EXAM OF SCIENTIFIC CULTURE

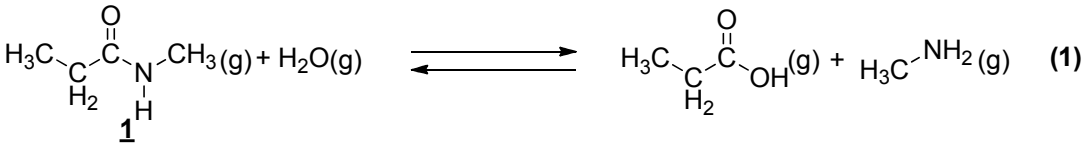
CHEMISTRY

PROBLEM 1:

The synthesis and degradation of proteins are two important processes in biology. The hydrolysis of a peptide bond presents characteristics that are directly related to the nature of this bond.

1.1 Chemical reactivity *in vitro*

The hydrolysis of N-methylpropanamide **1** in the gas phase results in the formation of propanoic acid and methylamine as shown in the balanced equation **(1)** below:

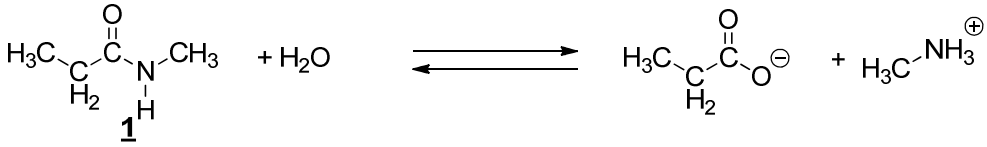


(1)

1.1.1 Knowing that the standard enthalpy of the hydrolysis of amide **1** $\Delta_{hyd}H°(1)$ is equal to 15 $kJ.mol^{-1}$, how would an increase in temperature influence this reaction?

1.1.2 Which would be the effect of an increase in the quantity of water at constant pressure on the extent of this reaction **(1)** at equilibrium?

The hydrolysis of **1** is now studied at 1 bar in a diluted solution at 300 K in two different solvents (water H_2O and chloroform CH_3Cl):



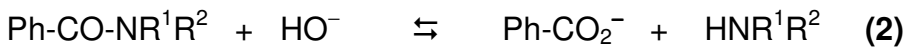
The equilibrium constant under these conditions equals to $K = 3$ in water (H_2O) as well as in chloroform ($CHCl_3$).

1.1.3 When the reaction is performed in chloroform with initially all components at a concentration of 1 $mol.L^{-1}$, what would be the final molar concentrations at equilibrium?

1.1.4 Does the molar concentration of the amide **1** at equilibrium change compared to the one calculated in the question before when the reaction is performed in water? Explain.

1.2 Kinetic aspects

The hydrolysis reaction of an amide is studied under basic aqueous conditions and the balanced equation **(2)** (Ph = C_6H_5) is given below:

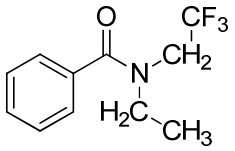


(2)

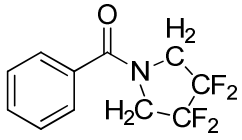
The pseudo first order apparent kinetic rate constant k_{hyd}, was measured in the presence of a large excess of hydroxide ion HO^-. K_{hyd} is the equilibrium constant of the reaction **(2)**.

1.2.1 Write the mechanism of the hydrolysis of the amide in basic medium **(2)**. Is the formation of the final products quantitative? Explain.

Now the hydrolysis of the amides **2** and **3**, shown below, is studied.



2



3

Data at 350 K :

Gibb's free energy of activation for hydrolysis:

Compound	**2**	**3**
$\Delta G^{\circ\neq}$ (kJ.mol^{-1})	125	105

1.2.2 Which of the two amides **2** or **3** is the better electrophile? Justify your answer.

1.2.3 Does the electrophilicity discussed in the question before agree with the values of the Gibb's free energy of activation for hydrolysis $\Delta G^{\circ\neq}$ given above?

