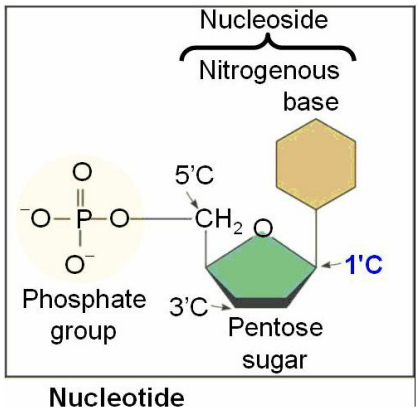
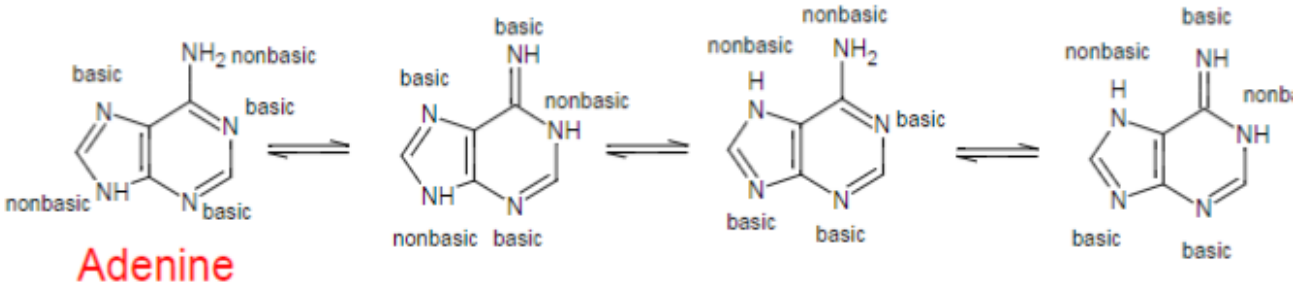
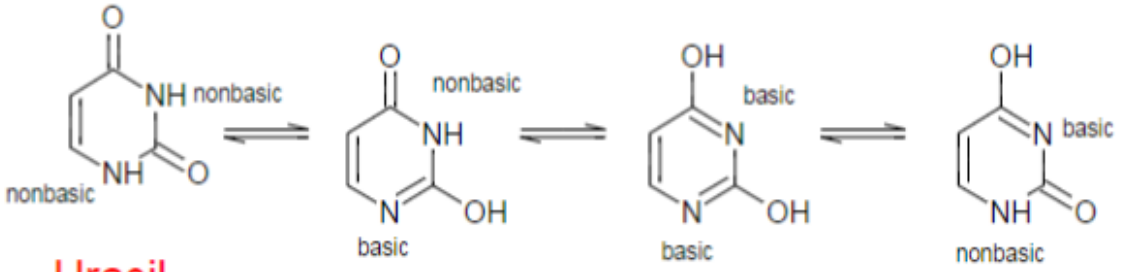
1. Structures – **Be able to draw**
    a. Purines – Double ring structures (Adenosine & Guanine)
    b. Pyrimidines – Single ring structures (Cytosine, Thymine, Uracil)
    c. Nucleosides (no phosphate group)
    d. Nucleotides (phosphate group included)



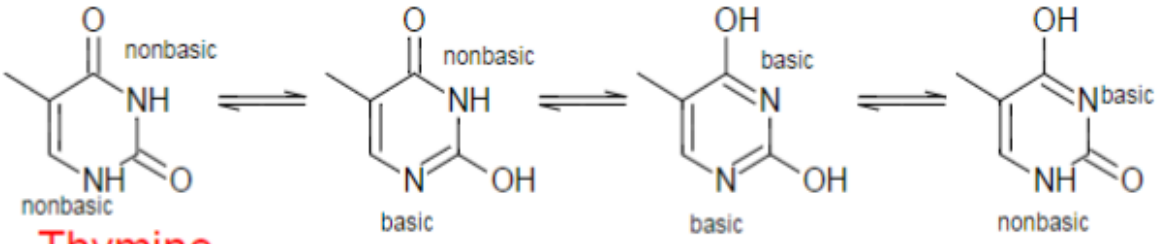
    e. Tautomers - 4 per nucleotide, 8 for guanine (including original). <u>No specific order required</u>. Hydrogens are moving to/from oxygen and nitrogen. Notice any nitrogen with an attached hydrogen will change, and any double bonded oxygen will change.
        i. For example take Cytosine: In 2 of its tautomers the NH_2 stays as an NH_2, for the last two it is NH with a double bond. The Oxygen is shown twice as double bonded and twice as an OH alcohol group. Mix and match these making the 4 possible combinations, and then complete the bonds on the nitrogen's. Remember **<u>every nitrogen will have 3 bonds that either come from a double bond inside the ring, or a bond to 1 or 2 hydrogen's.</u>**



