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Vienna University of Technology

Master Thesis

New Synthesis Pathways for a β-Lactamase Inhibitor

Institute of Applied Synthetic Chemistry

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by

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Date Signature

This thesis was conducted in cooperation with the Sandoz GmbH.

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I declare that I have developed and written the enclosed Master Thesis completely by myself, and have not used sources or means without declaration in the text. Any thoughts from others or literal quotations are clearly marked. The Master Thesis was not used in the same or in a similar version to achieve an academic degree or is being published elsewhere.

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Location, Date Daniel Himmelbauer

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Finally, I must express my very profound gratitude to my parents and to my girlfriend for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of working for and writing of this thesis.

List of Abbreviation

ANDA … Abbreviated New Drug Application

BOC … *tert*-Butyloxycarbonyl

CDI … Carbonyldiimidazole

DMAP … *N*,*N*-Dimethylpyridin-4-amine

DMF … Dimethylformamide

FDA … Food and Drug Administration

FMOC … Fluorenylmethoxycarbonyl

HPLC … High performance liquid chromatography

IBX … *o*-Iodoxybenzoic acid

NMP … *N*-Methyl-2-pyrrolidone

OB … Orange Book

PBP … Penicillin-binding protein

SET … Single electron transfer

TEA … Triethylamine

TFA … Trifluoracetyl

THF … Tetrahydrofuran

TiPS-Cl … Triisopropylsilylchloride

TLC … Thin layer chromatography

TP … Transpeptidase

Abstract

Bacterial strains came under enormous pressure due the overuse of β-lactam antibiotics. In a selective process only the resistant strains survived and new β-lactamase resistances were formed. Avibactam is the first non-β-lactam β-lactamase inhibitor which is used clinically since more than 20 years. Compared to its predecessors it has unique properties and a wider scope of activity against β-lactamases. By developing a new synthetic route for avibactam, the originator process patent can be circumvented and it is possible to be the first generic company on the market.

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1. Reaction scheme

1.1 Reaction scheme of the unsaturated hetero-cyclic ground structure

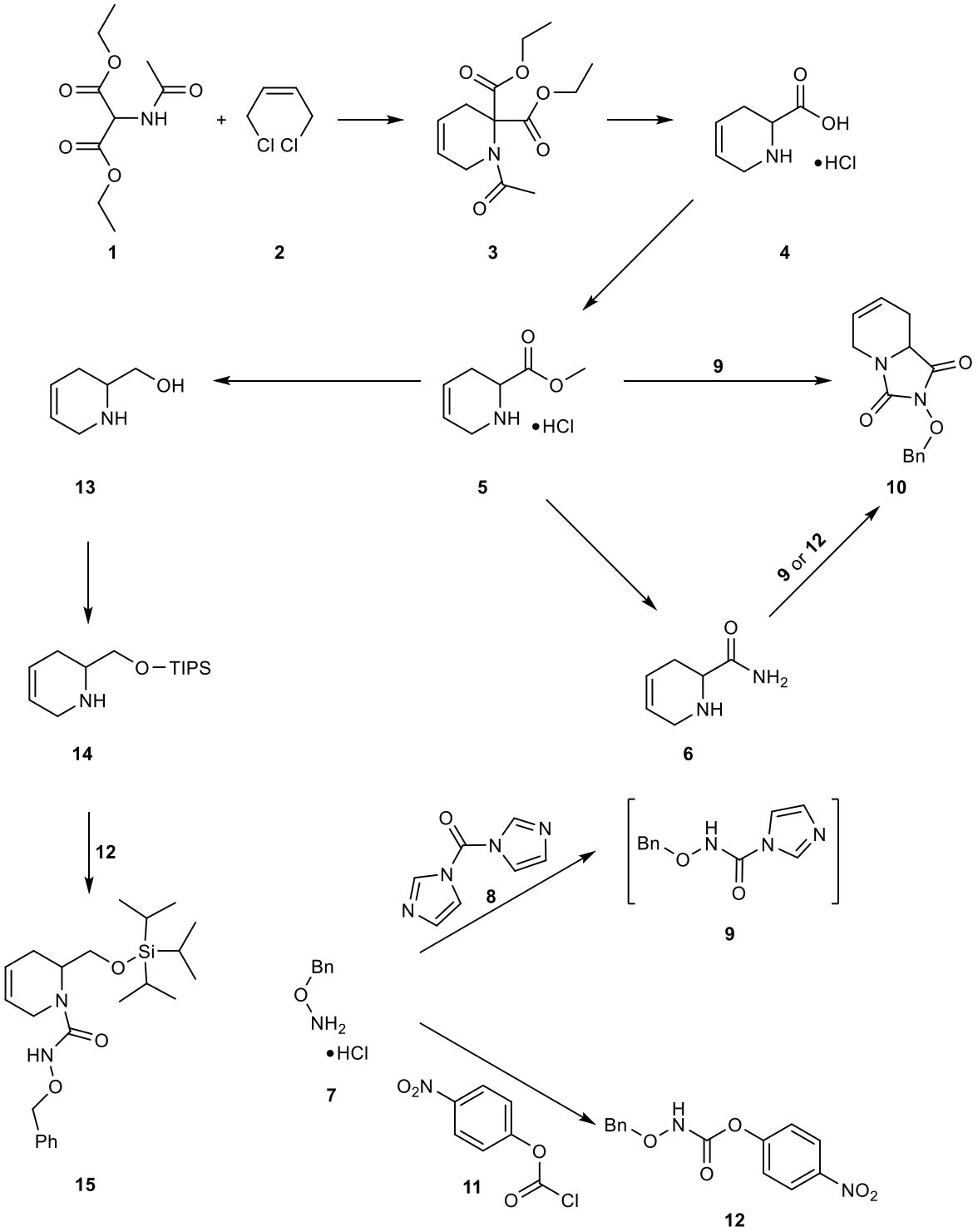


Fig. 1. Reaction scheme with Baikiain hydrochloride (**4**).

1.2 Reaction scheme of the saturated hetero cyclic ground structure

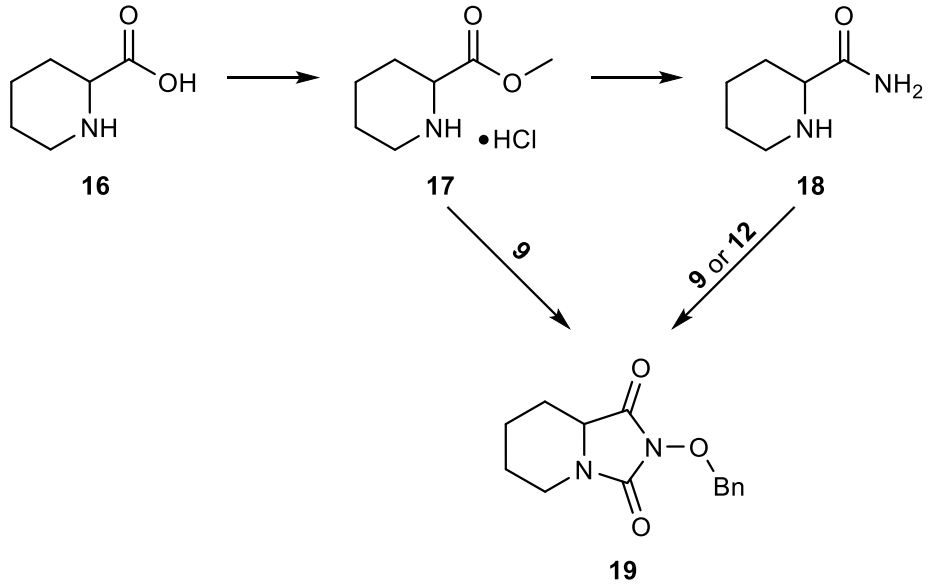


Fig. 2. Reaction scheme with the purchased pipecolic acid (**16**).

2. Introduction

The aim of this project was to find a new synthetic route for the non-β-lactam β-lactamase inhibitor avibactam (Fig. 3) to circumvent the process patent of the originator company.

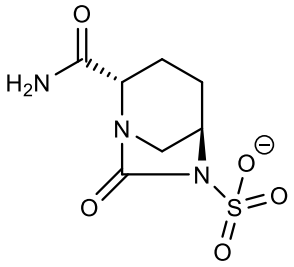


Fig. 3. Avibactam, modified from [1]

The topic of this introduction will be the fundamental mechanism of how β-lactam antibiotics like penicillin inhibit cell wall synthesis. Then the mechanism of the β-lactamase enzymes will be shortly discussed, followed by the comparison of β-lactam β-lactamase inhibitors with the new non-β-lactam β-lactamase inhibitor avibactam.

2.1 The mechanism of **β**-lactam antibiotics

The development and production of antibiotic agents lead to decades where the threat through infection diseases was highly reduced. However, in the last years the word bacterial resistance gained more attention. [2] Penicillin is the most known and common β-lactam antibiotic agent for the inhibition of the cell wall synthesis of a prokaryotic cell. It was discovered by Alexander Fleming 1928 (Nobel Prize 1945). Since Fleming's discovery numerous different generations and analogues have been developed and produced (Fig. 4).

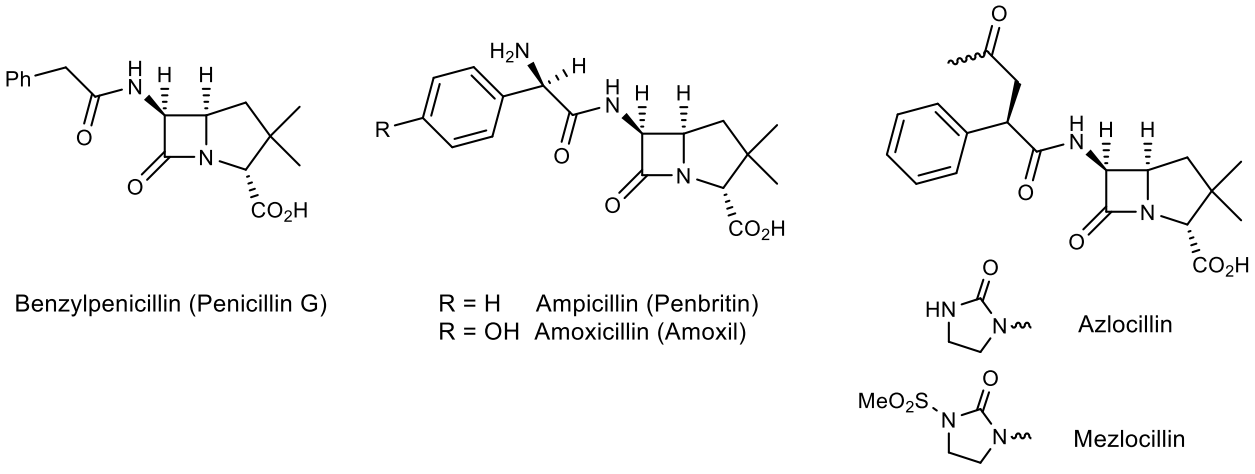


Fig. 4. Generations of penicilines from left to right, modified from [3].

The cell wall of a prokaryotic cell protects the organism from the influences of the environment. It is made of a polymeric peptidoglycan containing sugar units as backbone with peptide chains connected to them. The enzyme which plays an important role during the biosynthesis of the cell wall is called the penicillin-binding protein (PBP) and is located on the surface of the cell membrane. The PBPs can consist of a glycosyltransferase which is responsible for the polymerization of the sugar units and a transpeptidase (TP) for crosslinking them which makes the PBP bifunctional. Some of the PBPs exhibit only a transpeptidase activity and are therefore monofunctional. [4] The TP has a serine residue in the active site which is targeted by the β-lactam antibiotics. The hydroxyl functionality of the serine nucleophilicily attacks the carbonyl moiety of the β-lactam ring and binds the antibiotic agent covalently (Fig. 5). The active site of the enzyme is now inhibited and no further crosslinking is possible which leads to a fragile and unstable structure of the cell wall. [3]

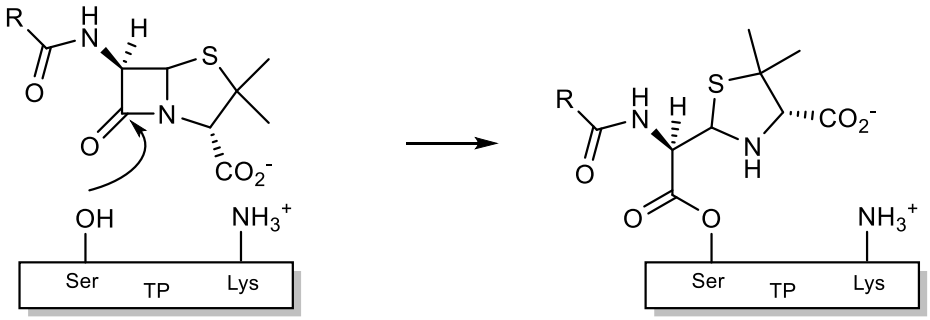


Fig. 5. Simpliefed binding scheme of penicillines in the active site of the TP, modified from [3].

2.2 β-Lactamase

The problem with bacteria is the high genetic variability they have due to mutation, conjunction, transformation or transduction[1]. [5] The ecosystem of the microorganism fell under enormous pressure through the high use of β-lactam agents in all areas of life. This resulted in a selective process where only the resistant strains survived and a lot of new variants of resistance were formed. [6] β-Lactamase enzymes are the most common and most important mechanism of a bacteria cell to protect itself against β-lactam antibiotics. [7] The first β-lactamase enzymes were described about 70 years ago [8] and can be found in both bacteria types, gram positive and gram negative (Fig. 6). In the first type they are located in the cell membrane and can be released into the environment. The second type gram negative bacteria pose a higher threat than the gram positive ones because the β-lactamase enzymes are mainly located in the periplasmic space[2] where they gather in high concentration. [3]

[1] "*Transformation, transduction, and conjugation differ in means for introducing DNA from donor cell into recipient cell*." [5]

[2] It is the space between the capsule and the cell wall and between the cell wall and the cell membrane [3].

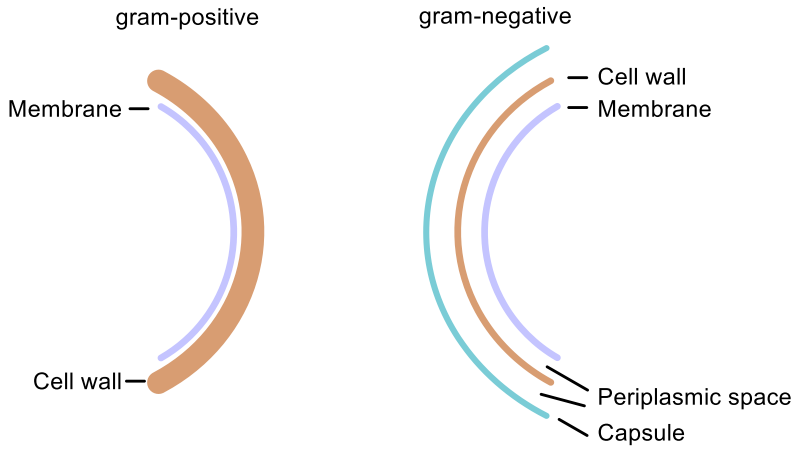


Fig. 6. Differences between gram-positive and gram-negative bacteria, modified from [3].

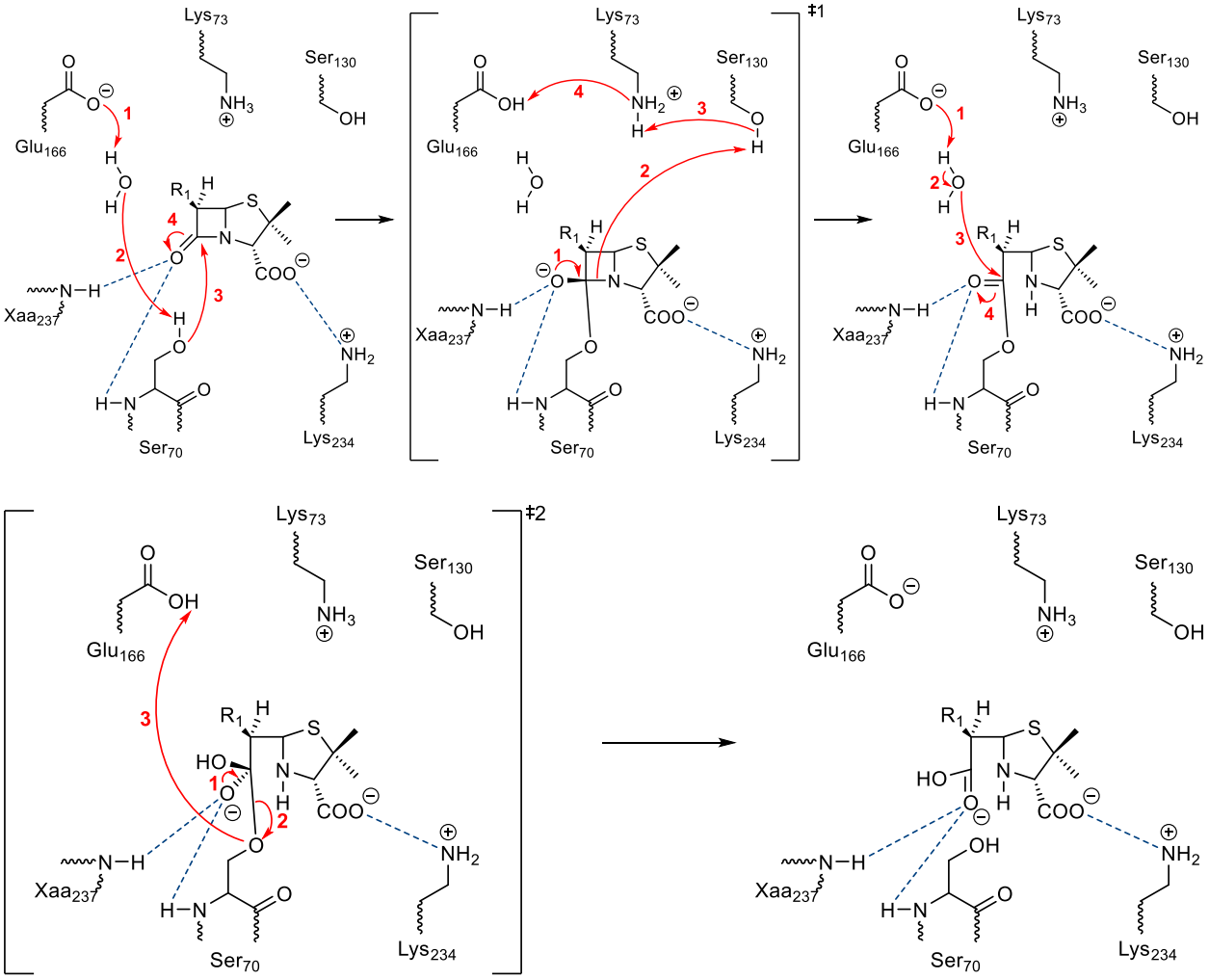


Fig. 7. "Proposed reaction mechanism for a penicillin β-lactam substrate and a class A serine β-lactamase enzyme", modified from [8]

The β-lactamase enzymes can be classified by the protein sequences (Ambler classification) in class A, B, C and D. While the classes A, C and D are containing serine in the active site, the class B is a little bit different. Instead of the serine residue it contains a Zn^{2+} ion which is coordinated to the active site. Even if the β-lactamase enzymes are different, the mechanism of resistance is fundamental the same. It covalently binds (at least in the serine enzymes) the β-lactam antibiotic, like the TP from the PBP, forming an acyl intermediate. But instead of staying covalently bounded to the active site the β-lactam antibiotic can be hydrolyzed due to a water molecule positioned in the active site (Fig. 7). [8]

2.3 β-Lactamase Inhibitors

There are two strategies for the development of β-lactam antibiotics to overcoming the threat by β-lactamase enzymes. The first one is to design new drugs which do not get hydrolyzed by β-lactamases or the second one is to develop specific β-lactamase inhibitors which block the enzyme and the antibiotic agent can complete its work. [8]

Clavulanic acid was one of the first β-lactamase inhibitors with Sulbactam and Tazobactam developed some years later (Fig. 8). They all show structure similarity to the β-lactam antibiotics due to the β-lactam ring with the attached five membered heterocycle. For clinical use they are combined with a partner β-lactam antibiotic, like Clavulanic acid is combined with Amoxicillin. They are very potent inhibitors against class A β-lactamases but poor against class B, C and D. The inhibition of the lactamase enzyme by Clavulanic acid, Sulbactam and Tazobactam is mechanism based and can be either transient or irreversibly inhibited. [6]

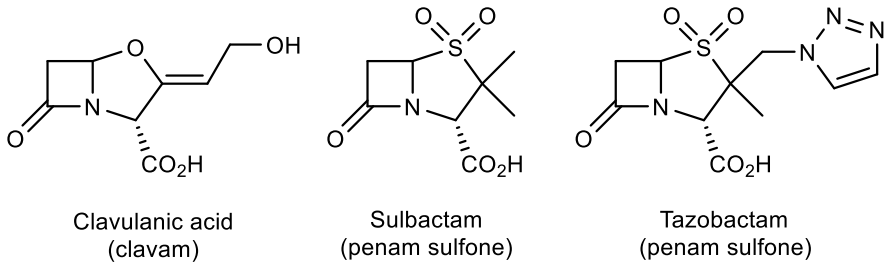


Fig. 8. β-lactamase inhibitors, modified from [6]

These inhibitors are nucleophilicily attacked by the serine residue in the active site of the lactamase enzyme like shown for penicillin in figure 7. But before the acylated intermediate is hydrolyzed the heterocyclic ring opens and a linear imine molecule is covalently bond to the serine. Now several intermediates can be formed from the linear product. The first ones would be a *cis*-enamine and a *trans*-enamine through tautomerisation of the imine forming the transient inhibition intermediates. The *trans*-enamine leads to the decarboxylated *trans*-enamine which is

irreversibly inactivated. The second ones would be irreversible inactivation products formed from the linear imine molecule (Fig. 9). [6] [8]

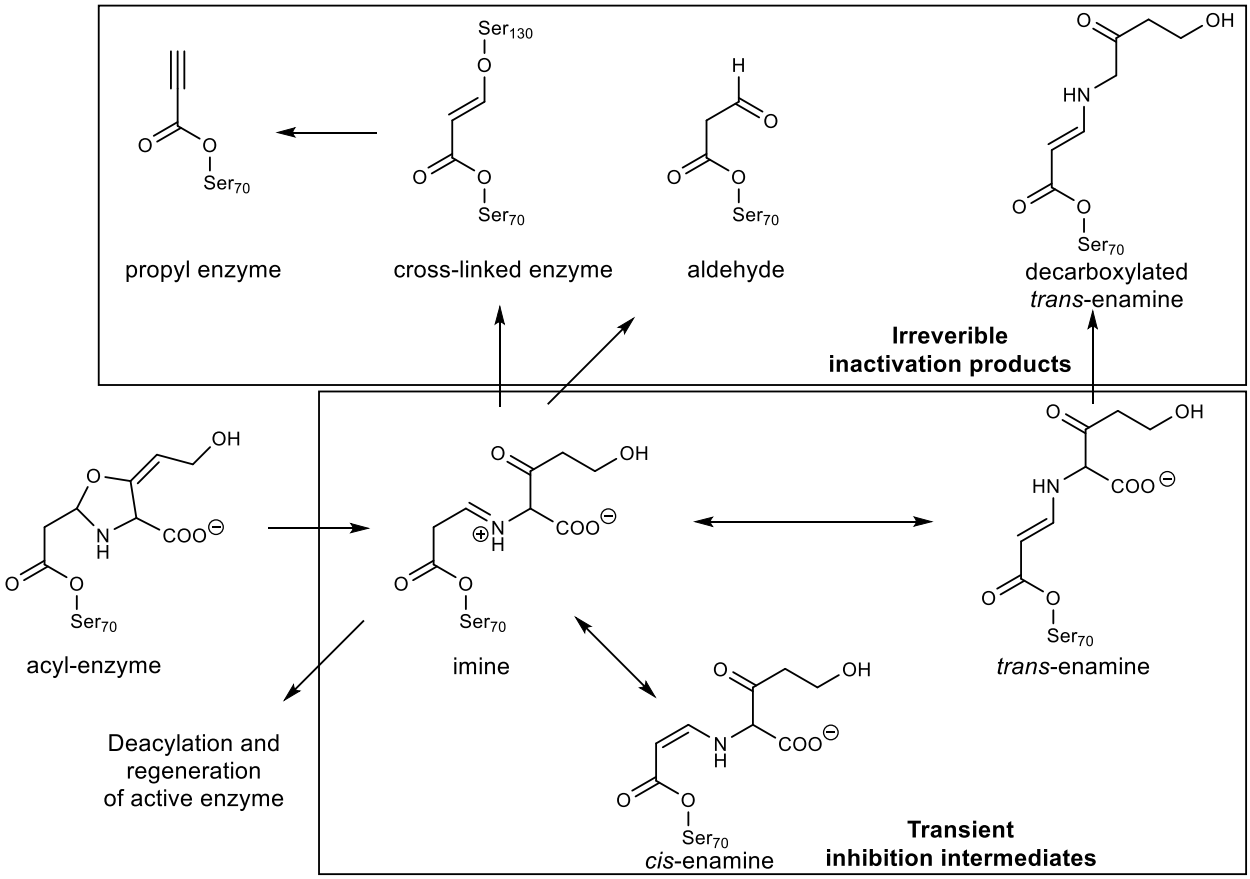


Fig. 9. "Proposed mechanism of inhibition of class A β-lactamases by clavulanate", modified from [8].

2.3.1 Avibactam

Avibactam is a non-β-lactam β-lactamase inhibitor (Fig. 3) and it's the molecule for which a new synthesis route should be developed to circumvent the originator patent. The main difference compared to the other inhibitors mentioned before is the absence of the β-lactam ring. Instead, it consists of a bicyclic structure with a five-membered urea ring and is a complete synthetic product. It is synthesized enantioselectively as the sodium salt which is water soluble. Furthermore, avibactam can effectively inhibit not only class A β-lactamases but class C and D as well, which is a big advantage compared to other β-lactamase inhibitors. [1] For clinical use it is combined with the cephalosporin[3] ceftazidime and sold in the USA under the brand name Avycaz® and in Europe as Zaviceftar®. This new drug is effective against a broad spectrum of

[3] "A group of β-lactam semi-synthetic antibacterial agents that target the bacterial transpeptidase enzymes." [3]

gram- positive and negative bacteria and is used to treat complicated intra-abdominal infections in combination with metronidazol such as complicated urinary tract infections. [9]

Avibactam inhibits the β-lactamase enzymes by a reversible mechanism which is completely different from the β-lactam β-lactamase inhibitors (Fig. 10). Similar to the reactions before, the nucleophilic serine residue in the active site of the enzyme attacks the carbon atom of the carbonyl functionality from the urea group, forming a carbamyl intermediate. The unique properties of this newly formed compound are that it can't be hydrolyzed like β-lactam agents and that the process is reversible. Because the six membered heterocycle ring doesn't open. The other nitrogen atom, containing the sulfonate group, can also perform a nucleophilic attack at the carbonyl group regaining the bicyclic structure of avibactam. The regained inhibitor can be either reused or released into the solution to inactivate other β-lactamase enzymes. These unique mode of action makes avibactam one of the most effective β-lactamase inhibitors against a broad spectrum of serine containing β-lactamase enzymes up to date. [10] [11]

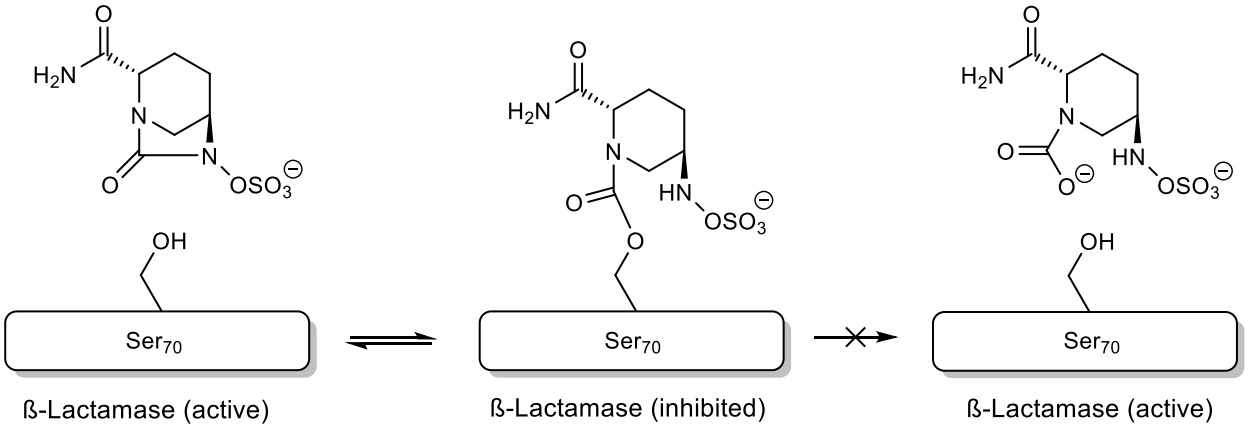


Fig. 10. Proposed mechanism of inhibition of class A ß-lactamases by Avibactam, modified from. [11]

2.4 Base and process patents of avibactam

Figure 11 shows the base patent and figure 12 the process patent for the synthesis of avibactam. The differences between these patents and the new developed route will be discussed in the following sections. In this section the important reaction steps in both patents will be described.

