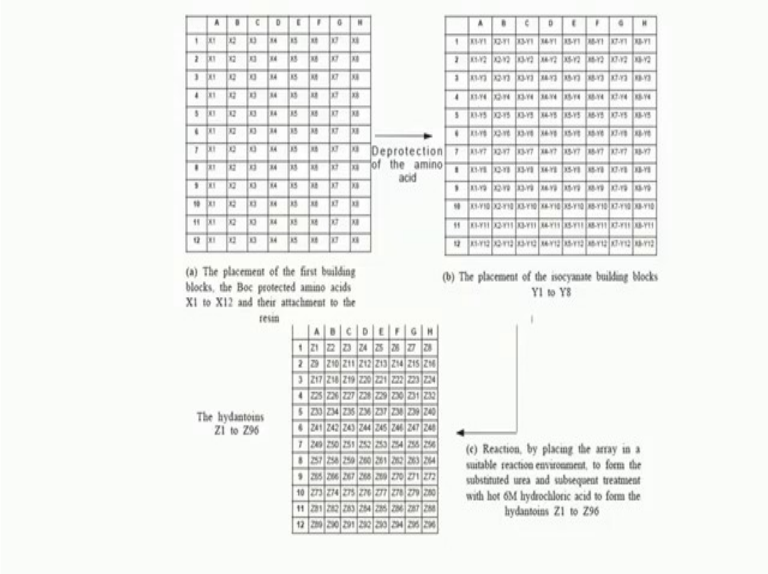
# Organic Chemistry In Biology And Drug Development
**Prof. Amit Basak**
**Department of Chemistry**
**Indian Institute of Technology, Kharagpur**

## Lecture - 43
## Combinatorial Chemistry

Welcome back to this course on Organic Chemistry in Biology and Drug Development. In the last session, we were discussing about combinatorial chemistry which is a technology that has been developed to produce a large library of compounds and then testing it through high throughput screening.

(Refer Slide Time: 00:54)



| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| 1 | X1 | X2 | X3 | X4 | X5 | X6 | X7 | X8 |
| 2 | X1 | X2 | X3 | X4 | X5 | X6 | X7 | X8 |
| 3 | X1 | X2 | X3 | X4 | X5 | X6 | X7 | X8 |
| 4 | X1 | X2 | X3 | X4 | X5 | X6 | X7 | X8 |
| 5 | X1 | X2 | X3 | X4 | X5 | X6 | X7 | X8 |
| 6 | X1 | X2 | X3 | X4 | X5 | X6 | X7 | X8 |
| 7 | X1 | X2 | X3 | X4 | X5 | X6 | X7 | X8 |
| 8 | X1 | X2 | X3 | X4 | X5 | X6 | X7 | X8 |
| 9 | X1 | X2 | X3 | X4 | X5 | X6 | X7 | X8 |
| 10 | X1 | X2 | X3 | X4 | X5 | X6 | X7 | X8 |
| 11 | X1 | X2 | X3 | X4 | X5 | X6 | X7 | X8 |
| 12 | X1 | X2 | X3 | X4 | X5 | X6 | X7 | X8 |

(a) The placement of the first building blocks, the Boc protected amino acids X1 to X12 and their attachment to the resin

Deprotection of the amino acid

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| 1 | X1-Y1 | X2-Y1 | X3-Y1 | X4-Y1 | X5-Y1 | X6-Y1 | X7-Y1 | X8-Y1 |
| 2 | X1-Y2 | X2-Y2 | X3-Y2 | X4-Y2 | X5-Y2 | X6-Y2 | X7-Y2 | X8-Y2 |
| 3 | X1-Y3 | X2-Y3 | X3-Y3 | X4-Y3 | X5-Y3 | X6-Y3 | X7-Y3 | X8-Y3 |
| 4 | X1-Y4 | X2-Y4 | X3-Y4 | X4-Y4 | X5-Y4 | X6-Y4 | X7-Y4 | X8-Y4 |
| 5 | X1-Y5 | X2-Y5 | X3-Y5 | X4-Y5 | X5-Y5 | X6-Y5 | X7-Y5 | X8-Y5 |
| 6 | X1-Y6 | X2-Y6 | X3-Y6 | X4-Y6 | X5-Y6 | X6-Y6 | X7-Y6 | X8-Y6 |
| 7 | X1-Y7 | X2-Y7 | X3-Y7 | X4-Y7 | X5-Y7 | X6-Y7 | X7-Y7 | X8-Y7 |
| 8 | X1-Y8 | X2-Y8 | X3-Y8 | X4-Y8 | X5-Y8 | X6-Y8 | X7-Y8 | X8-Y8 |
| 9 | X1-Y9 | X2-Y9 | X3-Y9 | X4-Y9 | X5-Y9 | X6-Y9 | X7-Y9 | X8-Y9 |
| 10 | X1-Y10 | X2-Y10 | X3-Y10 | X4-Y10 | X5-Y10 | X6-Y10 | X7-Y10 | X8-Y10 |
| 11 | X1-Y11 | X2-Y11 | X3-Y11 | X4-Y11 | X5-Y11 | X6-Y11 | X7-Y11 | X8-Y11 |
| 12 | X1-Y12 | X2-Y12 | X3-Y12 | X4-Y12 | X5-Y12 | X6-Y12 | X7-Y12 | X8-Y12 |

