UiT
THE ARCTIC
UNIVERSITY
OF NORWAY

Department of Chemistry, Faculty of Science and Technology

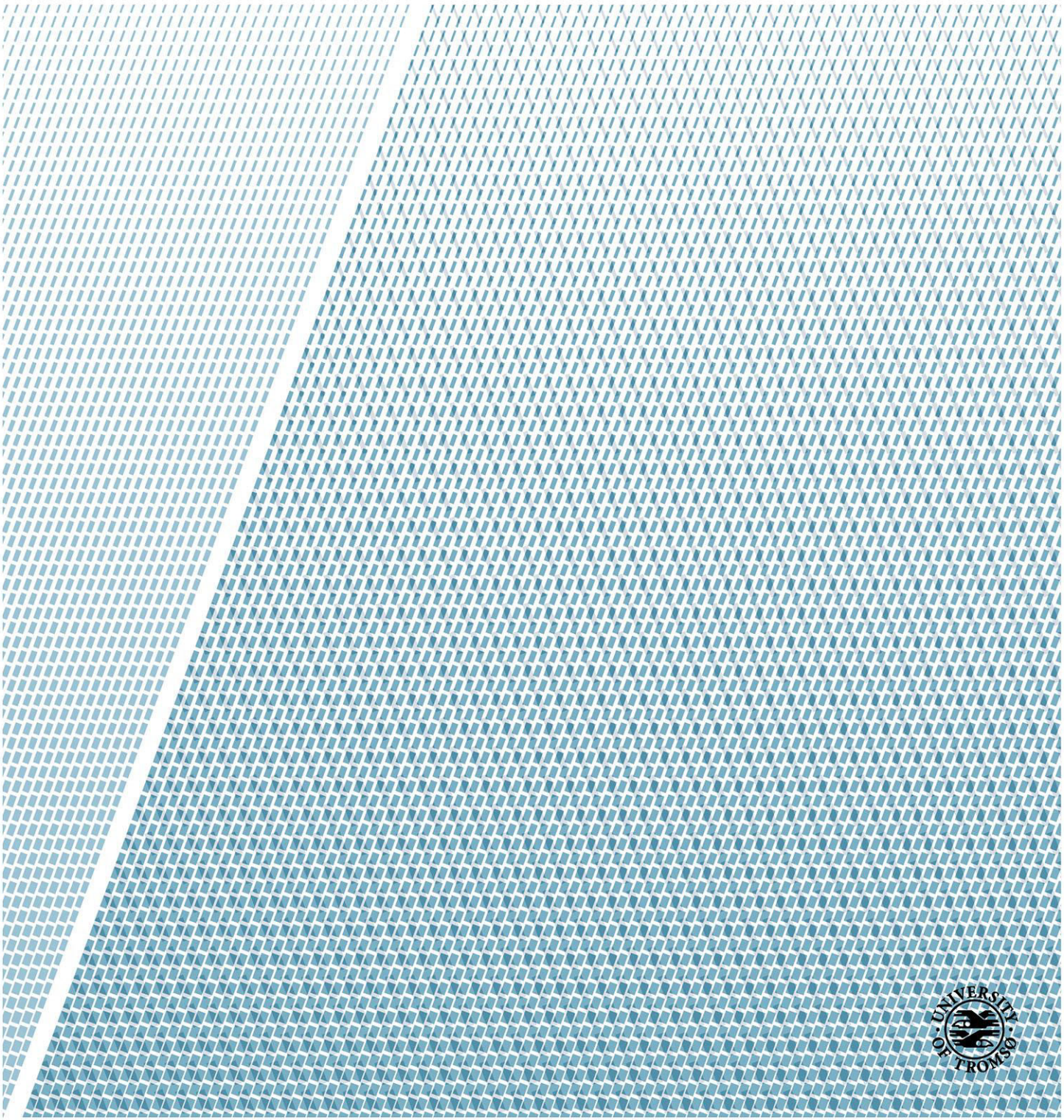
Studies Towards the Synthesis of DKP Analogues

Investigations of Bis-lactim Ether Reactivity with Aldehydes and Ketones

—

Arslan Kadysjev

KJE-3900 Master thesis in Organic Chemistry, May 2016



Acknowledgments

Throughout my master program there have been many people that offered support and guided me through my chemistry related work and other complications in life. Foremost I would like to thank my supervisor Associate Professor Annette Bayer at the University of Tromsø. In the last years she has given me a lot of constructive feedback as well as theoretical and practical help that I am very grateful for.

I would also like to thank my co-supervisors Associate Professor Jørn Hansen for offering me theoretical guidance; Dr. Magnus Engqvist and postdoctoral researcher Krisztian Bogar for supervision of my laboratory work.

A big thank you to the engineers Jostein Johansen, Truls Ingebrigsten and Arnfinn Kvarsnes at the University of Tromsø for help they provided with NMR, HRMS, GC-MS and IR.

Another thanks to Dr. Muhammad Zeeshan and Yngve Guttormsen for their advice and guidance with chemistry related work, their friendship and other help with diverse things.

I would also like to thank fellow students, department members and others who have helped me in any way throughout my master program.
Lastly, I thank my family Marat, Elena and my older brother Ernest for being there for me and for their moral support.

Abstract

A group of molecules with the name diketopiperazines (DKP) are called so, because of their diketopiperazine ring as the core structural unit. Mainly there are 3 types of diketopiperazies which only differ in in the position of the keto group, one type has the keto groups in 2,3- position; another in 2,5-; and the third in 2,6- position. The 2,5-isomer has attracted great interest, because of compounds that have the same core structure unit occur in nature. One good example of such compounds that have been studied lately is Barettin. Since DKPs have some similarities to peptides and are biologically active natural products, like Barettin, studies in synthesis of DKP analogues have been performed to use DKP analogues to improve the metabolic properties of peptides.

In this thesis, studies towards the synthesis of DKP analogues from the 2,5- isomer are described. The preparation of 2,5-diketopiperazine itself is described by two different methods. The first one is the more general approach and the second method is microwave assisted synthesis. Exploration of the use of Schöllkopf bis-lactime ethers in the synthesis of functionalized DKPs. The Schöllkopf ether was studied in a range of transformations through aldol condensation with aldehydes and ketones with various bases followed by dehydration.

Both 2,5-DKP syntheses and alkylation were found to be successful whereas the aldol condensation step turned out to be a little bit problematic. Through the course of the project a range of compounds were synthesized for analogue library.

Abbreviations

13C-NMR Carbon-13 nuclear magnetic resonance
1H-NMR Proton nuclear magnetic resonance
BuLi Butyl lithium
DBU 1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM Dichloromethane
DKP Diketopiperazine
DMF N,N-dimethylformamide
DMSO Dimethylsulfoxide
DNA Deoxyribonucleic acid
ESI Electrospray ionization
GC-MS Gas Chromatography with Mass Spectrometry detector
HRMS High Resolution Mass Spectrometry
IR Infrared (spectroscopy)
LDA Lithium diisopropylamide
LiHMDS Lithium hexamethyldisilazane
MeCN Acetonitrile
MTBE Methyl tert-butyl ether
MW Microwave
NMR Nuclear magnetic resonance
ppm Parts per million
tBuOK Potassium tertbutoxide
TFA Trifluoroacetic acid
THF Tetrahydrofuran
TLC Thin layer chromatography
TMP Magnesium chloro 2,2,6,6-Tetramethylpiperidide Lithium Chloride Complex
SN_2 Bimolecular nucleophilic substitution

Table of contents

Contents

1 Aims of the thesis

DKPs are a group of molecules that have diketopiperazine ring as their core structural unit. There are three types of DKPs which only differ in the position of the keto-group. In this project out of all these three, the focus was only on the 2,5-isomer, simply because it is the core structure of the natural product Barettin. Barettin is a natural product that was isolated from the marine sponge *Geodia barretti*[1]. Below are structures of Barettin and of how a general analogue would look like.

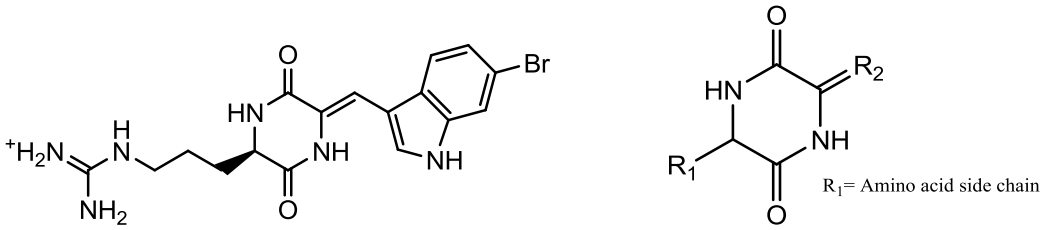


Figure 1. Barettin and the general structure of an analogue, R_2 is anything in this case.

There are many aspects of Barettin and its analogues that makes them so interesting. Foremost, studies have shown that Barettin has biological activity, which is a combination of anti-inflammatory and antioxidant activities [1]. This means that Barettin and its analogues with the same core structure, but different R_2 group can be used in the development of potential drug candidates in various medicinal areas and purposes. When you attempt to synthesize new molecules while using a previously tested procedure with similar structure, it does not necessarily go as planned. So another reason to make these compounds is for the sake of finding out how to do it, and by finding out how to synthesize different analogues we can create a compound library which will give us an overview over which analogues are possible to make and those that are not, as well as which analogues may or may not have biological activity.
The aims that were focused on and are presented in this project were:

- Explore the use of Schöllkopf bis-lactime ethers in the synthesis of functionalized DKPs.
- Can Schöllkopf bis-lactime ethers undergo aldoladdition or –condensation reactions?
- What type of substrates can be used in the reactions?
- Can the reactions be performed without loss of stereochemistry?

2 Background

2.1 Barettin

Barettin is a natural product that has 2,5-diketopiperazine as its core structural unit with arginine and tryptophan in 3,6 positions respectively. It was first isolated and described in 1986 from the marine sponge *Geodia barretti* that was harvested outside of Northern Norway. The molecular structure that was assigned to Barettin in 1986 is different than the one that was assigned later in 2002[2], but the molecule is in fact the same. This could be because of the equipment that was used in 1986, produced fragmentations of Barettin which led to misassigment of the correct structure. **Figure 2** presents these two structures.

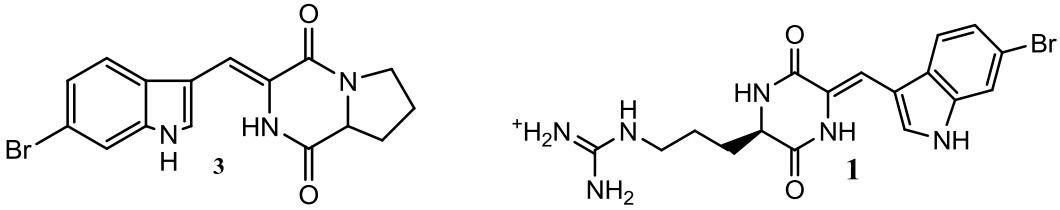


Figure 2. Structures of Barettin and from 1986 and 2002 respectively.

Today it is known that Barettin is a biologically active compound. Barettin has shown strong antioxidant activity in biochemical assays as well as in lipid peroxidation assays. Later it was also found that Barettin has yet another function, namely it was able to inhibit the secretion of inflammatory cytokines IL-1β and TNFα from LPS-stimulated THP-1 cells. Therefore Barettin could be a possible drug candidate to prevent the development of atherosclerosis[1]. As usual, most organisms do not produce large amounts of a particular chemical at once, simply because it is not needed, and sponges like *Geodia barretti* is no exception. Unless organisms have been specially modified in a lab. Since large amounts of Barettin could not be extracted from the sponge due to many reasons, a more synthetic approach had to be developed on how to synthesize it in a lab. Today there are several methods on how to do it, and just to mention some of them are the Schöllkopf method and Horner–Wadsworth–Emmons type reaction.[30-32,34]

2.2 Amino acids

A large portion of our cells, muscles and tissues is made up of amino acids, and there are 20 common amino acids. These amino acids have very important functions in the body like for example, they give the proper function to organs, gland, tendons and arteries. They are essential in repairing the body when it is damaged, they transport and store nutrient, giving cells their structure and many other functions which are no less important than others.[3]

But at a given point in time there are not many free amino acids that just travel from place to place within the body, amino acids are mostly used for construction of proteins which carry out the functions described above. About 20% of a human body consist only of proteins. The construction of proteins happen in ribosomes, where mRNA that carries the specific codon for a particular amino acid and tRNA that carries the anticodon and the appropriate amino acid come together to make a protein. This process is called translation.[3]

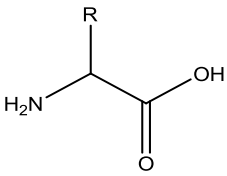


Figure 3. General structure of an amino acid in its un-ionized form. The R-group is the characteristic side chain that separates amino acids.

However, amino acids can also be synthesized in a lab and there are several way of doing it. Maybe one of the oldest methods is where you start by brominating the alpha carbon of a carboxylic acid. Then by using nucleophilic substitution with ammonia you can transform the alkyl bromide to the amino acid. Some other methods are the Strecker amino acid synthesis [4][5], where you treat an aldehyde with ammonia and potassium cyanide, which will give an α-amino nitrile. Then by hydrolyzing the nitrile you can get the α-amino acid. Other procedures with the use of asymmetric auxiliaries[6] or asymmetric catalysts[7] have been developed.

2.3 The Schöllkopf method

The Schöllkopf method or also commonly known as Schöllkopf bis-lactim amino acid synthesis is a method for the asymmetric synthesis of chiral amino acids that was established by Ulrich Schöllkopf in 1981[30-32]. This method involves the making of a dipeptide, cyclization, transformation of the keto groups into ethers, substitution of a glycine proton with something else and lastly breakage of the cyclization which is described more in detail below. The typical procedure involves a glycine as the substrate and a valine as the chiral auxiliary.

Two amino acids bind together to form a cyclic dipeptide which is also known as 2,5-diketopiperazine. An O-alkylation is performed with a Meerwein's salt as the alkylating agent, which result in the bis-lactim ether. Then one of the glycine protons is removed by a base, usually an organometallic base. The carbanion then reacts with an alkyl halide and it seems that this is the decisive step for stereoselectivity of the whole method. One face of the carbanion is shielded by the isopropyl group of valine, which creates steric hindrance for the alkyl halide; this forces the alkylation to happen on the opposite side of the isopropyl group which gives this reaction strong preference for just one enantiomer[12-13, 30-32]. But then again, the stereoselectivity might also be influenced by the bulkiness of an alkyl halide, or an aldehyde if it is used instead. In the final step, there is a possibility to first go from bis-lactim ether back to DKP with the help of a weak acid; or to separate the two amino acids through an acid hydrolysis directly from bis-lactim ether to form two amino acid esters with the help of a strong acid, as presented in **Figure 4** below.

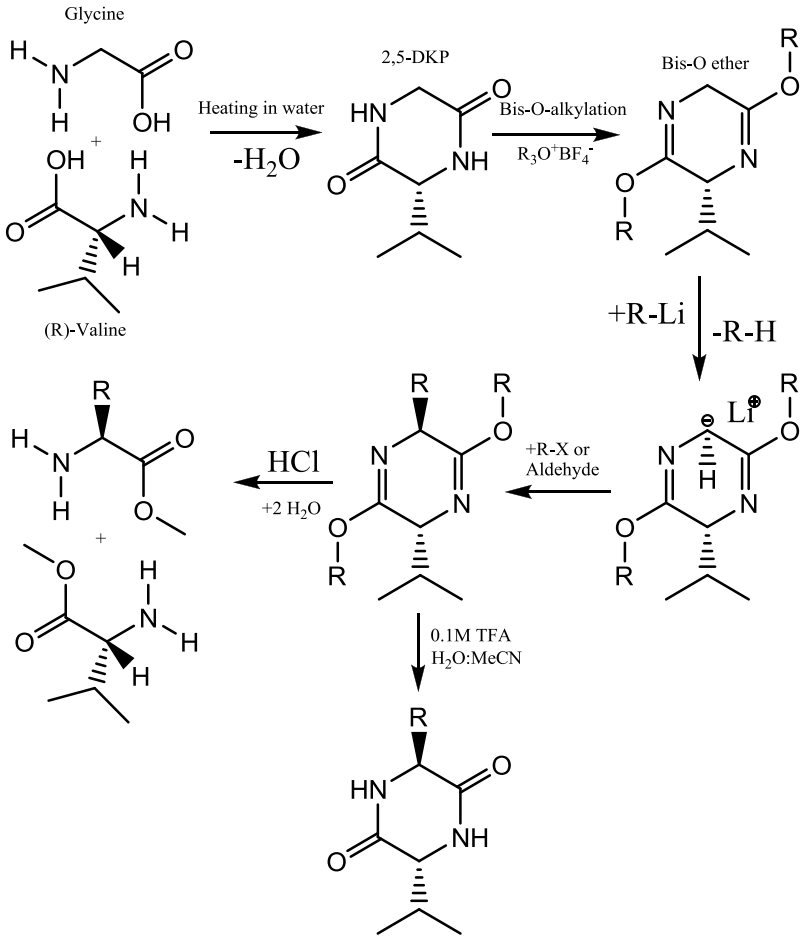


Figure 4. Reaction pathway of the Schöllkopf procedure.

2.3.1 Dipeptides

When the amine group of one amino acid reacts with the carboxylic group of another amino acid they become joined through an amide linkage. This newly formed bond is the peptide bond. This type of polymerization of amino acid is how proteins are made. **Figure 5** below presents the reaction mechanism of dipeptide formation.

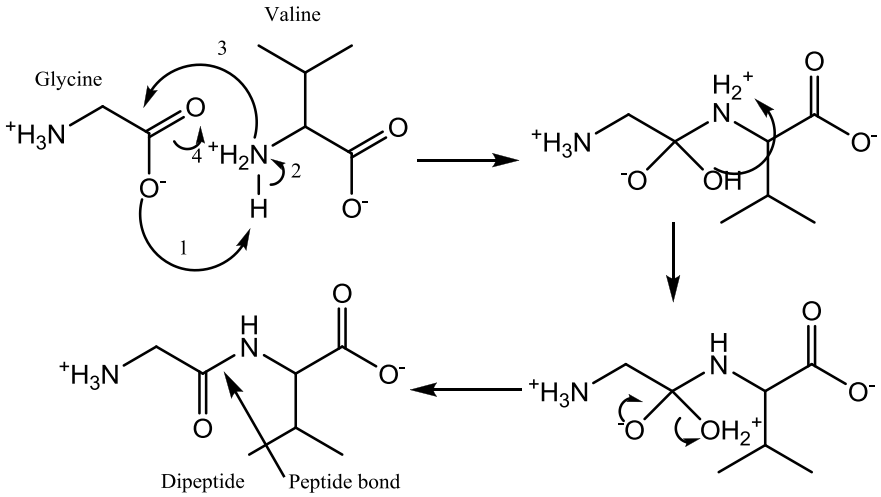


Figure 5. Glycine and Valine join together to form a dipeptide.

There is a common way to synthesize longer amino acid chain, the method is called solid-phase peptide synthesis. This method uses aromatic oxime derivatives (imines), **Figure 6,** of amino acid as activated units. These derivatives are added in a sequence onto the growing peptide chain, which is held together by a solid resin support system[8]. By varying the types and order of amino acids, with this method it is easily possible to synthesize long amino acid chains.

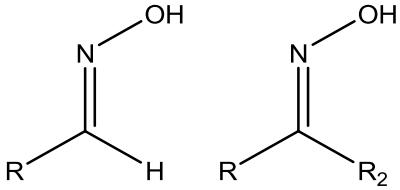


Figure 6. General structures of oximes. An aldoxime and ketoxime respectively.

2.3.2 Cyclization

The name cyclization gives away what this topic is about, namely the cyclization of molecules. In short, there are many ways to make a cyclic compound, it all depends on what kind of molecule do you want to make cyclic. Like for example, there is the Nazarov cyclization for the synthesis of cyclopentenones,[9] the radical cyclization which goes through the formation of radical intermediates,[10] or the Diels-Alder reaction.[11] However, we are interested in another type of cyclization, namely the one presented in **Figure 4**, the cyclization of a dipeptide which is presented below in **Figure 7**.

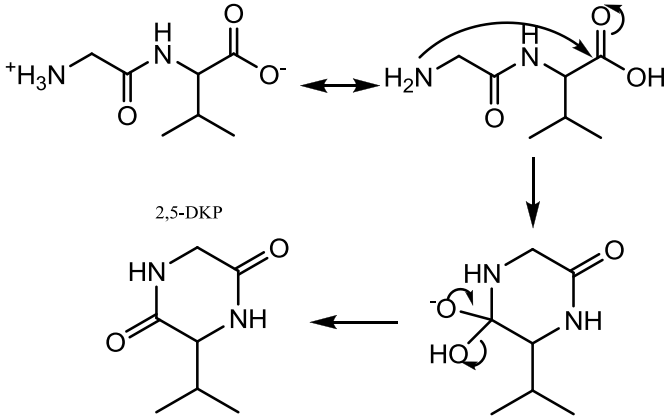


Figure 7. Reaction mechanism of the dipeptide cyclization into 2,5-DKP. Depending on which procedure is used, this reaction can be carried out in different solvents.[12][13] Some proton transfers are skipped in the figure.

The mechanism presented in **Figure 7** is the cyclization of the glycine-valine dipeptide. Depending on which procedure is used, this reaction can be carried out in different solvents.[12][13] But, this reaction method is not only applicable to glycine-valine dipeptides, it can also involve other amino acids.

2.3.3 Diketopiperazine

As mentioned earlier there are three isomers of diketopiperazine, one with the keto-groups in 2,3-, another with keto-groups in 2,5- and the third isomer with keto-groups in 2,6- positions presented in **figure 8**. All of them can be found in nature through biochemical synthesis, but they can also be synthesized in a lab.

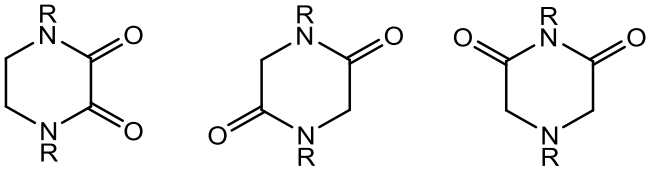


Figure 8. Diketopiperazine; 2,3-, 2,5- and 2,6-isomers.

All of the three isomers are frequently used in medicine and although the structures are quite similar, the areas in which each isomer I used in, vary. The 2,3-isomer for example can be found in antibiotics such as piperacillin, cefoperazone and bicyclomycin presented in **Figure 9**.[14]

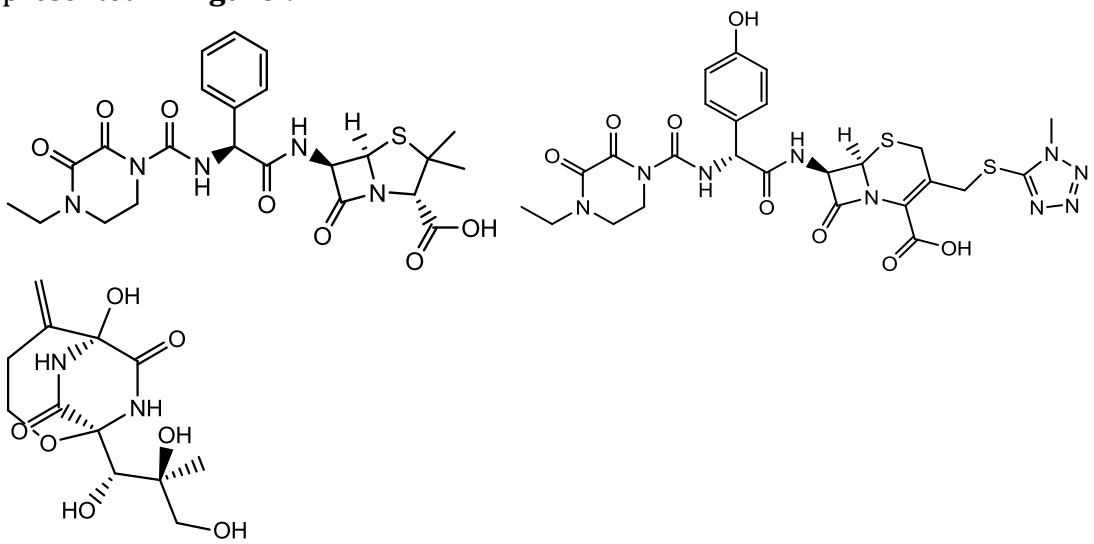


Figure 9. Structures of piperacillin, cefoperazone and bicyclomycin respectively.

