Essentials of Biomolecules: Nucleic Acids, Peptides and Carbohydrates
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Lecture-27
Peptide Coupling Agents, Solid Phase Synthesis, Peptide Based Therapeutics

Hello, everybody and welcome back. So, in the last lecture we have been talking about the synthesis of the peptides, short sequences of the peptides and we have started with the solution phase peptide synthesis and I have shown that you have to do certain amount of protection and deprotection chemistry in order to restrict the formation of the byproducts which are not desired ones. And I have shown how to protect the N-terminals as well as the acid functional groups.

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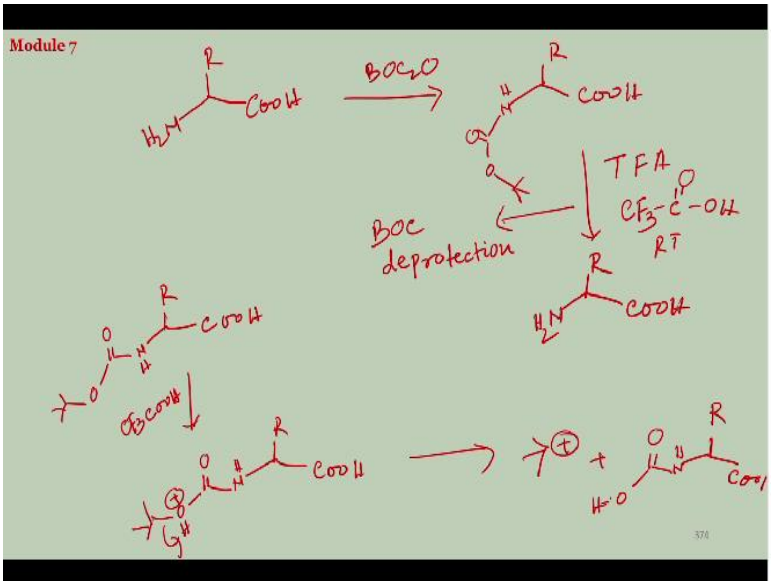

So, once again for the amine terminals if you want to protect the amino groups of the amino acids this is your amine, so amine group you can protect it either by using BOC anhydride that will give you this and then NH BOC O, tertiary butyl. This is the BOC protecting group that is usually used for the production or inactivation of the amine group. Now, how can you deprotect it. You can deprotect BOC usually we use a very mild condition for the deprotection of this.

You can use TFA, TFA is trifluoroacetic acid. The structure is CH 3 CO OH CH 3, CH 3 acetic acid CF 3 TFA means trifluoroacetic acid. If you treat this at room temperature, then your BOC

will be cleaved. How does it work. So I will write the product first. This and then you will get back your amino. So it is a deprotection. So, this is the BOC protection and here is the BOC deprotection. Now, R COOH this is your NH CO.

So, if you treat these CF 3 COOH this is an acid. So, basically it will protonate this oxygen here, this will be weight loss. So, this is acid based deprotection, once this is formed, now the reason why tertiary butyl group is used is because the tertiary carbocation are pretty stable. So, once this is protonated it will be attracting the electrons and it will give you the formation of a tertiary carbocation plus R COOH you have NH CO and OH.

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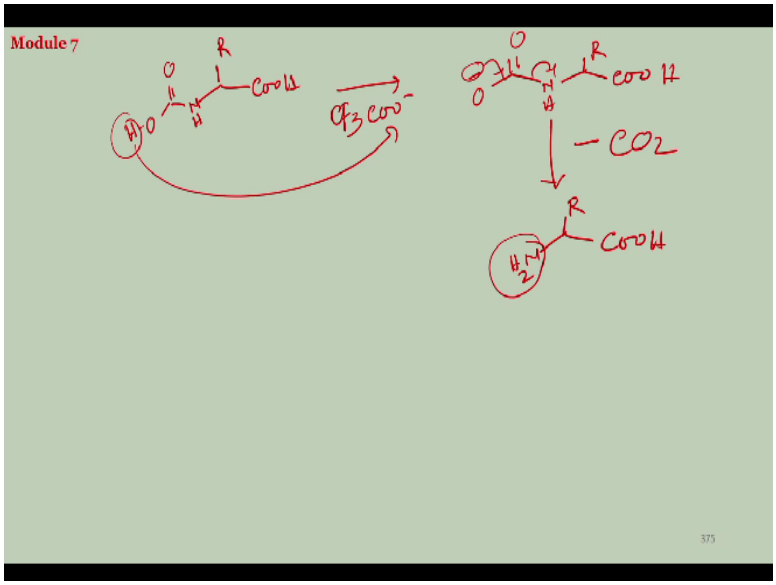

Now, you had formed initially if you see there was one proton transfer from the trifluoroacetic acid to this oxygen. So, this has become acetate anion CF 3 COO -. Now this again a proton transfer will happen from here to there and it will become a weight either a minus or it can happen instantly. So, the moment this is formed, this will easily decarboxylate minus CO 2. I have written it step vise first this, then this, it can happen at the same time.

That this will take up this proton and then this will go here they should be eliminated, so, minus CO 2 would be eliminated or carbon dioxide would be eliminated and that will give you the NH minus followed by the protonation, again will give you the amine back. So that is how deprotection of the BOC works. So, if you want your system if you want the deprotection to be

happening in acidic condition, then you use a BOC protection group. The second we have seen that you can use the FMOC chloride also right.

