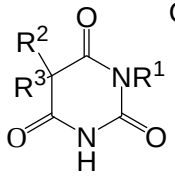
# 2. Synthesis of Nucleotides and Oligo-nucleotides

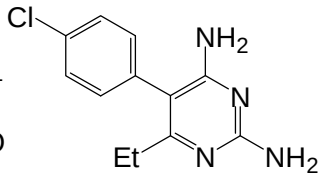
## 2.1 Synthesis of nucleosides

### 2.1.1 General information and synthesis of pyrimidines and purines

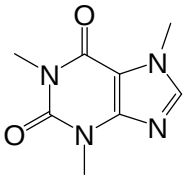
Many nucleotide analogs have strong biological effects. Many have antiproliferating activity and can be used for anti-viral and anti-cancer therapies. A few compounds with interesting biological activities are shown below.



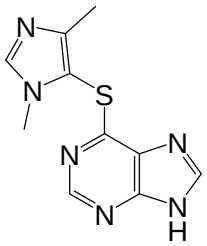
Barbiturate (Sedativa)



Antimalaria

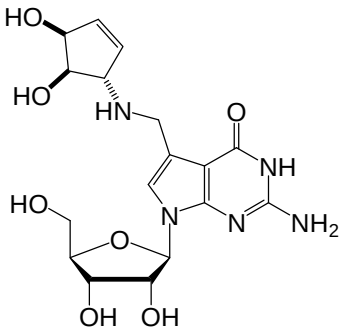


Caffeine

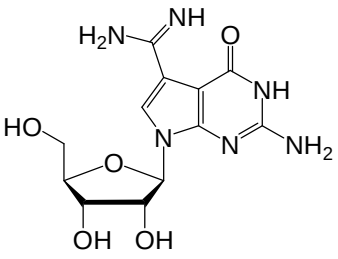


Azathioprine Immunosuppressant

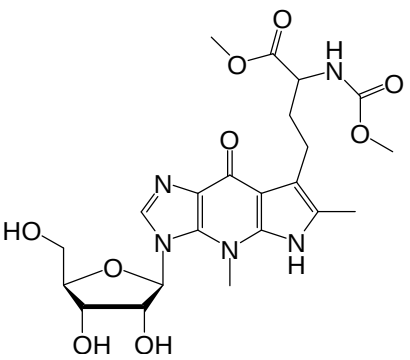
An important aspect of nucleic acid chemistry is the synthesis of new nucleosides and their incorporation into DNA and RNA. For example, efficient methods for the preparation of the unusual RNA bases Queusine, Wybutosine and Archaeosine are still missing.



Queuosine



Archaeosine

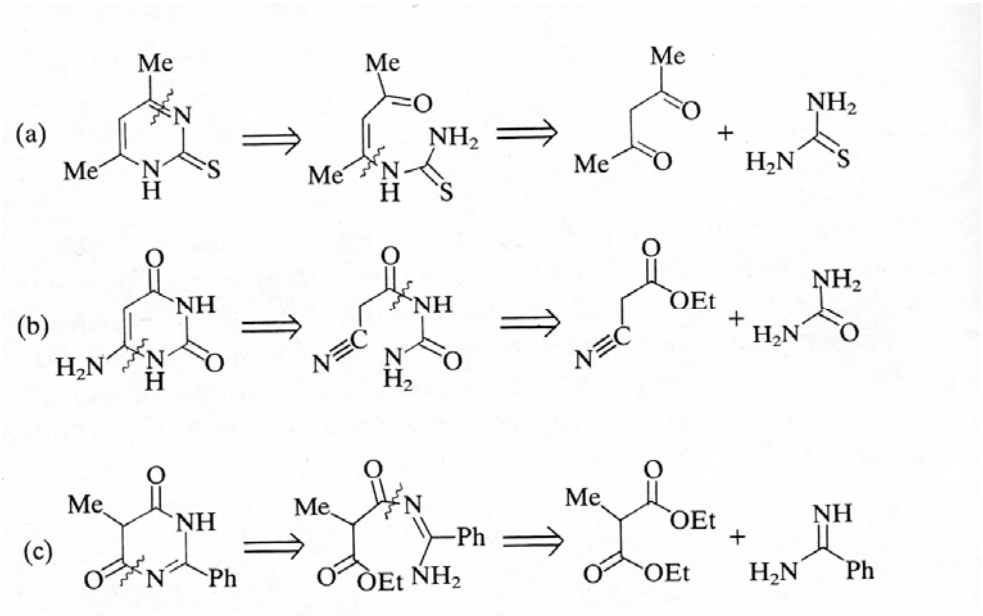


Wybutosine

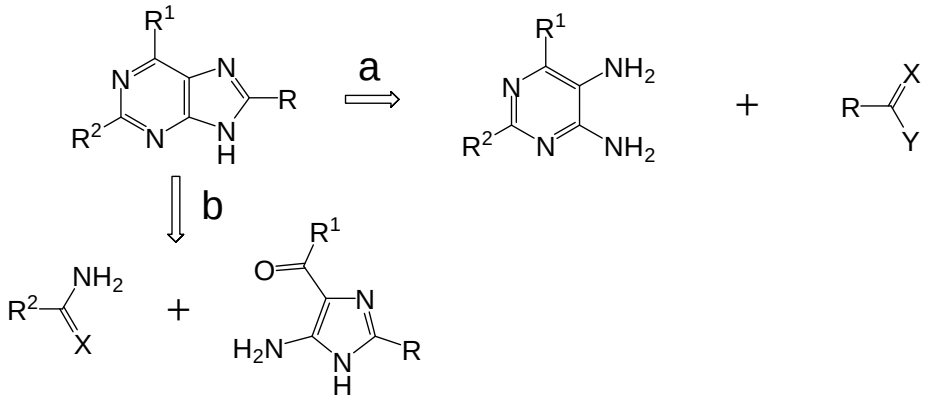
We therefore need efficient methods for the preparation of the heterocycles and for the synthesis of nucleosides and nucleotides. We also have to have good solid phase methods for the preparation of oligonucleotides.

**Synthesis of pyrimidines and purines**

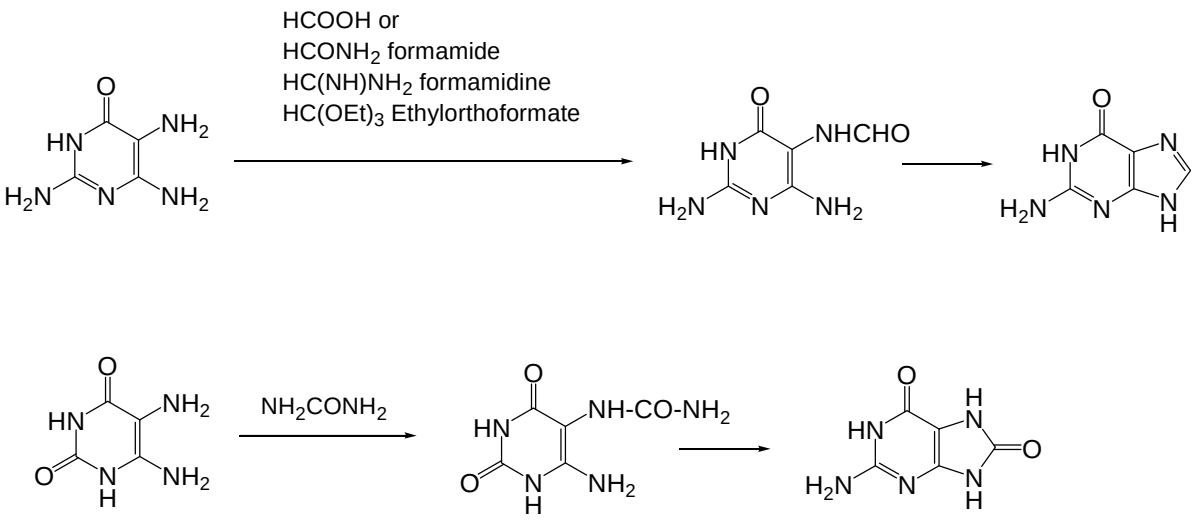
The synthesis of **pyrimidines** frequently includes the reaction of a C-C-C bis-electrophile with an N-C-N bis-nucleophile. Bis-electrophiles can be 1,3-diketones (a), 1,3-esternitriles (b) or even 1,3-diesters. As bis-nucleophiles function predominately thiourea (a), urea (b) or guanidine (c). The reaction conditions are in most cases rather harsh (a) conc. HCl, reflux or (b), and (c) NaOEt, EtOH, reflux.




The chemical synthesis of **purines** often proceeds along two different routes (a) or (b). Along route a, the imidazole ring is constructed at the pyrimidine ring. Route b forms the pyrimidine ring at the imidazole ring system.

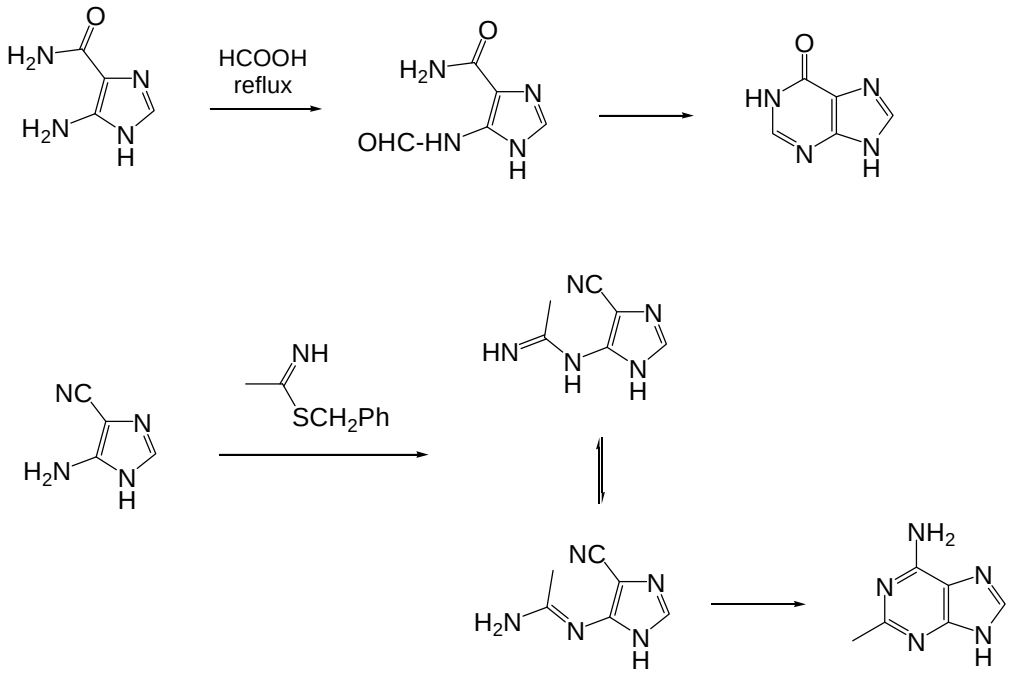


Route a is called Traube synthesis. Below are examples for this way to construct purine bases.



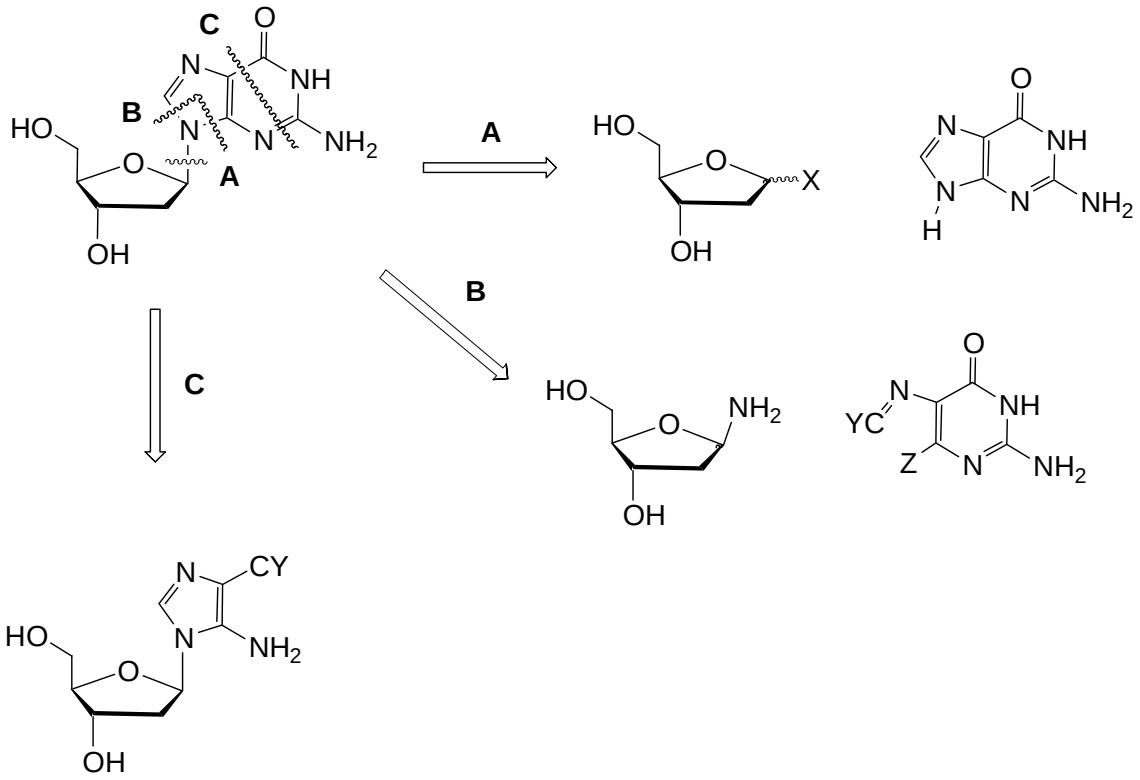

**Purine synthesis via the Traube way (a)**

Below are examples for the synthesis via route b.




These methods allow the synthesis of a variety of different pyrimidine and purine derivatives.

