

Magnesium pyrophosphates in enzyme mimics of nucleotide synthases and kinases and in their prebiotic chemistry

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Derivatives of ribosyl pyrophosphate have been synthesized, and examined with magnesium salts in the coupling of the ribose unit to various nucleophiles, including pyrazole and 2-chloroimidazole. Only with the magnesium salt present did they generate the ribosyl cation by binding to the leaving group and then couple the ribose derivative with nucleophiles. The role of magnesium salts in phosphorylation of methanol by ATP was also examined. Here a remarkable effect was seen: phosphorylation by ATP was slowed with low concentrations of Mg²⁺ but accelerated by higher concentrations. Related effects were also seen in the effect of Mg²⁺ on phosphorylation by ADP. The likely mechanisms explain these effects.

ATP | ADP | phosphoribosyl pyrophosphate | nucleosides

Enzyme mimics, lacking protein catalysts, can afford insight into biochemical mechanisms because frequently the essential chemical paths are similar, with the enzyme protein furnishing greater velocity and selectivity. A good example from our own work includes the mechanisms used when thiamine pyrophosphate is the coenzyme; thiamine itself can perform the essential catalysis, but more slowly and less selectively than with the enzyme (1, 2). As another example, our mimics of ribonuclease use the imidazole groups that are biochemically part of the enzyme itself to catalyze cleavage of RNA (3, 4). We here describe mimics of the enzymes that attach base groups to a ribose unit. We also describe mimics of kinases that transfer a phosphate group from ATP.

The synthesis of nucleotides in modern biochemistry involves 5-phosphoribosyl pyrophosphate, formed by direct transfer of the pyrophosphoryl group from ATP. This attaches to a nitrogenous base to generate a new carbon nitrogen bond by the displacement of the pyrophosphate group with assistance by Mg²⁺ (Fig. 1). For purine nucleotides the nucleophile is an ammonia molecule that is generated by cleaving an amide. This generates ribosylamine-5-phosphate, and the rest of the base is constructed around this amino group. For the synthesis of pyrimidine nucleotides the nucleophile is orotic acid, which later undergoes decarboxylation and other steps to generate the modern nucleotides. It seemed likely that binding to magnesium ions activates the departure of the pyrophosphate leaving group. Simple binding of pyrophosphate ions to Mg²⁺ is well studied (5).

Similarly a pyrophosphate group (ADP) is the leaving group in ATP phosphorylations by ATP in kinase reactions (6). Some studies have been reported on mimics for this process, and it seemed likely that in this case also the departure of the ADP leaving group would be assisted by binding to magnesium ions (7). However, we now find that a more complex mechanism is involved in the mimic, and thus likely in the prebiotic world and in the modern enzymatic process itself. Of course the exact structures now used in biochemistry may almost certainly have evolved from much simpler structures on prebiotic earth, but the same overall general chemistry is likely.

The prebiotic synthesis of nucleotides can involve the direct reaction of nucleobases with ribose, but in quite low yield (8). Another proposed route involves an arabinose amino-oxazoline intermediate that later inverts to ribonucleotide (9). Because the phosphate esters and anhydrides dominate the living world (10), and currently nucleotides are biosynthetically synthesized from phosphoribosyl pyrophosphate, we believe that nucleotides could have formed prebiotically by the reaction of ribose 1-pyrophosphate (RPP) or possibly the 5-phosphate derivative with the corresponding nucleobases in the presence of Mg²⁺ ions. D-Ribose can be available in the prebiotic world, and there are many credible ways in which phosphorylations could occur, with three phosphorylations leading to 5-phosphoribosyl pyrophosphate. We have examined such a nucleotide synthesis catalyzed by Mg²⁺. This is similar to the current biosynthetic pathways, but without the enzymes.