For the hydrolysis of amide **3**, $\Delta H^{\circ\neq}$(**3**) denotes the standard enthalpy of activation and $\Delta S^{\circ\neq}$(**3**) the standard entropy of activation.

1.2.4 How can the values of $\Delta H^{\circ\neq}$(**3**) and $\Delta S^{\circ\neq}$(**3**) be determined experimentally?

1.2.5 Express the rate of the reaction v as function of k_{hyd} and of the concentration of [**3**].

Now, we are interested in the reaction **(3)** which is performed in a buffered solution at pH = 7.4:

$$Ph\text{-}CO\text{-}NR^1R^2 + H_2O \leftrightarrows Ph\text{-}CO_2^- + H_2N^+R^1R^2 \quad \textbf{(3)}$$

1.2.6 The reaction **(3)** is better accomplished in the presence of a catalyst. Why?

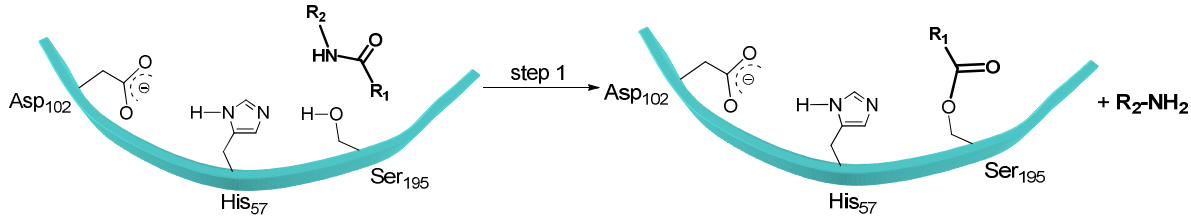
1.2.7 How does the presence of a catalyst influence the extent of the reaction **(3)** at equilibrium? Explain.

1.3 Hydrolysis of a peptide bond in biology and in the presence of a cobalt complex.

In biological media the hydrolysis of peptide bonds is catalyzed by enzymes. The peptidase α-chymotrypsin presents in its active site an arrangement of three amino acids which are important for catalysis: aspartate 102, histidine 57 and serine 195. The mechanism at the active site consists of two non-elementary steps, but only the first step is presented below. The activity of α-chymotrypsin is best at a pH of 8.

pKa values of the lateral chains of the three amino acids present in the active site of the enzyme:

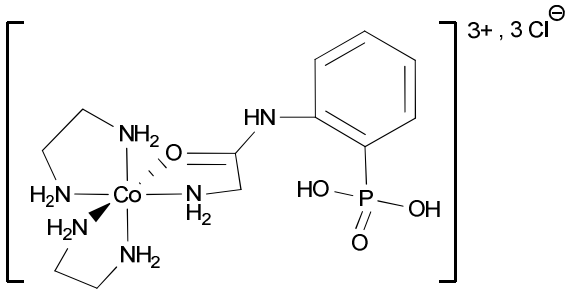
Aminoacid	**His**	**Asp**	**Ser**
pKa (lateral chain)	6.0	3.9	>16



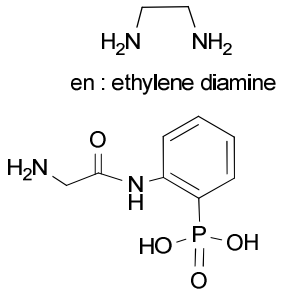
1.3.1 Write a mechanism for the first step and identify the role of each of the three amino acids Asp 102, His 57 and Ser 195 in this mechanism.

1.3.2 When the pH is lowered from 8 to 6 the activity of the α-chymotrypsin diminishes considerably. Propose an explanation for this observation.

