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A SIMPLE ONE FLASK SYNTHESIS OF NUCLEOSIDE 5'-TRIPHOSPHATES FROM UNPROTECTED NUCLEOSIDES VIA NUCLEOSIDE 5'-CYCLOTRIPHOSPHATES

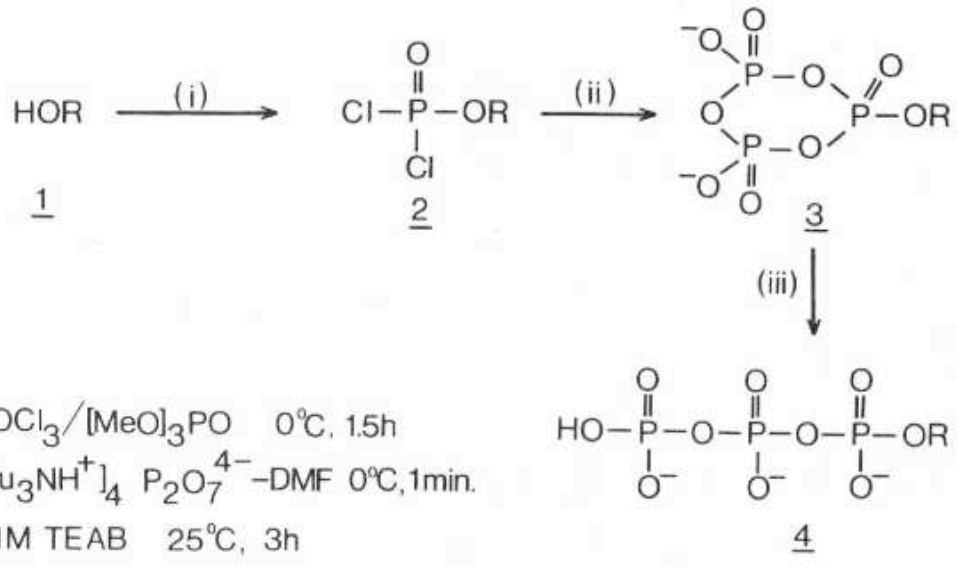
J.LUDWIG

Institute of Biophysics, Biological Research Centre
Hungarian Academy of Sciences, H-6701 Szeged, P.O.B. 521
(Hungary)

SUMMARY

Nucleoside 5'-phosphorodichloridates - resulting from the phosphorylation of unblocked nucleosides with $POCl_3$ in trimethyl phosphate - can be transformed in high yield into the corresponding nucleoside 5'-triphosphates, by a short treatment performed in situ with tri-n-butylammonium pyrophosphate in DMF followed by neutral hydrolysis. ATP,2'-dATP,3'-dATP,GTP, (E)BrVdUTP, (Z)BrVdUTP, (E)BrVUTP were synthesised using this procedure in 60-85% yield based on the starting nucleosides. The synthesis of pppA2'p5'A2'p5'A was also accomplished by the triphosphorylation of 3'-O-(o-nitrobenzyl)-adenilyl-(2'-5')-3'-O-(o-nitrobenzyl)-adenilyl-(2'-5')-3'-O-(o-nitrobenzyl)-adenosine and removal of the protecting groups by photolysis.

Phosphorylation of unprotected nucleosides with $POCl_3$ in trialkylphosphates gives predominantly nucleoside 5'-phosphorodichloridates (2)(ref.1). Compounds 2 can be transformed into nucleoside 5'-triphosphates (4) by a short treatment performed in situ with tri-n-butylammonium pyrophosphate in DMF under anhydrous conditions followed by neutral hydrolysis (refs.2-3).



1 2 3 4

(i) $POCl_3/[MeO]_3PO$ 0°C, 1.5h
(ii) $[Bu_3NH^+]_4\ P_2O_7^{4-}$–DMF 0°C, 1min.
(iii) 0.1M TEAB 25°C, 3h

Direct experimental proof for the formation of the intramolecular cyclisation product 3 has been obtained by ^{31}P NMR analysis of the reaction mixture. Based on comparison with the calculated abc spectrum the multiplet in Fig. 1 was assigned to compound 3 (ref. 4). The singlet at -19.43 ppm was assigned to inorganic cyclotriphosphate, resulting from the reaction of excess $POCl_3$ and pyrophosphate.

