One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis
Prof. N. Suryaprakash

CSIR Emeritus Scientist, Solid State and Structural Chemistry Unit
Indian Institute of Science – Bengaluru

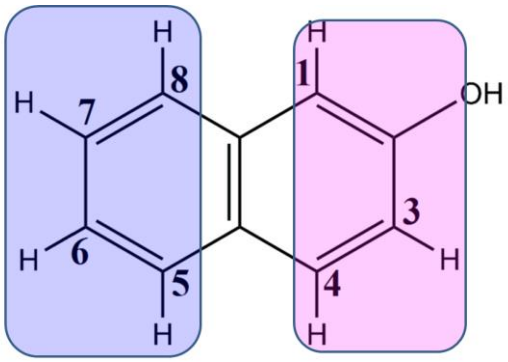
Lecture 58: 1D NOE, 1D TOCSY

Welcome all of you. In the last class, we started discussing about the steady state NOE experiment, a one dimensional NOE experiment, where you can acquire the data very fast because one dimensional is faster than two dimensional experiment, and you do not have to spend much time. Especially, if your molecules are not too big, small molecules and you want to get specific information, you do not need to spend time, large amount of time by doing a two dimensional experiment. On the other hand, if the molecules are simple and if you know the assignment, you can selectively irradiate a particular peak, saturate a particular peak and then get the NOE information by using difference NOE, steady state NOE. In fact, this is what I showed, some examples, how we can use it in a simple molecules, small molecules especially for the assignment of the particular substitution present in them like this thing. So, few examples we took last time.

In fact, in the case of pyridine substitution, where the para positions are R and R prime, one is the ethyl group, other is the methyl group, which is ethyl group, which is methyl group, we wanted to find out, we could do that. And then we took some isomers of molecule like thujone, alpha and beta isomers. We know whether it is endo or exo, if we know one of the protons which is endo, we hit it. I know which proton is close to it in the alpha isomer, which proton is close in spatial proximity to the H6 endo, then it is a beta isomer. I know which is the alpha isomer and which is beta isomer based on this close spatial proximity. And that NOE gets enhanced when I hit H6 endo. We saw that you are able to distinguish two isomers, alpha and beta thujones. And substituted naphthalene ring also we observed. And we see how we can do in the case of naphthalene substituted ring, one was CH2Cl, other was CH3. Based on the NOE enhancement on the same phenyl ring or a different phenyl ring, along with NOE for a CH3 group present, we could identify, pinpoint and say the both substituents are ortho to each other. Not only that they are on the same side of the one of the phenyl rings, and where it is we could easily fix it. So, two possible isomers were there we could identify them.

Like this varieties of isomers possibilities we could find out and we can rule out what is right, what is not right and get only particular structure. We did that even for one of the isomers, we had six possible isomers in the fluorine substituted molecule and then we wanted to know which is the correct isomer. Then by NOE, we could eliminate few of the

structures and finally, pinpoint and say only possible structure is this, because of the correlation we could see. So, like that we saw several examples. In this class, I will take another one or two examples of that 1D NOE, where bit more important things to especially for the assignment purpose of the peaks also. And also we took example for assignment of a particular peak in a five membered ring. But we will show in this naphthalene substituted molecule, how we can remove the ambiguity of the assignment.

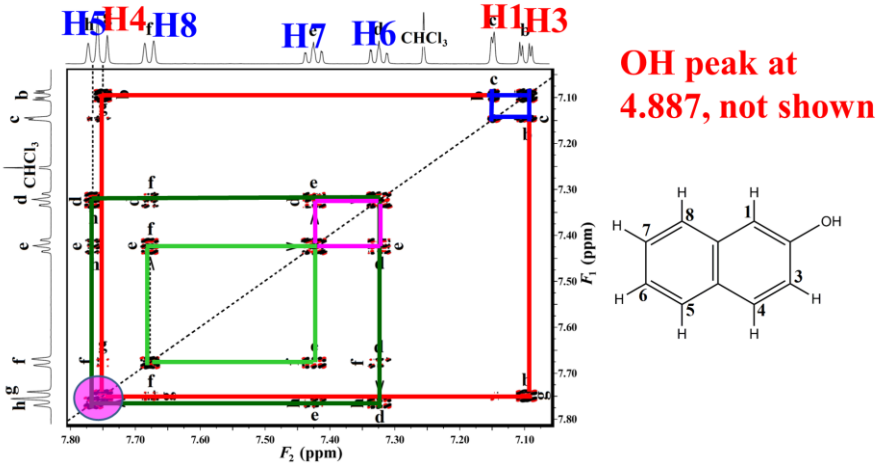


Two Different Spin Systems

Spin System 2: H8-H7-H6-H5

Spin System 1: H1-H3-H4

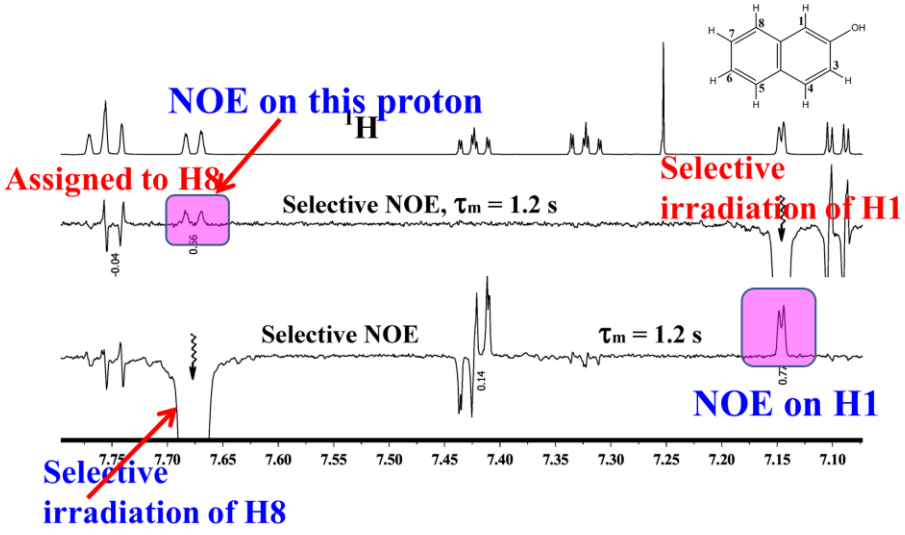
For example, I will start with this molecule here. It is you say 2 naphthalin, OH is here. Now, in this molecule there are two spin systems, one with 3 protons here H1, H3, H4, other is with 4 protons H5, H6, H7 and H8, there are two spin systems. And of course, 1D spectrum I am not going to show that. I know you people will be knowing by now. Otherwise, also you can see from the projection in this 2D spectrum.



