

# <sup>31</sup>P Chemical Shifts in NMR Spectra of Nucleotide Derivatives and Some Related Organophosphorus Compounds\*

By Inna Koukhareva, Ph.D.; TriLink BioTechnologies

Phosphorus Nuclear Magnetic Resonance (<sup>31</sup>P NMR) spectroscopy is widely used in the investigation of structure and chemical reactions of mono-, oligo and polynucleotides, their analogs, and derivatives. Many of the aspects of <sup>31</sup>P NMR spectroscopy were the subject of reviews [1], and systematic compilation of <sup>31</sup>P NMR data is presented in the literature [2].

The theory of <sup>31</sup>P NMR chemical shifts is very complex and is not very well developed compared to <sup>1</sup>H NMR. In this short introduction we cannot go into any details of <sup>31</sup>P NMR spectroscopy. Some facts below about <sup>31</sup>P NMR of nucleotide derivatives, including data presented in the tables, are brought here for informational purposes and are intended to help our customers in their routine work.

The Typical range of the <sup>31</sup>P chemical shifts of most known nucleotide derivatives covers the region from 200 ppm to -30 ppm. Many of P(III) derivatives resonate in a lower magnetic field (200 ppm to 50 ppm) compared to P(V) derivatives (70 ppm to -30 ppm). As one can deduce from the tables below, there is no unique correlation between <sup>31</sup>P NMR chemical shifts and the nature of the substituents on the phosphorus atom. However, a huge volume of the experimental data on nucleotides and their derivatives accumulated in <sup>31</sup>P NMR spectroscopy over 50 years, has helped introduce a simple set of rules that are useful in the interpretation of <sup>31</sup>P chemical shift changes [2].

In our work on the synthesis of different nucleotide derivatives (as well as other phosphorus containing compounds), <sup>31</sup>P NMR serves as one of the most important tools for evaluation of the structure, assurance of quality, and integrity of the products. When you are ordering our products you may request <sup>31</sup>P NMR spectra of the nucleotide derivatives to be recorded and sent to you with the compounds. <sup>31</sup>P NMR spectra are usually easy to interpret and some reference data presented in the Tables should help you in most cases. However, we are glad to assist you in the assignment of all the signals and in the complete interpretation of the <sup>31</sup>P NMR spectra.

## I. Pentavalent Phosphorus

### A. Phosphate mono-, di-, triesters and their mono- and diamides



X	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	δ, ppm	Solvent	Reference
O	-OH	-OH	5'-O-Nucleoside	1.2; 2.8-3.6; 0.4-1.2	H <sub>2</sub> O, pH 7.0; H <sub>2</sub> O; Pyridine	3 2 1
O	-OH	-OH	3'-O-Nucleoside	1.0	H <sub>2</sub> O, pH 7.0	3
O	3'-O-Nucleoside	-OH	5'-O-Nucleoside	-2.0-1.0	Pyridine	1
O	-OAryl	-OH	5'- or 3'-O-Nucleoside	-(5.0-6.0)	1,4-dioxane; Pyridine	1
O	-OAlkyl	-OAlkyl	5'- or 3'-O-Nucleoside	-(0.7-3.0)	Pyridine	1
O	-OAryl	-OAryl	5'- or 3'-O-Nucleoside (or -OAlkyl)	-7.7	Pyridine	1
O	-OAryl	-OAryl	5'- or 3'-O-Nucleoside	-(12.0-13.0)	Pyridine	1
O	-NH <sub>2</sub>	-OH	5'- or 3'-O-Nucleoside	8.8-9.3	D <sub>2</sub> O	1
O	-NH Alkyl	-OH	5'- or 3'-O-Nucleoside	5.0-6.5	Pyridine	1
O	-NH Aryl	-OH	5'- or 3'-O-Nucleoside	-1.0	Pyridine	1
O	5'-or 3'-O-Nucleoside	-NH <sub>2</sub>	5'-or 3'-O-Nucleoside	10.8-12.1	D <sub>2</sub> O, Pyridine	1
O	5'-or 3'-O-Nucleoside	-NHAlkyl	5'-or 3'-O-Nucleoside	8.4-9.2	Pyridine	1
O	5'-or 3'-O-Nucleoside	-NH Aryl	5'-or 3'-O-Nucleoside	2.6-2.8	Pyridine	1
O	5'-or 3'-O-Nucleoside	-NH Aryl	-OAryl	-(2.2-2.8)	Pyridine	1
O	5'-or 3'-O-Nucleoside	-NH Aryl	-NH Aryl	2.9	Pyridine	1
S	-OH	-OH	5'-or 3'-O-Nucleoside	43.5**	H <sub>2</sub> O	1
S	5'- O-Nucleoside	-OH	5'-or 3'-O-Nucleoside	55.0-57.0**	H <sub>2</sub> O	1, 3
S	5'- O-Nucleoside	-OAlkyl (-CNET)	3'- O-Nucleoside	69.0**	H <sub>2</sub> O	3

\*\*Denotes range of <sup>31</sup>P chemical shifts for two diastereomers

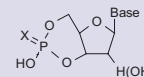
\* The <sup>31</sup>P chemical shifts are referenced to 85% H<sub>3</sub>PO<sub>4</sub> and positive values mean downfield shifts of <sup>31</sup>P signals. Only <sup>31</sup>P NMR spectra recorded with spin-spin decoupling {<sup>1</sup>H}-<sup>31</sup>P have been considered. The chemical shifts and coupling constants are reported as a range or as an average number from multiple data points.

