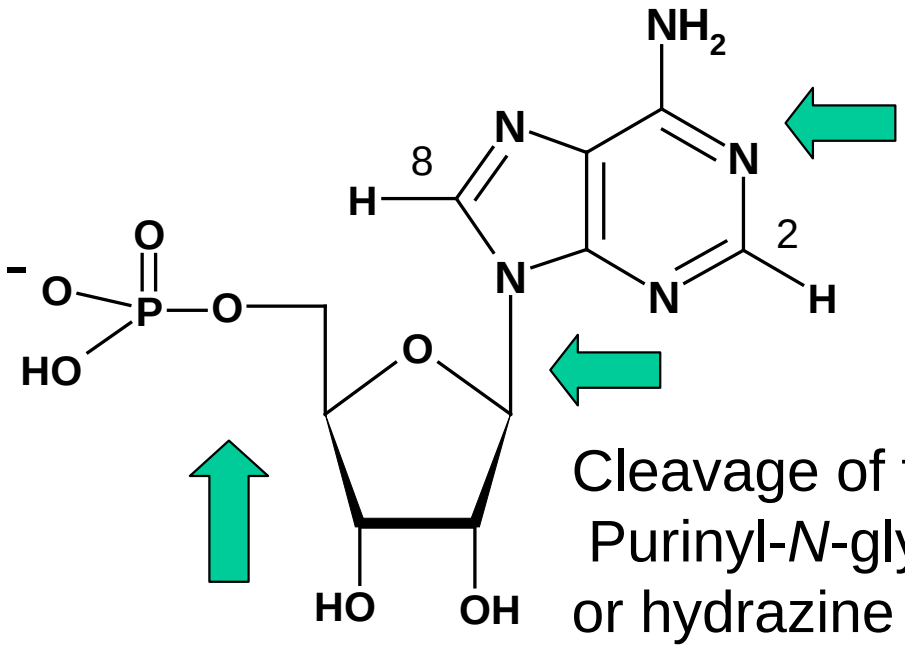
4. Chemical stability of nucleic acids



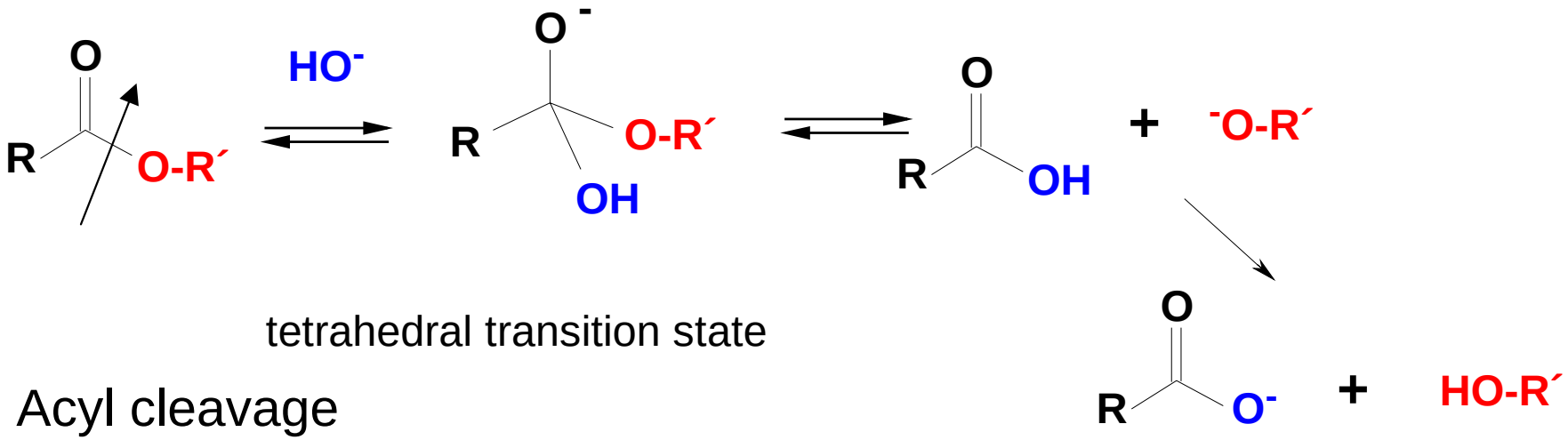
Heterocycle: stable
except for reactions with alkylating agents, nitrite and radicals

Cleavage of the *N*-glycosidic bond
Purinyl-*N*-glycosides are cleaved by acid or hydrazine treatment

Cleavage of the phosphodiester linkage:
RNA: hydrolyzed by 0.3 M KOH
DNA: stable

Chemical hydrolysis of phosphoesters

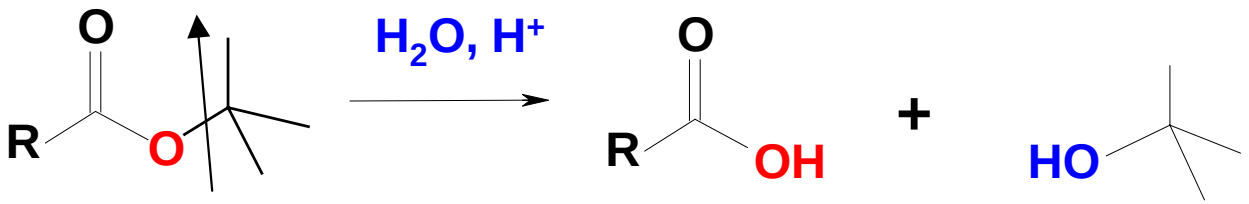
Comparison: Ester hydrolysis of carboxylic acid