Some metal complexes are also capable of hydrolyzing amide bonds. A cobalt complex associated to an amide type substrate R-PO_3H_2 is shown below:



$[Co(en)_2R\text{-}PO_3H_2](Cl)_3$



R-PO_3H_2

Data:

$[Co(en)_2R\text{-}PO_3H_2](Cl)_3$: $pKa_1 = 1.8$ and $pKa_2 = 5.6$

1.3.3 Indicate the oxidation state of the cobalt ion in this complex and give the electronic configuration of this ion in its ground state (Z(Co) = 27).

1.3.4 Knowing that this cobalt complex is diamagnetic, indicate whether this complex is high – or low spin. Explain.

The hydrolysis of the amide coordinated to the metal center is performed in a buffered solution at pH 7.2.

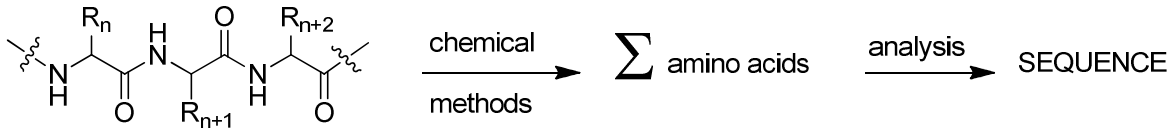
1.3.5 Identify the role played by the metal center in the hydrolysis of R-PO_3H_2.

1.3.6 What is the charge of the complex in this solution?

1.3.7 Propose an explanation for the fact that the reaction is faster at a pH of 7.2 than at a pH of 1.5.

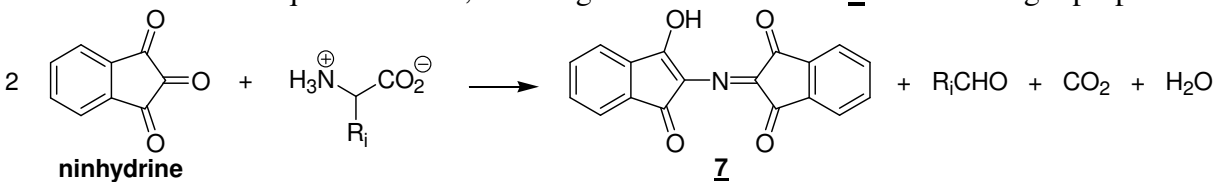
1.4 Protein sequencing

The sequencing of proteins consists in determining the number, the chemical nature and the order of all amino acids. In order to do so, a variety of chemical methods are available to liberate the acids for analysis.

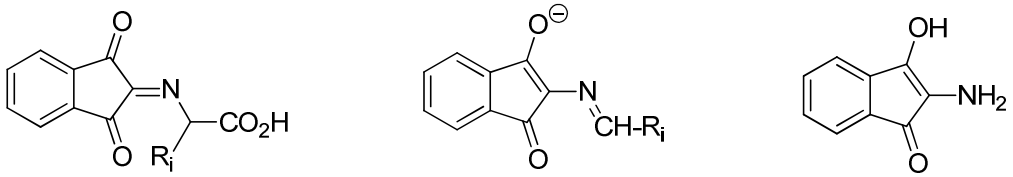


Reaction with ninhydrine

Ninhydrine is used as a chemical indicator for amino acids. This compound reacts with amino acids, as shown in the balanced equation below, resulting in the formation of **7** which is bright purple:



During the reaction three intermediate products (**4**, **5** and **6**) are formed:



1.4.1 In aqueous solution ninhydrine exists as two hydrated forms. Write the Lewis structure of these hydrated forms. Are both forms equally stable? Explain.

1.4.2 Write the mechanism under acidic catalysis of the formation of **4** starting form ninhydrine and the amino acid.

1.4.3 During the second step compound **4** decarboxylates in its basic form to give **5**. Write the mechanism of this decarboxylation and justify the facility of this reaction.

1.4.4 In the next step the imine **5** is hydrolyzed and results in the formation of **6**. Propose a mechanism with acidic catalysis for this step.

