One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis
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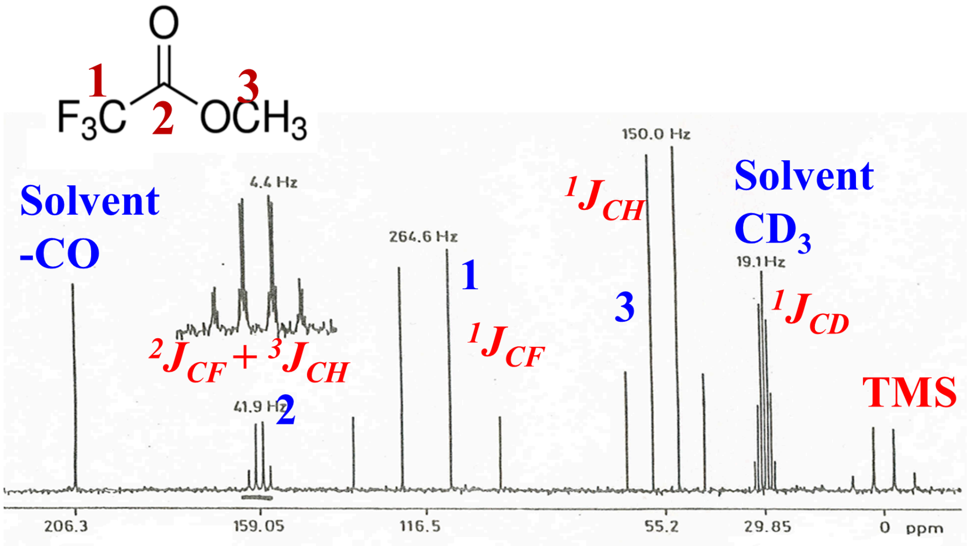
Lecture 27: 13C-NMR-IV

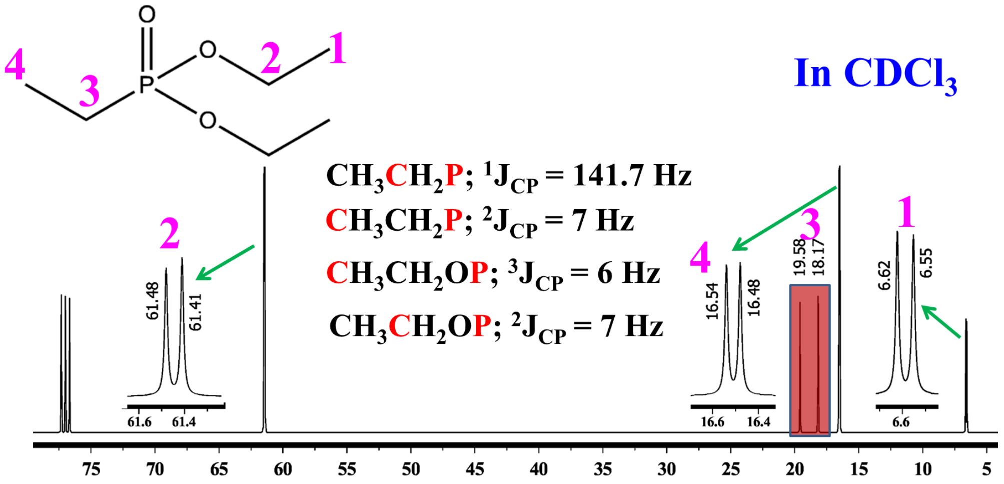
Welcome all of you. Since the last two or three classes, we have been discussing about carbon 13 NMR. Especially in the last class, we discussed about the analysis of the carbon 13 spectra, and varieties of nuclei. I also explained to you about substitution effect and how to predict the chemical shifts. It is possible, especially for the phenyl rings, I took the example and for different places or different positions of the phenyl ring, when the substitution is there, its effect at the ortho, metar, para position or ipso positions is already well documented. We can utilize these values from the table. Take benzene value as the base value and then add up all these values together, whether it is a add or subtract depending upon the contribution. And the final chemical shift value can be predicted, and we took several such examples. And afterwards, we also went to the analysis of the carbon 13 spectra, by while looking at the intensity pattern and the chemical shift positions. And we wanted to see the hetero-nuclear couplings in carbon 13 spectra. Especially, when you are looking at carbon 13 directly, when you are detecting carbon 13, though it is a dilute spin, the question of satellite does not arise at all. We are directly detecting the carbon 13 and it couplings with abundant spins like phosphorus and fluorine, we wanted to see. We took one or two examples of the molecule like 2-fluoropyridine and we wanted to find out what is the coupling constant, how we can analyze the spectrum of a carbon 13 spectra with proton decoupling, so that we are going to get only carbon fluorine couplings. We used that and analyzed. And of course, CF_3COOH is another thing which we took an example and the carbon directly attached to the fluorine was a quartet, we could make out and the COOH carbon also was a quartet because of two bond coupling. We knew how to analyze simple carbon 13 spectra from the information that is available from the multiplicity patterns and using chemical shift and we know how to extract the hetero-nuclear couplings. We can continue further now with another one or two examples before going further.

