Summer School of Science
28th July – 7th August
Požega, Croatia

Mimicking nature in a chemistry lab

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Abstract

Amino acids are one of the most important biomolecules in the Nature. All amino acids possess amino and carboxylic group, hydrogen atom and side chain attached on the central carbon atom. In the Nature exist twenty different amino acids that differ in the side chain and the structure of the side chain influences on the properties of amino acids like as solubility, polarity, separation, purification and reactivity. Nineteen amino acids are linear and only one amino acid is cyclic – proline. All natural amino acids have L relative configuration and *S* absolute configuration, except cysteine who has *R*. Amino acids are the building blocks for bigger structures like peptides and proteins. Here we reported about amino acids and synthesis of peptides, their detection, separation, purification and identification. We explained all process for the synthesis of tripeptide starting from the amino acids valine, phenylalanine and leucine.

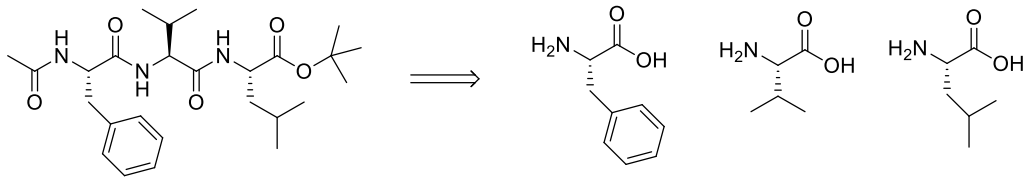
Contents

1. INTRODUCTION

The investigations in chemistry of biomolecules are very important because we can detect the way on which biomolecules participate in the processes in living beings. Furthermore, it's important to know the mechanism of reactions of biomolecules in the body that we can detect problems and treat diseases. However, for investigation on this area we should have knowledge, skills, money, equipment and most importantly, biomolecules. These molecules can be isolated from natural sources, but it is a very complicated process which takes much time and money and at the end, we get only a little amount of product. Since the amounts we get are insufficient for investigation, the process should be repeated a few times. In order to get more biomolecules, scientists have been discovered different methods for the synthetize of biomolecules in a laboratory, as peptides, proteins, nucleic acid, lipids etc.[1]

2. AIM

The aim of this project is learn how to prepare natural products in the laboratory with help of organic chemistry, organic synthesis. The main focus is learn all process of synthesis which include database searching, planning and conduction of synthesis, isolation and identification of compound(s). This project is based on the naturally occurring compounds - amino acids and synthesis of peptides. Our aim is preparation of tripeptide **5** starting from free amino acids – phenylalanine, valine and leucine and following the procedure for the synthesis of peptides (Scheme 1).



(*S*)-*tert*-butyl 2-((*S*)-2-((*S*)-2-acetamido-3-phenylpropanamido)-3-methylbutanamido)-4-methylpentanoate (**5**)

Scheme 1. Retrosynthesis of protected tripeptide.

3. THEORETICAL INTRODUCTION

3.1. Amino acids

In our cells exist different compounds which are important for life and good health. There we can find, for example, the DNA, different carbohydrates and proteins, which are the most important building blocks of a cell.[1] Every protein is made of different building blocks called **amino acids**. There are 20 different amino acids in the Nature which are crucial for life and are produced in the body, but some of them can't be produced and must be obtained through food. They call *essential amino acids* and include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Deficiency of some amino acid can leads to serious diseases, which are related with growth problems, hair loss, walking problems etc. To investigate and solve the problems related to proteins, peptides and amino acids, we can isolate them from Nature or prepare in a laboratory.

Amino acids or α-amino carboxylic acids are the group of organic compounds which consist of a central carbon atom (α-carbon), amino ($-NH_2$), carboxyl (-COOH) and R group (Figure 1).[2] The difference between the amino acids is in their side chain (R group) (Figure 2). All amino acids possess α-amino group, except proline who is a cyclic amino acid and possesses α-imino group.[3]

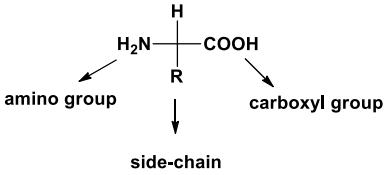


Figure 1. Structure of an amino acid.

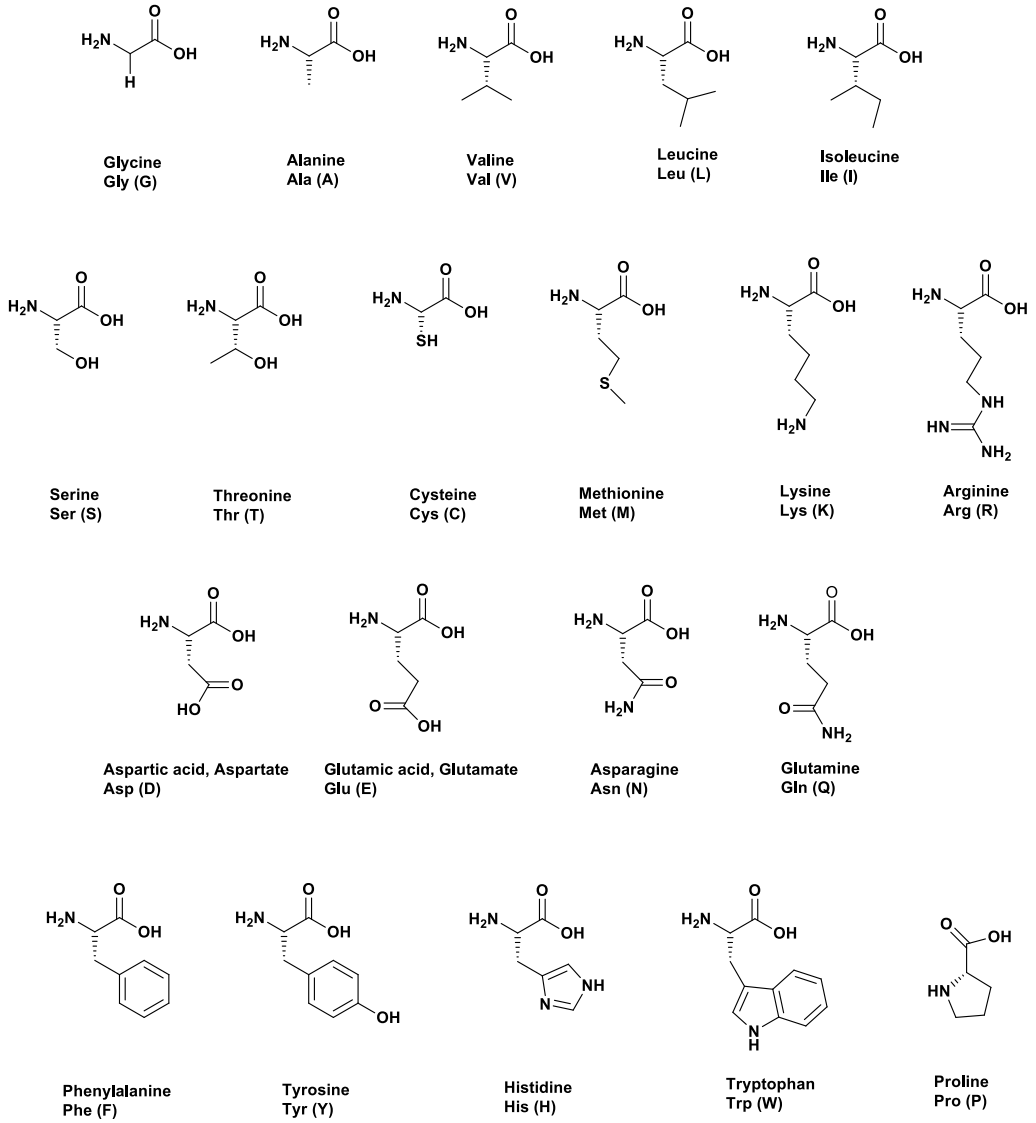


Figure 2. Structure of amino acids

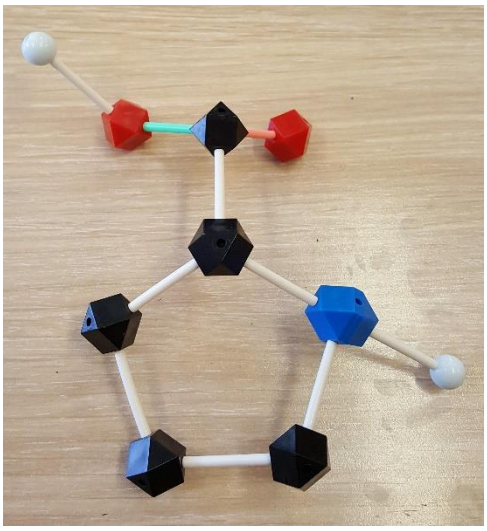


Figure 3. Amino acid proline made of plastic model in the Summer School.

3.2. Properties of amino acids

The properties of amino acids, like solubility in a solvent, possibility for some reactions, etc., depend of an amino and carboxylic group and side chain. All amino acids can be classified as acidic or basic and as hydrophilic or hydrophobic (Figure 4).[2]

