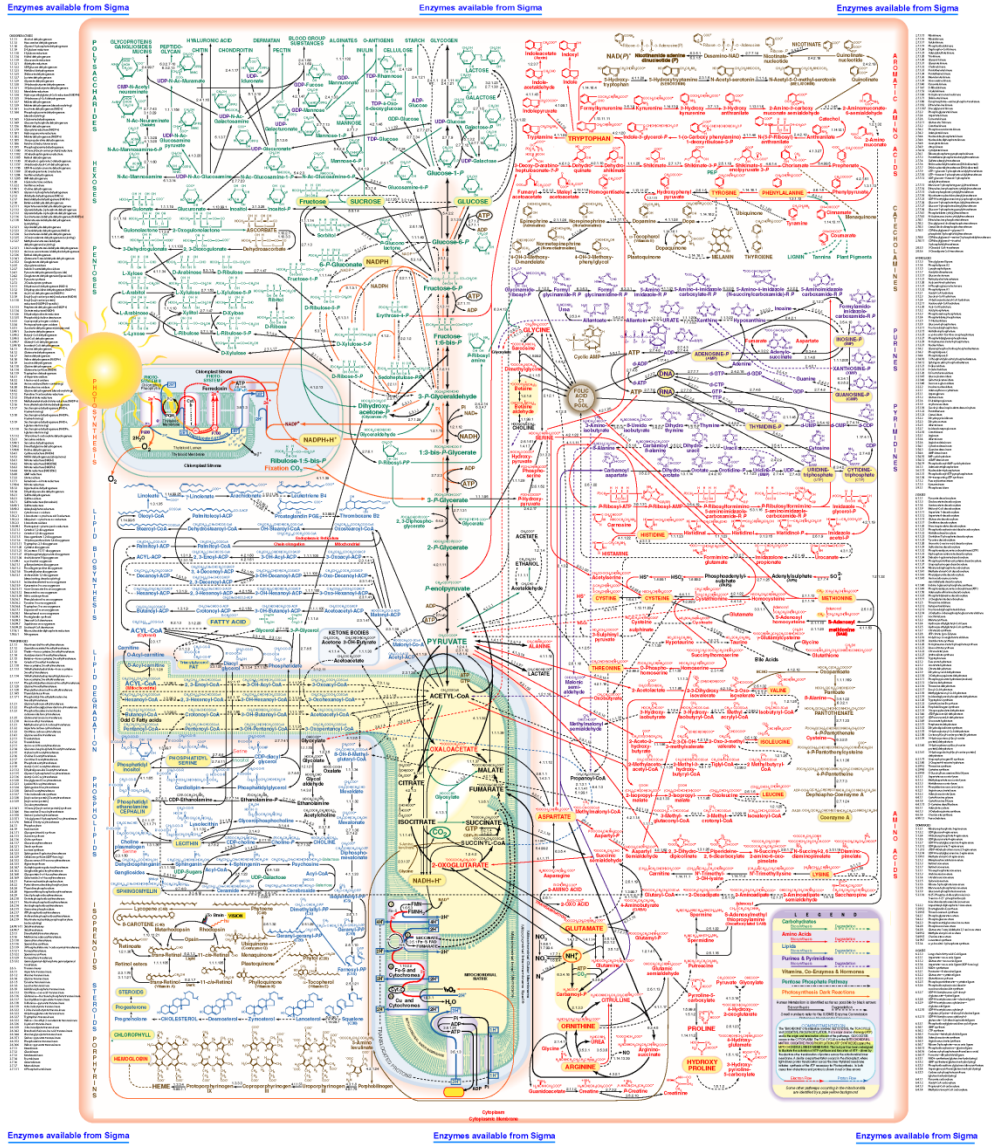
Four categories of enzymes

1. Group transfer reactions
2. Oxidations/reductions
3. Eliminations/isomerizations/ rearrangements
4. Reactions that make or break C-C bonds

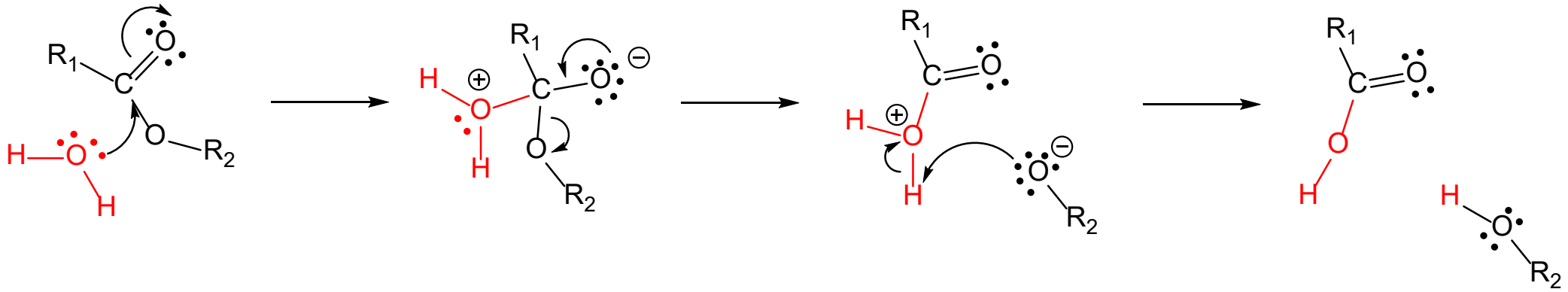


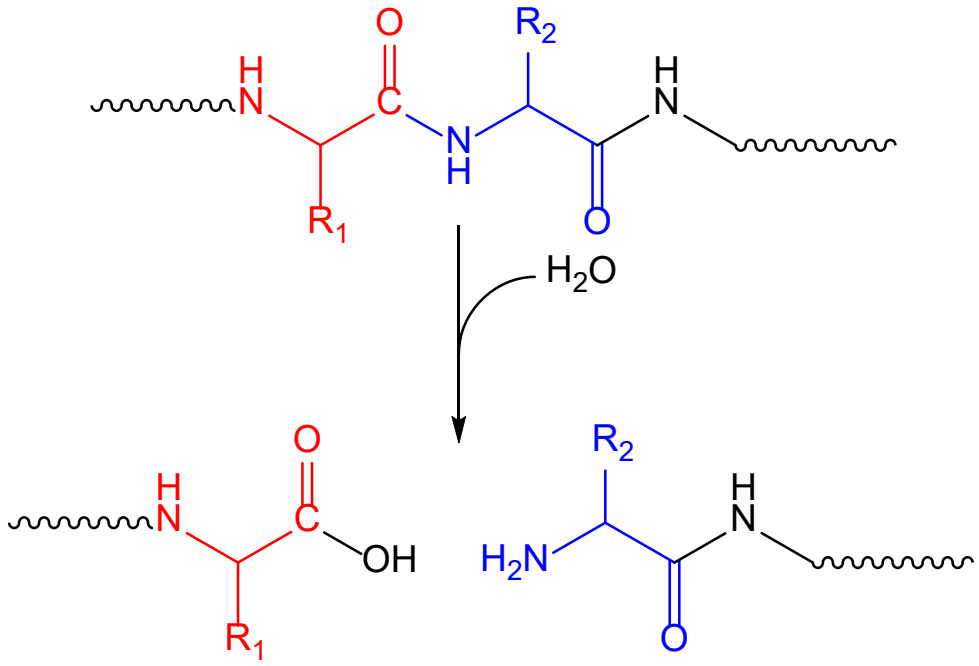
Enzyme-catalyzed group transfers

Transfer of an electrophilic group from one nucleophile to another

- Acyl group transfer
- Phosphoryl group transfer

Acyl group transfer to water: hydrolases





Scope of reactions/physiological functions of proteases

Digestive proteases- best characterized. Classes:

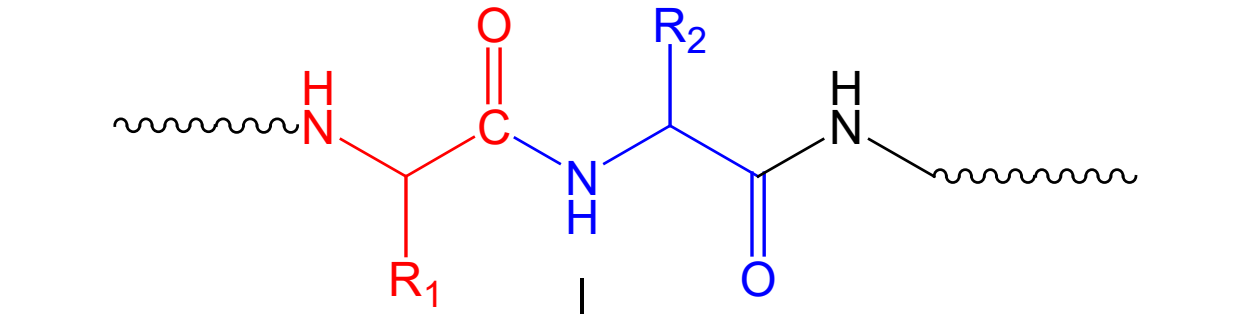
Chymotrypsin-catalyzed hydrolysis of peptide bonds: a case study that illustrates principles of enzyme catalysis

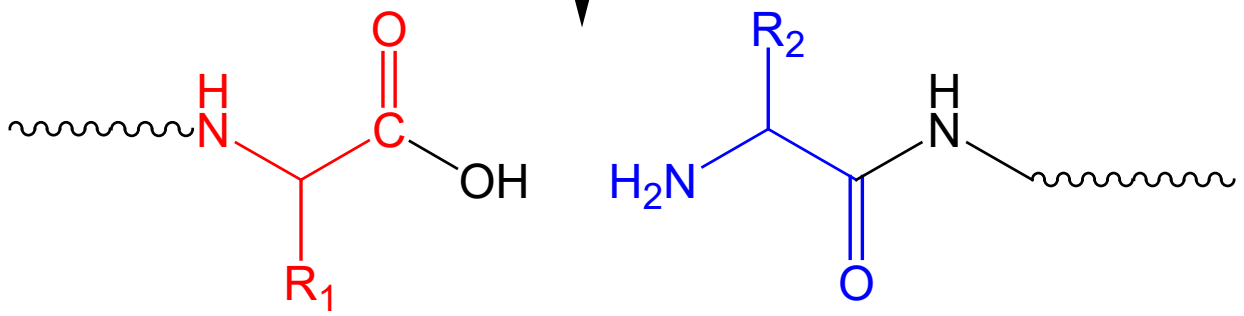
- 25,000 Mr protease
- zymogen → active form

- Cleaves peptide bonds adjacent to aromatic AAs
- A serine protease
- Serine proteases are the most thoroughly studied and understood class of enzymes

