A Thesis for the Degree of Ph.D. in Science

The Total Synthesis of Ikoamide and Odookeanynes, Highly *N*-Methylated Lipopeptides from Marine Cyanobacteria

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Contents

Abbreviations

Ac	acetyl
ACT	artemisinin-based combination therapy
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
Bu	butyl
COMU	ethyl 2-cyano-2-((dimethyliminio)(morpholino)methyloxyimino) acetate hexafluorophosphate
DCC	*N,N*′-dicyclohexylcarbodiimide
DCM	dichloromethane
DIPEA	*N,N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMP	Dess–Martin periodinane
DMSO	dimethyl sulfoxide
EDCI	3-[(ethylimino)methylidene]amino-*N,N*-dimethylpropan-1-amine
Et	ethyl
Fmoc	fluorenylmethoxycarbonyl
GIR	growth inhibition rate
HATU	1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate
HBTU	1-((dimethylamino)(dimethyliminio)methyl)-1*H*-benzo[d][1,2,3]triazole 3-oxide hexafluorophosphate
HMPA	hexamethylphosphoric triamide
HPLC	high performance liquid chromatography
HWE	Horner–Wadsworth–Emmons
KAHA	α-ketoacid-hydroxylamine
LAH	lithium aluminium hydride
Me	methyl
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NaHMDS	sodium hexamethyldisilazide
NMR	nuclear magnetic resonance
NRP	nonribosomal peptide
Ph	phenyl
PyBOP	(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
R	alkyl

SAR	structure–activity relationship
SPPS	solid phase peptide synthesis
TBS	*tert*-butyldimethylsilyl
TBTU	2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
TES	triethylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin-layer chromatography
TMS	trimethylsilyl
Ts	*p*-toluenesulfonyl

Chapter 1. Introduction

1-1. Natural organic compounds from marine organisms

The oceans have been closely intertwined with humanity since ancient times, serving various functions. As human activities increase in volume and quality, so does the utilization of the vast oceanic resources. Especially in recent years, with the progress of various investigations and studies on the oceans, people have become fascinated and intrigued by marine natural products with advanced functionalities. Natural products can be described as "compounds produced by living organisms." A group of compounds that are widely present and control the physiological functions of organisms is called primary metabolites, which are biosynthesized and metabolized by organisms for their survival. On the other hand, there is also a diverse group of substances called secondary metabolites, which represent the species of organisms and sometimes even individuals. Many marine secondary metabolites not only possess unique molecular structures but also exhibit incredibly potent biological activities. These marine natural products, which are distinctive in structure and biological activity, continue to impact organic chemistry and biology significantly.

The most notable discovery in marine natural product drug research to date can be traced back to the identification of nucleic acid analogs in sponges in 1950.[1] The investigation of novel nucleic acid-related compounds isolated from washed-up sponges led to the concept of using nucleic acid analogs as potential anticancer and antiviral drugs, garnering attention as potential antiviral agents against coronaviruses. However, this early accidental success did not have a follow-up, and subsequent research efforts have often been limited by the scarcity of natural supply and the challenges associated with evaluating their biological activities on a limited scale. Furthermore, artificially culturing marine organisms under laboratory conditions remains highly challenging due to their reliance on natural environments. This signifies that the development of marine natural compounds as pharmaceuticals is a highly challenging research concept, and unless both the obstacles of "discovery of candidate substances" and "ensuring an adequate supply" are overcome simultaneously, even getting off the starting line may be unattainable.

Therefore, providing a stable supply has always been a challenge in marine natural compound research, and organic synthetic chemistry has been utilized to address this issue. Organic synthetic chemistry enables the total synthesis of natural products to provide a quantitative supply. It facilitates the exploration of chemical biology using

natural products, such as preparing artificial analogs.

For example, in 1980, Kenneth L. Rinehart from the University of Illinois initiated chemical studies on *E. turbinata* and isolated ecteinascidins (Et743) as an anticancer component (Figure 1). Early studies found the active ingredient to be unstable and difficult to isolate, and several analogs were obtained before their structures were reported in 1990.[2] Ecteinascidins are isoquinoline alkaloids with structural similarities to the existing bacterial metabolite prodigiosin, which is also known for its structural motif in alkaloids derived from sponges, such as renieramycins. Therefore, considering the efficient purification of ecteinascidins from a large amount of tunicates, the authors developed a method that involved solvent fractionation, affinity partition chromatography, and open-column techniques, minimizing the use of HPLC. Subsequently, the Spanish company PharmaMar, involved in developing ecteinascidins, successfully provided compounds for clinical trials using cultured tunicates. The development of large-scale isolation methods has advanced further research on ecteinascidins. However, these methods still involve the isolation of compounds from natural or artificially cultivated marine organisms. In 1996, E. J. Corey and his team successfully achieved the total synthesis of the highly valuable anticancer drug ecteinascidin 743.[3]

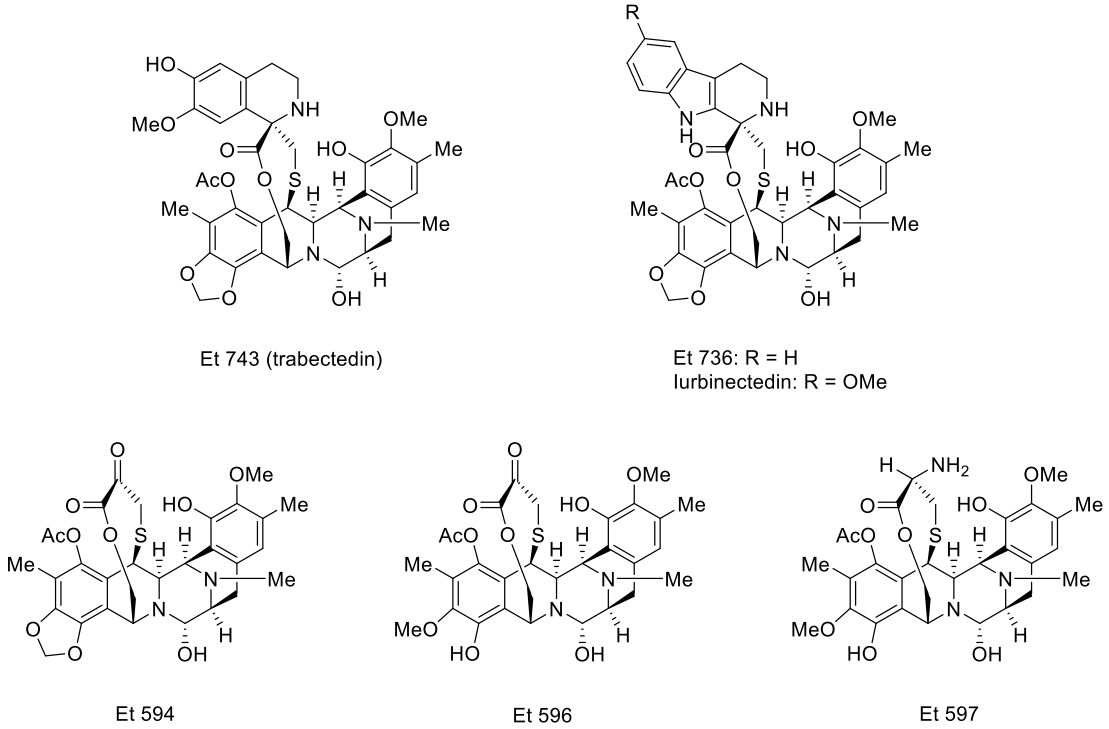


Figure 1. The structures of ecteinascidin 743 and its analogues.

Didemnin B is a cyclic peptide discovered by Rinehart from the Caribbean tunicate *Tridemnum solidum*.[81] *T. solidum* is a colonial ascidian that often covers corals in the shallow waters of the Caribbean reefs and is sometimes considered a harmful species. Rinehart and his colleagues conducted clinical trials of didemnin B as an anticancer agent in the early 1980s, and it was considered the top marine natural product for drug development (Figure 2). However, clinical trials were terminated due to the strong toxicity observed in phase 2 trials. Later, they discovered a derivative of didemnin B called dehydrodidemnin B (plitidepsin) in the Mediterranean tunicate *Aplidium albicans*. It was simply a conversion of the lactate at the end of didemnin B to pyruvate, but it exhibited more potent antitumor activity and even lower toxicity than didemnin B. In 1997, dehydrodidemnin B was successfully synthesized through total synthesis by Ernest Giralt and his team.[4] With the achievement of large-scale production through total synthesis, dehydrodidemnin B (commercially known as Aplidin) was developed as an anticancer drug and has been partially used for multiple myeloma. Plitidepsin inhibits protein synthesis in cancer cells by inhibiting a protein called EF-1A2,[5] which is required for ribosome translocation of aminoacyl-tRNAs during mRNA translation.[6] Interestingly, it has been reported that plitidepsin effectively treats novel coronavirus infections.[7] Since coronavirus replication is more sensitive to the process involving EF-1A2 than host cells, it exhibits potent anti-SARS-CoV-2 activity. These examples not only demonstrate the importance of organic synthesis chemistry but also highlight the significant potential of chemical biology and drug development.

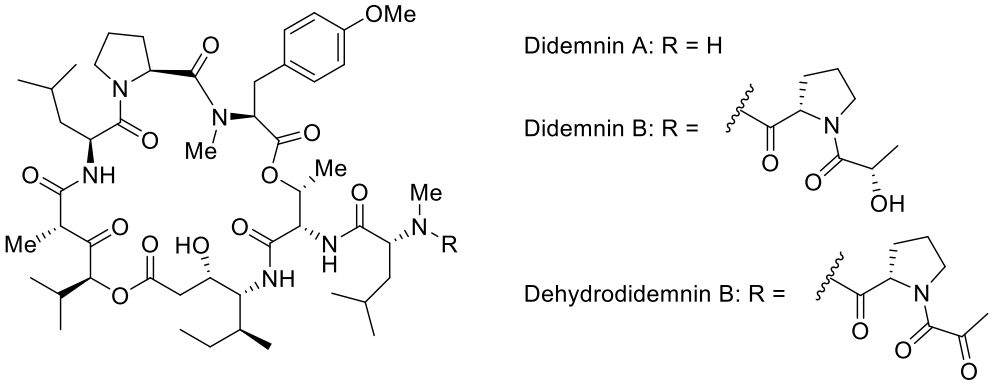


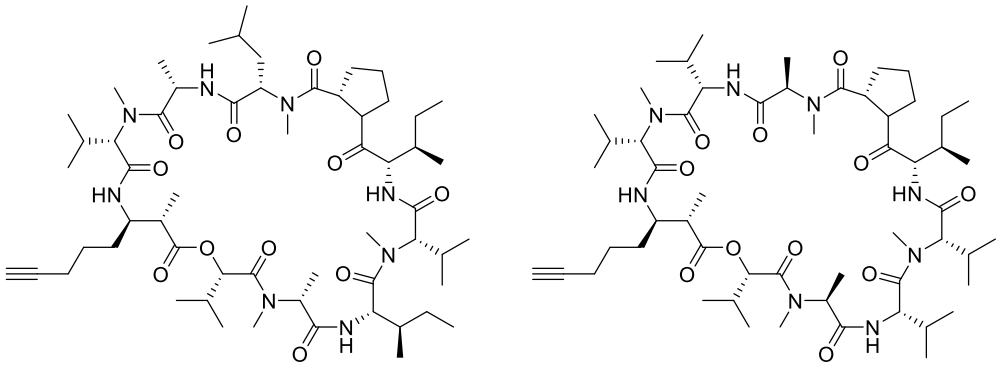
Figure 2. The structures of didemnin B and its analogues.

1-2. Cyanobacteria-derived peptides

Over the past 50 years, numerous compounds with astonishing and unique biological activities have been discovered from marine cyanobacteria originating from diverse habitats. These natural compounds exhibit antibacterial, antiviral, antifungal, enzyme inhibition, immunostimulant, cytotoxic, anti-plasmodial, anti-trypanosomal, anti-leishmanial, and insecticidal activities.[8,9,10] Among these antibiotic compounds, peptides and polyketide structural elements dominate.[11] These cyanobacteria-derived peptides can be mainly categorized into four distinct classes: depsipeptides, lipopeptides, cyclamides, and cyclic peptides. Depsipeptides and lipopeptides possess linear or cyclic structures, where a lipophilic fatty acid, aromatic acid, or glycosylated hydrocarbon tail is attached to the N-terminus of a short oligopeptide. These compounds are typically synthesized by nonribosomal peptide (NRP) synthetases or hybrid polyketide-NRP synthetases. However, in depsipeptides, one or more amide groups are substituted by ester groups.[12,13] Cyclamides are extensively modified peptides derived from ribosomes. This category includes hexameric and octameric cyclopeptides composed of alternately arranged hydrophobic and hydrophilic amino acids. The side chains can undergo heterocyclization, forming rings such as oxazole, oxazoline, thiazole, or thiazoline, resulting in relatively flat and disk-shaped structures.[14,15] Therefore, based on the origin, structural sequence, and antibiotic activity differences of cyanobacteria-derived peptide products, through the study of structure-activity relationship (SAR), it is possible to explore and develop new potential drugs or biocontrol agents, even through total synthesis.

1-2-1. Depsipeptides

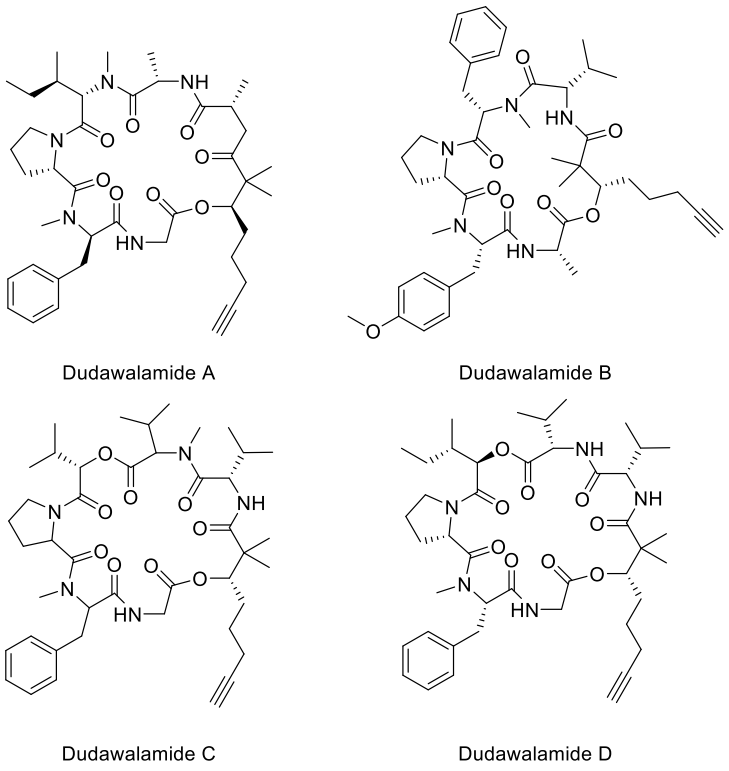
Since 2000, numerous depsipeptide natural products have been reported. For example, two cyclic depsipeptides, companeramides A and B, were isolated from *Leptolyngbya* spp. derived from coral reef limestone (Figure 3). These compounds share similar structural motifs, consisting of β-amino alkyoic acids and α-hydroxy alkanoic acid. These compounds exhibit distinct antimalarial activities against chloroquine-sensitive (D6) and chloroquine-resistant (Dd2 and 7G8) strains of *Plasmodium falciparum*, with IC_{50} values of 220-1100 nM, less than the chloroquine control, but without significant cytotoxicity against mammalian cells.[16]



Companeramide A Companeramide B

Figure 3. The structures of companeramides A and B.

Similarly, dudawalamides A–D, a family with a β-hydroxy alkynyl acid (Dhoya) from shallow marine habitat-derived *M. producens* (Figure 4),[17] exhibit broad spectrum activity against *P. falciparum*, *Leishmania donovani* and *Trypanosoma cruzi*. Interestingly, dudawalamides A and D show the most potent activity against *Plasmodium falciparum* (IC_{50} = 3.6 and 3.5 μM, respectively), while compound D is relatively effective against the other two parasites with IC_{50} values of 2.6 μM and a growth inhibition rate (GIR) of 60% at 10 μg/mL. Intriguing SAR analysis suggests that minor variations in residue configuration and sequence strongly influence the biological activity of these depsipeptides.



Dudawalamide A Dudawalamide B

Dudawalamide C Dudawalamide D

Figure 4. The structures of dudawalamides A–D.

Lagunamides A-C are members of the aurilide class isolated from the benthic shallow water-maintained *L. majuscule* (Figure 5).[18,19] These compounds exhibit significant antimalarial properties, with IC_{50} values of 0.19, 0.91, and 0.29 μM against *P. falciparum*, and weak anti-swarming activity against *Pseudomonas aeruginosa* at 100 ppm. Interestingly, the small differences between lagunamides A and B, reflecting variations in the polyketide moiety, result in enhanced antimalarial activity. Furthermore, it was found that the antimalarial activity of lagunamide A is similar to that of dolastatin 15 but lower than dolastatin 10.[82]

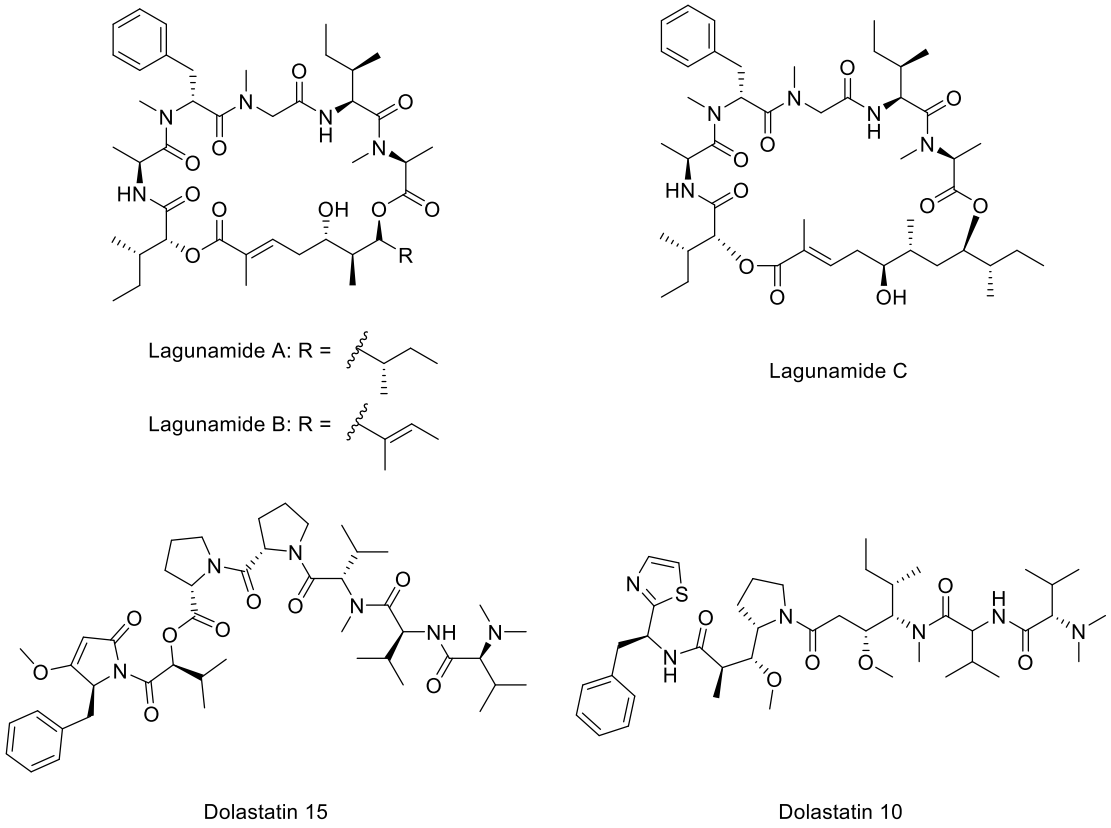


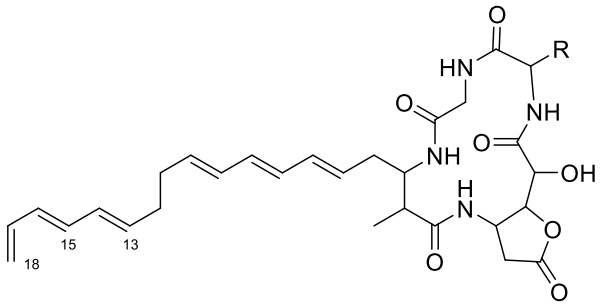
Figure 5. The structures of lagunamides A–C and dolastatins 15 and 10.

1-2-2. Lipopeptides

Lipopeptides are small molecules with diverse functions secreted into natural environments, where they may serve defensive roles against competing organisms or aid in interacting with the environment by establishing and maintaining symbiotic relationships in mixed habitats. Lipopeptides are amphiphilic molecules composed of short linear or cyclic peptides, linked to fatty acids through ester or amide bonds. The chain length of the fatty acid can vary, with or without various substituents, particularly hydroxyl or methyl branches. Amino acids in lipopeptides comprise proteinogenic amino acids, modified amino acids, and non-proteinogenic amino acids.

Due to the accelerated development of antibiotic resistance in bacteria and fungi as a result of the excessive use of broad-spectrum antibiotics to control human and plant pathogens, lipopeptides with antimicrobial activity (bacteriocins) have now gained significant interest as part of the search for new antibiotics. They present a considerable challenge in medicinal chemistry. Many bacteriocins have been shown to have broad-spectrum activity as they can rapidly kill bacteria and demonstrate synergistic effects with established antibiotics. However, challenges remain in translating research findings into clinical practice.

Taking cyclic lipopeptides as an example, anabaenolysins A–D were isolated from three benthic *Anabaena* strains (Figure 6).[20,21] These cyanobacterial lipopeptides consist of 2-(3-amino-5-oxotetrahydrofuran-2-yl)-2-hydroxyacetic acid and a polyunsaturated C_{18} β-amino acid. Biological assays have shown that they target cell membranes in an ergosterol-dependent manner and exhibit weak antifungal activity against *C. albicans*, *Aspergillus flavus* and *Aspergillus parasiticus*.[21,22] Surprisingly, the addition of hydrophilic cyclodextrin to hydrophobic anabaenolysins effectively enhances their antifungal activity.[21]



Anabaenolysin A: Δ^{13}, Δ^{15} saturated
Anabaenolysin B: Δ^{13}, Δ^{15}, Δ^{17} saturated
Anabaenolysin C: Δ^{17} saturated
Anabaenolysin D: Δ^{15}, Δ^{17} saturated

Figure 6. The structures of anabaenolysins A–D.

Brunsvicamides A–C were isolated from waste water by *Tychonema* sp (Figure 7).[23] The basic structure of brunsvicamides A and B is similar to those of mozamides, with only a methyl group difference, while brunsvicamide C differs in the *N*-methyl-*N′*-formylkynurenine moiety, which is believed to be derived from tryptophan through oxidation. These compounds inhibit *M. tuberculosis* protein tyrosine phosphatase B, a potential drug target for tuberculosis treatment (with IC_{50} values of 7.3 and 8.0 μM for brunsyicamides B and C, respectively). Analysis of a series of brunsvicamide analogs with different amino acid structures and stereochemistry indicates that D-Lys and L-Ile are crucial for inhibition.[24]

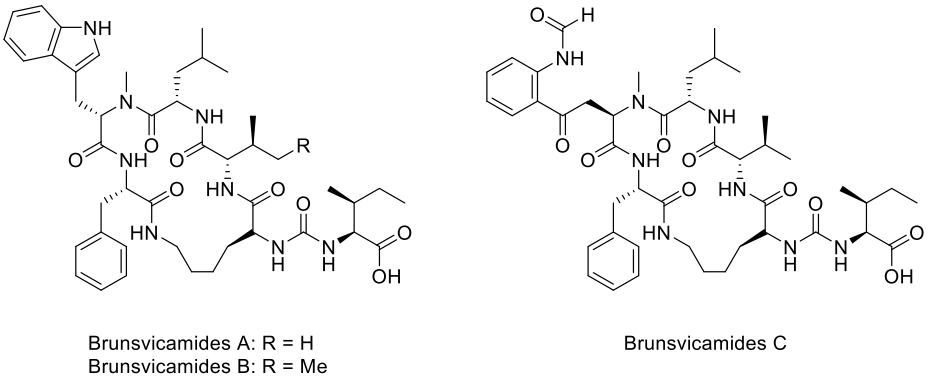


Figure 7. The structures of brunsvicamides A–C.

Another composition of lipopeptides is linear lipopeptides. Although cyclic lipopeptides offer higher rigidity and stability compared to linear lipopeptides, linear lipopeptides possess greater flexibility. Among the lipopeptides discovered so far and synthesized through total synthesis, linear lipopeptides constitute the majority. For example, carmabin A, dragomabin, and dragonamides A and E are derived from shallow water, diatom-covered, red, and mangrove root-derived *L. majuscule* (Figure 8). They share a similar structure consisting of a homologous alkynoic acid, *N*-methyl-Phe, and a terminal NH_2 group.[25-28] The former three compounds exhibit good activity against chloroquine-resistant *P. falciparum* (with IC_{50} values of 4.3, 6.0, and 7.7 μM, respectively),[26] while dragonamides A and E exhibit antileishmanial activity against *L. donovani* (with IC_{50} values of 5.1 and 6.5 μM, respectively).[28] However, non-aromatic analogs dragonamides B–D, show no activity indicating that the presence of aromatic amino acids at the C-terminus is essential for the antiparasitic activity of this compound series.[29]

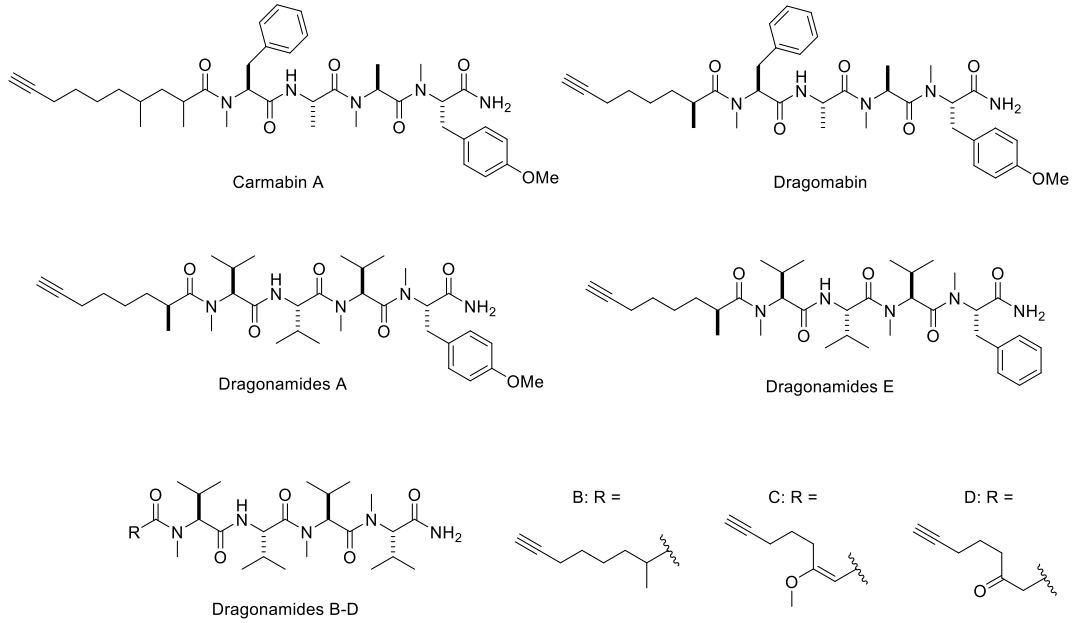


Figure 8. The structures of carmabin A, dragomabin, and dragonamides A–E.

Furthermore, the author must introduce the jahanyne family of linear lipopeptides discovered in Suenaga's laboratory. The jahanyne family was found in *Lyngbya sp*., with its peptide portion containing pyrrolidine derivatives, while the fatty acid portion is composed of similar structures but terminated with alkanoic, alkenoic, and alkynoic acids, respectively (Figure 9). The total synthesis of the jahanyne family has been achieved by Suenaga and his team,[30,31] and their inhibitory activity against cancer cell growth has been evaluated. The degree of unsaturation at the end of the fatty acid portion has been observed to influence the inhibitory activity against human cancer cells. This aspect will be discussed in detail in the following sections.

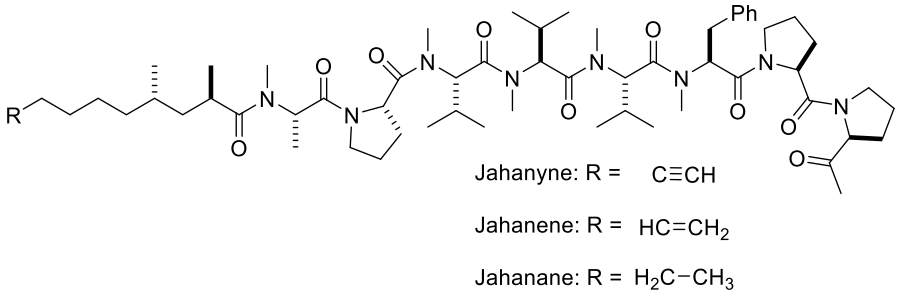


Figure 9. The structures of jahanyne family.

1-2-3. Cyclamides and cyclic peptides

Cyclamides are cyclopeptides with either six or eight amino acids, typically characterized by thiazole and oxazole rings which are thought to be cysteine and threonine derivatives, respectively. On the other hand, cyclic peptides are peptide chains that contain a cyclic sequence of amino acids, ranging in length from as short as two residues to several hundred residues. For example, cyclic cyclamides aerucyclamides A-D, derived from the freshwater *M. aeruginosa* PCC 7806, possess similar heterocyclic structures with an 18-membered ring (Figure 10).[32,33] The main difference lies in the presence of one oxazoline, one thiazoline, and one thiazole in aerucyclamides A and D, one oxazoline and two thiazoles in aerucyclamide B, and one oxazoline, one oxazole, and one thiazole unit in aerucyclamide C. Aerucyclamides A, B, C, and D demonstrate moderate selective antiparasitic activity, with lower IC_{50} values of 5.0, 0.7, 2.3, and 6.3 μM, respectively, against *P. falciparum*.