1.4.5 What type of reaction is the last step transforming **6** into **7**? Explain.

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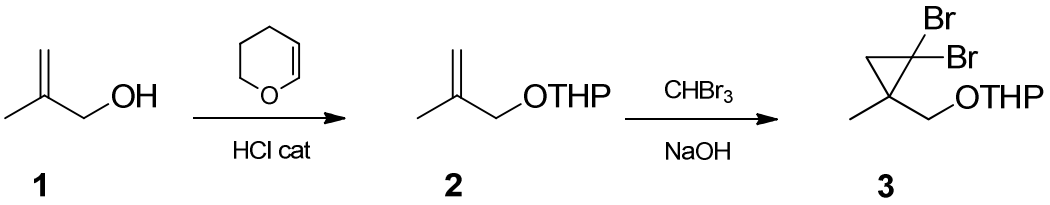
PROBLEM 2:

We examine the first steps of the synthesis of (±)-cycloclavin, which was reported by P. Wipf in 2011.



2.1 The synthesis starts with the protection of the alcohol in compound **1** upon using dihydropyran in an acidic medium.

2.1.1 The formula of the product **2** is $C_9H_{16}O_2$. Draw the structure of the product **2**.

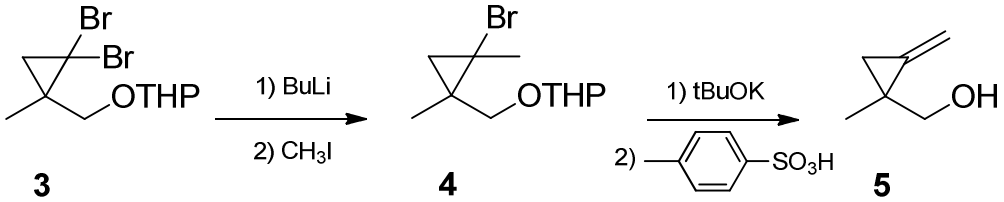
2.1.2 Propose a mechanism to account for the formation of the product **2**.

2.1.3 How do you explain the observed regioselectivity?

2.2 In the second step, the ether **2** is converted into the cyclopropane derivative **3**.

2.2.1. Upon treating bromoform $CHBr_3$ with sodium hydroxide, one observes the formation of an intermediate with CBr_2 formula. Propose a mechanism to account for its formation. Draw its Lewis structure. What is the name of such a species? What are its main properties?

2.2.2 Propose a mechanism accounting for the formation of the product **3**.



2.3 The cyclopropane derivative **3** is first treated with butyllithium and then with methyl iodide to yield the monobrominated derivative **4**. Propose a mechanism to account for the formation of the product **4**.

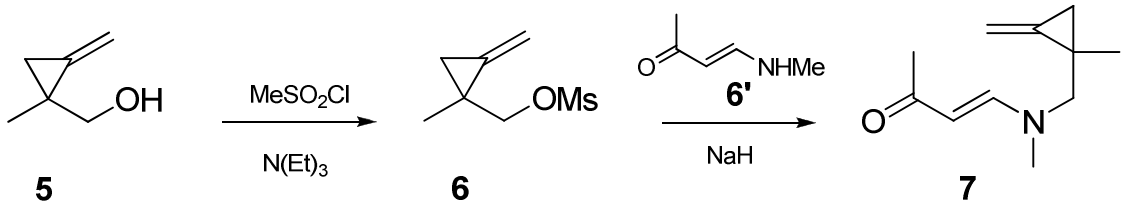
2.4 The brominated derivative **4** is subsequently treated with potassium tertiobutylate and then para-toluene sulfonic acid to give the alcohol **5**.

2.4.1 Draw the intermediate resulting from the treatment of the compound **4** by potassium tertiobutylate.

2.4.2 *A priori*, which mechanism is involved in the formation of the double bond in the product **5**? Justify your answer and describe this mechanism. How do you explain the observed regioselectivity?