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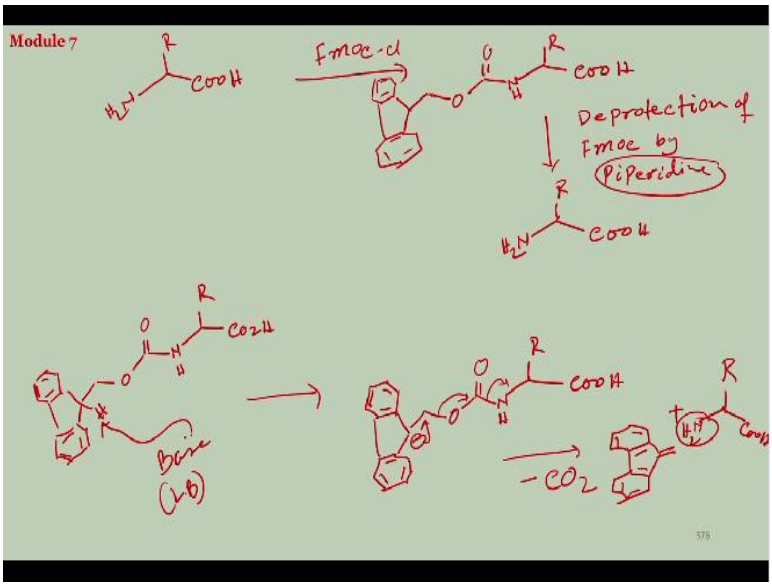

That is also amine petition NH 2 if you treat this with FMOC chloride then you have NH what is the structure of a FMOC, COO CH 2 and here you have. So, this is your FMOC part, this is the protection and that actually in activates the amine the NH group. Now how to deprotect it, deprotection of FMOC usually done by using mild base, we usually use piperidine, piperidine is a mild base, it is a Lewis base actually, so very mild base.

In presence of the bass, it can be deprotonated to give you the free amine. So, deprotection of FMOC occurs under basic condition, deprotonation of the BOC group we have seen occurred under acidic condition. So, this is a big difference and depending upon your requirement, what kind of peptide you are synthesizing, what is the sequence of your peptide sometimes you need to choose the acid level system.

Sometimes you need to choose the basic level system. So depending upon your requirements you can choose whether you want to go for FMOC chemistry or whether you want to go for BOC chemistry. So how that mechanism works. Double bond here. Here is another double bone, here is another double bone, there is another double bond. So it is aromatic system, fluorine system. So in presence of base Lewis base actually this is LB.

So here if you see this proton is actually pretty much acidic. Because negative charges here can be delocalized over the 3 rings. So that is a stable aromatic system and the negative charge can be delocalized. That makes this proton acidic. So if you have a base, lone pair of base will take of this proton, that will create a negative charge here. And that actually is the rate determining step. The attack of the base to this acidic proton is the rate determining step of this reaction.

So it generates a negative charge here, negative charge goes there, obviously, that comes here, goes here. So, again, it will release a molecule of carbon dioxide and it will form double bond this is the intermediate 4 side, this is the product that is formed, intermediate product that is formed again this will be reacting with something. If you have rather steps will give you a stable compound plus your desired free amine.

So that is how the FMOC is deprotected under using the piperidine. Now, usually when you do solution phase chemistry, FMOC chemistry is not that much preferred, BOC chemistry is more preferable compared to the FMOC chemistry because in the solution phase, you have piperidine there and FMOC is also released, piperidine is hard to be removed from your container. So, that is why this is a difficult part to remove piperidine even by depicted workup. So, in that sense BOC is better. So, next comes the acid we have talked about right.

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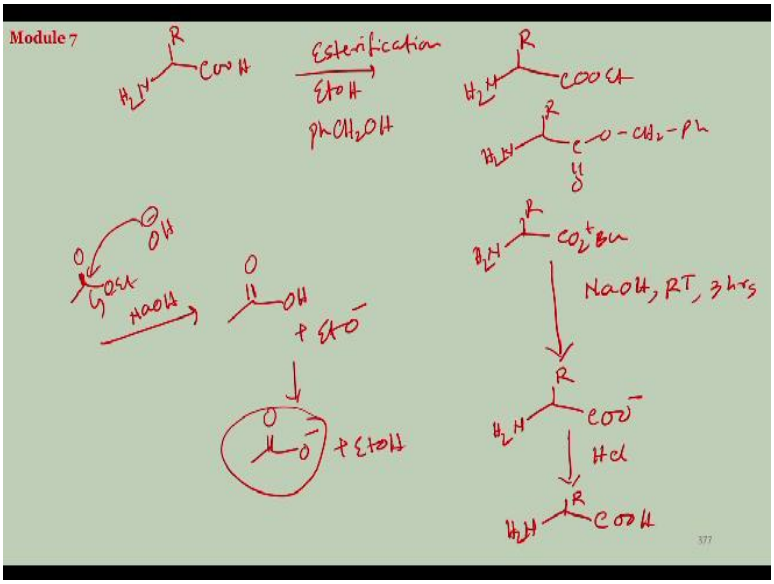

That you can use the esterification basically, you can do the esterification to this acid to produce the protected carboxylic acid. And I have mentioned that you can use either ethanol for the esterification or you can use the benzyl alcohol as well. That will give you CO 2Et or if you use this will give you NH 2 CO O CH 2 Ph or sometimes also you can use the tertiary butoxide production.

If you use tertiary butanol or the other reagent you can also go for CO 2 tertiary butyl also. So all these are actually as I have said that it can be removed by hydrogen palladium reduction. Moreover, apart from that, they can also be deprotected by simple ester hydrolysis that requires 2 steps. First step for the hydrolysis of ester what you need is sodium hydroxide, you need a strong base and it occurs at room temperature around about 3 hours.

If you keep then your ester would be hydrolyzed, if ester is hydrolyzed then what do you get, at the beginning you get a carboxylate anion, if you have an ester OEt for example, if you hydrolyze it base catalyzed hydrolysis if you remember sodium hydroxide then. So, basically it will cleave it. So, OH minus right, the reaction is this, you will have OH minus plus OH plus EtO minus obviously, this is acidic, so, this will transfer the proton here.

And in the equilibrium what you get is O minus plus ethanol that is what is your standard base catalyst hydrolysis reaction. So, that usually gives you a carboxylate anion. So, you will have a carboxylic anion first here and then you have to neutralize this using hydrochloric acid, we usually use one normal hydrochloric acid that will depend upon your substrate and how much amount is there.

So, you have to acidify it that will give you the carboxylic acid. So, it actually includes 2 reactions one is treatment with a strong acid sodium hydroxide followed by treatment with hydrochloric acid. So, base then comes acid. So, that is another way of deprotecting the ester group that is present in the amino acids and this is what we usually use in solution phase chemistry. Now, comes the coupling reagent that we have talked about, I have stopped there in the last lecture.