tetrahedral transition state

Acyl cleavage

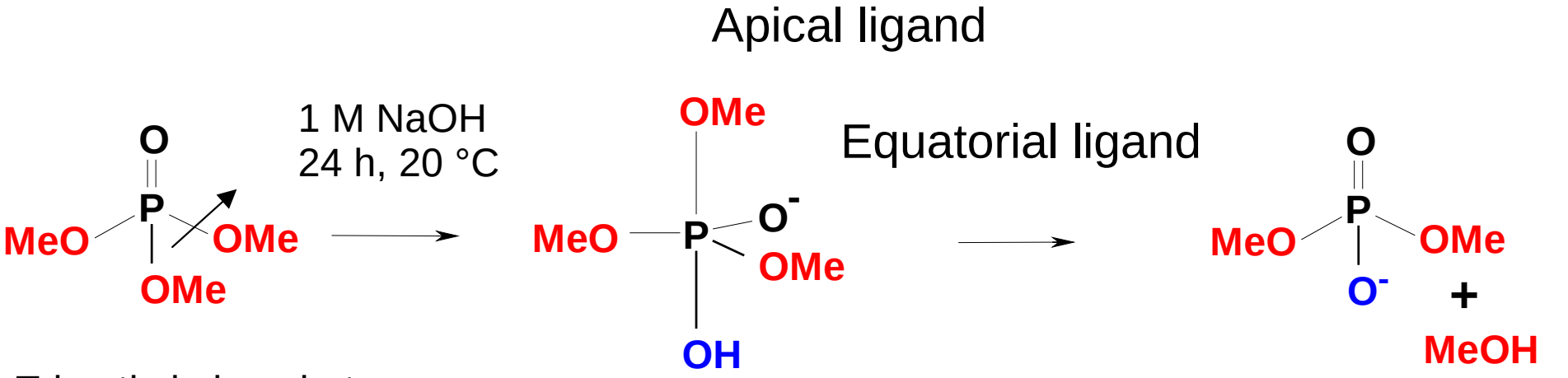
Proof: Reaction in ^{18}O labeled water



Alkyl cleavage in specific cases only

Chemical hydrolysis of phosphoesters

1.Phosphotriester



Trimethyl phosphate

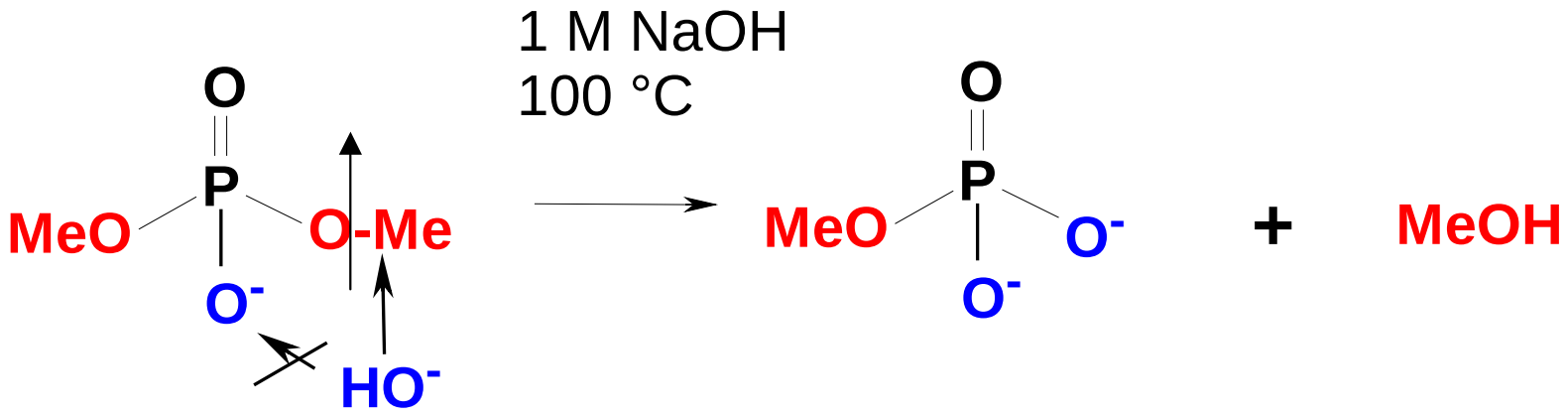
Dimethyl phosphate

Transition state: dsp^3-hybrid!