We synthesized ribose with protecting groups on the 2-, 3-, and 5-hydroxyl groups, so only the hydroxyl group on position 1 was available, and then synthesized protected ribose 1-phosphate (RP) and protected RPP as model compounds. To our knowledge, there were no previous reports of the synthesis of RPP derivatives nonenzymatically. For simple analogs we synthesized 2,3,5-tribenzoylribose 1-pyrophosphate and 2,3,5-Trisphenylpropylribose 1-pyrophosphate.

Synthesis of 2,3,5-Tribenzoylribose 1-Phosphate 5

1-O-Acetyl-2,3,5-Tris-O-benzoyl-β-D-ribofuranose **1**, on treatment with HBr-AcOH (11), afforded the corresponding ribosylbromide **2**, which on reaction with dibenzyl phosphate **3** formed 2,3,5-trisbenzoylribose dibenzylphosphate **4** (12). Compound **4** on reduction with Pd-C afforded the title compound **5** (Fig. 2).

Significance

Nucleotides are biosynthesized from a ribose pyrophosphate species, with enzymes and magnesium ions as catalysts. Magnesium ions alone can perform this process with synthetic ribose pyrophosphates, by binding to the pyrophosphate groups and linking cyclic nitrogen bases to the ribose. In a kinase mimic ATP transfers the terminal phosphate to receptors catalyzed by magnesium ions, binding to pyrophosphate groups, but magnesium ion at low concentrations inhibits the process, and only at higher concentrations is it a catalyst. This affords great insight into the mechanism of ATP phosphorylation, a fundamental biological process. Our enzyme mimics could well operate on prebiotic earth before enzymes were present. This work strengthens the picture of how important components of life could have been formed prebiotically.

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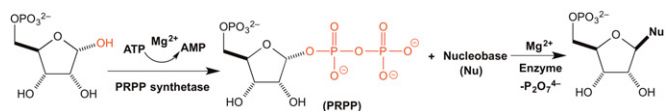


Fig. 1. Simple pathway for nucleotide synthesis. In modern biochemistry the immediately bound nucleophile is further modified.

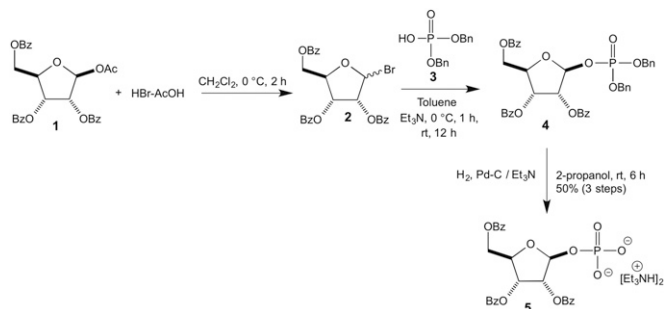


Fig. 2. Synthesis of 2,3,5-tribenzoylribose 1-phosphate 5.

Dibenzylphosphonate **6**, on reaction with *N*-chlorosuccinimide, afforded dibenzylphosphoryl chloride **7** (13), which on reaction with previously synthesized ribosyl phosphate **5** afforded the corresponding ribosyl dibenzylpyrophosphate derivative **8**. Compound **8** on reduction with Pd-C and triethylamine afforded the title compound **9** (Fig. 3).

We examined the reaction of RP **5** and RPP **9** with and without magnesium ions in methanol (used as solvent, Fig. 4). We found that 1-methylribose **10** was formed from ribosyl pyrophosphate with MgCl₂ in almost 50% yield in 48 h at room temperature with no significant increase after longer times. No methylribose was obtained in the absence of magnesium ions (Table 1, entry 3 and 4). Under our conditions no methylribose was obtained with RP both with and without magnesium ions (Table 1, entry 1 and 2).

