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FACULTY OF CHEMICAL TECHNOLOGY

INSTITUTE OF ORGANIC CHEMISTRY AND TECHNOLOGY

Study: Pharmacochemistry and medicinal materials

Synthesis and characterisation of chosen trifluoromethyl substituted salicylamides

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ZADÁNÍ BAKALÁŘSKÉ PRÁCE
(projektu, uměleckého díla, uměleckého výkonu)

Jméno a příjmení:	**Yasmin Kadyrbekova**
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Téma práce:	**Synthesis and characterization of chosen trifluoromethyl substituted salicylamides**
Zadávající katedra:	**Ústav organické chemie a technologie**

Zásady pro vypracování

Principles for working on this thesis:

1. Characterization of synthesis aliphatic salicylamides, alternatively structures of similar amides of carboxylic acids described in reference books.
2. Summary of individual methods of synthesis and their evaluation based on published results.
3. Synthesis suggestion of aliphatic amides based on salicylic acid, amino acid and trifluoromethyl aniline.
4. Experimental part should contain description of synthesis, isolation and characterization of the chosen derivates of salicylamides derived from various amino acids.
5. The results should be briefly evaluated and discussed from the chemistry point of view.
6. All points should be summarized to the form of bachelor thesis.

Rozsah pracovní zprávy:
Rozsah grafických prací:
Forma zpracování bakalářské práce: **tištěná**
Jazyk zpracování: **Angličtina**

Seznam doporučené literatury:

All available professional literature.

Vedoucí bakalářské práce: **doc. Ing. Aleš Imramovský, Ph.D.**
Ústav organické chemie a technologie

Datum zadání bakalářské práce: **26. února 2021**
Termín odevzdání bakalářské práce: **2. července 2021**

L.S.

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Acknowledgment

Here I would like to thank the supervisor of my thesis doc. Ing. Aleš Imramovský, Ph.D. for his direction and patience during work in a laboratory and help with working on this thesis. And last but not least I would like to thank my mother and grandmother for the support during my bachelor studies.

Summary

This bachelor's thesis is focused on the preparation of the series of compounds that are based on diamides derived from 5-chlorosalicylamide with different amino acid and 4-aminobenzotrifluoride.

In total were prepared five original compounds of diamides. Intermediates have -benyzl (or rather *O*-Bn) protecting group to prevent unwanted reaction leading to unwanted substances. After reduction using hydrogen with catalyst palladium on carbon (Pd-C) the benzyl protecting group is released to receive hydroxyl group and the final products are obtained.

Keywords

Salicylamides, synthesis of salicylamide's derivates, diamides, amino acids, biological activities

The list of abbreviation

Ac	acetyl
AcOH	acetic acid
Ala	alanine
API	Active Pharmaceutical Ingredient
BCG	Bacillus Calmette-Guerin
Bn	benzyl
BuOH	butanol
$CDCl_3$	deuterated chloroform
CHA	cyclohexane
DCC	*N*,*N*′-dicyclohexylcarbodiimide
DEE	diethyl ether
DMSO	dimethyl sulfoxide
EDC·HCl	(3-dimethylamino-propyl)-ethyl-carbodiimide hydrochloride
E. faecalis	*Enterococcus faecalis*
Et	ethyl
EtOAc	ethyl acetate
EtOH	ethanol
Et_2O	diethyl ether
Et_3N	triethylamine
HOBt·OH	1-hydroxybenzotriazole hydrate
Leu	leucine

MBC	Minimum Bactericidal Concentration
Me	methyl
MeCN	acetonitrile
MeOH	methanol
Met	methionine
MIC	Minimum Inhibitory Concentration
M. marinum	*Mycobacterium marinum*
M. smegmatis	*Mycobacterium smegmatis*
MRSA	*Methicillin-Resistant Staphylococcus Aureus*
MTB	*Mycobacterium Tubercle Bacilli/mycobacterium tuberculosis*
M. tuberculosis	*Mycobacterium tuberculosis*
Nleu	norleucine
Ph	phenyl
rt	room temperature
S. aureus	*Staphylococcus Aureus*
TB	tuberculosis
THF	tetrahydrofuran
TEA	triethylamine
TLC	thin-layer chromatography
Trp	tryptophane
VRE	*Vancomycin-Resistant Enterococcus*

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1. Introduction

As known salicylic acid and its derivates are used in many ways such as a medicine in dermatology to treat different skin problems, its derivates as a drug in pharmacy industry, as a preservative in food industry. The most famous derivate is the acetylsalicylic acid (***Image 1***) as known as Aspirin, ASA, Ascriptin, Aspirtab, Alpyrin, Algin, Bayer Aspirin, etc. Aspirin was studied by Bayer company hence it can be called a Bayer Aspirin, but their rights were either sold or lost. Acetylsalicylic acid, white crystalline drug, is usually taken orally to reduce pain, fever, or inflammation. Most commonly is used as analgetic-antipyretic. However, it can be used specifically to treat Kawasaki disease, pericarditis, and rheumatic fever. Its methyl esters are found in essential oils.

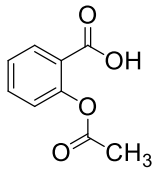


Image 1. *Acetylsalicylic acid*

Not only acetylsalicylic acid has shown its pharmaceutical properties but as well as methyl salicylate, Buklosamid, PAS (p-aminosalicylic acid), and others. The further studies of salicylic acid's derivates have shown that salicylamides and its derivates such as salicylanilide (***Image 2***) and others, have the promising results against cancerous cells and various bacteria and fungi. In this bachelor's thesis will be discussed salicylamides' derivates with antibacterial activities. Salicylanilide

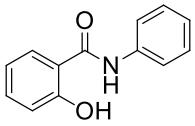


Image 2*. Salicylanilide*

It is very important to discover new structures that can lead to stronger drugs with antibacterial activities and the increasing resistance to bacterial strains for an example

Staphylococcus aureus, *Enterococcus faecalis* and various mycobacteria like tuberculosis. Why it is important? Because there are many reports of getting infected by these bacteria and statistics of the death rate around the world each year. Most cases of the diseases caused by these bacteria are registered in World Health Organisation (WHO). For an example, according to WHO every year 10 million people get ill by *Mycobacterium tuberculosis*. It is known to be preventable and curable disease. Unfortunately, 1.5 million people die from tuberculosis each year. Another good example is *Enterococcus faecalis*. It is found in healthy human body (specifically in gastrointestinal tracts) and can be used as probiotic. However, it can cause several diseases such as endocarditis and sepsis. Sepsis is very dangerous and occurs when the body's response to infection causes injury to its own tissues and organs. And it affects an estimated 49 million people and causes 11 million deaths globally every year.

It is difficult to fight against bacteria due to their resistance to antibiotics. Hence, we should discover and synthesise new drugs that can lead to new treatments.

This bachelor's thesis includes salicylic acid's derivates structure (alternatively salicylamide's derivates) and syntheses that has a promising antibacterial activity.

2. Aim of the work

The aim of the theoretical part of my bachelor's thesis is to briefly characterise synthesis of aliphatic salicylamides, alternatively structures of similar amides of carboxylic acids. This part also contains the general information about salicylic acid and salicylamides. Theoretical part, moreover, is about the synthesis suggestion of aliphatic amides based on salicylic acid, amino acid and trifluoromethyl aniline.

The next aim, which is presented in theoretical part, is biological activities of salicylamides. It is briefly written about their antibacterial activities, what kind of bacteria or fungi they eliminate and the mechanism.

The aim of the experimental part is to show the synthesis and isolation of the chosen derivates of 5-chlorosalicylamides derived from Trp, Met, Ala, NLeu, CHA amino acids.

For the isolation and purifying methods of intermediates should be used column chromatography, alternatively flash chromatography, or crystallization. The final products should be prepared in sufficient amount and purified, so they could be tested for antibacterial activities. However, due to COVID-19 outbreak and personal issues the final products are either not enough purified or the weight is too low for them to get tested.

3. Theoretical part

Theoretical part of the bachelor's thesis is focused on general information of the chosen derivates derived from salicylic acid. Hence it is important to know about salicylic acid's properties and syntheses as well as syntheses of salicylamides. There will be discussed known syntheses of 2-hydroxy-*N*-(arylalkyl)benzamides (diamides), which are the part of my experiments. Moreover, in my work the prepared compounds should have the antibacterial activities, which are briefly discussed in this paragraph.

3.1. Salicylic acid

2-hydroxybenzoic acid, $C_7H_6O_3$, is a white crystal needle that can melt at 156 °C and can sublimate if it is heated carefully. It is a strong acid with a typical sweet and sour taste for any other acids. Salicylic acid is soluble in fatty oils, alcohols, ethers, and hot water.[1] It is resistant to infection by fungi, bacteria, and viruses. Many plants like willow, poplar and meadowsweet have analgesic activity thanks to salicylic acid and its derivates, such as methyl salicylate, saligenin, salicin and many others (***Image 3***), which are found in these mentioned plants. The most popular drug that is derived from salicylic acid is acetylsalicylic acid, or as known as Aspirin.[2]

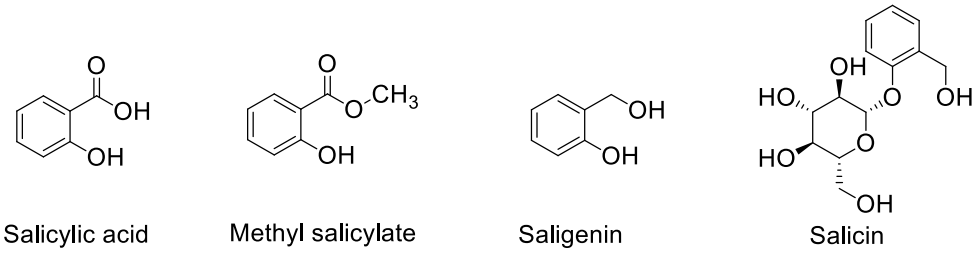
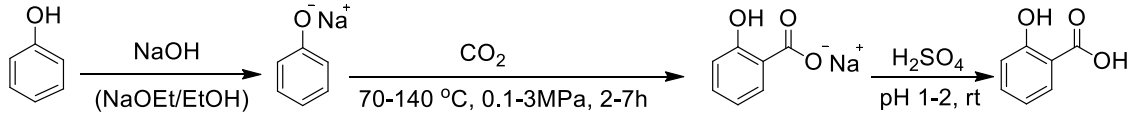


Image 3. *Salicylic acid and its derivates*

The first synthetic synthesis of salicylic acid was made by Hermann Kolbe and Rudolf Schmidt (or in some literatures Rudolf's last name can be written as Schmitt), hence the synthesis is named after them, which is Kolbe-Schmidt (Schmitt) reaction (***Scheme 1***). The reaction itself is based on heating sodium phenolate under high pressure while

bubbling carbon dioxide. After few hours, the obtained sodium salicylate is acidified by inorganic acid such as sulphuric acid until pH is around 1-2.[3]



Scheme 1. *Synthesis of salicylic acid by Kolbe-Schmidt reaction*

3.2. Salicylamides

Salicylamides are derivates of salicylic acids. The simplest of them is 2-hydroxybenzamide or salicylamide (Salamid, Dolomide, Urtosal), molecular formular $C_7H_7NO_2$ (***Image 4***). It is soluble in water, diethyl ether, ethanol, and propylene glycol. In medicine salicylamide is used in the same way as many derivates of salicylic acid as analgesic and antipyretic. As the drug it is usually take orally, is mostly absorbed in the human gastro-intestinal tract. Thanks to its capability to metabolise quickly and completely to inactive metabolites it is safe to take it *per os*. "It is distributed to most body tissues and rapidly excreted in urine, mainly as glucuronide and sulphate conjugates".[4]

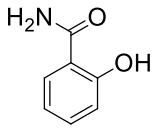
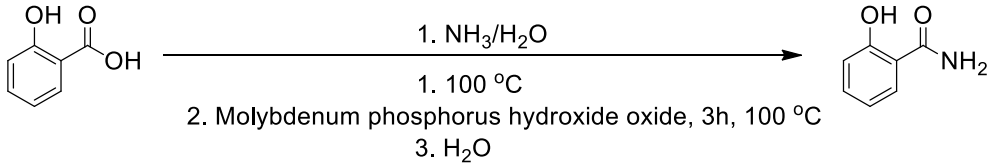


Image 4*. 2-hydroxybenzamide*

The efficient way to synthesise salicylamide is from salicylic acid, but it can be synthesised from different derivates of salicylic acid such as methyl salicylate, salicylaldehyde, salicylonitrile.

The synthesis from salicylic acid (***Scheme 2***) is economically and environmentally beneficial way that includes mixing salicylic acid with liquid ammonia and de-ionised water by evenly heating between 85 and 110 °C to make ammoniated solution, which is

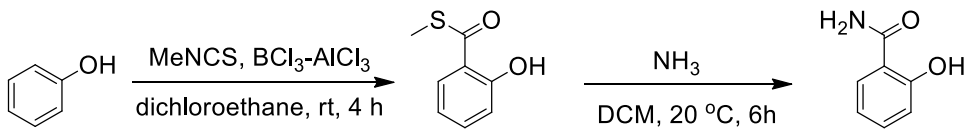
catalysed by molybdenum phosphorus hydroxide oxide, $HMoO_2P^{-6}$ (CAS: 11104-88-4), and dehydrated for few hours. After achieving the intermediate de-ionised water is added again and the intermediate is centrifuged. Afterwards the supernatant liquid is removed to obtain the final product.[5]



***Scheme 2**. Synthesis of salicylamide from salicylic acid*

As it was mentioned there are many ways to synthesise 2-hydroxybenzamide. One of the best yielded reactions showed syntheses with methyl thiosalicylate (***Scheme 3***). In the work, from where the reaction was taken, it is mentioned that they were trying to achieve not only 2-hydroxybenzamide but also *meta*-halogenated salicylamides and other *meta*-halogenated aromatic compounds including salicylic acids and methyl salicylates. The targeted substances for the sourced work are derivates of salicylic acid and oxadiazolium, which will be discussed later. [6]

For the 2-hydroxybenzamide specifically the reaction includes thioester that was synthesised from phenol using methyl thiocyanate with the presence of BCl_3-$AlCl_3$ in dichloroethane. After obtaining S-methyl-2-hydroxybenzothoate the reaction is continued by mixing this intermediate with ammonia in dichloromethane to achieve the final product. However, it can be synthesised directly from phenol using methyl cyanate and $AlCl_3$ in toluene, but it is not easily accessible and most likely the final product would be *N*-methylsalicylamide.[6]



***Scheme 3.** Synthesis of salicylamide from methytl thiosalicylate*

It is important to look in other structures of salicylamides. For my bachelor's thesis specifically, the main concern should be around *N*-substituted arylalkyl salicylamides derivates, but before I will touch that topic, I would like to mention some derivates of *N*-substituted aliphatic salicylamides and derivates of *N*-substituted aromatic salicylamide, which are mostly salicylanilides. Their syntheses will be discussed later in this work.

As it is mentioned in ***1. Introduction*** paragraph the bioactivities of salicylanilide (or *N*-phenylsalicylamide, 2-hydroxy-*N*-phenylbenzamide) should be studied more for discovering new potential drugs against various fungi, bacteria, and viruses. Generally, *N*-phenylsalicylamide, $C_{13}H_{11}NO_2$, is white, odourless crystal with the melting point 135 °C. It is very soluble in organic solvents (alcohols, ethers, benzene, chloroform), but is slightly soluble in water. It can be heated to decomposition; however, it is very dangerous due to its emission of toxic NO_x.[7]

In 1967 studies have shown that 45 analogues of salicylanilide have the bacteriostatic, tuberculostatic, fungistatic, ascaricidal, and molluscicidal effects. It is also shown that halogenated derivates of salicylanilide are effective against staphylococci (bacteriostatic effect) and filament dermatophytes (fungistatic effect).[8,9] It is reported in many studies that the overall activity on bacteria's walls is better against gram-positive than gram-negative.[8,10,11,12] In other words, salicylanilide and its derivates are effective against bacteria like *Mycobacterium tuberculosis* and can barely affect or have no effect on bacteria like *Pseudomonas pyocyanea*, *Candida albicans*, *Saccharomyces cerevisiae*, *Penicillium* strains, and *Aspergillus* strains.[8,9, 11-13]

More information of chosen bacteria will be discussed in the future chapters.

