POLITECNICO DI MILANO

Facoltà di Ingegneria Industriale e dell'Informazione

Corso di Laurea in
Ingegneria Chimica

SYNTHESIS OF POTENTIAL DDAH INHIBITORS FOR A SAR INVESTIGATION

Relatore: Prof. Alessandro SACCHETTI

Co-relatore: Prof. Matteo ZANDA

Tesi di Laurea di:

Isabella ROMANO Matr. 805528

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Chapter 1 INTRODUCTION

1.1 What is NO and what it causes

Nitric oxide (NO) is a highly reactive gaseous, tiny molecule just composed of two elements: nitrate and oxygen bound together, but it has only recently been discovered as a key biological molecule involved in the physiology of the human body.

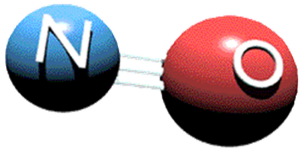


Figure 1.1 Nitrix Oxide

NO has been shown to be involved in many physiological processes, playing an important role in controlling the normal function of cells as well as in regulating larger scale processes as in the nervous and immune systems.
It is produced in various tissues and organs.
For example we can find NO in the digestive system, where it is responsible for the complete digestion of food and a healthy elimination.
NO improves blood flow in the brain as well, increasing oxygen quantity. Nitric oxide plays another important role in neurotransmission, promoting the transfer of nerve signals from one neuron to another. NO is particularly effective as a messenger because it spreads rapidly because its molecule is very small and apolar, but remains fairly localized because it is very reactive and is quickly destroyed.

NO is also produced in high amounts from specialized cells of the immune system called macrophages. They use it to kill pathogens, together with other reactive oxygen compounds, in the aftermath of a bacterial infection.

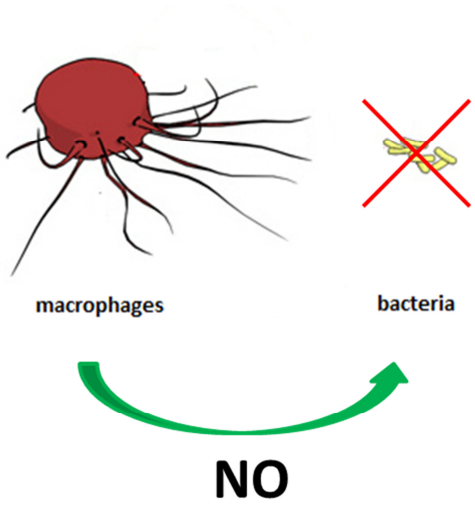


Figure 1.2 Macrophages use NO to kill bacteria

At the same time however, NO is a compound metaphorically considered a *double-edge sword* because a disregulation in its production leads to the development of several diseases. In this regard we can distinguish two cases, when the Nitric oxide is underproduced and when is overproduced.

In case its concentration is insufficient, it is associated with hypertension, pulmonary hypertension, coronary heart disease, heart failure, myocardial infarction and a low-level production has been found in malignant human breast, neuronal, gastric and many other cancers.

On the other hand, an excess in the NO synthesis is involved in disorders such as migraine, neurodegeneration (for example Parkinson, Alzheimer and sclerosis) and septic shock.

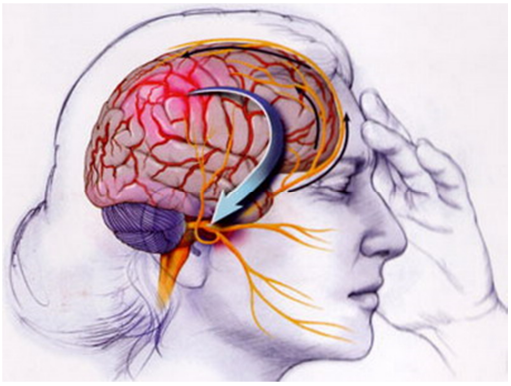


Figure 1.3 Migraine

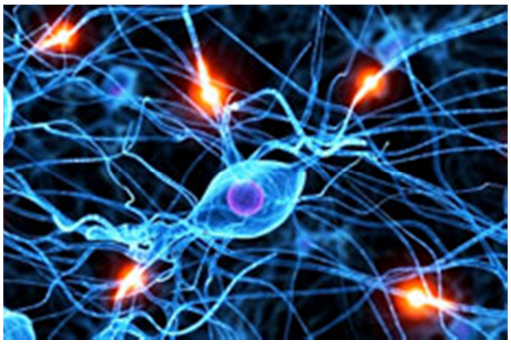


Figure 1.4 Nuerodegeneration

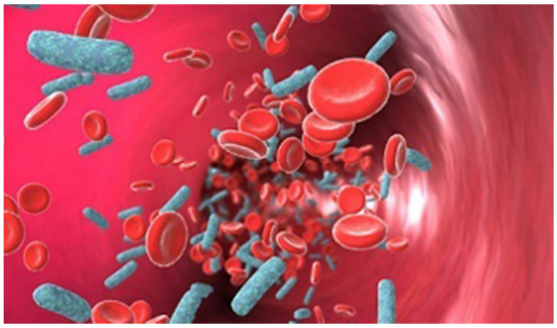


Figure 1.5 Septic shock

The damaging effects of this radical are reflected by the fact that high concentrations produced in a continuous way can rapidly react with superoxide anions (O_2-) which are simultaneously produced in the site of inflammation and generate peroxynitrite ($ONOO^-$), a potent oxidant that plays various pathophysiological roles in the development of inflammation.

Even more recently, research seems to indicate an involvement of NO in tumor angiogenesis.

As tumors grow, their original vascularization can be insufficient to supply the growing tissue mass.

Precisely this neovascularization is a key feature determining growth and metastasis of malignant tumors, where nitric oxide would promote tumor angiogenesis by upregulating VEGF (Vascular Endothelial Growth Factor).

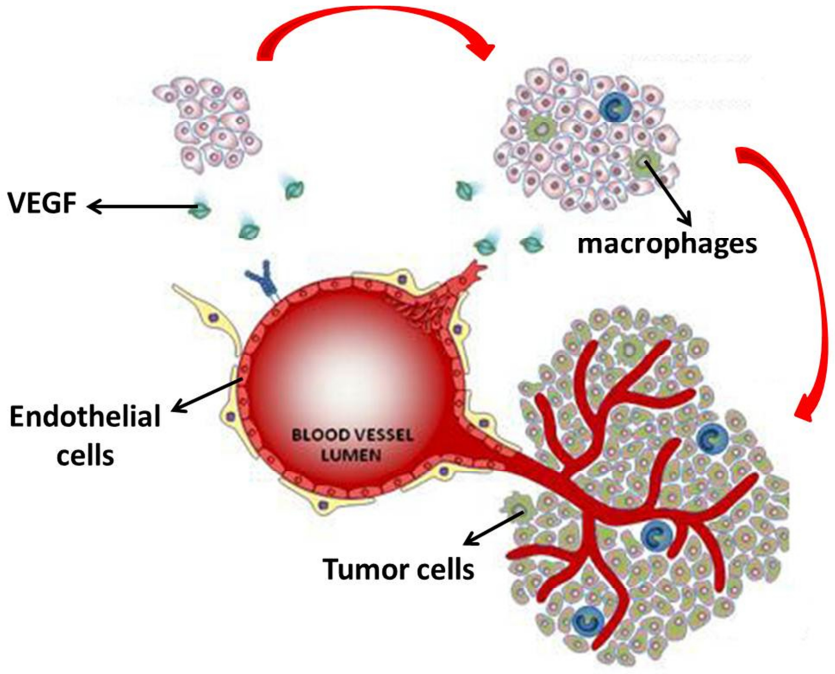


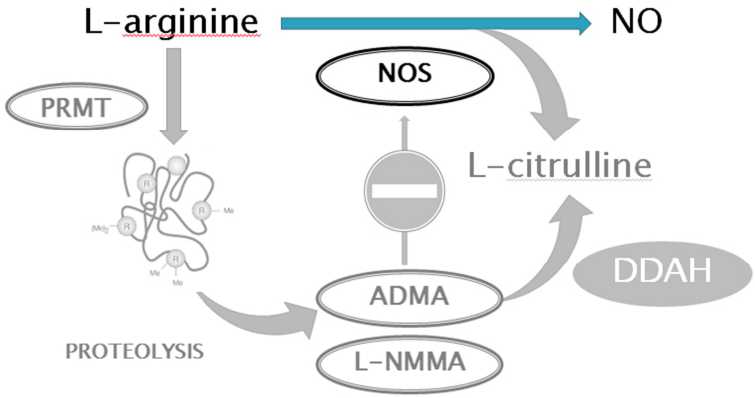
Figure 1.6 Tumor's angiogenesis

In view of all these studies and researches, we can assert that NO plays a very important role in the human body.

In the last decades, the attention has been focused on diseases of the nervous system, which represent a significant human, societal and economic burden in continuous growth in the elderly population and so, new pharmacotherapies are strongly needed.

1.2 How nitric oxide is formed in the human body

Before considering the modulation of the NO concentration, we must understand how it is produced by the body, and then locate the originating point.



Biosynthesis of NO is complex and involves an initial hydroxylation of the guanidine group ($H_2N(C=NH)NH$-) in the side chain of L-arginine (molecule identified in mammals), leading to the formation of N^G-hydroxy-L-arginine. This is followed by oxidation of the intermediate to form L-citrulline and NO.

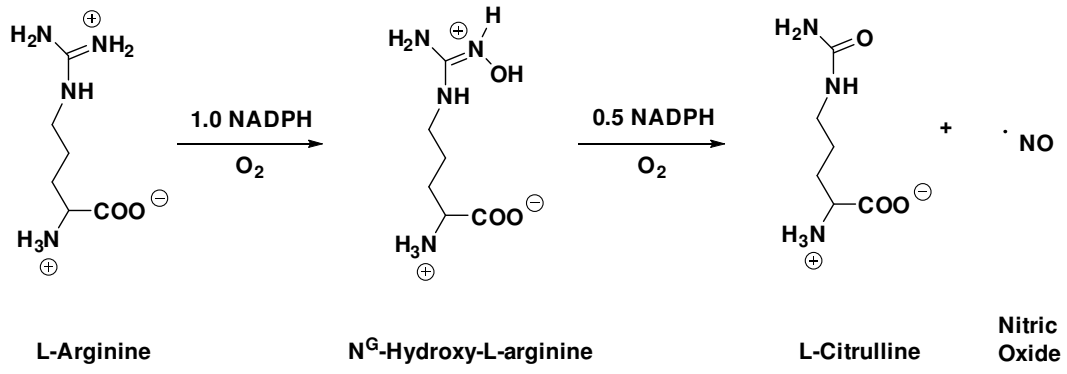


Figure 1.7 Synthesis of NO in the human body

The reaction is catalyzed by NOSs (Nitric Oxide Synthase), family of enzymes.
Mammalian cells produce three isoforms of NOS, which differ from one another because of the nitric oxide function they generate.

The neuronal NOS (nNOS) and endothelial NOS (eNOS) continuously produce low levels of NO that acts as a neurotransmitter and vasodilator, respectively.

The inducible NOS (iNOS), instead, produces greater amount of NO that is toxic and is used to attack pathogens.

All three of these enzymes are complex and are composed of several subunits (domains).

Generally speaking, in the Figure 1.8 we can see the subunit located at the top (oxidant subunit) whose role is to produce NO with the help of the heme group (in pink color), which in turn adds an oxygen atom from O_2 to nitrogen in the side chain of an amino acid arginine (in yellow color) converting it into citrulline.

The subunit at the bottom (reducing subunit) donates electrons to the top and contains a reducing coenzyme such as NADPH. It is a protein and an important cofactor in many biosynthesis pathways that control fundamental cellular processes. It generates reactive oxygen species. In this way, NADPH can transfer electrons across biological membranes (in green color).

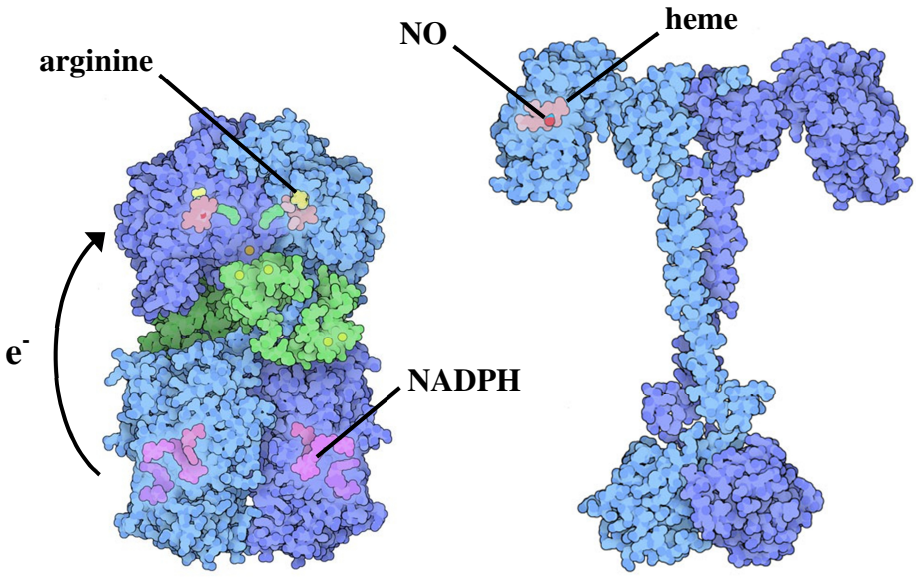


Figure 1.8 nNOS subunit (left), eNOS subunit (right)

1.3 How nitric oxide production is controlled

As mentioned before, NO biosynthesis is tightly regulated.

This natural regulation has suggested to the researchers new useful targets for therapeutics.

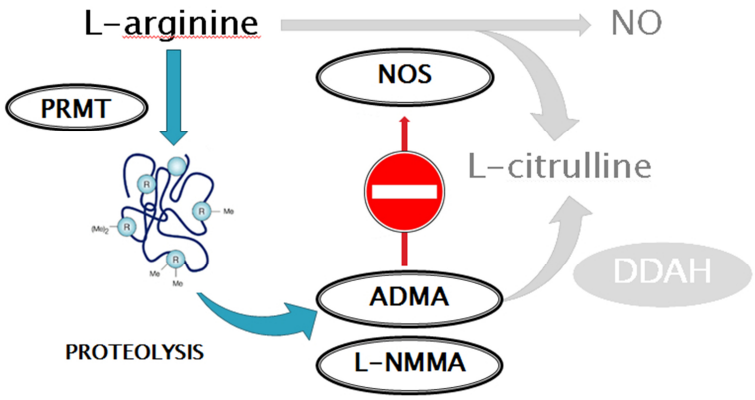
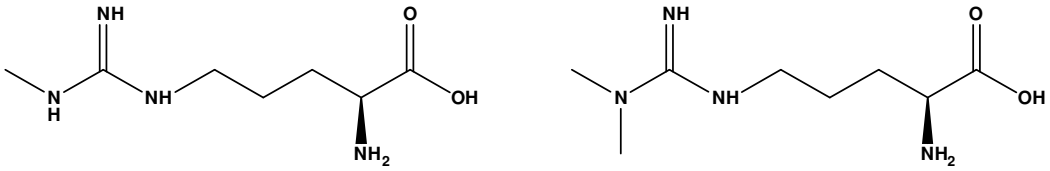


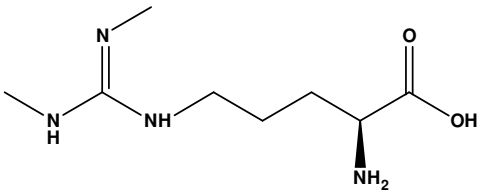
Figure 1.9 Inhibitor production

One such regulation mechanism involves pools of endogenous NOS inhibitors. The arginine analogues identified include: N^{ω}-methyl-L-arginine (L-NMMA), asymmetric N^{ω}, N^{ω} -dimethyl-L-arginine (ADMA) and symmetric N^{ω}, N'^{ω}-dimethyl-L-arginine (SDMA).



a)

b)



c)

Figure 1.10 a) L-NNMA b) ADMA c) SDMA

These methylated arginines are generated by PRMTs (class of enzymes called protein arginine methyltransferases), which have the task of adding one or two monomethyl groups to the guanidinium side chain of arginine residues. Then L-NMMA, ADMA and SDMA are released during proteolysis of these proteins containing methyleted arginine residues.

The L-NMMA and ADMA, but not SDMA, are competitive direct inhibitors of the nitric oxide synthase enzymes. The concentration of these methylated arginines is controlled by renal excretion and in particular by another enzyme: dimethylarginine dimethylaminohydrolase, **DDAH**, which hydrolyzes both substrates converting them into L-citrulline and either methylamine (substrate L-NMMA) and dimethylamine (substrate ADMA), thus relieving the inhibition of NOS and promoting NO biosynthesis.

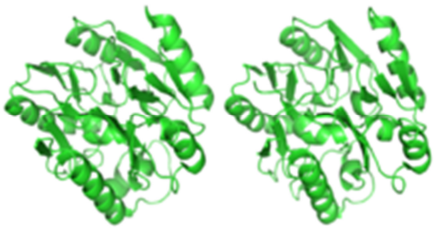


Figure 1.11 DDHA enzyme

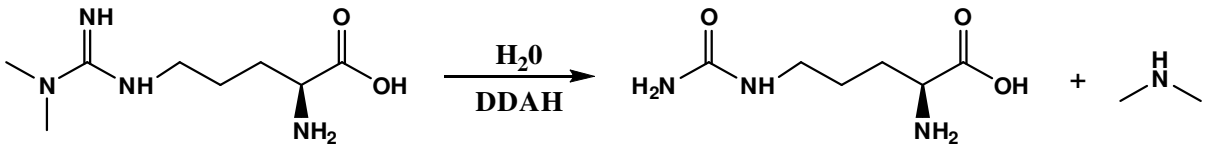


Figure 1.12 Hydrolysis catalyzed by DDAH

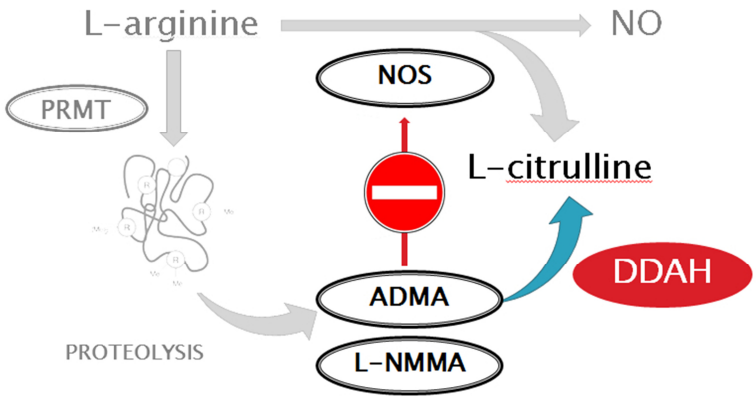


Figure 1.13 Methylated arginines inhibition

DDAH activity has been detected in a series of human tumors, in particularly in the brain.

The aim of further studies will be the pharmacological modification of DDAH as a mechanism for manipulating endogenous ADMA concentrations and regulating the production of nitric oxide in situations where alterations in its signalling have been shown to contribute to pathophysiology.

Chapter 2 STATE OF THE ART

2.1 From direct to indirect control

As above mentioned, several reports indicate that endogenous inhibitors of nitric oxide synthase, particularly asymmetric dimethylarginines regulate nitric oxide generation in several diseases.
Initially, the researchers decided to take advantage of the few differences between NOSs to create a drug that blocks one without affecting the others.
This would be very useful in treating certain diseases, such as Parkinson, Alzheimer and also multiple sclerosis where iNOS, (inducible nitric oxide synthase) plays a key role in their development. Unfortunately, however, the active sites of the three isoforms are practically identical. Studies are aimed at efforts to synthesize drugs that interact with other points where the enzyme shows differences.
As we saw in the NO pathway, it is possible to indirectly control its production, by interfering with the activity of DDAH, which proved to be a determinant key for ADMA and L-NMMA levels. This could be viewed as a desirable therapeutic strategy.

Analysing a certain quantity of human urine, dimethylarginine dimethylaminohydrolase was discovered. It was observed that there was a concentration of SDMA 30 times greater than ADMA and L-NMMA. This observation led McDermott to propose the existence of a catabolic pathway for ADMA and L-NMMA. A later study of the metabolism of ^{14}C-labelled ADMA and L-NMMA in rats confirmed that it is catabolized by an additional pathway leading to the formation of L-citrulline and their related amino acids.
To date, two isoforms of DDAH termed I and II have been identified in mammals.
DDAH I is expressed in brain, kidney, skeletal muscle, pancreas and liver; therefore its distribution is correlated with nNOS. DDAH II instead, has a more widespread distribution. In particular, we can find it in the heart, placenta, kidney and brain and is correlated with eNOS.

DDAH activity is the main pathway for ADMA elimination and its concentration within cells, because circulating ADMA is present at higher concentrations relative to L-NMMA and is often considered to be the principal inhibitor of NOS activity, even if it is important to point out that their concentrations differ from one another between tissues and organ systems.

Some examples can be endogenous methylarginines which may protect against neuroexcitotoxic injury by regulating neuronal NOS and might limit excess of NO by macrophages.

Exogenous L-NMMA has been shown to reverse hypotension of septic shock, prevent inflammation, reduce pain perception and treat headache.

Thus, experts have regarded a role for inhibitors of DDAH as a useful tool to study the relevant biological pathway and as potential therapeutic entities.

To this end, the next goal is to devise new selective inhibitors.

2.2 Selective substrate-based inhibitors

In an earlier study, examination of a small number of ADMA-like compounds known to be poor NOS inhibitors, during his studies, Beckenham UK, identified 2-amino-4-(N^G-methyl-guanidino) butanoic acid, **4124W**. One of the first exogenous molecules found to inhibit DDAH was a chain-shortened analogue of L-NMMA, a weak inhibitor with activity at millimolar concentrations and poor selectivity.

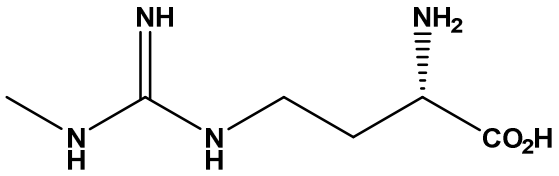


Figure 2.1 Compound 4124W

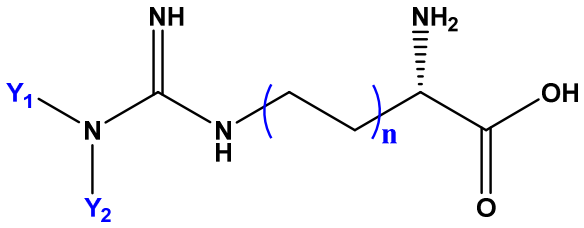
It has been shown that 4124W causes the continuous accumulation of endogenously generated ADMA in the human endothelial cells and DDAH acts to prevent its accumulation.

So, the 4124W compound represents the first point of reference for reversible inhibitor design.

The mechanism of the enzymes that act on the guanidine group is known, it seems logical to think that in all likelihood changes in this area can lead to an upsetting of the hydrolysis mechanism. Based on the structure of 4124W, Rossiter *et al*. have developed structural analogues of 4124W to improve DDAH inhibition and enhance selectivity for DDAH over NOS.

Modifications of 4124W which have been made include changing the number of carbon atoms in the middle alkyl chain and changing the Y groups on the guanidine moiety.

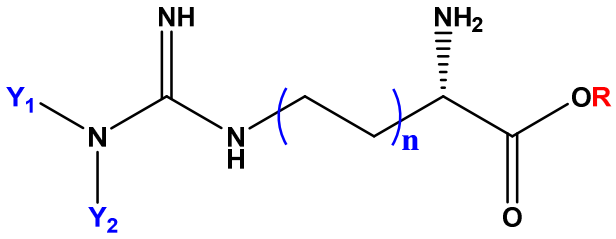
A series of analogues of this molecule are represented in Figure 2.2.



Y_1	Y_2
Me	H
Et	H
2-methoxyethyl	H
2-isopropoxyethyl	H
Me	Me
-piperidinyl-	

Figure 2.2 Compound 4124W mono-disubstituted

Other changes relate to the carboxyl group instead:



R
Me
Et
n-Pr
n-Bu
benzyl
i-Pr

Figure 2.3 Carboxyl group substituent

2.3 Numbers in comparison

IC_{50}, the half maximal inhibitory concentration, is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function.

This quantitative measure indicates how much of a particular substance (inhibitor) is needed to inhibit a given biological process (or component of a process, for example an enzyme or cell) by half. It represents the concentration of a substance that is required for 50% inhibition *in vitro*.

Since we are interested in the indirect control of the NO production acting on the inhibition of DDAH and not acting directly on NOSs, it is required that inhibitors have no direct effect on NOSs. The effect of inhibitors on eNOS activity was determined by measuring the conversion of [^{14}C]arginine to [^{14}C]citrulline.

The experimental data extrapolated from the experiments are reported in the table below.

Y_1	Y_2	$IC_{50,}\mu M$
Me	H	1510
Et	H	300
2-methoxyethyl	H	189
2-isopropoxyethyl	H	301
Me	Me	325
-piperidinyl-		264

Figure 2.4 IC_{50} data for N^G-substituted 2-Amino-4-guanidino-butanoic Acid

We can draw from Figure 2.4 the following conclusions:

- *N*-2-methoxyethyl substituent gave the best DDAH inhibition (IC_{50} 189 μM) of all the N-substituted analogues.

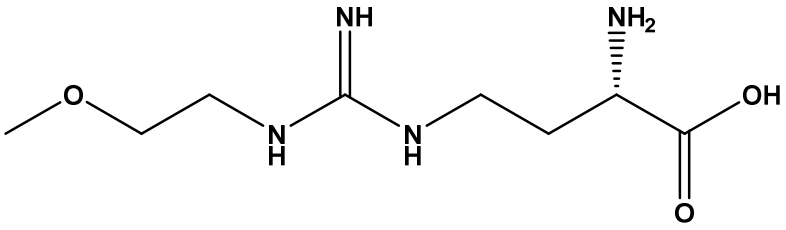


Figure 2.5 (S)-2-amino-4-(3-(2-methoxyethyl)guanidino)butanoic acid

- N,N-Disubstituted analogues were explored briefly, with the cyclic analogue pyperidinyl derivative having the highest activity (IC_{50} 264 μM).

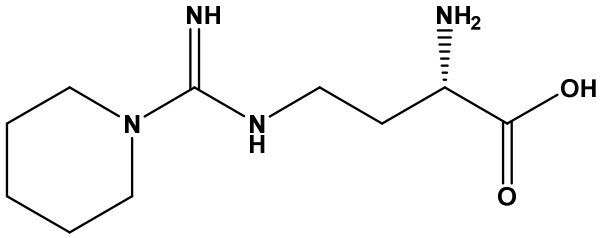


Figure 2.6 (S)-2-amino-4-(piperidine-1-carboximidamido)butanoic acid

- The N^G,N^G-dimethyl analogue with R_1=Me and R_1=Me, the corresponding chain-shortened version of ADMA, was also of reasonable potency (IC_{50} 325 μM), but inhibited eNOS in a preliminary screen.

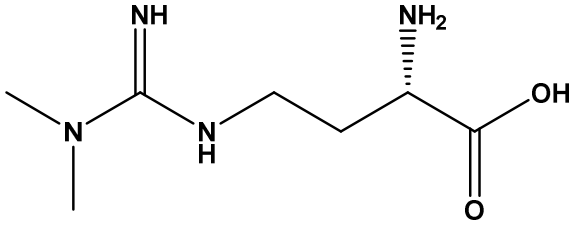


Figure 2.7 (S)-2-amino-4-(3,3-dimethylguanidino)butanoic acid

The N-2-methoxyethyl moiety was conserved for incorporation into other analogues due to its IC_{50} value.

Rossiter *et al.* focused on changes to the amino acid moiety. Ester of the compound in the Figure 2.5 were prepared.
In the table below are shown the inhibitions data.

R	$IC_{50,}\mu M$
Me	96
Et	159
n-Pr	111
n-Bu	113
benzyl	27
i-Pr	189

Figure 2.8 IC_{50} data for ester about *Figure 2.5*

We can draw the following conclusions:

- The 2-methoxyethyl group on the guanidine moiety and the benzyl ester on the acid was the most potent with IC_{50} = 27 μM out of a series of analogues with a two carbon length alkyl chain.

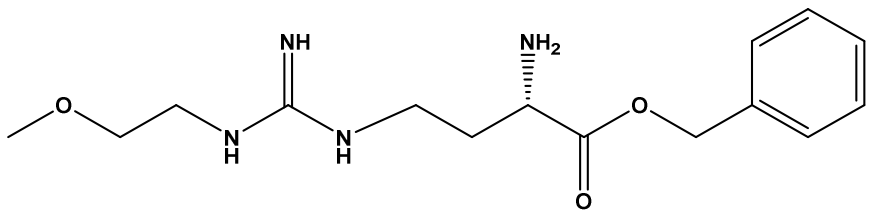


Figure 2.9 benzyl (S)-2-amino-4-(3-(2-methoxyethyl)guanidino)butanoate

- One carbon chain extension of compound in the Figure 2.5 gives free acid L-257 with IC_{50} = 22 μM. It has been demonstrated that this molecule has not any significant effect on NOSs.

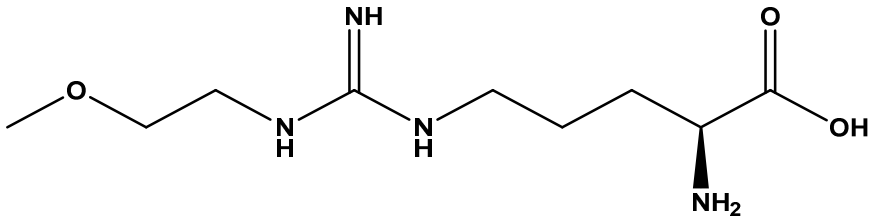


Figure 2.10 N^{w}-(2-methoxyethyl)-L-arginine

- Methyl ester, L-291 shows a inhibition IC_{50} = 20 μM.

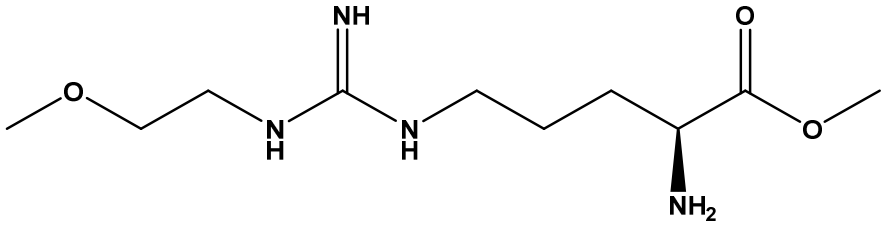


Figure 2.7 N^{w}-(2-methoxyethyl)-L-arginine methyl ester

We can conclude that among all the DDAH inhibitors reported in the literature, **L-257** has the highest selectivity towards NOSs. Using this molecule as a model, it is possible to synthesize a library of novel potential DDAH inhibitors, by varying the N-alkyl side chain or by introducing a carboxylate replacement.

2.4 From bioisosteric replacement to ZST316

More recent investigations into the binding pocket of the guanidine moiety by screening substituted guanidines amidines was carried out by Clement *et al.* Replacing the guanidine moiety of ornithine with amidine as a bioisostere showed improvement in activity of inhibitors. The definition of isostere is given by Langmuir. He said "compounds or groups of atoms having the same number of atoms and electrons". In a similar manner, the definition of bioisostere was given by Friedman "bioisostere are atoms or molecules that fit the broadest definition for isosteres and have the same type of biological activity". They represent one approach used by the medicinal chemist for the rational modification of compounds into safer and more clinically effective agents. In fact a compound with desired pharmacological activity may have undesirable characteristics that limit its bioavailability or structural features which adversely influence its metabolism and extraction from the body. It may also possess unwanted side effects or toxicity. Bioisosteres have been classified as either classical or nonclassical. The first ones are those which have similar steric and electronic features and have the same number of atoms of the substituent for which they are used as a replacement.

The nonclassical bioisosteres do not have the same number of atoms as the substituent for which they are used as a replacement. They are capable of maintaining similar biological activity by mimicking the spatial arrangement, electronic properties of the molecule that is critical for the preservation of biological activity. Why bioisosteric replacement?

There are a series of advantages, including:

- Greater selectivity
- Less side effects
- Decreased toxicity
- Increased stability

In the laboratories of the University of Aberdeen the replacement of the carboxylic group with a bioisosteric function and specifically with the following substituents was examined.

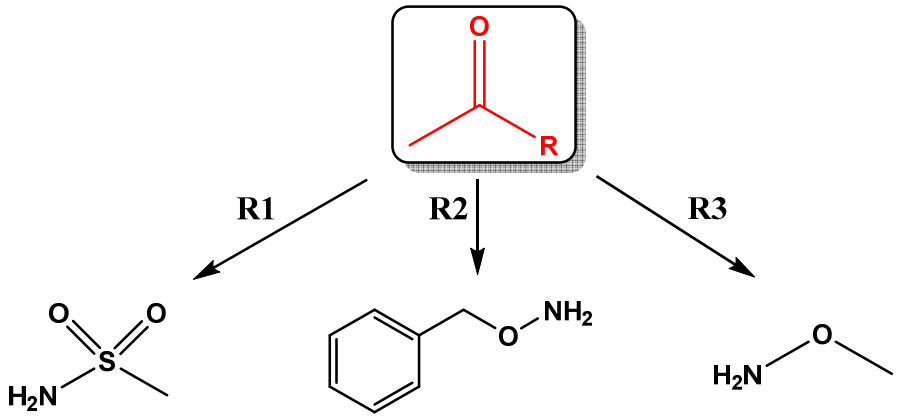


Figure 2.11 Non heterocyclic carboxylate bioisosteres

Other replacements involved the carboxylic group:

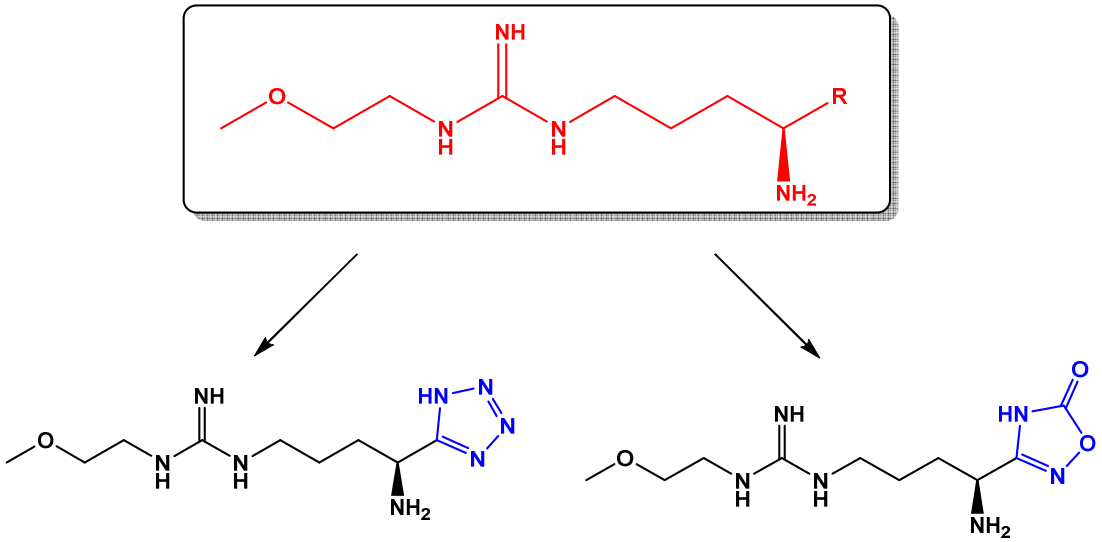


Figure 2.12 Heterocyclic structure carboxylate bioisosteres

Biological tests revealed that the bioisosteric replacement by a methyl acyl sulfonamide showed a 25-fold greater potency of inhibition then the compound L-257. Its selectivity is still to be assessed. We have evidence that modification of the methyl group may improve the potency and/or the selectivity.

From this observations, the project subject of this thesis, is born.

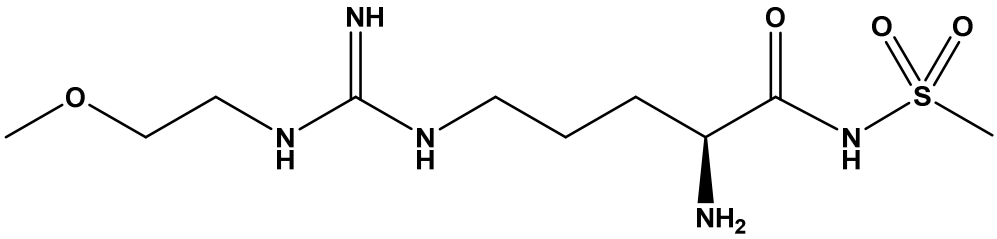
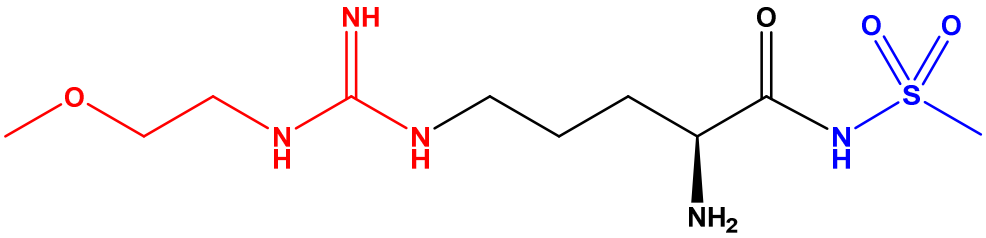


Figure 2.13 (S)-2-amino-5-(3-(2-methoxyethyl)guanidino)-N-(methyl-(methylene)sulfinyl)pentanamide

2.5 The aim of the project

The aim of my project is the creation of a small library of molecules which differ from one another because of the sulfonamide substituent.

From the studies conducted and the simulation of the molecular model it can be deduced that the left part of the compound ZST316 (red color) directly interacts with the enzyme pocket. It was decided to modify the substituent of the sulfonamide (in blue color) in order to improve interactions between DDAH and its inhibitors for a SAR investigation (Structure Activity Relationship).



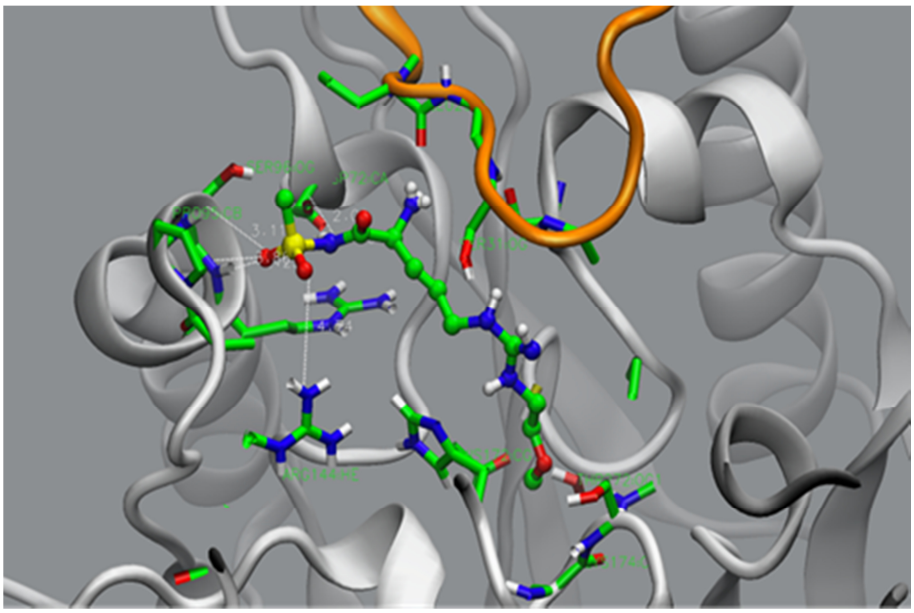


Figure 2.14 Representation of ZST316 binding mode with key residues

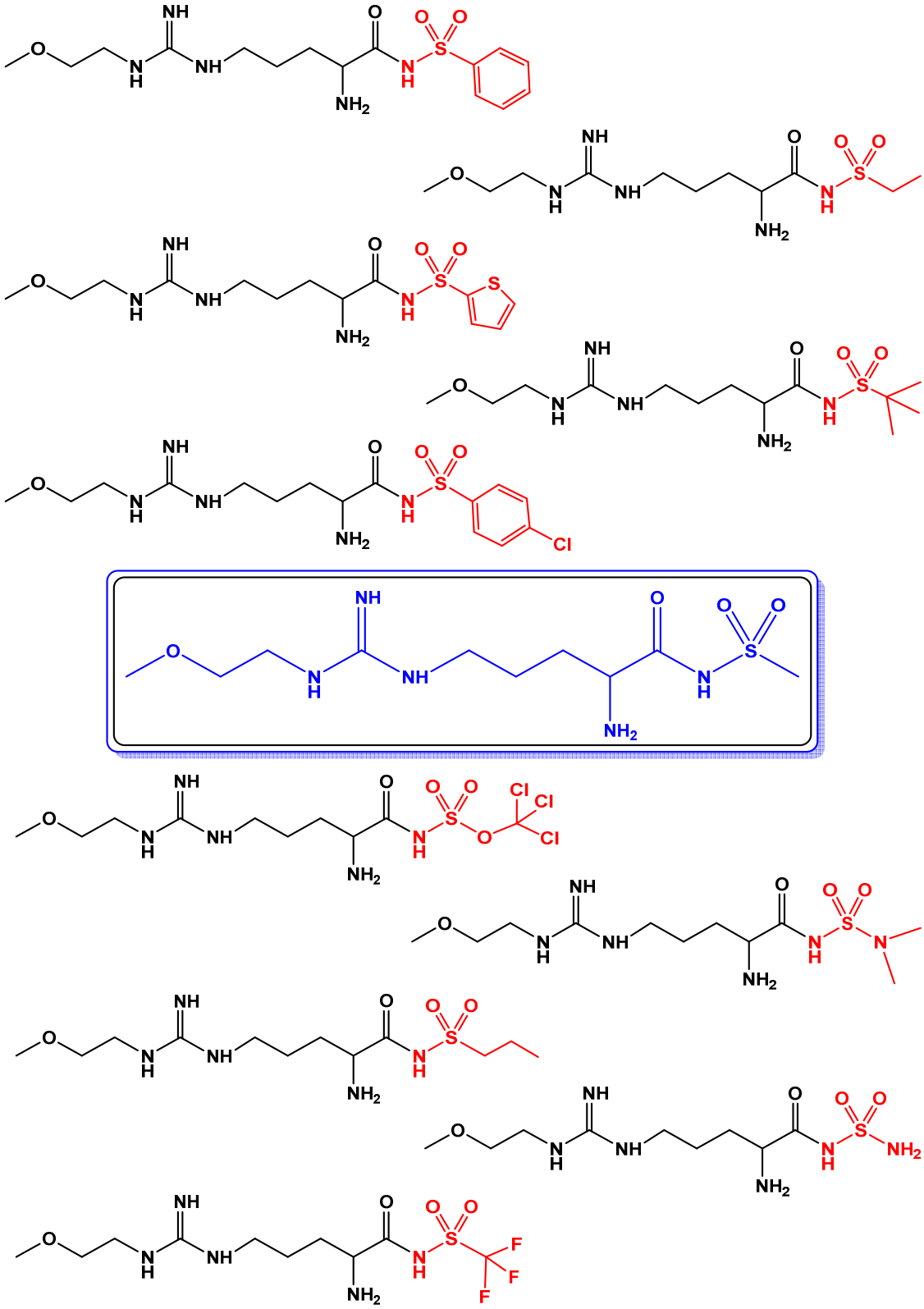
Legend:

- Oxygen
- Sulfur
- Nitrogen
- Carbon
- Hydrogen

In this way it will be possible to study the interactions of the new compounds with DDAH catalytic pocket and better understand the active configuration of the enzyme. The choice of the substituents was made so as to be able to analyze different molecular structures (longer chains, branched chains and rings) in the presence of elements with different electronegativity (for example chlorine and fluorine).

This strategy has been adopted with the aim of obtaining data to study the different situations of the enzyme-inhibitor interaction, in order to be able in the future, through biological tests, to better understand the effects of these molecules and to be able to improve the design of the inhibitor in order to increase the potency of inhibition and selectivity towards the DDAH.

For the synthesis of all these compounds the same procedure was followed and it was developed in the laboratories at the University of Aberdeen.



Chapter 3 RESULTS AND DISCUSSION

3.1 Operative choices

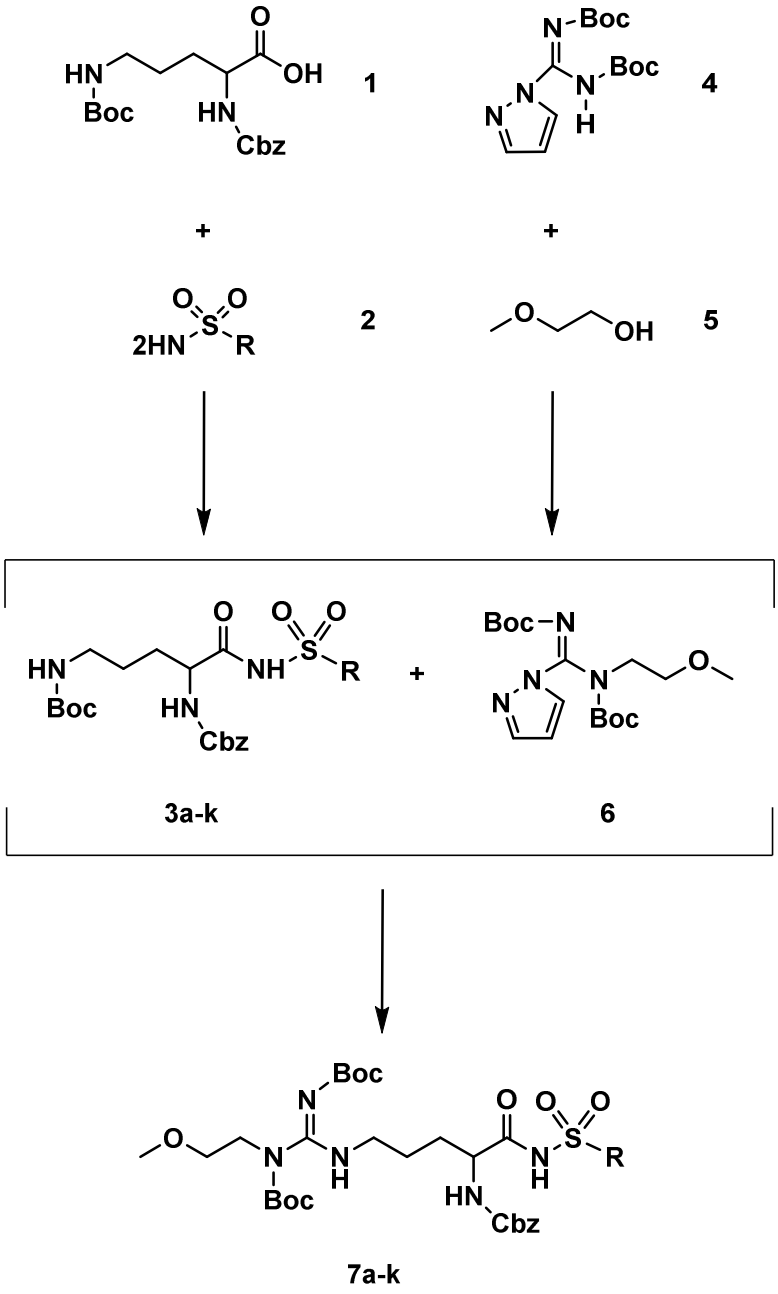


Figure 3.1 General scheme of the synthesis

The synthesis is divided into four steps.

The substituents used are presented in the figure below.

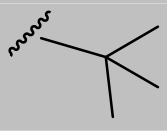
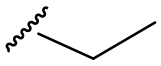
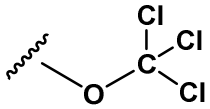
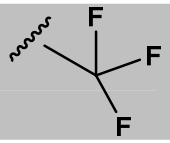
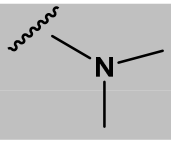
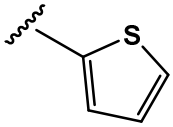
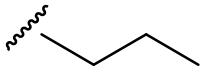
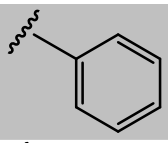
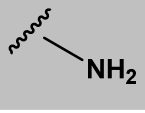
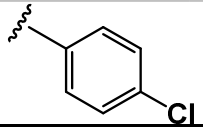
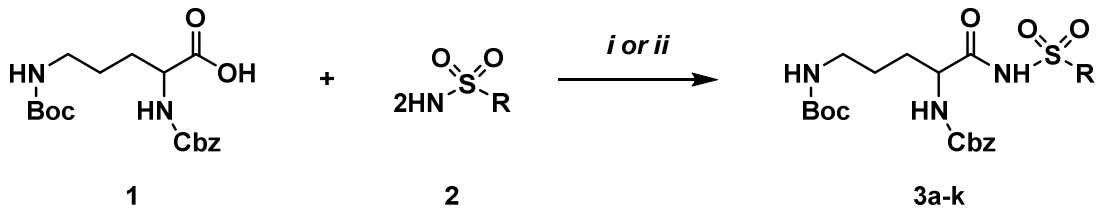
Entry	R	Entry	R
3a		**3g**	
3b		**3h**	
3c		**3i**	
3d		**3j**	
3e		**3k**	
3f			

Figure 3.2 The substituents used

3.1.1 Coupling reaction

The first step is the coupling between protected ornithine and desired sulfonamide.