We also examined the formation of a C–N bond of a cyclic nitrogen compound to the ribosyl pyrophosphate, but because the benzoyl protecting groups reacted in the model study we synthesized 2,3,5-Tris-phenylpropyl substituted RPP. Accordingly *D*-ribose **11**, on treatment with methanol and catalytic conc. sulfuric acid furnished the methyl ribofuranoside **12** (14), which on treatment with sodium hydride and phenylpropyl bromide **13** furnished 2,3,5-trisprotected methylribofuranoside, **14** (15). Compound **14** on treatment with HBr-AcOH (16) gave the corresponding ribosyl-bromide **15**, which on reaction with dibenzyl phosphate **3** gave ribose-dibenzylphosphate **16**. Compound **16** on reduction with Pd-C gave protected RP **17**, which on reaction with dibenzylphosphoryl chloride **7** yielded the corresponding ribosyl dibenzylpyrophosphate derivative **18**. Compound **18** on reduction with Pd-C produced the title compound **19** (Fig. 5).

We found that compound **19** (1 equiv, 0.05 M), MgI₂ (2 equiv, 0.1 M) in methanol at room temperature for 48 h afforded 78% yield of methyl ribofuranoside **14** in the presence of MgI₂ as additive, whereas there was no reaction in the absence of MgI₂ (used also with other nucleophiles as more soluble than MgCl₂).

We examined the reaction with two amine nucleophiles, pyrazole and 2-chloroimidazole, in dimethylformamide, the best solvent for all of the reactants (Fig. 6). The reaction of pyrazole (2 equiv, 0.2 M) and magnesium iodide (4 equiv, 0.4 M) with ribosyl pyrophosphate **19** (1 equiv, 0.1 M) at 60 °C for 40 h gave a 30% yield of ribose-pyrazole derivative **20**. The reaction gave no desired product in the absence of magnesium iodide. Similarly, the reaction of 2-chloroimidazole (8 equiv, 0.8 M) and magnesium iodide (4 equiv, 0.4 M) with ribosyl pyrophosphate **19**

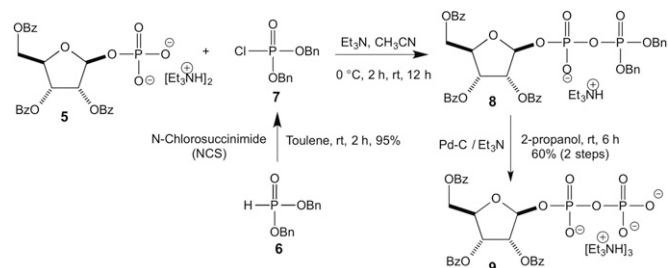


Fig. 3. Synthesis of 2,3,5-tribenzoylribose 1-pyrophosphate 9.

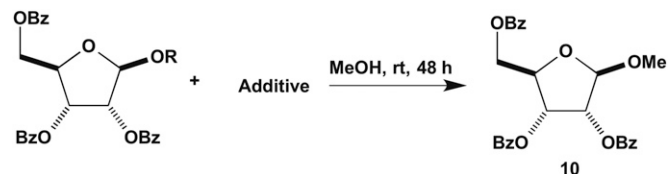


Fig. 4. Reaction of RP and RPP with methanol.

(1 equiv, 0.1 M) at 60 °C for 40 h afforded 48% yield of the ribose-imidazole derivative **21**. Only a trace of compound **21** was obtained in the absence of magnesium iodide (Table 2). We found that the relatively weak base pyrazole and 2-chloroimidazole added to the ribosyl unit but the more basic imidazole was not incorporated, nor was simple ammonia. Apparently the basic imidazole or ammonia binds to the magnesium ions, blocking catalysis, and with an excess of magnesium the imidazole or ammonia itself is bound and thus not a nucleophile.