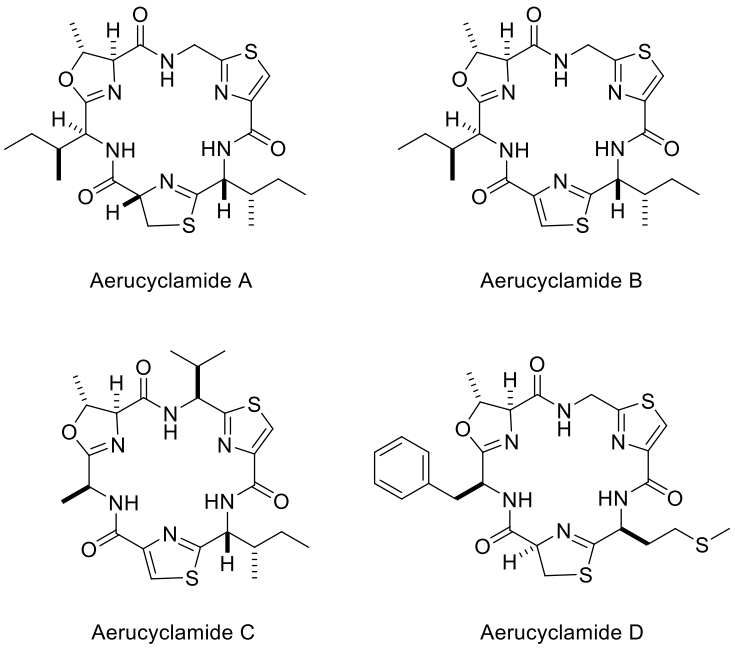


Figure 10. The structures of aerucyclamides A–D.

Balgacyclamides A and B, derived from *M. aeruginosa* EAWAG 251, possess an 18-membered ring consisting of one thiazole and two oxazoline moieties, as well as one thiazole and one oxazoline unit, respectively (Figure 11).[34] Balgacyclamides A and B exhibit micromolar IC_{50} activity against chloroquine-resistant *P. falciparum* (9.0 and 8.2 μM, respectively), but only weak activity against *T. b. rhodesiense* and *L. donovani*. Interestingly, it is worth noting that opening the oxazoline ring in balgacyclamide A does not significantly decrease its biological activity.

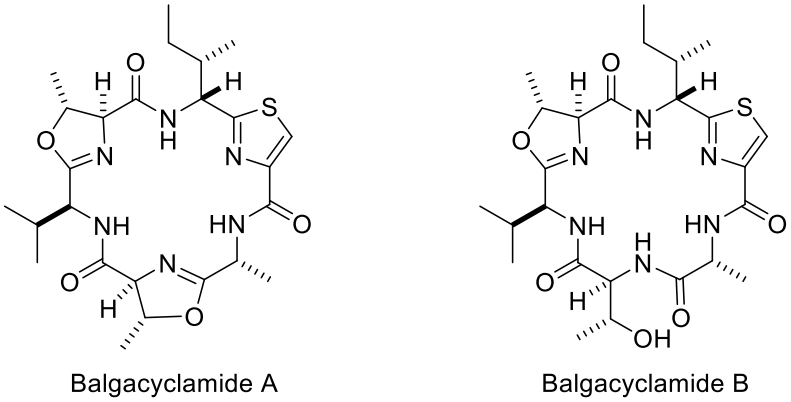


Figure 11. The structures of balgacyclamides A and B.

Finally, the typical cyclic peptides tolybyssidins A and B from T. *byssoidea* (EAWAG 195) are worth mentioning (Figure 12).[35] Both peptides contain the unusual amino acid dehydrohomoalanine (Dhha), as well as proteinogenic amino acids albeit with D- or L-

configuration. Tolybyssidins A and B inhibit the growth of the yeast *Candida albicans* at a concentration of 32 μg/mL for A and 64 μg/mL for B, respectively.

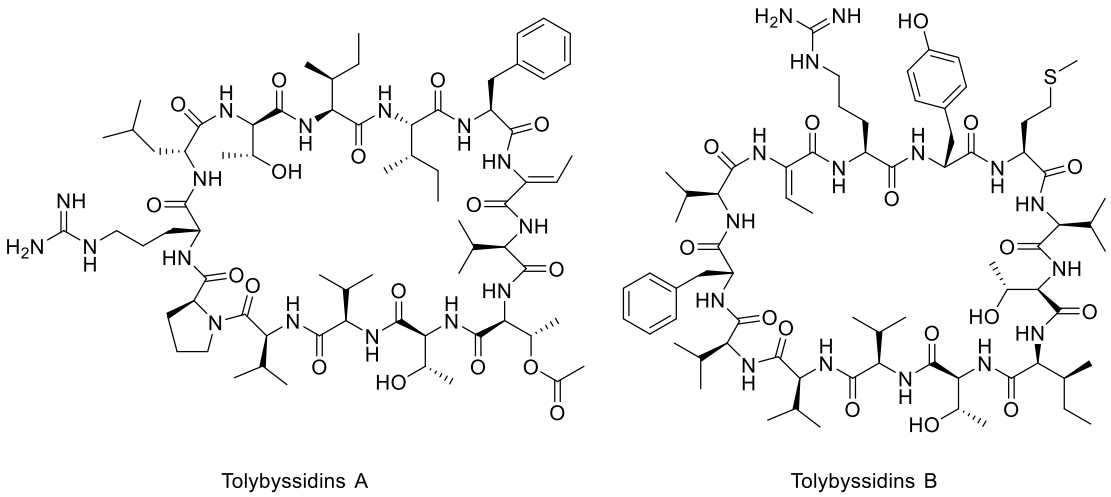


Figure 12. The structures of tolybyssidins A and B.

In summary, cyanobacteria offer a promising resource for antibiotic peptides with unique structures, including rare motifs and non-standard amino acid residues. However, many compounds derived from cyanobacteria exhibit cytotoxicity towards eukaryotic cells and erythrocytes, which limits their direct application as antibiotics. Therefore, conducting SAR analysis will be crucial in understanding their mode of action and facilitating the development of novel drugs and biopesticides with reduced toxicity. This can be achieved through drug design based on natural product pharmacophores, combinatorial biosynthesis, semi-synthesis, and total synthesis approaches. By harnessing these strategies, cyanobacterial-derived antibiotic peptides hold potential for the treatment of various diseases, including agricultural pests, parasitic diseases (such as malaria, trypanosomiasis, and leishmaniasis), dermatosis, tuberculosis, and influenza.

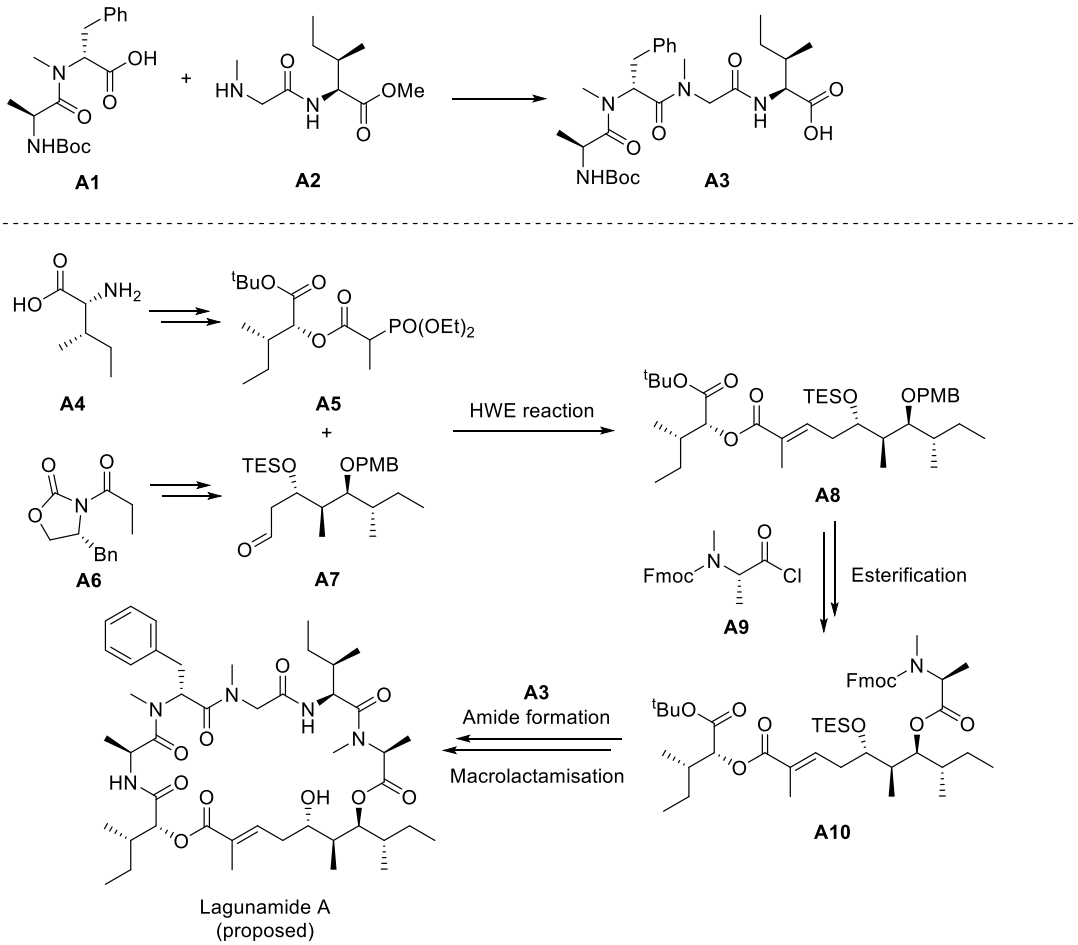
1-3. Total synthetic studies of cyanobacteria-derived peptides

The impact of total synthesis on society is immeasurable, with contributions ranging from advancements in organic chemistry to insights into specific influences and new chemistry through independent and creative research. Total synthesis, originating in the mid-19th century as a means of structural confirmation, has evolved into a scientific discipline with far-reaching implications. In its early stages, the limited analytical tools available to synthetic chemists necessitated following nature's footsteps for compound characterization. However, with the progress of science, chemical synthesis now offers unparalleled research opportunities for naturally occurring compounds with diverse chemical characteristics. In addition to their distinct three-dimensional structures and intrinsic biological properties, these compounds provide fertile ground for exploring structure-activity relationships through meticulous synthesis. Such investigations shed light on their biological functions and pave the way for the development of novel therapeutic agents with enhanced efficacy and selectivity. Moreover, total synthesis drives progress in medicinal chemistry, drug development, and the discovery of new chemical reactions that enable cleaner and more efficient processes. This multidisciplinary approach unifies various fields of chemical analysis, including inorganic chemistry, catalysis research, structural studies, chemical biology, and numerous other disciplines, making it one of the most significant areas of research in organic chemistry.

Many cyanobacteria-derived peptides introduced earlier have been extensively studied through total synthesis, providing abundant resources for exploring their biological activities, antibiotic properties and even developing new drugs. Given the significant interest in cyanobacteria-derived peptides, we have decided to conduct in-depth research on these peptides and their related total synthetic studies, as well as their contributions to biological activity testing and their value in research as novel antibiotics.

1-3-1. Total synthetic study of depsipeptides

For example, lagunamide A, mentioned earlier, was first synthesized through total synthesis by Ye and his team in 2012 (Scheme 1).[36] They dissected lagunamide A into upper and lower fragments. First, the upper tetrapeptide **A3** was obtained by coupling reagents to condense and modify two dipeptides. After preparing phosphonic diester **A5**, it was subjected to Horner-Wadsworth-Emmons condensation with aldehyde **A7** to yield the unsaturated ester **A8**. After further modification and esterification with **A9**, the lower triester **A10** was obtained. Finally, the removal of the Fmoc group and coupling reactions were carried out separately for tetrapeptide **A3** and triester **A10**, followed by the removal of protecting group and macrolactamization, resulting in the final product, lagunamide A. However, the synthesized product showed different ^{1}H and ^{13}C NMR spectra compared to the natural compound. They confirmed the incorrect structure of the original lagunamide A and synthesized six diastereomers of lagunamide A to determine the correct structure of the lagunamide A (Figure 13). After comparing them, they confirmed that the correct structure of lagunamide A should be lagunamide A **1e**. This exemplifies the intriguing aspect of total synthesis, where comparing the synthesized product with the natural compound allows verification. Total synthesis can help identify the correct structure and allow revalidation if they are not identical.



Scheme 1. Total synthesis of lagunamide A.

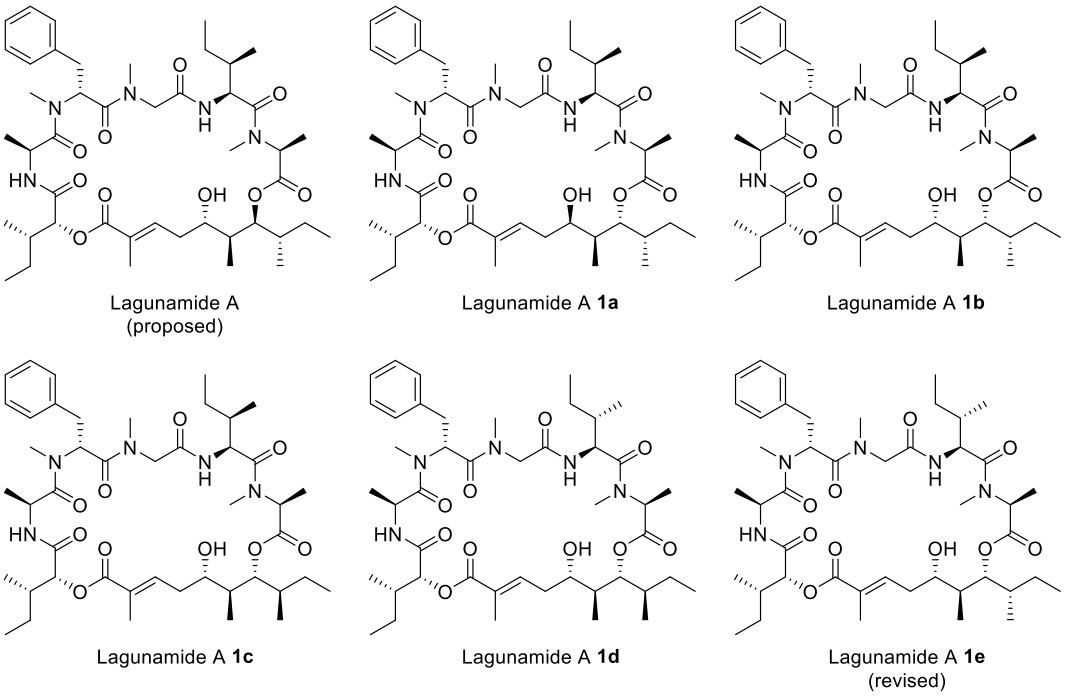
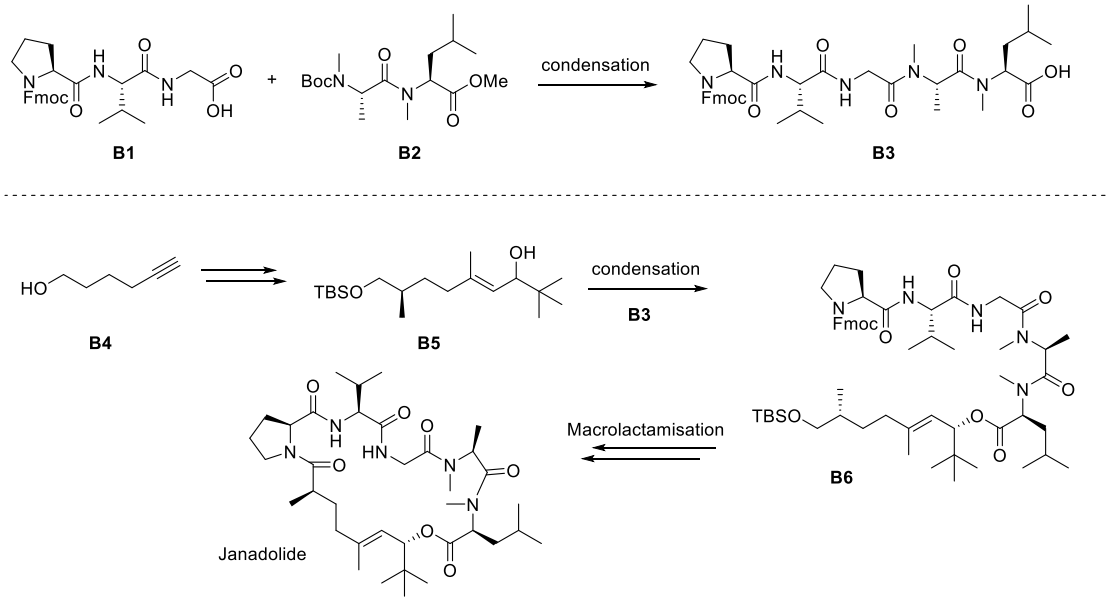


Figure 13. The structures of lagunamide A and **1a**–**1e**.

Additionally, janadolide, isolated from *Okeania* sp. by Suenaga and his team in 2016,[37] exhibits potent anti-trypanosomal activity without cytotoxicity to human cells (IC_{50} 47 nM). However, considering the limited supply from cyanobacteria sources, the team achieved the total synthesis of janadolide for the first time in 2018 (Scheme 2).[38] The tripeptide **B1** and dipeptide **B2** were coupled and modified using HATU coupling reagent to yield the corresponding pentapeptide **B3**. Subsequently, esterification with the fatty acid **B5** resulted in the janadolide precursor **B6**. Finally, deprotection and macrolactamization led to the formation of the ultimate product. However, dimers in the product suggested that the low yield of macrolactamization could be attributed to polymerization. The team attempted to construct the ring system through macrocyclization but did not obtain the desired product. This demonstrates the importance of macrolactamization as the crucial final step to avoid the excessive formation of unnecessary byproducts.

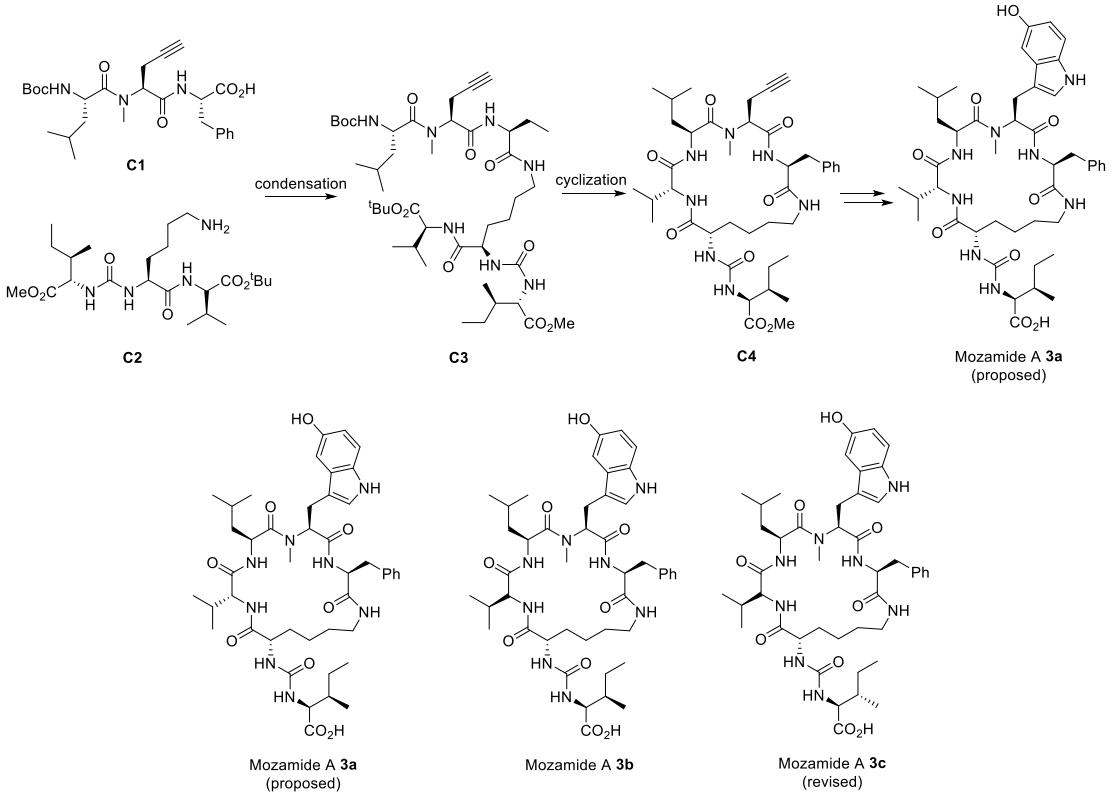


Scheme 2. Total synthesis of janadolide.

1-3-2. Total synthetic study of lipopeptides

Although there are fewer successful synthesis cases of cyclic lipopeptides compared to linear lipopeptides, the important role that cyclic lipopeptides play in drug research and development makes them an interesting and valuable target for total synthesis. Therefore, Kazmaier and his team referred to the cyclic lipopeptide mozamide A, which Faulkner and colleagues isolated from *Theonella* sponges collected in Mozambique in 1997.[39] They achieved the total synthesis in a convergent manner for the first time in 2019 and demonstrated that the initially proposed structure was inconsistent with the NMR data. After synthesizing two diastereomers, they compared their spectral data with the natural product and confirmed its correct structure (Scheme 3).[40]

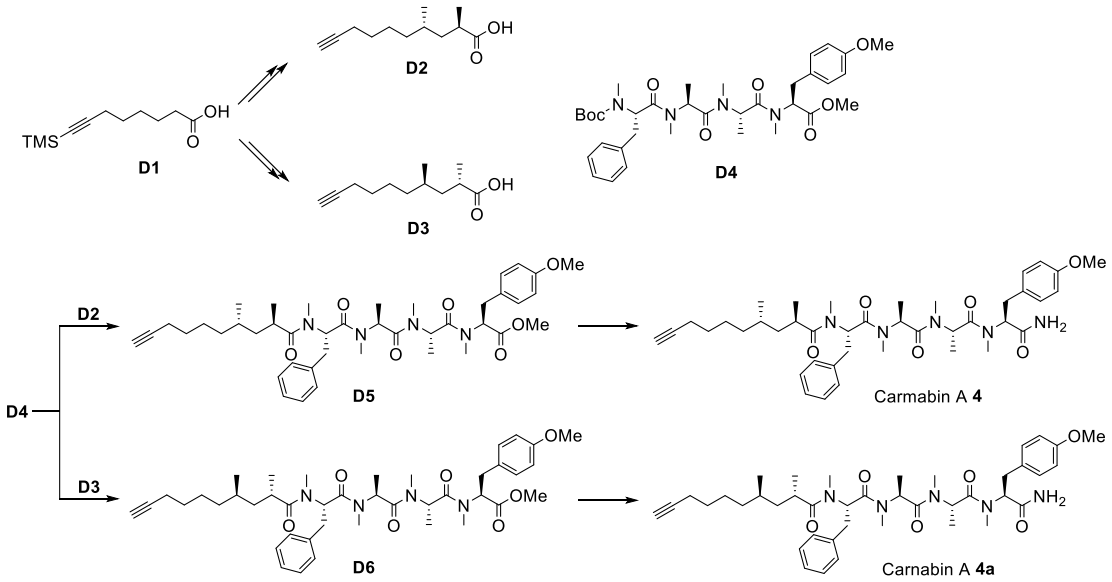
First, they performed a late-stage installation of 5-hydroxyindole onto the stannylated precursor based on retrosynthetic analysis, while the cyclic precursor was divided into two sets of tripeptides. After deprotection and coupling of the two sets of tripeptides **C1** and **C2** with a coupling reagent, a linear hexapeptide **C3** was obtained. After deprotection and cyclization with TBTU and DIPEA, a cyclic peptide **C4** with an alkyne moiety was obtained. Finally, the alkyne was subjected to Ru-catalyzed hydrostannation[41] and indole synthesis according to their sequence, resulting in the successful synthesis of mozamide A. Since the comparison of NMR data with the natural product showed poor consistency, they synthesized three candidate diastereomers for comparison with the natural product. The NMR spectrum of **3c** exhibited good consistency with that of mozamide A, leading to the conclusion that the actual structure of mozamide A is **3c**.



Scheme 3. Total synthesis of mozamide A **3a**–**3c**.

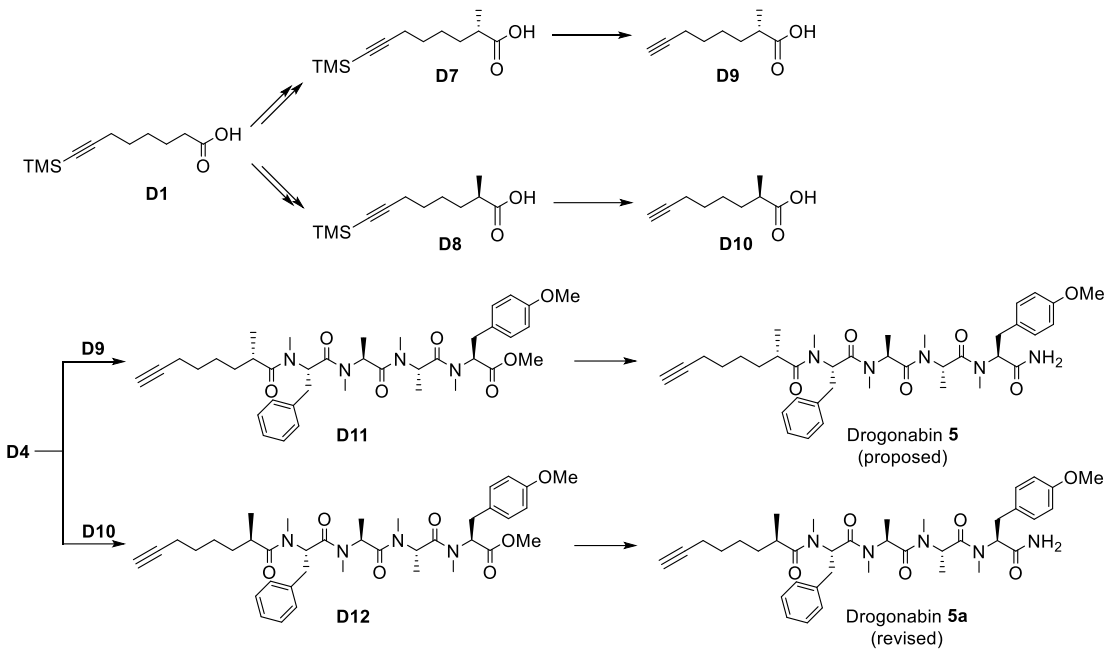
Compared to cyclic lipopeptides, numerous successful total synthetic studies have been done on linear lipopeptides. Although relatively easier, research on peptides remains an indispensable factor. For example, carmabin A and dragomabin, mentioned earlier, possess structurally similar highly *N*-methylated amino acids and lipid chains and exhibit good activity against chloroquine-resistant *Plasmodium falciparum*. However, due to their scarcity, the medicinal chemistry and mode of action were yet to be explored. Moreover, Gerwick et al. could not obtain the liberated moiety by degradation of carmabin A without sufficient material, making it challenging to determine the configuration of the chiral center through comparison with synthetic isomers.[26] Furthermore, the synthesis of adequate quantities of carmabin A and dragomabin, hindered by their unknown stereochemical configurations and conformational isomers induced by *N*-methylation, further impeded research on these peptides as potential lead compounds and their mode of action. Therefore, the achievement of total synthesis may provide new clues for drug discovery and facilitate further medical research. Chen and their team accomplished the total synthesis of carmabin A and dragomabin, along with the absolute stereochemical determination of the former, in 2018.[42]

Due to the identical tetrapeptide regions in carmabin A and dragomabin, and the possibility of acylation at the C-terminal amide through esterification, they fragmented carmabin A and dragomabin into protected tetrapeptides and respective fatty acid fragments (Scheme 4). Firstly, the fatty acid portion of carmabin A was constructed using a previously developed synthetic route,[43] achieving good stereoselectivity with 2*S*,4*R* and 2*R*,4*S* configurations on the fatty acid **D2** and **D3** fragments, respectively. Next, for the tetrapeptide part, the protected tetrapeptide **D4** was obtained through repeated coupling under the same conditions. Subsequently, the deprotection of tetrapeptide **D4** and its coupling reactions with fatty acids **D2** and **D3** yielded the precursors. Finally, treatment with ammonia led to the formation of carmabin A **4** and **4a**. Through careful comparison, the spectral data of compound **4** were consistent with the originally reported data for the natural product within the error range. Therefore, the absolute configuration of carmabin A was determined to be **4**.



Scheme 4. Total synthesis of carmabin A and **4a**.

Next, they turned to the fatty acid portion of dragomabin (Scheme 5), following a similar approach. After the first methylation, they removed the TMS protecting group and chiral auxiliary to obtain the corresponding fatty acid **D9**. Subsequently, the deprotection of the tetrapeptide and coupling reactions were carried out, followed by treatment with ammonia to generate dragomabin **5**, with the same reported stereochemistry. However, the ^{1}H NMR and optical rotation did not match the reported values. To address this, they prepared fatty acid **D10** using the same procedure as above and coupled it with the tetrapeptide to obtain **5a**. It was indeed found that the spectral data of **5a** were consistent with those of natural dragomabin. Therefore, the structure of dragomabin was revised, as shown in **5a**.



Scheme 5. Total synthesis of drogomabins **5** and **5a**.

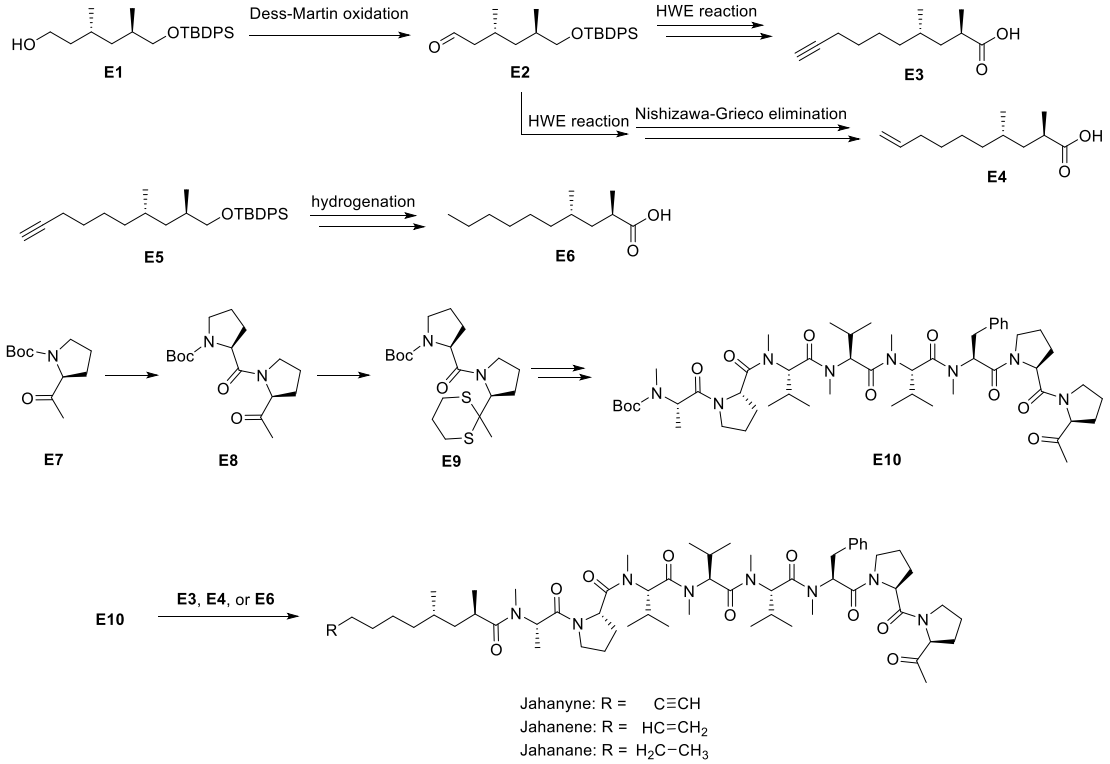
In the context of the total synthesis of linear peptides, I would like to introduce the work of Suenaga and his team, who first isolated jahanyne in 2015, as mentioned earlier. However, due to the limited availability of the natural product, the details of jahanyne's biological activity remained unclear. In 2018, the team also isolated two new analogs, jahanene and jahanane, from *Okeania* sp. and determined their structures.[44] Therefore, achieving the total synthesis of the jahanyne family and elucidating the structure-activity relationship of the fatty acid chain's terminal structure has become highly important and valuable for further research.