OH peak at 4.887, not shown

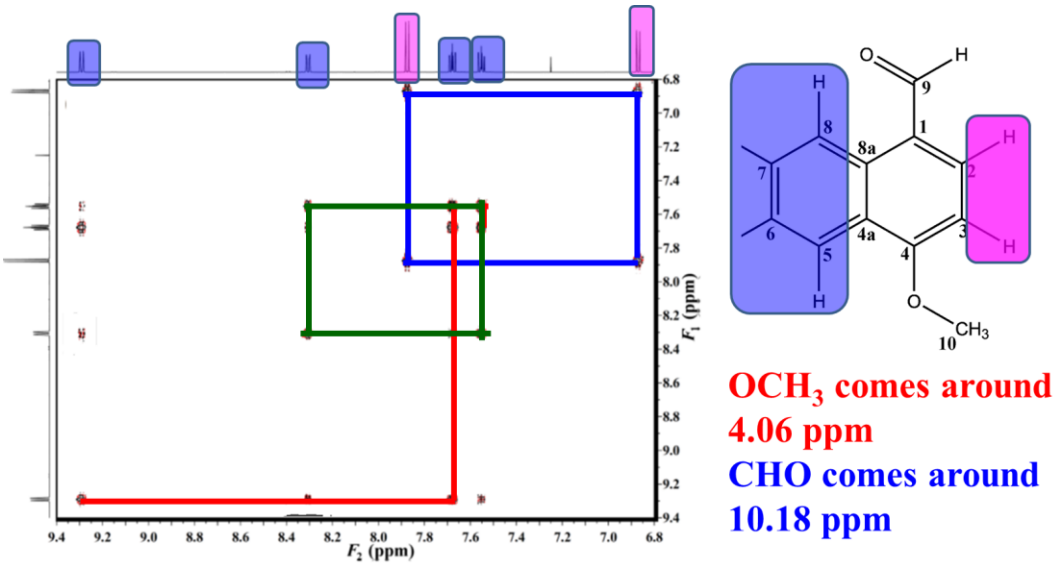
The OH peak is not shown, and this is H1 proton, I will say. This proton experience only one meta coupling and one para coupling, that is all. It is a very small splitting, meta coupling is of the order 1 to 1.5 Hz, and the para coupling is 0.5 Hz. If it is not resolved,

you get only a doublet, small doublet. This is I would say possibly H1. And then start with that and then next is B, of course, next is H3, H3 is this one, why it is H3? I will say this is H3 because this experiences one ortho coupling and one meta coupling, this is doublet of doublet. I can identify this one. And you go further, you can complete this square and you can identify other proton also, that is H4. So, all the 3 protons we can assign. And of course, there is a overlap here, this H4 has to be a doublet and para coupling if it is not seen, you can see here it is as a doublet. Of course, one of the components of the doublet is overlapped there. As a consequence, you will not see clear doublet, but it looks like a triplet, but other doublet is from the different proton. So, this how we can assign all the 3 protons of this phenyl group. Of course, remaining other protons are here, we can start assigning from there. See easily, we can start going, I would say one of the protons is here, 5, looking at the multiplicity pattern, it is a doublet, this is large doublet that too, this could be an ortho proton coupling, and other meta coupling is partially resolved. With that idea I would say this is H5, I do not know it could be H8 also. I will start assuming that it is H5, then complete this square and I will say correlated peak, this is H6, complete this square, then I will say H7. From H6, then I will go further, and I will complete this square, I will make the complete assignment, and this is H8. OK. Now, I could make the assignment based on the cross correlated peaks from the COSY spectrum, very easily I could see that here. I assumed that one is H5, what guaranteed this is H5, why not H8? Why that H8 also should have a similar pattern. Similarly, H7, H6 should have a identical pattern, even in this phenyl ring. Interestingly this will also be a doublet of doublet of doublet, identical pattern here also. And these two will also have identical pattern. Now, if I started with this assumption as H5, I am not sure whether H5 or H8, so there i an ambiguity here, spin-system assignment could be H6, H8, H7, H5 or alternately H5, H, H7 and H6, that is also possible. So, in which case how to resolve the ambiguity, we can resort to selective NOE experiment here, that is the steady state NOE experiment. We can do that.

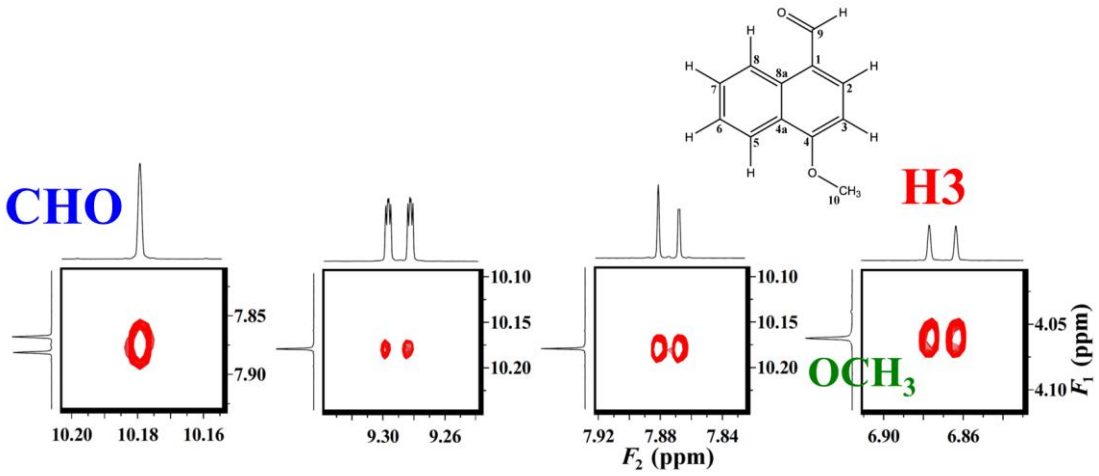


For example, C8-H8 bond if you consider it is parallel to this bond C1-H1. So, if I irradiate this, there is a possibility you will get the NOE here, that is a possibility. And similarly if you consider H4, H4 is close to H5. Of course these two are far away from H8. If I hit this, you cannot get the NOE for this, if I hit this you cannot get NOE for this, H4 is close to H5, and not to H8. This is basically looking at the structure you know. So, with this idea we will start doing the selective NOE. The selective NOE. What we will do, one of the peak I know, for in this ring I have already assigned. Let us say I am hitting this proton, selective irradiation of proton H1, this one. If I hit that proton, where do you expect the NOE? This is a one dimensional spectrum, conventional spectrum. When you hit that proton and take the difference, you see there is some enhancement here, on this proton. What is that proton? If I hit this one, if the enhancement is here, it cannot be this proton, because from the multiplicity pattern I know it is a doublet. So, it cannot be this one, this cannot be H5. This is because H1 when I selectively irradiate, I am getting enhancement here, it must be H8, enhancement on a phenyl proton that must be H8. So, I can assign that easily, assignment problem is solved, ambiguity is resolved. I do not know whether I have to start with this as H5 or H8. Now, I know it is H8. And to confirm that I will irradiate H8, then where do you get the enhancement you will see, on the this one H1 and other ring, H1 proton of the other phenyl ring. It clearly confirms this H8. But also there is enhancement for the neighboring proton from H8 this one, then you know what is this one this is H7, assignment problem became simple. You can now rule out the ambiguity. That is what we do and then selective NOE experiment confirms assignment. What is the assignment. What was confusing, the ambiguity was there, now, I can say this is the possibility H5, H8, H7, H6. You go systematically from the left to the right in the particular frequency order. Then I will know this proton is here, this is next here, next in the increasing frequency or decreasing delta, high field.