(b) The placement of the isocyanate building blocks Y1 to Y8

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| 1 | Z1 | Z2 | Z3 | Z4 | Z5 | Z6 | Z7 | Z8 |
| 2 | Z9 | Z10 | Z11 | Z12 | Z13 | Z14 | Z15 | Z16 |
| 3 | Z17 | Z18 | Z19 | Z20 | Z21 | Z22 | Z23 | Z24 |
| 4 | Z25 | Z26 | Z27 | Z28 | Z29 | Z30 | Z31 | Z32 |
| 5 | Z33 | Z34 | Z35 | Z36 | Z37 | Z38 | Z39 | Z40 |
| 6 | Z41 | Z42 | Z43 | Z44 | Z45 | Z46 | Z47 | Z48 |
| 7 | Z49 | Z50 | Z51 | Z52 | Z53 | Z54 | Z55 | Z56 |
| 8 | Z57 | Z58 | Z59 | Z60 | Z61 | Z62 | Z63 | Z64 |
| 9 | Z65 | Z66 | Z67 | Z68 | Z69 | Z70 | Z71 | Z72 |
| 10 | Z73 | Z74 | Z75 | Z76 | Z77 | Z78 | Z79 | Z80 |
| 11 | Z81 | Z82 | Z83 | Z84 | Z85 | Z86 | Z87 | Z88 |
| 12 | Z89 | Z90 | Z91 | Z92 | Z93 | Z94 | Z95 | Z96 |

The hydantoins Z1 to Z96

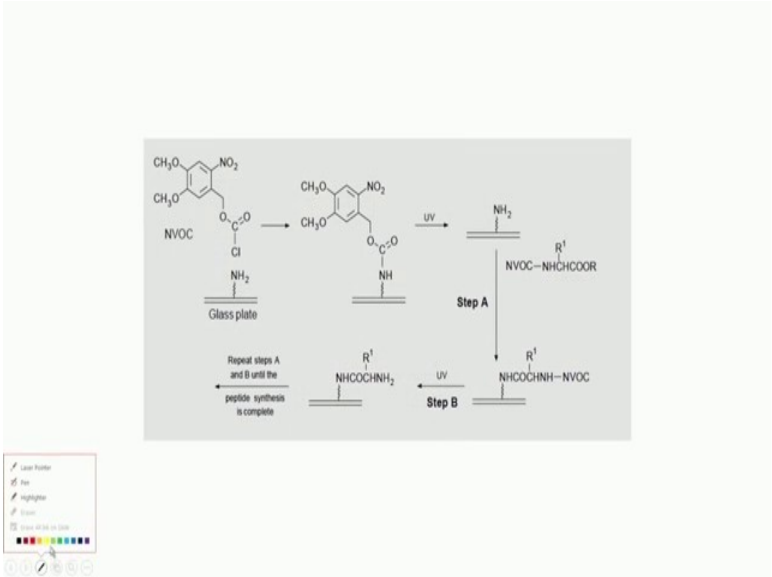
(c) Reaction, by placing the array in a suitable reaction environment, to form the substituted urea and subsequent treatment with hot 6M hydrochloric acid to form the hydantoins Z1 to Z96

And the whole exercise is done to have a quick access to the hit compound, so that the hit compound can be found, which would pave the way for finding the lead compound; and ultimately lead optimization. This process, usually in earlier days, it was taking lot of time because the biological screening was taking a lot of time. So, even if you make few compounds per day or per week that would have been sufficient for a testing within the time framed by the biologist.

Now, because of the advent of high throughput screening, there is a demand that you produce lot of compounds in a particular day or two. And then get it tested as quickly as possible, because the whole idea of medicinal chemistry or drug development is that one should really very quickly know what are the failure compounds which are going to fail or which are failing in the high throughput screening and try to pick out the lead compounds or the hit compounds as early as possible without wasting much time and energy.