In the retrosynthetic analysis, the jahanyne family could be disassembled into three fatty acids with varying degrees of unsaturation and a highly methylated heptapeptide. The three fatty acids with different degrees of unsaturation could be prepared from known alcohol derived from (*S*)-Roche ester. Simultaneously, the modified heptapeptide could be derived from several amino acids and a ketone at the C-terminus.

The synthetic study began with the fatty acids (Scheme 6), where the known alcohol **E1**[45] was transformed into the aldehyde **E2** through Dess-Martin oxidation. Subsequent Horner-Wadsworth-Emmons reaction and a series of reactions yielded the fatty acid **E3** with an alkyne at the terminal position. On the other hand, the fatty acid **E4** with an alkene at the terminal position was synthesized starting from the intermediate aldehyde **E2** and underwent HWE reaction, Nishizawa−Grieco elimination,[46] and a series of reactions. Finally, the saturated fatty acid **E6** was synthesized from the intermediate

alkyne **E5** derived from alkyne **E3**. After catalytic hydrogenation and subsequent deprotection and oxidation steps, the fatty acid **E6** was obtained.

On the other hand, the modified heptapeptide was synthesized starting from the known ketone **E7**[47], which was coupled with *N*-Boc-L-proline to form the amide **E8**. The ketone group in **E8** was protected as a dithioacetal group to prevent undesired side reactions. Subsequently, the corresponding modified amino acids were sequentially introduced using HATU as the coupling reagent. Finally, the desired modified heptapeptide **E10** was obtained through the cleavage of the dithioacetal by iodine. After deprotection of the heptapeptide **E10**, it was coupled separately with the fatty acids **E3**, **E4**, and **E6** to obtain the final products, jahanyne, jahanene, and jahanane, respectively. Not only was the total synthesis of the jahanyne family achieved for the first time, but the growth inhibitory activity of natural and synthetic jahanyne family compounds against human cancer cells by the MTT assay, establishing the structure-activity relationship of the jahanyne family partially.



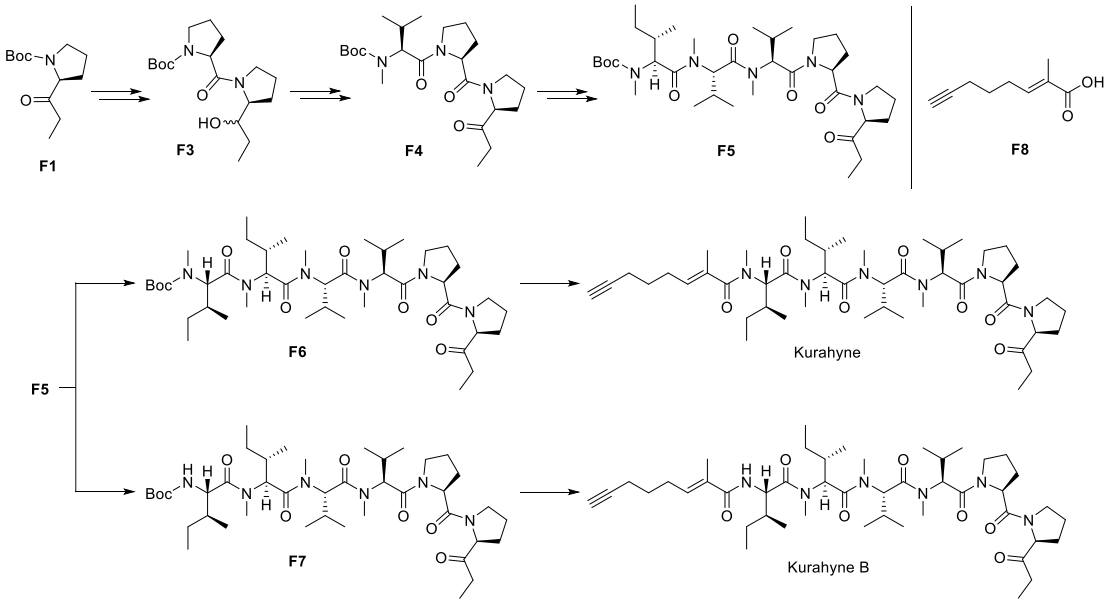
Scheme 6. Total synthesis of the jahanyne family.

Similarly, to introduce kurahyne and kurahyne B, discovered and isolated by Suenaga and his team. Kurahyne is a peptide that induces cell apoptosis and was isolated from the marine cyanobacterium *Lyngbya* sp. in 2014.[48] Later, a new analog called kurahyne B was isolated from the marine cyanobacterium *Okeania* sp. in 2015.[49] The overall structures of both compounds were elucidated through spectroscopic analysis, and their

absolute configurations were established through total synthesis.[49] It was found that kurahyne and kurahyne B exhibit the same level of biological activity inhibiting human cancer cell growth.

The structural analysis discovered that kurahyne B is a demethylated analog of kurahyne, where the N-terminal *N*-Me-Ile of kurahyne replaces Ile. Therefore, it was assumed that they share the same absolute configuration. The first total synthesis of kurahyne and kurahyne B was performed to validate this assumption. Starting from the known ethyl ketone **F1** at the C-terminus, the corresponding amino acids were sequentially condensed (Scheme 7). To avoid undesired cyclization products resulting from direct condensation of the dipeptide, the keto group was first reduced to the corresponding alcohol **F3**, followed by condensation and oxidation to obtain the modified dipeptide **F4**. In the synthesis of the modified pentapeptide, a more efficient coupling reagent, COMU, was used due to poor reactivity. The corresponding pentapeptides **F6** and **F7** were obtained. Finally, they were individually coupled with carboxylic acid **F8**[50] to yield kurahyne and kurahyne B.

The growth inhibitory activity of kurahyne and kurahyne B against human cancer cells, HeLa and HL60, was evaluated using an MTT assay.[48,49] The measured data indicated that both compounds exhibited comparable levels of inhibition against human cancer cell growth. This not only achieved the first total synthesis of kurahyne and kurahyne B but also confirmed the inhibition of human cancer cell growth.

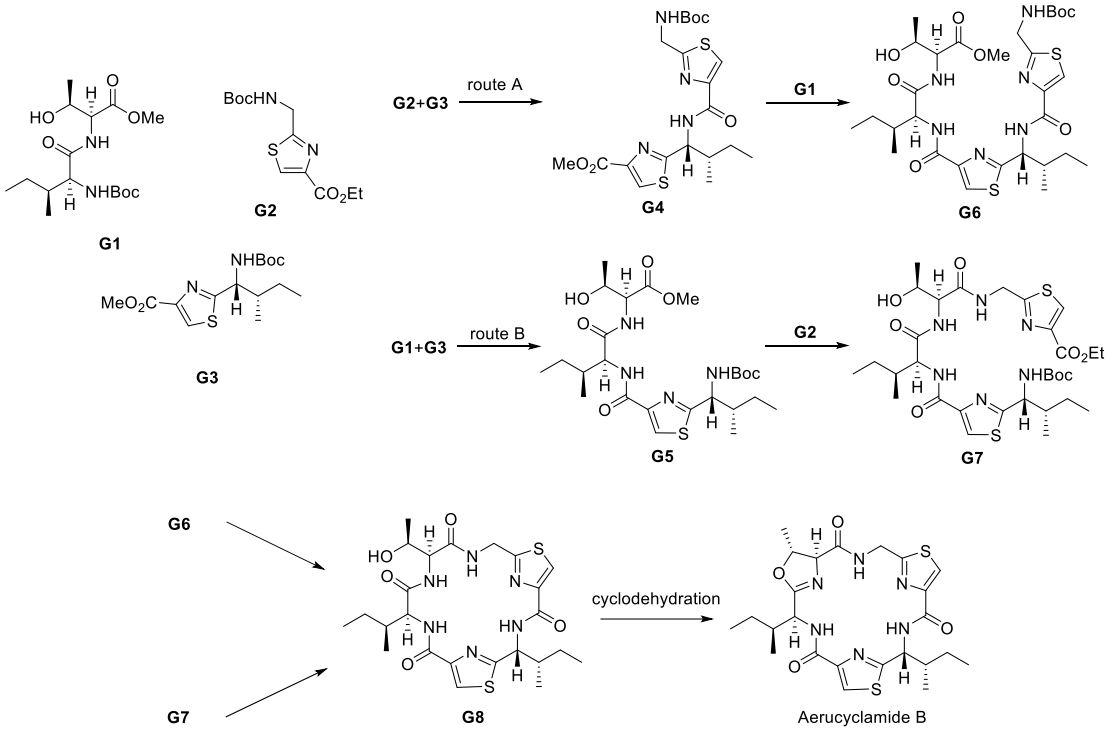


Scheme 7. Total synthesis of kurahyne and kurahyne B.

1-3-3. Total synthetic study of cyclamides and cyclic peptides

There have been numerous cases of total synthesis for cyclamides and cyclic peptides. One of these examples is the previously mentioned aerucyclamides A-D.[14] In 2008, Gademann and colleagues isolated these compounds from the toxic freshwater cyanobacterium Microcystis aeruginosa PCC 7806. Among the four compounds, aerucyclamide B exhibited the highest activity, with a submicromolar IC_{50} value against *Plasmodium falciparum* K1. Furthermore, this compound displayed significant selectivity for the parasite compared to the L6 rat myoblast cell line. In 2013, Serra and his team accomplished the total synthesis of aerucyclamide B for the first time.[51]

In the retrosynthetic analysis, the structure was initially divided into two thiazoles and one dipeptide unit, which were then assembled to form the macrocycle (Scheme 8). Subsequently, a cyclodehydration reaction was conducted to obtain the oxazoline ring. The corresponding dipeptide **G1** was synthesized using HBTU as the coupling reagent. Thiazole **G2** was prepared following known methods,[52] while thiazole **G3** was obtained through the cyclodehydration of β-hydroxythioamides and further oxidation methodology. A series of reactions led to the formation of the imidazole **G3**. In the study of macrocycle assembly, two reaction pathways were explored to obtain intermediate compounds **G6** and **G7** aiming to investigate the cyclization point's influence on the formation. The desired macrocyclic compound **G8** was obtained with a higher yield from **G7** compared to the yield obtained from the precursor **G6**, indicating the importance of the choice of cyclization point in this particular case. Finally, the final product, aerucyclamide B, was obtained through a cyclodehydration reaction.

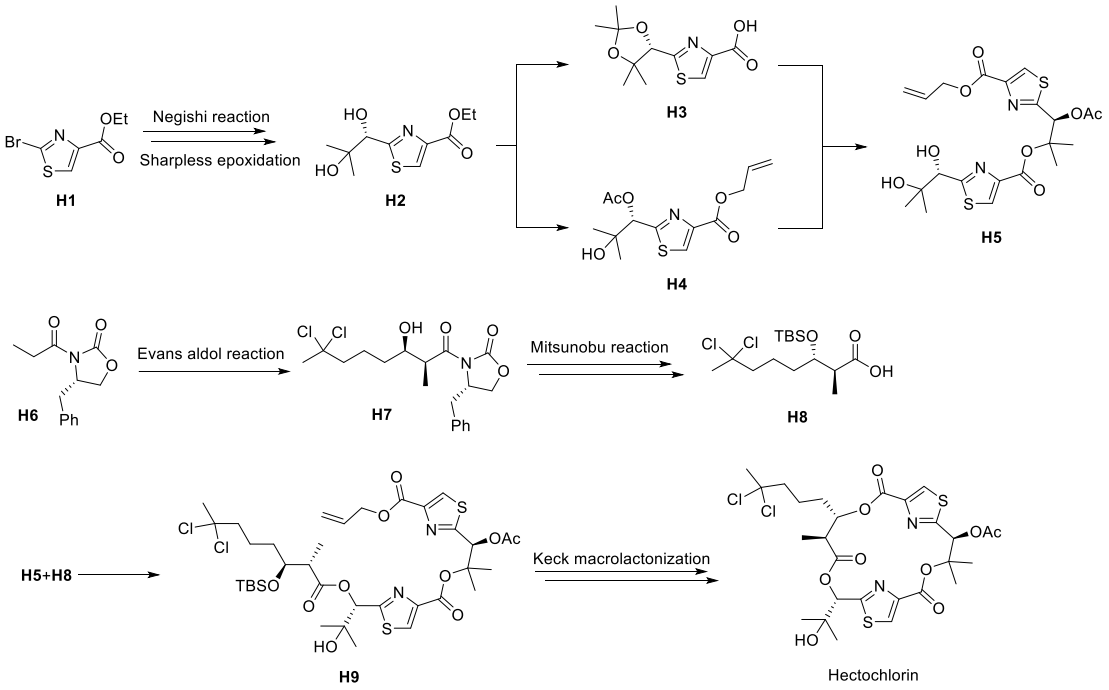


Scheme 8. Total synthesis of aerucyclamide B.

Another novel natural fungicide, hectochlorin, isolated from *Lyngbya majuscula*, is worth mentioning.[53] Oliver and his team discovered its activity against various pathogens during crop disease screening. Subsequently, they conducted in-depth research on hectochlorin and developed a synthetic route for hectochlorin and its analogs. In 2002, they successfully achieved the total synthesis of hectochlorin.[54] Their retrosynthetic analysis dissected hectochlorin into one aldol subunit and two thiazole subunits (Scheme 9). There were synthetic examples for the aldol subunit for the (2*R*,3*S*) portion,[55] and incorporating a hydroxyl inversion approach was necessary to obtain the (2*S*,3*S*) configuration. By coupling two appropriately protected thiazole precursors, they obtained the bis-thiazole diol intermediate, and upon introducing the aldol subunit, they achieved the final cyclization to form hectochlorin.

Firstly, the synthesis of the thiazole subunit **H2** was carried out via Negishi reaction and Sharpless epoxidation starting from 2-bromo-4-ethoxycarbonylthiazole **H1**. The resulting **H2** was then differentially protected to yield two thiazole coupling precursors. Subsequently, the two thiazole precursors were coupled using DCC and DMAP, resulting in bis-thiazole diol **H5**. The preparation of the aldol subunit involved the boron enolate of (*S*)-(+)-4-benzyl-3-propionyl-2-oxazolidinone, which was subjected to an Evans aldol reaction to obtain the (2*S*,3*R*) precursor **H7**. Subsequently, the Mitsunobu condition was employed to invert and protect the secondary alcohol

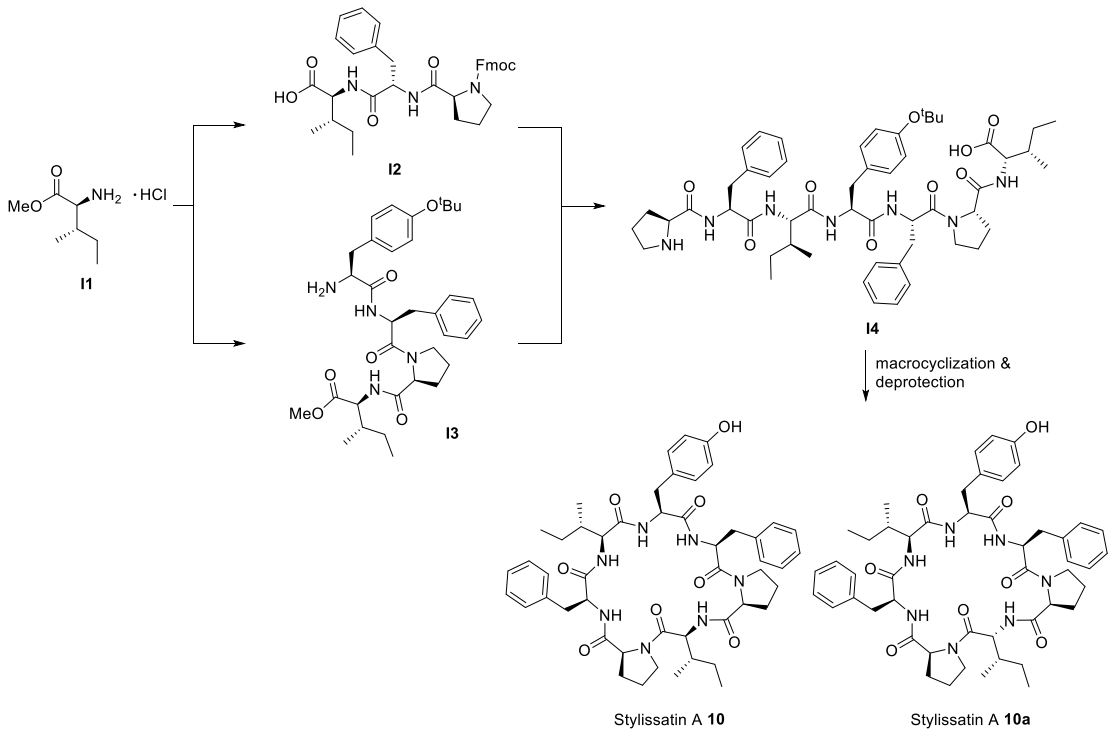
resulting in the aldol subunit **H8**. Finally, coupling of the bis-thiazole diol **H5** with the aldol subunit **H8** led to the linear triester **H9**, which upon deprotection, underwent Keck macrolactonization conditions to yield the final cyclized product hectochlorin.



Scheme 9. Total synthesis of hectochlorin.

The extensive biological properties and complex chemical structures of natural cyclic peptides have attracted the interest of many research laboratories. They are primarily composed of several amino acids and exhibit various variations. Therefore, I would like to introduce stylissatin A, a cyclic heptapeptide rich in proline, isolated from the marine sponge *Stylissa massa* in Papua New Guinea.[56] The total synthesis of stylissatin A was first achieved by Kigoshi and his team in 2015 (Scheme 10).[57]

Stylissatin A was disconnected into a tripeptide **I2** and a tetrapeptide **I3**, which could be prepared from readily available amino acid derivatives. Firstly, L-isoleucine methyl ester hydrochloride is the starting material for both peptides. After condensation with the corresponding modified amino acids and subsequent deprotection, the tripeptides **I2** and **I3** were obtained separately. Then, they were coupled and modified to yield the linear peptide **I4**. In the crucial macrocyclization step, DEPBT coupling reagent was selected to result in a relatively fast reaction, albeit with low diastereoselectivity. Finally, by removing the protecting groups, stylissatin A and its D-*allo*-Ile epimer **10a** were obtained.



Scheme 10. Total synthesis of stylissatin A and **10a**.

In summary, marine cyanobacterial secondary metabolites possess various structures and play a crucial role as important bioorganic compounds with significant therapeutic potential. Many of these compounds can serve as potential lead compounds or candidate molecules. Total synthesis plays a vital role in confirming chemical structures and has also facilitated research on the biosynthesis and chemoenzymatic synthesis of peptides. Furthermore, it provides a method for synthesizing various peptide derivatives, aiding in screening a large number of compounds for drug discovery.

Synthetic chemistry has developed rapidly in the past few decades, with sustainable synthetic methods such as catalytic amide formation or peptide coupling (native chemical ligation, Ser/Thr ligation, and KAHA ligation) emerging, showing great potential in modern peptide synthesis. Moreover, there is still ample room for transferring synthetic methods developed for small organic molecules to the total synthesis of natural peptides. Other methodologies (cross couplings, cyclizations, olefinations, click reactions, etc.) may also be helpful in the total synthesis of some natural peptides. In fact, for the total synthesis of complex natural peptides, the fusion of multiple strategies (SPPS, novel synthetic methods, convergent synthesis, liquid-phase synthesis, solid-phase synthesis, and biomimetic synthesis) is encouraged to achieve better results. These remarkable works demonstrate the application of multiple strategies in peptide synthesis.

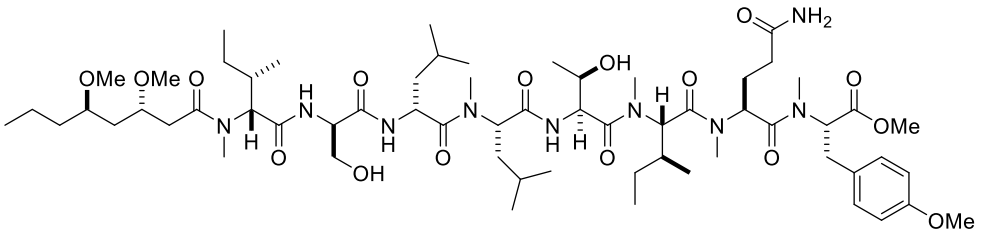
With an increasing number of peptides discovered in natural organisms, numerous naturally occurring peptides will be artificially synthesized in organic chemistry, and total synthesis research will continue to play a significant role in this field.

Chapter 2. Total synthesis of ikoamide

2-1. Introduction

In Africa, malaria caused by the parasitic protozoan *Plasmodium falciparum* remains a severe infectious disease even today. The current mainstream treatment approach is artemisinin-based combination therapy (ACT). However, unfortunately, reports of artemisinin-resistant strains of *P. falciparum* emerged in 2007, posing a significant threat to public health.[58] Even more alarming is the discovery of another artemisinin-resistant strain in Uganda.[59] With 90% of malaria cases occurring in Africa, this further complicates the control of the epidemic. Therefore, the development of new antimalarial drugs is urgently needed.

In the previous introduction, we learned about the potential of marine cyanobacteria as lead compounds for drug discovery, particularly as promising sources for antimalarial agents. In 2020, our research team studied the secondary metabolites of the marine cyanobacterium *Okeania* sp. and discovered an antimalarial compound which was named ikoamide (**1**) after Iko-pier near the collection site of the cyanobacteria (Figure 14).[60] Structurally, ikoamide (**1**) is a linear lipopeptide consisting of a fatty acid portion with two methoxy groups and eight α-amino acids, including five *N*-methylated amino acids and three D-amino acids. Compound **1** is the first natural product demonstrated to possess a 3,5-dimethoxyoctanoic acid moiety. Furthermore, **1** showed potent antimalarial activity with an IC_{50} value of 0.14 μM. On the other hand, **1** did not show growth-inhibitory activity against HeLa cells or HL60 cells at 10 μM. Based on these results, ikoamide (**1**) selectively inhibited the growth of malarial parasites.



Ikoamide (**1**)

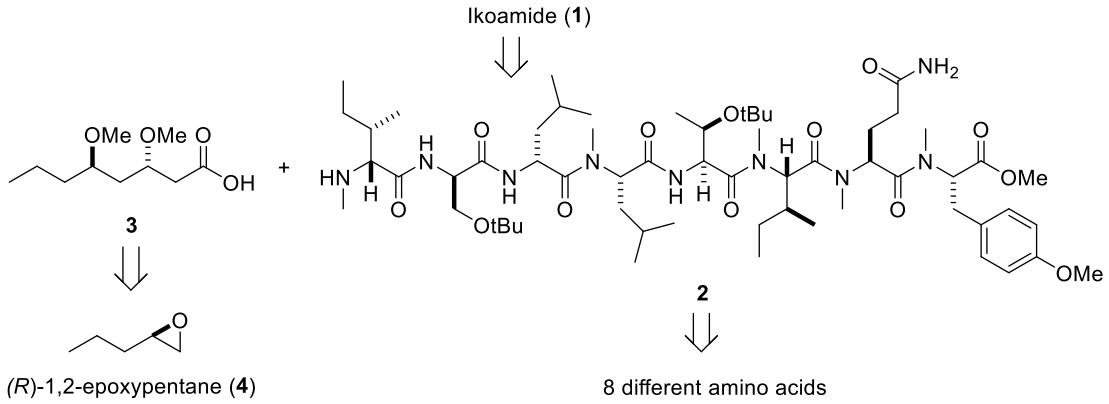
Okeania sp.

Structure
-5 *N*-Methylated amino acid
-3 D-form amino acid
-3,5-dimethoxyoctanoic acid moiety

Figure 14. The structure of ikoamide (**1**).

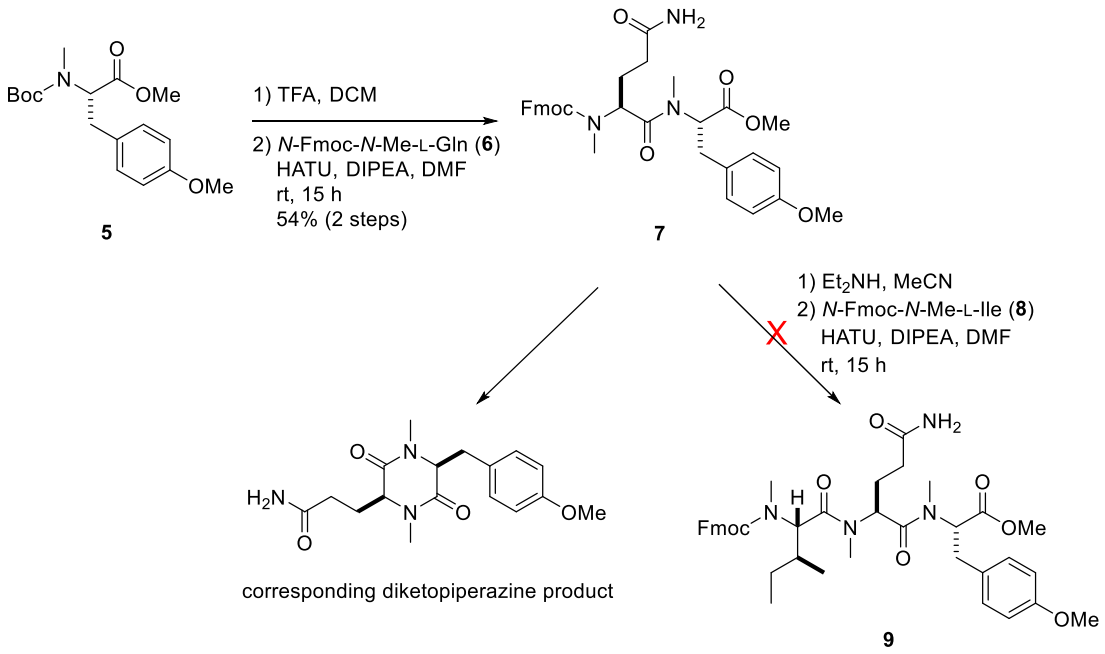
2-2. Total synthesis and biological activity of ikoamide

The retrosynthetic analysis of ikoamide (**1**) involves the coupling between octapeptide **2** and fatty acid **3** (Scheme 11). The protected octapeptide **2** can be derived from the corresponding amino acids, while the fatty acid **3** could be synthesized from (*R*)-1,2-epoxypentane (**4**). Octapeptide **2** contains five *N*-methylamide groups, and due to the low reactivity of *N*-methylamine, we need to use amino acid derivatives protected with carbamate groups to avoid epimerization. Additionally, the presence of rotamers complicates NMR, making the detection of epimers challenging if a fragment coupling strategy is employed. Therefore, we chose a linear synthetic route for octapeptide **2**. Furthermore, we have selected Fmoc instead of Boc as the protecting group, mainly because we used *tert*-butyl protecting groups for the hydroxyl groups of Thr and Ser, which are unstable during the Boc deprotection of octapeptide **2**.



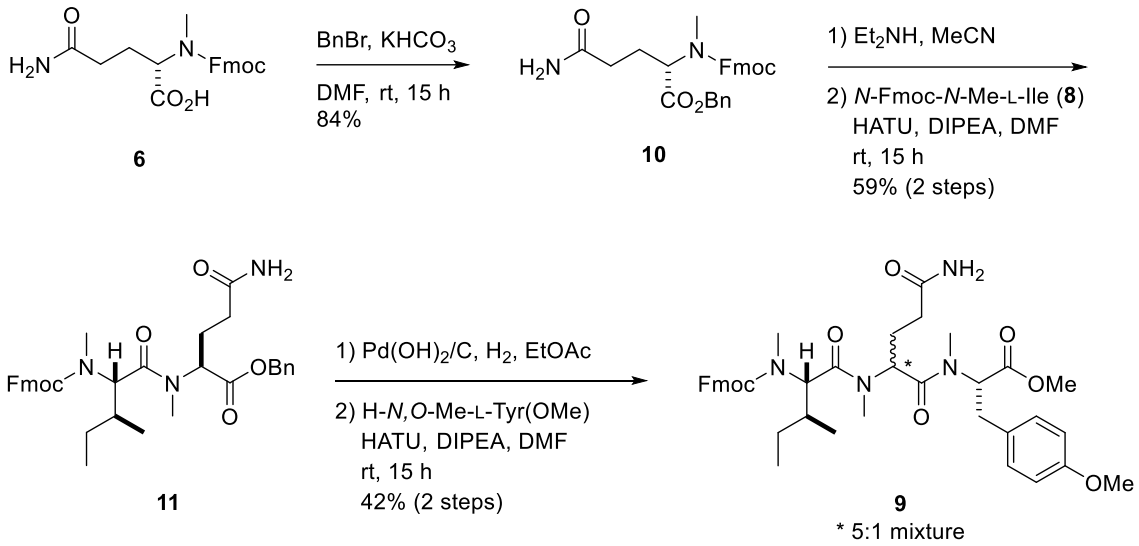
Scheme 11. Retrosynthetic analysis of ikoamide (**1**).

Based on the retrosynthetic analysis, our synthesis started with the preparation of octapeptide **2** (Scheme 12). The deprotected *N*-Boc-*N*,*O*-dimethyl-L-Tyr methyl ester (**5**)[61] is coupling with *N*-Fmoc-*N*-Me-L-Gln-OH (**6**)[62] to obtain the dipeptide **7** (yield: 54%). Subsequently, deprotection of **7** and then coupling with *N*-Fmoc-*N*-Me-L-Ile (**8**)[63] were performed, but the expected tripeptide **9** was not formed due to undesired side reactions leading to the corresponding dioxopiperazine product instead of coupling with **8**.