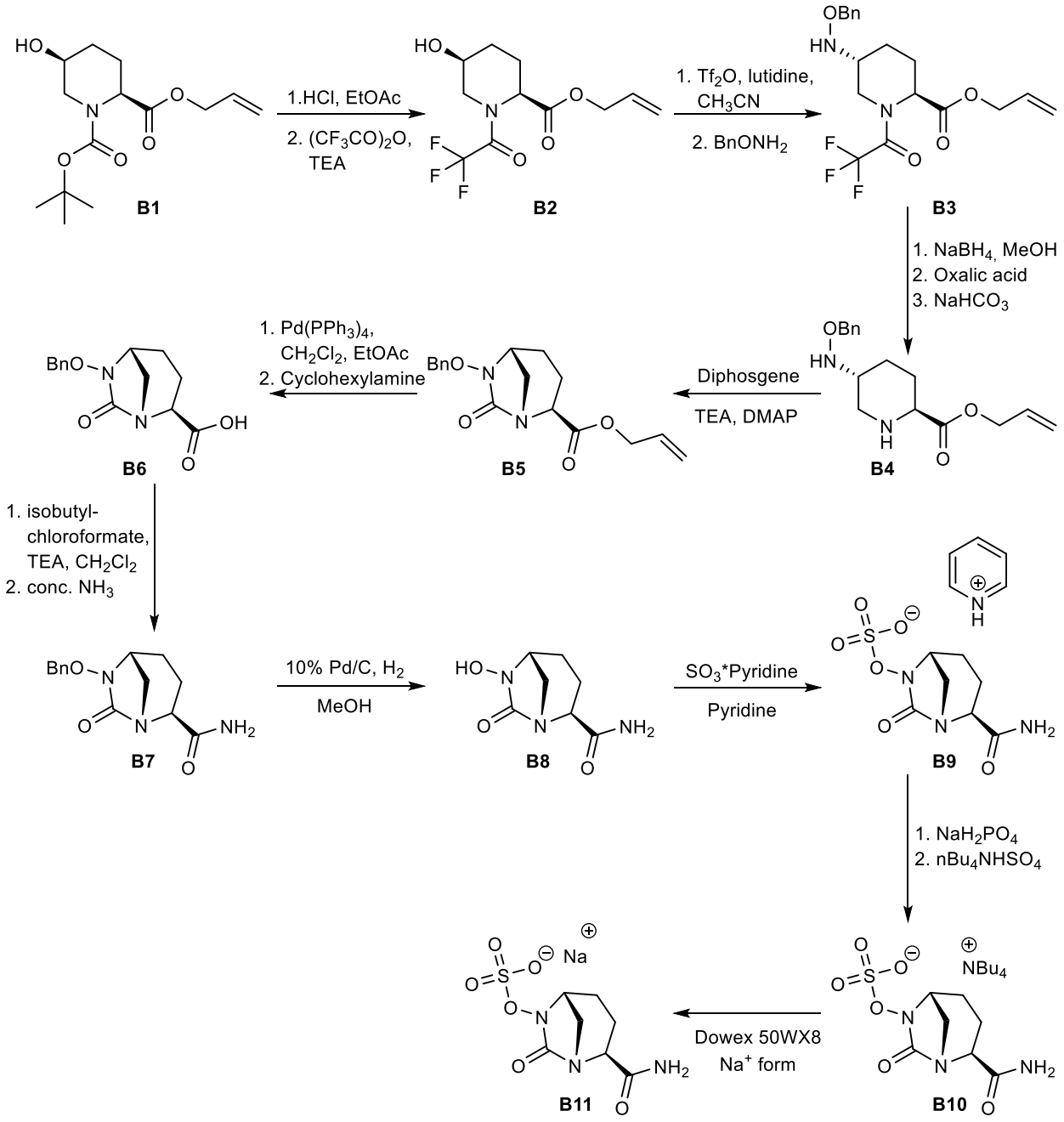


Fig. 11. Base patent of avibactam, modified from [12]

The base patent starts with a racemic mixture of substance **B1** where under acidic conditions the *tert*-butyloxycarbonyl (BOC) protecting group was removed and replaced with the trifluoracetyl

(TFA) protecting group. In the next step *O*-benzylhydroxylamine is introduced in the *meta* position to the TFA group, which leads to substance **B3.** The next two reaction steps are crucial because they lead to the ground structure of avibactam. First the TFA protecting group was removed and substance **B4** isolated followed by carboxylation of the piperidine nitrogen with diphosgene. In the same step cyclisation to the urea derivative **B5** occurred. Afterwards, the ester is converted into the amide **B7**. In the last steps the benzyloxy group was hydrogenated and the sodium salt of avibactam is obtained with an overall yield of 24% after sulfonation and ion exchange. [12]

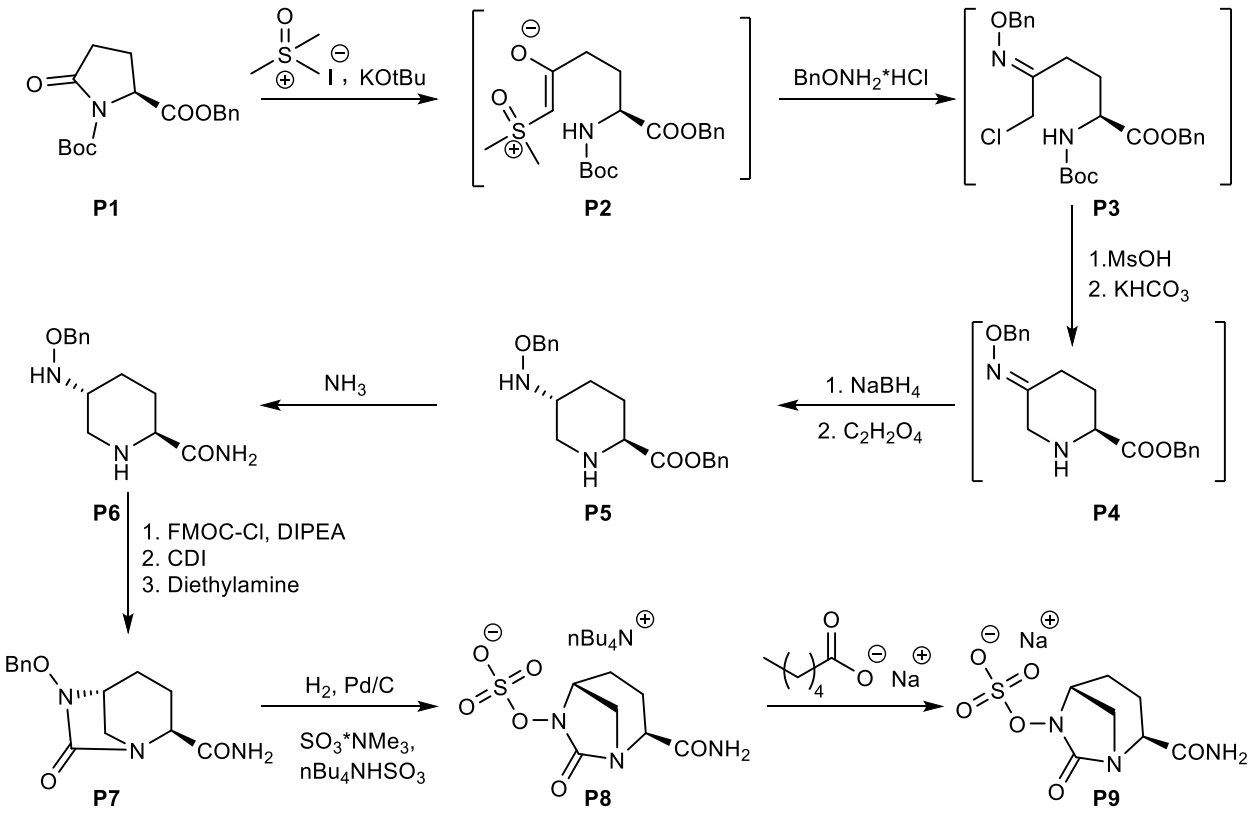


Fig. 12. Process patent of avibactam, modified from [13]

In the process patent the intermediates between starting material **P1** and compound **P5** don't need to be isolated. Then similar to the base patent the ester is converted to the amide. The urea compound **P7** is synthesized by connecting fluorenylmethoxycarbonyl (FMOC) to the piperidine nitrogen as protecting group. Then the nitrogen in the meta position is carboxylated. Afterwards the protecting group is removed and the piperidine nitrogen can attack the carboxyl group resulting in cyclization. Imidazole is acting as leaving group. The final reaction steps are similar to the base patent. The benzyloxy group was hydrogenated and after ion exchange the sodium salt of avibactam was isolated with an overall yield of 50%. [13]

2.5 Retrosynthetic analysis of avibactam

In this section a simplified retrosynthetic analysis of avibactam will be discussed.

The first disconnections shown in Figure 13 lead to the synthons **S1a** and **S1b**. The chosen polarity for the synthons was in both cases simple, due to the nitrogen atoms which carries a lone pair of electrons. For the carbon atom of **S1a** it was more difficult to find an appropriate substituent to carry the partial positive charge. Therefore, the C-C single bond was converted into a C-C double bond. The reason was due to the published *o*-iodoxybenzoic acid (IBX) mediated cyclisation reaction published by K. C. Nicolaou *et. al.* in which the synthesis of cyclized urea derivatives is described by forming a C-N bond from olefins. [14]

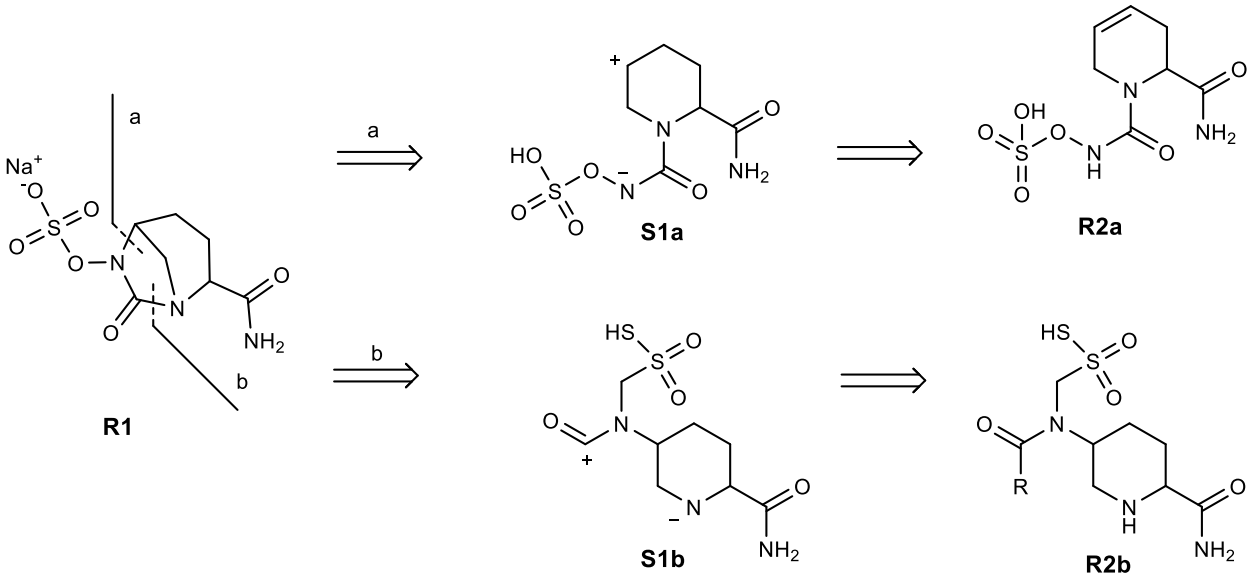


Fig. 13. Retrosynthetic analysis of Avibactam, part one.

The disconnection continued with **R2a** between the nitrogen atom from the six membered heterocycle and the carbonyl atom from the urea group. This lead to two synthons which could be easily transformed into literature known compounds (Fig. 14). **S2a** could be transformed *eg.* into **9** after adding imidazole as a leaving group for the positive polarized carbonyl atom and a functional group interconversion (FGI) of the sulfonate group to benzoyl. The synthesis of **9** was published by Jeffrey L. Romine *et. al.* from carbonyldiimidazole (CDI) and [(aminooxy)methyl]-benzene. [15]

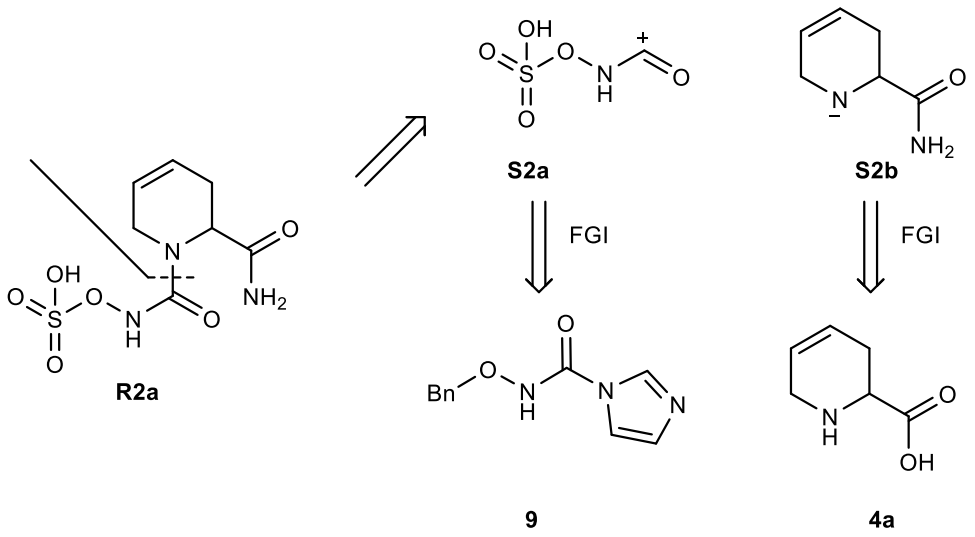


Fig. 14. Retrosynthetic analysis of Avibactam, part two.

The other synthon **S2b** could be transformed after FGI into Baikiain, (**4a**), a literature-well known compound which can be found and isolated from the plant *Baikiaea plurijuga*. [16] Several syntheses of Baikiain and its hydrochloride salt were reported by Albert W. Burgstahler *et. al.* [16], Claus Herdeis *et. al.* [17] and Edward Leete *et. al.* [18] for example.

To come back to substance **R2b** the carbonyl group must contain a good leaving group. So the residue R could be like in substance **9** imidazole. If the C-N bond is disconnected this would lead to similar synthons already discussed before and further lead to similar starting materials.

2.5.1 Synthetic route for avibactam

In this section the overall chosen synthetic route is summarized (Fig. 15). This was the "guideline" for synthesis of avibactam and to circumvent the originator patent. The results will be discussed in the next section.

The first aim was the synthesis of Baikiain (**4a**), from diethyl acetamidomalonate (**1**), and (cis)-1,4-dichloro-2-buten (**2**), which is a simple starting material and doesn't infringe with the before mentioned patents. [17] [16] [18] The next step was to make a carboxylic ester followed by the conversion into the amide. [19] This is similar to the process patent where the ester is converted into the amide. Then the idea was to use *N*-(benzyloxy)-1*H*-imidazole-1-carboxamide which was synthesized from CDI and *O*-benzylhydroxylamine for the synthesis of the urea derivative (**16**). [15] Key intermediate **16** should be cyclized by a radical or cationic cyclisation induced by 2-iodoxybenzoic acid to form the cyclic urea derivative, (**17**). [14] Sulfonation in analogy to the originator patents of the final intermediate **17** yields avibactam. [13]

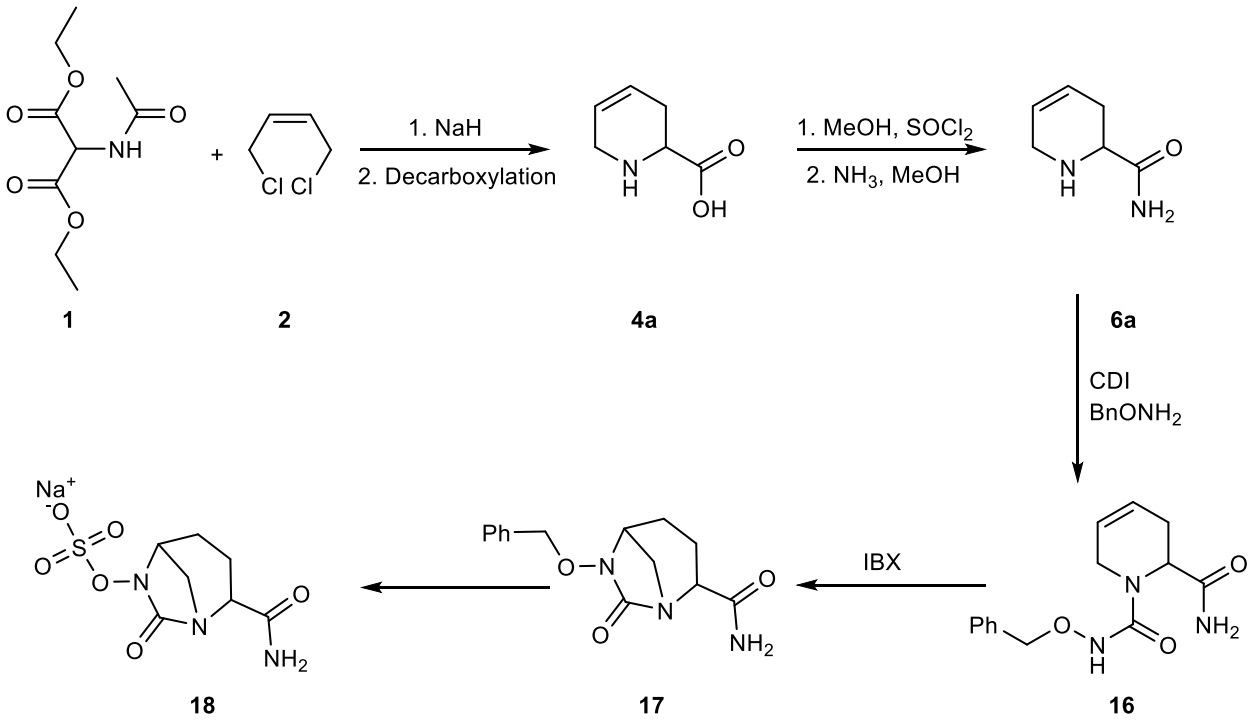


Fig. 15. "Guideline" for the synthesis of Avibactam.

2.5.2 Benefits for challenging the originator patent

A generic company can sell a generic drug when the patent and/or other exclusive rights of the originator company expires. [20] But there is the chance for a generic company to get a semi-exclusivity of 180 days where the said company can sell its generic drug together with the brand drug from the originator. This can be achieved by the first generic company who files a paragraph IV Abbreviated New Drug Application (ANDA) to the Food and Drug Administration (FDA). In order to do this the generic company must challenge the originators patent listed in the Orange Book (OB). Another requirement is that the ANDA is filed one year before the exclusivity rights of the said originator company expires. The originator patent can be challenged when the generic product is not infringing with the originators brand or the originators patent is invalid. The last requirement is that the generic company must be the first one who files the ANDA. [21]

3. Results and Discussion:

3.1 Synthesis of Baikiain hydrochloride

For the synthesis of Baikiain hydrochloride there are known reaction published by Claus Herdeis *et. al.* [17], Albert W. Burgstahler *et. al.* [16] and Edward Leete *et. al.* [18].

The cheap and commercially available diethyl acetamidomalonate (**1**) was chosen as a starting material. The two H-atoms where one was located at the acidic C-atom between the two carboxylic esters and the other one at the N-atom had relative low pK_a values. They could be easily deprotonated by a strong base like NaH, followed by a nucleophilic attack at the second starting material the (cis)-1,4-dichloro-2-buten (**2**). With the two equally positive polarized carbon atoms, **2** was an ideal reactant and it provided the heterocyclic product with the double bond which was crucial for the final reaction step (Fig. 16). [16] [18]

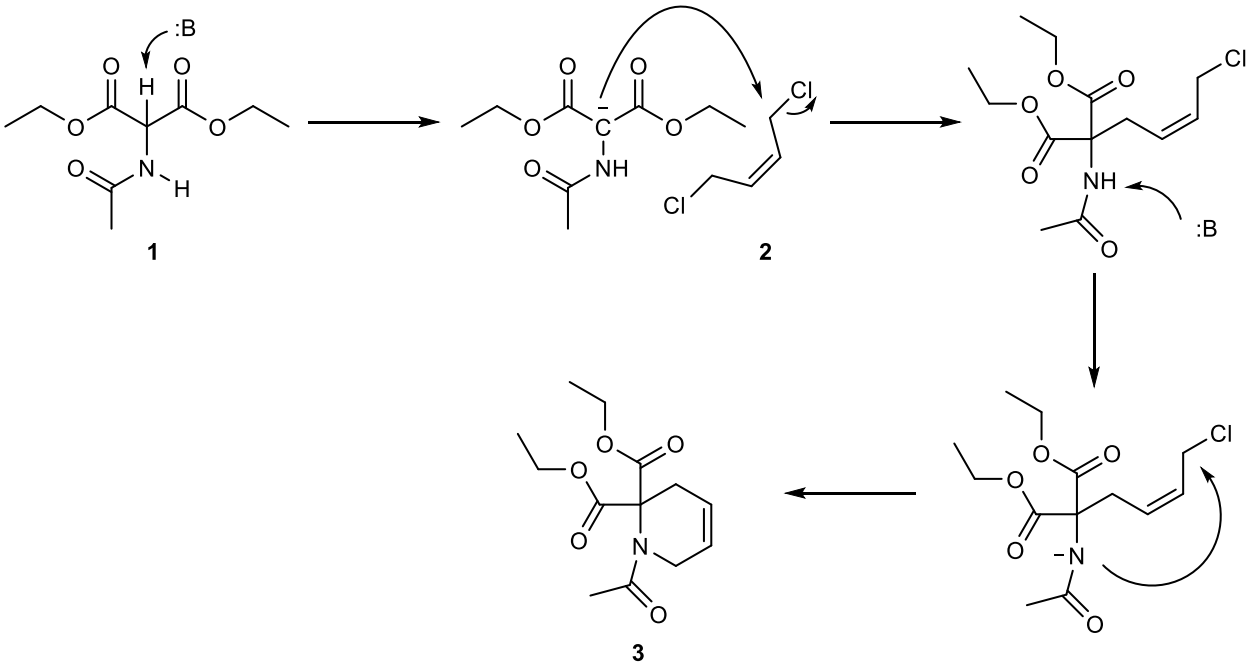


*Fig. 16: Proposed reaction mechanism for diethyl 1-acetyl-3,6-dihydropyridine-2,2(1H)-dicarboxylate (**3**).* [16]

The reaction was carried out in *N*-methyl-2-pyrrolidone (NMP) and gave the diethyl 1-acetyl-3,6-dihydropyridine-2,2(1*H*)-dicarboxylate (**3**) as a highly viscous brownish oil which was purified by silica gel chromatography. [18]

The Baikiain hydrochloride (**4**) was obtained by treating **3** with HCl and AcOH to start a decarboxylation and deacetylation reaction (Fig. 17). [17] [18]

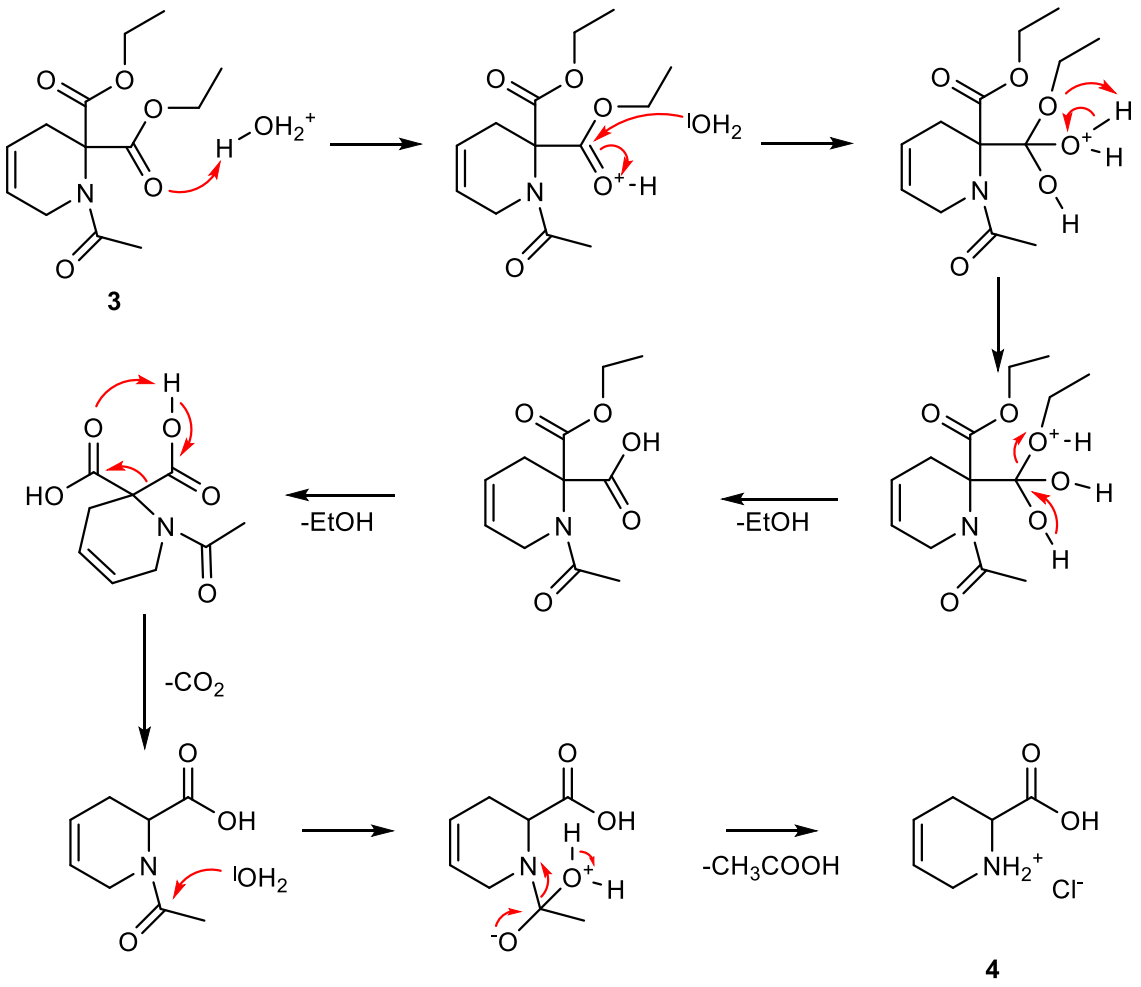


Fig. 17*: Proposed reaction mechanism for decarboxylation and deacetylation reaction of diethyl 1-acetyl-3,6-dihydropyridine-2,2(1H)-dicarboxylate (**3**) with HCl and AcOH.* [22]

After purification with active carbon and recrystallization Baikiain hydrochloride was obtained with an overall yield of 32%.

To use this reaction in an industrial process there has to be more done. First of all, increasing the overall yield. This could be achieved by replacing NMP by another solvent and reducing the formation of byproducts. The problem with NMP was, for example, that the product couldn't be extracted completely from the solvent due to its miscibility with other polar organic solvents and water. Furthermore, removing the solvent by distillation under reduced pressure wasn't an option either because of its high boiling point. [23] During the reaction control with HPLC the formation of an unwanted byproduct could be observed which was removed by silica gel chromatography.

Another very important point is that for the decarboxylation and deacetylation reaction no starting material is present. Therefore, it has to be checked that the starting material is completely converted or removed during the extraction or purification process. If diethyl acetamidomalonate is present during the decarboxylation and deacetylation step, the amino acid glycine will be formed (Fig. 18).

