MECHANOCHEMICAL SYNTHESIS OF ANTIHISTAMINE ACTIVE PHARMACEUTICAL INGREDIENT CINNARIZINE

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF ENGINEERING AND SCIENCE
OF BILKENT UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF
MASTER OF SCIENCE
IN
CHEMISTRY

By
Rana Uzunlar
February 2025

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We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

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ABSTRACT

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M.Sc. in Chemistry

Advisor: Bilge Baytekin

February 2025

Mechanochemical methods are recognized as an appealing, greener methods for producing various molecular compounds. It has gained significance across multiple disciplines, such as chemistry and material science, due to its eco-conscious nature, allowing it to be performed without solvents or with only minimal amounts of solvent. Mechanochemical approaches also stand out as a prominent synthetic approach, enabling efficient and rapid processes compared to conventional synthesis approaches. Their synthetic capabilities through a range of solid-state transformations, yielding diverse compounds including inorganic, organic, polymeric, metal-organic-framework, and organometallic materials, have been proved over the decades. Yet another captivating illustration of mechanochemical reactions is seen in the effective pharmaceutical drugs and medicine preparations. This upward trajectory is evident in the increasing adoption of solid-state methodologies within pharmaceutical material science. These mechanochemical attempts are branched under a recently emerging field, namely “medicinal mechanochemistry,” and have been quite efficient in the synthesis of active pharmaceutical ingredients (APIs) and drugs such as paracetamol. They are also employed for the alteration of APIs through processes like the formation of salts or cocrystals. However, a synthetic pathway of many APIs in which mechanochemistry is

used as a bimolecular solid-state reaction initiator, is not available yet. Here, we show that utilizing ball milling of the reactants through a simple S_N2 reaction a first-generation H_1 antihistaminic API, cinnarizine, can be synthesized in moderate yields (25-50%). The milling and reaction parameters are explored in terms of product yields and a comparison with a conventional synthetic route is also provided. Milling produces cinnarizine in 1-60 minutes, compared to conventional organic synthesis, 4-24 hours. This route brings an innovative perspective to synthesizing widely used APIs in medicinal mechanochemistry and its potential applications in industry.

Keywords: Ball milling, mechanochemical synthesis, antihistamines, cinnarizine.

ÖZET

ANTİHİSTAMİNİK İLAÇ ETKEN MADDESİ SİNNARİZİNİN MEKANOKİMYASAL SENTEZİ

Rana Uzunlar

Kimya Bölümü, Yüksek Lisans

Tez Danışmanı: Bilge Baytekin

Şubat 2025

Mekanokimyasal metotlar, çeşitli moleküler bileşiklerin üretilmesi için geleneksel sentez yöntemlerine göre daha çevreci bir yöntem olarak kabul edilmektedir. Çevreye duyarlı doğası nedeniyle, çözücüler olmadan veya yalnızca minimum miktarda çözücüyle gerçekleştirilmesine olanak sağlaması nedeniyle bu metotlar kimya ve malzeme bilimi gibi birçok disiplinde önem kazanmıştır. Mekanokimyasal yaklaşımlar aynı zamanda geleneksel sentez yaklaşımlarına göre daha verimli ve hızlı süreçlere olanak sağlayan, öne çıkan sentetik yaklaşımlar olarak öne çıkmaktadır. Bu prosedürler ile inorganik, organik, polimerik, metal-organik-yapılar ve organometalik malzemeler de dahil olmak üzere çeşitli bileşiklerin üretimi on yıllar boyunca başarıyla gerçekleştirilmiştir. Mekanokimyasal reaksiyonların başka etkileyici örnekleri de aktif farmasötik bileşen (AFB) ve ilaç preparatlarında görülmektedir. Farmasötik malzeme hazırlanmasında katı hal metodolojilerinin giderek daha fazla benimsendiği görülmektedir. Bu mekanokimyasal girişimler, yakın zamanda ortaya çıkan bir alan olan "tıbbi mekanokimya" altında dallanmıştır ve AFB'lerin ve parasetamol gibi ilaçların sentezinde oldukça etkili olmuştur. Ayrıca bu yöntemler AFBlerin, tuzlarının veya kokristallerin oluşumu gibi işlemler yoluyla değiştirilmesi için de kullanılırlar. Bununla birlikte, birçok AFB'nin mekanokimyanın bimoleküler katı hal reaksiyon başlatıcısı

olarak kullanıldığı bir sentez yolu henüz mevcut değildir. Bu çalışmada, bilyeli öğütücü ile basit bir S_N2 tepkimesi yoluyla tepkiyenlerin birinci nesil antihistaminik AFB olan sinarizinin orta verimlerde (%25-50) mekanokimyasal olarak sentezlenebileceği gösterilmiştir. Öğütme ve reaksiyon parametreleri, ürün verimleri açısından incelenmekte ve ayrıca geleneksel sentetik yolla bir karşılaştırma da sunulmaktadır. Öğütme ile 1-60 dakikada ulaşılan verimlere, geleneksel organik sentezle 4-24 saatte ulaşılabilmektedir. Sunulan bu yeni metot, yaygın olarak kullanılan AFB'lerin ve bunların endüstrideki potansiyel uygulamalarının sentezlenmesine yenilikçi bir bakış açısı getirmektedir.

Anahtar Kelimeler: Bilyeli değirmen, mekanokimyasal sentez, antihistaminikler, sinarizin

ACKNOWLEDGEMENT

To begin with, I would like to express my heartfelt gratitude to my advisor, Dr. Bilge Baytekin, for her greatest support, guidance, trust and never-ending knowledge of science. Her way of thinking and viewing the world from quite a different perspective always amazes me and is the reason why I want to work with her in the first place. Just like her name, she is one of the "Bilge"st person I know. Learning from her experience during all these years we have been together was extremely valuable for my professional growth and journey in science. Not only her knowledge in chemistry but also her kind soul motivates me every day all day to believe, as scientists, we can all be hardworking but also considerate and rich at heart to our colleagues at the same time. So, thank you sincerely for everything Hocam.

Next, I would like to thank my thesis committee, Prof. Engin Umut Akkaya and Assoc. Prof. Çağatay Dengiz for their kind acceptance of evaluating my thesis, their valuable feedback, and the scientific discussions we held.

I am sincerely indebted to the generous support from Assist. Prof. Yunus Emre Türkmen for his discussions and fruitful comments about my thesis work. I would also like to express my thanks to the rest of the members of our faculty, Bilkent Chemistry Department, since they built and shaped my background in chemistry with their dedication to teaching and being examples of up-to-date scientists.

I want to thank every member of the Baytekin Research Group I had a chance to meet, past and present. Thank you for providing me with a supportive and healthy environment in which successful research can only be done. Getting to know all of you and working together has been a pleasure. I also wish to thank Türkmen Group for their

useful tips in organic applications and sharing enjoyable times. My special thanks to Merve Temel, whose presence I cherish deeply. She has been a wonderful friend who provides a great ambiance in the university during these years. Without your kindness, help, and amazing friendship, I would be lost.

Finally, I owe my deepest gratitude to my one and only family: my dad, my mom and sister. Thank you for everything from the very bottom of my heart. Thank you for your unconditional love, eternal support, and endless patience. There cannot be enough words to describe their meaning for me. . I would like to thank my father, Murat Uzunlar, for always supporting me, for trusting me throughout my life but also always secretly keeping an eye on me. I would like to thank my mother, Akile Uzunlar, for always being there no matter what, for always listening and giving the best advice to me, and for always providing a better life for me. I would like to thank my sister sincerely, Ceyda Uzunlar, for being my best friend forever, for being the joy of my life and for always making me smile. Thank you, and I love you.

Dedicated to my beloved family...

List of Abbreviation

APCI-MS	Atmospheric pressure chemical ionization mass spectrometry
API	Active pharmaceutical ingredient
ATR	Attenuated total reflectance
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
ESI-MS	Electrospray ionization mass spectrometry
EtOAc	Ethyl acetate
FTIR	Fourier transform infrared
HR-MS	High-resolution mass spectrum
MeOH	Methanol
NMR	Nuclear magnetic resonance
R*f*	Retention factor
SS	Stainless-steel
TLC	Thin-layer chromatography
TMS	Trimethylsilyl
UV	Ultraviolet

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1. Introduction

1.1. Green Chemistry

Remarkable advancements in industry and technology were made in the last century, highlighting the critical role of innovations in human prosperity. However, the adverse effects of rapid industrialization and consumption growth on the environment were overlooked for many years. For instance, unchecked disposal of waste constantly increases the pollution of soil, air, and water sources on both surface and underground. Despite strict regulations on waste management in several countries currently, according to reports of the US Environmental Protection Agency, hazardous waste production in the United States ranged from 20 to 30 million tons from 2001 to 2019[1] **(Figure 1)**.

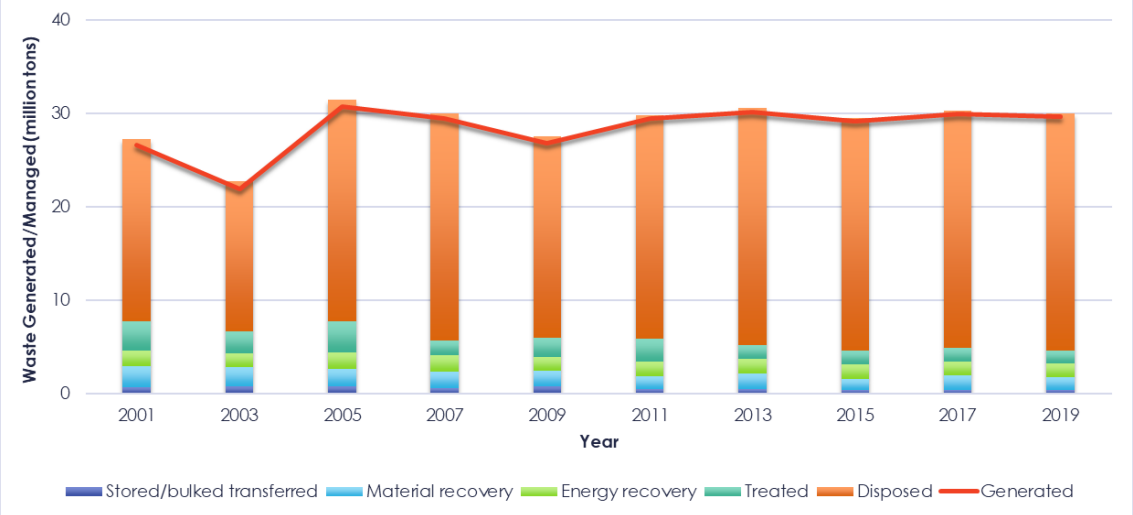


Figure 1: Hazardous waste generation and management in the U.S., 2001-2019 (Adapted from[1])

Consequently, scientists globally are tackling the problem of developing greener and eco-conscious methods that promote long-term sustainability and have minimal environmental impact. That's where Green Chemistry comes into play.

Implementing more stringent yet essential policies to monitor industrial activities in several chemical areas, including pharmaceuticals and fine chemistry, becomes unavoidable. The introduction of these new regulations prompts the generation of new, cleaner, and more efficient synthetic approaches that follow the "12 principles of green chemistry"[2] **(Figure 2)**.

Figure 2: 12 Principles of Green Chemistry

1.2. Mechanochemistry

Mechanochemical reactions are defined as chemical reactions that are induced by the direct absorption of mechanical energy[3].

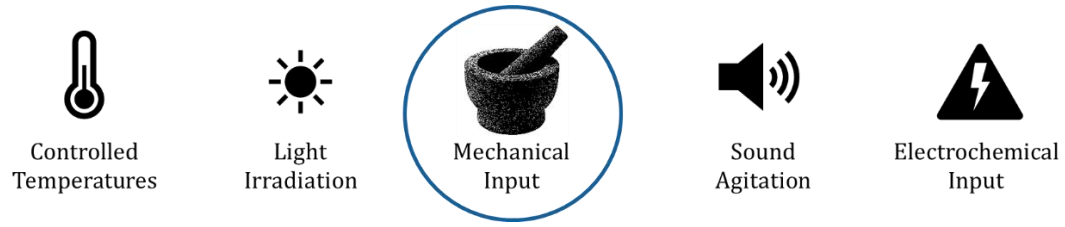


Figure 3: Various external factors to induce and proceed with a reaction and, mechanochemistry being one of them

Mechanochemical preparations, initiating chemical reactions with the absorption of mechanical force, becomes a green alternative to conventional synthesis since it fulfills many sustainable chemistry principles. In other words, mechanochemistry goes hand in hand with green chemistry because mechanochemical reactions give scientists a playground to achieve what they require in a green chemistry application, such as less hazardous chemical synthesis, safer solvents & reaction conditions, safer chemicals & products, an increase in energy efficiency, and prevention of waste. Especially, solvent-free mechanochemical reactions driven by physical forces, such as grinding, milling, or extrusion, have arisen as a feasible option instead of conventional synthesis in which toxic bases and solvents are used as the medium. Besides avoiding the usage of solvents and bases/acids, this approach also offers major benefits by preventing workups and multistep processes, reducing long times for reaction and waste generation[4,5].

The input force in mechanochemical processes can be generated through automated or manual grinding or by employing various milling devices featuring planetary or vibrational configurations. Grinding, milling, and extrusion are commonly preferred in mechanochemistry. Ball mills open a possibility for solvent-free reactions due to their closed system environment and can replace stirrers in

mechanochemical procedures. Additionally, flexibility in adjusting parameters such as time or frequency of milling is quite advantageous, considering the use of ball milling in mechanochemistry. Twin-screw extrusion is another method that is mostly favored in the industry to execute a broad selection of mechanochemical reactions[6-8].

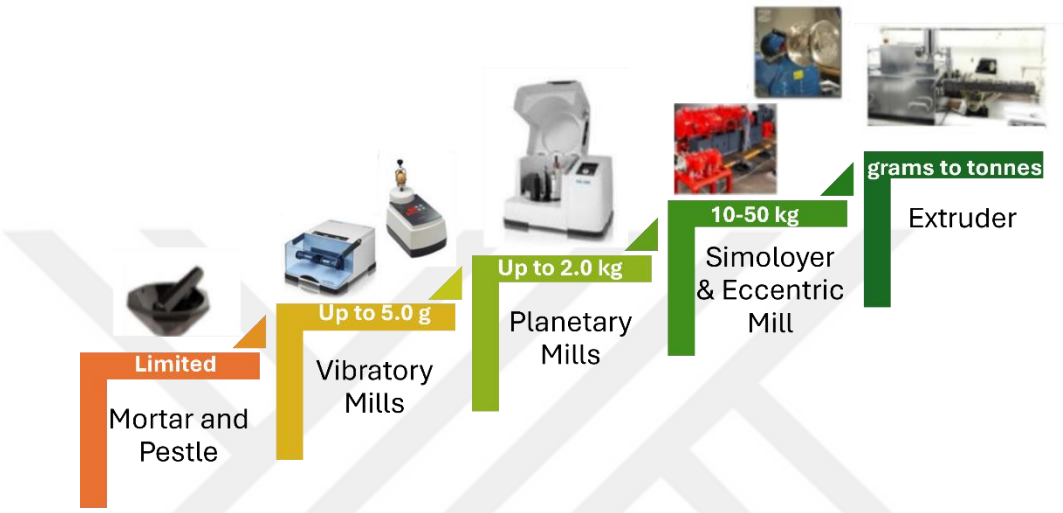


Figure 4 : Upscaling of mechanochemical instruments (Adapted from[9])

In organic mechano-synthesis, planetary or vibrational ball mills are utilized. In both types of ball mills, sample holders, known as jars, are filled with reactants and balls for milling. In the case of planetary ball mills, jars are spun on their axes while the main disk beneath them rotates in the reverse direction. In this type of mill, the high rotational speed of the jars causes the generation of shearing forces that carry out homogenous mixing and activation of reactants mechanically. On the other hand, the working principle for the vibrational ball mills is different from that of planetary ones since the jar vibrates at a set frequency. As the jar is shaken, reactants mix with the collision of balls and compounds, resulting in downsizing particles and mechanical activation of reactants, hence the mechanochemical

reaction. Due to this horizontal movement, they also go with the name “shaker” or “mixer” mills[10].

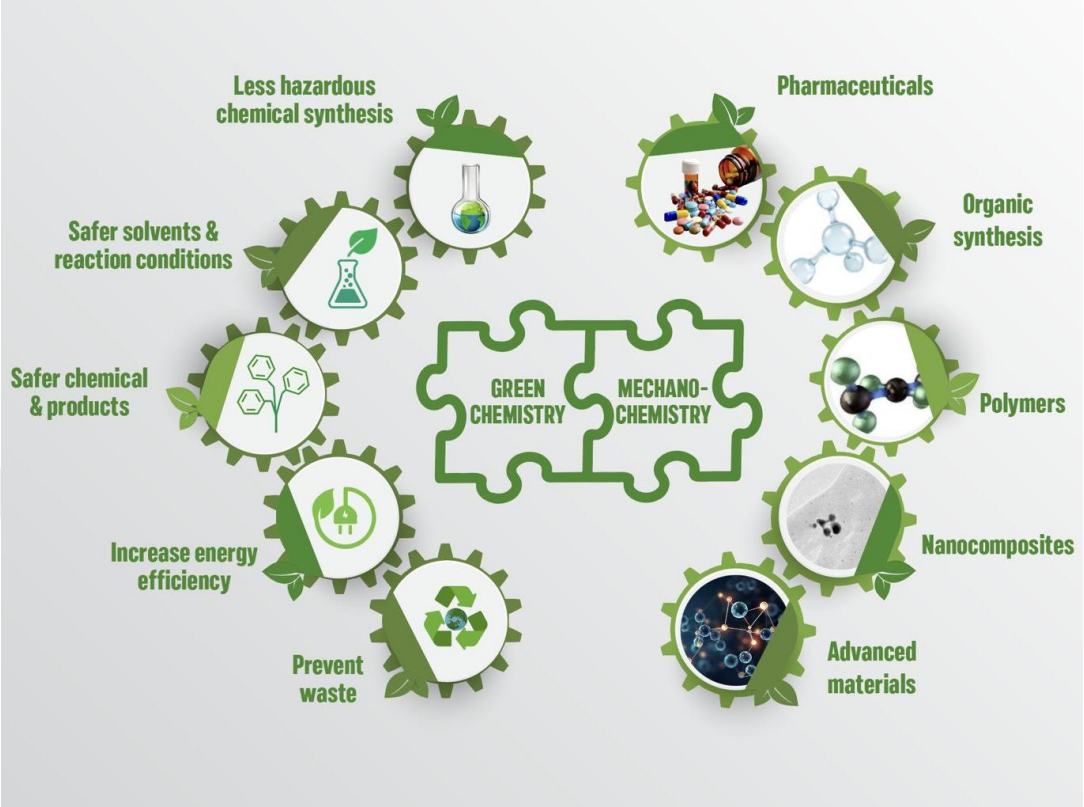


Figure 5: Green Chemistry and Mechanochemistry connection

Mechanochemistry has the potential and flexibility to be combined with various research areas such as pharmaceuticals, organic chemistry, polymers chemistry, nanocomposites and advanced materials since it can be integrated into green chemistry practices[11]. For instance, our research group, Baytekin Group, previously used polymer mechanochemistry to develop some polymer/metal nanocomposites mechanochemically. The mechanochemically obtained morphological changes and radicals on cellulose via its cryo-milling[12] helped preparation of some catalytically active and antibacterial cellulose metal nanocomposites[13]. Similarly, the group displayed also an alternative way to

produce antibacterial textiles via ultrasonication, which were also prepared by the mechanochemical formation of radicalic species[14].

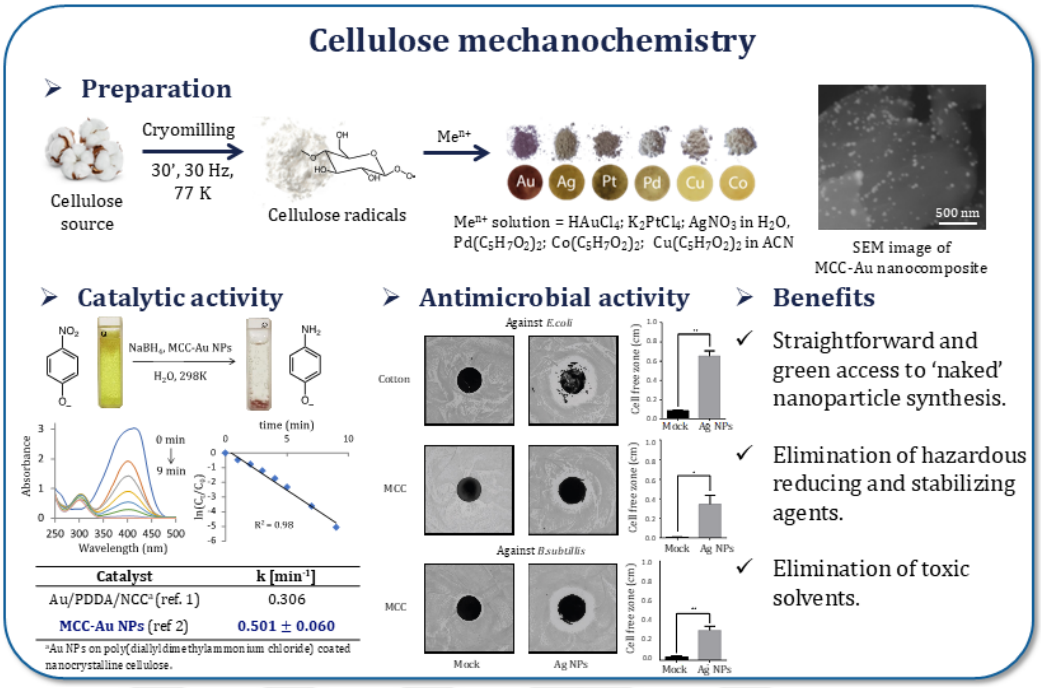
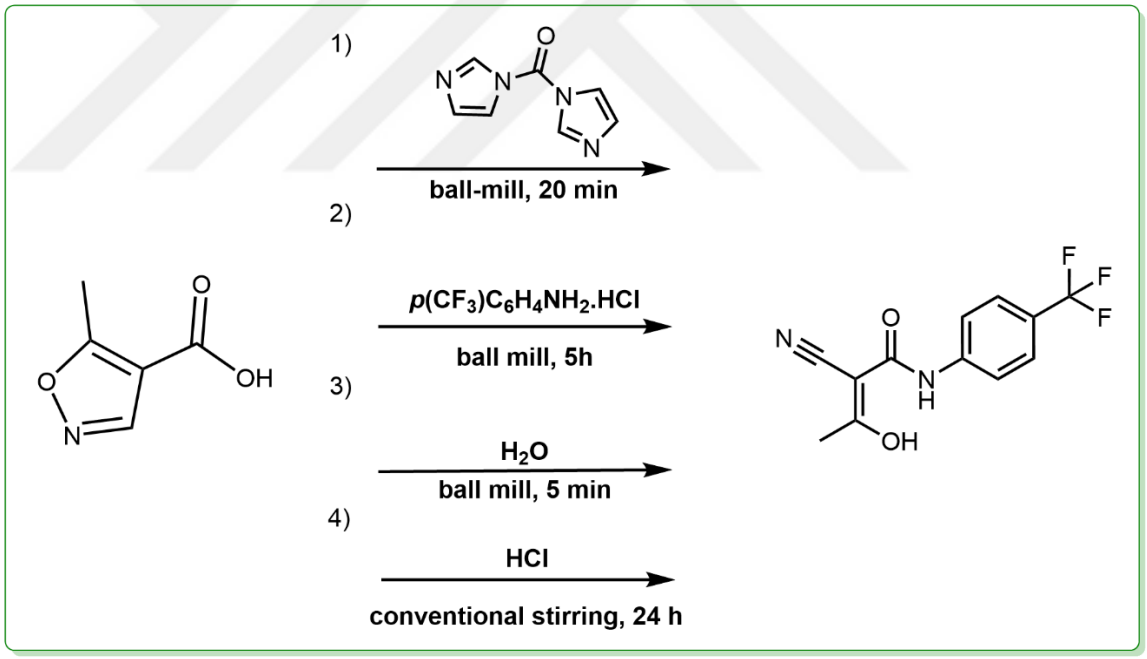


Figure 6: Previously performed cellulose mechanochemistry work by our group[14]

1.3. Medicinal Mechanochemistry

In recent years, mechanochemistry has drawn notable attention from diverse scientific disciplines, such as material science, chemistry, and medicine. The convergence of mechanochemistry, organic, and medicinal chemistry led to a newly emerging interdisciplinary field called "Medicinal Mechanochemistry". This new area of research has generated fresh prospects for drug design. The synthesis of **active pharmaceutical ingredients (APIs)** and their pharmaceutically relevant derivatives and fragments could be generated through mechanochemical preparations with exceptional outcomes. The mechanochemical approach concurrently enhanced the efficiency and sustainability of the processes [15-17].

There are many mechanochemical API preparation methods reported in the literature. Dantrolene used as skeletal muscle myorelaxant, nitrofurantoin used as antibacterial agent[18], bismuth subsalicylate used for diarrhea treatment[19], teriflunomide used against multiple sclerosis[20], phenytoin used as anti-seizure medicine[21], axitinib used for renal cell carcinoma treatment[22] and leu-enkephalin[23] were prepared with high yields via ball-milling. To elaborate one example, Teriflunomide was generated by ball milling successfully with a yield of 81%. In this preparation no solvent other than water was used **(Scheme1)**. Mechanochemical reaction, in which no solvent was used, results in the faster acylation of aniline hydrochlorides compared to the conventional wet synthesis method[20].



Scheme 1: Various reaction examples of sustainable mechanosynthesis of Teriflunomide via ball milling.

1.4. Antihistamines

Allergic diseases are one of the most general inflammatory disorders across the globe[24-26]. These diseases include hives, hay fever (allergic rhinitis), conjunctivitis and allergic reactions[27].

Broad prevalence of the allergic disease created a high worldwide demand for the development of related treatments and antihistaminic drugs. Antihistamines are described as prescribed medications to treat symptoms of allergic reactions of various types. A large collection of antihistamines was developed with the recognition of histamine as a key mediator in allergic conditions. This library of compounds was categorized into four subclasses, each based on their targeted histamine receptor. They are labeled as H_1- H_2- H_3- H_4-antihistamines. H_1- and H_2-antihistamines are the most comprehensive subclasses, while H_3- H_4-antihistamines subclasses consist of relatively newer/newly generated compounds, some of which still require clinical approval[28].

First-generation antihistamines representatives include alkylamines (e.g., pheniramine, chlorphenamine, chlorpheniramine, dexchlorpheniramine, brompheniramine, cyclizine, diphenylpyraline), ethanolamines (e.g., diphenhydramine), piperazines (e.g., cinnarizine), and tricyclics (e.g., trimeprazine, cyproheptadine)[28]. They have poor receptor selectivity and can cross the blood-brain barrier, which may lead to side effects as drowsiness, sedation, or sleepiness[29]. Nonetheless, first-generation H_1-antihistamines such as chlorpheniramine, diphenhydramine, cyclizine, or diphenylpyraline are still successfully used to treat severe allergic reactions. New-generation antihistamines cross the blood-brain barrier much less in comparison to the first-generation ones[30]

They selectively bind to peripheral histamine H_1 receptors, which results in a noticeable decrease of side effects such as sedation[30]. However, drowsiness can be accelerated upon a higher dosage of these drugs. The most consumed examples of new-generation antihistamines are cetirizine (Zyrtec®), loratadine (Alavert®, Claritin®), and fexofenadine (Allegra®)[30].

1.4.1. Preparation of Antihistamine APIs

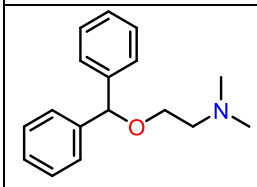
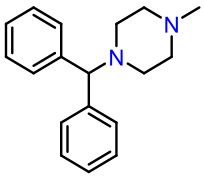
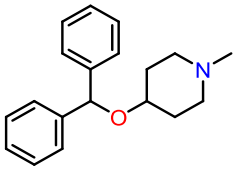
Like many other APIs, first generation antihistamine APIs are also prepared through conventional, solvent-based synthesis. The conventional synthetic routes are already producing large quantities of these drugs. However, the 'cost' of these syntheses (like all other solvent-based syntheses) is in the use of (non-green) solvents, which must be removed after synthesis. In most cases other not benign chemicals are also used in the preparation of these compounds (Some examples in **Table 1**).

A mechanochemical synthesis of an antihistaminic API using (cryo) milling approach has not been reported in the literature, yet. Recently, our group has successfully synthesized antihistaminic APIs, diphenhydramine, cyclizine, and diphenylpyraline using this approach **(Table 1)**. The results of these work proved that the mechanochemical approach, especially cryomilling can be very efficient for synthetic preparation of these APIs. It can also be proposed that it is extremely beneficial to choose a cryo temperature in the synthesis of some of these compounds.

The 'green'ness of the conventional and mechanochemical (cryomilling) approaches can be roughly compared by a software[31] (Merck MiliporeSigma's DOZN 2.0 Green Chemistry Evaluator). The computation processes the solvent and

the reactant green index, the duration of the reaction, and the energy consumption during the reaction and separation, and provides a greenness score for the given method. The lower values depict greener synthesis. For diphenhydramine (DPH) synthesis (**Table 1**), the ball-milling method scores much less than the solvent-based conventional synthesis leading to the same compound. It is no surprise that the newly discovered cryo approach is greener, since it takes only a few minutes to complete the reaction with 91% yield through this route in comparison to hours of reflux in 98% in the conventional route.

Table 1: First generation antihistamine APIs previously prepared in Baytekin RG. The yield and reaction time comparison of conventional route, cryo milling and room temperature milling.

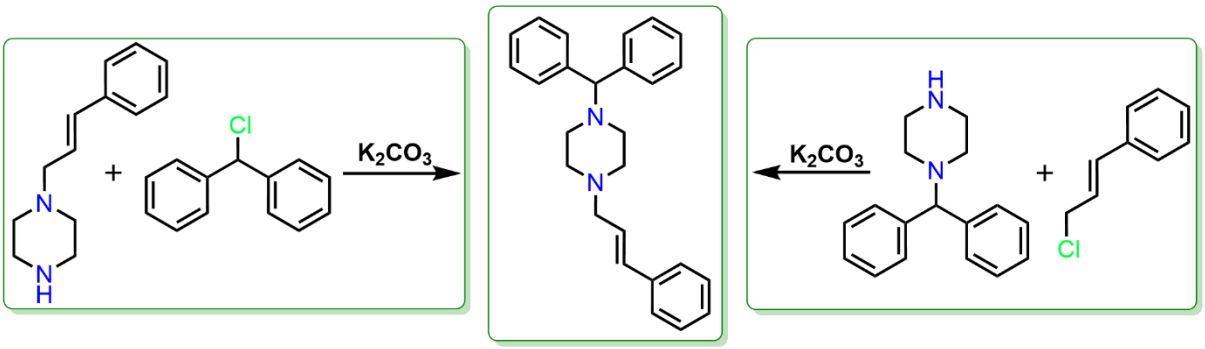
Previously synthesized APIs in Baytekin Research Group	**Conventional synthesis** ***highest reported yield and reaction time***	**Room temperature milling** ***highest yield and reaction time***	**Cryo temperature milling** ***highest yield and reaction time***
 **Diphenhydramine**	**98%** in 8 hours with catalyst and reflux[32]	**90%** in 1 minute no solvent	**91%** in 5 minutes no solvent
 **Cyclizine**	**99 %** in 24 hours with catalyst, solvent and under pressure[33]	**63%** in 20 minutes no solvent	**84%** in 1 hour no solvent
 **Diphenylpyraline**	**85.5%** in 5 hours with solvent use, refluxing[34]	**84%** in 10 minutes no solvent	**29%** in 1 hour no solvent

1.4.2. Cinnarizine

Cinnarizine is a first-generation antihistamine that can act as a calcium channel blocker, H1 receptor antagonist, anti-allergic agent, vestibular suppressant, and antiemetic. Cinnarizine effectively treats nausea and vomiting caused by motion sickness[35], and other conditions like vertigo[36], Ménière's disease[37], or chemotherapy[38]. Motion sickness is a common epidemiological phenomenon[39] and everyone may suffer from motion sickness at one point in life[40]. Cinnarizine is not available in the USA, but it is highly popular in Europe as an anti-motion sickness drug[41]. Seasickness is the most well-known form of motion sickness and nausea is its main symptom. In fact, the word "nausea" originated from the Greek word naus, meaning a ship or a boat[42]. Hence, Cinnarizine was highlighted as the most-used drug in the British Royal Navy[43]. It can be found in the pharmacology market in the brand names Stugeron®, Stunarone®, and Cinarin®. Even though it is generally well tolerated, mild to severe side effects may be observed due to prescribed Cinnarizine. Possible complications include drowsiness during the daytime, dry mouth, headache, skin problems, weight gain, and muscle rigidity[44]. Some studies even showed that it caused a risk of drug-induced parkinsonism, especially in elderly patients, particularly women, and in individuals who have been using it for an extended period[45].