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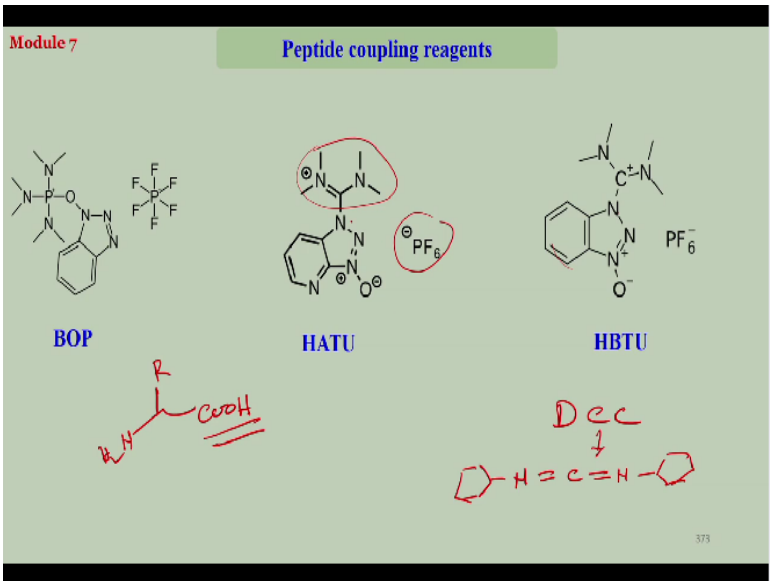

So, there are several types of coupling reagent and the variations are because of different factors like some will induce more decimalisation. Some only induce more decimalisation, less decimalisation and so on. And there are other factors does involve which I might be talking about. So, out of the several, coupling reagents, this is one of them. So with a simple structure and then you have BOP, widely used.

HATU is very widely used. HBTU is also quite well known and popular. So I will show you 2 mechanisms. One is for DCC, how it occurs with DCC and then with the HATU, H A T U and as I have mentioned in the last lecture, that all these coupling agents are end to activate the carboxylic group.

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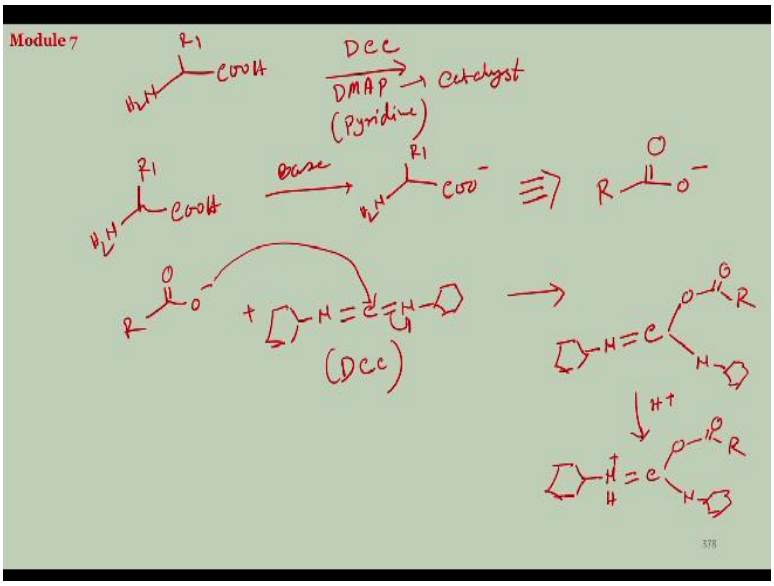

So if you have an amine group, you have this amino acid R 1 and you treat this with DCC and DCC always requires a slide again a mild base. And you can choose different types of mild base. In this case I have chosen DMAP, it is a pyridine derivative, you can look up the structure, DMAP is a mild base, which has a pyridine might in it. So, at first what happens because of the presence of the base and DMAP is used as a catalyst.

You would not need equivalent quantity, you need just a little amount of it. So in presence of the base, which is DMAP in this case, you get a anion here, so instead of writing the amino acid all the time in general, I am writing it as a just the carboxyl part. And here is R that means that all of it. So you have now the anion methane. Now comes the DCC. This is the structure of the DCC dicyclocarbodiimide is one of these eliminated and need to take up the proton.

So, what do you have now C O COR here you have N and the cyclohexane ring, here you have this, that is one of the intermediate. Now, in presence of a slight amount of acid H plus, this gets protonated. So, this should be NH plus and have this.

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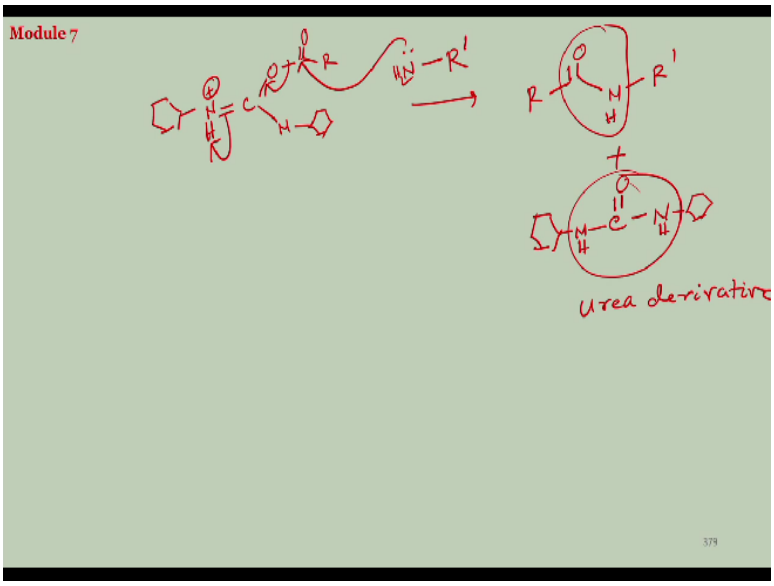

C should be O, CO R and here there was N and the cyclohexane part this N cyclosin and these N is protonated. Now we have the amine. Let us say you have taken another amino acid, I am just writing R for the amino acid part, it is R prime, R dash here. So now this is the activated carbonyl activated complex. So this would attack the carbonyl carbon. This would come here and this is the driving force for doing that.

