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Optimizing phenylethylphosphonamidates for the inhibition of prostate-specific membrane antigen

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Abstract—A series of eight *N*-2-phenylethylphosphonyl derivatives of glutamic acid was prepared to determine if the inhibitory potency of a phenylethylphosphonyl derivative of glutamic acid against prostate-specific membrane antigen (PSMA) could be improved through rational substitutions on the phenyl ring. The design of these eight analogs was based upon the Topliss batchwise approach. Of the inhibitors from the first generation, the 3,4-dichlorophenyl analog exhibited the greatest improvement over the lead compound which was an unsubstituted phenyl derivative, while the 4-methoxyphenyl analog was essentially void of inhibitory potency against PSMA in single-dose studies. From the potency ranking order of the first generation, the parameter most important to the pharmacophore was determined to be $\pi + \sigma$. Attempts to optimize further the potency of inhibitors by preparing a second generation of compounds did not result in structures with greater potency than that of the 3,4-dichlorophenyl analog from the first generation. Based upon K_i values, the 3,4-dichlorophenyl analog represented a potency improvement of nearly one order of magnitude. These results confirm further the usefulness of the Topliss approach to analog development when large library synthesis cannot be achieved readily.

1. Introduction

One of the most common types of cancer among men over the age of 50 is prostate cancer. The mortality rate from this prevalent malignancy is second only to lung cancer.[1] In the United States alone, it is estimated that over 230,000 new cases of prostate cancer will be diagnosed this year.[2] Its incidence is associated with age, family history, and lifestyle factors,[3] and varies strikingly among ethnic, racial, and national groups with noteworthy high rates of both incidence and mortality among African Americans.[4] One notable discovery in prostate cancer research has been the identification of an overexpressed membrane-bound cell surface protein on prostate cancer cells, namely, prostate-specific membrane antigen (PSMA). PSMA, also known as folate hydrolase I (FOLH1) and glutamate carboxypeptidase II (GCP II), is a 750-amino acid type II transmembrane glycoprotein[5] and was discovered during the development of the LNCaP cell line, one which retains most of the known features of prostate cancer.[6]

Although PSMA is primarily expressed in normal human prostate epithelium, the importance of this enzyme lies with the fact that it is upregulated and strongly expressed in prostate cancer cells[7] and the endothelium of tumor-associated neovasculature of multiple nonprostatic solid malignancies.[8] Therefore, it is not surprising that PSMA has attracted a great deal of attention as a target for immunotherapy.[9] In addition to its immunological importance, it has been demonstrated that PSMA possesses two, yet poorly understood, enzymatic activities: the hydrolytic cleavage and liberation of glutamic acid from both γ-glutamyl derivatives of folic acid[10] and the neuropeptide NAAG[11] (*N*-acetylaspartylglutamate). Despite the identification of PSMA's enzymatic activities, questions of medical interest remain to be answered for PSMA, especially with regard to its role in prostate cancer. We have hypothesized that development and use of tight-binding inhibitors of PSMA enzyme may not only help reveal the role of this enzyme, but may be ultimately translated into chemotherapeutic strategies.

Keywords: Prostate-specific membrane antigen; PSMA; Glutamate carboxypeptidase II; Phosphonamidate; Topliss.
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Until recently,[12] there was no crystal structure of PSMA and thus the development of specific inhibitors relied upon rational strategies to identify and exploit binding sites within or near the architecture of its active site. In our previous work, we identified the presence of a hydrophobic-binding site remote from the catalytic center of PSMA using a series of phenylalkylphosphonamidate derivatives of glutamic acid[13] The focus of this work was to refine the structure of the moderately potent phenylethylphosponamidate derivative of glutamic acid (**1**, Fig. 1) to enhance its inhibitory potency against PSMA.

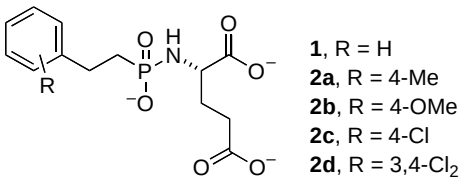
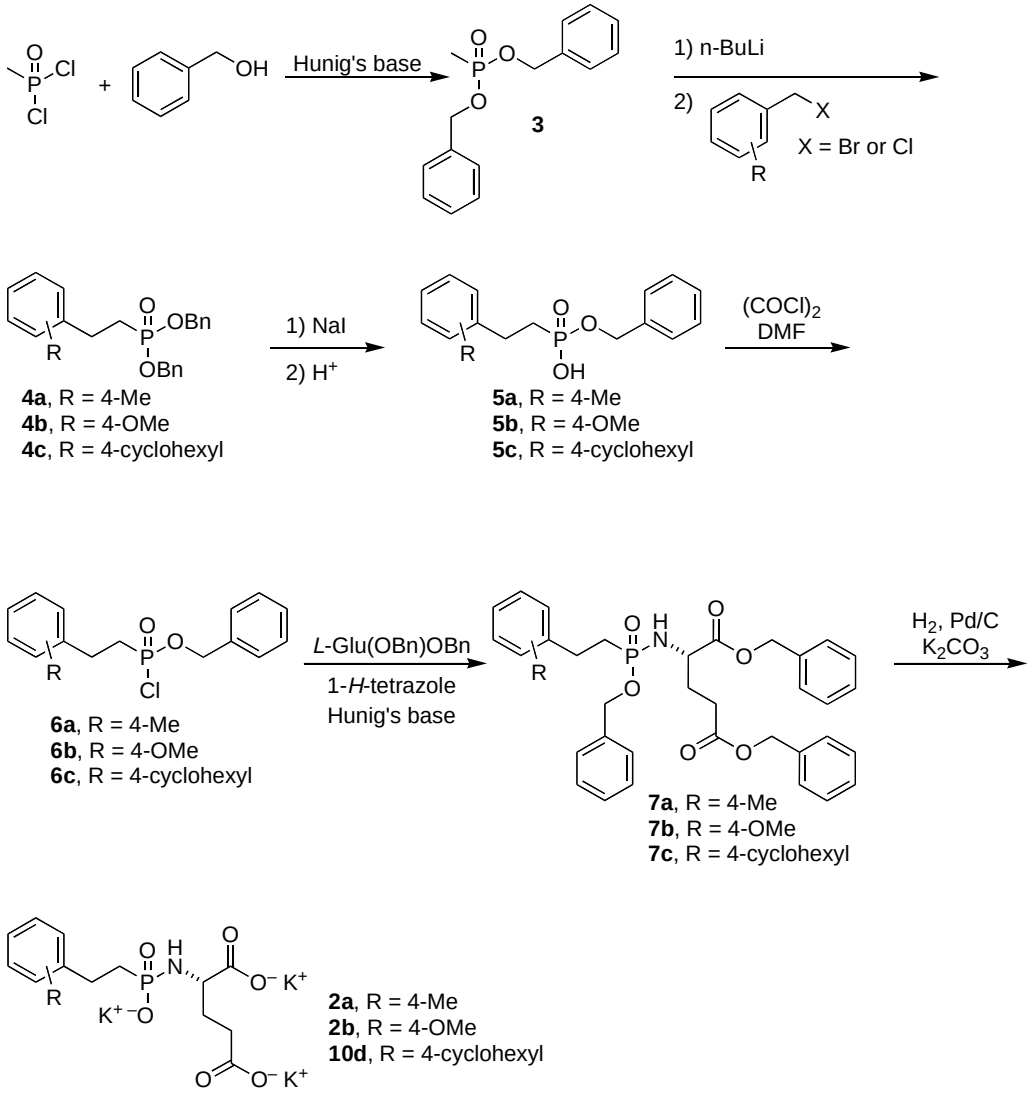


Figure 1. First generation PSMA inhibitors.

