Development of a novel method for synthesis of β-lactams by the use of diazoacetamides

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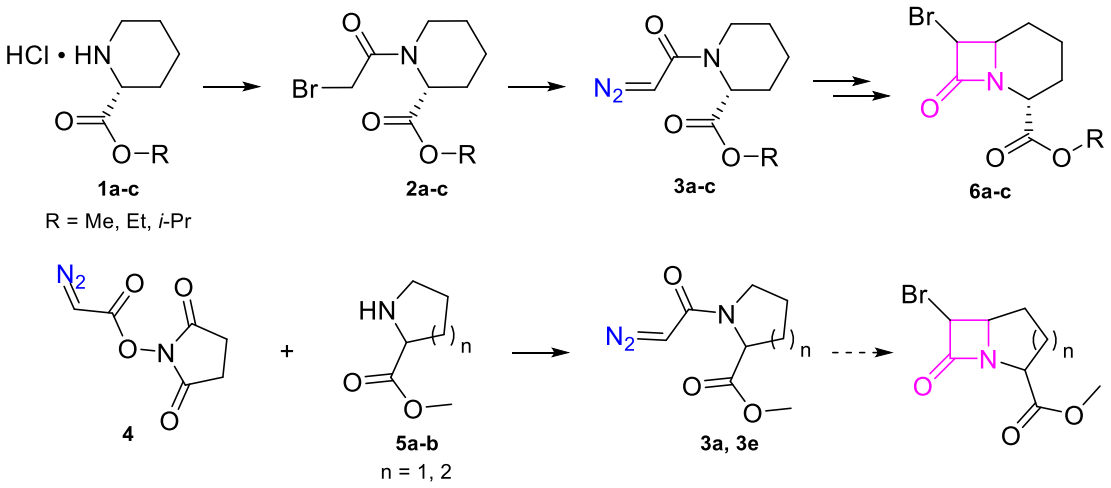
Ida-Helene Kågen Spydevold
Oslo, May 2022

Abstract

Due to the increase in antibacterial resistance, the world is in need of new, powerful antibacterial agents and synthetic methods to halter this trend. This study focused on broadening the scope of a new synthesis of β-lactams developed by Kaupang and Konradsen. The method uses carbenes generated from α-bromodiazoacetamides in an intramolecular C-H insertion reaction to generate the β-lactam moiety. With this new method, it could be possible to synthesize new β-lactam molecules. This creates an opportunity of discovering molecules with novel antibacterial properties beyond what is known today.

The method was successfully applied in the syntheses of the diazoacetamides with a methyl, ethyl or isopropyl ester. Only the β-lactam with the ethyl ester was successfully synthesized from the diazoacetamides. Consequently, not enough data was available to determine how the product distribution in this synthetic step was affected by the ester.

Additional focus of this thesis was on improving the synthesis of the diazoacetamides, due to a difficult purification and moderate yields. Several test reactions were conducted in order to develop a one-step synthesis of the diazoacetamides. A one-step reaction could avoid the problems in the original procedure, in addition to making the β-lactam formation more effective. The test reaction using 2,5-dioxypyrrolidin-1-yl 2-diazoacetate and methyl D-prolinate as reactants seems promising, but more work is necessary to develop the procedure further.



Abbreviations

ACN	Acetonitrile
Ala	Alanine
ATR	Attenuated Total Reflectance
Cat.	Catalyst
COSY	Correlation Spectroscopy
DBU	1,8-Diazabicyclo-[5.4.0]undec-7-ene
DCC	*N*,*N*'-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DEPT	Distortionless Enhancement by Polarization Transfer
DMAP	4-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
DMPU	1,3-Dimethyl-1,3-diazinan-2-one
DMSO	Dimethyl sulfoxide
DTH	*N*,*N*'-Ditosylhydrazine
Eqv.	Equivalents
Err	Error
ESI	Electrospray ionization
Hex	Hexane
HMBC	Heteronuclear Multiple Bond Correlation Spectroscopy
HR-MS	High Resolution Mass Spectrometry
HSQC	Heteronuclear Single Quantum Correlation Spectroscopy
IR	Infrared spectroscopy
IS	Internal standard
m/z	Mass-to-charge ratio
MS	Mass spectrometry
NAG	*N*-Acetylglucosamine
NAM	*N*-Acetylmuramic acid
NBP	*N*-Bromophthalimide
NBS	*N*-Bromosuccinimide
NM	Not measurable
NMR	Nuclear Magnetic Resonance Spectroscopy
PBP	Penicillin-Bonding Proteins
Sat.	Saturated
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TMG	1,1,3,3-Tetramethylguanidine

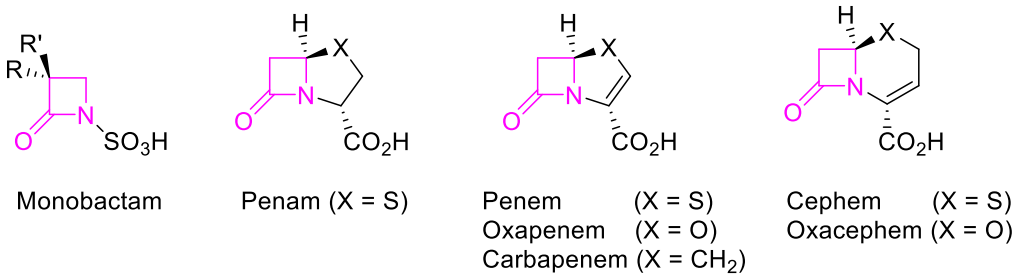
Table of content

1 Introduction

Antibiotics are essential tools for treating human diseases caused by bacteria.[1] However, an ongoing increase in resistance against antibacterial agents constitutes a growing problem to worldwide human health. Simultaneously, fewer introductions of new antibiotics aggravates the situation further. Consequently, the world is in need of new, powerful antibacterial agents, as well as new methods, to halter this trend.[1-2] The Bonge-Hansen group contributes to this problem by developing new methods to synthesize molecules of potential antibacterial properties. One example is the group's work on developing a new method to synthesize β-lactams using diazo compounds and carbenes, in the hope that this might lead to new β-lactam molecules with antibacterial properties.

1.1 β-lactam antibiotics

β-lactam antibiotics are a collective term used for antibacterial agents consisting of a four-membered cyclic amide, where the *N*-atom is attached to the β-carbon relative to the carbonyl group.[3] The β-lactams are divided into several classes depending on their core structure. Monobactams consist solely of the four-membered amide, while the other classes are bicyclic with the four-membered amide fused to a 5- or 6-membered ring. This second ring can be saturated or unsaturated, contain heteroatoms or solely consist of carbon atoms.[4] **Figure 1.1** illustrates some of the different classes, with the β-lactam moiety marked in pink.

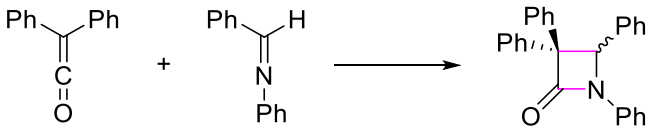


Monobactam | Penam (X = S) | Penem (X = S), Oxapenem (X = O), Carbapenem (X = CH_2) | Cephem (X = S), Oxacephem (X = O)

***Figure 1.1**: Different classes of β-lactams, where the β-lactam moiety is marked in pink.*[4]

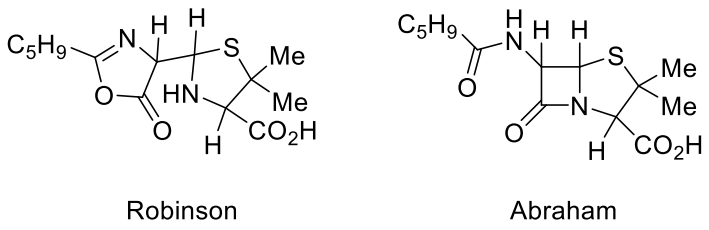
1.1.1 Discovery of the β-lactams

The first reported synthesis of a β-lactam was by Staudinger in 1907, where he formed the ring by a [2+2] cycloaddition between a ketene and an imine (**Scheme 1.1)**.[5] However, the antibacterial effect of the β-lactams were not known until Fleming's accidental discovery of penicillin in the late 1920s.[6] While working with the bacterium *staphylococcus*, some of his petri dishes were contaminated with mold, and he observed that the bacteria did not grow close to the mold. Fleming determined that the mold contained a substance with antibacterial properties, naming it penicillin after the fungi species *penicillium*, which the mold originated from.[6]



***Scheme 1.1**: [2+2] cycloaddition of a ketene and an imine to form the β-lactam ring.[5] The bonds formed in the reaction are marked in pink.*

Fleming's work laid the foundation for the successful method of large-scale production and purification of penicillin-producing mold presented by Florey and Chain in 1941.[7] With access to larger doses of penicillin, clinical trials on humans started in the 1940s. By the end of the Second World War, the use of penicillin as an antibacterial agent increased rapidly.[8] The structure of penicillin however, was yet to be determined. Robinson and Abraham both suggested a structure for the penicillin molecule in 1943 (**Figure 1.2**).[9] The breakthrough came in 1949, when Hodgkin and Rogers-Low managed to determine the penicillin structure using X-ray crystallography,[10,11] confirming Abraham's suggestion.[9] Today, several β-lactam antibiotics exists, the molecule Fleming discovered being part of the penicillin subgroup of the penam class.[4]



Robinson Abraham

***Figure 1.2**: Robinson's (left) and Abraham's (right) suggestions for the structure of penicillin.[9]*

1.1.2 Biological activity

β-lactam antibiotics target the cell wall synthesis in bacteria. Prokaryotes, unlike eukaryotes, contain a layer made of peptidoglycan called the cell wall outside of the cell membrane.[4] The cell wall is essential for the shape, as well as protecting the interior of the bacteria.[8] Chains of alternating *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM) builds up the peptidoglycan layer.[4,8,12] The NAM-residues have a peptide subunit that covalently cross-links the chains together, either directly or by a short peptide bridge between the subunits of other NAM-residues.[12] **Figure 1.3** simply illustrates the peptidoglycan structure.

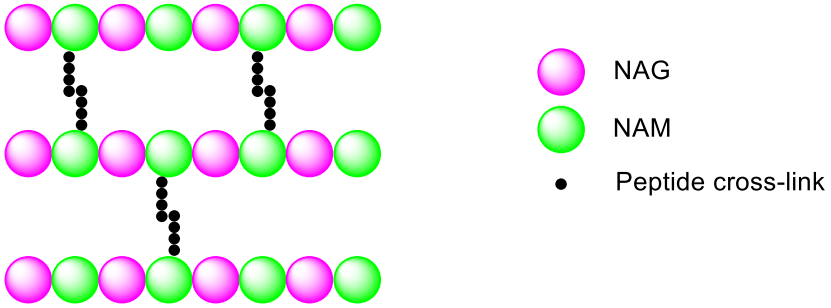
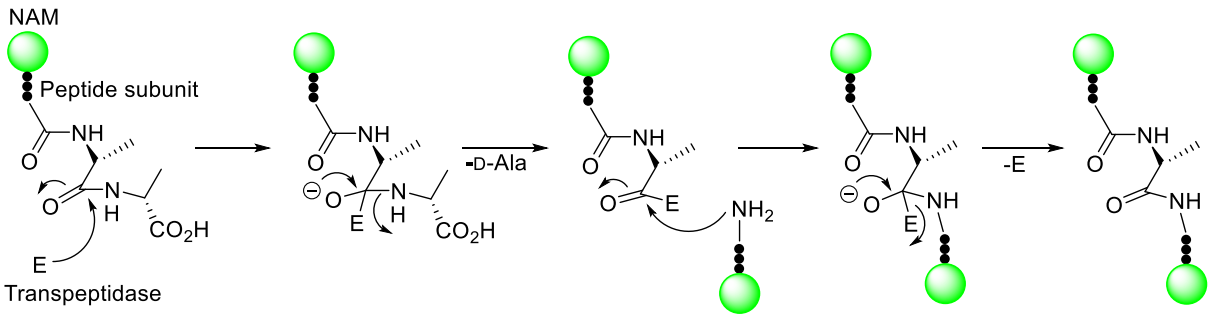


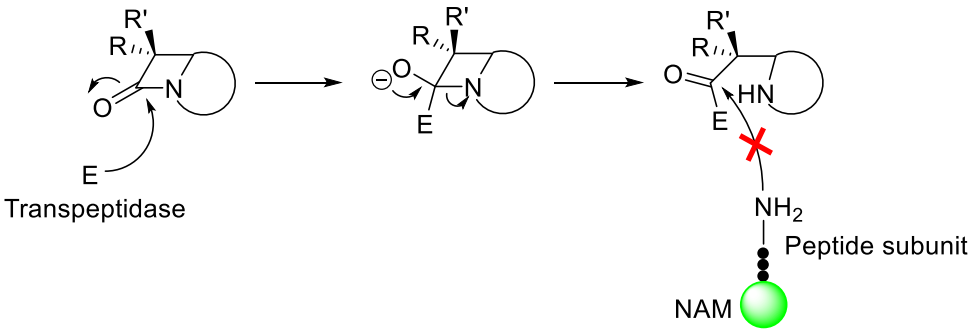
***Figure 1.3**: Simple illustration of the peptidoglycan structure of the cell wall.[4, 12]*

As bacteria grows, a constant synthesizing and reorganization of the peptidoglycan layer follows.[8] The last step of cell wall synthesis involves the cross-linking of the chains (**Scheme 1.2**). Transpeptidase enzymes, commonly called Penicillin-Bonding Proteins (PBPs), catalyze this process.[4,8] They recognize the end of the peptide subunit of NAM, a characteristic D-Ala-D-Ala. The transpeptidase cleaves the peptide bond between the two amino acids by binding to the carbonyl in the second to last D-Ala amino acid. This releases the terminal D-Ala. The cross-linking completes with nucleophilic attack of the acylated enzyme by a neighboring peptide subunit, thus regenerating the enzyme.[4]



***Scheme 1.2**: Illustration of the cross-linking mechanism, where E is the transpeptidase enzyme, the green circles are NAM-molecules, and the black circles the rest of the peptide subunit.[4]*

The antibacterial effect of β-lactam antibiotics originates from their ability to bind irreversibly to transpeptidase.[4,8,12] The β-lactam moiety resembles the D-Ala-D-Ala in the peptide subunit, causing the enzyme to bind to the carbonyl in the antibiotic instead (**Scheme 1.3**).[4,8] The resulting acylated enzyme is stable, and the bicyclic ring hinders the incoming nucleophilic attack by the neighboring peptide subunit.[4,12] This hindrance of further cross-linking terminates the cell wall synthesis, and results in lysis and death of the bacteria.[4] Consequently, the β-lactam moiety is essential for the antibacterial effect, as the transpeptidase would not bind to the β-lactam antibiotics otherwise.



***Scheme 1.3**: Illustration of how β-lactam antibiotics bind irreversibly to transpeptidase, terminating further cell wall synthesis. E is the transpeptidase enzyme, the green circle is a NAM-molecule, and the black circles the rest of the peptide subunit.*[4]

1.2 Synthesis of the β-lactam ring

Due to continuous work with β-lactam antibiotics for decades, a numerous synthetic methods are available for construction of the β-lactam ring.[13] The following section presents a selection of these methods. Throughout the section, the bonds formed in the reactions will be specified by using the numbering of the β-lactam ring in **Figure 1.4**.

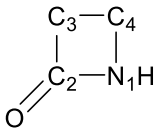
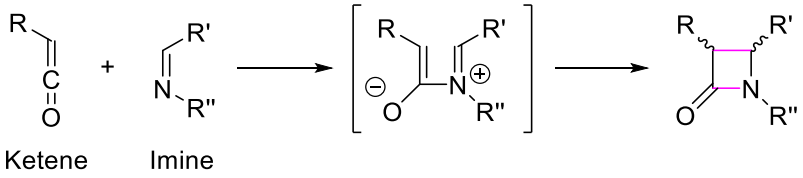


***Figure 1.4**: The numbering of the β-lactam ring used in the following sections.*[13]

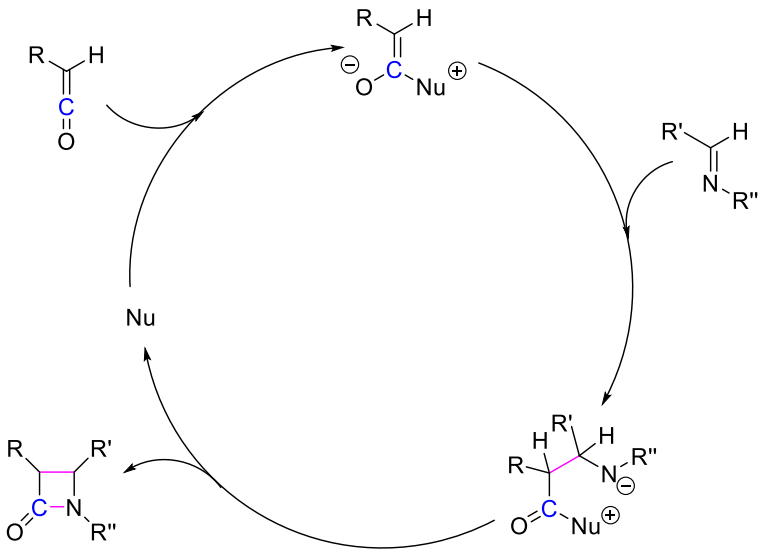
1.2.1 Miscellaneous synthetic methods

Of all the different methods of β-lactam synthesis, the Staudinger reaction was the first, and is still one of the most frequently used.[5,13,14] The readily available starting materials, an imine and a ketene, go through a [2+2] cycloaddition to form the *N*1-C2 and C3-C4 bonds (**Scheme 1.4**).[13,14] Strictly speaking, this is generally accepted as a step-wise reaction, but often referred to a as a [2+2] cycloaddition.[14] The reaction starts with the nucleophilic imine nitrogen attacking the carbonyl of the ketene. This creates a zwitterionic intermediate, which forms the β-lactam by ring closure, terminating the reaction.[13-15]



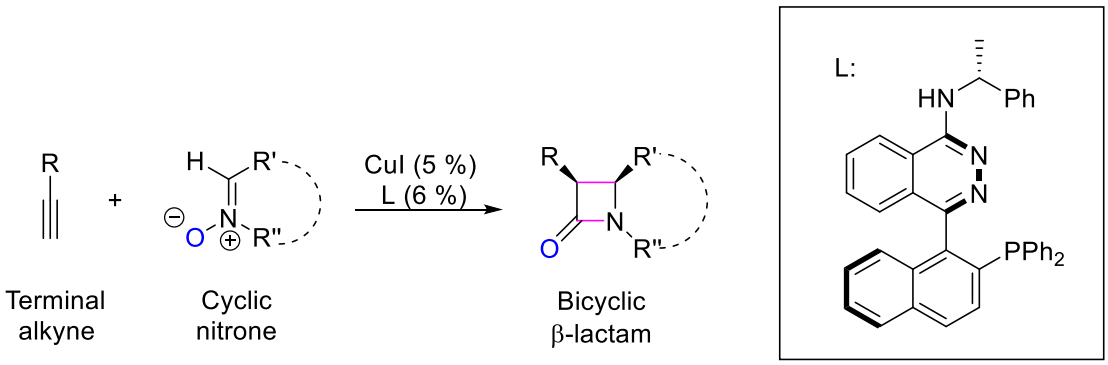
***Scheme 1.4**: The Staudinger reaction, a step-wise [2+2] cycloaddition.[5] The bonds formed in the reaction are marked in pink.*

In presence of a stronger nucleophile than the imine, however, the reaction changes to a so-called umpolung version of the Staudinger reaction (**Scheme 1.5**). The stronger nucleophile attacks the ketene, creating an enolate, which in turn reacts with the imine by a nucleophilic addition.[13,15] Ring closure and release of the nucleophile provides the β-lactam. Due to the regeneration of the nucleophile, this version of the Staudinger reaction creates an opportunity of a catalytic asymmetric cycle.[15]



***Scheme 1.5**: The umpolong version of the Staudinger reaction, where a stronger nucleophile than the imine is present.[15] The bonds formed in the reaction are marked in pink.*

The Staudinger reaction was the leading method for β-lactam-ring synthesis for many years, before several new metal-catalyzed methods were developed.[13] One example is the Kinugasa reaction.[16] This approach forms the *N*1-C2 and C3-C4 bonds by a Cu-catalyzed reaction between a nitrone and a terminal alkyne (**Scheme 1.6**), but the exact mechanism remains unknown.[13,15,16] Several versions and modifications of the reaction exists, due to decades of research.[14,15] **Scheme 1.6** illustrates an example presented by Furman *et al.*, where they used a cyclic nitrone to create a bicyclic β-lactam.[13,17] As most of the existing β-lactam antibiotics are bicyclic,[3] this serves as a powerful addition to the existing synthetic methods.

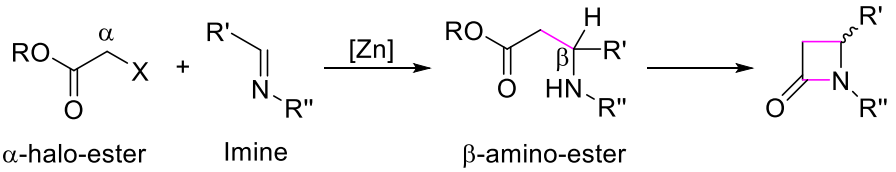


***Scheme 1.6**: A version of the Kinugasa reaction presented by Furman* et al.*, where they used a cyclic nitrone to create a bicyclic β-lactam by forming the* N*1-C2 and C3-C4 bonds (marked in pink).[13, 17]*

Ring-expansion from an aziridine ring to a four-membered β-lactam ring by insertion of carbon monoxide is another example of metal-catalyzed β-lactam synthesis (**Scheme 1.7**).[13,15] This method forms the *N*1-C2 and the C2-C3 bonds of the β-lactam ring. The catalyst decides the stereochemistry of the product β-lactam molecule, which is a useful property in syntheses of molecules with antibacterial properties.[13] When using a Rh-catalyst, the CO-insertion occurs at the more hindered side of the aziridine ring, resulting in retention of the stereochemistry.[13,18,19] Using a Co-catalyst however, results in inversion of the stereochemistry, as the insertion occurs at the less hindered side of the ring.[13,20]

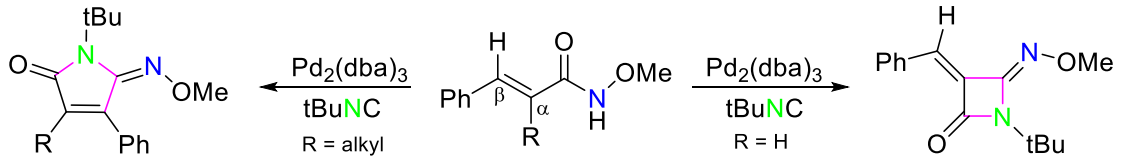
***Scheme 1.7**: Ring-expansion from an aziridine ring to a four-membered β-lactam ring by CO-insertion. The catalyst decides the stereochemistry of the product.[13] The bonds formed in the reaction are marked in pink.*

The aza-Reformatsky reaction is a metal-catalyzed reaction forming the *N*1-C2 and C3-C4 bonds. This is a Zn-catalyzed reaction where an α-halo-ester is transformed to a β-amino-ester by reacting with an imine (**Scheme 1.8**).[13] Ring-closure by forming an amide bond gives the β-lactam moiety. Several enantioselective and diastereoselective methods exists.[13-15] In addition, it is possible to create bicyclic β-lactam molecules by using cyclic imines.[13]



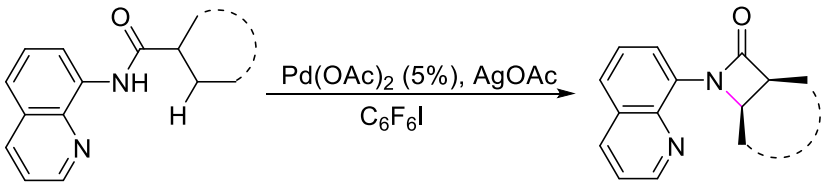
***Scheme 1.8**: The aza-Reformatsky reaction, where an α-halo-ester reacts with an imine to create a β-amino-ester, which gives the β-lactam moiety by an intramolecular ring-closure.[13] The bonds formed in the reaction are marked in pink.*

In recent years, newer methods such as C-H activation adds to the numerous synthetic methods of creating the β-lactam ring.[13] In a study by Yu *et al.* from 2016, a novel method for synthesis of 4-imino-β-lactam by a Pd-catalyzed C-H activation of an α,β-unsaturated amide were presented (**Scheme 1.9**).[21] This was the first example of using metal-catalyzed C-H activation to synthesize this molecule.[13] Activation of the α-C-H bond followed by ring closure is believed to be part of the mechanism. When substituting the α-H with an alkyl, the authors observed the formation of a γ-lactam ring instead of a β-lactam ring, which supports the proposed mechanism.[13,21]



***Scheme 1.9**: Pd-catalyzed C-H activation of an α,β-unsaturated amide, where R dictates the outcome of the reaction.[13, 21] The bonds formed in the reaction are marked in pink.*

Wu *et al.* reported another way of synthesizing β-lactams using C-H activation in 2016.[22] By utilizing methods first described by Chen *et al.*[23] and Daugulis *et al.*[24], the authors developed a synthesis using quinoline as a directing group in a Pd-catalyzed intramolecular reaction of an amide (**Scheme 1.10**).[22] The *N*1-C4 bond was formed by activation of a C(sp^3)-H bond. Pentafluoroiodo-benzene and AgOAc turned out to be essential in this method.[13,22]



***Scheme 1.10**: Pd-catalyzed C-H activation of an amide with quinoline as a directing group.[13, 22] The bond formed in the reaction is marked in pink.*

A widely used method for synthesis of bicyclic β-lactams is the metal catalyzed C-H insertion of carbenes generated from diazo molecules.[13] However, before going in detail on this method, an introduction to diazo compounds and carbenes are in order. The next section describes diazo compounds, followed by carbenes in **Section 1.2.3**. The in depth description of the metal catalyzed C-H insertion is located in **Section 1.2.4**.

1.2.2 Diazo compounds

Diazo compounds consists of a dinitrogen functional group, a $R_2C{=}N_2$.[25,26] The first synthesis of a diazo molecule, which were ethyl diazoacetate, was in 1883.[26.27] At the time of the publication, it was not clear whether a cyclic or a linear form was the correct structure for the dinitrogen group (**Figure 1.5**).[26] This remained unclear until 1957, when Clusius and Lüthi managed to prove experimentally that the linear form was the correct structure of the diazo group.[26,28] The linear form of diazo compounds consists of several resonance structures, where the two illustrated in **Figure 1.5** contributes the most to the true structure.[26]

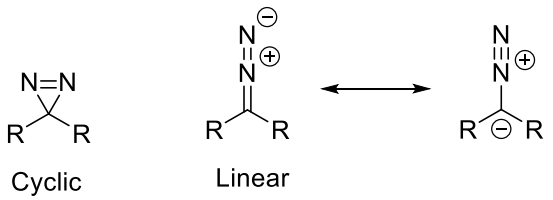
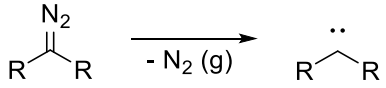


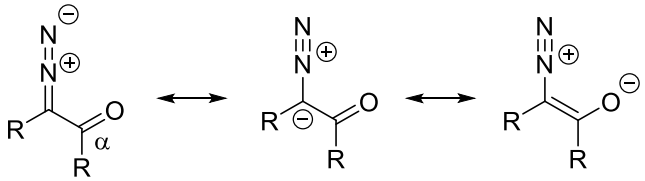
***Figure 1.5**: The two proposed structures of the diazo functional group, and the two most important resonance structures of the linear form.*[26]

Today, diazo compounds serves as powerful tools in a broad spectrum of syntheses as precursors to carbenes (**Section 1.2.3**) by release of nitrogen gas (**Scheme 1.11**).[25,26] However, caution is necessary when working with these molecules.[25,29] Diazo compounds are generally toxic, potentially explosive and thermally labile. An example is diazomethane, which explodes at the slightest provocation such as direct sunlight or chipped glassware.[25,29,30] These properties limits the use of diazo compounds in the industry.[29]



***Scheme 1.11**: Diazo compounds serves as precursors to carbenes (**Section 1.2.3**) by release of nitrogen gas.*[26]

The introduction of electron withdrawing groups α to the diazo-carbon stabilizes the diazo compounds and makes them safer to handle.[29,30] Additional resonance forms for these kinds of molecules might explain the better stability. Using α-diazocarbonyl as an example shows that the negative charge at the diazo-carbon can delocalize onto the oxygen atom of the carbonyl (**Scheme 1.12**).[26,30] This decreases the nucleophilicity of the diazo-carbon and adds double bond character between the diazo-carbon and the carbonyl-carbon. Such molecules are less likely to decompose by release of nitrogen gas, thus being more stable and safer to handle.[29]



***Scheme 1.12**: The additional resonance forms might explain the better stability of α-diazocarbonyls.*[26]

1.2.3 Carbenes

One of the useful features of diazo compounds derives from their ability to generate carbenes by release of nitrogen gas (**Scheme 1.11**).[25,26] Carbenes are neutral, divalent carbon atoms with six valence electrons, which makes them highly reactive.[31-33] Two of the valence electrons are located in non-bonding orbitals. Depending on the spin state of these electrons, the carbene is either in a singlet or a triplet state (**Figure 1.6**).[31,33] If the electrons occupy the same orbital with opposing spins, the carbene is in a singlet state. When occupying two different orbitals with parallel spins, the carbene is in a triplet state.[31] These differences effects the overall properties of the carbene molecule. While a singlet state carbene is ambiphilic due to both a lone pair and an empty orbital, a triplet state has a more diradical character.[31,33]

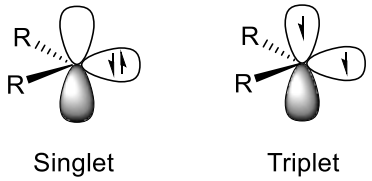
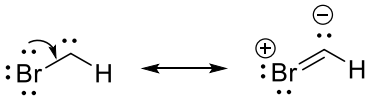


***Figure 1.6**: The location and the spin state of the valence electrons decide whether the carbene is in a singlet and triplet state.[31]*

The high reactivity of carbenes results in short half-lives.[33] However, the stability is highly affected by the substituents.[33,34] Both inductively electron withdrawing groups through σ-bonds and π-donating groups stabilize carbenes, thus increasing the half-live.[31,33] Halogens are both σ-electron withdrawing and π-donating species, as illustrated by bromine in **Scheme 1.13**. Steric substituents might contribute to the stabilization of carbenes as well.[33] There are a few examples of carbene molecules that are stable enough to be isolated and analyzed, even though this is not possible with most carbenes.[31,33]

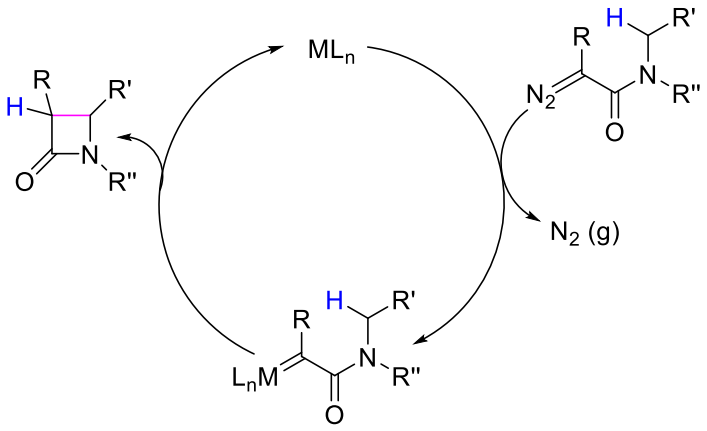


***Scheme 1.13**: Halogens are both σ-electron withdrawing and π-donating species.[31]*

The most common methods of generating carbenes are by thermally decomposition or photolysis of diazo compounds.[31,33,34] Due to their high reactivity, they serve as both reactive reagents and intermediates at the same time in a ton of different syntheses.[32-34] Some examples includes addition to alkenes and insertion into C-H bonds.[31,33]

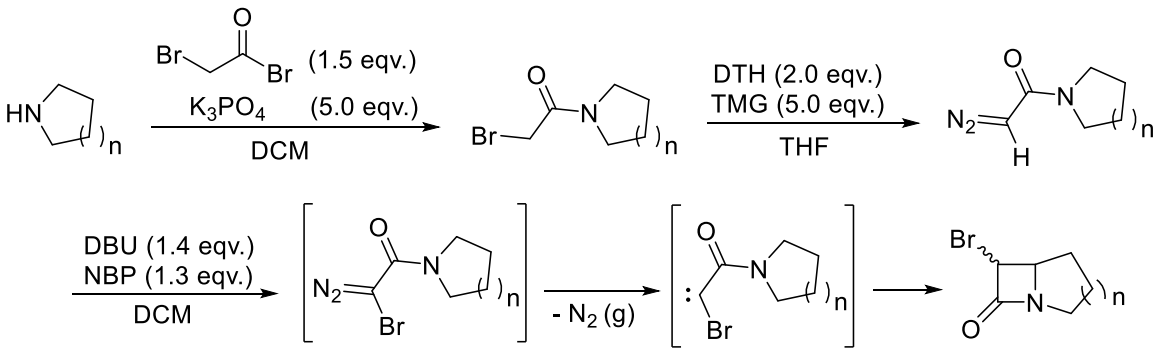
1.2.4 Synthesis of β-lactams using diazo and carbene chemistry

Diazo compounds and carbenes are important tools in many syntheses of β-lactams.[14,15] A well-studied and important method is the metal-catalyzed C-H insertion of diazoamide carbenes (**Scheme 1.14**).[13-15] Activation of the diazoamide by a metal generates a carbenoid, a metal stabilized carbene complex.[13,35] This carbenoid can insert into a C-H bond in an intramolecular manner to form the β-lactam ring.[13,15] Although several transition metals can activate the diazo molecule, rhodium has showed the best reactivity.[13] A normal challenge of these kinds of procedures are competing reaction pathways. With several available C-H bonds, the more thermodynamically favorable five and six membered lactam rings might compete with the four membered β-lactam ring.[13-15] However, the outcome of these reactions heavily depends on the steric and electronic effects of the desired C-H bond, the ligands of the catalyst and the substituent attached to the diazo-carbon.[13,15]



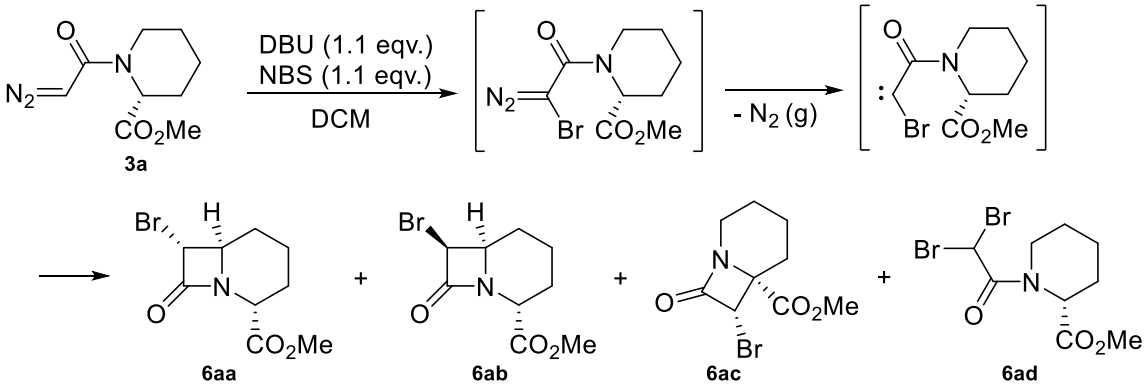
***Scheme 1.14**: A metal-catalyzed C-H insertion of a diazoamide.[15] M = Metal, L = Ligand.*

Despite the numerous variations of the metal catalyzed β-lactam synthesis using diazo compounds, there are no reports of using halogenated diazoamides.[36] In general, little research of α-halodiazoamides exists. This knowledge gap was the foundation of the novel research by Kaupang, an earlier member of the Bonge-Hansen group. By utilizing methods for halogenating diazo molecules developed by the group,[37] Kaupang examined the halogenation of diazoamides in order to synthesize bicyclic β-lactams.[36] **Scheme 1.15** illustrates the method with a general cyclic amine. Kaupang experienced that the yield was better when the α-bromodiazoacetamide decomposed thermally to the carbene, followed by C-H insertion and ring-closure, without the use of a catalyst. This opened a path of a possible metal and catalyst free method for synthesis of bicyclic β-lactams.[36]



***Scheme 1.15**: Kaupang's method to synthesize bicyclic β-lactams by halogenating diazoacetamides.*[36]

Konradsen of the Bonge-Hansen group took on the work of developing this metal-free method further.[38] Kaupang's work only included symmetric amine rings, but in order to get a method with a broader scope, Konradsen introduced a methyl ester alpha to the amine.[36,38] He observed and identified four products in the last synthetic step,[38] which was a bit surprising (**Scheme 1.16**).

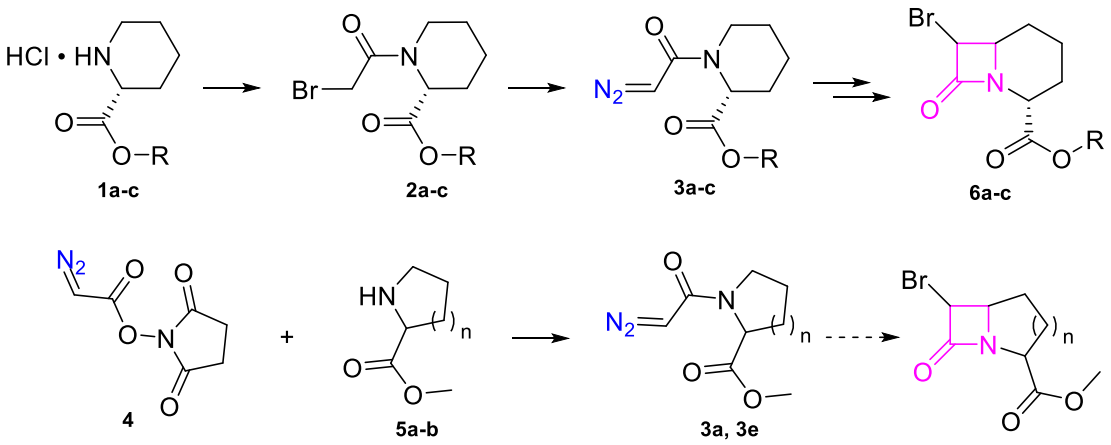


***Scheme 1.16**: Konradsen's work resulted in four identified products.*[36, 38]

When Kaupang used symmetric amines, the carbene could insert into the C-H bonds on either side of the nitrogen and still get the same product. This was not the case with Konradsen's substrate.[38] Nevertheless, considering the halo-carbenes preference of more electron-rich C-H bonds,[39,40] the group thought that the ring-closure would be selective and give **6aa** and/or **6ab**. Due to the identification of **6ac**, this turned out not to be true. Further work and calculations of Konradsen revealed that the partial double-bond character of the amide was responsible for the unfavorable ring-closure. When oriented in a way that put the carbene close to the ester side of the nitrogen, it was more energetically favored to form **6ac** by C-H insertion than rotate and insert on the other side of the nitrogen.[38] Therefore, it would be beneficial to develop conditions where the rotation of the amide bond places the carbene away from the ester, thus favoring the formation of **6aa** and **6ab**.

2 Aim of the study

This thesis is a continuation of Kaupang's and Konradsen's work of developing a new synthesis of β-lactams with the use of carbene and diazo chemistry. The goal of the Bonge-Hansen group is to develop and optimize new and broad procedures that can give new molecules with potential antibacterial properties. This thesis builds on the work by Konradsen in an attempt to broaden the scope of the procedure (**Scheme 2.1**). Additionally, this study focuses on solving problems in the original reaction route by attempting to decrease the number of synthetic steps.



***Scheme 2.1**: This thesis is a continuation of the work by Kaupang and Konradsen, where developing a new synthesis of β-lactams is the main goal.*

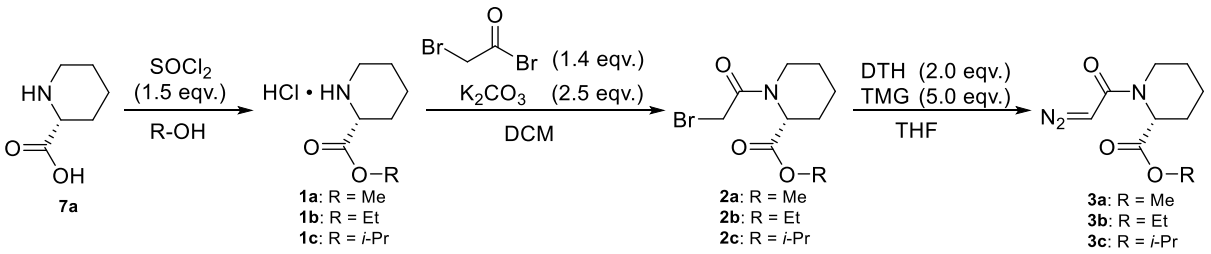
Summarized, the overall goals of this thesis are as follows:

- Synthesize β-lactams by reproducing the work of Konradsen with different esters.
- Improve the synthesis of **3a** by developing and optimize a new, one-step reaction.
- Investigate how different ester groups affect the distribution of products in the β-lactam formation step.

3 Results and discussion

3.1 Diazoacetamides with different esters

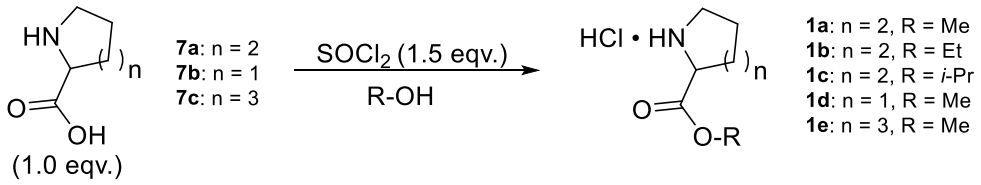
This section presents the work related to the synthesis of the diazoacetamides **3a-c**, using the method developed by Kaupang and Konradsen (**Scheme 3.1**).[36,38] The method was first reproduced using the methyl ester, and then tested with the ethyl and isopropyl esters.



***Scheme 3.1**: The synthesis of the diazoacetamides **3a-c**, a method developed by Kaupang and Konradsen.*[36, 38]

3.1.1 Esterification of the starting materials

The very first step in the syntheses of the diazoacetamides was the introduction of the ester groups by a Fischer esterification (**Scheme 3.2**).[41] A solution of the appropriate alcohol and thionyl chloride was prepared according to a procedure by Dave *et al.*,[42] giving an acidic solution. The addition of **7a**, **7b** or **7c** resulted in the transformation of the carboxylic acids to the esters. Methanol, ethanol and isopropanol served as the source of methyl, ethyl and isopropyl for **1a-e**. Of all the substrates, only **7b** and **1d** had a *S*-configuration, the rest had a *R*-configuration.



***Scheme 3.2**: The esterification of the starting materials **7a-c**, using a procedure by Dave* et al.*[42]*

When checking the crude mixtures with NMR after ended reaction time and purification, it was discovered that both the starting material and the desired product were present in all the entries. This was difficult to discover with other deuterated solvents than MeOD-d_4. The 7-membered ring **1e** illustrates the difference between the starting material and the product the best (**Figure 3.1**).

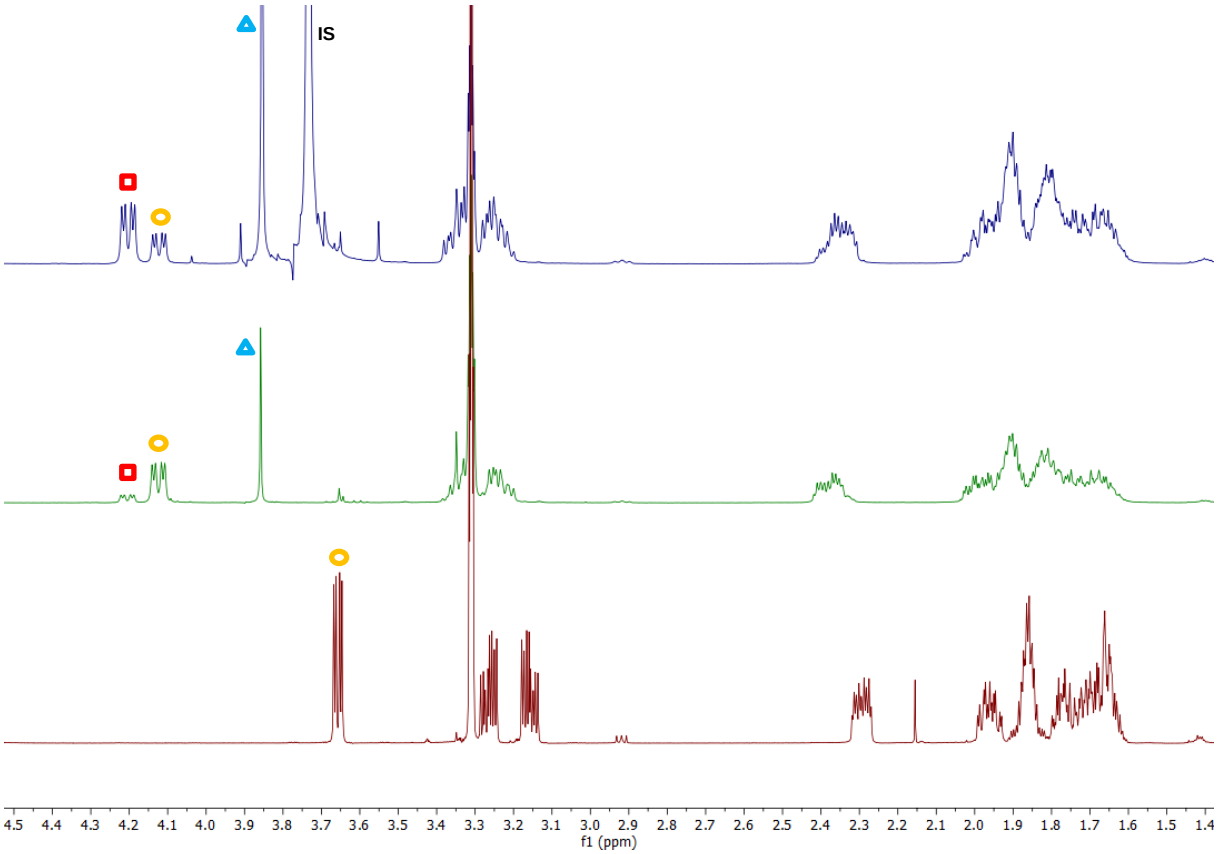
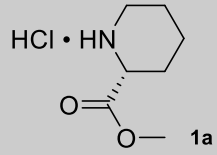
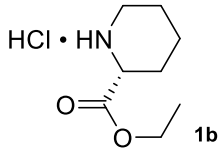
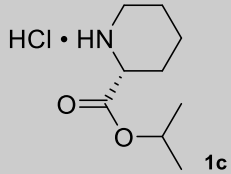
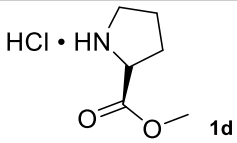
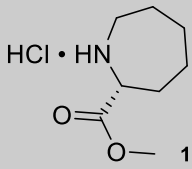


Figure 3.1*: The starting material* ***7c*** *(red spectrum) and the* ***1e****-crude midway (green spectrum) and at the end of the reaction (blue spectrum). Yellow circle = signal from starting material, red square = signal from product, blue triangle = methyl ester signal, IS = internal standard.*

The bottom red spectrum is the starting material **7c**, while the green and blue represents the **1e**-crude midway and at the end of the reaction respectively. As the only change in the reaction is the transformation of a carboxylic acid to a methyl ester, a direct comparison between the signals is possible. Due to the methyl ester signal (blue triangle) in both the blue and green spectra, the formation of product is apparent. Next, consider the two signals marked with a red square and a yellow circle. In the starting material **7c**, only one such signal is present. It belongs to the α-hydrogen of the ester group. In the reaction mixtures however, there are two such signals, but notice the difference between the green and the blue spectrum. From the spectrum recorded midway in the reaction to the one recorded at the end, the red square signal increases while the yellow circle signal decreases. This strongly suggests that the circle signal belongs to the starting material, hence decreasing as more **7c** converts to **1e**. Even though the circle signal of the pure starting material do not align with the corresponding signal in the crude mixture, the rest of the signals in the azepane ring match. This further supports that the red square signal belongs to the product.

The arguments presented above applies to all the conducted esterifications of **7a-c**. Consequently, the yields of the mixtures were calculated by using the ratio between the two signals in the NMR spectra. As presented by **Table 3.1**, the yields vary significantly. Due to overlapping signals in the NMR spectrum of **1c**, it was not possible to calculate the yield. In fact, it was hard to determine the formation of any product at all. However, when reacting it with 2-bromoacetyl bromide in the next synthetic step, the product was the desired **2c** (**Section 3.1.2**). Therefore, the mixture did contain **1c**, but it is not possible to calculate how much.

Table 3.1: The yields of the esterifications of **7a-c**, calculated from the ratio between product and starting material in the NMR spectra.

Product	Yield (%)
 **1a**	51
 **1b**	21
 **1c**	NM[a]
 **1d**	~90
 **1e**	65

[a] Not measurable

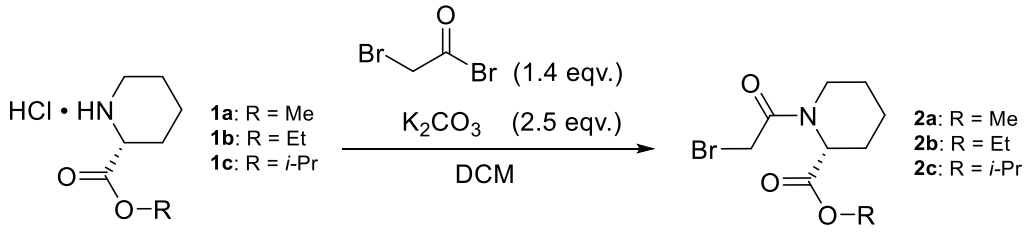
Next, consider the yield of **1d** at ~90%. When examining the NMR spectrum, at first glance it appears as a pure sample without any starting material left. However, the yield is over 100% if presuming a pure sample. A closer look at the NMR spectrum revealed that there was some starting material left, but not much. The signals used to calculate the yields in the other esterifications were satisfactory separated (**Figure 3.1**), but that was not the case with **1d**. It was possible to determine the presence of both **1d** and **7b**, but the signals overlapped enough

that a proper calculation of the yield was not possible. If using the integrals of the other signals however, the ratio between the product and the mixture gave **1d** in approximately 90% yield. This is not an error-proof calculation, but an indication of the yield of **1d** in the mixture.

The methyl esterifications of compound **7a**, **7b** and **7c** varies from 51% to about 90%. Considering that 5-membered rings are more reactive than 6- and 7-membered rings, it makes sense that **1d** has the highest yield. There is a slight difference in the yield of **1a** and **1e**. The two esterifications were conducted using the same procedure, the only difference being that the methanol solution was heated in the synthesis of **1e**. Seemingly, this was positive for the yield. Therefore, it would be beneficial to try this in the preparation of **1a** as well, but also in the preparation of **1b**. As larger alcohols are less reactive in esterification reactions, it makes sense that **1a** was synthesized in a larger yield than **1b**. Heating of the ethanol mixture might provide enough energy to compensate for the reactivity and give a higher yield than at ambient temperature. The same arguments are relevant for the isopropyl ester. Although **1c** was prepared by heating of the isopropanol solution, it is not possible to determine from this entry how the yield was affected by the heating compared to the other esterifications. It is likely however, that the yield was better than it would have been without the heating, due to the less reactivity of isopropanol compared to methanol.

3.1.2 Bromoacetylation of 1a-c

The second step in the synthesis of the diazoacetamides with different esters was the bromoacetylations of **1a-c** (**Scheme 3.3**). 2-bromoacetyl bromide served as the source of bromoacetyl, and K_2CO_3 was added as base.

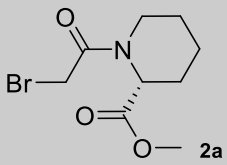
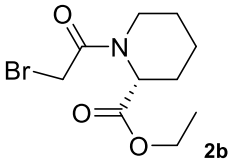
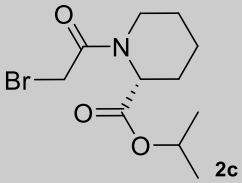


***Scheme 3.3**: The synthesis of **2a-c** from **1a-c**.*

Table 3.2 displays the yields of the bromoacetylations of **1a-c**, in addition to the total yields of the esterification and bromoacetylation steps. As it was not possible to calculate the yield of **1c** (**Section 3.1.1**), the total yield of the two steps is the only way to display the yield of **2c**. It is rather low, at 17%, but both **2a** and **2b** follows the same trend with 32% and 18% respectively. Considering the bromoacetylation steps alone however, both **2a** and **2b** were formed in good yields. Hence, it can be assumed that the bromoacetylation step of **1c** follows

the same trend, and that the esterification step effects the overall low yield the most. An explanation for the slightly better total yield for the methyl ester might be the better yield in the esterification.