So, what you now get is R prime or right from the R part R CO NH R prime, you have your peptide bond form plus you have the side product that is this, if this is NH 2, then this is urea. So, this is a derivative of urea, that comes out as a byproduct of the peptide coupling. So, this is your coupled peptides. So, that one.

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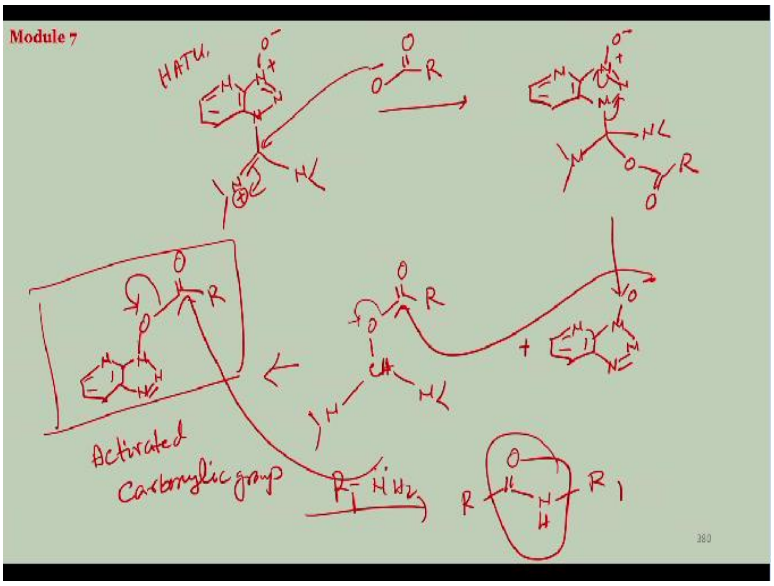

And now, if you use the HATU, how that mechanism goes there, HATU has the structure cyclic triazine O minus that makes this plus and here dimethyl group N N dimethyl and here is N N dimethyl. This is aromatic. This is the structure of H A T U HATU, same I consider that you have taken the acid. General acid instead of writing the amino acid, you have the carboxylic acid and the carboxylic acid is existing as carboxylate anion that you can find already I have shown you how to obtain this.

Again, for this also you need a little bit of amount of Lewis base. So, you have a negative charge here. You have an electron deficient carbon here. First attack is this, this is O COR N, this becomes a neutral now. Now once this is formed is another factor that this is electro positive, this nitrogen is positively charged. So, this will drag the electron density towards it, this forces this bond with it. So, what do you form, you form this.

This becomes O minus, this becomes neutral and here you have a double bond plus you have this carbon and you have O COR, you had a CH of course here. Next is this negative charge attacks the carbonyl carbon and this opens here. So you have this is eliminated as an again a urea derivative. So you have O double bond O here, here now to develop the new bond with a ring and ester. This is your activated carboxylic group here I will finish.

Now, if you have the amine, which is R NH 2 this will react it will come back again, it will be regenerated to some extent, though minus would be relieved and you will have your this is R, this is R 1 CO NH and R 1, your peptide bond is formed. That is the rough sketch of that mechanism for how the coupling reagents are involved in forming the peptide bond or inactivating the carboxylic group okay. So, now this is for the chemistry. Now, we will be coming to the solid phase peptide synthesis.

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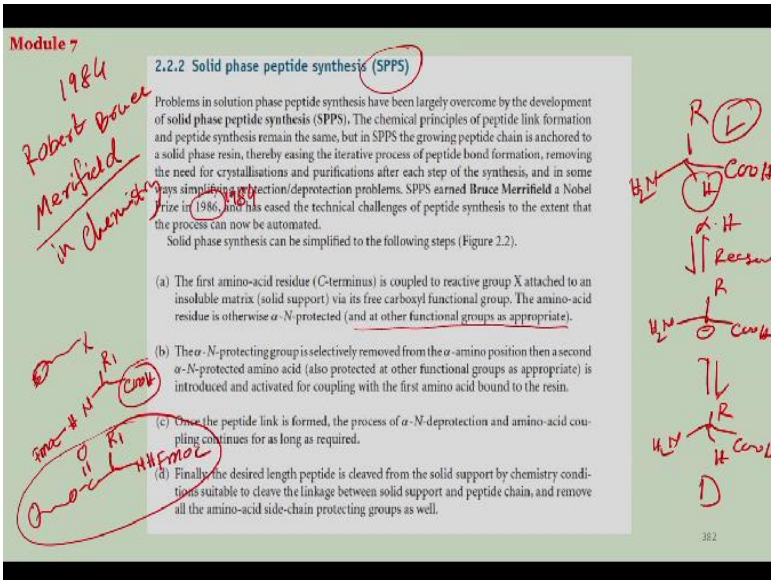

Solid phase peptide synthesis are in short we call it SPPS is of course, as you know is the synthesis of peptide how peptides can be synthesized, but we do not do it in solution phase rather in solid phase. Solution phase, the problem is, I had shown that when you are going for multiple steps, more number of amino acid residues in the peptide. In every step, you are forming multiple products, and all of them would be carried forward if you cannot purify them in each step.

Then all of them would be carried forward to the next step. And then will multiply. So, you will have a number of side reactions going on, apart from your desired product. That is problem 1, second problem is the problem of regimization. If you see this is your amino acid here, there is hydrogen right alpha hydrogen, alpha H. So, whenever you are doing the reactions of peptide coupling, in the solution phase, you are using a set of reagents.

You are using sometimes acids, sometimes base along with the coupling reagent. And depending upon your side chain, this proton has some kind of acidity. So, in presence of reagent transiently, I should write it as reversible reaction. It forms sometimes slight amount of carbon ion can be generated depending upon your reagent. And depending upon the acidity of the alpha hydrogen, the moment it generates, of course, it is not very stable.

So, it will come back to the this phase, it will take up the proton back and will give you the amino acid. But in the meantime it changes its configuration. If this is of L configuration, here there is no configuration. So, when this is taking of the proton back, it can go to L in that case you will get your original amino acid or it can go to the D configuration, because the H + can come from either direction.

