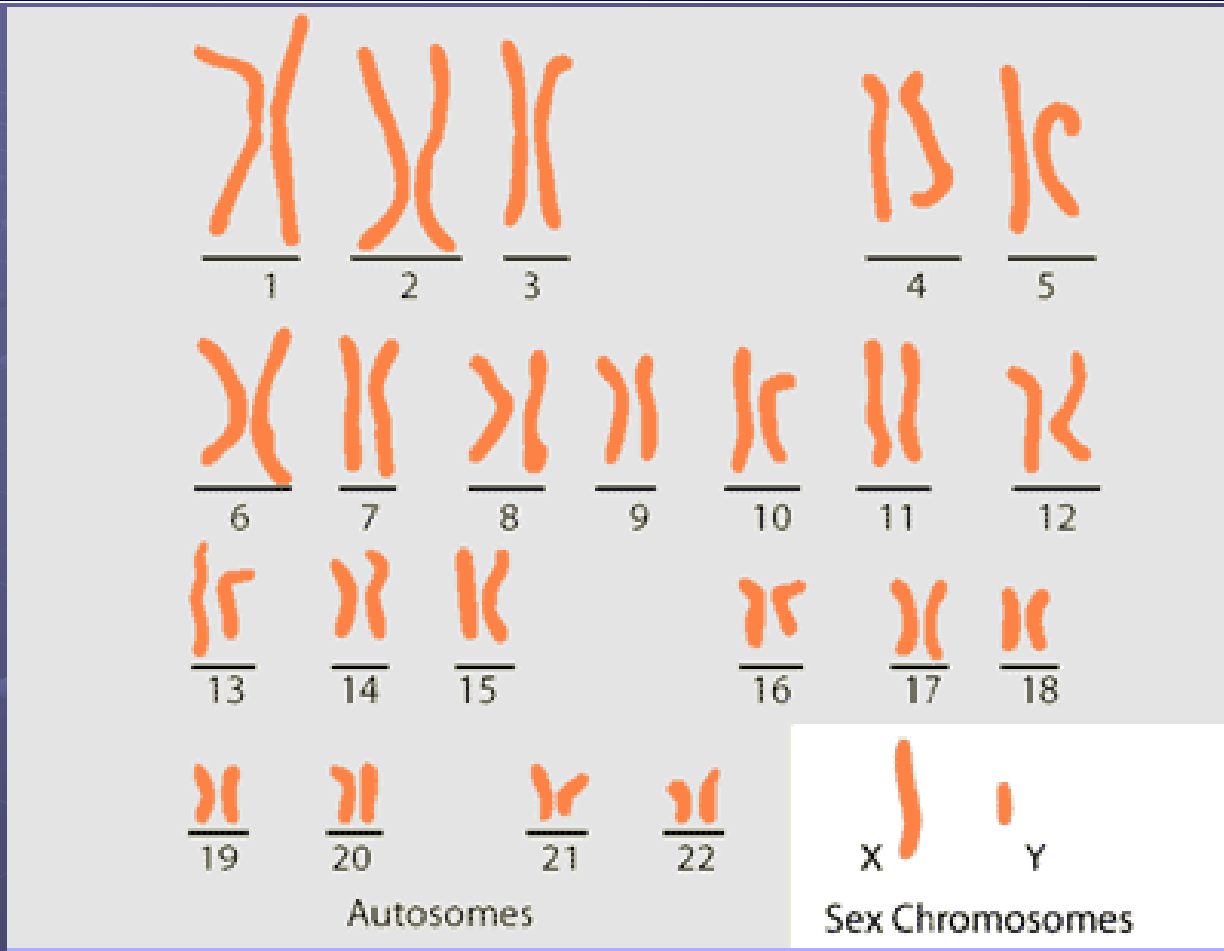
CHEMICAL SYNTHESIS OF BIOPOLYMERS

1. Genomics



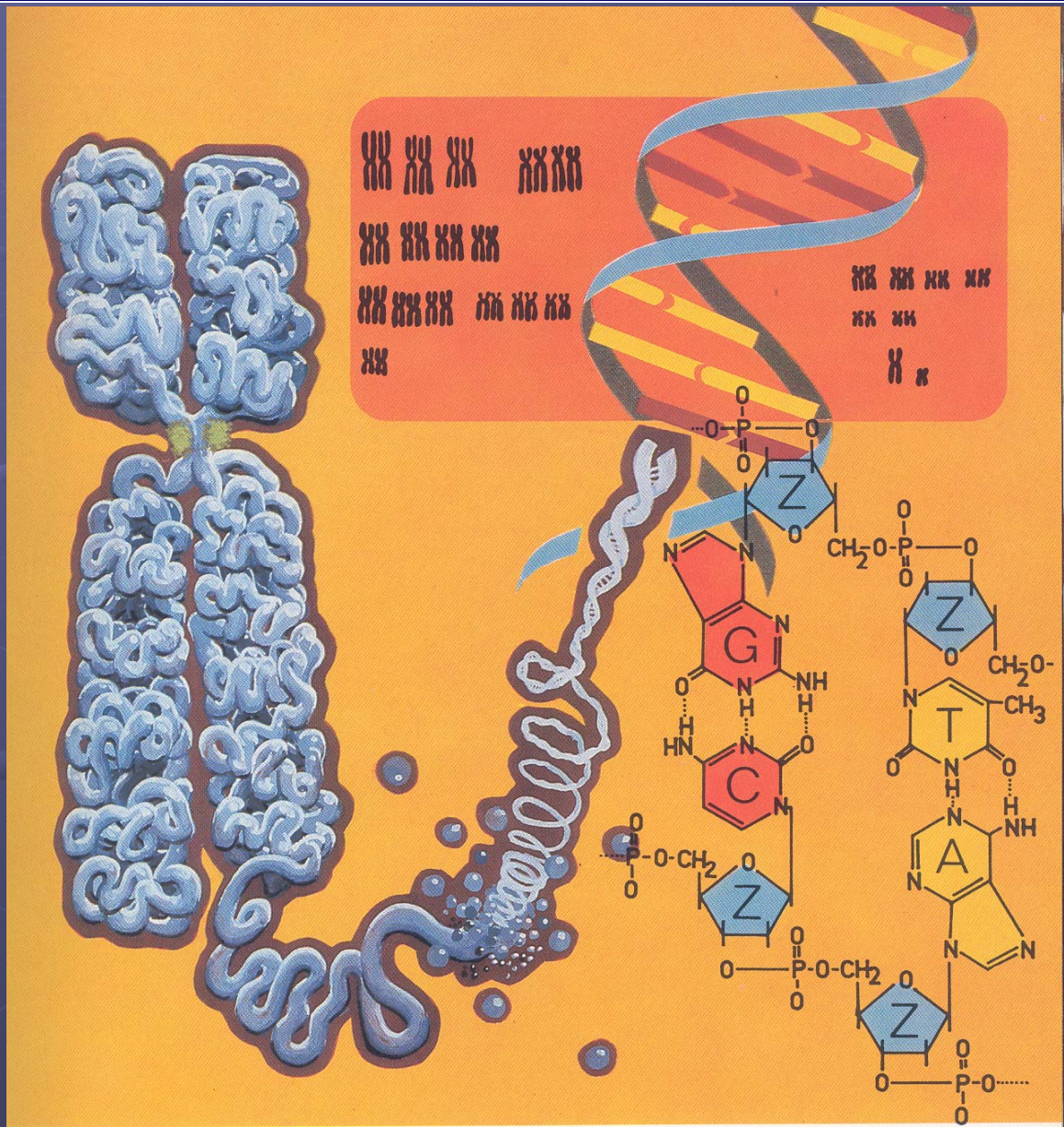
The Human Genome

The Human Genome is the total of the genetic information that is held in each human cell. It is usually made up of 46 chromosomes: 22 pairs of autosomes and 1 pair of sex chromosomes, which are usually X and X for females and X and Y for males.

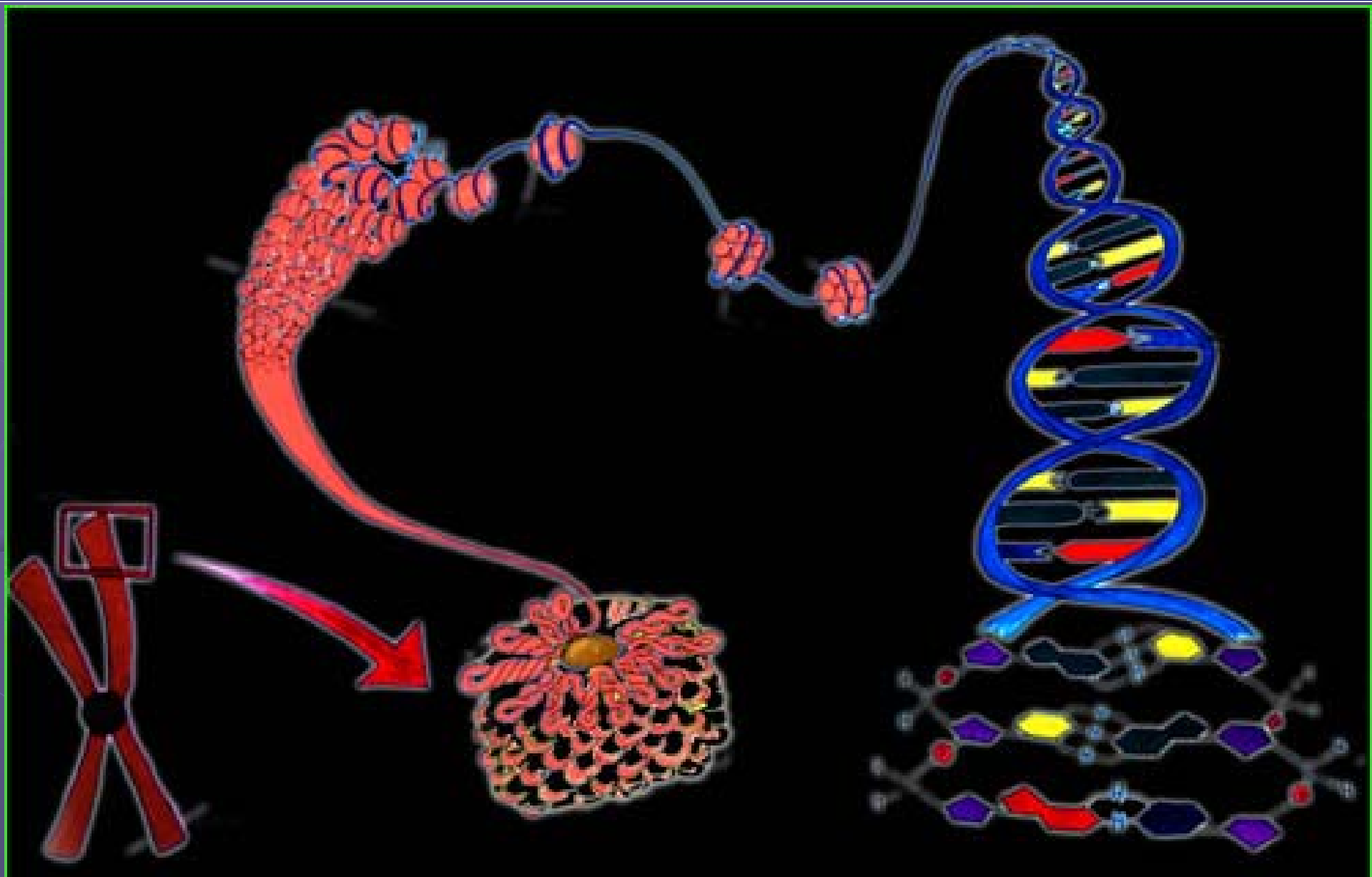
CHEMICAL SYNTHESIS OF BIOPOLYMERS

1.1 Structure and Chemistry

The architect's plans for protein synthesis are located at specific places of the DNA: **genes**.
The entirety of all genes of an organism is named **genome**.

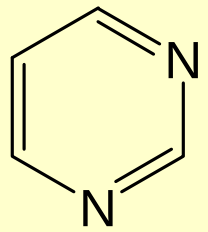


CHEMICAL SYNTHESIS OF BIOPOLYMERS

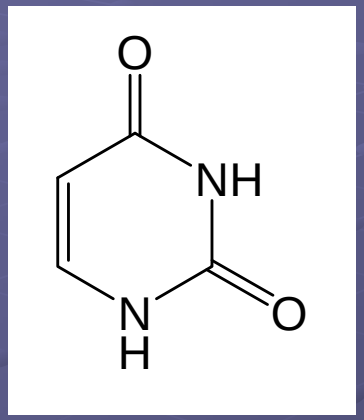


CHEMICAL SYNTHESIS OF BIOPOLYMERS

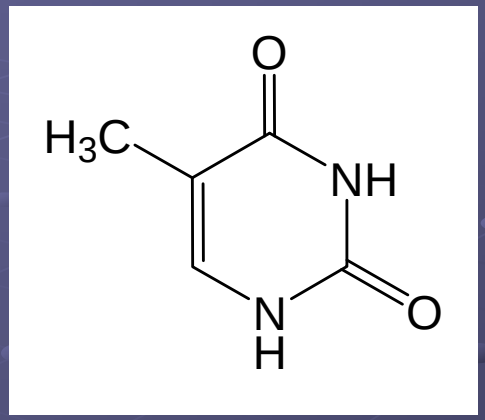
Nucleobasen



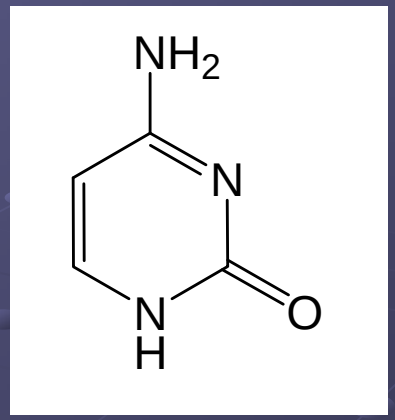
pyrimidine



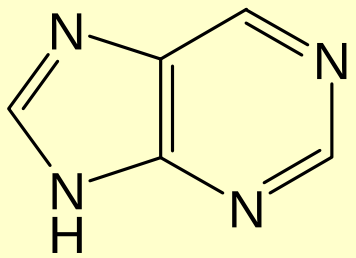
uracil
(RNA)



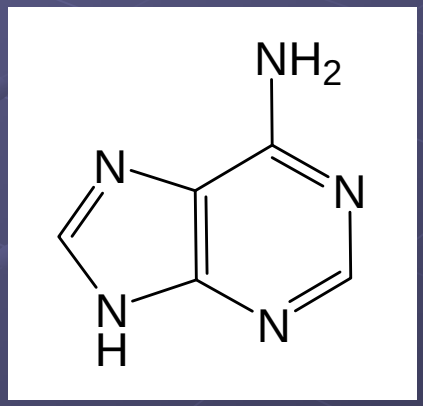
thymine
(DNA)



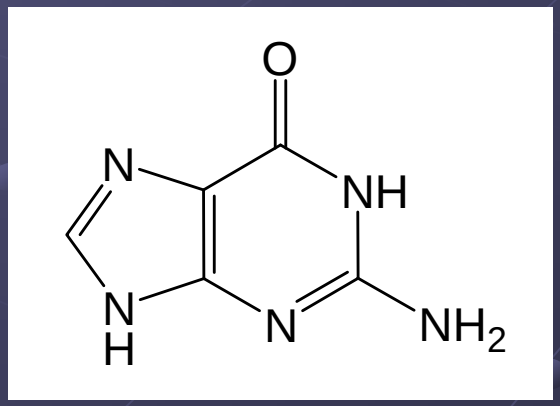
cytosine
(DNA,RNA)



purine



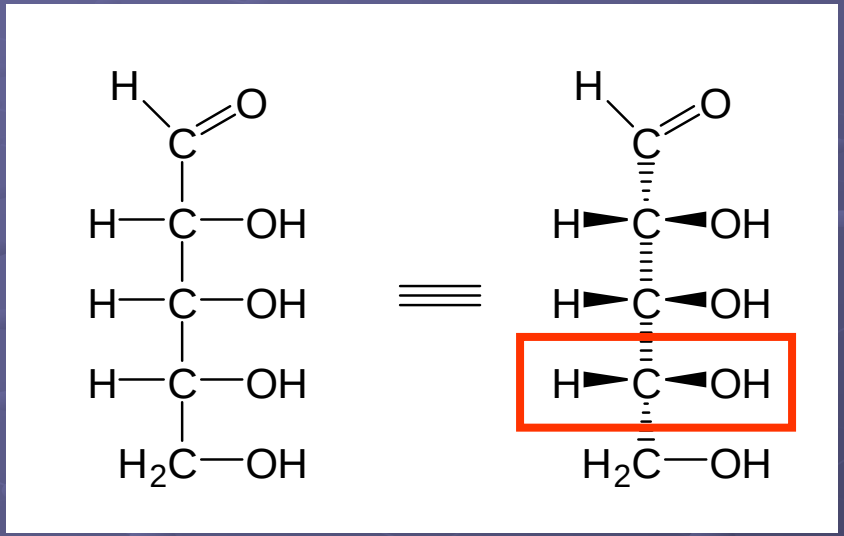
adenine
(DNA,RNA)



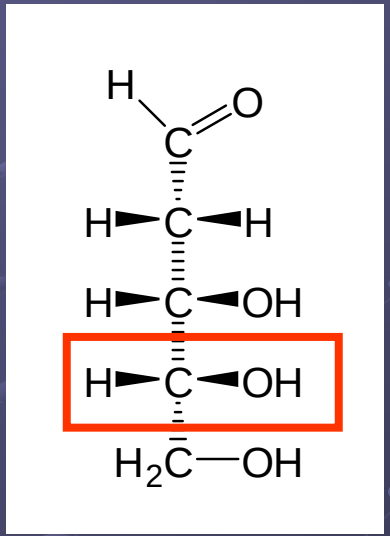
guanine
(DNA,RNA)

CHEMICAL SYNTHESIS OF BIOPOLYMERS

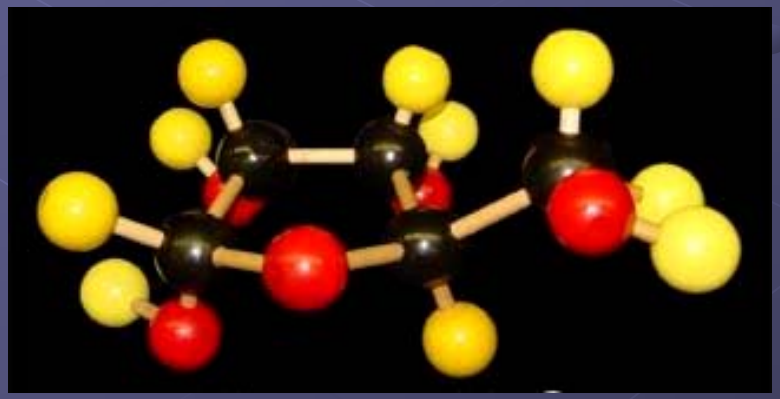
Pentosen

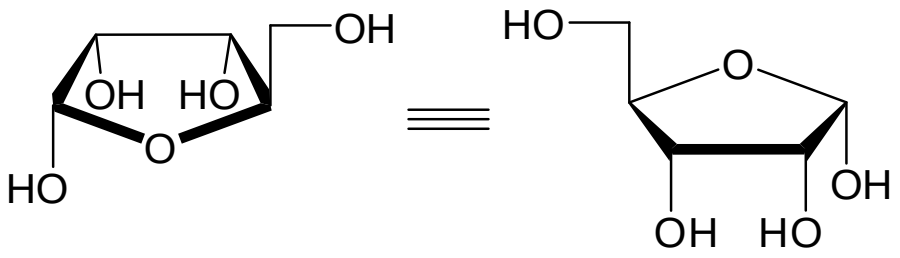


D-ribose
(RNA)



2-deoxy-D-ribose
(DNA)