Phosphoryl cleavage

Chemical hydrolysis of phosphoesters

2.Phosphodiester



Dimethyl phosphate

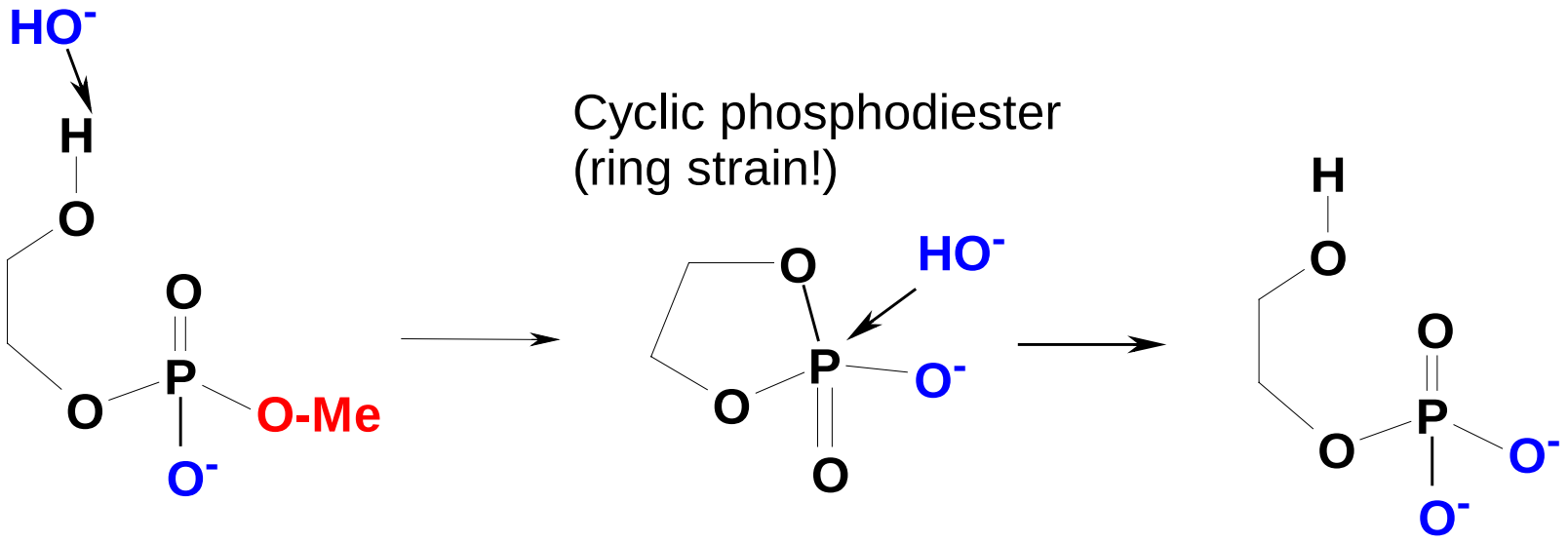
Methyl phosphate

Alkyl cleavage

Half life time: 16 days!

Chemical hydrolysis of phosphoesters

2.Phosphodiester



2-Hydroxyethyl-methyl-phosphate

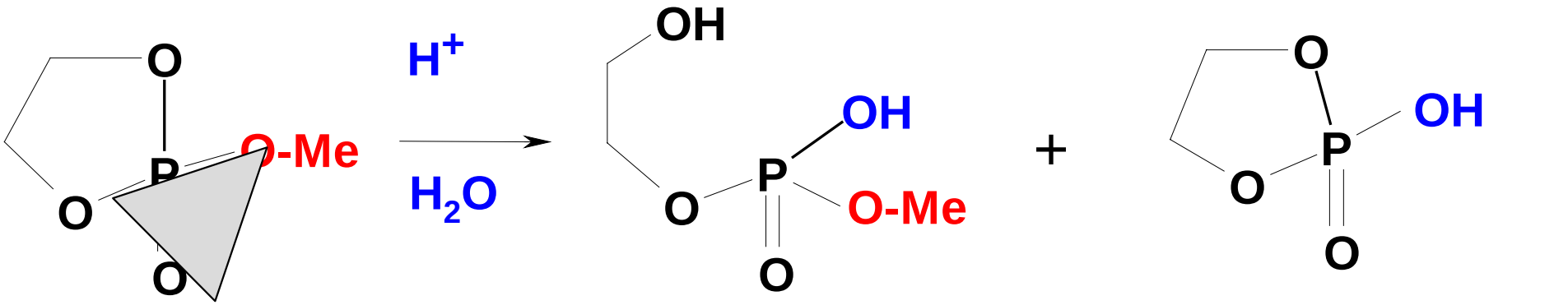
2-Hydroxyethyl phosphate

Δ H = -26.8 kJ/mol

Cf: Dimethyl phosphate: -7.5 kJ/ mol

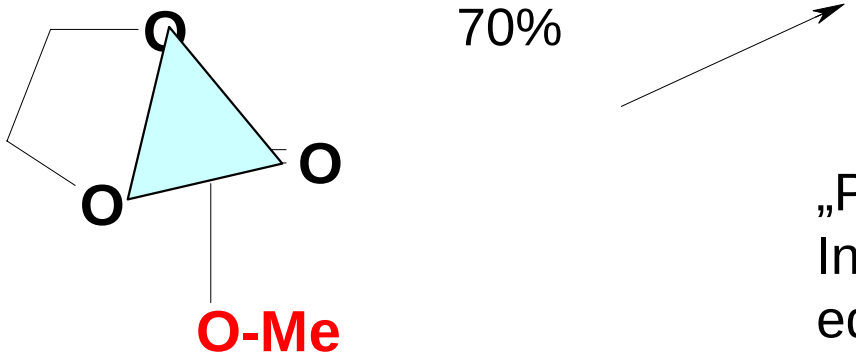
Half life time at 25 °C 25 min!

