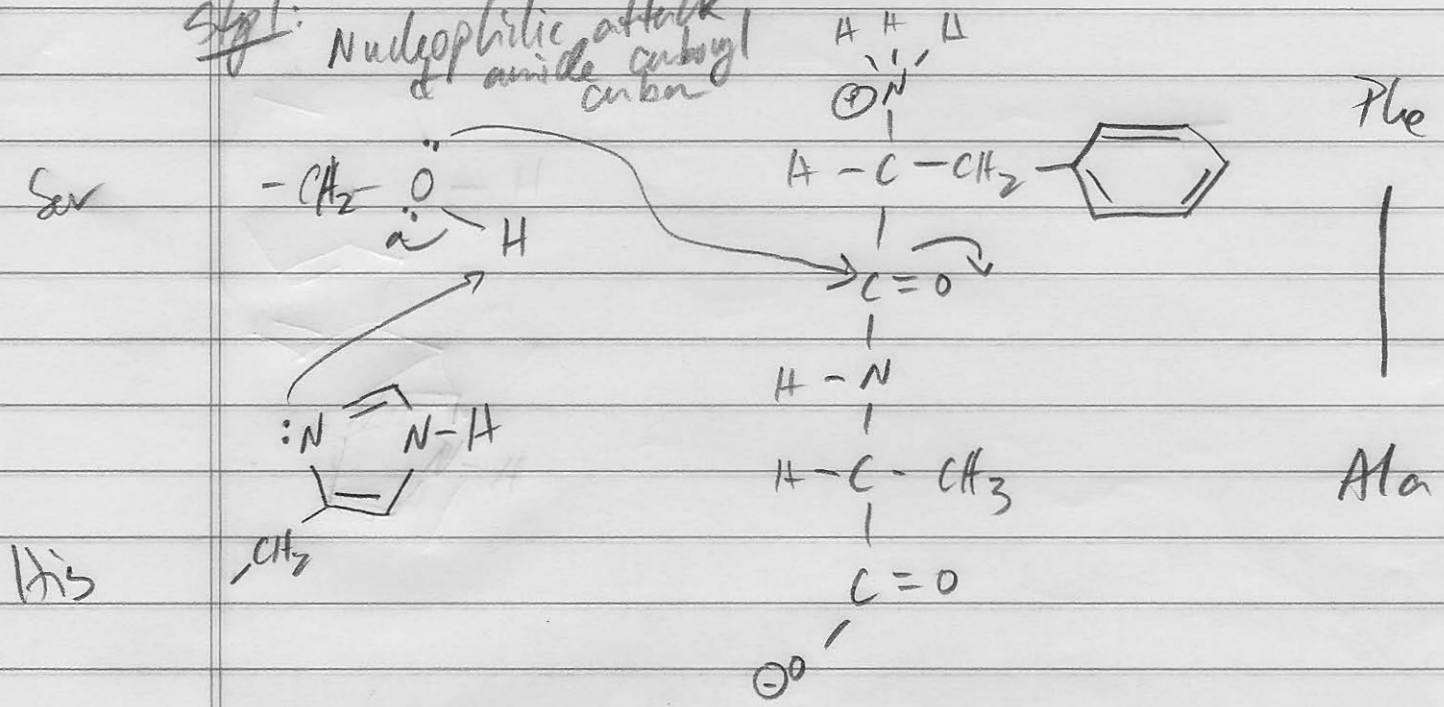
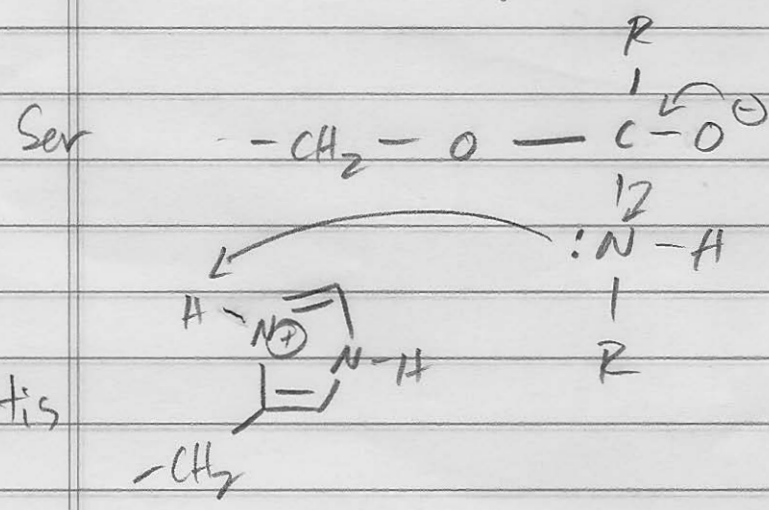
Problem Set 3 key