1 2 3a-k

Figure 3.3 Coupling between ornithine and sulfonamide. i e ii

This same mechanism has been followed for the synthesis of the compound ZST316 in the laboratories of the University of Aberdeen. The procedure is reported in the literature (R. Rönn et al. / *Bioorg. Med. Chem. 14* (**2006**) 544-559; Patent WO2012/166665 A2, **2012**).

In this step, we use two different methods with two different coupling reagents (CDI and EDCl), reported in the Figure 3.4.

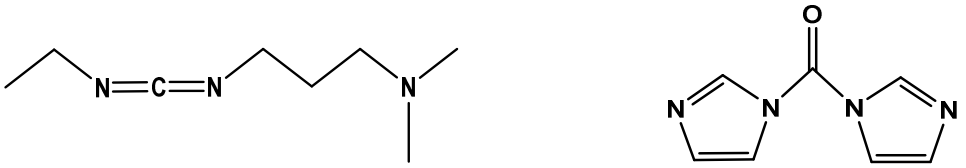


Figure 3.4 Coupling reagents: EDCl (left) CDI (right)

The choice of synthesizing the same molecule with two different methods (under different operating conditions) at the end of the project, allowed us to compare the yields of this step to optimize the synthesis in the future.

Both ornithine and the desired sulfonamides are commercially available.

Amid the starting commercial materials tested, this ornithine **1** was the most suitable, but not all combinations of the protecting groups have been tried.

i) The procedure using EDCl is the following:
In a round bottom flask of 50 mL, Z-Orn-(Boc)-OH (1 mmol), EDCl (1.2 Eq) and dry DCM solvent were added in this order. Then DMAP was added (4 Eq). Then the sulfonamide was added (1.2 Eq) in small amounts.

ii) The procedure using CDI is the following:
Into a two-neck flask, CDI (2 Eq) was added under N_2 atmosphere to a solution of Z-Orn-(Boc)OH (1 mmol) in dry THF solvent. The mixture was stirred at room temperature for 1 hour.
Then, the desired sulfonamide (1.5 Eq) and DBU (1.5 Eq) were added to the solution. The resulting mixture was stirred for 16 hours. The formation of the compounds 3a-k was verified via NMR analysis.

The mechanisms of coupling with EDCl and CDI are presented in the next figures.

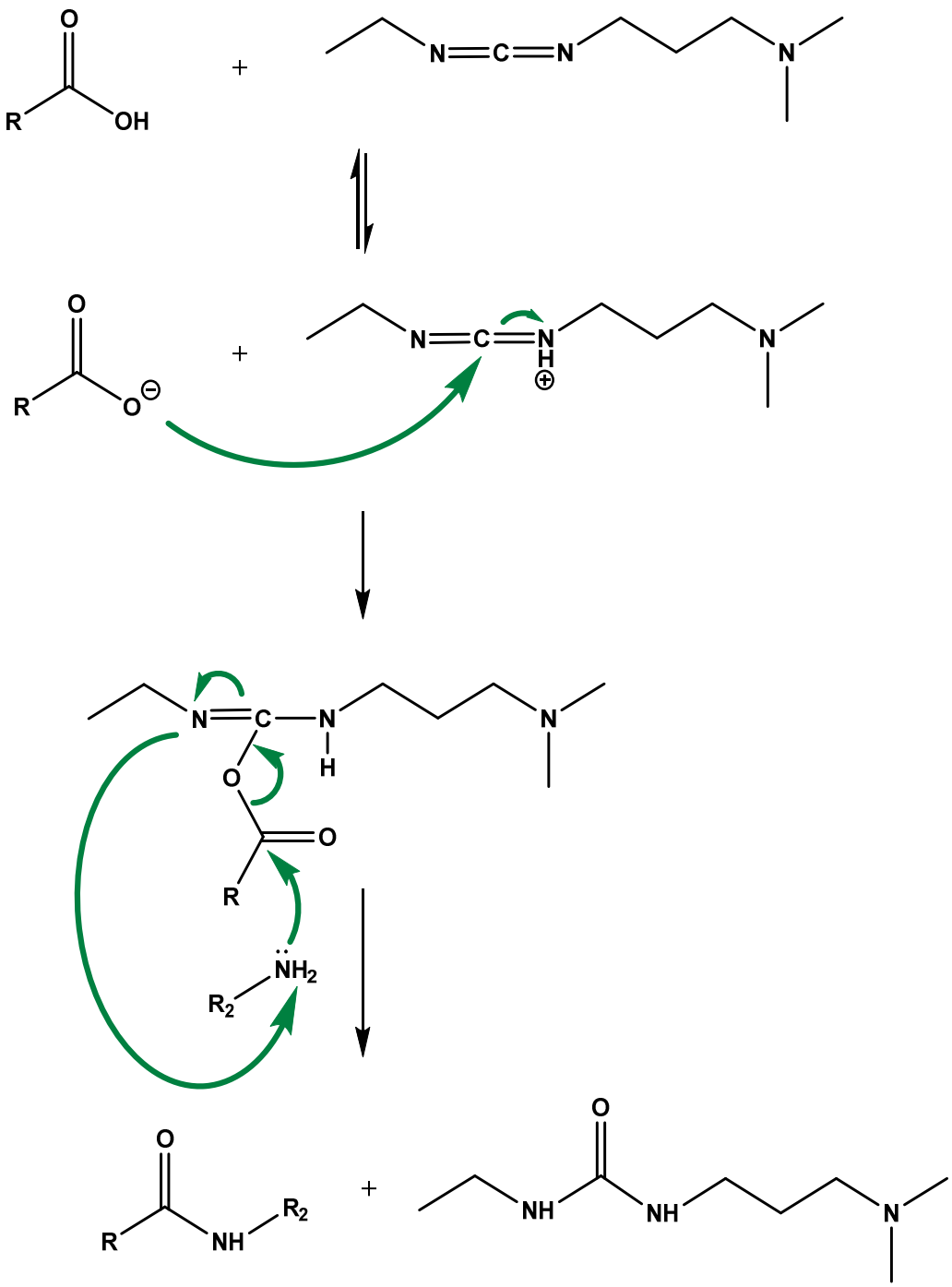


Figure 3.5 Reaction mechanism with EDCl

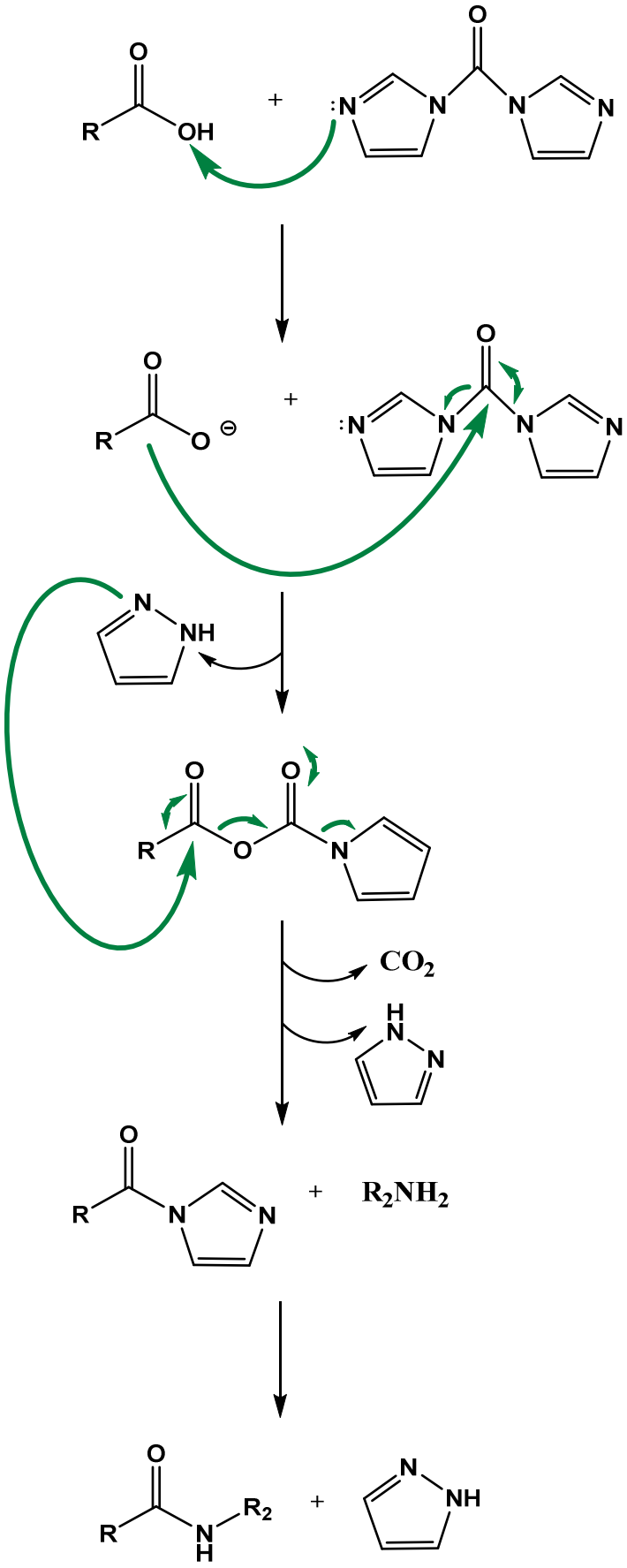


Figure 3.6 Reaction mechanism with CDI

The yields obtained from both steps are synthesized in Figure 3.7.

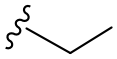
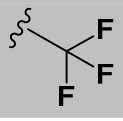
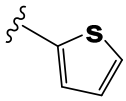
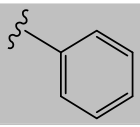
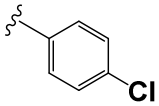
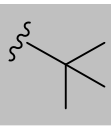
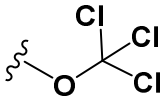
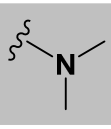
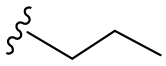
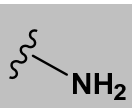
Entry	R	Yield (i)	Yield (ii)
3a		40%	51%
3b		28%	99%
3c		35%	16%
3d		-	93%
3e		51%	54%
3f		43%	45%
3g		84%	76%
3h		34%	76%
3i		82%	83%
3j		74%	82%
3k		-	75%

Figure 3.7 Percentage yields of first step for both methods

Generally speaking there are no great differences between entries, with the exception of the number 3b, 3c, 3d and 3h. For most of the products, the method using the CDI

as coupling reagent appears to be the best in terms of percentage of yielded products.

With regard to the entry 3k by the method *i*), the synthesis failed because of the prevalent formation of the following bisaduct.

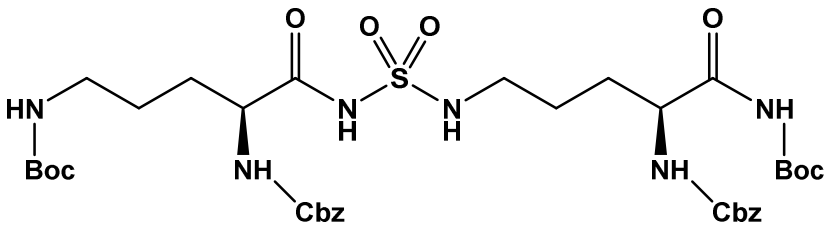


Figure 3.8 Bisaduct formed for the entry 3k

Unlike the other substituents, this sulphamide is formed on both sides by functional groups NH_2. The coupling synthesis is complicated, because both groups tend to react and hence could lead to bisaduct, decreasing the selectivity of the desired product.

For entry 3b the yield reported is quantitative, because it was noted from TLC that the impurity of the solution was minimal and a possible purification would have been useless.

3.1.2 Mitsunobu reaction

The second step is the preparation of a stock of guanilating reagent under *"Mitsunobu"* conditions, starting from the commercially available acid number 4. The scheme of preparation is proposed below.

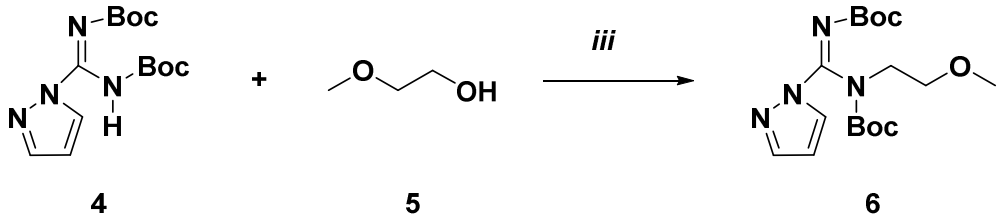


Figure 3.9 Mitsunobu reaction

This procedure make it possible to convert an alcohol (in the our case 2-methoxyethan-1-ol) into a variety of functional groups, such as an ester, using triphenylphosphine and an azodicarboxylate as diethyl azodicarboxylate (DEAD) or diisopropyl azodicarboxylate (DIAD). In our synthesis we only use the DIAD because it presents less hazards connected with its use and transport**.**
The yields of this step are quantitative. The reaction was monitored by TLC before being interrupted.

The procedure is reported in the literature (S. Rossiter *et al*. *J. Med. Chem.*, **2005**, 48, 4670-4678) and is the following:
Diethyl azodi-carboxylate (3mmol) was added dropwise at 0 °C to the solution of N,N'-bis-tert-butoxycarbonylpyrazole-1-carboxamide (2 mmol), triphenylphoshine (3 mmol), 2-methoxyethanol (4 mmol) in dry THF solvent, stirring for 30 mins. Then the ice bath was removed and the mixture was stirred at room temperature for 16 hours.
Subsequent purification was made by silica gel chromatography.
The formation of the product was verified via NMR analysis

The reaction mechanism is presented in the next figure.

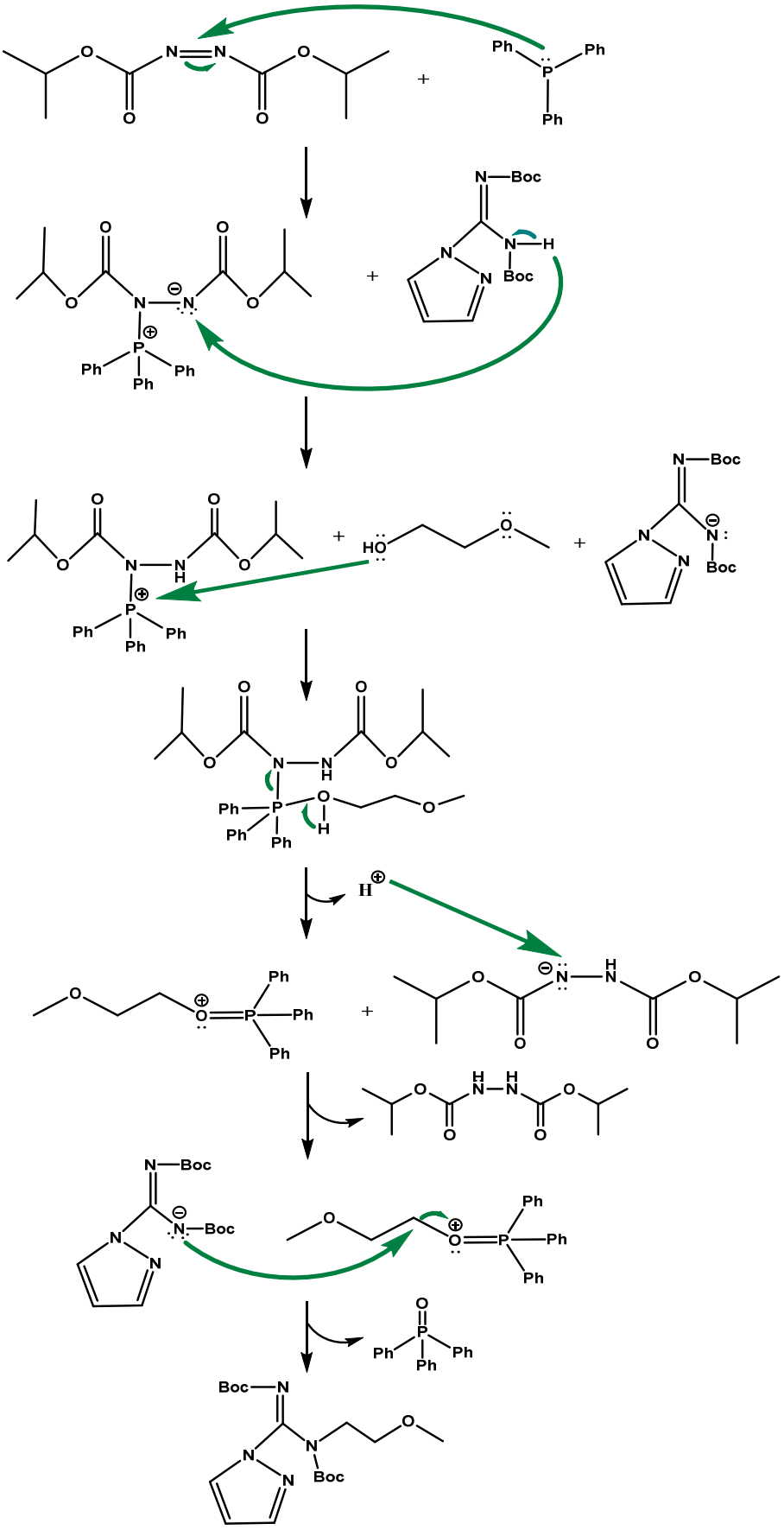


Figure 3.10 Reaction mechanism of Mitsunobu reaction

3.1.3 Deprotection and guanidilation

The third step of the synthesis is developed in two continuous stages.

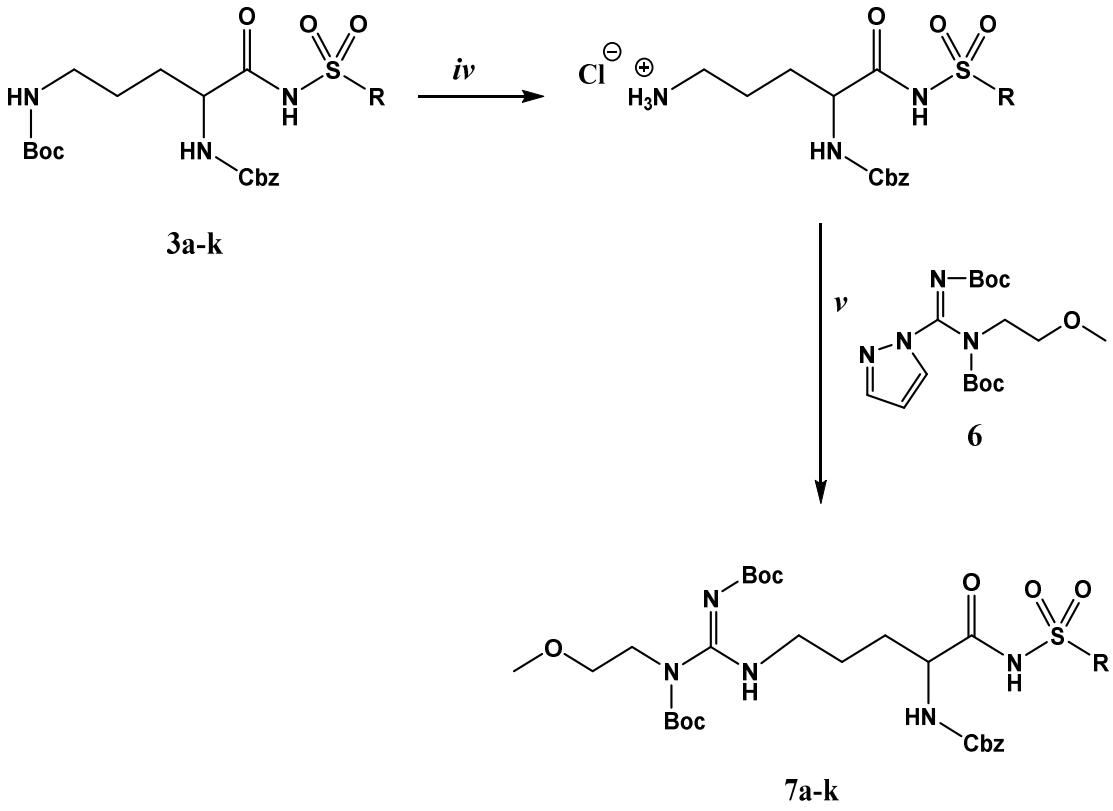


Figure 3.11 Deprotection and Guanidination

The first consists in the deprotection of the Boc protecting group that is located laterally to the molecular chain. The second part involves the reaction with the guanilating reagent number 6, achieving the introduction of the desired guanidin group. The intermediate product is not isolated and purified.

All substrates were appropriately protected to facilitate purification of the intermediates.

The procedure is reported in literature (Van Ameijde J. *et al.Org Biomol. Chem*., **2010**, 8, 1629,1639), and we used a HCl 4N solution in dioxane, which is readily commercially available.

HCl-dioxane 4M (2 mL) at room temperature for 1 hour was added to the solution of acyl sulfonamide of ornithine (mmol obtained from the previous step).

Solvent was then completely evaporated once checked total deprotection of the starting material by TLC.

Then DIPEA (2 Eq) dropwise in small amounts and Mitsunobu product (1 Eq) in solution of DCM (2 mL) were added to the mixture at room temperature for 24 hours

The purification was made by silica gel chromatography, the formation of the deprotected and guanidilated molecule was verified via NMR analysis.

The deprotection mechanism is presented in the following figure.

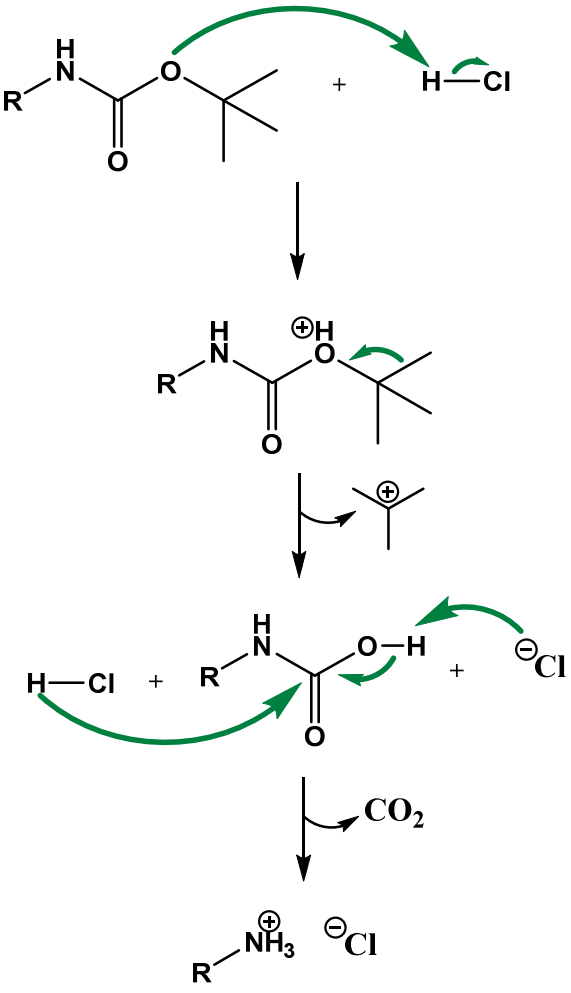


Figure 3.12 Reaction mechanism of deprotection

The table shows the yields for this step.

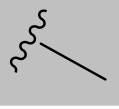
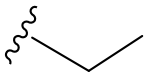
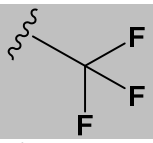
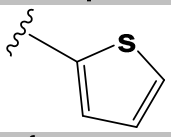
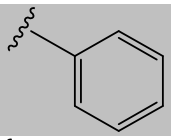
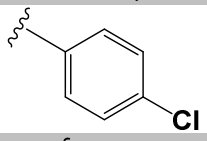
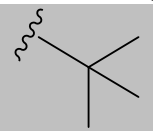
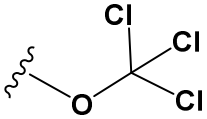
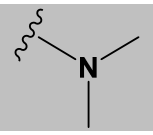
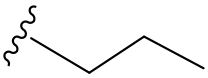
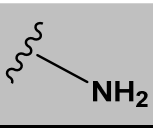
Entry	R	Yield (v)
7a		42%
7b		26%
7c		23%
7d		92%
7e		26%
7f		43%
7g		-
7h		-
7i		-
7j		-
7k		-

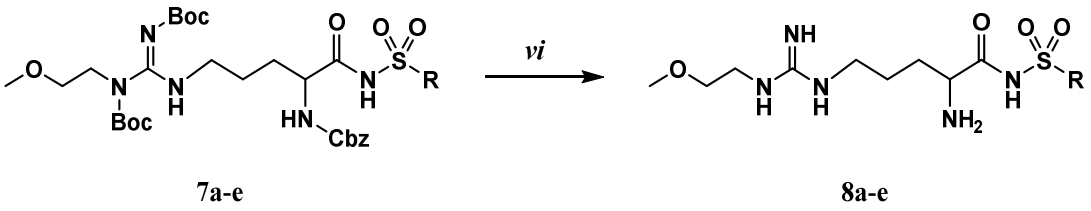
Figure 3.13 Yields of deprotection and guanidination reaction

All the yields are less than 50%. This is because in the reaction process there are competing molecular reactions, *intra* and *inter* molecular. The first one is favored and lead to the closure of the molecule, forming the lactam following the expulsion

of the sulfonamide, while the *inter* molecular reaction goes on to give the desired product.

The purification by silica gel chromatography for the molecules from *g* to *k* was difficult. The NMR spectra were very dirty after the purifications, so they have not been deprotected to give the final product.

3.1.4 Total deprotection



7a-e **8a-e**

Figure 3.14 Total Deprotection

In the final step, the molecules 7a-e were totally deprotected.

The procedure is reported in literature (R. J. Worthington *et al.Org. Biomol. Chem.* **2007**, 5, 249-259) and is the following:
7a-e (mmol obtained to the previous step) TFA (2 mmol) and TFSMA (12 Eq) were added at room temperature for 1 hour to the starting material. The solution was washed with DCM. The aqueous phase was extracted with distilled water. The formation of our final product was verified via mass spectrometry.
Then the aqueous solution was lyophilized and purified by HPLC to obtain pure 8a-e for the future biological experiments, thanks to which they will be tested towards inhibition activity and selectivity.

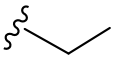
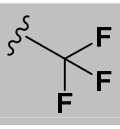
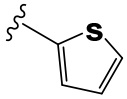
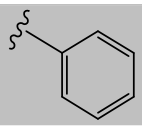
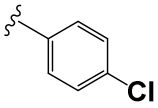
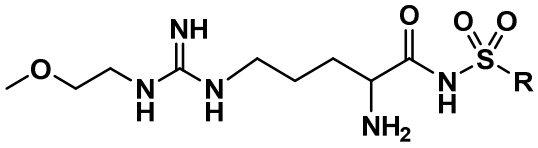
Entry	R	Yield (i)
8a		99%
8b		99%
8c		99%
8d		99%
8e		99%
8f		99%

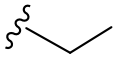
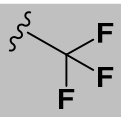
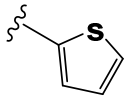
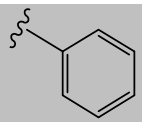
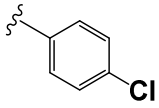
Figure 3.15 Percentage yields of final step

3.1.5 Molecular docking studies

In order to gather information on the mode of binding of the target compounds on the receptor, we performed molecular docking studies. The receptor was first modelled starting from the crystal stricture of dimethylarginine dimethylaminohydrolase I (DDAH 1) in complex with citrulline (pdb code: 2C6Z, 1.6 Å resolution). Docking studies were performed with VINA as implemented in the Yasara software. Results are reported as binding energy as obtained from the VINA algorithm. For each ligand the corresponding complex was further optimized by steepest descent minimization with the AMBER03 force field and the binding energy was calculated with the same force field. Results are reported in Figure 3.16.



8a-k

Entry	R	Binding Energy from VINA (kcal/mol)	Binding Energy AMBER03 after energy minimization (kcal/mol)
8a		6.6390	40.7371
8b		6.6070	39.57628
8c		6.7690	43.97497
8d		7.1210	49.80056
8e		7.5270	46.38689
8f		7.0210	50.11847

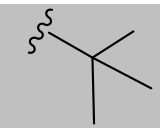
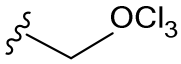
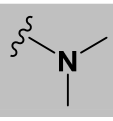
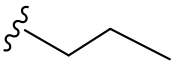
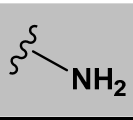
8g		6.0420	41.40755
8h		5.5990	47.16841
8i		6.3670	39.04125
8j		6.7890	43.037
8k		6.9500	34.46103

Figure 3.16 Results from molecular docking of **8a-k** on DDAH1 receptor model

The higher binding energy as calculated both from Vina and Amber03 have been obtained for the compounds bearing a terminal aromatic ring. Compounds **8d-f** are predicted to be more active than the reference compound **8a**. Inspection of the poses reveals the role of the aromatic rings in positioning the sulphoxide moiety toward the formation of a number of stabilizing hydrogen bonds with Arg-144 and Arg-97. Moreover the aromatic groups occupy a lipophilic pocket comprising the residues Leu-69 and Cys-72. For compound **8k** an high binding energy is calculated by VINA docking on rigid receptor, whereas a much lower binding energy is predicted after energy minimization. This result highlights the need for further optimization of the ligand-receptor complex geometry for example by molecular dynamics calculations.

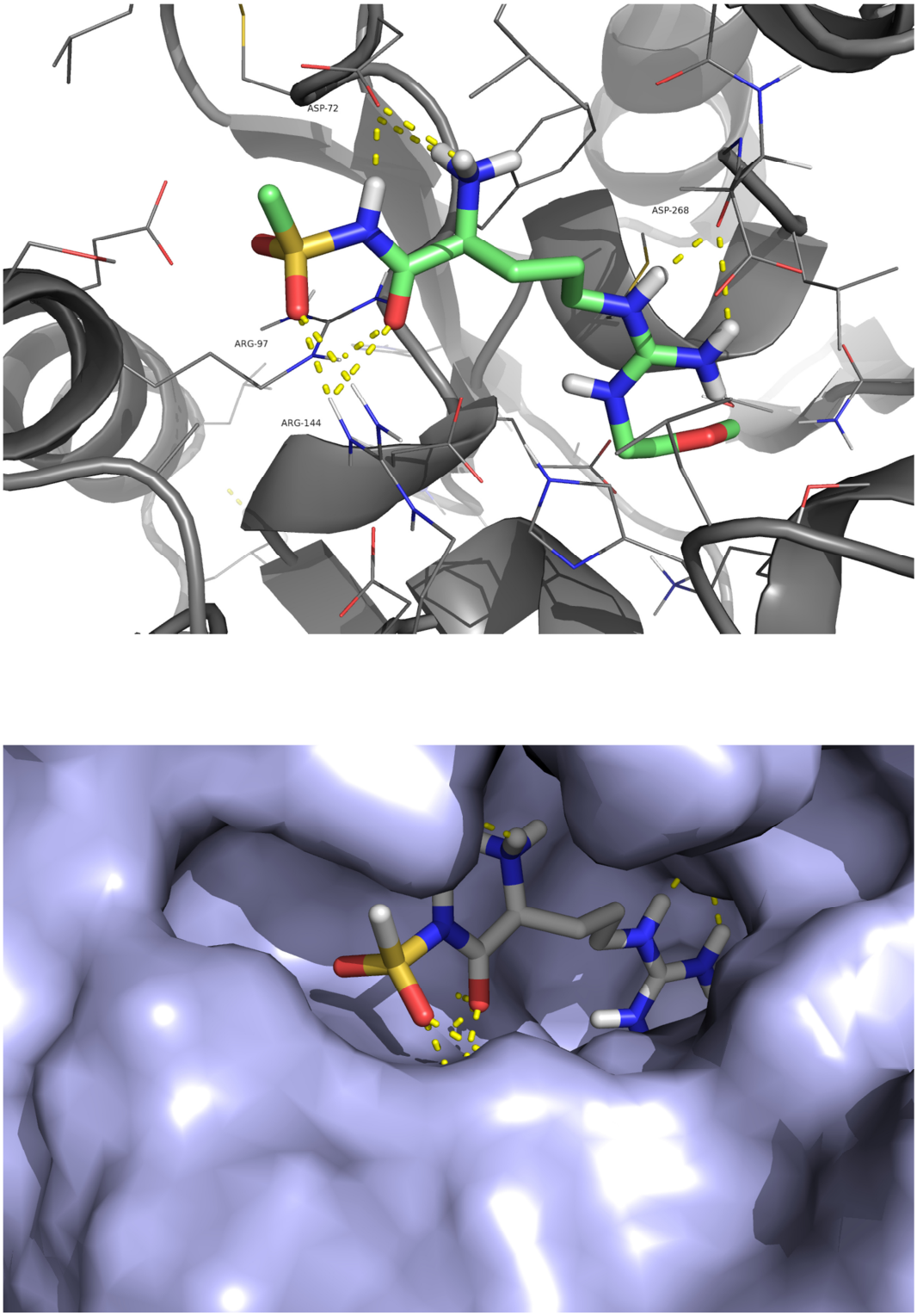


Figure 3.17 Docking pose for reference compound **8a**

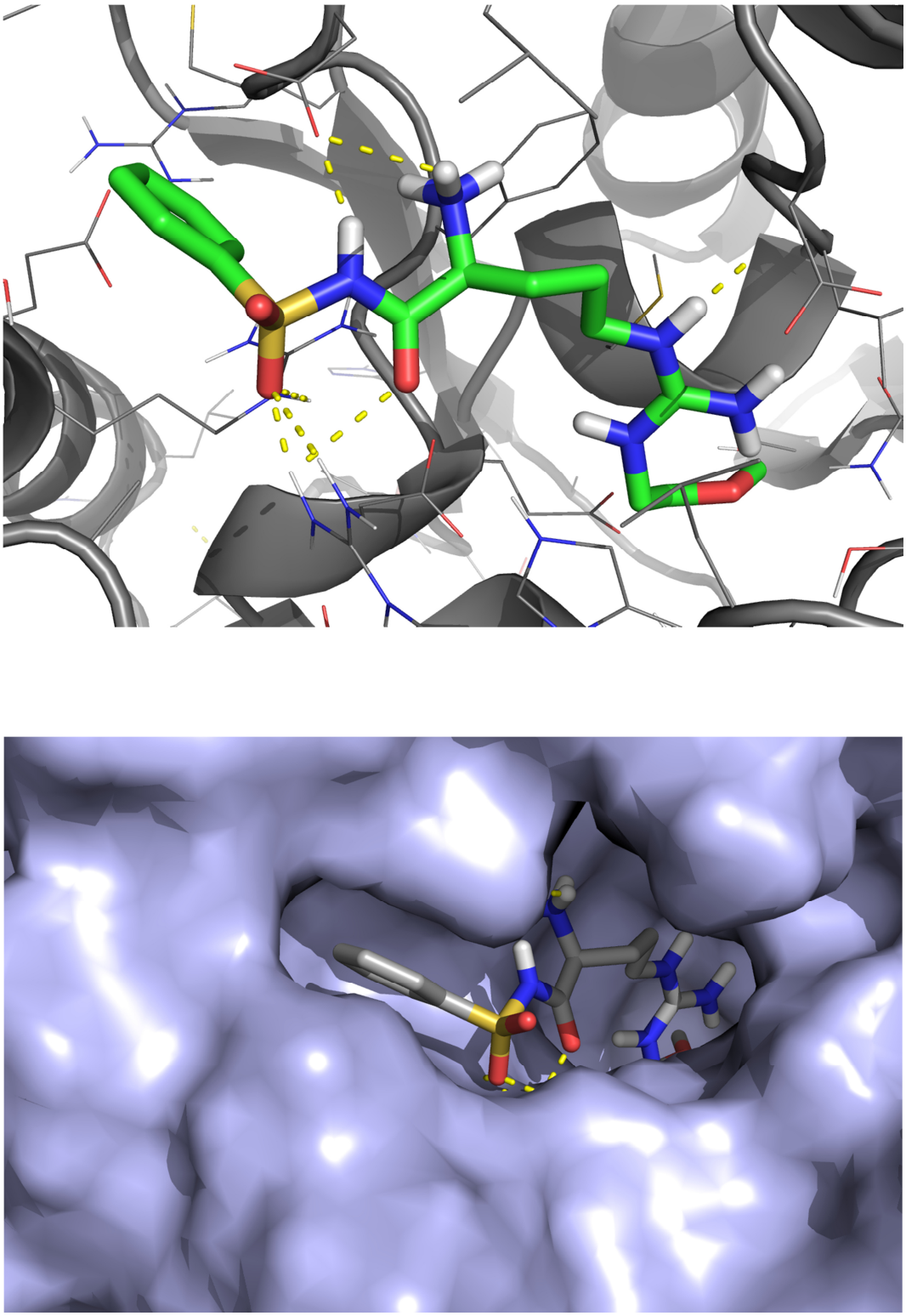
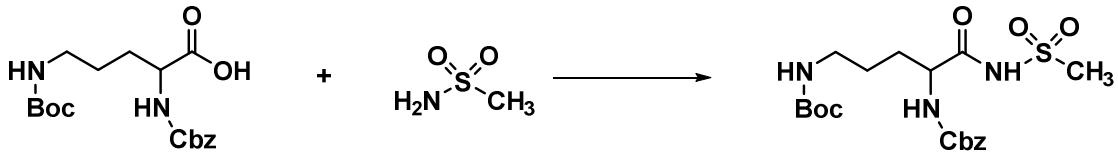


Figure 3.18 Docking pose for compound 8e

Chapter 4 EXPERIMENTAL SECTION

4.1 Procedure for the synthesis of 3a-k using EDCI as coupling reagent

4.1.1 Compound 3a



1 2 3a

Z-Orn-(Boc)-OH (1mmol, 366 mg), EDCl (1.2 mmol, 186 mg) and 5 mL of dry DCM. were added in this order in a round bottom flask of 50 mL. Then DMAP (4 mmol, 489 mg) and 3 mL of dry DCM were added. Then methane sulfonamide and 2 mL of dry DCM were added (1.2 mmol, 114 mg) in small amounts. The mixture was stirred at room temperature for 16 hours. The solution was washed first with citric acid (5% w) (x2), then with distilled water (x2) and brine (x1). The aqueous phase was extracted with DCM. The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum. Subsequent purification was made by silica gel chromatography (MeOH/DCM = 1:99).

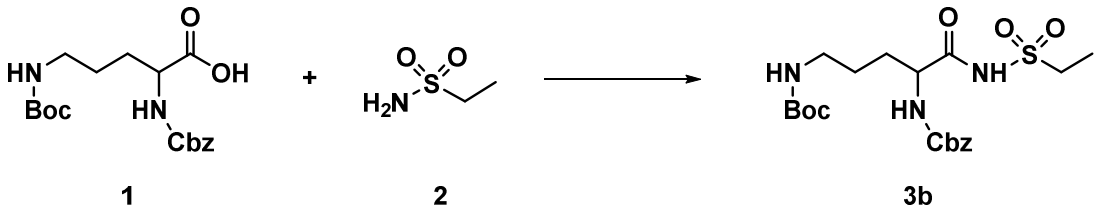
Benzyl tert-butyl (5-(methylsulfonamido)-5-oxopentane-1,4-diyl)dicarbamate.

3a. Yield 42%

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.33 – 7.29 (m, 5H), 5.07 (m, 2H), 4.28 (s, 1H), 3.04 (m, 3H), 1.90 (m, 2H), 1.68 – 1.59 (m, 2H), 1.51 (m, 2H), 1.43 – 1.40 (m, 9H)

HRMS (ESI) calc for $C_{19}H_{29}N_3O_7S$ 443.17, found 466 (M+23)

4.1.2 Compound 3b



1 2 3b

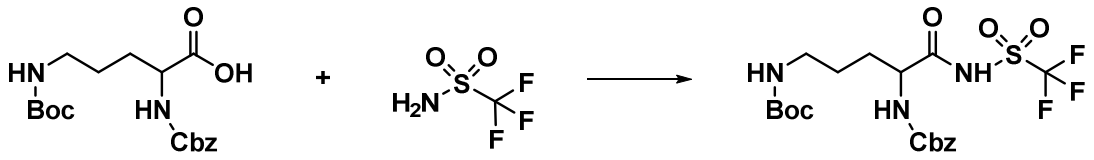
Z-Orn-(Boc)-OH (1mmol, 366 mg), EDCl (1.2 mmol, 186 mg) and 5 mL of dry DCM were added in this order to a round bottom flask of 50 mL. Then DMAP (4 mmol, 489 mg) and 3 mL of dry DCM were added. Then ethane sulfonamide (1.2 mmol, 131 mg) in small amounts and 2 mL of dry DCM were added. The mixture was stirred at room temperature for 16 hours. The solution was washed first with HCl (x2), then with distilled water (x2) and brine (x1). The aqueous phase was extracted with DCM. The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum. Subsequent purification was made by silica gel chromatography (MeOH/DCM = 5:95).

Benzyl tert-butyl (5-(ethylsulfonamido)-5-oxopentane-1,4-diyl)dicarbamate. 3b.
Yield = 28%
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.32 (m, 5H), 5.09 (s, 1H), 5.07 (s, 1H), 4.40 (s, 1H), 3.24 (m, 2H), 3.05 (m, 2H), 1.85 (s, 1H), 1.64 (s, 1H), 1.54 (m, 2H), 1.42 (m, 9H), 1.28 (m, 3H).

^{13}C NMR (101 MHz, CDCl3) δ 156.90, 156.51 (2H), 136.11, 128.75, 128.51 (2H), 128.18, 128.06 (2H), 126.99, 79.77, 77.32, 67.11, 47.63, 39.19, 29.68, 28.43, 26.19, 7.85.

HRMS (ESI) calc for $C_{20}H_{31}N_3O_7S$ 457.54, found 480.20 (M+23)

4.1.3 Compound 3c



1 2 3c

Z-Orn-(Boc)-OH (1mmol, 366 mg), EDCl (1.2 mmol, 186 mg) and 5 mL of dry DCM were added in this order to a round bottom flask of 50 mL. Then DMAP (4 mmol, 489 mg) and 3 mL of dry DCM were added. Then trifluoro methane sulfonamide (1.2 mmol, 179 mg) in small amounts and 2 mL of dry DCM were added. The mixture was stirred at room temperature for 16 hours. The solution was washed first with citric acid (5% w) (x2), then with distilled water (x2) and brine (x1). The aqueous phase was extracted with DCM. The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum. Subsequent purification was made by silica gel chromatography (MeOH/DCM = 3:97).

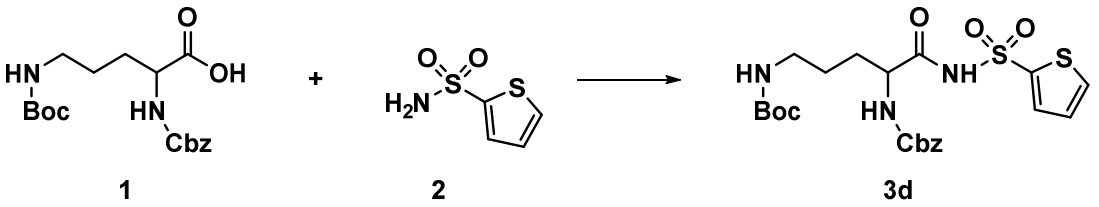
Benzyl tert-butyl (5-oxo-5-((trifluoromethyl)sulfonamido)pentane-1,4-diyl)dicarbamate. 3c. Yield = 35%

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.39 – 7.23 (m, 5H), 5.04 (m, 2H), 3.01 (m, 1H), 2.01 (m, 2H), 1.61 (m, 2H), 1.47 (m, 2H), 1.41 (m, 9H).

^{13}C NMR (101 MHz, CDCl3) δ 156.55, 156.21, 135.97, 128.83, 128.45, 128.11, 127.94, 121.36, 118.14, 81.42, 79.74, 77.25, 67.17, 57.12, 39.98, 39.61, 29.70, 28.30, 25.16.

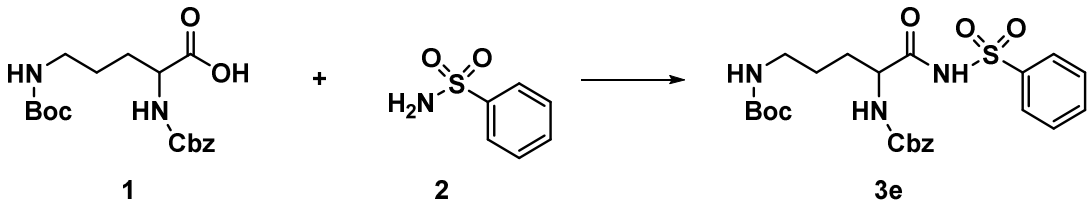
HRMS (ESI) calc for $C_{19}H_{26}F_3N_3O_7S$ 497.49, found 520.00 (M+23)

4.1.4 Compound 3d



Z-Orn-(Boc)-OH (0.8 mmol, 293 mg), EDCl (0.96 mmol, 149 mg) and 5 mL of dry DCM were added in this order to a round bottom flask of 50 mL. Then DMAP (3.2 mmol, 391 mg) and 3 mL of dry DCM were added. Then thiopene sulfonamide (0.96 mmol, 157 mg) in small amounts and 2 mL of dry DCM were added. The mixture was stirred at room temperature for 16 hours. The solution was washed first with citric acid (5% w) (x2), then with distilled water (x2) and brine (x1). The organic phase was extracted with ethyl acetate. After checking the reagent solution with TLC, the only resulting component was the starting material. The reaction did not work.

4.1.5 Compound 3e



Z-Orn-(Boc)-OH (1 mmol, 366 mg), EDCl (1.2 mmol, 186 mg) and 5 mL of dry DCM were added in this order to a round bottom flask of 50 mL. Then DMAP (4 mmol, 489 mg) and 3 mL of dry DCM were added. Then benzene sulfonamide (1.2 mmol, 189 mg) in small amounts and 2 mL of dry DCM were added. The mixture was stirred at room temperature for 16 hours. The solution was washed first with citric acid (5% w) (x2), then with distilled water (x2) and brine (x1). The aqueous phase was extracted with DCM. The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum. Subsequent purification was made by silica gel chromatography (hexane/ethyl acetate = 7:3).

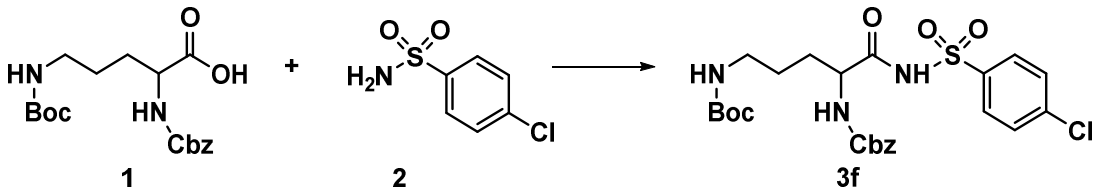
Benzyl tert-butyl (5-oxo-5-(phenylsulfonamido)pentane-1,4-diyl)dicarbamate. 3e. Yield = 51%.

^{1}H NMR (400 MHz, Chloroform-d) δ 8.02 (m, 2H), 7.61 – 7.42 (m, 3H), 7.30 (m, 5H), 5.07 (m, 2H), 4.49 (s, 1H), 3.30 (s, 0H), 2.98 (m, 2H), 1.79 (m, 2H), 1.65 – 1.50 (m, 2H), 1.47 (m, 9H).

^{13}C NMR (101 MHz, CDCl3) δ 157.19, 156.47 (2H), 136.03 (2H), 128.78 (2H), 128.50 (4H), 128.16 (2H), 128.01, 80.05, 77.27, 67.16, 38.66, 30.00, 29.70, 29.36, 28.47, 26.24, 22.70.

HRMS (ESI) calc for $C_{24}H_{31}N_3O_7S$ 505.59, found 528.20 (M+23)

4.1.6 Compound 3f



Z-Orn-(Boc)-OH (0.33 mmol, 122 mg), EDCl (0.4 mmol, 62 mg) and 2 mL of dry DCM were added in this order to a round bottom flask of 50 mL. Then DMAP (1.33 mmol, 163 mg) and 1 mL of dry DCM were added. Then 4-chlorobenzene sulfonamide (0.4 mmol, 77 mg) in small amounts and 1 mL of dry DCM were added. The mixture was stirred at room temperature for 16 hours. The solution was washed first with citric acid (5% w) (x2), then with distilled water (x2) and brine (x1). The aqueous phase was extracted with DCM. The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum. Subsequent purification was made by silica gel chromatography (MeOH/DCM = 2:98).