The design of the target analogs presented herein was based on the batchwise method of the Topliss operational approach of modulating the hydrophobic and electronic parameters of the phenyl ring of phenylethylphosponamidate **1**.[14,15] As defined by this approach, the first set of analogs of the unsubstituted phenyl derivative were the 4-methylphenyl **2a**, 4-methoxyphenyl **2b**, 4-chlorophenyl **2c**, and the 3,4-dichlorophenyl **2d** analogs (Fig. 1).[15] Assay results of this initial set of analogs were expected to reveal the favorable electronic (σ) and hydrophobic (π) requirements of the pharmacophore. Based upon a ranked order of potency for the initial set of compounds, a specific combination of σ and π factors were identified, which subsequently defined a second generation of derivatives in the optimization process.

2. Results and discussion

The synthetic route for preparing both the 4-methylphenyl **2a** and 4-methoxyphenyl **2b** analogs is shown in Scheme 1. This method utilized chemistry that was



Scheme 1. Synthetic route for nonhalogenated analogs.

established for the preparation of the unsubstituted analog **1**.[16] In this sequence, methylphosphonic dichloride was treated with benzyl alcohol and *N*-*N*-diisopropylethylamine to produce the dibenzyl ester **3**.[17] After deprotonation of **3** with *n*-BuLi and treatment with the appropriate benzyl halide, phosphonates **4** were obtained. Selective monodealkylation of one benzyl ester with sodium iodide produced phosphonic acids **5**. Treatment of **5** with oxalyl chloride gave the phosphonyl chlorides **6**, which were immediately coupled to the dibenzyl ester of L-glutamic acid to afford the phosphonamidate precursors **7**. Complete deprotection of the benzyl esters by hydrogenolysis yielded the final products **2** as tripotassium salts.

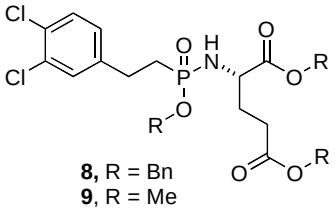
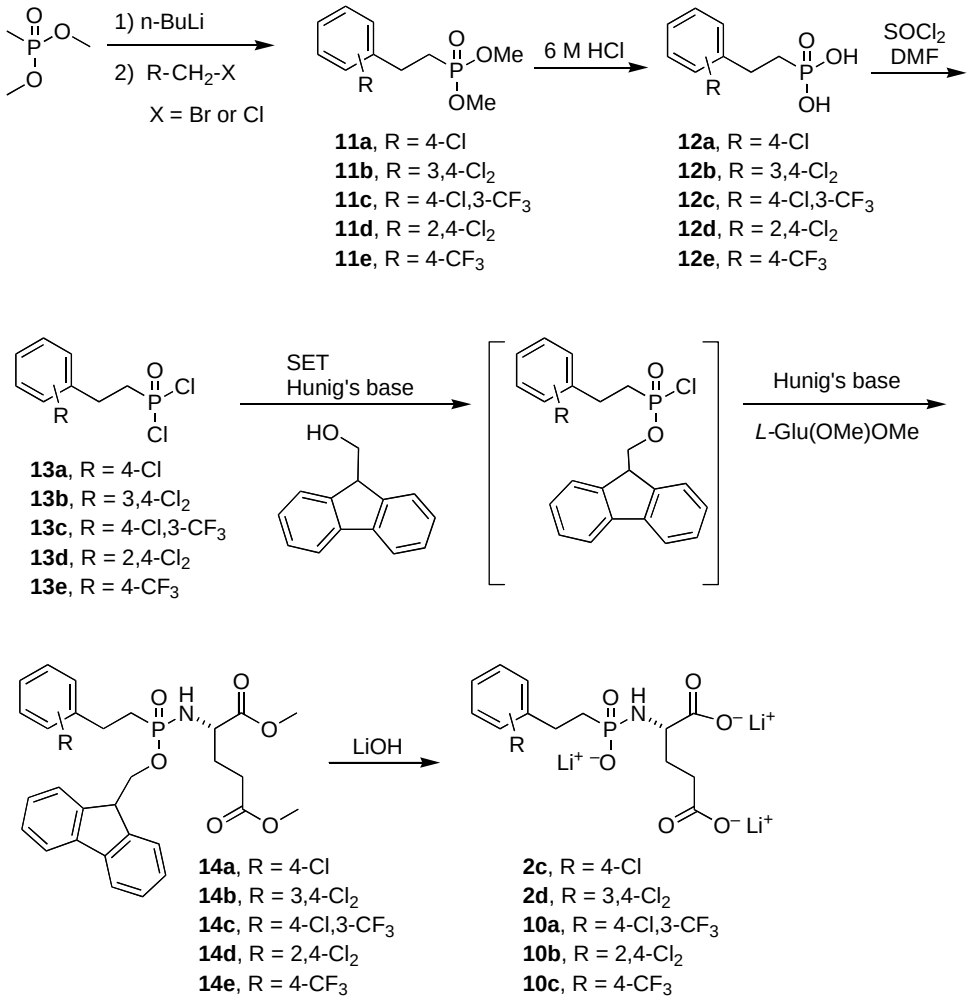


Figure 2.

Initial attempts to employ the method outlined in Scheme 1 to synthesize the 4-chlorophenyl, **2c**, and 3,4-dichlorophenyl, **2d**, inhibitors resulted in partial dehalogenation of the phenyl ring during the hydrogenolysis of the final deprotection step. The loss of chlorine was also observed when Pearlman's catalyst, $Pd(OH)_2$, was used in attempts at the hydrogenolysis of the benzyl groups of the protected precursor **8** (Fig. 2). These problems prompted an investigation into alternative conditions to circumvent dehalogenation during deprotection and allow the removal of all protecting groups in a final synthetic step.

Although sequential treatment of **8** with trimethylsilyl iodide and water[18] deprotected the carboxylate esters, it failed to remove the benzyl ester of the phosphonamidate. Refluxing **8** in the presence of trimethylsilyl bromide in CCl_4 and subsequent treatment with water and K_2CO_3 also failed to give the desired deprotected product. Similar results were also observed for the fully protected methyl ester analog **9** (Fig. 2). Based upon these results, our attention turned toward alternative protecting groups for inhibitors **2c** and **2d**. The acid labile nature of the phosphorus–nitrogen[19] provided an additional constraint upon the selection of suitable



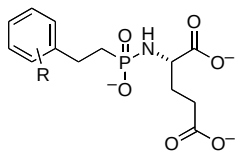
Scheme 2. Synthetic route to prepare halogen substituted analogs.

protecting groups and therefore, *tert*-butyl esters were precluded. As such, basic hydrolysis in the final deprotection step was considered plausible and was initially attempted with trimethyl esters **9** prepared through an analogous synthetic route to that described in Scheme 1. Attempts to hydrolyze the three methyl esters of **9** by treatment with lithium hydroxide failed to result in complete deprotection of the molecule, as evidenced by ^{1}H and ^{31}P NMR. We observed that the phosphorus methyl ester could not be fully hydrolyzed with excess base or extended reaction times. We theorized that the electron-donating characteristics of the nitrogen in the phosphonamidate precluded hydrolysis of the phosphonic methyl esters. More severe basic conditions were not attempted to preserve the stereochemical integrity of the glutamate α-carbon.

We had previously examined phosphonamidate protecting groups that could be conveniently deprotected by treatment with mild base.[20,21] Specifically, we confirmed that the 9-fluorenylmethyl and the 2-cyanoethyl phosphorus esters could be easily deprotected by using lithium hydroxide. Thus, our synthetic strategy was redesigned to incorporate the 9-fluorenylmethyl ester (Scheme 2). Dimethyl methylphosphonate was deprotonated with *n*-BuLi and subsequently alkylated with an appropriately substituted benzyl halide to give dimethyl phosphonates **11a** and **11b**. The phosphorus dimethyl esters were both hydrolyzed by refluxing in 6 M HCl to afford the phosphonic acids **12a** and **12b**. Upon refluxing in thionyl chloride with a catalytic amount of DMF, dichlorides **13a** and **13b** were obtained. Addition of 9-fluorenylmethanol in the presence of a catalytic amount of 5-ethylthio-1H-tetrazole (SET), followed by addition of the dimethyl ester of glutamic acid, produced the precursors **14a** and **14b**. Although minor amounts of difluorenylphosphonate side-products were sometimes observed, the desired intermediates were isolated by reversed-phase chromatography. Hydrolysis of the mixed esters **14a** and **14b** with lithium hydroxide gave the remaining first-generation inhibitors **2c** and **2d** as lithium salts.