Table 3.2: The yield of **2a-b** in the bromoacetylation, and the total yield of **2a-c** in two steps from **7a-c**.

Product	Yield bromoacetylation (%)	Total yield (%)
 **2a**	I: 83[a] II: 63[b]	II: 32
 **2b**	87	18
 **2c**		17

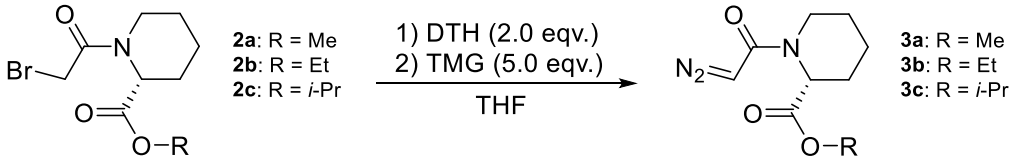
[a] Used pure **1a** as starting material

[b] Used a mixture of **1a** and **7a** as starting material

Both commercially available **1a** and the homemade mixture of **1a** and **7a** served as the starting material in the synthesis of **2a**. As displayed in **Table 3.2**, there is a difference in the yield depending which of these were used. Pure **1a** produced **2a** with 83% yield, which is not far from the 94% reported by Konradsen.[38] The yield droped to 63% when using the mixture. A competition between **1a** and **7a** as nucleophiles might explain this observation, as the nitrogen atom in both the piperidine rings may attack 2-bromoacetyl bromide. As the NMR spectra show no sign of these impurities, unreacted **7a** and the bromoacylated **7a** are most likely removed in the extraction. Considering the still good yield of 63% however, it is apparent that more **1a** reacts than **7a**. Nevertheless, it would be beneficial to start with pure reactants to avoid this competition completely and get as high yields as possible.

3.1.3 The synthesis of the diazoacetamides with different esters

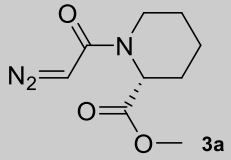
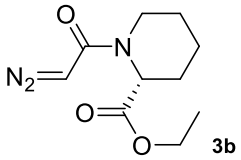
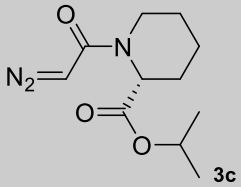
The following section covers the synthesis of the diazoacetamides **3a-c** from **2a-c** (**Scheme 3.4**). This step introduces the diazo functional group, which is essential for the β-lactam formation in the next step (**Section 3.3**).



***Scheme 3.4**: The synthesis of the diazoacetamides **3a-c**.*

The yields from several syntheses of **3a-c** are displayed in **Table 3.3**.

Table 3.3: The yields of the diazoacetamides **3a-c** in different entries.

Product	Yield (%)
 **3a**	I: 48 II: 32 III: 47 IV: 12 V: 36
 **3b**	I: 29 II: 59
 **3c**	I: 36 II: 34

The yields varies from 12% for **3a** to 59% for **3b**. This illustrates one of the problems with this synthetic step. When purifying the crude mixture with the silica plug, the fractions were collected by color. As it is possible to observe the diazoacetamides as a yellow band on the column, this might not seem too problematic. In reality however, this acts as a source of losing the product. TLC was used to investigate this in one of the syntheses of **3b** (entry II). It turned out that both the fractions before and after the yellow band on the column contained the desired product, despite being colorless to the naked eye. Collecting both the yellow and colorless fractions gave **3b** in 59% yield, which is considerably better than the other entries. This

suggests that collecting the fractions by color is not an optimal solution without the use of TLC. It is likely that the yields of the other entries might have been better with a different conduction of the purification. In addition, a better collection could result in less variation of the yields, which is a problematic challenge with the original purification method.

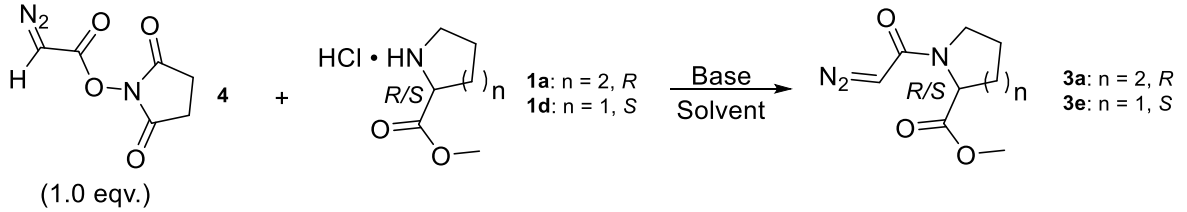
Another thing worth mentioning is the difference in yield depending on the used mmol scale. Konradsen reported getting **3a** in 76% yield on a 4.6 mmol scale, and 66% yield after an additional purification step not conducted in this thesis.[38] The best entry of **3a** in **Table 3.3** is 48% on a 0.4 mmol scale, which is notably lower. From these two numbers alone, it may seem like a larger mmol scale is beneficial. However, when conducting the reaction on a 0.8 mmol scale, the yields dropped to 12% and 36% for **3a** respectively. When synthesizing **3c** on a 0.8 mmol scale however, the yield of 34% was in accordance of the 36% yield obtained on a 0.4 mmol scale. It seems like a difference in personal skills might have an impact on the yield rather than the mmol scale. In addition, as the purification step probably results in large differences in the yield, this might explain the difference between the entries in **Table 3.3** and Konradsen's work as well.[38]

In addition to varying yields, probably due to how the fractions are collected, it is difficult to get the pure products. Despite the silica plug, impurities from DTH is visible in the NMR spectra. Konradsen reported the same problem for **3a**, but did not suggest any solution to the problem.[38] It would be beneficial to have a procedure giving the diazoacetamides as pure as possible. As Konradsen reported several products in the forming of the β-lactams,[38] pure **3a-c** as starting materials would make the NMR spectra less complex.

To summarize the content of **Section 3.1**, compound **3a** has successfully been synthesized by using the method developed by Kaupang and Konradsen. Additionally, it has been proved that the method works when changing the ester to ethyl and isopropyl, giving **3b** and **3c**. Heating of the reaction mixtures might increase the yield of **1a-e** and avoid the mixture of product and starting material. The bromoacetylation step works well with good yields, but the synthesis of the diazoacetamides have several challenges with the purification.

3.2 Development of a one-step synthesis of diazoacetamides

A big part of this thesis was to improve the synthesis of the diazoacetamides. With the current method developed by Kaupang and Konradsen,[36,38] the purification of **3a-c** serves as a thorn in the side. The formation of byproducts without a proper way to remove them is a weakness of the method, as well as the moderate yields. In addition, it would be beneficial to have a broad method with the possibility to change both the ring size and the atoms in the ring. When developing the procedure, Kaupang tested the conditions using a ring containing both nitrogen and sulfur. He experienced that the sulfur caused problems in the bromoacetylation step, as the combination of sulfur and the halogen resulted in decomposition of the product, terminating further synthesis.[43] This illustrates a limitation of the method. As many existing β-lactam antibiotics contain sulfur in the bicyclic skeleton of the molecules,[3] this narrows the scope of the method. Consequently, the group wanted to improve the synthesis of the diazoacetamides by developing a new procedure. A one-step reaction using 2,5-dioxypyrrolidin-1-yl 2-diazoacetate (**4**) as the source of the diazo functional group was proposed (**Scheme 3.5**).

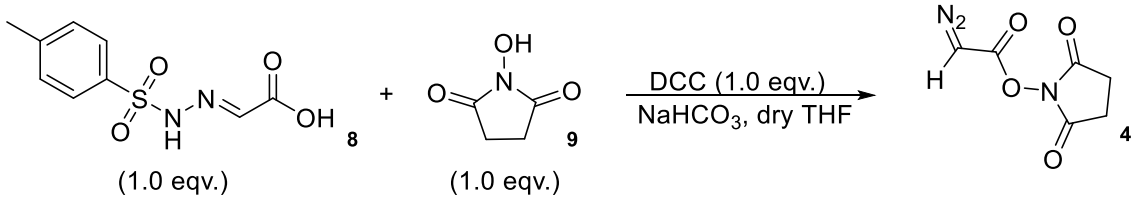


***Scheme 3.5**: The proposed one-step synthesis of diazoacetamides using **4** as the source of the diazo functional group.*

The proposed method has a number of potential advantages. Firstly, it is a one-step reaction, making it one step shorter than the current method. This reduces the total number of steps in the synthesis of the β-lactams, potentially making the overall synthesis more productive. Next, consider the width of the scope. Introduction of the diazo group directly from **4** removes the need of an initial bromoacetylation step, which efficiently avoids the problems with sulfur-containing rings in combination with halogens.[43] This creates an opportunity to synthesize diazoacetamides like **3a-c**, but with different ring-sizes and atoms in the rings as well. In addition, this one-step reaction might solve the purification problem of the diazoacetamides in the original procedure. The use of different reagents might make the separation of the product from impurities and byproducts easier.

3.2.1 Synthesis of compound 4

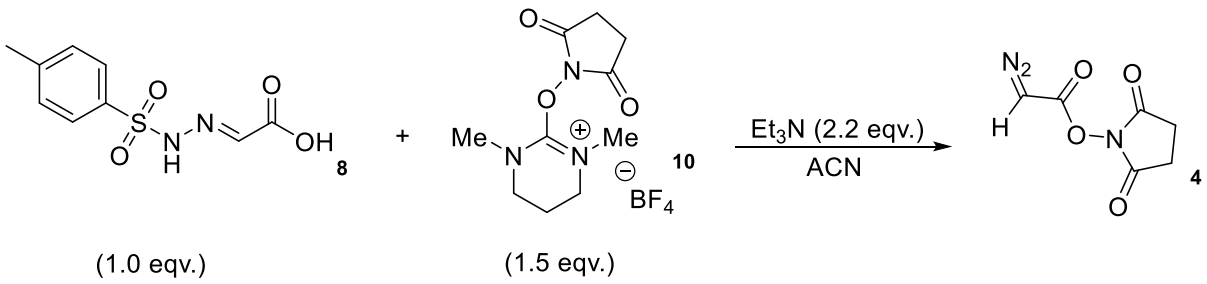
In order to test the proposed method (**Scheme 3.5**), compound **4** had to be accessible. Using a procedure described by Badet *et al*.,[44] **4** was synthesized using compound **8** and **9** in combination with DCC (**Sceme 3.6**).



***Scheme 3.6**: Synthesis of compound **4**, a procedure by Badet* et al.*[44]*

The staring material **9** is cheap and readily available, and **8** is easily synthesized in very good yields from readily available reagents.[45] However, the procedure consists of one extraction, a silica plug, a recrystallization and about 24 hours of stirring time. This makes it a time consuming procedure to implement, in addition to poor yields. Badet *et al*. reported a yield of 65% for **4**,[44] but the members of the Bonge-Hansen group have not managed to reproduce this, getting stable yields around 25%. Despite getting perfectly pure product, the poor yields and the execution time of the procedure is not ideal if **4** is to be used in the new synthesis of the diazoacetamides (**Scheme 3.5**). A prerequisite for the new method to work ideally is a well-functioning synthesis of **4** in good yields and a shorter execution time. Hence, a modification of this synthesis is a necessity.

Other members of the group are currently working on improving the reaction illustrated in **Scheme 3.7**.



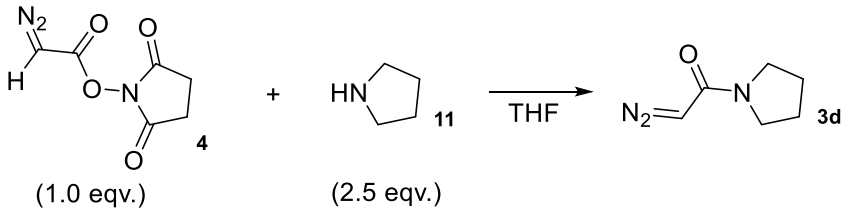
***Scheme 3.7**: A synthesis of compound **4** that is currently under development in the Bonge-Hansen group.*

This procedure uses **8** and **10** with triethylamine as base to generate **4**. A procedure described by Nájera *et al*. was used to synthesize **10**. The product was completely pure, but the yields obtained in the group was lower than the reported yields.[46] Using **10** in combination with **8** gave the desired product in only 1.5 hours, but the purification turned out to be a challenge.

The eluent used in the silica plug was not ideal, as **4** co-eluted with byproducts that were not removed in the extraction prior to the silica plug. It is crucial to find a proper way of purifying the product to justify the use of this procedure instead of the original one by Badet *et al*.[44] Despite the current development of the method in the group, the original method was used to synthesize **4** in this thesis. The new procedure was applied briefly, but as the synthesis of **4** was not a focus in this work, the original method was easier to conduct in order to have a supply of **4**.

3.2.2 Development of a synthesis of diazoacetamides using 4

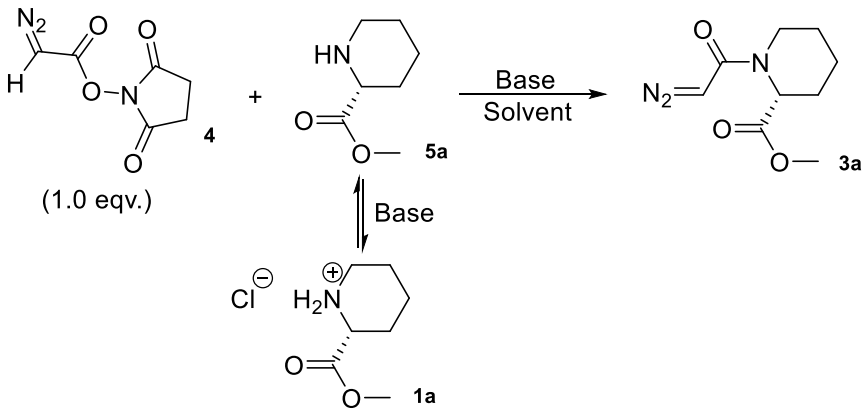
Initial work in the group used compound **11** to test the proposed reaction with **4** (**Scheme 3.8**). This gave the desired diazoacetamide **3d** with an IS-yield of 39% in only 35 min, which shows that the reaction worked as intended. Considering the aim was to develop an improved and broader method than the one developed by Kaupang and Konradsen,[36,38] the next step was to test the reaction with amino esters like **1a** as substrates.



***Scheme 3.8**: Synthesis of the diazoacetamide **3d** by the use of **4** and **11**.*

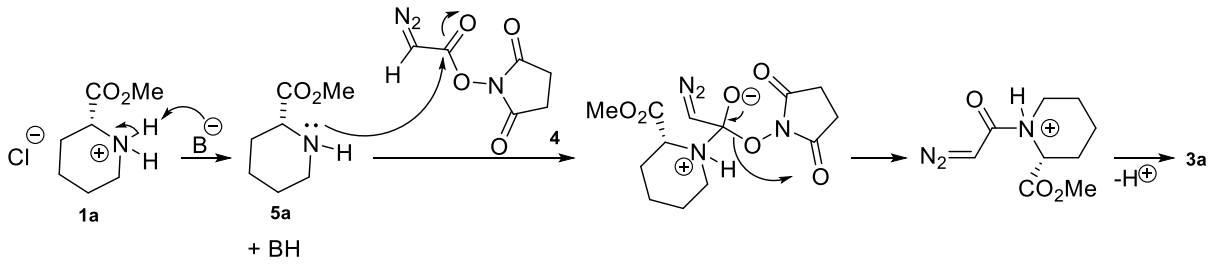
Synthesis of diazoacetamide 3a using 4 and 1a

As a start, the new method was tested using **1a** as the substrate. This made a direct comparison between the new and the original synthesis of **3a** possible. Several test reactions with various mild bases, solvents, temperature and equivalents were conducted (**Scheme 3.9**). Compound **4** served as the limiting reagent on a 0.10-0.20 mmole scale in all the test reactions. The amino ester **1a** was added in excess, varying from 1.50 to 2.20 eqv. Only commercially available **1a** was used in these reactions, thus avoiding the problem with the homemade mixture of ester and carboxylic acid (**Section 3.1.1**). The addition of base was to deprotonate **1a** to give **5a**, creating an equilibrium. As **5a** was removed by reacting with **4**, the thought was that the equilibrium would shift towards **5a** and favor the formation of the product.



***Scheme 3.9**: Several test reactions were conducted in order to synthesize **3a** from **1a** and **4**.*

The proposed mechanism for the reaction involves the nucleophilic attack of the lone pair of the **5a**-nitrogen on the carbonyl in **4** (**Scheme 3.10**). The deprotonated form of **9** serves as a leaving group when regenerating the carbonyl, providing the protonated form of **3a**. Removal of the proton by a base give **3a** as the product.



***Scheme 3.10**: Proposed mechanism for the reaction between **1a** and **4**.*

Despite numerous attempts varying the reaction conditions, the procedure did not work as intended when using **1a** as the substrate. When comparing NMR spectra from the test reactions with **3a** synthesized with the original method, it was difficult to get a conclusive answer. The characteristic signals of the piperidine ring of **3a**, which are visibly different from the ring signals of **1a**, seemed not to be present in the spectra from the test reactions. Because of excess **1a** however, the signals of **3a** might be overlapping with the starting material and potentially other byproducts, making them hard to find. In some of the test reactions, there were a few signals matching characteristic signals from the desired product, but none of them matched all of the signals at once. In addition, the signals that matched **3a** were of low intensity. It is therefore not possible to confirm the formation of **3a**, or confirm that is was not formed, from the NMR spectra alone. An IR spectrum of one of the test reactions have the characteristic diazo-stretch around 2100 cm^{-1} (**Figure 3.2**),[26] but this is not enough evidence to confirm the formation of the diazoacetamide. Regardless of the formation of **3a** or not, if it was formed at all, it was not much. Consequently, the reaction did not work as intended.

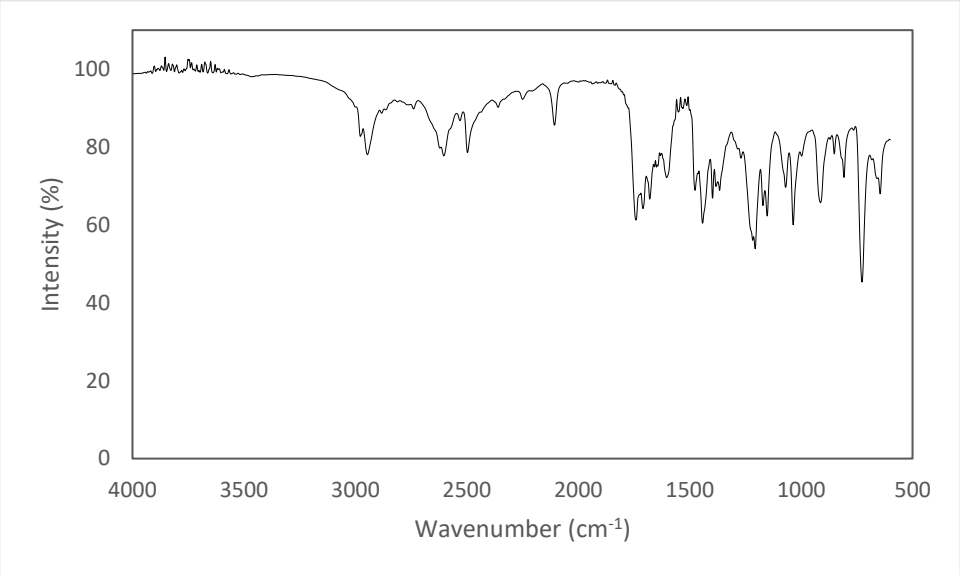
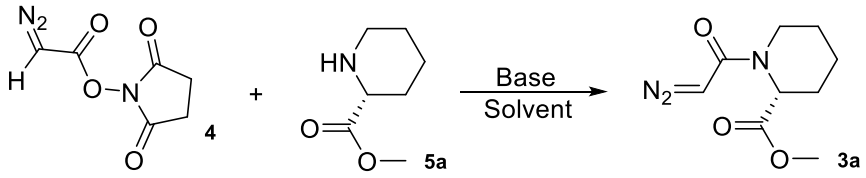


***Figure 3.2**: IR spectrum of one of the test reactions with **1a** and **4**, showing the characteristic diazo-stretch around 2100 cm^{-1}.*[26]

Synthesis of diazoacetamide 3a using 4 and 5a directly

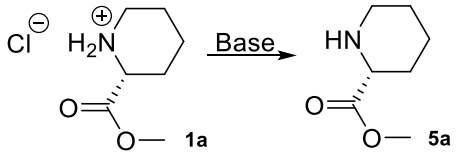
Initial work in the group indicated that the reaction might work better when using **5a** directly in the reaction with **4** (**Scheme 3.11**), instead of generating **5a** from **1a** in the reaction. If the formation of **5a** was not ideal and the equilibrium was favoring **1a**, this might explain the lack of product. To test this, **1a** had to be desalted in order to generate **5a**.



***Scheme 3.11**: Proposed synthesis of the diazoacetamide **3a**, using **4** and **5a**.*

The desalting process was tested with different bases to get the yields as high as possible. Compound **1a** was solved in water and transferred to a separatory funnel with DCM. The different bases were added directly to the separatory funnel, to hopefully separate **1a** from **5a** as fast as possible and increasing the yield and purity. **Table 3.4** summarizes the findings from these tests.

Table 3.4: The yields of the desalting of **1a** with different bases.



Base used	Yield (%)
1M K_3PO_4	79
Sat. K_3PO_4	21
DBU	0
Solid $NaHCO_3$	89

Initially, the yields were unexpectedly low, 35% being the highest. Then it was discovered that the homemade **1a** was in fact a mixture of **1a** and the starting material **7a** (**Section 3.1.1**). Considering that the desalted product **5a** was completely pure, this was not a problem with the exception of getting false numbers for the yields. Recalculating the yields with the mixture of starting materials taken into account gave a completely different picture. DBU still gave 0% yield, as the NMR spectrum showed no signals that could be recognized. The characteristic methyl ester signal was gone, which indicates that DBU might destroy the ester. Both adjusting the pH with 1M K_3PO_4 and saturating the water phase by adding solid $NaHCO_3$ gave very good yields. The latter was inspired by a procedure described by Jamison *et al*.[47] Solid $NaHCO_3$ was chosen as the best method to desalt **1a**, as it gave the best yield and had a short reaction time.

With **5a** at hand, the proposed reaction with **4** and **5a** was tested under different conditions (**Scheme 3.11**). When using DBU as base, no sign of the product was visible in the NMR spectra. Using triethylamine seemed promising however, as some of the signals in the NMR spectrum aligned with characteristic signals of **3a** (**Figure 3.3**).

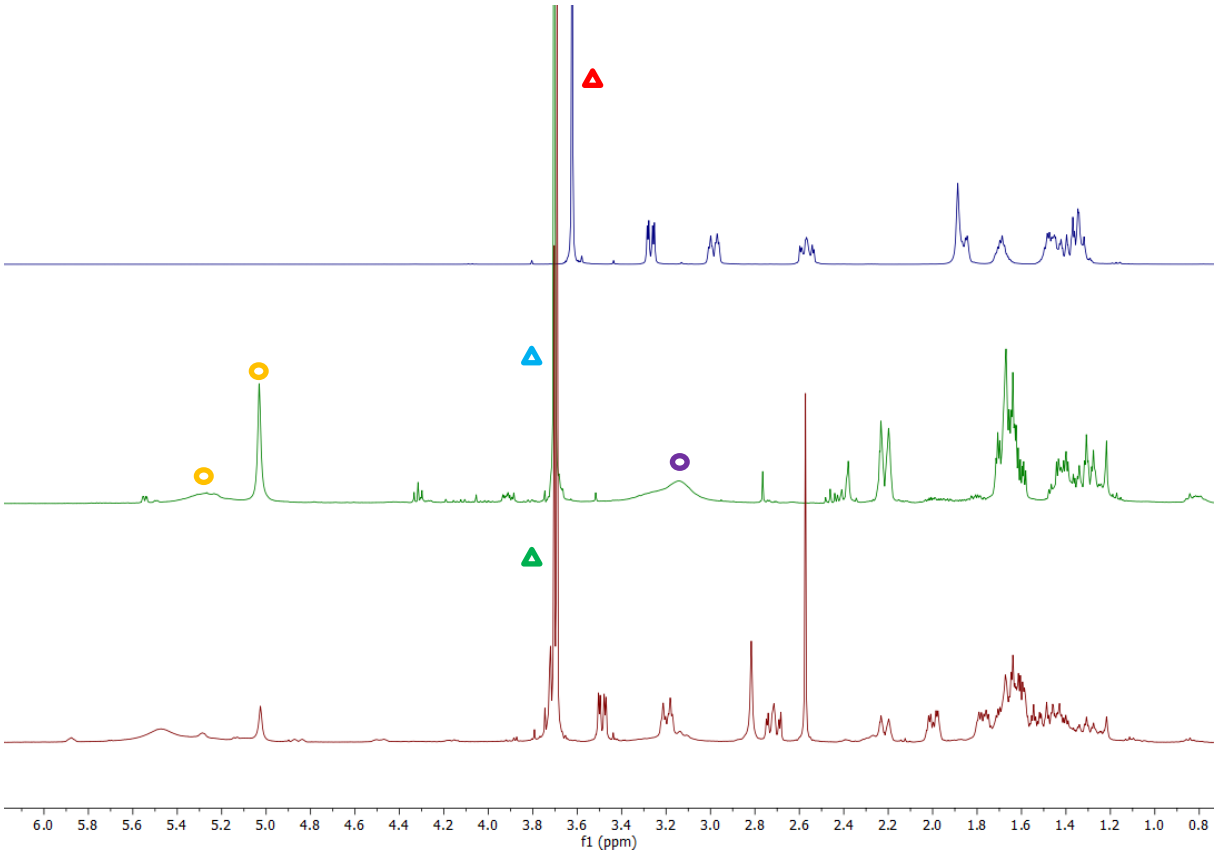


***Figure 3.3**: Comparison of signals in the NMR spectra of **5a** (blue spectrum), **3a** (green spectrum) and a test reaction (red spectrum). The circles are characteristic signals of **3a**, and the triangles are methyl ester signals.*

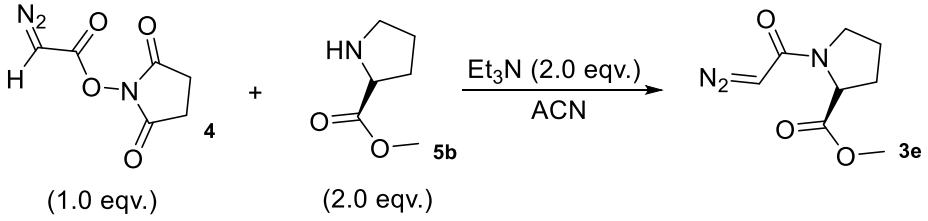
The blue spectrum is the starting material **5a**, the green spectrum is the diazoacetamide **3a** made with the original method, and the red spectrum is one of the test reactions using Et_3N as base. In the green spectrum, two easily recognizable signals of **3a** are marked with yellow circles. Looking closely at the test reaction spectrum, there seems to be two such signals there. The methyl ester signal (green triangle) align perfectly with the methyl signal of **3a** (blue triangle) and not with the methyl ester signal of the starting material (red triangle). With excess of **5a** in the reaction however, there should be an ester signal in the red spectrum aligning with the red triangle signal as well, but that is not the case. Since a different concentration or a mixture of molecules can change the chemical shift of a signal slightly, the green triangle signal is most likely the methyl signal of **5a**. It is therefore not possible to tell if the source of the green triangle signal is from **5a** only, or a mixture of **5a** and a product that might be **3a**. The additional signals from excess of starting material in the red spectrum makes it harder to identify characteristic signals of the diazoacetamide as well. The purple circle signal is an example, where overlapping signals makes it difficult to tell if it is present in the red spectrum or not.

Based on the arguments given above, it is not possible to confirm the presence of **3a** from the NMR spectra alone. The discussed signals might indicate the formation of **3a**, but it is not

enough to confirm the suspicion. Hence, it did not help switching **1a** with **5a** in the reaction. The proposed reaction using **4** to generate diazoacetamides is not working as intended under the current conditions. However, the initial test reactions show that the method works when using the **11** as the substrate. It might be the larger ring size, the presence of the ester or both that hinders the easy formation of **3a** observed when working with **11**. More work is needed to understand the reaction, before it can be used to synthesize diazoacetamides.

Synthesis of diazoacetamide 3e using 4 and 5b

In order to get a more conclusive answer whether the ring size, the presence of the ester group or both were the contributing factor to the lack of **3a** as product, a last test reaction using **5b** was conducted (**Scheme 3.12**).



***Scheme 3.12**: Proposed synthesis of the diazoacetamide **3e**.*

Compound **5b** was chosen as the substrate because it had the same ester as **5a** and the same ring size as **11**. Triethylamine and **5b** were both added in two equivalents excess. TLC was used to monitor the reaction. The initial thought was to wait until all **4** was consumed, but the reaction was stopped after 24 hours, despite the presence of **4** on the TLC plate. A comparison between the NMR spectra of the test reaction and the starting material **5b** is illustrated in **Figure 3.4**.

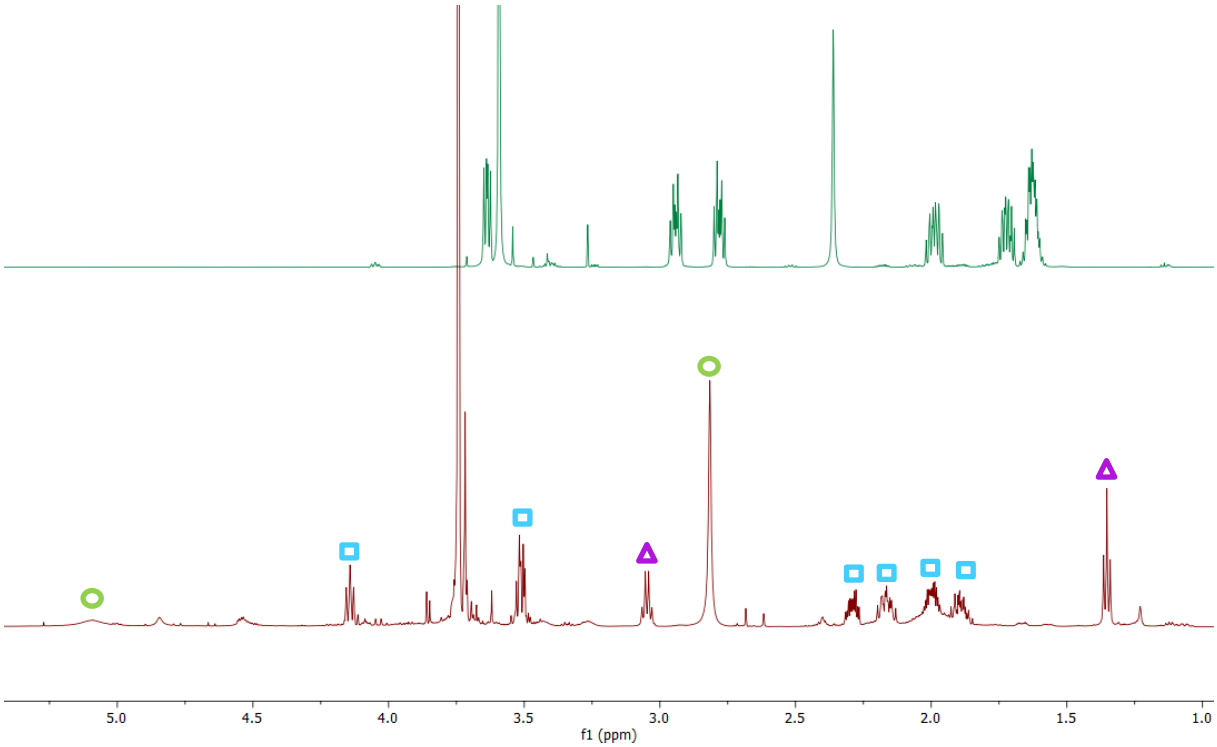


***Figure 3.4**: Comparison of the signals in the NMR spectra of **5a** (green spectrum) and a test reaction with **5a** and **4** (red spectrum). Green circles = **4**, purple triangles = Et_3N, blue squares = signals belonging to the same product.*

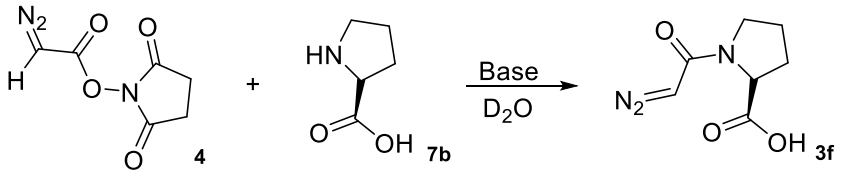
It is apparent from the NMR spectra that something happened in the reaction. The ring signals of **5b** (green spectrum) differs from the signals in the test reaction (red spectrum), which indicates the formation of a new molecule or molecules. Surprisingly however, it seems like all the **5b** molecules reacted, despite the limiting reactant **4** still being present (green circles). This indicates competing reaction paths and different products.

Identifying the products in the test reaction was not an easy task. By using 2D-NMR spectra, the signals belonging to the same molecule were identified. The orange circles belongs to remaining **4**, and the purple triangles belongs to remaining trimethylamine (**Figure 3.4**). As for the products, the most dominant signals marked in blue squares belongs to the same molecule. These signals could originate from the desired diazoacetamide **3e**, but not enough data could be interpreted from the NMR spectra to give a conclusive answer. The lack of the methyl signal was an important contributor to this, as it most likely overlapped with the signal of the internal standard at δ 3.74. As a result, there were no obvious signals that could be used as a reference in the calculation of the internal standard yield. The reaction seems promising however, as one of the masses in the HR-MS analysis of the test reaction matches the mass of **3e**. Consequently, there is enough physical data to confirm the formation of a product. Both the NMR spectra and the HR-MS analysis implies the formation of **3e**, but more work is needed

for conformation. A further investigation of this procedure would be beneficial, as it might reveal more details of the actual products of the reaction, in addition to its limitations.

3.2.3 Development of a synthesis of diazoacetamides in water

An important aspect of developing a new synthesis of the diazoacetamides was the idea of a simple and general method, with as few synthetic steps as possible. The esterification of the carboxylic acid (**Section 3.1.1**) is a necessary step in the original method by Kaupang and Konradsen, as it protects the carboxylic acid in the synthetic steps leading to the β-lactam molecule.[36,38] Regeneration of the carboxylic acid is necessary at some point in the process however, as most bicyclic β-lactam antibiotics have this functional group.[3] With this in mind, a new method for synthesizing diazoacetamides was proposed, using the carboxylic acid directly with water as solvent (**Scheme 3.13**).



***Scheme 3.13**: Proposed synthesis of the diazoacetamide **3f**, using **4** and **7b** with water as solvent.*

Water as solvent has a number of advantages, as it is easily accessible, cheap and environmental friendly. In addition, there is no longer any need for the protecting ester group. Deprotonation of the carboxylic acid under basic conditions gives the carboxylate, with the negative charge delocalized between the two oxygen atoms. This makes the carboxylate a poorer nucleophile than the lone pair of the nitrogen in **7b**. Consequently, the carboxylate will not compete with the nitrogen in the reactions or affect the mechanism significantly. Getting this procedure to work would shorten the total synthesis of the β-lactam molecules with three steps, as the protecting and regenerating of the carboxylic acid are two steps by itself.

Na_2CO_3 or $NaHCO_3$ served as the base in the test reactions. Two equivalents excess were necessary to deprotonate both the amine and the carboxylic acid. Compound **4** and **7b** both served as the limiting reactant on a 0.1, 0.25 and 0.5 mmol scale. Ideally, **4** would be the limiting reactant as **7b** is a cheap and readily available substance, while **4** is a much more expensive reagent in terms of reaction time and yield (**Section 3.2.1**). Problems with removing excess of **7b** however, lead to the use of **4** in excess as well.

In order to measure the yield by internal standard directly, deuterated water served as solvent in all the test reactions. With IS-yields as high as 94% and a reaction time of less than two hours, the method seemed very promising. Unfortunately, these yields were not correct. By a coincidence, it was discovered that the reaction mixture changed from yellow to a colorless solution. This happened within the timespan of finishing the reaction and measuring the sample on the NMR. Since the diazoacetamides **3a-c** appears as yellow oils when using the original method, this raised questions. Additionally, the NMR tubes contained several air bubbles, making it difficult to get good quality spectra. To determine if the product was **3f** or not, one of the test reactions was checked with IR (**Figure 3.5**).

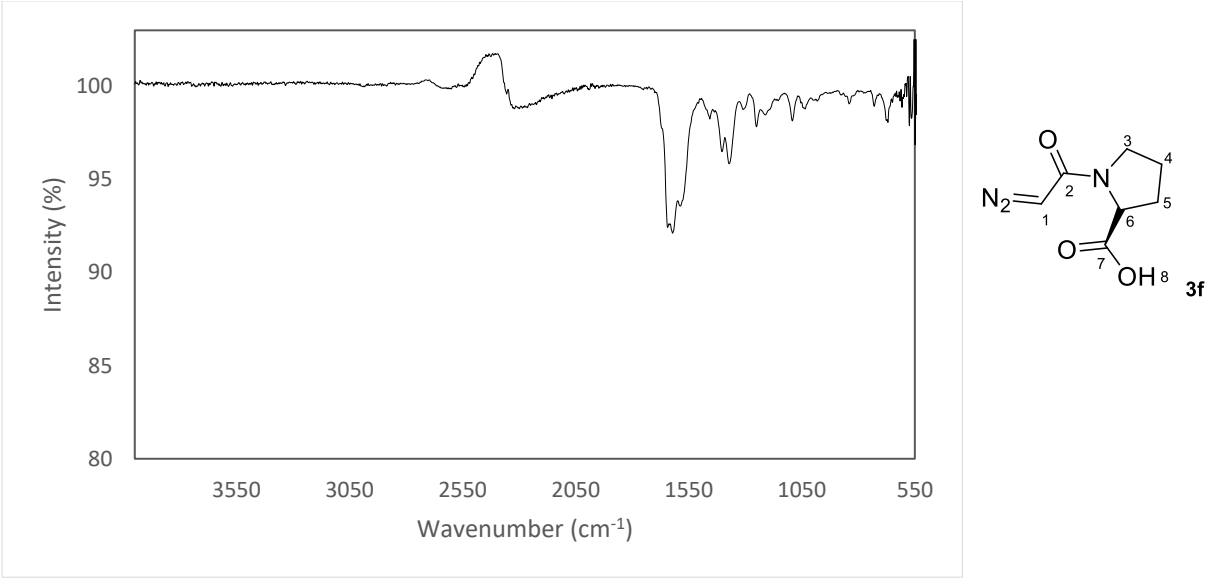


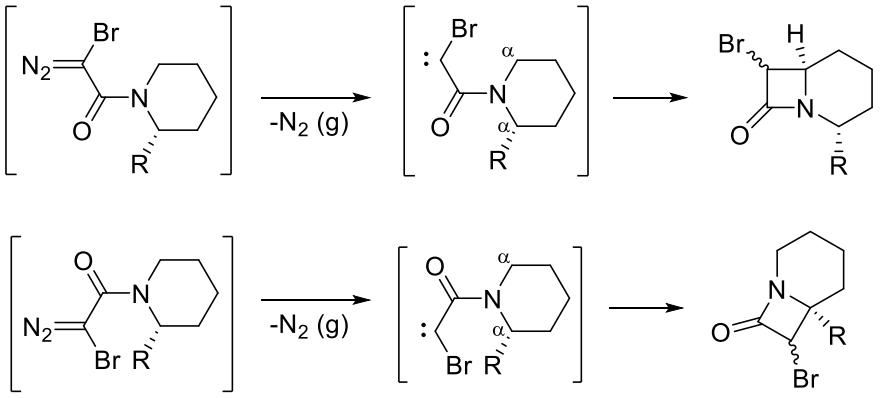
Figure 3.5*: An IR spectrum of one of the test reactions where* ***4*** *and* ***7b*** *were used to synthesize* ***3f****.*

The lack of the characteristic diazo-stretch around 2100 cm^{-1} clearly confirmed that the desired molecule was not present.[26] Decomposition of diazo molecules happens by release of nitrogen gas, which might explain the observed air bubbles in the NMR tubes. The content of the sample is therefore unknown, but based on the NMR spectra, it seems like the pyrrolidine ring and the ester are intact. This was part of the reason why the decomposition was not discovered sooner, as H-6 was used to calculate the internal standard yield (**Figure 3.5**). Ideally, the characteristic H-1 signal of the **3f** would have been used, but with D_2O as solvent, this signal was not usable. From these observations, it appears as the diazo functional group is not stable in water. The method do not work under the current reaction conditions, and if water as solvent is the cause of the decomposition, there is no need of further investigation into this procedure.

To summarize, four slightly different methods for the synthesis of diazoacetamides in one step by the use of **4** have been presented in **Section 3.2**. Water as solvent to skip the protection of the carboxylic acid do not work, as observations indicate that the diazo functional group decomposes in water. It seems like the 6-membered rings **1a** and **5a** do not produce the desired product, and if they do, it is not much. The most promising method is the one using **5b**, as the physical data supports the formation of the diazoacetamide **3e**. More work is in order to confirm this however, in addition to a better understanding of the mechanism and the impact of the reagent.

3.3 Investigation of how different esters affects the synthesis of the β-lactams

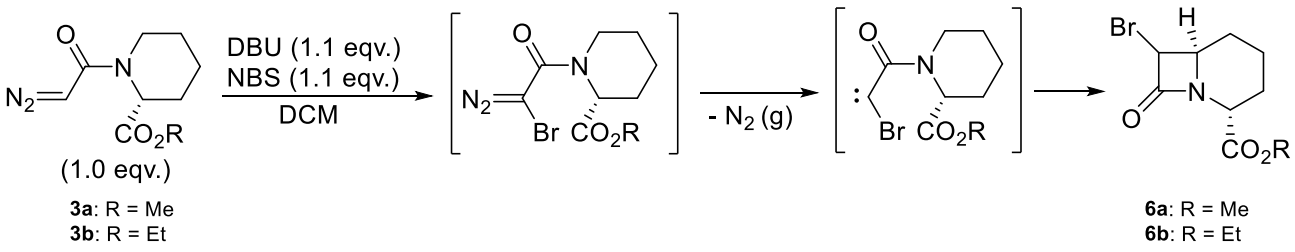
Konradsen's work lead to the identification of four products in the β-lactam formation step, where one of them was the result of an unfavorably C-H insertion. Calculations revealed that the orientation of the amide bond was the cause of the formation of the different molecules.[38] Due to the partial double bond character of the amide bond, the carbene would be located closer to one of the two α-carbons of the nitrogen (**Scheme 3.14**). Konradsen determined that it was more energetically favored to do the C-H insertion at the ester side rather than rotate the amide bond and insert on the other side.[38] Consequently, the group wanted to explore the possibility that the ester group could affect the orientation of the amide bond, and thus the distribution of the products.



***Scheme 3.14**: The partial double bond of the amide affects the orientation of the carbene, giving two different products due to insertion into different C-H bonds.[38]*

3.3.1 The reaction conditions

Brominating the diazoacetamides **3a-b** gives unstable molecules that decomposes to form carbenes by release of nitrogen gas.[38] The carbenes forms the β-lactams by an intramolecular C-H insertion (**Scheme 3.15**).



***Scheme 3.15**: The synthesis of the β-lactams **6a-b** from the diazoacetamides **3a-b**.*

This reaction requires good technical skills and practice to conduct. After adding DBU and NBS to a cold mixture of the diazoacetamide and DCM, the brominated diazoacetamide spontaneously decomposes to the very reactive carbene. Consequently, it is important to remove excess of the starting materials as fast as possible to avoid unfavorable side reactions with the carbenes. As a result, the stirring time must be a compromise between how much diazoacetamide have time to react with the starting materials versus the generated carbene. Konradsen reported the stirring time to be about one minute, before transferring the reaction mixture to a silica plug.[38] Minimizing the contact time on the silica plug reduces the risk of alternative reaction paths for the carbene as well. This illustrates that several factors in the execution of the procedure itself affects the outcome of the reaction.

Konradsen reported a characteristic orange color of the reaction mixture when adding NBS, which faded to a paler orange over time due to the generation of product.[38] The orange color was observed in the entries of this thesis as well. When pulled though the silica plug however, in some entries the color had changed to yellow when it should have kept the orange color. In other entries, too much eluent was used, contaminating the product with starting materials pulled through the column, or the product was left in the column because of too little eluent. This indicates that an optimization of the purification is necessary, in order to make it easier to conduct.

Two test reactions with alternative purification methods were tried with **3b** as the diazoacetamide in order to optimize the procedure. The first entry used catalytic amounts of DBU instead of the usual 1.1 equivalents. Instead of the silica plug, the reaction mixture was purified by extraction after thermally decomposition at ambient temperature. Presuming that catalytic amounts of DBU would decrease the number of unwanted side reactions with the carbene significantly, the silica plug was no longer necessary. The second test reaction used 1.1 equivalents of DBU as in the original procedure, but was extracted with a cold thiosulfate solution directly after two minutes of stirring time. Both methods produced the desired product **6ba** (**Figure 3.6**), in addition to byproducts (**Section 3.3.2**).

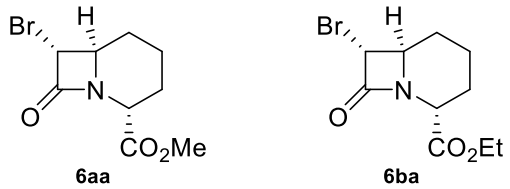
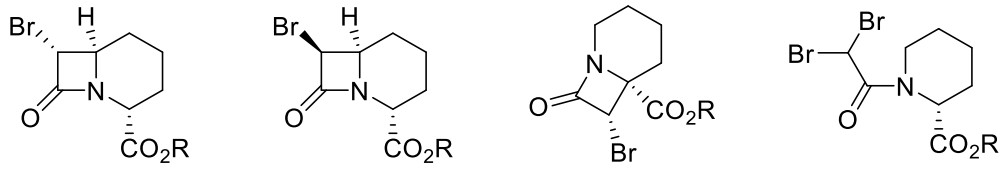


***Figure 3.6**: The desired products in the β-lactam formation step.*

The internal standard yield of the **6ba** was 11% and 23% for the first and second test reactions respectively. Konradsen reported an IS-yield of 38% for **6aa**,[38] which is slightly better than the entry using cold thiosulfate. There might be a difference in reactivity between the methyl and ethyl esters as well, but more work is in order to establish this. The two purification alternatives show that the removal of the silica plug is possible. However, more work is necessary in order to optimize and simplify the execution of the purification.

3.3.2 Distribution of the products

Konradsen identified four products in the β-lactam formation step when working with the methyl ester (**Figure 3.7**).[38] Three compounds, **6ba-6bc**, were identified in the two test reactions with **3b** (**Section 3.3.1**). Initially, the product distribution of both the ethyl and isopropyl esters were to be compared with that of the methyl ester. Unfortunately, only the ethyl ester was successfully synthesized to the β-lactam within the available time. Hence, a brief discussion comparing the methyl and ethyl ester follows, using the reported product distribution of the methyl ester from Konradsen.



6aa: R = Me
6ba: R = Et

6ab: R = Me
6bb: R = Et

6ac: R = Me
6bc: R = Et

6ad: R = Me
6bd: R = Et

***Figure 3.7**: Konradsen identified four products (**6aa-6ad**) when working with the methyl ester.[38] The three correspondingly molecules **6ba-6ac** were identified in the test reactions with the ethyl ester.*

By using the structural elucidation of **6aa-6ac** reported by Konradsen,[38] the identification of the products from the test reactions was possible. A comparison between the NMR spectra of the test reactions and a spectrum of **6aa** and **6ac** from Konradsen[38] is illustrated in **Figure 3.8**.

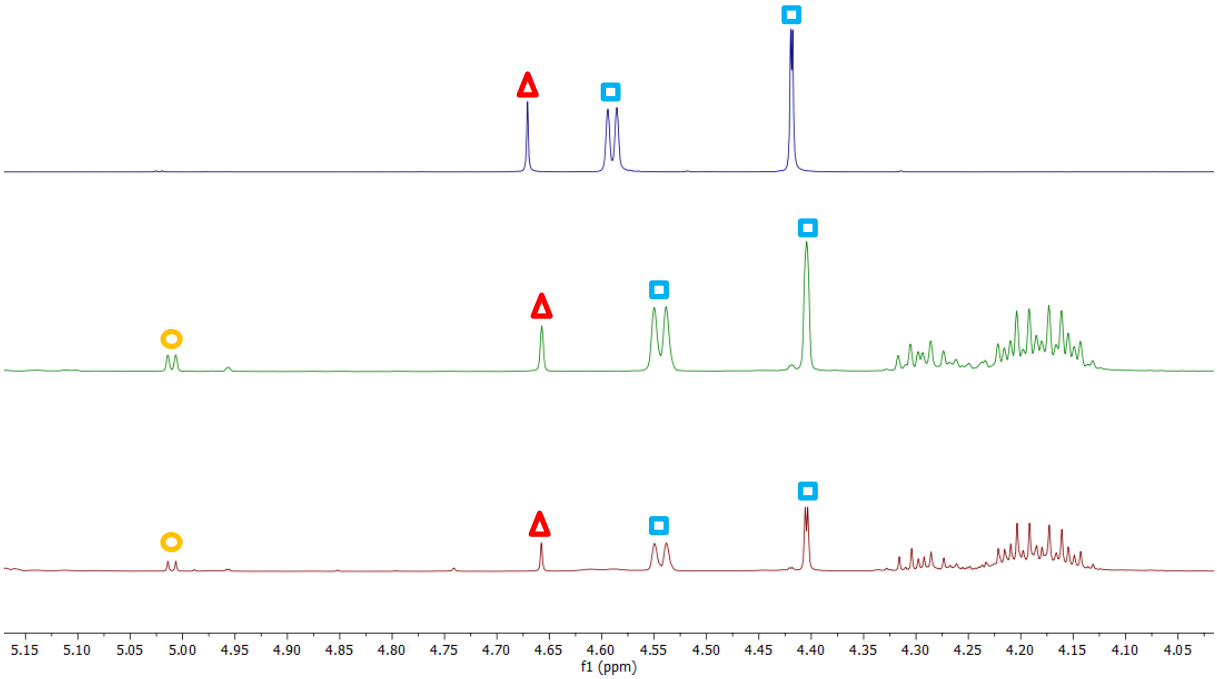
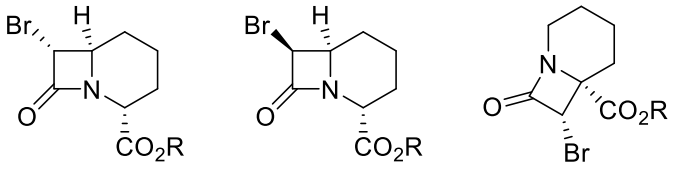


***Figure 3.8**: A comparison of the characteristic signals of **6aa** and **6ac** (blue spectrum) from Konradsen[38] with the spectra of the test reactions with the ethyl ester (red spectrum = cat. DBU, green spectrum = extraction with cold thiosulfate). Red triangles = **6ac**/**6bc** signal, blue squares = **6aa**/**6ba** signals, yellow circles = **6bb** signals.*

The characteristic signals of **6aa** and **6ac** are displayed in the blue spectrum. As all of these signals originates from the core structure of the β-lactams, a direct comparison with the spectra from the test reactions was possible. The triangle signals originates from **6ac**/**6bc** and the square signals from **6aa**/**6ba** respectively. Konradsen reported **6ab** to have a characteristic core signal around δ 5.05,[38] which align well with the yellow circle signals and indicates the presence of **6bb** in both the test reactions.

Table 3.5 display the total yield of β-lactam formation from the two test reactions, in addition to the yield of the methyl ester β-lactam reported by Konradsen.[38] The relative yields of the three products in each entry are displayed as well. Compound **6aa**/**6ba** are the desired products, and are fortunately the main product in all three entries. As Konradsen reported having problems with separating **6ac** from **6aa**,[38] it would be beneficial to have as little formation of **6ac** and **6bc** as possible. Seemingly, all three entries have more or less the same distribution of products. However, it is not possible to establish the relationship between the ester and the distribution of products without more data. More esters of different sizes are needed in order to collect more data of which a relationship can be established more conclusively.

Table 3.5: The total yield of β-lactam formation in the two test reactions with the ethyl ester, in addition to the methyl ester reported by Konradsen.[38] The relative yield of the three products is displayed for each entry.