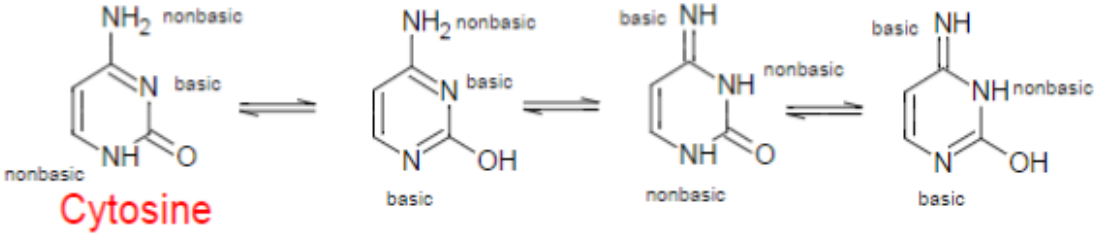
Adenine



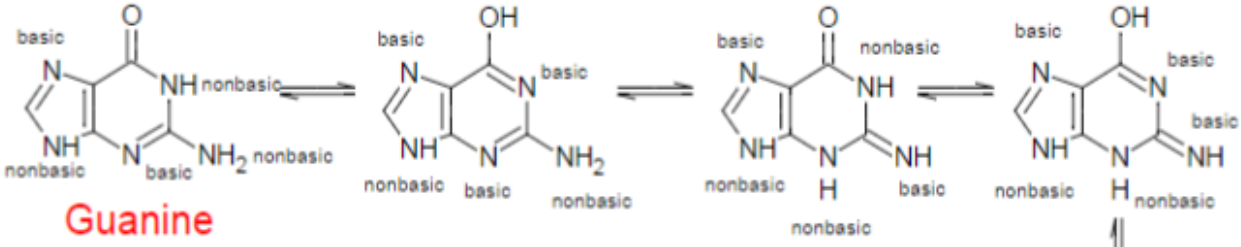
Uracil



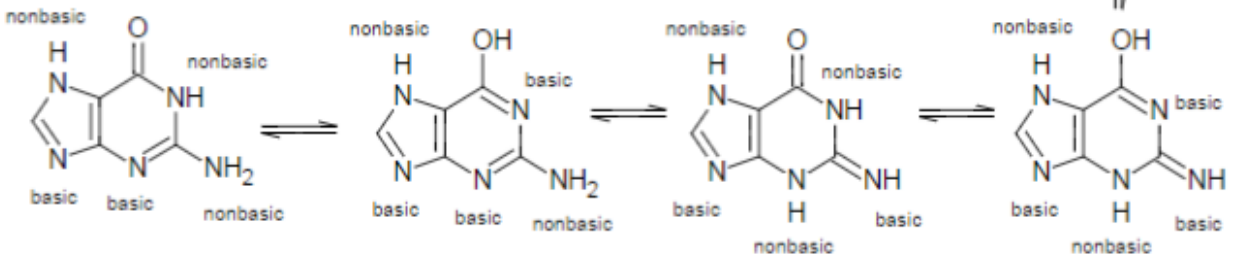
Thymine



Cytosine

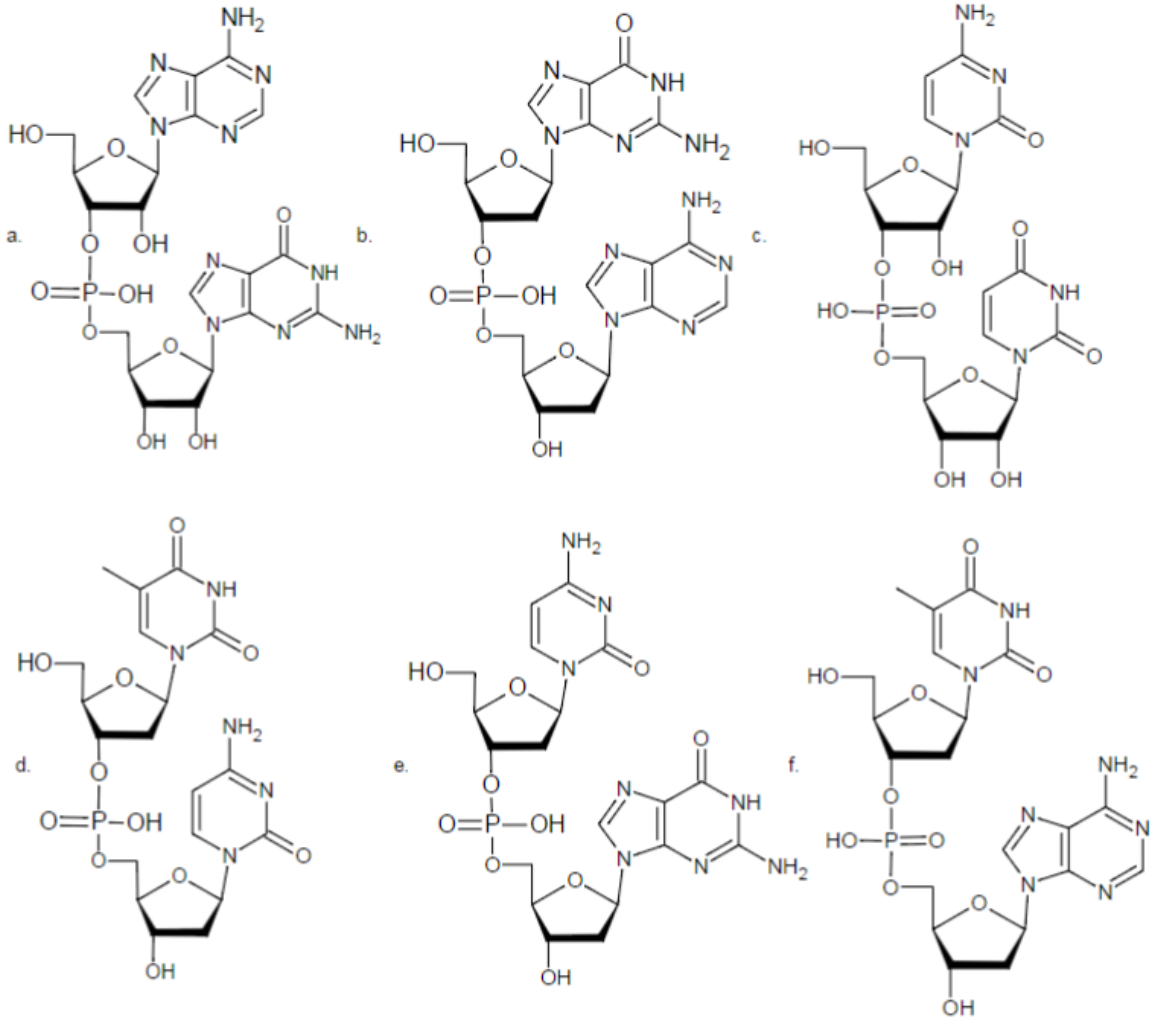


Guanine

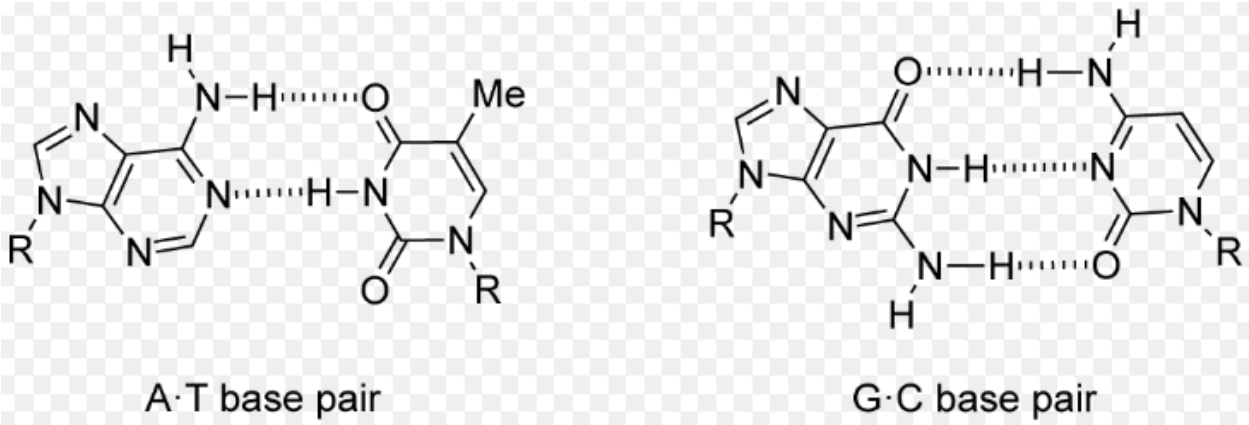


2. Phosphodiester linkages
    a. Be able to draw dinucleotides
        i. If small “d” in front of nucleotide, then no hydroxyl (OH) group on the #2 carbon of ribose ring.
        ii. Phosphodiester bonds will be between the Oxygen on the #3 carbons of one amino acid, and the #5 carbon of the other.
        iii.
            a. AG
            b. dGdA
            c. CU
