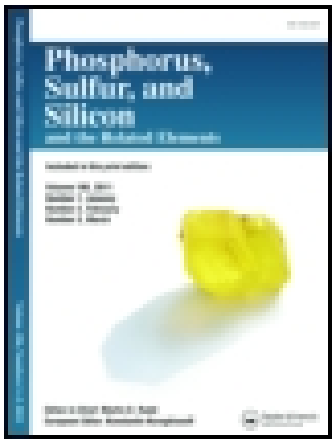

Phosphorus, Sulfur, and Silicon and the Related Elements

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# Oxathiaphospholane Approach to the Synthesis of Conjugates of Amino Acids Methyl Esters with Nucleosides

Janina Baraniak [a] , Renata Kaczmarek [a] , Ewa Wasilewska [a] & Wojciech J. Stec [a]

[a] Department of Bioorganic Chemistry , Centre of Molecular and Macromolecular Studies Polish Academy of Science, Department of Bioorganic Chemistry , Sienkiewicza 112, Łódź, 90-363, Poland

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# OXATHIAPHOSPHOLANE APPROACH TO THE SYNTHESIS OF CONJUGATES OF AMINO ACIDS METHYL ESTERS WITH NUCLEOSIDES

*Janina Baraniak, Renata Kaczmarek, Ewa Wasilewska, and Wojciech J. Stec*
*Department of Bioorganic Chemistry, Centre of Molecular and Macromolecular Studies Polish Academy of Science, Department of Bioorganic Chemistry, Sienkiewicza 112, 90-363 Łódź, Poland*

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*Based upon 1,3,2-oxathiaphospholane chemistry, 5′-O-derivatization of nucleosides with the O-methyl esters of amino acids was performed and corresponding conjugates were obtained in satisfactory yield.*

*Keywords:* Conjugates; N- and O-phosphorothioylated amino acids; nuclesides; oxathiaphospholane ring opening condensation

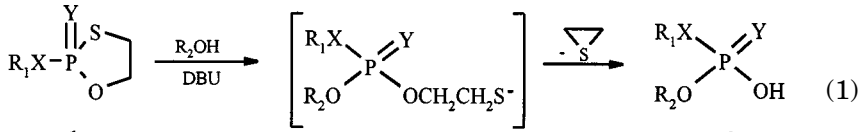
In the light of early recognized importance of enzymatic *O*-phosphorylation of hydroxyl groups of serine, threonine, or tyrosine in the peptides sequence for their biological function,[1] chemical phosphorylation of proteins become a matter of challenge for numerous chemical establishment.

Independently, discovery of nucleoproteins in which the hydroxy groups of L-amino acids serine, threonine, or tyrosine are conveniently bound *via* phosphate linkages to 5′-end of nucleic acids,[2] initiated extensive search for new methods of their chemical synthesis. On the other side, natural products containing phosphoramidate linkage between 5′-*O*-nucleoside and amino acid have been identified.[3] Therefore, an attention has been attracted to the development of a new method of synthesis of nucleoside and amino acid conjugates *via* phosphoramidate linkage.[4] Accessibility of such hybrids has opened the door to the exploration of peptide-oligonucleotide conjugates as antisense constructs.

In this laboratory, a novel method of synthesis of phosphodiesters and phosphoramidoesters has been designed. This method is based upon attachment of 1,3,2-oxathiaphospholane moiety to hydroxyl or

Address correspondence to J. Baraniak. E-mail: baraniak@bio.cbmm.lodz.pl

amino-groups and their P-oxidation or P-sulfurization, providing intermediate like **1** (Eq. 1, Y=O, S).




**1** **3** (1)

If R carries chiral auxiliary, compound of type **1** may be separated into diastereomers. DBU-assisted 1,3,2-oxathiaphospholane ring opening condensation of **1** with alcohols provides in high yields phosphorothioate diesters or phosphorothioate amidoesters, respectively, and if diastereomerically pure **1** is used, aforementioned compound can be prepared in stereocontrolled manner. Examples of successful application of OTP methodology to the synthesis of phosphorothioate diesters and amidoesters are numerous and include the first stereocontrolled synthesis of oligo(nucleoside phosphorothioate)s,[5] dinucleoside N3′-P5′ phosphates and phosphorothioates,[6] dinucleoside 3′,5′-boranophosphates,[7] or bis-adenosylphosphorothioylated polyols.[8]

In this communication we wish to present our recent results on 1,3,2-oxathiaphosphorothioylation of O-methyl esters of amino acids. Their treatment with an equimolar amount of 2-chloro-1,3,2-oxathiaphospholane (**2**) in pyridine solution in the presence of elemental sulfur provided 2-N-(carbomethoxyaminoacido)-[2-thiono-1,3,2-oxathiaphospholanes] (**1**, Y=S, $R_1X$=amino acid O-methyl ester). Compounds **1** were isolated from the reaction mixture by silica gel column chromatography with satisfactory yields and were characterized by means of $^1H$ NMR, $^{31}P$ NMR, and FAB-MS analysis (Table I).