Salicylanilide and its derivates interact with the cell mitochondria of bacteria or even any parasitic-like organism.[14] There is a possibility that this effect can be influenced by hydrophobic groups on the anilide ring and electron-accepting group on the salicylic moiety.[11]

I would like to mention that the Imramovský Research Group has found potential antibacterial agents in other salicylamide's derivates like 2-hydroxy-*N*-[1-(2-hydroxyphenylamino)-1-oxoalkan-2-yl]benzamide. They are also known as diamides

thanks to their characteristic skeleton (***Image 5***).[12] These diamides are focused on this bachelor's thesis, but mostly their syntheses. Certainly, the syntheses will be described later in this work. However, I would like to note that the activity of these diamides can vary according to type of the amino acid group they have.[12,15] They can even have the same activity as the proteasomal inhibitors that leads to anticancer activity.[15]

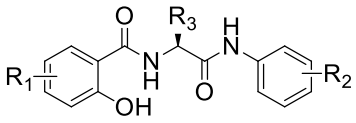


Image 5. *Structure of diamides*

Aside from diamides many derivates of *N*-substituted aromatic salicylamides (***Image 6***) are registered for medical use and not only. For an example antimycin itself has no specific used, but antimycin 1 and antimycin 3 are antibiotics and can be used as insecticide and miticide.[16] There are also halogenated salicylanilides such as Niclosamide, 5-chlorosalicylanilide, Closantel and 5-chloro-*N*-(4-(trifluoromethyl)phenyl)-2-hydroxybenzamide. Nicloasmide and Closantel are mostly anthelmintics that are good against tapeworms or nematodes, when the other two are antimycobacterial.[9,14]

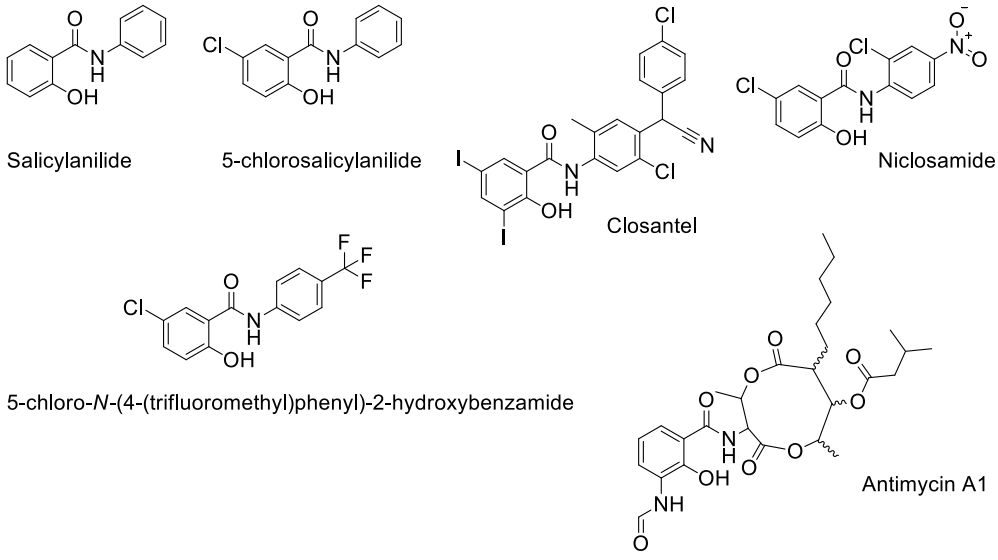


Image 6. *N-substituted aromatic salicylamides*

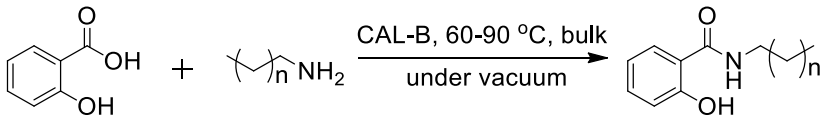
For this bachelor's thesis it is important to discuss about 5-chlor-*N*-substituted derivates. They have indeed similar properties to salicylanilide such as antibacterial activity. And it has been reported in some studies that chlorine at the position 5 has connection to the activity of these salicylanilides or even diamides.[12,17] The further explanation will be in the bioactivity chapter.

Syntheses of *N*-substituted aromatic salicylamides will be discussed later in this work.

3.2.1. Synthesis of *N*-substituted aliphatic amides of salicylic acid

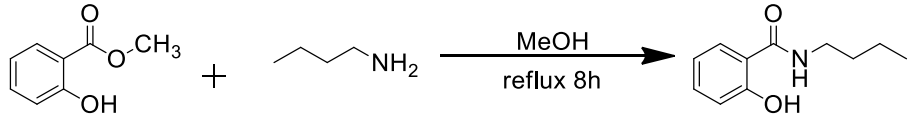
In this chapter is shown different syntheses of aliphatic salicylamides. The simplest aliphatic salicylamide, 2-hydroxybenzamide, is discussed in the previous paragraph. The other aliphatic salicylamides have in amino group one or two hydrogens substituted with aliphatic chain.

The longest aliphatic chain with the excellent yield results (vary between 75.6 % and 83.5 %) can be achieved by using Candida antarctica lipase B (***Scheme 4***). The general synthesis is based on reaction between phenolic acids (in this case salicylic acid) and aliphatic amines. The specific of this reaction is that it does not need any solvent with activating agents.[18]



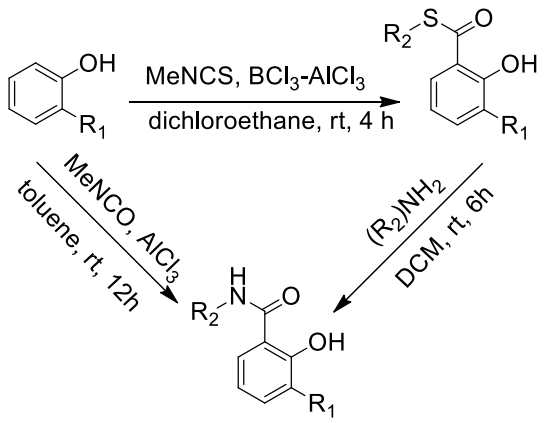
Scheme 4. *Synthesis of aliphatic salicylamides with the long aliphatic chain from salicylic acid*

The other long aliphatic chain derivate can be obtained using butylamine and methyl salicylate (***Scheme 5***), which are dissolved in methanol. The mixture is heated at reflux for 8 hours while stirring. After control of presence of the reactants the methanol should be distilled, and what is remained after distillation should be acidified by adding an aqueous hydrochloric acid. Then follows extraction with dichloromethane. For the best result, the mixture needs to be purified by silica gel column chromatography.[19]



Scheme 5. *Synthesis of aliphatic salicylamides with the long aliphatic chain from methyl salicylate*

Some aliphatic salicylamides can be synthesised using the same method as the one that is mentioned in the previous paragraph in ***Scheme 3***. For the direct syntheses *N*-substituted aliphatic salicylamides can be used *meta*-halogenated phenols or using the two-step method from *meta*-halogenated thioesters, from which synthesise the targeted products (***Scheme 6***). [6]

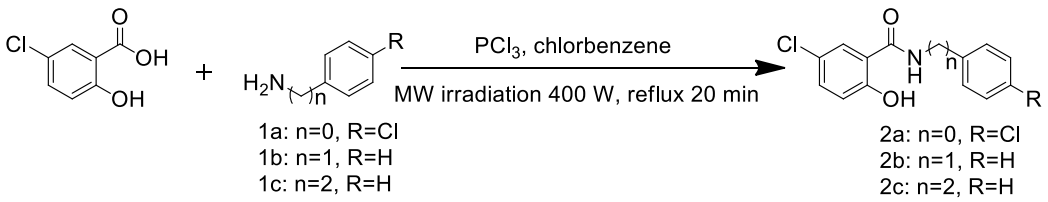


Scheme 6. *Synthesis of aliphatic salicylamides from different meta-halogenated phenols*

As it was mentioned before for the direct syntheses of aliphatic salicylamides the *meta*-halogenated phenols react with methyl cyanate and aluminium chloride (aluminium trichloride) in toluene. For this pathway R_2 is always Me group, so when R_1 = H the yield is 79 %, when R_1 = F the yield is 81 %, when R_1 = Cl the yield is 94 %, when R_1 = Br the yield is 87%. It is reported that this way of synthesis of *N*-substituted derivates is indeed better than the two-step way which includes synthesis thioesters at first and then the aliphatic salicylamides at last. And for the second path it is better to produce with R_2 being a -H group, which makes it *meta*-halogen-2-hydroxybenzamide. So, the results are for R_1 = H the yield is 79 %, for R_1 = F the yield is 63 %, for R_1 = Cl the yield is 51 %, for R_1 = Br the yield is 53 %.[6]

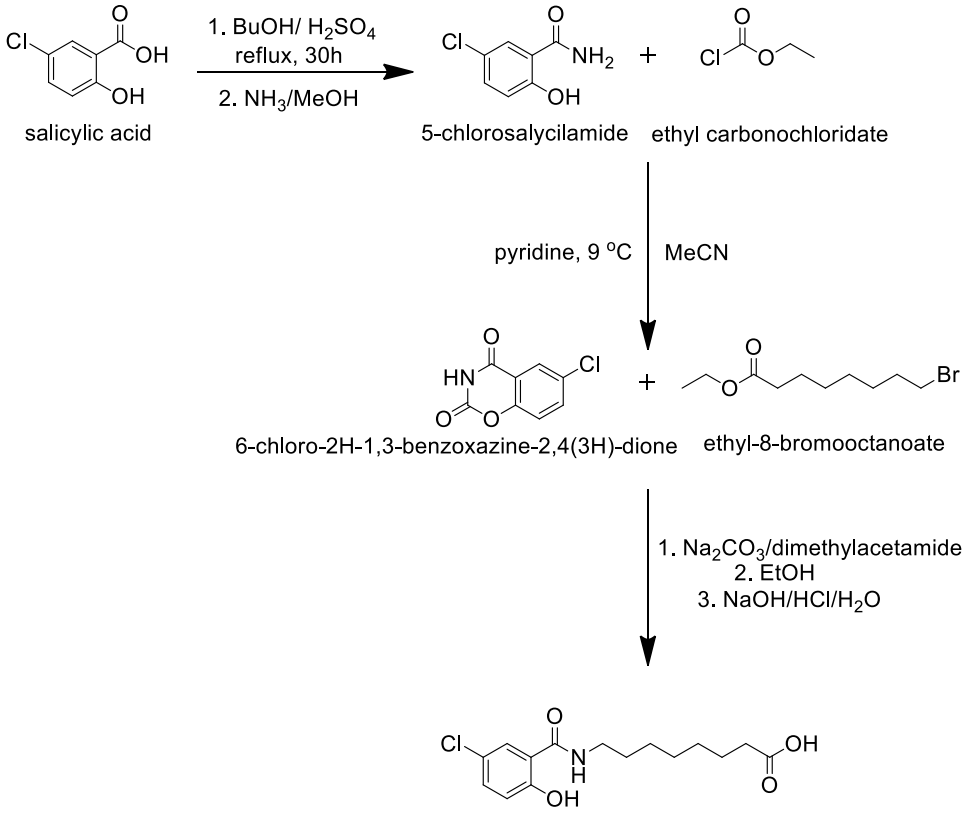
The other aliphatic salicylamides that I would like to discuss are based on 5-chlorosalicylic acid. As it is mentioned in the previous chapter the substitution of chlorine at the position 5 on salicylic acid's moiety can increase the suppression of defecated

immune cells.[17] One of the quickest synthesis of these derivates is reported using microwave-assisted way (***Scheme 7***).[20]



Scheme 7*. Synthesis of salicylamides using microwave-assisted way*

From 5-chlorosalycilyc acid can be synthesised *N*-(5-chlorosalicyloyl)-8-aminocaprylic acid by 3-step reaction that is used in pharmaceutical industry (***Scheme 8***). It is one of the fastest and high yielded preparation of this derivate.[21]



Scheme 8*. Synthesis of N-(5-chlorosalicyloyl)-8-aminocaprylic acid*

In the first step salicylic acid is mixed with n-butanol using Dean stark trap, while poring sulphuric acid. The rection mixture is set to reflux for 30 hours. Afterwards the reaction should be cooled and charged with deionised water, keeping the room temperature. Next it is washed by sodium bicarbonate and deionised water until all aqueous phase is removed. After that the reactor is set to distillation to eliminate remaining n-butanol by heating the mixture between 140 °C and 150 °C and gently lowering pressure to 500 mm Hg, which is approximately 66.6612 kPa. Adding the anhydrous methanol, the headspace of the reactor is flushed with anhydrous ammonia by barley letting it to be bubbled that is set for 18-24 hours. In the end the mixture was distilled, acidified, filtrated, and dried to achieve the final product, which is 5-chlorosalycilamide. [21]

In the second step acetonitrile is cooled to 9 °C and then 5-chlorosalycilamide can be added before pyridine. After two hours the mixture reacts with ethyl chloroformate (chloroformic acid ethyl ester), however, the temperature should remain and cannot be over 14 °C. Next the reaction is heated to 85-94 °C for 6 hours, while collecting distillate for monitoring the conversion. In the end the reaction is cooled and dried to receive intermediate (6-chloro-2H-1,3-benzoxazine-2,4(3H)-dione) that should be stored without exposing to water. [21]

In the third last step cooling reactor is charged with anhydrous sodium carbonate then is added 6-chloro-2H-1,3-benzoxazine-2,4(3H)-dione and ethyl-8-bromooctanoate, which is set to 65-75 °C. The reaction should be finished in 7 hours. After that the reactor is cooled to 45 °C and the mixture is washed by ethanol several times, but for the last one the temperature is reduced even more down to 8 °C, which yields ethyl 8-(6-chloro-2H-1,3-benzoxazine-2,4(3H)-dionyl)octanoate. To eliminate ethanol is added sodium hydroxide with purified water, after which the reaction is set to reflux from 68 °C up to 98 °C. Over 4 hours the mixture was cooled down to room temperature and it is acidified by hydrochloric acid with purified water. However, pH is needed to be adjusted by sodium hydroxide solution to achieve pH around 2-4. In the end the mixture is crystalised and then is dried to yield *N*-(5-chlorosalicyloyl)-8-aminocaprylic acid.[21]

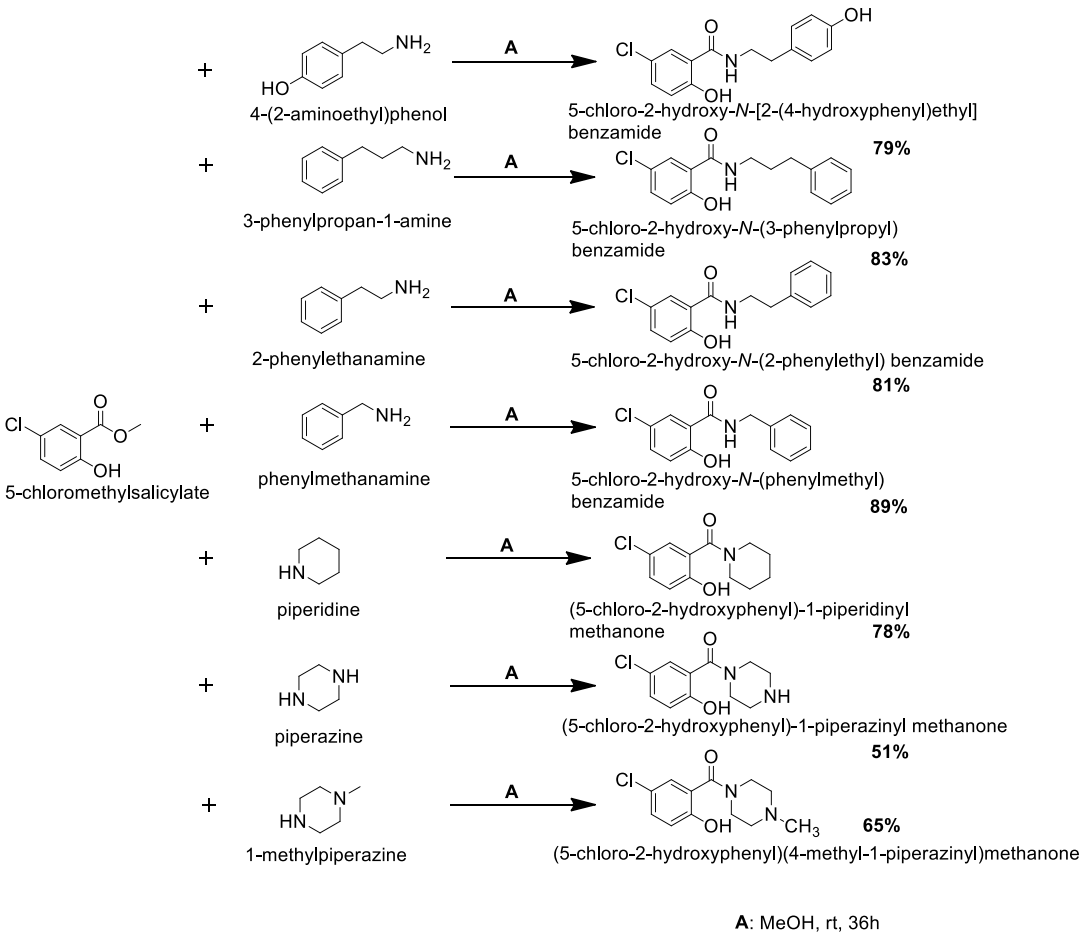
I would like to describe syntheses (***Scheme 9***) from the work, where it is reported about 5-chlor salicylic acid's derivates having antitumor and anti-inflammatory agents, which were briefly discussed in the ***3.2. Salicylamides*** paragraph.[17] The names of compounds are given according to the article.

The procedure is the same for every reaction that are shown below. However, to obtain methyl 5-chlorsalicylate they used 5-chlorsalicylic acid in methanol and concentrated H_2SO_4 to mix it. After that the mixture was set to reflux for one hour, after which it was evaporated. And dissolved mixture in ethyl acetate was washed with 5 % $NaHCO_3$.[17]

For the following reactions methyl 5-chlosalicylate is dissolved in methanol and to the solution is added either phenylalkylamine or hetero nonaromatic cycle (piperidine, piperazine, methylpiperazine). The reaction is let to stir for 36 hours. Afterwards, the mixture is evaporated. For washing process with 1 M HCl and 5 % $NaHCO_3$ the mixture is dissolved in ethyl acetate. The next it is dried over anhydrous Na_2SO_4. In the end the ethyl acetate is needed to be removed by flash evaporate to obtain to the corresponding amine N-(5-chlorosalicyloyl)phenylalkylamine. [17]

As it is shown the best yielded are 5-chloro-2-hydroxy-*N*-(phenylmethyl)benzamide with 89 % and 5-chloro-2-hydroxy-*N*-(3-phenylpropyl)benzamide with 83 %. Overall, 5-chloro-2-hydroxy-*N*-(phenylalkyl)benzamide are better yielded than (5-chloro-2-hydroxyphenyl)-1-piperidinylmethanone and (5-chloro-2-hydroxyphenyl)-1-piperazinylmethanone. However, the result of piperidine derivate is significantly better than the piperazine one. To obtain better results piperazine derivate needs to be *N*-substituted.[17]

This reaction is similar to what I have used in the experimental part.



