Mechanisms in Organic Chemistry
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Lecture-27
Isotope Labelling

So welcome back. In the last class we had looked at equilibrium isotope effects and this is seen due to the fact that isotopic substitution makes a shift in the equilibrium.

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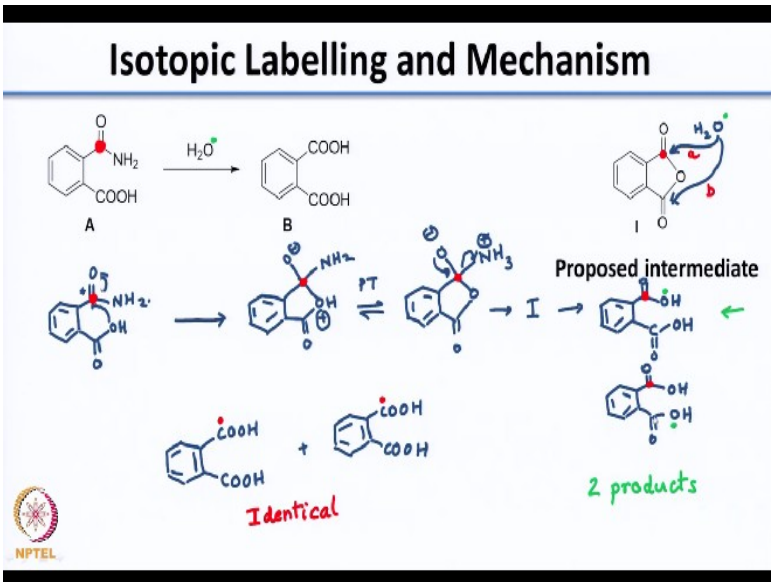

We had seen that very very small shifts in the equilibrium are also observable and we could get very small perturbations also to be measurable and this could be used to determine the nature of the intermediates. So we had seen the example where you had seen a case where you had a rapid equilibrium versus one where you had a symmetric intermediate which is resonance stabilized. Then towards the end of the class we had started looking at the experiment called as isotope labelling.

So here what is done is a particular position in the reactant is labelled and then we follow where the label grows in the product. So we had looked at 2/3 examples of how this can be used to determine the mechanism and before we stopped the class I had asked you to think of the mechanism for this reaction. Now I will give you a hint if you have written several mechanisms what is proposed is that it goes via this anhydride intermediate shown here.

So if any of your mechanisms do not have the anhydride intermediate, I suggest you press the pause button and then try to work out a mechanism where you get this anhydride intermediate and another hint I will tell you is that the neighboring carboxylic acid has a role to play. So let us see if you got the mechanism correctly. So I am going to write out the carboxylic acid group because then it will be easier for you to follow what is going on.

Now the intermediate shown here indicates that you have a nucleophilic attack by the neighboring group at this electrophilic position. So one way you can do it is you can show this add in to give you, so you generate this and now you need to get from here to the intermediate shown here, so after a proton transfer you will generate a species where you have, then you have, to give you the intermediate I, now the intermediate I is symmetric.

So once you have your intermediate I, you can have your water attack at either of these positions so it can either attack here or it can attack here and essentially what you will get would be, since it is symmetric, again after a bunch of proton transfers, so you get your product. Now to figure out whether this is what is happening or you can imagine that one can always argue that no, the water is probably directly attacking the carbonyl of the amide.

So to distinguish between these 2 mechanisms what was done was that the carbon of the amide was labelled and the oxygen of water was labelled. Alright? So you had these 2 labels, now if you have these 2 labels how will that help you in the reaction? So look carefully at the intermediates and see what the label has done. What the label has done is, it has now again made it unsymmetric.

So when you get intermediate I, the labelling of the carbon makes this unsymmetrical. So here also because this is labelled, so now let us see how this label helps. So if you have the intermediate I and as I said you can have say pathway A or pathway B. Now if it follows pathway A, the product would be this right? And if it follows pathway B and again remember here that you have also labelled your oxygen.

So, if you have pathway A you will get this product whereas if you follow pathway B the product you get would be where you have this as the labelled carbon and this as the labelled oxygen. Now looking at this you would get 2 different products. So now that you get 2 different products you can say that it goes via a symmetric intermediate. Had it been a direct attack of the water to $CONH_2$, this would have been the only product.