Chymotrypsin

- Cleaves (hydrolyzes) peptide bonds adjacent to aromatic AAs

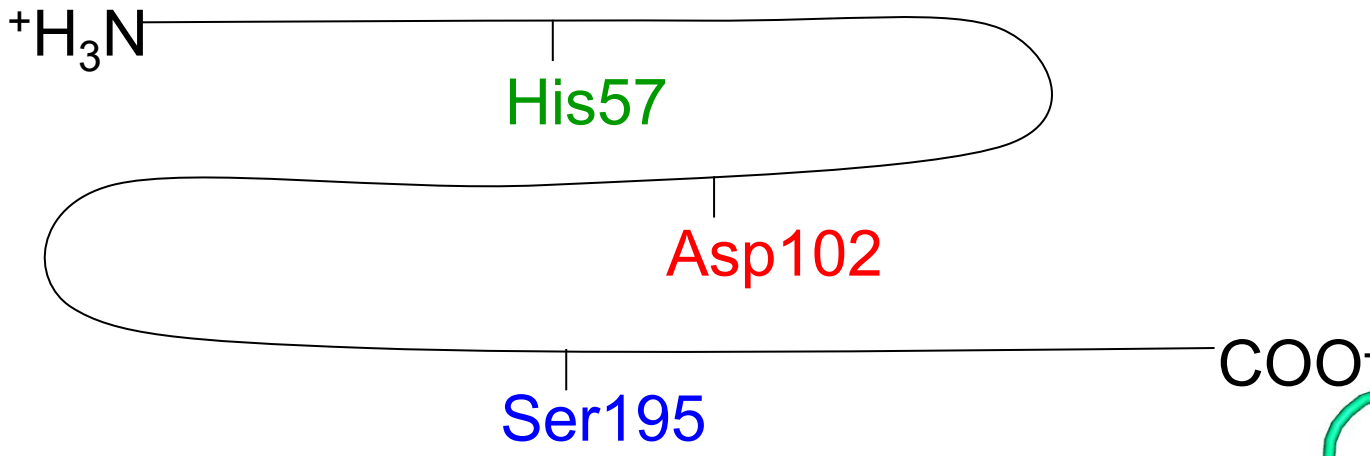


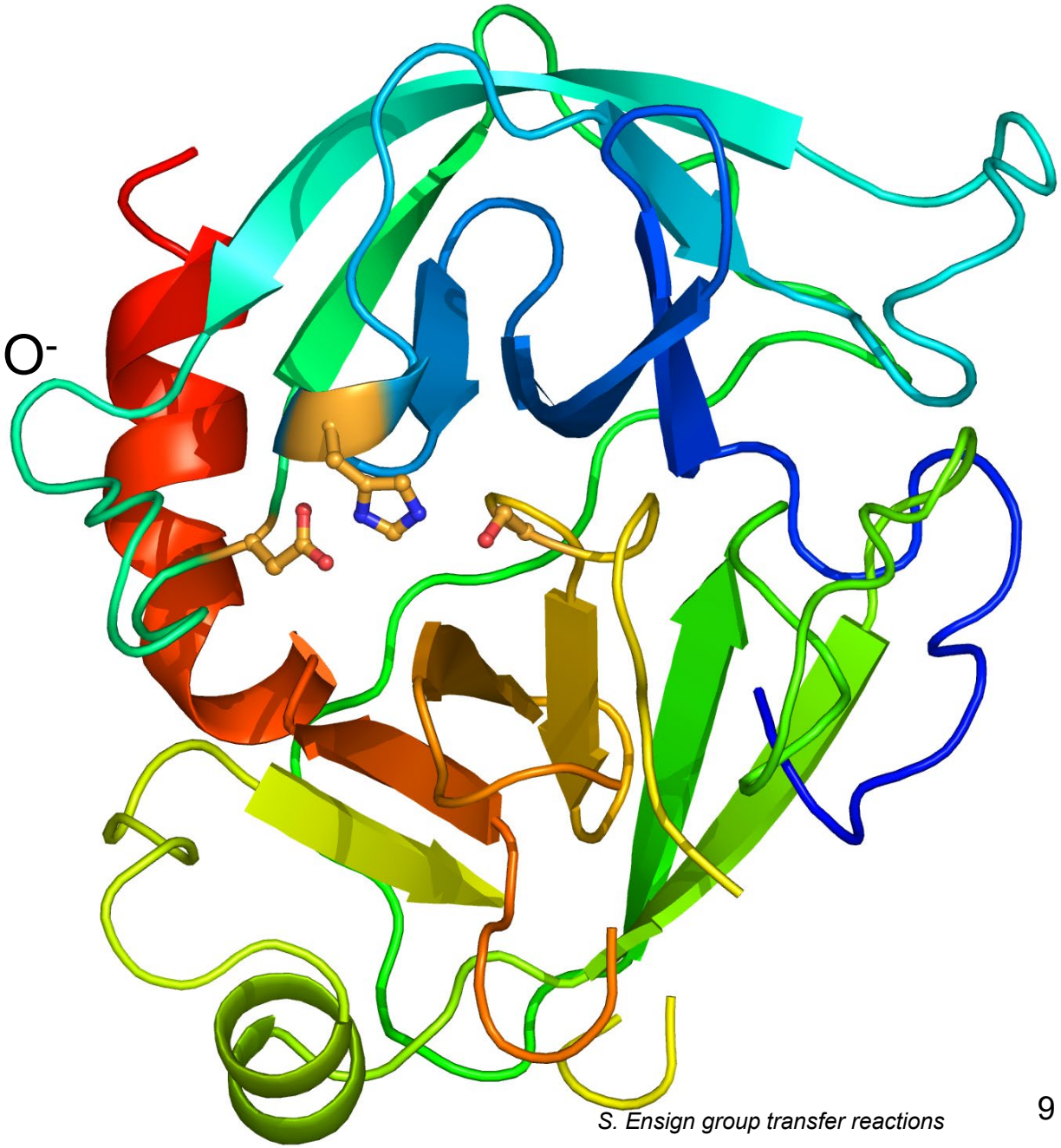


R_1 = Phe, Tyr, Trp

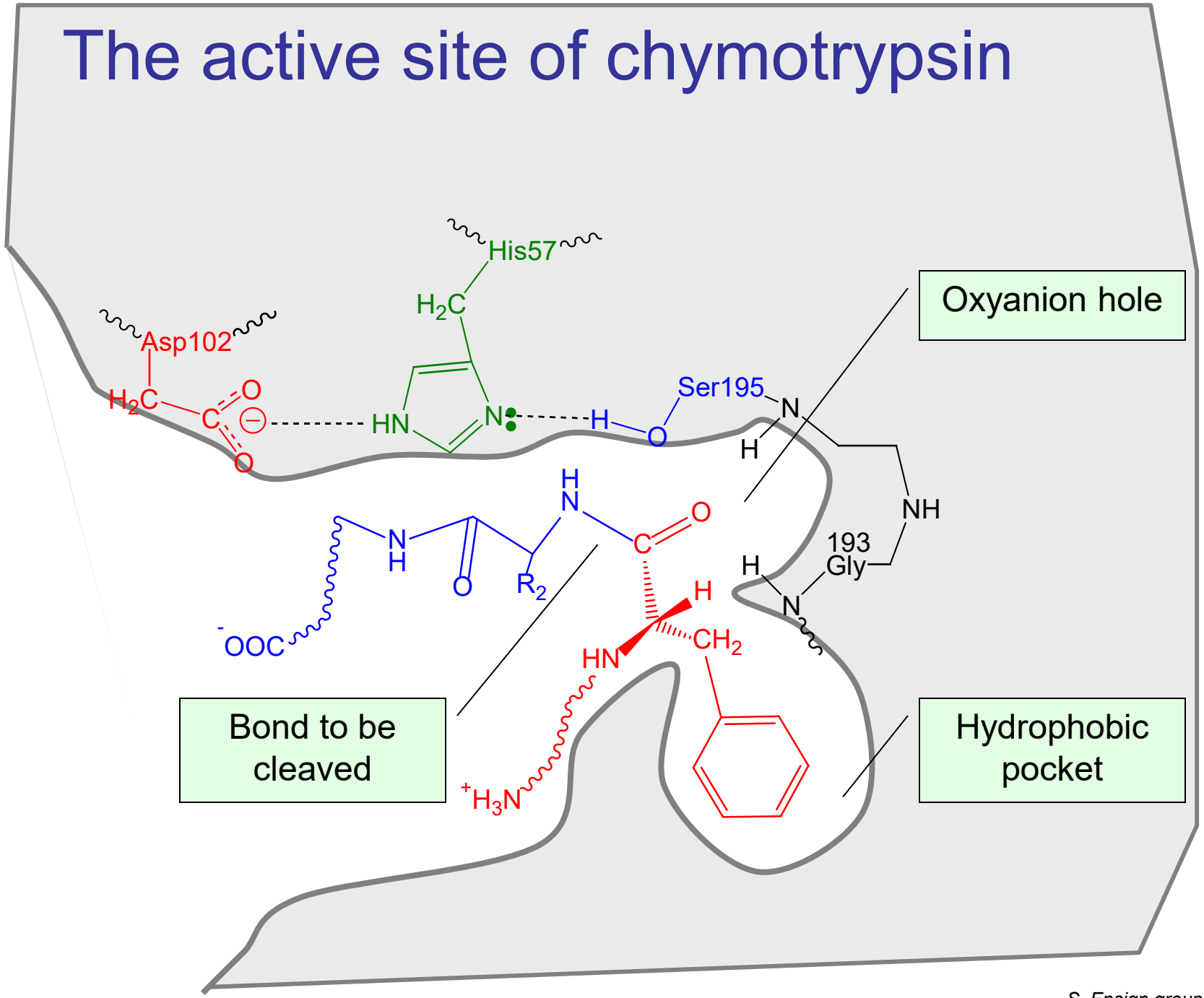
Serine proteases: serine is a nucleophile. Attack of serine on peptide bond generates a covalent acyl-enzyme intermediate

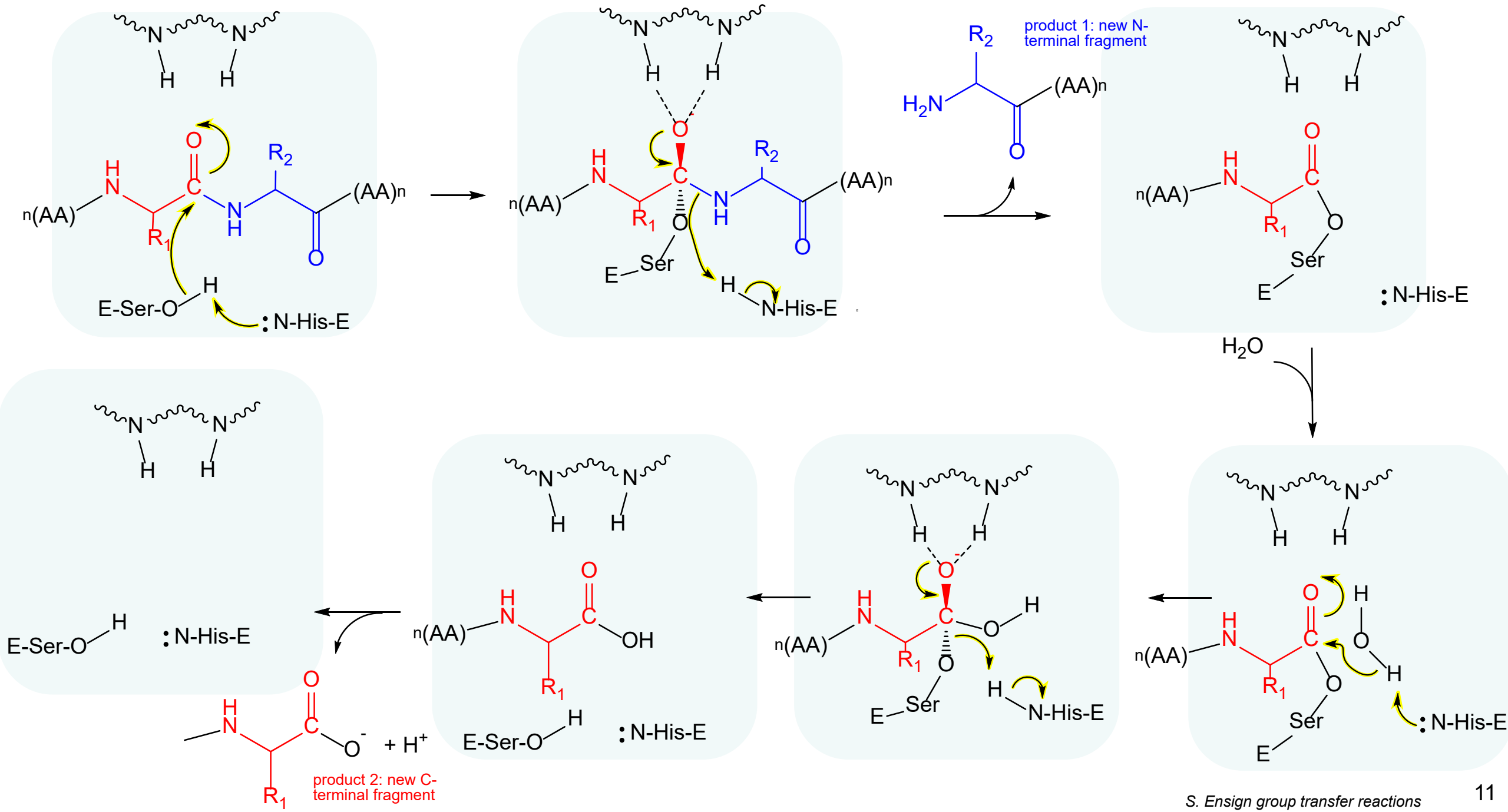
Critical amino acid residues at the active site of chymotrypsin





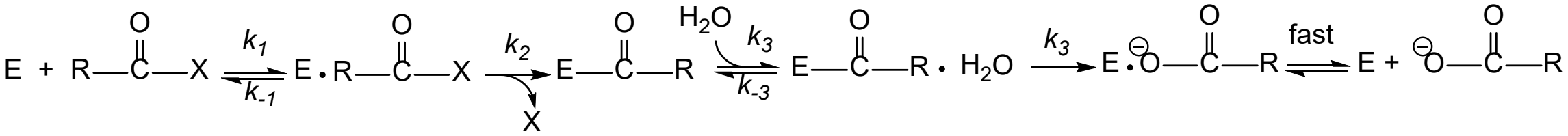
The active site of chymotrypsin



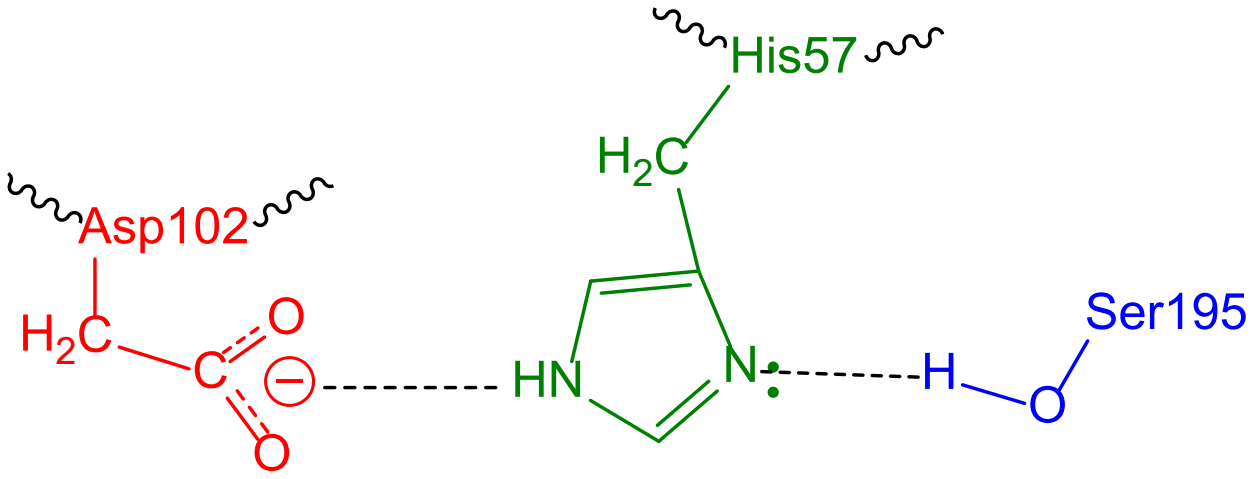

product 1: new N-terminal fragment
product 2: new C-terminal fragment
E-Ser-O
: N-His-E
Ser
E
n(AA)
(AA)n
H2O
+ H+

Catalytic principles observed in chymotrypsin

- Ping-pong kinetic mechanism:

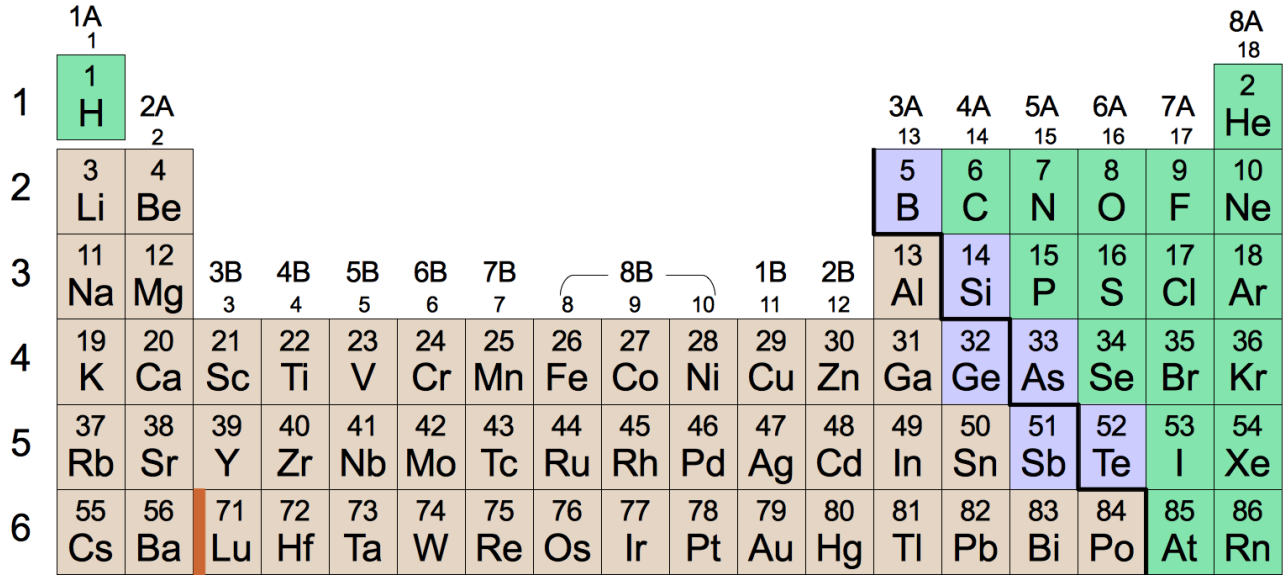


- His57: 2x general base, 2x general acid
- Two tetrahedral intermediates (transition states)
- Oxyanion hole stabilizes tetrahedral intermediates (TS)
- The pKa of “free” serine is 13.6! (6.6 units above phys.): how can it serve as a nucleophile?


His57
H2C
Asp102
H2C
C
O
O
HN
N
H
O
Ser195

Enzyme-catalyzed group transfers

- Acyl group transfer
- Phosphoryl group transfer



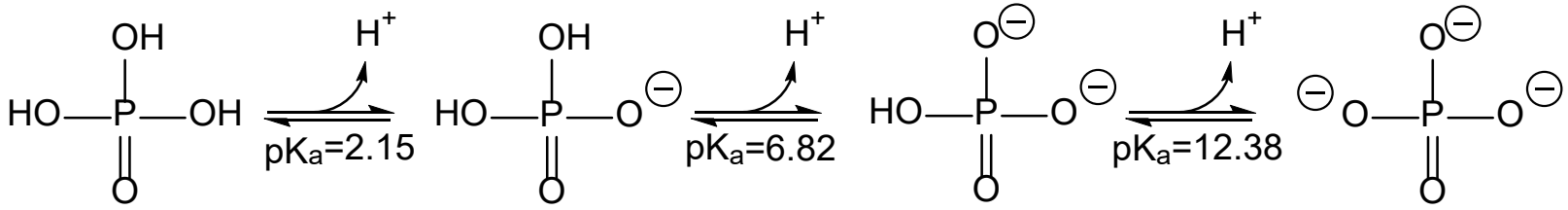
	1A 1								8B							8A 18
1	1 H	2A 2											3A 13	4A 14	5A 15	6A 16	7A 17	2 He
2	3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne
3	11 Na	12 Mg	3B 3	4B 4	5B 5	6B 6	7B 7	8	9	10	1B 11	2B 12	13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
4	19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
5	37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
6	55 Cs	56 Ba	71 Lu	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn

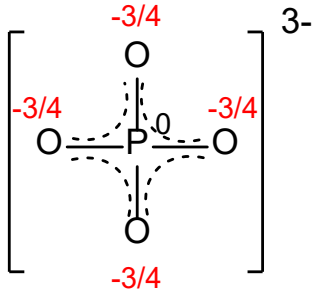
Purposes of phosphorylated compounds

- Convert a poor leaving group to a good one
- Stabilizing structural element
- Increase H_2O solubility of hydrophobic molecules
- Offer reactive handle to biological catalysts
- Enzyme regulation

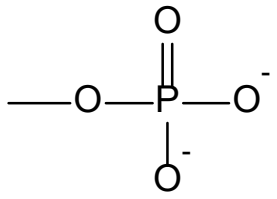
Phosphoryl group transfer: nomenclature

Inorganic phosphate:

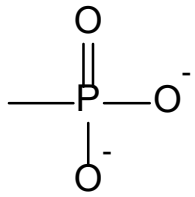




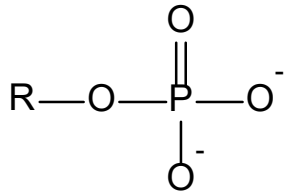
A phosphate group:



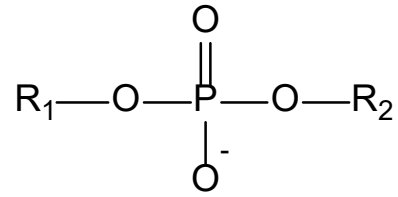
A phosphoryl group:



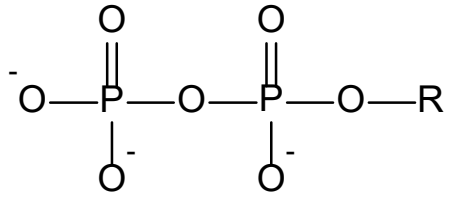
A phosphomonoester:



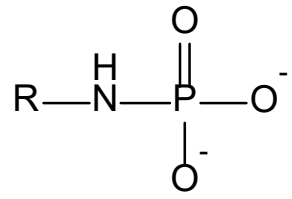
A phosphodiester:



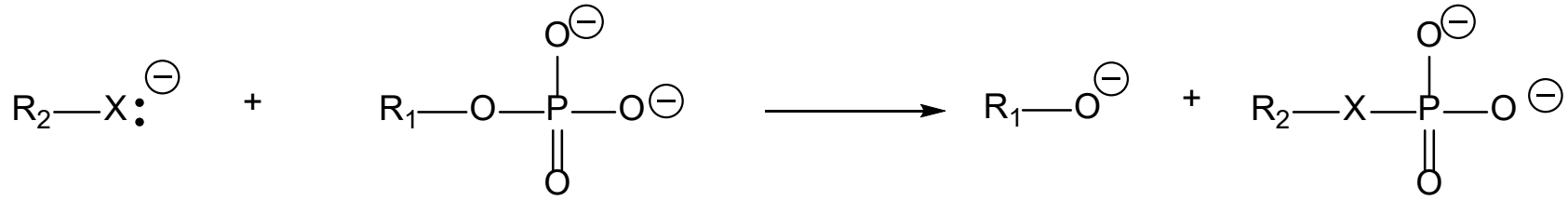
A phosphoanhydride:



A phosphoramidate:



Phosphoryl group transfer



Nucleophile	Product
H_2O	Inorganic phosphate
R_2—OH	Alkyl phosphate
R_2—C(=O)—O$^-$	Acyl phosphate
R_2—NH_2	phosphoramidate
R_2O—P(=O)(O$^-$)—O$^-$	phosphoanhydride

Scope of “phosphoryl” transfer reactions

Phosphoryl transfer

- Phosphatase — Water is the acceptor
- Kinase — A nucleophile other than water is the acceptor
- Mutase — A nucleophile other than water is the acceptor

Phosphoester transfer

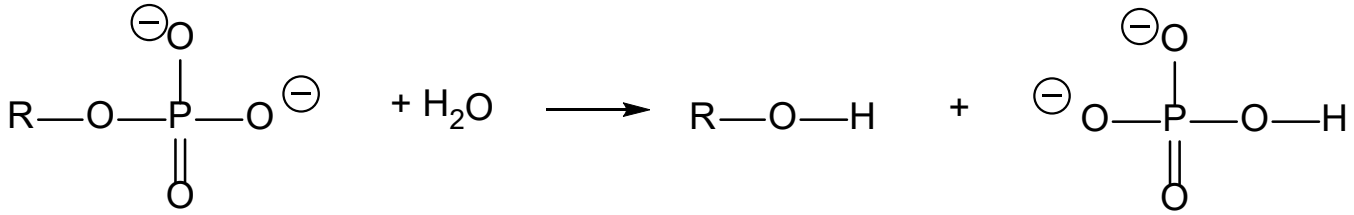
- Phosphodiesterase — Water is the acceptor
- Nucleotidyl transferase — A nucleophile other than water is the acceptor

Phosphorolysis

- Phosphorylase

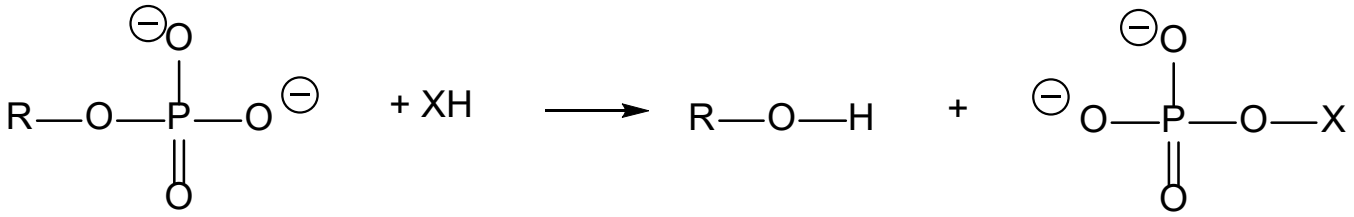
Scope of phosphoryl transfer reactions

- Phosphatase
  - S/T; Y; dual specific
  - Acid and alkaline (small molecules)



Scope of phosphoryl transfer reactions

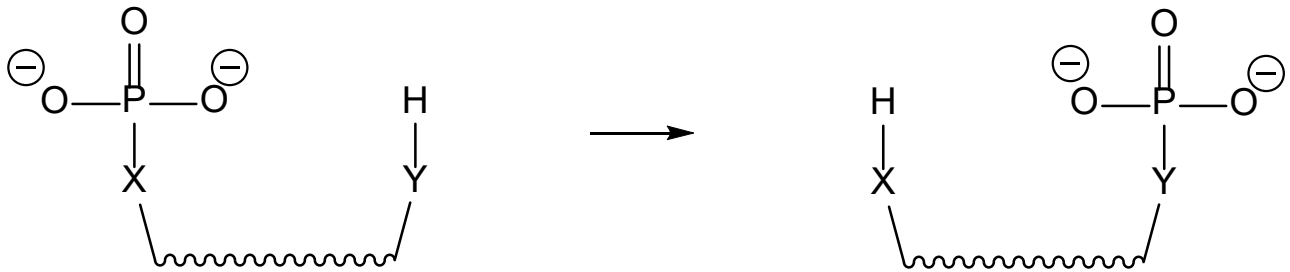
- Kinase
  - S/T, H, Y, D, C, small molecules



Important examples from central metabolism: hexokinase, phosphofructokinase, pyruvate kinase, creatine kinase, protein kinase a, phosphorylase kinase

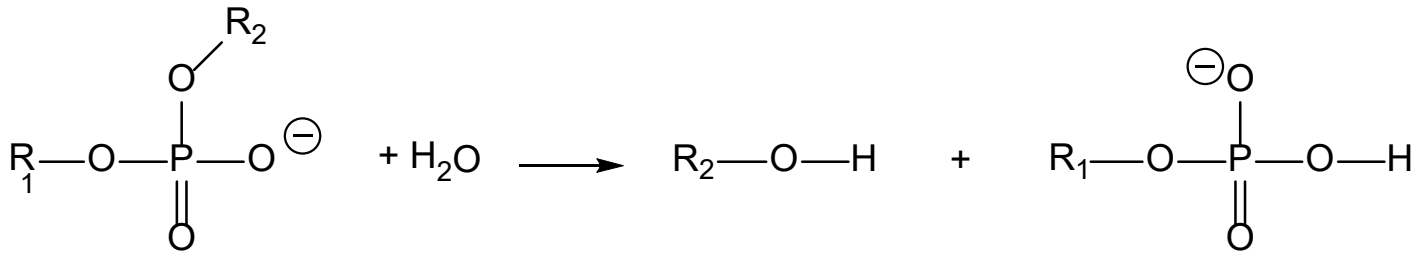
Scope of phosphoryl transfer reactions

- Mutase



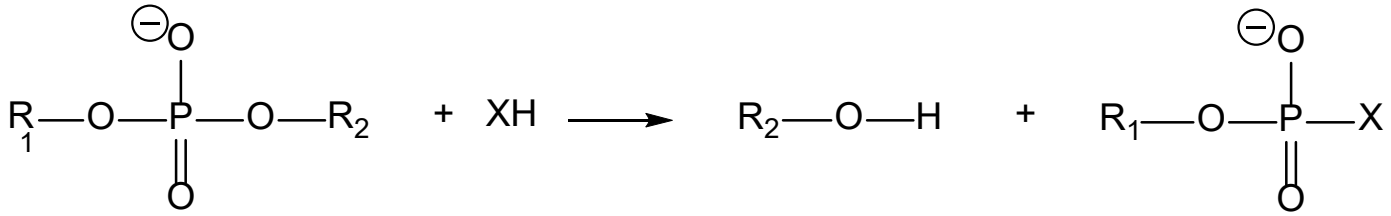
Alkylphosphoryl group (phosphoester) transfer reactions