① Substrate: Phe - Ala

Serine Protease: Catalytic Amino Acids: Ser and His

Step 1: Nucleophilic attack of amide carbonyl carbon





Rearrangement of tetrahedral intermediate and cleavage of amide bond!

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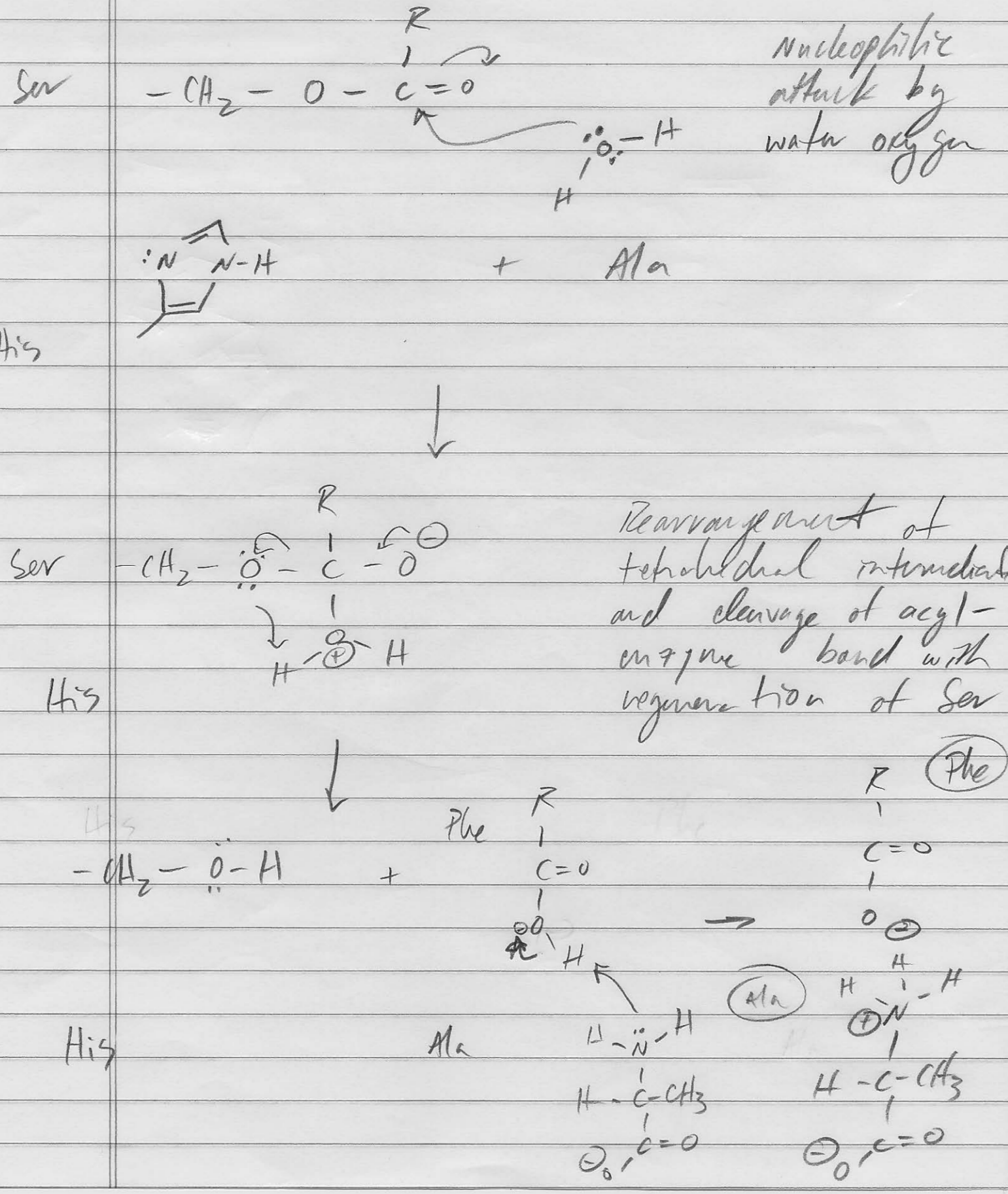
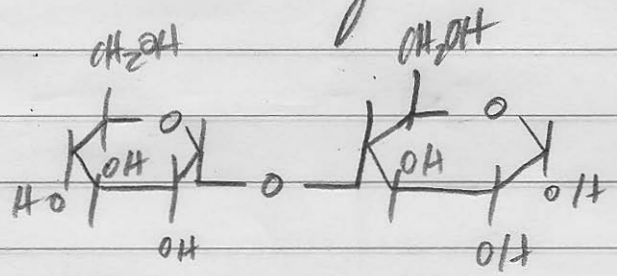
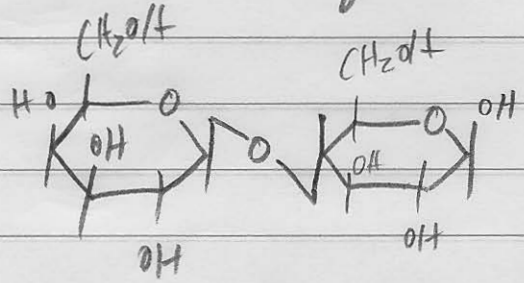