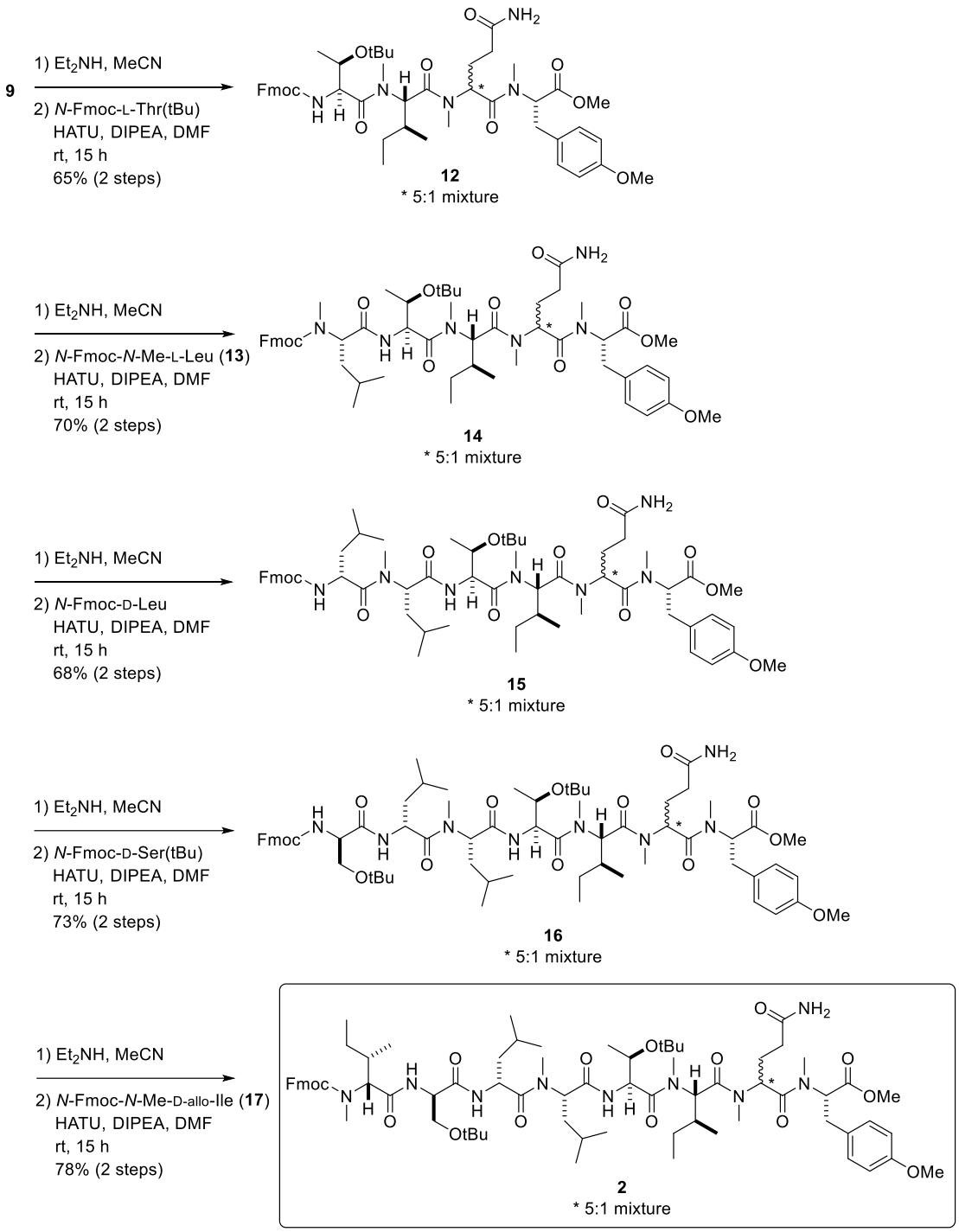
Scheme 12. Synthesis of tripeptide **9**.

Therefore, we revised the synthetic scheme as illustrated in Scheme 13. The starting amino acid was changed to Gln at position 2, and the carboxylic acid group of *N*-Fmoc-*N*-Me-L-Gln-OH (**6**) was protected with benzyl bromide to obtain the corresponding ester **10**. Coupling with *N*-Fmoc-*N*-Me-L-Ile (**8**) resulted in the corresponding dipeptide **11** with a yield of 59%. Subsequently, the benzyl group of **11** was removed under hydrogenolysis with palladium hydroxide on activated charcoal, and the resulting free carboxylic acid was directly coupled with deprotected **5** at a moderate rate to provide the tripeptide **9** (yield: 42%). Due to the epimerization during the coupling reaction, an inseparable mixture of 5:1 diastereomers was obtained in the *N*-Me-Gln portion. At this stage, the diastereomeric purity concerning the *N*-Me-Gln moiety could not be determined because of the complexity of NMR spectra due to the existence of rotamers in a solution. Therefore, we continued the subsequent condensation reaction using a diastereomeric mixture. To improve the low yields, we attempted to use alternative reaction reagents such as EDCI and HBTU instead of HATU. In the experimental comparison, the yield using HBTU was 33%, while EDCI yielded less than 5%. Based on these results, HATU can achieve a better yield. Therefore, only HATU was used as the coupling reagent in the subsequent reaction.



Scheme 13. Synthesis of tripeptide **9**.

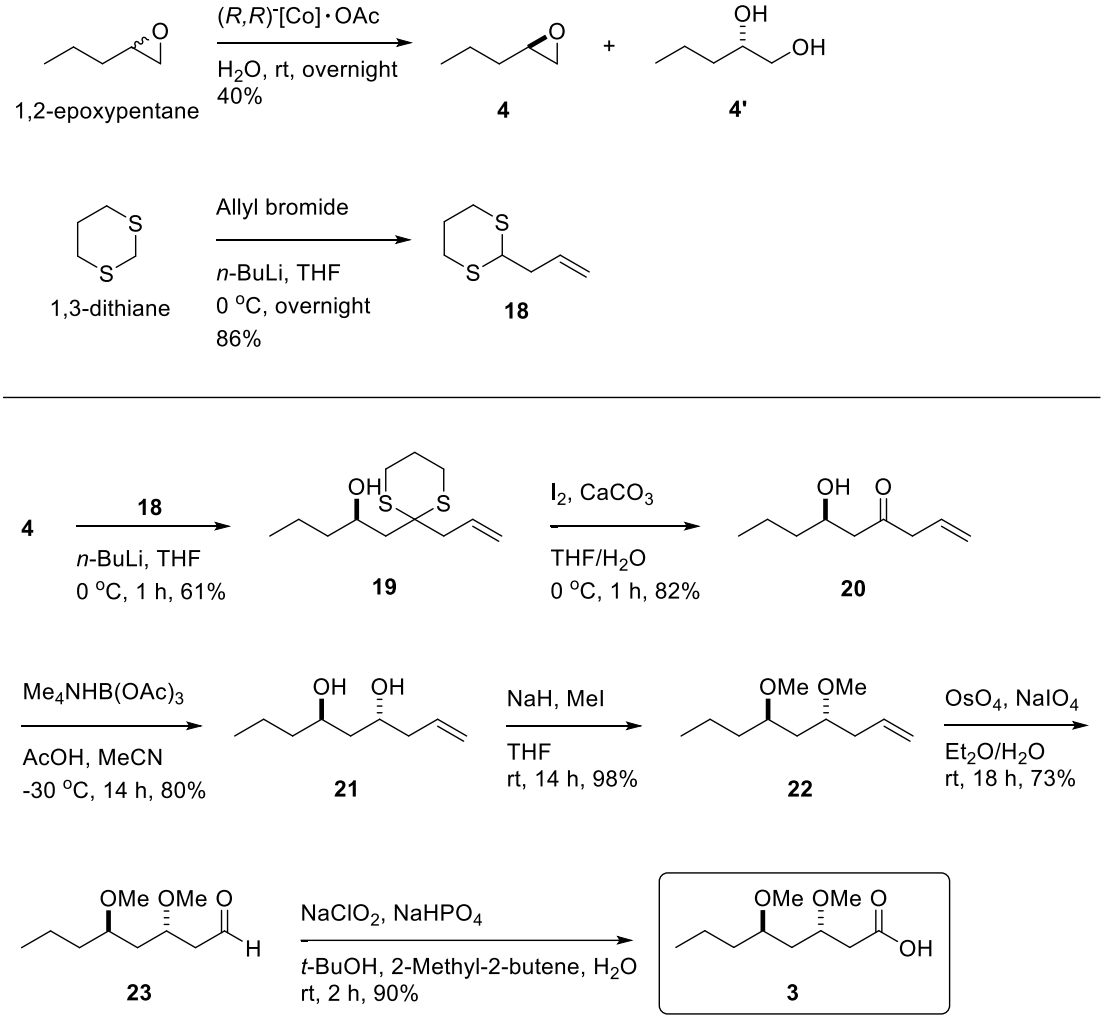
To avoid unwanted side reactions in the subsequent reactions, the amino groups were protected with Fmoc group of both Thr and Ser, while the hydroxyl groups were protected with a *tert*-butyl group. The commercially available samples of protected D-Leu, L-Thr, and D-Ser were used directly. Then, in Scheme 14, continued the reaction with tripeptide **9** (5:1 mixture), and after removal of the Fmoc group of tripeptide **9** by diethylamine and acetonitrile to free amine group, coupling reaction with the protected Thr using HATU gave tetrapeptide **12** in 65% yield. Then, under this condition, coupling reaction with *N*-Fmoc-*N*-Me-L-Leu (**13**)[64] afforded pentapeptide **14** in 70% yield, which was further coupled with *N*-Fmoc-D-Leu to give hexapeptide **15** in 68% yield. Using this coupling condition, protected Ser and *N*-Fmoc-*N*-Me-D-*allo*-Ile (**17**)[65] were successively introduced, providing heptapeptide **16** and octapeptide **2** in 73% and 78% yields, respectively. The decreased yields were attributed to the growing hindrance at the N-termini of **9**, **10**, **11**, and **14**. After synthesizing octapeptide **2**, all peptide products were analyzed by HPLC, following the synthesis of hexapeptide **15**, each product could be purified by HPLC to obtain a diastereomerically pure sample (see the Experimental section for details).



Scheme 14. Synthesis of octapeptide **2**.

Our synthesis of the chiral 3,5-dimethoxyocatanoic acid **3** is summarized in Scheme 15. First, the kinetic resolution of 1,2-epoxypentane using catalytic hydrolysis gave (*R*)-1,2-epoxypentane (**4**) (yield 40%).[66] Allyl-1,3-dithiane (**18**) was obtained in 86% yield from the lithium derivative of 1,3-dithiane and allyl bromide.[67] Next, the reaction of (*R*)-1,2-epoxypentane (**4**) with dithiane **18** using the Corey-Seebach reaction[68] afforded alcohol **19** with a yield of 61%. The dithiane hydrolysis of **19** gave ketone **20**[68] (yield

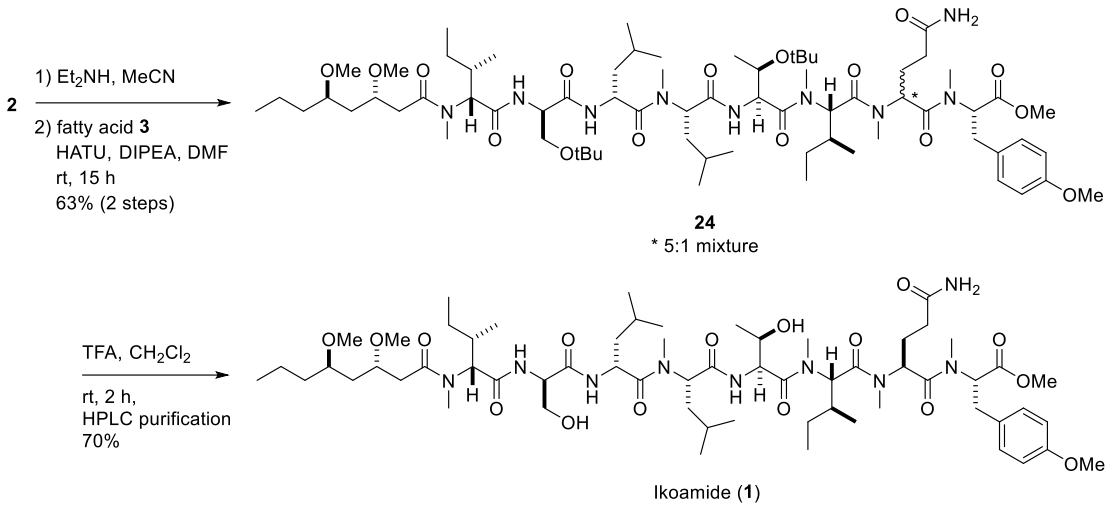
82%), which was further converted into the known diol **21** by Evans-Saksena reduction[69] with a yield of 80%. The stereoselectivity of the reduction was 8:1, and the undesired syn diol could be removed by chromatography. The subsequent methylation of **21** gave dimethoxy alkene **22** (yield 98%), which was then oxidized to form aldehyde **23** (yield 73%). The aldehyde **23** was further oxidized to produce the desired fatty acid **3** with a yield of 90%. The NMR data for **3** agreed with those of the same compound reported in the literature.[60]



Scheme 15. Synthesis of fatty acid **3**.

Following the synthesis of octapeptide **2** and fatty acid **3**, we carried out the final step of the total synthesis of ikoamide (**1**) (Scheme 16). After Fmoc-deprotection of **2** by diethylamine and acetonitrile, the resulting secondary amine was condensed with fatty acid **3** to provide precursor **24** in 63% overall yield over two steps. Removal of the *tert*-butyl group by TFA gave ikoamide (**1**) in 70% yield. All physical data for the synthetic ikoamide (**1**) were well-correlated with those of the natural compound except for

exchangeable protons in ^{1}H NMR.[70] Thus, we achieved the total synthesis of ikoamide (**1**) and confirmed its chemical structure.



Scheme 16. Synthesis of ikoamide (**1**).

Since the natural ikoamide has been proven to possess antimalarial activity, we evaluated the antimalarial activity against *Plasmodium falciparum* of synthesized ikoamide (**1**). Also, we evaluation of antitrypansomal activity against *Trypanosoma brucei rhodesience* (Table 1). The synthesized **1** demonstrated similar antimalarial activity with an IC_{50} value of 0.024 μM, comparable to the natural compound. Furthermore, synthesized **1** exhibited significant antitrypansomal activity, with an IC_{50} value of 1.3 μM. This highlights the potential of ikoamide (**1**) as a compound with antimalarial and antitrypanosomal properties.

Table 1. Bioactivity of ikoamide (**1**).

Compound	Antitrypanosomal activity (IC_{50}, μM)[a]	Antimalarial activity (IC_{50}, μM)[b]
Nature ikoamide	N/A	0.14
Synthetic ikoamide (**1**)	1.3	0.024

[a] against *Trypanosoma brucei rhodesiense*. Pentamidine (positive control): IC_{50} = 0.006 μM. n = 3.

[b] against *Plasmodium falciparum*. Chloroquine (positive control): IC_{50} =0.047 μM. n = 3.

In summary, we have achieved a total synthesis of ikoamide (**1**) from 8 amino acid derivatives and (*R*)-1,2-epoxypentane (**4**) with the longest linear sequence of 18 steps and an overall yield of 1.1%. This method provides not only an effective route for synthesizing ikoamide (**1**), but also a route for the synthesis of structurally related compounds for SAR studies.

Chapter 3. Total synthesis of odookeanynes A and B

3-1. Introduction

Secondary metabolites from marine cyanobacteria have attracted widespread attention due to their unique structures and biological activities. These compounds serve as valuable repositories of chemical diversity, with many of them explored as potential lead compounds in drug discovery efforts and investigated for promising biological and antiviral properties. In addition to toxicity studies, marine organisms have also been of interest for their bioactivity as potential antidiabetic[71] and antiobesity[72] agents. Therefore, studying natural compounds as potential therapeutic agents for diabetes and obesity treatment is expected to encourage innovation in biology and pharmacy.

In the previous introduction, ikoamide (**1**) possessed potent antimalarial activity, highlighting its potential as a therapeutic agent. In the ongoing search for novel bioactive substances, Teruya and his team, in their study, two acetylene-containing lipopeptides, odookeanynes A (**25**) and B (**26**)[73], obtained from *Okeania* sp. marine cyanobacterium (Figure 15). Their structures share similarities with jahanyne[44] and kurahyne,[48,49] which contain 2,4-dimethyldec-9-ynoic acid (fatty acid) and 2-(1-oxo-ethyl)pyrrolidine or 2-(1-oxo-propyl)pyrrolidine (Opp) moieties and have been previously reported and synthesized. Since similar lipopeptides with fatty acids at the *N*-terminal have been shown to exert antiparasitic activity, similar activity is expected for odookeanynes A (**25**) and B (**26**). Further investigations into the synthesis of odookeanynes A (**25**) and B (**26**) and their analogs may lead to discoveries in drug development. In addition, they found that in the presence of insulin, isolated odookeanynes A (**25**) and B (**26**) significantly enhanced the adipocyte differentiation of 3T3-L1 cells. Increasing small insulin-sensitive adipocytes by promoting adipocyte differentiation should improve the treatment of type 2 diabetes and obesity.[73] Due to the scarcity of natural products, only 17.7 mg of odookeanyne A and 15.0 mg of odookeanyne B were isolated from 150 g of *Okeania* sp. marine cyanobacterium, testing the bioactivity related to odookeanynes has been challenging.

We have accomplished the total synthesis of odookeanynes A (**25**) and B (**26**) for the first time. Furthermore, due to the demonstrated antitrypansomal activity of structurally related peptide compounds such as almiramide[77] and doragonamide[78], the synthesized ikoamide (**1**) in the previous chapter showed remarkable results not only in antimalarial activity but also in antitrypansomal activity. Therefore, we also conducted the same

antimalarial and antitrypansomal activity testing for the synthesized odookeanynes A (**25**) and B (**26**).

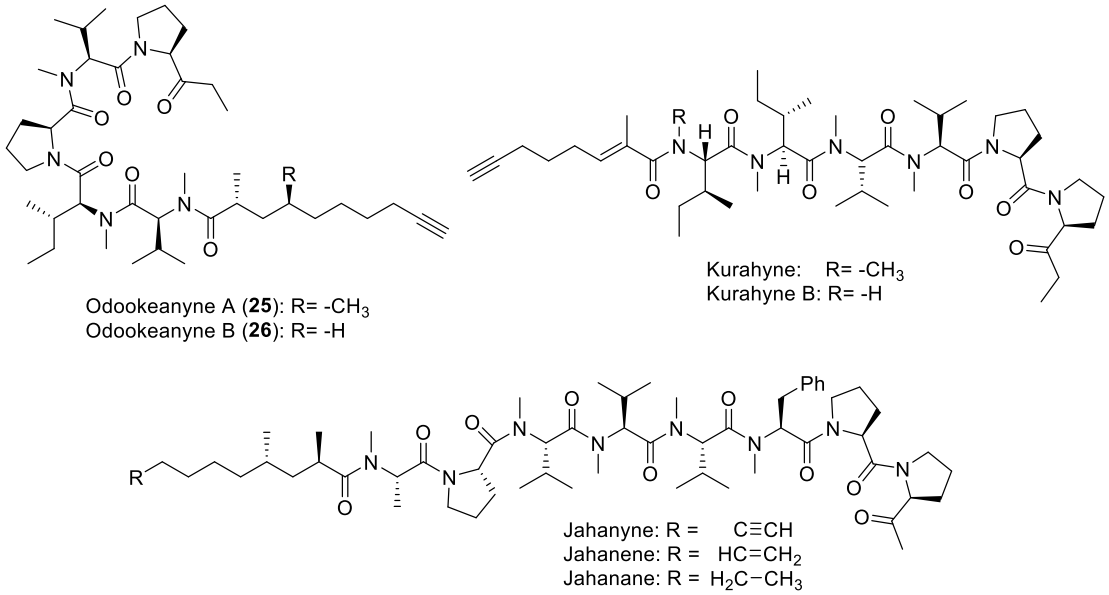
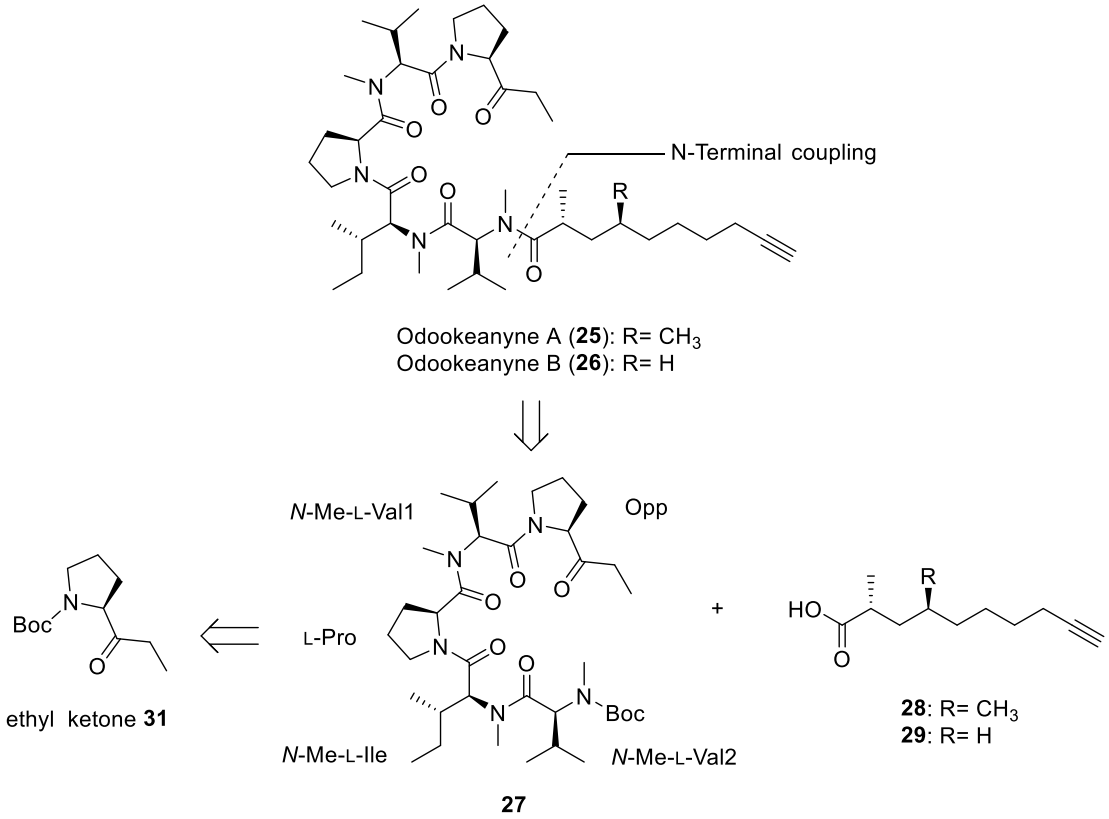


Figure 15. Structures of odookeanynes A (**25**) and B (**26**), jahanyne family, kurahyne, and kurahyne B.

3-2. Total synthesis and biological activity of odookeanynes

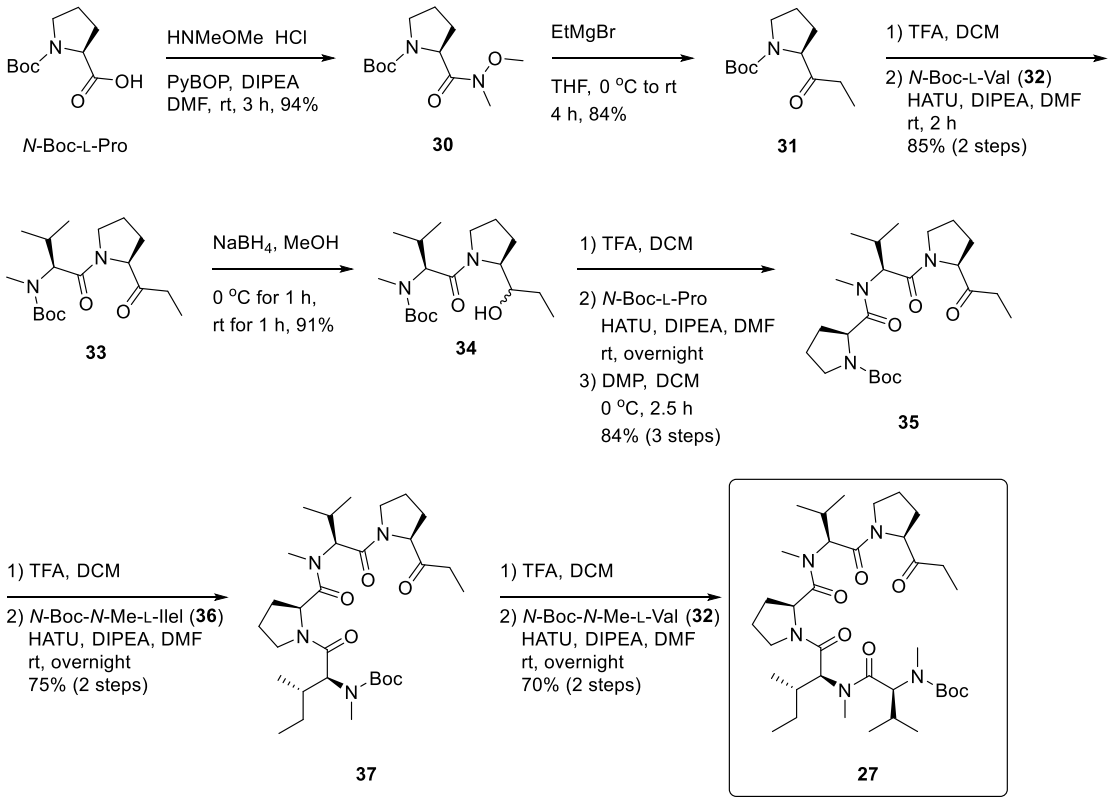
According to retrosynthetic analysis (Scheme 17), odookeanynes A (**25**) and B (**26**) are composed of modified tetrapeptide **27** and two kinds of acetylene-containing carboxylic acid **28**[43] and **29**. Modified tetrapeptide **27** could be prepared from ethyl ketone **31**[49] by condensation reactions. According to a previous report, carboxylic acid **28** could be prepared in seven steps from the commercially available starting material[43] Since the synthesis route for carboxylic acid **29** has not been reported, we intend to refer to the synthetic pathway for carboxylic acid **28**, as they have similar structures. According to previous reports, ethyl ketone **31** could be prepared[49] and other *N*-Me amino acids could be easily prepared from the corresponding amino acids. These moieties could be connected in a stepwise manner, starting from ethyl ketone **31**.



Scheme 17. Retrosynthetic analysis of odookeanynes A (**25**) and B (**26**).

Due to structural similarities, we referenced the synthesis method of kurahyne,[48,49] using only HATU as the coupling reagent, which proved to be an effective way to obtain the target product. First, *N*-Boc-L-Pro underwent two steps of reaction to obtain the known ethyl ketone **31**[49] with a yield of 79% in two steps. Then, compound **31** was deprotected and coupled with *N*-Boc-*N*-Me-L-Val (**32**) via a condensation reaction using HATU as coupling reagents, resulting in compound **33** with a yield of 85%.

However, due to the deprotected Val amino group, which could undergo intramolecular nucleophilic attack on the Opp ketone moiety, an undesired cyclization product was expected to form during the direct coupling of **33** with *N*-Boc-L-Pro. Therefore, compound **33** was reduced using sodium borohydride to obtain the corresponding alcohol **34**, which existed as an inseparable mixture of epimers (in a ratio of 2:1). Subsequently, alcohol **34** was deprotected and coupled with *N*-Boc-L-Pro, followed by oxidation using DMP to afford the modified dipeptide **35** with a three-step overall yield of 84%. Next, modified dipeptide **35** was deprotected and coupled with *N*-Boc-*N*-Me-L-Ile (**36**) to give tripeptide **37** in 75% yield. Finally, the Boc group of tripeptide **37** was removed, and the deprotected amine was coupled with **32** again to obtain the modified tetrapeptide **27** with a yield of 62% (Scheme 18). As the chain length increases, the yield is expected to decrease gradually, resulting in reduced reaction efficiency. Furthermore, during the deprotection mediated by TFA and coupling process basic conditions, the keto moiety in Opp epimerization was not observed.

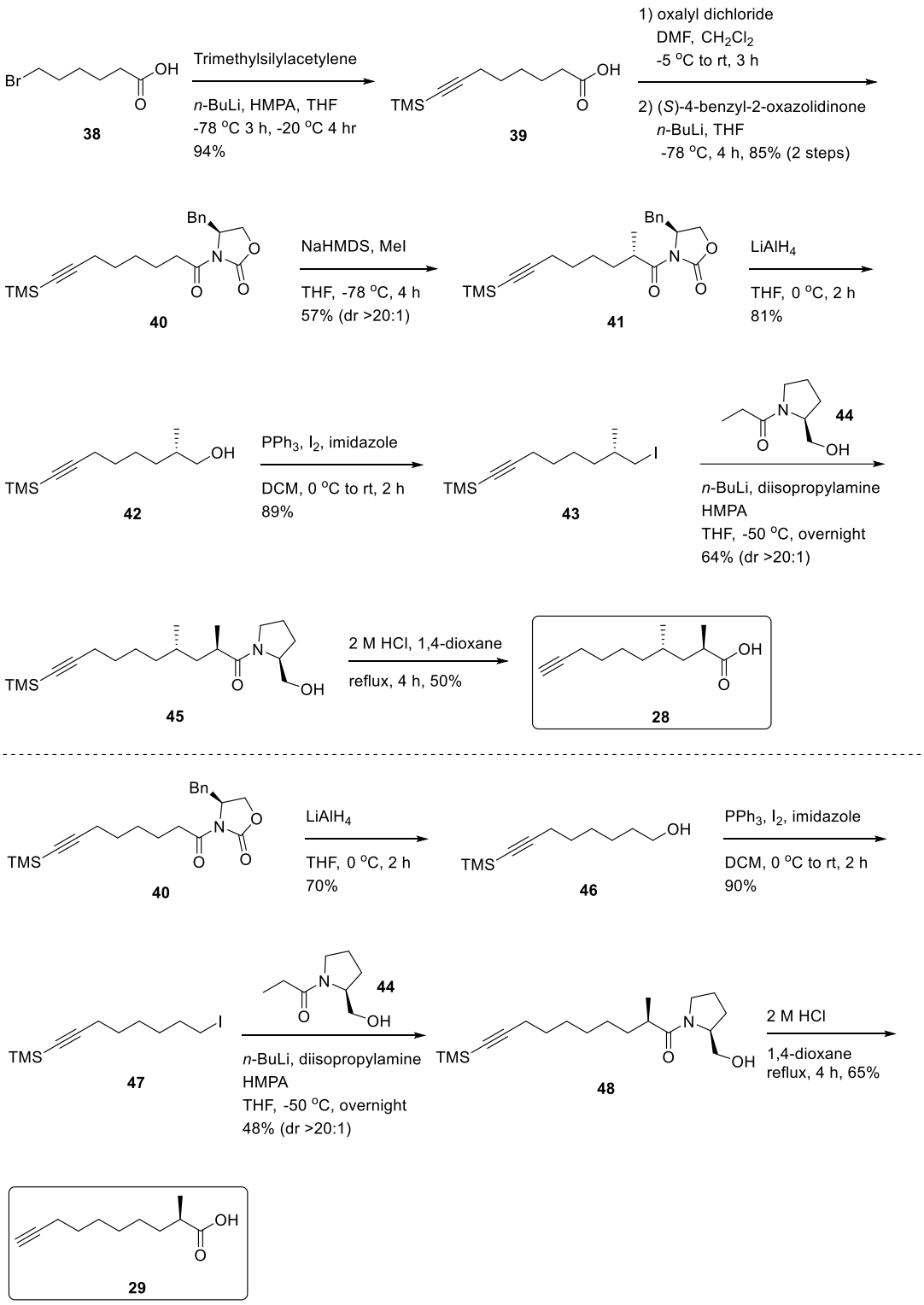


Scheme 18. Synthesis of tetrapeptide **27**.

