

## Synthesis of P-Chiral, Phosphorothioic Acid Analogs of N-Phospholeucinamide

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**Abstract:** A diastereomeric mixture of *R<sub>p</sub>*- and *S<sub>p</sub>*- N-[O-methyl phosphorothioic acid]leucinamides were synthesized by thiophosphorylation of leucinamide followed by monodealkylation of the phosphate methyl ester. Individual diastereomers were prepared by a sequence utilizing selective protection of the thioic acid moiety as the *S*-benzyl group, chromatographic separation, and deprotection.

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Some of the more potent inhibitors of metallopeptidases contain a phosphorus-based, transition state (TS) moiety at the scissile or reactive carboxamide bond. Several synthetic and naturally occurring examples of these transition state inhibitors have been studied including: phosphoramidon (1),<sup>1</sup> N-phospholeucinamide (2),<sup>2</sup> and benzyloxycarbonyl-N-phosphoro-*l*-leu-*l*-ala (not shown).<sup>3</sup> The extent and bonding orientation by these transition state inhibitors to metalloenzymes

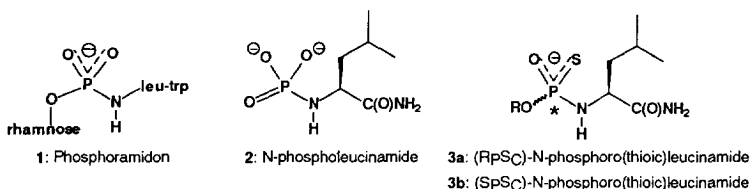
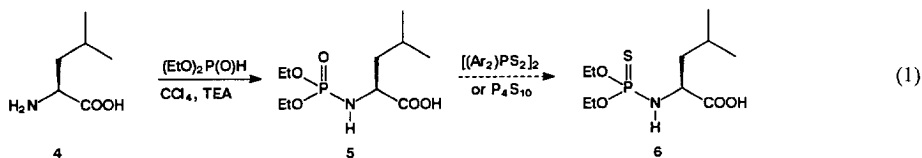


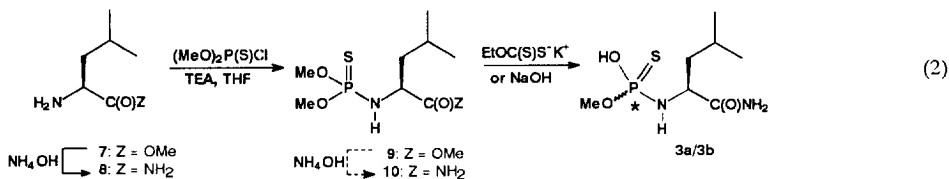
Figure 1.

depends greatly on the inhibitor structure. Phosphoramidon 1 (a phosphoramidate) binds the metal in the enzyme active site via monodentate ligation whereas N-phospholeucinamide 2 and benzyloxycarbonyl-N-phosphoro-*l*-leu-*l*-ala (phosphoramidic acids) interact via bidentate ligation.<sup>4</sup> Therefore, the interaction between metalloenzyme and TS inhibitor is highly dependent on the phosphorus group structure. It occurred to us that a hybrid form of these two inhibitor binding modes may be useful to study metalloenzyme specificity, namely, a N-phospholeucinamide analog that binds with monodentate ligation. Moreover, if the phosphate ligating group could be prepared in stereoisomeric form, dual probes of enzyme mechanism could be envisioned. This paper describes the preparation of novel, P-chiral (\*), transition state analogs 3a and 3b in which one of the phosphate oxygens has been substituted for a sulfur atom (thiophosphate) (Fig. 1). The rationale for the sulfur substitution is three-fold: (a) a thiophosphate anion will interact differently than a phosphate anion with various metal atoms contained in the enzyme active site, (b) a thiophosphate monoester generates a P-chiral center of asymmetry, and (c) thiophosphates can exist in either thionate [-P(S)OH] or thiolate [-P(O)SH] tautomeric forms offering two modes of ligation. Simple phosphorus esters were chosen for 3a and 3b (R = Me, Et) that would minimize steric complications as an inhibitor and lock the P-chiral center. Using these guidelines, the two main challenges to prepare 3a and 3b were to introduce the P-chiral group and to identify a reliable preparation of the individual diastereomers.