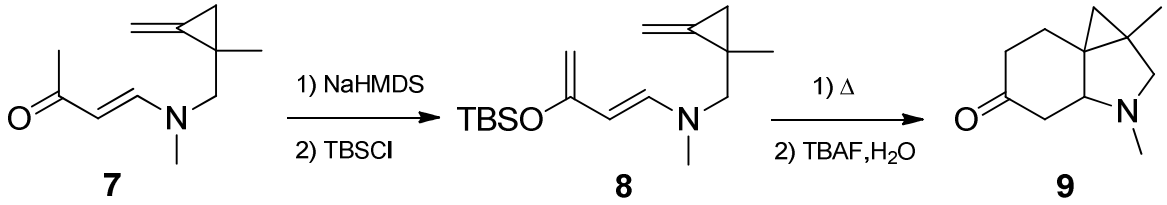
2.4.3 What is the role of para-toluene sulfonic acid to give the alcohol **5**?

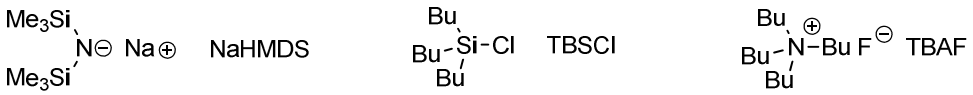
2.5 Justify the relevance of protecting the alcohol function in the reactant **1** to achieve the synthesis of the product **5**.



2.6 The product **5** is treated by mesyl chloride ($MeSO_2Cl$) in the presence of triethylamine to obtain the product **6**. Considering the next step (**6** → **7**), what is the objective of the conversion **5** → **6**?

2.7 The reaction of sodium hydride on the amine **6'** yields an intermediate, which reacts with the mesylate **6** to provide the enamine **7**. What is the mechanism involved in this reaction?





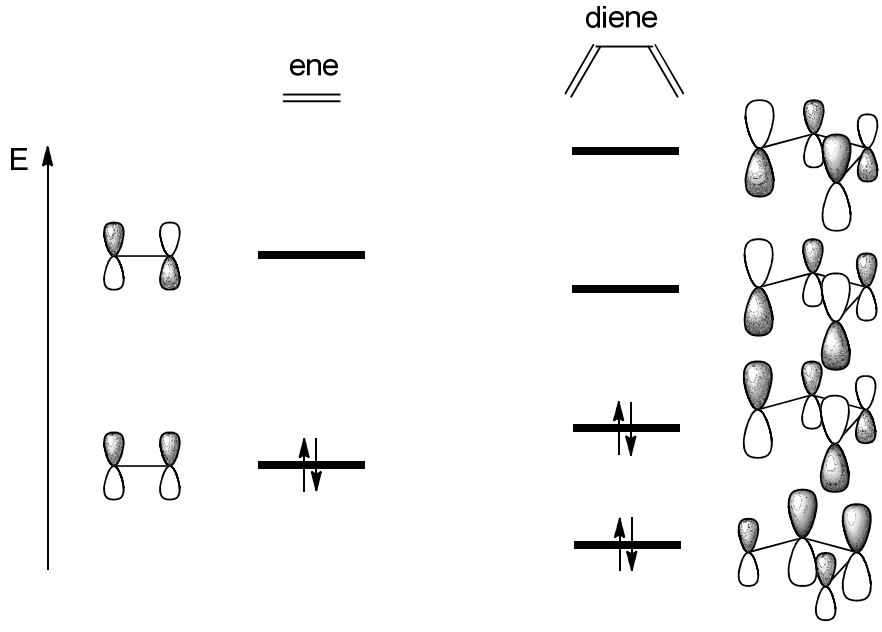
2.8 Treated by the amidure (NaHMDS) and then by tributylsilyl chloride (TBSCl), the reactant **7** yields the product **8**.

2.8.1 Among the two elements Cl and Si, which one is the most electronegative? Justify your answer.

2.8.2 Propose a mechanism to account for the formation of the product **8**.

2.9 In a first step, the product **8** is heated in solution to yield an intermediate.

2.9.1 In this step, the reaction involves the diene and ene moieties of the molecule **8** which respective diagrams of wave functions of molecular orbitals and energies are pictured below.