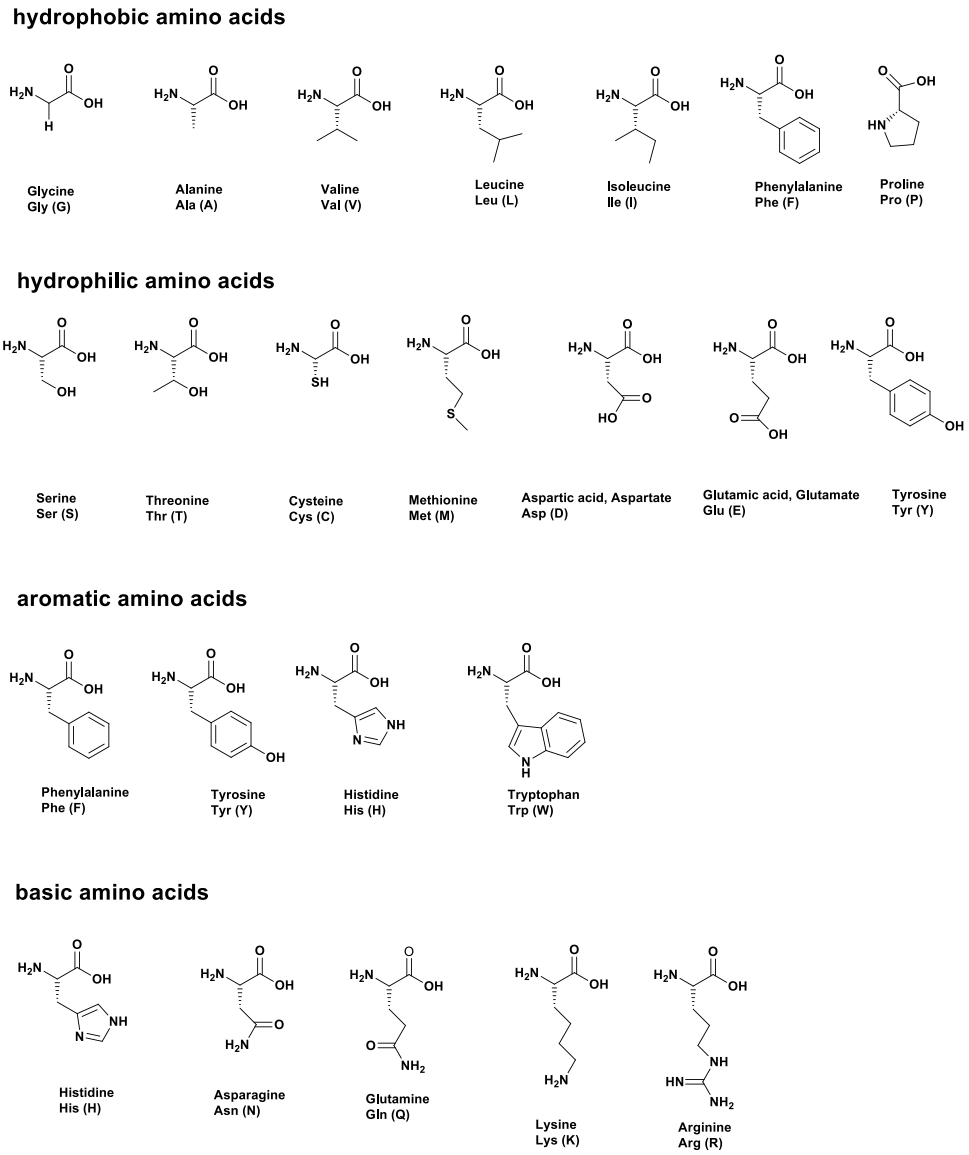
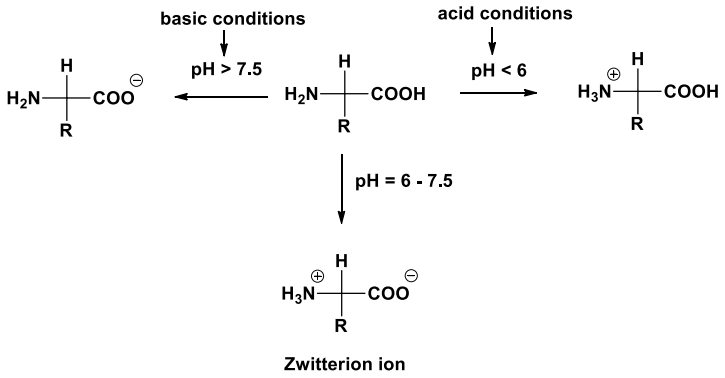


Figure 4. Classification of amino acids according to the side chain group.

Free amino and carboxylic group show different properties in acidic, basic (alkaline) and neutral medium as well as in water (Scheme 2).[2] In neutral medium, amino acid has a free amino (-NH_2) and carboxyl (-CO_2H) group, but if that neutral medium is water than amino group is protonated and yielding a positive charge on nitrogen atom (-NH_3^+) and carboxyl group is deprotonated and yielding negative charge on oxygen atom (-CO_2^-) (Scheme 2). The name of that shape is a Zwitterion ion and exist only in water or in water solution. If amino acid is in acidic medium (pH < 6) than amino group is protonated (-NH_3^+) and the carboxyl group is in neutral shape (-CO_2H), but if we have basic (alkaline) medium (pH > 7.5) than amino group is in neutral shape (-NH_2) and carboxyl group is deprotonated (-CO_2^-). These shapes and

properties of amino acids are very important for reactions of amino acids in a different medium, especially in the body where a medium for all reactions is water. The group in the side chain can be protonated and deprotonated, too.



Scheme 2. Properties of amino acids in different medium.

3.3. Stereochemistry of amino acids

In the term of stereochemistry, all natural amino acids, except glycine, are chiral molecules which means they have at least one chiral carbon atom – carbon atom bind with four different groups.[1] All amino acids have absolute and relative configuration. The relative configuration is determined by comparing it with a compound of known relative configuration (standards), like L-glyceraldehyde or L-milk acid. If the amino acid rotates the plane of polarized light on the same side like standards (left), than has the same relative configuration like standards (L relative configuration). All amino acids have L-relative configuration.

Absolute configuration is determined by CIP rules (Cahn-Ingold-Prelog). In the chiral molecule, each group which is attached on the chiral atom has a priority according to its atomic number. The group with higher atomic number has higher priority. If there are two identical atoms attached on chiral center, the next atom in the two substituents is examined. If the priority follows the clockwise direction, the absolute configuration is *R* (*lat. rectus*), but if the priority follows the direction opposite than clockwise, than it is *S* (*lat. sinister*) configuration (Figure 5). All natural amino acids have *S* absolute configuration except cysteine which has *R* configuration.

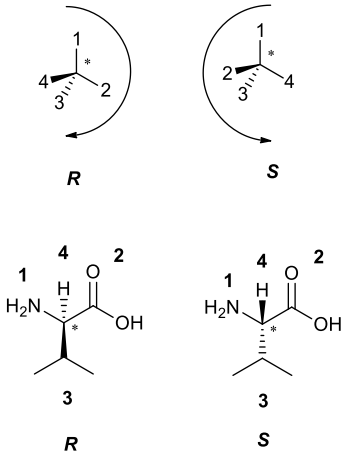


Figure 5. Determination of the absolute configuration.

3.4. Chromatography

Chromatography is analytical technique that enables separation, purification and identification of closely related components of a mixture for qualitative and quantitative analysis.[4] First chromatography was made and discovered by Russian botanist Mikhail Tswett who worked on separation of plant pigments, such as chlorophylls and xanthophylls. He put the solution of leafage in a glass column packed with calcium carbonate and separated pigments was appeared as a color bands on the column.[5]

Today, we use different types of chromatography what depends of the stationary and mobile phase. Stationary phase could be silica gel, aluminum or C-18 and fill in the column of glass or metal or could be inflict on glass or aluminum plates. Mobile phase could be gas (helium, hydrogen, nitrogen) or liquid (solvent(s)). Like a mobile phase we use organic solvents – ethanol, ethyl acetate, acetone, dichloromethane, petroleum ether, diethyl ether, hexane… or inorganic – water.

The types of chromatography are:

- column chromatography – gas chromatography (GC), high performance liquid chromatography (HPLC), ion-exchange chromatography, silica gel column chromatography

- thin layer chromatography (TLC) - the stationary phase is silica gel inflict on the aluminum or glass plate and mobile phase is solvent or mixture of solvents. The mobile phase rise on the paper by capillary forces and separate the components.

Chromatography is based on the following principles:[6]

1. crude mixture of desire compound can be dissolved in solvent and put in the column on the crude stationary phase or can be evaporated together with silica gel and put in the column with silica gel
2. oil mixture of desire compound can be direct add on the silica gel
3. the mobile phase pass through the column under the influence of gravity or pump and eluate our desire compound
4. some components of the mixture stay longer on the stationary phase and they move slowly, while others pass rapidly (the speed of moving component through column depend of the molecular characteristic like adsorption, partition, affinity for silica gel and polarity). The purification and separation on silica gel depend of the polarity of compounds for separation and polarity of solvents. For purification of polar compounds we use polar solvent(s) and for purification of nonpolar compounds we use nonpolar solvent(s).

3.5. Extraction

Extraction is an analytical technique that is based on separation of desired substance(s) between two immiscible phases – organic and aqueous phase (Figure 6).[4,5] The typical aqueous phase is aqueous solution of sodium chloride ($NaCl_{(aq)}$), ammonium chloride ($NH_4Cl_{(aq)}$), sodium bicarbonate ($NaHCO_{3(aq)}$), citric acid ($C_6H_8O_{7(aq)}$) or only distilled water. As an organic phase we usually use dichloromethane (DCM, CH_2Cl_2), ethyl acetate (EtOAc, $CH_2CO_2CH_2CH_3$), hexane (C_6H_{14}) or diethyl ether ($CH_3CH_2OCH_2CH_3$). With this technique we can separate inorganic impurities from organic compound(s). Extraction is performed in the separating funnel in which we add a mixture of our compound, aqueous solution and the solvent in which we want to transfer our compound(s). The separating funnel is shaken strongly and left to separate the layers. The solvent with higher density is the down layer and should be released through the bottom side of the separating funnel. A solvent of lower density is the

upper layer and should be poured through the upper side of the separating funnel. In this process the organic compound(s) is/are transport/s in the organic phase and everything other, what is insoluble in organic phase, stay into a water phase. The same process should be repeated three times. After extraction the organic layers need to be washed with the solution of sodium chloride and dried over the medium for drying like anhydrous sodium sulfate (Na_2SO_4) or anhydrous magnesium sulfate ($MgSO_4$).

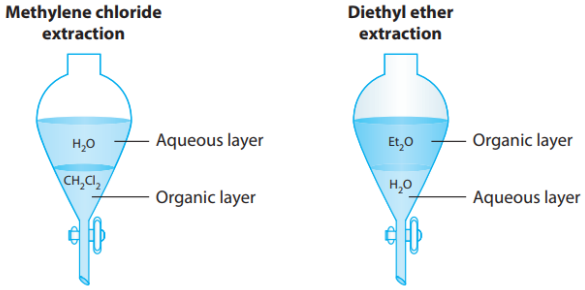


Figure 6. Two types of the layer in the extraction.

3.6. Mass spectrometry

Mass spectrometry is an analytical technique in which molecules are ionized and separate according to the mass.[1] For detection of the structure is important to know the molecular mass of product(s). In the MS device, the sample translates into gas state and in vacuum, electrons are ejected from the molecule. On this way appears molecular cation (M^+) which matches to the molecular mass of compound plus mass of hydrogen atom or sodium atom (M^+ = M (molecule) + M (H^+) or M (Na^+) and this is positive MS spectrum. Furthermore, exists the negative MS spectrum which matches to the molecular mass of compound minus molecular mass of hydrogen atom (M^- = M (molecule) – M (H^+)).

3.7. Nuclear Magnetic Resonance

NMR spectroscopy is one of the technique for detecting real structure of organic compounds.[1] The data about the structure of molecule is given by probe of magnetic performance of atoms inside of the molecule. This technique can be only use with the nucleus which possess only odd mass number like ^{1}H, ^{11}B, ^{13}C, ^{15}N, ^{19}F and ^{31}P. For analysis we need to put our sample (liquid or solid, from 1 to 10 mg) in a glass tube and add between 0.7 mL to 1.5 mL of deuterated solvent (solvent which doesn't possess protons like as $CDCl_3$ ($CHCl_3$ chloroform), CD_3OD (CH_3OH methanol) and CD_3CN (CH_3CN acetonitrile)). The glass tube is put in the NMR device between poles of powerful magnets (300 MHz and 600 MHz for ^{1}H analysis and 75 MHz and 151 MHz for ^{13}C analysis) and the tube rotates rapidly about 5 minutes for the ^{1}H NMR spectra or 30 minutes for the ^{13}C NMR spectra.

3.8. Protective groups

Synthetic organic chemistry is based on the concourse of reagents and catalysts to achieve the clean formation of new bonds.[7] In this step is important to prevent the formation of undesired bonds and side reactions and enable the reaction only on the one reactive side of the compound. It's important to plan all synthesis very well and select a properly good protecting group which must fulfill a number of requirements like as:[8]

1. should be easily introduced with nontoxic and available reagents
2. should be stable to a broad range of reaction conditions
3. it must react selectively in good yield to give a protected substrate that is stable
4. must be selectively removed in good yield by nontoxic and available reagents

In the peptide synthesis is necessary protect and deprotect functional groups a few times and because of that is important to plan all synthesis very well. It is necessary to protect α-carboxyl and α-amino group because they are the most important functional groups in amino acids, peptides and proteins and can react so rapidly. If it’s necessary, we can protect the side chain, too.