Once obtained in sufficient quantity, the first four analogs **2a**–**2d** along with the parent inhibitor **1** were assayed for inhibition against purified PSMA, as described previously.[13] A single-dose experiment in which both the inhibitor and substrate concentrations were 1.0 μM provided the relative potencies for the first generation inhibitors (Table 1). Two striking results were observed; the 4-methoxyphenyl substitution of **2b** abrogated the inhibitory potency of the lead compound **1**, while the 3,4-dichlorophenyl derivative **2d** elicited complete inhibition under the conditions of this single-dose experiment. An examination of the individual σ and π values of the phenyl substitutions of the first generation inhibitors indicated that both positive π and σ values were favorable in terms of potency enhancement (Table 1). The 3,4-dichlorophenyl analog **2d** possessed the greatest positive σ and π values of the series and was found to be the most potent analog in this series. The 4-methoxyphenyl analog **2b**, which had both negative π and σ values, was devoid of any inhibitory activity

Table 1. Inhibition of PSMA by first generation analogs



Inhibitor	R	π[15]	σ[15]	% Inhibition[a]
1	H	0	0	71
2a	4-Me	0.56	−0.17	74
2b	4-OMe	−0.02	−0.27	0
2c	4-Cl	0.71	0.23	85
2d	3,4-Cl_2	1.25	0.52	100

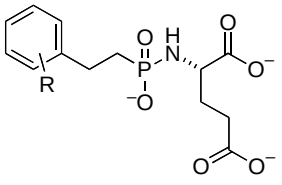
[a] 1 μM inhibitor and 1 μM substrate.

under the conditions examined. It was concluded that hydrophobicity alone could not account for the enhancement of potency because we had previously found that simply increasing the number of methylenes between the phosphonamide and the phenyl ring did not offer as great an enhancement of potency[16] as seen with the 3,4-dichlorophenyl inhibitor **2d**. A combination of factors seemed to be responsible for the enhanced potency of the 3,4-dichlorophenyl (**2d)** and the 4-chlorophenyl (**2c**) analogs. Some contribution of electronic effects, more likely than not, had a significant role in enhancing the binding of the inhibitors to PSMA's active site.

The potency ranking for this first generation of inhibitors was 3,4-Cl_2 > 4-Cl > 4-Me > H > 4-OMe. Upon comparison with the Topliss batchwise operational scheme, $\pi + \sigma$ was determined to be the dominant parameter in selecting the second set of derivatives.[15] Consequently, the analogs of a subsequent generation demanded by this approach were: 4-Cl, 3-CF_3; 3-CF_3, 4-NO_2; 4-CF_3; 2,4-Cl_2; 4-cyclopentyl; and the 4-cyclohexyl derivatives. Of these six suggested compounds, the following set of four were prepared as representatives: 4-Cl, 3-CF_3; 4-CF_3; 2,4-Cl_2; and 4-cyclohexyl. Incidentally, the 3-CF_3, 4-NO_2 analog was attempted using the strategies outlined in Scheme 2, but several attempts resulted in complex mixtures or decomposition.

The halogenated analogs **10a**–**c** from the second generation were prepared, as described in Scheme 2. The 4-cyclohexyl inhibitor **10d** was prepared utilizing the benzyl-protecting group method outlined in Scheme 1. While the other substituted benzyl halides were commercially available, the 4-cyclohexyl benzyl halide,[22–24] which was key to the alkylation step in Scheme 1, was prepared from 4-cyclohexyl benzoic acid (see Fig. 3).

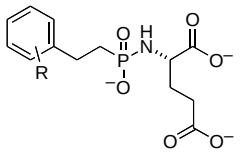
Once prepared, the second generation of inhibitors was first assayed in a single-dose experiment for relative



10a, R = 4-Cl, 3-CF_3
10b, R = 2,4-Cl_2
10c, R = 4-CF_3
10d, R = 4-cyclohexyl

Figure 3. Second generation PSMA inhibitors.

Table 2. PSMA inhibition by second generation analogs



Inhibitor	R	π[15]	σ[15]	% Inhibition[a]	K_i[b]
2d	3,4-Cl_2	1.25	0.52	62	24 (5.4)
10a	4-Cl, 3-CF_3	1.59	0.66	41	42 (5.9)
10b	2,4-Cl_2	1.42	0.46	41	46 (4.0)
10c	4-CF_3	0.88	0.54	30	85 (8.9)
10d	4-Cyclohexyl	2.51	−0.22	21	96 (11)

[a] 100 nM inhibitor and 1 μM substrate.
[b] Standard error in parenthesis.

inhibitory potency against purified PSMA (Table 2). Percent inhibition of PSMA was determined at a concentration 10-fold lower than that used in the screening of the first generation inhibitors. Dixon analyses using two substrate concentrations (1.0 and 2.0 μM) were conducted for inhibitors **2d** and **10a–d**, and the resulting K_i values are presented in Table 2. Inhibitor concentrations varied from 0.1 to 0.4 μM and representative data for inhibitor **2d** are shown in Figure 4. It was observed that no second generation inhibitors exhibited potency greater than the 3,4-dichlorophenyl analog **2d** of the first generation. Despite these results, all four analogs offered considerable improvement in the inhibition of PSMA's enzymatic activity compared to the lead compound **1** (K_i = 159 nM). All second generation inhibitors possessed positive π values. The inhibition data of this group confirm that lipophilicity is favored in the pharmacophore, as suggested by the results of the first set of compounds, as well as our previous investigation which examined longer alkyl linkers between the central phosphorus and the phenyl ring.[13] While a positive σ value is confirmed to be favored for inhibition, it may not be as important as lipophilicity. For example, the 4-cyclohexyl analog, which possesses a considerably large positive π value with a negative σ value, exhibits significant inhibition of PSMA's enzymatic activity.

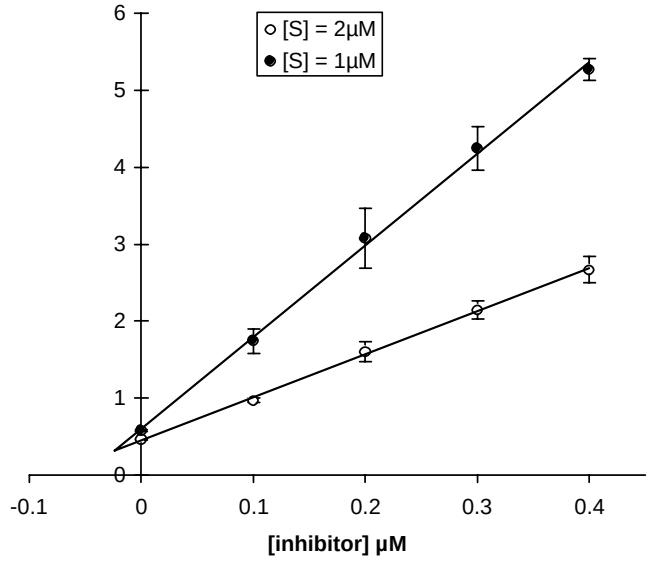


Figure 4. Dixon analysis for phosphonamidate **2d**.

3. Conclusion

Based upon a Topliss batchwise method, a series of substituted 2-phenylethylphosphonamidate inhibitors of PSMA was synthesized and assayed for inhibitory potency. Two general synthetic strategies were developed to prepare both halogenated and nonhalogenated analogs. Based upon the potency ranking order of the first generation of inhibitors, the parameter most important to the pharmacophore was determined to be $\pi + \sigma$. Attempts to optimize further the potency by refining the structures in a second generation of inhibitors did not improve the inhibitory potency. For the phenylethyl scaffold of these phosphonamidates, the 3,4-dichlorophenyl analog afforded the greatest inhibitory potency against PSMA.

