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Oxathiaphospholane Approach to *N*- and *O*-Phosphorothioylation of Amino Acids

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A method of highly efficient synthesis of *N*- and *O*-phosphorothioylated amino acids was developed. *N*- and *O*-(2-Thiono-1,3,2-oxathiaphospholanyl)amino acid methyl esters (**3**) were prepared in high yields in reaction of amino acid methyl esters with 2-chloro-1,3,2-oxathiaphospholane in pyridine in the presence of elemental sulfur. Compounds **3** were converted in high yield into the corresponding methyl or benzyl phosphorothioamides **6** and **7** by DBU-assisted treatment with methanol or benzyl alcohol. When 3-hydroxypropionitrile was used instead of methanol or benzyl alcohol, the corresponding 2-cyanoethylphosphorothioamidates **4** were obtained in high yield, from which the 2-cyanoethyl group was removed with concentrated ammonium hydroxide. The oxathiaphospholane methodology was also applied for the phosphorylation of amino acids. Thus, 2-oxo-1,3,2-oxathiaphospholane derivatives **10** were prepared by oxidation of compounds **3** with SeO_2. Compounds **10** were transformed into the corresponding phosphate diesters or amidoesters upon treatment with 3-hydroxypropionitrile in the presence of DBU. The DBU-assisted oxathiaphospholane ring-opening process in **3** and **10** did not cause any measurable C-racemization of phosphorothioylated/phosphorylated amino acids.

Introduction

Protein phosphorylation is widely recognized as an important posttranslational modification that regulates protein functions in living cells,[1] by switching cellular activities from one stage to another, and regulates gene expression, cell proliferation, and cell differentiation.[2] In most cases tyrosine as well as serine/threonine kinases and corresponding phosphatases are involved in this process.[3] *N*-Phosphorylated proteins and amino acids also play important roles in the regulation of enzyme activity and protein biosynthesis.[4] The molecular basis of protein regulation induced by phosphorylation has attracted interest recently. However, the isolation of phosphorylated peptides from biological sources for functional studies is usually not feasible, and therefore efficient chemical phosphorylation methods are still appreciated.

O-Phosphorylated derivatives of serine, threonine, and tyrosine have been extensively studied, whereas limited information is available for other amino acids modified by a phosphate group due to paucity of such derivatives. Therefore, many research groups are involved in the development of synthetic methods for the preparation of phosphoamino acids and phosphopeptides in order to get a deeper insight into the influence of phosphorylation on the structure and function of the peptides.[5−9]

In general, introduction of phosphate moiety to esters of amino acids or peptides has been accomplished employing dialkyl or diaryl phosphorochloridates.[10] However, the most effective and versatile procedures for these purposes were based on P^{III} chemistry, originally developed for the solid-phase synthesis of oligodeoxynucleotides.[11] A variety of different protecting groups of phosphate function (benzyl,[6a,7b] 4-chlorobenzyl,[7c] allyl,[5b] 2-cyanoethyl,[9a] *tert*-butyl[6a]) were introduced. Alternatively, monoprotected amino acids or peptide phosphates were synthesized using H-phosphonate chemistry.[9b]

Phosphorothioate analogues of the phosphate were originally developed for the modification of nucleotides

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and oligodeoxynucleotides.[12] Only a few examples of phosphorothioylated amino acids or peptides obtained by chemical synthesis have been reported.[7b,13,14.] Replacement of the phosphate linked to an amino acid residue with a phosphorothioate is expected to lead to derivatives with increased stability, which may be useful in the investigation of the process of protein phosphorylation/dephosphorylation. Moreover, some of the well-known inhibitors of metallopeptidases such as *N*-phospholeucinamide[15] should increase their inhibitory activity by substitution of one of the phosphate oxygen atoms with sulfur. The basis for the enhanced inhibitory potency of such compounds, especially against zinc metallopeptidases, is presumably due to favorable zinc−sulfur interactions within the enzyme active sites.[16]

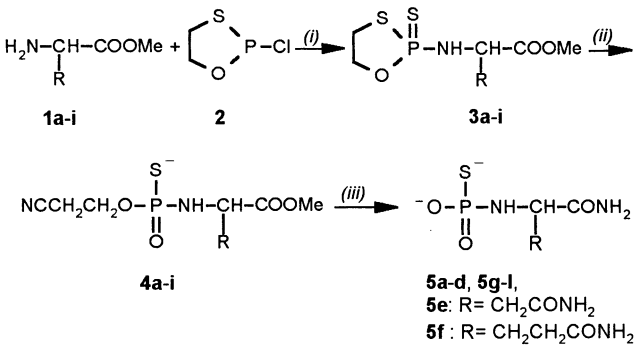
In this paper we present application of the oxathiaphospholane methodology [17] to the synthesis of *N*-phosphorothioylated amino acids as well as *O*-phosphorothioylated derivatives of hydroxyamino acids, i.e., serine, threonine and tyrosine. Although the approach had been designed for the stereocontrolled synthesis of oligo(nucleoside phosphorothioate)s, we demonstrated its application to the synthesis of N3′ → P5′ dinucleotides.[18] Recently, on the basis of this methodology, conjugates of L-phenylalanine and L-tryptophan with AZT-5′-*O*-phosphorothioate and phosphorodithioate have been prepared.[19]

Results and Discussion

Our synthetic strategy consists of three steps: (i) *O*- or *N*-derivatization of the corresponding amino acid methyl esters with oxathiaphosphitylating reagent, (ii) opening of 2-thiono-1,3,2-oxathiaphospholane ring of amino acid derivatives with 3-hydroxypropionitrile, and (iii) removal of *β*-cyanoethyl protecting group. Attempts at oxathiaphosphorylation of amino acids possessing free carboxyl function were unsuccessful due to formation of unknown byproducts.

N-Oxathiaphosphorothioylation of L-amino acid methyl esters (**1a**−**i**) was achieved by reaction with 2-chloro-1,3,2-oxathiaphospholane (**2**) in pyridine solution in the presence of elemental sulfur (Scheme 1). The resulting *N*-(2-thiono-1,3,2-oxathiaphospholanyl)amino acid methyl esters (**3a**−**i**) were isolated from the reaction mixture by silica gel column chromatography in satisfactory yields (75−95%) and characterized by ^{31}P NMR and FAB-MS analysis (Table 1). By virtue of asymmetry at phosphorus atom, compounds **3** appeared as the mixtures of two diastereomers in the ratio ca. 1:1 except for proline derivative **3i**, which was found to be a 41:59 mixture. In the case of tryptophan derivative **3h**, diastereomers were separated by crystallization. The absolute configuration at the phosphorus atom of individual species has been assigned by X-ray analysis, and the diastereomer absorbing at lower field in the ^{31}P NMR spectrum has been assigned as R_P. Relevant data will be published elsewhere.

SCHEME 1[a]



[a] Reagents: (i) S_8, pyridine; (ii) $HOCH_2CH_2CN$, DBU; (iii) NH_4OH.

TABLE 1. Physicochemical Characteristics of Compounds 3a−l

entry	R	cmpd	^{31}P NMR (δ, ppm)[a]	FAB-MS *m*/*z* (M − 1)	yield (%)[b]
1	CH_3	**3a**	96.70; 97.34	240	83
2	$CH(CH_3)_2$	**3b**	96.66; 97.91	268	78
3	$CH_2CH(CH_3)_2$	**3c**	96.79; 95.61	282	87
4	CH_2Ph	**3d**	96.50; 95.59	316	89
5	CH_2COOMe	**3e**	97.38; 96.52	298	95
6	CH_2CH_2COOMe	**3f**	97.66; 96.10	312	80
7	$CH_2CH_2SCH_3$	**3g**	96.71; 96.28	300	95
8	CH_2-indolyl-3	**3h**	96.54; 95.52	355	75
9	proline	**3i**	96.50; 94.30	266	87
10	CH_2	**3j**	105.49; 105.55	356	34(83)[c]
11	$CH(CH_3)$	**3k**	104.00; 103.89	370	92
12	$CH_2C_6H_4$	**3l**	100.52; 100.50	432	75

[a] In $CDCl_3$. [b] Yield of isolated products. [c] Reaction was performed in CH_2Cl_2 in a presence of *N*,*N*-diisopropylethylamine.