Entry	R =	Total yield (%)	Relative yield of products (%)		
1[a]	Me	54	70	13	17
2	Et	14	79	7	14
3	Et	31	74	10	16

[a] Values reported by Konradsen.[38]

4 Conclusion and future work

The method developed by Kaupang and Konradsen has successfully been applied in the syntheses of **2a-c** and **3a-c**. Reactions with both **3a** and **3b** were conducted in order to synthesize **6a** and **6b**, but only **6b** was successful.
More data is needed in order to establish the relationship between the ester group and the product distribution in the forming of the β-lactams. Seemingly, the ethyl ester give roughly the same product distribution as the methyl ester. However, an optimization of the purification is in order for a better procedure and comparison between the esters.

Despite several test reactions with compound **4** in an attempt to develop a one-step synthesis of the diazoacetamides, only one procedure seems to give the desired product. Water as solvent seemingly leads to the decomposition of the diazo functional group, and formation of **3a** cannot be confirmed when using either **1a** or **5a** as substrate. When reacting **4** with **5b**, spectroscopic data are in agreement with the formation of compound **3e**, but more work is needed for confirmation.

In order to develop the method further, it would be beneficial to continue the work of the one-step synthesis of the diazoacetamides. Conclusive identification of the product in the reaction between **4** and **5b** will give important information for the further development.
As the work with synthesizing the β-lactam with the isopropyl ester was not finished, it would be of interest to continue this work. Additionally, it would be informative to investigate other esters as well. This could give a more conclusive answer regarding the influence of the esters in the product distribution of the β-lactam formation step.

5 Experimental section

5.1 General information

All reagents and solvents were used as delivered from Sigma-Aldrich, VWR, Fluorochem and BLDpharm, unless stated otherwise. The deuteated solvents were purchased from Cambridge Isotope Laboratories. DCC was recrystallized before use. Hexane, DCM and EtOAc were distillated before use. Distillated type 2 water was used in all reactions and work-up. A MB SPS-800 solvent Purification System from MBraun were used to dry DMF, THF and DCM.

Thin Layer Chromatography (TLC) was performed on 60 F_{254} silica gel coated alumina plates or 60 RP-18 F_{254} silica gel coated alumina plates from Merck. pH was measured using MColorpHast™ pH-paper or MQuant pH 0-14 Universal indicator, both from Merck.

NMR spectra were recorded using Bruker Avance DPX300, AVII400, AVIII400, AVNEO400, AVI600 or AVII600. Chemical shifts (δ) are given in parts per million (ppm) and coupling constants (*J*) in Hertz (Hz). The following deuterated solvents and solvent signals were used: $CDCl_3$ (δ 7.24/ 77.0), DMSO-d_6 (δ 2.50/ 39.52), MeOD-d_4 (δ 3.31/ 49.00), D_2O (δ 4.65).[48] Multiplicities were abbreviated as followed: s (singlet), d (doublet), t (triplet), q, (quartet), quint (quintet), sept (septet), m (multiplet), bx (broad signal of x, e.g. bd - broad doublet), dd (doublet of doublets), td (triplet of doublets). The assigning of ^{1}H- and ^{13}C-shift were done using DEPT135 or DEPT135Q, HSQC, HMBC and COSY. These spectra are located in the **Appendix**.

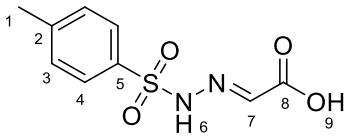
Mass spectra were obtained on a Bruker maXis II ETD spectrometer (ESI, positive ionization mode unless stated otherwise), by Erlend Steinvik, Lina Aarsbog or Sverre Løyland (University of Oslo).

A Stuart SMP10-melting point instrument was used to measure the melting point of the compounds.

The FTIR spectra were recorded in ATR (Bruker ATR A225/Q) on a Vertex 80 Bruker infrared spectrophotometer. It was equipped with a DTGS detector, and 32 interferograms (recorded at 4 cm^{-1} resolution) were typically averaged for each of the spectra.

5.2 Synthesis of starting materials

5.2.1 2-(4-methylbenzenesulfonamido)imino acetic acid (8)



Compound **8** was prepared based on a procedure by Nelson *et al*.[45] Glyoxylic acid monohydrate (10.00 g, 108.7 mmol, 1.0 eqv.) was solved in type 2 water (110 mL), using an oil bath (65˚C) until everything dissolved. A 2.5 M HCl-solution (66 mL) was added to a flask containing 4-methylbenzenesulfonohydrazide (20.35 g, 109.3 mmol, 1.0 eqv.), using an oil bath (65˚C) until solvation. This solution was added to the flask containing glyoxylic acid monohydrate, resulting in precipitation of a white solid. The mixture was stirred a couple of minutes before transferring the flask to an ice bath, followed by isolation of the precipitate by vacuum filtration. After washing the crystals with ice cold type 2 water, the solvent was evaporated *in vacuo*. The crude product was recrystallized by adding EtOAc until solvation during heating in an oil bath (77˚C). Hexane was added until saturation, before the flask was transferred to an ice bath, resulting in precipitation. The crystals were isolated by vacuum filtration and the solvent evaporated *in vacuo*, giving **8** as a white solid.

Yield: 77% (20.4140 g, 84.28 mmol)

^{1}H-NMR (400 MHz, DMSO-d_6); Mayor rotamer: δ 13.06 (bs, 1H, H-9/H-6), 12.28 (bs, 1H, H-6/H-9), 7.71 (d, *J* = 8.3 Hz, 2H, H-4), 7.44 (d, *J* = 8.1 Hz, 2H, H-3), 7.18 (s, 1H, H-7), 2.39 (s, 3H, H-1), Minor rotamer: δ 13.06 (bs, 1H, H-9/H-6), 12.28 (bs, 1H, H-6/H-9), 7.55 (d, *J* = 8.2 Hz, 2H, H-4), 7.37 (d, *J* = 7.9 Hz, 2H, H-3), 7.18 (s, 1H, H-7), 2.37 (s, 3H, H-1)

^{13}C-NMR (151 MHz, DMSO-d_6); Mayor rotamer: δ 163.5 (C=O, C-8), 144.0 (C, C-2), 137.4 (CH, C-7), 135.7 (C, C-5), 129.9 (CH, C-3), 127.1 (CH, C-4), 21.0 (CH_3, C-1),
Minor rotamer: δ 163.5 (C=O, C-8), 144.0 (C, C-2), 137.4 (CH, C-7), 135.7 (C, C-5), 129.4 (CH, C-3), 124.4 (CH, C-4), 20.7 (CH_3, C-1)

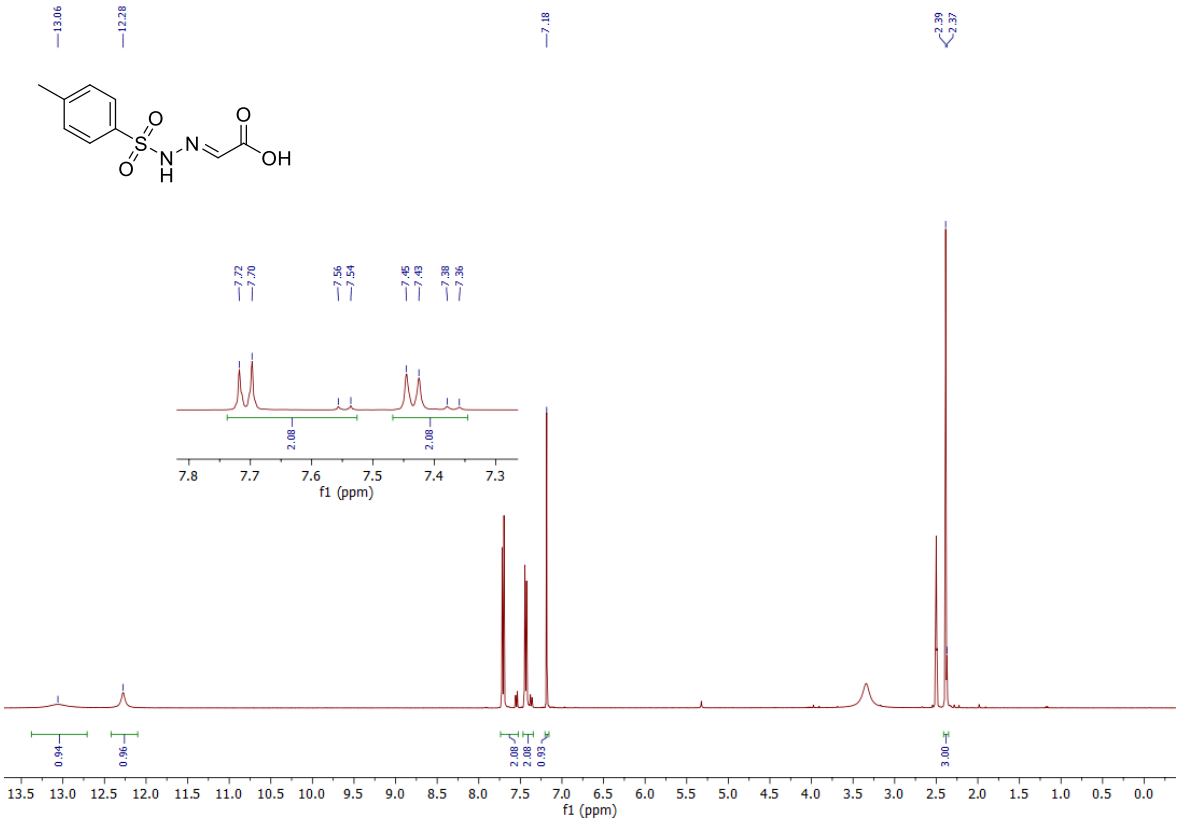
HR-MS (ESI, MeOH) *m/z*: Calculated for 287.0073 $[M - H + 2Na]^+$, found 287.0073 for $C_9H_9N_2O_4SNa_2$ (err: 0.1 ppm)

MS (ESI, MeOH) *m/z*: 287 $[M - H + 2Na]^+$ (100%), 265 $[M + Na]^+$ (17%)

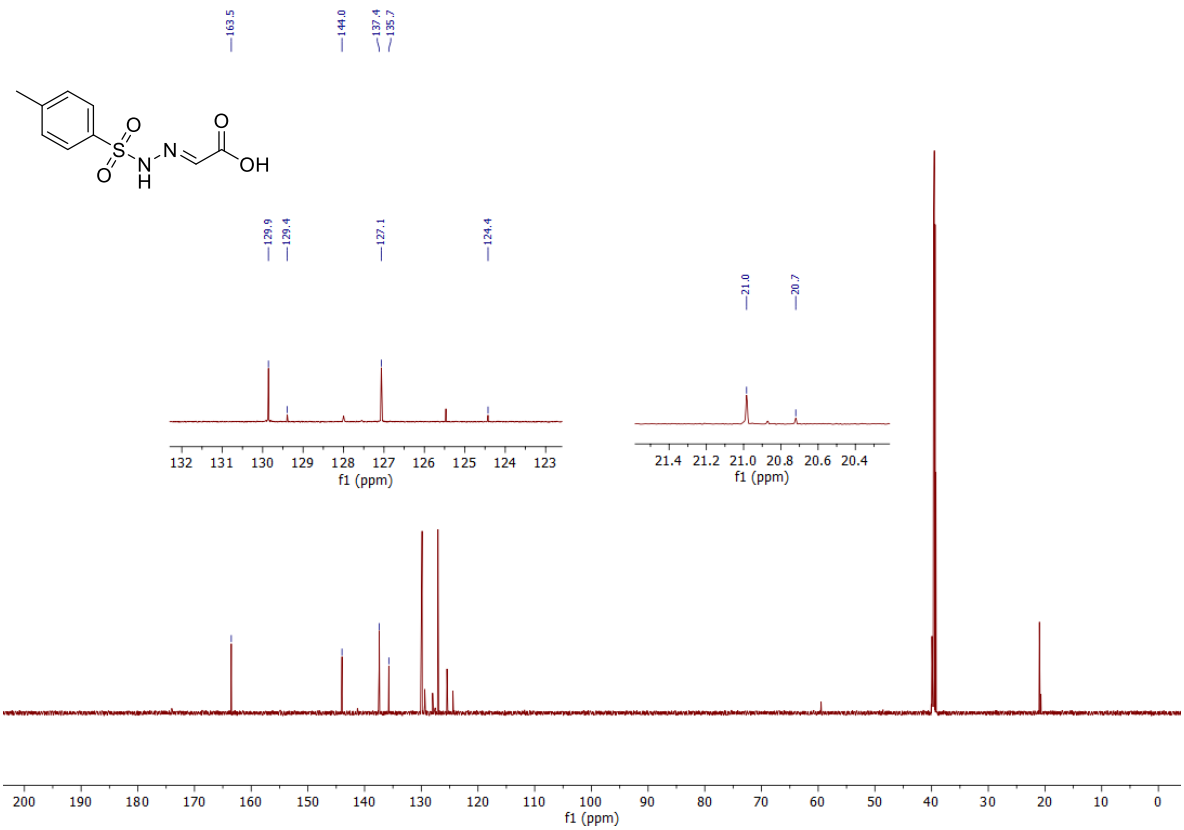
Melting point: 139-146˚C

This compound is reported in literature.[45]

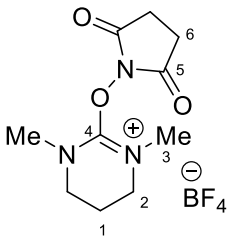
^{1}H NMR spectrum of 8 (400 MHz, DMSO-d_6)



^{13}C NMR spectrum of 8 (151 MHz, DMSO-d_6)



5.2.2 2-((2,5-dioxopyrrolidin-1-yl)oxy)-1,3-dimethyl-3,4,5,6-tetrahydropyrimidin-1-ium tetrafluoroborate (10)



Compound **10** was prepared based on a procedure by Nájera.[46] A solution of DMPU (5.0875 g, 39.69 mmol, 1.0 eqv.), DMF (0.3 mL) and dry DCM (40 mL) was added dropwise to a flask of oxalyl chloride (4.20 mL, 49.0 mmol, 1.2 eqv.). The mixture stirred at ambient temperature for 1 h and then at 40-41˚C for 24 h using an oil bath. After evaporation of the solvent *in vacuo*, the mixture was washed by solving it in dry DCM (10 mL) followed by evaporation under reduced pressure three times. The mixture was dissolved in ACN (40 mL), added $NaBF_4$ (5.2692 g, 47.99 mmol, 1.2 eqv.) and stirred at ambient temperature for 24 h before adding *N*-hydroxysuccinimide (**9**) (5.0984 g, 44.31 mmol, 1.1 eqv.). The flask was placed in a water bath before adding Et_3N (6.70 mL, 48.3 mmol, 1.2 eqv.). After removal of the water bath, the mixture stirred at ambient temperature for 5 h followed by 1 h at 45˚C using an oil bath. The mixture was filtrated through a layer of celite using vacuum filtration, and the solvent evaporated *in vacuo*. The crude mixture was recrystallized by solving it in MeOH:*i*-PrOH (1:1) (15 mL) and again using MeOH:*i*-PrOH (1:5) (60 mL).[49] The solution was stirred in a cold water bath for 2 h, before isolating the precipitate by vacuum filtration and evaporation under reduced pressure, giving **10** as a white solid.

Yield: 26% (5.7395 g, 18.33 mmol)

^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.53 (t, *J* = 5.9 Hz, 4H, H-2), 3.15 (s, 6H, H-3), 2.82 (s, 4H, H-6), 1.99 (quint, *J* = 5.9 Hz, 2H, H-1)

^{13}C-NMR (151 MHz, DMSO-d_6): δ 170.1 (C=O, C-5), 155.9 (C, C-4), 49.0 (CH_2, C-2), 38.2 (CH_3, C-3), 25.7 (CH_2, C-6), 20.0 (CH_2, C-1)

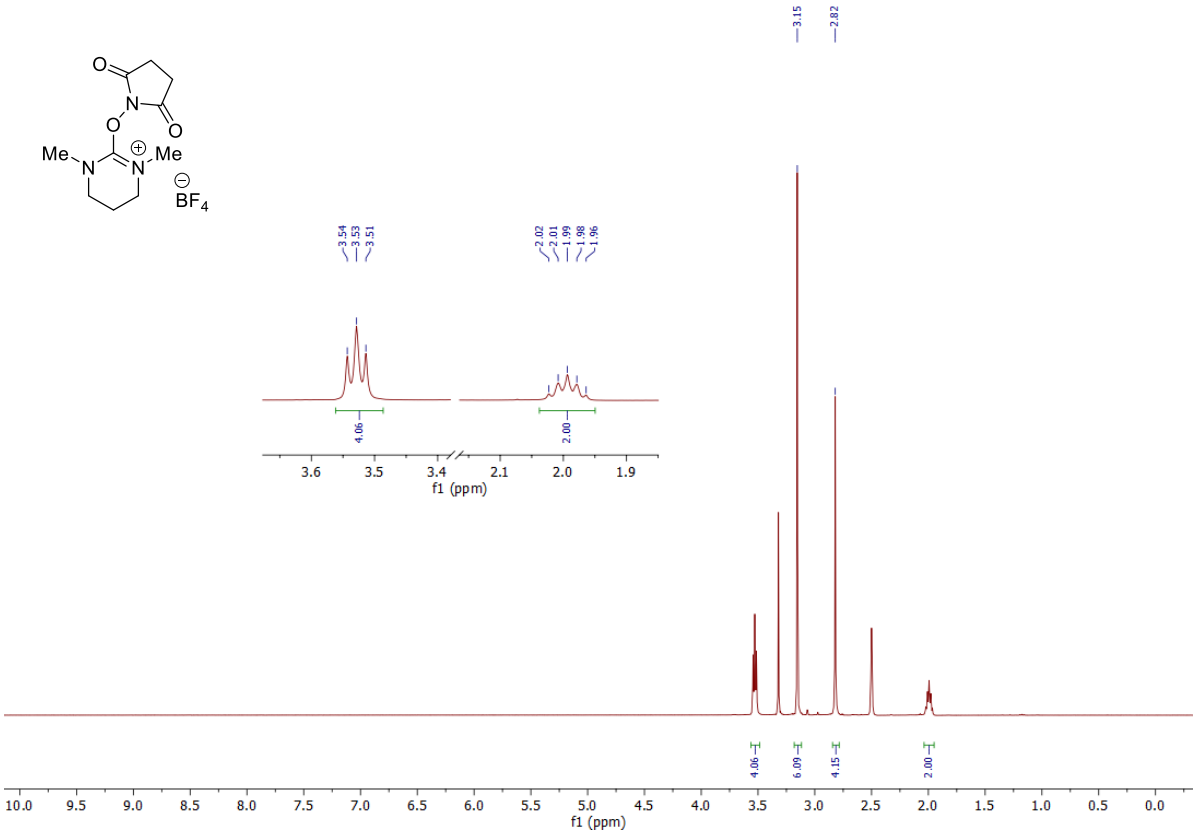
HR-MS (ESI, H_2O) *m/z*: Calculated for 151.0843/ 226.1185; found 151.0842 for $C_6H_{12}N_2ONa$ (err: -0.7 ppm)/ 226.1186 for $C_{10}H_{16}O_3N_3$ (err: 0.7 ppm)

MS (ESI, H_2O) *m/z*: 151 $[C_6H_{12}N_2O + Na]^+$ (100%), 279 (13%), 226 $[M]^+$ (8%)

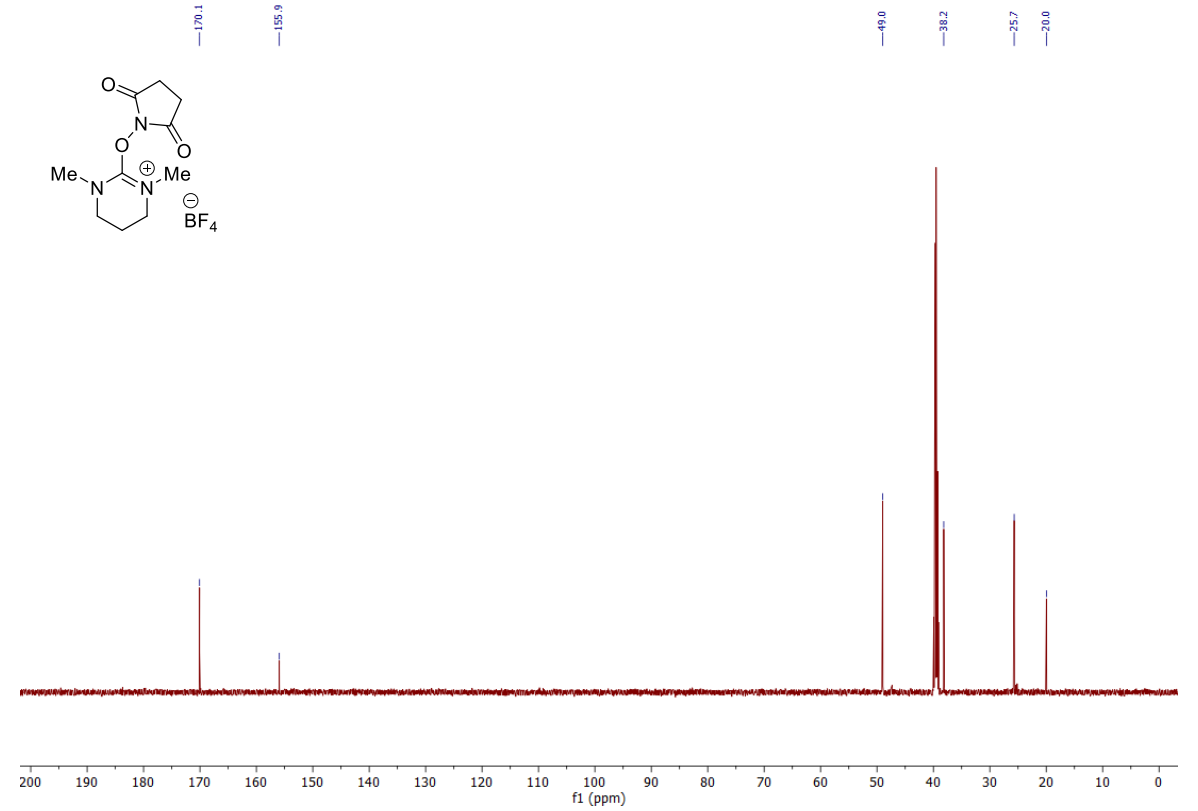
Melting point: 142-146˚C

This compound is reported in literature.[46]

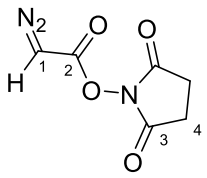
^{1}H NMR spectrum of 10 (400 MHz, DMSO-d_6)



^{13}C NMR spectrum of 10 (151 MHz, DMSO-d_6)



5.2.3 2,5-dioxypyrrolidin-1-yl 2-diazoacetate (4)



Compound **4** was prepared based on a procedure by Badet *et al*.[44] Compound **8** (2.4224 g, 10.00 mmol, 1.0 eqv.), **9** (1.1511 g, 10.00 mmol, 1.0 eqv.) and $NaHCO_3$ (843.9 g, 10.05 mmol, 1.0 eqv.) were dissolved in THF (15 mL). The reaction flask was cooled down in an ice bath before DCC (2.0607 g, 10.09 mmol, 1.0 eqv.) solved in THF (35 mL) was added dropwise to the solution, resulting in precipitation. The mixture stirred at ambient temperature for 20 h 45 min, followed by 1 h in an ice bath. The precipitate was filtrated off, and the filtrate was left in the refrigerator overnight, resulting in more precipitation. After removing the precipitation by filtration, the solvent was evaporated *in vacuo*. The crude mixture was dissolved in EtO_2 (15 mL) and extracted with 5 x 20 mL type 2 water. The collected water phases were washed with 3 x 30 mL DCM, followed by concentration of the DCM-phases *in vacuo*. After dissolving the crude mixture in DCM, it was purified by a silica plug, using EtOAc:DCM (1:10) as eluent. The fractions were monitored using TLC, with EtOAc:DCM (1:10) as the eluent, and the ones containing **4** were collected, filtrated and evaporated *in vacuo*. Recrystallization was done by solving the crude mixture in DCM (5 mL) and adding hexane (5 mL) carefully along the edge of the reaction flask, creating two phases. The DCM phase was evaporated slowly under reduced pressure without stirring, which resulted in precipitation. After carefully transferring the hexane-phase to a new flask, the precipitate was evaporated to dryness, giving **4** as a yellow solid. Some precipitation had appeared in the flask containing the hexane-phase the next day. Removal of the solvent and evaporation to dryness resulted in **4** as a yellow solid.

Yield: 27% (492.9 mg, 2.692 mmol)

^{1}H-NMR (600 MHz, $CDCl_3$): δ 5.09 (bs, 1H, H-1), 2.82 (s, 4H, H-4)

^{13}C-NMR (151 MHz, $CDCl_3$): δ 169.2 (C=O, C-2 + C-3), 45.0 (CH, C-1), 25.5 (CH_2 C-4)

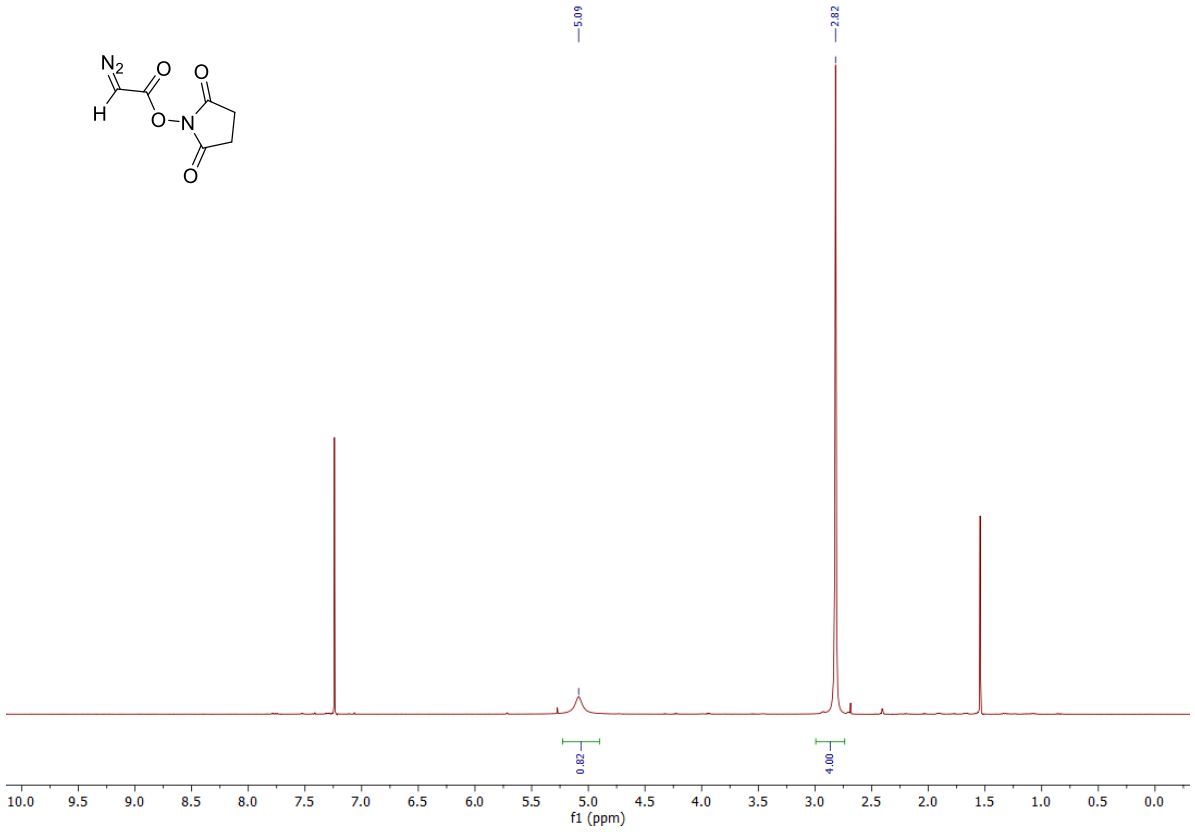
HR-MS (ESI, ACN) *m/z*: Calculated for 206.0172 $[M + Na]^+$; found 206.0172 for $C_6H_5N_3O_4Na$ (err: 0.2 ppm)

MS (ESI, ACN) *m/z*: 206 $[M + Na]^+$ (100%), 138 (14%), 164 (7%)

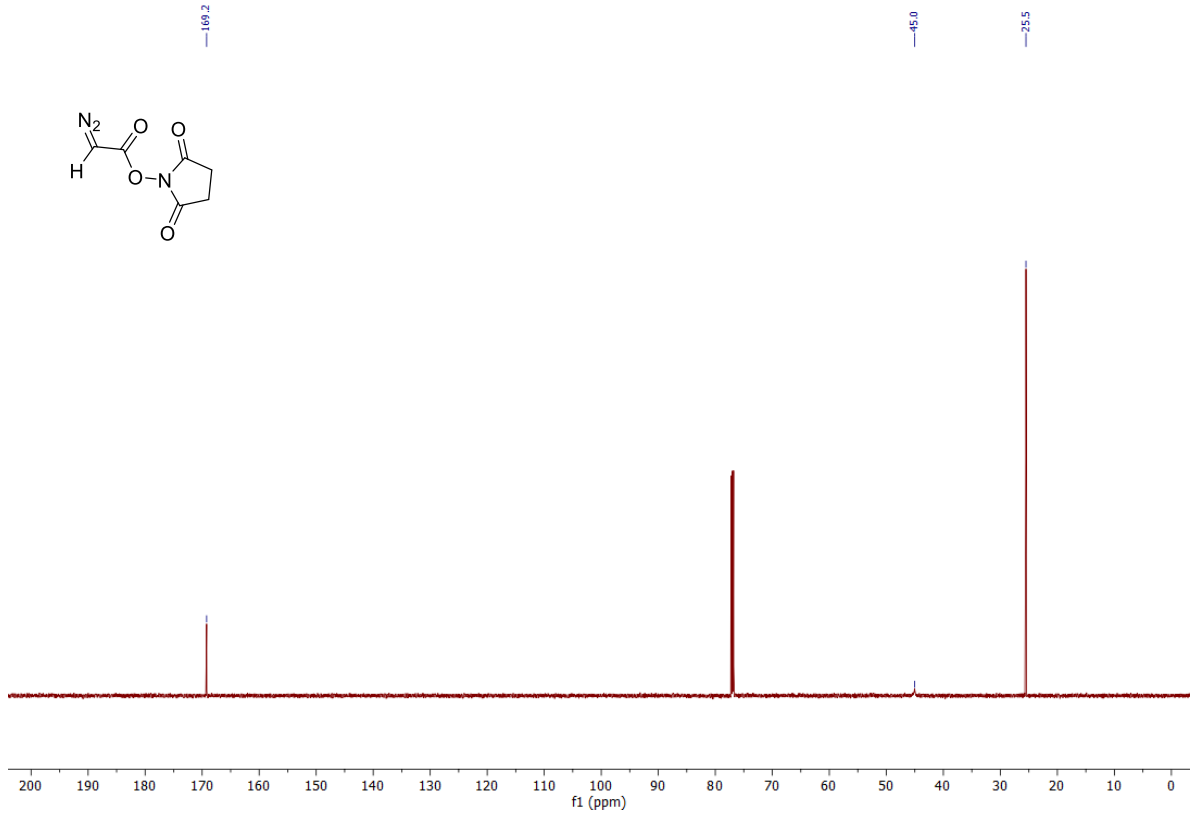
IR (ATR): Wavenumber (cm^{-1}); 2149 ($C=N_2$), 1726 (C=O)

This compound is reported in literature.[44]

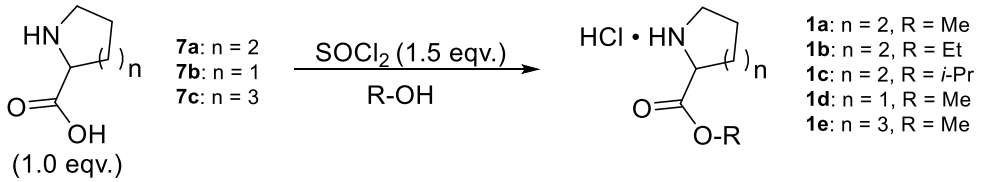
^{1}H NMR spectrum of 4 (600 MHz, $CDCl_3$)



^{13}C NMR spectrum of 4 (151 MHz, $CDCl_3$)

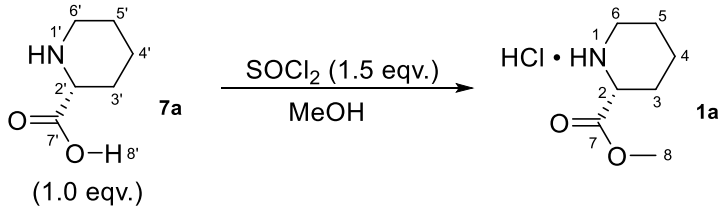


5.3 General procedure for the syntheses of the hydrochloride esters 1a-e



The preparation of **1a-e** was based on a procedure by Dave *et al*.[42] R-OH was cooled down to ~0˚C in an ice bath, before thionyl chloride (1.5 eqv.) was added dropwise to the solution. D-(+)-pipecolic acid (**7a**), L-proline (**7b**) or (*R*)-azepane-2-carboxylic acid (**7c**) was added, the ice bath removed, and the solution was left stirring at ambient temperature, at 45˚C or at 70˚C overnight. The solvent was evaporated *in vacuo*, and the resulting crude mixture washed with EtOAc, giving the product **1a-c** of varying yield and purity.

5.3.1 (*R*)-Methyl piperidine-2-carboxylate hydrochloride (1a)



Compound **1a** was synthesized according to **Procedure 5.3**, using MeOH (50 mL), $SOCl_2$ (0.80 mL, 11 mmol, 1.4 eqv.) and **7a** (1.0002 g, 7.759 mmol, 1.0 eqv.). The solution was stirred at ambient temperature for 17 h 25 min, followed by evaporation of the solvent under reduced pressure. The crude mixture was washed with 3 x 20 mL EtOAc, which was evaporated *in vacuo* between each adding, giving a mixture of **1a** and **7a** (1.3539 g in total) as a white salt.

Yield: 51% (713 mg, 3.97 mmol) (Calculated from NMR)

^{1}H-NMR (600 MHz, MeOD-d_4): δ 4.04 (dd, *J* = 11.6 Hz, 3.6 Hz, 1H, H-2), 3.95 (dd, *J* = 11.6 Hz, 3.6 Hz, 1H, H-2'), 3.85 (s, 3H, H-8), 3.44-3.38 (m, 2H, H-6 + H-6'), 3.08-3.01 (m, 2H, H-6 + H-6'), 2.32-2.26 (m, 2H, H-3 + H-3'), 1.93-1.87 (m, 4H, H-4 + H-4'), 1.78-1.62 (m, 6H, H-3 + H-3' + H-5 + H-5')

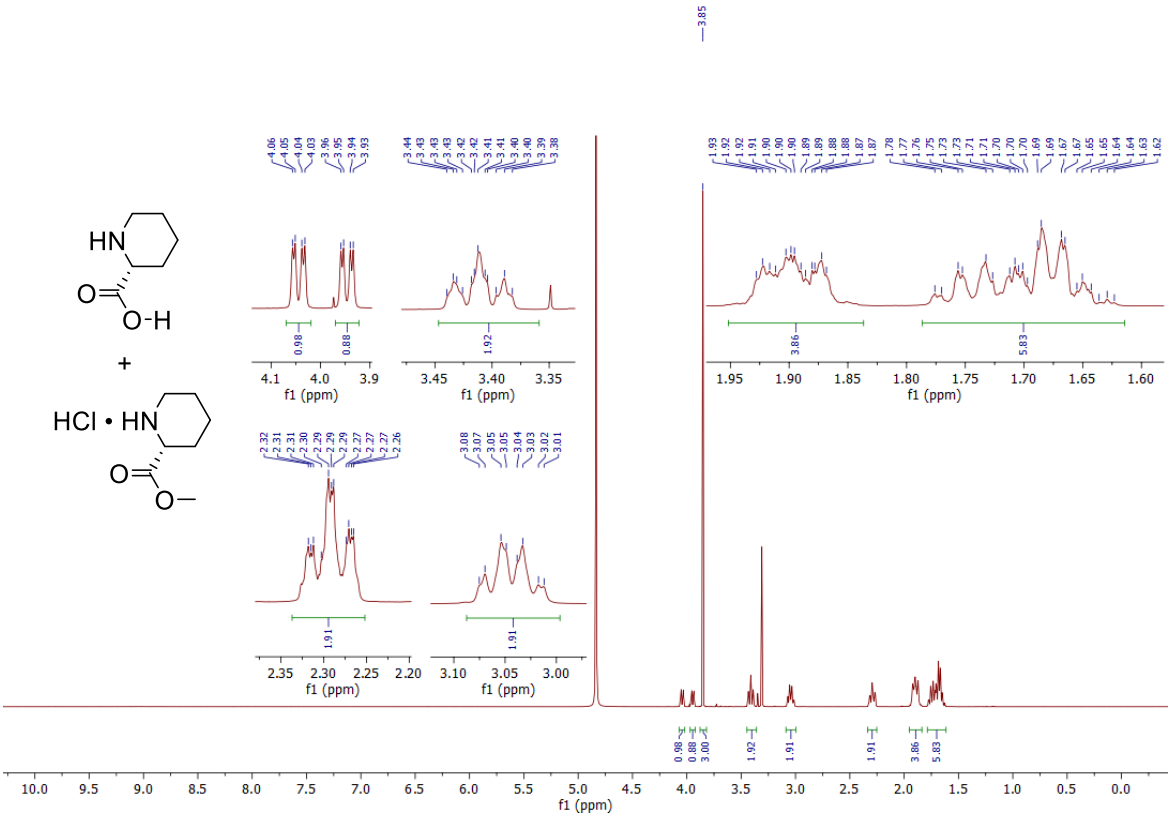
^{13}C-NMR (151 MHz, MeOD-d_4): δ 171.2 (C=O, C-7'), 170.3 (C=O, C-7), 57.9 (CH, C-2 + C-2'), 53.7 (CH_3, C-8), 45.2 + 45.0 (CH_2, C-6 + C-6'), 27.3 + 27.1 (CH_2, C-3 + C-3'), 22.9 (CH_2, C-5 + C-5'), 22.9 + 22.7 (CH_2, C-4 + C-4')

HR-MS (ESI, H_2O) *m/z*: Calculated for 144.1019 $[M - Cl]^+$; found 144.1019 for $C_7H_{14}NO_2$ (err: -0.0 ppm)

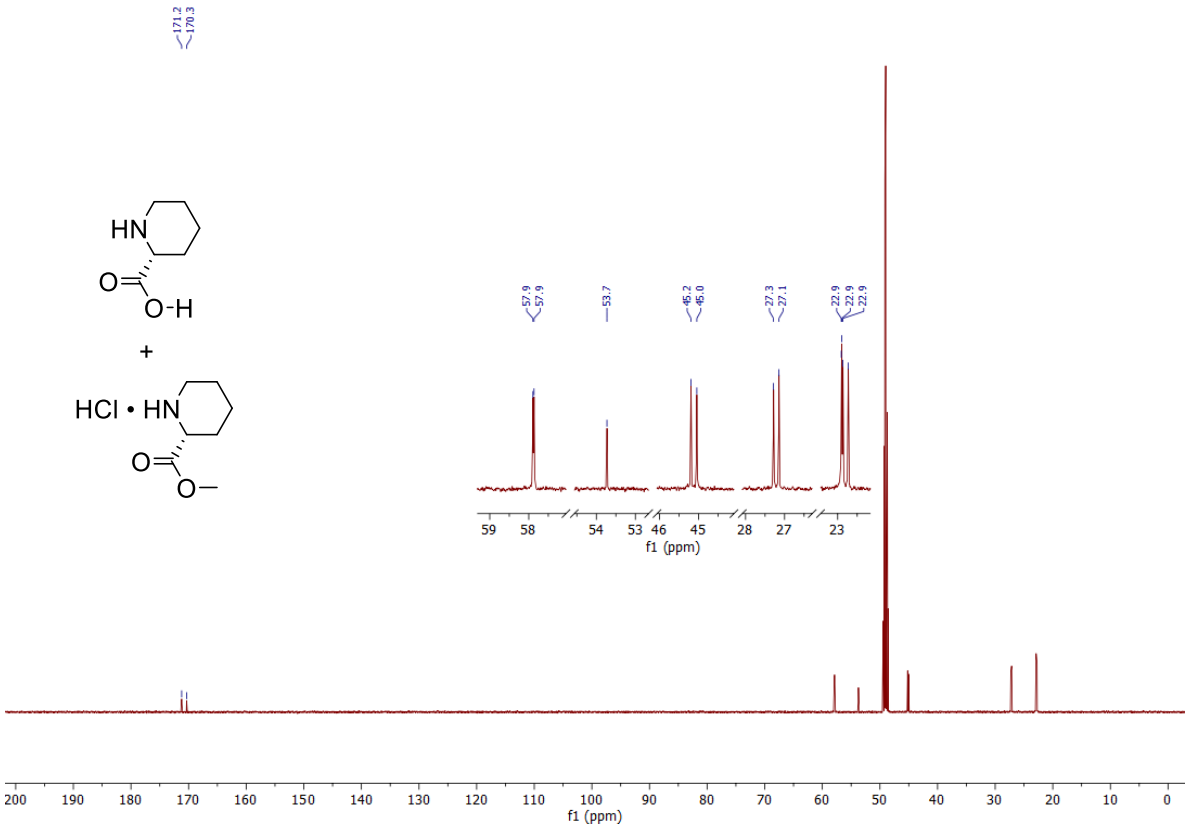
MS (ESI, H_2O) *m/z*: 144 $[M - Cl]^+$ (100%), 152 $[C_6H_{11}NO_2 + Na]^+$ (37%)

This compound is reported in literature.[50]

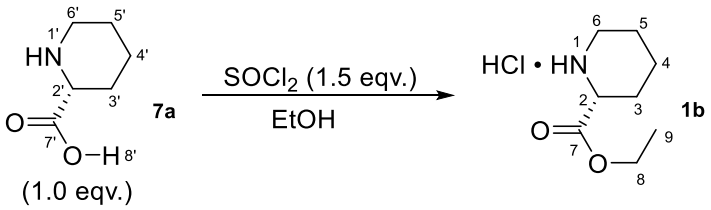
^{1}H NMR spectrum of the mixture of 1a and 7a (600 MHz, MeOD-d_4)



^{13}C NMR spectrum of the mixture of 1a and 7a (151 MHz, MeOD-d_4)



5.3.2 (*R*)-Ethyl piperidine-2-carboxylate hydrochloride (1b)



Compound **1b** was synthesized according to **Procedure 5.3**, using EtOH (50 mL), $SOCl_2$ (0.80 mL, 11 mmol, 1.4 eqv.) and **7a** (1.0066 g, 7.795 mmol, 1.0 eqv.). The solution was stirred at ambient temperature for 19 h 35 min, followed by evaporation of the solvent under reduced pressure. The crude mixture was washed with 3 x 20 mL EtOAc, which was evaporated *in vacuo* between each adding, giving a mixture of **1b** and **7a** (783.9 mg in total) as a white salt.

Yield: 21% (320 mg, 1.66 mmol) (Calculated from NMR)

^{1}H-NMR (600 MHz, MeOD-d_4): δ 4.31 (q, *J* = 7.1 Hz, 2H, H-8), 4.01 (dd, *J* = 11.6 Hz, 3.5 Hz, 1H, H-2), 3.93 (dd, *J* = 11.6 Hz, 3.5 Hz, 1H, H-2'), 3.44-3.38 (m, 2H, H-6 + H-6'), 3.06-3.00 (m, 2H, H-6 + H-6'), 2.33-2.27 (m, 2H, H-3 + H-3'), 1.95-1.85 (m, 4H, H-4 + H-4'), 1.77-1.62 (m, 6H, H-3 + H-3' + H-5 + H-5'), 1.32 (t, *J* = 7.1 Hz, 3H, H-9)

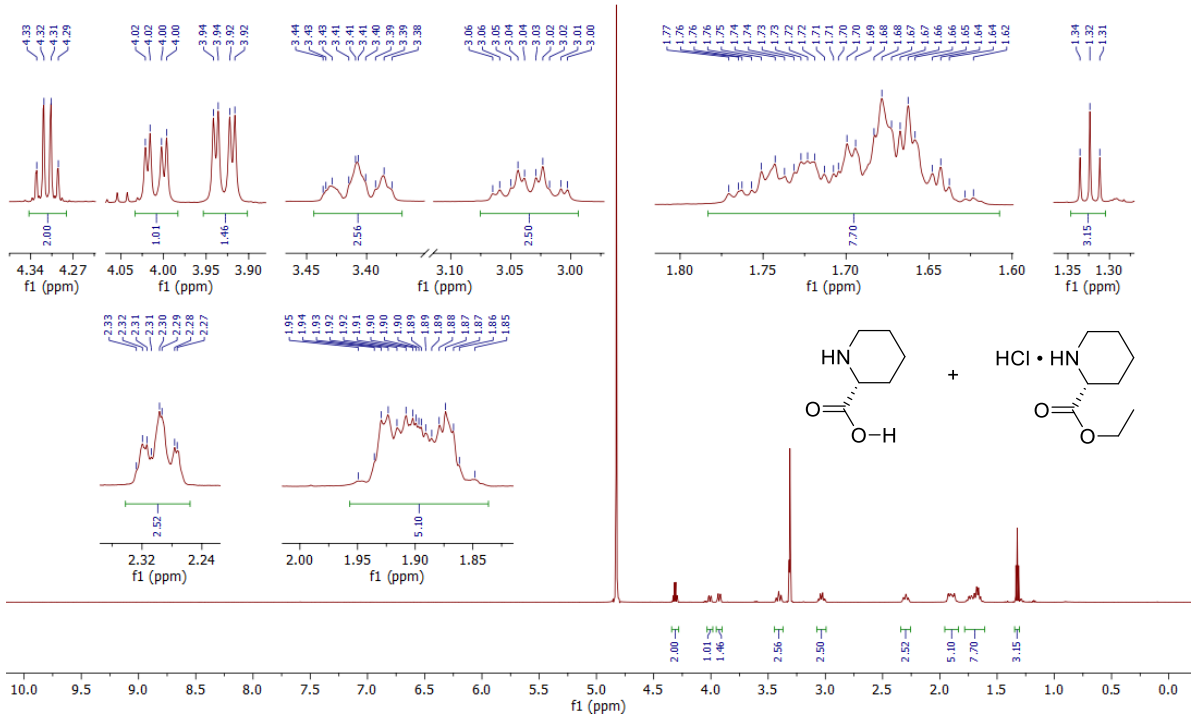
^{13}C-NMR (151 MHz, MeOD-d_4): δ 171.3 (C=O, C-7'), 159.5 (C=O, C-7), 63.7 (CH_2, C-8), 58.0 (CH, C-2 + C-2'), 45.2 + 45.0 (CH_2, C-6 + C-6'), 27.3 + 27.2 (CH_2, C-3 + C-3'), 22.9 (CH_2, C-5 + C-5'), 22.8 (CH_2, C-4 + C-4'), 14.3 (CH_3, C-9)

HR-MS (ESI, H_2O) *m/z*: Calculated for 158.1176 $[M - Cl]^+$; found 158.1179 for $C_8H_{16}NO_2$ (err: -0.1 ppm)

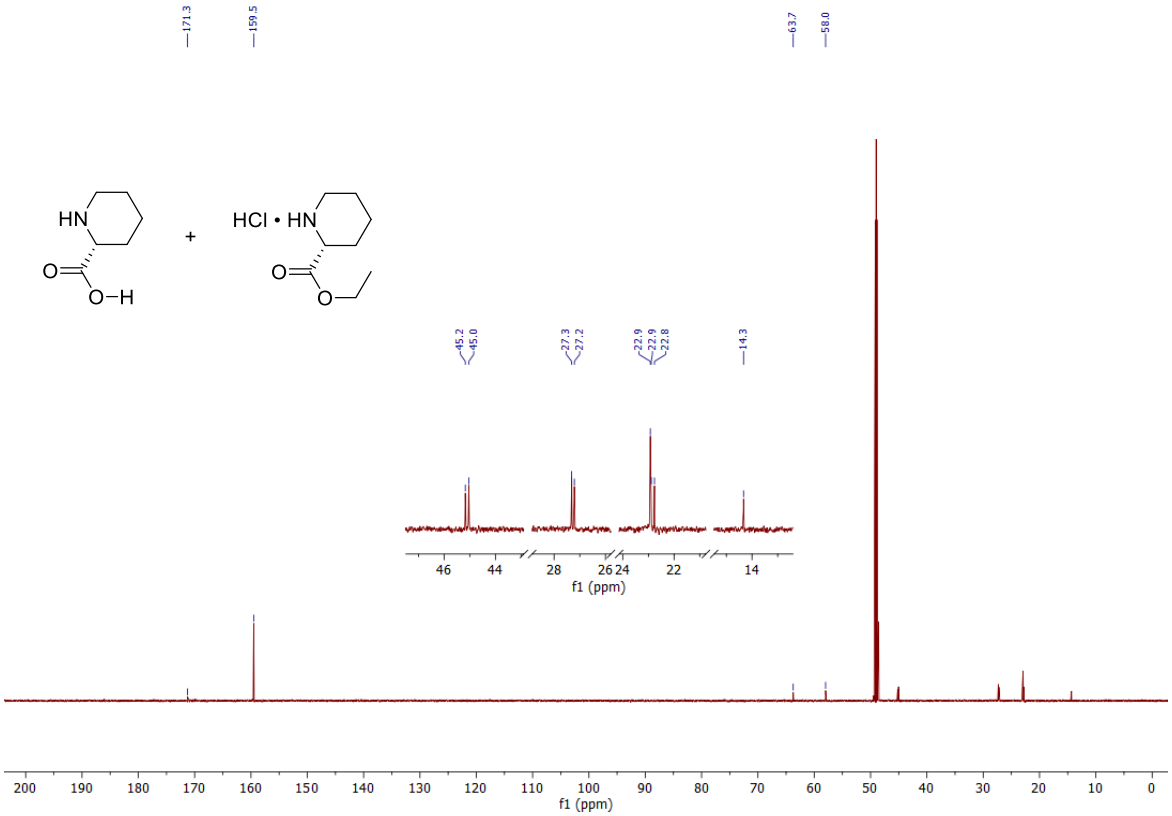
MS (ESI, H_2O) *m/z*: 158 $[M - Cl]^+$ (100%), 152 $[C_6H_{11}NO_2 + Na]^+$ (58%)

This compound is reported in literature.[51]

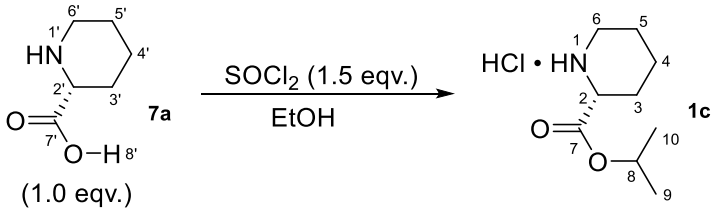
^{1}H NMR spectrum of the mixture of 1b and 7a (600 MHz, MeOD-d_4)



^{13}C NMR spectrum of the mixture of 1b and 7a (151 MHz, MeOD-d_4)



5.3.3 (*R*)-Isopropyl piperidine-2-carboxylate hydrochloride (1c)



Compound **1c** was synthesized according to **Procedure 5.3**, using *i*-PrOH (50 mL), $SOCl_2$ (0.80 mL, 11 mmol, 1.4 eqv.) and **7a** (1.0035 g, 7.771 mmol, 1.0 eqv.). The solution was stirred at 70˚C using a heating block for 20 h 15 min, followed by evaporation of the solvent under reduced pressure. The crude mixture was washed with 3 x 20 mL EtOAc, which was evaporated *in vacuo* between each adding. Next, 20 mL EtOAc was added, the content stirred vigorously a couple of minutes followed by removal of the solvent using a pipette. This was repeated two more times. Evaporation under reduced pressure resulted in a mixture of **1c** and **7a** as a brown syrup.