Chemically known as 1-benzhydryl-4-[(*E*)-3-phenylprop-2-enyl]piperazine, Cinnarizine belongs to the family of piperazine derivatives and appears as a white powder[46]. It was first prepared by Janssen Pharmaceutical Companies in 1959[47]. There were initially two approaches yielding cinnarizine. The first method was a reaction of 1-trans-cinnamylpiperazine and benzhydryl chloride in an alkaline

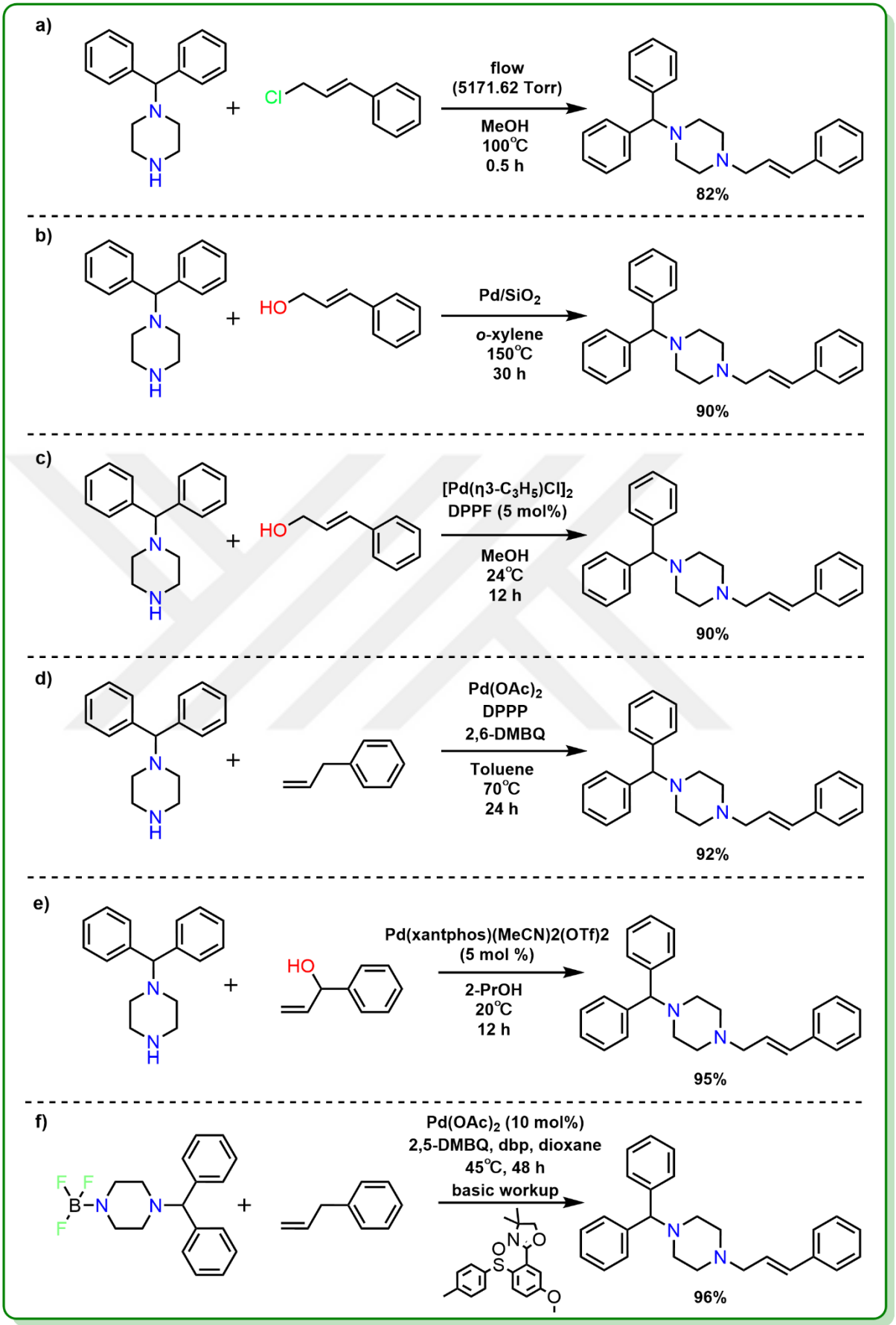
medium. The second method was the addition of cinnamyl chloride to 1-benzhydryl piperazine in the presence of potassium carbonate. **(Scheme 2)**



Scheme 2: Two different methods patented by Janssen Pharmaceutical Companies for cinnarizine synthesis.

A considerable amount of literature has been published on the synthesis of cinnarizine over the years **(Scheme 3)**. In 2016, a multi-step synthetic sequence was developed by Borukhova et al. in a flow reactor[48]. In the last step, they injected cinnamyl chloride into a stream of 1-(diphenylmethyl)piperazine and generated cinnarizine with 82% yield in 30 minutes at 100 °C under 5171.62 Torr pressure in the presence of methanol **(Scheme 3a)**. Another reported cinnarizine synthesis was carried out by Alshammari et al. through Pd/SiO_2 catalysis in the reaction of 1-(diphenylmethyl)piperazine with cinnamyl alcohol in the *o*-xylene at 150 °C for 30 hours to access 90% yield[49] **(Scheme 3b)**. Jing et al., on the other hand, used $[Pd(\eta^3\text{-allyl})Cl]_2$/DPPF catalytic system in the medium with the same starting materials, 1-(diphenylmethyl)piperazine and cinnamyl alcohol, and after 12 hours in room temperature succeeded 90% yield of cinnarizine[50] **(Scheme 3c)**. Additionally, Jin et al. proved that cinnarizine can be synthesized with a yield of 92% by conducting the amination of allylbenzene, with 1-(diphenylmethyl)piperazine in a reaction of Pd(OAc)(5.0 mol%), DPPP (10mol%)

and 2,6-DMBQ in toluene at 70 °C for 24 hours[51] **(Scheme 3d)**. In a recently reported synthesis, Wang et al. involved the addition of a palladium complex ligated with Xantphos as a catalyst in the reaction of 1-phenyl-2-propen-1-ol and 1-(diphenylmethyl)piperazine in isopropanol for 12 hours in the room temperature. In this reaction cinnarizine was obtained with 95% yield[52] **(Scheme 3e)**. In an elegant work reported by Ali et al., cinnarizine was synthesized through the reaction of cyclizine fragments and allylbenzene as a result of allylic C-H amination cross-coupling using AACC catalysis in toluene for 48 hours at 45 °C and after basic workup 96% of yield was achieved[53] **(Scheme 3f)**.



Scheme 3: Selected cinnarizine wet syntheses reported in literature.

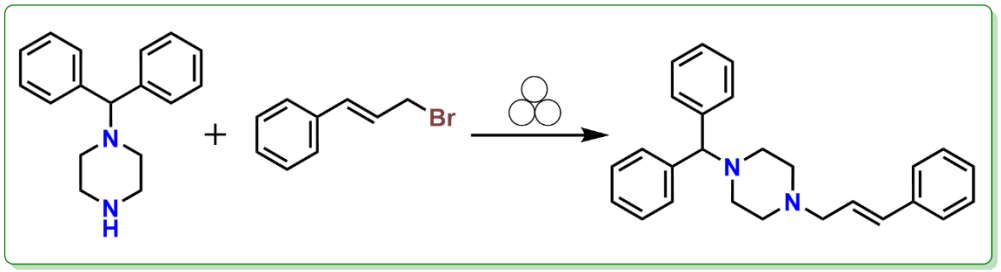
All of the above discussed methodologies have a similar common traditional way of using solvent, bases and/or catalysts at high temperatures for a long time. Furthermore, most of them required column chromatography, which causes lots of solvent consumption, for the isolation of the product.

1.5. Aim

As shown in the previous section, cryomilling can be a better synthetic alternative to conventional methods for some first-generation antihistamines in terms of reaction yields, reaction time, and synthetic greenness. The cryomilling method developed for the compounds listed above does not contain organic solvents or bases during the synthesis. Since it does not produce many side products, there is no large amount of solvent requirement in the work-up and purification of the APIs formed. Therefore, it is viable to proceed with the exploration of the method in preparation of other synthetic compound(s).

On the other hand, the reasons for the cryo method's higher efficiency are still not known. These unexpectedly high efficiencies (especially for DPH in **Table 1**) were attributed to lower the melting point of the reactants in this case. Under cryo conditions, the reactants change to the solid phase, and this change opens a new, solid-state reaction pathway towards the reaction completion, which cannot be attained at room temperature milling or conventional synthesis in a solvent. However, with the increasing number of examples, the reactants' melting point vs. reaction yield correlation seemed less relevant – yet this hypothesis needs more data to be significant. In summary, there is a need for more case studies with the cryo preparation of other compounds. It is necessary to try similar reactions, first, to understand the possibilities and limitations of the method and, second, to discover

(if there are any) differences between the synthetic pathways of (cryo)milling and the conventional method.



Scheme 4: The target reaction of this work – A milling reaction of cinnamyl bromide with diphenylmethyl piperazine yielding cinnarizine.

In this work, a cryomilling preparation method for cinnarizine is explored. A one-step reaction leading to the product is performed in a cryomill without using any solvents or bases. A simple yet effective approach is used to understand the cryomilling reaction of cinnarizine. In this approach, the reaction parameters are altered, and the changes in the yield are monitored. Cinnarizine was selected from the library of first-generation antihistamine APIs since it has a chemical structure similar to the previously prepared ('cryo efficient') diphenhydramine. Therefore, the cinnarizine preparation reaction may also help resolve the missing mechanistic puzzle from the previous reactions. A one-step reaction was searched from a library of available starting materials that resembled the previous reactions we successfully performed in the cryomill to obtain the other antihistamines. The reaction involving cinnamyl bromide with diphenylmethyl piperazine stood as the best choice since these reactants were commercially available, and the nucleophilic attack resembled the previous cases. On the other hand, in the previous case, the nucleophiles were attacking the carbon with two phenyl substituents (bromodiphenyl methane) in an S_N1 fashion, and in this time, the attack is on a 1^o cation, and the reaction may take

place with a more S_N2 character. Nevertheless, the commercial availability of this pair of reactants led us to choose them to act in the formation of our target compound. The following parts discuss the effectiveness of this change, presenting the yields obtained through it.

Finally, considering the global demand for antihistamines, the advantages of the proposed cryo mechanochemical route is discussed as a plausible alternative for synthesizing cinnarizine in a new and green way.

2. Experimental

2.1. Analytical Techniques

NMR Spectroscopy

All NMR spectra were recorded with Bruker Avance III (100 or 400 MHz) and processed with MestReNova. All the signal data were in δ values (ppm) and coupling constants (J) were in Hertz (Hz). The instrument was calibrated for both ^{1}H-NMR and ^{13}C-NMR using internal standard TMS (0.0 ppm) or residual deuterated solvent signal $CHCl_3$ in $CDCl_3$ (7.26 ppm) for the former, and $CDCl_3$ (77.16 ppm) for the latter. All measurements were done at room temperature. The following parameters and their abbreviations were used to report the NMR spectra: chemical shifts (ppm), integrations, multiplicities (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, dd = doublet of doublets, dt = doublet of triplet, m = multiplet, br = broad).

Mass Spectrometry

ESI Q-TOF LC/MS and APCI Q-TOF LC/MS: Samples were measured with an Agilent 6224 High Resolution Quadrupole Mass Time-of-Flight Liquid Chromatography/Mass Spectrometry equipped with a Multimode Ionization (MMI) source that has both Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI) modes. Sample solutions in methanol (MeOH) were introduced into the ion source through a syringe pump with a flow rate of 5 μL/min. Then, flow rate of solvent was adjusted to 0.5 μL/min. All the remaining parameters were set for maximum abundance of the relative $[M+H]^+$and $[M-H]^-$.

ATR-FTIR Spectroscopy

ATR-FTIR: Attenuated Total Reflection - Fourier Transform Infrared (ATR-FTIR) spectra were obtained by a Bruker Alpha II Platinum instrument.

2.2. Solvents and Other Chemicals

All the solvents for chromatography, extraction and filtration were obtained as puriss. p.a., ACS reagent and used without further distillation.

Starting materials 1-(diphenylmethyl)piperazine and cinnamyl bromide were supplied from Thermo Fischer Scientific and Sigma Aldrich, respectively. Reference cinnarizine was purchased from TCI Chemicals. All chemicals were used as received without further purification.

Thin Layer Chromatography (TLC) was performed on aluminum-backed plates pre-coated with silica gel (Merck Silica Gel PF-254) and silica gel (mesh size: 60-200) was used for column chromatography.

2.3. Cinnarizine Pestle and Mortar Reaction

The cinnarizine preparation starting with 1-(diphenylmethyl)piperazine and cinnamyl bromide was initially performed with a classical pestle and mortar system. An agate mortar was charged with 1-(diphenylmethyl)piperazine (1.0 mmol, 252.36 mg, 1.0 equiv.) and cinnamyl bromide (1.0 mmol, 252.36 mg, 1.0 equiv.). An agate pestle was used to hand mill the two reactants for 5 minutes with moderate pressing and continuous mixing with the pestle **(Figure 7)**.

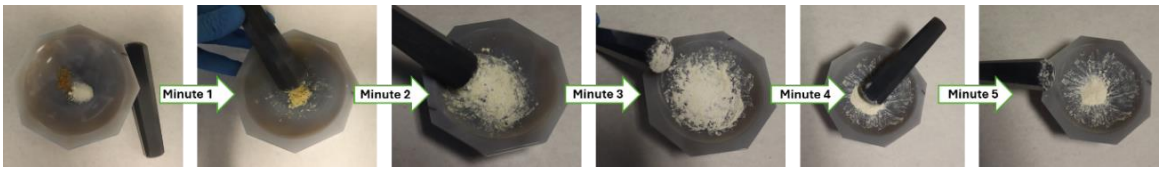


Figure 7: The images of the grinding experiment in chronological order

2.4. Cryomilling Procedures and Parameters

All mechanochemical reactions were conducted in a cryomill (Retsch). The mill was operated at room temperature and cryogenic temperature (-196 °C). The cryogenic temperature was attained for the whole duration of the reactions performed at this temperature by a cooling system that continuously cycles liquid nitrogen from a tank attached to the instrument **(Figure 8)**. A brief 5 minutes of cooling step was inserted before the milling step in the milling program to ensure the reactions start at the cryo temperature.

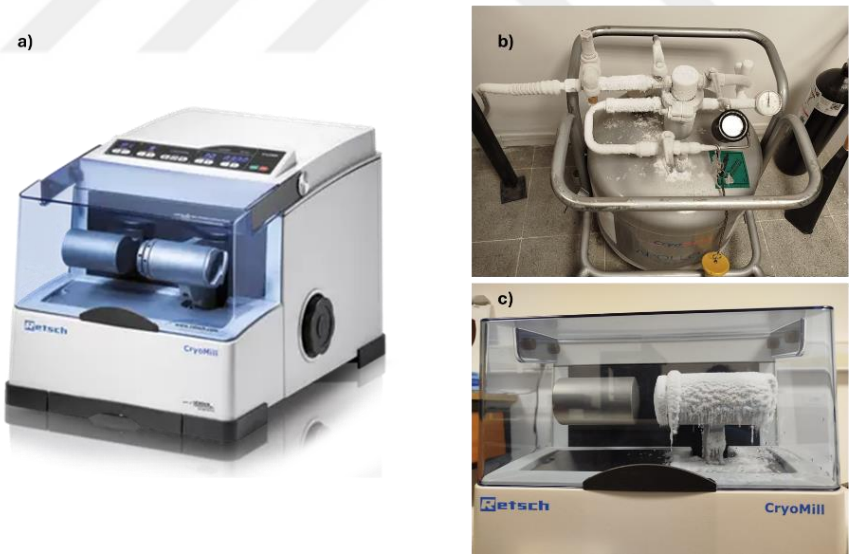


Figure 8 : The images of a) cryomill instrument (adapted from[54]), b) liq. N_2 tank connected to instrument, c) frozen jar holder with the jar inside.

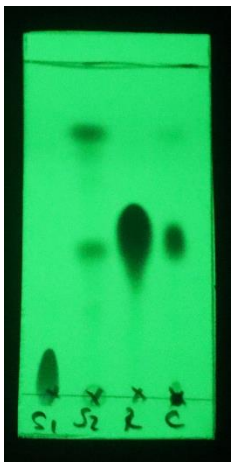
The millings were carried out in 25 mL zirconium dioxide (zirconia, ZrO_2) coated or stainless steel (SS) milling jars. The same material milling balls (6 balls, each 10.06 mm in diameter) in these jars were equipped for milling **(Figure 9)**.

Figure 9 : The image of zirconia and stainless-steel milling materials.

In a typical cryomilling procedure, 1-(diphenylmethyl)piperazine (1.0 mmol, 252.36 mg, 1.0 equiv.) and cinnamyl bromide (1.0 mmol, 252.36 mg, 1.0 equiv.) were ball milled for the given durations at 30 Hz in 25 mL zirconia or SS jar with 6 identical same-material balls at cryo temperature (77 K) or room temperature (298 K).

Once it's finished, the reaction progress was monitored by TLC, the retention factors were calculated to opt for an ideal eluent system for the separation.

TLC Visualization



Left Image: TLC of crude mixture of 20 min. of cryomilling under UV light with 254 nm.

Spots from left to right:
1st spot S1 (Starting Material 1): 1-(Diphenylmethyl)piperazine
2nd spot S2 (Starting Material 2): Cinnamyl Bromide
3rd spot R (Reference): Cinnarizine Commercial
4th spot C (Crude): Crude Mixture collected after milling

Mobile Phase: EtOAc:Hexanes (1:1) with 1.5% Et_3N, $Rf = 0.46$

The collection of the crude mixture from the milling media and after treatment

At the end of the given milling period, the milling jar was treated carefully to collect all powder materials. The yellowish-white fine powder mixture was collected by washing the jar and the balls with DCM (total volume 10 mL) with a glass pipette several times. The solvent was then evaporated in vacuum, and the obtained solid was subjected to ^{1}H-NMR analyses to estimate the yield (termed as '**NMR yield**') with the addition of 1,3,5-trimethoxybenzene as internal standard via peak integration.

The separation of the cinnarizine product was attained by column chromatography using the dry loading method and gradient elution (SiO_2; EtOAc:Hexanes with 1.5% Et_3N = 1:4 → 1:1 → DCM: 5 % MeOH with 1.5% Et_3N)

2.5. Cinnarizine Conventional Wet Synthesis Procedure

The procedure was adapted from a patent[55] in which 1-(diphenylmethyl)piperazine (1.0 mmol, 252.36 mg) was taken into a 50 mL oven-dried double neck round bottom flask, and 25 mL hexane was added into the flask. The solution was heated to 70 °C and stirred under reflux for 1.5 h. Anhydrous Na_2CO_3 (3.3 mmol, 353.29 mg) was added into the reaction medium, and after 15 minutes of stirring, cinnamyl bromide (1.0 mmol, 197.07 mg) was also put into the flask. The reaction mixture was stirred for 4.0 h at 70-75 °C. After TLC monitoring the reaction, suction filtration was applied to the mixture by washing the flask with 25 mL hexane. The filtrate was evaporated, and the obtained crude mixture was separated by column chromatography. Isolated cinnarizine was reported as 85 %.

The same procedure was also tried with 1.0 h of stirring of the reactants, and after column purification, the yield was reported as 32 %.

3. Results and Discussion

The mechanochemical milling for the preparation of cinnarizine was handled in 4 parts:

1) Preparation of cinnarizine at room temperature in a mortar/pestle system.

2) Preparation of cinnarizine via (cryo)milling and exploration of the effect of the milling parameters on the reaction yields.

3) Preparation of cinnarizine via (cryo)milling and exploration of the effect of the leaving group on the reaction yields. Involvement of a base in the milling reaction.

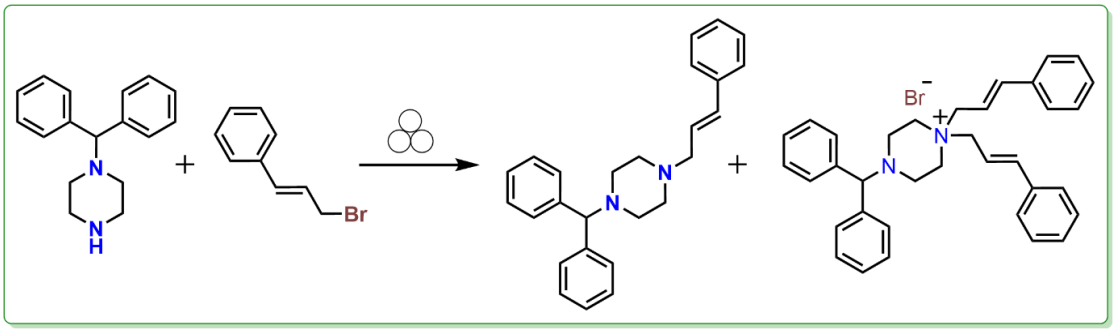
4) Preparation of the product via literature reported conventional method.

3.1. General method of the product purification, the product and side product identification, and the reaction yield definitions

Figure 10 : A typical view of the milling materials and the reaction mixture after milling. The mixture was collected carefully and completely from the jar and milling balls.

In all the above preparations 1-3, detailed below, the reaction mixture was purified to obtain the product after the milling. Although the same target product formed in all trials, the side product's presence and amount forced minor alterations in the purification method, such as the change in the chromatographic solvent system. However, the separation method remained the same: After milling, the first step was the collection of the powder product mixture from the milling material. TLC and a ^{1}H-NMR analysis were performed on the gathered crude powder at this stage. The peak integrals from the target cinnarizine were compared with those of the internal NMR standard, 1,3,5-trimethoxybenzene (see Figure 11 for the peak assignment of cinnarizine). The yield calculated (see Appendix D for details of the calculation), prior to any further purification, is termed '**NMR yield**'. On the other hand, the yield of the final purified material obtained is called '**isolated yield**'.

It should be emphasized that the isolated yields reported in this work can be improved by further optimization or a total alteration of the purification procedure, for which we tried a previously reported crystallization[55]. Nevertheless, only a rough comparison of the yields in the different trials were needed in this study, which were attained successfully as displayed in the following sections.



Scheme 5: The reaction leading to desired main product, cinnarizine, and undesired side product, 4-(diphenylmethyl)-1,1-bis[(E)-3-phenyl-prop-2-enyl]piperazine bromide (BIS).

The TLC and column chromatography of the crude mixture revealed the formation of a single side product together with cinnarizine. It is later identified by ^{1}H-NMR and mass spectrometry as 4-(diphenylmethyl)-1,1-bis[(*E*)-3-phenyl-prop-2-enyl]piperazine bromide (Scheme 5), which will be abbreviated as '**BIS**' in the text after this point. BIS has an extra cinnamyl 'arm' in its structure, due to a reaction of the target cinnarizine with cinnamyl bromide following the formation of cinnarizine. The two compounds, cinnarizine and BIS, can be differentiated by their characteristic NMR signals and their integrations (**Figure 11)**.

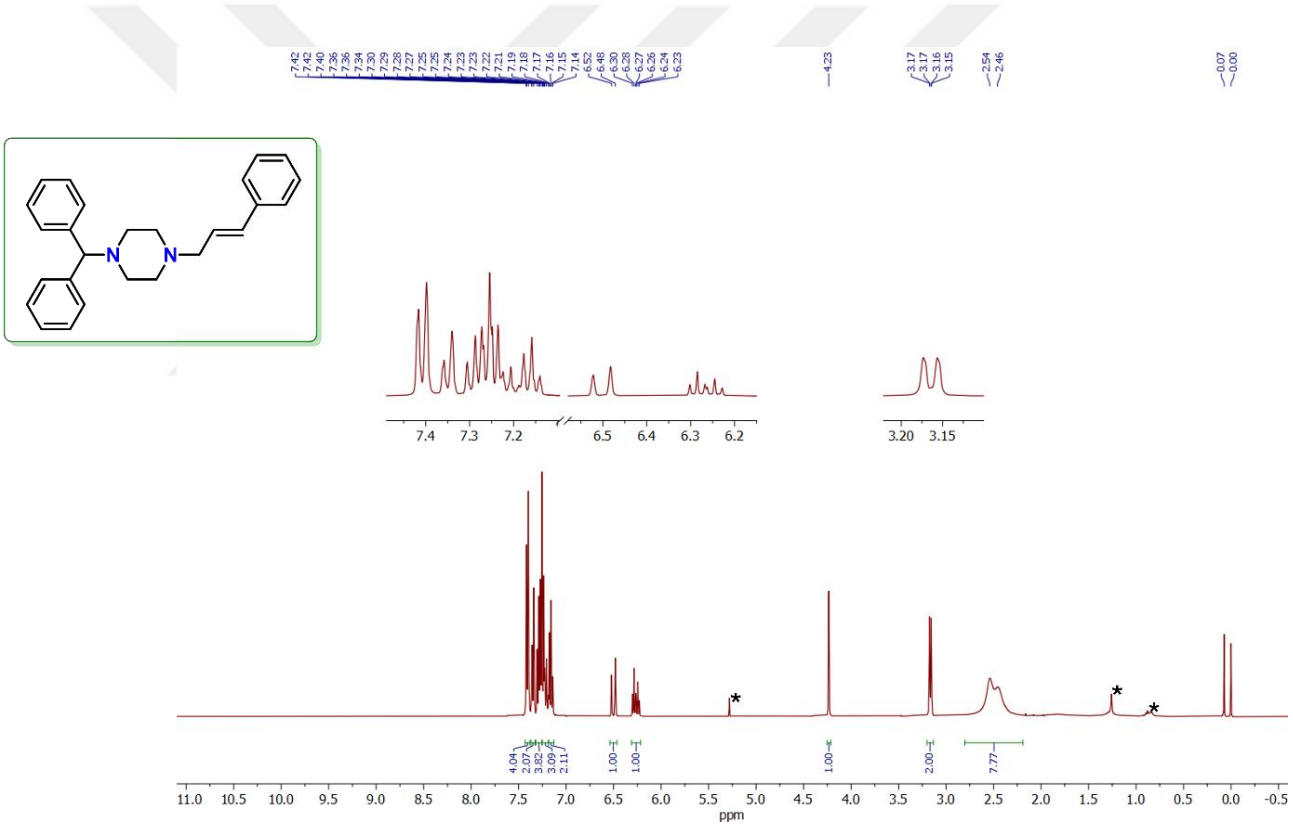
Cinnarizine has 4.23 ppm singlet and 2.54 ppm broad singlet signals for protons of the methylene group on the diphenylmethyl piperazine fragment and of the piperazine fragment, respectively, with an integration ratio of 1:8. BIS has two different broad singlets for the same protons, at 4.59 ppm and 3.52 ppm - 2.76 ppm, respectively, with a ratio of 1:8. Broad signals for the piperazine protons were expected since cyclic piperazine has no fixed chair conformation due to ring flips of axial and equatorial groups.

Additionally, cinnarizine has a doublet at 6.50 ppm , a doublet of triplet at 6.26 ppm (J=15.8 Hz) which are the signals for protons on the alkene group of cinnamyl fragment, and a doublet of doublet at 3.16 ppm (J= 6.8 Hz) belongs to the proton adjacent to alkene group, with a ratio of 1:1:2. BIS has these characteristic signals at 7.03 ppm, 6.55 ppm and 4.25 ppm for the same protons, respectively, with a ratio of 2:2:4 (**Figure 11)**. Examining the proton ratios of both product and side product for this region, integrations of BIS are two times that the integrations of cinnarizine. This is expected since BIS has two cinnamyl "arm"s while cinnarizine has only one. Furthermore, the shift towards the low field for the BIS signals can be explained by

the fact that BIS is in its salt form, and nitrogen, which is bonded to the cinnamyl "arm"s on the piperazine group, is positively charged.

Lastly, the integration of the aromatic region protons verified the expected number of protons stemming from the phenyl groups for both cinnarizine and BIS and these values were used to determine the formation and quantification of the BIS side product in the crude mixture.

The formation of BIS reduces the yield of the cinnarizine obtained, obviously, since the formation of BIS consumes the previously formed cinnarizine.



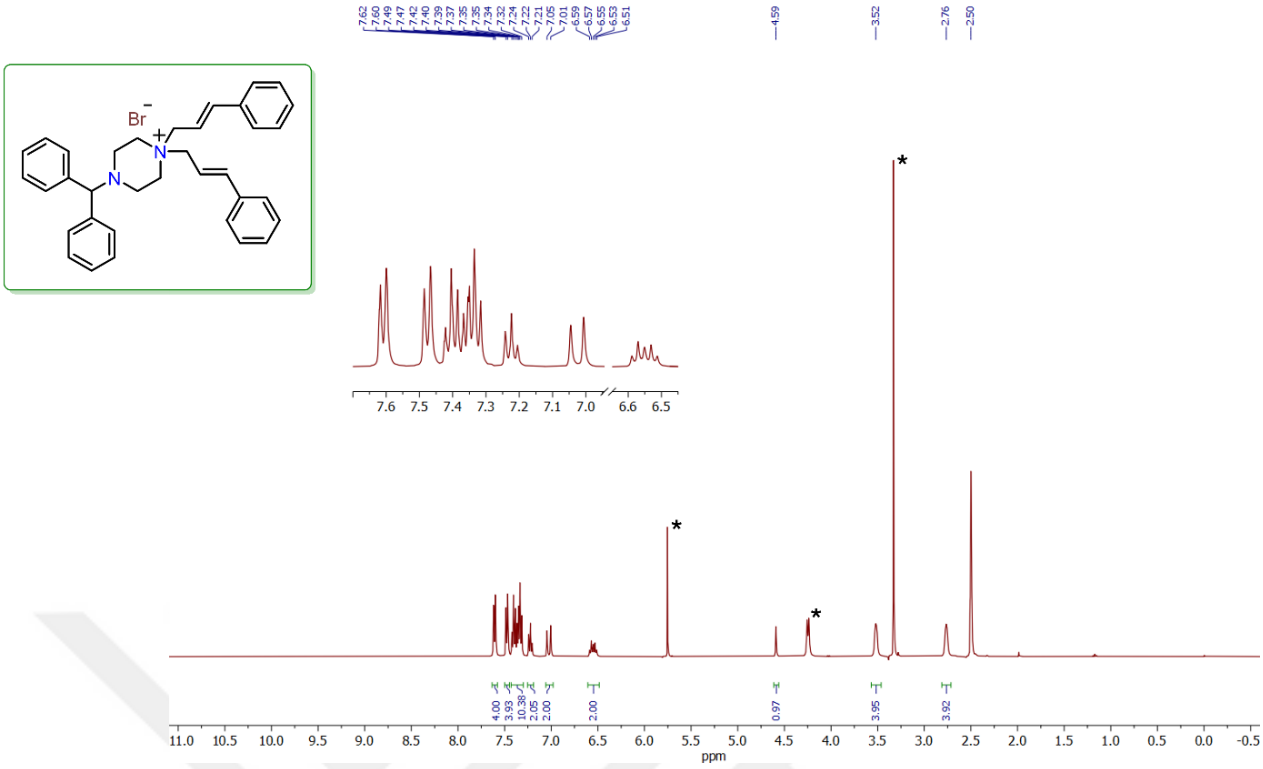


Figure 11 : Differentiation of the target product cinnarizine from side product BIS using their ^{1}H NMR spectra. Solvent peaks such as DCM, EtoAc, Hexane, and H_2O were indicated with the * symbol.

3.2. Preparation of cinnarizine at room temperature in a mortar/pestle system

As mentioned above in the experimental section, an agate mortar/pestle was used to hand mill the two reactants for the given periods with moderate pressing and continuous mixing with the pestle. Even with this simple method, the choice of the milling material can be critical in some reactions. An agate mortar and pestle were employed in this work. Agate is a natural mineral known for its hardness (Mohs scale 6.5-7.0) and resistance to chemical attack. The polished surfaces of agate are ideal for retrieving the formed product and cleaning the milling equipment. However, the most crucial feature of agate in this work is that it is a

milling medium in which no side reactions can be initiated by an electron transfer to or from it, i.e., the ones that could be suspected when a metal mortar was used. Finally, this material does not cause static charging when easily charged powdery reactants are used, such as plastic mortars.

In a typical reaction with a mortar pestle system, the powder reactants (an equimolar ratio of a total of 449.43 mg) were mixed and pressed against each other manually in consecutive cycles for 5 min. This sort of preparation is far from attaining control of mixing and milling parameters such as the extent, homogeneity, and force of mixing. Nevertheless, the mortar pestle system has proven successful in providing some initial results implying whether a reaction is likely to occur at ambient conditions. In this case, the initial trials with a mortar provided that cinnarizine was formed with this method according to the ^{1}H-NMR spectrum of crude mixture **(Figure 12)**. This is an indication that a (cryo)milling reaction has a promising feasibility, and the product can be separated from the milling material.

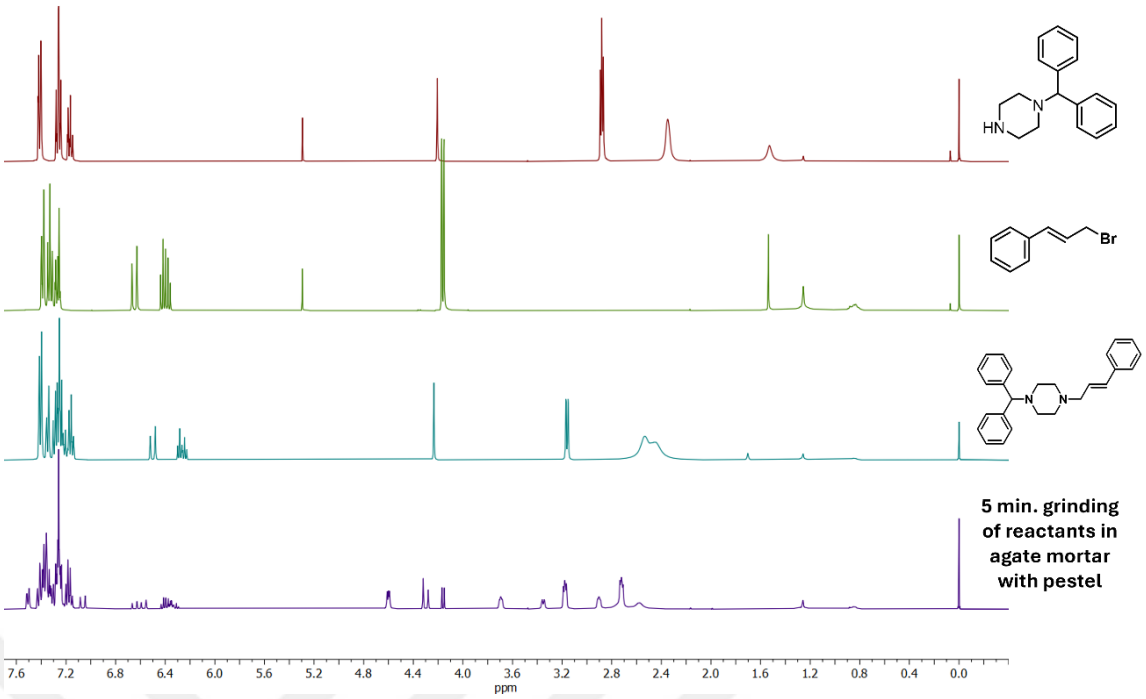


Figure 12: ^{1}H-NMR spectra of commercial reactants 1-(diphenylmethyl) piperazine, cinnamyl chloride and commercial cinnarizine as a reference respectively and H-NMR spectrum of crude mixture after 5 min. of grinding and crushing of reactants in an agate mortar with an agate pestle.