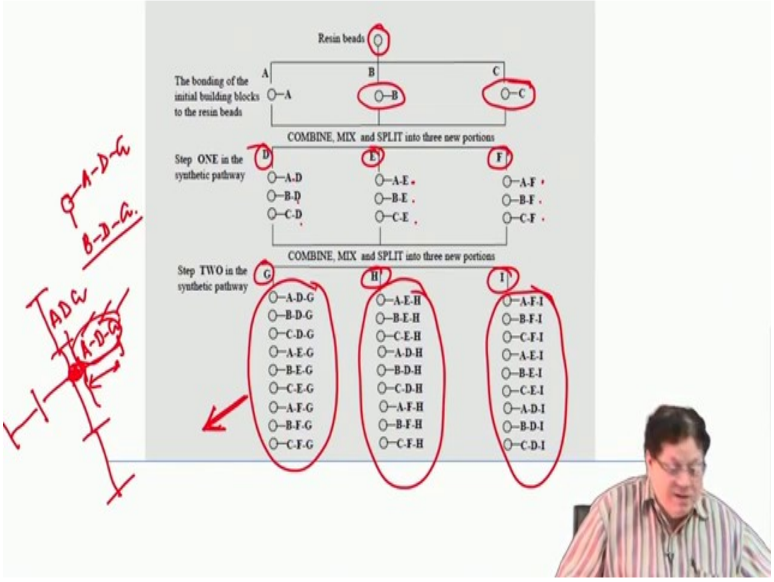
So, towards that end, this combinatorial chemistry was developed. This is basically a synthetic technology in which a large library of compounds are made. Basically there are two techniques, one is called parallel synthesis and the other is mix and split method. In parallel synthesis, this is basically a 96 well plate, in each well there is a bead, there are few beads, and then you have produced a particular type of compound, it’s not that in a particular well you have a mixture of compounds. There are several beads.

(Refer Slide Time: 03:03)



Suppose if I take this groove, this groove will have ultimately in the final product which is represented by Z1, but Z1 is basically a combination of X1-Y1, and then you are adding this making the hydantoins, basically it is a two-step process. The first step is that adding the Y1 to the X1 and then you did some reaction; you apply some reaction condition like heating with hot 6M HCl, so that this is a particular type of combinatorial chemistry where you are making hydantoins. And what I am trying to say that each well has only a defined compound and you know what is the structure of that compound.

(Refer Slide Time: 04:05)



On the other hand, if you go to the other technology which is called mix and split, in this case you get a much larger library of compounds, but the problem is that in a particular well or if you are doing it in test tubes or your container, we will have beads where each bead is connected to a particular compound, but you do not know the identity of the compound. It is a collection of beads of different compounds. But only one thing you know that each bead is only connected to one particular type of compound, but you do not know which bead it is.

So, let us start from there where we ended. So, in this mix and split, you take the resin bead attached with the functionality. And then you attach; you divided into three pots. And in this pot, you add A; and in the other pot, you add B and in the third pot, you add C. So, you have resin bead here attached to a resin bead B, and here resin bead is attached to C.

But then you mix these two, take all of them together and then split. So, what will happen? Each of these beads in a particular well or test tube, will have A, will have attachment to B and it will have also attachment with C. So, that means, there are beads which are attached to A, there are beads which are attached to B, there are beads which are attached to C. Now, you basically you have distributed into 3 pots or 3 wells and your adding D in the first pot, E in the second and F in the third.

So, in the process, you are getting here A-D, B-D and C-D. Here you will get A-E, B-E, C-E and so on. So, in the third one, since you are adding F, so A-F, B-F and C-F; then you again mix it and split. So, when you spilt your each well will have beads which are connected to A-D, B-D, C-D, A-E, B-E, C-E as well as A-F, B-F and C-F. Now, what you are doing you have again split it into 3, 3 pots and then add G. So, when in one pot you are adding G, in the other pot you are adding H and in the third one you are adding I.

So, there will be 9 different compounds attached to the beads, attached to separate beads; you must understand this that a particular bead will not have say A-D-G and also attached to B-D- G, that will not happen; because you are covering the functionality (whatever number of functional groups attached to the bead) as they are all attached to either A or B or C, when you started the synthesis. So, each bead is connected to one type of compound not a mixture of compounds.