            d. dTdC
            e. dCdG
            f. dTdA

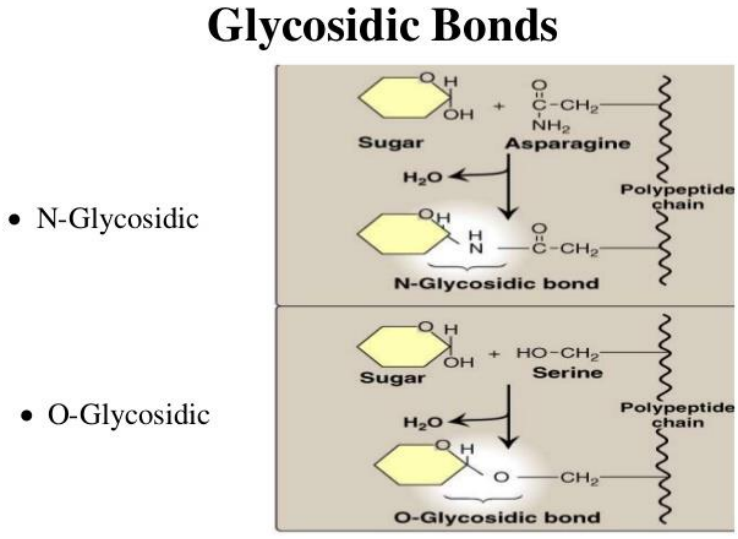
1. Dinucleotide Structures



3. Watson Crick base pairing – **this will 100% be on the test as the first question. (replace R with H in picture below for test. R represents where each nucleic acid would attach to a sugar)**



4. Basic nitrogen's have a double bond attached to them. That's all the understanding required for this test. The actual reason is because when a nitrogen is bonded to a hydrogen in a ring, its non-bonding electrons are involved in the resonance of the ring, so they cannot be donated (definition of a Lewis base). Also you can think like this. If the nitrogen that has a double bond uses its free electrons to bind with another element, it would cause the Nitrogen to have a +1 charge, BUT that nitrogen is able to get rid of that charge by breaking its double bond to a single bond, and regaining its non-bonding electrons and neutral charge. Once again you don't need this understanding for the test.
5. Glycosidic Bonds are formed via hydrolysis (the removal of H_2O). Know mechanism of hydrolysis if you don't already. Can be achieved with acid or base.



a.
b. DNA vs RNA stability in mild base – DNA more stable due to lack of hydroxyl on carbon #2.