3.3. Preparation of cinnarizine via (cryo)milling and exploration of the effect of the milling parameters on the reaction yield

In an identical manner to the mortar pestle system, an equimolar mixture (the ideal stoichiometric ratio of the reactants), which is of a total of 450 mg of the reactants 1-(diphenylmethyl) piperazine and cinnamyl bromide was charged in the jar of the milling apparatus. In addition to the conventional synthetic parameters such as the duration of the reaction and the temperature, the milling material and frequency of milling should be considered in milling reactions. Finally, the total

amount of material charged in the jar is also another parameter – which we optimized for our 25 mL jars and 6 milling balls as ca. 500 mg for previous similar reactions.

In **Table 2**, the parameters used in the mortar/pestle reaction (entry 0) and the milling experiments, entry 1-12, and the parameter values are displayed.

Table 2: Different reaction conditions[a] and corresponding yields for (cryo)milling preparation of cinnarizine with the target method. [a]Reaction conditions: 1.0 mmol (252.36 mg, 1.0 equivalent) of 1-(diphenylmethyl) piperazine and 1.0 mmol (197.07 mg, 1.0 equivalent) of cinnamyl bromide were grinded with pestle and mortar (Entry 0) or ball-milled at 30 Hz in a zirconium oxide coated or stainless-steel jar with 6 identical same material balls (Entries 1-12) for the stated duration at the given temperature. [b]NMR yield: Refers to the approximate product yield in the crude mixture obtained through calculations from the signals of the NMR internal standard. [c]Isolated Yield: Refers to the isolated product yield obtained after the work-up of crude.

Entry	Milling Material	Milling frequency (Hz)	Duration (min)	T (K)	NMR Yield (%)[b] Cinnarizine	Isolated Yield (%)[c] Cinnarizine	NMR Yield (%)[b] BIS	Isolated Yield (%)[c] BIS
0	Mortar& Pestle	-	5 min	298	23.2%	-	69.6%	-
1	ZrO_2	30 Hz	1 min	298	21.4%	25.6%	64.3%	14.0%
2	ZrO_2	30 Hz	10 min	298	31.8%	27.5%	38.3%	17.8%
3	ZrO_2	30 Hz	20 min	298	21.7%	27.9%	49.6%	29.7%
4	ZrO_2	30 Hz	30 min	298	25.9%	27.3%	48.3%	23.2%
5	ZrO_2	30 Hz	60 min	298	25.3%	26.6%	51.0%	24.0%
6	ZrO_2	30 Hz	1 min	77	27.6%	23.2%	51.0%	28.9%
7	ZrO_2	30 Hz	10 min	77	36.8%	25.4%	46.1%	10.7%
8	ZrO_2	30 Hz	20 min	77	30.7%	29.3%	44.6%	24.8%
9	ZrO_2	30 Hz	30 min	77	30.4%	25.6%	49.8%	22.4%
10	ZrO_2	30 Hz	60 min	77	18.7%	23.5%	50.1%	25.0%
11	SS	30 Hz	20 min	298	25.53%	20.9%	62.5%	30.6%
12	SS	30 Hz	20 min	77	26.3%	22.9%	56.4%	13.4%
13 (base trial)	ZrO_2	30 Hz	20 min	77	46.9%	45.5%	27.2%	10.6%

Milling material

For the identical reasons discussed for using agate mortar (an inorganic material) in the previous section, using of a stainless steel (SS) jar for this reaction was initially hesitated. The -unknown, if any- intermediates, transition states could be prone to an electron transfer to or from the metal milling material. Therefore, the experiments focused using a zirconia jar and ball set. A few trials (Entries **11** and **12**) with SS jar showed no significant change in the NMR yield with this material. However, since the SS balls are heavier (4.04 g vs 2.17 g) than the ZrO_2 balls, the impact momentum should be higher in the reaction and that can be the reason for the higher NMR yield of BIS in the reactions with SS balls.

Milling frequency and duration

Based on the previous experience with the preparations of similar organic compounds in our research group, the milling frequency was kept at 30 Hz for all entries, **1-13**. The milling time, previously found as a more significant parameter, was varied between 1-60 min in the experiments. Both at room temperature and under cryo conditions, higher NMR yields are obtained when the milling duration increases from 1 to 10 minutes, as can be expected from a higher number of collisions of reactants in the jar. On the other hand, a decrease in the reaction yield was observed when prolonged durations, 30 min or 60 mins were used, presumably because of the side reaction yielding the BIS product, which consumes the formed cinnarizine. Here, it is noteworthy that even milling for 1 min yields ca. 25 % cinnarizine at the given temperatures – which can never be the case for any conventional reaction (Also, see in the following text, in section **3.4.**, a 'conventional' trial we report for a comparison).

Milling temperature

As we introduced in the **Aim** part of this thesis, the most exciting-to-monitor parameter for this work is the milling temperature. Previously, it was observed that the effect of milling temperature was more pronounced when the melting points of the reactants were low. The effect was the most dominant (higher yields at cryo temperature) for the liquid reactants at room temperature. However, the number of trials was not statistically significant to conclude a theory in general. Here, our reactants diphenylmethyl piperazine (m.p. 90-93 °C) and cinnamyl bromide (26-29°C) are solids at room temperature, although the latter one still melts easily at slightly higher ambient temperatures. Therefore, if the 'melting point hypothesis' is followed, it can be expected that this reaction might be slightly affected by a decrease in temperature, in the direction of higher yields. When the NMR yields reported in **Table 2** are compared for entries **1-5** (room temperature milling) and **6-10** (cryomilling), it is observed that this is the case. The milling produced 20-25% product at room temperature vs. 27-30% at the cryo temperature. Of course, it should be added that the side reaction consuming cinnarizine may also be affected by the change in temperature. It can be reported that the yield of BIS formation is less affected by the change in the milling temperature. Finally, it is worth stressing that the highest yield (36.8%) of cinnarizine was obtained via cryomilling for 10 minutes.

3.4. Preparation of cinnarizine via (cryo)milling and the exploration of the effect of different leaving group on the reaction yields. Involvement of a base in the milling reaction

The change of the leaving group when the identical milling conditions to entry **8** was used with a change in the reactant from cinnamyl bromide to cinnamyl chloride, the milling reaction did not yield any product as verified by ^{1}H-NMR results **(Figure 13)**. Considering the given reaction works (conventionally) via an S_N2 mechanism, in which the nucleophile attacks the carbon adjacent to the leaving group and the halogen leaves in a concerted fashion, it can be expected that using a bromide reactant (with a better leaving group) is a better choice for a faster reaction. This significant difference in the reaction yield with a change of the leaving group also signifies that the reaction presumably proceeds via S_N2 pathway, also in the mechanochemical preparation.

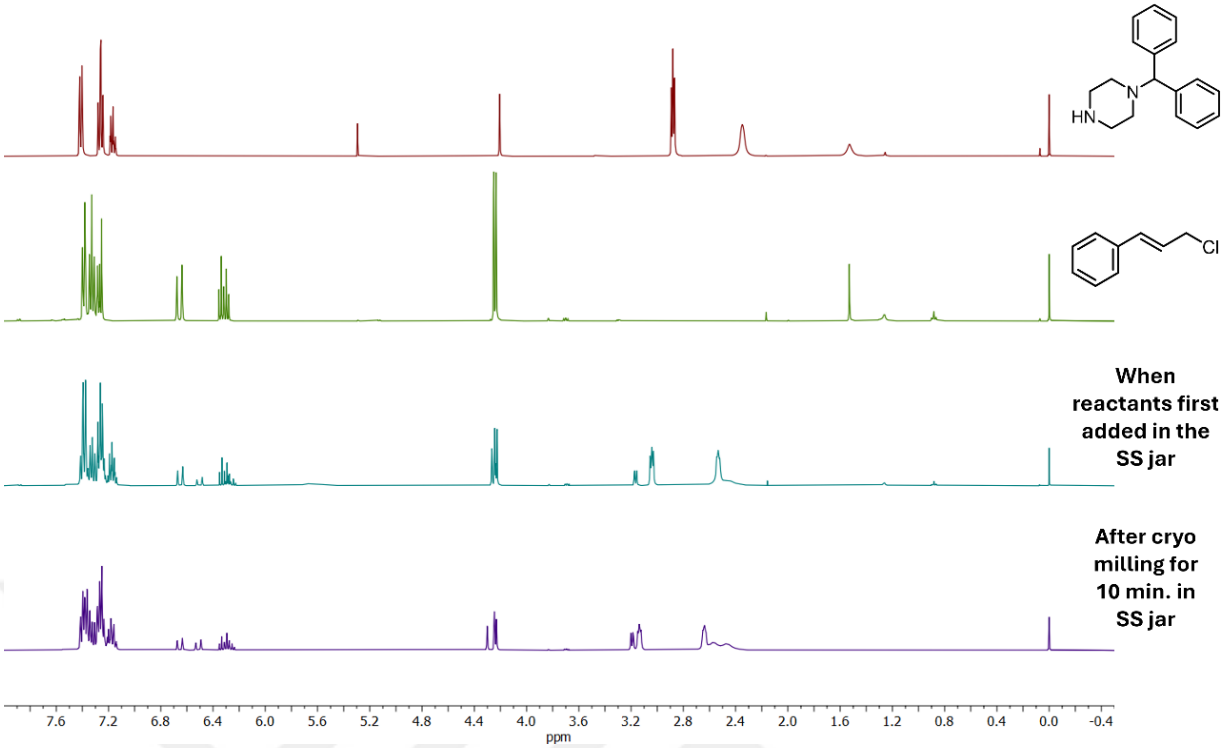


Figure 13 : ^{1}H-NMR spectra of commercial reactants 1-(diphenylmethyl) piperazine and cinnamyl chloride and their mixture when they were first charged into the SS jar for the milling compared with the spectrum of the mix after cryomilling for 10 minutes.

The addition of a base

The typical conventional methods use a base to keep the (amine) nucleophile deprotonated through the reaction. In the first attempts to perform a nucleophilic reaction in our group (in the synthesis of previous APIs), the base was forgotten. Nevertheless, the later trials with an addition of base under the same conditions yielded almost the same amount of material. In mechanochemical green methods, it is important to avoid any additional chemical if a higher green-value of the method is desired. Therefore, this serendipitous observation led to the subsequent reactions to be performed despite of a lack of a base. On the other hand, to compare

our mechanochemical method with the conventional one and to assess the effect of the presence of the base in this solid-state mechanochemical reaction, experiments were added to **Table 2** as entry **13**. The results showed that the addition of an equimolar amount of anhydrous sodium carbonate (Na2CO3) base into the jar with the reactants yielded 45.5 % isolated cinnarizine which was one and a half times more than the yield obtained with the no-base trial **(Table 2** entry **8)**.

3.5. Preparation of the product via literature reported conventional method

As shown above, in the results of the milling experiments, the target compound cinnarizine can be obtained in moderate yields in less than 10 minutes using the milling apparatus. The studies on the conventional synthesis of cinnarizine starting with the same reactants report 4 hours of refluxing at 75 °C for a complete reaction, resulting in ca. 90% yield[55].

These rare reports need to be tested to better compare the conventional and the mechanochemical methods in terms of yield, duration, and greenness. Therefore, we performed a conventional synthetic preparation, refluxing the reactants in 25 mL hexane, for 4 hours, followed by the same identification and purification steps for cinnarizine. As a result, we determined that cinnarizine can be produced with 85.3% yield under these conditions. On the other hand, the same procedure was also applied with only stirring for 1 h and after purification a yield of 31.8% was recorded. Remembering that even a 1-minute ball milling (Entries **1** and **6**) can also yield a similar result, the mechanochemical method stands out as a viable alternative to the conventional one.

A note on the comparison of mechano vs. conventional synthesis.

Although a rough comparison can be made (as was made in the previous paragraph) between the yields of conventional synthesis and the mechanochemical milling preparations, many other factors must be considered for the 'best' method. The first advantage of using the above-displayed milling method for the preparation of cinnarizine is the absence of any toxic solvents, which are needed to mix and reflux the reactants in conventional synthesis. Also, no base was used in the above-given method, which is another plus on the side of the new method. The duration of the synthesis, together with the much-reduced energy consumption, adds to the benefits of the milling. On the other hand, the purification step, identical to both methods, is unavoidable also for the studied mechanochemical method leading to cinnarizine.

4. Conclusion

In this work, a new one-step mechanochemical reaction of cinnamyl bromide with diphenylmethyl piperazine yielding cinnarizine was explored. The previous studies in our group implied that the (cryo)milling could be a better synthetic alternative to conventional methods for some first-generation antihistamines in terms of reaction yields, reaction time, and synthetic greenness. The milling does not require any solvents and may run without a base to yield the product in moderate to excellent yields. Here, we explored the effect of milling conditions on the yield of the obtained cinnarizine. Although there wasn't a single most effective parameter to report, the most important factors affecting the reaction yield were reported as the milling duration and the temperature. Using stainless-steel milling material instead of ZrO_2 enhanced the yield of the side product, too. Other than these milling parameters, the reaction conditions, such as adding the base of change of the leaving group in the cinnamyl halides, were found to be more effective in the direction expected from a conventional wet synthesis running with an S_N2 mechanism. As there was only a slight difference between the yields of cryo and temperature milling, it is hard to conclude whether the reaction is affected by a phase change in the reactant material. However, the competing rates of cryomilling imply that a solid-state pathway is still producing the cinnarizine in moderate yields without a base.

The separation of the product turned more cumbersome than is reported in the literature. Since the isolated yield was not consistent, the completion of the reaction was followed by NMR yields, the values of which seemed more reliable. As a next step, the separation procedure will be improved, and a better separation of the product and report of the yields will be provided. Also, the stoichiometric ratio of the base

will be explored together with the initial reactant mixture with the lower stoichiometric ratios of the cinnamyl bromide. The proposed changes will help reduce the side product's yield and increase the cinnarizine yield.

In summary, it is viable to proceed with the exploration of the (cryo)milling method in preparation of other synthetic compounds. With every new example, some mysteries are unveiled, yet others appear to be explored.

Finally, considering the global demand for antihistamines, the proposed cryo mechanochemical route's advantages stand as a reasonable alternative for synthesizing cinnarizine in a new and green way – if and once the procedure can be optimized and suited for upscale synthesis.

5. References

[1] "Report on the Environment (ROE) | US EPA," can be found under https://cfpub.epa.gov/roe/indicator.cfm?i=54, n.d.

[2] P. T. Anastas, J. C. Warner, Green Chemistry: Theory and Practice, Oxford University Press, New York 1998.

[3] K. Horie, M. Barón, R. B. Fox, J. He, M. Hess, J. Kahovec, T. Kitayama, P. Kubisa, E. Maréchal, W. Mormann, R. F. T. Stepto, D. Tabak, J. Vohlídal, E. S. Wilks, W. J. Work, Pure and Applied Chemistry 2004, 76, DOI 10.1351/pac200476040889.

[4] J. Andersen, J. Mack, Green Chemistry 2018, 20, DOI 10.1039/c7gc03797j.

[5] T. Friščić, C. Mottillo, H. M. Titi, Angewandte Chemie - International Edition 2020, 59, DOI 10.1002/anie.201906755.

[6] G. W. Wang, Chem Soc Rev 2013, 42, DOI 10.1039/c3cs35526h.

[7] D. Tan, T. Friščić, European J Org Chem 2018, 2018, DOI 10.1002/ejoc.201700961.

[8] D. E. Crawford, J. Casaban, Advanced Materials 2016, 28, DOI 10.1002/adma.201505352.

[9] E. Colacino, V. Isoni, D. Crawford, F. García, Trends Chem 2021, 3, 335–339.

[10] J. L. Do, T. Friščić, ACS Cent Sci 2017, 3, DOI 10.1021/acscentsci.6b00277.

[11] J. Batteas, K. G. Blank, E. Colacino, F. Emmerling, T. Friščí, F. F. Friščí, J. Mack, J. Moore, M. E. Rivas, W. Tysoe, RSC Mechanochemistry 2025, 2, 10–19.

[12] Ö. Laçin, J. Kwiczak-Yiğitbaşı, M. Erkan, Ş. C. Cevher, B. Baytekin, Polym Degrad Stab 2019, 168, DOI 10.1016/j.polymdegradstab.2019.108945.

[13] J. Kwiczak-Yiğitbaşi, Ö. Laçin, M. Demir, R. E. Ahan, U. Ö. Ş. Şeker, B. Baytekin, Green Chemistry 2020, 22, DOI 10.1039/c9gc02781e.

[14] J. Kwiczak-Yiğitbaşı, M. Demir, R. Erdem Ahan, S. Canlı, U. Özgür Şafak Şeker, B. Baytekin, ACS Sustainable Chemistry & Engineering 2020, 8, 18879–18888.

[15] A. Delori, T. Frišić, W. Jones, CrystEngComm 2012, 14, DOI 10.1039/c2ce06582g.

[16] E. Colacino, A. Porcheddu, C. Charnay, F. Delogu, React Chem Eng 2019, 4, DOI 10.1039/c9re00069k.

[17] D. Tan, L. Loots, T. Friščić, Chemical Communications 2016, 52, DOI 10.1039/c6cc02015a.

[18] E. Colacino, A. Porcheddu, I. Halasz, C. Charnay, F. Delogu, R. Guerra, J. Fullenwarth, Green Chemistry 2018, 20, DOI 10.1039/c8gc01345d.

[19] V. André, A. Hardeman, I. Halasz, R. S. Stein, G. J. Jackson, D. G. Reid, M. J. Duer, C. Curfs, M. T. Duarte, T. Friščić, Angewandte Chemie 2011, 123, DOI 10.1002/ange.201103171.

[20] T. X. Métro, J. Bonnamour, T. Reidon, J. Sarpoulet, J. Martinez, F. Lamaty, Chemical Communications 2012, 48, DOI 10.1039/c2cc36352f.

[21] L. Konnert, B. Reneaud, R. M. De Figueiredo, J. M. Campagne, F. Lamaty, J. Martinez, E. Colacino, Journal of Organic Chemistry 2014, 79, DOI 10.1021/jo5017629.

[22] J. Yu, Z. Hong, X. Yang, Y. Jiang, Z. Jiang, W. Su, Beilstein Journal of Organic Chemistry 2018, 14, DOI 10.3762/bjoc.14.66.

[23] J. Bonnamour, T. X. Métro, J. Martinez, F. Lamaty, Green Chemistry 2013, 15, DOI 10.1039/c3gc40302e.

[24] T. Yao, J. Yan, Y. Li, J. Wang, M. Qiao, X. Hu, X. Shi, S. Cao, F. Qiu, RSC Adv 2020, 10, DOI 10.1039/d0ra06763f.

[25] C. Mastrorilli, D. Posa, F. Cipriani, C. Caffarelli, Pediatric Allergy and Immunology 2016, 27, DOI 10.1111/pai.12681.

[26] L. B. Grabenhenrich, T. Keil, A. Reich, H. Gough, J. Beschorner, U. Hoffmann, C. P. Bauer, J. Forster, A. Schuster, D. Schramm, O. Nitsche, F. Zepp, Y. A. Lee, R. Bergmann, K. Bergmann, U. Wahn, S. Lau, Journal of Allergy and Clinical Immunology 2015, 136, DOI 10.1016/j.jaci.2015.03.040.

[27] F. E. R. Simons, in Journal of Allergy and Clinical Immunology, 2003.

[28] A. M. Mahdy, N. R. Webster, Anaesthesia & Intensive Care Medicine 2011, 12, 324– 329.

[29] R. Luisa, F. Fabiana, S. Isola, International Journal of Allergy Medications 2019, 5, DOI 10.23937/2572-3308.1510037.

[30] L. K. Golightly, L. S. Greos, Drugs 2005, 65, DOI 10.2165/00003495-200565030- 00004.

[31] "DOZNTM Quantitative Green Chemistry Evaluator," can be found under https://www.sigmaaldrich.com/TR/en/services/software-and-digital-platforms/dozn-tool?srsltid=AfmBOoo3QOOhcwbL1aU0LIsbRoGlZrk1szxQJ1MU6mMh6WJD7gY Mz_3z, n.d.

[32] "CN 104892435 A - Preparation method of diphenhydramine | The Lens," can be found under https://www.lens.org/lens/patent/161-790-770-324-405/frontpage?l=en, n.d.

[33] J. Wang, W. Wang, X. Yang, J. Liu, H. Huang, M. Chang, Sci China Chem 2023, 66, 518–525.

[34] "US 2751388 A - Process for preparing benzhydryl ethers | The Lens," can be found underhttps://www.lens.org/lens/patent/190-656-989-477566/frontpage?l=en, n.d.

[35] G. Towse, J Laryngol Otol 1980, 94, 1009–1015.

[36] C. P. Pianese, L. O. V. Hidalgo, R. H. González, C. E. Madrid, J. E. C. Ponce, A. M. Ramírez, L. M. Morán, J. E. P. Arenas, A. T. Y. G. Rubio, J. O. Uribe, J. Abiuso, E. Hanuch, J. Alegría, C. Volpi, R. Flaskamp, A. P. Sanjuán, J. M. G. Gómez, J. Hernández, A. Pedraza, D. Quijano, C. Martínez, J. R. Castañeda, O. J. C. Guerra, G. V. F, Otology and Neurotology 2002, 23, DOI 10.1097/00129492-200205000- 00023.

[37] D. Schremmer, I. Bognar-Steinberg, W. Baumann, J. Pytel, Clin Drug Investig 1999, 18, DOI 10.2165/00044011-199918050-00003.

[38] C. H. Wilder-Smith, J. Schimke, B. Osterwalder, H. J. Senn, Acta Oncol (Madr) 1991, 30, 731–734.

[39] C. P. Alcañiz Escandell, J. A. Román Ivorra, Drugs Context 2019, 8, 2019-9–4.

[40] D. G. Herron, American Journal of Nursing 2010, 110, 49–51.

[41] J. Hargreaves, Practitioner 1981, 226.

[42] Oxford English Dictionary 2023, DOI 10.1093/OED/7338125647.

[43] B. J. W. Pingree, R. J. Pethybridge, Pharmaceut Med 1989, 4.

[44] "Side effects of cinnarizine - NHS," can be found under https://www.nhs.uk/medicines/cinnarizine/side-effects-of-cinnarizine/, n.d.

[45] H. A. G. Teive, A. R. Troiano, F. M. B. Germiniani, L. C. Werneck, Parkinsonism Relat Disord 2004, 10, DOI 10.1016/j.parkreldis.2003.12.004.

[46] "The Merck index : an encyclopedia of chemical and drugs," can be found under https://librarycatalog.bilkent.edu.tr/client/en_GB/default/search/detailnonmodal/ent:$002f$002fSD_ILS$002f0$002fSD_ILS:141274/ada?qu=merck+index&lm=UNIVERSITY, n.d.

[47] "US 2882271 A - | The Lens," can be found under https://www.lens.org/lens/patent/065-569-784-217-659/frontpage?l=en, n.d.

[48] S. Borukhova, T. Noël, V. Hessel, ChemSusChem 2016, 9, 67–74.

[49] A. S. Alshammari, K. Natte, N. V. Kalevaru, A. Bagabas, R. V. Jagadeesh, J Catal 2020, 382, 141–149.

[50] J. Jing, X. Huo, J. Shen, J. Fu, Q. Meng, W. Zhang, Chemical Communications 2017, 53, 5151–5154.

[51] Y. Jin, Y. Jing, C. Li, M. Li, W. Wu, Z. Ke, H. Jiang, Nature Chemistry 2022 14:10 2022, 14, 1118–1125.

[52] M. Wang, Y. Xie, J. Li, H. Huang, Synlett 2014, 25, 2781–2786.

[53] S. Z. Ali, B. G. Budaitis, D. F. A. Fontaine, A. L. Pace, J. A. Garwin, M. C. White, Science (1979) 2022, 376, 276–283.

[54] "Cryomill - the perfect mill for cryogenic grinding - RETSCH," can be found under https://www.retsch.com/products/milling/ball-mills/mixer-mill-cryomill/, n.d.

[55] "CN 103254152 A - New synthesis method of cinnarizine | The Lens," can be found under https://www.lens.org/lens/patent/029-390-636-123110/frontpage?l=en, n.d.

6. Appendices

6.1. Appendix A: 1H-NMR and 13C-NMR Spectra

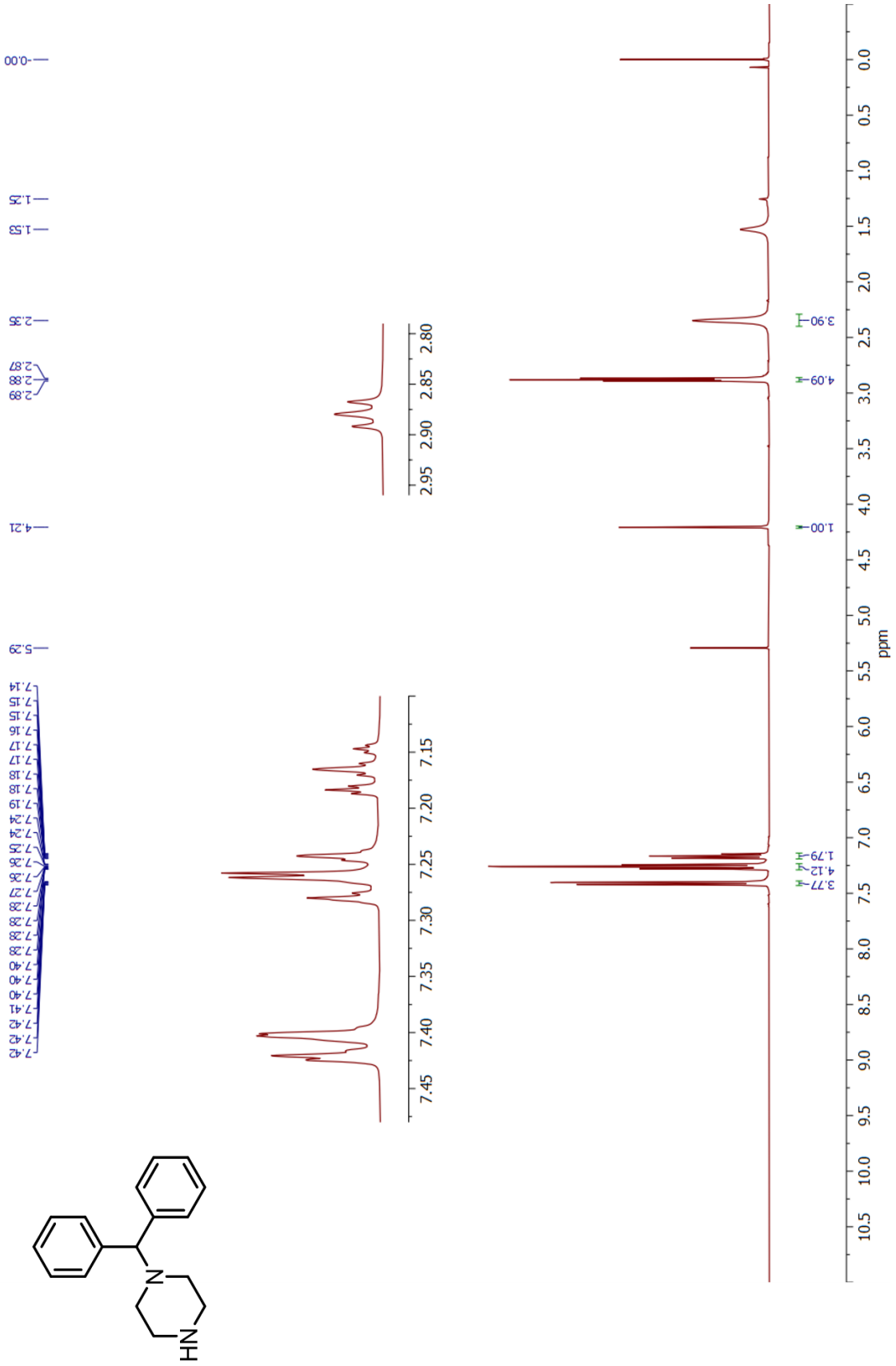


Figure 14: ^{1}H-NMR spectrum of commercial starting material 1-(diphenylmethyl)piperazine in $CDCl_3$

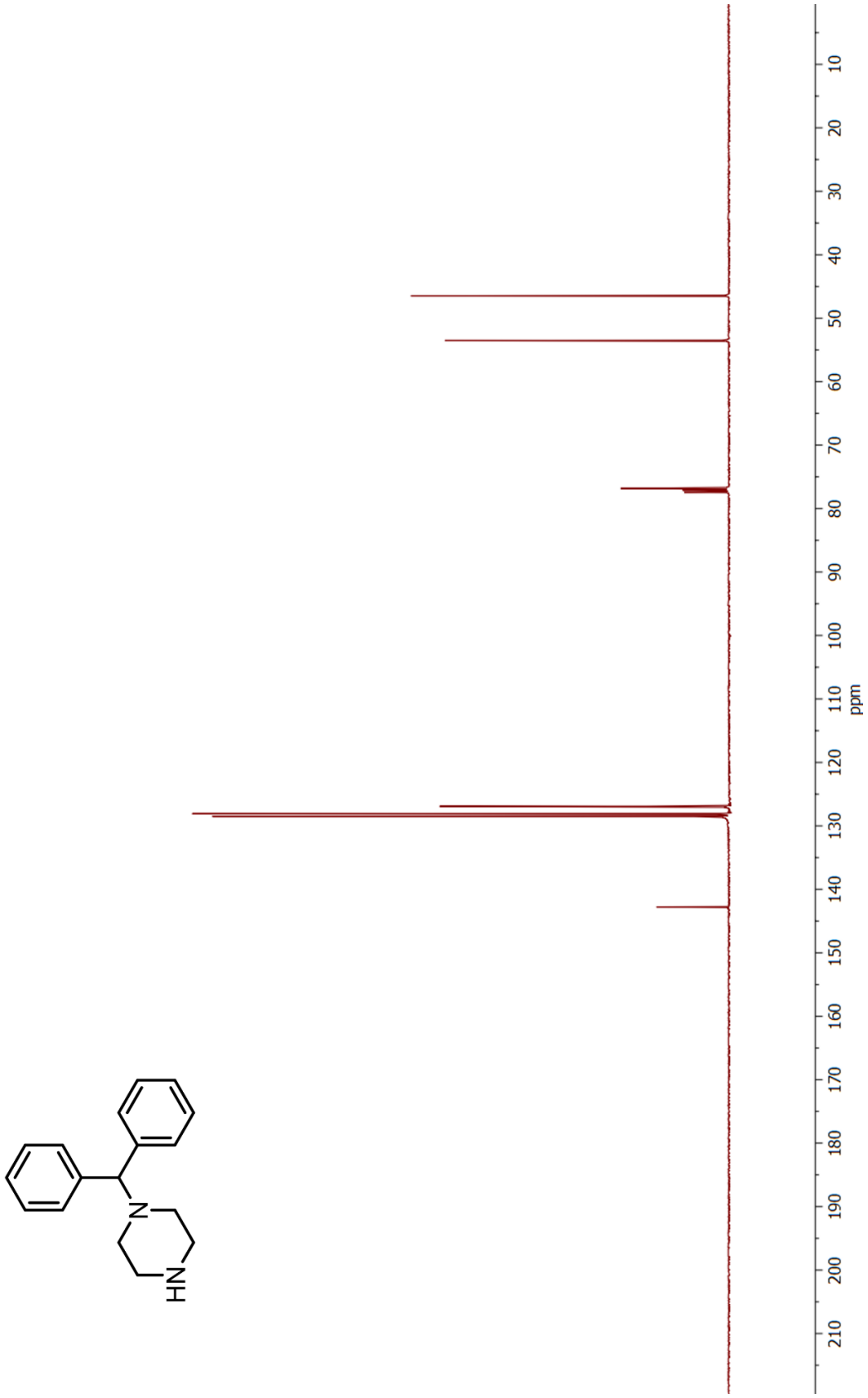


Figure 15: ^{13}C-NMR spectrum of commercial starting material 1-(diphenylmethyl)piperazine in $CDCl_3$

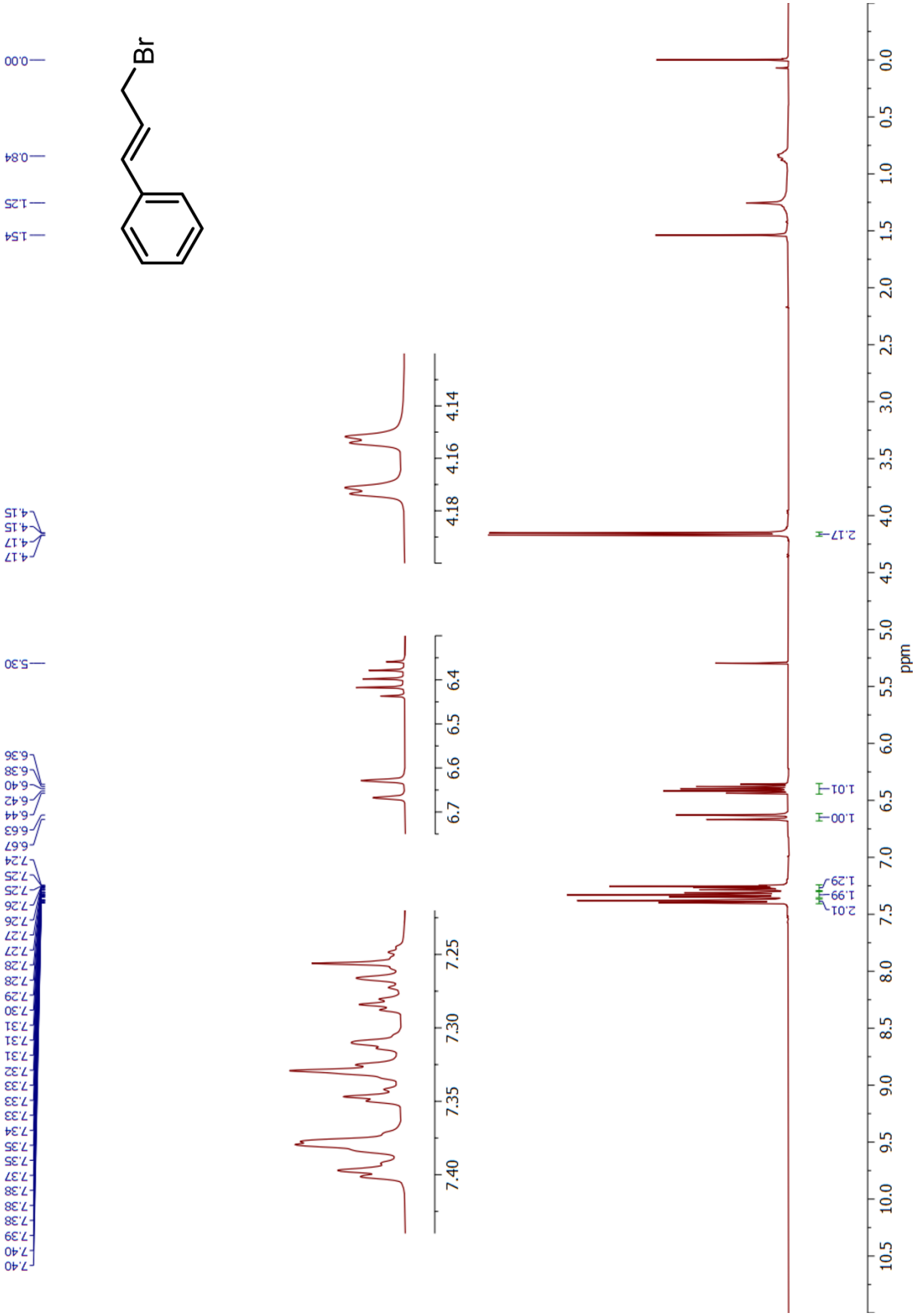


Figure 16: ^{1}H-NMR spectrum of commercial starting material cinnamyl bromide in $CDCl_3$