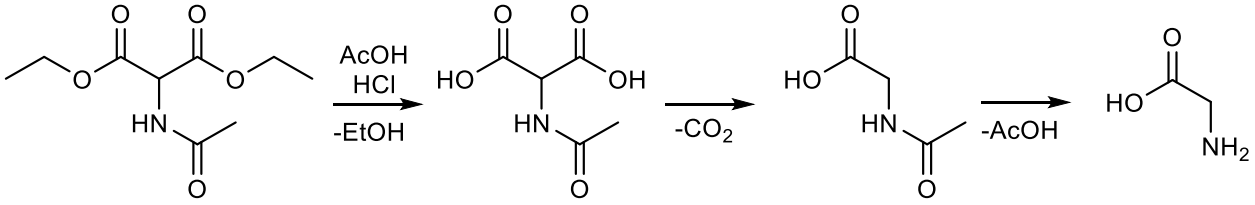


Fig. 18*: Formation of glycine by decarboxylation and deacetylation of the starting material diethyl acetamidomalonate.*

From an environmental point of view the reaction could be criticized of its low atom efficiency[4] which is 42%. This number was calculated under consideration of a perfect stoichiometric reaction but we have to keep in mind that the yield at this point was only 32%. To summarize, a lot of work has to be done to make this reaction environmental and economical profitable.

The reason why this route was chosen is that it provides a simple and small molecule (Baikiain hydrochloride) as starting material.

3.2 The use of pipecolic acid as trail material

Pipecolic acid is a commercial available substance which is very similar to Baikiain hydrochloride (Fig. 19).

Fig. 19*: Left Baikiain hydrochloride and right pipecolic aicd.*

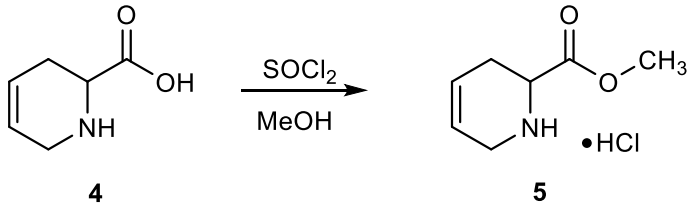
Pipecolic acid was used for the first esterification reactions, the followed conversion into the amide and experiments for the syntheses of the urea derivatives due to its similarity with Baikiain hydrochloride. When the reaction was successful with pipecolic acid the same reaction conditions

[4] Is a measure of waste generated by a reaction and is calculated by the molecular weight of the desired product divided by the total molecular weight of all products. [34]

were used for Baikiain hydrochloride. Pipecolic acid was used as model substrate for the first experiments because the overall reaction for Baikiain hydrochloride was very time consuming and the yield was very low.

Figure 2 shows all the successful reactions which worked with pipecolic acid. In the following section all the non-working reactions with pipecolic acid will be discussed. Please note, if a reaction was successful with both substances (Baikiain hydrochloride & pipecolic acid) as starting material only the Baikiain hydrochloride one will be discussed.

3.3 Esterification of Baikiain hydrochloride



4 **5**

Fig. 20*: Synthesis of Baikiain ester hydrochloride (**5**).*

In Figure 20 the synthesis of methyl 1,2,3,6-tetrahydropyridine-2-carboxylate hydrochloride (**5**) (in further text referred as Baikiain ester hydrochloride) is shown. In the first experiments it was tried to obtain the free ester. Therefore, Baikiain hydrochloride was suspended in MeOH and $SOCl_2$ was added. After 24 hours the reaction mixture was concentrated and neutralized with aqueous NaOH, $KHCO_3$ or K_2CO_3 to pH 7.5-9. The product was extracted with ethyl acetate and obtained as a brownish oil. The first problem was that the neutralization step was very sensitive which led to very different yields between 0 – 86%. The second problem was that a signal in the ^{1}H-NMR at 3.63 ppm was found which could be corresponding to dimethyl sulfite. [24] Because this impurity couldn't be removed easily due the instability of the ester and the irreproducible reaction we decided to precipitate the ester as hydrochloride in ether. This was obtained by concentration of the reaction mixture under reduced pressure until a white solid precipitated. The solid was filtered off and washed with absolute diethylether. In ether the solubility of the ester hydrochloride was low and the rest of the product precipitated. Furthermore, the impurity corresponding to the signal at 3.63 remained in the ether and the ester was obtained as hydrochloride with a high purity. [19] [25] The reaction was simple and gave the product as white crystalline solid with a good yield of 88%.

3.4 Synthesis of Baikiain amide

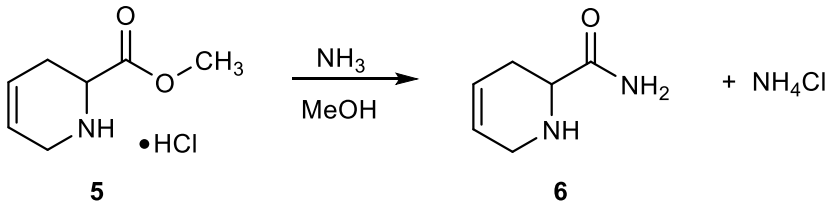


Fig. 21*: Synthesis of Baikiain amide (**6**).*

Figure 21 shows the synthesis of 1,2,3,6-tetrahydropyridine-2-carboxamide (**6**) (in further text referred as Baikiain amide). Therefore, Baikiain ester hydrochloride (**5**) was dissolved in an methanolic ammonia solution (7 M). After complete conversion the solvent was removed under reduced pressure leaving a mixture of product **6** and NH_4Cl as white crystalline solid. For the next experiments the NH_4Cl contaminated Baikiain amide was used because the effort for the separation of about 1 g product were too high and we supposed that the NH_4Cl wouldn't interfere with the next reaction step. The concentration of **6** was 76% which was measured with a quantitative ^{1}H-NMR where 1,2,4,5-tetrachloro-3-nitrobenzene was used as internal reference standard. The yield was good with 85% but the reaction time with 48 h was very long. The reaction would probably be faster if a higher pressure would be used. [19]

3.5 Synthesis of the Urea derivative

For the synthesis of the urea derivative (**16**) shown in figure 22, different approaches were pursued.

3.5.1 Direct urea derivative synthesis

The first strategy was to synthesize the urea derivative direct with the Baikiain ester (Fig. 23) because this functional group might be more favorable than the amide for the next reaction step.

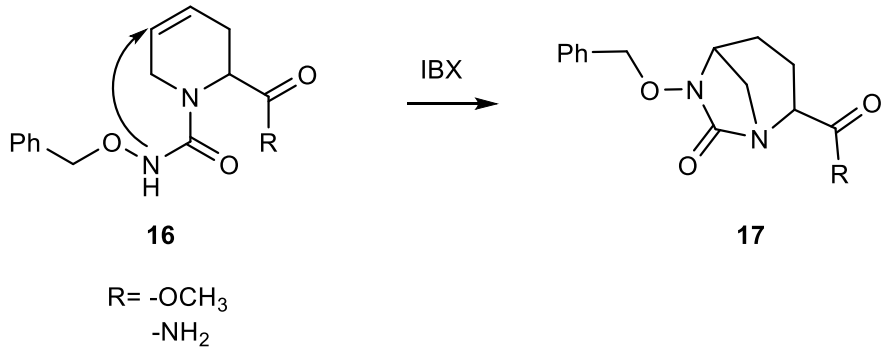


*Fig. 22: Target compound **16** (urea derivative) and next reaction step with IBX.* [14]

Where a IBX mediated cyclisation reaction should be carried out between the -NH of the urea group and the unsaturated carbon of the pyridine ring. The -NH_2 of the amide group could interfere with this reaction (Fig. 22). [14]

For the synthesis of the urea derivative with the Baikiain ester, N-(benzyloxy)-1*H*-imidazole-1-carboxamide (**9**) was synthesized *in situ* from *O*-benzylhydroxylamine hydrochloride (**7**) ($BnONH_3$.HCl) and CDI. The non-isolated intermediate **9** was added to the Baikiain ester hydrochloride which was treated with Et_3N as base in absolute tetrahydrofuran (THF). The idea was that the deprotonated nitrogen of the Baikiain ester (**5**) performs a nucleophilic attack at the carboxylic carbon atom of **9**. The problem was that a second nucleophilic attack occurred to form the cyclic product **10** (Fig. 23).

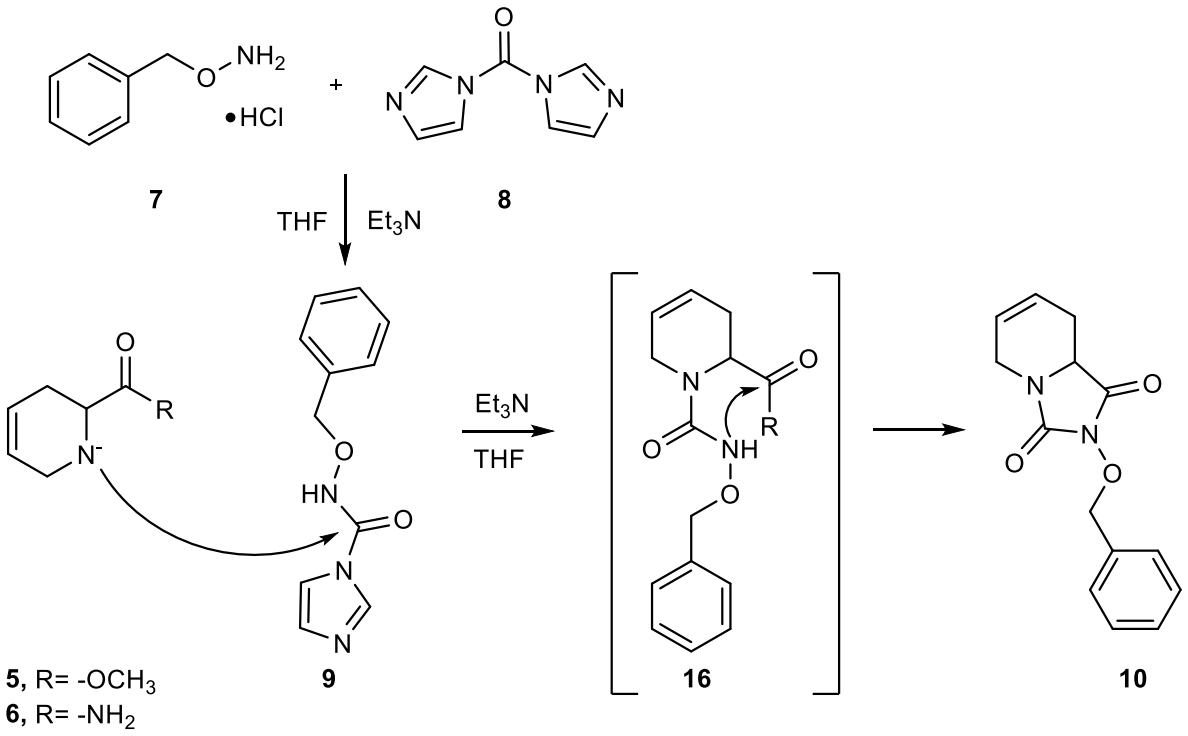


*Fig. 23: Formation of the unwanted cyclic urea-derivative **10**.*

In the ^{1}H-NMR and ^{13}C-NMR the signal for the methyl group was clearly missing. Since we already expected a second nucleophilic attack at the carboxylic atom, the reaction was repeated with Baikiain amide (**6**). The idea behind this was that amides are less electrophilic and that -NH_2 is a very bad leaving group. We thought that the nucleophilic attack can be avoided and no ring closure takes place. But still under basic conditions the nucleophilic attack occurred forming the same cyclic product **10**. Even if it is very unusual that the -NH_2 is acting as a leaving group, the small distance of both substituents to each other could be one of the reasons why the cyclisation

reaction is favored. Thermodynamically the formation of the hydantoin is energetically favorable. The yield of **10** was with the Baikiain ester (48%) better than with Baikian amide (30%). [15]

3.5.2 Synthesis of 4-nitrophenyl(benzyloxy)carbamate

In the same time, it was tried to replace *N*-(benzyloxy)-1*H*-imidazole-1-carboxamide (**9**) with a more stable compound. Like mentioned before **9** had to be synthesized *in situ* and handled carefully under inert gas conditions. Another disadvantage was that **9** had to be added very slowly to the reaction mixture to reduce the probability to form the dimer or trimer of itself (Fig. 24). [15]

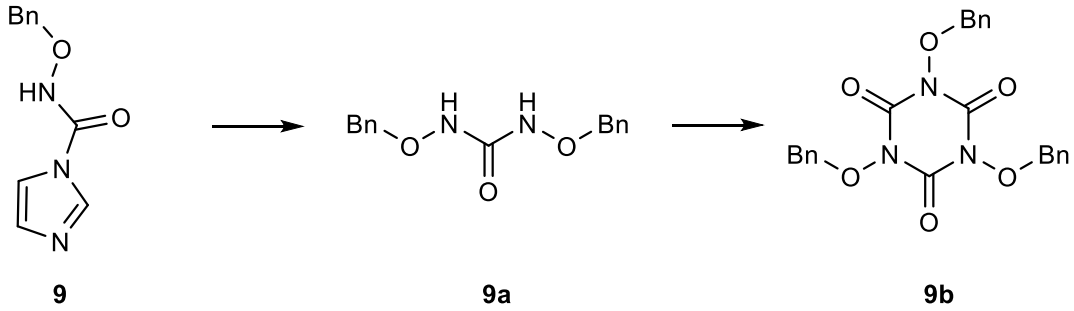


Fig. 24*: Formation of the dimer **9a** and the trimer **9b** of compound **9**.*

To overcome the disadvantage of **9**, 4-nitrophenyl(benzyloxy)carbamate (**12**) was synthesized from $BnONH_3$.HCl and 4-nitrophenyl chloroformate (**11**) with pyridine as base (Fig. 25). [26]

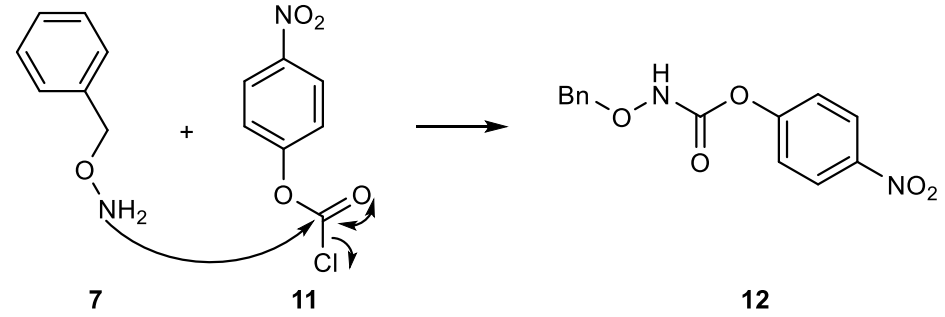


Fig. 25: Synthesis of 4-nitrophenyl(benzyloxy)carbamate (**12**).

4-Nitrophenyl(benzyloxy)carbamate (**12**) is a white crystalline solid, which was purified by recrystallization in ethyl acetate and the reaction with Baikiain amide could be performed in CH_2Cl_2 under atmospheric conditions. Absolute water free conditions were not necessary anymore which reduced the preparative effort. In both cases the product **10** was synthesized but with 4-nitrophenyl(benzyloxy)carbamate the overall reaction time was shorter. The disadvantage of **12** was the toxic byproduct nitrophenol. Furthermore, the reaction with Baikiain amide (**6**) and

the carbamate (**12**) doesn't work with Et_3N as base. Instead *N*,*N*-dimethylpyridin-4-amine (DMAP) had to be used as nucleophilic catalyst. [15] [27]

3.5.3 Urea derivative synthesis with triphosgen

Albert Defion *et. al.* [28] published the synthesis of a similar urea derivative like our target structure **16**. Instead of an unsaturated pyridine ring they used a saturated pyrrolidine. We tested their reaction conditions for the synthesis of the carbamoyl chloride intermediate **22** and the urea derivative **16a** (Fig. 26). Instead of Baikiain ester (**5**) methyl pipecolinate (**20**) was used as test substance. [28]

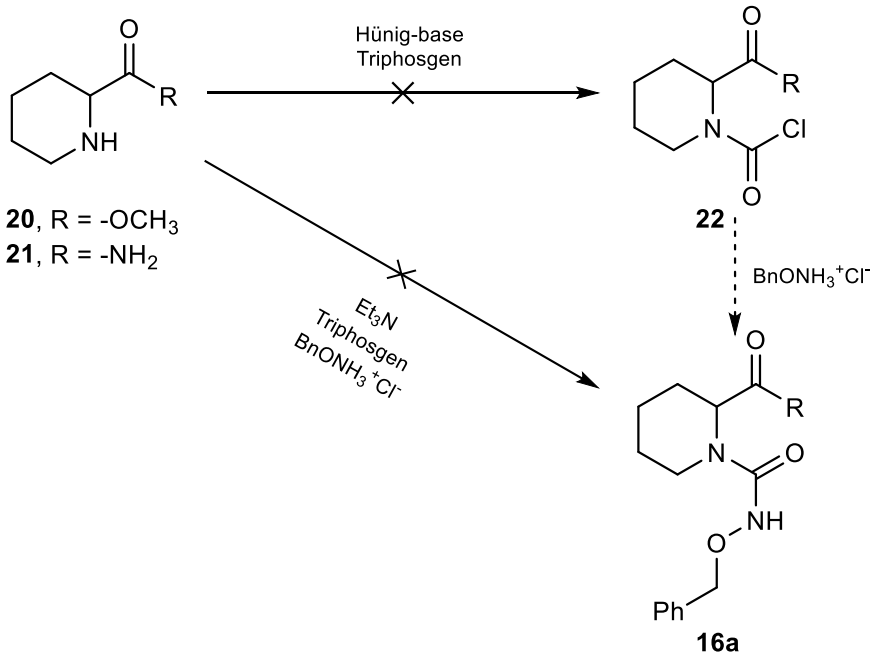


Fig. 26: Urea derivative synthesis with triphosgen.

For the synthesis of the carbamoyl chloride intermediate **22** the methyl pipecolinate (**20**) was dissolved in CH_2Cl_2 and treated with Hünig-base. Then at about 0 °C triphosgen was added and the reaction mixture stirred for two days. The starting material was consumed and a wide range of different products were synthesized but the target product couldn't be isolated. [29] For the direct synthesis of the urea derivative **16a,** Et_3N was used instead of the Hünig base and the reaction was tested this time with methyl pipecolinate (**20**) and with 2-piperidinecarboxamide (**21**) as starting materials. The reactant was treated with Et_3N in CH_2Cl_2 and then triphosgene was added. After stirring for 24 hours the $BnONH_3$.HCl was added to the reaction mixture. [28] Disappointingly the starting material was consumed and the target product couldn't be isolated.

3.5.4 Urea derivative synthesis by elimination of the highly reactive carbonyl group

Because the reaction with triphosgene didn't work and the reaction with the Baikiain ester/amide with the *N*-(benzyloxy)-1*H*-imidazole-1-carboxamide or the 4-nitrophenyl (benzyloxy)carbamate substituent always lead to the wrong cyclized product **10**, we decided to neutralize the reactive carbonyl group by steric hindrance.

The first strategy was to synthesize a more steric hindered ester **24** (Fig. 27). Again pipecolic acid was used as test substrate and it was treated as described for Baikiain ester hydrochloride in figure 27 with tert-butanol and $SOCl_2$. The reaction mixture was stirred for four days but no conversion of the reactant could be observed. [30]

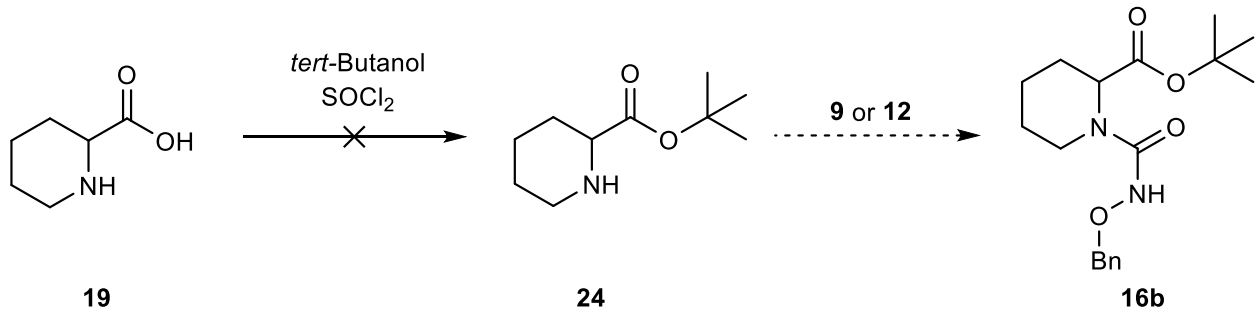


Fig. 27*: Synthesis of 2-methyl-2-propanyl 2-piperidinecarboxylate (**24**).*

If the reaction was successful, the next reaction step would have been to synthesize the urea derivative **16b** with one of the above mentioned reagents **9** or **12**.

Because of time constrains and the fact that the esterification with *tert*-butanol was unsuccessful we decided to remove the carbonyl group of the Baikian ester by reducing it with $LiAlH_4$ to an alcohol. The reaction worked with 89% yield (*Fig.* 28). [31]

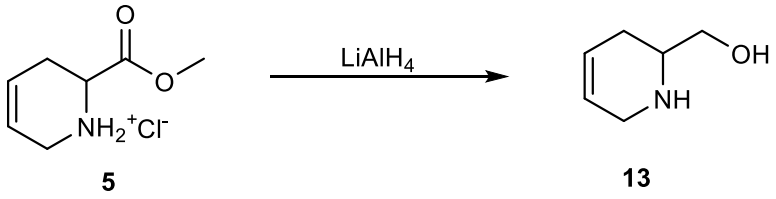


Fig. 28*: Reduction of Baikiain ester hydrochloride (**5**).*

In the next step the hydroxyl functionality of **13** was protected with triisopropylsilylchlorid (TiPS-Cl). The purpose of this bulky substituent was to prevent a cyclisation of the hydroxyl functionality onto the urea moiety which will be formed in the next reaction step.

The (1,2,3,6-tetrahydropyridin-2-yl)methanol (**13**) was dissolved in dimethylformamide (DMF) with TiPS-Cl and DMAP. The reaction mixture was stirred for six days until a conversion of the reactant could be observed. [32]

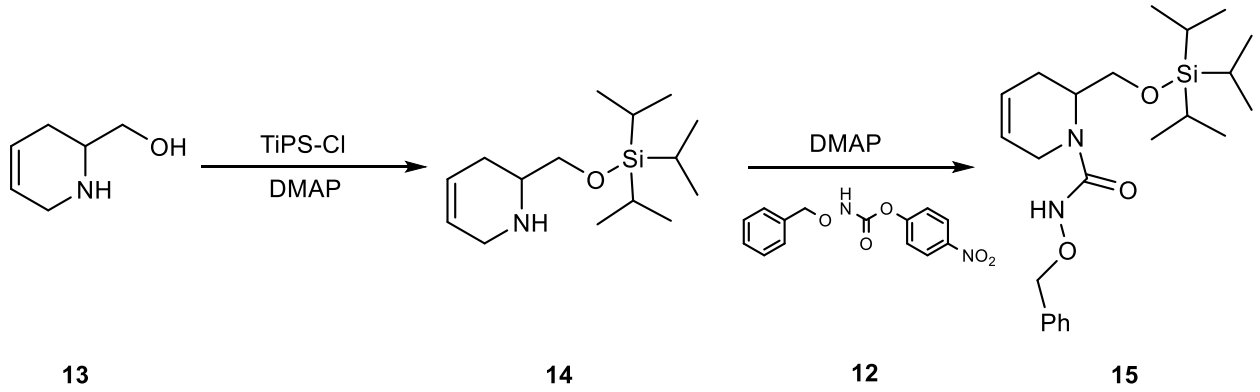


Fig. 29*: Successful synthesis of the urea derivative* ***15****.*

Subsequently the protected alcohol **14**, was reacted with 4-nitrophenyl(benzyloxy)carbamate (**12**) (Fig. 29). Instead of Et_3N we used DMAP again as base and catalyst. The reactants were added together and the mixture was stirred for one hour. [27] After extraction the urea derivative **15** was obtained as colorless oil with a yield of 65%.

3.6 IBX mediated cyclisation reaction

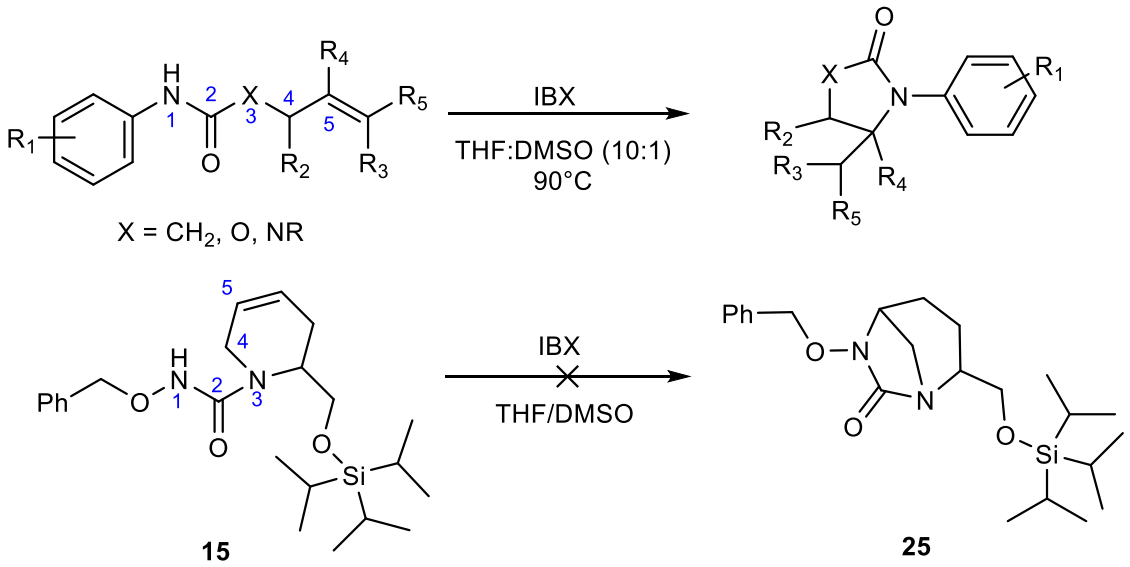


Fig. 30*: Comparison of a) Overall IBX mediated cyclisation reaction by* K. C. Nicolaou *et. al., modified from* [14] with b) *IBX mediated cyclisation reaction of the urea derivative* ***15***

The cyclisation of the urea derivative **15**, is the key step of our synthetic approach. The reaction would lead to the core structure of avibactam in a single step yielding the advanced intermediate **25**. K. C. Nicolaou *et. al.* [14] introduced IBX mediated cyclisation reactions where a N-C bonds are formed from amides and unsaturated carbon atoms to synthesize cyclized urea derivatives by a single electron transfer (SET) mechanism. Therefore, the urea derivative was suspended in THF/DMSO with the IBX. Identical reaction conditions to K. C. Nicolaou's work were used for the reaction (*Fig.* 30). The concentration of the reactant was 0.025 M and two equivalents of IBX were used. The reaction mixture was heated to reflux and after 4.5 h another two equivalents of IBX were added. After two days of stirring under reflux and another two days at room temperature the starting material was consumed, but no product could be found. It is important to mention here that only one experiment could be performed due the lack of time for this project. [14]

4. Conclusion

A lot of work has to be done to create a new synthetic route for the synthesis of avibactam. Baikiain hydrochloride is an ideal starting material since it resembles a core structural element of avibactam, the heterocyclic piperidine ring system and the amide functionality. However, the low yield of the overall synthesis of Baikiain hydrochloride and its low atom efficiency have to be improved for an industrial process. The second problem is the (cis)-1,4-dichlor-2-buten with its toxic and carcinogenic properties.

The formation of the unwanted cyclic urea derivative **10** were the NH_2 of the amide group is acting as leaving group was a very surprising observation (Fig. 23). This lead to an unexpected result and a new synthetic approach was started where the electrophilic carbonyl group was reduced to an alcohol. Because no Baikian hydrochloride was left, the Baikiain ester hydrochloride was reduced to the alcohol with $LiAlH_4$. To avoid the esterification step it could be tried to reduce the Baikiain hydrochloride direct to the alcohol. To avoid the intramolecular cyclisation completely the bulky TiPS-Cl was used as protecting group for the alcohol.

With the highly reactive carbonyl group removed from Baikiain we were able to synthesize the urea-derivative **15** shown in figure 29. The disadvantage in this reaction was the formation of nitrophenol which is a toxic and carcinogenic compound. If we consider such a reaction in an overall up scaled process the use of reagent **9** would be a better choice. In this case imidazole would be formed which is less harmful than nitrophenol.

Due to the limited time for this project only one experiment could be performed for the IBX mediated cyclisation reaction which was unsuccessful.