In summary, the results suggest that enhancement of inhibitory potency against PSMA is not solely dependent upon lipophilicity. Rather, some attenuation of substituent electronegativity along with lipophilicity leads to enhanced potency. These results suggest that a binding site remote from the catalytic center of PMSA does not simply respond to hydrophobic interactions, but may also accommodate dipole interactions. From the homologation exercise in a separate project completed recently,[16] it was found that a pentamethylene linker provides the optimal distance between the central phosphorus atom and the tethered phenyl ring. Because optimization of the phenylethylphosphonamidate derivative described herein leads to a significant increase of PSMA inhibition, we expect that a similar refinement of structure can be achieved for the more potent phenylpentylphosphonamidate derivative and such studies are currently underway.

4. Experimental

4.1. Synthesis

Solvents and chemicals from commercial sources were used without purification. Chromatography was performed with Biotage normal-phase flash columns

containing 32–63 μm silica and with Biotage reversed-phase C_{18} flash columns. Proton, carbon, and phosphorus NMR spectra were recorded on a Bruker DRX or Varian Gemini 300 Series NMR spectrometer at 300.13 MHz for ^{1}H, 75.48 MHz for ^{13}C, and 121.4 for ^{31}P. Proton and carbon are reported in relation to tetramethylsilane used as an internal standard. The chemical shifts of ^{31}P NMR spectra are reported in relation to 85% H_3PO_4. Combustion analyses were performed by Quantitative Technologies Inc., Whitehouse, NJ. High-resolution mass spectra (FAB) were performed by the University of Notre Dame Mass Spectrometry Facility, Notre Dame, IN 46556-5670.

4.1.1. Dibenzyl methylphosphonate (3). A solution of 1.00 g (7.5 mmol, 1.0 equiv) of methyl phosphonic dichloride in 15 mL of dry benzene was prepared. While stirring under nitrogen atmosphere, 44.2 mg (0.6 mmol, 0.08 equiv) of 1-*H*-tetrazole was added. The solution was cooled to 0 °C and 1.65 mL (15.9 mmol, 2.1 equiv) of benzyl alcohol was added dropwise, followed by the dropwise addition of 2.75 mL (15.8 mmol, 2.1 equiv) of *N*-*N*-diisopropylethylamine. The reaction mixture was allowed to warm to room temperature overnight. After 16 h, the mixture was rotary evaporated and flash chromatographed using 30% ethyl acetate/hexanes ($R_f = 0.14$) as eluent to give **3** as a lightly yellow colored oil (84%). ^{1}H NMR ($CDCl_3$) δ: 1.48 (d, $J = 17.6$ Hz, 3H), 5.05 (m, 4H), 7.35 (m, 10H). ^{13}C NMR ($CDCl_3$) δ: 10.72, 12.64, 67.12, 67.20, 128.10, 128.61, 128.79, 136.50, 136.58. ^{31}P NMR ($CDCl_3$) δ: 31.94.

4.1.2. General procedure for dibenzyl 2-phenylethylphosphonates (4). A solution of 0.98 g (3.6 mmol, 1.0 equiv) of dibenzyl methylphosphonate **3** in 20 mL of dry THF was cooled to −78 °C. While stirring under nitrogen atmosphere, 1.71 mL (4.3 mmol, 1.2 equiv) of 2.5 M *n*-BuLi in hexanes was added dropwise. After addition of *n*-BuLi, 0.84 g (4.6 mmol, 1.3 equiv) of benzyl halide was added. The reaction solution was allowed to warm to room temperature. After 16 h, 30 mL of brine was added and the mixture was extracted with Et_2O (3× 50 mL). The combined organic extracts were dried with $MgSO_4$ and filtered. The filtrate was rotary evaporated and flash chromatographed producing dibenzyl phenylphosphonates as light yellow oils.

4.1.3. Dibenzyl 2-(4-methyl)phenylethylphosphonate (4a). Chromatography conditions: 30% ethyl acetate/hexanes, $R_f = 0.46$. Yield: 72%. ^{1}H NMR ($CDCl_3$) δ: 2.11 (m, 2H), 2.31 (s, 3H), 2.83 (m, 2H), 5.02 (m, 4H), 6.99 (d, $J = 7.7$ Hz, 2H), 7.07 (d, $J = 7.7$ Hz, 2H), 7.34 (m, 10H). ^{13}C NMR ($CDCl_3$) δ: 20.98, 27.21, 28.00, 28.06, 29.04, 67.18, 67.26, 73.98, 128.09, 128.14, 128.60, 128.79, 129.41, 136.06, 136.58, 137.80. ^{31}P NMR ($CDCl_3$) δ: 32.29.

4.1.4. Dibenzyl 2-(4-methoxy)phenylethylphosphonate (4b). Chromatography conditions: 30% ethyl acetate/hexanes, $R_f = 0.18$. Yield: 49%. ^{1}H NMR ($CDCl_3$) δ: 2.05 (m, 2H), 2.83 (m, 2H), 3.76 (m, 3H), 4.94 (m, 4H), 6.80 (m, 2H), 7.02 (m, 2H), 7.32 (m, 10H). ^{13}C NMR ($CDCl_3$) δ: 27.37, 27.66, 29.21, 33.41, 55.26, 55.31, 67.19, 67.27, 113.86, 114.10, 128.17, 128.63, 128.68, 128.83, 129.21, 130.30, 131.57, 131.71, 132.90, 133.14, 136.60, 136.68, 158.31, 158.37. ^{31}P NMR ($CDCl_3$) δ: 32.32.

4.1.5. Dibenzyl 2-(4-cyclohexyl)phenylethylphosphonate (4c). Chromatography conditions: 30% ethyl acetate/hexanes, $R_f = 0.23$. Yield: 31%. ^{1}H NMR ($CDCl_3$) δ: 1.36 (m, 5H), 1.81 (m, 5H), 2.07 (m, 2H), 2.46 (m, 1H), 2.84 (m, 2H), 5.02 (m, 4H), 7.03 (d, $J = 7.7$ Hz, 2H), 7.11 (d, $J = 7.7$ Hz, 2H), 7.36 (m, 10H). ^{13}C NMR ($CDCl_3$) δ: 26.13, 26.89, 27.07, 27.98, 28.04, 28.90, 34.49, 44.17, 67.18, 67.27, 127.15, 128.12, 128.59, 128.79, 136.58, 138.15, 138.39, 146.42. ^{31}P NMR ($CDCl_3$) δ: 32.39.

4.1.6. General procedure for benzyl 2-phenylethylphosphonic acid (5). A stirring solution of 0.78 g (2.1 mmol, 1.0 equiv) of dibenzyl 2-phenylethylphosphonate **4** and 0.38 g (2.5 mmol, 1.2 equiv) of NaI in 15 mL of 2-butanone was refluxed for 4.5 h under a nitrogen atmosphere. The solvent was rotary evaporated. The salt was collected by vacuum filtering, washed with cold acetone, and dissolved in 1:1 brine mixture (20 mL total) of brine and 1 M HCl. The solution was extracted with ethyl acetate (3× 50 mL). The combined organic extracts were dried with $MgSO_4$ and filtered. The filtrate was rotary evaporated and dried under vacuum to give benzyl 2-phenylethylphosphonic acids as light yellow oils.

4.1.7. 2-(4-Methyl)phenylethylphosphonic acid monobenzyl ester (5a). Yield: 65%. ^{1}H NMR ($CDCl_3$) δ: 1.99 (m, 2H), 2.25 (m, 3H), 2.83 (m, 2H), 5.01 (m, 2H), 6.97 (m, 4H), 7.29 (m, 5H). ^{13}C NMR ($CDCl_3$) δ: 20.95, 28.26, 29.28, 66.45, 67.21, 127.79, 128.03, 128.14, 128.59, 128.79, 129.26, 135.64. ^{31}P NMR ($CDCl_3$) δ: 32.32.

4.1.8. 2-(4-Methoxy)phenylethylphosphonic acid monobenzyl ester (5b). Yield: 41%. ^{1}H NMR ($CDCl_3$) δ: 2.06 (m, 2H), 2.87 (m, 2H), 3.77 (s, 3H), 5.05 (m, 2H), 6.81 (d, $J = 8.2$ Hz, 2H), 7.07 (d, $J = 8.8$ Hz, 2H), 7.32 (m, 5H). ^{13}C NMR ($CDCl_3$) δ: 27.48, 27.42, 27.30, 29.17, 55.31, 66.69, 66.60, 113.82, 114.10, 127.96, 128.78, 128.56, 129.18, 130.27, 132.88, 133.12, 136.45, 158.33. ^{31}P NMR ($CDCl_3$) δ: 34.61.