Neighboring group participation
Anchimeric assistance



2-Hydroxyethyl-methyl phosphate

Ethylene-phosphate

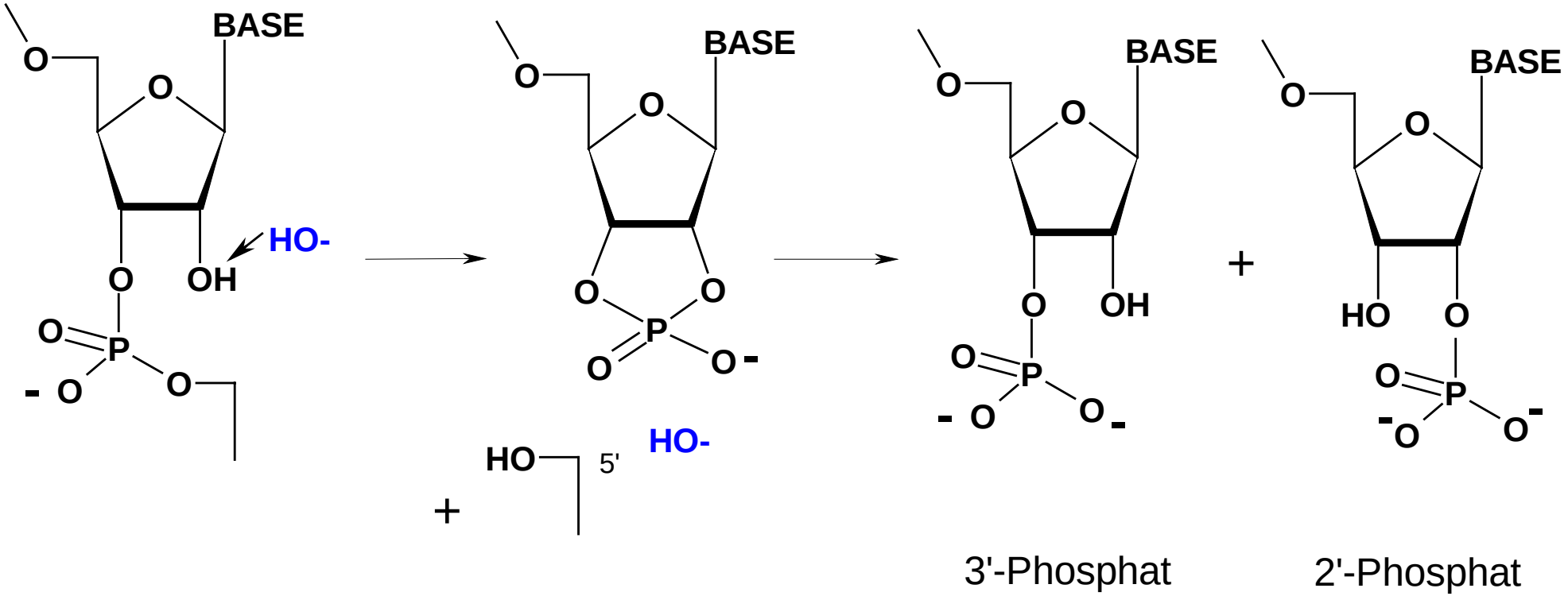


„Pseudorotation“:
Interchange of apical and equatorial ligands

Leaving group may only be displaced from an apical position (longest bond)