Avibactam has two stereo centers. The first one is at the carbon atom which contains the amide group and the other one is the carbon atom next to the sulfonated nitrogen atom (Fig. 3). The stereochemistry of the target molecule was neglected during the project, due to the short time frame. The main focus was laid on an overall successful racemic synthesis. However, it was considered that the enantiomers of Baikiain could be separated by chiral resolution.

5. Experimental

5.1 General

All solvents were purchased from Sigma Aldrich with HPLC purity if not stated otherwise.

Table 1: Overview Chemicals

Substance	Purchased from	CAS Number	Concentration
O-Benzylhydroxylamine hydrochloride	Fluorochem Aldrich	2687-43-6	--- 99%
1,1'-Carbonyldiimidazole	Aldrich	530-62-1	---
(cis)-1,4-Dichlor-2-buten	Aldrich	1476-11-5	95%
Diethyl acetamidomalonate	Aldrich	1068-90-2	98%
4-(Dimethylamino)pyridine	Fluka Analytical	1122-58-3	≥ 99%
2-Iodoxybenzoic acid	Aldrich	61717-85-6	45%
Lithiumaluminum hydride	Aldrich	16853-85-3	95%
4-Nitrophenyl chloroformate	Aldrich	7693-46-1	96%
Sodium hydride	Aldrich	7646-69-7	60%
Triisopropylsilyl chloride	Aldrich	13154-24-0	97%
Thionyl chloride	Aldrich	7719-09-7	≥ 99%

5.2 Chromatography

Thin layer chromatography (TLC) or high performance liquid chromatography (HPLC) were used for reaction control.

5.2.1 TLC

Silicagel 60 F_{254} Aluminium sheets 5 x 7.5 cm

A potassium permanganate solution (3 g $KMnO_4$, 20 g K_2CO_3, 5 ml NaOH (5%), 300 ml H_2O), the Hanessian stain or a ninhydrin solution (1.5 g ninhydrin, 5 ml AcOH, 500 ml EtOH (95 %)) were used to dye the TLC.

5.2.2 HPLC-System

Device: Agilent Technologies 1200 Series

Column: YMC-Triart C18, 150*4.6 mm, 3 µm

Eluents: **A**: 1000 g H_2O + 3.884 g sulfamic acid/ **B**: 250 g H_2O + 3.884 g sulfamic acid + 586.5 g acetonitrile

Method: Gradient: 0 min 97% A, 5 min 40% A, 9 min 0% A, 12 min 0% A, 12.01 min 97% A, 16 min 97% A

Detector:	UV/VIS, 210 nm, BW 4, Ref 360/100, Peak width: > 0.1 min, Slit: 4 nm
Flow:	1.0 ml/min
Injection Volume:	5 µl
Oven temperature:	40 °C
Solvent for samples:	Acetonitrile/ H_2O 1:1

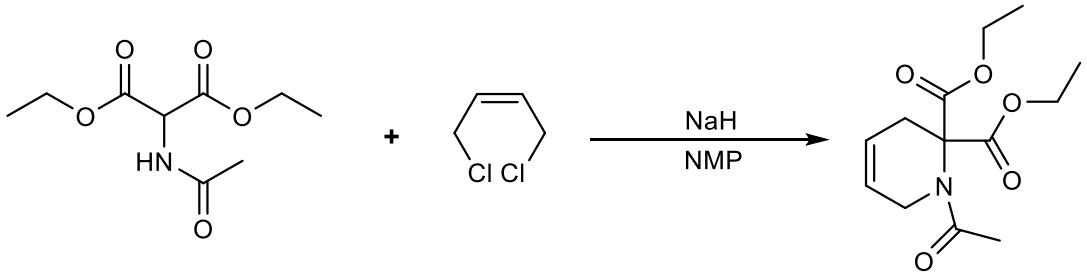
5.3 NMR

Device:	Bruker, 400 MHz
Software:	Topspin 3.1

5.4 Experiments with pipecolic acid

All the reactions with pipecolic acid mentioned in figure 2 work equivalent with Baikiain hydrochloride. Therefore, the experiments in which pipecolic acid was used will not be described because the procedure is the same as for Baikiain hydrochloride.

5.5 Diethyl 1-acetyl-3,6-dihydropyridine-2,2(1H)-dicarboxylate (3)



1 **2** **3**

Diethyl acetamidomalonate (**1**) (10.0 g, 46.0 mmol, 1.0 eq.) was dissolved in NMP (50 ml) in a dried 100 ml 3-necked flask under inert gas. The solution was cooled with an ice-bath to 2 – 5 °C and NaH (1.9 g, 60%, 47.5 mmol, 1.0 eq.) was added in small portions. Afterwards (cis)-1,4-dichlor-2-butene (**2**) (5.7 g, 46.0 mmol, 1.0 eq.) was added rapidly to the stirred reaction mixture. After one hour the second equivalent of NaH (1.9 g, 60%, 47.5 mmol, 1.0 eq.) was added in small portions. Then the reaction mixture was stirred over night at room temperature.

The next day the reaction mixture was cooled with an ice-bath and HCl (120 ml, 1 M) was added. The resulting solution was extracted with ethyl acetate (3 x ~100 ml) and washed with water and brine. The combined organic layers were dried over Na_2SO_4 and evaporated under vacuum.

The crude product was purified by a silica gel 60 (120 g) column chromatography (Heptane/EtOAc 1:1). The yield of **3** as orange, high viscous, oil like liquid was 7.9 g (29.3 mmol, 64%).

^{1}H-NMR (400 MHz, CD_3OD) δ [ppm] = 5.84 (m, 1 H, C***H***=CH), 5.68 (m, 1 H, CH=C***H***), 4.22 (m, 4 H, O-C***H$_2$***-), 4.01 (m, 2 H, N-C***H$_2$***-), 2.85 (m, 2 H, CH-C***H$_2$***-C), 2.16 (s, 3 H, -CO-C**H$_3$**), 1.26 (m, 6 H, -CH_2-C***H$_3$***)

HPLC: τ_R = 8.4 min

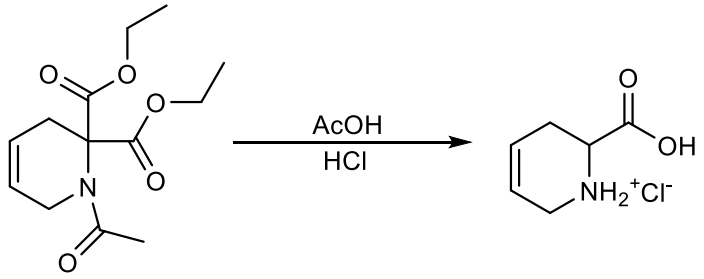
R_f (Heptane/EtOAc 1:1) = 0.30

Literature data for comparison from S. Kotha and K. Singh: [33]

^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm] = 1.29 (6H, t, J = 7.1 Hz), 2.10 (3H, s), 2.86–2.89 (2H, m), 4.03–4.05 (2H, m), 4.30 (4H, q, J = 7.0 Hz), 5.68–5.72 (1H, m), 5.84–5.90 (1H, m). [33]

^{13}C NMR (100.6 MHz, $CDCl_3$) δ [ppm] = 14.1, 22.4, 32.3, 45.6, 62.2, 66.7, 123.0, 123.1, 168.3, 172.7. [33]

5.6 Baikiain hydrochloride (4)



3 **4**

Compound **3** (7.9 g, 29.3 mmol, 1.0 eq.) was submitted into a 250 ml round-bottom flask and dissolved in AcOH (75 ml, conc.), HCl (45 ml, conc.) and the resulting yellow solution was stirred under reflux for three hours. Once the mixture was cooled to room temperature, the acids were removed under reduced pressure.

The crude orange/ brown solid was dissolved in water and stirred with activated carbon and filtrated. The resulting colorless liquid was evaporated under vacuum at 50 °C and white crystals were obtained and recrystallized in MeOH/EtOH 2.5:1.

The yield of Baikiain hydrochloride (**4**) as white crystals was 2.4 g (14.7 mmol, 50%).

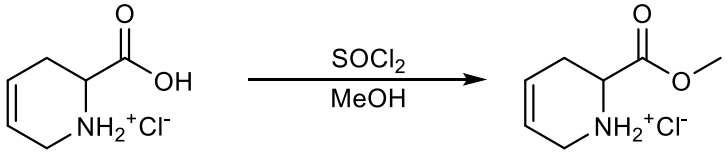
^{1}H-NMR (400 MHz, CD_3OD) δ [ppm] = 6.01 (m, 1 H, -CH_2-C***H***=CH-), 5.81 (m, 1 H, -CH=C***H***-CH_2-N-), 4.18 (dd, 1 H, *J* = 10.7, 5.4 Hz, -C***H***-), 3.76 (m, 2 H, -C**H_2**-N-), 2.76 (m, 1 H, -C**H_2**-CH), 2.52 (m, 1 H, -C**H_2**-CH-).

^{13}C-NMR (400 MHz; CD_3OD) δ [ppm] = 171.1 (***C***=O), 125.8 (-CH=***C***H-CH_2-N-), 121.5 (-CH_2-***C***H=CH-), 54.4 (-CH_2-***C***H-N-), 43.2 (=CH-***C***H_2-N-), 26.4 (=HC-***C***H_2-CH-).

HPLC/MS: τ_R = 2.2 min; m/z = 128 (M+H);

R_f (*i*-PrOH/H_2O/AcOH 6:1:1) = 0.43

5.7 Baikiain ester hydrochloride (5)



4 **5**

Compound **4** (0.49 g, 3.00 mmol, 1.0 eq.) was charged in a dried 25 ml round-bottom flask and suspended in MeOH (10 ml, anhyd.) under inert gas and cooled with an ice-bath. Thionyl chloride (0.44 ml, 1.67 g/cm^3, 6.18 mmol, 2.1 eq.) was added slowly and after complete addition the reaction mixture was stirred 24 h at room temperature.

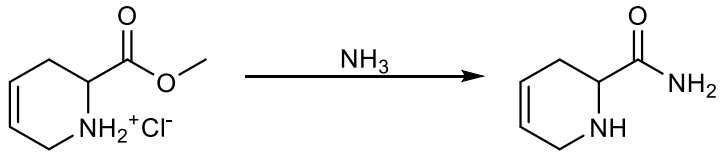
The clear colorless solution was concentrated in vacuum until a white solid precipitated. The precipitate was filtrated and washed with diethyl ether (anhyd.). The yield of Baikiain ester hydrochloride (**5**) as white crystals was 0.47 g (2.65 mmol, 88%)

^{1}H-NMR (400 MHz, CD_3OD) δ [ppm] = 6.00 (m, 1 H, -CH_2-C***H***=CH-), 5.82 (m, 1 H, -CH=C***H***-CH_2-N-), 4.28 (dd, 1 H, *J* = 10.6, 5.3 Hz, -C***H***-), 3.88 (s, 3 H, O-C***H$_3$***), 3.78 (m, 2 H, -C**H$_2$**-N-), 2.74 (m, 1 H, -C**H$_2$**-CH), 2.53 (m, 1 H, -C**H$_2$**-CH-).

^{13}C-NMR (400 MHz; CD_3OD) δ [ppm] = 170.2 (***C***=O), 125.4 (-CH=***C***H-CH_2-N-), 121.4 (-CH_2-***C***H=CH-), 54.3 (-CH_2-***C***H-N-), 53.9 (-O***C***H_3), 43.2 (=CH-***C***H_2-N-), 26.1 (=HC-***C***H_2-CH-).

R_f (*i*-PrOH/H_2O/AcOH 6:1:1) = 0.45

5.8 Baikiain amide (6)



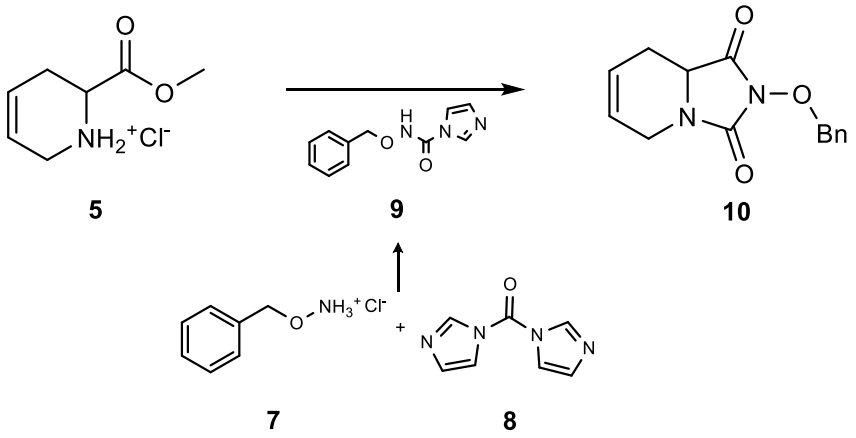
5 **6**

Compound **5** (1.14 g, 6.97 mmol, 1.0 eq.) was charged in a 100 ml round-bottom flask and dissolved in NH_3/MeOH (50 ml, 7 M). The reaction mixture was allowed to stand for 48 h. Afterwards the solvent was removed in vacuum and the yield of Baikiain amide (**6**) as white solid was 0.98 g with a purity of 76.0% (5.90 mmol, 85%).

^{1}H-NMR (400 MHz, CD_3OD) δ [ppm] = 6.00 (m, 1 H, -CH_2-C***H***=CH-), 5.81 (m, 1 H, -CH=C***H***-CH_2-N-), 4.28 (dd, 1 H, *J* = 11.3, 4.9 Hz, -C***H***-), 3.72 (m, 2 H, -C**H$_2$**-N-), 2.71 (m, 1 H, -C**H$_2$**-CH), 2.45 (m, 1 H, -C**H$_2$**-CH-).

^{13}C-NMR (400 MHz, CD_3OD) δ [ppm] = 172.2 (***C***=O), 125.7 (-CH=***C***H-CH_2-N-), 121.7 (-CH_2-***C***H=CH-), 55.2 (-CH_2-***C***H-N-), 43.2 (=CH-***C***H_2-N-), 27.6 (=HC-***C***H_2-CH-).

5.9 2-(Benzyloxy)-8,8a-dihydroimidazo[1,5-a]pyridine-1,3(2H,5H)-dione (10) from Baikiain ester hydrochloride



THF (8 ml, anhyd.) and Et_3N (0.23 ml, 0.73 g/cm^3, 1.66 mmol, 1.0 eq.) were added to *O*-Benzylhydroxylamine hydrochloride (**7**) (262 mg, 1.64 mmol, 1.0 eq.) in a dried round-bottom flask and stirred for 10 min under inert gas. Then the solution was vacuum filtrated under an inert gas flow and the filter cake was washed two times with THF (5 ml, anhyd.). The filtrate was added dropwise to a solution of CDI, (264 mg, 1.63 mmol, 1.0 eq.) and THF (13 ml, anhyd.) under inert gas at 0 °C and then stirred for 60 min at 0 °C.

Afterwards Baikiain ester hydrochloride (**5**) (313 mg, 1.76 mmol, 1.1 eq.) dissolved in CH_2Cl_2 (13 ml) with Et_3N (0.24 ml, 0.73 g/cm^3, 1.73 mmol, 1.1 eq.) was added to the reaction mixture and stirred for another 60 min at room temperature.

Then the reaction mixture was diluted with EtOAc (10 ml) and washed with water and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuum until a solid precipitated.

The crude product was purified by a silica gel 60 (20 g) column chromatography (Heptane/EtOAc 1:4) and afterwards dissolved in a very small amount of ethyl acetate and precipitated in heptane. The yield of **8** as white crystalline solid was 200 mg (0.77 mmol, 48%).

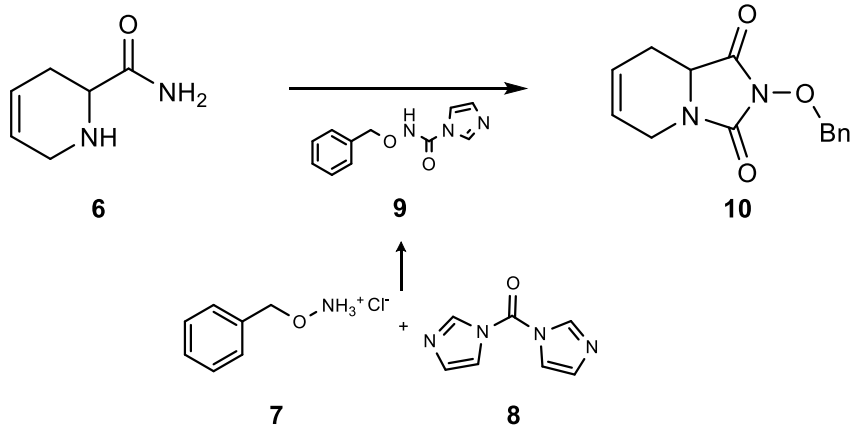
^{1}H-NMR (400 MHz, $CDCl_3$) δ [ppm] = 7.51 (m, 2 H, ***H***$_{Ar}$), 7.38 (m, 3 H, ***H***$_{Ar}$), 5.85 (m, 1 H, -CH_2-C***H***=CH-), 5.78 (m, 1 H, -CH=C***H***-CH_2-N-), 5.16 (s, 2 H, -O-C***H$_2$***-Ph), 4.31 (m, 1 H, =CH-C**H$_2$**-N-), 3.87 (dd, 1 H, *J* = 10.9, 5.3 Hz, -CH_2-C***H***=CH-), 3.68 (m, 1 H, =CH-C**H$_2$**-N-), 2.56 (m, 1 H, =HC-C***H$_2$***-CH-), 2.07 (m, 1 H, =HC-C***H$_2$***-CH-).

^{13}C-NMR (400 MHz, $CDCl_3$) δ [ppm] = 167.6 (-CH-***C***O-N-), 151.6 (-N-***C***O-N-), 133.6 (***C***$_{Ar.}$), 130.2 (***C***$_{Ar.}$), 129.5 (***C***$_{Ar.}$), 128.6 (***C***$_{Ar.}$), 123.6 (-CH=***C***H-CH_2-N-), 122.5 (-CH_2-***C***H=CH-), 79.5 (-O-***C***H_2-Ph), 51.7 (-CH_2-***C***H-N-), 39.2 (=CH-***C***H_2-N-), 25.9 (=HC-***C***H_2-CH-).

HPLC/MS: τ_R = 9.0 min; m/z = 259.1 (M+H), 517.1 (2M+H)

R_f (Heptane/EtOAc 1:4) = 0.67

5.10 2-(Benzyloxy)-8,8a-dihydroimidazo[1,5-a]pyridine-1,3(2H,5H)-dione (10) from Baikiain amide



THF (6 ml, anhyd.) and Et_3N (50 µl, 0.73 g/cm^3, 0.36 mmol, 1.1 eq.) were added to *O*-benzylhydroxylamine hydrochloride (**7**) (57 mg, 0.36 mmol, 1.1 eq.) in a dried round-bottom flask and stirred for 10 min under inert gas. Then the solution was vacuum filtrated under an inert gas flow and the filter cake was washed three times with THF (2 ml, anhyd.). The filtrate was added dropwise to a solution of CDI (55 mg, 0.34 mmol, 1.0 eq.) and THF (6 ml, anhyd.) under inert gas at 0 °C and then stirred for 45 min at 0 °C.

Afterwards Baikiain amide (**6**) (42 mg, 0.33 mmol, 1.0 eq.) suspended in CH_2Cl_2 (5 ml) was added to the reaction mixture and stirred for another 45 min at room temperature.

Then the reaction mixture was diluted with ethyl acetate (5 ml) and washed with water and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuum until a solid precipitated.

The crude product was purified by a silica gel 60 (4 g) column chromatography (Heptane/EtOAc 1:4). The yield of **8**, as white crystalline solid was 26 mg (0.10 mmol, 30%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ [ppm] = 7.50 (m, 2 H, ***H***$_{Ar}$), 7.37 (m, 3 H, ***H***$_{Ar}$), 5.84 (m, 1 H, -CH_2-C***H***=CH-), 5.77 (m, 1 H, -CH=C***H***-CH_2-N-), 5.15 (s, 2 H, -O-C***H$_2$***-Ph), 4.30 (m, 1 H, =CH-C**H$_2$**-N-), 3.86

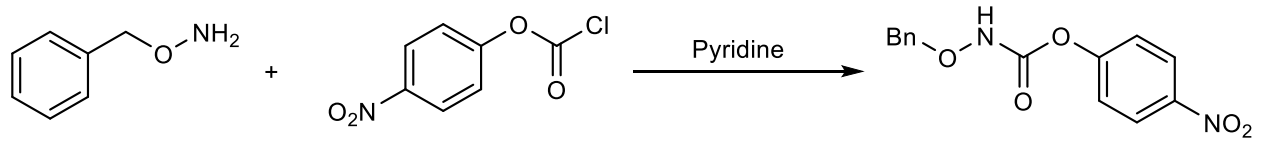
(dd, 1 H, *J* = 10.8, 5.2 Hz, -CH_2-C***H***=CH-), 3.67 (m, 1 H, =CH-C**H_2**-N-), 2.55 (m, 1 H, =HC-C***H_2***-CH-), 2.06 (m, 1 H, =HC-C***H_2***-CH-).

^{13}C-NMR (400 MHz, $CDCl_3$) δ [ppm] = 167.6 (-CH-***C***O-N-), 151.6 (-N-***C***O-N-), 133.6 (***C***$_{Ar.}$), 130.2 (***C***$_{Ar.}$), 129.5 (***C***$_{Ar.}$), 128.6 (***C***$_{Ar.}$), 123.6 (-CH=***C***H-CH_2-N-), 122.5 (-CH_2-***C***H=CH-), 79.5 (-O-***C***H_2-Ph), 51.7 (-CH_2-***C***H-N-), 39.2 (=CH-***C***H_2-N-), 25.9 (=HC-***C***H_2-CH-).

HPLC/MS: τ_R = 9.0 min; m/z = 259.1 (M+H), 517.1 (2M+H)

R_f (Heptane/EtOAc 1:4) = 0.66

5.11 4-Nitrophenyl (benzyloxy)carbamate (12)



7 **11** **12**

Pyridine (2.7 ml, 0.98 g/cm^3, 33.5 mmol, 1.0 eq.) and CH_2Cl_2 (65 ml) were added to *O*-benzylhydroxylamine hydrochloride (**7**) (5.28 g, 33.1 mmol, 1.0 eq.) in a three-necked flask and stirred at room temperature. Then 4-nitrophenyl chloroformate (**11**) (6.65 g, 33.0 mmol, 1.0 eq.) dissolved in CH_2Cl_2 (40 ml) was added via dropping funnel and the reaction mixture was stirred six hours under reflux.

After cooling to room temperature the reaction mixture was diluted with CH_2Cl_2 (60 ml) and washed sequentially with HCl (100 ml, 1 M), H_2O (100 ml), $NaHCO_3$ (100 ml, 1 M), H_2O (100 ml) and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuum until a solid precipitated. The crude product was recrystallized with ethyl acetate. The yield of **12** as white crystalline solid was 6.46 g (22.4 mmol, 67.9%).

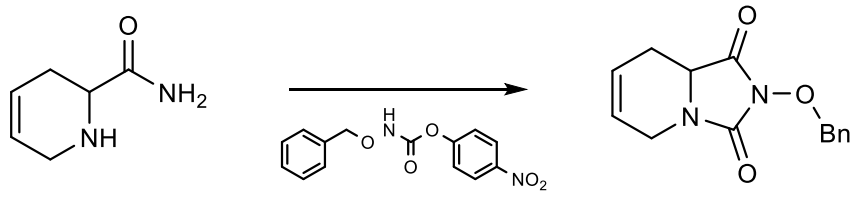
^{1}H-NMR (400 MHz, $CDCl_3$) δ [ppm] = 8.24 (m, 2 H, ***H***$_{Ar}$), 7.83 (s, 1 H, N***H***), 7.41 (m, 5 H, ***H***$_{Ar}$), 7.31 (m, 2 H, ***H***$_{Ar}$), 4.98 (s, 2 H, C***H_2***)

^{13}C-NMR (400 MHz, $CDCl_3$) δ [ppm] = 155.2 (-N-***C***O-O-), 145.3 (***C***$_{Ar}$), 134.9 (***C***$_{Ar}$), 129.4 (***C***$_{Ar}$), 128.8 (***C***$_{Ar}$), 125.3 (***C***$_{Ar}$), 122.0 (***C***$_{Ar}$), 79.2 (-***C***H_2).

HPLC: τ_R = 10.3 min

R_f (Hexane/EtOAc 1:1) = 0.66

5.12 2-(Benzyloxy)-8,8a-dihydroimidazo[1,5-a]pyridine-1,3(2H,5H)-dione (10) with 4-nitrophenyl (benzyloxy)carbamate



6 **12** **10**

Baikiain amide (**6**) (131 mg, 76%, 0.789 mmol, 1.0 eq.) and DMAP (107 mg, 0.876 mmol, 1.1 eq.) were charged in a reaction vial and suspended in CH_2Cl_2 (5 ml). Then 4-nitrophenyl (benzyloxy)carbamate (**12**) (224 mg, 0.777 mmol, 1.0 eq.) was added to the reaction mixture and stirred at room temperature for two hours.

Afterwards the reaction mixture was extracted with two times with NaOH (~10 ml, 1 M) and the organic layer was dried with Na_2SO_4 and concentrated in vacuum. The crude product was purified by a silica gel 60 (10 g) column chromatography (Hexane/EtOAc/MeOH 14:5:1). The yield of **10** as white crystalline solid was 76 mg (0.294 mmol, 37%).

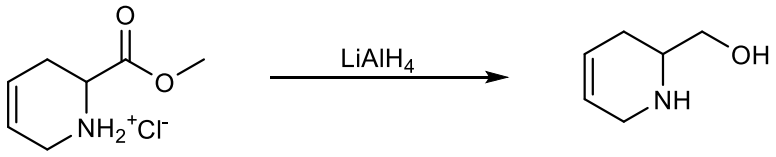
^{1}H-NMR (400 MHz, MeOD) δ [ppm] = 7.49 (m, 2 H, ***H***$_{Ar}$), 7.37 (m, 3 H, ***H***$_{Ar}$), 5.85 (m, 1 H, -CH_2-C***H***=CH-), 5.79 (m, 1 H, -CH=C***H***-CH_2-N-), 5.10 (s, 2 H, -O-C***H$_2$***-Ph), 4.21 (m, 1 H, =CH-C**H$_2$**-N-), 4.01 (dd, 1 H, *J* = 10.9, 5.3 Hz, -CH_2-C***H***=CH-), 3.68 (m, 1 H, =CH-C**H$_2$**-N-), 2.50 (m, 1 H, =HC-C***H$_2$***-CH-), 2.09 (m, 1 H, =HC-C***H$_2$***-CH-).

^{13}C-NMR (400 MHz, MeOD) δ [ppm] = 169.6 (-CH-***C***O-N-), 153.0 (-N-***C***O-N-), 135.1 (***C***$_{Ar.}$), 131.2 (***C***$_{Ar.}$), 130.3 (***C***$_{Ar.}$), 129.5 (***C***$_{Ar.}$), 124.4 (-CH=***C***H-CH_2-N-), 123.5 (-CH_2-***C***H=CH-), 80.3 (-O-***C***H_2-Ph), 52.9 (-CH_2-***C***H-N-), 39.9 (=CH-***C***H_2-N-), 26.5 (=HC-***C***H_2-CH-).

HPLC: τ_R = 9.3 min

R_f (Hexane/EtOAc/MeOH 14:5:1) = 0.75

5.13 (1,2,3,6-Tetrahydropyridin-2-yl)methanol (13)



5 **13**

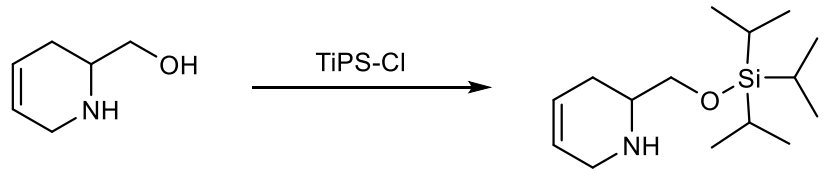
$LiAlH_4$ (161 mg, 4.24 mmol, 3.7 eq.) was suspended in THF (15 ml, anhyd.) and at 0 °C Baikiain ester hydrochloride (**5**) (205 mg, 1.15 mmol, 1.0 eq.) was added in small portions. The reaction mixture was stirred under reflux for three hours. Afterwards it was again cooled to 0 °C and a Na_2SO_4- solution (~2 ml, saturated) was slowly added. The reaction mixture was vacuum filtrated and the filter cake was washed with methyl *tert*-butyl ether. The filtrate was dried with Na_2SO_4 and the organic solvent removed under reduced pressure. The yield of **13** as a white solid was 115 mg (1.02 mmol, 89%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ [ppm] = 5.75 (m, 2 H, C***H***=C***H***), 3.66 (dd, 1 H, *J* = 10.8, 3.7 Hz, -C***H$_2$***-OH), 3.43 (m, 1 H, -C***H$_2$***-OH), 3.39 (m, 2 H, =CH-C**H$_2$**-N-), 2.91 (dddd,1 H, *J* = 8.4, 9.6, 4.2, 4.1 Hz, -CH_2-C***H***(CH_2)-N-), 1.96 (m, 1 H, =HC-C***H$_2$***-CH-), 1.87 (m, 1 H, =HC-C***H$_2$***-CH-).