The 2,5-isomer as described above is the core structure of Barettin which has some medicinal application. The 2,5-diketopiperazines has also other biological activities including antifungal,[15] antiviral,[16] antitumor[17] and antibacterial[18] activities. In some biochemical synthesis this isomer is also frequently generated as degradation products in the syntheses of oligopeptides.[14] Another application is in commercialized drugs such as Tadalafil, Gliotoxin, Retosiban, Epelsiban and Aplaviroc.
Lastly, the 2,6-isomer of DKPs has been of much interest in medicinal chemistry as antiproliferative agents through the inhibition of DNA topoisomerase II.[14]

2.3.4 Bis-O-alkylation and dealkylation

The idea behind bis-o-alkylation of diketopiperazines is quite simple if we look at the structure. First the nitrogen atoms would have to be deprotonated, the resulting structure will be stabilized by resonance. Now that we have negatively charged oxygens, an alkylation is possible with the appropriate reagent. The reagents can be for example alkyl halides of R-X type or Meerwein salts like $Me_3O^+BF_4^-$.[13] The mechanism is presented in **Figure 10**.

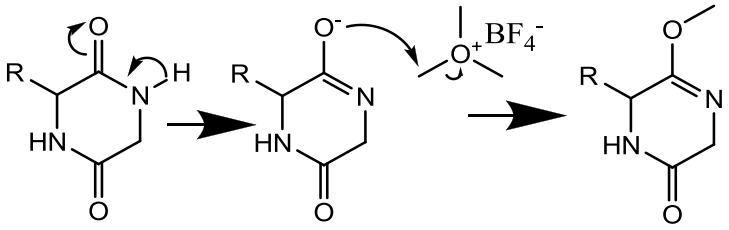


Figure 10. Reaction mechanism of bis-o-methylation. Reaction has only been shown on one side for simplicity.

If we now want to go back, a possible dealkylation reaction might be something like treating the product from **Figure 10** with an acid in water which will result in substitution where the methoxy-groups are exchanged with hydroxyl-groups. The resulting structure from this step is an isomer of the starting material. However, according to some studies that was performed,[19-21] if a strong acid such as HCl is used, then there is high chance to open the ring between nitrogen and carbonyl. While on the other hand, low concentrated TFA[20-21] or TMS-I[19] could be used to restore DKP from bis-lactim ether as shown in **Figure 11**.

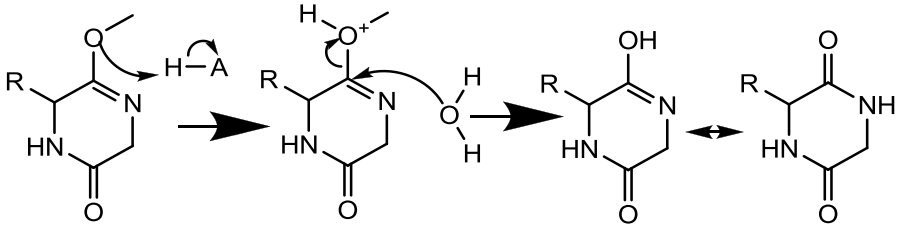


Figure 11. A possible reaction mechanism of the dealkylation process. The mechanism is shown only on one side for simplicity.

2.4 Importance of different bases

As one would expect, different bases have different strength and that makes them unique to one another, but all of them have a pH above 7. There is an enormous amount of different bases, everything from nonorganic, organic, inorganic, oxide mixtures and many other. The use of each base depends on how easily you can remove a proton that you want to remove, the more acidic and accessible the protons are in a molecule the easier is for them to be removed by a base.

The relevant bases that are used in this project are mostly strong/super bases of the R^-Li^+ type, BuLi, LiTMP, LDA and LiHMDS and RO^- type, Cs_2SO_3 and K-$tBuO^-$. Previously performed studies[19-23] have shown that these bases have the effect of deprotonation of diketopiperazines and other similar structures. The most important difference between these two types is the strength, R^-Li^+ are considered to be superbases and are stronger than RO^- type of bases. Another difference that is worth to mention is how big or bulky each of these bases are. This difference is only relevant in some cases and the reason for that is, it all depends on how easily the protons are accessible in a molecule. For example, a commonly used base that has a four carbon chain and not bulky, is BuLi ($pK_a \approx 50$[24]). While others of the same type like LiTMP, LDA and LiHMDS are quite bulky bases.

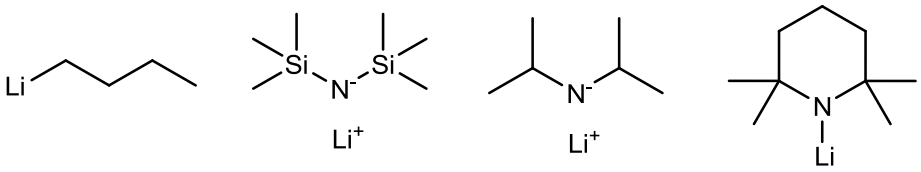


Figure 12. BuLi, LiHMDS, LDA and LiTMP respectively. Note that LiHMDS exists only as a cyclic trimer and BuLi as a tetramer/hexamer. The structures above are a simple representations of those.

2.5 Aldol addition or condensation

An aldol addition reaction is where an enol or an enolate ion reacts with a compound that has a carbonyl group to form a β-hydroxyaldehyde or β-hydroxyketone depending on which starting material is used. If this product is reacted further with either acid or base, a dehydration would happen, and this is the condensation part of the reaction.

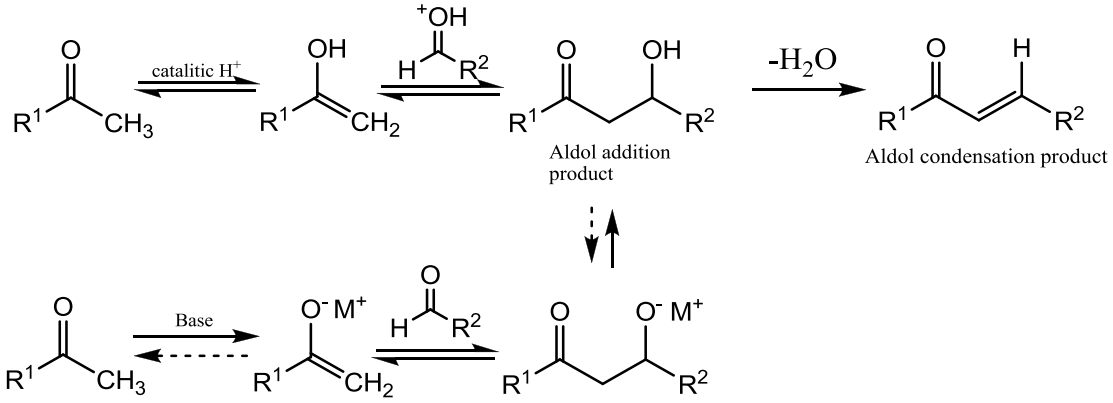


Figure 13. Aldol addition reaction with aldol condensation as the final product under basic and acidic conditions.

Studies have been performed on the use of various 2,5-diketopiperazines as the starting material in aldol condensation reactions.[22-23][25] The 2,5-DKPs used in those studies were more "activated" by having an acetyl group on both nitrogen atoms. This modification would prevent the formation of enolate ions and thereby activating/making the CH_2 protons more acidic, the base would easily remove one of the protons. Also by having an oxygen atom near the deprotonation site from an acetyl-group is good, especially if an R^-Li^+ base is used, since the oxygen would coordinate the base.

In this project we are using a bis-lactim ether similar to that in **figure 10 ,** The difference is that we use an ethyl- instead of a methyl group. Studies[26-28] have shown that even without those acetyl groups on the nitrogen atoms the reaction still works, and the general procedure is very similar. Whether there are acetyl-groups on the nitrogen atoms or not it cannot be looked at from the perspective of a general aldol condensation reaction. Because unlike the general aldol condensation reaction where an enol reacts with a protonated carbonyl of an aldehyde under acidic conditions, an aldol condensation where bis-lactim ether is used instead of an enol, would have to be under basic conditions. Otherwise there is a chance that the ether oxygen would be protonated and fall off like in **figure 11.** The reaction mechanism of aldol condensation is presented in **Figure 14**.

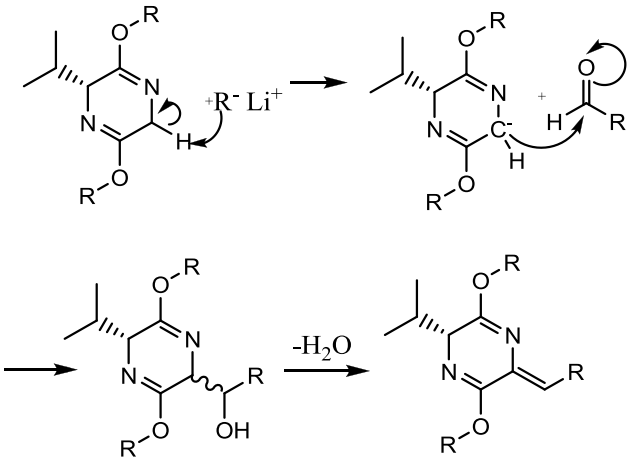


Figure 14. A possible reaction mechanism of an aldol addition/condensation reaction between bis-lactim ether and an aldehyde.

2.6 Alkylation

An alkylation reaction involves the transfer of an alkyl group from one molecule to another. There are several ways in which an alkyl group can be transferred to another molecule and that is through a carbocation, carbanion, carbene or a free radical. There are many variants of alkylating agents, and to mention just some of them are organometallic compounds, alkyl halides, Meerwein' salts and etc.; but, probably the most common way to do it is by using alkyl halides (R-X) as alkylating agents. Alkylation with alkyl halides usually happen with reagents that have the halogen atom is on either primary or secondary carbon atom, that way it would be more accessible for a nucleophile as shown in **Figure 15**.

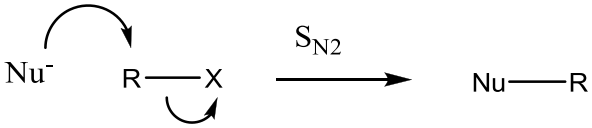


Figure 15. An alkylation reaction with a general alkyl halide as the alkylating agent.

If one would like to react a 2,5-diketopiperazine with an alkyl halide[29] instead of an aldehyde, the reaction mechanism would pretty much be the same except for the condensation step that is present in the aldol condensation reaction in **Figure 13**. General reaction mechanism of a bis-lactim ether reacting with an alkyl halide is presented in **Figure 16**.

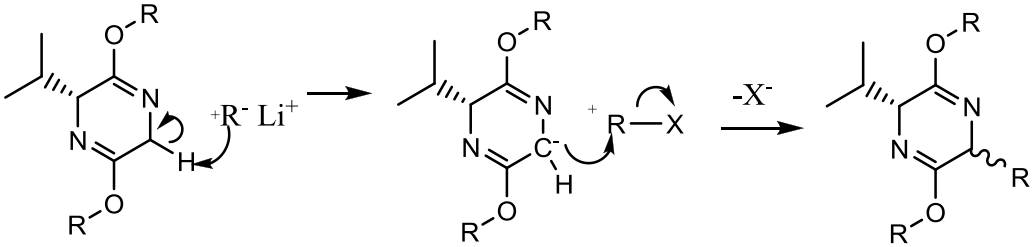
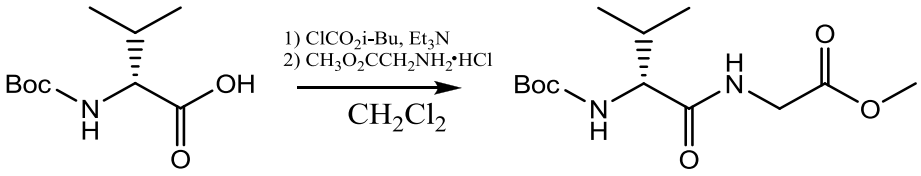


Figure 16. Reaction mechanism for alkylation of DKP.

3 Results and Discussion

3.1 Synthesis of the dipeptide (5)



4 **5**

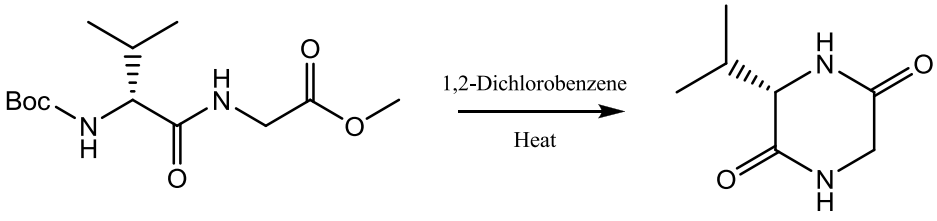
Scheme 1. Synthesis of Boc-N-valine-glycine-OMe (**5**)

Synthesis of the dipeptide **5** was done by first reacting Boc-D-valine **4** with isobutyl chloroformate and triethylamine in dichloromethane. Then, after some time reacted with a mixture of glycine methyl ester, triethylamine and dichloromethane as described above in **Scheme 1** and reported in literature.[12-13] Reaction gave a whitish solid product and recrystallization from cyclohexane resulted in pure white powder. The pure product was analyzed by ^{1}H and ^{13}C NMR, and spectra were in full agreement with reported data in literature.[12-13] The yield was 76%.
Note that some of the product was lost during the workup due to accident, so the yield could be higher.

3.2 Cyclization of the dipeptide (5)

Cyclization of the dipeptide **5** was performed by two different syntheses. The first synthesis was the general conventional heating process, and the second synthesis involved cyclization in a microwave. The main differences between these two procedures are the reaction times and solvents in which the reactions were carried out, other than that the idea of cyclization of the dipeptide is the same.

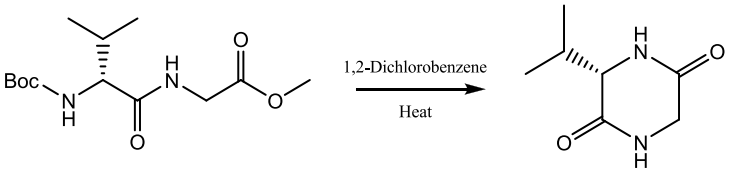
3.2.1 Cyclization of 5 by conventional heating.



5 **6**

Scheme 2. Synthesis of 2,5-diketopiperazine (**6**) by conventional heating

To synthesize 2,5-DKP **6**, dipeptide **5** was mixed with 1,2-dichlorobenzene, heated and stirred overnight. Then cooled down, filtered, washed with TMBE and left to dry at room temperature, **Scheme 2**. This experiment was performed one more time, where some of the conditions were different, to make more of 2,5-DKP **6,** and to see if it could be possible to increase the yield. Reaction conditions and results are presented in **Table 1**.
Table 1. Different reaction conditions and results for synthesis of (**6**).

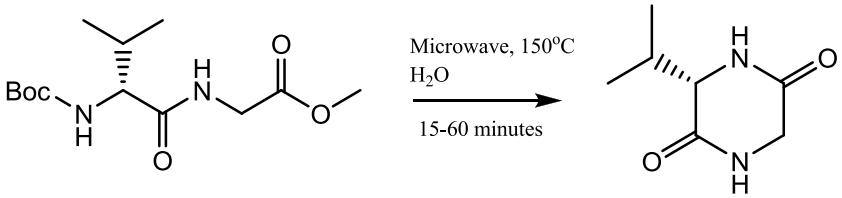


5 **6**

Entry	Temperature	Reaction time	Crude yield
a	172°C	20-21 hours	35%
b	151°C	20-21 hours	30%

The yield for conventional heating reaction reported in literature[13] is 69%, while the yields from this reaction method was only 35%[a] and 30%[b], which is almost twice as less of un-pure product. The ^{1}H NMR spectra gave weak signals for the peaks, probably due to small amounts of stuff used in the analysis, but they were mostly the same. One peak at 2.10 ppm (m, 1H) reported in the literature[13] is especially difficult to see on my spectra since it overlaps with something else. The splitting of some signals (multiplets) reported in the literature are not consistent with what my analysis gave, [13] this might be because the product used for ^{1}H NMR analysis was not pure. However the signals are in the same region with very similar ppm values.
As for purification of the product, 2,5-DKP **6** from this reaction, it was decided to wait until more was synthesized, and until synthesis of **6** was performed with microwave. Since the microwave synthesis gave better results, the product from this reaction method was never purified.

3.2.2 Cyclization of 5 by microwave irradiation

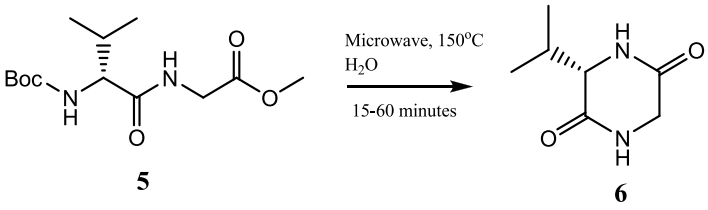


5 **6**

Scheme 3. Synthesis of 2,5-diketopiperaznie (**6**) by microwave irradiation

Dipeptide **5** was transferred to a special microwave vial with a magnetic stirring bar, filled with water, locked and run at 600 rpm, 150-170°C and for 15-60 minutes. After the reaction was done, the reaction mixture was cooled down to room temperature, and then water was evaporated on a rotary evaporator. The resulting product was a white solid, a recrystallization was attempted from EtOH:$CHCl_3$ (3:1) to yield 28% pure 2,5-DKP (**6**). This reaction was carried out additional times under different conditions for optimization and for synthesis of more compound (**6**). **Table 2** below presents the details of conditions used and which results they gave.

Table 2. Different reaction conditions and results for microwave assisted synthesis of (**6**).



5 **6**

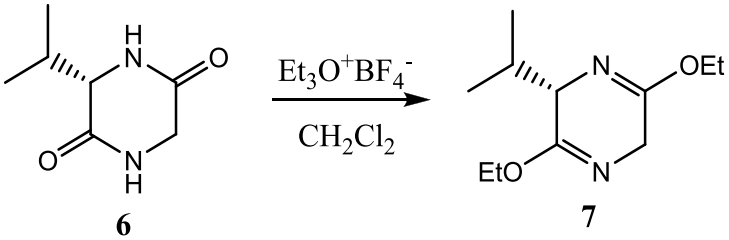
Entry	Temperature	Reaction time	Crude yield
a	150°C	15 minutes	28%
b*	170°C	60 minutes	95%
c*	160°C	2x30 minutes	96%
d*	150°C	75 minutes	89%

* is the syntheses after optimization,

The procedure used in the literature did not work for me,[12] in the article it was reported a yield of about 75%, while when I used the same conditions I only managed to get 28%[a]. So, an optimization of the reaction had to be done. Optimization of the procedure was rather straight forward, dipeptide **5** was first put into the microwave and turn on for 30 minutes hold-time. A sample was then taken out and analyzed by ^{1}H NMR, it was clear that there was still some unreacted starting material. The same was done after 15 more minutes and then again after another 10 minutes. After total of about 55 minutes reaction time, ^{1}H NMR analysis showed that pretty much everything of starting material was gone. Entries b-d show very good yields, even higher than that in literature.[12] The ^{1}H and ^{13}C NMR data were in full agreement with the published data. [12-13]

There was a lot of problems in the attempts to purify DKP **6**. The recrystallization from EtOH:$CHCl_3$ (3:1) as described in the article[12] did not work. ^{1}H NMR analysis showed that there was still small amounts of starting material and other impurities. This is probably due to insolubility of compounds **5** and **6** in EtOH:$CHCl_3$. It was found that compounds **5** and **6** have a good solubility only in water, it was tried out and showed no success. Since ^{1}H NMR analysis of the crude product 2,5-DKP (**6**) showed only small amounts of impurities, it was decided that it was good enough for the next step.

3.3 O-Ethylation of the 2,5-DKP (6) to form the bis-lactim ether (7)



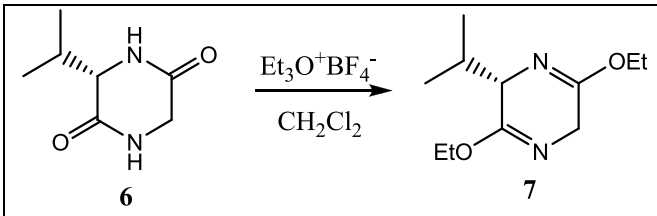
6 **7**

Scheme 4. Synthesis of bis-lactim ether (**7**)

Bis-lactim ether **7** was prepared by reaction between DKP **6** and a solution of $Et_3O^+BF_4^-$ in 1M methylene chloride, in the presence of CH_2Cl_2 as solvent, **Scheme 4**. This reaction was run several times and none of them gave good results. Even though ^{1}H NMR analyses of the crude products showed the characteristic signals for bis-lactim ether **7**, They were in such small amounts, that the column chromatography purification always gave bis-lactim ether **7** with impurities. Mostly, those impurities were either ethylation only on one side and ring opening.

The reagent used in this reaction is very sensitive to moisture in the air, and since the bottle with the chemical wasn't that new, it was thought that the reagent was mostly destroyed. A miscalculation in the amounts of $Et_3O^+BF_4^-$ solution needed for the reaction, might have been the cause of bad results. Note that this was not noticed when the reactions were performed, and since this reaction was attempted several times, this error is in all of them. As a result a new bottle of $Et_3O^+BF_4^-$ in its solid form was used instead.
The reaction procedure for the reagent $Et_3O^+BF_4^-$ in its solid form is exactly the same and follows **Scheme 4**. These reactions produced far better results, whether the reasons for that was due to a completely new reagent or the correct calculations is uncertain, but most likely both. After the synthesis and purification of bis-lactim ether **7,** ^1H and ^{13}C NMR analyses were run and the spectral data was consistent with the literature data.[12-13] Purification was done by silica gel column chromatography with pentane:EtOAc (95:5) used as solvent that gradually increased in polarity after a while. Several reactions have been performed, **Table 3** below presents the conditions and results.

Table 3. Different reaction conditions and results for synthesis of (**7**).



6 **7**

Entry	Temperature	Reaction time	Yield
a	R.T.	144 hours	27%
b	30°C	72 hours	48%
c	30°C	70 hours	43%
d	30°C	72 hours	47%
e	30°C	72 hours	45%

Note that entry **a** was the first reaction, hence the low yield. In entries **b-e** some portions of the product was lost in purification. All yields represent pure bis-lactim ether **7**.

3.4 Aldol condensation and alkylation reactions of (7) with different bases.

The reactions between bis-lactim ether (**7**) and aldehydes/alkyl halides with different bases are described below. All the reactions were carried out in dry equipment and under nitrogen gas. Reactions between 2,5-DKPs, bis-lactim ethers and aldehydes as well as alkyl halides have been reported,[26-29,33] but in all those reactions the DKPs had had additional substrates on both nitrogen atoms and/or one of the glycine protons were substituted with a halogen before it was reacted with an aldehyde. However the focus in this project was only the reaction between bis-lactim ether **7** and aldehydes, and an alkyl halide just for testing. The exact reactions that are described below have not been reported earlier. Reactions under similar conditions are grouped together and summarized in **Tables 5** and **6**. All of the possible reaction products from aldol addition, condensation and alkylation reactions are presented in **Figure 17** below.