Yield: Not possible to measure

^{1}H-NMR (400 MHz, MeOD-d_4): δ 5.13 (sept, *J* = 6.3 Hz, 1H, H-8), 3.98-3.92 (m, 2H, H-2 + H-2'), 3.42-3.38 (m, 2H, H-6 + H-6'), 3.06-3.00 (m, 2H, H-6 + H-6'), 2.33-2.25 (m, 2H, H-3 + H-3'), 1.93-1.84 (m, 4H, H-4 + H-4'), 1.79-1.63 (m, 6H, H-3 + H-3' + H-5 + H-5'), 1.33-1.28 (m, 6H, H-9 + H-10)

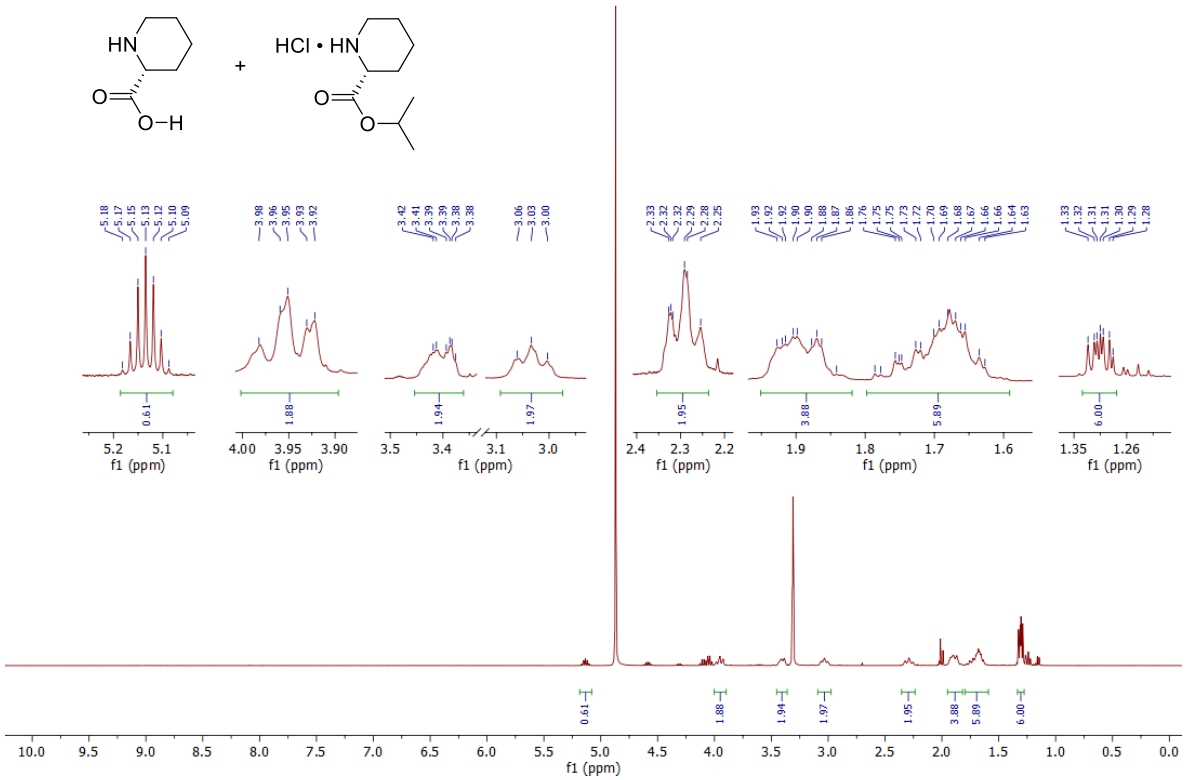
^{13}C-NMR (100 MHz, MeOD-d_4): δ 171.2 (C=O, C-7'), 169.5 (C=O, C-7), 72.0 (CH, C-8), 58.0 + 57.8 (CH, C-2 + C-2'), 45.2 + 45.0 (CH_2, C-6 + C-6'), 27.3 + 27.2 (CH_2, C-3 + C-3'), 22.9 + 22.8 (CH_2, C-5 + C-5'), 21.8 (CH_2, C-4 + C-4'), 18.4 + 15.4 (CH_3, C-9 + C-10)

HR-MS (ESI, H_2O) *m/z*: Calculated for 172.1331 $[M - Cl]^+$; found 172.1332 for $C_9H_{18}NO_2$ (err: 0.4 ppm)

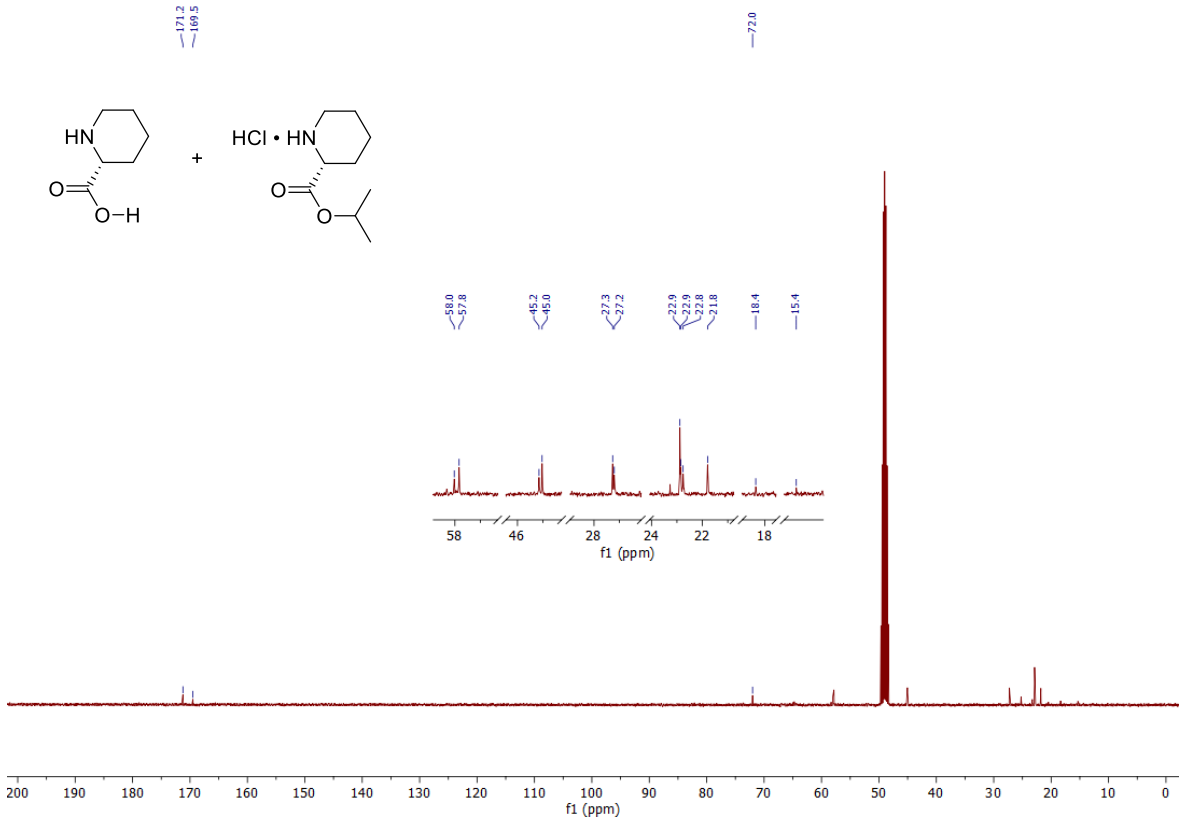
MS (ESI, H_2O) *m/z*: 130 [**7a** + H]$^+$ (100%), 172 $[M - Cl]^+$ (51%)

This compound is not reported in literature.

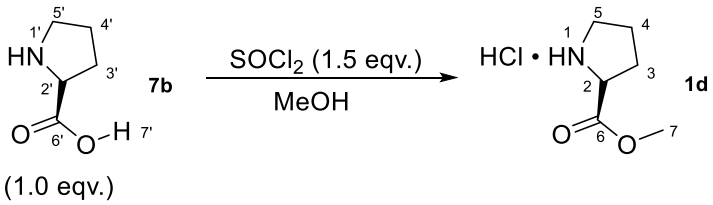
^{1}H NMR spectrum of the mixture of 1c and 7a (400 MHz, MeOD-d_4)



^{13}C NMR spectrum of the mixture of 1c and 7a (100 MHz, MeOD-d_4)



5.3.4 (*S*)-Methyl pyrrolidine-2-carboxylate hydrochloride (1d)



Compound **1d** was synthesized according to **Procedure 5.3**, using MeOH (50 mL), $SOCl_2$ (0.95 mL, 13 mmol, 1.5 eqv.) and **7b** (1.0017 g, 8.702 mmol, 1.0 eqv.). The solution was stirred at ambient temperature for 19 h 15 min, followed by evaporation of the solvent under reduced pressure. The crude mixture was washed with 3 x 20 mL EtOAc, which was evaporated *in vacuo* between each adding, followed by further evaporation under reduced pressure. This gave a mixture of **1d** and **7b** (1.4560 g in total) as a white, wax-like oil.

Yield: ~90% (Assumption based on NMR, see **Section 3.1.1** for details)

^{1}H-NMR (400 MHz, MeOD-d_4): δ 4.47-4.37 (m, 1H, H-2), 3.86 (s, 3H, H-7), 3.45-3.33 (m, 2H, H-5), 2.48-2.39 (m, 1H, H-3), 2.19-1.99 (m, 3H, H-3 + H-4)

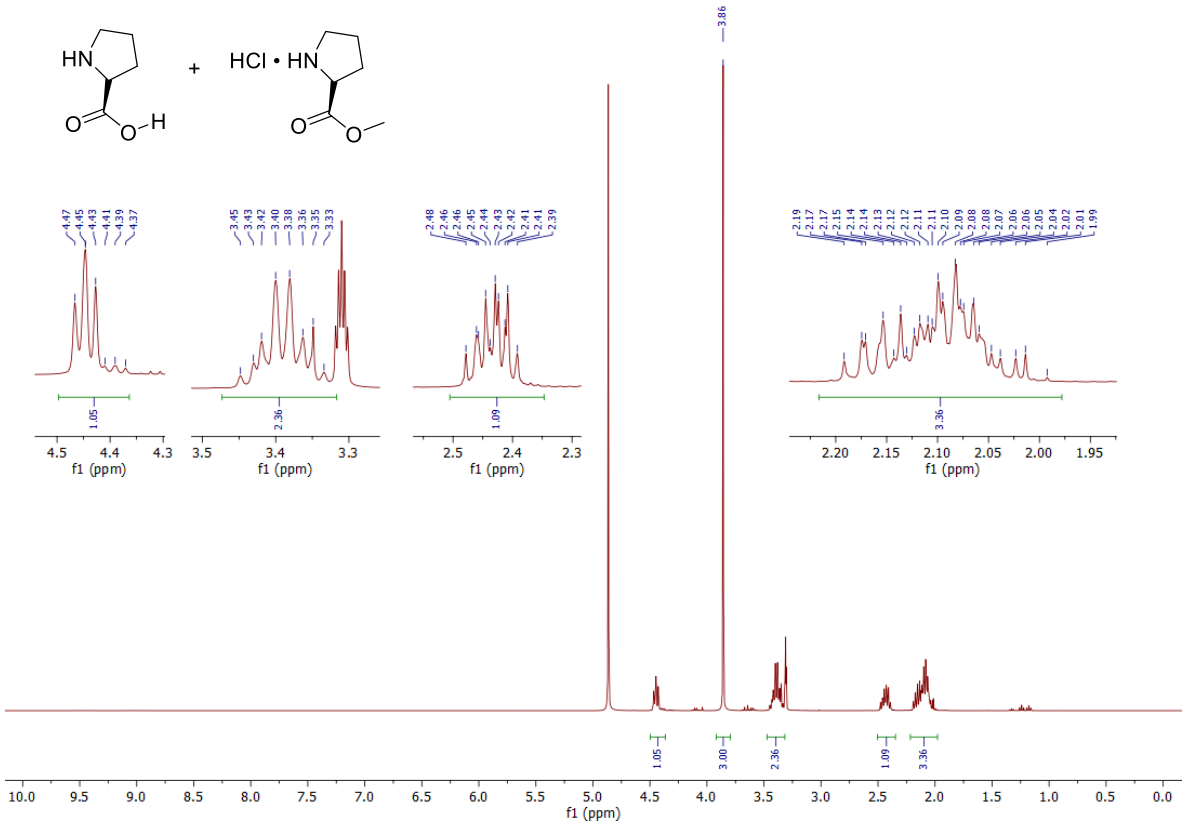
^{13}C-NMR (100 MHz, MeOD-d_4): δ 170.5 (C=O, C-6), 60.7 (CH, C-2), 53.9 (CH_3, C-7), 47.2 (CH_2, C-5), 29.3 (CH_2, C-3), 24.5 (CH_2, C-4)

HR-MS (ESI, H_2O) *m/z*: Calculated for 130.0878 $[M - Cl]^+$; found 130.0863 for $C_6H_{12}NO_2$ (err: -12.0 ppm)

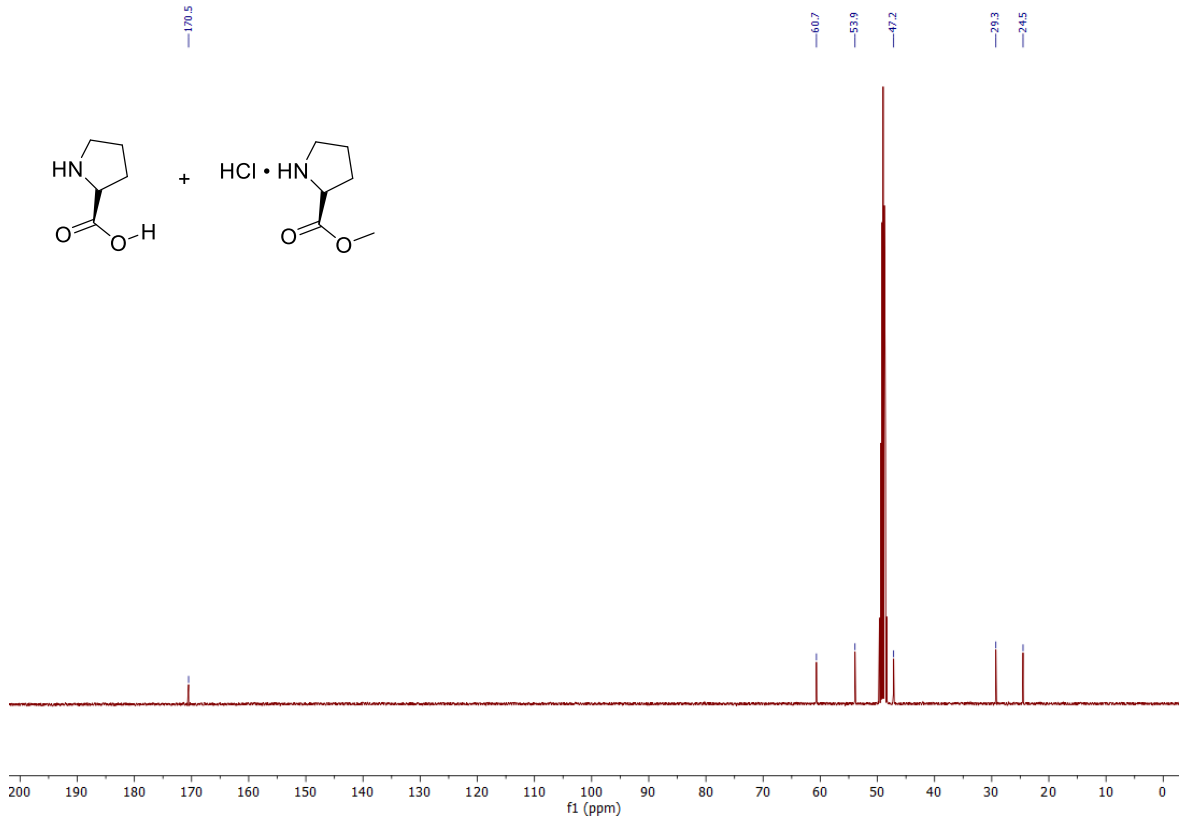
MS (ESI, H_2O) *m/z*: 130 $[M - Cl]^+$ (100%), 152 $[M - HCl + Na]^+$ (19%)

This compound is reported in literature.[52]

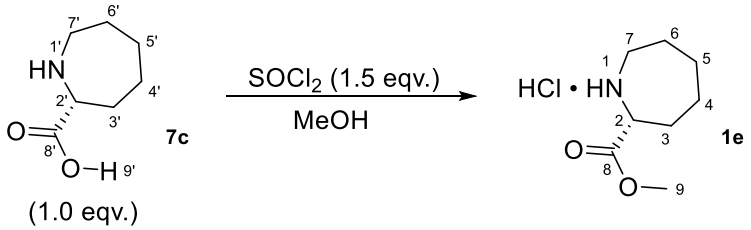
^{1}H NMR spectrum of the mixture of 1d and 7b (400 MHz, MeOD-d_4)



^{13}C NMR spectrum of the mixture of 1d and 7b (100 MHz, MeOD-d_4)



5.3.5 (*R*)-Methyl azepane-2-carboxylate hydrochloride (1e)



Compound **1e** was synthesized according to **Procedure 5.3**, using MeOH (25 mL), $SOCl_2$ (0.15 mL, 2.1 mmol, 1.5 eqv.) and **7c** (201.5 mg, 1.41 mmol, 1.0 eqv.). The solution was stirred at ambient temperature for 20 h 50 min, followed by evaporation of the solvent under reduced pressure. The procedure was repeated, but the stirring was done at 45˚C using a heating block. After 13 h 55 min, the solvent was evaporated under reduced pressure, followed by washing of the crude mixture using 3 x 10 mL EtOAc, evaporating the solvent *in vacuo* between each adding. This resulted in a mixture of **1e** and **7c** (265.9 mg in total) as an orange salt.

Yield: 65% (177 mg, 0.91 mmol) (Calculated from NMR)

^{1}H-NMR (400 MHz, MeOD-d_4): δ 4.20 (dd, *J* = 9.8 Hz, 3.7 Hz, 1H, H-2), 4.12 (dd, *J* = 9.7 Hz, 3.7 Hz, 1H, H-2'), 3.85 (s, 3H, H-9), 3.38-3.33 (m, 2H, H-7 + H-7'), 3.28-3.20 (m, 2H, H-7 + H-7'), 2.40-2.31 (m, 2H, H-3 + H-3'), 2.03-1.63 (m, 13H, H-3 + H-3' + H-4 + H-4' + H-5 + H-5' + H-6 + H-6')

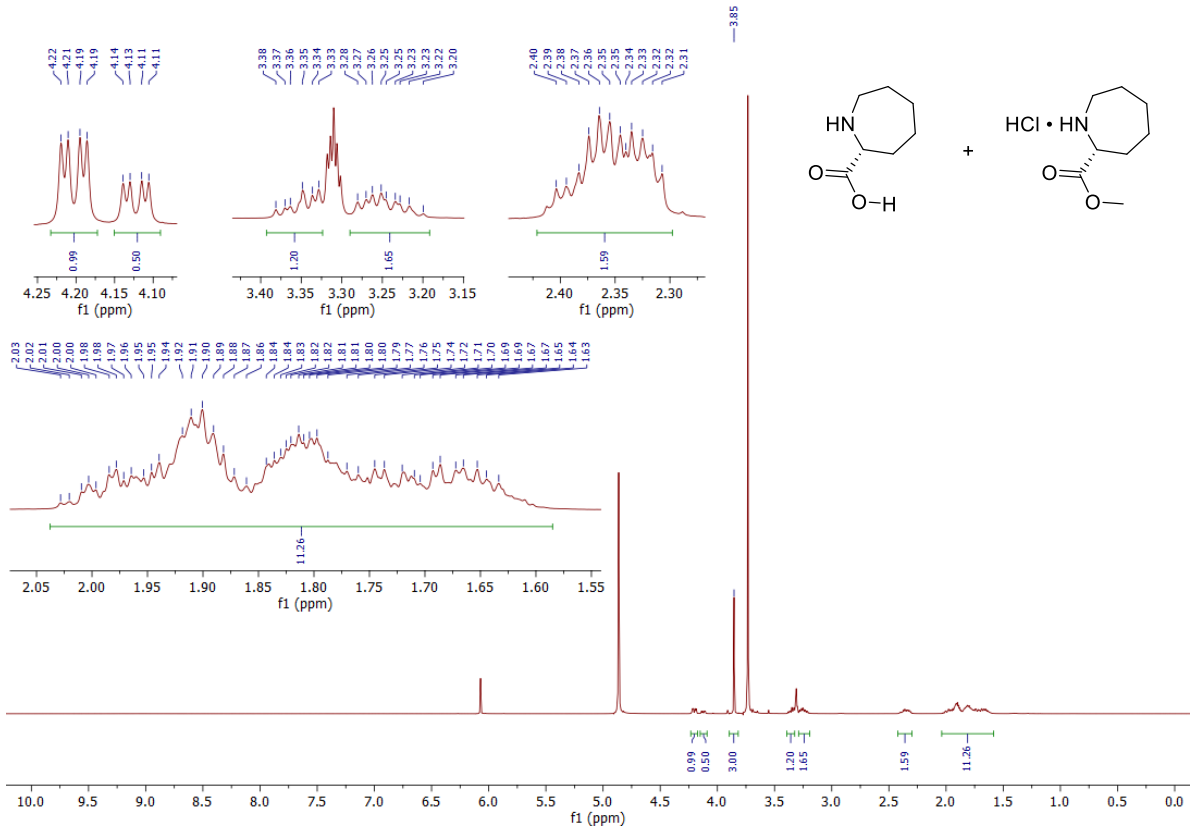
^{13}C-NMR (151 MHz, MeOD-d_4): δ 172.0 (C=O, C-8'), 171.2 (C=O, C-8), 60.5 (CH, C-2 + C-2'), 53.9 (CH_3, C-9), 46.9 + 46.8 (CH_2, C-7 + C-7'), 29.7 (CH_2, C-3 + C-3'), 27.3 + 27.2 (CH_2, C-6 + C-6'), 26.1 + 26.0 (CH_2, C-5 + C-5'), 25.9 (CH_2, C-4 + C-4')

HR-MS (ESI, H_2O) *m/z*: Calculated for 158.1175 $[M - Cl]^+$; found 158.1176 for $C_8H_{16}NO_2$ (err: 0.1 ppm)

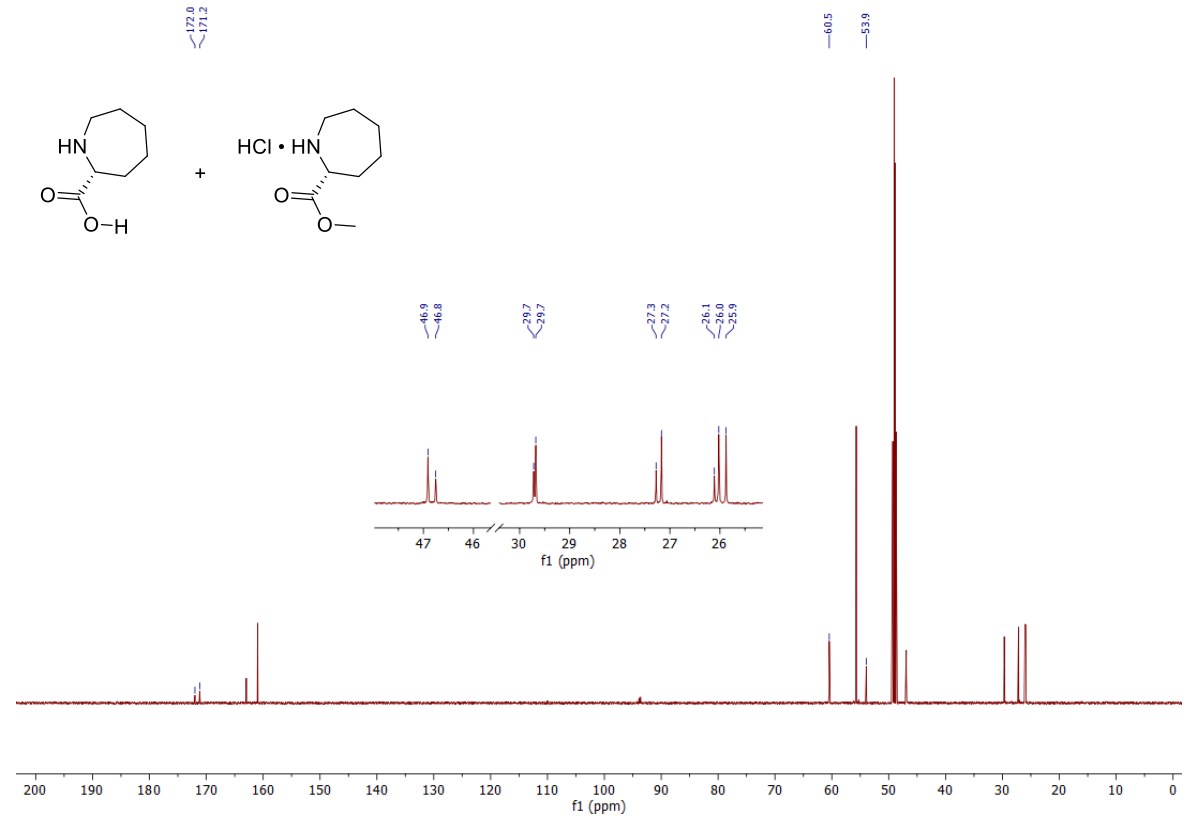
MS (ESI, H_2O) *m/z*: 158 $[M - Cl]^+$ (100%), 166 $[C_7H_{13}NO_2 + Na]^+$ (24%)

This compound is not reported in literature.

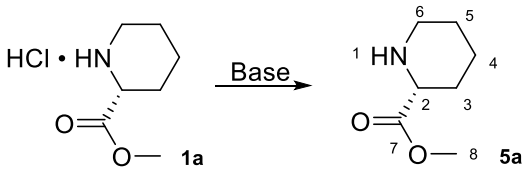
^{1}H NMR spectrum of the mixture of 1e and 7c (400 MHz, MeOD-d_4)



^{13}C NMR spectrum of the mixture of 1e and 7c (151 MHz, MeOD-d_4)



5.4 Desalting to get methyl (*R*)-piperidine-2-carboxylate (5a)



The following section describes several methods for the desalting of **1a** to get **5a**.

Method I

A mixture of **1a** and **7a** (100.8 mg in total), of which 38% was **1a** (38 mg, 0.21 mmol, 1.0 eqv.), was solved in type 2 water (2 mL) and transferred to a separatory funnel. After adding DCM (6 mL), the water phase was adjusted to pH 12 using a 1M K_3PO_4-solution (pH 13), mixing well between each adding. The phases were extracted, followed by washing of the water phase with 4 x 6 mL DCM and 5 x 10 mL DCM. The organic phases were collected, dried using $MgSO_4$, filtrated and evaporated to dryness under reduced pressure, giving **5a** as a colorless oil.

Yield: 79% (24.0 mg, 0.17 mmol)

Method II

A mixture of **1a** and **7a** (99.2 mg in total), of which 38% was **1a** (37 mg, 0.21 mmol, 1.0 eqv.), was dissolved in type 2 water (1 mL) and transferred to a separatory funnel. After adding saturated K_3SO_4 (2 mL) and DCM (6 mL), the phases were extracted and the water phase washed with 4 x 6 mL DCM. The collected organic phases were dried using Na_2SO_4, filtrated and evaporated to dryness under reduced pressure, giving **5a** as a colorless oil.

Yield: 21% (6.2 mg, 0.04 mmol)

Method III

A mixture of **1a** and **7a** (99.0 mg in total), of which 38% was **1a** (37 mg, 0.21 mmol, 1.0 eqv.), was transferred to a reaction flask. Addition of DBU (83 µL, 0.56 mmol, 2.8 eqv.) made **1a** dissolve, but not everything. The mixture stirred for 5 min before it was transferred to a separatory funnel and washed with 5 x 10 mL type 2 water. After the organic phase was dried using $MgSO_4$, it was filtrated and concentrated *in vacuo*. The NMR spectrum showed no traces of **5a**.

Yield: 0%

Method IV

This method was inspired by a procedure described by Jamison *et al*.[47] A mixture of **1a** and **7a** (198.3 mg in total), of which 38% was **1a** (75 mg, 0.42 mmol, 1.0 eqv.), was solved in type 2 water (2 mL) and transferred to a separatory funnel. After adding DCM (6 mL), solid $NaHCO_3$ was added in portions until saturation of the water phase, stirring the phases between each adding. The phases were extracted, followed by washing of the water phase using 9 x 6 mL DCM. The organic phase was dried using $MgSO_4$, filtrated and evaporated to dryness under reduced pressure, giving **5a** as a colorless oil.

Yield: 89% (53.4 mg, 0.37 mmol)

^{1}H-NMR (600 MHz, $CDCl_3$): δ 3.68 (s, 3H, H-8), 3.33 (dd, *J* = 10.3 Hz, 3.3 Hz, 1H, H-2), 3.04 (dt, *J* = 11.7 Hz, 3.8 Hz, 1H, H-6), 2.65-2.61 (m, 1H, H-6), 1.93-1.91 (m, 2H, H-3 + H-1), 1.77-1.73 (m, 1H, H-4), 1.55-1.47 (m, 2H, H-3 + H-5), 1.45-1.37 (m, 2H, H-4 + H-5)

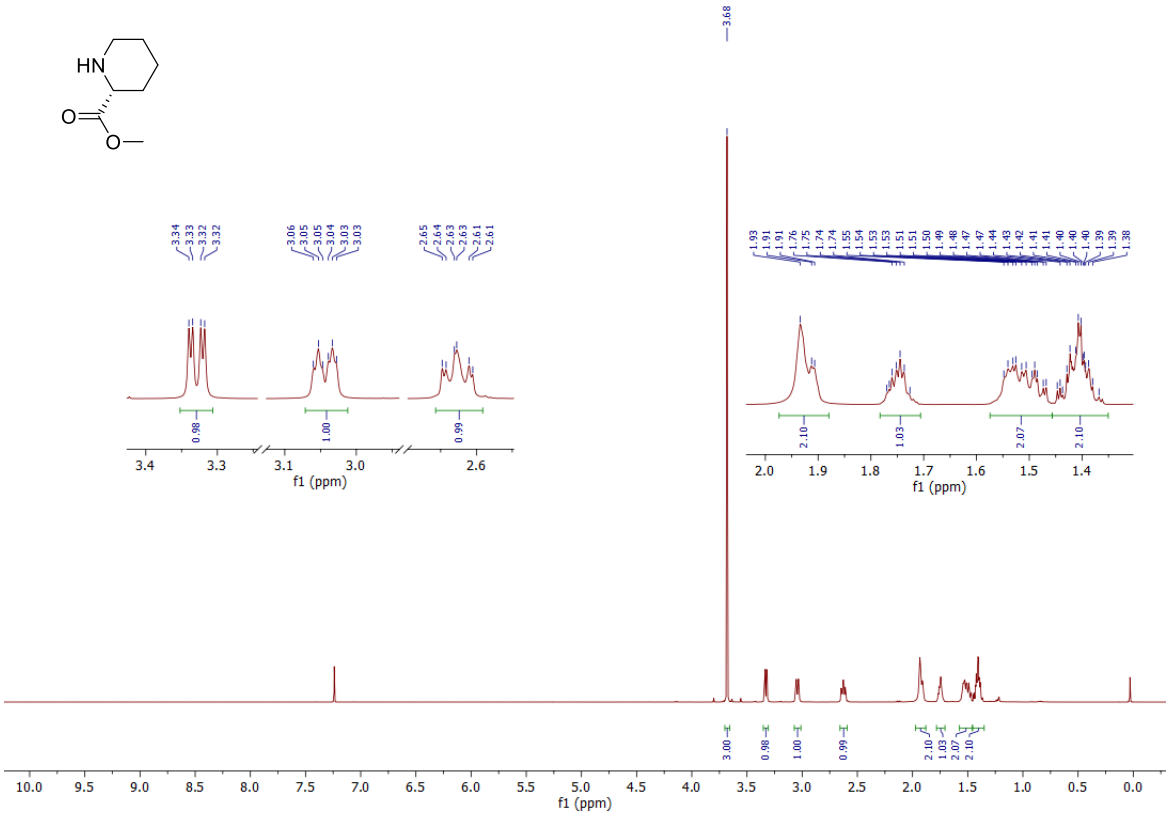
^{13}C-NMR (151 MHz, $CDCl_3$): δ 174.0 (C=O, C-7), 58.6 (CH, C-2), 51.8 (CH_3, C-8), 45.6 (CH_2, C-6), 29.2 (CH_2, C-3), 25.9 (CH_2, C-5), 24.1 (CH_2, C-4)

HR-MS (ESI, ACN) *m/z*: Calculated for 144.1018 $[M + H]^+$; found 144.1019 for $C_7H_{14}NO_2$ (err: 1.0 ppm)

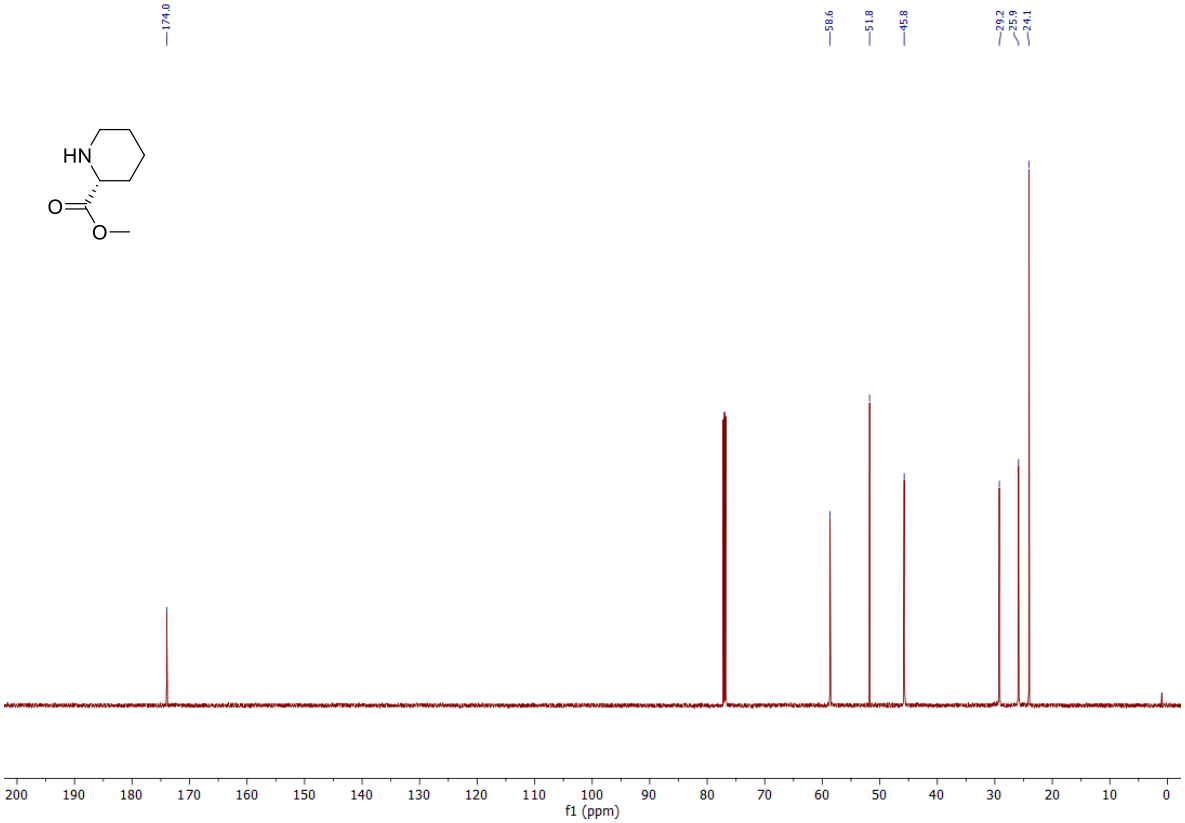
MS (ESI, ACN) *m/z*: 144 $[M + H]^+$ (100%)

This compound is reported in literature.[53]

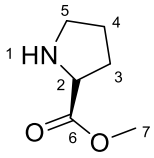
^{1}H NMR spectrum of 5a (600 MHz, $CDCl_3$)



^{13}C NMR spectrum of 5a (151 MHz, $CDCl_3$)



5.5 Desalting to get methyl D-prolinate (5b)



This method was inspired by a procedure described by Jamison *et al*.[47] A mixture of **1d** and **7b** (188.2 mg in total), of which ~90% was **1d** (169 mg, 1.03 mmol, 1.0 eqv.), was solved in type 2 water (2 mL) and transferred to a separatory funnel. DCM (6 mL) was added, followed by addition of solid $NaHCO_3$ in portions until saturation of the water phase. The phases were stirred between each portion. After extraction of the phases, the water phase was washed with 9 x 6 mL DCM. The collected organic phases were dried with $MgSO_4$, filtrated and concentrated *in vacuo*, giving **5b** as a colorless oil.

Yield: 55% (73.2 mg, 0.57 mmol)

^{1}H-NMR (600 MHz, $CDCl_3$): δ 3.64 (dd, *J* = 8.7 Hz, 5.8 Hz, 1H, H-2), 3.59 (s, 3H, H-7), 2.96-2.92 (m, 1H, H-5), 2.80-2.76 (m, 1H, H-5), 2.36 (s, 1H, H-1), 2.02-1.96 (m, 1H, H-3), 1.75-1.69 (m, 1H, H-3), 1.66-1.59 (m, 2H, H-4)

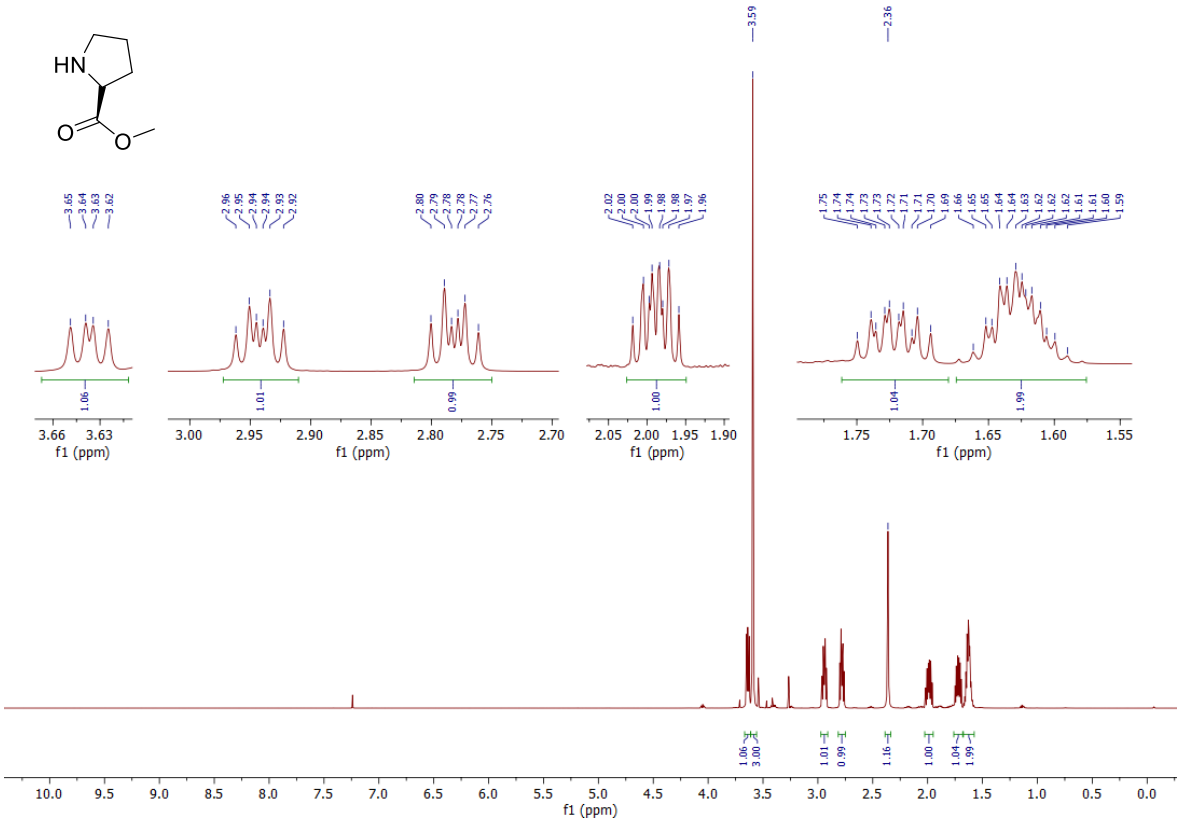
^{13}C-NMR (151 MHz, $CDCl_3$): δ 175.6 (C=O, C-6), 59.4 (CH, C-2), 51.7 (CH_3, C-7), 46.7 (CH_2, C-5), 29.9 (CH_2, C-3), 25.2 (CH_2, C-4)

HR-MS (ESI, ACN) *m/z*: Calculated for 130.0859 $[M + H]^+$; found 130.0863 for $C_6H_{12}NO_2$ (err: 2.6 ppm)

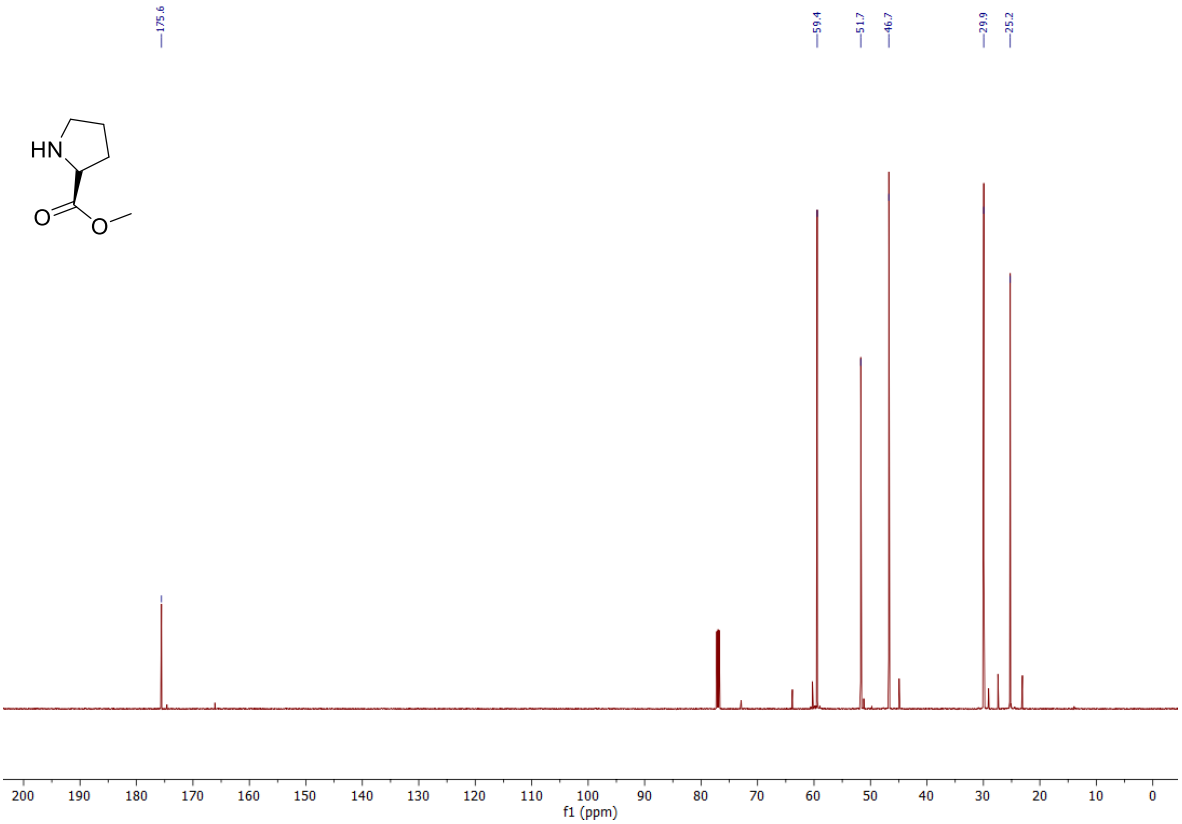
MS (ESI, ACN) *m/z*: 130 $[M + H]^+$ (100%), 91 (26%)

This compound is reported in literature.[54]

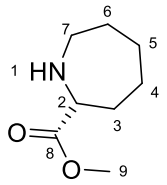
^{1}H NMR spectrum of 5b (600 MHz, $CDCl_3$)



^{13}C NMR spectrum of 5b (151 MHz, $CDCl_3$)



5.6 Desalting to get methyl (*R*)-azepane-2-carboxylate (5c)



This method was inspired by a procedure described by Jamison *et al*.[47] A mixture of **1e** and **7c** (149.2 mg in total), of which 66% was **1e** (99 mg, 0.51 mmol, 1.0 eqv.), was solved in type 2 water (2 mL) and transferred to a separatory funnel. DCM (6 mL) was added, followed by addition of solid $NaHCO_3$ in portions until saturation of the water phase. The phases were mixed well between each portion. After extraction of the phases, the water phase was washed with 9 x 6 mL DCM. The collected organic phases were dried with $MgSO_4$, filtrated and concentrated *in vacuo*, giving **5c** as a yellow oil.

Yield: 59% (47.3 mg, 0.30 mmol)

^{1}H-NMR (600 MHz, $CDCl_3$): δ 3.65 (s, 3H, H-9), 3.47 (dd, *J* = 9.3 Hz, 4.9 Hz, 1H, H-2), 3.00-2.96 (m, 1H, H-7), 2.70-2.65 (m, 1H, H-7), 2.43 (bs, 1H, H-1), 2.05-2.00 (m, 1H, H-3), 1.70-1.62 (m, 2H, H-3 + H-4), 1.60-1.49 (m, 5H, H-4 + H-5 + H-6)

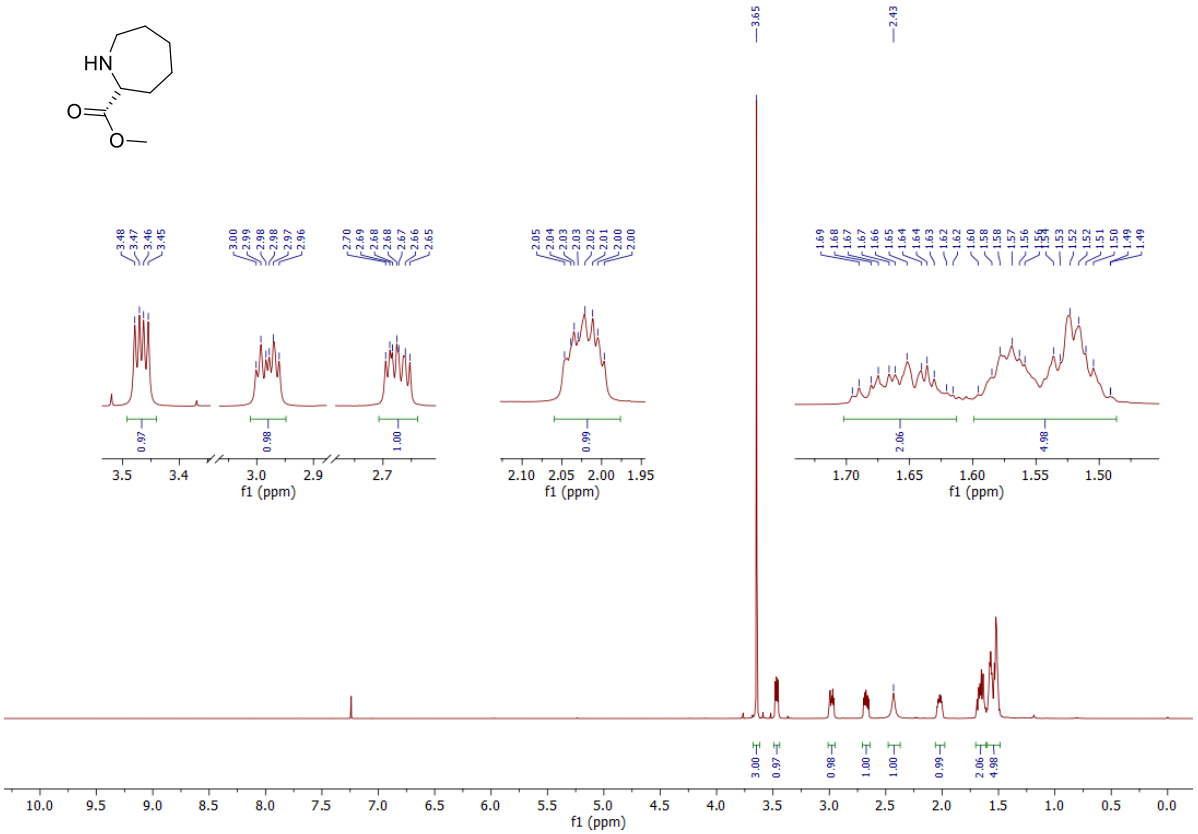
^{13}C-NMR (151 MHz, $CDCl_3$): δ 175.2 (C=O, C-8), 60.6 (CH, C-2), 51.8 (CH_3, C-9), 46.6 (CH_2, C-7), 32.8 (CH_2, C-3), 31.6 (CH_2, C-6), 27.5 (CH_2, C-5), 25.4 (CH_2, C-4)

HR-MS (ESI, ACN) *m/z*: Calculated for 158.1176 $[M + H]^+$/ 180.0996 $[M + Na]^+$; found 158.1176 for $C_8H_{16}NO_2$ (err: -0.4 ppm)/ 180.0995 for $C_8H_{15}NO_2Na$ (err: -0.4 ppm)

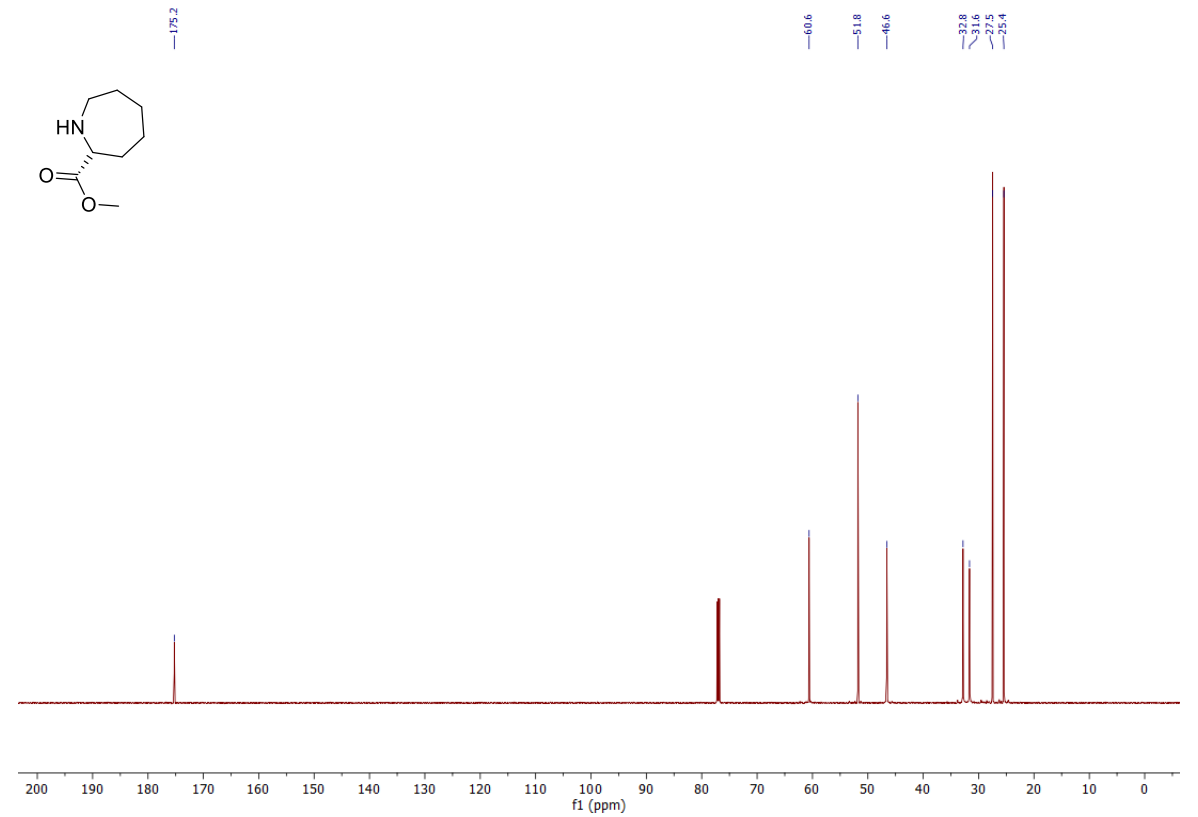
MS (ESI, ACN) *m/z*: 158 $[M + H]^+$ (100%), 180 $[M + Na]^+$ (48%)

This compound is not reported in literature.

