# 14.8: DNA SYNTHESIS

**OBJECTIVES**

After completing this section, you should be able to

- describe, briefly, the steps required for chemical synthesis of DNA segments.

DNA must be synthesized to study genes, the sequence of genomes, and many other studies. This occurs in two fashions, by polymerase chain reaction (PCR) and chemical synthesis. PCR is covered in Section 28.8. Here we will focus on chemical synthesis of short DNA segments, which which are called oligonucleotides. Oligonucleotide synthesis is the chemical synthesis of relatively short fragments of nucleic acids, both DNA and RNA with a defined chemical structure (sequence). The technique is extremely useful in current laboratory practice because it provides a rapid and inexpensive access to custom-made oligonucleotides of the desired sequence.

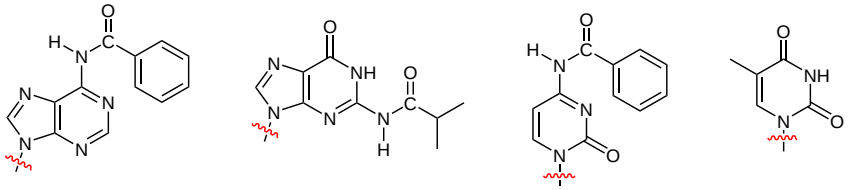
The synthesis of DNA is more difficult than peptide synthesis (Section 26-8) because of the complexity of nucleotide monomers. Commercial automated DNA synthesizers are available which allow for DNA segments to be made quickly and at a low cost. DNA synthesizers typically uses solid-phase techniques similar to the Merrifield solid-phase peptide synthesizer. Nucleotides are protected then covalently bonded to a solid support. Nucleotides are sequentially coupled to the growing oligonucleotide chain in the order required by the sequence of the product. Upon the completion of the chain assembly, the product is released from the solid phase to solution, it is then deprotected, and collected. The occurrence of side reactions sets practical limits for the length of synthetic oligonucleotides (up to about 200 nucleotide residues) because the number of errors accumulates with the length of the oligonucleotide being synthesized. Products are often isolated by HPLC to obtain the desired oligonucleotides in high purity. Typically, synthetic oligonucleotides are single-stranded DNA or RNA molecules around 15–25 bases in length.

## DNA CHEMICAL SYNTHESIS

The synthesis of DNA involves five steps:

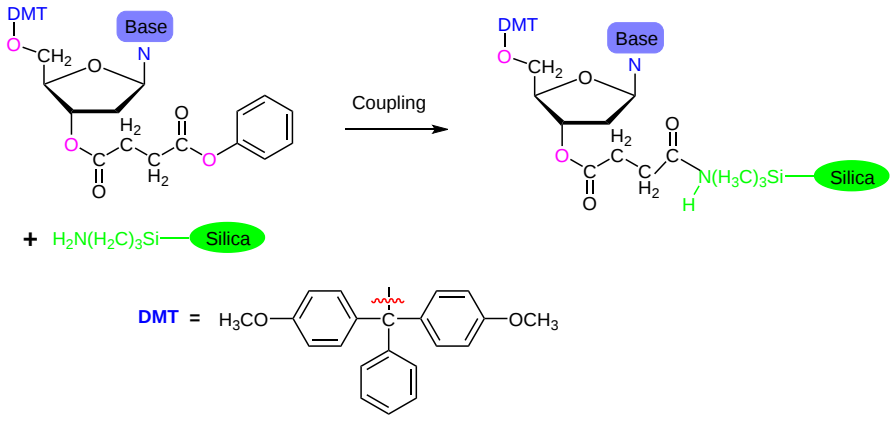
### *STEP 1*

Nucleosides used for this synthesis are modified with a linking agent at the 3' hydroxyl group of deoxyribose. The 5' hydroxyl group of the nucleosides is protected with *p*-dimethoxytrityl (DMT) ether. The amine groups on the nucleoside's heterocyclic bases are also protected. The amines of adenine and cytosine bases are protected with benzoyl groups. Guanine's amine are protected by a isobutyryl group and thymine has no amine groups so protection is not required.



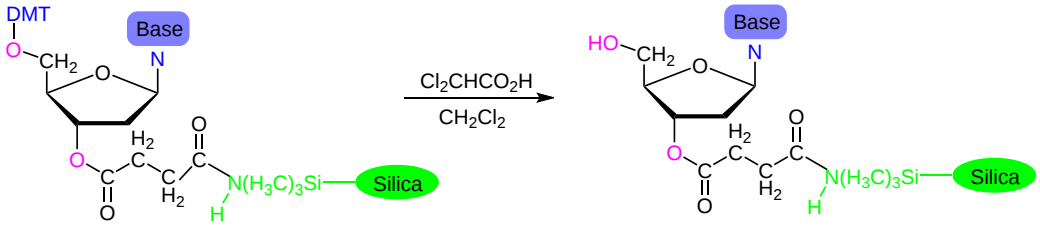
**N-Protected Adenine** **N-Protected Guanine** **N-Protected Cytosine** **Thymine**

The solid phase support used for DNA synthesis is commonly silica ($SO_2$) spheres which have been functionalized with (3-aminopropyl)triethoxysilane such that an amine group is available for reaction on the surface. A protected nucleoside is coupled to the solid phase support through an ester linkage with the 3' hydroxyl group of the nucleoside and an amide linkage with amine group from the silica surface.