4.1.9. 2-(4-Cyclohexyl)phenylethylphosphonic acid monobenzyl ester (5c). Yield: 73%. ^{1}H NMR ($CDCl_3$) δ: 1.40 (m, 5H), 1.84 (m, 5H), 2.16 (m, 2H), 2.52 (m, 1H), 2.97 (m, 2H), 5.13 (m, 2H), 7.19 (m, 4H), 7.40 (m, 5H). ^{13}C NMR ($CDCl_3$) δ: 26.22, 26.85, 26.97, 27.07, 27.94, 27.99, 28.93, 34.32, 34.57, 44.23, 66.81, 66.90, 127.21, 127.56, 128.03, 128.17, 128.57, 128.81, 136.50, 136.58, 138.25, 138.50, 146.13. ^{31}P NMR ($CDCl_3$) δ: 34.82.

4.1.10. General procedure for benzyl 2-phenylethylphosphonamidates (7). A solution of 0.15 g (0.50 mmol, 1.0 equiv) of benzyl 2-phenylethylphosphonic acid **5** in 10 mL of dry CH_2Cl_2 was cooled to 0 °C. While stirring under a nitrogen atmosphere, 52 μL (0.61 mmol, 1.2 equiv) of oxalyl chloride and two drops of DMF were added. The cooling bath was removed 30 min later and the solution was stirred at room temperature for 1 h. The reaction solution was rotary evaporated, dried under vacuum, and then redissolved in 10 mL of dry

THF. While stirring under nitrogen atmosphere, 17 mg (0.25 mmol, 0.5 equiv) of 1-*H*-tetrazole was added. The solution was cooled to 0 °C and a solution of 0.17 g (0.52 mmol, 1.0 equiv) of *L*-Glu(OBn)OBn dissolved in 1 mL of dry THF was added, followed by the addition of 0.11 mL (0.65 mmol, 1.3 equiv) of *N*-*N*-diisopropylethylamine. The reaction mixture was allowed to warm to room temperature and stirred under nitrogen. After 15 h, the mixture was rotary evaporated and flash chromatographed to give benzyl 2-phenylethylphosphonamidates as clear colorless oils.

4.1.11. *N*-{Benzyloxy[2-(4-methyl)phenylethyl]phosphinyl}-L-glutamic acid dibenzyl ester (7a). Chromatography conditions: a step gradient 30% and 50% ethyl acetate/hexanes. Yield: 32%. ^{1}H NMR ($CDCl_3$) δ: 2.03 (m, 4H), 2.32 (s, 3H), 2.42 (m, 2H), 2.87 (m, 2H), 3.18 (m, 1H), 4.09 (m, 1H), 5.01 (m, 6H), 7.05 (m, 4H), 7.33 (m, 15 H). ^{13}C NMR ($CDCl_3$) δ: 21.01, 28.16, 29.27, 29.39, 29.62, 30.01, 30.95, 31.10, 53.13, 53.25, 65.36, 65.45, 65.56, 65.64, 66.58, 67.43, 127.81, 128.01, 128.17, 128.20, 128.42, 128.50, 128.52, 128.59, 128.76, 128.79, 128.86, 129.42, 129.46, 135.35, 135.92, 135.97, 136.02, 136.85, 136.92, 138.01, 138.23, 172.36, 172.40, 173.65, 173.72. ^{31}P NMR ($CDCl_3$) δ: 33.36, 34.05. Anal. Calcd for $C_{35}H_{38}NO_6P$: C, 70.10; H, 6.39; N, 2.34. Found: C, 69.68; H, 6.32; N, 2.31.

4.1.12. *N*-{Benzyloxy[2-(4-methoxy)phenylethyl]phosphinyl}-L-glutamic acid dibenzyl ester (7b). Chromatography conditions: 50% ethyl acetate/hexanes, R_f = 0.30. Yield: 29%. ^{1}H NMR ($CDCl_3$) δ: 1.98 (m, 4H), 2.41 (m, 2H), 2.82 (m, 2H), 3.03 (m, 1H), 3.77 (s, 3H), 4.05 (m, 1H), 5.03 (m, 6H), 6.79 (m, 2H), 7.04 (m, 2H), 7.28 (m, 15H). ^{13}C NMR ($CDCl_3$) δ: 28.19, 29.62, 29.95, 30.05, 30.15, 30.25, 30.51, 30.58, 31.75, 53.61, 53.72, 55.81, 65.83, 65.92, 66.04, 66.16, 67.07, 67.94, 114.51, 114.47, 128.15, 128.33, 128.83, 128.87, 128.94, 129.13, 129.21, 129.57, 129.61, 133.45, 133.68, 135.62, 136.21, 137.10, 137.19, 158.63, 172.93, 173.01, 173.65, 173.69. ^{31}P NMR ($CDCl_3$) δ: 33.36, 34.05. Anal. Calcd for $C_{35}H_{38}NO_7P$: C, 68.28; H, 6.22; N, 2.28. Found: C, 68.30; H, 6.24; N, 2.25.

4.1.13. *N*-{Benzyloxy[2-(4-cyclohexyl)phenylethyl]phosphinyl}-L-glutamic acid dibenzyl ester (7c). Chromatography conditions: 50% ethyl acetate/hexanes, R_f = 0.39. Yield: 29%. ^{1}H NMR ($CDCl_3$) δ: 1.35 (m, 5H), 1.81 (m, 5H), 2.06 (m, 4H), 2.43 (m, 3H), 2.88 (m, 2H), 3.19 (m, 1H), 4.11 (m, 1H), 5.02 (m, 6H), 7.10 (m, 4H), 7.29 (m, 15H). ^{13}C NMR ($CDCl_3$) δ: 26.17, 26.81, 26.92, 28.11, 28.16, 29.16, 29.29, 29.63, 29.69, 30.02, 30.08, 30.84, 31.00, 34.28, 34.54, 44.20, 53.13, 53.24, 65.38, 65.46, 65.56, 65.64, 66.58, 67.43, 127.17, 127.22, 127.52, 127.79, 128.00, 128.22, 128.38, 128.42, 128.49, 128.51, 128.59, 128.59, 128.76, 128.85, 135.36, 135.93, 138.36, 146.35, 146.41, 172.65, 172.73, 173.40. ^{31}P NMR ($CDCl_3$) δ: 33.43, 34.14. Anal. Calcd for $C_{40}H_{46}NO_6P$: C, 71.95; H, 6.94; N, 2.10. Found: C, 71.60; H, 6.93; N, 2.09.

4.1.14. General procedure for 2-phenylethylphosphonamidates (2a–b, 10d). A solution of 0.08 g (0.14 mmol, 1.0 equiv) of benzyl 2-phenylethylphosphonamidate **7** in 2 mL THF and 1 mL water was prepared. While stirring, 31 mg (0.23 mmol, 1.7 equiv) of K_2CO_3 was added, followed by the addition of 17 mg of 10% Pd on carbon. The mixture was purged with $H_{2(gas)}$ and stirred under a balloon of H_2 for 3 h. The mixture was filtered through Celite and the filtrate was concentrated under reduced pressure to give 2-phenylethylphosphonamidates as light yellow solids.

4.1.15. *N*-{Hydroxy[2-(4-methyl)phenylethyl]phosphinyl}-L-glutamic acid tripotassium salt (2a). Yield: 96%. ^{1}H NMR (D_2O) δ: 1.76 (m, 4H), 2.20 (m, 2H), 2.26 (s, 3H), 2.69 (m, 2H), 3.45 (m, 1H), 7.16 (d, J = 8.7 Hz, 4H). ^{13}C NMR (D_2O) δ: 23.75, 32.80, 34.62, 36.25, 37.75, 60.38, 131.93, 133.01, 139.70, 143.78, 144.02, 185.73, 186.91. ^{31}P NMR (D_2O) δ: 26.47. FAB-HRMS: Calcd for $C_{14}H_{17}K_2NO_6P$ $(M-K)^-$ 404.0068. Found 404.0074.