Indicate on the preceding diagram the most favorable orbital overlap(s) to yield a reorganization of the chemical bonds. Justify your answer.

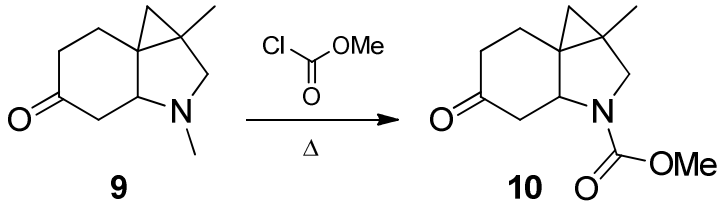
2.9.2 Draw the intermediate derivative obtained after the first heating step.

2.10 The intermediate obtained in the preceding question is subsequently treated by tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) in the presence of water traces to give the product **9**.

2.10.1 The fluorine gives a very strong bond with silicon. Propose a mechanism to account for the formation of the product **9**?

2.10.2 Justify the use of tetrabutylammonium fluoride (TBAF) instead of potassium fluoride (KF) to obtain the product **9**?

2.11 The last investigated step involved the demethylation of the amine function, which subsequently yields the carbamate **10**.



9 **10**

2.11.1 Draw the positively charged intermediate resulting from the nucleophilic attack of the tertiary amine on the Cl(CO)OMe chloride.

2.11.2 Show how this intermediate can account for the formation of the product **10**.

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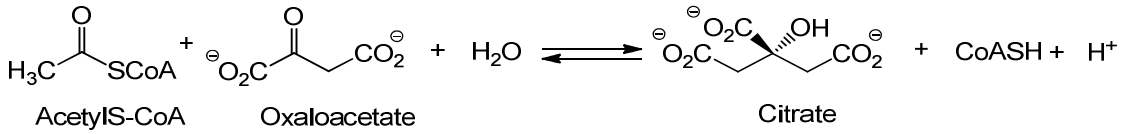
CHEMISTRY

PROBLEM 3:

3.1. The Krebs cycle assures a large part of the energetic needs of the cell, thanks to the electrons present in the C-H and C-C bonds of acetylcoenzyme A (CH_3-CO-SCoA). Globally, this coenzyme is oxidized into carbon dioxide with the intervention of other coenzymes, such as FAD.

3.1.1 Complete the redox equation below and calculate the oxidation state of the carbon atoms of acetylcoenzyme A and carbon dioxide.

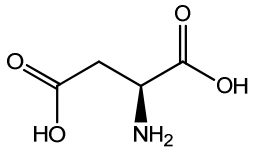
The first step in the Krebs cycle is catalyzed by *citrate synthetase*, as shown below:



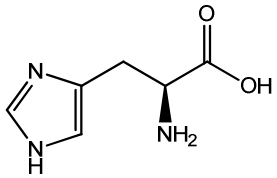
3.1.2 This step can be decomposed into three reactions: (a) enolisation of acetylS-CoA, (b) condensation of the formed enol onto oxaloacetate and (c) hydrolysis of the thioester function. Propose a mechanism for each reaction under acidic catalysis conditions.

Actually, an X-ray diffraction study of *citrate synthetase* has shown that two amino acids, the carboxylate of aspartate 375 and the imidazole of histidine 274, participate in the catalytic activity of the enzyme.

3.1.3 These residues taking part in the catalysis present lateral chains that can be ionized. The pKa values are: 2.1; 3.9 and 9.8 for aspartic acid and 1.7; 6.0 and 9.1 for the imidazole cycle of histidine. Assign these pKa values to the different acid-base functions of the amino acids shown below.



