Rules of Chemospecificity of Nucleophilc Ring-opening of Dithia-/Oxathia-Phospholane Towards the Selective Synthesis of Nucleoside 5'-*O*-P_α-Thio/Dithio/Trithio-Phosphate Ester Conjugates

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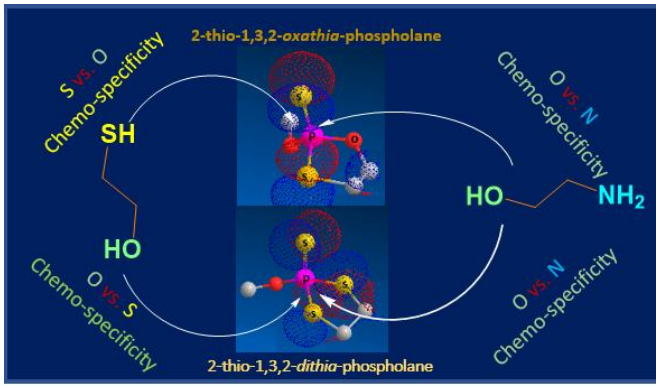


Abstract

DBU-assisted nucleophilic ring-opening of both uridine-5'-(2-thio-1,3,2-*dithia*-phospholane), **3**, and uridine-(2-thio-1,3,2-*oxathia*-phospholane), **8**, lasted 2 min at RT and resulted in quantitative yields of uridine-5'-phosphoro-di/trithioate esters. Furthermore, it was selective for alcohol and thiol vs. amine nucleophiles. Yet, reaction of mercaptoethanol with **3**, was chemo-specific for the oxygen vs. sulfur nucleophile, while for the reaction of mercaptoethanol with **8**, the opposite chemo-specificity was observed, probably related to the steric hindrance in the former case. The observed chemospecificity opens facile avenue for the synthesis of nucleoside-5'-*O*-P_α-thio/dithio/trithio-phosphate ester derivatives.

Introduction

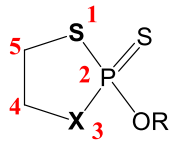
2-Thio-1,3,2-*dithia*phospholane, **1**, and 2-thio-1,3,2-*oxathia*phospholane, **2**, ring systems are useful as synthetic synthons for the formation of nucleoside 5′-*O*-P_α-*thio*- or -*dithio*-phosphate analogs or oligonucleotides bearing phosphorothioate or dithioate linkages.[1]

Nucleoside 5′-*O*-P_α-(thio)triphosphate analogs,[2] have gained wide application in biochemistry and cell biology.[3] These compounds have been used also for the enzymatic synthesis of DNA and RNA fragments possessing chiral phosphorothioate internucleotide bonds. The related phosphoro*di*thioate analogs have been used as lubricants and fuel additives.[4] During the last decade, oligodeoxynucleotide analogs containing phophorodithioate linkages (dithioate DNA) have received increasing attention for biochemical and therapeutic applications as well.[4] This is because dithioate DNA/RNA is achiral, isoelectronic with respect to normal DNA, and nuclease resistant. Several methods for the synthesis of these analogs have been developed.[5] Recently we have reported the

synthesis and application of nucleoside-5′-*O*-P_α-thio/dithio-triphosphate analogs as inhibitors of nucleotide pyrophosphatase/phosphodiesterase 1 (NPP1), for the potential treatment of CPPD disease which accompanies osteoarthritis.[1a-c, 6] Importantly, we found that nucleoside 5′-*O*-$P_{\alpha,\alpha}$-dithio-triphosphate analogs are reasonably stable under acidic and basic, and under oxidizing conditions.[1a, b, 5a, 7]

The synthetic usefulness of ring systems **1** and **2** for the formation of a variety of research tools and therapeutic agents based on nucleoside 5′-*O*-P_α-*thio*- or -*dithio*- or -*trithio*-phosphate ester, has attracted our attention with a view to exploring the chemoselectivity of the ring opening of both 2-thio-1,3,2-*dithia*phospholane and 2-thio-1,3,2-*oxathia*-phospholane rings by various nucleophiles. Establishing the chemoselectivity rules is required for learning the scope of the reaction and then for application of the rules for synthesizing useful nucleotide-conjugates towards various uses.[8]

Here we report the investigation of the reactivity and chemo-selectivity of uridine-5'-*O*-(2-thio-1,3,2-*dithia*phospholane) vs. uridine-5'-*O*-(2-thio-1,3,2-*oxathia*phospholane) with a variety of mono- or bi-functional nucleophiles, with a view to establish the rules of reactivity of the above phospholane rings.