But since you are getting 2 products it indicates that it is going via the symmetric intermediate I. Now my question to you is had I not labelled the oxygen would I still be able to get this information? So had you not label the oxygen what would be the products possible? You would have gotten, let us put the labels, so this carbon is labelled, this carbon is labelled. Since the oxygen is not labelled both these products would again be identical.

So you will not be able to figure out what would be the nature of the intermediate. So that is why when you design experiments or when you hypothetically try to figure out what the mechanism is, you have to draw it out and think of all possibilities. So in this case we have seen that you require the labelling of the water oxygen as well as the labelling of the carbon for you to figure out their relative position.

So what we saw in this case is that because we had labelled both you were able to distinguish between this product which has the 2 labels next to each other versus the other product where the labelled oxygen is next to a carbon which is not labelled. So this is a very nice illustration of how while designing these experiments one has to be very smart in picking which places to label and how the label will help you actually distinguish the mechanism.

Otherwise if you had just put one label you would not have been able to clearly distinguish between the 2 mechanisms i.e. direct attack versus formation of intermediate I. Now this isotopic labelling has also been used to understand mechanisms in biology. So always we have also been looking at how you can apply this to understanding biology right. So let us look at an example.

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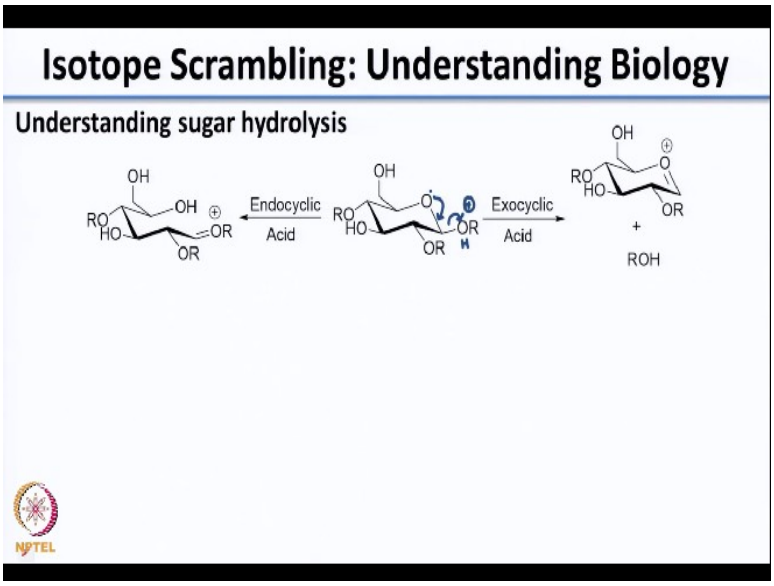

So sugar hydrolysis in the body, now this takes place using enzymes, but there are 2 pathways that are proposed. One is called as an exocyclic pathway and an endocyclic pathway. In the exocyclic pathway you have cleavage of the exocyclic bond. I will draw up show the arrows so that you understand what is going on. So you have this lone pair on oxygen. So you can imagine this coming in and this will go out of course this is in an acidic medium.

So this is probably protonated. So the exocyclic it gives you this intermediate shown here and ROH. In the end of cyclic bond cleavage what happens is you are now pushing electrons from the other oxygen.

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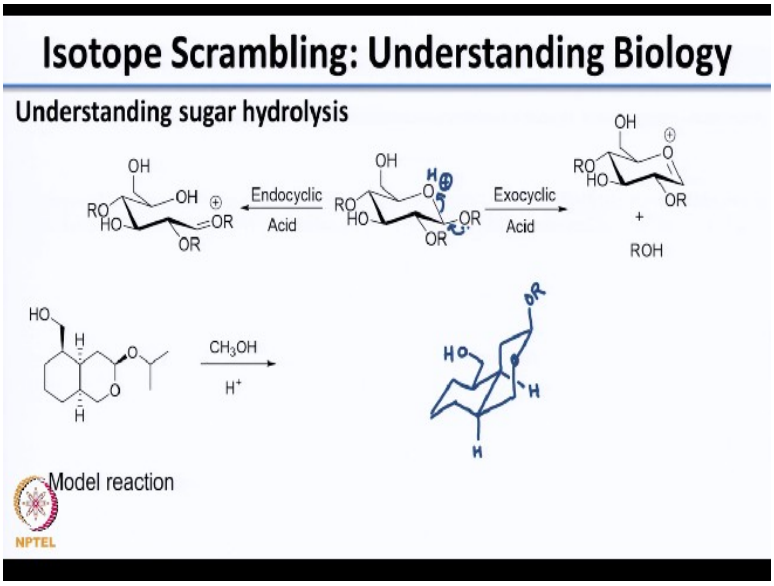

