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POTASSIUM PEROXYMONOSULFATE (OXONE) - AN EFFICIENT OXIDIZING AGENT FOR PHOSPHOTHIO COMPOUNDS

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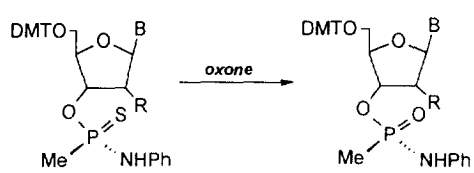
Abstract: Potassium peroxymonosulfate (oxone) is demonstrated as a versatile chemoselective and stereospecific oxidizing agent for phosphothio compounds. Its application in nucleotide chemistry is presented.

Conversion of organophosphates bearing P=S or P=Se bonds into their oxo-derivatives (P=O) has attracted the attention of several research establishments involved in stereochemical correlations aiming at the elucidation of organic and enzymatic reaction mechanisms,[1] studies on the metabolism of organophosphorus pesticides,[2] or detoxification of organophosphorus warfare agents.[3]

In this communication we demonstrate that potassium peroxymonosulfate (oxone[4]) can be efficiently used for P(S)→P(O) conversion, which occurs with **retention of configuration at the phosphorus atom**. It has been found that Ph_3PS dissolved in THF and stirred with 2 molar equivalents of oxone in a buffered solution of sodium acetate (pH ~7) is quantitatively converted into Ph_3PO within 30 minutes.[5] From studies coming from this laboratory it is known that the conversion of P(Se)→P(O) proceeds with high yields and with the retention of configuration at the phosphorus atom.[6]

The same stereochemistry of P(S)→P(O) conversion has been observed in the case of nucleoside derivatives: [Rp]-5'-O-DMT-nucleoside 3'-O-methanephosphonoanilidothioates (**1**) under treatment with oxone are transformed into the corresponding [Sp]-5'-*O*-DMT-nucleoside 3'-*O*-methanephosphonoanilidates (**2**).[7] Both ^{31}P NMR and ^{1}H NMR data confirm that these oxidations are fully stereospecific (Scheme 1).



FAST - *[Rp] - 1* FAST - *[Rp] - 2*

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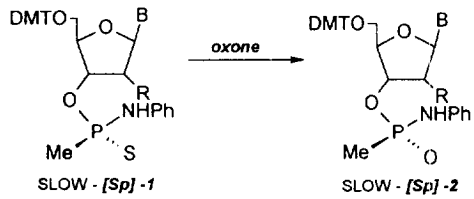

This conversion has been achieved under the standard conditions with good yields, without affecting nucleobases and protective groups (Table 1).

Table 1. Properties of 5'-*O*-DMT-Nucleoside 3'-*O*-Methanephosphonoanilidates (**2**) Prepared from **1** via oxone Oxidation

Methanephosphonoanilidates (2)		FAB⁻(FAB⁺)MS	Calcd Exact Mass	^{31}P NMR ($CDCl_3$) [ppm]	^{1}H NMR ($CDCl_3$)[a]	Yield [%][b]
^{DMT}T	FAST	796.3	796.247	29.93	1.66, $^{2}J_{P-H}$=16.88	
	SLOW	796.3			1.70, $^{2}J_{P-H}$=16.92	
$^{DMT}C^{Bz}$	FAST	785.3	786.282	29.68	1.65, $^{2}J_{P-H}$=16.85	82
	SLOW	785.3		29.52	1.68, $^{2}J_{P-H}$=16.89	85
$^{DMT}A^{Bz}$	FAST	808.3(810.4)	809.285	30.01	1.70, $^{2}J_{P-H}$=16.84	85
	SLOW	808.3(810.4)		29.86	1.72, $^{2}J_{P-H}$=16.77	80
$^{DMT}G^{ibu}$	FAST	790.4	791.286	30.12	1.65, $^{2}J_{P-H}$=16.84	80
	SLOW	790.4		30.63	1.70, $^{2}J_{P-H}$=16.85	75
$^{DMT}U_{2'OMe}$	FAST	712.3	713.250	31.34	1.65, $^{2}J_{P-H}$=16.85	90[c]
	SLOW	712.3		31.58	1.71, $^{2}J_{P-H}$=17.00	90[c]
$^{DMT}C^{ibu}{}_{2'OMe}$	FAST	781.3 (783.5)	782.12	31.05	1.65, $^{2}J_{P-H}$=16.88	92[c]
	SLOW	781.4 (783.5)		31.72	1.68, $^{2}J_{P-H}$=16.91	75
$^{DMT}A^{Bz}{}_{2'OMe}$	FAST	838.3(840.4)	839.296	30.54	1.70, $^{2}J_{P-H}$=16.85	70
	SLOW	838.3(840.4)		29.96	1.76, $^{2}J_{P-H}$=16.95	81
$^{DMT}G^{ibu}{}_{2'OMe}$	FAST	820.3	821.307	30.69	1.67, $^{2}J_{P-H}$=16.85	80
	SLOW	820.3		30.97	1.73, $^{2}J_{P-H}$=16.92	>80

[a]Selected data for signals of P-CH_3 (d: in ppm, *J* in Hz). [b]Isolated yields. [c]NMR yield >98%.

