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Synthesis of individual glutamate-containing phosphonamidothionate stereoisomers

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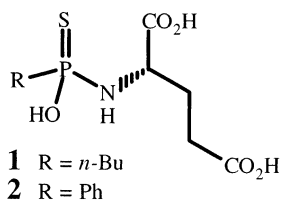
Abstract—The acquisition of the individual stereoisomers of chiral phosphonothioic acids is anticipated to reveal the significance of phosphorus stereochemistry upon the inhibition of metallocarboxypeptidases as well as their utility as chiral and stereoselective inhibitory probes. Two methods for preparing individual glutamate-containing phosphonamidothioic acid diastereomers have been identified. One method achieves the resolution through fractional crystallization of the intermediate 9-fluorenemethoxy phosphonamidates while the second does so through chromatographic resolution of intermediate β-(acylmercapto)ethyl phosphonamidothiolates.

Recent research efforts in our laboratory have been aimed at developing potent competitive inhibitors for the general class of enzymes known as glutamate carboxypeptidases (GCP). Examples of such metallopeptidase include *N*-acetylated-alpha-linked-acidic dipeptidase (NAALADase),[1] prostate-specific membrane antigen (PSMA),[2] pteroylpoly-glutamate hydrolase (PPH),[3] and carboxypeptidase G (CPG).[4] The acquisition of inhibitors for such enzymes is expected to further the understanding of the biological role of these metallocarboxypeptidases as well as to serve in the elucidation of germane active site features. In addition, inhibitors of CPG_2, an enzyme closely related to CPG and one for which the crystal structure is known, have been recently sought for the use in inhibiting non-tumor-localized enzyme in ADEPT strategies.[5]



1 R = *n*-Bu
2 R = Ph

Figure 1. Phosphonamidothionate inhibitors of GCP.

Based upon our preliminary evidence, compounds containing the phosphonamidothionate motif (Fig. 1) show strong promise as potent tetrahedral-intermediate analog inhibitors of metallopeptidases with the unique value of probing enzyme active-site architecture with complementary chiral phosphorus centers.[6] The basis for the enhanced inhibitory potency of such compounds, especially against zinc–metallopeptidases, is presumably due to favorable zinc–sulfur interactions within enzyme active sites.[7]

The focus of the present study was to explore methodology which would allow for the procurement of the individual diastereomers of phosphonamidothionates such as **1** and **2**. With the acquisition of such individual stereoisomers, the significance of phosphorus stereochemistry of chiral phosphonothioic acids upon the inhibition of metallocarboxypeptidases as well as their utility as chiral and stereoselective inhibitory probes can be ultimately addressed.

For the purposes of this work, two specific analogs **1** and **2** were targeted as representative glutamate-containing phosphonamidothionates. The rationale for the selection of the hydrocarbon ligands to phosphorus was based upon the known capability of some GCP's to hydrolyze a variety of alkyl and aryl amides of glutamic acid.[4] Indeed, we recently noted that for this glutamate carboxypeptidase, both methotrexate and *N*-[4-(4-nitrophenyl)butanoyl] glutamic acid exhibited very similar kinetic profiles as substrates, the later substrate having a slightly lower K_m and higher V_{max}.[8]

Keywords: chiral phosphonothioic acids; chiral phosphonamidothiolates; glutamate; β-(acylmercapto)ethyl; 9-fluorenemethoxy.