So, that will change the stereochemistry of the amino acid sometimes. So, you will have D L mixture that is called the regimization. So, although you have started with a pure L amino acid, there is always a high chance of getting a D L mixture of peptides, that you do not want, that can be minimized in solid phase chemistry, if you do the peptide coupling in solid phase. So, that is another advantage of doing the solid phase chemistry.

Third one is of course, the ease of purification that every step you can just wash away we have seen in DNA case that when you have been doing the solid phase DNA synthesis, because the reaction is happening on a solid support, all the other reagents that you use on top of it after the restoration over, you can just remove them by washing. So, those are the advantages of solid phasing peptide synthesis.

And by the way, solid phase peptide synthesis has earned a Nobel Prize in 1984 and Robert Bruce Merrifield is the person who received a Nobel Prize for his solid phase peptide synthesis protocol in chemistry, he is very famous person actually, Professor Merrifield, he has developed many other techniques not only the solid phase peptide synthesis, but overall solid phase organic synthesis. So, the steps of the solid phase peptide synthesis can be divided into these 4 roughly. The first step this is wrong, this is wrong it should be 1984.

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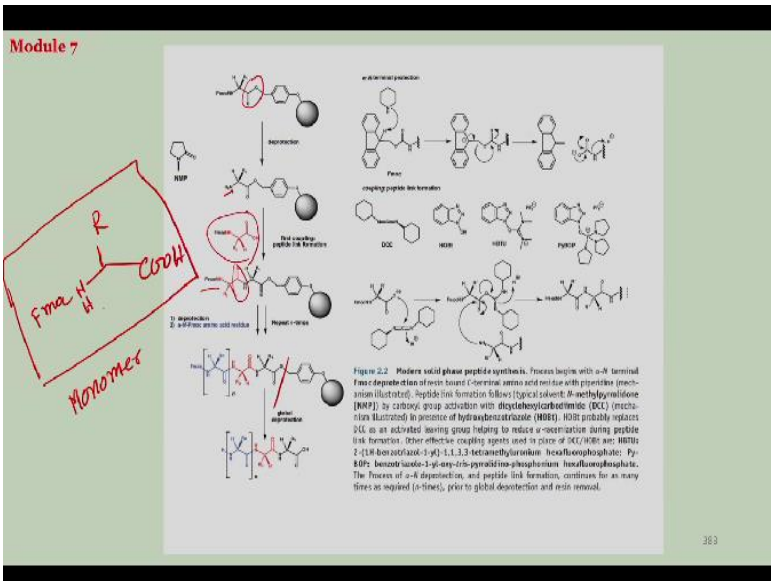

So, this slide as well as the next slide, this picture I have picked up these are actually the book content from the Miller and Kaner okay. So the first step of the solid phase synthesis is that the first amino acid residue is coupled to reactive group X attached to an insoluble matrix. I will explain, via it is free carboxyl functional group, the amino acid residue is otherwise alpha N protected and other functional groups as appropriate.

This means the side chains should be appropriately protected also. That is the fourth step, this basically says that in the fourth step, if you have the solid support S this has a linker, it is connected to a functional group X and then you attach these with first amino acid let us do a FMOC chemistry here. You attach it with your first amino acid through the carboxyl, so that you get, you can do the other way around also, but we usually do it this way O CO, this is R NH FMOC.

This is very important. So that is how the first amino acid would be attached with the solid support. Now, your amino acid is on solid content which has the amine group protected FMOC and will all the time we use the same monomer from now onwards. So, the N-protecting group is selectively removed of course, you have to do the reaction further. So, next step is the removal of FMOC group as I have shown before that it can be removed by using piperidine from the alpha position.

And then our second alpha N-protected amino acid is introduced and activated for coupling with the first amino acid bound to the region, so this should be coupled. Then, once the peptide link is formed the process of alpha N deprotection and amino acid coupling continues for as long as required. So, you can recycle the steps, that is the completion of a cycle here one reaction is finished, then you add the second monomer.

Then the third monomer and it can go on, once everything is finished, finally, the desired length peptide is cleaved, you need only your peptides. So, it has to be removed from the solid support is cleaved from the solid support by chemistry conditions suitable to cleave the linkages between solid support and peptide chain. So, depending upon what is your solid support which kind of solid support you have used and which kind of linker you have here.

You can choose the appropriate chemistry to break this bond and remove all the amino acid side chains and put it in groups as well. And in the last part, you also have need to do whatever the protecting groups you have used on the side chains. So, these 4 are the primary steps for the synthesis involved in the solid phase peptide synthesis. This is the rough sketch that you have the solid phase.

This is a linker actually and that is how the first amino acid is connected, here there was 1 chloride. So, if you use this acid then it will have first this formation. So, first amino acid is attached here with it is FMOC protection, the next step is the rotation of the FMOC. So, that you get a free amine group here and now you add the monomer. This is your monomer, that is very important.

The monomer is the free acid and FMOC protected amine and every time you have to use this monomer only where the acid would be free. This is what acid NH FMOC, this is your monomer for peptide synthesis, solid phase peptide synthesis. So, you use this along with the coupling reagent, then you will have the peptide bond formation, this peptide bond and again FMOC. Further in the next step again you cleave their FMOC, add another monomer with a different amino acid and continue it.

So, once you reach your number of sequences, once you finish adding all the amino acids stepwise, then finally, you have to need to cleave this bond here, that is a global deprotection to remove the solid phase to get your acid C terminal back, and at the same time, it is a single step basically, where you can cleave this bond as well as all the protecting groups that you have used on the amino acid side chains.

