

**REACTIVITY AND SELECTIVITY OF THE REACTION OF *o,o*-DIETHYL
2,4-DINITROPHENYL PHOSPHATE AND THIONOPHOSPHATE WITH
THIOLS OF LOW MOLECULAR WEIGHT**

J. G. Santos, M. E. Aliaga, K. Alarcón, A. Torres, D. Céspedes and P.
Pavez.*

Facultad de Química. Pontificia Universidad Católica de Chile. Casilla
306, Santiago 6094411, Chile.

Author Information

*Corresponding author. Tel.: +56-223541743; fax: +56-226864744; e-
mail: ppavezg@uc.cl

Present address: Facultad de Química, Pontificia Universidad Católica de
Chile, Av. Vicuña Mackenna 4860, Santiago 6094411, Chile.

Table of contents	Page
Figure S1: Sequence of ^{31}P NMR spectra for the reaction of <i>O,O</i> -diethyl 2,4-dinitrophenyl phosphate (1) with N-acetyl cysteine in D_2O - Acetonitrile 60:40 %v/v.	S4
Figure S2: Sequence of ^{31}P NMR spectra for the reaction of <i>O,O</i> -diethyl 2,4-dinitrophenyl phosphate (1) with L-cysteine in D_2O - Acetonitrile 60:40 %v/v.	S5
Figure S3: Sequence of ^{31}P NMR spectra for the reaction of <i>O,O</i> -diethyl 2,4-dinitrophenyl phosphate (1) with Homocysteine in D_2O - Acetonitrile 60:40 %v/v.	S6
Figure S4: Sequence of ^{31}P NMR spectra for the reaction of <i>O,O</i> -diethyl 2,4-dinitrophenyl phosphate (1) with Glutathione in D_2O - Acetonitrile 60:40 %v/v.	S7
Figure S5: Sequence of ^{31}P NMR spectra for the reaction of <i>O,O</i> -diethyl 2,4-dinitrophenyl phosphate (1) with Penicillamine in D_2O - Acetonitrile 60:40 %v/v.	S8
Figure S6: Sequence of ^{31}P NMR spectra for the reaction of <i>O,O</i> -diethyl 2,4-dinitrophenyl thionophosphate (2) with L-cysteine in D_2O -Acetonitrile 60:40 %v/v.	S9
Figure S7: ^{31}P -NMR spectrum of compound 3 , obtained in the reaction of <i>O,O</i> -diethyl chlorophosphate with NaOH in D_2O - Acetonitrile 60:40 %v/v.	S10
Figure S8: ^{31}P -NMR spectrum of compound 4 , obtained in the reaction of <i>O,O</i> -diethyl chlorothionophosphate with NaOH in D_2O - Acetonitrile 60:40 %v/v.	S11
Figure S9: ^1H NMR spectra for the reaction of 1-Chloro-2,4-dinitrobenzene with N-acetylcysteine in D_2O - Acetonitrile 60:40 %v/v.	S12
Figure S10: ^1H NMR spectra for the reaction of 1-Chloro-2,4-dinitrobenzene with Penicillamine in D_2O - Acetonitrile 60:40 %v/v.	S13
Figure S11: ^1H NMR spectrum of compound 8 formed in the reaction of 1 with L-Cys at very long time, in D_2O - Acetonitrile 60:40 %v/v.	S14
Figure S12: ^1H NMR spectrum of the mixture of compounds 7 and 8 formed during the reaction of 1 with L-Cys, in D_2O - Acetonitrile 60:40 %v/v.	S15
Figure S13: Sequence of ^1H NMR spectra for the reaction of <i>O,O</i> -diethyl 2,4-dinitrophenyl phosphate (1) with Homocysteine in D_2O - Acetonitrile 60:40 %v/v.	S16
Figure S14: Sequence of ^1H NMR spectra for the reaction of <i>O,O</i> -diethyl 2,4-dinitrophenyl phosphate (1) with Glutathione in D_2O - Acetonitrile 60:40 %v/v.	S17
Table S1: Chemical shifts of 1-Chloro-2,4-dinitrobenzene, 2,4-dinitrophenol, compounds 1 and 2 and those attributed to the compounds 3-8 .	S18

Table S2: Kinetics data for the 1 and 2 reactions with Cys 0.05M, $\mu = 0.2$ M(KCl) at $25 \pm 0.1^\circ\text{C}$.	S19
Table S3: Experimental conditions and k_{obs} values for the reactions <i>O,O</i> -diethyl 2,4-dinitrophenyl phosphate (1) with N-acetylcysteine (NAC) in aqueous ethanol 44wt. % , 37.0°C , $\mu = 0.2$ M in KCl.	S20
Table S4: Experimental conditions and k_{obs} values for the reactions <i>O,O</i> -diethyl 2,4-dinitrophenyl phosphate (1) with L-Cysteine (Cys) in aqueous ethanol 44wt. % , 37.0°C , $\mu = 0.2$ M in KCl.	S20
Table S5: Experimental conditions and k_{obs} values for the reactions <i>O,O</i> -diethyl 2,4-dinitrophenyl phosphate (1) with Homocysteine (Hcys) in aqueous ethanol 44wt. % , 37.0°C , $\mu = 0.2$ M in KCl.	S21
Table S6: Experimental conditions and k_{obs} values for the reactions <i>O,O</i> -diethyl 2,4-dinitrophenyl phosphate (1) with Glutathione (GSH) in aqueous ethanol 44wt. % , 37.0°C , $\mu = 0.2$ M in KCl.	S21
Table S7: Experimental conditions and k_{obs} values for the reactions <i>O,O</i> -diethyl 2,4-dinitrophenyl phosphate (1) with D-Penicillamine (Pen) in aqueous ethanol 44wt. % , 37.0°C , $\mu = 0.2$ M in KCl.	S22
Table S8: Experimental conditions and k_{obs} values for the reactions <i>O,O</i> -diethyl 2,4-dinitrophenyl thionohosphate (2) with L-Cysteine (Cys) in aqueous ethanol 44wt. % , 37.0°C , $\mu = 0.2$ M in KCl.	S22
Table S9: Experimental conditions and k_{obs} values for the reactions <i>O,O</i> -diethyl 2,4-dinitrophenyl thionohosphate (2) with Glutathione (GSH) in aqueous ethanol 44wt. % , 37.0°C , $\mu = 0.2$ M in KCl.	S23
Table S10: Experimental conditions and k_{obs} values for the reactions <i>O,O</i> -diethyl 2,4-dinitrophenyl thionohosphate (2) with Homocysteine (Hcys) in aqueous ethanol 44wt. % , 37.0°C , $\mu = 0.2$ M in KCl.	S23
Table S11: Experimental conditions and k_{obs} values for the reactions <i>O,O</i> -diethyl 2,4-dinitrophenyl thionohosphate (2) with N-acetylcysteine (NAC) in aqueous ethanol 44wt. % . 37.0°C . $\mu = 0.2$ M in KCl.	S24
Table S12: Experimental conditions and zero-order k_{obs} values for the reaction <i>O,O</i> -diethyl 2,4-dinitrophenyl phosphate (1) with L-Cysteine (Cys) in aqueous ethanol 44wt. % , 37.0°C , $\mu = 0.2$ M in KCl	S24