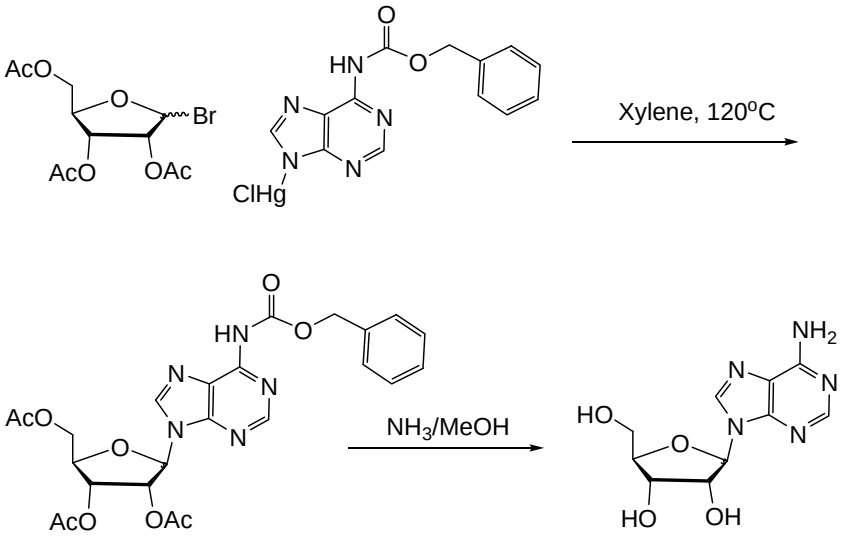
For the preparation of nucleosides, these heterocycles have to be attached to the C1' position of the sugar. The synthesis of nucleosides can be performed in three different ways (A, B and C). Route A involves a nucleophilic substitution with the leaving group attached to the sugar and the base acting as the nucleophile. Route B uses a sugar containing a nucleophile on C1' and the base as the electrophile. Route C achieves the synthesis of nucleosides by construction of the heterocycle in the presence of the sugar moiety. This way is elegant but also difficult because the sugars rarely withstand the rather harsh condition often employed in heterocyclic chemistry.



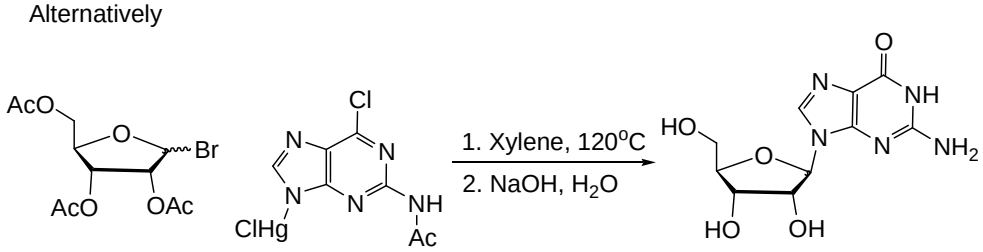

### 2.1.2 Synthesis of nucleosides using route A

Old methods for the construction of nucleosides use heavy metal salts of the bases, predominantly $Ag^{+}$ or $Hg^{II+}$-salts. These heavy metal salts are reacted with sugars bearing in C1' position either a chloro- or bromo-substituent. These methods, which are called Fischer-Helferich or Koenigs-Knorr are today still in use primarily for the synthesis of guanosine derivatives. Other potentially nucleophilic positions in the molecules require protection. Problematic with the method is the low solubility of the $Hg^{II+}$ and $Ag^{+}$ salts and the fact that the halogen sugars are rapidly hydrolyzed. The

mechanisms of the reactions are very complex. One obtains often the kinetically determined *O*-alkylated products first, which then rearrange under the harsh condition.

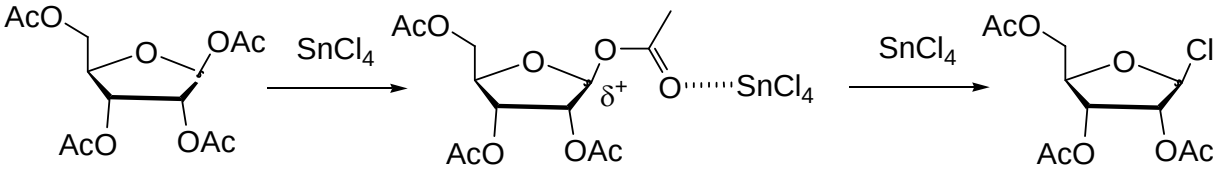



Alternatively

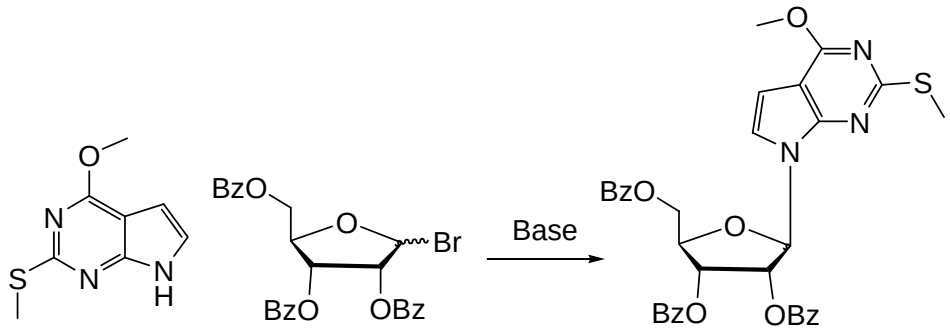



**Fischer-Helferich or Koenigs-Knorr method**

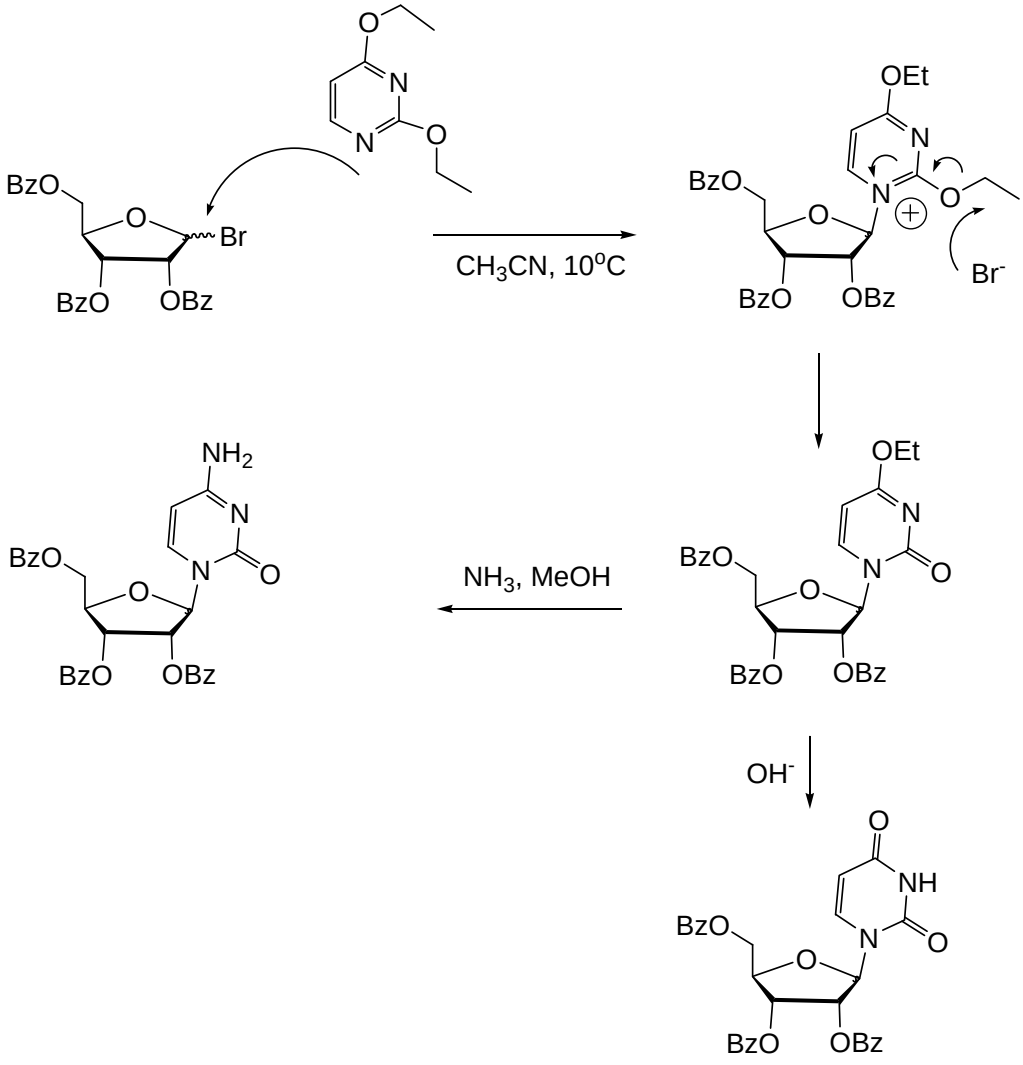
The procedure gives in general the correct regiochemistry. N1 of pyrimidines and N9 of the purines are reacting. A variation of the reaction uses instead of the halogen sugar the fully acetylated sugars, which are activated upon addition of a Lewis acid such as $SnCl_4$ or $TiCl_4$ generating the halogen sugar *in situ*.



Instead of the heavy metal salts also alkylated bases can be used. In these species the nucleophilicity of the sp-lone pairs is used for the reaction. This method carries the name Hilbert-Johnson nucleosidation.

