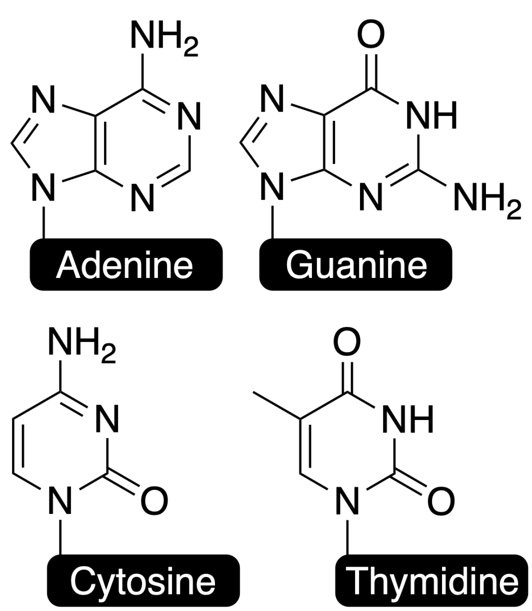
2024 WUCT: Chemistry of Cancer

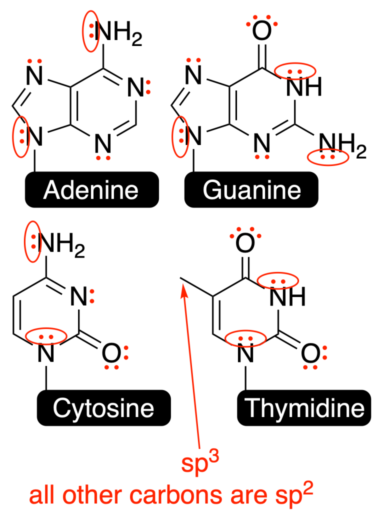
This exam consists of 6 questions and is worth 100 points. You will complete this exam as a team of two. You will have 1 hour to take the exam. The only allowed resources for this exam are a calculator and the provided equation sheet. You may NOT use any other notes or books. You must show your work and box your final answer to receive credit for a problem. NOTE: If you get the answer to an early part of a question incorrect but later use that answer for a subsequent part of the question, you can still earn full credit for those subsequent parts. Please write your answer in the designated space on the answer sheet. If you need additional space for a problem, you may use the blank scratch page at the end of the exam. Make sure to clearly indicate in the problem's designated space where the rest of your work can be found. Any work anywhere other than the exam or the scratch page will not be graded. Dark pencil or pen is preferred.

<u>Problem #1</u>: *(26 points)*

DNA (deoxyribonucleic acid) is the 'coding system' of life, writing the instructions for the production, use, and export of life's biomolecules through the arrangement of four letters (A, G, C, and T). The letters of this code correspond to the nitrogenous bases represented by the following chemical structures. Note that the bond from N to the box with the name of the base represents a N–C bond.



a. Show all lone pairs on nitrogen and oxygen atoms. ***(2 points)*** **(+0.5 pts per fully correct base, 2 pts total)**
b. Circle the lone pairs involved in resonance. ***(2 points)*** **(+0.5 pts per fully correct base, 2 pts total)**
c. Label the hybridization of each carbon(s). ***(2 points)*** **(+0.5 pts per fully correct base, 2 pts total)**



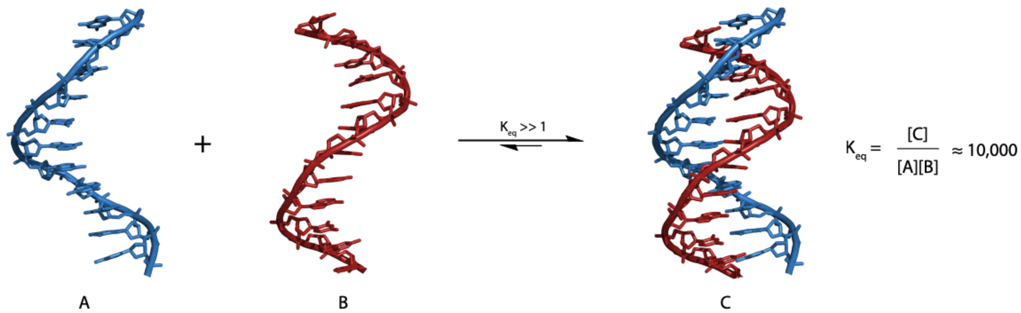
d. The nitrogenous bases in DNA hydrogen bond with each other to form a double-stranded superstructure. This process of forming double-stranded DNA is called hybridization (distinct from the linear combination of atomic orbitals). In the early 1950s, James Watson, Francis Crick, and Rosalynd Franklin performed pioneering research that culminated in the discovery that AT (adenine-thymine) and GC (guanine-cytosine) base pairs are exclusive. This Watson-Crick-Franklin model explains how both strands of double-stranded DNA can encode the same information.

   i. What is hydrogen bonding? ***(2 points)***

   An attractive interaction between a hydrogen atom that is bound to a highly electronegative atom (F, O, N) and another F, O, or N atom.

   +1 point for explanation of hydrogen bonding as an attractive interaction between a hydrogen atom bound to a highly electronegative atom and another highly electronegative atom.
   +1 point for specifying that the highly electronegative atoms involved in hydrogen bonding are F, O, or N.

ii. The following figure illustrates the hybridization of DNA as an equilibrium between individual DNA strands A and B and the double-stranded DNA C.



