7.29 (m, 9H), 7.45−7.65 (m, 6H), 7.70−7.81 (m, 2H), 7.84−7.93 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 167.7, 145.2, 134.6, 132.3, 62.1 (d, J = 109), 35.0 (d, J = 95), 16.9 (d, J = 6); ^{31}P NMR ($CDCl_3$) δ 41.2; MS (DCI/NH_3) m/z 525 (MH^+, 0.5), 447 (3), 243 (100); HRMS (CI/NH_3) calcd for $C_{31}H_{29}N_2O_4PH$ 525.1943, found 525.1926.

***O*-Ethyl [*N*-(Trifluoroacetyl)-*N*-(4-phthalimidobutyl)-4-aminobutyl]-[(*N′*-tritylamino)methyl]phosphinate (21).** To a stirred solution of **11a** (1.0 g, 2.3 mmol) and **17** (0.58 g, 2.3 mmol) in dry toluene (50 mL) was added $BF_3\cdot Et_2O$ (30 μL, 0.24 mmol), and the reaction solution was heated overnight at reflux temperature under N_2. The mixture was cooled to rt and diluted with EtOAc (150 mL). After usual workup, the crude product was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 100:0 to 99:1) to afford 1.29 g (81%) of the product as a syrup: ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3H, J = 7), 1.55−1.86 (m, 8H), 1.95−2.00 (m, 3H), 2.43−2.62 (m, 2H), 3.38−3.49 (m, 4H), 3.72 (t, 2H, J = 6.2), 3.42−4.08 (m, 2H), 7.15−7.35 (m, 9H), 7.44 (d, 6H, J = 7.5), 7.67−7.75 (m, 2H), 7.82−7.87 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 168.43, 156.7 (q, J = 41 Hz), 144.8, 144.75, 134.2, 134.1, 134.0, 132.1, 132.0, 128.6, 128.3, 128.1, 126.8, 126.7, 123.4, 123.3, 116.6 (q, J = 290 Hz),

71.6, 71.5, 60.9, 60.8, 60.7, 47.2, 46.6, 46.4, 41.4 (d, $^1J_{CP}$ = 102 Hz), 37.2, 37.1, 29.9 (d, $^2J_{CP}$ = 13.8 Hz), 28.0 (d, J = 14.4 Hz), 26.7 (d, $^1J_{CP}$ = 93 Hz), 26.0, 25.9, 25.8, 24.0, 19.1, 19.0, 18.9, 18.8, 16.8, 16.7; ^{31}P NMR ($CDCl_3$) δ 54.4, 54.0; MS (FAB) *m/z* 734 (MH^+, 1.4), 656 (1.5), 243 (100), 91 (15); HRMS (FAB) calcd for $C_{40}H_{43}F_3N_3O_5PH$ 734.2971, found 734.2935. Anal. Calcd for $C_{40}H_{43}F_3N_3O_5P\cdot0.5H_2O$: C, 64.67; H, 5.98; N, 5.66. Found: C, 64.32; H, 5.93; N, 5.58.

***O*-Ethyl [*N*-Tosyl-*N*-(4-phthalimidobutyl)-4-aminobutyl][(*N*′-tritylamino)methyl]phosphinate (22)** was obtained as a syrup in 70% yield in a similar way as for **21**: ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3H, J = 7), 1.55−1.70 (m, 8H), 1.95−2.95 (br, 1H), 2.38 (s, 3H), 2.40−2.52 (m, 2H), 3.05−3.20 (m, 4H), 3.68 (t, 2H, J = 7), 3.90−4.05 (m, 2H), 7.15−7.28 (m, 11H), 7.46 (d, 6H, J = 8), 7.64−7.75 (m, 4H), 7.78−7.88 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 168.5, 144.9, 143.3, 136.9, 134.2, 132.2, 129.8, 128.7, 128.2, 127.3, 126.8, 123.4, 71.6 (d, J = 16), 60.87 (d, J = 6), 48.2, 41.4 (d, J = 102), 37.4, 30.3 (d, J = 14), 26.9 (d, J = 92), 26.2, 25.9, 21.6, 19.1, 16.9; ^{31}P NMR ($CDCl_3$) δ 54.5; MS (FAB) *m/z* 792 (MH^+, 4), 243 (100), 165 (20), 91 (21); HRMS (FAB) calcd for $C_{45}H_{50}N_3O_6PSH$ 792.3236, found 792.3198. Anal. Calcd for $C_{45}H_{50}N_3O_6PS\cdot1.5H_2O$: C, 65.99; H, 6.54; N, 5.13. Found: C, 65.90; H, 6.16; N, 4.98.