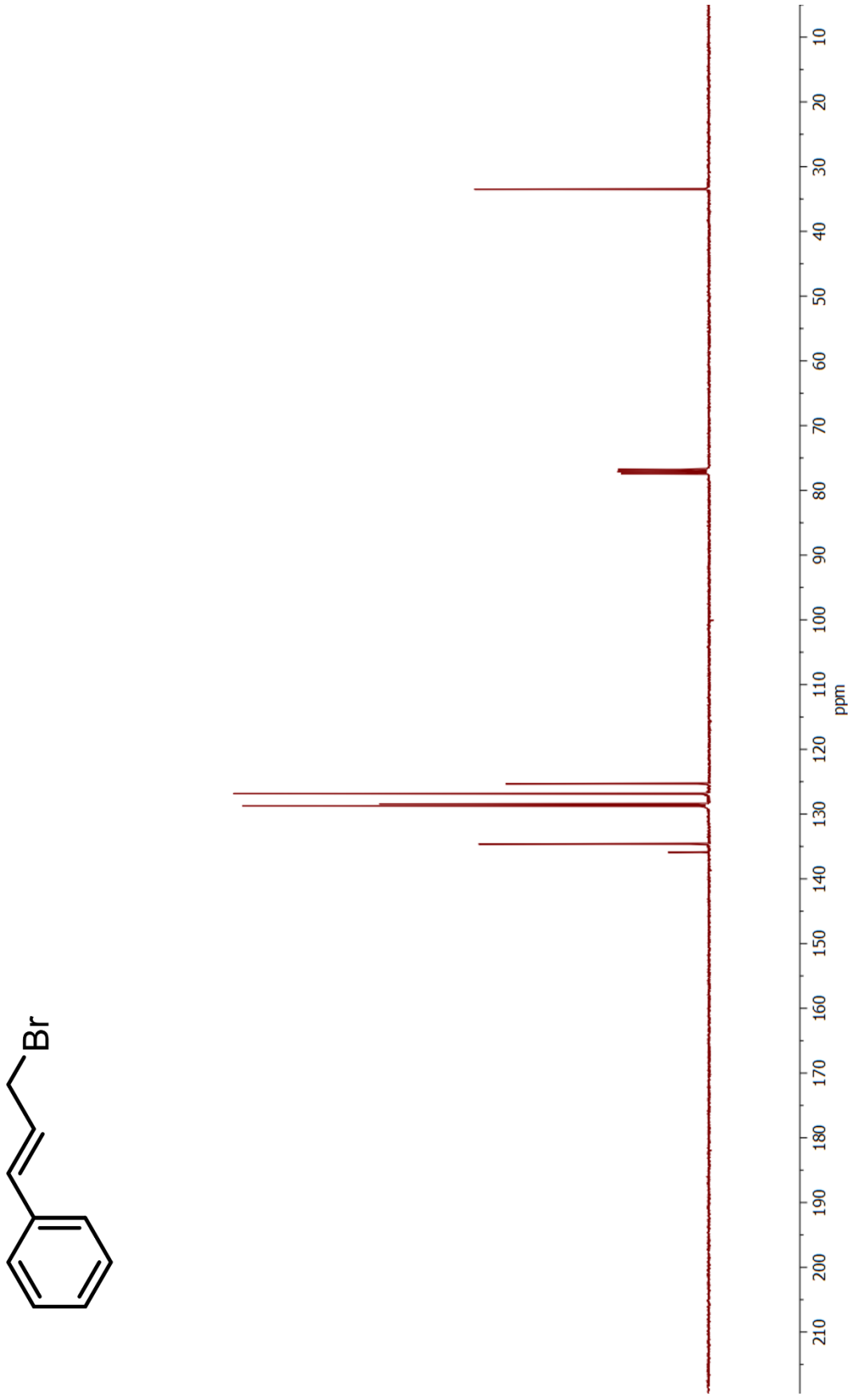


Figure 17: ^{13}C-NMR spectrum of commercial starting material cinnamyl bromide in $CDCl_3$

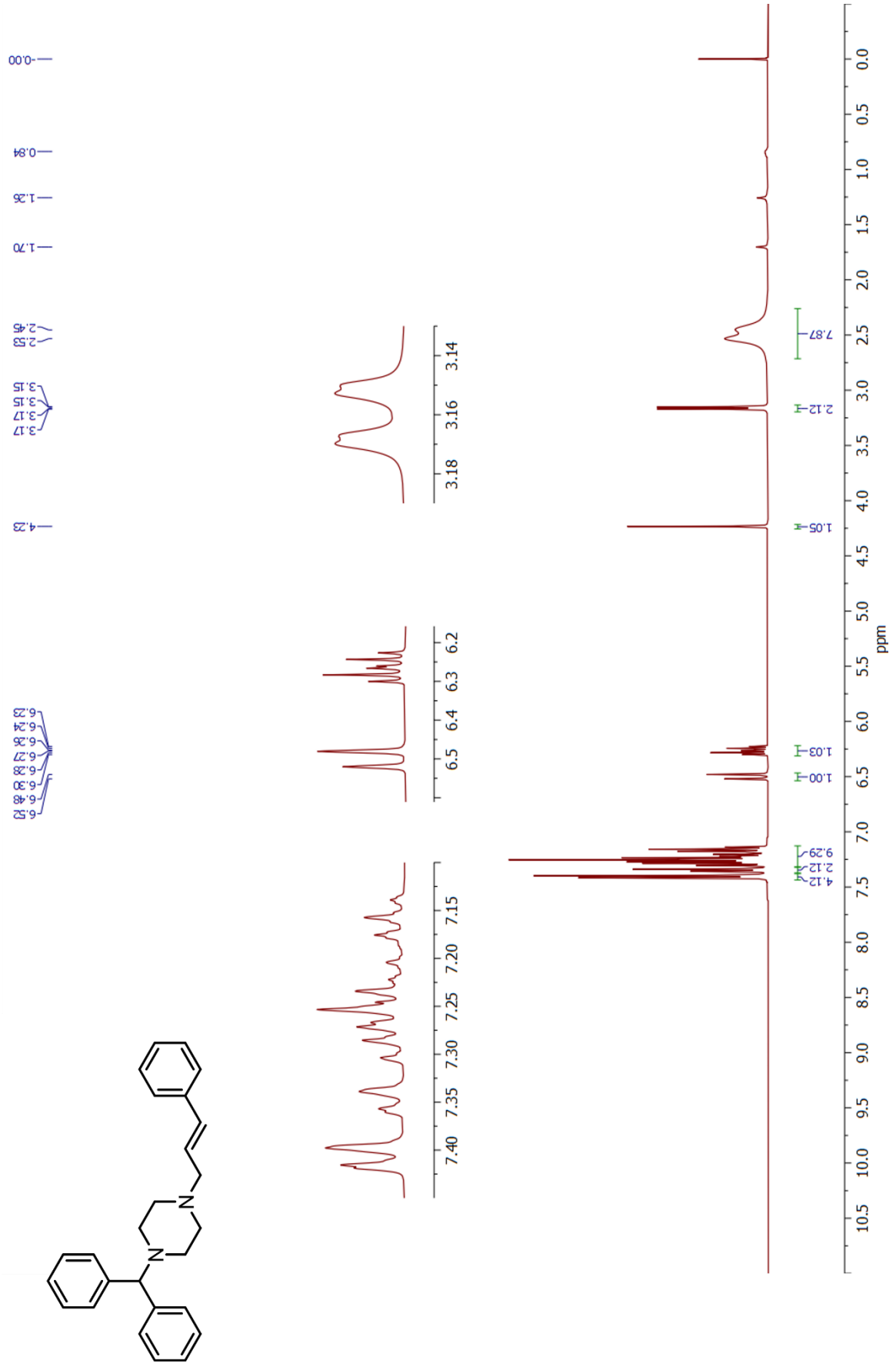


Figure 18: ^{1}H-NMR spectrum of the commercial cinnarizine in $CDCl_3$

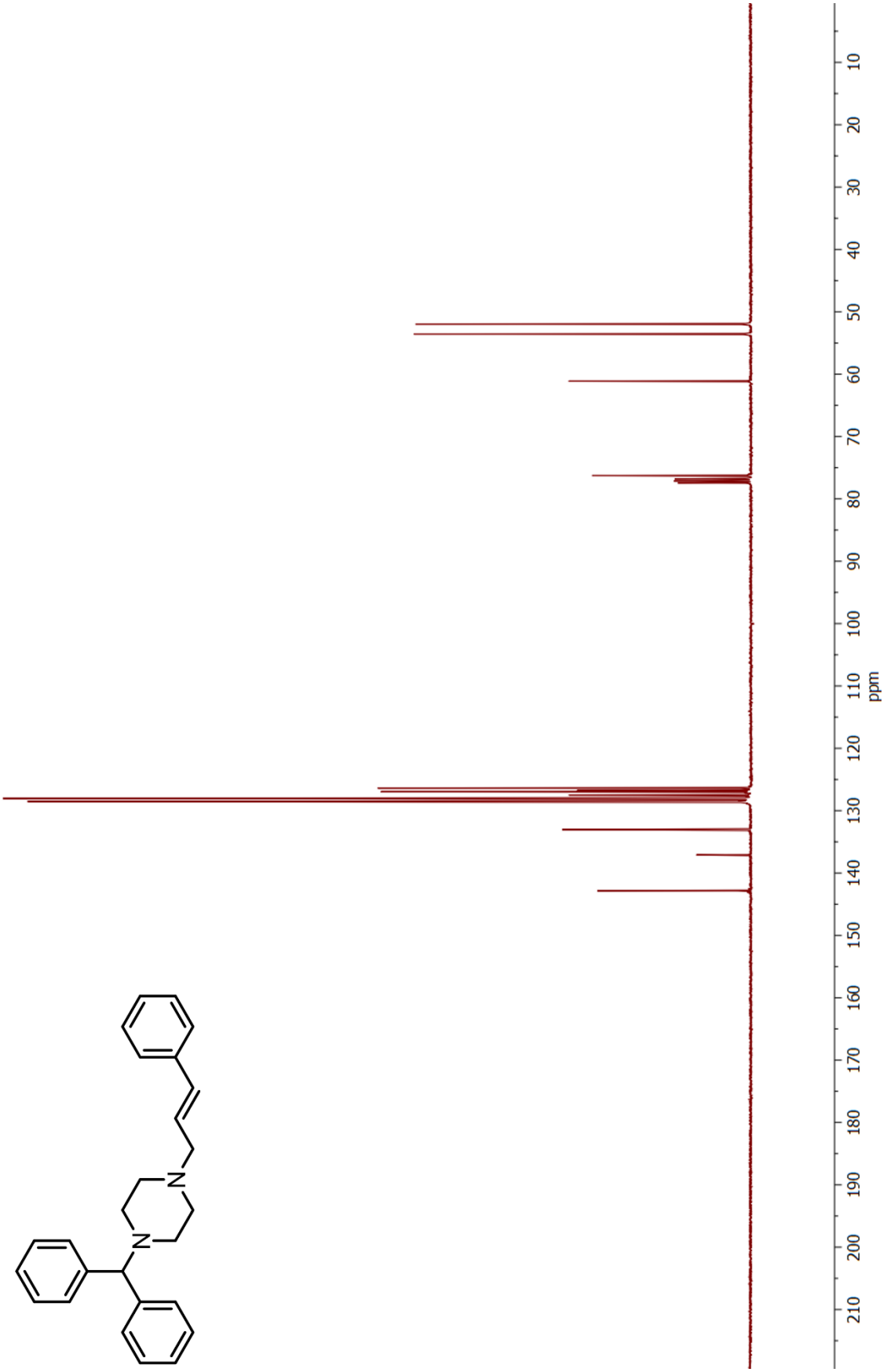


Figure 19: ^{13}C-NMR spectrum of the commercial cinnarizine in $CDCl_3$

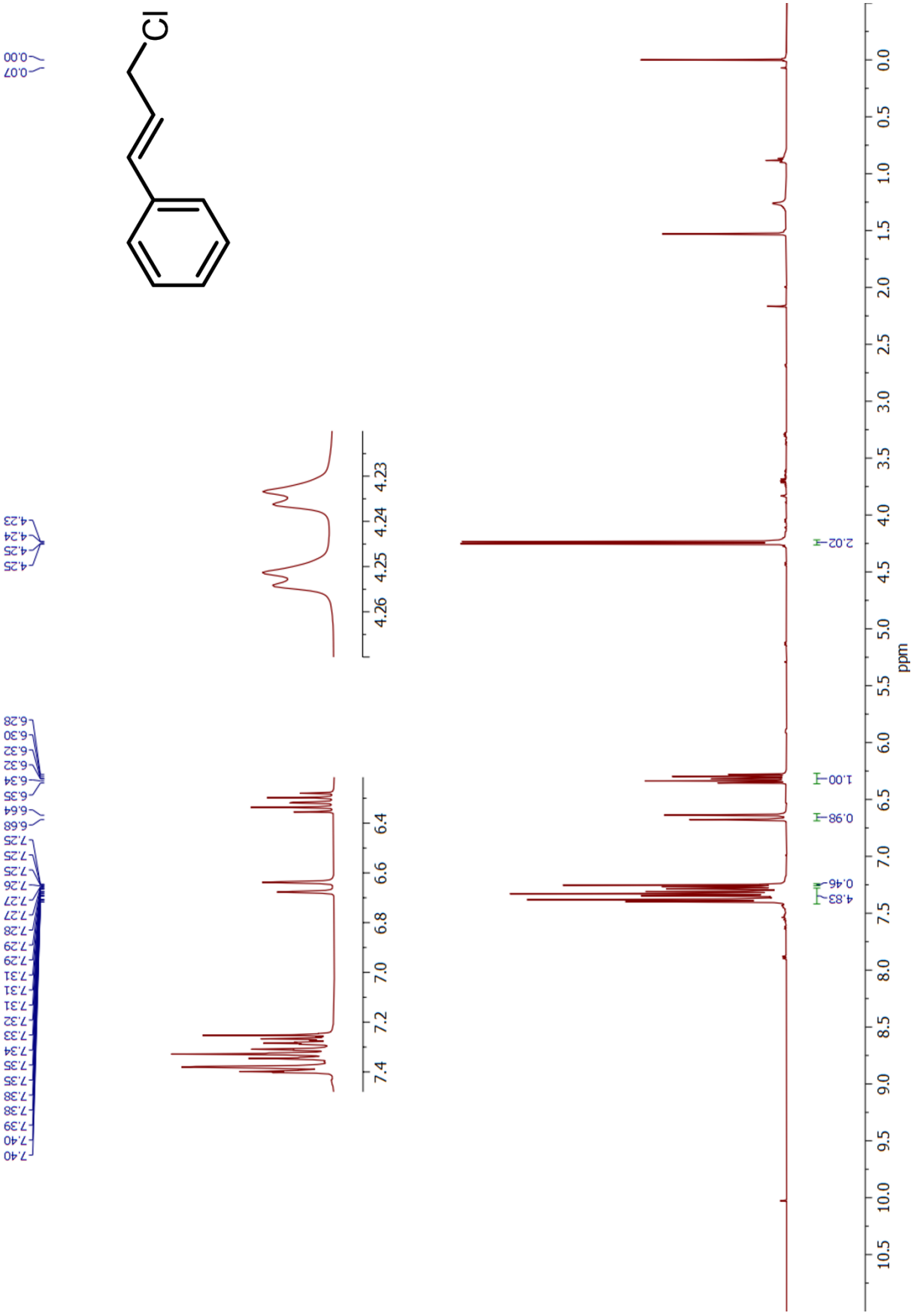


Figure 20: ^{1}H-NMR spectrum of commercial starting material cinnamyl chloride in $CDCl_3$

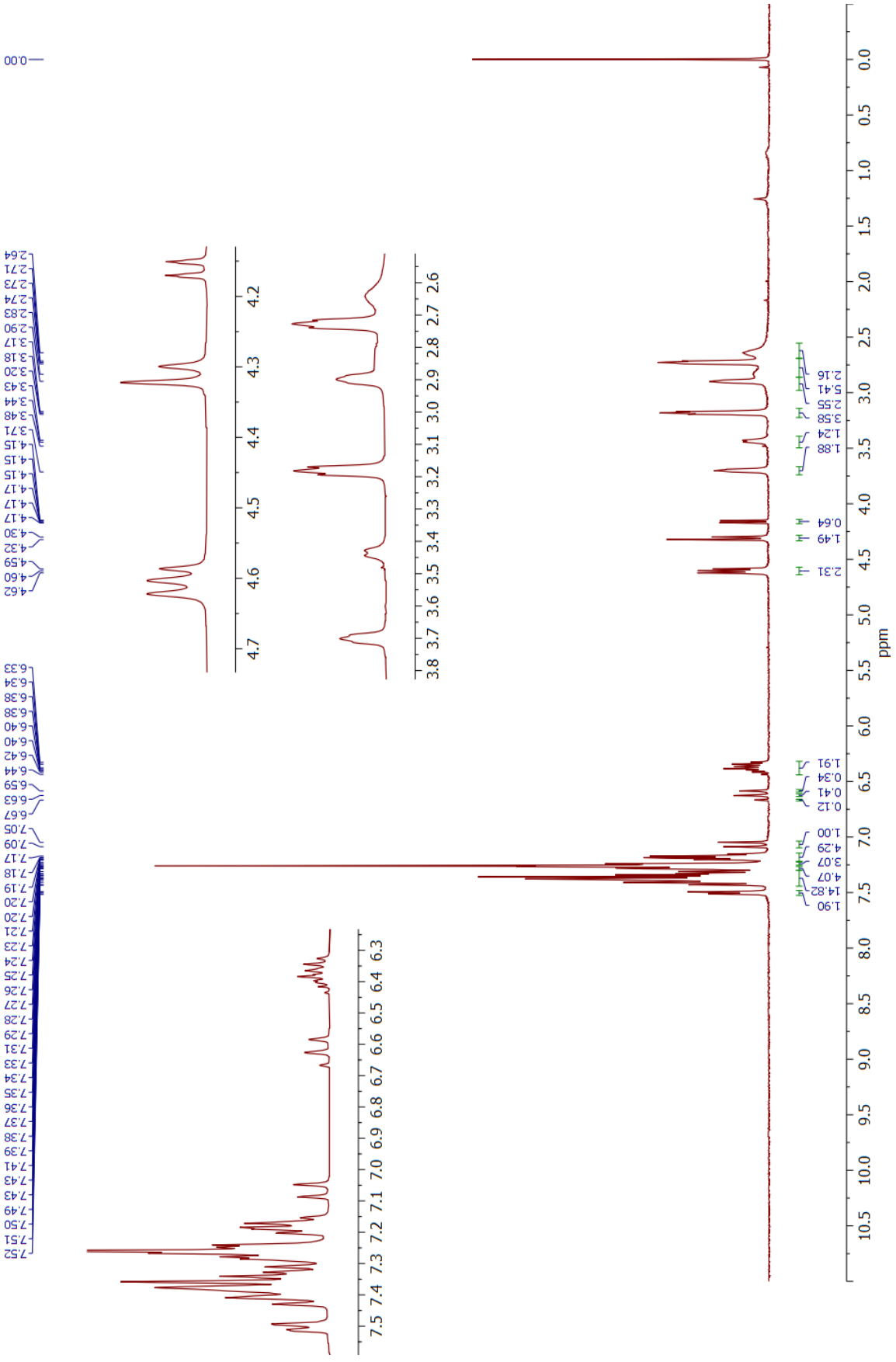


Figure 21: ^{1}H-NMR spectrum of the crude mixture of entry 1 of Table 2 in $CDCl_3$

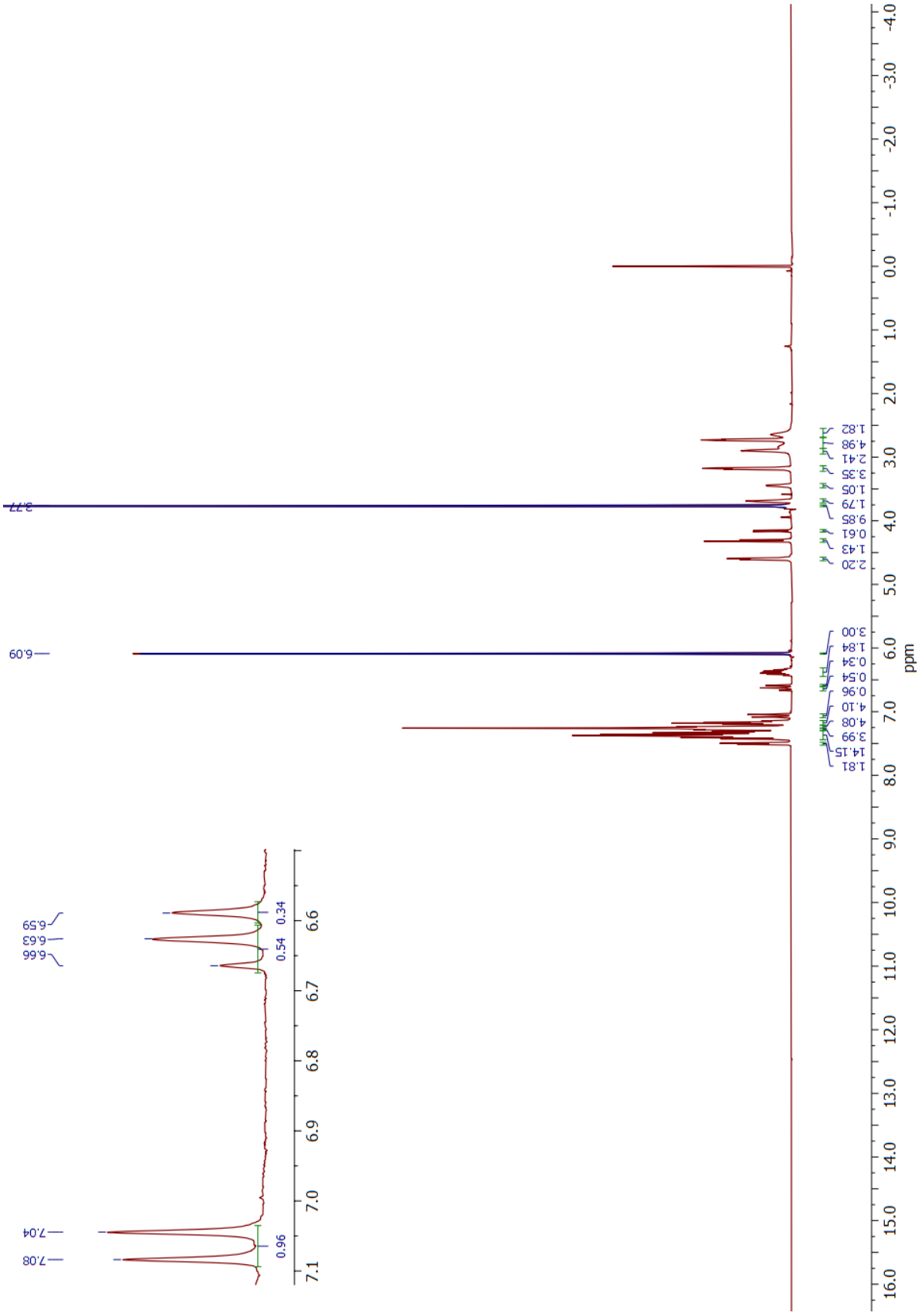


Figure 22: ^{1}H-NMR spectrum of the crude mixture with 1,3,5-Trimethoxybenzene (Internal Standard) of entry 1 of Table 2 in $CDCl_3$

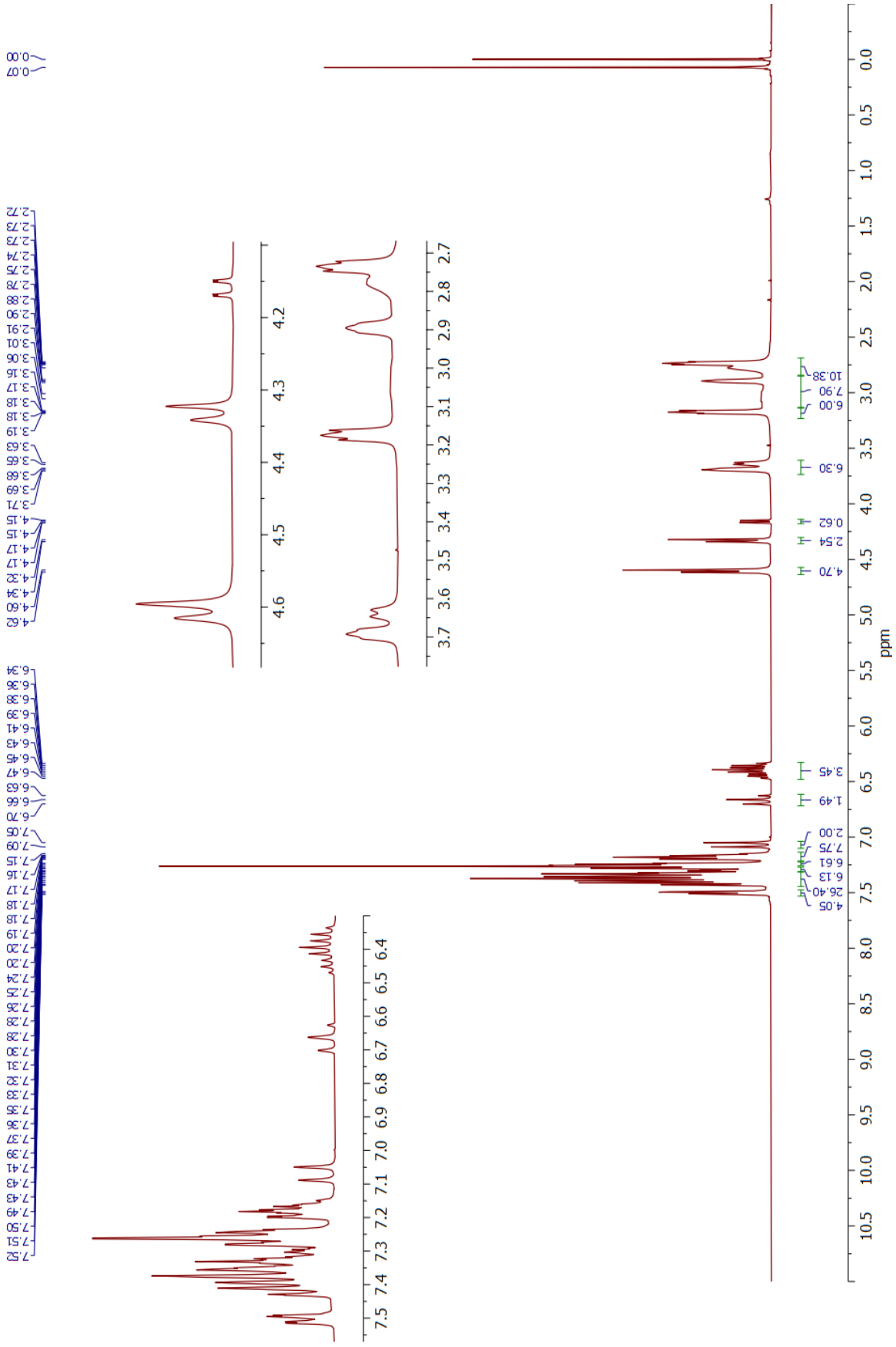


Figure 23: ^{1}H-NMR spectrum of the crude mixture of entry 2 of Table 2 in $CDCl_3$

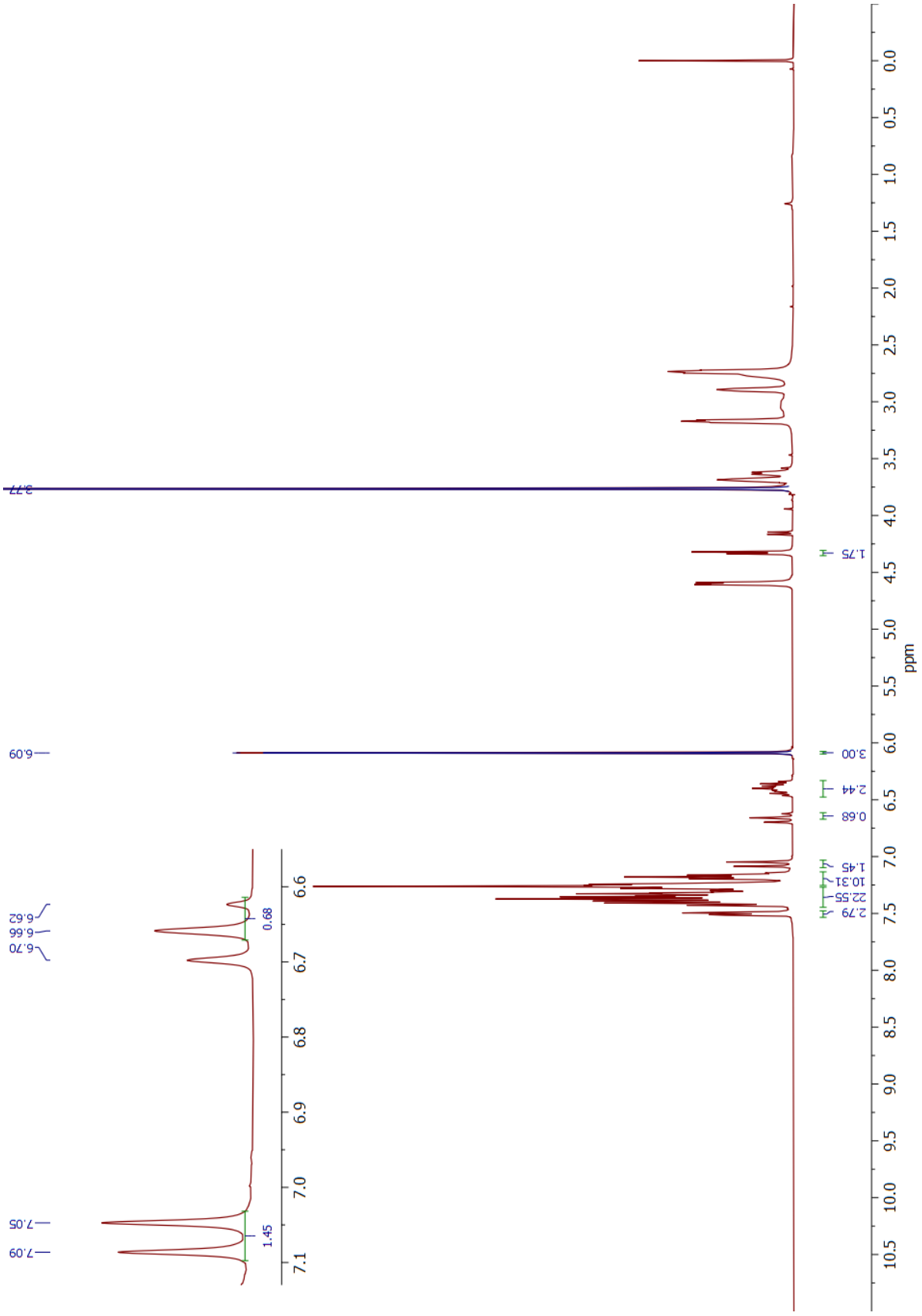


Figure 24: ^{1}H-NMR spectrum of the crude mixture with 1,3,5-Trimethoxybenzene (Internal Standard) of entry 2 of Table 2 in $CDCl_3$