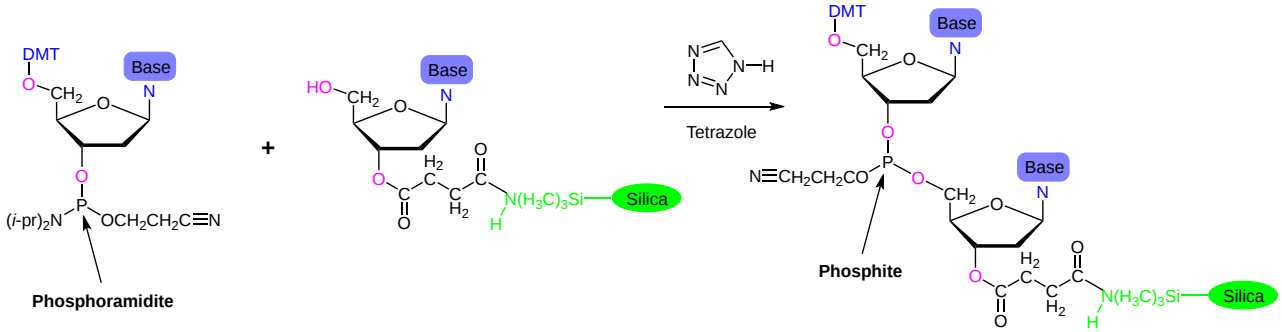
### STEP 2

Reaction with with dichloroacetic acid removes the DMT protecting group from the 5' hydroxyl of the nucleoside attached to the silica surface. The *p*-dimethoxytrityl leaving group forms a relatively stable dimethoxytrityl cation which is both tertiary and benzylic. The reaction proceeds rapidly through a $S_N1$ mechanism.



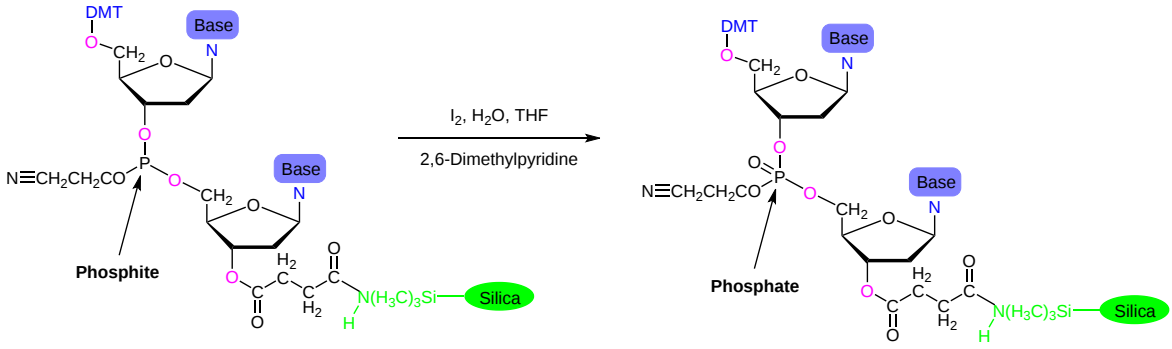

### STEP 3

The nucleoside attached to the silica surface is then reacted with a protected nucleoside which has a **phosphoramidite** functional group [$R_2NP(OR)_2$] attached to the 3' hydroxyl group of its deoxyribose moiety. In addition, one of the phosphorus oxygen atoms of the phosphoramidite group is protected with a beta-cyanoethyl group (-$OCH_2CH_2CN$). The two nucleosides are coupled in a reaction which uses acetonitrile as a polar aprotic solvent, tetrazole as a heterocyclic amine catalyst, and produces a product with a phosphite functional group [$P(OR)_3$].



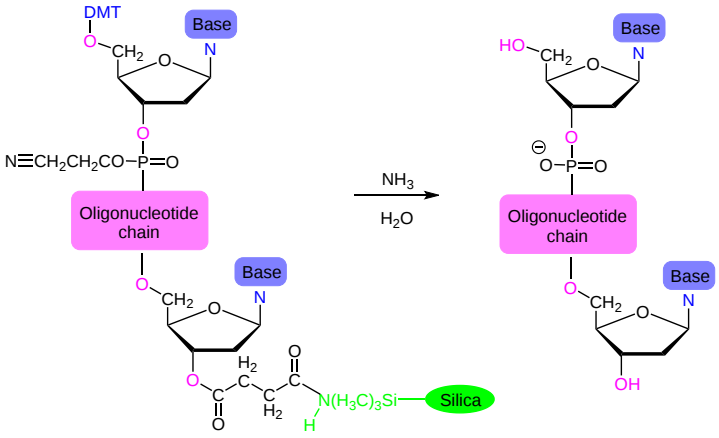

### STEP 4

Next the phosphite product of the previous step is oxidized to a phosphate by reaction with iodine ($I_2$) along with 2,6-dimethylpyridine in aqueous tetrahydrofuran (THF). Additional nucleosides can now be added by repeating the **phosphoramidite oligodeoxynucleotide synthesis cycle** of (1) DMT deprotection, (2) phosphoramidite coupling, and (3) oxidation to a phosphite.




### STEP 5

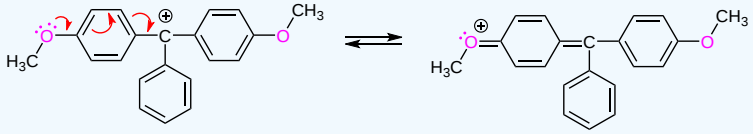
After the oligonucleotide chain of the desired sequence has been made, the final step is the removal of all the protecting groups and the linkage to silica by reaction with aqueous ammonia ($NH_3$).




## EXERCISE 14.8.1

Draw a mechanism which shows why dimethoxytrityl cation produced by the cleavage of a *p*-dimethoxytrityl protecting group is exceptionally stable.

**Answer**



## EXERCISE 14.8.2

When the beta-cyanoethyl protecting group is cleaved with aqueous ammonia, Acrylonitrile, $H_2CCHCN$ , is also produced as a by-product. Draw a mechanism for the reaction.

**Answer**