Synthesis of *N*-Phosphonamidothionate Derivatives of Glutamic Acid

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Introduction

Although *N*-phosphonothionylamino acids have not commonly been considered as potential enzyme inhibitors, the analogous phosphonamidates are well-known to function as potent tetrahedral-intermediate analogue inhibitors of metallopeptidases.[1] With such phosphonamidate inhibitors, active-site metal ions, specifically Zn^{2+}, have been shown to coordinate with the phosphonyl oxygen ligands analogous to the putative bidentate complex of the enzymatic transition-state or tetrahedral intermediate.[2] Although the formations of Zn^{2+}−oxygen complexes are favorable, the coordination of Zn^{2+} with sulfur is of a more covalent nature.[3] Therefore, the simple replacement of a phosphonyl oxygen with a sulfur atom is anticipated to provide more potent inhibitors of metallopeptidases due to enhanced chelation of active-site metals. In addition, *N*-phosphonothionylamino acids are unique to their respective phosphonamidate counterparts as they possess a chiral center at phosphorus that may be invaluable for probing active-site architecture. Although *N*-thiophosphonylamino groups have been greatly overlooked as potentially potent, transition-state analogue, or tetrahedral-intermediate inhibitors of metalloproteases, progress toward developing synthetic strategies for phosphonamidothionates has recently been pioneered.[4]

Our main objective in this investigation was to synthesize putative inhibitors of glutamate carboxypeptidases such as prostate specific membrane antigen (PSMA),[5] N-acetylated α-linked acidic dipeptidase (NAALADase),[6] and carboxypeptidase G (CPG),[7] all of which function to hydrolytically cleave terminal glutamate residues. Investigations thus far have indicated that these enzymes are metallopeptidases with the greatest information gathered for a form of CPG due to the recent acquisition of its crystal structure confirming the presence of zinc(II) in the active site.[8] In previous studies with a partially purified form of CPG, we observed that *N*-phosphonamidate derivatives of glutamic acid (**1**, Figure 1) were indeed competitive inhibitors, albeit weak.[9] Based on that limited success, we have proposed the phosphonamidothionates (**2**) as putative and potent inhibitors of glutamate carboxypeptidases.

Results and Discussion

Due to the susceptibility of the phosphorus−nitrogen bond to acidic conditions, it was desired to use a base-labile protecting group on phosphorus such as the 2-cyanoethoxy ligand, which has seen limited use for the preparation of phosphonamidothionates[10] but extensive use in the synthesis of nucleoside phosphorodithioates.[11] Base-labile protecting groups for the carboxylic acids of glutamic were also chosen with the anticipation that all the protecting groups could be conveniently removed in one final step. On the basis of these considerations, the intermediate targets **4** (Scheme 1) were pursued.

Because we had recently developed a convenient method for the preparation of the intermediate phosphoramidate esters **3** in a one-pot, two-step reaction,[12] we sought to prepare the series of phosphonamidothionate esters **4** through path A in Scheme 1. Although **3a** and **3b** could be prepared via this route, their preparation generally suffered low yields while good yields (greater than 60%) were obtained for the intermediates **3c** and **3d**. These intermediates were subsequently thionated with Lawesson's reagent to provide the respective phosphonamidothionate esters **4c** and **4d**.

To circumvent the difficulties encountered in path A for the preparation of **4a**, a more direct route was pursued. Thus, methylphosphonothioic dichloride was employed in a direct and sequential thiophosphonylation of 3-hydroxypropionitrile and glutamic acid dimethyl ester to give the phosphonamidothionate ester **4a** (Scheme 1, path B). Due to the ready availability of phenylphosphonothioic dichloride, path B was also explored for the preparation of **4d** and was found to be superior to path A. Alternatively, recent methodology which was successful for the preparation of nucleoside methanephosphonothioanilidates was applied to the preparation of **4b** (Scheme 1, path C).[13] The results from this three-step, one-pot sequence provided **4b** in good yield. It should be noted that through all three pathways delineated the