1 X = S R = nucleoside
2 X = O R = nucleoside

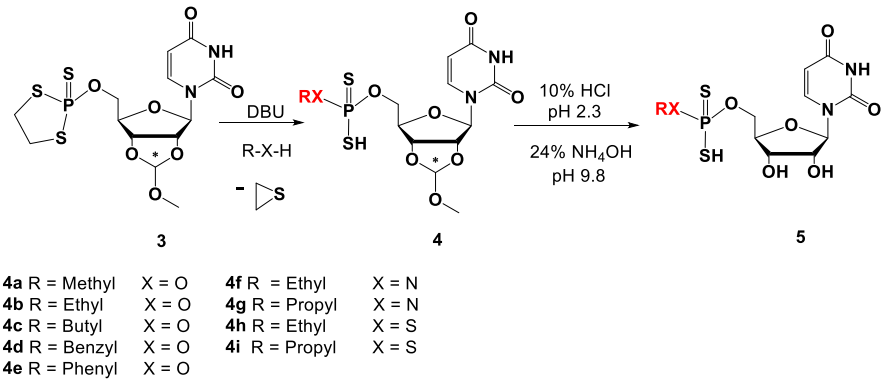
Results

Reactions of uridine-5'-(2-thio-1,3,2-*dithia*phospholane), 3, with alcohols, thiols, and amines

For comparison of the reactivity and the chemoselectivity of both nucleoside-5'-(2-thio-1,3,2-*dithia*phospholane) and nucleoside-5'-(2-thio-1,3,2-*oxathia*phospholane) with nucleophiles, we have used uridine as a simple nucleoside model.

A diastereomeric mixture of uridine-2′,3′-methoxymethylidene-5′-O-(2-thio-1,3,2-*dithia*phospholane), **3**, treated with 2 equivalents of dry methanol in the presence of an

equimolar amount of DBU in dry acetonitrile, quantitatively resulted in uridine 2′,3′-methoxymethylidene-5'-*O*-phosphorodithioate-methyl ester, **4a,** after 2 min at RT (Table 1, Scheme 1).



Scheme 1. General ring-opening reaction of uridine-5'-(2-thio-1,3,2-*dithia*phospholane), **3**, with alcohols, thiols, and amines in the presence of DBU.

Likewise, reactions of **3** with other alcohols such as: ethanol, n-butanol, benzyl alcohol, and phenol, were rapid (lasting up to 5 min) at RT resulting in quantitative yields of uridine-2′,3′-methoxymethylidene-*O*-5'-phosphorodithioate ester analogs, **4b-e,** and phosphoro-trithioate ester analogs, **4h-i**, when thiol nucleophiles were used. Compounds **4a–e** and **4h-i** were deprotected followed by ion-exchange chromatographic purification, to give the free nucleotides **5a–e** at more than 95 % yield (Table 1).

Table 1. Data for nucleotides 5a–e, and 5h-i: % yield, ^{31}P NMR chemical shift, and HPLC Rt

Compound	Nucleophile [a]	Yield [b]	^{31}P NMR chemical shift (ppm)[c]	Rt (min)[d]
5a	Methanol	>95%	116.9	3.3
5b	Ethanol	>95%	116.9	6.8
5c	Butanol	>95%	113.2	14.5
5d	Benzyl alcohol	>95%	114.0	18.4
5e	Phenol	>95%	110.7	13.7
5f	Ethylamine	n.r.	n.r.	-
5g	Propylamine	n.r.	n.r.	-
5h	Ethanethiol	>95%	117.9	2.8
5i	Propanethiol	>95%	118.1	2.9

[a] General procedure for the synthesis of **5a–i**: To a solution of uridine-2′,3′-methoxymethylidene-5′-O-(2-thio-1,3,2-*dithia*phospholane) (0.34 g, 5 mmol) in acetonitrile (10 mL) the appropriate nucleophile (10 mmol) was added followed by DBU (0.76 g , 5 mmol). When the reaction was completed (as indicated by ^{31}P NMR), the solvent was evaporated and the residue was treated with 10% HCl, pH 2.3, for 3 h, and then with 24% NH_4OH, pH 9.8, for 45 min. The aqueous solution was freeze dried and separated by liquid chromatography on Sephadex® DEAE A-25 column, using a linear gradient of ammonium bicarbonate (0.02–0.4 M). The products were obtained as a white solid at high yields as mentioned in Table 1.

[b] n.r. - no reaction

[c] ^{31}P NMR spectrum in D_2O, at 242.9 MHz and 300K.

[d] Conditions: RP-18 reverse phase column (Phenomenex, Kinetex 2.6u C_{18} 100A, 100 X 4.6 mm), flow rate 1 mL/min, detection at 260 nm, applying isocratic elution with 80% triethylammonium-acetate (0.3 M, pH 7.4) : 20 % acetonitrile.

Unlike alcohols, the treatment of **3** with amines, e.g., ethylamine and propylamine, did not result in any product under the reaction conditions mentioned above. No reaction took place even upon increasing the reaction's temperature (up to reflux), changing the solvent to DMF, pyridine, DCM, or THF, or upon the addition of Et_3N.