We also examined the possible role of Mg²⁺ in phosphorylations by ATP (adenosine triphosphate) **21** (Fig. 7). We performed the reaction in 50% vol/vol of water/methanol (where MgCl₂ is quite soluble) and compared the yields of methyl phosphate with various concentrations of added MgCl₂ under the same conditions. The results are listed in Table 3; the yield as a function of time is shown in Table 4 and plotted in Fig. 8. The findings were at first sight remarkable. Under our standard conditions the presence of 10 equivalents of MgCl₂ (0.25 M) led to a decrease in the product formed, so Mg²⁺ was an inhibitor. With higher concentrations of Mg²⁺ there was an increase in product formed, so here the Mg²⁺ furnished positive catalysis. These results are not unexpected, considering the likely roles the magnesium ions play.

In ATP **22** phosphate #3, the terminal phosphate, is transferred as phosphate #1 and #2 are pyrophosphate leaving groups, and this should be accelerated by Mg²⁺ binding of pyrophosphates. However, the first Mg²⁺ would surely preferentially bind to the pyrophosphate unit of phosphates #2 and #3. Phosphate #3 will bind more strongly because it is a dianion, the others are monoanions, and the result is that transfer of phosphate #3 is inhibited because it will require breaking up the magnesium-linked #2–#3 pyrophosphate group. Thus, the

Table 1. Reaction of RP and RPP in methanol with and without MgCl₂

Entry	Starting material	Additive	Yield, %
1	(OBz) ₃ RP (5)	No additive	No reaction
2	(OBz) ₃ RP (5)	MgCl ₂	Trace
3	(OBz) ₃ RPP (9)	No additive	No reaction
4	(OBz) ₃ RPP (9)	MgCl ₂	50

Compound **5** or **9** (1 equiv, 0.05 M) and MgCl₂ (2 equiv, 0.1 M) for entry 2 and 4.