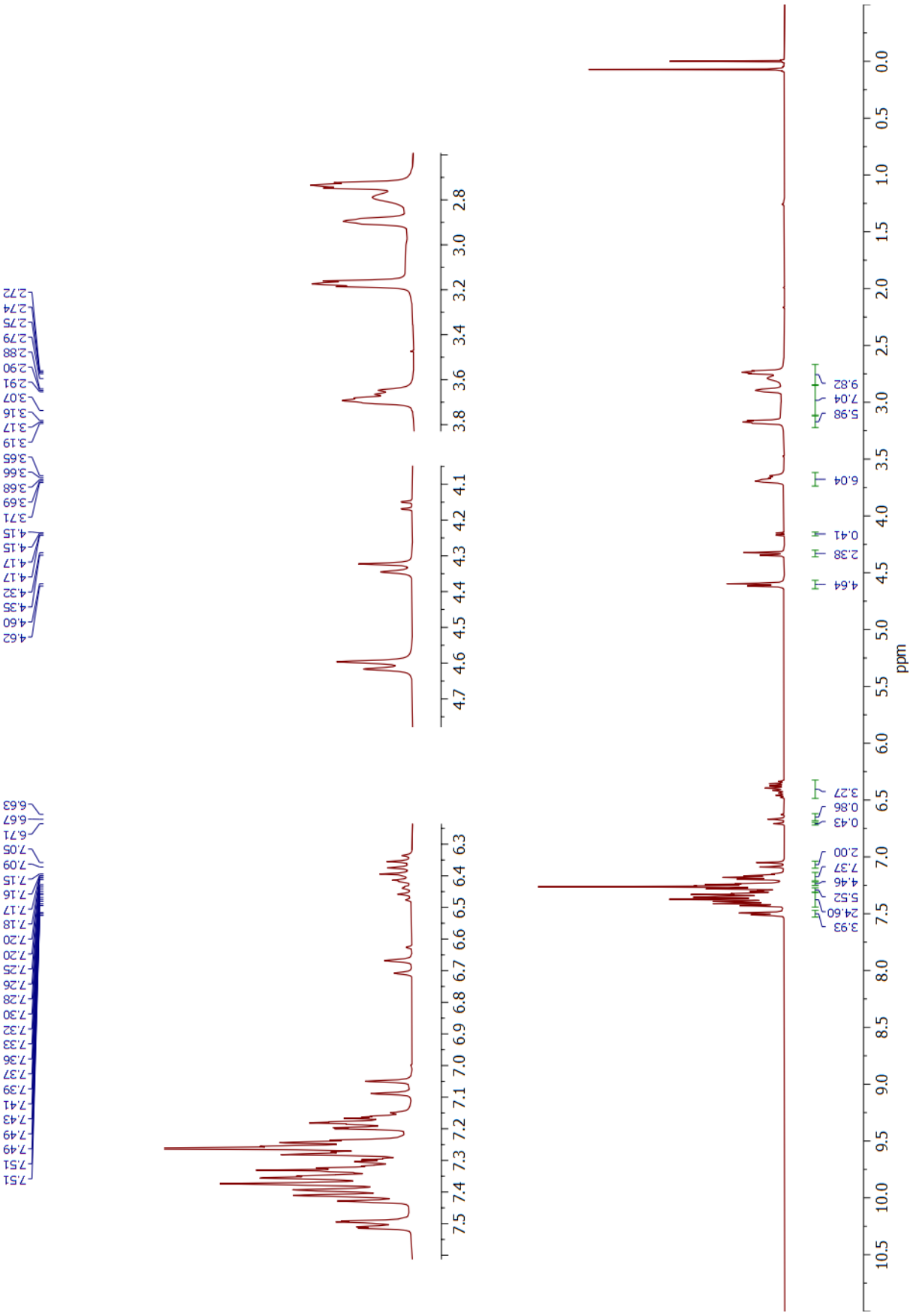


Figure 25: ^{1}H-NMR spectrum of the crude mixture of entry 3 of Table 2 in $CDCl_3$

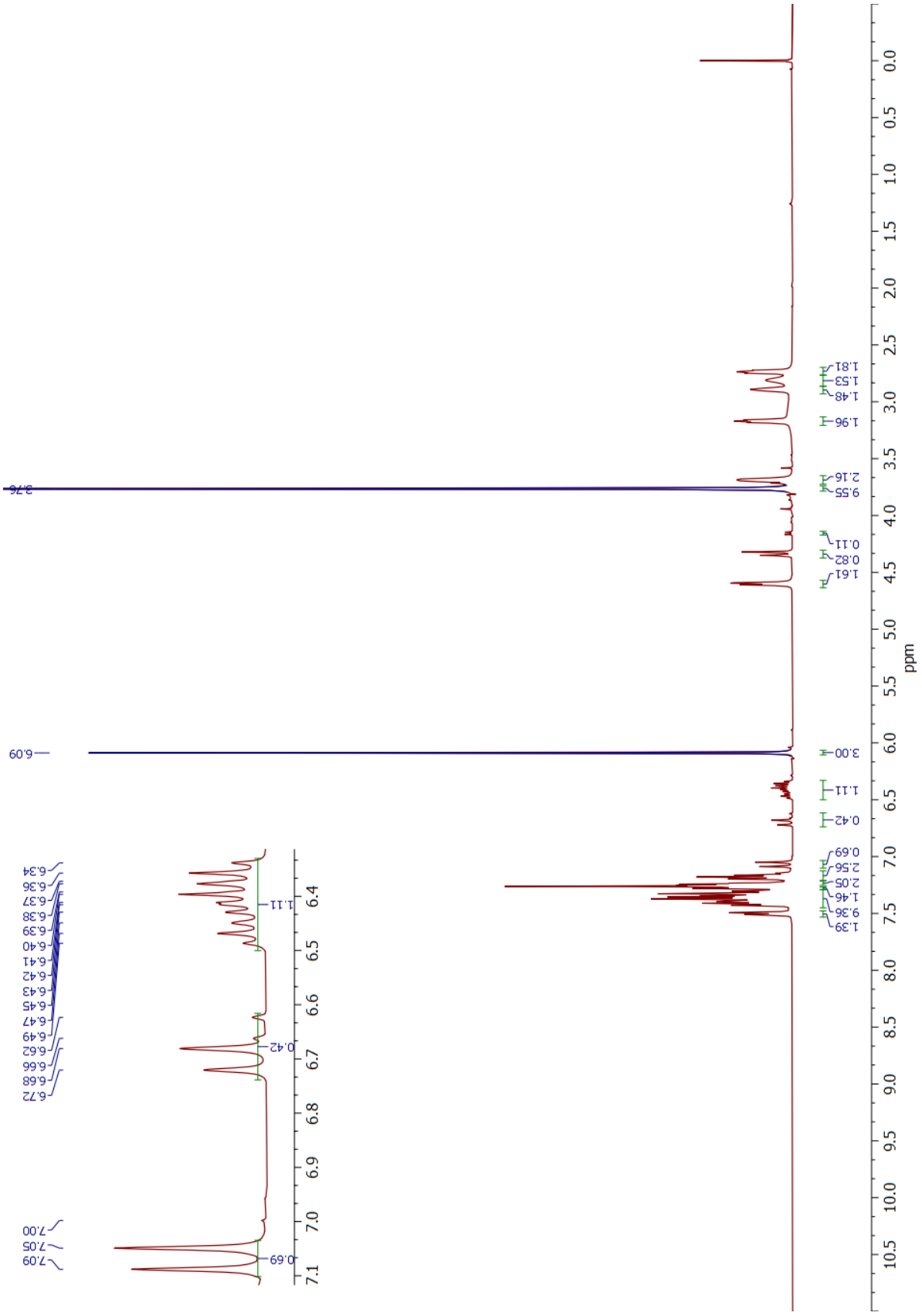


Figure 26: ^{1}H-NMR spectrum of the crude mixture with 1,3,5-Trimethoxybenzene (Internal Standard) of entry 3 of Table 2 in $CDCl_3$

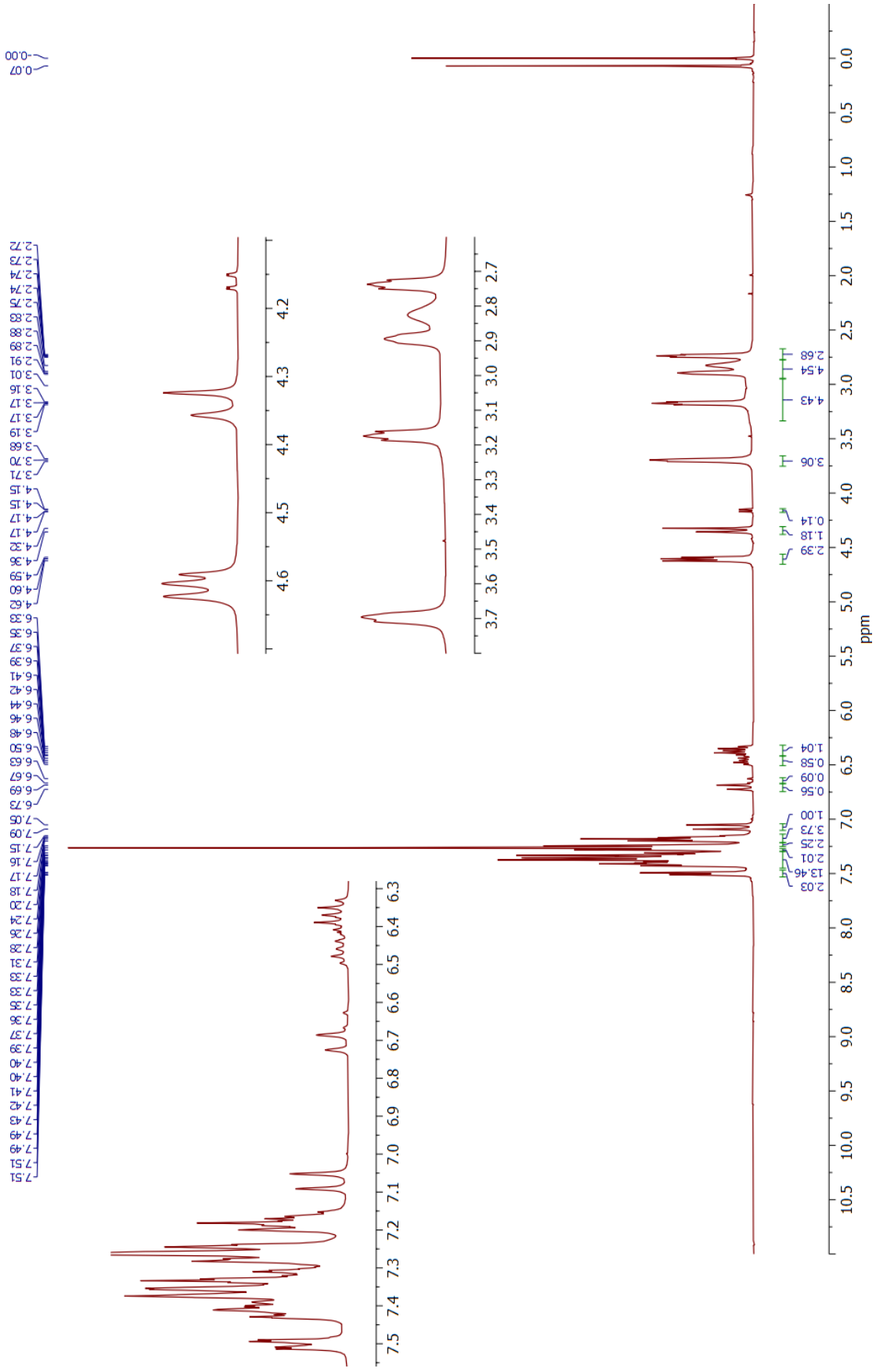


Figure 27: ^{1}H-NMR spectrum of the crude mixture of entry 4 of Table 2 in $CDCl_3$

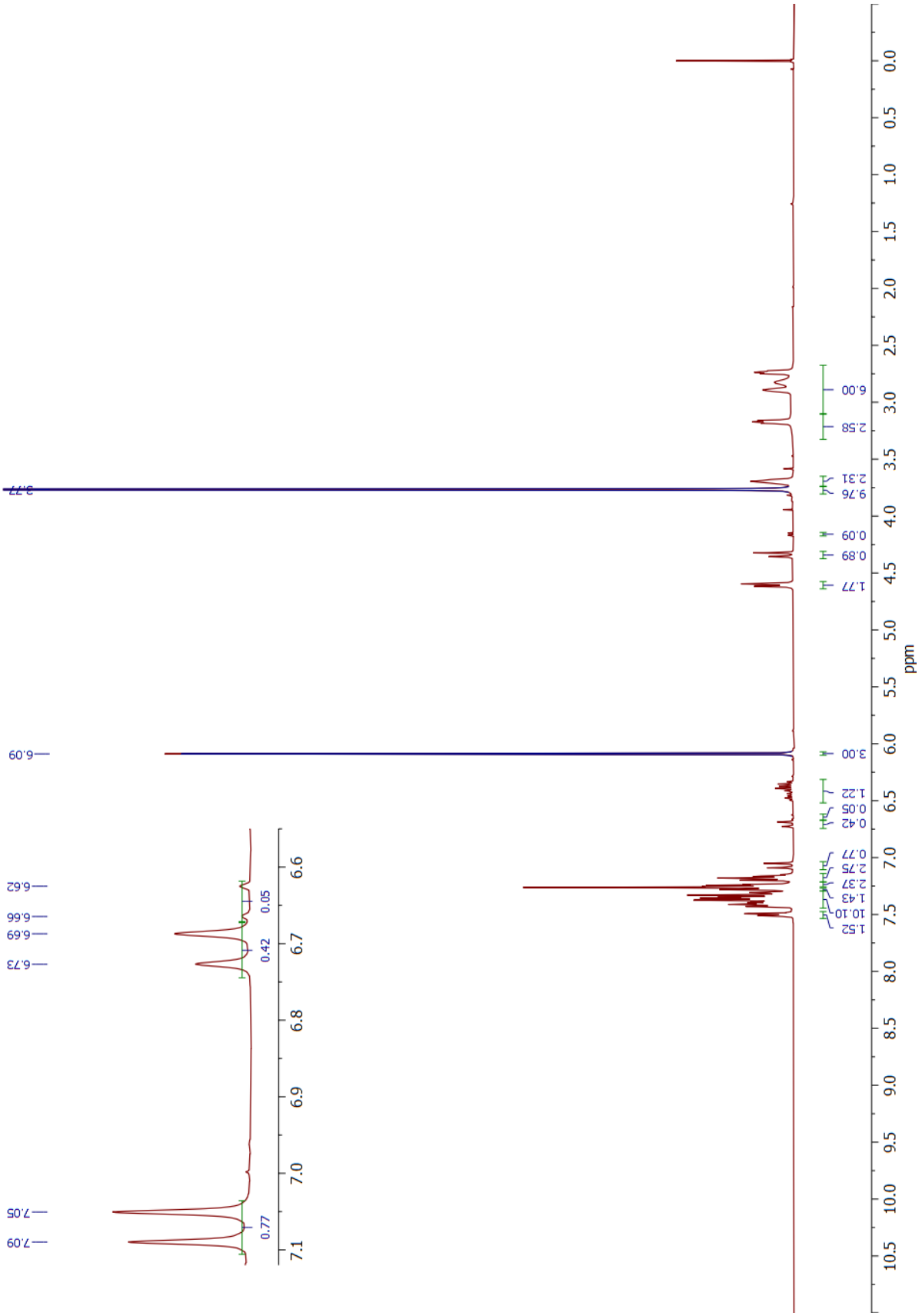


Figure 28: ^{1}H-NMR spectrum of the crude mixture with 1,3,5-Trimethoxybenzene (Internal Standard) of entry 4 of Table 2 in $CDCl_3$

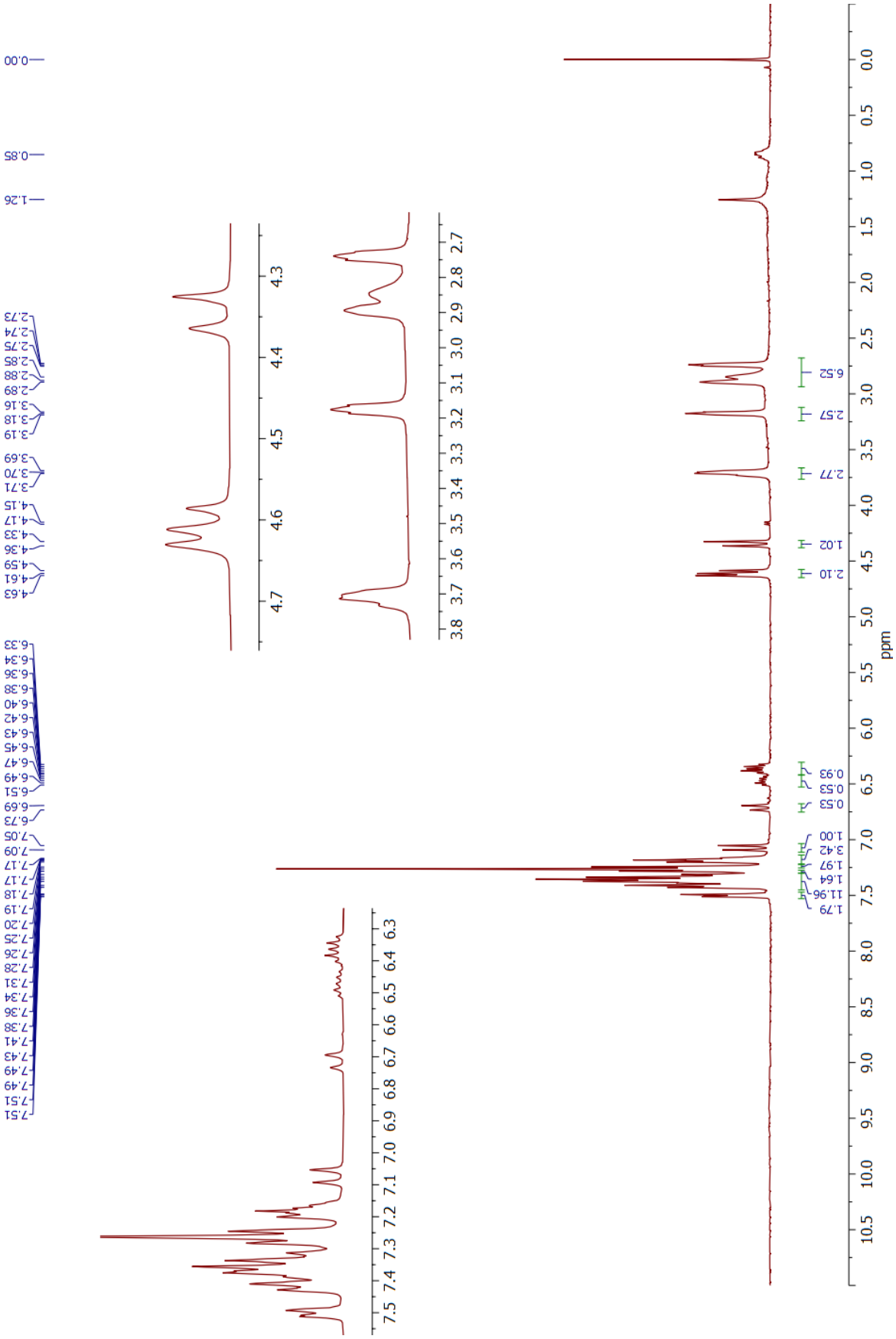


Figure 29: ^{1}H-NMR spectrum of the crude mixture of entry 5 of Table 2 in $CDCl_3$

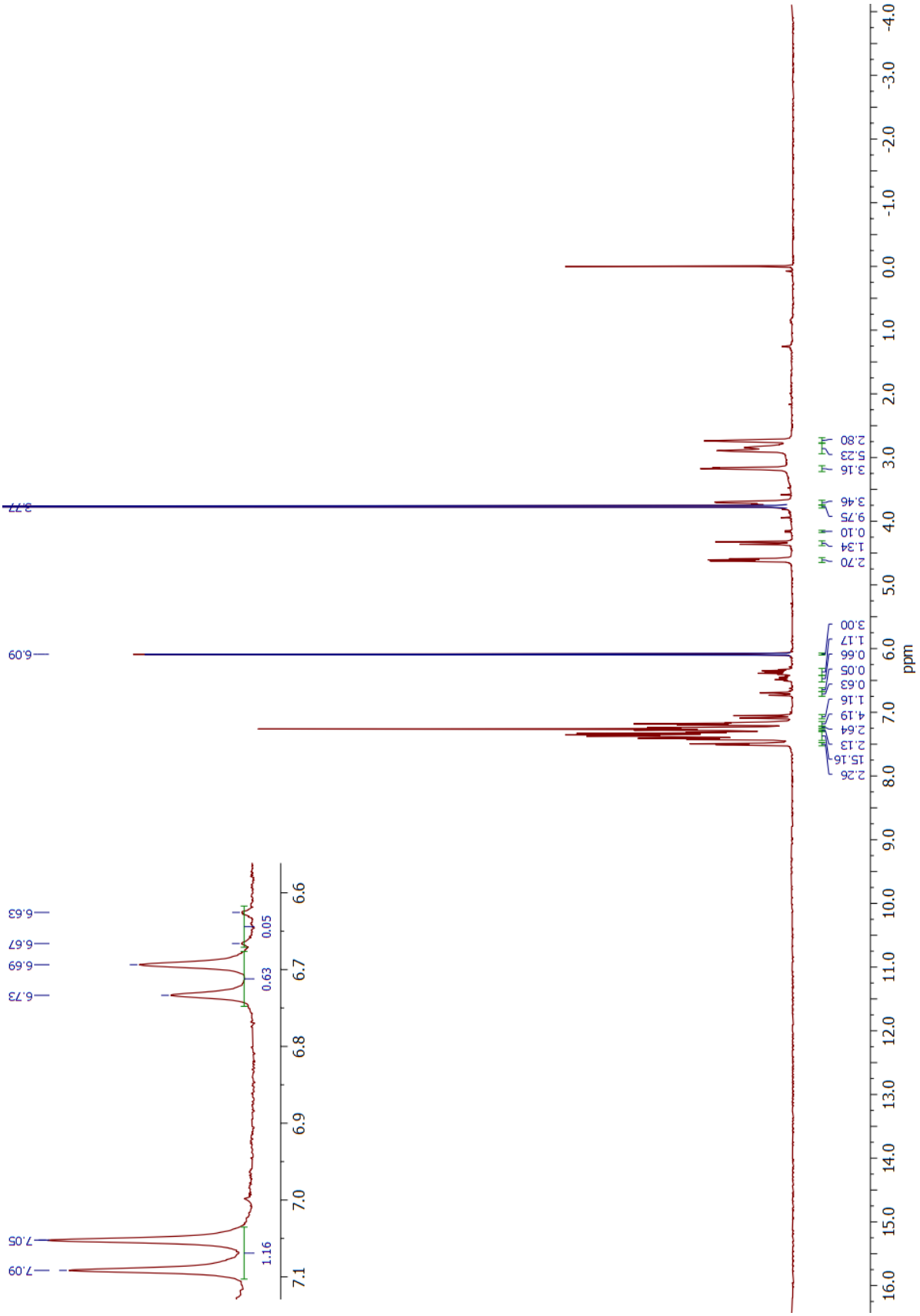


Figure 30: ^{1}H-NMR spectrum of the crude mixture with 1,3,5-Trimethoxybenzene (Internal Standard) of entry 5 of Table 2 in $CDCl_3$

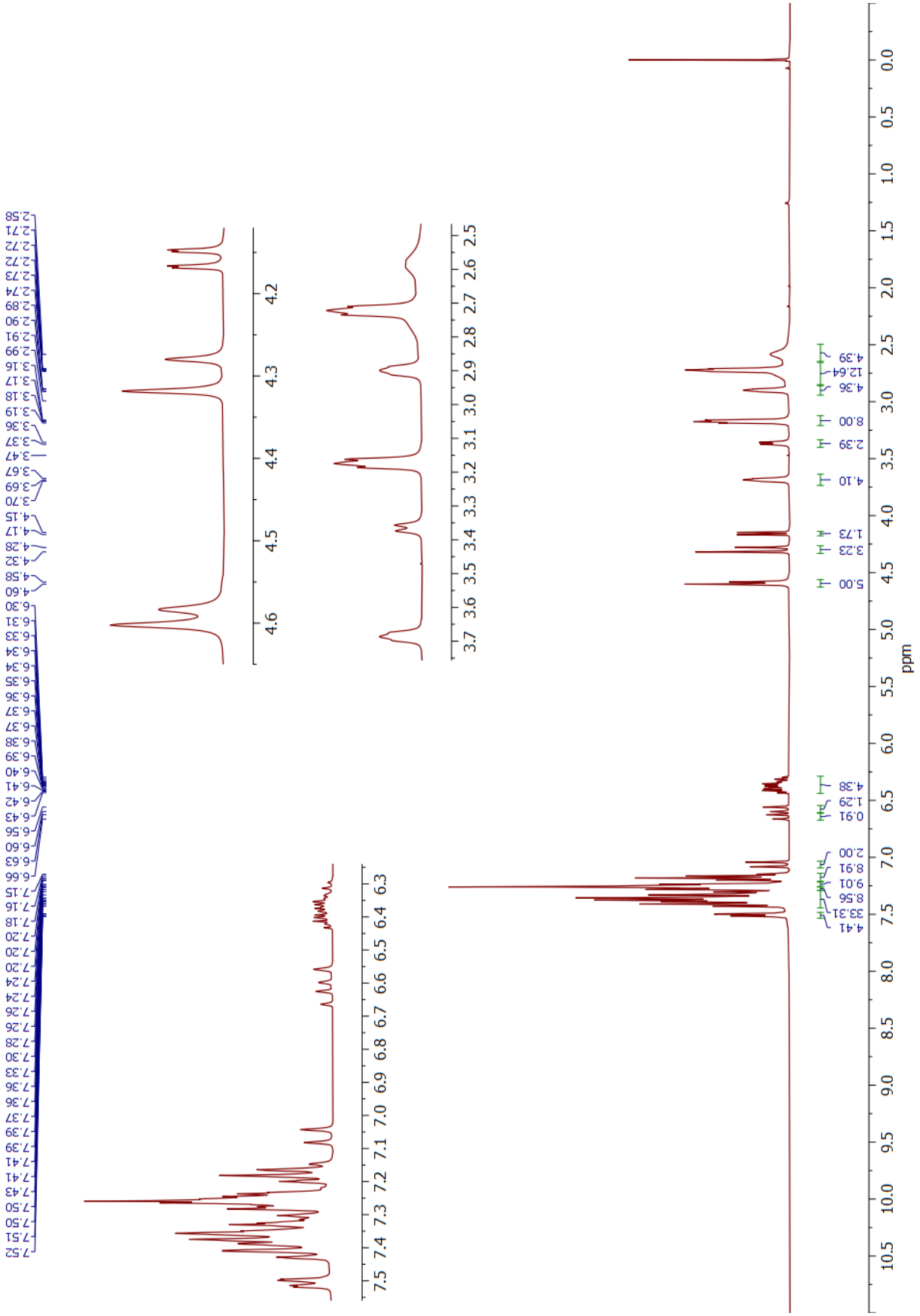


Figure 31: ^{1}H-NMR spectrum of the crude mixture of entry 6 of Table 2 in $CDCl_3$

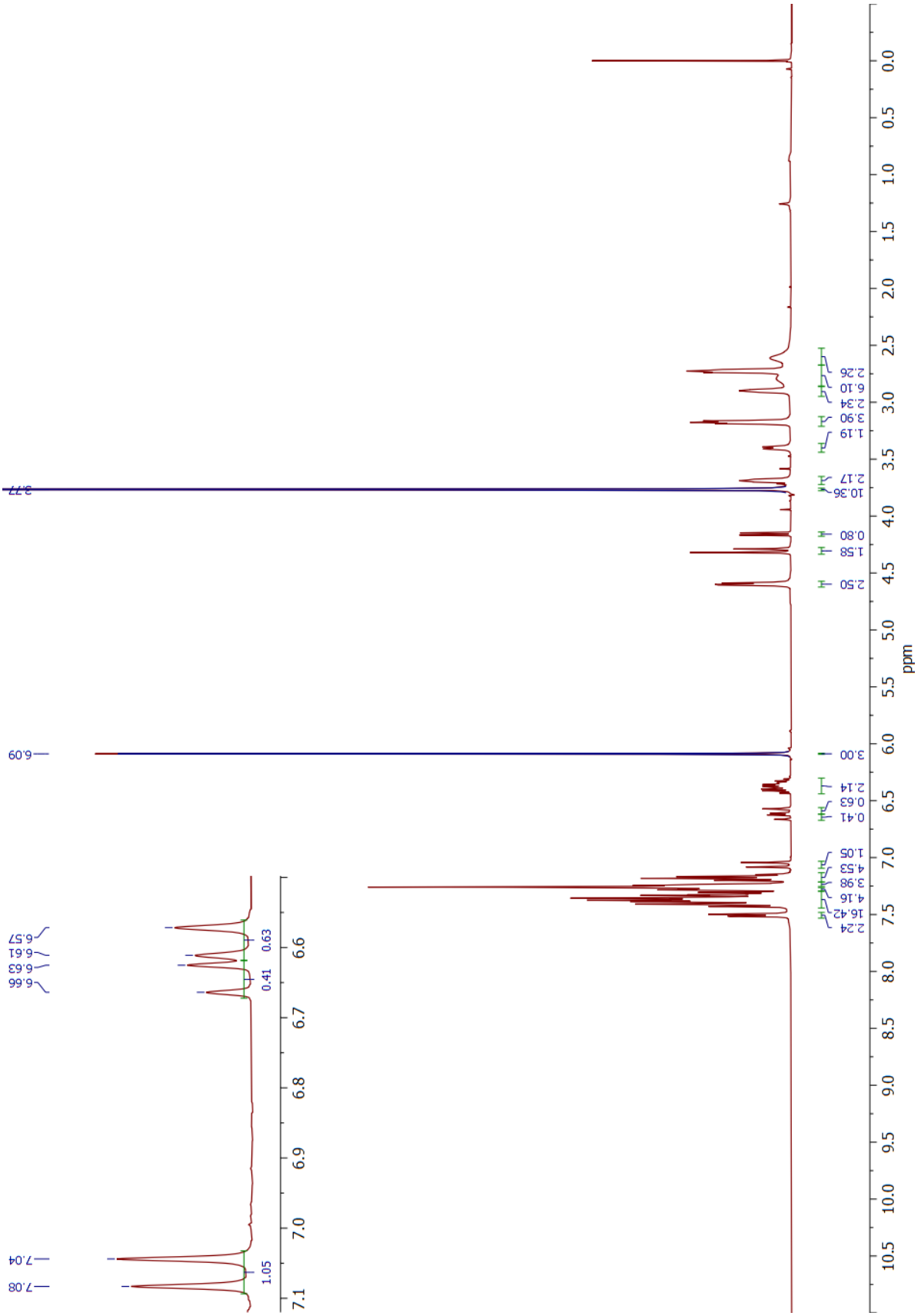


Figure 32: ^{1}H-NMR spectrum of the crude mixture with 1,3,5-Trimethoxybenzene (Internal Standard) of entry 6 of Table 2 in $CDCl_3$

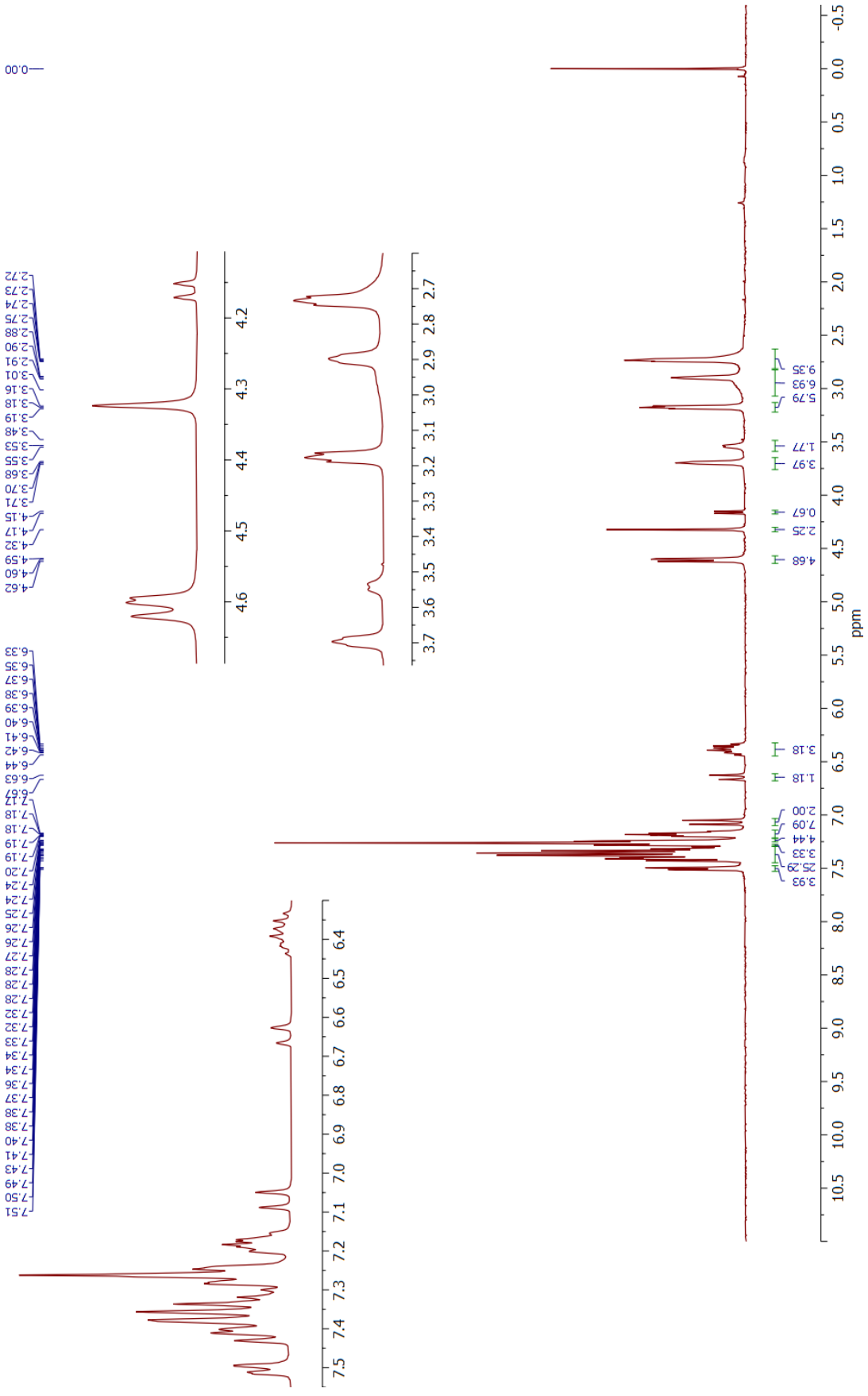


Figure 33: ^{1}H-NMR spectrum of the crude mixture of entry 7 of Table 2 in $CDCl_3$