Our initial strategy sought to introduce a dialkoxy phosphoramidate group at the amino position, conduct a P=O to P=S exchange, and monodealkylate the diester to furnish a separable, diastereomeric mixture of **3a** and **3b**. Thus, *l*-leucine (**4**) was converted into N-[O,O-diethyl phosphoryl]leucine (**5**) in 70% yield by reaction with diethyl phosphite<sup>6</sup> (Eqn. 1). Unfortunately, all attempts to form the thiophosphoryl analog **6** failed including numerous variations in conditions with Lawesson's reagent  $[(Ar_2PS_2)_2]$ ,<sup>7</sup> P<sub>4</sub>S<sub>10</sub> or elemental sulfur. The corresponding N-(diethoxy phosphoryl)tryptophan derivative was prepared, which also failed to undergo thionation despite improved solubility under the reaction conditions. Direct displacement of one of the ethyl esters of **5** with NaSH<sup>8</sup> or Na<sub>2</sub>S also failed to produce **3a/3b**.



To circumvent the problems associated with the free carboxylic acid and thionation exchange reaction, a direct thiophosphorylation of methyl leucinate hydrochloride (**7**) was attempted (Eqn. 2). Dimethyl chlorothiophosphate was reacted with **7** to afford methyl N-[O,O-dimethyl thiophosphoryl]leucinate (**9**) in 75% yield which showed a typical phosphoramidothionate <sup>31</sup>P signal at 75.8 ppm.<sup>9</sup> However, reaction of carboxyester **9** with NH<sub>4</sub>OH failed to provide carboxamide **10**. The <sup>31</sup>P NMR of the crude reaction indicated several peaks likely resulting from parallel or sequential reaction of NH<sub>4</sub>OH at the phosphorus diester.

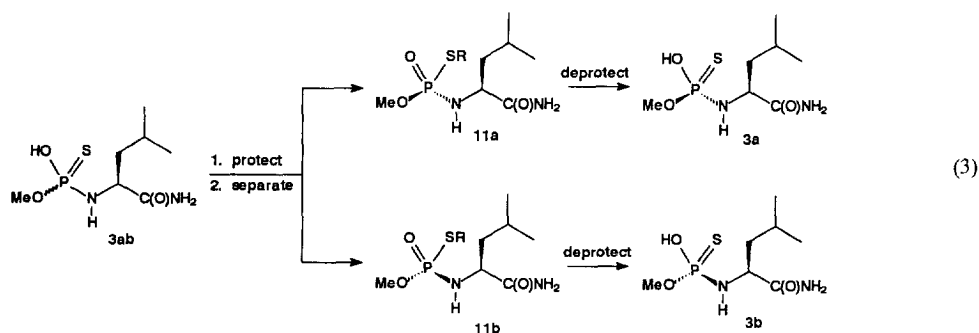


Leucinamide (**8**) was next chosen as a starting point for introduction of the phosphorus group despite possible competing reactions at the amine and amide sites. Leucinamide (**8**) was reacted with dimethyl chlorophosphate  $[(MeO)_2P(O)Cl]$  to give a complex mixture of products but reaction of **8** with dimethyl chlorothiophosphate  $[(MeO)_2P(S)Cl]$  gave a 70% yield of phosphoramidothionate **10** (<sup>31</sup>P = 75.5 ppm). The reduced phosphorylating ability of the thionate reagent likely led to greater selectivity at the amine. With the phosphoramidate linkage installed and the carboxamide in place, only conversion of the phosphorus ester to the thioic acid and separation of the diastereomers remained. Dealkylation of **10** was conducted successfully with potassium ethyl xanthate  $(EtOC(S)S^-K^+)$ <sup>10</sup> to afford **3a** and **3b** as a mixture of diastereomers in 81% yield following neutralization with 10% HCl (Eqn. 2). Similarly, hydrolysis of **10** with 1M NaOH in methanol afforded **3a** and **3b** after neutralization in 82% yield. The diastereomeric mixture of **3a** and **3b** displayed <sup>31</sup>P NMR signals at 66.5 ppm and 68.3 ppm (1:1 ratio), which likely indicates the predominant thionate (P=S) tautomer. To our dismay, all efforts to conduct a preparative separation of the diastereomers on silica gel or alumina failed. In most instances, only a 1-5% diastereomer enrichment was accomplished at the expense of a 50% loss in material (decomposition and/or reaction on the column). Compounds **3a** and **3b** are highly polar materials ( $R_f = 0.05$ , MeOH:EtOAc 1:4) that gave only modest separation on normal or reverse phase HPLC; therefore, no attempt to separate **3a** and **3b** using preparative HPLC was tried.