- Phosphodiesterase
  - DNAse, RNAse, phospholipase
- An alkylphosphoryl group is transferred



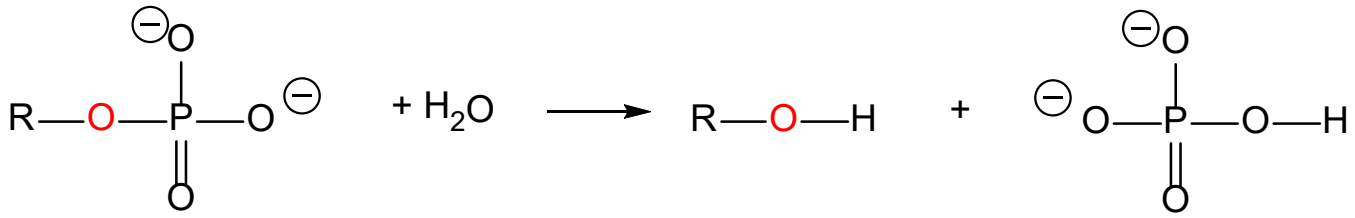
Alkylphosphoryl group (phosphoester) transfer reactions

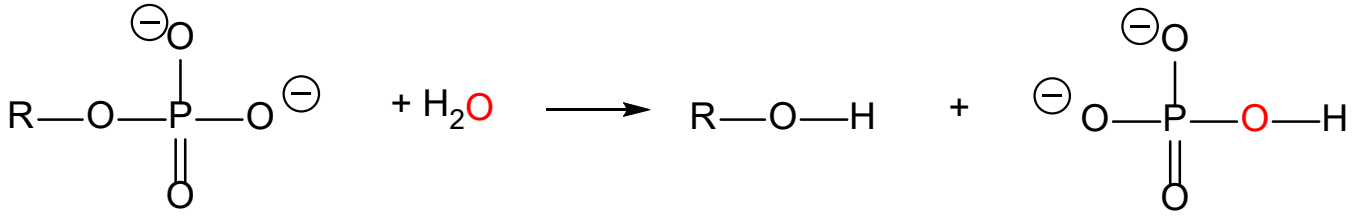
- Nucleotidyl transferase
  - Example: ADP ribosyl transferase



Chemistry of phosphoryl group transfer

- Which bond is broken? Isotopic labelling:

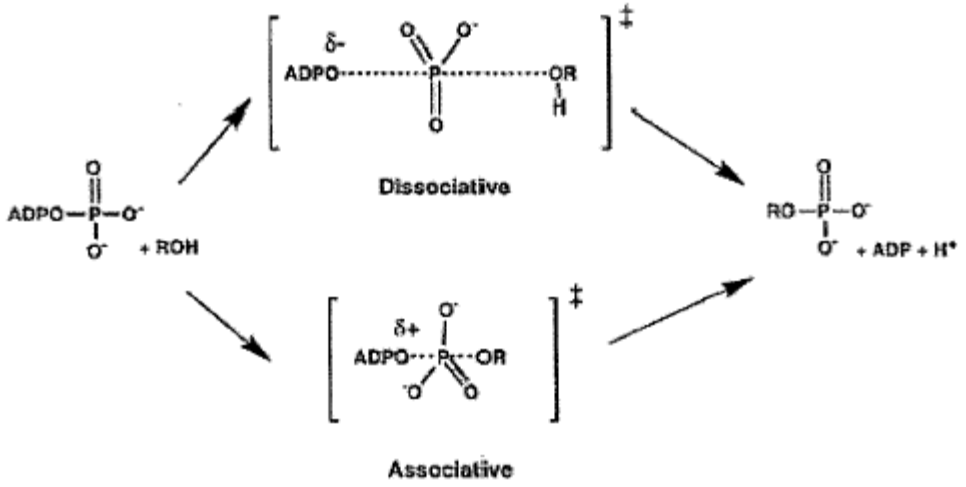




- A phosphoryl group is transferred, NOT a phosphate group

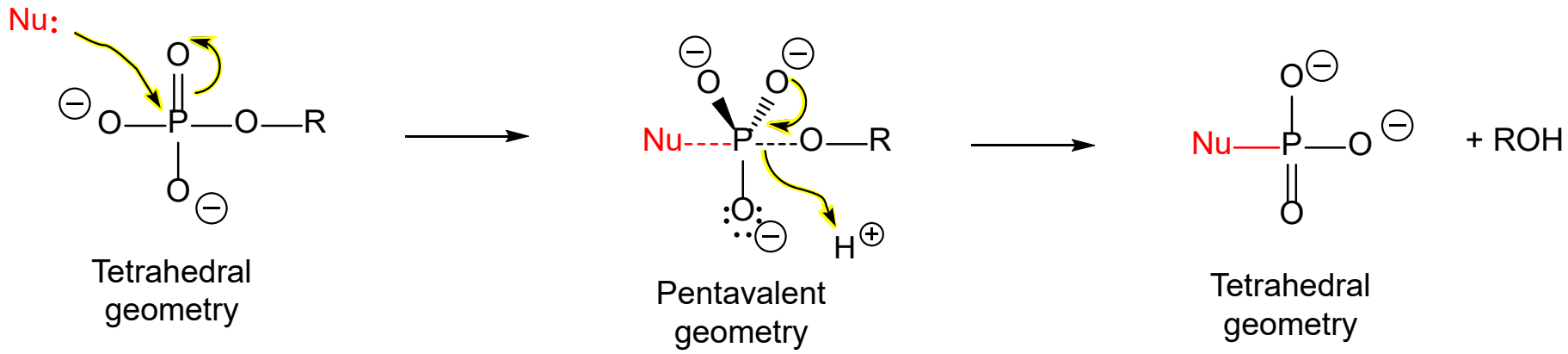
Chemistry of phosphoryl group transfer

- Possible mechanisms for phosphoryl group displacement
  - Dissociative (S_N1)
  - Associative (S_N2)

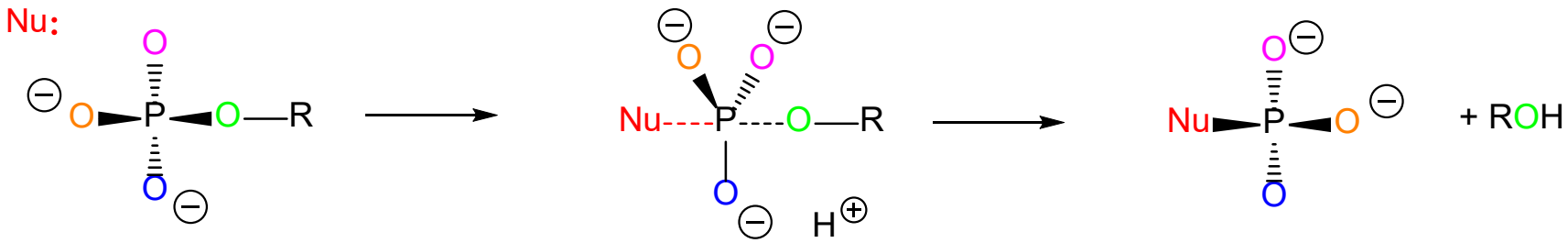


Associative mechanism (S_N2)

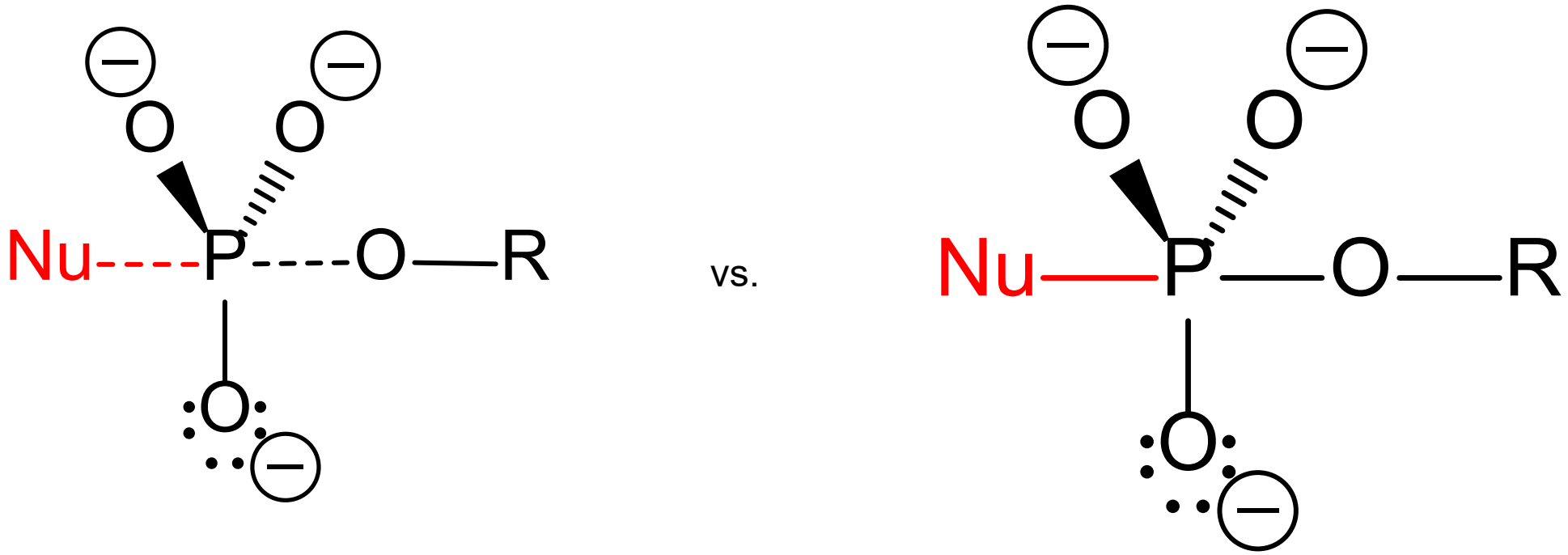
- Pentavalent *intermediate* or *transition state*?



- Stereochemical consequences?

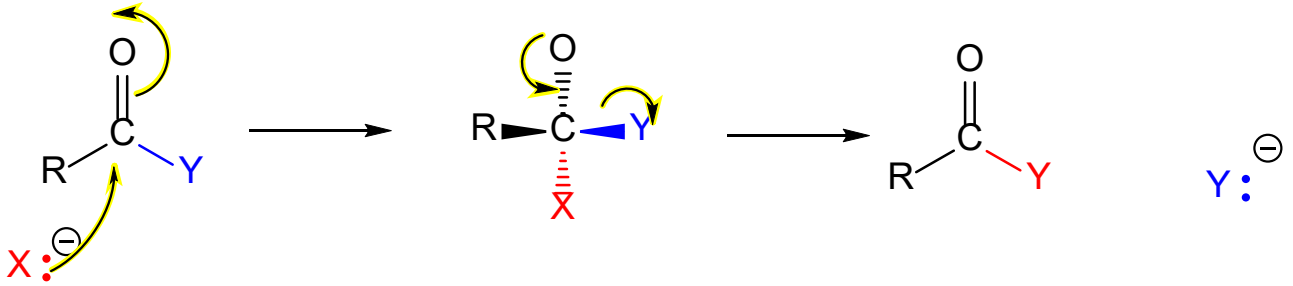


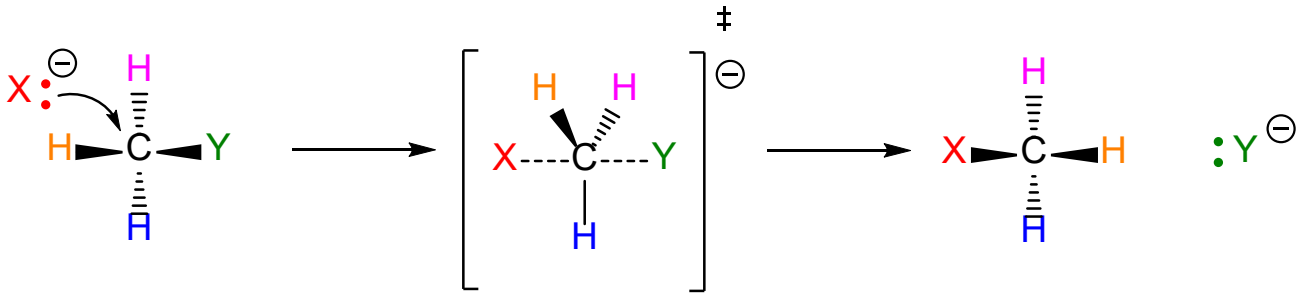
Phosphoryl transfer reactions occur with inversion. This can be proven using “chiral” substrates with three isotopes of O present