^{13}C-NMR (400 MHz, $CDCl_3$) δ [ppm] = 127.0 (-CH=***C***H-CH_2-N-), 125.0 (-CH_2-***C***H=CH-), 65.9 (***C***H_2-OH), 54.1 (-CH_2-***C***H(CH_2)-N-), 44.7 (=CH-***C***H_2-N-), 27.6 (=HC-***C***H_2-CH-).

R_f (EtOAc/MeOH/H_2O/NH_3 aq. Solution (conc.) 4:2:2:0.1) = 0.22

5.14 2-(((Triisopropylsilyl)oxy)methyl)-1,2,3,6-tetrahydropyridine (14)



13 **14**

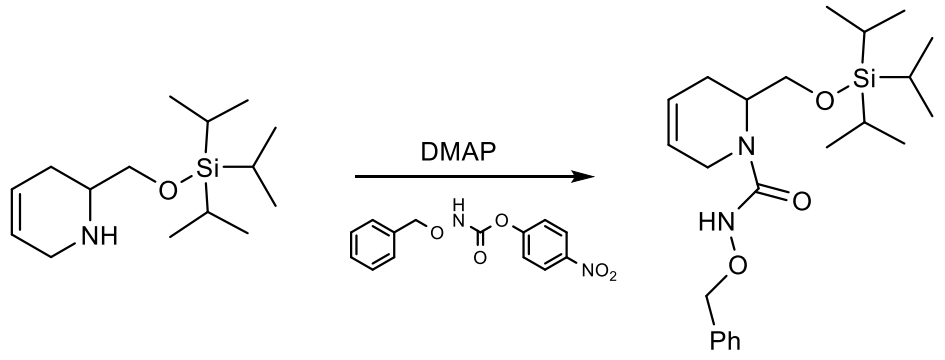
(1,2,3,6-Tetrahydropyridin-2-yl)methanol (**13**) (111 mg, 0.981 mmol, 1.0 eq.) was dissolved in DMF and DMAP (240 mg, 1.96 mmol, 2.0 eq.) and TiPS-Cl (378 mg, 1.96 mmol, 2.0 eq.) were added at 0°C. The reaction mixture was stirred for six days at room temperature, then diluted with CH_2Cl_2 and extracted one time with H_2O. The organic phase was dried with Na_2SO_4 and the organic solvent removed under reduced pressure. The crude product was purified by a silica gel 60 (18 g) column chromatography (grd. Heptane/EtOAc 4:1 → EtOAc/MeOH (5%)). The yield of **14** as a colorless oil was 79 mg (0.293 mmol, 30%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ [ppm] = 5.73 (m, 2 H, C***H***=C***H***), 3.72 (dd, 1 H, *J* = 9.6, 3.9, -C***H$_2$***-OH), 3.57 (dd, 1 H, *J* = 9.6, 7.7, -C***H$_2$***-OH), 3.42 (m, 2 H, =CH-C**H$_2$**-N-), 2.89 (m, 1 H, -C***H$_2$***-O-), 2.48 (s, 1 H, N-***H***), 1.91 (m, 2 H, =HC-C***H$_2$***-CH-), 1.06 (m, 21 H, (C***H***(C***H$_3$***)$_2$)$_3$Si).

^{13}C-NMR (400 MHz, $CDCl_3$) δ [ppm] = 126.5 (-CH=***C***H-CH_2-N-), 124.9 (-CH_2-***C***H=CH-), 67.3 (***C***H_2-O-), 54.4 (-CH_2-***C***H(CH_2)-N-), 45.0 (=CH-***C***H_2-N-), 27.8 (=HC-***C***H_2-CH-), 18.1 (***C***H_3), 12.0 (***C***H).

R_f (EtOAc) = 0.35

5.15 N-(Benzyloxy)-2-(((triisopropylsilyl)oxy)methyl)-3,6-dihydropyridine-1(2H)-carboxamide (15)



14 **12** **15**

2-(((Triisopropylsilyl)oxy)methyl)-1,2,3,6-tetrahydropyridine (**14**) (79 mg, 0.293 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (6 ml) and DMAP (39 mg, 0.319 mmol, 1.1 eq.) dissolved in CH_2Cl_2 (1 ml) and 4-Nitrophenyl(benzyloxy)carbamate (**12**) dissolved in CH_2Cl_2 (1 ml) were added quickly to the stirred solution. The reaction mixture was stirred for one hour at room temperature and then extracted five times with a $NaHCO_3$- solution (~25 ml, 8.3%). The organic Phase was dried with Na_2SO_4 and the organic solvent removed under reduced pressure. The crude product was purified by a silica gel 60 (5 g) column chromatography (Heptane/EtOAc 32%). The yield of **15** as a colorless oil was 80 mg (0.191 mmol, 65%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ [ppm] = 7.78 (s, 1 H, N-***H***), 7.33 (m, 5 H, $\boldsymbol{H}_{Ar}$), 5.67 (m, 2 H, C***H***=C***H***), 4.84 (dd, 2 H, *J* = 45.7, 11.8 Hz, -C$\boldsymbol{H_2}$-Ph), 4.28 (m, 1 H, =CH-C$\mathbf{H_2}$-N-), 4.15 (m, 1 H, -CH_2-C***H***(CH_2)-N-), 3.70 (t, 1 H, *J* = 9.5 Hz, -C$\boldsymbol{H_2}$-O-Si-), 3.53 (m, 2 H, -C$\boldsymbol{H_2}$-O-Si-…=CH-C$\mathbf{H_2}$-N-), 2.36 (m, 1 H, =HC-C$\boldsymbol{H_2}$-CH-), 1.97 (m, 1 H, =HC-C$\boldsymbol{H_2}$-CH-), 1.01 (m, 21 H, (C***H***(C$\boldsymbol{H_3}$)$_2$)$_3$Si).

^{13}C-NMR (400 MHz, $CDCl_3$) δ [ppm] = 160.5 (***C***=O), 137.0 (O-CH_2-$\boldsymbol{C}_{Ar.}$), 128.8 ($\boldsymbol{C}_{Ar.}$), 128.4 ($\boldsymbol{C}_{Ar.}$), 128.1 ($\boldsymbol{C}_{Ar.}$), 123.0 (-CH=***C***H-CH_2-N-), 122.3 (-CH=***C***H-CH_2-N-), 77.8 (-CH_2-***C***H=CH-), 64.1 (***C***H_2-O-Si), 50.2 (-CH_2-***C***H(CH_2)-N-), 39.8 (=CH-***C***H_2-N-), 25.4 (=HC-***C***H_2-CH-), 18.0 (***C***H_3), 11.8 (***C***H).

R_f (Heptane/EtOAc 32%) = 0.38

Literaturverzeichnis

1. **George G. Zhanel, et. al.** *Ceftazidime-Avibactam: a Novel Cephalosporin/β-lactamase Inhibitor Combination.* s.l. : Drugs 73, 159-177, 2013.

2. **Cesar A. Arias and Barbara E. Murray.** *Antibiotic-Resisntant Bugs in the 21st Century - A Clinical Super-Challenge.* s.l. : N. ENGL. J. MED. 360, 5, 439-443, 2009.

3. **Graham L. Patrick.** *An Introuction to Medicinal Chemistry.* New York : Oxford University Press Inc., 2009. 978-0-19-923447-9.

4. **Hongbaek Cho, Tsuyoshi Uehara, Thomas G. Bernhardt.** *Beta-Lactam Antibiotics Induce a Lethal Malfunctioning of the Bacterial Cell Wall Synthesis Machinery.* s.l. : Cell 159, 1300-1311, 2014.

5. **Samuel Baron et. al.** *Medical Microbiology. 4th edition.* Galveston : University of Texas Medical Branch at Galveston, 1996. 0-9631172-1-1.

6. **Christian Therrien, Roger C. Levesque.** *Molecular basis of antibiotic resistance and β-lactamase inhibition by mechanism-based inactivators: perspectives and future directions.* s.l. : FEMS Mirobiology Reviews, Vol. 24, 251-262, 2000.

7. **Karen Bush and George A. Jacoby.** *Updated Functional Classification of β-Lactamases.* s.l. : ANTIMICROBIAL AGENTS AND CHEMOTHERAPY 969-976, 2010.

8. **Sarah M. Drawz and Robert A. Bonomo.** *Three Decades of β-Lactamase Inhibitors.* s.l. : American Society for Microbiology, 23, 160-201, 2010.

9. **Allergan.** ***AVYCAZ (ceftazidime and avibactam) for Injection, for intravenous use.*** **[Online] [Zitat vom: 10. 07 2016.] http://www.allergan.com/assets/pdf/avycaz_pi.**

10. Dustin T. King, et.al. *Molecular Mechanism of Avibactam-Mediated β-Lactamase Inhibition.* s.l. : ACS Infect. Dis., Vol. 1, 175-184, 2015.

11. Sushmita D. Lahiri, et. al. *Structural Insight into Potent Broad-Spectrum Inhibition with Reversible Recyclization Mechanism: Avibactam in Complex with CTX-M-15 and Pseudmonas aeruginosa AmpC β-Lactamases.* s.l. : Antimicrobal Agents and Chemotherapy, Vol. 57, No. 6, 2496-2505, 2013.

12. Maxime Lampilas, Romainville (FR), et al. *AZABICYCLIC COMPOUNDS, PREPARATION THEREOF AND USE AS MEDICINES, IN PARTICULAR AS ANTIBACTERIAL AGENTS. US 2003/0199541 A1* US, 23. 10 2003.

13. BOYD, John Alistair [GB/GB], et. al. *PROCESS FOR PREPARING HETEROCYCLIC COMPOUNDS INCLUDING TRANS-7-OXO-6-(SULPHOOXY)-1,6-DIAZABICYCLO[3,2,1]OCTANE-2-CARBOXAMIDE AND SALTS THEREOF. WO 2012/172368 A1* US, 20. 12 2012 .

14. K. C. Nicolaou, P. S. Baran, Y.-L. Zhong, S. Barluenga, K. W. Hunt, R. Kranich, and J. A. Vega. *Iodine (V) Reagents in Organic Synthesis. Part 3. New Routes to Heterocyclic Compounds via o-Iodoxybenzoic Acid-Mediated Cyclization: Generality, Scope, and Mechanism.* s.l. : J. AM. CHEM. SOC. VOL. 124, NO. 10, 2233-2244, 2002.

15. Jeffrey L. Romine, Scott W. Martin, Nicholas A. Meanwell, James R. Epperson. *Synthesis of 3-Hydroxypyrimidine-2,4-diones. Addition of Anilines to Benzyloxy Isocyanate Synthons to Givy N-Hydrxyureas.* s.l. : SYNTHESIS, 846-850, 1994.

16. Aiman, Albert W. Burgstahler and Charles E. *A Direct Synthesis of DL-Baikiain.* s.l. : American Chem. Soc., 25, 489-492, 1960.

17. Engel, Claus Herdeis and Wolfram. *An Efficient Synthetic Method for the Preparation of (±)-Baikiain and its Derivatives.* Weinheim : Arch. Pharm. 326, 297-301, 1993.

18. Edward Leete and Mark E. Mueller. *Biomimetic Synthesis of Anatabine from 2,5-Dihydropyridine Produced by the Oxidative Decarboxylation of Baikiain.* s.l. : J. AM. Chem. Soc. 104, 6440-6444, 1982.

19. Hardtmann, Goetz E., Dr., Morristown, N.J., US, Houlihan, William J., Dr., Mountain Lakes, N.J., US und Giger, Rudolf K.A., Dr., Muttenz, CH. *Tetracyclische Chinazolinone. DE 3702943 A1* Deutschland, 31. 01 1987. Offenlegungsschrift.

20. World Health organisation. [Online] 2016. [Zitat vom: 22. 06 2016.] http://www.who.int/trade/glossary/story034/en/.

21. Kurt R. Karst. FDA | Law Blog The official blog of Hyman, Phelps McNamara, P.C. ***Like a Bad Penny, Pre-MMA 180-Day Exclusivity Keeps Turning Up!*** **[Online] 17. 02 2014. [Zitat vom: 24. 06 2016.] http://www.fdalawblog.net/fda_law_blog_hyman_phelps/2014/02/like-a-bad-penny-pre-mma-180-day-exclusivity-keeps-turning-up.html.**

22. Clayden, Jonathan. *Organic Chemistry second Edition.* New York : Oxford University Press, 2012. 978-0-19-927029-3.

23. Safety Data Sheet according to Regulation (EC) No. 1907/2006. ***1-Methyl-2-pyrrolidinone.*** **[Online] 17. 02 2015. [Zitat vom: 05. 06 2016.] http://www.sigmaaldrich.com.**

24. Sigma Aldrich . Sigma Aldrich a part of Merck. [Online] [Zitat vom: 13. 06 2016.] http://www.sigmaaldrich.com/spectra/fnmr/FNMR003536.PDF.

25. Ramachandran and Li. *The Synthesis of L-Valyl-L-Lysyl-L-valyl-L-tyrosyl-L-proline.* s.l. : Ramachandran and Li, 28, 173-177, 1963.

26. Samant, Manoj P. et al. *Novel Analogues of Degarelix Incorporating Hydroxy-, Methoxy-, and Pegylated-Urea Moieties at Positions 3, 5, 6 and the N-Terminus. Part III.* s.l. : Journal of Medicinal Chemistry, 49(12), 3536-3543, 2006.

27. Dennis A. Parrish et al. *A convient method for the synthesis of N-hydroxyureas.* s.l. : Tetrahedron Letters, 46, 8841-8843, 2005.

28. Albert Defoin, Agnès Brouillard-Poichet, and Jacques Streith. *Asymmetric Diels-Alder cycloaddition with Chiral Carbamoyl Dienophiles.* s.l. : HELVETIVA CHIMICA ACTA Vol. 75, 1992.

29. Wood, Richard Delarey et al. *Preparation of benzamide compounds as glutamate receptor modulators and therapeutic agents. 20090239919* USA, 24. 09 2009. Pat. Appl. Publ.

30. Fish, Paul V. et al. *Selective Urokinase-Type Plasminogen Activator Inhibitors. 1-(7-Sulfonamidoisoquinoliyl)guanidines.* s.l. : Journal of Medicinal Chemistry, 50(10), 2341-2351, 2007.

31. Gerasyuto, Aleksey l. et al. *A Formal [3 + 3] Cycloaddition Reaction. 5. An Enantioselective Intramolecular Formal Aza-[3 + 3] Cycloaddition Reaction Promoted by Chiral Amine Salts.* s.l. : Journal of Organic Chemistry, 70(11), 4248-4256, 2005.

32. Robert F. Cunico and Lewis Bedell. *The Triisopropylsilyl Group as a HYdrxyl-Protecting Function.* s.l. : J. Org. Chem. 45, 4797-4798, 1980.

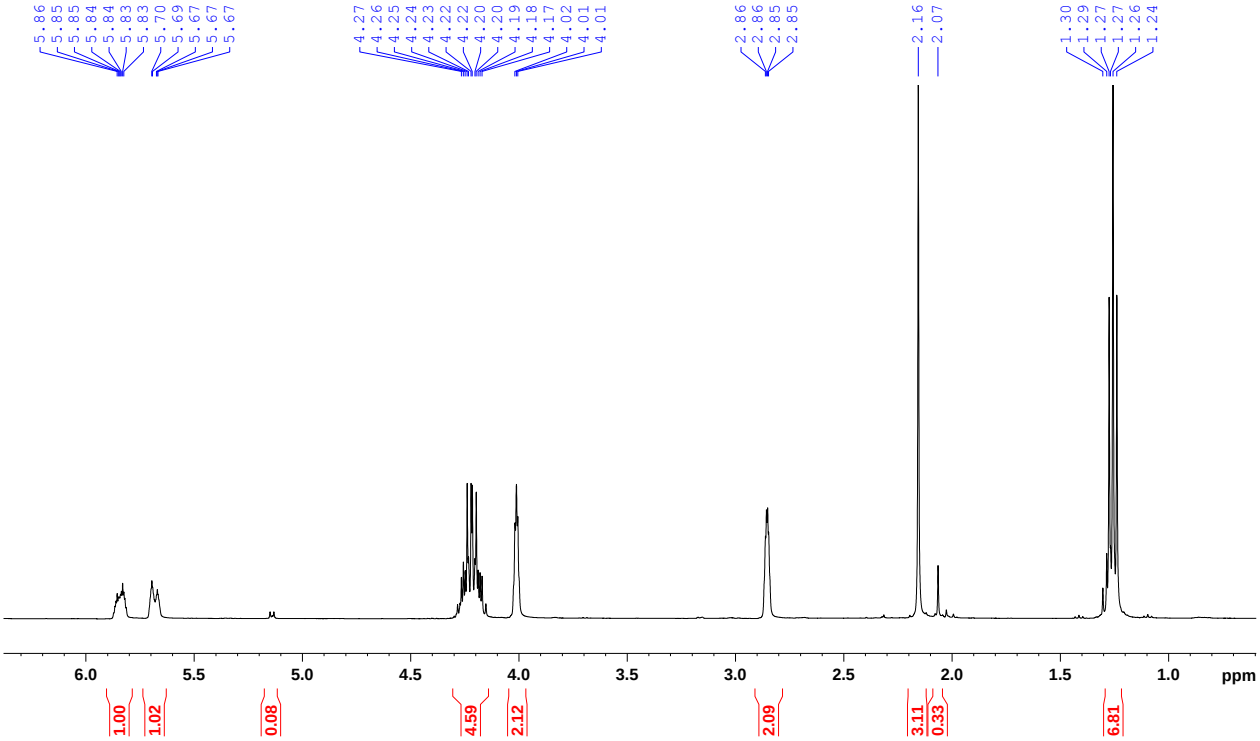
33. Sambasivarao Kotha and Kuldeep Singh. *N-Alkylation of diethyl acetamidomalonate: synthesis of constrained amino acid derivatives by ring-closing metathesis.* s.l. : Tetrahedron Letters, Vol. 45, 9607–9610, 2004.

34. Roger A. Sheldon. *Atom efficiency and catalysis in organic synthesis.* s.l. : Pure and Applied Chemistry 72, 1233-1246, 2000.

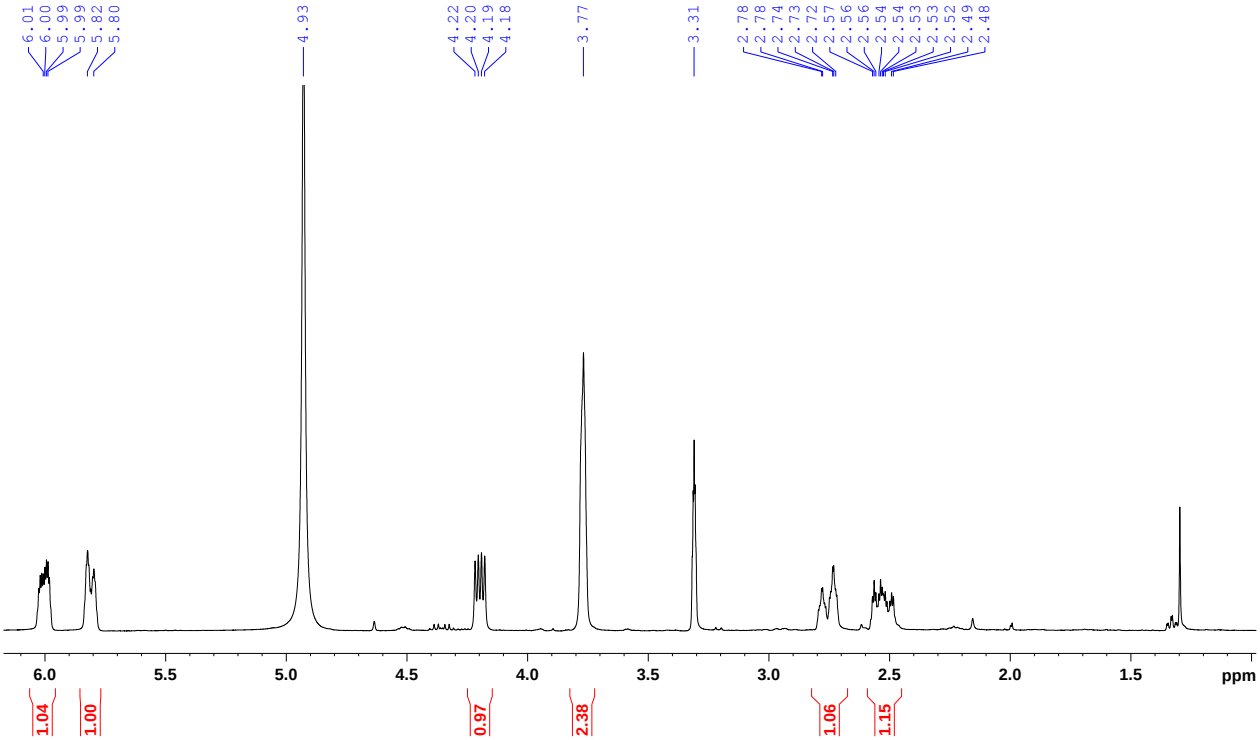
7. Appendix

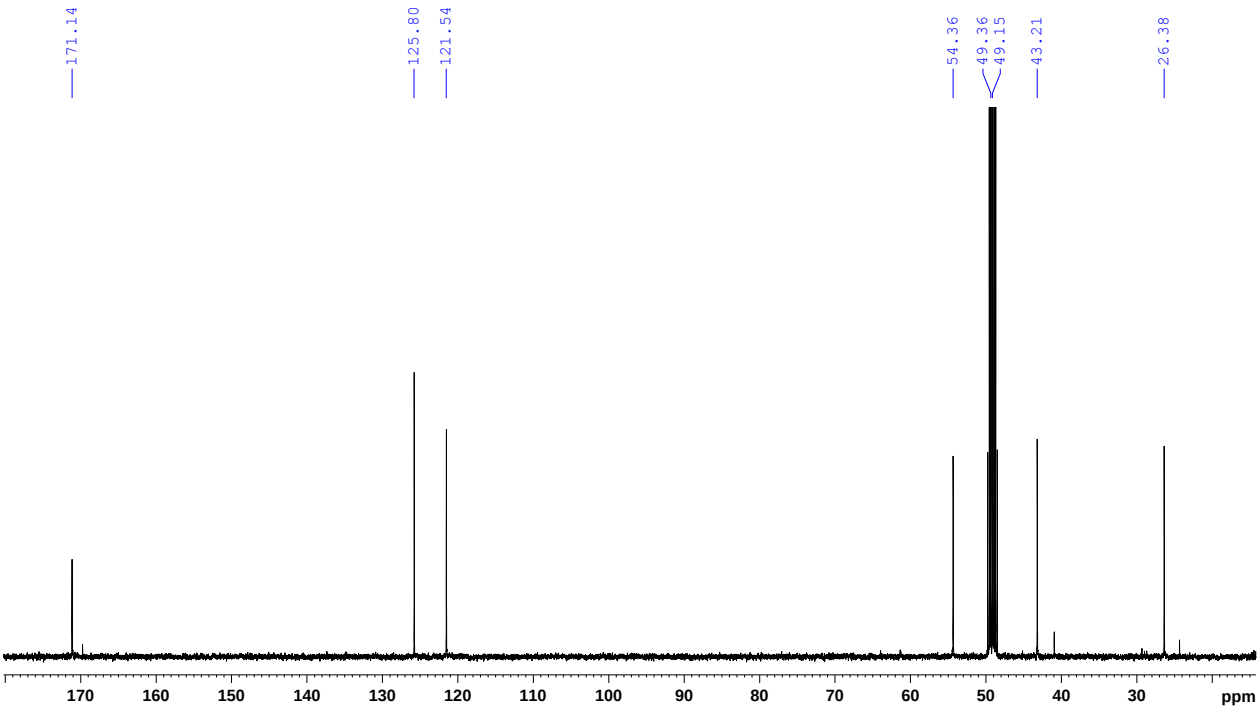
7.1 NMR's

7.1.1 Diethyl 1-acetyl-3,6-dihydropyridine-2,2(1H)-dicarboxylate (3)

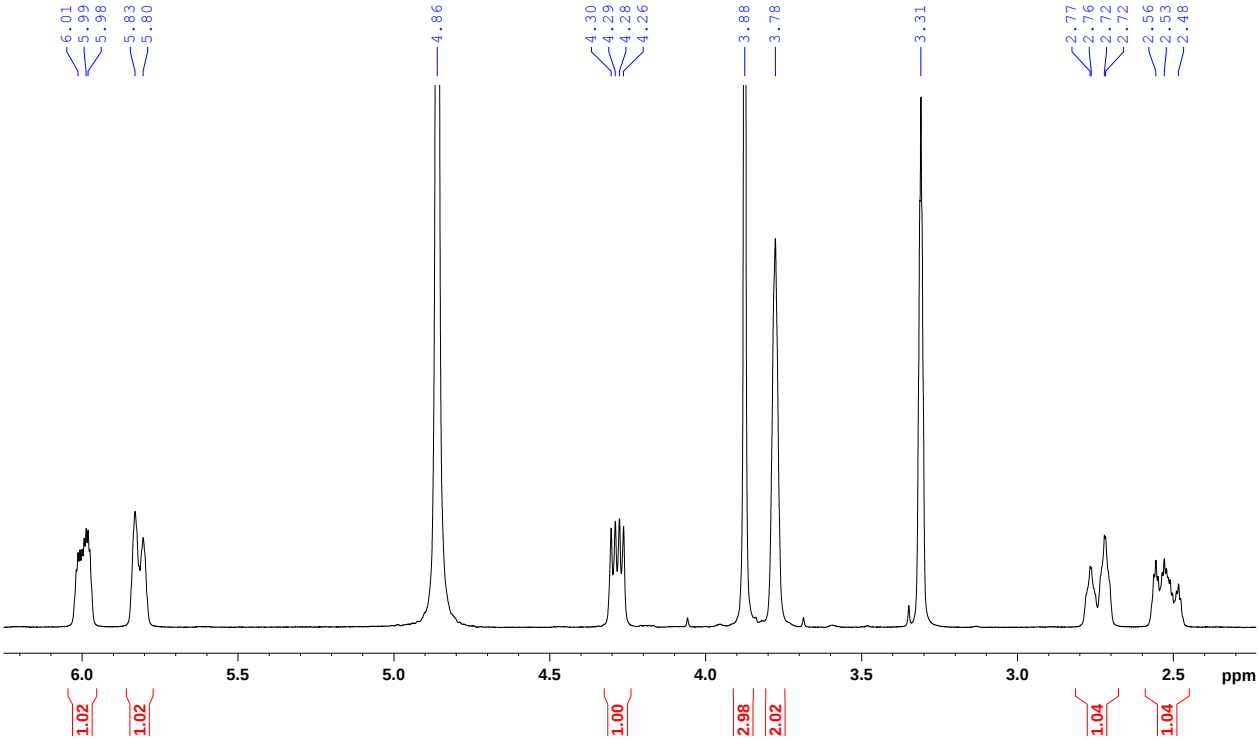


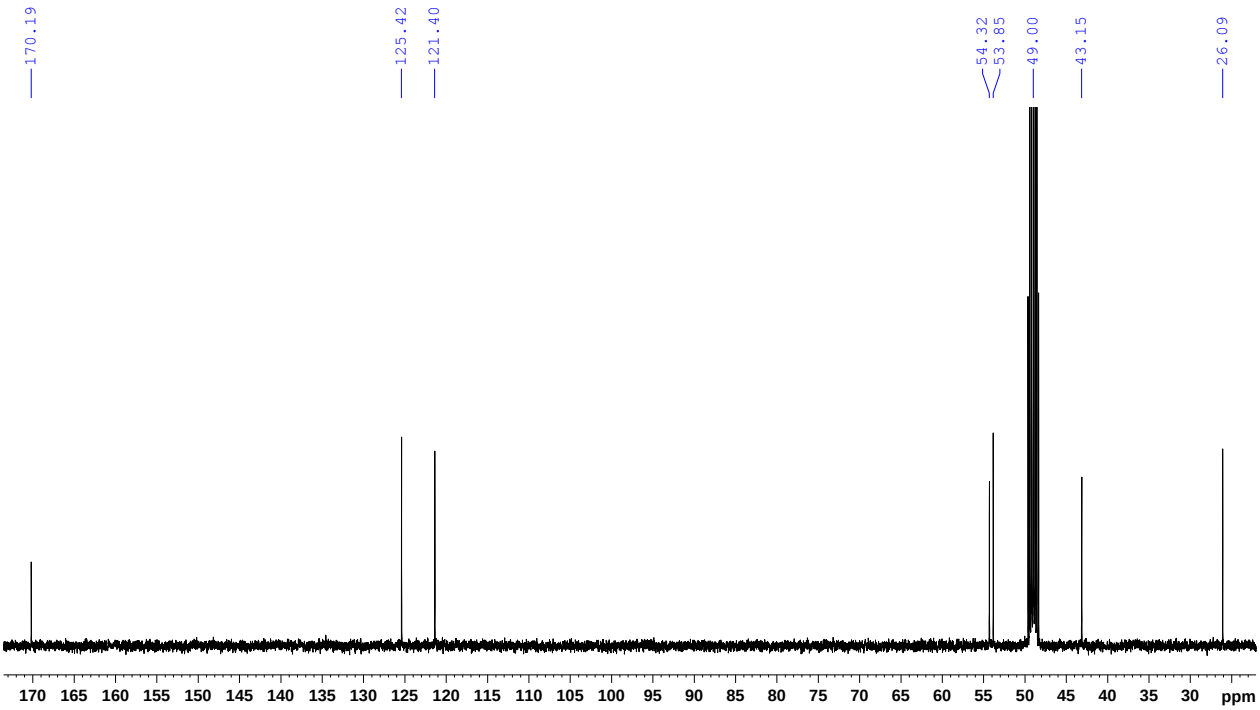
7.1.2 Baikiain hydrochloride (4)