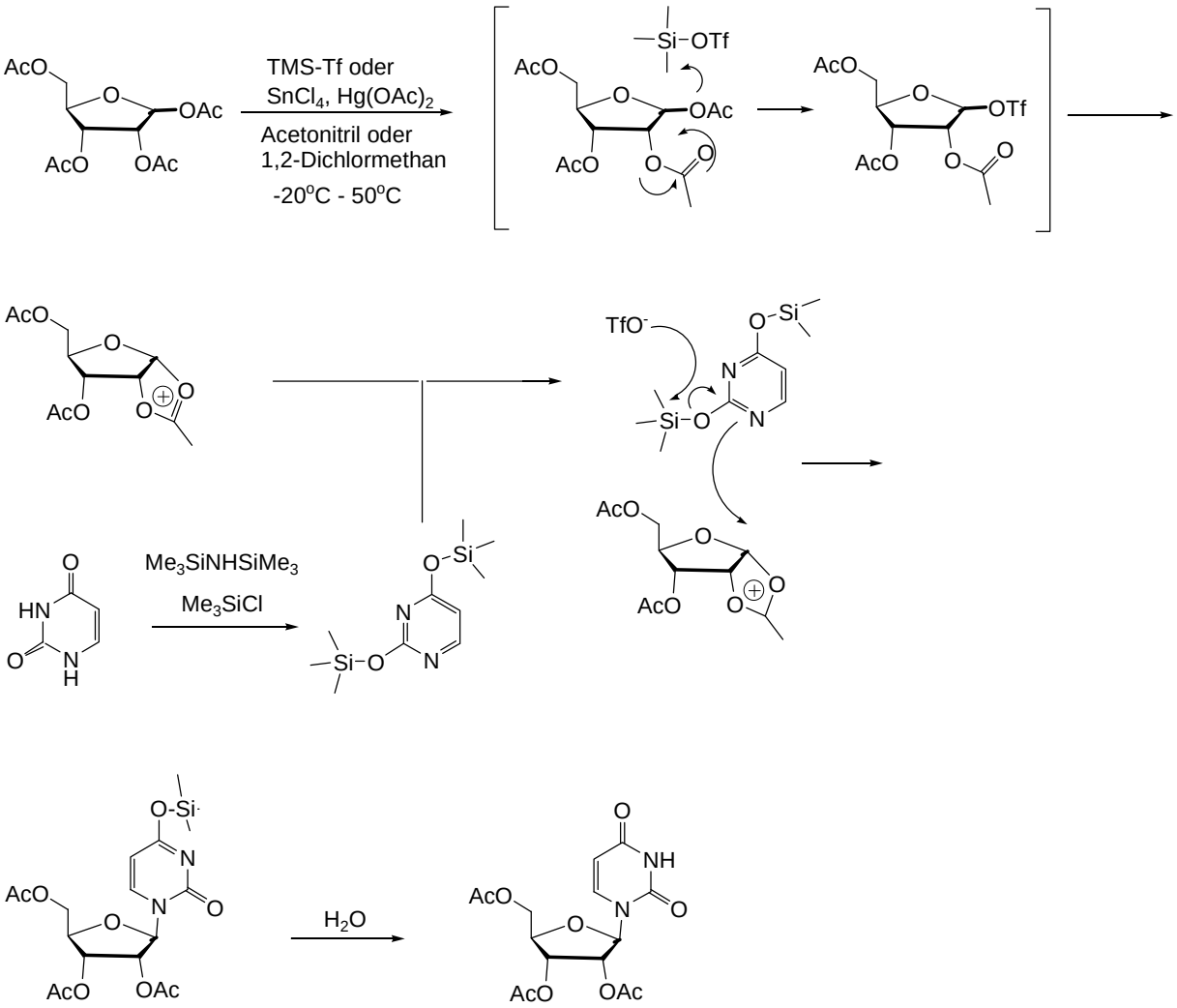



Direct nucleosidation gives first the quatary salt, which than eliminates




**Hilbert Johnson nucleosidation**

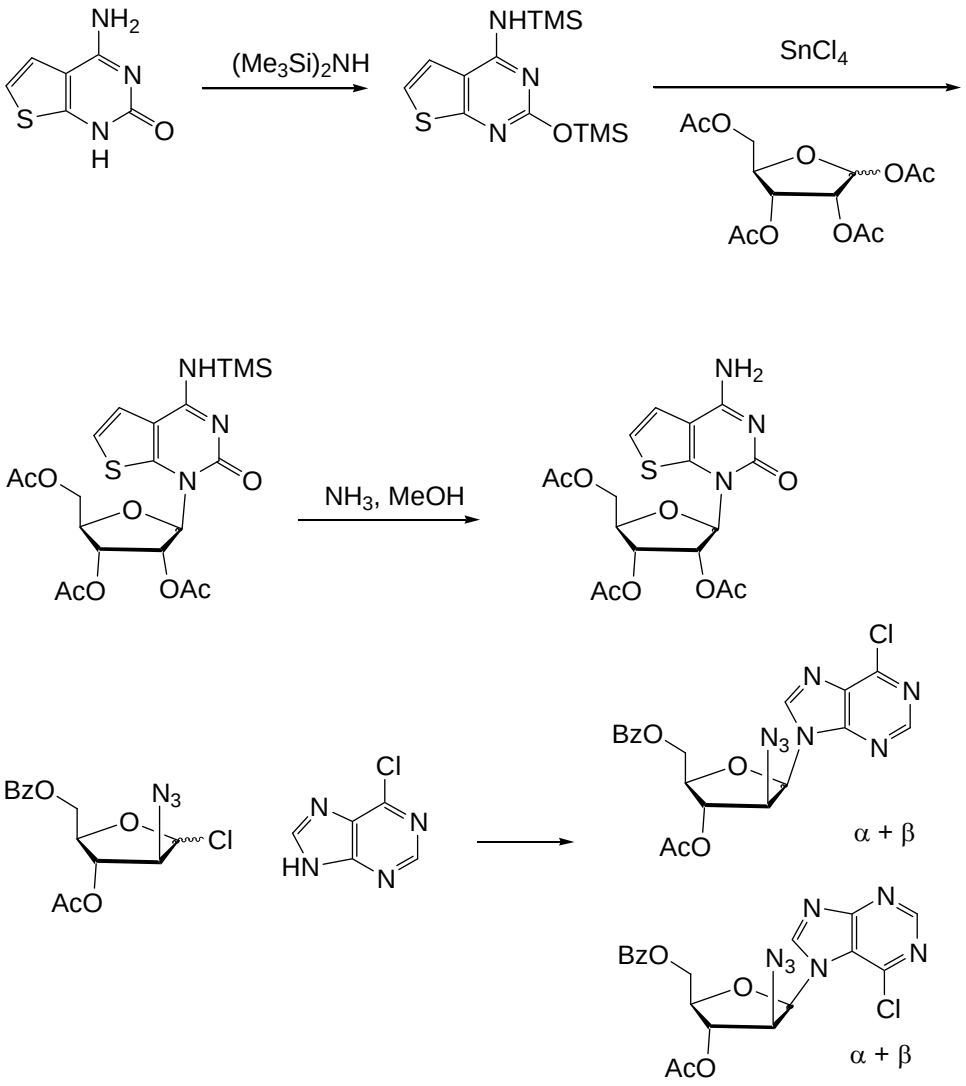
A modern version of the Hilbert-Johnson method is the silyl-Hilbert-Johnson method also called Vorbrüggen nucleosidation. Here the nucleobases are not alkylated but silylated, which has the additional advantage that the bases become nicely soluble. The silylation is best performed with hexamethyldisilazane (HMDS) in the presence of a small, catalytic amount of $Me_3SiCl$. Alternatively one can use bis(trimethylsilyl)acetamide [BSA] ($(Me_3Si)_2NCOCH_3$) The silylated base reacts subsequently with the peracetylated sugars, which are *in situ* converted into the halogen sugars or sugar triflates using a Lewis acid such as TMS-Tf or $SnCl_4$. The Tf-anion or the complex $SnCl_4$….$^-$OAc are helping with the desilylation of the base during the reaction.




**Vorbrüggen nucleosidation**

Depending on the conditions, the reaction proceeds in an $S_N2$ type manner with already strong $S_N1$ contribution. The transition state has consequently a strong oxycarbenium ion character. The glycosylation yields therefore a mixture of the α– and the β–products.

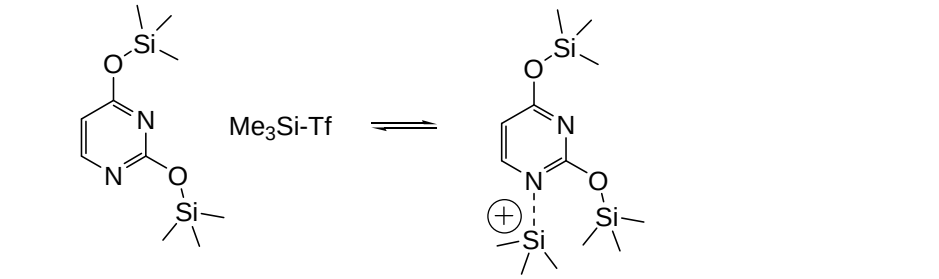
The reaction towards the α– to β–products depends strongly of the nature and the configuration of the group attached at C2‘. Acetate or a benzoate at C2’ are able to stabilize the oxycarbenium ion (neighbouring group effect). The incoming base will in such case be always reacting *trans* relative to this group. This 1,2-*trans* rule is called Bakers rule.




**Examples for nucleosidations**

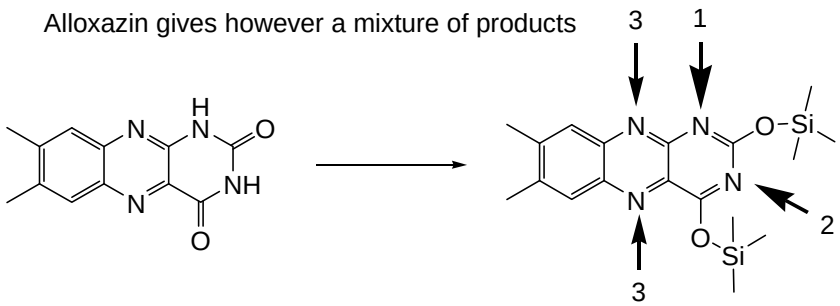
The nucleobase will always react with is most nucleophilic *N*-atom. So not only the stereochemistry at C1' but also the regiochemistry of the heterocycles is a problem during the reaction. Predictin,g which of the many N-atoms of some heterocycles will finally react is very difficult. Examples are shown below.

The Lewis acid used for the glycosylation plays an important role. First, it is required to activate the mostly peracetylated sugar. But secondly it will also form a complex with all basic centres present in the reaction mixture and therefore also with the centres at the heterocycle, which we want to react. Of course, the nucleophilic centre, which is mostly nucleophilic and therefore *per se* the most reactive centre is also the one which will be most strongly complexes and consequently deactivated by the Lewis acid. The problem of the reaction is finding a Lewis acid that is just strong enough to activate the sugar without complexation of two much of the heterocycle.




70% adenosine

Alloxazin gives however a mixture of products

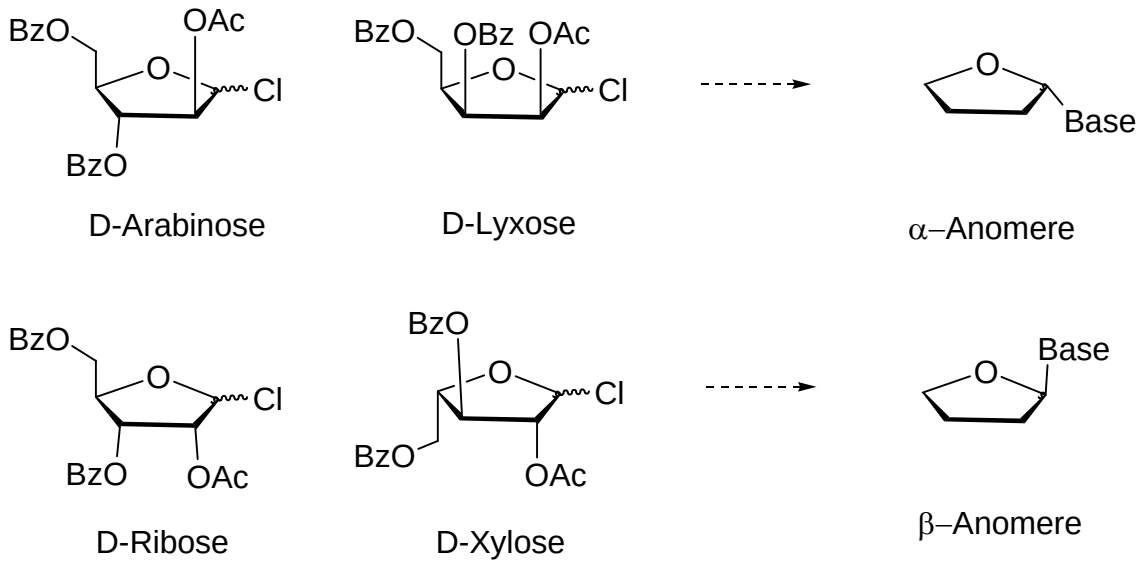



**Further examples for Vorbrüggen nucleosidations**

TMS-Tf is such a weak Lewis acid. If, however, reaction is wanted at the second best nucleophilic centre, it may help to increase the Lewis acid strength in order to complex the most reactive centre giving the second best a chance for reaction ($SnCl_4$). Another option is working in slightly nucleophilic solvents. In addition glycosylation reactions are mostly equilibrium reactions. So heating the reaction for a while may initiate a rearrangement giving rise to the thermodynamically most stable nucleoside (see below). The Vorbrüggen method works spectacularly well for pyrimidine bases, but also purine glycosylations can be achieved.

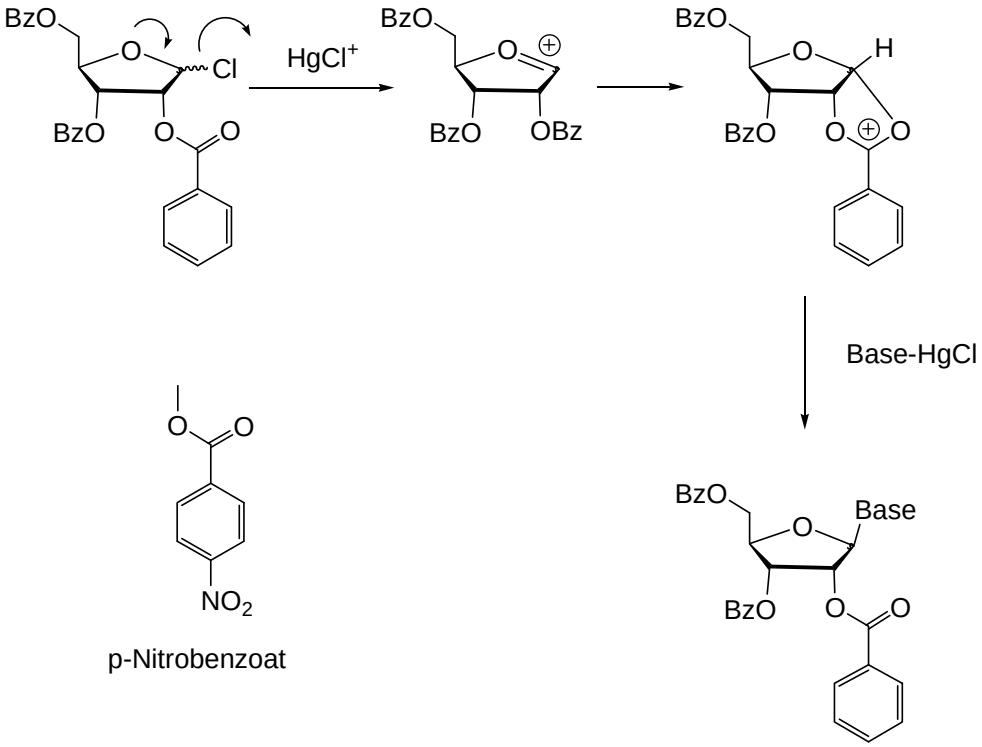
### 2.1.3 Bakers rule determines the stereochemistry at the anomeric centre

The problem of the glycosylation reaction is controlling the regio- and stereochemistry of the reaction. If a 1,2-trans relation of the two groups at C1' and C2' is desired, then Bakers rule allows synthetic access. The oxycarbeniumion will be stabilized by bridging, forcing the incoming nucleophile to react from the opposite face of the molecule. This rule gives the products shown below. The 1'-chloro-D-arabinose and 1'-chloro-D-lyxose will react (e.g. acetyl protected) with a base to give mainly the $\alpha$-anomers. 1'-chloro-D-ribose and the 1'-chloro-D-xylose will react to give the $\beta$-anomers.



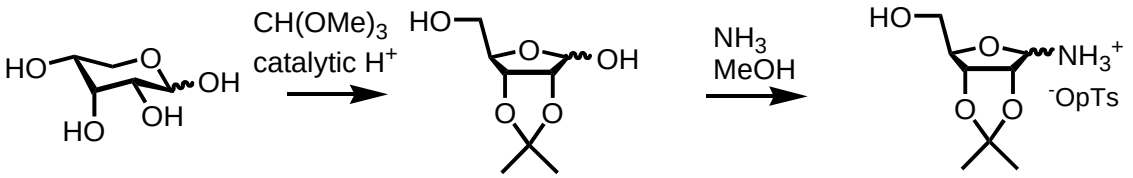

**Bakers Rule**

The neighbouring group effect is particularly well shown if one uses instead of benzoyl the *p*-nitrobenzoyl protecting group at C2'. The electron withdrawing effect of the *p*-nitro group reduces the ability to stabilize the oxycarbenium ion. Consequently, control over the stereochemistry at C1' should be reduced. This is indeed observed. One obtains less of the desired β-anomer.

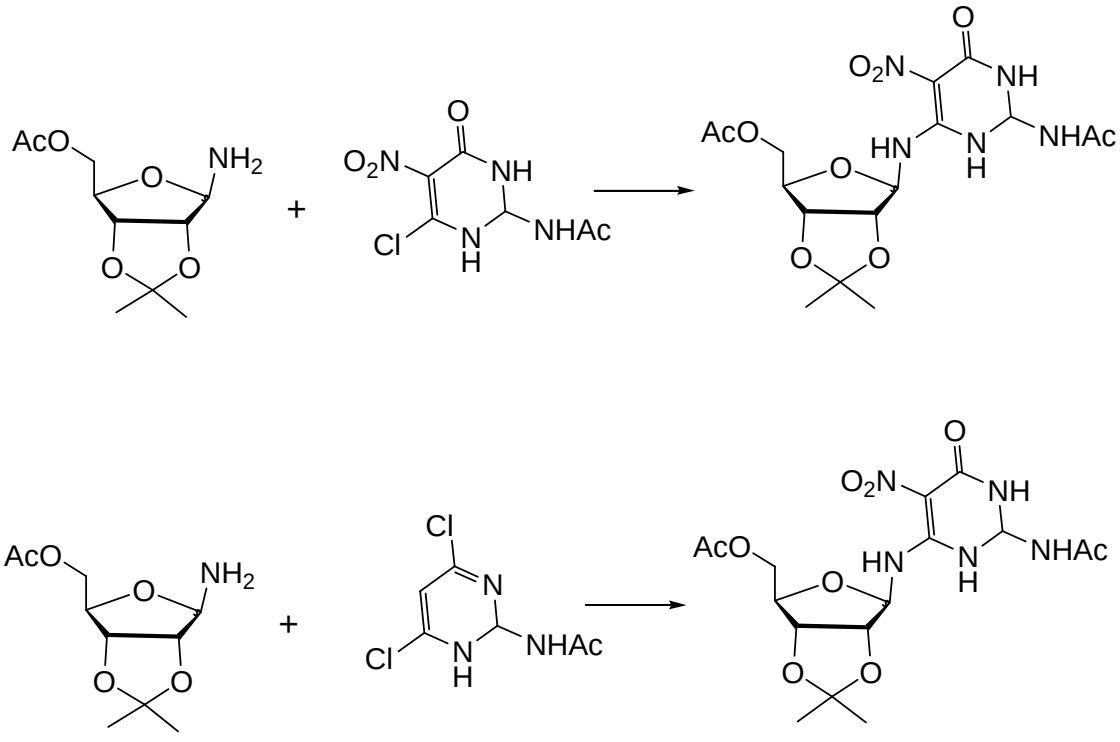



### 2.1.3 Syntheses based on Strategy B

One can exploit that the nucleobases are in principle very electron deficient aromatic systems amenable to nucleophilic (instead or electrophilic) aromatic substitutions. Attacking bases with a nucleophile requires converting the sugar into the 2'-amino sugar. Starting from D-(-)-ribose, one changes the configuration of the sugar first by converting the ribose into the acetonite protected D-(-)-ribofuranose, which upon treatment with ammonia gives the 1'-aminosugar.