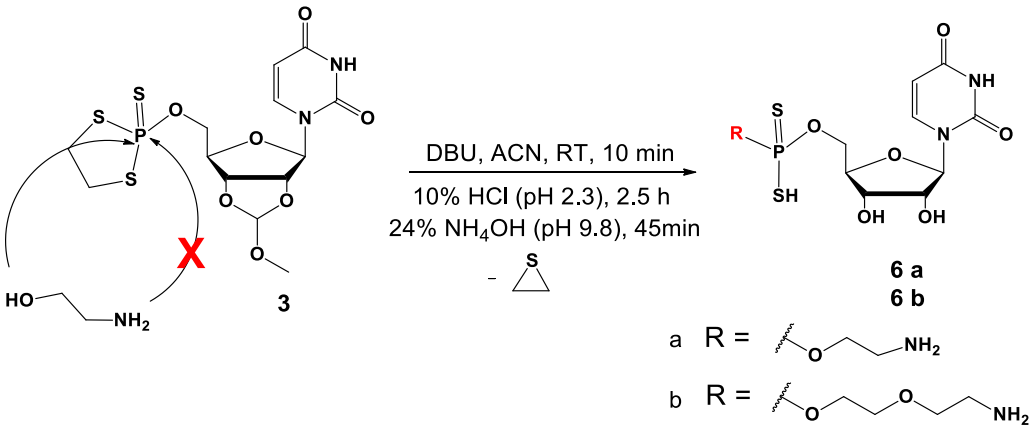
Yet, thiol-nucleophiles such as ethanethiol and propanethiol, reacted with **3** under the above reaction conditions just like alcohols to give **5h** and **5i** in a quantitative yield (Table 1, Scheme 1, SI, Fig. 1).

Selective nucleophilic reaction with bi-functional nucleophiles, usually requires protecting groups for the undesired strong nucleophile group to avoid its participation in the reaction.

In case of ethanol amine, usually, specific reaction with oxygen group vs. amine group usually requires protection of the amine group, or application of special reaction

conditions, to reduce the nucleophilicity of the amine group. In these approaches priority is given to a selective reaction of the weaker nucleophile.

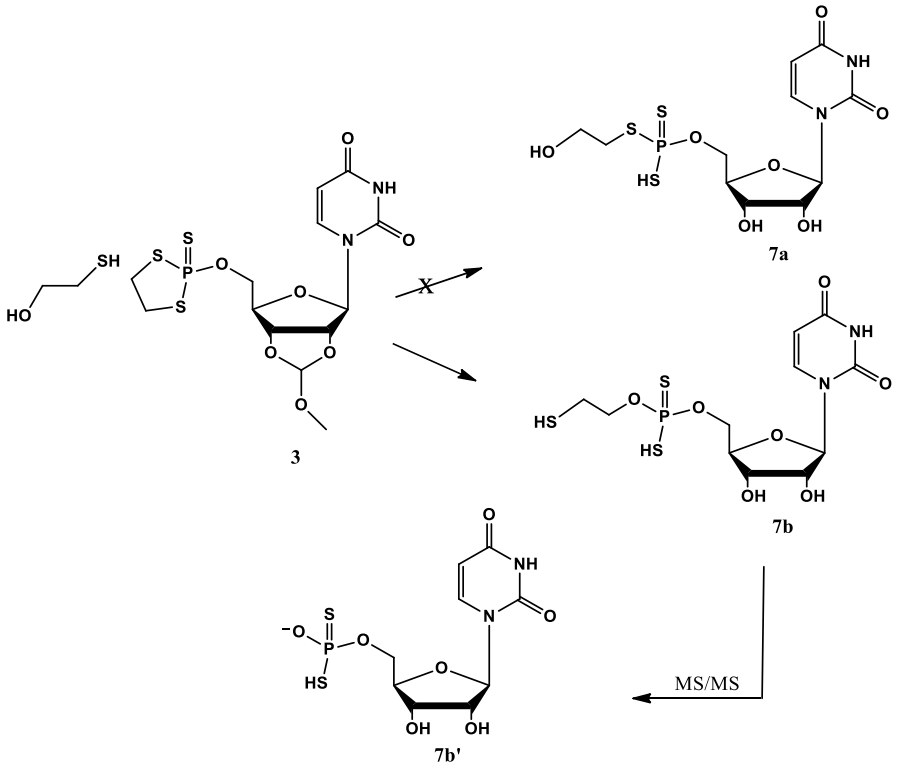
Based on the above findings (Scheme 1 and Table 1), we treated **3** with nucleophiles such as ethanol amine and 2-(2-aminoethoxy)-ethanol, without any amine protection under the conditions mentioned in Table 1. Expectedly, the reaction with **3** and ethanolamine and 2-(2-aminoethoxy)-ethanol was chemo-specific giving rise to **6a** and **6b** due to dithiaphospholane ring opening by the alcohol function only (Scheme 2). This chemo-specificity has been proven by HR-MS/MS, which clearly showed (by the fragmentation of products **6a** and **6b**) that the oxygen atom is involved in ring opening (SI, Fig. 2).



Scheme 2. Chemospecificity of alcohol vs. amine in the reaction between uridine-5'-(2-thio-1,3,2-*dithia*phospholane) and ethanolamine or 2-(2-aminoethoxy)-ethanol.