4.1.16. *N*-{Hydroxy[2-(4-methoxy)phenylethyl]phosphinyl}-L-glutamic acid tripotassium salt (2b). Yield: quantitative. ^{1}H NMR (D_2O) δ: 1.63 (m, 4H), 2.09 (m, 2H), 2.58 (m, 2H), 3.34 (m, 1H), 3.67, (s, 3H), 6.81 (d, J = 8.1 Hz, 2H), 7.11 (d, J = 8.1 Hz, 2H). ^{13}C NMR (D_2O) δ: 30.17, 32.52, 34.03, 34.10, 35.58, 56.95, 58.22, 115.71, 115.71, 130.90, 137.39, 137.63, 158.51, 183.59, 184.76. ^{31}P NMR (D_2O) δ: 26.38. FAB-HRMS: Calcd for $C_{14}H_{17}K_2NO_7P$ $(M-K)^-$ 420.0017. Found 420.0032.

4.1.17. *N*-{Hydroxy[2-(4-cyclohexyl)phenylethyl]phosphinyl}-L-glutamic acid tripotassium salt (10d). Yield: quantitative. ^{1}H NMR (D_2O) δ: 1.22 (m, 5H), 1.67 (m, 9H), 2.12 (m, 2H), 2.58 (m, 1H), 2.62 (m, 2H), 3.38 (m, 1H), 7.15 (s, 4H). ^{13}C NMR (D_2O) δ: 27.03, 27.80, 30.40, 32.15, 33.80, 35.36, 35.42, 44.93, 57.99, 128.44, 129.65, 141.95, 142.19, 147.82, 183.32, 184.51. ^{31}P NMR (D_2O) δ: 26.44. FAB-HRMS: Calcd for $C_{19}H_{25}K_2NO_6P$ $(M-K)^-$ 472.0694. Found 472.0704.

4.1.18. General procedure for 2-phenylethylphosphonic acid dimethyl ester (11). Compounds were prepared as described in the general procedure for compound **3** using methyl dimethylphosphonate and benzyl halide.

4.1.19. 2-(4-Chloro)phenylethylphosphonic acid dimethyl ester (11a). Chromatography conditions: a step gradient of 75% ethyl acetate/hexanes and pure ethyl acetate. Yield: 25%. ^{1}H NMR ($CDCl_3$) δ: 1.95 (m, 2H), 2.89 (m, 2H), 3.75 (m, 6H), 7.14 (d, J = 8.2 Hz, 2H), 7.27 (d, J = 8.2 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ: 22.44, 24.29, 24.71, 49.32, 125.56, 126.28, 127.23, 129.04, 135.92, 136.14. ^{31}P NMR ($CDCl_3$) δ: 33.20.

4.1.20. 2-(3,4-Dichloro)phenylethylphosphonic acid dimethyl ester (11b). Chromatography conditions: a step gradient of 75% ethyl acetate/hexanes and pure ethyl acetate. Yield: 33%. ^{1}H NMR ($CDCl_3$) δ: 2.04 (m, 2H), 2.88 (m, 2H), 3.74 (m, 6H), 7.05 (d, J = 7.7 Hz, 1H), 7.33 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 25.38, 27.25, 27.71, 27.76, 52.45, 52.54, 127.73, 128.55, 130.25, 130.46, 130.70, 131.15. ^{31}P NMR ($CDCl_3$) δ: 32.64.

4.1.21. 2-(4-Chloro-3-trifluoromethyl)phenylethylphosphonic acid dimethyl ester (11c). Chromatography conditions: a step gradient of 75% ethyl acetate/hexanes and pure ethyl

acetate. Yield: 39%. ^{1}H NMR ($CDCl_3$) δ: 2.05 (m, 2H), 2.95 (m, 2H), 3.73 (2s, 6H), 7.32 (d, J = 8.2 Hz, 1H), 7.44 (d, J = 8.2 Hz, 1H), 7.53 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 25.37, 27.24, 27.87, 27.92, 52.45, 52.54, 127.37, 127.44, 131.80, 132.75, 139.89, 140.11. ^{31}P NMR ($CDCl_3$) δ: 32.42.

4.1.22. 2-(2,4-Dichloro)phenylethylphosphonic acid dimethyl ester (11d). Chromatography conditions: a step gradient of 75% ethyl acetate/hexanes and pure ethyl acetate. Yield: 11%. ^{1}H NMR ($CDCl_3$) δ: 2.04 (m, 2H), 2.97 (m, 2H), 3.75 (m, 6H), 7.18 (m, 2), 7.36 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 23.57, 25.43, 26.30, 26.35, 52.42, 52.51, 127.49, 129.58, 131.24, 133.21, 136.97. ^{31}P NMR ($CDCl_3$) δ: 32.75.

4.1.23. 2-(4-Trifluoromethyl)phenylethylphosphonic acid dimethyl ester (11e). Chromatography conditions: a step gradient of 75% ethyl acetate/hexanes and pure ethyl acetate. Yield: 35%. ^{1}H NMR ($CDCl_3$) δ: 2.06 (m, 2H), 2.97 (m, 2H), 3.71 (2s, 6H), 7.32 (d, J = 8.4 Hz, 2H), 7.55 (d, J = 8.2 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ: 25.36, 27.22, 28.34, 28.39, 52.41, 52.51, 125.68, 125.74, 128.59, 144.90. ^{31}P NMR ($CDCl_3$) δ: 32.86.

4.1.24. General procedure for 2-phenylethylphosphonic acid (12). A suspension of 0.80 g (2.8 mmol) of **11** in 16 mL of 6 M HCl was refluxed for 16 h. The mixture was cooled to room temperature and an oil formed. The mixture was extracted with Et_2O (3× 50 mL). The combined organic extracts were dried with $MgSO_4$ and filtered. The filtrate was rotary evaporated and dried under vacuum to give **12** as a clear colorless oil.

4.1.25. 2-(4-Chloro)phenylethylphosphonic acid (12a). ^{1}H NMR ($CDCl_3$) δ: 2.08 (m, 2H), 2.88 (m, 2H), 7.11 (d, J = 8.2 Hz, 2H), 7.25 (d, J = 8.2 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ: 27.63, 27.7, 28.65, 128.80, 128.92, 129.55, 130.55, 132.43. ^{31}P NMR ($CDCl_3$) δ: 34.55.

4.1.26. 2-(3,4-Dichloro)phenylethylphosphonic acid (12b). ^{1}H NMR ($CDCl_3$) δ: 2.10 (m, 2H), 2.89 (m, 2H), 7.04 (d, J = 8.2 Hz, 1H), 7.31 (s, 1H), 7.37 (d, J = 8.2 Hz, 1H). ^{13}C NMR ($CDCl_3$): 29.46, 33.19, 127.45, 128.23, 130.07, 130.26, 130.52, 130.95, 132.33, 132.49. ^{31}P NMR ($CDCl_3$) δ: 34.53.

4.1.27. 2-(4-Chloro-3-trifluoromethyl)phenylethylphosphonic acid (12c). Yield: 83%. ^{1}H NMR ($CDCl_3$) δ: 2.09 (m, 2H), 2.94 (m, 2H), 7.32 (d, J = 8.8 Hz, 1H), 7.44 (d, J = 8.8 Hz, 1H), 7.54 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 25.98, 27.67, 127.38, 127.45, 131.84, 132.73, 139.69, 139.92. ^{31}P NMR ($CDCl_3$) δ: 33.84.

4.1.28. 2-(2,4-Dichloro)phenylethylphosphonic acid (12d). Yield: 87%. ^{1}H NMR ($CDCl_3$) δ: 2.09 (m, 2H), 2.95 (m, 2H), 7.23 (m, 2H), 7.37 (m, 1H). ^{13}C NMR ($CDCl_3$) δ: 25.88, 25.96, 29.45, 127.25, 129.34, 129.40, 130.89, 132.27, 132.55. ^{31}P NMR ($CDCl_3$) δ: 32.77.