This sugar can be reacted with nucleobases as shown below in the presence of weak bases (to free the amine). In the example below the heterocycle comprises quasi a vinylogous acid chloride. The reaction mechanism follows therefore an addition elimination process.

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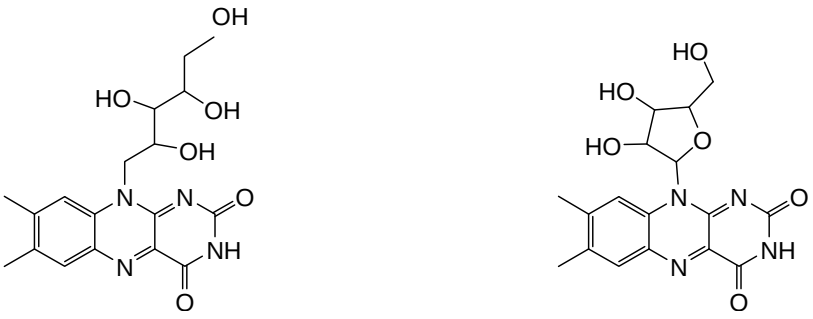
**Synthesis of nucleosides based on an addition elimination reaction sequence**

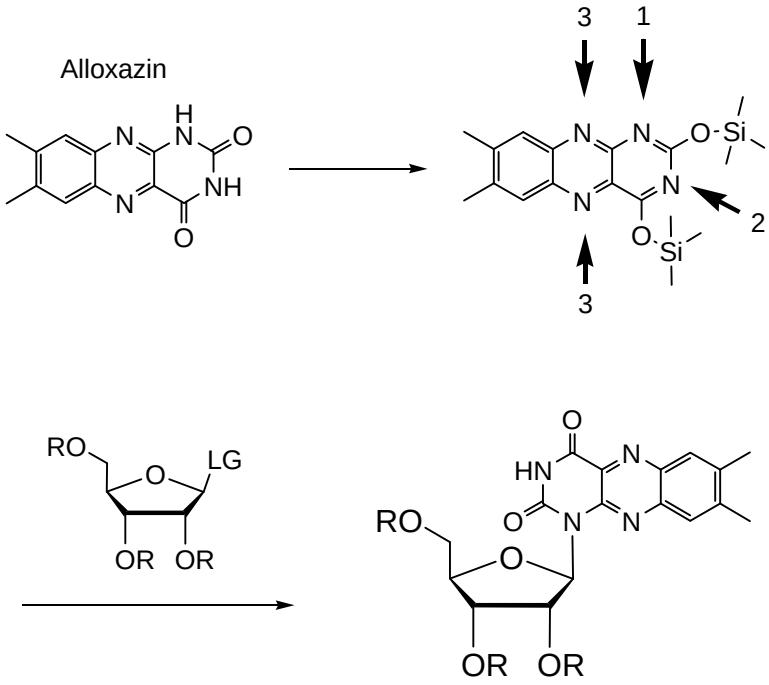
### 2.1.4 Synthesis of the heterocycle at the sugar

Due to the problems associated with controlling the regiochemistry and the stereochemistry at the anomeric centre, complex heterocycles as nucleobases require synthesis of the heterocyclus directly at the sugar moiety. A good example is the problem to create a flavin – vitamin $B_2$- type nucleobase. Vitamin $B_2$ is always

attached to an open chain ribose unit. The question was asked, what happens if we create a Vit $B_2$ derivative with the sugar adopting the ribofuranosidic from. Can the Vit $B_2$ then act as a nucleobase?

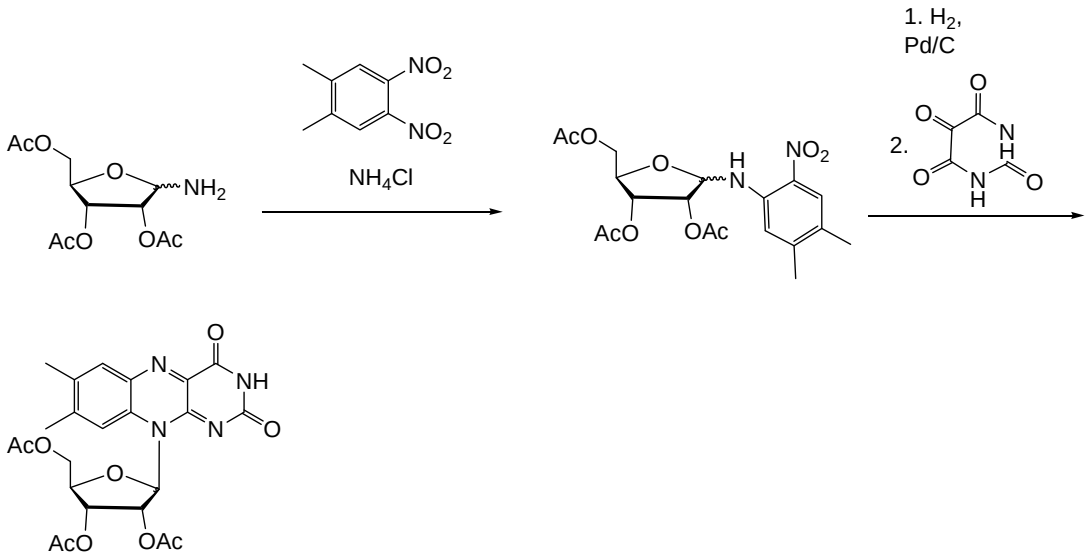



Use of the Vorbrüggen method allowed, however, only preparation of the alloxazine nucleobase, which shows no catalytic properties any more.




**Alloxazine nucleobases**

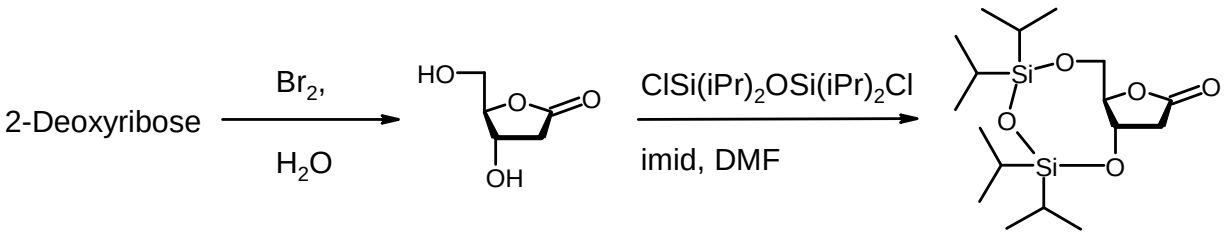
The strategy that finally allowed synthesis of this compound is depicted below:




**Vitamin $B_2$ nucleobase**

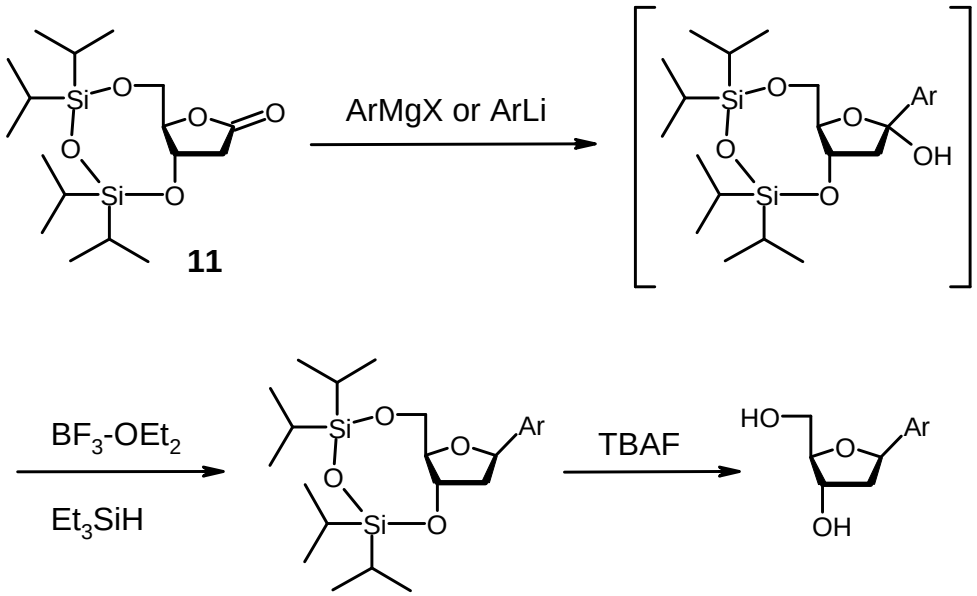
**Synthesis of C-nucleosides**

Many C-nucleosides are excellent natural products antibiotics. In comparison to the normal nucleosides, they contain no acetal unit any more and are hence much more stable compared to normal nucleosides and nucleotides. These are particularly unstable if the heterocyclus is readily protonated, which generates a good leaving group. One synthetic route to C-nucleosides uses ribonolactone as an intermediate. This is generated from e.g. 2'-deoxyribose by oxidation of the anomeric OH group with bromine. Protection with the special protecting group, which locks simultaneously the 5' and 3'-OH-groups together in a seven membered ring gives the protected ribonolactone ready for C-nucleoside synthesis. This involves reaction with a C-nucleophile, either the lithiated base or the Grignard reagent.



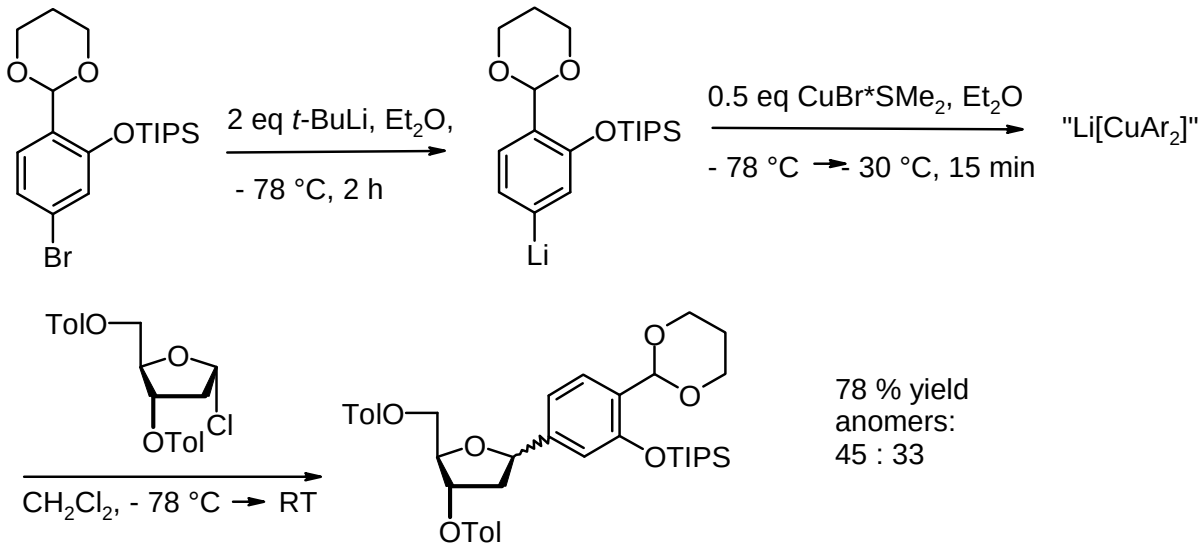

**Synthesis of ribonolactone**

The intermediate semi acetal is reduced with $Et_3Si$-H in the presence of a Lewis acid. Final deprotection is achieved with tetrabutylammoniumfluoride (TBAF). This procedures is generally yielding the C-nucleosides in fair yields.

