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Biomimetic One-Pot Synthesis of Nucleotide Phosphates

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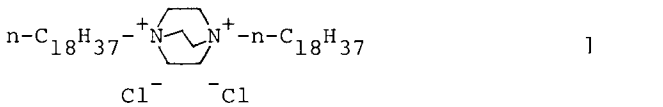
A strongly hydrophobic rigid diammonium, 1, an efficient extracting and transporting phase transfer reagent with high specificity for the pyrophosphate grouping, was used to synthesize ADP, ATP or ADP-NH_2 in a hydrophobic medium. Thus, practically pure ADP-NH_2 was obtained in 65 % yield within 2 min.

Since nucleotides are important key compounds in metabolism, respiration, biosynthesis, regulation or many other biological processes, their syntheses are of particular significance and have been attempted extensively by chemical or biological methods as summarized in Table I and II.

The authors have been investigating hydrophobic rigid diammonium ion 1 recognizing a pyrophosphate grouping specifically in a nonpolar medium in order to gain more insights into the polar recognition of nucleotides based on the simplified model system.

Until now discriminating extraction[1,2,3] and highly selective transport[3,4] of ADP or ATP were successfully carried out by the use of this "pyrophosphatophile."



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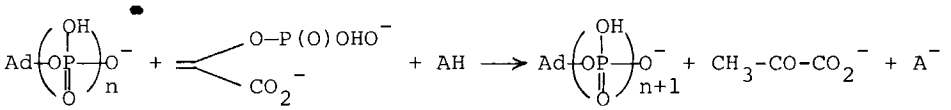
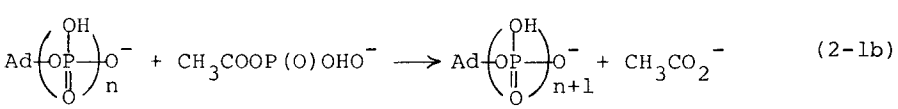
These successes have driven us to utilize 1 as a catalyst for the *biomimetic synthesis* of ADP or ATP starting from AMP (eqn 2-2), mimicking the corresponding biosyntheses (2-1). Since an anion is remarkably activated by a phase transfer reagent in a hydrophobic environment, the

biosynthesis:

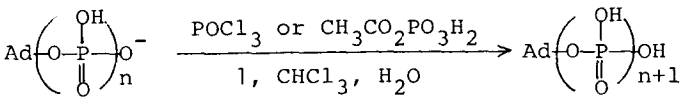


(2-1a)



(2-1b)

biomimetic synthesis:



(2-2)

Table I. Typical Examples of Nucleotide synthesis by Chemical Methods. (AS-S-M; adenosine-5-p-morphoridate)

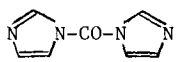
reactant	condition	product	ref
AMP	H_3PO_4, DCC, $(n\text{-BU})_3N$, pyridine, 20°, 48 h	ATP + ADP	5
AS-5-M	$\{(n\text{-BU})_3NH^+\}_2PO_4^=$, pyridine, r.T., 2-48 h	ADP + ATP	6
AMP	Bu_2PSBr, $H_2PO_4^-(n\text{-Bu})_3NH^+$, AgOAc, pyridine, r.T., 2 h	ADP	7
AMP	BU_2PSBr, AgOAc, $HPO_3OPO_3H^=\{(n\text{-Bu})_3NH^+\}_2$, r.T., 2 h	ATP	7
ADP	 H_3PO_4, DMF, r.T., 36 h	ATP	8
AMP	$(PhO)_2POCl$, $(n\text{-BU})_3N$, $HPO_4^=\{(n\text{-Bu})_3NH^+\}_2$	ADP	9

Table II. Typical Examples of Nucleotide Synthesis by Biochemical Methods.

reactant	condition	product	ref
ADP	carbamylphospho kinase, KCNO, NaH_2PO_4, Mg^{2+}	ATP	10
ADP	acetate kinase, acetyl phosphate	ATP	11
ADP	pyruvate kinase, phosphoenol puruvate	ATP	12
ADP	Ca^{2+}(or Na^+,K^+) dependent ATPase, H_3PO_4, Mg^{2+},Ca^{2+}(or Na^+,K^+)	ATP	13
ADP	ATPase(chloroplast) ΔpH 2 ∿ ΔpH 4, H_3PO_4, Mg^{2+},K^+	ATP	14

biomimetic synthesis (eqn 2-2) were attempted in chloroform in the presence of 1 in expectation that 1 may act as a highly discriminating phase transfer reagent.