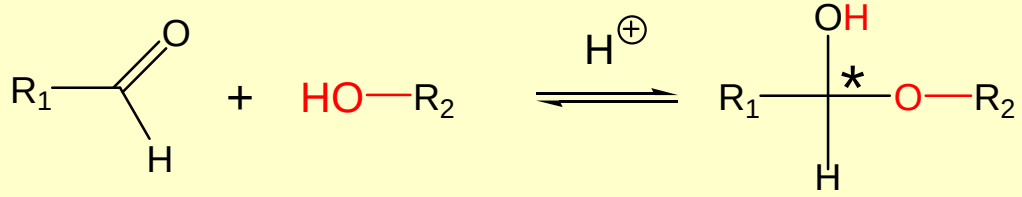


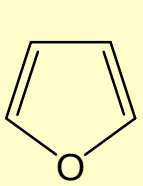


α-D-ribofuranose

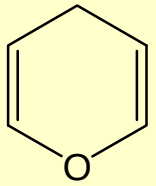
CHEMICAL SYNTHESIS OF BIOPOLYMERS

Chemie der Kohlenhydrate



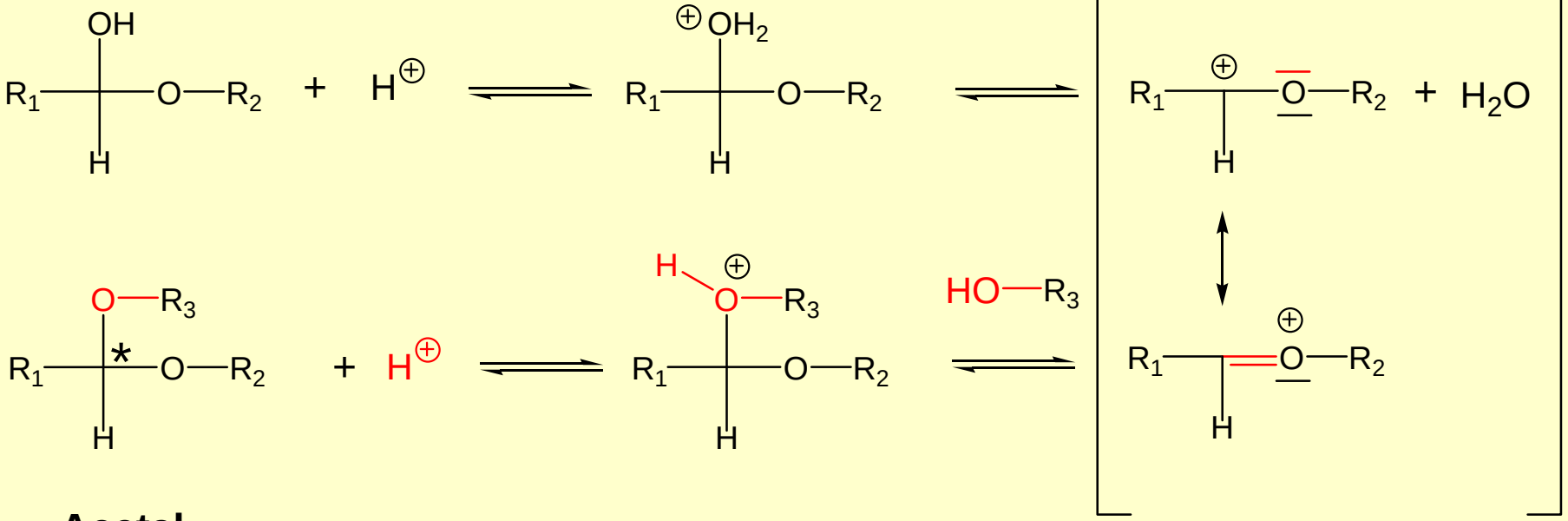


Furan



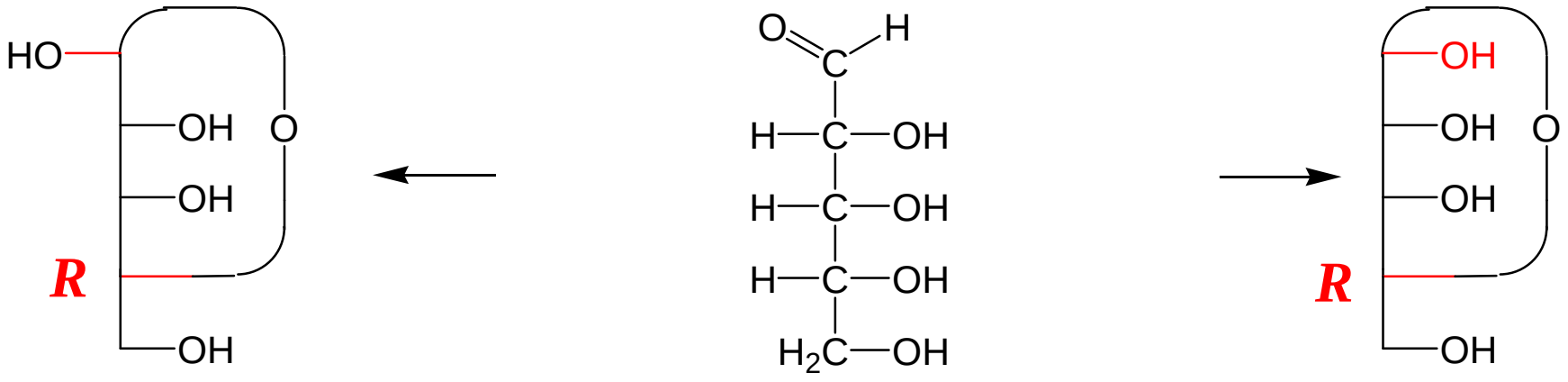
Pyran

Halbacetal

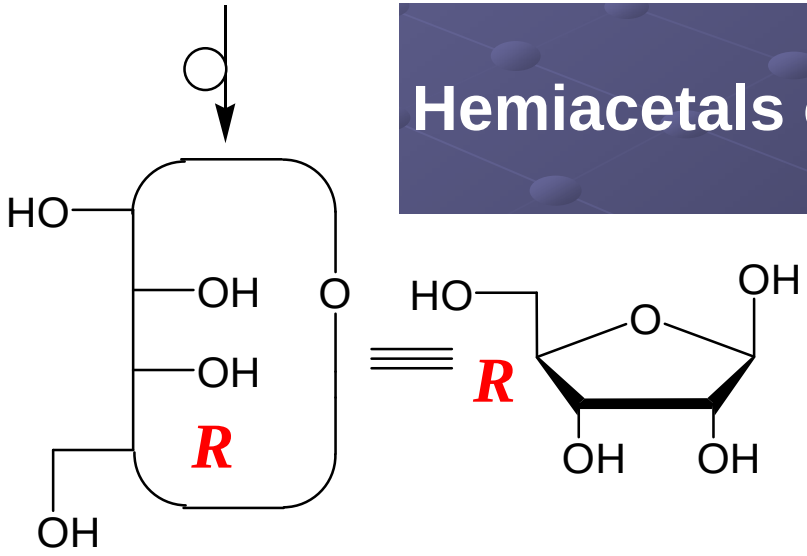


Acetal

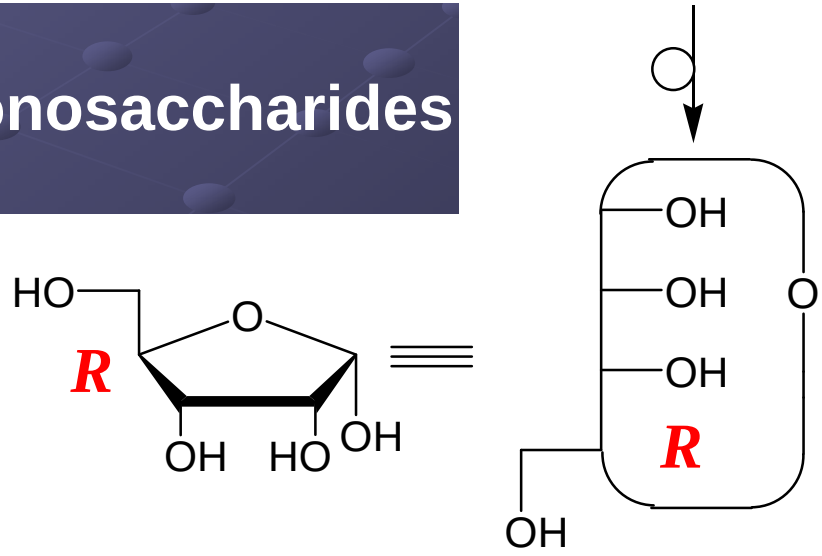
CHEMICAL SYNTHESIS OF BIOPOLYMERS



Hemiacetals of Monosaccharides

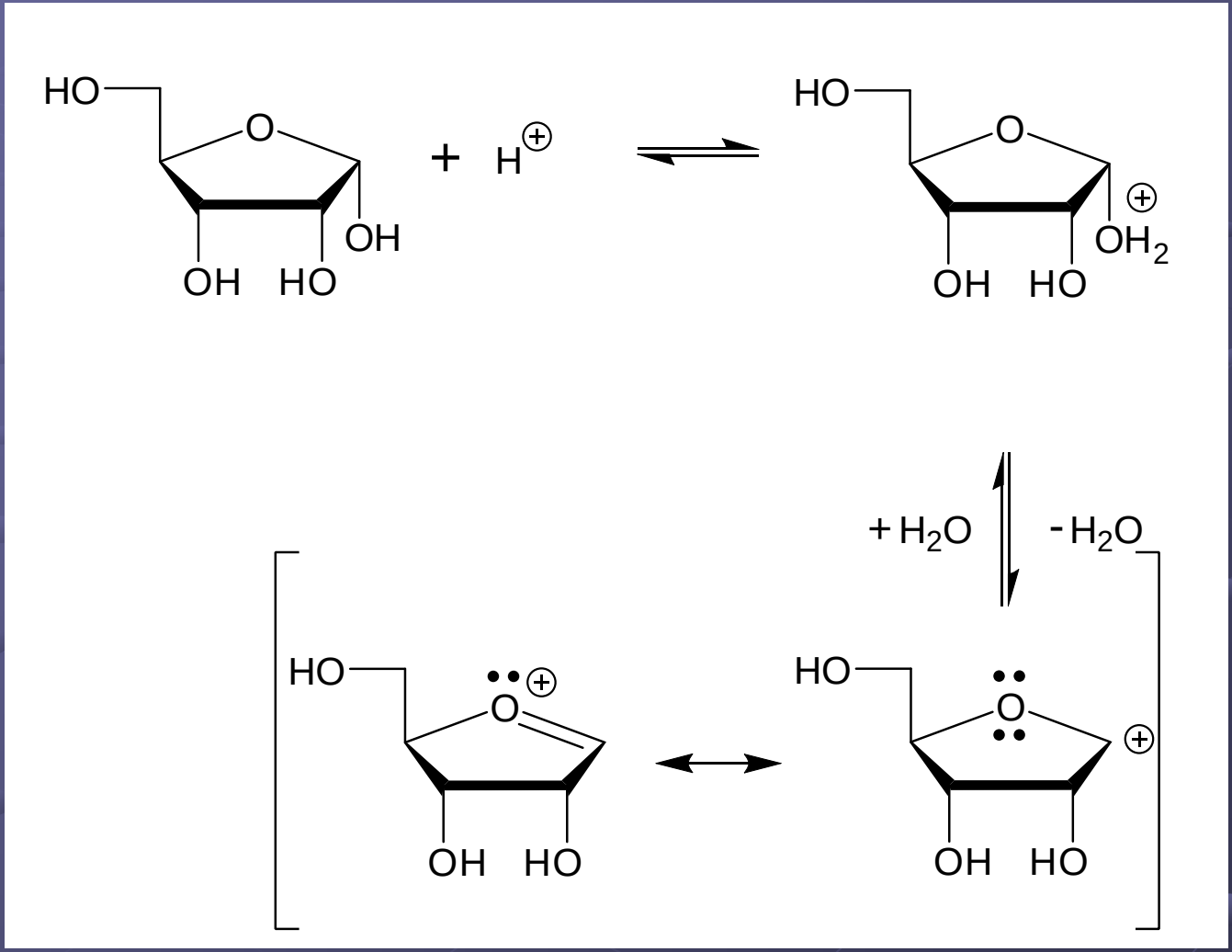


β-D-ribofuranose

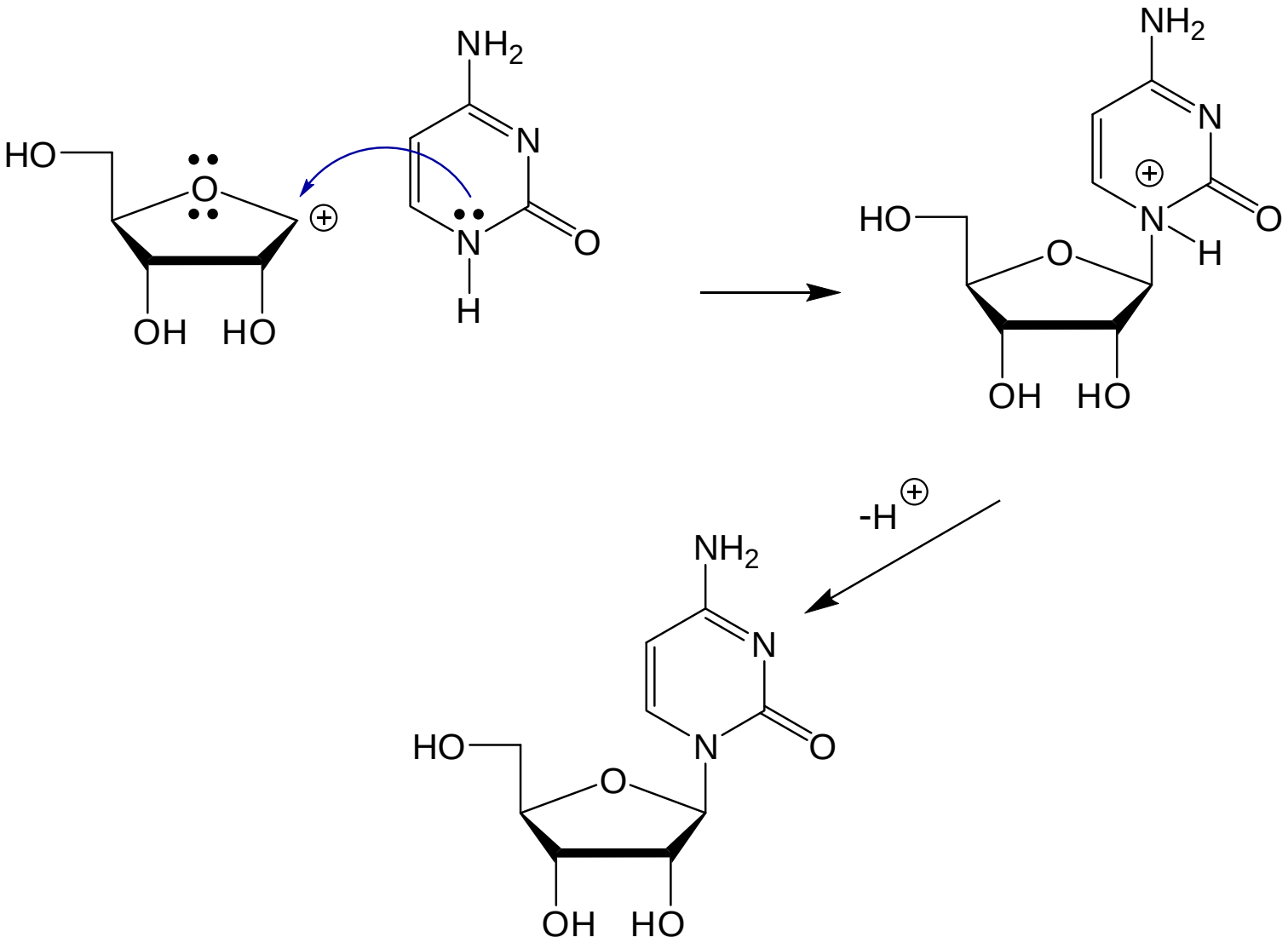


α-D-ribofuranose

CHEMICAL SYNTHESIS OF BIOPOLYMERS



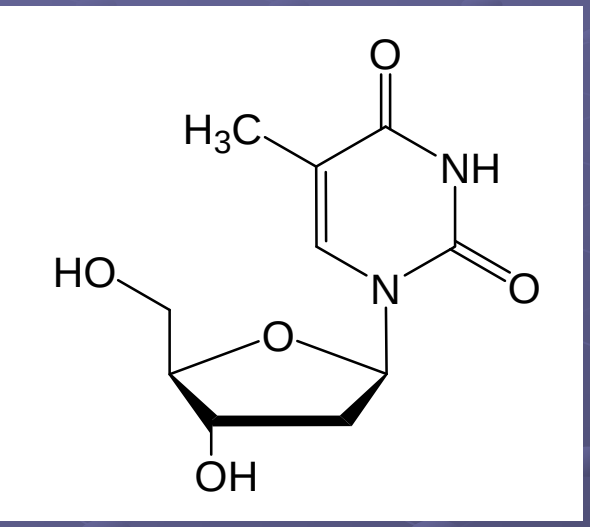
CHEMICAL SYNTHESIS OF BIOPOLYMERS



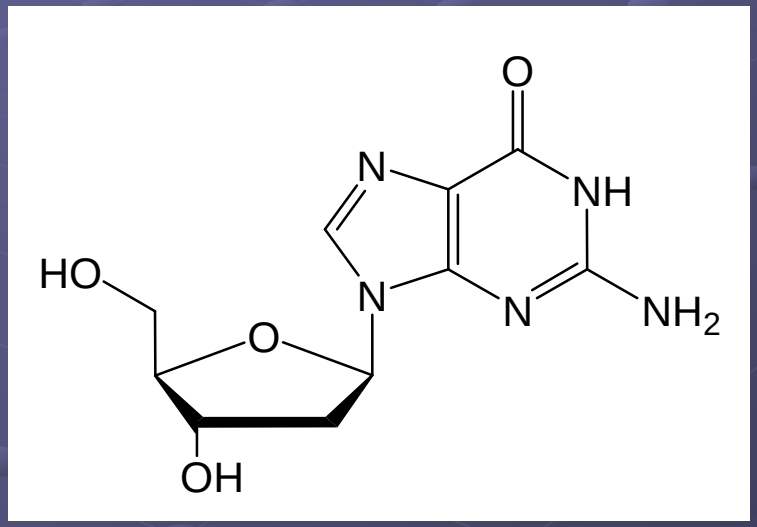
CHEMICAL SYNTHESIS OF BIOPOLYMERS

Nucleosides

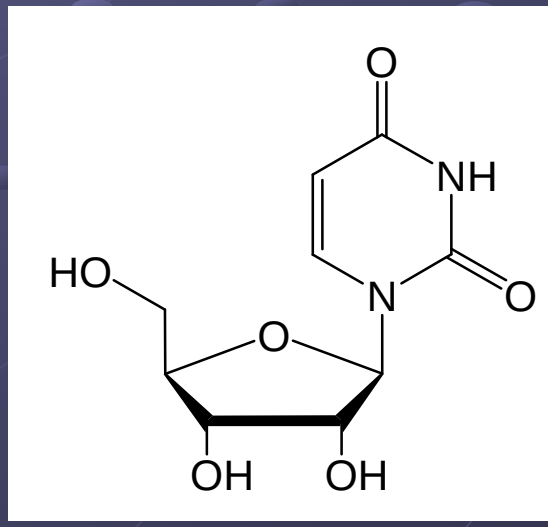
The purines and pyrimidines are bonded to the anomeric carbon of the furanose ring In a β-*N*-glycosidic linkage.



thymidine (T)



deoxy-guanosine (dG)

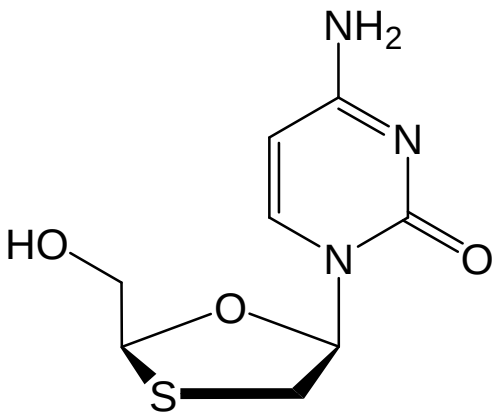


uridine (U)

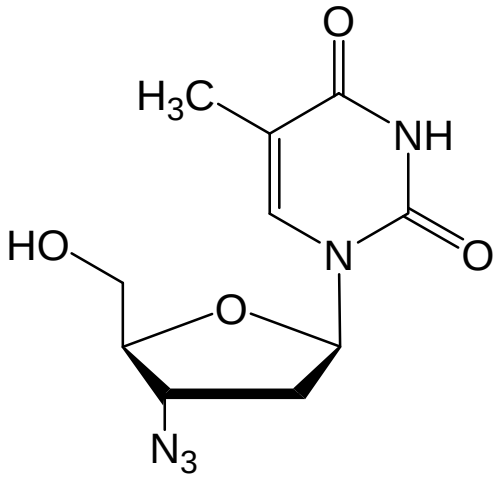
In the case of pyrimidine bases the nucleosides get the ending –***idine***, for example **cytidine**, **uridine** or **thymidine**, while the purine bases marked by the ending ***–osine***, for example **adenosine** and **guanosine**.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

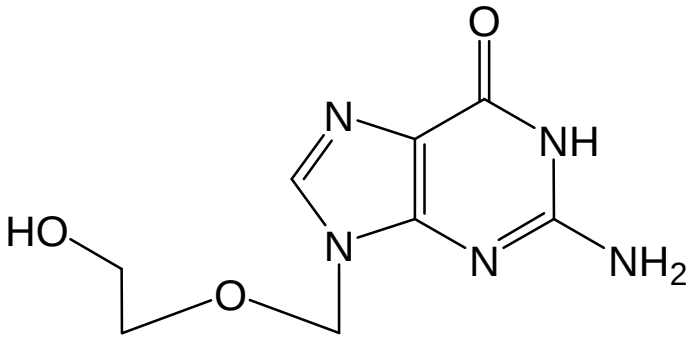
Nucleoside Analogues



thiacytidine



azidothymidine



aciclovir

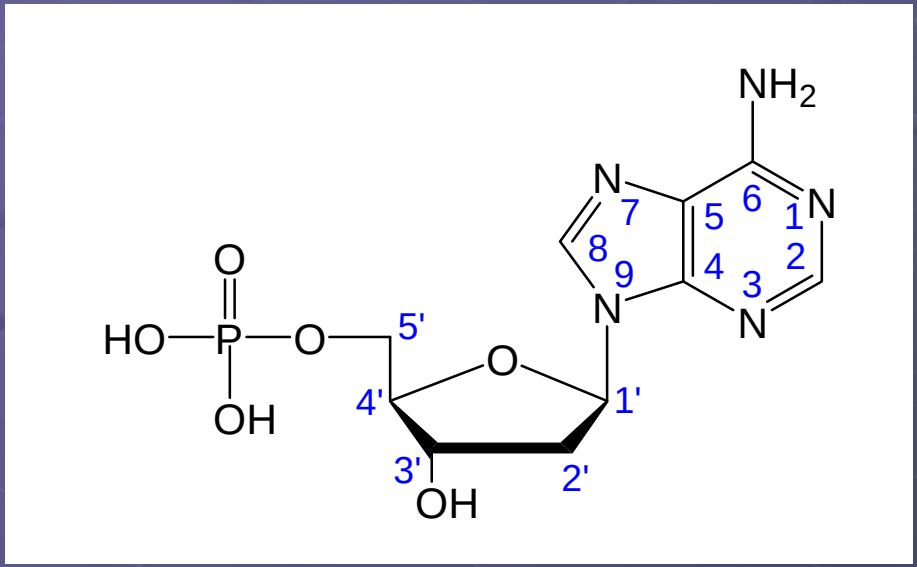
drugs against HIV

drug against herpes infection

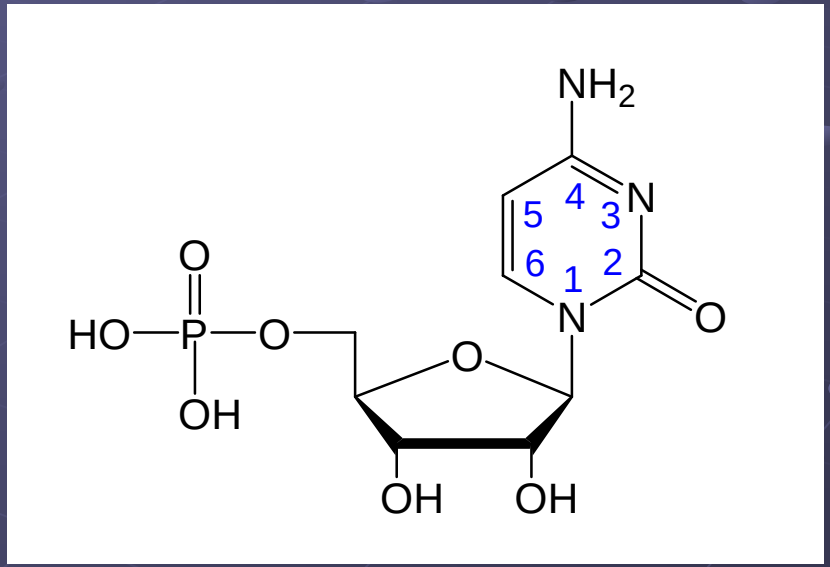
CHEMICAL SYNTHESIS OF BIOPOLYMERS

Nucleotides

A **nucleotide** is a nucleoside with either the 5‘- or 3‘-OH group bonded in an ester linkage to phosphoric acid.