Products **6a** and **6b** may find potential as antibacterial and antiviral drugs since amino ethoxy group ($NH_2CH_2CH_2O$-) is known as an important moiety in such drugs.[9]

Since both alcohols and thiols react with uridine-2-thio-1,3,2-dithiaphospholane, **3**, a mixture of products **7a** and **7b** was expected in the reaction of **3** with mercaptoethanol. Surprisingly, the reaction of **3** with mercaptoethanol showed complete chemo-selectivity for the alcohol vs. thiol nucleophile (Scheme 3).

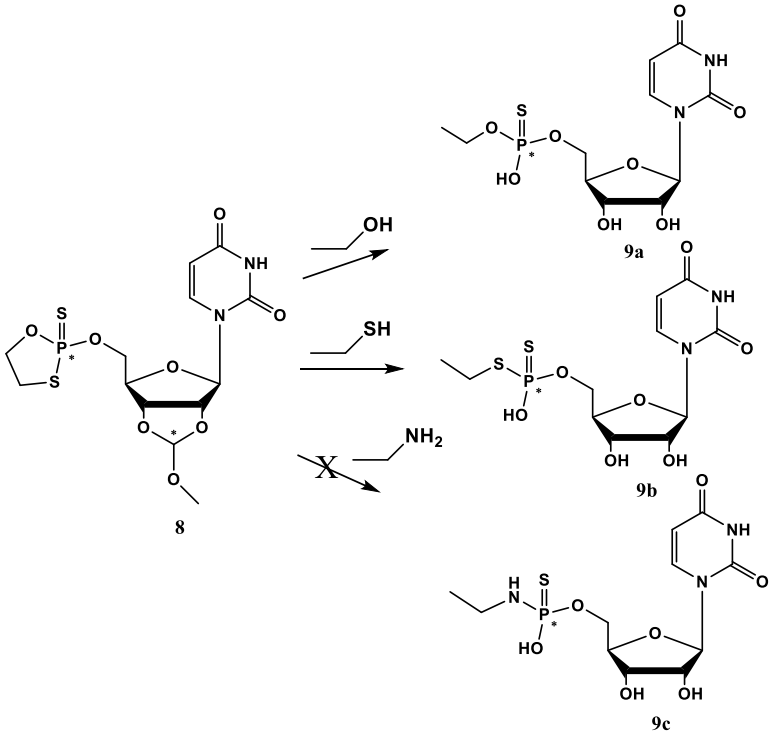


Scheme 3. Chemo-selectivity of uridine-5'-(2-thio-1,3,2-*dithia*phospholane) for alcohol vs. thiol in the reaction with mercaptoethanol.

^{31}P NMR signals of **5h** (product of reaction of **3** with ethanethiol) and **5b** (product of reaction with ethanol) were surprisingly similar (117.9 and 116.9 ppm for **5h** and **5b**, respectively). Hence, to decipher the chemo-selectivity of the reaction of **3** with mercaptoethanol (Scheme 3), namely, the formation of either **7a** or **7b**, we applied high resolution tandem mass spectrometry (HR-MS/MS) technique, to determine the chemo selectivity and final structure of the reaction product. Via HR-MS/MS experiment we capture the molecule, and by using collision gas we fragment it. Analysis the spectrum reveals a signal of 354.982 Da **7b'** which indicates a chemical bond of P-O rather than P-S, meaning formation of **7b**. This clearly supports the chemo selectivity of the reaction (SI, Fig. 3).

Furthermore, we investigated the scope of the reaction of uridine-5'-(2-thio-1,3,2-*oxathia*phospholane, **8**, with ethanol, ethanethiol, and ethylamine under the same reaction conditions mentioned in Table 1 (Scheme 4). Reaction with ethanol and ethanethiol,

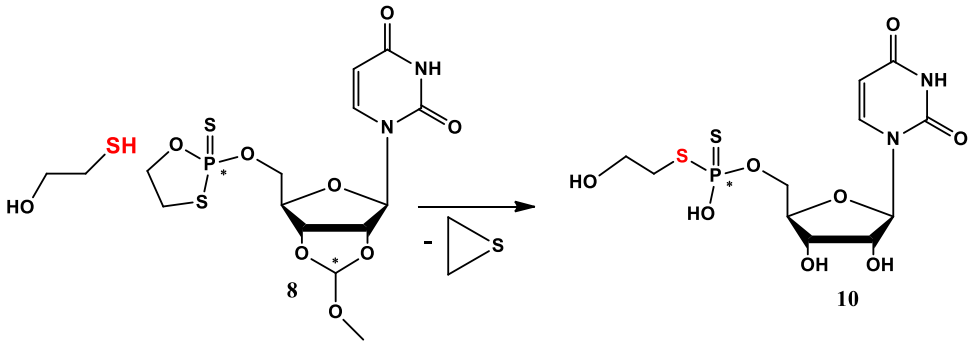
resulted in products **9a** and **9b** (^{31}P NMR signal at 57 and 75 ppm, respectively. SI, Fig. 4 A, B), while no reaction occurred with ethylamine (Scheme 4).