Our prior success with the separation of P-chiral thioic acids via fractional crystallization of alkaloid salts<sup>11</sup> prompted a brief investigation of forming alkaloid salts of **3a** and **3b**. Compound **10** was reacted with an equimolar amount of strychnine (THF or MeCN) to form a diastereomer mixture in about 70% conversion (by <sup>31</sup>P NMR). Recrystallization of the amorphous solids and foams did not lead to any diastereomer enrichment. Similar negative results were observed using (R)- or (S)- $\alpha$ -methylbenzyl amine.

In light of these results, a protecting group for the P-chiral thioic acid was needed that would decrease the molecule polarity to aid separation, not racemize the carbon or phosphorus stereocenter, and be chemoselective. The development of a suitable phosphorothioic acid protecting group could also benefit future studies that wish to install thioic acids as TS analogs. Modification of the thioic acid mixture **3a/3b** by trityl, *tert*-butyl, trialkylsilyl, and 9-fluorenylmethyl groups were attempted but all gave poor yield (0-8%). Formation of disulfides (R = SPh, SMe, Eqn. 3) were tried next since simple hydride reduction should regenerate the thioic acid. Neither oxidative coupling of **3a/3b** with thiophenol nor reaction of **3a/3b** with methyl methanethiosulfonate afforded the corresponding disulfide. We also attempted to install a  $\beta$ -cyanoethyl protecting group but thioic acids **3a/3b** did not undergo Michael addition to acrylonitrile in the presence or absence of TEA.

Installation of a benzyl group was considered next as a possible protecting group because benzyl groups are commonly cleaved by catalytic hydrogenolysis. Potential poisoning by the released sulfhydryl group of **3a/3b**, however, suggested that a *p*-methoxybenzyl group would better serve our needs because it can be removed under mild acid conditions. Compounds **3a/3b** were reacted with *p*-methoxybenzyl bromide (TEA, THF)<sup>12</sup> to form the diastereomeric S-*p*-methoxybenzyl thioesters **11a** and **11b** (R = CH<sub>2</sub>PhOMe; <sup>31</sup>P  $\delta$  32.2./32.4 ppm), which were separable on silica gel (5% MeOH/EtOAc). The <sup>31</sup>P chemical shift is consistent with P=O formation and alkylation at the sulfur group.<sup>9</sup> Despite this important advance, we were unable to deprotect the *p*-methoxybenzyl group using a variety of conditions (CF<sub>3</sub>COOH, HgOAc<sub>2</sub>, AlCl<sub>3</sub>-anisole, etc.).



During the course of our studies with S-benzyl protecting groups, the unsubstituted S-benzyl derivative **11a** and **11b** (R = -CH<sub>2</sub>Ph; <sup>31</sup>P  $\delta$  33.8/34.5 ppm) were prepared in 45% yield by reaction of **3a/3b** with benzyl bromide. The diastereomers separated cleanly but as expected, hydrogenolysis failed to furnish the thioic acids and mostly starting material was recovered. Several alternatives to hydrogenolysis were attempted which resulted in using dissolving metal reduction for possible deprotection despite concern that the metal-ammonia combination could displace one of the phosphorus ligands. We were pleased to find that reaction of either **11a** or **11b** (R = -CH<sub>2</sub>Ph) with sodium in ammonia at -78°C gave clean formation of **3a** and **3b** in 85% yield (100% by <sup>31</sup>P NMR) after chromatography.<sup>13</sup> We were also gratified to find that only a single diastereomer formed with no contamination by other diastereomer ensuring that the stereochemical integrity at phosphorus for this transformation had been maintained. The configuration at phosphorus has not yet been established and are tentatively assigned.