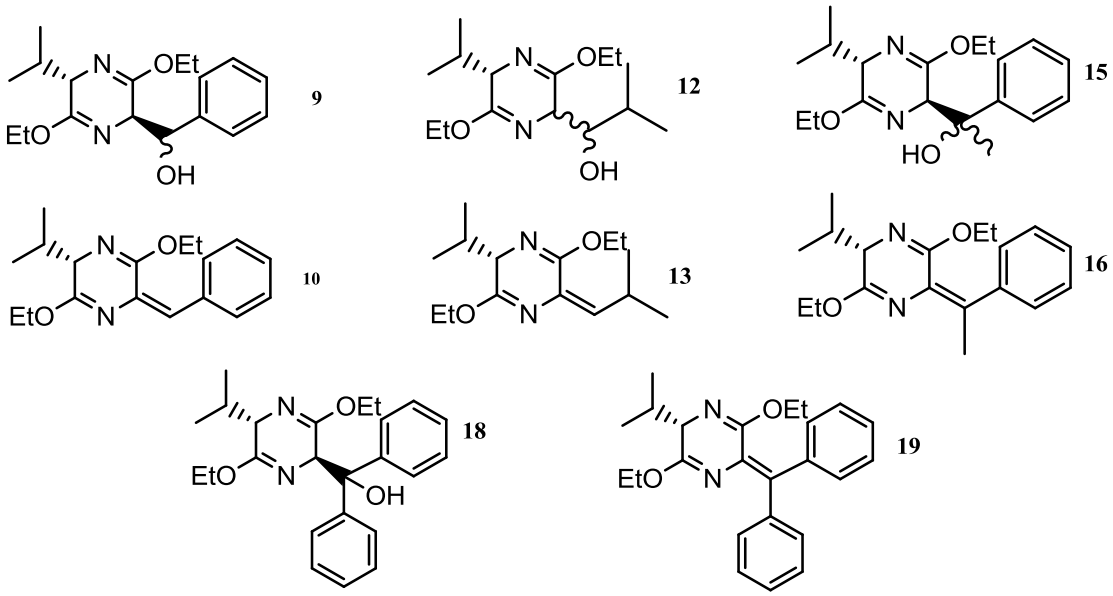
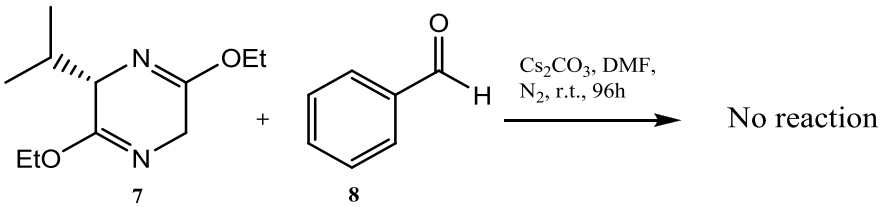


Figure 17. A summary of all possible products from the different reactions.

In all aldol addition/condensation reactions that actually worked, it is observed that the major product is the addition product. This does make sense since most of the base is used up in aldol addition. To actually get the condensation product, more of the base should have been added to the reaction, but this wasn't done. The general procedure used to make all of the compounds that are presented in **Figure 17** was the same and will only be described once. The differences between these reactions are the amounts of starting material used and the different bases, which are presented in sections **3.4.1 - 3.4.11** and **Schemes 5 – 14**.

Bis-lactim ether **7** was mixed together with dry DMF/THF, left either at room temperature or cooled down to -78°C and then a base was added. The reaction was then stirred for some time and then an aldehyde was added. The addition of bases and aldehydes was done slowly with syringes. After that the reaction was run either for 3 hours or overnight under N_2. All the reactions were monitored by TLC and from that decided either to stop or continue to reactions. In most of the reactions it was observed that THF evaporated, and the reaction mixtures became slimy or gel-like mixtures. This was solved by the addition of more dry THF.

3.4.1 Synthesis of aldol addition 9 and condensation 10 products with Cs_2CO_3 as the base.

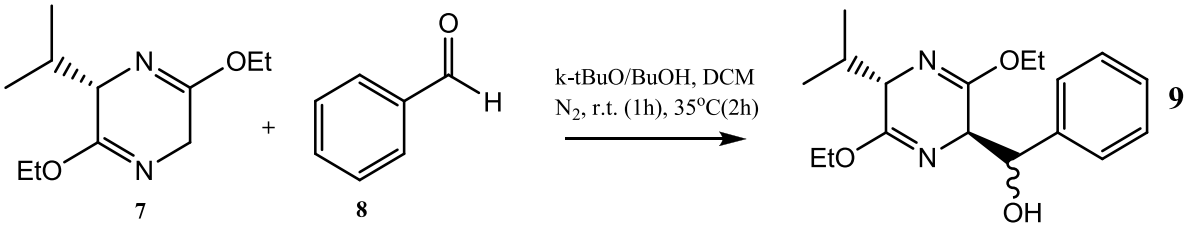


Scheme 5. Synthesis of aldol addition **9** and condensation **10** products with **Cs_2CO_3** as the base.

TLC tests of the reaction mixture showed no differences and after 96 hours the reaction was stopped. ^{1}H NMR and HRMS analyses of the crude product showed only the starting

material, decomposed starting material and benzaldehyde. It seems like Cs_2CO_3 base was not strong enough to take one of the glycine protons, so there was no reaction.

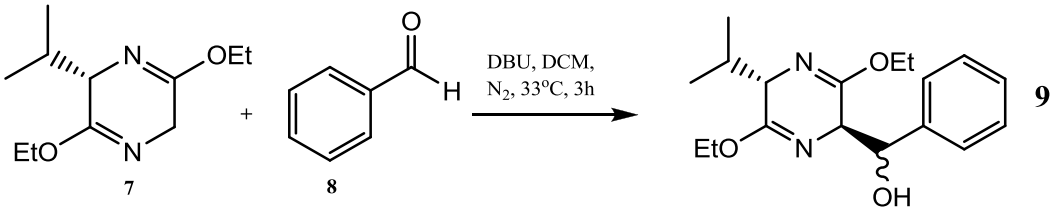
3.4.2 Synthesis of aldol addition product 9 with k-tBuO/BuOH as the base.



Scheme 6. Synthesis of aldol addition product **9** with **k-tBuO/BuOH** as the base.

TLC showed some results, there was too many spots, so it was impossible to determine whether the reaction produced the wanted product or decomposition of starting material. ^{1}H NMR and HRMS analyses of the crude product showed mostly starting material, decomposed starting material, benzaldehyde and very small amounts of compounds **9**. In the HRMS spectra small amounts of aldol condensation product **10** was observed, but in ^{1}H NMR there was nothing. Purification with silica gel column chromatography was attempted, but due to the very small amounts of the desired product, it could not be isolated and was lost in the purification.
Another similar reaction was carried out, but this time DCM was not used and gave somewhat better results. In the ^{1}H NMR and HRMS analyses of the crude, there was no more starting material and benzaldehyde, only different versions of the decomposed starting material and small amounts of aldol addition product **9**, HRMS showed that aldol condensation product **10** was not present at all. Just as the previous reaction, we were unable to purify aldol addition product **9** due to very small amounts of it. There can be many reasons why these reactions worked so badly, whether the base just wasn't strong enough or it reacted with bis-lactim ether in some other ways, but one thing is for sure is that k-tBuO/BuOH solution used in these reactions was quite old. Improvement of the reaction could definitely be something like increasing the reaction time and using more of the base.

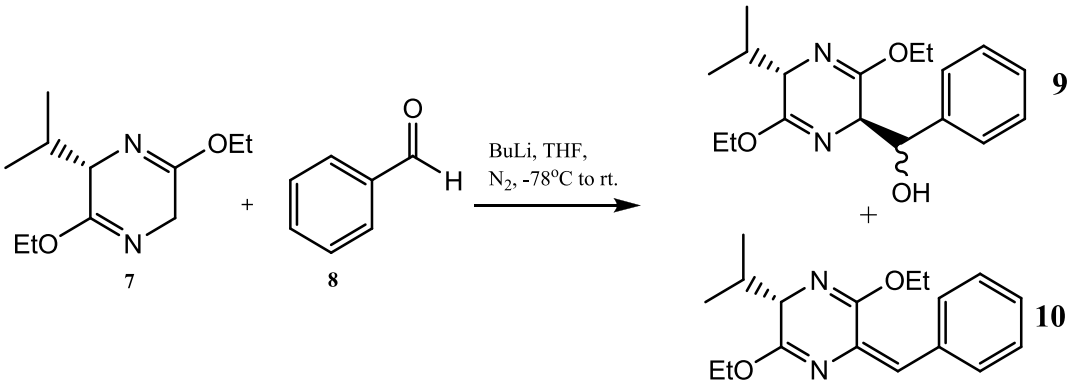
3.4.3 Synthesis of aldol addition product 9 with DBU as the base.



Scheme 7. Synthesis of aldol addition product **9** with **DBU** as the base.

TLC Showed that the starting material was not disappearing and there was no visible changes in the reaction mixture, so it was stopped after about 3 hours. ^{1}H NMR and HRMS analyses of the crude product showed a lot of starting material, decomposed starting material, benzaldehyde and very little of aldol addition product **9**. As we can see from the proton and mass spectra, the reaction was stopped prematurely and definitely required more reaction time, and probably even more DBU since it was more than 2 years old.

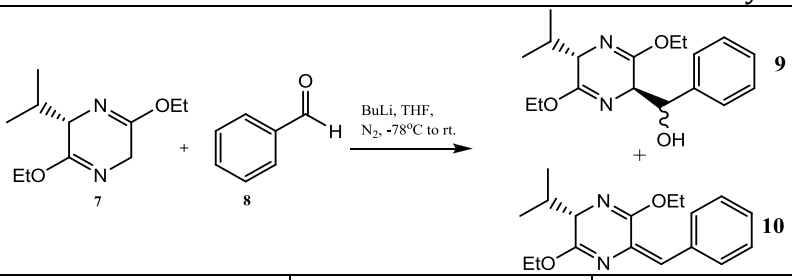
3.4.4 Synthesis of aldol addition product 9 and aldol condensation product 10 with BuLi as the base.



Scheme 8. Synthesis of aldol addition product **9** and aldol condensation product **10** with **BuLi** as the base.

This reaction was carried out twice under different conditions, **Table 4** below presents conditions and results.

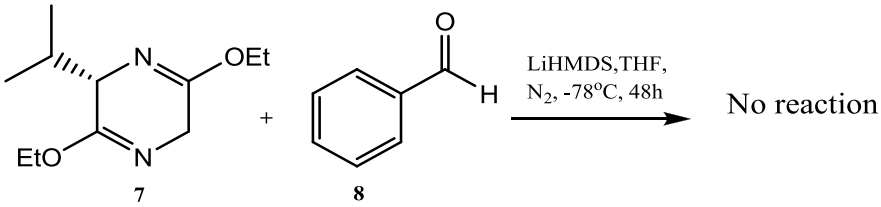
Table 4. Different reaction conditions and results for synthesis of (**9**) and (**10**).



Entry	Temperature	Reaction time	Yield
a	-78°C the whole time.	1 hour after addition of BuLi, then 2 more hours after addition of benzaldehyde	Below 1% of pure product.
b	-78°C for 2 hours at the start, then left in an open cooling bath starting at -78°C which increased to RT. overnight	1 hour after addition of BuLi, then 1 more hour at -78°C after addition of benzaldehyde. Then left it overnight.	Only **9** was observed, but 2 isomers of it, 27% of one and 35% of the other isomer.

^{1}H NMR and HRMS analyses show the characteristic signals for both compounds **9** and **10**, as well as some degradation of the starting material, which was expected. The yield for entry **b** is higher than that for entry **a**, this is also expected. The reason for that is the reaction time for entry **b** is longer than for **a**. Another important factor to be considered is that BuLi used in entry **b** was of better quality.

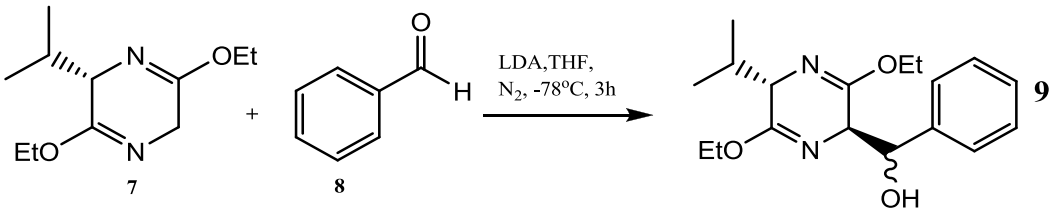
3.4.5 Synthesis of aldol addition product 9 and aldol condensation product 10 with LiHMDS as the base.



Scheme 9. Synthesis of aldol addition product **9** and aldol condensation product **10** with **LiHMDS** as the base.

The reaction was monitored by TLC and after about 48 hours there was no difference in TLC tests so the reaction was stopped, worked up and analyzed. ^{1}H NMR and HRMS analyses showed the characteristic signals for starting material, decomposition of it and benzaldehyde. Even HRMS did not show trances of compounds **9** and **10**. Maybe LiHMDS is not strong enough or is too bulky to come close to the glycine protons.

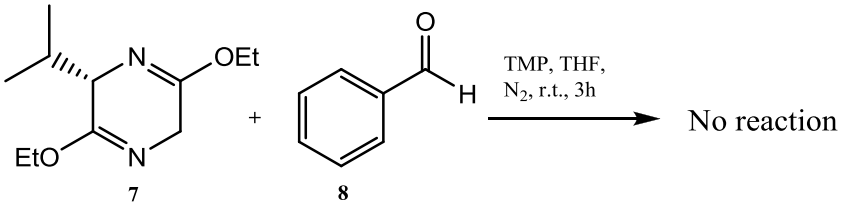
3.4.6 Synthesis of aldol addition product 9 with LDA as the base.



Scheme 10. Synthesis of aldol addition product **9** with **LDA** as the base.

TLC showed some change, but also showed starting material. As for the visible change of reaction mixture, it was difficult to see it, since LDA mixture was very brown. So when LDA was added the reaction mixture became dark brown. ^{1}H NMR and HRMS showed the characteristic signals for both aldol addition product **9**. ^{1}H NMR also revealed that there are 2 isomers of the product which yielded about 1% of one isomer and about 8% of the other. So, even though the reaction time was short some of the desired product still formed. However, this reaction could use some improvements like increasing the reaction time and using a fresher LDA.

3.4.7 Synthesis of aldol addition product 9 and aldol condensation product 10 with TMP as the base.

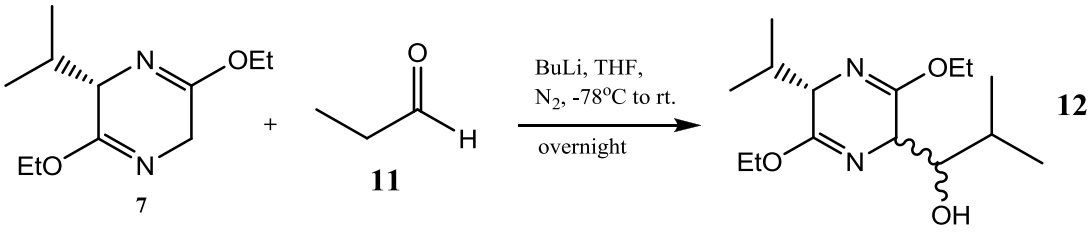


Scheme 11. Synthesis of aldol addition product **9** and aldol condensation product **10** with **TMP** as the base.

TLC tests during the reaction did not show much difference from starting mixture. ^{1}H NMR and HRMS analyses revealed that this reaction did not work, all that was observed

in the spectra was starting material, degradation of it and benzaldehyde. This is probably because TMP wasn't strong enough to take one of the glycine protons or it could have been that TMP is just too bulky and have problems getting to the glycine proton, just like LiHMDS. Or maybe TMP complex was just old and got destroyed. And like some of the other reactions, this one also could use some improvements.

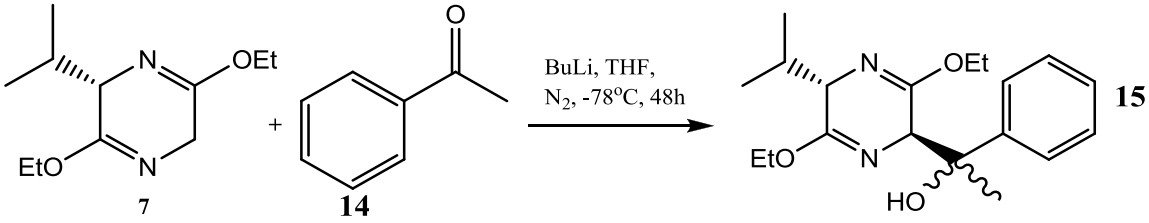
3.4.8 Synthesis of aldol addition product 12 with BuLi as the base.



Scheme 12. Synthesis of aldol addition product **12** with **BuLi** as the base.

Compound **7** was first mixed with THF and cooled down to -78°C, and then BuLi was added slowly with a syringe and stirred for a while. Later isobutyraldehyde was added with a syringe as well and left the reaction in a -78°C open cooling bath. This was so that the reaction would still run at -78°C for a few more hours and then automatically by itself go to room temperature. The reaction was left overnight under N_2. ^1H, ^{13}C NMR and HRMS show clear characteristic signals for compound **12**. Isobutyraldehyde is smaller than benzaldehyde in size, therefore the isopropyl group from the valine part of the bis-lactim ether will have less influence over on which side isobutyraldehyde will bind. So, unless there is a condensation reaction done afterwards there is a possibility for both isomers. Compared to benzaldehyde which is bigger and is forced to bind on the opposite side of the isopropyl group. However, from ^1H NMR and COSY it was determined that there was only 1 isomer which yielded in 10% of pure aldol addition product **12**.

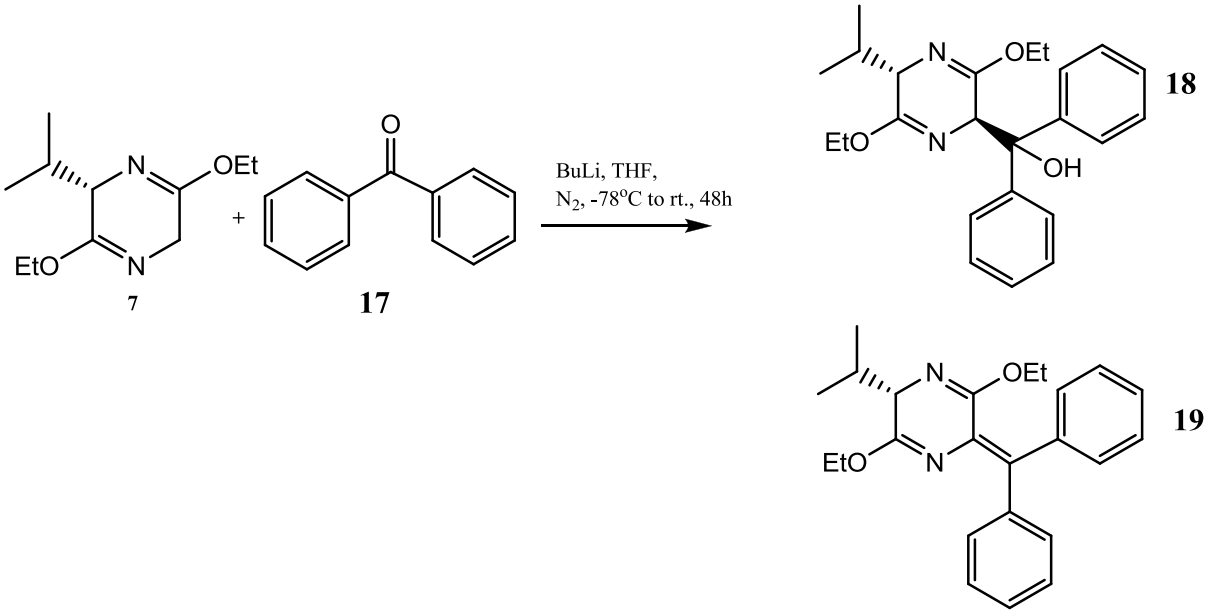
3.4.9 Synthesis of aldol addition product 15 with BuLi as the base.



Scheme 13. Synthesis of aldol addition product **15** with **BuLi** as the base.

The procedure is exactly the as for other reactions. The reaction was monitored by TLC and after 48h there was no difference on TLC tests, so it was stopped and worked up. ^1H NMR and HRMS analyses showed signals characteristic for compound **15**, some unreacted starting material was also observed. ^1H NMR also revealed that there are two isomers in the product and the total yield of them both are 54%.

3.4.10 Synthesis of aldol addition product 18 and condensation product 19 with BuLi as the base.



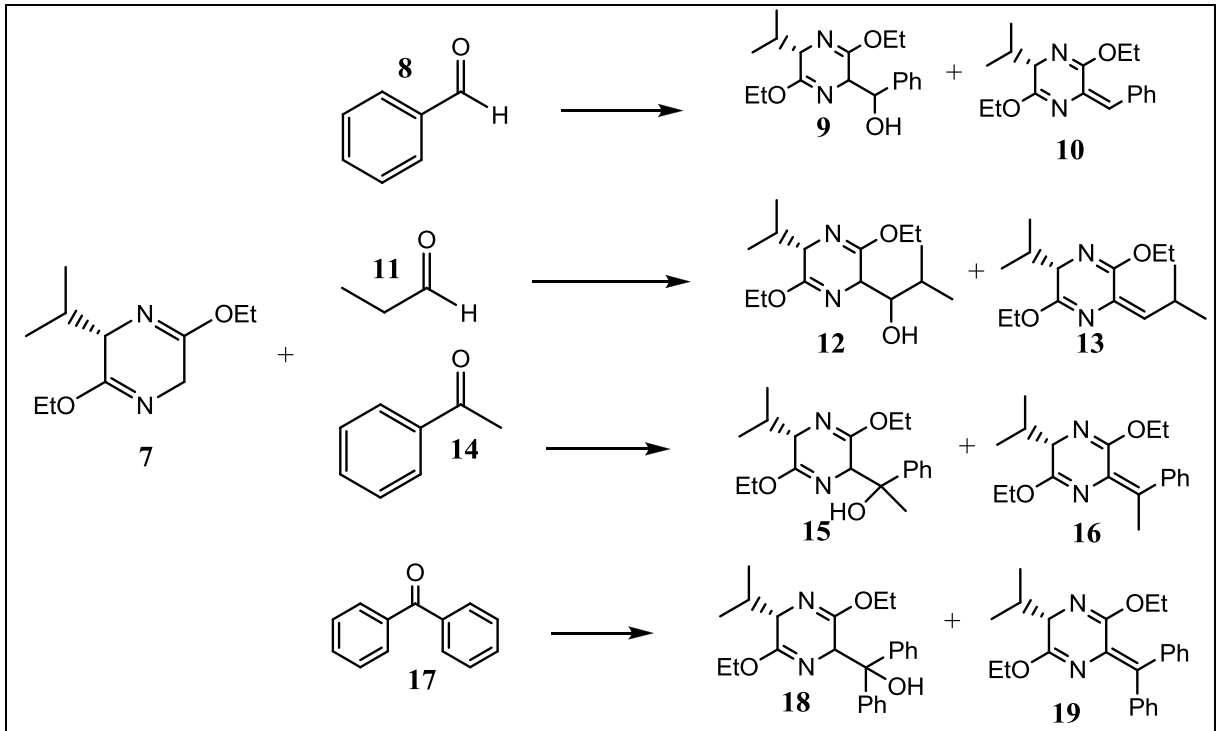
Scheme 14. Synthesis of aldol addition product **18** and condensation product **19** with **BuLi** as the base.

The same procedure and the exact same conditions were used as for the experiment that is described in section 3.4.8. ^{1}H NMR and HRMS analyses revealed that there was still some unreacted starting material left, something of compounds **18** and some trace amounts of compound **19** in HRMS. ^{1}H NMR spectra also shows some indications that there are also 2 isomers with the total yield of 60%.

3.4.11 Summary of aldol addition/condensation reactions.

In all aldol addition/condensation reactions we see that the amounts of aldol addition products are higher than the amounts of the condensation product, and this makes sense since the base is most likely used up and there is no way to make the condensation reaction. A solution to this could be to add more of the same base used in the reaction, especially in those reactions where there is still some unreacted starting material left. That would also improve the yield altogether. **Table 5** below presents a summary of all aldol addition/condensation reactions.