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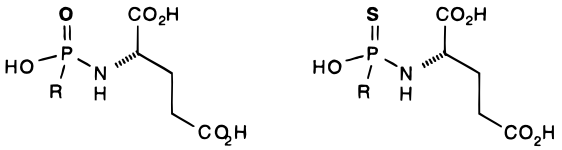

phosphonamidate **1** phosphonamidothionate **2**

Figure 1. Structures of phosphorus-containing glutamate carboxypeptidase inhibitors.

phosphonamidothionate esters **4** were provided as a mixture of diastereomeric mixtures, racemic at phosphorus. Because chromatographic resolution of diastereomeric mixtures of **4** proved to be difficult, further attempts for the resolution of these compounds have been postponed.

Although 2-cyanoethyl phosphorus esters are traditionally removed with aqueous ammonia, we explored the possibility of removing this group with LiOH, the same conditions that would be employed to hydrolyze the C-terminal methyl esters. Indeed, we found that in the case of our phosphonamidothionate ester intermediates **4** complete deprotection occurred to give the target phosphonamidothionates **2** as the trilithium salts. It is noteworthy to mention that of the two cosolvents explored for this dual deprotection with LiOH, it was found that in methanol the reaction was complete after 18 h, while in acetonitrile the reaction was still incomplete after 72 h. It was noted that upon complete deprotection of phosphonamidothionate esters **4** to the phosphonamidothionates **2**, the diastereomeric ratios were not altered.

Ultimately, it is anticipated that these compounds will prove to be more potent inhibitors of metallopeptidases than analogous phosphonamidates, and preliminary results for **1a** and **1d** with the CPG and PSMA have indicated as much. More detailed investigations into the mode and kinetics of inhibition are currently underway, and the results will be forthcoming. In summary, we have identified three distinct strategies for the preparation of simple amino acid-containing phosphonamidothionates. On the basis of preliminary evidence, such compounds containing the phosphonamidothionate motif show strong promise as potent tetrahedral-intermediate analogue inhibitors of metallopeptidases with the unique value of probing enzyme active sites with complementary chiral phosphorus centers.

Experimental Section

General Methods. All solvents used in reactions (benzene, CH_2Cl_2, THF), 3-hydoxypropionitrile, diisopropylethylamine (DEA), and triethylamine (TEA) were freshly distilled prior to use. Sulfur was recrystallized from toluene. All other reagents were used as supplied unless otherwise stated. Liquid (flash)[14] chromatography was carried out using silica gel 60 (230−400 mesh). ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a Bruker DRX 300 MHz NMR spectrometer. ^{1}H NMR chemical shifts are relative to TMS (δ = 0.00 ppm), $CDCl_3$ (δ = 7.24 ppm), or CD_3OD (δ = 4.87 and 3.31 ppm). ^{13}C NMR chemical shifts are relative to CD_3OD (δ = 49.15 ppm). ^{31}P NMR chemical shifts are relative to 85% H_3PO_4 (δ = 0.00 ppm). Combustion analyses were performed by Quantitative Technologies Inc., Whitehouse, NJ. High-resolution mass spectra (FAB) were performed by the UW Medicinal Chemistry Mass Spectrometry Center, Seattle, WA. High-resolution mass spectra (ESI) were performed by the UC Berkeley Mass Spectrometry Facility, Berkeley, CA.

General Procedure for Path A (Procedure A). A flask was charged with 1*H*-tetrazole (18.2 mg, 0.26 mmol) and benzene (9 mL) under an $Ar_{(g)}$ atmosphere followed by the dropwise addition of an alkylphosphonic dichloride (2.86 mmol), and the temperature was reduced to 4 °C. To the stirring reaction mixture were sequentially added 3-hydroxypropionitrile (0.19 g, 2.60 mmol) and diisopropylethylamine (0.5 mL, 2.86 mmol) dropwise via syringe. The reaction mixture was stirred and allowed to warm to room temperature until 3-hydroxypropionitrile was consumed (approximately 2 h) as monitored by TLC. Glutamic acid dimethyl ester hydrochloride (0.61 g, 2.86 mmol) and DEA (1.0 mL, 5,72 mmol) were dissolved in benzene (8 mL) and added dropwise to the reaction mixture, and the resulting mixture was allowed to stir for an additional 3 h. The reaction mixture was concentrated in vacuo, and the resulting oil was directly purified by flash chromatography to give the phosphonamidate esters **3**. The phosphonamidothionate esters **4** were prepared by refluxing a solution of **3** (0.72 mmol) and Lawesson's reagent (0.16 g, 0.4 mmol) in toluene (6 mL) for 3 h. The reaction mixture was concentrated in vacuo, and the resulting oil was purified by flash chromatography.