2-deoxy-adenosine-5‘-monophosphate



cytidine-5‘-monophosphate

The **nucleotides of RNA** are more precisely called **ribonucleotides**, whereas the **nucleotides of DNA** are called **deoxyribonucleotides**.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

1.2 Chemical Synthesis of Nucleosides

It remains more economical to produce the major nucleosides by degrading nucleic acids than by total synthesis.

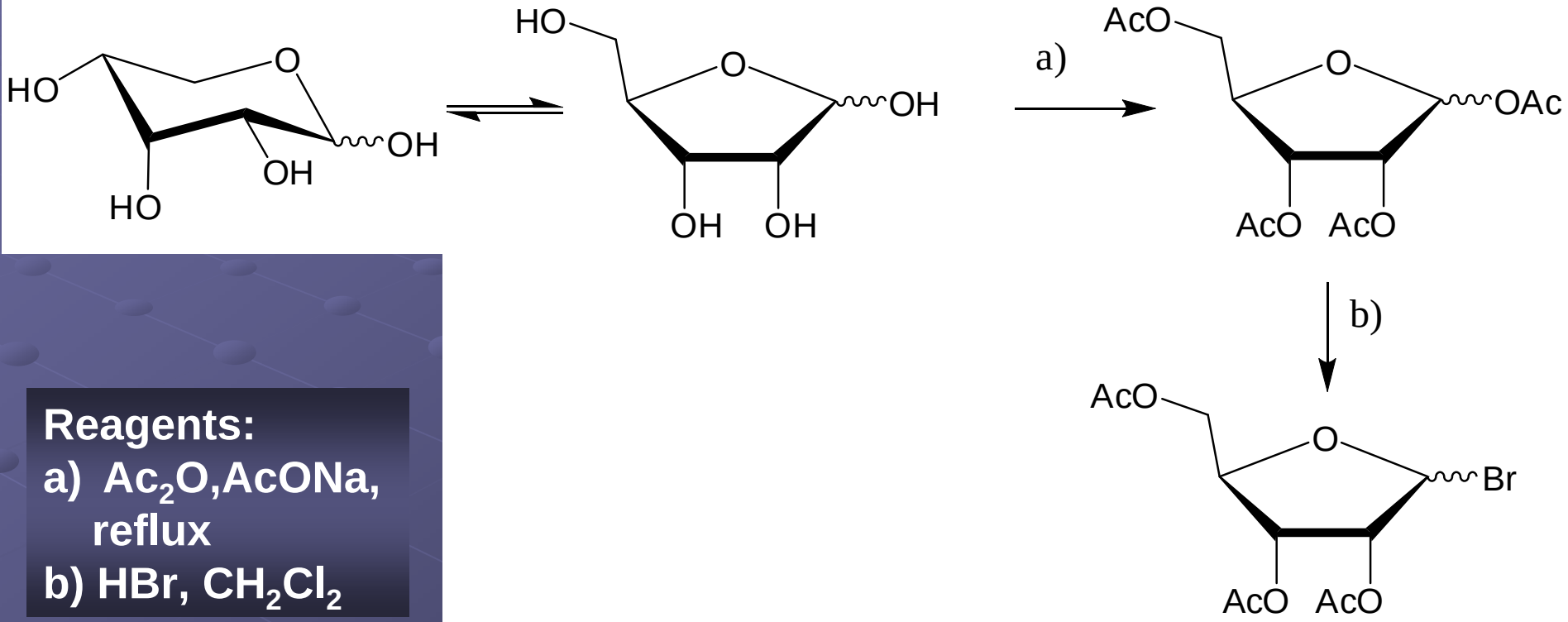
Modified nucleosides are widely distributed naturally, therefore chemical synthesis makes sense.

Fischer, Helferich, Königs and **Knorr** introduced the use of a heavy metal salt of a purine to catalyse the nucleophilic displacement of a halogen substituent from C-1 of an acylated sugar.

Hermann Emil Fischer
Nobel Prize in Chemistry
1902

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Synthesis of D-ribose tetraacetate and of the corresponding bromide

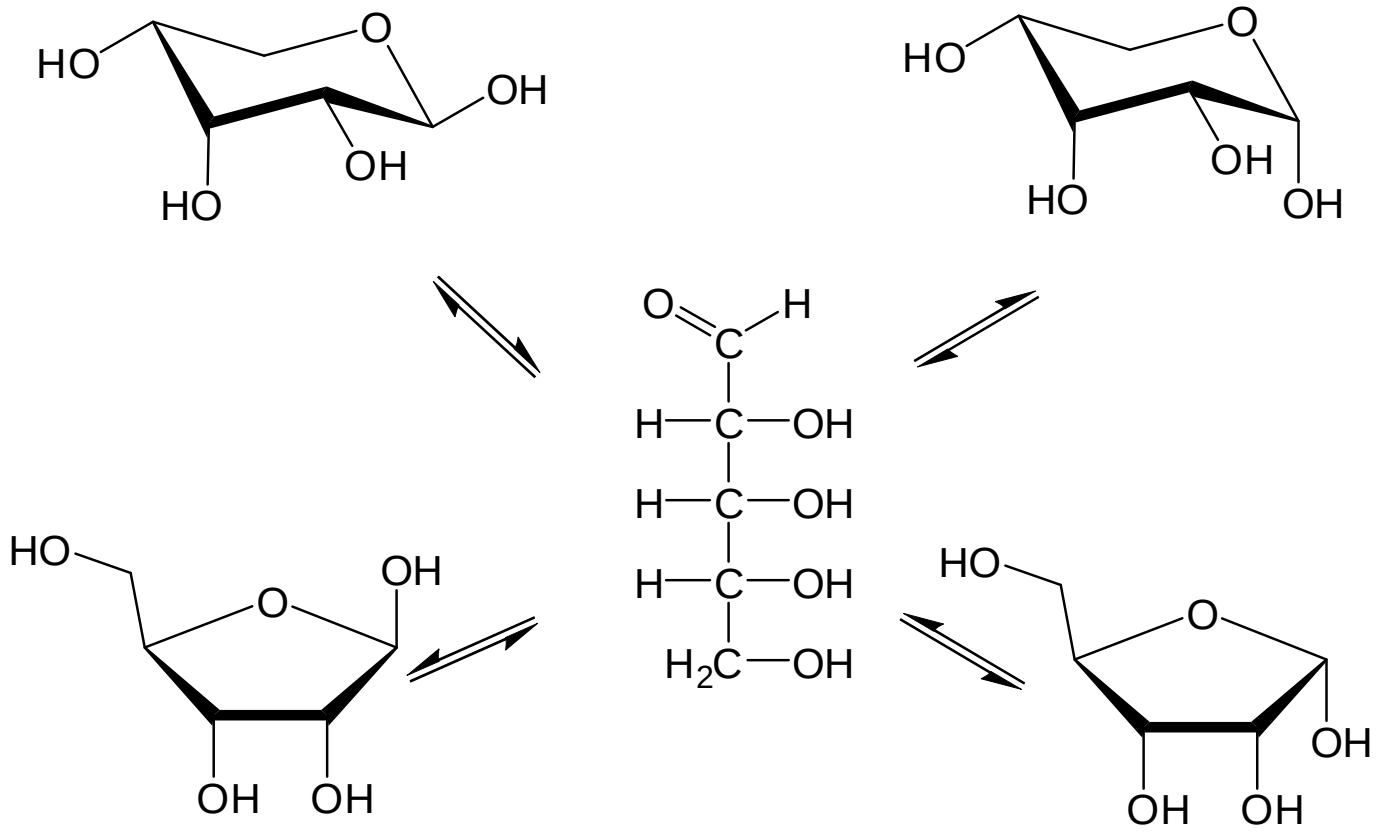


Reagents:

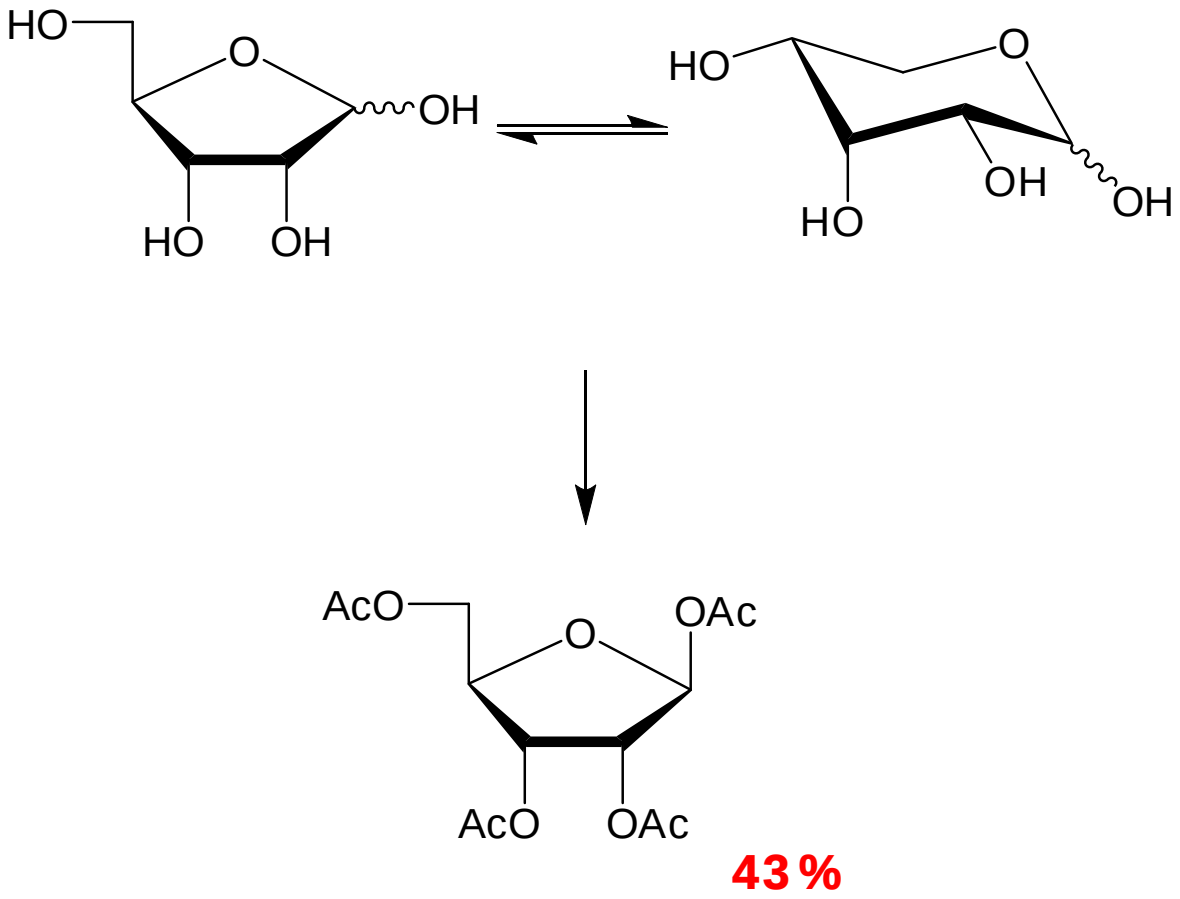
a) Ac_2O,AcONa, reflux

b) HBr, CH_2Cl_2

CHEMICAL SYNTHESIS OF BIOPOLYMERS



CHEMICAL SYNTHESIS OF BIOPOLYMERS



Prof. Dr. Helmut Zinner

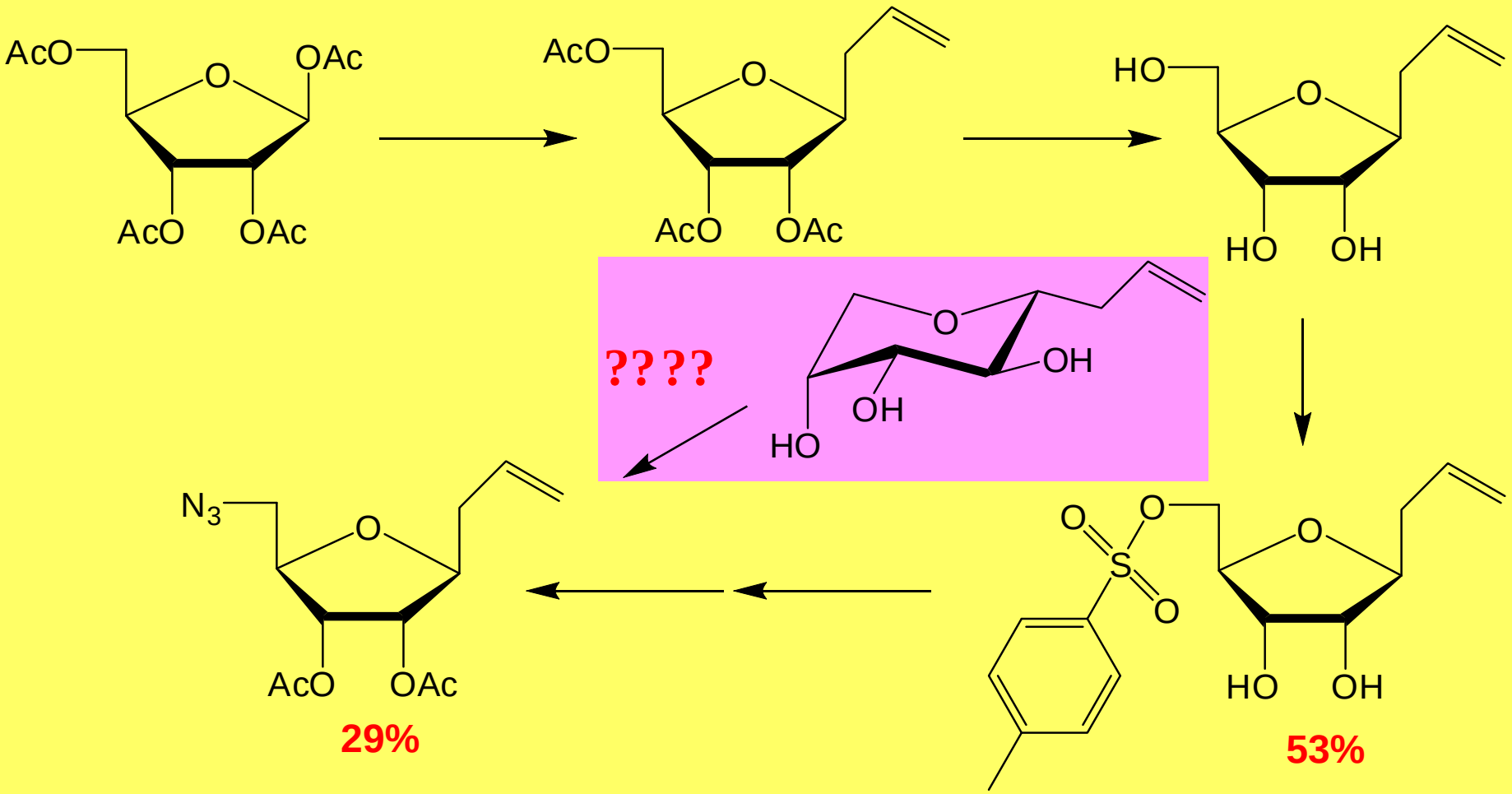
Zinner, H. *Chem. Ber.* **1953,** *86*, 817-824.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Tetra-*O*-acetyl-β-D-ribopyranose can be obtained at 0 °C by acetylation with acetic anhydride in pyridine in more than 80% yield.

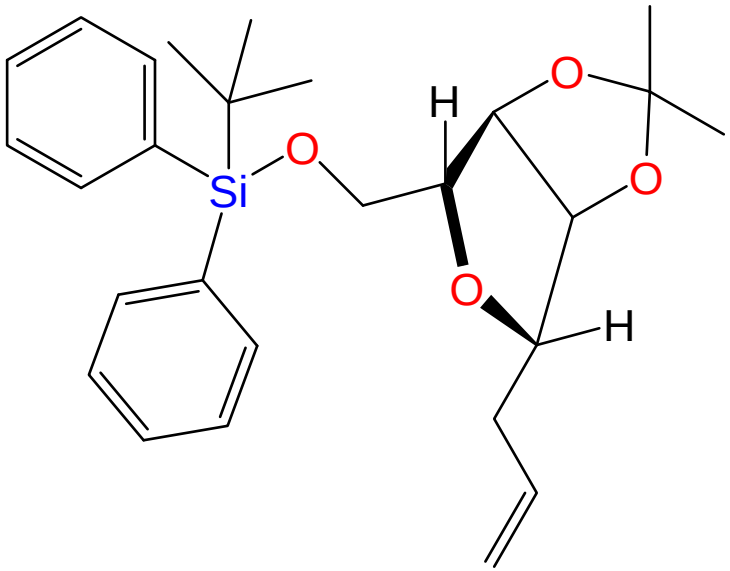
Tetra-*O*-acetyl-β-D-ribofuranose can be obtained after heating under reflux for 3 min in a mixture of acetic anhydride and sodium acetate by fractionated crystallization in 43% yield.

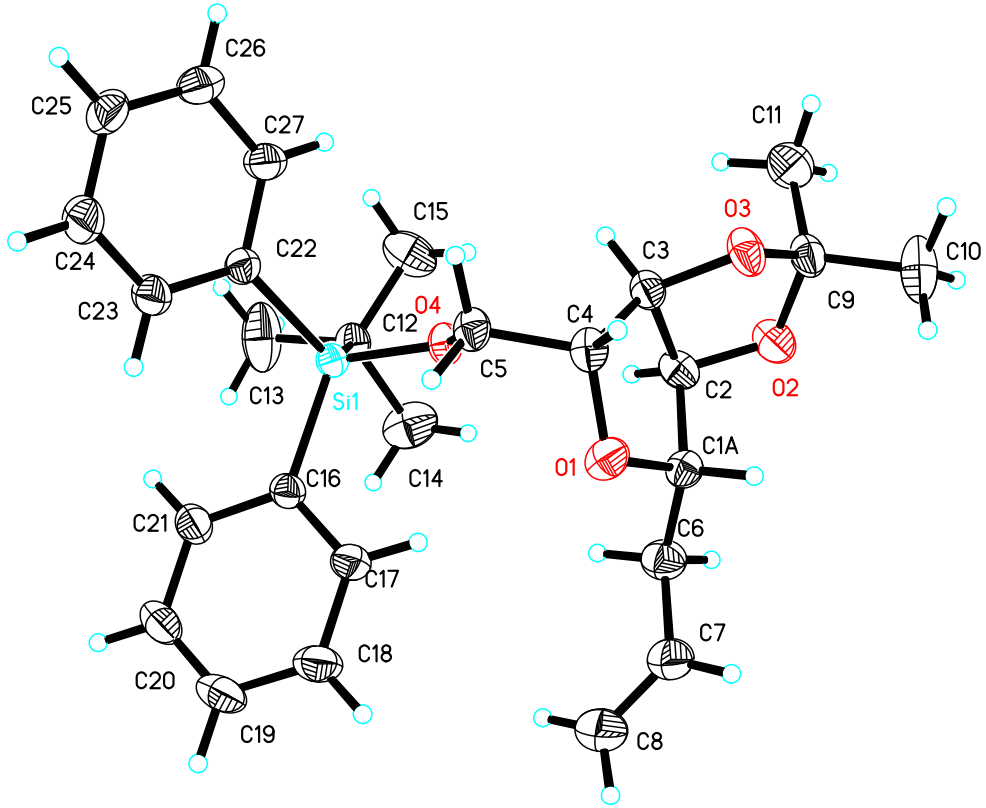
CHEMICAL SYNTHESIS OF BIOPOLYMERS



McDevitt, J. P.; Lansbury, P. T. Jr. *J. Am. Chem. Soc.* **1996**, *118*, 3818-3828.

