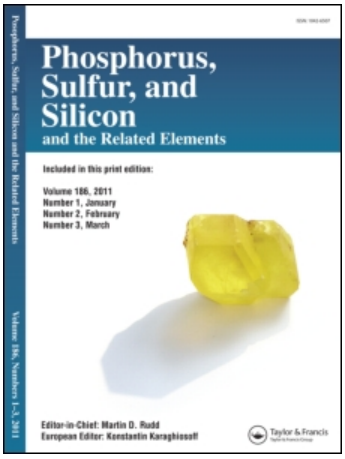


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# STEREOCHEMISTRY OF DBU-ASSISTED REACTION OF NUCLEOSIDE 3'-O-(2-THIONO-1.3.2-OXATHIAPHOSPHOLANES) WITH 5'-HYDROXYNUCLEOSIDES

WOJCIECH J. STEC,[a] BOLESLAW KARWOWSKI,[a] PIOTR GUGA,[a] KONRAD MISIURA,[a] MICHAŁ WIECZOREK,[b] JAROSŁAW BŁASZCZYK[b]

[a]Polish Academy of Sciences, Centre of Molecular and Macromolecular Studies, Department of Bioorganic Chemistry, Sienkiewicza 112, 90-363 Łódź, Poland; [b]Technical University of Łódź, Institute of Technical Biochemistry, Stefanowskiego 4/10, 90-924 Łódź, Poland

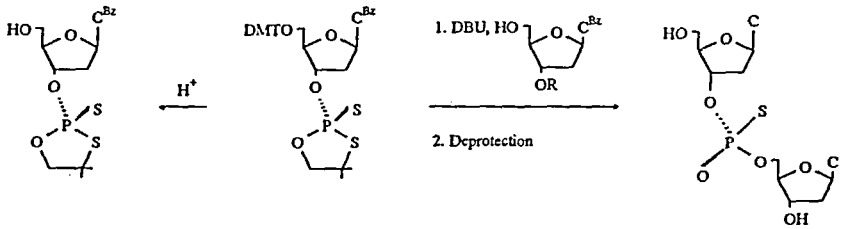


It has been demonstrated that reaction of diastereomerically pure 5'-*O*-DMT-nucleoside-3'-*O*-(2-thiono-1.3.2-oxathiaphospholanes) with 5'-*O*-unprotected nucleosides occurs, assisted by DBU, with high stereospecificity providing diastereomerically pure dinucleoside(3',5')phosphorothioates.[1] Model studies involving 2-*N*(α-naphthylethyl)amino-2-thiono-1.3.2-oxathiaphospholane evidently indicated that DBU-assisted methanolysis provides *O*-methyl-*N*(α-naphthylethyl)-phosphoroamidothioate with net *retention* of configuration.[2] This result, promptly documented by X-ray crystallography of substrates and products of aforementioned conversion, allowed to anticipate that 1.3.2-oxathiaphospholane ring-opening process occurs *via* "adjacent" type mechanism involving an attack of MeOH on phosphorus collinear with endocyclic P-O bond; pentacoordinate phosphorane intermediate before collapse has to undergo the single pseudorotation process, placing endocyclic P-S bond in apical position. Such analysis allowed for implication that reaction of 5'-*O*-DMT-nucleoside-3'-*O*-(2-thiono-1.3.2-oxathiaphospholane) with 5'-unprotected nucleoside or nucleotide occurs according the same rules and prediction, that diastereomerically pure oxathiaphospholane substrate for [Rp]-dinucleoside (3',5')-phosphorothioate has to be of [Rp]-configuration.[2]

In this report we present experimental evidence that oxathiaphospholane substrate has the same absolute configuration of P-atom as that of resulting dinucleoside (3',5')phosphorothioate. 5'-*O*-DMT-$N^4$-benzoylcytidine-3'-*O*-(2-thiono-4,4,-dimethyl-1.3.2-oxathiaphospholane) (1) has been prepared in tetrazole-catalyzed reaction of 5'-*O*-DMT-$N^4$-benzoylcytidine with 2-*N*,*N*-diisopropylamino-4,4-dimethyl-1.3.2-oxathiaphospholane[3], followed by oxidation with elemental sulphur. Column chromatography on silica gel 60H with ethyl acetate/butyl acetate (2:1, v/v) as eluting system gave pure FAST-eluted diastereomer of 1 (FAST-1);

$^{31}$P NMR: 107.76 ($CD_3CN$); MS FAB$^+$: 840.3 $(M+K)^+$, MS FAB$^-$: 839.4 $(M-K)^-$. This isomer of **1** was reacted with $N^4$-benzoylcytidine anchored *via* its 3'-*O*-group on a solid support LCA-CPG[4] performed in the presence of a 200-fold molar excess of DBU to give, after cleavage from the support and base deprotection[5] [Rp]-dicytidine (3',5')-phosphorothioate (**2**). Identification of this product was based upon its comparison with genuine sample of [Rp]-**2**.[6]

SCHEME 1




[Rp]-**3** [Rp]-**1** [Rp]-**2**

DMT = 4,4'-dimethoxytrityl; C = Cytosin-1-yl; $C^{Bz}$ = $N^4$-Benzoylcytosin-1-yl; R = linker to solid support

Independently, FAST-**1** was treated with p-toluenesulphonic acid/methylene chloride for removal of the dimethoxytrityl protective group and after purification on chromatographic column (silica gel 60 with chloroform/methanol = 90:10 as eluting system), gave $N^4$-benzoylcytidine 3'-*O*-(2-thiono-4,4-dimethyl-1.3.2-oxathiaphospholane) (**3**). The product was dissolved in toluene/methylene chloride/diethyl ether (10:2:0.5, v/v) and left for slow crystallization. The crystals collected (m.p. 119-121°C; $^{31}$P NMR ($CD_3CN$): 107.57 ppm; MS FAB$^+$: 498.2, MS FAB$^-$: 496.1) were subjected for X-ray analysis which showed that the absolute configuration at phosphorus is [Rp] (Fig.1). Therefore, the absolute configuration of the parent FAST-**1** must be also [Rp]. This result is consistent with the mechanism we proposed earlier,[2] indicating that substrates **1** and resulting products **2** are of the same absolute configuration of phosphorus atom. Results of *ab initio* calculations performed by Taira and Uchimaru[7] are in favor of this mechanism (data not presented).