Scheme 4. Reaction of uridine-5'-(2-thio-1,3,2-*oxathia*phospholane), **8**, with ethanol, ethanethiol, and ethylamine under the reaction conditions mentioned in Table 1.

Both uridine-2-thio-1,3,2-*oxathia*phospholane, **8**, and uridine-2-thio-1,3,2-*dithia*-phospholane, **3**, reacted with ethanol and ethanethiol but not with amines. Therefore, we expected that the reaction between **8** and mercaptoethanol will be chemo-selective to oxygen vs. sulfur as in the case of **7b** (Scheme 3, SI, Fig. 4)

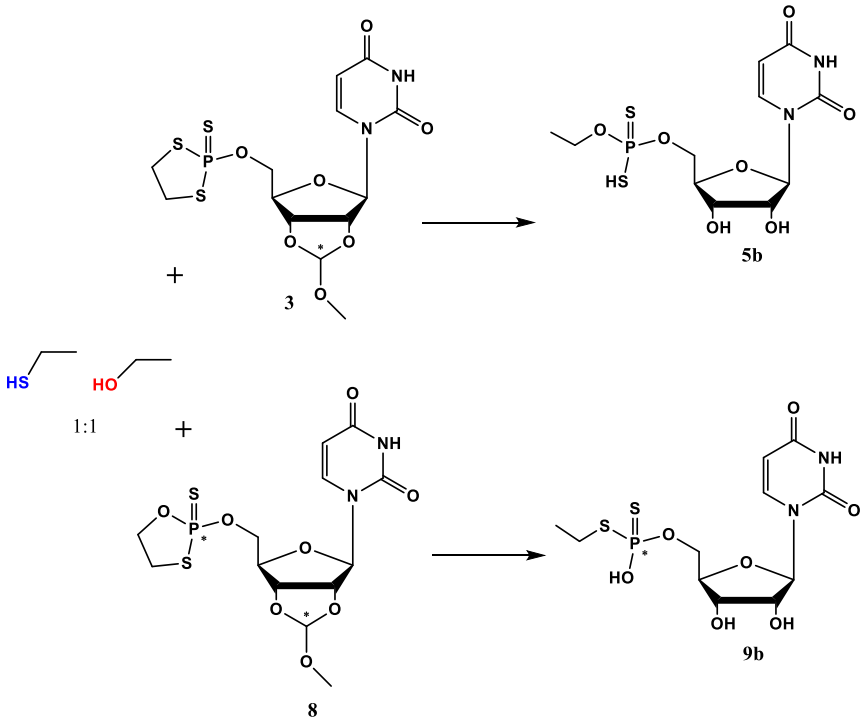
Surprisingly, we observed a selective formation of product **10**, indicating preferential chemoselectivity for thiols vs. alcohols in the reaction of **8** with mercaptoethanol (Scheme 5).



Scheme 5. Chemo-selectivity for thiol vs. alcohol nucleophile in the reaction between uridine-5'-(2-thio-1,3,2-*dithia*phospholane) and mercaptoethanol.

^{31}P NMR chemical shifts of **9a** (formed upon reaction of uridine-2-thio-1,3,2-*oxathia*phospholane, **8**, with ethanol), and **9b** (formed upon reaction with ethanethiol), are 57 and 75 ppm, respectively. However, reaction of uridine-5'-(2-thio-1,3,2-*oxathia*phospholane) with mercaptoethanol forms product **10** (^{31}P NMR δ 75 ppm) indicating complete chemo-selectivity to sulfur vs. oxygen nucleophile (Scheme 5, SI, Fig. 5).

Interestingly, reaction of **8** with 1:1 solution of ethanethiol and ethanol, resulted in one product, **9b**, as observed in ^{31}P NMR spectrum, while a similar reaction of **3** with 1:1 solution of ethanethiol and ethanol resulted only in **5b** (Scheme 6, SI, Fig. 5).



Scheme 6. Reaction of **3** and **8** with a 1:1 solution of ethanethiol and ethanol.

Conclusion

In this study we searched for a facile approach for the preparation of various nucleoside-5'-*O*-thio/dithio/triphosphate-conjugates. Indeed, DBU-assisted nucleophilic ring-opening of both uridine-5'-(2-thio-1,3,2-*dithia*-phospholane), **3**, and uridine-(2-thio-1,3,2-*oxathia*-phospholane), **8**, lasted 2 min at RT and resulted in quantitative yields of uridine-5'-phosphoro-di/trithioate esters.

Furthermore, we investigated the susceptibility of uridine-5'-(2-thio-1,3,2-*dithia*-phospholane), **3**, and uridine-5'-(2-thio-1,3,2-*oxathia*-phospholane), **8**, to various nucleophiles such as aliphatic alcohols, phenols, thiols, amines, and difunctional nucleophiles. We have shown that alcohols and thiols are nucleophiles capable of opening both *dithia*-phospholane and *oxathia*-phospholane, in the presence of DBU, while reaction with amines did not occur at all. As expected, reaction of **3** with ethanol amine showed chemo-specificity for oxygen vs. amine (Scheme 5).