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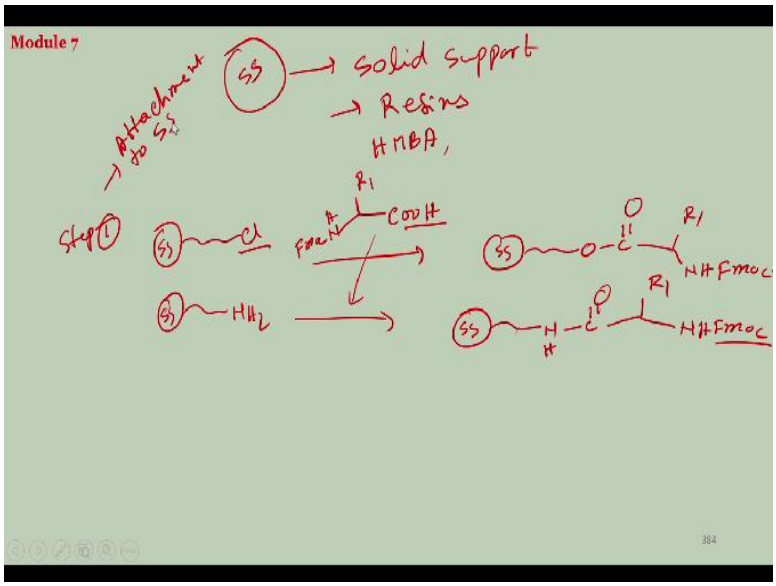

So, if I write down the steps, let us say you have taken our solid support yes solid support. This is usually there are raisins. There are different types of raisins available for peptide synthesis, like HMBA and others. They have different functional groups. They are the different resins are they vary in their functional groups, that are there. So in general, I will take here 1 X, let us say chloro, it can be a chloro, it can even be an amine R 1 NH FMOC.

So this is step 1, this is the reaction between this chloro and this carboxylic acid. So you will have O CO R 1 NH FMOC, if you use amine, then you can have a peptide coupling NH CO. So, you have to use the peptide coupling reagent here, if you have an amine group, then it will have the amide bond here. In that case you will have a hard time cleaving this. For in those cases it cleaves that somewhere else and you get NH 2 amide free at the C terminal NH FMOC. So, that is your first step.

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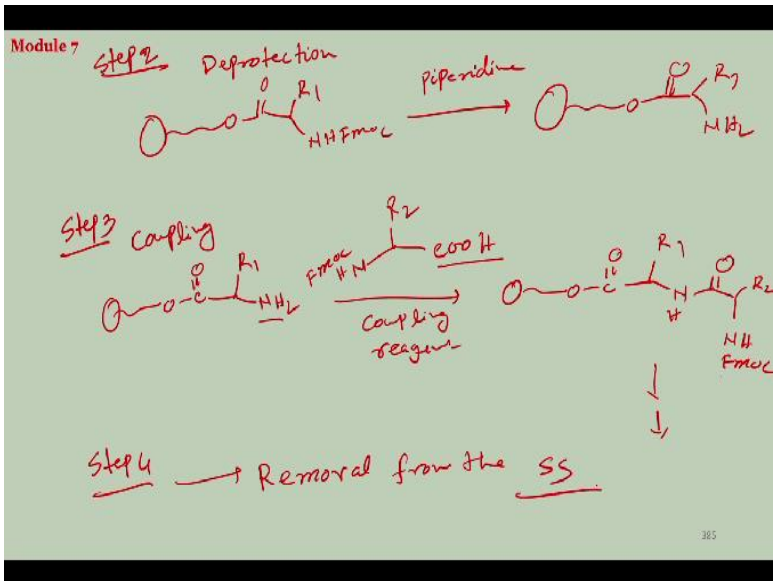

Step 2 is deprotection. So step 1 is actually attachment to solid support. Step 2 is the deprotection, the solid support is there, I am taking the first on OCO NH FMOC, you have to use piperidine, since you are doing the solid phase reaction piperidine use is not a problem because once you remove this, all the piperidine that is present in solution can be washed away O CO R and free NH 2, that is step 2. Step 3 is coupling.

So solid phase O CO R NH 2. Now you add your second amino acid is R 1 R 1, R 1, R 2 FMOC protected, so that this amine does not react. So now we have acid free here, you have amine free here and they can be coupled and this is your monomer. So, every time you will use the same different amino acid but the same FMOC protected and free acid. This is an coupling reagent O CO R NH, CO R 2 NH FMOC.

And likewise you can continue and continue. Finally, step 4 is removal from the solid support and that reagent various so I am not writing that, that is how you can get your short sequences of peptides. So this is all about the solid phase peptide synthesis. And now, I will move on to the other part that the peptide based therapeutics.

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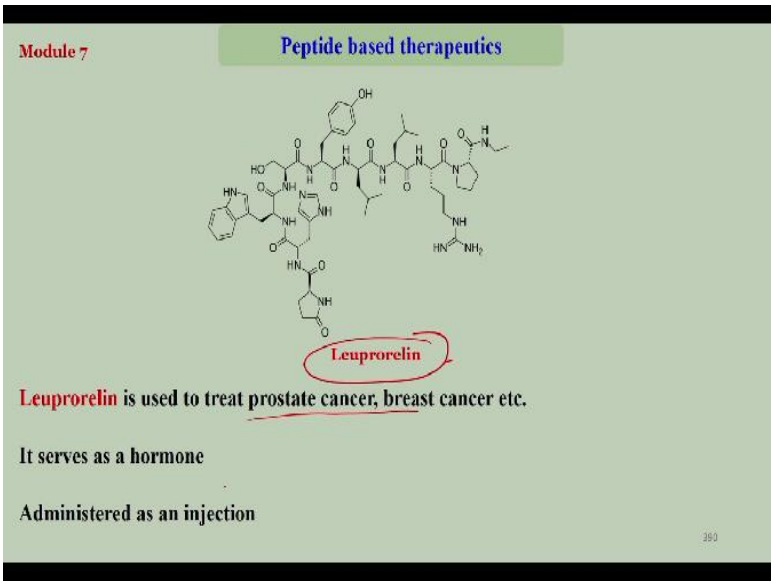

What would the peptides do in understanding or in their applications in understanding biological properties or in their applications. And we have already seen some of them that I have talked about in the last lecture, that there are many peptides that show targeting efficiency such as Creka peptide, RGD Creka, tumor targeting RGD and NGR, these 2 were tumor homing, then polylysine containing or polyarginine containing peptide sequences were good for self intuition and known as self encrypting peptides.

We have similarly shown that blood brain barrier peptides are also there. So, these peptides are indirect applications for as therapeutics because they themselves may not have or they themselves are not that much efficient in curing a disease, these are not the medicines, these are not the drugs, but they help the drugs or other stuffs to be carried over to specific positions and to get into that cells.