Alkaline hydrolysis of DNA and RNA

DNA: stable (1 h at 100 °C, 1 M NaOH
RNA: hydrolyzed at RT in 0.1 M NaOH



Enzymatic hydrolysis of RNA

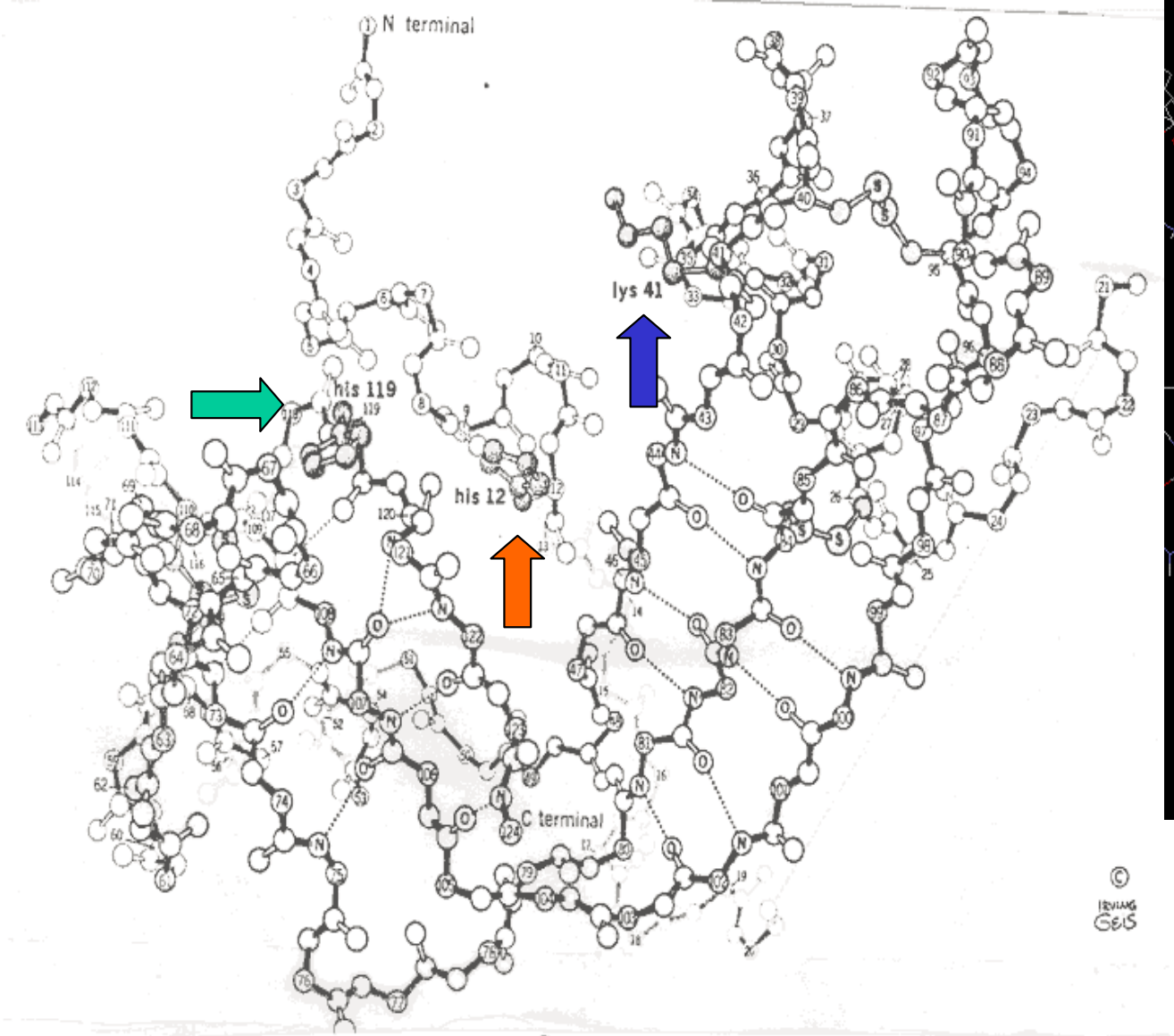
Ribonucleases

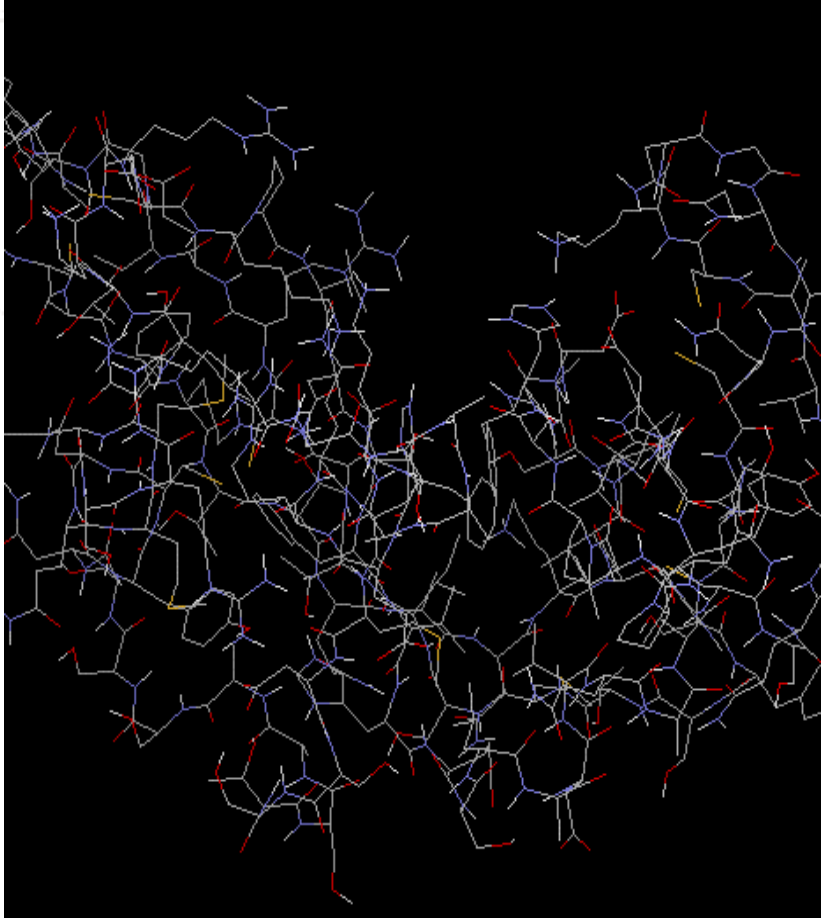
Non-specific cleavage or specific cleavage of bases
Ribonuclease A: Pyrimidin-Nucleotides (C,U)
Ribonuclease T_1 (*Aspergillus oryzae):* Guanine (in *syn*-conformation)

Ribonuclease P (Ribozymes)

Ribonuklease A

Isolation from bovine pancreas
Many crystal structures (124 entries, *e.g.* pdb-codes: 1AFK, 1H1H, 1QHC, 1RND)
First „NMR-structure“, 4 Nobel prizes
M: 13.680, 124 amino acids
Chemical syntheses: 1969 Merrifield (0.4 mg), 1979 Yajima (3 mg)





RNAse A

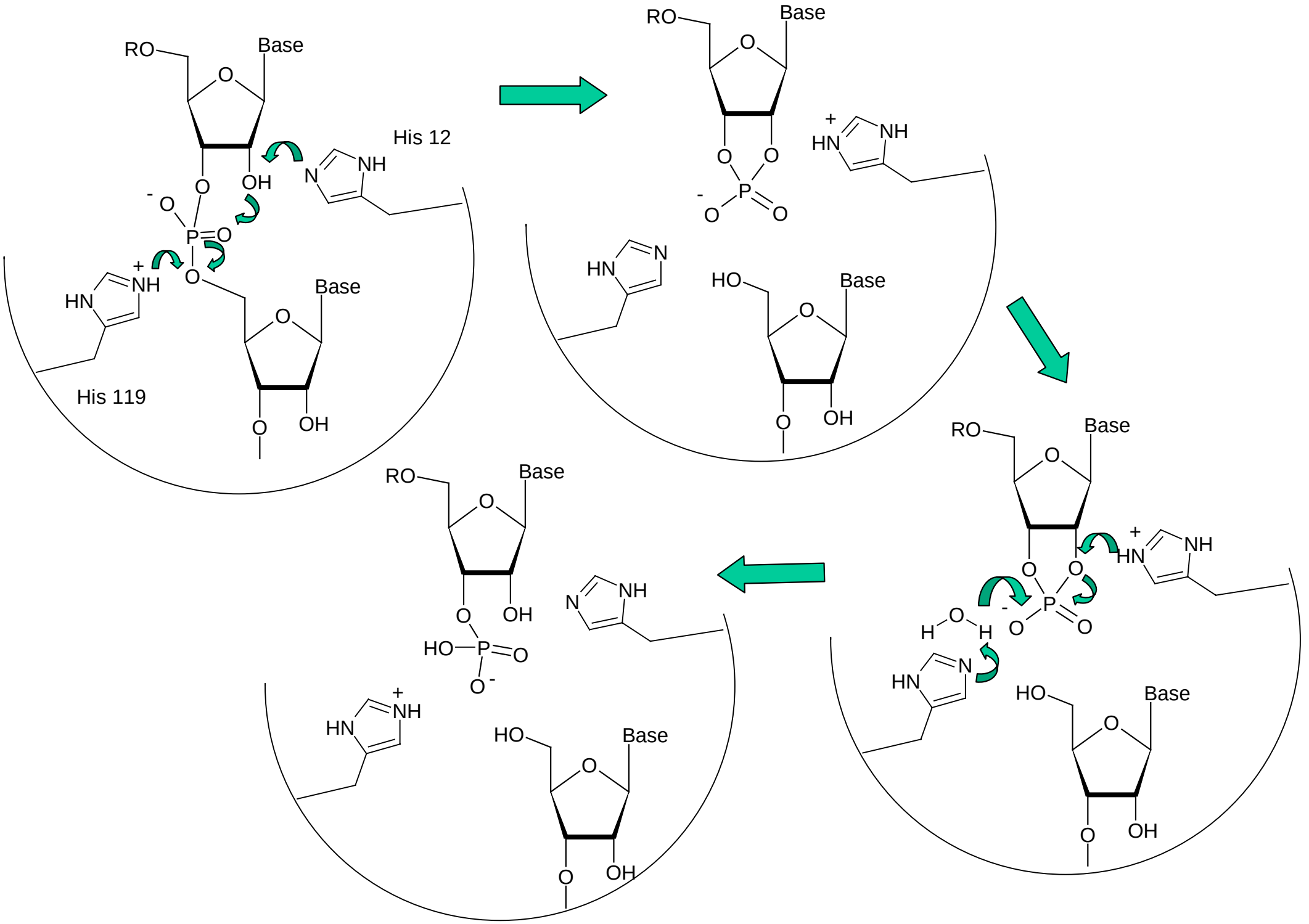
Catalytic centre:

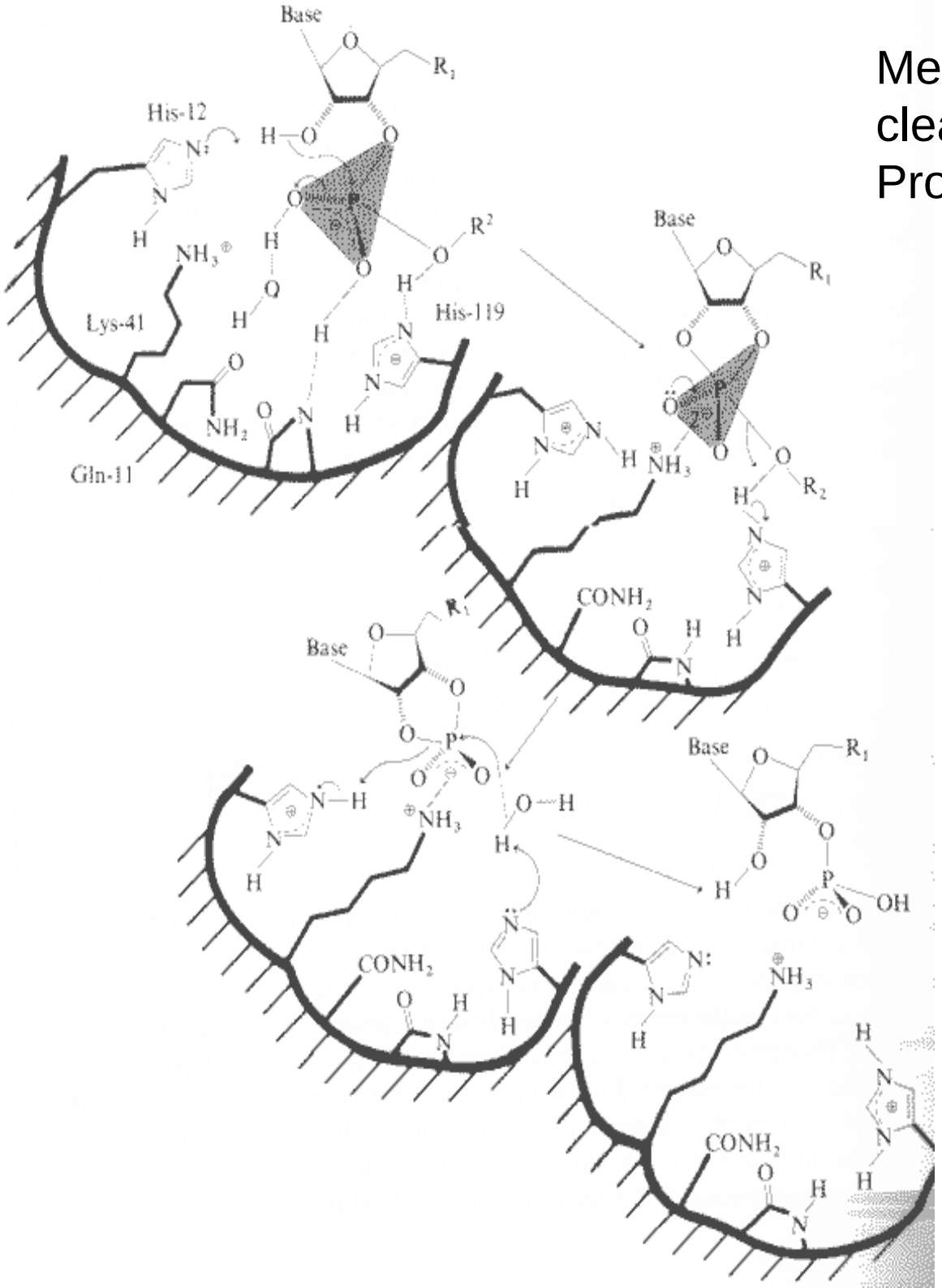
His 12

His 119

Lys 41

Mechanism of ribonuclease A reaction





Mechanism of phosphodiester-cleavage
Product: 3`-Phosphate

Major steps:
Transesterification - hydrolysis

General acid-base catalysis (histidine)

Ribozymes – catalytically active ribonucleic acids - metalloenzyme

Discovered in 1982

2 Groups

35 – 155 Nucleotides: Hammerhead, Hairpin, Hepatitis Delta
Formation of 2´,3`-cyclo-phosphates and 5´-OH (analogous to RNAse A)