Protection of *α*-carboxyl group is taking place in acid medium and with methanol to making methyl ester, benzyl alcohol to making benzyl ester and 2-methylpropene to making *tert*-butyloxycarbonyl protection (Figure 7). Deprotection of the mentioned groups is taking place in different medium like alkaline medium, in the flow of hydrogen with palladium on the carbon and in acid medium. For protection of *α*-amino group we use Boc group (*tert*-butyloxycarbonyl), Z group (benzyloxycarbonyl) and Fmoc group (9-fluorenylmethoxycarbonyl) (Figure 8). For protection with Boc and Z group we need strong alkaline medium (inorganic base) and with Fmoc we use mild alkaline medium (organic base). For deprotection use trifluoroacetic acid (TFA), flow of the hydrogen gas with palladium on the carbon and piperidine or triethylamine (TEA).

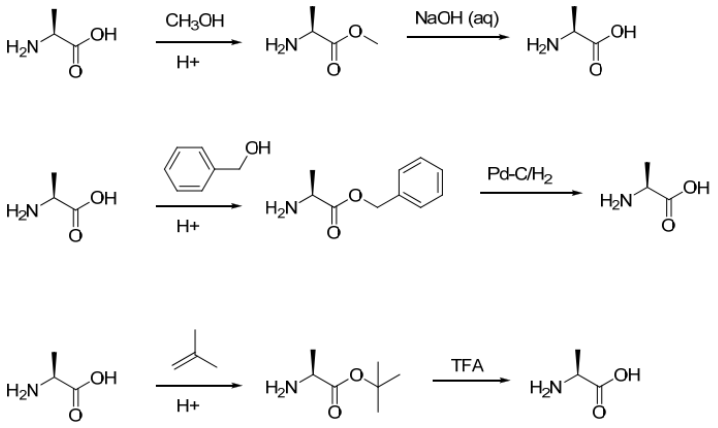


Figure 7. Protection and deprotection of *α*-carboxyl group.

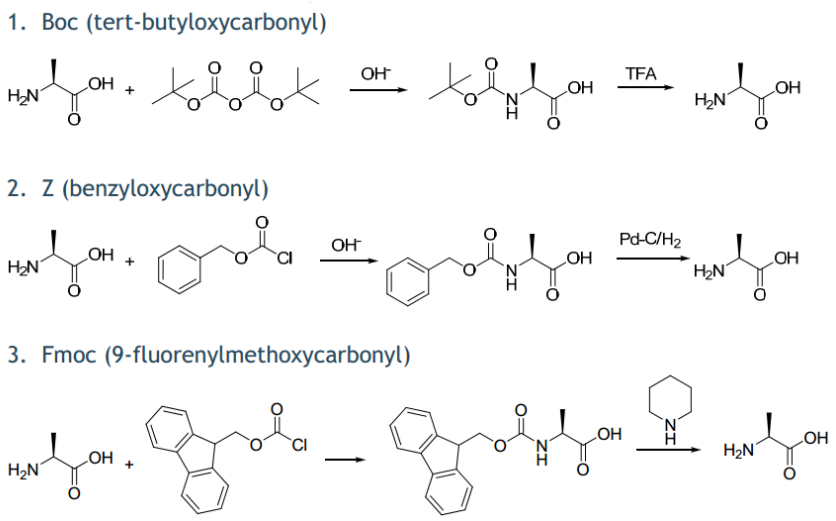


Figure 8. Protection and deprotection of α-amino group.

3.9. Condensation reagents

After protection, the second step is activation of α-carboxyl group and coupling between first and second amino acid. The activation of carboxyl group is important because reaction between α-carboxyl group and α-amino group is very slow if condensation reagent isn't present.[1] The most frequently used groups for activation are groups based on amide bond – *N*,*N*'-dicyclohexylcarbodiimide (DCC) or *N*,*N*'-diisopropylcarbodiimide (DIC) in combination with *N*-hydroxysuccinimide (NHS) or groups based on the ester bond – phsophonium salts (HOBt, BOP) and uronium salts (HATU) (Figure 8).

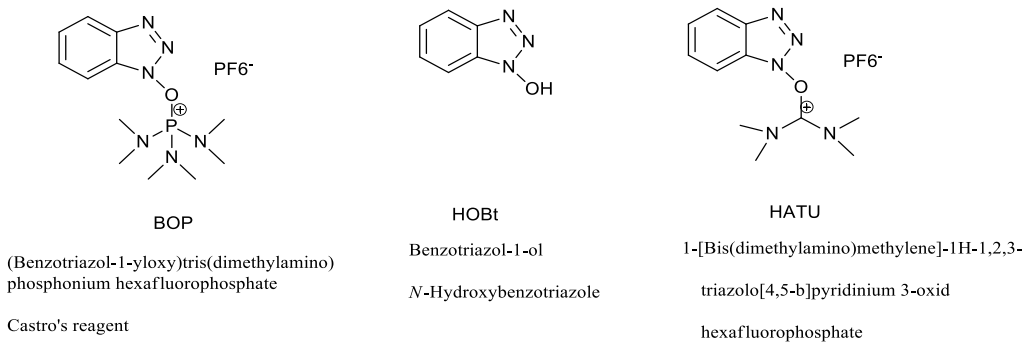


Figure 9. Phsophonium and uranium salts.

3.10. Dipeptides and tripeptides

Peptides are molecules that consist of between 2 and 10 amino acids that are held together by the peptide bond (Figure 10). They are fundamental components of cells that carry out important biological functions, like as regulation of the activities of other molecules. The name of peptide chain comes from the name of amino acids that are the building blocks of a peptide. For example, if peptide is constructed of three amino acids (Val-Ser-Ala) than the full name is valyl-seryl-alanine, so, the two first amino acids have a sequel –yl and the last amino acid has a normal name. Polypeptides are molecules that consist of between 10 to 100 amino acids. If in the chain are more than 100 amino acids, the name of the structure is a protein.

Peptides are formed by linking α-amino group of one amino acid and α-carboxyl group of another amino acid. The bond between carbon atom from carboxyl group and nitrogen atom from amino group is called peptide bond and this bond is planar and there's no rotation between these two atoms. Only the bonds ψ and φ can make rotation.

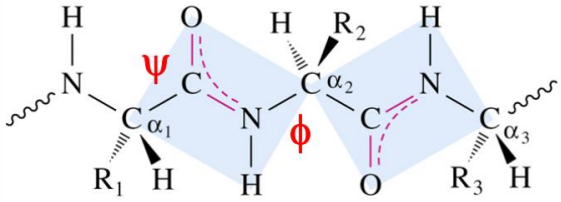


Figure 10. Peptide bond.

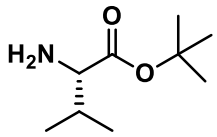
4. EXPERIMENTAL PART

Solvents and free amino acids for reactions are commercially available. Systematic compounds names are generated using program ChemDraw Ultra (version 12,0,2,1076) following IUPAC conventions.

4.1. Detection of amino acids by TLC

1. Put in the glass about 10 ml of solvent(s)
2. Take a TLC plates and draw a straight line about 1 cm from the bottom
3. Draw a small point(s) on straight line and mark the point(s)
4. Take a small amount of your sample and put in the Eppendorf tube and dissolve in some solvent(s)
5. With capillary tube take the solution of your compound and put a little amount on the one point. If you have a more samples repeat the procedure.
6. Put the plate in the glass and when the mobile phase is about 1 cm from the end of plate, take it out and dry it with hairdryer or on the air
7. If your compound contains aromatic ring(s) or double/triple bond in conjugation they can be observed under ultraviolet light (UV). So, take your plate with the tweezer and put under the UV light. On the place where is component you can see purple or blue point(s) and round off with the pencil.
8. If your compound doesn't contain conjugation or aromatic ring(s), take a plate and put in the solution of potassium permanganate or ninhydrine. Dry a plate with the heat-gun and on the place where is compound you can see yellow, blue, red or orange point(s). Always, first check the plate under the UV light before dying.
9. Determine the position of compound(s).

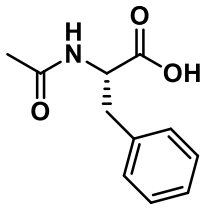
4.2. Synthesis of (*S*)-*tert*-butyl 2-amino-3-methylbutanoate (1)



1

(*S*)-2-amino-3-methylbutanoic acid (0.017 mol, 2 g) was dissolved in 20 mL of *tert*-butyl acetate on the cool bath. Perchloric acid ($HClO_4$) (1.6 eq, 0.027 mol, 1.67 mL) was added slowly and the reaction mixture was stirred for 24 hours on room temperature. The reaction was extracted once with distilled water. Organic layer was extracted three times with 5M hydrochloride acid (HCl) and potassium carbonate (K_2CO_3) was added in collected water layers until pH = 9 (basic). Mixture was extracted three times with ethyl acetate and organic layers were dried over anhydrous sodium sulfate (Na_2SO_4).[1]

4.3. Synthesis of (*S*)-2-acetamido-3-phenylpropanoic acid (2)

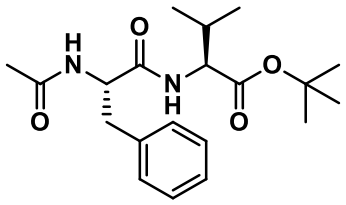


2

Phenylalanine (0.012 mol, 2 g) was dissolved in 20 mL of 0.5M sodium hydroxide (NaOH) in the cool bath. The trimethylamine (2.8 eq, 0.034 mol, 4.7 mL) was added and then acetic anhydride (1.4 eq, 0.017 mol, 1.6 mL). The reaction mixture was stirred for 2 hours and was extracted once with diethyl ether. In the water layer was added 6M HCl to do pH = 2. The mixture was extracted three times with ethyl acetate and organic layers were dried over anhydrous sodium sulfate (Na_2SO_4).

[1] The yield of reaction wasn't calculated because we hadn't the rotavapory in the school for moving solvent from the flask. The same reason is for all other reactions.

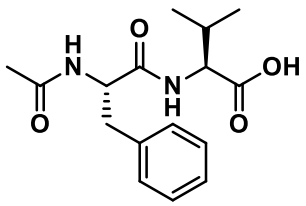
4.4.Synthesis of (*S*)-*tert*-butyl 2-((S)-2-acetamido-3-phenylpropanamido)-3-methylbutanoate (3)



3

(*S*)-2-acetamido-3-phenylpropanoic acid (0.0965 mmol, 20 mg) was dissolved in dichloromethane and was added *N*-methylmorpholine (NMM, 1.1 eq, 0.106 mmol, 12 μL). The reaction mixture was stirred for a two minutes and was added HATU (1.1 eq, 0.106 mmol, 40 mg). The reaction mixture was stirred over the night on a room temperature. Ammonium chloride was added and the reaction mixture was extracted with ethyl acetate and the organic layers were dried over anhydrous sodium sulfate (Na_2SO_4).