And now, I will take the example of coupled carbon 13 spectrum of methyl trifluoroacetate in CD_3OD and this is the carbon 13 spectrum and every peak here is genuine peak. Now, our job is to identify and make the assignment which peak correspond to which. Of course, methyl is this one trifluoro, methyl trifluoroacetate and of course, we are looking at the carbon 13 spectrum fully coupled, it is not decoupled. So, that means you have fluorine carbon couplings and also proton carbon couplings both are simultaneously present. That is why I took this example, so that we should know how to interpret such spectrum.

Where do you start with? Of course, there is a quartet at 0 ppm. Obviously, that was the reference we used, that is TMS and TMS CH3 protons split that carbon into a quartet that is what I told you already for different carbon having different protons attached CH3, CH2, CH and quaternary carbon how we get the multiplicity patterns? quartet, triplet, doublet and singlet, all these things I discussed.

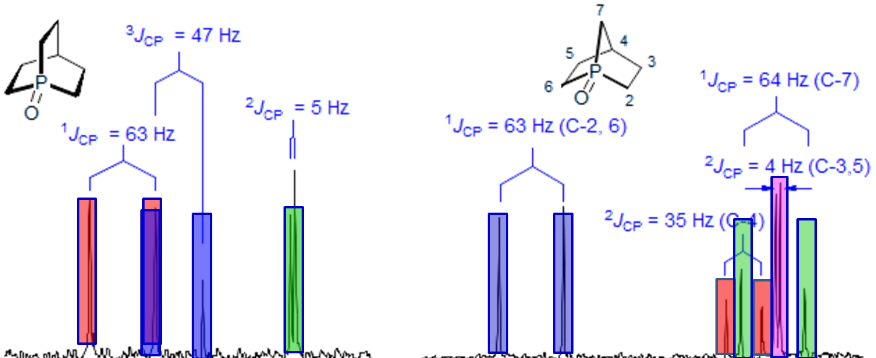


Obviously, this is a TMS which is giving a quartet. What about this one? This is CD3 part of the acetone, this CD3COCD3. So, that is a multiplicity pattern which you are seeing, this is solvent peak. Then what are the remaining peaks? Of course, remaining peaks, you can look at it, this is a CF3 carbon and it has a long range coupling; this is CF3 carbon. This is a one bond coupling, between carbon fluorine which is quite large; about 264 Hz and a large split quartet, quartet coming because of three fluorine atoms that are chemically equivalent. So, this one carbon is going to be a quartet. I fairly assume this is a one bond carbon fluorine coupling quite large and this is CF3 group coupling with carbon. What is the next one? If you look at the next one, you can you can start making the assignment, if this is carbon 1, I would say this is carbon 3. That is carbon 3, it is fine. Why is it a quartet? See remember there may be 4 bond coupling with the fluorine which may not be reflected in the spectrum, which may not be there at all. So, this carbon is a quartet because of its coupling with CH3 protons, 3 protons as a consequence is a quartet. What you get here, if you measure, is the CH coupling, here you got CF coupling. So, that is fine and if you look at the carbon 2, something interesting is happening. Look at the carbon 2 it is a quartet fine. I can say this this could be because of the 2 bond fluorine coupling; and also it could be coupling with these 3 protons you do not know, but I would fairly say it is 2, but if you expand this one you can see each line is a quartet. It is quartets of quartet, fantastic. So, that means this carbon experiencing coupling with both CF3 group and CH3 group. So, this is a coupling because of the fluorine, it is a large quartet of the order of 42 Hz approximately; and each line is a quartet because of the long range coupling with CH3 protons. That is very clearly understandable here. We can make assignment of course, this is TMS, this is solvent, what is this last peak left over. This is solvent carbon CO which is not split because of any other reason. That is a solvent carbon. And very clearly we could assign all the peaks. Here is a simple molecule and we could get both heteronuclear couplings carbon proton and carbon fluorine here. This is 1JCF; this is 1JCH; this 2JCF plus 3JCH, this is 1JCD; TMS and this.



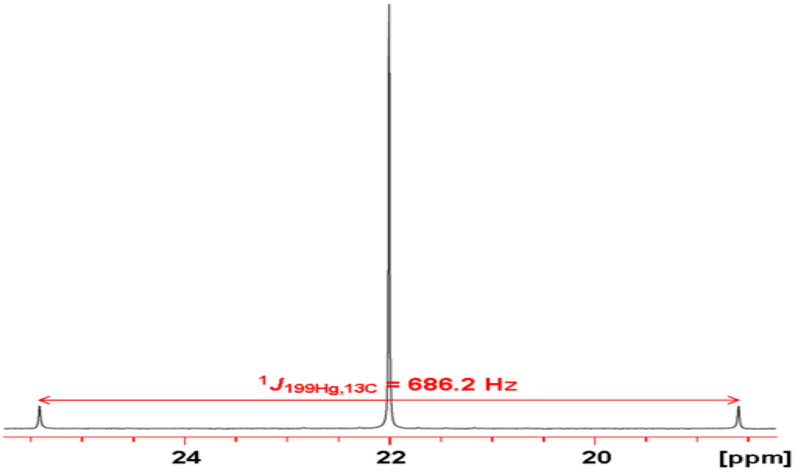
We will go further. So far we saw the fluorine couplings. What happen if there is a phosphorus present, an another example of a simple molecule I am taking. This is a molecule with a spectrum of diethyl diethyl ethyl phosphonate. This is a molecule and now we can see there are 3CH3, 1 and 2; and 3, and 3 CH2 groups are present and a phosphorus is here. And if you look at the spectrum carefully, and look at the title here it is proton decoupled. So, there is no carbon proton coupling present here, if at all there is a multiplicity coming it is because of coupling with phosphorus that we should know. So, each of them is expanded here. See this is a doublet, this is a doublet, and this is a doublet and of course, this is obviously 3 line pattern, of equal intensity because of coupling with deuterium, this is CDCl3 solvent, that is also clear. So, there are 4 different carbons here and