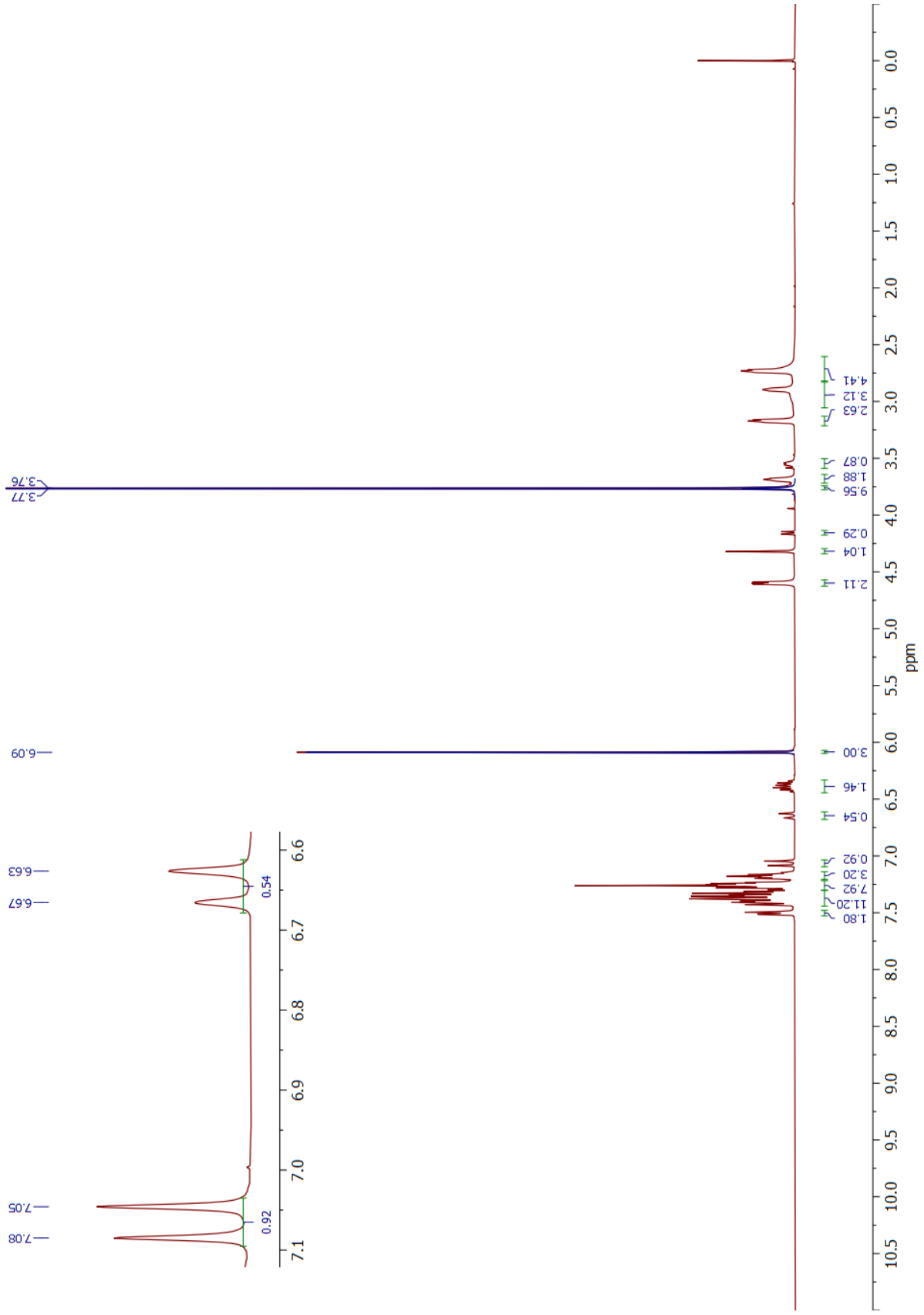


Figure 34: ^{1}H-NMR spectrum of the crude mixture with 1,3,5-Trimethoxybenzene (Internal Standard) of entry 7 of Table 2 in $CDCl_3$

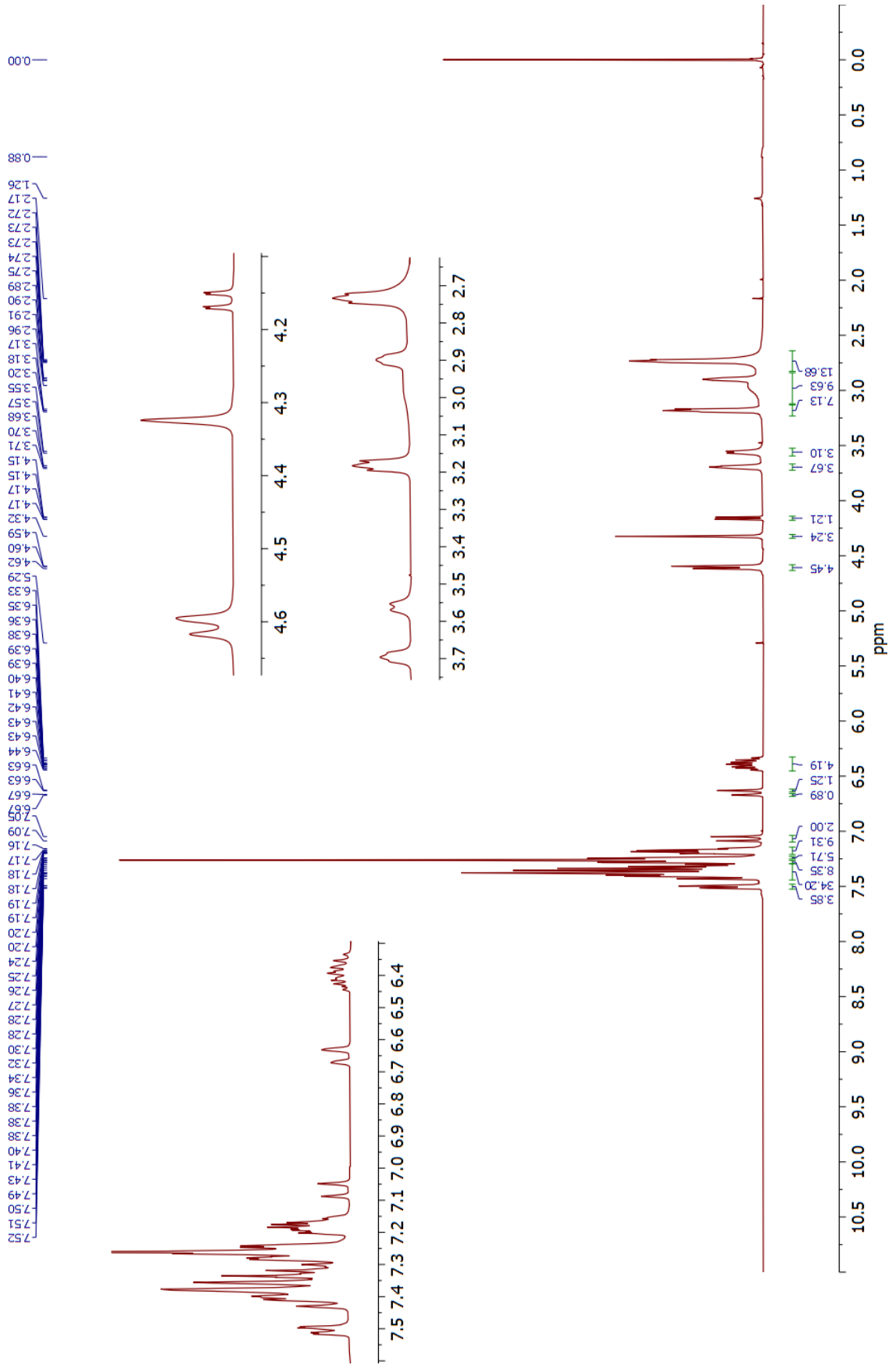


Figure 35: ^{1}H-NMR spectrum of the crude mixture of entry 8 of Table 2 in $CDCl_3$

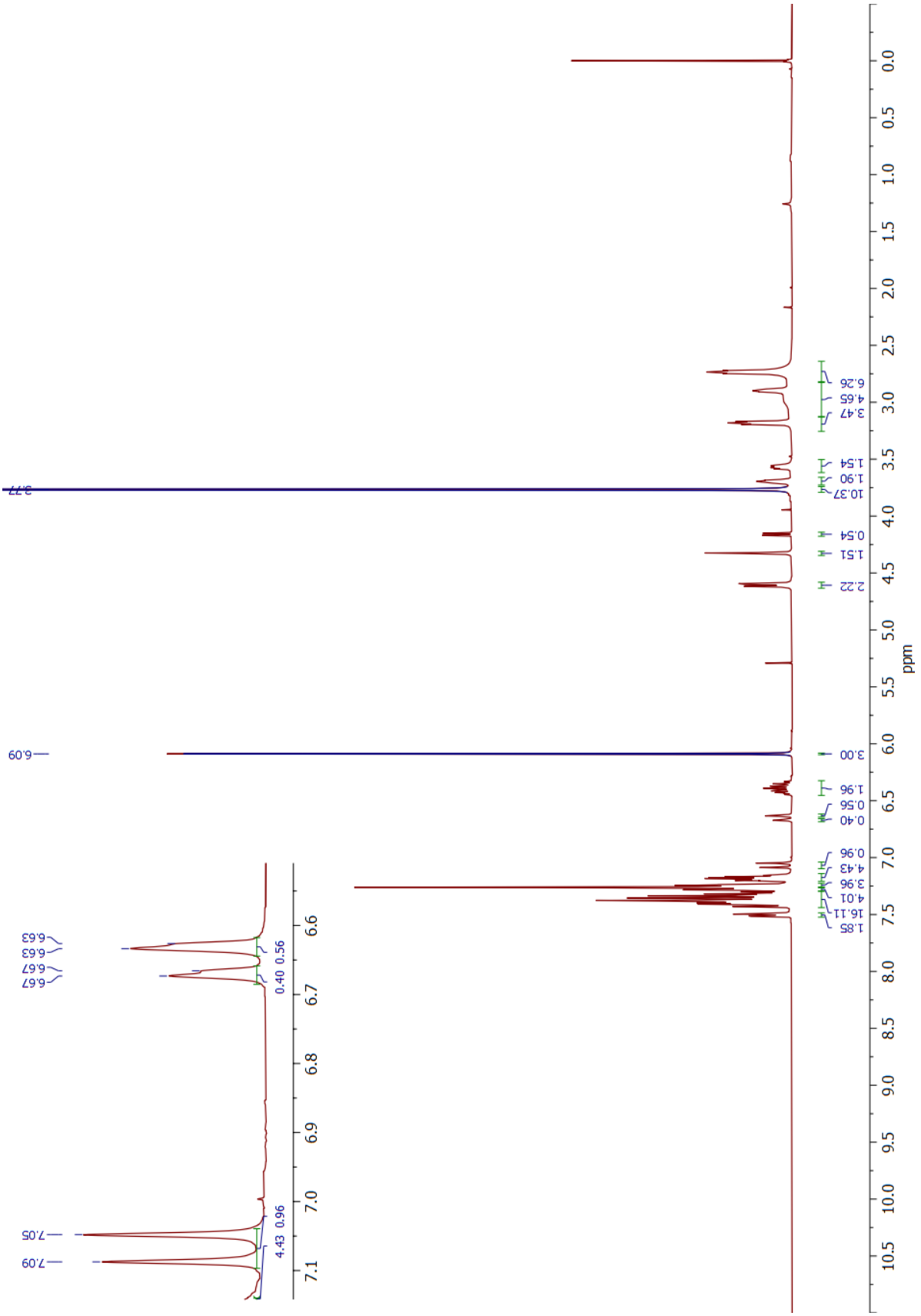


Figure 36: ^{1}H-NMR spectrum of the crude mixture with 1,3,5-Trimethoxybenzene (Internal Standard) of entry 8 of Table 2 in $CDCl_3$

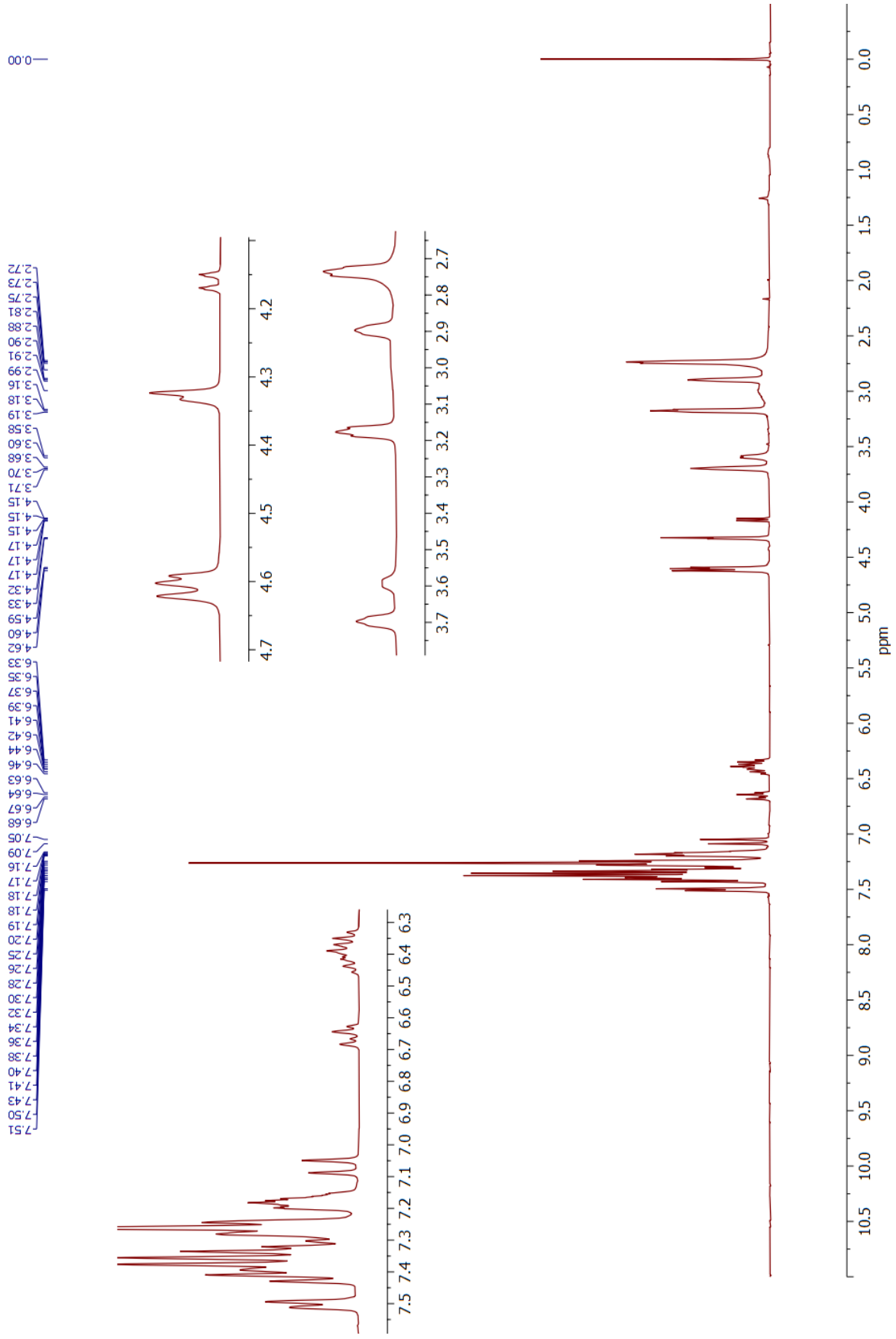


Figure 37: ^{1}H-NMR spectrum of the crude mixture of entry 9 of Table 2 in $CDCl_3$

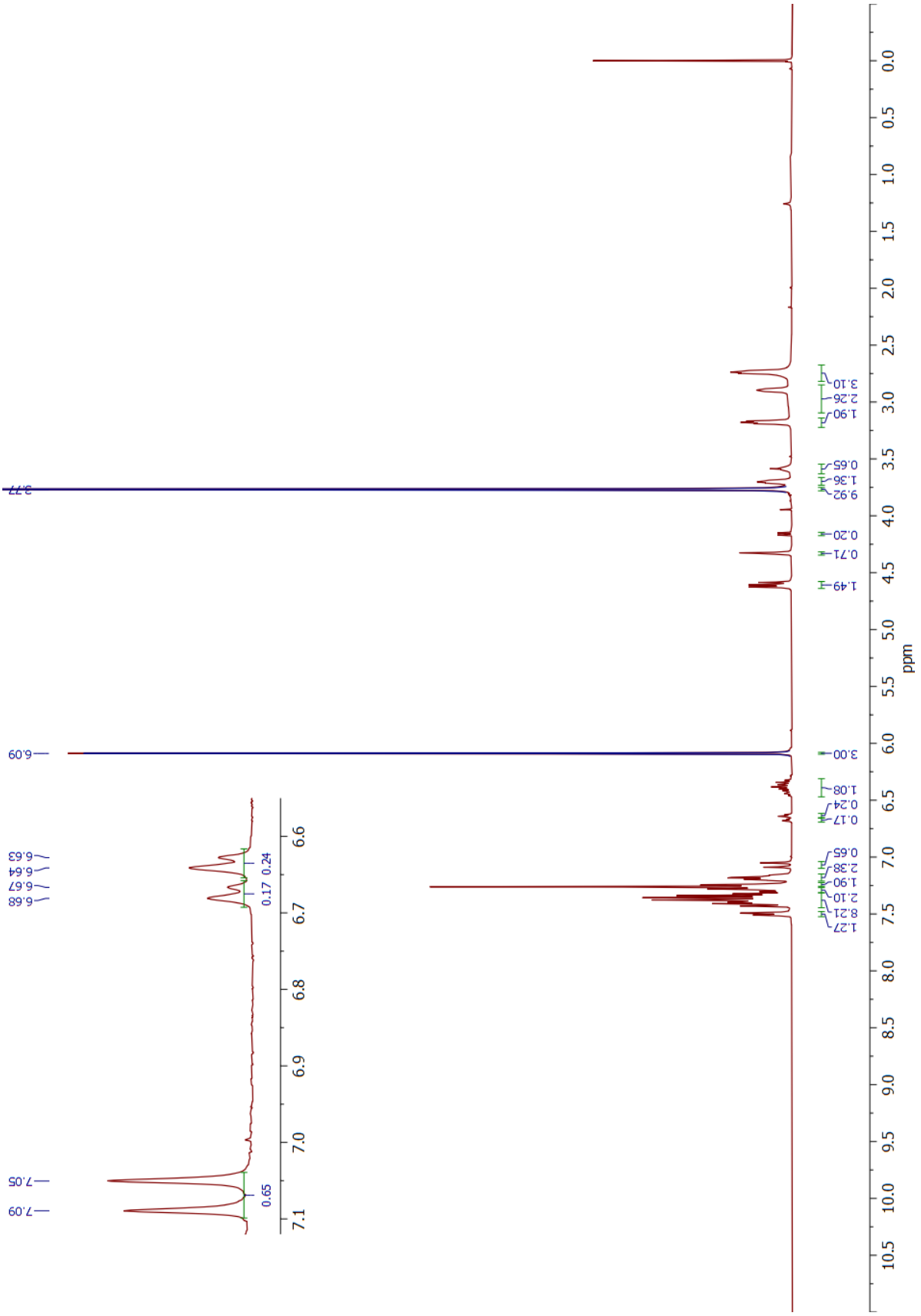
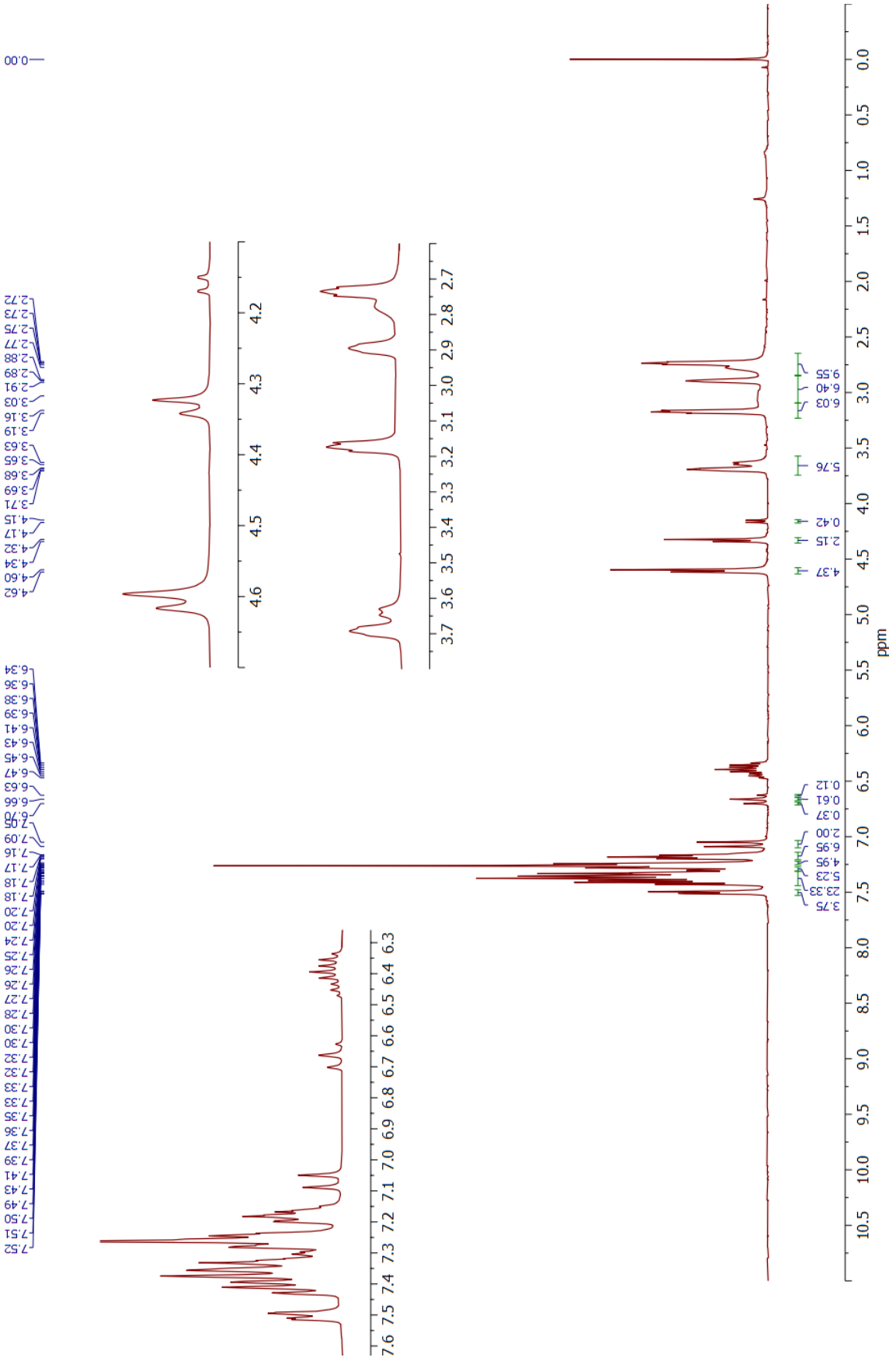


Figure 38: ^{1}H-NMR spectrum of the crude mixture with 1,3,5-Trimethoxybenzene (Internal Standard) of entry 9 of Table 2 in $CDCl_3$



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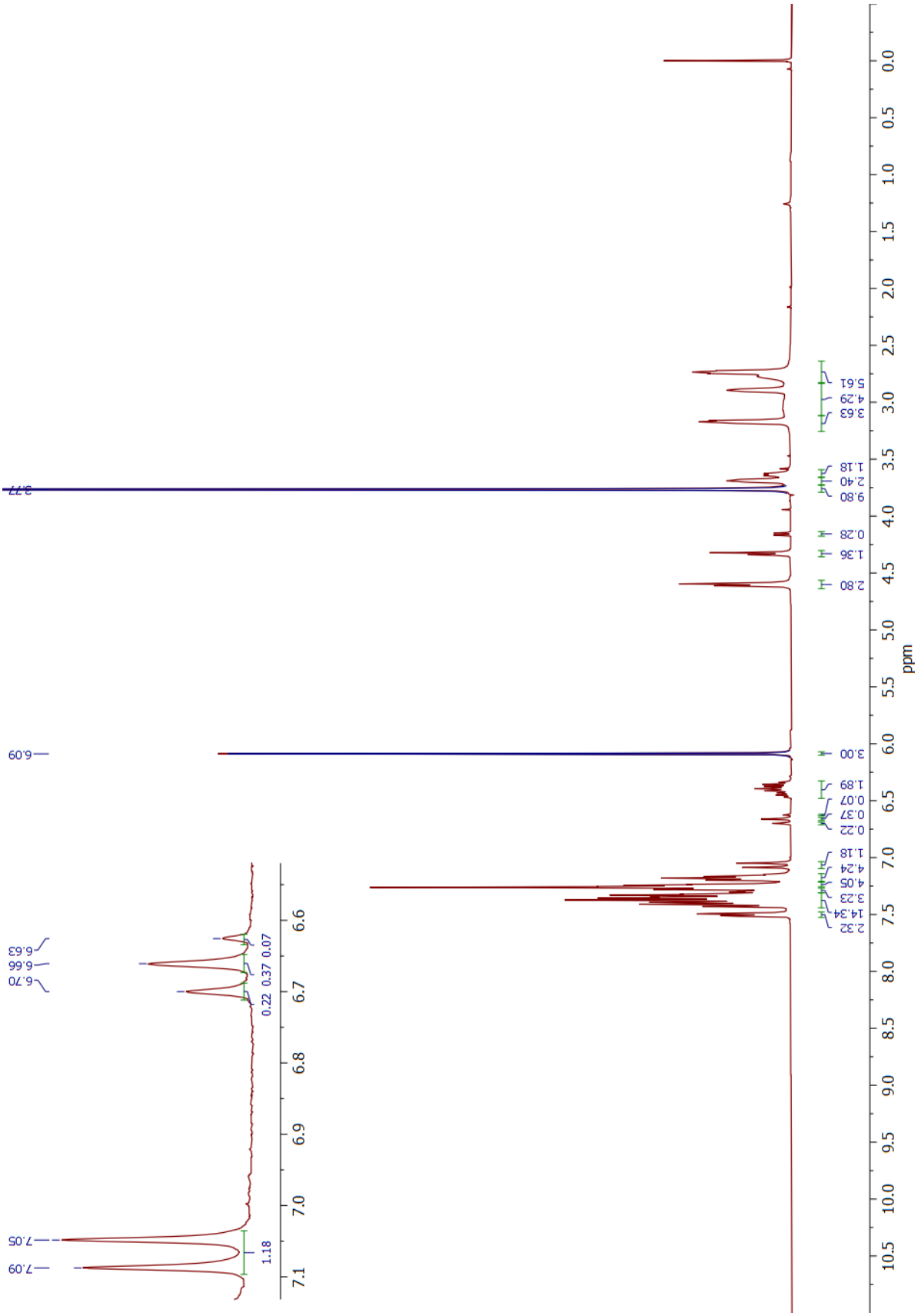

Figure 40: ^{1}H-NMR spectrum of the crude mixture with 1,3,5-Trimethoxybenzene (Internal Standard) of entry 10 of Table 2 in $CDCl_3$

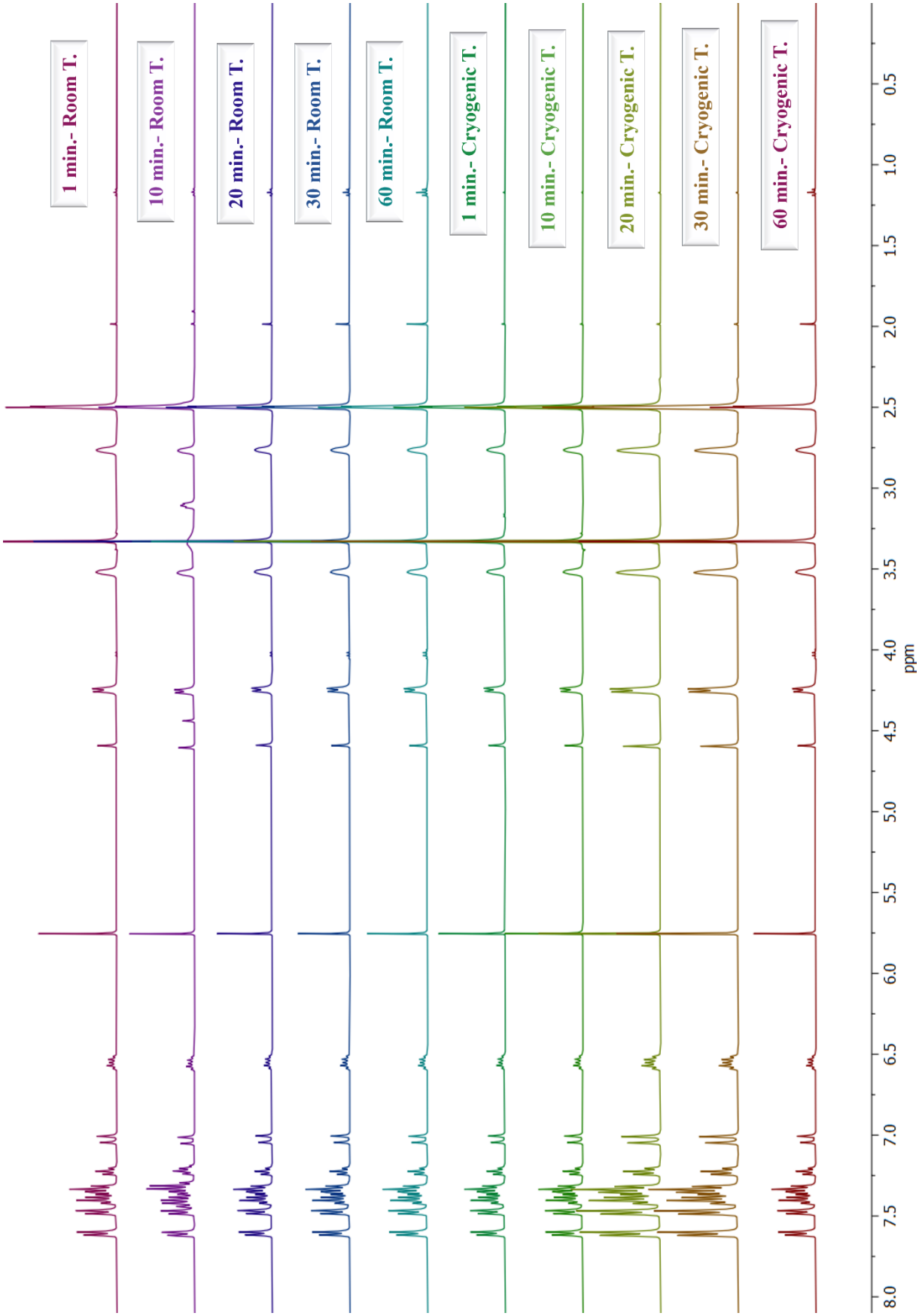

Figure 41: ^{1}H-NMRs of the isolated cinnarizine from all milling experiments

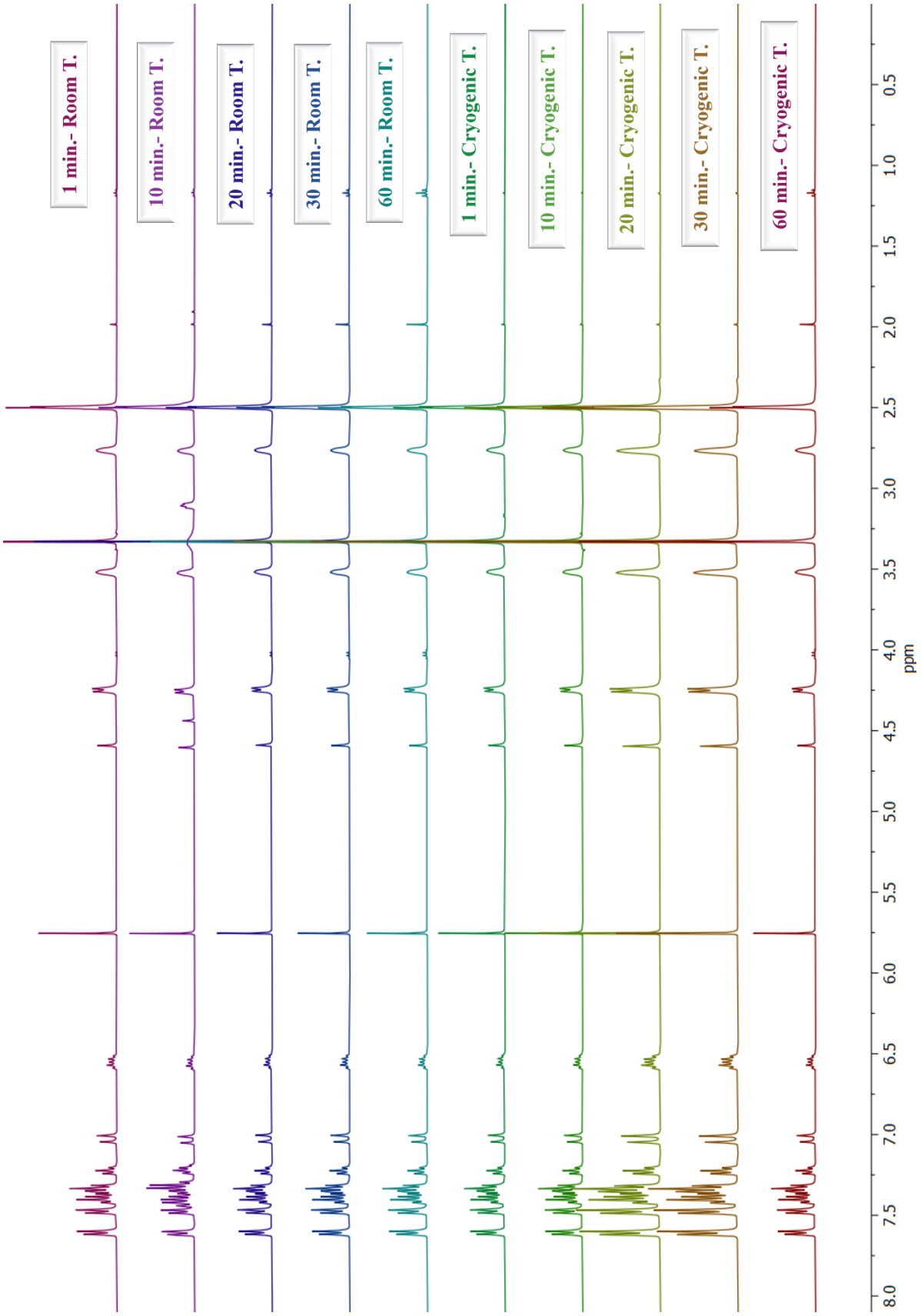

Figure 42: ^{1}H-NMRs of the isolated 4-(Diphenylmethyl)-1,1-bis[(E)-3-phenyl-prop-2-enyl]piperazine Bromide from all milling experiments