Table 5. Summary of all aldol addition/condensation reactions.



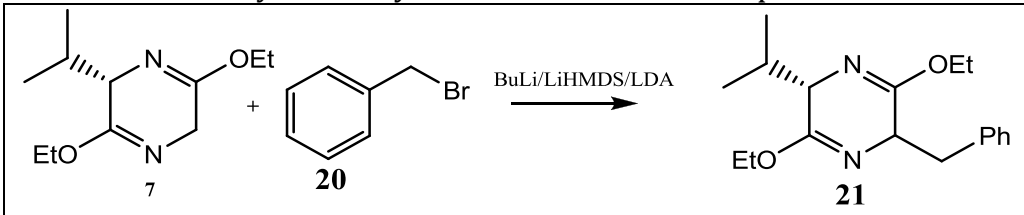
Entry	Starting material	Base	Aldehyde	Temperature	Reaction time	Yield
A	**7**	**Cs_2CO_3**	Benzaldehyde	r.t.	96 hours	No reaction
B	**7**	**ktBuO/BuOH**	Benzaldehyde	r.t. (1h) and 30°C (2h)	3 hours	Very low, lost in purification
C	**7**	**DBU**	Benzaldehyde	33°C	3 hours	Very low, lost in purification
D	**7**	**BuLi**	Benzaldehyde	-78°C	3 hours and overnight	Below 1% (from 3h) and 62% (from o.n.) of pure product
E	**7**	**LiHMDS**	Benzaldehyde	-78°C	48 hours	No reaction
F	**7**	**LDA**	Benzaldehyde	-78°C	3 hours	Total of 9%
G	**7**	**TMP complex**	Benzaldehyde	r.t.	3 hours	No reaction
H	**7**	**BuLi**	Isobutyraldehyde	-78°C to r.t.	overnight	10%
I	**7**	**BuLi**	Acetophenone	-78°C to r.t.	overnight	54%
J	**7**	**BuLi**	Benzophenone	-78°C to r.t.	overnight	60%

(h = hours; r.t. = room temperature; o.n. = overnight)

3.5 Alkylation reactions between bis-lactim ether and alkyl halides.

This section will only summarize the alkylation reactions shortly since the goal of them was just to test if the different bases were still working, because as mentioned earlier some of them was quite old. Reaction conditions for alkylation with different bases were mostly the same, **Table 6** below presents what is different and which results were gathered.

Table 6. Summary of all alkylation reactions with compound **7.**



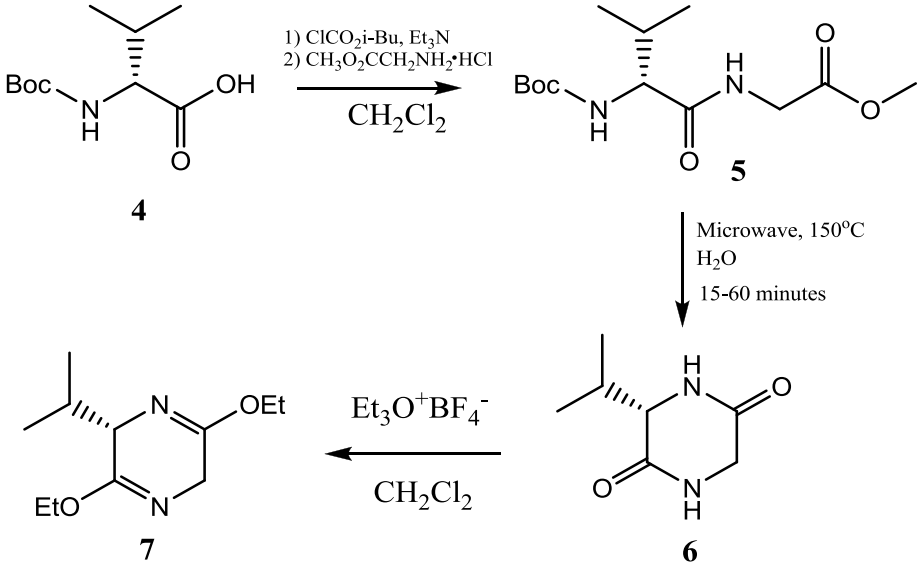
Entry	Starting material	Base	Alkyl halide	Temperature	Reaction time	Yield
A	**7**	**BuLi**	Benzyl bromide	-78°C	3 hours	63%
B	**7**	**LiHMDS**	Benzyl bromide	-78°C to r.t.	Overnight	3%
C	**7**	**LDA**	Benzyl bromide	-78°C to r.t.	Overnight	15%

(r.t. =room temperature)

From the yields in **Table 6** we can see that the reaction where BuLi was used is the best. This can be influenced by many things, maybe LiHMDS and LDA were just old bases, which they were, and were mostly destroyed, while BuLi was fresher and gave better results. Another factor can be the size of the bases. BuLi compared to the other two are a lot less bulky which allows it to get to glycine protons a lot more easily.

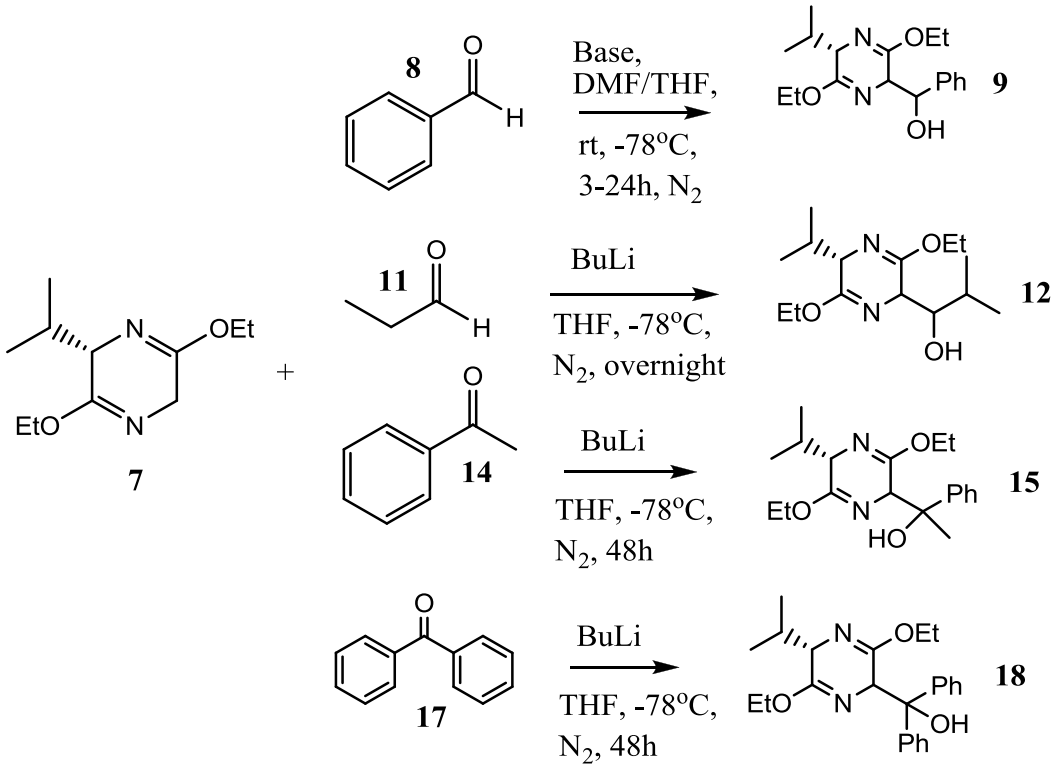
4 Conclusion

The first part of the thesis, sections 3.1-3.3, describes my work towards the synthesis of bis-lactim ether **7** using the Schöllkopf approach. This synthesis is also thoroughly described in literature. And overall, results that were obtained from the reactions, are what was expected, even though there were some complications in syntheses due to minor errors. Especially the purification of the 2,5-diketopiperazine **6**.
A number of reactions of bis-lactim ether **7** synthesis have been performed to make an optimization, from the results we can see that the yield ranged from 43% to 48% of pure bis-lactim ether **7**, this is a very small yield range, and that shows consistency in the synthesis.



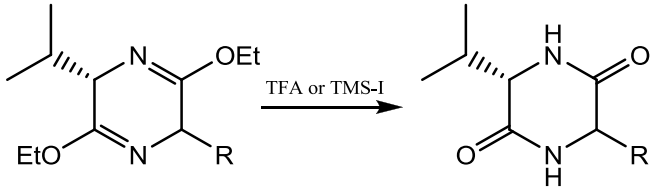
Scheme 15. Summary of the synthesis of bis-lactim ether **7**.

In the aldol addition and condensation reactions we observed that most of the formed product was the aldol addition product, and that goes for all the different aldehydes and ketones used, and that is expected since we did not use large amounts of base. The yields in **Table 5** show us that the best base for this type of reactions is BuLi, despite whether the substrate is an aldehyde or ketone. The other bases like Cs_2CO_3, DBU and BuOK are perhaps just not strong enough to take of the glycine protons. The other lithium bases, LiHMDS, LDA and TMP complex, were not that good either. A reaction with them either did not happen at all, or produced very low yields. LDA gave only 9% of aldol addition product **9**. However, we know that the alkylation reaction of bis-lactim ether **7** should work, and **Table 6** presents the results of those alkylation reactions. We see that LiHMDS and LDA work quite badly compared to BuLi, and this tells us two things. LiHMDS and LDA were either too old and were pretty much destroyed reagents and/or they were too bulky to be able to come close to the glycine protons. **Scheme 16** presents the aldol addtition reactions and product.



Scheme 16. Summary of aldol addition reactions with their respective products.

From analyses of the aldol addition products of reactions with benzaldehyde, acetophenone and benzophenone we could also observe that there were 2 isomers. Where one OH is in the R- and the other in the S-conformation. We were unable to purify these two isomers since they moved through a silica gel column at the same rate. Due to lack of time there was not put that much effort into purification of these isomers. Further work might be done by someone else in the future to purify these isomers as well as try to go back from substituted bis-lactim ether to ketone version of the molecule, as presented in **Scheme 17**.



Scheme 17. Synthesis of substituted 2,5-DKP from substituted bis-lactim ether.

5 References

[1] Lind KF, Hansen E, Østerud B, et al. Antioxidant and Anti-Inflammatory Activities of Barettin. *Marine Drugs*. 2013;11(7):2655-2666.
[2] S. Sölter, R. Dieckmann, M. Blumenberg, W. Francke., Barettin, revisited?. *Tetrahedron Letters.*, 2002, 43(18), pp 3385-3386.
[3] Book
[4] Shibasaki, M., Kanai, M. and Mita, T. 2008. The Catalytic Asymmetric Strecker Reaction. Organic Reactions. 70:1:1–119.
[5] J. Huang, E. J. Corey. A New Chiral Catalyst for the Enantioselective Strecker Synthesis of α-Amino Acids. *Org. Lett.*, 2004, 6 (26), pp 5027–5029.
[6] F. A. Davis, R. E. Reddy, P. S. Portonovo,. Asymmetric strecker synthesis using enantiopure sulfinimines: A convenient synthesis of α-amino acids. *Tetrahedron Letters.*, 1994, 35(50), pp 9351-9354.
[7] H. Ishitani, S. Komiyama, Y. Hasegawa, S. Kobayashi., Catalytic Asymmetric Strecker Synthesis. Preparation of Enantiomerically Pure α-Amino Acid Derivatives from Aldimines and Tributyltin Cyanide or Achiral Aldehydes, Amines, and Hydrogen Cyanide Using a Chiral Zirconium Catalyst. *J. Am. Chem. Soc.*, 2000, 122 (5), pp 762–766
[8] L. A. Carpino., 1-Hydroxy-7-azabenzotriazole. An efficient peptide coupling additive., *J. Am. Chem. Soc.*, 1993, 115 (10), pp 4397–4398.
[9] A. J. Frontier, C. Collison., The Nazarov cyclization in organic synthesis. Recent advances., Tetrahedron., 2005, 61(32), pp 7577-7606.
[10] Giese, B., Kopping, B., Göbel, T., Dickhaut, J., Thoma, G., Kulicke, K. and Trach, F. 2004. Radical Cyclization Reactions. Organic Reactions. 48:2:301–856.
[11] K. C. Nicolaou, S. A. Snyder, T. Montagnon, G. Vassilikogiannakis., The Diels-Alder Reaction in Total Synthesis., *Angew. Chem. Int. Ed.* 2002, 41, 1668-1698.
[12] A.-C. Carlsson, F. Jam, M. Tullberg, Å. Pilotti, P. Ioannidis, K. Luthman, M. Grøtli., Microwave-assisted synthesis of the Schöllkopf chiral auxiliaries: (3S)- and (3R)-3,6-dihydro-2,5-diethoxy-3-isopropyl-pyrazine., *Tetrahedron Letters*, 2006, 47, pp 5199-5201.
[13] J. Chen, S. P. Corbin, N. J. Holman., An Improved Scale Synthesis of the Schöllkopf Chiral Auxiliaries: (2R)- and (2S)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine., Organic Process Research & Development., 2005, 9, pp 185-187.
[14] C. J. Dinsmore, D. C. Beshore., Recent advances in the synthesis of diketopiperazines., *Tetrahedron*, 2002 58(17), pp 3297-3312.
[15] D. R. Houston, B. Synstad, V. G. H. Eijsink, M. J. R. Stark, I. M. Eggleston, D. M. F. van Aalten., Structure-Based Exploration of Cyclic Dipeptide Chitinase Inhibitors., *J. Med. Chem.*, 2004, 47 (23), pp 5713–5720.
[16] S. Sinha, R. Srivastava, Prof. E. De Clercq, Dr. R. K. Singh., Synthesis and Antiviral Properties of Arabino and Ribonucleosides of 1,3-Dideazaadenine, 4-Nitro-1, 3-dideazaadenine and Diketopiperazine., *Nucleosides, Nucleotides and Nucleic Acids*., 2004, 23(12), pp 1815-1824.
[17] Nicholson, B.; Lloyd, G. K.; Miller, B. R.; Palladino, M. A.; Kiso, Y.; Hayashi, Y.; Neuteboom, S. T.C., NPI-2358 is a tubulin-depolymerizing agent: in-vitro evidence for activity as a tumor vascular-disrupting agent., *Anti-Cancer Drugs*, 2006, 17(1), pp 25-31.
[18] O. S. Kwon, S. H. Park, B.-S. Yun, Y. R. Pyun, C.-J. Kim., Cjcclo(Dehydroala-L-Leu), an α-Glucosidase Inhibitor from Penicillium sp. F7Q6l4., *The Journal of Antibiotics*., 2000, 53, pp 954-958.

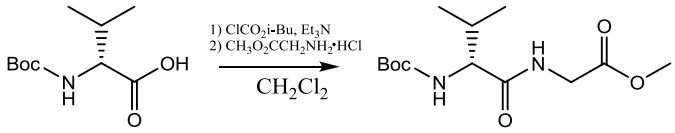
[19] H. J. Lim, J. C. Gallucci, T. V. RajanBabu., Annulated Diketopiperazines from Dipeptides or Schöllkopf Reagents via Tandem Cyclization-Intramolecular N-Arylation., *ORGANIC LETTERS*., 2010, Vol. 12, No. 9, pp 2162-2165
[20] M. Andrei, J. Efskind, T. Viljugrein, C. Rømming, K. Undheim., Chemoselective rhodium(II)–carbenoid cyclisation reactions in the stereoselective construction of rigidified cyclic a-amino acid derivatives., *Tetrahedron: Asymmetry.,* 2004, 15, pp 1301–1313.
[21] M. Andrei, C. Römming, K. Undheim., Rh(II)-catalysed carbenoid cyclisations in a stereoselective approach to a-quaternary pipecolic acid derivatives., *Tetrahedron: Asymmetry*., 2004, 15, pp 1359–1370.
[22] J. F. González, E. de la Cuesta, C. Avendaño., Improvements in Aldol Reactions with Diketopiperazines., *Synthetic Communications*., 2004, 34(9), 1589-1597.
[23] Y. Yamazaki, K. Tanaka, B. Nicholson, G. Deyanat-Yazdi, B. Potts, T. Yoshida, A. Oda, T. Kitagawa, S. Orikasa, Y. Kiso, H. Yasui, M. Akamatsu, T. Chinen, T. Usui, Y. Shinozaki, F. Yakushiji, B. R. Miller, S. Neuteboom, M. Palladino, K. Kanoh, G. K. Lloyd, Y. Hayashi., Synthesis and Structure–Activity Relationship Study of Antimicrotubule Agents Phenylahistin Derivatives with a Didehydropiperazine-2,5-dione Structure., *J. Med. Chem.,* 2012, 55(3), pp 1056–1071.
[24] http://www.uwindsor.ca/people/jgreen/sites/uwindsor.ca.people.jgreen/files/pka_bases.pdf (09.05.2016)
[25] (a) C. Gallina, A. Liberatori., A new synthesis of 1-acetyl-3-arylidene(alkylidene) piperazine-2,5-diones., *Tetrahedron Letters*., 1973, 14(14), pp 1135-1136; (b) C. Gallina, A. Liberatori., Condensation of 1,4-diacetylpiperazine-2,5-dione with aldehydes., *Tetrahedron.*, 1974, 30(5), pp 667-673.
[26] G. Cremonesi, P. D. Croce, F. Fontana, C. Fiorelli, C. La Rosa., Stereoselective synthesis of β,ε-dihydroxy-α-amino acids by ring opening of 4,5-dihydroisoxazolyl derivatives., *Tetrahedron: Asymmetry*., 2008, 19(24), pp 2850-2855.
[27] M. Andrei, K. Undheim., Stereoselective synthesis of functionalised cycloalkene α-quaternary α-amino acid derivatives., *Tetrahedron: Asymmetry*., 2004, 15(1), pp 53-63.
[28] G. Cremonesi, P. D. Croce, F. Fontana, A. Forni, C. La Rosa., Stereoselective synthesis of β-hydroxy-α-amino acids β-substituted with non-aromatic heterocycles., *Tetrahedron: Asymmetry*., 2008, 18(14), pp 1667-1675.
[29] H. D. Toop, A. S. Don, J. C. Morris., Synthesis and biological evaluation of analogs of AAL(S) for use as ceramide synthase 1 inhibitors., *Org. Biomol. Chem.*, 2015,13, 11593-11596
[30] U. Schöllkopf, U. Groth, C. Deng., Enantioselective Syntheses of (R)-Amino Acids Using L-Valine as Chiral Agent., *Angew. Chem. Int. Ed.*, 1981, 20(9), pp 798-799.
[31] U. Schöllkopf, W. Hartwig, U. Groth., Enantioselective Synthesis of α-Methyl-α-aminocarboxylic Acids by Alkylation of the Lactim Ether of cyclo-(L-Ala-L-Ala)., *Angew. Chem. Int. Ed.*, 1979, 18(11), pp 863-864.
[32] U. Schöllkopf, T.Tiller, J. Bardenhagen., Asymmetric synthesis via heterocyclic intermediates - XXXIX[1]: Asymmetric synthesis of (enantiomerically and diastereomerically virtually pure) methyl 2-amino-4,5-epoxy-3-hydroxy-alkanoates and methyl 2-amino-3-hydroxy-4,5-methylene-alkanoates by the bislactimether method., *Tetrahedron.*, 1988, 44(17), pp 5293-5305.
[33] A. D. Borthwick., 2,5-Diketopiperazines: Synthesis, Reactions, Medicinal Chemistry, and Bioactive Natural Products., *Chem. Rev.*, 2012, 112 (7), pp 3641–3716.

[34] A.-N. Johnson, J. Bergman, M. Sjögren, LL Bohlin., Synthesis of barettin., *Tetrahedron*., 2004, 60(4), pp 961-965.

6 Experimental section

All reagents were purchased from Sigma Aldrich Co. and used as received. Dry DMF and THF was obtained from water free bottles with molecular sieves and under inert gas. All glassware used under inert conditions were heated in oven at 105°C overnight. Column chromatography was performed using silica gel 35-70 micron from Grace GmbH. Reactions monitored by TLC was run on 60 F254 silica gel plates and visualized with UV and stains.
NMR spectra were recorded on Varian Mercury-400 plus and Ascend™ 400 Bruker with samplecase NMR spectrometers. Chemical shift values (δ) are reported in parts per million (ppm) relative to chloroform. All NMR spectra were processed with MestReNova v8.0.1-10878. All molecular structures were made in ChemDraw Ultra 11.0.
Progress of some experiments was also monitored by GC-MS, it was done on a Thermo Scientifinc Trace GC Ultra with a Thermo Scientific ITQ 1100 detector. HRMS spectra were recorded LTQ Orbitrap XL in positive electrospray ionization (ESI) mode. IR spectra were obtained on a Agilent Technologies Cary 630 FT-IR spectrometer. Absorptions are given in reciprocal centimetres. Microwave irradiation was in a Microwave synthesis reaction Monowave 300 from Anton Paar.

6.1 (R)-methyl 2-(2-(tert-butoxycarbonylamino)-3-methylbutanamido)acetate (5)

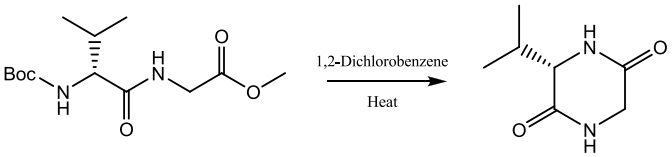


4 **5**

A 250 mL round bottom flask with 105 mL CH_2Cl_2 was cooled down on ice together with Boc-Val-OH (7.98 g, 36.7 mmol) and Et_3N (3.73 g, 36.9 mmol), which was then closed and mixed. Then 4.75 mL of isobutyl chloroformate was added dropwise with a syringe during 30 minutes, and after that, this mixture was stirred for about 35 minutes on ice. At the same time in another flask, glycine methyl ester hydrochloride (4.61 g, 36.7 mmol) and Et_3N (3.74 g, 37.0 mmol) were mixed in 105 ml CH_2Cl_2 and stirred for 35 minutes at room temperature. After those 35 minutes, the mixture with glycine methyl ester was added to the first mixture with Boc-Val-OH dropwise during 1 hour and 45 minutes with the help of an addition funnel. After the addition was complete, the reaction mixture stirred for about 19 hours at room temperature and then washed with water (3 * 120 mL) and about 50 mL BRINE, then dried with Na_2SO_2 and concentrated under vacuum to yield 10.2 g (97%) crude.
The crude was purified by recrystallization from cyclohexane to yield pure (R)-methyl 2-(2-(tert-butoxycarbonylamino)-3-methylbutanamido)acetate (**5**) as white powder (8.02 g, 76%). NOTE: during collection of the product after recrystallization some of it was lost due to a small accident, but not much was lost.

^{1}H-NMR (MeOD, 400 MHz): δ 0.93 (3H, d, J = 8 Hz), δ 0.98 (3H, d, J = 8 Hz), δ 1.43 (9H, d, J = 12 Hz), δ 2.19 (1H, m), δ 3.76 (3H, s), δ 3.96-4.07 (3H, m), δ 5.02 (1H, s), δ 6.47 (1H, s).
^{13}C-NMR ($CDCl_3$, 100 MHz): Too much noise, difficult to see.

6.2 Synthesis of (S)-3-isopropylpiperazine-2,5-dione (6) by conventional heating



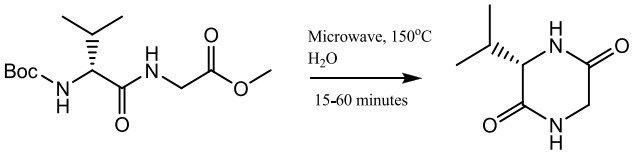
5 6

(R)-methyl 2-(2-(tert-butoxycarbonylamino)-3-methylbutanamido)acetate (**5**) (0.99 g, 3.43 mmol) was transferred to a round bottom flask containing 10 mL 1,2-dichlorobenzene. A reflux system was set up; the mixture was put on stirring and heated to about 172°C and left it like that overnight, about 19 hours. After that, the reaction mixture was cooled down to about 50°C and 6 mL of TBME was added, and then cooled down to room temperature. Since the product was a solid and it precipitated in the reaction, the reaction mixture was just filtered through a sintered glass filter and washed with about 10 mL TBME. The resulting solid had a light-brown color and was left in the hood to dry at room temperature. The crude from the reaction yielded un-pure (S)-3-isopropylpiperazine-2,5-dione (**6**) 0.190 g (1.22 mmol, 35%). This reaction was performed one more time under the same conditions and it yielded un-pure (S)-3-isopropylpiperazine-2,5-dione (**6**) (0.164 g, 1.05 mmol, 31%).