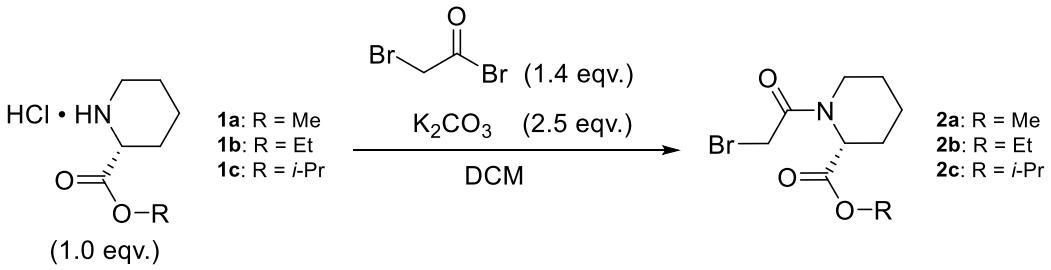
^{1}H NMR spectrum of 5c (600 MHz, $CDCl_3$)



^{13}C NMR spectrum of 5c (151 MHz, $CDCl_3$)

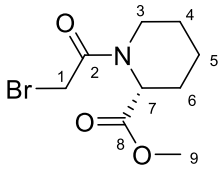


5.7 General procedure for the synthesis of “R”-(*R*)-1-(2-bromoacetyl)piperidine-2-carboxylate



A solution of **1a**, **1b** or **1c** (1.0 eqv.), K_2CO_3 (2.5 eqv.) and dry DCM was cooled down in an ice bath before adding 2-bromoacetyl bromide (1.4 eqv.) dropwise. The mixture was stirred at ambient temperature for 2-3 hours, followed by purification by extracting with sat. $NaHCO_3$-solution, sat. NaCl-solution and DCM. The organic phase was dried using $MgSO_4$, filtrated and evaporated *in vacuo*, giving the product **2a**, **2b** or **2c** of varying yield.

5.7.1 Methyl-(*R*)-1-(2-bromoacetyl)piperidine-2-carboxylate (2a)



Compound **2a** was synthesized according to **Procedure 5.7**, using commercially bought **1a** (396 mg, 2.20 mmol, 1.0 eqv.), K_2CO_3 (769 mg, 5.57 mmol, 2.5 eqv.), 2-bromoacetyl bromide (0.27 mL, 3.1 mmol, 1.4 eqv.) and dry DCM (10 mL). The reaction mixture stirred at ambient temperature for 3 h, resulting in a weak pink solution with white precipitate. The mixture was extracted with 2 x 10 mL sat. $NaHCO_3$-solution and 1 x 10 mL sat. NaCl-solution, followed by washing of the collected water phases with 2 x 10 mL DCM. The collected organic phases were washed with 1 x 20 mL NaCl-solution, dried using $MgSO_4$, filtrated and evaporated *in vacuo*, giving **2a** as a yellow oil.

Yield: 83% (483 mg, 1.83 mmol)

^{1}H-NMR (600 MHz, $CDCl_3$);

Mayor rotamer: δ 5.29 (d, *J* = 5.7 Hz, 1H, H-7), 3.93 (d, *J* = 10.7 Hz, 1H, H-1), 3.84 (d, *J* = 10.8 Hz, 1H, H-1), 3.77-3.75 (m, 1H, H-3), 3.71 (s, 3H, H-9), 3.30 (td, *J* = 13.2 Hz, 3.1 Hz, 1H, H-3), 2.29-2.22 (m, 1H, H-6), 1.74-1.70 (m, 2H, H-4 + H-5), 1.68-1.61 (m, 1H, H-6), 1.58-1.50 (m, 1H, H-4), 1.37-1.29 (m, 1H, H-5)
Minor rotamer: δ 4.61 (d, *J* = 5.5 Hz, 1H, H-7), 4.47 (d, *J* = 13.7 Hz, 1H, H-3), 3.93 (d, *J* = 10.7 Hz, 1H, H-1), 3.84 (d, *J* = 10.8 Hz, 1H, H-1), 3.71 (s, 3H, H-9), 2.70 (td, *J* = 13.1 Hz, 2.8 Hz, 1H, H-3), 2.29-2.22 (m, 1H, H-6), 1.74-1.70 (m, 2H, H-4 + H-5), 1.68-1.61 (m, 1H, H-6), 1.58-1.50 (m, 1H, H-4), 1.37-1.29 (m, 1H, H-5)

^{13}C-NMR (151 MHz, $CDCl_3$);

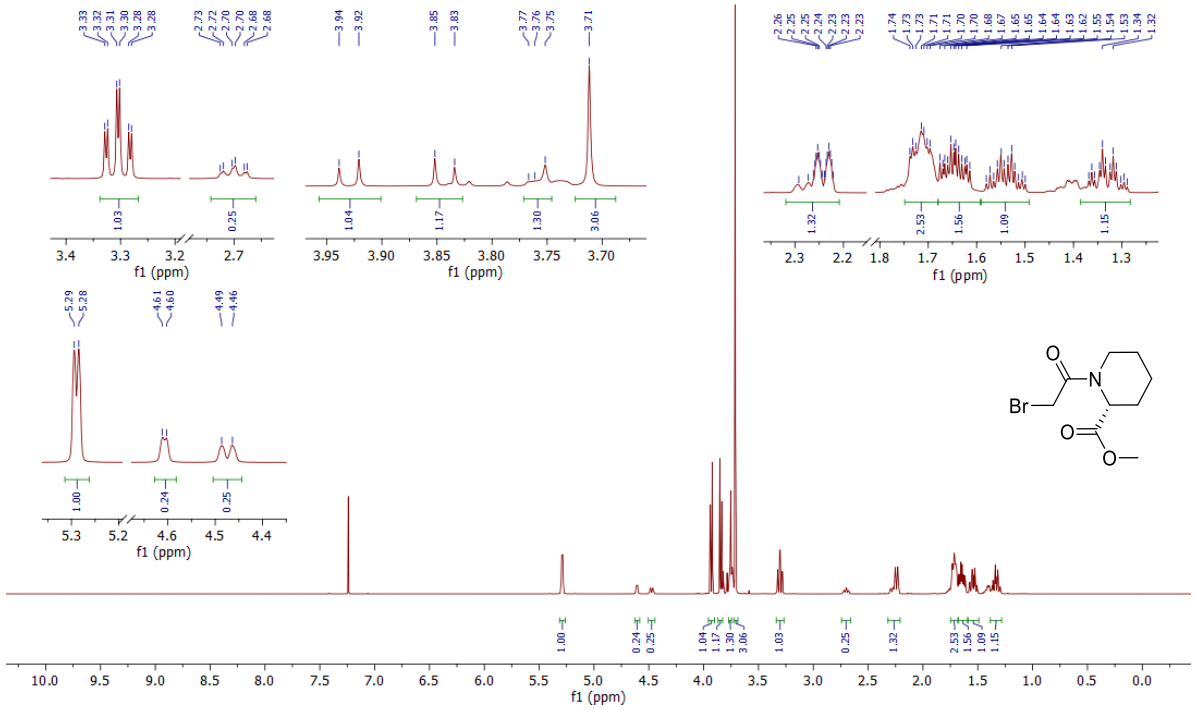
Mayor rotamer: δ 171.3 (C=O, C-8), 166.7 (C=O, C-2), 52.3 (CH, C-7), 52.3 (CH_3, C-9), 44.7 (CH_2, C-3), 26.4 (CH_2, C-6), 25.9 (CH_2, C-1), 25.0 (CH_2, C-4), 20.7 (CH_2, C-5)
Minor rotamer: δ 170.8 (C=O, C-8), 166.4 (C=O, C-2), 57.1 (CH, C-7), 52.3 (CH_3, C-9), 40.0 (CH_2, C-3), 27.0 (CH_2, C-6), 25.9 (CH_2, C-1), 24.3 (CH_2, C-4), 20.6 (CH_2, C-5)

HR-MS (ESI, MeOH) *m/z*: Calculated for 286.0049 $[M + Na]^+$; found 286.0049 for $C_9H_{14}O_3N^{79}BrNa$ (err: 0.2 ppm)

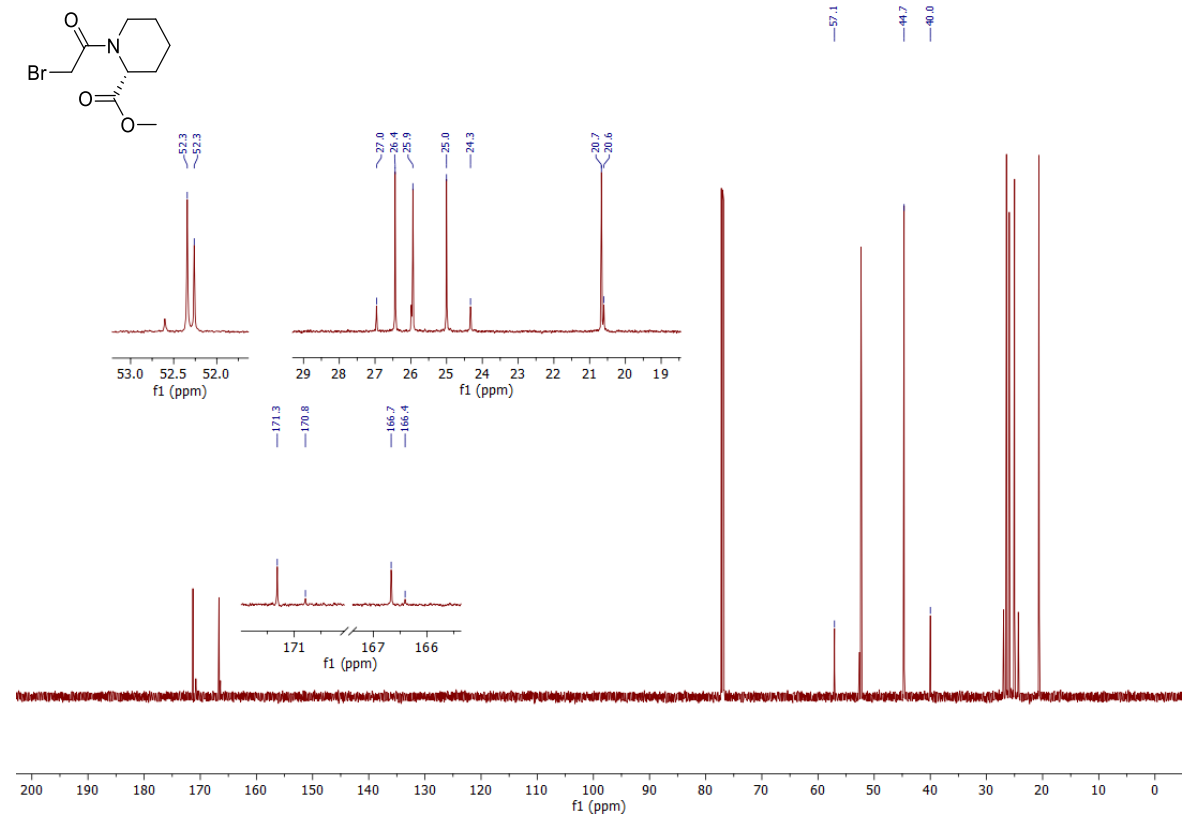
MS (ESI, MeOH) *m/z*: 286 $[M + Na]^+$ (100%), 288 $[M + Na]^+$ (94%)

This compound has previously been reported.[38]

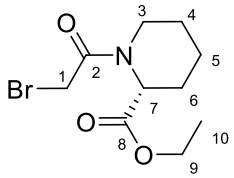
^{1}H NMR spectrum of 2a (600 MHz, $CDCl_3$)



^{13}C NMR spectrum of 2a (151 MHz, $CDCl_3$)



5.7.2 Ethyl-(R)-1-(2-bromoacetyl)piperidine-2-carboxylate (2b)



Compound **2b** was synthesizes according to **Procedure 5.7**. A solution of K_2CO_3 (956.3 mg, 6.921 mmol, 6.1 eqv.), 2-bromoacetyl bromide (0.34 mL, 3.9 mmol, 3.5 eqv.), a mixture of **1b** and **7a** (534.4 mg in total) of which 41% was **1b** (219 mg, 1.13 mmol, 1.0 eqv.), and dry DCM (10 mL) was stirred at ambient temperature for 2 h 30 min. The mixture was extracted with 3 x 10 mL sat. $NaHCO_3$-solution and 2 x 10 mL sat. NaCl-solution, followed by washing of the collected water phases with 3 x 10 mL DCM. The collected organic phases were washed with 2 x 20 mL sat. NaCl-solution, dried with $MgSO_4$, filtrated and the solvent evaporated *in vacuo*, giving **2b** as a yellow oil.

Yield: 87% (274.5 mg, 0.987 mmol)

^{1}H-NMR (600 MHz, $CDCl_3$); Mayor rotamer: δ 5.27 (d, *J* = 5.5 Hz, 1H, H-7), 4.22-4.16 (m, 2H, H-9), 3.93 (d, *J* = 10.7 Hz, 1H, H-1), 3.85 (d, *J* = 10.7 Hz, 1H, H-1), 3.75 (bd, *J* = 13.4 Hz, 1H, H-3), 3.33 (td, *J* = 13.2 Hz, 3.1 Hz, 1H, H-3), 2.27-2.24 (m, 1H, H-6), 1.74-1.71 (m, 2H, H-4 + H-5), 1.68-1.53 (m, 2H, H-4 + H-6), 1.35-1.33 (m, 1H, H-5), 1.29-1.24 (m, 3H, H-10)
Minor rotamer: δ 4.58 (d, *J* = 4.3 Hz, 1H, H-7), 4.48 (d, *J* = 13.9 Hz, 1H, H-3), 4.22-4.16 (m, 2H, H-9), 3.93 (d, *J* = 10.7 Hz, 1H, H-1), 3.85 (d, *J* = 10.7 Hz, 1H, H-1), 2.72 (td, *J* = 13.1 Hz, 3.5 Hz, 1H, H-3), 2.27-2.24 (m, 1H, H-6), 1.74-1.71 (m, 2H, H-4 + H-5), 1.68-1.53 (m, 2H, H-4 + H-6), 1.35-1.33 (m, 1H, H-5), 1.29-1.24 (m, 3H, H-10)

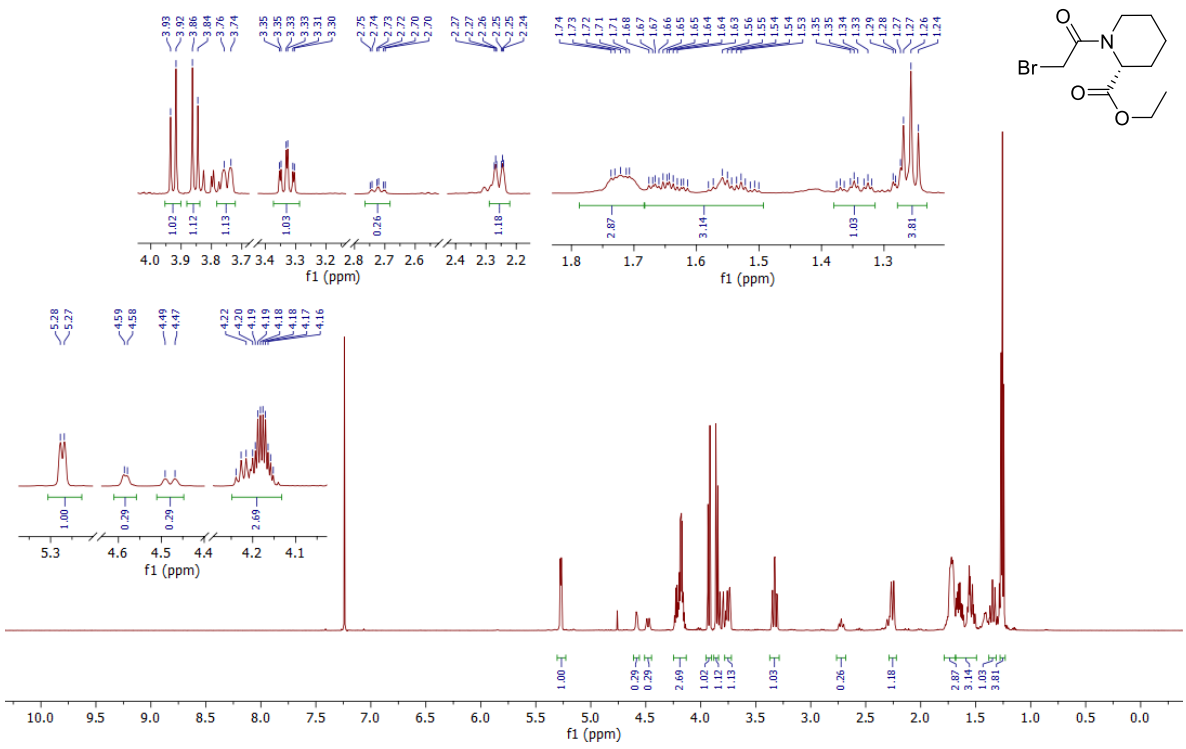
^{13}C-NMR (151 MHz, $CDCl_3$); Mayor rotamer: δ 170.8 (C=O, C-8), 166.6 (C=O, C-2), 61.3 (CH_2, C-9), 52.4 (CH, C-7), 44.7 (CH_2, C-3), 26.5 (CH_2, C-6), 26.0 (CH_2, C-1), 25.1 (CH_2, C-4), 20.7 (CH_2, C-5), 14.2 (CH_3, C-10).
Minor rotamer: δ 170.3 (C=O, C-8), 166.5 (C=O, C-2), 61.7 (CH_2, C-9), 57.1 (CH, C-7), 40.0 (CH_2, C-3), 27.0 (CH_2, C-6), 26.0 (CH_2, C-1), 24.4 (CH_2, C-4), 20.6 (CH_2, C-5), 14.2 (CH_3, C-10)

HR-MS (ESI, ACN) *m/z*: Calculated for 300.0204 $[M + Na]^+$; found 300.0206 for $C_{10}H_{16}O_3N^{79}BrNa$ (err: 0.4 ppm)

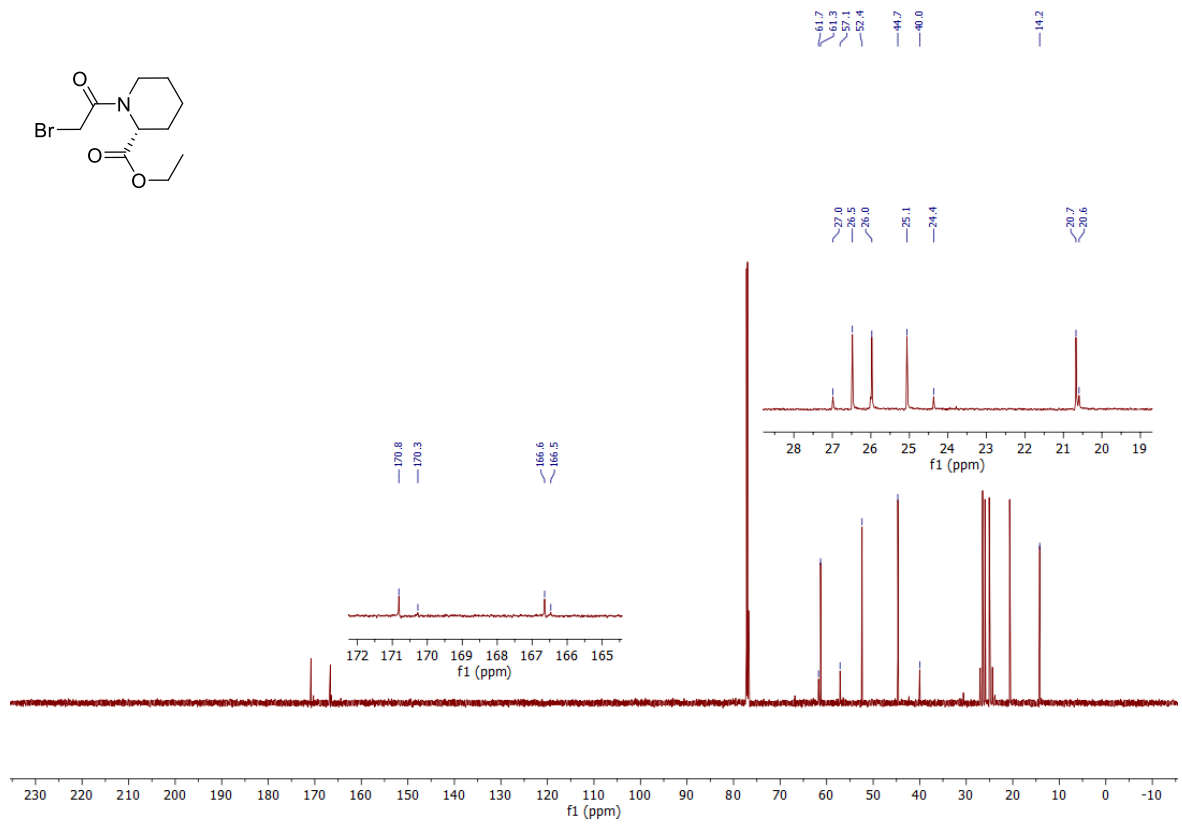
MS (ESI, ACN) *m/z*: 300 $[M + Na]^+$ (100%), 302 $[M + Na]^+$ (96%)

This compound is not reported in literature.

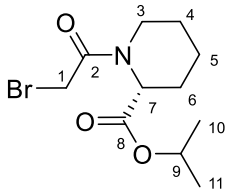
^{1}H NMR spectrum of 2b (600 MHz, $CDCl_3$)



^{13}C NMR spectrum of 2b (151 MHz, $CDCl_3$)



5.7.3 Isopropyl-(R)-1-(2-bromoacetyl)piperidine-2-carboxylate (2c)



Compound **2c** was synthesized according to **Procedure 5.7**, using a mixture of **1c** and **7a** (1.2728 g in total), K_2CO_3 (2.1147 g, 15,30 mmol.), 2-bromoacetyl bromide (0.75 mL, 8.6 mmol) and dry DCM (25 mL). The reaction mixture stirred at ambient temperature for 3 h 10 min. After this, it was extracted with 2 x 25 mL sat. $NaHCO_3$-solution and 1 x 25 mL sat. NaCl-solution, followed by washing of the collected water phases with 2 x 25 mL DCM. The organic phases were collected, washed with 1 x 50 mL sat. NaCl-solution, dried using $MgSO_4$, filtrated and evaporated *in vacuo*, giving **2c** as a yellow oil.

Yield: 17% (380 mg, 1.30 mmol) (Calculated over two steps from **7a**, **Section 5.3.3**)

^{1}H-NMR (600 MHz, $CDCl_3$); Mayor rotamer: δ 5.23 (d, *J* = 5.5 Hz, 1H, H-7), 5.05 (m, 1H, H-9), 3.91 (d, *J* = 10.7 Hz, 1H, H-1), 3.85 (d, *J* = 10.7 Hz, 1H, H-1), 3.74 (bd, *J* = 14.6 Hz, 1H, H-3), 3.33 (td, *J* = 13.2 Hz, 3.0 Hz, 1H, H-3), 2.26-2.23 (m, 1H, H-6), 1.73-1.70 (m, 2H, H-4 + H-5), 1.66-1.52 (m, 2H, H-4 + H-6), 1.33-1.31 (m, 1H, H-5), 1.26-1.22 (m, 6H, H-10 + H-11)
Minor rotamer: δ 5.05 (m, 1H, H-9), 4.54 (d, *J* = 5.4 Hz, 1H, H-7), 4.47 (d, *J* = 13.4 Hz, 1H, H-3), 3.91 (d, *J* = 10.7 Hz, 1H, H-1), 3.85 (d, *J* = 10.7 Hz, 1H, H-1), 2.72 (td, *J* = 13.1 Hz, 3.3 Hz, 1H, H-3), 2.26-2.23 (m, 1H, H-6), 1.73-1.70 (m, 2H, H-4 + H-5), 1.66-1.52 (m, 2H, H-4 + H-6), 1.33-1.31 (m, 1H, H-5), 1.26-1.22 (m, 6H, H-10 + H-11)

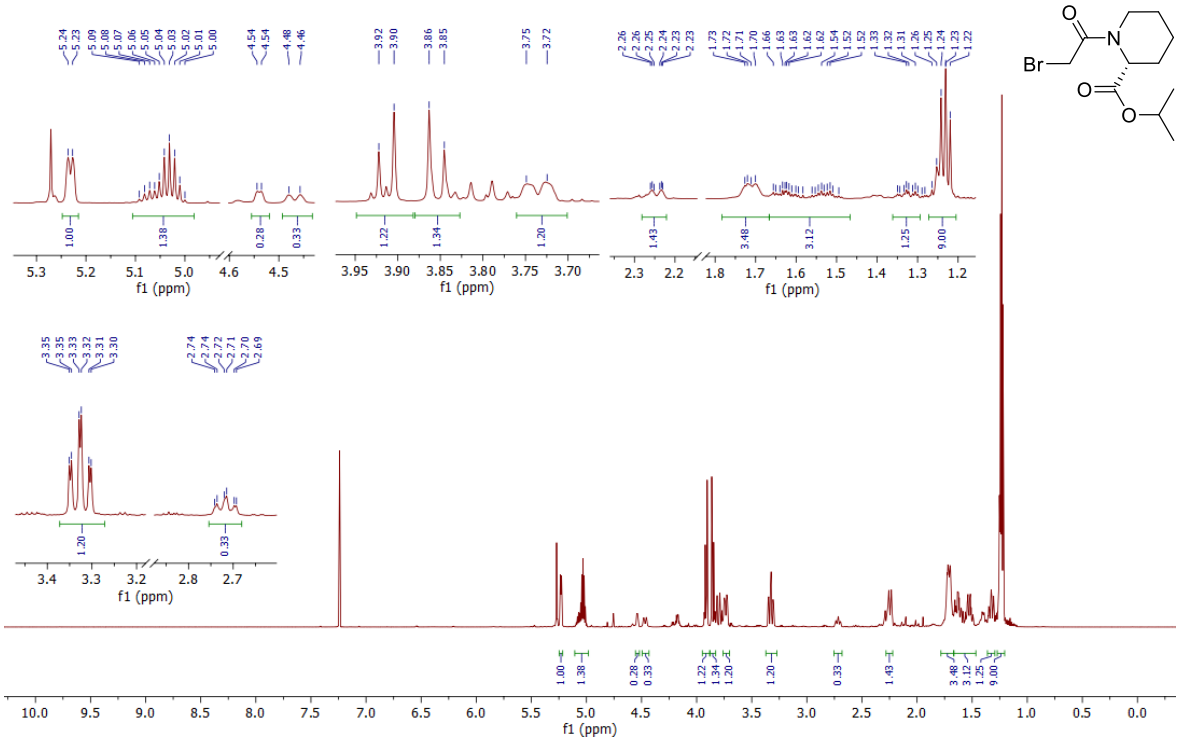
^{13}C-NMR (151 MHz, $CDCl_3$); Mayor rotamer: δ 170.2 (C=O, C-8), 166.6 (C=O, C-2), 68.9 (CH, C-9), 52.4 (CH, C-7), 44.7 (CH_2, C-3), 26.5 (CH_2, C-6), 26.0 (CH_2, C-1), 25.1 (CH_2, C-4), 21.8 (CH_3, C-10 + C-11), 20.6 (CH_2, C-5)
Minor rotamer: δ 169.7 (C=O, C-8), 166.6 (C=O, C-2), 69.5 (CH, C-9), 57.2 (CH, C-7), 40.0 (CH_2, C-3), 27.0 (CH_2, C-6), 26.0 (CH_2, C-1), 24.4 (CH_2, C-4), 20.5 (CH_2, C-5), 14.2 (CH_3, C-10 + C-11)

HR-MS (ESI, ACN) *m/z*: Calculated for 314.0359 $[M + Na]^+$; found 314.0362 for $C_{11}H_{18}O_3N^{79}BrNa$ (err: 1.0 ppm)

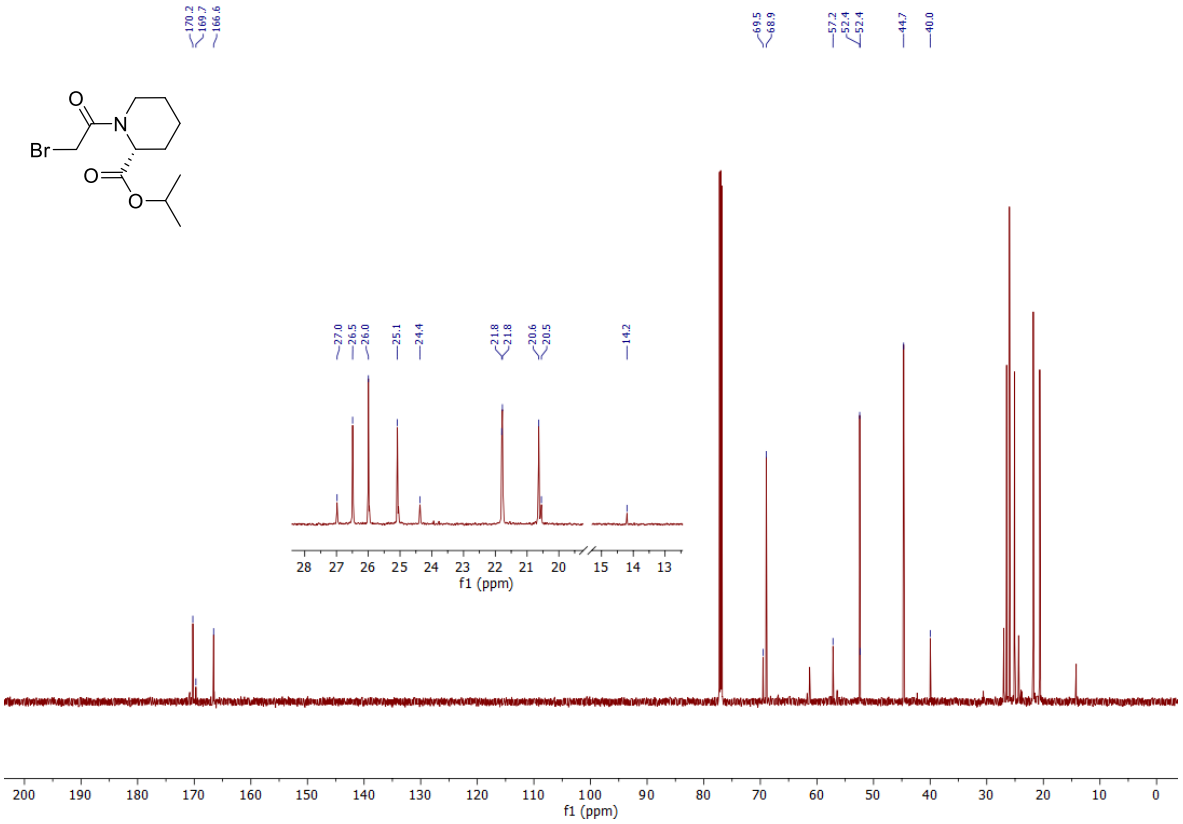
MS (ESI, ACN) *m/z*: 314 $[M + Na]^+$ (100%), 316 $[M + Na]^+$ (98%)

This compound is not reported in literature.

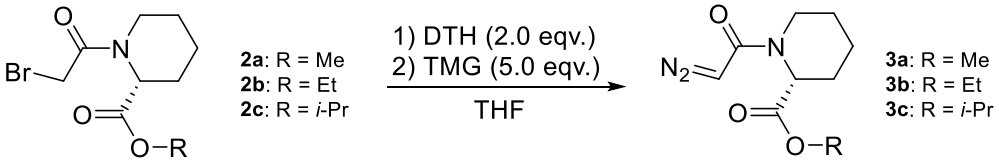
^{1}H NMR spectrum of 2c (600 MHz, $CDCl_3$)



^{13}C NMR spectrum of 2c (151 MHz, $CDCl_3$)

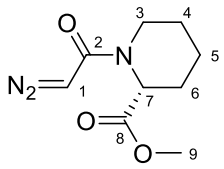


5.8 General procedure for the synthesis of “R”-(*R*)-1-(2-diazoacetyl)piperidine-2-carboxylate



Compound **2a**, **2b** or **2c** (1.0 eqv.) solved in THF was cooled down in an ice bath before addition of DTH (2.0 eqv.). Thereafter, TMG (5.0 eqv.) solved in THF was added dropwise to the reaction mixture, resulting in a yellow solution with white precipitate. After stirring at ambient temperature for 2-3 h, the mixture was filtrated and the filtrate concentrated under reduced pressure. The crude mixture was purified using a silica plug with Hex:DCM (1:1) as eluent, collecting the fractions by color. The yellow fractions were collected and evaporated *in vacuo*, giving the product **3a**, **3b** or **3c** of varying yield and purity.

5.8.1 Methyl-(*R*)-1-(2-diazoacetyl)piperidine-2-carboxylate (3a)



Compound **3a** was synthesized according to **Procedure 5.8**, using THF (5 mL), **2a** (99.0 mg, 0.375 mmol, 1.0 eqv.), DTH (259.0 mg, 0.761 mmol, 2.0 eqv.) and TMG (210.0 mg, 1.82 mmol, 4.9 eqv.) solved in THF (3 mL). The reaction stirred at ambient temperature for 2 h, followed by filtration and evaporation to dryness of the filtrate. The crude mixture was solved in some THF and purified using a silica plug (1 cm x 2.5 cm) with Hex:DCM (1:1) as eluent. The fractions were collected by color, where the yellow ones were collected, filtrated and concentrated *in vacuo*, giving **3a** as a yellow oil.

Yield: 48% (38.0 mg, 0.18 mmol)

^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.27 (bs, 1H, H-7), 5.03 (s, 1H, H-1), 3.70 (s, 3H, H-9), 3.14 (bs, 2H, H-3), 2.25-2.19 (m, 1H, H-6), 1.72-1.58 (m, 3H, H-4 + H-5 + H-6), 1.48-1.22 (m, 2H, H-4 + H-5)

^{13}C-NMR (100 MHz, $CDCl_3$): δ 171.8 (C=O, C-8), 166.3 (C=O, C-2), 52.6 (bs, CH, C-7), 52.2 (CH_3, C-9), 46.8 (CH, C-1), 43.0 (bs, CH_2, C-3), 26.6 (CH_2, C-6), 24.9 (CH_2, C-4), 20.7 (CH_2, C-5)

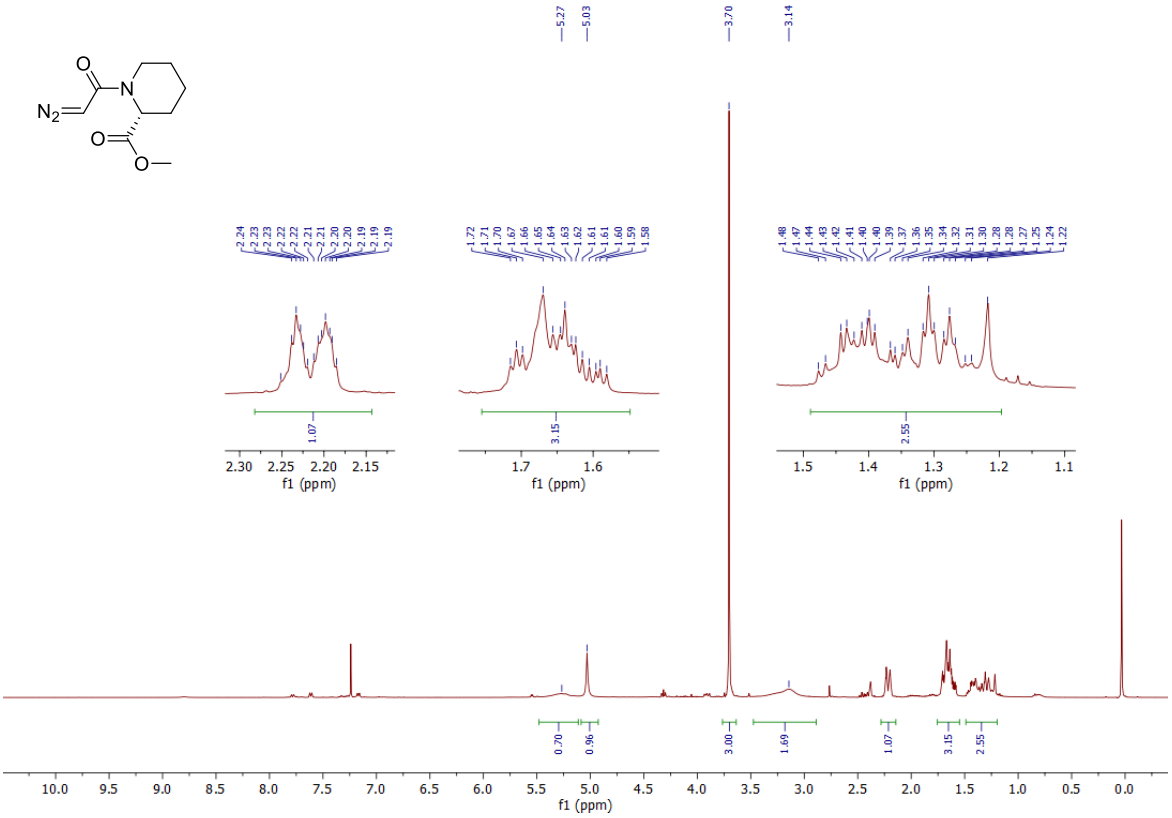
HR-MS (ESI, ACN) *m/z*: Calculated for 234.0849 $[M + Na]^+$; found 234.0849 for $C_9H_{13}O_3N_3Na$ (err: 0.2 ppm)

MS (ESI, ACN) *m/z*: 234 $[M + Na]^+$ (100%)

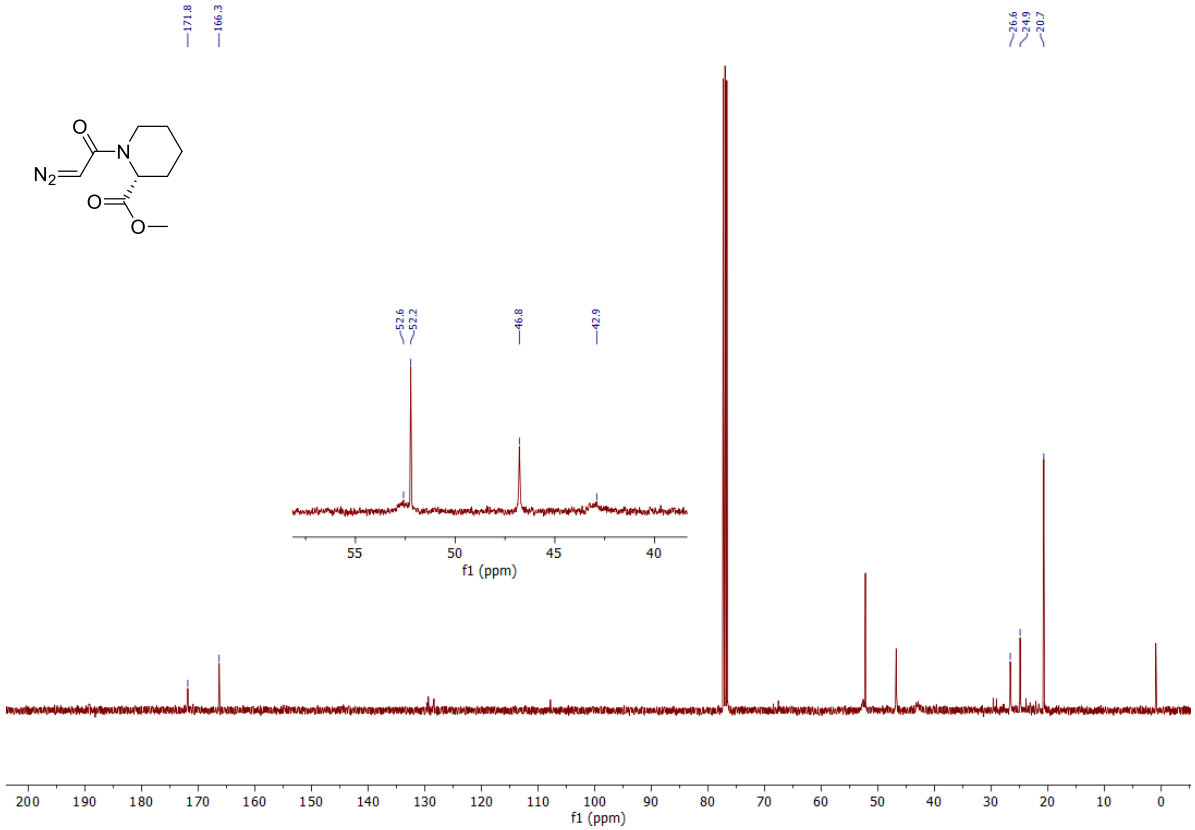
IR (ATR): Wavenumber (cm^{-1}); 2102 ($C=N_2$), 1738 (C=O)

This compound has previously been reported.[38]

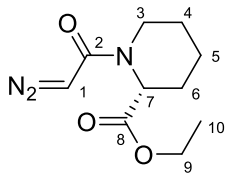
^{1}H NMR spectrum of 3a (400 MHz, $CDCl_3$)



^{13}C NMR spectrum of 3a (100 MHz, $CDCl_3$)



5.8.2 Ethyl (*R*)-1-(2-diazoacetyl)piperidine-2-carboxylate (3b)



Compound **3b** was synthesized according to **Procedure 5.8**, using THF (15 mL), **2b** (349.6 mg, 1.28 mmol, 1.0 eqv.), DTH (859.0 mg, 2.52 mmol, 2.0 eqv.) and TMG (732.4 mg, 6.36 mmol, 5.1 eqv.) solved in THF (8 mL). The reaction mixture stirred at ambient temperature for 2 h 15 min, followed by filtration of the mixture and concentration of the filtrate *in vacuo*. The crude mixture was dissolved in some THF and purified using a silica plug (2 cm x 2.5 cm) with Hex:DCM (1:1) as eluent. The fractions were collected by color and checked using TLC (Hex:DCM (1:1)), filtrated and evaporated *in vacuo*, giving **3b** as a yellow oil.

Yield: 59% (166.8 mg, 0.741 mmol)

^{1}H-NMR (600 MHz, $CDCl_3$): δ 5.24 (bs, 1H, H-7), 5.05 (s, 1H, H-1), 4.17-4.12 (m, 2H, H-9), 3.13 (bs, 2H, H-3), 2.22-2.18 (m, 1H, H-6), 1.69-1.58 (m, 3H, H-4 + H-5 + H-6), 1.44-1.46 (m, 1H, H-4), 1.34-1.27 (m, 1H, H-5), 1.22 (t, *J* = 7.1 Hz, 3H, H-10)

^{13}C-NMR (151 MHz, $CDCl_3$): δ 171.2 (C=O, C-8), 166.3 (C=O, C-2), 61.1 (CH_2, C-9), 52.7 (bs, CH, C-7), 46.6 (CH, C-1), 43.0 (bs, CH_2, C-3), 26.7 (CH_2, C-6), 24.9 (CH_2, C-4), 20.6 (CH_2, C-5), 14.1 (CH_3, C-10)

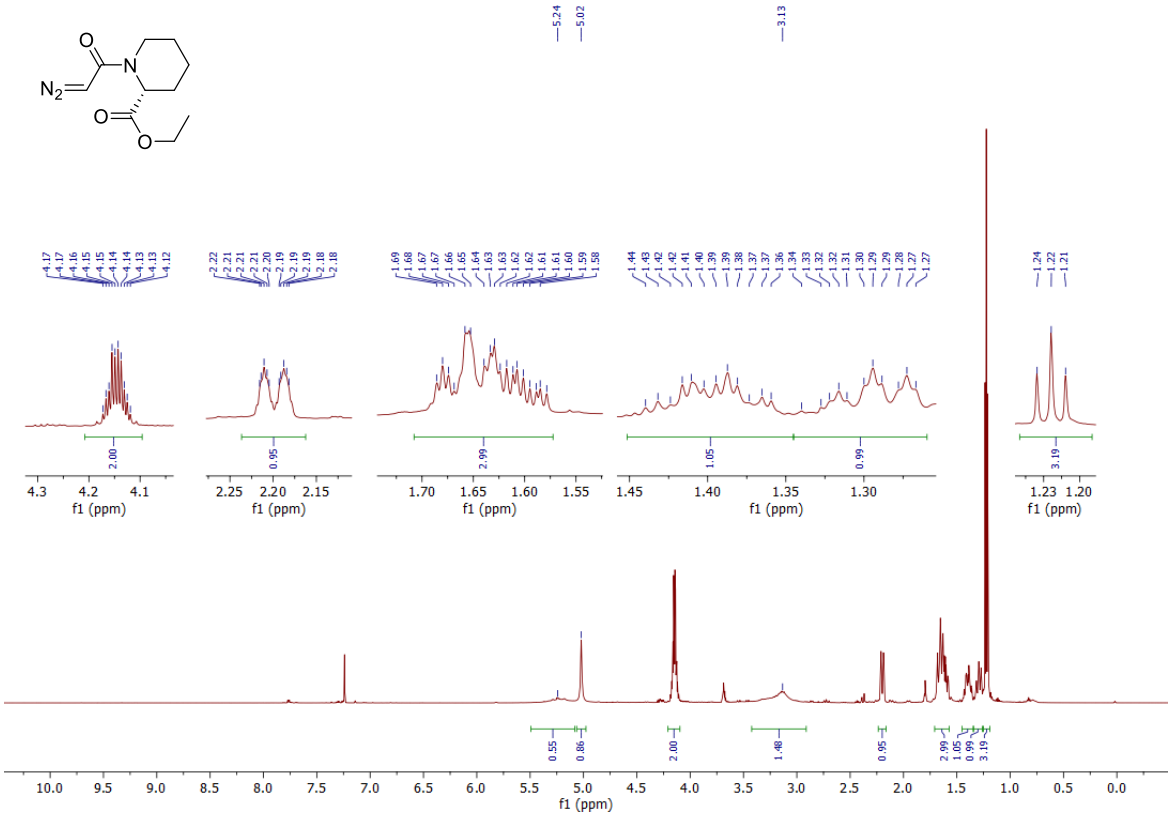
HR-MS (ESI, ACN) *m/z*: Calculated for 248.1006 $[M + Na]^+$; found 248.1006 for $C_{10}H_{15}O_3N_3Na$ (err: -0.2 ppm)

MS (ESI, ACN) *m/z*: 248 $[M + Na]^+$ (100%), 192 (12%)

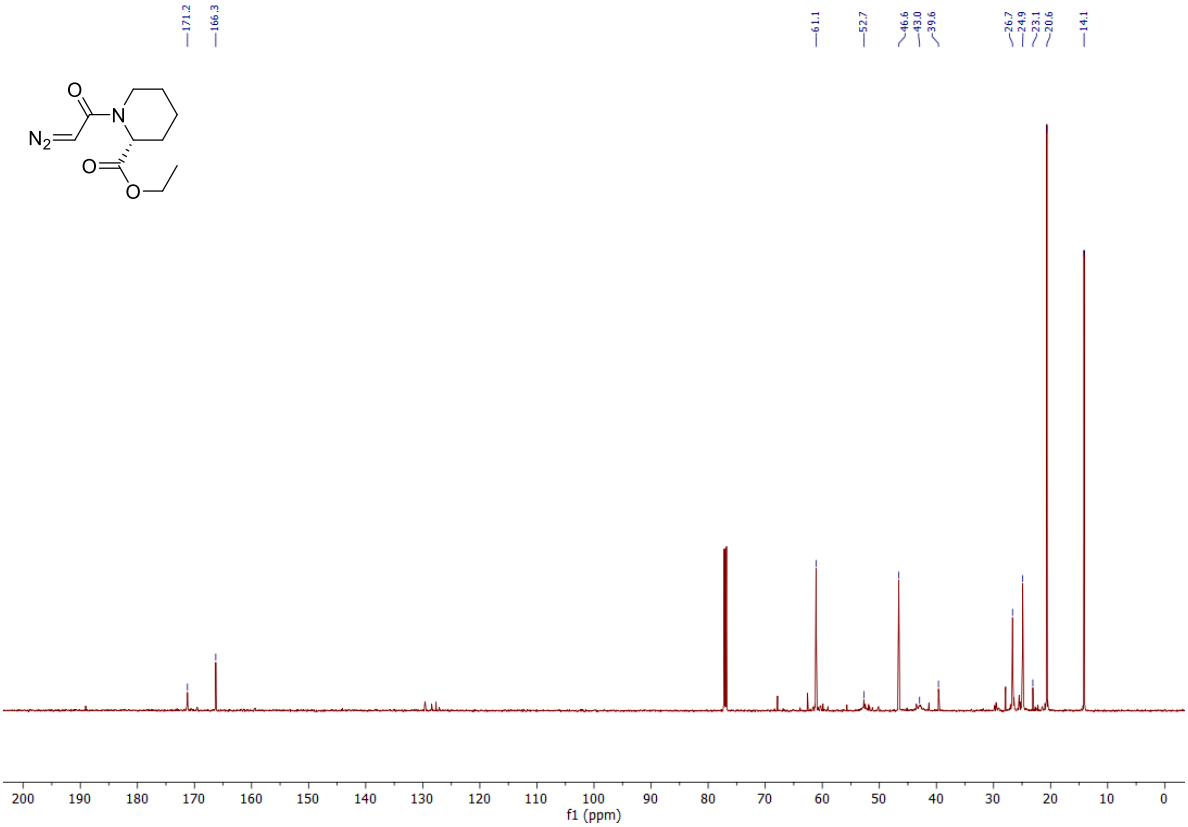
IR (ATR): Wavenumber (cm^{-1}); 2100 ($C{=}N_2$), 1736 (C=O)

This compound is not reported in literature.

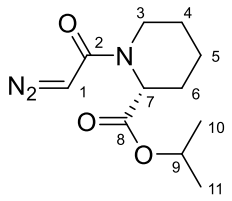
^{1}H NMR spectrum of 3b (600 MHz, $CDCl_3$)



^{13}C NMR spectrum of 3b (151 MHz, $CDCl_3$)



5.8.3 Isopropyl (*R*)-1-(2-bromoacetyl)piperidine-2-carboxylate (3c)



Compound **3c** was synthesized according to **Procedure 5.8**, using THF (5 mL), **2c** (109.2 mg, 0.374 mg, 1.0 eqv.), DTH (231.8 mg, 0.681 mmol, 1.8 eqv.), and TMG (204.2 mg, 1.77 mmol, 4.7 eqv.) solved in THF (3 mL). The reaction mixture stirred at ambient temperature for 2 h 40 min, followed by filtration of the mixture and concentration of the filtrate *in vacuo*. The crude mixture was solved in some THF and purified using a silica plug (2 cm x 2.5 cm) with Hex:DCM (1:1) as eluent. The fractions were collected by color, filtrated and evaporated under reduced pressure, giving **3c** as a yellow oil.