Briefly explain the significance of the equilibrium constant, considering the position of the equilibrium and the relative rates of the forward and reverse reactions. ***(2 points)***

The equilibrium lies far to the right. **(+1 point)**
The rate of the forward reaction is significantly greater than the rate of the reverse reaction. **(+1 point)**

iii. Categorize the following arguments as describing entropy vs enthalpy and favoring single-stranded (ss) vs double-stranded (ds) DNA in the following table. Multiple arguments may occupy the same cell. Argument E has been categorized in the table as an example. ***(4 points)***

1. Argument A: Single-stranded DNA is more flexible than double-stranded DNA.
2. Argument B: Hydrogen bonding between DNA bases and each other.
3. Argument C: Hydrogen bonding between DNA bases and water
4. Argument D: Van der Waals interactions between stacked bases (imagining DNA as a twisted ladder, these interactions are between adjacent rungs of the ladder).
5. Argument E: Purging of water from between bases (a process known as the hydrophobic effect).

	Favors ssDNA	Favors dsDNA
Enthalpic Argument	C	B, D
Entropic Argument	A	E

+1 point for each correct categorization

iv. Provide a brief rationale for your categorization of these thermodynamic arguments. Argument E has been rationalized for you as an example. ***(4 points)***

Argument E: The 'interior' of DNA is hydrophobic and thus causes water to form an ordered framework around it. Forming a double-strand purges water from the interior, disrupting this ordered framework and causing an increase in *entropy*.

Argument A: Since dsDNA is more rigid, it is more ordered, decreasing entropy.
Argument B: The formation of hydrogen bonds between DNA strands and each other are stabilizing and associated with a decrease in enthalpy.
Argument C: The formation of hydrogen bonds between DNA strands and water are stabilizing and associated with a decrease in enthalpy.
Argument D: Van der Waals interactions between 'rungs' of the DNA 'ladder' are stabilizing and associated with a decrease in enthalpy.

+1 point for each correct rationale (+4 points total)
-0.5 points for each incorrect rationale

v. Explain qualitatively why the increase in entropy seen with argument E favors dsDNA. ***(5 points)***

$\Delta G = \Delta H - T\Delta S$ **(+1 point)**
Increase in entropy will decrease ΔG **(+1 point)**
$\Delta G = - RTlnK_{eq}$ **(+1 point)**
A decrease in ΔG results in a larger K_{eq} **(+1 point)**
A larger K_{eq} causes the equilibrium to shift towards the formation of double-stranded DNA, so dsDNA is favored **(+1 point)**

e. A common technique in cancer research is the polymerase chain reaction (PCR) that allows for the exponential amplification of a particular DNA sequence of interest. PCR cycles between several temperatures to separate ("melt") the strands, join ("anneal") the strands, and provide the optimal conditions for the enzymatic synthesis of new DNA. The specifics of the PCR protocol are not important for this question, though.

   i. Sequences rich in G (guanine) and C (cytosine) bases relative to A (adenine) and T (thymine) bases require a higher "melting" temperature. Explain this observation. ***(2 points)***

      G-C pair has more hydrogen bonds than A-T pair: G (guanine) and C (cytosine) bases use three hydrogen bonds to achieve canonical Watson-Crick-Franklin base-pairing while A (adenine) and T (thymidine) only use two hydrogen bonds. More hydrogen bonds in the G-C base pair makes it stronger, requiring a higher energy input to break the bonds, thereby resulting in a higher melting temperature for sequences rich in G and C bases compared to those rich in A and T bases.

      +1 point for saying that G-C pair has more hydrogen bonds than A-T pair
      +1 point for saying that more hydrogen bonds in the G-C base pair makes it stronger, requiring a higher energy input to break the bonds, thereby resulting in a higher melting temperature for sequences rich in G and C bases compared to those rich in A and T bases.

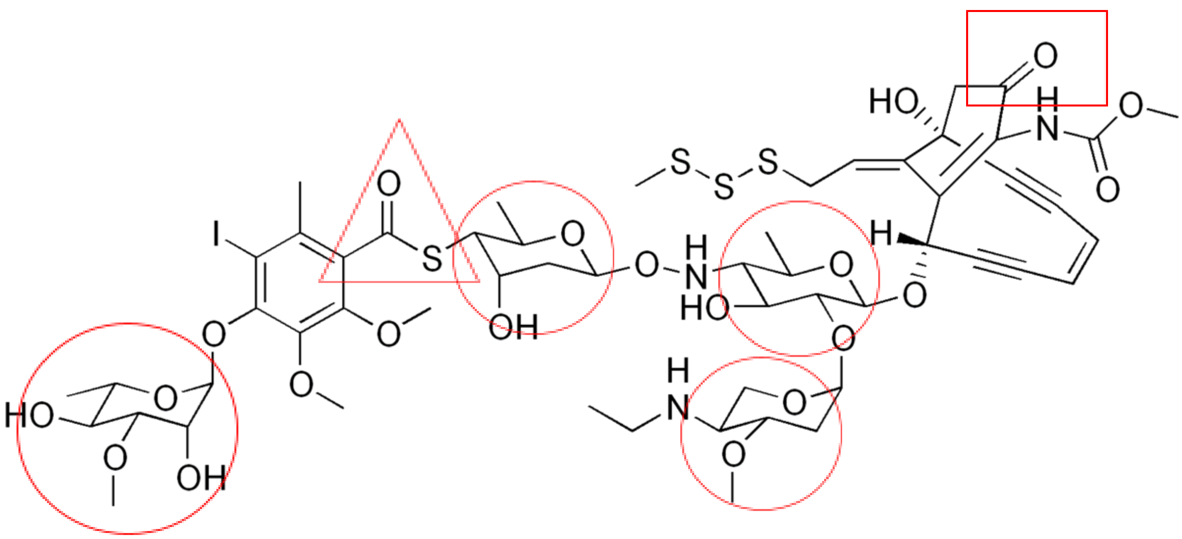
   ii. Given the following DNA sequences:

      Sequence A: ATATCGCGATCGATAGCGCTA
      Sequence B: GCATCGCGATCGATCGATCGC

      Calculate the % GC content for sequence A and sequence B. ***(1 point)***

      Sequence A: 47.62% **(+0.5 points)**
      Sequence B: 61.90% **(+0.5 points)**

<u>Problem #2</u>: *(14 points)*
Calicheamicin is a natural product that is synthesized by the bacterium *Micromonospora echinospora*. Calicheamicin is a highly toxic compound that has been used in cancer therapy for over a decade; more specifically, calicheamicin has been developed to specifically target solid acute myeloid leukemia (AML) tumors. The toxicity of calicheamicin compounds derives from its ability to bind to the minor groove of DNA strands and upon cyclization of its enediyne, generate a highly reactive diradical that can ultimately cause double stranded DNA breaks.



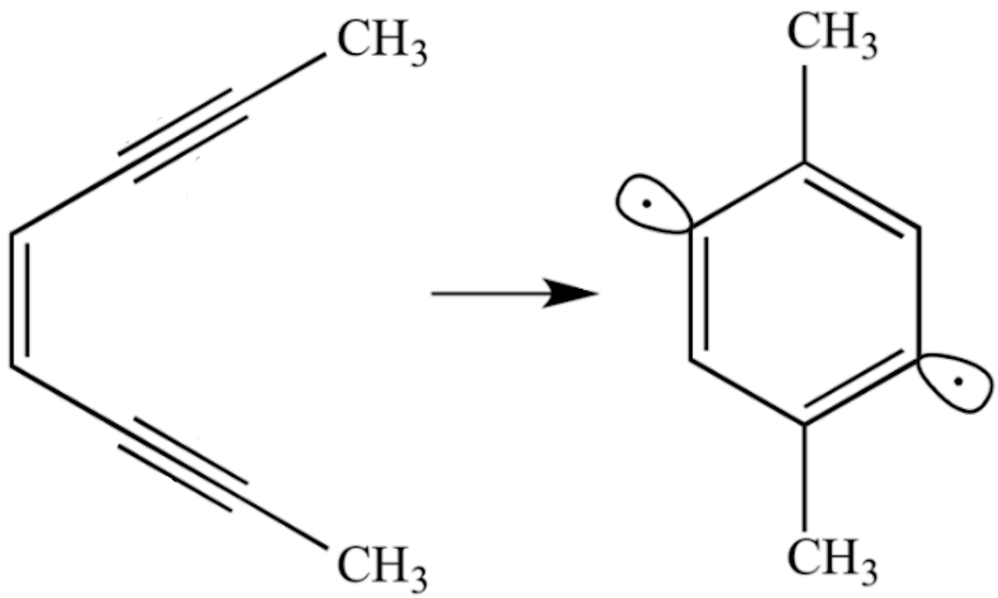
a. Identify the following functional groups within calicheamicin: ***(4 points)***
   i. Draw a triangle around a thioester **(+1 point)**
   ii. Draw two circles around two sugars **(+1 point for each correct; +2 points total)**
   iii. Draw a box around a carbonyl **(+1 point)**

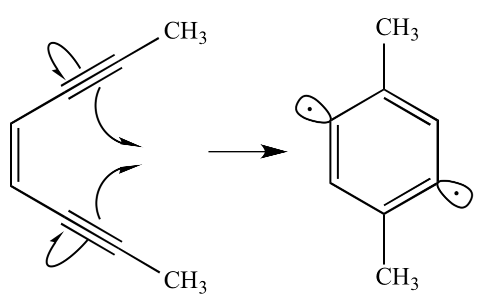
b. One of the driving factors of the cyclization of the enediyne into benzene is the formation of an aromatic compound. Define aromaticity. ***(2 points)***

   Aromaticity is a characteristic exhibited by fully conjugated planar ring molecules, where their resonance stabilization is stronger than their fully conjugated linear counterparts. This heightened stabilization arises from the delocalization of π electrons.

   +1 point for mentioning fully conjugated planar ring molecules
   +1 point for mentioning that stabilization arises from the delocalization of π electrons

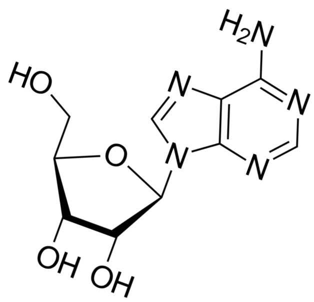
c. The enediyne on calicheamicin can undergo a reaction known as the Bergman cyclization to become the diradical benzene (shown below). Propose an arrow pushing mechanism to account for the formation of this structure, where arrows represent movement of electrons. (Hint: Use half arrows to denote the movement of single electrons) ***(2 points)***

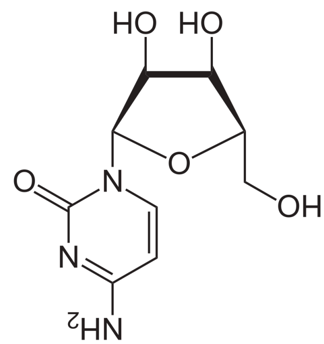


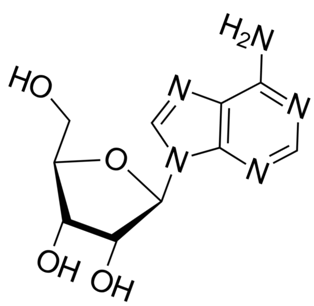


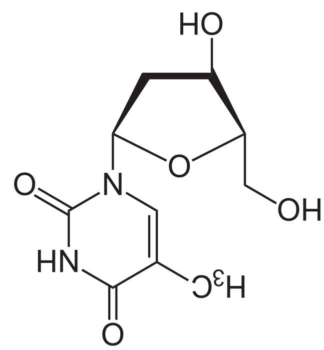
d. Upon the formation of the highly reactive diradical benzene, calicheamicin is able to induce double stranded DNA breaks. Let's say, upon attempting to repair one of the double stranded breaks caused by calicheamicin, the cell makes a mistake and causes a mismatched base pair of A→C, instead of A→T.