***Scheme 9**. Syntheses of N-(5-chlorosalicyloyl)phenalkanyl- and heterocyclicamine*

In the end I would like to mention the synthesis of salicylamides using carbodiimides (***Scheme 10***). They have been reported being as *Plasmodium falciparum* dihydroorotate dehydrogenase (PfDHODH) inhibitors. For this work I will mention the syntheses that are derived from methyl salicylate. The used carbodiimide is dicyclohexyl carbodiimide (DCC), which is shown in the ***Image 7***.[22]

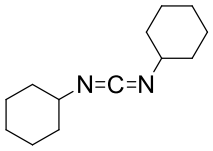


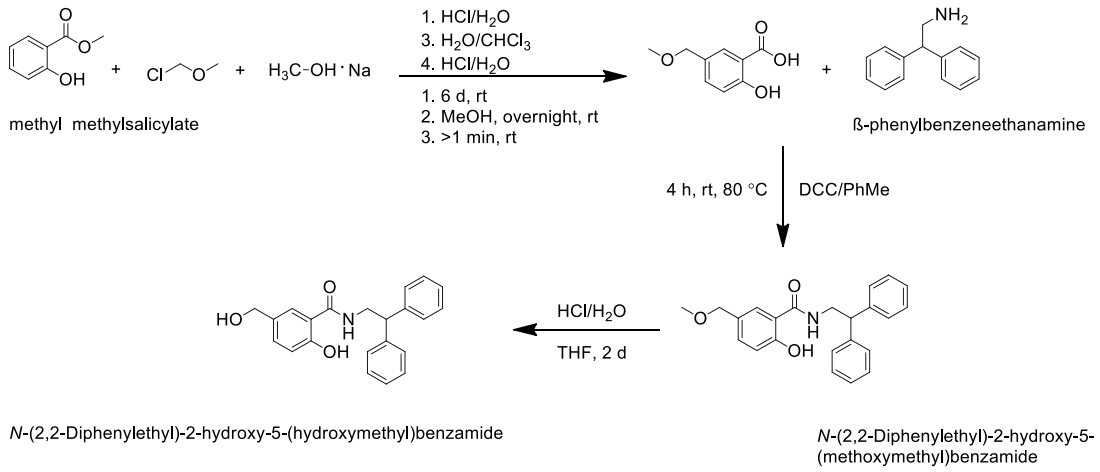
***Image** 7. DCC*

Salicylic acid methyl ester (98 mmol) is stirred in concentrated HCl (37 %) and is added chloromethyl methyl ether (254 mmol). Then the reaction mixture is stirred for 4 days and is added second portion of chloromethyl methyl ether (56 mmol). The mixture is stirred for another 2 days. The crude product is isolated by filtration and is dried in vacuum. The product is recrystallised twice from petroleum ether (2x100 mL) to obtain 5-chloromethyl-2-hydroxy-benzoic acid methyl ester, which is in the next step stirred with 0.5 M NaOMe in MeOH (5 mL) at room temperature overnight. After the solvent was removed, $CHCl_3$ (20 mL) and water (10 mL) are added to the mixtire while stirring to accomplish the hydrolysis of the ester. Then the solution is acidified with 5 M HCl (0.5 mL). The organic phase is extracted with 1 M $NaHCO_3$ (20 mL). The mixture is again acidified with 5M HCl (2 mL). The mixture is extracted with $CHCl_3$ and then dried with Na_2SO_4. The solvent is evaporated to obtain 2-hydroxy-5-methoxymethyl-benzoic acid.[22]

A mixture of benzoic acid derivative (1.2 mmol), 2,2-diphenylethylamine (1.4 mmol), dicyclohexyl carbodiimide (1.05 mmol) and toluene (3 mL) are stirred together. The reaction mixture is then heated to 80 °C for 4 hours. After these hours, the mixture is cooled to 40 °C. Afterwards, the formed *N*,*N*'-dicyclohexylurea is filtered. For purification is used column chromatography with toluene solution on silica gel and with heptane/EtOAc (at a ratio of 8:1 to 2:1) as eluent to obtain *N*-(2,2-diphenylethyl)-2-hydroxy-5-(methoxymethyl)benzamide.[22]

For the last step *N*-(2,2-diphenylethyl)-2-hydroxy-5-(methoxymethyl)benzamide (0.28 mmol) is dissolved in THF (10 mL) and is added to 5M HCl (40 mL). The reaction mixture is stirred vigorously for 2 days. After the time has passed, the residue is extracted with $CHCl_3$ (2x50 mL). The combined organic phase is washed with water (50 mL) and is dried using Na_2SO_4. Then the solvent is evaporated. For purification is used column

chromatography on silica gel with heptane/EtOAc (at a ratio of 4:1 to 1:1) as eluent to obtain the final product (2,2-diphenylethyl)-2-hydroxy-5-(hydroxymethyl)benzamide.[22]

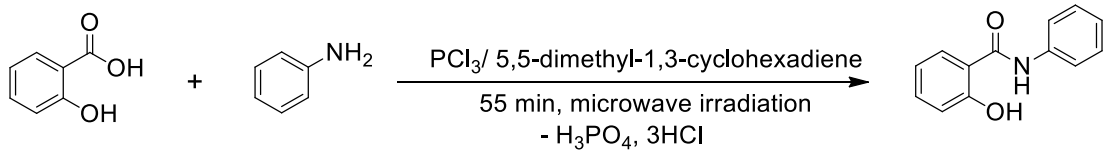


***Scheme 10**. Synthesis of salicylamides using carbodiimides*

3.2.2. Synthesis of N-substituted aromatic amides of salicylic acid

The aromatic salicylamides previously were discussed in the chapter ***3.2. Salicylamides***. Therefore, most of them are salicylanilides, hence in this chapter the main focused is on their syntheses.

The simplest salicylanilide is 2-hydroxy-*N*-phenylbenzamide (***Image 2***). The synthesis is based on reaction between salicylic acid and aniline. In 1968 there was the synthesis showed the 73 % yield using P_2O_5 as condensing agent, $AlCl_3$ as catalyst, and xylene as solvent.[23] But recent experiments showed better results and one of them uses the microwave irradiation way (***Scheme 11***). It is very simple reaction that has a good yield and overall reduced reaction time.[24]

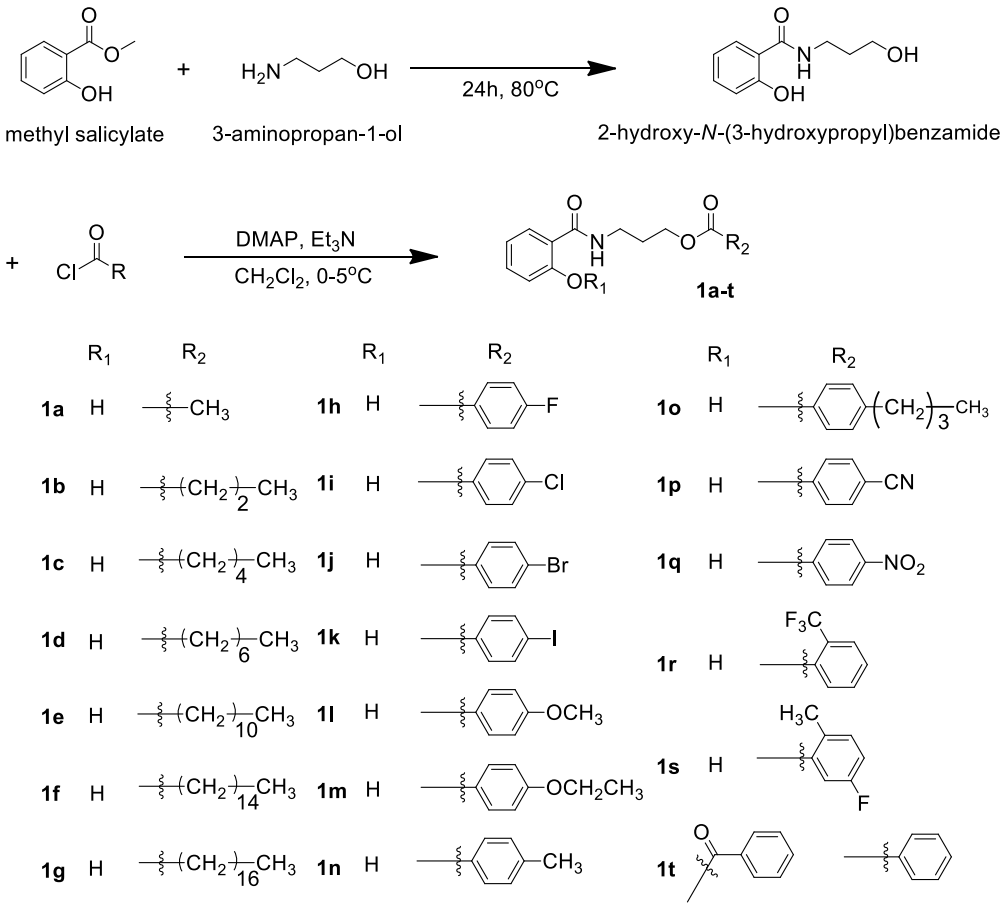


***Scheme 11**. Synthesis of 2-hydroxy-N-phenylbenzamide*

In one of recent studies has reported some inhibitory activity on polyphenoloxidase (PPO) of various salicylanilide's analogues like fatty acid 3-(2-hydroxy-benzoylamino)-propyl esters (**1a-1g**) and substituted-benzoic acid 3-(2-hydroxy-benzoylamino)-propyl esters (**1h-1t**).[25]

The procedure of the syntheses of following derivates **1a-t** is the same (***Scheme 12***). Before them the intermediate 2-hydroxy-*N*-(3-hydroxypropyl)benzamide is synthesised as first. For that methyl salicylate and 3-amino-1-propanol are added in the same round flask to stir them for 24 hours at 80 °C, controlling the reaction by using TLC. After the reaction is finished the mixture is extracted with ethyl acetate and tert-pentyl alcohol (at a ratio of 1:1). The organic phase is dried over anhydrous $MgSO_4$ and evaporated in vacuum. In the end the substance is purified by silica gel column chromatography (petroleum ether/ethyl acetate at a ratio of 1:5) to achieve the final product, which is 2-hydroxy-*N*-(3-hydroxypropyl)benzamide. The yield is 80 %.[25]

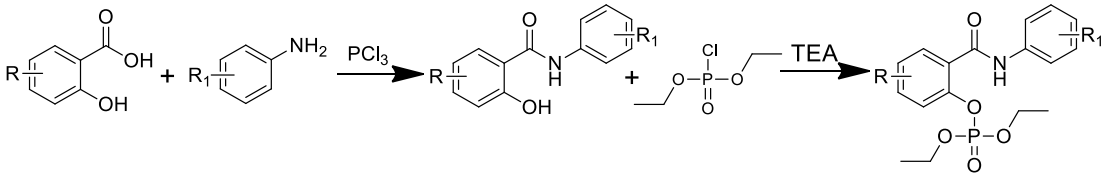
The intermediate (2-hydroxy-*N*-(3-hydroxypropyl)benzamide), DMAP (4-dimethylaminopyridine) and Et_3N are dissolved in dried CH_2Cl_2 solution in the ice bath. The reaction is let to be stirred overnight. Afterwards, the mixture is evaporated in vacuum and, what is left in the flask, is purified by a silica gel column chromatography with petroleum ether/ethyl acetate (at a ratio of 3:1). Here products are divided by their physical characteristic: one group is yellow oil (**1a-d** and **1r**) and the second is solid, which are recrystallized from ethyl acetate to give colourless crystal (**1e-t**) in yield of 59–88 %. However, the best compound that has shown promising anti-browning and antimicrobial properties to be applied in the food industry is **1q**.[25]



***Scheme 12**. Synthesis of derivatives of salicylanilide*

In the 1981 was reported a potential salicylamide antiplaque agents and one of them is dibromsalan.[26] However, in 2014 were synthesised a series of 27 salicylanilide diethyl phosphates, where one of intermediates is dibromsalan.[27]

The following series of substituted diethyl [(2-phenylcarbamoyl)phenyl] phosphates are synthesised in two steps (***Scheme 13***). The R on salicylic acid is either chlorine at the position 4 or 5, or it is bromine at the position 4 (4-Cl, 4-Br, 5-Cl). The R_1 on aniline is halogens at the positions 3 or 4 (3-Cl, 4-Cl, 3-Br, 4-Br, 3-F, 4-F), can be dihalogen at positions 3 and 4 (3,4-diCl), or it is trifluoromethyl group at positions 3 or 4 (3-CF3, 4-CF3). Specifically for 4/5-chloro and 5-bromosalicylic acids with substituted anilines the condensation is done in a microwave reactor. Synthesised salicylanilides are esterified by diethyl chlorophosphate in triethylamine (TEA) and dichloromethane. In the end the mixture is isolated and purified giving within the range of 11–78 %.[27]



Scheme 13. *Syntheses of substituted diethyl [(2-phenylcarbamoyl)phenyl] phosphates*

3.2.3. Synthesis of diamides

This chapter is important to this bachelor's thesis. In the experimental part I will show my work that is closely related to syntheses of 2-hydroxy-*N*-(arylalkyl)benzamides or so called diamides (***Image 5***). Here I would like to continue this topic and describe their syntheses as it was promised.

As I have mentioned in paragraph ***3.2. Salicylamides*** Imramovský Research Group have had experiments with diamides. In 2006 was reported process of salicylanilide esterification, where they obtained Z-amino acid esters with substituted salicylanilides. Further experiments showed their capability to form cycles like benzoxazepines and nine-membered ring (***Image 8***).[11]

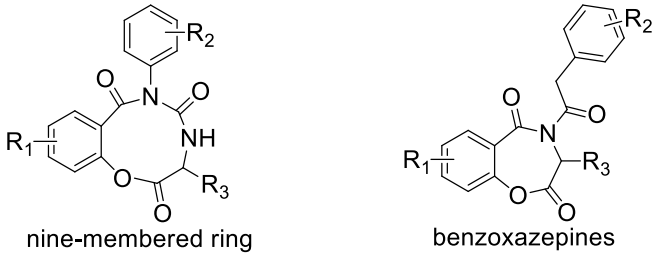


Image 8. *Cyclisation possobilities of Z-amino acid esters with substituted salicylanilides*

From Z-amino acid esters can be obtained diamides as well as from the Leuch's anhydride of L-phenylalanine with the proper salicylanilide salt (***Scheme 14***).[11]

For synthesis of hydrobromide salt a solution of hydrogen bromide in acetic acid (33%) needs to be added to *N*-benzyloxycarbonyl-protected esters (or Z-amino acid esters) while stirring it at room temperature for 30 min. When the gas (in this case carbon dioxide) evolution ceased, which can be monitored visually: the suspension turns into a clear brown solution, dry diethyl ether (DEE) can be added to the mixture. The next the reaction mixture is collected by filtration, then it is washed with DEE three times and afterwards is dried. The isolated crystals should be suspended in dry chloroform at room temperature, filtered and the left chloroform is evaporated in vacuum at room temperature. The yield is about 90 %.[11]

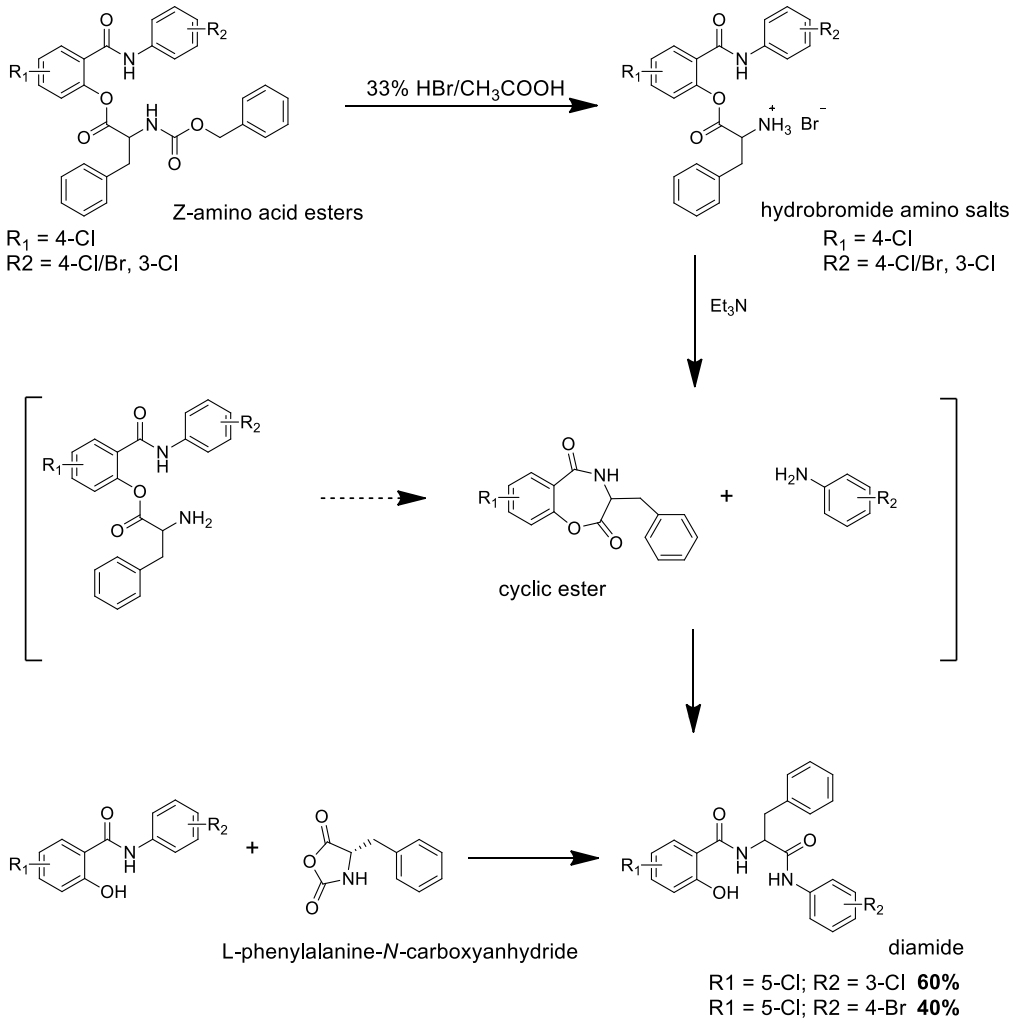
For synthesis of diamide can be used the pathway that includes synthesis of hydrobromide salt. In the same flask triethylamine needs to be added to hydrobromide salt, which was made in the previous step, in dry chloroform and the mixture is stirred at room temperature for 30 min. Afterwards, the insoluble material is filtered off and the filtrate should be purified by using either a Chromatotron® Harrison Research Model 7924T (toluene/ethyl acetate at a ratio of 4:1) or flash chromatography (toluene/ethyl acetate at a ratio of 9:1).[11]

The second pathway includes synthesis of the Leuch's anhydride of L-phenylalanine. The reactant's synthesis is not shown in the ***Scheme 14***, but I would like to briefly describe it, which is given in the same article.[11]

For the Leuch's anhydride synthesis a solution of bis(trichloromethyl)carbonate in tetrahydrofuran (THF) is added to a suspension of L-phenylalanine in THF. The reaction mixture is stirred for 3 hours at 50 °C under an argon atmosphere. When reaction is done, which is monitored visually (the suspension becomes a transparent solution), the solvent is evaporated under reduced pressure. The produced oil is recrystallized from a mixture of THF/petroleum ether and then it is dried at room temperature. The yield is 78 %. The given product is then used in the synthesis that is described in the ***Scheme 14***.[11]

Finally, for the diamide's synthesis a solution of 5-chloro-*N*-(3-chlorophenyl)-2-hydroxybenzamide potassium salt, cetyltrimethylammonium bromide and a solution of L-phenylalanine-*N*-carboxyanhydride (all mentioned solutions are in THF) are added. The reaction is performed under direct sonication at 40 °C for 1 hour. The solvent is then evaporated under reduced pressure, afterwards the residue is dissolved in dry ethyl acetate and heated at reflux for 1 hour. Insoluble material is filtered off, and the filtrate is evaporated and purified by flash chromatography (toluene/ethyl acetate at a ratio of 4:1).[11]

The yields of diamide's vary (between 40 and 60 %) according to their R_1 and R_2 groups.[11]



***Scheme 14**. Two pathway syntheses of diamide*

Another reported synthesis in October 2013, which I have mentioned in ***3.2. Salicylamides*** includes works from Imramovský Research Group, described the syntheses of diamides using substituted 2-(benzyloxy)chlorobenzoic acid and L-amino acid tert-butyl ester hydrochloride (***Scheme 15***). These diamides have shown potential anti-cancer activity.[15] When the similar report (that was also previously mentioned) in November the same year detected diamides with antibacterial activity.[12]

First, I would like to describe syntheses of the earlier registered article that is shown in ***Scheme 15***.