We can go for the conformation of another molecule. You understood now how we can utilize the selective NOE to make the unambiguous assignment of protons in the molecule containing phenyl rings. We can go for the next one, the conformation of 4-Methoxy-1-Naphthaldehyde in CDCl3. Here we use COSY for identifying the peaks, similar peak. Here OCH3 is here, CHO is here. Again two protons which are ortho to each other. Of course, we analyzed this molecule, remember we analyzed the protons spectrum. This is a identifiable spin system. This has to be from this group. Then obviously thee are protons 2 and 3. Then remaining 4 protons are here. This is doublet of a doublet, doublet of a doublets and two triplets like patterns. The two triplet like pattern has to come from here and other 2 from this. So, assignment can be made by using COSY. Of course, OCH3 is there, CHO is there. They are not shown. They are far away. That is not important right now. And there are two possible ways of assigning the doublet, triplet, triplet, doublet. This could be H8 or H5 or this could be H7 or H6.

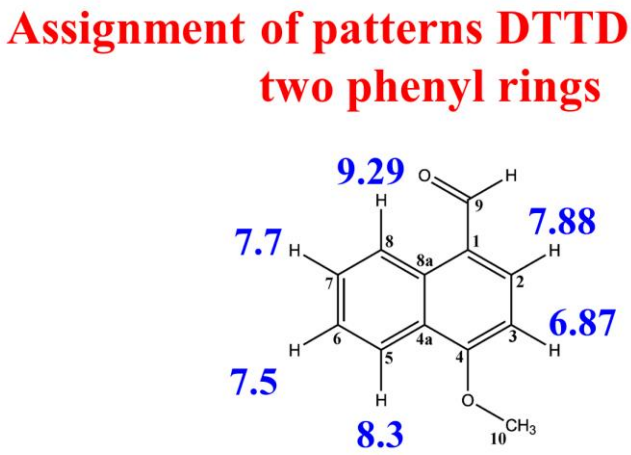


Similar to what we saw in the previous example. How do we do that? We can resort to 1D or also do a 2D. This is a simple molecule. There is no need of a 2D, but still if you do the 2D what we see is here.

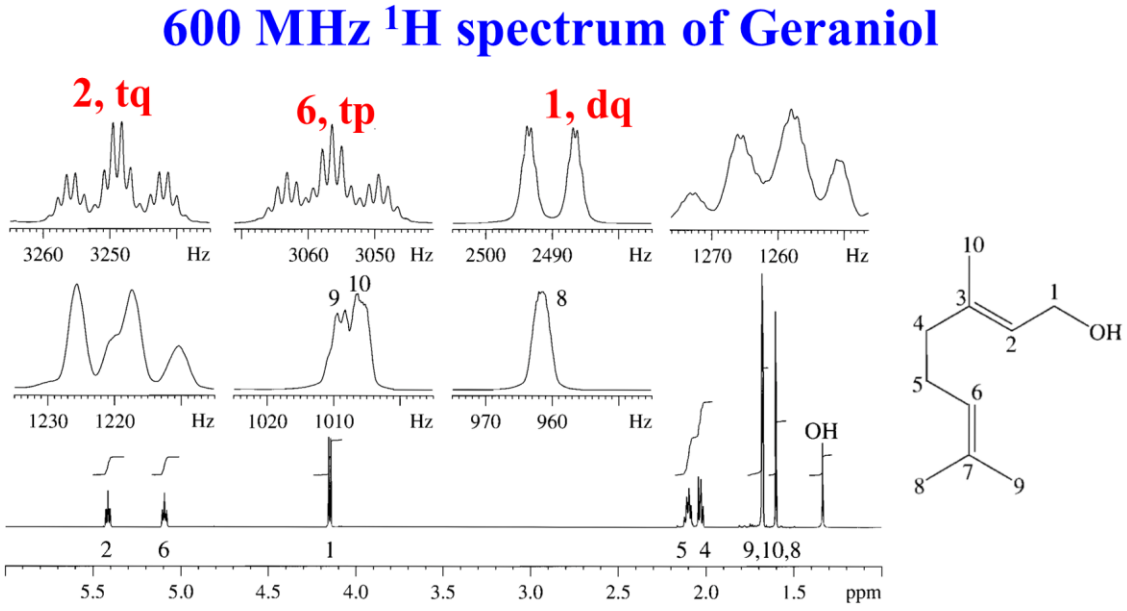


We are going to see OCH3 which gives a cross peak to proton doublet at 6.87. This one, this has to be CH3. OCH3 is giving this doublet. This has to be H3. We can make the assignment for peak coming at 6.87 ppm for H3 proton. Further, you can see CHO shows correlation to proton at 7.88 ppm here. This CHO is here. This is giving correlation to proton at 7.8 ppm. This is a doublet. We know doublet of a doublet. This has to be H2. So, H2 and H3 we can start making the assignment already. And of course, CHO also shows correlation to proton doublet, another one at 9.2 ppm. And this is assigned to H8 because this is the only possible, which is in close spatial proximity. It cannot be anything else. So, we can assign this peak which is coming here as H8. Then you can rule

out the possibilities. This ambiguity will not be there now. So, we can make the assignment very clearly like this, which proton is which.