So I will erase this and write how the endocyclic pathway works, so the flow of arrows is essentially the reverse here than what we had seen in the first case. So in the endocyclic pathway again probably you can imagine this being protonated. So you have this coming in and this bond cleaving, so you have the cycle being broken. Now if you were to determine which pathway is taking place, is it the endocyclic pathway or is it the exocyclic pathway?

A model system which was chosen was this molecule, so this is your model reaction. This is a decaline like system. Let us try to write the stable conformation for this, so that you are able to visualize what is going on. So I do not know if you have had exposure to writing the confirmations for decaline, if not it would be a good idea to practice. I think we had done it before for the trans decaline.

Now we will do it for the cis-decaline, so here the ring junction is cis. So go ahead and try to write the conformation press the pause button on the video and try to write the stable conformation for this molecule. So you can check your answers, so first I will write the chair form. So I am forming cis junction here, remember these are my hydrogens, this is the axial hydrogen. So now let us draw it.

So this is my equatorial, this is my axial, so then I just need to connect the two and I get my cis decaline alright? And here now I will redraw the hydrogen I had erased. This is my cis ring junction. So now I have drawn the skeleton of the molecule with the cis ring junction. Now let us put the substituents. So from the ring junction at the 1,2,3 position I have this OR group and it is going up. So I will write it like this alright?

And then at the adjacent position I have the CH_2OH group, it is again going up, so I will write it like this. So this is what the conformation looks like for the molecule given here. Now this was chosen as the model reaction, so now let us try to write, I am missing an oxygen here because we are here. So now let us try to write the mechanism for this reaction using our model reaction. So here we are doing the endocyclic as well as exocyclic hydrolysis with in the presence of methanol.

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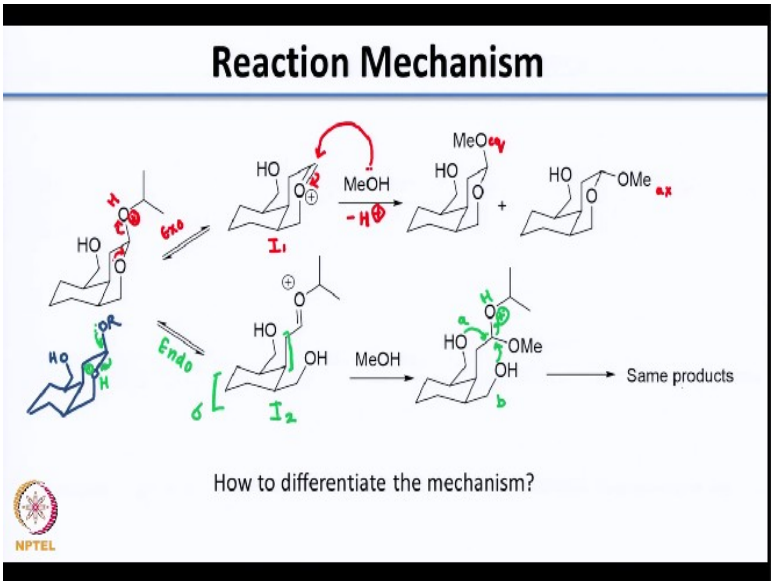

So this is what it will look like. So first is the exocyclic mechanism. So in the exocyclic mechanism, again the first step let us say would be protonation. So once this is protonated you can imagine these lone pairs coming in and once this lone pair comes in this will leave it will give you this intermediate, let us call this I_1.