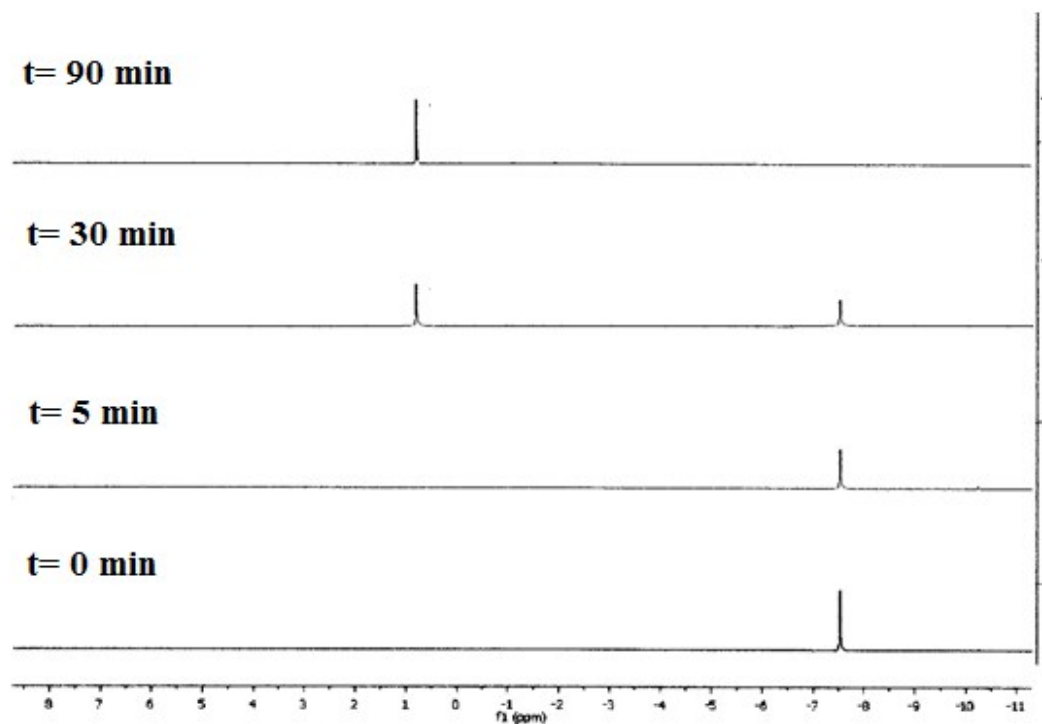


Figure S1: Sequence of ^{31}P NMR spectra for the reaction of *O,O*-diethyl 2,4-dinitrophenyl phosphate (**1**) with N-acetyl cysteine in D_2O - Acetonitrile 60:40 %v/v, 0.1M in biothiol, 0.006M the substrate and $\text{pH}=\text{p}K_a$ of corresponding thiol group

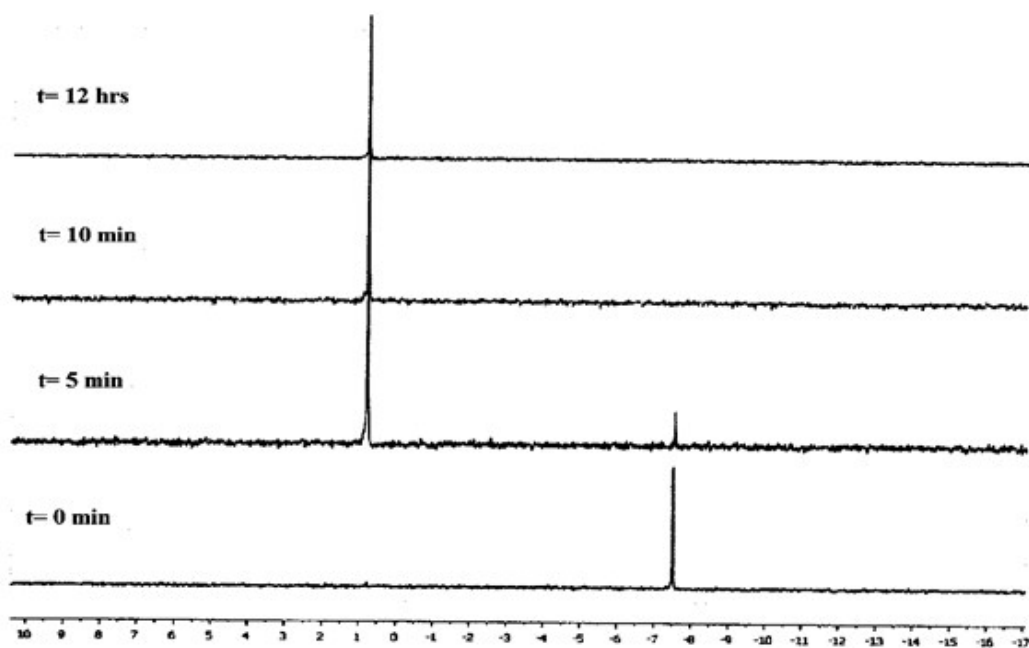


Figure S2: Sequence of ^{31}P NMR spectra for the reaction of *O,O*-diethyl 2,4-dinitrophenyl phosphate (**1**) with L-cysteine in D_2O - Acetonitrile 60:40 %v/v, 0.1M in biothiol, 0.006M the substrate and $\text{pH}=\text{p}K_a$ of corresponding thiol group

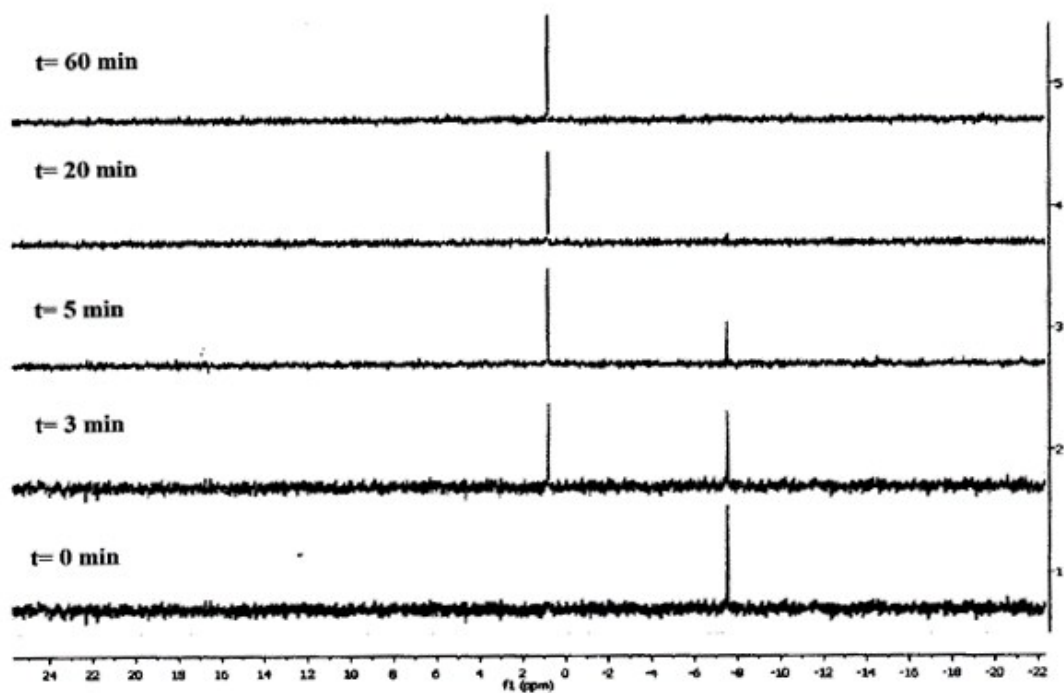


Figure S3: Sequence of ^{31}P NMR spectra for the reaction of *O,O*-diethyl 2,4-dinitrophenyl phosphate (**1**) with Homocysteine in D_2O - Acetonitrile 60:40 %v/v, 0.1M in biothiol, 0.006M the substrate and $\text{pH}=\text{p}K_a$ of corresponding thiol group.