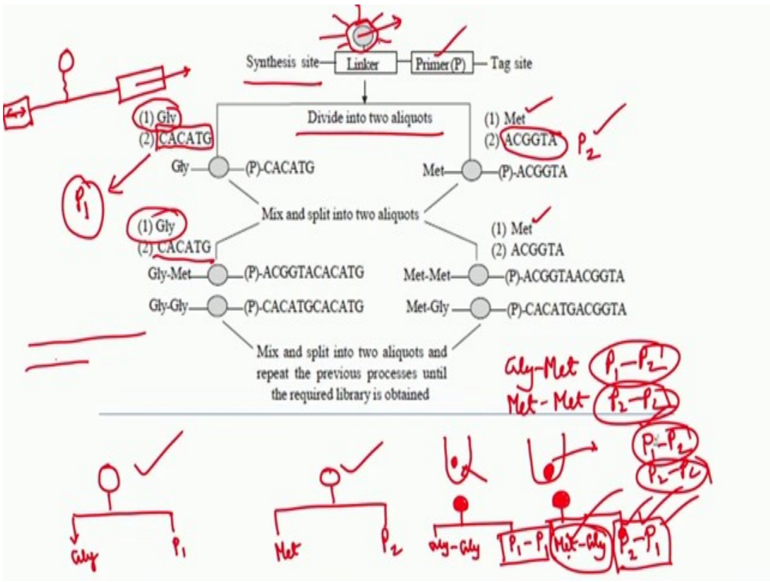
So, now you have 9 compounds here attached to the beads; here also 9 compounds; and here also 9 compounds. Now, you test these beads containing the compounds and see if there is any bioactivity in any one of these wells or pots, wherever you are doing the reaction. Suppose there is some activity shown by this cluster, the next thing is that you do not know actually know which bead is connected to what, but what you know is that out of these beads, at least some compounds are bioactive. So, the problem now is to basically know what is the compound attached to a particular bead; see you can individually separate these beads and also test its bioactivity.

And then suppose I get a bead which shows bioactivity, but I do not know what is attached here, whether it is A-D-G or B-D-G or C-D-G; so, how to do that? Now, you can say that I will take the bead, and then detach whatever compounds are there; remember one bead does not have only one valency, they are polyvalent beads that means, from one bead you can get several molecules of these, but all are same compound; if it is A-D-G, then all are A-D-G here. So, there is no scrambling of the structure of the compound that is attached to a particular bead. But the big question is how to know, what is the compound that is attached to a particular bead; because these beads are not colored, it is not that blue beads are always having ADG, red beads are having other compounds, and each bead is of same colour and everything.

Now, we have to basically deconvolute; you have a bead, which is attached to a compound and that is showing some bioactivity. Now, the task is how to know what compound is attached to the bead? One way is that you break this bond between the bead and the terminal end and see the sequence of these different entities A-D-G or it is B-D-G, you can check that.

But if these are some compounds which are not very easy to do the sequencing; you know sequencing can be done usually on peptides and as well as for nucleic acids, these are easier ones that you can do using Edman degradation or Sangers method as needed. For other compounds, if it has different types of entities A, B, C are different entities, then it will be very difficult to really know what is the structure of the compound that is attached to a particular bead. So, how to know the structure of the compound attached to a bead? There is a technique which is called tagging technique, I will show you what is that.

(Refer Slide Time: 10:38)


Synthesis site — Linker — Primer (P) — Tag site

Divide into two aliquots

(1) Gly
(2) CACATG
Gly—○—(P)-CACATG

(1) Met
(2) ACGGTA
Met—○—(P)-ACGGTA

Mix and split into two aliquots

(1) Gly
(2) CACATG
Gly-Met—○—(P)-ACGGTACACATG
Gly-Gly—○—(P)-CACATGCACATG

(1) Met
(2) ACGGTA
Met-Met—○—(P)-ACGGTAACGGTA
Met-Gly—○—(P)-CACATGACGGTA

Mix and split into two aliquots and repeat the previous processes until the required library is obtained

This is the bead to start with, I said that these are polyvalent that means, there are lot of binding sites here, where I can add A, B or C that is my reacting partner. So, when I have this bead, I have the bead attached to a linker and that linker is distributed in two channels, at one site, you can do one type of reaction and in the other site, you can do the synthesis that you are interested in.

Maybe an example will demonstrate it and it will be easier to understand what I am saying about the tagging method. So, you have a bead, you can forget about the linker for the time being. So, the bead has reactive sites in one reactive site, you are adding A, B, C, D, E, F and on the other site you are adding something which is easy to sequence.