Interestingly, reaction of mercaptoethanol with uridine-5'-(2-thio-1,3,2-*dithia*-phospholane), **3**, showed chemo-specificity for the oxygen vs. sulfur nucleophile, while reaction of mercaptoethanol with uridine-5'-(2-thio-1,3,2-*oxathia*-phospholane), **8**, resulted in the opposite chemo-selectivity for sulfur vs. oxygen nucleophile.

Reaction of either uridine-5'-(2-thio-1,3,2-*dithia*-phospholane), **3**, or uridine-5'-(2-thio-1,3,2-oxathia-phospholane), **8**, with 1:1 ethanol: ethanethiol solution, resulted in the same chemo-selectivity mentioned above.

These results were rather puzzling, especially when considering the enthalpy change of nucleophilic ring-opening reactions of 2-thio-1,3,2-*dithia*-phospholane and 2-thio-1,3,2-*oxathia*-phospholane by ethanol, Schemes 1 and 4, respectively. Specifically, formation of P-O bond with the concomitant dissociation of P-S bond due to ring opening by an alkoxide[10] is equivalent for either 2-thio-1,3,2-*dithia- or oxathia*-phospholane (SI, Scheme 1).

This puzzle was more pronounced in the competitive reactions, O vs. S nucleophilic attack of either mercaptoethanol or 1:1 mixtures of ethanol and ethanethiol (Schemes 3, 5, and 6), where uridine-5'-(2-thio-1,3,2-*oxathia*-phospholane), **8**, was chemo-selective to ethanethiol vs. ethanol, and uridine-5'-(2-thio-1,3,2-*dithia*-phospholane), **3**, was chemo-selective to ethanol vs. ethanethiol.

Hence, we hypothesize that the reason for this chemo-specificity is the following. Generally, the nucleophilicity of the thiol group is higher than that of oxygen atom.[11] Yet, the atomic radii of the two sulfur atoms (3p orbitals) in 2-thio-1,3,2-*dithia*-phospholane ring is larger than the one sulfur atom and one oxygen atom in 2-thio-1,3,2-*oxathia*-phospholane,. Although ethanethiol is more nucleophilic than ethanol, the larger atomic radius of the former, results in steric hindrance between the of ethanethiol and the of 2-thio-1,3,2-*dithia*-phospholane. As a result, 2-thio-1,3,2-*dithia*-phospholane ring is chemo-selective for ethanol vs. ethanethiol. However, the reaction of 2-thio-1,3,2-*oxathia*-phospholane ring is selective to ethanethiol vs. ethanol, as a result of less steric hindrance between the 2-thio-1,3,2-*oxathia*-phospholane ring and the ethanethiol, thus allowing the reaction of the stronger nucleophile.

Experimental

General

All commercial reagents were used without further purification, unless otherwise noted. All air- and moisture sensitive reactions were conducted in flame-dried, nitrogen-flushed, two-neck flasks sealed with rubber septa, and the reagents were introduced with a syringe. All reactants for moisture-sensitive reactions were dried overnight in a vacuum oven. Progress of the reactions was monitored by TLC using precoated Merck silica gel plates (60F-253). Reactants and products were visualized using UV light. Compounds were characterized by NMR using a Bruker DPX-400 or DMX-600 spectrometer. ^{1}H NMR spectra were recorded at 400 or 600 MHz. Nucleotides were also characterized by ^{31}P NMR in D_2O at 161.96 MHz. High-resolution mass spectra were recorded on an AutoSpec-ESI mass spectrometer. Nucleotides were analyzed using electron spray ionization (ESI) on a Q-TOF microinstrument (Waters). Primary purification of the nucleotides was achieved on a Combiflash RF+ Teledyne ISCO system using a column of Sephadex DEAE-A25, swollen in 1 M $NaHCO_3$ at 4 °C for 24 h. The resin was washed with deionized water before use. LC separation was monitored by UV detection at 260 nm and 220 nm. Final purification of the nucleotides was achieved on an HPLC (Merck-Hitachi) system using a semipreparative reversed-phase column [Gemini 5u C-18 110A, 250 mm × 10 mm, 5 μm (Phenomenex, Torrance, CA)]. The details of the solvent system gradients used for the separation of each product are provided below. The purity of the nucleotides was evaluated on an analytical reversed-phase HPLC column system [Gemini 5u C-18 110A, 150 mm × 3.60 mm, 5 μm (Phenomenex)] in two solvent systems, I and II. Solvent system I consisted of (A) 100 mM triethylammonium acetate (TEAA) (pH 7) and (B) CH_3CN. Solvent system

II consisted of (A) 46 mM PBS (pH 7.4) and (B) CH_3CN. The products, obtained as Na^+ salts, were generally ≥95% pure.