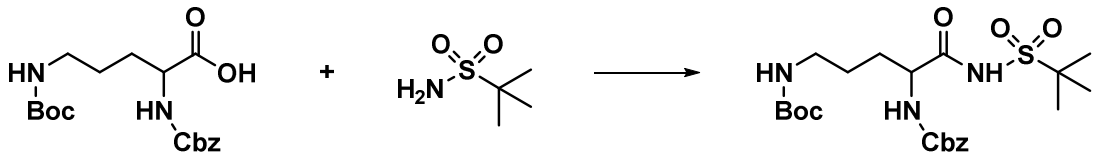
Benzyl tert-butyl (5-((4-chlorophenyl)sulfonamido)-5-oxopentane-1,4-diyl)dicarbamate. 3f. Yield = 43%

^{1}H NMR (400 MHz, Chloroform-d) δ 7.95 – 7.89 (m, 2H), 7.78 (m, 2H), 7.34 – 7.28 (m, 5H), 5.04 (m, 3H), 3.00 (m, 2H), 1.79 (m, 2H), 1.53 (m, 2H), 1.46 (m, 9H).

^{13}C NMR (101 MHz, CDCl3) δ 157.25, 156.52 (2H), 136.03 (2H), 128.97 (4H), 128.51 (2H), 128.19 (2H), 128.01 (2H), 77.24, 67.38, 67.13, 29.70, 28.44 (3H), 26.29, 22.69.

HRMS (ESI) calc for $C_{24}H_{30}ClN_3O_7S$ 540.03, found 563.54 (M+23)

4.1.7 Compound 3g



1 2 3g

Z-Orn-(Boc)-OH (1mmol, 366 mg), EDCl (1.2 mmol, 186 mg) and 5 mL of dry DCM were added in this order to a round bottom flask of 50 mL. Then DMAP (4 mmol, 489 mg) and 3 mL of dry DCM were added. Then 2-methylpropane-2-sulfonamide (1.2 mmol, 165 mg) in small amounts and 2 mL of dry DCM were added. The mixture was stirred at room temperature for 16 hours. The solution was washed first with citric acid (5% w) (x2), then with distilled water (x2) and brine (x1). The aqueous phase was extracted with DCM. The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum. Subsequent purification was made by silica gel chromatography (MeOH/DCM = 3:97).

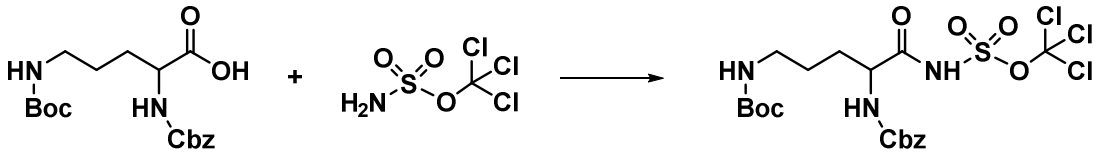
Benzyl tert-butyl (5-((1,1-dimethylethyl)sulfonamido)-5-oxopentane-1,4-diyl)dicarbamate. 3g. Yield = 84%

^{1}H NMR (400 MHz, Chloroform-d) δ 7.33 (m, 5H), 5.15 – 5.01 (m, 3H), 3.08 (m, 2H), 1.85 (m, 2H), 1.51 (m, 2H), 1.42 (m, 9H), 1.33 (m, 9H).

^{13}C NMR (101 MHz, CDCl3) δ 156.80, 156.57, 136.12, 128.47 (2H), 128.32, 128.13, 128.05 (2H), 79.53, 77.41, 67.07, 41.12, 39.35, 29.64, 28.44 (3H), 26.14, 24.07 (3H).

HRMS (ESI) calc for $C_{22}H_{35}N_3O_7S$ 485.60, found 508.23 (M+23)

4.1.8 Compound 3h



Z-Orn-(Boc)-OH (0.8 mmol, 293 mg), EDCl (0.96 mmol, 149 mg) and 4 mL of dry DCM were added in this order to a round bottom flask of 50 mL. Then DMAP (3.2 mmol, 391 mg) and 2 mL of dry DCM were added. Then the trichloromethyl sulfamate (0.96 mmol, 219 mg) in small amounts and 2 mL of dry DCM were added. The mixture was stirred at room temperature for 16 hours. The solution was washed first with citric acid (5% w) (x2), then with distilled water (x2) and brine (x1). The aqueous phase was extracted with DCM. The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum. Subsequent purification was made by silica gel chromatography (MeOH/DCM = 3:97).

Trichloromethyl (2-(((benzyloxy)carbonyl)amino)-5-((tert-butoxycarbonyl)amino)pentanoyl)sulfamate. 3h. Yield = 34%.

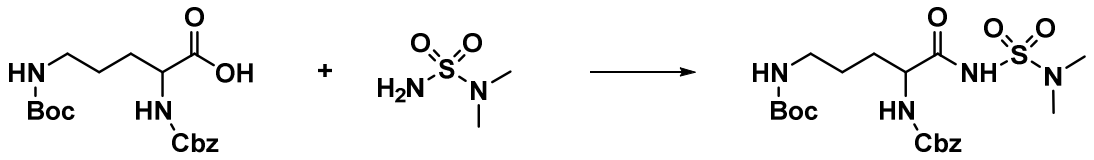
^{1}H NMR (400 MHz, DMSO-d6) δ 7.34 (m, 5H), 5.01 (m, 2H), 4.61 (s, 1H), 3.35 (m, 2H), 2.51 (m, 2H), 1.50 (m, 2H), 1.44 (m, 9H).

.

^{13}C NMR (101 MHz, DMSO) δ 156.23, 155.98, 137.70, 128.76 (2H), 128.10, 128.00, 96.08, 79.64, 78.51, 78.44, 77.75, 77.48, 65.55, 57.13, 28.76 (3H), 26.68.

HRMS (ESI) calc for $C_{19}H_{26}Cl_3N_3O_8S$ 462.84, found 485.95 (M+23)

4.1.9 Compound 3i



1 2 3i

Z-Orn-(Boc)-OH (0.8 mmol, 293 mg), EDCl (0.96 mmol, 149 mg) and 4 mL of dry DCM were added in this order to a round bottom flask of 50 mL. Then DMAP (3.2 mmol, 391 mg) and 2 mL of dry DCM were added. Then the N,N-dimethyl sulfonamide (0.96 mmol, 149 mg) in small amounts and 2 mL of dry DCM were added. The mixture was stirred at room temperature for 16 hours. The solution was washed first with citric acid (5% w) (x2), then with distilled water (x2) and brine (x1). The aqueous phase was extracted with DCM. The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum. Subsequent purification was made by silica gel chromatography (MeOH/DCM = 5:95).

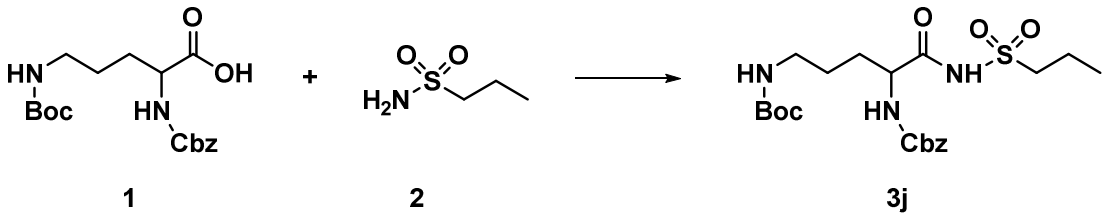
Benzyl tert-butyl (5-((N,N-dimethylsulfamoyl)amino)-5-oxopentane-1,4-diyl)dicarbamate. 3i. Yield = 82%

^{1}H NMR (400 MHz, DMSO-d6) δ 7.36 (m, 5H), 5.04 (m, 2H), 4.00 (s, 1H), 3.33 (m, 2H), 2.79 (m, 6H), 1.70 (m, 2H), 1.50 (m, 2H), 1.38 (m, 9H).

^{13}C NMR (101 MHz, CDCl3) δ 171.60, 156.99, 156.57, 136.06, 128.51 (2H), 128.19, 128.00, 79.74, 77.38, 67.20, 53.86, 38.95, 38.19, 29.58, 28.42 (3H), 27.94, 26.31.

HRMS (ESI) calc for $C_{20}H_{32}N_4O_7S$ 472.56, found 495.20 (M+23)

4.1.10 Compound 3j



1 2 3j

Z-Orn-(Boc)-OH (0.8 mmol, 293 mg), EDCl (0.96 mmol, 149 mg) and 4 mL of dry DCM were added in this order to a round bottom flask of 50 mL. Then DMAP (3.2 mmol, 391 mg) and 2 mL of dry DCM were added. Then the propane sulfonamide (0.96 mmol, 118 mg) in small amounts and 2 mL of dry DCM were added. The mixture was stirred at room temperature for 16 hours. The solution was washed first with citric acid (5% w) (x2), then with distilled water (x2) and brine (x1). The aqueous phase was extracted with DCM. The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum. Subsequent purification was made by silica gel chromatography (MeOH/DCM = 5:95).

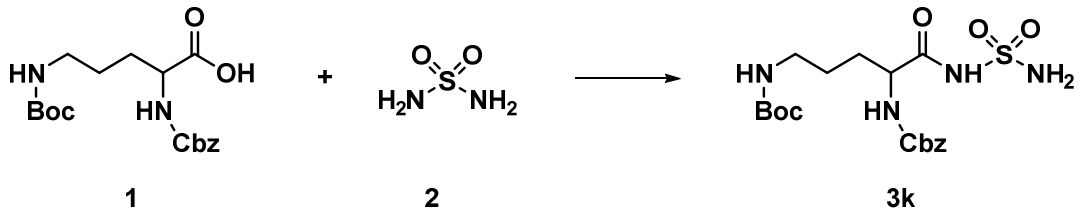
Benzyl tert-butyl (5-oxo-5-(propylsulfonamido)pentane-1,4-diyl)dicarbamate. 3j. Yield = 74%

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.33 (m, 5H), 5.12 – 5.07 (m, 2H), 4.47 (s, 1H), 3.23 – 3.01 (m, 4H), 1.90 – 1.74 (m, 4H), 1.55 (m, 2H), 1.43 (m, 9H), 1.01 (m, 3H).

^{13}C NMR (101 MHz, CDCl3) δ 172.80, 157.06, 156.58, 136.03, 128.53, 128.21, 128.00, 79.87, 77.34, 67.24, 56.82, 54.88, 54.22, 38.92, 29.45, 28.42, 26.27, 17.56, 16.81, 12.74, 12.70.

HRMS (ESI) calc for $C_{21}H_{33}N_3O_7S$ 471.57, found 494.34 (M+23)

4.1.11 Compound 3k

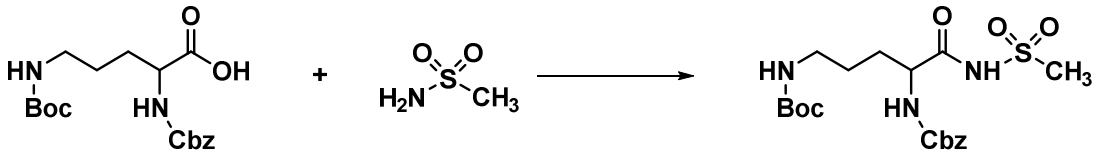


1 2 3k

Z-Orn-(Boc)-OH (0.8 mmol, 293 mg), EDCl (0.96 mmol, 149 mg) and 4 mL of dry DCM were added in this order to a round bottom flask of 50 mL. Then DMAP (3.2 mmol, 391 mg) and 2 mL of dry DCM were added. Then the sulfonamide (0.96 mmol, 92 mg) in small amounts and 2 mL of dry DCM were added. The mixture was stirred at room temperature for 16 hours. The solution was washed first with citric acid (5% w) (x2), then with distilled water (x2) and brine (x1). The aqueous phase was extracted with DCM. The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum. Subsequent purification was made by silica gel chromatography (MeOH/DCM = 2:98). The solution did not work. The product is only bisaduct.

4.2 Procedure for the synthesis of 3a-k using CDI as coupling reagent

4.2.1 Compound 3a



1 2 3a

In a two-necked flask, CDI (2 mmol, 324 mg) was added under N_2 atmosphere to a solution of Z-Orn-(Boc)OH (1 mmol, 366 mg) in dry THF solvent (20 mL). The mixture was stirred at room temperature for 1 hour.

Then, methane sulfonamide (1.5 mmol, 143 mg) and DBU (1.5 mmol, 228 mg) were added.. The resulting mixture was stirred for 16 hours.

The solution was washed first with citric acid (5% w) (x2), then with distilled water (x2) and brine (x1). The aqueous phase was extracted with DCM. (x2). The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum.

Purification was made by silica gel chromatography (MeOH/DCM = 1:99).

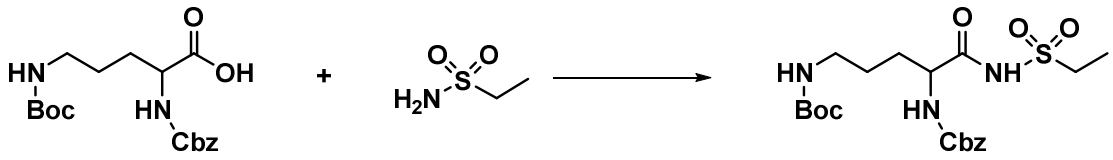
Benzyl tert-butyl (5-(methylsulfonamido)-5-oxopentane-1,4-diyl)dicarbamate.

3a. Yield = 51%

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.33 – 7.29 (m, 5H), 5.07 (m, 2H), 4.28 (s, 1H), 3.04 (m, 3H), 1.90 (m, 2H), 1.68 – 1.59 (m, 2H), 1.51 (m, 2H), 1.43 – 1.40 (m, 9H)

HRMS (ESI) calc for $C_{19}H_{29}N_3O_7S$ 443.17, found 466 (M+23)

4.2.2 Compound 3b



1 2 3b

In a two-necked flask, CDI (0.67 mmol, 108 mg) was added under N_2 atmosphere to a solution of Z-Orn-(Boc)OH (0.88 mmol, 312 mg) in dry THF solvent (7 mL). The mixture was stirred at room temperature for 1 hour.

Then, ethane sulfonamide (0.5 mmol, 55 mg) and DBU (0.5 mmol, 76 mg) were added to the solution. The resulting mixture was stirred for 16 hours.

The solution was washed first with HCl (x2), then with distilled water (x2) and brine (x1). The aqueous phase was extracted with DCM. (x2). The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum.

Purification by silica gel chromatography was not carried out.

Benzyl tert-butyl (5-(ethylsulfonamido)-5-oxopentane-1,4-diyl)dicarbamate. 3b.

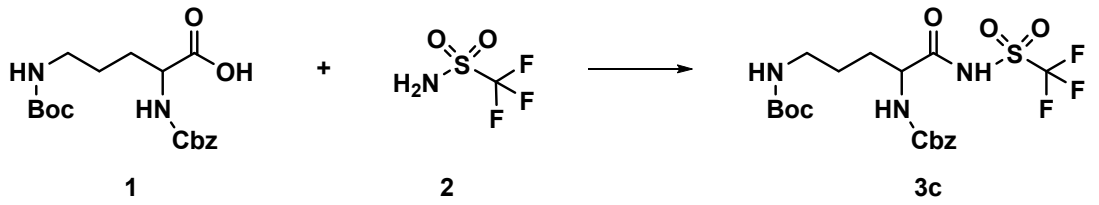
Yield = 99%

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.32 (m, 5H), 5.09 (s, 1H), 5.07 (s, 1H), 4.40 (s, 1H), 3.24 (m, 2H), 3.05 (m, 2H), 1.85 (s, 1H), 1.64 (s, 1H), 1.54 (m, 2H), 1.42 (m, 9H), 1.28 (m, 3H).

^{13}C NMR (101 MHz, CDCl3) δ 156.90, 156.51 (2H), 136.11, 128.75, 128.51 (2H), 128.18, 128.06 (2H), 126.99, 79.77, 77.32, 67.11, 47.63, 39.19, 29.68, 28.43, 26.19, 7.85.

HRMS (ESI) calc for $C_{20}H_{31}N_3O_7S$ 457.54, found 480.20 (M+23)

4.2.3 Compound 3c



1 2 3c

In a two-necked flask, CDI (0.67 mmol, 108 mg) was added under N_2 atmosphere to a solution of Z-Orn-(Boc)OH (0.33 mmol, 122 mg) in dry THF solvent (7 mL). The mixture was stirred at room temperature for 1 hour.

Then, trifluoro sulfonamide (0.5 mmol, 300 mg) and DBU (0.5 mmol, 76 mg) were added to the solution. The resulting mixture was stirred for 16 hours.

The solution was washed first with citric acid (5% w) (x2), then with distilled water (x2) and brine (x1). The aqueous phase was extracted with DCM. (x2). The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum. Purification was made by silica gel chromatography (MeOH/DCM = 3:97).

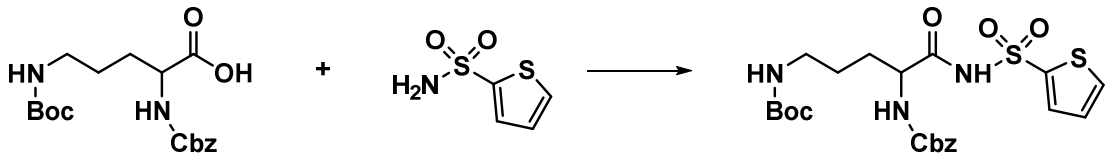
Benzyl tert-butyl (5-oxo-5-((trifluoromethyl)sulfonamido)pentane-1,4-diyl)dicarbamate. 3c. Yield = 16%

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.39 – 7.23 (m, 5H), 5.04 (m, 2H), 3.01 (m, 1H), 2.01 (m, 2H), 1.61 (m, 2H), 1.47 (m, 2H), 1.41 (m, 9H).

^{13}C NMR (101 MHz, CDCl3) δ 156.55, 156.21, 135.97, 128.83, 128.45, 128.11, 127.94, 121.36, 118.14, 81.42, 79.74, 77.25, 67.17, 57.12, 39.98, 39.61, 29.70, 28.30, 25.16.

HRMS (ESI) calc for $C_{19}H_{26}F_3N_3O_7S$ 497.49, found 520.00 (M+23)

4.2.4 Compound 3d



In a two-necked flask, CDI (2 mmol, 324 mg) was added under N_2 atmosphere to a solution of Z-Orn-(Boc)OH (1 mmol, 366 mg) in dry THF solvent (20 mL). The mixture was stirred at room temperature for 1 hour.

Then, thiophene sulfonamide (1.5 mmol, 245 mg) and DBU (1.5 mmol, 228 mg) were added to the solution. The resulting mixture was stirred for 16 hours.

The solution was washed first with citric acid (5% w) (x2), then with distilled water (x2) and brine (x1). The aqueous phase was extracted with DCM. (x2). The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum. Purification was made by silica gel chromatography (MeOH/DCM = 2:98).

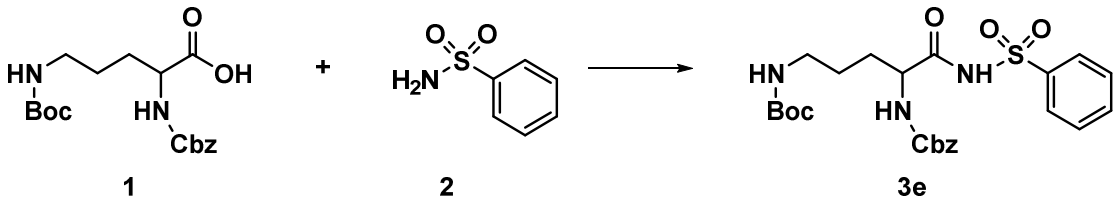
Benzyl tert-butyl (5-oxo-5-(thiophene-2-sulfonamido)pentane-1,4-diyl)dicarbamate. 3d. Yield = 93%

^{1}H NMR (400 MHz, DMSO-*d*) δ 7.75 (m, 5H), 7.55 (s, 1H), 7.35 (s, 1H), 6.73 (s, 1H), 5.00 (m, 2H), 3.82 (s, 1H), 2.85 (m, 2H), 2.51 (m, 2H), 1.64 – 1.55 (m, 2H), 1.37 (m, 9H).

^{13}C NMR (101 MHz, CDCl3) δ 156.55, 156.21, 135.97, 128.83, 128.45, 128.11, 127.94 (2H), 121.36, 118.14, 81.42, 79.74, 77.25, 67.17, 57.12, 39.98, 39.61, 29.70, 28.30 (3H), 25.16.

HRMS (ESI) calc for $C_{22}H_{29}N_3O_7S_2$ 511.61, found 534.10 (M+23)

4.2.5 Compound 3e



1 2 3e

In a two-necked flask, CDI (0.67 mmol, 108 mg) was added under N_2 atmosphere to a solution of Z-Orn-(Boc)OH (0.33 mmol, 122 mg) in dry THF solvent (7 mL). The mixture was stirred at room temperature for 1 hour.

Then, benzene sulfonamide (0.5 mmol, 79 mg) and DBU (0.5 mmol, 76 mg) were added to the solution. The resulting mixture was stirred for 16 hours.

The solution was washed first with citric acid (5% w) (x2), then with distilled water (x2) and brine (x1). The aqueous phase was extracted with DCM. (x2). The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum. Purification was made by silica gel chromatography (MeOH/DCM = 3:97).

Benzyl tert-butyl (5-oxo-5-(phenylsulfonamido)pentane-1,4-diyl)dicarbamate.

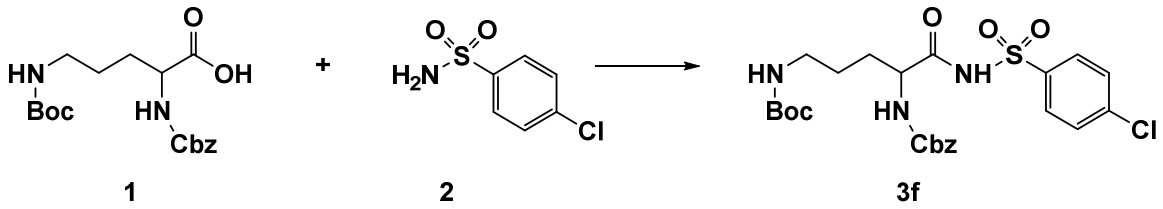
3e. Yield = 54%

^{1}H NMR (400 MHz, Chloroform-d) δ 8.02 (m, 2H), 7.61 – 7.42 (m, 3H), 7.30 (m, 5H), 5.07 (m, 2H), 4.49 (s, 1H), 3.30 (s, 0H), 2.98 (m, 2H), 1.79 (m, 2H), 1.65 – 1.50 (m, 2H), 1.47 (m, 9H).

^{13}C NMR (101 MHz, CDCl3) δ 157.19, 156.47 (2H), 136.03 (2H), 128.78 (2H), 128.50 (4H), 128.16 (2H), 128.01, 80.05, 77.27, 67.16, 38.66, 30.00, 29.70, 29.36, 28.47, 26.24, 22.70.

HRMS (ESI) calc for $C_{24}H_{31}N_3O_7S$ 505.59, found 528.20 (M+23)

4.2.6 Compound 3f



1 2 3f

In a two-necked flask, CDI (0.67 mmol, 108 mg) was added under N_2 atmosphere to a solution of Z-Orn-(Boc)OH (0.33 mmol, 122 mg) in dry THF solvent (7 mL). The mixture was stirred at room temperature for 1 hour.

Then, 4-chlorobenzene sulfonamide (0.5 mmol, 77 mg) and DBU (0.5 mmol, 76 mg) were added to the solution. The resulting mixture was stirred for 16 hours.

The solution was washed first with citric acid (5% w) (x2), then with distilled water (x2) and brine (x1). The aqueous phase was extracted with DCM. (x2). The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum. Purification was made by silica gel chromatography (MeOH/DCM = 2:98).

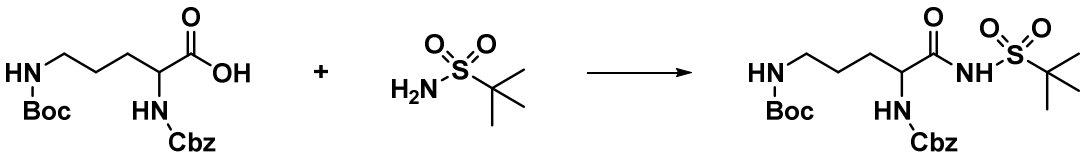
Benzyl tert-butyl (5-((4-chlorophenyl)sulfonamido)-5-oxopentane-1,4-diyl)dicarbamate. 3f. Yield = 45%

^{1}H NMR (400 MHz, Chloroform-d) δ 7.95 – 7.89 (m, 2H), 7.78 (m, 2H), 7.34 – 7.28 (m, 5H), 5.04 (m, 3H), 3.00 (m, 2H), 1.79 (m, 2H), 1.53 (m, 2H), 1.46 (m, 9H).

^{13}C NMR (101 MHz, CDCl3) δ 157.25, 156.52 (2H), 136.03 (2H), 128.97 (4H), 128.51 (2H), 128.19 (2H), 128.01 (2H), 77.24, 67.38, 67.13, 29.70, 28.44 (3H), 26.29, 22.69.

HRMS (ESI) calc for $C_{24}H_{30}ClN_3O_7S$ 540.03, found 563.47 (M+23)

4.2.7 Compound 3g



1 2 3g

In a two-necked flask, CDI (2 mmol, 324 mg) was added under N_2 atmosphere to a solution of Z-Orn-(Boc)OH (1 mmol, 366 mg) in dry THF solvent (20 mL). The mixture was stirred at room temperature for 1 hour.

Then, methylpropane sulfonamide (1.5 mmol, 206 mg) and DBU (1.5 mmol, 228 mg) were added to the solution. The resulting mixture was stirred for 16 hours.

The solution was washed first with citric acid (5% w) (x2), then with distilled water (x2) and brine (x1). The aqueous phase was extracted with DCM. (x2). The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum. Purification was made by silica gel chromatography (MeOH/DCM = 3:97).

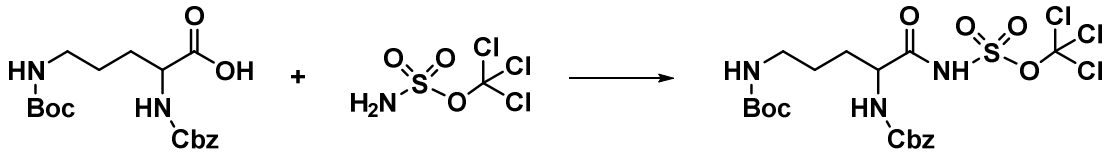
Benzyl tert-butyl (5-((1,1-dimethylethyl)sulfonamido)-5-oxopentane-1,4-diyl)dicarbamate. 3g. Yield = 76%

^{1}H NMR (400 MHz, Chloroform-d) δ 7.33 (m, 5H), 5.15 – 5.01 (m, 3H), 3.08 (m, 2H), 1.85 (m, 2H), 1.51 (m, 2H), 1.42 (m, 9H), 1.33 (m, 9H).

^{13}C NMR (101 MHz, CDCl3) δ 156.80, 156.57, 136.12, 128.47 (2H), 128.32, 128.13, 128.05 (2H), 79.53, 77.41, 67.07, 41.12, 39.35, 29.64, 28.44 (3H), 26.14, 24.07 (3H).

HRMS (ESI) calc for $C_{22}H_{35}N_3O_7S$ 485.60, found 508.94 (M+23)

4.2.8 Compound 3h



1 2 3h

In a two-necked flask, CDI (1.6 mmol, 260 mg) was added under N_2 atmosphere to a solution of Z-Orn-(Boc)OH (0.8 mmol, 293 mg) in dry THF solvent (20 mL). The mixture was stirred at room temperature for 1 hour.

Then, trichloromethyl sulfamate (1.2 mmol, 274 mg) and DBU (1.2 mmol, 183 mg) were added to the solution. The resulting mixture was stirred for 16 hours.

The solution was washed first with citric acid (5% w) (x2), then with distilled water (x2) and brine (x1). The aqueous phase was extracted with DCM. (x2). The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum. Purification was made by silica gel chromatography (MeOH/DCM = 3:97).

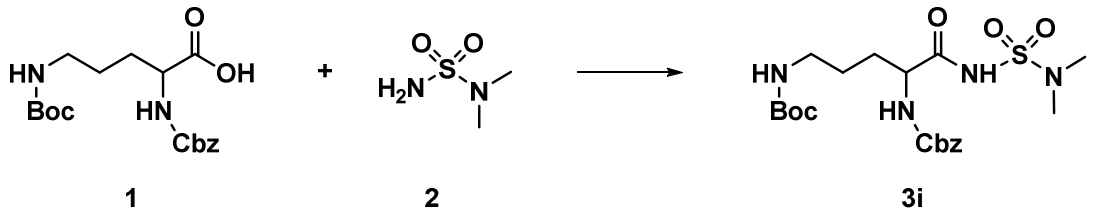
Trichloromethyl (2-(((benzyloxy)carbonyl)amino)-5-((tert-butoxycarbonyl)amino)pentanoyl)sulfamate. 3h. Yield = 76%

^{1}H NMR (400 MHz, DMSO-d6) δ 7.34 (m, 5H), 5.01 (m, 2H), 4.61 (s, 1H), 3.35 (m, 2H), 2.51 (m, 2H), 1.50 (m, 2H), 1.44 (m, 9H).

^{13}C NMR (101 MHz, DMSO) δ 156.23, 155.98, 137.70, 128.76 (2H), 128.10, 128.00, 96.08, 79.64, 78.51, 78.44, 77.75, 77.48, 65.55, 57.13, 28.76 (3H), 26.68.

HRMS (ESI) calc for $C_{19}H_{26}Cl_3N_3O_8S$ 462.84, found 485.46 (M+23)

4.2.9 Compound 3i



1 2 3i

In a two-necked flask, CDI (0.6 mmol, 108 mg) was added under N_2 atmosphere to a solution of Z-Orn-(Boc)OH (0.3 mmol, 120 mg) in dry THF solvent (7 mL). The mixture was stirred at room temperature for 1 hour.

Then, N,N-dimethyl Sulfamide (0.5 mmol, 62 mg) and DBU (0.5 mmol, 76 mg) were added to the solution. The resulting mixture was stirred for 16 hours.

The solution was washed first with citric acid (5% w) (x2), then with distilled water (x2) and brine (x1). The aqueous phase was extracted with DCM. (x2). The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum. Purification was made by silica gel chromatography (MeOH/DCM = 3:97).

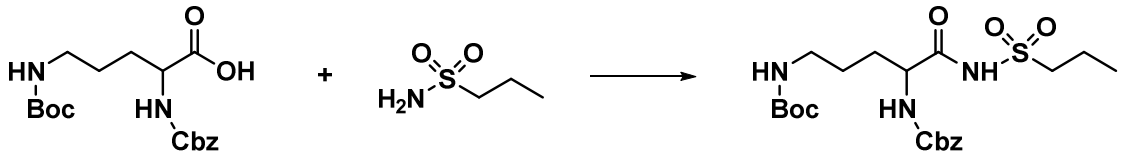
Benzyl tert-butyl (5-((N,N-dimethylsulfamoyl)amino)-5-oxopentane-1,4-diyl)dicarbamate. 3i. Yield = 83%

^{1}H NMR (400 MHz, DMSO-d6) δ 7.36 (m, 5H), 5.04 (m, 2H), 4.00 (s, 1H), 3.33 (m, 2H), 2.79 (m, 6H), 1.70 (m, 2H), 1.50 (m, 2H), 1.38 (m, 9H).

^{13}C NMR (101 MHz, CDCl3) δ 171.60, 156.99, 156.57, 136.06, 128.51 (2H), 128.19, 128.00, 79.74, 77.38, 67.20, 53.86, 38.95, 38.19, 29.58, 28.42 (3H), 27.94, 26.31.

HRMS (ESI) calc for $C_{20}H_{32}N_4O_7S$ 472.56, found 495.20 (M+23)

4.2.10 Compound 3j



1 2 3j

In a two-necked flask, CDI (1.6 mmol, 260 mg) was added under N_2 atmosphere to a solution of Z-Orn-(Boc)OH (0.8 mmol, 293 mg) in dry THF solvent (20 mL). The mixture was stirred at room temperature for 1 hour.

Then, propane sulfonamide (0.96 mmol, 118 mg) and DBU (1.2 mmol, 183 mg) were added to the solution. The resulting mixture was stirred for 16 hours.

The solution was washed first with citric acid (5% w) (x2), then with distilled water (x2) and brine (x1). The aqueous phase was extracted with DCM. (x2). The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum. Purification was made by silica gel chromatography (MeOH/DCM = 5:95).

Benzyl tert-butyl (5-oxo-5-(propylsulfonamido)pentane-1,4-diyl)dicarbamate.

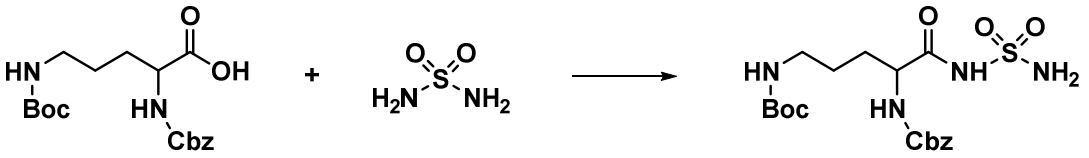
3j. Yield = 82%

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.33 (m, 5H), 5.12 – 5.07 (m, 2H), 4.47 (s, 1H), 3.23 – 3.01 (m, 4H), 1.90 – 1.74 (m, 4H), 1.55 (m, 2H), 1.43 (m, 9H), 1.01 (m, 3H).

^{13}C NMR (101 MHz, CDCl3) δ 172.80, 157.06, 156.58, 136.03, 128.53, 128.21, 128.00, 79.87, 77.34, 67.24, 56.82, 54.88, 54.22, 38.92, 29.45, 28.42, 26.27, 17.56, 16.81, 12.74, 12.70.

HRMS (ESI) calc for $C_{21}H_{33}N_3O_7S$ 471.57, found 494.27 (M+23)

4.2.11 Compound 3k



1 2 3k

In a two-necked flask, , CDI (1.6 mmol, 260 mg) was added under N_2 atmosphere to a solution of Z-Orn-(Boc)OH (0.8 mmol, 293 mg) in dry THF solvent (20 mL). The mixture was stirred at room temperature for 1 hour.

Then, sulphamide (0.96 mmol, 92 mg) and DBU (1.2 mmol, 183 mg) were added to the solution. The resulting mixture was stirred for 16 hours.

The solution was washed first with citric acid (5% w) (x2), then with distilled water (x2) and brine (x1). The aqueous phase was extracted with DCM. (x2). The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum. The purification was made by silica gel chromatography (MeOH/DCM = 5:95).

Benzyl tert-butyl (5-oxo-5-(sulfamoylamino)pentane-1,4-diyl)dicarbamate. 3k.

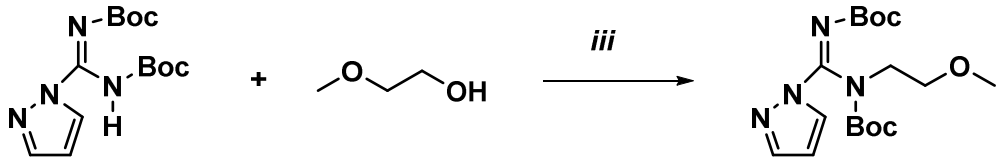
Yield = 75%

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.31 (m, 5H), 5.07 (m, 2H), 4.37 (s, 1H), 3.07 (m, 2H), 1.73 (m, 2H), 1.52 (m, 2H), 1.41 (m, 9H).

^{13}C NMR (101 MHz, CDCl3) δ 175.70, 172.83, 158.67, 156.81, 135.91, 128.54, 128.23, 128.11, 79.77, 77.27, 67.39, 58.43, 54.48, 39.37, 29.69, 29.13, 28.42, 25.99.

HRMS (ESI) calc for $C_{18}H_{28}N_4O_7S$ 444.17, found 467.1 (M+23)

4.3 Procedure for the synthesis of 6



4 5 6

To a solution of N,N'-bis-tert-butoxycarbonylpyrazole-1-carboxamidine (2 mmol, 620 mg), triphenylphosphine (3 mmol; 787 mg) and 2-methoxyethanol (4 mmol, 304 mg), diethyl azodi-carboxylate (3 mmol, 607 mg) was added dropwise stirring at 0 °C under N_2 atmosphere.

After 30 mins the ice bath was removed and the mixture was stirred at room temperature for 16 hours. The solution was concentrated under vacuum.

The purification was made by silica gel chromatography (n-Hexane/Ethyl acetate = 85:15).

Tert-butyl (E)-(((tert-butoxycarbonyl)imino)(1H-pyrazol-1-yl)methyl)(2-methoxyethyl)carbamate. 6.

Yield = quantitative

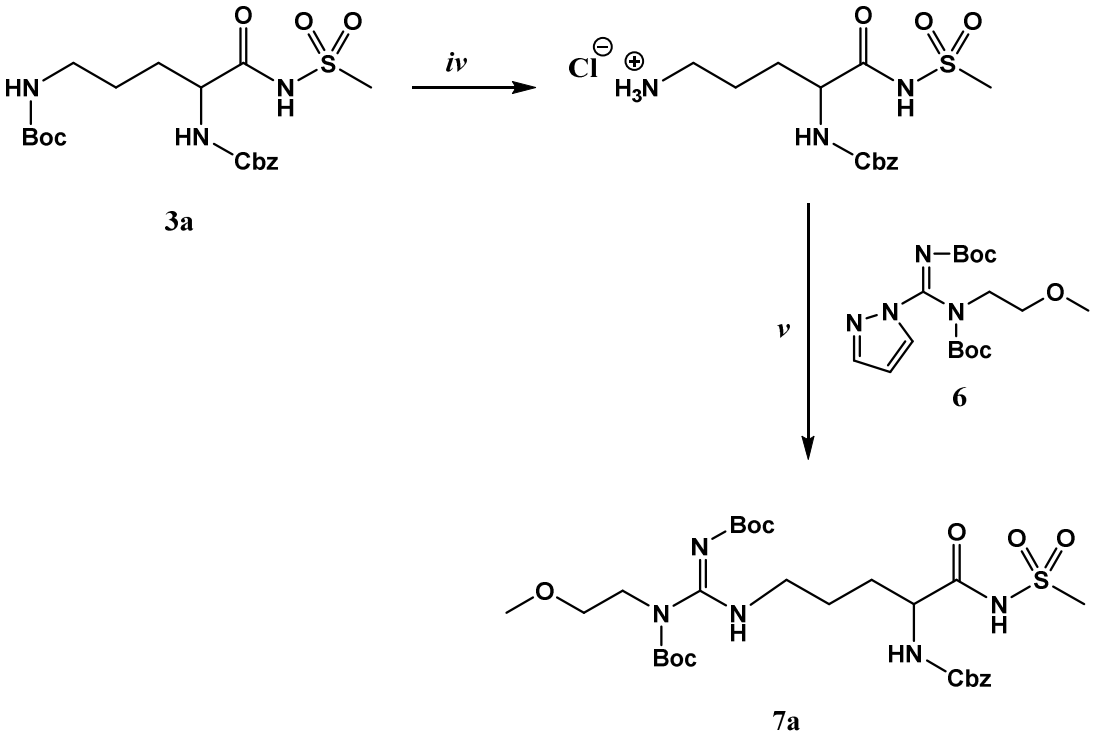
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.82 (s, 1H), 7.55 (s, 1H), 6.28 (s, 1H), 3.78 (d, *J* = 5.6 Hz, 1H), 3.52 (d, *J* = 5.6 Hz, 2H), 3.14 (s, 3H), 1.37 (s, 9H), 1.14 (s, 9H).

^{13}C NMR (101 MHz, CDCl3) δ 157.30, 152.24, 144.48, 142.86, 129.86, 108.59, 82.49, 81.91, 77.48, 69.88, 58.21, 47.51, 29.49, 27.77, 27.55, 21.93, 21.84.

HRMS (ESI) calc for $C_{17}H_{28}N_4O_5$ 368.43, found 491.87 (M+23)

4.4 Procedure for the synthesis of 7a-k

4.4.1 Compound 7a



HCl-dioxane 4M (2 mL) was added at room temperature for 1 hour to a solution of acyl sulfonamide of ornithine 3a (0.93 mmol, 412 mg).

Then DIPEA (1.84 mmol, 238 mg) dropwise in small amounts, Mitsunobu product (0.93 mmol, 342 mg) in solution of DCM (2 mL) were added to the mixture at room temperature for 24 hours.

The purification was made by silica gel chromatography (MeOH/DCM = 5:95).

7a.

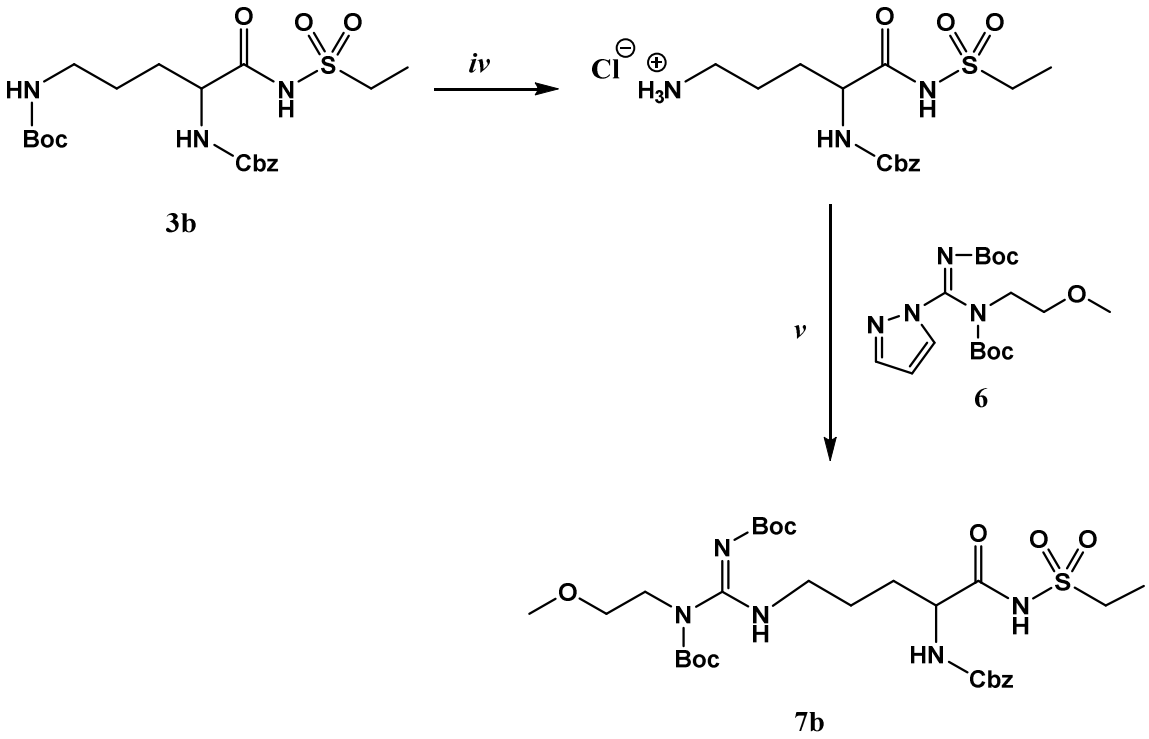
Yield = 42%

^{1}H NMR (400 MHz, Chloroform-d) δ 7.28 – 7.24 (m, 5H), 5.02 (m, 2H), 4.25 (s, 1H), 3.70 (m, 2H), 3.59 (m, 2H), 3.45 (m, 2H), 3.27 (m, 3H), 3.01 (m, 3H), 1.62 (m, 4H), 1.41 – 1.39 (m, 18H).

^{13}C NMR (101 MHz, CDCl3) δ 156.17, 152.07, 136.39, 128.40, 127.97, 127.95, 82.55, 79.85, 77.41, 70.59, 66.71, 58.61, 55.63, 54.08, 42.30, 40.79, 31.84, 29.91, 29.60, 29.27, 28.14, 28.11, 24.97, 22.61, 18.44, 17.43, 14.07, 12.07.

HRMS (ESI) calc for $C_{28}H_{45}N_5O_{10}S$ 643.75, found 644.3 (M+1)

4.4.2 Compound 7b



3b

HCl-dioxane 4M (2 mL) was added at room temperature for 1 hour to the solution of acyl sulfonamide of ornithine 3b (0.88 mmol, 412 mg).

Then DIPEA (1.1 mmol, 144 mg) dropwise in small amounts and Mitsunobu product (0.7 mmol, 258 mg) in solution of DCM (2 mL) were added to the mixture at room temperature for 24 hours.

The purification was made by silica gel chromatography (MeOH/DCM = 2:98).

7b.

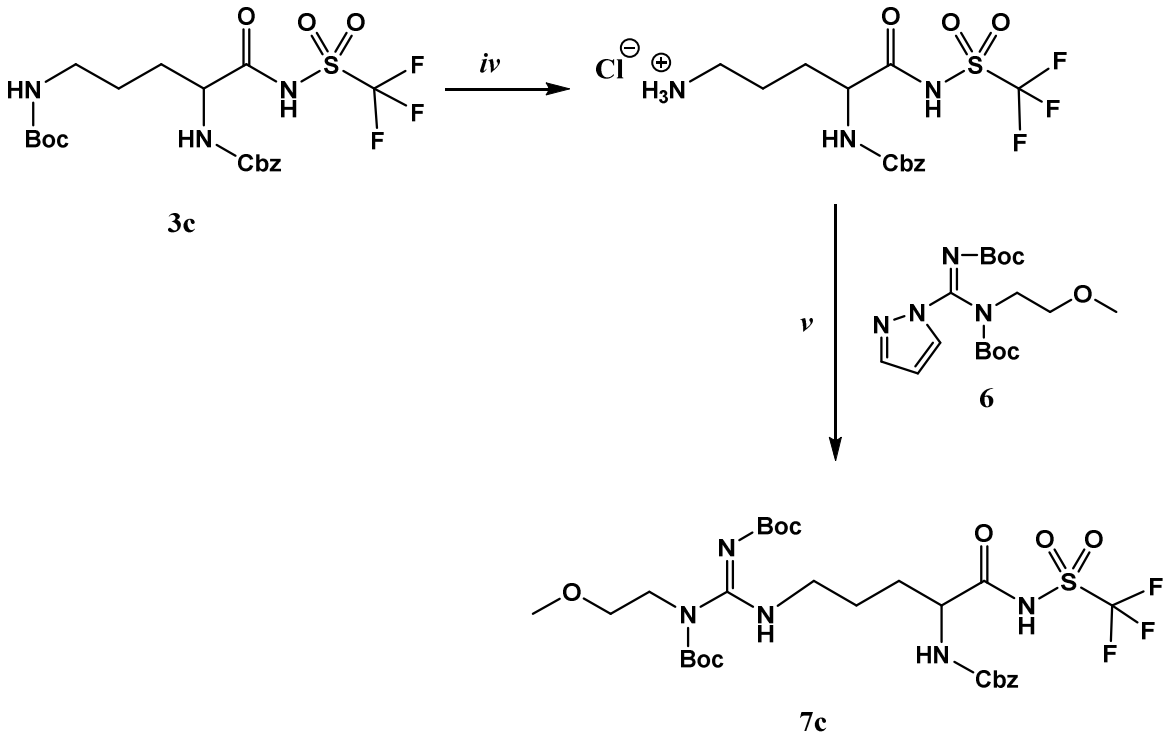
Yield = 26%

^{1}H NMR (400 MHz, Chloroform-d) δ 7.26 – 7.21 (m, 5H), 5.01 (m, 2H), 4.25 (s, 3H), 3.65 (m, 2H), 3.45 (m, 4H), 3.25 (m, 3H), 1.59 (m, 4H), 1.43 – 1.36 (m, 18H), 1.23 (m, 3H).

^{13}C NMR (101 MHz, CDCl3) δ 173.93, 156.43, 152.26, 136.15, 128.48 (2C), 128.14, 128.03 (2C), 82.77, 80.00, 77.32, 70.64, 67.04, 63.00, 58.51, 55.09, 47.54, 29.66, 29.32, 28.15 (7C), 25.11, 7.82.

HRMS (ESI) calc for $C_{29}H_{47}N_5O_{10}S$ 657.78, found 658.30 (M+1)

4.4.3 Compound 7c



HCl-dioxane 4M (2 mL) was added to the solution of acyl sulfonamide of ornithine 3c (0.765 mmol, 380 mg) at room temperature for 1 hour.

Then DIPEA (1 mmol, 129 mg) dropwise in small amounts and Mitsunobu product (0.7 mmol, 258 mg) in solution of DCM (2 mL) were added to the mixture at room temperature for 24 hours.

The purification was made by silica gel chromatography (MeOH/DCM = 3:97).

7c.