CHEMICAL SYNTHESIS OF BIOPOLYMERS





CHEMICAL SYNTHESIS OF BIOPOLYMERS

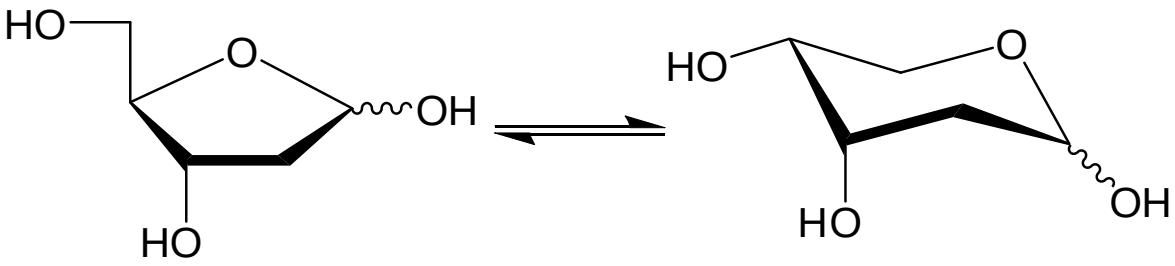
…. from the chaos a voice spoke to me:

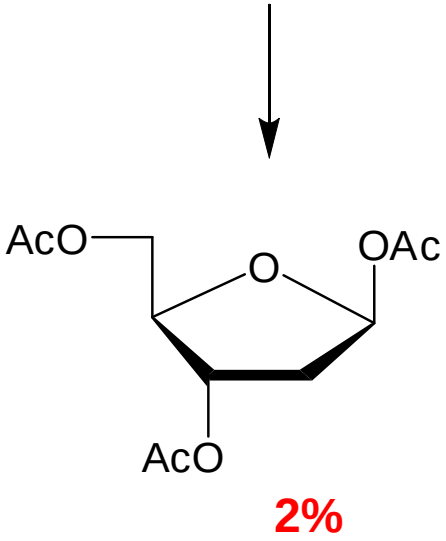
“Smile and be happy, things could always be worse!”

…. and I smiled and was happy, and they got worse”

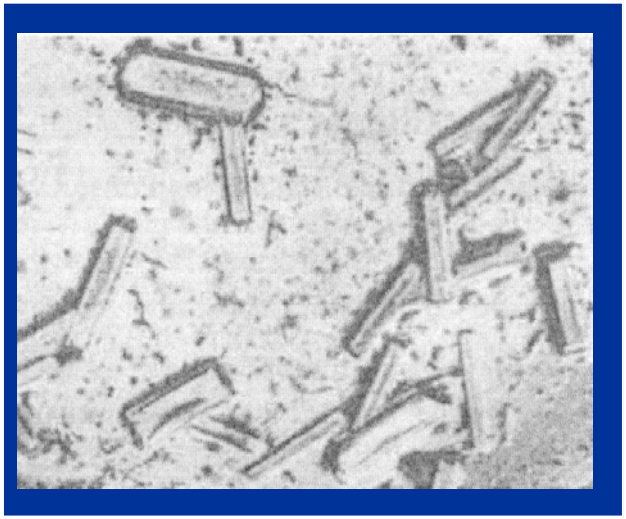
1,3,5-tri-*O*-acetyl-2-deoxy-β-D-ribofuranose was obtained by short acetylation at higher temperature after 18 months in **2% yield** by collection of crystals with a specific form.

CHEMICAL SYNTHESIS OF BIOPOLYMERS



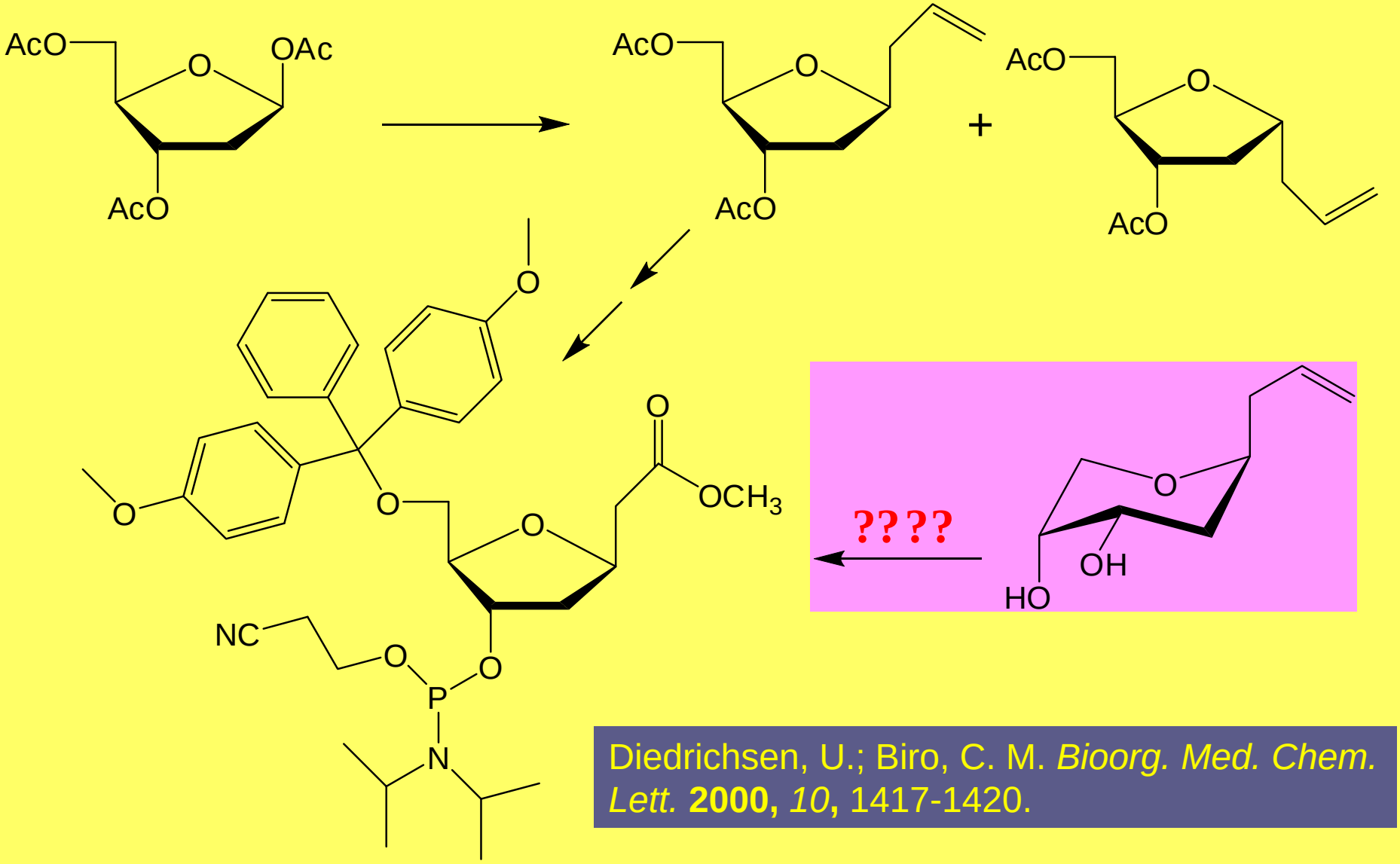


2%



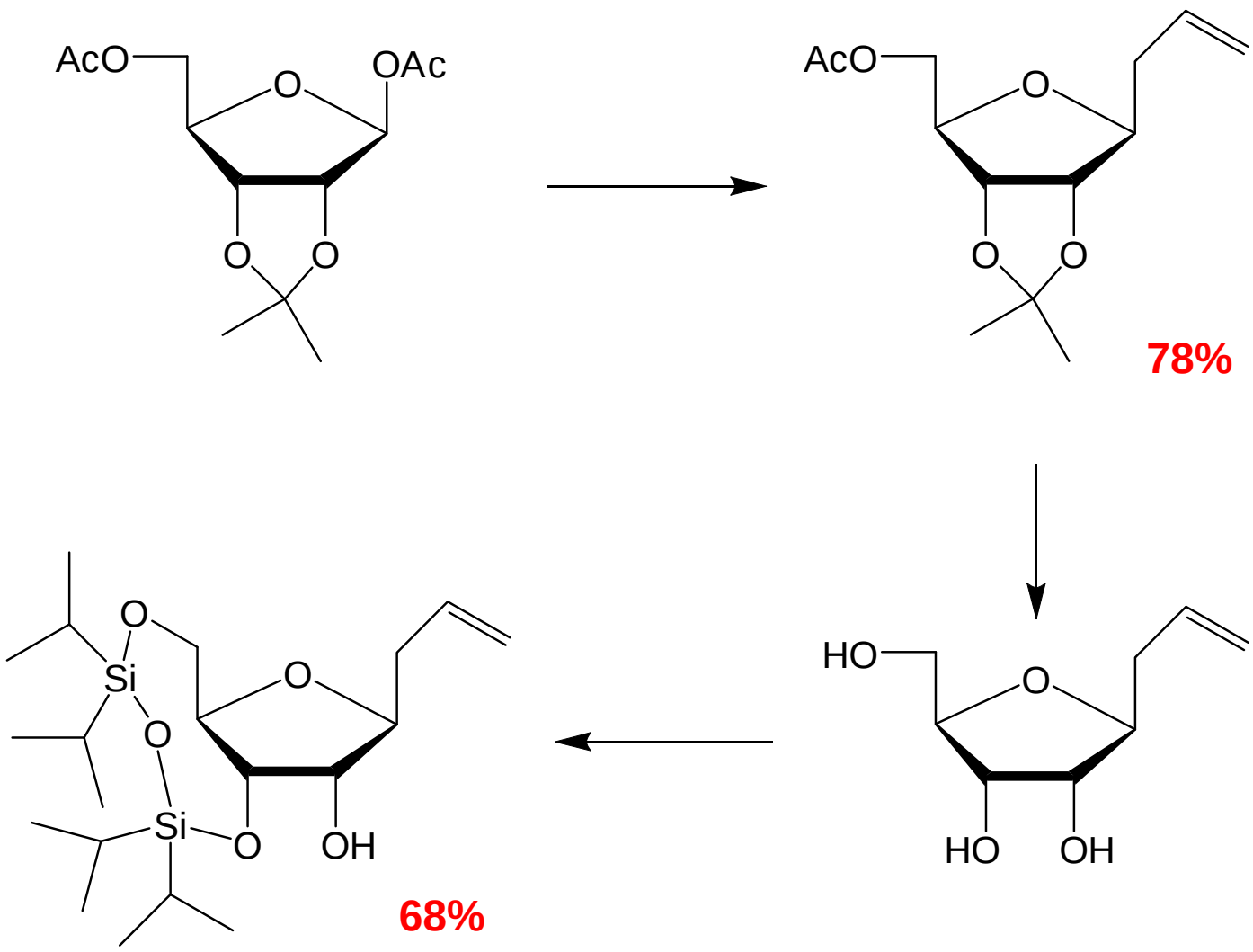
Venner, H.; Zinner, H. *Chem. Ber.* **1960,** *93*, 137-140.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

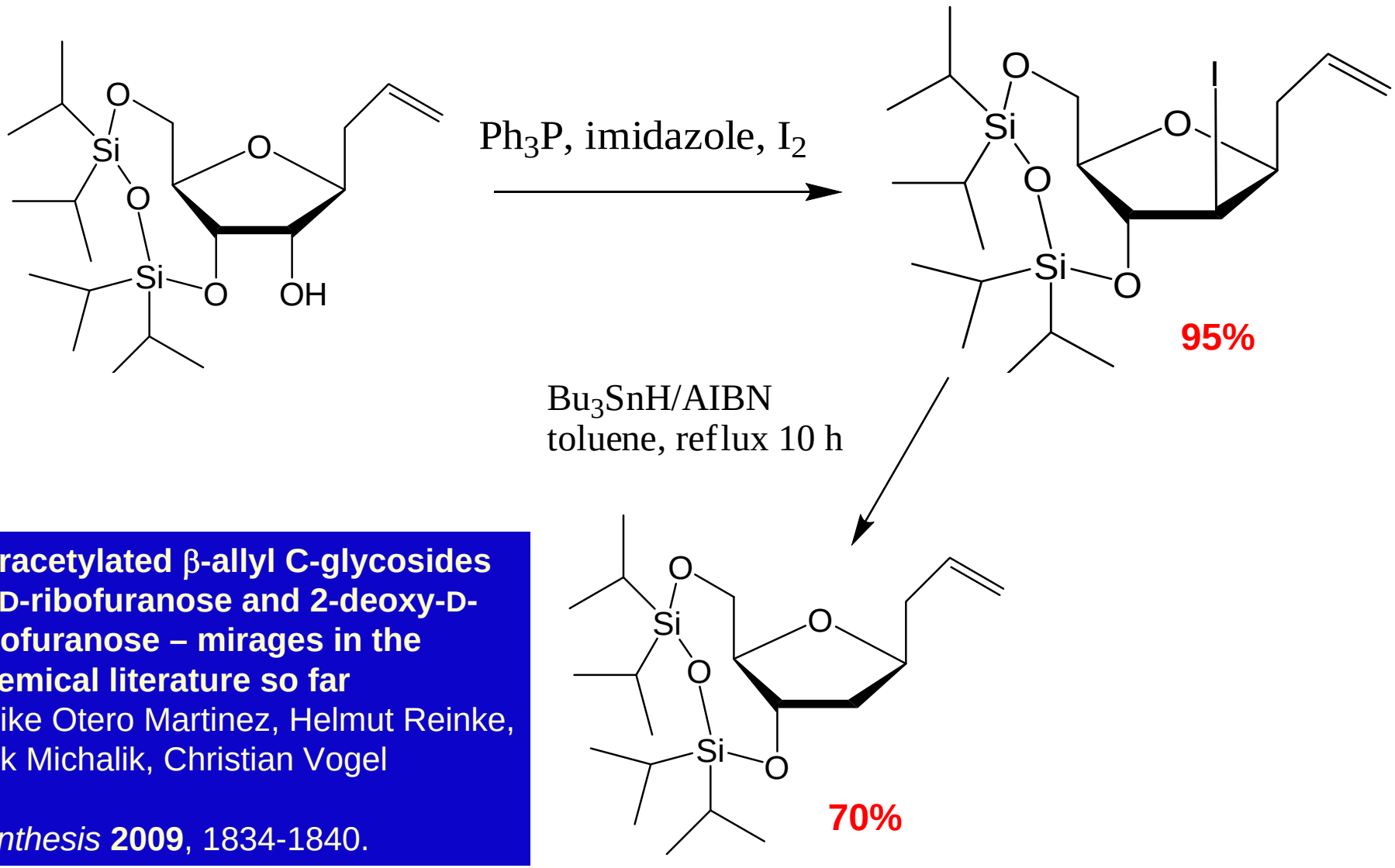


Diedrichsen, U.; Biro, C. M. *Bioorg. Med. Chem. Lett.* **2000,** *10***,** 1417-1420.

CHEMICAL SYNTHESIS OF BIOPOLYMERS



CHEMICAL SYNTHESIS OF BIOPOLYMERS

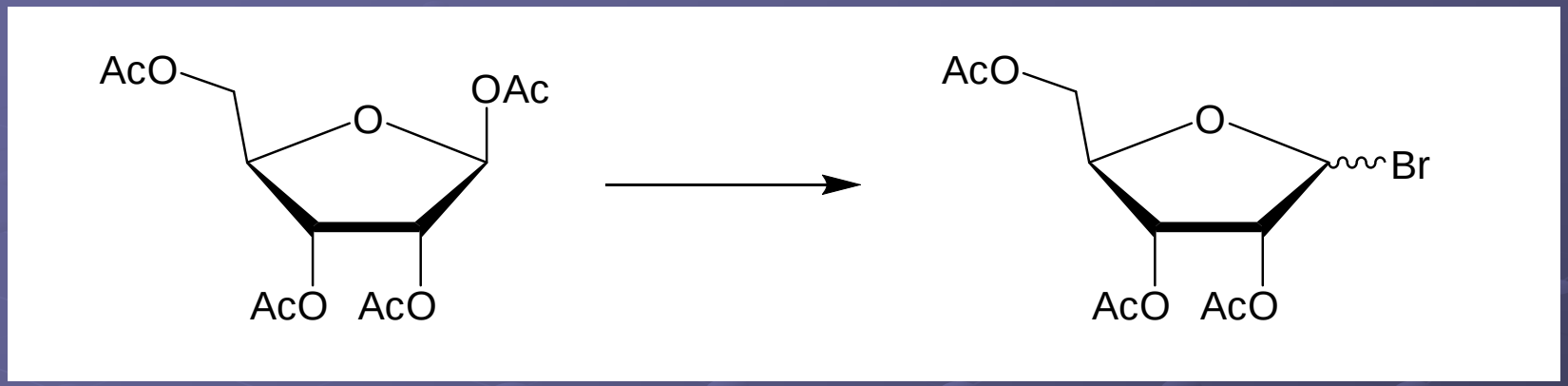


Peracetylated β-allyl C-glycosides of D-ribofuranose and 2-deoxy-D-ribofuranose – mirages in the chemical literature so far

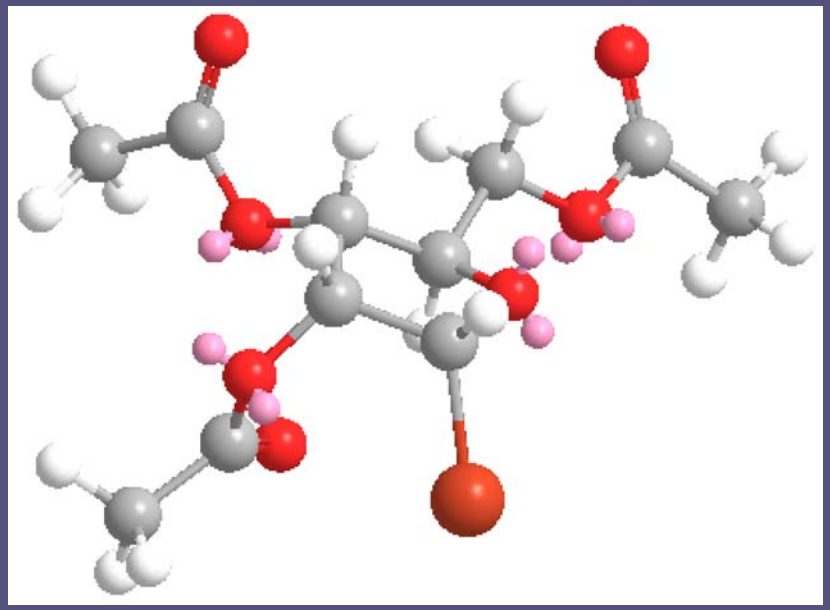
Heike Otero Martinez, Helmut Reinke, Dirk Michalik, Christian Vogel

Synthesis **2009**, 1834-1840.