4.1.29. 2-(4-Trifluoromethyl)phenylethylphosphonic acid (12e). Yield: 94%. ^{1}H NMR ($CDCl_3$) δ: 2.14 (m, 2H), 2.99 (m, 2H), 7.32 (d, J = 7.7 Hz, 2H), 7.56 (d, J = 8.2 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ: 28.09, 125.74, 125.79, 128.58, 129.47. ^{31}P NMR ($CDCl_3$) δ: 34.40.

4.1.30. General procedure for 9-fluorenylmethyl 2-phenylethylphosphonamidates (14). A solution of 0.10 g (0.41 mmol, 1.0 equiv) of **12** in 3 mL of thionyl chloride with two drops of DMF was refluxed under nitrogen atmosphere for 3.5 h. The yellow reaction solution was rotary evaporated and dried under vacuum to give a yellow oil, which was redissolved in 1.5 mL of dry benzene. While stirring under nitrogen, 6.6 mg (0.051 mmol, 0.1 equiv) of 5-ethylthio-1H-tetrazole was added. After cooling to 0 °C, a solution of 69 mg (0.35 mmol, 0.9 equiv) of 9-fluorenylmethanol and 71 μL (0.41 mmol, 1.0 equiv) of *N*,*N*-diisopropylethylamine in 2 mL of dry benzene was added dropwise. The bath was removed after 15 min and the mixture was stirred at room temperature for 2.5 h. The mixture was cooled back to 0 °C and a solution of 81 mg (0.46 mmol, 1.1 equiv) of L-Glu(OMe)OMe and 85 μL (0.46 mmol, 1.2 equiv) of *N*-*N*-diisopropylethylamine in 2 mL of dry benzene was added dropwise. The mixture was allowed to warm to room temperature. After 17 h, 30 mL of ethyl acetate was added. The solution was washed with 10% w/v citric acid (2× 5 mL). The organic layer was dried with $MgSO_4$ and filtered. The filtrate was rotary evaporated. The crude material was flash chromatographed to give the products as clear colorless oils.

4.1.31. *N*-{9-Fluorenylmethoxy[2-(4-chloro)phenylethyl]phosphinyl}-L-glutamic acid dimethyl ester (14a). Chromatography conditions: a step gradient of 50% and 75% ethyl acetate/hexanes. Yield: 6%. ^{1}H NMR ($CDCl_3$) δ: 1.94 (m, 4H), 2.33 (m, 2H), 2.71 (m, 2H), 3.67 (m, 6H), 3.93 (m, 1H), 4.20 (m, 2H), 4.50 (m, 1H), 7.05 (m, 2H), 7.34 (m, 6H), 7.60 (m, 2H), 7.77 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 27.42, 27.76, 27.82, 29.03, 29.51, 29.59, 29.66, 29.72, 29.82, 30.33, 30.69, 30.77, 36.13, 48.15, 48.25, 48.35, 51.79, 51.85, 52.45, 52.55, 52.97, 53.03, 53.37, 65.50, 65.59, 120.17,120.22, 124.98, 125.04, 125.25, 125.31, 127.31, 128.01, 128.04, 128.07, 128.12, 128.85, 129.59, 129.65, 132.28, 141.62, 141.68, 141.74, 143.36, 143.58, 143.92, 144.05, 173.21, 173.35, 173.96, 174.13. ^{31}P NMR ($CDCl_3$) δ: 32.42, 33.02. Anal. Calcd for $C_{29}H_{31}ClNO_6P$: C, 62.65; H, 5.62; N, 2.52. Found: C, 62.24; H, 5.16; N, 2.31.

4.1.32. *N*-{9-Fluorenylmethoxy[2-(3,4-dichloro)phenylethyl]phosphinyl}-L-glutamic acid dimethyl ester (14b). Chromatography conditions: a step gradient of 50% and 75% ethyl acetate/hexanes. Yield: 28%. ^{1}H NMR ($CDCl_3$) δ: 1.92 (m, 4H), 2.36 (m, 2H), 2.64 (m, 2H), 3.66 (m, 6H), 3.92 (m, 1H), 4.19 (m, 2H), 4.55 (m, 1H), 6.95 (m, 1H), 7.31 (m, 7H), 7.60 (m, 2H), 7.78 (m, 1H). ^{13}C NMR ($CDCl_3$) δ: 27.59, 27.65, 28.78, 29.57, 29.72, 29.83, 30.53, 48.14, 48.24, 48.35, 51.90, 52.61, 53.01, 65.51, 65.60, 120.25, 124.91, 125.27, 127.36, 127.71, 127.75, 128.06, 128.11, 128.16, 130.21, 130.66, 132.60, 141.66, 143.31, 143.52. ^{31}P NMR ($CDCl_3$) δ: 31.97, 32.54. Anal. Calcd for $C_{29}H_{30}Cl_2NO_6P$: C, 58.99; H, 5.12; N, 2.37. Found: C, 58.86; H, 5.08; N, 2.48.

4.1.33. *N*-{9-Fluorenylmethoxy[2-(4-chloro-3-trifluoromethyl)phenylethyl]phosphinyl}-L-glutamic acid dimethyl ester (14c). Chromatography conditions: 75% EtOAc/hexanes, R_f = 0.44; followed by reverse phase (C_{18}) with 25% H_2O/acetonitrile, R_f = 0.44. Yield: 8%. ^{1}H NMR

($CDCl_3$) δ: 1.94 (m, 4H), 2.34 (m, 2H), 2.68 (m, 2H), 3.67 (m, 6H), 3.94 (m, 1H), 4.26 (m, 2H), 4.56 (m, 1H), 7.22 (m, 7H), 7.60 (m, 2H), 7.77 (d, J = 7.7 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ: 27.68, 28.80, 29.54, 29.75, 29.84, 48.15, 48.25, 51.86, 52.59, 53.03, 65.55, 100.21, 120.22, 124.88, 124.94, 125.21, 125.26, 127.35, 128.06, 128.12, 128.17, 131.77, 132.77, 141.71, 141.78, 143.33, 173.21, 173.37. ^{31}P NMR ($CDCl_3$) δ: 31.77, 32.33. Anal. Calcd for $C_{30}H_{30}ClF_3NO_6P$: C, 57.75; H, 4.85; N, 2.24 N. Found: C, 57.76; H, 4.56; N, 2.11.

4.1.34. *N*-{9-Fluorenylmethoxy[2-(2,4-dichloro)phenylethyl]phosphinyl}-L-glutamic acid dimethyl ester (14d). Chromatography conditions: a step gradient of 50% and 75% ethyl acetate/hexanes. Yield: 26%. ^{1}H NMR ($CDCl_3$) δ: 1.97 (m, 4H), 2.35 (m, 2H), 2.82 (m, 2H), 3.68 (m, 6H), 3.97 (m, 1H), 4.26 (m, 2H), 4.51 (m, 1H), 7.18 (m, 2H), 7.37 (m, 5H), 7.62 (m, 2H), 7.77 (d, J = 7.1 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ: 26.27, 26.31, 27.10, 27.18, 28.79, 28.92, 29.57, 29.62, 29.77, 29.82, 48.12, 48.22, 48.32, 51.77, 51.82, 52.53, 52.99, 53.09, 65.61, 65.70, 120.19, 120.22, 125.02, 125.06, 125.27, 125.35, 127.31, 127.51, 128.00, 128.04, 128.07, 128.11, 129.52, 131.22, 133.07, 133.10, 134.56, 137.20, 141.56, 141.61, 141.66, 141.73, 143.33, 143.54, 143.87, 143.98, 173.20, 173.31, 173.93. ^{31}P NMR ($CDCl_3$) δ: 32.20, 32.79. Anal. Calcd for $C_{30}H_{30}ClF_3NO_6P$: C, 58.99; H, 5.12; N, 2.37. Found: C, 59.05; H, 5.02; N, 2.41.