Yield: 36% (32.3 mg, 0.14 mmol)

^{1}H-NMR (600 MHz, $CDCl_3$): δ 5.20 (bs, 1H, H-7), 5.04-4.99 (m, 2H, H-1 + H-9), 3.15 (bs, 1H, H-3), 2.22-2.17 (m, 1H, H-6), 1.69-1.56 (m, 3H, H-4 + H-5 + H-6), 1.44-1.36 (m, 1H, H-4), 1.32-1.26 (m, 1H, H-5), 1.24-1.20 (m, 6H, H-10 + H-11)

^{13}C-NMR (151 MHz, $CDCl_3$): δ 170.7 (C=O, C-8), 166.3 (C=O, C-2), 68.7 (CH, C-9), 52.6 (bs, CH, C-7), 46.6 (CH, C-1), 42.9 (bs, CH_2, C-3), 26.7 (CH_2, C-6), 24.9 (CH_2, C-4), 21.7 (CH_3, C-10/ C-11), 20.6 (CH_2, C-5), 14.2 (CH_3, C-11/ C-10)

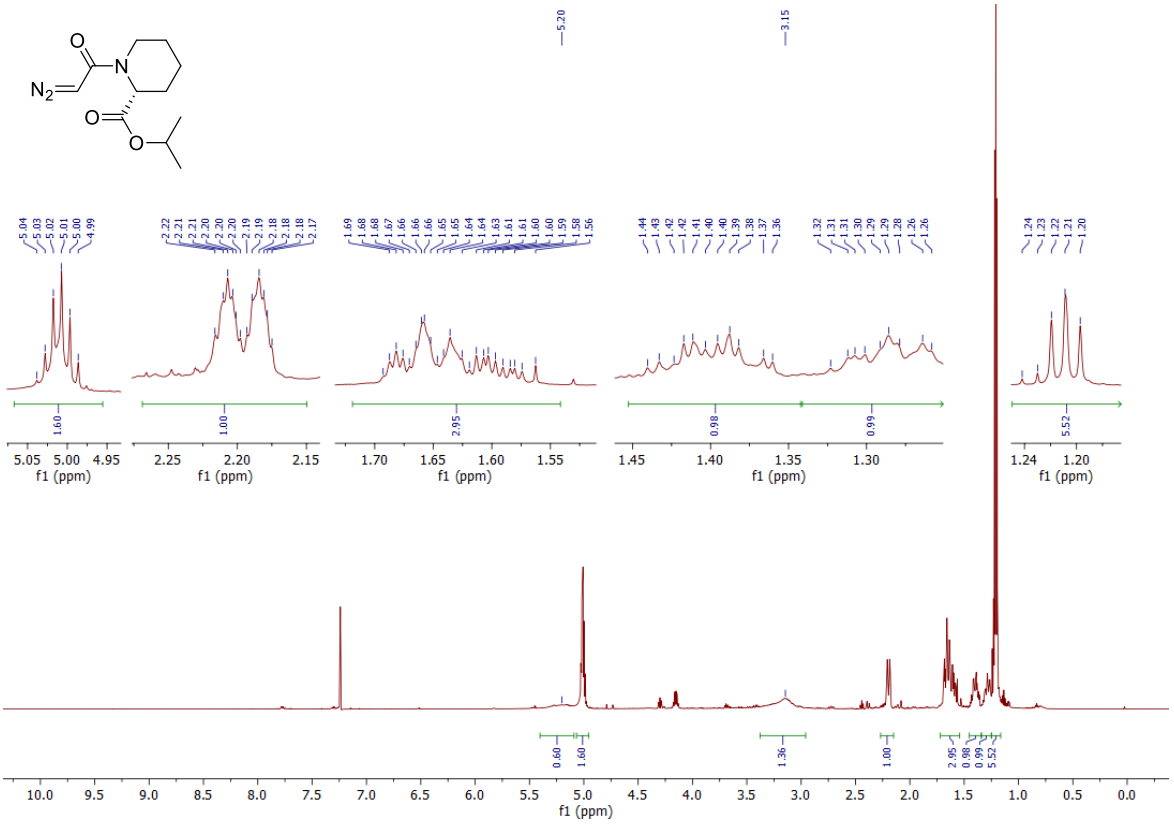
HR-MS (ESI, ACN) *m/z*: Calculated for 262.1161 $[M + Na]^+$; found 262.1162 for $C_{11}H_{17}O_3N_3Na$ (err: 0.5 ppm)

MS (ESI, ACN) *m/z*: 262 $[M + Na]^+$ (100%), 164 (19%). 206 (18%)

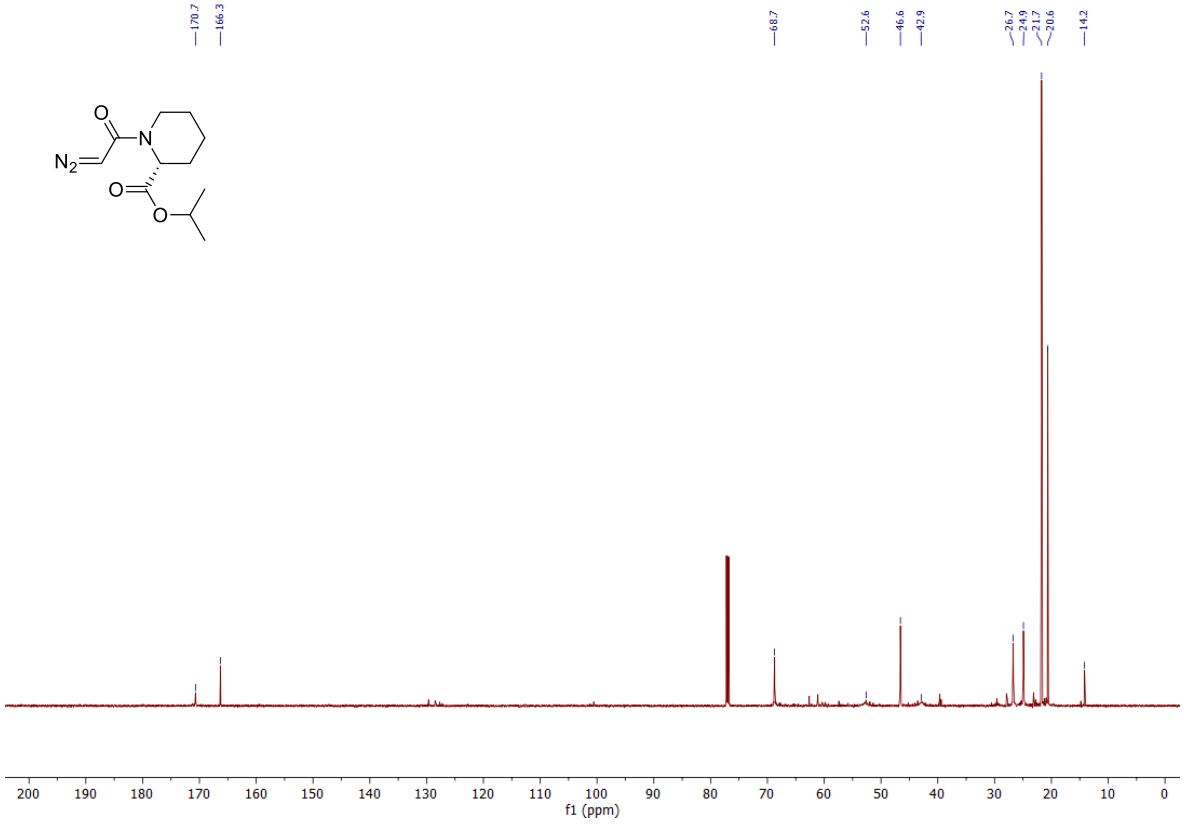
IR (ATR): Wavenumber (cm^{-1}); 2102 ($C{=}N_2$), 1728 (C=O)

This compound is not reported in literature.

^{1}H NMR spectrum of 3c (600 MHz, $CDCl_3$)

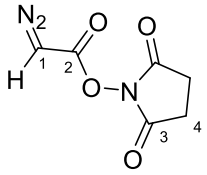


^{13}C NMR spectrum of 3c (151 MHz, $CDCl_3$)



5.9 Attempted syntheses

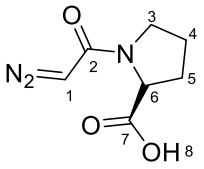
2,5-dioxypyrrolidin-1-yl 2-diazoacetate (4)



Compound **8** (251.6 mg, 1.04 mmol, 1.0 eqv.) and **10** (485.5 mg, 1.55 mmol, 1.5 eqv.) were solved in ACN (5 mL), then cooled down in an ice bath. Et_3N (0.32 mL, 2.3 mmol, 2.2 eqv.) was added dropwise, resulting in a yellow-orange solution. The mixture stirred for 1 h 20 min in the ice bath, followed by evaporation of the solvent *in vacuo*. The crude mixture was extracted with DCM and a sat. NaCl-solution, purified by a silica plug (2 cm x 6 cm) with DCM:Hex (1:1), DCM and EtOAc:DCM (1:20) as eluents, and recrystallized using chloroform and heptane. After another silica plug with EtOAc:Hex (1:1) as the eluent, giving 3.5 mg of **4** that was not pure.

This compound is reported in literature.[44]

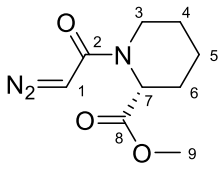
(2-diazoacetyl)-L-proline (3f)



A series of tests where **7b** was solved in D_2O, followed by addition of Na_2CO_3 or $NaHCO_3$ and **4** were conducted. Both **7b** and **4** were used as the limiting reactant, and the tests were conducted at a 0.1, 0.25 or 0.5 mmol scale. The reactions were monitored by TLC, using EtOAc:DCM (1:10) as eluent, but this worked only when **4** was the limiting reactant. In some of the tests, the pH of the reaction mixture was adjusted, followed by extraction with DCM, EtOAc or Et_2O, while others were extracted only. The product seemed to decompose in the water.

This compound is not reported in literature.

Methyl (*R*)-1-(2-diazoacetyl)piperidine-2-carboxylate (3a)

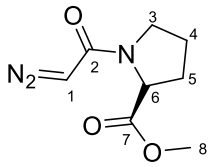


A series of tests where **1a** was solved in ACN or DMF, followed by addition of Et_3N or DBU and **4** were conducted. A 0.1 or 0.2 mmol scale was used, with **4** as the limiting reactant, at ambient temperature or at 35˚C. The reactions were monitored by TLC, using EtOAc:DCM (1:1) as eluent. The formation of product could not be confirmed in any of the tests.

Another series of tests used **5a** solved in ACN, followed by addition of Et_3N or DBU and **4**. A 0.1 mmol scale was used, and the tests were conducted at ambient temperature. In some of the tests, Et_3N was used as the base, with catalytic amounts of DBU or DMAP. The reactions were monitored using TLC, with EtOAc:DCM (1:10) as eluent. The formation of product could not be confirmed in any of the tests.

This compound has previously been reported.[38]

Methyl (2-diazoacetyl)-D-prolinate (3d)



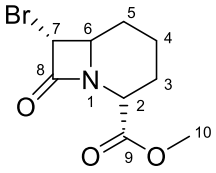
Et_3N (30 µL, 0.22 mmol, 2.1 eqv.) was added to a solution of **5b** (22.8 mg, 0.177 mmol, 1.7 eqv.) and ACN (0.5 mL). Compound **4** (18.6 mg, 0.10 mmol, 1.0 eqv.) was added after some stirring. The mixture stirred at ambient temperature and was checked with TLC (EtOAc:DCM (1:10)) regularly. The reaction was stopped after 23 h 10 min. EtOAc (10 mL) was added and the solution transferred to a separatory funnel, followed by extraction with 3 x 3 mL sat. NaCl-solution. The organic phase was dried using $MgSO_4$, filtrated and evaporated to dryness under reduced pressure. What seems to be **3d** was formed, but it could not be confirmed.

HR-MS (ESI, ACN) *m/z*: Calculated for 220.0692 $[M + Na]^+$; found 220.0693 for $C_8H_{11}N_3O_3Na$ (err: 0.3 ppm)

MS (ESI, ACN) *m/z*: 220 $[M + Na]^+$ (100%)

This compound is reported in literature.[55]

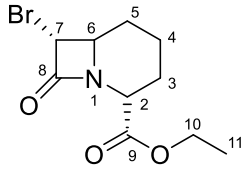
Methyl (2*R*)-7-bromo-8-oxo-1-azabicyclo[4.2.0]octane-2-carboxylate (6aa)



DBU (62.6 mg, 0.411 mmol, 1.1 eqv.) solved in DCM (1 mL) was added to a cold solution of **3a** (76.5 mg, 0.362 mmol, 1.0 eqv.) and DCM (8 mL), followed by addition of NBS (72.9 mg, 0.410 mmol, 1.1 eqv.). After about 1 min of stirring, the reaction mixture was transferred to a silica plug (2.0 cm x 2.0 cm) and separated using cold DCM as eluent. Three large fractions were collected and left at ambient temperature to observe any change of color. The fractions were filtrated, evaporated *in vacuo* and checked with NMR. No traces of product was observed from this entry, but in another entry on a 0.19 mmol scale, traces of product were observed. In a third entry, the reaction mixture stirred in an acetone bath for 30 min, before it was transferred to a silica plug and separated by using vacuum. The fraction was left at ambient temperature to observe any change of color, before it was filtrated and evaporated under reduced pressure. There were too many signals in the NMR spectrum to determine the presence of product.

This compound has previously been reported.[38]

Ethyl (2*R*)-7-bromo-8-oxo-1-azabicyclo[4.2.0]octance-2-carboxylate (6ba)



Method I

3b (70.3 mg, 0.312 mmol, 1.0 eqv.) was solved in cold DCM (8 mL) before a solution of DBU (9.4 mg, 0.062 mmol, cat.) in cold DCM (1 mL) and NBS (61.2 mg, 0.344 mmol, 1.1 eqv.) were added. The mixture stirred at ambient temperature for thermally decomposition. After 1 h 45 min, the solution was transferred to a separatory funnel and extracted with 5 x 10 mL sat. $NaHCO_3$-solution. The organic phase was dried using $MgSO_4$, filtrated and evaporated *in vacuo*, giving a mixture of **6ba**, **6bb** and **6bc** as an orange-yellow oil.

IS-yield: 11% of **6ba** (9.3 mg, 0.034 mmol)

HR-MS (ESI, ACN) *m/z*: Calculated for 298.0048 $[M + Na]^+$; found 298.0049 for $C_{10}H_{14}O_3N^{79}BrNa$ (err: 0.3 ppm)

MS (ESI, ACN) *m/z*: 298 $[M + Na]^+$ (100%), 300 $[M + Na]^+$ (97%)

Method II

3b (63.2 mg, 0.281 mmol, 1.0 eqv.) was solved in cold DCM (8 mL) before a solution of DBU (56.1 mg, 0.369 mmol, 1.3 eqv.) in cold DCM (1 mL) and NBS (60.2 mg, 0.338 mmol, 1.2 eqv.) were added. After 2 min of stirring, the mixture was transferred to a separatory funnel and extracted with 3 x 13 mL cold 0.5 M $Na_2O_3S_2$-solution. The organic phase was left at ambient temperature for thermally decomposition, before it was dried using $MgSO_4$, filtrated and evaporated *in vacuo*, giving a mixture of **6ba**, **6bb** and **6bc** as an orange-yellow oil.

IS-yield: 23% of **6ba** (17.6 mg, 0.064 mmol)

HR-MS (ESI, ACN) *m/z*: Calculated for 298.0048 $[M + Na]^+$; found 298.0049 for $C_{10}H_{14}O_3N^{79}BrNa$ (err: 0.4 ppm)

MS (ESI, ACN) *m/z*: 298 $[M + Na]^+$ (100%), 300 $[M + Na]^+$ (97%)

This compound is not reported in literature.

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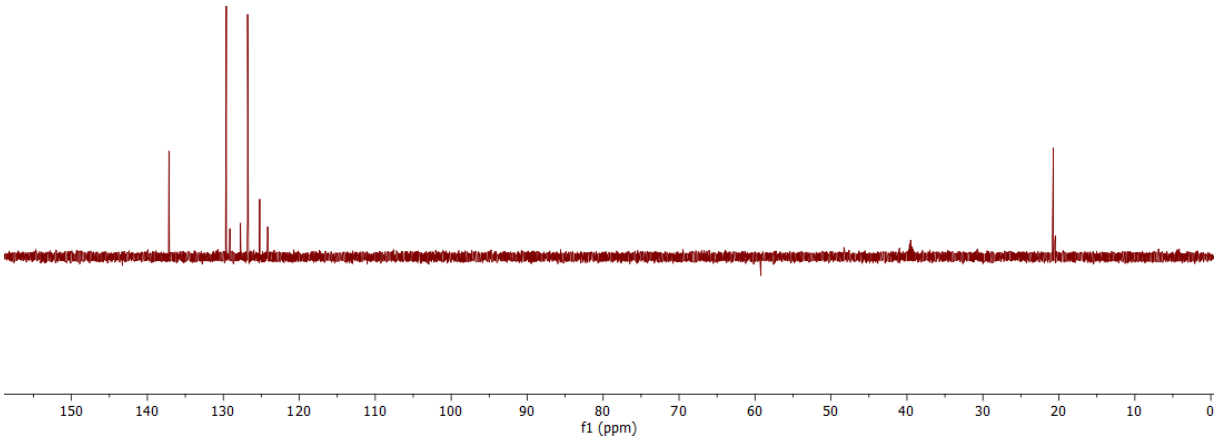
Appendix

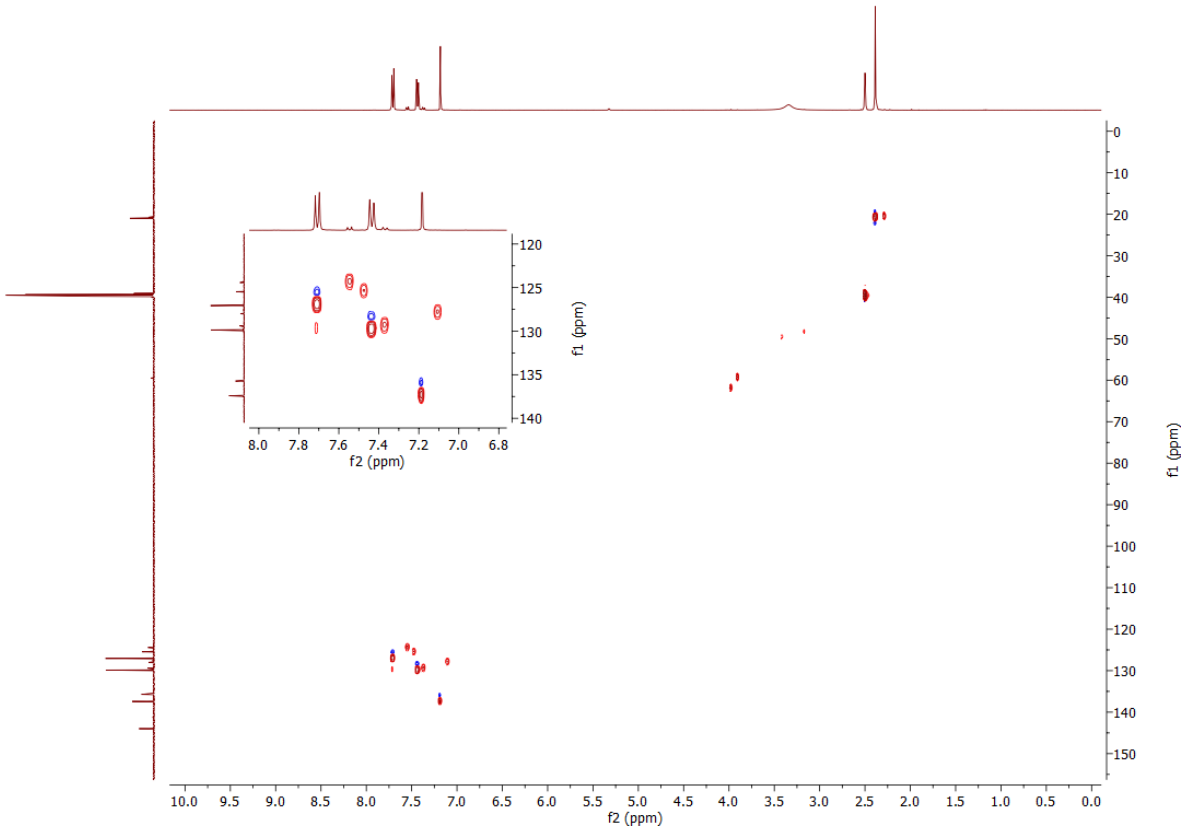
A.1 NMR spectra

A.1.1 2-(4-methylbenzenesulfonamido)imino acetic acid (8)

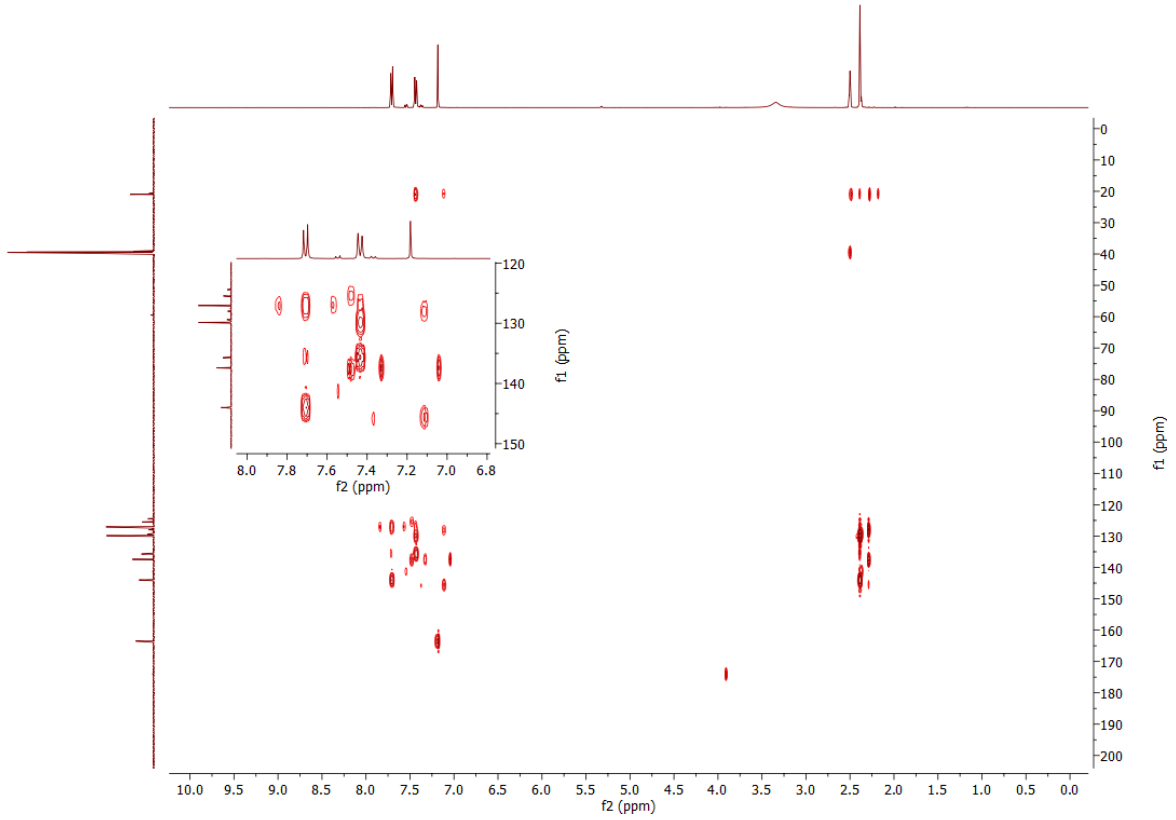
DEPT135 spectrum of 8 (151 MHz, DMSO-d_6)



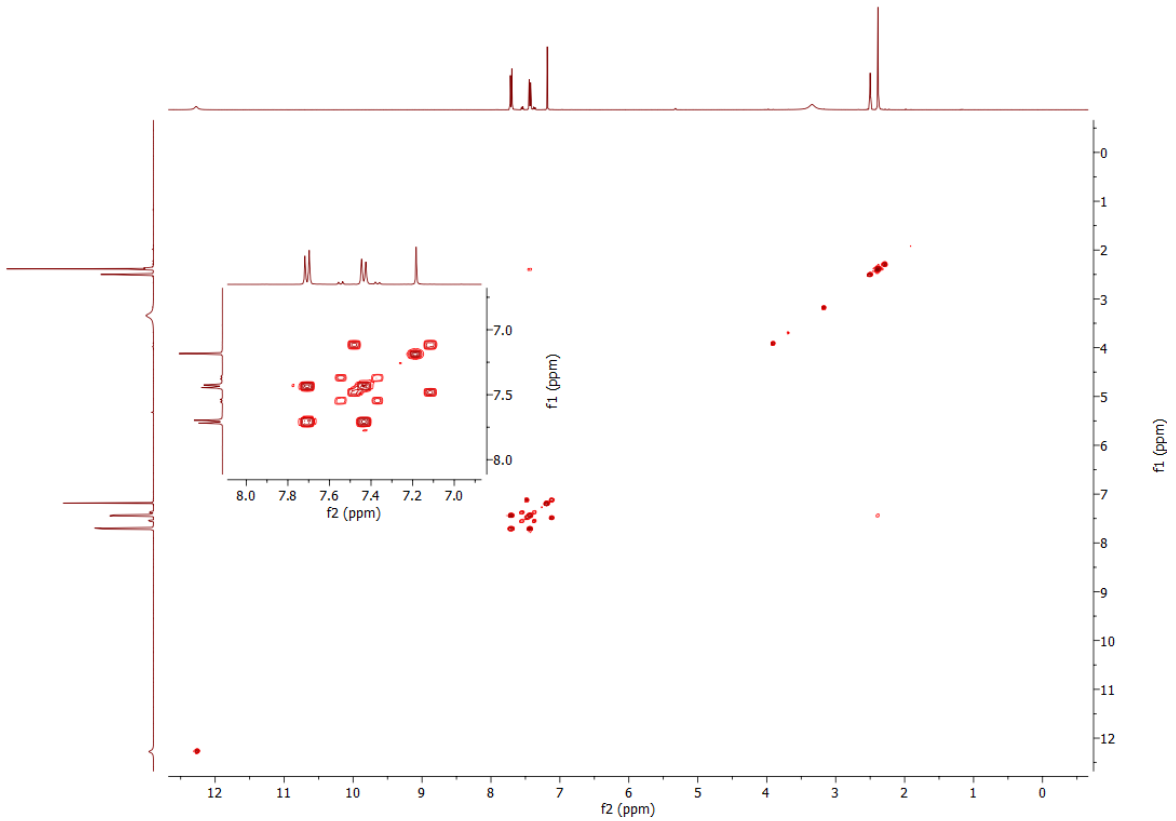
HSQC spectrum of 8 (600 MHz, DMSO-d_6)



HMBC spectrum of 8 (600 MHz, DMSO-d_6)

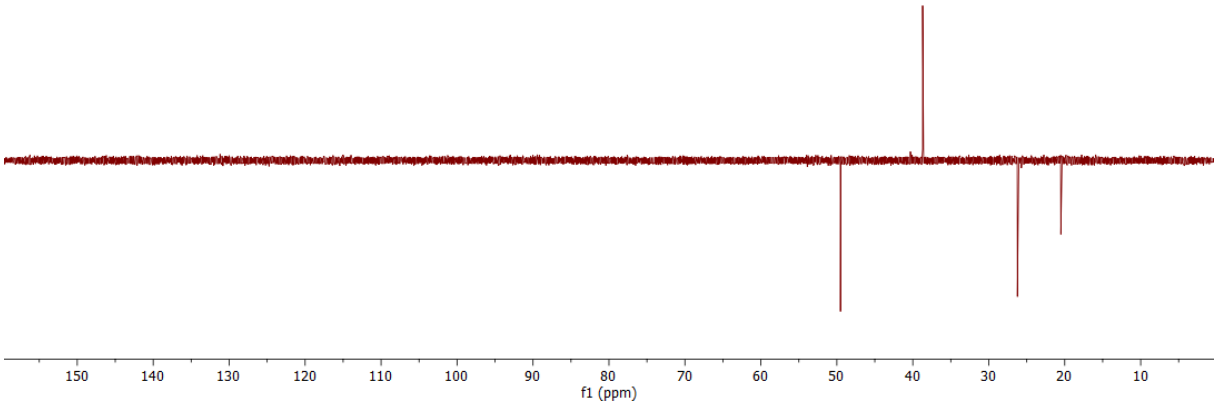


COSY spectrum of 8 (600 MHz, DMSO-d_6)

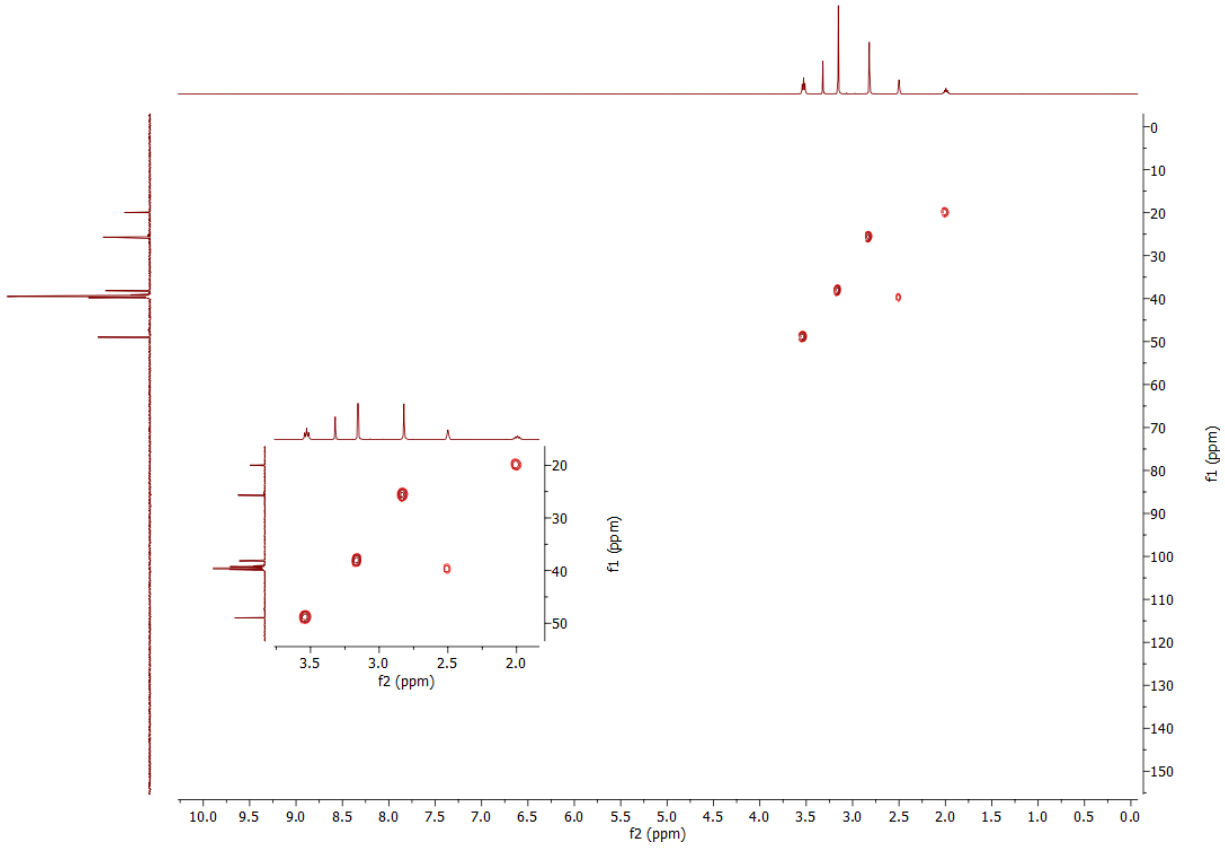


A.1.2 2-((2,5-dioxopyrrolidin-1-yl)oxy)-1,3-dimethyl-3,4,5,6-tetrahydropyrimidin-1-ium tetrafluoroborate (10)

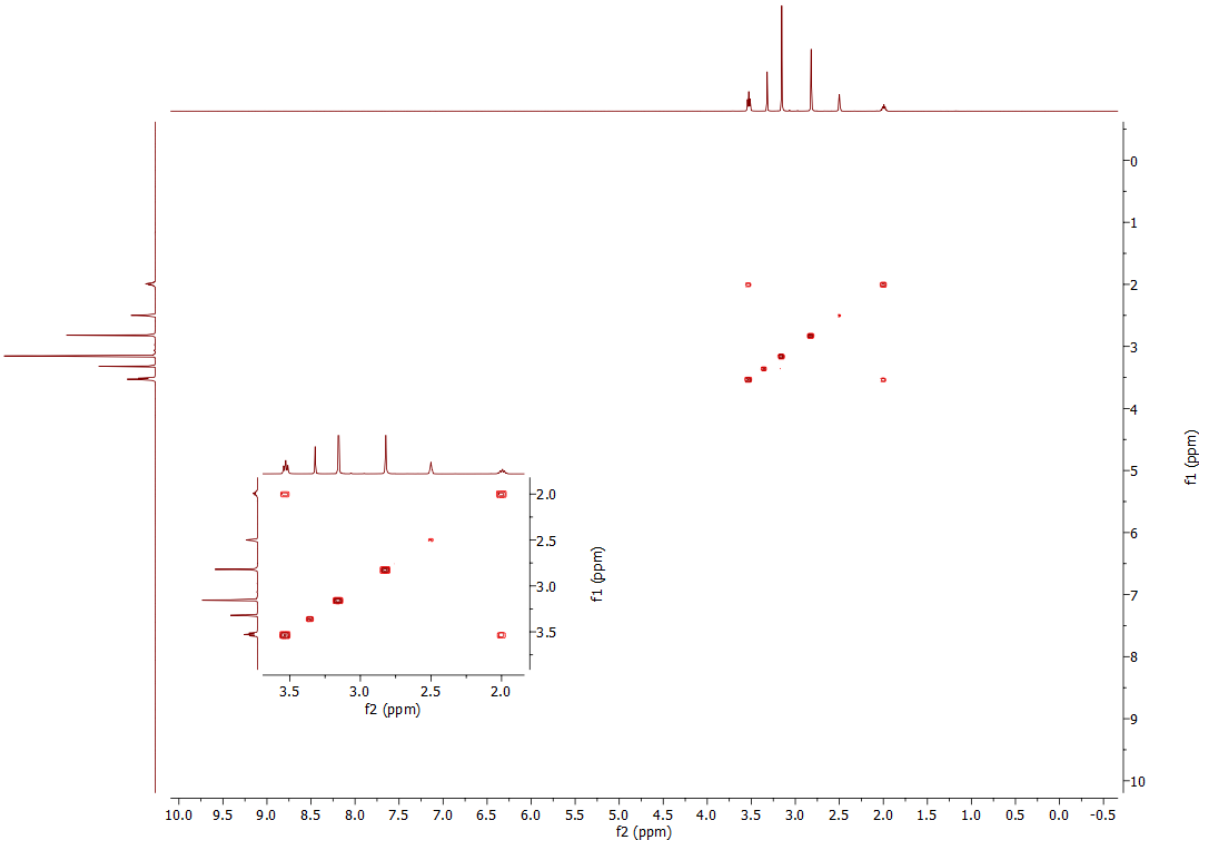
DEPT135 spectrum of 10 (151 MHz, DMSO-d_6)



HSQC spectrum of 10 (600 MHz, DMSO-d_6)

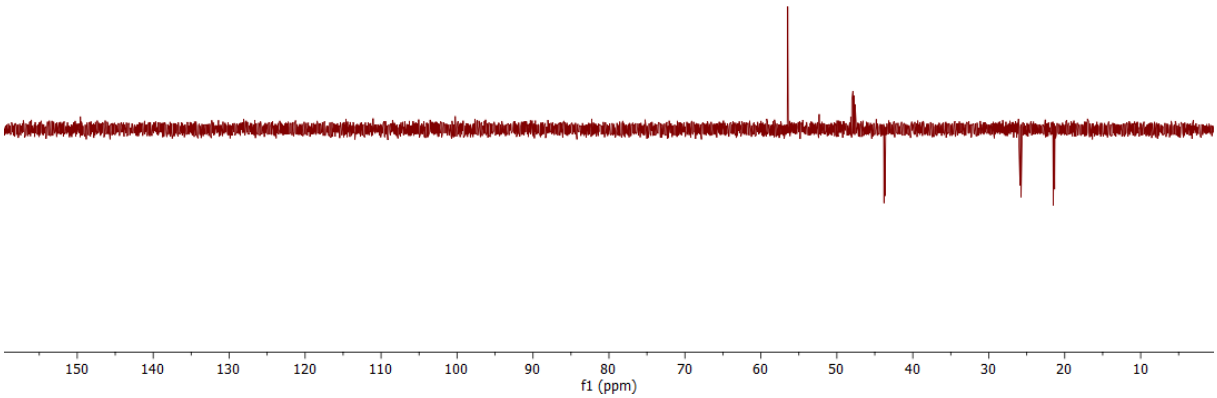


COSY spectrum of 10 (600 MHz, DMSO-d_6)

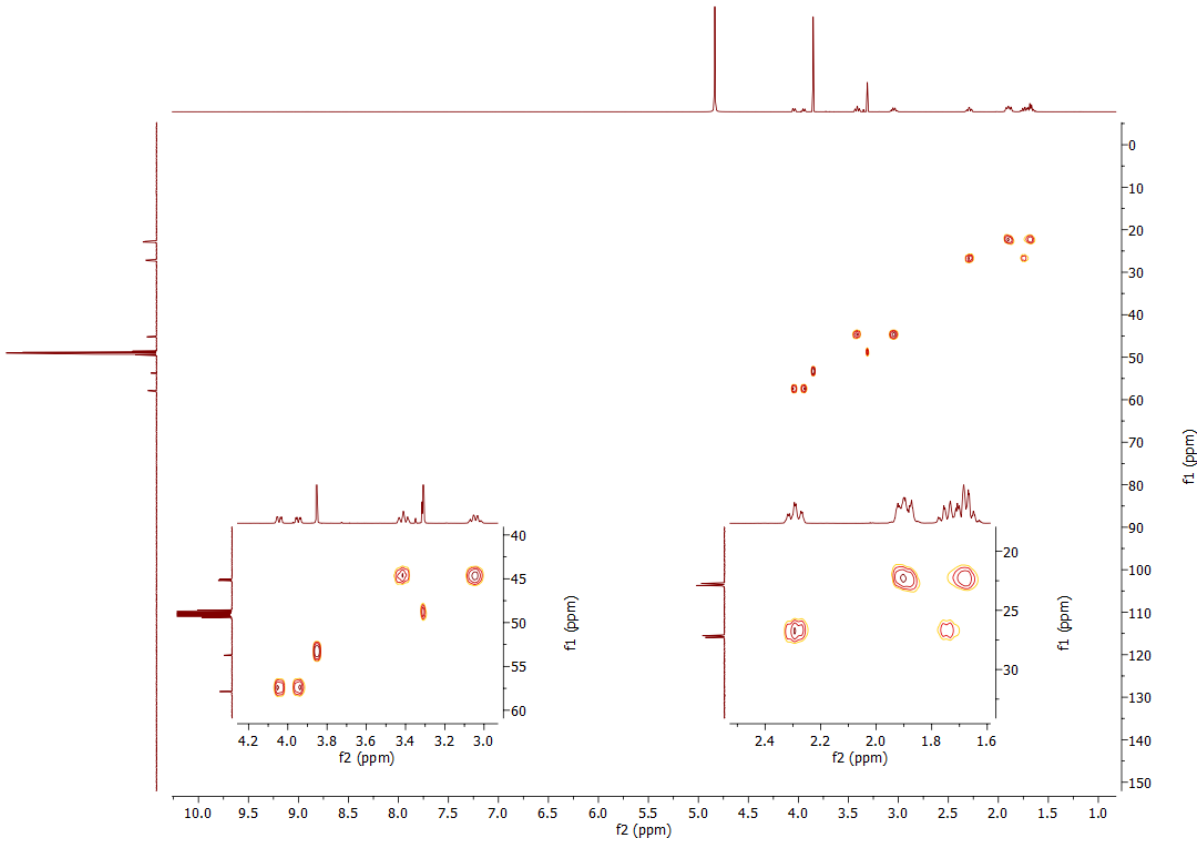


A.1.3 (*R*)-Methyl piperidine-2-carboxylate hydrochloride (1a)

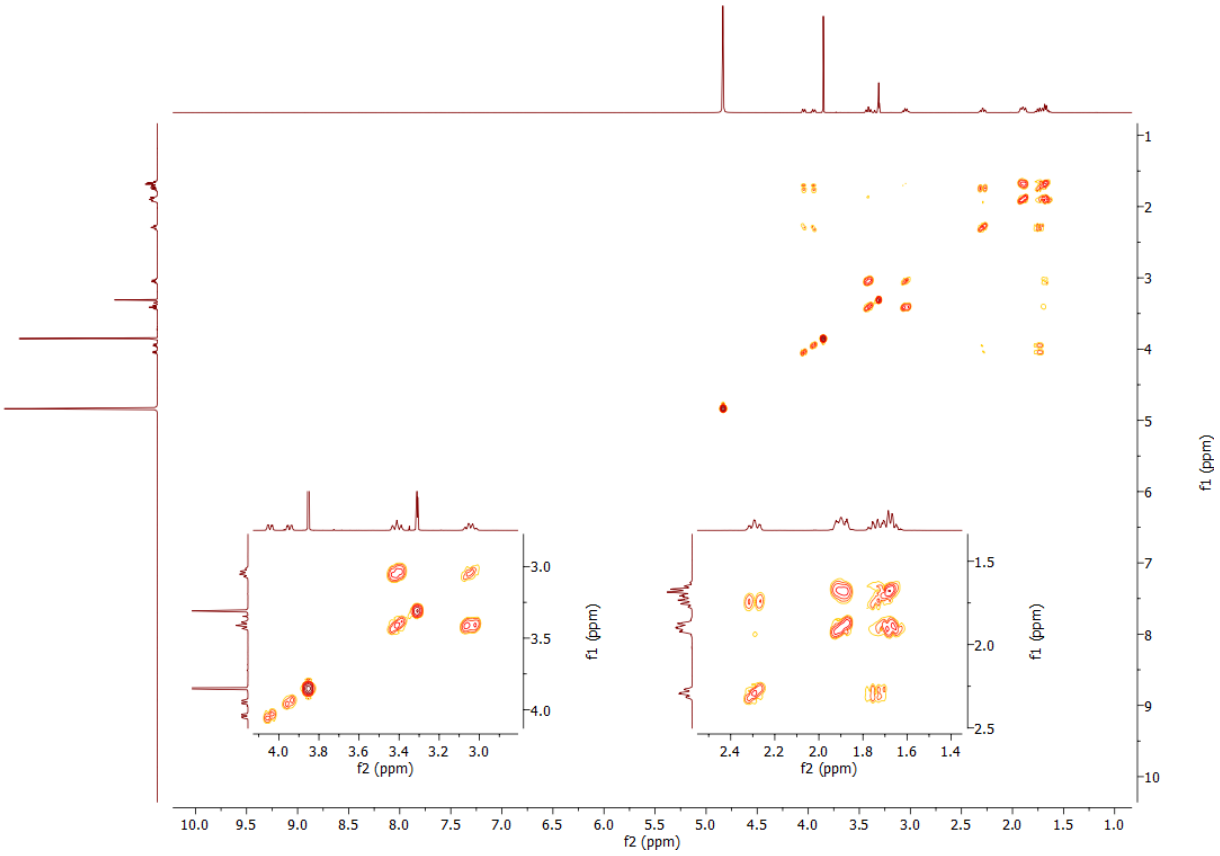
DEPT135 spectrum of 1a (151 MHz, MeOD-d_4)



HSQC spectrum of 1a (600 MHz, MeOD-d_4)

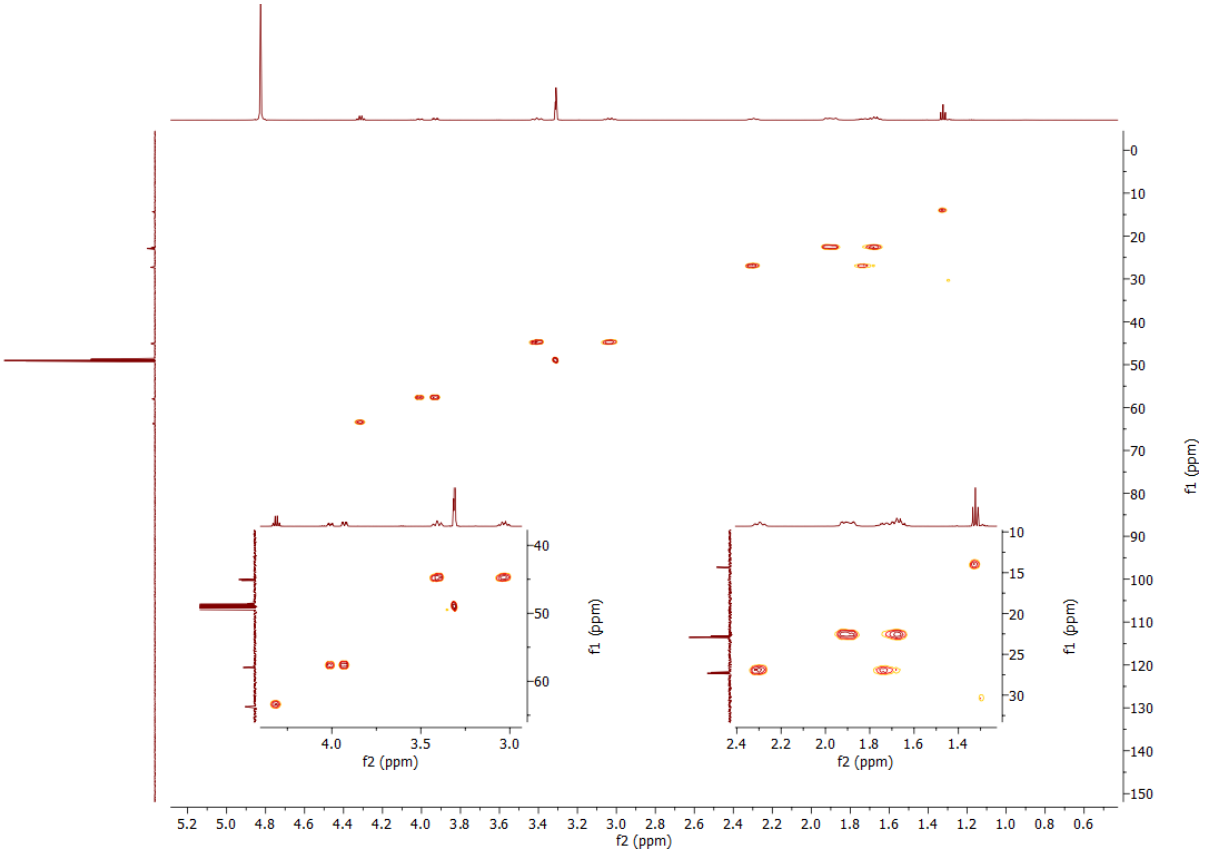


COSY spectrum of 1a (600 MHz, MeOD-d_4)



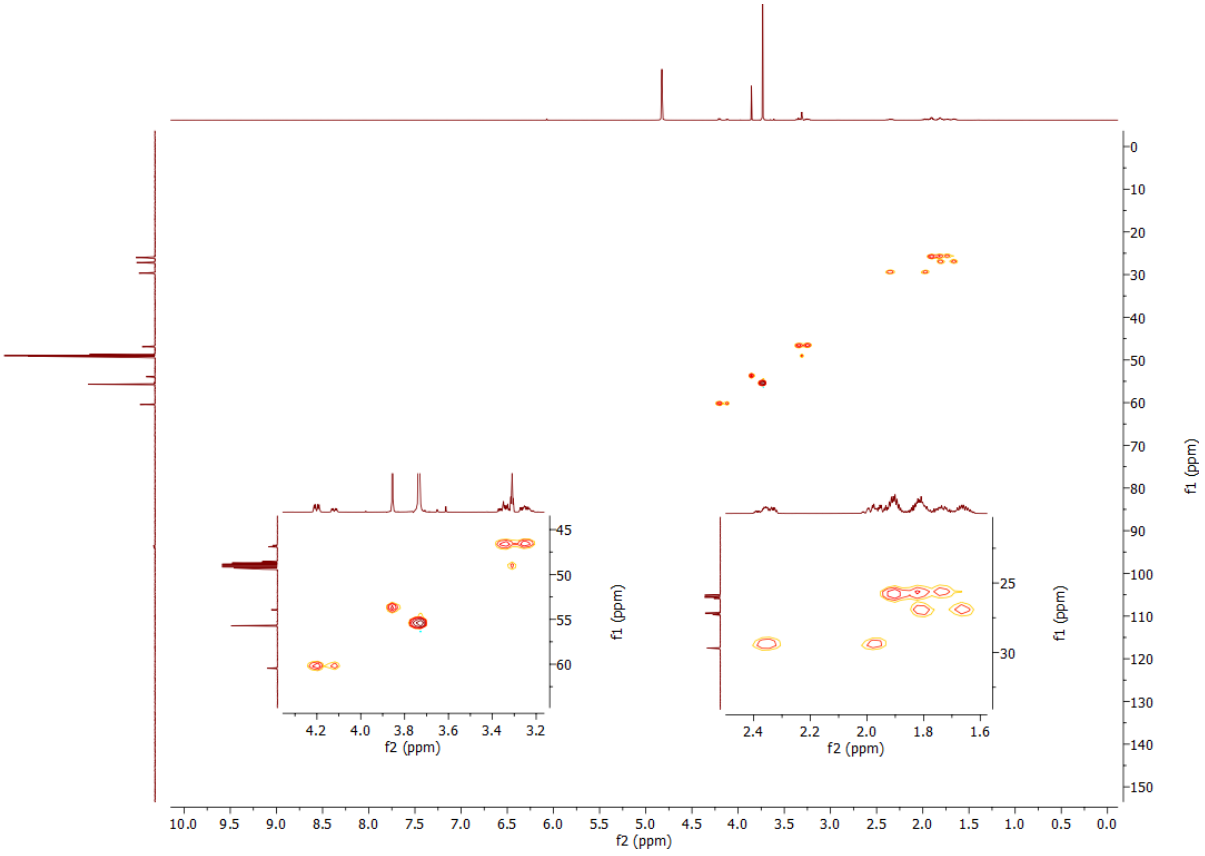
A.1.4 (*R*)-Ethyl piperidine-2-carboxylate hydrochloride (1b)

HSQC spectrum of 1b (600 MHz, MeOD-d_4)



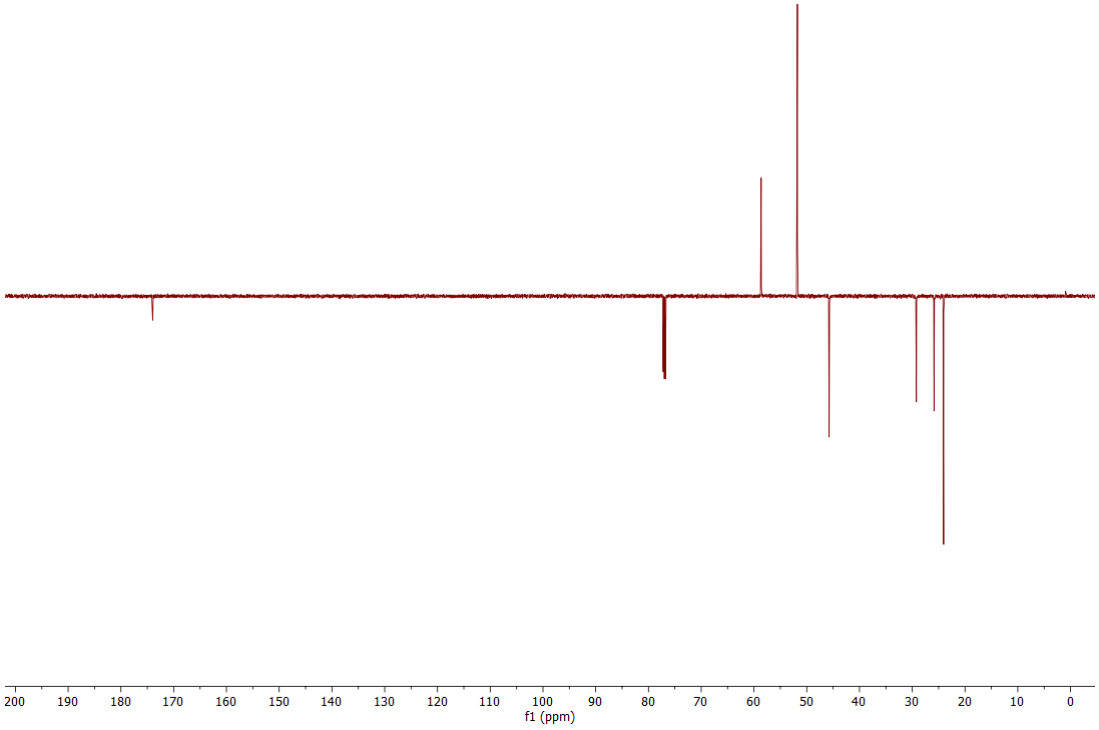
A.1.5 (*R*)-Methyl azepane-2-carboxylate hydrochloride (1e)

HSQC spectrum of 1e (600 MHz, MeOD-d_4)

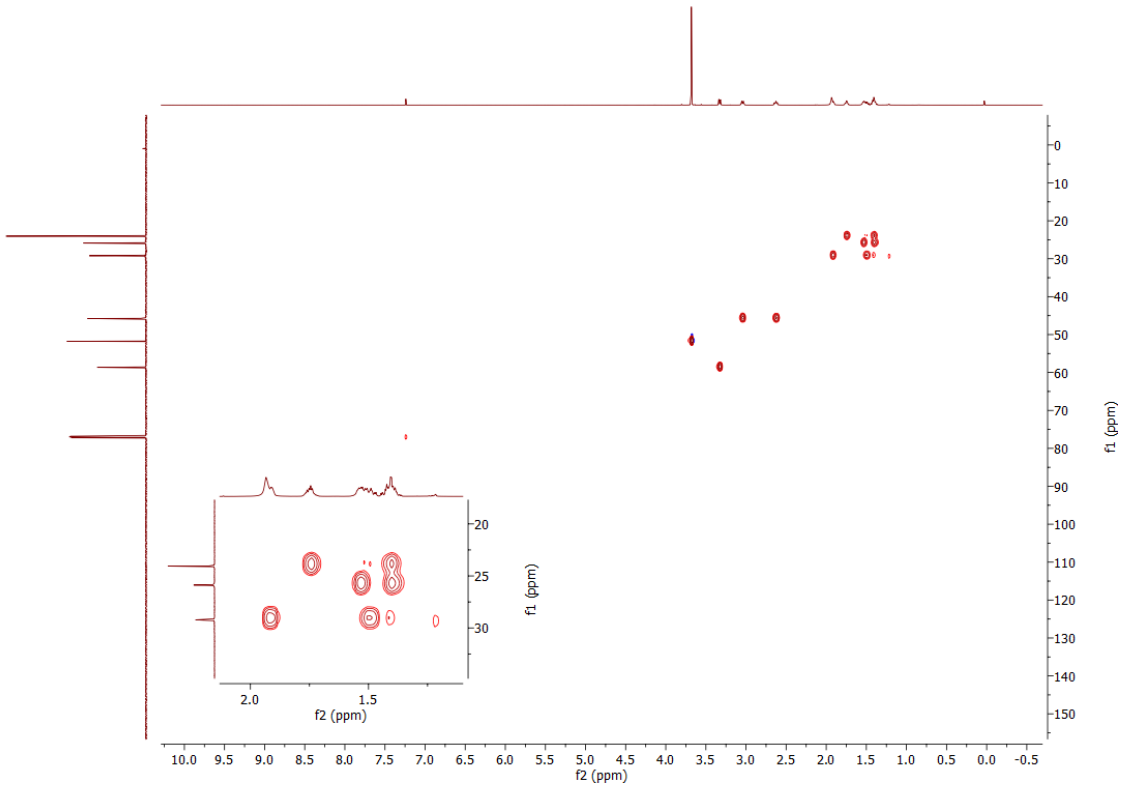


A.1.6 Methyl (*R*)-piperidine-2-carboxylate (5a)