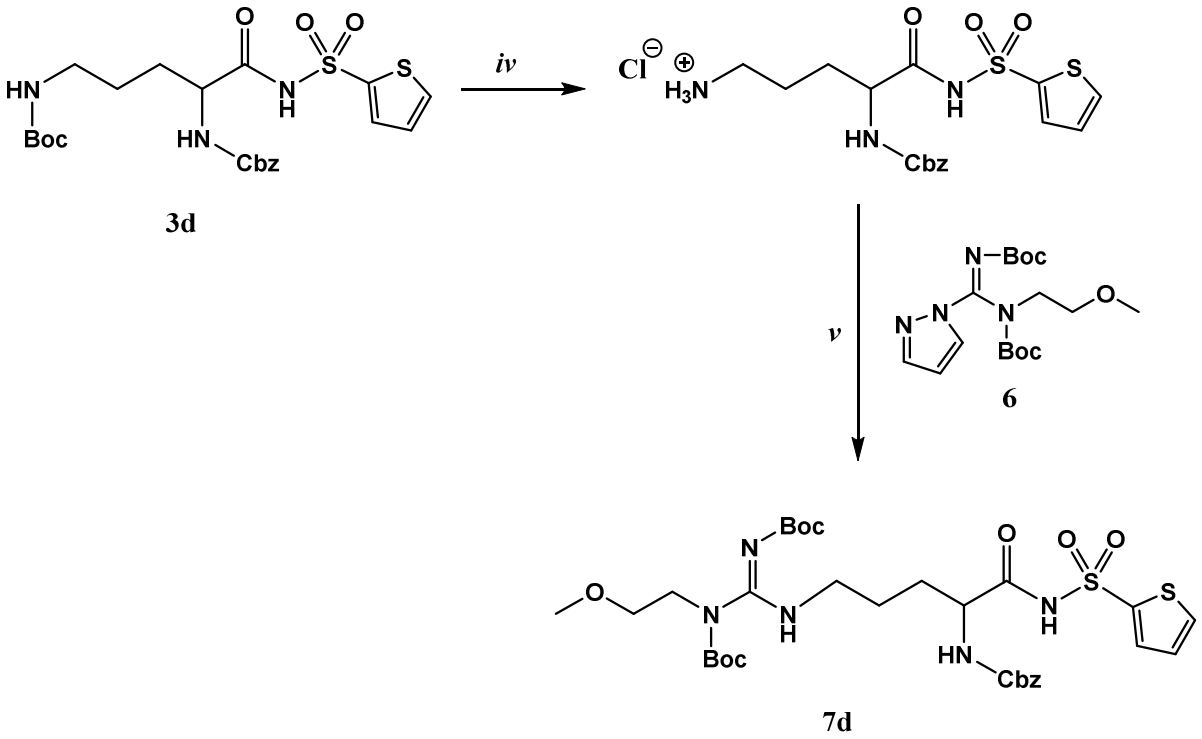
Yield = 23%

^{1}H NMR (400 MHz, Chloroform-d) δ 7.32 – 7.28 (m, 5H), 5.11 – 4.97 (m, 2H), 4.21 (s, 1H), 3.72 (m, 2H), 3.51 (m, 2H), 3.34 (m, 2H), 3.27 (m, 3H), 1.72 (m, 2H), 1.51 (m, 2H), 1.47 – 1.42 (m, 18H).

^{13}C NMR (101 MHz, CDCl3) δ 180.32, 156.38, 136.35, 128.41 (2C), 127.97, 127.90 (3C), 121.57, 118.38, 83.33, 77.28, 70.56, 66.77, 58.82, 56.88, 30.03, 29.68, 28.06, 28.04 (7C), 14.11.

HRMS (ESI) calc for $C_{28}H_{42}F_3N_5O_{10}S$ 697.72, found 698.30 (M+1)

4.4.4 Compound 7d



HCl-dioxane 4M (2 mL) was added to the solution of acyl sulfonamide of ornithine 3d (1.17 mmol, 596 mg) at room temperature for 1 hour.

Then DIPEA (1.5 mmol, 194 mg) dropwise in small amounts and Mitsunobu product (1.17 mmol, 431 mg) in solution of DCM (2 mL) were added to the mixture at room temperature for 24 hours.

The purification was made by silica gel chromatography (MeOH/DCM = 2:98).

7d.

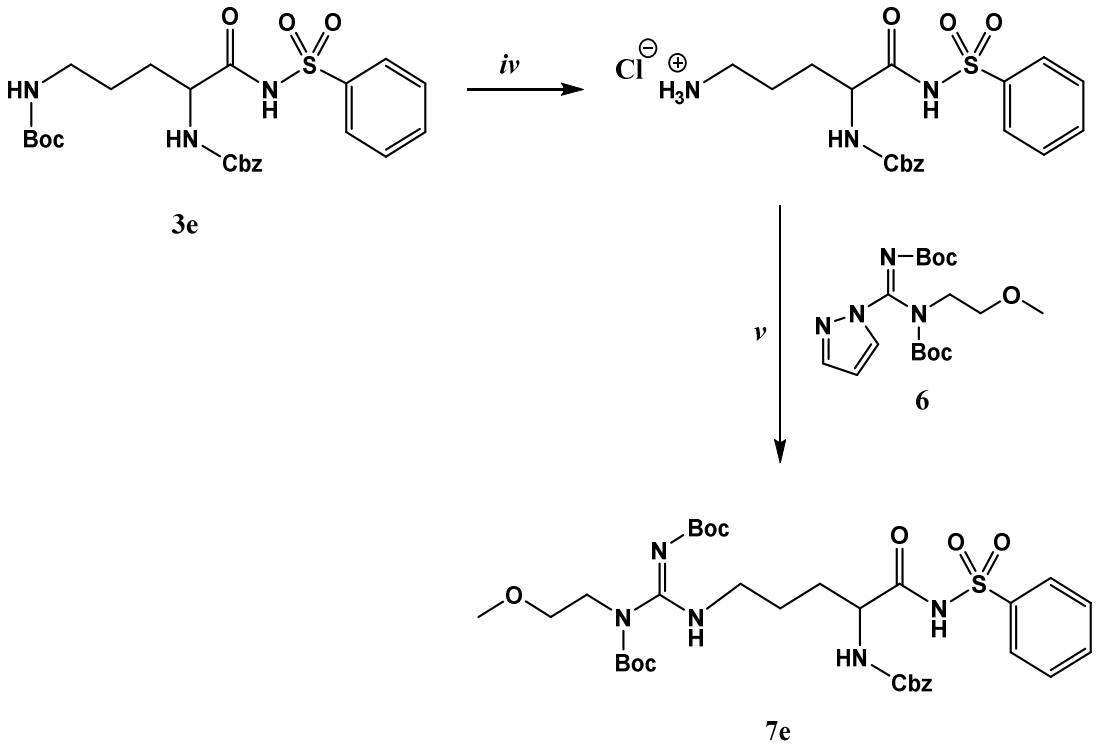
Yield = 92%

^{1}H NMR (400 MHz, DMSO-d6) δ 7.66 (s, 1H), 7.37 – 7.30 (m, 5H), 7.11 (m, 2H), 5.00 (m, 2H), 3.94 (m, 3H), 3.52 – 3.44 (m, 2H), 3.44 – 3.41 (m, 2H), 3.22 (m, 3H), 3.06 (m, 2H), 1.53 – 1.42 (m, 20H).

^{13}C NMR (101 MHz, DMSO) δ 156.29 (2C), 152.46, 137.36, 133.37, 132.94, 128.79 (2C), 128.24, 128.17 (2C), 127.41 (2C), 81.11, 79.64, 78.16, 70.25, 65.91, 58.49, 55.57, 47.14, 29.39, 28.37 (7C), 28.27, 25.05.

HRMS (ESI) calc for $C_{31}H_{45}N_5O_{10}S_2$ 711.85, found 712.20 (M+1)

4.4.5 Compound 7e



HCl-dioxane 4M (2 mL) was added to the solution of acyl sulfonamide of ornithine 3e (0.34 mmol, 180 mg) at room temperature for 1 hour. Then DIPEA (0.5 mmol, 65 mg) dropwise in small amounts and Mitsunobu product (0.3 mmol, 110 mg) in solution of DCM (2 mL) were added to the mixture at room temperature for 24 hours.

The purification was made by silica gel chromatography (MeOH/DCM = 2:98).

7e.

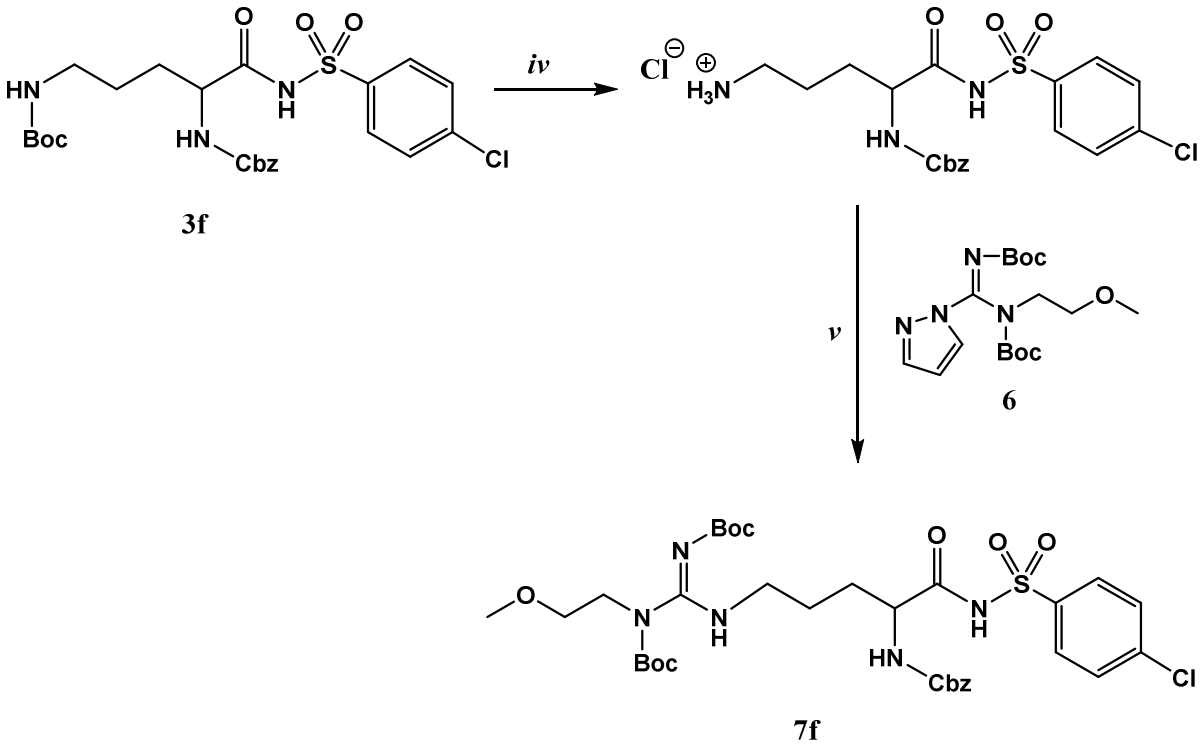
Yield = 26%

^{1}H NMR (400 MHz, Chloroform-d) δ 8.02 (m, 2H), 7.54 (s, 1H), 7.45 (m, 2H), 7.33 – 7.28 (m, 5H), 5.03 (m, 3H), 3.75 (m, 2H), 3.53 (m, 2H), 3.29 (m, 3H), 1.67 – 1.52 (m, 4H), 1.50 – 1.44 (m, 20H).

^{13}C NMR (101 MHz, CDCl3) δ 156.31, 152.22, 136.17, 133.13, 128.69, 128.47, 128.09, 127.99, 127.84, 82.83, 80.11, 77.30, 70.64, 66.98, 58.34, 54.60, 33.69, 31.92, 30.14, 29.68, 29.35, 28.17, 28.16 (7C), 26.69, 24.91, 22.68, 14.12.

HRMS (ESI) calc for $C_{33}H_{47}N_5O_{10}S$ 705.82, found 706.30 (M+1)

4.4.6 Compound 7f



HCl-dioxane 4M (2 mL) was added to the solution of acyl sulfonamide of ornithine 3f (0.463 mmol, 250 mg) at room temperature for 1 hour.

Then DIPEA (1 mmol, 129 mg) dropwise in small amounts and Mitsunobu product (0.46 mmol, 169 mg) in solution of DCM (2 mL) were added to the mixture at room temperature for 24 hours.

The purification was made by silica gel chromatography (MeOH/DCM = 1:99).

7f.

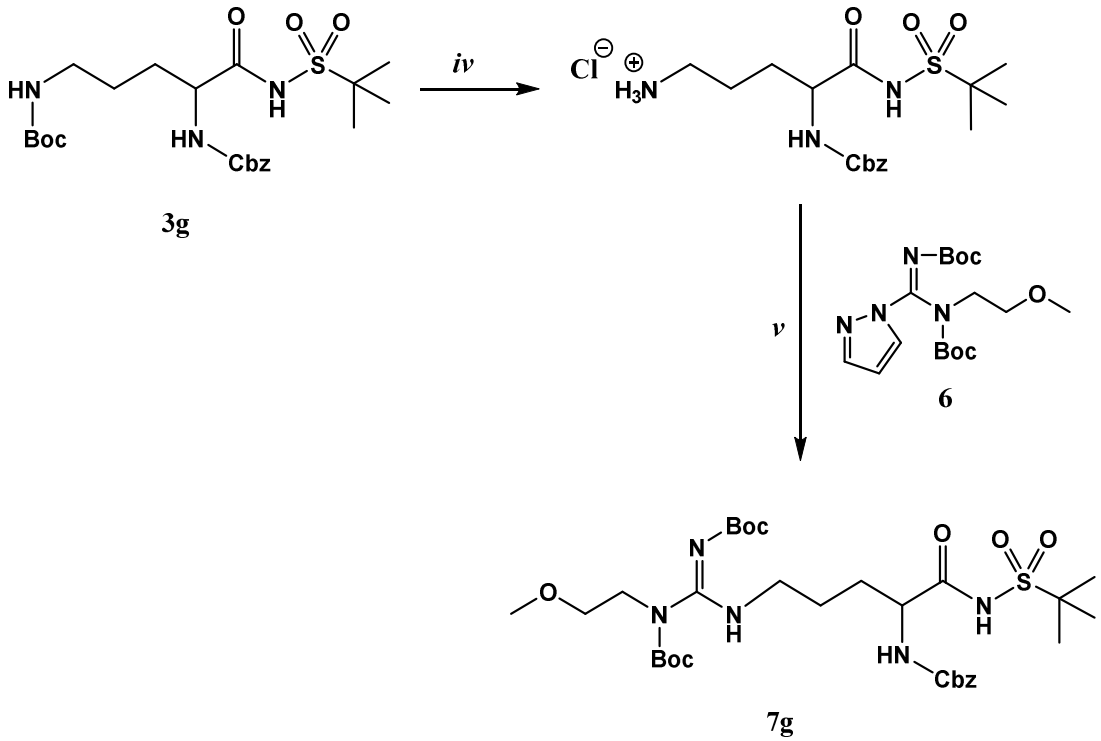
Yield = quantitative

^{1}H NMR (400 MHz, Chloroform-d) δ 7.91 (m, 2H), 7.36 (m, 2H), 7.31 – 7.27 (m, 5H), 5.02 (m, 2H), 4.26 (s, 3H), 3.79 – 3.61 (m, 2H), 3.50 (m, 2H), 3.27 (m, 3H), 1.68 – 1.50 (m, 4H), 1.47 – 1.43 (m, 18H).

^{13}C NMR (101 MHz, CDCl3) δ 156.23, 152.01, 138.67, 136.34, 128.91, 128.74, 128.44 (2C), 128.02, 127.94, 82.85, 80.27, 77.32, 70.61, 66.75, 58.70, 58.56, 55.41, 54.45, 42.61, 30.01, 29.67, 28.44, 28.16, 28.14 (7C), 24.87, 12.12.

HRMS (ESI) calc for $C_{33}H_{46}ClN_5O_{10}S$ 740.27, found 740.20

4.4.7 Compound 7g



HCl-dioxane 4M (2.5 mL) was added to the solution of acyl sulfonamide of ornithine 3g (0.7 mmol, 339 mg) at room temperature for 1 hour.

Then DIPEA (1 mmol, 129 mg) dropwise in small amounts and Mitsunobu product (0.7 mmol, 257 mg) in solution of DCM (2 mL) were added to the mixture at room temperature for 24 hours.

The solution was washed first with citric acid (5% w) (x1), then with distilled water (x1) and brine (x1). The organic phase was extracted with ethyl acetate (x2). The aqueous layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum.

The purification was made by silica gel chromatography (MeOH/DCM = 5:95).

7g.

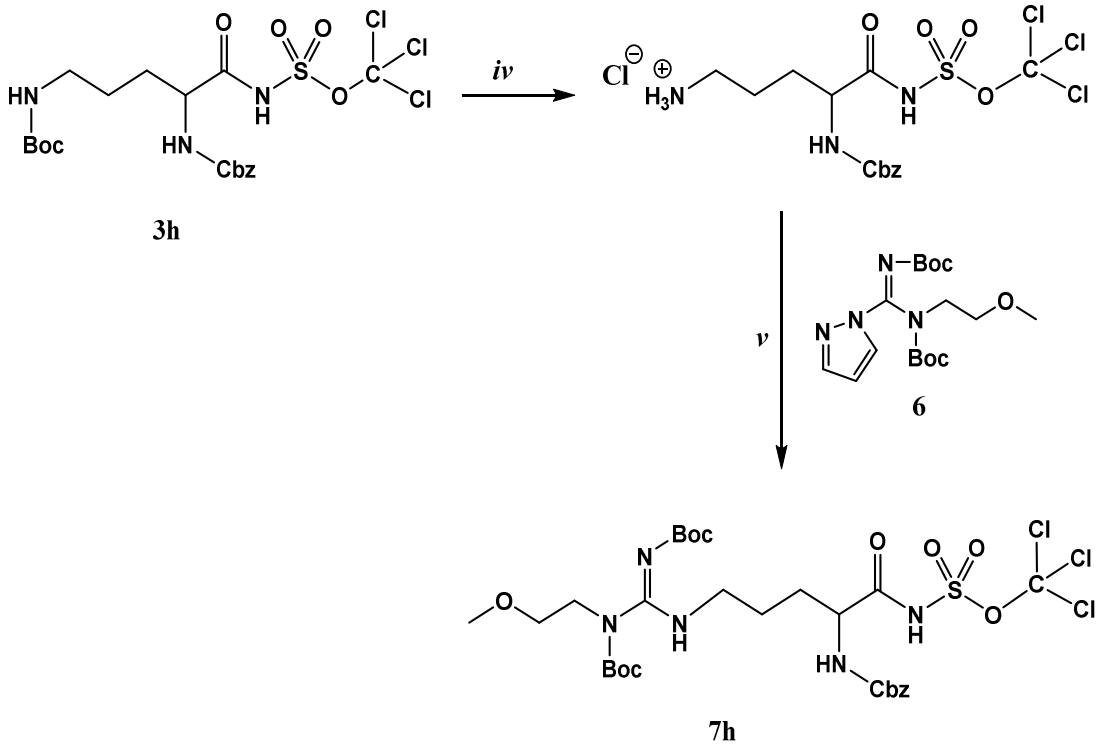
Yield = quantitative

^{1}H NMR (400 MHz, Chloroform-d) δ 7.34 – 7.31 (m, 5H), 5.10 (m, 2H), 4.31 (m, 3H), 3.78 (m, 2H), 3.54 (m, 2H), 3.37 – 3.27 (m, 11H), 1.72 (m, 2H), 1.51 (m, 2H), 1.48 – 1.45 (m, 9H), 1.42 – 1.38 (m, 9H).

^{13}C NMR (101 MHz, CDCl3) δ 156.50 (2C), 152.33, 136.14, 128.48 (2C), 128.14, 128.04 (2C), 82.79, 79.86, 77.30, 70.61, 67.05 (2C), 61.49, 58.44, 55.33, 53.45, 29.67, 28.16 (7C), 25.06, 24.18 (3C).

HRMS (ESI) calc for $C_{31}H_{51}N_5O_{10}S$ 685.83, found 686.20 (M+1)

4.4.8 Compound 7h



HCl-dioxane 4M (2.5 mL) was added to the solution of acyl sulfonamide of ornithine 3h (0.76 mmol, 577 mg) at room temperature for 1 hour.

Then DIPEA (1.5 mmol, 194 mg) dropwise in small amounts and Mitsunobu product (0.7 mmol, 257 mg) in solution of DCM (2 mL) were added to the mixture at room temperature for 24 hours.

The purification was made by silica gel chromatography (MeOH/DCM = 2:98).

7h.

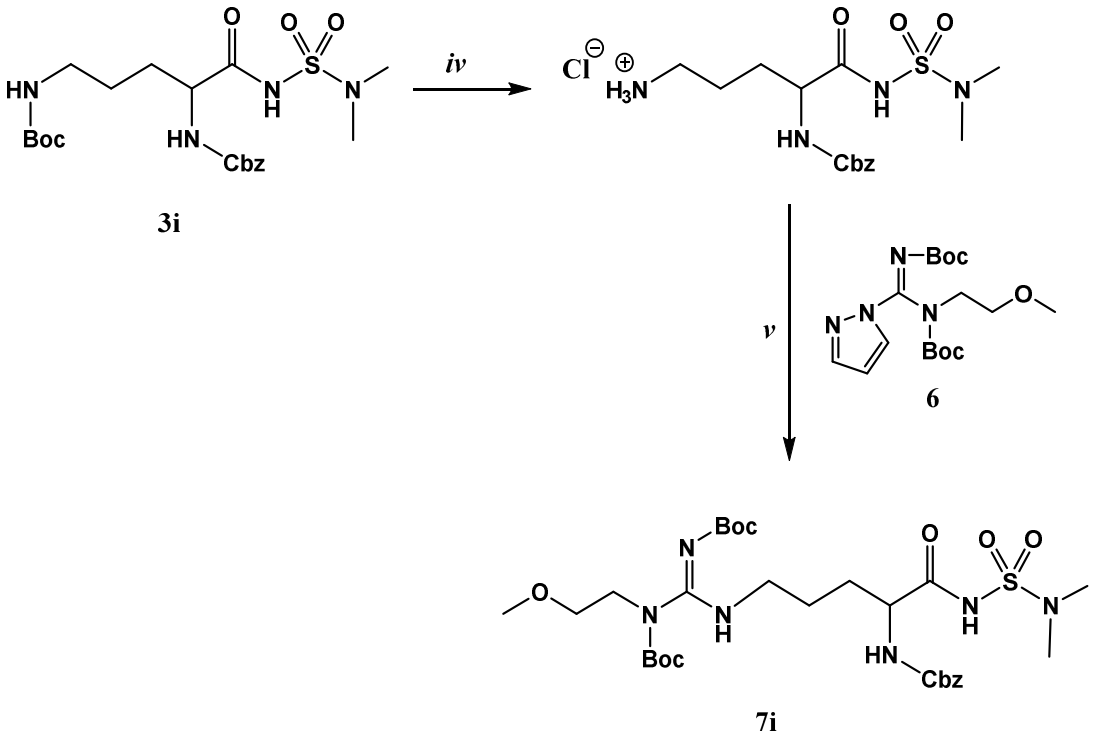
Yield = quantitative

^{1}H NMR (400 MHz, Chloroform-d) δ 7.33 – 7.31 (m, 5H), 5.13 – 5.00 (m, 2H), 4.21 (m, 3H), 3.78 (m, 2H), 3.38 – 3.31 (m, 5H), 1.77 (m, 2H), 1.53 (m, 2H), 1.49 – 1.46 (m, 18H).

^{13}C NMR (101 MHz, CDCl3) δ 156.30 (2C), 136.44, 128.44 (2C), 127.98 (3C), 127.89, 94.35, 78.24, 77.27, 70.54, 66.73, 59.14, 58.89, 56.41, 30.35, 30.23, 29.68, 28.12 (7C), 27.91.

HRMS (ESI) calc for $C_{28}H_{42}Cl_3N_5O_{11}S$ 763.08, found 764.95 (M+1)

4.4.9 Compound 7i



3i

7i

HCl-dioxane 4M (2 mL) was added to the solution of acyl sulfonamide of ornithine 3i (0.65 mmol, 290 mg) at room temperature for 1 hour.

Then DIPEA (1 mmol, 129 mg) dropwise in small amounts and Mitsunobu product (0.6 mmol, 220 mg) in solution of DCM (2 mL) were added to the mixture at room temperature for 24 hours.

The solution was washed first with citric acid (5% w) (x1), then with distilled water (x1) and brine (x1). The aqueous phase was extracted with DCM (x2). The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum.

The purification was made by silica gel chromatography (MeOH/DCM = 1:99).

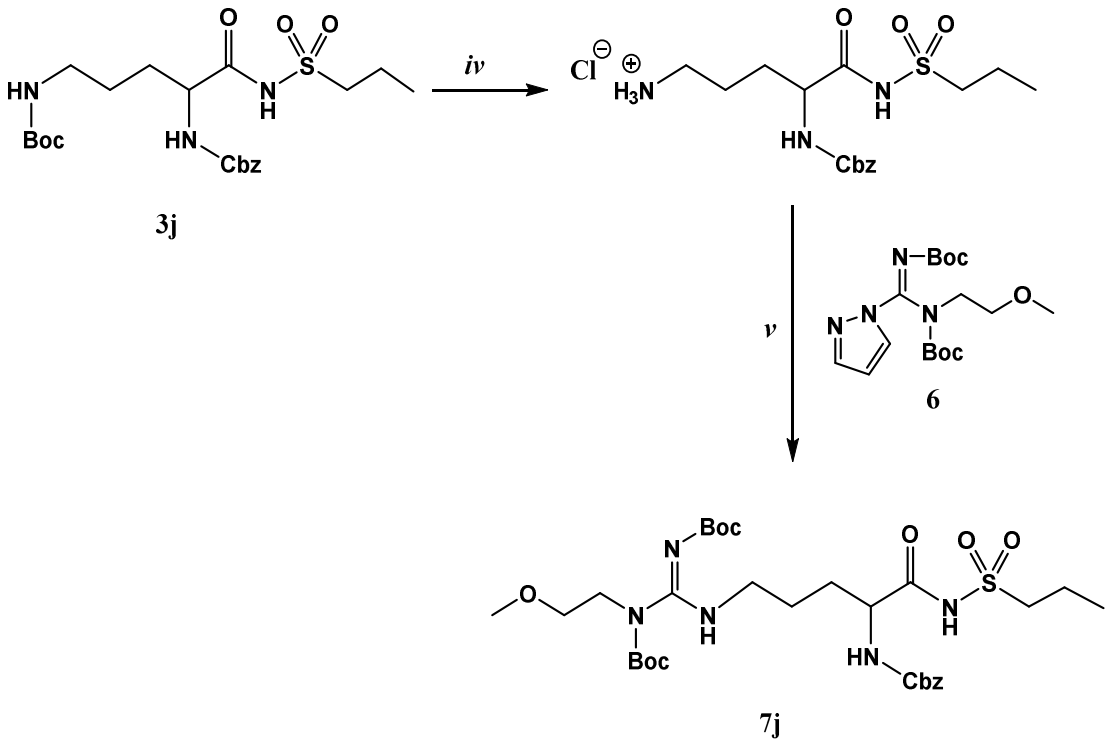
7i.

Yield = quantitative

^{1}H NMR (400 MHz, CDCl3) δ 7.31 - 7.28 (m, 5H), 5.07 - 5.02 (m, 2H), 4.25 (s, 1H), 3.67 (m, 2H), 3.40 (m, 2H), 3.23 (m, 5H), 2.78 (m, 6H), 1.79 - 1.77 (m, 2H), 1.52 - 1.51 (m, 2H), 1.48 - 1.38 (m, 18H).

HRMS (ESI) calc for $C_{29}H_{48}N_6O_{10}S$ 672.80, found 673.74 (M+1)

4.4.10 Compound 7j



HCl-dioxane 4M (2 mL) was added to the solution of acyl sulfonamide of ornithine 3j (0.59 mmol, 278 mg) at room temperature for 1 hour.

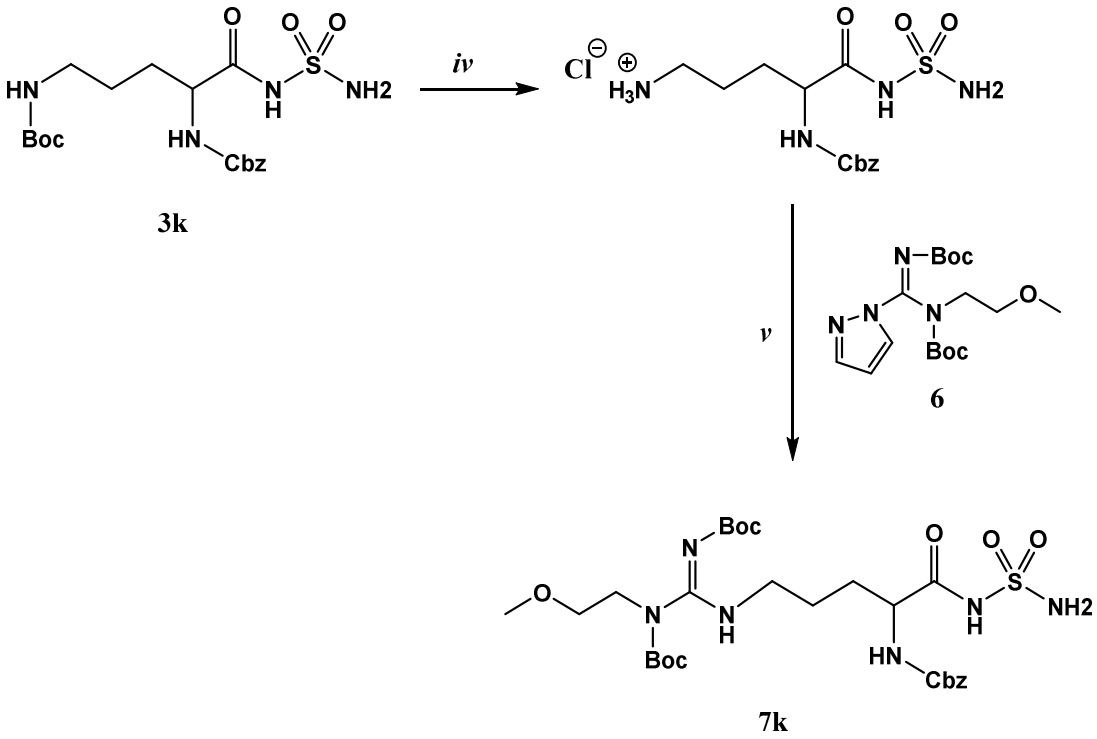
Then DIPEA (1 mmol, 129 mg) dropwise in small amounts and Mitsunobu product (0.6 mmol, 220 mg) in solution of DCM (2 mL) were added to the mixture at room temperature for 24 hours.

The solution was washed first with citric acid (5% w) (x1), then with distilled water (x1) and brine (x1). The aqueous phase was extracted with DCM (x2). The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum.

The purification was made by silica gel chromatography (MeOH/DCM = 2:98).

HRMS (ESI) calc for $C_{30}H_{49}N_5O_{10}S$ 671.81, found 694.58 (M+23)

4.4.11 Compound 7k



3k

6

7k

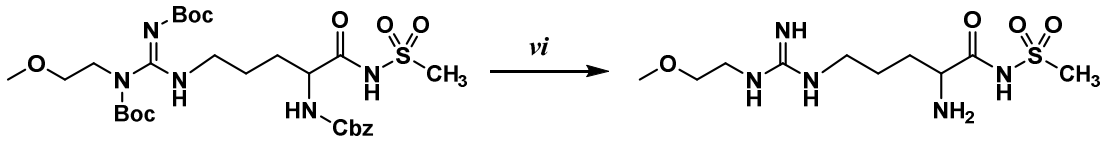
HCl-dioxane 4M (2 mL) was added to the solution of acyl sulfonamide of ornithine 3k (0.83 mmol, 372 mg) at room temperature for 1 hour.

Then DIPEA (1.2 mmol, 155 mg) dropwise in small amounts and Mitsunobu product (0.83 mmol, 305 mg) in solution of DCM (2 mL) were added to the mixture at room temperature for 24 hours.

The solution was washed first with citric acid (5% w) (x1), then with distilled water (x1) and brine (x1). The aqueous phase was extracted with DCM (x2). The organic layer was dried with Na_2SO_4 , filtered and the solution was concentrated under vacuum. The reaction did not work.

4.5 Procedure for the synthesis of 8b-f

4.5.1 Compound 8a



7a **8a**

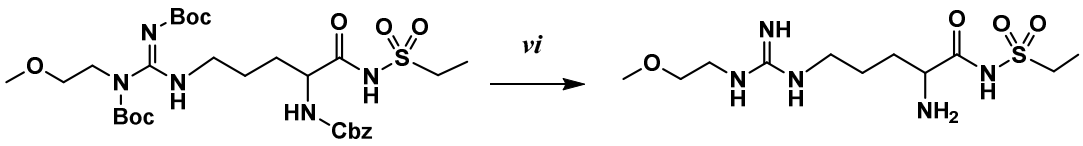
To the starting material 7a (0.42 mmol, 271 mg), TFA (2 mL) and TFSMA (5.04 mmol, 756 mg) were added at room temperature for 1 hour,
The solution was washed with distillated water (x2). The organic layer was extracted with DCM. The formation of the final product was verified via mass spectrometry. Then the aqueous solution was lyophilized and purified by HPLC.

2-amino-5-(3-(2-methoxyethyl)guanidino)-N-(methylsulfonyl)pentanamide. 8a.
Yield = quantitative
^{1}H NMR (400 MHz, D2O) δ 3.94 (t, J = 6.0 Hz, 1H, CH), 3.53 (t, J = 5.0 Hz, 2H, CH2N), 3.35-3.29 (m, 5H, OCH2, CH3), 3.25-3.15 (m, 5H, NCH2, CH3O), 1.98-1.84 (m, 2H, CH2), 1.74-1.52 (m, 2H, CH2).

^{13}C NMR (100 MHz, D2O) δ 172.0 (CO), 156.2 (CN3), 70.3 (CH2N), 58.2 (CH3), 54.0 (CH), 41.1 (OCH2), 40.3 (NCH2), 40.2 (CH3O), 27.5 (CH2), 23.4 (CH2).

HRMS (ESI) calc for $C_{10}H_{23}N_5O_4S$ 309.39, found 310.10 (M+1).

4.5.2 Compound 8b



7b **8b**

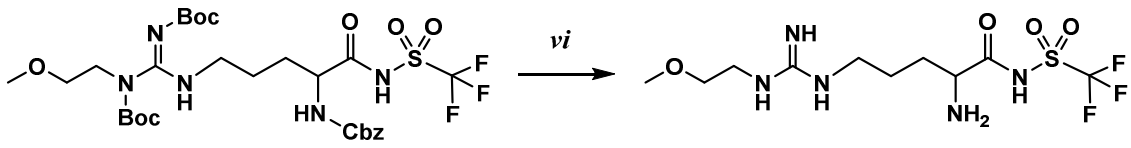
To the starting material 7b (0.177 mmol, 116 mg), TFA (2 mL) and TFSMA (2.121 mmol, 318 mg) were added at room temperature for 1 hour.
The solution was washed with distilled water (x2). The organic layer was extracted with DCM. The formation of the final product was verified via mass spectrometry. Then the aqueous solution was lyophilized and purified by HPLC.

2-amino-N-(ethylsulfonyl)-5-(3-(2-methoxyethyl)guanidino)pentanamide. 8b.
Yield = quantitative
^{1}H NMR (400 MHz, DMSO) δ 8.38 (2H), 7.61 (1H), 3.97 - 3.89 (2H), 3.44 - 3.38 (4H), 3.31 (1H), 3.29 (3H), 3.19 - 3.14 (2H), 1.81 - 1.75 (2H), 1.55 - 1.50 (2H), 1.27 (3H).

^{13}C NMR (101 MHz, DMSO) δ 169.77, 158.54, 70.42, 58.56, 53.03 (2C), 41.26 (2C), 28.28, 24.47, 8.18.

HRMS (ESI) calc for $C_{11}H_{25}N_5O_4S$ 323.41, found 324.20 (M+1).

4.5.3 Compound 8c



7c **8c**

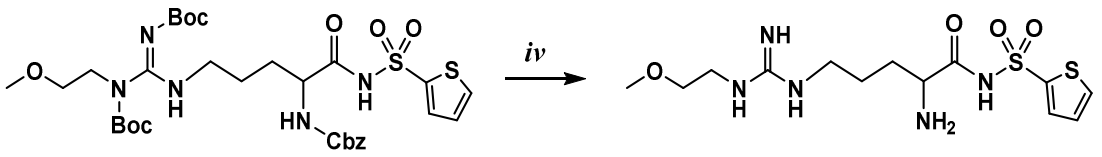
To the starting material 7c (0.174 mmol, 122 mg), TFA (2 mL) and TFSMA (2.088 mmol, 313 mg) were added at room temperature for 1 hour.
The solution was washed with distilled water (x2). The organic layer was extracted with DCM. The formation of the final product was verified via mass spectrometry. Then the aqueous solution was lyophilized and purified by HPLC.

2-amino5(3(2methoxyethyl)guanidino)N((trifluoromethyl)sulfonyl)pentanamide.
8c. Yield = quantitative
^{1}H NMR (400 MHz, DMSO) δ 7.95 (2H), 7.74 (1H), 3.62 - 3.57 (2H), 3.44 - 3.41 (4H), 3.31 (1H), 3.29 (3H), 1.78 - 1.75 (2H), 1.59 - 1.53 (2H).

^{13}C NMR (101 MHz, DMSO) δ 173.69, 158.61, 158.30, 70.43, 58.54, 55.08, 41.24, 35.27, 24.72 (2C).

HRMS (ESI) calc for $C_{10}H_{20}F_3N_5O_4S$ 363.36, found 364.10 (M+1)

4.5.4 Compound 8d



7d **8d**

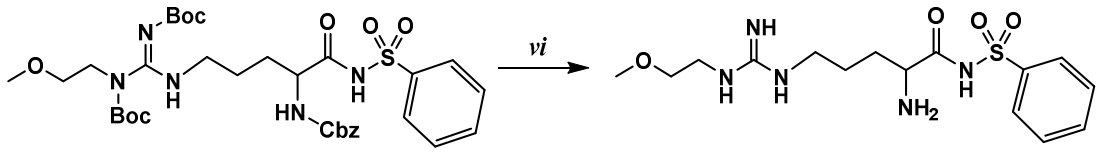
To the starting material 7d (0.317 mmol, 226 mg), TFA (2 mL) and TFSMA (3.804 mmol, 571 mg) were added at room temperature for 1 hour.
The solution was washed with distilled water (x2). The organic layer was extracted with DCM. The formation of the final product was verified via mass spectrometry. Then the aqueous solution was lyophilized and purified by HPL.

2-amino-5-(3-(2-methoxyethyl)guanidino)-N-(thiophen2ylsulfonyl)pentanamide.
8d. Yield = quantitative
^{1}H NMR (400 MHz, DMSO) δ 7.77 (1H), 7.76, 7.39 (1H), 7.08 - 7.07 (2H), 3.45 - 3.41 (2H), 3.34 - 3.33 (2H), 3.32 - 3.31 (2H), 3.31 - 3.29 (3H), 3.24 (1H), 1.78 - 1.76 (2H), 1.55 - 1.47 (2H).

^{13}C NMR (101 MHz, DMSO) δ 175.64, 154.29, 125.89 (2C), 122.47 (2C), 70.45, 60.29, 50.33, 42.40, 36.26, 26.58 (2C).

HRMS (ESI) calc for $C_{13}H_{23}N_5O_4S_2$ 377.48, found 378.10 (M+1)

4.5.5 Compound 8e



7e **8e**

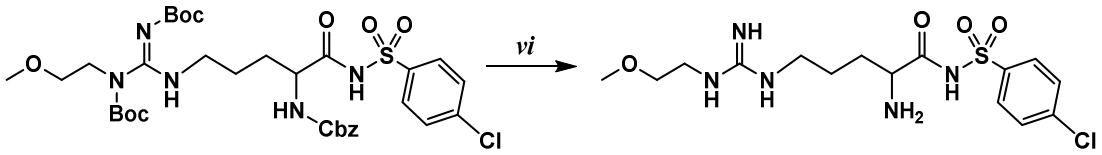
To the starting material 7e (0.215 mmol, 226 mg), TFA (2 mL) and TFSMA (2.58 mmol, 387 mg) were added at room temperature for 1 hour.
The solution was washed with distilled water (x2). The organic layer was extracted with DCM. The formation of the final product was verified via mass spectrometry. Then the aqueous solution was lyophilized and purified by HPLC.

2-amino-5-(3-(2-methoxyethyl)guanidino)-N-(phenylsulfonyl)pentanamide. 8e.
Yield = quantitative
^{1}H NMR (400 MHz, DMSO) δ 8.04 (1H), 7.91 - 7.89 (2H), 7.63 (1H), 7.61 - 7.59 (2H), 3.66 (2H), 3.45 - 3.42 (2H), 3.32 - 3.31 (2H), 3.31 - 3.29 (3H), 3.26 (1H), 1.71 (2H), 1.45 (2H).

^{13}C NMR (101 MHz, DMSO) δ 158.76, 158.44, 158.12, 156.22, 128.98, 127.77, 70.44, 58.57, 53.68, 41.27, 40.73, 39.67, 39.46, 28.39, 24.51.

HRMS (ESI) calc for $C_{15}H_{25}N_5O_4S$ 371.46, found 372.20 (M+1)

4.5.6 Compound 8f



7f **8f**

To the starting material 7f (0.2 mmol, 146.3 mg), TFA (2 mL) and TFSMA (2.4 mmol, 192 mg) were added at room temperature for 1 hour.
The solution was washed with distilled water (x2). The organic layer was extracted with DCM. The formation of the final product was verified via mass spectrometry. Then the aqueous solution was lyophilized and purified by HPLC.

2-amino-N-((4-chlorophenyl)sulfonyl)-5-(3-(2-methoxyethyl)guanidino)pentanamide. 8f. Yield = quantitative

^{1}H NMR (400 MHz, DMSO) δ 7.98 (1H), 7.88 - 7.86 (2H), 7.59 - 7.57 (2H), 3.65 (2H), 3.44 - 3.42 (2H), 3.32 - 3.31 (2H), 3.29 (3H), 3.11 - 3.10 (1H), 1.73 - 1.68 (2H), 1.45 (2H).

^{13}C NMR (101 MHz, DMSO) δ 171.18, 158.17, 156.23, 129.73, 128.83, 70.44, 58.57, 53.97, 41.27, 40.78, 40.67, 40.46, 39.43, 28.46, 24.59.