Fatty acid **28** was prepared according to a previous report (Scheme 19).[43] Compound **38** was treated with *n*-BuLi, and trimethylsilylacetylene to afford **39** in 94% yield.[74] Subsequent activation of **39** using oxalyl dichloride and amidation with the Evans auxiliary furnished compound **40** in 85% yield. Diastereoselective methylation of the sodium enolate generated from **40** to afford **41** in 57% yield (according to ^{1}H NMR, dr

of >20:1).[75] Reduction of compound **41** with $LiAlH_4$ yielded alcohol **42** with an 81%. In the Appel reaction,[76] alcohol **42** was reacted with iodine to yield the corresponding alkyl iodide **43** with a good yield of 89%. Subsequently, employing the modified Evans conditions described in the literature[43], the alkyl iodide **43** was subjected to diastereoselective alkylation with the resulting enolate derived from **44**, affording compound **45** with a yield of 64% (according to ^{1}H NMR, dr of >20:1). Finally, compound **45** was treated with 2 M HCl to hydrolyze the TMS group and the amide bond. In this experiment, we attempted to add 1,4-dioxane to achieve a homogeneous reaction, but it seemed to have little effect on improving the yield. The corresponding known fatty acid **28** was obtained with a yield of 50%.

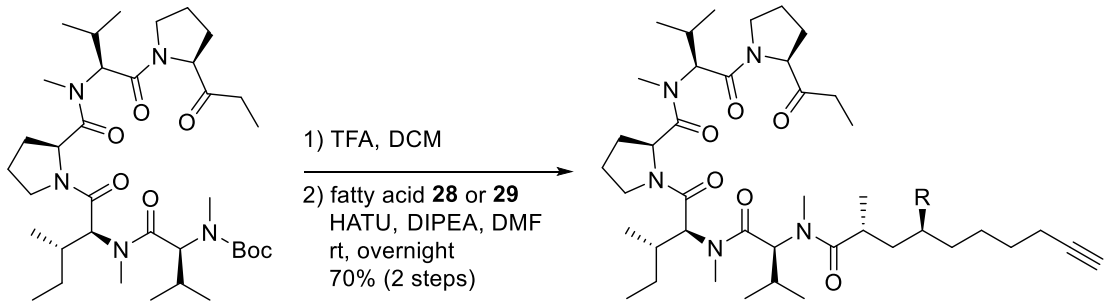
For the synthesis of fatty acid **29**, we used a synthetic approach similar to that for fatty acid **28** (Scheme 19). Starting from the intermediate compound **40**, reduction using $LiAlH_4$ afforded the corresponding alcohol **46** with a yield of 70%. Subsequently, the Appel reaction resulted in formation of the corresponding alkyl iodide **47** with a yield of 90%.[76] The reaction between iodide **47** and compound **44** yielded product **48** (48% yield, according to ^{1}H NMR, dr of >20:1). Finally, treatment with 2 M HCl for hydrolysis furnished the fatty acid **29** with a yield of 65%.



Scheme 19. Synthesis of fatty acids **28** and **29**.

Having completed the synthesis of modified tetrapeptide **27** and fatty acids **28** and **29**, we then carried out the final step of the total synthesis of odookeanynes A (**25**) and B (**26**) (Scheme 20). After deprotection of **27**, the resulting secondary amine was condensed with **28** to provide odookeanyne A (**25**) in 80% overall yield for two steps.

Similarly, odookeanyne B (**26**) was synthesized by the reaction with fatty acid **29**, with a yield of 75%. All of the spectroscopic data of the synthetic odookeanynes A (**25**) and B (**26**) matched the corresponding values of the natural compounds.[73] There are no epimers in the final product. Therefore, no epimerization in OPP has occurred in the deprotection and condensation reactions. Thus, we achieved the total synthesis of the odookeanynes A (**25**) and B (**26**) and confirmed their structures.



27

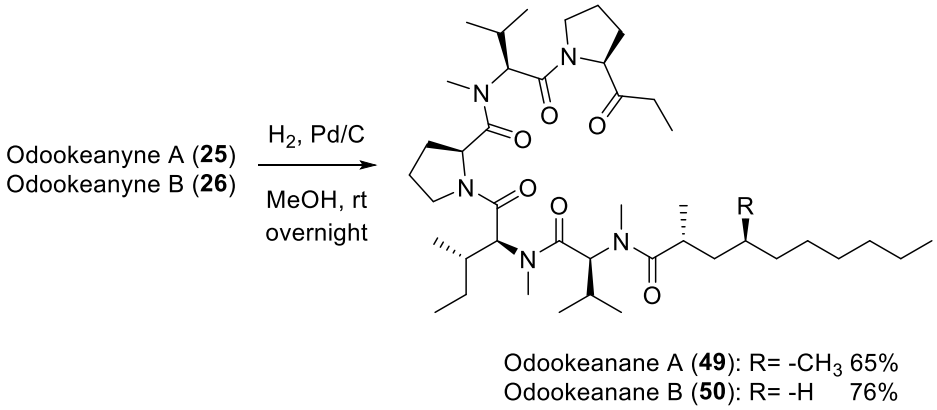
Odookeanyne A (**25**): R= -CH_3 80% (2 steps)
Odookeanyne B (**26**): R= -H 75% (2 steps)

Scheme 20. Synthesis of odookeanynes A (**25**) and B (**26**).

Due to the demonstrated antiparasitic activity of structurally similar peptide compounds such as almiramide[77] and doragonamide[78], we decided to conduct similar experiments on the synthesized odookeanynes. We evaluated the antitrypanosomal activity of synthesized odookeanynes by a method similar to that reported previously.[79] To our surprise, both compounds exhibited remarkably potent antitrypanosomal activity, with odookeanyne A (**25**) and odookeanyne B (**26**) showing IC_{50} values of 21.3±6.7 nM and 68.7±15.3 nM, respectively (Table 2). Odookeanyne A (**25**) has slightly more potent antitrypanosomal activity than odookeanyne B (**26**). We speculate that γ-methyl group in the fatty acid chain may be responsible for this difference in activity. To clarify the importance of acetylene moiety against the activity, we conducted hydrogenation reactions on both odookeanynes A (**25**) and B (**26**), resulting in saturated fatty acid portions of odookeananes A (**49**) and B (**50**) (Scheme 21). Subsequently, we performed antitrypanosomal activity tests on A (**49**) and B (**50**). Surprisingly, we found that the low unsaturation at the terminus of the fatty acid moiety significantly enhanced antitrypanosomal activity. The antitrypanosomal activity of odookeanane A (**49**) was 59% growth inhibition at 0.3 nM, and odookeanane B (**50**) IC_{50} value was 2.0 nM.

Additionally, we conducted antimalarial activity testing on four synthetic compounds.[80] The antimalarial activity of odookeanynes A and B was much lower than its antitrypanosomal activity, with IC_{50} values for both 1.2±0.4 μM and 1.0±0.5 μM.

Furthermore, based on the results of the antimalarial activity testing for odookeananes A and B, the degree of unsaturation of fatty acid moiety didn't affect their antimalarial activities, with IC_{50} values of 1.0 μM and 0.55 μM, respectively. This data suggest that odookeanynes might interact with a trypanosomal-specific target, resulting in its lower antimalarial activity than its antitrypanosomal activity. Furthermore, this prediction is also supported by the fact that a γ-methyl group of fatty acid moiety did not enhance the antimalarial activity, unlike antitrypanosomal activity. This indicates that odookeanynes exhibit selective antitrypanosomal activity and are potentially antitrypanosomal drugs.



Scheme 21. Synthesis of odookeananes A (**49**) and B (**50**)

Table 2. Antitrypanosomal and antimalarial activities of odookeanynes and odookeananes.

Compound	Antitrypanosomal activity (IC_{50}, nM)[a]	Antimalarial activity (IC_{50}, μM)[b]
Odookeanine A (**25**)	21.3±6.7[d]	1.2±0.4[d]
Odookeanyne B (**26**)	68.7±15.3[d]	1.0±0.5[d]
Odookeanane A (**49**)	<0.3[c]	1.0[e]
Odookeanane B (**50**)	2.0[e]	0.55[e]

[a] against *Trypanosoma brucei rhodesiense*. Pentamidine (positive control): IC_{50} = 6.1 nM. n = 3.

[b] against *Plasmodium falciparum*. Chloroquine (positive control): IC_{50} =47 nM. n = 3.

[c] 59% growth inhibition at 0.3 nM. n = 1.

[d] n = 3

[e] n = 1

In summary, we have achieved the first total synthesis of odookeanynes A (**25**) and B (**26**) with overall yields of 2.5% in 14 steps for odookeanyne A (**25**) and 3.6% in 13 steps for odookeanyne B (**26**). A biological evaluation using the synthesized odookeanynes revealed their hidden potential as antitrypanosomal compounds. The synthetic pathway and antiparasitic activity discovered and applied in this study are expected to provide new insights into this class of compounds.

Chapter 4. Summary

In this research and development, we investigated the biological activities of the natural compounds ikoamide and odookeanynes and discovered their potential as antimalarial drugs as well as for the treatment of type 2 diabetes and obesity. However, due to limited natural resources, we could not conduct extensive biological activity evaluations. Therefore, we conducted total synthesis studies on these two natural lipopeptides.

Firstly, we achieved the total synthesis of ikoamide (**1**). Although diastereomers that cannot be separated were formed from tripeptide to pentapeptide, the subsequent purification by HPLC allowed us to obtain pure products starting from hexapeptide. Using (*R*)-1,2-epoxypentane as the starting material, a series of reactions led to the desired 3,5-dimethoxyoctanoic acid. Finally, coupling the octapeptide with the fatty acid successfully resulted in the synthesis of ikoamide (**1**). In terms of biological activity evaluation, we not only confirmed that the synthesized ikoamide exhibited potent antimalarial activity similar to the natural compound, with an IC_{50} value of 0.024 μM. We also conducted antitrypanosomal activity testing and found that ikoamide (**1**) demonstrated significant antitrypanosomal activity with an IC_{50} value of 1.3 μM.

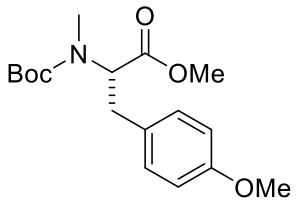
Secondly, we accomplished the total synthesis of odookeanynes A (**25**) and B (**26**). Using ethyl ketone as a starting material and coupling it with respective amino acids, we obtained the desired modified tetrapeptide product. For the fatty acid portion, we referred to the method by Chen et al.[43] and extended its application to obtain the corresponding acetylenic acids. Successful coupling resulted in the total synthesis of odookeanynes A (**25**) and B (**26**). Regarding biological activity evaluation, we discovered that odookeanynes exhibited remarkable antitrypanosomal activity with IC_{50} values of A: 21.3±6.7 nM and B: 68.7±15.3 nM. This suggests that odookeanyne may interact with specific targets in trypanosomes, exhibiting selective antitrypanosomal activity and holding potential as antitrypanosomal drugs.

In the future, we aim to create novel antimalarial and antitrypanosomal drugs through the total synthesis and biological activity evaluation of more natural peptides. Additionally, our goal is to explore various biological activities and, given the limited natural resources, confirm the structures of natural compounds through total synthesis and provide sufficient quantities of products for more extensive and valuable biological activity evaluations. This will contribute to the development of more effective tools for the treatment of related diseases.

Chapter 5. Experimental section

General Experimental Procedures. Infrared (IR) spectra were recorded on a Bruker ALPHA instrument. All nuclear magnetic resonance (NMR) spectral data were recorded on JEOL ECS-400 spectrometers for 1H (400 MHz) and 13C{1H} (100 MHz) at room temperature. Chemical shifts are reported δ values in parts per million relative to the solvent signal ($CDCl_3$; δ = 7.26 ppm for 1H NMR, δ = 77.00 ppm for 13C NMR), and coupling constants are in hertz (Hz). The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, dt = double triplet, q = quartet, dq = double quartet, m = multiplet. All chemicals and solvents used in this study were of the best grade and available from a commercial source (Nacarai Tesque). Reactions were monitored by thin-layer chromatography (TLC), which was performed on E. Merck precoated silica gel 60F254 (Merck). To detect compounds, the plate was irradiated with UV (254 nm), then immersed in a 7% ethanol solution of phosphomolybdic acid, and heated on a hot plate. To elevate the temperature, reaction mixtures were heated using an oil bath. Silica gel 60N (irregular, 63−212 μm) was used for open column chromatography unless otherwise noted. All nonaqueous reactions were performed under an atmosphere of nitrogen using oven-dried glassware under an N2 atmosphere and a standard syringe in septa techniques.

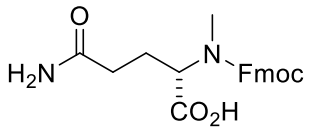
Total Synthesis Procedures



5

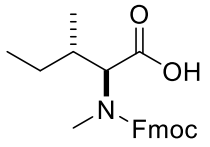
Methyl (*S*)-2-((*tert*-butoxycarbonyl)(methyl)amino)-3-(4-methoxyphenyl) propanoate (5): A solution of *N*-Boc-L-Tyr-OH (5.63 g, 20.0 mmol) in anhydrous DMF (70 mL) was treated with NaH (3.2 g of 60% in oil, 80.0 mmol) and the mixture was stirred for 20 minutes before being treated with iodomethane (12.5 mL, 200 mmol). The reaction was stirred at room temperature overnight before being quenched with the addition of saturated aqueous NH_4Cl (100 mL) and extracted with EtOAc (70 mL×3). The combined organic layers were washed with saturated aqueous NH_4Cl (70 mL), saturated aqueous $Na_2S_2O_3$ (70 mL), and brine (70 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (hexanes/EtOAc, 5:1 v/v) to give **5** (6.68 g, 20.0 mmol, 99% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) (major rotamers) δ 7.06 (d, *J* = 8.8 Hz, 2H), 6.80 (d, *J* =

8.8 Hz, 2H), 4.86 (dd, *J* = 5.2, 10.8 Hz, 0.5H), 4.44 (dd, *J* = 4.8, 10.4 Hz, 0.5H), 3.74 (s, 3H), 3.71 (s, 1.5H), 3.69 (s, 1.5H), 3.21 (m, 1H), 2.90 (m, 1H), 2.69 (s, 3H), 1.35 (s, 4.5H), 1.32 (s, 4.5H); $^{13}C\{^{1}H\}$ NMR $CDCl_3$, 100 MHz) δ 171.8, 171.5, 158.3, 158.1, 155.7, 154.8, 131.5, 129.9, 129.8, 129.5, 129.3, 113.8, 113.7, 113.4, 80.1, 79.8, 63.0, 61.7, 59.5, 55.1, 52.0, 34.5, 34.0, 32.5, 31.8, 28.3, 28.12, 28.07. Spectroscopic data are in agreement with those reported in the literature.



6

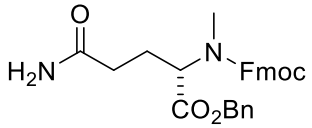
***N*²-(((9H-fluoren-9-yl)methoxy)carbonyl)-*N*²-methyl-L-glutamine (6):** A solution of *N*-Fmoc-L-Gln(Trt)-OH (9.16 g, 15.0 mmol) in DMF (5 mL) and toluene (100 mL) was added paraformaldehyde (30 g) and TsOH (0.5 g). The reaction was stirred at 140 °C for 2 h under reflux. The solution was cooled to room temperature and diluted with EtOAc (100 mL). The whole mixture was successively washed with saturated aqueous $NaHCO_3$ (70 mL×2), water (70 mL), and brine (70 mL), dried over Na_2SO_4, and concentrated to give crude amine. To a solution of the above crude amine in CH_2Cl_2 (40 mL) was added triethylsilane (9.6 mL, 60 mmol) and trifluoroacetic acid (58 mL, 750 mmol). The reaction mixture was stirred at room temperature overnight before being diluted with toluene and evaporated the solution under reduced pressure 3 times. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 10:1 v/v) to give **6** (5.52 g, 14.4 mmol, 96% yield) as a white solid: ^{1}H NMR (d^6-DMSO, 400 MHz) (major rotamers) δ 7.89 (d, *J* = 6.8 Hz, 2H), 7.64 (d, *J* = 7.2 Hz, 2H), 7.43-7.39 (m, 2H), 7.35-7.28 (m, 2H), 7.25 (brs, 1H, NH), 6.78 (brs, 1H, NH), 4.48 (m, 1H), 4.32-4.28 (m, 2H), 4.24 (m, 1H), 2.76 (s, 3H), 2.28-1.80 (m, 4H); $^{13}C\{^{1}H\}$ NMR (d^6-DMSO, 100 MHz) (major rotamer) δ 173.8, 172.6, 156.3, 143.9, 140.9, 127.9, 127.3, 125.3, 120.3, 67.1, 58.4, 46.8, 31.7, 30.8, 24.2. Spectroscopic data are in agreement with those reported in the literature.



8

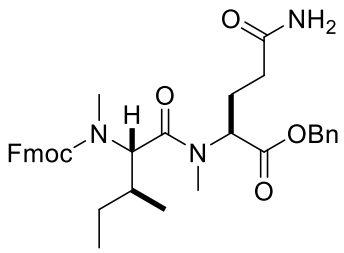
***N*-(((9H-fluoren-9-yl)methoxy)carbonyl)-*N*-methyl-L-isoleucine (8):** A solution of *N*-Fmoc-L-Ile-OH (10.6 g, 30.0 mmol) in toluene (100 mL) were added

paraformaldehyde (35 g) and TsOH (1.2 g). The reaction mixture was stirred at 160 °C for 2 h under reflux, cooled to room temperature, and diluted with EtOAc (100 mL). The mixture was successively washed with saturated aqueous $NaHCO_3$ (70 mL×2), water (70 mL), and brine (70 mL), dried over Na_2SO_4, and concentrated. To a solution of the above crude compound in CH_2Cl_2 (30 mL) were added triethylsilane (19.2 mL, 120 mmol) and trifluoroacetic acid (46 mL, 600 mmol). The reaction was stirred at room temperature overnight before being diluted with toluene and evaporated the solution under reduced pressure 3 times. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 30:1 v/v) to give **8** (11.0 g, 30.0 mmol, 99% yield) as a white solid: ^{1}H NMR (d^6-DMSO, 400 MHz) (major rotamers) δ 7.87 (d, *J* = 8.0 Hz, 2H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.41-7.38 (m, 2H), 7.33-7.29 (m, 2H), 4.46-4.32 (m, 2H), 4.28-4.24 (m, 2H), 2.69 (s, 3H), 1.90-1.71 (m, 1H), 1.24-1.04 (m, 1H), 0.96-0.91 (m, 1H), 0.86 (d, *J* = 7.2 Hz, 3H), 0.79 (t, *J* = 7.2 Hz, 3H); ^{13}C{^{1}H} NMR (d^6-DMSO, 100 MHz) (major rotamer) δ 172.1, 156.1, 143.9, 140.9, 127.7, 127.2, 125.0, 120.2, 66.8, 62.3, 46.8, 32.7, 30.0, 24.6, 15.8, 10.5. Spectroscopic data are in agreement with those reported in the literature.



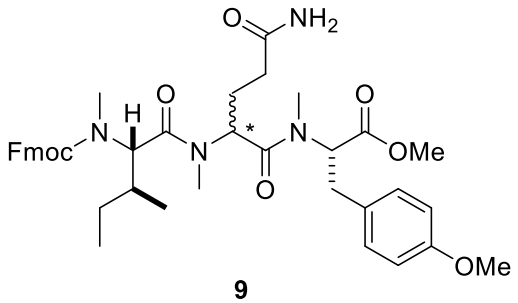
10

Benzyl *N*^{2}-(((9H-fluoren-9-yl)methoxy)carbonyl)-*N*^{2}-methyl-L-glutaminate (10): A solution of **6** (5.7 g, 14.8 mmol) in anhydrous DMF (70 mL) was added benzyl bromide (3.5 mL, 29.6 mmol) and potassium bicarbonate (4.4 g, 44.4 mmol). The reaction was stirred at room temperature overnight and then diluted with EtOAc (70 mL). The whole mixture was successively washed with water (50 mL), saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH, 20:1 v/v) to give **10** (5.9 g, 12.5 mmol, 84% yield) as a white solid: $[\alpha]_D^{25}$ −14.0 (*c* 1.06, $CHCl_3$); ^{1}H NMR (d^6-DMSO, 400 MHz) (major rotamer) δ 7.89 (d, *J* = 7.2 Hz, 2H), 7.62 (d, *J* = 8.0 Hz, 2H), 7.42-7.28 (m, 9H), 7.25 (brs, 1H), 6.79 (brs, 1H), 5.07 (m, 2H), 4.59 (dd, *J* = 4.8, 11.2 Hz, 1H), 4.33-4.15 (m, 3H), 2.75 (s, 3H), 2.14-1.88 (m, 4H); ^{13}C{^{1}H} NMR (d^6-DMSO, 100 MHz) (major rotamer) δ 173.4, 170.7, 156.1, 143.8, 143.8, 140.8, 140.8, 135.9, 128.6, 128.5, 128.1, 127.9, 127.9, 127.8, 127.7, 127.3, 127.2, 125.1, 125.0, 120.2, 120.2, 67.1, 66.2, 58.6, 46.7, 31.2, 31.2, 24.0; IR (neat) 3346, 1739, 1684, 1451, 1402 cm^{-1}; HRMS (ESI-TOF) *m/z*: [M + Na]$^+$ Calcd for $C_{28}H_{28}N_2O_5Na$ 495.1896; found, 495.1913.



11

Benzyl N^2-(*N*-(((9H-fluoren-9-yl)methoxy)carbonyl)-*N*-methyl-L-isoleucyl)-N^2-methyl-L-glutaminate (11): A solution of **10** (5.8 g, 12.3 mmol) in MeCN (15 mL) and Et_2NH (10 mL) was stirred at room temperature for 1 h and concentrated, giving the corresponding crude amine. To a solution of the above amine in dry DMF (7 mL) were added **8** (5.5 g, 15 mmol), HATU (7.0 g, 18.5 mmol), and DIPEA (12.9 mL, 74 mmol) cooled at 0 ˚C. The reaction mixture was then stirred at room temperature overnight and diluted with EtOAc (80 mL). The whole mixture was washed successively with 10% aq. citric acid (50 mL), water (50 mL), saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH, 30:1 v/v) to give dipeptide **11** (4.3 g, 14.7 mmol, 59% yield) as a white solid: $[\alpha]_D^{24}$ −82.2 (*c* 1.02, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, a mixture of rotamers) δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.57 (d, *J* = 7.6 Hz, 2H), 7.41-7.27 (m, 9H), 5.18-5.03 (m, 2H), 4.91-4.69 (m, 2H), 4.47 (m, 1H), 4.24 (t, *J* = 6.6 Hz, 1H), 4.19 (m, 1H), 2.95 (s, 2H), 2.79 (s, 2H), 2.64 (s, 2H), 2.36 (m, 1H), 2.12-1.95 (m, 4H), 1.27 (m, 1H), 0.98 (m, 1H), 0.88 (t, *J* = 7.4 Hz, 3H), 0.79 (d, *J* = 6.4 Hz, 3H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 174.0, 172.2, 171.2, 170.1, 156.8, 144.0, 143.7, 141.6, 141.3, 135.1, 128.6, 128.5, 128.4, 128.3, 127.7, 127.6, 127.3, 127.0, 124.83, 124.80, 124.1, 120.0, 119.8, 67.4, 67.2, 58.5, 56.3, 47.2, 33.5, 31.7, 30.5, 29.3, 24.5, 24.0, 15.1, 10.7; IR (neat) 3345, 2964, 1739, 1688, 1451, 1402 cm^{-1}; HRMS (ESI-TOF) *m*/*z*: [M + Na]$^+$ Calcd for $C_{35}H_{41}N_3O_6Na$ 622.2893; found, 622.2919.

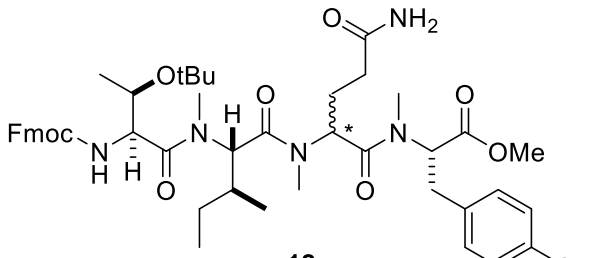


9

* 5:1 mixture

Methyl (5*S*,8*S*,11*S*)-8-(3-amino-3-oxopropyl)-5-((*S*)-*sec*-butyl)-1-(9H-fluoren-9-yl)-11-(4-methoxybenzyl)-4,7,10-trimethyl-3,6,9-trioxo-2-oxa-4,7,10-

triazadodecan-12-oate (9): A suspension of dipeptide **11** (6.0 g, 10.0 mmol) and 20% palladium hydroxide on activated charcoal (1.8 g) in EtOAc (30 mL) was stirred under an atmosphere of hydrogen at room temperature overnight. The mixture was filtered to remove the catalyst, and the filtrate was evaporated under reduced pressure. The resulting residue was dried under a vacuum to give the crude carboxylic acid as a white solid. To a solution of **5** (6.5 g, 20 mmol) in CH_2Cl_2 (20 mL) and trifluoroacetic acid (23 mL) was stirred at room temperature for 1 h and concentrated, giving the corresponding crude amine (TFA salt). To a solution of combining the above carboxylic acid and amine in dry DMF (2 mL) were added HATU (5.7 g, 15 mmol), and DIPEA (10.5 mL, 60 mmol) cooled at 0 ˚C. The reaction mixture was then stirred at room temperature overnight, and diluted with EtOAc (80 mL). The whole mixture was washed successively with 10% aq. citric acid (50 mL), water (50 mL), saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH, 20:1 v/v) to give tripeptide **7** (3.0 g, 4.23 mmol, 42% yield) as a white solid. This was an inseparable mixture of the diastereomers for *N*-Me-Gln (the ratio is 5:1): $[\alpha]_D^{24}$ −65.8 (*c* 1.34, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, a mixture of rotamers) δ 7.75 (d, *J* = 7.2 Hz, 2H), 7.55 (d, *J* = 6.8 Hz, 2H), 7.41-7.28 (m, 4H), 7.05 (d, *J* = 8.8 Hz, 2H), 6.80 (d, *J* = 8.4 Hz, 2H), 5.45-5.32 (m, 2H), 4.98 (m, 1H), 4.70-4.48 (m, 2H), 4.22 (m, 1H), 3.78-3.74 (m, 6H), 3.54 (m, 1H), 3.29 (m, 1H), 2.99-2.35 (m, 9H), 2.16-1.90 (m, 5H), 1.27-1.23 (m, 2H), 0.90-0.82 (m, 3H), 0.72-0.57 (m, 3H); ^{1}H NMR (d^6-DMSO, 400 MHz, a mixture of rotamers) 7.86 (d, *J* = 7.6 Hz, 2H), 7.61 (d, *J* = 7.6 Hz, 2H), 7.40-7.28 (m, 4H), 7.13-6.98 (m, 2H), 6.88-6.69 (m, 2H), 5.18-5.00 (m, 2H), 4.83 (m, 1H), 4.67 (m, 1H), 4.51 (m, 1H), 4.40-4.23 (m, 2H), 3.72-3.57 (m, 6H), 3.49 (m, 1H), 3.12 (m, 1H), 2.82-2.36 (m, 9H), 2.11-1.60 (m, 5H), 1.43-1.08 (m, 2H), 0.81-0.74 (m, 2H), 0.65-0.51 (m, 2H), 0.29-0.15 (m, 2H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 174.2, 170.6, 169.9, 169.6, 158.6, 156.5, 143.71, 143.66, 141.32, 141.27, 130.5, 129.8, 129.6, 127.8, 127.7, 127.2, 127.0, 124.74, 124.70, 120.0, 119.8, 114.4, 113.9, 67.2, 66.1, 59.6, 59.2, 55.2, 52.4, 47.3, 33.5, 32.9, 32.4, 29.5, 29.0, 24.6, 24.1, 15.6, 10.7; IR (neat) 3349, 3010, 2962, 1742, 1690, 1641, 1514, 1451, 1400 cm^{-1}; HRMS (ESI-TOF) *m*/*z*: [M + Na]$^+$ Calcd for $C_{40}H_{50}N_4O_8Na$ 737.3526; found, 737.3556.