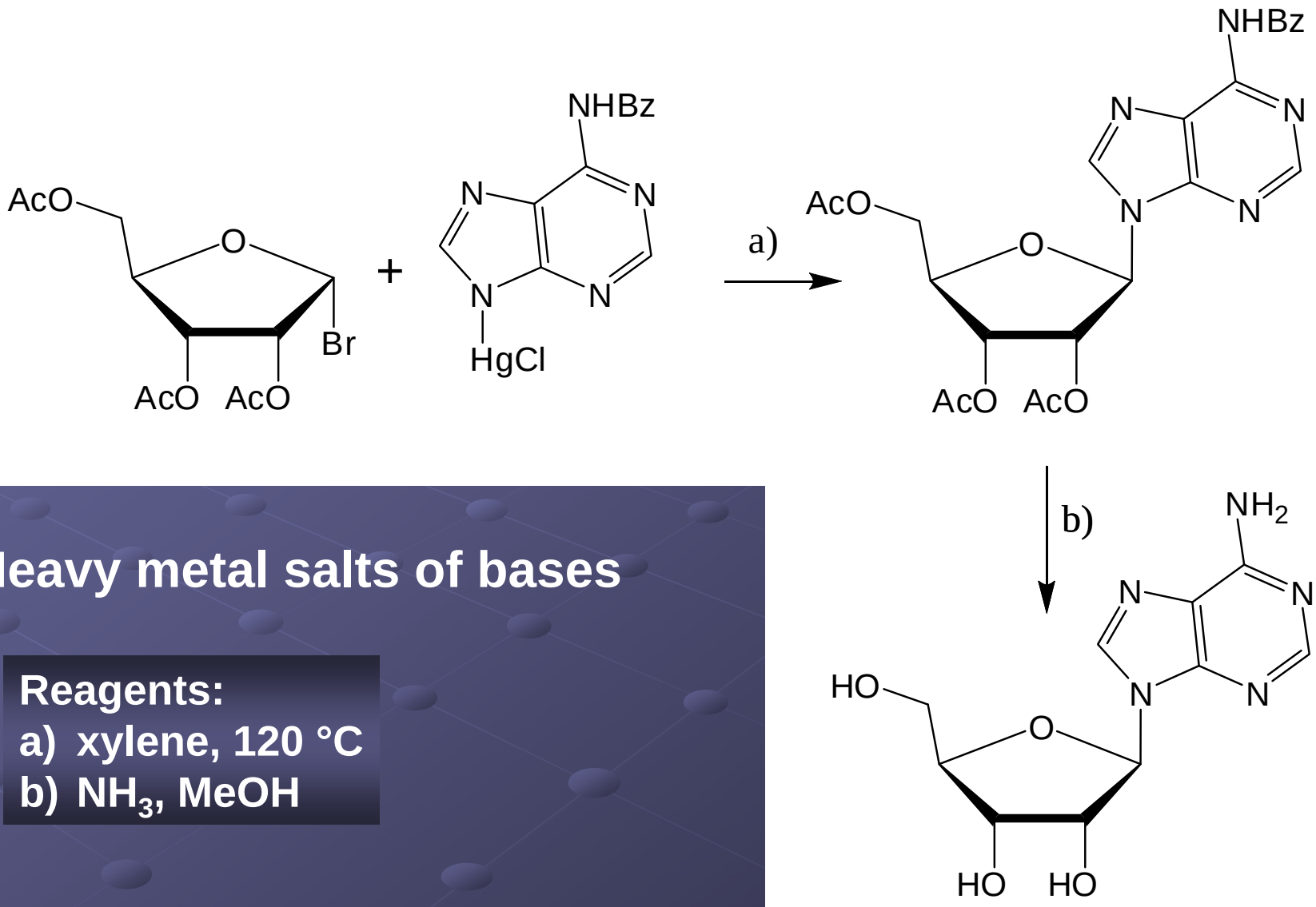
CHEMICAL SYNTHESIS OF BIOPOLYMERS



The corresponding **tri-*O*-acetyl-D-ribofuranosyl bromide** or **chloride** are usually obtained as an α/β-mixture.



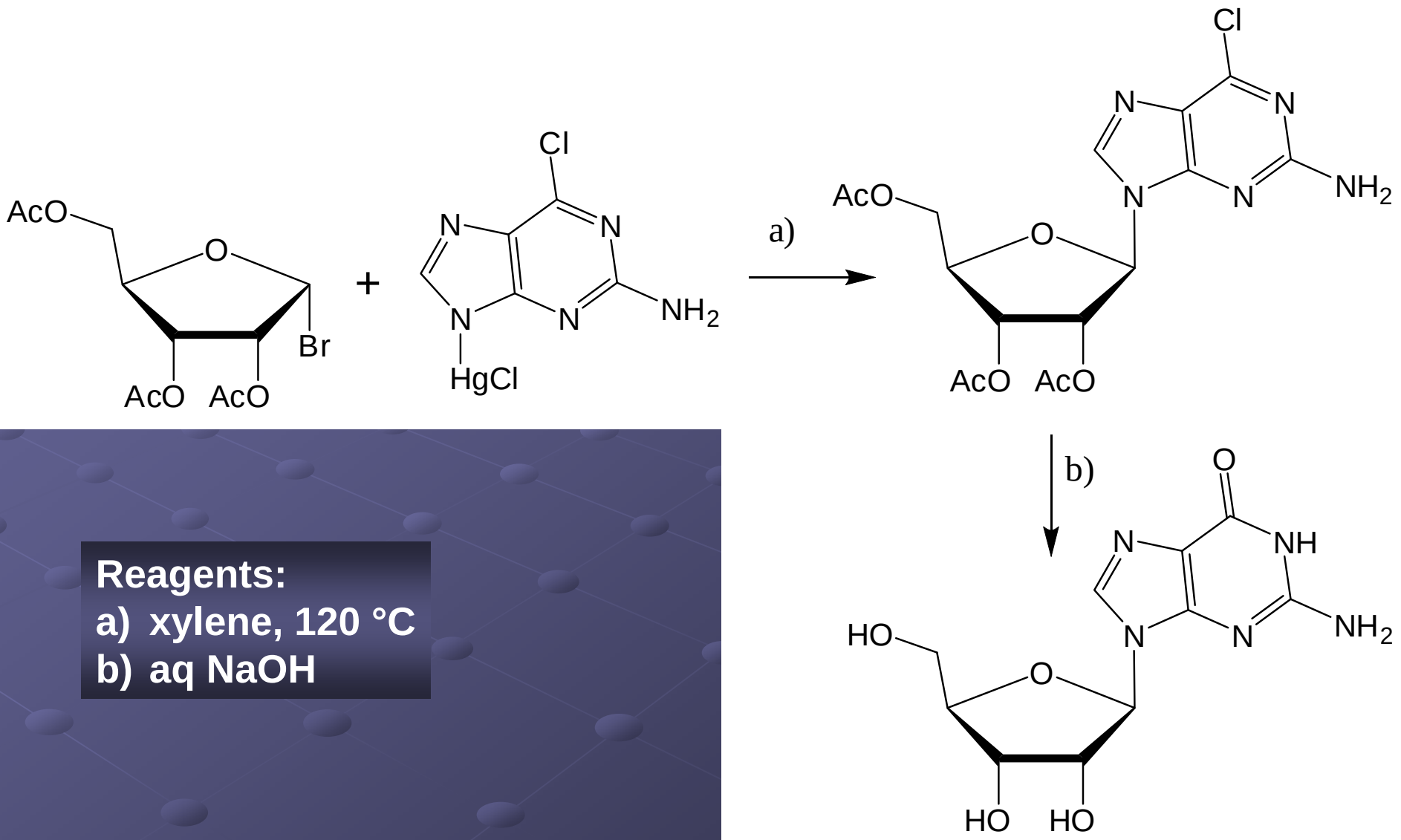
CHEMICAL SYNTHESIS OF BIOPOLYMERS



Heavy metal salts of bases

Reagents:
a) xylene, 120 °C
b) NH_3, MeOH

CHEMICAL SYNTHESIS OF BIOPOLYMERS



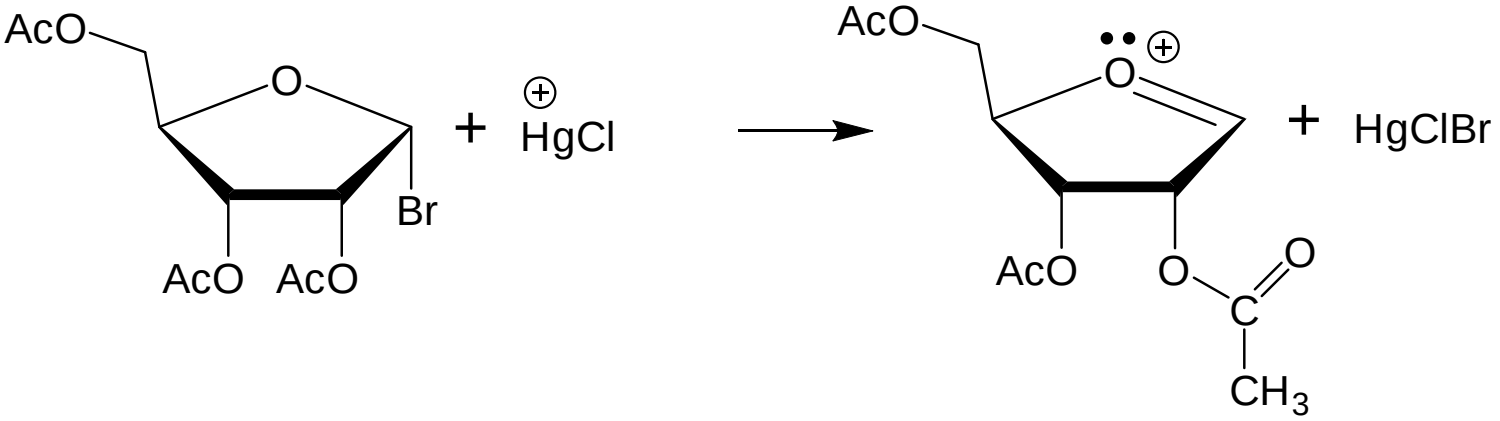
Reagents:
a) xylene, 120 °C
b) aq NaOH

CHEMICAL SYNTHESIS OF BIOPOLYMERS

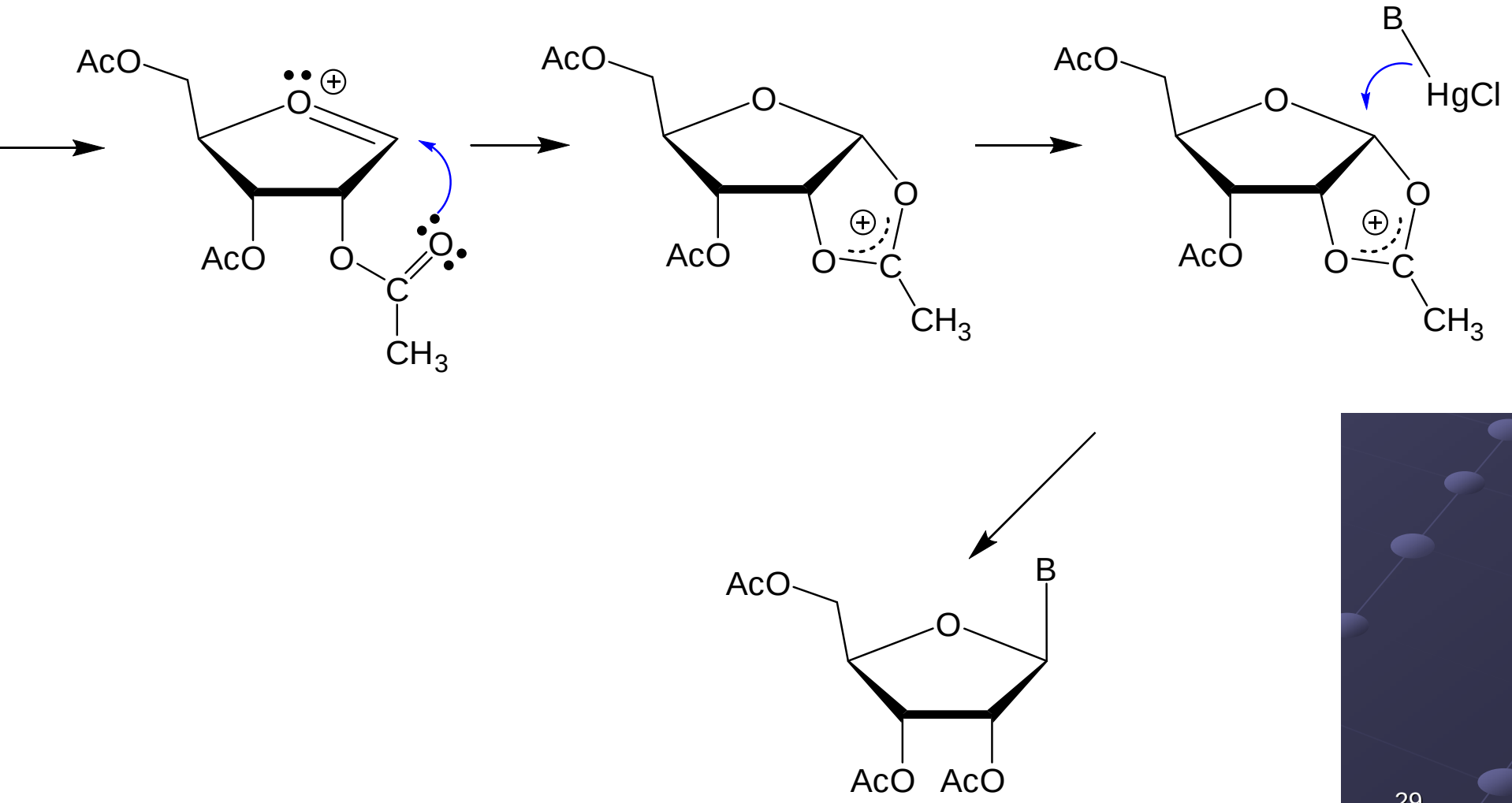
Control of anomeric stereochemistry

Sugars with a 2-acyloxy substituent on condensation with a base invariably give *N*-glycosides that have the 1,2-*trans*-configuration.

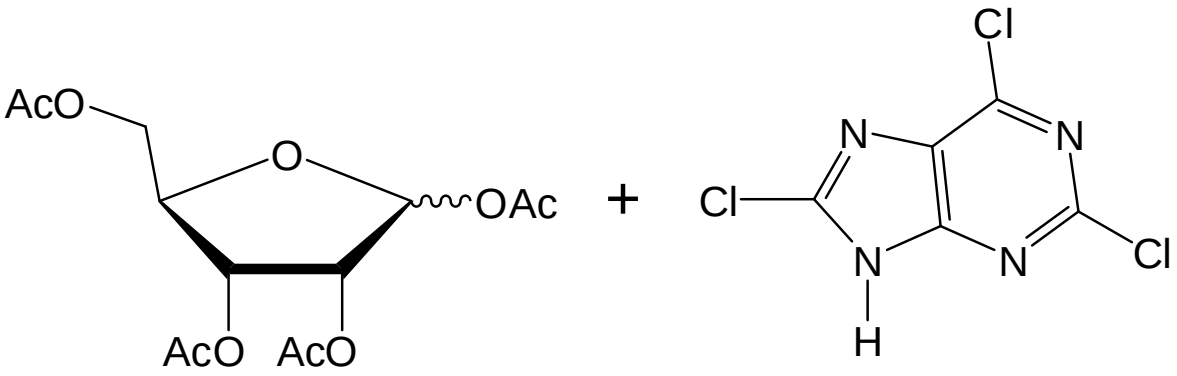
For this stereoselectivity neighbouring group participation is responsible.



CHEMICAL SYNTHESIS OF BIOPOLYMERS



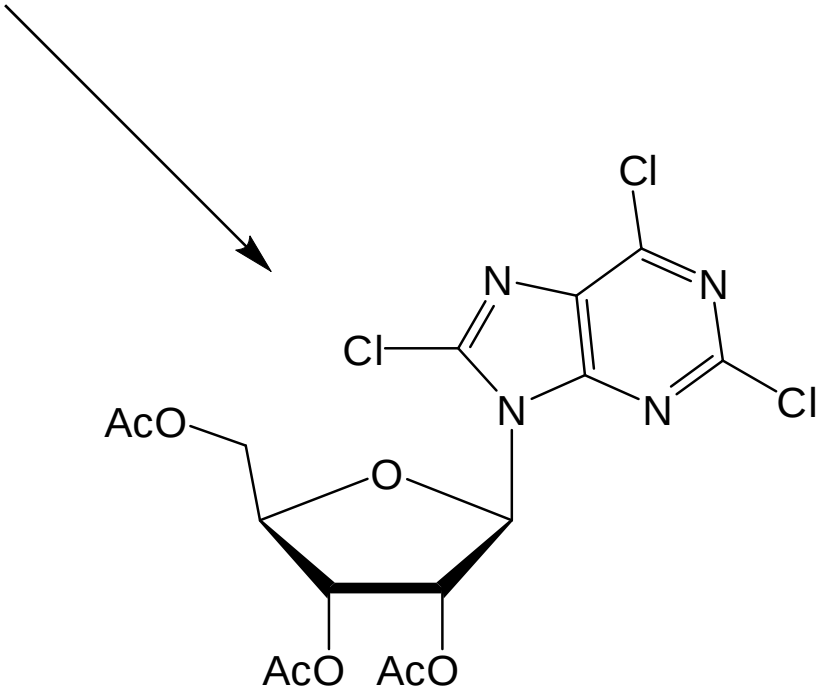
CHEMICAL SYNTHESIS OF BIOPOLYMERS



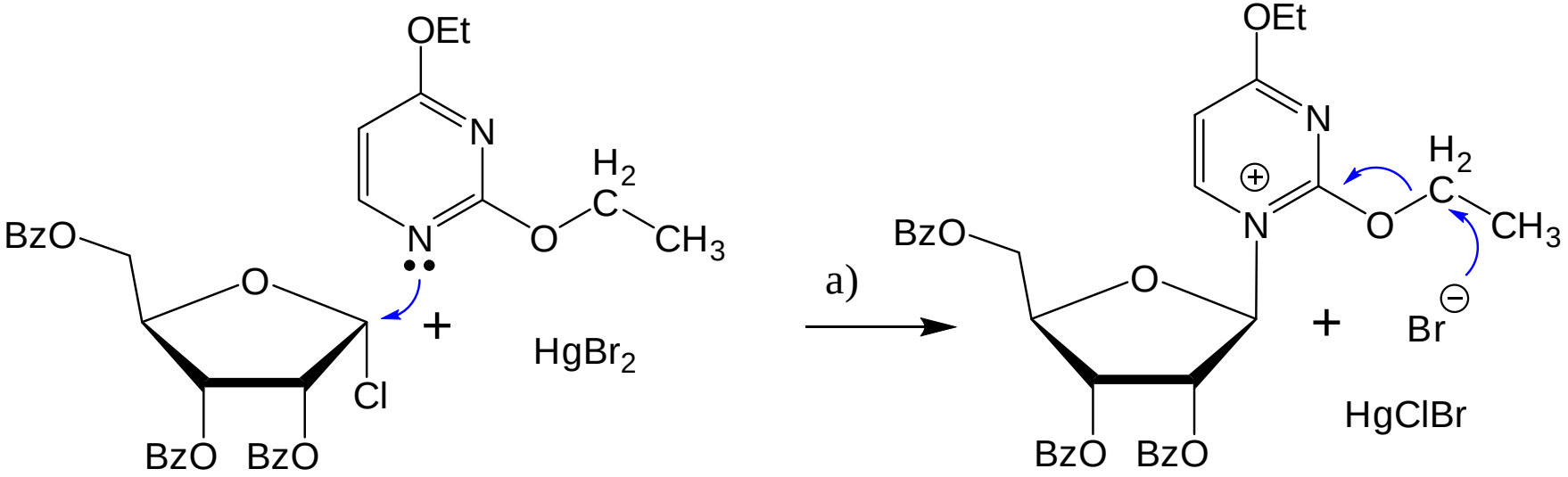
Fusion synthesis of nucleosides

Reagents:
melt at 150 °C

This method is at its best for **weakly basic heterocycles** having **low melting points**.