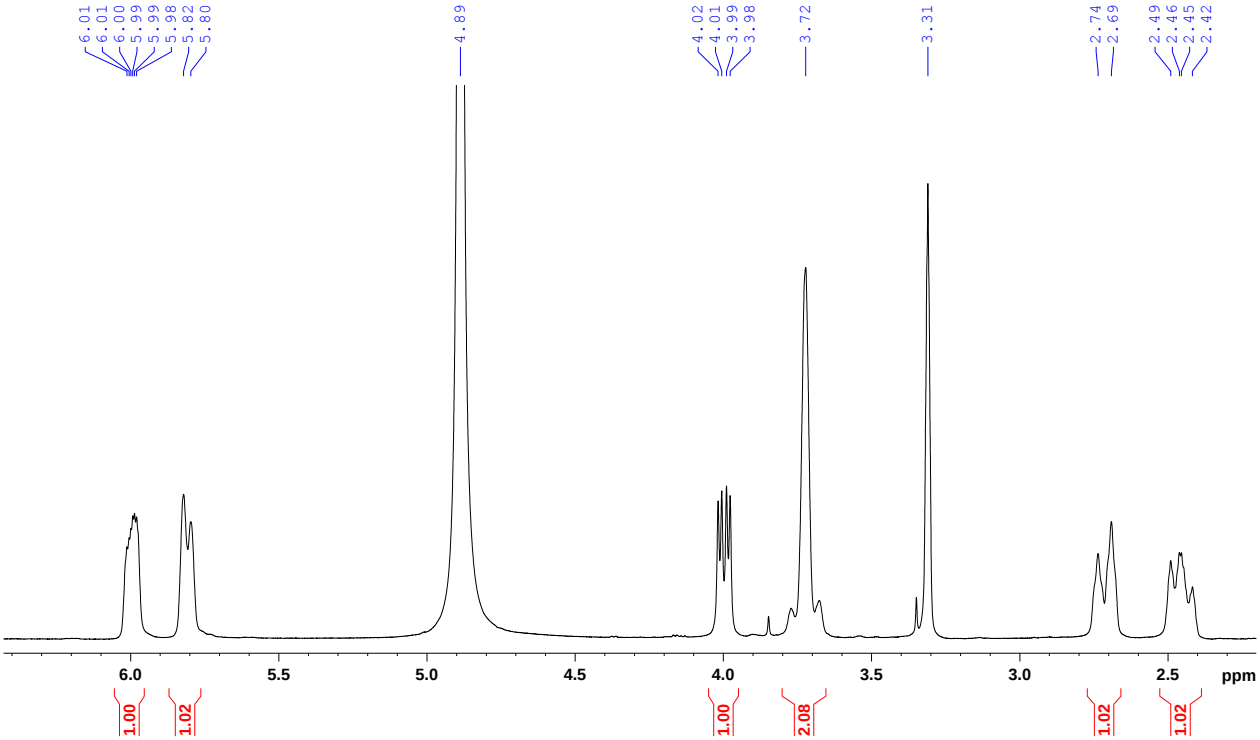


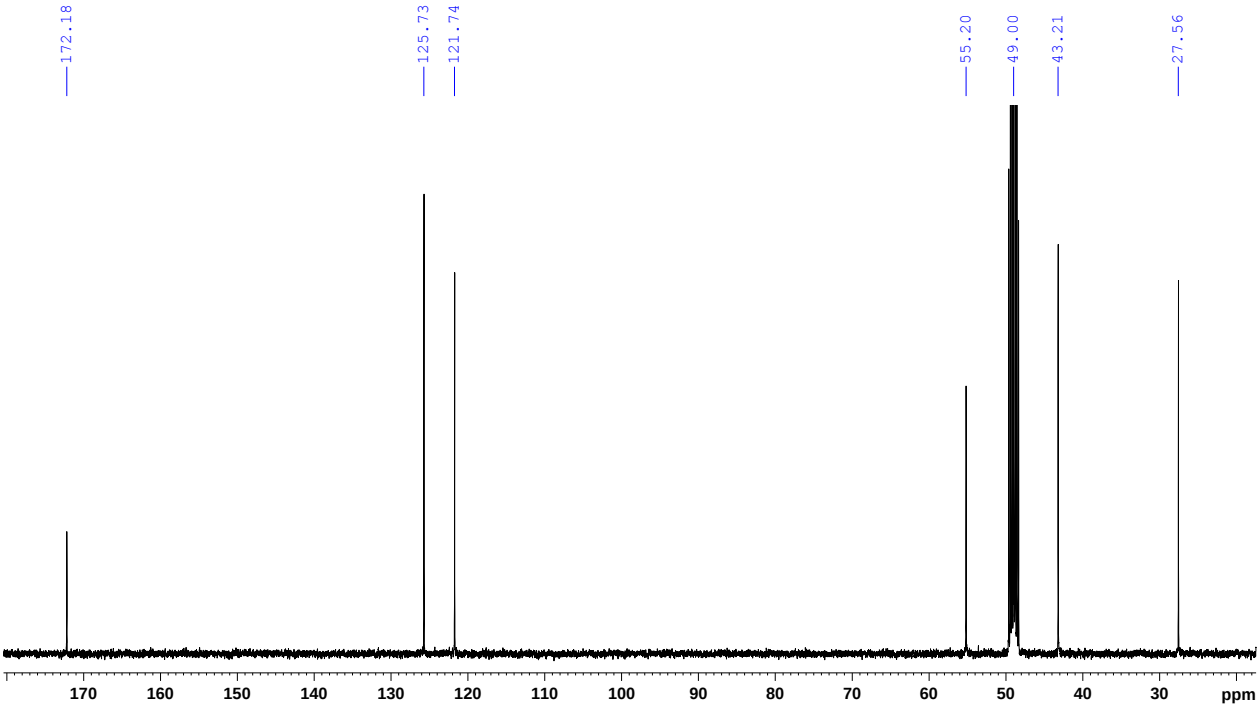
7.1.3 Baikiain ester hydrochloride (5)



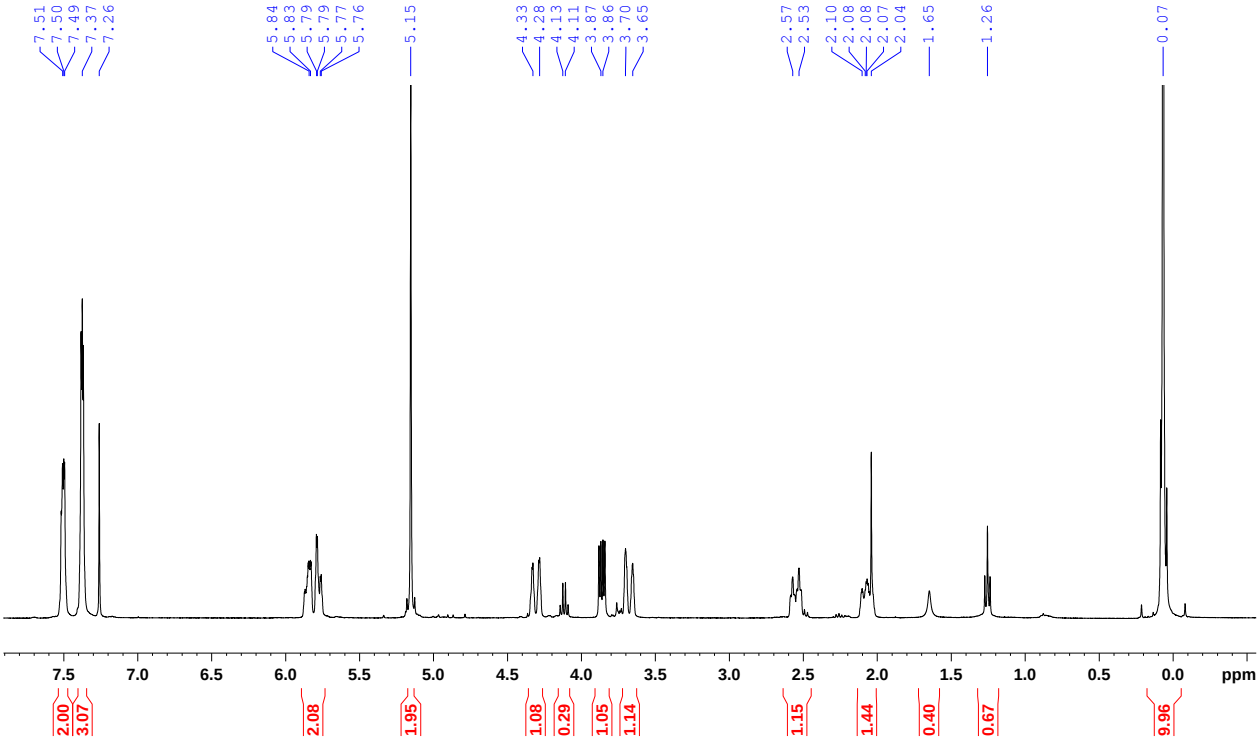


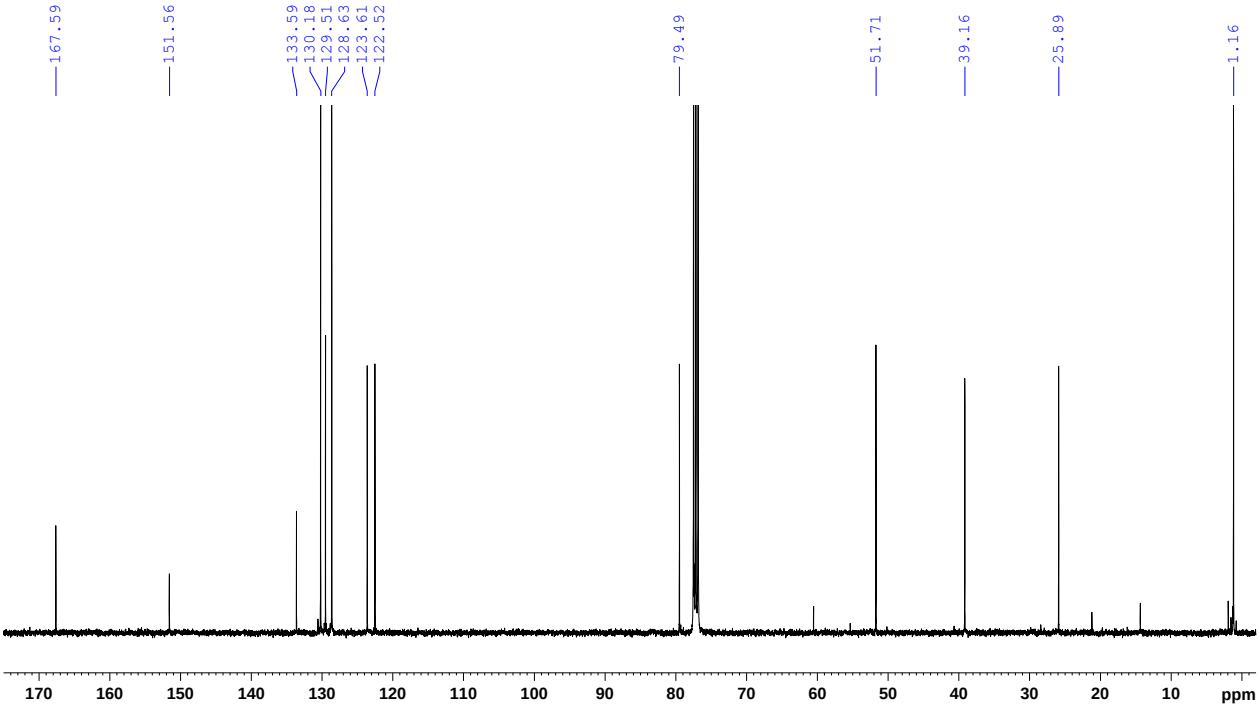
7.1.4 Baikiain amide (6)



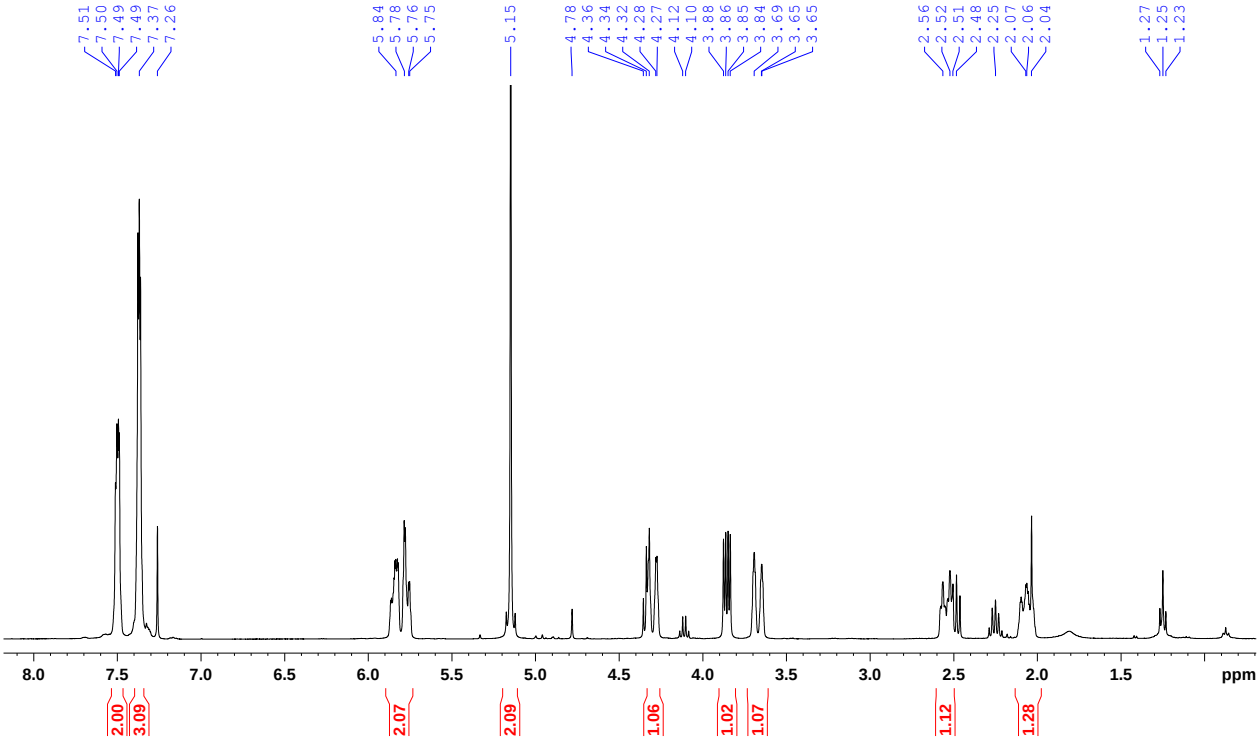


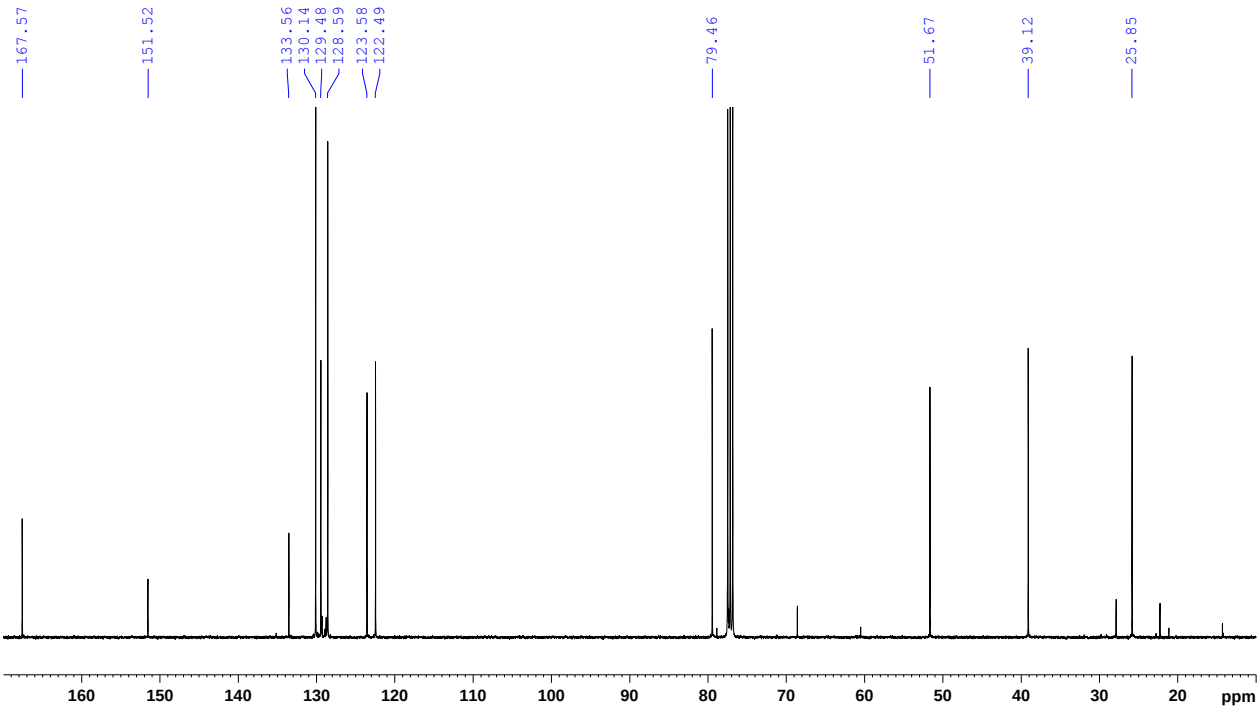
7.1.5 2-(Benzyloxy)-8,8a-dihydroimidazo[1,5-a]pyridine-1,3(2H,5H)-dione (10) from Baikiain ester hydrochloride



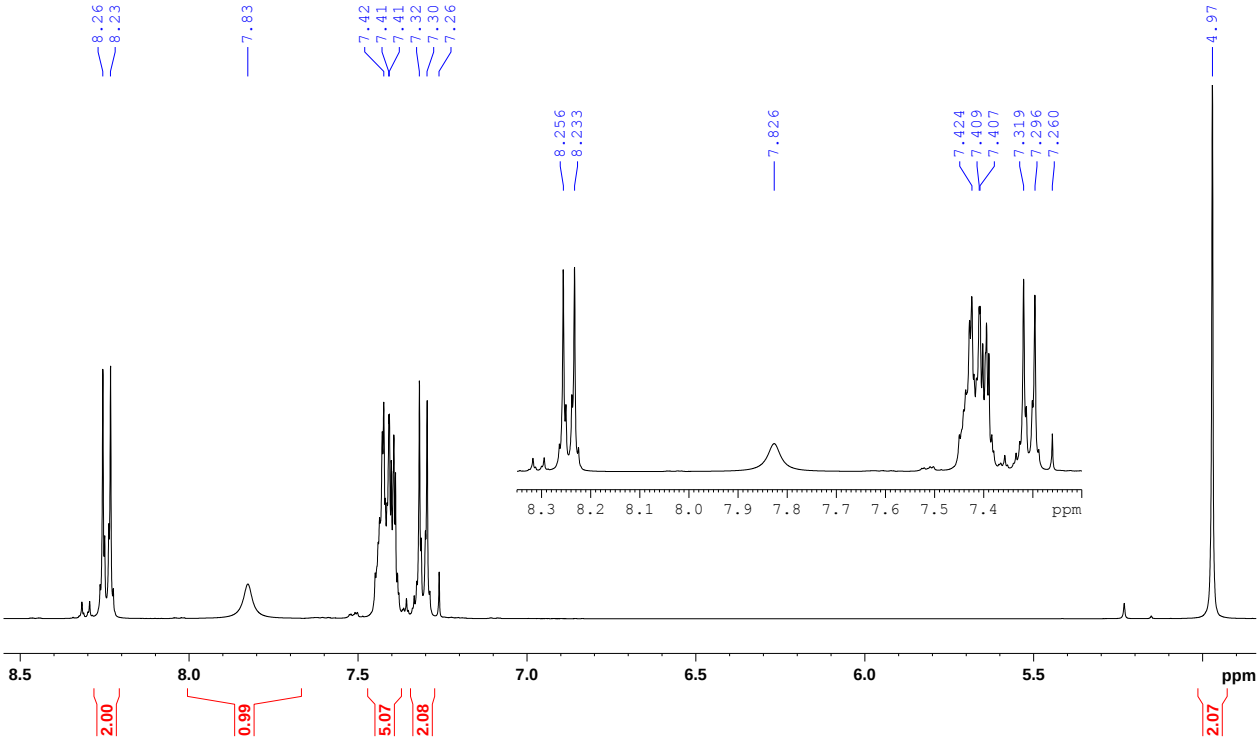


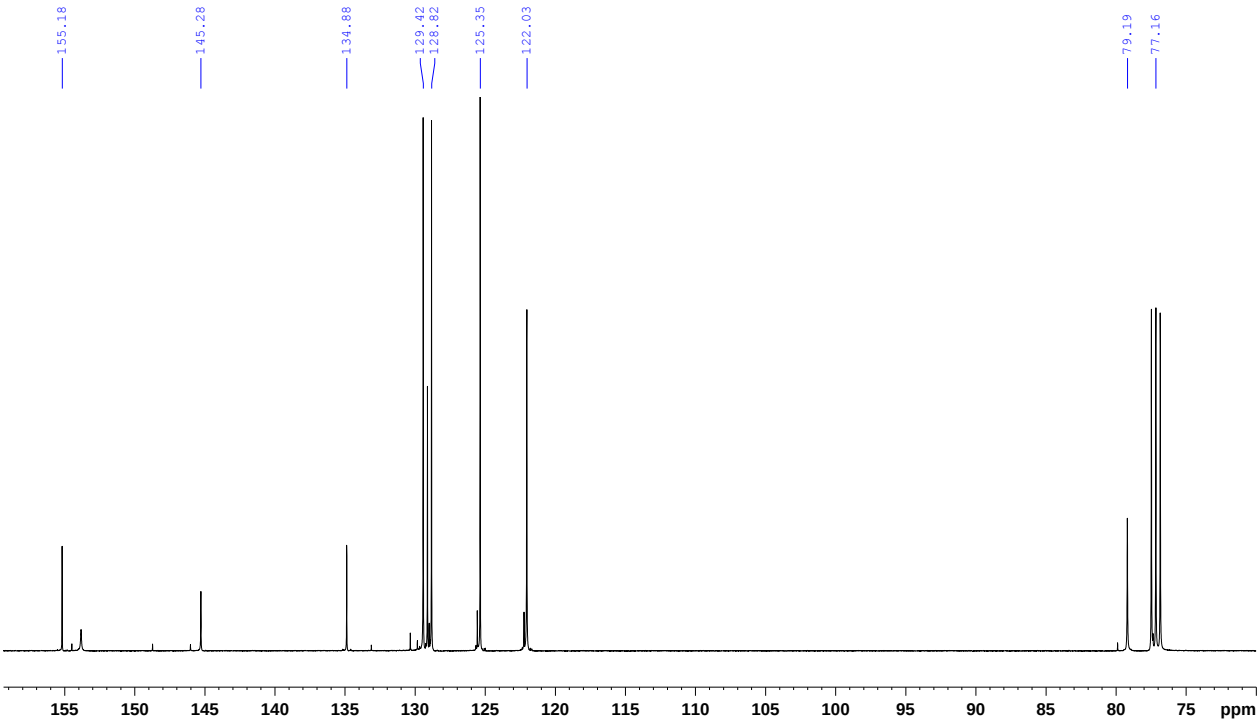
7.1.6 2-(Benzyloxy)-8,8a-dihydroimidazo[1,5-a]pyridine-1,3(2H,5H)-dione (10) from Baikiain amide



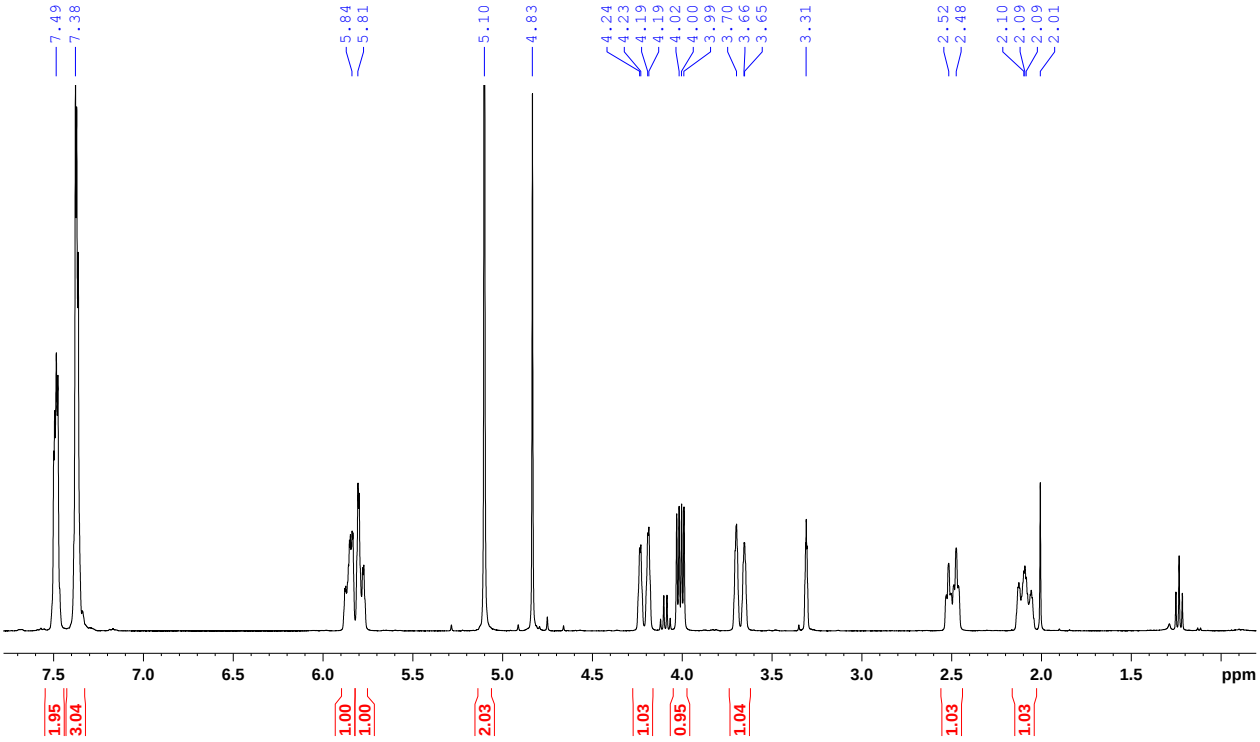


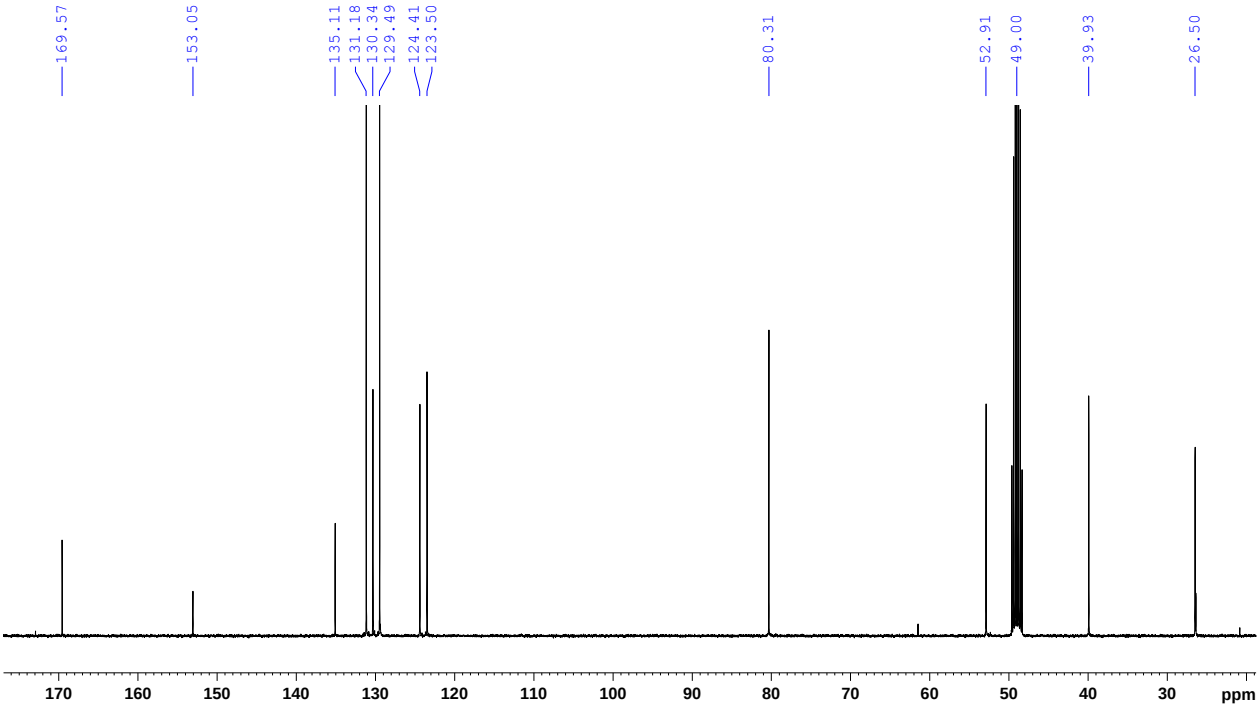
7.1.7 4-Nitrophenyl (benzyloxy)carbamate (12)