^{13}C {^{1}H} spectrum



each of them is a doublet here. Of course, I have written 1 2 3 and 4 this also there we will see now. The first one is a doublet that is because of coupling with this phosphorus 1 2 3 bond coupling is there is a doublet. Of course, phosphorus is spin half nucleus. As a consequence carbon peak is split into a doublet. Now this is another one this is carbon 2 and of course, this is also having a coupling with phosphorus, 2 bond coupling. Look at this one, this is the third one which is directly bonded to phosphorus. The carbon 3 which is expanded here, and that splitting is quite larger, you can see this is also a doublet very easy. And last one, left over if you expand this one this is carbon 4 here. And that also has 2 bond coupling with phosphorus. Of course, protons are decoupled. So, each peak is a doublet from the coupling strength you can also make the assignment very easily because 1 bond coupling is quite large, this is that one. And then of course, 2 bond and then 3 bond like this we can make the assignment. And you can measure the couplings also. So, you can very easily see that 1 bond carbon proton coupling here is about 140 Hz and of course, 2 bond is 7 Hz, 3 bond is 6 Hz and another 2 bond with other carbon is 7 Hz.

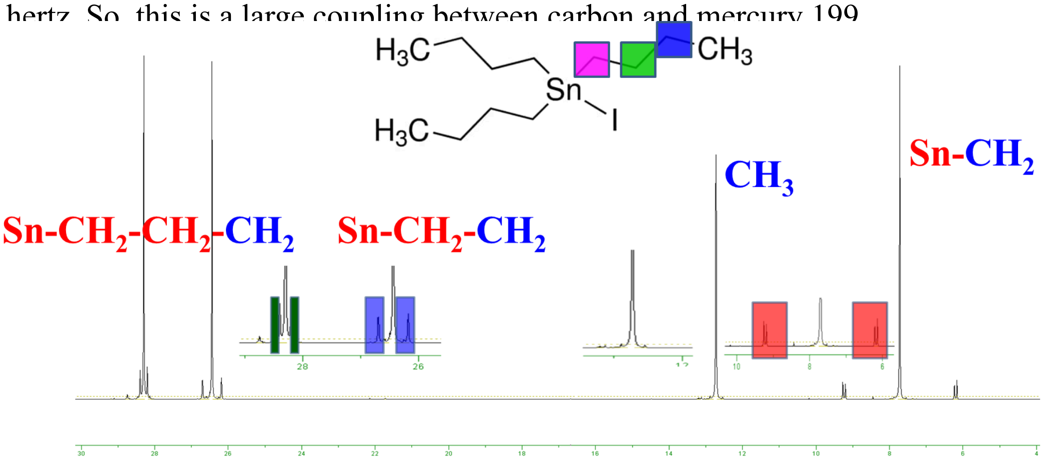
Continuing further we will take another example of 2 molecules where we can extract carbon 13 and phosphorus couplings. In this molecule again this is a proton decoupled spectrum. As I have been always telling you if the nucleus is put in the flower bracket that means that nucleus is decoupled with the nuclei which you are detecting. So, we are detecting carbon 13 decoupling proton. Obviously, carbon proton couplings are not present in the spectrum. So, now we can look at it very carefully. Tere are 2 molecules, look at this molecule and this olecule. Of course, we will start with this molecule which is highlighted here and this molecule we have ^{1}J coupling is there; ^{1}J carbon phosphorus coupling; it is quite large and is of the order of 65 Hz; approximately this correspond to carbon 7. And of course, there is also another 1JCP which is for carbons 2 and 6 both of them having 1 bond couplings. And it is here of the similar order of the order of 63 or 64 Hz. These are carbon 2 and 6 are identical. So, we are getting like this. then what about this one? this is a 2 bond coupling; this is 2 bond coupling with carbon 4. You can see here 2 bond coupling; and then 3 you can see other coupling with carbon 3 and 5. Again it is 2 bond coupling. So, each of the carbon here is a doublet because of its coupling