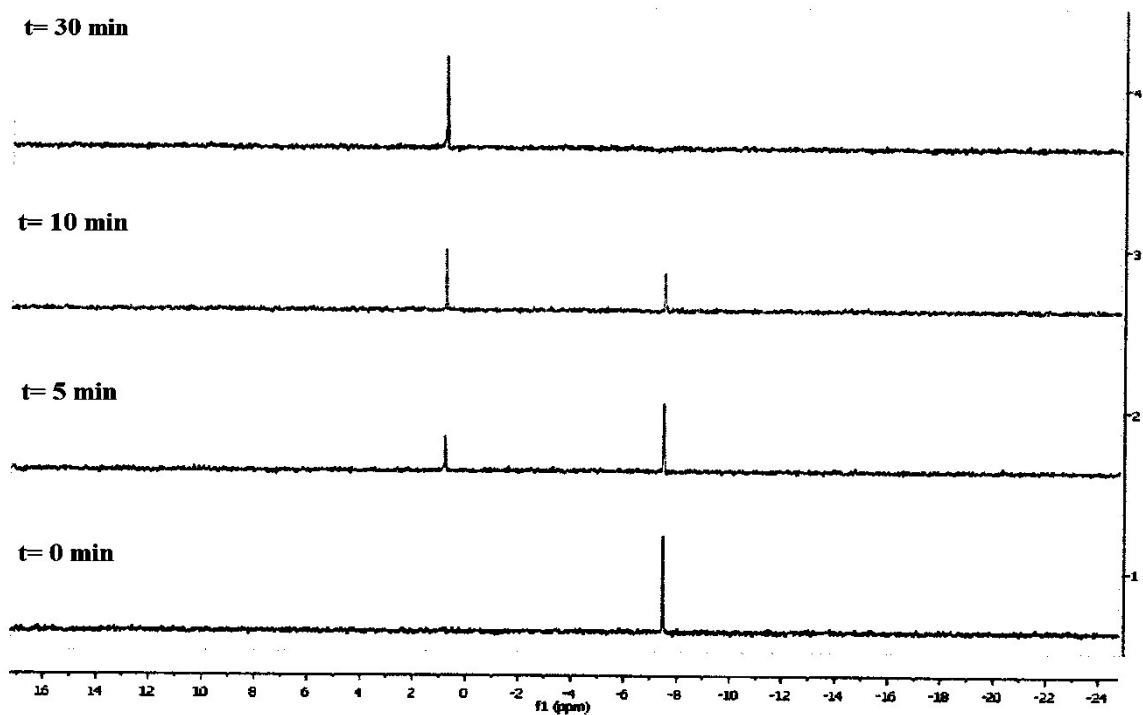


Figure S4: Sequence of ^{31}P NMR spectra for the reaction of *O,O*-diethyl 2,4-dinitrophenyl phosphate (**1**) with Glutathione in D_2O - Acetonitrile 60:40 %v/v, 0.1M in biothiol, 0.006M the substrate and $\text{pH}=\text{p}K_a$ of corresponding thiol group.

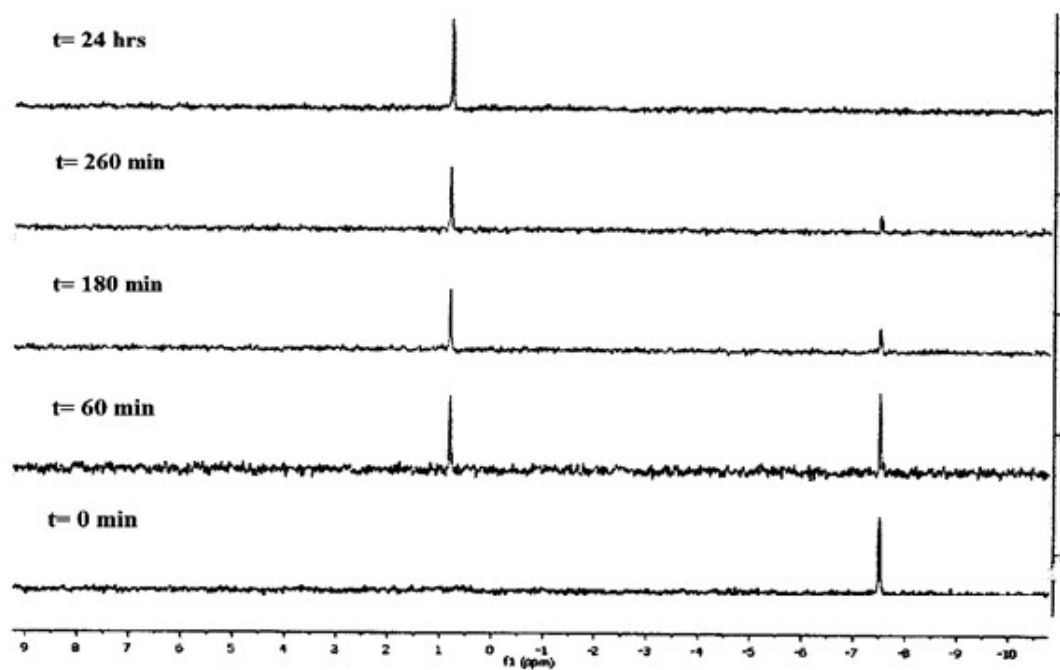


Figure S5: Sequence of ^{31}P NMR spectra for the reaction of *O,O*-diethyl 2,4-dinitrophenyl phosphate (**1**) with Penicillamine in D_2O - Acetonitrile 60:40 %v/v, 0.1M in biothiol, 0.006M the substrate and $\text{pH}=\text{p}K_a$ of corresponding thiol group.