Now, we will see there are many number of peptides that actually can act as medicine itself. They are also in the market, you can find many of them, many of the peptide based medicines in the market lots, some most of them are present as injections. So, they are themselves medicines or drugs. So, this peptide leuprorelin has this structure, it is a peptide of course you can see short sequence.

If you start from here, this is a prolene, this is argentine, this is leucine, another leucine, this is tyrosine, this is serine, this is tryptophan, this is your histidine and this is not a natural amino acid. So, this is a modification part, this is slightly modified peptide. This molecule is used as anti cancer agent to treat prostate cancer, breast cancer and other types of cancer also, and it is a marketized drug. So it basically acts as a kind of hormone. And this is administered as an injection.

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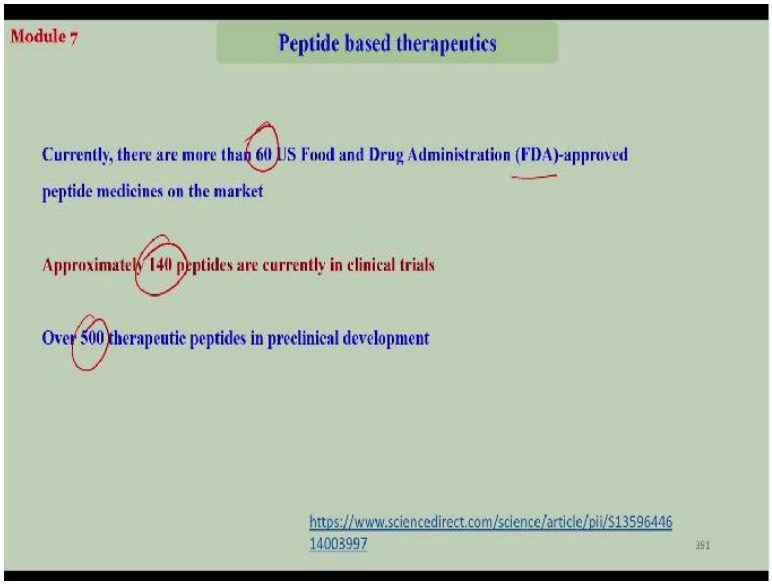

Currently, there are around 60 peptides that are used as medicines that are FDA approved medicines present in the market. And they have a huge turnover billions of dollars actually. For example, this drug has an annual turnover of around 7 billion dollar. So, 60 number of peptide based molecules or peptide based therapeutic agents are present in the market and they are used as medicines.

Approximately 140 peptides are currently in clinical trials and more than 500 peptides are in preclinical development. That means they have shown the efficacy, they have shown the activities, but they are not yet into the last stages of clinical trials, they are in the at the beginning of their clinical trial. So, these informations I have obtained from this journal article. So, I work also on the peptide chemistry and we also have developed some of the peptides. I am not showing it here, some of the peptides for different forms which can act as therapeutics.

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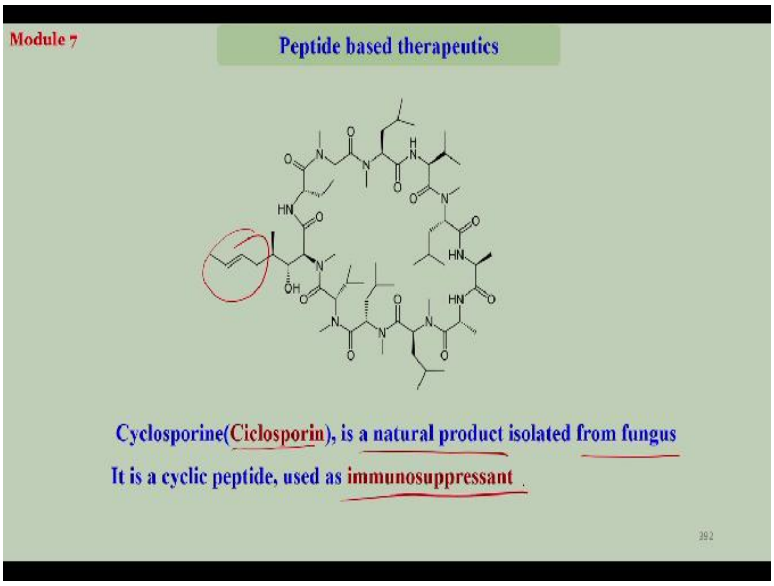

So, this is another example of another peptide named as cyclosporine or this is also named as ciclosporin. This is basically a cyclic peptide with a modification cyclic peptide, this is not only a synthesized peptide, this is also a natural product, it is obtained from natural source from a certain fungus and that shows that is used as immunosuppressant drug.

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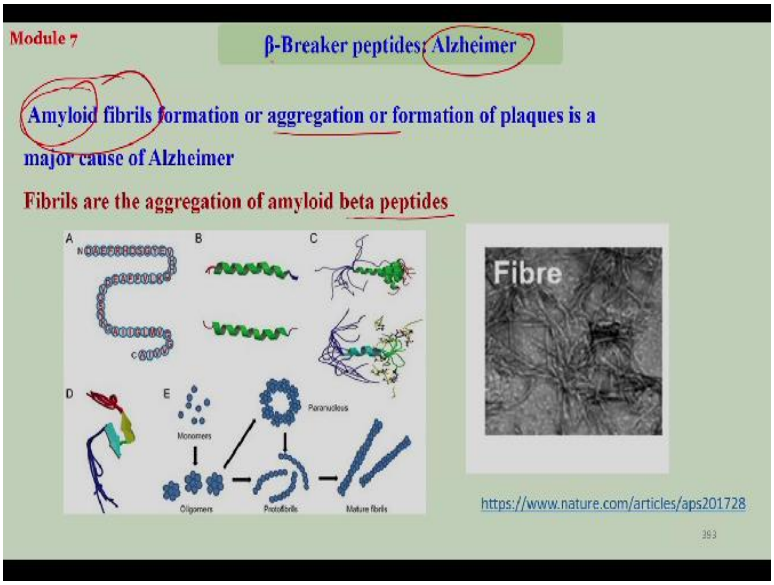

And very importantly nowadays a very hot topic of in the peptide chemistry, in the peptide designing is to break the protein aggregation which is a major cause of Alzheimer's disease. And this type of peptides are known as beta breaker peptides. So, Alzheimer is a notorious disease, which usually happens in old age, and one of the cause of Alzheimer is the formation of amyloid fibrils. Fibrils means fiber formation.