So once you have this intermediate you can have the methanol. So you can have the methanol add in and of course we are showing a deprotonation to give you two possible products. You can have the equatorial product where you have the $methanol_{equatorial}$ and you can have the axial product we will call it as $methanol_{axial}$ alright? So now let us look at the endocyclic pathway. So in the end of cyclic pathway now the flow of electrons would be the opposite.

So you would have these electrons come here, maybe I can draw it again, so that it is easier for you to see. I am just going to write it as OR. So now in this case what is happening is that so you have protonation here. So you can imagine this coming here and this bond leaving to give you intermediate 2. Now what is very interesting about intermediate 2 is that if you try to write the elements of symmetry,

so can you try to identify if there are any elements of symmetry present in intermediate 2? So if you are not able to see it, there is a mirror plane here. So there is a mirror plane here, so it is a sigma plane. So because of this mirror plane both of these OH groups are identical right? The molecule is identical both of these OH groups are identical. So now in the next part of the mechanism what is happening? You have your methanol adding in here.

Once the methanol adds in you can again think of a protonation here and either of these OH groups, so this could be a or b can add in to give you again the same products. So you will not be able to distinguish the products based on this model substrates, because essentially you are getting the same product. So how do you know whether it is endocyclic or exocyclic? So what you would see is to distinguish between these 2 what was done was this was a clever trick.

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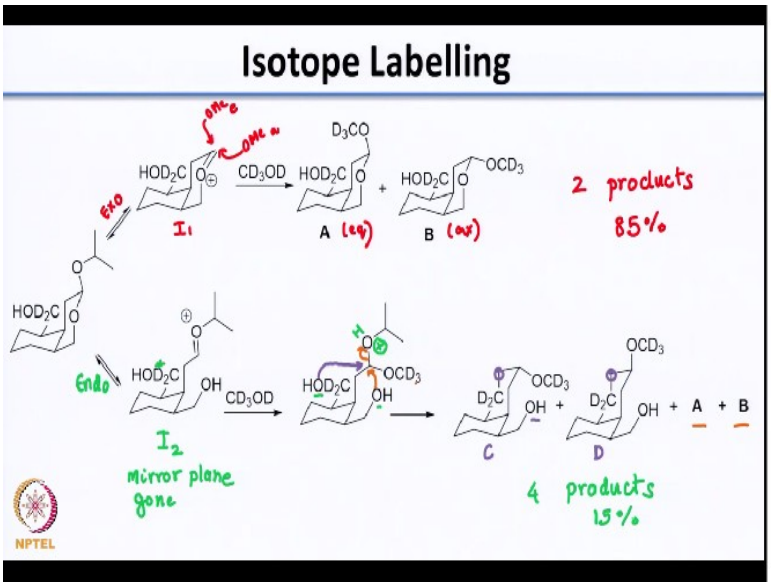

This position was isotopically labelled. So you have CD_2OH. So now the mechanisms are similar I am not going to push the arrows. So this is your exo, this is your endo and in the case of exocyclic you have intermediate 1. Again, intermediate 1 can lead to two products either the equatorial one which will come from the equatorial side, so that would be the equatorial or you can have it come from this side which would be the OMe which is axial.

So you get product A and B. So A comes from the equatorial attack and B comes from the axial attack. Now when you look at the endocyclic pathway, with the endocyclic pathway what has

happened is now you have generated intermediate I_2 and the mirror plane that we had earlier has now disappeared because one side you have CD_2OH and the other side you have CH_2OH.

So introduction of this CD_2OH has destroyed the mirror plane. So the mirror plane is gone. So the next step again is like before, where you add the OCD_3 and then you get this second structure. Now once you get the second structure now both these OHs are not identical. Alright? So if you have this OH coming back in, so now you have these 2 OHs which are different. So let us look at what happens in the next step because they are different.

So you are protonating this. Now once you protonate, so you have your leaving group here these 2 OHs are different. So I am going to use color to make it easier for you to understand as to what is going on. So if this OH group reacts here and then you have this leaving what you will essentially get is product A and product B. So it can either be equatorial, the new OCD_3 can either be equatorial or axial but it would be identical. Okay?

