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Synthesis of Nucleoside α-Thiotriphosphates via an Oxathiaphospholane Approach†

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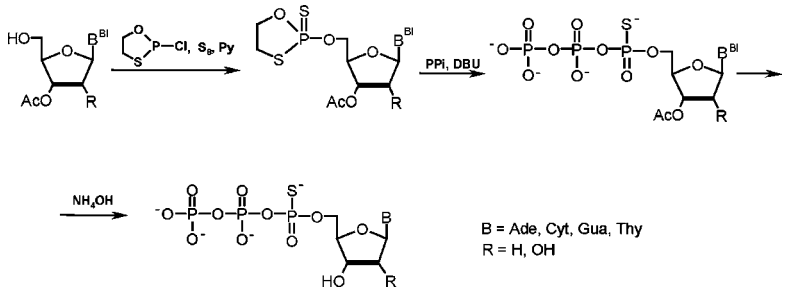
ABSTRACT



Nucleoside 5′-*O*-(α-thiotriphosphates) were obtained in reactions of the appropriate nucleoside 5′-*O*-(2-thio-1,3,2-oxathiaphospholanes) with pyrophosphate in the presence of DBU. The presented method allows also for preparation of α-seleno congeners and corresponding α-modified diphosphates.

Nucleoside 5′-*O*-(α-thiotriphosphates) (**1**), obtained for the first time by Eckstein and Gindl in the late 1960s[1] in the reaction of an appropriate 5′-*O*-phosphorothioimidazolidate with inorganic pyrophosphate, gained wide application in biochemistry and in molecular and cell biology.[2] These compounds were used for the enzymatic synthesis of DNA and RNA fragments possessing phosphorothioate internucleotide bonds. DNA and RNA polymerases accept as substrates only S_P-diastereoisomers of **1** to provide oligo(deoxyribo-[3] and -ribonucleoside[4] phosphorothioate)s with R_P configuration at P-chiral centers, as first pointed out by Eckstein. Besides their wide application for synthesis of phosphorothioate-labeled oligonucleotides, nucleoside 5′-*O*-(α-thiotriphosphates) (**1**) were used as tools for the study of the mode of action of several enzymes, e.g., myosine, AMV reverse transcriptase, DNA-dependent DNA polymerase and RNA polymerase, adenylate and guanylate cyclases, RNA ligase, various kinases, synthetases, and transferases. Compounds **1** were also employed in DNA sequencing, mutagenesis, labeling hybridization probes, and recently for studies on nucleotide receptors.[5]

In parallel with their broad applications, numerous efforts have been made to develop other methods for preparing of

† This paper is dedicated to Professor Marvin H. Caruthers on the occasion of his 65th birthday.

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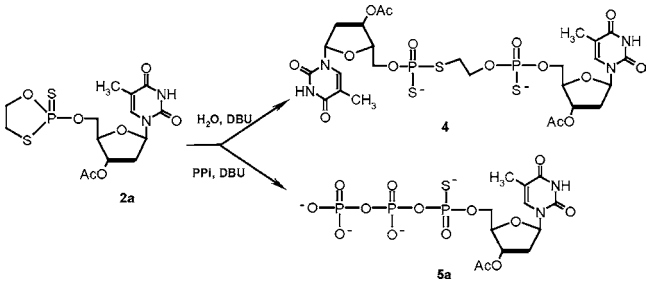
1.[6] In 1989, Eckstein and Ludwig described a new and efficient procedure for the synthesis of **1** employing tricoordinated phosphorus compounds as phosphitylating reagents.[7] Condensation of appropriately protected nucleosides and 2-chloro-4*H*-1,3,2-benzodioxaphosphorine resulted in an intermediate, which without isolation, reacted first with bis(tri-*n*-butylammonium) pyrophosphate followed by an addition of elemental sulfur, providing, after deprotection, the desired α-thiotriphosphates **1** with 60−75% yield. The Ludwig−Eckstein method was also applied for the synthesis of some α-thiotriphosphates on a solid support.[8]

The successful synthesis of various biophosphates using oxathiaphospholane methodology (OTP)[9] prompted us to examine its use for the synthesis of nucleoside 5′-*O*-(α-thiotriphosphates) (**1**). Early results[10] on the opening of the 2-thio-1,3,2-dithiaphospholane ring system with inorganic pyrophosphate leading to nucleoside 5′-*O*-α-dithiotriphosphate provided evidence that ring-opening condensation with pyrophosphate is feasible, albeit the yield of desired product was rather discouraging. Results of our recent studies on the synthesis of nucleoside 5′-*O*-α-thiotriphosphates via an OTP approach are presented in this communication.