At first should be synthesised amino acid salicylate esters from substituted acetyl or benzyloxy salicylic acid and amino acid ester hydrochloride. Amino acid moiety varies. In their work was used different (*S*) and (*R*) isomers of alanine, leucine, phenylalanine, cyclohexylalanine and indol-alanine.[15]

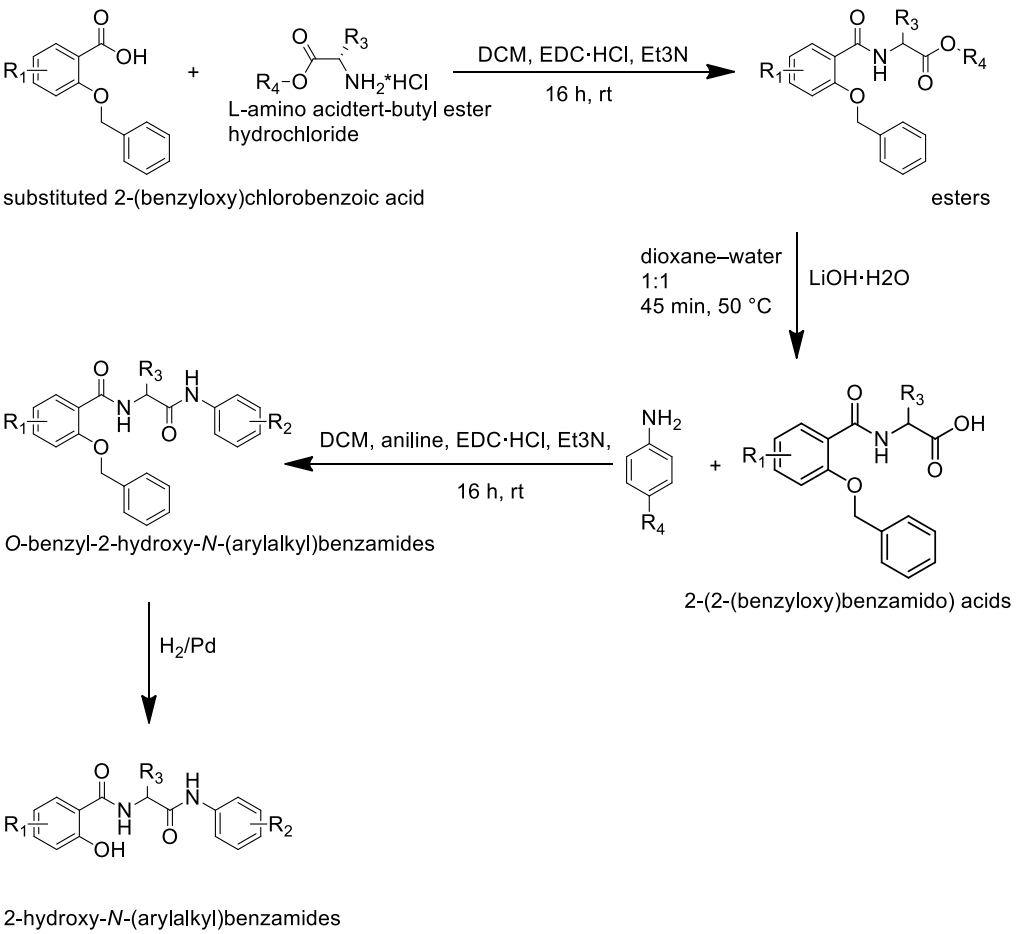
Therefore, quantitatively is added amino acid ester hydrochloride to a stirred solution of substituted acetyl or benzyloxy salicylic acid in dichloromethane. The solution is then cooled. When it approaches the desired temperature (5 °C), EDC·HCl and HOBt·H2O are added to the mixture. Triethyl amine is added dropwise. The clear mixture is stirred at the same temperature for 30 min. After additional stirring at room temperature overnight, the reaction mixture is washed three times with dichloromethane. The next step is extraction with saturated solution of sodium hydrogen carbonate ($NaHCO_3$), 5 % water solution of citric acid ($C_6H_8O_7$), and brine (NaCl), then dried over anhydrous Na_2SO_4. Afterwards, the organic solvent is evaporated under reduced pressure and residue is purified by silica gel column chromatography (EtOAc/hexane at a ratio of 1:1) to give desired esters as colourless oils.[15]

The next procedure includes synthesis of amino acid *O*-benzyl-salicylate acids. To a stirred solution of esters, which were prepared from the previous steps, in mixture of 1,4-dioxane/H_2O (at a ratio of 2:1) is added $LiOH·H_2O$ at 50 °C. When esters are disappeared from the mixture (monitoring with TLC), to the reaction is added HCl and the residues is washed three times with EtOAc. Organic phases are collected and dried over anhydrous

Na_2SO_4. And to obtain *O*-benzyl salicyloic acids the solvent is evaporated under reduced pressure.[15]

The following general procedure for synthesis of 2-*O*-benzyloxy-*N*-(arylalkyl)benzamides includes reaction of chosen aniline and amino acid derivate, which was described in the previous steps. To a stirred solution of *O*-benzyl salicyloic acids in dichloromethane quantitatively is added a chosen aniline. The solution is then cooled. When it approaches the desired temperature (5 °C), EDC·HCl and HOBt·H2O are added to the mixture. Triethyl amine is added dropwise. The clear mixture was stirred at the same temperature for 30 min. After additional stirring at room temperature overnight, the reaction mixture is washed three times with dichloromethane. The next step is extraction with saturated solution of sodium hydrogen carbonate ($NaHCO_3$), 5 % water solution of citric acid ($C_6H_8O_7$), and brine (NaCl), then dried over anhydrous Na_2SO_4. Afterwards, the organic solvent is evaporated under reduced pressure and residue is purified by silica gel column chromatography (EtOAc/hexane at a ratio of 1:1) to give desired 2-*O*-benzyloxy-*N*-(arylalkyl)benzamides.[15]

For the last procedure for synthesis of 2-hydroxy-*N*-(arylalkyl)benzamides needs the previous described compound (2-benzyloxy-*N*-(arylalkyl)benzamide) to be dissolved in EtOAc. The mixture is bubbled with hydrogen using catalyst (10 % Pd on carbon) at the standard atmospheric pressure (1 atm) for 12 hours. Palladium on carbon is filtered off, and the filtrate is evaporated under reduced pressure. The residue is purified by silica gel column chromatography (EtOAc/hexane at a ratio of 1:3.5) to give the desired 2-hydroxy-*N*-(arylalkyl)benzamides.[15]



Scheme 15. *Synthesis of diamides using substituted 2-(benzyloxy)chlorobenzoic acid and L-amino acid tert-butyl ester hydrochloride*

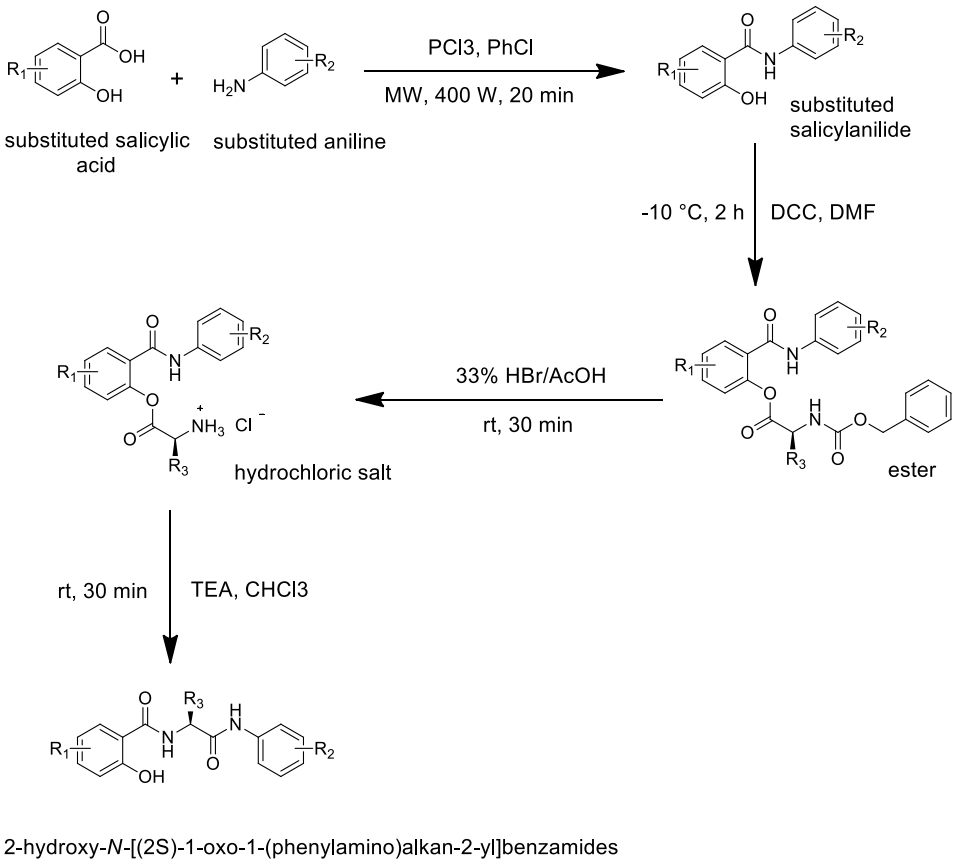
For the different approach of synthesis diamides, which was published in November 2013, is used substituted salicylic acid and appropriate aniline (***Scheme 16***).[12]

For preparation of salicylamides it needs a mixture of substituted salicylic acid, appropriate amine, and phosphorus trichloride in chlorobenzene to be stirred under microwave irradiation in the microwave reactor (400 W input power) for 20 min. After that time, the reaction mixture is left in the fridge overnight. To obtain substituted salicylanilide the residue needs to be collected by filtration and recrystallized from ethanol.[12]

The next step is preparation of amino acid salicylanilide esters. *N*-benzyloxycarbonyl protected amino acid and substituted salicylanilide are dissolved in dry DMF. Afterwards the mixture should be cooled to -10 °C, and then *N*,*N*-dicyclohexylcarbodiimide can be added in three portions for more than 1 hour. The mixture is then stirred for 3 hours at the same temperature and stored at 4 °C for 20 hours. The residue is removed by filtration, and the solvent is evaporated in vacuum. In the end the product is crystallized from ethyl acetate/hexane.[12]

The final step is preparation of diamides. A 33 % solution of hydrogen bromide in acetic acid is gently while stirring added to *N*-benzyloxycarbonyl-protected ester, which was synthesised in the previous step. The suspension is let to stir at room temperature for 30 min. When the gas (carbon dioxide) evolution, which can be monitored visually: the suspension turns into a clear brown solution, is ceased, dry diethyl ether is added. The residue is collected by filtration, then is washed with diethyl ether and dried. Obtained hydrobromide salts are suspended in dry chloroform, and triethylamine is added at room temperature. The reaction is let to stir for 30 min. After that time, insoluble material is filtered off, and the filtrate ispurified by column chromatography (ethyl acetate/hexan at a ratio of 1:1).[12]

At the end of this chapter, I would like to note that in the experimental part will be described more syntheses of diamides.



***Scheme 16**. Synthesis of diamides from substituted salicylic acid and aniline*

3.3. Biological activities of salicylamides

I have mentioned in the chapter ***3.2. Salicylamides*** that the studied salicylamides have some biological activities such as antibacterial, anti-fungi, and anti-cancer. It is important to look at the mechanism of inhibition of chosen bacteria as well as their definition and why they are dangerous. In this chapter will be briefly discussed biological activities of salicylic acid and salicylamide's derivates.

3.3.1. Biological activities of salicylic acid

Salicylic acid as its own is mostly used in cosmetic medicine as a dermatologic drug to treat skin. The form of the drug is usually gel, crème or paste. These forms of salicylic acid should be at high concentration in the form of a stable suspension and contain a polydimethylsiloxane with terminal hydroxyl groups as a stabilising agent.[28] To penetrate skin the cosmetic agent should be water-soluble, poorly water-soluble, or water-insoluble. The intradermal or transdermal delivery system is useful in treating skin conditions such as cellulite, hyperpigmentation, skin aging, and acne.[29] These diseases can be treated if a medicine is only applied directly on them, on a skin's surface. Hence it is very important to make salicylic acid to penetrate skin.

Acne is a dermatological disorder that is caused by *Propionibacterium acnes*. This bacterium digests sebum and keratin and irritates the affected hair follicle resulting in further inflammation.[30,31] Salicylic acid has been found to decrease skin lipids and has anti-inflammatory properties. In other words, salicylic acid decreases sebocyte lipogenesis by downregulating the adenosine monophosphate-activated protein kinase (AMPK)/sterol response element-binding protein-1 (SREBP-1) pathway and reduces inflammation by suppressing the NF-κB (protein complex that controls transcription of DNA, cytokine production and cell survival) pathway in these cells. This acid also decreases the cell viability of human SEB-1 sebocytes by increasing apoptosis of the cells.[31]

Salicylic acid's derivates are globally known such as Aspirin, methyl salicylate, saligenin, salicin that are shown in ***Image 3***. Hence, they will not be discussed in this paragraph and the other reason is that they are not the main concern in this work.

3.3.2. Biological activities of salicylamides derivates

In case of antibacterial activity, it is reported in several works that the salicylamide's derivates inhibits growth of gram-positive bacteria.[8,10-12] And I have mentioned briefly an interesting interaction of salicylanilide and its derivatives in the chapter ***3.2. Salicylamides***. They can interact with parasite's mitochondria, and they effect in some way bacteria's energy metabolism by uncoupling oxidative phosphorylation, which prevents the production of adenosine triphosphate (ATP).[14] The main target is indeed the antibacterial properties. Therefore, I will be describing specifically the influence on gram-positive bacteria and what are they.

For my work's purposes I would like to reference mostly to Imramovský Research Group studies. Their studies have already been mentioned earlier in this bachelor's thesis. They have shown a promising result of antimicrobial activity from salicylanilide carbamates against bacteria such as *M. marinum, M. smegmatis, M. tuberculosis, E. faecalis* and *S. aureus*. The Imramovksy's research group have been experimenting on various salicyl derivates to discover their antimicrobial activities and to examine how do these activities change depending on the different substitutes.[11,12,15]

As I have mentioned before in chapter ***3.2. Salicylamides*** some studies have discovered that substitution of chlorine at the position 5 in salicylic acid's moiety in salicylanilides (the simple structure is shown in ***Image 2***) can increase the ability of salicylic acid to inhibit the expression of NF-κB activity. It can lead to new anti-inflammatory and antitumor drugs that can treat diseases with NF-κB activity.[17] And these compounds as well as diamides (***Image 5***) substituted by chlorine in C_5 of the salicylic ring have higher antibacterial potential than substituted by chlorine in C_4 position of the salicylic ring. And on top of that antimycobacterial activity of diamides is significantly influenced by electron-withdrawing substituents (like $-CF_3$) at the position 4 in aniline's moiety.[12]

Here I would like to discuss about *Staphylococcus Aureus* and its strains.

Staphylococcus Aureus is a pathogenic bacterium which is carried by live chickens and turkeys.[32,33] The bacterium is usually found in bird's ears, nasal cavity, throat or even

in skin as it is found in human's bodies. The toxins in *S. aureus* cannot be destroyed by heating a meat (only by cooling process) which can cause foodborne disease. Hence it is a big issue for the food industry. [32-34] Not only people are infected by handling a meat of an infected dead animal but local farmers and workers in slaughterhouses are exposed, who are most likely will get ill. It is important to check sanitary in those industries and in the worst cases, an infected person should be treated well.

However, the concerning factor of *S. aureus* is the resistance to multidrug, especially MRSA's strains.[33-35] *Methicillin-resistant S. aureus* is a nosocomial pathogen and an increasingly frequent cause of community-acquired infection.[32] Currently there are laboratory experiments and tests to produce drugs for treating and preventing MRSA infection.[32] Diamides are the good example of this kind of drugs.[12]

Here I would like to discuss about *Enterococcus faecalis* and its strains.

E. faecalis is a gram-positive commensal bacterium commonly isolated from the skin and gastrointestinal tract of human.[36,37] "*E. faecalis* is a nonmotile, catalase-negative, facultative anaerobic microbe that can grow at temperatures between 10 and 45 °C, and survive at 60 °C for 30 min."[36] And in humans it can cause disease such as endocarditis, urinary tract infections and infection of root canal of teeth, as well as meningitis. The issue with this bacterium is that it has a multiple antibiotic resistance for drugs like aminoglycosides, aztreonam, cephalosporins, clindamycin, penicillin including oxacillin, and trimethoprim/sulfamethoxazole.[36,37] Due to its multi-antimicrobial resistance, teicoplanin and especially vancomycin are the last defence for infections caused by *E. faecalis*.[36]

One of the *Enterococcus faecalis*'s strains is known as Vancomycin-Resistant Enterococcus (referred to as glycopeptide-resistant enterococci) is known to be nosocomial pathogens.[32,38] As enterococcus's strain VRE can be involved in infections of the urinary tract, surgical wounds, and the bloodstream.[32] The source of vancomycin-resistant enterococcus is likely from farm animals as a result of use of avoparcin, a glycopeptide antibiotic. After many researches of VRE were developed new antibiotics such as linezolid and daptomycin and development of new forms will not stop.[39]

Here I would like to discuss about *Mycobacteria* family.