HRMS (ESI) calc for $C_{15}H_{24}ClN_5O_4S$, 405,90, found 406.67 (M+1)

4.6 NMR spectra

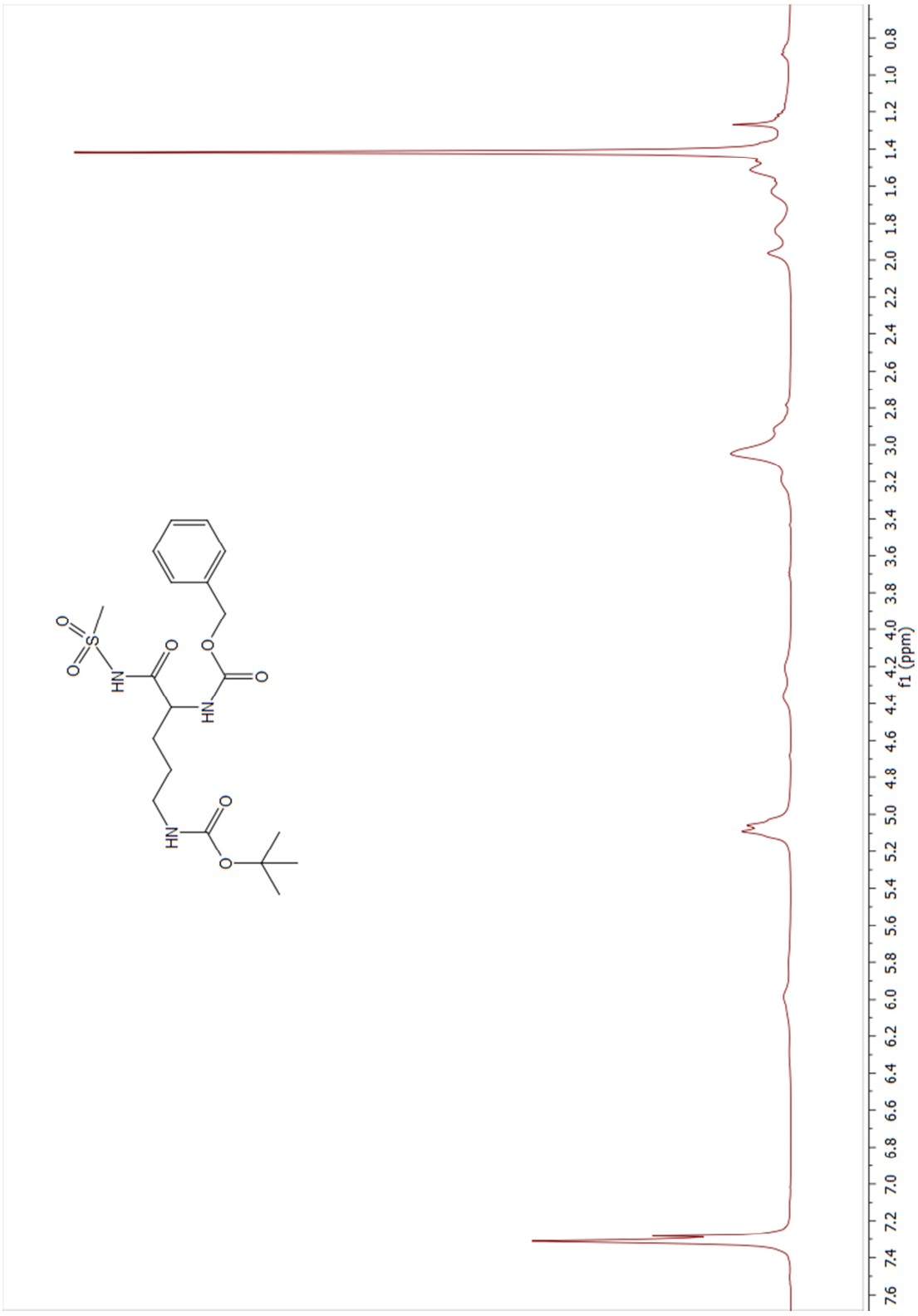


Figure 4.1 ^{1}H NMR spectrum of 3a

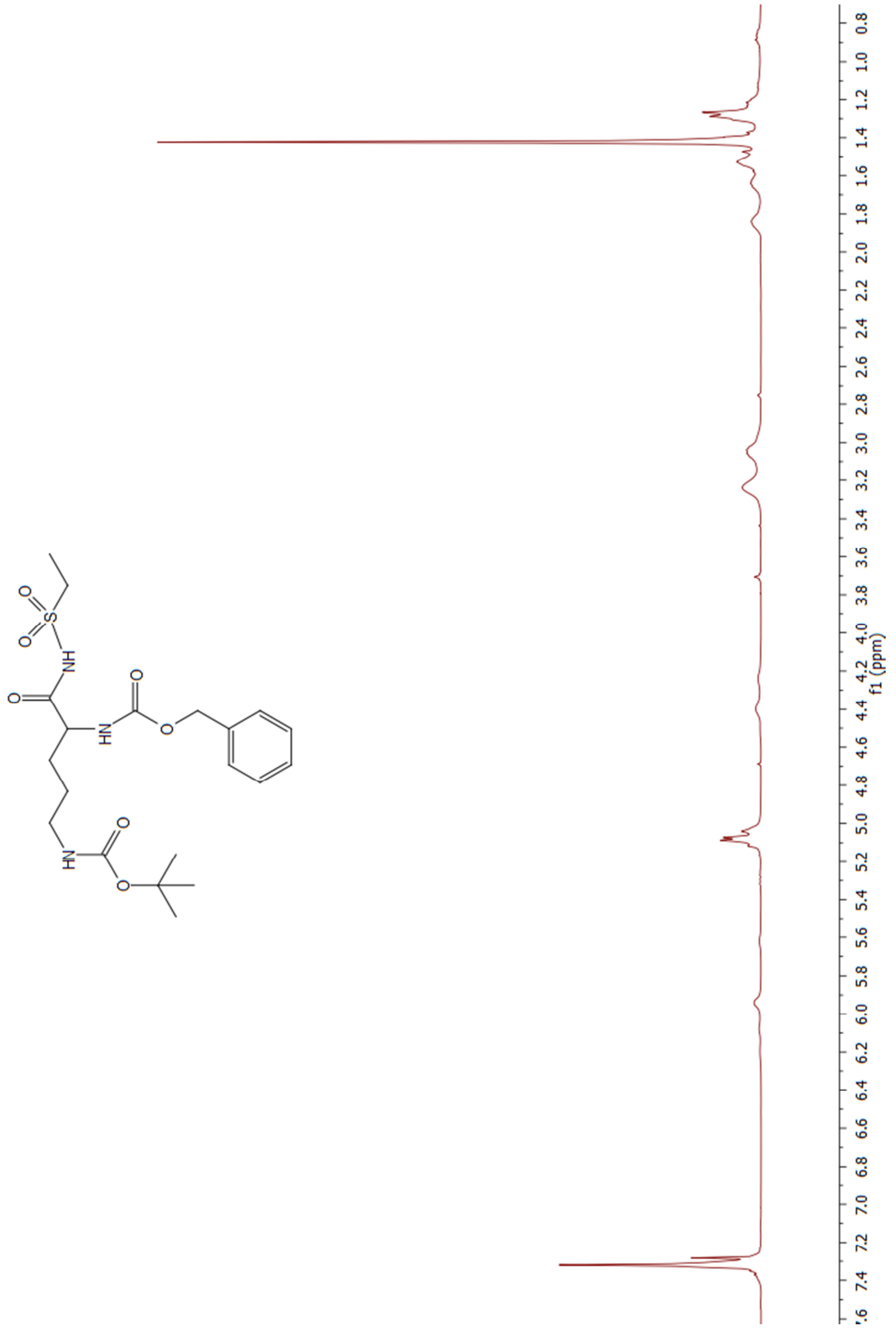


Figure 4.2 ^{1}H NMR spectrum of 3b

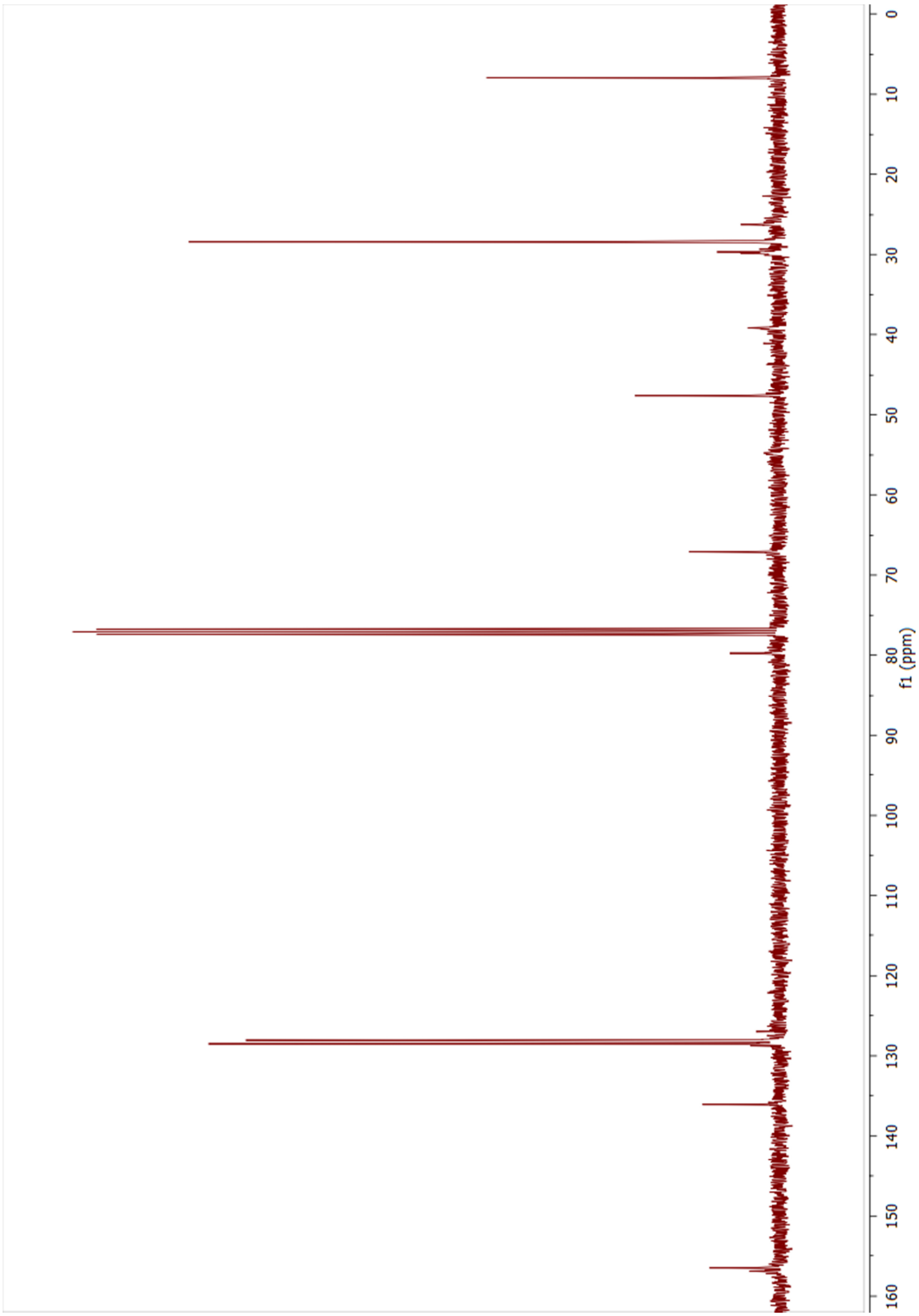


Figure 4.3 ^{14}C NMR spectrum of 3b

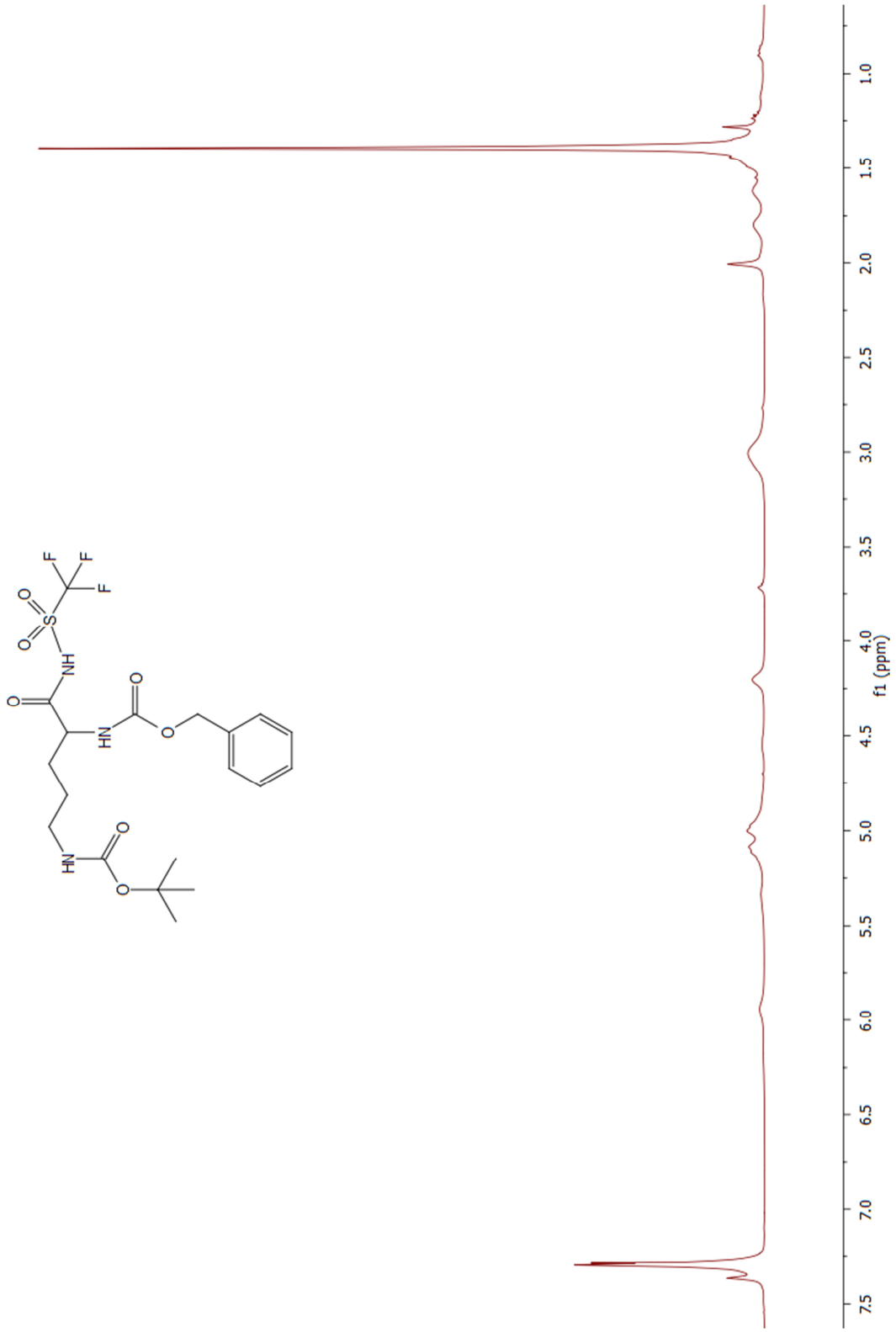


Figure 4.4 ^{1}H NMR spectrum of 3c

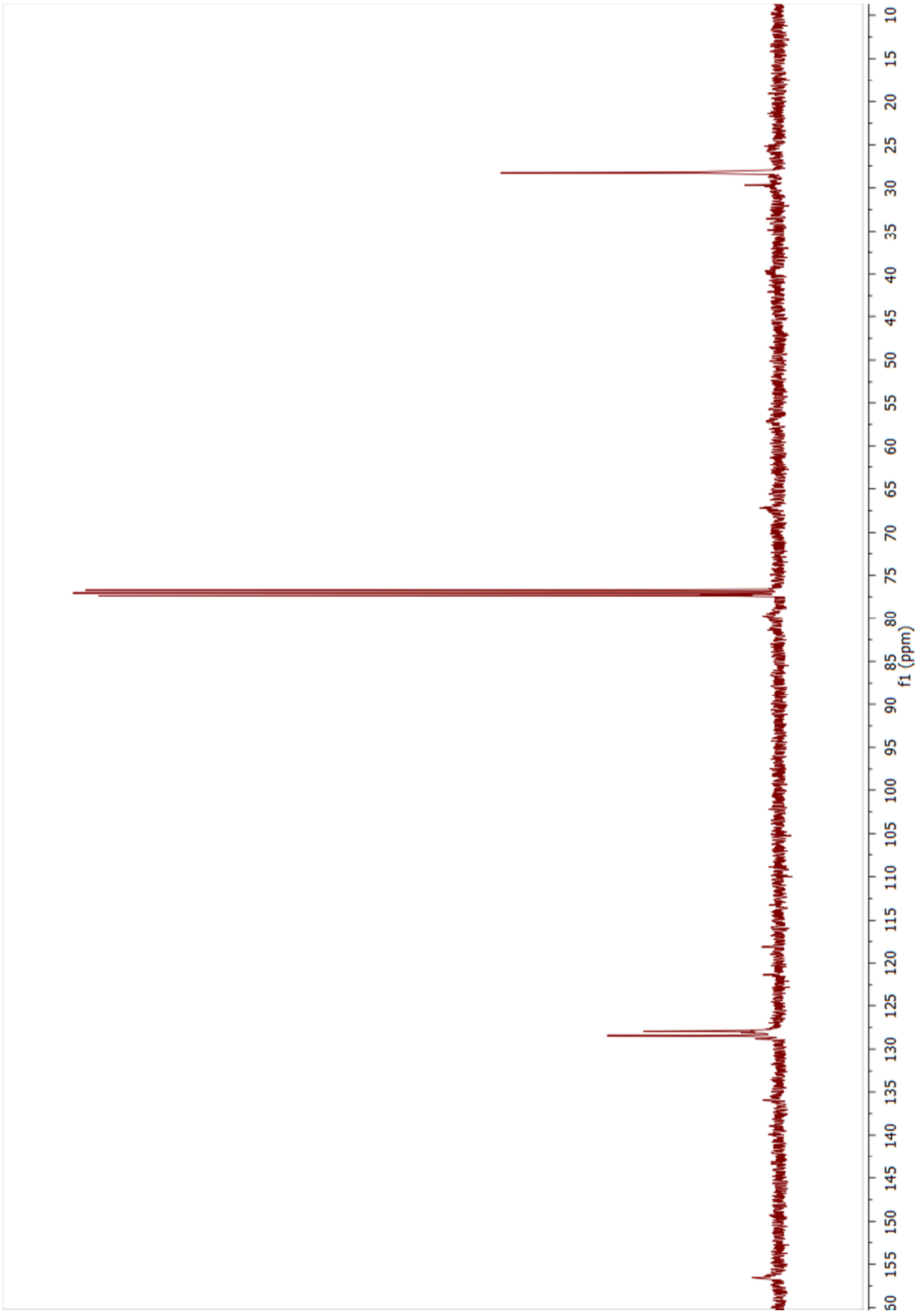


Figure 4.5 ^{14}C NMR spectrum of 3c

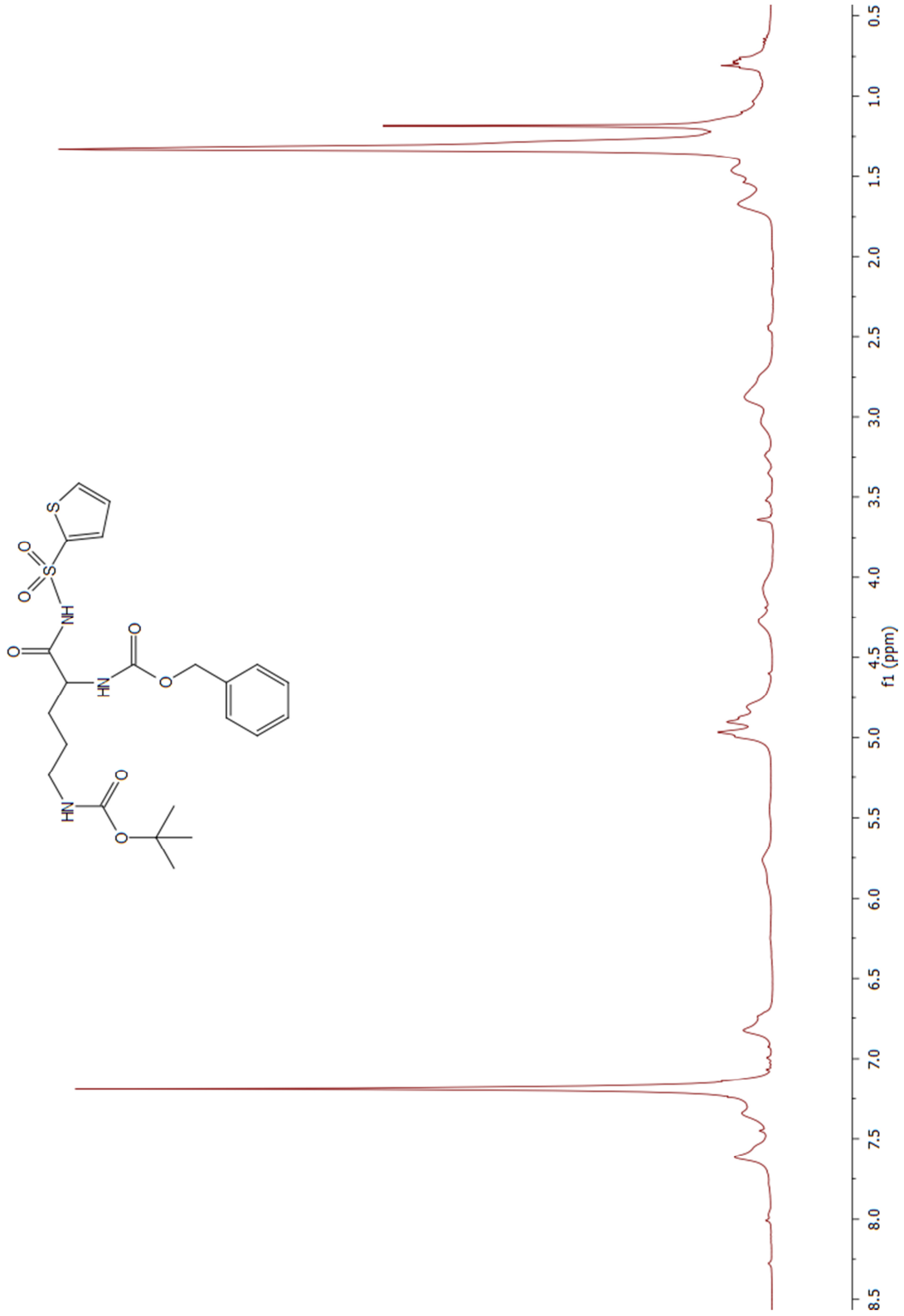


Figure 4.6 ^{1}H NMR spectrum of 3d

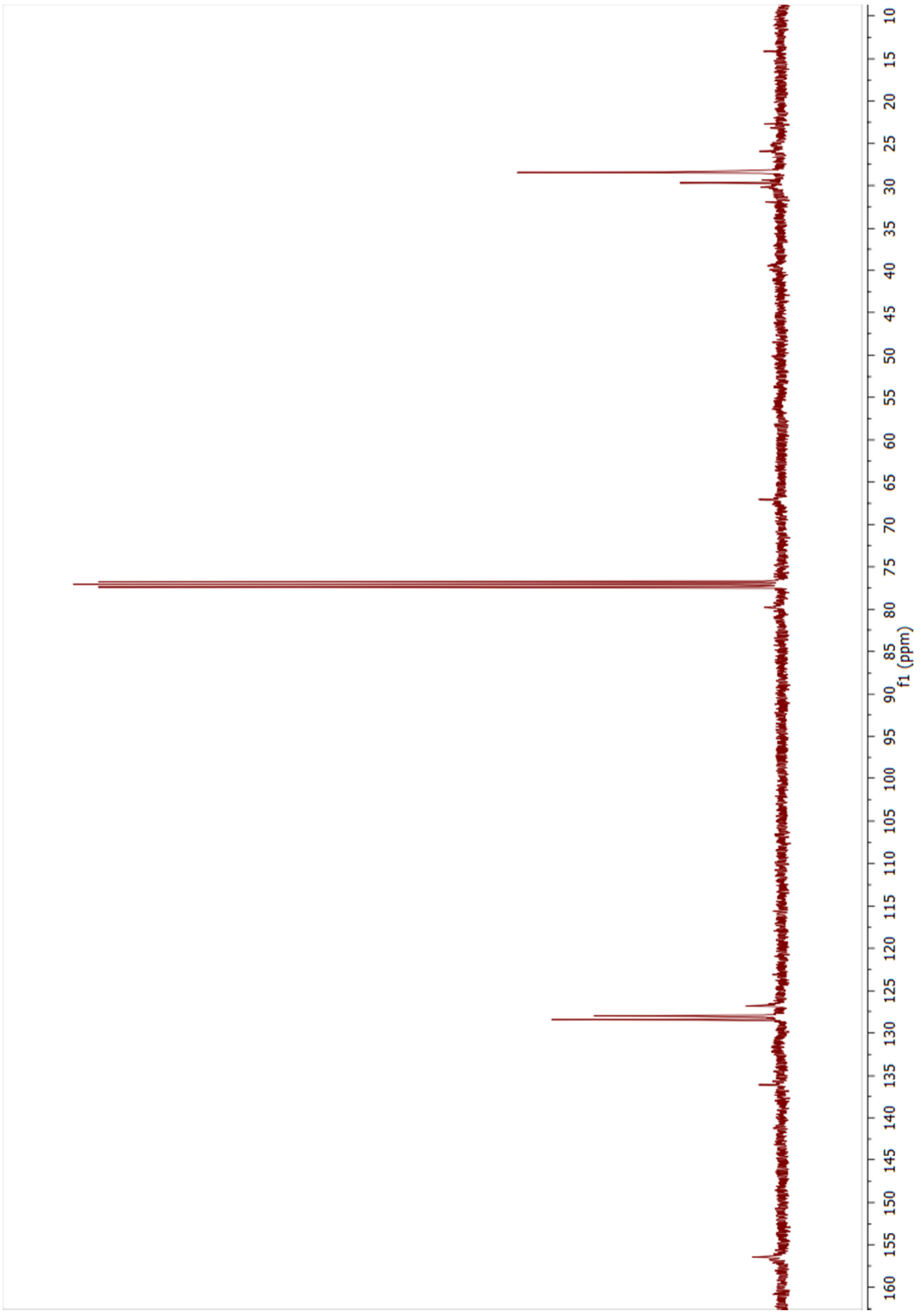


Figure 4.7 ^{14}C NMR spectrum of 3d

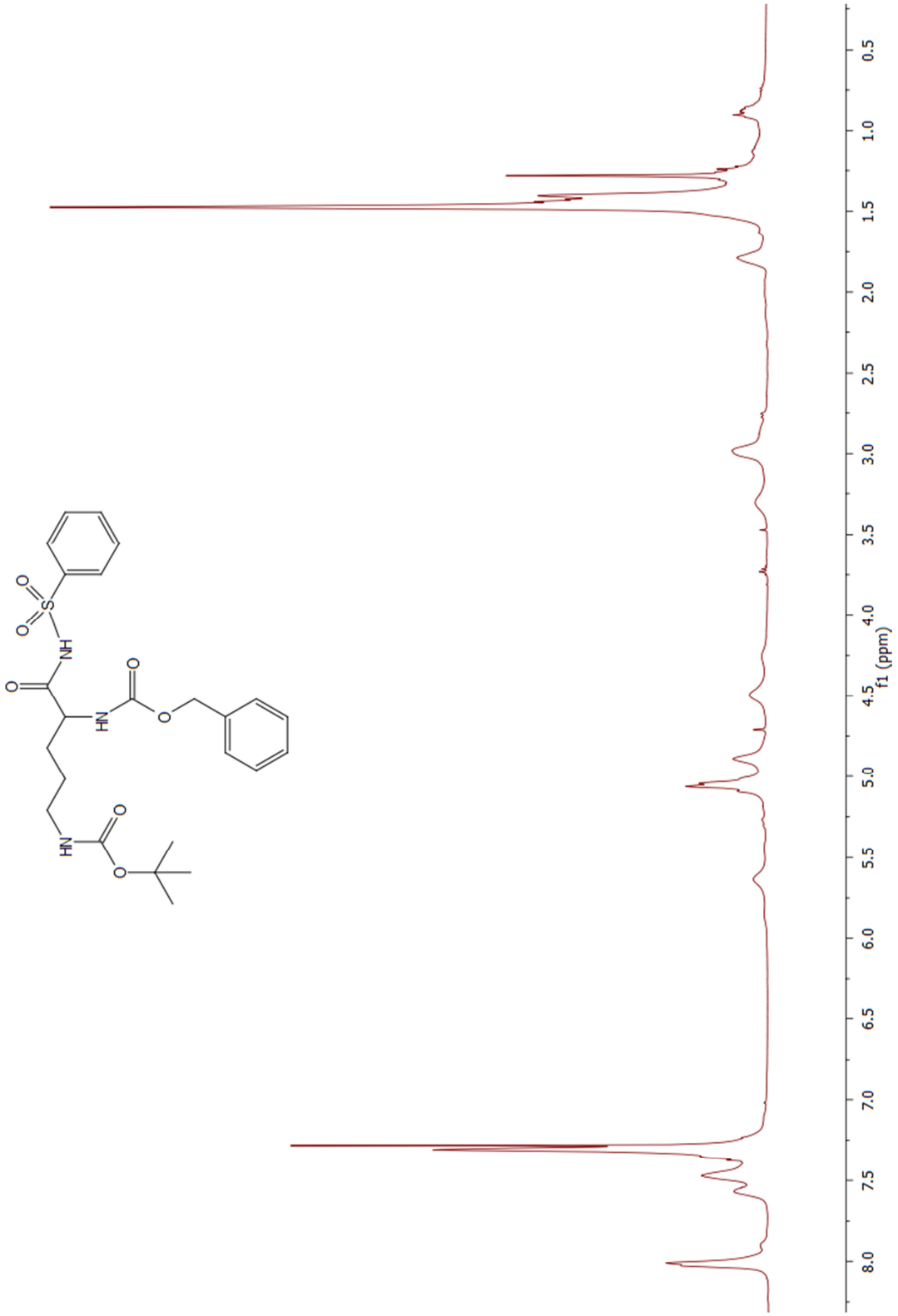


Figure 4.8 ^{1}H NMR spectrum of 3e

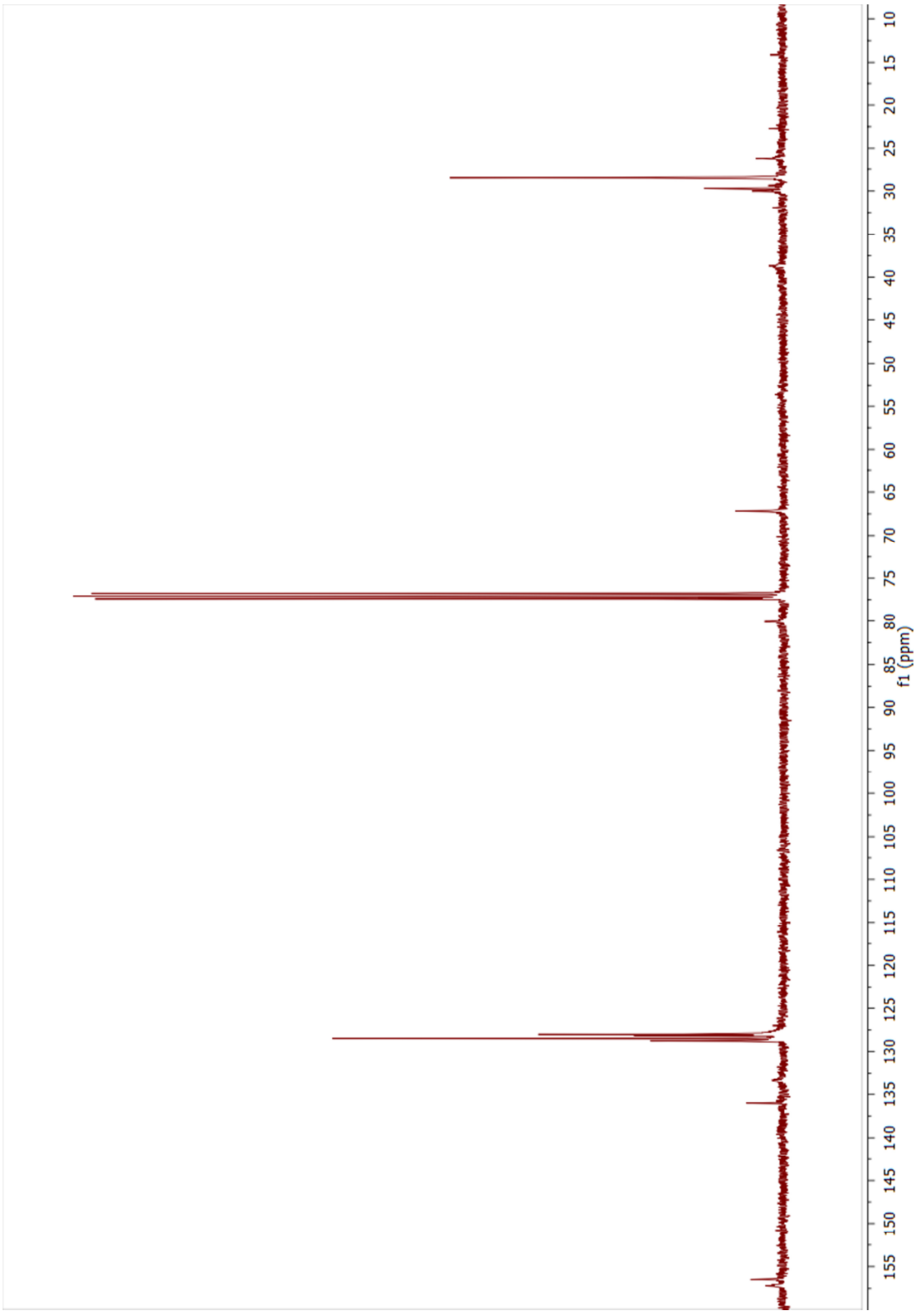


Figure 4.9 ^{14}C NMR spectrum of 3e

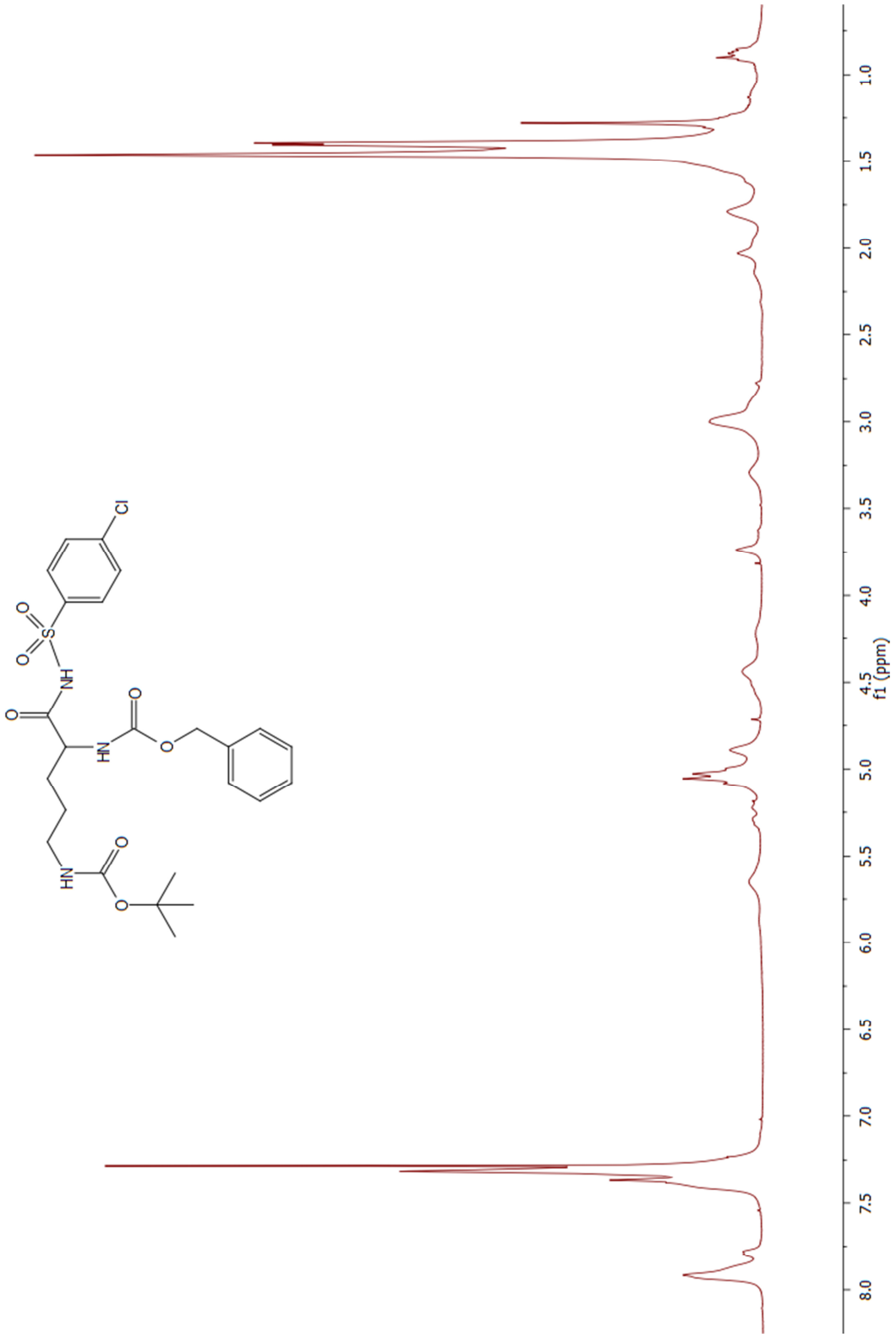


Figure 4.10 ^{1}H NMR spectrum of 3f

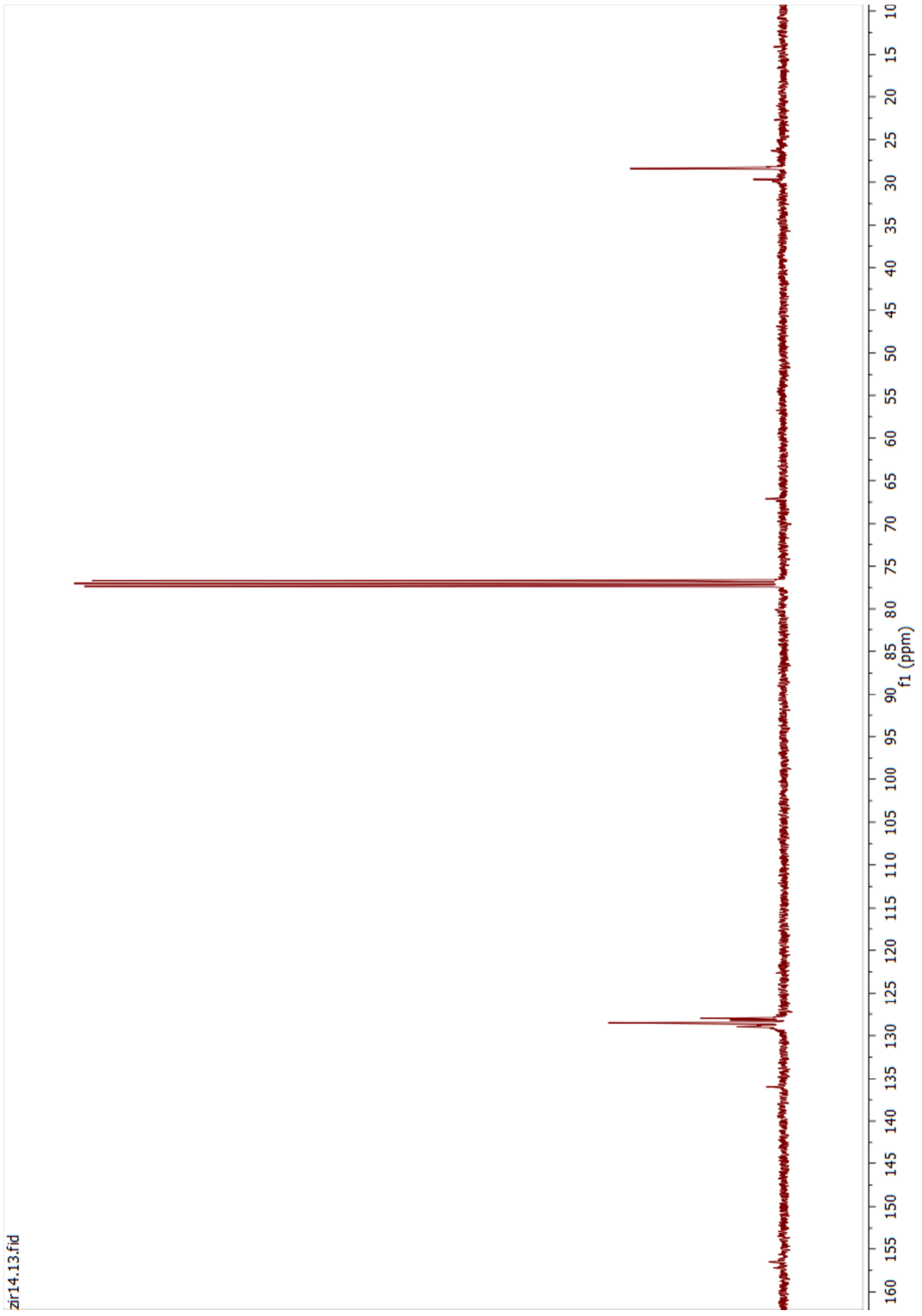


Figure 4.11 ^{14}C NMR spectrum of 3f

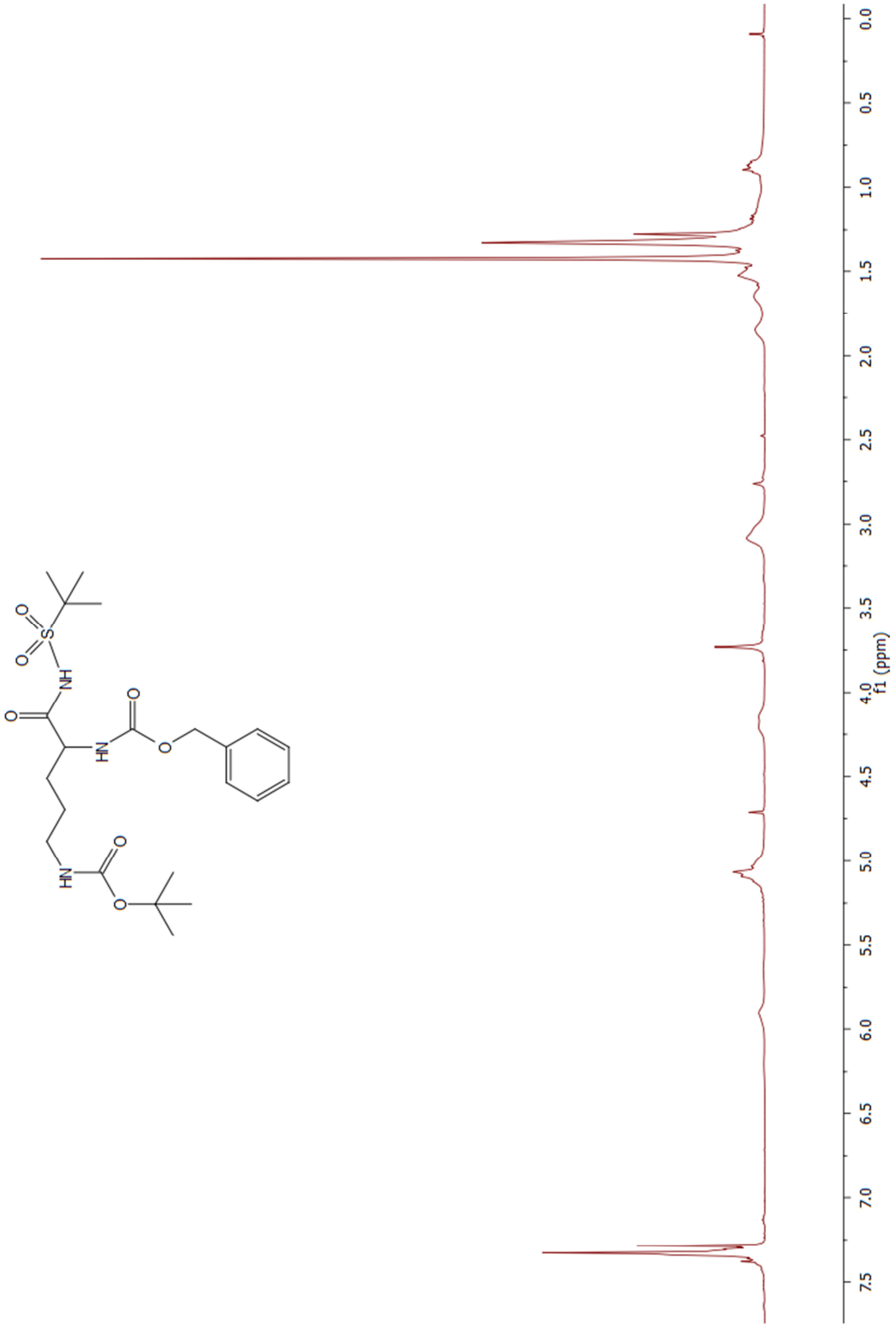


Figure 4.12 ^{1}H NMR spectrum of 3g

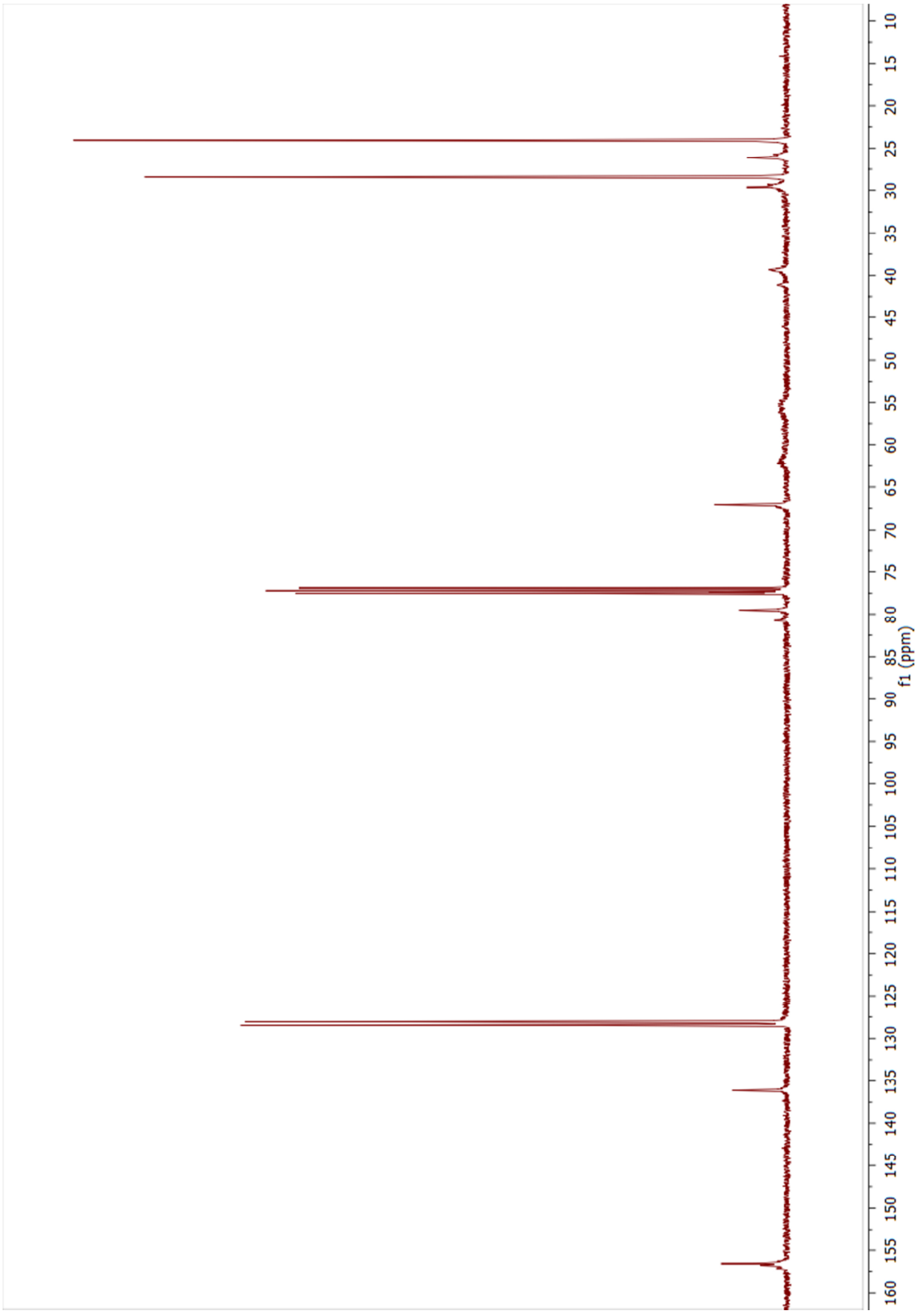


Figure 4.13 ^{14}C NMR spectrum of 3g

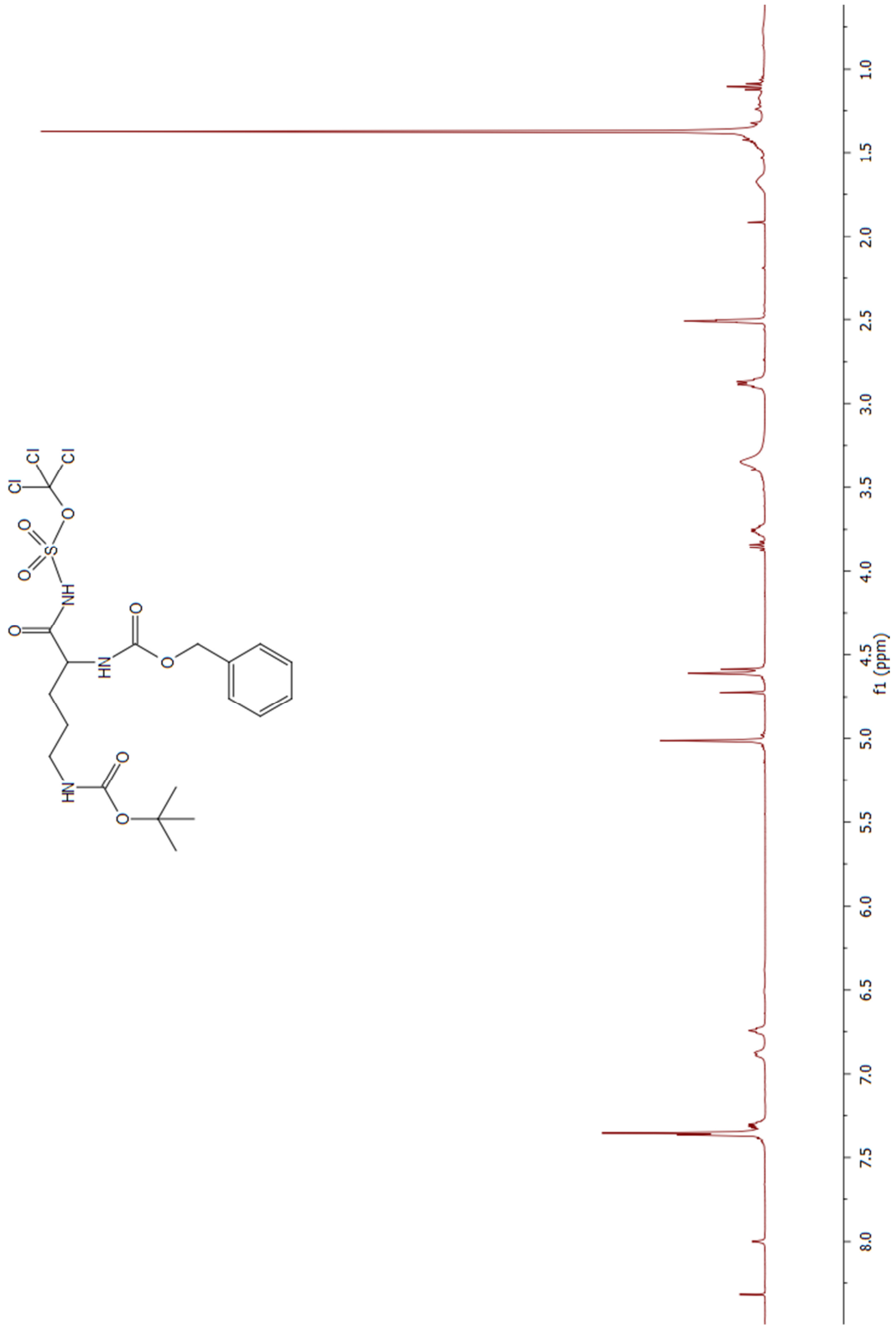


Figure 4.14 ^{1}H NMR spectrum of 3h

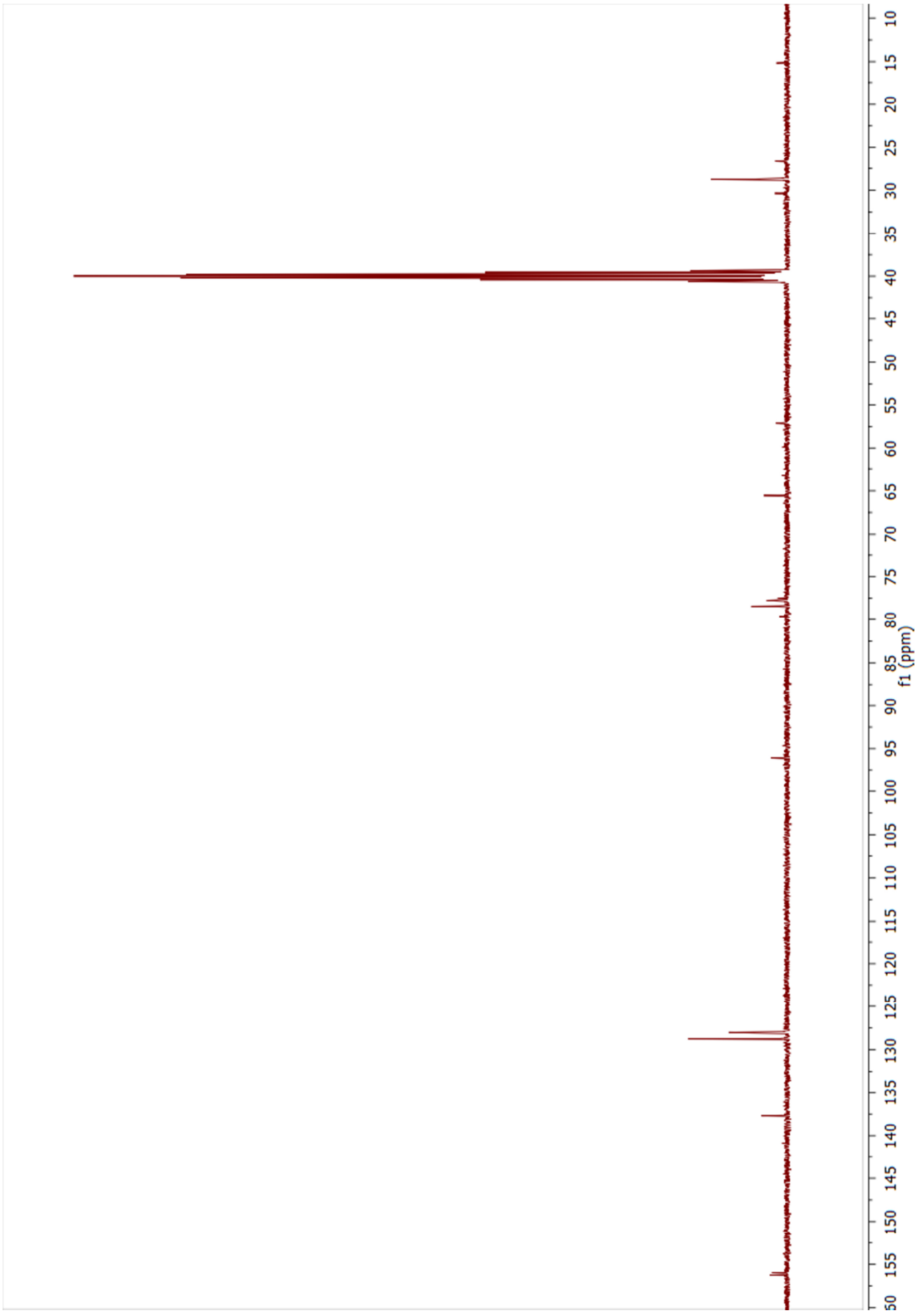


Figure 4.15 ^{14}C NMR spectrum of 3h

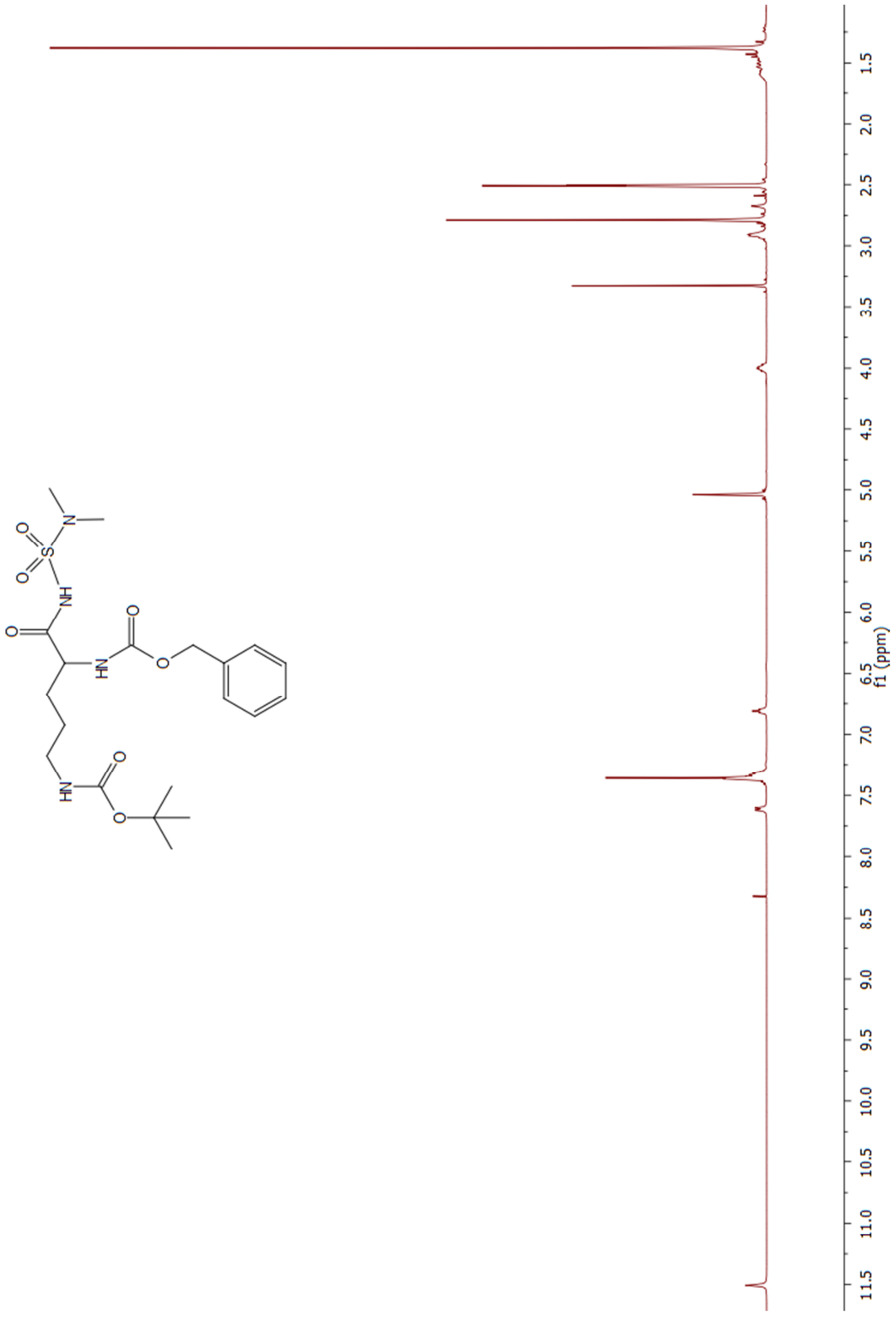


Figure 4.16 ^{1}H NMR spectrum of 3i

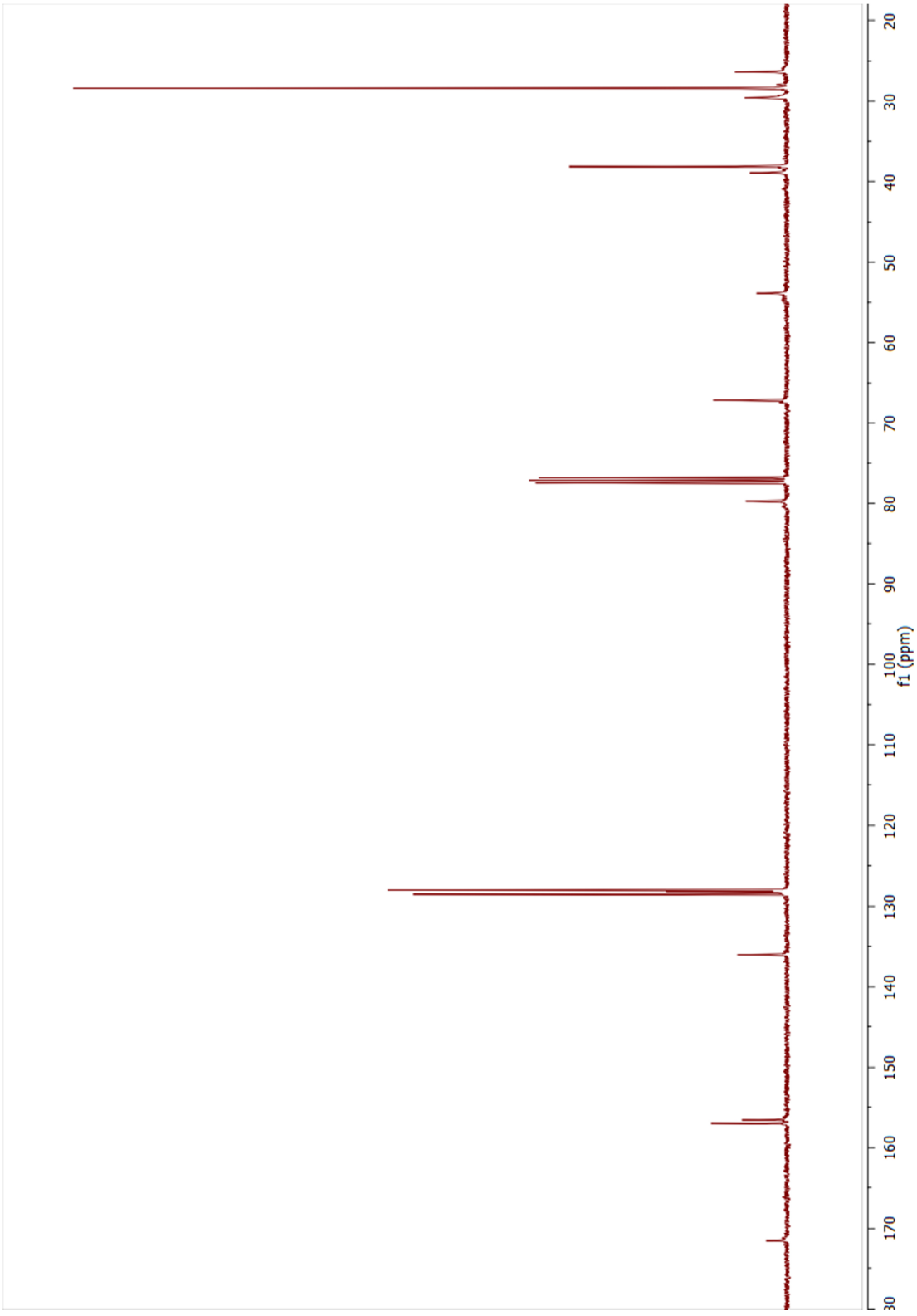


Figure 4.17 ^{14}C NMR spectrum of 3i

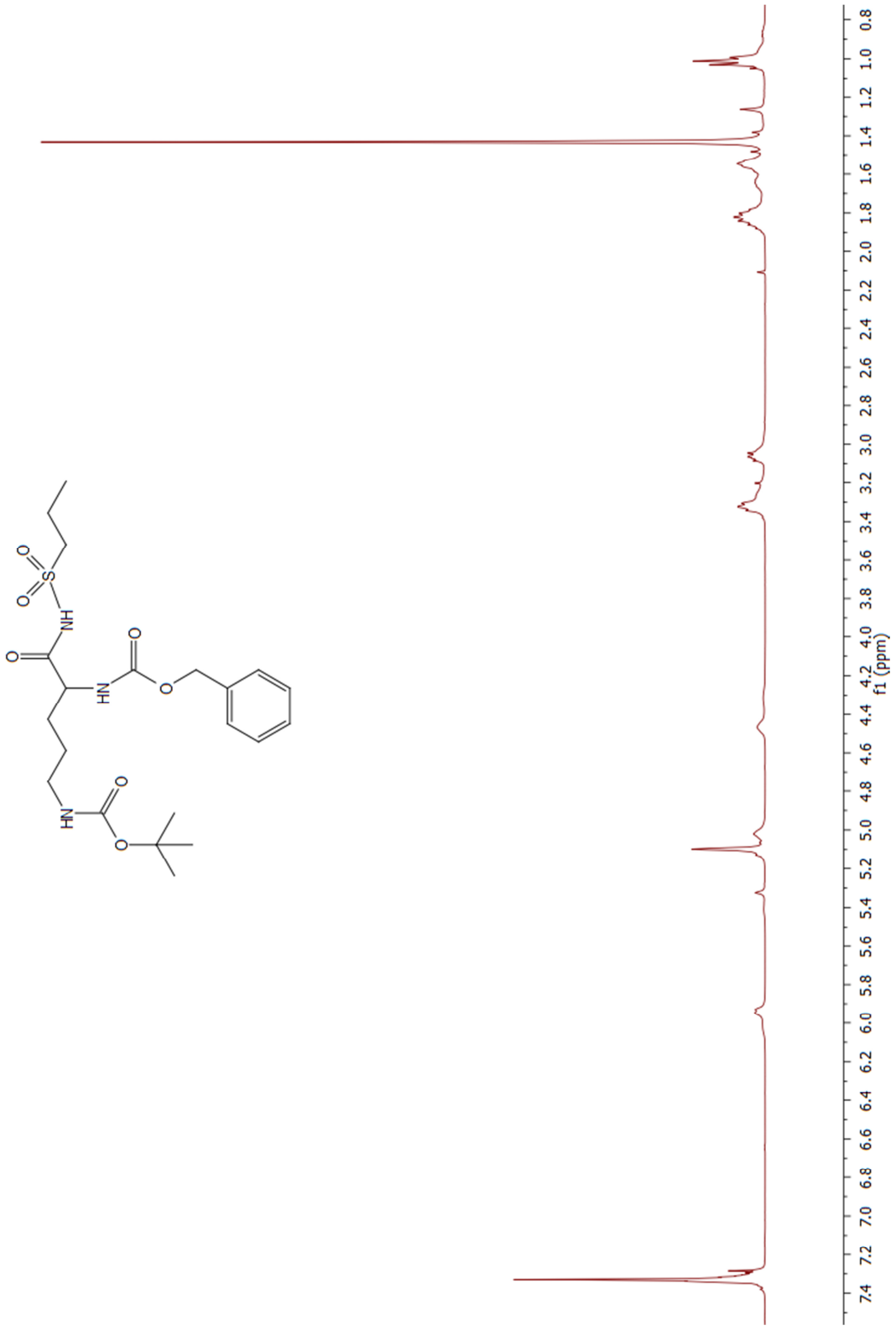


Figure 4.18 ^{1}H NMR spectrum of 3j

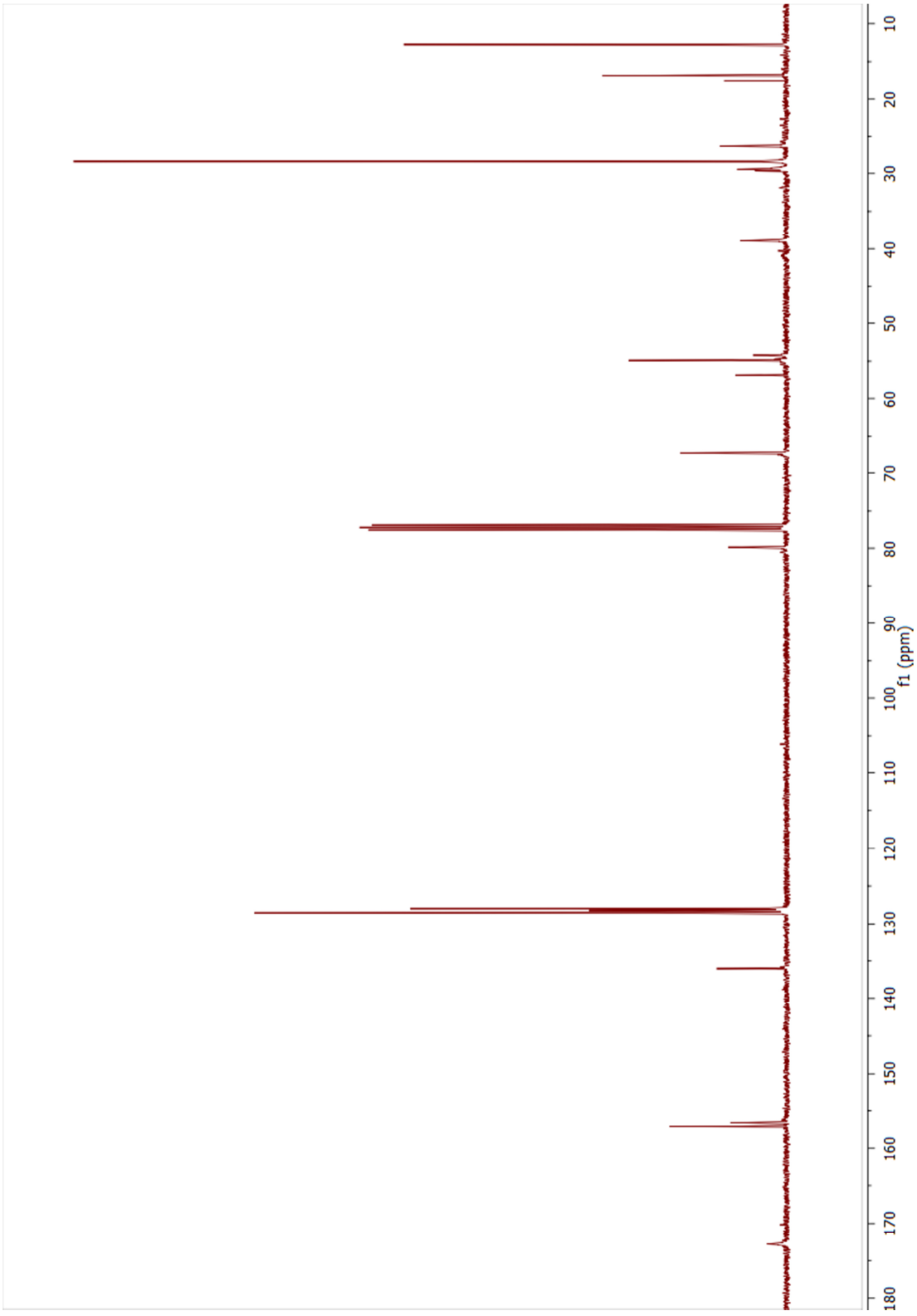


Figure 4.19 ^{14}C NMR spectrum of 3j

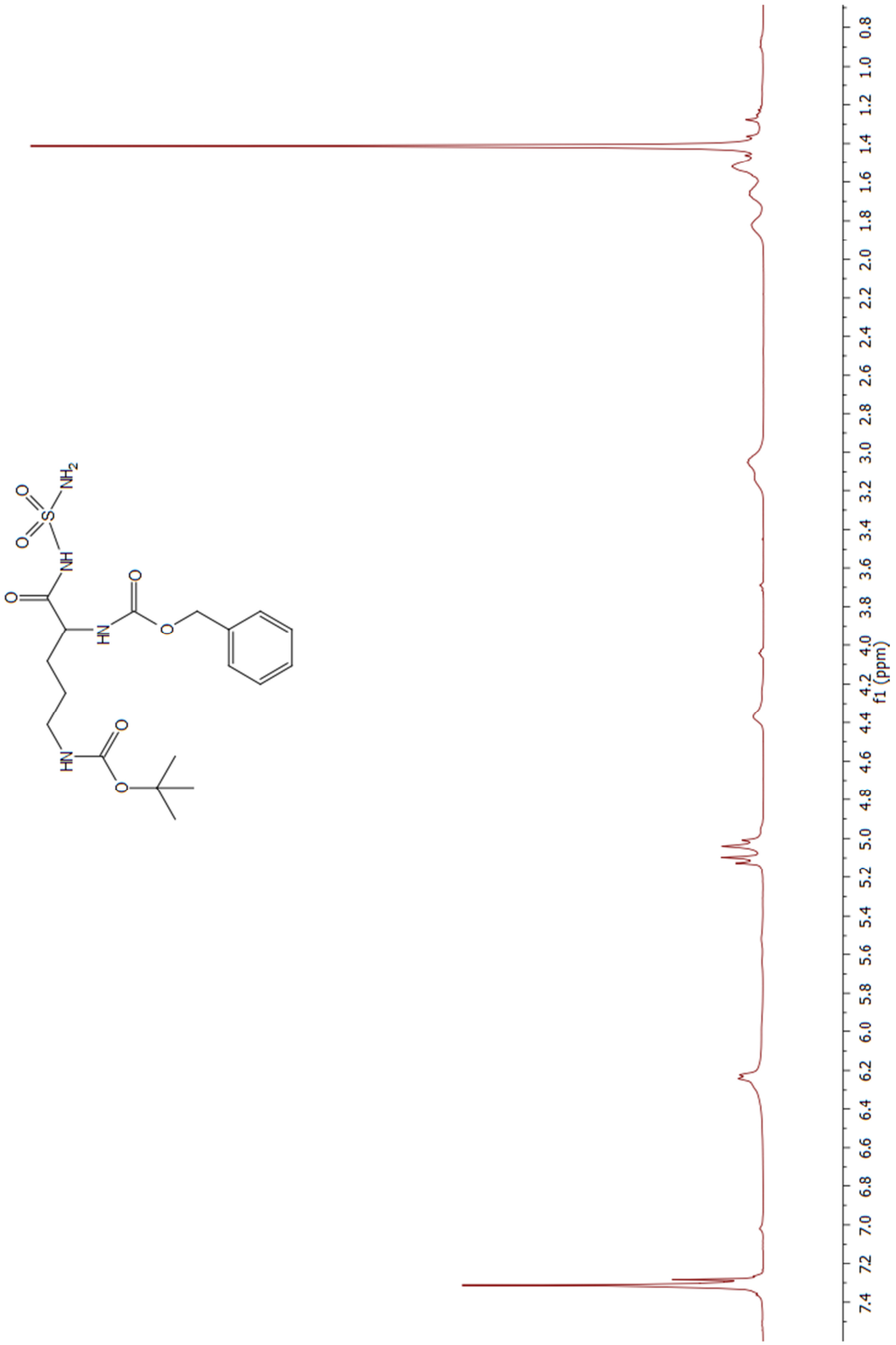


Figure 4.20 ^{1}H NMR spectrum of 3k

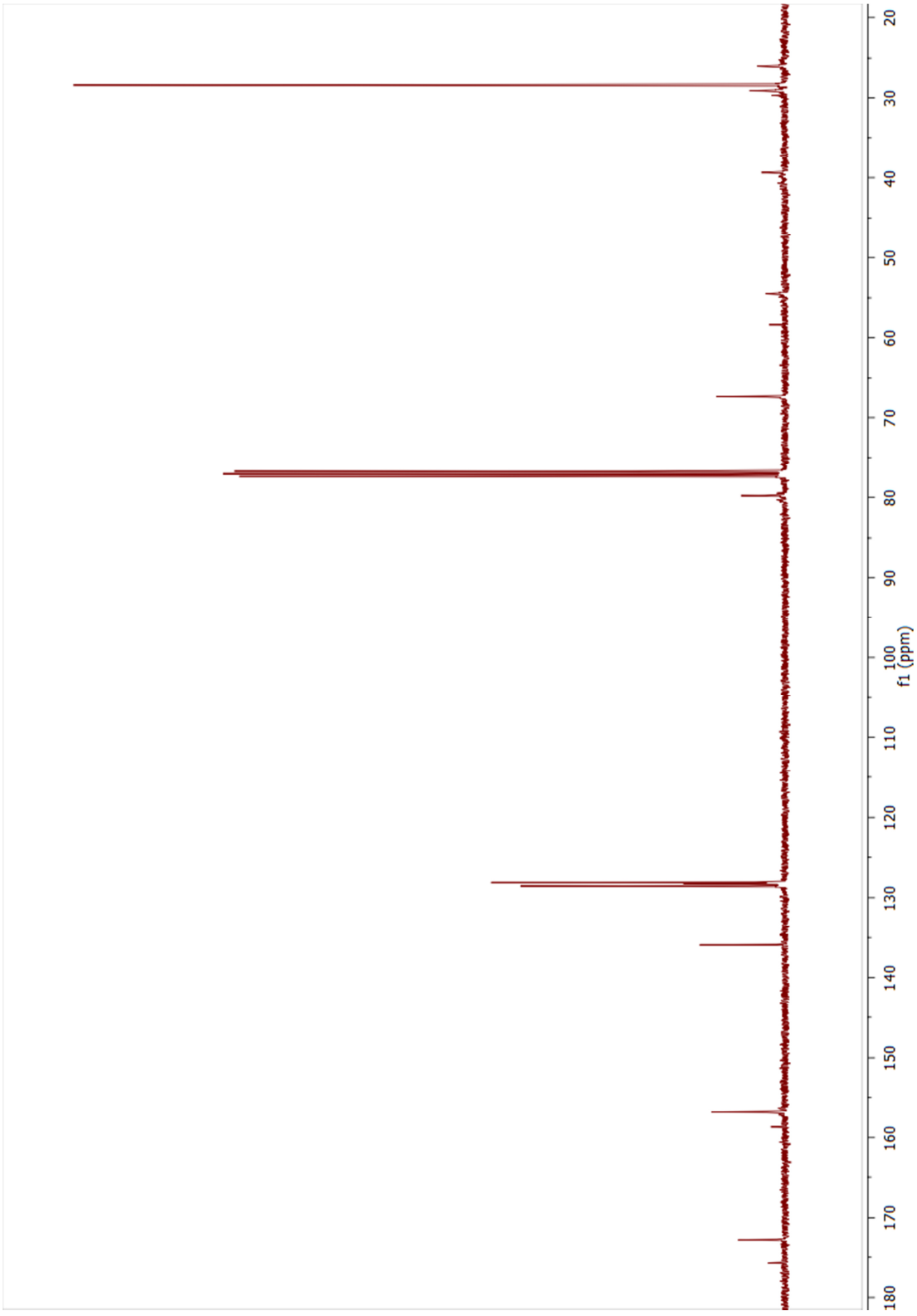


Figure 4.21 ^{14}C NMR spectrum of 3k

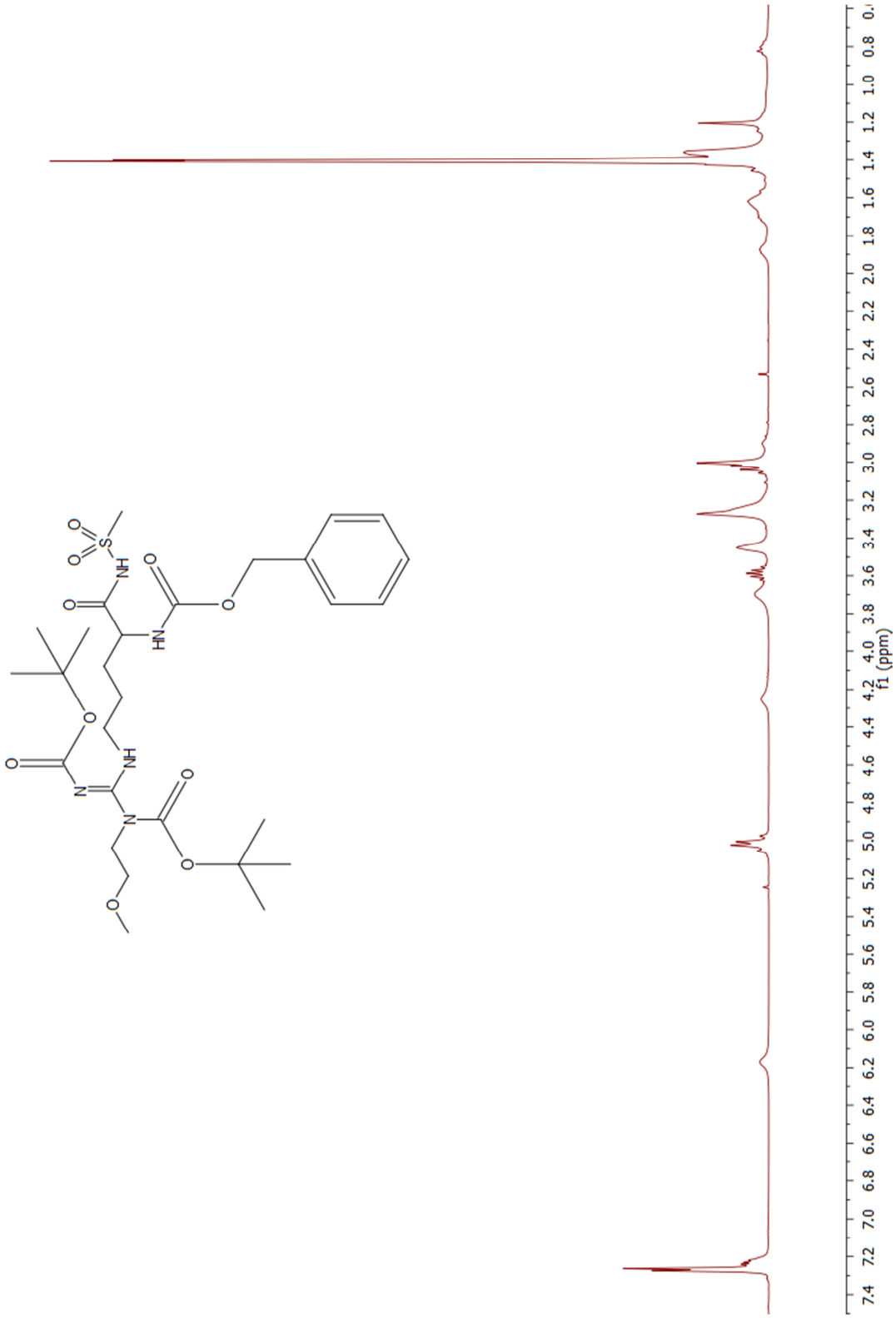


Figure 4.22 ^{1}H NMR spectrum of 7a

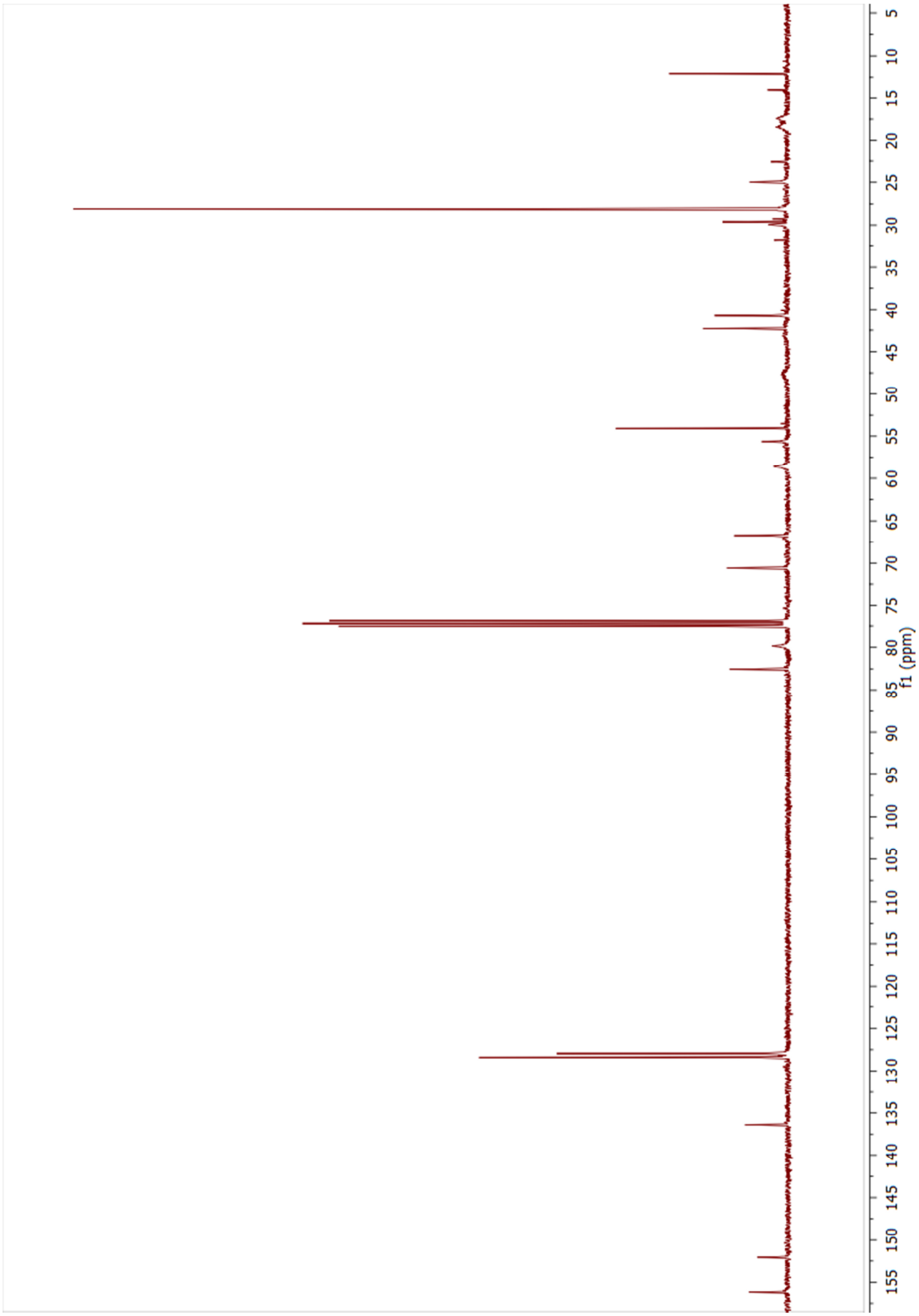


Figure 4.23 ^{14}C NMR spectrum of 7a

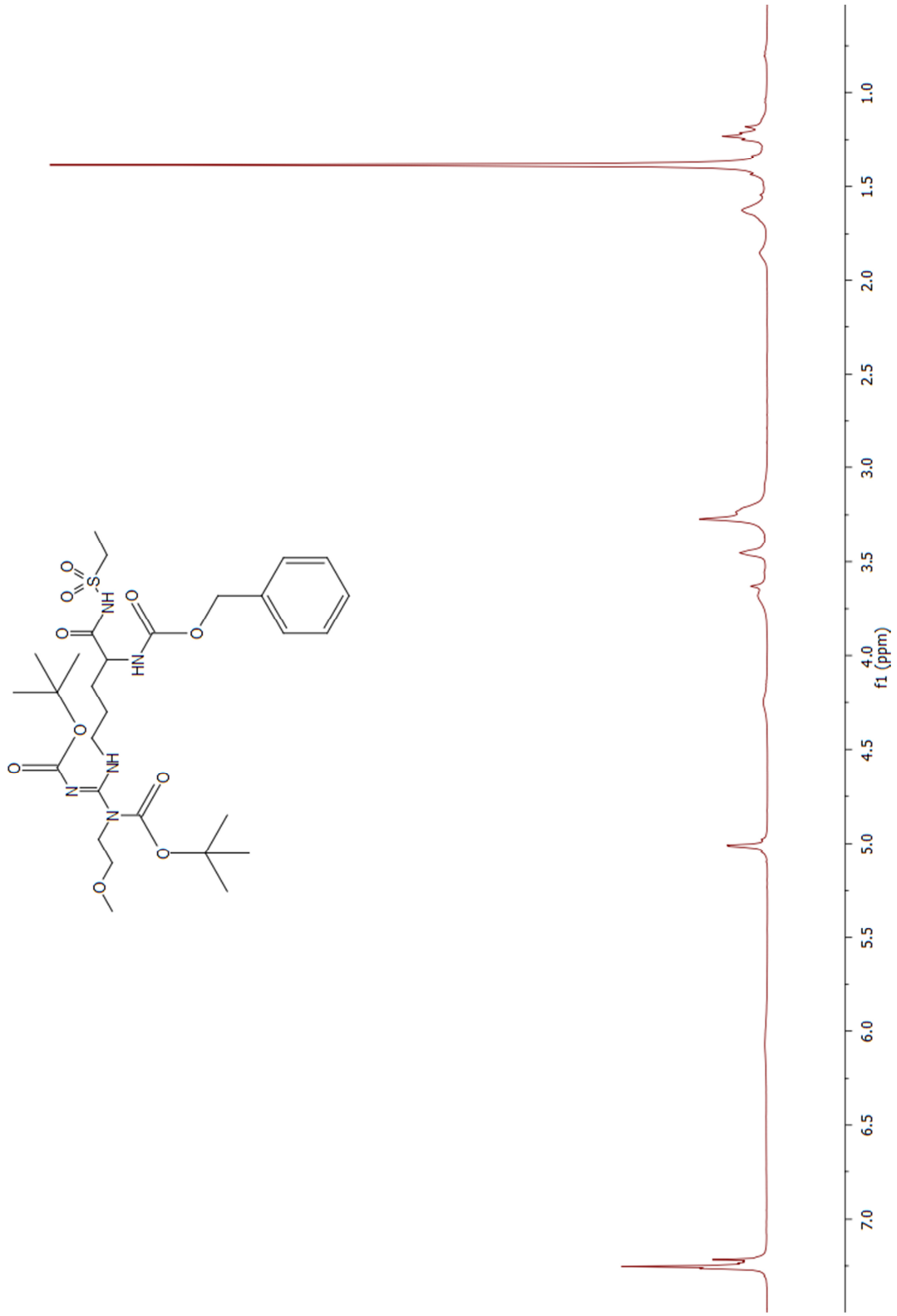


Figure 4.24 ^{1}H NMR spectrum of 7b

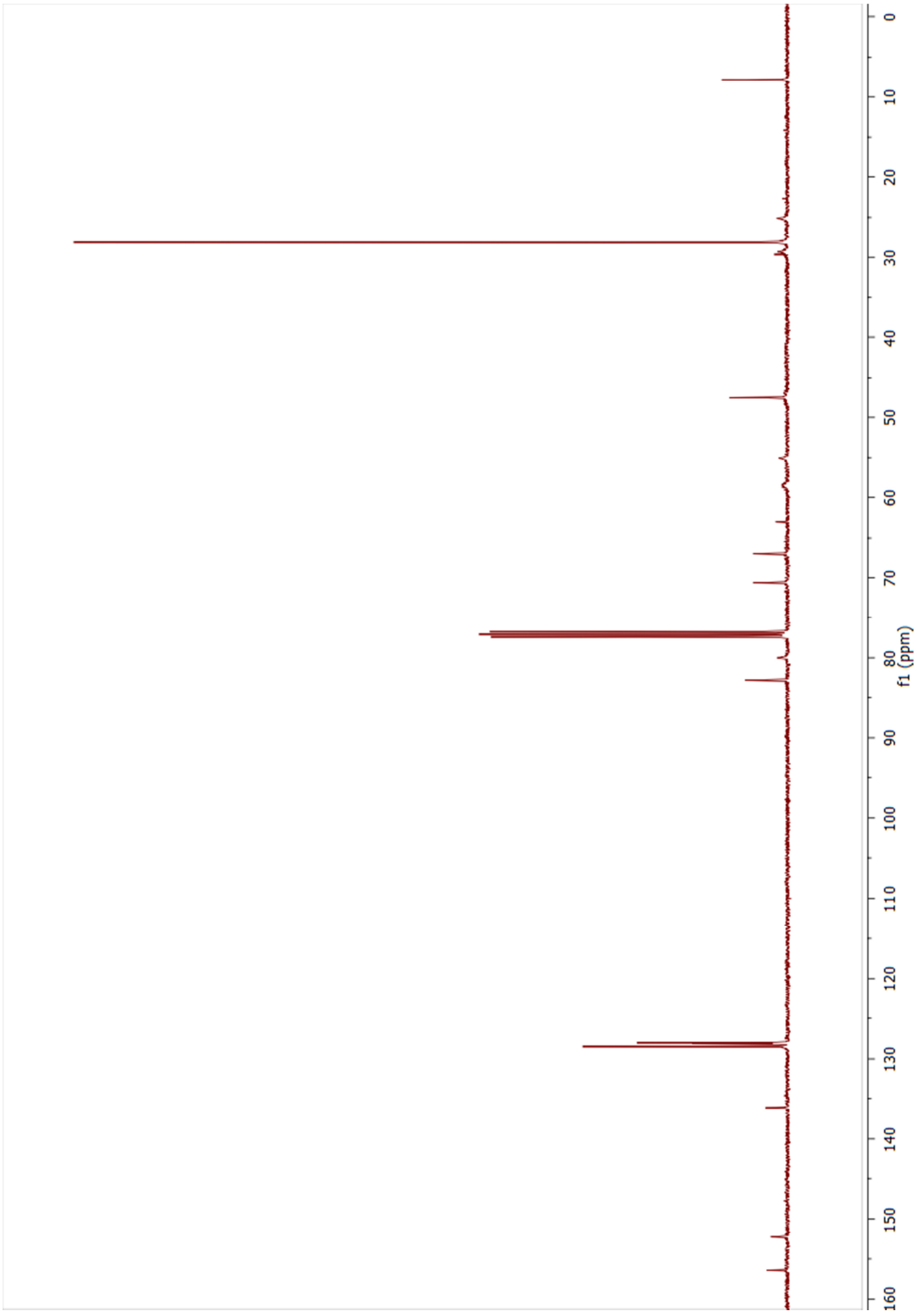


Figure 4.25 ^{14}C NMR spectrum of 7b

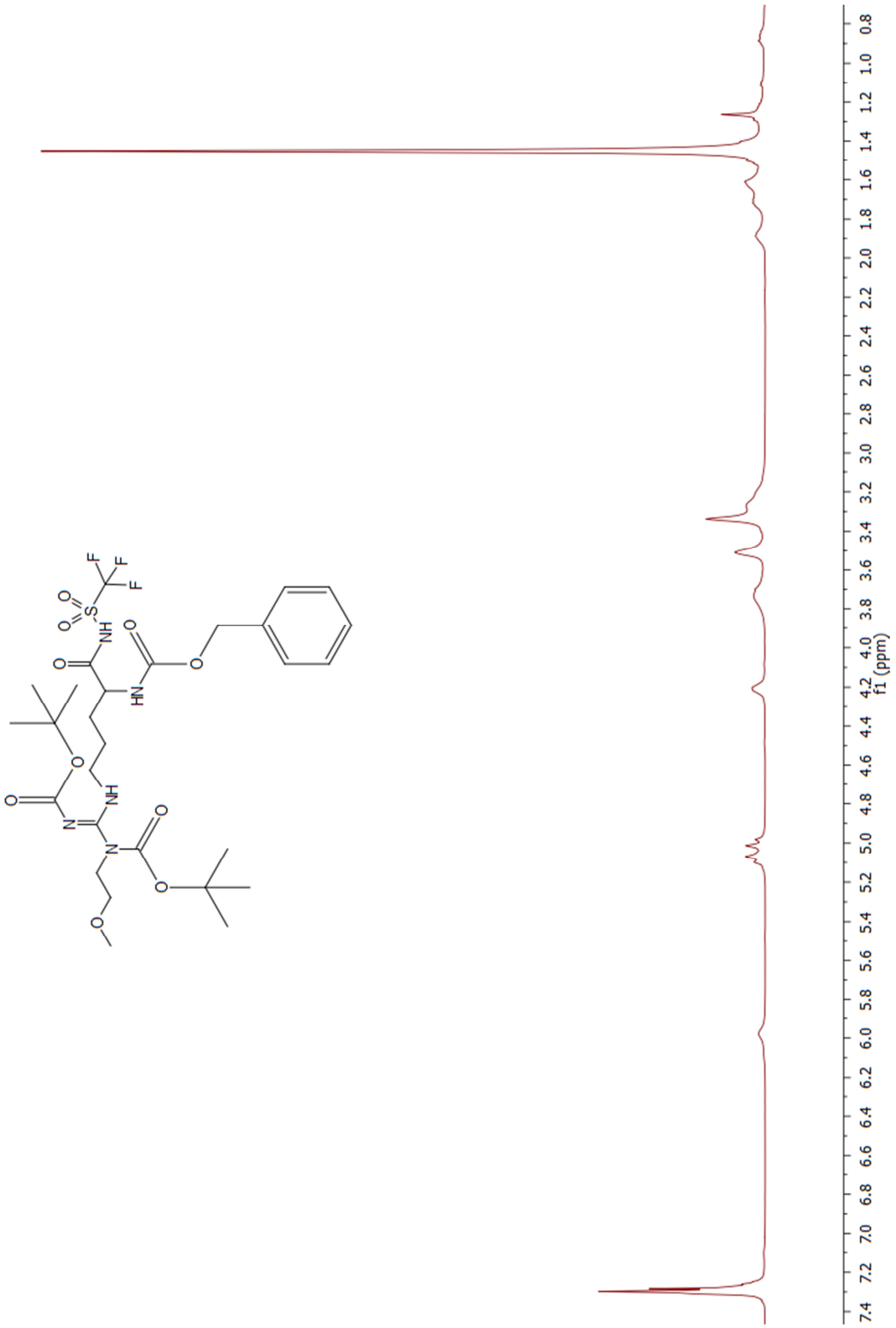


Figure 4.26 ^{1}H NMR spectrum of 7c

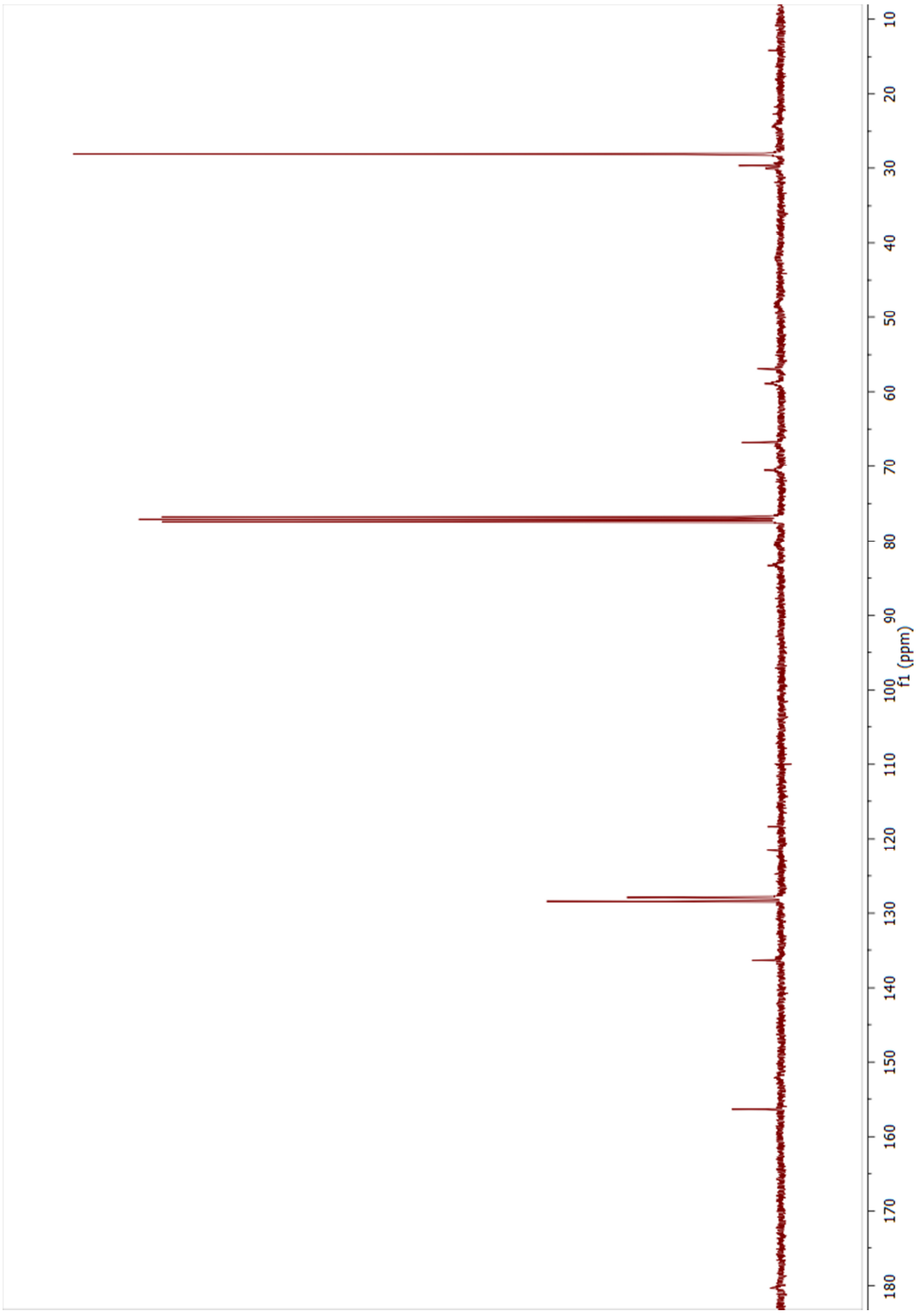


Figure 4.27 ^{14}C NMR spectrum of 7c

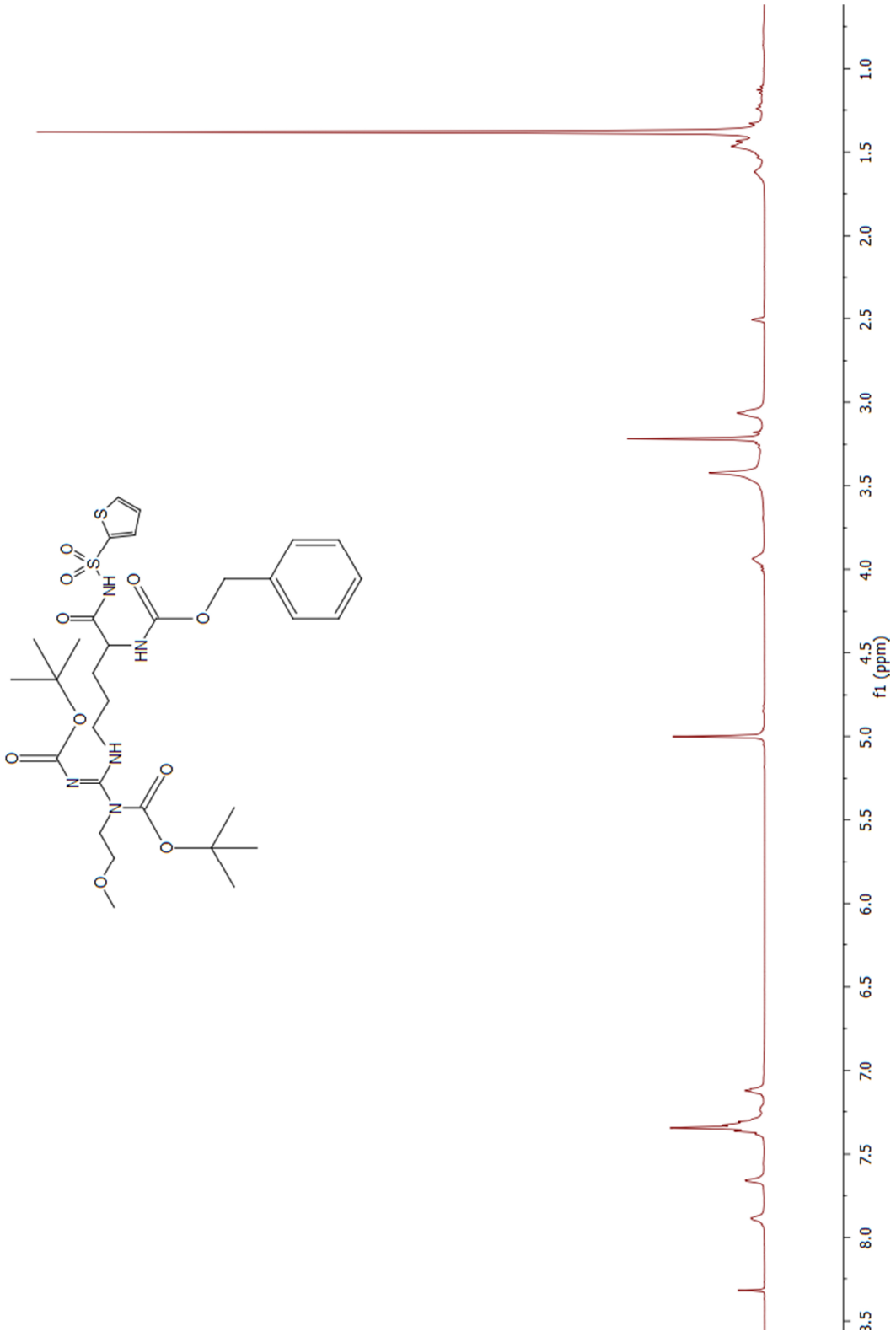


Figure 4.28 ^{1}H NMR spectrum of 7d

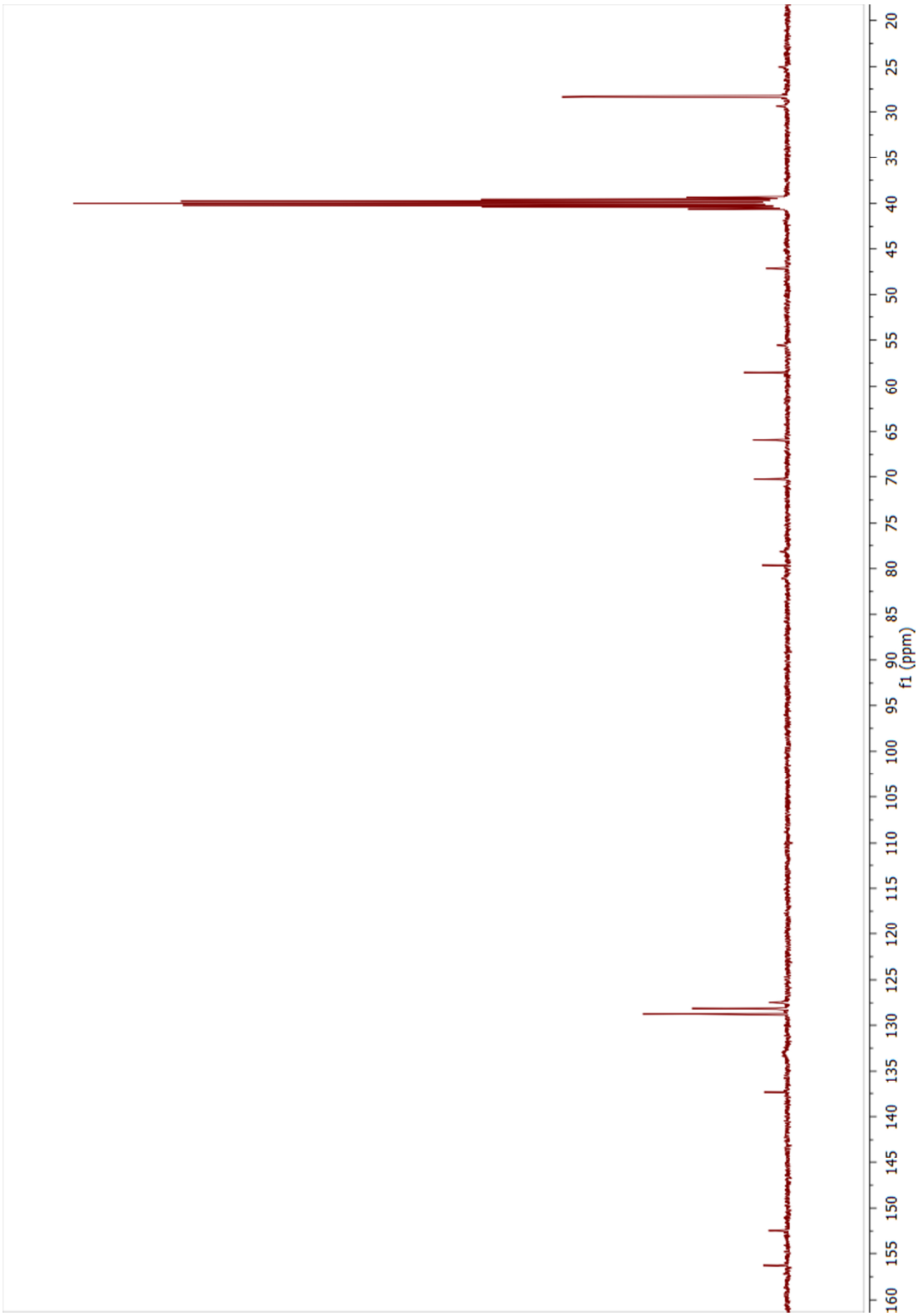


Figure 4.29 ^{14}C NMR spectrum of 7d

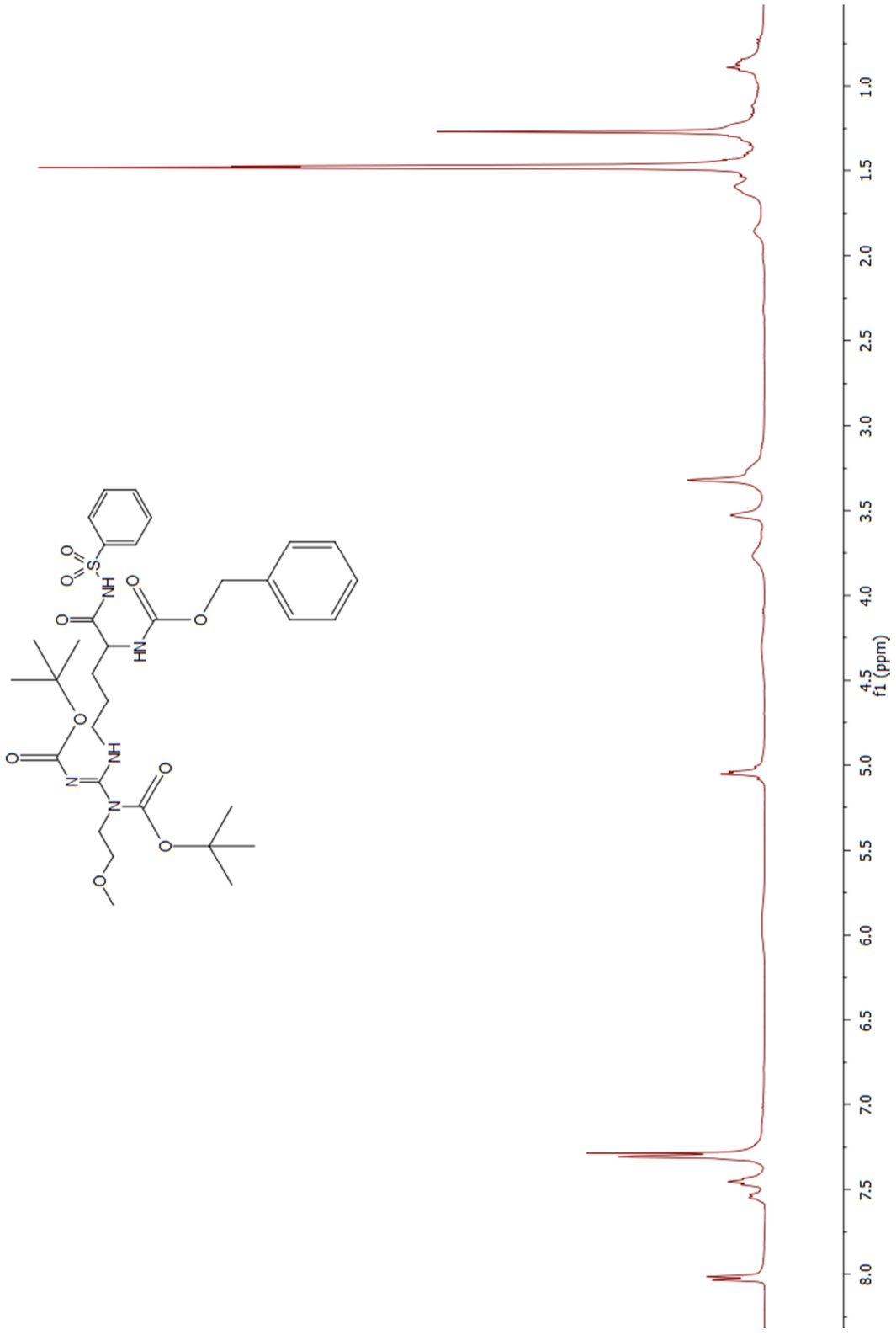


Figure 4.30 ^{1}H NMR spectrum of 7e

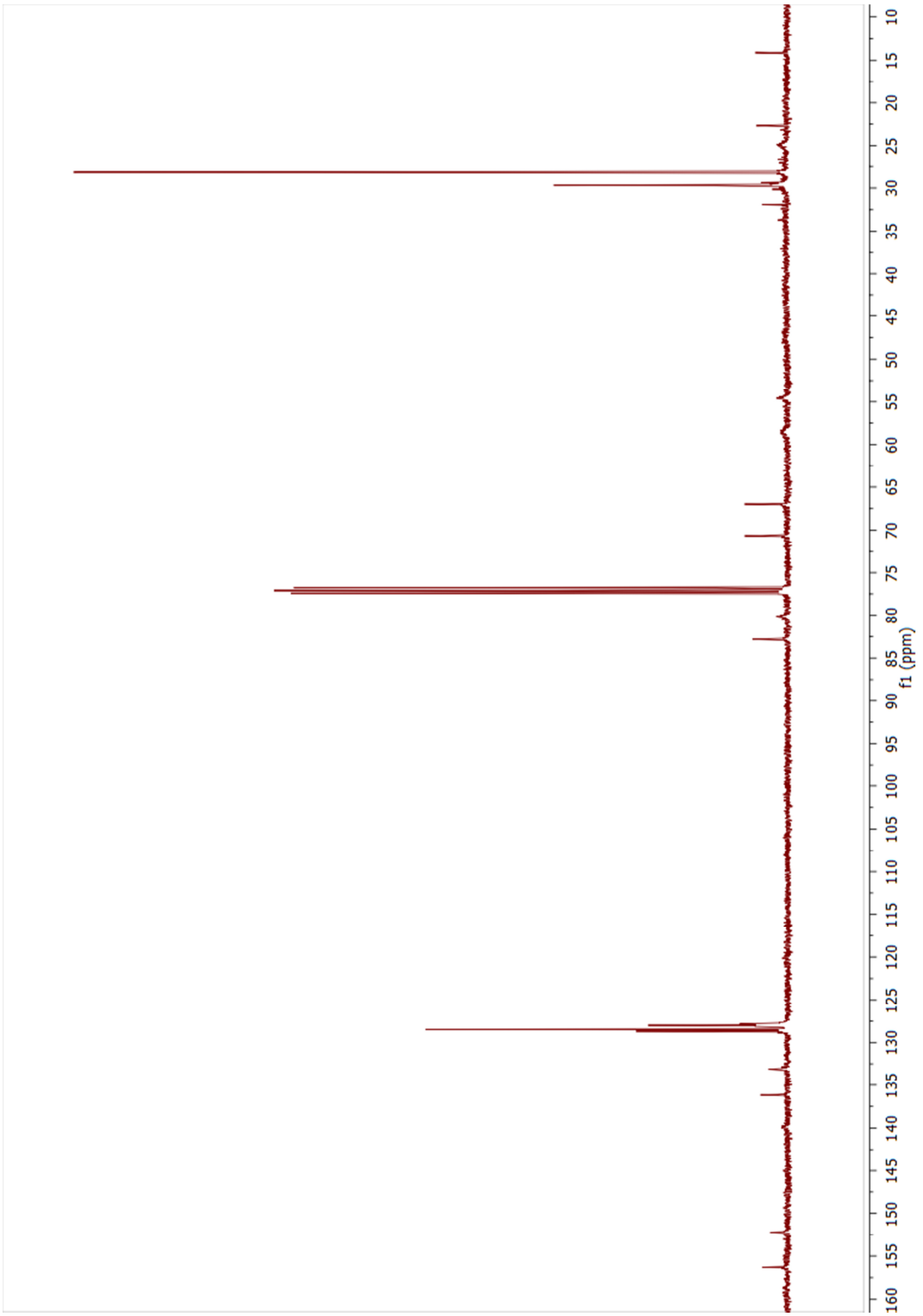


Figure 4.31 ^{14}C NMR spectrum of 7e

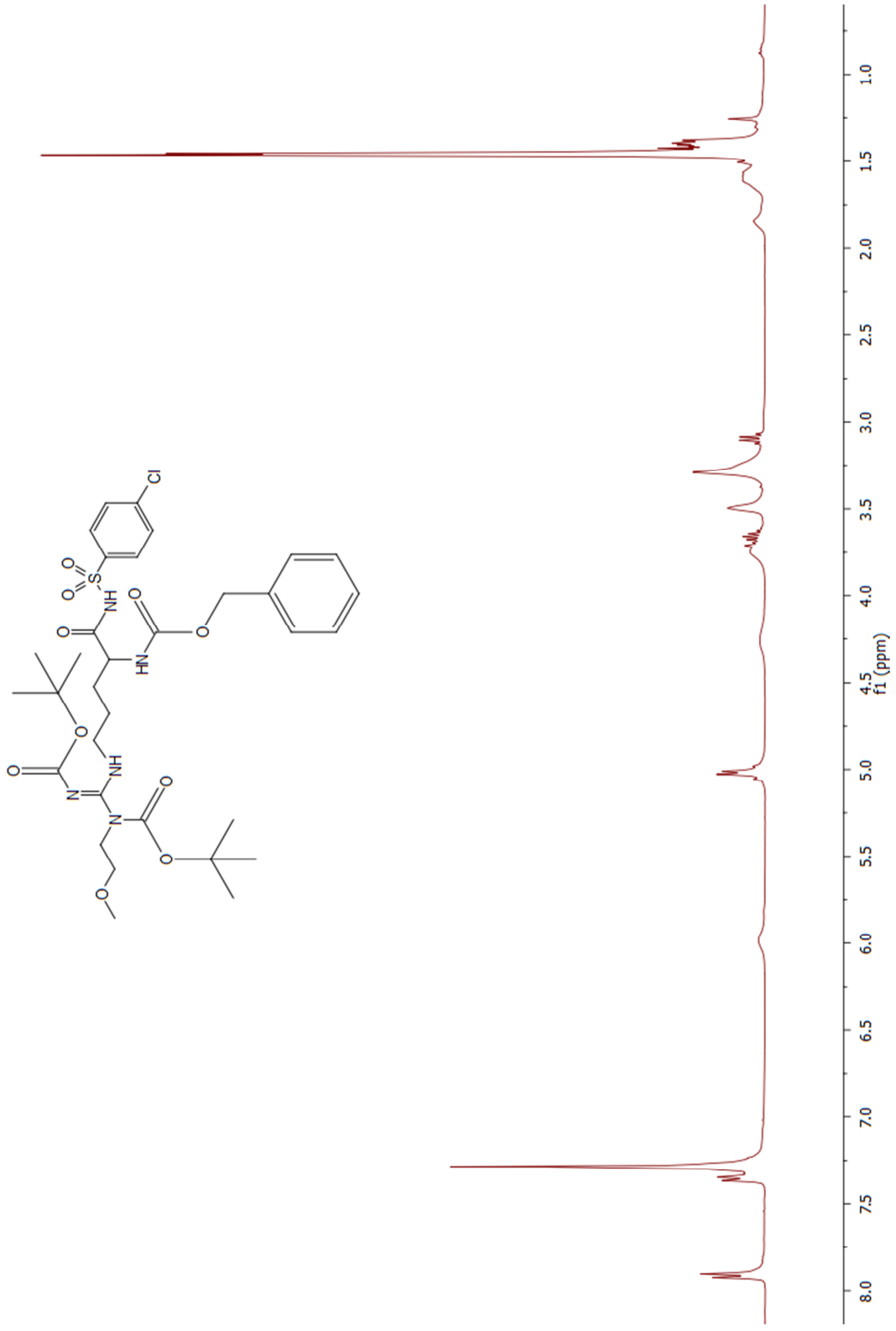


Figure 4.32 ^{1}H NMR spectrum of 7f

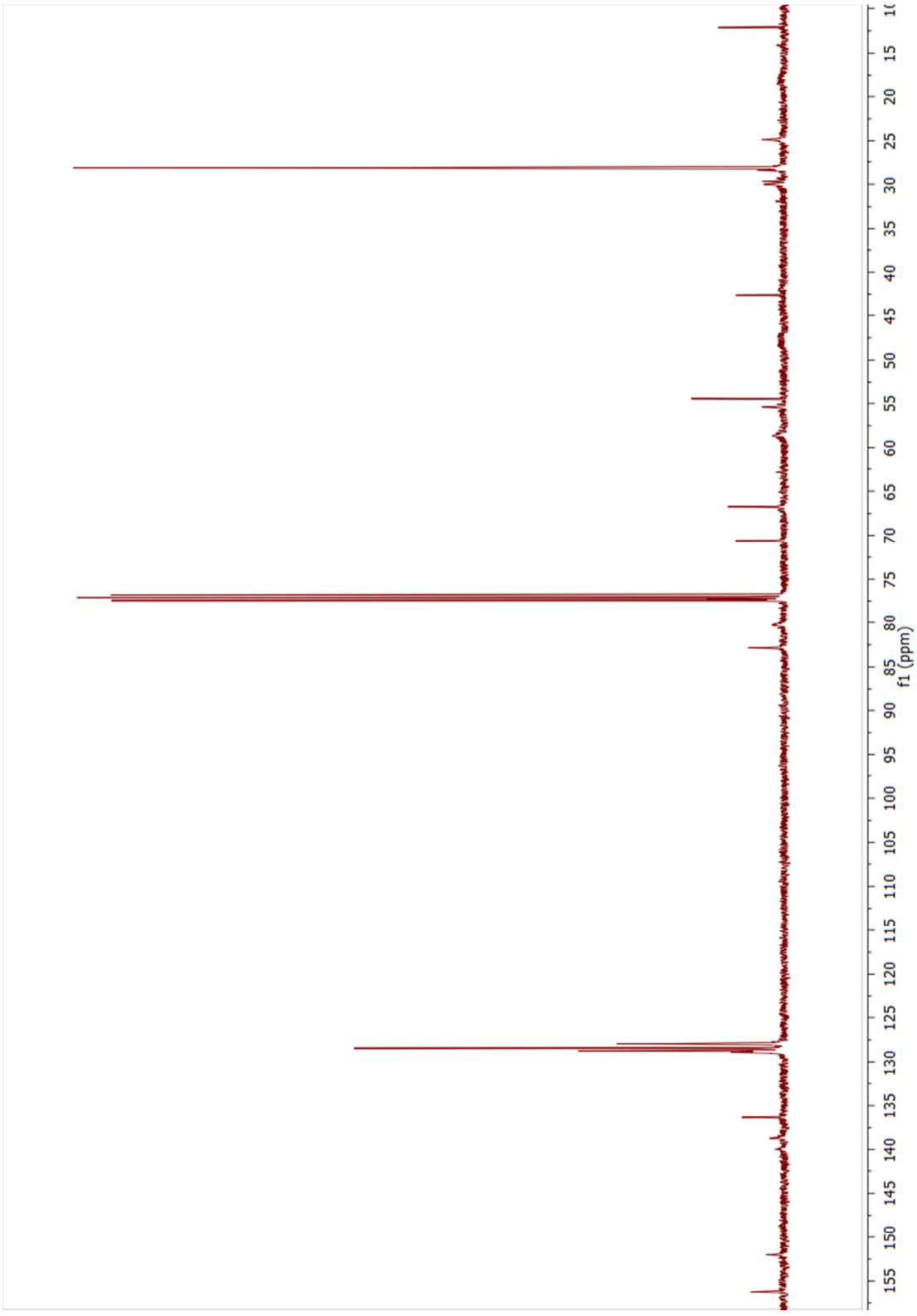


