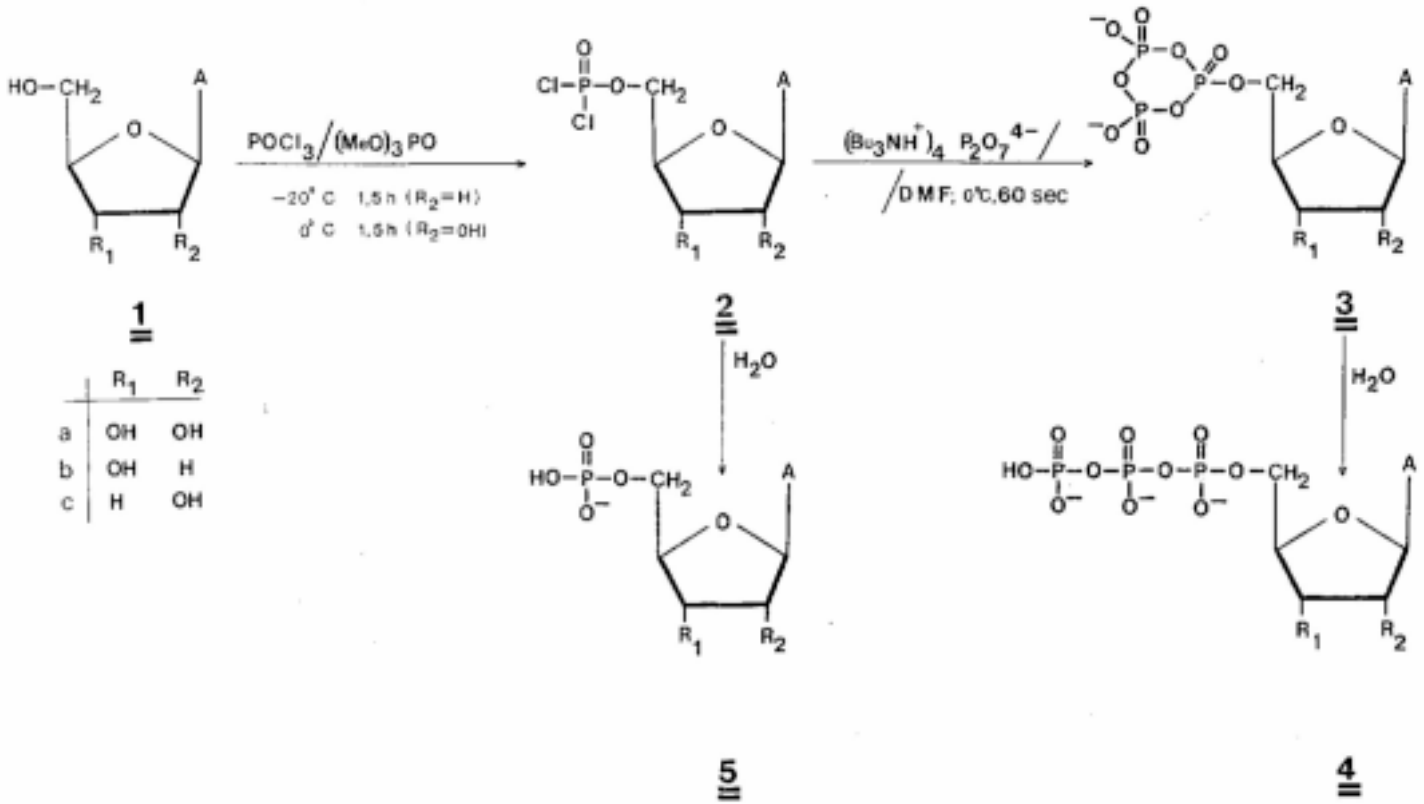
A SIMPLE ONE FLASK SYNTHESIS OF NUCLEOSIDE 5'–TRIPHOSPHATES FROM UNPROTECTED NUCLEOSIDES VIA NUCLEOSIDE 5'–CYCLOTRIPHOSPHATES

J. LUDWIG

INSTITUTE OF BIOPHYSICS, BIOLOGICAL RESEARCH CENTRE

HUNGARIAN ACADEMY OF SCIENCES, H–6701 SZEGED, HUNGARY

The Yoshikawa reaction, ie. phosphorylation of unblocked nucleosides with $POCl_3$ in trialkylphosphates gives predominantly nucleosides 5'-phosphorodichloridates (2). 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 anhydrons conditions, followed by neutral hydrolysis.