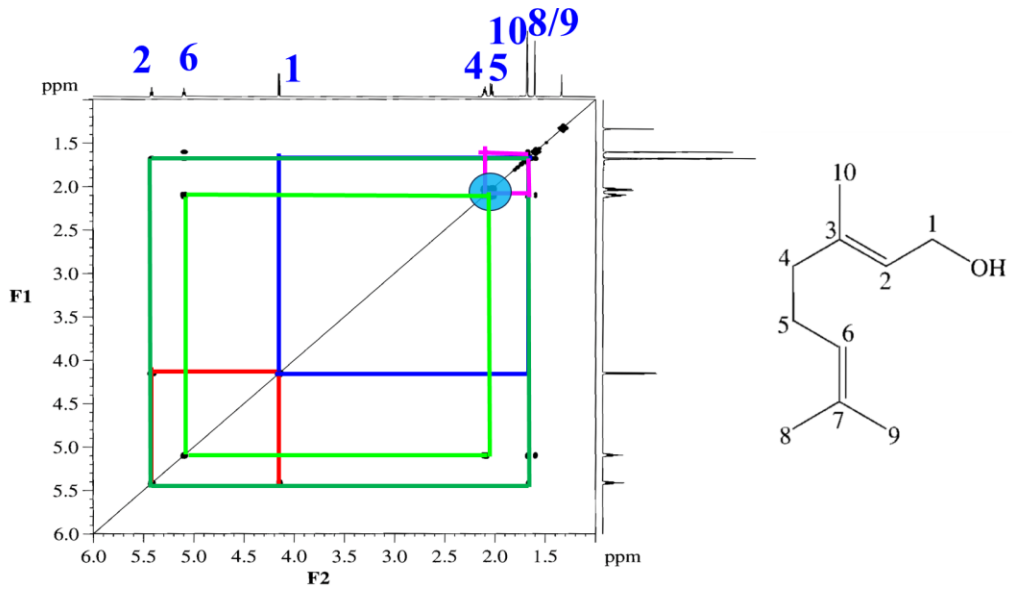


Another interesting part is this one. Look at this molecule. This is a molecule called geroniol. It is a 1D spectrum. They are all expanded here. For example, if you look at this proton, this one, this is a doublet because of this and a quartet because of a long range coupling, doublet of a quartets. Similarly, all these things can be assigned.

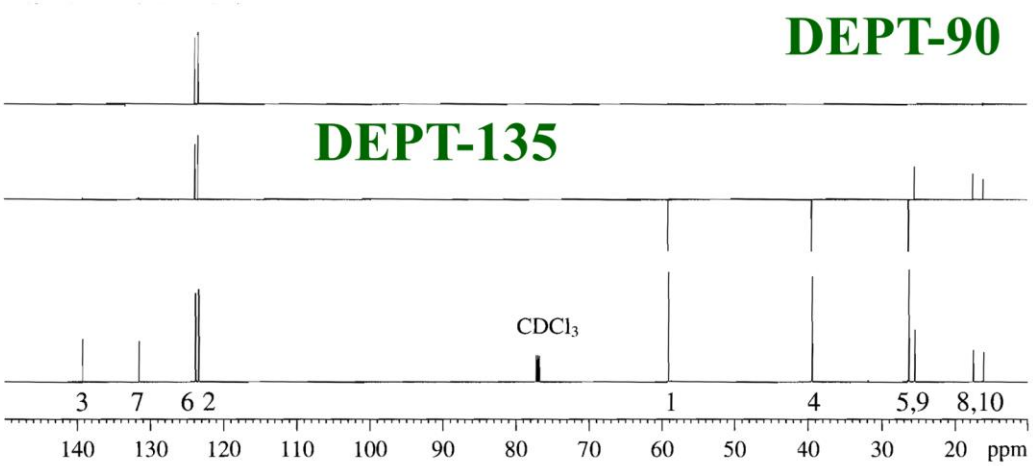


Now, the question for us is what is the conformation of this geraniol? What is the conformation of this molecule? For that, we can make the assignment using the COSY. All the peaks have been assigned. There is no question. Everything has been clearly assigned. Systematically, we know which is 1, and then I showed you it should be a

doublet and a quartet. From that, we started assigning for all the peaks. Everything can be clearly assigned here using the COSY.

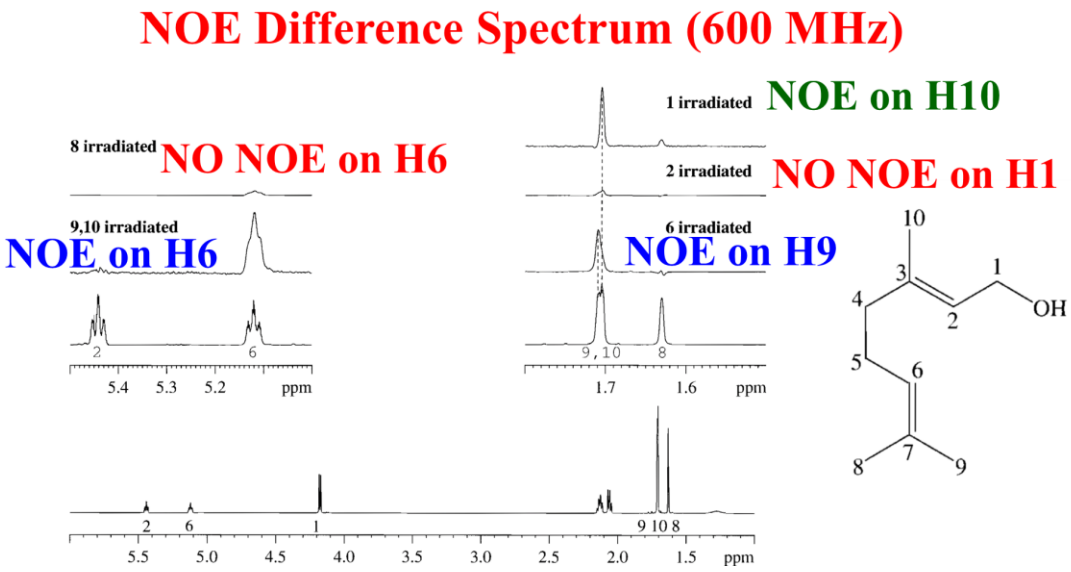


Start with 1. I told you. 1 is very clear. unambiguously, I know that. From 1, you can go to 2. From 2, you can go to other things. Very easily, from 1, you came to 2. Then, we can go to 10. Then, from 10, you can go to 4 and then, also you can go to other things very easily. 4 to 5, 5 to 6, 6 to 7 like that. Of course, we can also identify how many protons are there, how many CH3s are there, CH2 and CH are there by using DEPT-90 and DEPT-135.



This experiment confirms there are 3 CH3s. Remember the DEPT experiment, I told you. It confirmed there are 3 CH3s and 2 CHs are here and CH2s are negative 3 CH2s and 2 CHs. Of course, there are 2 quaternaries which have no effect. So, you can see them from

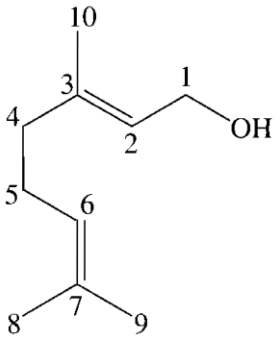
the normal spectrum. You can identify the types of protons CH3, CH2 because this is a simple molecule. We can make all the assignments of protonated carbons and distinguish correct chemical shift of proton 8 and 9, everything by using INADEQUATE. Assume that I have done everything. That is not important. Assignment can be done. I have already told you how to do that by combining varieties of NMR experiments, 1D, 2D, COSY, HMQC, HMBC, etc. Quickly I went through, and assuming that you know how to do the assignment using COSY, HSQC, HMBC. Of course, methyl edited HSQC will identify the CH2 protons and then DEP135 also you can do, And I could identify all the protonated carbons, odd protonated and then even protonated carbons, everything. Now, my question is what is real conformation of the molecule? How do you find out the real conformation of this molecule? First, we can do selective NOE for this molecule.