Given the structures of adenosine, cytosine, and thymine below, draw the possible H bonds that can occur between adenosine and cytosine, and the H bonds that can occur between adenosine and thymine: ***(2 points)***

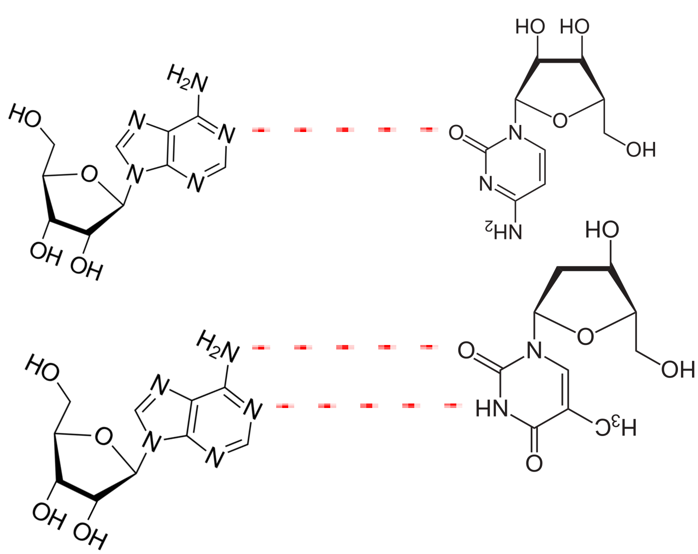








Answer: **+1 point for each correct drawing of H bonds in a pair**



e. Given your proposed H-bonds, why does A normally pair with T in DNA? ***(3 points)***

A normally pairs with T in DNA because it results in 2 hydrogen bonds between the two nucleotides whereas an adenosine molecule can only form 1 hydrogen bond with cytosine. Adenosine pairing with Thymine results in more stable bonding.

+1 point for identifying that A can form 2 H-bonds with T
+1 point for identifying that A can only form 1 H-bond with C
+1 point for saying that A-T pair results in more stable bonding

<u>Problem #3</u>: *(17 points)*
Breast cancer is one of the most common death causes in the US. Over 200,000 cases are identified each year. Breast cancer starts from cells within the breast growing out of control. If diagnosed in a timely manner, breast cancer is commonly treated by removing the cancerous breast. According to the Center of Disease Control and Prevention of the United States, the most common cause for breast cancer is inherited mutation in BRC1 and BRC2 genes.

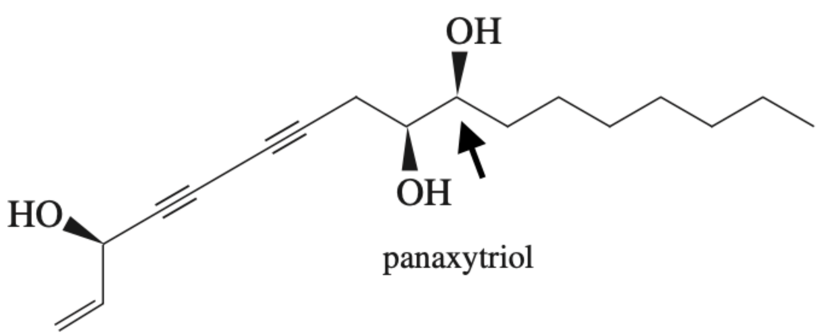
A lot of cancer research involves the study of genes and DNA. Genes are sections in DNA that are responsible for encoding protein.

a. Propose three biochemical interactions that stabilize the double helix structure of DNA? ***(4 points)***

   Hydrophobic interaction, Hydrogen bond, ionic interaction, salt bridge, etc.

   +4 points if 3 interactions are correct
   +3 points if 2 interactions are correct
   +2 points if 1 interaction is correct

b. The alkyne functional group is an important class of organic chemicals that can have many pharmaceutical and industrial uses. To start you off, alkyne is a hydrocarbon molecule consisting of at least one carbon-carbon triple bond. An example of alkyne, as we would discuss here, is called panaxytriol. This is a medicine for alleviating the side effects of chemotherapy.



panaxytriol

Indicate what type of the carbon this is as pointed by the arrow. ***(1 point)***
Circle one answer from (primary/secondary/tertiary). **(+1 point)**

c. Typically, if a strong nucleophile is present in the solution of alkyl halide, a reaction called nucleophilic substitution reaction will occur via an S_N2 mechanism. However, for this panaxytriol molecule above, nucleophilic substitution reaction will not take place. Explain your reasoning in no more than 3 sentences. ***(2 points)***

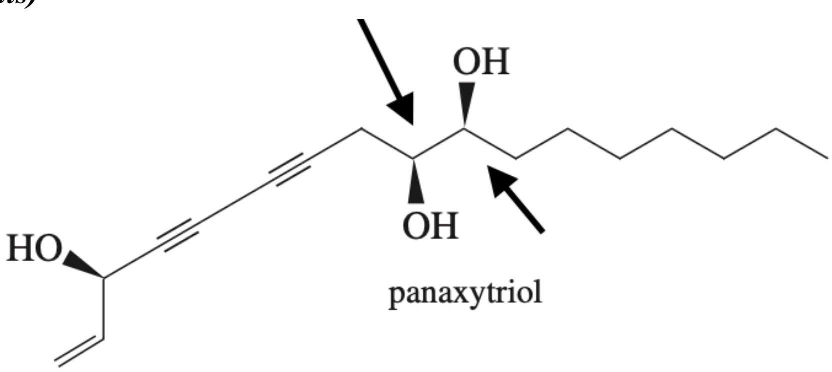
OH^- is not a good leaving group.