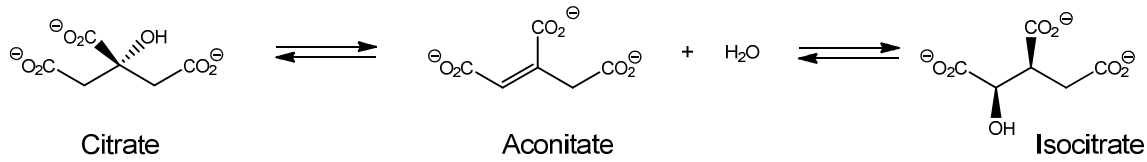
Aspartic acid



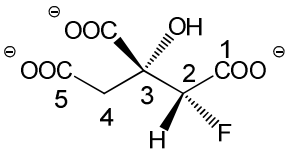
Histidine

3.1.4 Knowing that in the active site of the enzyme the lateral chains of aspartic acid and histidine are present as CO_2^- and ImH_2^+, respectively, and taking their pKa values as unchanged in the protein, indicate approximately at which pH the protein functions the best. Justify your answer.

Then the enzyme *aconitase* isomerizes citrate into isocitrate by forming aconitate as an intermediate:



One of the configuration isomers of 2-fluorocitrate, shown below, is an inhibitor of the enzyme aconitase.

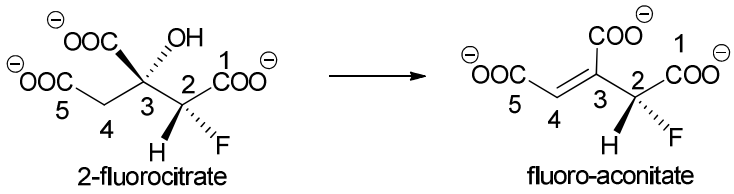


3.1.5 How many configuration stereoisomers exist of 2-fluorocitrate? Justify your answer.

3.1.6 This molecule presents three carboxylate groups. Which of the three functions is the less basic? Justify your answer.

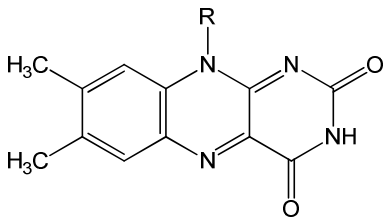
3.1.7 Indicate the absolute configuration of the asymmetric carbon atoms of 2-fluorocitrate. Justify your answer.

The 2-fluorocitrate undergoes several reactions until finally *in fine* a competitive inhibitor of *aconitase* is produced. The first reaction is a dehydration resulting in the formation of fluoro-aconitate.



3.1.8 What is the configuration of the double bond of fluoro-aconitate shown above? Justify your answer.

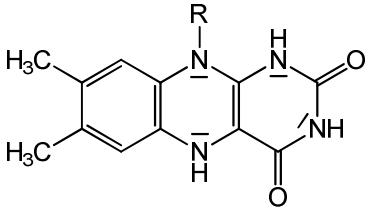
3.2 One of the important cofactors in the Krebs cycle is a flavine cofactor (FAD). Its structure is presented below:



3.2.1 Propose the hybridization state of the four nitrogen atoms of FAD and indicate the nature of the orbital containing the non-bonding electron pair.

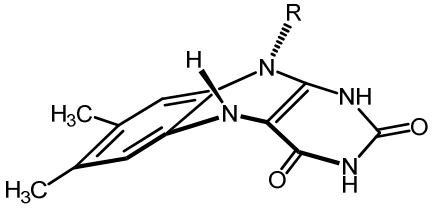
3.2.2 FAD is an aromatic compound. Justify this character by showing, among other possibilities, the number of electrons involved in the aromaticity.

FAD can be reduced into $FADH_2$ whose Lewis structure is shown below:

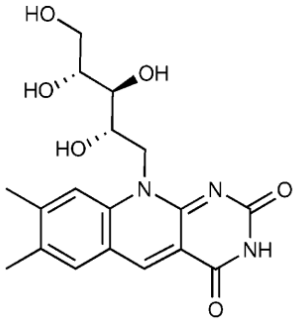


3.2.3 Assuming that the three cycles of $FADH_2$ are coplanar; would this tricyclic system be aromatic? Justify your answer.