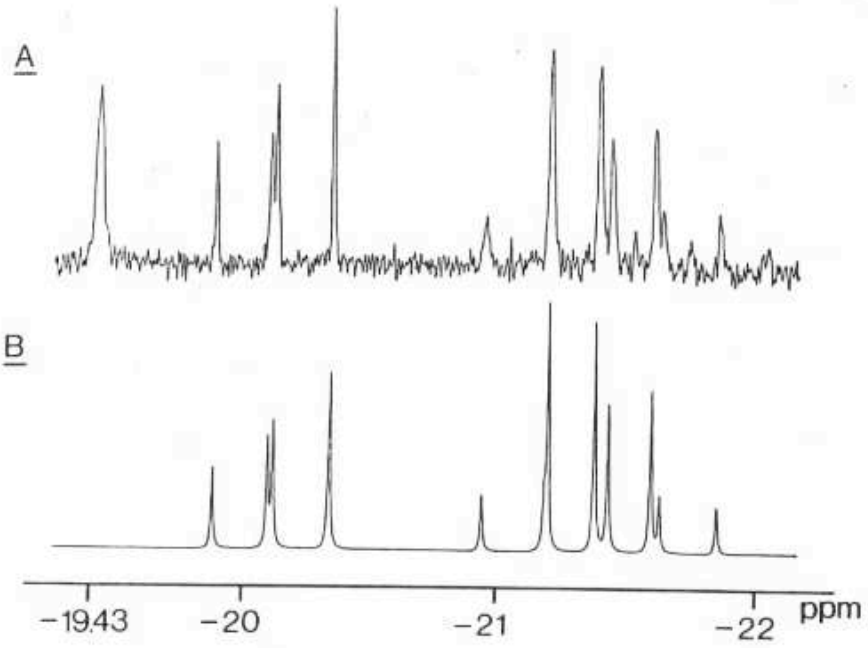


Fig. 1. A Actual ^{31}P NMR spectrum (middle phosphate region) of the reaction mixture after step (ii) (R=adenosin-5'-yl) in DMF: $(MeO)_3PO$ = 4 : 1 at 101.24 MHz
B Theoretical spectrum of compound 3
Calculated NMR parameters: δ_1 = -20.22 ppm, δ_2 = -21.31 ppm, δ_3 = -21.67 ppm; J_{12} = 23.84 Hz, J_{13} = 24.35 Hz, J_{23} = 25.90 Hz

As demonstrated by following the time course of the appearance of its reaction product with morpholine (P^3-morpholino-P^1--adenosinyl-5'-triphosphate) the formation of 3 was completed within 60 sec. 3 was stable under the reaction conditions for at least 2 days, however the presence of excess pyrophosphate resulted in its slow conversion into p_5A. Addition of aqueous buffer (step iii) (Scheme) led to the quantitative formation of ATP. (^{31}P NMR δ (D_2O) -8.53 ppm /d/, J = 20.81 Hz; -9.11 ppm /d/, J = 19.95 Hz; -20.87 ppm /dd/, J = 19.57 Hz). The remaining signals in the ^{31}P NMR spectrum of the hydrolysis mixture were assigned to pyrophosphate (δ = -8.44 ppm) /s/ and

cyclotriphosphate (δ = -19.55 ppm /s/).

In a typical experimental procedure $POCl_3$ (0.26 mmol) was pipetted into a suspension of adenosine (0.2 mmol) in dry $(MeO)_3P{=}O$ (0.5 ml) and the mixture was stirred at 0^oC for 1.5 h. A mixture of 0.5 M tetrakis-tri-n-butylammonium pyrophosphate in anhydrous DMF (0.3mmol), DMF (1.7%) and tri-n-butylamine (0.3 mmol) was added under vigorous stirring. After 1 min 0.1 M aqueous $Et_3N.H_2CO_3$, pH = 7.4 was added (10 ml) and after standing for 3 h at 0^oC, the reaction mixture was applied onto a DE-32(HCO_3^-) column. Elution was performed with a linear gradient of $Et_3N.H_2CO_3$. ATP was isolated in 85% yield based on starting adenosine. 2'dATP (yield 79%) and 3'dATP (yield 70%) were obtained using essentially the same procedure except that phosphorylation was performed at -20^oC in the case of 2'-deoxyadenosine.

For the synthesis of /Z/-5-Bromovinyl-2'-deoxyuridine--5'-triphosphate (5), /E/-5-Bromovinyl-2'-deoxyuridine-5'--triphosphate (6), /E/-5-Bromovinyl-uridine-5'-triphosphate (7) the phosphorylation of the nucleosides was performed at 0^oC for 12-15 h. $POCl_3$ has been used in 2.2 fold and pyrophosphate in 5 fold excess. Products were isolated by DE-32(HCO_3^-) ion exchange chromatography. Yields and ^{31}P NMR data are presented in Table 1.

TABLE 1
Yields and ^{31}P NMR data for compounds 5-7

Compound	Yield	^{31}P NMR (δ) ppm		
		P^{α}	P^{β}	P^{γ}
(Z)BrVdUTP (5)	64.5%	-10.46/d/ J=19.12Hz	-20.88/dd/ J=19.72Hz	-5.13/d/ J=19.79Hz
(E)BrVdUTP (6)	70%	-10.43/d/ J=19.64Hz	-20.93/dd/ J=19.77Hz	-5.23/d/ J=20.03Hz
(E)BrVUTP (7)	63%	-10.59/d/ J=20.15Hz	-21.14/dd/ J=19.76Hz	-6.07/d/ J=19.76Hz

This simple one flask method has also been employed for the

insertion of the 5'-triphosphate group into 2'-5'-oligoadenylate. A 3'-O-protected trimer has been used as starting material in order to avoid isomerisation of the interribonucleotide linkage during phosphorylation. Thus phosphorylation of 3'-O-(o-nitrobenzyl)-adenilyl-(2'-5')-3'-O-(o-nitrobenzyl)-adenilyl-(2'-5')--3'-O-(o-nitrobenzyl)-adenosine [A(NB)pA(NB)pA(NB)] with excess $POCl_3$ in $(MeO)_3P=O$ and in situ treatment of the resulting 5'-phosphorodichloridate with pyrophosphate, followed by hydrolysis gave pppA(NB)pA(NB)pA(NB) in 60% yield. [^{31}P NMR δ(dioxane:H_2O=2:1) 0.30 /s/; 0.13 /s/; -8.83 /d/, J=18.4 Hz; -9.58 /d/, J=18.9 Hz, -20.80 /dd/, J=19.2 Hz]. o-nitrobenzyl ether groups were removed by photolysis and the pppA2'p5'A2'p5'A formed was characterised by enzymatic degradations.

The use of imidodiphosphate instead of pyrophosphate in step (ii) resulted in the formation of $\beta\gamma$-imidotriphosphate derivatives. pNHppG [^{31}P NMR $\delta(D_2O)$ 1.29 /d/ (P_1), -6.64 /dd/ (P_2), -8.17 /d/ (P_3), J_{12} = 20.6 Hz, J_{23} = 5.5 Hz] was obtained in 66% isolated yield.

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