+2 points for saying that OH^- is not a good leaving group
+1.5 points for saying that the molecule is too bulky
+1 points for saying that this is not an alkyl halide

d. There exists one way that you can activate the S_N2 nucleophilic substitution reaction for this molecule. Write it out briefly. (Hint: Consider attempting to make a better nucleophile or make a better electrophile.) ***(2 points)***

Rubric:
Answers containing "Adding acid" will receive full credit.
Answers containing "Use a stronger nucleophile" will receive 1 pt.
Answers containing "Use a stronger electrophile" will receive 0.5 pt.

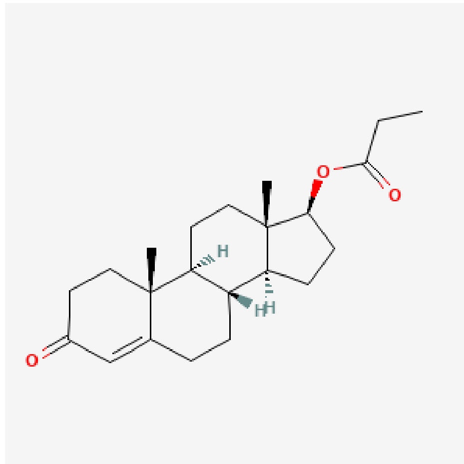
e. Reflect back to your answer in part b, and observe the structure of this molecule. Assuming the condition in part d is fulfilled, which carbon pointed by the arrow is the site where the substitution reaction is going to happen? Circle the top or bottom arrow. ***(2 points)***



panaxytriol

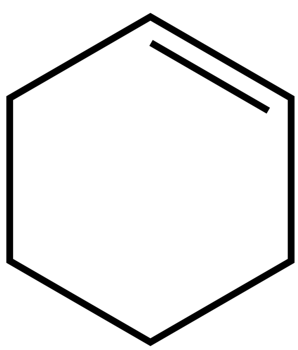
+2 points if "bottom" was circled

f. Males can also develop breast cancer. In the past few decades, the incidence of male breast cancer rose by 40% (Noone et al., 2018). A key piece of information discovered by researchers was the decline in levels of androgen (a male hormone) and abnormal increase levels of estrogen. Here the structure of androgen:

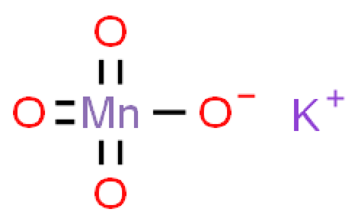


Androgens have a fairly complicated structure, but we are going to focus only on one part of this structure. As you may have noted, the top right end of the molecule is a carboxylic acid. In organic chemistry, we have many different methods to synthesize carboxylic acids. One of the most commonly-used methods, and a price-friendly one, is cleaving alkenes to obtain a carboxylic acid.

Here, we will provide you with a structure, a cyclohexene, and $KMnO_4$ that you could work on to produce a carboxylic acid using a cyclohexene. Please fill in an electron-pushing mechanism using arrows to show how carboxylic acid is produced using these two molecules? ***(4 points)***