Figure 4.33 ^{14}C NMR spectrum of 7f

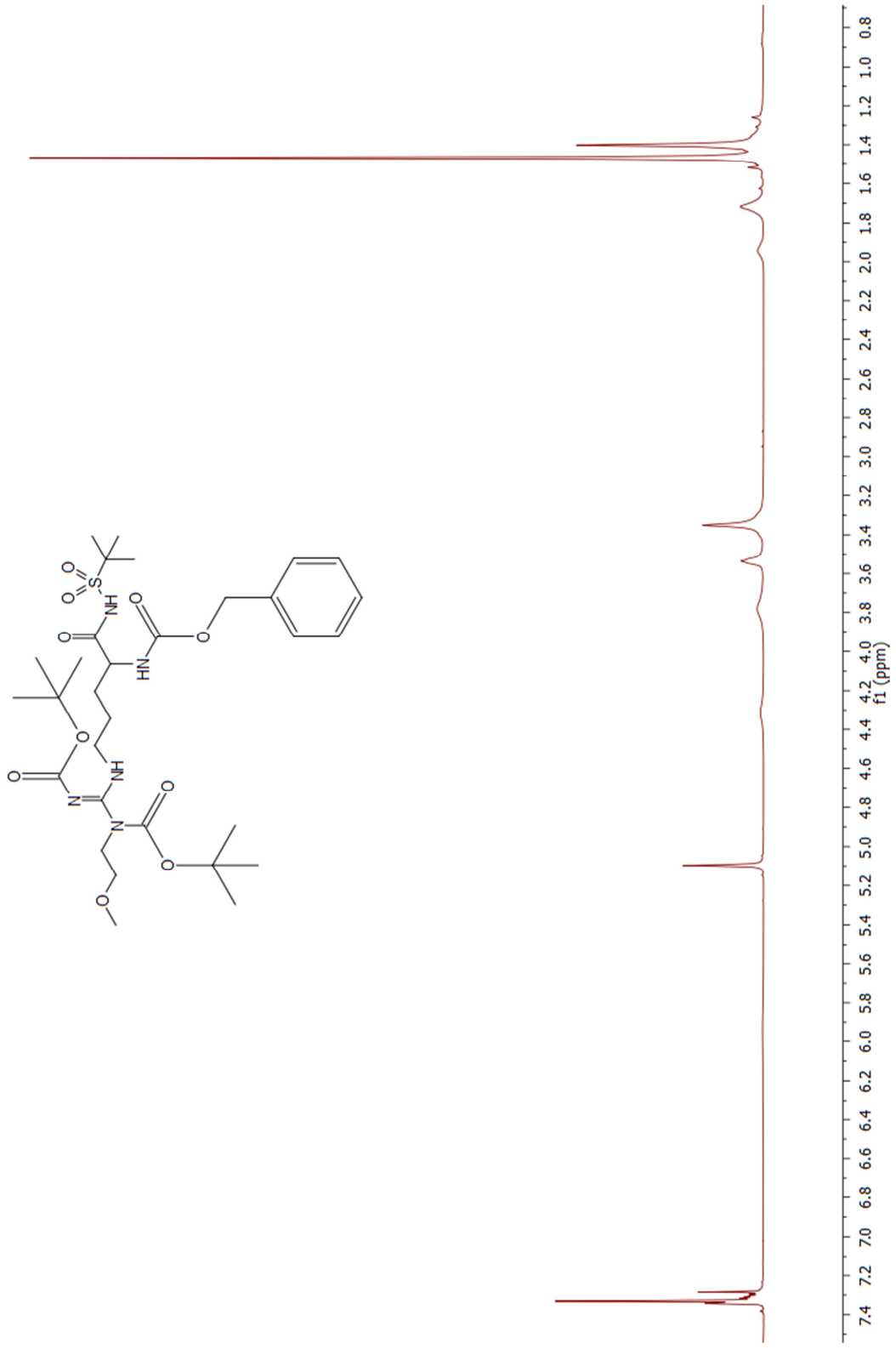


Figure 4.34 ^{1}H NMR spectrum of 7g

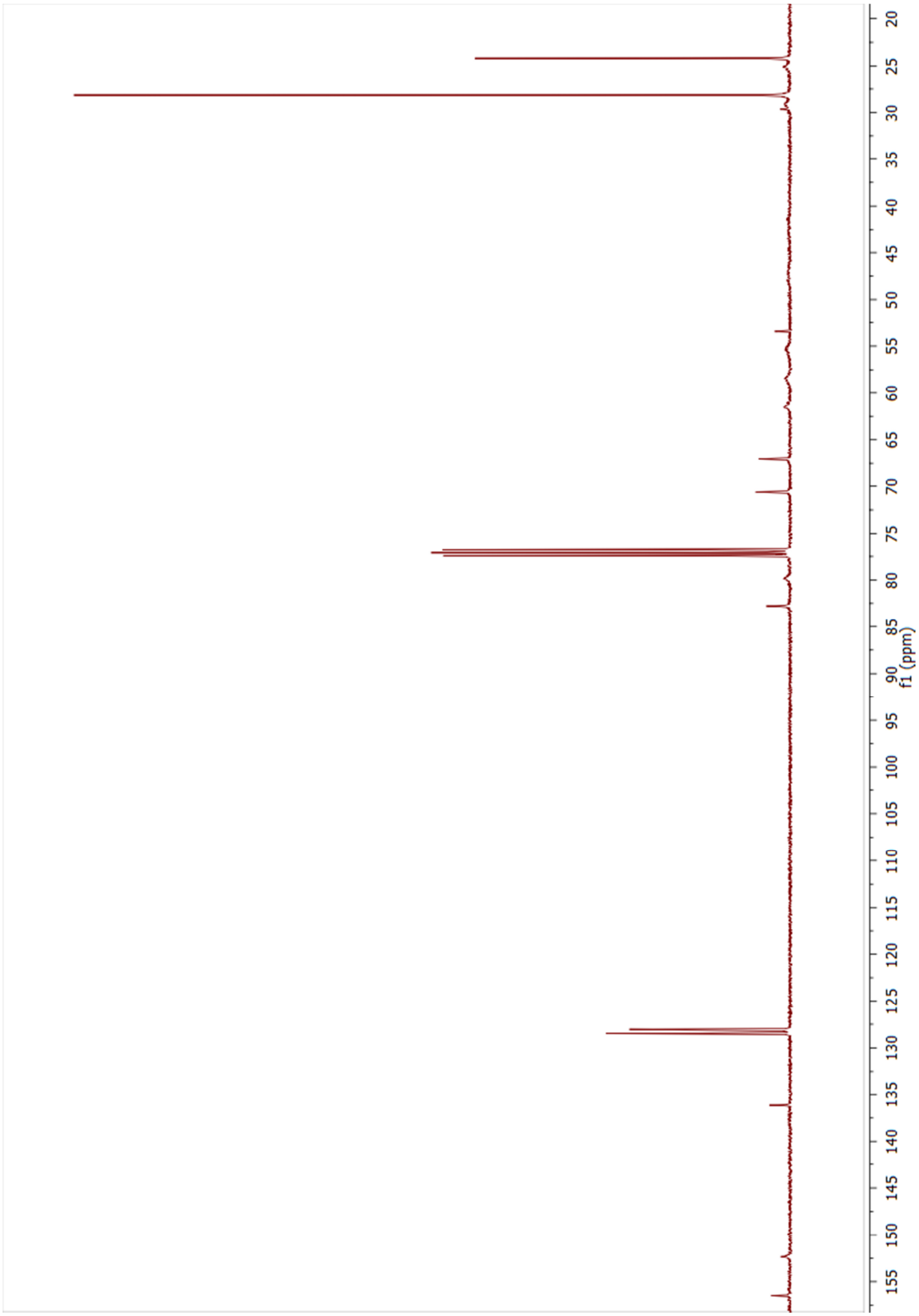


Figure 4.35 ^{14}C NMR spectrum of 7g

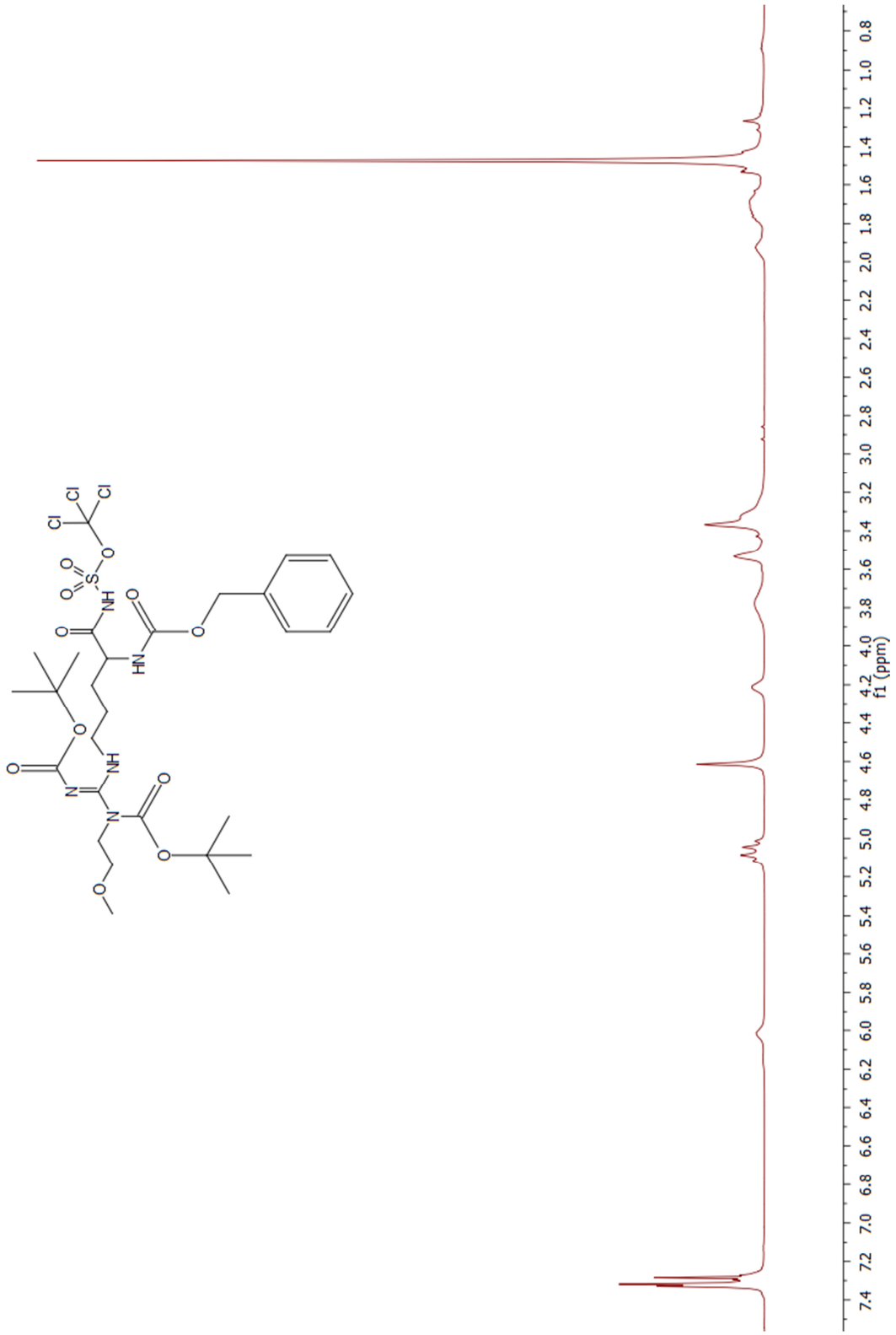


Figure 4.36 ^{1}H MNR spectrum of 7h

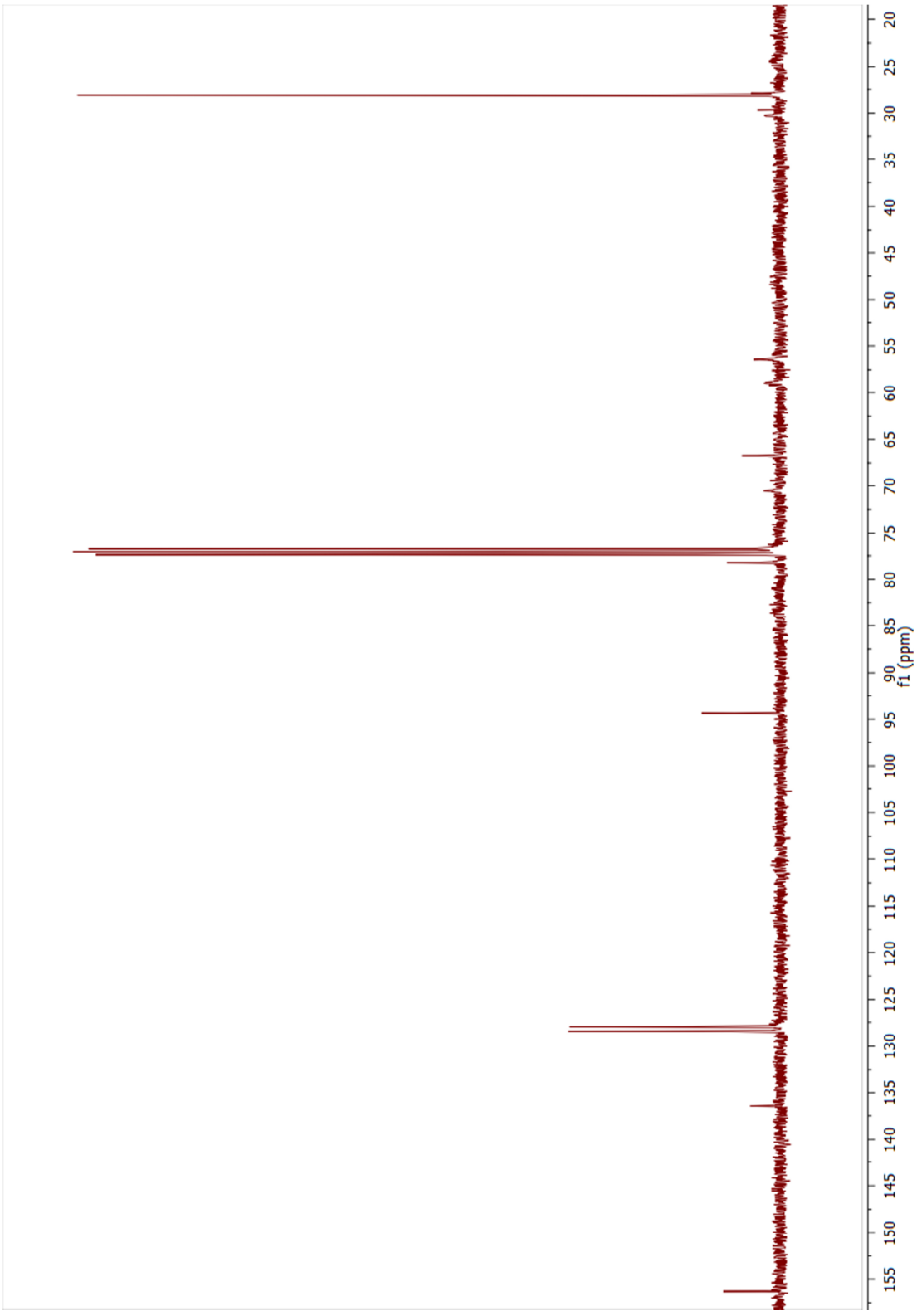


Figure 4.37 ^{14}C MNR spectrum of 7h

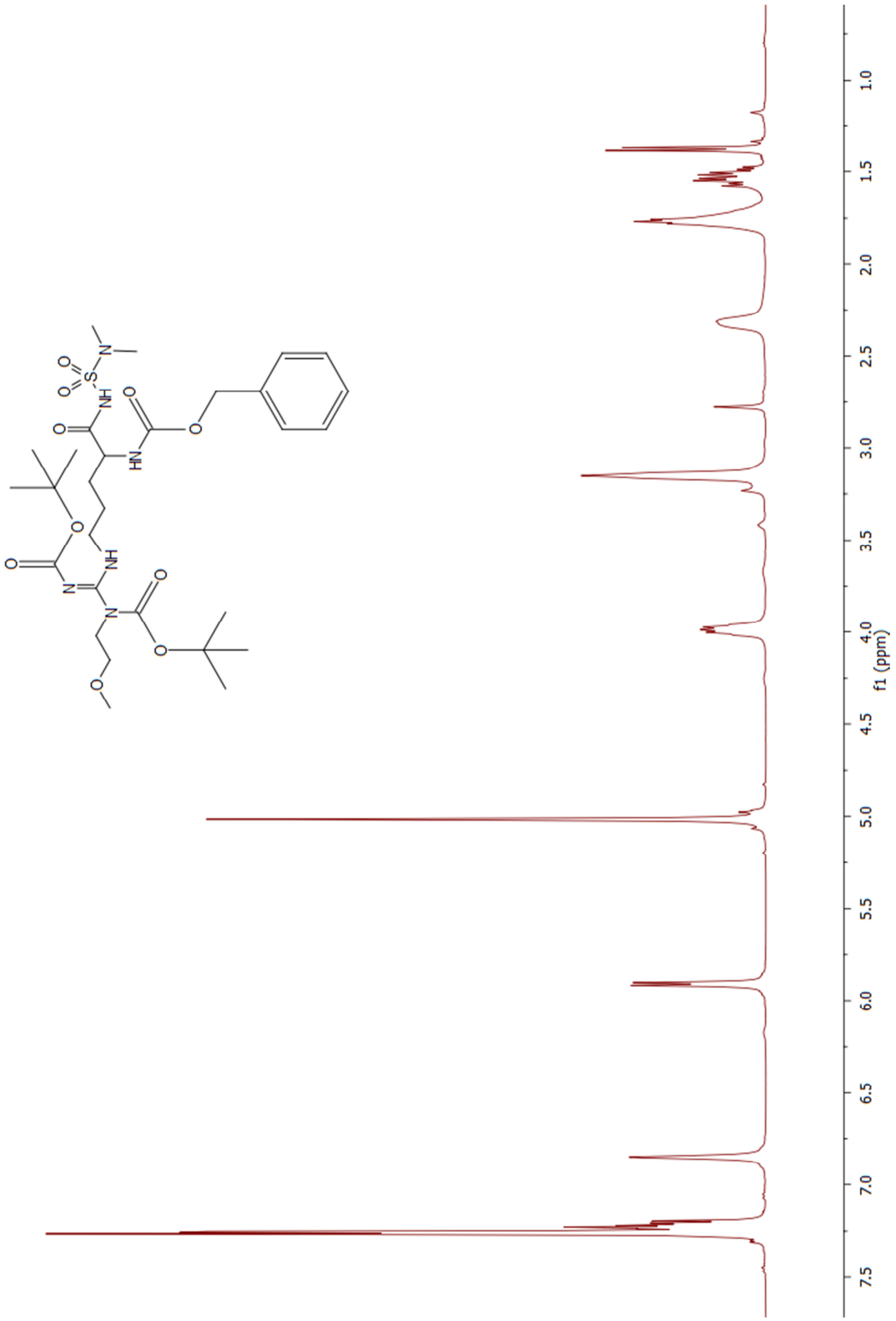


Figure 4.38 ^{1}H NMR spectrum of 7i

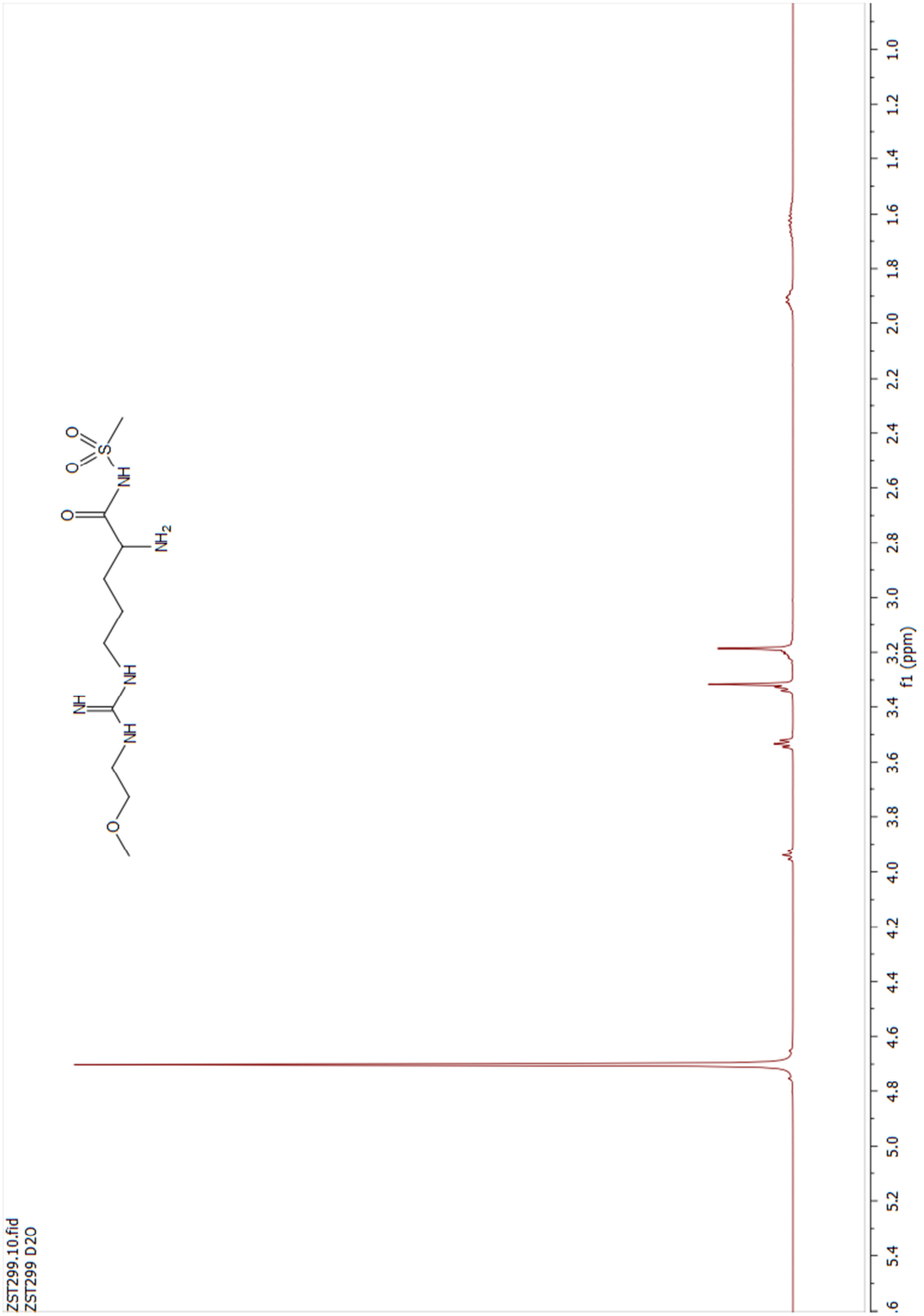


Figure 4.39 ^{1}H NMR spectrum of 8a

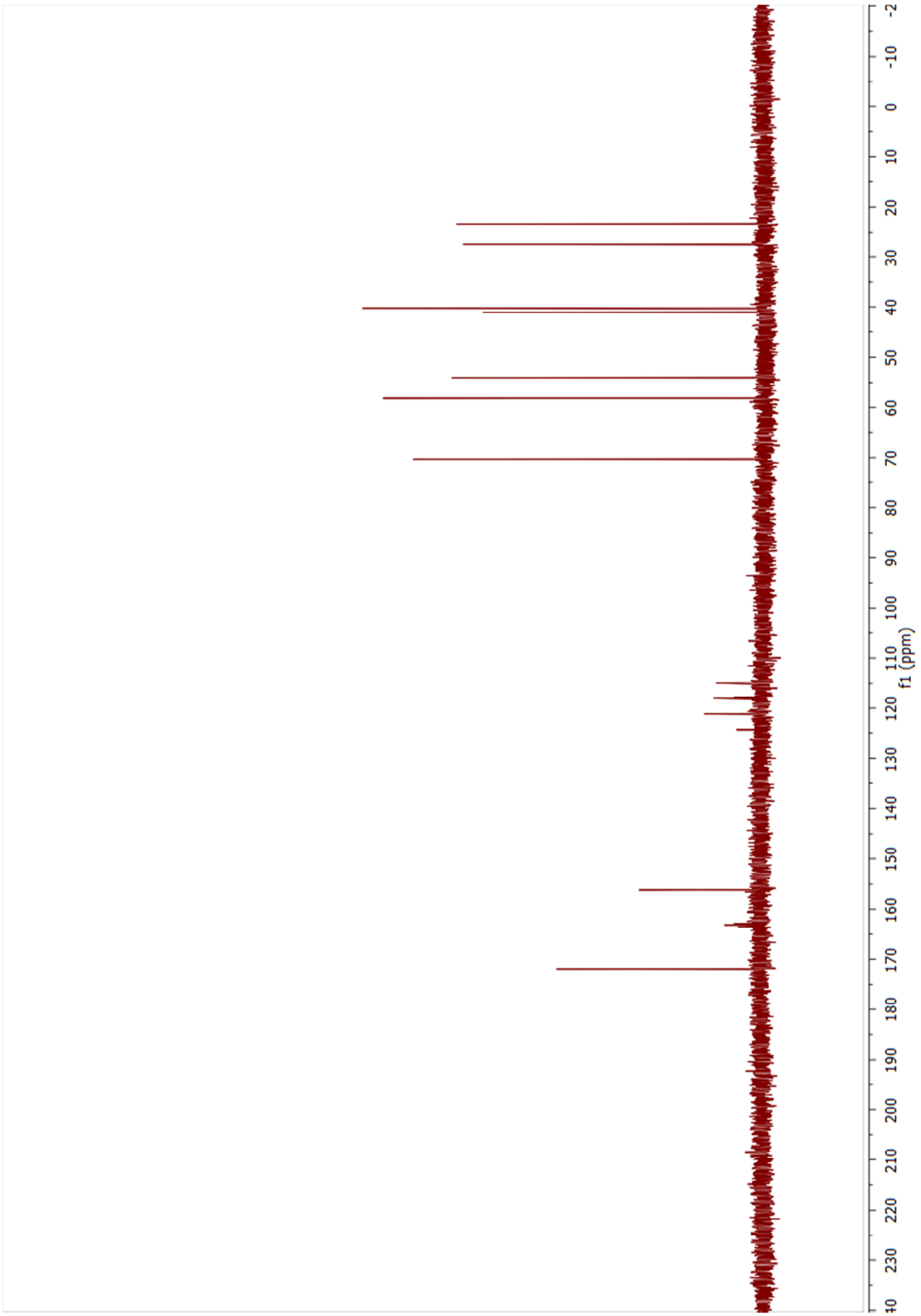


Figure 4.40 ^{14}C NMR spectrum of 8a

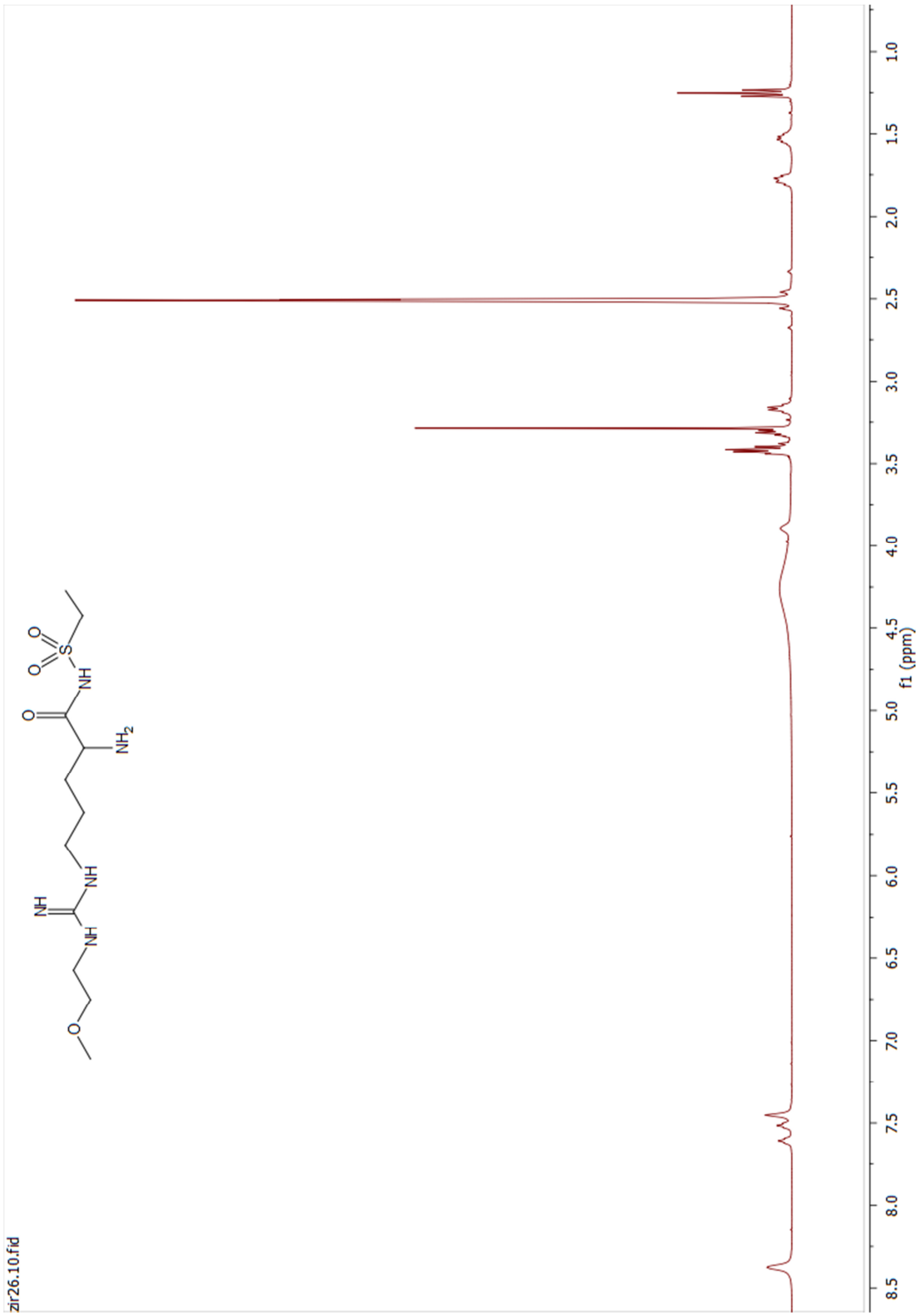


Figure 4.41 1H NMR spectrum of 8b

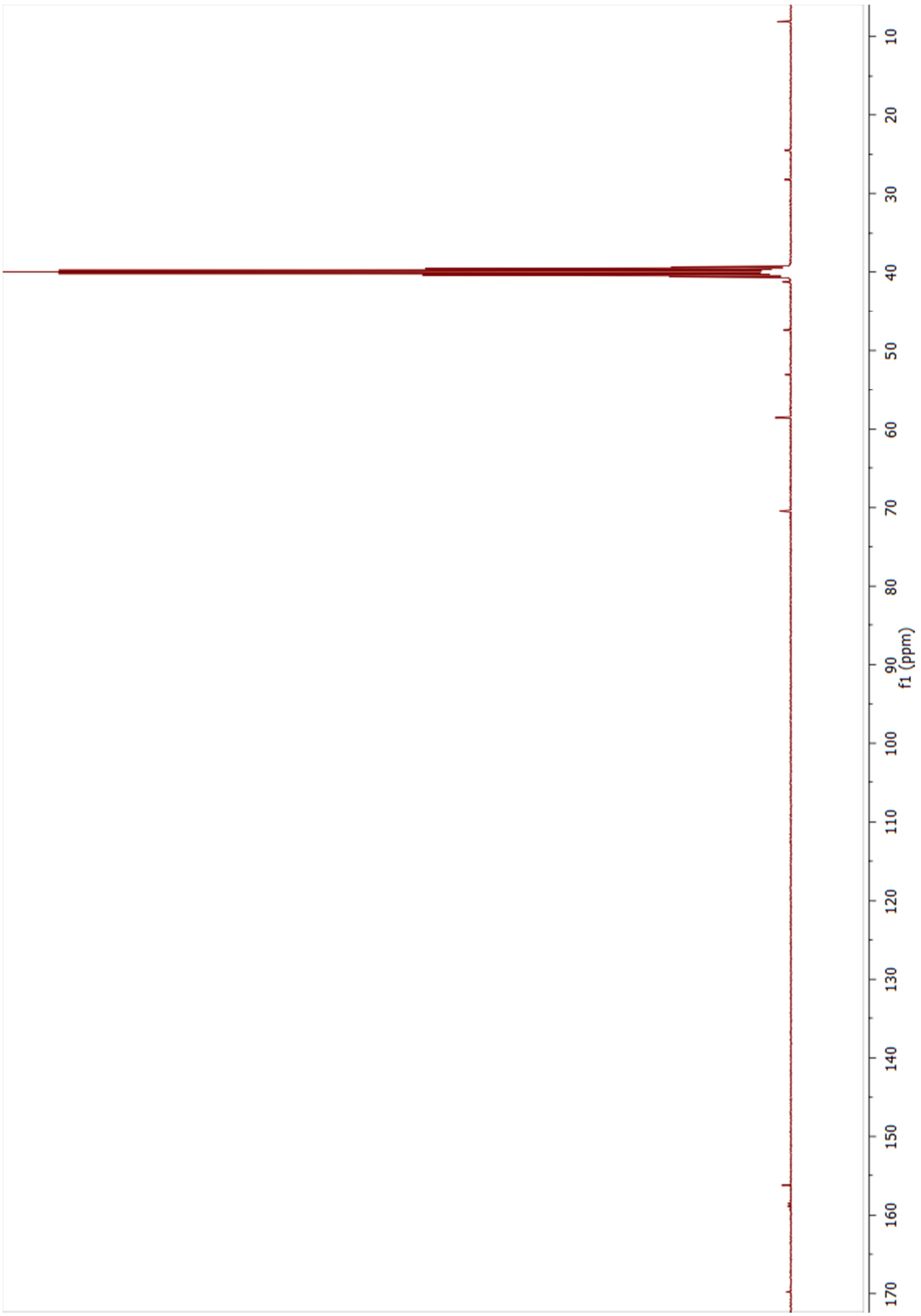


Figure 4.42 ^{14}C NMR spectrum of 8b

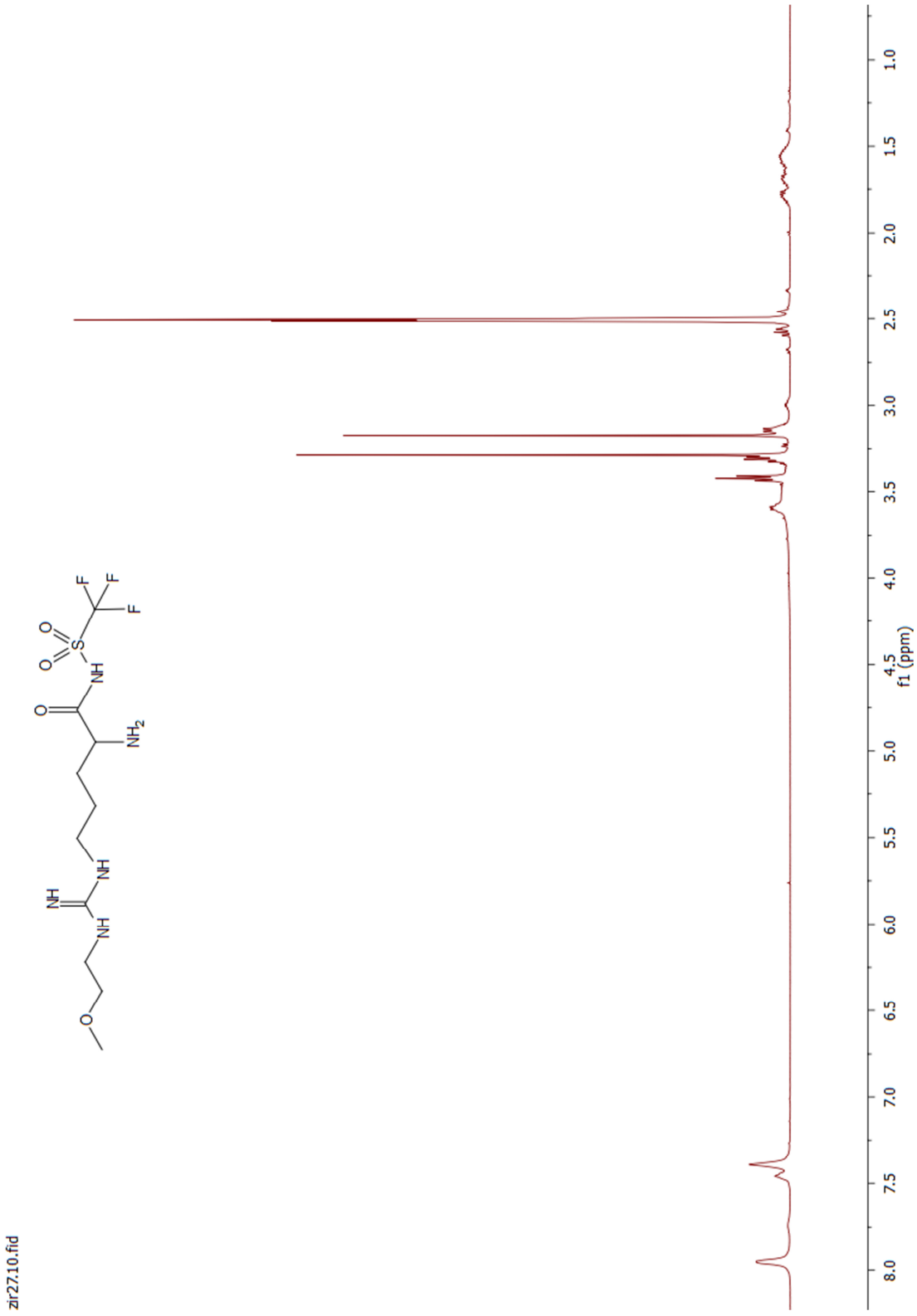


Figure 4.43 ^{1}H NMR spectrum of 8c

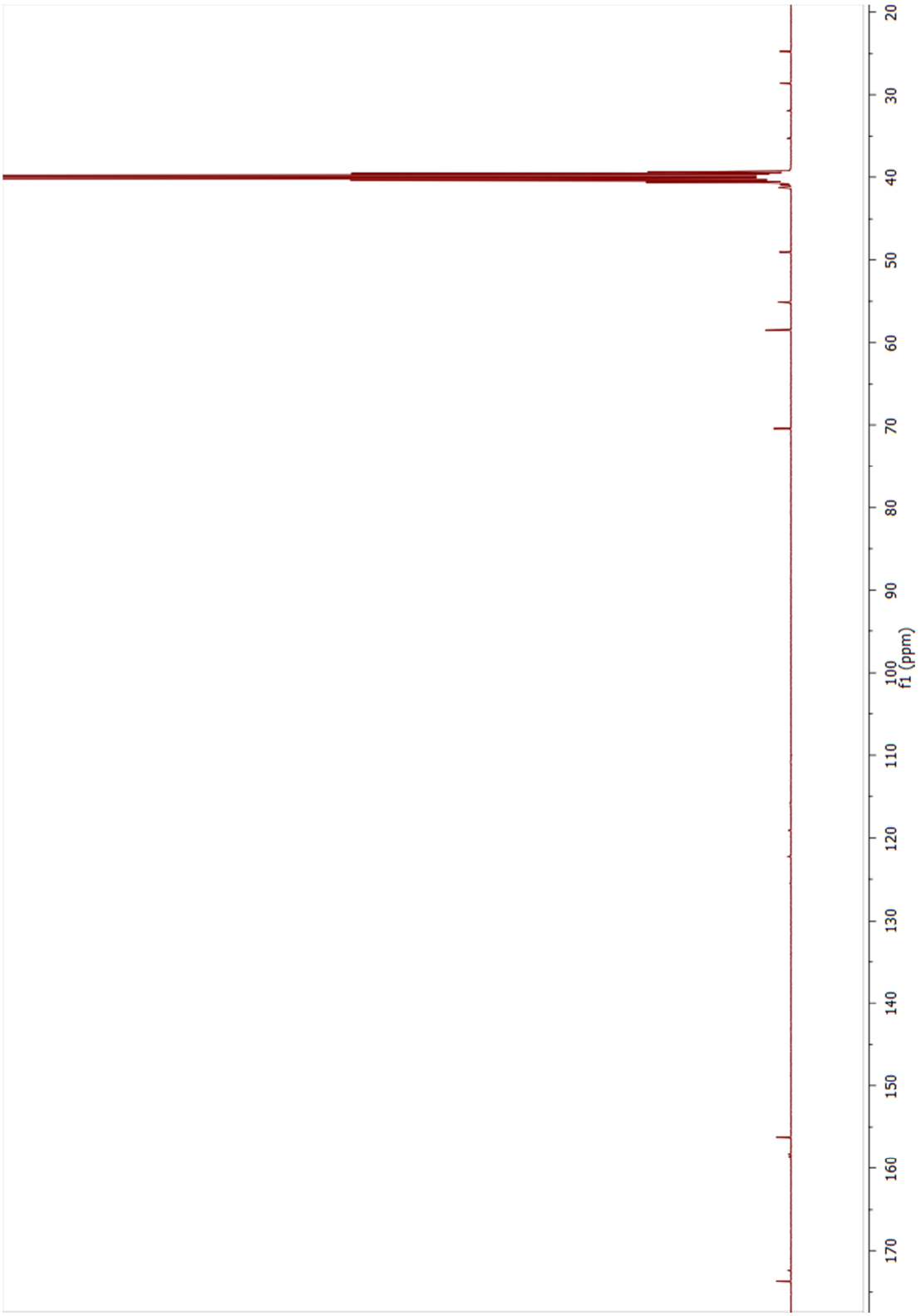


Figure 4.44 ^{14}C NMR spectrum of 8c

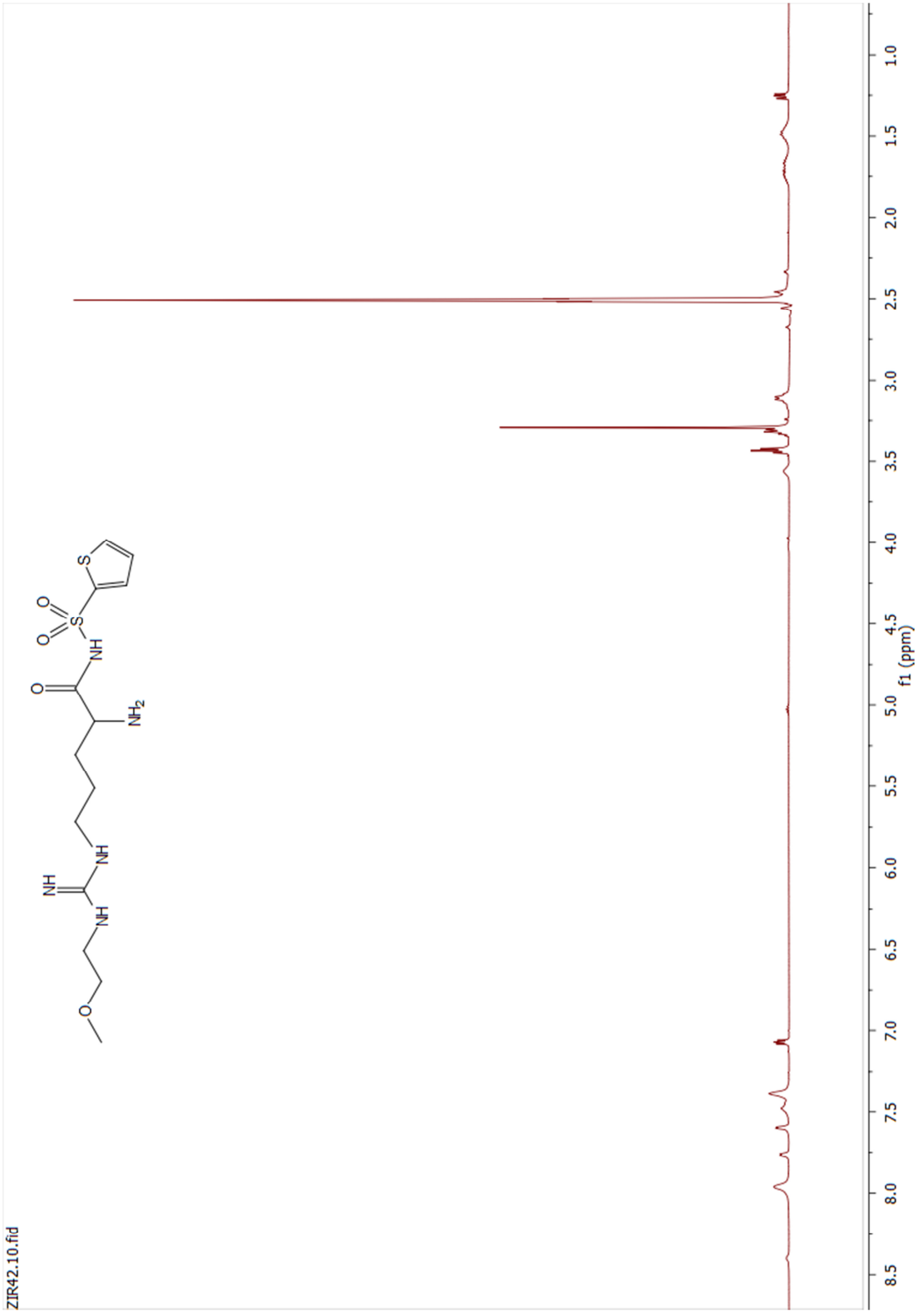


Figure 4.45 ^{1}H NMR spectrum of 8d

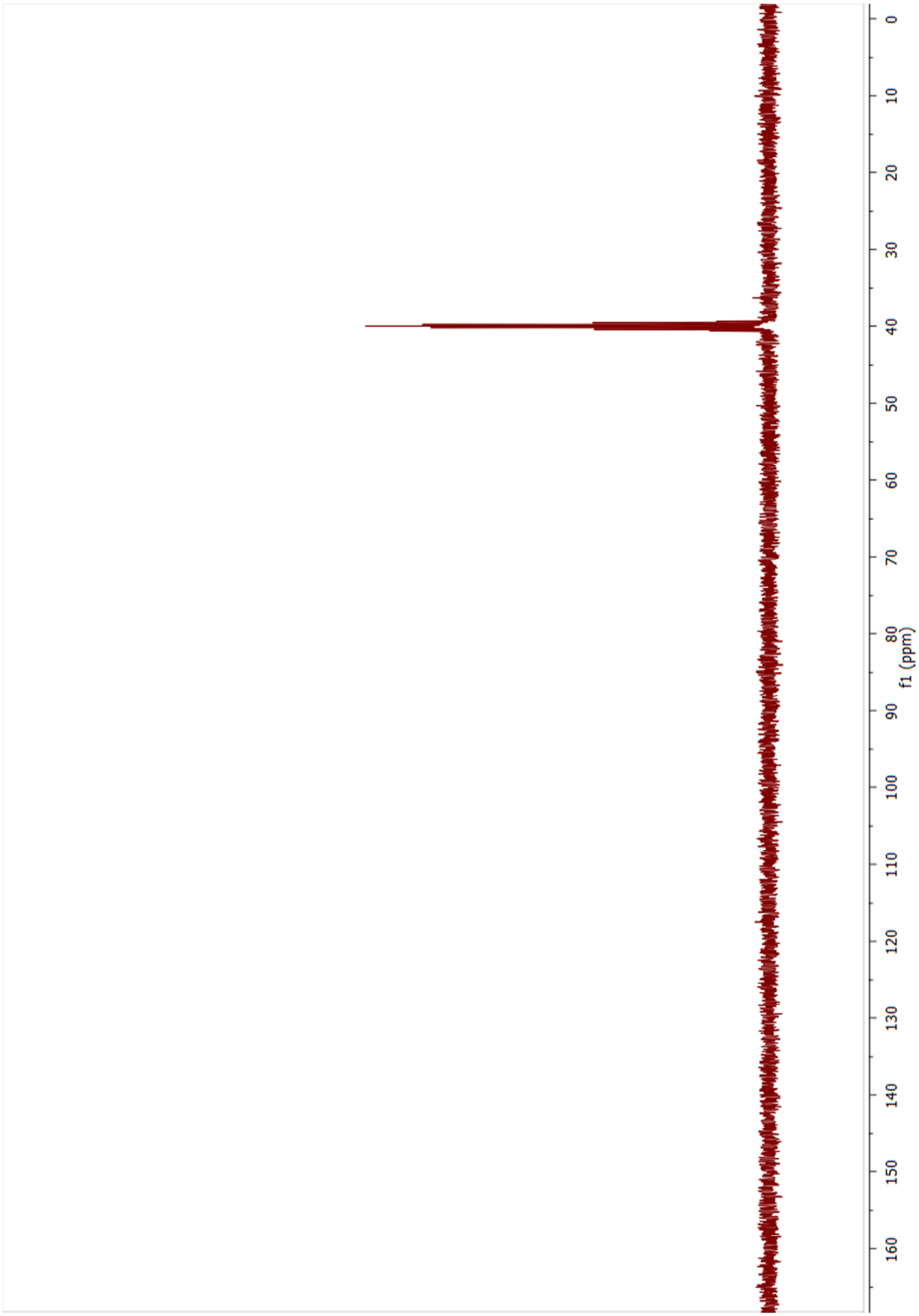


Figure 4.46 ^{14}C NMR spectrum of 8d

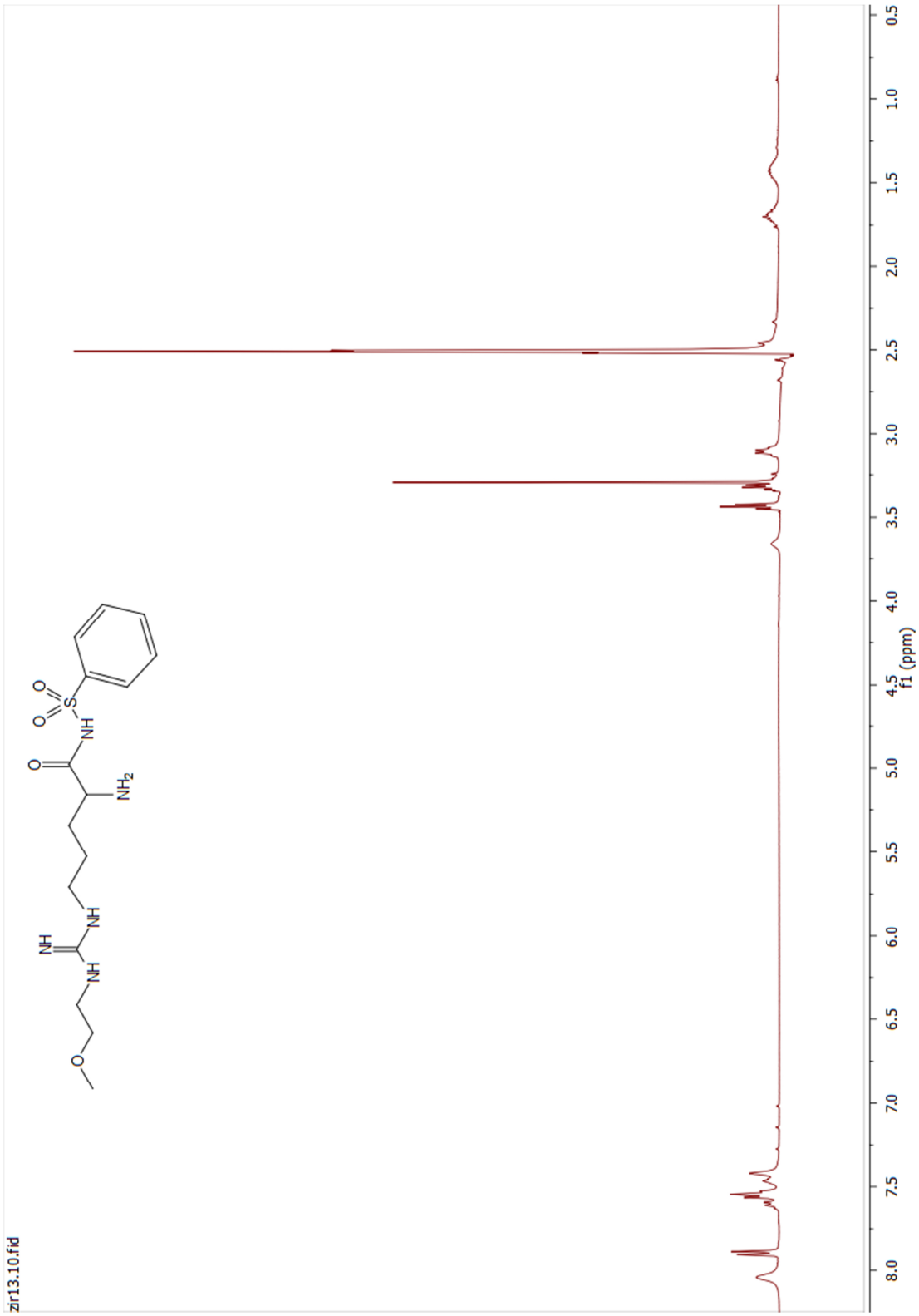


Figure 4.47 ^{1}H MNR spectrum of 8e

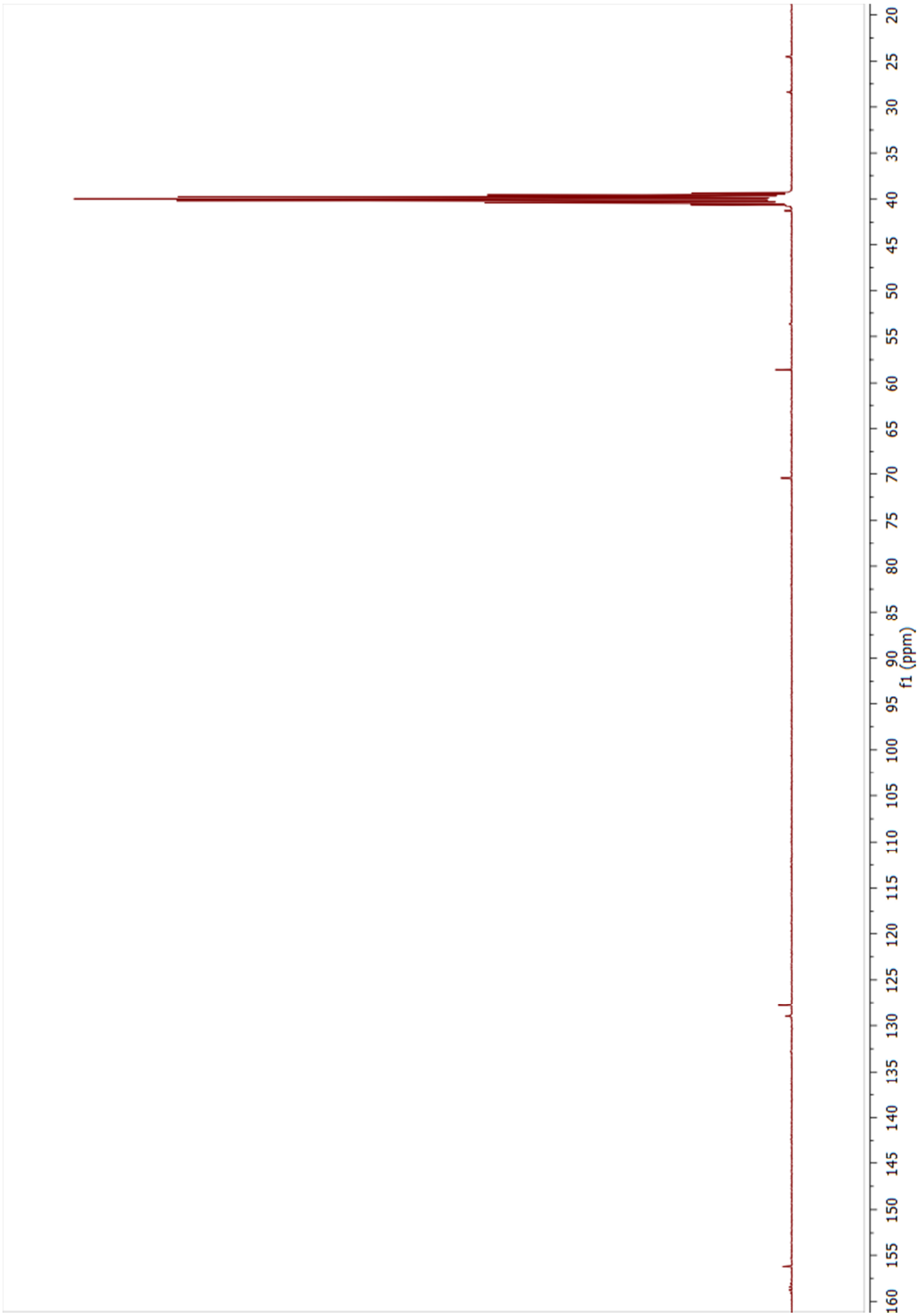


Figure 4.48 ^{14}C MNR spectrum of 8e

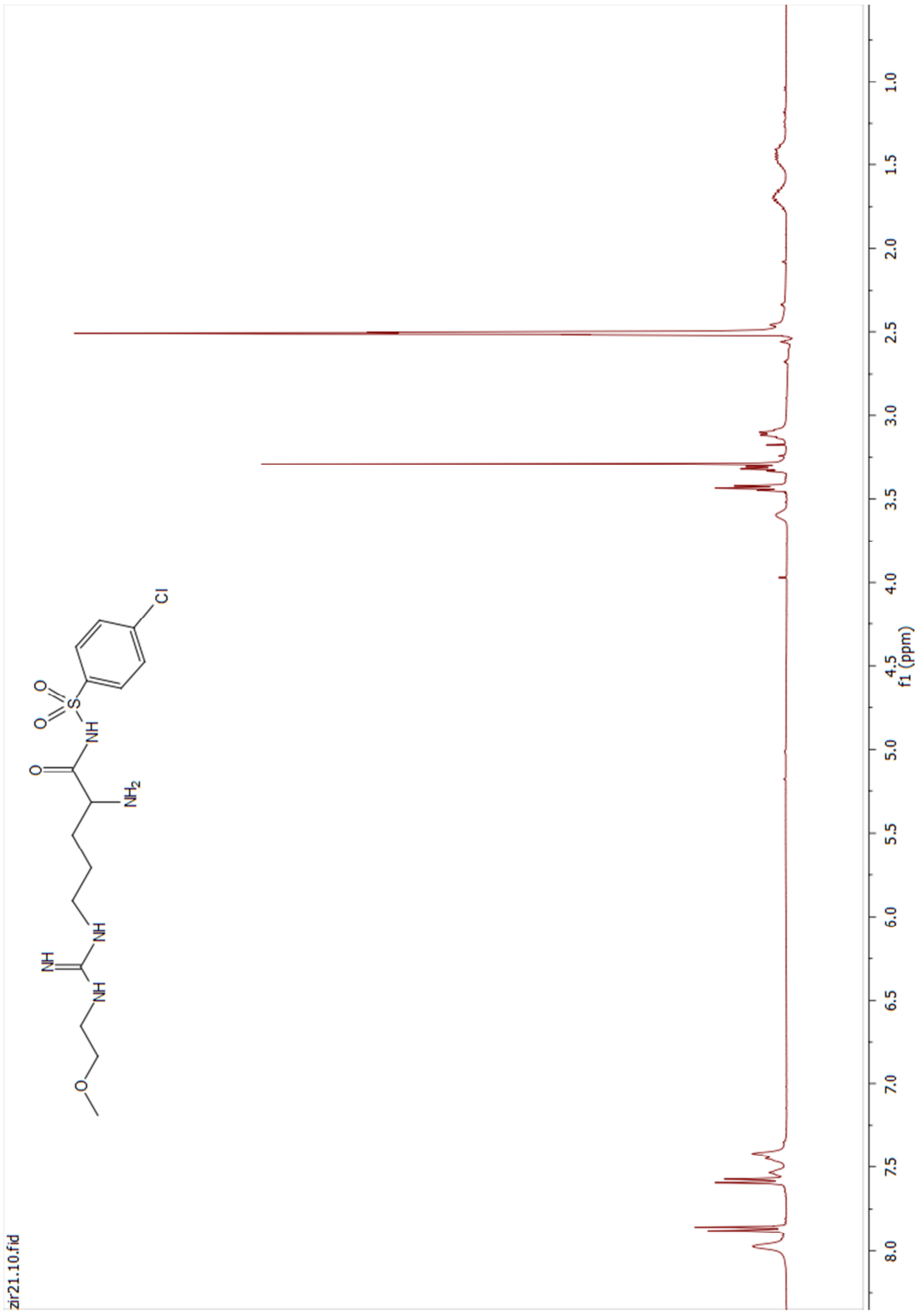


Figure 4.49 ^{1}H NMR spectrum of 8f

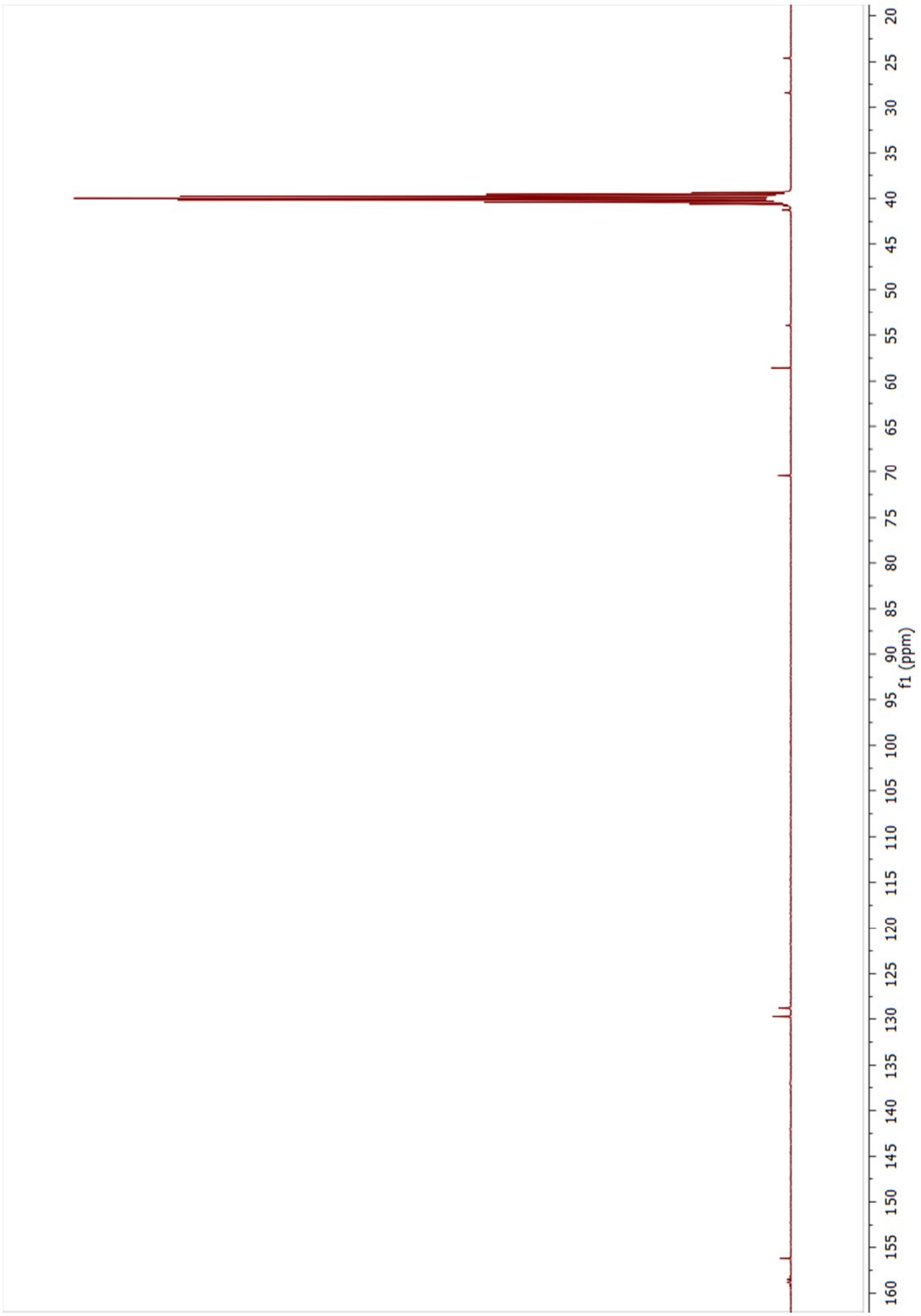


Figure 4.50 ^{14}C NMR spectrum of 8f

Chapter 5 TOOLS FOR THE ANALYSIS

5.1.1 NMR spectroscopy

The NMR (Nuclear Magnetic Resonance) spectroscopy is the best method based on the magnetic properties of the nuclei of certain atoms and isotopes.

The purpose of NMR is substantially to derive information about the resonance frequency of the active cores. The most used atoms are hydrogen, carbon-13 and nitrogen-15. There are two methods to operate.

In continuous wave NMR spectroscopy, an electromagnet generates the magnetic field necessary to induce the splitting between the energy levels of the active atoms in the sample. At the same time, an emitter of electromagnetic waves bombards the electromagnet at a precise frequency. The resonance frequency of an active nucleus is directly proportional to the applied field. As the field increases, resonance frequencies also increase.

The second method, used by most modern spectrometers, consists in keeping constant the field but increasing the frequency of the incident radio wave.

The cores of atoms with an odd number of protons and/or an odd number of neutrons rotate on their axes (spin) and have an angular momentum equal to $\mathbf{I} = (1/2)\ n$.

When a core with spin is placed inside a magnetic field, the core is subjected to forces that make it rotate in order to align itself with the external magnetic field.