Ser
Nucleophilic attack by water oxygen
His
+ Ala
Rearrangement of tetrahedral intermediate and cleavage of acyl-enzyme bond with regeneration of Ser
His
Phe
Ala
His
Ala

(2) a) Maltose = glucose α-1,4-glucose

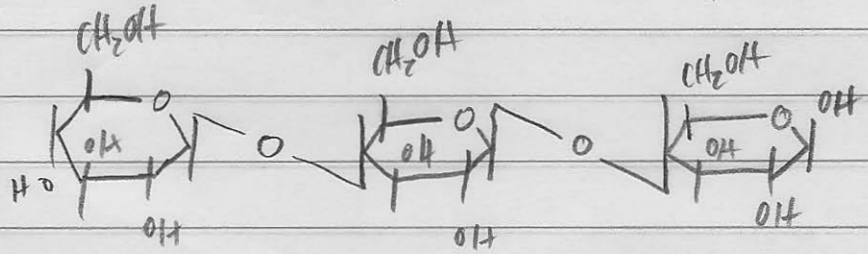


b) Lactose = galactose β-1,4-glucose

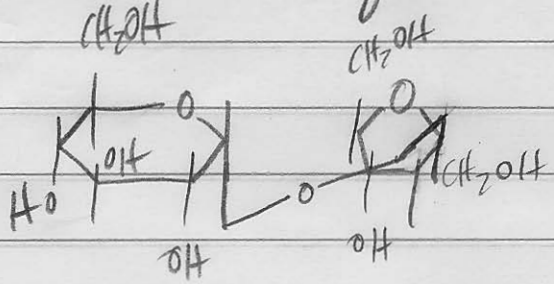
(make the glucose β)