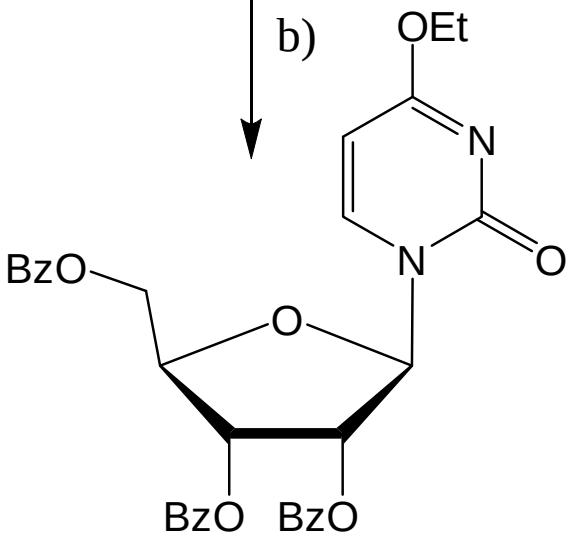


CHEMICAL SYNTHESIS OF BIOPOLYMERS

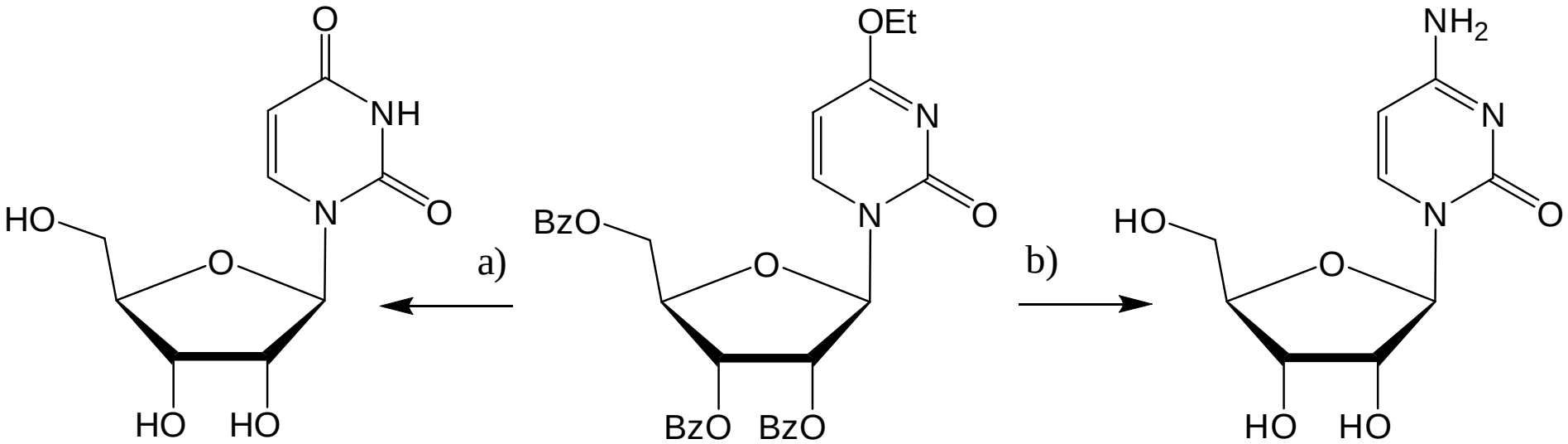


The quaternization procedure (Hilbert and Johnson)

Reagents:
a) CH_3CN 10 °C
b) CH_3CN reflux



CHEMICAL SYNTHESIS OF BIOPOLYMERS



Reagents:
a) aq NaOH
b) NH_3, MeOH

Such condensations frequently give mixtures of α- and β-anomers although the use of $HgBr_2$ increases the proportion of the β-anomer.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

The silyl base procedure

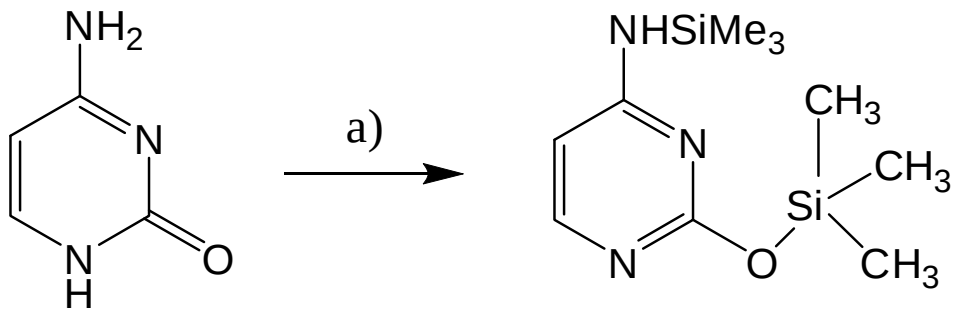
This method was developed independently by **Nishimura**, by **Birkofer**, and by **Wittenburg.**

Prof. Dr. E. Wittenburg

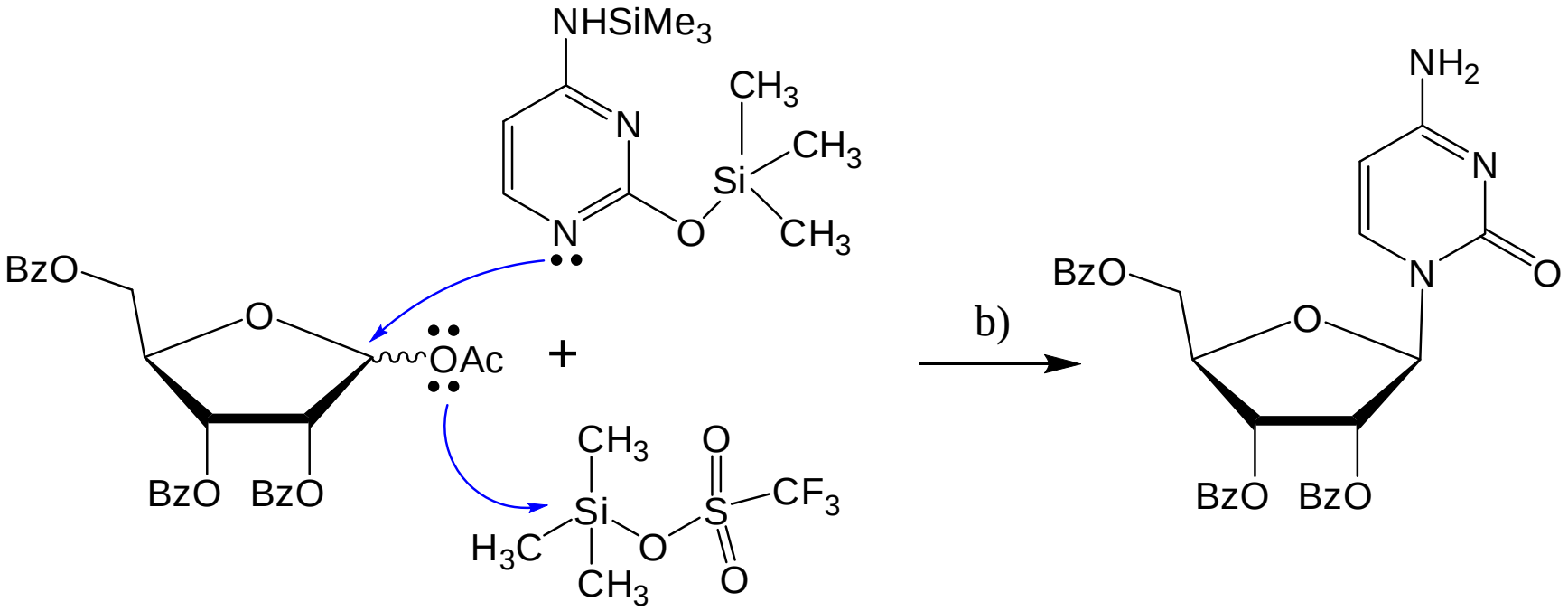
Advantages:

- The silylated bases are easily prepared.
- They react smoothly with sugars in homo-geneous solution.
- Typically, the reaction is carried out in **acetonitrile** or **1,2-dichloro-ethane** at around **-20° to +50 °C** in the presence of **$SnCl_4$**, **$Hg(OAc)_2$**, **trimethylsilyl triflate** or **trimethylsilyl perchlorate** as Lewis acid.
- They give intermediate products that can easily be converted into modified bases.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

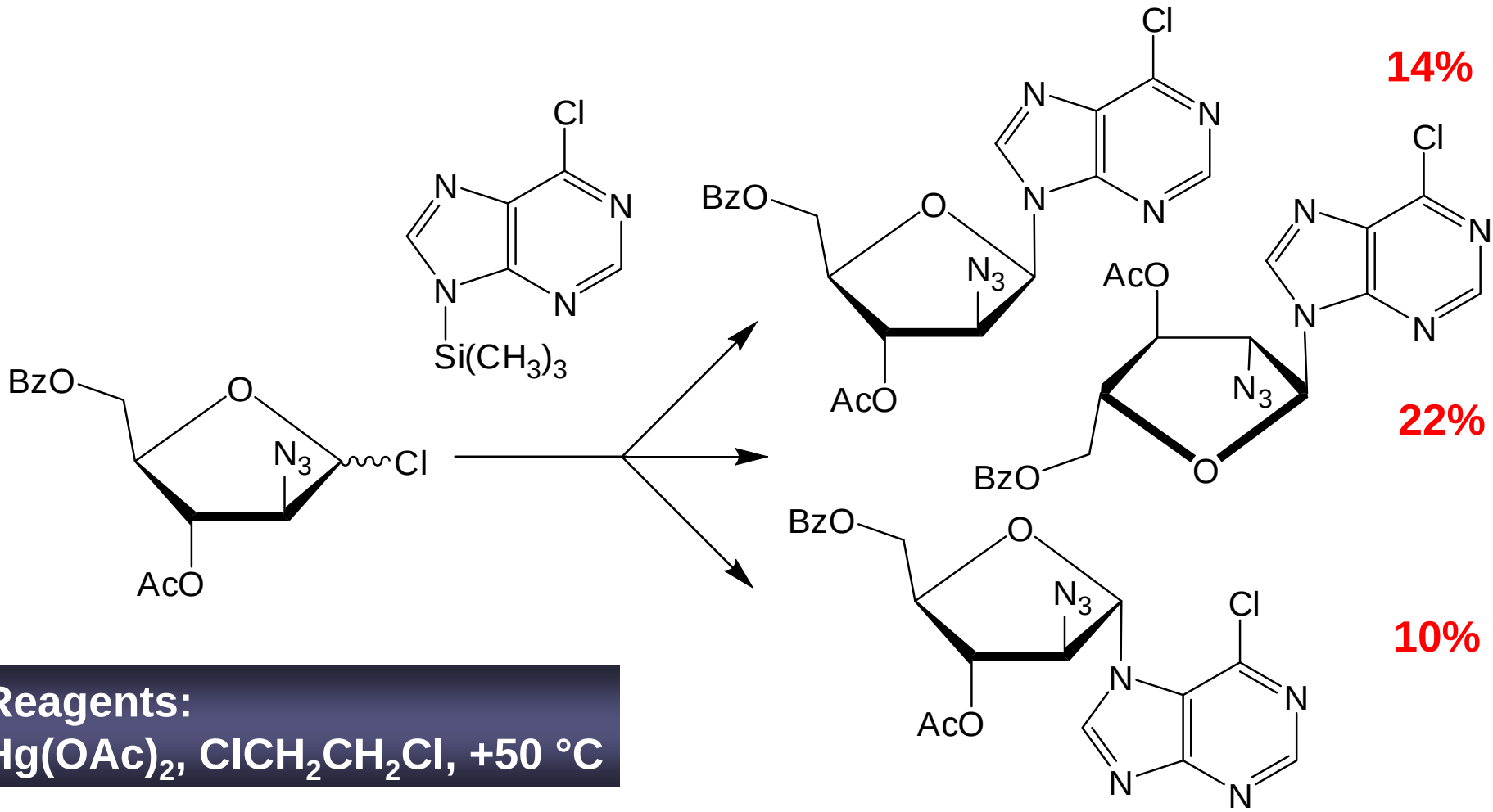


Reagents:
a) $CH_3C(=O)N(SiMe_3)_2$
b) H_2O



CHEMICAL SYNTHESIS OF BIOPOLYMERS

In some cases, the silyl base procedure suffers from a **lack of** precise control of **regio- and stereoselectivity**.



Reagents:
$Hg(OAc)_2$, $ClCH_2CH_2Cl$, +50 °C

CHEMICAL SYNTHESIS OF BIOPOLYMERS

The transglycosylation procedure

This method is particularly effective for transferring sugars from pyrimidines (π-deficient heterocycles) to more basic purines (π-excessive heterocycles).

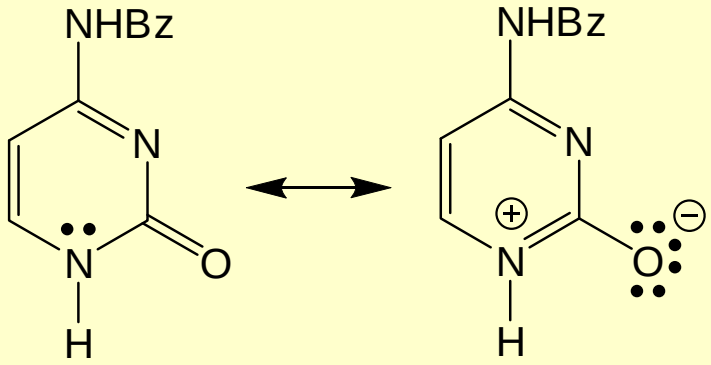
The reaction has all the features of an S_N1 ionization process.

Therefore, transglycosylation is now a favoured method for the preparation of **α-anomers of pyrimidine nucleosides** from their natural isomers.

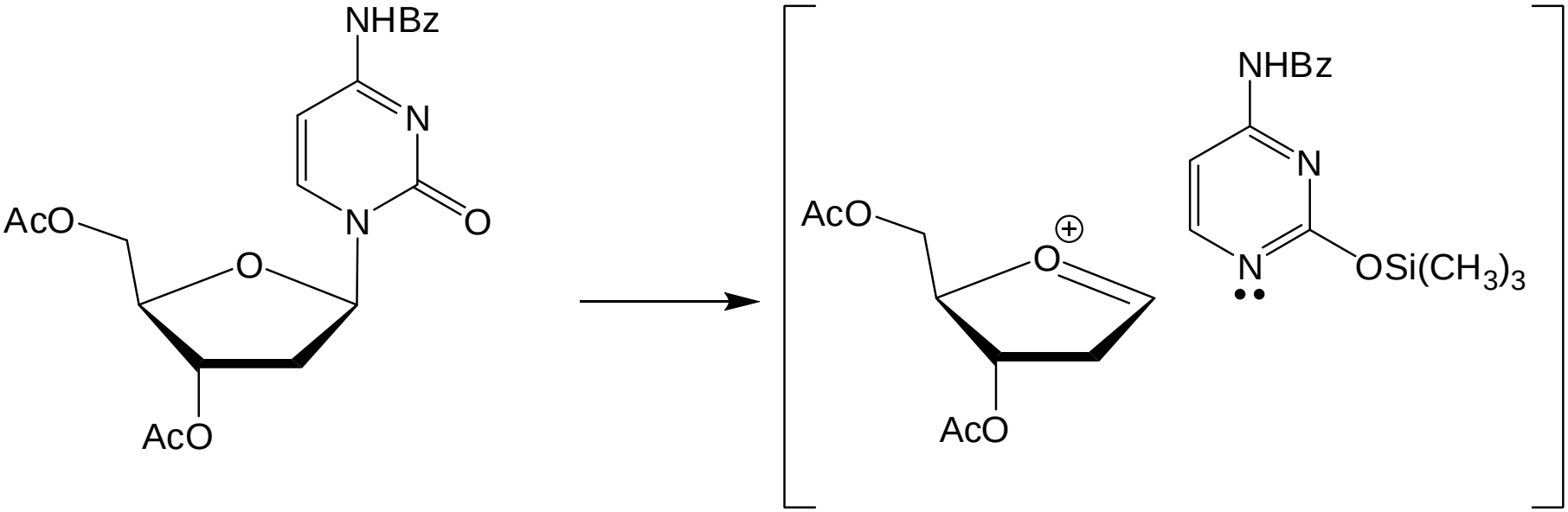
On the other hand, transglycosylation provides a useful synthesis of **α-anomers of pyrine nucleosides**.

However, **only experience** is able to predict the **thermodynamically favoured regioselectivity** of these processes.

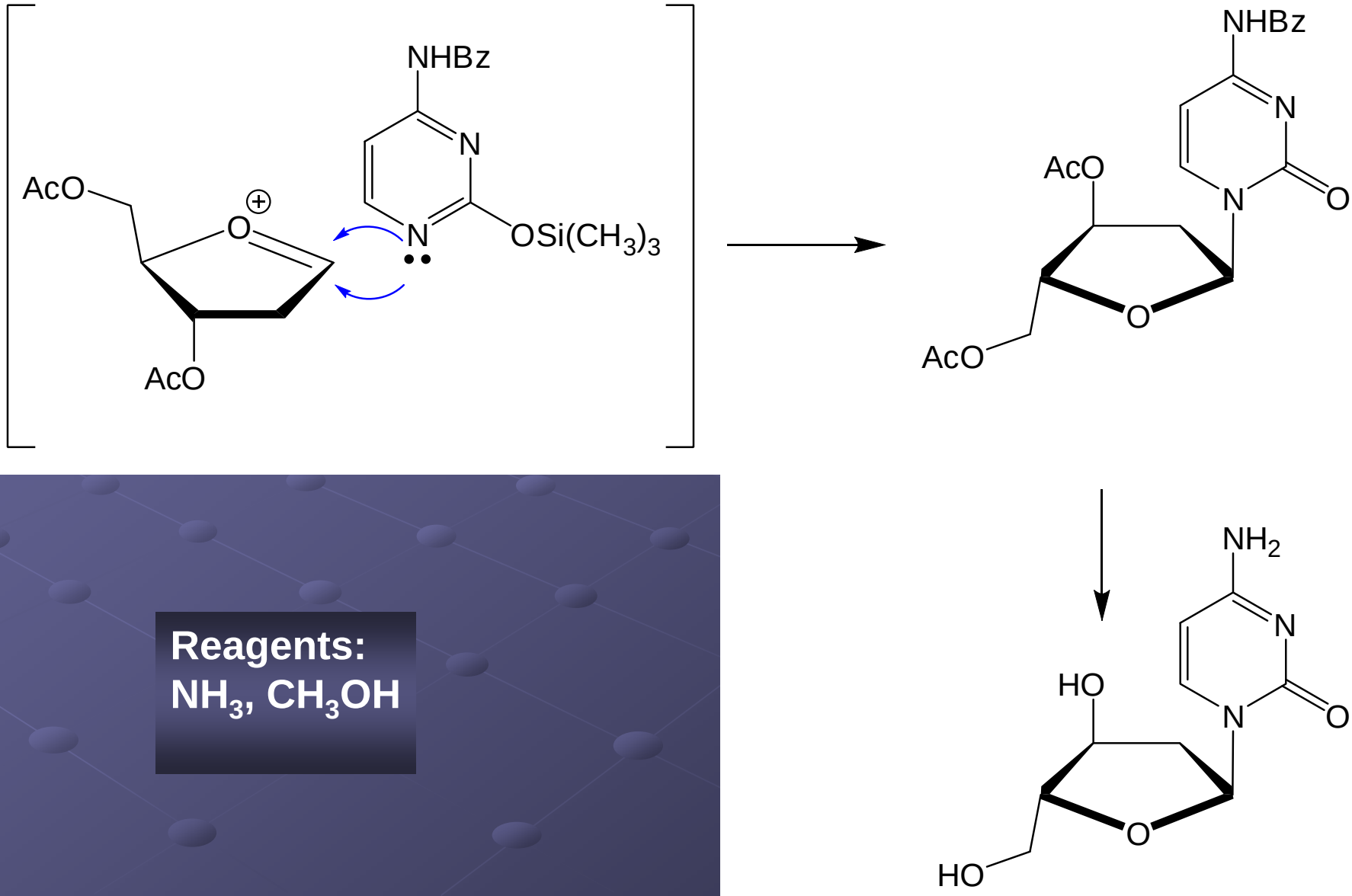
CHEMICAL SYNTHESIS OF BIOPOLYMERS



Reagents:
$H_3C(C{=}O)NSi(CH_3)_3$, $CF_3SO_3Si(CH_3)_3$

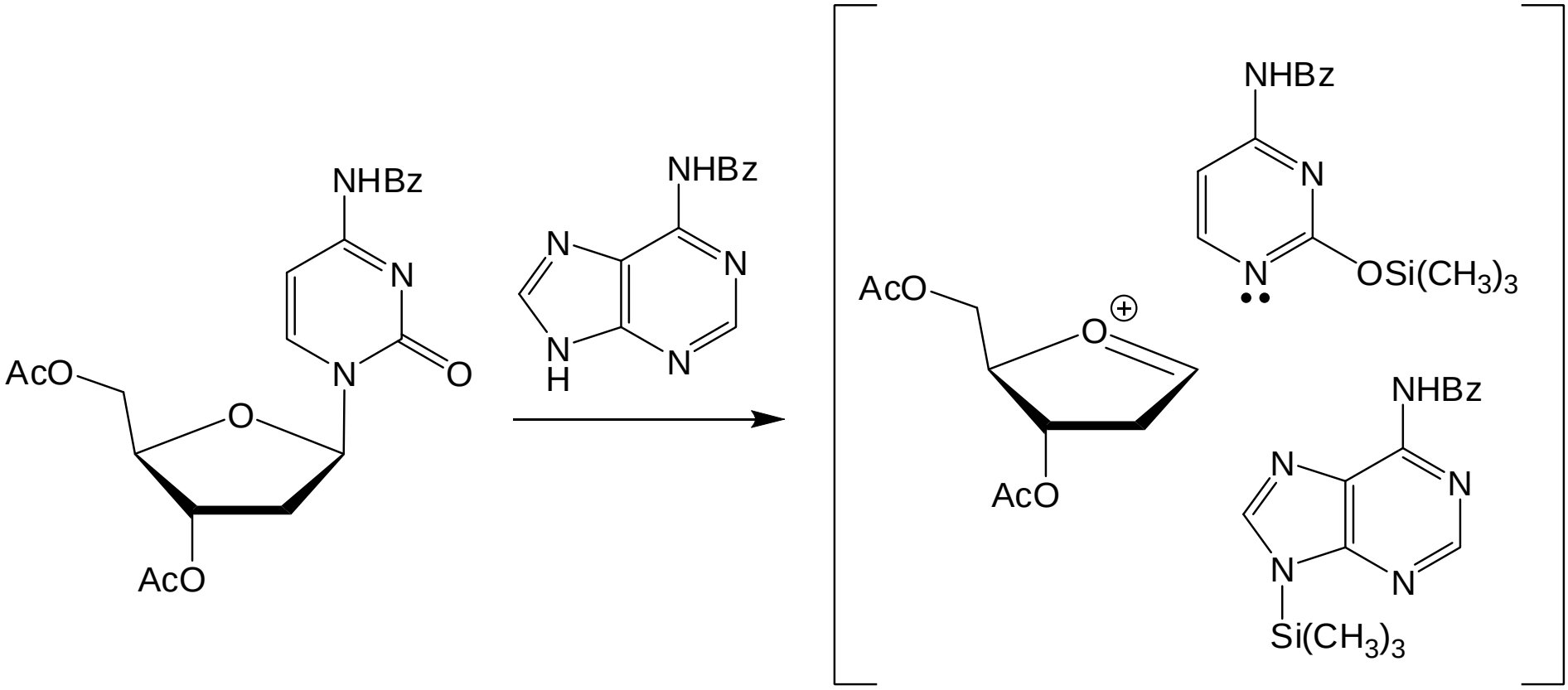


CHEMICAL SYNTHESIS OF BIOPOLYMERS



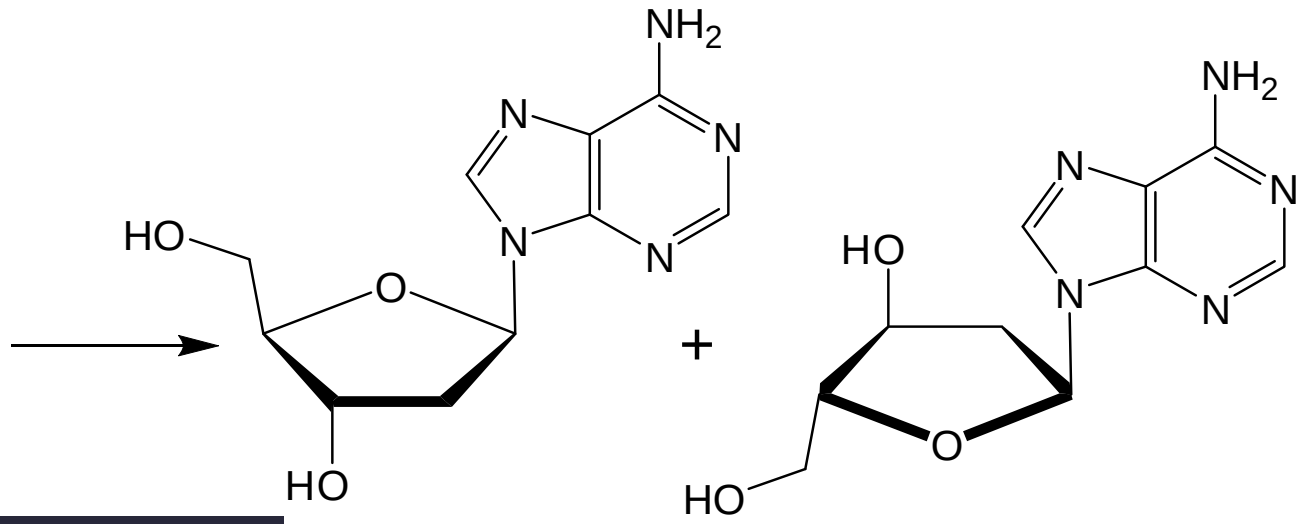
Reagents:
NH_3, CH_3OH

CHEMICAL SYNTHESIS OF BIOPOLYMERS



Reagents:
$H_3C(C{=}O)NSi(CH_3)_3$, $CF_3SO_3Si(CH_3)_3$

CHEMICAL SYNTHESIS OF BIOPOLYMERS



Reagents:
NH_3, CH_3OH

Transglycosylation can be also catalysed by enzymes.
(bacterial *N*-deoxyribofuranosyl transferase or intact *E*.coli cells immobilezed in a gel matrix)