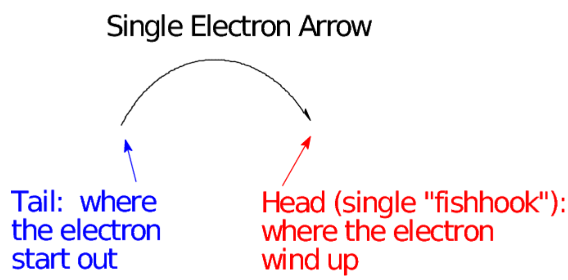


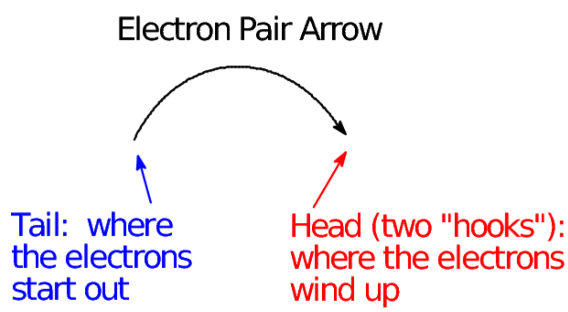
Cyclohexene



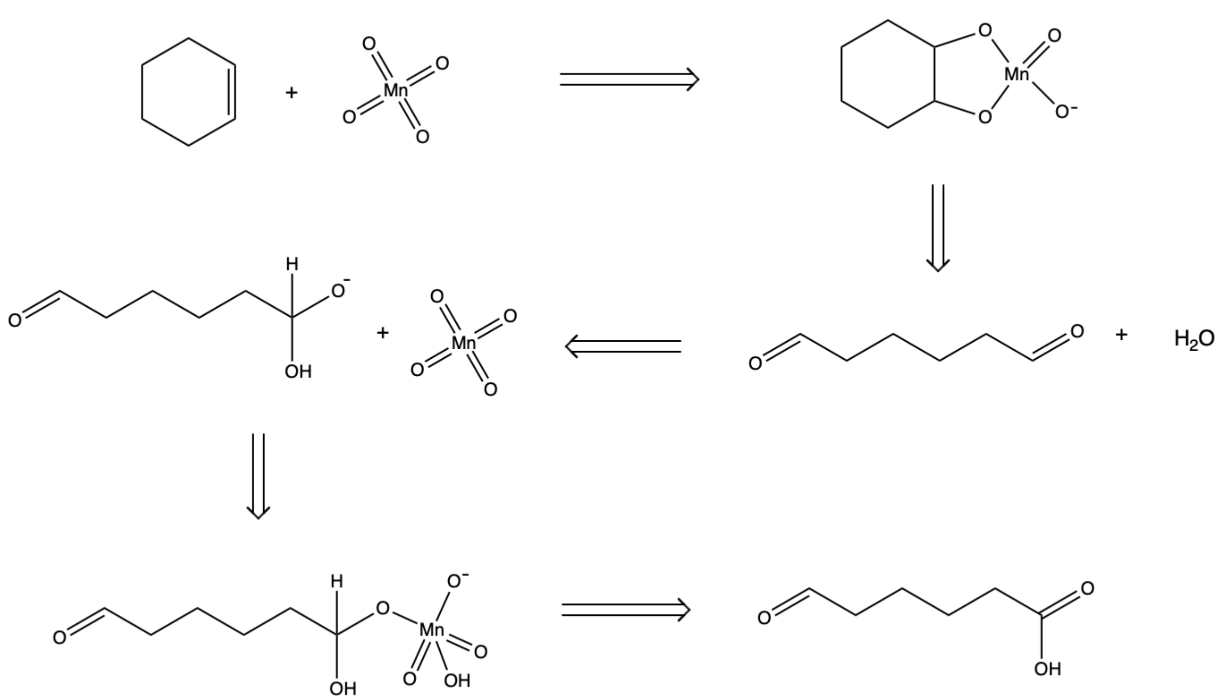
Potassium Permanganate

Standard Conventions of drawing electron-pushing mechanism:
A fish-hooked arrow indicates the movement of one electron, while a full arrow indicates the movement of an electron pair (2 e-). Illustration shown below, please look carefully and use the correct arrow for your intention and for the correct mechanism.





Illustrations from: Oregon State University Website: https://sites.science.oregonstate.edu/~gablek/CH334/Chapter2/elec_push.html



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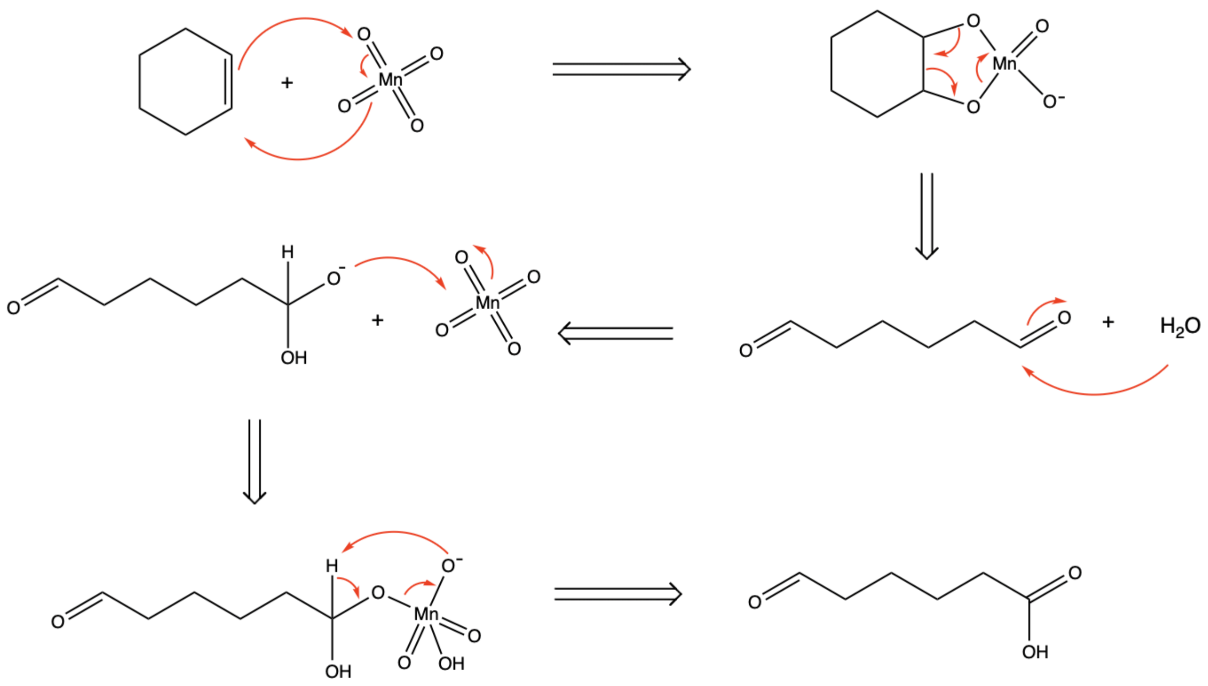