To obtain *N*-[*O*-(2-cyanoethyl)phosphorothioyl]amino acid methyl esters (**4a**−**i**), the oxathiaphospholane ring in **3a**−**i** was opened with 3-hydroxypropionitrile in the presence of DBU [20] (Scheme 1). In this reaction the formation of intermediates, *N*-[*O*-(2-cyanoethyl)-*O*-(2-mercaptoethyl)-phosphorothioyl]amino acid methyl esters, was expected. They should undergo fast elimination of ethylene sulfide, providing the desired products **4a**−**i**. Indeed, examination of the reaction mixtures by ^{31}P NMR after 3 h revealed in each case full conversion of **3a**−**i** into a pair of diastereomeric organophosphorus products with the chemical shifts characteristic for *O*-alkyl-amidophosphorothioate.[18] The crude compounds **4a**−**i** were purified by silica gel column chromatography and their structures were verified by FAB mass spectrometry.

The derivatives **4a**−**i** were converted into *N*-phosphorothioate amino acids (**5a**−**i**) by overnight treatment with

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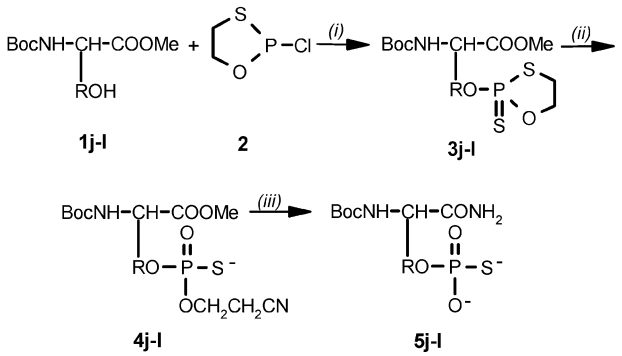
SCHEME 2[a]



[a] Reagents: (i) S_8, pyridine; (ii) $HOCH_2CH_2CN$, DBU; (iii) NH_4OH.

concentrated aqueous ammonia at room temperature. This process also converts carboxymethyl function into carboxamide (Scheme 1). Compounds **5a**−**i** were isolated from the reaction mixture by means of Sephadex LH-20 gel filtration. Their structures were confirmed by FAB mass spectrometry.

Analogous procedure has been applied for *O*-phosphorothioylation of *N*-(*tert*-butoxycarbonyl)-protected serine, threonine, and tyrosine methyl esters (**1j**−**l**). The synthesis of corresponding *O*-oxathiaphospholane derivatives **3j**−**l** together with their subsequent condensation with 3-hydroxypropionitrile leading to compounds **4j**−**l**, and final deprotection providing **5j**−**l** is depicted in Scheme 2.

It is noteworthy to mention that when serine derivative **1j** was oxathiaphosphitylated under standard conditions with **2** in pyridine as a solvent and HCl scavenger, desired compound **3j** was isolated in 34% yield (Table 1). However, when this reaction was performed in methylene chloride in the presence of *N*,*N*-diisopropylethylamine, the yield increased to 83%.

The methodology presented in this paper allowed us to obtained not only amino acid derivatives with unprotected phosphorothioic moiety but also their monoalkyl phosphorothioate species. When the 2-thiono-1,3,2-oxathiaphospholane ring in **3c** and **3f** has been opened by means of methanol treatment for 3 h, the corresponding *N*-(*O*-methylphosphorothioyl)amino acid methyl esters (**6c, 6f**) were obtained in almost quantitative yield (Scheme 3). Also *N*-(*O*-benzylphosphorothioyl)amino acid methyl esters (**7c, 7f**) were obtained in high yield under treatment of compounds **3c** and **3f** with benzyl alcohol, but longer time (12h) was needed to complete this reaction. Amino acid methyl esters in **6** and **7** were converted into corresponding litium salts (**8** and **9**) by means of LiOH/MeOH.[14] Compound **6c**, considered a potential inhibitor of metallopeptidases, was synthesized earlier in 75% yield by phosphorothioylation of leucinamide followed by monodealkylation of the phosphorothioate methyl ester.[21] Hence, our strategy is highly efficient and convenient for the synthesis of this class of compounds.

The introduction of a phosphate function into amino acids based on the above-described methodology is also possible. The oxathiaphospholane derivatives **3** possess sulfur atom in the both exo- and endocyclic position, but

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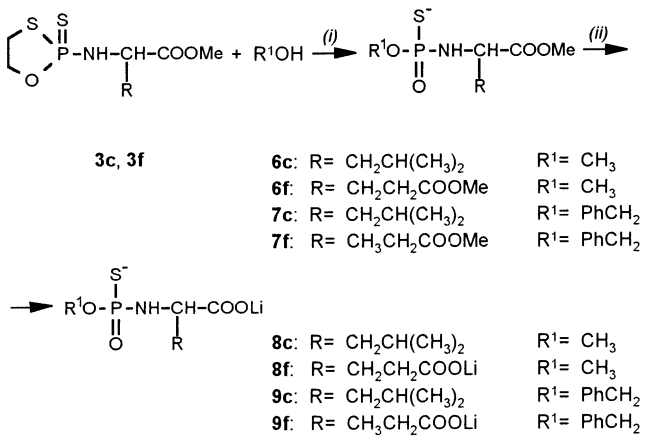
SCHEME 3[a]



[a] Reagents: (i) DBU/CH_3CN; (ii) LiOH/MeOH.

then sulfur atom present in the resulting phosphorothioamino acids **5** originates from exocyclic sulfur in **3**. Replacement of this exocyclic sulfur atom by oxygen gives rise to **10**, which in the condensation reaction with 3-hydroxypropionitrile followed by elimination of episulfide should provide compound possessing a monoalkylphosphoramidate bond. Attempts at oxidation of intermediately formed P^{III} 1,3,2-oxathiaphospholane derivatives using anhydrous *tert*-butyl hydroperoxide in benzene solution gave a mixture of products containing the desired **10** and a considerable amount of unidentified side products. Therefore, we turned our attention to the oxidation of 2-thiono derivatives **3** into 2-oxo **10** by means of selenium dioxide.[22]

When *N*-(2-thiono-1,3,2-oxathiaphospholanyl)amino acid methyl esters (**3**) were treated in acetonitrile solution with SeO_2 for 15 min, quantitative transformation of P═S compounds **3** into P═O derivatives **10** was observed.[23] Since the reaction was clean and no side products were formed, derivatives **10** were used for further reactions after only filtration through a small volume of silica gel in order to remove an excess of selenium dioxide. The compounds **10** were then converted into the corresponding phosphate derivatives **11** by treatment with 3-hydroxypropionitrile/DBU reagents (Scheme 4).

A major problem in amino acids chemistry is associated with racemization of the amino acid or its derivative. Since the oxathiaphospholane ring-opening process in **3** is catalyzed by a strong base such as DBU, it was necessary to check if racemization of phosphorylated derivatives occurred during this reaction. ^{31}P NMR technique was used for this purpose, and 2-thiono-1,3,2-oxathiaphospholane derivatives of racemic alanine (**3a**), leucine (**3c**), and glutamic acid (**3f**) were prepared as model compounds. ^{31}P NMR spectra recorded for L-**3** as well as for the corresponding D,L-**3** showed in both cases two signals because L-**3** exists as a pair of diastereomers identical with two enantiomeric pairs present in D,L-**3**. The ring-opening condensation of D,L-**3** with 3-hydroxypropionitrile/DBU provided isomers of D,L-**4**, which also