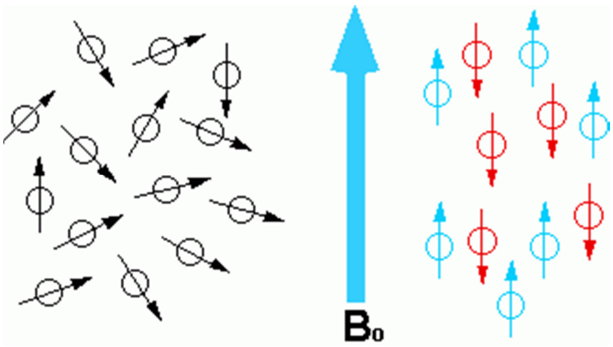


Figure 5.1 Cores into a magnetic field B_0

The active cores are immersed in a chemical environment. There are atoms, molecules around them. The NMR signal of a core is shifted in the spectrum to higher or lower frequencies depending on its chemical environment.

This is because electrons are charged particles. When they are immersed in a magnetic field, they move and generate a small magnetic field opposite to the much stronger one which was applied. This secondary magnetic field shields the nucleus.

If the electron density is large, the shielding is greater. The cores that are in an electron-rich environment feel a magnetic field and will undergo transition to a lower frequency compared to electron-poor cores. The resulting shift in the NMR signal is called *chemical shift*. Protons and carbons adjacent to electronegative atoms are shielded.

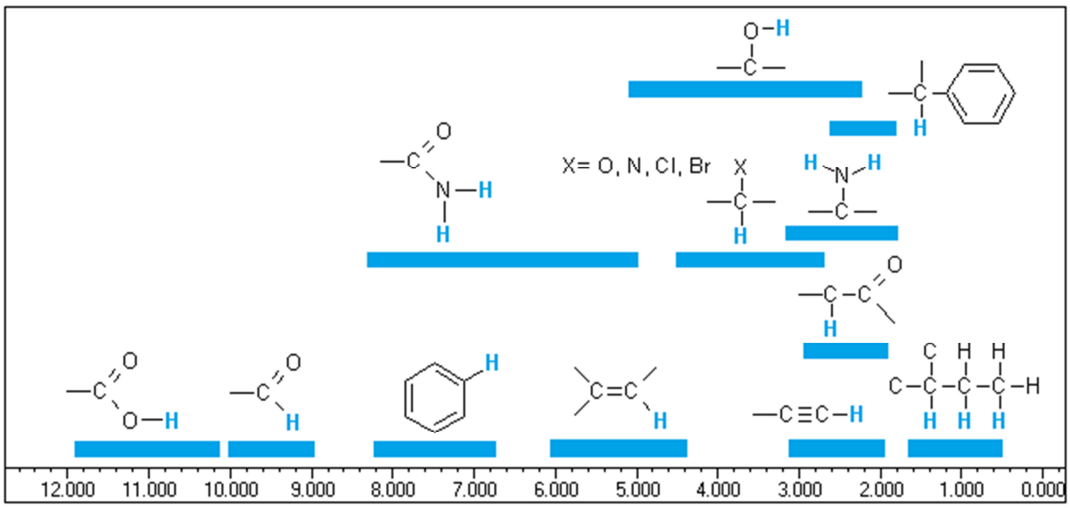


Figure 5.2 ^{1}H-NMR table of chemical shifts

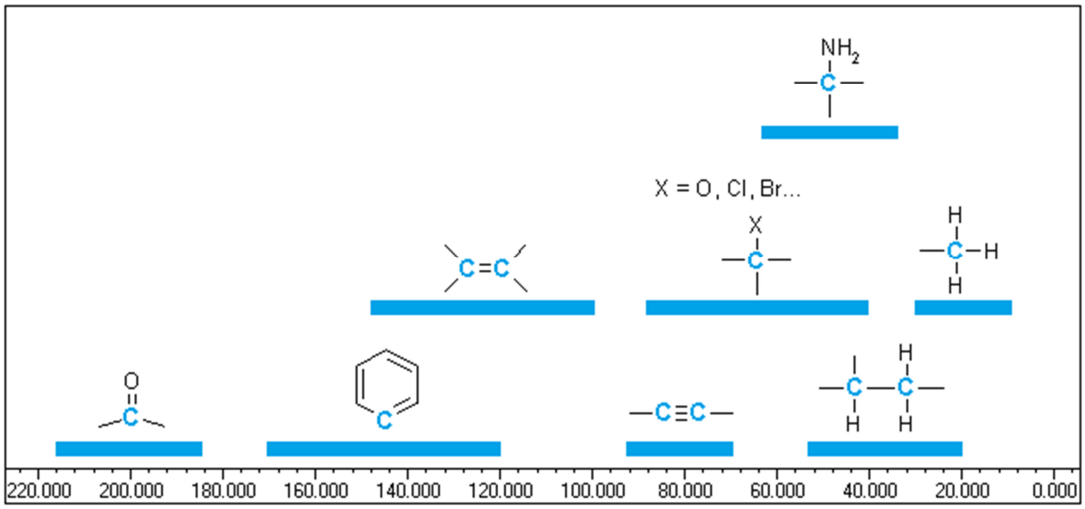


Figure 5.3 ^{13}C-NMR table of chemical shifts

In ^{1}H-NMR the intensity of the absorbance is proportional to the number of protons generating the signal. The area under the peak (the integral) is directly proportional to the number of that type of protons in the molecule.

5.1.2 Mass Spectrometry

Mass spectrometry (MS) is an analytical chemistry technique that helps identify the amount and type of chemicals present in a sample by measuring the mass-to-charge ratio and abundance of gas-phase ions. A mass spectrum is a plot of the ion signal as a function of the mass-to-charge ratio. The spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and molecules, and to elucidate the chemical structures of molecules. Mass spectrometry works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios.

In a typical MS procedure, a sample is ionized, for example by bombarding it with electrons. This may cause some of the sample's molecules to break into charged fragments. These ions are then separated according to their mass-to-charge ratio, typically by accelerating them and subjecting them to an electric or magnetic field. The ions are detected by a mechanism capable of detecting charged particles. Results

are displayed as spectra of the relative abundance of detected ions as a function of the mass-to-charge ratio.

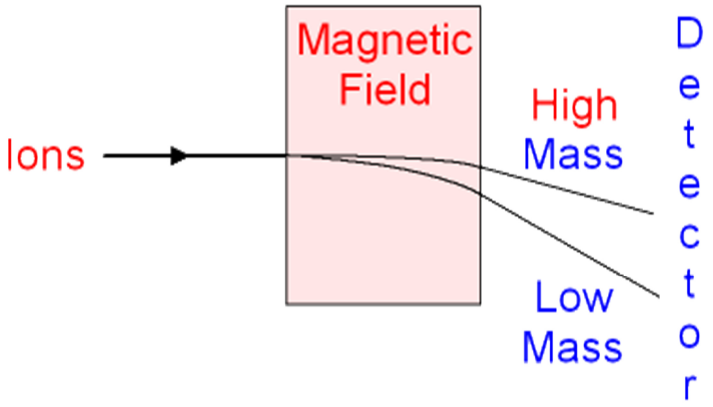


Figure 5.4 Simplified diagram of mass spectroscopy

5.1.3 High Performance Liquid Chromatography-HPLC

It is a chromatographic technique that separates two or more compounds present in a solvent exploiting the equilibrium of affinity between a "stationary phase" placed inside the chromatographic column and a "mobile phase" flowing through it.