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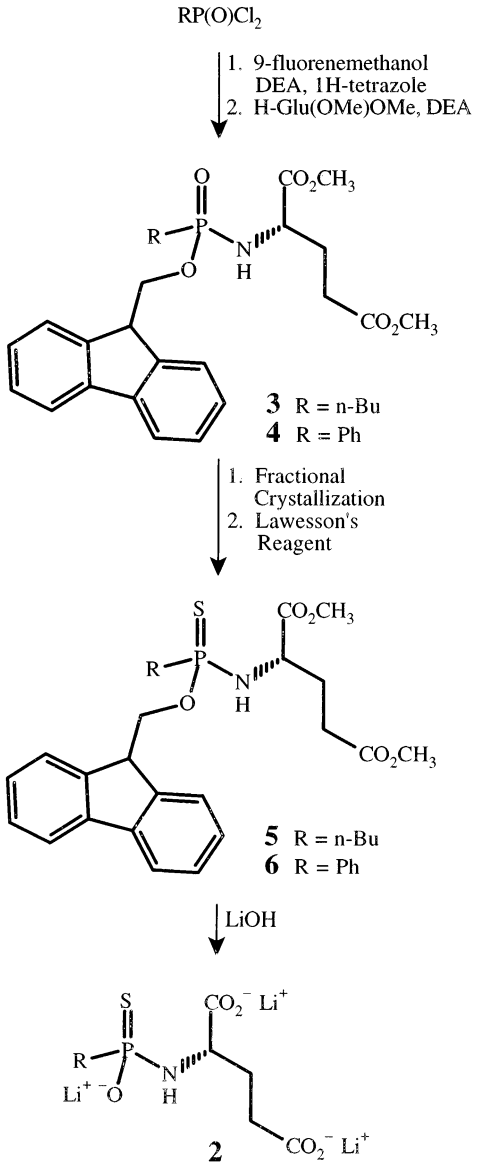
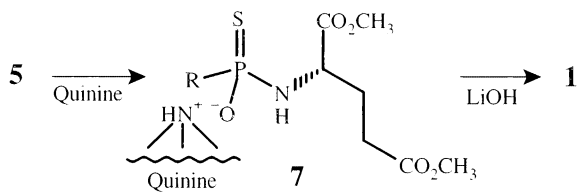
In a previous report, phosphonamidothionates **1** and **2** were prepared utilizing a 2-cyanoethoxy ligand as a protecting group on phosphorus. Although this group could be conveniently removed simultaneously with the glutamate methyl esters in mild basic conditions, it afforded no chromatographic resolution of its synthetic precursors.[6] It was envisioned that a larger ligand could impart either greater chromatographic resolution of the chiral phosphorus center or allow synthetic intermediates to exists as solids allowing for separation of phosphorus diastereomers through fractional crystallization. The selection of the phosphorus protecting group was further constrained by the requirement of being removed conveniently with the glutamate methyl esters under mild basic conditions in a final step. It was noted that the 9-fluorenemethoxy group had been previously used as a phosphorus protecting group[9] with the potential for meeting the established selection criteria. As such, it was incorporated into the synthetic strategy outlined in Scheme 1.

Phosphonamidates **3** and **4** were conveniently prepared via the 1*H*-tetrazole catalyzed two-step reaction, which could be fractionally recrystallized to provide both individual diastereomers (**3a**, **3b** and **4a**, **4b**) with good yields.[10] Stereospecific thionation with Lawesson's reagent was envisioned to provide the individual respective isomers (**5a**, **5b** and **6a**, **6b**) with retention of the phosphorus configuration,[11] which could be further deprotected with LiOH to provide the individuals **1** and **2**. This tactic of sequential thionation[12] and deprotection[13] worked as planned for phenyl congener **2** (Scheme 1). However, problems were encountered in the thionation step with Lawesson's reagent for the butyl analog. It was found that, the conversion of the individual isomers of **3a** and **3b** to **5a** and **5b**, respectively, did not occur with absolute stereospecificity but occurred with approximately 10% configuration inversion at the phosphorus center giving a mixture of two isomers.



Scheme 1. Synthesis and resolution of *O*-(9-fluorenemethyl) phosphonamidothionates.

The problem of the slight racemization of the phosphorus configuration that occurred during the respective thionation of **3a** and **3b** to **5a** and **5b**, was solved by submitting these 90:10 diastereomeric mixtures of **5** to a two-step deprotection procedure (Scheme 2). Following treatment of **5** with quinine in refluxing methanol, the 9-fluorenemethoxy group was removed and the resulting quinine salt mixture was recrystallized to give the single isomers **7a** and **7b**.[14] Final deprotection with LiOH afforded the target individual isomers **1a** and **1b** as single diastereomers (Scheme 2).[13]



Scheme 2. Quinine resolution of phosphonamidothionates.