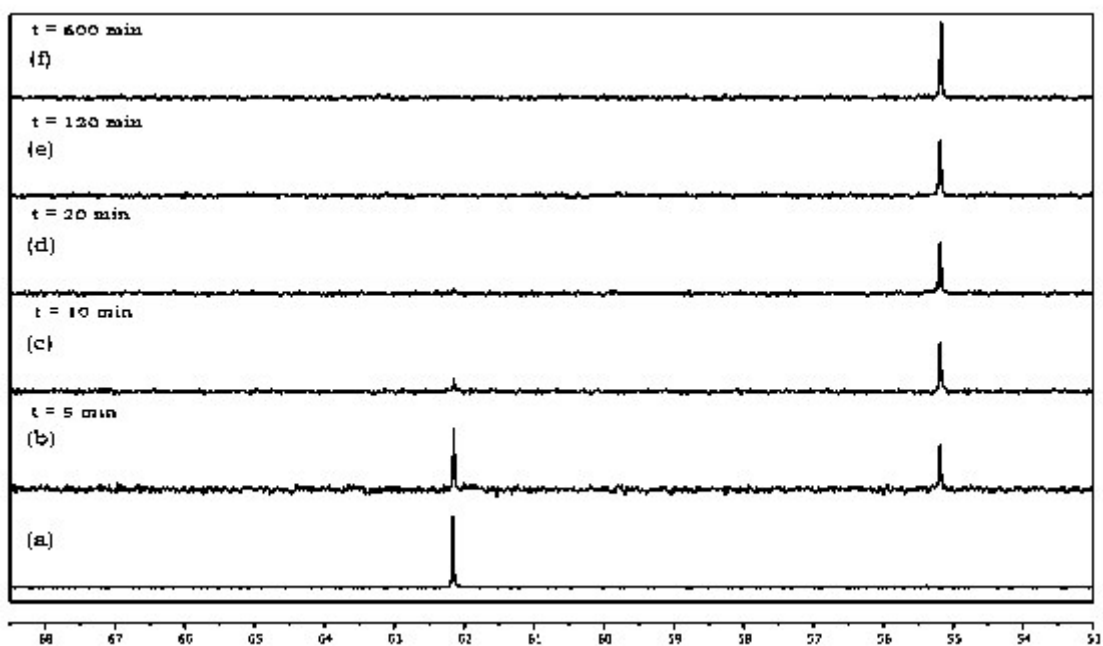


Figure S6: Sequence of ^{31}P NMR spectra for the reaction of *O,O*-diethyl 2,4-dinitrophenyl thionophosphate (**2**) with L-cysteine in D_2O -Acetonitrile 60:40 %v/v, 0.1M in biethiol, 0.006M the substrate and $\text{pH}=\text{p}K_a$ of corresponding thiol group.

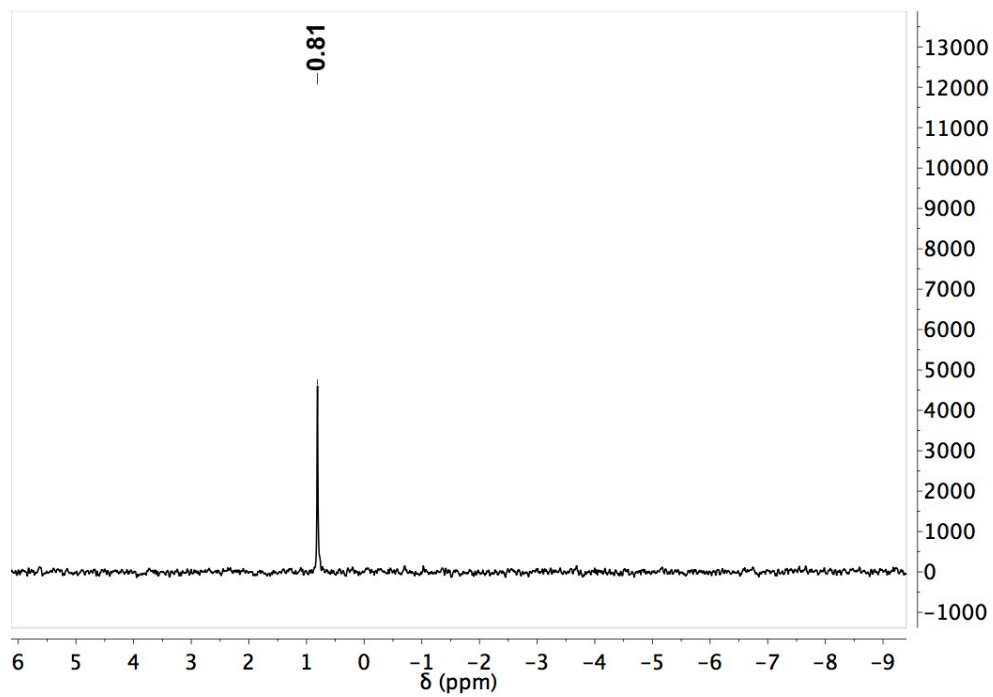


Figure S7: ^{31}P -NMR spectrum (400MHz) of compound **3**, obtained in the reaction of *O,O*-diethyl chlorophosphate with NaOH in D_2O - Acetonitrile 60:40 %v/v.

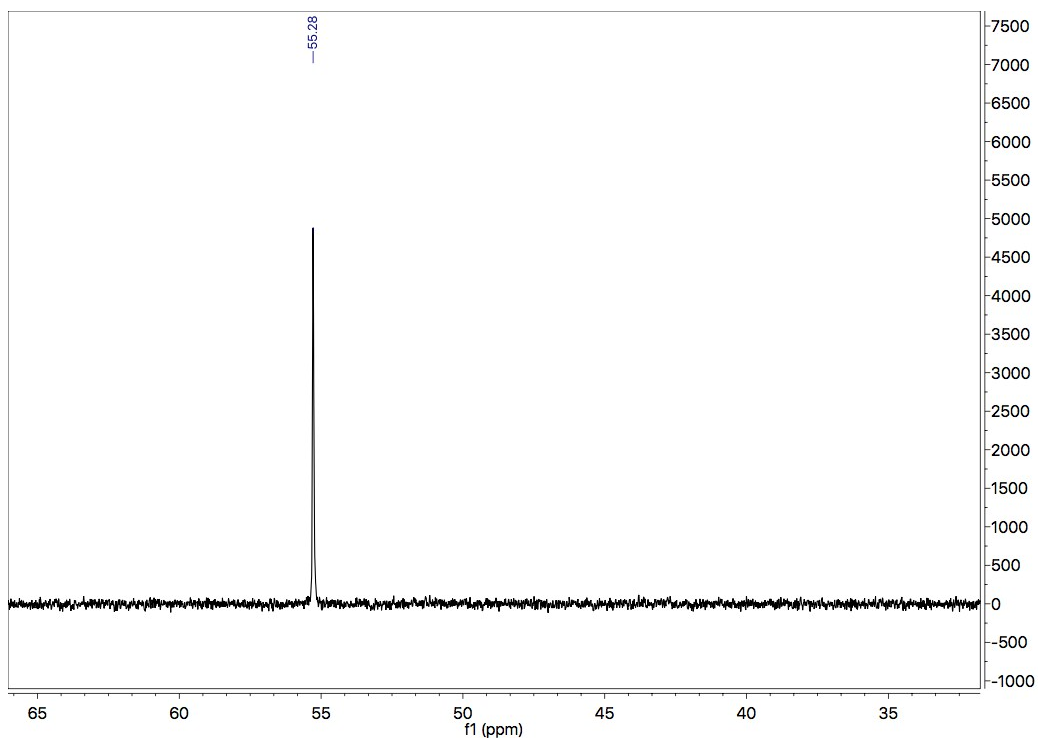


Figure S8: ^{31}P -NMR spectrum (400MHz) of compound **4**, obtained in the reaction of *O,O*-diethyl chlorothionophosphate with NaOH in D_2O - Acetonitrile 60:40 %v/v.