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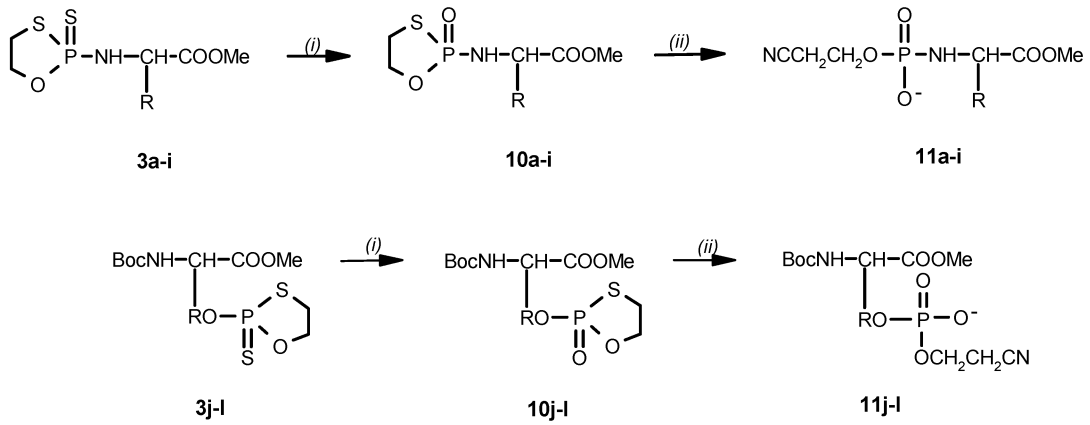
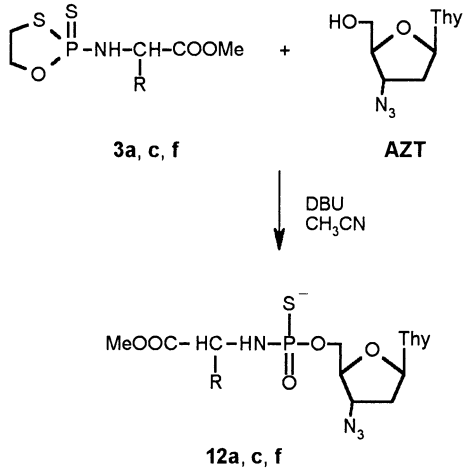
(23) Potencially this process can be used for stereospecific conversion of pure diastereoisomer of **3** into analogue **10**, as described for 3′-*O*-(2-thiono-1,3,2-oxathiaphospholanyl)nucleosides.[22]

SCHEME 4[a]



[a] Reagents: (i) SeO_2 in CH_3CN; (ii) $HOCH_2CH_2CN$, DBU.

SCHEME 5



formed two enantiomeric pairs. However, when derivatization of D,L-**3** was performed with AZT (Scheme 5), four diastereomers of conjugates **12** were visible. The same reaction performed for L-**3** provided two diastereomers. From these model experiments we concluded that the oxathiaphospholane ring-opening process catalyzed by DBU in **3** did not cause racemization in resulting phosphorothioylated amino acids **4**. Another possibility of racemization should be considered for the ammonolysis process and the treatment with LiOH. To test whether these steps may result in amino acid racemization, conjugates **12** formed from L-**3** were treated independently with aqueous ammonia and LiOH/MeOH. ^{31}P NMR spectra recorded after 12 h did not reveal any extra signals derived from D-analogues, indicating no racemization during ammonolysis and lithium hydroxide treatment. The result is in agreement with earlier literature reports that the ammonia treatment does not cause racemization of any amino acid.[24]

Conclusions

We developed a new strategy for the phosphorothioylation of amino acids using 2-thiono-1,3,2-oxathiaphospholane derivatives of amino acid methyl esters (**3**) as precursors. These intermediates are obtained in a facile and efficient way and are stable and versatile compounds that can be stored for a long time. As can be seen from the results summarized in Table 1, the oxathiaphosphorothioylation reaction works well not only for derivatization of hydroxyl groups in the side chain of amino acids but also for functionalization of the amino group. We demonstrated that oxathiaphospholane derivatives **3** can be considered as intermediates for condensation reaction with various alcohols. This reaction, based on DBU-assisted nucleophilic attack of alcohol on the phosphorus atom of the oxathiaphospholane ring in **3**, furnished phosphorothioate derivatives **4**, **6**, and **7** in high yields. The oxathiaphospholane strategy is of particular interest, since it provides a facile route to compounds possessing unprotected phosphorothioate moiety. If 3-hydroxypropionitrile was used as the alcohol, the 2-cyanoethyl group was easily removed from resulting **4** in the following ammonolysis process, leading to phosphorothioamino acids **5**. We confirmed by ^{31}P NMR analysis that the presence of DBU in the oxathiaphospholane ring-opening reactions did not induce the racemization of phosphorothioylated derivatives of amino acids.

We also demonstrated that the oxathiaphospholane strategy employing 2-oxo-1,3,2-oxathiaphospholanes derivatives **10** is suitable for phosphorylation of amino acids and is a general route to the synthesis of phosphodiesters, phosphoamidoesters, and phosphoamidates of amino acid methyl esters, as well as their phosphorothioate congeners. It is worthy to mention that it can also be expanded to the preparation of phosphorylated amino acids labeled in phosphate moiety with oxygen isotope, since ^{18}O-labeled 1,3,2-oxathiaphosphitylating reagent had been synthesized in our laboratory.[22]

In summary, we developed a new strategy for facile introduction of phosphate and phosphorothioate moiety into amino acids. Application of the oxathiaphospholane methodology to phosphorothioylation of peptides is currently in progress.

Experimental Section

General Methods. ^{31}P, ^{1}H, and ^{13}C NMR spectra (ppm) were measured on a 200 MHz spectrometer unless stated otherwise. ^{31}P NMR shift values were assigned relative to H_3PO_4 as an external standard. ∗Refers to the presence of

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diastereoisomeric peaks in the NMR spectrum. Analytical thin-layer chromatography (TLC) was performed on precoated (0.25 mm thickness) glass plates (silica gel 60 F-254). Spots were visualized by UV light (254 nm) if possible and by staining plates with either $PdCl_2$ or ninhydrin. Column chromatography was performed using Kieselgel-60 230−400 mesh silica gel. All nonvolatile compounds were routinely dried under high vacuum. Anhydrous solvents and other liquid reagents were transferred by syringe. Acetonitrile was distilled from P_2O_5 after being refluxed for several hours and stored over CaH_2. Pyridine was distilled from CaH_2 after being refluxed for several hours and stored over CaH_2. Other solvents were dried according to known methods and distilled prior to use.[25] 2-Chloro-1,3,2-oxathiaphospholane was prepared as previously reported.[17]

General Procedure for the Synthesis of *N*- or *O*-(2-Thiono-1,3,2-oxathiaphospholanyl)amino Acid Methyl Esters (3). To a solution of amino acid methyl ester **1** (1 mmol) in 5 mL of dry pyridine was added elemental sulfur (2 mmol) followed by dropwise addition of 2-chloro-1,3,2-oxathiaphospholane (**2**) (1 mmol). The reaction mixture was stirred at room temperature for 12 h. Then the solvent was removed in vacuo and the residue was triturated with acetonitrile (10 mL). Undissolved sulfur was filtered and the filtrate was condensed in vacuo. The residue was dissolved in 2−3 mL of chloroform and applied to a silica gel column (2.5 × 18 cm). The column was eluted with chloroform for compounds **3a,b**, **3d**−**l** and with methanol in chloroform (0 → 5%) for **3c**.

Appropriate fractions were combined and evaporated in vacuo to give desired compounds **3a**−**l**. Their chemical shifts in ^{31}P NMR, FAB-MS and yields are summarized in Table 1.

***N*-(2-Thiono-1,3,2-oxathiaphospholanyl)alanine Methyl Ester (3a).** ^{1}H NMR ($CDCl_3$) δ: 4.46−4.28 (m, 2H), 4.17−3.99 (m, 2H), 3.75* and 3.73* (s, 3H), 3.51−3.38 (m, 2H), 1.46* and 1.41* (d, 3H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$) δ: 173.41, 170.64, 68.69, 68.38, 52.49, 51.42, 51.21, 36.98, 36.72, 20.60 (J = 6.8 Hz), 20.40 (J = 6.4 Hz).

***N*-(2-Thiono-1,3,2-oxathiaphospholanyl)valine Methyl Ester (3b).** ^{1}H NMR ($CDCl_3$) δ: 4.50−4.26 (m, 2H), 4.00−3.76 (m, 2H), 3.73* and 3.72* (s, 3H), 3.55−3.37 (m, 2H), 2.13−2.00 (m, 1H), 0.96* and 0.92* (d, 6H, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$) δ: 172.61, 172.47, 68.79, 68.38, 61.11, 61.02, 52.11, 37.02, 36.71, 31.88 (J = 5.9 Hz), 31.64 (J = 6.0 Hz), 18.82, 17.70.