First attempts to obtain **1** in reaction of 3′-*O*-acetylthymidine 5′-*O*-(2-thio-1,3,2-oxathiaphospholane) (**2a**, mixture of diastereoisomers, dr ca. 1:1) with tris(tetra-*n*-butylammonium) hydrogen pyrophosphate (**3**) in the presence of an equimolar amount of DBU[9a,11] failed. The total consumption of **2a** was observed after 30 min. Analysis of ^{31}P NMR spectra[12] of the reaction mixture revealed the presence of two groups of signals at ca. 67 and 56 ppm that suggested the formation of "dimeric" product **4** as a result of ring-opening nucleophilic substitution in **2a** with water[11] (Scheme 1).

Scheme 1. Ring-Opening Condensation of 3′-*O*-Acetylthymidine 5′-*O*-(2-Thio-1,3,2-oxathiaphospholane) with Pyrophosphate



Since the above reaction was performed under strictly anhydrous conditions[13] (oven-dried glass equipment, argon atmosphere, acetonitrile with less than 20 ppm of water), the only source of water could come from a solution of pyrophosphate **3**. Reagent **3** was obtained from potassium pyrophosphate by cation exchange on Dowex 50WX8 and neutralization of pyrophosphoric acid with tetra-*n*-butylammonium hydroxide, and the resulting solution was lyophilized.[14] Prepared in this way, pyrophosphate **3** exists, however, as a hydrate.[15] Removal of water by the method described by Poulter[14] (crystallization from ethyl acetate or coevaporation from acetonitrile solutions) appeared to be insufficient to obtain **3** dry enough to produce 3′-*O*-acetylthymidine 5′-*O*-(α-thiotriphosphate) (**5a**) in its reaction with **2a**. Sufficiently dry **3** was prepared by pretreatment of acetonitrile solution of **3** with 3 Å molecular sieves before its reaction with a diastereomeric mixture (ca. 1:1) of **2a**. A 10% molar excess of DBU was used, and after 2 h, the resonance signal from **2a** vanished (^{31}P NMR assay), providing **5a** with 58% NMR yield. Compound **5a** was isolated from the reaction mixture by DEAE-Sephadex chromatography in 32% yield (as calculated on the basis of starting **2a**), and its structure was proved by ^{31}P NMR and FAB-MS. Applicability of this procedure for the synthesis of **1** was examined using 5′-*O*-(2-thio-1,3,2-oxathiaphospholanes) of all eight common deoxyribo- and ribonucleosides **2a**−**h** (Scheme 2). Starting from suitably protected nucleosides[16] in a reaction with 2-chloro-1,3,2-oxathiaphospholane[9c,17] in the presence of elemental sulfur in a pyridine solution, we obtained compounds **2a**−**h** with excellent yields. Oxathiaphospholanes **2a**−**h** (mixture of diastereomers, ca. 1:1) reacted with a dry solution of **3** (as described above) in the presence of DBU (10% molar excess), providing protected nucleoside 5′-*O*-α-thiotriphosphates **5a**−**h** in 48−78% yield, as estimated by ^{31}P NMR (Table 1). No stereoselectivity was observed in these reactions since both diastereomers of **5a**−**h** were formed in an equimolar ratio. Deprotection reactions were performed by the use of concentrated ammonia solution, and conditions

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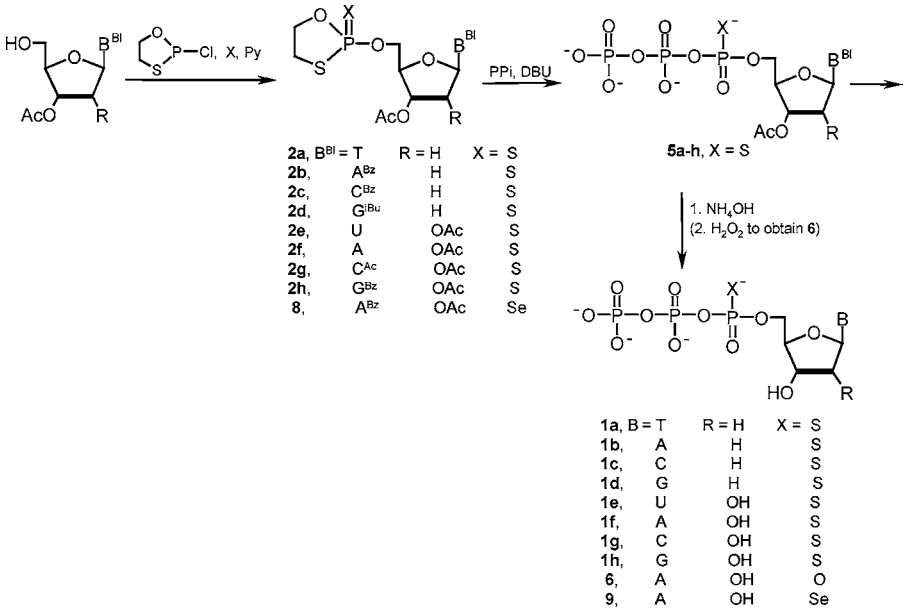
Scheme 2. Synthesis of Nucleoside 5′-*O*-(α-Thiotriphosphates)



for **5a**−**h** were optimized for each compound by monitoring the progress of deprotection using HPLC.