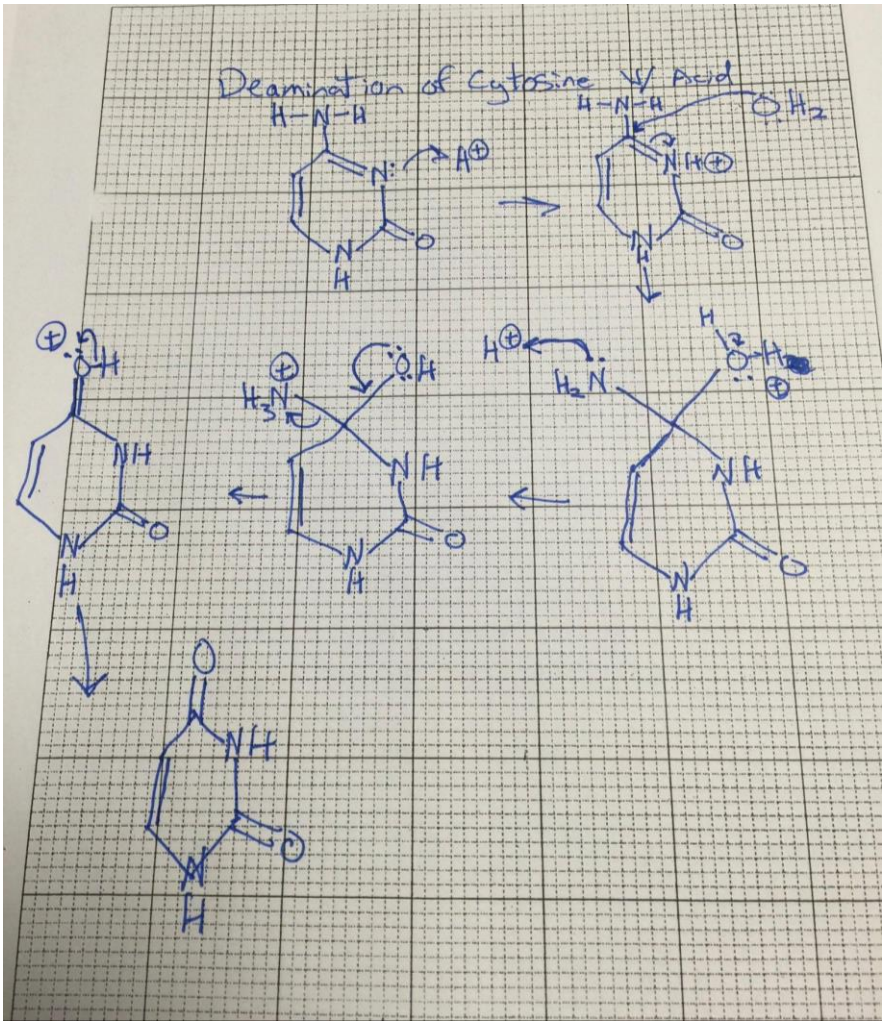
RNA, however, is a stable molecule as the presence of negative charge (–ve) on sugar-phosphate backbone protects it from attack by Hydroxyl ions (OH–) that

would lead to Hydrolytic cleavage. But, the presence of 2'-Hydroxyl (-OH) group makes the RNA susceptible to Base-catalyzed hydrolysis. Moreover, RNA is also prone to Auto-Hydrolysis when it is single stranded. This spontaneous cleavage reaction takes place in **basic solutions**, where free hydroxyl ions in solution can easily deprotonate the 2'-Hydroxyl (-OH) group of the Ribose sugar.

However, if this 2'-Hydroxyl (-OH) group is removed from the ribose sugar then the rate of such base- catalyzed hydrolysis are decreased by approximately 100 fold under extreme conditions.

Thus, the presence of 2'-Hydroxyl (-OH) group on every nucleotide of RNA makes it labile and easily degradable.