***N*-(2-Thiono-1,3,2-oxathiaphospholanyl)leucine Methyl Ester (3c).** ^{1}H NMR ($CDCl_3$) δ: 4.49−4.36 (m, 1H), 4.28−4.21 (m, 1H), 4.13−4.08* and 4.04−3.96* (m, 1H), 3.72* and 3.71* (s, 3H), 3.54−3.49 (m, 1H), 3.45−3.40 (m, 1H), 1.83−1.74 (m, 1H), 1.63−1.49 (m, 2H), 1.00 (t, 1H, J = 6.0 Hz), 0.94 (t, 6H, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$) δ: 173.58, 68.80, 68.42, 54.35, 54.08, 52.28, 42.96, 36.98 (J = 5.9 Hz), 24.27, 22.64, 21.84, 21.56.

***N*-(2-Thiono-1,3,2-oxathiaphospholanyl)phenylalanine Methyl Ester (3d).** ^{1}H NMR ($CDCl_3$) δ: 7.39−7.12 (m, 5H), 4.47−4.23 (m, 3H), 4.21−3.91 (m, 2H), 3.70* and 3.69* (s, 6H), 3.47−3.29 (m, 2H), 3.11−3.05 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 148.24, 136.99, 135.10, 129.10, 128.25, 126.87, 68.40, 68.13, 56.48, 52.06, 39.80 (J = 4.5 Hz), 36.69, 36.43.

***N*-(2-Thiono-1,3,2-oxathiaphospholanyl)aspartic Acid Dimethyl Ester (3e).** ^{1}H NMR ($CDCl_3$): δ 4.51−4.28 (m, 4H), 3.75* and 3.68* (s, 3H), 3.52−3.40 (m, 2H), 3.06−2.77 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 171.36, 171.12, 170.74, 170.58, 68.60, 68.38, 52.56, 52.50, 51.85, 51.70, 37.84 (J = 3.9 Hz), 37.52 (J = 4.9 Hz), 36.71, 36.41.

***N*-(2-Thiono-1,3,2-oxathiaphospholanyl)glutamic Acid Dimethyl Ester (3f).** ^{1}H NMR ($CDCl_3$) δ: 4.40−4.27 (m, 2H), 4.17−4.09 (m, 2H), 3.74 (s, 3H), 3.66 (s, 3H), 3.51−3.39 (m, 2H), 2.52−2.41 (m, 2H), 2.12−2.06 (m, 1H), 2.00−1.89 (m, 1H). ^{13}C NMR ($CDCl_3$) δ: 172.80, 172.30, 68.62, 68.22, 54.67, 54.35, 52.35, 51.43, 36.81, 36.52, 29.34, 29.04, 28.41.

***N*-(2-Thiono-1,3,2-oxathiaphospholanyl)methionine Methyl Ester (3g).** ^{1}H NMR ($CDCl_3$) δ: 4.58−4.46 (m, 1H), 4.44−4.22 (m, 2H), 4.21−4.03 (m, 2H), 3.77* and 3.76* (s, 3H), 3.63−3.40 (m, 2H), 2.66−2.55 (m, 2H), 2.09* and 2.08* (s, 3H), 2.04−1.89 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 172.74, 68.89, 68.51, 54.58, 54.08, 52.61, 37.15, 36.90, 33.13 (J = 6.9 Hz), 32.86 (J = 6.9 Hz), 29.79, 15.31, 15.13.

***N*-(2-Thiono-1,3,2-oxathiaphospholanyl)tryptophan Methyl Ester (3h).** ^{1}H NMR ($CDCl_3$) δ: 8.09 (bs, 1H), 7.57 (d, 1H), 7.39−7.35 (m, 1H), 7.24−7.06 (m, 3H), 4.38−4.06 (m, 4H), 3.67 (s, 3H), 3.44−3.26 (m, 4H). ^{13}C NMR ($CDCl_3$) δ: 170.10, 123.40, 123.13, 122.22, 119.64, 118.55, 118.20, 111.27, 109.57, 68.70, 68.35, 56.37, 56.11, 52.50, 36.97, 30.02.

***N*-(2-Thiono-1,3,2-oxathiaphospholanyl)proline Methyl Ester (3i).** ^{1}H NMR ($CDCl_3$) δ: 4.58−4.29 (m, 3H), 3.74* and 3.73* (s, 3H), 3.54−3.31 (m, 4H), 2.26−1.95 (m, 4H). ^{13}C NMR ($CDCl_3$) δ: 173.95, 68.47, 68.15, 62.31 (J = 6.7 Hz), 61.74 (J = 6.6 Hz), 52.25, 47.94, 47.67, 37.04, 36.67, 30.90 (J = 9.2 Hz), 25.24 (J = 8.2 Hz).

***O*-(2-Thiono-1,3,2-oxathiaphospholanyl)-*N*-(*tert*-butoxycarbonyl)serine Methyl Ester (3j).** ^{1}H NMR ($CDCl_3$) δ: 4.57−4.37 (m, 5H), 3.74* and 3.73* (s, 3H), 3.55−3.41 (m, 2H), 1.45 (s, 10H). ^{13}C NMR ($CDCl_3$) δ: 169.76, 154.94, 80.32, 70.67, 68.12 (J = 6.5 Hz), 53.65 (J = 7.4 Hz), 52.71, 36.33, 28.20.

***O*-(2-Thiono-1,3,2-oxathiaphospholanyl)-*N*-(*tert*-butoxycarbonyl)threonine Methyl Ester (3k).** ^{1}H NMR ($CDCl_3$) δ: 5.33−5.13 (m, 2H), 4.55−4.29 (m, 3H), 3.78* and 3.77* (s, 3H), 3.55−3.41 (m, 2H), 1.51−1.39 (m, 12H). ^{13}C NMR ($CDCl_3$)δ: 169.90, 155.38, 79.97, 70.31, 70.12, 57.54, 52.36, 36.33, 36.16, 27.88, 18.46, 17.95.

***O*-(2-Thiono-1,3,2-oxathiaphospholanyl)-*N*-(*tert*-butoxycarbonyl)tyrosine Methyl Ester (3l).** ^{1}H NMR ($CDCl_3$)-δ: 7.27−7.11 (m, 4H), 4.98−4.41 (m, 3H), 3.70 (s, 3H), 3.48−3.31 (m, 2H), 3.09−3.02 (m, 2H), 1.41 (s, 10H). ^{13}C NMR ($CDCl_3$) δ: 171.91, 154.80, 149.64 (J = 10.0 Hz), 133.61, 130.26, 130.12, 121.42, 79.79, 70.95, 54.23, 52.08, 37.63, 36.49, 28.09.

General Procedure for Synthesis of *N*- or *O*-[*O*-(2-Cyanoethyl)phosphorothioyl]amino Acid Methyl Esters (4). To a solution of **3** (1 mmol) in 10 mL of dry acetonitrile was added 3-hydroxypropionitrile (2 mmol) followed by DBU (1.2 mmol). The reaction mixture was stirred for 3 h at room temperature. Then the solvent was evaporated, and the residue was dissolved in a mixture of chloroform/methanol (50:1, v/v) and chromatographed on a silica gel column (2.5 × 18 cm; eluent 2% → 22% methanol in chloroform) to give compounds **4a**−**l**.

***N*-[*O*-(2-Cyanoethyl)phosphorothioyl]alanine Methyl Ester (4a).** Yield 73%. ^{31}P NMR ($CDCl_3$/CD_3OD) δ: 58.30, 58.20. ^{1}H NMR ($CDCl_3$/CD_3OD) δ: 4.05−3.95 (m, 3H), 3.69* and 3.67* (s, 3H), 3.36−3.30 (d, 2H), 2.72−2.62 (m, 2H), 1.33−1.30 (t, 3H, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$/CD_3OD) δ: 177.05, 176.24, 117.87, 59.98, 52.37, 52.24, 51.00, 50.20, 20.46 (J = 6.0 Hz), 20.21, 19.36. FAB-MS m/z: (M − 1) 251.