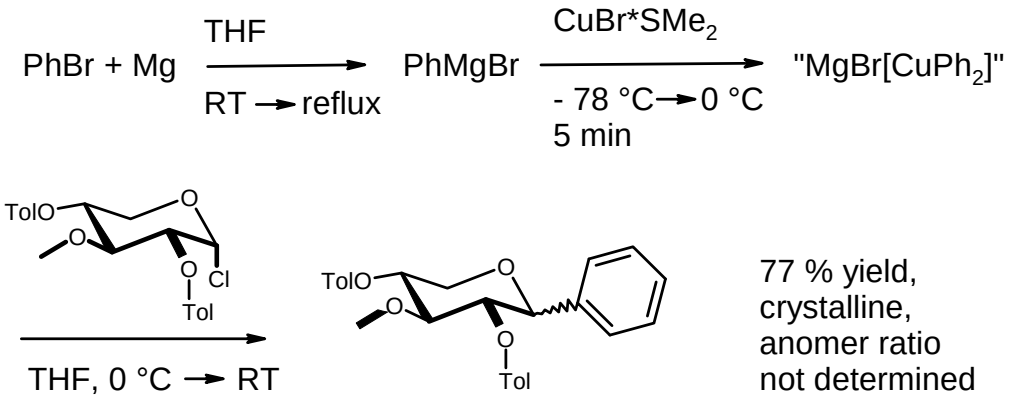



**Synthesis of C-nucleosides**

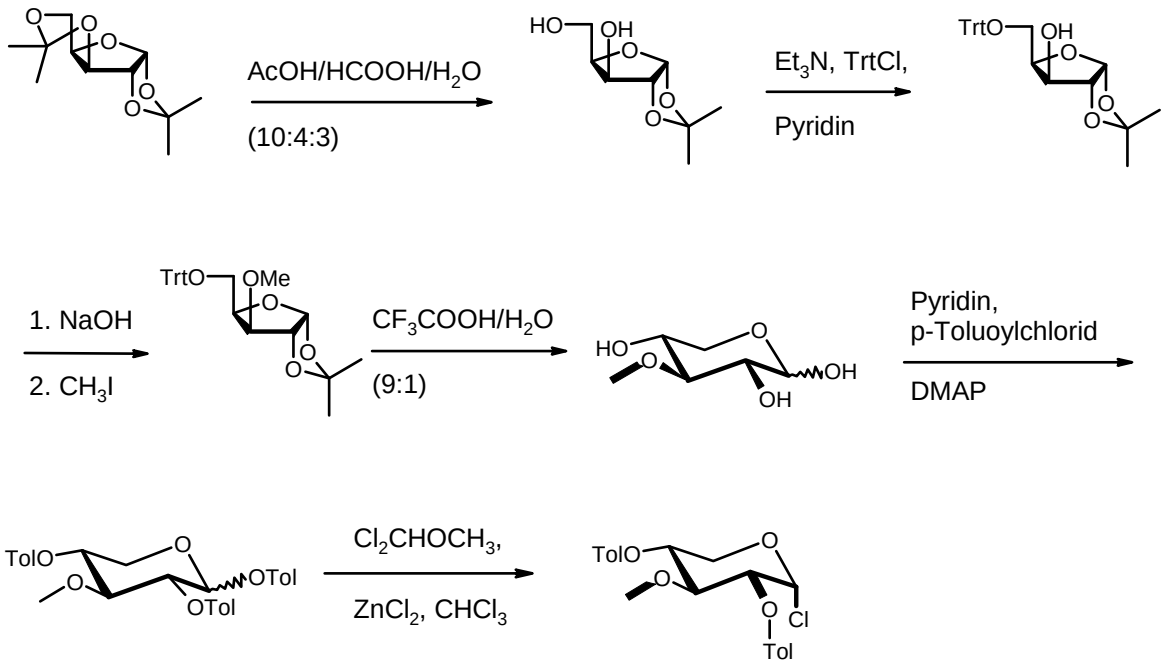
In our hand, however, the reaction of a cuprate with a C1' halogen sugar is working more efficiently. The base is first lithiated and then transmetallated to give the cuprate, which then reacts with the C1'-halogen sugar in a nucleophilic substitution.



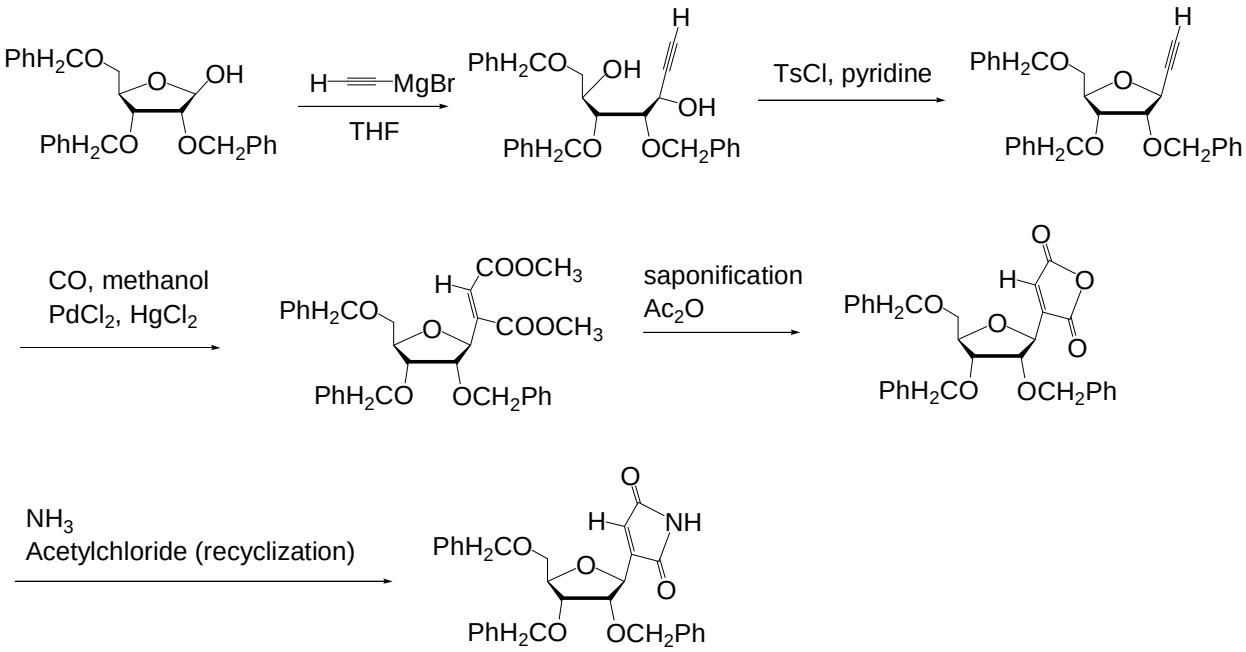
This methodology is also allowing synthesis of C-nucleosides of pyranosidic sugars as shown in the example below.




How does one get the pyranosidic xylose sugar starting material? The reaction sequence below exemplifies the type of chemical transformations needed to prepare the sugar. Starting material is diacetone-(D)-glucose.



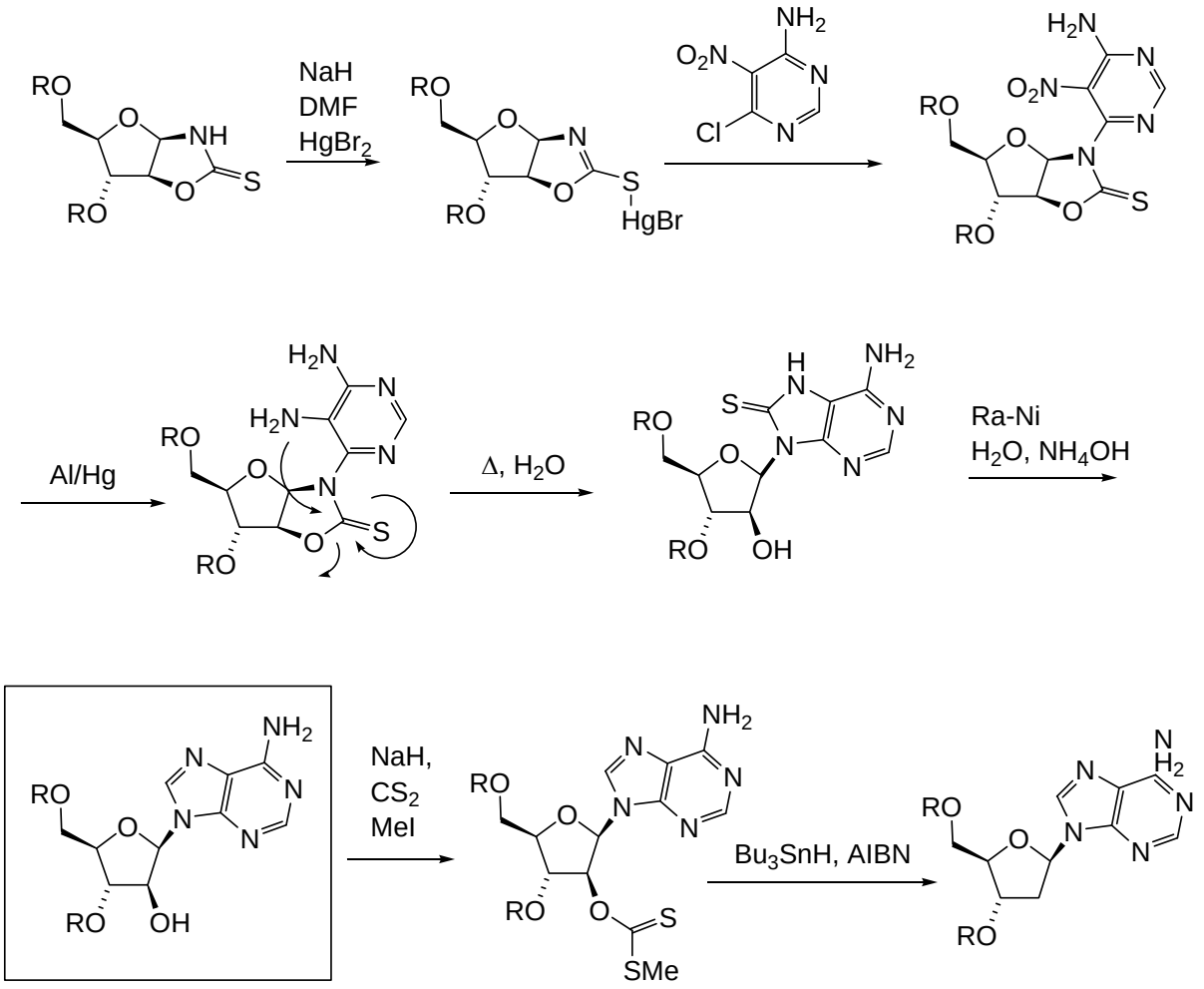
Another approach is exemplified by the total synthesis of showdomycin an antibiotic from the organism *Streptomyces showdoensis*.




**Total synthesis of the antibiotic showdomycin**

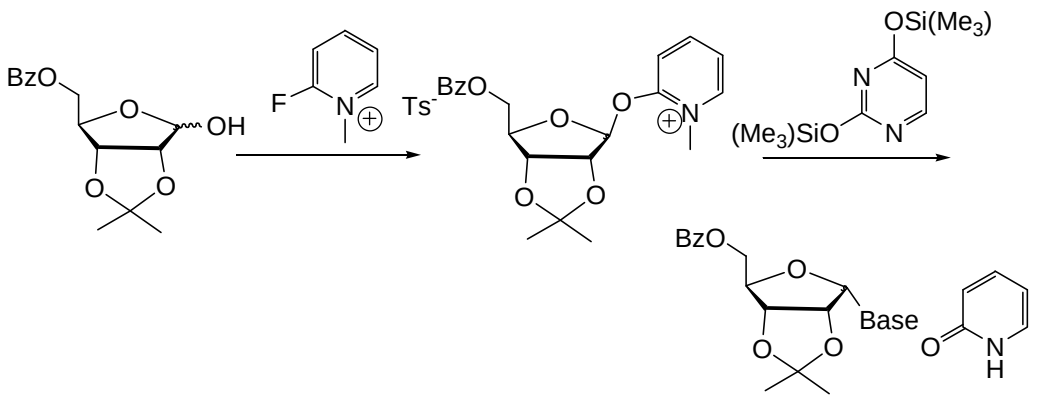
### 2.1.5 Stereoselective synthesis of α- and β-nucleosides („anti-Baker“)

A couple of methods exist for the stereoselective synthesis of nucleosides not accessible via Bakers rule. Selective preparation of β-anomers is for example possible using the oxazilidine-method. Depicted is the synthesis of arabinofuranosyl-adenine. Furthermore, the Barton McCombie method is frequently employed for the deoxygenation of the 2'-OH group.




**Stereoselective synthesis of D-(-)-arabinoadenosine with relative configuration 1,2-*syn*.**

A stereoselective synthesis of α-anomeric sugar is possible using 2-fluoro-1-methylpyridinium tosylate. Reaction of 5-*O*-benzoyl-2,3-*O*-isopropylideneribofuranose with 2-fluoro-1-methylpyridinium tosylate in the presence of a tertiary amine give almost exclusively the β-anomer of the 2-hydroxy-*N*-methylpyridiniumribofuranoside. Reaction with a silylated base proceeds exclusively via $S_N2$ to give the α-products together with a small amount of the β-product due to competing $S_N1$.




**Stereoselective synthesis of α-anomeric sugars with 1,2-*syn* configuration**

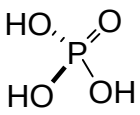
# 2.2 Synthesis of the Nucleotides

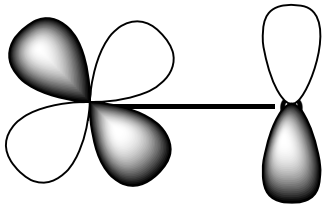
### 2.2.1 The chemistry of P (V)

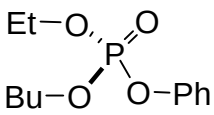
In biology, only the P in the oxydation state +5 is of relevance. This phosphor carries four “ligands”.

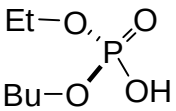
**The P-O single bonds** are $sp^3$ hybrid orbitals and they are about 1.6 Å long.

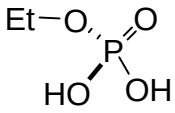
**The „P=O“ bonds** are 1.46 Å long. They are $p_\pi d_\pi$ hybrid orbitals. However the double bond does not add to the bond strength. It is better to write $P^+$-$O^-$

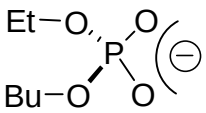




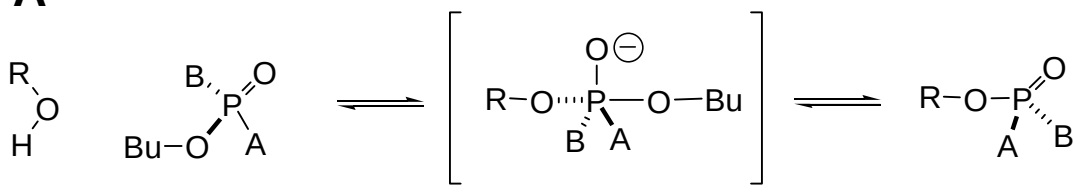

The typical **Triesters** such as PO(OEt)(OBu)(OPh) are soluble in almost all organic solvents. Here the P is a chiral centre.

The **Diesters** such as PO(OEt)(OBu)(OH) are water soluble compounds with a p$K_a$-value of 1.5. They are strongly acidic and hence deprotonated in water (for comparison: HOAc: p$K_a$ = 4.75). The charge is distributed among the two oxygen’s (of course the reason for the low p$K_a$-value).

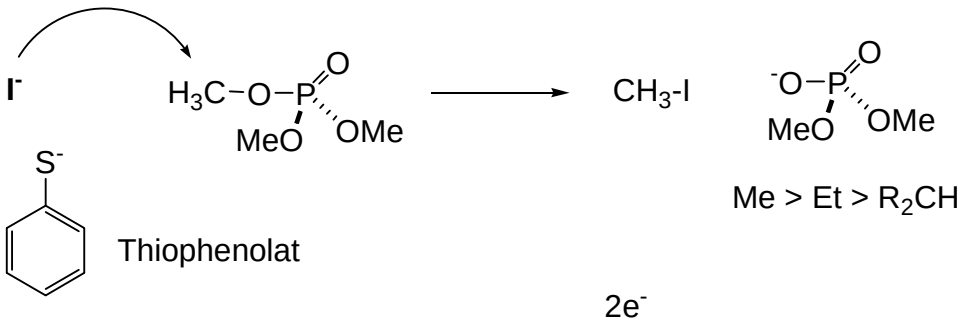
The **Monoester** such as $PO(OEt)(OH)_2$ are also water soluble with p$K_a$-values of 1.6 und 6.6. Under physiological condition they exist as a mixture of mono- and dianions .