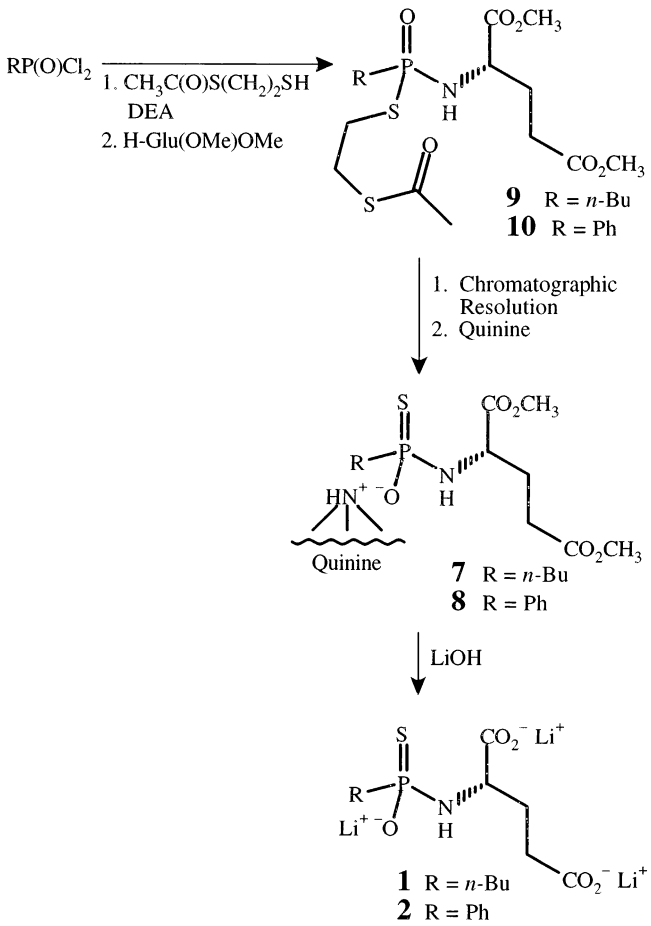
While working towards the preparation of a related series of phosphonamidothiolates, it was discovered that the individual diastereomers of β-(acylmercapto)ethyl phosphonamidothiolates **9** and **10** were readily resolved by column chromatography. The phosphonamidothiolates **9** and **10** were readily prepared[15] from the corresponding phosphonyl dichloride in a sequential reaction with $CH_3COS(CH_2)_2SH$[16] and $Glu(OMe)_2$ and were subsequently and conveniently resolved into two single diastereomers (**9a**, **9b** and **10a**, **10b**) by silica-gel chromatography. Treatment of **9** and **10**, respectively, with quinine in refluxing methanol removed the protecting group from the sulfur ligand to phosphorus.[14] Subsequent treatment with LiOH/MeOH hydrolyzed the glutamate methyl esters to afford **1** and **2** as single isomers.[13]

It should be noted that the two-step deprotection procedure (first with quinine then with LiOH) provides **1** and **2** in essentially pure form without any undesired

Table 1. ^{31}P NMR chemical shift data

Compound	R	^{31}P NMR (δ)
1a	*n*-Bu[a]	69.02
1b	*n*-Bu[a]	69.31
2a	Ph[a]	56.28
2b	Ph[a]	57.28
3a	*n*-Bu	35.66
3b	*n*-Bu	36.24
4a	Ph	22.58
4b	Ph	23.39
5a	*n*-Bu	91.11
5b	*n*-Bu	91.61
6a	Ph	78.11
6b	Ph	78.33
7a	*n*-Bu[a]	70.48
7b	*n*-Bu[a]	70.82
8a	Ph[a]	58.80
8b	Ph[a]	59.13
9a	*n*-Bu	50.02
9b	*n*-Bu	52.41
10a	Ph	37.38
10b	Ph	39.21

[a] Chemical shifts were obtained in CD_3OD and externally referenced to H_3PO (85%) in CD_3OD, all others were obtained in $CDCl_3$ and externally referenced to H_3PO (85%) in $CDCl_3$.



Scheme 3. Synthesis and resolution of β-(acylmercapto)ethyl phosphonamidothionates.

formation of the corresponding phosphonamidothionate; the major byproduct when **9** or **10** was treated with LiOH directly without the pre-treatment with quinine. Based upon these observations, it was hypothesized that direct treatment of **9** or **10** with LiOH resulted in significant displacement of $CH_3COS(CH_2)_2SH$ rather than the desired deprotection. Therefore, when **9** or **10** was first treated with quinine in methanol, the resultant thiophosphonamidate anion served to protect the phosphorus center from the attack of hydroxide in the subsequent deprotection with LiOH.