***N*-[*O*-(2-Cyanoethyl)phosphorothioyl]valine Methyl Ester (4b).** Yield 96%. ^{31}P NMR ($CDCl_3$/CD_3OD) δ: 58.03, 57.71. ^{1}H NMR ($CDCl_3$/CD_3OD) δ: 4.06−3.87 (m, 2H), 3. 65* and 3.64* (s, 3H), 3.62−3.53 (m, 3H), 3.32−3.04 (m, 1H), 2.68−2.60 (m, 2H), 0.89−0.79 (m, 6H). ^{13}C NMR ($CDCl_3$/CD_3OD) δ: 175.78, 175.46, 117.83, 60.37, 60.11, 59.84, 59.75, 51.84, 51.78, 31.84 (J = 5.2 Hz), 19.41 (J = 8.0 Hz), 18.80, 17.66. FAB-MS m/z: (M − 1) 279.

***N*-[*O*-(2-Cyanoethyl)phosphorothioyl]leucine Methyl Ester (4c).** Yield 96%. ^{31}P NMR ($CDCl_3$/CD_3OD) δ: 59.14, 58.25. ^{1}H NMR ($CDCl_3$/CD_3OD) δ: 4.06−3.96 (m, 3H), 3.77* and 3.71* (s, 3H), 3.60−3.40 (m, 2H), 2.77−2.74 (m, 2H), 1.82−1.76 (m, 1H), 1.54−1.49 (m, 2H))0.94* and 0.93* (d, 9H, J = 6.6 Hz). ^{13}C NMR ($CDCl_3$/CD_3OD) δ: 177.44, 176.41, 117.81, 59.88, 53.96, 53.15, 52.08, 51.92, 43.54 (J = 5.0 Hz),43.02 (J

(25) Perrin, D. D.; Armarego, W. L. F. In *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon Press: New York 1988.

= 5.0 Hz), 24.32, 22.50, 22.39, 21.83, 21.46, 19.36 (J = 8.4 Hz). FAB-MS m/z: (M − 1) 293.

***N*-[*O*-(2-Cyanoethyl)phosphorothioyl]phenylalanine Methyl Ester (4d).** Yield 73%. ^{31}P NMR ($CDCl_3/CD_3OD$) δ: 57.25, 56.73. ^{1}H NMR ($CDCl_3/CD_3OD$) δ: 7.26−7.01 (m, 5H), 4.20−3.95 (m, 3H), 3.70−3.60 (m, 1H), 3.53* and 3.51* (s, 3H), 3.45−3.29 (m, 1H), 2.94−2.69 (m, 2H), 2.37−2.28 (q, 2H, J = 5.8 Hz). ^{13}C NMR ($CDCl_3/CD_3OD$) δ: 175.45, 174.82, 136.59, 129.12, 128.07, 126.48, 117.65, 59.33, 56.72, 55.94, 51.75, 39.96 (J = 7.0 Hz), 39.64 (J = 7.0 Hz), 18.97 (J = 8.2 Hz). FAB-MS m/z: (M − 1) 327.

***N*-[*O*-(2-Cyanoethyl)phosphorothioyl]aspartic Acid Dimethyl Ester (4e).** Yield 96%. ^{31}P NMR ($CDCl_3/CD_3OD$) δ: 57.72, 56.82. ^{1}H NMR ($CDCl_3/CD_3OD$) δ: 4.30−4.19 (m, 2H), 4.02−3.91 (m, 2H), 3.66* and 3.65* (s, 3H), 3.60* and 3.58* (s, 3H), 3.55−3.50 (m, 2H), 2.81−2.74 (m, 2H), 2.65−2.63 (m, 2H). ^{13}C NMR ($CDCl_3/CD_3OD$) δ: 174.05, 173.68, 171.87, 171.71, 117.84, 59.88, 59.82, 52.56, 52.50, 51.84, 51.72, 51.13, 38.32, 19.32 (J = 8.4 Hz). FAB-MS m/z: (M − 1) 309.

***N*-[*O*-(2-Cyanoethyl)phosphorothioyl]glutamic Acid Dimethyl Ester (4f).** Yield 73%. ^{31}P NMR ($CDCl_3/CD_3OD$) δ: 58.30, 58.20. ^{1}H NMR ($CDCl_3/CD_3OD$) δ: 4.22 (bs, 1H), 4.00−3.92 (m, 2H), 3.66* and 3.64* (s, 3H), 3.61* and 3.58* (s, 3H), 3.56 (bs, 1H), 3.28 (d, 1H), 2.69−2.58 (m, 2H), 2.43−2.33 (m, 2H), 2.04−1.97 (m, 1H), 1.85−1.78 (m, 1H). ^{13}C NMR ($CDCl_3/CD_3OD$) δ: 175.13, 175.08, 174.73, 174.19, 173.72, 117.73, 59.74, 59.68, 54.06, 53.79, 52.08, 51.97, 51.61, 51.43, 29.93, 29.65, 29.06, 28.96 (J = 6.0 Hz), 19.18 (J = 8.0 Hz). FAB-MS m/z: (M − 1) 323.

***N*-[*O*-(2-Cyanoethyl)phosphorothioyl]methionine Methyl Ester (4g).** Yield 89%. ^{31}P NMR ($CDCl_3/CD_3OD$) δ: 58.57, 57.85. ^{1}H NMR ($CDCl_3/CD_3OD$) δ: 4.18−3.97 (m, 3H), 3.72* and 3.69* (s, 3H), 3.38 (bs, 1H), 3.24 (bs, 1H), 2.75−2.62 (m, 2H), 2.57−2.52 (q, 2H, J = 7.4 Hz), 2.04* and 2.03* (s, 3H), 1.99−1.94 (m, 1H), 1.91−1.82 (m, 1H). ^{13}C NMR ($CDCl_3/CD_3OD$) δ: 176.73, 175.48, 117.99, 60.24, 60.11, 54.84, 53.65, 52.61, 52.38, 33.42, 33.87, 29.95, 29.79, 19.47 (J = 8.0 Hz), 15.12. FAB-MS m/z: (M − 1) 311.

***N*-[*O*-(2-Cyanoethyl)phosphorothioyl]tryptophan Methyl Ester (4h).** Yield 93%. ^{31}P NMR ($CDCl_3/CD_3OD$) δ: 57.15, 56.11. ^{1}H NMR ($CDCl_3/CD_3OD$) δ: 8.15 (bs, 1H), 7.49 (d, 1H), 7.46−7.31 (m, 1H), 7.28−7.01 (m, 3H), 4.34−4.23 (m, 2H), 4.15−4.05 (m, 1H), 3.64* and 3.59* (s, 3H), 3.28−3.20 (m, 2H), 2.38−2.13 (m, 2H). ^{13}C NMR ($CDCl_3/CD_3OD$) δ: 176.16, 175.60, 123.41, 122.43, 121.54, 118.91, 118.14, 117.89, 117.76, 111.24, 109.58, 59.53, 55.96, 54.95, 52.02, 30.07, 29.62, 18.92 (J = 8.0 Hz). FAB-MS m/z: (M − 1) 366.

***N*-[*O*-(2-Cyanoethyl)phosphorothioyl]proline Methyl Ester (4i).** Yield 88%. ^{31}P NMR ($CDCl_3/CD_3OD$) δ: 58.77, 56.99. ^{1}H NMR ($CDCl_3/CD_3OD$) δ: 4.23−4.18 (m, 1H), 4.07−3.97 (m, 2H), 3.65* and 3.63* (s, 3H), 3.59−3.54 (m, 2H), 3.31−3.17 (m, 1H), 2.70−2.59 (m, 2H), 2.10−2.03 (m, 1H), 1.85−1.75 (m, 3H). ^{13}C NMR ($CDCl_3/CD_3OD$) δ: 177.39, 176.46, 117.92, 117.79, 61.65 (J = 7.0 Hz), 59.71 (J = 4.0 Hz), 52.12, 51.92, 47.53, 46.65, 31.33 (J = 6.8 Hz), 30.97 (J = 7.5 Hz), 25.50 (J = 7.4 Hz), 25.29 (J = 7.2 Hz), 19.33 (J = 8.0 Hz). FAB-MS m/z: (M − 1) 277.