One of the most dangerous bacteria from this group is *Mycobacterium tuberculosis*. *M. tuberculosis* is a bacterium (bacillus) with facultative intracellular pathogen, often replicating in macrophages.[40] It is s transmitted commonly from human to human by the aerosol route (by inhalation of infected droplet into the lungs). It is a causative agent of tuberculosis.[40,41,42] The bacillus is mostly stored in the human's alveoli with caseation (active inflammation lesion).[40,42] The disease caused by this bacteria effects not only lungs but can also be spread in lymph nodes, bones and serous membrane, the most serious form is tuberculous meningitis.[42]

TB can be treated with the magnificent amount of antibiotics for a long period of time (due to TB's ability to obtain a resistance to the drugs), which certainly depends on case to case. For an example, it is important to know what is a patient condition and if they have any other diseases that can lead to more harmful condition.[41,42] However the spread of MTB, potentially the tuberculosis, can be prevented with the vaccine. The most popular one is Bacillus Calmette-Guerin or BCG, which contains live attenuated bacteria derived from one of the related bacteria to mycobacterium tuberculosis that is *Mycobacterium bovis*. Unfortunately there are many issues with the vaccine and one of the them is the necessity using the TB skin test for screening for TB exposure, which gives a false positive tuberculin skin test in the most cases.[40,42] That is why we need to find other solution to treat infections caused by *Mycobacterium tuberculosis*.

The other representative to this family is *Mycobacterium smegmatis* that is specifically belonged to acid-fast actinomycetes, which means this bacterium do not freely decolorised with acid-alcohol after staining with hot solutions of carbol fuchsin.[43,44] It can synthesise three [NiFe]-hydrogenases, which are enzymes that catalyse the reversible oxidation, belonging to three different group.[45,46] This bacterium is aerobic, which leads to the fact that aerobic actinomycetes cause various infections in human such as s mycetoma, lymphocutaneous, abscess or cellulitis, primary pulmonary infection, and much more.[45,47] In the last studies *M. smegmatis* (and its mutated strain) is experimented

on its capability of producing H_2 thanks to [NiFe]-hydrogenase. And it has shown that only two enzymes (hydrogenase 1 and hydrogenase 2) are capable of it.[45]

The last mycobacterium I would like to note is *Mycobacterium marinum* that is very similar to *M. smegmatis* and belongs to non-tuberculosis bacteria. As being mycobacterium it can be spread through biofilms, sewage, ground and surface waters, air, and aerosolized water and can cause nosocomial infections, skin lesions, various pulmonary diseases, and lymphadenitis. This bacterium colonizes in water hence it is dangerous to be in the infected area nor consume the water. What makes it problematic is that mycobacterium is also resistant to disinfection and can survive in hot water.[48]

4. Experimental part

In this part of bachelor's thesis is focused on going though individual experiments, thanks to them this work can be established at the first place. I will describe in details synthetic pathways, which have led to obtaining intermediates and final products.

This part is divided to results and the discussion and the experimental part, where I will describe the syntheses.

At first, I will briefly summarise all materials, methods, and apparatuses, which I have used in university's laboratory, and which have been used for purification and characterisation of my prepared compounds.

Every commercially available solvents, chemical agents, and other chemicals, which were used for syntheses, were bought from Sigma-Aldrich, Acros Organics, TCI, Merck or Fluorochem companies. The purification of these chemicals was not needed for the reaction use.

For the column chromatography was used silica gel (SiO_2 60 Å, size of the particle's 0,060-0,200 mm, Acros Organics). Used solvent was EtOAc/hexane phase or their regenerated mixture, alternatively any commercially available solvents.

Thin-layer chromatography was executed on aluminium small plates covered with silica gel (SiO_2 60 F254 (Merck)) with visualisation under UV lamp (254 or 360 nm). For the compounds that could not be visually monitored under UV lamp was used 5% solution of phosphomolybdic acid ($H_3PO_4.12MoO_3$) in ethanol.

Melting points were measured on capillary melting-point apparatus Buchi B-545.

^1H and ^{13}C spectra of nuclear magnetic resonance (NMR) were measured by supervisor of this bachelor's thesis. The measurement was run at the laboratory's temperature and on the Bruker AVANCE III 400 apparatus for frequencies 400,13 alternatively 100,62 MHz (^{13}C) or on Bruker Ascend™ 500 apparatus for frequencies500,13 MHz (^1H) alternatively 125,76 MHz (^{13}C). Chemical's shifts are given in ppm unit measure in relation to remaining signals of $CDCl_3$, DMSO-*d*$_6$ or D_2O solvents.

Unfortunately, my knowledge of working with NMR spectra is very feeble. Hence, their signals will not be described in this bachelor's thesis.

Elementary analysis (also written as CHN analysis) was executed by service engineer on Thermo Scientific Flash 2000 Organic elementar analyser.

Mass spectra were measured on mass spectrometer MALDI with high resolution LTQ Orbitrap XL (Thermo Fisher Scientific, Bremen, Germany) and with nitrogen UV laser (337 nm, 60 Hz). For measuring was used dried droplet method. Spectra were measured in positive or negative ion mode in normal mass range (m/z 50-2000) with resolution 100 000 at m/z = 400. For the laser position option was used pre-defined motion spiral scheme. As matrix was used 0,2 M solution of 2,5-dihydroxybenzoic acid (DHB) in the mixture of MeCN:H_2O (95:5) or trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) in MeCN. Molar ratio of matrix: sample was always approximately 40:1. Final spectrum is a ratio of the entire measurement.

5. Results and the discussion

At first, in this chapter I would like to describe synthetic pathways leading to intermediates, which will be used for targeted final products. Reaction pathways for intermediates preparation are executed in our laboratory relatively often and it can be considered as a routine matter. Some of the prepared intermediates were used for the next steps without purification. And some were purified and only after that were used for the next reactions. All obtained intermediates were put through elementary analysis and ^{1}H NMR spectroscopy. Obtained results were corresponded to values described in literature and as well to results of similar experiments that were executed by students from the previous years.[12,15,49]

Next in this chapter will be focused on original molecules, which are the main cause of the whole bachelor's thesis. It is about five compounds that are derived from diamides and varies in amino acids. Method of preparation of these compounds were based on knowledge described in the literature.[12,15] *O*-benzyl protected compounds were purified using column chromatography or crystallisation. They were characterised by ^{1}H a ^{13}C NMR spectroscopy (but the description of them is not in this work due to feeble knowledge of working with them), mass spectroscopy with high resonance and elementary analysis. These compounds were also used to prepare final products. Final products were prepared based on the literature and experimental skills of my supervisor doc. Ing. Aleš Imramovský. [12,15] And I also used the works from other students. They were characterised by ^{1}H a ^{13}C NMR spectroscopy (again the description of them is not in this work), mass spectroscopy with high resonance and elementary analysis.

Below there is ***Image 9***, where are shown general structures of prepared substances of this work. The numbers (**6-11**) are corresponded to ***Tables 1-4*** and numbers **12a-e** are corresponded to the chapter ***5.6. Preparation of targeted diamides*** and ***6. Synthesis and characterisation.*** Hence, the R is norleucine (**a**), cyclohexylalanine(**b**), methionine (**c**), tryptophane (**d**) or alanine (**e**) according to their codes **a-e**. Alanine in the **6a-d** is missing because I used alanine that was prepared by other students.

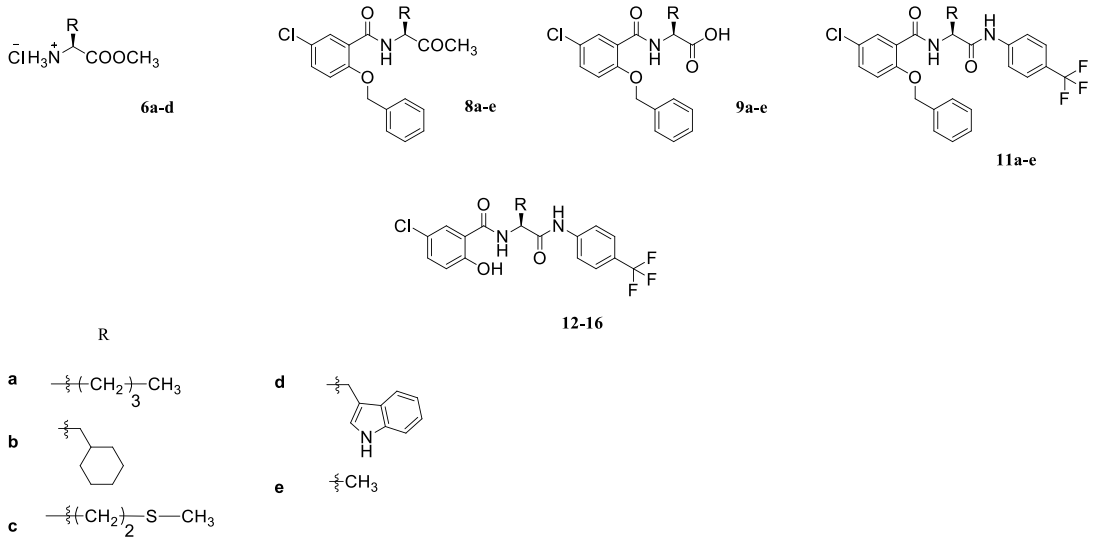


***Image 9**. Structures of prepared compounds*

5.1. Preparation of *O*-benzyl protected 5-chlorsalicylic acid (4)

To prepare all intermediates was at first used 2-(benzyloxy)-5-chlorbenzencarboxyl acid (**4**). The scheme of this synthesis is shown on ***Scheme 17***. The method was taken from the used literature.[50]

The amount of 2-(benzyloxy)-5-chlorbenzencarboxyl acid (**4**) was enough for the purposes of this work. However, I would use another student's prepared acid (**4**), if mine was completely used. The new syntheses were not urgent to make by me since in the laboratory was decent amount. Purification of the final product was not done thanks to the good results received from elementary (CHN) analyses and TLC.

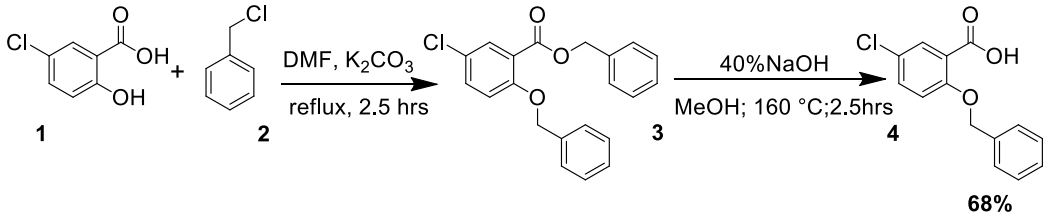
For this preparation was used cleaned boiling flask (in sizes from 250 mL to 1 L), pH papers, Büchner funnel, Petri dish, aluminium small plates covered with silica gel for TLC, separatory funnel, magnetic stirrer, magnetic stirring bar, and stand.

The purpose of this reaction was to protect hydroxy group on carboxyl group of salicylic acid moiety by substituting proton with benzyl group.

For the reaction was used 0.1158 mol of commercial 5-chlorosalicylic acid (**1**), which did not need any purification. To obtain intermediate (**3**) 5-chlorosalicylic acid (**1**) was turned into salt using 0.5215 mol of K_2CO_3 and appropriate amount of dimethylformamide (DMF) to dissolve. Then the substitution of halogen in benzylchloride (**2**) could be done by heating the mixture to reflux for 2.5 hours to achieve the targeted intermediate (**3**). After the reaction was extracted by brine and water, the solvent, in this case was used DCM, was evaporated using RVO, which yielded yellow oil intermediate product (**3**), which was used in the next step.[50]

Because in 5-chlorosalicylic acid (**1**) are two acidic nucleophiles the substitution of proton applies for hydroxy group on the aromatic cycle and on carboxyl group. That is why the intermediate product (**3**) should be hydrolysed (was used here 40% solution of NaOH) and subsequently acidified (was used HCl) to pH = 1 to obtain the targeted product of 2-(benzyloxy)-5-chlorbenzencarboxyl acid (**4**). The second step was used from the same literature as was used in the previous step.[50] Recrystallised 2-(benzyloxy)-5-chlorbenzencarboxyl acid (**4**) yielded 68.33 %, which is approximately 68 %.

The final product was tested by using CHN analysis and was measured the melting point, which was 121,1-121,8 °C.



Scheme 17. *Synthesis of O-benzyl protected 5-chlorsalicylic acid*

5.2. Preparation of methyl esters hydrochloride salts of appropriate acids (6a-d)

In the previous chapter I described the synthesis of 2-(benzyloxy)-5-chlorbenzencarboxyl acid (**4**), which was further used to synthesise methyl esters hydrochloride salts of appropriate acids (**6a-d**). The synthesis is shown in ***Scheme 18***. The method was taken from the used literature.[15,51]

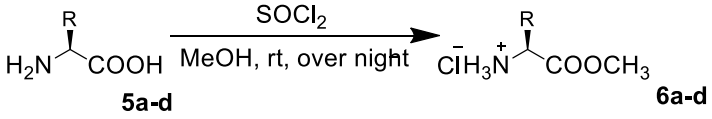
For this preparation was used cleaned boiling flask (in sizes from 250 mL to 500 mL), pH papers, Büchner funnel, aluminium small plates covered with silica gel for TLC, separatory funnel, magnetic stirrer, magnetic stirring bar, and stand. Purification of the final products were not done thanks to the good results received from elementary analyses and TLC. However, the analysis of **6c** is missing, but the explanation will be later in this chapter. The analysis of **6d** was not good, but me and my colleague made the agreement that it was the residue of thionyl chloride. Thus, I did not further purify it, but instead washed it with DCM. By my mistake I did not send it again to service engineer to analyse it if there was still residue of $SOCl_2$. That is why there is not any further results of this compound.

For the preparing salts of chosen amino acids **5a-d** (1 equivalent) was used methanol with thionyl chloride (1.2 equivalent). Amino acids in this work were bought from Sigma-Aldrich, which were norleucine (**a**), cyclohexylalanine(**b**), methionine (**c**), tryptophane (**d**) and alanine (**e**). Thionyl chloride as being a source of chloride ions, provides organochlorine compounds, in this case is acyl chloride and hydrochloride on amine group. The chlorine in the acyl group is substituted by methyl group to obtain targeted acids methyl esters hydrochloride salts **6a-d**. In general the whole reaction is esterification.[15,51]

This reaction was done delicately due to very reactive and exothermic $SOCl_2$. Thus, at the beginning, the temperature of the reaction was cooled externally by ice and NaCl to 0 °C. Afterwards, was carefully drop by drop added $SOCl_2$ while monitoring the temperature, which should not have been surpassed 5 °C. When the whole $SOCl_2$ was added, the reaction was left mixing at the same temperature for 30 minutes. After that the

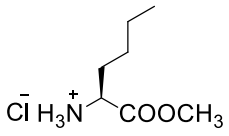
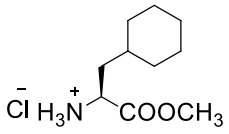
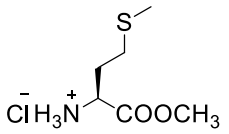
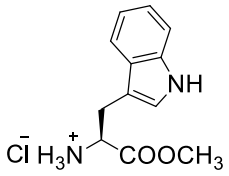
mixture was heated to reflux for 2 hours. Next the reaction was cooled, and the solvent was evaporated using RVO. The residue was washed three times with DCM until thionyl chloride was not present in the product (controlling with pH paper). All steps were followed by the used literature.[15,51] In the end were obtained white crystals (**6a,b,c**), pink crystals (**6d**) with the yields that are shown in the ***Table 1***.

The methyl ester hydrochloride salt of L-alanine (that could have been numbered as 6e) was synthesised by other students. The analyses were done for most compounds, except for the L-methionine (**6c**), which was the latest compound during my last days in the laboratory and then started my exam season in June, when I could not go to the laboratory. Thus, I could not properly finish analyses and I must confess that I did not spend enough time to fully complete experiments with L-methionine (**6-12c**).



Scheme 18. *Synthesis of methyl esters of 5-chlor-O-benzyl-salicylic-L-amino acids*

Table 1. *The yields of the chosen methyl esters*

Compound	R	Structrure	Yields
6a	-Nleu		**98.5%**
6b	-CHA		**93.45%**
6c	-Met		**97.9%**
6d	-Trp		**99.8%**

Melting points: 133.9-134.8 °C (**6a**), 148-152.5 °C (**6b**), 200.4-200.6 °C (**6d**) and measuring **6c** was not done due to absence in the laboratory, because of the exam season in June.

Elementary analyses: for $C_7H_{16}ClNO_2$ (**6a**) was estimated: C 46.28; H 8,88; N 7.71; was received: C 46.13±0.06; H 8.98±0.03; N 7.74±0.04.

for $C_{10}H_{20}ClNO_2$ (**6b**) was estimated: C 54.17; H 9.09; N 6.32; was received: C 54.25±0.08; H 9.15±0.01; N 6.34±0.08.

for $C_6H_{14}ClNO_2S$ (**6c**) was not done due to the reason I mentioned above.

for $C_{12}H_{15}ClN_2O_2$ (**6d**) was estimated: C 64.69; H 6.20; N 3.59; was received: C 56.63±0.12; H 5.88±0.06; N 10.71±0.06 (residue of $SOCl_2$)

NMR analyses were done for **6a**, but my knowledge of reading NMR are not enough to describe them in this work. Hence, they will be not discussed. I will leave the numbers of NMR analyses if someone needs it in the future. All of them are in the university's database. NMR: for **6a** ^{1}H [856], ^{13}C [857]. By my mistake (specifically my lack of focus on these compounds) for others **6b-d** analyses were not done and due to my absence in the last in the laboratory due to the exam season.