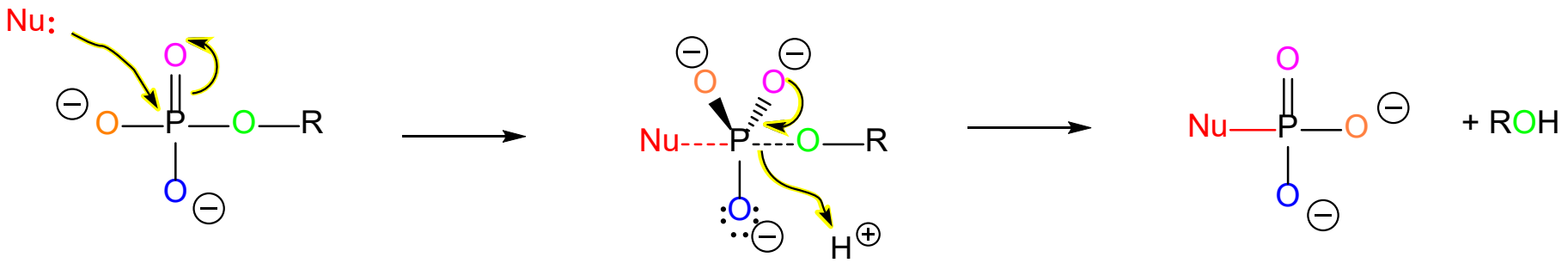

Nu
P
O
R
vs.
Nu
P
O
R

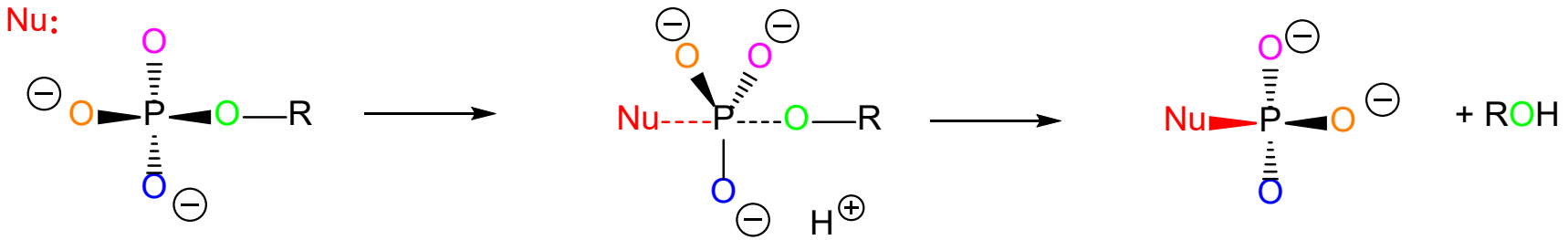
Associative mechanism (S_N2)

- Compare to displacements at sp^2 and sp^3 C centers









Rules for formation and decomposition of pentacoordinate intermediates of relevance to enzyme mechanisms

- No pentavalent “intermediate” has ever been directly observed or isolated

Science 2003: 299, 2067-2071

The Pentacovalent Phosphorus Intermediate of a Phosphoryl Transfer Reaction

Sushmita D. Lahiri,[1] Guofeng Zhang,[2] Debra Dunaway-Mariano,[2]* Karen N. Allen[1]*

Enzymes provide enormous rate enhancements, unmatched by any other type of catalyst. The stabilization of high-energy states along the reaction coordinate is the crux of the catalytic power of enzymes. We report the atomic-resolution structure of a high-energy reaction intermediate stabilized in the active site of an enzyme. Crystallization of phosphorylated β-phosphoglucomutase in the presence of the Mg(II) cofactor and either of the substrates glucose 1-phosphate or glucose 6-phosphate produced crystals of the enzyme–Mg(II)–glucose 1,6-(bis)phosphate complex, which diffracted x-rays to 1.2 and 1.4 angstroms, respectively. The structure reveals a stabilized pentacovalent phosphorane formed in the phosphoryl transfer from the C(1)O of glucose 1,6-(bis)phosphate to the nucleophilic Asp8 carboxylate.

PNAS 2006: 103, 14732-14737

A Trojan horse transition state analogue generated by MgF_3^- formation in an enzyme active site

Nicola J. Baxter[†], Luis F. Olguin[‡], Marko Goličnik[‡§], Guoqiang Feng[¶], Andrea M. Hounslow[†], Wolfgang Bermel[‖], G. Michael Blackburn[¶], Florian Hollfelder[‡††], Jonathan P. Waltho[†,††], and Nicholas H. Williams[¶††]

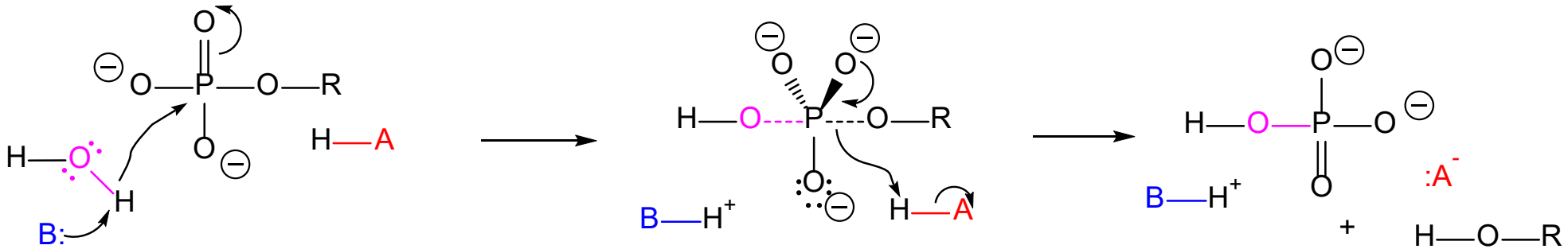
[†]Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield S10 2TN, United Kingdom; [‡]Department of Biochemistry, University of Cambridge, Cambridge CB2 1GA, United Kingdom; [¶]Centre for Chemical Biology, Department of Chemistry, University of Sheffield, Sheffield S3 7HF, United Kingdom; and [‖]Bruker BioSpin GmbH, Silberstreifen 4, 76287 Rheinstetten, Germany

Edited by Perry A. Frey, University of Wisconsin, Madison, WI, and approved July 26, 2006 (received for review May 30, 2006)

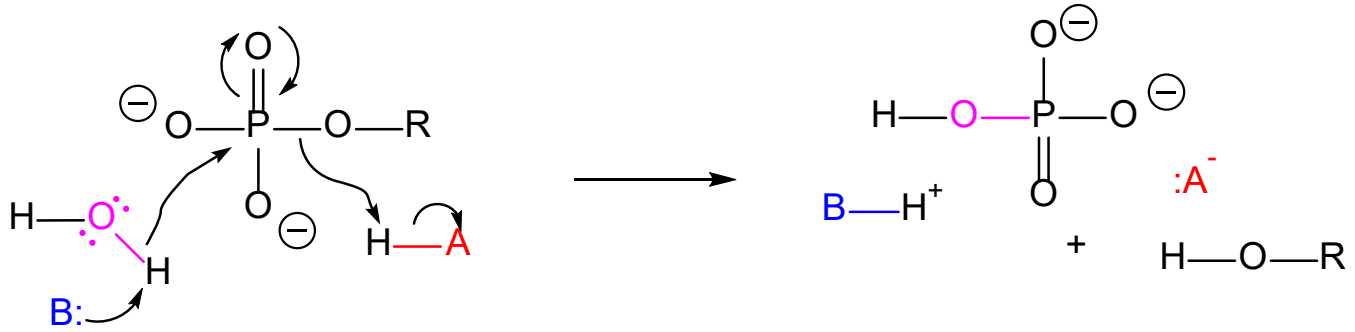
- Nucleophiles approach and leaving groups depart from axial positions
- 4-6 member rings will span axial-equatorial positions
- Electronegative ligands prefer axial positions, electropositive ligands prefer equatorial positions

Phosphoryl group transfer to water: phosphatase

Mechanism showing the pentavalent transition state:

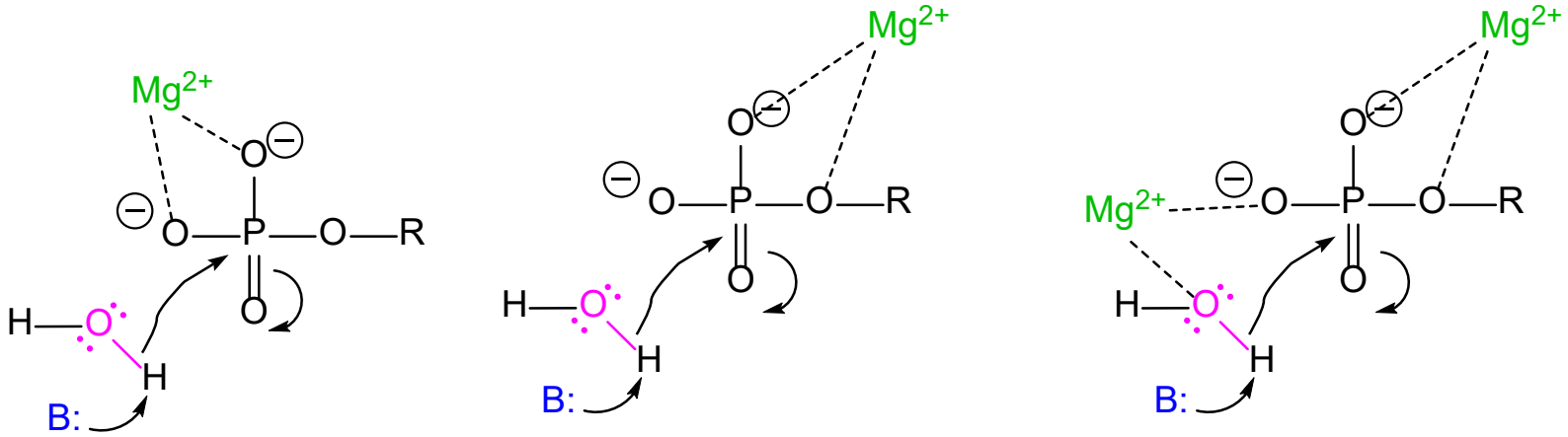


Mechanism “shortcutting” the pentavalent transition state



Mechanistic considerations: identity of general acids/bases? Charge shielding and stabilization?

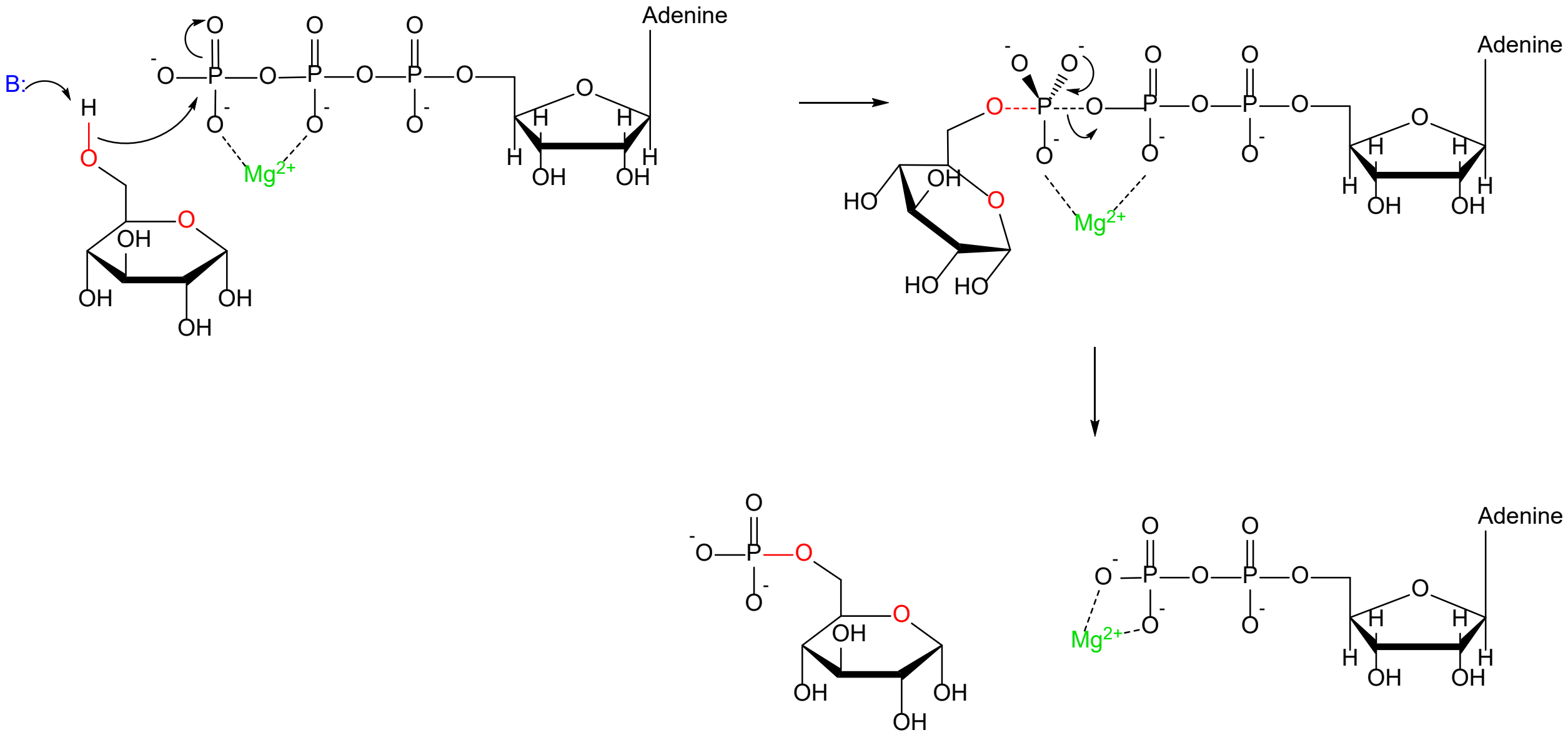
Possible roles for a metal ion in charge shielding/stabilization