***O*-[*O*-(2-Cyanoethyl)phosphorothioyl]-*N*-(*tert*-butoxycarbonyl)serine Methyl Ester (4j).** Yield 97%. ^{31}P NMR ($CDCl_3/CD_3OD$) δ: 59.17, 58.22. ^{1}H NMR ($CDCl_3/CD_3OD$) δ: 4.40 (bs, 1H), 4.25−4.09 (m, 2H), 4.07−3.94 (m, 2H), 3.68* and 3.67* (s, 3H), 3.57 (bs, 1H), 3.29−3.25 (qw, 1H, J = 1.6 Hz), 2.68−2.61 (m, 2H), 1.35 (s, 9H). ^{13}C NMR ($CDCl_3/CD_3OD$) δ: 171.03, 155.74, 117.53, 80.21, 65.73, 60.74, 53.86, 52.43, 28.00, 19.23 (J = 7.0 Hz). FAB-MS m/z: (M − 1) 367.

***O*-[*O*-(2-Cyanoethyl)phosphorothioyl]-*N*-(*tert*-butoxycarbonyl)threonine Methyl Ester (4k).** Yield 70%. ^{31}P NMR ($CDCl_3/CD_3OD$) δ: 58.22, 57.36. ^{1}H NMR ($CDCl_3/CD_3OD$) δ: 4.82 (bs, 1H), 4.18−4.00 (m, 2H), 3.74 (bs, 2H), 3.68* and 3.67* (s, 3H), 3.27−3.24 (qw, 1H, J = 1.6 Hz), 2.67−2.60 (t, 2H, J = 6.4 Hz), 1.35 (s, 9H), 1.27* and 1.23* (s, 3H, J = 6.4 Hz). ^{13}C NMR ($CDCl_3/CD_3OD$) δ: 172.16, 150.75, 117.65, 80.22, 72.67, 60.82, 60.46, 58.13, 52.44, 27.98, 19.32 (J = 8.0 Hz), 18.31. FAB-MS m/z: (M − 1) 381.

***O*-[*O*-(2-Cyanoethyl)phosphorothioyl]-*N*-(*tert*-butoxycarbonyl)tyrosine Methyl Ester (4l).** Yield 74%. ^{31}P NMR ($CDCl_3/CD_3OD$) δ: 53.17, 53.12. ^{1}H NMR ($CDCl_3/CD_3OD$) δ: 6.95 (dd, 4H, J = 8.4 Hz), 4.42−4.36 (t, 1H, J = 5.6 Hz), 4.23−4.04 (m, 2H), 3.61* and 3.60* (s, 3H), 3.59−3.52 (m, 2H), 2.94−2.89 (m, 2H), 2.67−2.59 (q, 2H, J = 6.0 Hz), 1.31 (s, 9H). ^{13}C NMR ($CDCl_3/CD_3OD$) δ: 172.36, 150.95, 131.60, 129.85, 120.83, 117.51, 79.96, 61.16 (J = 10.0 Hz), 54.34, 52.05, 37.24, 27.97, 19.33 (J = 8.0 Hz). FAB-MS m/z: (M − 1) 443.

General Procedure for Synthesis of *N*- or *O*-(Phosphorothioyl)amino Acid Amides (5). Compound **4** (1 mmol) was suspended in 10 mL of 20% aqueous ammonia and left for 24 h at room temperature in a tighthy closed vessel. Then the ammonia was evaporated, and the residue was dissolved in a mixture of MeOH/H_2O (6:1, v/v) and purified on a Sephadex LH-20 gel filtration column (eluent MeOH/H_2O, 6/1, v/v). Compounds **5a**−**l** were more than 92% pure according to analytical HPLC [Econosphere C-18 column (4.6 × 250 mm), 1.0 mL/min flow rate; buffer A, 0.1 M TEAB pH 7.5; buffer B, 40% CH_3CN in 0.1 M TEAB; gradient from 0 → 50% B over 20 min, 50% → 100% over 3 min, then isocratic].

***N*-(Phosphorothioyl)alaninamide (5a).** Yield 77%. ^{31}P NMR (D_2O) δ: 47.49. ^{1}H NMR (CD_3OD) δ: 3.53−3.37 (m, 1H), 1.56 (d, 3H, J = 7.2 Hz). ^{13}C NMR (CD_3OD) δ: 188.12, 50.25, 21.32 (J = 3.2 Hz). FAB-MS m/z: (M − 1) 183.

***N*-(Phosphorothioyl)valinamide (5b).** Yield 84%. ^{31}P NMR (D_2O) δ: 47.05. ^{1}H NMR (CD_3OD) δ: 3.30−3.07 (m, 1H), 2.15−2.00 (m, 1H), 0.95−0.83 (m, 6H). ^{13}C NMR (CD_3OD) δ: 187.15, 58.45, 33.21 (J = 6.5 Hz), 21.08, 18.13. FAB-MS m/z: (M − 1) 211.

***N*-(Phosphorothioyl)leucinamide (5c).** Yield 81%. ^{31}P NMR (D_2O) δ: 49.34. ^{1}H NMR (CD_3OD) δ: 3.54−3.45(m, 1H), 2.95−2.87 (m, 1H), 1.72−1.63 (m, 2H), 0.99−0.91 (m, 6H). ^{13}C NMR (CD_3OD) δ: 186.02, 55.13, 47.21 (J = 6.1 Hz), 26.10, 24.31, 22.05. FAB-MS m/z: (M − 1) 225.

***N*-(Phosphorothioyl)phenylalaninamide (5d).** Yield 72%. ^{31}P NMR (D_2O) δ: 46.01. ^{1}H NMR (CD_3OD) δ: 7.25−7.15 (m, 5H), 3.95 (m, 1H), 2.95 (m, 2H). ^{13}C NMR (CD_3OD) δ: 183.12, 136.21, 129.42, 127.92, 126.31, 55.73, 40.21. FAB-MS m/z: (M − 1) 259.

***N*-(Phosphorothioyl)asparticdiamide (5e).** Yield 83%. ^{31}P NMR (D_2O) δ: 47.35. ^{1}H NMR (CD_3OD) δ: 3.50−3.38 (m, 1H), 2.12−1.97 (m, 2H). ^{13}C NMR (CD_3OD) δ: 191.05, 187.12, 52.31, 38.34. FAB-MS m/z: (M − 1) 226.

***N*-(Phosphorothioyl)glutamicdiamide (5f).** Yield 77%. ^{31}P NMR (D_2O) δ: 47.83. ^{1}H NMR (CD_3OD) δ: 3.48−3.37 (m, 1H), 2.18−2.04 (m, 2H), 1.84−1.60 (m, 2H). ^{13}C NMR (CD_3OD) δ: 184.21, 182.12, 53.18, 36.45, 34.02. FAB-MS m/z: (M − 1) 240.

***N*-(Phosphorothioyl)methioninamide (5g).** Yield 80%. ^{31}P NMR (D_2O) δ: 41.97. ^{1}H NMR (CD_3OD) δ: 3.49−3.35 (m, 1H), 2.35−2.21 (m, 2H), 2.13 (s, 3H), 1.97−1.91 (m, 2H). ^{13}C NMR (CD_3OD) δ: 185.12, 53.00, 34.61, 29.27, 15.61. FAB-MS m/z: (M − 1) 243.

***N*-(Phosphorothioyl)tryptophanylamide (5h).** Yield 80%. ^{31}P NMR (D_2O) δ: 49.78. ^{1}H NMR (CD_3OD) δ: 7.51−7.10 (m, 3H), 7.05−6.95 (m, 2H), 3.92 (m,1H), 3.18 (m, 2H). ^{13}C NMR (CD_3OD) δ: 187.15, 135.90, 127.61, 124.12, 122.30, 119.00, 118.42, 111.22, 108.89, 55.18, 30.72. FAB-MS m/z: (M − 1) 298.

***N*-(Phosphorothioyl)prolinamide (5i).** Yield 81%. ^{31}P NMR (D_2O) δ: 47.17. ^{1}H NMR (CD_3OD) δ: 3.95−3.87 (m, 1H), 3.64−3.52 (m, 2H), 3.29−3.15 (m, 2H), 2.08−1.97 (m, 2H). ^{13}C NMR (CD_3OD) δ: 182.13, 63.21, 49.21, 30.59 (J = 9.2 Hz), 25.14. FAB-MS m/z: (M − 1) 209.