Suppose, I have separated the beads into two aliquots; I am interested in making peptides. So, in one aliquot I added glycine; so, the glycine gets attached at the synthesis site. And at the same time I add some bases, in this case, it is CACATG. So, I add a base, but for understanding you can say that I add something which is denoted as $P_1$, here. And here on the other aliquot, I add methionine, a different amino acid, and I add another set of bases, which is denoted by $P_2$. So, what will happen that this bead which now can be represented that it has got glycine here and on the other side, because I am adding glycine and then also I am adding this $P_1$, which is a combination of bases, the bases that are present in DNA.

So, glycine and on the other valent hand you are having this $P_1$ and in this other aliquot, you have methionine and here you are adding the $P_2$ (another collection of bases). So, then again you mix and split, if you mix and split and then suppose I add again glycine here, so, what I will get? I have two containers. So, when I mix and split, so here the beads will have both the characteristic this as well as that, because I have mixed it and then splitted it.

So, when I added glycine what I will get? Remember whenever I add glycine, I have the same set of bases $P_1$. So, this bead will have glycine and then if I have added another glycine and on the other side at the same time, I add this $P_1$; so $P_1$ will be attached to $P_1$. I also have methionine here so methionine and that will be attached to glycine and in the tagging site that is my $P_2$, and then $P_2$ will be attached to $P_1$, so this is the scenario. Now, suppose I stop at the dipeptide. I see that one bead is giving some activity, then I am interested what I have added here; whether it is methionine-glycine or it is glycine-glycine. I know that if it is methionine-glycine, I will have $P_2$-$P_1$; and if it is glycine-glycine, I will have $P_1$-$P_1$.

So, I make the complementary base sequence and see which gets attached to this base sequence represented by $P_2$ and $P_1$; we call that primer. What is primer? Primer is that if you have a sequence of bases you already know that and if you give the complementary base, then they are going to go and hybridize. So, if it is $P_1$-$P_1$, you make a set of primer which is complementary to $P_1$-$P_1$ and another set of primer which is complementary to $P_2$-$P_1$.

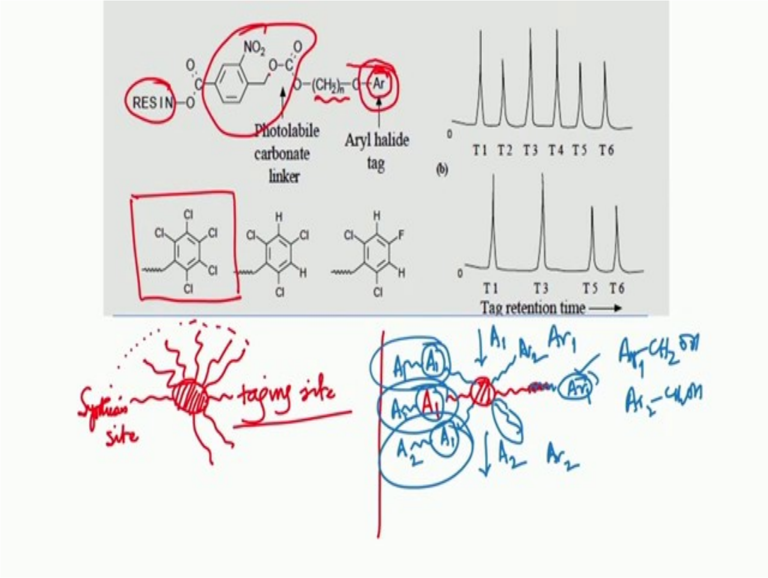
So, now you see that which primer is actually giving the hybridization and then if you see that they are complementary to $P_2$-$P_1$ and hybridizing with that bead tagging site, then you know that it must be having methionine glycine. And once you know that then you can separately make methionine glycine and do the bioassay. Now you know that the first amino acid has to be methionine, so you can take methionine and then other amino acids you can vary, and then optimize the hit.

So, basically you are synthesizing your compounds, at the same time you are adding a tagging entity. In this case, our example was a particular type of base sequence, because the base sequence is easy to detect by synthesis of the corresponding primers and see whether it is hybridizing or not.

So, from that hybridization result, we can tell that what is the tagging code; it is $P_1$ $P_2$-$P_1$ or it is $P_1$-$P_1$; and then you can actually detect the contents of the other test tubes; you are adding again methionine. So, it will be here, it will be glycine and then methionine, so that will be $P_1$-$P_2$ and the other cases $P_2$-$P_2$ that means, when the bead is connected to glycine I know that the it is attached $P_1$ on the tagging site; and when I added the methionine, so that $P_1$ will be attached to $P_2$, because with methionine I add $P_2$.