^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.86 (3H, d, J = 4 Hz), δ 0.93 (3H, d, J = 4 Hz), δ 2.08 (1H, m), δ 3.52 (1H, m), δ 3.62 and 3.80 (AB, 2H, J = 16 Hz), δ 7.99 (1H, s), δ 8.17 (1H, s).

Due to low yields in both reactions, it was decided to leave the purification of (S)-3-isopropylpiperazine-2,5-dione (**6**) from these reactions after it was performed with the assistance of a microwave. The experiment of microwave assisted synthesis of (S)-3-isopropylpiperazine-2,5-dione (**6**) showed better results both in the color of the crude product and the yield was more than twice as much, see section 3.2.2. So it was decided to leave the purification of this crude product altogether.

6.3 Microwave synthesis of (S)-3-isopropylpiperazine-2,5-dione (6)

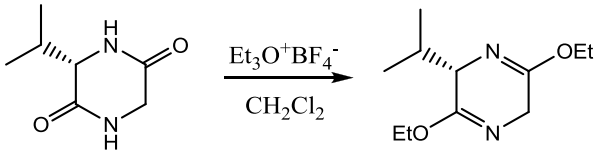


5 6

(R)-methyl 2-(2-(tert-butoxycarbonylamino)-3-methylbutanamido)acetate (**5**) (0.995 g, 3.45 mmol) was transferred to a special G30 microwave vial with a magnetic stirring bar. Then 10 mL of distilled H_2O was added and the vial was closed with a special lock designed for the microwave. The reaction was run at 150°C, 600 rpm and 15 minutes. After the reaction was done, the MW machine cooled the mixture down to 55 °C and it was taken out and it saw mixture was completely transparent. The mixture was transferred to a round bottom flask and set to evaporation under vacuum. Reaction resulted in white solid that was recrystallized from EtOH/$CHCl_3$ (3:1) to yield (S)-3-isopropylpiperazine-2,5-dione (**6**) 0.154 g (0.000986 mmol, 29%). Additional syntheses of (S)-3-isopropylpiperazine-2,5-dione (**6**) were performed the same way, but with different reaction conditions like time and temperature, **Table 2** in section 3.2.2 presents the conditions used and results obtained.

^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.97 (3H, d, J = 4 Hz), δ 1.04 (3H, d, J = 4 Hz), δ 2.21-2.29 (1H, m), δ 3.72-3.75 (1H, m), δ 3.83 (1H, d, J = 20), δ 4.00 (1H, d, J = 20).
HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_7H_{12}N_2O_2$: 156.09; Found for $C_7H_{12}N_2O_2 + H_2O$: 175.1080

6.4 Synthesis of (S)-3,6-diethoxy-2-isopropyl-2,5-dihydropyrazine (7) with $Et_3O^+BF_4^-$ solution.

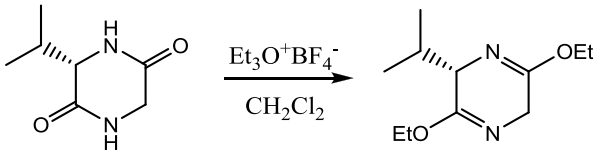


6 **7**

(S)-3-isopropylpiperazine-2,5-dione (**6**) (0.195 g, 1.25 mmol) was transferred to a 25 mL round bottom flask with 10 mL CH_2Cl_2, a reflux system was set up and the mixture was set to stir. Then $Et_3O^+BF_4^-$ (1M in methylene chloride) solution (0.75 mL, 4.04 mmol) was added to the stirring mixture, and the reaction stirred at room temperature for 7 days. After that, the reaction mixture was transferred to a 20 mL $NaHCO_3$ and 20 mL DCM solution that was cooled down to about 5°C beforehand. During the addition of reaction mixture to $NaHCO_3$:DCM solution, the pH was constantly kept at about 8-9 by the addition of small portions of 3M NaOH. The phases were then separated, and the aqueous phase extracted with DCM (2 x 25 mL). The organic phases were then collected together and washed with saturated NaCl solution, dries with $MgSO_4$ and concentrated on a rotary evaporator. The resulting crude, yellow-brown oil was collected and saved without any further purification. This reaction was performed several times; the only difference is the amount of chemicals and reaction times that were used. Due to small amounts of product it was lost during the column chromatography purification.

^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.88 (3H, d, J = 4 Hz), δ 1.00 (3H, d, J = 4 Hz), δ 1.27 (6H, t, J = 16), δ 2.18-2.25 (1H, m), δ 3.87-3.97 (3H, m, NCH and NCH_2), δ 4.05-4.16 (4H, m).
^{13}C-NMR ($CDCl_3$, 100 MHz): δ 170.51, 160.11, 61.97, 58.97, 50.07, 32.88, 18.80, 16.59, 14.67.

6.5 Synthesis of (S)-3,6-diethoxy-2-isopropyl-2,5-dihydropyrazine (7) with $Et_3O^+BF_4^-$ (pure solid)



6 **7**

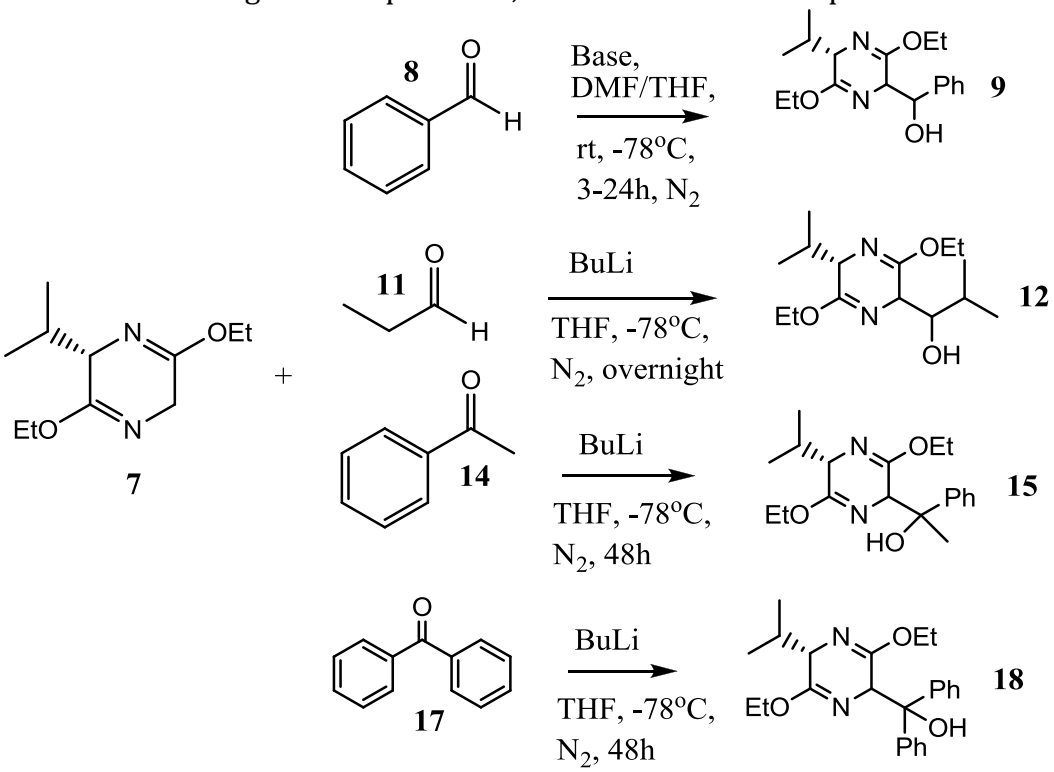
(S)-3-isopropylpiperazine-2,5-dione (**6**) (0.250 g, 1.60 mmol) and $Et_3O^+BF_4^-$ (pure solid) (1.019 g, 5.36 mmol) was transferred to a 100 mL round bottom flask with about 20 mL CH_2Cl_2, and the mixture was put to stir at room temperature. In 24 hours a TLC test was done to see the reaction progress, there was still a lot of starting material left. In 72 hours the same was done and the same was observed. After a total of 144 hours a TLC test showed that there was no more starting material in the mixture and the reaction was stopped. The reaction mixture was slowly added to a cold (0-5°C) solution of 40 mL $NaHCO_3$ and 40 mL CH_2Cl_2 that was stirring vigorously, 15 mL of 3M NaOH was also

added slowly at the same time to keep the pH at 8-9. The phases were then separated, the water phase was washed with 2x40 mL CH_2Cl_2. The organic phases were then collected and washed with BRINE, then dried with $MgSO_4$ and concentrated on a rotary evaporator. The crude was then purified with a silica gel column chromatography, pentane:EtOAc (90:10) as the solvent which increased in polarity after a while, to yield 0.091 g (0.43 mmol, 27%). This reaction was performed several times, the yield from them were 43-48% as it is presented in **Table 3**.

^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.72 (3H, d, J = 8 Hz), δ 0.98 (3H, d, J = 8 Hz), δ 1.21-1.24 (6H, m), δ 2.15-2.21 (1H, m), δ 3.85-3.94 (3H, m), δ 3.97-4.19 (4H, m).
^{13}C-NMR ($CDCl_3$, 100 MHz): δ 164.39, 161.89, 61.10, 60.78, 46.85, 32.63, 19.11, 17.10, 14.40
HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{11}H_{20}N_2O_2$ $[M+H]^+$: 213.1580; Found: 213. 1593

6.6 Aldol addition, condensation and alkylation of (S)-3,6-diethoxy-2-isopropyl-2,5-dihydropyrazine (7) with different bases

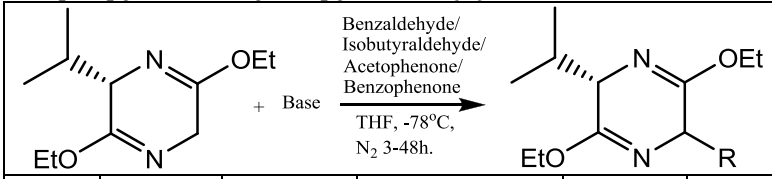
Note; the aldol addition, condensation and alkylation reactions of **7** were carried out in the same way, but with different agents and different results. Thus will only be described for one general experiment, but the results will be presented for all reactions.



Bis-lactim ether **7** was mixed with reaction solvent DMF/THF, and then either left at room temperature or cooled down to -78°C. Then a base was added slowly with a syringe, and after addition the reaction mixture was allowed to stir for about 1 hour at -78°C. Then benzaldehyde, isobutyraldehyde, acetophenone, benzophenone or benzyl bromide was added with a syringe slowly to the reaction mixture, and then left in the same cooling bath at -78°C which was open. So that the reaction would still go at -78°C for some hours and then heat up to room temperature overnight by itself. In the work up, the reaction mixture was quenched with either water/ammonia solution but mostly by phosphate buffer with pH 7. Then diethylether or DCM was added and mixed very

well and the phases were separated. The water phase was washed twice with diethylether or DCM (about 10-20 mL). The organic phases were then collected and washed with BRINE, then dried with Na_2SO_4, filtered and concentrated on rotary evaporator. The crude was then purified on a silica gel column with pentane:EtOAc (95:5) as the starting solvent which after a while was increased in polarity. All the specific conditions and results from these reactions are presented in **Table 7** below.

Table 7. Summary of Aldol addition, condensation and alkylation of (S)-3,6-diethoxy-2-isopropyl-2,5-dihydropyrazine (**7**) with different bases.



Entry	Bis-lactim ether	Base	Substrate	Solvent	Temperature	Reaction time	Yield
A	0.107g, 0.50 mmol	**Cs_2CO_3**, (0.222g, 0.68 mmol)	Benzaldehyde (0.060 g, 0.57 mmol)	DMF	Rt.	96h	No reaction
B	0.050 g, 0.24 mmol	**k-tBuO /BuOH**, 0.3 mL	Benzaldehyde (0.031 g, 0.29 mmol)	DCM	Rt. (1h), 35°C (2h)	3h	Very low, lost in purification
C	0.057 g, 0.27 mmol	**DBU**, 0.053 g, 0.35 mmol	Benzaldehyde (0.025 g, 0.24 mmol)	DCM	33°C	3h	Very low, lost in purification
D	0.057 g, 0.27 mmol	**BuLi**, 0.14mL, 2.7M in heptane	Benzaldehyde, 0.040 mL	THF	-78°C	Overnight	0.053g, 62%
E	0.042 g,0.20 mmol	**LiHMDS** 0.36mL, 1M in THF	Benzaldehyde, 0.035 mL	THF	-78°C	3h	No reaction
F	0.059 g,0.28 mmol	**LDA**, 0.18mL, 2M in THF	Benzaldehyde, 0.08 mL	THF	-78°C	3h	0.008 g, 9%
G	0.056 g, 0.26 mmol	**TMP**, 0.36mL, 1M in	Benzaldehyde, 0.08 mL	THF	Rt.	3h	No reaction

		THF/toluene					
H	0.054 g, 0.25 mmol	**BuLi**, 0.13mL, 2.7M in Heptane	Isobutyraldehyde, 0.050 mL	THF	-78°C for about 5 hours, then rt.	overnight	0.007g, 10%
I	0.057 g, 0.27 mmol	**BuLi**, 0.13mL, 2.7M in Heptane	Acetophenone, 0.046g, 0.38 mmol	THF	-78°C for about 5 hours, then rt.	48h	0.048g, 54%
J	0.057 g, 0.27 mmol	**BuLi**, 0.13mL, 2.7M in Heptane	Benzophenone, 0.053 g, 0.29 mmol	THF	-78°C for about 5 hours, then rt.	48h	0.064g, 60%
K	0.048 g, 0.23 mmol	**BuLi**, 0.22mL, 1.6M in hexane	Benzyl bromide, 0.040 mL	THF	-78°C	3h	0.043g, 63%
L	0.046 g, 0.22 mmol	**LiHMDS** 0.36mL, 1M in THF	Benzyl bromide, 0.040 mL	THF	-78°C for 30 min, then at rt.	Overnight	0.002g, 3%
M	0.047 g, 0.22 mmol	**LDA**, 0.18mL, 2M in THF	Benzyl bromide, 0.040 mL	THF	-78°C for 30 min, then at rt.	Overnight	0.010g, 15%

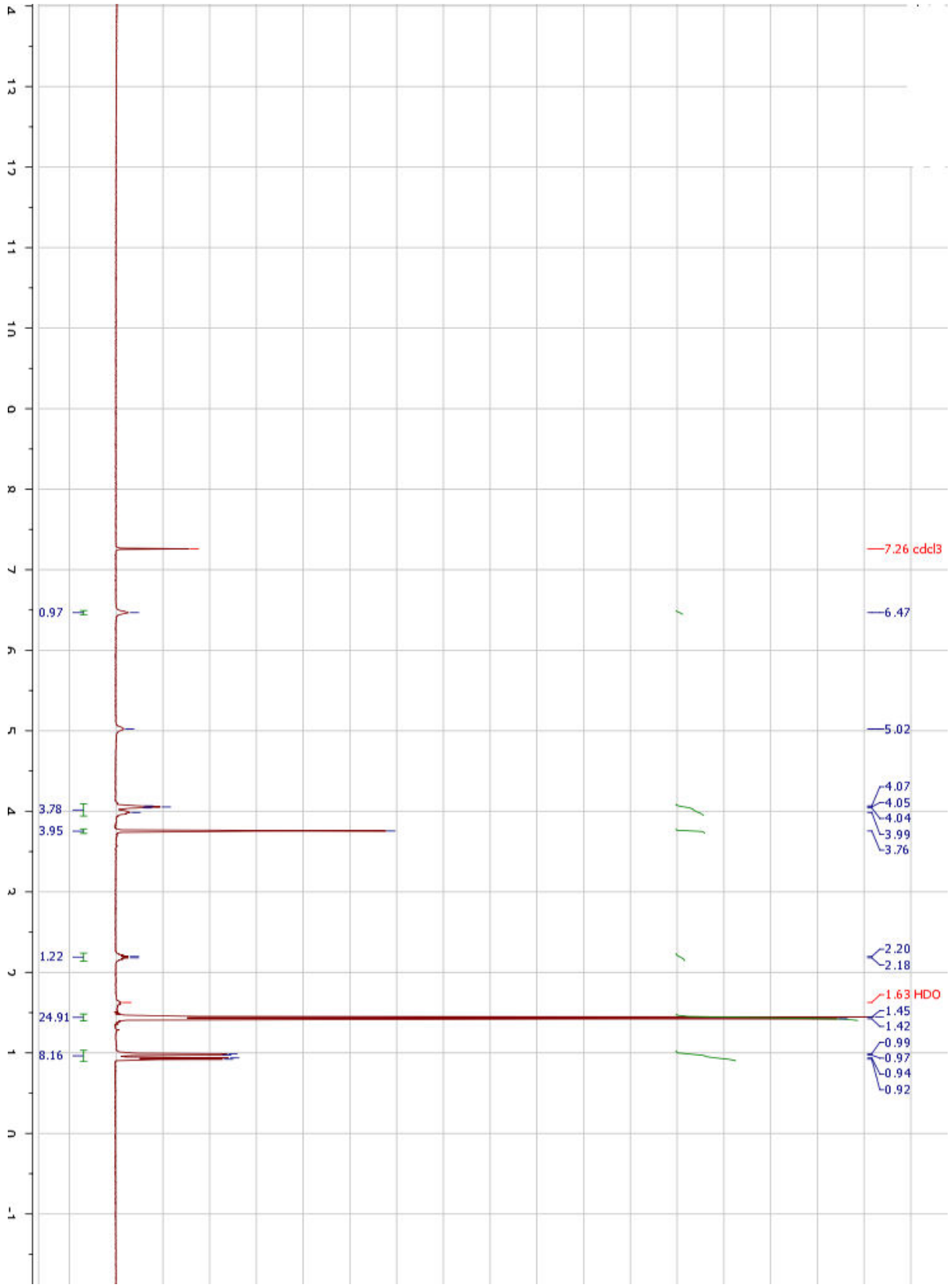
^{1}H-NMR for (**9**) ($CDCl_3$, 400 MHz): δ 0.77 (3H, d, J = 8 Hz), δ 1.01 (3H, d, J = 8 Hz), δ 1.25-1.38 (6H, m), δ 2.18-2.27 (1H, m), δ 3.75-3.77 (1H, t, J = 8 Hz), δ 4.10-4.27 (5H, m), δ 5.08 (1H, m), δ 7.16-7.40 (5H, m). (5H, m).
^{13}C-NMR for (**9**) ($CDCl_3$, 100 MHz): δ 165.62, 161.13, 141.61, 127.89, 127.65, 126.76, 126.66, 74.54, 61.09, 60.72, 50.48, 31.91, 19.06, 16.87, 14.40, 14.34
HRMS for (**9**) (ESI) m/z: $[M+H]^+$ calculated for $C_{18}H_{24}N_2O_3$ $[M+Na]^+$: 341.1798; Found: 341.1839

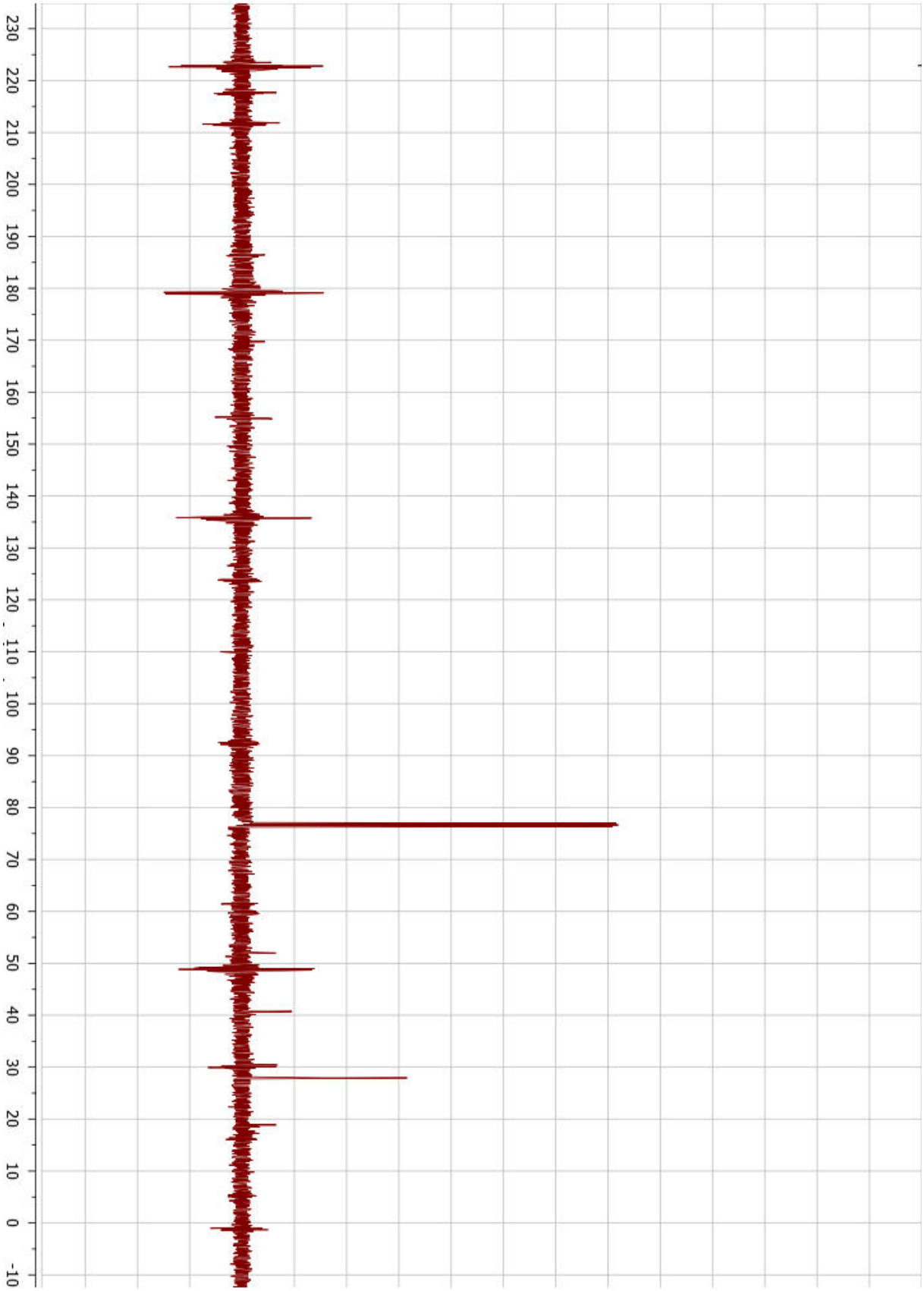
^{1}H-NMR for (**12**) ($CDCl_3$, 400 MHz): δ 0.73 (3H, d, J = 8 Hz), δ 0.98 (3H, d, J = 4 Hz), δ 1.03 (3H, d, J = 4 Hz), δ 1.04 (3H, d, J = 4 Hz), δ 1.21-1.33 (4H, m), δ 1.94-2.03 (1H, m), δ 2.22-2.30 (1H, m), δ 3.93-3.96 (1H, m), δ 4.04-4.05 (1H, m), δ 4.07-4.25 (5H, m).
^{13}C-NMR for (**12**) ($CDCl_3$, 100 MHz): δ 165.30, 162.13, 77.57, 60.97, 60.89, 57.42, 32.23, 31.18, 19.63, 19.45, 19.21, 19.12, 17.03, 14.50, 14.46
HRMS for (**12**) (ESI) m/z: $[M+H]^+$ calculated for $C_{15}H_{26}N_2O_3$ $[M+H]^+$: 285.2180; Found:

285.2169
^{1}H-NMR for (**15**) ($CDCl_3$, 400 MHz): δ 0.58 (3H, d, J = 8 Hz), δ 0.9 (3H, d, J = 4 Hz), δ 1.13-1.36 (6H, m), δ 2.09-2.22(1H, m), δ 3.04 (1H, t, J = 8.0 Hz), δ 3.91-4.25 (4H, m), δ 4.65 (3H, s), δ 7.17-7.38 (5H, m). ^{13}C-NMR for (**15**) ($CDCl_3$, 100 MHz): No carbon specter for this molecule. HRMS for (**15**) (ESI) m/z: $[M+H]^+$ calculated for $C_{19}H_{26}N_2O_3$ $[M+H]^+$: 333.2180; Found: 333.2166
^{1}H-NMR for (**18**) ($CDCl_3$, 400 MHz): δ 0.53 (3H, d, J = 4.0 Hz), δ 0.81 (3H, d, J = 8.0 Hz), δ 0.87 (3H, t, J = 12.0), δ 1.01 (3H, t, J = 12.0), δ 2.00-2.08 (1H, m), δ 3.29 (1H, t, J = 8.0), δ 3.73-3.92 (5H, m), δ 4.69 (1H, d, J = 4.0), δ 7.07-6.68 (10H, m). ^{13}C-NMR for (**18**) ($CDCl_3$, 100 MHz): δ 196.83, 164.41, 160.88, 144.31, 144.29, 137.71, 132.51, 130.16, 128.38, 127.82, 127.75, 127.46, 127.11, 126.91, 80.75, 63.92, 61.08, 60.94, 60.87, 31.49, 19.19, 16.86, 14.31, 13.97 HRMS for (**18**) (ESI) m/z: $[M+H]^+$ calculated for $C_{24}H_{30}N_2O_3$ $[M+K]^+$: 433.3283; Found: 433.1887

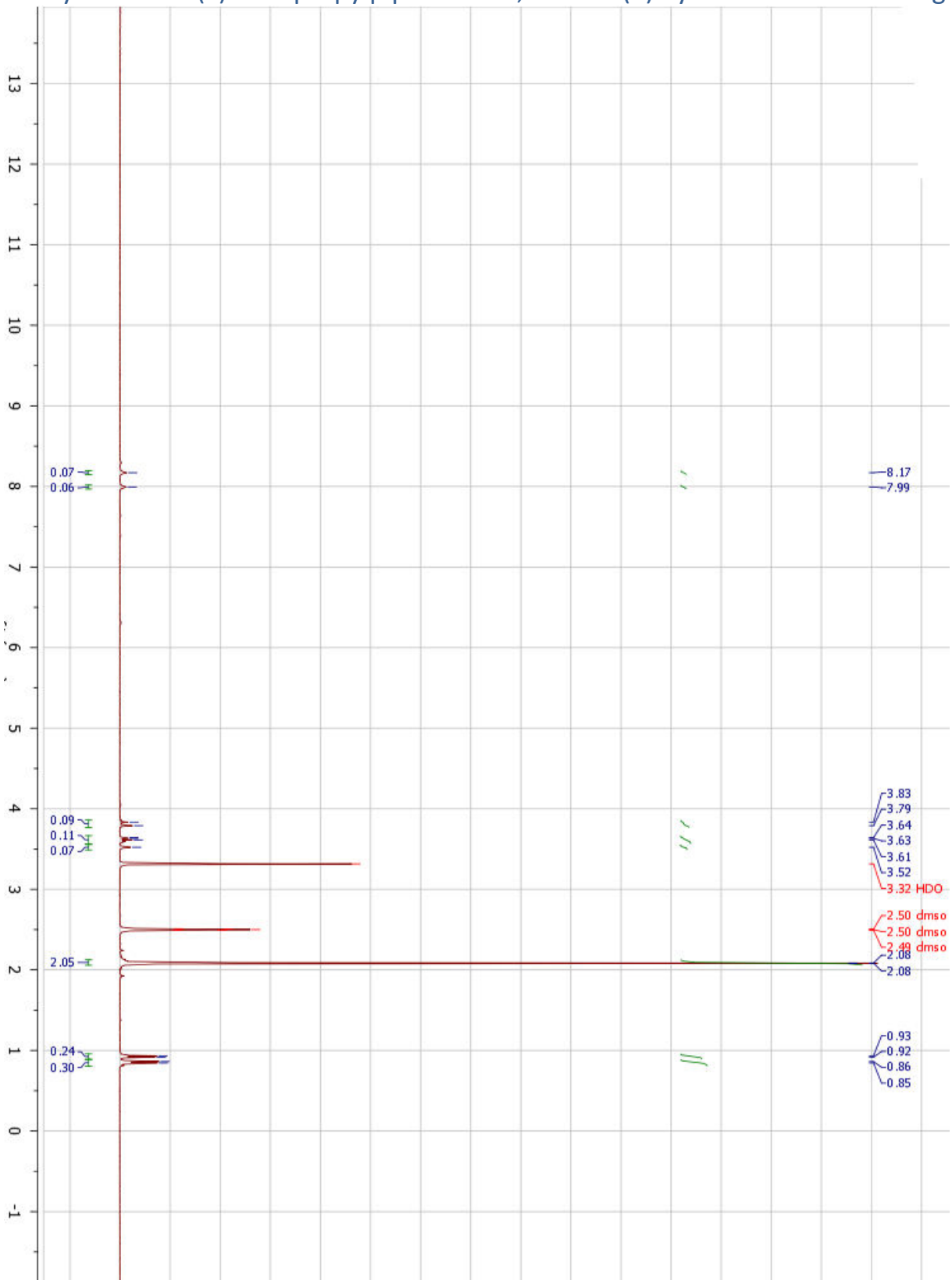
7 Spectra of molecules

7.1 (R)-methyl 2-(2-(tert-butoxycarbonylamino)-3-methylbutanamido)acetate (5)

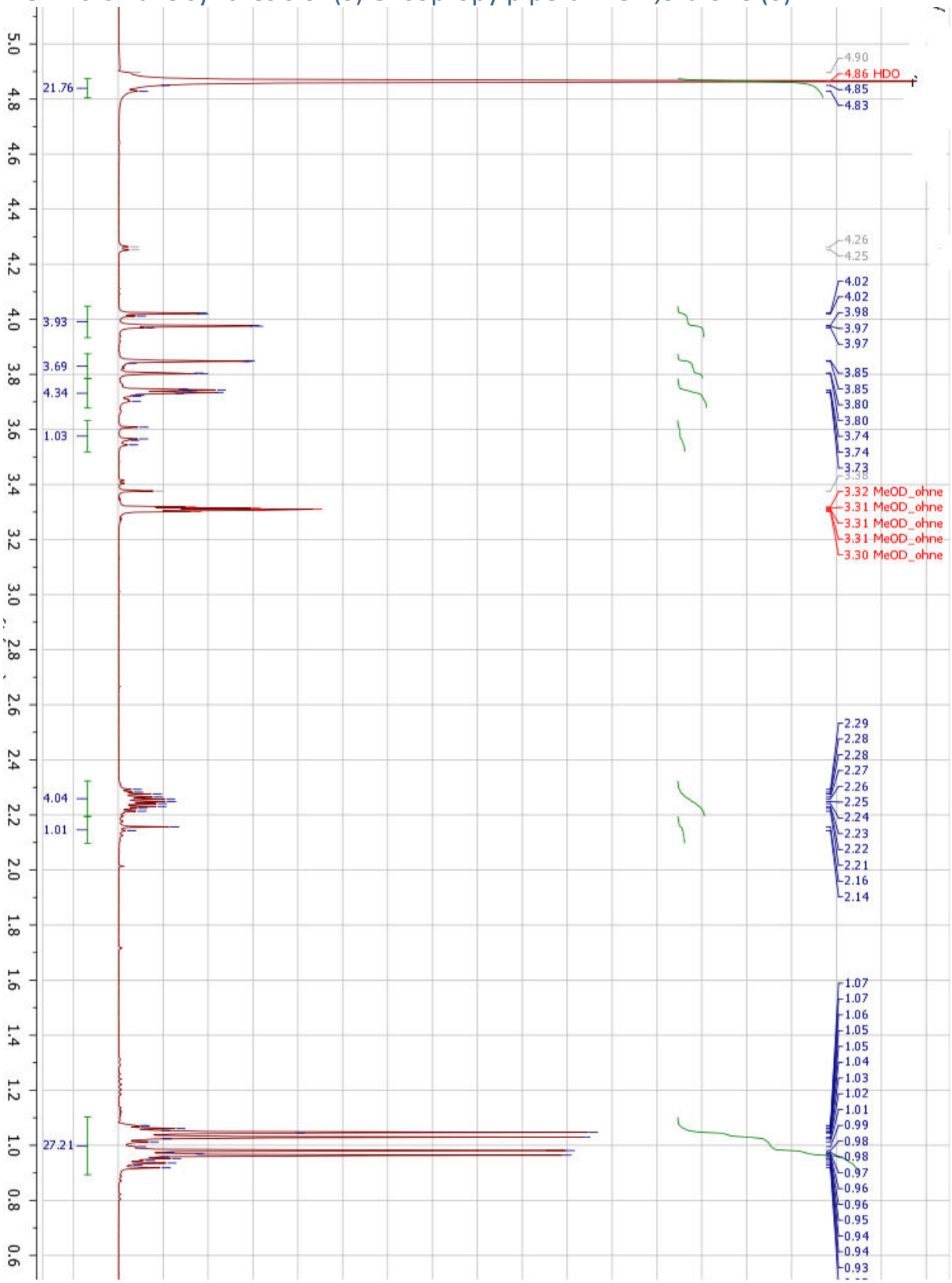

7.26 cdcl3
6.47
5.02
4.07
4.05
4.04
3.99
3.76
2.20
2.18
1.63 HDO
1.45
1.42
0.99
0.97
0.94
0.92
0.97
3.78
3.95
1.22
24.91
8.16

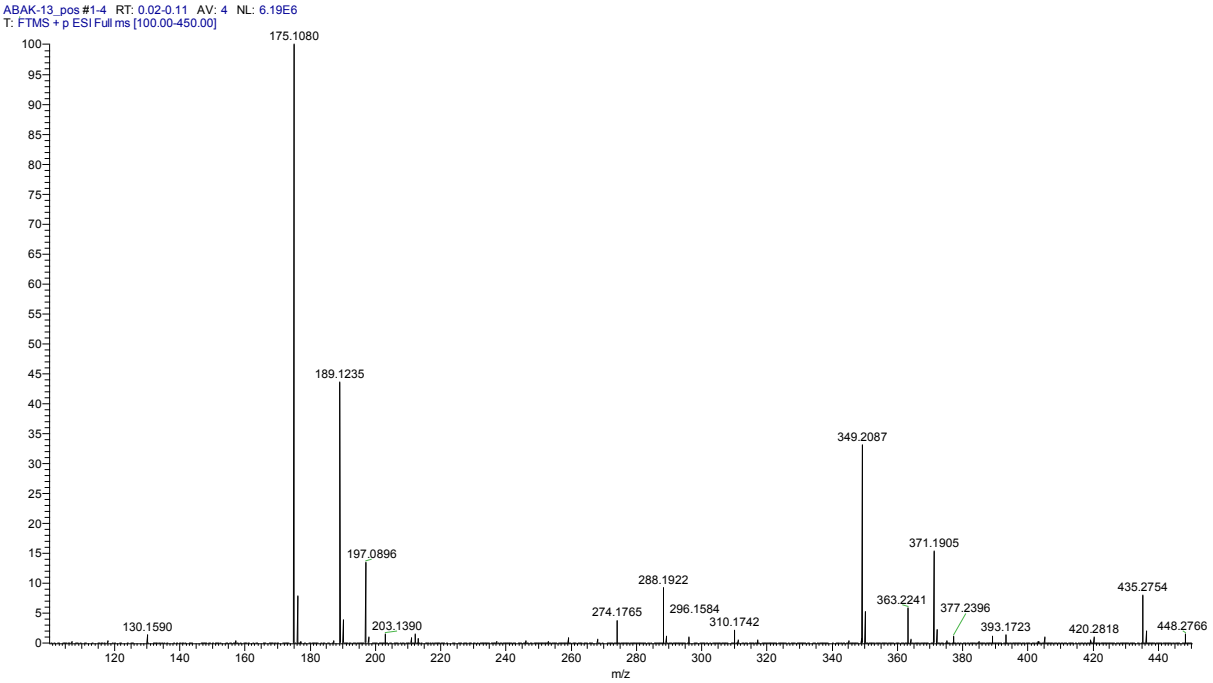

230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

7.2 Synthesis of (S)-3-isopropylpiperazine-2,5-dione (6) by conventional heating

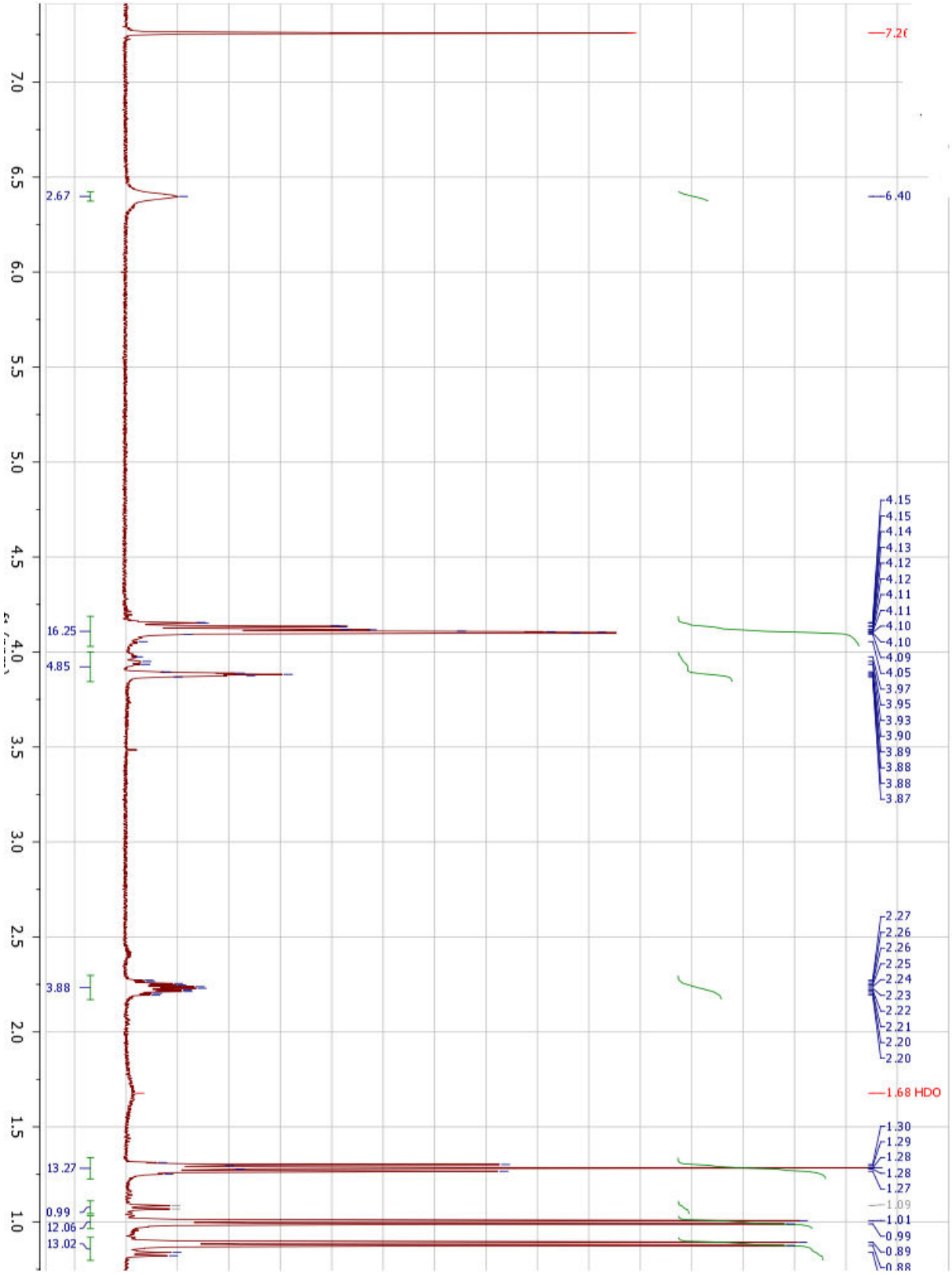


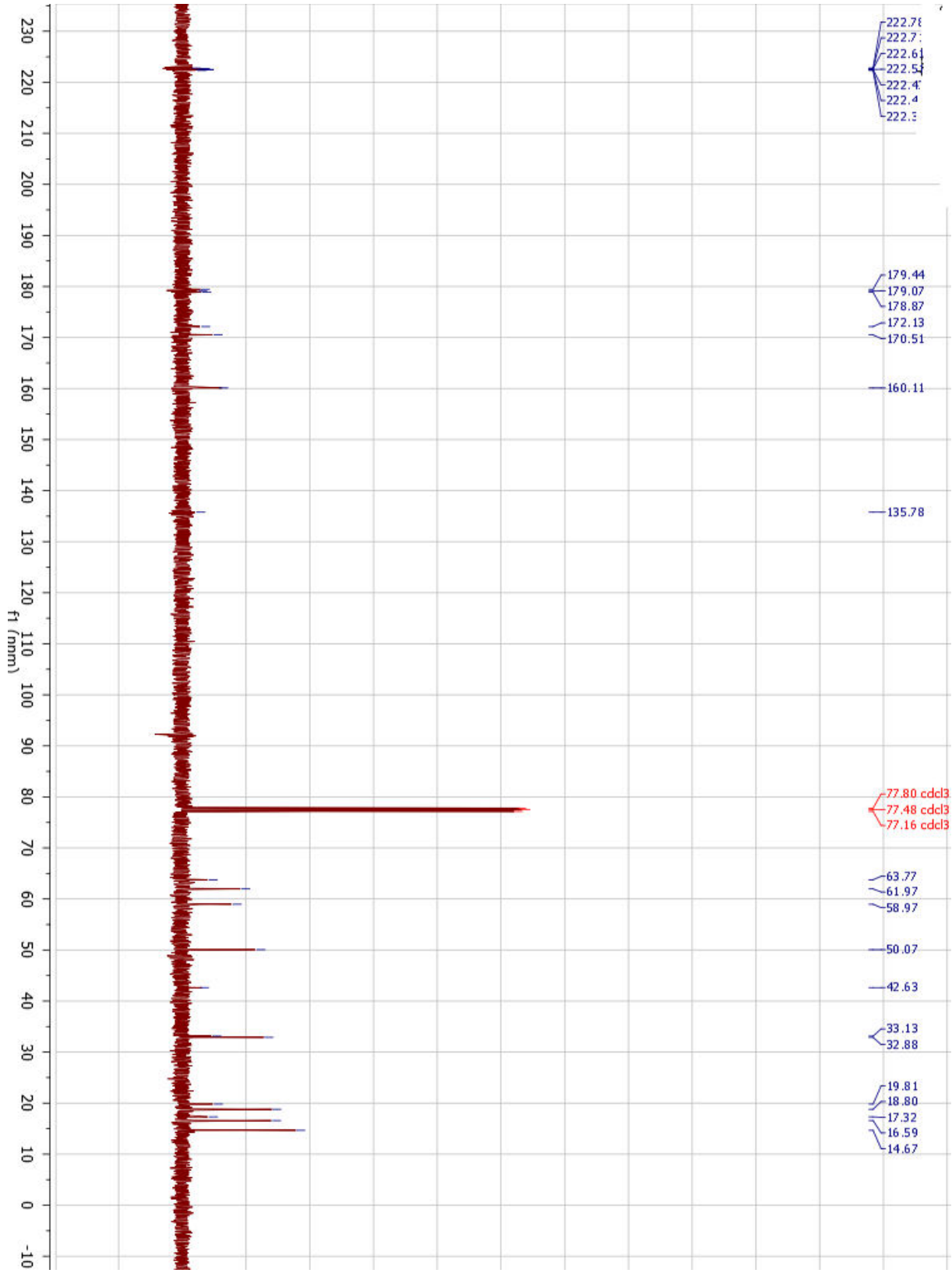
7.3 Microwave synthesis of (S)-3-isopropylpiperazine-2,5-dione (6)



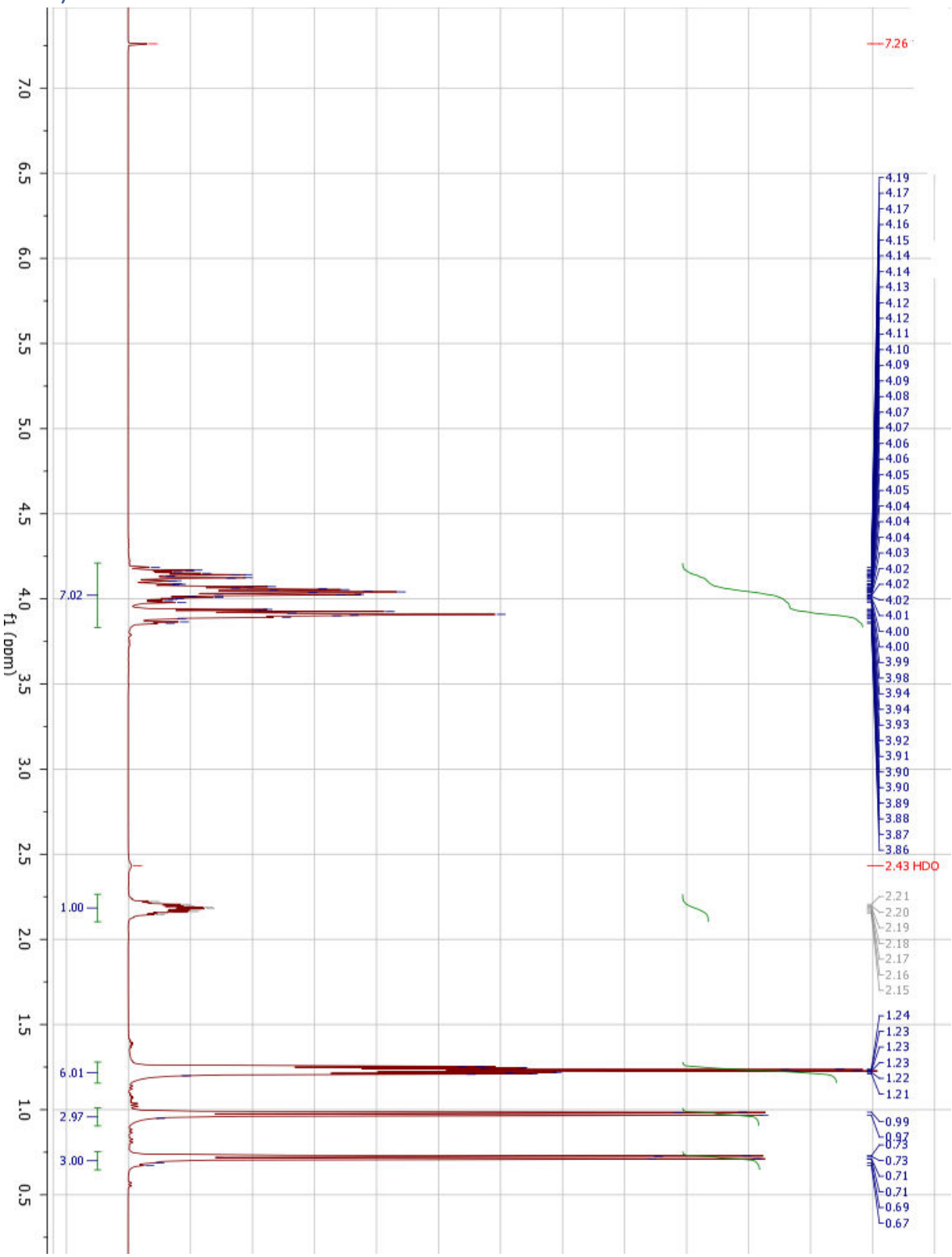

ABAK-13_pos #1-4 RT: 0.02-0.11 AV: 4 NL: 6.19E6
T: FTMS + p ESI Full ms [100.00-450.00]
175.1080
189.1235
197.0896
130.1590
203.1390
274.1765
288.1922
296.1584
310.1742
349.2087
363.2241
371.1905
377.2396
393.1723
420.2818
435.2754
448.2766
m/z