Obtained crude **1a**−**h** were purified by DEAE-Sephadex chromatography. For **1d** and **1h**, an additional purification by RP-HPLC was required to obtain analytically pure compounds since obtained samples were slightly contaminated with the aforementioned "dimers" of structure **4**. The yields of obtained **1a**−**h** as a ca. 1:1 mixture of diastereomers are presented in Table 1. Their structures were proved by MALDI-MS and by ^{31}P NMR spectra, identical with those reported in the literature.[7] At this stage, this new synthesis of nucleoside 5′-*O*-(α-thiotriphosphates) was not optimized, especially with respect to conditions of their isolation.[18] Inspection of data presented in Table 1 indicates the dramatic difference between the yield of formation of protected triphosphates **5a**−**h** with those obtained for isolated final products **1a**−**h**. Apparently, the purification procedure requires further improvements.

We demonstrated that obtained nucleoside 5′-*O*-α-thiotriphosphates can be simply oxidized into the appropriate triphosphates by means of Oxone[19] and hydrogen peroxide. As an example, **1f** was converted using these reagents into

Table 1. Yields and Physicochemical Characteristics of Nucleoside 5′-*O*-(α-Thiotriphosphates) **1a**−**h**

compd	yield of condensation (**5a**−**h**, ^{31}P NMR)	conditions[a] of DEAE-Sephadex purification	total yield	HPLC[b], t_R [min]	MALDI-MS
1a (dTTPαS)	58	0.4→0.8	18	7.16, 7.55	497
1b (dATPαS)	70	0.1→0.7	17	8.07, 8.30	506
1c (dCTPαS)	78	0.4→0.8	27	5.85, 6.23	482
1d (dGTPαS)	48	0.2→0.7	10[c]	7.20, 7.39	522
1e (UTPαS)	52	0.4→1.0	12	7.36, 7.82	499
1f (ATPαS)	71	0.05→0.7	27	8.02, 8.45	522
1g (CTPαS)	73	0.2→0.7	30	7.35, 7.75	498
1h (GTPαS)	68	0.05→0.7	16[c]	6.95, 7.40	538

[a] Initial and final concentration of triethylammonium bicarbonate buffer. [b] HPLC conditions: Econosphere C18, 5 μm, 250 × 4.6 mm column eluted with 0.1 M TEAB, pH 7.5, with 0→60% acetonitrile in 20 min. [c] After RP-HPLC purification.

ATP (**6**) in 63 and 65% yield, respectively. We have also found that oxathiaphospholane **2a** reacted with bis(tetra-*n*-butylammonium) hydrogen phosphate, and the appropriate thymidine 5′-*O*-α-thiodiphosphate (**7**)[6c] was obtained with 24% yield. Very recently, Ramsay−Shaw reported that oxathiaphospholane chemistry was successfully employed for an efficient synthesis of deoxy- and ribonucleoside 5′-*O*-α-boranodiposphates.[20]

Since our aim was to study the scope and limitations of OTP methodology, it was also tempting to apply this procedure for the synthesis of other analogues of nucleoside tri- and diphosphates, including α-selenotriphosphates. The lack of general and sufficient methodology of the preparation of this class of compounds was stressed in a recent review.[21] It is also worth mentioning that chimeric PSe/PO oligonucleotides, first described by us in 1984,[22] have been recently suggested to be invaluable tools for X-ray structure analysis of nucleic acids.[23] Therefore, the seleno analogue of **2f**, namely, 2′,3′-*O*,*O*-diacetyladenosine 5′-*O*-(2-seleno-1,3,2-oxathiaphospholane) (**8**), was synthesized in a manner similar to that employed for the preparation 2-thio compounds **2a**−**h** and used as a substrate for preparation of adenosine 5′-*O*-α-selenotriphosphate **9** and corresponding -diphosphate **10** with 31 and 23% yields, respectively. The evidence presented here that ring-opening condensation of oxathiaphospholanes **2** with pyrophosphate and phosphate ions provides α-modified polyphosphates was corollary for further development of OTP methodology toward the synthesis of numerous analogues of nucleoside polyphosphates as well as disubstituted pyro- and polyphosphates. These classes of compounds recently have drawn great attention due to their possible medical application.[24] In this context, the stereocontrolled synthesis of such P-chiral analogues that has to be preceded by elaboration of efficient separation of diastereomers of compounds **2** and **8** remains challenging.

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Supporting Information Available: Experimental procedures and characterization data for compounds **1a**−**h**, **2a**−**h**, **4**, and **6**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Since some of the amount of **1a**−**h** can be lost during purification, the ratio of crude compouds to Sephadex should be optimized each time.

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