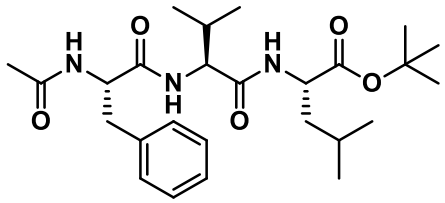
4.5. Synthesis of (*S*)-2-((*S*)-2-acetamido-3-phenylpropanamido)-3-methylbutanoic acid (4)



4

(*S*)-*tert*-butyl 2-((S)-2-acetamido-3-phenylpropanamido)-3-methylbutanoate (0.055 mmol, 20 mg) was dissolved in ethyl acetate and was added 5M hydrochloride acid. The reaction mixture was stirred over the night on room temperature. Saturated solution of NaCl were added and the reaction mixture was extracted with ethyl acetate. The organic layers were dried over anhydrous sodium sulfate (Na_2SO_4).

4.6. Synthesis of (*S*)-*tert*-butyl 2-((*S*)-2-((*S*)-2-acetamido-3-phenylpropanamido)-3-methylbutanamido)-4-methylpentanoate (5)



5

S)-2-((*S*)-2-acetamido-3-phenylpropanamido)-3-methylbutanoic acid (0.098 mmol, 30 mg) was dissolved in dichloromethane with a drop of dimethylformamide (DMF) and was added *N*-methylmorpholine (NMM, 1.1 eq, 0.107 mmol, 12 μL). The reaction mixture was stirred for a 2 minutes and was added HATU (1.1 eq, 0.107 mmol, 40 mg) and after 1 minute (*S*)-*tert*-butyl 2-amino-4-methylpentanoate (1 eq, 0.098 mmol, 18 mg). The reaction mixture was stirred over a night and extracted with ammonium chloride and ethyl acetate. The organic layers were dried over anhydrous sodium sulfate (Na_2SO_4).

5. RESULTS AND DISCUSSION

5.1. Detection of amino acids

5.1.1. Thin Layer Chromatography (TLC)

For qualitative detection of amino acids we used crude silica gel supported on aluminum plate (TLC plates) and four different mixture of solvents: 1) ethyl acetate/petroleum ether = 2/1; 2) petroleum ether/ethyl acetate/acetic acid = 2/1/0.1; 3) petroleum ether/ethyl acetate = 2/1; 4) ethyl acetate/acetic acid = 1/1 (Figure 11). With these mixture of solvents we tried to investigate the behavior of different amino acids in different polarity of solvents. We used ten different amino acids (glycine, valine, arginine, serine, threonine, lysine, glutamate, glutamine, tryptophan and proline) dissolved in a mixture of methanol/water = 2/1.

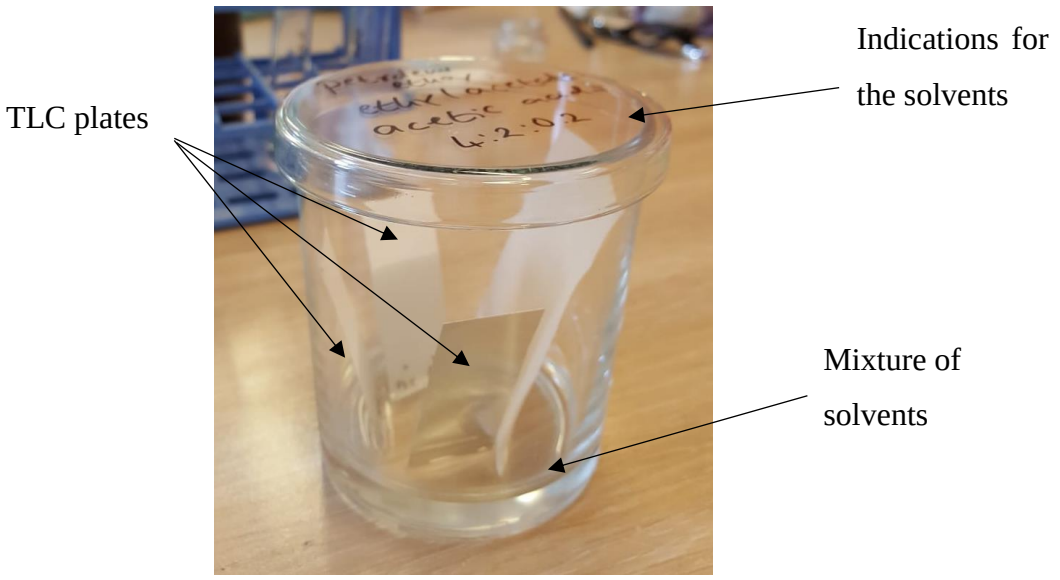


Figure 11. TLC plates.

After the chromatography, we put the plate in the ninhydrine which reacts with free amino group in the amino acid and enable detection. Ninhydrin is an organic compound for qualitative detection of amino acids.[9] The structure is based on six-five membered fused ring, two carbonyl and two hydroxyl group. On the figure 12 are presented TLC plates with different amino acids in all four mixtures. The reaction with the nihydrine produces purple dye with all

the amino acids except for the proline that produces a yellow dye because it`s the only amino acid with a cyclic structure and has different mechanism with ninhydrine. If the compounds contain aromatic ring(s) or double/triple bond in conjugation they can be observed under ultraviolet light (UV), like tryptophan, phenylalanine, histidine and tyrosine. In the first three mixture of solvents, all amino acids are at the start of the TLC plate because the mixture of solvent isn't so polar and amino acids can't travel more at the TLC plate. In the last mixture where we have acetic acid, the amino acids achieved the plates more then with the other solvents because amino acids are polar molecules and acetic acid is polar, and can carries the amino acids higher on the TLC plate.

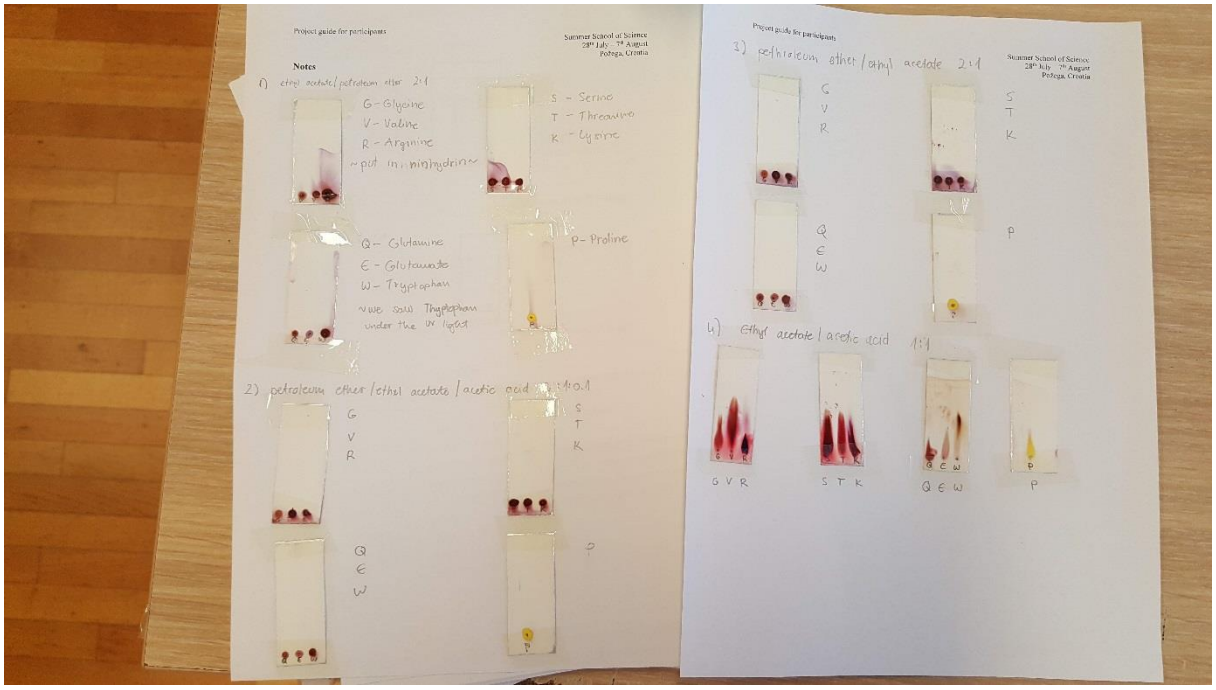


Figure 12. Results of the TLC.

5.1.2. MS of amino acids

As described in the theoretical introduction for detection of the structure is important to know molecular mass of product. For valine it's 117.08 g/mol. In the positive part of the graph we have to search for the value of the molecular mass plus the mass of hydrogen (118.09 g/mol) and the molecular mass plus the mass of sodium (140.07 g/mol). In the negative part we have to search for the value of the molecular mass of the product minus the mass of hydrogen (116.07 g/mol). In this graph we found only one value in positive part, but it is enough to say that there is valine (Figure 13).

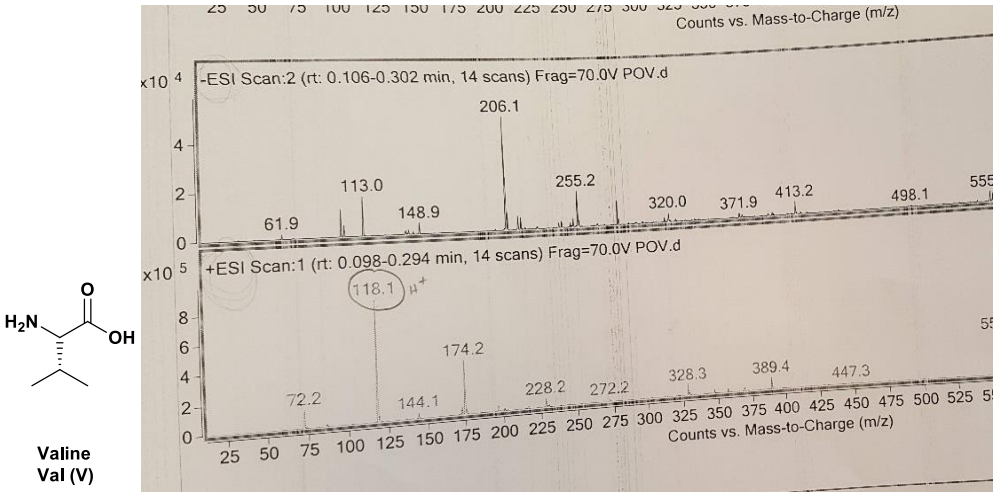


Figure 13. MS spectra of valine.

We did the same process also for lysine. The molecular mass is 146.11 g/mol, the positive values are 147.12 g/mol with the hydrogen ion and 169.10 g/mol with the sodium ion, the negative value is 145.10 g/mol. In this case, only in the negative part of spectrum we found the signal (Figure14).