How do you do that? We will irradiate this proton, I am confident of that, because I knew where it comes. I will irradiate that proton and then there is NOE on proton 10. This is proton 10. If you irradiate this, there is a NOE here. And to confirm that you irradiate 2. When you irradiate 2 here, there is no NOE on H1. They are not on the same side. That is the conclusion. If they were on the same side, irradiation of 2 would have given the NOE for this. When I irradiate 2, there is no NOE on H1, whereas when I irradiate H1, there is NOE on proton 10, that means H10 and this one are on the same side. That is fine. Go to the next one, irradiated 8 here. There is no NOE on this proton 6. There is single proton here. There is no NOE on proton 6. On the other hand irradiate 9 or 10 because they are close by, both you have to irradiate simultaneously. Then you are going to see NOE and proton 6. That is interesting. Here if you irradiate, there is no NOE on this, but if you irradiate this, you have a NOE here. What does it mean? Proton 6 and 9 are on the same side of the double bond. To can confirm that you irradiate H6, you get the NOE on the

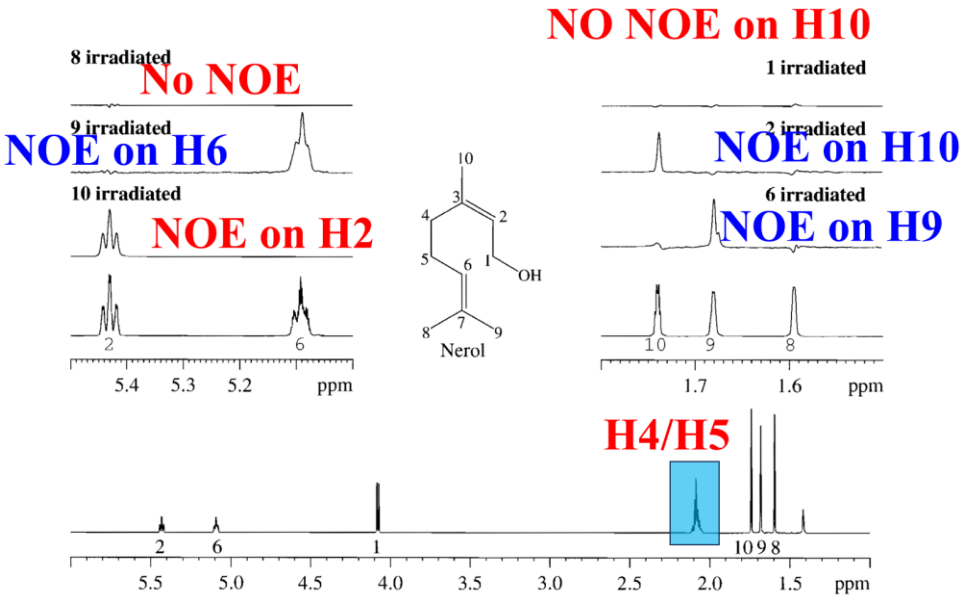
proton 9. That shows the proton, H6 and H9 are cis to each other. They are on the same side of the double bond. So, with the NOE difference on this geraniol molecule, what is the conclusion we can draw? NOE on H10 when H1 is irradiated There is no NOE or some weak weight you can see on H1 when H2 is irradiated. That confirms H10 and H2 are on the same side. Another thing there is NOE on H6 when H8 is irradiated and NOE on H6 when this is irradiated H9, NOE on H9, of course when H6 is irradiated. This confirms H6 and H9 are cis to each other and H8 and H6 are trans to each other. You can get the geometry of this molecule. The correct conformation of this geraniol from NOE experiment,difference NOE experiment is this. This is because these two are trans to each other, these two are cis to each other and this and these are on the same side of the same side of the double bond.

T



here is isomer of this molecule interestingly that is called Nerol. Other one is called geroniol, this is Nerol. See this is the isomer of this. What is the structure of this one?

Can we get the NOE without going into assignments and everything quickly? Same I will irradiate proton 1 here.

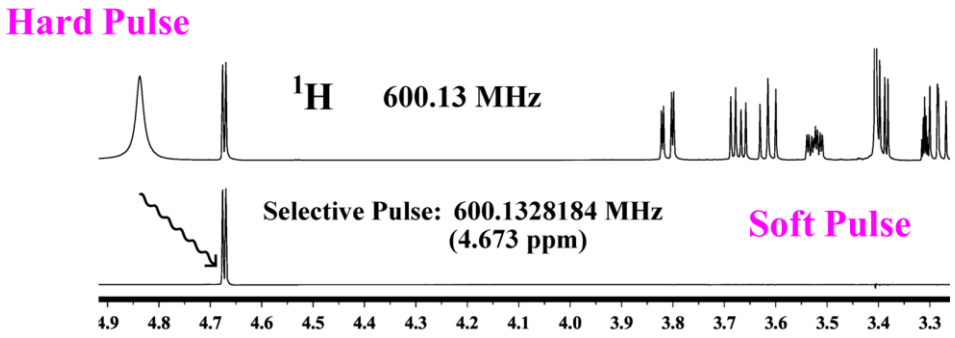


No NOE on H10 interestingly. Remember in the previous geroniol, there when we irradiated H1 was there was NOE on 10, but here there is no NOE, on the other isomer of this molecule. Irradiate 2, there is NOE on H10, very good NOE you see, on H10, there is NOE. What does it confirm? This H2 and H10 are on the same side of the double bond, that confirms. Come here, irradiate proton 9, there is NOE on H6, this one. And irradiate H6, there is NOE on H9 the converse is also true. That means H6 and H9 are on the same side of the double bond. If they are trans to each other like we saw in previous many examples there would not have been any NOE. So, that means these two are on the same side of the double bond. And at the same time, if I irradiate proton 10 there is no NOE on proton 2. That is another interesting thing. If I irradiate here, there is no NOE here. So, from this what you can conclude from the NOE difference spectrum, there is NOE on H10 when H2 is irradiated there is NOE on H2 when H10 is irradiated. It confirms they are cis to each other, H10 and H2, proton 2 and proton 10 are cis to each other. The other NOE you see, NOE on H10 when H6 is irradiated, NOE on H6 when H10 is irradiated, they are cis to each other, again. So, it confirms H6 and H9 are cis to each other and H8 and H6 are trans to each other. Two isomers of the same molecule, how we can get the structural information very easily just by doing 1D difference NOE.