This mechanism implies that DBU assists the reaction as the strong base.[8] However, evidence was reported recently that chlorobis(diisopropylamino)phosphane and DBU in acetonitrile solution form a cationic phosphane[9] and evidently DBU acts

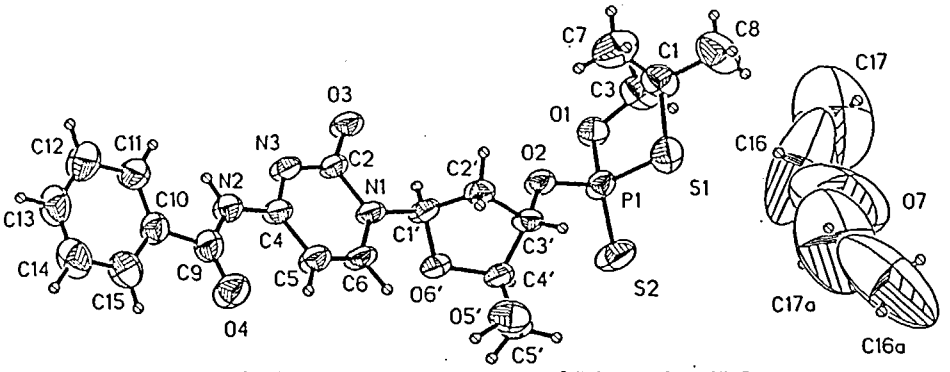
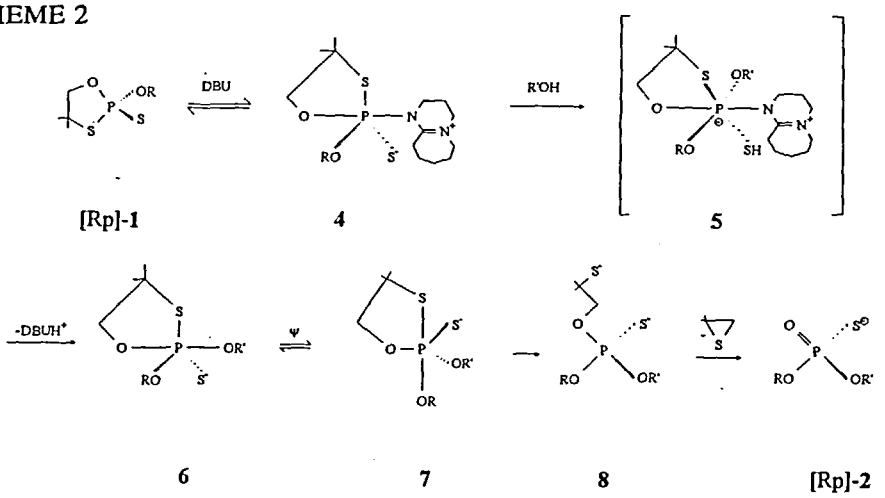



Fig.1. Molecular structure of "Fast-related"-3.

in this system as a nucleophile. This conclusion is consistent with our earlier observation that DBU, generally accepted as "non-nucleophilic strong base",[8] may participate in the process of nucleophilic substitution at tetracoordinate phosphorus atom.[10] For example, the DBU-promoted epimerization of nucleoside-3'-*O*-(*O*-4-nitrophenyl methanephosphonates) has been explained by an attack of DBU on phosphorus and the release of 4-nitrophenoxy anion. Such a function of the DBU would explain the observed phenomenon that nonbicyclic amidine bases such as 1,8-bis(dimethylamino)naphthalene, *N,N,N',N'*-tetramethylethylenediamine, tetramethylguanidine, 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine are far less effective than DBU in promoting reaction between **1** and 5'-*O*-unprotected nucleosides.[3] We therefore formulate the further hypothesis that, in the process of 1.3.2-oxathiaphospholane ring opening condensation, the function of DBU is not that of deprotonation of the alcohol; it must act as a nucleophile, which in the first stage attacks at phosphorus in the 1.3.2-oxathiaphospholane ring system from the side opposite to endocyclic P-O bond (Scheme 2). The resulting pentacoordinate trigonal bipyramide **4** is stabilized *via* attractive interactions between negatively charged sulphur and positively charged bridgehead nitrogen atom. This intermediate undergoes slow reaction with alcohol *via* addition-elimination mode and hexacoordinate tetragonal bypiramide transition state **5** with negative charge located at phosphorus atom. Elimination of the protonated DBU generates intermediate **6** which undergoes pseudorotation, as indicated in Scheme 2. Collapse of **7** *via* cleavage of the P-S bond followed by elimination of episulfide gives the phosphorothioate diester of [Rp]-configuration.

One question remains unanswered. Why does replacement of the protonated DBU by the alkoxide ligand occur with retention of configuration? This remains obscure since the rules of ligand exchange at hexacoordinate phosphorus are not fully understood.[11]

SCHEME 2




R = 3'-nucleoside; R' = 5'-nucleoside



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