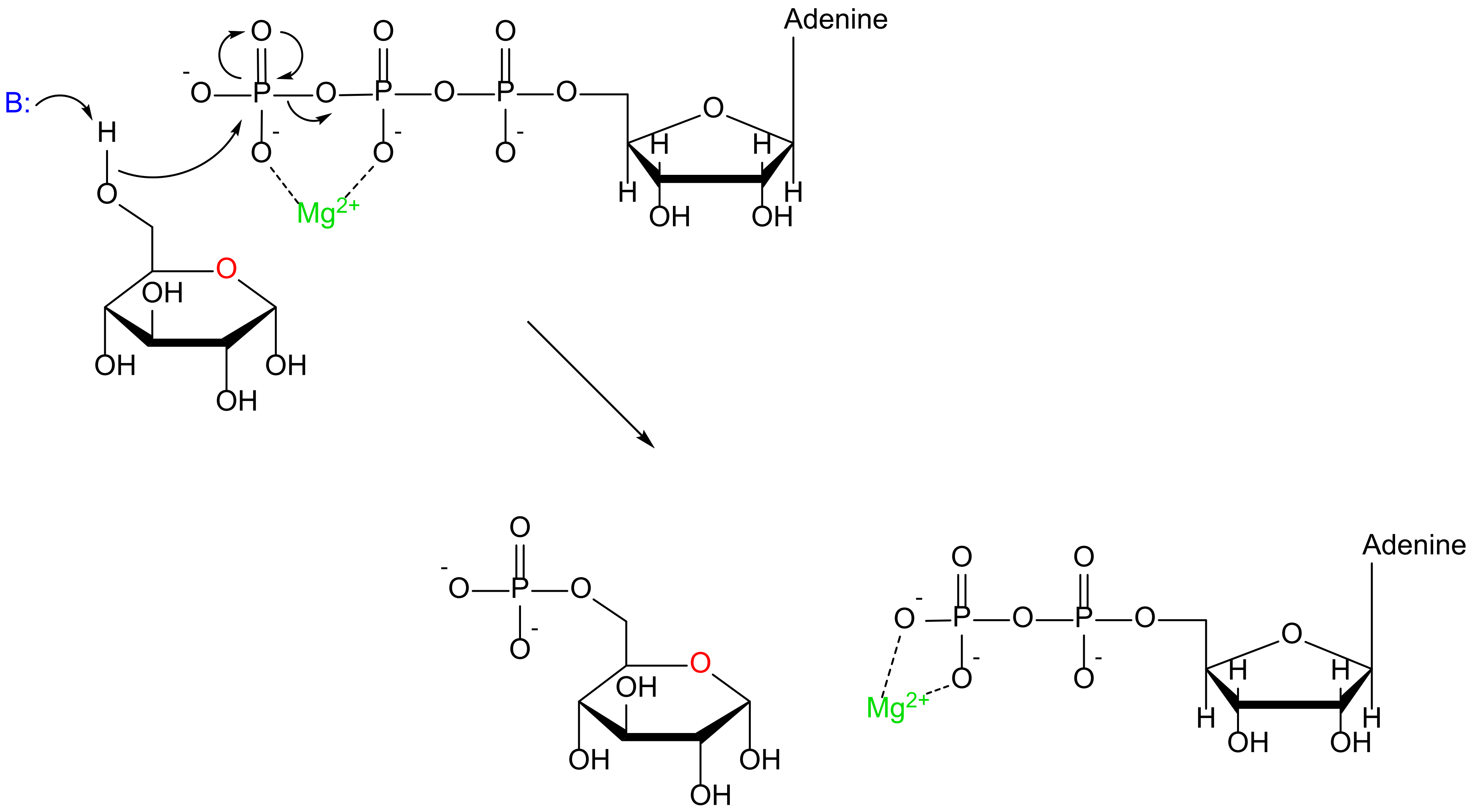
Examples of phosphoryl group transfer

- Transfer of a phosphoryl group from ATP to an alcohol (hexokinase)
- Transfer of a phosphoryl group from one position to another in a molecule (phosphoglyceromutase)
- Substrate level phosphorylation (phosphoglycerate kinase, pyruvate kinase)
- Converting a poor leaving group to a good leaving group (glutamine synthetase)

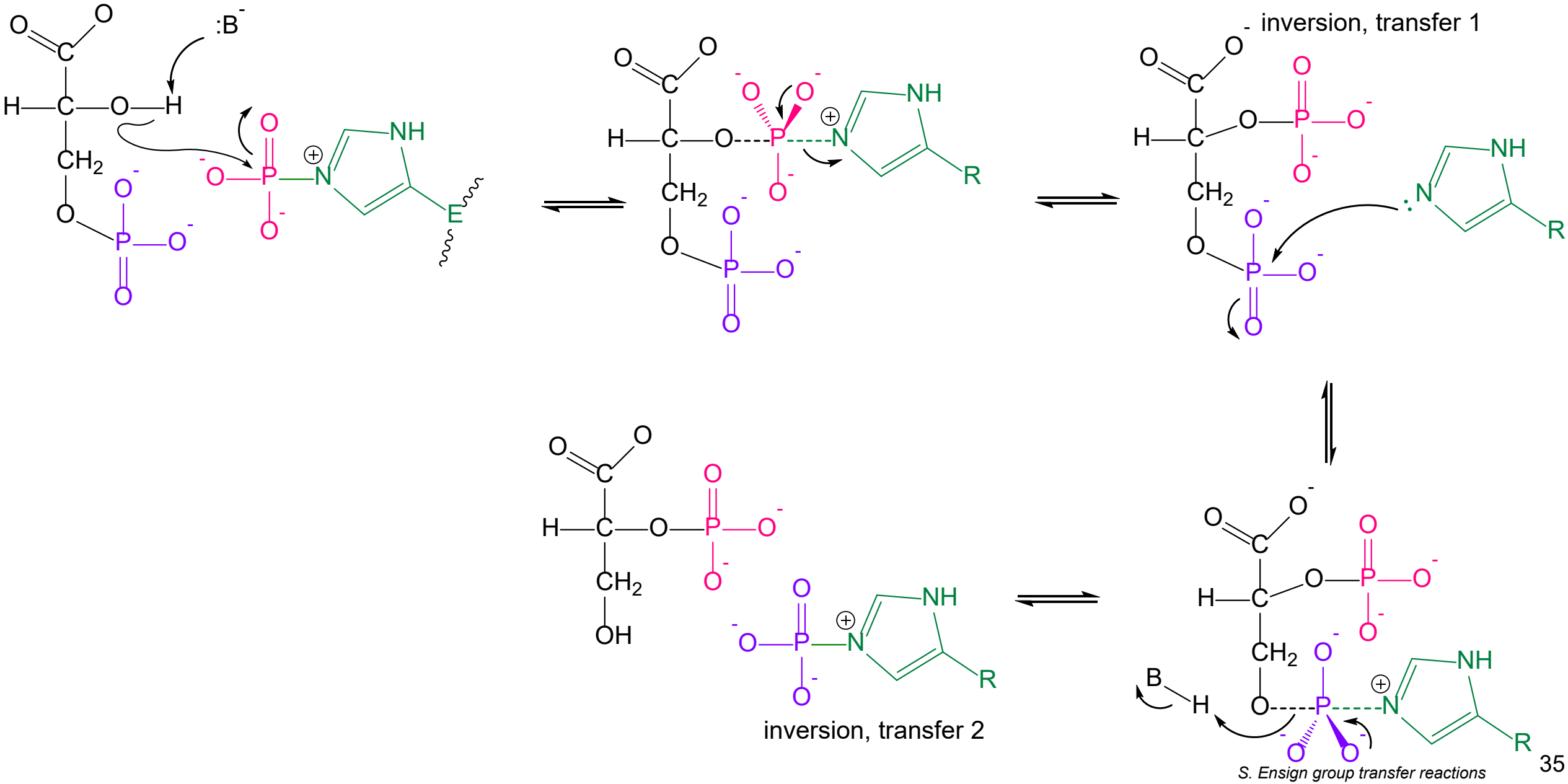
Hexokinase mechanism showing pentavalent TS