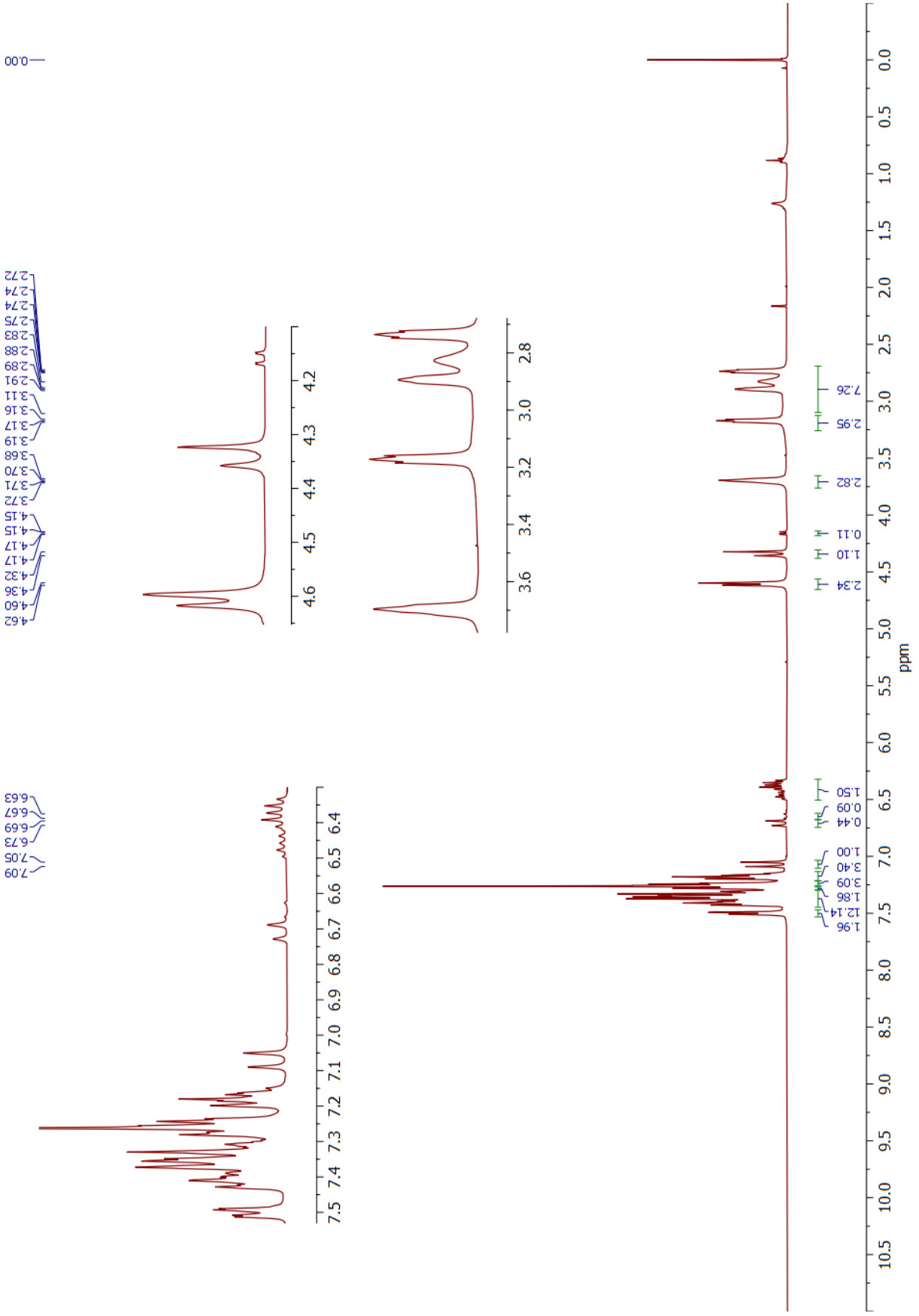

Figure 43: ^{1}H-NMR spectrum of the crude mixture of entry 11 of Table 2 in $CDCl_3$

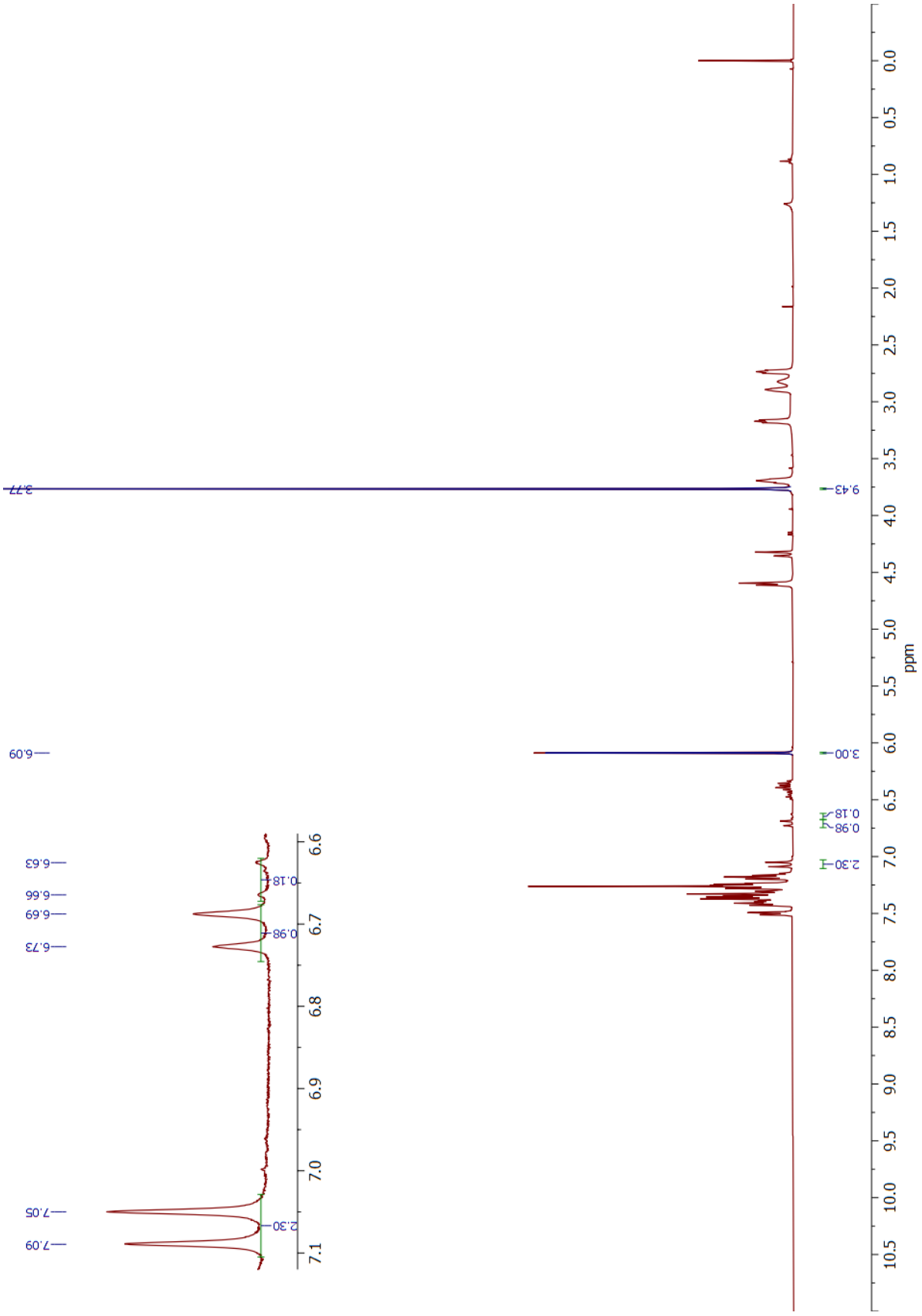

Figure 44: ^{1}H-NMR spectrum of the crude mixture with 1,3,5-Trimethoxybenzene (Internal Standard) of entry 11 of Table 2 in $CDCl_3$

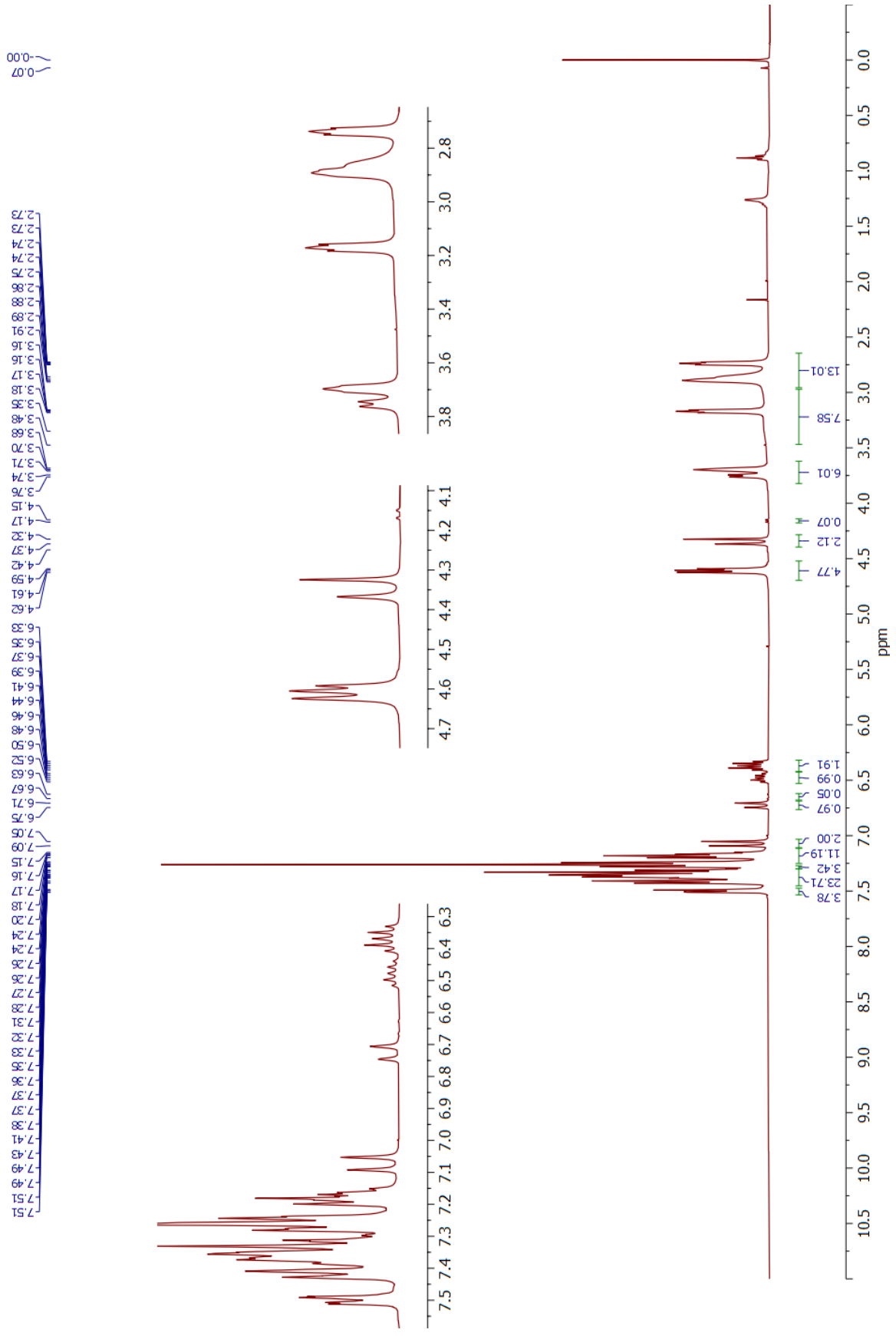

Figure 45: ^{1}H-NMR spectrum of the crude mixture of entry 12 of Table 2 in $CDCl_3$

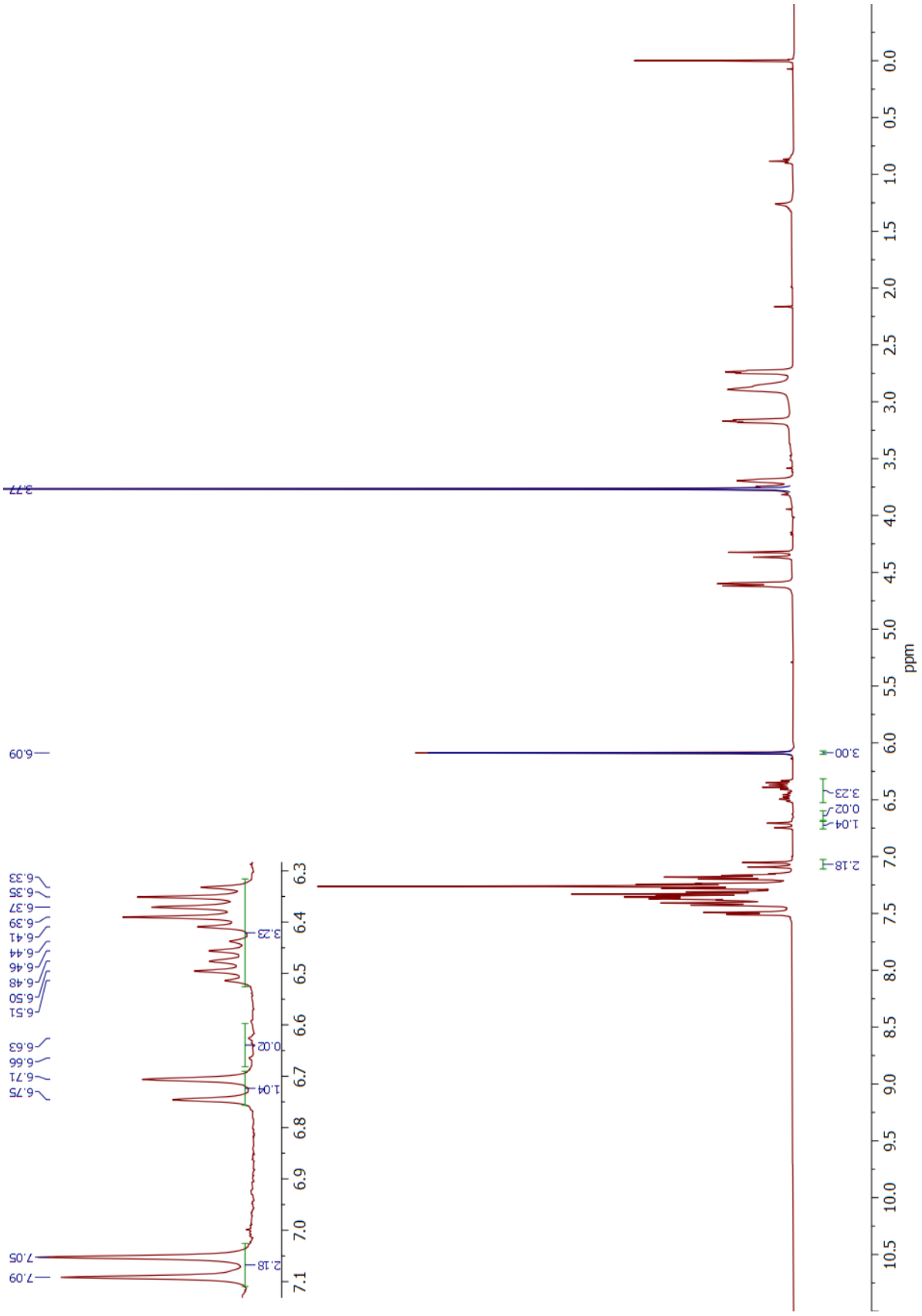

Figure 46: ^{1}H-NMR spectrum of the crude mixture with 1,3,5-Trimethoxybenzene (Internal Standard) of entry 12 of Table 2 in $CDCl_3$

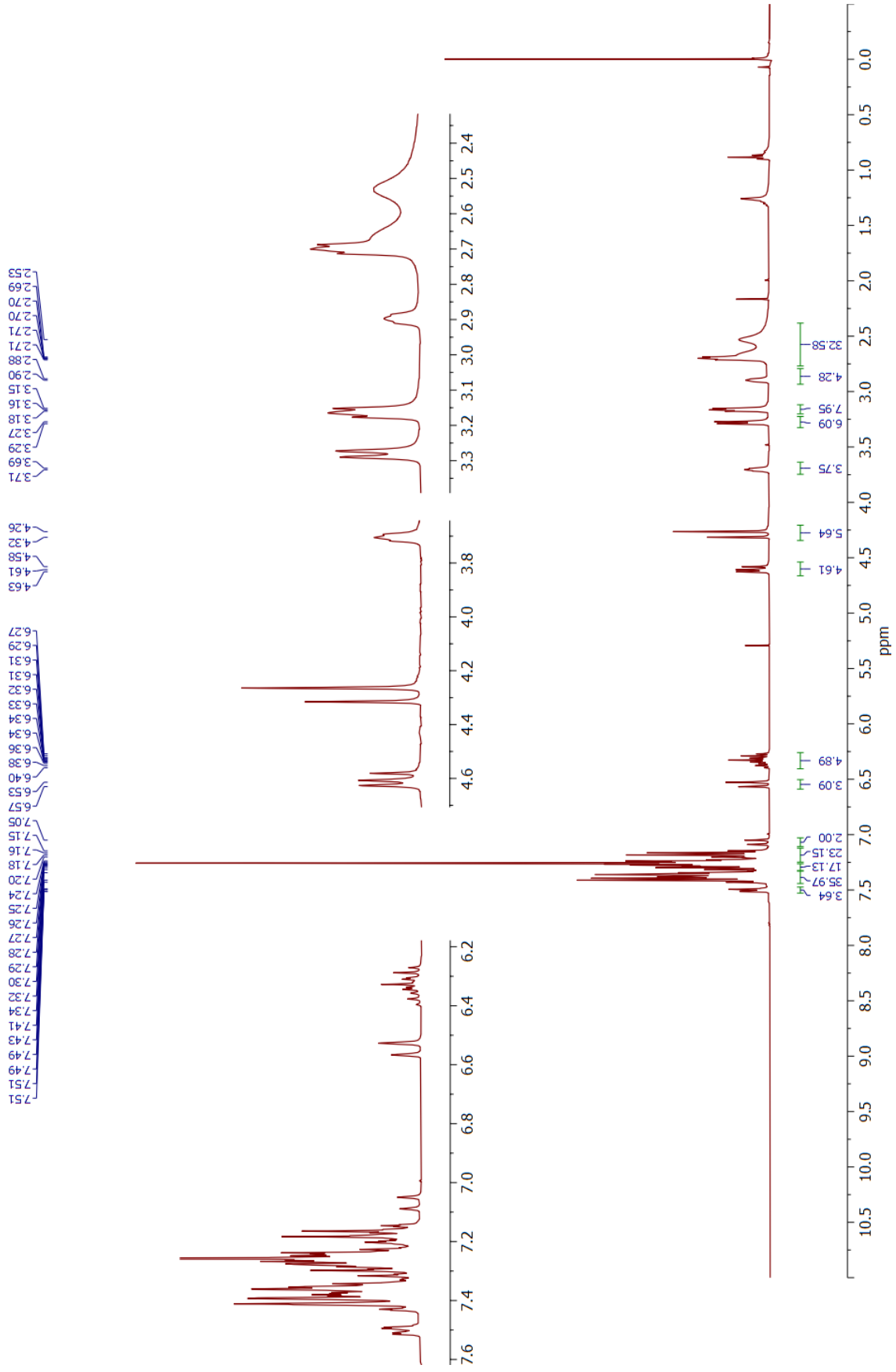

Figure 47: ^{1}H-NMR spectrum of the crude mixture of entry 13 of Table 2 in $CDCl_3$

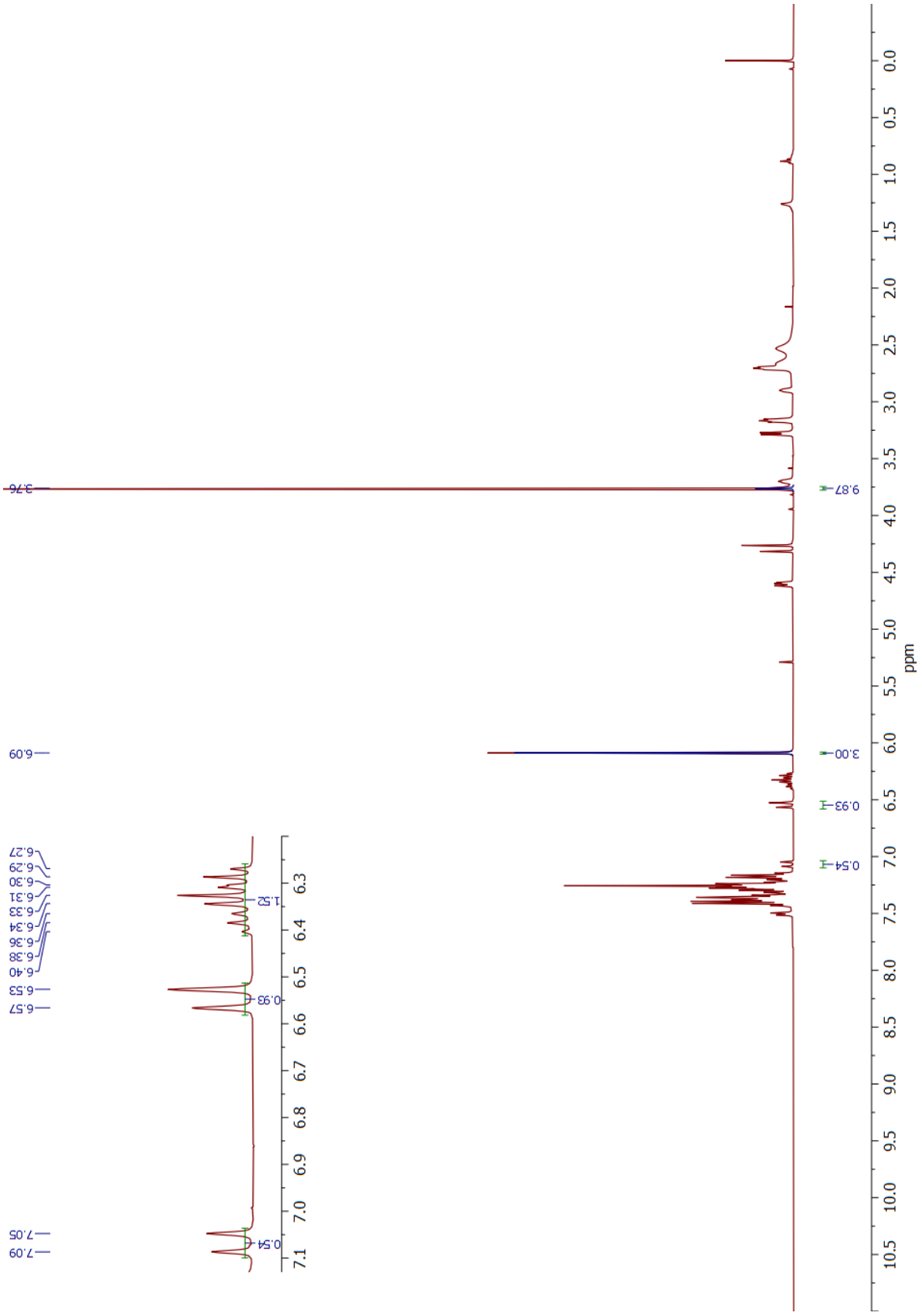

Figure 48: ^{1}H-NMR spectrum of the crude mixture with 1,3,5-Trimethoxybenzene (Internal Standard) of entry 13 of Table 2 in $CDCl_3$

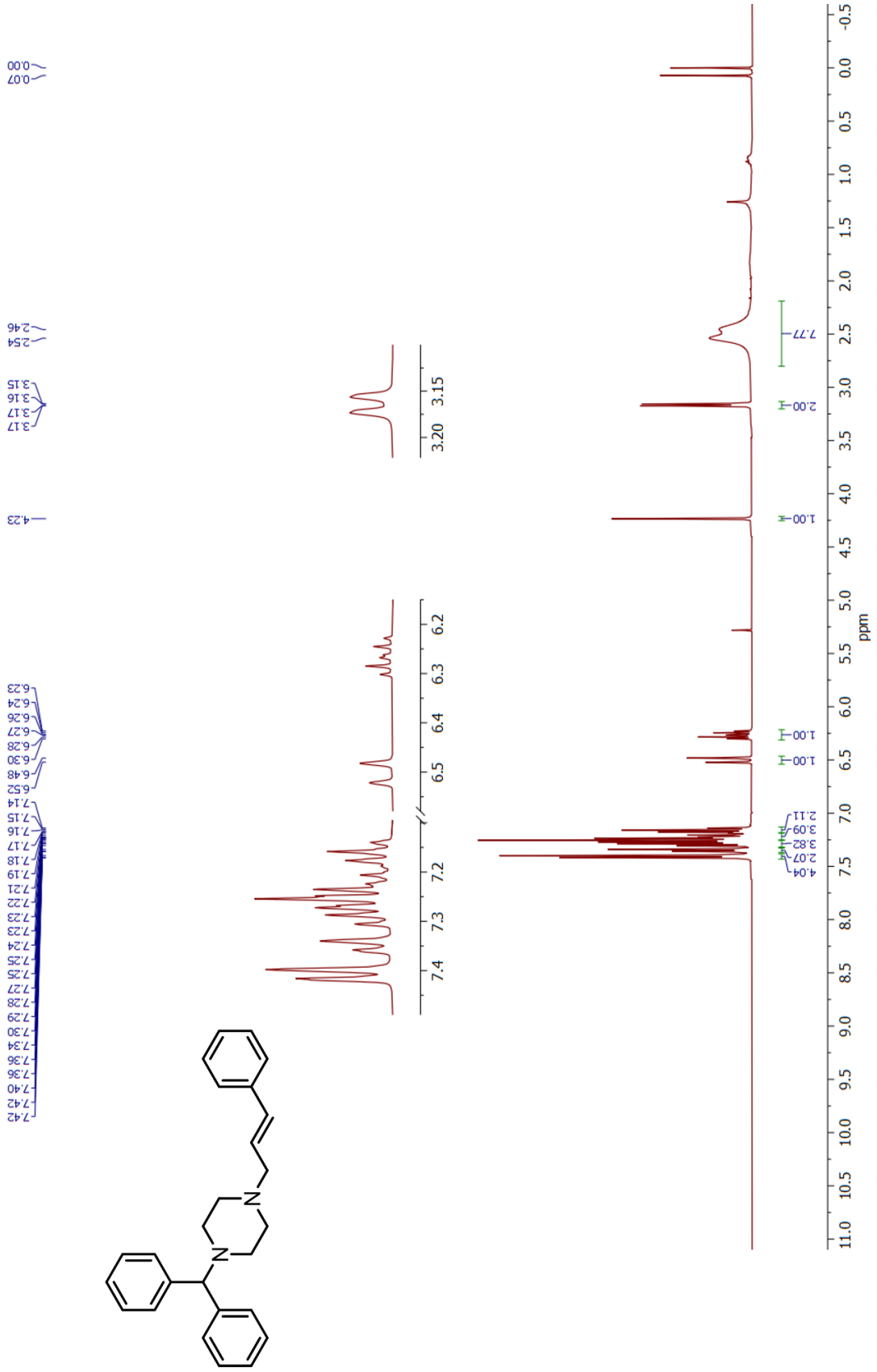

Figure 49: ^{1}H-NMR spectrum of the purified cinnarizine product of entry 8 of Table 2 in $CDCl_3$

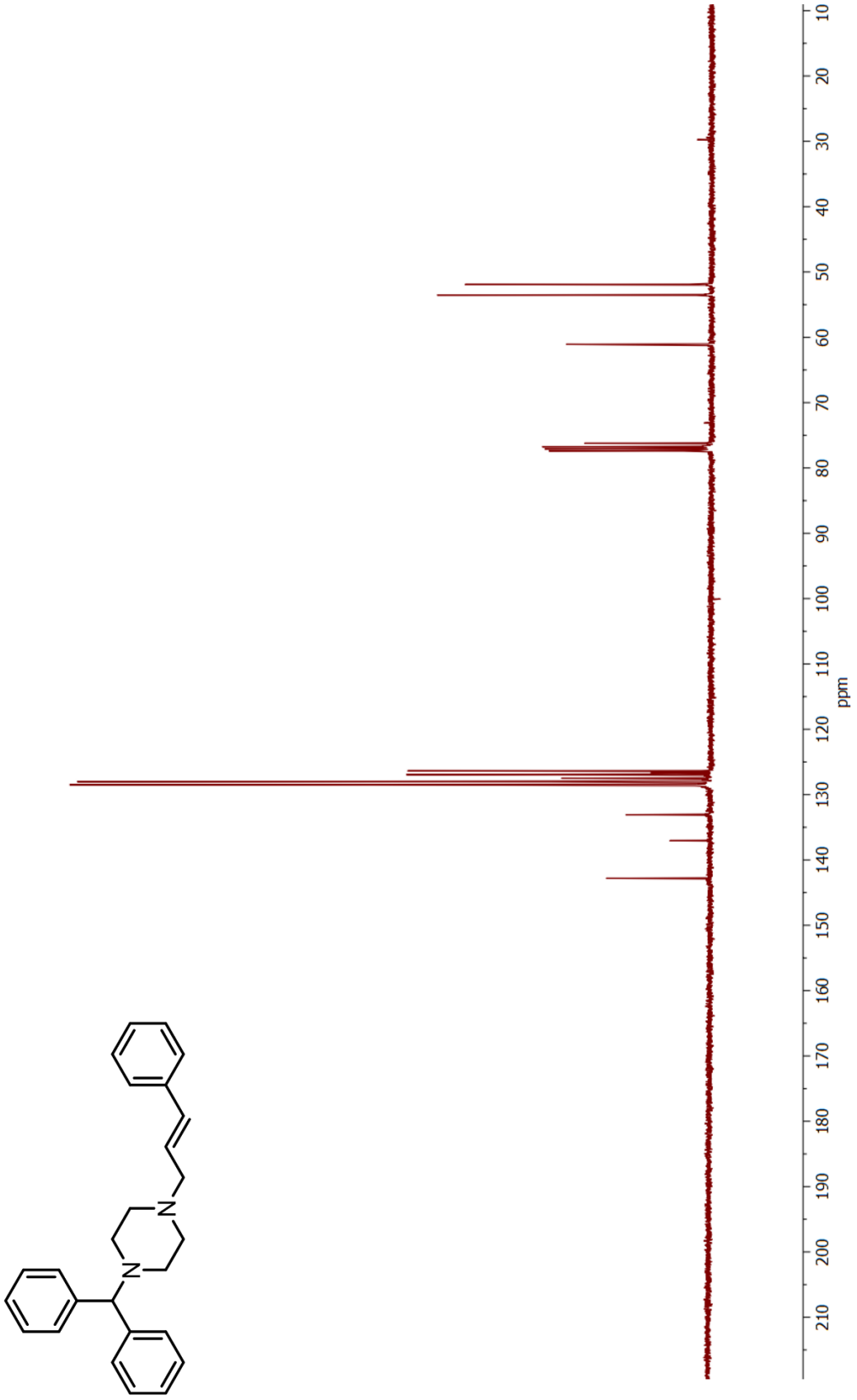

Figure 50: ^{13}C-NMR spectrum of the purified cinnarizine product of entry 8 of Table 2 in $CDCl_3$

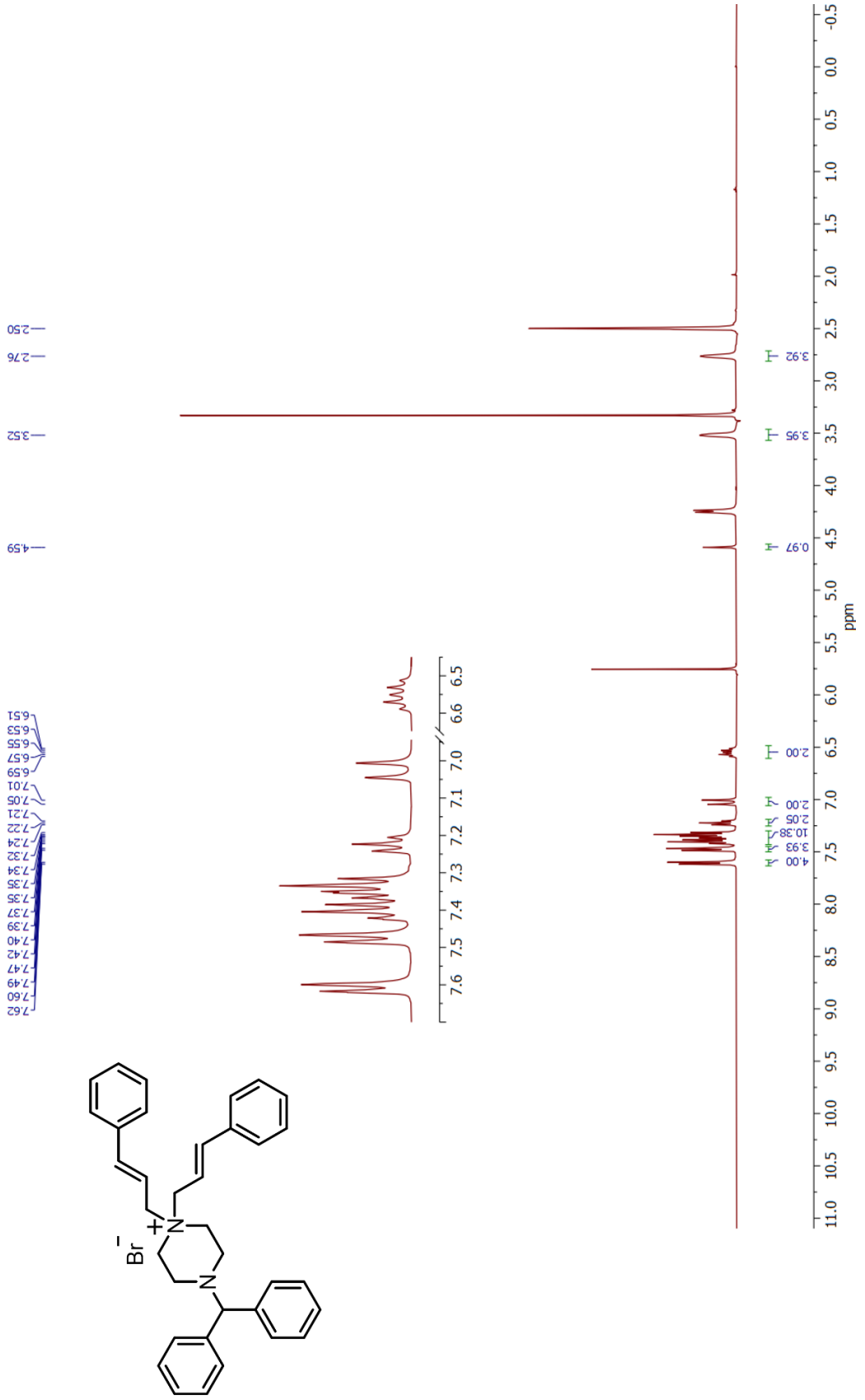

Figure 51: [1]H-NMR spectrum of the purified BIS side product of entry 8 of Table 2 in DMSO-*d6*