Thus, the S-benzyl group used in combination with dissolving metal reduction appears a suitable protecting group sequence for P-chiral phosphorothioic acids. With diastereomerically pure **3a** and **3b** in hand, the interaction of these interesting asymmetric, transition state analogs with metalloenzymes can be explored. This will be the subject of a future investigation.

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13. **N-[(R)-(O-methyl hydrogen thiophosphoryl)]-(l)-leucineamide (3a) and N-[(S)-(O-methyl hydrogen thiophosphoryl)]-(l)-leucineamide (3b).** Approximately 25 mL of anhydrous ammonia was condensed at  $-78^\circ\text{C}$  (dry ice in acetone) into a cold trap receiver. A portion of the condensed liquid ammonia (15 mL) was transferred to a flask containing either N-[(R)- or N-[(S)-(O-methyl S-benzyl thiophosphoryl)]-(L)-leucinamide (200 mg, 0.61 mmole) at  $-78^\circ\text{C}$  (not totally soluble). The flask was held at  $-78^\circ\text{C}$  whereupon 10 mg portions of freshly cut sodium was added. The first few pieces briefly generated a blue color that disappeared with swirling as the reaction warmed. As subsequent portions of sodium were added, the starting material (which collected on the flask sides) began to dissolve. When a blue color persisted the reaction was stirred an additional 5 min at  $-78^\circ\text{C}$ , solid  $\text{NH}_4\text{Cl}$  was added, the ice bath removed, and the reaction warmed to room temperature with evaporation of the liquid ammonia (water trap). To the semi-solid reaction, ethyl ether and 5% NaOH were added to remove the remaining organic impurities. The layers were separated and the organic layer was discarded. The aqueous layer was carefully neutralized by 5% HCl to pH 2, and extracted thrice with ethyl acetate (15 mL). The combined EtOAc layers were dried over  $\text{MgSO}_4$  and evaporated to afford 125 mg of a white foam. Anal. calcd for  $\text{C}_7\text{H}_{17}\text{N}_2\text{O}_3\text{SP}$ : C, 34.97; H, 7.08; N, 11.66. Found: C, 35.35; H, 7.31; N, 11.31. **Data for (3a):** Yield: 85%.  $R_f$  = 0.049 (20% MeOH / EtOAc).  $[\alpha]_D^{25}$  = -2.58 (c, 2.1,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR:  $\delta$  0.91 (d, J = 6.7 Hz, 3H), 1.52-1.61 (m, 2H), 1.79-1.83 (m, 1H), 3.65 (d, J = 13.6 Hz, 3H), 3.83-3.90 (m, 1H), 7.04 (br, s, 1H) and 7.20 (br, s, 1H).  $^{13}\text{C}$  NMR:  $\delta$  21.3, 23.1, 24.5, 42.9 (d, J = 7.1 Hz), 53.1 (d, J = 4.9 Hz), 54.1 and 179.2.  $^{31}\text{P}$  NMR (in  $\text{CDCl}_3$ ):  $\delta$  66.5 (J = 12.8 Hz coupled  $^1\text{H}$  spectrum). **Data for (3b):** Yield: 83%.  $R_f$  = 0.049 (20% MeOH / EtOAc).  $[\alpha]_D^{25}$  = -8.24 (c, 1.00,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (9:1,  $\text{CDCl}_3$ : $d_6$ -DMSO):  $\delta$  0.50 (d, J = 6.6 Hz, 3H), 1.04-1.10 (m, 2H), 1.28-1.40 (m, 1H), 3.14 (d, J = 13.8 Hz, 3H), 3.25-3.35 (m, 1H), 6.05 (br, s, 1H) and 6.74 (br, s, 1H).  $^{13}\text{C}$  NMR (9:1,  $\text{CDCl}_3$ : $d_6$ -DMSO):  $\delta$  21.5, 23.1, 24.3, 43.0 (d, J = 7.1 Hz), 52.9 (d, J = 4.8 Hz), 54.5 and 177.6.  $^{31}\text{P}$  NMR (9:1,  $\text{CDCl}_3$ : $d_6$ -DMSO):  $\delta$  68.3 (J = 12.8 Hz, coupled  $^1\text{H}$  spectrum).

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