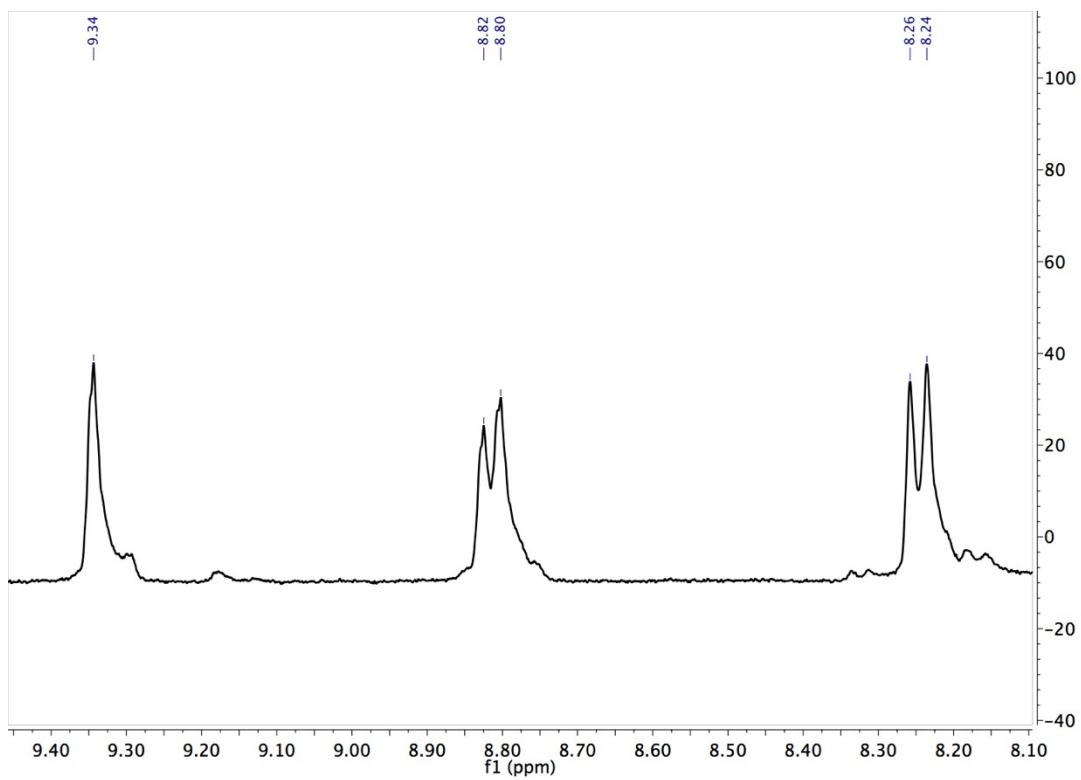


Figure S9: ¹H NMR spectra for the reaction of 1-Chloro-2,4-dinitrobenzene with N-acetylcysteine in D₂O- Acetonitrile 60:40 %v/v.

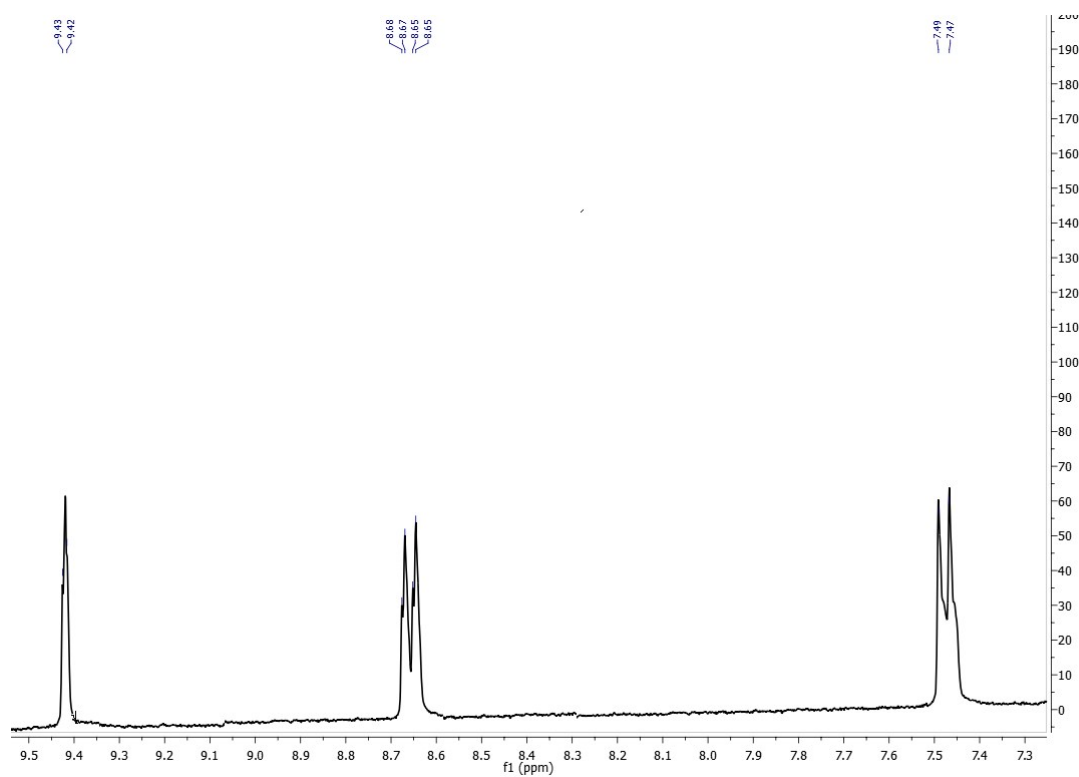


Figure S10: ¹H NMR spectra for the reaction of 1-Chloro-2,4-dinitrobenzene with Penicillamine in D₂O- Acetonitrile 60:40 %v/v.