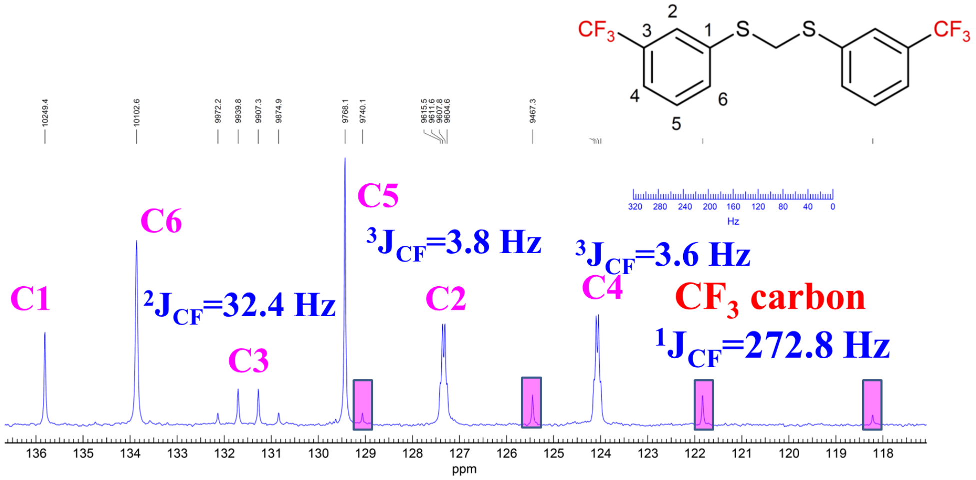
with phosphorus. Very easily you can assign, and extract the coupling information by doing proton decoupling. If there was no decoupling this would have been a very complex spectrum; very difficult to extract both carbon proton and carbon phosphorus couplings.

Now, coming to this molecule again 1 bond coupling is of the similar order 64 Hz. This is one bond coupling and then you can have another onr 2 bond coupling; another 2 bond, 3 bond coupling is there. There are 3 different types; 1 bond coupling, 1 bond coupling and 2 bond and then 3 bond coupling. Everything you can easily measure it. This is between the coupling of carbon with abundant spins phosphorus and fluorine. What about coupling between carbon and low abundant spins? carbon itself is a dilute spin. When I say I when I am detecting that we do not have to worry about it. When we are directly detecting the abundant spin, we have worry about satellites. Now, we are directly detecting, but can that give satellites, because of coupling to other heteronuclei which is less abundant? Of course, it can give and give rise to satellites.

Let us take one or two examples of that look at this molecule this is dimethyl mercury, the neat sample is taken, it is not dissolved in any solvent and carbon-13 NMR if you see we have mercury here. The mercury has several isotopes; usually mercur 199 is what we have to consider, mercury 201 and others are very difficult to detect. So, mercury 119 is the nuclei of our choice, most of the time. So, that is going to give rise satellites. The abundance is of approximately 17 percent and that can couple to carbon 13 and give rise to satellites. See this is the main peak coming because of the carbon attached to CH_3 and this carbon of course, we have decoupled proton is coupled to mercury; Hg199. And look at this, it fairly big intensity satellites, because the abundance is close to 17 percent and so mercury coupled to carbon here is appearing as satellites. So, measure the separation between these two satellites you are going to get one bond carbon mercury coupling, which is fairly large and is of the order of 686 Hz. I told you in some cases of some heteronuclei the coupling can be of the order several hundreds even thousand Hz, several thousand hertz. So, this is a large coupling between carbon and mercury 199

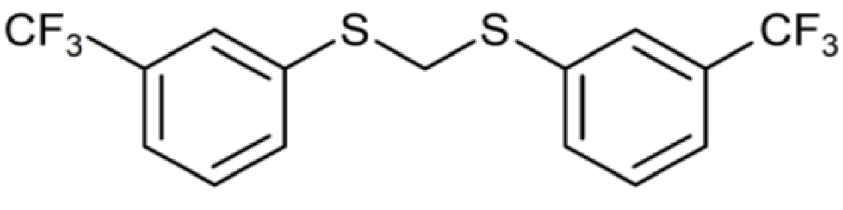


We will go to the other molecule it is a carbon 13 NMR of tributyltiniodide here. The tin has two isotopes interestingly tin 115, of course, 115 is also there that is very very low, we can ignore it. The 117 and 119 are the two things which we have to consider. Both are interestingly spin half nuclei and abundance is almost equal, 8.6 and 7.7 percent, nearly equal. You can say both of them are approximately 8 percent. And in this molecule if you are looking at the carbon 13 spectrum of it, look at it what is the type of spectrum you are going to get. First of course, this is a CH3 peak and you can see this is a CH3 group, and there is multiplicity being shown here. So, that means it is not decoupled. I mean it is coupled there is a multiplicity which if you expand you can see it is not shown here. So, this is CH3 carbon, we know that one. And of course, next is this one CH2 carbon attached to tin directly bonded to tin and this interestingly if you look at this one if you expand this CH2 carbon directly attached to tin, this is expanded here, you can see two peaks on either side. They are satellites of tin coupling to this carbon CH2. They are nearly of equal intensity, as I said natural abundance is almost similar. And you know the couplings are different one is tin 117 coupling to carbon other is tin 119 coupling to carbon. Which is which we can find out very easily that is not a difficult job thing. Now, next come to this carbon; this carbon is like this; and it is this CH2 carbon. Again if you see on either side you are going to see satellites and there are two peaks here. So, you can very clearly see and of course, this is if you go further another CH2 is there; this CH2 is coupled to tin and that coupling is here; and it is not very well resolved to show coupling between both the different types of tin 117, 119; whereas it is clearly seen here. Can we make use of carbon 13 NMR for the structural assignment, simply without using proton NMR? At times very easily just looking at the carbon 13 NMR you can arrive at the structure of the molecule. How do we do that? Let us take the example of this molecule. This is a carbon 13 NMR proton decoupled spectrum of this molecule there is a name for this molecule; first we have to make the assignment of all these peaks. First thing we have the challenge to identify the position of CF3. Whether CF3 is here, here, here or here, we do not know. Can we make an assignment or get the structure of the molecule based on this