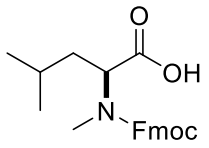


12

* 5:1 mixture

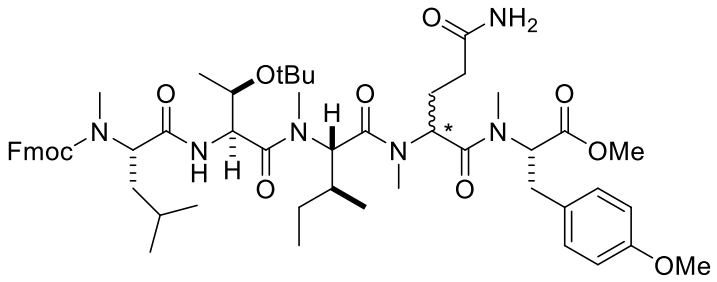
Methyl (5*S*,8*S*,11*S*,14*S*)-11-(3-amino-3-oxopropyl)-5-((*R*)-1-(*tert*-butoxy)ethyl)-8-((*S*)-*sec*-butyl)-1-(9H-fluoren-9-yl)-14-(4-methoxybenzyl)-7,10,13-trimethyl-3,6,9,12-tetraoxo-2-oxa-4,7,10,13-tetraazapentadecan-15-oate (12): A solution of tripeptide **9** (3.0 g, 4.2 mmol, a 5:1 mixture of the diastereomers) in MeCN (10 mL) and Et_2NH (5 mL) was stirred at room temperature for 1 h and concentrated, giving the corresponding crude amine. To a solution of the above amine in dry DMF (1.5 mL) was added *N*-Fmoc-L-Thr(tBu)-OH (3.4 g, 8.5 mmol), HATU (4.0 g, 10.6 mmol), and DIPEA (4.4 mL, 25.4 mmol) at 0 ˚C. The reaction mixture was then stirred at room temperature overnight, and diluted with EtOAc (80 mL). The whole mixture was washed successively with 10% aq. citric acid (50 mL), water (50 mL), saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH, 30:1 v/v) to give tetrapeptide **12** (2.4 g, 2.76 mmol, 65% yield) as a white solid. This was an inseparable mixture of the diastereomers (the ratio is 5:1): $[\alpha]_D^{25}$ −71.5 (*c* 0.28, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, a mixture of rotamers) δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.58 (d, *J* = 7.2 Hz, 2H), 7.42-7.28 (m, 4H), 7.09 (d, *J* = 8.6 Hz, 2H), 6.83 (d, *J* = 8.8 Hz, 2H), 6.39 (brs, 1H), 5.81 (brs, 1H), 5.68 (m, 1H), 5.38-5.22 (m, 2H), 5.09 (m, 1H), 4.68 (m, 1H), 4.39 (m, 1H), 4.22 (m, 1H), 3.90 (m, 1H), 3.78 (s, 3H), 3.76 (s, 3H), 3.33 (m, 1H), 3.12 (s, 3H), 3.04 (s, 3H), 2.96 (m, 1H), 2.77 (s, 3H), 2.13-2.05 (m, 3H), 1.81-1.64 (m, 4H), 1.19 (s, 9H), 0.99 (d, *J* = 6.4 Hz, 3H), 0.88-0.84 (m, 3H), 0.77 (t, *J* = 7.4 Hz, 3H); ^{1}H NMR (d^6-DMSO, 400 MHz, a mixture of rotamers) 7.87 (d, *J* = 7.6 Hz, 2H), 7.71 (d, *J* = 7.6 Hz, 2H), 7.51-7.38 (m, 2H), 7.31-7.27 (m, 2H), 7.17-7.06 (m, 2H), 6.89-6.79 (m, 2H), 6.68 (brs, 1H), 5.21 (brs, 1H), 5.11 (m, 1H), 4.90 (m, 1H), 4.64 (m, 1H), 4.46 (m, 1H), 4.30 (m, 1H), 4.20-4.17 (m, 2H), 3.73-3.57 (m, 6H), 3.51 (m, 1H), 2.97-2.88 (m, 4.5H), 2.81 (m, 1H), 2.72-2.64 (m, 4.5H), 2.26-2.07 (m, 3H), 1.97-1.48 (m, 4H), 1.08 (s, 9H), 0.98-0.86 (m, 3H), 0.77-0.63 (m, 6H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz, a mixture of rotamers) δ 174.4, 170.8, 170.7, 170.3, 169.7, 158.7, 155.5, 143.9, 141.3, 130.5, 129.9, 129.7, 127.7, 127.0, 125.1, 120.0, 114.1, 114.0, 75.1, 68.8, 66.9, 60.4, 58.6, 57.3, 56.2, 55.3, 52.5, 47.1, 33.4, 32.8, 31.6, 31.1, 30.8, 29.7, 28.1, 28.02, 27.96, 24.9, 24.1, 17.9, 15.8, 10.9; IR (neat) 3326, 2972, 1719, 1635, 1514, 1451, 1406 cm^{-1}; HRMS (ESI-TOF) *m*/*z*: $[M + Na]^+$ Calcd for $C_{48}H_{65}N_5O_{10}Na$ 894.4629;

found, 894.4661.



13

***N*-(((9H-fluoren-9-yl)methoxy)carbonyl)-*N*-methyl-L-leucine (13):** A solution of *N*-Fmoc-L-Leu-OH (3.54 g, 10.0 mmol) in toluene (70 mL) were added paraformaldehyde (15 g) and TsOH (0.4 g). The reaction mixture was stirred at 160 ˚C for 2 h under reflux, cooled to room temperature, and diluted with EtOAc (100 mL). The mixture was successively washed with saturated aqueous $NaHCO_3$ (70 mL×2), water (70 mL), and brine (70 mL), dried over Na_2SO_4, and concentrated. To a solution of the above crude compound in CH_2Cl_2 (10 mL) were added triethylsilane (6.4 mL, 40 mmol) and trifluoroacetic acid (15.3 mL, 200 mmol). The reaction was stirred at room temperature overnight before being diluted with toluene and evaporated the solution under reduced pressure 3 times. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 30:1 v/v) to give **13** (3.50 g, 9.52 mmol, 95% yield) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.79 (d, *J* = 8.0 Hz, 2H), 7.64 (d, *J* = 7.6 Hz, 2H), 7.44-7.29 (m, 4H), 5.00 (m, 0.5H), 4.63 (m, 0.5H), 4.56-4.45 (m, 2H), 4.28 (m, 1H), 2.90 (s, 3H), 1.79 (t, *J* = 7.2 Hz, 2H), 1.67-1.49 (m, 1H), 1.00 (t, *J* = 6.8 Hz, 3H), 0.91 (d, *J* = 6.4 Hz, 1.5H), 0.79 (d, *J* = 6.4 Hz, 1.5H); ^{13}C{^{1}H} NMR $CDCl_3$, 100 MHz) (major rotamer) δ 177.6, 157.1, 143.7, 141.2, 127.6, 127.0, 124.9, 119.9, 67.7, 56.5, 47.2, 37.1, 30.2, 24.8, 23.2, 21.1. Spectroscopic data are in agreement with those reported in the literature.

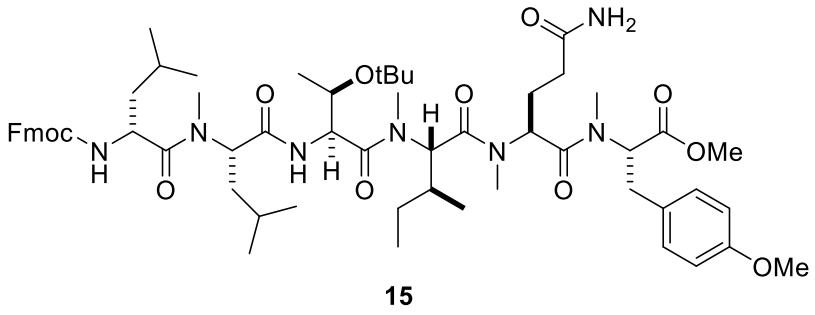


14

* 5:1 mixture

Methyl (5*S*,8*S*,11*S*,14*S*,17*S*)-14-(3-amino-3-oxopropyl)-8-((*R*)-1-(*tert*-butoxy) ethyl)-11-((*S*)-*sec*-butyl)-1-(9H-fluoren-9-yl)-5-isobutyl-17-(4-methoxybenzyl)-4,10,13,16-tetramethyl-3,6,9,12,15-pentaoxo-2-oxa-4,7,10,13,16-pentaazaoctadecan-18-oate (14): A solution of tetrapeptide **12** (2.4 g, 2.8 mmol, a 5:1

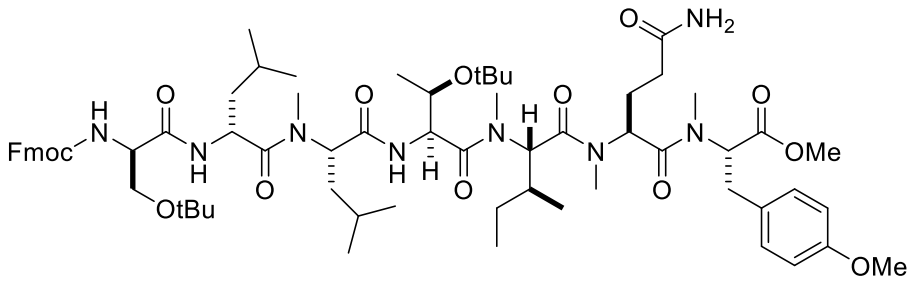
mixture of the diastereomers) in MeCN (4 mL) and Et_2NH (2 mL) was stirred at room temperature for 1 h and concentrated, giving the corresponding crude amine. To a solution of the above amine in dry DMF (1 mL) were added **13** (2.0 g, 5.5 mmol), HATU (2.6 g, 6.9 mmol), and DIPEA (2.9 mL, 16.5 mmol) cooled at 0 ˚C. The reaction mixture was then stirred at room temperature overnight, and diluted with EtOAc (50 mL). The whole mixture was washed successively with 10% aq. citric acid (30 mL), water (30 mL), saturated aqueous $NaHCO_3$ (30 mL) and brine (30 mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH, 30:1 v/v) to give pentapeptide **14** (1.97 g, 1.97 mmol, 70% yield) as a white solid. This was an inseparable mixture of the diastereomers (the ratio is 5:1): $[\alpha]_D^{25}$ −121.0 (*c* 0.29, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, a mixture of rotamers, signals due to minor rotamers in brackets) δ 7.76 (d, *J* = 8.0 Hz, 2H), 7.58 (d, *J* = 6.8 Hz, 2H), 7.39 (m, 2H), 7.31 (m, 2H), [7.14, 7.07] (d, *J* = 8.8 Hz, 2H), [6.88, 6.80] (d, *J* = 8.8 Hz, 2H), 6.37 (brs, 1H), 5.78 (brs, 1H), 5.54 (brs, 1H), 5.34 (dd, *J* = 5.6, 8.0 Hz, 1H), 5.27 (d, *J* = 10.4 Hz, 1H), 5.06 (m, 1H), 4.84 (dd, *J* = 6.4, 12.0 Hz, 1H), 4.63 (m, 1H), 4.49 (dd, *J* = 7.6, 10.4 Hz, 1H), 4.39 (m, 1H), 4.26 (m, 1H), 3.77 (s, 3H), 3.74 (s, 3H), 3.33 (m, 1H), 3.16 (s, 1.5H), 3.10 (s, 1.5H), 3.04 (s, 1.5H), 2.99 (s, 1.5H), 2.86 (s, 1.5H), 2.83 (s, 1.5H), 2.80 (s, 1.5H), 2.75 (s, 1.5H), 2.69 (m, 1H), 2.41 (m, 1H), 2.11-2.04 (m, 2H), 1.77-1.75 (m, 2H), 1.67-1.62 (m, 3H), 1.45 (m, 1H), 1.16 (s, 9H), 0.97-0.90 (m, 6H), 0.83 (m, 6H), 0.74 (t, *J* = 6.4 Hz, 3H); ^{1}H NMR (d^6-DMSO, 400 MHz, a mixture of rotamers) 7.88 (d, *J* = 7.6 Hz, 2H), 7.61 (d, *J* = 7.2 Hz, 2H), 7.40 (t, *J* = 7.6 Hz, 2H), 7.31 (t, *J* = 7.6 Hz, 2H), [7.12, 7.09] (d, *J* = 8.4 Hz, 2H), [6.87, 6.81] (d, *J* = 8.8 Hz, 2H), 6.66 (brs, 1H), 5.21-5.11 (m, 2H), 4.88 (m, 1H), 4.74-4.62 (m, 2H), 4.41-4.26 (m, 3H), 3.71 (s, 3H), 3.64 (s, 3H), 3.15 (m, 1H), 2.89 (s, 3H), 2.71 (s, 3H), 2.64 (s, 3H), 2.18 (s, 2H), 1.97-1.87 (m, 2H), 1.76 (m, 1H), 1.55-1.46 (m, 2H), 1.32-1.13 (m, 2H), 1.03 (s, 8H), 0.94-0.92 (m, 2H), 0.87-0.77 (m, 9H), 0.68 (d, *J* = 6.4 Hz, 3H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz, a mixture of rotamers) δ 174.3, 173.9, 170.8, 170.3, 170.2, 170.0, 169.8, 169.5, 158.7, 157.0, 143.9, 143.8, 141.3, 130.5, 129.6, 128.4, 127.7, 127.0, 124.9, 120.0, 114.4, 114.0, 75.1, 74.9, 68.1, 67.8, 61.1, 58.7, 57.8, 57.2, 56.7, 55.4, 55.2, 53.7, 52.7, 52.4, 47.2, 36.6, 34.2, 33.5, 32.8, 32.5, 31.8, 31.6, 31.0, 30.3, 29.7, 28.0, 27.9, 24.9, 24.7, 24.0, 23.7, 23.1, 21.9, 18.0, 15.7, 11.0, 10.8; IR (neat) 3340, 2964, 1742, 1684, 1636, 1514, 1456, 1401 cm^{-1}; HRMS (ESI-TOF) *m*/*z*: $[M + Na]^+$ Calcd for $C_{55}H_{78}N_6O_{11}Na$ 1021.5626; found, 1021.5616.



15

Methyl (5*R*,8*S*,11*S*,14*S*,17*S*,20*S*)-17-(3-amino-3-oxopropyl)-11-((*R*)-1-(*tert*-butoxy)ethyl)-14-((*S*)-*sec*-butyl)-1-(9H-fluoren-9-yl)-5,8-diisobutyl-20-(4-methoxybenzyl)-7,13,16,19-tetramethyl-3,6,9,12,15,18-hexaoxo-2-oxa-4,7,10,13,16,19-hexaazahenicosan-21-oate (15): A solution of pentapeptide **14** (1.97 g, 1.97 mmol, a 5:1 mixture of the diastereomers) in MeCN (4 mL) and Et_2NH (2 mL) was stirred at room temperature for 1 h and concentrated, giving the corresponding crude amine. To a solution of the above amine in dry DMF (1 mL) were added *N*-Fmoc-D-Leu-OH (1.4 g, 3.9 mmol), HATU (1.8 g, 4.8 mmol), and DIPEA (2.0 mL, 11.6 mmol) at 0 ˚C. The reaction mixture was then stirred at room temperature overnight, and diluted with EtOAc (50 mL). The whole mixture was washed successively with 10% aq. citric acid (30 mL), water (30mL), saturated aqueous $NaHCO_3$ (30mL) and brine (30 mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH, 30:1 v/v) to give hexapeptide **15** (1.5 g, 1.3 mmol, 68% yield, a 5:1 mixture of the diastereomers) as a white solid. A part of the obtained hexapeptide **12** (20 mg) was purified by HPLC [Cosmosil 5C_{18}-MS-II, (ϕ20 × 250 mm); flow rate, 5.0 mL/min; detector, UV 215 nm; solvent 90% aqueous MeOH] to afford an analytical sample of diastereomerically pure hexapeptide **12** (7.7 mg, T_R= 41.74 min) and its diastereomer (1.2 mg, T_R= 40.75 min). **12**: $[\alpha]_D^{26}$ −78.7 (*c* 0.39, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, a mixture of rotamers, signals due to minor rotamers in brackets) δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.59 (d, *J* = 6.8 Hz, 2H), 7.40 (t, *J* = 8.0 Hz, 2H), 7.30 (t, *J* = 8.0 Hz, 2H), [7.12, 7.07] (d, *J* = 8.8 Hz, 2H), [6.88, 6.80] (d, *J* = 8.4 Hz, 2H), 6.34 (brs, 1H), 5.79 (brs, 1H), 5.76 (brs, 1H), 5.56 (m, 1H), 5.33 (dd, *J* = 5.6, 8.4 Hz, 1H), 5.23 (d, *J* = 10.8 Hz, 1H), 5.10-5.02 (m, 2H), 4.85-4.73 (m, 2H), 4.42 (m, 1H), 4.28-4.19 (m, 2H), 3.79 (s, 1.5H), 3.78 (s, 1.5H), 3.77 (s, 1.5H), 3.74 (s, 1.5H), 3.31 (m, 1H), 3.14 (s, 1.5H), 3.09 (s, 1.5H), 3.03 (s, 1.5H), 3.02 (s, 1.5H), 2.93 (s, 1.5H), 2.93 (m, 1H), 2.86 (s, 1.5H), 2.75 (s, 3H), 2.13-1.97 (m, 3H), 1.86-1.61 (m, 6H), 1.53 (m, 1H), 1.42-1.36 (m, 2H), 1.25 (m, 1H), 1.12 (s, 9H), 1.01 (s, 1.5H), 1.00 (s, 1.5H), 0.95 (s, 3H), 0.93 (s, 3H), 0.91-0.70 (m, 9H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz, a mixture of rotamers) δ 174.3, 174.1, 174.0, 173.9, 170.8, 170.6, 170.3, 170.2, 170.1, 169.8, 169.7, 169.5, 158.7, 158.2, 156.1, 143.9, 143.7, 141.2, 130.5, 129.9, 129.6, 127.7, 127.0, 125.1, 120.0, 114.4, 114.0, 75.0, 74.9, 74.6, 68.2, 67.0, 61.1, 58.7, 57.9,

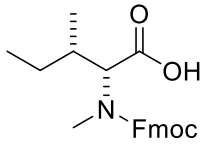
57.3, 56.4, 55.3, 54.5, 54.0, 53.6, 52.7, 52.4, 49.8, 47.2, 42.8, 42.6, 36.5, 36.4, 33.6, 33.4, 32.8, 32.5, 31.8, 31.5, 31.0, 30.8, 30.5, 30.3, 29.7, 28.1, 28.0, 27.9, 24.9, 24.6, 24.0, 23.7, 23.3, 23.1, 21.8, 18.7, 18.0, 15.7, 15.5, 11.1, 10.9, 10.7; IR (neat) 3308, 2960, 1721, 1631, 1514, 1451, 1408 cm^{-1}; HRMS (ESI-TOF) *m*/*z*: $[M + Na]^+$ Calcd for $C_{61}H_{89}N_7O_{12}Na$ 1134.6467; found, 1134.6420.



16

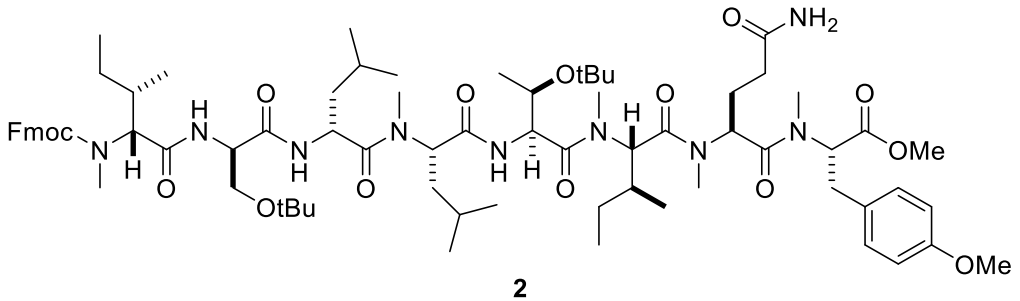
Methyl (5*R*,8*R*,11*S*,14*S*,17*S*,20*S*,23*S*)-20-(3-amino-3-oxopropyl)-14-((*R*)-1-(*tert*-butoxy)ethyl)-5-(*tert*-butoxymethyl)-17-((*S*)-*sec*-butyl)-1-(9H-fluoren-9-yl)-8,11-diisobutyl-23-(4-methoxybenzyl)-10,16,19,22-tetramethyl-3,6,9,12,15,18,21-heptaoxo-2-oxa-4,7,10,13,16,19,22-heptaazatetracosan-24-oate (16): A solution of hexapeptide **15** (1.5 g, 1.3 mmol, a 5:1 mixture of the diastereomers) in MeCN (4 mL) and Et_2NH (2 mL) was stirred at room temperature for 1 h and concentrated, giving the corresponding crude amine. To a solution of the above amine in dry DMF (1 mL) were added *N*-Fmoc-*D*-Ser(tBu)-OH (1.0 g, 2.6 mmol), HATU (1.2 g, 3.3 mmol), and DIPEA (1.4 mL, 7.9 mmol) cooled at 0 ˚C. The reaction mixture was then stirred at room temperature overnight, and diluted with EtOAc (40 mL). The whole mixture was washed successively with 10% aq. citric acid (20 mL), water (20 mL), saturated aqueous $NaHCO_3$ (20mL) and brine (20 mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH, 30:1 v/v) to give heptapeptide **16** (1.2 g, 0.95 mmol, 73% yield, a 5:1 mixture of the diastereomers) as a white solid. A part of the afford heptapeptide **16** (15 mg) was purified by HPLC [Cosmosil $5C_{18}$-MS-II, (ϕ20 × 250 mm); flow rate, 5.0 mL/min; detector, UV 215 nm; solvent 95% aqueous MeOH] to obtain an analytical sample of diastereomerically pure heptapeptide **13** (5.4 mg, T_R= 21.97 min) and its diastereomer (1.4 mg, T_R= 20.86 min). **13**: $[\alpha]_D^{26}$ −59.7 (*c* 0.27, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz, a mixture of rotamers, signals due to minor rotamers in brackets) δ 7.76 (d, *J* = 7.2 Hz, 2H), 7.58 (d, *J* = 7.6 Hz, 2H), 7.35 (m, 2H), 7.30 (m, 2H), [7.13, 7.04] (d, *J* = 8.8 Hz, 2H), [6.89, 6.80] (d, *J* = 8.8 Hz, 2H), 6.35 (brs, 1H), 6.13 (d, *J* = 7.2 Hz, 1H), 5.92 (d, *J* = 6.0 Hz, 1H), 5.80 (d, *J* = 6.4 Hz, 1H), 5.76 (brs, 1H), 5.34 (dd, *J* = 5.2, 8.4 Hz, 1H), 5.11 (dd, *J* = 5.6 Hz, 10.0 Hz, 1H), 5.05 (d, *J* = 10.8 Hz, 1H), 4.96-4.84 (m, 2H), 4.65-4.63 (m, 1H), 4.44 (dd, *J* = 7.6, 11.2 Hz, 1H), 4.40 (m, 2H), 4.31 (m ,1H), 4.24 (m, 1H),

3.77 (s, 3H), 3.70 (s, 3H), 3.34-3.25 (m, 2H), 3.12 (s, 3H), 3.09 (s, 3H), 2.99-2.80 (m, 2H), 2.75 (s, 3H), 2.50 (s, 3H), 2.05 (m, 1H), 1.75-1.37 (m, 12H), 1.25 (s, 9H), 1.21-1.20 (m, 3H), 1.17 (s, 9H), 1.00-0.93 (m, 6H), 0.88-0.81 (m, 6H), 0.75-0.71 (m, 3H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz, a mixture of rotamers) δ 174.3, 174.0, 173.6, 170.8, 170.6, 170.4, 170.2, 170.0, 169.8, 158.7, 158.6, 156.2, 143.9, 143.7, 141.3, 130.4, 129.7, 127.7, 127.1, 125.1, 120.0, 114.4, 114.0, 75.0, 74.8, 74.4, 74.3, 68.4, 68.2, 67.1, 61.6, 61.1, 57.9, 57.1, 55.4, 55.2, 54.8, 54.4, 54.1, 53.9, 53.4, 53.2, 52.6, 52.5, 52.4, 48.3, 47.1, 41.9, 36.7, 34.2, 33.5, 32.9, 32.7, 31.9, 31.6, 31.2, 31.1, 30.9, 30.2, 29.8, 29.7, 28.1, 28.0, 27.4, 27.3, 24.9, 24.7, 24.1, 23.7, 23.3, 23.2, 22.0, 21.9, 21.8, 21.7, 18.4, 18.1, 15.9, 15.7, 11.1, 10.8; IR (neat) 3316, 2968, 2872, 1741, 1635, 1514, 1409 cm^{-1}; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{68}H_{102}N_8O_{14}Na$ 1277.7413; found, 1277.7431.



17

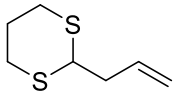
***N*-(((9H-fluoren-9-yl)methoxy)carbonyl)-*N*-methyl-D-*allo*-isoleucine (17):** A solution of *N*-Fmoc-D-*allo*-Leu-OH (1.06 g, 3.0 mmol) in toluene (50 mL) were added paraformaldehyde (5.0 g) and TsOH (0.12 g). The reaction mixture was stirred at 160 °C for 2 h under reflux, cooled to room temperature, and diluted with EtOAc (50 mL). The mixture was successively washed with saturated aqueous $NaHCO_3$ (30 mL×2), water (30 mL), and brine (30 mL), dried over Na_2SO_4, and concentrated. To a solution of the above crude compound in CH_2Cl_2 (5 mL) were added triethylsilane (1.9 mL, 12 mmol) and trifluoroacetic acid (4.6 mL, 60 mmol). The reaction was stirred at room temperature overnight before being diluted with toluene and evaporated the solution under reduced pressure 3 times. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 30:1 v/v) to give **17** (0.96 g, 2.62 mmol, 87% yield) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) (major rotamers) δ 7.76 (d, *J* = 7.2 Hz, 2H), 7.62-7.58 (m, 2H), 7.42-7.30 (m, 4H), 4.59-4.43 (m, 3H), 4.26 (m, 1H), 2.91 (s, 3H), 1.98 (m, 1H), 1.51 (m, 1H), 1.16 (m, 1H), 0.96 (t, *J* = 7.2 Hz, 3H), 0.88 (d, *J* = 6.8 Hz, 3H); $^{13}C\{^{1}H\}$ NMR $CDCl_3$, 100 MHz) (major rotamer) δ 175.6, 157.3, 143.7, 141.3, 127.7, 127.0, 124.9, 120.0, 67.9, 63.5, 62.5, 47.2, 33.8, 31.5, 30.5, 26.1, 15.1, 11.1. Spectroscopic data are in agreement with those reported in the literature.



2

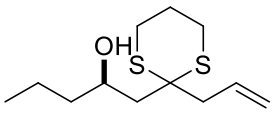
Methyl (5*R*,8*R*,11*R*,14*S*,17*S*,20*S*,23*S*,26*S*)-23-(3-amino-3-oxopropyl)-17-((*R*)-1-(*tert*-butoxy)ethyl)-8-(*tert*-butoxymethyl)-5,20-di((*S*)-*sec*-butyl)-1-(9H-fluoren-9-yl)-11,14-diisobutyl-26-(4-methoxybenzyl)-4,13,19,22,25-pentamethyl-3,6,9,12,15,18,21,24-octaoxo-2-oxa-4,7,10,13,16,19,22,25-octaazaheptacosan-27-oate (2): A solution of heptapeptide **16** (1.2 g, 1.0 mmol, a 5:1 mixture of the diastereomers) in MeCN (3 mL) and Et_2NH (1.5 mL) was stirred at room temperature for 1 h and concentrated, giving the corresponding crude amine. To a solution of the above amine in dry DMF (0.7 mL) were added **17** (0.7 g, 1.9 mmol), HATU (0.9 g, 2.4 mmol), and DIPEA (1.0 mL, 5.7 mmol) cooled at 0 ˚C. The reaction mixture was then stirred at room temperature overnight, and diluted with EtOAc (40 mL). The whole mixture was washed successively with 10% aq. citric acid (20 mL), water (20 mL), saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH, 30:1 v/v) to give octapeptide **2** (1.0 g, 0.74 mmol, 78% yield, a 5:1 mixture of the diastereomers) as a white solid. A part of the obtained octapeptide **2** (22 mg) was purified by HPLC [Cosmosil 5C_{18}-MS-II, (ϕ20 × 250 mm); flow rate, 5.0 mL/min; detector, UV 215 nm; solvent 95% aqueous MeOH] to afford an analytical sample of diastereomerically pure octapeptide **2** (8.7 mg, T_R= 32.91 min) and its diastereomer (1.5 mg, T_R= 31.31 min). **2**: $[\alpha]_D^{26}$ −44.9 (*c* 0.44, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, a mixture of rotamers, signals due to minor rotamers in brackets) δ 7.77 (d, *J* = 7.2 Hz, 2H), [7.58, 7.57] (d, *J* = 8.0 Hz, 2H), 7.40 (t, *J* = 7.2 Hz, 2H), 7.31 (t, *J* = 8.0 Hz, 2H), [7.13, 7.04] (d, *J* = 8.8 Hz, 2H), [6.87, 6.81] (d, *J* = 8.8 Hz, 2H), 6.37 (brs, 2H), 5.93 (brs, 1H), 5.79 (brs, 2H), 5.33 (dd, *J* = 5.2, 8.4 Hz, 1H), 5.11 (d, *J* = 11.2 Hz, 1H), 5.04-4.96 (m, 2H), 4.88 (dd, *J* = 5.2, 8.4 Hz, 1H), 4.70 (m, 1H), 4.53-4.44 (m, 2H), 4.33 (m, 1H), 4.27-4.24 (m, 2H), 3.79 (s, 1.5H), 3.78 (s, 1.5H), 3.72 (s, 1.5H), 3.68 (s, 1.5H), 3.33-3.27 (m, 2H), 3.11 (s, 3H), 3.05 (s, 1.5H), 3.04 (s, 1.5H), 2.94 (s, 1.5H), 2.90 (s, 1.5H), 2.85 (s, 1.5H), 2.84 (s, 1.5H), 2.81-2.76 (m, 2H), 2.70 (s, 1.5H), 2.52 (s, 1.5H), 2.11-2.00 (m, 3H), 1.83 (m, 1H), 1.70-1.60 (m, 6H), 1.53-1.48 (m, 3H), 1.43-1.38 (m, 2H), 1.25 (m, 1H), 1.17 (s, 9H), 1.15 (s, 9H), 1.13 (s, 3H), 0.98-0.90 (m, 12H), 0.86-0.81 (m, 9H), 0.76-0.72 (m, 3H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz, a mixture of rotamers) δ 174.3, 173.3, 170.7, 170.5, 170.3, 169.8, 169.7, 169.4, 158.7,

158.6, 157.2, 143.9, 141.3, 130.5, 129.7, 127.7, 127.0, 125.0, 120.0, 114.4, 114.0, 75.0, 73.9, 68.4, 67.8, 64.0, 61.1, 60.7, 59.2, 55.3, 54.6, 53.5, 52.6, 52.3, 48.1, 47.2, 42.6, 42.4, 36.6, 34.2, 33.5, 32.9, 32.7, 31.8, 31.6, 31.0, 30.8, 30.2, 29.9, 28.0, 27.3, 26.1, 24.9, 24.5, 24.1, 23.4, 23.1, 21.8, 15.7, 14.7, 14.2, 11.5, 10.8; IR (neat) 3316, 2965, 2874, 1743, 1635, 1514, 1452, 1407 cm^{-1}; HRMS (ESI-TOF) *m*/*z*: $[M + Na]^+$ Calcd for $C_{75}H_{115}N_9O_{15}Na$ 1404.8410; found, 1404.8409.