Interestingly, *O,O,O*-trimethyl phosphorothioate was completely oxidized under the standard conditions into *O,O,O*-trimethyl phosphate within 30 min, whereas the time required for complete conversion of potassium *O,O*-dimethyl phosphorothioate under identical conditions (pH 7, room temperature) into potassium *O,O*-dimethyl phosphate was 18 hours.

In spite of the rather slow rate of oxidation of anionic phosphorothioates, this result was attractive from the point of a long-standing interest in conversion of oligo(nucleoside phosphorothioate)s (PS-oligos) into "natural" oligonucleotides[8,9] under conditions that do not affect nucleobases nor the sugar-phosphate backbone. Analysis of natural oligonucleotides (e.g., sequencing or cloning) is much more reliable than that of PS-oligos. Among the many reagents described as effective oxidants of phosphothioates or phosphoselenoates, including bromine, BrCN, HNO_3, N_2O_4, H_2O_2, $KMnO_4$, chloral or organic peracids[10] only iodine/water[8,11] or sodium periodate[9] withstand the requirements of nondestructive oxidation of PS-oligos. A literature search indicated that oxone has been used for the oxidation of oligonucleotide analogues with internucleotide dimethylene sulfide functions leading to oligonucleotide analogues with a dimethylene sulfone groups replacing the phosphodiester linkages. Efficient sulfide→sulfone oxidation occurred at room temperature without affecting nucleobases, when a methanol solution of sulfide was stirred with ca. 0.6 M solution of oxone in 0.1 M sodium acetate buffer at pH 4.5 for 30 min.[12]

Relatively nondestructive conditions for the oxidation of PS-Oligos were found (0.05 M oxone, CH_3COONa buffer, pH 7.5, 48-60 h) and tested with **3**, the 20-mer phosphorothioate analogue of $^{5'}$-d[CCC TGC TCC CCC CTG GTC CC]-$^{3'}$ (**4**). Reactions were monitored by ^{31}P NMR (Figure 1).[13] The complete oxidation has been demonstrated by means of ^{31}P NMR, but it can be observed (autoradiogram, Figure 2, lane 5) that the oxidation reaction is also accompanied by a partial degradation of the oligomer **4.**

Analogous results were obtained, when the sequence **3** was modified by introduction of two adenosines instead of cytidines $^{5'}$-d[CCC TGC TCA CCA CTG GTC CC]-$^{3'}$ (**5**)].[14]

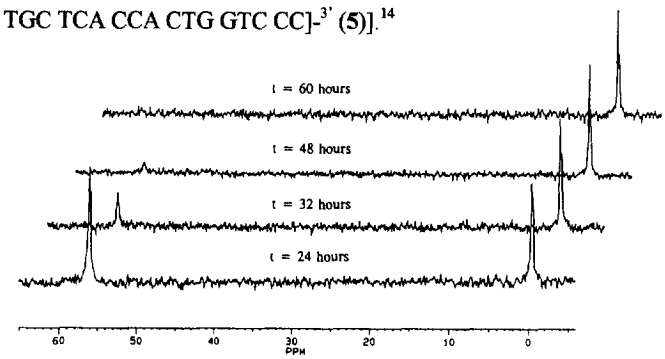

Figure 1. ^{31}P NMR spectra (121.47 MHz, ^{1}H decoupled) for the oxidation of (**3**) with 0.05 M oxone in D_2O at room temperature.

The purity of the products was checked by HPLC analysis and Gel Electrophoresis. The product resulting from oligonucleotides (**3**) and (**5**), PO-Oligos (**4**) and (**6**), were degraded with *DNase I* and, separately, with endonuclease from *Serratia marcescens*. The pattern of enzymatically generated products was compared with that obtained by analogous degradation of a genuine samples (**4**) and (**6**) (Figure 2).