Thus, 200 ml of the aqueous solution of AMP (typical concentration, 1.1×10^{-4}M) adjusted to pH 8 was gently stirred with 200 ml of the chloroform solution of $1 \cdot 2Cl^-$ and the organic phase was separated. Into 10 ml of this organic phase was added a large excess amount of $POCl_3$ and K_2CO_3 or other suitable base ($NaHCO_3$, Cs_2CO_3 or urea) with external cooling. After appropriate reaction time, the organic phase was treated with 10 ml of the aqueous solution of $NaClO_4$ (1.0×10^{-2}M) at pH 5 - 6. Then the aqueous solution was analyzed for AMP, ADP and ATP by means of high-pressure liquid chromatography (Bonda Pack AX/corasil). The results were summarized in Table III.

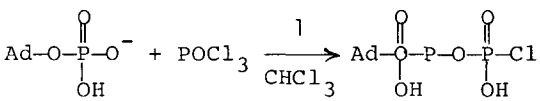
Table III. Biominetic syntheses of nucleotides using $POCl_3$ in chloroform in the presence of "pyrophosphatophile" 1[a].

base	AMP(% after 1 h)	ADP(% after 1h)	ATP(% after 1h)
K_2CO_3	42 ± 8	23 ± 2	12 ± 2
Cs_2CO_3	52 ± 2	20 ± 1	11 ± 6
urea [b]	49	21	10

a. average value of several experiments b. single experiment

Under mild reaction conditions, ADP was obtained as a major product together with unreacted AMP. But under more drastic conditions, further phosphorylation took place to give a considerable amount of ATP, as well as ADP. The presence of a suitable base is essential for the successful phosphorylation. Vigorous agitation did not affect the yield of phosphorylation product appreciably.

It is concluded, from these experiments, that the AMP anion extracted by 1[1,2] from the aqueous solution to the chloroform solution seems to be considerably activated by desolvation, being satisfactorily reactive toward $POCl_3$ to form the corresponding mixed anhydride chloride, 4, which then is readily converted to ADP (and further to ATP) by a small amount of water present in the system. Successful phosphorylation of AMP was also observed with acetyl phosphate.



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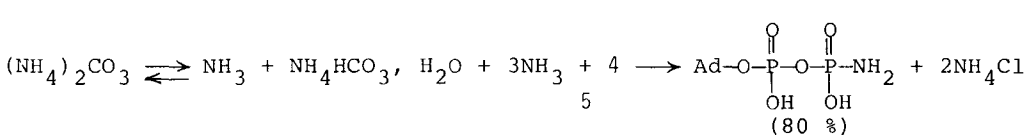
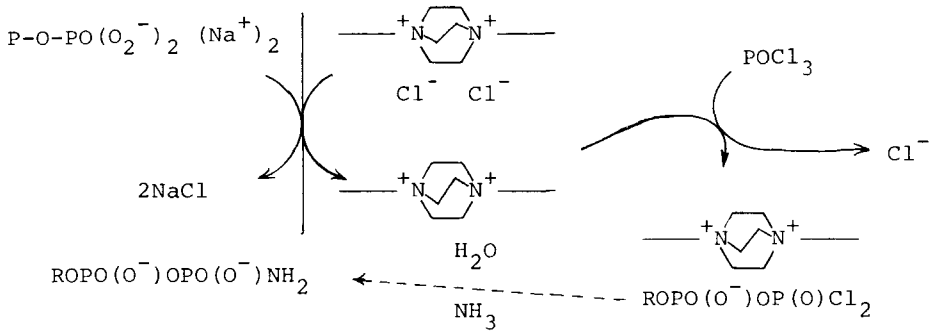
Usual phase transfer reagents were much less effective for the phosphorylation than 1.

Hydrolysis of 4 competes with hydrolytic cleavage of ATP or ADP to ADP or AMP, giving ATP or ADP in a steady state quantity. Thus, ammonium carbonate was used as a buffering base as well as an amination reagent to collapse 4 effectively. Very rapid formation of the product (within 2 min) in a good yield (ca 65% based on AMP used and more than 80% based on AMP consumed) was observed, which was determined as the corresponding phosphoramide, 5, based on the HPLC analysis and ^{31}P-NMR compared with authentic samples.[15] ^{31}P-NMR; ($NaOD-D_2O$), doublets at 0.7 and 10.2 ppm (J, 20.3 Hz).



Thus, present direct phosphorylation of AMP phosphate or pyrophosphate by use of "pyrophosphatophile" 1 is useful for the synthesis of variety of derivatives of ADP and ATP, just shown in the case of ADP-amide 5. The present synthesis has another great advantage of

getting products in very pure states compared with previous procedures (eg., after usual work and before careful purification, the present procedure gave ADP-NH_2 > 95% purity but the reported DCC procedure[1a] only gave ADP-NH_2 in ca 50% purity based on ^{31}P NMR and HPLC). The detailed results will be reported in a full-length article.



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