***O*-(Phosphorothioyl)-*N*-(*tert*-butoxycarbonyl)serinamide (5j).** Yield 83%. ^{31}P NMR (D_2O) δ: 46.83. ^{1}H NMR (CD_3OD) δ: 3.31−3.17 (m, 1H), 2.95−2.80 (m, 2H), 1.44 (s, 9H). ^{13}C NMR (CD_3OD) δ: 183.99, 155.17, 84.07, 65.12, 56.42, 28.01. FAB-MS m/z: (M − 1) 299.

***N*-(Phosphorothioyl)-*N*-(*tert*-butoxycarbonyl)threoninamide (5k).** Yield 80%. ^{31}P NMR (D_2O) δ: 48.52. ^{1}H NMR (CD_3OD) δ: 3.43−3.33 (m, 1H), 3.01−2.86 (m, 1H), 1.44 (s, 9H), 1.32 (d, 3H, J = 6.2 Hz). ^{13}C NMR (CD_3OD) δ: 183.12, 156.12, 84.00, 68.15, 59.47, 28.15, 19.31. FAB-MS *m/z*: (M − 1) 313.

***N*-(Phosphorothioyl)-*N*-(*tert*-butoxycarbonyl)tyrosinamide (5l).** Yield 77%. ^{31}P NMR (D_2O) δ: 43.92. ^{1}H NMR (CD_3OD) δ: 7.21−6.88 (m,4H), 3.80−3.62 (m, 1H), 2.90−2.85 (m, 2H), 1.37 (s, 9H). ^{13}C NMR (CD_3OD) δ: 183.77, 156.21, 155.47, 130.12, 126.74, 115.32, 86.22, 56.14, 40.12, 28.00. FAB-MS *m/z*: (M − 1) 375.

***N*-(*O*-Methylphosphorothioyl)leucine Methyl Ester (6c).** Reaction of compound **3c** (1 mmol) with methanol (1.5 mmol) in the presence of DBU (1.1 mmol) was performed as described for **4**. Yield 96%. ^{31}P NMR ($CDCl_3$) δ: 63.64, 60.10. ^{1}H NMR ($CDCl_3$) δ: 3.97 (bs, 1H), 3.80 (bs, 1H), 3.62* and 3.55* (s, 3H), 3.44* and 3.38* (d, 3H, J = 12.7 Hz), 1.88−1.45 (m, 1H), 1.39−1.33 (m, 2H), 0.79−0.75 (qw, 6H). ^{13}C NMR ($CDCl_3$) δ: 178.29, 176.26, 52.57, 52.28 (J = 4.8 Hz), 51.61, 43.40 (J = 6.0 Hz), 24.07, 22.59, 21.78. FAB-MS *m/z*: (M − 1) 254.

***N*-(*O*-Methylphosphorothioyl)leucine Dilithium Salt (8c).** Compound **6c** (O.5 mmol) was dissolved in methanol (3.0 mL), into which was added a 1.0 M aqueous solution of LiOH (2.5 mL). The solution was stirred at room temperature for 12 h and then filtered. The solvent was evaporated in vacuo, and the residue was resuspended in anhydrous methanol and filtered through 0.2 μm Teflon membrane to provide **8c**. ^{31}P NMR (CD_3OD) δ: 59.43, 59.04. ^{1}H NMR (CD_3OD) δ: 3.68−3.57 (m, 1H), 3.51* and 3.49* (d, 3H, J = 12.9 Hz), 1.69−1.59 (m, 2H), 1.46−1.37 (m, 2H), 0.91−0.86 (qw, 6H). ^{13}C NMR (CD_3OD) δ: 58.96 (J = 4.6 Hz), 55.43, 54,0.81, 47.17, 27.11, 26.06, 24.96, 24.70. FAB-MS *m/z*: (M+1) 254, (M-Li) 246.

***N*-(*O*-Methylphosphorothioyl)glutamic Acid Dimethyl Ester (6f).** Reaction of compound **3f** (1 mmol) with methanol (1.5 mmol) and DBU (1.1 mmol) was performed as described for **4**. Yield 96%. ^{31}P NMR ($CDCl_3$) δ: 60.59, 59.18. ^{1}H NMR ($CDCl_3$) δ: 3.96 (bs, 1H), 3.67* and 3.65* (s, 3H), 3.61* and 3.59* (s, 3H), 3.58−3.56 (m, 1H), 3.47* and 3.44* (d, 3H, J = 13.0 Hz), 2.45−2.37 (m, 2H), 2.04−1.97 (m, 1H), 1.87−1.79 (m, 1H). ^{13}C NMR ($CDCl_3$) δ: 174.13, 173.79, 53.86, 52.32 (J = 4.9 Hz), 51.72, 51.58, 30.14, 29.78. FAB-MS *m/z*: (M − 1) 284.

***N*-(*O*-Methylphosphorothioyl)glutamic Acid Trilithium Salt (8f).** The reaction was carried out according to the procedure described in the case of **8c**. Compound **8f**: ^{31}P NMR (CD_3OD) δ: 60.52, 60.32. ^{1}H NMR (CD_3OD) δ: 3.68−3.56 (m, 1H), 3.45* and 3.42* (d, 3H, J = 13.1 Hz), 2.23−2.15 (m, 2H), 1.87−1.76 (m, 4H). ^{13}C NMR (CD_3OD) δ: 59.74 (J = 4.7 Hz), 57.70, 54.84, 42.05, 36.61, 34.36. FAB-MS *m/z*: (M − 1) 274, (M − Li) 268.

***N*-(*O*-Benzylphosphorothioyl)leucine Methyl Ester (7c).** Reaction of compound **3c** (1 mmol) with benzyl alcohol (1.5 mmol) in the presence of DBU (1.1 mmol) was performed as described for **4** except that the reaction mixture was stirred for 12 h at room temperature. Yield 90%. ^{31}P NMR ($CDCl_3$) δ: 59.15, 57.30, ^{1}H NMR ($CDCl_3$) δ: 7.29−7.17 (m, 5H), 4.88 (bs, 1H), 4.78 (bs, 1H), 4.01* and 3,83* (bs, 1H), 3.58* and 3.54* (s, 3H), 3.33 (s, 1H), 1.69−1.60 (m, 1H), 1.43−1.35 (m, 2H), 0.80 (t, 6H, J = 6.0 Hz). ^{13}C NMR ($CDCl_3$) δ: 176.63, 137.82, 137.76, 128.07, 127.56, 127.37, 127.29, 68.71 (J = 5.9 Hz), 52.00, 51.80, 43.56 (J = 6.0 Hz), 22.51, 22.35, 21.98, 21.57. FAB-MS *m/z*: (M − 1) 330.

***N*-(*O*-Benzylphosphorothioyl)leucine Dilithium Salt (9c).** The reaction was carried out according to the procedure described in the case of **8c**. Compound **9c**: ^{31}P NMR (CD_3OD) δ: 58.49, 57.54. ^{1}H NMR (CD_3OD) δ: 7.36−7.27 (m, 5H), 4.82 (s, 2H), 3.61−3.56 (m, 2H), 1.58−1.51 (m, 1H), 1.32−1.29 (m, 2H), 0.79−0.76 (q, 6H, J = 3.0 Hz). ^{13}C NMR (CD_3OD) δ: 131.37, 130.70, 69.63, 56.77, 51.60, 47.24, 27.04, 24.96, 24.81, 24.68. FAB-MS *m/z*: (M − 1) 322, (M − Li) 316.

***N*-(*O*-Benzylphosphorothioyl)glutamic Acid Dimethyl Ester (7f).** Reaction of compound **3f** (1 mmol) with benzyl alcohol (1.5 mmol) in a presence of DBU (1.1 mmol) was performed as described for **4** except that the reaction mixture was stirred for 12 h at room temperature. Yield 96%. ^{31}P NMR ($CDCl_3$) δ: 59.37, 58.34. ^{1}H NMR ($CDCl_3$) δ: 7.28−7.16 (m,5H), 4.96−4.80 (t, 1H), 4.81−4.76 (t, 1H), 4.99 (d, 1H), 3.58−3.55 (m, 6H), 3.37−3.12 (m, 1H), 2.36 (t, 2H), 1.81 (bs, 1H), 1.79 (bs, 1H). ^{13}C NMR ($CDCl_3$) δ:175.12, 174.51, 137.50, 137.12, 128.15, 127.32, 127.10, 126.92, 68.43 (J = 5.9 Hz), 52.47, 51.43, 51.03, 29.92, 29.14. FAB-MS *m/z*: (M − 1) 360.