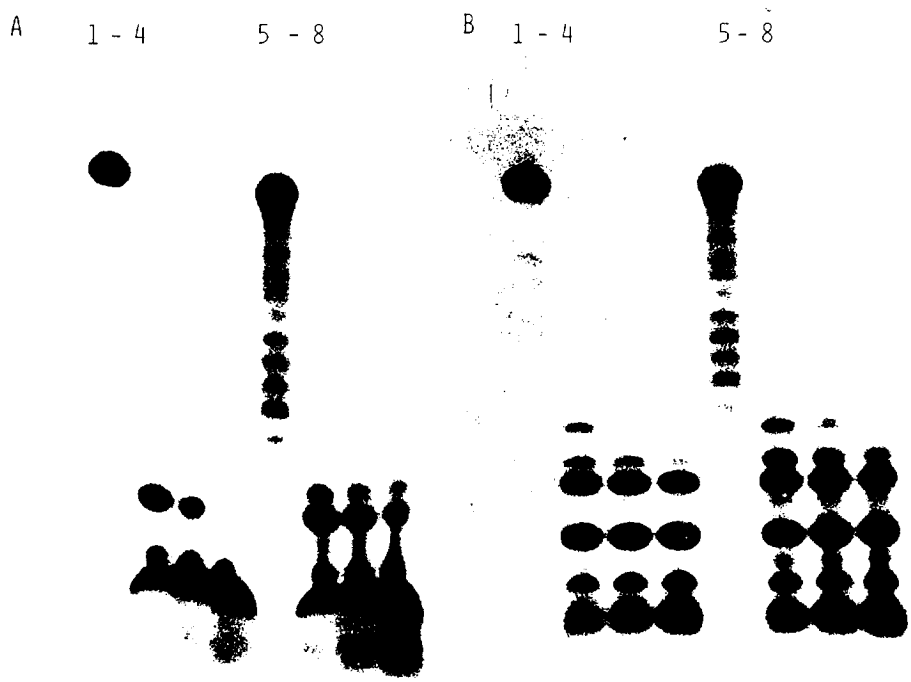

Figure 2. A) Autoradiogram of degradation products of (**4**) after treatment with *DNase* I. The 5'-^{32}P-labeled oligonucleotide (**4**) (3.35 mM) was dissolved in 40 mL of 5 mM CH_3COONa, 10 mM $MgCl_2$, 2 mM $CaCl_2$ and incubated with *DNase* I (200 units of the enzyme). 10 mL aliquots of the reaction mixture were removed for analysis, heat-denaturated, and loaded on 20% polyacrylamide gel (denaturating 7 M urea).

Lanes 1-4: oligonucleotide (**4**) (genuine sample) after 0, 10, 20, and 60 min of incubation, respectively.

Lanes 5-8: oligonucleotide (**4**) [obtained from (**3**)] after 0, 10, 20, and 60 min of incubation, respectively.

B) Autoradiogram of degradation products of (**4**) after treatment with *Sma endonuclease* and 20% polyacrylamide gel (denaturing 7 M urea).

The oligonucleotides were labeled at the 5'-end with ^{32}P. Solutions (40 mL) buffered to pH 8.5

(25 mM Tris) and containing 4 mM $MgCl_2$ and 3.35 mM oligonucleotide (**4**) were incubated with 4 units of the enzyme. 10 mL aliquots of the reaction mixture were removed for analysis, heat-denaturated, and loaded on PAGE.

Lanes 1-4: oligonucleotide (**4**) (genuine sample) after 0, 10, 20, 40, and 60 min of incubation, respectively.

Lanes 5-8: oligonucleotide (**4**) [obtained from (**3**)] after 0, 10, 20, and 60 min of incubation, respectively.

It is known that *DNase* I[15] and *Sma endonuclease*[16], used in concentrations as described in discussed experiments, do not digest internucleotide phosphorothioate linkages. Therefore, the similar pattern of degradation of the genuine sample of **4** with that of the product of enzymatic degradation of **4** obtained from oxone-treatment of **3** indicate the exhaustive conversion of **3**→**4**.

In conclusion, oxone can be used for selective, stereoretentive oxidation of a wide variety of P(S) compounds, including nucleoside methanephosphonothioates and oligo(nucleoside phosphorothioate)s.

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4. The commercially available oxone (Caro's salt, Aldrich) is represented by the formula: $2KHSO_5{\cdot}KHSO_4{\cdot}K_2SO_4$.
5. *General procedure:* Into a vigorously stirred solution of the corresponding substrate (0.1 mmol) in THF/MeOH (1:1 v/v, 2 mL), a buffered solution of oxone (2 mL, 0.1 M, pH 7- 7.5) was added in one portion at room temperature. After the reaction was complete (TLC or ^{31}P NMR assay), aqueous $Na_2S_2O_3$ (0.065 M, 2 mL) was added, with stirring continued for 2 min, followed by extraction of the reaction mixture (3 times) with $CHCl_3$. The combined organic extracts were dried ($MgSO_4$) and solvents were

concentrated in vacuum.

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13. (**3**): ^{31}P NMR, δ: 55.86 ppm (D_2O); (**4**): ^{31}P NMR, δ: -0.24 ppm (D_2O).
14 Oligodeoxynucleotides **5** and **6**. Were synthesized on an ABI 391 synthesizer, using standard phosphoramidite chemistry (1mmol scale), and S-Tetra for sulfurization step (Stec, W. J; Uznanski, B.; Wilk, A.; Hirsbein, B. I.; Fearon, K. L., Bergor, B. J. *Tetrahedron Lett.* **1993**, *34*, 5317-5320). The oxidation was followed by HPLC (ODS Hypersil, 0-40% MeCN in 0.1 M. TEAB, 15 min, flow 3 mL/min.): R_t = 11.51.min (**5**), and R_t = 8.92 .min for (**6**).
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