***O*-Ethyl [*N*-(Benzyloxycarbonyl)-*N*-[4-[*N*′-(benzyloxycarbonyl)amino]butyl]-4-aminobutyl][(*N*″-tritylamino)-methyl]phosphinate (23).** A solution of **21** (1.24 g, 1.69 mmol) and hydrazine monohydrate (0.84 mL, 16.8 mmol) in MeOH (40 mL) was stirred at rt for 48 h. Volatile materials were then removed under reduced pressure, and the residue was partitioned between CH_2Cl_2 (140 mL) and NH_4OH (50 mL). The aqueous layer was separated and extracted with CH_2Cl_2 (2 × 50 mL). Combined CH_2Cl_2 extracts were dried (Na_2SO_4). Removal of solvents afforded an oil that, after being dried in vacuo, was redissolved in dry CH_2Cl_2 (8 mL). DMAP (50 mg) and DIEA (0.52 g, 4.06 mmol) were then added followed by the addition of benzyl chloroformate (0.69 g, 4.06 mmol) at 0 °C. The mixture was then stirred at rt overnight. Volatile components were removed under reduced pressure. After usual workup, the crude product was purified by column chromatography on silica gel ($CHCl_3$ to a mixture of MeOH/$CHCl_3$ (99:1)) to afford 1.09 g (82%) of **23** as an oil: ^{1}H NMR (320 K, DMSO-d_6) δ 1.16 (t, 3H, J = 7), 1.34−1.61 (m, 8H), 1.74−1.85 (m, 2H), 2.42−2.43 (m, 2H), 2.96−3.04 (q, 2H), 3.15−3.24 (m, 4H), 3.85−3.97 (m, 2H), 5.00 (s, 2H), 5.04 (s, 2H), 6.95−7.11 (br, ca. 1H), 7.15−7.46 (m, 26H); ^{13}C NMR ($CDCl_3$) δ 156.5, 156.2, 144.8, 136.9, 136.8, 128.6, 128.1, 127.9, 127.3, 126.9, 126.7, 67.0, 66.6, 60.7, 47.1, 46.7, 41.3 (d, J = 108 Hz), 40.7, 29.6, 26.9 (d, J = 92 Hz), 27.2, 25.6, 19.0, 16.7; ^{31}P NMR (DMSO-d_6) δ 54.2; MS (FAB) *m/z* 776 (MH^+, 1.3), 532 (1.1), 243 (100), 91 (41); HRMS (FAB) calcd for $C_{46}H_{54}N_3O_6PH$ 776.3829, found 776.3837. Anal. Calcd for $C_{46}H_{54}N_3O_6P\cdot1.5H_2O$: C, 68.80; H, 7.17; N, 5.23. Found: C, 68.85; H, 6.87; N, 5.15.