## I. Pentavalent Phosphorus (continued)

### B. Nucleoside 3',5'-cyclophosphate

X	$\delta$ , ppm	Solvent	Reference
O	-(1.5-2.0)	D <sub>2</sub> O, pH7	1
S	52.0-55.0**	Pyridine	1

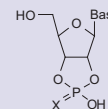
\*\*Denotes range of <sup>31</sup>P chemical shifts for two diastereomers



### C. Nucleoside 2',3'-cyclophosphate

X	$\delta$ , ppm	Solvent	Reference
O	17.6-18.5	DMF; Pyridine; MeOH	1
S	75.0-76.0**	H <sub>2</sub> O	1

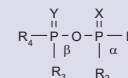
\*\*Denotes range of <sup>31</sup>P chemical shifts for two diastereomers



### D. Pyrophosphate derivatives of nucleosides

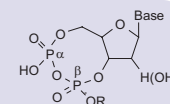
X	Y	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	$\delta_{\alpha}$ , ppm	$\delta_{\beta}$ , ppm	J $\alpha\beta$ , Hz	Solvent	Reference
O	O	5'-O-Nucleoside	-OH	-OH	-OH	-10.0	-6.0	20.7	H <sub>2</sub> O	3
O	O	3'-O-Nucleoside	-OH	-OH	-OH	-(10.0-11.0)	-(5.0-6.0)	21.4	H <sub>2</sub> O	3
O	O	5'-O-Nucleoside	-OH	-OH	5'-or 3'-O-Nucleoside	-(10.0-11.0)	-10.0	n/a	Pyridine	1
O	O	5'-O-Nucleoside	-OH	-OAryl	-OH	-11.2	-16.6	17.0	Pyridine	1
O	O	5'-O-Nucleoside	-OAryl	-OAryl	5'-O-Nucleoside	-19.0	-19.0	n/a	Pyridine	1
O	O	5'-O-Nucleoside	-OH	-OH	-NH <sub>2</sub>	-11.3	-0.8	20.0	D <sub>2</sub> O, pH10	1
O	O	5'-O-Nucleoside	-OH	-NHAryl	5'-O-Nucleoside	-11.2	-6.8	17.5	Pyridine	1
O	O	5'-O-Nucleoside	-NHAryl	-NHAryl	5'-O-Nucleoside	-(7.0-7.4)	-(7.0-7.4)	n/a	Pyridine	1
S	O	5'-O-Nucleoside	-OH	-OH	-OH	42.1**	-6.3**	30.8	H <sub>2</sub> O	1
O	S	5'-O-Nucleoside	-OH	-OH	-OH	-11.7**	34.0**	31.2	H <sub>2</sub> O	1

\*\*Denotes range of <sup>31</sup>P chemical shifts for two diastereomers



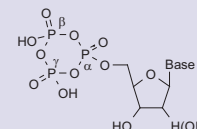
### E. Nucleoside 3',5'-cyclopyrophosphate

R	$\delta_{\alpha}$ , ppm	$\delta_{\beta}$ , ppm	J $\alpha\beta$ , Hz	Solvent	Reference
H	-13.7	-14.4	27.6	DMF	1
3'-O-Nucleoside	-13.0	-15.0	22.0	H <sub>2</sub> O, pH 9	1



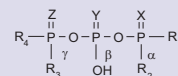
### F. Nucleoside 5'-O-trimetaphosphate

$\delta_{\alpha}$	$\delta_{\beta}$	$\delta_{\gamma}$	J $\alpha\beta$ , Hz	J $\beta\gamma$ , Hz	J $\alpha\gamma$ , Hz	Solvent	Reference
-23.0	-24.0	-24.6	23.5	23.0	23.7	Pyridine	1



## I. Pentavalent Phosphorus (continued)

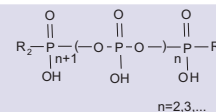
## G. Triphosphate derivatives of nucleosides