MALDI (HRMS m/z) analyses were not done for any of the mentioned compounds. I was told that those compounds are usually clean (without any secondary products) and the presence of them is 100 %. I did not consider that these analyses would be useful for this work, which is absolutely my mistake.

5.3. Preparation of methyl esters of 5-chlor *O*-benzyl-salicylic-L-amino acids (8a-e)

In the previous chapter I described the synthesis of 2-(benzyloxy)-5-chlorbenzencarboxyl acid (**4**) and methyl esters of 5-chlor-*O*-benzyl-salicylic-L-amino acids (**6a-d**), which was further used to synthesise methyl esters of (2*S*)-2-[[2-(benzyloxy)-5-chlorobenzoyl]benzamido) acids (**8a-e**), the general synthesis of which is shown in the ***Scheme 19***. For compounds **8a-d** it is a two-step reaction and for **8e** it is one-step. The method was followed by the used literature.[15]

The first step for **8a-d** contained the transformation of 1 equivalent of previously prepared methyl ester salts **6a-d** into methyl esters **7a-d** using 1 equivalent of K_2CO_3. The process was followed by the used literature.[15]

The second step contained reaction of prepared methyl esters **7a-d** with 1 equivalent of 2-(benzyloxy)-5-chlorbenzencarboxyl acid (**4**), which synthesis was discussed in this work, using 1.1 equivalent of $HOBt \cdot H_2O$ with 1.2 equivalent of EDC·HCl to suppress racemisation and activate carboxyl group. In this step the amine group of methyl ester acid acted as a base that took proton from hydroxyl group on 2-(benzyloxy)-5-chlorbenzencarboxyl acid (**4**), which resulted ammonium salt. To obtain the final product **8a-e** the salts were dehydrated by heat.[15]

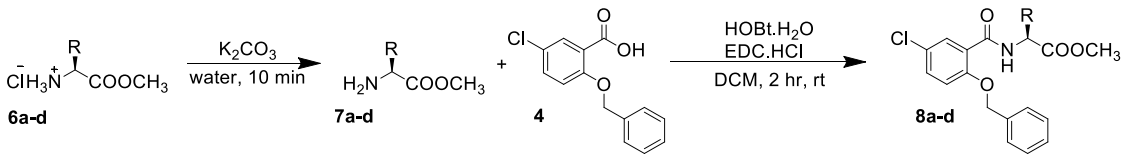
After the reflux, the mixtures were extracted with $NaHCO_3$, 5 % of citric acid, brine, and water. Then the solvent was dried and evaporated using RVO.[15] In the end were obtained yellow oils **8a-b**, orange crystals **8d** and white crystals **8c** of (2*S*)-2-[[2-(benzyloxy)-5-chlorobenzoyl]benzamido) acids (**8a-d**). Some of them **8a-c** were used without any purification in the next experiments thanks to the good results received from elementary, NMR and MALDI analyses and according to TLC they were also pure. But **8d** showed worse result (especially it was visually seen on TLC). Thus, it needed purification using column chromatography on silica gel with EtOAc/hexane phase. Yield before purification was 61.5 % and after was 33.2 %, which resulted the yield to dropped by 54 %. Their yields are shown in the ***Table 2***.

And to obtain **8e** compound was used a different method of preparation (***Scheme 20***). However, the literature was used the same as it was for the preparation of **8a-d** compounds.[15] The different method was used, because the previous one with L-alanine showed worse yields than the one I would discuss here.

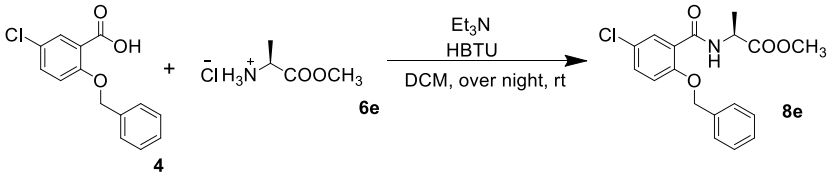
To substituted hydroxy group on 2-(benzyloxy)-5-chlorbenzencarboxyl acid (**4**) was used 1 equivalent L-alanine acid tert-butyl ester hydrochloride (**6e**) with 2 equivalents of Et_3N as a base, which resulted the salt triethylamine hydrochloride, and 1.2 equivalent of HBTU to activate carboxyl group.[15]

To achieve purer compound was used extraction with saturated solution of $NaHCO_3$, 5% of citric acid and 10 % of HCl.[15] However, the residue still needed to purify (elementary analysis showed bad results). For that step was used column chromatography on silica gel with EtOAc/hexane phase (at a ratio of 3:1). Were obtained white crystals of (2*S*)-2-[[2-(benzyloxy)-5-chlorobenzoyl-L-alanine]benzamido) acid (**8e**). The yield was 68%.

The final products were tested by using NMR spectroscopy (^1H and ^{13}C were measured in DMSO at 400 MHz and 500 MHz), but my knowledge of reading NMR are not enough to describe them in this work. Hence, they will be not discussed below. But I will write the numbers of ^1H and ^{13}C. Therefore, others could find them in the university's database.

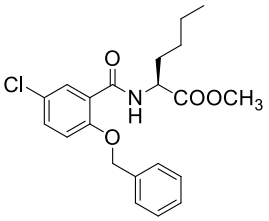
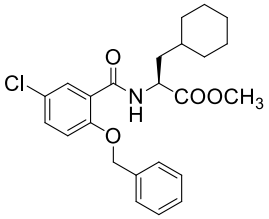


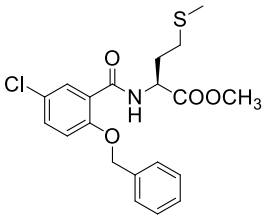
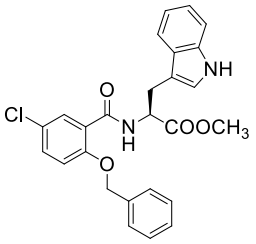
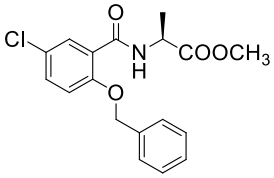
Scheme 19. *Synthesis of methyl esters of 2-(2-(benzyloxy)benzamido) acids*



Scheme 20. *Synthesis of methyl (2S)-2-[[2-(benzyloxy)-5-chlorobenzoyl]amino]propanoate*

Table 2. *The yields of methyl esters of 2-(2-(benzyloxy)benzamido) acids*

Compound	R	Structure	Yields
8a	-Nleu		**63.75%**
8b	-CHA		**65%**

8c	-Met		**70.6%**
8d	-Trp		**33.2%**
8e	-Ala		**68%**

Melting points: were not measured due to my lack of focus measuring their melting points and my absence in the last days in the laboratory due to the exam season.

Elementary analyses: for $C_{21}H_{24}ClNO_4$ (**8a**) was not done due to the missing sample. By my mistake I could not done it before it had been missing.

for $C_{24}H_{28}ClNO_4$ (**8b**) was estimated: C 67.05; H 6.56; N 3.26; was received: C 66.94±0.22; H 6.68±0.04; N 3.20±0.01.

for $C_{20}H_{22}ClNO_4S$ (**8c**) was estimated: C 58.89; H 5.44; N 3.43; S 7.86; was received: C 58.97±0.05; H 5.55±0.03; N 3.30±0.01; S 7.42±0.02.

for $C_{26}H_{23}ClN_2O_4$ (**8d**) was estimated: C 67.46; H 5.01; N 6.05; was received: C 67.57±0.04; H 5.09±0.02; N 6.03±0.04.

for $C_{18}H_{18}ClNO_4$ (**8e**) was estimated: C 64.69; H 6.20; N 3.59; was received: C 66.32±0,30; H 6.60±0,06; N 3.47±0,4.

NMR analyses were done for **8a-d**, but are not described due to the reason I mentioned above. NMR for **8a**: ^{1}H [858] and ^{13}C [859]; for **8b**: ^{1}H [849] and ^{13}C [850]; for **8c**: ^{1}H [428] and ^{13}C [429]; for **8d**: ^{1}H [758] and ^{13}C [759]. **8e** was not done due to my lack of focus on completing analysis for this compound and my absence in the last in the laboratory due to the exam season.

MALDI (HRMS m/z) analyses: for $C_{21}H_{24}ClNO_4$ (**8a**) and for $C_{24}H_{28}ClNO_4$ (**8b**) were not measured due to the missing sample of **8a** and the measuring of **8b** was not done due to my lack of focus on completing analysis for this compound and my absence in the last in the laboratory due to the exam season.

for $C_{20}H_{22}ClNO_4S$ (**8c**) was estimated: 408.10364 $[M+H]^+$; 430.08558 $[M+Na]^+$; 446.05952 $[M+K]^+$; was received: 418.10610 $[M+H]^+$; 430.05896 $[M+Na]^+$; 446.05896 $[M+K]^+$.

for $C_{26}H_{23}ClN_2O_4$ (**8d**) was estimated: 463.14246 $[M+H]^+$; 485.12440 $[M+Na]^+$; 501.09834 $[M+K]^+$; was received: 463.14191 $[M+H]^+$; 485.12386 $[M+Na]^+$; 501.09779 $[M+K]^+$.

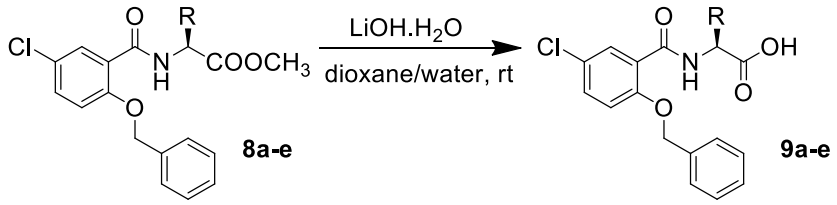
for $C_{18}H_{18}ClNO_4$ (**8e**) was estimated: 348.10027 $[M+H]^+$; 370.08221 $[M+Na]^+$; 386.05615 $[M+K]^+$; was received: 348.09971 $[M+H]^+$; 370.08166 $[M+Na]^+$; 386.05559 $[M+K]^{+\cdot}$

5.4. Preparation of 2-(2-(benzyloxy)benzamido) acids

The methyl esters of (2*S*)-2-[[2-(benzyloxy)-5-chlorobenzoyl]benzamido) acids (**8a-e**) were next used for preparation of 2-(2-(benzyloxy)benzamido) acids (**9a-e**) that is shown in the ***Scheme 21***.[15]

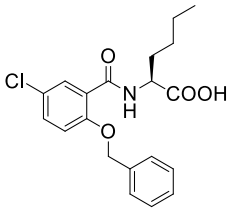
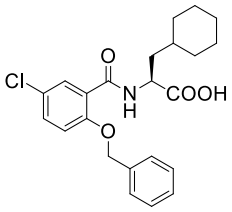
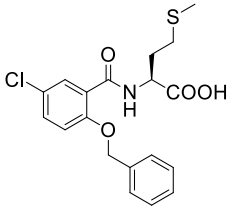
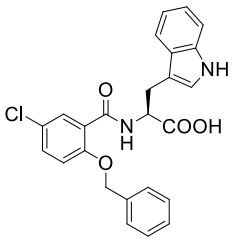
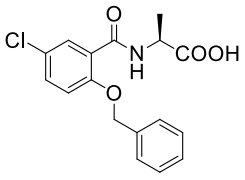
1 equivalent of methyl esters (**8a-e**) was hydrolysed by 3 equivalents of LiOH with dioxane as a solvent and subsequently acidified by HCl to pH = 1 to obtain appropriated acids. The method was followed from the used literature.[15]

In the end was obtained bright yellow oil **9a,b**, white crystals **9e** and bright orange crystals **9d**. They were used without any purification thanks to TLC and purity was confirmed by NMR, MALDI and CHN analyses. Their yields are shown in the ***Table 3***.



Scheme 21. *Synthesis pf (2S)-2-[[2-(benzyloxy)-5-chlorobenzoyl]benzamido) acids*

Table 3. *The yields of 2-(2-(benzyloxy)benzamido) acids*

Compound	R	Structure	Yields
9a	-Nleu		**97.3%**
9b	-CHA		**92.4%**
9c	-Met		**75.5%**
9d	-Trp		**78.27%**
9e	-Ala		**71%**

Melting points: were not measured due to my lack of focus measuring their melting points and my absence in the last days in the laboratory due to the exam season.

Elementary analyses: for $C_{20}H_{22}ClNO_4$ (**9a**) was estimated: C 63.91; H 5.90; N 3.73; was received: C 62.77/63.69/64.63/63.70; H 6.20±0.08; N 3.38±0.10.

for $C_{23}H_{26}ClNO_4$ (**9b**) was estimated: C 66.42; H 6.30; N 3.37; was received: C 62.92/63.98/65.55; H 6.11±0.16; N 3.16±0.09.

for $C_{19}H_{20}ClNO_4S$ (**9c**) was estimated: C 57.94; H 5.12; N 3.56; S 8.14; was received: C 57.99±0.41; H 5.03±0.07; N 3.27±0.10; S 7.56±0.16.

for $C_{25}H_{21}ClN_2O_4$ (**9d**) was estimated: C 66.89; H 4.72; N 6.24; was received: C 65.24±0.06; H 4.91±0.03; N 5.52±0.03 (residue H_2O).

for $C_{17}H_{16}ClNO_4$ (**9e**) was estimated: C 61.18; H 4.83; N 4.20; was received: C 61.66±0.01; H 4.84±0.04; N 4.40±0.03.

If the analyses were not very good the products still were used in the next experiments, but were just washed with DCM, because MALDI and TLC confirmed that they were indeed targeted compounds. The only reason the elementary analyses could look worse was because the products were probably not completely dried.

NMR analyses were done for **9a-e**, but are not described due to the reason I mentioned above. NMR for **9a**: ^{1}H [424] and ^{13}C [425]; for **9b**: ^{1}H [851] and ^{13}C [852]; for **9d**: ^{1}H [296] and ^{13}C [768]. for **9e**: ^{1}H [295] and ^{13}C [767]. **9c** was the last compound that I was preparing in my last days in the laboratory. And it was not done due to my absence in the last in the laboratory due to the exam season.

MALDI (HRMS m/z) analyses: for $C_{20}H_{22}ClNO_4$ (**9a**) was estimated: 376.13157 $[M+H]^+$; 398.11351 $[M+Na]^+$; 414.087415 $[M+K]^+$; was received: 376.13101 $[M+H]^+$; 398.11296 $[M+Na]^+$; 414.08689 $[M+K]^+$.

for $C_{23}H_{26}ClNO_4$ (**9b**) was estimated: 416.16231 $[M+H]^+$; 454.11819 $[M+K]^+$; 438.14426 $[M+Na]^+$; was received: 416.16345 $[M+H]^+$; 452.16119 $[M+K]^+$; 438.14548 $[M+Na]^+$.

for $C_{19}H_{20}ClNO_4S$ (**9c**) the sample was delivered to service engineer, but the results were not received by the time this work should have already been done.

for $C_{25}H_{21}ClN_2O_4$ (**9d**) was estimated: 449.12681 $[M+H]^+$; 471.10875 $[M+Na]^+$; 487.08269 $[M+K]^+$; was received: 449.12626 $[M+H]^+$; 471.10821 $[M+Na]^+$; 487.08214 $[M+K]^+$.

for $C_{17}H_{16}ClNO_4$ (**9e**) was estimated: 334.08462 $[M+H]^+$; 356.06656 $[M+Na]^+$; 372.04050 $[M+K]^+$; was received: 334.08406 $[M+H]^+$; 356.06601 $[M+Na]^+$; 372.03994 $[M+K]^+$.

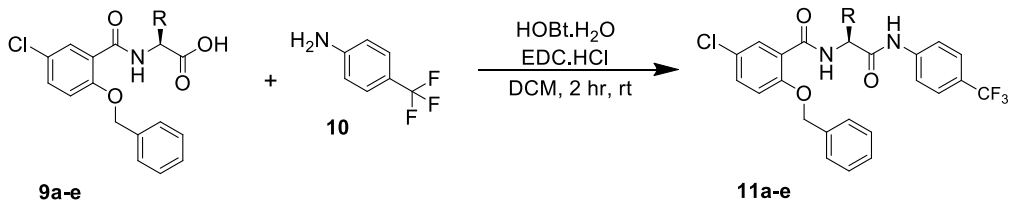
5.5. Preparation of *O*-benzyl protected diamides

Before I will discuss individually the original final products there is still the last method left (***Scheme 22***). The prepared 2-(2-(benzyloxy)benzamido) acids (**9a-e**) reacted with trifluoro-p-toluidine (**10**) to obtain the key intermediate (**11a-e**). The method was followed by the used literature.[15]

The preparation is the same as it was described in the second step of synthesis of methyl esters of 2-(2-(benzyloxy)benzamido) acids (**8a-d**) that is also shown in the ***Scheme 19***. The method was used the same as before.[15] Instead of methyl ester acids for this reaction was used 1 equivalent of previously prepared 2-(benzyloxy)-5-chlorbenzencarboxyl acid (**9a-e**) that reacted with 1.2 equivalent of trifluoro-p-toluidine (**10**) using the same reagents HOBt·H_2O and EDC·HCl with the same equivalents.[15]

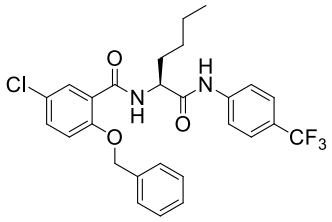
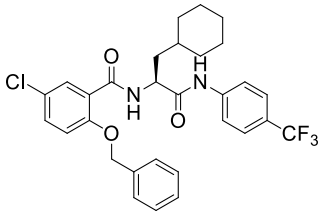
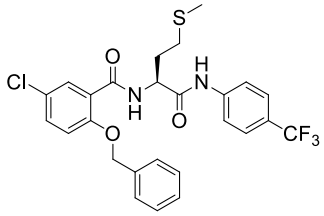
Was used again extraction with $NaHCO_3$, 5 % of citric acid, brine, and water.[15] In the end were obtained orange crystals **11d** and white crystals **11a,b,e** of *O*-benzyl-2-hydroxy-*N*-(arylalkyl)benzamides (**11a-e**). They were purified using column chromatography on silica gel with EtOAc/hexane phase. Their yields are shown in the ***Table 4***.[15]

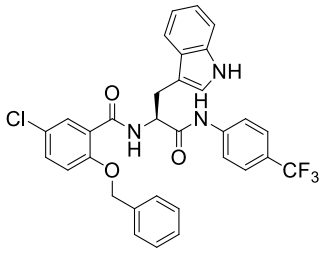
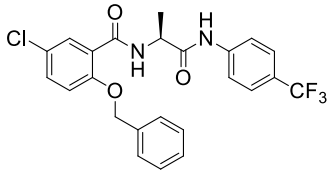
The **11c** compound was not finished due to limited time and my absence in the laboratory during exam season in June 2021.



