Lecture 22B • 06/12/12

[lab quiz – Merrified resin, BOC, DCC][exam 3 – amino acids, d/l, pI, acid-base, electrophoresis, ion-exchange chromatography, ninhydrin, synthesis of amino acids, protein structure, sequencing, Edman degradation][ACS exam][clear as mud]

[methionine][writing amino acids with amino end left is standard][tyrosine][indole][tryptophan – not enough in turkey to truly make you sleepy, related to serotonin]

Strecker synthesis [need to cover HVZ]

[It] starts with an aldehyde, from which a very simple imine is generated. We take a trace of acid and ammonia and we make an imine. Pretend for just a moment that we didn't do this step; pretend that we still had a carbonyl. If I then showed you sodium cyanide under acidic conditions; if this was a carbonyl, what would you have said the type of reaction that would be possible? [You would make a] cyanohydrin. Same thing does happen, even if we have the imine. You can end up with, since we're in acidic conditions, an ammonium group, then right next to that, a nitrile. If you hydrolyze it – strong acid, heat, and water – that nitrile turns into a carboxylc acid that you still have the amino group on, so you've made an alpha-amino acids. The one drawback is the same drawback that we had with the phthalimidomalonic ester synthesis: we're only able to make enantiomers this way. [separating enantiomers using biological agents]

Separating diastereomers

Let's say that we had the following: methyl 2-oxo[propanoate]. When we use sodium borohydride [on a] ketone that's also an ester, what happens? Just the ketone [is reduced] because the ester's not reactive enough. But, is there any control of stereochemistry in this reaction? Is there any way to make just one version of this molecule or the other? Right now, where the carbonyl is, the ketone, we have a carboxylic acid on one side, a methyl group, and then when we reduce, we're going to have a hydrogen and make an alcohol, which, since we have four different substituents, is there a way to control how that happens? It's planar, so the hydrogen could attack from either side. What happens when we do the second set of conditions here? Assume they're not strong enough to dehydrate; assume that all we're doing is hydrolyzing this compound. If we really wanted to be safe I could say this: saponify, and then use gentle acid work-up. You'll get two materials, both of them carboxylic acids, both of them only having one stereocenter. They're enantiomers. What physical properties do they share in common? Everything, except for [two], except for optical rotation [and the new property]. So can these easily be separated? Optical rotation is something that appears only when you shine light through it [can light be used to separate compounds]. No, they cannot be easily separated, because we use things like chromatography ... there are such things as chiral columns, columns that are filled with a chiral material that therefore can separate based on size. When we do things like melting point, these have the same melting points; you can't separate them. Same boiling point, same index of refraction, same density, everything except their shape.

But what if we did this: that that mixture of compounds, and react them with something like this. That's just an amine, not an amino acid, but it's a chriral amine, and it's going to react with both compounds, cause you'll have an acid-base reaction. Since you react them at the same time, it doesn't matter which the amino group ends up getting connected to. The amino group's only got one stereoconfiguration, so whichever molecule it ended up with, it'd be the same counterion. What is the configuration of that nitrogen stereocenter, R or S? R. The structure on the left where you had a wedge, is that stereocenter R or S? It is also R. Which means the other structure is S, because this is the mirror image. These two structures, these two salts, together are diastereomers. Diastereomers have different physical properties from each other, so at this point, if they stay in salt form, they behave differently enough where now they could be separated. This is using what is known as a chiral auxiliary – some other molecule that's thrown in to differentiate the behavior of these two enantiomers.

[enantiotopic protons] If we have a compound that is in achiral solvents, maybe it won't have any differentiation of behavior under NMR; it is when we put it in chiral solvents that we then have an issue – that would have been the case for enantiotopic protons. Here, we have enantiomers, which are identical to each other, until they interact with some other chiral unit, which turns them now not into enantiomers any more, but into diastereomers. Diastereomers have different physical properties, which, if you use the right kind of salt, you get enough difference that you can separate the two compounds. In this specific example, since all I did was make a salt, we could acidify afterwards, release the carboxylic acid, and we've separated out the two enantiomers. This is enantiomeric separation using an auxiliary.