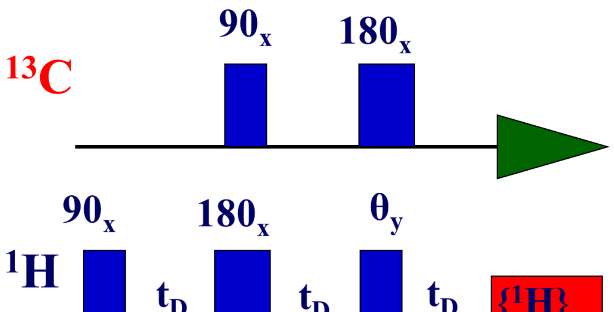


Of course, first assignment we know this is CDCl3 because the CDCl3 gives three lines of equal intensity because of carbon coupling to deuterium that I have been telling you. And if you look at it, this is CH2 carbon is sitting at the center between two sulfurs, it is CH2 carbon I can say fairly there is no other coupling possible for this one. So, it is that one, fine. That is a CH2 carbon. The remaining part, is this low field region and is expanded here. If you carefully see a lot of information you can get out of it. If you see there are four peaks here of intensity 1331. What is this one? this is carbon of CF3 group carbon 13 of CF3 group, the three fluorines are splitting this carbon into quartets of intensity pattern 1331 that is what it is. So, we can make the assignment for this to the center of this quartet is the chemical shift of CF3 carbon. So, we can make the assignment of that, one bond coupling is quite large also. And the next is there are the three other carbons C1, C6 and C5; here C1, C6 and C5 and all of them appear to be singlets. Of course, C1 is a quaternary carbon, is very weak in intensity that we can make out from the intensity itself; and C5 and C6 are here and they are singlets. That clearly shows this long range coupling of fluorine to these two carbons are not there. That is one conclusion you can draw. But what about other carbons 2, 3 and 4. Look at it this is a carbon 3, I would say which is directly attached to CF3 group, this carbon and this is also a quartet because of these three fluorines. This is a large coupling, here large quartet, but this is two bond coupling, and is quite small, but still it is a quartet and measurable quantity. I would fairly say that is, I can make the assignment confidently as C3; and of course, C2 and C4 both of them are separated by 1, 2, 3 bonds away; two bonds couplings are there and they are again the quartets. Each of them is expanded here, you can see that. If you see four peaks here; four peaks here 1, 3, 3, 1 quartet. So, this is two bond; this is three bond CF coupling. Look at it three bond CF coupling is more or less of the order of 3 to 4 Hz, very small and it is not well result, but still you can make out the quartet structure in both of them. So, I can confidently say from three bond coupling this is C2 and C4 which is C2, C4 of course, I already told you, how to do that we can make the rough estimate of that. And this is again two bond coupling we can clearly say this is C3 a sort of assignment is made. This clearly tells me there is one CF3 group and there are six carbons present in this molecule. From the analysis there is one CF3 group and there are the all these remaining six carbons; five here and CH2 in the previous one I showed you. What are the observations we can draw? the conclusion here is the structure must be symmetrical. Why do I say that? If the structure is not symmetrical here look at it then the CF3 group if it is somewhere here without any symmetry; then the number of carbons would have been more, otherwise we would have got CF3 two peaks; because of symmetry, chemical equivalence is there and only one CF3 group is observed with quartet, that is fine.