General Procedure for Path B (Procedure B). A solution of 3-hydroxypropionitrile (0.43 g, 6 mmol) and triethylamine (0.9 mL, 6 mmol) was added via syringe to a stirring solution of an alkylphosphonothioic dichloride (6 mmol) in methylene chloride (7 mL) at −5 °C. The resulting solution was warmed to 20 °C and stirred 3 h. A solution L-glutamic dimethyl ester hydrochloride (0.14 g, 6.6 mmol) and triethylamine (2.5 mL, 18 mmol) in methylene chloride (20 mL) was added to the reaction mixture and stirred for an additional 3 h, after which time the reaction mixture was diluted with methylene dichloride (20 mL), washed with 20 mL of 2 M H_2SO_4, dried with Na_2SO_4, and concentrated in vacuo. The resulting crude product was obtained as a viscous yellow oil that was purified by flash chromatography.

***N*-[2-Cyanoethoxy(*n*-butyl)phosphinyl]-L-glutamic Acid Dimethyl Ester (3c). 3c** was prepared using procedure A and purified by flash chromatography (MeOH/ethyl acetate 2.5:97.5 v/v, R_f = 0.19) to give a colorless oil (0.52 g, 1.49 mmol, 58% yield). ^{1}H NMR ($CDCl_3$) δ: 0.91 (t, J = 7.26 Hz, 3H), 1.40 (dt, J = 7.2, 14.6 Hz, 2H), 1.52−1.57 (m, 2H), 1.70−1.77 (m, 2H), 1.91−2.21 (dm, 2H), 2.39−2.45 (m, 2H), 2.67−2.74 (m, 2H), 3.12−3.19 (m, 1H), 3.67 (s, 3H), 3.75 (s, 3H), 4.02−4.21 (dm, 3H). ^{31}P NMR ($CDCl_3$) δ: 36.27, 37.16.

***N*-[2-Cyanoethoxy(phenyl)phosphinyl]-L-glutamic Acid Dimethyl Ester (3d). 3d** was prepared using procedure A and purified by flash chromatography (MeOH/ethyl acetate 10:90 v/v, R_f = 0.44) to give a colorless oil (0.43 g, 1.16 mmol, 55% yield). ^{1}H NMR ($CDCl_3$) δ: 1.75−2.12 (dm, 2H), 2.33−2.40 (m, 2H), 2.72−2.79 (m, 2H), 3.60 and 3.66 (s, 3H), 3.62 and 3.67 (s, 3H), 3.86−4.10 (m, 1H), 4.17−4.34 (dm, 2H), 7.43−7.58 (m,3H), 7.74−7.87 (m, 2H). ^{31}P NMR ($CDCl_3$): δ 19.90, 20.93; 23.07, 23.84.

***N*-[2-Cyanoethoxy(methyl)phosphinothioyl]-L-glutamic Acid Dimethyl Ester (4a). 4a** was prepared using procedure B and purified by flash chromatography to give a colorless oil (0.87 g, 2,7 mmol, 45% yield). ^{1}H NMR ($CDCl_3$) δ: 1.83 and 1.86 (d, J = 15.3 Hz, 3H), 1.91−2.19 (m, 2H,), 2.39−2.48 (m, 2H), 2.66−2.77 (m, 2H), 3.39−3.56 (m, 1H), 3.68 and 3.69 (s, 3H), 3.75 and 3.76 (s, 3H), 4.21−4.24 (dm, 3H). ^{31}P NMR ($CDCl_3$) δ: 86.12, 86.30. Anal. Calcd for $C_{11}H_{19}N_2O_5PS$: C, 40.98; H, 5.90; N, 8.69. Found: C, 40.81; H, 6.00, N, 8.65.