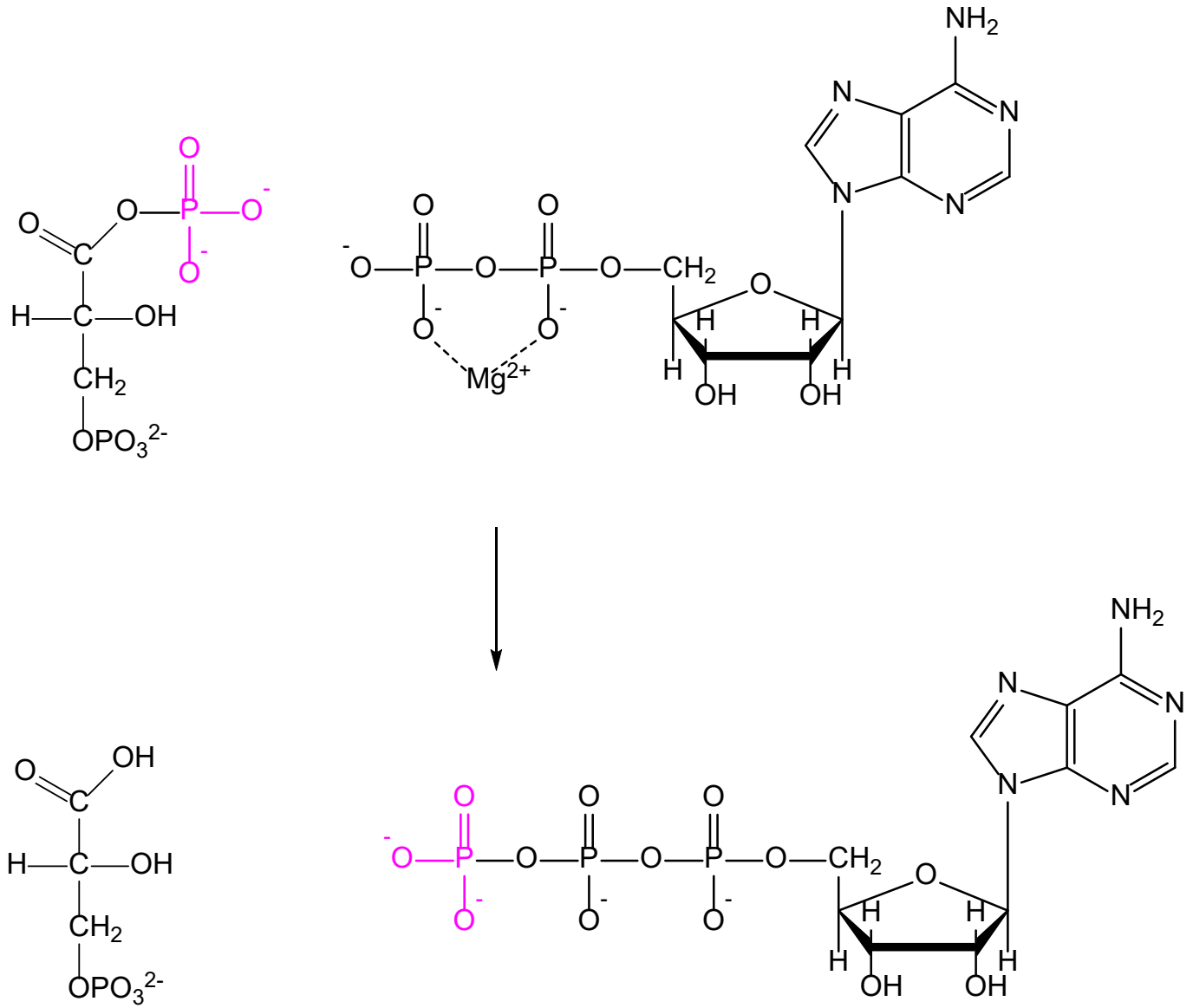
Hexokinase “shortcut” mechanism



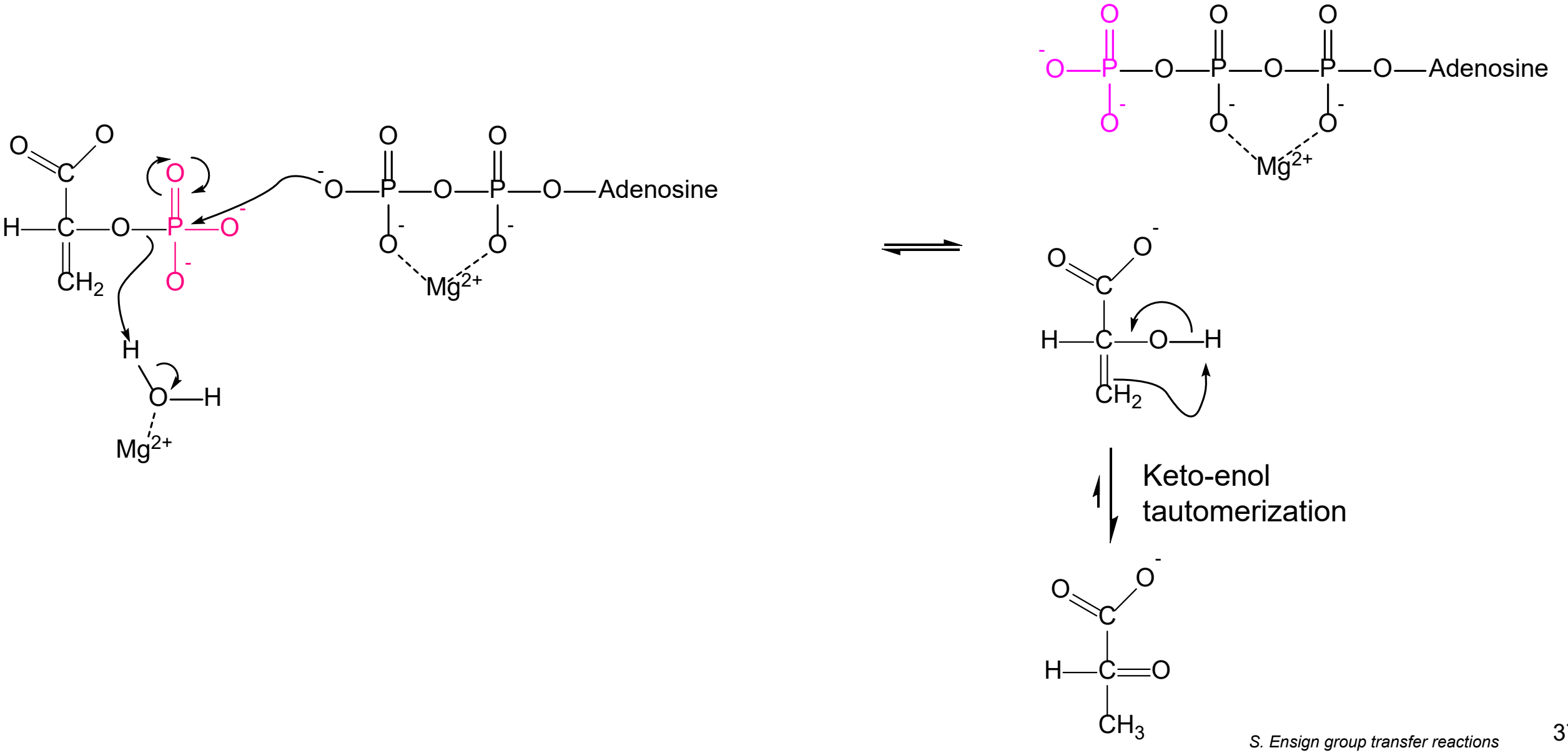
Phosphoglyceromutase



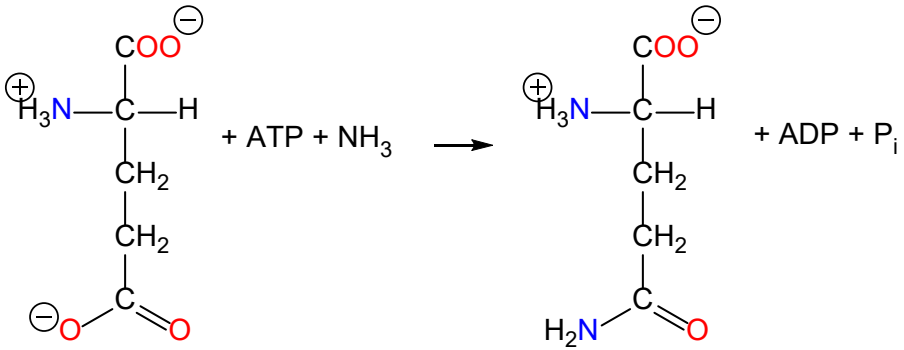
Substrate level phosphorylation: phosphoglycerate kinase

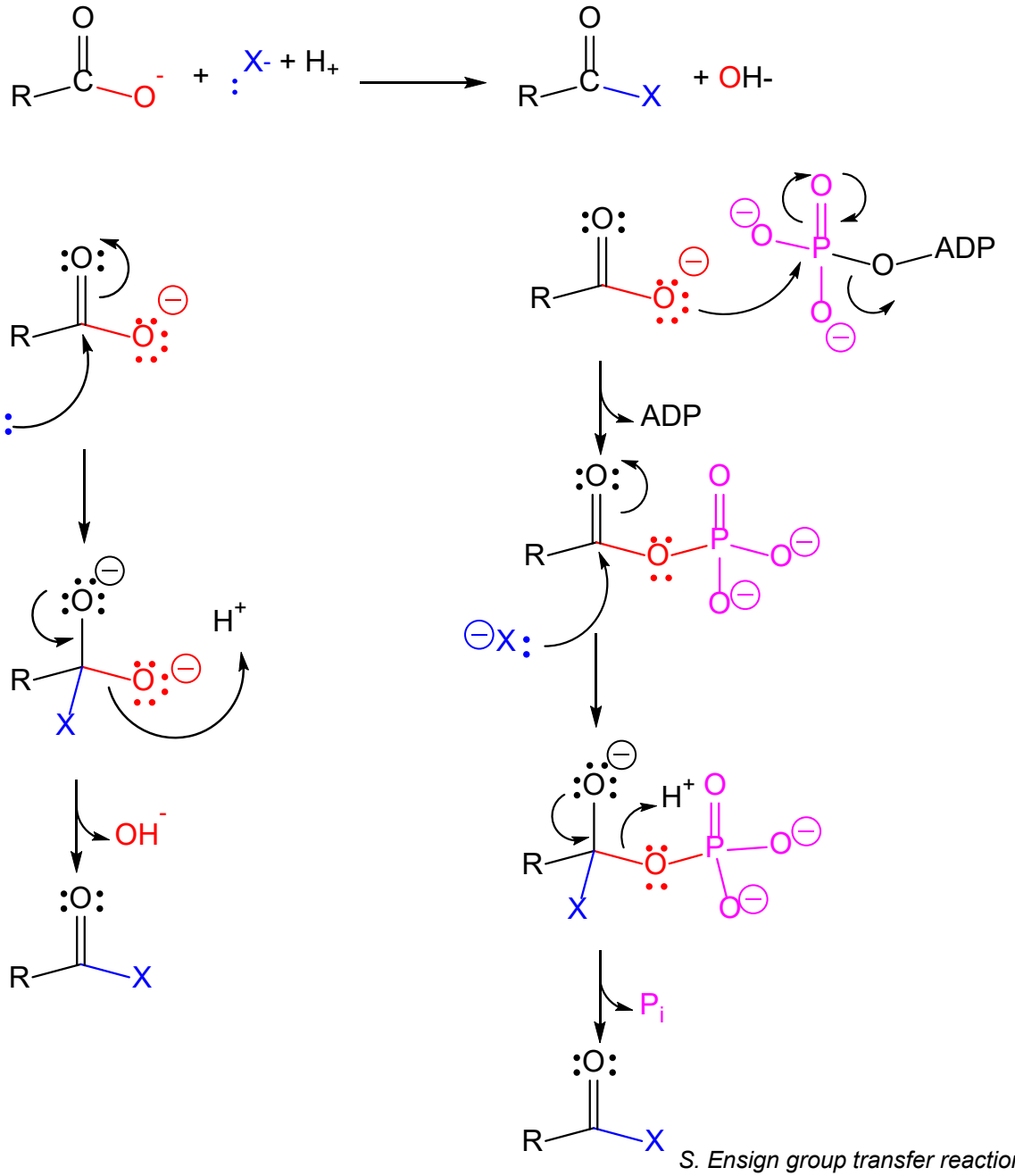


Substrate level phosphorylation: pyruvate kinase



Converting a poor leaving group to a good leaving group: glutamine synthetase

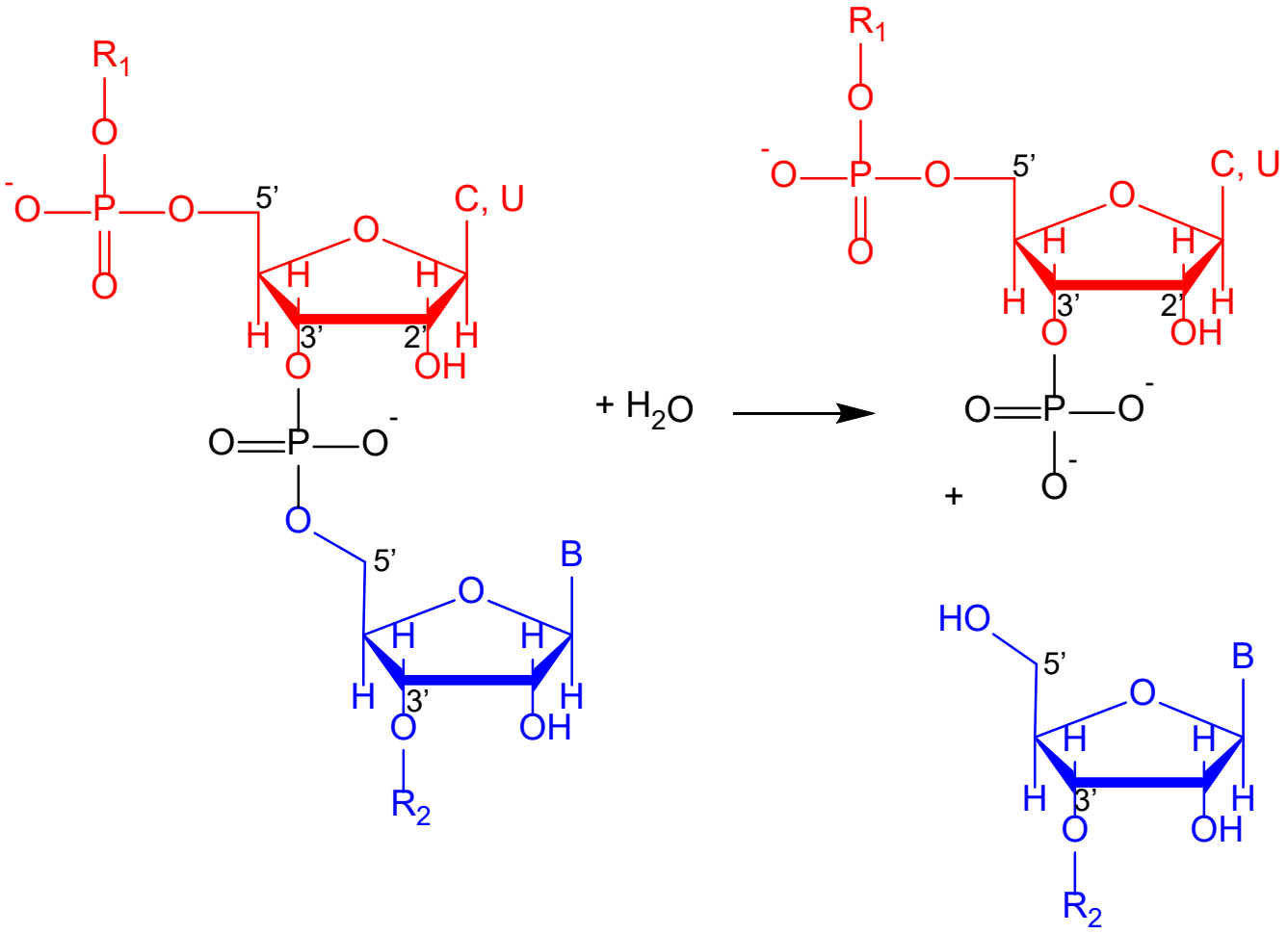




Phosphester group transfer: ribonuclease A

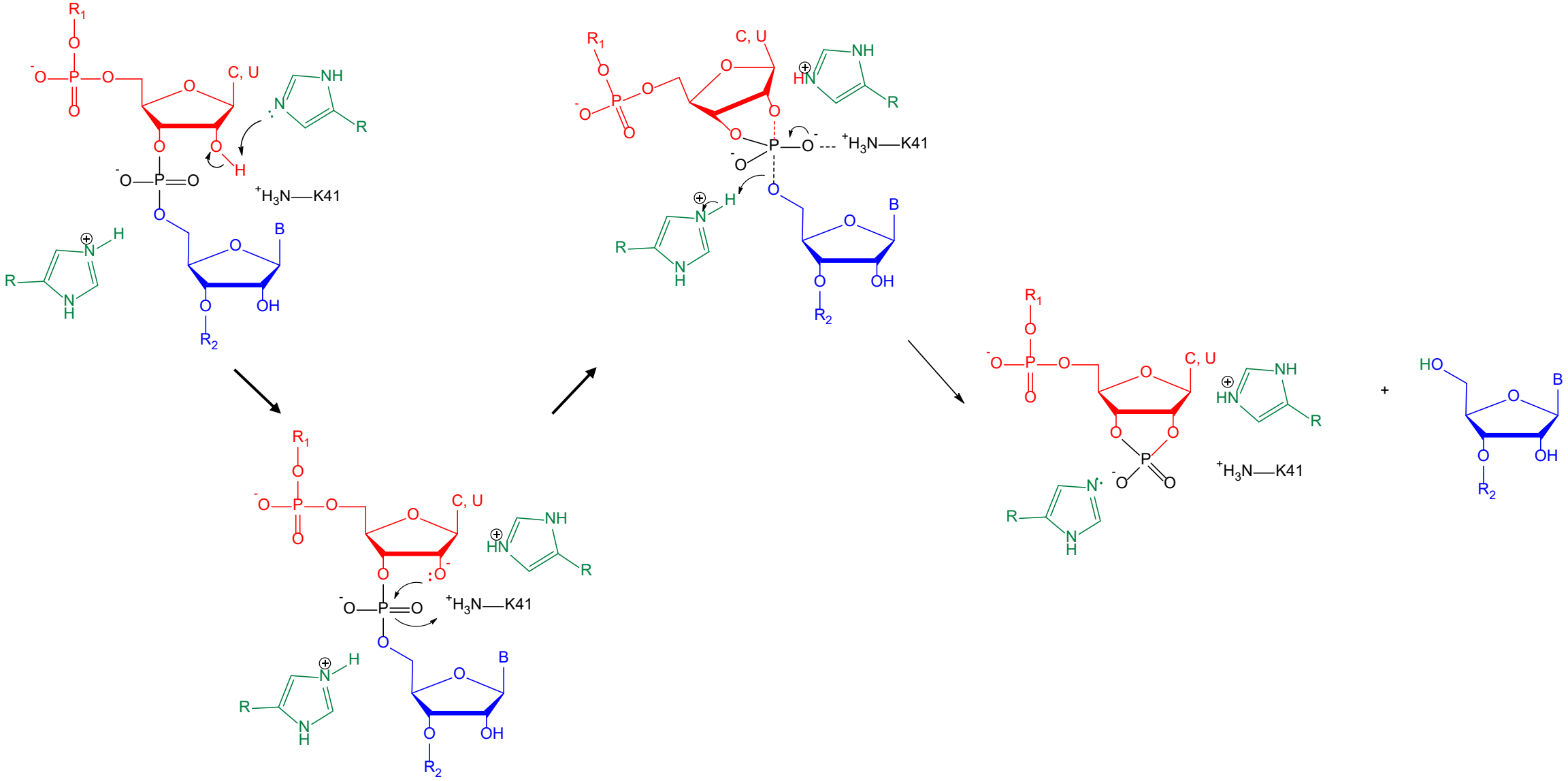
- 2’,3’-cyclic phosphate intermediate
- Two pentavalent transition states
  - cyclization, hydrolysis
  - 5-member ring spans axial, equatorial positions
- Two base mechanism
- In-line associative mechanism proposed for both the cyclization and hydrolysis steps