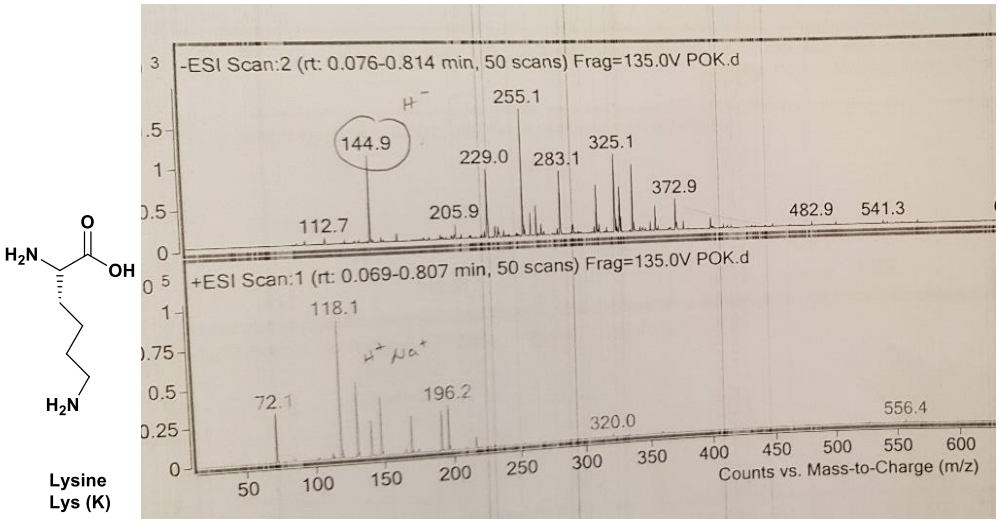
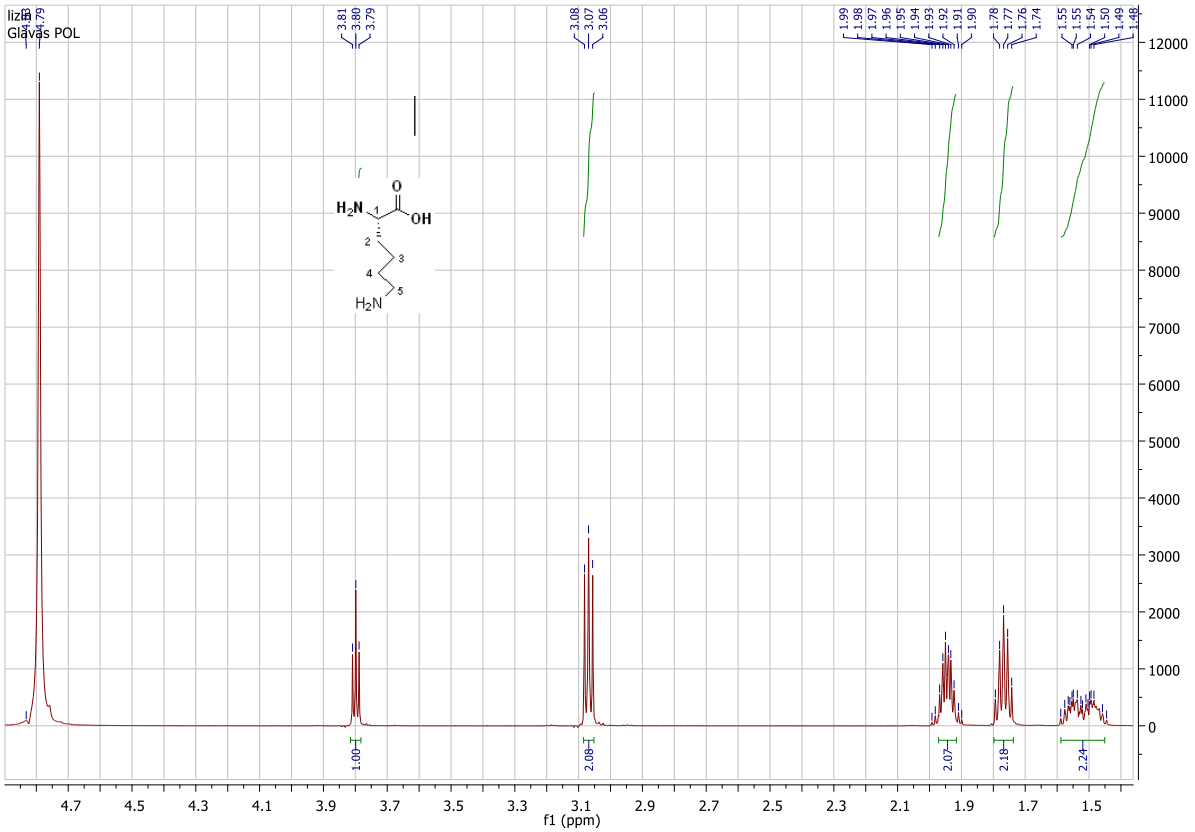


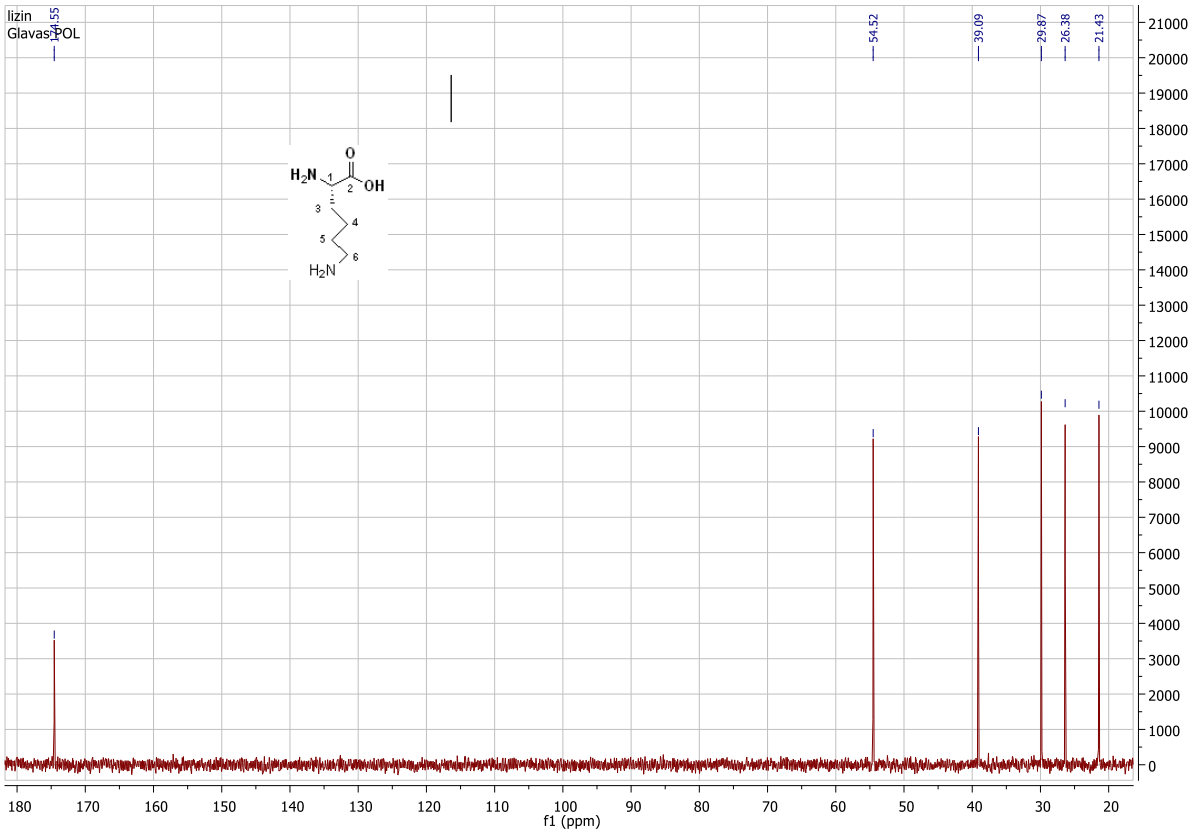
Figure 14. MS spectra of lysine.

5.1.3. NMR of amino acids

At the figure 15 are presented the ^{1}H and ^{13}C NMR spectra of lysine made in deuterium water. All hydrogen and carbon atoms fit to the signals in spectra. In the spectrum isn't show the free amino groups and free carboxylic group because sometimes these atoms are invisible and we need a longer recording of NMR to see them. The correct values of shifts are presented below the NMR spectrum.



^{1}H NMR (D_2O, 300 MHz) δ / ppm: 3.81 – 3.79 (1H, H1), 3.08 – 3.06 (2H, H5), 1.99 – 1.90 (2H, H2), 1.78 – 1.76 (2H, H4), 1.55 – 1.48 (2H, H3).

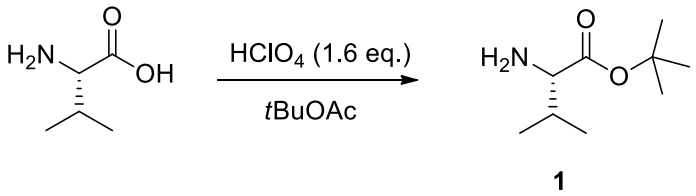


^{13}C NMR (D_2O, 300 MHz) δ / ppm: 176.6 (C2), 54.5 (C1), 39.1 (C6), 29.9 (C3), 26.4 (C5), 21.4 (C4).

Figure 15. ^{1}H and ^{13}C NMR spectra of lysine.

5.2. Protection of α-carboxyl group

During the coupling of the compounds, it's important to protect groups which we don't want to react in the reaction an on that way avoid the side reactions. For the synthesis of dipeptide, first we protected carboxyl group on valine. For protection we used *tert*-butoxy group in acidic medium (Scheme 3).

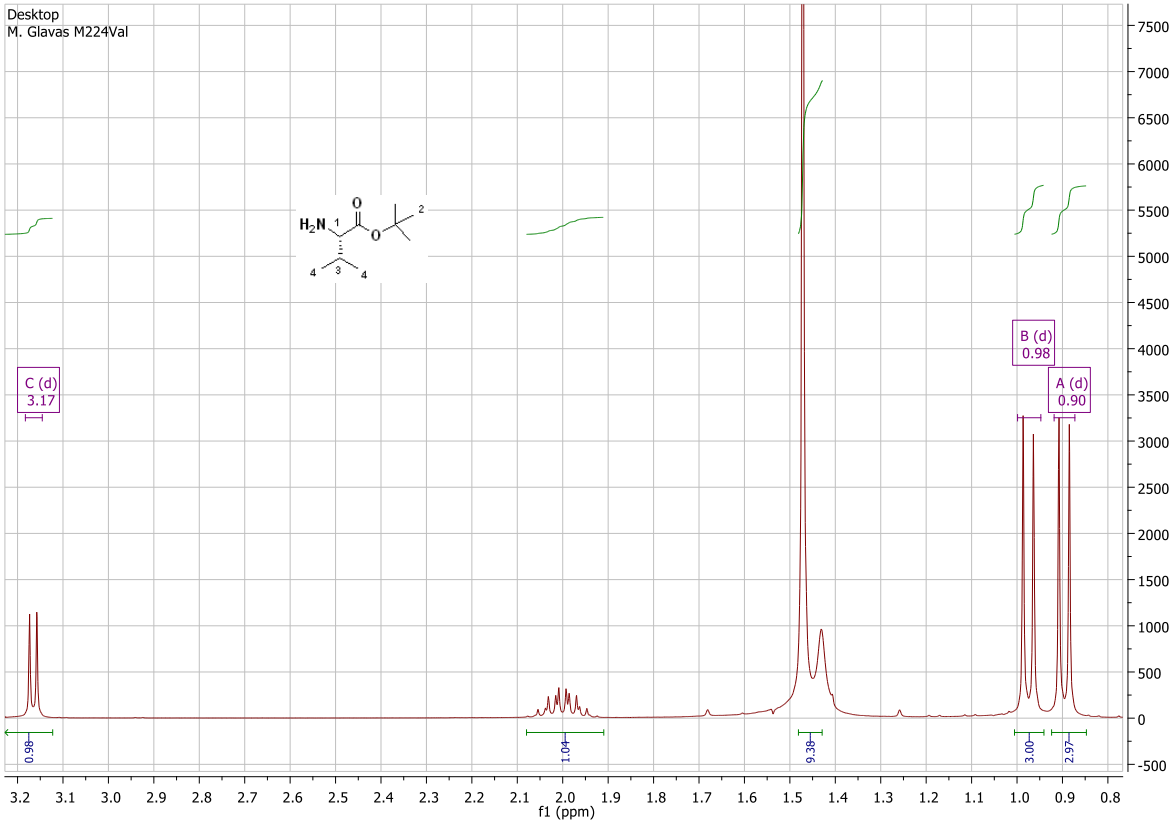


Scheme 3. Synthesis of protected valine.