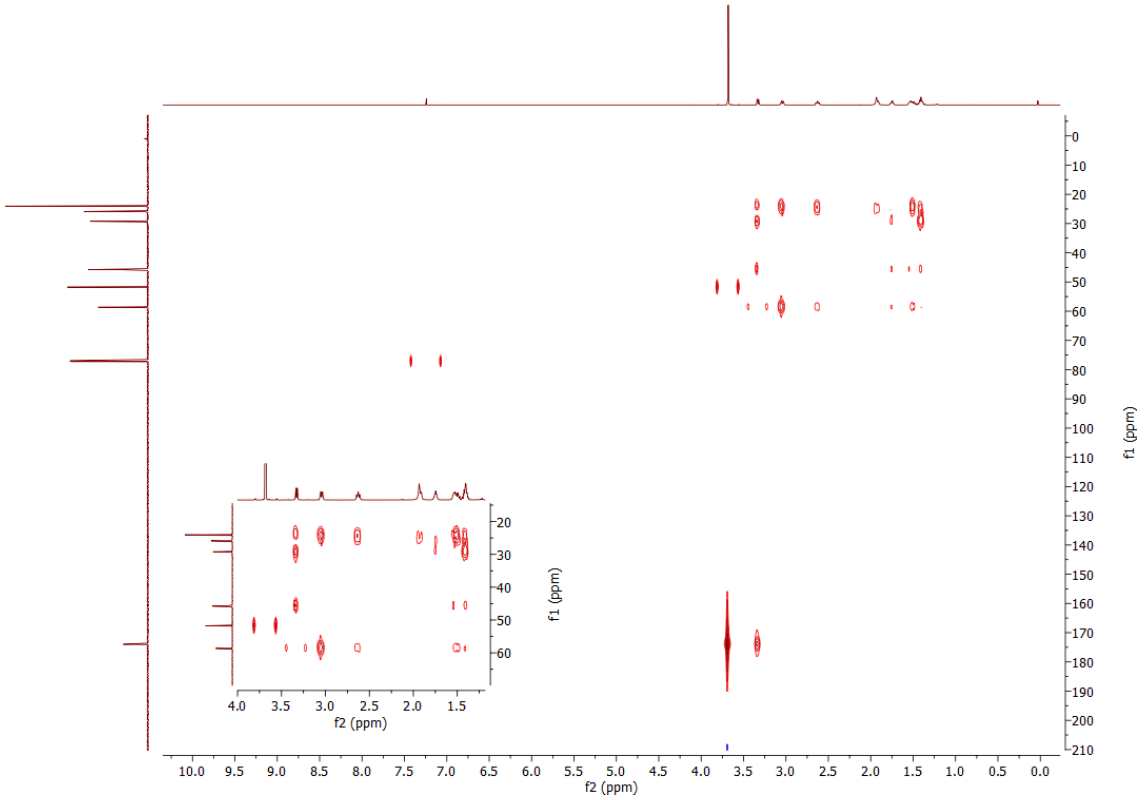
DEPT135Q spectrum of 5a (151 MHz, $CDCl_3$)



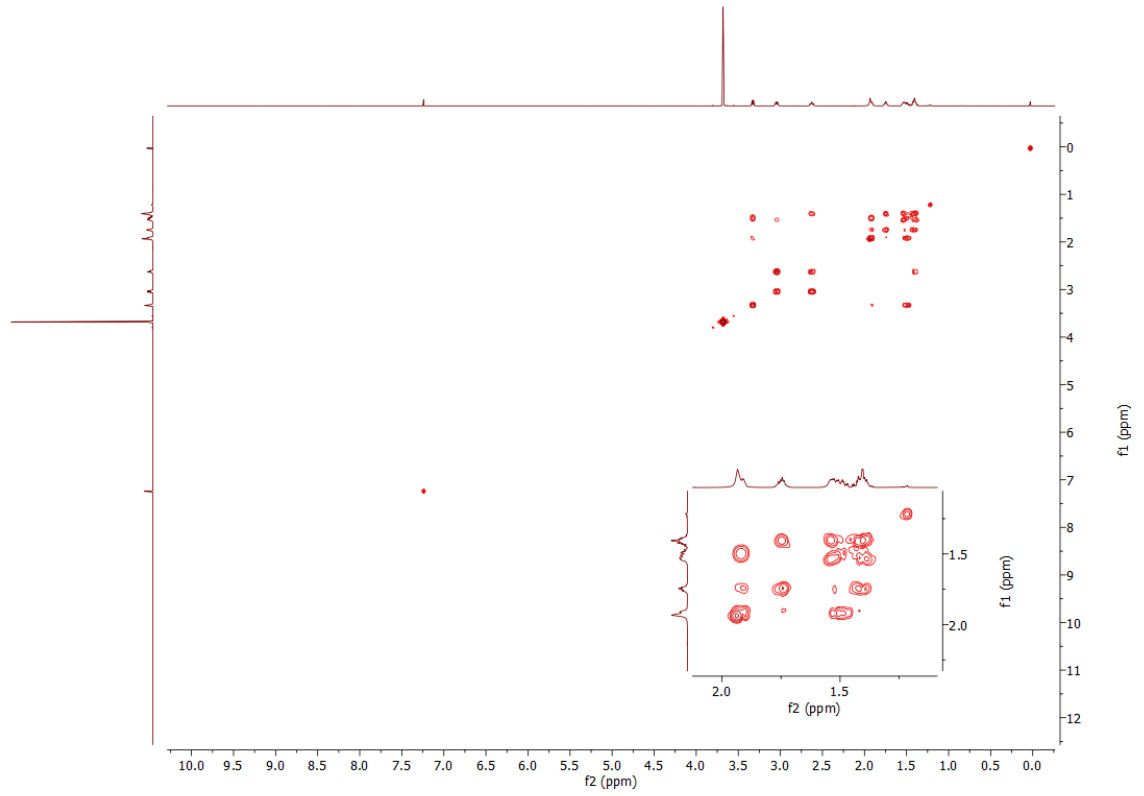
HSQC spectrum of 5a (600 MHz, $CDCl_3$)



HMBC spectrum of 5a (600 MHz, $CDCl_3$)

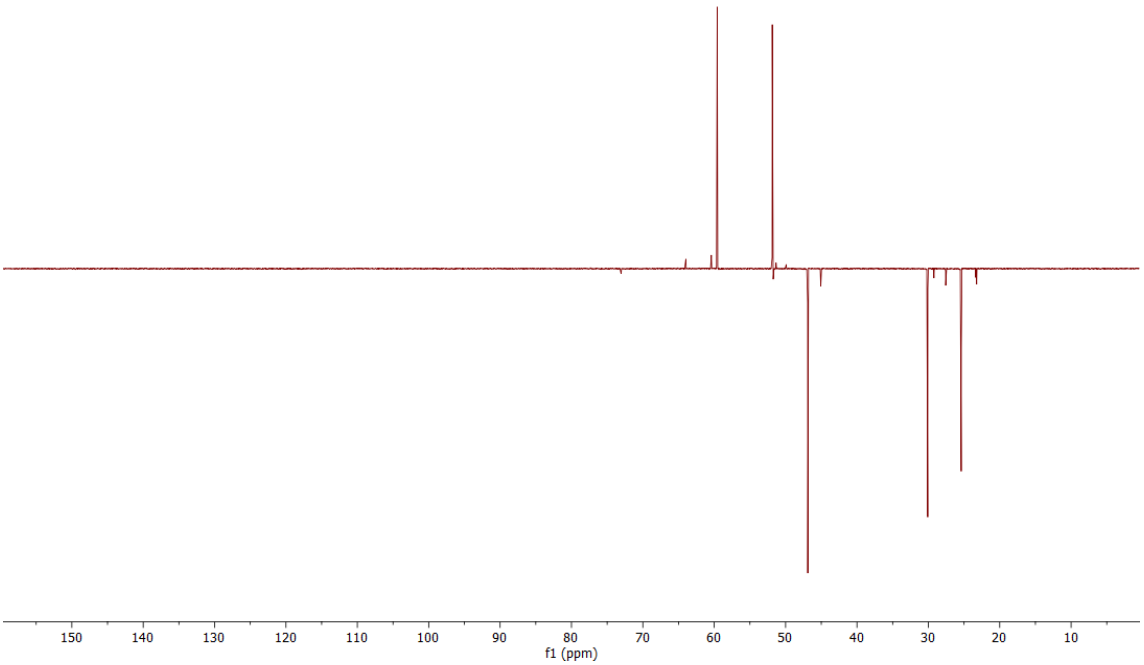


COSY spectrum of 5a (600 MHz, $CDCl_3$)

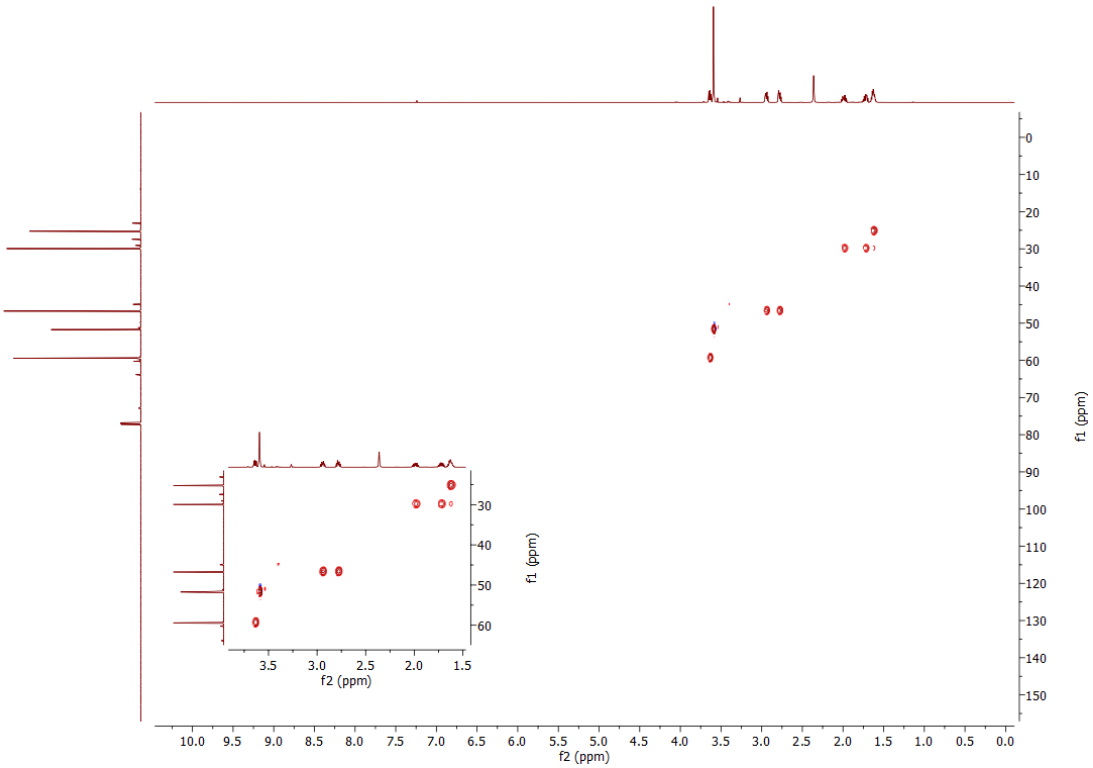


A.1.7 Methyl D-prolinate (5b)

DEPT135 spectrum of 5b (151 MHz, $CDCl_3$)

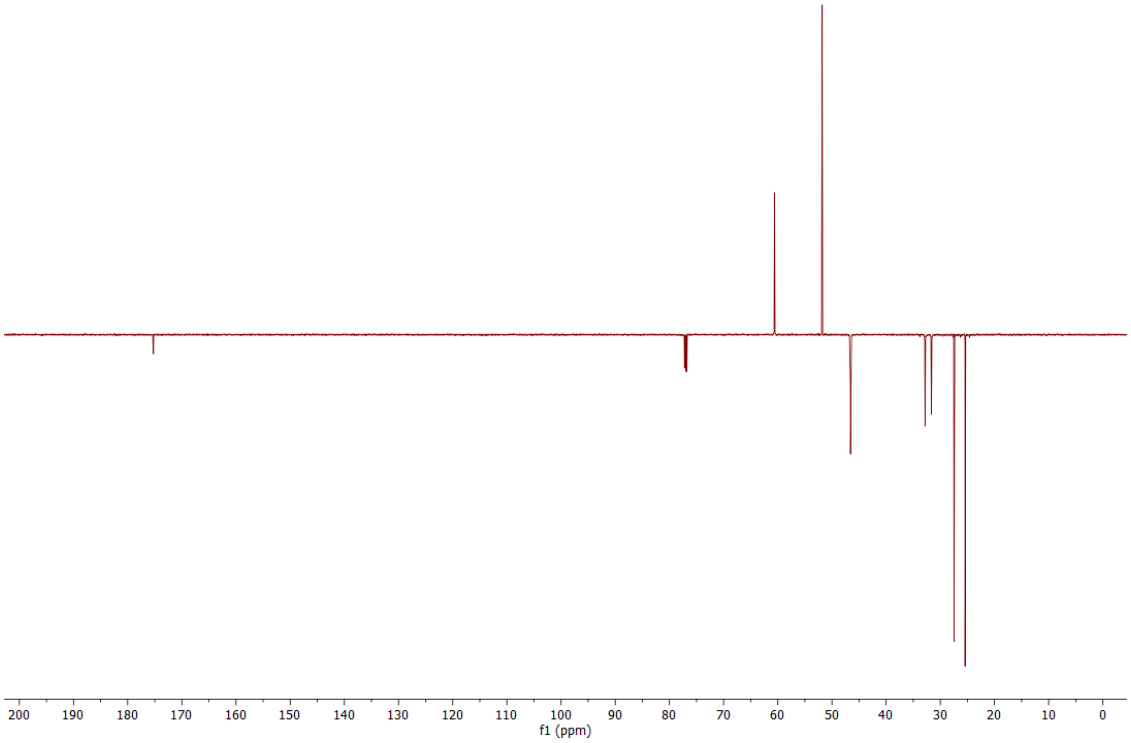


HSQC spectrum of 5b (600 MHz, $CDCl_3$)

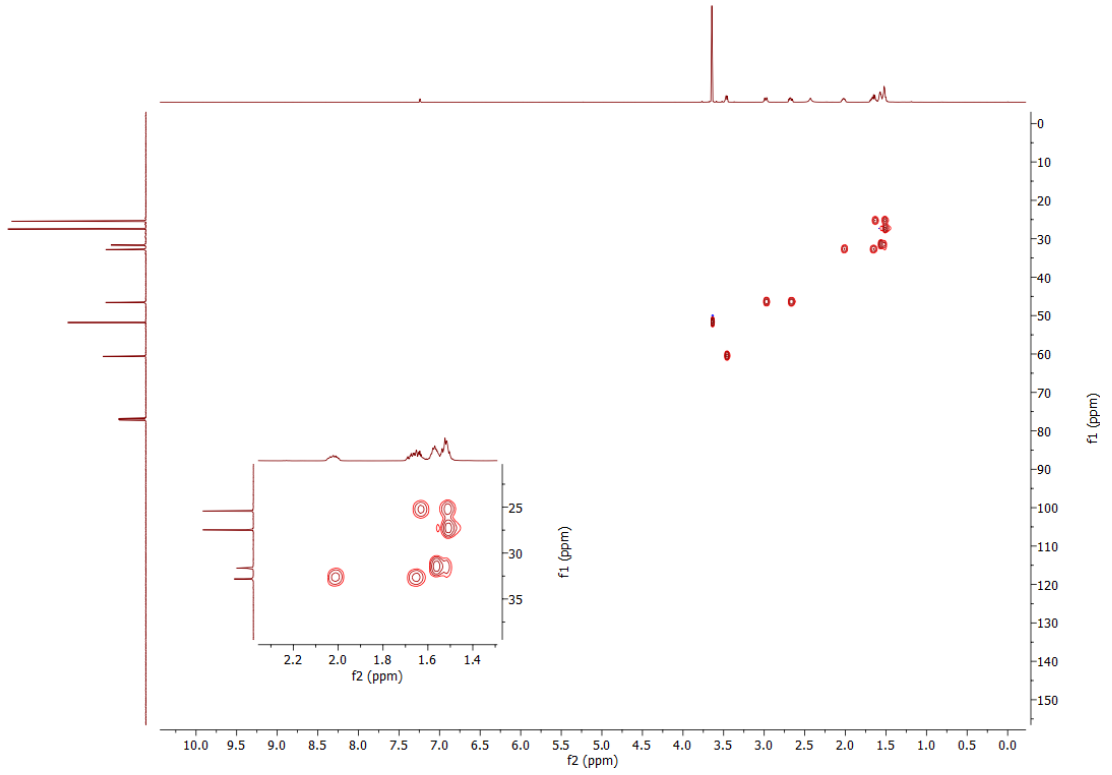


A.1.8 Methyl (*R*)-azepane-2-carboxylate (5c)

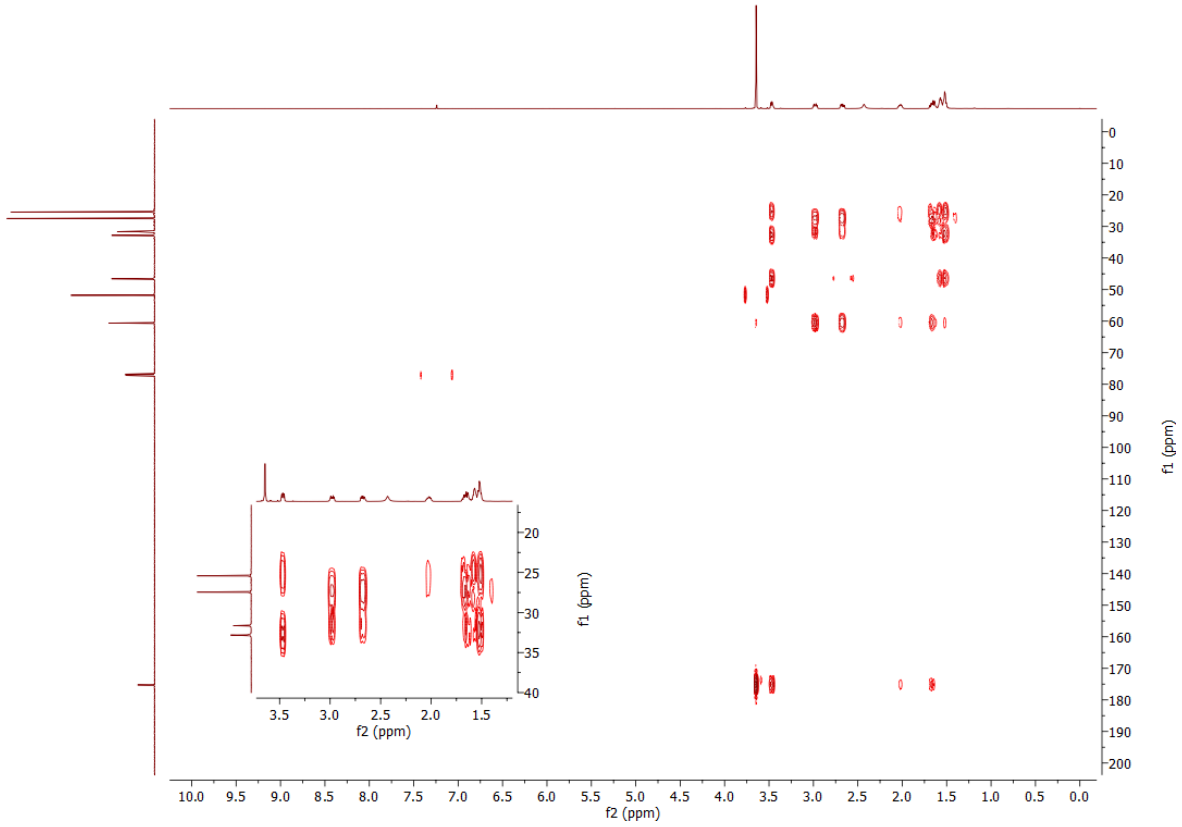
DEPT135Q spectrum of 5c (151 MHz, $CDCl_3$)



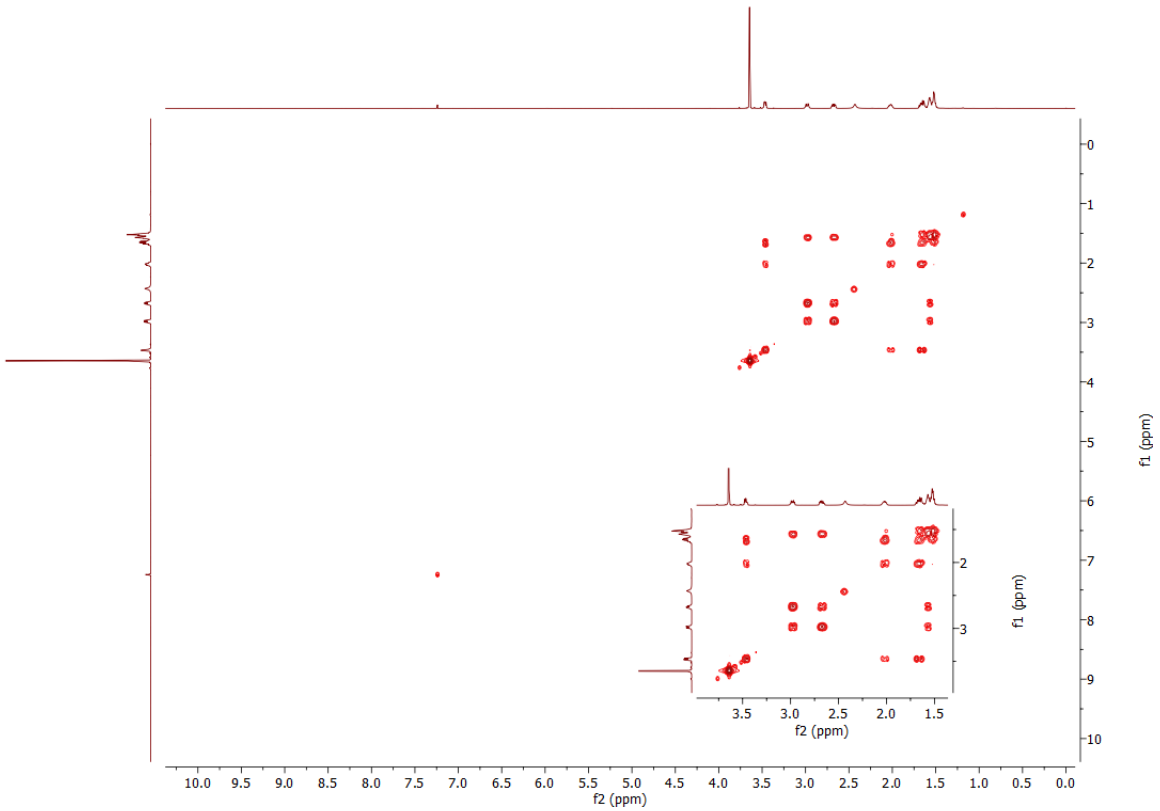
HSQC spectrum of 5c (600 MHz, $CDCl_3$)



HMBC spectrum of 5c (600 MHz, $CDCl_3$)

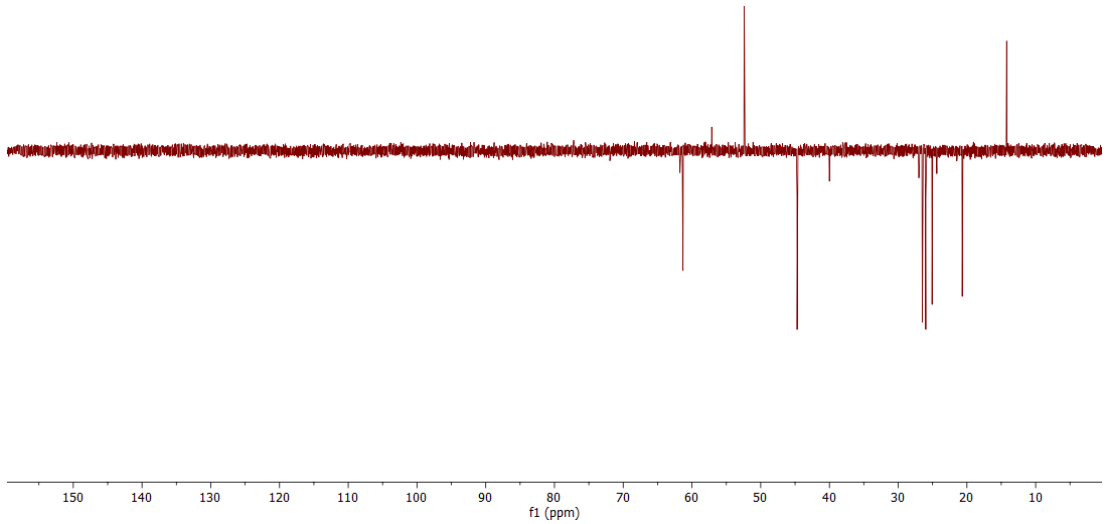


COSY spectrum of 5c (600 MHz, $CDCl_3$)

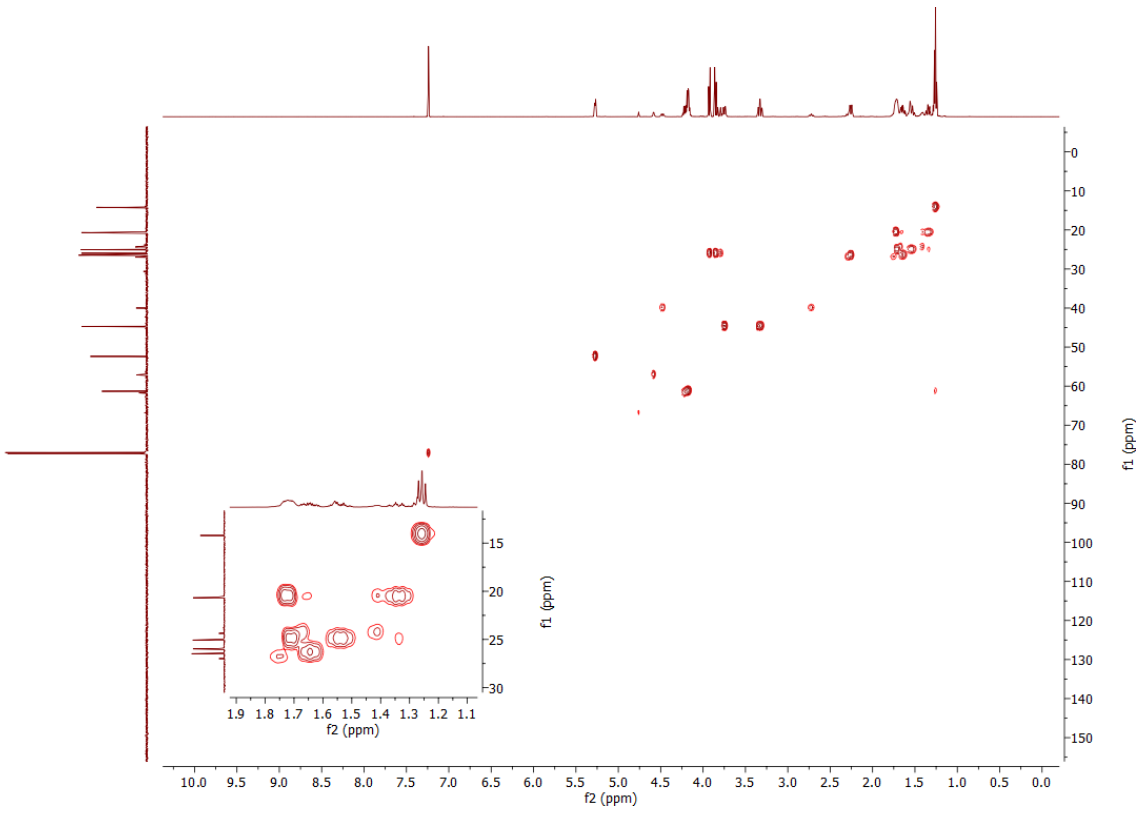


A.1.9 Ethyl-(*R*)-1-(2-bromoacetyl)piperidine-2-carboxylate (2b)

DEPT135 spectrum of 2b (151 MHz, $CDCl_3$)

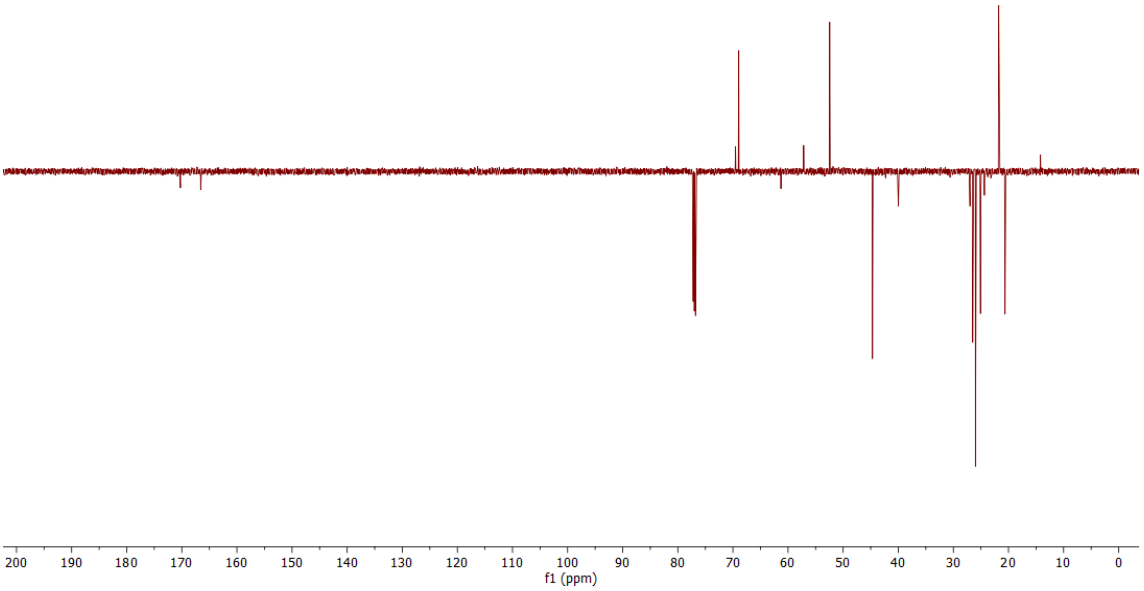


HSQC spectrum of 2b (600 MHz, $CDCl_3$)

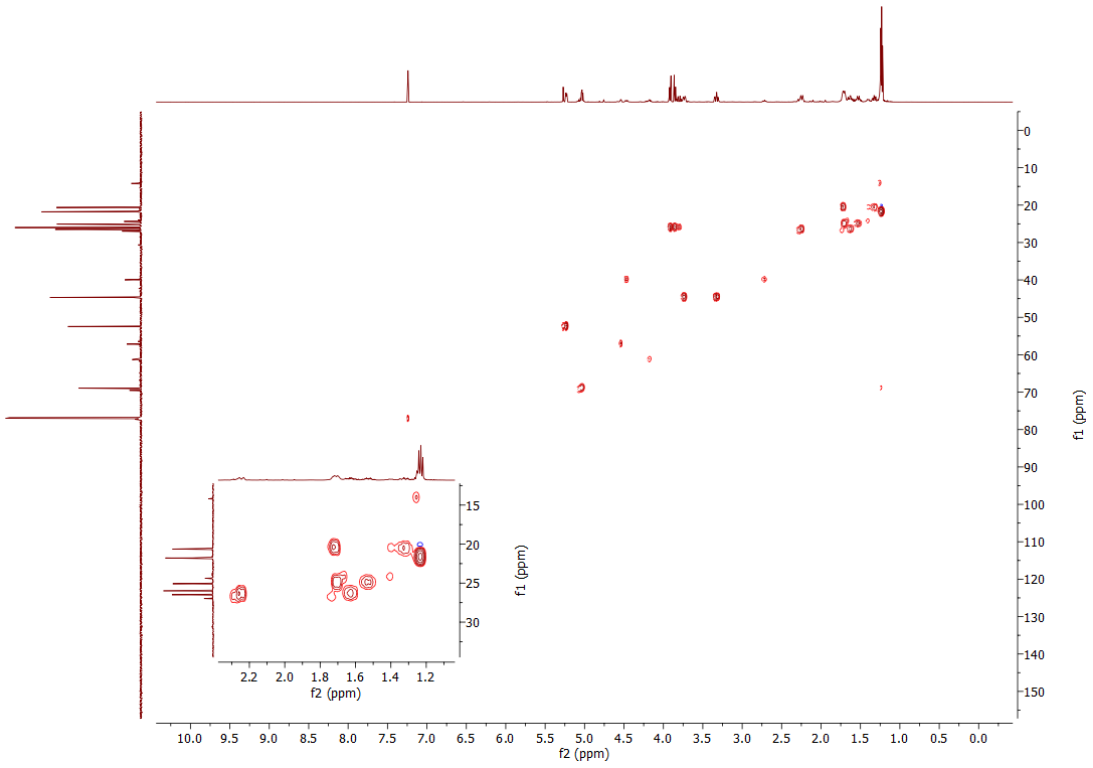


A.1.10 Isopropyl-(*R*)-1-(2-bromoacetyl)piperidine-2-carboxylate (2c)

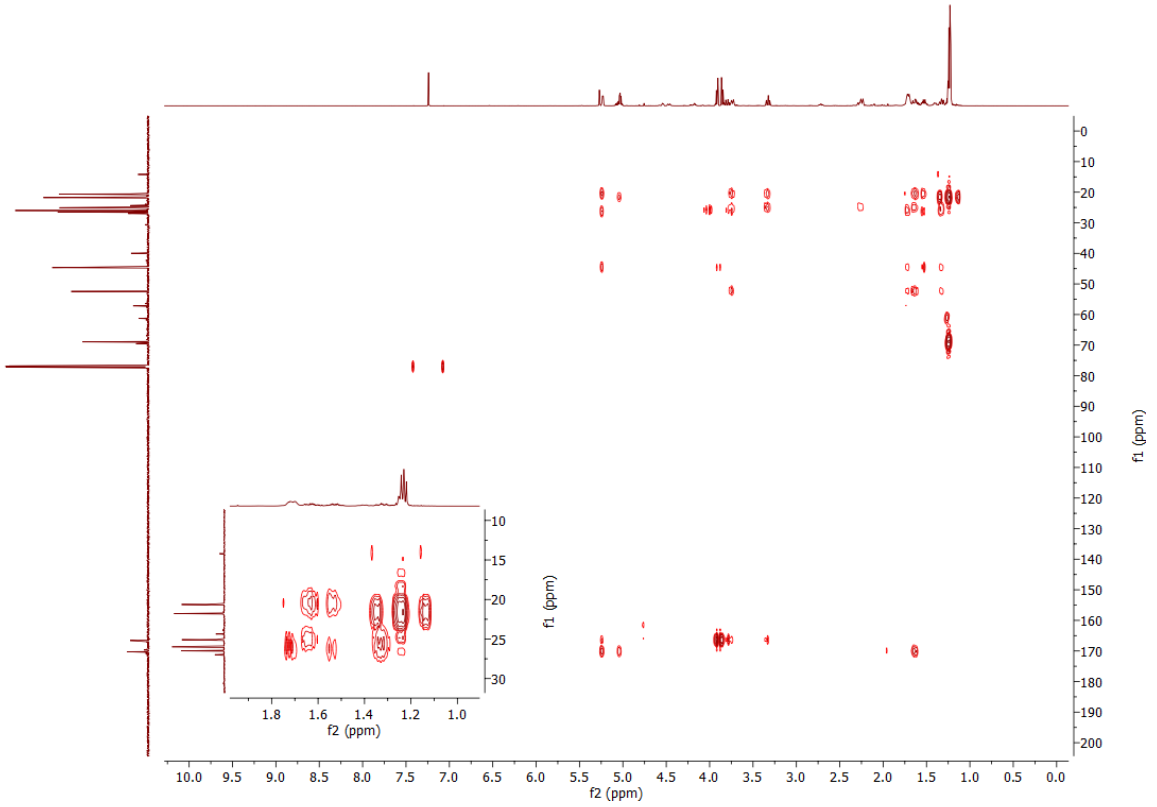
DEPT135Q spectrum of 2c (151 MHz, $CDCl_3$)



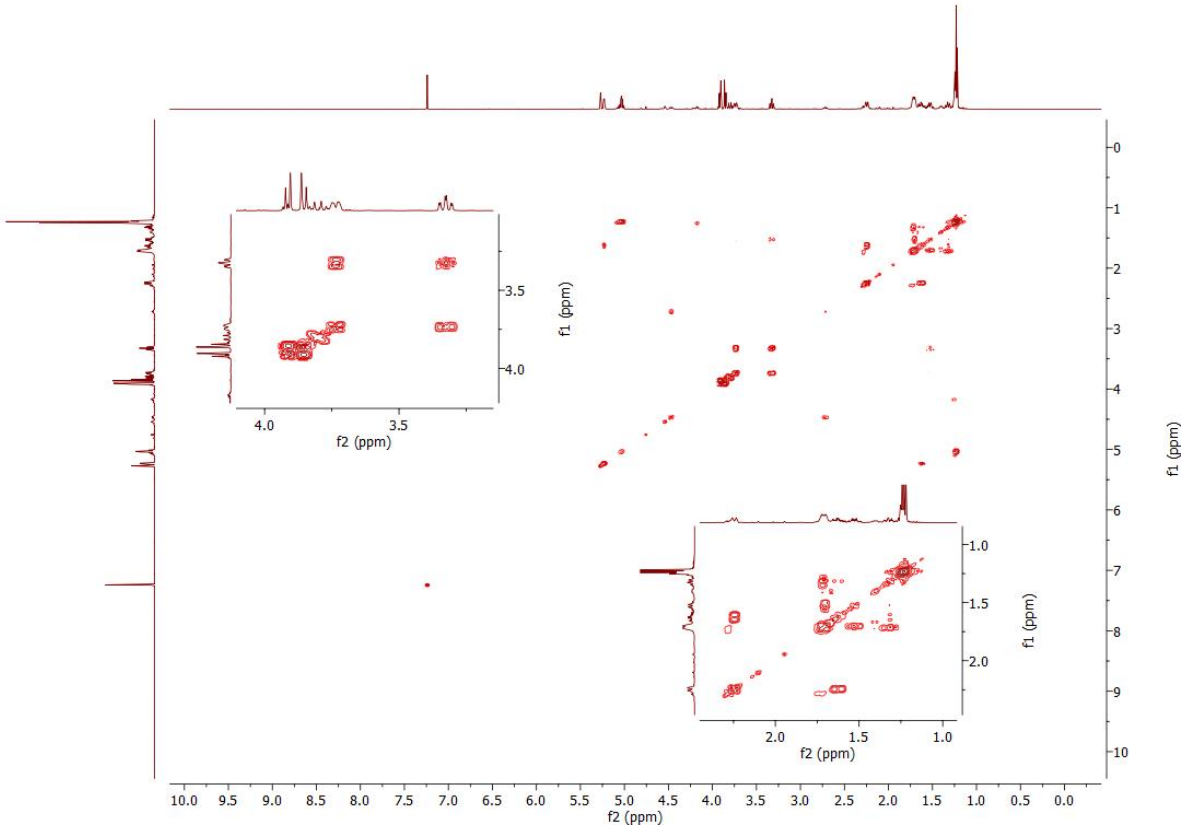
HSQC spectrum of 2c (600 MHz, $CDCl_3$)



HMBC spectrum of 2c (600 MHz, $CDCl_3$)

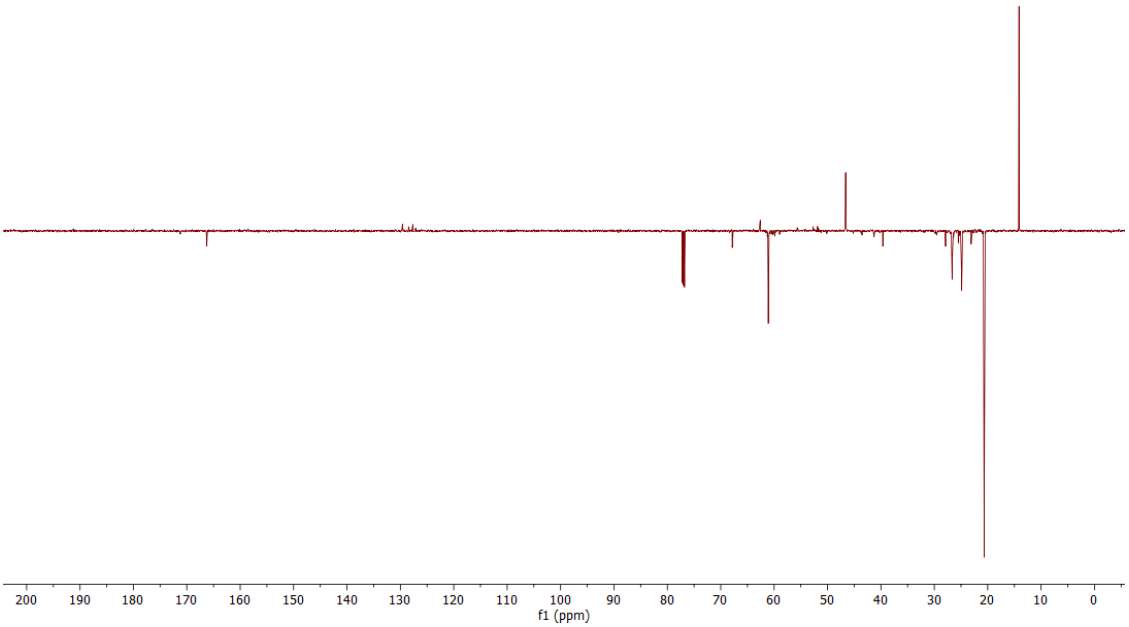


COSY spectrum of 2c (600 MHz, $CDCl_3$)

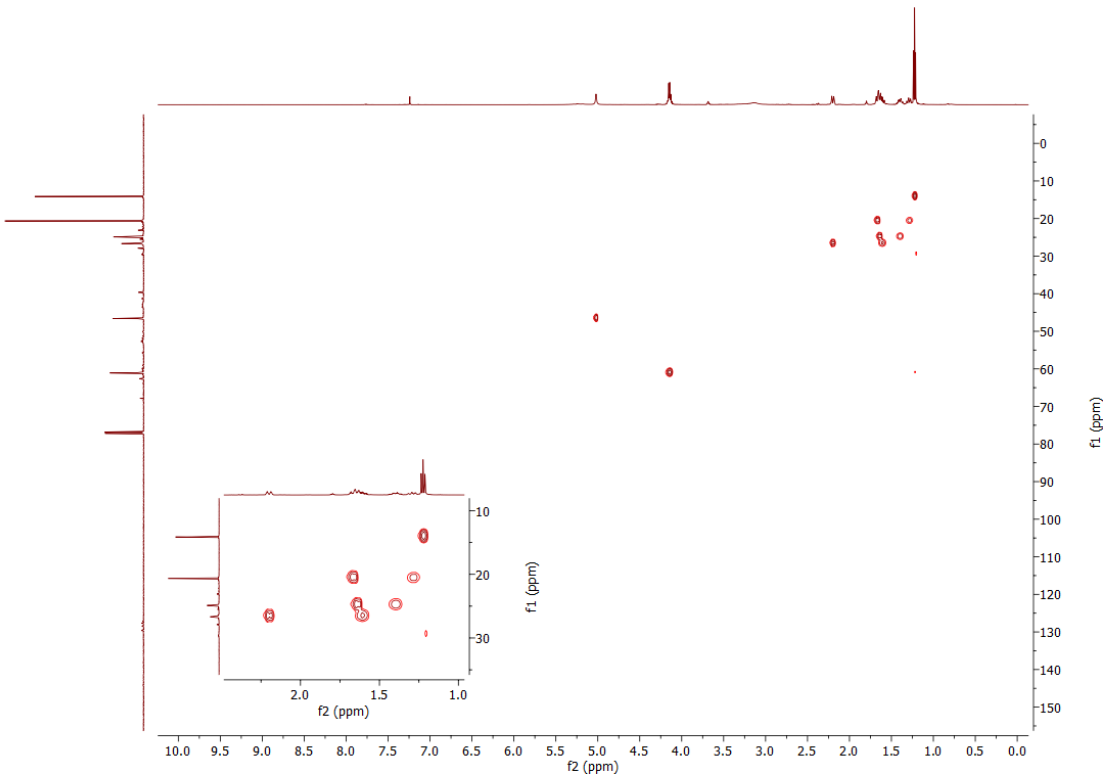


A.1.11 Ethyl (*R*)-1-(2-diazoacetyl)piperidine-2-carboxylate (3b)

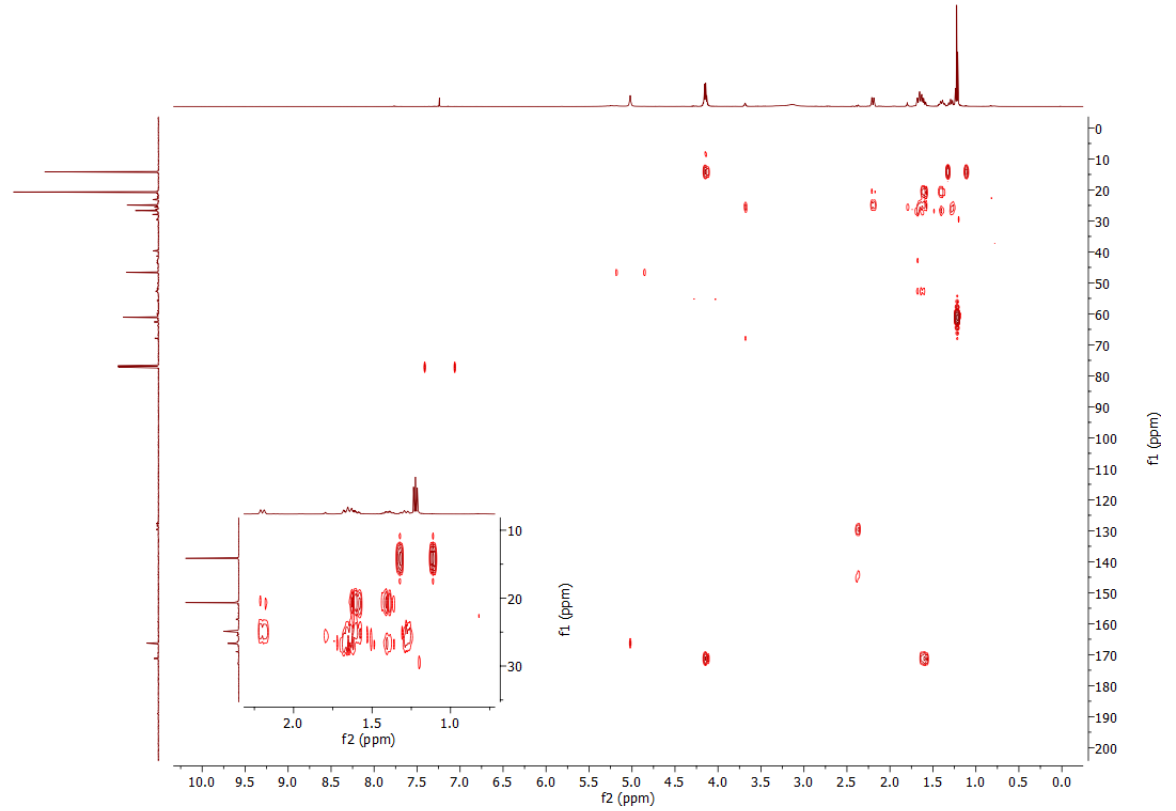
DEPT135Q spectrum of 3b (151 MHz, $CDCl_3$)



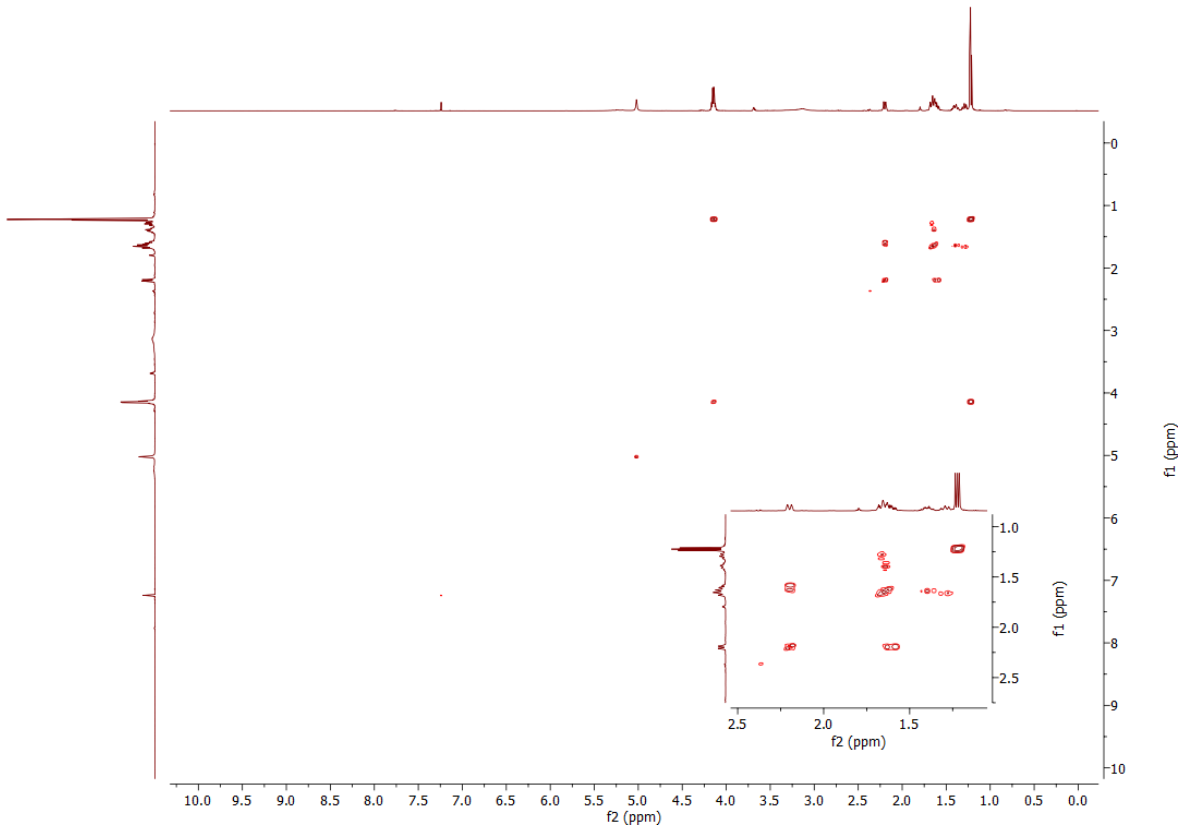
HSQC spectrum of 3b (600 MHz, $CDCl_3$)



HMBC spectrum of 3b (600 MHz, $CDCl_3$)