X	Y	Z	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	δ <sub>α</sub> , ppm	δ <sub>β</sub> , ppm	δ <sub>γ</sub> , ppm	J <sub>αβ</sub> , Hz	J <sub>βγ</sub> , Hz	Solvent	Reference
O	O	O	-OH	-OH	-OH	-OH	-5.3(d)	-18.6(t)	-5.3(d)			H <sub>2</sub> O	3
O	O	O	5'-O-Nuc.	-OH	-OH	-OH	-(10.0-11.0)(d)	-(20.0-23.0)(t)	-(5.0-7.7)(d)	20.0	20.0	H <sub>2</sub> O, pH 7.0	1, 2, 3
O	O	O	5'-O-Nuc.	-OH	-OH	5'-O-Nuc.	-11.0(d)	-22.6(t)	-11.0(d)	n/a	n/a	H <sub>2</sub> O, pH 7.0	3
O	O	O	5'-O-Nuc.	-OH	-OH	-OArly	-14.0	-24.5	-19.7	n/a	n/a	DMF:Pyridine=1:1	1
O	O	O	5'-O-Nuc.	-Alkyl	-OH	-OH	25.0(d)**	-(23.5-24.0)**	-9.7(d)**	21.8	19.5	D <sub>2</sub> O	3, 5, 6
O	O	O	5'-O-Nuc.	-OH	-OH	-NHAlkyl	-11.8	-22.0	-3.6	22.5	22.5	DMSO: Pyridine=1:1	1
O	O	O	5'-O-Nuc.	-OH	-OH	-NHArly	-11.4	-23.2	-10.2	n/a	n/a	H <sub>2</sub> O, pH 11	1
S	O	O	5'-O-Nuc.	-OH	-OH	-OH	42.0-44.0**	-(21.1-22.0)**	-(4.7-9.6)**	23.0-31.8	19.3-20.5	H <sub>2</sub> O, pH 7.0	3, 4
O	S	O	5'-O-Nuc.	-OH	-OH	-OH	-11.4**	30.0**	-6.0**	6.5	27.3	H <sub>2</sub> O, pH 7.0	1
O	O	S	5'-O-Nuc.	-OH	-OH	-OH	-10.6	-22.0	35.0	19.6	29.0	H <sub>2</sub> O, pH 7.0	1

\*\*Denotes range of <sup>31</sup>P chemical shifts for two diastereomers

## H. Polyphosphate derivatives of Nucleosides



N	R <sup>1</sup>	R <sup>2</sup>	δ <sub>P<sub>1</sub></sub> , ppm	δ <sub>P<sub>n</sub></sub> , ppm	δ <sub>n+1</sub> , ppm	J <sub>1,2</sub> , Hz	J(n+1)n, Hz	Solvent	Reference
2	5'-O-Nuc.	-OH	-11.0 (d)	-22.7(d)	-10.3	19.0-20.0	19.0-20.0	H <sub>2</sub> O	3
2, 3	5'-O-Nuc.	5'-O-Nuc.	-(10.8-11.2)(d)	-22.7(d)	-(10.8-11.2)	19.0-20.0	19.0-20.0	H <sub>2</sub> O, pH 7.0	1, 3

## II. Trivalent Phosphorus Compounds

R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	δ, ppm	Solvent	Reference
3'-O-Nucleoside	-Cl	-OAlkyl	165.0-170.0	***	2
3'-or 5'-O-Nucleoside	-N(Alkyl) <sub>2</sub>	-OAlkyl	149.0-150.0**	CD <sub>3</sub> CN; CDCl <sub>3</sub>	3
-OAlkyl	-N(Alkyl) <sub>2</sub>	-N(Alkyl) <sub>2</sub>	131.0-138.0	***	2
-Cl	-Cl	-Cl	215.0-220.0	***	2
-OAlkyl	-Cl	-Cl	177.0-180.0	***	2
-OArly	-OArly	-Cl	153.0-157.0	***	2
-OArly	-Cl	-Cl	176.0-184.0	***	2
-OArly	-N(Alkyl) <sub>2</sub>	-N(Alkyl) <sub>2</sub>	131.0	***	2
-OArly	-OArly	-N(Alkyl) <sub>2</sub>	141.0	***	2
3'-or 5'-O-Nucleoside	3'-or 5'-O-Nucleoside	-OAlkyl	140.0-141.0**	CDCl <sub>3</sub>	1

\*\*Denotes range of <sup>31</sup>P chemical shifts for two diastereomers

\*\*\*See ref.2 for solvent information



## References

- A.V. Lebedev, A.I. Rezvukhin, Nucl. Acids Res., 1984, V.12, N14 5547-5566.
- M.M. Crutchfield, et. al, <sup>31</sup>P Nuclear Magnetic Resonance in Topics in Phosphorus Chemistry. New York, Intescience Publishers, 1967.
- TriLink Biotechnologies, Inc. data.
- J. Ludwig, F. Eckstein, J. Org. Chem., 1989, V.54, N3, 631-635.
- Higuchi H., Endo T., Kaji A., 1990, Biochemistry, 29, 8747-8753.
- Victorova L.S., Dyatkina N.B., Mozzherin D.F., Atkazhev A.M., Crayevsky A.A., Kukhanova M.K., Nucl. Acids Res., 1992, V.20, N4, 783-789.