1

	R_1	R_2
a	OH	OH
b	OH	H
c	H	OH

2

3

5

4

^{31}P NMR (I), AND CHEMICAL EVIDENCE (II)

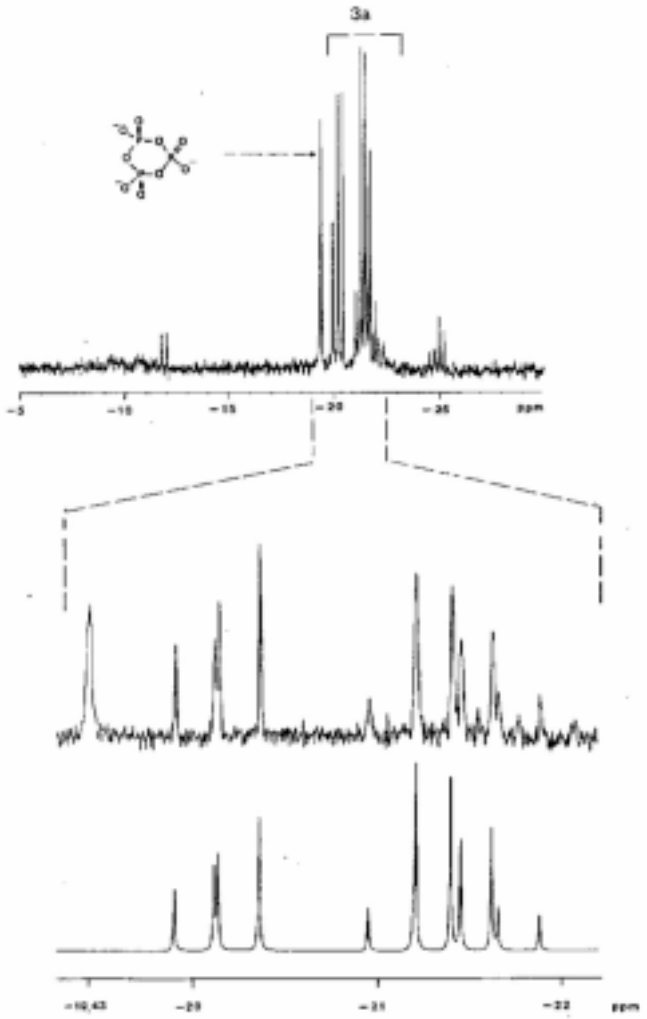
FOR THE FORMATION OF 3a

A

^{31}P NMR spectrum of the reaction mixture in DMF · $[MeO]_3PO$ = 4 · 1 , before hydrolysis

Molar ratio of starting materials:

3a · $POCl_3$ · pyrophosphate = 1 · 1.3 · 1.3



B1 Expanded middle phosphate region

B2 Calculated abc spectrum of 3a

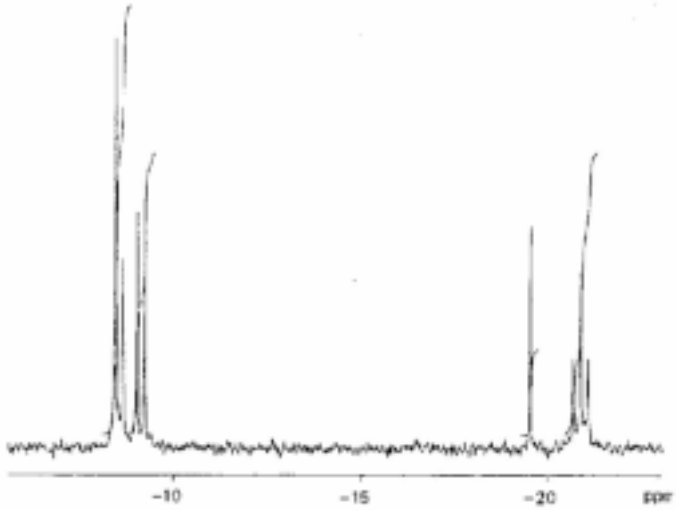
Spectral parameters: δ_1 = −20,22 ppm J_{12} = 23,84 Hz

δ_2 = −20,31 ppm J_{13} = 24,35 Hz

δ_3 = −21,67 ppm J_{23} = 25,90 Hz

c

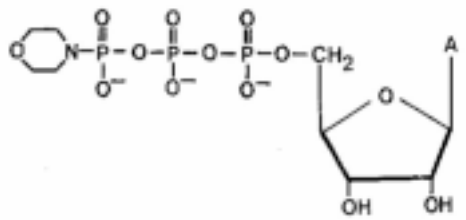
^{31}P NMR spectrum of the reaction mixture in D_2O, after hydrolysis



ATP [4a] : δ = -8.53 /d/, J = 20.81 Hz
-9.11 /d/, J = 19.95 Hz
-20.87 /dd/, J = 19.57 Hz
pyrophosphate : δ = -8.44 /s/
and cyclotriphosphate : δ = -19.55 /s/ were found in ≈ 1 : 0.2 : 0.1 molar ratio