It is noteworthy to mention that the resolution of the individual isomers was conveniently monitored by ^{31}P NMR (Table 1). All 'a' and 'b' isomers were identified as intermediates in the synthesis of **1a** or **2a** and **1b** or **2b**, respectively. Determination of the absolute configuration of the phosphorus center in these isomers is currently underway.

In summary, the preparation of the individual diastereomers of glutamate-containing phosphonamidothioic acids has been achieved through two methods; fractional crystallization and chromatography. The success of these methods relied upon the 9-fluorenemethoxy or the β-(acetylmercapto)ethylthio groups as phosphorus protecting ligands. Although targets **1** and **2** could be obtained as individual isomers by the fractional recrystallization method (Schemes 1 and 2), this procedure was quite time consuming. On the other hand, the procurement of the individual diastereomers of **1** and **2** through the chromatographic resolution of phosphonamidothiolates **9** and **10** (Scheme 3) was rapid and more convenient. With the acquisition of the individual stereoisomers of compounds such as **1** and **2**, the significance of phosphorus stereochemistry of chiral phosphonothioic acids upon the inhibition of metallocarboxypeptidases as well as their utility as chiral and stereoselective inhibitory probes can now be examined.

Acknowledgements

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10. Experimental procedures for compounds **3** and **4.** A solution of 9-fluorenemethanol (0.53 g, 2.7 mmol) and DEA (0.52 ml, 3.0 mmol) in benzene (6.0 ml) was added via syringe to a stirring solution of alkylphosphonic dichloride (0.42 ml, 3.0 mmol) and 1*H*-tetrazole (0.02 g, 0.27 mmol) in benzene (10 ml) under an argon atmosphere at 4°C. The resulting solution was warmed to room temperature and stirred for 3 h, followed by the addition of a solution of L-glutamic acid dimethyl ester (0.57 g, 3.2 mmol) and DEA (0.59 ml, 3.4 mmol) in benzene (6.0 ml). The solution was stirred for another 3 h and then filtered and concentrated in vacuo to afford a yellow oil, which was purified by flash chromatography (EtOAc:hexane 2:1, v/v) to give the ester **3** (62%) and **4** (60%). Phosphonamidates **3** and **4** were fractionally recrystallized from acetone and hexane to afford the individual diastereoisomers, respectively.
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12. Experimental procedures for compounds **5** and **6**. Refluxing a solution of **3** or **4** (0.66 mmol) and Lawesson's reagent (0.37 mmol) in toluene (16 ml) for about 2 h followed by concentration in vacuo gave a yellow oil, which was purified by flash chromatography to give **5** (89%) and **6** (84%) [CH_2Cl_2: EtOAc 50:1 v/v (**5a**: R_f= 0.43. **5b**: R_f=0.42. **6a**: R_f=0.46. **6b**: R_f=0.44)].
13. General experimental procedures for compounds **1** and **2**. Phosphonamidothionates **7** and **6** or **8** (0.1 mmol) were dissolved in methanol (0.6 mL), into which was added a 1.0 M aqueous solution of LiOH (0.36 mL). The solution was stirred at room temperature for 15 h and then filtered. The solvent was evaporated in vacuo to give **1** and **2** as white residue, which was further purified by re-suspension in anhydrous methanol and filtration (0.2 μm Teflon membrane) to provide the desired pure trilithium salts **1** (68%) and **2** (81%).

N-[hydroxy(*n*-butyl)phosphinothioyl]-L-glutamic acid trilithium salt (**1a**). ^{1}H NMR (CD_3OD): δ 0.80 (t, J=7.2 Hz, 3H), 1.25 (m, 2H), 1.40–1.59 (m, 2H), 1.61–1.73 (m, 2H), 1.74–1.92 (m, 2H), 2.08–2.27 (m, 2H), 3.58 (dt, J=10.8 Hz, J=6.3 Hz, 1H); ^{13}C NMR (CD_3OD): δ 12.96, 23.87 (d, J=18.70 Hz), 26.43, 32.74, 34.12, 37.54 (d, J=93.98 Hz), 56.98, 180.91 (d, J=3.38 Hz), 181.99; FABHRMS (M–Li) calcd 294.0729. Found 294.0728 for $C_9H_{15}Li_2NO_5PS$.