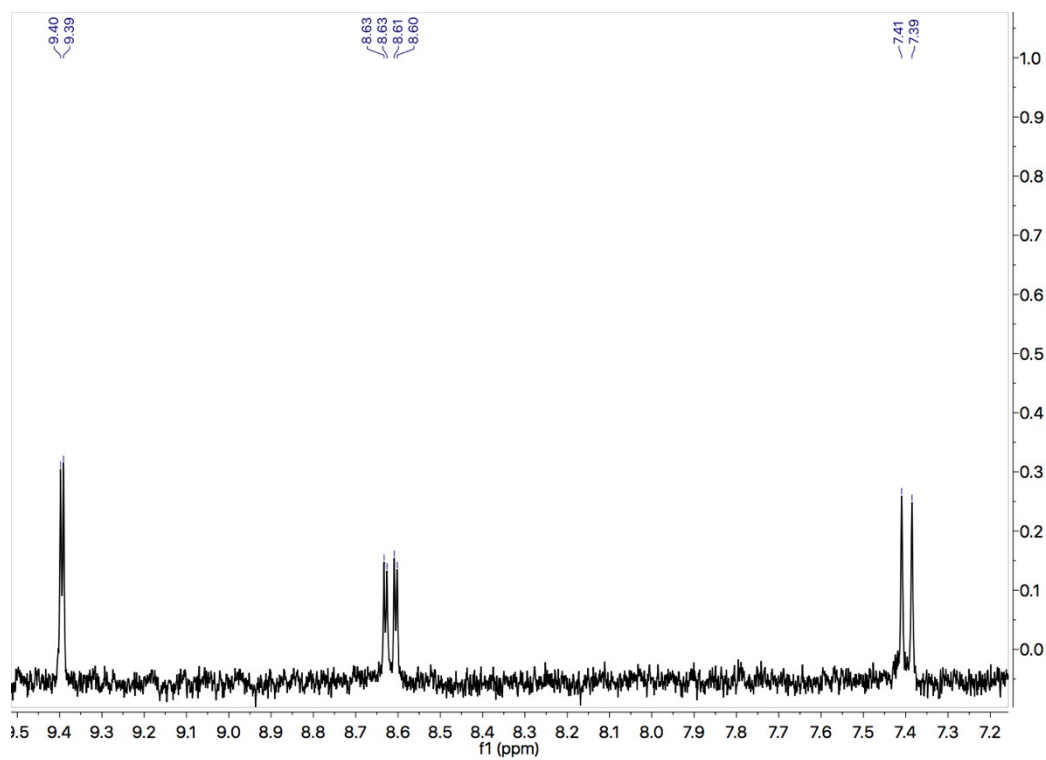


Figure S11: ^1H NMR spectrum of compound **8** formed in the reaction of **1** with L-Cys at very long time, in D_2O - Acetonitrile 60:40 %v/v.

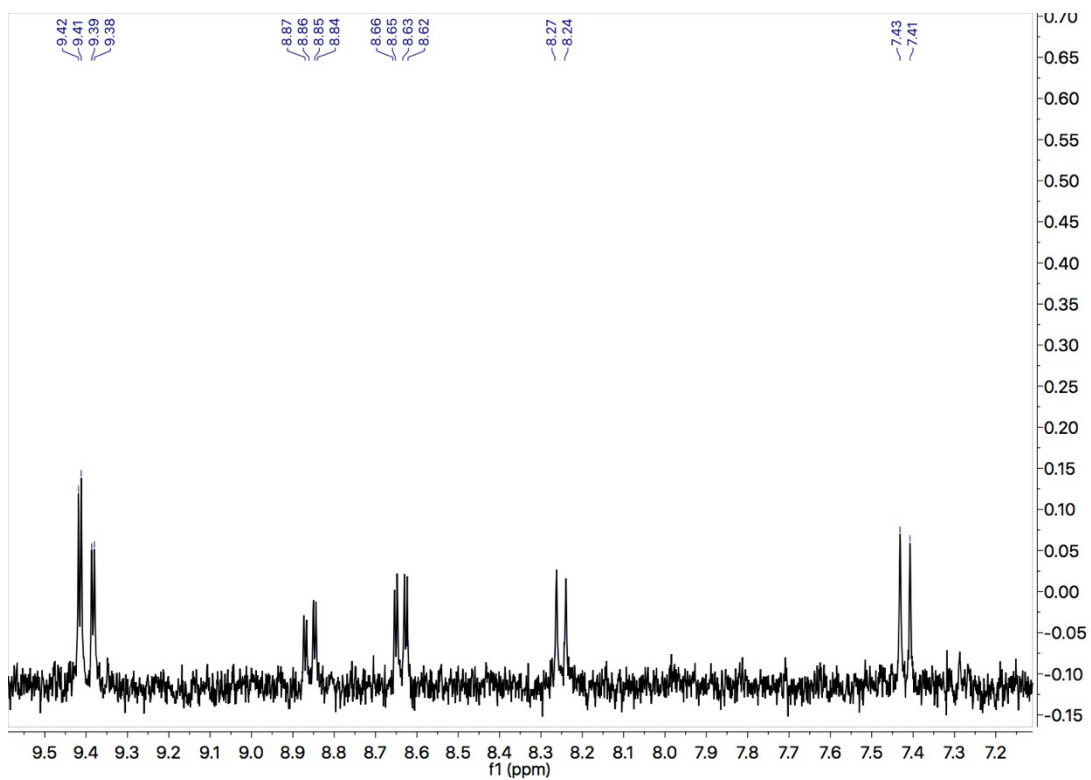


Figure S12: ¹H NMR spectrum of the mixture of compounds **7** and **8** formed during the reaction of **1** with L-Cys, in D₂O- Acetonitrile 60:40 %v/v.

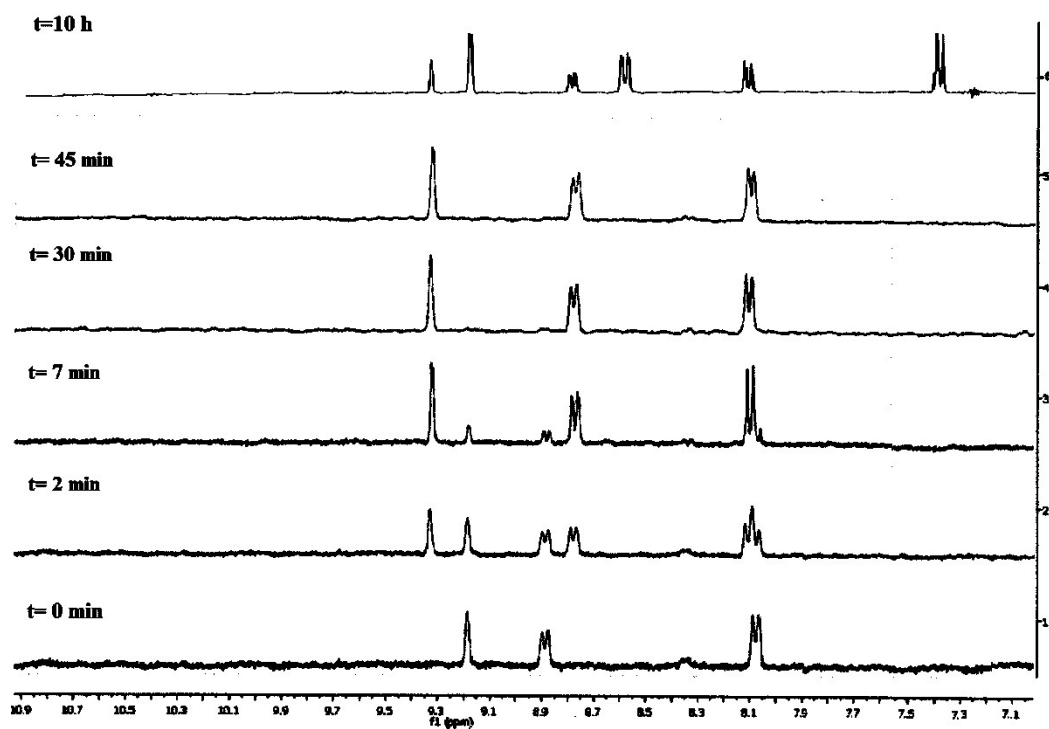


Figure S13: Sequence of ¹H NMR spectra for the reaction of *O,O*-diethyl 2,4-dinitrophenyl phosphate (**1**) with Homocysteine in D₂O- Acetonitrile 60:40 %v/v.