***Scheme 22**. Synthesis of O-benzyl-2-hydroxy-N-(arylalkyl)benzamides*

***Table 4**. The yields of O-benzyl-2-hydroxy-N-(arylalkyl)benzamides*

Compound	R	Structure	Yields
11a	-Nleu		**49.85%**
11b	-CHA		**40%**
11c	-Met		**- %**

11d	-Trp		**51.2%**
11e	-Ala		**12%**

Melting points: were not measured due to my lack of focus measuring their melting points and my absence in the last days in the laboratory due to the exam season.

Elementary analyses: for $C_{27}H_{26}ClF_3N_2O_3$ (**11a**) was estimated: C 62.49; H 5.05; N 5.40; was received: C 62.63±0.40; H 5.14±0.03; N 5.14±0.04.

for $C_{30}H_{30}ClF_3N_2O_3$ (**11b**) was estimated: C 64.46; H 5.41; N 5.01; was received: C 63.59±0.02; H 5.60±0.03; N 4.87±0.06.

for $C_{26}H_{24}ClF_3N_2O_3S$ (**11c**) was not done due to incomplete substance.

for $C_{32}H_{25}ClF_3N_3O_3$ (**11d**) was estimated: C 64.92; H 4.26; N 7.10; was received: C 63.67±0.57; H 4.61±0.02; N 6.92±0.08 (residue H_2O).

for $C_{24}H_{20}ClF_3N_2O_3$ (**11e**) was estimated: C 60.45; H 4.23; N 5.87; was received: C 60.08±0.22; H 4.15±0.12; N 5.48±0.02.

NMR analyses were done for **11a-d**, but are not described due to the reason I mentioned above. NMR for **11a**: ^{1}H [439], ^{13}C [963] and ^{19}F [964]; for **11b**: ^{1}H [853], ^{13}C [855] and ^{19}F [854]; for **11d**: ^{1}H [409], ^{13}C [410] and ^{19}F [958]. for **11e**: ^{1}H [307], ^{13}C [782] and ^{19}F [781]. **11c** was the last compound that I was preparing in my last days in the

laboratory. And it was not done due to my absence in the last in the laboratory due to the exam season.

MALDI (HRMS m/z) analyses: for $C_{27}H_{26}ClF_3N_2O_3$ (**11a**) was estimated: 519.16624 $[M+H]^+$; 541.14818 $[M+Na]^+$; 557.12212 $[M+K]^+$; was received: 519.16568 $[M+H]^+$; 541.14763 $[M+Na]^+$; 557.12156 $[M+K]^+$.

for $C_{30}H_{30}ClF_3N_2O_3$ (**11b**) was estimated: 559.19698 $[M+H]^+$; 581.17893 $[M+Na]^+$; 597.15286 $[M+K]^+$; was received: 559.19861 $[M+H]^+$; 581.18054 $[M+Na]^+$; 597.15460 $[M+K]^+$.

for $C_{26}H_{24}ClF_3N_2O_3S$ (**11c**) was not done due to incomplete substance, which I could not finish due to the reasons above.

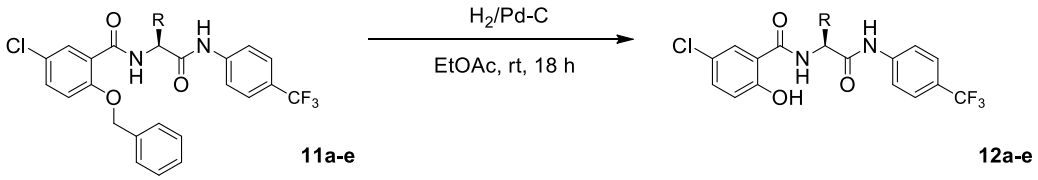
for $C_{32}H_{25}ClF_3N_3O_3$ (**11d**) was estimated: 592.161478 $[M+H]^+$; 614.14342 $[M+Na]^+$; 630.11618 $[M+K]^+$; was received: 592.16093 $[M+H]^+$; 614.14288 $[M+Na]^+$; 630.11681 $[M+K]^+$.

for $C_{24}H_{20}ClF_3N_2O_3$ (**11e**) was estimated: 477.11928 $[M+H]^+$; 499.10122 $[M+Na]^+$; 515.07516 $[M+K]^+$; was received: 477.11873 $[M+H]^+$; 499.10068 $[M+Na]^+$; 515.07461 $[M+K]^+$.

5.6. Preparation of targeted diamides

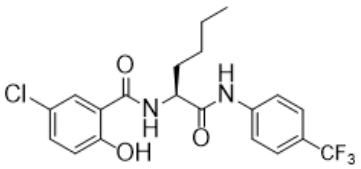
In this chapter I would like to discuss the syntheses of targeted diamides (**12a-e**) that were prepared from the *O*-benzyl protected diamides (**11a-e**). Each diamide will be discussed individually in the next chapter ***6. Synthesis and characterisation***, where will be also discussed their yields and analyses. The general synthesis of targeted diamides is shown in the ***Scheme 23***.[15]

In the last step the protected benzyl group was finally removed by using hydrogen with palladium on carbon as a catalyst. The method was followed by the used literature.[15]



Scheme 23. *Synthesis of diamides*

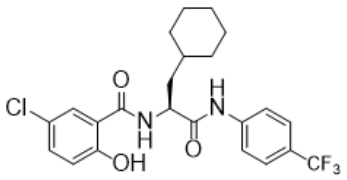
6. Synthesis and characterisation



(*S*)-5-chloro-2-hydroxy-*N*-(1-oxo-1-((4-(trifluoromethyl)phenyl)amino)hexan-2-yl)benzamide (12a)

In 250 mL of boiling flask was filled with 0.2193 g of the reactant (**11a**), which was dissolved in 50 mL EtOAc and was added 1 teaspoon of Pd/C. Afterwards the mixture was bubbled by hydrogen using hydrogenator under atmospheric pressure. The reaction was left over night.

After reactants were ceased (detection by using TLC), the catalyst was filtrated using 3 folded filter papers. The product was isolated using column chromatography. For this was used 1 g of silica gel for the sample and 109.81 g of silica gel for the column in the phase EtOAc/hexane at a ratio of 1:6. During the process were found two products of **12.1a** (the first elute) and **12.2a** (the second elute). After CHN analysis it was confirmed that the targeted product is **12.1a**. Yielded 0.1832 g (**76.1 %**) of white crystals. **Melting point**: was not measured due to my absence in the last in the laboratory due to the exam season. **NMR analysis**: ^{1}H NMR [441] in DMSO 5mm 500MHz; ^{13}C NMR [442] in DMSO 5mm 500MHz; ^{19}F NMR [960] in DMSO 5mm 400MHz. The further explanation of the NMR analysis will not be written due to the lack of knowledge of reading the spectroscopy. **HRMS (MALDI) m/z**: for $C_{20}H_{20}ClF_3N_2O_3$ was estimated: 429.11928 $[M+H]^+$; 451.10122 $[M+Na]^+$; 467.07516 $[M+K]^+$; was received: 429.11873 $[M+H]^+$; 451.10068 $[M+Na]^+$; 467.07461 $[M+K]^+$. **Elementary analysis**: for $C_{20}H_{20}ClF_3N_2O_3$ was estimated: C 56.02; H 4.70; N 6.53; was received: C 55.01±0.05; H 5.00±0.01; N 5.98±0.01.

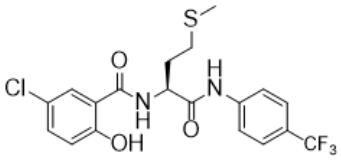


(S)-5-chloro-*N*-(3-cyclohexyl-1-oxo-1-((4-(trifluoromethyl)phenyl)amino)propan-2-yl)-2-hydroxybenzamide (12b)

In 250 mL of boiling flask was filled with 1.1915 g of the reactant **11b**, which was dissolved in 100 mL EtOAc and was added 1

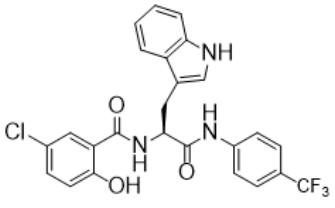
teaspoon of Pd/C. Afterwards the mixture was bubbled by hydrogen using hydrogenator under atmospheric pressure. The reaction was left over night.

After reactants were ceased (detection by using TLC), the catalyst was filtrated using 3 folded filter papers. The product was isolated using column chromatography. For this was used 5 g of silica gel for the sample and 198.863 g of silica gel for the column in the phase EtOAc/hexane at a ratio of 1:7. During the process were found two products which are **12.1b** (the first elute) and **12.2b** (the second elute). After CHN analysis it was confirmed that the targeted product is **12.1b**. Yielded 0.072 g (**7.2 %**) of white crystals **Melting point**: was not measured due to my absence in the last in the laboratory due to the exam season. **NMR analysis**: ^{1}H NMR in $CDCl_3$; ^{13}C NMR in $CDCl_3$; ^{19}F NMR in $CDCl_3$ the sample was condensed. The left amount was not enough to do another NMR analysis. There was not enough time to synthesis the new compound due to the COVID 19 restrictions and my absence in June due to exam season. **HRMS (MALDI) m/z**: for $C_{23}H_{24}ClF_3N_2O_3$ was estimated: 469.15058 $[M+H]^+$; 491.13252 $[M+Na]^+$; 507.10646 $[M+K]^+$; was received: 469.15003 $[M+H]^+$; 491.13198 $[M+Na]^+$; 507.10591 $[M+K]^+$. **Elementary analysis**: for $C_{23}H_{24}ClF_3N_2O_3$ was estimated: C 58.91; H 5.16; N5.97; was received: C 59.87 ± 0.19; H 5.68 ± 0.06; N 5.29 ± 0.22. (There is probably the residue of solvents).



(*S*)-5-chloro-2-hydroxy-*N*-(4-(methylthio)-1-oxo-1-((4-(trifluoromethyl)phenyl)amino)butan-2-yl)benzamide (12c)

The method of preparation would be as it was described in the literature.[15] The product (**12c**) was not finished due to limited time and my absence in the laboratory during exam season in June 2021.

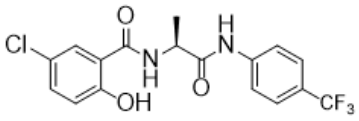


(*S*)-*N*-(3-(1H-indol-3-yl)-1-oxo-1-((4-(trifluoromethyl)phenyl)amino)propan-2-yl)-5-chloro-2-hydroxybenzamide (12d)

In 250 mL of boiling flask was filled with 1.148 g of the reactant **11d**, which was dissolved in

100 mL EtOAc and was added 1 teaspoon of Pd/C. Afterwards the mixture was bubbled by hydrogen using hydrogenator under atmospheric pressure. The reaction was left over night. The following day the reaction was monitored using TLC and according to it the reactants were not vanished. Overall, the spots on the used chromatography looked very messy. After the week, the TLC showed the same results. Thus, I discussed with the supervisor whether to continue or stop the process. It was agreed to stop the process.

Unfortunately, there was no time to redo from the start the whole synthesis of the product **12d** with given circumstances.



(*S*)-5-chloro-2-hydroxy-*N*-(1-oxo-1-((4-(trifluoromethyl)phenyl)amino)propan-2-yl)benzamide (12e)

In 100 mL of boiling flask was filled with 0.114 g of **11e**, which was dissolved in 20 mL of tetrahydrofuran (THF) and was transformed to cuvette. Afterwards was added 1 tsp. (teaspoon) of Pd/C. The next day the reaction was controlled with thin-layer chromatography (TLC) and was visualized using mixture 1:1 EtOAc/hexane, however, there still were reactants. The reaction mixture was bubbled by hydrogen using hydrogenator under atmospheric pressure. According to TLC the reactants were not detected. The catalyser was filtrated using 3 paper filters and the product was isolated using flask chromatography (in before was an attempt of crystallization, but it was not successful). Yielded 0.03 g (**33 %**) of white crystals. **HRMS (MALDI) m/z**: for $C_{17}H_{14}ClF_3N_2O_3$ was measured, but the results were not found. **Elementary analysis**: for $C_{17}H_{14}ClF_3N_2O_3$ was estimated: C 52.79; H 3.65; N 7.24; was received: C 59.91±0.02; H 4.99±0.04; N 7.20±0.05.

After MALDI analysis the service engineer commented that the compound **12e** was something else. And it was corresponded to elementary analysis, where it was agreed that the prepared compound was a derivate without chlorine.

Unfortunately, there was no time to redo from the start the whole synthesis of the product **12e** with given circumstances.

7. Conclusion

Theoretical part of the bachelor's thesis is focused on description of salicylamides. Firstly, it is briefly mentioned the description of salicylic acid. After that there are discussed aromatic salicylamides, mostly about salicylanilide, and aliphatic salicylamides. Apart of their descriptions, in this part is also discussed about their biological activities and importantly about their syntheses. Many possibilities of syntheses are shown and every each of them has their own benefits. The main focused is on syntheses of diamides, specifically 2-hydroxy-*N*-(arylalkyl)benzamides, thanks to them this work could be established. They are very important to know because they have potential antibacterial activities. In this part is also explained how each substitute can influence the bioactivity of the diamides. The significant group is chlorine at the position 5 on the salicylic acid's moiety. And the variety of amino acids can also lead to different bioactivity. Many bacteria have resistance to antibiotics. Hence, we should discover more drugs that can help with the treatment.

In the end of the theoretical part is dedicated on biological activities. Firstly, is described bioactivity of salicylic acid that has mainly anti-acne activity. Salicylic acid's derivates, however, are not described, but are briefly mentioned their generic drug's names and properties. Most concern is over antibacterial activities of salicylamides even though many of them have anti-cancer activity specifically proteasomal inhibition. In this part is also briefly described chosen bacteria and what kind of diseases or infections they can cause. That is another reason why there should be more studies regarding salicylamide's derivates.

Experimental part describes syntheses of intermediates that are followed by syntheses of the desired products. Firstly, is shown the synthesis of 2-(benzyloxy)-5-chlorbenzencarboxyl acid that is derived from 5-chlorsalicylic acid. From the prepared acid are obtained methyl esters of (2*S*)-2-[[2-(benzyloxy)-5-chlorobenzoyl]benzamido) acids using chemical agents HOBt·H_2O and EDC·HCl. Afterwards, is followed the preparation of 2-(2-(benzyloxy)benzamido) acids from methyl esters by adding LiOH monohydrate and then acidifying them with HCl. For the key

intermediate of *O*-benzyl-2-hydroxy-*N*-(arylalkyl)benzamides the synthesis needs the prepared 2-(2-(benzyloxy)benzamido) acids and trifluoro-p-toluidine mixed in together using chemical agents HOBt·H_2O and EDC·HCl. Therefore, in the end can be obtained final product of 2-hydroxy-*N*-(arylalkyl)benzamides by hydrogenation of *O*-benzyl-2-hydroxy-*N*-(arylalkyl)benzamides using catalyst Pd on carbon.

8. Literature

Citation is done according to ACS norms.

[1] Rouette, H. *Encyclopedia of Textile Finishing* [online]; Woodhead Publishing: Aachen, 2001; p 2. https://app.knovel.com/web/view/khtml/show.v/rcid:kpETF00001/cid:kt003VXSO1/viewerType:khtml//root_slug:encyclopedia-textile/url_slug:salicylic-acid?b-content-type=encyclopedia&b-q=Salicylic%20acid&include_synonyms=no&sort_on=default&page=2&view=collapsed&zoom=1&q=Salicylic%20acid (accessed July 03, 2021).

[2] Cammack, R.; Attwood, T. K.; Campbell, P. N.; Parish, J. H.; Smith, A. D.; Stirling, J. L.; Vella, F. *Oxford Dictionary of Biochemistry and Molecular Biology*, 2nd ed. [online]; Oxford University Press: Oxford, 2006; p 598. https://app.knovel.com/web/view/khtml/show.v/rcid:kpODBMBE01/cid:kt008ODCI1/viewerType:khtml//root_slug:oxford-dictionary-biochemistry/url_slug:salicylic-acid?b-content-type=dictionary&b-q=Salicylic%20acid&include_synonyms=no&sort_on=default&page=2&view=collapsed&zoom=1&q=Salicylic%20acid (accessed July 03, 2021).

[3] Chen, X.; Chen, H. Preparation of salicylic acid by Kolbe-Schmitt reaction. Zhejiang University. CN 101613270A, Dec 30, 2009.

[4] Gangolli, S. *Dictionary of Substances and Their Effects*, 3rd ed. [online]; Royal Society of Chemistry: Cambridge, 2005; p S5. https://app.knovel.com/web/view/html/show.v/rcid:kpDSTEDOS3/cid:kt002PA3V6/viewerType:html//root_slug:dictionary-substances/url_slug:salicylamide?b-q=salicylamides&include_synonyms=no&q=salicylamides%20&sort_on=default&page=1 (accessed July 03, 2021).

[5] Wang, C.; Wang C. Process for preparation of salicylamide. Zhenjiang Gaopeng Pharmaceutical CO. CN105503640 (A). April 20, 2016.

[6] Dobler, M. R. A short and efficient preparation of methyl-[1,2,4]oxadiazolium derivatives with plant-inducing activity. *Organic and Biomolecular Chemistry* **2004**, *2* (7), 963-964.

[7] Lewis, R. J., Sr. *Sax's Dangerous Properties of Industrial Materials*, 12th ed. [online]; John Wiley & Sons: New Jersey, 2012; p 3919. https://app.knovel.com/web/view/pdf/show.v/rcid:kpSDPIME0E/cid:kt00ANMQJ6/viewerType:pdf//root_slug:saxs-dangerous-properties/url_slug:salicylanilide-sah500?cid=kt00ANMQJ6&b-q=Salicylanilide&include_synonyms=no&q=Salicylanilide%20&sort_on=default (accessed July 04, 2021).