The hydroxyacids shown above are enantiomers, which means they cannot be easily separated, since all their physical properties are identical with the exception of optical rotation [and new property]. We throw another compound at them, something that's got a chiral center. For this to work, it either needs to be chemically-bonded, so it becomes truly one molecule, or in this case we could use salt formation, because the counter-ion, the ammonium ion is the same for both salts, so if exchange occurs, it doesn't matter. If a chiral auxiliary is reacted with the two acids, diastereomers are formed. Since diastereomers have different physical properties, the salts can be separated.

Add acid for the carboxylic acids to be reprotonated. Since we've separated out the diastereomers, turned it back to carboxylic acids, that means we've separated out the enantiomers. [enzymes]

Protein structure

There's four forms of structure: primary, secondary, tertiary, quaternary. Each one of them is a different level of organization. The most fundamental level of organization is simply what order do amino acids appear in a protein. We list these typically from the amino to the acid terminus.

We've seen the Edman degradation, which can be used to pull one amino acid from the chain off at a time. The reason we would do that is to come up with the sequence. [However,] you cannot do an infinite number of degradations. There are other techniques that can be used to cleave a peptide chain at different places. WIth enough information, you can put those pieces back together, but if you're able to separate the smaller pieces, sequence those smaller pieces, then by extension you've sequenced the larger protein structure. Cleaving a peptide chain at a methionine unit. Let me draw up a portion of an amino acid chain that has methionine on it. This can react with the compound cyanogen bromide, which is the name for this molecule. It looks like a cyanide or a nitrile, except we have a bromine instead of a hydrogen or a methyl group or an alkyl group attach. It turns out that sulfur can undergo an Sn2 reaction, even if it only has alkyl groups on it like this. Sulfur's much less electronegative than oxygen, much more able to handle positive charge; it's also big and squishy, which [lead's to] sulfur's ability to perform well in Sn2 reactions, because it's able to delocalized or give off some of its electron density to start that process of bond formation more easily than the small, compact, greedy oxygen. This is just a plain Sn2 substitution, and it's not so bad even though we ended up with a positive-charged sulfur.

What does this reaction do? It makes the sulfur a leaving group. We have this nitrogen you can see mechanism arrow coming from; this you can imagine being due to the fact that nitrogen easily delocalizes with the carbonyl, this is why amides are not terribly reactive. That positively-charged sulfur [can come near to] that carbonyl oxygen that amide is in delocalization with. That carbonyl can open up and intramolecularly attack to kick off the sulfur as a leaving group. When you do this, you end up with a derivative of a lactone. It turns out that, once you've done this reaction, you hydrolyze it with acid and water, and it goes through a two-step degradation: first, it goes from being the nitrogen equivalent of a lactone to just a plain, old lactone. Notice that the rest of the protein chain is cleaved at this point. Of course, under these kinds of acidic conditions, eventually the lactone is going to open up to give us a carboxylic acid and an alcohol. What matters is the fact that we cleaved the chain. We made a smaller piece; we could go try to sequence this piece. Knowing we had done this disconnection, eventually stitch these back together though logic; that gives us the larger protein structure.

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A few more amino acids:

Synthesis of amino acids
Strecker synthesis

The hydroxyacids shown above are enantiomers, which means they cannot be easily physically separated since their physical properties are identical (with the exception of optical rotation, which cannot be used to separate them). If a chiral auxiliary (extra molecule) is reacted with two acids, diastereomers are formed. Since diastereomers have different physical properties, they can be separated. Once the original carboxylic acids are recovered, they will have been effectively separated.

Protein structure
1° – sequence – the order of amino acids
Cleavage at methionine

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Structures *(remaining structures identical to lecture 22A)*

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