And then whenever there is glycine-methionine, then the tagging sequence will be $P_1$-$P_2$; and whenever it is methionine-methionine, the tagging sequence will be $P_2$-$P_2$. So, you can get four different tagging sequence attached to the bead, and from the tagging sequence you can tell what is the peptide sequence in the desired compound.

(Refer Slide Time: 18:58)



There are different tagging techniques. I will not go into very details, but I will show another tagging technique. And instead of having this nucleotide bases, you can have other types of tagging systems. Like in this case it is said that resin bead.

In resin bead, you know that there is a synthesis site, where you do the synthesis and there is a tagging site, where you do the tagging. So, whenever you add one component, you have to add the corresponding tag. Now, this tag earlier I told you about the base nucleotide bases, someone has devised this that whenever there is a synthesis done, on the other tagging site, you are adding this nitrobenzyloxycarbonyl attached to a linker that is attached to a substituted aromatic moiety. Again I repeat, the bead has synthesis sites and bead has tagging sites.

One more important point is that the synthesis site is not just one, there may be several synthesis sites and there may be more tagging sites also. Basically when you do the synthesis, you maintain the concentration at such a level, so that the synthesis sites are more or less covered. Leaving the tagging sites, tagging sites are also reactive entities. So, we have to be careful that the tagging site are left free.

Suppose, I put $A_1$ in the tagging site, I put this *via ortho*-nitro benzyloxycarbonyl group. This *ortho*-nitro benzyloxycarbonyl is also photo labile. So, if you shine light here, this goes off and carbon dioxide is liberated, then you are generating aryl halide. So, this is the mechanism that forms the aldehyde and this loses the carbon dioxide and finally, this aryl tag comes out as the benzyl system $CH_2OH$.

So, basically there is a linker which is photo labile group and then you have this aromatic ring $Ar_1$. Now, there are other tagging sites here and there are also synthesis site here. So, you maintain it in such a way that all the synthesis sites are blocked with $A_1$.

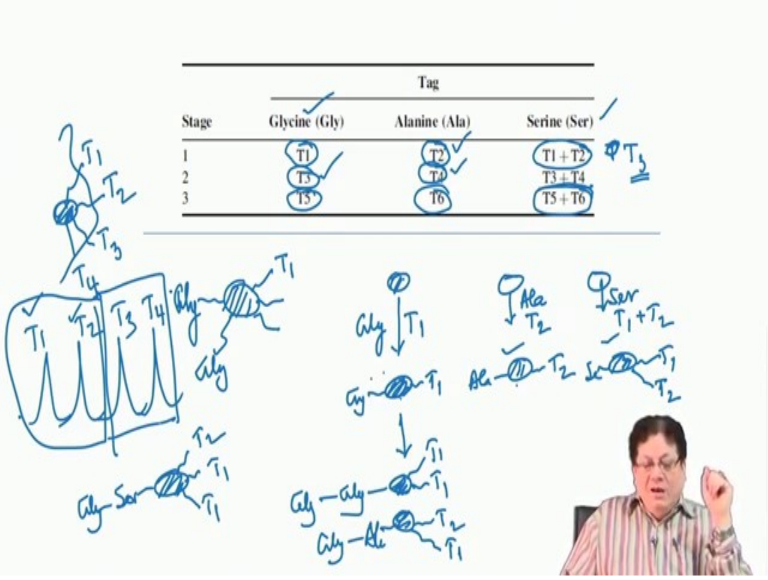
You maintain such a concentration that some of the tagging sites are still vacant. So, whenever you are adding $A_1$, you are adding this benzyloxycarbonyl with Ar at the terminal end with a different $Ar_1$ aromatic aryl ring; some of the tagging sites still vacant. So, when you do the next reaction, which means, in the first reaction you are adding $A_1$ and you are also adding $Ar_1$, $Ar_1$ means via this benzyloxycarbonyl.

And then you are adding now $A_2$; so, when you add $A_2$, you add $Ar_2$. So, $A_2$ will be attached to $A_1$ and this $Ar_1$ actually ends there, because Ar does not react with another Ar. So, because of some of the tagging sites are now vacant, so now this will be connected to $Ar_2$. Still some tagging sites are left vacant; it is just a calibration of the concentration that will work here, so this is a little tricky that you maintain the concentration in such a way that your synthesis sites are all or filled up, but the tagging reactive sites are still free.