18

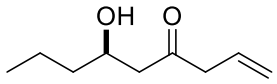
2-allyl-1,3-dithiane (18): A solution of 1,3-dithiane (6.02 g, 50 mmol) in THF (40 mL) was cooled to -40 ˚C and kept under a N_2 atmosphere. To the mixture was dropwise added n-BuLi (62.5 mL,100 mmol). The resulting solution was stirred at -15 ˚C for 4 h and the cooled to -75 ˚C followed by addition of allyl bromide (13 mL, 150 mmol). The mixture was stirred overnight when the temperature gradually rose from -75 ˚C to 0 ˚C. The reaction was quenched by addition of water (50 mL). The aqueous phase was extracted with EtOAc (50 mL×2). The combined organic layers were washed with brine (70 mL), dried over Na_2SO_4, and concentrated. The crude oil was distilled under reduced pressure to give **18** (6.85 g, 42.8 mmol, 86% yield) as a colorless oil: bp 235.9 ˚C/101 kPa; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.83 (m, 1H), 5.13 (d, *J* = 15.2 Hz, 1H), 5.09 (d, *J* = 10.4 Hz, 1H), 4.07 (t, *J* = 7.2 Hz, 1H), 2.89-2.77 (m, 4H), 2.50-2.46 (m, 2H), 2.09 (m, 1H), 1.83 (m, 1H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 133.6, 117.8, 46.9, 39.6, 30.3, 25.6. Spectroscopic data are in agreement with those reported in the literature.



19

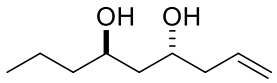
(*R*)-1-(2-allyl-1,3-dithian-2-yl)pentan-2-ol (19): A solution of **18** (4.8 g, 30 mmol) in THF (30 mL) was added dropwise a 1.6 M solution of n-BuLi in hexane (37.5 mL, 60 mmol) cooled at 0 ºC under an argon atmosphere, and the resulting mixture was stirred for 30 min before (*R*)-1.2-epoxypentane **4** (2.8 g, 33 mmol) was added. After stirring at 0 ºC for 1 h, the reaction mixture was diluted with saturated aqueous NH_4Cl and EtOAc. The layers were separated, and the aqueous layer was extracted with EtOAc (50 mL×3). The combined organic layers were washed with brine (80 mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography

on silica gel (hexanes/EtOAc, 8:1 v/v) to give **19** (4.6 g, 18.4 mmol, 61% yield) as a colorless oil: $[\alpha]_D^{27}$ −12.9 (*c* 6.39, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 5.82 (m, 1H), 5.10-5.05 (m, 2H), 3.90 (m, 1H), 2.95-2.59 (m, 6H), 2.15 (dd, *J* = 8.8, 15.6 Hz, 1H), 1.99-1.79 (m, 3H), 1.47-1.23 (m, 4H), 0.85 (t, *J* = 7.0 Hz, 3H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz) δ 132.2, 118.7, 67.9, 51.1, 44.6, 43.9, 39.8, 26.1, 25.8, 24.6, 18.5, 13.9; IR (neat) 3447, 3075, 2956, 2931, 2871, 1638, 1439, 1422 cm^{-1}; HRMS (ESI-TOF) *m*/*z*: $[M + Na]^+$ Calcd for $C_{12}H_{22}OS_2Na$ 269.1010; found, 269.0998.



20

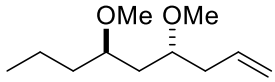
(*R*)-6-hydroxynon-1-en-4-one (20): A stirred mixture of alcohol **19** (4.6 g, 18.5 mmol) and $CaCO_3$ (18.5 g, 185 mmol) in THF/H_2O (4/1, 100 mL) cooled at 0 ºC was added I_2 (18.8 g, 74 mmol). The reaction mixture was stirred for 1 h, quenched with saturated aqueous NaS_2O_3 (100 mL), and diluted with EtOAc. The layers were separated, and the aqueous layer was extracted with EtOAc (80 mL×3). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel (hexanes/EtOAc, 5:1 v/v) to give **20** (2.4 g, 15.2 mmol, 82% yield) as a colorless oil: $[\alpha]_D^{27}$ −42.4 (*c* 1.81, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 5.89 (m, 1H), 5.20 (d, *J* = 10.0 Hz, 1H), 5.15 (d, *J* = 17.6 Hz, 1H), 4.04 (m, 1H), 3.19 (d, *J* = 7.2 Hz, 2H), 2.65-2.50 (m, 2H), 1.52-1.30 (m, 4H), 0.91 (t, *J* = 7.2 Hz, 3H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz) δ 209.9, 129.9, 119.3, 67.2, 48.6, 48.4, 38.5, 18.6, 13.9; IR (neat) 3436, 3081, 2960, 2933, 2874, 1710, 1639, 1466 cm^{-1}; HRMS (ESI-TOF) *m*/*z*: $[M + Na]^+$ Calcd for $C_9H_{16}O_2Na$ 179.1048; found, 179.1038.



21

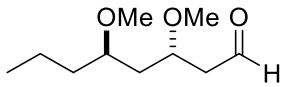
(4*R*,6*R*)-non-1-ene-4,6-diol (21): A solution of tetramethylammonium triacetoxyborohydride (10.4 g, 39.6 mmol) in anhydrous MeCN (25 mL) and acetic acid (25 mL) was stirred at room temperature for 30 min and then cooled to -30 °C. To the mixture a solution of ketone **20** (0.77 g, 4.9 mmol) in anhydrous MeCN (25 mL) was added. The reaction mixture was stirred at -30 ˚C for 14 h and quenched with saturated aqueous Na/K tartrate (70 mL). The layers were separated, and the aqueous layer was extracted with dichloromethane (50 mL×3), and the combined organic

extracts were washed with saturated aqueous $NaHCO_3$ (80 mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel (hexanes/EtOAc, 1:1 v/v) to give **21** (0.62 g, 3.95 mmol, 80% yield) as a colorless oil: $[\alpha]_D^{24}$ −10.9 (*c* 0.13, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 5.82 (m, 1H), 5.15 (d, *J* = 15.6 Hz, 1H), 5.14 (d, *J* = 12.4 Hz, 1H), 4.04-3.93 (m, 2H), 2.35-2.22 (m, 2H), 1.52 (t, *J* = 9.8 Hz, 2H), 1.48-1.24 (m, 4H), 0.94 (t, *J* = 7.2 Hz, 3H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz) δ 134.7, 117.5, 68.5, 68.0, 41.84, 39.4, 18.8, 13.9; IR (neat) 3355, 3077, 2958, 2934, 2873, 1830, 1642, 1434 cm^{-1}; HRMS (ESI-TOF) *m*/*z*: $[M + Na]^+$ Calcd for $C_9H_{18}O_2Na$ 181.1205; found, 181.1199.



22

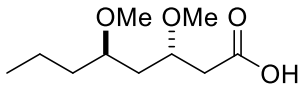
(4*R*,6*R*)-4,6-dimethoxynon-1-ene (22): A solution of diol **21** (0.62 g, 3.9 mmol) in THF (10 mL) was added NaH (60% in oil, 0.6 g, 16 mmol), and the mixture was stirred at room temperature for 30 minutes before being treated with iodomethane (2.5 mL, 40 mmol). The mixture was stirred at room temperature overnight before being quenched with the addition of saturated aqueous NH_4Cl (50 mL), and extracted with EtOAc (50 mL×3). The organic phase was washed with saturated aqueous NH_4Cl (80 mL) and brine (80 mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel (hexanes/EtOAc, 10:1 v/v) to give **22** (0.71 g, 3.81 mmol, 98% yield) as a colorless oil: $[\alpha]_D^{25}$ −46.8 (*c* 1.18, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 5.80 (m, 1H), 5.06 (d, 1H, *J* = 16.8 Hz), 5.05 (d, 1H, *J* = 10.4 Hz), 3.43 (m, 1H), 3.36 (m, 1H), 3.35 (s, 3H), 3.32 (s, 3H), 2.28-2.21 (m, 2H), 1.50 (t, *J* = 6.8 Hz, 2H), 1.43-1.24 (m, 4H), 0.90 (t, *J* = 7.4 Hz, 3H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz) δ 134.5, 117.1, 77.4, 77.0, 56.7, 56.6, 39.5, 38.0, 36.1, 18.2, 14.3; IR (neat) 3735, 2931, 1734, 1457 cm^{-1}; HRMS (ESI-TOF) *m*/*z*: $[M + Na]^+$ Calcd for $C_{11}H_{22}O_2Na$ 209.1518; found, 209.1513.



23

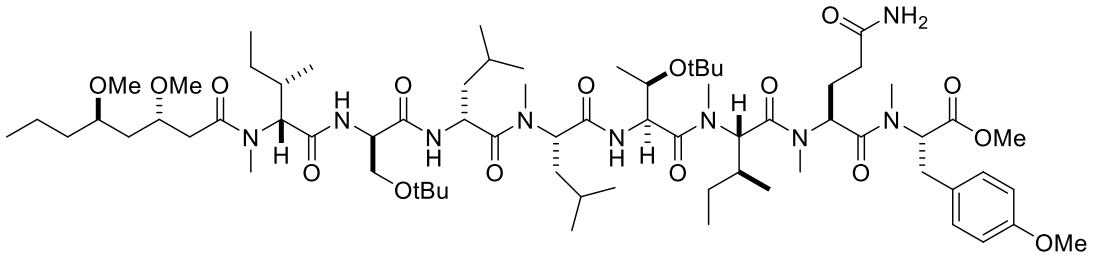
(3*S*,5*R*)-3,5-dimethoxyoctanal (23): A solution of alkene **22** (0.56 g, 3.0 mmol) in Et_2O/H_2O (1/1, 120 mL) was added of 2 wt % solution of OsO_4 in H_2O (0.6 mL, 5 mol %) and $NaIO_4$ (1.6 g, 7.5 mmol). The reaction mixture was stirred at room temperature for 18 h and diluted with EtOAc. The layers were separated, and the aqueous layer was

extracted with EtOAc (50 mL×3). The combined organic layers dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel (hexanes/EtOAc, 3:1 v/v) to give **23** (0.41 g, 2.18 mmol, 73% yield) as a colorless oil: $[\alpha]_D^{25}$ −50.6 (*c* 0.05, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.76 (t, *J* = 2.4 Hz, 1H), 3.85 (m, 1H), 3.32 (s, 3H), 3.30 (m, 1H), 3.28 (s, 3H), 2.56 (dd, *J* = 2.4, 6.0 Hz, 2H), 1.64 (m, 1H), 1.53-1.44 (m, 2H), 1.41-1.26 (m, 3H), 0.87 (t, *J* = 7.4 Hz, 3H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 201.3, 77.0, 73.5, 57.0, 56.3, 48.4, 39.9, 35.6, 18.0, 14.2; IR (neat) 2958, 2934,m 2874, 2826, 2727, 1726, 1459 cm^{-1}; HRMS (ESI-TOF) *m*/*z*: [M + Na]$^+$ Calcd for $C_{10}H_{20}O_3Na$ 211.1310; found, 211.1314.



3

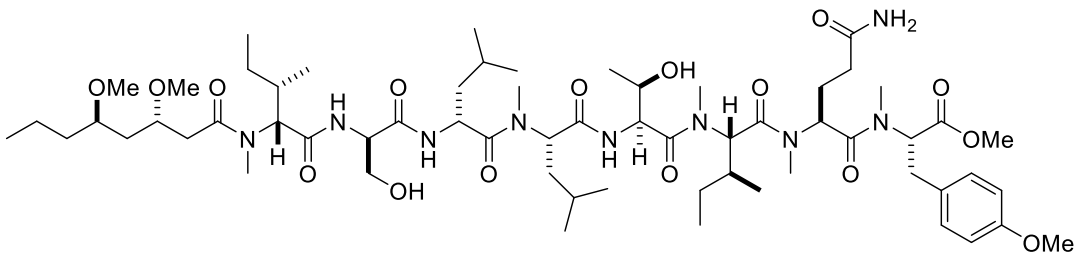
(3*S*,5*R*)-3,5-dimethoxyoctanoic acid (**3**). A solution of aldehyde **23** (0.45 g, 2.2 mmol) in *tert*-butyl alcohol (11 mL) and 2-methyl-2-butene (2.3 mL, 22 mmol) at room temperature was added a solution of sodium chlorite (1.0 g, 11 mmol) and sodium dihydrogen phosphate (0.5 g, 4.4 mmol) in water (11 mL). The reaction mixture was stirred vigorously for 2 h, diluted with brine (10 mL), and acidified to pH 3 with 1 M HCl. The aqueous was extracted with CH_2Cl_2 (30 mL×3). The combined organic layers dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH, 30:1 v/v) to give **3** (0.40 g, 1.96 mmol, 90% yield) as a colorless oil: $[\alpha]_D^{27}$ −183.7 (*c* 0.29, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.80 (m, 1H), 3.38 (s, 3H), 3.36 (m, 1H), 3.32 (s, 3H), 2.60-2.46 (m, 2H), 1.68-1.12 (m, 6H), 0.90 (t, *J* = 7.4 Hz, 3H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 176.0, 77.3, 74.8, 57.2, 56.3, 39.7, 39.4, 35.7, 18.1, 14.2; IR (neat) 3429, 2958, 2828, 1714, 1458 cm^{-1}; HRMS (ESI-TOF) *m*/*z*: [M + Na]$^+$ Calcd for $C_{10}H_{20}O_4Na$ 227.1259; found, 227.1265.



24

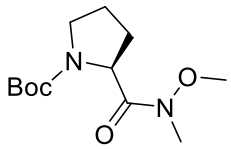
Methyl (3*R*,5*S*,9*R*,12*R*,15*R*,21*S*,24*S*,27*S*,30*S*)-27-(3-amino-3-oxopropyl)-21-((*R*)-1-(*tert*-butoxy)ethyl)-12-(*tert*-butoxymethyl)-9,24-di((*S*)-*sec*-butyl)-15,18-

diisobutyl-5-methoxy-30-(4-methoxybenzyl)-8,17,23,26,29-pentamethyl-7,10,13,16,19,22,25,28-octaoxo-3-propyl-2-oxa-8,11,14,17,20,23,26,29-octaazahentriacontan-31-oate (24): A solution of octapeptide **2** (0.29 g, 0.25 mmol, a 5:1 mixture of the diastereomers) in MeCN (3 mL) and Et_2NH (1.5 mL) was stirred at room temperature for 1 h and concentrated, giving the corresponding crude amine. To a solution of the above amine in dry DMF (0.5 mL) was added fatty acid **3** (0.10 g, 0.5 mmol), HATU (0.24 g, 0.6 mmol), and DIPEA (0.26 mL, 1.5 mmol) cooled at 0 ˚C. The reaction mixture was then stirred at room temperature overnight, and diluted with EtOAc (20 mL). The whole mixture was washed successively with 10% aq. citric acid (10 mL), water (10 mL), saturated aqueous $NaHCO_3$ (10 mL) and brine (10 mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH, 30:1 v/v) to give compound **24** (0.21 g, 0.16 mmol, 63% yield, a 5:1 mixture of the diastereomers) as a white solid. A part of the obtained compound **24** (20 mg) was purified by HPLC analysis [Cosmosil 5C_{18}-MS-II, (ϕ20 × 250 mm); flow rate, 5.0 mL/min; detector, UV 215 nm; solvent 90% aqueous MeOH] to afford an analytical sample of diastereomerically pure compound **24** (7.0 mg, T_R= 42.33 min) and its diastereomer (1.3 mg, T_R= 38.64 min). **24**: $[\alpha]_D^{26}$ −42.8 (*c* 0.35, $CHCl_3$); ^{1}H NMR (d_6-Acetone, 400 MHz, a mixture of rotamers) δ 7.43 (d, *J* = 7.6 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.17 (d, *J* = 8.8 Hz, 2H), 7.05 (d, *J* = 8.0 Hz, 1H), 6.92 (brs, 1H, NH), 6.88 (d, *J* = 8.8 Hz, 2H), 6.68 (brs, 1H), 6.45 (brs, 1H), 6.13 (brs, 1H), 6.05 (brs, 1H), 5.39 (dd, *J* = 6.4, 8.0 Hz, 1H), 5.13 (dd, *J* = 6.4, 8.8 Hz, 1H), 5.11 (d, *J* = 10.4 Hz, 1H), 5.07 (dd, *J* = 4.8, 11.6 Hz, 1H), 4.97 (m, 1H), 4.89 (m, 1H), 4.71 (m, 1H), 4.61 (m, 1H), 4.47 (m, 1H), 3.88 (m, 1H), 3.82 (s, 3H), 3.75 (m, 1H), 3.71 (m, 1H), 3.70 (s, 3H), 3.35 (m, 1H), 3.33 (s, 3H), 3.29 (s, 3H), 3.25 (m, 1H), 3.10 (s, 3H), 3.09 (s, 3H), 3.07 (m, 1H), 3.02 (s, 3H), 2.81 (s, 3H), 2.79 (m, 1H), 2.48 (s, 3H), 2.35 (m, 1H), 2.17 (m, 1H), 2.11 (m, 1H), 2.02 (m, 1H), 1.99 (m, 1H), 1.96 (m, 1H), 1.89 (m, 1H), 1.75 (m, 1H), 1.71-1.67 (m, 2H), 1.66 (m, 1H), 1.63-1.60 (m, 2H), 1.59 (m, 1H), 1.56-1.53 (m, 2H), 1.51 (m, 1H), 1.46 (m, 1H), 1.38-1.33 (m, 2H), 1.31 (m, 1H), 1.18 (m, 18H), 1.16 (d, *J* = 6.4 Hz, 3H), 1.02 (m, 1H), 0.99 (m, 3H), 0.97 (m, 3H), 0.95 (m, 1H), 0.94 (m, 3H), 0.92 (m, 3H), 0.91 (m, 3H), 0.88 (m, 3H), 0.85 (m, 3H), 0.81 (m, 3H), 0.79 (m, 3H). $^{13}C\{^1H\}$ NMR (d_6-Acetone, 100 MHz, a mixture of rotamers) δ 174.13, 174.07, 172.8, 171.6, 171.2, 170.84, 170.77, 170.5, 170.3, 170.2, 159.6, 131.3, 130.8, 114.7, 77.9, 76.3, 75.0, 74.0, 69.1, 62.4, 61.6, 60.3, 57.9, 57.5, 56.3, 55.6, 55.3, 54.4, 54.3, 53.8, 53.7, 52.3, 48.7, 42.8, 40.9, 39.7, 37.6, 36.7, 34.1, 33.7, 33.6, 33.4, 32.3, 31.9, 31.3, 30.9, 30.6, 28.6, 27.7, 27.0, 25.7, 25.6, 25.2, 23.7, 23.6, 22.0, 21.9, 19.3, 18.9, 16.3, 15.1, 14.6, 11.9, 11.3; IR (neat) 3317, 2963, 2934, 2874, 1744, 1633, 1515, 1469, 1408 cm^{-1}; HRMS (ESI-TOF) *m*/*z*: $[M + Na]^+$ Calcd for $C_{70}H_{123}N_9O_{16}Na$ 1368.8986; found, 1368.9017.



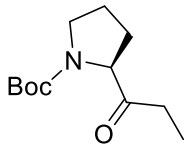
Ikoamide (**1**)

Ikoamide (**1**). A solution of compound **24** (0.10 g, 74.3 μmol, a 5:1 mixture of the diastereomers) in dichloromethane (2 mL) and trifluoroacetic acid (1 mL) was stirred at room temperature for 2 hours and concentrated to give the final product **ikoamide** (**1**) (0.1 g, a 5:1 mixture of the diastereomers). A part of the obtained **ikoamide** (**1**) (25 mg) was purified by HPLC [Cosmosil 5C_{18}-MS-II, (ϕ20 × 250 mm); flow rate, 5.0 mL/min; detector, UV 215 nm; solvent 85% aqueous MeOH] to give diastereomerically pure **ikoamide** (**1**) (11.4 mg, T_R= 47.30 min, 46%, conversion yield) as a white solid and its diastereomer (2.1 mg, T_R= 37.68 min). **1**: $[\alpha]_D^{27}$ −61.4 (*c* 0.57, $CHCl_3$); ^{1}H NMR (d_6-Acetone, 400 MHz, a mixture of rotamers) δ 8.02 (d, *J* = 5.2 Hz, 1H), [7.49, 7.39] (d, *J* = 6.4 Hz, 1H), [7.23, 7.15] (d, *J* = 8.6 Hz, 2H), 7.05 (d, *J* = 9.2 Hz, 1H), 6.98 (brs, 1H), [6.92, 6.87] (d, *J* = 8.4 Hz, 2H), 6.68 (brs, 1H), 5.39 (dd, *J* = 5.2, 9.2 Hz, 1H), 5.23 (dd, *J* = 6.0, 9.6 Hz, 1H), 5.13 (d, *J* = 11.2 Hz, 1H), 5.10 (dd, *J* = 5.2, 12.0 Hz, 1H), 4.82 (m, 1H), 4.79 (m, 1H), 4.76 (m, 1H), 4.36 (m, 1H), 4.08 (m, 1H), 3.87 (m, 1H), 3.79 (s, 3H), 3.75 (m, 1H), 3.69 (s, 3H), 3.63 (m, 1H), 3.36 (m, 1H), 3.32 (s, 3H), 3.28 (s, 3H), 3.24 (m, 1H), 3.11 (s, 3H), 3.09 (m, 1H), 3.09 (s, 3H), 3.06 (m, 1H), 3.02 (s, 3H), 2.81 (s, 3H), 2.76 (dd, *J* = 6.8, 15.6 Hz, 1H), 2.62 (s, 3H), 2.40 (dd, *J* = 6.0, 15.2 Hz, 1H), 2.11 (m, 1H), 2.05 (m, 1H), 2.02 (m, 1H), 1.98 (m, 1H), 1.93 (m, 1H), 1.89 (m, 1H), 1.79 (m, 1H), 1.74-1.70 (m, 2H), 1.69 (m, 1H), 1.66-1.61 (m, 2H), 1.58 (m, 1H), 1.57-1.54 (m, 2H), 1.52 (m, 1H), 1.47 (m ,1H), 1.38-1.30 (m, 2H), 1.28 (m, 1H), 1.14 (d, *J* = 6.4 Hz, 3H), 1.10 (m, 1H), 0.98 (m, 3H), 0.96 (m, 3H), 0.95 (m, 1H), 0.93 (m, 3H), 0.91 (m, 3H), 0.88 (m, 3H), 0.86 (m, 3H), 0.84 (m, 3H), 0.79 (m, 3H), 0.77 (m, 3H); ^{13}C{^{1}H} NMR (d_6-Acetone, 100 MHz, a mixture of rotamers) δ 175.4, 173.3, 173.0, 171.9, 171.6, 171.0, 170.9, 170.8, 170.5, 159.7, 159.5, 131.4, 130.7, 130.1, 114.9, 114.7, 114.6, 77.9, 76.5, 67.6, 62.4, 61.5, 61.2, 60.0, 57.5, 57.2, 56.3, 55.9, 55.6, 55.4, 53.3, 52.8, 52.5, 52.3, 49.9, 41.0, 40.7, 39.6, 36.7, 34.0, 33.7, 33.5, 32.2, 31.6, 31.2, 30.8, 26.8, 25.8, 25.5, 25.0, 23.7, 23.4, 22.3, 21.6, 20.6, 18.9, 15.8, 14.9, 14.6, 11.7, 11.1; IR (neat) 3308, 2960, 2934, 2874, 1743, 1634, 1515, 1465, 1408 cm^{-1}; HRMS (ESI-TOF) *m*/*z*: $[M + Na]^+$ Calcd for $C_{62}H_{107}N_9O_{16}Na$ 1256.7734; found, 1256.7729.



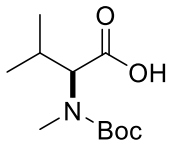
30

***tert*-butyl (*S*)-2-(methoxy(methyl)carbamoyl)pyrrolidine-1-carboxylate (30):** A solution of *N*-Boc-L-Pro-OH (4.31 g, 20.0 mmol) and *N*,*O*-dimethylhydroxylamine hydrochloride (2.93 g, 30 mmol) in DMF (70 mL) was added PyBOP (15.6 g, 30 mmol) and DIPEA (10.5 mL, 60 mmol) under a N_2 atmosphere. The reaction mixture was stirred at room temperature for 3 h, and diluted with EtOAc (100 mL). The whole mixture was washed successively with saturated aqueous $NaHCO_3$ (70 mL×2), water (70 mL×2) and 1 M $KHSO_4$ (70 mL×2), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel (MeOH/$CHCl_3$, 1:30 v/v) to give **30** (4.86 g, 18.8 mmol, 94% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.64 (m, 0.5H), 4.59 (m, 0.5H), 3.77 (s, 1.5H), 3.71 (s, 1.5H), 3.62-3.34 (m, 2H), 3.19 (s, 3H), 2.25-1.80 (m, 4H), 1.45 (s, 4.5 H), 1.41 (s, 4.5H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 154.5, 153.9, 79.5, 79.4, 61.3, 61.2, 56.8, 56.5, 46.9, 46.6, 30.5, 29.6, 28.5, 28.4, 24.0, 23.4. Spectroscopic data are in agreement with those reported in the literature.



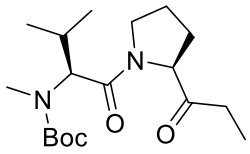
31

***tert*-butyl (*S*)-2-propionylpyrrolidine-1-carboxylate (31):** To a solution of **30** (4.86 g, 18.8 mmol) in THF (70 mL) cooled at 0 ˚C under N_2 was added a 1 M solution of EtMgBr in THF (56.5 mL 56.5 mmol) dropwise. The reaction was stirred at room temperature for 4 h before being quenched with the addition of saturated aqueous NH_4Cl (50 mL) at 0 ˚C and then extracted the EtOAc (70 mL×3). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 1:4 v/v) to give **31** (3.59 g, 15.8 mmol, 84% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.23 (dd, *J* = 4.4, 9.2 Hz, 0.5H), 4.15 (dd, *J* = 5.6, 8.8 Hz, 0.5H), 3.46-3.29 (m, 2H), 2.46-2.26 (m, 2H), 2.05 (m, 1H), 1.81-1.65 (m, 3H), 1.35 (s, 3H), 1.29 (s, 6H), 0.96 (t, *J* = 7.6 Hz, 3H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 210.5, 154.3, 153.6, 79.7, 79.4, 64.8, 64.3, 46.7, 46.5, 32.1, 31.4, 29.9, 28.8, 28.2, 28.0, 24.2, 23.5, 7.3, 7.2. Spectroscopic data are in agreement with those reported in the literature.



32

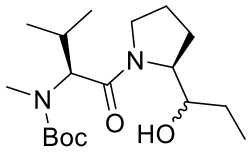
***N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-valine (32):** A solution of *N*-Boc-L-Val-OH (6.52 g, 30.0 mmol) in THF (100 mL) cooled at 0 ˚C. The mixture was treated with NaH (4.8 g of 60% in oil, 120 mmol) and stirred at 0 ˚C for 30 minutes before being treated with iodomethane (18.7 mL, 300 mmol). The reaction was stirred at room temperature overnight before being quenched with the addition of water (80 mL) and acidified by 10% aqueous citric acid to pH 3, then extracted with EtOAc (70 mL×3). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (MeOH/$CHCl_3$, 1:30 v/v) to give **32** (7.27 g, 29.9 mmol, 99% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.17 (d, *J* = 10.4 Hz, 0.5H), 4.09 (d, *J* = 10.8 Hz, 0.5H), 2.86 (s, 3H), 2.25 (m, 1H), 1.45 (s, 9H), 1.02 (d, *J* = 6.4 Hz, 3H), 0.91 (d, *J* = 7.2 Hz, 3H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 176.5, 175.2, 157.0, 155.6, 80.9, 80.6, 65.8, 65.0, 32.7, 31.1, 28.3, 27.8, 27.4, 20.1, 19.7, 19.1, 19.0. Spectroscopic data are in agreement with those reported in the literature.



33

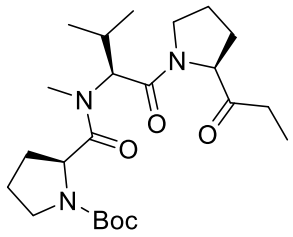
***tert*-butyl methyl((*S*)-3-methyl-1-oxo-1-((*S*)-2-propionylpyrrolidin-1-yl)butan-2-yl)carbamate (33):** A solution of ethyl ketone **31** (3.41 g, 15.0 mmol) in CH_2Cl_2 (15 mL) and trifluoroacetic acid (10 mL) was stirred at room temperature for 1 h and concentrated, giving the corresponding crude amine (TFA salt). To a solution of the above amine in dry DMF (6.5 mL) were added **32** (4.16 g, 18.0 mmol), HATU (7.41 g, 19.5 mmol), and DIPEA (10.5 mL, 60.0 mmol) cooled at 0 ˚C. The reaction mixture was then stirred at room temperature for 2 h, and diluted with EtOAc (80 mL). The whole mixture was washed successively with 10% aq. citric acid (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine (50 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/Hexane, 1:1 v/v) to give compound **33** (4.36 g, 12.8 mmol, 85% yield) as a colorless oil: $[\alpha]_D^{27}$ -117.3 (*c* 2.75, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.57 (d, *J* = 11.2 Hz, 0.7H), 4.53 (dd, *J* = 5.6, 8.8 Hz, 1H), 4.34 (d, *J* = 10.4 Hz, 0.3H), 3.86 (m, 0.7H), 3.66 (m, 1.3H),

2.80 (s, 2H), 2.77 (s, 1H), 2.66-2.45 (m, 2H), 2.28-2.08 (m, 2H), 2.03-1.86 (m, 2H), 1.78 (m, 1H), 1.46 (s, 3H), 1.43 (s, 6H), 1.06 (t, J = 7.2 Hz, 3H), 0.96 (d, J = 6.4 Hz, 2H), 0.92 (d, J = 7.2 Hz, 1H), 0.85 (d, J = 6.4 Hz, 3H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz) δ 209.0, 169.8, 168.7, 156.4, 155.4, 80.2, 79.2, 64.3, 64.2, 62.2, 60.6, 47.5, 46.9, 33.6,33.5, 29.5, 29.0, 28.4, 28.3, 28.1, 27.5, 27.4, 24.9, 24.8, 19.5, 19.1, 18.4, 7.4; IR (neat) 2972, 2875, 1726, 1688, 1643 cm^{-1}; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{18}H_{32}N_2O_4Na$ 363.2260; found, 363.2268.