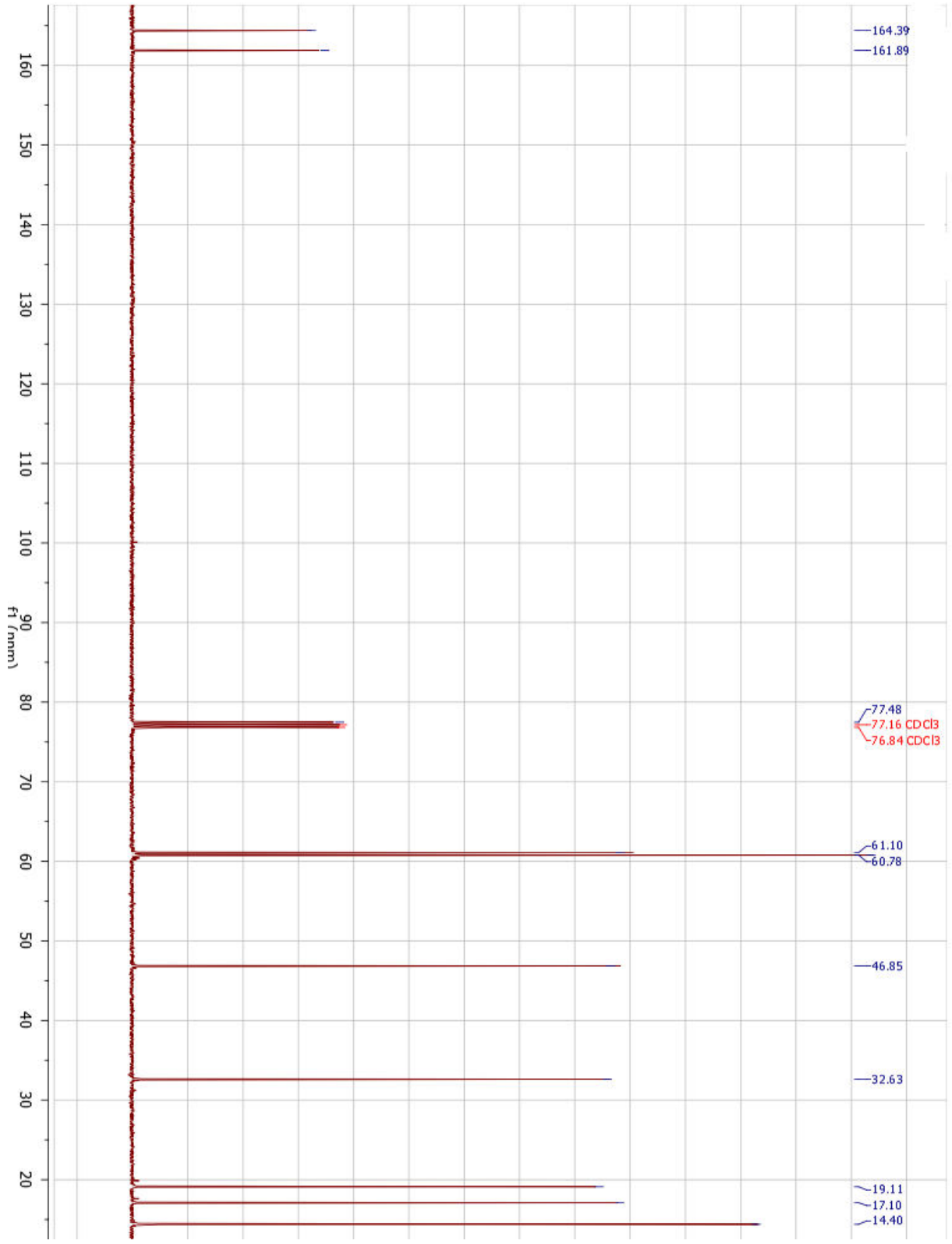
7.4 (S)-3,6-diethoxy-2-isopropyl-2,5-dihydropyrazine (7) with $Et_3O^+BF_4^-$ solution.

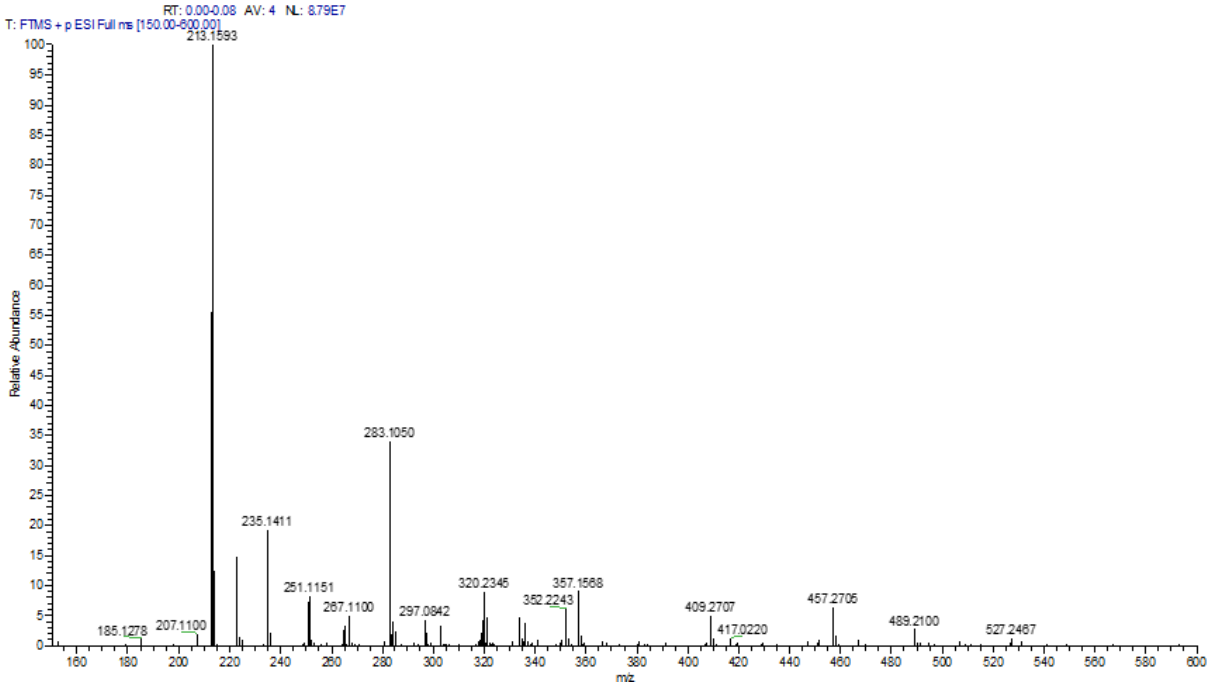



222.78
222.7
222.61
222.5
222.4
222.4
222.3
179.44
179.07
178.87
172.13
170.51
160.11
135.78
77.80 cdcl3
77.48 cdcl3
77.16 cdcl3
63.77
61.97
58.97
50.07
42.63
33.13
32.88
19.81
18.80
17.32
16.59
14.67
f1 (ppm)

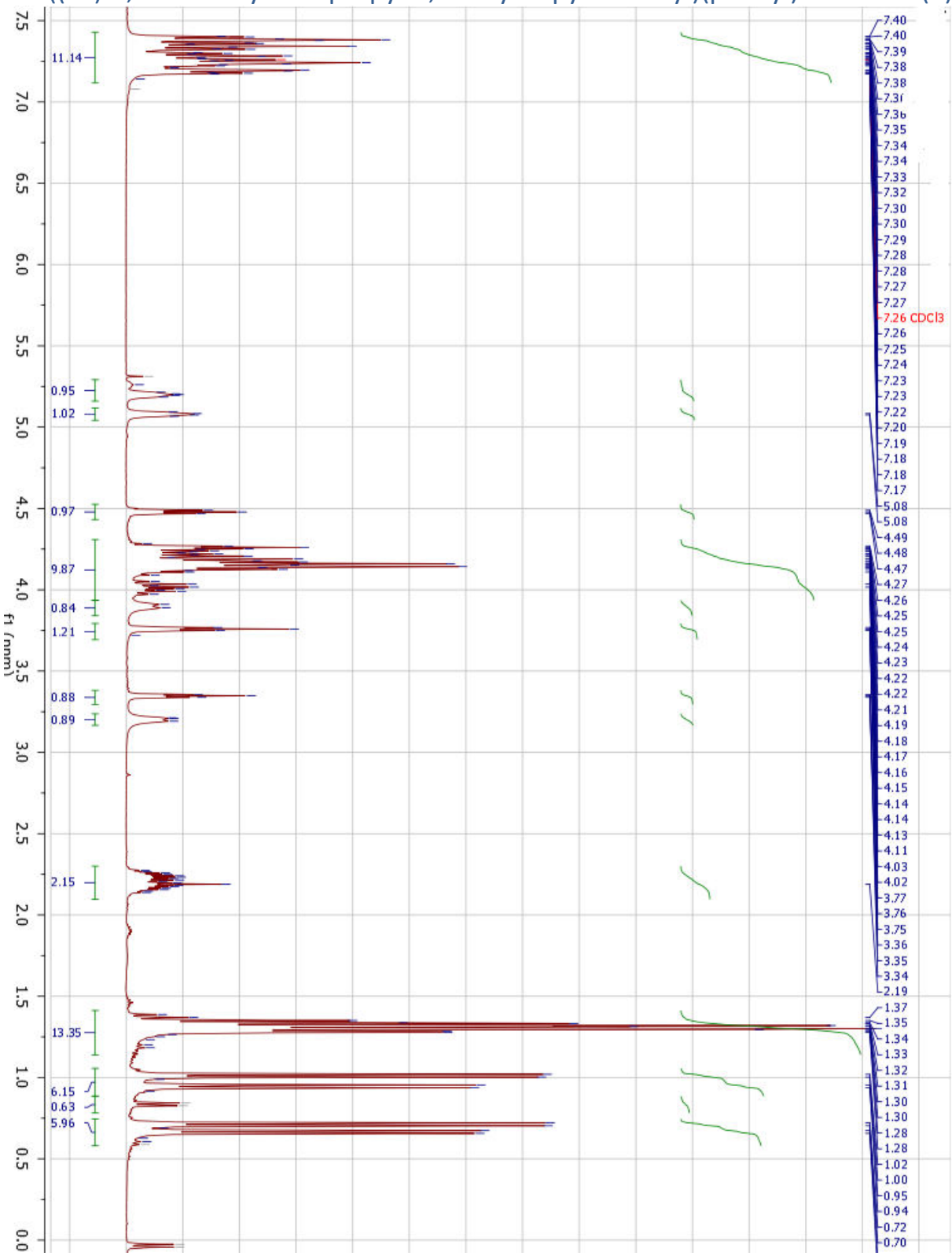
7.5 (S)-3,6-diethoxy-2-isopropyl-2,5-dihydropyrazine (7) with $Et_3O^+BF_4^-$ (pure solid)

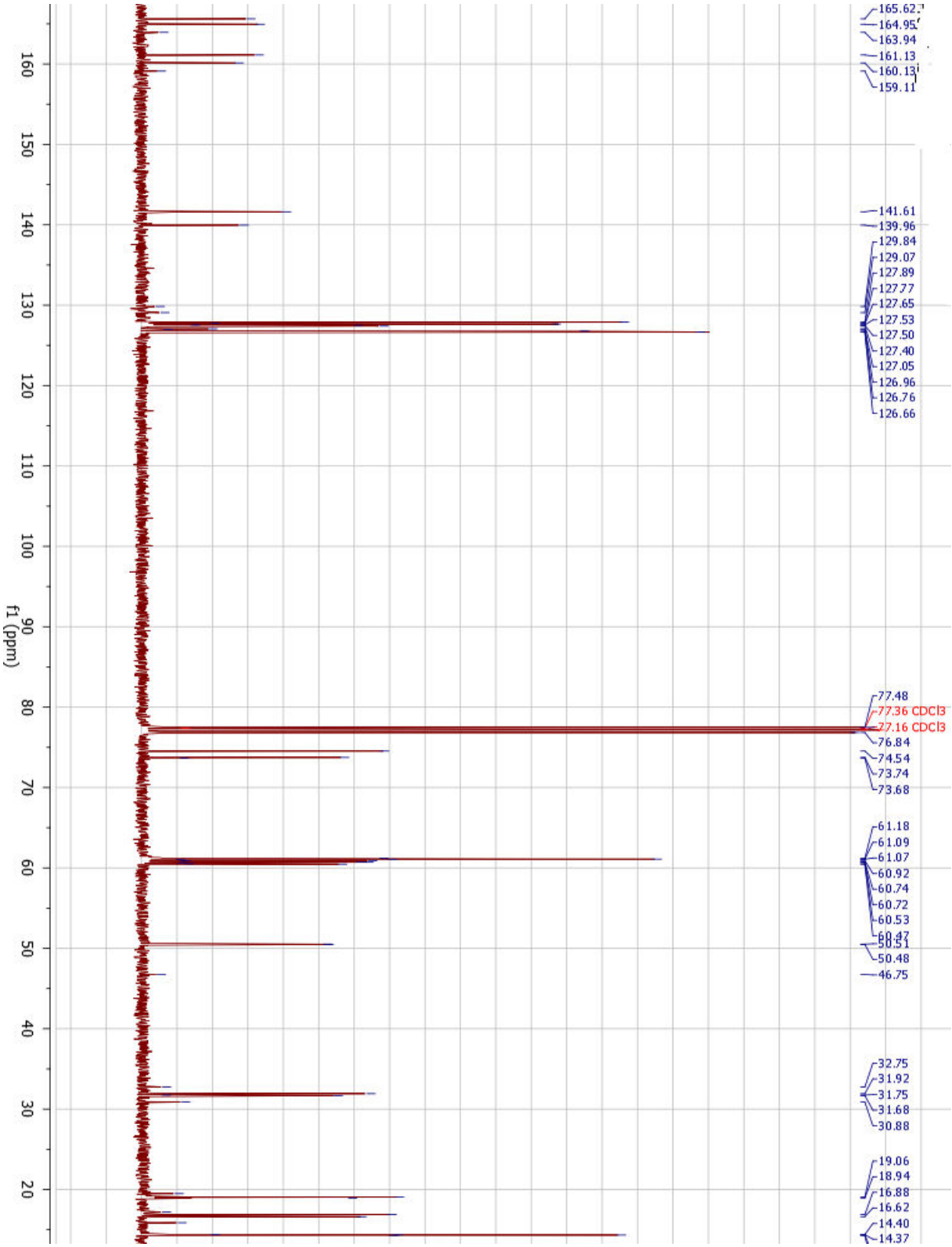


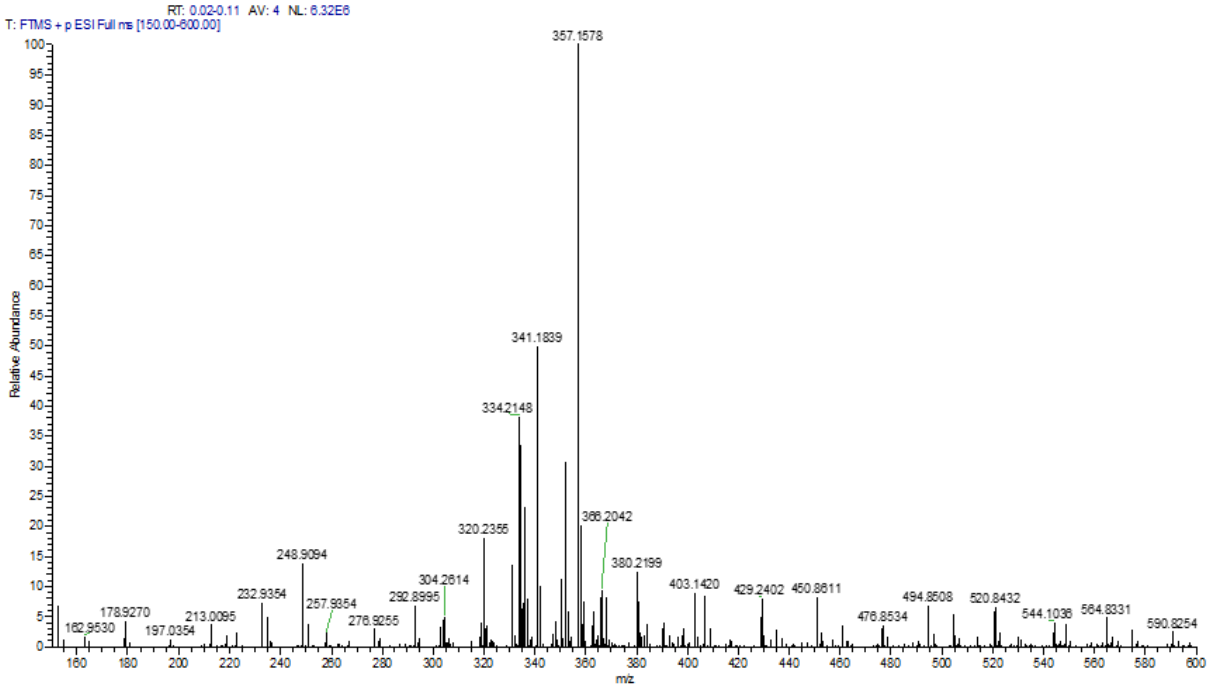

164.39
161.89
77.48
77.16 CDCl3
76.84 CDCl3
61.10
60.78
46.85
32.63
19.11
17.10
14.40
f1 (ppm)


RT: 0.00-0.08 AV: 4 NL: 8.79E7
T: FTMS + p ESI Full ms [150.00-600.00]
213.1593
283.1050
235.1411
251.1151
267.1100
297.0842
320.2345
357.1568
352.2243
409.2707
457.2705
489.2100
527.2467
185.1278
207.1100
417.0220
Relative Abundance
m/z

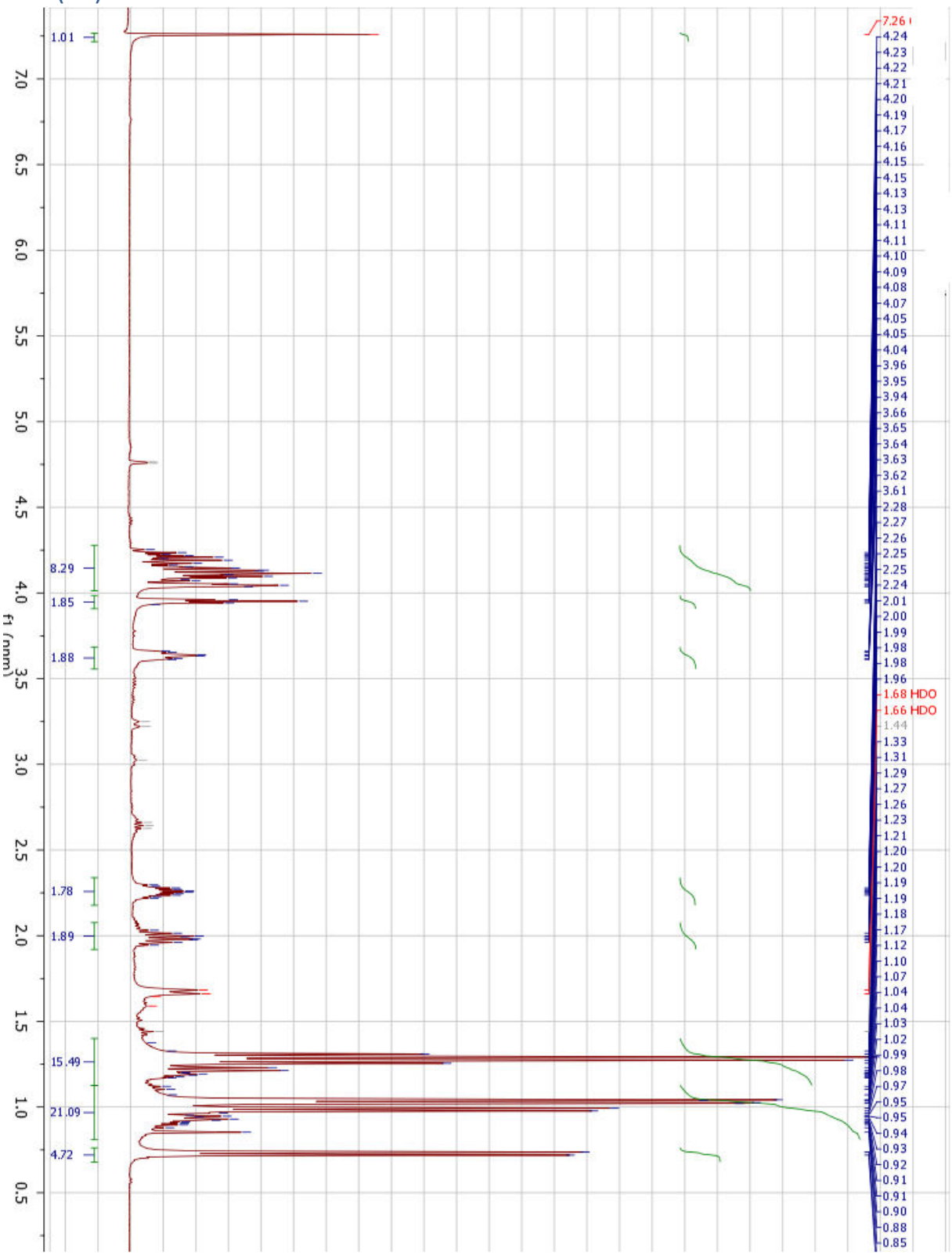
7.6 ((5S)-3,6-diethoxy-5-isopropyl-2,5-dihydropyrazin-2-yl)(phenyl)methanol (9)