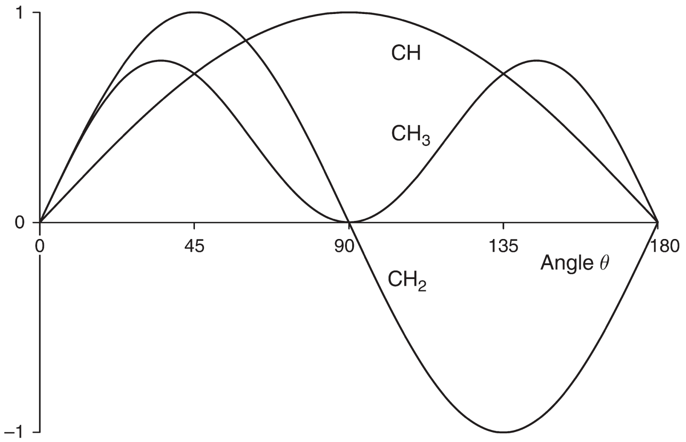
The CF3 is also not in the para position. Why do I say that? if it was to be in the para position the symmetry would have been reduced. For example instead of here if CF3 would have been here, then what would have happened? this carbon this carbon has chemical equivalence and this and this carbon has chemical equivalence then the number of carbon peaks would have reduced; I said we one CF3 and six carbons are there. If there was a symmetry if it was in para position, with respect to S, I am talking with respect to sulfur in which case you would have got one CF3 and only four carbon peaks; not six. The very fact we are getting six carbon peaks, one another conclusion I can draw is this this is not in the para position. We have done already two conclusions one there is only single CF3 and there is a symmetry here because of that we are getting single CF3 and this CF3 is also not in the para position with respect to sulfur otherwise the number of carbons what we observe would have reduced instead of six we would have got four peaks. Let us go further. The CF3 is also not in the ortho position I will say. Here it is also not ortho with respect to this, why? very easily you can come to a conclusion this CF3 is coupled with this and also these two carbons. If it were to be here this carbon would have coupled to fluorine. Otherwise the CS carbon would have been split by CF3 group into a quartet, the long range coupling with CF3 this carbon 1 is the carbon attached to S sulfur is still a singlet. That means I can conclude easily, and say CF3 is not ortho to CS carbon. So it is not para, it is not ortho to CS carbon, then what is the other option? it must be meta to CS carbon. So the correct structure from the carbon-13 spectrum interpretation I can draw is this one. So that means by simply looking at the carbon-13 spectrum, the number of carbons and this multiplicity pattern for each of the carbon, that are coupled to fluorine I could draw a conclusion ,this is the structure of this molecule very easily. So we can utilize that idea.



Going further I want to tell you one important experiment called DEPT. This involves what is called a polarization transfer. As I said yesterday polarization transfer I am going to discuss that later, because I have not discussed this polarization transfer and NOE which I introduced yesterday while doing the decoupling NOE factor I said these two terms I mentioned but not discussed in detail which I am going to do it in next class or subsequent classes. But right now understanding of that is not important but please remember there will be a polarization transfer which means it will transfer the polarization or the magnetization from abundant spins to rare spins. When we do that what will happen? the dilute spins intensity will go up, the signal will enhance the signal intensity will go up. It is like robbing the rich paying the poor. Take the magnetization from proton and give it carbon which is dilute spin. And now using polarization transfer technique and also by using an experiment called DEPT an experimental sequence, called distortionless enhancement by polarization transfer. Using this it is possible for us to identify different carbons attached to different protons. For example I can point out what are the carbons attached to CH3, what are the carbons with CH2 protons, carbon attached to one proton, and quaternary carbon. So all CH3, CH2, CH and quaternary carbons; only four types of carbons would be possible; and they can be identified by using DEPT, the distortionless enhancement by polarization transfer. So the two new concepts, I told you we were NOE and Polarization transfer, we will discuss in the subsequent classes. What is the need of a DEPT? Look at this molecule which we discussed earlier also, carbon 13 spectrum. This is coupled; this is decoupled. Even in the decoupled spectrum so many peaks are there, what can you make out of this? In the first approximation I will say this is a quartet peak this is a CH3 carbon, I would say further I would say this is there are two doublets here, then I would say, there are these are two CHs, this could be a CH which is a doublet. Beyond that from this region what can we say except one or two carbons here? What can we say about these carbons? what is the multiplicity pattern which is CH3 which is CH2 which is CH? So it is very difficult to identify. Only in this molecule a couple of CH3 and couple of CH2 will be observed we can identify. What about other carbons? How do you identify them? For that we use a pulse sequence called DEPT. The DEPT is an experiment, very simple experiment. What we do is we apply carbon 13 pulse, 90, 180 and then on the proton channel 90, 180 and theta pulse. This is the sequence.



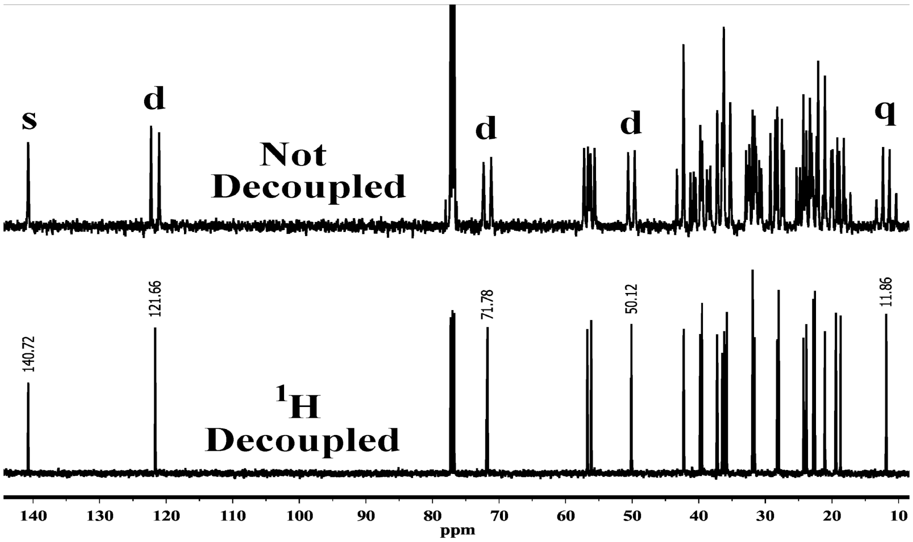
Do not worry about how it works and everything, because as we have discussed this in one of the previous courses. What is important is angle theta of the last pulse on the proton. On the proton there are three pulses we are worried about on the last pulse the theta degree pulse; and proton and this angle with which we are going to tilt the magnetization using this theta is called a flip angle of the pulse. The flip angle can be different, we can keep it constant and vary it. So what we can do is we can do the polarization transfer by transferring proton magnetization to carbon 13. And the advantages we can get the experimental time is reduced by 1/16; very interesting. See if an experiment takes 16 hours, by using DEPT you can do the polarization transfer we can reduce it to 1 hour, great reduction in the experimental time we can do that. So how do you identify carbons, we have to do three experiments in principle 2 are enough 90 and 135, but in some books and others people mention three experiments. I am explaining all the three, but practically you require only two experiments. So in the third proton pulse is there, a flip angle plus theta. which I showed in the pulse program we can do three experiments by keeping angle theta as 45 degree, 90 degree and 135 degree. Three experiments we can do; and based on these three experiments the phase and the intensity of the carbon peaks vary. They depend upon the flip angle of the last pulse, depending upon whether you have 45, 90 or 135, the phase of the peak whether it is positive or negative and about this intensity, whether it is 0 or maximum we can define, we can understand. And this pulse sequence tells us based on the intensity and the phases which carbon is which. This is graphically I am showing like this.