So you would get these 2 and you would have CD_2OH lying untouched. Now what happens if the CD_2OH interacts? So here what will happen is, let us use a different color, say purple, now when this interacts here, you have a new ring being formed and the OH will remain as it is because now it is not interacting so it will remain in the background as it is. The CD_2OH has interacted to form, an oxygen is missing here.

so there will be an oxygen here, there will be an oxygen here. So it forms these 2 products let us call them C and D as well and these 2 products can be distinguished from A and B because you have this label of deuterium. So in the earlier cases C and D and A and B were identical because all of them when hydrogen. But in this case because you have the deuterium labelling you have C and D which are different from A and B.

So the endocyclic pathway gives you 4 products, whereas the exocyclic pathway gives you only 2 products. So using this model system and analyzing the ratio of the products you can figure out whether it is the exocyclic pathway or the endocyclic pathway and based on this analysis what

was found was that it goes predominantly by the exocyclic pathway almost 85% of the reaction and only 15% of it goes by the endocyclic pathway.

So you can do the analysis of the 4 products formed and what was seen was probably that the proportion of product C and D would have been very less in the reaction mixture. So they could say that once the reaction was done since C and D were very less it went mainly by the exocyclic pathway. So this is how isotope labelling can be used very beautifully to illustrate or show what the reaction mechanism could be even in biology.

So all the examples we have seen so far whenever we have tried to understand biology is we have chosen model systems which could be replicated in lab where the model systems are a good indication of how the biological system will work. If you choose a bad model system then you cannot extrapolate and say that oh even in biology it will probably be working like this. So, so far we have looked at several methods of using isotopes either you can study the kinetics or you can attach a label which will tell you what is the mechanism of the reaction.

Now let us look at some other methods that could be used to determine what is the nature of the intermediate. Intermediates are highly reactive, so if you want to determine the nature of the reagents you cannot just easily be able to isolate an intermediate and say this is the intermediate because you cannot, they would not stay in solution that long, we were studying kinetics at that point also I told you some have very very small lifetimes.

So unless there is some way to either stabilize the intermediate you will not be able to see it. So a trick that people use is they trap the intermediate so that you can see what the intermediate looks like. So you add what is called as a trapping agent which will trap the intermediate so that you can see what it looks like. So points to consider while choosing a trap. So obviously a trap would be something that would react with the intermediate.

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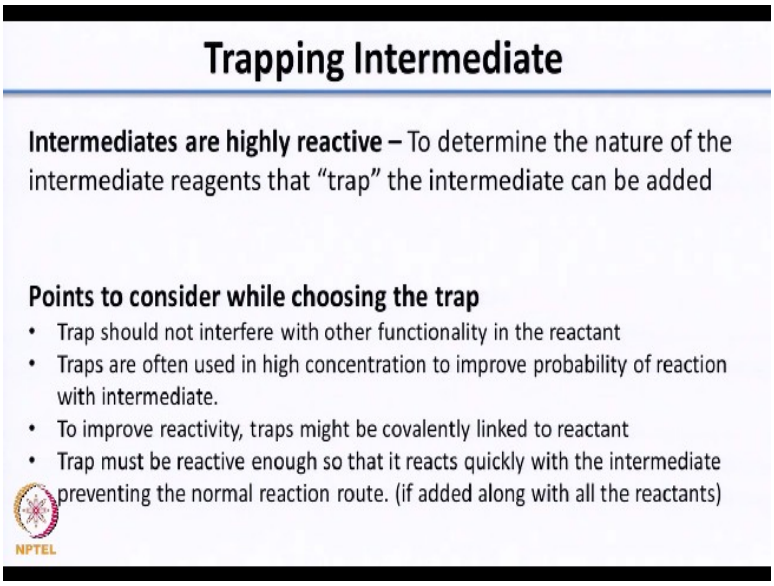

So it should have an affinity with the intermediate, but then the trap should not interfere with other functionality in the reactants. So you cannot have a trap which would be reacting with other parts of the reactant because that will again not give you good information. The other thing is it might have to be used in higher concentration because you are trying to improve probability of reaction of the trap with the intermediate.