[8] Jeney, E.; Zsolnai, T. Antimicrobial, ascaricidal, and molluscicidal activities of salicylanilide analogs. *Zentralblatt fuer Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Abteilung 1: Medizinisch-Hygienische Bakteriologie, Virusforschung und Parasitologie, Originale* **1967**, *202* (4), 547-562.

[9] Lee, I.; Gruberb, T. D.; Samuels, A.; Yun, M.; Nam, B.; Kang, M.; Crowley, K.; Winterroth, B.; Boshoff, H. I.; Barry, C. E. III. Structure–activity relationships of antitubercular salicylanilides consistent with disruption of the proton gradient via proton shuttling. *Bioorganic & Medicinal Chemistry* **2013**, *21* (1), 114-126.

[10] Hamada, Y.; Takeuchi, I.; Ito, Y.; Matsui, S.; Ito, T. On the antimicrobial activity and syntheses of salicylanilide derivatives. *Yakugaku Zasshi* **1981**, *101* (7), 633-641.

[11] Imramovský, A.; Vinšová, J.; Férriz, J. M.; Kuneš, J.; Pour, M.; Doležal, M. Salicylanilide esterification: unexpected formation of novel seven-membered rings. *Tetrahedron Letters* **2006**, *47* (29), 5007-5011.

[12] Pauk, K.; Zadrazilova, I.; Imramovsky, A.; Vinsova, J.; Pokorna, M.; Masarikova, M.; Cizek, A.; Jampilek, J. New derivatives of salicylamides: Preparation

and antimicrobial activity against various bacterial species. *Bioorganic & Medicinal Chemistry* **2013**, *21* (21), 6574-6581.

[13] Baranyai, Z.; Kratky, M.; Vosatka, R.; Szabo, E.; Senoner, Z.; David, S.; Stolarikova, J.; Vinsova, J.; Bosze, S. In vitro biological evaluation of new antimycobacterial salicylanilide-tuftsin conjugates. *European Journal of Medicinal Chemistry* **2017**, *133*, 152-173.

[14] Vardanyan, R.; Hruby, V. *Synthesis of Best-Seller Drugs* [online]; Elsevier: Arizona, 2016; p 754-755. https://app.knovel.com/web/view/khtml/show.v/rcid:kpSBSD0007/cid:kt010V0R15/viewerType:khtml//root_slug:synthesis-best-seller/url_slug:salicylanilides?b-q=Salicylanilide&include_synonyms=no&sort_on=default&page=6&view=collapsed&zoom=1&q=Salicylanilide (accessed July 04, 2021).

[15] Imramovský, A.; Jorda, R.; Pauk, K.; Řezníčková, E.; Dušek, J; Hanusek, J.; Kryštof, V. Substituted 2-hydroxy-N-(arylalkyl)benzamides induce apoptosis in cancer cell lines. *European Journal of Medicinal Chemistry* **2013**, *68*, 253-259.

[16] Pohanish, R. P. *Sittig's Handbook of Toxic and Hazardous Chemicals and Carcinogens* 7th ed. [online]; Elsevier: Oxford, 2017; p 281-282. https://app.knovel.com/web/view/khtml/show.v/rcid:kpSHTHCC21/cid:kt011E5173/viewerType:khtml//root_slug:sittigs-handbook-toxic/url_slug:antimycin-a?b-q=antimycin%20A&include_synonyms=no&sort_on=default&page=281&view=collapsed&zoom=1&q=antimycin%20A (accessed July 04, 2021).

[17] Kim, J.; Kang, S.; Hong, S.; Yum, S.; Kim, Y. M.; Jung, Y. Structure-activity relationship of salicylic acid derivatives on inhibition of TNF-α dependent NFκB activity: Implication on anti-inflammatory effect of N-(5-chlorosalicyloyl) phenethylamine against experimental colitis. *European Journal of Medicinal Chemistry* **2012**, *48*, 36-44.

[18] Kaushik, P.; Shakil, N. A.; Kumar, J.; Singh, B. B. Lipase-Catalyzed Solvent-Free Amidation of Phenolic Acids. *Synthetic Communications* **2015**, *45* (5), 579-587.

[19] Kondo, H.; Kuwana, Y.; Takada, H.; Chigrinov, V. G.; Kwok, H. Azo compound, composition for optical alignment film using same, and method for producing optical alignment film. Dainippon INK & Chemicals and Univ Hong Kong Science & Techn. WO 2006003893 A1. Jan 12, 2006.

[20] Imramovský, A.; Pauk, K.; Padelkova, Z.; Hanusek, J. Crystal Structure of the 5-Chloro Salicylamides: Three Different Types of the H-bonding Influenced Linear Chain Formation in the Solid State. *Crystals* **2012**, *2* (2), 349-361.

[21] Bay, W. E.; Corvino J. P. Method of preparing salicylamides. Emisphere technologies INC. WO0192206A1. Dec 06, 2001.

[22] Fritzson, I.; Bedingfield, P. T. P.; Sundin, A. P.; McConkey, G.; Nilsson, U. J. N-Substituted salicylamides as selective malaria parasite dihydroorotate dehydrogenase inhibitors. *MedChemComm* **2011**, *2* (9), 895-898.

[23] Radmilovic Rovira, N. Synthesis of salicylanilide. *Anales de la Facultad de Quimica y Farmacia (Universidad de Chile)* **1968**, *19*, 80-83.

[24] Lu, C.; Zhao, B.; Jiang, Y.; Ding, H.; Yang, S. One-Pot Synthesis of Salicylanilides by Direct Amide Bond Formation from Salicyclic Acid Under Microwave Irradiation. *Synthetic Communications* **2011**, *41* (9), 1257-1266.

[25] Fang, H.; Guo, H.; Chen, H.; Zhang, J.; Hong, Z.; Fang, M. Novel derivatives of salicylanilide: Synthesis, characterization, PPO inhibitory activity and cytotoxicity. *Journal of Molecular Structure* **2021**, *1223*, 128995.

[26] Coburn, R. A.; Batista, A. J.; Evans, R. T.; Genco, R. J. Potential salicylamide antiplaque agents: in vitro antibacterial activity against Actinomyces viscosus. *Journal of Medicinal Chemistry* **1981**, *24* (10), 1245-1249.

[27] Vinšová, J.; Kozic, J.; Krátký, M.; Stolaříková, J.; Mandíková, J.; Trejtnar, F.; Buchta, V. Salicylanilide diethyl phosphates: Synthesis, antimicrobial activity and cytotoxicity. *Bioorganic and Medicinal Chemistry* **2014**, *22* (2), 728-737.

[28] Buxade Vinas, A.; Albreda Plaza, M.; Canovas Ubera, M.; Conchillo Teruel. A. Pharmaceutical composition containing micronized salicylic acid for topical use at high concentration, and its corresponding method and use. Laboratorios Vinas S.A. ES 2 575 655 A1. June 30, 2016.

[29] Levin, G.; Sacks, H.; Rudaev, S. Transdermal delivery system for cosmetic agents. Transpharma Medical LTD. US 2003-60480229. WO 2004/112689 A2. Dec 29, 2004.

[30] Weinberger, G. I.; Nagel, R. H.; Brown, R. A. Topical compositions for the treatment of dermatological disorders. Evologie LLC. WO 2012/177433 A1. Dec 27, 2012.

[31] Lu, J.; Cong, T.; Wen, X.; Li, X.; Du, D.; He, G.; Jiang, X. Salicylic acid treats acne vulgaris by suppressing AMPK/SREBP1 pathway in sebocytes. *Experimental Dermatology* **2019**, *28* (7), 786-794.

[32] Yates, M. V.; Nakatsu, C. H.; Miller, R. V.; Pillai, S. D. *Manual of Environmental Microbiology*, 4th ed [online]; American Society for Microbiology (ASM): Washington, 2016; p 2.1.1-7. https://app.knovel.com/web/view/khtml/show.v/rcid:kpMEME0014/cid:kt0110T511/viewerType:khtml//root_slug:manual-environmental/url_slug:staphylococcus-aureus?b-q=Staphylococcus%20aureus&sort_on=default&b-subscription=true&b-group-by=true&page=9&b-sort-on=default&b-content-type=all_references&include_synonyms=no&view=collapsed&zoom=1&q=Staphyl Staphyl%20aureus (accessed July 06, 2021).

[33] Mead, G. C. *Food Safety Control in the Poultry Industry* [online]; CRC Press LLC: Boca Raton and Woodhead Publishing Limited: Cambridge, 2005; p 27-28.

https://app.knovel.com/web/view/khtml/show.v/rcid:kpFSCPI001/cid:kt010XSBW4/viewerType:khtml//root_slug:food-safety-control-in/url_slug:food-safet-introduction?b-q=Staphylococcus%20aureus%20subsp.%20aureus&sort_on=default&b-group-by=true&page=7&b-sort-on=default&b-content-type=all_references&include_synonyms=no&view=collapsed&zoom=1&q=Staphyl Staphyl%20aureus%20subsp.%20aureus (accessed July 06, 2021).

[34] Purslow, P. P. *New Aspects of Meat Quality - From Genes to Ethics* [online]; Elsevier: Duxford, 2017; p 204-205. https://app.knovel.com/web/view/khtml/show.v/rcid:kpNAMQFGEH/cid:kt011G0OJC/viewerType:khtml//root_slug:new-aspects-meat-quality/url_slug:staphylococcus-aureus?b-q=Staphylococcus%20aureus&sort_on=default&b-subscription=true&b-group-by=true&page=14&b-sort-on=default&b-content-type=all_references&include_synonyms=no&view=collapsed&zoom=1&q=Staphylococcus%20aureus (accessed July 06, 2021).

[35] Motarjemi, Y.; Moy, G. G; Todd, E. CD. *Encyclopedia of Food Safety* [online]; Elsevier: San Diego, 2014; p 197. https://app.knovel.com/web/view/khtml/show.v/rcid:kpEFS00001/cid:kt00C6BTL1/viewerType:khtml//root_slug:encyclopedia-food-safety/url_slug:staphylococcus-aureus?b-q=MRSA&sort_on=default&b-group-by=true&page=32&b-sort-on=default&b-content-type=all_references&include_synonyms=no&view=collapsed&zoom=1&q=MRSA (accessed July 06, 2021).

[36] Barros-velázquez, J. *Antimicrobial Food Packaging* [online]; Elsevier: London, 2016; p 21-23. https://app.knovel.com/web/view/khtml/show.v/rcid:kpAFP0001P/cid:kt010WNHJ4/viewerType:khtml//root_slug:antimicrobial-food-packaging/url_slug:enterococcus-faecalis?b-

q=Enterococcus%20faecalis&include_synonyms=no&sort_on=default&page=11&vi
vi=collapsed&zoom=1&q=Enterococcus%20faecalis (accessed July 06, 2021).

[37] Griffiths, M. *Understanding Pathogen Behaviour - Virulence, Stress Response and Resistance* [online]. CRC Press LLC: Boca Raton and Woodhead Publishing Limited: Cambridge, 2005; p 580. https://app.knovel.com/web/view/khtml/show.v/rcid:kpUPBVSRR1/cid:kt010XPCW1/viewerType:khtml//root_slug:understanding-pathogen/url_slug:enterococcus-faecalis?b-q=Enterococcus%20faecalis&include_synonyms=no&sort_on=default&page=32&view=collapsed&zoom=1&q=Enterococcus%20faecalis (accessed July 06, 2021).

[38] Garcia, L. S. *Clinical Microbiology Procedures Handbook* 3rd ed. [online]; American Society for Microbiology (ASM): Washington, 2010; p 13.18.1. https://app.knovel.com/web/view/khtml/show.v/rcid:kpCMPHVE03/cid:kt0091UIK2/viewerType:khtml//root_slug:clinical-microbiology/url_slug:screening-vancomycin?b-q=Vancomycin-Resistant%20Enterococcus&include_synonyms=no&sort_on=default&page=38&view=collapsed&zoom=1&q=Vancomycin-Resistant%20Enterococcus (accessed July 06, 2021).

[39] Motarjemi, Y.; Adams, M. *Emerging Foodborne Pathogens* [online]; Woodhead Publishing Limited: Cambridge, 2006; p 582-584. https://app.knovel.com/web/view/khtml/show.v/rcid:kpEFP00005/cid:kt003WODI3/viewerType:khtml//root_slug:emerging-foodborne-pathogens/url_slug:infections-caused-by?b-q=VRE&b-taxonomy=11%40%40enterococci%40%4031&include_synonyms=no&sort_on=default&page=405&view=collapsed&zoom=1&q=VRE%20%2B%20(enterococci) (accessed July 06, 2021).

[40] Fleming, D. O.; Hunt, D. L. *Biological Safety - Principles and Practices* 4th ed [online]; American Society for Microbiology (ASM): Washington, 2006; p 112-113.

https://app.knovel.com/web/view/khtml/show.v/rcid:kpBSPPE003/cid:kt008XA011/viewerType:khtml//root_slug:biological-safety-principles/url_slug:mycobacterium-tuberculosis?b-q=Mycobacterium%20tuberculosis&include_synonyms=no&sort_on=default&page=20&view=collapsed&zoom=1&q=Mycobacterium%20tuberculosis (accessed July 06, 2021).

[41] International Food Information Service. *Dictionary of Food Science and Technology* 2nd ed [online]; International Food Information Service (IFIS Publishing): Shinfield, 2009; p 434. https://app.knovel.com/web/view/khtml/show.v/rcid:kpDFSTE001/cid:kt006QG633/viewerType:khtml//root_slug:dictionary-food-science/url_slug:tuberculosis?b-q=Mycobacterium%20tuberculosis&include_synonyms=no&sort_on=default&page=20&view=collapsed&zoom=1&q=Mycobacterium%20tuberculosis (accessed July 06, 2021).

[42] Mehta, S. C.; Kar, A. *Pharmaceutical Pharmacology* [online]; New Academic Science: London, 2014; p 973-980. https://app.knovel.com/web/view/khtml/show.v/rcid:kpPP000032/cid:kt011NOQX1/viewerType:khtml//root_slug:pharmaceutical-pharmacology/url_slug:tuberculosis-tb?b-q=Mycobacterium%20tuberculosis&include_synonyms=no&sort_on=default&page=2&view=collapsed&zoom=1&q=Mycobacterium%20tuberculosis (accessed July 06, 2021).

[43] Lacroix, C. *Protective Cultures, Antimicrobial Metabolites and Bacteriophages for Food and Beverage Biopreservation* [online]; Woodhead Publishing: Cambridge, 2011; p 109-110. https://app.knovel.com/web/view/khtml/show.v/rcid:kpPCAMBFB6/cid:kt00927663/viewerType:khtml//root_slug:protective-cultures-antimicrobial/url_slug:spectrum-inhibition-mode?b-q=mycobacterium%20smegmatis%20AND%20acid%20fast%20bacteria&include_s

ynonyms=no&sort_on=default&page=10&view=collapsed&zoom=1&q=mycobacterium%20smegmatis%20AND%20acid%20fast%20bacteria (accessed July 06, 2021).

[44] Reddy, C. A.; Beveridge, T. J.; Breznak, J. A.; Marzluf, G. A.; Schmidt, T. M.; Snyder, L. R. *Methods for General and Molecular Microbiology* 3rd ed. [online]; American Society for Microbiology (ASM): Washington, 2007; p 27-28. https://app.knovel.com/web/view/khtml/show.v/rcid:kpMGMME001/cid:kt0090JFO2/viewerType:khtml//root_slug:methods-general-molecular/url_slug:acid-fast-staining?b-content-type=all_references&b-q=acid-fast%20actinomycetes&include_synonyms=no&sort_on=default&page=9&view=collapsed&zoom=1&q=acid-fast%20actinomycetes (accessed July 06, 2021).

[45] Rögner, M. *Biohydrogen* [online]; De Gruyter: Berlin, 2015; p 82-84. https://app.knovel.com/web/view/khtml/show.v/rcid:kpB0000005/cid:kt010ZHL18/viewerTyp:khtml//root_slug:biohydrogen/url_slug:o2-insensi-physiological?b-q=Mycobacterium%20smegmatis&include_synonyms=no&sort_on=default&page=22&view=collapsed&zoom=1&q=Mycobacterium%20smegmatis (accessed July 06, 2021).

[46] Sawers, R. G.; Pinske, C. NiFe-Hydrogenase Assembly. *Encyclopedia of Inorganic and Bioinorganic Chemistry* **2017**, (188), 1-11.

[47] Garcia, L. S. *Clinical Microbiology Procedures Handbook* 3rd ed. [online]; American Society for Microbiology (ASM): Washington, 2010; p 6.1.1-6.3.3.4. https://app.knovel.com/web/view/khtml/show.v/rcid:kpCMPHVE03/cid:kt0091U9B1/viewerType:khtml//root_slug:clinical-microbiology/url_slug:specimen-examination?b-content-type=all_references&b-q=acid-fast%20actinomycetes&include_synonyms=no&sort_on=default&page=2&view=collapsed&zoom=1&q=acid-fast%20actinomycetes (accessed July 06, 2021).

[48] Lehr, J.; Keeley, J.; Lehr, J. *Water Encyclopedia: Domestic, municipal, and industrial supply and waste disposal* [online]; John Wiley & Sons: New Jersey, 2005; p 278.

https://app.knovel.com/web/view/khtml/show.v/rcid:kpWEV00004/cid:kt003ZE3X1/viewerType:khtml//root_slug:water-encyclopedia-volumes/url_slug:health-effects-microbial?b-content-type=definitions&b-q=Mycobacterium%20marinum&include_synonyms=no&sort_on=default&page=22&view=collapsed&zoom=1&q=Mycobacterium%20marinum (accessed July 06, 2021).

[49] Jorda, R.; Dusek, J.; Reznickova, E.; Pauk, K.; Magar, P. P.; Imramovsky, A.; Krystof, V. Synthesis and antiproteasomal activity of novel O-benzyl salicylamide-based inhibitors built from leucine and phenylalanine. *European Journal of Medicinal Chemistry* **2017**, *135*, 142-158.

[50] Gaudioso, L. A.; Weglarz, M. A. Process for the synthesis of exochelins. US6063919A. Aug 14, 1998.

[51] Zhang, P.; Wang, G.; Liu, J.; Xu, F.; Zhao, Z.; Wang, W.; Wang, J.; Wang, G.; Wu, P. Three new metabolites from the endophytic fungus Climacocystis montana isolated from the root bark of Paeonia ostia. *Phytochemistry Letters* **2018**, *26*, 50 – 54.