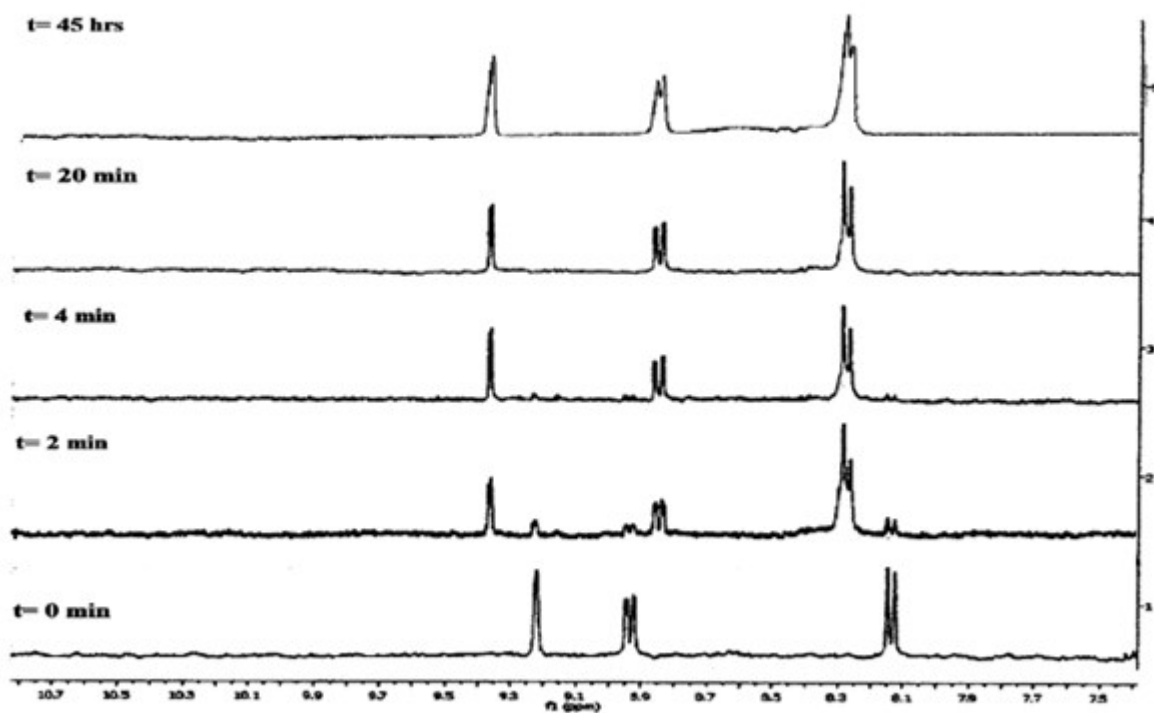


Figure S14: Sequence of ^1H NMR spectra for the reaction of *O,O*-diethyl 2,4-dinitrophenyl phosphate (**1**) with Glutathione in D_2O - Acetonitrile 60:40 %v/v.

Table S1: Chemical shifts of 1-Chloro-2,4-dinitrobenzene, 2,4-dinitrophenol, compounds **1** and **2** and those attributed to the compounds **3-8**.

Compound	NMR- ¹ H δ (ppm)	NMR- ³¹ P δ (ppm)
<i>O,O</i> -diethyl 2,4-dinitrophenyl phosphate (1)	8.11 (d, $J_1=9.2$ Hz, 1H), 8.90 (dd, $J_1=9.2$ Hz, $J_2=2.8$ Hz, 1H), 9.19 (d, $J_2=2.8$ Hz, 1H)	-7.52
<i>O,O</i> -diethyl 2,4-dinitrophenyl thionophosphate (2)	8.11 (d, $J_1=9.2$ Hz, 1H), 8.90 (dd, $J_1=9.2$ Hz, $J_2=2.8$ Hz, 1H), 9.17 (d, $J_2=2.8$ Hz, 1H)	62.2
<i>O,O</i> -diethylphosphoric acid (3)		0.81
<i>O,O</i> -diethylthiophosphoric acid (4)		55.3
1-(<i>S</i> -acetylcysteine) 2,4-dinitrobenzene (5)	8.25 (d, $J_1=8.8$ Hz, 1H), 8.81 (dd, $J_1=8.8$ Hz, $J_2=2.1$ Hz, 1H), 9.34 (d, $J_2=2.1$ Hz, 1H).	
1-(<i>N</i> -penicillamine) 2,4-dinitrobenzene (6)	7.48 (d, $J_1=9.6$ Hz, 1H), 8.65 (dd, $J_1=9.6$ Hz, $J_2=2.7$ Hz, 1H), 9.40 (d, $J_2=2.7$ Hz, 1H).	
1-(<i>S</i> -cysteil) 2,4-dinitrobenzene (7)	8.25 (d, $J_1=9.1$ Hz, 1H), 8.80 (dd, $J_1=9.1$ Hz, $J_2=2.5$ Hz, 1H), 9.31 (d, $J_2=2.5$ Hz, 1H).	
1-(<i>N</i> -cysteil) 2,4-dinitrobenzene (8)	7.4 (d, $J_1=9.7$ Hz, 1H), 8.63 (dd, $J_1=9.7$ Hz, $J_2=2.4$ Hz, 1H), 9.40 (d, $J_2=2.4$ Hz, 1H).	
2,4-dinitrophenol	7.01 (d, $J_1=9.6$ Hz, 1H-Ar), 8.35 (dd, $J_1=9.6$ Hz, $J_2=3.2$ Hz, 1H), 9.08 (d, $J_2=2.8$ Hz, 1H)	
1-chloro-2,4-dinitrobenzene	8.34 (d, $J_1=8.8$ Hz, 1H), 8.83 (dd, $J_1=8.8$ Hz, $J_2=2.8$ Hz, 1H), 9.16 (d, $J_2=2.4$ Hz, 1H)	

Table S2: Kinetics data for the **1** and **2** reactions with Cys 0.05M, $\mu = 0.2$ M(KCl) at $25 \pm 0.1^\circ\text{C}$.

pH	$10^4 k_{\text{obs}}/\text{s}^{-1}$	
	1	2
6.01	6.50	0.21
6.55	16.9	0.66
6.94	36.3	2.00
7.49	110	4.86
7.98	295	12.6
8.52	565	17.1
9.07	773	22.5
10.07	1000	25.1
10.56	1050	-

Table S3: Experimental conditions and k_{obs} values for the reactions *O,O*-diethyl 2,4-dinitrophenyl phosphate (**1**) with N-acetylcysteine (NAC) in aqueous ethanol 44wt. % , 37.0 °C, $\mu = 0.2$ M in KCl.