6. Number 6 is skipped in skillz.
7. Deamination – Loss/removal of the amine (NH_2) from the nucleic acid.
    a. Protonate amino group to make it a good leaving group. It is replaced with a double bonded oxygen.
    b. Attach carbon it's attached to with oxygen of water.
    c. There are multiple mechanisms possible, here is one:



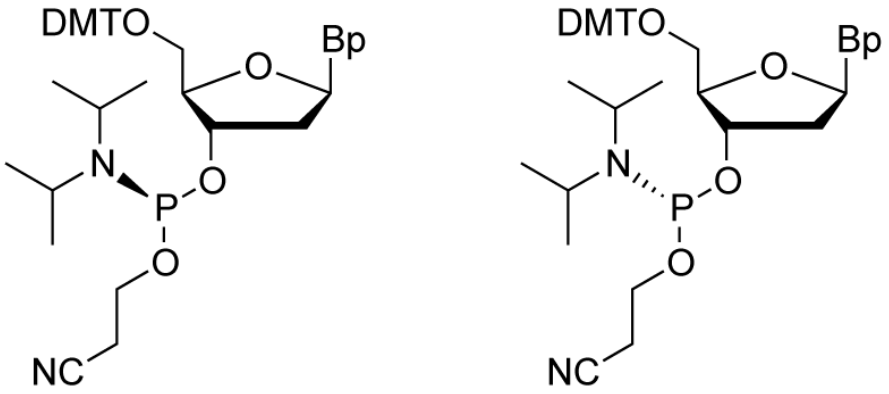
8. Template Strand: CATTCGGAA
   Coding Strand : TTCCGAATG
   mRNA : UUCCGAAUG
   These are all shown in the 5' to 3' direction. Notice that the coding strand is 'backwards' compared to the template strand, and that the mRNA sequence is the same as the coding strand with the Thymine (T) changed to Uracil (U).
9. DNA codons
    a. Multiple codons can code for the same amino acid
        i. 64 possible codons that code for 20 amino acids
    b. A codon is 3 nucleotides in length
    c. Stop codons are : UGA UAA UAG
        i. U Go Away = UGA
        ii. U Are Away = UAA
        iii. U Are Gone = UAG
10. PCR – Polymerase Chain Reaction
    a. **Step 1: Denaturing** – Double stranded DNA is heated to separate it into two single strands. (Breaks the hydrogen bonds between the strands)
    b. **Step 2: Annealing** – the temperature is lowered to enable the DNA primers to attach to each single strand.
    c. **Step 3: Extending** – the temperature is raised and the new strand of DNA is made by the Taq polymerase enzyme. (the DNA polymerase of Taq is very stable at high temperatures, which means it can withstand the temperatures needed to denature in step 1)
    d. Repeat….. (each repetition doubles the amount. The total # of products is given by 2^n where n = number of times run)
    e. When finished, electrophoresis can be used to check the quantity and size of the DNA fragments produced.
11. Sanger Sequencing (Classical)
    a. 4 reactions to add each ddNTP – add ddATP, add ddGTP, add ddTTP, add ddCTP.
    b. Primers labeled with radioisotope 32p
    c. DNA polymerase
    d. Gel electrophoresis (must have single base resolution – ability to tell exact amount of nucleotides i.e. 10 vs 11)
    e. 4 separations (one for each ddNTP) read manually

    Sanger sequencing (modern)

    a. 1 reactions to add all ddNTP
    b. Primers unlabeled. Dideoxynucleotides (ddNTP) labeled with different fluorescent dye.
    c. DNA polymerase

d. Capillary electrophoresis (much higher voltage, faster)
e. One separation (read automatically, laser fluorescence)

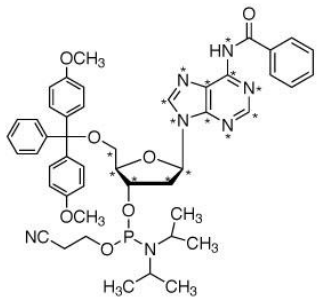
12. Solid Phase DNA synthesis
    a. General form of ANY phosphoramidite:



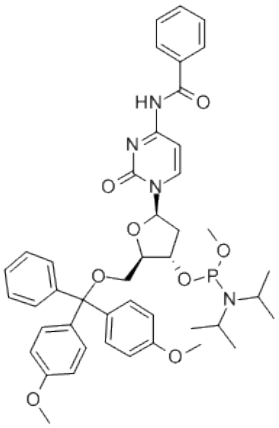
Bp = protected base or T

    b. Adenine, Cytosine, and Guanine need to have their amino (NH_2) group protected:

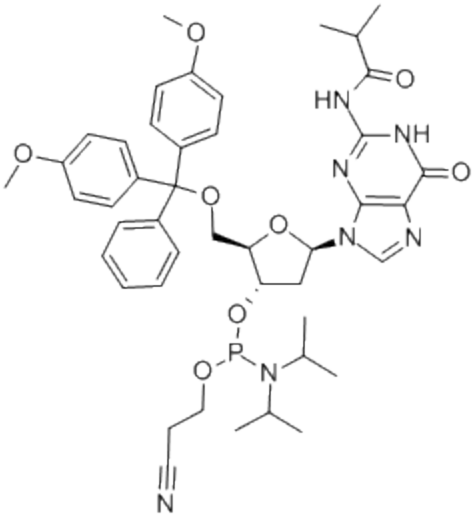
Adenine:



Cytosine:

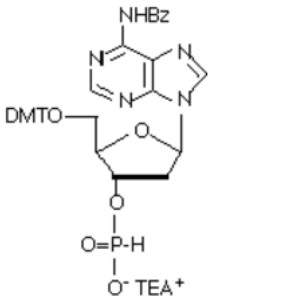


Guanine:

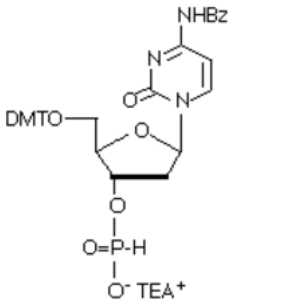


Note: Thymine does not need protection as it lacks an amino group.

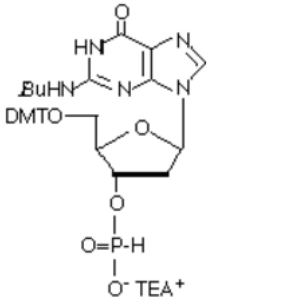
H- Phosphonates – vary slightly, doubt they will be asked, if so just draw this for whatever one