***N*-[2-Cyanoethoxy(ethyl)phosphinothioyl]-L-glutamic Acid Dimethyl Ester (4b; Path C).** A solution of 3-hydroxypropionitrile (0.21 g, 3 mmol) in THF (5 mL) was added via syringe to a stirring solution of dichloroethylphosphine (0.43 g, 3.3 mmol) and triethylamine (0.5 mL, 3.3 mmol) in THF (15 mL) at −40 °C. The resulting solution was stirred 0.5 h and then allowed to warm to ambient temperature. A solution of L-glutamic acid dimethyl ester (0.7 g, 3.9 mmol) and triethylamine (1.0 mL, 6.6 mmol) in THF (5 mL) was added to the reaction mixture, followed by the addition of sulfur (0.15 g, 4.8 mmol). The solution was stirred overnight, filtered, concentrated in vacuo, and purified by flash chromatography (hexane/ethyl acetate/THF 60:40:1 v/v, R_f = 0.23) to give **4b** as a colorless oil (0.33 g, 0.98 mmol, 33% yield). ^{1}H NMR ($CDCl_3$) δ: 1.62 and

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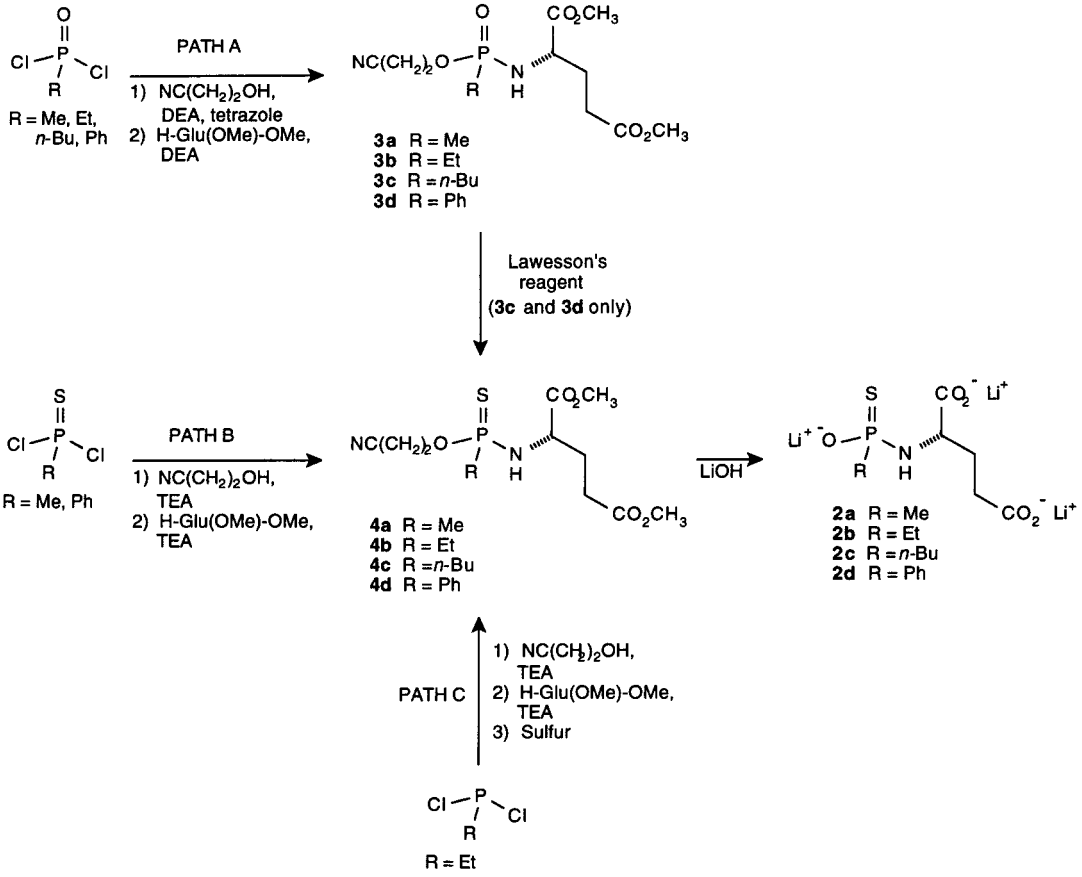
Scheme 1. Synthesis of *N*-Phosphonamidothionate Derivatives of Glutamic Acid