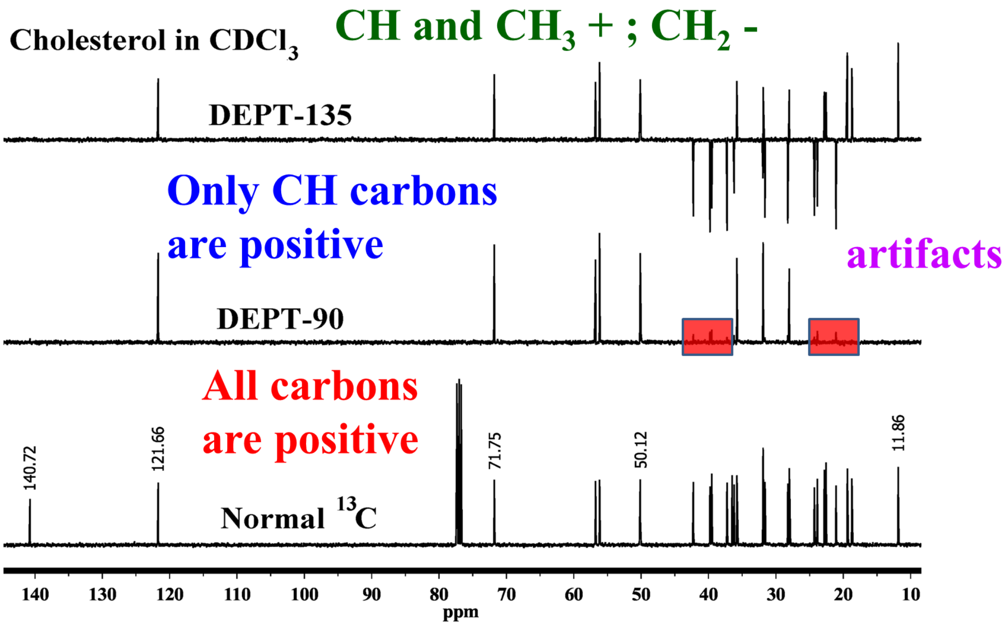
For example if I do a flip angle pulse with a DEPT experiment, the last proton pulse keeping 45 degrees; remember CH, CH3 and CH2 carbons all are positive intensity. And the other hand I would I do a DEPT experiment keeping flip angle exactly 90. Now you see CH2 and CH3 intensity goes to 0, only single proton attach carbon, that will have full intensity. Only CH2 and CH3 goes to 0; and CH carbon have full intensity. Go further and 135 degrees you make it, interestingly CH3 and CH would be positive and CH2 is negative. So all you have to do is two experiments; 90 and 135 and a conventional carbon 13 spectrum. You will be able to identify all the three carbons fairly easily.

Experiment	**Quaternary Carbon**	**CH**	**CH_2**	**CH_3**
Conventional ^{13}C spectrum	+	+	+	+
DEPT-45	0	+	+	+
DEPT-90	0	+	0	0
DEPT-135	0	+	-	+

This is the table I have prepared for you this is a conventional spectrum all carbons are positive intensity if you do DEPT 45 you do not see quarternary carbon because there is no polarization transfer. There must be directly attached carbon for polarization transfer. All other carbons are positive. If you go to DEPT 90 only CH is positive. all others are 0 in intensity. f you do DEPT 135, CH is positive, CH2 is negative CH3 is positive, that is all, we have to remember. Then do an experiment on this cholesterol.



When you do that experiment this is what I showed, a conventional experiment not decoupled fully coupled spectrum. And this is decoupled experiment. We do not know which carbon is which; whether it is CH2 carbon, CH3 carbon or CO carbon or quarternary carbon. We will see now the DEPT experiment of that. Very interesting.