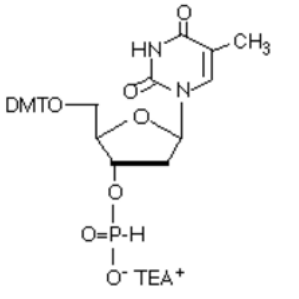
dA-H-Phosphonate



dC-H-Phosphonate



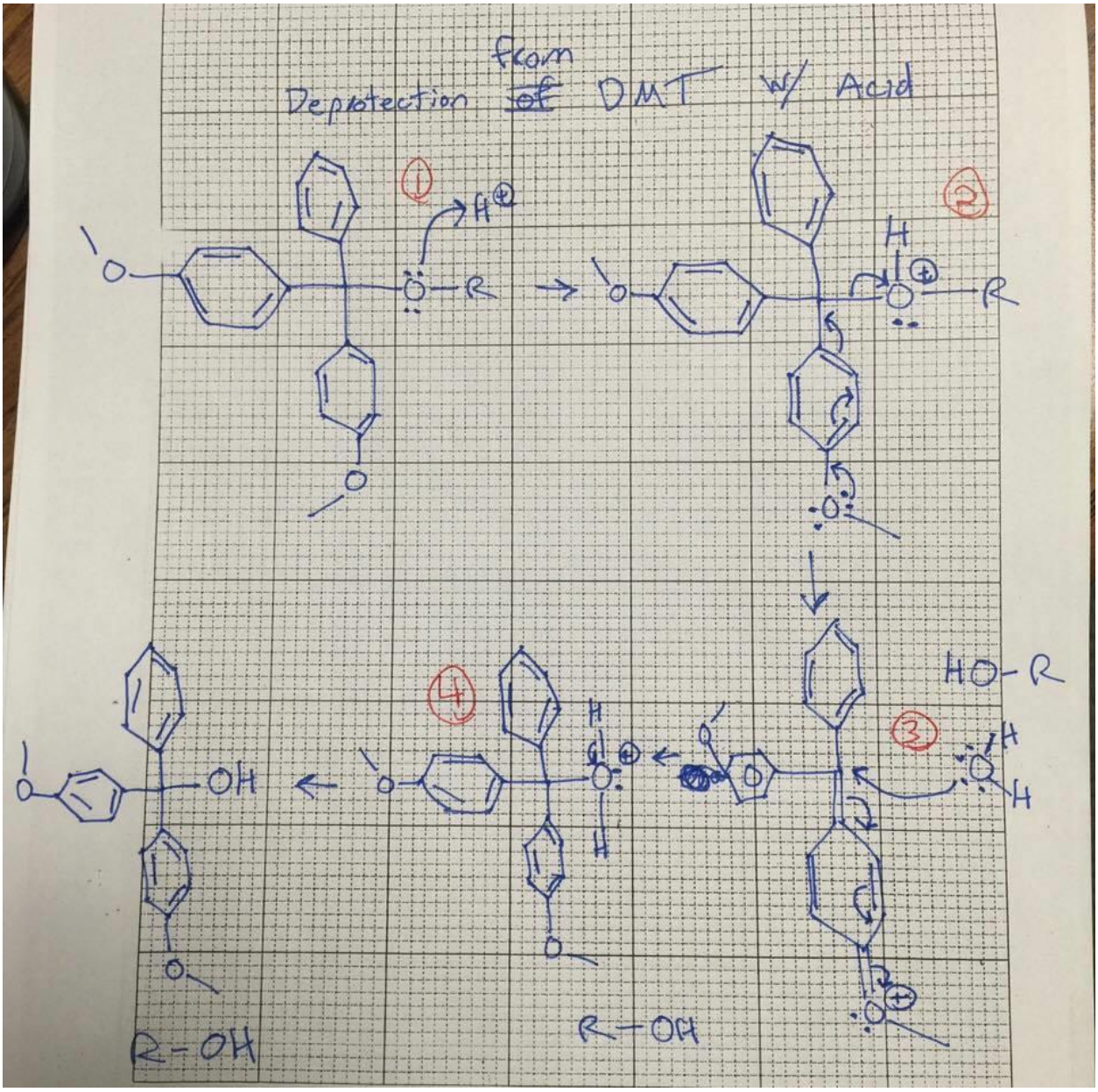
dG-H-Phosphonate



dT-H-Phosphonate

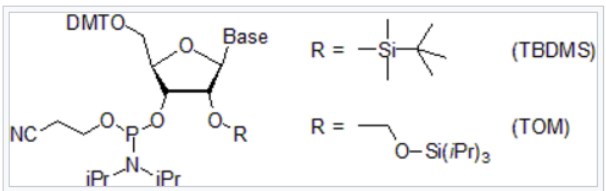
Notice the “TEA” on the phosphate group and the “BZ” attached to the amino group on the nucleic acid.

C. Deprotection from DMTO:



D. Protection of RNA (doubtful to be on test but who knows)

- In RNA synthesis, the 2'-hydroxy group is protected with TBDMS (*t*-butyldimethylsilyl) group.[46][47][48][49] or with TOM (tri-*iso*-propylsilyloxymethyl) group,[50][51] both being removable by treatment with fluoride ion.



2'-*O*-protected ribonucleoside phosphoramidites.

13. Nucleic acid separations
    a. Electrophoresis – separates molecules by size as they are pulled through a gel by an electric field.
        i. PAGE – polyacrylamide gel electrophoresis – used for **small proteins and DNA fragments**
        ii. Agarose gels have greater range of separation and are used for **large protein and dna fragments**.
        iii. Ethidium bromide is an intercalcating dye, which means it inserts itself between the bases that are stacked in the center of the DNA helix. One ethidium bromide molecule binds to one base. As each dye molecule binds to the bases the helix is unwound to accommodate the strain from the dye.
    b. Affinity Chromatography – method of separating biochemical mixtures based on a highly specific interaction such as between antigen and antibody, enzyme and substraight, or receptor and ligand.
        i. Wessel test definition: A DNA fragment was attached to a support and used to isolate a **specific** RNA. This is an example of Affinity Chromotography.
    c. Anion Exchange Chromatography – process that separates substances based on their charges using an ion-exchange resign containing positively charged groups. Anion exchange resin will bind to negatively charged molecules.