1.24 (dt, J = 7.6 Hz, 3H), 1.93−2.14 (dm, 4H,), 2.40−2.46 (m, 2H), 2.69−2.74 (m, 2H), 3.36−3.55 (dm, 1H), 3.69 and 3.70 (s, 3H), 3.76 and 3.77 (s, 3H), 4.05−4.30 (dm, 3H). ^{31}P NMR ($CDCl_3$) δ: 93.47, 93.88. Anal. Calcd for $C_{12}H_{21}N_2O_5PS$: C, 42.81; H, 6.29; N, 8.33. Found: C, 42.88; H, 6.17; N, 8.20.

***N*-[2-Cyanoethoxy(*n*-butyl)phosphinothioyl]-L-glutamic Acid Dimethyl Ester (4c). 4c** was prepared using procedure A and purified by flash chromatography (hexanes/ethyl acetate 5:2 v/v, R_f = 0.23) to give a colorless oil (0.15 g, 0.42 mmol, 58% yield). ^{1}H NMR ($CDCl_3$) δ: 0.93(t, J = 7.2 Hz, 3H), 1.41 (dt, J = 7.2, 14.4 Hz, 2H), 1.58−1.62 (m, 2H), 1.91−2.00 (dm, 4H), 2.39−2.45 (m, 2H), 2.66−2.72 (m, 2H), 3.47 (m, 1H), 3.68 and 3.69 (s, 3H), 3.75 and 3.76 (s, 3H), 4.18−4.27 (dm, 3H). ^{31}P NMR (300 Hz, $CDCl_3$) δ: 91.82, 92.22. Anal. Calcd for $C_{14}H_{25}N_2O_5PS$: C, 46.14; H, 6.87; N, 7.69. Found: C, 46.20; H, 6.96; N, 7.57.

***N*-[2-Cyanoethoxy(phenyl)phosphinothioyl]-L-glutamic Acid Dimethyl Ester (4d). 4d** was prepared using procedures A and B and purified by flash chromatography (hexanes/ethyl acetate 2:1 v/v R_f = 0.24) to give a colorless oil (0.12 g, 0.32 mmol, 44% yield from procedure A; 1.06 g, 2.76 mmol, 46% yield from procedure B. ^{1}H NMR ($CDCl_3$) δ: 1.89−2.18 (dm, 2H,), 2.29−2.40 (m, 2H), 2.74−2.81 (m, 2H), 3.63 and 3.64 (s, 3H), 3.65 and 3.66 (s, 3H), 3.85−3.98 (m, 1H), 4.23−4.31 (dm, 2H), 7.46−7.54 (m, 3H), 7.85−7.90 (m, 2H). ^{31}PNMR ($CDCl_3$) δ: 78.22, 78.47. Anal. Calcd for $C_{16}H_{21}N_2O_5PS$: C, 49.99; H, 5.47; N, 7.29. Found: C, 50.26; H, 5.51. N, 7.18.

General Procedure for Phosphonamidothioic Acids 2. Phosphonamidothionate ester **4** (0.5 mmol) was dissolved in methanol (2 mL) to which was added aqueous lithium hydroxide (2 mL, 1.0 M). The resulting solution was stirred at room temperature for 18 h and then filtered. The solvent was evaporated in vacuo to give **2** as a white residue. The residue was resuspended in anhydrous methanol, filtered (0.2 μm Teflon membrane), and concentrated in vacuo to give the trilithium salt of the desired product 2 as a white solid.