Ribonuclease A



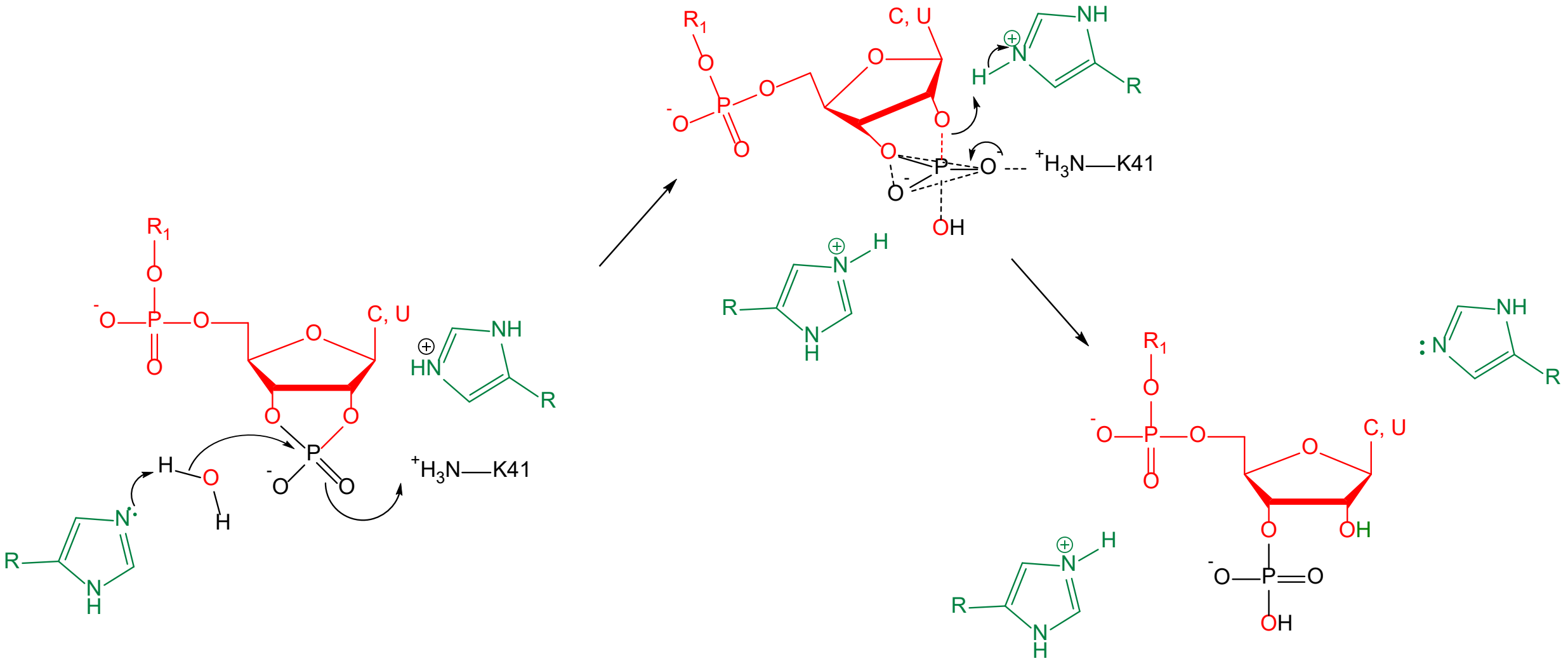
Ribonuclease A

- Formation of a 2’3’-cyclic phosphate intermediate



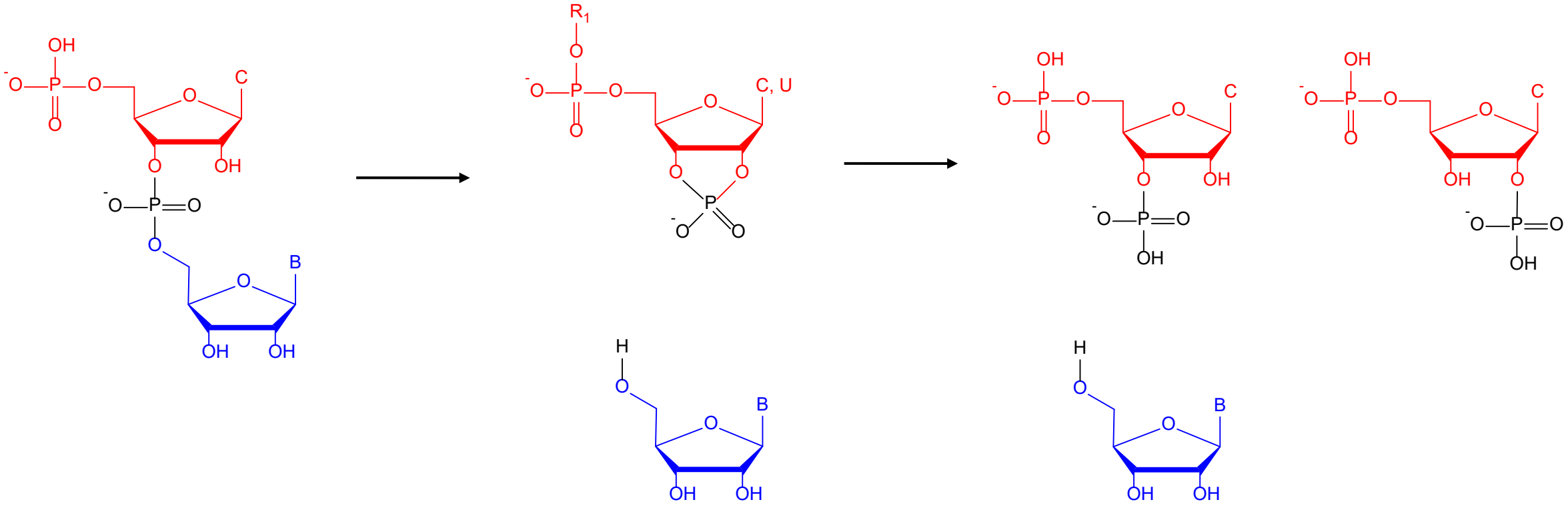
Ribonuclease A

- Hydrolysis of the 2’3’-cyclic phosphate intermediate



Evidences for the mechanism

- model (nonenzymatic) systems
  - Polynucleotides with KOH: mix of 2' and 3' phosphates. No 5' phosphate.
  - Deoxypolynucleotides with KOH
  - Cyclic intermediate with KOH



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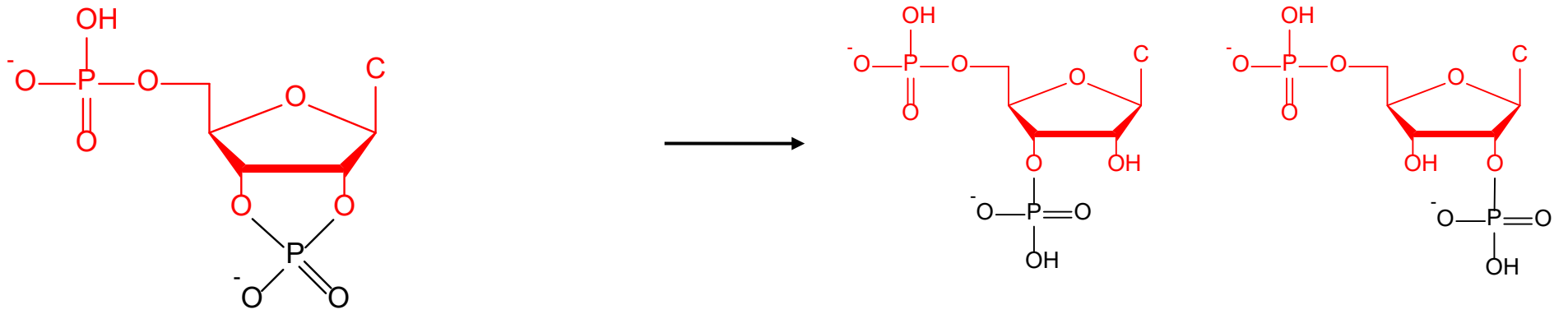

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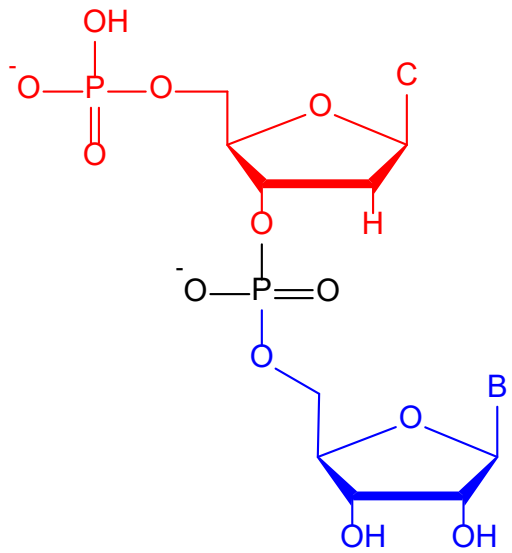
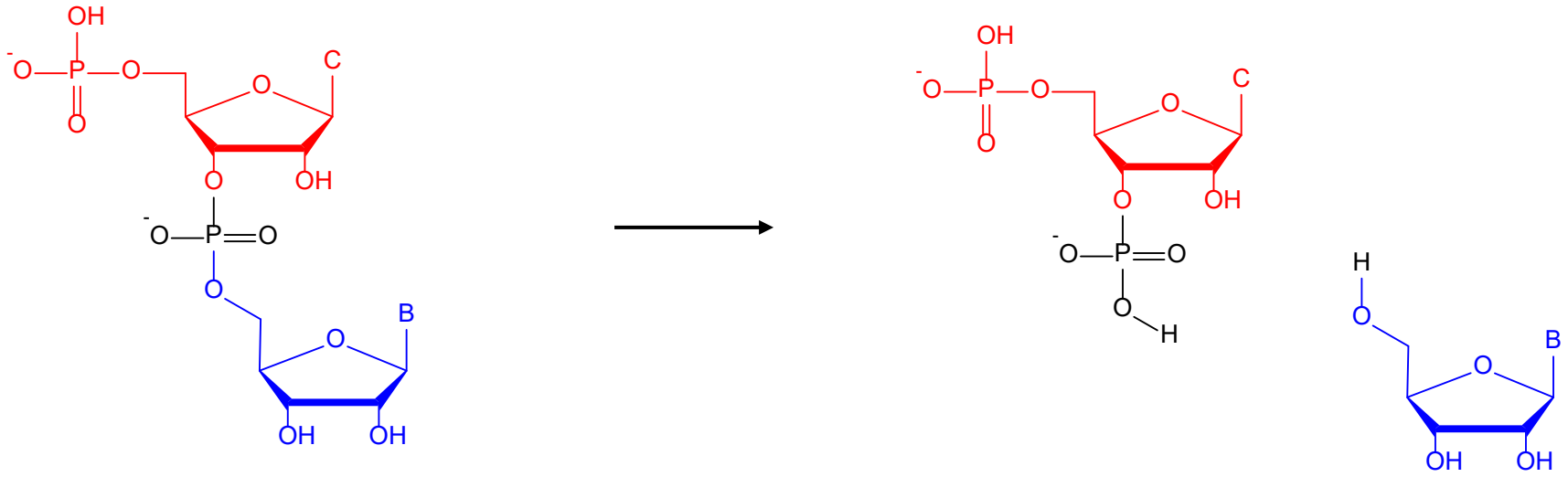
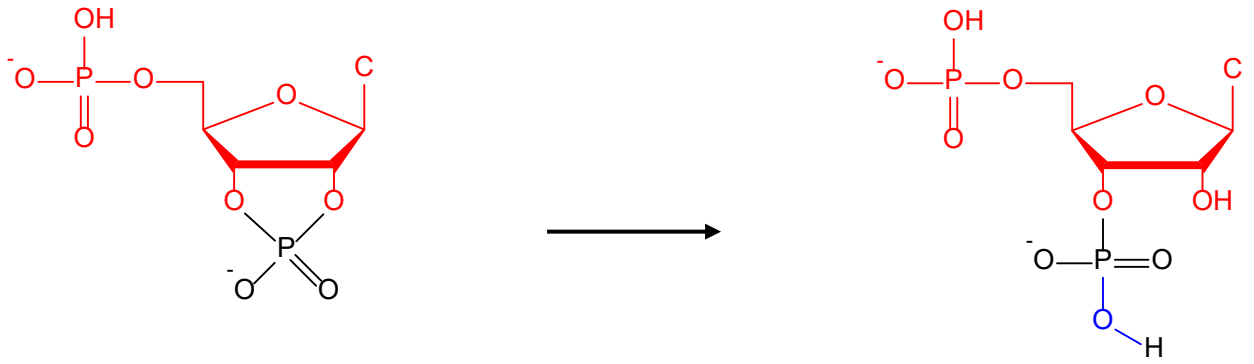


Evidences for the mechanism

- Enymatic activity with:
  - pCpN (poor substrate)



  - 5’-phosphocytidine 2’-3’cyclic monophosphate



Purposes of phosphorylated compounds

- Convert a poor leaving group to a good one
- Stabilizing structural element
- Increase H_2O solubility of hydrophobic molecules
- Offer reactive handle to biological catalysts
- Enzyme regulation