You got the point now, how we could use 1D difference NOE. I think we have given lot of examples, lot of things we have discussed. There is no point in discussing further with these things, continuing with only steady state NOE experiments. I have given N number of examples as how to make the assignments based on the different substituents in the phenyl group, regiospecificity and cis-trans conformation. All those things can be done by a selective experiment. Only thing you have to be very careful about frequency selective excitation. For simple molecules you can do. You cannot do this experiment and a big protein. It is impossible to do that without disturbing the neighboring peaks because spectrum will be very complex. In such cases, you have to do transient NOE, by doing 2D NOESY. I am showing this in simple molecules, if it is a simple organic molecule you are working with, you do not have to spend time, you can do the data acquisition faster by doing steady state NOE experiments. With this, I am going to stop with this NOE.

I will go to a new topic now. That is another one-dimensional experiment and we will discuss that. That is called one-dimensional TOCSY experiment. Identical experiment similar to, remember we discussed TOCSY experiment, 2D TOCSY. What would we do? Similar to COSY, we apply 90 degree pulse and then mixing pulse in the transverse plane. What is going to happen there? Chemical shifts are refocused, J couplings are present and the spins lose their identity. There will not be any chemical shifts. When there is a Hartmann Hahn matching condition, I told you there is a transfer of magnetization among all the coupled spins. That is a TOCSY experiment. And I showed

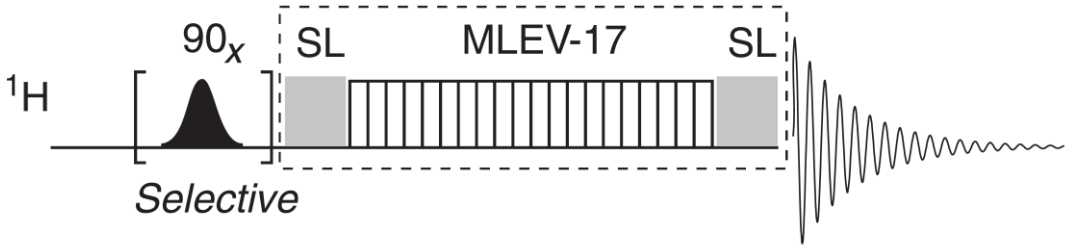
you also, in TOCSY experiment what happens? It depends upon the mixing time and then the magnetization will traverse to all through the coupled spin system one after the other. So, it depends upon the mixing time, mixing period and also strength of the coupling. Both are important. If the coupling strength is larger, you will have a stronger peak and if this proton is very far away, very weak, but still forms a part of the coupled spin system, then also the cross peak intensity will be little less. So, this information is very important. You can do this thing. The TOCSY cross peaks depends upon the mixing time and also the strength of the coupling. That we can do for a big molecule, again a twodimensional experiment. Very simple molecule if you have, why do you have to do the 2D experiment? Can you not do this data acquisition faster by doing a one dimensional experiment, a selective experiment? We can do that. I will show you that. Now, what do you do in a selective excitation and a non-selective excitation? I think we already know that.



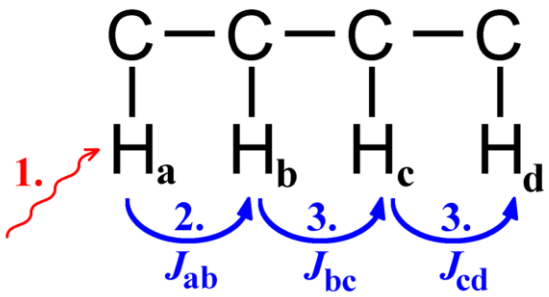
If you do not know, the selective excitation means you can apply RF pulse, so that only particular peak is excited, that is detected. All other peaks are suppressed, that is the beauty of NMR. See, this is the called application of a soft pulse. Soft pulse means, selectively you can apply pulse, so that only particular peak is excited. All other are suppressed. This is called selective excitation. But this is non-selective excitation. We can do this by applying a hard pulse, 90 degree pulse. All the, spins, all the protons which are in the entire range of chemical shifts get excited. That is a hard pulse. Non-selective excitation. This is non-selective, this is selective. Selective is always done by soft pulse. This is what you should remember that.

With this, we can do TOCSY in a different way. What does one-dimensional selective TOCSY does? Remember in the TOCSY experiment, we are going to apply hard pulse and then we have a mixing and then vary t1 and then collect the signal. That is okay. But here, we do not want to apply hard pulse. We apply a selective pulse. And a pulse which is shown with a shape like this means, we are applying a selective pulse on a particular peak. It could be frequency selective or band selective. You can select one bunch of

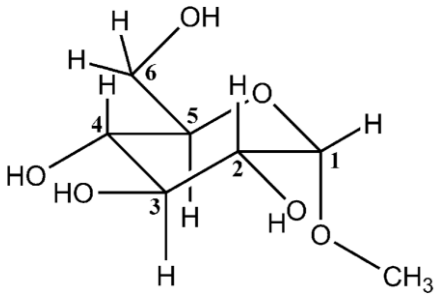
frequencies or a single frequency, does not matter.This is called selective pulse. Whereas, if you write a pulse this in any pulse sequence, it is a hard pulse. Please remember that.



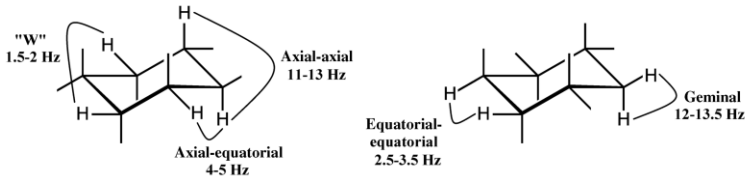
So, we can apply a selective 90 pulse here and then afterwards, do the isotropic mixing, start collecting the signal. Very simple experiment. If you do this experiment, you can do it faster. For example, I will apply a selective pulse to excite one of the peaks in spectrum. Then I apply a mixing pulse and transfer of magnetization of this proton to other proton that are J coupled takes place, as I told you depending upon the duration of the mixing and the strength of the coupling. This is what is going to happen. Transfer is very efficient to the protons that have large J couplings. I select a particular proton, let us say A, it has a large J coupling with B, more than 10, 15, Hz. Let us say 15 Hz. Then transfer for this is more. If it is coupled to other one, let us say with J coupling of 1 Hz, transfer to this is not efficient. Transfer to the other proton, magnetization transfer is efficient only if the coupling is large with the selected proton. That is what we should know. Alright. I mean the least efficient of the proton is that has a small J coupling. And if they are not part of the coupled spin system, there is no magnetization transfer. This also we know. That already in the 2D talks also we discussed. The magnetization transfer takes place among the protons that forms the part of the coupled spin system. If they are not coupled among themselves, there is no transfer of magnetization. We saw that, I showed you the 4 x 100 meter relay example where particular spin system only can get the magnetization transfer like batons getting transferred from only for a particular team members, not for other teams of the different countries. We saw that in one of the slides. So, intensity of the peaks also depends upon the number of transfers and the efficiency of each transfer. That depends upon magnitude of J. Sequential transfer of magnetization among coupled spins goes like this.