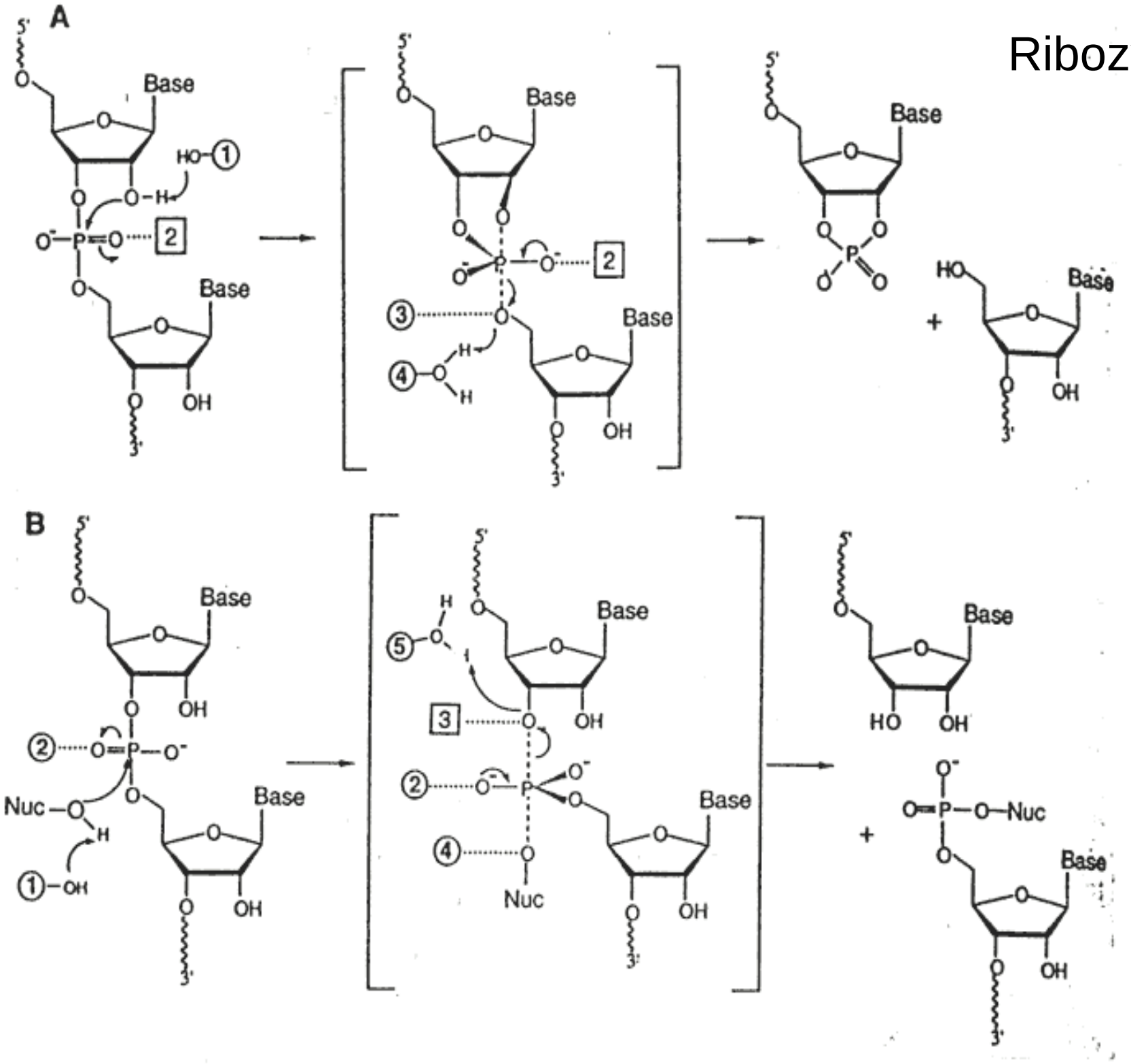
100- 3000 Nucleotides: RNase P, Group I and II Introns
Formation of 5`-phosphate (and 3´-OH)

Self modification, except RNase P (processes t-RNA Precursor)