The negatively charged mono- and diesters are extremely stable particularly towards hydrolysis. Any attacking nucleophile has to pass along the negative charge. The triesters in contrast are rapidly hydrolyzed, due the lack of any protecting negative charge, using three different reaction pathways (A, B und C).

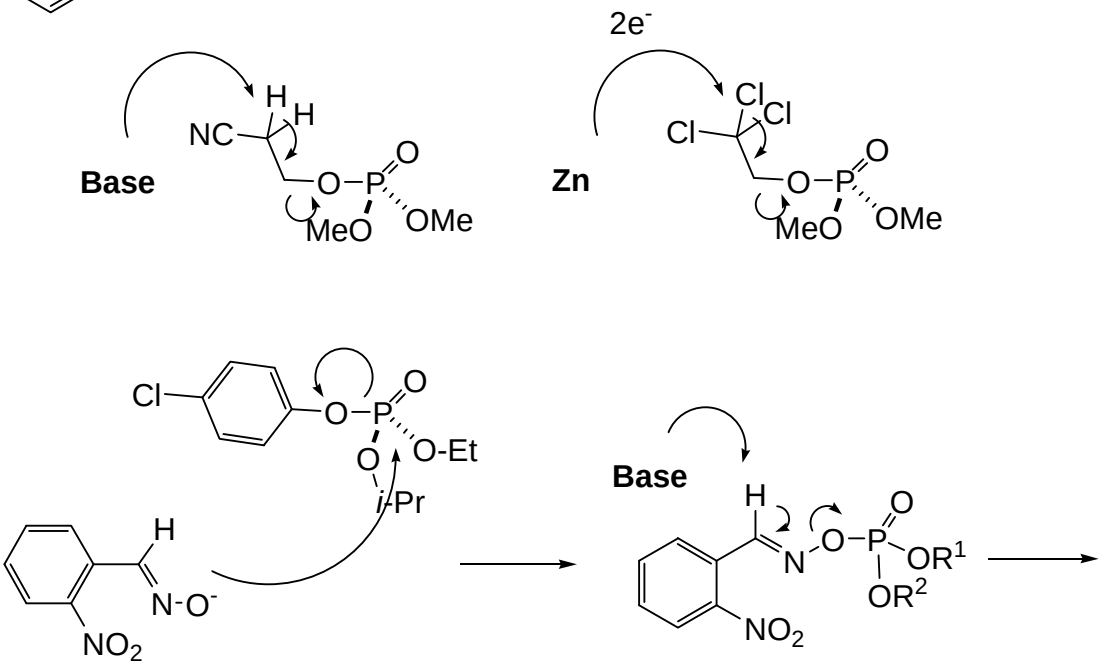
**A**

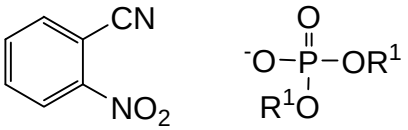



**B**




**C**



Pathway A describes a reaction following a typical $S_N2P$-Mechanismus. It is an associative mechanism, in which the nucleophile firstly attaches to the phosphor followed by elimination of the leaving group. In such a process, pseudorotation is frequently slower than decomposition of the intermediate so that chiral information is partially transferable. The mechanism is very fast if the leaving group is a phenol as in an arylesters of P(V). It is of course the low p$K_a$-value of the phenolate (p$K_a$-value 10) which make this anion a better leaving group in comparison to a normal alcoholate (p$K_a$-value about 15). To cleave aryl esters one uses frequently oximate-anions as the nucleophiles. These decompose later upon base treatment in a β-eliminination as described below. Typical arylesters used as protecting group for DNA synthesis are *p*-chlorophenolester.

Pathway B describes how very soft nucleophiles can attack a phosphotriester. They do not react with the P-centre but attack the alkyl group of the ester. In this reaction the phosphotriester behaves like a methyl iodide with the phosphodiester being the leaving group. The reaction is a typical $S_N2$-Reaktionen. This reaction is extremely well suited to deprotect phosphotriester. Deprotection in this way is fast and efficient with methylesters. Ethylester or secondary centres react much slower. So the sequence of reactivity is: Me > Et > $R_2CH$.
A typical application of the reaction is the deprotection of phosphoric acid methyl esters with thiophenolat.

Pathway C describes a β-elimination of alkylphosphat-triesters. This reaction works well when the β-C-Atom of the ester group bears an electron withdrawing substituent which acidifies the neighbouring H-atoms. This is for example the case with the cyanoethyl-group. This cyanoethyl group is today one of the most widely used protecting group in oligonucleotide chemistry. Another example is the trichlorethylester-protecting group, which can be removed reductively with Zn.
β-Elimination allows also cleavage of the oximate esters.

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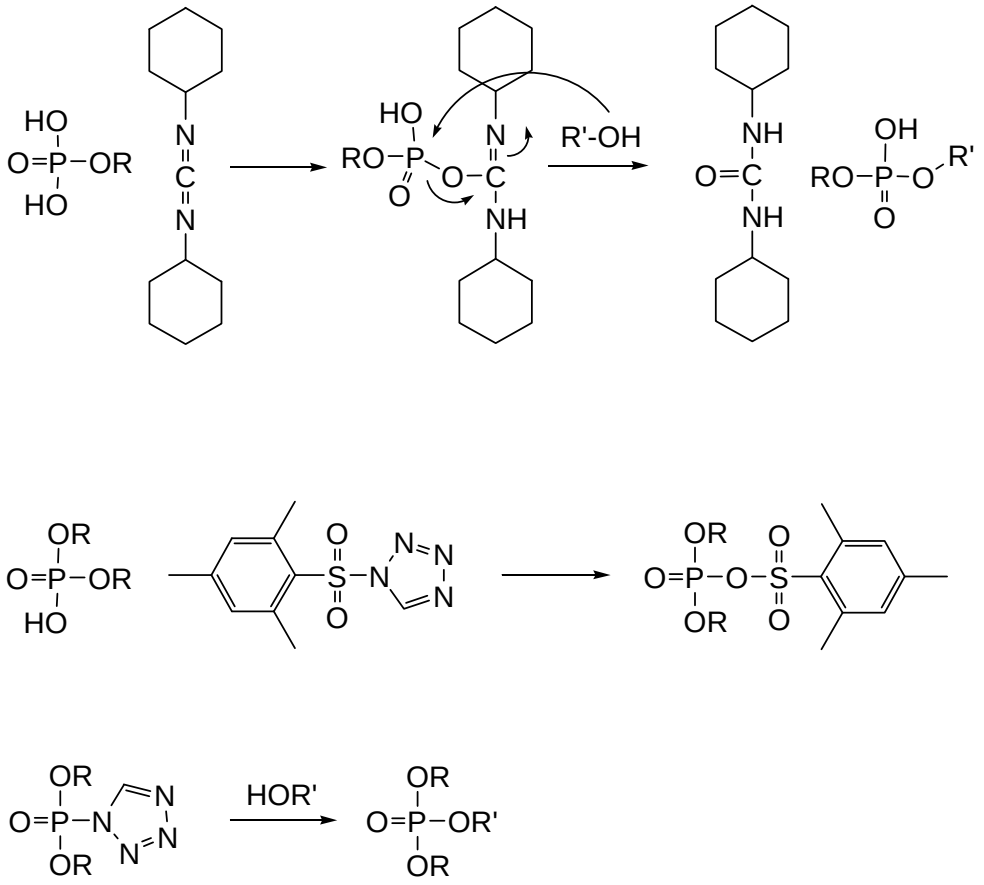
### 2.2.2 Synthesis of phosphate esters

If we prepare phosphate esters starting with phosphor compounds in the oxidation state +V, one way to create the ester is the usage of dicyclohexylcarbodiimide (DCC) in analogy to carboxylic ester synthesis. In the first step activation of the mono- or diesters with DCC is achieved as shown below. Then the attack by the nucleophile is followed. DCC is actually active enough to create mono and diesters. Triesters are not preparable using this method.

Further activation of the diesters to give triesters can be performed with mesitylene sulfonylchloride or with the tetrazolides and nitrotriazolides. Azolide anions have the advantage that they are only weakly nucleophilic. Already the $Cl^-$ can cause problems yielding cleavage of the phosphate triesters.

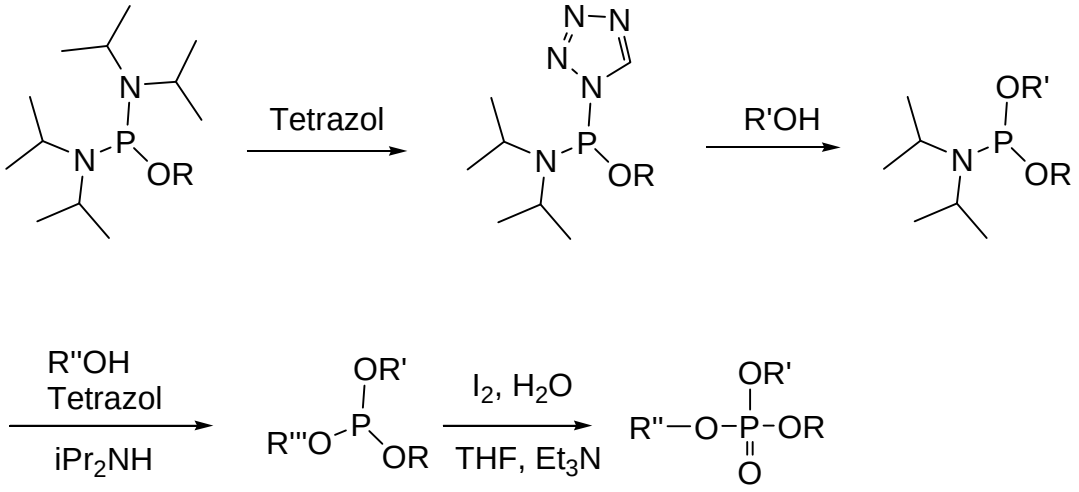



Today many synthesis of triesters proceed via phosphor in the oxidation state P(III) and consequently via phosphite esters. One exploits that P(III)-species are much more reactive (compare $PCl_3$ with $POCl_3$). A true break through in the field of making

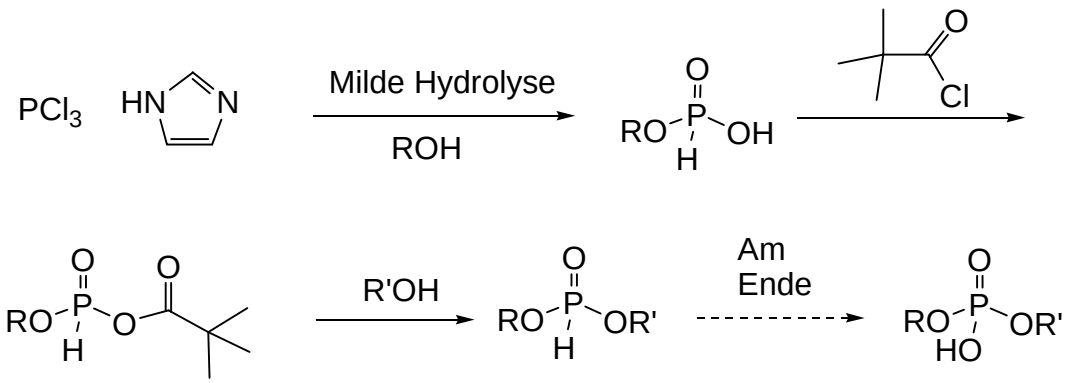
phosphate triesters was the development of the phosphoramidite chemistry by Caruthers und Matteucci (Fig. **A** below).

Here one reacts a diamide of phosphoric acid with tetrazole. The acidity of the tetrazole is sufficient to protonate the amide N-atoms. The protonated amides are extremely good leaving groups so that tetrazole can attack the phosphorous forming a tetrazolide intermediate. This procedure allows *in situ* activation of a stable amide precursor! The tetrazolides react quickly with nucleophiles such as alcohols to give the triester. One can stop the reaction starting from monoesters at the level of diesters by controlling the stochiometry. The resulting phosphate triester are rather instable. They are finally oxidized to the phosphate esters using iodine or *tert*-butylhydroperoxide.