Fiber means they are not soluble in the body if the proteins give becomes fibers strong fibers, they do not become soluble and they cannot function. So, they will be deposited in the brain cells. So, Alzheimer is the basically the problem in the brain cells. So, proteins get deposited in the form of fibers in the brain cells. So, that stacks it up actually. And this is the amyloid proteins which actually most of the times it is the amyloid proteins, which forms the fibers.

So, basically aggregation of the peptides, you have seen earlier, how the beta sheets structures are formed in proteins with the stacking layered by layered stacking interactions right. So, those kinds of aggregations are responsible for the formation of the fibers. Here you can see if you have this kind of, this is the primary sequence. This is the folded structure and they it forms they start aggregating under certain conditions.

And form the fibrils and then you have the fiber formation basically that does not move that is not becomes insoluble. This is a picture that shows how the fibers are formed. So, fibrils are the aggregation of amyloid beta peptides, beta is a part of the protein structures. I have also mentioned this before now, whole protein structure when you consider the whole protein may not have fully beta sheet structure or fully alpha helix structure.

A part of the protein will remain as alpha helix or other parts of the protein may stay as beta sheet structures. So, in this case amyloid beta peptide the part where the beta sheet conformation occurs that is responsible for the aggregations because of the layered structures. So, the cure is that to break this fiber formation either to break this fiber formation or to prevent such kind of fibers to be formed.

So, both ways and nowadays people are developing, trying to develop good peptides, good peptide sequences, synthesized peptide sequences, which can block the formation of the aggregation or after a aggregation which can break the aggregation also. So those are called the beta breaker peptides. There are some popular ones.

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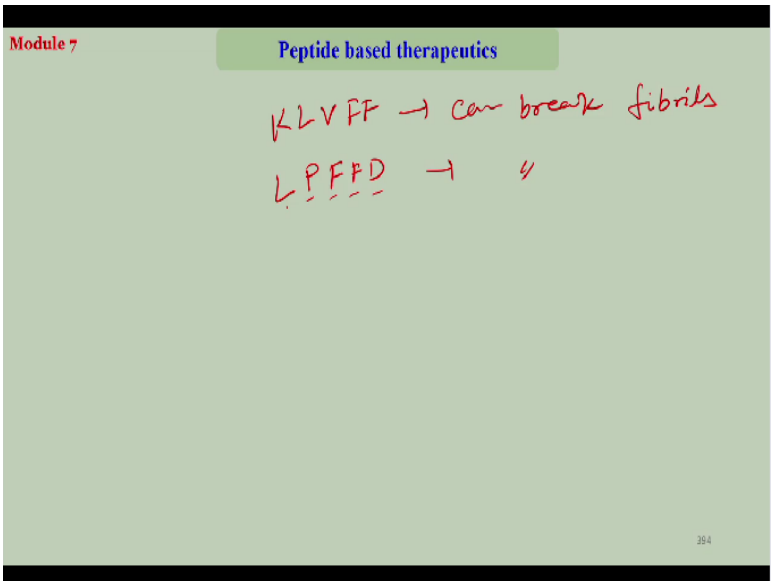

Of course, none of them has come to market yet. For example KLVFF is one kind of peptide that shows that has the tendency to break, that can break fiber formation, that can break the fibrils. So, K is your lysine, L is leucine, V is valine, phenylalanine, phenylalanine. Similarly, LPFFD also does the same thing, they break up fibrils or they are inhibitor of fibril formations, L is leucine, P is prolene, F is phenylalanine, phenylalanine, aspartic acid.

So, there are many other examples available in the literature you can go through it. So, with this I will conclude the synopsis of module 6 and 7.

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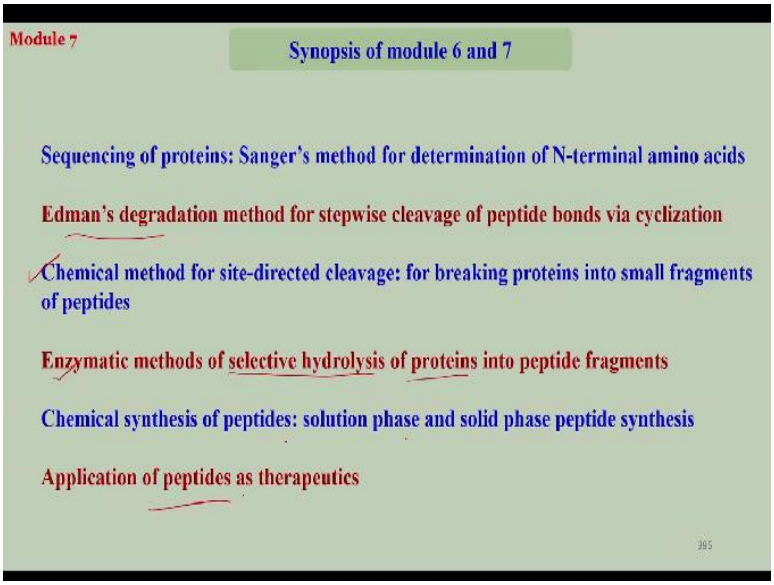

Module 6 I have not shown that the summary. So, in this 2 modules combined what we have talked about that we have seen how the sequencing of proteins can be done using the Sanger's method as well as using the Edman's degradation method and out of this Edman's degradation method was used for stepwise cleavage of peptide bonds. So, it is the key method that is used for protein sequencing.

Then we have seen how we can break the full protein into its fragments, shorter fragments, and there are chemical methods for it for site specific cleavage, there are enzymatic methods for it where the selective hydrolysis happens or selective hydrolysis of the peptide bond occurs. So that you can break a protein into it is smaller peptide fragments. And then now we have seen the chemical synthesis of peptides.

Both solution phase and solid phase peptide synthesis have been shown. And finally, we have given a little bit of idea, or a little bit of the application but how the peptides can be used as therapeutics. So this is all about this module 6 and 7. And the next lecture we will start the new module. Thank you.