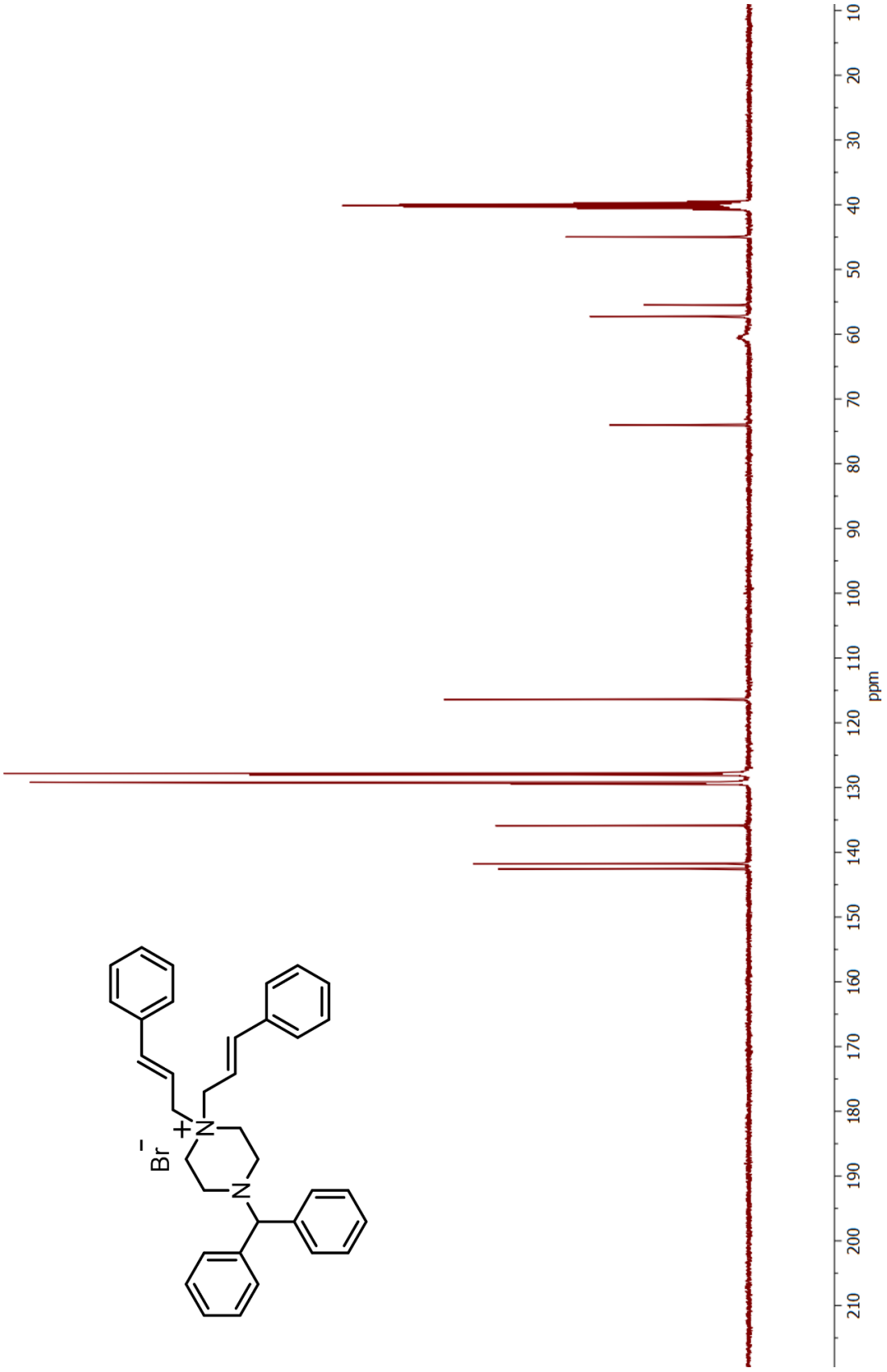

Figure 52: ^{13}C-NMR spectrum of the purified BIS side product of entry 8 of Table 2 in DMSO-d6

6.2. Appendix B: HRMS Spectra

Cinnarizine

Chemical Formula: $C_{26}H_{28}N_2$

Molecular Weight: 368.514

HRMS (APCI+): m/z calculated for $C_{26}H_{28}N_2$ $[M+H]^+$: 369.23253, found: 369.23253, 369.23253, 369.23286, 369.23253, 369.23253, 369.23253, 369.23260, 369.23306, 369.23314, 369.23250, 369.23196,369.23196 respectively for **Figure 53-64**.

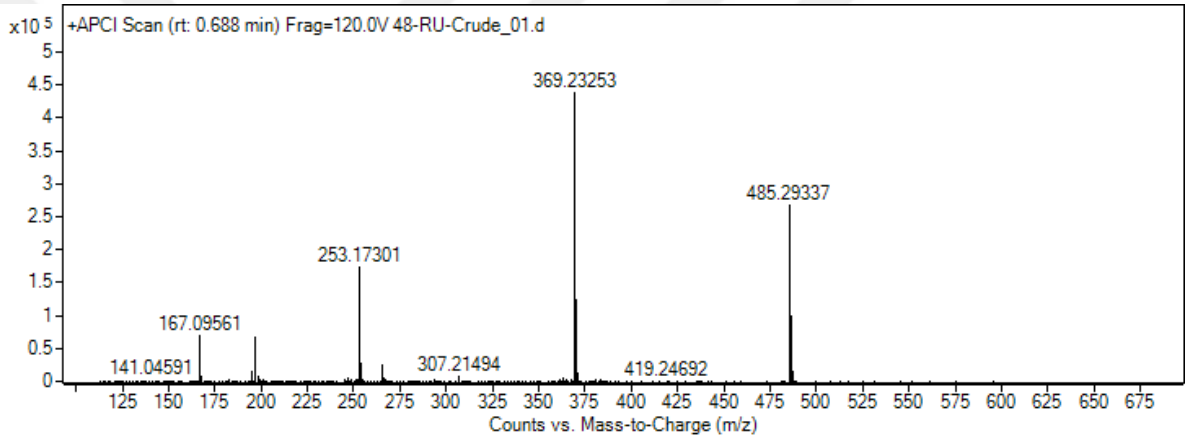

Figure 53: APCI-HRMS spectrum of crude mixture of entry 1 from Table 2

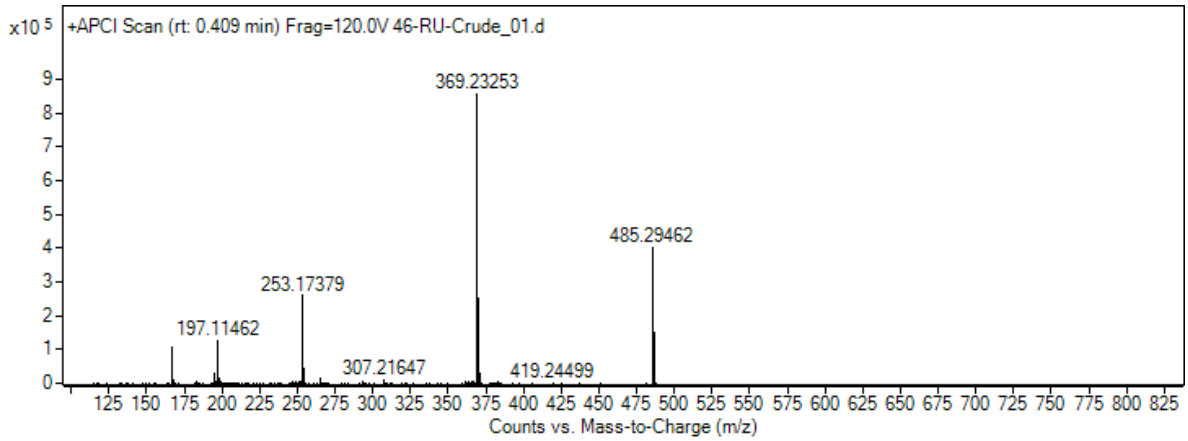

Figure 54: APCI-HRMS spectrum of crude mixture of entry 2 from Table 2

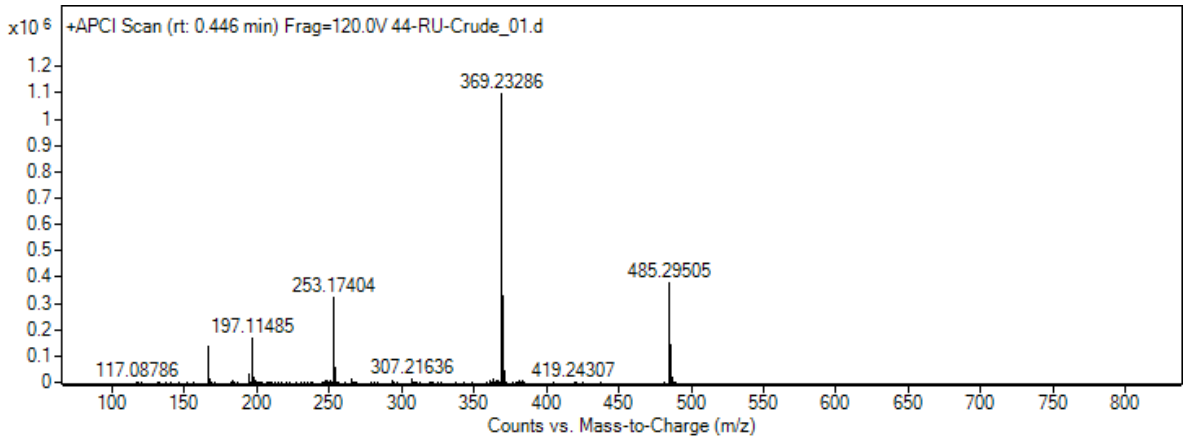

Figure 55: APCI-HRMS spectrum of crude mixture of entry 3 from Table 2

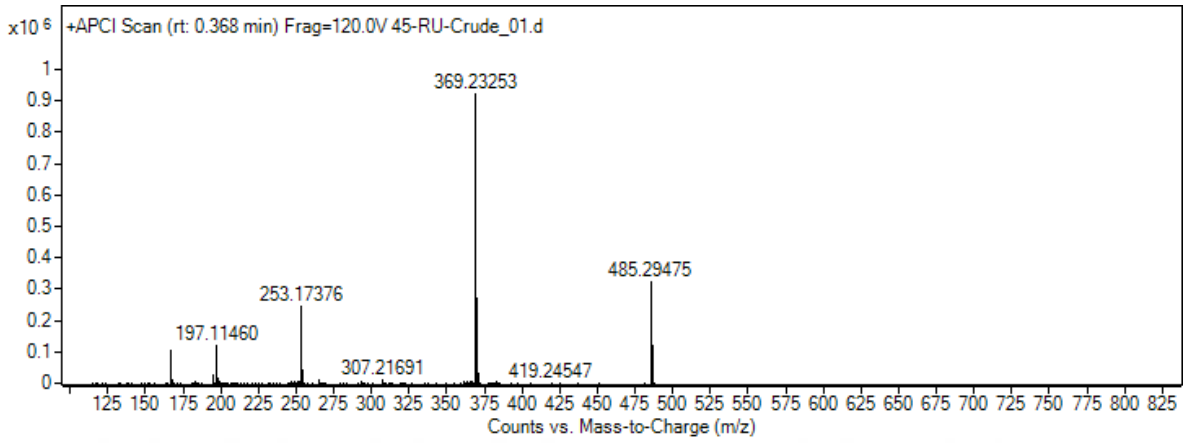

Figure 56: APCI-HRMS spectrum of crude mixture of entry 4 from Table 2

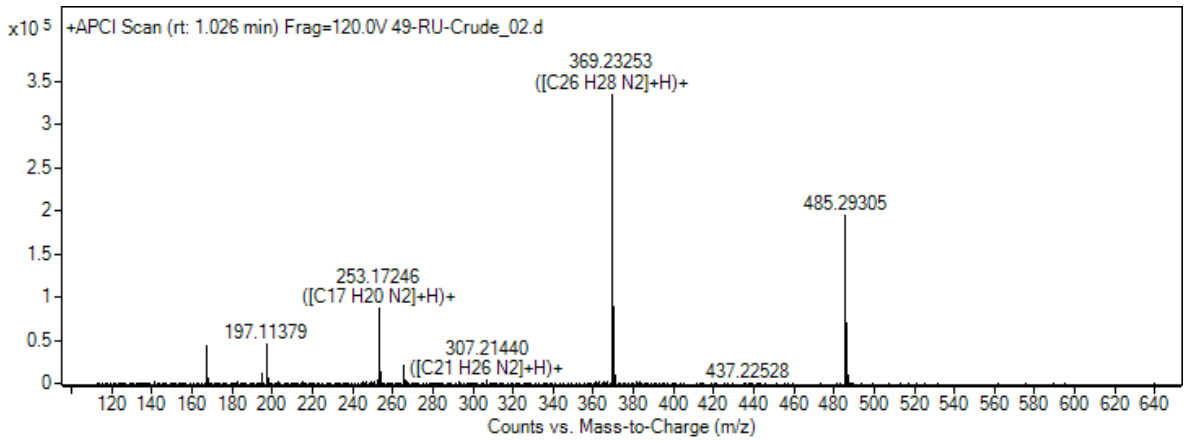

Figure 57: APCI-HRMS spectrum of crude mixture of entry 5 from Table 2

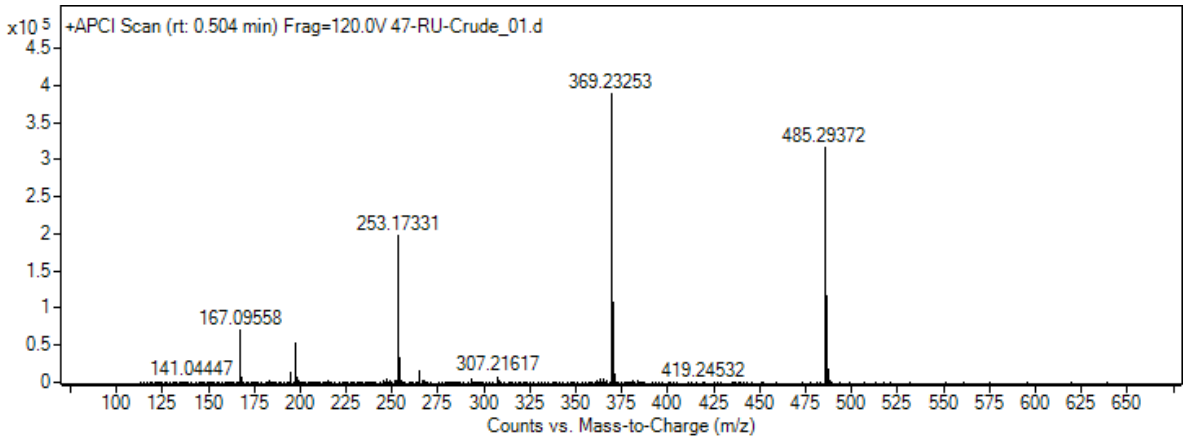

Figure 58: APCI-HRMS spectrum of crude mixture of entry 6 from Table 2

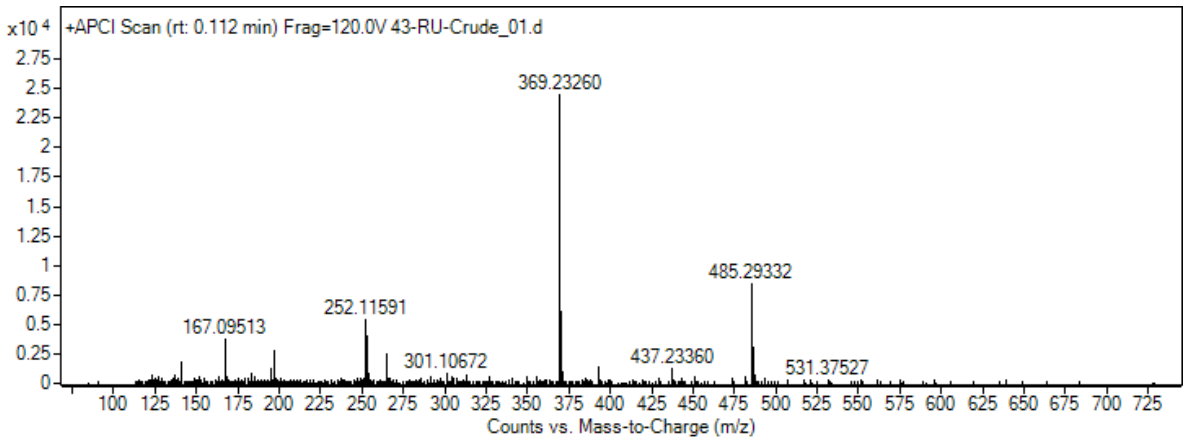

Figure 59: APCI-HRMS spectrum of crude mixture of entry 7 from Table 2

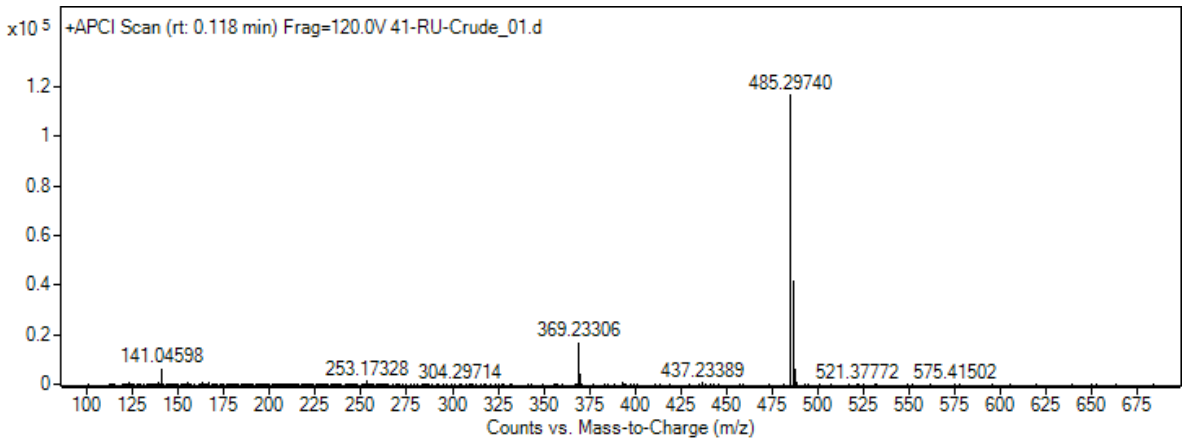

Figure 60: APCI-HRMS spectrum of crude mixture of entry 8 from Table 2

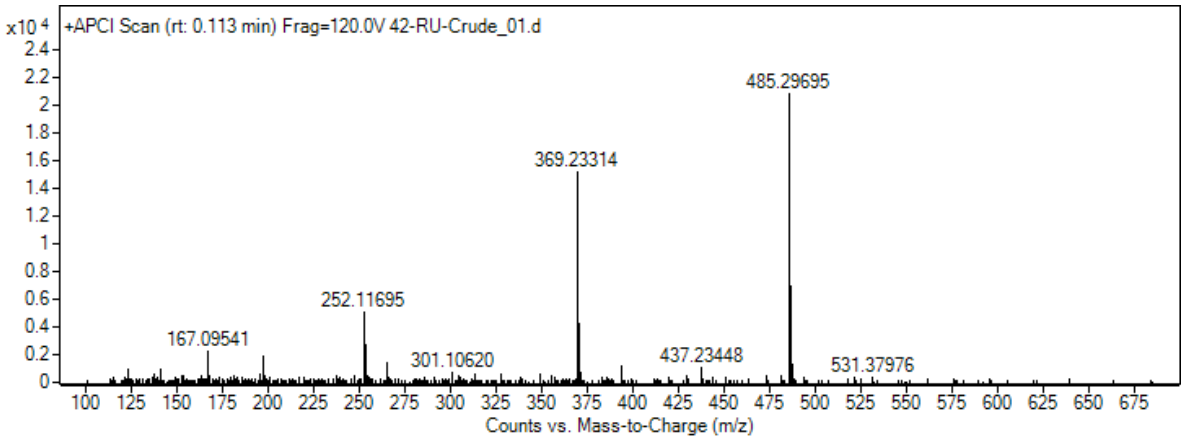

Figure 61: APCI-HRMS spectrum of crude mixture of entry 9 from Table 2

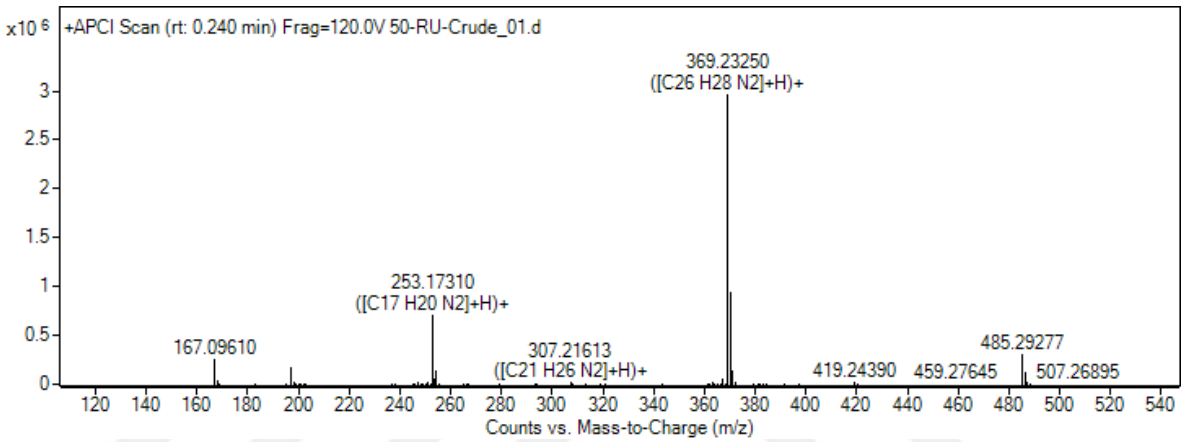

Figure 62: APCI-HRMS spectrum of crude mixture of entry 10 from Table 2

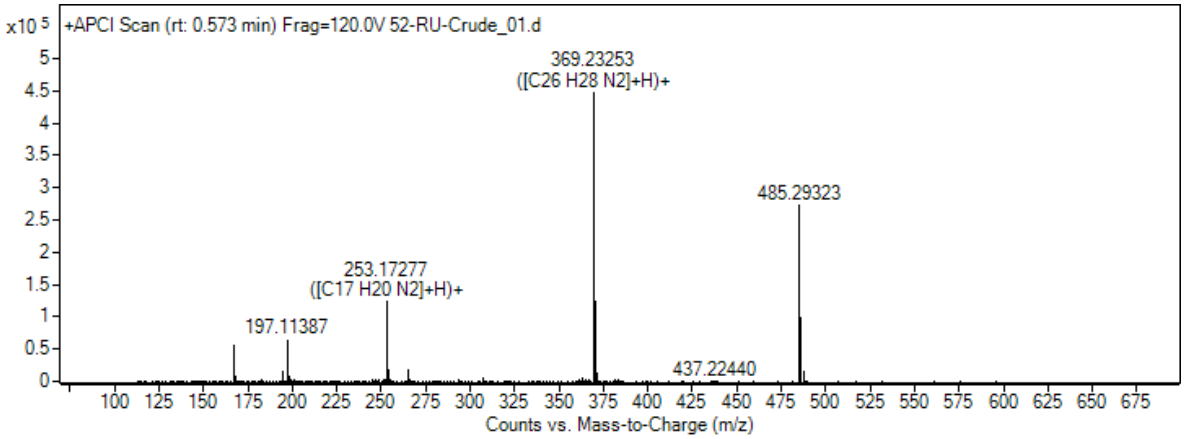

Figure 63: APCI-HRMS spectrum of crude mixture of entry 11 from Table 2

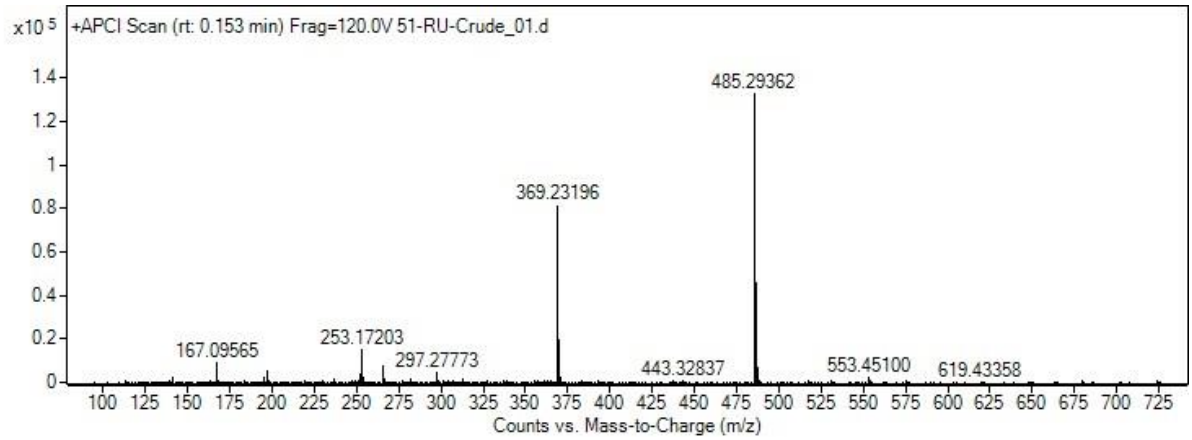

Figure 64: APCI-HRMS spectrum of crude mixture of entry 12 from Table 2

6.3. Appendix C: ATR Spectra

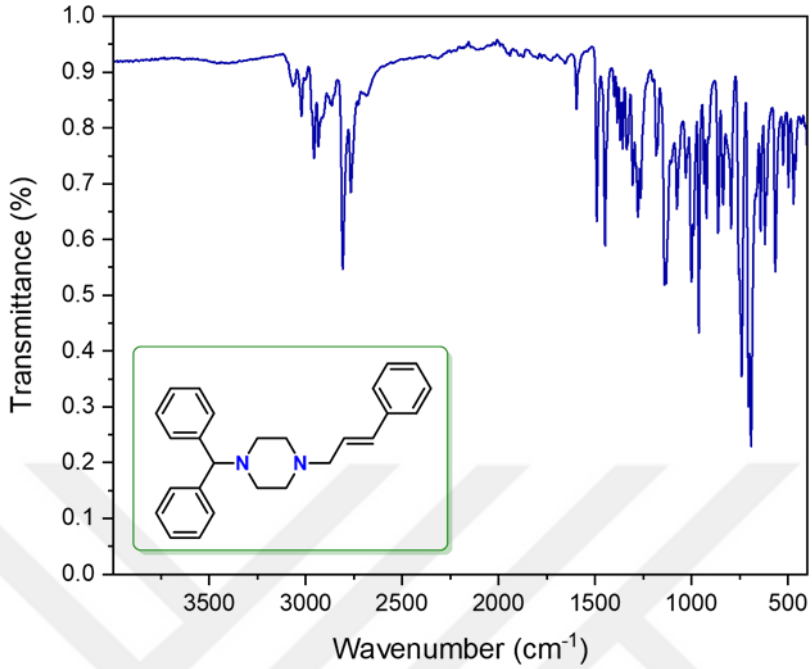

Figure 65: IR spectrum of of the purified cinnarizine product of entry 8 of Table 2

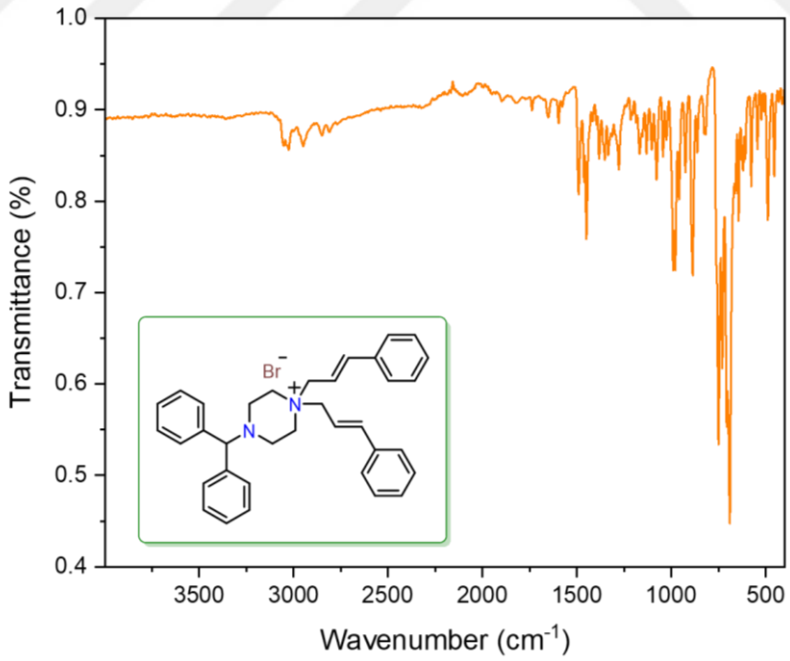

Figure 66: IR spectrum of of the purified BIS side product of entry 8 of Table 2

6.4. Appendix D: Calculations of NMR yields

All the NMR yield calculations of the product (Cinnarizine) and side product (4-(Diphenylmethyl)-1,1-bis[(E)-3-phenyl-prop-2-enyl]piperazine bromide) were done by addition of an Internal Standard (1,3,5-Trimethoxybenzene) with a known weight into the Crude NMR samples.

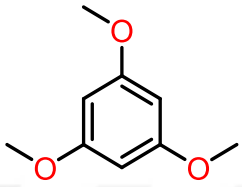

Figure 67: Structure of 1,3,5-trimethoxybenzene

1,3,5-Trimethoxybenzene exhibited 2 characteristic peaks, a singlet with the integral of 3H at 6.08 ppm corresponding to aromatic protons and another singlet with the integral of 9H at 3.77 ppm corresponding to protons on methoxy groups. In all calculations, its signal at 6.08 ppm was considered, and its mol was found for each case since the amount of it added to the crude NMR sample was recorded.

The following equation was used to calculate the NMR yields,

$$\frac{n_P}{n_{IS}} = \frac{(\frac{I_P}{N_P})}{(\frac{I_{IS}}{N_{IS}})} \quad (1)$$

where n_P and n_{IS} were the number of moles of product and internal standard respectively, I_P and I_{IS} referred to the integration of the unique product and internal standard signals, respectively, and N_P and N_{IS} indicated the number of protons corresponded to the chosen signal.

The following is an example of the calculations performed to obtain an estimate product yield out of crude mixture. Doublet signals around 6.70 ppm were

selected as unique product peak and its integral is used as I_P in the calculations. For the cases, in which there are 3 or 4 peaks were existed that region, to increase reliability of the calculations, NMR yield of cinnamyl bromide remained inside the crude were calculated and subtracted from the NMR yield of product, cinnarizine, because both have doublets coming from the same region and there is a probability of signal overlapping. This way was applied because no other unique peak of the product was found and assigned in the crude NMR. For cinnamyl bromide, doublet of doublet signal at 4.16 ppm was opted. For the internal standard, singlet at 6.08 ppm was preferred and it's integral is used as I_{IS}. Additionally, with the same method, NMR yield (%) for side product, BIS, was determined. Doublet signals at 7.07 ppm was chosen as the unique peak only coming from BIS and calculations are made accordingly.

It is important to note that the margin of error could be high for NMR yields due to weighing internal standards in less than 10 milligrams and the difficulty of assigning product signals in crude mixture NMR spectra.

Calculations for Entry 8 of Table 2

6.20 mg of internal standard was added to the crude NMR sample.

$$MW_{IS} = 168.19\ g/mol$$

$$n_{IS} = (6.20\ \times\ 10^{-3})g\ \times MW_{IS} =\ 3.68 \times\ 10^{-5}\ mol$$

$$\frac{n_P}{0.0191\ mmol} = \frac{\left(\frac{I_P}{N_P}\right)}{\left(\frac{I_{IS}}{N_{IS}}\right)} = \frac{\left(\frac{0.44}{1\text{H}}\right)}{\left(\frac{3.00}{3\text{ H}}\right)}$$

$$n_P = 0.016192\ mmol = 1.61\ \times 10^{-4}\ mol$$

$$MW_P = 368.514\ {}^{g}/_{mol}$$

$$n_P \times MW_P = 0.0059669\ g = 5.9669\ mg$$

In the NMR sample, 20.07 mg crude was used and the total crude after dried in the vacuum was recorded as 389.5 mg. From the proportions,

$$\frac{19.42\ mg}{403.04\ mg} = \frac{5.96\ mg}{x\ mg}$$

$$x = 123.84\ mg\ of\ product\ estimated\ in\ the\ crude$$

Therefore, the NMR yield percent was found,

$$\frac{123.84\ mg}{403.0\ mg} \times 100 = 30.7\ \%$$