N-[hydroxy(*n*-butyl)phosphinothioyl]-L-glutamic acid trilithium salt (**1b**). ^{1}H NMR (CD_3OD): δ 0.85 (t, J=7.5 Hz, 3H), 1.31 (m, 2H), 1.51–1.76 (dm, 4H), 1.80–1.96 (m, 2H), 2.13–2.31 (m, 2H), 3.64 (dt, J=11.1 Hz, J=6.0 Hz, 1H); ^{13}C NMR (CD_3OD): δ 14.43, 25.10 (d, J=18.45 Hz), 27.91, 34.22 (d, J=3.75 Hz), 35.64, 39.44 (d, J= 119.18 Hz), 58.40, 182.38 (d, J=4.65 Hz), 183.32; FABHRMS (M–Li) calcd 294.0729. Found 294.0705 for $C_9H_{15}Li_2NO_5PS$.

N-[hydroxy(*n*-butyl)phosphinothioyl]-L-glutamic acid trilithium salt (**2a**). ^{1}H NMR (CD_3OD): δ 1.57–1.77 (m, 2H), 1.88–2.14 (dm, 2H), 3.27 (dt, J=6.3 Hz, J=5.7 Hz, 1H), 6.98–7.08 (m, 3H), 7.57–7.64 (dm, 2H); ^{13}C NMR (CD_3OD): δ 34.19, 35.35, 58.50, 128.54, 128.71, 130.20, 131.80, 131.94, 144.51 (d, J=128.6 Hz), 181.62 (d, J= 5.10 Hz), 183.69; FABHRMS (M–Li) calcd 314.0416. Found 314.0433 for $C_{11}H_{11}Li_2NO_5PS$.

N-[hydroxy(*n*-butyl)phosphinothioyl]-L-glutamic acid trilithium salt (**2b**). ^{1}H NMR (CD_3OD): δ 1.45–1.58 (m, 2H), 1.81–1.83 (m, 2H), 3.28 (dt, J=6.0 Hz, J=2.8 Hz, 1H), 6.96–7.02 (m, 3H), 7.57–7.64 (dm, 2H); ^{13}C NMR (CD_3OD): δ 33.72 (d, J=4.65 Hz), 35.55, 58.55, 128.45, 128.62, 130.24, 131.92, 132.04, 144.40 (d, J=131.4 Hz), 182.18 (d, J=3.75 Hz), 183.27. FABHRMS (M–Li) calcd 314.0416. Found 314.0411 for $C_{11}H_{11}Li_2NO_5PS$.

14. Experimental procedures for compounds **7** and **8**. Refluxing a solution of **5**, **9** or **10** (0.45 mmol) and quinine (146 mg, 0.45 mmol) in methanol (5 ml) for 24 h followed by concentration in vacuo gave a yellow oil, which was recrystallized from acetone and hexane (3:10 v/v) to give compounds **7** from **5** or **9** and **8** from **10**.
15. Experimental procedures for compounds **9** and **10**. Into a flask charged with 1*H*-tetrazole (0.02 g, 0.3 mmol) and alkylphosphonic dichloride (3.3 mmol) in benzene (10.0 mL) were added sequentially ethanedithiol monoacetate (0.41 g, 3.0 mmol) and a solution of diisopropylethylamine (0.52 mL, 3.0 mmol) in benzene (5.0 mL) via syringe at 4°C under an argon atmosphere. The solution was allowed to warm to room temperature and stirred until ethanedithiol monoacetate was consumed (approximately 3 h) as monitored by TLC. Glutamic acid dimethyl ester (0.58 g, 3.3 mmol) and DEA (0.59 mL, 3.4 mmol) in benzene (5.0 mL) was added dropwise to the reaction mixture and allowed to stir for an additional 3 h. The reaction mixture was concentrated in vacuo and purified by flash chromatography to give **9** (65%) and **10** (60%) [EtOAc:hexane 4:1 v/v (**9a**: R_f=0.35. **9b**: R_f=0.25. **10a**: R_f=0.36. **10b**: R_f=0.27)].
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