II

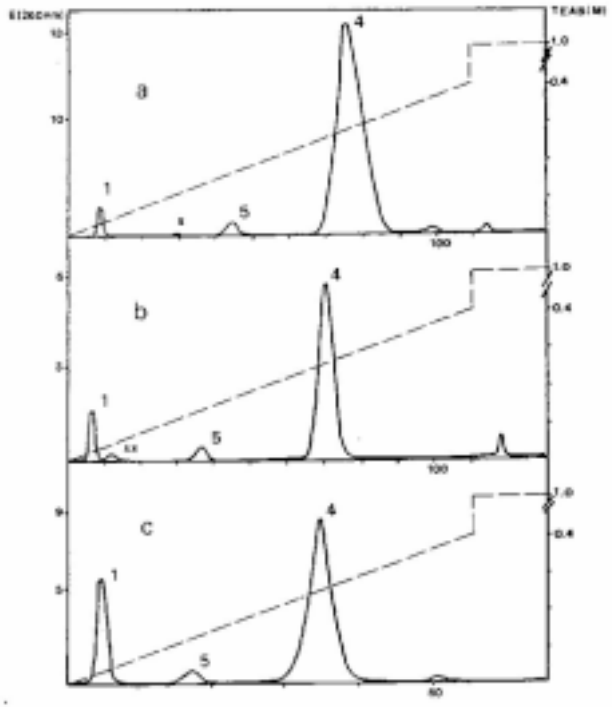
Compound 6a was formed, in the same yield as ATP upon treatment of the reaction mixture with morpholine instead of H_2O



6a

ISOLATION OF ATP (a), 2'dATP (b) 3'dATP (c) BY [DE-32 HCO_3^-]

ION EXCHANGE CHROMATOGRAPHY.

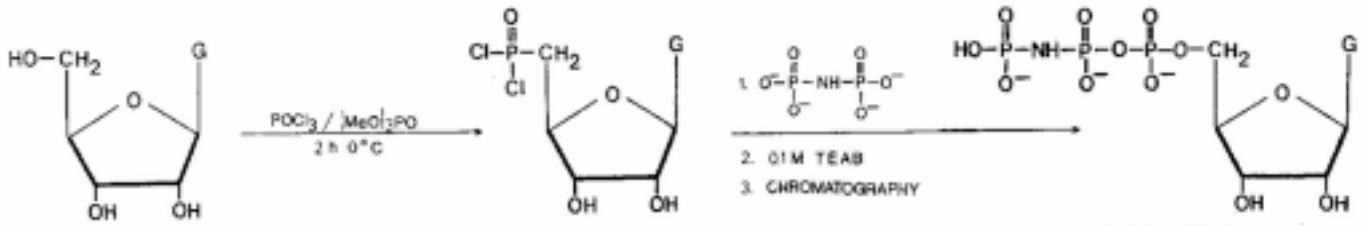


Percentage product distribution

	1	5	4	adenosine-2,3'cyclo-phosphate	adenine
a	4 %	4.5 %	86 %	0.5 %*	—
b	11 %	3 %	78 %	—	2 %**
c	19 %	4 %	70 %	—	—

SYNTHESIS OF 5'-GUANILYL IMIDODIPHOSPHATE

Due to the short reaction time (60 sec) significantly improved yields may be obtained in the synthesis of less stable nucleoside 5'-triphosphate analogues.



Molar ratio of starting materials:

Guanosine : $POCl_3$: Imidodiphosphate = 1 : 1.5 : 1.5

YIELD 66 %

^{31}P NMR δ (D_2O) ppm

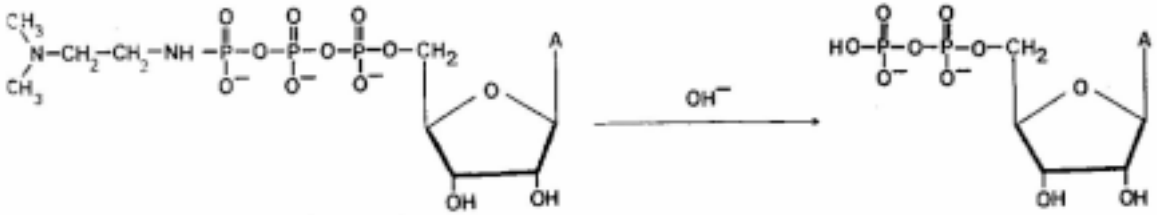
1.29 (d) (P_1)

-8.64 (dd) (P_2)

-8.17 (d) (P_3) J_{12} = 20.6 Hz

J_{23} = 5.5 Hz

SYNTHESIS OF ADP



INSERTION OF THE 5'-TERMINAL TRIPHOSPHATE

GROUP INTO 2'–5' OLIGOADENYLATE