I do the DEPT experiment. It is normal carbon 13; all are positive; do DEPT 90; when you do DEPT 90 what did I say? you will get only CH carbons; all are CH carbons here. You do DEPT 135 what did I say? here look at in this table DEPT 135 if you do CH is positive CH3 is positive and CH2 is negative. See here in the DEPT135 CH and CH3 are positive and all CH2 are negative, I can say even in the crowded spectrum, these are all CH2 and these are all CH and CH3 from the DEPT 135. Now combination of these two experiments taking the information from this this and this you can do complete assignment of all the carbons whether it is CH3 carbon , CH2 carbon or CH carbon can be done. Okay. So this is what it is, the expanded region of that, if I show, you will clearly appreciate the importance of this one. Look at this one, here, here, there are two carbons one is positive and the negative. This must be CH2; this could be CH here; look at it, very close by and you will not find the difference especially here see one is positive other negative. One is CH the other could be CH2. So this you will not be able to make out, but if you look at the region of chemical shift, you will say it could be CH3 or it could be CH2, you do not know. So only chemical shift information alone will not be able to tell you which carbon is this; but in the DEPT experiment I am able to simply take care of this thing, and I can say which are the carbons which is attached to single proton or three protons and two protons. All CH2 will be negative in intensity with DEPT135 all

CH and CH3 are positive intensity. That is how there are few experimental requirements, the DEPT is most important.

The 90 degree pulse has to be perfect like this. Only with exact 90 degree pulse only CH carbons will be seen. If it is slightly deviating let us say instead of 90 you make 95 degree, see already CH peaks appear, negative CH2 peaks you can start getting. The CH2 peaks negative which in principle you have to get only CH peaks; and all other peaks should go to 0 for 90. But you know other peaks start coming up, it can confuse you. Secondly also you can go to this one, this instead of 95 make it 85 degree, all other peaks would not come, but if it is more than 90 CH2 peaks will be negative but here CH2 peaks will be positive for less than 90. If it is 85 degree even they are coming it will confuse you to be CH carbons. So precise 90 degree is very important. Little bit of mis-tuning of the 90 degree you get into problems. So these are all CH2 carbons which appears positive.

The probe tuning is very very important for DEPT. The probe has to be tuned when the samples are changed from polar to nonpolar, etc. And of course there are two types of probes in the NMR, the direct detection probe where 13C coil is inside, proton coil is outside. In the inverse probe proton coil is inside and carbon 13 coil is outside; and so very important for all those things. So for a direct probe tuning is not very critical here directly you see carbons. But the inverse probe tuning is very critical, if you do not do it properly you will make a mistake and you do DEPT 90, the slight variation in the angle you may get the CH2 peaks positive or negative instead of only CH2 peaks. That get confused. So DEPT experiment might fail with this thing; and also the setting of the angle is extremely important. For example average CH coupling in all the experiments we take to be 150 Hz. It need not be, it can be more it can be less also. But what happens if the CH coupling is large very large, and deviate significantly. This what happens. Look at it, this is the case where carbon 13 and this is a carbon 1 here interestingly this carbon CH coupling is very large about 240 and 250 Hertz. As a consequence what is going to happen? this CH peak in the DEPT 90 we should see only CH peak, you know, that is not appearing here. That is what happens if you go to DEPT135 also. If there is missing, this type of errors will happen. And if you go like this for example this one, this will also come. This starts appearing so some of these thing there will be a big problem, If you look at this carbon CH2, the carbon 2 look at this intensity. What is happening is mistuning of 90 degree or if the angle, the J coupling is deviating significantly from the set value of 150 Hertz; there could be experimental artifacts. So precise setting of the J value is important. Sometimes J value is different what you should do is you to do two three experiments okay. That is what it is. So advantage of DEPT or INEPT, which I have not explained to you, I will show you later. This is APT and this is DEPT135. DEPT has a lot of advantages signal intensity is much better here, okay. Well there are lots of things we can discuss about it. DEPT135 is much better than APT because of the intensity, there is a polarization transfer here okay. In APT there is no polarization transfer only in DEPT there is a polarization transfer , that is the advantage. And lastly I wanted to tell you one thing about how we can utilize DEPT in getting the structure of some one or two molecules. Since the time is getting up I am going to stop here. we will come back and just explain that one next and then continue further with the analysis of the other spectra. So, in this class already we discussed lot of things about the analysis of the spectra of carbon; how do you get the heteronuclear couplings when the abundance spin is present, when the carbon is couple the rare spin, dilute spin like selenium that we saw, and many of the other examples like for example DEPT experiment DEPT 90 135 DEPT 45 how we can analyze you know in a coupled region to identify the carbons attached to single proton, two protons or three protons etcetera based on the intensity of the the phases. It is a DEPT experiment where the polarization transfer is involved, as a consequence signal intensity goes up and because signal intensity goes up the experimental time drastically gets reduced. These are the advantages of that, In addition to identification of different carbons, we can speed up the experiment and I showed some of the examples of how we have to perfectly set the 90 degree in DEPT experiment and how missetting of the J value can give us the problems. These are all certain tricky issues which one should remember. With this I am going to stop, I will come back and continue with the remaining part later. Thank you very much