A substance more similar to the stationary phase than to the mobile phase takes more time to run through the chromatographic column. The sample to be analyzed is injected at the beginning of the chromatographic column where it is pushed through the stationary phase from the mobile phase by applying pressure. To obtain high efficiency in separation it is necessary that the particle size of the filler is very small.
At the end of the column a detector (IR, UV-VIS, mass spectrometer) is applied and is connected to a computer that allows a continuous analysis of the output of the column, and then is able to quantify and/or identify the substances injected through the appropriate chromatogram. The main advantages of this technique are a greater accuracy and precision.
The model used at the University of Aberdeen is *Agilent 1260 Infinity*, the column is *Luna C18(2) 10x250mm 5um 100 Å-Phenomenex.*

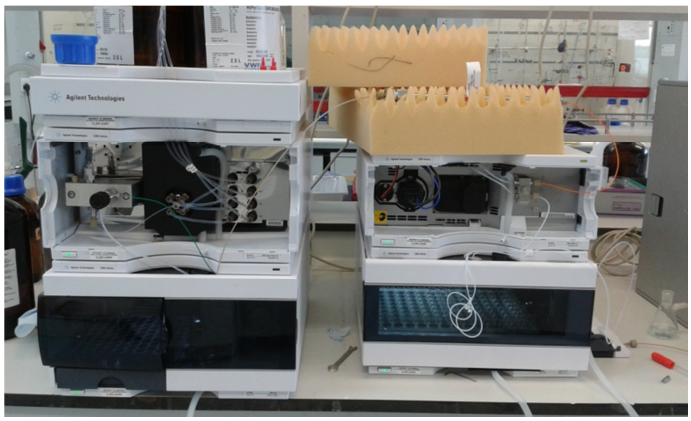


Figure 5.5 HPLC used at the University of Aberdeen

Chapter 6 **CONCLUSIONS**

In order to improve the yield (*i*) of the 3d (in the first step) you must repeat the synthesis by changing operating conditions.

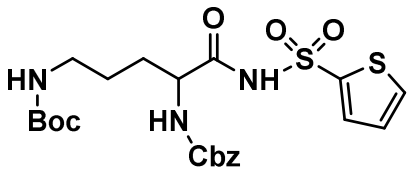


Figure 6.1 Compound 3d

For the product 3k, in the coupling reaction with EDCl, we can try to protect the -NH_2- group on the outside of the molecular chain with a protecting group so as to avoid the formation of bisaduct and facilitate the coupling reaction between the sulfonamide and ornithine.

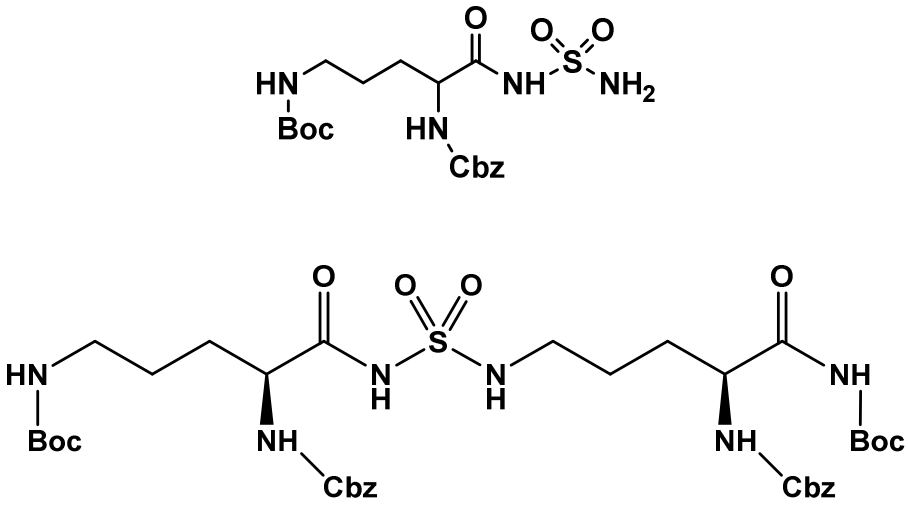


Figure 6.2 Compound 3k (above), the bisaduct (below)

For the future works we will provid for the purification of molecules 7 from *g* to *k*, which have given the most problems in the guanidination (*iv*) and deprotection (*v*) steps, because the main byproduct is still present after silica gel chromatography purification.

A first attempt to purification via column chromatographic under specific conditions of eluent mixture (DCM / MeOH) have failed to obtain the pure products. A further investigation is needed to find the right conditions for these purifications.

The our aim is obtain the products pure for biological experiments. The required purity is very high (99%).

The biological experiments will be performed at the Department of Clinical Pharmachology at Flinders University in Adelaide (South Australia). The molecules will be tested to determine their inhibition activity and selectivity towards the DDAH.

Figure 6.3 Inhibition profile characterization

Only in case of identification of potent inhibitors, they will be used for several *in vitro* tests.

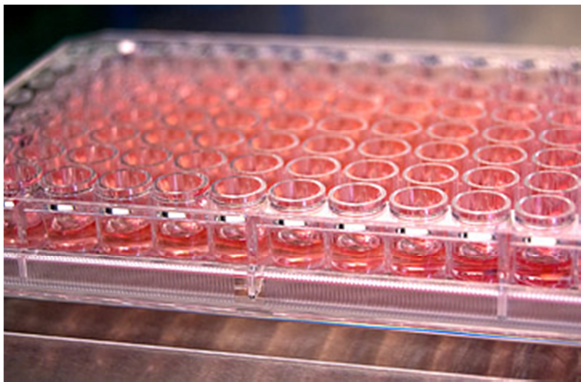


Figure 6.4 *In vitro* test

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