-If lone pair electrons were omitted, as long as charges are correctly shown, no credit will be deducted.
-If a test taker draws out the mechanism from two separate chemicals to the formation of a 5-membered ring (The 3rd step in the mechanism above.) Award 2 points.
-If a testaker did not draw any arrows, desired products and intermediates are not formed. No point will be awarded.
-If a testaker did not draw any arrows, but obtained the final product directly, award 2 points.
-If a test taker did not draw any arrows, obtained the 5-membered ring but not the product, award 1 point.
-If a test taker cleaves the double bond, and shows another MnO4-will attack from below but failed to show the final product, award 3 points.
-If a test taker forms a five-membered ring and cleaves the double bond but not the final product, award 2 points.
-Correct mechanism and correct product produced will be awarded full credit. (4 points.)
-Correct mechanism, correct product, but the 5-membered ring did not show up. A test taker will receive 3 points.

g. What other functional group along with the carboxylic acid will be produced in this reaction? ***(2 points)***

Aldehyde **(+2 points)**

Problem #4: ***(16 points)***

Extracellular acidity is a common feature of many solid tumors, similar to hypoxia. This feature develops due to increased metabolic reprogramming and the use of glycolysis and lactic acid fermentation to provide enough energy to the rapidly proliferating tumor cells.

a. Write the chemical equation of the acid dissociation reaction for lactic acid given the following chemical formula of lactic acid: $CH_3CH(OH)COOH$. ***(1 point)***

$CH_3CH(OH)COOH_{(aq)} + H_2O_{(l)} \rightarrow CH_3CH(OH)COO^-_{(aq)} + H_3O^+_{(aq)}$

+1 point for the correct chemical equation

b. If the pH of a 1.1 M solution of lactic acid at equilibrium is measured to be 1.91, calculate the acid dissociation constant of lactic acid. ***(4 points)***

ICE table **(+1 point)**

$CH_3CH(OH)COOH_{(aq)} + H_2O_{(l)} \rightarrow CH_3CH(OH)COO^-_{(aq)} + H_3O^+_{(aq)}$

	$CH_3CH(OH)COOH$	$CH_3CH(OH)COO^-$	H_3O^+
I	1.1	0	0
C	-x	+x	+x
E	~1.1	x	x

$K_a \frac{[CH_3CH(OH)COO^-][H_3O^+]}{[CH_3CH(OH)COOH]} = \frac{x^2}{1.1}$ **(1 point)**

$x = 10^{-1.91} = 0.01240967364$ **(1 point)**

$K_a = 1.4*10^{-4}$ **(1 point)**

+1 point for correctly using the ICE table
+1 point for setting up the correct equation
+1 point for obtaining the correct x
+1 point for correct final answer

c. Imagine that a physician is able to find the pH of the ventricular space in a patient's brain who has been diagnosed with brain cancer. If the pH is 5.6, calculate the value of $[OH^-]$. ***(2 points)***

$pOH = 14 - pH = 14 - 5.6 = 8.4$

$[OH^-] = 10^{-8.4} = 3.98 \times 10^{-9}$

+1 point for correct pOH or correct $[H_3O^+]$

+1 point for correct final answer

d. Calculate the concentration of lactic acid using the acid dissociation constant you calculated in part b and the pH value from part c . ***(2 points)***

$[H_3O^+] = 10^{-5.6} = 0.00000251188$

$K_a = 0.00000251188^2 / (x-0.00000251188) = 1.4*10^{-4}$

x = 0.0179 M

+1 point for correct $[H_3O^+]$

+1 point for correct final answer

e. Normal cells have a typical pH of 7.4. What is the difference in concentration in H^+ ions between a normal cell and a tumor cell in the brain. ***(2 points)***

$10^{-5.6} - 10^{-7.4} = 0.00000251188 - 3.98107171e\text{-}8 = 0.00000247206$ M

+1 point for showing work, +1 point for correct final answer and units

f. Current research has explored the potential for sodium bicarbonate to decrease the acidity of the tumor microenvironment. A group of MIT researchers added sodium bicarbonate to the mice's drinking water. They found that reducing the tumor's acidity also reduced metastasis, or the spread of the cancer.

For the purposes of this question, you will use sodium hydroxide to achieve the same effect. What is the pH after 50 mL of 5 M $NaOH$ was added to 1 L of the mice's drinking water? Using the concentration of lactic acid you gauged in part d, and the assumption that 1.13 mL of the drinking water will reach the 15 mL of fluid in the ventricular space. Is this sufficient to bring the cells back to their typical pH? ***(5 points)***

[NaOH] in drinking water = 0.05 * 5 mol / 1.05 L = 0.238 M

0.00113 L * 0.238 M = 0.000269 mol +1

$[CH_3CH(OH)COOH]$ = 0.0179 M * 0.015L = 0.000269 mol +1

Essentially equivalence point so all acid was converted to its conjugate base

$[CH_3CH(OH)COO^-]$ = 0.000269 mol / 0.16113 L = 0.00166 M +1

$K_b = [OH^-][CH_3CH(OH)COOH]/[CH_3CH(OH)COO^-]$

$7.1 \times 10^{-11} = x^2 / 0.00166$ +1

$x = [OH^-] = 3.43 * 10^{-7}$ M

$pOH = -\log(3.43 * 10^{-7}) = 6.465$

pH = 14 - 6.465 = 7.535 +1

Yes +1

Problem #5: ***(14 points)***

A common way solid tumors can be imaged is through Positron Emission Tomography (PET) scans. As the name suggests, a small amount of radioactive tracer is injected into the patient, and it goes through radioactive decay. The emitted particles are then detected, forming the scan. Carbon-11 is commonly used. It follows the decay:

$$^{11}C \rightarrow {}^{11}B + \nu + e^{+}$$

where ν is a neutrino and e^{+} is a positron.