***N*-(*O*-Benzylphosphorothioyl)glutamic Acid Trilithium Salt (9f).** The reaction was carried out according to the procedure described in the case of **8c**. Compound **9f**: ^{31}P NMR (CD_3OD) δ: 59.14, 58.37. ^{1}H NMR (CD_3OD) δ: 7.47−7.33 (m, 5H), 4.91−4.84 (m, 3H), 3.75−3.58 (m, 1H), 2.26−2.16 (m, 2H), 1.90−1.80 (m, 2H). ^{13}C NMR (CD_3OD) δ: 131.47, 130.80, 130.55, 69.82, 59.71, 59.54, 36.58, 34.62. FAB-MS *m/z*: (M − Li) 344.

General Procedure for the Synthesis of *N*- or *O*-[*O*-(2-Cyanoethyl)phosphoryl]amino Acid Methyl Esters (11). (A) Synthesis of *N*- or *O*-(2-Oxo-1,3,2-oxathiaphospholanyl)amino Acid Methyl Esters (10). Compound **3** (1 mmol) was dissolved in dry acetonitrile (10 mL), and then SeO_2 (2 mmol) was added. The reaction mixture was stirred for 15 min at room temperature. At this point, the ^{31}P NMR control showed full disappearance of the substrate and quantitative formation of 2-oxo derivative **10** (two signals absorbing at 44−48 ppm). Then the reaction mixture was passed through a dry silica gel column (ca. 1 g) to absorb traces of dissolved SeO_2. **(B) Condensation of 10 with 3-Hydroxypropionitrile.** Crude compound **10** in acetonitrile solution was reacted with 3-hydroxypropionitrile and DBU, providing **11**. The reaction was carried out according to general procedure described for compounds **4**.

***N*-(2-Oxo-1,3,2-oxathiaphospholanyl)alanine Methyl Ester (10a).** ^{31}P NMR (CD_3CN) δ: 46.26, 45.84. FAB-MS *m/z*: (M − 1) 224.

***N*-[*O*-(2-Cyanoethyl)phosphoryl]alanine Methyl Ester (11a).** Yield 70%. ^{31}P NMR ($CDCl_3/CD_3OD$) δ: 4.92. ^{1}H NMR ($CDCl_3/CD_3OD$) δ: 4.05−3.95 (m, 3H), 3.69 (s, 3H), 3.36−3.30 (d, 2H), 2.72−2.62 (m, 2H), 1.33−1.30 (t, 3H, J = 6.8 Hz). ^{13}C NMR ($CDCl_3/CD_3OD$) δ: 177.05, 176.24, 117.87, 59.98, 52.37, 52.24, 51.00, 50.20, 20.46 (J = 6.0 Hz), 20.21, 19.36. FAB-MS *m/z*: (M − 1) 235.

***N*-(2-Oxo-1,3,2-oxathiaphospholanyl)leucine Methyl Ester (10c).** ^{31}P NMR (CD_3CN) δ: 47.73, 46.80. FAB-MS *m/z*: (M − 1) 266.

***N*-[*O*-(2-Cyanoethyl)phosphoryl]leucine Methyl Ester (11c).** Yield 71%. ^{31}P NMR ($CDCl_3/CD_3OD$) δ: 5.12. ^{1}H NMR ($CDCl_3/CD_3OD$) δ: 4.18−4.01 (m, 3H), 3.71 (s, 3H), 3.75−3.52 (m, 2H), 2.78−2.71 (m, 2H), 1.90−1.82 (m, 1H), 1.60−1.53 (m, 2H), 0.93 (d, 6H, J = 6.6 Hz). ^{13}C NMR ($CDCl_3/CD_3OD$) δ: 177.40, 176.61, 118.11, 59.88, 53.15, 52.08, 51.92, 44.14 (J = 5.2 Hz), 43.02 (J = 5.0 Hz), 24.32, 22.90, 22.39, 21.83, 19.36 (J = 8.2 Hz). FAB-MS *m/z*: (M − 1) 277.

***N*-(2-Oxo-1,3,2-oxathiaphospholanyl)aspartic Acid Dimethyl Ester (10e).** ^{31}P NMR (CD_3CN) δ: 47.36, 47.16. FAB-MS *m/z*: (M − 1) 282.

***N*-[*O*-(2-Cyanoethyl)phosphoryl]aspartic Acid Dimethyl Ester (11e).** Yield 72%. ^{31}P NMR ($CDCl_3/CD_3OD$) δ: 4.45. ^{1}H NMR ($CDCl_3/CD_3OD$) δ: 4.36−4.25 (m, 2H), 4.11−3.99 (m, 2H), 3.66 (s, 3H), 3.61 (s, 3H), 3.58−3.54 (m, 2H), 2.88−2.76 (m, 2H), 2.66−2.61 (m, 2H). ^{13}C NMR ($CDCl_3/CD_3OD$) δ: 174.32, 173.99, 172.17, 171.71, 117.64, 59.88, 59.77, 52.76, 52.61, 51.84, 51.90, 51.75, 38.32, 19.32. FAB-MS *m/z*: (M − 1) 293.

***N*-(2-Oxo-1,3,2-oxathiaphospholanyl)tryptophan Methyl Ester (10h).** ^{31}P NMR (CD_3CN) δ: 47.42, 46.66. FAB-MS *m/z*: (M − 1) 339.

***N*-[*O*-(2-Cyanoethyl)phosphoryl]tryptophan Methyl Ester (11h).** Yield 68%. ^{31}P NMR ($CDCl_3/CD_3OD$) δ: 5.10. ^{1}H NMR ($CDCl_3/CD_3OD$) δ: 8.10 (bs, 1H), 7.50 (d, 1H), 7.48−7.32 (m, 1H), 7.27−7.00 (m, 3H), 4.32−4.25 (m, 2H), 4.18−4.08 (m, 1H), 3.60 (s, 3H), 3.28−3.21 (m, 2H), 2.36−2.15 (m, 2H). ^{13}C NMR ($CDCl_3/CD_3OD$) δ: 176.10, 175.83, 123.01, 122.13, 121.56, 119.01, 118.34, 118.07, 117.78, 111.34, 109.78, 59.53, 55.72, 54.85, 52.34, 30.56, 29.62, 18.73. FAB-MS *m*/*z*: (M − 1) 350.

***O*-(2-Oxo-1,3,2-oxathiaphospholanyl)-*N*-(*tert*-butoxycarbonyl)threonine Methyl Ester (10k).** ^{31}P NMR (CD_3CN) δ: 45.36, 45.30. FAB-MS *m*/*z*: (M − 1) 354.

***O*-[*O*-(2-Cyanoethyl)phosphoryl]-*N*-(*tert*-butoxycarbonyl)threonine Methyl Ester (11k).** Yield 62%. ^{31}P NMR ($CDCl_3/CD_3OD$) δ: 0.39. ^{1}H NMR ($CDCl_3/CD_3OD$) δ: 4.79 (bs, 1H), 4.17−4.02 (m, 2H), 3.74 (bs, 2H), 3.65 (s, 3H), 3.25−3.20 (m, 1H), 2.64−2.55 (t, 2H, J = 6.2 Hz), 1.32 (s, 9H), 1.24 (s, 3H, J = 6.2 Hz). ^{13}C NMR ($CDCl_3/CD_3OD$) δ: 172.56, 151.00, 117.82, 80.56, 72.67, 60.88, 60.36, 58.43, 52.44, 28.05, 19.48, 18.30. FAB-MS *m*/*z*: (M − 1) 365.

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Supporting Information Available: Experimental procedure and ^{31}P NMR spectra for compounds **12a**, **12c**, and **12f** obtained in DBU catalyzed reactions of L-**3a**, D,L-**3a**, L-**3c**, D,L-**3c**, L-**3f**, and D,L-**3f** with AZT. This material is available free of charge via the Internet at http://pubs.acs.org.

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