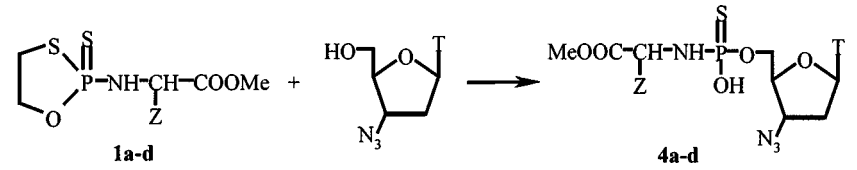
**TABLE I** The Physicochemical Characteristics of Compounds **1a–g** and **4b**, **4d**

| Comp. | Z | $^{31}P$ NMR [ppm] | FAB-MS [M-1] | Yield [%] |
|---|---|---|---|---|
| **1a** | $-CH_3$ | 96.7; 97.3 | 241 | 83 |
| **1b** | $-CH_2Ph$ | 96.5; 95.6 | 316 | 89 |
| **1c** | $-CH_2CH_2COOMe$ | 97.7; 96.1 | 313 | 80 |
| **1d** | $-CH_2$-indoyl-3 | 96.5; 95.5 | 355 | 75 |
| **1e** | $-CH_2-$ | 105.6; 105.5 | 356 | 62 |
| **1f** | $-CH(CH_3)$ | 104.0; 103.9 | 370 | 92 |
| **1g** | $-CH_2C_6H_4-$ | 100.52 | 432 | 73 |
| **4b** | $-CH_2Ph$ | 58.5; 58.2 | 523 | 87 |
| **4d** | $-CH_2$-indoyl-3 | 58.5; 58.4 | 562 | 92 |

Compounds **1** were subjected to the DBU-assisted ring-opening condensation process by means of 5′-hydroxyl group of nucleosides. The cleavage of endocyclic P—S bond was followed by fast elimination of episulfide leading to nucleosido-5′-aminoacidophosphoramidothioates (**4**). DBU is the reagent of choice for acceleration of the 1,3,2-oxathiaphospholane ring opening condensation process, although the bases, such as imidazole or DMAP, can be used as the catalysts for this condensation.

Elaborated procedure has been applied first for the synthesis of phosphorothioate conjugates of amino acids with antiviral nucleosides.[9] It has been known from the literature that the higher the activity against HIV-1 replication in PBMCs cells, then the parent, AZT, exhibits phosphate derivatives of L-tryptophan and L-phenylalanine.[10] Therefore, phosphorothioate analogues **4b** and **4d** have been obtained.

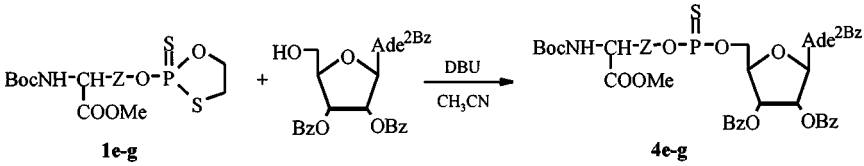
AZT and 2′,3′-dideoxythymidine (D4T) were chosen as antiviral nucleosides[8] (Eq. 2).




(2)

In the case of tryptophan, diastereomeric OTP derivatives **1d** were separated *via* crystallization, and the absolute configuration at phosphorus atom of diastereomer absorbing at lower field in $^{31}$P NMR spectrum has been assigned by X-ray analysis as $R_P$. Therefore, compound **4d** prepared from $R_P$-**1d** has $S_P$-configuration. At present, phosphorothioate conjugates of amino acids with AZT and D4T (**4**) are tested as inhibitors of HIV-1 transcriptase.[11]

The second type of conjugates involves phosphodiester linkage between 5′-hydroxyl group of nucleoside and hydroxyl group of amino acid side chains. The *O*-oxathiaphospholane derivatives of appropriately amino- and carboxyl-protected threonine and tyrosine (**1e–g**) were condensed with nucleosides (Eq. 3).




(3)

The essential problem in the synthesis of that type of conjugate is their stability under basic conditions, casually used for the removal of nucleoside-protecting groups.[9] Compounds **4** were treated with conc. aqueous ammonia for 24 h. The desired removal of benzoyl protecting group was accompanied by concomitant conversion of carboxymethyl into carboxamide function; decomposition of phosphorothioate linkage involving 5′-O-nucleoside and hydroxyl side chain of aforementioned amino acids were not observed.

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