General procedure for the synthesis of 5a–i:

The appropriate nucleophile (10 mmol) was added to a solution of uridine-2′,3′-methoxymethylidene-5′-O-(2-thio-1,3,2-dithiaphospholane) (0.34 g, 5 mmol) in acetonitrile (10 mL). Next, DBU (5 mmol) was added with stirring. The progress of the reaction was monitored by ^{31}P-NMR. When the reaction was complete, the solvent was evaporated and the residue was treated first with 10% HCl, pH 2.3, for 3 h, and then with 24% NH_4OH, pH 9.8, for 45 min. The aqueous phase was freeze dried and the residue was separated by liquid chromatography on Sephadex® DEAE A-25 column with a linear gradient of 0-0.5 M NH_4HCO_3 buffer (pH 7.5). After repeated freeze-drying of the relevant fraction, the product was obtained at 90-98% overall yield as a white solid.

Uridine- 5'-*O*- phosphorodithioate-methyl ester, **5a**

Compound **5a** was prepared following the above general procedure starting from uridine-2′,3′-methoxymethylidene-5′-O-(2-thio-1,3,2-dithiaphospholane) (0.21 g, 0.5 mmol) and methanol (0.032 g, 1 mmol). The product was obtained as a white solid (0.17 g) at 97 % yield; ^{1}H NMR (D_2O, 400 MHz) δ: 8.04 (d, 1H), 6.01 (d, 1H), 5.93 (d, 2H), 4.27 (m, 5H), 3.64 (d, 3H) ppm. ^{31}P NMR (D_2O, 161.96 MHz) δ: 116.9 (s, 1P) ppm. ^{13}C NMR (D_2O, 100.61 MHz) δ: 172, 159, 144, 105, 91, 85, 76, 72, 67, 55 ppm. HRMS ESI (negative): m/z calculated for $C_{10}H_{15}N_2O_7P_1S_2^{2-}$: 368.9958, found 368.9978. The following purity data

were obtained on an analytical column: t_R 3.3 min (97% purity) using solvent system I with a TEAA/CH_3CN isocratic elution at 80:20 over 20 min at a flow rate of 1 mL/min.

Uridine- -5'-*O*-phosphorodithioate-ethyl-ester, 5b

Compound **5b** was prepared following the above general procedure starting from uridine-2′,3′-methoxymethylidene-5′-O-(2-thio-1,3,2-dithiaphospholane) (0.21 g, 0.5 mmol) and ethanol (0.046 g, 1 mmol). The product was obtained as a white solid (0.187 g) at 98 % yield; ^{1}H NMR (D_2O, 400 MHz) δ: 8.05 (d, 1H), 5.93 (d, 1H), 5.86 (d, 2H), 4.30 (m, 5H), 4.01 (q, 2H), 1.26 (t, 3H) ppm. ^{31}P NMR (D_2O, 161.96 MHz) δ: 116.9 (s, 1P) ppm. ^{13}C NMR (D_2O, 100.61 MHz) δ: 166, 151, 142, 102, 89, 83, 74, 70, 64, 63, 15 ppm. HRMS ESI (negative): m/z calculated for $C_{11}H_{17}N_2O_7P_1S_2^1$: 383.0136, found 383.0134. The following purity data were obtained on an analytical column: t_R 6.8 min (98% purity) using solvent system I with a TEAA/CH_3CN isocratic elution at 80:20 over 20 min at a flow rate of 1 mL/min.

Uridine- 5'-*O*-phosphorodithioate-butyl-ester, 5c

Compound **5c** was prepared following the above general procedure starting from uridine-2′,3′-methoxymethylidene-5′-O-(2-thio-1,3,2-dithiaphospholane) (0.21 g, 0.5 mmol) and butanol (0.074 g, 1 mmol). The product was obtained as a white solid (0.197 g) at 96 % yield; ^{1}H NMR (D_2O, 400 MHz) δ: 8.18 (d, 1H), 6.09 (d, 1H), 6.02 (d, 1H), 4.30 (m, 5H), 4.1 (t, 2H), 1.7 (m, 2H), 1.4 (m, 2H), 0.99 (t, 3H) ppm. ^{31}P NMR (D_2O, 161.96 MHz) δ: 113.2 (s, 1P) ppm. ^{13}C NMR (D_2O, 100.61 MHz) δ: 167, 153, 142, 103, 89, 83, 74, 70, 67, 65, 61, 32, 19, 13 ppm. HRMS ESI (negative): m/z calculated for $C_{13}H_{21}N_2O_7P_1S_2^{1-}$: 411.0425, found 411.0448. The following purity data were obtained on an analytical

column: t_R 14.5 min (94 % purity) using solvent system I with a TEAA/CH_3CN isocratic elution at 80:20 over 20 min at a flow rate of 1 mL/min.