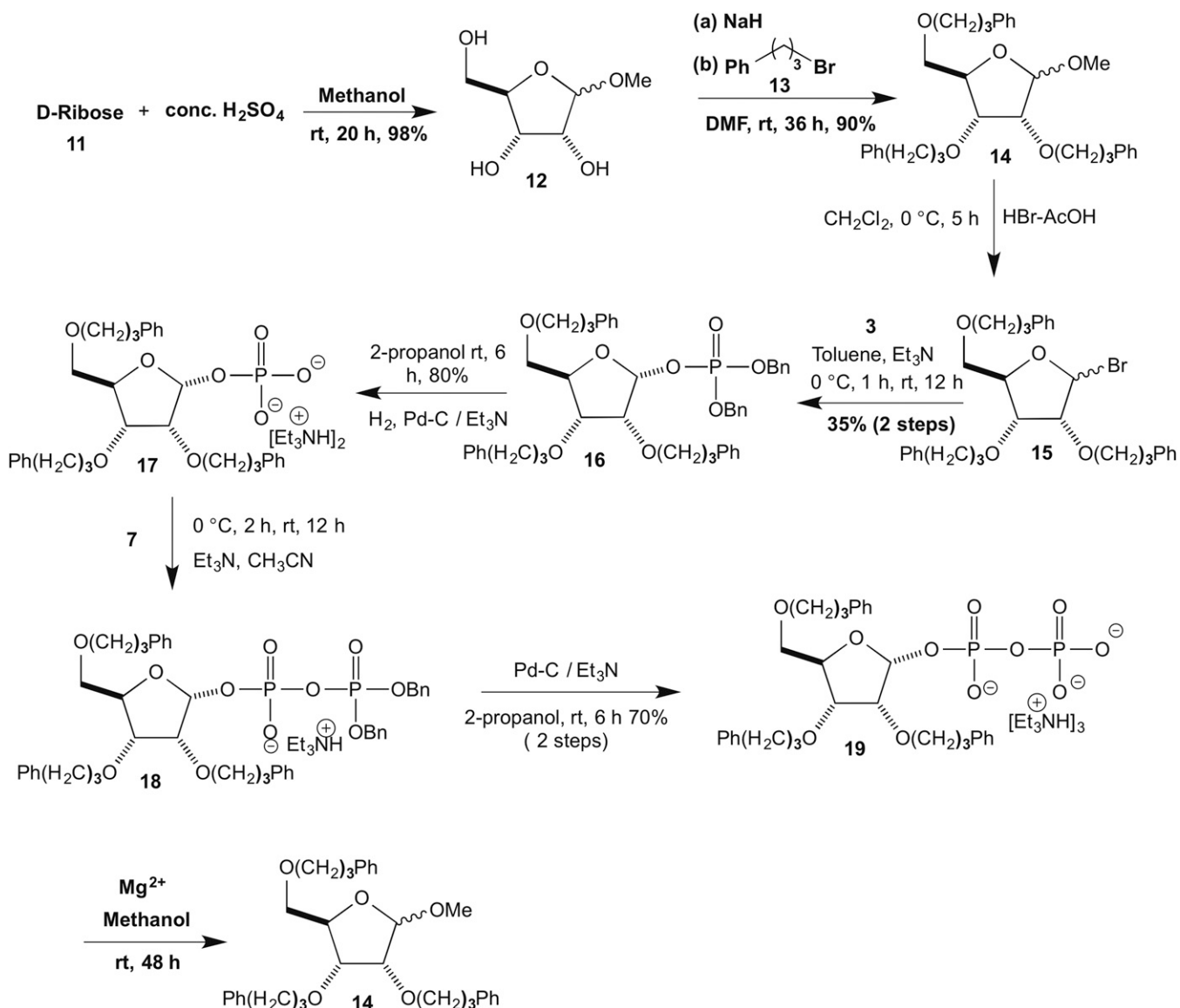


Fig. 5. Synthesis of phenylpropyl substituted RPP **19** and its reaction with methanol using Mg^{2+} catalyst.

phosphate transfer is slowed. However, with increasing Mg^{2+} concentrations a second magnesium can then bind in the #1–#2 position, furnishing the driving force anticipated when a magnesium-bound pyrophosphate is the leaving group. At some point this can apparently be enough to overcome the inhibition effect from the binding at the first #2–#3 pyrophosphate site.

To add to this picture, we examined phosphorylation of methanol by ADP, under the same conditions. As Table 5 shows, ADP is a phosphorylating agent even without added Mg^{2+} , and has this role to some extent in modern enzyme-catalyzed biology, but it is poorer than ATP (Table 3). Addition of Mg^{2+} has a significantly smaller effect, and here there is also a decrease in yield at low concentration when the chelate is present and then an increase

at higher concentrations. Its affinity for the first magnesium ion should be less than for ATP, and at high concentrations a second Mg^{2+} is bound. It does not have a pyrophosphate leaving group, but breaking up a chelated magnesium pyrophosphate with added magnesium ion could activate phosphorylation simply by partially neutralizing the negative charges that make the phosphorus less reactive.

Table 2. Reaction of RPP **19** with pyrazole and with 2-chloroimidazole nucleophiles in the presence and absence of magnesium iodide

Entry	Nucleophiles	Additive	Product	Yield, %
1*	Pyrazole	No additive	20	—
2*	Pyrazole	MgI_2	20	30
3 [†]	2-Chloroimidazole	No additive	21	Trace
4 [†]	2-Chloroimidazole	MgI_2	21	48

*Compound **19** (0.1 M), MgI_2 (0.4 M), pyrazole (0.2 M), DMF (solvent).

[†]Compound **19** (0.1 M), MgI_2 (0.4 M), 2-chloroimidazole (0.8 M), DMF (solvent).

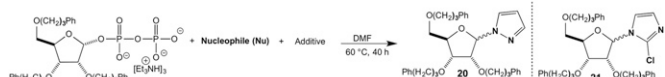


Fig. 6. Reaction of RPP **19** with pyrazole and with 2-chloroimidazole.