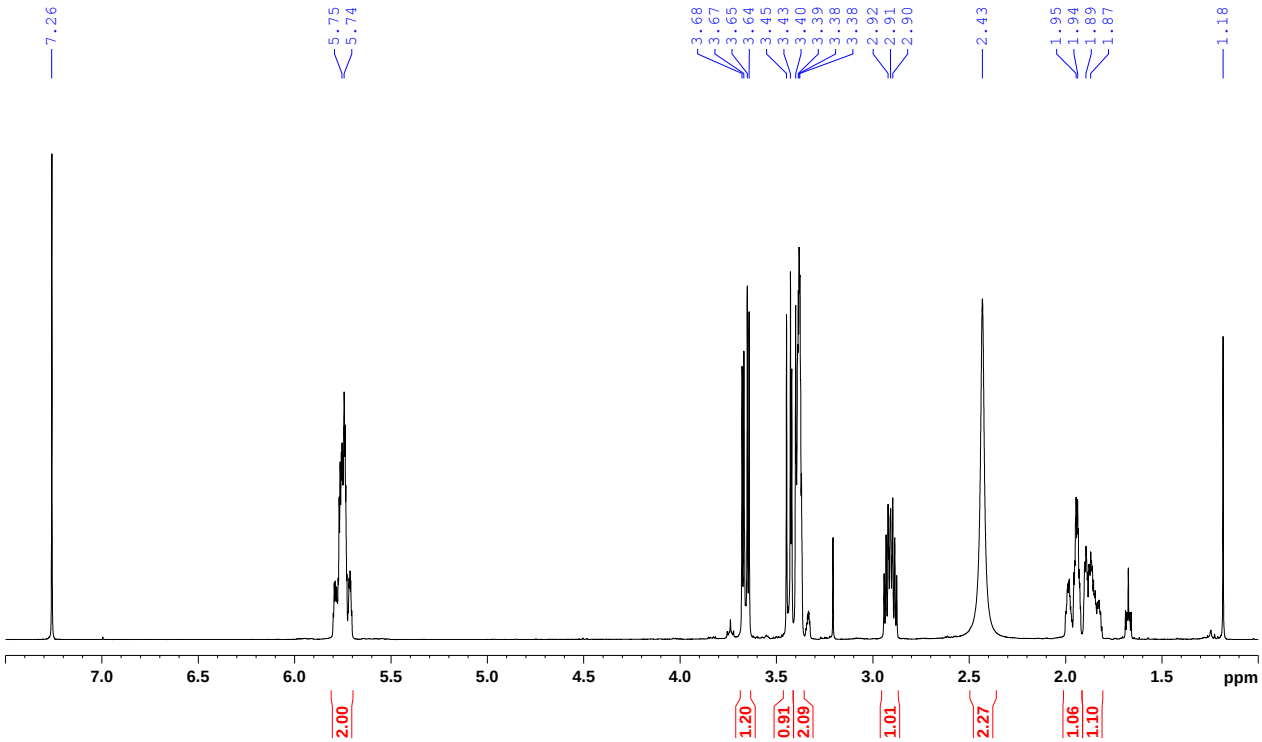


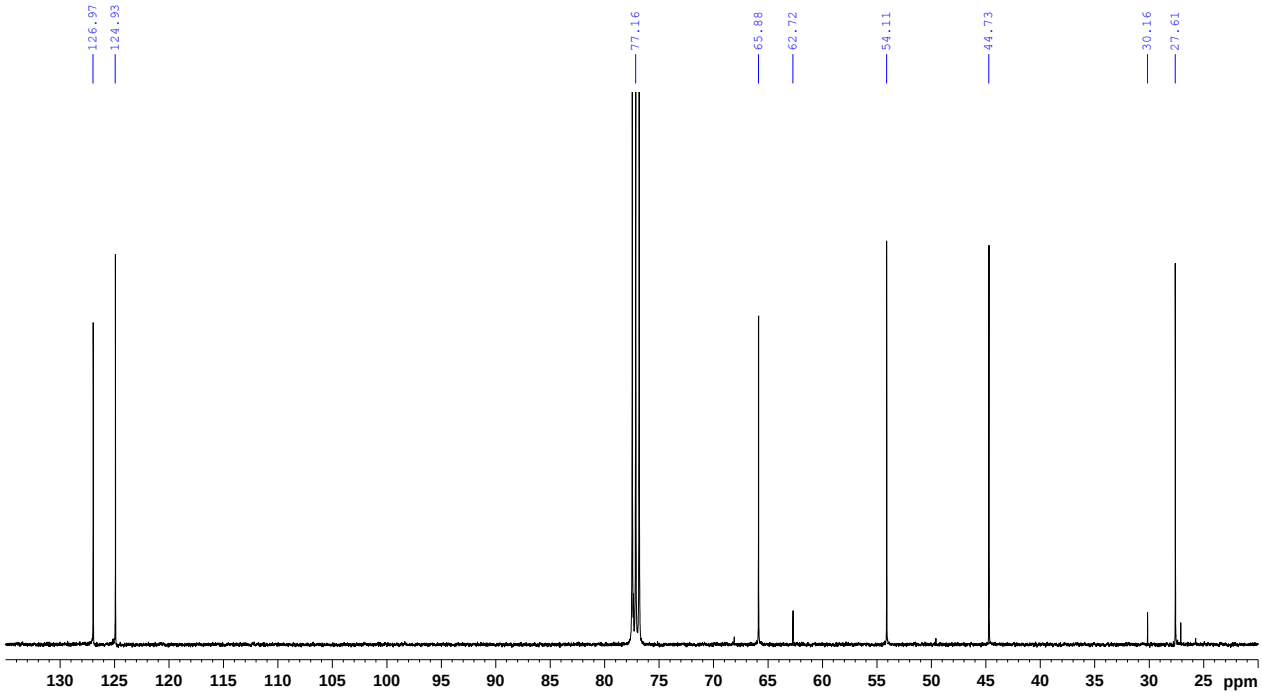
7.1.8 2-(Benzyloxy)-8,8a-dihydroimidazo[1,5-a]pyridine-1,3(2H,5H)-dione (10) with 4-nitrophenyl (benzyloxy)carbamate



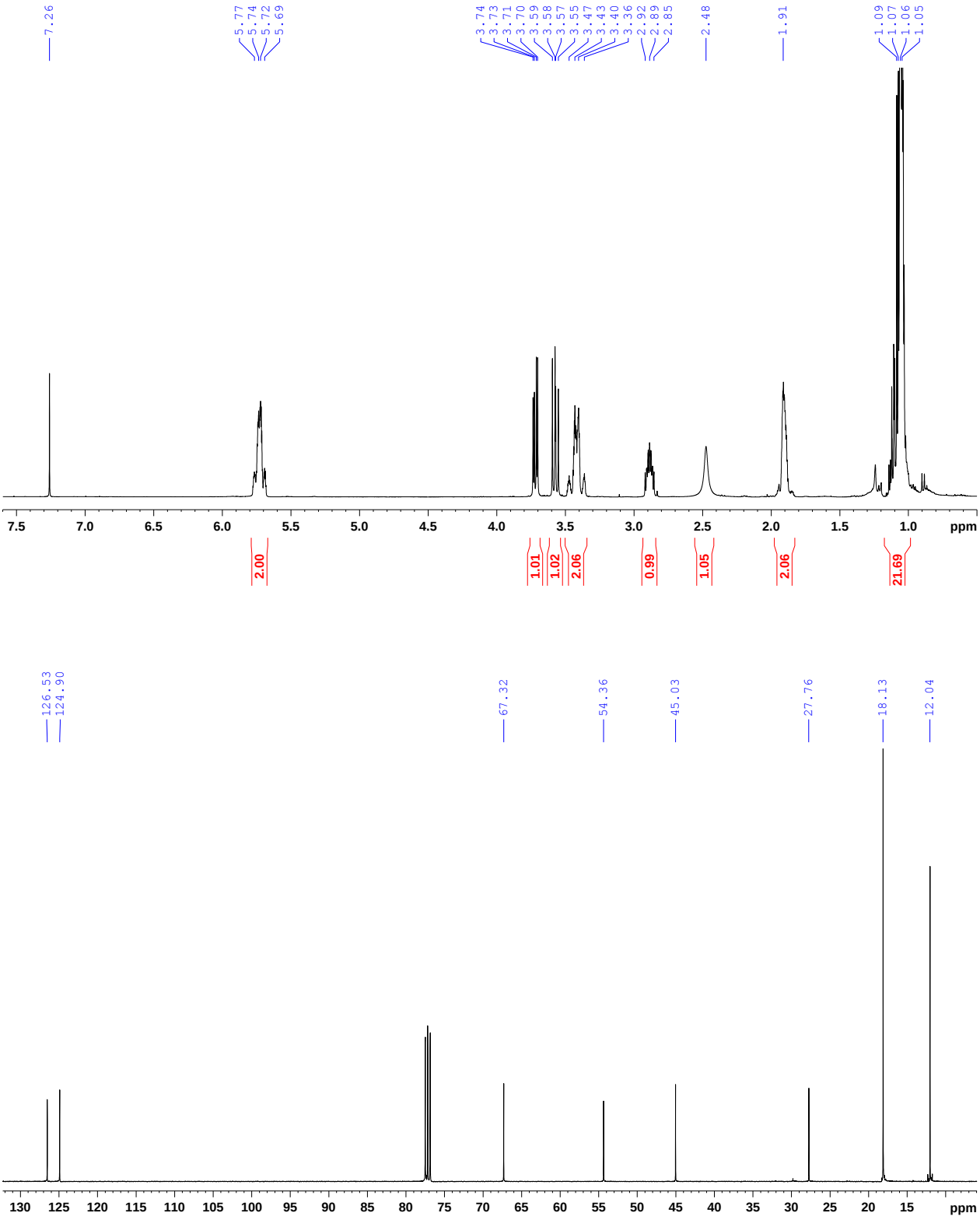


7.1.9 (1,2,3,6-Tetrahydropyridin-2-yl)methanol (13)

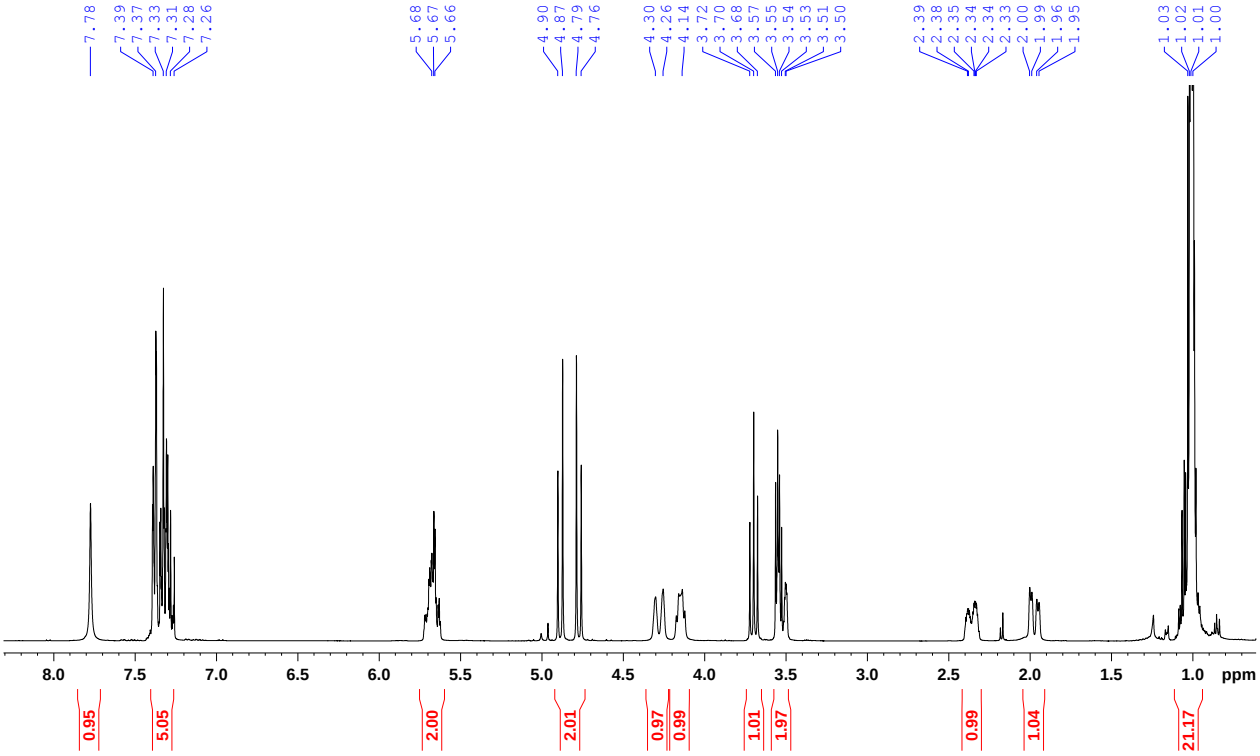


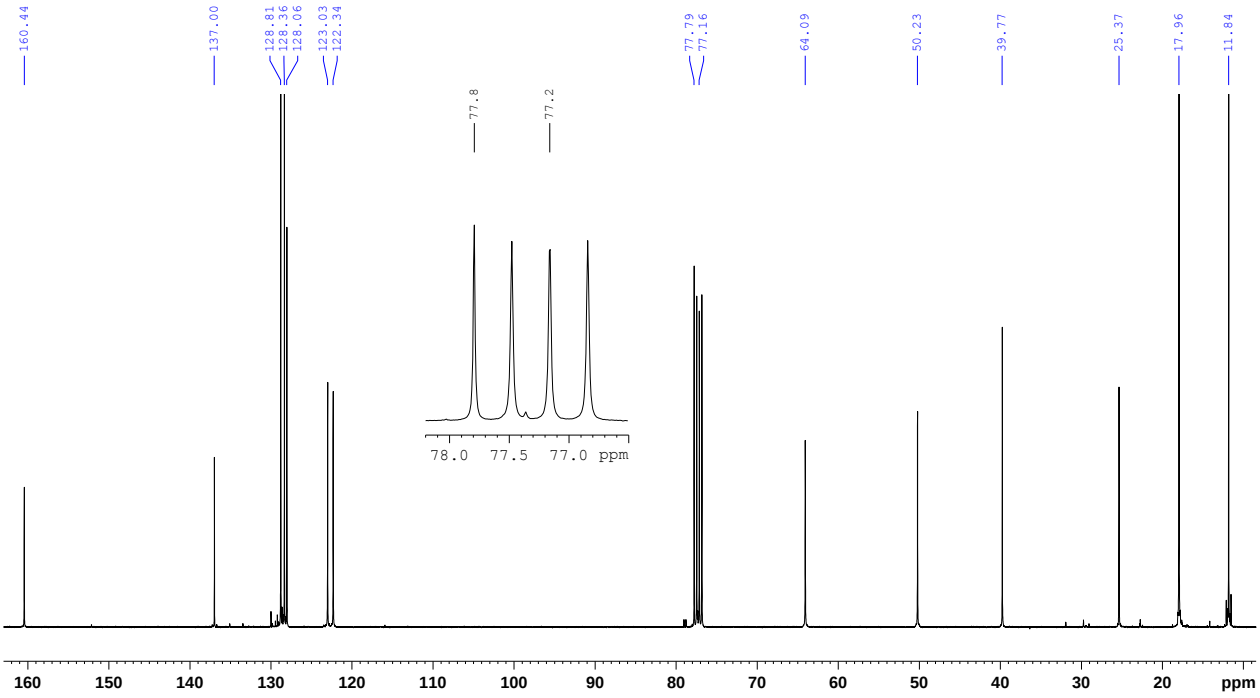


7.1.10 2-(((Triisopropylsilyl)oxy)methyl)-1,2,3,6-tetrahydropyridine (14)



7.1.11 N-(Benzyloxy)-2-(((triisopropylsilyl)oxy)methyl)-3,6-dihydropyridine-1(2H)-carboxamide (15)





7.2 Quantitative ^{1}H-NMR

Baikiain amide (6)

m(Amid)=8,2 mg
m(TCNM)=4,4 mg
The purity of the Internal Standard (TCNB) is 99.72 % (w/w)

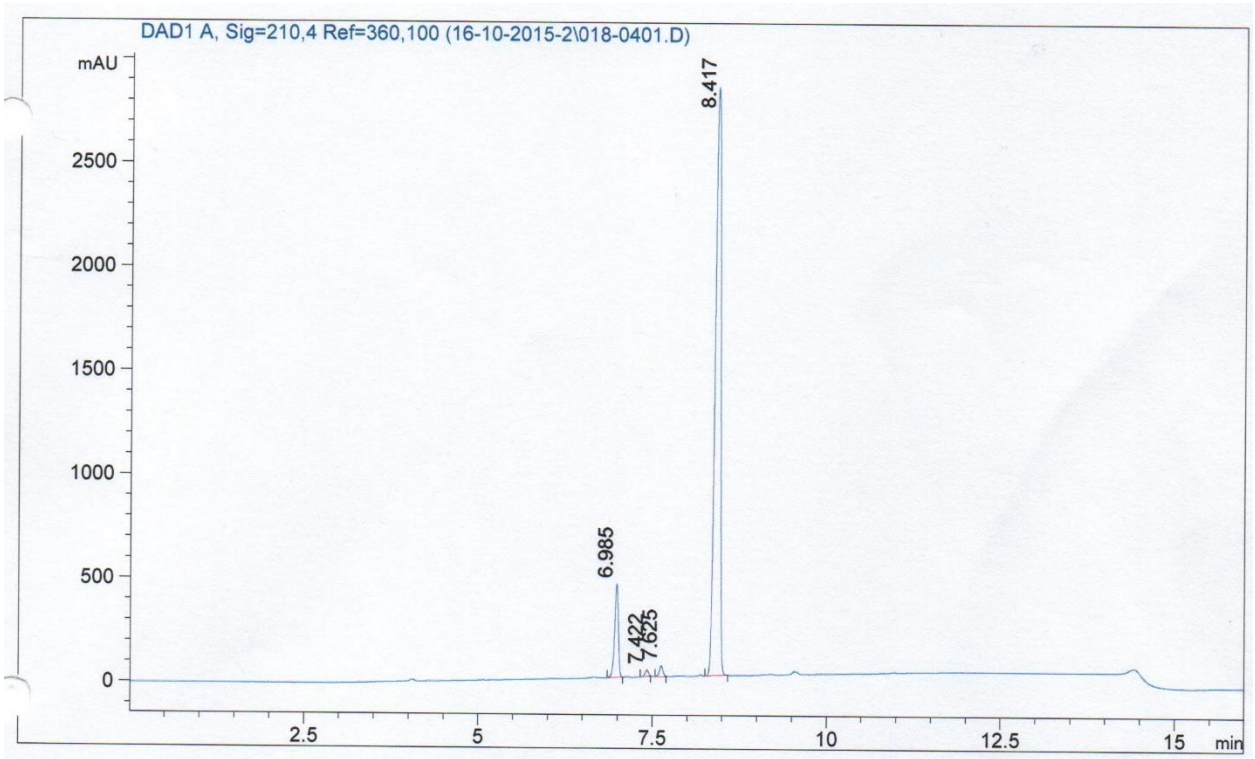
Component	Assay % (w/w)	Integral	Number of H	Mw (g/mol)	Component mass (mg)
Baikiainamid	76.654	1.0064	1	126.160	8.2000
Baikiainamid	76.163	1.0000	1	126.160	8.2000
Baikiainamid	75.176	0.9870	1	126.160	8.2000
Baikiainamid	76.638	2.0125	2	126.160	8.2000
Baikiainamid	75.415	0.9902	1	126.160	8.2000
Baikiainamid	76.082	0.9989	1	126.160	8.2000
TCNB	99.720	0.3397	1	260.880	4.4000

The purity of 'Baikiainamid' is 76.021 % (w/w), SD= 0.61 % (w/w), RSD= 0

No	Low	High	Slope	Bias	Integral	Obs-Calc	Number of H
1	8.511	8.458	0.000	0.000	0.3397	0.000	1
2	5.941	5.828	0.000	0.000	1.0064	-0.006	1
3	5.768	5.668	0.000	0.000	1.0000	0.000	1
4	3.905	3.800	0.000	0.000	0.9870	0.014	1
5	3.662	3.482	0.000	0.000	2.0125	-0.011	2
6	2.704	2.580	0.000	0.000	0.9902	0.011	1
7	2.329	2.191	0.000	0.000	0.9989	0.002	1

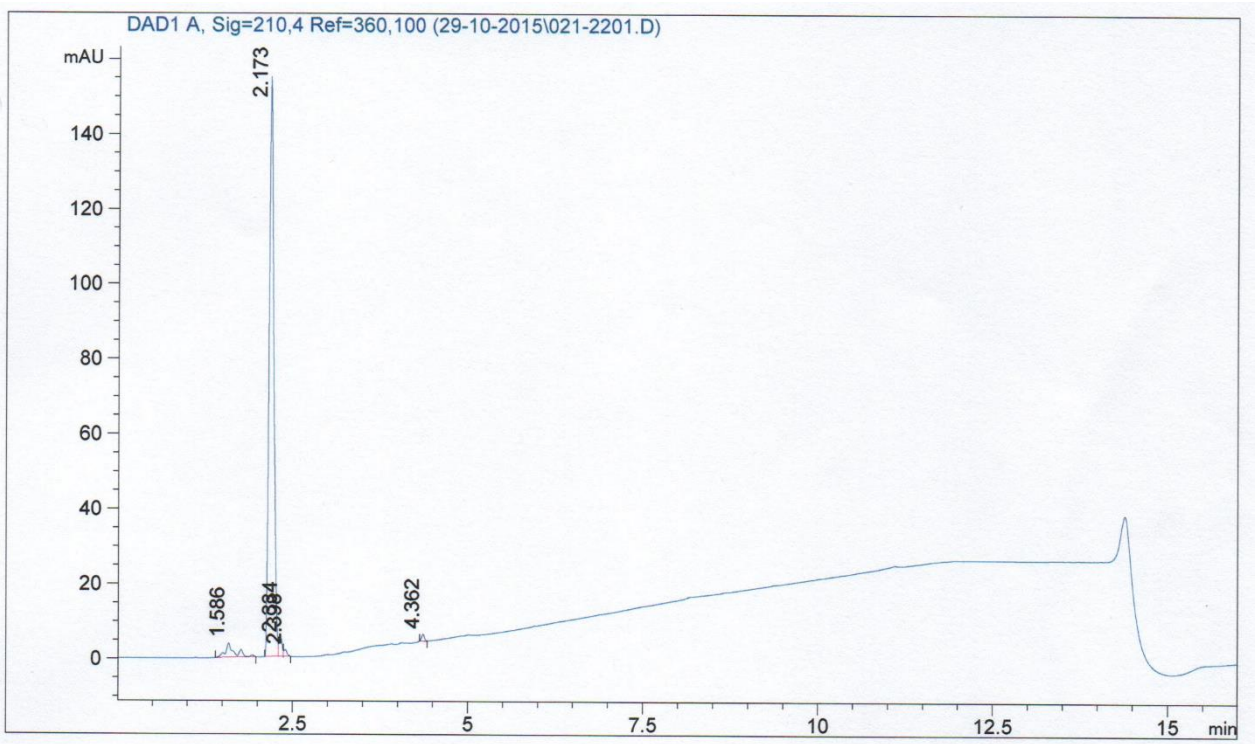
7.3 HPLC spectra

7.3.1 Diethyl 1-acetyl-3,6-dihydropyridine-2,2(1H)-dicarboxylate (3)



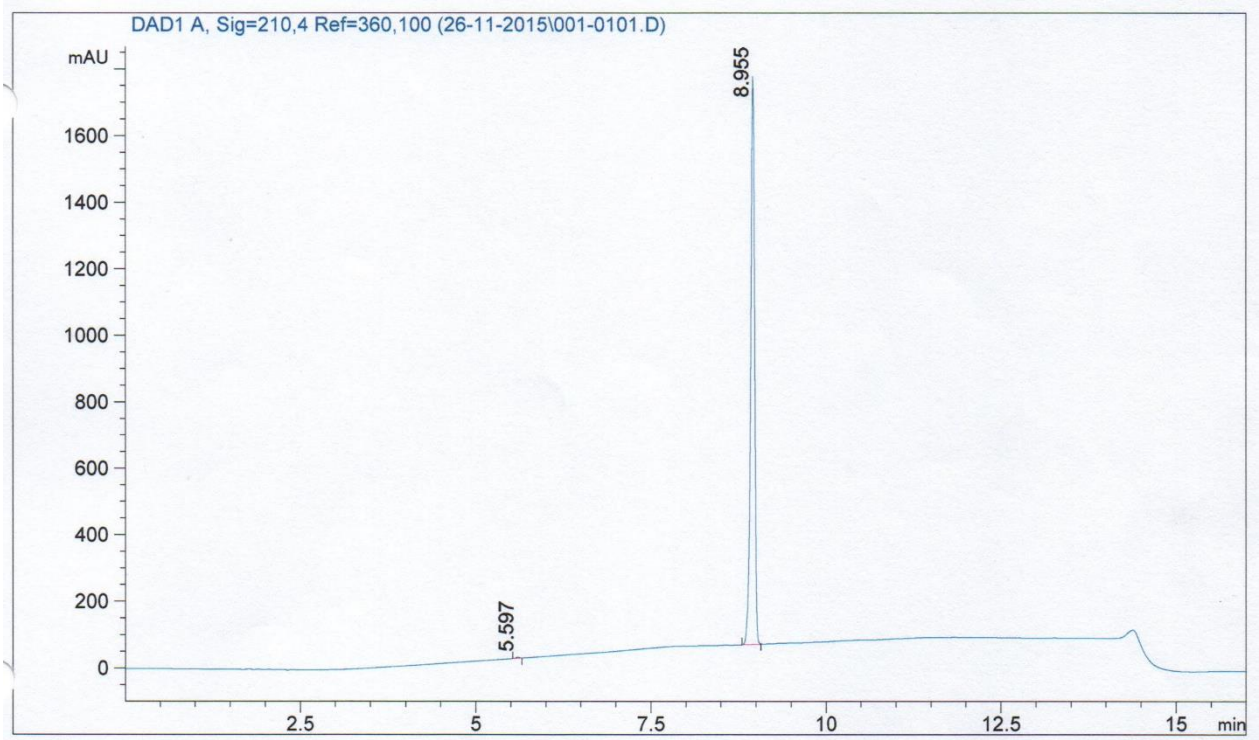
Ret.-Zeit	Substanzname	Peak - Fläche	Gehalt g/kg	Peakfl. in %
6.98		1633.0	0.00	9.27
7.42		103.4	0.00	0.59
7.63		189.8	0.00	1.08
8.42		15687.1	0.00	89.06
		17613.3		