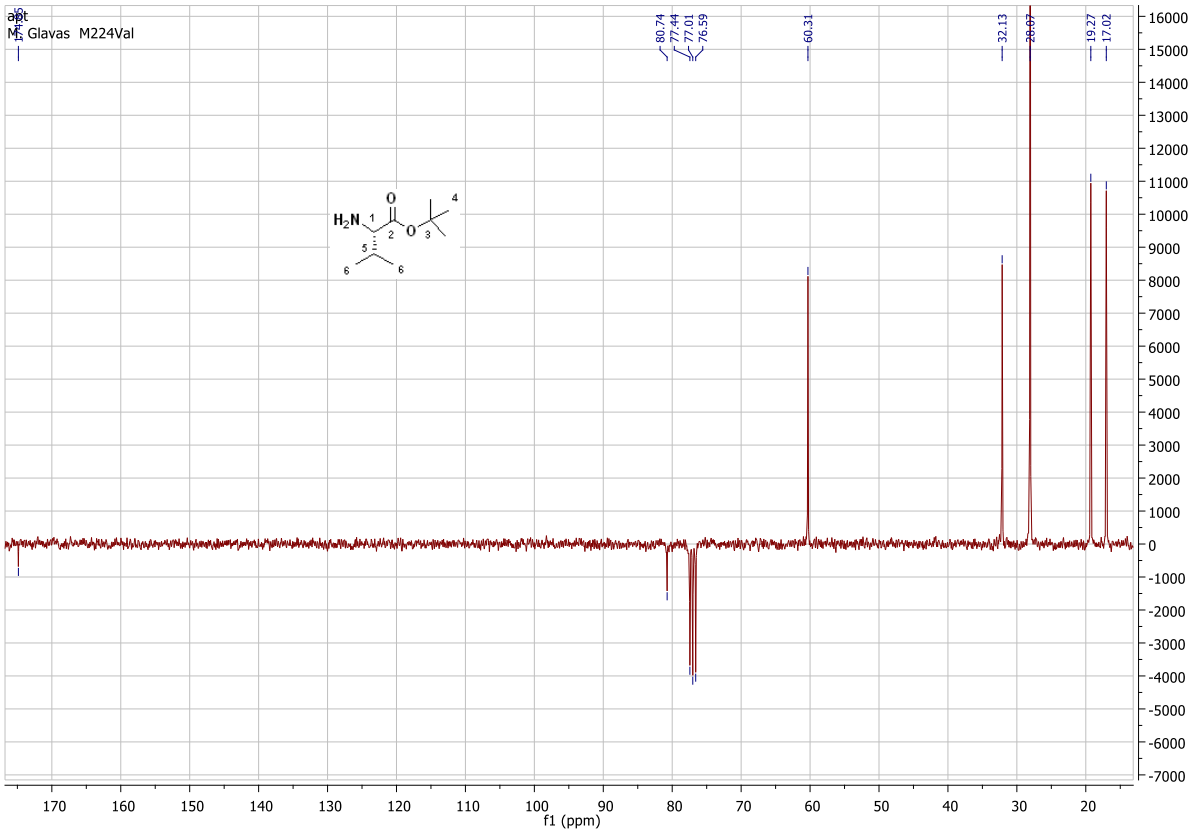
The results obtained by TLC are shown below (Figure 16). The difference between height of the dot that shows valine without and the one with protection group indicates difference in polarity and, consequently, difference in solubility in the mobile phase. Since the mobile phase is non-polar (petroleum ether/ethyl acetate =5/5), protected valine, which is less polar than the non-protected one, stays on the bottom phase and protected valine travels higher. This difference indicates that a new compound has been synthetized and that does not contain the starting material. TLC plate immersed in potassium permanganate shows position of valine and protected valine (left TLC plate). Since there is a free amino group in the compound, it can be seen when coloured in ninhydrine (right TLC plate).

Figure 16. TLC of protected and non-protected valine.

The results obtained by NMR in spectra of ^{1}H and ^{13}C are shown on figures below (Figure 17) and all shifts are correct and match to all hydrogen and carbon atoms.



^{1}H NMR ($CDCl_3$, 300 MHz) δ / ppm: 3.17 – 3.16 (1H, H1), 2.05 – 1.95 (1H, H3), 1.54 – 1.43 (9H, H2), 0.99 – 0.96 (3H, H4), 0.91 – 0.88 (3H, H4).

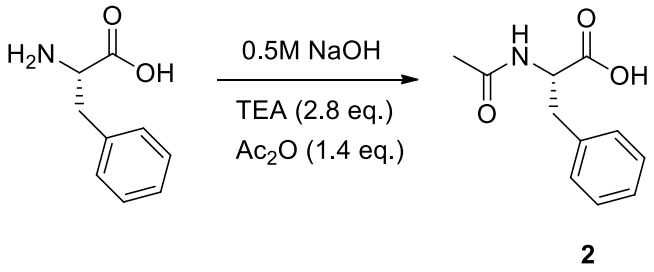


^{13}C NMR ($CDCl_3$, 300 MHz) δ / ppm: 174.85 (C2), 80.74 (C3), 60.31 (C1), 32.13 (C5), 28.07 (C4), 19.27 (C6), 17.02 (C6).

Figure 17. ^{1}H and ^{13}C NMR spectra of protected valine.

5.3. Protection of α-amino group

After the synthesis of protected valine on the α-carboxyl side, the next step was protection of phenylalanine on the α-amino side. In this reaction we protected the amino group of phenylalanine with the acetyl group (Scheme 4).



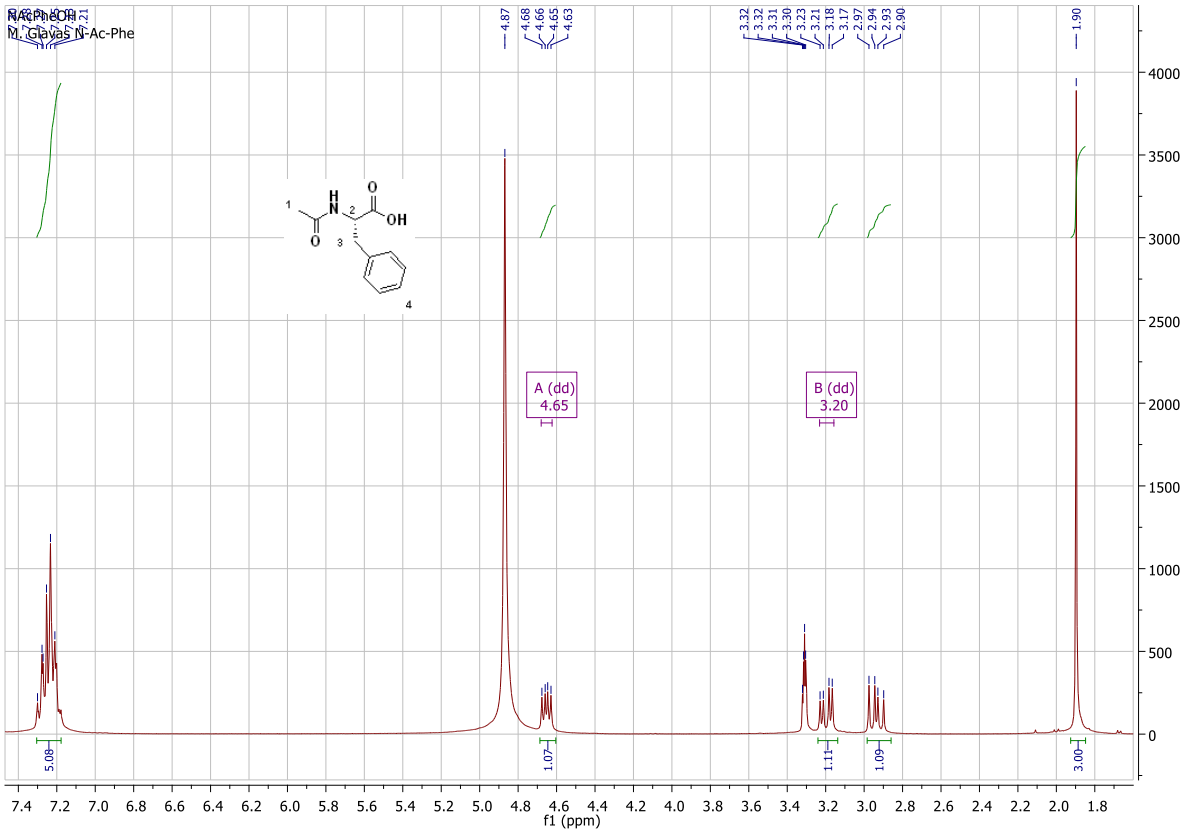
2

Scheme 4. Synthesis of protected phenylalanine.