Uridine- 5'-*O*-phosphorodithioate-benzyl-ester, 5d

Compound **5d** was prepared following the above general procedure starting from uridine-2′,3′-methoxymethylidene-5′-O-(2-thio-1,3,2-dithiaphospholane) (0.21 g, 0.5 mmol) and benzyl alcohol (0.108 g, 1 mmol). The product was obtained as a white solid (0.213 g) at 96 % yield; ^{1}H NMR (D_2O, 400 MHz) δ: 8 (d, 1H), 7.4, (m, 5H), 5.8 (d, 1H), 5.7 (d, 1H), 5.01 (d, 2H), 4.20 (m, 3H), 3.9 (m, 2H) ppm. ^{31}P NMR (D_2O, 161.96 MHz) δ: 114.01 (s, 1P) ppm. ^{13}C NMR (D_2O, 100.61 MHz) δ: 165, 151, 142, 135, 127, 126, 121, 103, 89, 83, 74, 70, 67, 61 ppm. HRMS ESI (negative): m/z calculated for $C_{16}H_{19}N_2O_7P_1S_2^{1-}$: 445.0325, found 445.0300. The following purity data were obtained on an analytical column: t_R 18.4 min (99% purity) using solvent system I with a TEAA/CH_3CN isocratic elution at 80:20 over 20 min at a flow rate of 1 mL/min.

Uridine- 5'-*O*-phosphorodithioate-phenyl-ester, 5e

Compound **5e** was prepared following the above general procedure starting from uridine-2′,3′-methoxymethylidene-5′-O-(2-thio-1,3,2-dithiaphospholane) (0.21 g, 0.5 mmol) and phenol (0.901 g, 1 mmol). The product was obtained as a white solid (0.198 g) at 98 % yield; ^{1}H NMR (D_2O, 400 MHz) δ: 8.3 (d, 1H), 7.2, (m, 5H), 5.8 (d, 1H), 5.7 (d, 1H), 4.11 (m, 3H), 3.7 (m, 2H) ppm. ^{31}P NMR (D_2O, 161.96 MHz) δ: 110.7 (s, 1P) ppm. ^{13}C NMR (D_2O, 100.61 MHz) δ: 165, 151, 142, 135, 127, 126, 121, 103, 89, 83, 74, 67, 61 ppm. HRMS ESI (negative): m/z calculated for $C_{16}H_{19}N_2O_7P_1S_2^{1-}$: 433.0311, found 433.0315. The following purity data were obtained on an analytical column: t_R 13.7 min (99% purity)

using solvent system I with a TEAA/CH_3CN isocratic elution at 80:20 over 20 min at a flow rate of 1 mL/min.

Uridine- 5'-*O*-phosphorodithioate-ethylthio-ester, **5h**

Compound **5h** was prepared following the above general procedure starting from uridine-2′,3′-methoxymethylidene-5′-O-(2-thio-1,3,2-dithiaphospholane) (0.21 g, 0.5 mmol) and ethanethiol (0.062 g, 1 mmol). The product was obtained as a white solid (0.199 g) at 99 % yield; ^{1}H NMR (D_2O, 400 MHz) δ: 8.1 (d, 1H), 5.9 (m, 2H), 4.20 (m, 5H), 2.8 (t, 2H), 1.3 (t, 3H) ppm. ^{31}P NMR (D_2O, 161.96 MHz) δ: 117.9 (s, 1P) ppm. ^{13}C NMR (D_2O, 100.61 MHz) δ: 166, 151, 142, 102, 89, 83, 74, 70, 64, 43 ppm. HRMS ESI (negative): m/z calculated for $C_{11}H_{17}N_2O_6P_1S_3^{1-}$: 398.9905, found 398.9902. The following purity data were obtained on an analytical column: t_R 2.8 min (99% purity) using solvent system I with a TEAA/CH_3CN isocratic elution at 80:20 over 20 min at a flow rate of 1 mL/min.

Uridine-O-5'-phosphorodithioate- propylthio-ester, **5i**

Compound **5i** was prepared following the above general procedure starting from uridine-2′,3′-methoxymethylidene-5′-O-(2-thio-1,3,2-dithiaphospholane) (0.21 g, 0.5 mmol) and propanethiol (0.076 g, 1 mmol). The product was obtained as a white solid (0.199 g) at 95 % yield; ^{1}H NMR (D_2O, 400 MHz) δ: 8.2 (d, 1H), 6.04 (m, 2H), 4.30 (m, 5H), 4.1 (t, 2H), 1.7 (t, 2H), 0.9 (t, 3H) ppm. ^{31}P NMR (D_2O, 161.96 MHz) δ: 118.1 (s, 1P). ^{13}C NMR (D_2O, 100.61 MHz) δ: 167, 153, 142, 103, 89, 83, 74, 70, 64, 43, 33, 13 ppm. HRMS ESI (negative): m/z calculated for $C_{12}H_{19}N_2O_6P_1S_3^{1-}$: 413.9912, found 413.9907. The following purity data were obtained on an analytical column: t_R 2.9 min (97% purity) using solvent

system I with a TEAA/CH_3CN isocratic elution at 80:20 over 20 min at a flow rate of 1 mL/min.

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