**A**



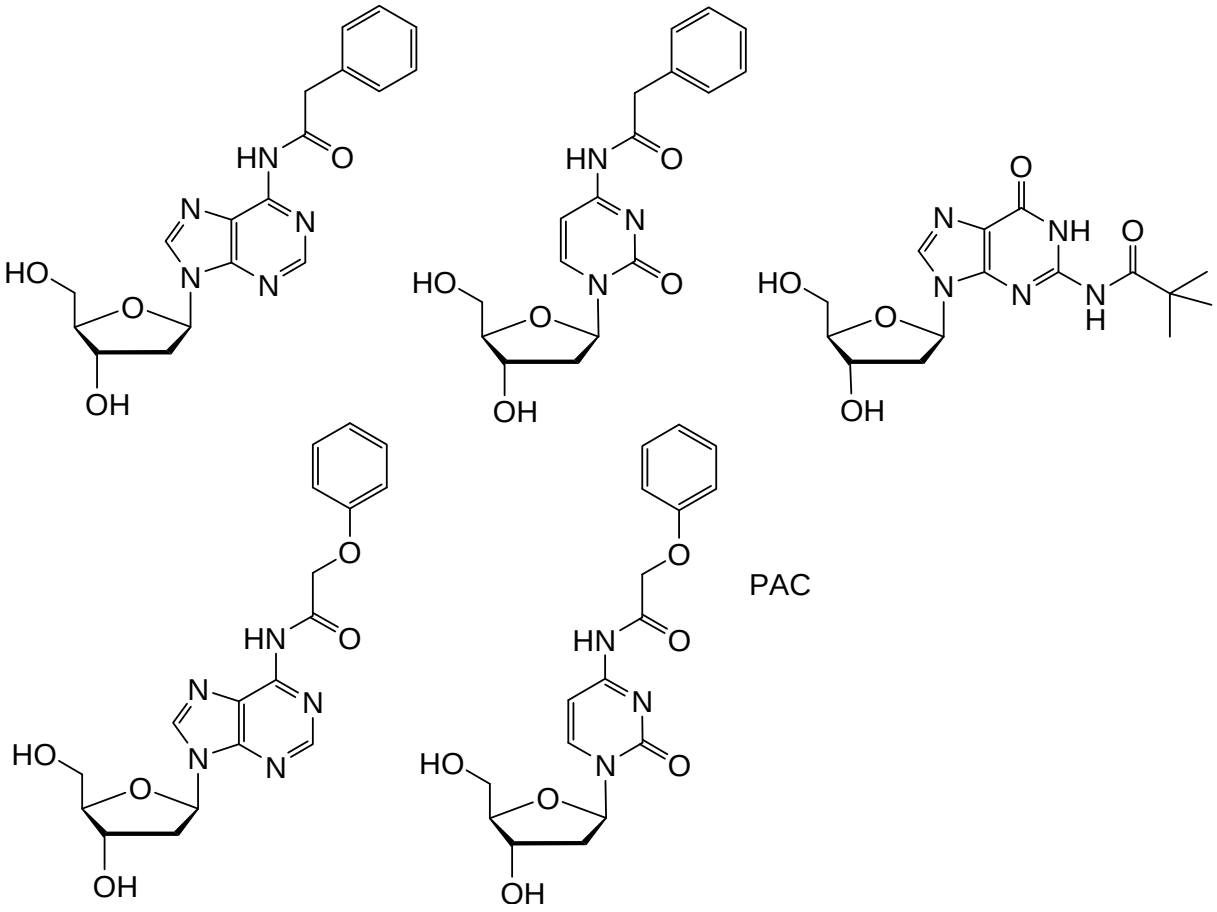

**B**



Based on result of the Todd-group also H-phosphonate chemistry has become popular in recent years. (Figure **B** above). First, reaction of $PCl_3$ with imidazole followed by mild hydrolysis gives H-phosphonates, which are tautomers of phosphite monoesters. In contrast to phosphite monoesters, however, these H-phosphonates are relatively stable. The H-phosphonates can be activated as mixed P(III)-carboxylic acid anhydrides. In use are bulky acid chlorides for the activation such as pivaloyl acid chloride and adamantoyl acid chloride. These anhydrides react quickly with alcohols to give H-phosphonate diesters. These can be oxidized again with either iodine or *tert*-butylhydroperoxide to give the phosphate diesters.

### 2.2.3 Solid phase synthesis

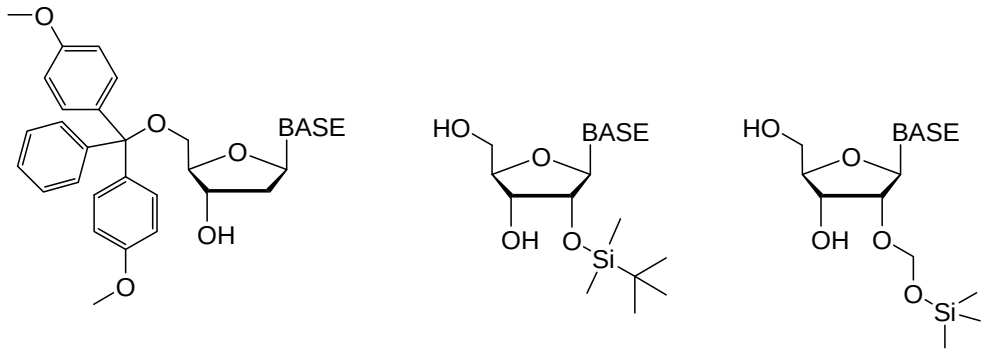
In DNA and RNA solid phase synthesis we have to form phosphodiester bonds between nucleosides. This requires attack of an activated phosphor species on one unit by an hydroxyl group of the second unit. All other, even only weakly nuleophilic groups, have to be protected with permanent protecting groups that will be removed at the end of oligonucleotide synthesis. Standard protecting group are benzoyl for the 6-amino group at adenine, benzoyl at the 4-amino group at cytosine and *iso*-butyryl for the 2-amino group of guanine. At the end of DNA synthesis, these groups are being removed with an aqueous conc. $NH_3$/ethanol mixture at 50°C over night. One can replace the benzoyl group by phenoxyacetyl moieties (PAC), which gives nucleobases, which are a little easier to deprotect. DNA prepared using phenoxyacetyl/iso-butyryl groups are deprotected using $NH_3$/ethanol mixture at r.t over night.




**Base protecting groups used for solid phase DNA synthesis**

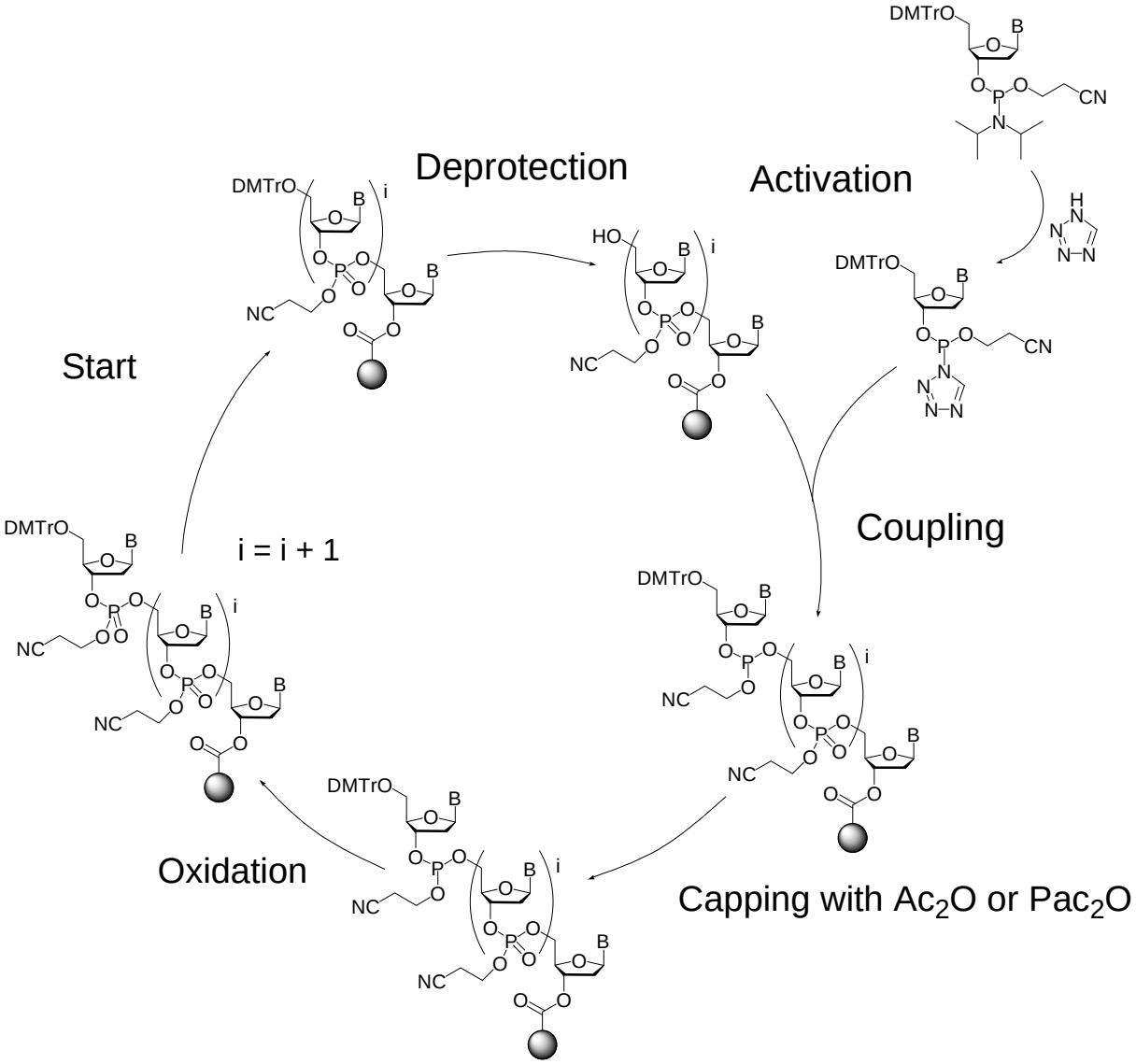
For DNA synthesis sequential formation of a phosphodiester group between the 3'OH and the 5'OH has to be performed. To this end, we have to install a temporary protecting group either at 3'OH or 5'OH, which has to be cleaved in every step during the DNA synthesis. Today most common is to attach this group to the 5'OH group (primary). In use are the trityl-group (Trt), the monomethoxytrityl group (MMT), and the dimethyoxytrityl group (DMT). The groups are cleaved in an E1-type mechanism with acid. Since acid is causing depurinations one has to use mild acids. The ability to cleave the three protecting groups with acid is increasing by a factor of ten in the order Trt < MMT < DMT. Most common is therefore today the use of the DMT group and the cleavage of this group during DNA synthesis with $CH_2ClCOOH$ or $CHCl_2COOH$.

For RNA synthesis we need in addition a permanent protecting group at the 2'OH group. For this, either the TBDMS-protecting group or the TOM-protecting group are in use, which are after DNA synthesis cleaved with fluoride.



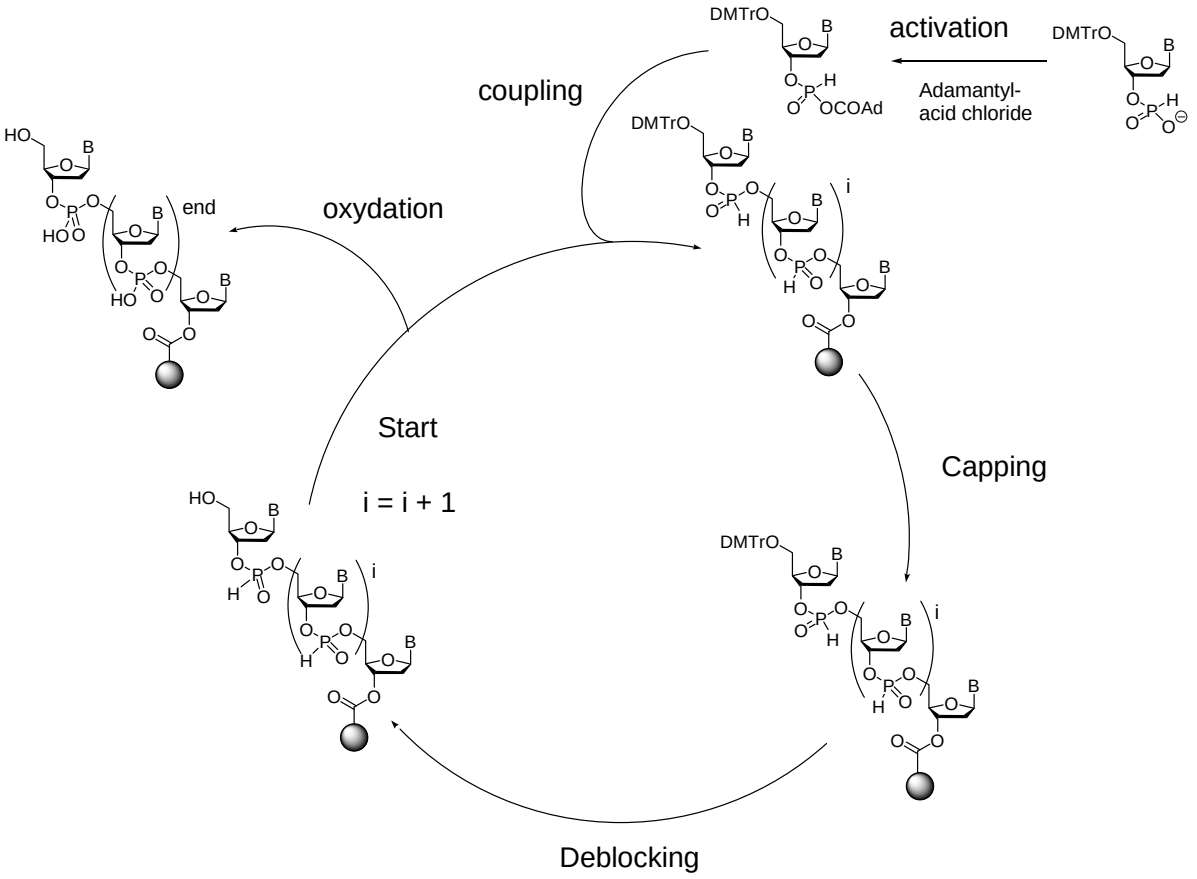

**DMT protecting group on 5'OH and TBDMS and TOM groups at 2'OH**

The solid phase procedure combines now the chemistry discussed above. The first example is the phosphoramidite DNA synthesis scheme.




**Phosphoramidite coupling protocol**

A very similar sequence is employed during H-phosphonate synthesis. Here however, oxidation is not performed after each synthesis cycle but at the end of the DNA synthesis. Oxidation can be problematic because it is performed under slightly basic conditions which may cause already cleavage of the diester. This can be a problem if modifications are introduced into DNA, which may have for steric reasons a slower oxidation kinetics.