CHEMICAL SYNTHESIS OF BIOPOLYMERS

1.3 Chemistry of esters of phosphorus acid

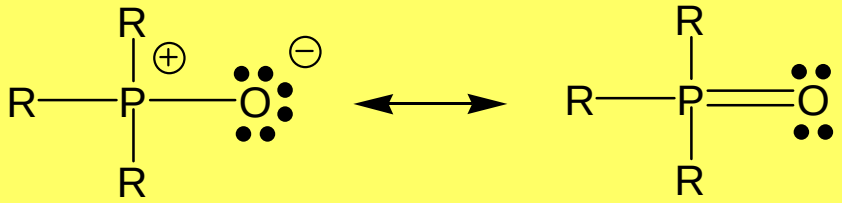
The **orthophosphoric acid**, H_3PO_4, has a coordination number 4. Orthophosphates are **tetrahedral** at phosphorus.

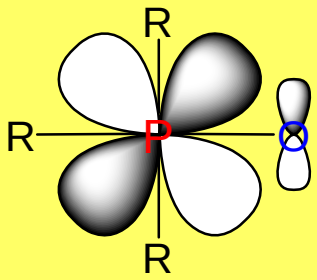
The single P-O-bonds are 0.16 nm long, whereas the P-O-double bond is shorter, 0.146 nm.

The double bond involves a phosphorus d-orbital in a p_π-d_π interaction.

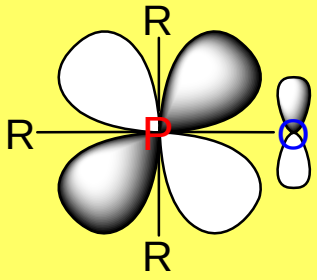
Because phosphorus has five 3d-orbitals it can participate to more than one oxygen ligand.

Bonding to neutral nitrogen ligands is rather weak so the nitrogen remains moderately basic.



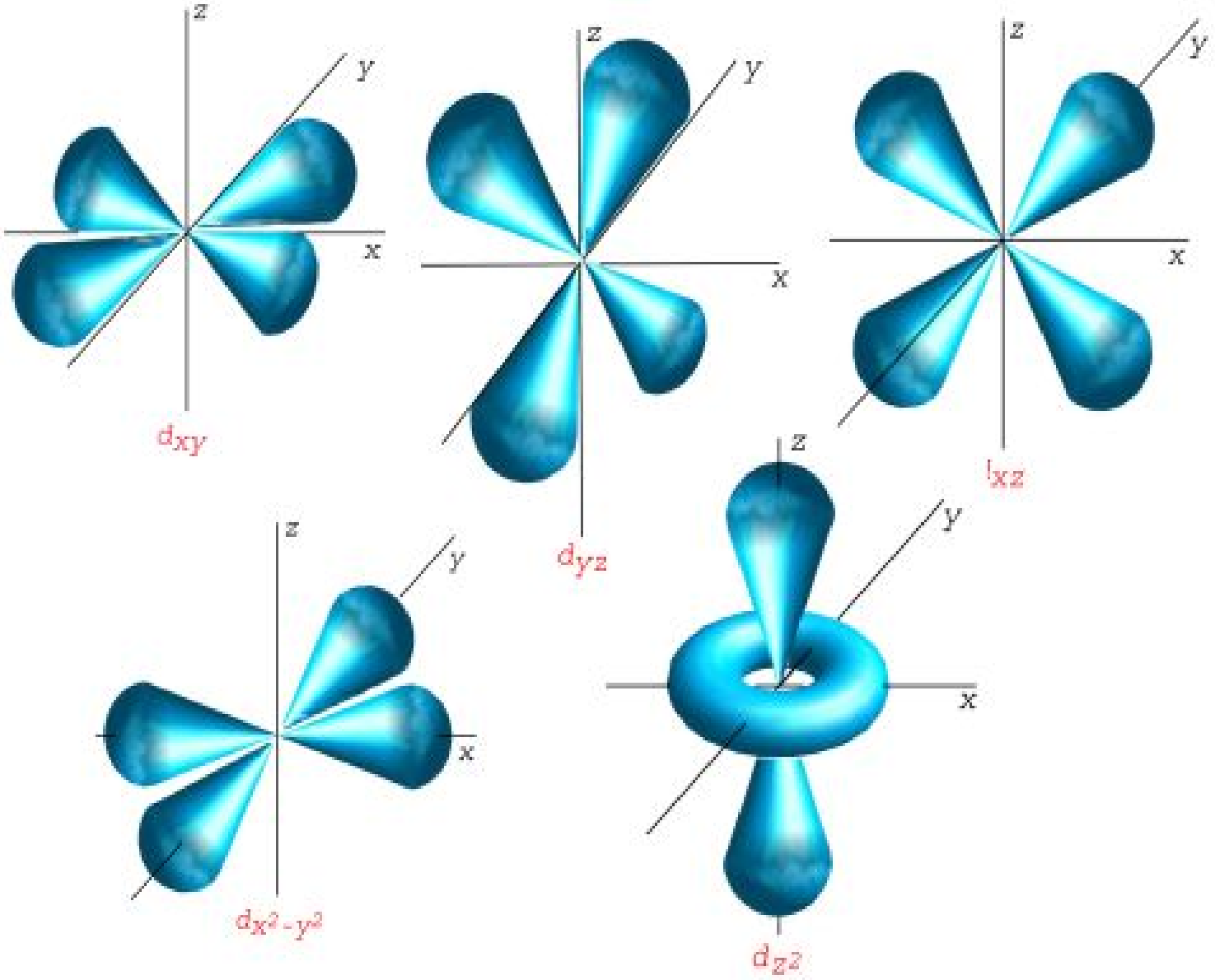


$3d_{xy}$



$3d_{xz}$

CHEMICAL SYNTHESIS OF BIOPOLYMERS

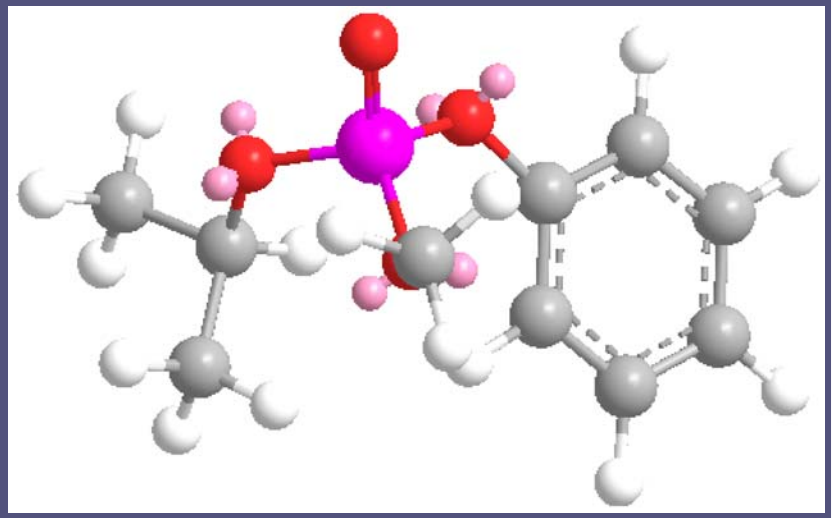


CHEMICAL SYNTHESIS OF BIOPOLYMERS

Phosphate triesters

They are soluble in many organic solvents.

They are sufficiently stable to be purified by chromatography



When all three ester groups are different, the phosphorus atom is a centre of asymmetry and optical isomers are possible.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

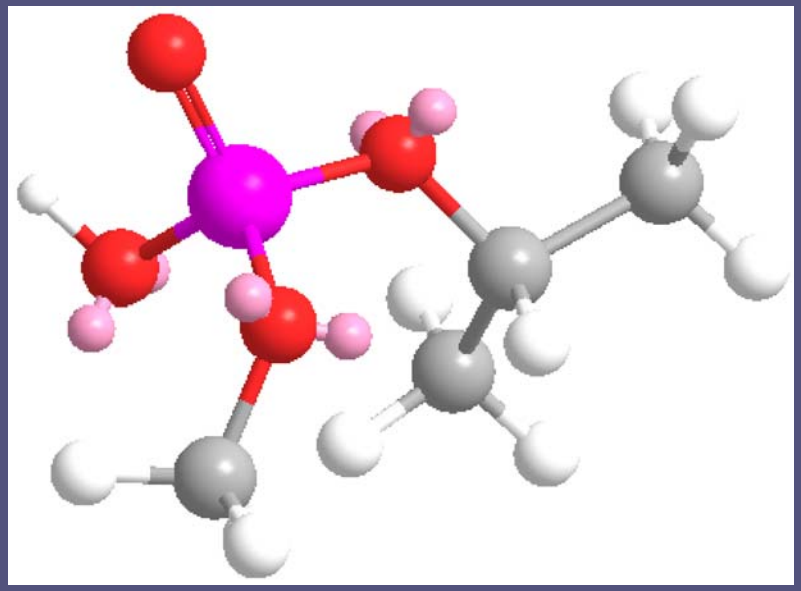
Phosphate diesters

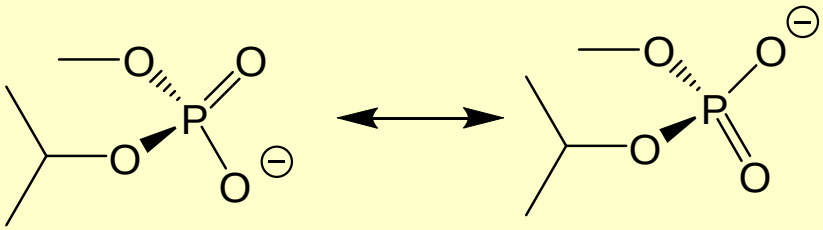
Two hydrogens are replaced by alkyl or aryl groups, while the remaining OH ligand is strongly acid (pK_a ~ 1.5)

Consequently, phosphate diesters exist as monoanions at pH > 2, and are usually water-soluble.

The negative charge is shared equally between the two unsubstituted oxygens.

The phosphorus atom is a pro-chiral centre.

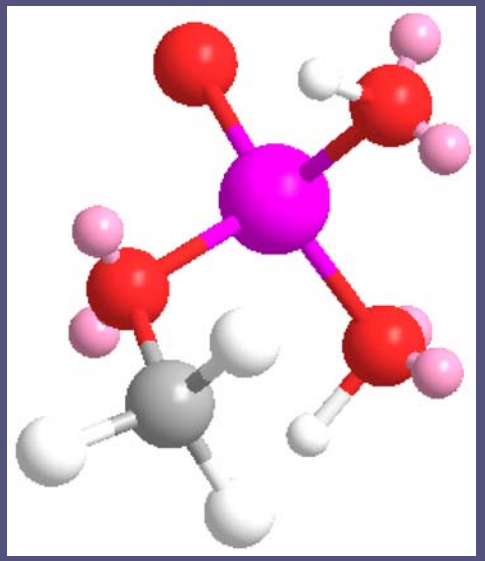




CHEMICAL SYNTHESIS OF BIOPOLYMERS

Phosphate monoesters

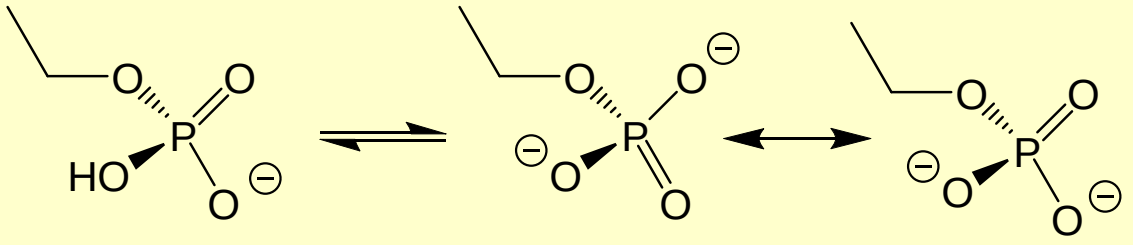
They have two ionizable OH groups (pK_a1 ~1.6 and pK_a2 ~ 6.6)



Consequently, there is an equilibrium in neutral solution (effectively from pH 5 to pH 8) between the monoanion and the dianion.

In the monoanion, the hydrogen atom translocates rapidly between the three oxygen making them all equivalent in solution.

The equivalent oxygens share the negative charge in both mono- and dianions and there is partial double bonding to each.



CHEMICAL SYNTHESIS OF BIOPOLYMERS

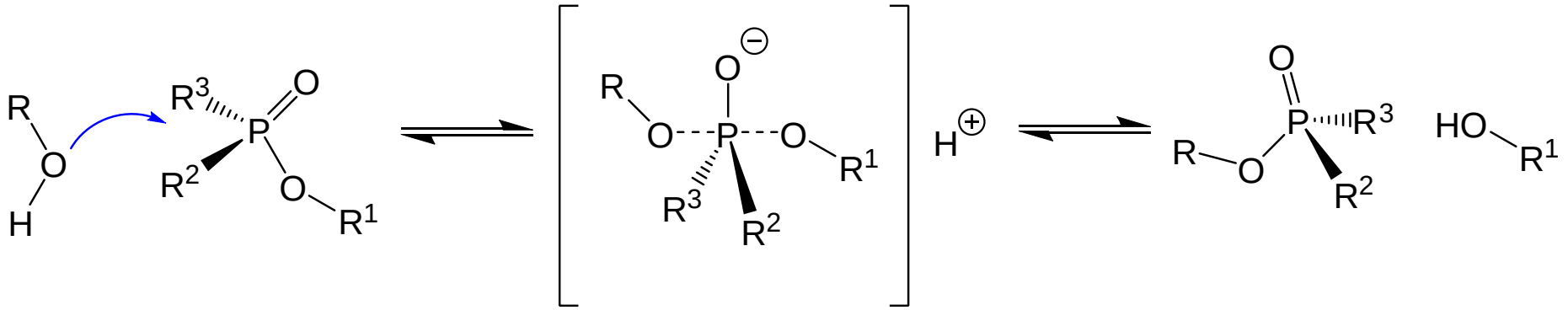
Hydrolysis of phosphate esters

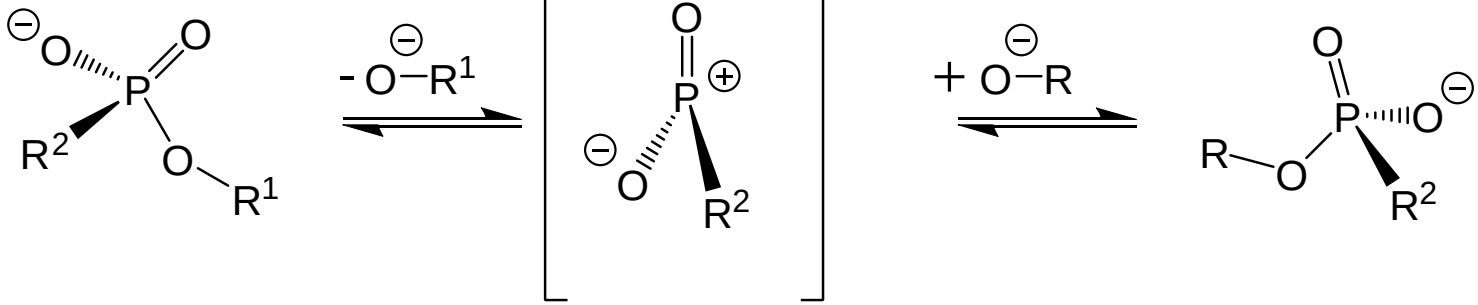
The **great stability of phosphate diesters** to hydrolysis under physiological conditions is an essential feature of the chemistry of nucleic acids and intrinsic to life itself.

Studies of **mechanisms for their hydrolysis** have shown that both C-O and P-O cleavage could be involved.

In the case of P-O-cleavage, associative, S_N2 (**P**), mechanisms are more common than dissociative ones, S_N1 (**P**), and they are usually linked to inversion of configuration at phosphorus.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

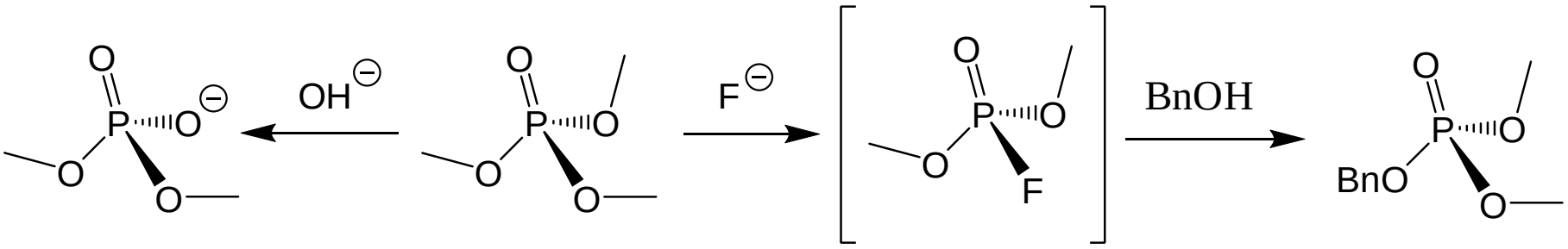




CHEMICAL SYNTHESIS OF BIOPOLYMERS

Hydrolysis of phosphate triesters

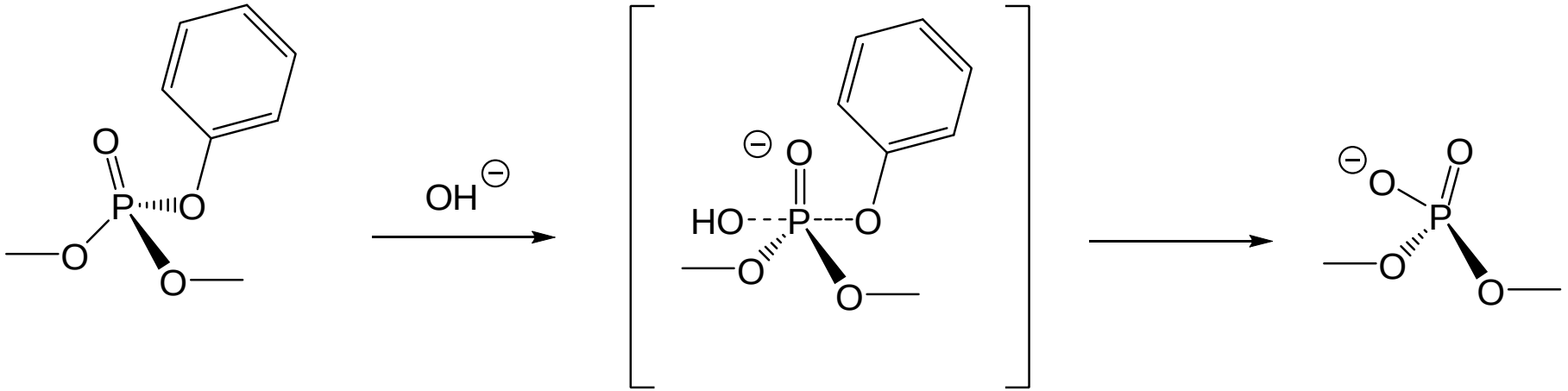
Trimethyl phosphate is slowly hydrolysed in alkaline solution in an **$S_N2(P)$** process. Fluoride ions react similar and can be used in transesterification.