See you have your reaction going once the intermediate is formed it would like to do whatever it does with your reaction mixture. So it might be reacting with another molecule but your trap has to catch the intermediate before it reacts with the other molecule. So the trap has to be quite reactive at the same time to improve the reactivity or to improve the probability of reaction what is done is you increase the concentration of the trap.

So if you increase the concentration of the trap once the intermediate is formed there is a greater chance that it will meet the trap and not the reactant. A simple analogy, I told you a lot of times you can think of molecules to behave just like us. So suppose you are going to a meeting right and in the meeting you have several people who are there. Alright? Now suppose now let us say this meeting is a reunion of your school.

So you are going back to your school reunion. Alright? and usually if you have a school reunion you will be trying to meet all possible friends from your batch because you want to interact with

them. Now if the reunion is open to all batches it will be very difficult for you to spot someone from your batch and make a handshake and say it is a meeting of 1000 people. So you will have to keep going around and looking for your batch mate.

Whereas suppose your school made an announcement that we are having this reunion specifically for your batch, say the batch of 2012 or 2015 he is saying this is a special reunion for your batch and it is also open to other people. So now when you come to the meeting there is a greater chance for you to find your batch mates because you have a greater number of your batch mates within the same meeting.

So it is the same thing with an intermediate and a trap. If you have many traps around the intermediate there is a greater chance for the trap to form a bond with the intermediate and the intermediate will not react with anything else in your flask. Another trick that people use is they covalently linked the trap to your reactant. So covalently linking the trap means what? As soon as the intermediate is formed since the trap in the neighborhood it will come and react with your intermediate.

So by covalently linking what are you doing? Your intermediate is form and your trap will come and directly trap it. So there is no chance for anyone from far away coming and trying to interact with your intermediate. So this is another trick and again if the trap is not super reactive, so if you are a trap, again in your class reunion and if you are just sitting in one corner of the room there is no chance that you would meet all your batch mates because you are very unreactive

and sitting in one corner of the room. Same thing in a react, so the trap has to be reactive that once the intermediate is formed the trap will actually interact with it, it cannot be something which will just be so dormant or so lazy that you do not get your trapped intermediate. So having these concepts in mind, in the next class we will look at examples of how you can trap different intermediates.

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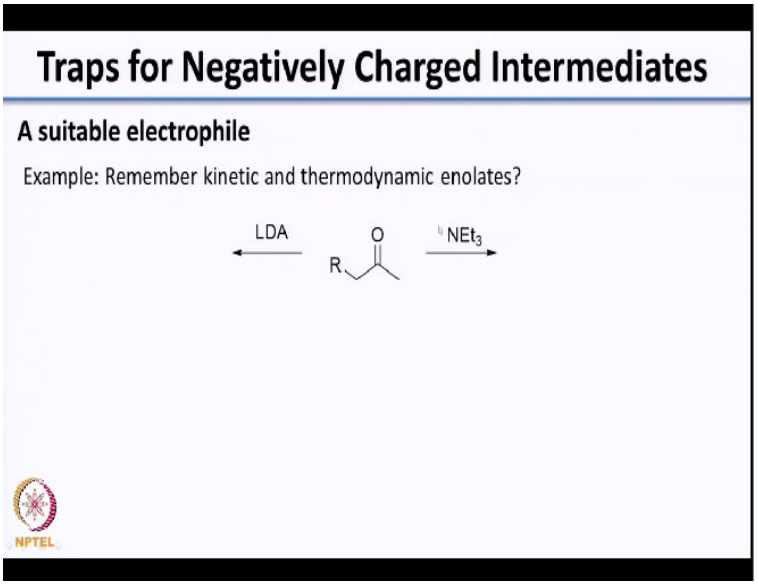

But before going as usual I will leave you with a question to think about remember we had done kinetic and thermodynamic enolates. So in the next class we will start with traps for negatively charged intermediates and what I want you to think of is, I have shown you this reaction on the screen, actually you need not think of it you have already studied it. So I am sure you will be able to write the answer.

So I want you to write the structure of enolate that is formed based on the reagents given. So in one case the reagent is LDA which is a base, the other reagent is triethylamine which is also a base. So I want you to write the structure of both of these enolates and the other hint I will give you is this reaction is done at low temperature. So with this in mind please write the structure of the enolates and I will see you in the next lecture. Thank you.