c) Cellobiose = glucose-β-1,4-glucose-β-1,4-glucose

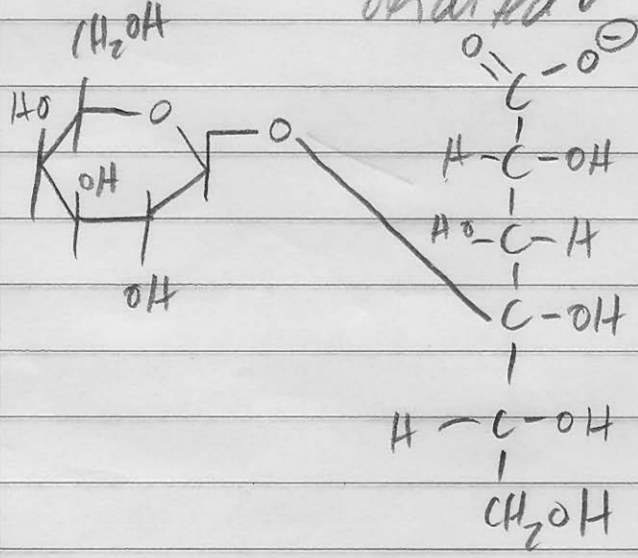


d) Sucrose = glucose α-1,2-fructose

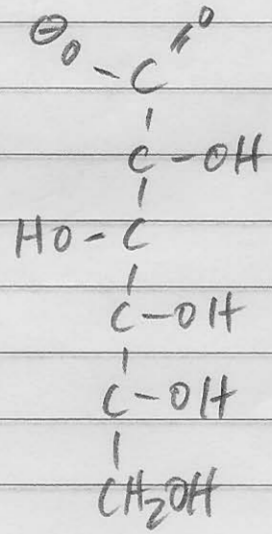


(3) a) Sucrose is not a reducing sugar
(The ketone group of fructose is in a bond)
(The aldehyde group of glucose is in a bond)

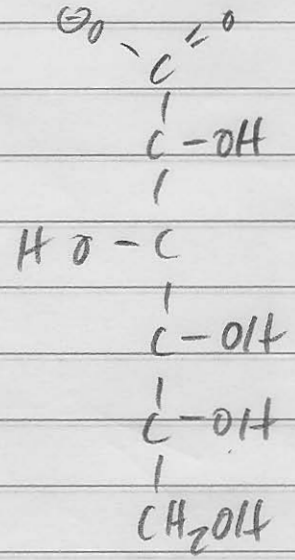
b) Lactose: The aldehyde of glucose will be oxidized



c) α-D-fructose
The ketose will isomerize into an aldose giving you:

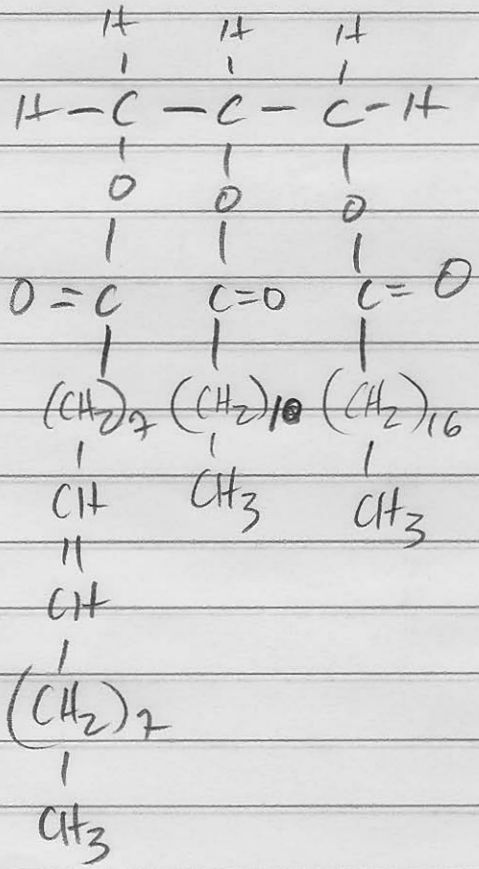


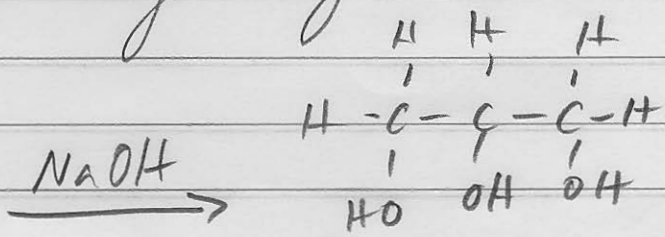
d) β-D-glucose

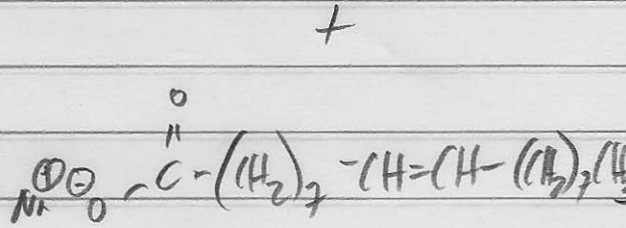


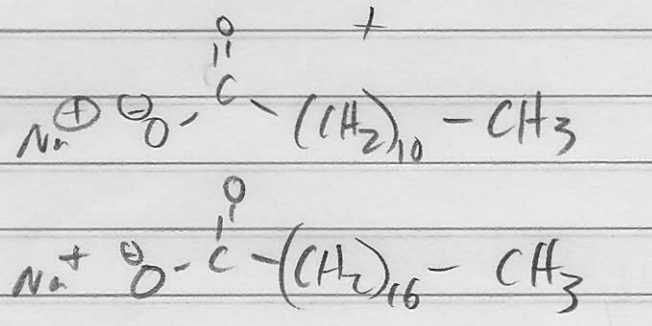
e) Trehalose (glucose α-1,1-glucose) is not a reducing sugar.

④









oleic lauric Palmitic

(5) a) $K_{cat}{}_{W.T.} = \frac{V_m}{[E_T]}$ $\quad K_{cat}{}_{mut} = 0.25\, K_{cat}{}_{W.T.}$

If the $[E_T]$ is the same for the comparison (and it has to be or you couldn't compare anything) then you can say:

$$K_{cat}{}_{mut} = \frac{V_m{}_{mut}}{[E_T]} \qquad K_{cat}{}_{WT} = \frac{V_m{}_{WT}}{[E_T]}$$

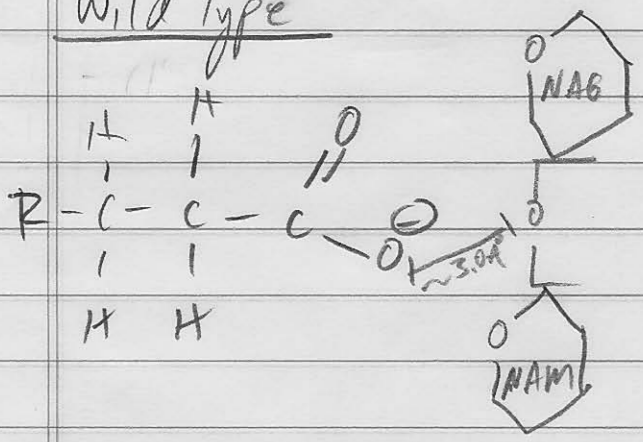
$$\frac{V_m{}_{mut}}{[E_T]} = 0.25 \left(\frac{V_m{}_{WT}}{[E_T]}\right)$$

$$\boxed{V_m{}_{mut} = 0.25\, V_m{}_{WT}}$$

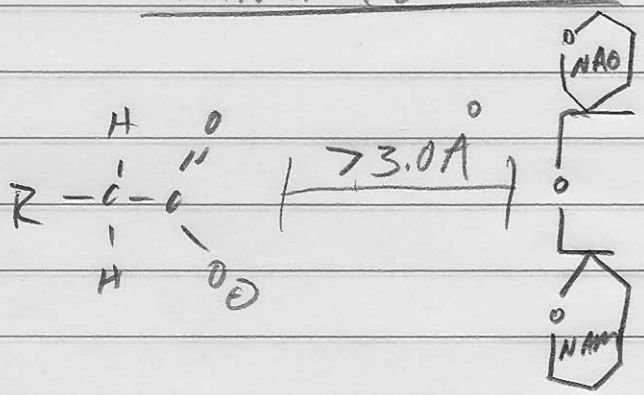
b) No. The mutation is to a catalytic residue, not a residue involved in substrate binding so Km should be unaffected.

c) The mutation will place the ~~glutamate~~ carboxylate group farther away from the glycosidic bond oxygen by a full $-CH_2-$ group.

Wild Type



Mutant (E → D)



d) The catalytic aspartate helps stabilize the carbocation intermediate formed in the N-Acetyl-muramic acid after the glycosidic bond is cleaved until water can attack the carbon.