[NAC] _T /M	$10^3 k_{\text{obs}} / \text{s}^{-1}$		
	pH: 7.0 $F_{\text{N}(\text{S}^-)}: 0.0032$	pH: 7.5 $F_{\text{N}(\text{S}^-)}: 0.0099$	pH: 8.0 $F_{\text{N}(\text{S}^-)}: 0.0307$
0.10	7.18	20.1	56.7
0.08	6.14	16.2	45.2
0.06	5.10	13.3	33.5
0.04	3.16	8.15	22.7
0.02	1.69	4.99	11.3
0.01	8.16	2.06	5.48

Table S4: Experimental conditions and k_{obs} values for the reactions *O,O*-diethyl 2,4-dinitrophenyl phosphate (**1**) with L-Cysteine (Cys) in aqueous ethanol 44wt. % , 37.0 °C, $\mu = 0.2$ M in KCl.

[L-Cys] _T /M	$10^2 k_{\text{obs}} / \text{s}^{-1}$		
	pH: 7.0 $F_{\text{N}(\text{S}^-)}: 0.0736$	pH: 7.5 $F_{\text{N}(\text{S}^-)}: 0.2008$	pH: 8.0 $F_{\text{N}}: 0.4427$
0.10	2.04	4.21	10.5
0.08	1.56	3.82	7.62
0.06	1.15	2.64	5.71
0.04	0.824	2.55	4.72
0.02	0.783	1.28	2.88
0.01	0.237	0.600	1.55

Table S5: Experimental conditions and k_{obs} values for the reactions *O,O*-diethyl 2,4-dinitrophenyl phosphate (**1**) with Homocysteine (Hcys) in aqueous ethanol 44wt. % , 37.0 °C, $\mu = 0.2$ M in KCl.

[H-Cys] _T /M	$10^3 k_{\text{obs}} / \text{s}^{-1}$		
	pH: 7.0 $F_{\text{N(S-)}}: 0.0532$	pH: 7.5 $F_{\text{N(S-)}}: 0.1510$	pH: 8.0 $F_{\text{N(S-)}}: 0.3599$
0.01	1.16	2.32	4.81
0.008	0.903	1.92	3.91
0.006	0.740	1.55	3.05
0.004	0.535	1.13	2.10
0.002	0.421	0.789	1.28
0.001	0.344	0.530	0.874

Table S6: Experimental conditions and k_{obs} values for the reactions *O,O*-diethyl 2,4-dinitrophenyl phosphate (**1**) with Glutathione (GSH) in aqueous ethanol 44wt. % , 37.0 °C, $\mu = 0.2$ M in KCl.

[GSH] _T /M	$10^3 k_{\text{obs}} / \text{s}^{-1}$		
	pH: 7.0 $F_{\text{N(S-)}}: 0.0187$	pH: 7.5 $F_{\text{N(S-)}}: 0.0568$	pH: 8.0 $F_{\text{N(S-)}}: 0.1600$
0.12	10.7		
0.08	4.30		
0.06	4.10		
0.05		14.0	
0.04	3.41	12.6	
0.03		10.1	22.3
0.02	1.90	7.08	16.3
0.01	0.961	4.73	10.2
0.005		2.41	3.52

Table S7: Experimental conditions and k_{obs} values for the reactions *O,O*-diethyl 2,4-dinitrophenyl phosphate (**1**) with D-Penicillamine (Pen) in aqueous ethanol 44wt. % , 37.0 °C, $\mu = 0.2$ M in KCl.

	$10^4 k_{\text{obs}} / \text{s}^{-1}$
[Pen] _T /M	pH: 10.0 $F_{\text{N}(\text{NH}_2)}$: 0.24
0.01	15.7
0.008	13.4
0.006	9.64
0.004	6.22
0.002	3.62
0.001	2.25

Table S8: Experimental conditions and k_{obs} values for the reactions *O,O*-diethyl 2,4-dinitrophenyl thionohosphate (**2**) with L-Cysteine (Cys) in aqueous ethanol 44wt. % , 37.0 °C, $\mu = 0.2$ M in KCl.

	$10^5 k_{\text{obs}} / \text{s}^{-1}$	
$10^3 [\text{Cys}]_{\text{T}} / \text{M}$	pH: 6.9 F_{N} : 0.059	pH: 7.4 F_{N} : 0.167
15	57.7	107
10	45.5	86.0
8.0	49.6	75.5
6.0	31.5	55.4
4.0		47.3
2.5		35.0

Table S9: Experimental conditions and k_{obs} values for the reactions *O,O*-diethyl 2,4-dinitrophenyl thionohosphate (**2**) with Glutathione (GSH) in aqueous ethanol 44wt. % , 37.0 °C, $\mu = 0.2$ M in KCl.

$10^3 [\text{GSH}]_{\text{T}}/\text{M}$	$10^4 k_{\text{obs}}/\text{s}^{-1}$	
	pH: 6.9 $F_{\text{N}}: 0.015$	pH: 7.4 $F_{\text{N}}: 0.046$
15	9.86	23.9
10	6.95	17.5
8.0	5.93	
6.0	4.58	11.8
4.0	2.67	8.13
2.5	1.60	
1.0	0.673	1.66

Table S10: Experimental conditions and k_{obs} values for the reactions *O,O*-diethyl 2,4-dinitrophenyl thionohosphate (**2**) with Homocysteine (Hcys) in aqueous ethanol 44wt. % , 37.0 °C, $\mu = 0.2$ M in KCl.

$10^3 [\text{Hcys}]_{\text{T}}/\text{M}$	$10^4 k_{\text{obs}}/\text{s}^{-1}$	
	pH: 6.9 $F_{\text{N}}: 0.043$	pH: 7.4 $F_{\text{N}}: 0.124$
15	6.13	14.5
10	4.31	9.92
8.0	3.24	8.19
6.0	2.73	6.48
4.0	1.81	4.27
2.5		2.66
1.0	1.28	1.87

Table S11: Experimental conditions and k_{obs} values for the reactions *O,O*-diethyl 2,4-dinitrophenyl thionohosphate (**2**) with N-acetylcysteine (NAC) in aqueous ethanol 44wt. %. 37.0 °C. $\mu = 0.2$ M in KCl.

$10^3 [\text{NAC}]_{\text{T}}/\text{M}$	$10^5 k_{\text{obs}}/\text{s}^{-1}$	
	pH: 6.9 $F_{\text{N}}: 0.0025$	pH: 7.4 $F_{\text{N}}: 0.0099$
15	40.4	138
10	31.4	80.3
8.0	24.7	69.2
6.0	20.5	54.1
4.0	11.0	32.9
2.5	6.56	20.5
1.0	1.76	8.02

Table S12: Experimental conditions and zero-order k_{obs} values for the rearrangement reaction of **7** in aqueous ethanol 44wt. % , 37.0 °C, $\mu = 0.2$ M in KCl

[Cys]/M	$10^5 k_{\text{obs}}/\text{mol s}^{-1}$			
	pH=7.5	pH=8.0	pH=8.5	pH=9.0
0.1		1.54		1.35-2.1
0.16	1.3-2.1	1.67-1.78	1.67	1.75-1.79
0.22	1.3-1.6	1.94		1.32
0.28	1.4	1.38	1.0-2.08	1.02-1.19
0.34	1.1	1.57	1.16	1.03-1.71
0.40	1.7	1.36-1.75	1.36-1.48	1.86
0.5		1.08	1.06	1.88
0.6	1.1-2.1	1.2-1.41	1.20-1.24	