So, one site is occupied by $Ar_1$ via this benzyloxycarbonyl, and then the next adjacent site you put a $Ar_2$, when you do the second reaction. And if you do a third reaction you can consider $Ar_3$, because still some tagging sites are left empty, so you can put $Ar_3$. Now, after everything is done, you shine light and when you shine light your benzyloxycarbonyl falls off.

So, what you will get is the $Ar_1$-$CH_2OH$, $Ar_2$-$CH_2OH$, then you will get $Ar_3$-$CH_2OH$. Now, you do a HPLC, what will happen? Each tag is different, so each tag will show its profile in the chromatogram. And depending on the number of tags or number of peaks corresponding to different tags, you can identify what is the sequence of these entities $A_1$, $A_2$, $A_3$.

(Refer Slide Time: 26:16)



| | Tag | | |
|---|---|---|---|
| Stage | Glycine (Gly) | Alanine (Ala) | Serine (Ser) |
| 1 | T1 | T2 | T1 + T2 |
| 2 | T3 | T4 | T3 + T4 |
| 3 | T5 | T6 | T5 + T6 |

Whenever you add glycine, you have these tagging sites, so you add $T_1$. So, basically you are adding glycine, so you are adding $T_1$. These beads are distributed suppose in three pots. So, in the first bead you are adding glycine and you are adding a tag $T_1$.

In the second container, you are adding alanine and you are adding the tag, tag means representing the aromatic ring, because *ortho*-nitro benzyloxycarbonyl is common and then the linker is also common. So, the substitution pattern is different in the aromatic ring, so you add $T_2$. And then here you add suppose serine and when you add serine, you do not need to add another tag $T_3$, but you can add $T_1$ and $T_2$ both.

So, what will happen here the bead will have glycine and then $T_1$, and this bead will have alanine and $T_2$, and in case of serine you have $T_1$, $T_2$.

Now, suppose I stop here, I mix the beads, and then I distribute. So, I can now mix it and then split it into 3, and then I add another glycine. So, in this pot, because I have mixed all these, so it will have glycine and whenever I add glycine, I add $T_1$. So, whenever there is glycine, it will only have $T_1$. I repeat, each pot now will have all the 3 components.

So, I will have alanine here and this alanine will be attached to glycine, but $T_2$ is already attached to the alanine bead. Whenever I add glycine I add $T_1$, so that will have $T_2$ and $T_1$. And then I have the third one that is serine. Serine already has $T_1$ as well as $T_2$. Now, I have added glycine, I add only $T_1$.

Now, if you stop at the stage one, we have mixed it and I want to know the compound attached to each bead; whether it is attached to glycine, whether it is attached to serine or whether it is attached to alanine. How do you decide? You separate the bead and then strip off this by shining light, so $T_1$, $T_2$s will fall off, containing different aromatic rings.

Now, you push it into the HPLC, you get a chromatogram and then you match with your reference one that where the retention times of each of these tags are there. Now suppose I stopped at the first stage; question is whether the bead is attached to glycine or it is attached to alanine or it attached to serine, how do I know? I just strip off this $T_1$, $T_2$ all these tags and then push it into the HPLC or GC.

I will get the different peaks corresponding to this $T_1$, $T_2$, $T_3$. And what happens that if the bead is attached to only glycine, I will see only $T_1$, because whenever I added glycine I add only $T_1$. If I see that there is $T_2$ coming out as demonstrated by HPLC, then I know that the bead is attached to alanine. If I see that both $T_1$ and $T_2$ are coming, then I know that it is serine.

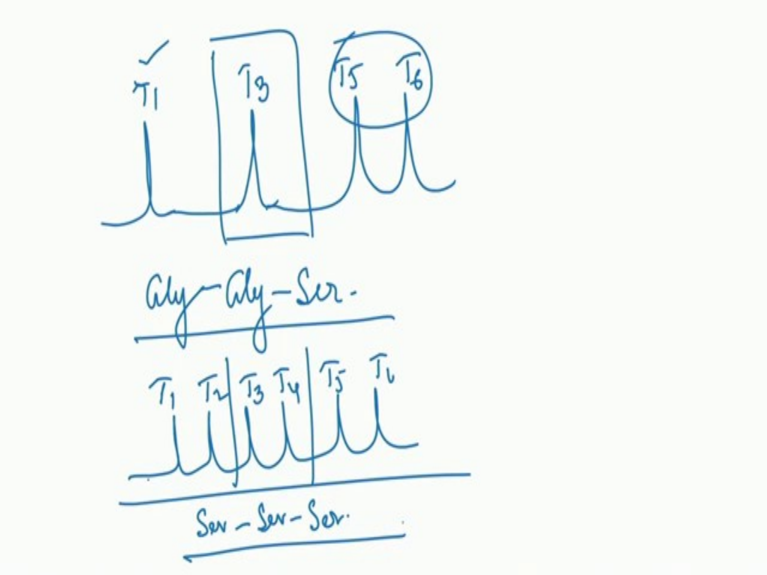
So, basically this process allows you to combine these $T_1$, $T_2$ and $T_1$ plus $T_2$; you are not adding another extra $T_3$. So; that means if you want to discriminate between 3 substrates, in this case glycine, alanine and serine, you need two tags. One separate tag for glycine, a separate tag for alanine and a combined tag for the serine.