7.3.2 Baikiain hydrochloride (4)



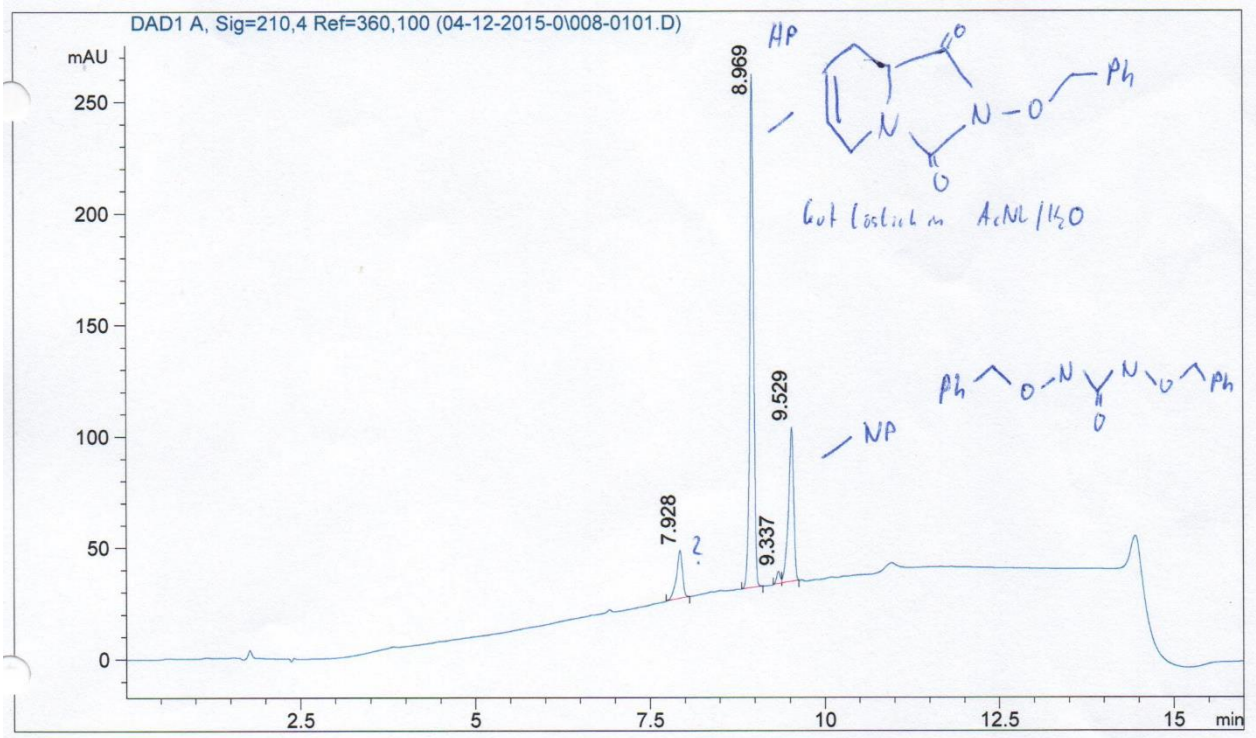
Ret.-Zeit	Substanzname	Peak - Fläche	Gehalt g/kg	Peakfl. in %
1.59		36.1	0.00	4.58
2.17		725.4	0.00	92.08
2.33		14.8	0.00	1.88
2.40		5.4	0.00	0.69
4.36		6.0	0.00	0.76
		787.7		

7.3.3 2-(Benzyloxy)-8,8a-dihydroimidazo[1,5-a]pyridine-1,3(2H,5H)-dione (10) from Baikiain ester hydrochloride



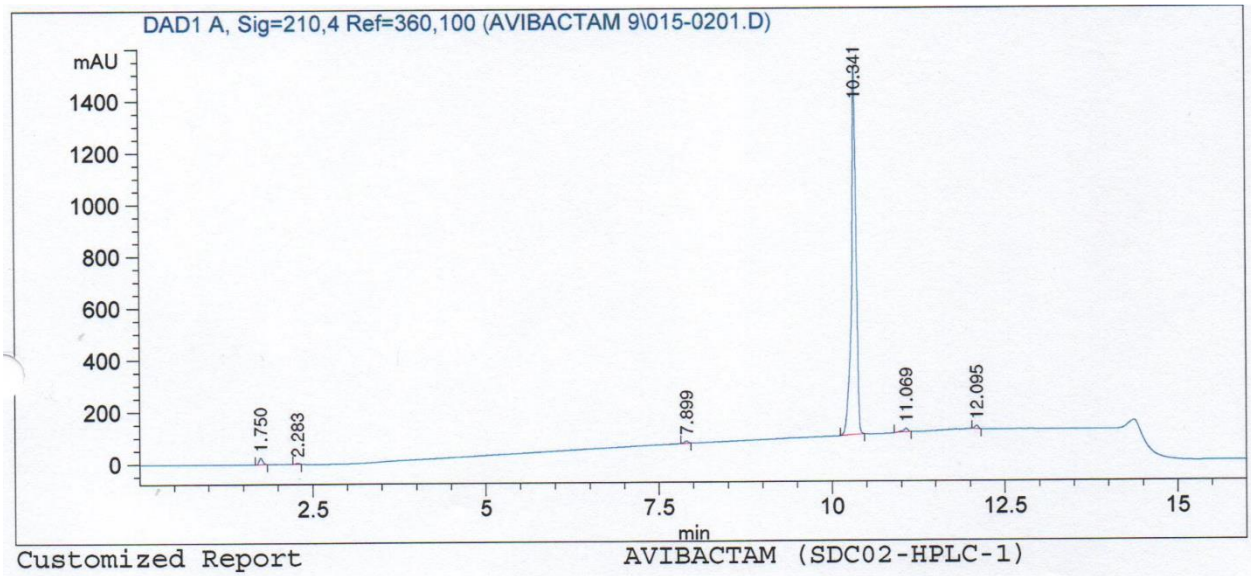
Ret.-Zeit	Substanzname	Peak - Fläche	Gehalt g/kg	Peakfl. in %
5.60		10.5	0.00	0.16
8.96		6610.1	0.00	99.84
		6620.6		

7.3.4 2-(Benzyloxy)-8,8a-dihydroimidazo[1,5-a]pyridine-1,3(2H,5H)-dione (10) from Baikiain amide



Ret.-Zeit	Substanzname	Peak - Fläche	Gehalt g/kg	Peakfl. in %
7.93		131.1	0.00	9.36
8.97		888.9	0.00	63.44
9.34		20.7	0.00	1.48
9.53		360.4	0.00	25.72
		1401.2		

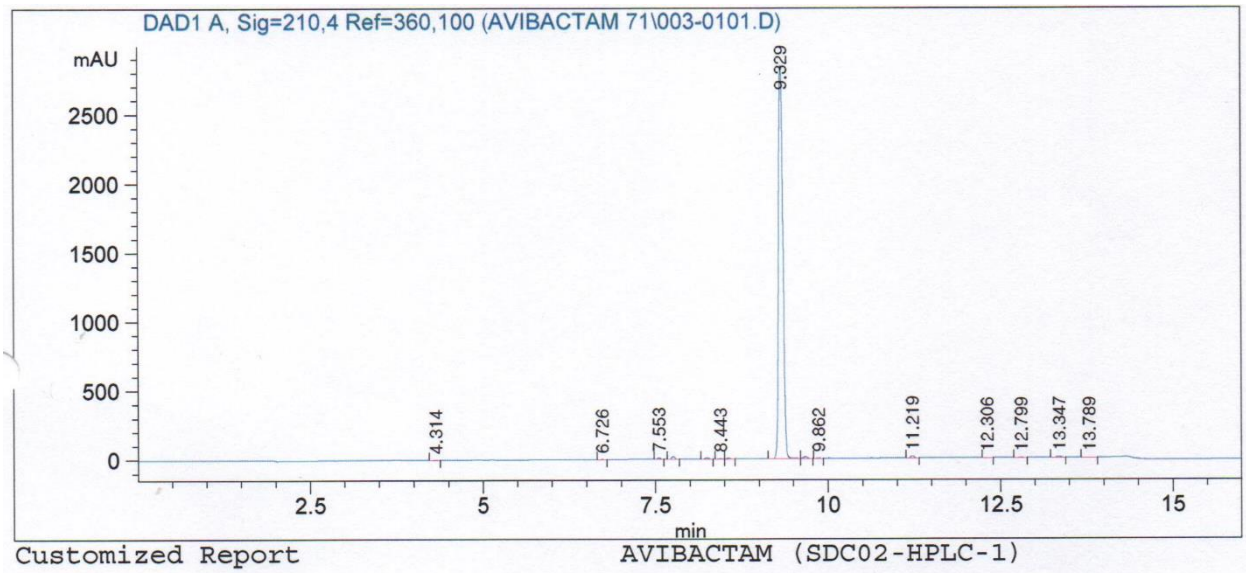
7.3.5 4-Nitrophenyl (benzyloxy)carbamate (12)



Customized Report AVIBACTAM (SDC02-HPLC-1)

Nr.	RT [min]	Area	Area %	Name
1	1.75	91.85	1.35	
2	2.28	14.53	0.21	
3	7.90	35.55	0.52	
4	10.34	6564.24	96.19	
5	11.07	73.02	1.07	
6	12.10	45.28	0.66	
Total		6824.47	100.00	

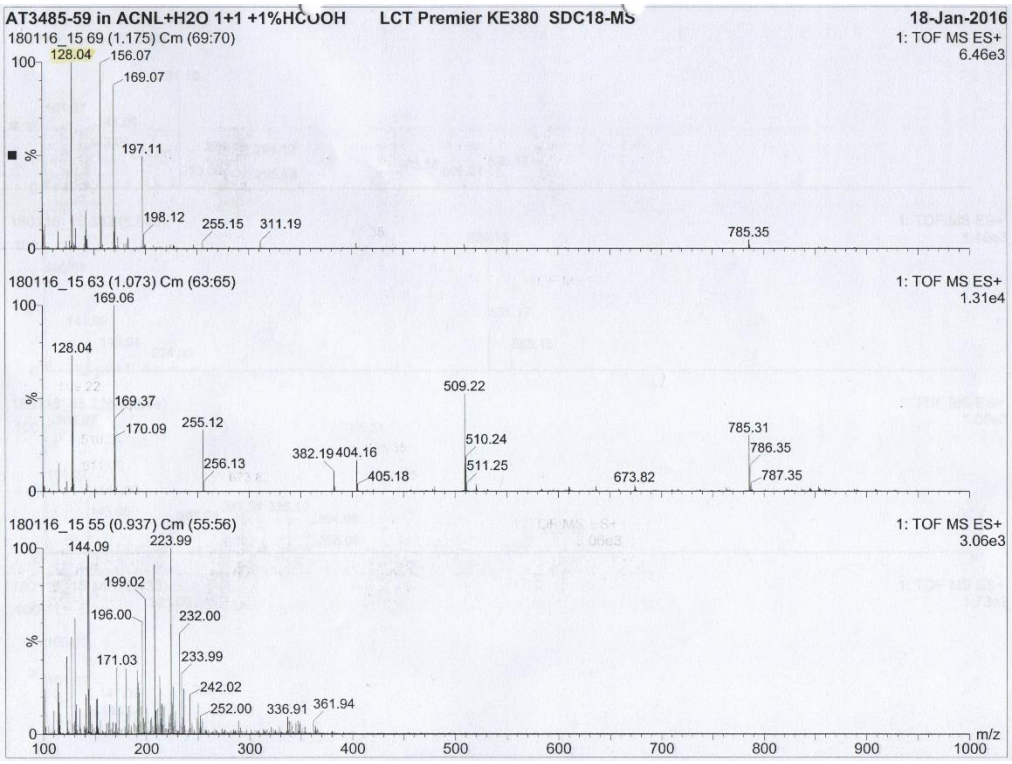
7.3.6 2-(Benzyloxy)-8,8a-dihydroimidazo[1,5-a]pyridine-1,3(2H,5H)-dione (10) with 4-Nitrophenyl (benzyloxy)carbamate

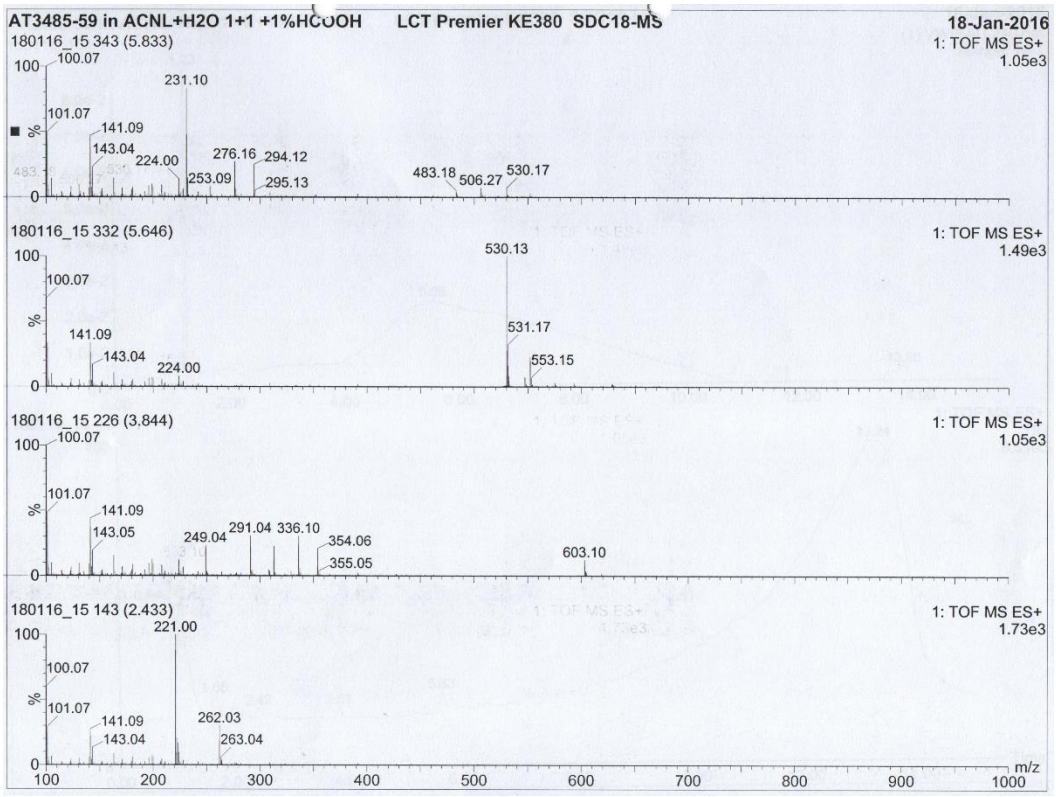


Nr.	RT [min]	Area	Area %	Name
1	4.31	2.88	0.02	
2	6.73	2.85	0.02	
3	7.55	29.38	0.23	
4	7.76	58.53	0.45	
5	8.25	27.87	0.22	
6	8.44	10.35	0.08	
7	8.56	21.25	0.17	
8	9.33	12559.51	97.56	
9	9.67	41.12	0.32	
10	9.86	6.18	0.05	
11	11.22	49.22	0.38	
12	12.31	5.08	0.04	
13	12.80	10.38	0.08	
14	13.35	40.81	0.32	
15	13.79	8.03	0.06	
Total		12873.44	100.00	

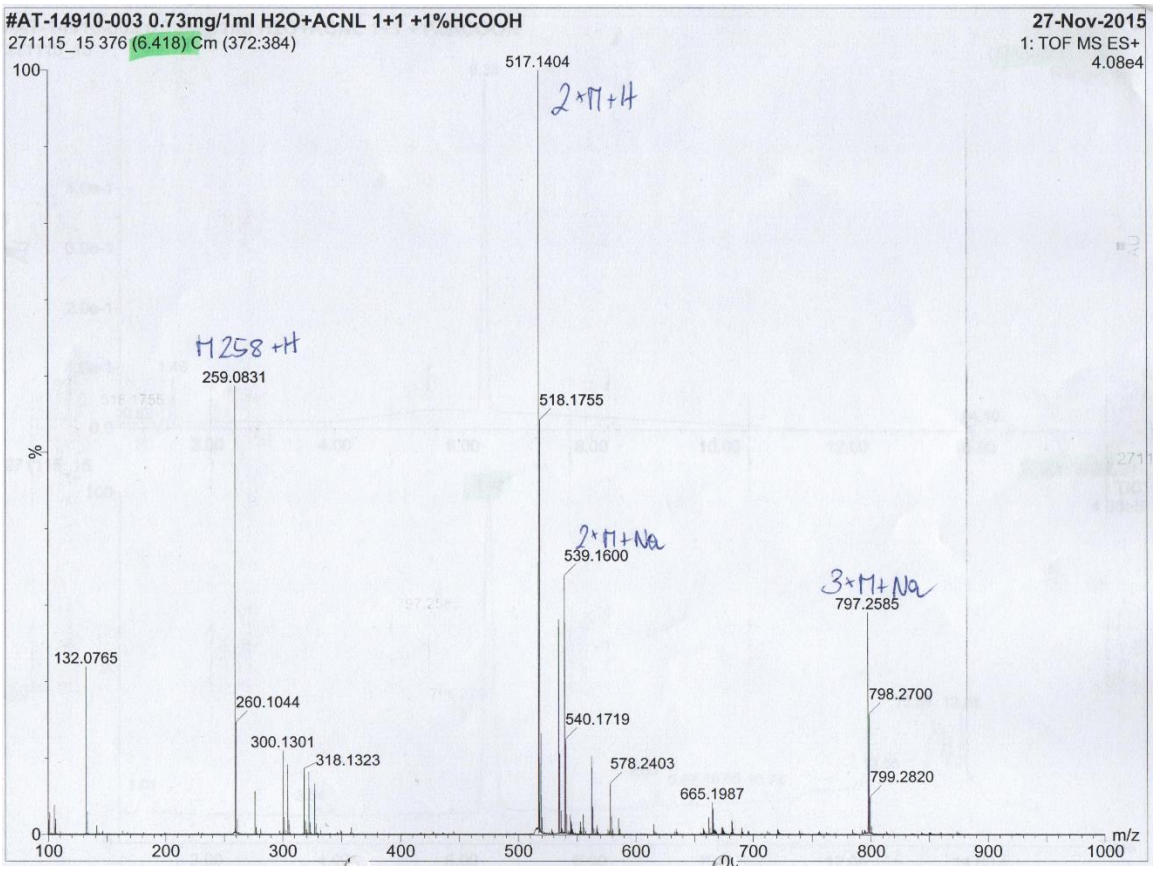
7.4 Mass spectrometry

7.4.1 Baikiain ester hydrochloride (4)

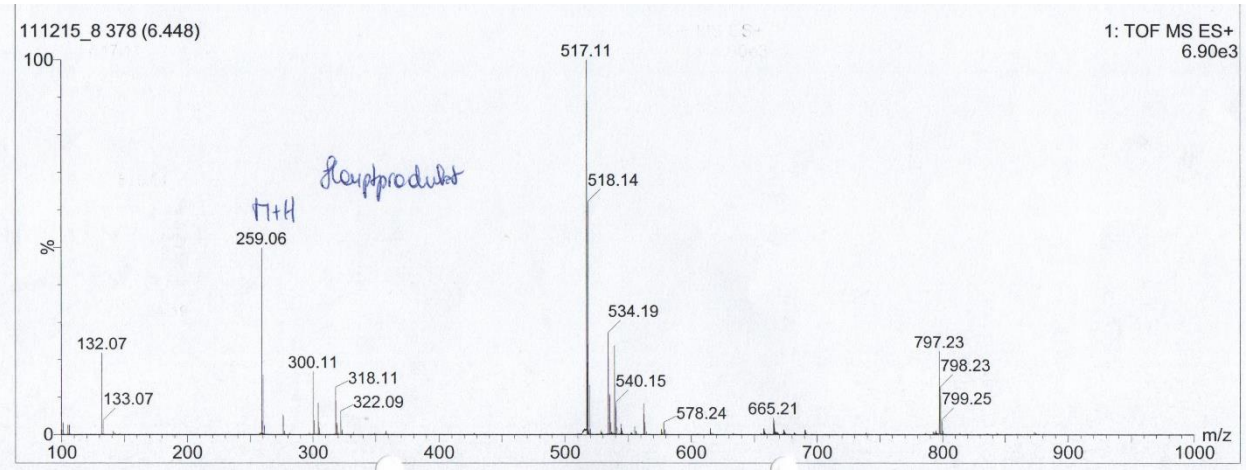




7.4.2 2-(Benzyloxy)-8,8a-dihydroimidazo[1,5-a]pyridine-1,3(2H,5H)-dione (10) from Baikiain ester hydrochloride

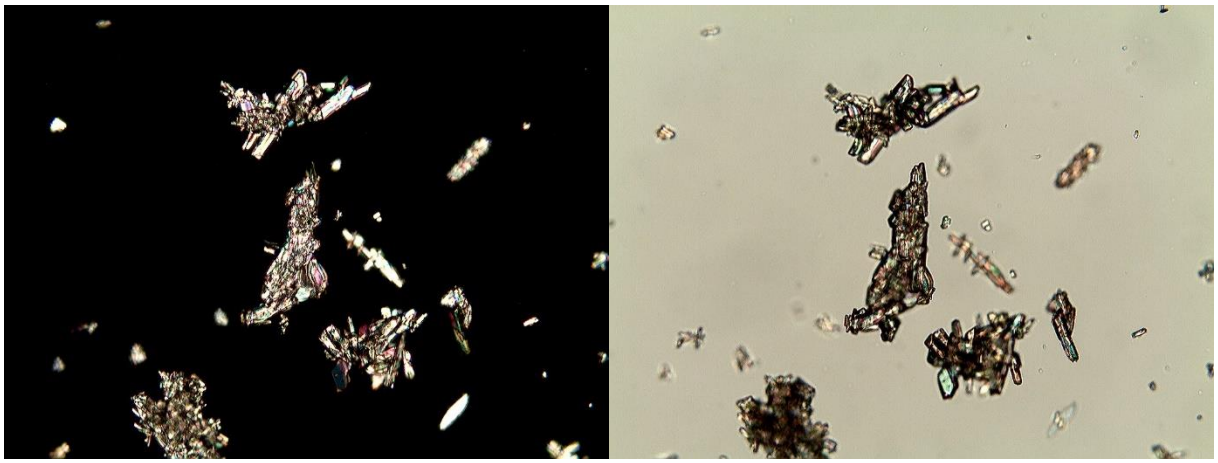


7.4.3 2-(Benzyloxy)-8,8a-dihydroimidazo[1,5-a]pyridine-1,3(2H,5H)-dione (10) from Baikiain amide

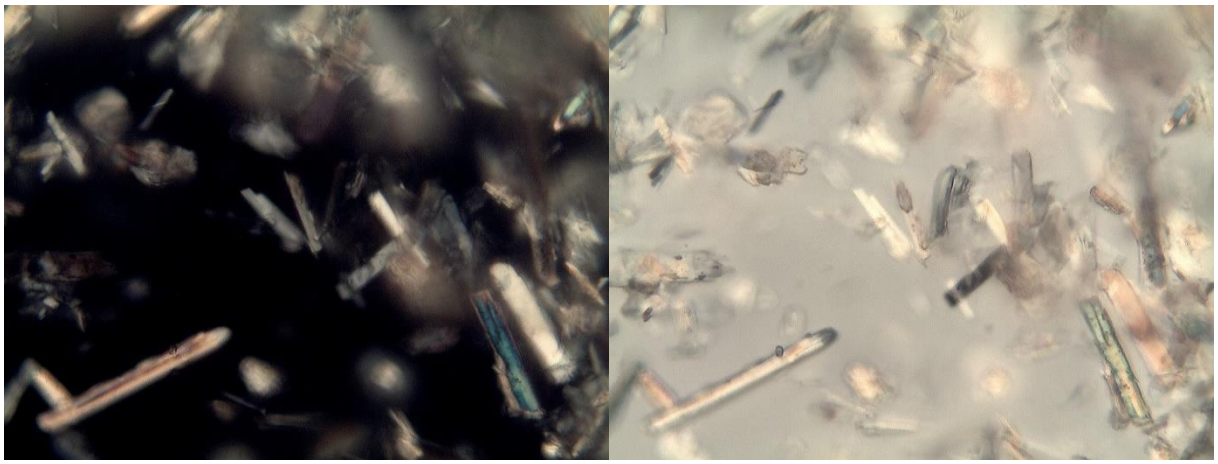


7.5 Petrographic microscope analysis

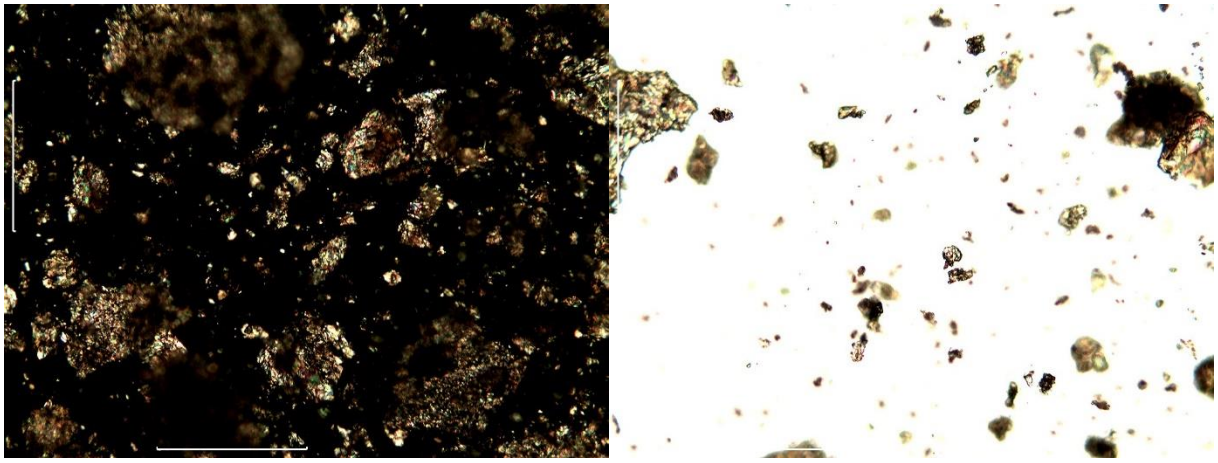
7.5.1 Baikiain hydrochloride (4)



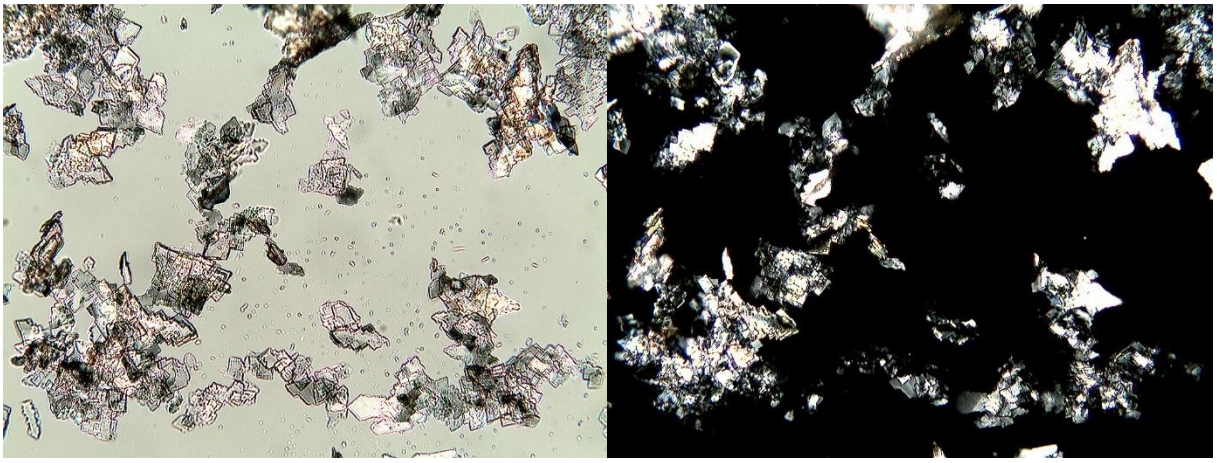
7.5.2 Baikiain ester hydrochloride (5)



7.5.3 Baikiain amide (6)



7.5.4 2-(Benzyloxy)-8,8a-dihydroimidazo[1,5-a]pyridine-1,3(2H,5H)-dione (10) from Baikiain ester hydrochloride



7.5.5 4-Nitrophenyl (benzyloxy)carbamate (12)