With $H_2{}^{18}O$ as solvent, no isotope exchange (**^{18}O**) is observed **into the P=O** group of **unreacted ester** and **no Me^{18}OH** is formed.

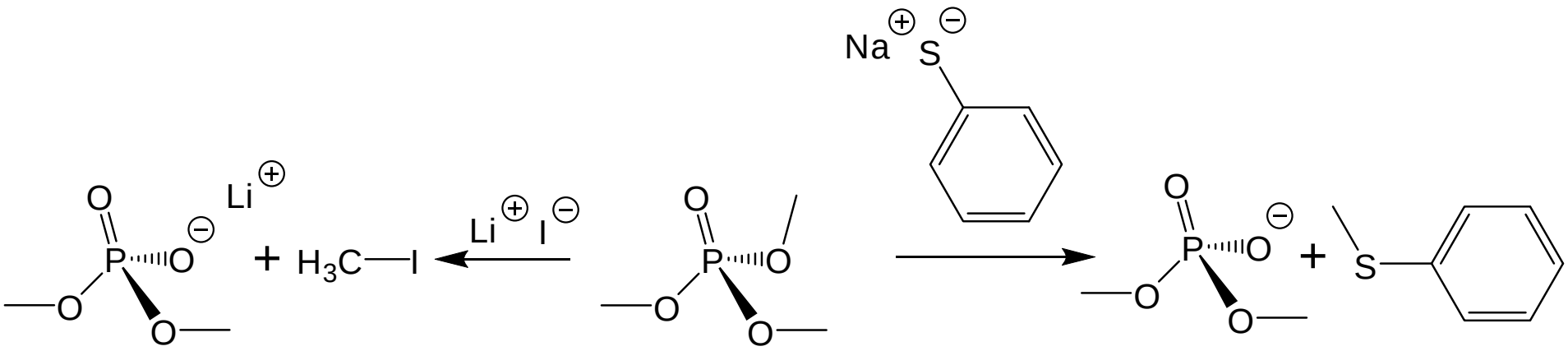
CHEMICAL SYNTHESIS OF BIOPOLYMERS

It is also possible to achieve **selective, nucleophilic displacement [$S_N2(P)$]** of the **phenolic residue in** a **dialkyl aryl phosphate** on account of its better leaving group ability.



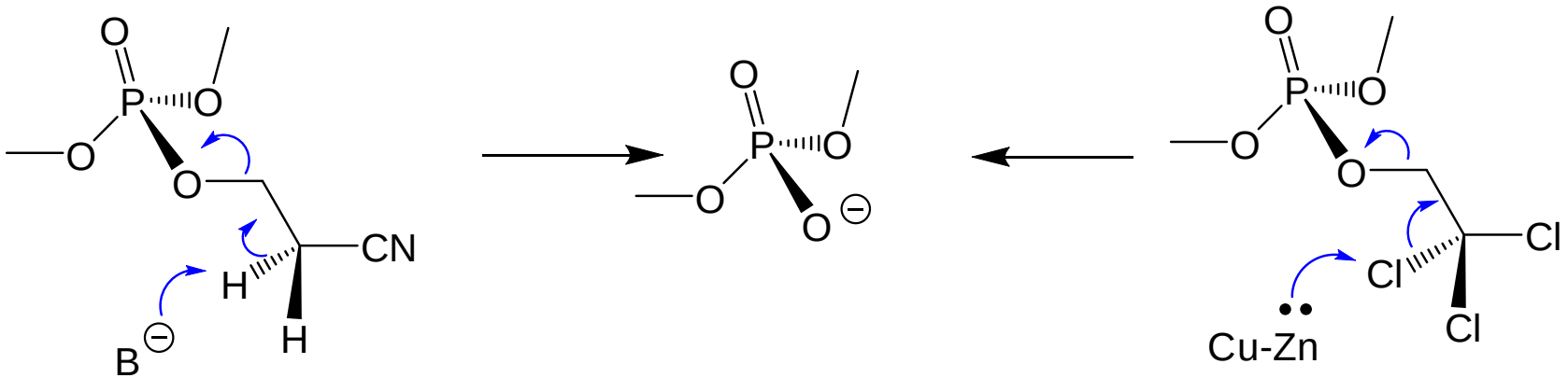
CHEMICAL SYNTHESIS OF BIOPOLYMERS

Soft nucleophiles such $RS^{\ominus}$, $Br^{\ominus}$, or $I^{\ominus}$ dealkylate with **C-O cleavage**. This behaviour is used in the **thiophenolate deprotection** in **oligonucleotide synthesis.**



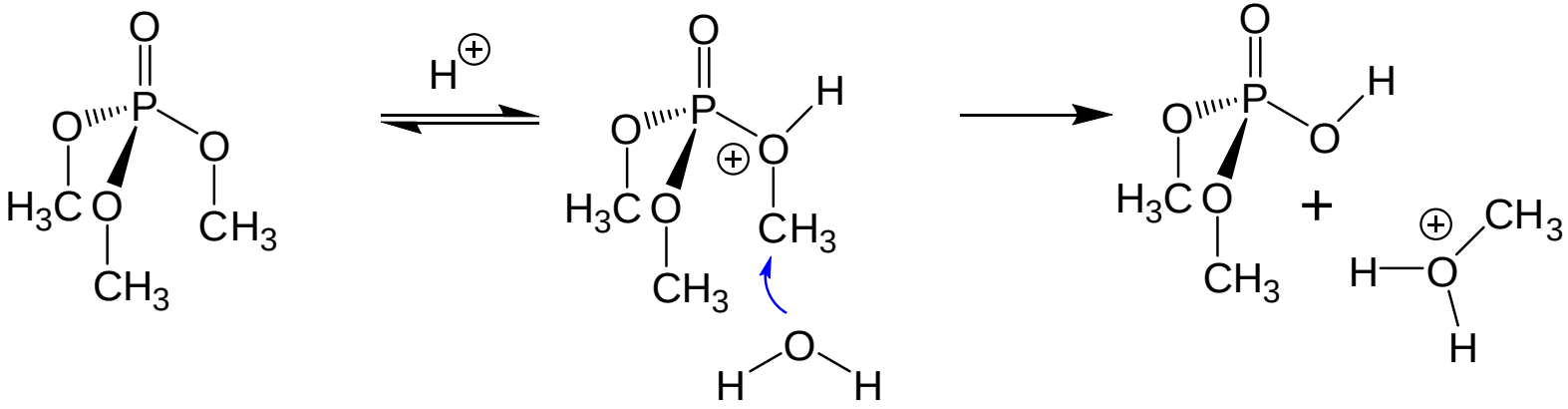
CHEMICAL SYNTHESIS OF BIOPOLYMERS

Selective deprotection of phosphate triesters can be achieved by **β-elimination** using the **2-cyanoethyl** ester or **2,2,2-trichloroethyl ester** as **temporary protecting groups**.



CHEMICAL SYNTHESIS OF BIOPOLYMERS

Trimethyl phosphate is hydrolysed **extremely slowly** in **neutral** and **in acidic conditions** with **C-O cleavage**.



CHEMICAL SYNTHESIS OF BIOPOLYMERS

Hydrolysis of phosphate diesters

At **pH > 2**, phosphate diesters exist as their monoanions, which are stable in boiling water.

That means, phosphate diesters hydrolysed extremely slowly **in neutral and in acidic conditions** with **C-O cleavage** which is comparably to triesters.

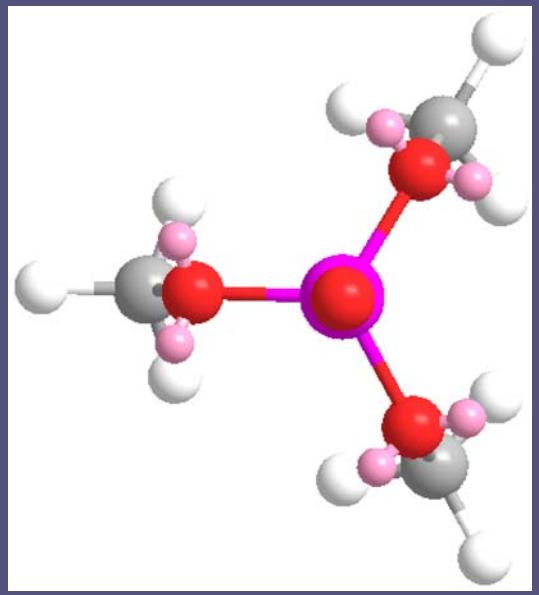
In **strong alkaline conditions**, diesters hydrolyse more slowly than triesters; (because of anion - anion repulsion) with predominant **C-O-cleavage** (>90%).

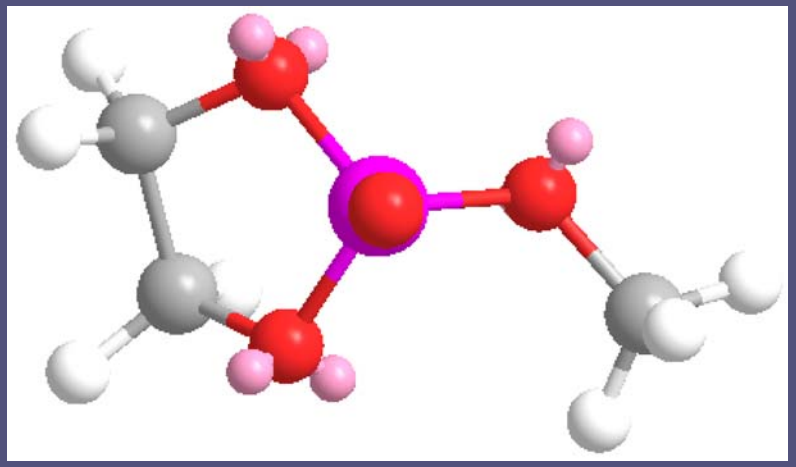
As expected, **aryl esters** are rather more reactive than alkylesters. But then a **selective, nucleophilic displacement [$S_N2(P)$]** occurs at phosphor.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Cyclic phosphates of 1,2-diols (5-membered ring) hydrolyse some **10^7 times faster** than do their acyclic or 6- and 7-membered cyclic derivatives.

A high percentage of this acceleration is attributed **to relief of strain** in the 5-membered cyclic ester, which has a 98° O-P-O angle.
(114.5 ° in acyclic esters)

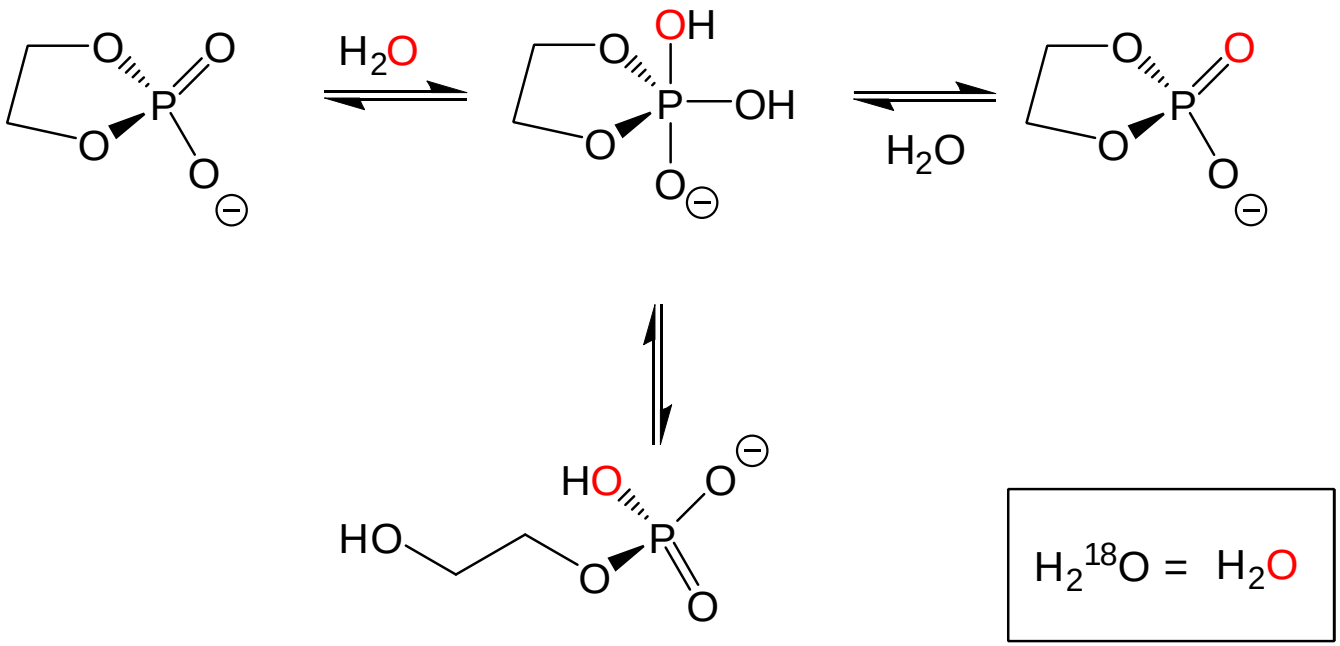




CHEMICAL SYNTHESIS OF BIOPOLYMERS

The ring strain accelerates **ring closure**, **ring opening** (both **endocyclic substituion**), and **exocyclic P-O cleavage**.

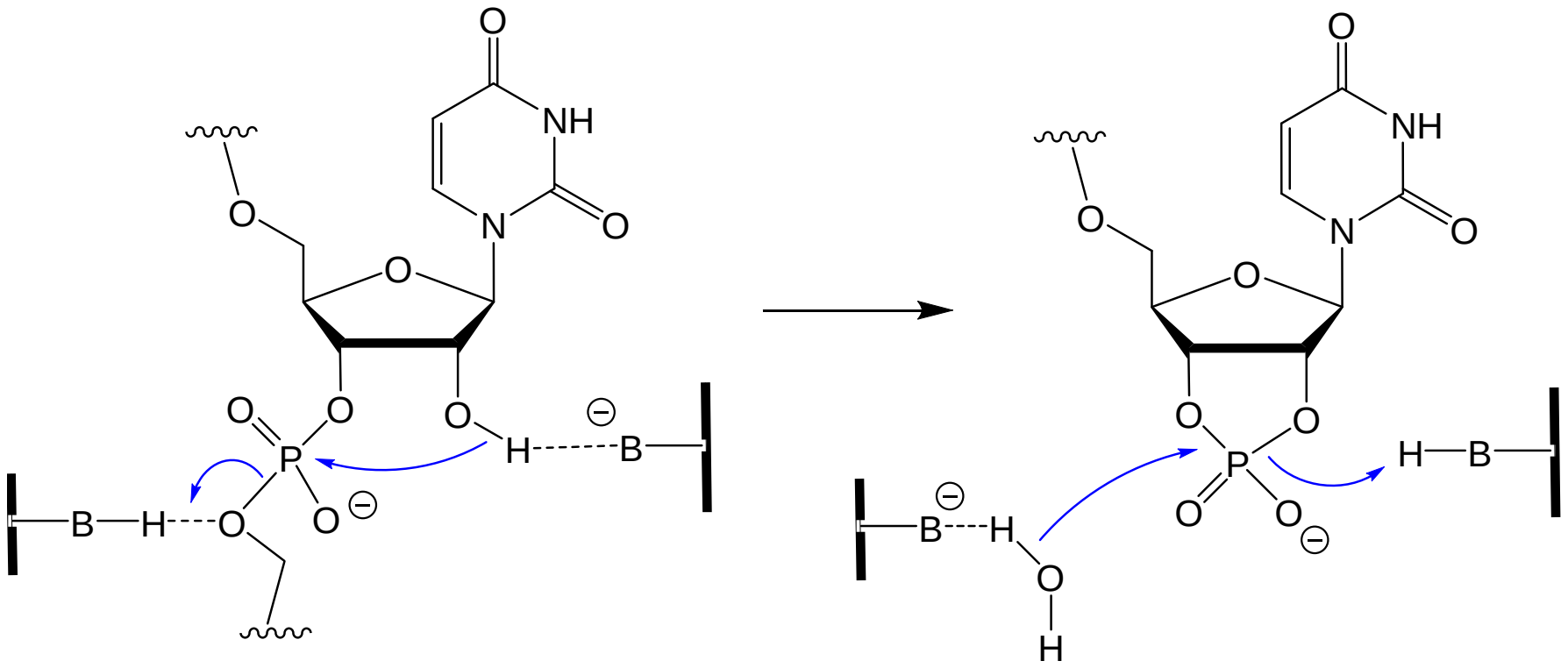
In both **acidic and alkaline conditions**, these reactions involve an **addition-elimination mechanism** (associative process).



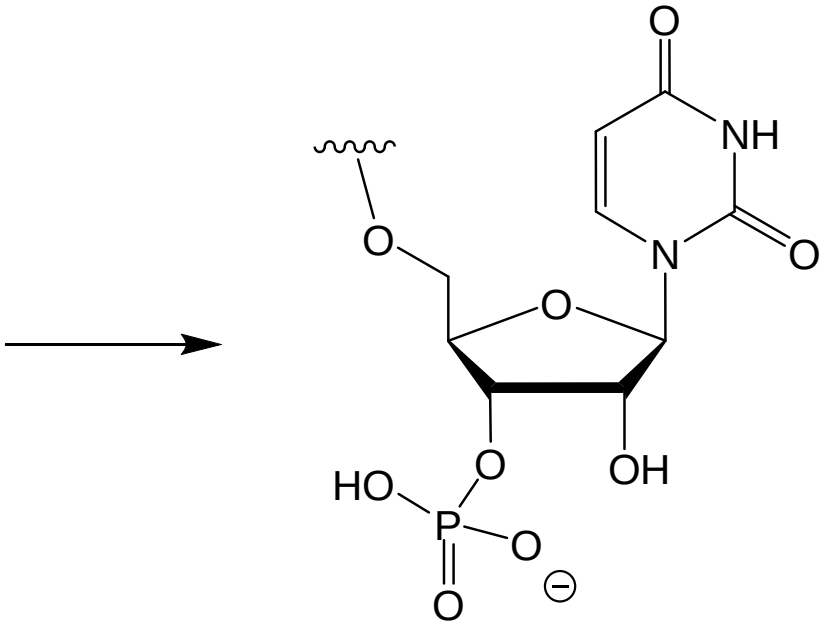
CHEMICAL SYNTHESIS OF BIOPOLYMERS

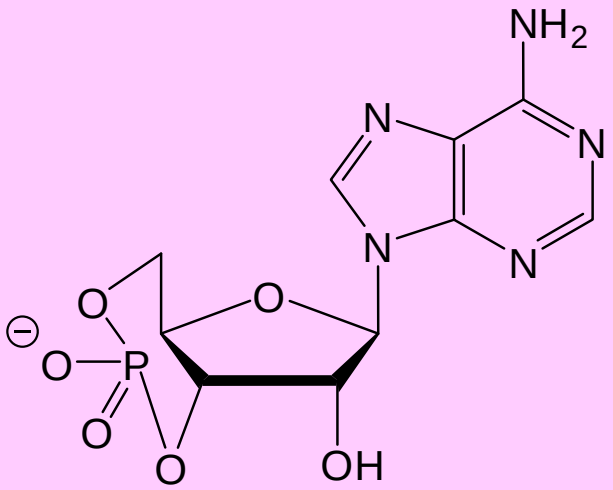
This phenomenon is involved in the **hydrolysis of RNA by ribonucleases or by alkali**.

In both cases, the 5-membered 2',3'-cyclic phosphates are formed by the displacement of the 5'-O-nucleoside residue and then followed by ring-opening.



CHEMICAL SYNTHESIS OF BIOPOLYMERS





It must be emphasized that this **remarkable reactivity is exclusive to 5-membered cyclic phosphate esters**.

That contrasts totally with the stability of 6-ring cyclic phosphates: **3',5'-cAMP** acts as the second messenger in cell signalling. This key role is dependent on its **kinetic stability to non-enzymatic hydrolysis**.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Hydrolysis of phosphate monoesters

At low pH, hydrolysis of phosphate monoesters proceeds via the **conjugate acid** as described for triesters (**C-O-cleavage**).

Monoesters are **very resistant to alkaline hydrolysis** as a result of anionic repulsion.

Under neutral conditions phosphate monoesters show an **unusually large reactivity**. This proceeds by **P-O- cleavage** and has all the **characteristics of a dissociative process (S_N1).**