4.1.35. *N*-{9-Fluorenylmethoxy[2-(4-trifluoromethyl)phenylethyl]phosphinyl}-L-glutamic acid dimethyl ester (14e). Chromatography conditions: 75% EtOAc/hexanes, R_f = 0.43. Yield: 38%. ^{1}H NMR ($CDCl_3$) δ: 1.96 (m, 4H), 2.34 (m, 2H), 2.76 (m, 2H), 3.65 (m, 6H), 4.96 (m, 1H), 4.23 (m, 2H), 4.54 (m, 1H), 7.22 (m, 2H), 7.37 (m, 4H), 7.59 (m, 4H), 7.78 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 28.22, 28.27, 28.32, 28.37, 28.84, 29.51, 29.58, 29.64, 29.74, 29.83, 30.51, 30.58, 48.16,48.26, 48.36, 51.79, 51.84, 52.56, 53.02, 53.08, 65.53, 65.62, 120.18, 120.23, 120.27, 124.94, 125.00, 125.22, 125.28, 125.67, 125.72, 127.33,128.03, 128.07, 128.10, 128.15, 128.57,128.62, 141.66, 141.78,143.33, 143.56, 143.88, 144.00, 173.34, 173.96. ^{31}P NMR ($CDCl_3$) δ: 32.20, 32.78. Anal. Calcd for $C_{30}H_{31}F_3NO_6P$: C, 61.12; H, 5.30; N, 2.38. Found: C, 60.68; H, 5.08; N, 2.69.

4.1.36. General procedure for 2-phenylethylphosphonamidates (2c–d, 10a–c). A solution of 80 mg (0.14 mmol, 1.0 equiv) of **14** in 0.8 mL MeOH was prepared. While stirring, a solution of 21 mg (0.51 mmol, 3.8 equiv) of $LiOH{\cdot}H_2O$ dissolved in 0.5 mL water was added. After stirring the mixture at rt for 3.5 h, the solvent was removed by rotary evaporation. The crude material was resuspended in MeOH and was filtered through a 0.2 μm Whatman teflon membrane. The filtrate was rotary evaporated and resuspended in 4 mL water. The mixture was washed with CH_2Cl_2 (3× 2 mL). The aqueous was concentrated under reduced pressure and was dried under vacuum to give the product as a light yellow solid.

4.1.37. *N*-{Hydroxy[2-(4-chloro)phenylethyl]phosphinyl}-L-glutamic acid trilithium salt (2c). Yield: quantitative. ^{1}H NMR (D_2O) δ: 1.63 (m, 4H), 2.05 (m, 2H), 2.57 (m, 2H), 3.30 (m, 1H), 7.08 (d, J = 8.4 Hz, 2H), 7.15 (d, J = 8.4 Hz, 2H). ^{13}C NMR (D_2O) δ: 30.20, 31.87, 33.46, 33.62, 33.69, 35.26, 57.89, 129.71, 131.00, 132.19, 142.88, 143.12, 169.68, 183.30, 183.34, 184.50. ^{31}P NMR (D_2O) δ 26.23. FAB-HRMS: Calcd for $C_{13}H_{14}ClLi_2NO_6P$ $(M-Li)^-$ 360.0567. Found 360.0542.

4.1.38. *N*-{Hydroxy[2-(3,4-dichloro)phenylethyl]phosphinyl}-L-glutamic acid trilithium salt (2d). Yield: 84%. ^{1}H NMR (D_2O) δ: 1.72 (m, 4H), 2.16 (m, 2H), 2.70 (m, 2H), 3.43 (m, 1H), 7.15 (d, J = 6.3 Hz, 1H), 7.40 (m, 2H). ^{13}C NMR (D_2O) δ: 28.91, 30.42, 32.01, 32.54, 32.61, 34.14, 45.66, 56.78, 128.23, 128.89, 130.10, 130.37, 131.43, 143.71, 143.95, 182.09, 183.24. ^{31}P NMR (D_2O) δ: 26.16. FAB-HRMS: Calcd for $C_{13}H_{13}Cl_2Li_2NO_6P$ $(M-Li)^-$ 394.0178. Found 394.0158.

4.1.39. *N*-{Hydroxy[2-(4-chloro-3-trifluromethyl)phenylethyl]phosphinyl}-L-glutamic acid trilithium salt (10a). Yield: 97%. ^{1}H NMR (D_2O) δ: 1.68 (m, 4H), 2.06 (m, 2H), 2.67 (m, 2H), 3.37 (m, 1H), 7.35 (m, 2H), 7.55 (s, 1H). ^{13}C NMR (D_2O) δ: 30.19, 31.61, 33.20, 33.65, 35.29, 57.92, 128.78, 132.60, 134.49, 143.75, 183.34, 184.50. ^{31}P NMR (D_2O) δ: 25.75. FAB-HRMS: Calcd for $C_{14}H_{13}ClF_3Li_2NO_6P$ $(M-Li)^-$ 428.0441. Found 428.0432.

4.1.40. *N*-{Hydroxy[2-(2,4-dichloro)phenylethyl]phosphinyl}-L-glutamic acid trilithium salt (10b). Yield: 82%. ^{1}H NMR (D_2O) δ: 1.70 (m, 4H), 2.12 (m, 2H), 2.74 (m, 2H), 3.42 (m, 1H), 7.16 (m, 2H), 7.31 (s, 1H). ^{13}C NMR (D_2O) δ: 28.56, 29.84, 31.44, 33.80, 35.27, 128.62, 130.12, 132.53, 132.99, 135.17, 140.24, 140.48, 183.23, 184.57. ^{31}P NMR (D_2O) δ: 25.83. FAB-HRMS: Calcd for $C_{13}H_{13}Cl_2Li_2NO_6P$ $(M-Li)^-$ 394.0178. Found 394.0163.

4.1.41. *N*-{Hydroxy[2-(4-trifluoromethyl)phenylethyl]phosphinyl}-L-glutamic acid trilithium salt (10c). Yield: 74%. ^{1}H NMR (D_2O) δ: 1.79 (m, 4H), 2.17 (m, 2H), 2.77 (m, 2H), 3.42 (m, 1H), 7.37 (d, J = 7.8 Hz, 2H), 7.56 (d, J = 7.8 Hz, 2H). ^{13}C NMR (D_2O) δ: 30.83, 31.73, 33.33, 33.76, 33.83, 35.40, 58.05, 126.78, 127.67, 128.56, 130.02, 148.82, 149.06, 183.43, 184.56. ^{31}P NMR (D_2O) δ: 25.92. FAB-HRMS: Calcd for $C_{14}H_{14}F_3Li_2NO_6P$ $(M-Li)^-$ 394.0831. Found 394.0809.

4.2. PSMA inhibition assay

Inhibition studies were performed as described previously with only minor modifications[16] and are briefly presented here. Working solutions of the substrate (*N*-[4-(phenylazo)benzoyl]-glutamyl-γ-glutamic acid, PABGγG) and all inhibitors were made in Tris buffer (50 mM, pH 7.4) containing 150 mM NaCl. Working solutions of purified PSMA[16] were appropriately diluted in Tris buffer (50 mM, pH 7.4) to provide no more than 15% conversion of substrate to product in the absence of inhibitor. A typical incubation mixture (final volume 250 μL) was prepared by the addition of either 25 μL of an inhibitor solution or 25 μL Tris buffer (50 mM, pH 7.4) to 175 μL Tris buffer (50 mM, pH 7.4) in a test tube. A volume of 25 μL of the PSMA working solution was added to the above solution. The enzymatic reaction was initiated by the addition of 25 μL PABGγG (10 or 20 μM). In all

cases, the final concentration of PABGγG was either 1 or 2 μM, while the final inhibitor concentration varied from 0.1 to 0.4 μM. The reaction was allowed to proceed for 15 min with constant shaking at 37 °C and was terminated by the addition of 25 μL methanolic TFA (2% trifluoroacetic acid by volume in methanol), followed by vortexing. The quenched incubation mixture was then buffered by the addition of 25 μL K_2HPO_4 (0.1 M), vortexed, and centrifuged (10 min at 7000*g*). An 85 μL aliquot of the resulting supernatant was subsequently quantified by HPLC as previously described.[16]

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