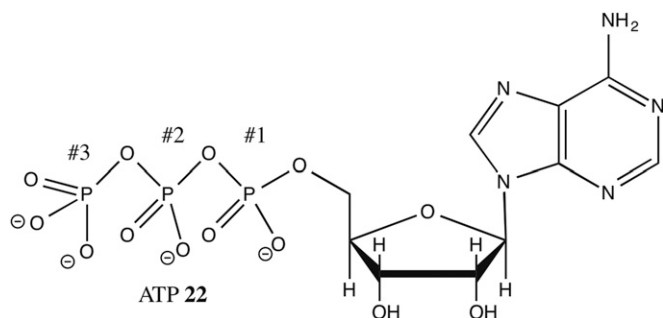


Fig. 7. Structure of ATP with the phosphate residues numbered.

Table 3. Phosphorylation of methanol with ATP

Entry	Phosphorylating agent	Concentration of Mg ²⁺ , M	Yield, %*
1	ATP.2Na	No MgCl ₂	36 ± 1
2	ATP.2Na	0.25	23 ± 1
3	ATP.2Na	1.0	32 ± 1
4	ATP.2Na	2.0	50 ± 0.5

ATP (0.025 M), MgCl₂, H₂O:MeOH (1:1), 60 °C, 48 h.
*NMR yield.

Table 4. Rate of methanol phosphorylation with ATP

Entry	Time, h	Yield, %*
1	12	8
2	24	15.6
3	36	23.7
4	48	30
5	60	36.2

ATP (0.025 M), MgCl₂ (1 M), H₂O:MeOH (1:1), 60 °C.
*NMR yield.

Conclusion

Here we see the other aspect of magnesium cation binding, activating a phosphate group by simple charge neutralization. This effect could also play a role with ATP, but we think binding the 1–2 pyrophosphate leaving group, as we invoked for ATP, is the more likely explanation there.

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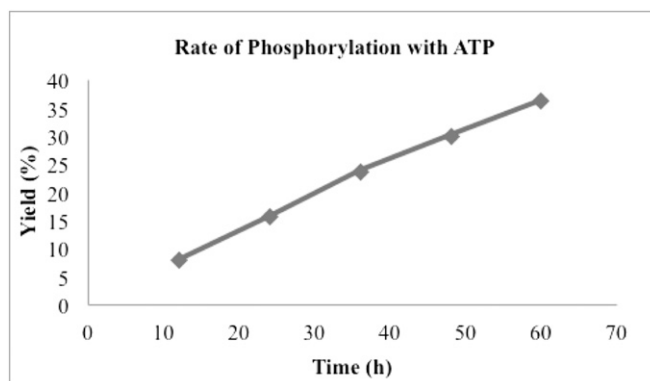


Fig. 8. Rate of methanol phosphorylation at 1.0 M MgCl₂ concentration with ATP (0.025 M) at 60 °C.

Table 5. Phosphorylation of methanol with ADP

Entry	Phosphorylating agent	Concentration of Mg ²⁺ , M	Yield, %*
1	ADP.2Na	No MgCl ₂	10.5 ± 0.5
2	ADP.2Na	0.25	8.5 ± 0.5
3	ADP.2Na	1.0	12.3 ± 0.2
4	ADP.2Na	2.0	16.8 ± 0.2

ADP (0.025 M), MgCl₂, H₂O:MeOH (1:1), 60 °C, 48 h.
*NMR yield.

How could enzymes play roles in these processes to accelerate them? In both the systems binding of the substrates would of course help, but with ATP we propose an extra effect. The reason that the first magnesium ion is an inhibitor is that it should spontaneously go to the 2–3 pyrophosphate group, but an enzyme could bind it selectively to favor the magnesium 1–2 pyrophosphate structure. There the binding of the pyrophosphate leaving group would be fully effective, as in our ribosyl pyrophosphate systems. Thus, we propose that our results could be clues to prebiotic chemistry where enzymes did not yet exist, and they give insights into the modern enzyme systems for which they are simple mimics.

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