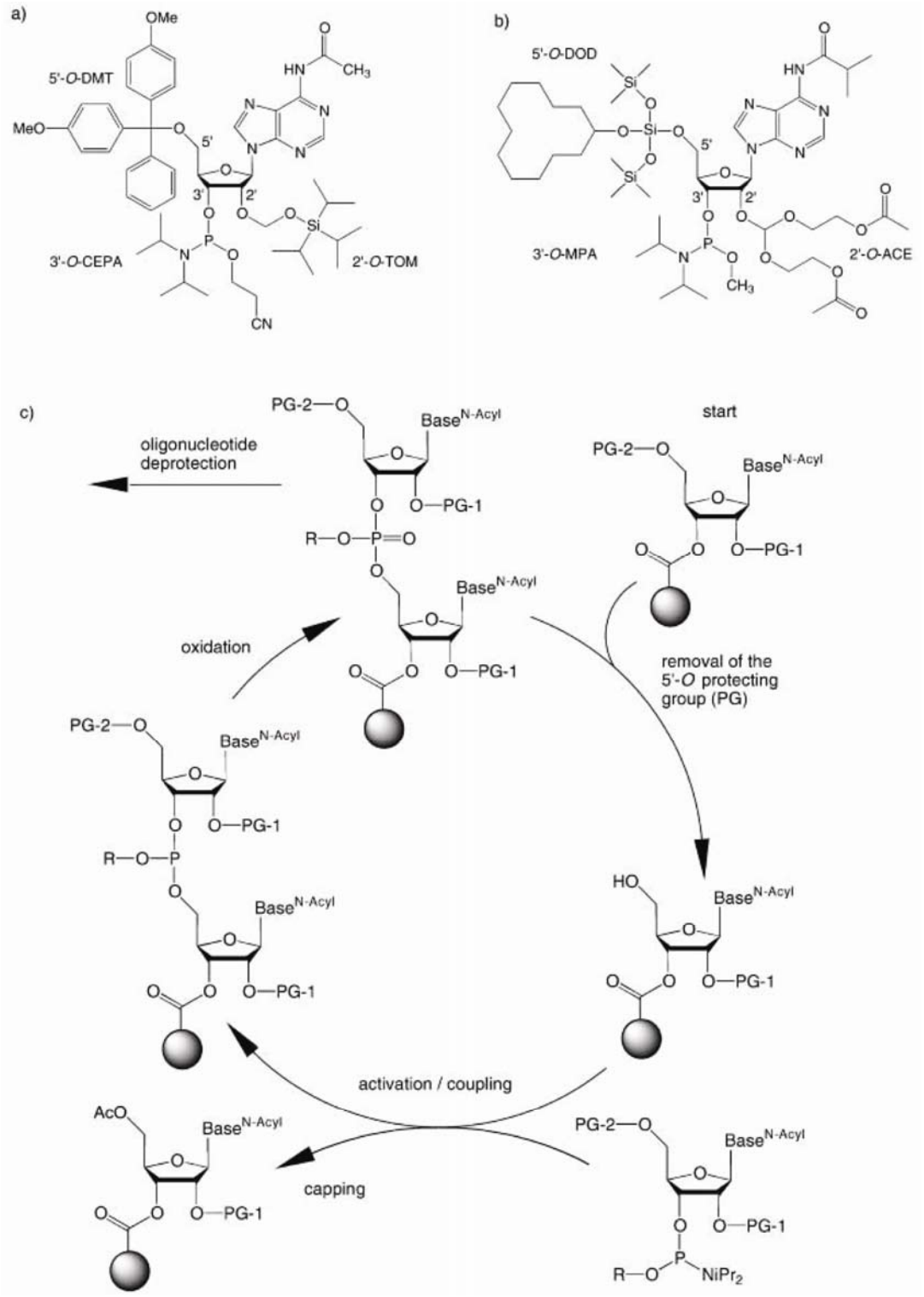



**H-Phosphonate DNA synthesis scheme**

**New developments**

DNA synthesis is today very efficient and in fact the basis for modern biotechnology, where DNA primers for PCR are routinely needed. For chemisty however, the synthesis conditions are still rather harsh. Particularly the deprotection required strongly basic conditions. Here, the use for more base labile protecting groups helps but still the incorporation of very base sensitive building blocks remains a challenge. Another problem is RNA synthesis which still less efficient compared to DNA

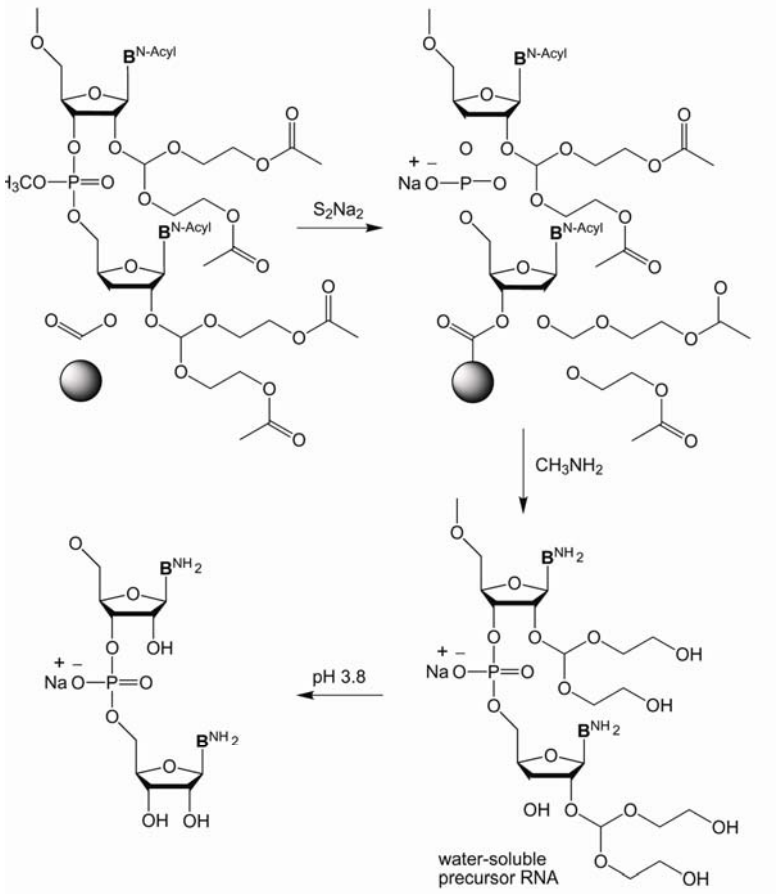
(coupling yields hardly extend beyond 97%) which limits the length of the RNA that can be synthesized. Here the TOM and the 2'*O*-ACE methodologies are new developments. The TOM methodology is along the line of standard chemistry. The 2'-OH protecting group is sterically a little less hindered which increases the coupling yields. The ACE method is a completely new technology.




For the precise reaction conditions used in both synthesis cycles see the table below.

Table 1. Conditions for a complete coupling cycle in the 2′-*O*-TOM method and in the 2′-*O*-ACE method, and for the subsequent deprotection of the synthesized oligoribonucleotide.

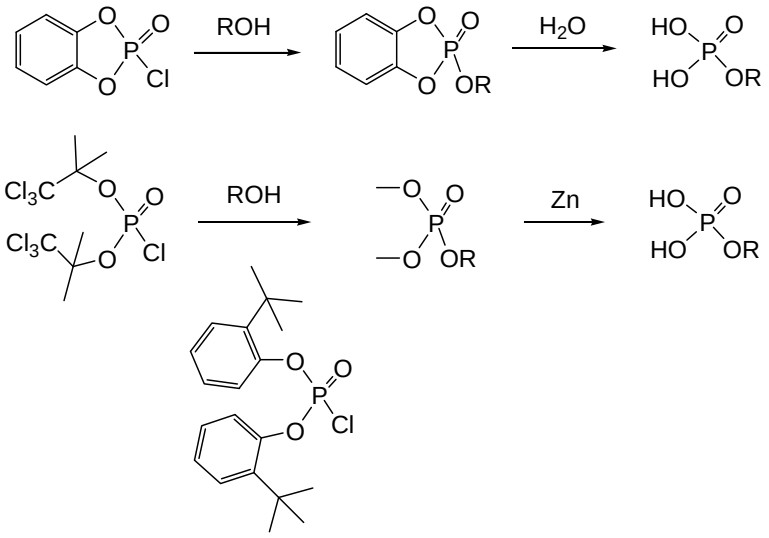
| | 2′-*O*-TOM method (1.5 μmole scale) | 2′-*O*-ACE method (0.2 μmole scale) |
|---|---|---|
| Removal of the 5′-*O*-PG | 4% dichloroacetic acid in 1,2-dichloroethane, 90 s | 1.1 M HF/2.9 M triethylamine in dimethylformamide, 35 s |
| Activation/Coupling | 0.25 M benzylthiotetrazole (65 equiv)/0.1 M cyanoethyl-phosphoramidite (6 equiv) in $CH_3CN$, 90 s | 0.5 M ethylthiotetrazole (75 equiv)/0.1 M methylphosphor-amidite (15 equiv) in $CH_3CN$, 90 s |
| Capping | $Ac_2O$/2,6-lutidine/THF (1/1/8; v/v) and *N*-methylimidazole/THF (16/84; v/v), 60 s | $Ac_2O/CH_3CN$ (1/9; v/v) and *N*-methylimidazole/$CH_3CN$ (1/9; v/v), 30 s |
| Oxidation | $I_2/H_2O$/pyridine/THF (3/2/20/75; w/w), 45 s | 1 M *tert*-butylhydroperoxide in toluene, 45 s |
| Oligonucleotide deprotection | 1) 10 M $MeNH_2$ in $EtOH/H_2O$; 1–24 h, 25–33 °C<br>2) 1 M $Bu_4NF \cdot 3H_2O$ in THF; 1–50 h, 25 °C<br>3) 1 M Tris · HCl, $H_2O$, pH 7.4 | 1) 1 M disodium-2-carbamoyl-2-cyanoethylene-1,1-dithiolate trihydrate ($S_2Na_2$) in DMF; 15 min<br>2) 40% $MeNH_2$ in $H_2O$; 1 h, 55 °C<br>3) 100 mM tetramethylethylendiamine/acetic acid, pH 3.8; 30 min, 60 °C |

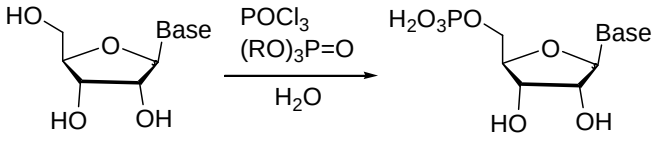



Scheme 2. Deprotection of an oligoribonucleotide synthesized by the 2′-*O*-ACE method proceeds via a water-soluble precursor RNA which cannot form strong secondary structures and is therefore easy to analyze by HPLC.

### 2.2.4 Synthesis of other biologically relevant phosphates

Important is also the synthesis of phosphate monoesters. They are commonly prepared by two different methods. One method involves hydrolysis of triesters. Catechol-phosphoric acid chloride was developed for this purpose. This reagent reacts rapidly with alcohols to give the corresponding triesters. Due to the higher reactivity of the two aryl ester groups they can be cleaved selectively with already water. In order to prepare selectively phosphate monoesters of 5'OH groups, sterically bulky reagents were developed, which react preferentially with primary OH groups. Useful reagents are *bis*(2-*tert*-butylphenyl)phosphorchloridate (hydrolysis with $H_2O$) or *bis*(2,2,2,-trichloro-1,1-dimethylethyl)phosphorchloridate (cleavage with Zn).



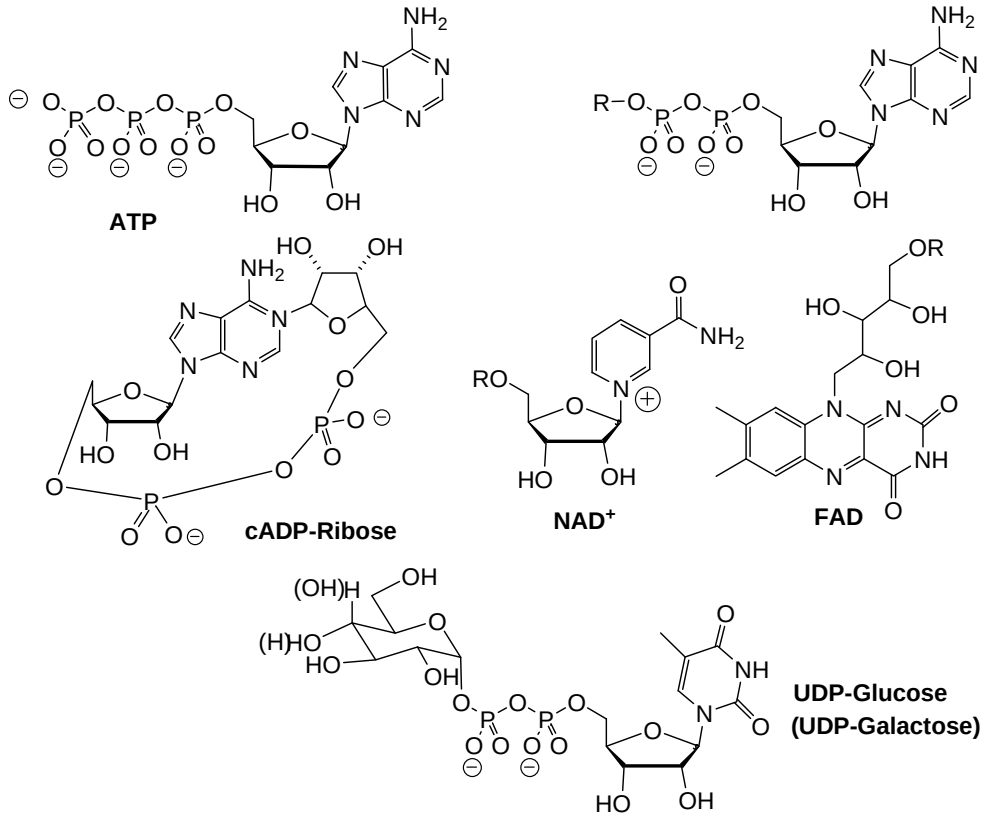




oder AcCN, Pyridin, $H_2O$ 80% Ausbeute, >90% Selektivität

The second approach was developed by Yoshikawa and Sowa-Ouchi. Here, phosphoroxychloride is reacted in the presence of a phosphoric acid triester with the alcohol (Yoshikawa). Subsequent hydrolysis gives the monoesters. Also reaction of phosphoroxychloride with the alcohol in a acetonitrile/pyridine/$H_2O$ mixture (Sowa-Ouchi) provides the monoester in 80% yield.

### 2.2.4 Synthesis of condensed phosphates and pyrophosphates

The examples below show how important condensed phosphates are in nature. ATP serves as the major energy depot in our cells. Compounds like $NAD^+$ and FAD transfer as coenzymes hydride equivalents, shuttle electron and activate oxygen. The biosynthesis of sugars requires UDP-precursors such as UDP-glucose or UDP-galactose. cADP-ribose is regulating the Ca-level in our cells and an important second messenger.




The synthesis of these compounds requires activation of the monoesters e.g. with DCC. Reaction with morpholine or diisopropylamine gives the corresponding amidates, which can be converted with diphosphates to triphosphates. Direct reaction of the monophosphates with diphenylphosphorchloridate gives after hydrolysis also the diphosphates. The diphenyloxy-protected diphosphate can be reacted with phosphate to given even the triphosphate in an $S_N2$-type reaction.

Important is to avoid phosphotriester intermediates because they are so rapidly hydrolysed. Poulter reacted the 5‘OH tosylates directly with diphosphate or even

triphosphate, which also enabled synthesis of compounds containing condensed phosphates.