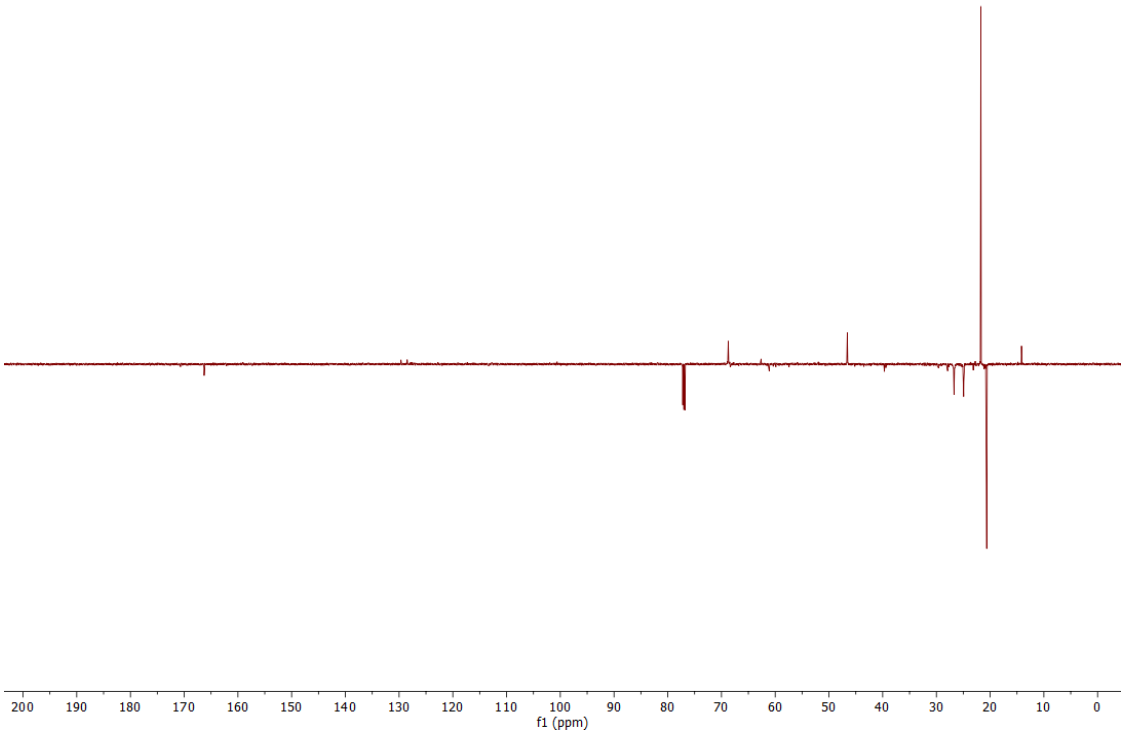


COSY spectrum of 3b (600 MHz, $CDCl_3$)

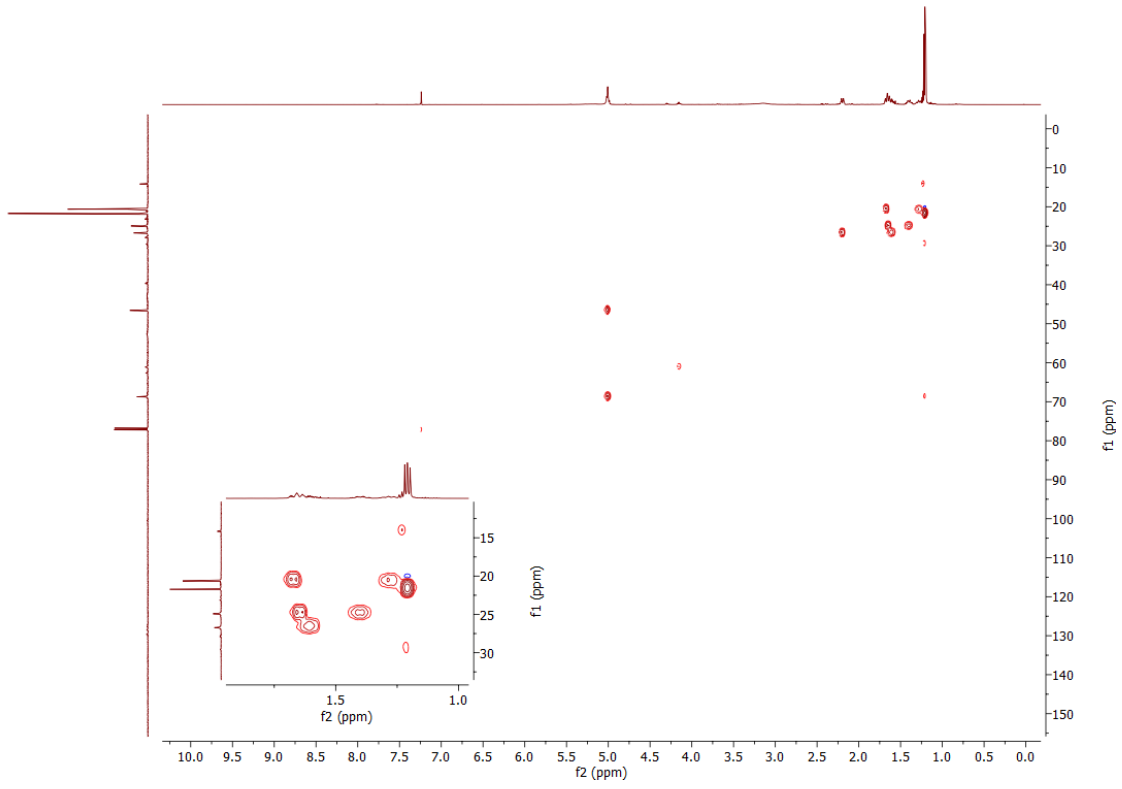


A.1.12 Isopropyl (*R*)-1-(2-bromoacetyl)piperidine-2-carboxylate (3c)

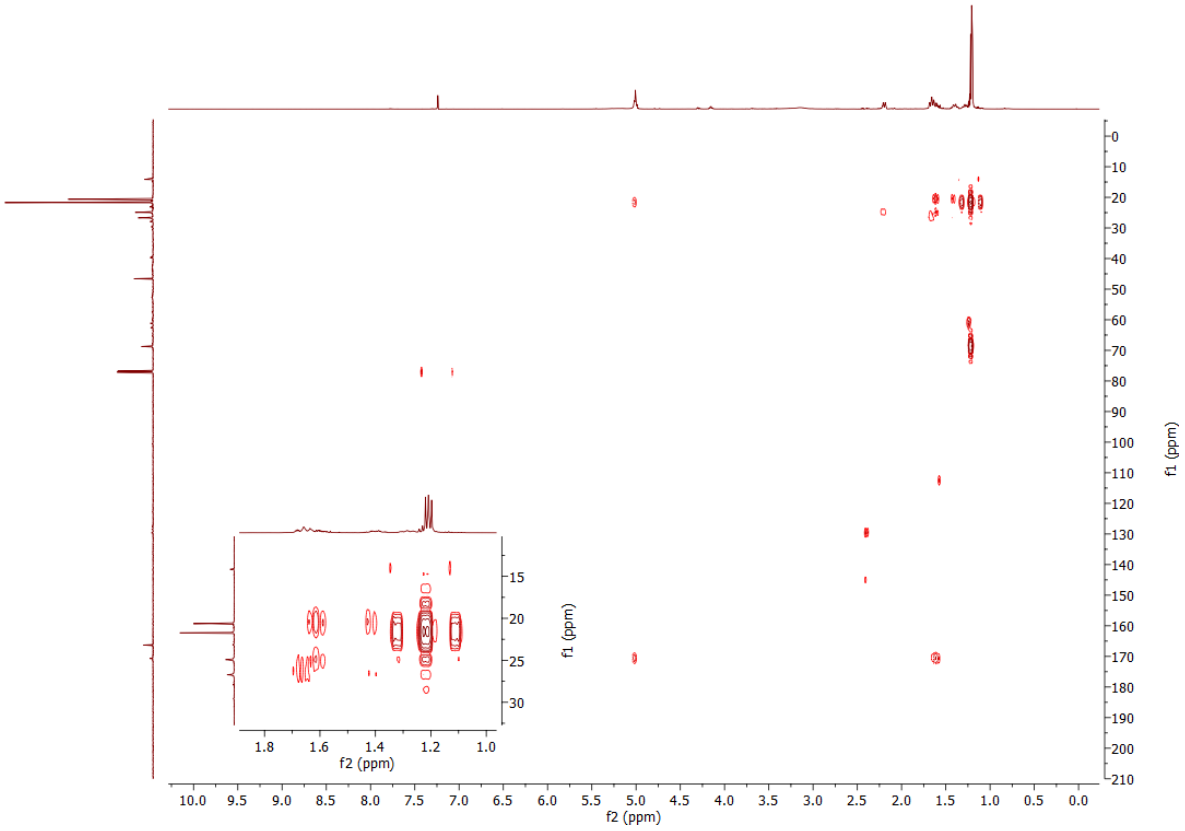
DEPT135Q spectrum of 3c (151 MHz, $CDCl_3$)



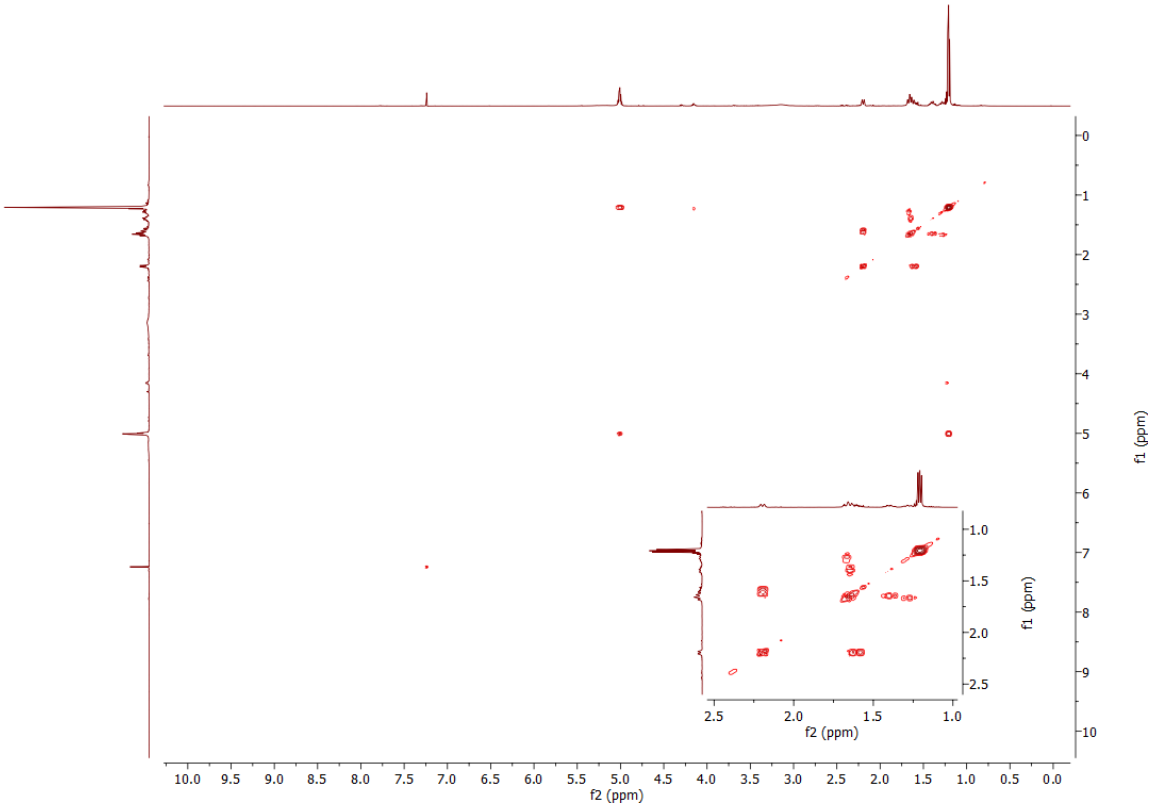
HSQC spectrum of 3c (600 MHz, $CDCl_3$)



HMBC spectrum of 3c (600 MHz, $CDCl_3$)

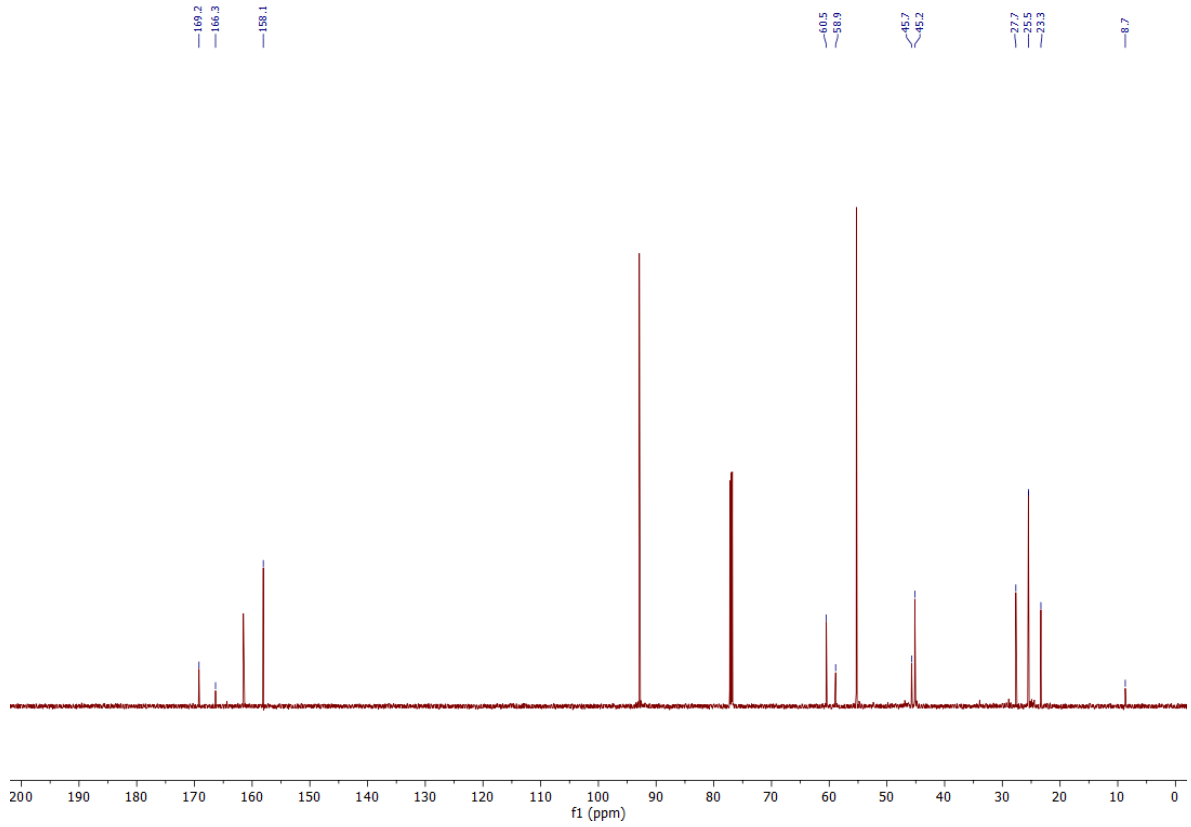


COSY spectrum of 3c (600 MHz, $CDCl_3$)

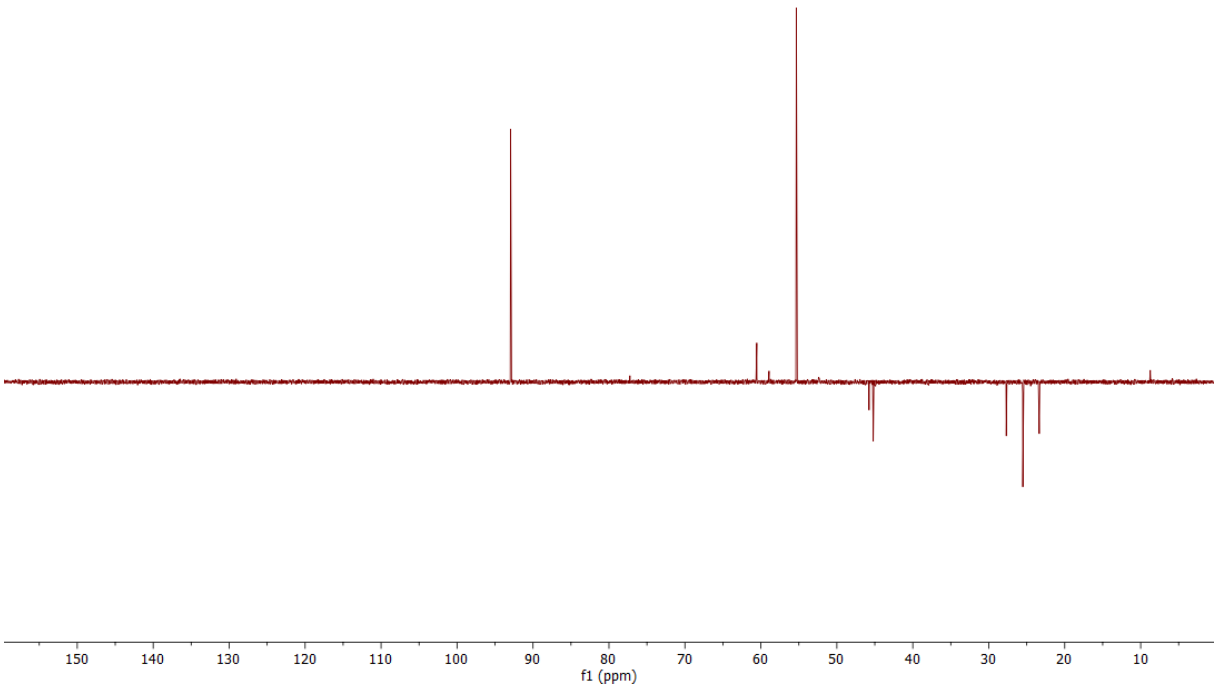


A.1.13 Test reaction for the synthesis of methyl (2-diazoacetyl)-D-prolinate (3d)

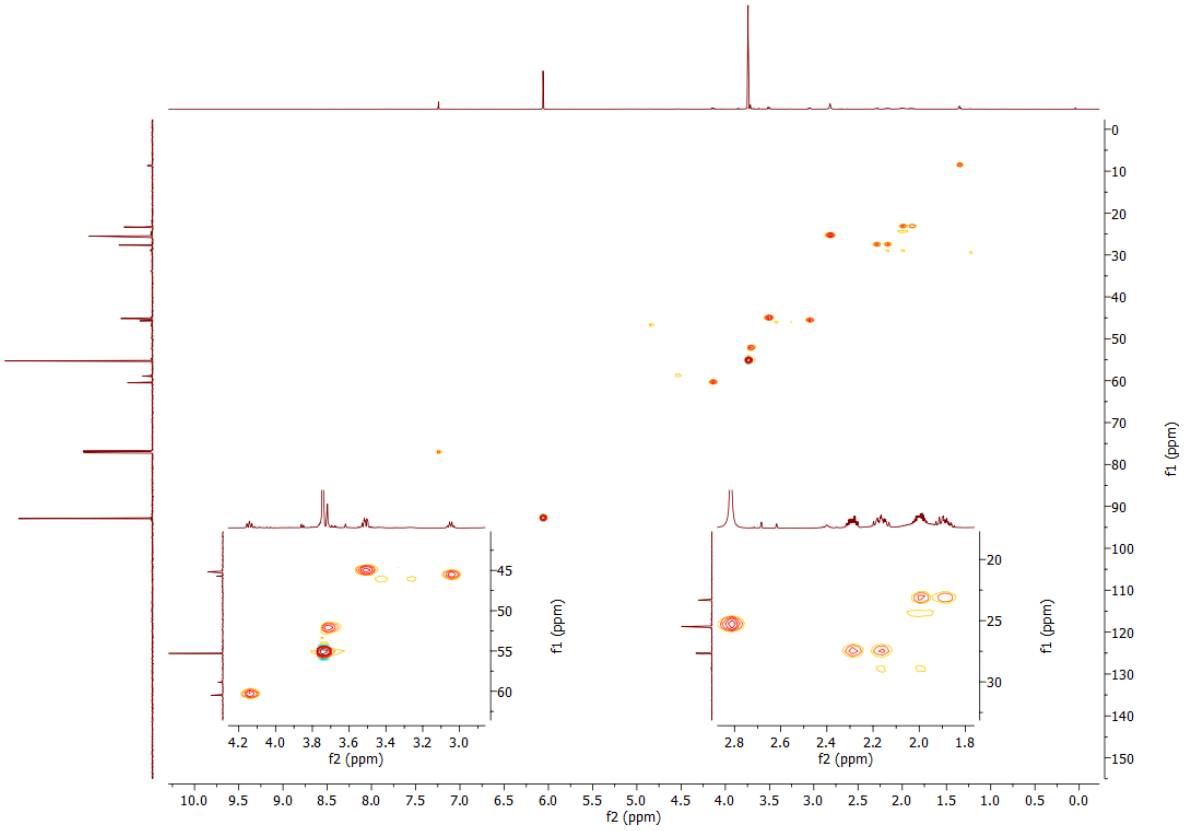
^{13}C NMR spectrum of the test reaction to synthesize 3d (151 MHz, $CDCl_3$)



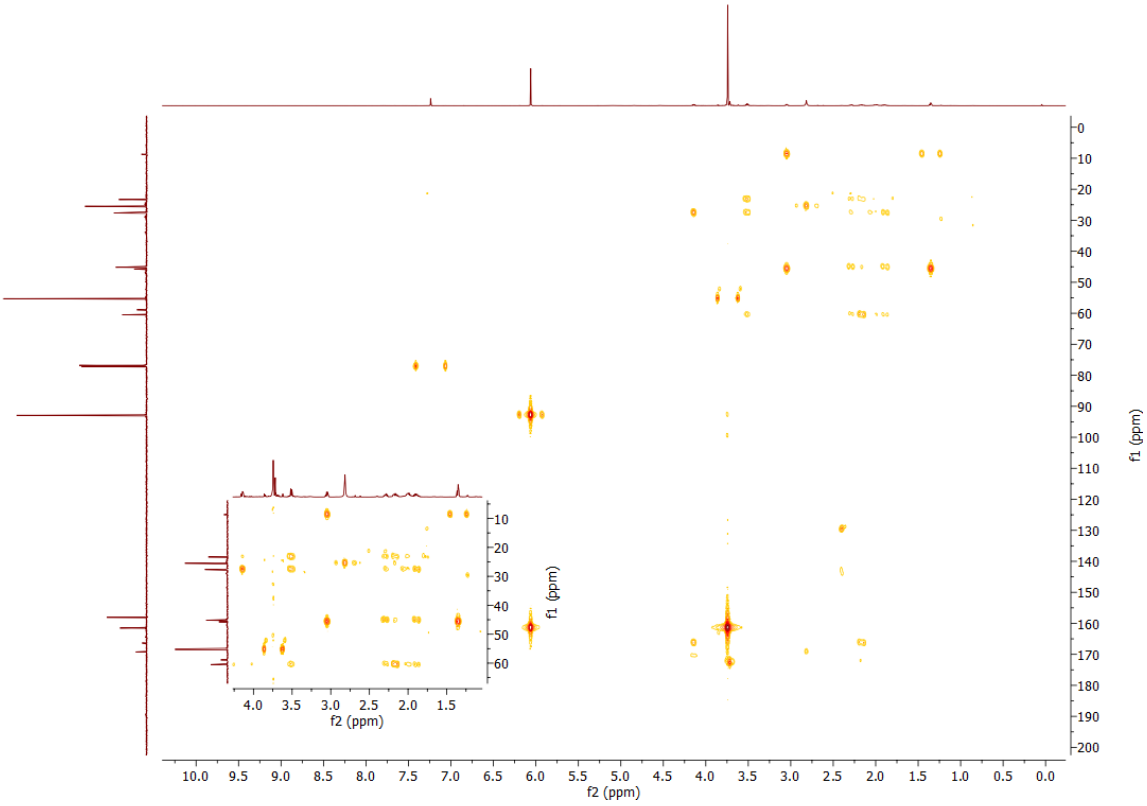
DEPT135 spectrum of the test reaction to synthesize 3d (151 MHz, $CDCl_3$)



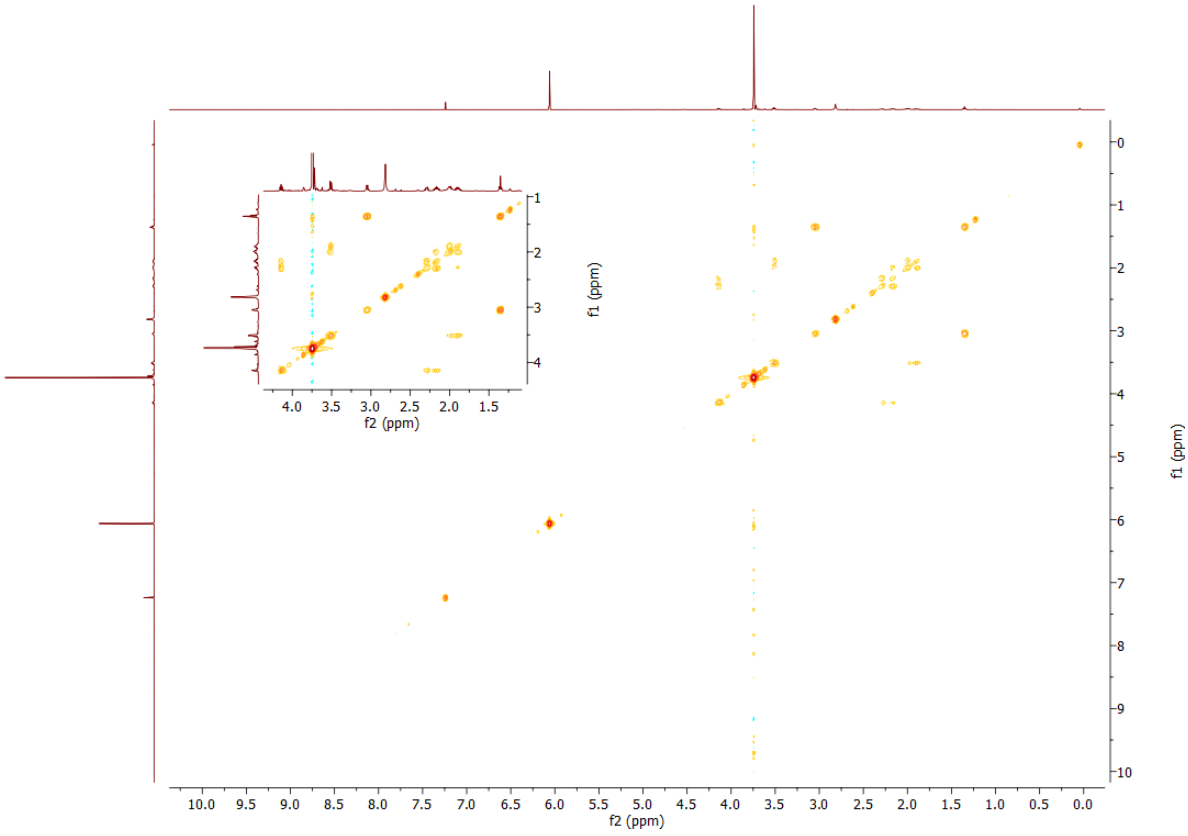
HSQC spectrum of the test reaction to synthesize 3d (600 MHz, $CDCl_3$)



HMBC spectrum of the test reaction to synthesize 3d (600 MHz, $CDCl_3$)

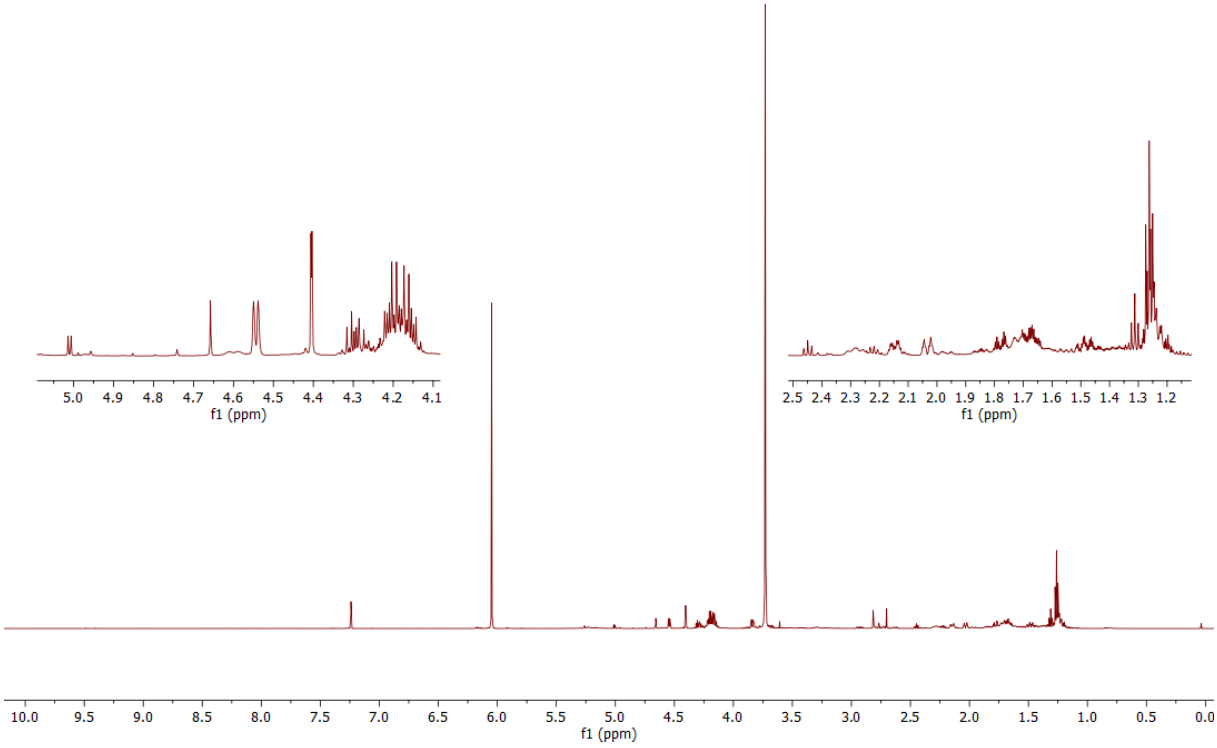


COSY spectrum of the test reaction to synthesize 3d (600 MHz, $CDCl_3$)

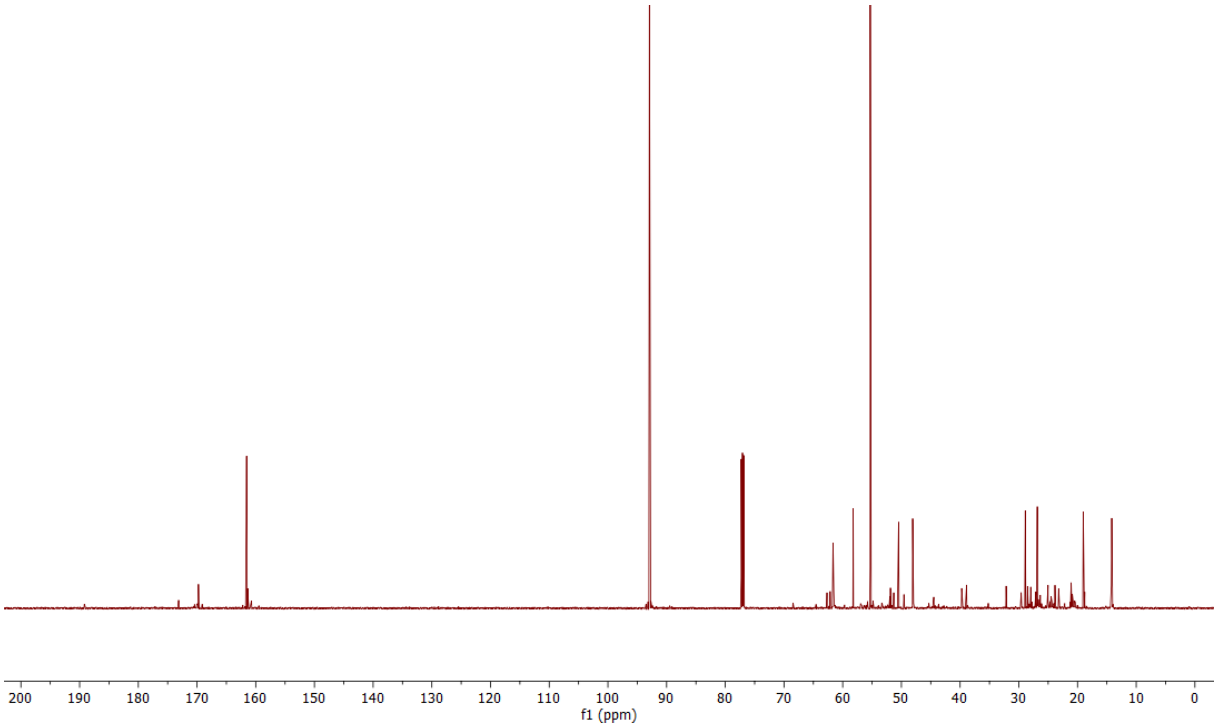


A.1.14 Test reaction for the synthesis of ethyl (2R)-7-bromo-8-oxo-1-azabicyclo[4.2.0]octance-2-carboxylate (6b)

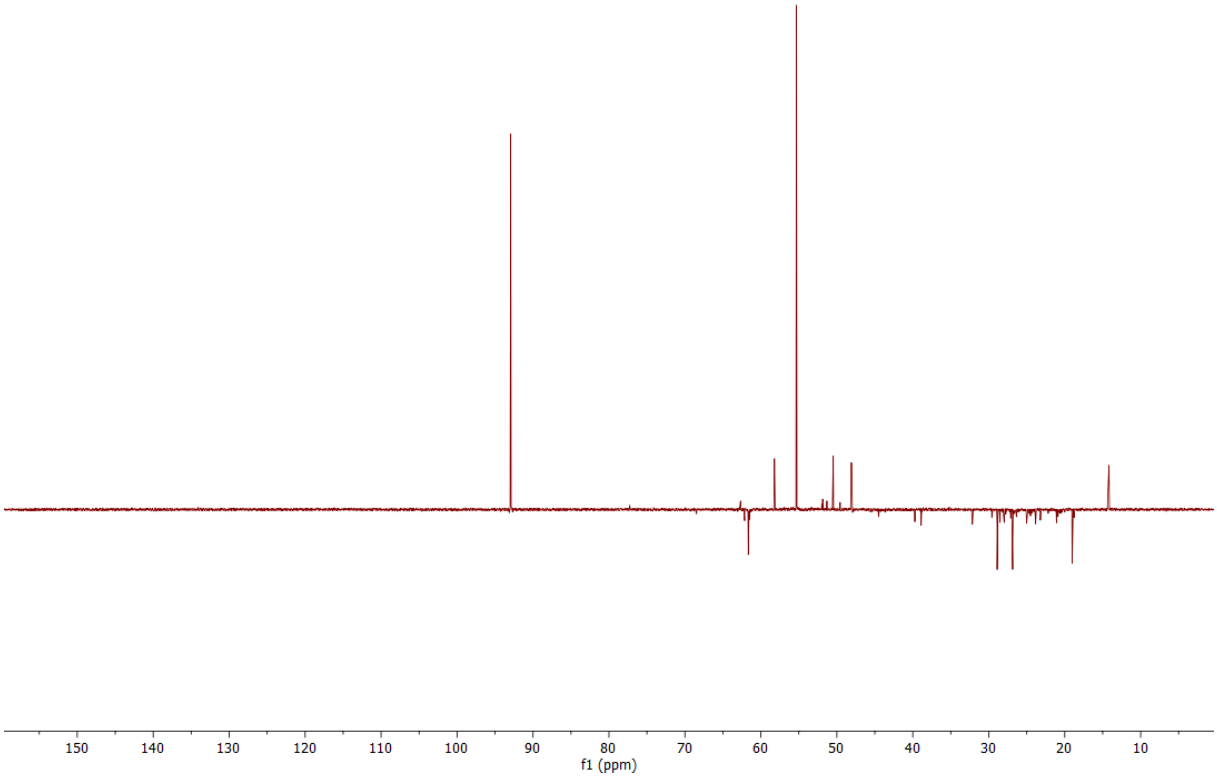
^{1}H NMR spectrum of one of the test reactions to synthesize 6b (600 MHz, $CDCl_3$)



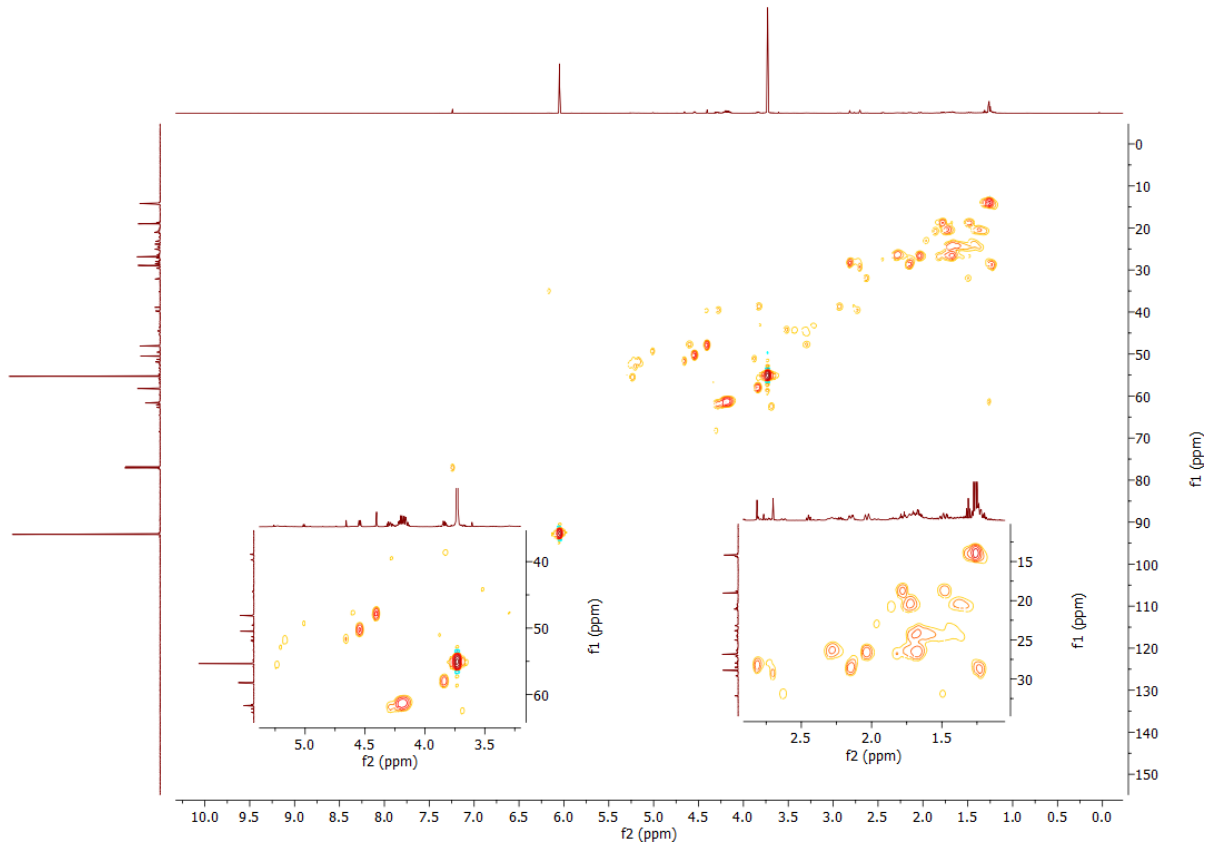
^{13}C NMR spectrum of one of the test reactions to synthesize 6b (151 MHz, $CDCl_3$)



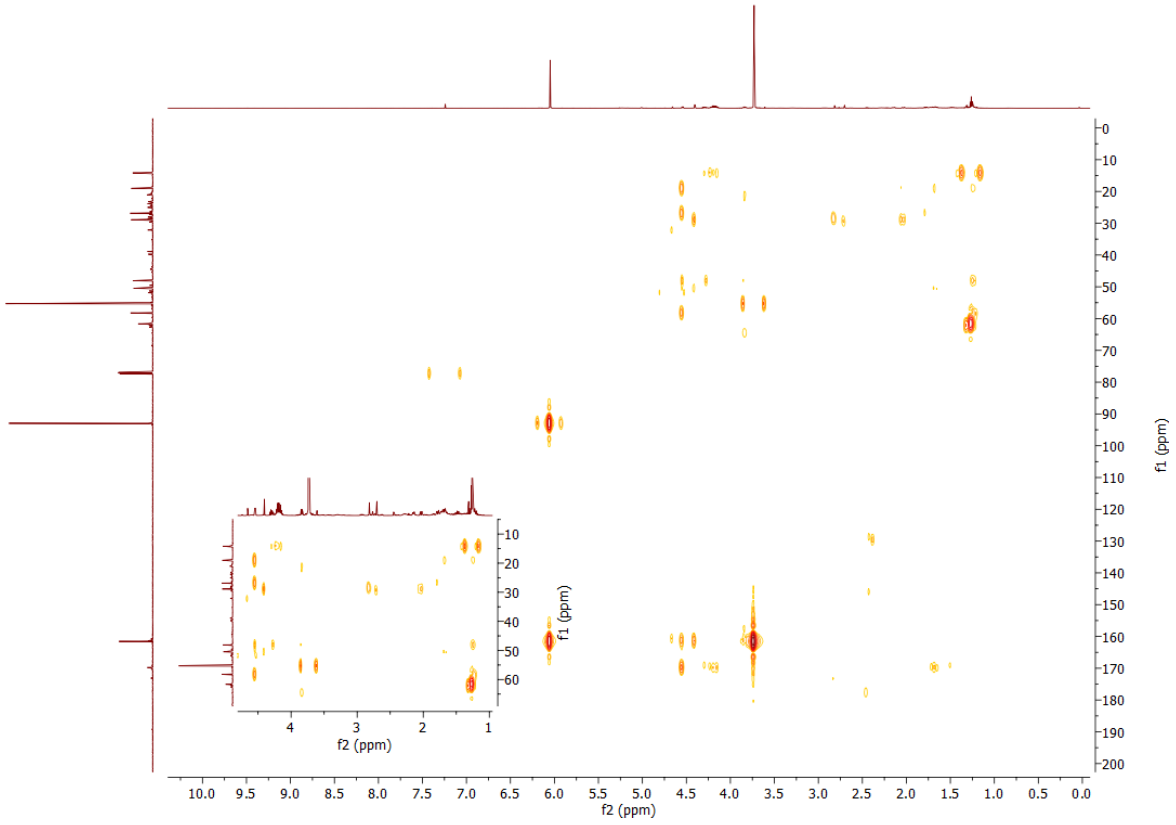
DEPT153 spectrum of one of the test reactions to synthesize 6b (151 MHz, $CDCl_3$)



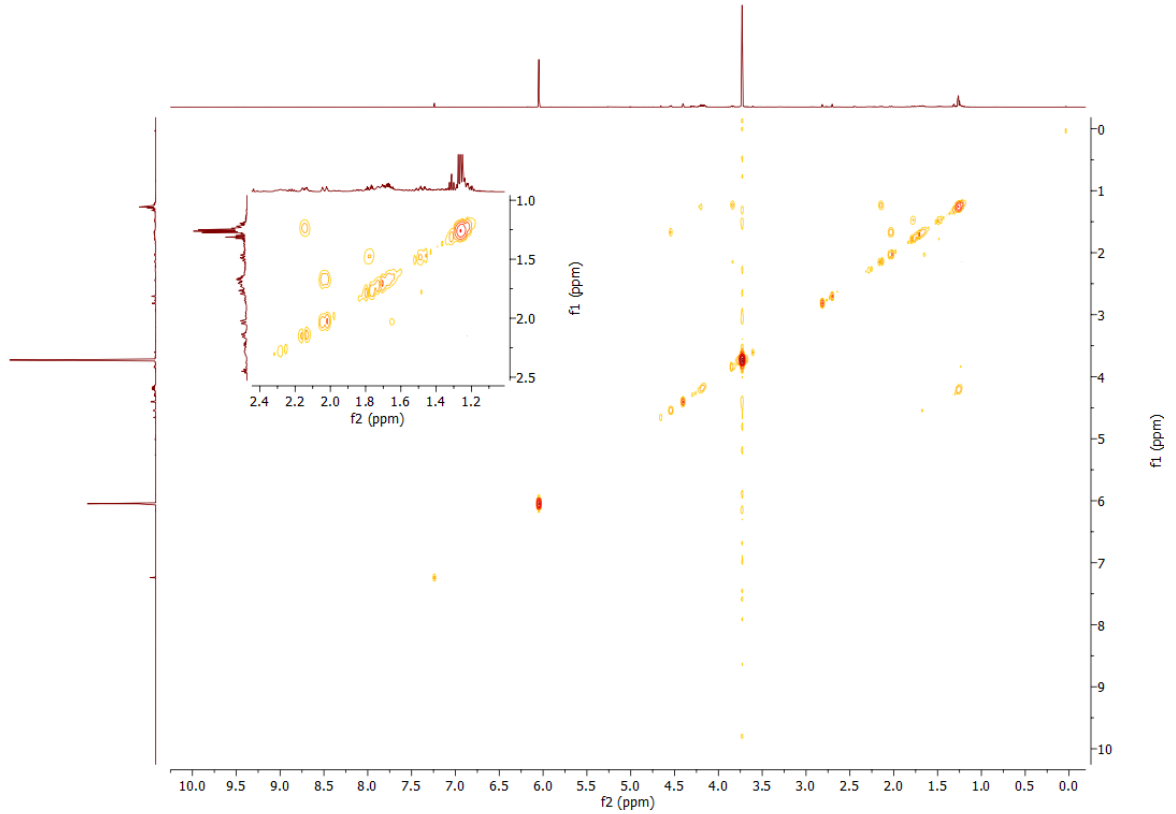
HSQC spectrum of one of the test reactions to synthesize 6b (600 MHz, $CDCl_3$)



HMBC spectrum of one of the test reactions to synthesize 6b (600 MHz, $CDCl_3$)

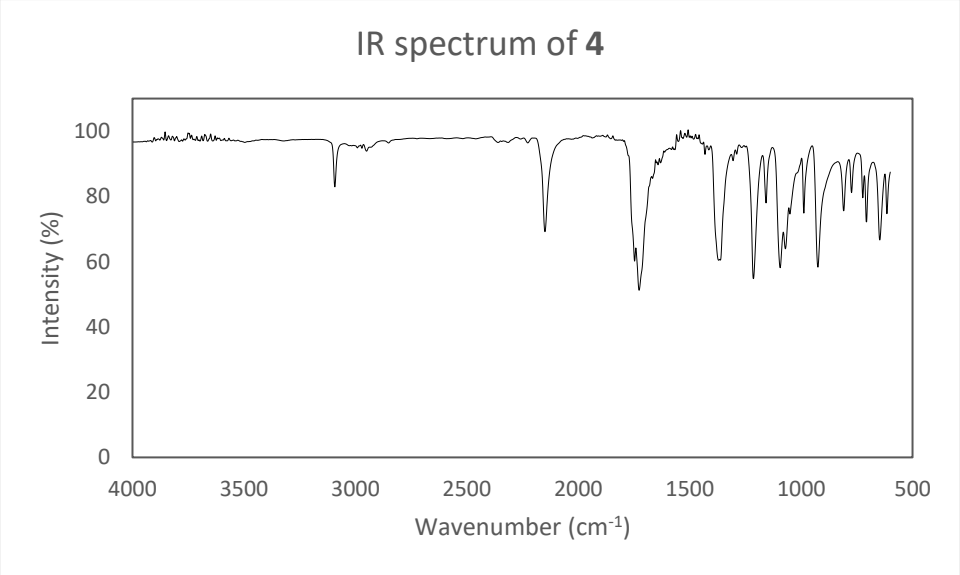


COSY spectrum of one of the test reactions to synthesize 6b (600 MHz, $CDCl_3$)

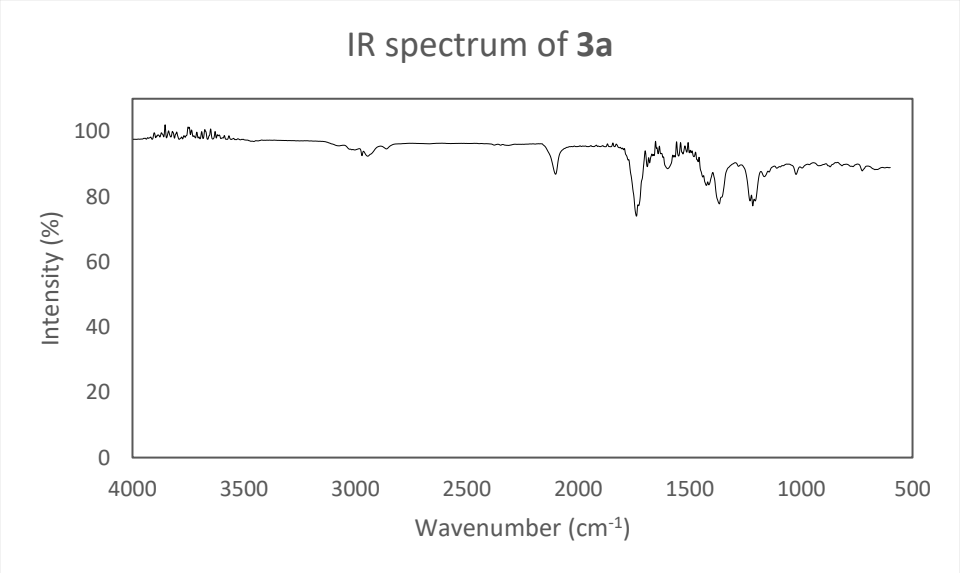


A.2 IR spectra

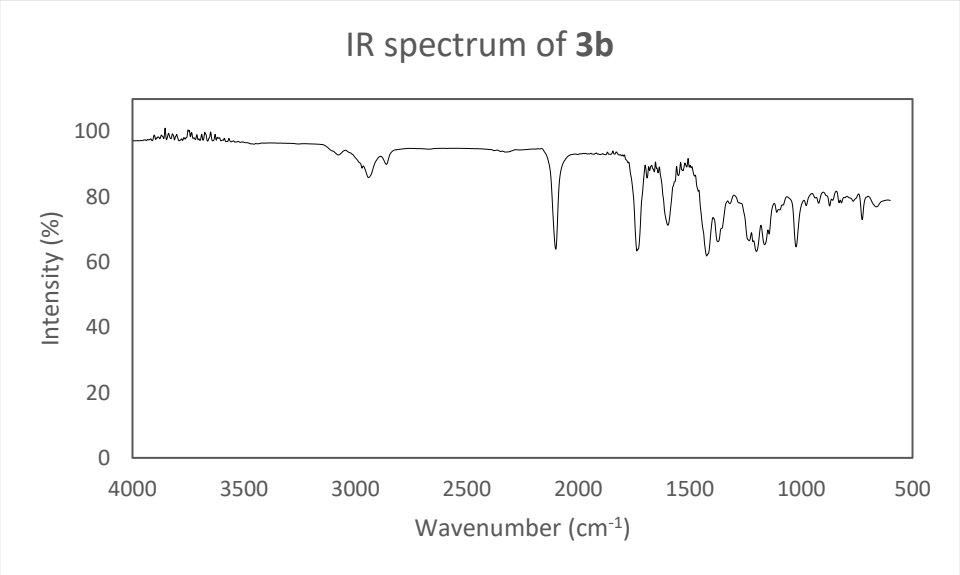
A.2.1 2,5-dioxypyrrolidin-1-yl 2-diazoacetate (4)



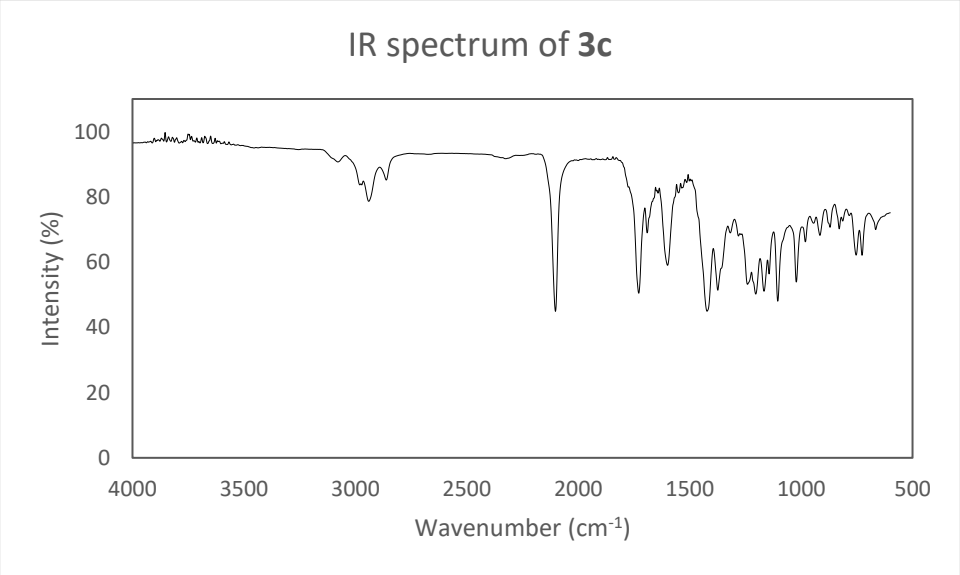
A.2.2 Methyl-(*R*)-1-(2-diazoacetyl)piperidine-2-carboxylate (3a)



A.2.3 Ethyl-(*R*)-1-(2-diazoacetyl)piperidine-2-carboxylate (3b)



A.2.4 Isopropyl-(*R*)-1-(2-diazoacetyl)piperidine-2-carboxylate (3c)



A.2.5 Test reaction for the synthesis of methyl (2-diazoacetyl)-D-prolinate (3d)