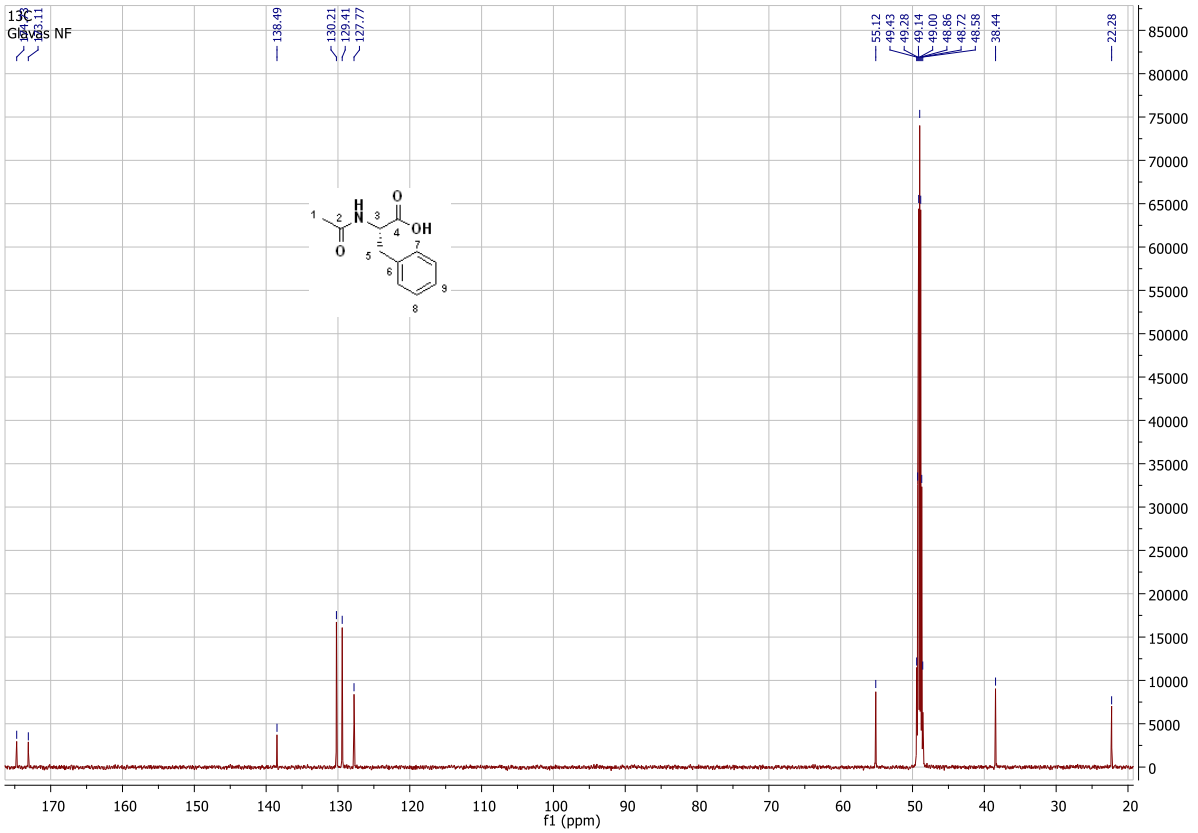
The results obtained by TLC are shown on the Figure 18. The difference between heigh of the dot that represents non-protected phenylalanine and the protected one indicates difference in polarity. As explained in the previous experiment, in the non-polar mobile phase, non-polar compounds travel better, but the difference is not that significant as in Figure 16. Protected phenylalanine is more non-polar than non-protected phenylalanine but both have a big phenyl ring who has a big influence on polarity. The mixture of solutions petroleum ether/ethyl acetate = 1/1 are at the same time polar and non-polar and because of influence of the big phenyl ring, free phenylalanine is on the start and protected is a little more above the start.

Figure 18. Comparison of non-protected and protected phenylalanine on TLC plate

The results obtained by NMR in spectra of ^{1}H and ^{13}C are shown on figures below (Figure 19). All shifts are correct and match to all hydrogen and carbon atoms.



^{1}H NMR (CD_3OD, 300 MHz) δ / ppm: 7.30 – 7.21 (5H, H4), 4.68 – 4.63 (1H, H2), 3.32 – 3.17 (1H, H3), 2.97 – 2.90 (1H, H3), 1.90 (3H, H1).

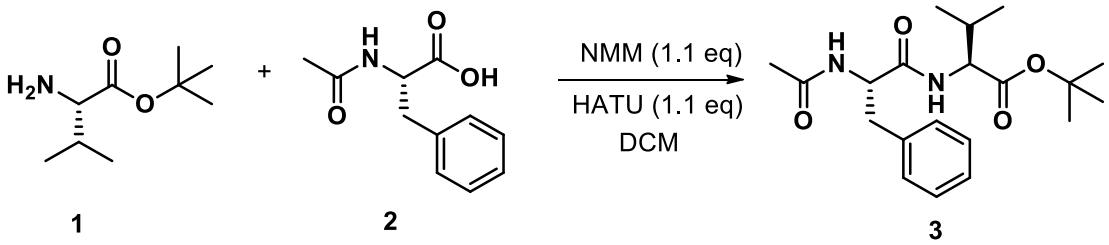


^{13}C NMR (CD_3OD, 300 MHz) δ / ppm: 174.73 (C2), 173.11 (C4), 138.49 (C6), 130.21 (C7), 129.41 (C8), 127.77 (C9), 55.12 (C3), 38.44 (C5), 22.28 (C1).

Figure 19. ^{1}H and ^{13}C NMR spectra of protected phenylalanine.

5.4.Synthesis of dipeptide phenylalanyl-valine

After protection of amino and carboxyl group, which we don't need in reaction, the nest step was synthesis of dipeptide. The reaction was set up with protected valine **1** and phenylalanine **2** using NMM like a base for keep the amino group in the basic shape and HATU like a condensation reagent (Scheme 5).



1 2 3

Scheme 5. Synthesis of dipeptide.

After the synthesis, product was compared with starting materials on a TLC plate (Figure 20). Protected valine shows the biggest trace due to its higher concentration in the solution. It travels better on the plate because it's more polar than protected phenylalanine. The dipeptide is more polar again, almost as valine. Along with the synthetized compounds, some impurities can be seen on the plate. All three samples are visible under the UV-light because of conjugated bounds and aromatic ring.

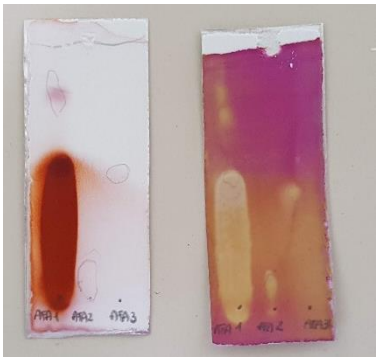
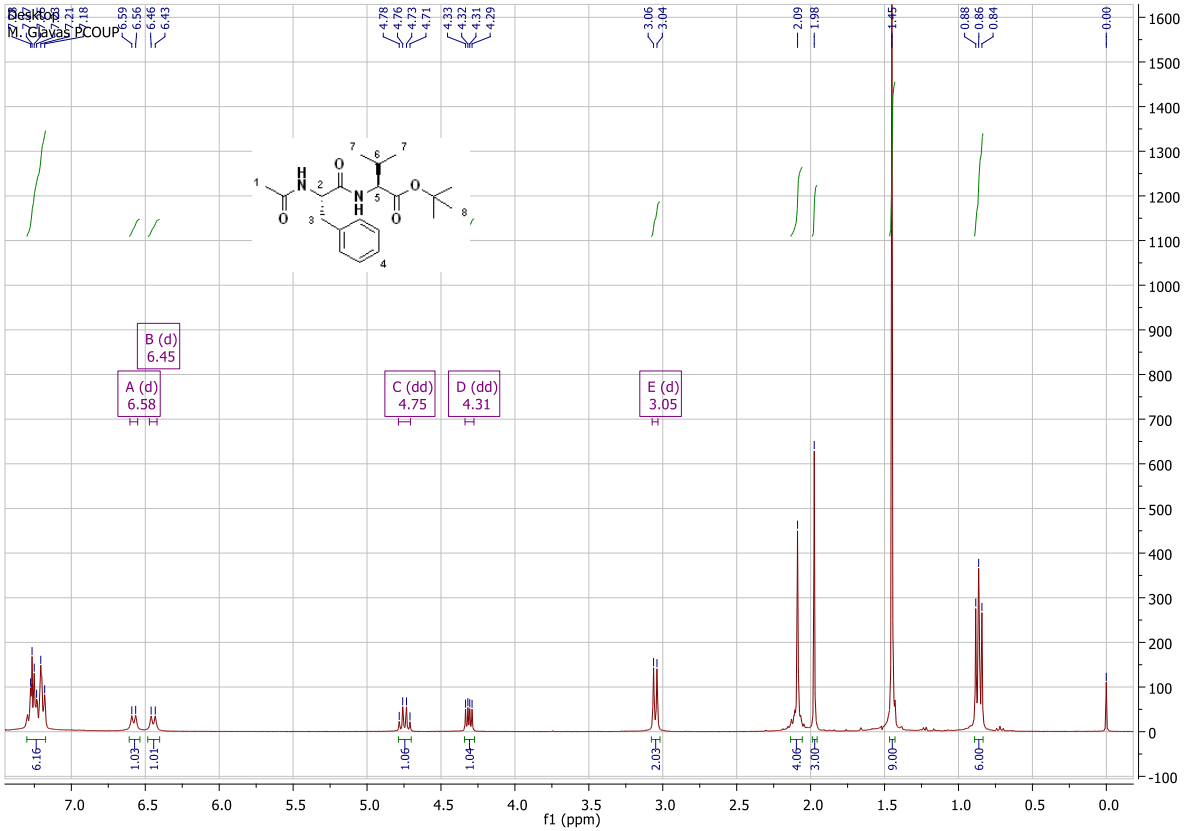
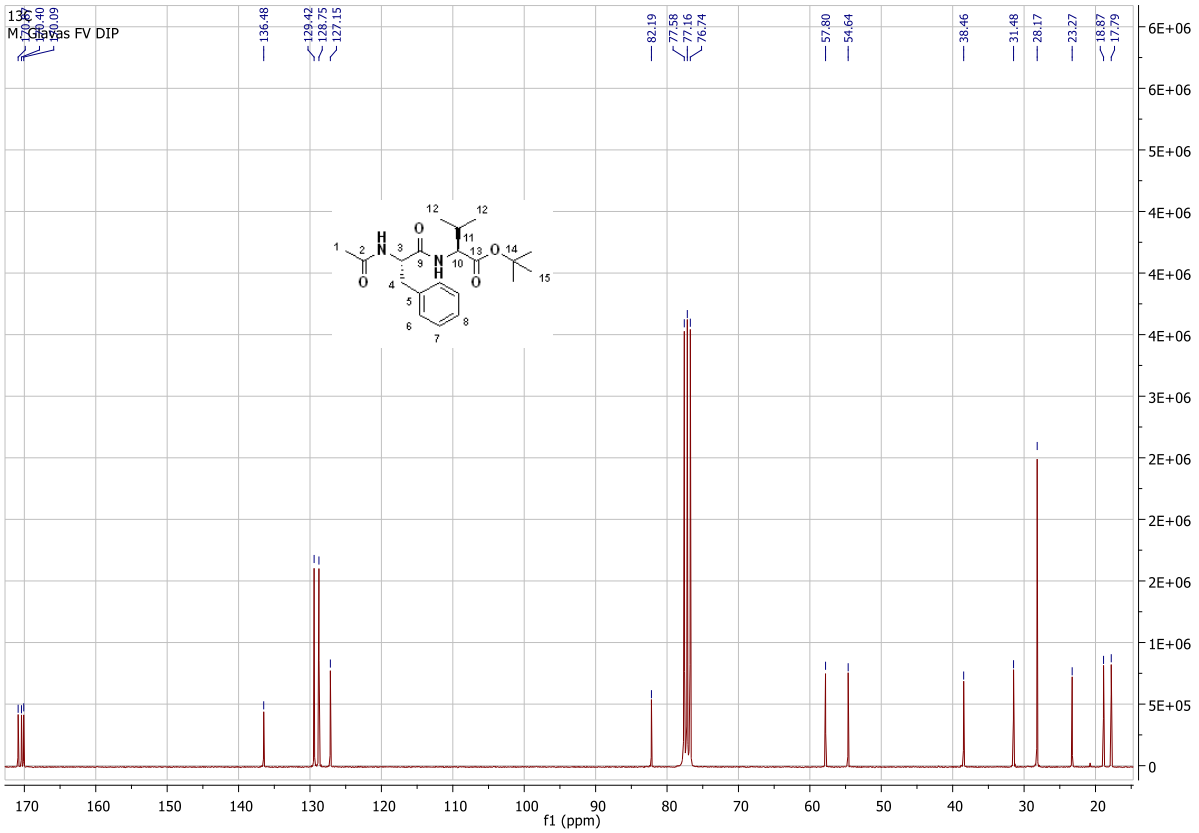


Figure 20. Comparison between dipeptide **3** and starting reagents (ninhydrine (left), potassium permanganate (right)).