3.2.4 Actually, the three cycles of $FADH_2$ are not coplanar. Indicate the state of hybridization of the four nitrogen atoms and the nature of the orbital containing the non-bonding electron pair.

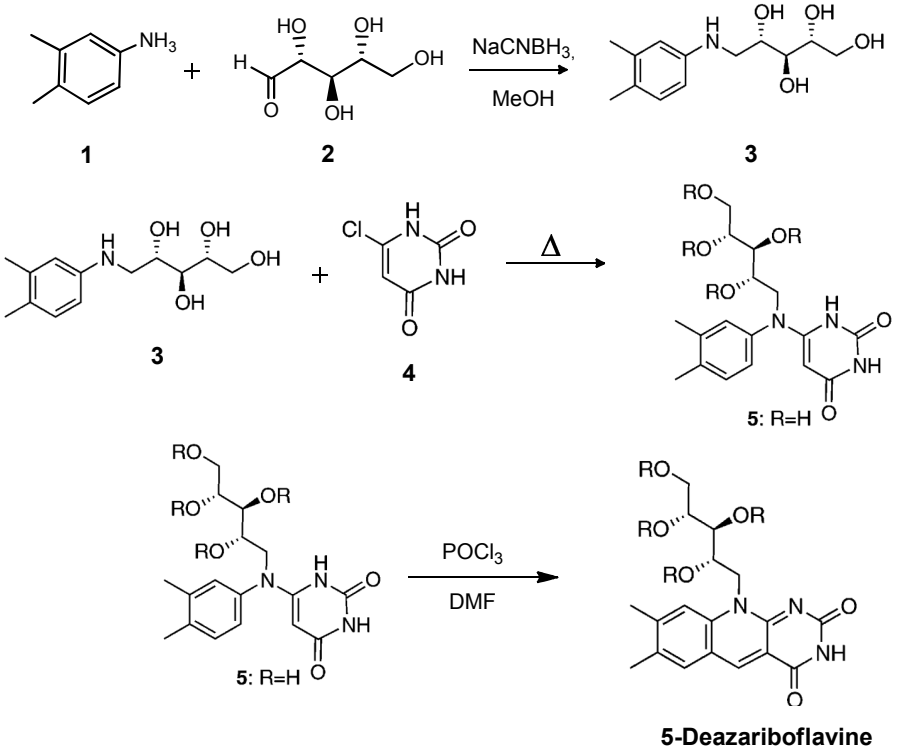


3.3 Flavin cofactors participate in catalysis *via* a large number of mechanisms. The synthesis of analogues of this molecule is therefore important for the study of these mechanisms. The 5-Deazariboflavine was synthesized with this aim:



5-Deazariboflavine

The general scheme of the synthesis of 5-Deazariboflavine is shown below:

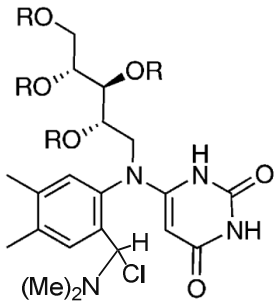


3.3.1 Write the mechanism of the transformation of **1** and **2** into **3**. What is the role of $NaCNBH_3$ in this reaction? Explain.

3.3.2 Identify the most electrophilic carbon in 6-chlorouracil **4**. Justify your answer.

3.3.3 Write the mechanism responsible for the coupling between **3** and **4** to form **5**.

In the last step, **5** is transformed into 5-Deazariboflavine. During this step an intermediate **I** is formed, shown below:



I

The reactive **I',** species responsible for the formation of this intermediate **I** through reaction with **5,** is formed *in situ* by reaction between DMF ($(CH_3)_2NCHO$) and $POCl_3$.

3.3.4 Write the mechanism of this reaction occurring between $P(=O)Cl_3$ and DMF giving **I'**.

3.3.5 Write the reaction between **I'** and **5** resulting in the formation of the intermediate **I**.

3.3.6 Propose a mechanism for the last step resulting in the formation of 5-Deazariboflavine.