a. If ^{15}O went through the same decay, give the products. ***(2 points)***

   $^{15}O \rightarrow {}^{15}N + \nu + e^{+}$
   +1 for N product
   +1 for others

b. A scientist is preparing a PET scan tracer and leaves out 4.50 g of ^{15}O. Five minutes later they come back and only 0.830 g of ^{15}O remain. The rest has decayed into ^{15}N. How much time would it take for only half of the ^{15}O to decay in seconds? ***(2 points)***

   0.830g = 4.5*(0.5)^(300s/t) , t = 123 s
   +1 for correct answer
   +1 for work

c. What you found is called the half-life of ^{15}O. Generalize it into an equation that takes the inputs of the half life, $t_{1/2}$, time, t, and an initial concentration, C_0, to produce the final concentration after the given time, C(t). ***(2 points)***

   $C(t) = C_0*(0.5)$^$(t/t_{1/2})$

d. Say a scientist is using ^{11}C as the radioactive tracer. It has a half life of 20.4 minutes. If the PET scan takes 36 minutes,

   i. How many half lives have gone by? ***(1 point)***

      36/20.4 = 1.8 half lives (1 pt)

   ii. If you start with a total of 1 g of ^{11}C, how much is left? ***(1 point)***

      1*(0.5)^(1.7647) = 0.29 g

e. The emitted positrons are detected to create the image. Using resolution in your response, why might species that emit lower energy positrons be better for PET scans as opposed to higher energy ones. ***(2 points)***

Lower energy positrons allow for higher resolution images to be taken (1pt) as higher energy positrons can be missed by the detector (1pt)

f. Some tracers are designed to be glucose analogs. Knowing that metabolic production is increased in cancer cells which consumes glucose, describe how a PET scan detects cancer tumors (i.e. where the tracer will collect and why) and what appears on the scan. ***(3 points)***

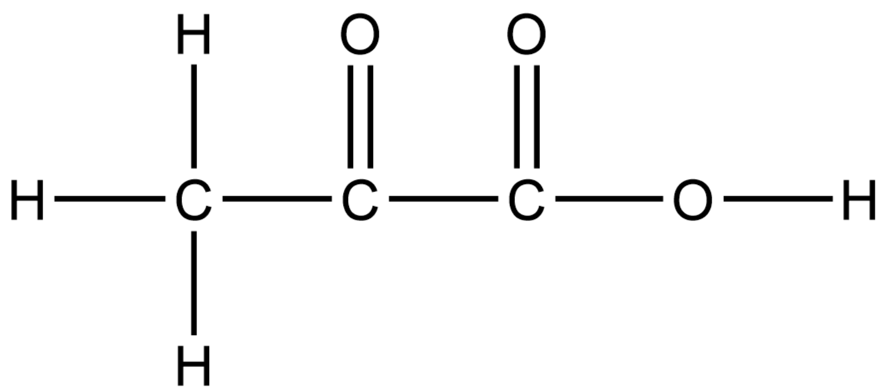
As a glucose analog, **the tracer will collect where glucose collects** (1pt). This means that **in cancer cells where there is increased glucose consumption, the tracer will build up** there and emit positrons (1pt). The emitted positrons will be detected, meaning **a bright signal spot will show up** on the scan where the tumor is (1pt).

g. Given the detection mechanism explored in part f, what kinds of cancer might not show up on a PET scan? ***(1 point)***

Low activity cancers where glucose production is not increased OR liquid state cancers that are more dispersed in the body. (1pt for either answer).

Problem #6: ***(14 points)***

Cancer cells require lots of glucose. Glucose is digested inside cells into a molecule called pyruvic acid. The structure of pyruvic acid is shown below:

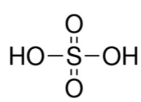


a. On the figure, label the hybridization of all carbon atoms. ***(3 points)***

Right to Left: sp^3, sp^2, sp^2

+1 point for each correct hybridization of a carbon atom (+3 points total)

b. Pyruvic acid has a pK_a of 2.49. Which of the following acids do you expect to have the most similar pK_a value? ***(2 points)***

i. 

ii. HCl

iii. HI

iv. Acetic Acid **(+2 points)**

c. The pH of the inside of the cell is buffered to be about 7.38. Which of the following buffers would you use to mimic this pH? The pK_a values of the conjugate acid in each pair is given. ***(2 points)***

1. $\frac{H_2SO_4}{HSO^{4-}}$ $pK_a = -3$
2. $\frac{CH_3CO_2H}{CH_3CO_2^-}$ $pK_a = 4.76$
3. $\frac{H_3PO_4}{H_2PO_4^-}$ $pK_a = 2.15$
4. $\frac{HCN}{CN^-}$ $pK_a = 9.20$

$\frac{H_2PO_4^-}{HPO_4^{2-}}$ $pK_a = 7.20$ **(+2 points)**

d. At this pH, calculate the ratio of $\frac{[A^-]}{[HA]}$ for the pair $\frac{H_2PO_4^{2-}}{HPO_4^{2-}}$-, where A^- represents the conjugate base, and HA is the conjugate acid. ***(4 points)***

Henderson-hasselbalch equation: $pH = pka + log \frac{[A-]}{[HA]}$

$7.38 = 7.20 + log \frac{[A-]}{[HA]}$

$\frac{[A-]}{[HA]} = 10^{0.18} = 1.51$

(**+4 points**)

e. Pyruvate is produced inside cells. At physiological pH, do you expect to measure more pyruvate inside or outside the cells? Explain. ***(3 points)***

At cellular pH, pyruvate is in its negatively charged form (**+1**). The cell membrane is impermeable to most charged compounds (**+1**), so none of the pyruvate can cross the membrane and exit the cell (**+1**).