The results obtained by NMR in spectra of ^{1}H and ^{13}C are shown on figures below (Figure 21). All shifts are correct and matches to the hydrogen and carbon atoms.



^{1}H NMR ($CDCl_3$, 300 MHz) δ / ppm: 7.28 – 7.18 (6H, H4), 6.59 – 6.43 (1H, NH), 6.46 – 6.56 (1H, NH), 4.78 – 4.71 (1H, H2), 4.33 – 4.29 (1H, H5), 3.06 – 3.04 (2H, H3), 1.98 (3H, H1), 1.45 (9H, H8), 0.88 – 0.84 (6H, H7).

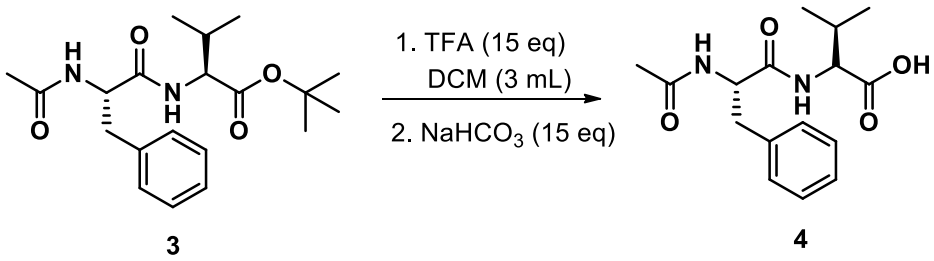


^{13}C NMR ($CDCl_3$, 300 MHz) δ / ppm: 170.87 (C2), 170.40 (C9), 170.09 (C13), 136.48 (C5), 129.42 (C7), 128.75 (C6), 127.15 (C8), 82.19 (C14), 57.80 (C10), 54.64 (C3), 38.46 (C4), 31.48 (C11), 28.17 (C15), 23.27 (C1), 18.87 (C12), 17.79 (C12).

Figure 21. ^{1}H and ^{13}C spectra NMR of dipeptide **3**.

5.5. Deprotection of dipeptide

For reaction with the new amino acid and preparing the tripeptide, we made deprotection on dipeptide **3** (Scheme 6) and after deprotection our dipeptide had a free carboxyl group. With that free group the dipeptide can make peptide bond with another amino acid or a chain of them which have a free amino group. We can synthetize peptides or proteins by the reactions of protection and deprotection of amino and carboxyl groups.



Scheme 6. Deprotection of dipeptide.

To detect if we have our compound after the reaction we use MS spectra. Our dipeptide has 306.36 g/mol. In the positive spectra we have to look for the value of the molecular mass plus the mass of hydrogen atom (307.37 g/mol) and the same molecular mass plus the mass of sodium atom (329.35 g/mol). In the negative part we have to look for the value of the molecular mass of the product minus the mass of a hydrogen atom (305.35 g/mol). In this graph only the molar mass + molar mass of hydrogen atom we found.

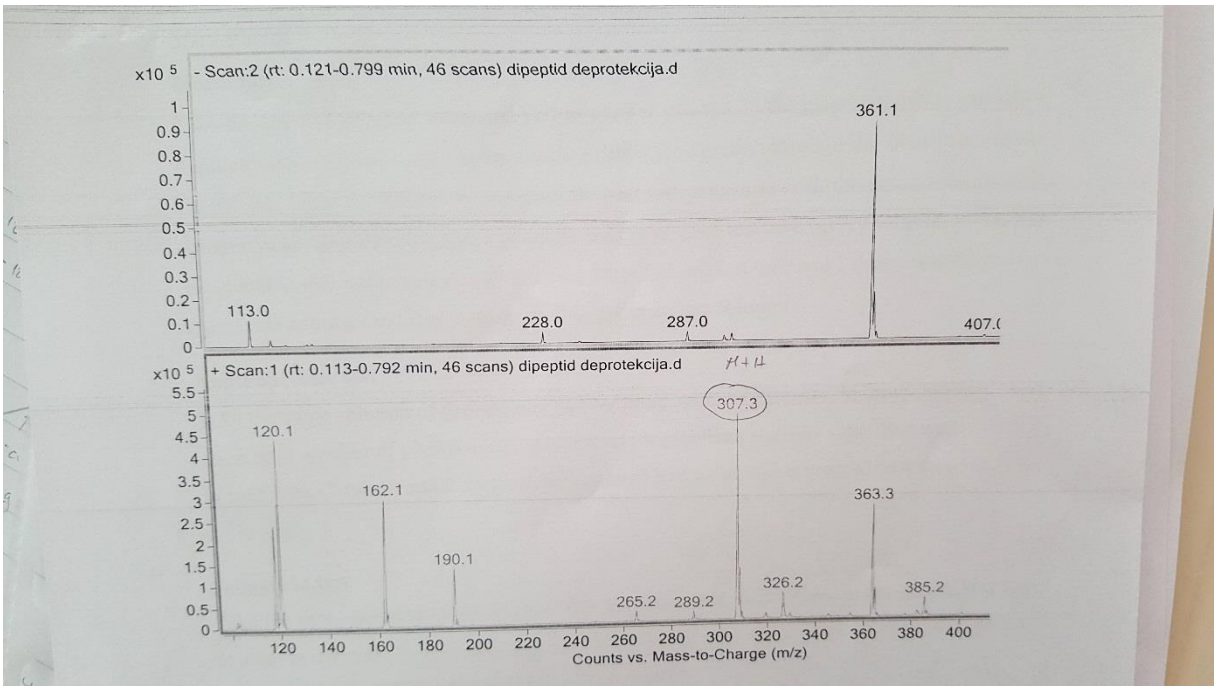
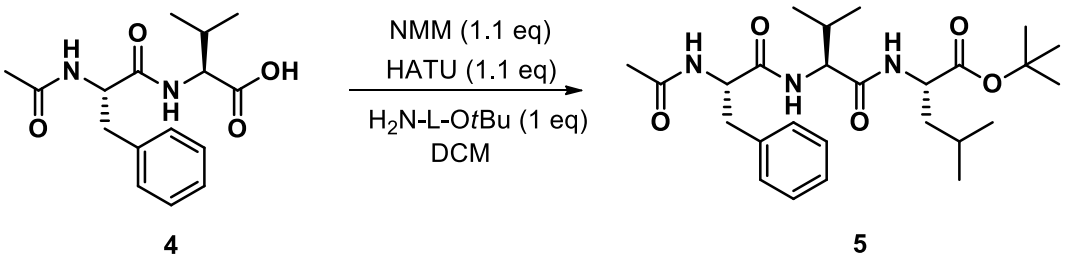


Figure 22. MS spectrum of dipeptide.

5.6.Synthesis of tripeptide

The last step was synthesis of tripeptide phenylalanyl-valyl-leucine (Scheme 7). Compound **4** was reacted with HATU for activation of carboxyl group and then was added leucine with the protection on the carboxyl side. NMM was used like a base for keep the amino group in the basic shape.



Scheme 7. Synthesis of tripeptide.

To detect if we have our tripeptide after the reaction we use MS spectra. Our tripeptide it's 475.62 g/mol. In the positive spectra we have to look for the value of the molecular mass plus the mass of hydrogen atom (476.63 g/mol) and the same molecular mass plus the mass of sodium atom (498.61 g/mol). In the negative part we have to look for the value of the molecular mass of the product minus the mass of a hydrogen atom (474.61 g/mol). In this spectra we found two values in the positive MS spectrum.

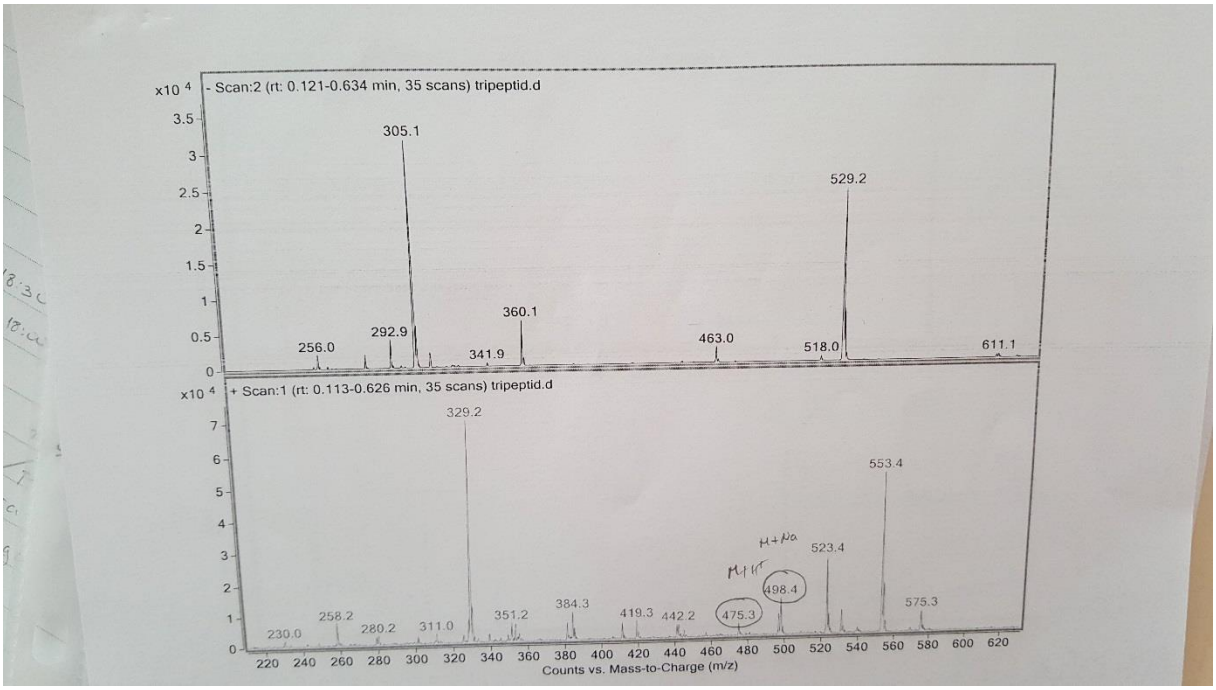


Figure 23. MS spectrum of the tripeptide.

6. CONCLUSION

In this chemistry project we prepare tripeptide (*S*)-*tert*-butyl 2-((*S*)-2-((*S*)-2-acetamido-3-phenylpropanamido)-3-methylbutanamido)-4-methylpentanoate (**5**) from the starting amino acids – phenylalanine, valine and leucine. The synthesis started from the natural phenylalanine and valine with the free amino and carboxyl group. In the first step amino group of phenylalanine and carboxyl group of valine were protected with the protection group. In the second step, free amino group of valine reacted with the free carboxyl group of phenylalanine and we prepared dipeptide (*S*)-*tert*-butyl 2-((*S*)-2-acetamido-3-phenylpropanamido)-3-methylbutanoate (**3**). After deprotection on carboxyl group of valine we set up new reaction with leucine. In this step we prepared tripeptide **5**.

Follow the same procedure for planning the synthesis and set up the reactions of peptides, we can prepare different combinations of peptides, oligopeptides or proteins in the lab. On this way, we are independent of the Nature and don't need to use natural sources. All compounds can be prepared in the lab in the bigger amount than we can isolate from the natural sources.

7. REFERENCES

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