***N*-[Hydroxy(methyl)phosphinothioyl]-L-glutamic Acid Trilithium Salt (2a).** Yield: 0.12 g, 0.46 mmol, 93%. ^{1}H NMR (CD_3OD) δ: 1.29 and 1.30 (d, J = 14.1 Hz, 3H), 1.54−1.65 (m, 2H), 1.94−2.11 (m, 2H), 3.31−3.4 (m, 1H). ^{13}C NMR (CD_3OD) δ: 25.34 (d, J = 18.9 Hz), 26.60(d, J = 20.7 Hz), 33.95, 35.60, 58.42, 58.55, 182.50 and 182.55(d, J = 3.6 Hz), 183.35, 183.45. ^{31}P NMR (CD_3OD) δ: 62.63, 63.35. ESI-HRMS: calcd for $C_6H_9Li_2NO_5PS$ (M − Li)$^-$ 252.0259, found 252.0259.

***N*-[Hydroxy(ethyl)phosphinothioyl]-L-glutamic Acid Trilithium Salt (2b).** Yield: 0.12 g, 0.43 mmol, 86%. ^{1}H NMR (D_2O) δ: 0.99 and 1.06 (dt, J = 7.6, 4.8 Hz, 3H), 1.69−1.83 (m, 4H), 2.15−2.21 (m, 2H), 3.48 and 3.62 (dt, J = 6.4, 10.7 Hz, 1H). ^{13}C NMR (D_2O) δ: 8.94(d, J = 5.1 Hz), 9.16(d, J = 4.4 Hz), 30.33, 31.37, 31.58, 32.63, 33.57, 33.71 (d, J = 6.0 Hz), 35.36, 35.49, 58.26, 58.36, 183.35, 183.40, 184.58, 184.62. ^{31}P NMR (D_2O) δ: 69.87, 71.42. FAB-HRMS: calcd for $C_7H_{11}Li_2NO_5PS$ (M − Li)$^-$ 266.0416, found 266.0416.

***N*-[Hydroxy(*n*-butyl)phosphinothioyl]-L-glutamic Acid Trilithium Salt (2c).** Yield: 0.14 g, 0.46 mmol, 93%. ^{1}H NMR (CD_3OD) δ: 0.78 and 0.79 (t, J = 7.3 Hz, 3H), 1.21−1.27 (m, 2H), 1.47−1.52 (m, 2H), 1.54−1.64 (m, 2H), 1.76−1.81 (m, 2H), 2.13−2.19 (m, 2H), 3.50−3.60 (m, 1H). ^{13}C NMR (CD_3OD) δ: 14.46, 25.21, 25.46, 27.87, 27.91, 34.13, 34.18, 35.57, 38.44 and 39.71(d, J = 20.9 Hz), 58.33, 58.37, 182.44, 182.49, 183.36, 183.51. ^{31}P NMR (CD_3OD) δ: 68.30, 68.70. ESI-HRMS: calcd for $C_9H_{15}Li_2NO_5PS$ (M - Li)$^-$ 294.0729, found 294.0736.

***N*-[Hydroxy(phenyl)phosphinothioyl]-L-glutamic Acid Trilithium Salt (2d).** Yield: 0.15 g, 0.47 mmol, 93%. ^{1}H NMR (CD_3OD) δ: 1.49−1.61 (m, 2H), 1.78−2.15 (m, 2H), 3.18−3.26 (m, 1H), 6.93−6.98 (m, 3H), 7.50−7.57 (m, 2H). ^{13}C NMR (CD_3OD) δ: 33.70 and 34.17(d, J = 5.9 Hz), 35.32, 35.53, 128.49, 128.54, 128.67, 128.72, 128.96, 130.21, 130.38, 131.12, 131.78, 131.92, 132.06, 181.57, 181.65, 183.37, 183.69. ^{31}P NMR (CD_3OD) δ: 55.58, 56.54. ESI-HRMS: calcd for $C_{11}H_{11}Li_2NO_5PS$ (M − Li)$^-$ 314.0416, found 314.0415.

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Supporting Information Available: NMR spectra of **2a**−**d** and **3c**,**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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