***O*-Ethyl [*N*-(Benzyloxycarbonyl)-*N*-[4-[*N*′-(benzyloxycarbonyl)amino]butyl]-4-aminobutyl][(α-*O*-benzyl-Z-γ-glutamylalaninyl)amino]methyl]phosphinate (25).** A solution of **23** (0.4 g, 0.52 mmol) in 1 M HCl/MeOH (20 mL) was heated to reflux for 20 min. Volatile components were then removed under reduced pressure, and the residue, a syrup-like semisolid, was dried in vacuo. This material was triturated with ether and the ether layer was then carefully decanted. This process was repeated for several times. The resultant residue was dried completely in vacuo and then was dissolved in CH_2Cl_2 (25 mL) and cooled to 0 °C. To this stirred solution were added **24**[8] (0.23 g, 0.52 mmol), PyBOP (0.27 g, 0.52 mmol), and finally DIEA (0.27 g, 2.1 mmol). The mixture was then stirred at 0 °C for 20 min and at rt for 2 h. After usual workup, the crude product was purified by column chromatography on silica gel (gradient elution from 100% $CHCl_3$ to a mixture of $CHCl_3$/MeOH (97:3)) to afford 0.4 g (80%) of **25** as an oil: R_f 0.3 (3% MeOH/$CHCl_3$); ^{1}H NMR (320 K, DMSO-d_6) δ 1.05−1.17 (m, 6H), 1.34−1.57 (m, 8H), 1.58−1.70 (m, 2H), 1.74−1.89 (m, 1H), 1.93−2.05 (m, 1H), 2.23 (t, 2 H, J = 7.2), 2.95−3.04 (m, 2H), 3.30−3.55 (m, 2H), 3.89−3.98 (m, 2H), 4.08−4.18 (m, 1H), 4.20−4.34 (m, 1H), 4.95−5.14 (m, 8H), 6.95−7.08 (br, 1H), 7.32 (bs, 20 H), 7.50−7.65 (br, 1H), 7.77−7.85 (m, 1H), 7.91−8.00 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 172.8, 172.0, 156.5, 136.7, 136.3, 135.4, 128.6, 128.5, 128.3, 128.1, 127.8, 67.2, 67.0, 66.6, 61.2, 61.1, 53.8, 48.9, 46.5, 40.7, 39.5, 36.9 (d, J = 98.5 Hz), 32.0, 29.4 (d, J = 72 Hz), 27.8, 27.2, 25.8, 25.4, 18.8, 18.2, 16.6; ^{31}P NMR (DMSO-d_6) δ 51.8; MS (FAB) *m/z* 958 (MH^+, 7), 920 (2), 119 (6), 91 (100); HRMS (FAB) calcd for $C_{50}H_{64}N_5O_{12}PH$ 958.4367, found 958.4373. Anal. Calcd for $C_{50}H_{64}N_5O_{12}P\cdot H_2O$: C, 61.51; H, 6.83; N, 7.18. Found: C, 61.16; H, 6.74; N, 7.12.

[*N*-(4′-Aminobutyl)-4-aminobutyl][[(γ-glutamylalaninyl)amino]methyl]phosphinic Acid (2a). A solution of **25** (0.35 g, 0.36 mmol) in 30% HBr/AcOH (25 mL) was stirred at rt for 48 h. Volatile components were then removed on a rotary evaporator with a bath temperature not exceeding 40 °C. The residue was dissolved in MeOH (20 mL), following which propylene oxide was added dropwise to give a pH 6 solution. Solvents were removed under reduced pressure, and the product was purified by ion-exchange chromatography on an AG 50W-X2 cation-exchange resin (100−200 mesh, NH_4^+ form) eluting isocratically with 0.1 M NH_4HCO_3 buffer to afford 123 mg (77%) of **2a** as a hygroscopic solid: ^{1}H NMR (D_2O) δ 1.36 (d, 3H, J = 7.2), 1.41−1.59 (m, 4H), 1.62−1.78 (m, 6H), 2.00−2.12 (m, 2H), 2.43 (t, 2H, J = 6.5), 2.90−3.17 (m, 6H), 3.35 (d, 2H, J = 9.3), 3.68 (t, 1H), 4.24 (q, 1H); ^{13}C NMR (CD_3OD) δ 176.5, 175.8, 175.1, 56.1, 51.2, 48.2, 48.1, 40.5 (d, J = 98 Hz), 40.2, 32.9, 30.4, 28.6 (d, J = 93 Hz), 28.3 (d, J = 16 Hz), 26.1, 24.2, 20.1, 17.6; ^{31}P NMR (D_2O) δ 39.7; MS (FAB) *m/z* 438 (MH^+, 89), 155 (24), 121 (12), 89 (100); HRMS (FAB) calcd for $C_{17}H_{36}N_5O_6PH$ 438.2481, found 438.2482; reversed-phase HPLC[56] t_R = 7.7 min. Anal. Calcd for $C_{17}H_{36}N_5O_7P\cdot0.5H_2CO_3\cdot H_2O$: C, 43.19; H, 8.09; N, 14.40. Found: C, 43.29; H, 7.94; N, 14.40.

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Supporting Information Available: NMR spectral data of **8b**,**c**, **9b**,**c**, **10c**, **14**, and **20** (17 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.

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