Let us say I irradiated proton A. Selectively, I excite this proton. Then, let us say there is a J coupling between this and this. There is a transfer of magnetization between this and this. Then, this is coupled to this. There is a transfer of magnetization. This is coupled to this. So, sequentially it starts going one after the other. Supposing, I give very small time and do not give enough time for the spins to propagate, that is the magnetization to propagate. It will not propagate completely. Then, it will restrict only to this. Give more time, it will propagate to this. Give further time, it will propagate to this, if they are among the coupled spin system. So, that is also very important. This is how the polarization transfer takes place. So, we will see how we can do the selective TOCSY on a particular molecule. This is a molecule, methyl alpha glucoside.

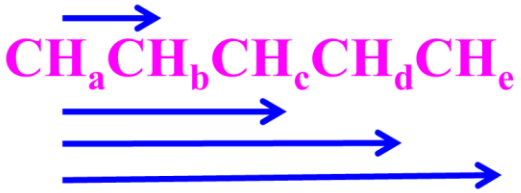


We want to see can we use one dimensional TOCSY selectively and then, selectively excite them and then, keep on doing the isotropic mixing with a different time. See, how the magnetization gets transferred. Systematically, we can start the assignment. We can do that and before that, I want to give you one idea.



Please remember, always in such type of molecules like this, sugar molecules and others, glucose, axial-axial coupling is larger always. Let us say, 11 to 15 or 14 Hz like that. Axial-equatorial couopling is next, that is 4 to 5 Hz and equatorial-equatorial coupling is small. This information is needed because sometimes, if you want to identify, let us say, protons here, both are like they have a geminal relationship. If you want to identify which is which, then coupling information we can utilize, because if there are axial-axial relationship, then you will see J coupling is larger and from the separation you can assign that. So, this information just to tell you how we can use. But since it is a glucoside molecule which I have taken, methyl glucoside here, you know, methyl alpha glucoside

molecule. These sugars generally are unbranched carbon chains. They are not branched. They assume they are all linear chain and most carbons are CH2s generally. So, you can assume them to be linear spin system, because they are not branched. If you remember this structure of the sugar, they would have seen C-C-C-C, would have written OH - H, H-OH like that. That is a structure of the sugar which we know how we used to learn in our college days. So, you can consider it as a linear spin system unbranched. In such spin system, TOCSY mixing is like a diffusion process. That means, if I start with this, it will come here, it will come here, it will come like that. It diffuses one after the other like a diffusion process. How it happen? Diffusion goes slowly from one place to another place like that. This is what happens.

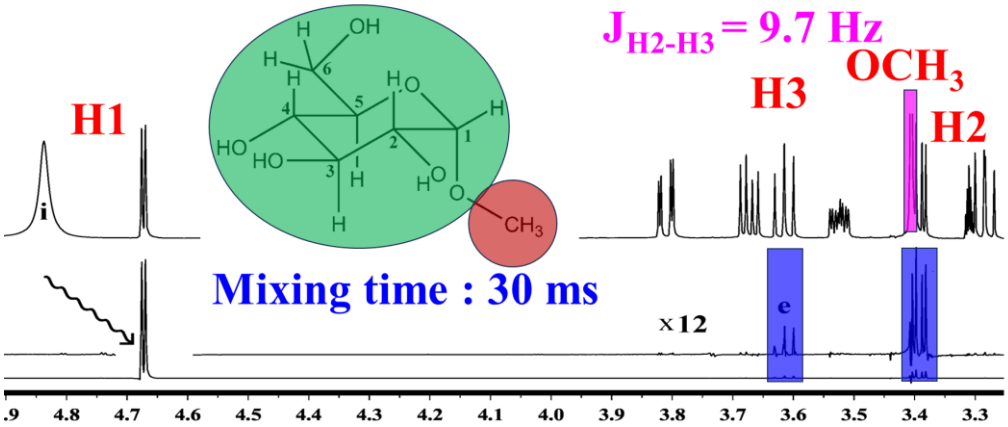


If I start a TOCSY in a sugar molecule like this, a linear chain. Let us say I am hitting proton A. I am selectively exciting proton A. Then magnetization will start with A, it immediately gives to B for a particular time if I give mixing time, It gives its magnetization to immediate couple neighbor B. I increase little more time. It goes to C, it goes like this. Then it from A to C and then give more time A to B, A to C and A to D and if you give enormous time, it can go up to E. But if you give beyond that what will happen? Remember in the TOCSY, I was telling you it is a cyclic process. It keeps oscillating. Magnetization goes forward and backward. Then it will come back. So, you should give optimum mixing time. This is what happens. You ask me a question, why should I start to here? What happens if I start with C, proton C? Possible.

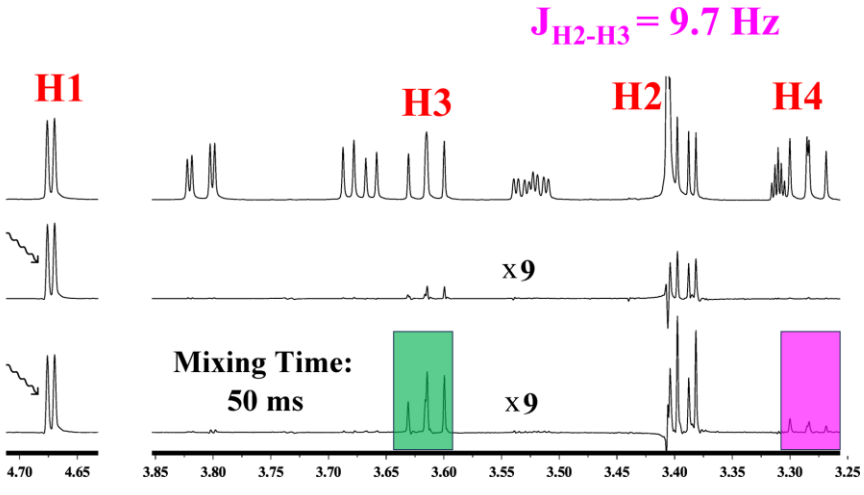
Then magnetization initially is given to let us say B and D. If I start with C, it will give to B and D here. Give more time, it will go to D and E, A and E with longer mixing time. The magnetization get transferred to other protons. This is how polarization transfer takes place.