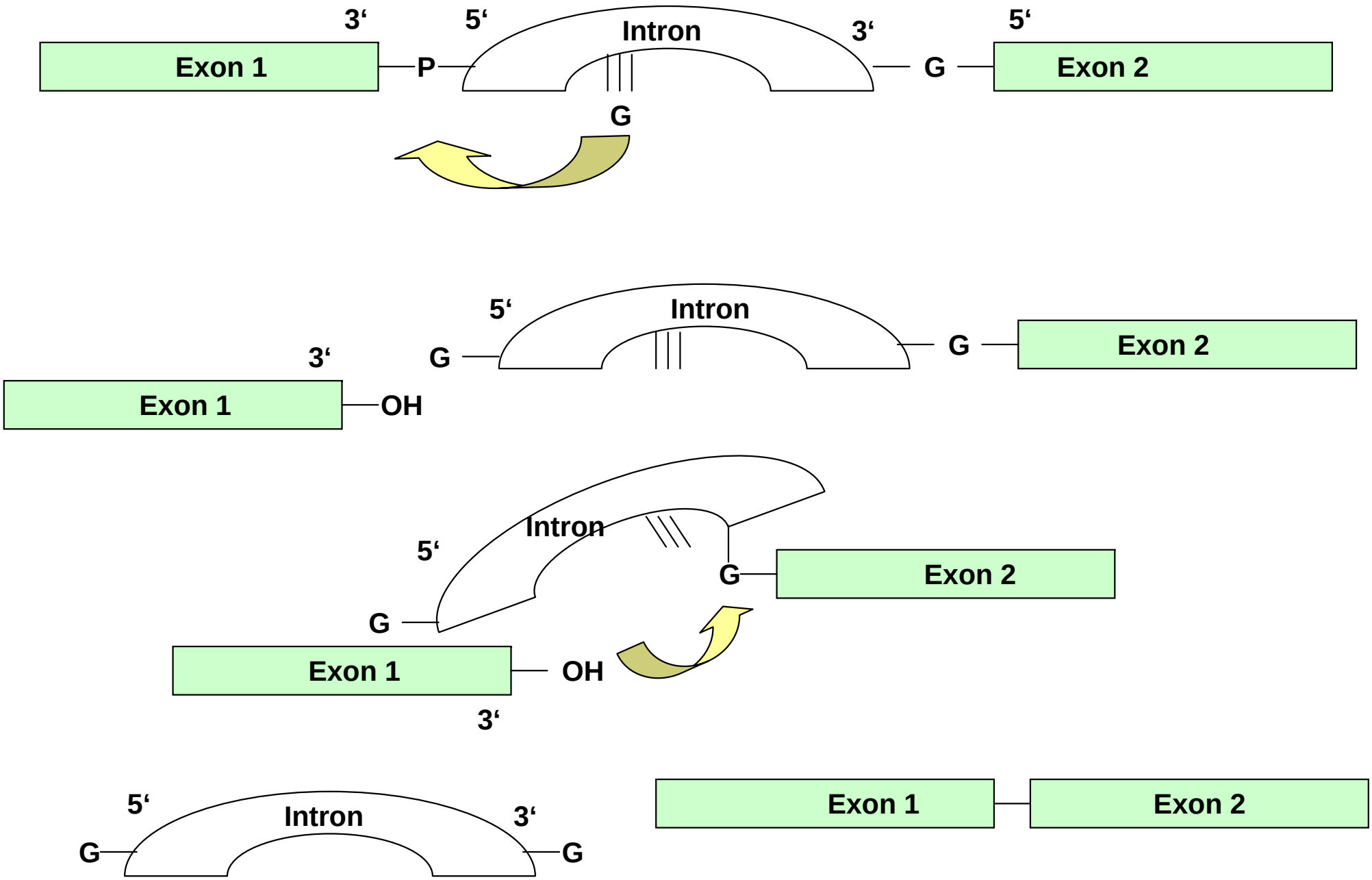
Reaction rate enhancement: ~ 10^{11}

Essential: Mg^{2+}

Ribozyme



Group I Intron-Splicing



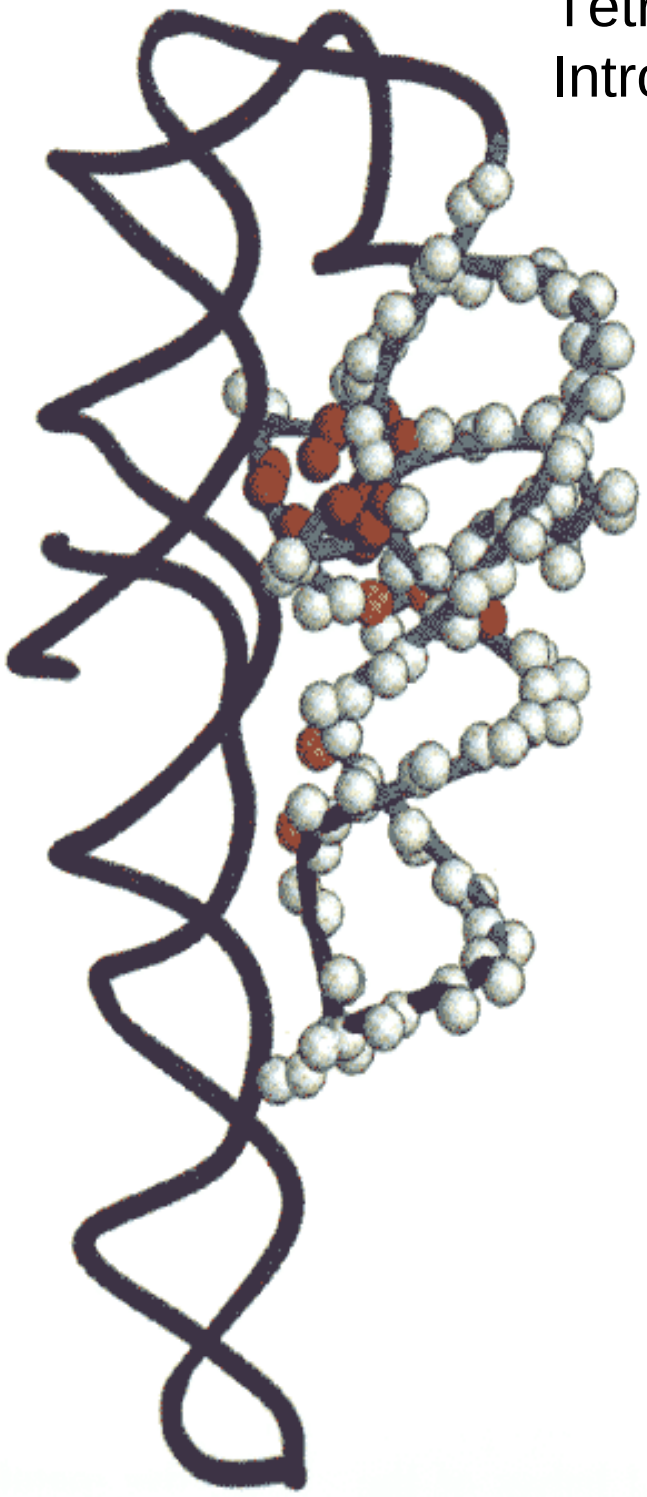
Magnesium:
Coordination of O only (Mn $^{2+}$ also with N)
6 Ligands (Ca $^{2+}$ > 6)

pKa of water lowered to 11.4

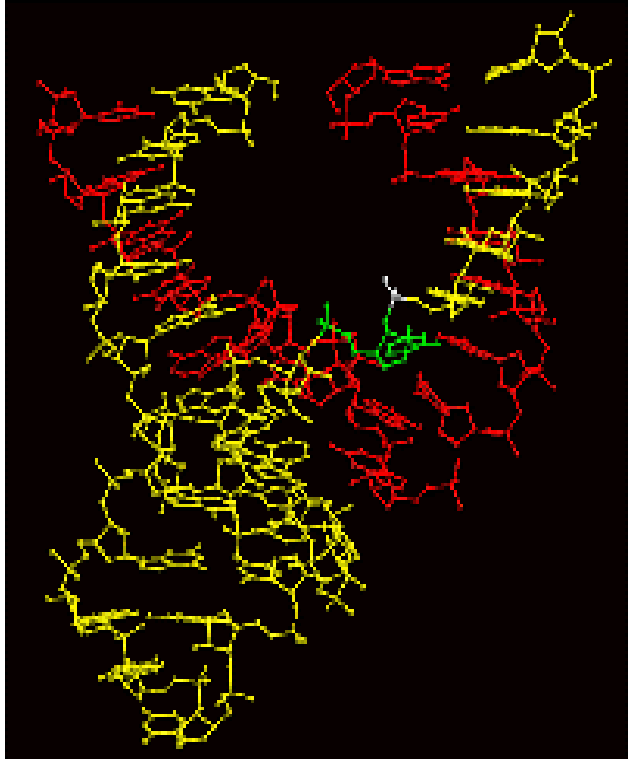
Smallest catalytically active RNA:

UUU
Hydrolysis of GAAA in the presence of Mn^{2+}

Weakest codon-anticodon binding interaction



Tetrahymena Group I Intron



Hammerhead-RNA