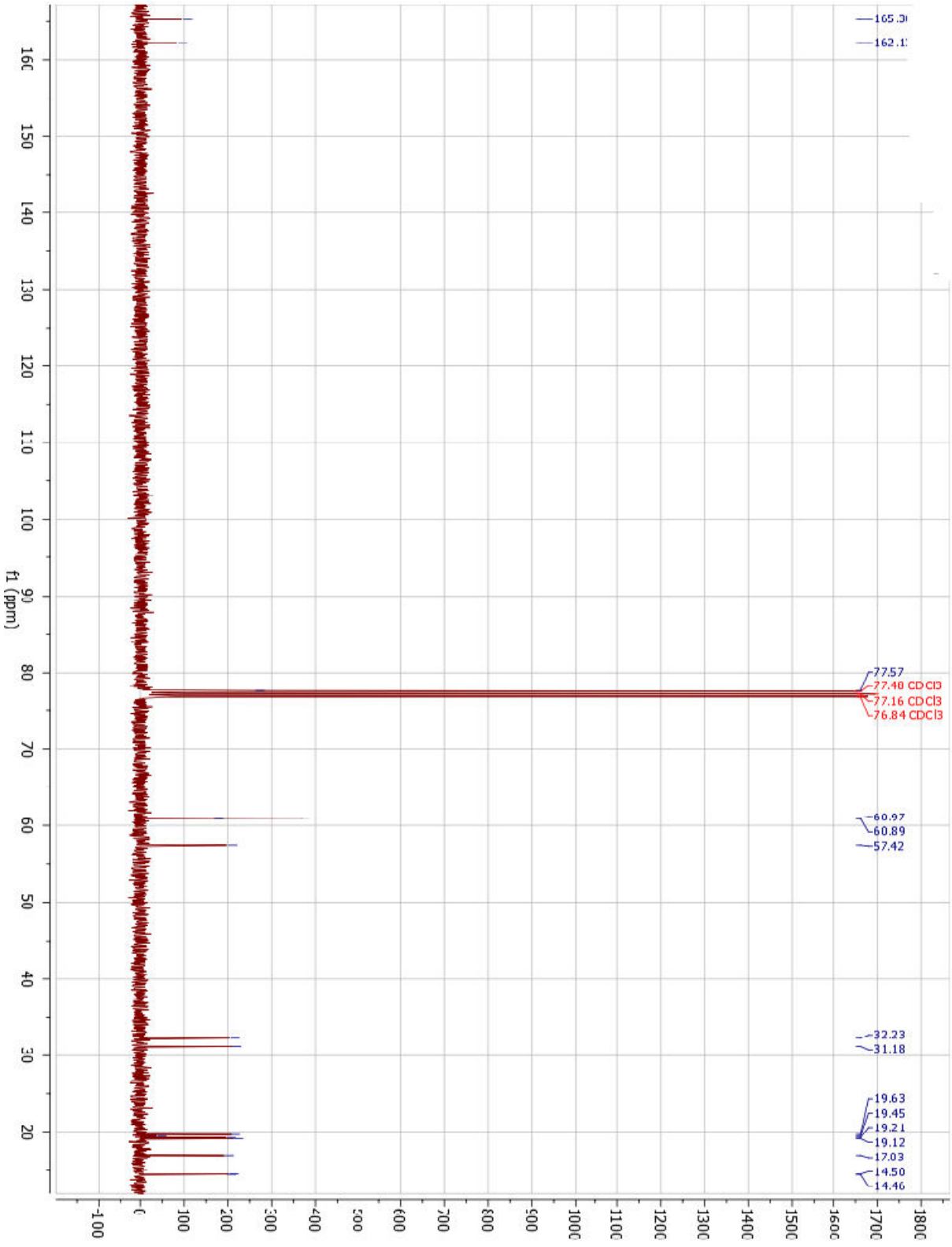


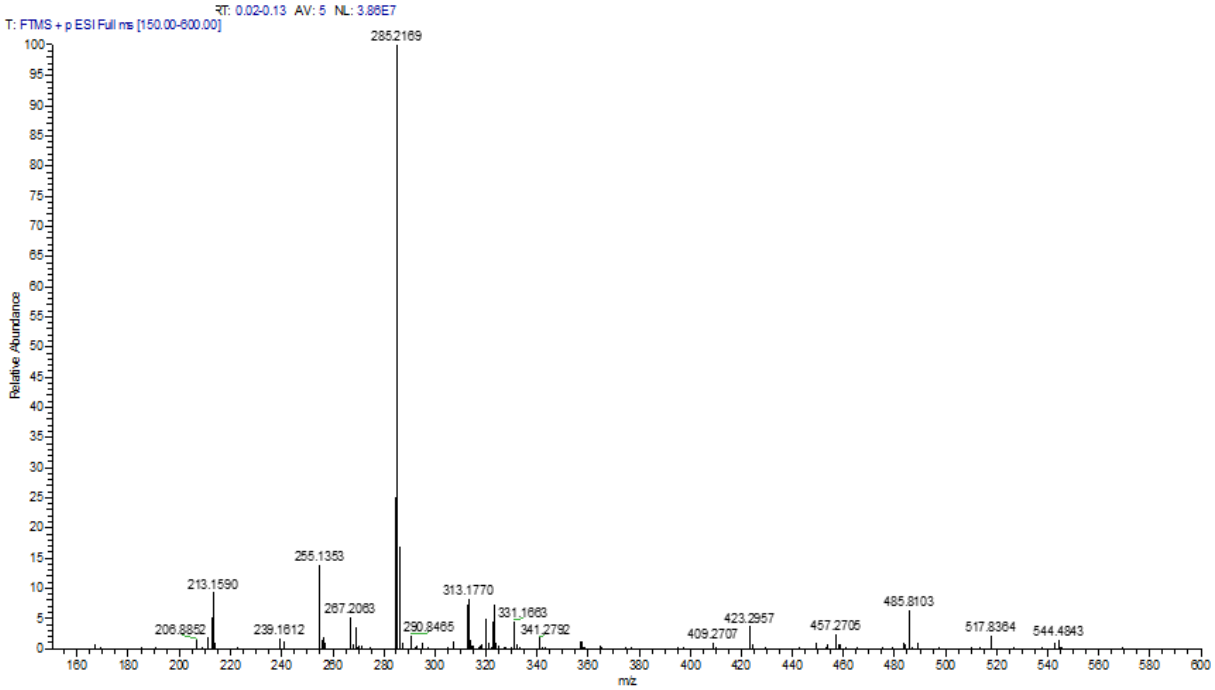

f1 (ppm)
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
165.62
164.95
163.94
161.13
160.13
159.11
141.61
139.96
129.84
129.07
127.89
127.77
127.65
127.53
127.50
127.40
127.05
126.96
126.76
126.66
77.48
77.36 CDCl3
77.16 CDCl3
76.84
74.54
73.74
73.68
61.18
61.09
61.07
60.92
60.74
60.72
60.53
50.51
50.48
46.75
32.75
31.92
31.75
31.68
30.88
19.06
18.94
16.88
16.62
14.40
14.37


RT: 0.02-0.11 AV: 4 NL: 6.32E6
T: FTMS + p ESI Full ms [150.00-600.00]
Relative Abundance
m/z
357.1578
341.1839
334.2148
366.2042
320.2355
380.2199
248.9094
304.2614
403.1420
429.2402
450.8611
494.8508
520.8432
544.1036
564.8331
590.8254
476.8534
232.9354
292.8995
257.9854
276.9255
178.9270
213.0095
162.9530
197.0354

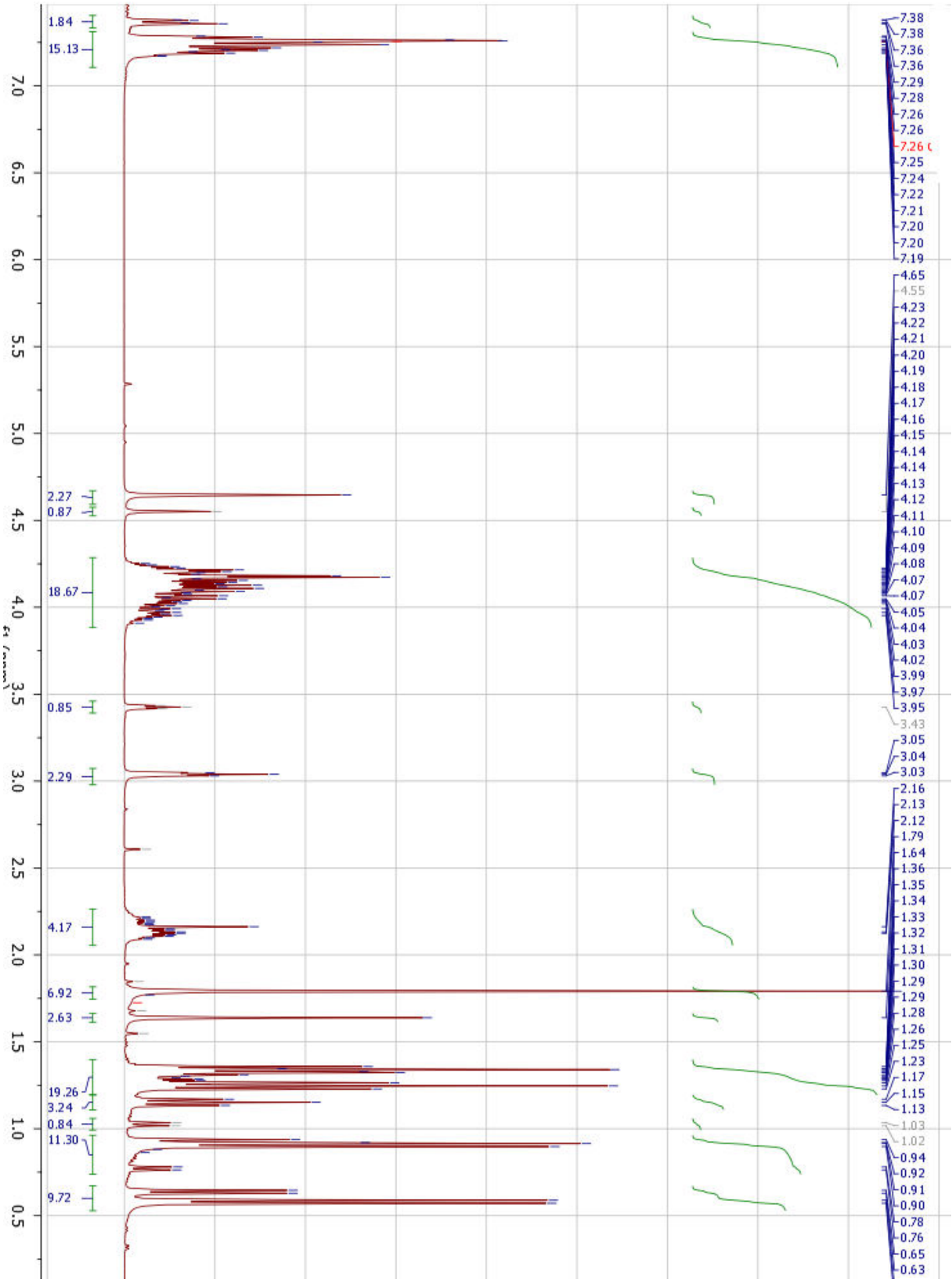
7.7 1-((5S)-3,6-diethoxy-5-isopropyl-2,5-dihydropyrazin-2-yl)-2-methylpropan-1-ol (12)

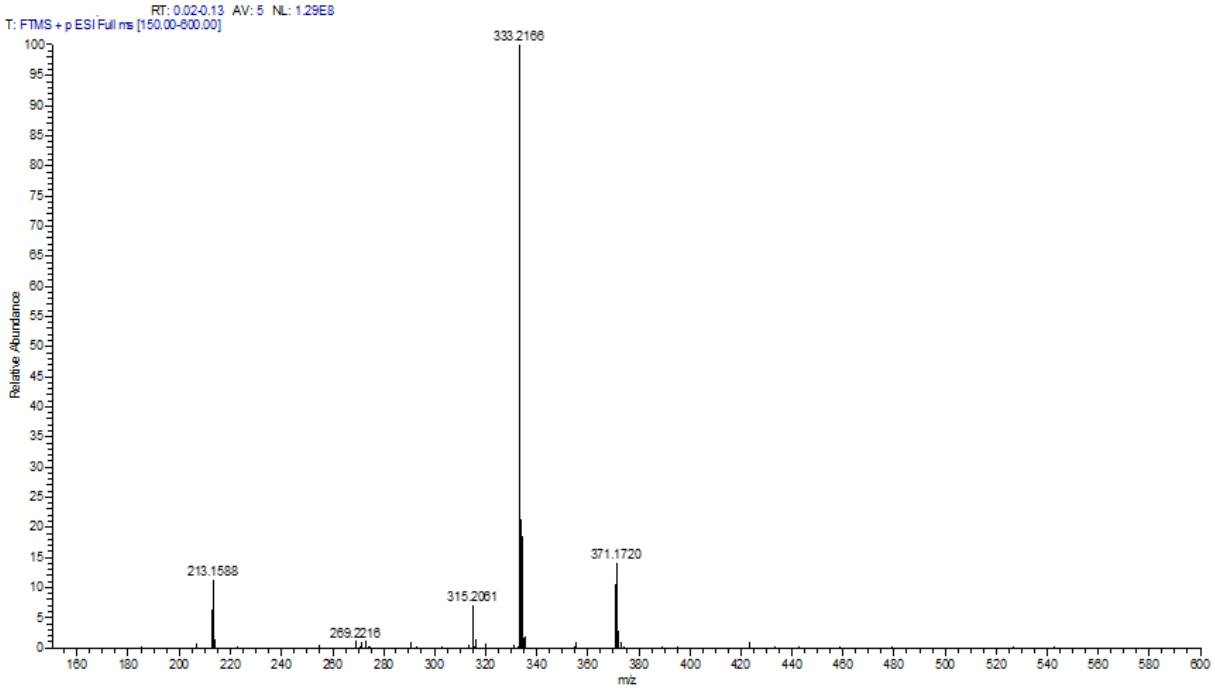



165.3
162.1
77.57
77.40 CDCl3
77.16 CDCl3
76.84 CDCl3
60.97
60.89
57.42
32.23
31.18
19.63
19.45
19.21
19.12
17.03
14.50
14.46
f1 (ppm)

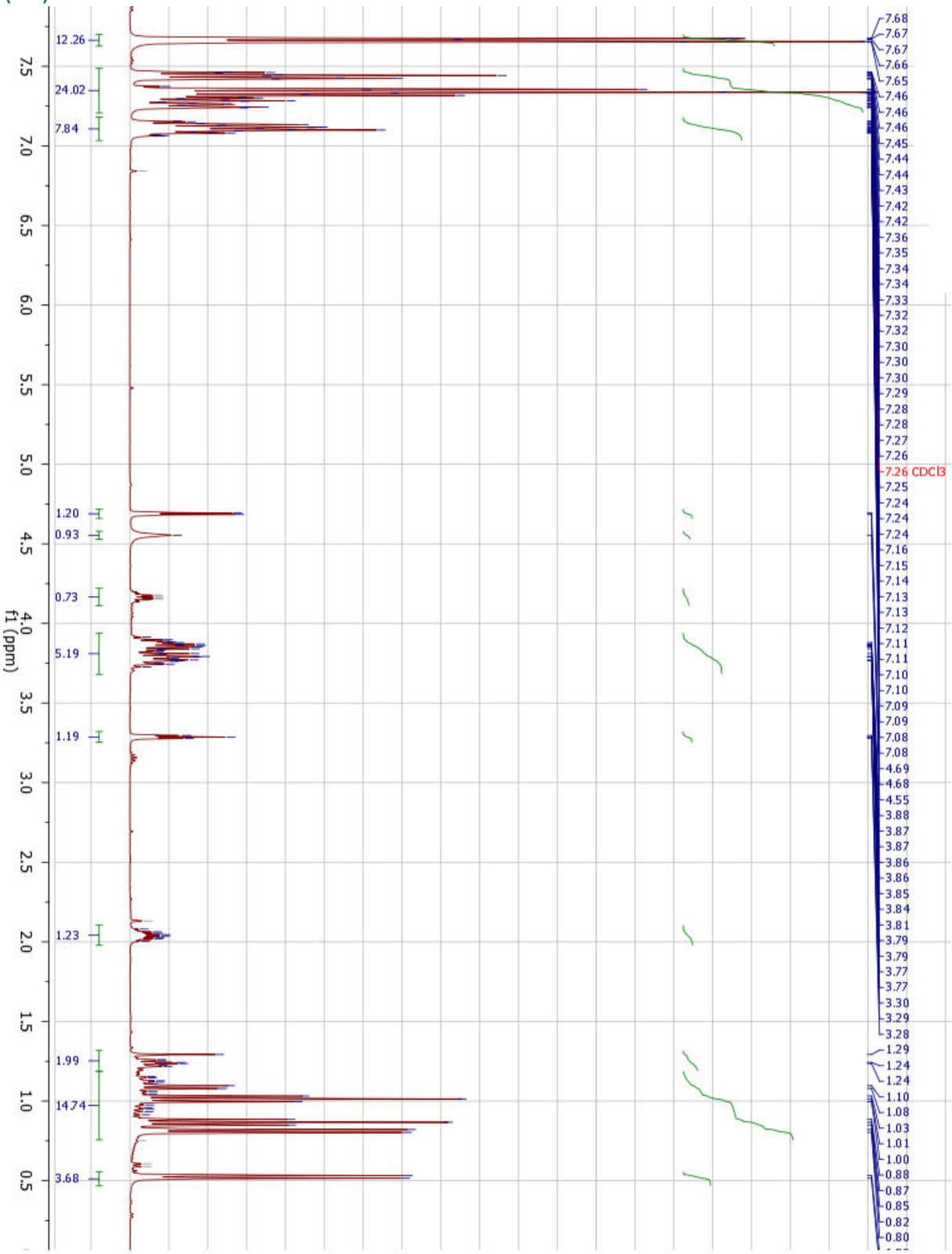

RT: 0.02-0.13 AV: 5 NL: 3.86E7
T: FTMS + p ESI Full ms [150.00-600.00]
285.2169
213.1590
255.1353
267.2063
206.8852
239.1612
290.8465
313.1770
331.1663
341.2792
409.2707
423.2957
457.2705
485.8103
517.8364
544.4843
Relative Abundance
m/z

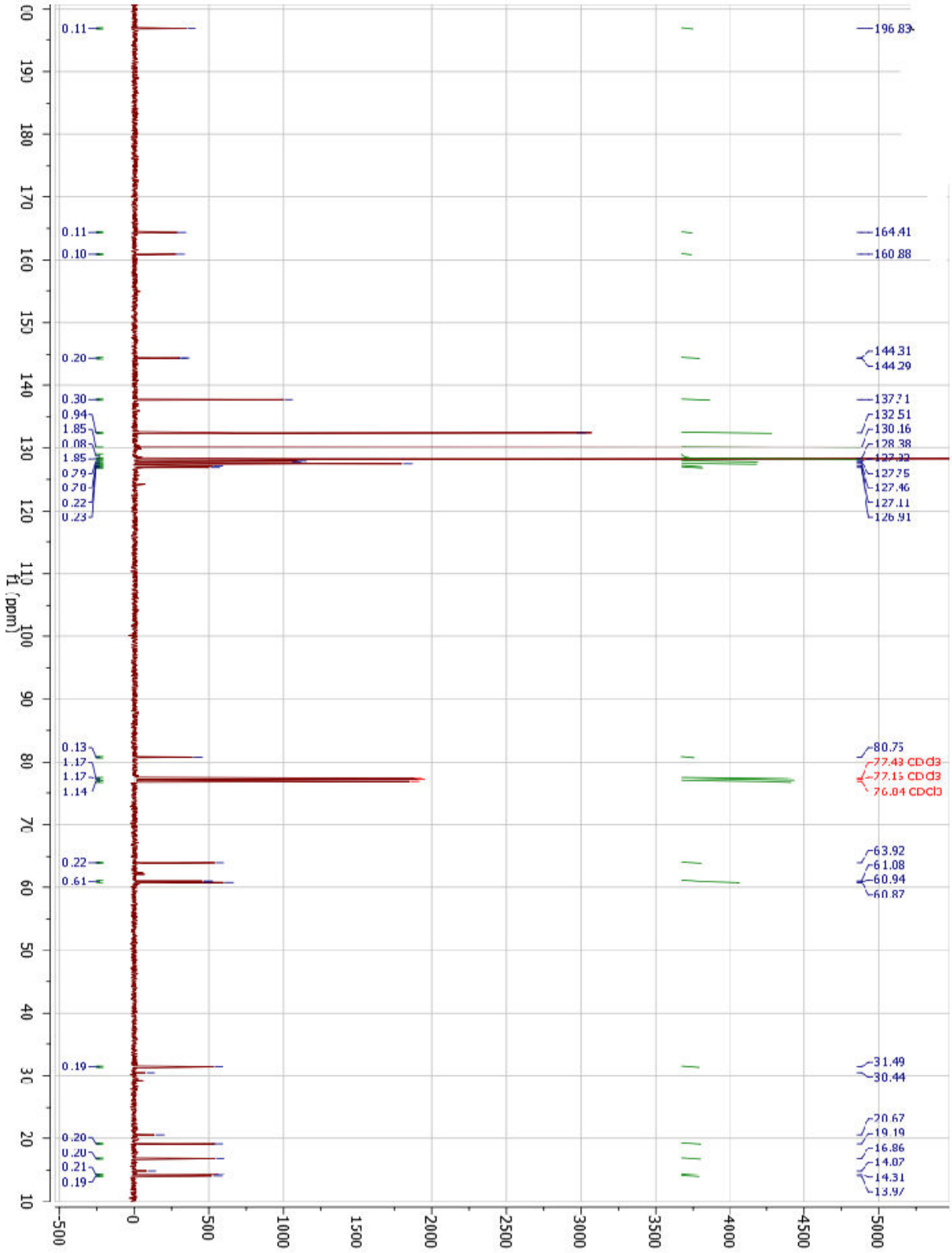
7.8 1-((5S)-3,6-diethoxy-5-isopropyl-2,5-dihydropyrazin-2-yl)-1-phenylethanol (15)

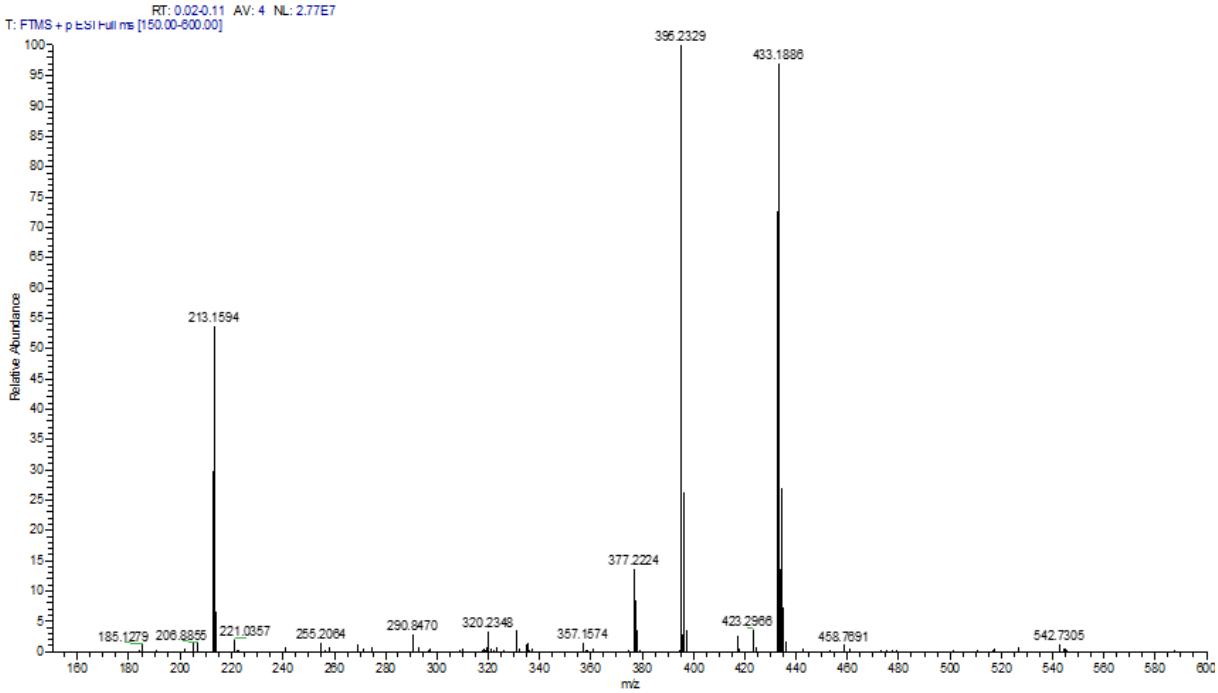



RT: 0.02-0.13 AV: 5 NL: 1.29E8
T: FTMS + p ESI Full ms [150.00-600.00]
333.2166
371.1720
213.1588
315.2061
269.2216
Relative Abundance
m/z

7.9 ((5S)-3,6-diethoxy-5-isopropyl-2,5-dihydropyrazin-2-yl)diphenylmethanol (18)




f1 (ppm)
0.11
0.11
0.10
0.20
0.30
0.94
1.85
0.08
1.85
0.79
0.70
0.22
0.23
0.13
1.17
1.17
1.14
0.22
0.61
0.19
0.20
0.20
0.21
0.19
196.83
164.41
160.88
144.31
144.29
137.71
132.51
130.16
128.38
127.33
127.75
127.46
127.11
126.91
80.75
77.43 CDCl3
77.15 CDCl3
76.84 CDCl3
63.92
61.08
60.94
60.87
31.49
30.44
20.67
19.19
16.86
14.07
14.31
13.97


RT: 0.02-0.11 AV: 4 NL: 2.77E7
T: FTMS + p ESI Full ms [150.00-600.00]
Relative Abundance
m/z
395.2329
433.1886
213.1594
377.2224
185.1279
206.8855
221.0357
255.2064
290.8470
320.2348
357.1574
423.2966
458.7891
542.7305