Now, we will start with one experiment like this. I am going to selectively excite this proton. What is this proton? If you see that, there are two spin systems here, one OCH3 is

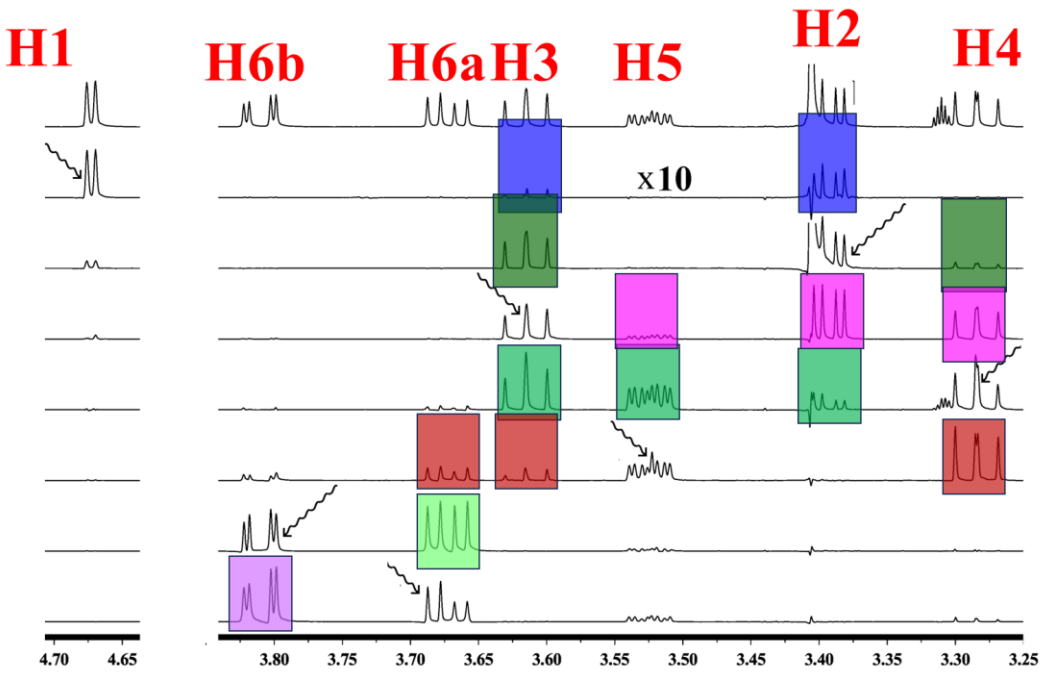
Second is other system. I will start with irradiating one of them. And that is OCA3, make assignment is made. This is H1. What is H1 proton? See, if I have to make the assignment of the sugar molecules, always I start with an anomeric proton because anomeric proton comes between 4.5 to 5.5 ppm and is usually coupled to one single proton and will be a doublet.



And depending upon the coupling strength, we can even say which is alpha and which is beta isomer. Beta isomer has a large coupling than alpha, J coupling. So, I know there is only one peak is coming here doublet in the downfield around 4.7 ppm. That must be anomeric proton H1. I will start with that. I will selectively irradiate proton H1 and then do the mixing. Mixing time of 30 milliseconds is given. See what is happening. There is enhancement for proton E and also OCH3. OCH3 is here. So, that is okay. And I will see the enhancement at both the places. Little bit of enhancement is there for H3 also, because if you enhance vertical intensity a little bit more, you can see this, there is a small enhancement here. Not only OCH3 and also little bit very close to that. So, magnification if you do and you can see. And you ask me a question, why do not you see H2 because J coupling is very weak. That is why I said you have to understand the J coupling very important. Axial-axial J coupling is larger than equatorial. That is what I told you. So, we will do it quickly. Same thing we do irradiate proton 1 instead of mixing time 30 ms, we give 50 milliseconds.



See this is enhanced more because now it has transferred more from H1 to H2 to H3 it is going. See it and then slowly you can see bit of magnetization to other protons also. Remember I started with H1 went to H2 and then if you increase the mixing time enhance the signal intensity you can see little bit of magnetization to H3 and for this one, H4 also. Slowly it is enhanced because I am increasing the mixing time. We can identify this in a different way. You can selectively irradiate proton 1 and I saw the enhancement here between 2 and 3. I selectively irradiated proton 2, then you see the enhancement in 3. I selectively irradiated now proton 3, you will see the enhancement at proton 4, and of course 2 is also there because they are close by.



See 2 and 4 and also little bit weak you can see the enhance the intensity here, this could be proton 5. That you can confirm by hitting proton 4. Then you will see the enhancement for protons 3 and 5, because they are next to each other, and also small enhancement here 2 which is far away. I irradiated proton 5, you see enhancement of proton, other 2 protons of course 4 is there, 3 is also there, and there is also enhancement on another proton 6. Proton 6 has 2 protons, A and B. Both you can see enhancement here. So, if you irradiate proton one of them you can see the enhancement is other one, H6 and HA, and minute peak enhancement for the 5, 4 also 6a. This how selectively I irradiated the proton, kept on increasing the mixing time. and you see the propagation of the magnetization from one spin to other spin. Alternately selectively irradiate one of them and see how the magnetization is transferred. So, slowly step by step you can keep irradiating one by one and see where there is enhancement in the intensity. You can make

the complete assignment, that is possible, like this. So, with this I think time is up, I am going to stop here. So, what I tried to explain today is I started with more examples of the 1D difference in NOE. We saw how difference in NOE is going to help you in making the assignment, few more examples we took. And then afterwards assignment of the particular peak in the phenyl position, everything we understood. Then I also told you it is possible that all sorts of 2D experiment what we have, such as 2D, TOCSY, HSQC, COSY, NOESY everything can be done in a 1D way.

Same one dimension version is possible for many of them. ROSEY, ROSEY also one dimension version is possible. But then it is not possible to do for a bigger molecule. For a small molecule it is possible to do that. That is what we saw that. So, I showed in the TOCSY selective excitation of one of them and give a mixing time, the magnetization getting transferred to other protons. Increasing in the mixing time, it goes to the next proton it depends upon the mixing time and also strength of the coupling. And alternately go to different protons, step by step every time we a selectively excite one proton see where the enhancement is there. If there is transfer of magnetization that peak start coming up, like that systematically you can go and make the assignment of all the protons by a simple one dimensional experiment. This is is a one dimensional TOCSY, this makes helps you in making assignment. With this I am going to stop here. I think we have discussed enough. In the next class I will continue with little bit of TOCSY and then another one or two different topics, I will try to cover. I think another one hour time two classes will be there maybe I will try to cover little bit more about TOCSY and if possible pure shift in the next two classes. I will stop with this. Thank you very much.