So, if you carry on the synthesis of glycine, you introduce $T_3$ another tag. So, whenever you are adding the second glycine you add $T_3$, when you are adding the second alanine your adding $T_4$, when you are adding the serine you are adding $T_3$ plus $T_4$. And if you make a tripeptide, then when you add glycine you add $T_5$, when you add alanine you get you add $T_6$, and when you add serine, $T_5$ plus $T_6$ ok, so that means, you have made

9 compounds, but you have used only 6 tags; you are not using 9 tags; because you have a combination of tags. So, now suppose I see that in the bead I strip off, the tags attached are here $T_1$, $T_2$ and suppose I also see $T_3$ and I also see $T_4$. I stop at the $T_3$ and $T_4$.

I take the HPLC chromatogram and what I see that I could see $T_1$, I could see $T_2$, I will see $T_3$ and I will see $T_4$. So, if I see $T_1$ and both $T_2$, because they are involved in only the first step of the synthesis that means, it must be serine as the first amino acid. Because, if the first amino acid had been glycine I would not have seen the $T_2$. If I only see $T_2$, then I know that the first amino acid is alanine, but I am seeing $T_1$ plus $T_2$ if that be the case that means, the first amino acid is serine. What about the second amino acid, the second amino acid I will see what are by tags $T_3$ or $T_4$ or a both $T_3$ and $T_4$. So, here I am seeing both $T_3$ and $T_4$ if that be the case that means, the second one is also serine, so that means I am having serine-serine linkage.

(Refer Slide Time: 34:48)



And so now I can develop a problem like this, suppose I see $T_1$ I do not see $T_2$, I see $T_3$, I am making the tripeptide now; and then I see $T_5$ and $T_6$. So, what is the sequence of the amino acids? In the first one I am seeing only the $T_1$, now $T_1$ is given only when the glycine is there. And then $T_3$ second one, the possibilities are that I will see only $T_3$ or I will see only $T_4$ or I will see $T_3$ plus $T_4$. Here I see only $T_3$, so it must be glycine.

And then I see $T_5$ and $T_6$ together, if I see both together that is the third step that means, it is serine, so that is the sequence of the compound attached to a particular bead. So, this techniques actually simplifies if you increase the number of amino acids, but proportionately you are not increasing the number of tags. Basically it is like a binary system that if you have 0 and 1, you can get a combination out of only 0, 1.

On the other hand, suppose if you have $T_1$, $T_2$, $T_3$, $T_4$, $T_5$, $T_6$, then what will be the sequence of your peptide it will be $T_4$, $T_5$, $T_6$. So, I get $T_1$ plus $T_2$ that means it is serine; I get $T_3$ plus $T_4$ that means this is also serine; and I get $T_5$ plus $T_6$ that means that is also serine so that is the peptide tripeptide.

There are other methods of doing this; I just mentioned two methods, one is this primer based method where you add the DNA base sequences attached as the tagging entity. And then finally, you add the primer and then see which primer is hybridizing with the nucleotide sequence that is attached to a bead as the tag.

And the second one you are taking a photo labile, *ortho*-nitro benzyloxycarbonyl attached through a linker to an aromatic ring, these aromatic rings are differently substituted. So, they will have different retention times, so that acts as $T_1$, $T_2$, $T_3$, $T_4$, $T_5$, $T_6$ and the trick is that you use a two tags for three components that reduces the number of tags, because after all that will be expensive. If you need the similar number of tags, so that will be economically more expensive than if you reduce the number of tags.

Next we will go to some medicinal aspects, but before that we have now discussed the drug discovery process, in general what is done, what are the different steps, how you do that; and the second thing that we did is the combinatorial chemistry that is the requirement for the day that how to get a large library of compounds, and then get it tested very quickly. And also how to know the structure of the compound that you are generating which is attached to a particular bead.

Thank you.