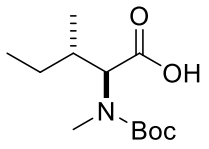
34

***tert*-butyl ((*S*)-1-((*S*)-2-(1-hydroxypropyl)pyrrolidin-1-yl)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (34):** A solution of compound **33** (4.33 g, 12.7 mmol) in dry MeOH (30 mL) was added $NaBH_4$ (1.45 g, 38.4 mmol) under a N_2 atmosphere, and the reaction solution was stirred at 0 ˚C for 1 h and at room temperature for 1 h. After the completion of the reaction, saturated aqueous NH_4Cl (50 mL) was added to the reaction mixture, and the mixture was extracted with EtOAc (50 mL×3). The combined organic layer was washed with brine (50 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (MeOH/$CHCl_3$, 1:30 v/v) to give alcohol **34** (3.98 g, 11.6 mmol, 91% yield) as a 2:1 mixture of diastereomers as a colorless oil: $[\alpha]_D^{28}$ -120.9 (*c* 2.67, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) (major rotamers) δ 4.57 (d, J = 11.2 Hz, 1H), 4.15 (m, 1H), 4.02 (m, 0.5H), 3.73 (m, 0.5H), 3.52-3.34 (m, 2H), 2.81 (s, 1.5H), 2.79 (s, 1.5H), 2.27 (m, 1H), 2.06-1.87 (m, 2H), 1.85-1.62 (m, 2H), 1.56 (m, 1H), 1.46 (s, 3H), 1.43 (s, 6H), 1.36 (m, 1H), 1.00 (t, J = 7.2 Hz, 3H), 0.95 (d, J = 6.4 Hz, 3H), 0.85 (d, J = 6.8 Hz, 3H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz) δ 173.1, 172.1, 171.1, 156.4, 155.3, 80.3, 80.0, 74.8, 74.6, 64.2, 63.9, 63.1, 62.9, 62.7, 62.6, 61.6, 61.3, 48.7, 48.0, 47.6, 47.0, 29.6, 29.0, 28.4, 28.3, 28.0, 27.9, 27.5, 27.4 ,27.3, 27.1, 25.3, 25.1, 24.6, 24.3, 24.2, 19.8, 19.6, 19.5, 19.2, 18.34, 18.26, 18.2; IR (neat) 3444, 2967, 2934, 2876, 1688, 1625 cm^{-1}; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{18}H_{34}N_2O_4Na$ 365.2416; found, 365.2408.



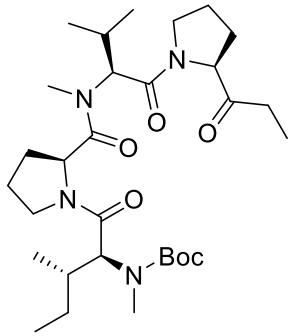
35

tert-butyl (*S*)-2-(methyl((*S*)-3-methyl-1-oxo-1-((*S*)-2-propionylpyrrolidin-1-yl)butan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (35): A solution of alcohol **34** (3.95 g, 11.6 mmol) in CH_2Cl_2 (15 mL) and trifluoroacetic acid (10 mL) was stirred at room temperature for 1 h and concentrated, giving the corresponding crude amine (TFA salt). To a solution of the above amine in dry DMF (6.5 mL) were added *N*-Boc-L-Pro (3.75 g, 17.4 mmol), HATU (7.07 g, 18.6 mmol), and DIPEA (12.1 mL, 69.6 mmol) cooled at 0 ˚C. The reaction mixture was then stirred at room temperature overnight, and diluted with EtOAc (80 mL). The whole mixture was washed successively with 10% aq. citric acid (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine (50 mL), dried over Na_2SO_4, and concentrated to afford crude modified dipeptide alcohol (5.22 g). To a solution of modified dipeptide alcohol (5.22 g) in CH_2Cl_2 (30 mL) was added DMP (9.84 g, 23.2 mmol) at 0 ˚C. The reaction mixture was then stirred at 0 ˚C for 2.5 h, diluted with saturated aqueous $Na_2S_2O_3$ (100 mL), and extracted with EtOAc (50 mL×3). The combined organic layer was washed with saturated aqueous $NaHCO_3$ (80 mL) and brine (80 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (MeOH/$CHCl_3$, 1:30 v/v) to give modified dipeptide **35** (4.25 g, 9.72 mmol, 84% yield in 3 steps) as a white solid: $[\alpha]_D^{28}$ -134.1 (*c* 3.37, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) (major rotamers) δ 5.01 (dd, *J* = 6.4, 11.2 Hz, 1H), 4.59 (m, 1H), 4.51 (t, *J* = 6.4 Hz, 1H), 3.89 (m, 1H), 3.68-3.37 (m, 3H), 3.10 (s, 1.5H), 3.04 (s, 1.5H), 2.63-2.46 (m, 2H), 2.29-2.18 (m, 2H), 2.14-2.07 (m, 2H), 2.04-1.81 (m, 3H), 1.78-1.70 (m. 2H), 1.41 (s, 4.5H), 1.38 (s, 4.5H), 1.05 (t, *J* = 6.8 Hz, 3H), 1.02 (d, *J* = 6.4 Hz, 3H), 0.90 (d, *J* = 6.4 Hz, 3H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 208.8, 208.7, 173.7, 173.2, 169.6, 169.5, 154.1, 153.8, 79.6, 79.3, 64.2, 59.3, 57.2, 56.4, 47.65, 47.57, 46.7, 46.6, 33.4, 30.5, 30.3, 30.1, 29.5, 28.4, 27.4, 27.3, 24.9, 24.3, 22.9, 19.3, 19.0, 18.8, 18.7, 7.3; IR (neat) 2974, 2877, 1724, 1696, 1639 cm^{-1}; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{23}H_{39}N_3O_5Na$ 460.2787; found, 460.2801.



36

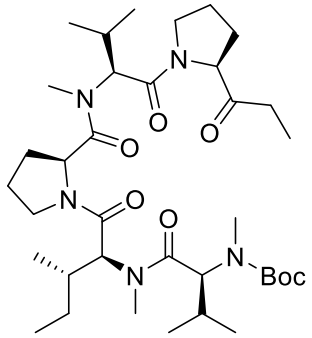
***N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-isoleucine (36):** A solution of *N*-Boc-L-Ile-OH (3.47 g, 15.0 mmol) in THF (100 mL) cooled at 0 ˚C. The mixture was treated with NaH (6.0 g of 60% in oil, 150 mmol) and stirred at 0 ˚C for 30 minutes before being treated with iodomethane (9.4 mL, 150 mmol). The reaction was stirred at room temperature overnight before being quenched with the addition of water (80 mL) and acidified by 10% aqueous citric acid to pH 3, then extracted with EtOAc (70 mL×3). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (MeOH/$CHCl_3$, 1:30 v/v) to give **36** (3.48 g, 14.2 mmol, 95% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.24 (d, *J* = 9.2 Hz, 0.5H), 4.12 (d, *J* = 10.4 Hz, 0.5H), 2.87 (s, 3H), 2.06 (m, 1H), 1.47 (s, 9H), 1.37 (m, 1H), 1.08 (m, 1H), 0.98 (d, *J* = 6.8 Hz, 3H), 0.90 (t *J* = 7.2 Hz, 3H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 174.8, 174.7, 156.4, 155.8, 80.5, 80.3, 63.2, 62.4, 33.3, 30.8, 30.4, 28.0, 24.8, 15.7, 15.5, 10.5, 10.2. Spectroscopic data are in agreement with those reported in the literature.



37

***tert*-butyl methyl((2*S*,3*S*)-3-methyl-1-((*S*)-2-(methyl((*S*)-3-methyl-1-oxo-1-((*S*)-2-propionylpyrrolidin-1-yl)butan-2-yl)carbamoyl)pyrrolidin-1-yl)-1-oxopentan-2-yl) carbamate (37):** A solution of modified dipeptide **35** (4.19 g, 9.7 mmol) in CH_2Cl_2 (15 mL) and trifluoroacetic acid (10 mL) was stirred at room temperature for 1 h and concentrated, giving the corresponding crude amine (TFA salt). To a solution of the above amine in dry DMF (6 mL) were added **36** (3.57 g, 14.6 mmol), HATU (5.89 g, 15.5 mmol), and DIPEA (10.1 mL, 58.2 mmol) cooled at 0 ˚C. The reaction mixture was then stirred at room temperature overnight, and diluted with EtOAc (80 mL). The whole mixture was washed successively with 10% aq. citric acid (50 mL), saturated

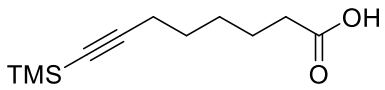
aqueous $NaHCO_3$ (50 mL), and brine (50 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (MeOH/$CHCl_3$, 1:30 v/v) to give modified tripeptide **37** (4.10 g, 7.26 mmol, 75% yield) as a colorless oil: $[\alpha]_D^{29}$ -166.9 (*c* 4.21, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) (major rotamers) δ 5.01 (d, *J* = 10.8 Hz, 1H), 4.80 (m, 1H), 4.63 (d, *J* = 11.2 Hz, 1H), 4.46 (m, 1H), 3.91-3.53 (m, 4H), 3.134 (s, 1.5H), 3.125 (s, 1.5H), 2.78 (s, 1.5H), 2.74 (s, 1.5H), 2.62-2.43 (m, 2H), 2.25-2.06 (m, 4H), 2.02-1.88 (m, 4H), 1.86-1.71 (m, 4H), 1.44 (s, 4.5H), 1.42 (s, 4.5H), 1.05 (t, *J* = 7.2 Hz, 3H), 0.96 (d, *J* = 6.4 Hz, 3H), 0.89-0.80 (m, 9H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz) δ 208.7, 173.1, 169.4, 169.3, 168.3, 156.2, 155.3, 80.1, 79.6, 64.3, 61.0, 59.34, 59.29, 59.2, 56.4, 56.3, 47.6, 47.5, 46.8, 33.44, 33.38, 33.2, 30.4, 29.7, 29.0, 28.4, 28.3, 27.4, 24.9, 24.4, 24.3, 19.0, 18.8, 15.5, 15.1, 10.8, 10.7, 7.4; IR (neat) 2969, 2877, 1727, 1687, 1639 cm^{-1}; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{30}H_{52}N_4O_6Na$ 587.3785; found, 587.3766.



27

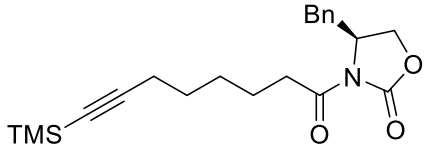
***tert*-butyl methyl((*S*)-3-methyl-1-(methyl((2*S*,3*S*)-3-methyl-1-((*S*)-2-(methyl((*S*)-3-methyl-1-oxo-1-((*S*)-2-propionylpyrrolidin-1-yl)butan-2-yl)carbamoyl)pyrrolidin-1-yl)-1-oxopentan-2-yl)amino)-1-oxobutan-2-yl)carbamate (27):** A solution of modified tripeptide **10** (4.02 g, 7.1 mmol) in CH_2Cl_2 (15 mL) and trifluoroacetic acid (10 mL) was stirred at room temperature for 1 h and concentrated, giving the corresponding crude amine (TFA salt). To a solution of the above amine in dry DMF (5 mL) were added **32** (2.47 g, 10.7 mmol), HATU (4.34 g, 11.4 mmol), and DIPEA (7.45 mL, 42.8 mmol) cooled at 0 ˚C. The reaction mixture was then stirred at room temperature overnight, and diluted with EtOAc (80 mL). The whole mixture was washed successively with 10% aq. citric acid (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine (50 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (MeOH/$CHCl_3$, 1:30 v/v) to give modified tetrapeptide **27** (2.99 g, 4.40 mmol, 62% yield) as a colorless oil: $[\alpha]_D^{29}$ -187.2 (*c* 4.27, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) (major rotamers) δ 5.10 (d, *J* = 11.2 Hz, 1H), 5.01 (d, *J* = 10.8 Hz, 1H), 4.78 (m, 1H), 4.67 (d, *J* = 11.2 Hz, 1H),

4.50 (dd, *J* = 5.6, 8.4 Hz, 1H)), 3.93 (m, 1H), 3.77-3.52 (m, 3H), 3.12 (s, 3H), 3.07 (s, 1.5H), 3.05 (s, 1.5H), 2.73 (s, 2H), 2.71 (s, 1H), 2.60-2.45 (m, 2H), 2.33-2.18 (m, 2H), 2.14-2.02 (m, 4H), 1.99-1.70 (m, 7H), 1.43 (s, 4.5H), 1.42 (s, 4.5H), 1.05 (t, *J* = 7.2 Hz, 3H), 0.96 (d, *J* = 6.4 Hz, 3H), 0.90-0.79 (m, 15H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz) δ 208.73, 208.69, 173.0, 172.9, 171.4, 170.5, 169.4, 168.9, 168.5, 156.2, 155.1, 80.2, 79.8, 64.3, 61.4, 59.5, 59.2, 57.8, 57.7, 56.4, 47.6, 33.4, 33.2, 30.6, 30.4, 29.3, 29.0, 28.3, 28.2, 27.4, 27.2, 24.92, 24.86, 23.9, 20.0, 19.5, 19.0, 18.8, 18.2, 18.1, 15.1, 14.9, 10.5, 10.4, 7.4; IR (neat) 2967, 2876, 2460, 1726, 1689, 1637 cm^{-1}; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{36}H_{63}N_5O_7Na$ 700.4625; found, 700.4620.



39

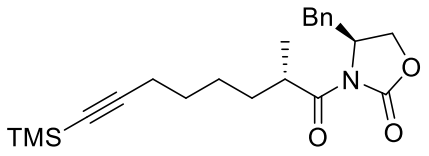
8-(trimethylsilyl)oct-7-ynoic acid (39): To a solution of trimethylethynylsilane (49.6 mL, 348.2 mmol) in THF (150 mL) cooled at −78 ˚C under argon was added a 2.3 M solution of n-BuLi in cyclohexane (156.9 mL 360.9 mmol) dropwise, and the mixture was stirred at −78 ˚C for 1 h. A solution of compound **38** (25.0 g, 128.1 mmol) in THF (50 mL) was slowly added dropwise to the reaction mixture before HMPA (98 mL) in THF (10 mL) was introduced. After addition, stirring was continued at −78 ˚C for 2 h and then at −20 ˚C for 4 h. The reaction was quenched with saturated aqueous NH_4Cl (200 mL). EtOAc (300 mL) was added, and the layers were separated. The organic layer was washed with brine (200 mL×2), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/Hexane, 1:10 v/v) to give compound **39** (25.7 g, 121.0 mmol, 94% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.35 (t, *J* = 7.6 Hz, 2H), 2.21 (t, *J* = 7.6 Hz, 2H), 1.64 (m, 2H), 1.53 (m, 2H), 1.44 (m, 2H), 0.13 (s, 9H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz) δ 180.3, 107.1, 84.6, 33.9, 28.13, 28.10, 24.1, 19.6, 0.10. Spectroscopic data are in agreement with those reported in the literature.



40

(*S*)-4-benzyl-3-(8-(trimethylsilyl)oct-7-ynoyl)oxazolidin-2-one (40): To a solution of compound **39** (19.1 g, 90.0 mmol) and DMF (0.20 mL) in DCM (200 mL) cooled at −5 ˚C under argon was added oxalyl dichloride (15.5 mL, 180.0 mmol) dropwise, and

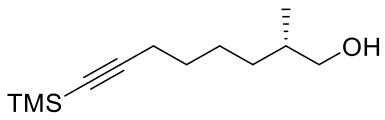
the mixture was warmed to room temperature and stirred for 3 h. The mixture was concentrated, and then THF (50 mL) was added and the mixture was concentrated again to afford the crude acyl chloride and used directly. To the solution of (*S*)-4-Benzyl-2-oxazolidinone (20.7 g, 117.0 mmol) in THF (200 mL) was added n-BuLi (2.3 M, 54.8 mL, 126.0 mmol) dropwise at −78 ˚C under argon. After the mixture had been stirred at −78 ˚C for 2 h, the crude acyl chloride in THF (50 mL) was added dropwise at −78 ˚C. After the mixture had been stirred at −78 ˚C for 2 h, the reaction was quenched with saturated aqueous NH_4Cl (200 mL). EtOAc (300 mL) was added, and the layers were separated. The organic layer was washed with brine (200 mL×2), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/Hexane, 1:5 v/v) to give compound **40** (28.3 g, 76.3 mmol, 85% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.34-7.19 (m, 5H), 4.66 (m, 1H), 4.21-4.14 (m, 2H), 3.28 (dd, *J* = 3.6, 13.6 Hz, 1H), 3.02-2.85 (m, 2H), 2.76 (dd, *J* = 9.6, 13.6 Hz, 1H), 2.24 (t, *J* = 6.8 Hz, 2H), 1.71 (m, 2H), 1.57 (m, 2H), 1.48 (m, 2H), 0.14 (s, 9H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 173.1, 153.4, 135.2, 129.3, 128.9, 127.3, 107.2, 84.4, 66.1, 55.1, 37.8, 35.3, 28.3, 28.2, 23.6, 19.6, 0.10. Spectroscopic data are in agreement with those reported in the literature.



41

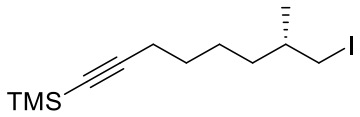
(*S*)-4-benzyl-3-((*S*)-2-methyl-8-(trimethylsilyl)oct-7-ynoyl)oxazolidin-2-one (41): A solution of compound **40** (16.7 g, 45.0 mmol) in THF (150 mL) cooled at −78 ˚C under argon was added a 1.9 M solution of NaHMDS in THF (30.8 mL, 58.5 mmol) dropwise. After the mixture had been stirred at −78 ˚C for 1 h, CH_3I (14.0 mL, 225.0 mmol) was added dropwise at −78 ˚C. After the mixture had been stirred at −78 ˚C for 3 h, the reaction was quenched with saturated aqueous NH_4Cl (100 mL). EtOAc (200 mL) was added, and the layers were separated. The organic layer was washed with brine (200 mL×2), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/Hexane, 1:5 v/v) to give compound **41** (9.82 g, 25.5 mmol, 57% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.34-7.20 (m, 5H), 4.69-4.64 (m, 1H), 4.22-4.15 (m, 2H), 3.74-3.66 (m, 1H), 3.26 (dd, *J* = 3.2, 13.2 Hz, 1H), 2.76 (dd, *J* = 9.6, 13.2 Hz, 1H), 2.21 (t, *J* = 7.6 Hz, 2H), 1.75 (m, 1H), 1.58-1.35 (m, 5H), 1.22 (d, *J* = 6.8 Hz, 3H), 0.14 (s, 9H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 177.1, 153.0, 135.2, 129.4, 128.9, 127.3, 107.2, 84.5, 66.0, 55.3, 37.8, 37.5, 32.7, 28.4, 26.3, 19.7, 17.3, 0.12. Spectroscopic data are in agreement with those reported in the

literature.



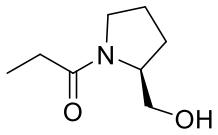
42

(*S*)-2-methyl-8-(trimethylsilyl)oct-7-yn-1-ol (42): A solution of compound **41** (9.79 g, 25.4 mmol) in THF (80 mL) cooled at 0 ˚C under argon was added $LiAlH_4$ powder (1.16 g, 30.5 mmol) little by little. After the mixture had been stirred at 0 ˚C for 2 h, the reaction was quenched with EtOAc (10 mL). Water (3 mL) was added followed by Na_2SO_4 (10 g), and the mixture was stirred for 0.2 h at room temperature then filtered, and the filtrate was concentrated. The residue was purified by column chromatography on silica gel (EtOAc/Hexane, 1:5 v/v) to give compound **41** (4.36 g, 20.6 mmol, 81% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.49-3.37 (m, 2H), 2.20 (t, *J* = 7.2 Hz, 2H), 1.60 (m, 1H), 1.51-1.32 (m, 5H), 1.23 (t, *J* = 7.2 Hz, 1H), 1.13-1.06 (m, 1H), 0.89 (d, *J* = 7.2 Hz, 3H), 0.12 (s, 9H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 107.5, 84.4, 68.1, 35.5, 32.4, 28.6, 25.9, 19.7, 16.4, 0.10. Spectroscopic data are in agreement with those reported in the literature.



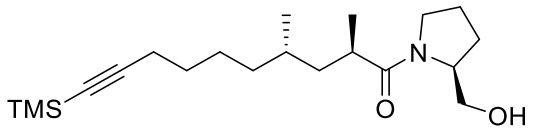
43

(*S*)-(8-iodo-7-methyloct-1-yn-1-yl)trimethylsilane (43): A solution of PPh_3 (6.92 g, 26.4 mmol) in DCM (50 mL) cooled at 0 ˚C under argon were added I_2 (7.21 g, 28.4 mmol) and imidazole (1.80 g, 26.4 mmol). After the mixture had been stirred at 0 ˚C for 15 min, a solution of compound **42** (4.31 g, 20.3 mmol) in DCM (10 mL) was added dropwise at 0 ˚C. After addition, stirring was continued at room temperature for 2 h, and diluted with DCM (50 mL). The mixture was washed with saturated aqueous $Na_2S_2O_3$ (100 mL×2) and brine (100 mL×2), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (Hexane) to give compound **43** (5.82 g, 18.1 mmol, 89% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.22 (dd, *J* = 4.8, 9.6 Hz, 1H), 3.16 (dd, *J* = 6.0, 9.6 Hz, 1H), 2.23 (t, *J* = 6.8 Hz, 2H), 1.56-1.34 (m, 6H), 1.24 (m, 1H), 0.97 (d, *J* = 6.4 Hz, 3H), 0.14 (s, 9H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 107.3, 84.6, 35.8, 34.5, 28.4, 25.9, 20.5, 19.7, 17.7, 0.16. Spectroscopic data are in agreement with those reported in the literature.



44

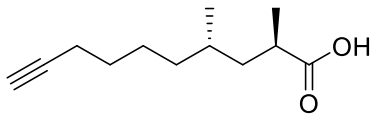
(*S*)-1-(2-(hydroxymethyl)pyrrolidin-1-yl)propan-1-one (44): A solution of L-prolinol (5.06 g, 50.0 mmol) in CH_2Cl_2 (80 mL) cooled at 0 ˚C under N_2 was added propionic anhydride (9.6 mL, 75 mmol) dropwise. After the mixture had been stirred at 0 ˚C for 3 min, diluted with CH_2Cl_2 and evaporated the solution under reduced pressure 3 times. The residue was purified by column chromatography on silica gel (EtOAc) to give compound **44** (7.97 g, 49.9 mmol, 99% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.09 (m, 1H), 3.54-3.32 (m, 4H), 2.22 (q, *J* = 7.6 Hz, 2H), 1.96-1.72 (m, 3H), 1.54 (m, 1H), 1.03 (t, *J* = 7.6 Hz, 3H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 174.7, 66.6, 60.7, 47.7, 27.9, 24.1, 8.6. Spectroscopic data are in agreement with those reported in the literature.



45

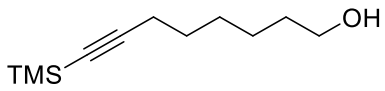
(2*R*,4*S*)-1-((*S*)-2-(hydroxymethyl)pyrrolidin-1-yl)-2,4-dimethyl-10-(trimethylsilyl)dec-9-yn-1-one (45): A solution of diisopropylamine (8.14 mL, 58.1 mmol) in THF (50 mL) cooled at −10 ˚C under argon was added a 2.3 M solution of n-BuLi in cyclohexane (23.8 mL, 54.8 mmol) dropwise. After addition, the reaction mixture was warmed to room temperature, stirred for 40 min, and then cooled to −10 ˚C, and then a solution of compound **44** (3.91 g, 24.9 mmol) in THF (10 mL) was added dropwise. After the mixture had been stirred for 1 h, HMPA (10.11 mL, 58.1 mmol) was introduced. The reaction mixture was cooled to −78 ˚C, and a solution of compound **43** (5.35 g, 16.6 mmol) in THF (10 mL) was added dropwise at −78 ˚C. After the mixture had been stirred at −50 ˚C for 4 h, the reaction was quenched with saturated aqueous NH_4Cl (50 mL), and the mixture was extracted with EtOAc (30 mL×3). The combined organic layers were washed with brine (50 mL×2), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/Hexane, 1:1 v/v) to give compound **45** (3.76 g, 10.7 mmol, 64% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.21 (m, 1H), 3.65-3.45 (m, 4H), 2.61 (m, 1H), 2.19 (t, *J* = 6.8 Hz, 2H), 2.06-1.82 (m, 3H), 1.57 (m, 1H), 1.51-1.21 (m, 9H), 1.10 (d, *J* = 6.8 Hz, 3H), 0.84 (d, *J* = 6.0 Hz, 3H), 0.12 (s, 9H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 178.5, 107.4, 84.4, 67.7, 61.1, 47.8, 40.5, 36.7, 35.7, 30.2, 28.7, 28.2, 25.9, 24.4, 19.7, 19.3, 17.2, 0.12. Spectroscopic data are in agreement with those reported in

the literature.



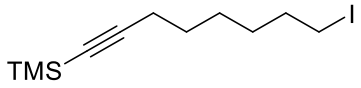
28

(2*R*,4*S*)-2,4-dimethyldec-9-ynoic acid (28): A solution of compound **45** (3.73 g, 10.6 mmol) in HCl (2 M, 40 mL) and 1,4-dioxane (10 mL) was refluxed for 4 h, and then the reaction mixture was extracted with EtOAc (50 mL×3). The organic layer was washed with brine (50 mL×2), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/Hexane, 1:3 v/v) to give compound **28** (1.05 g, 5.33 mmol, 50% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.53 (m, 1H), 2.18 (td, *J* = 7.6, 2.8 Hz, 2H), 1.94 (t, *J* = 2.8 Hz, 1H), 1.58-1.29 (m, 9H), 1.15 (d, *J* = 6.8 Hz, 3H), 0.87 (d, *J* = 6.0 Hz, 3H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 183.8, 84.6, 68.2, 40.6, 37.1, 36.3, 30.2, 28.6, 25.9, 19.2, 18.3, 16.8. Spectroscopic data are in agreement with those reported in the literature.



46

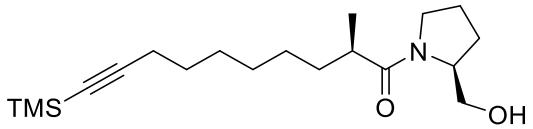
8-(trimethylsilyl)oct-7-yn-1-ol (46): A solution of compound **40** (9.29 g, 25 mmol) in THF (80 mL) was added $LiAlH_4$ (1.14 g, 30 mmol) batchwise under argon at 0 ˚C. After the mixture had been stirred for 2 h at 0 ˚C, the reaction was quenched with EtOAc (10 mL). Water (3 mL) was added followed by Na_2SO_4 (10 g), and the mixture was stirred for 0.2 h at room temperature than filtered, and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/Hexane, 1:5 v/v) to give compound **46** (3.47 g, 17.5 mmol, 70% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.62 (t, *J* = 7.2 Hz, 2H), 2.20 (t, *J* = 6.8 Hz, 2H), 1.59-1.47 (m, 4H), 1.44-1.32 (m, 4H), 0.12 (s, 9H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 107.5, 84.4, 62.8, 32.5, 28.5, 25.2, 19.7, 0.1; IR (neat) 3627, 3169, 2174, 1651 cm^{-1}; HRMS (ESI-TOF) *m/z*: [M + Na]$^+$ Calcd for $C_{11}H_{22}OSiNa$ 221.1338; found, 221.1345.



47

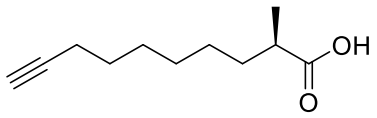
(8-iodooct-1-yn-1-yl)trimethylsilane (47): A solution of PPh_3 (5.90 g, 22.5 mmol) in DCM (50 mL) were added I_2 (6.14 g, 24.2 mmol) and imidazole (1.77 g, 26.0 mmol) at 0 ˚C under argon. After the mixture had been stirred for 15 min at 0 ˚C, compound **46**

(3.43 g, 17.3 mmol) in DCM (10 mL) was added dropwise at 0 ˚C. After addition, stirring was continued at room temperature for 2 h, and diluted with DCM (50 mL). The whole mixture was washed with saturated aqueous $Na_2S_2O_3$ (70 mL×2) and brine (70 mL×2), dried over Na_2SO4, and concentrated. The residue was purified by column chromatography on silica gel (Hexane) to give compound **47** (4.81 g, 15.6 mmol, 90% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.18 (t, *J* = 7.6 Hz, 2H), 2.22 (t, *J* = 6.8 Hz, 2H), 1.86-1.79 (m, 2H), 1.57-1.50 (m, 2H), 1.42-1.39 (m, 4H), 0.14 (s, 9H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 107.3, 84.5, 33.3, 29.9, 28.3, 27.6, 19.7, 7.0, 0.1; IR (neat) 2957, 2935, 2858, 2174, 1460 cm^{-1}; HRMS (ESI-TOF) *m/z*: [M + Na]$^+$ Calcd for $C_{11}H_{21}ISiNa$ 331.0355; found, 331.0339.



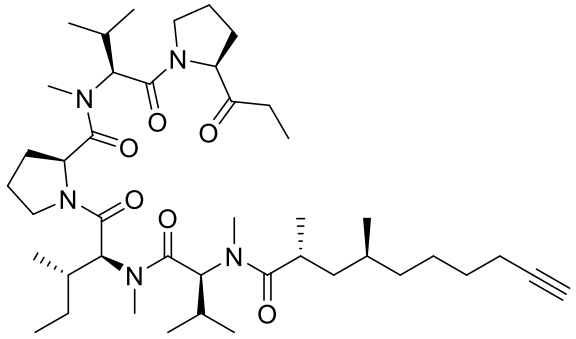
48

(*R*)-1-((*S*)-2-(hydroxymethyl)pyrrolidin-1-yl)-2-methyl-10-(trimethylsilyl)dec-9-yn-1-one (48): A solution of diisopropylamine (7.61 mL, 54.3 mmol) in THF (40 mL) was added n-BuLi (2.3 M, 22.24 mL, 51.2 mmol) dropwise at −10 ˚C under argon. After addition, the reaction mixture was warmed to room temperature, stirred for 40 min, and then cooled to −10 ˚C, and then compound **44** (3.66 g, 23.3 mmol) was added dropwise. After the mixture had been stirred for 1 h, HMPA (9.44 mL, 54.3 mmol) was introduced. The reaction mixture was cooled to −78 ˚C, and compound **47** (4.77 g, 15.5 mmol) in THF (10 mL) was added dropwise at −78 ˚C. After the mixture had been stirred at −50 ˚C for 4 h, the reaction was quenched with saturated aqueous NH_4Cl (50 mL), extracted with EtOAc (30 mL×3). The organic layer was washed with brine (50 mL×2), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/Hexane, 1:1 v/v) to give compound **48** (1.67 g, 4.96 mmol, 48% yield) as a colorless oil: $[\alpha]_D^{29}$ -35.6 (*c* 2.85, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.23 (m, 1H), 3.65-3.44 (m, 4H), 2.53 (m, 1H), 2.18 (t, *J* = 7.2 Hz, 2H), 2.05-1.82 (m, 3H), 1.64-1.44 (m, 4H), 1.41-1.21 (m, 7H), 1.12 (d, *J* = 6.8 Hz, 3H), 0.12 (s, 9H); ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz) δ 178.3, 107.5, 84.3, 67.8, 61.0, 47.8, 38.1, 33.5, 29.1, 28.6, 28.5, 28.3, 27.4, 24.4, 19.8, 17.7, 0.1; IR (neat) 3404, 2933, 2858, 2173, 1622 cm^{-1}; HRMS (ESI-TOF) *m/z*: [M + Na]$^+$ Calcd for $C_{19}H_{35}O_2SiNa$ 360.2335; found, 360.2349.



29

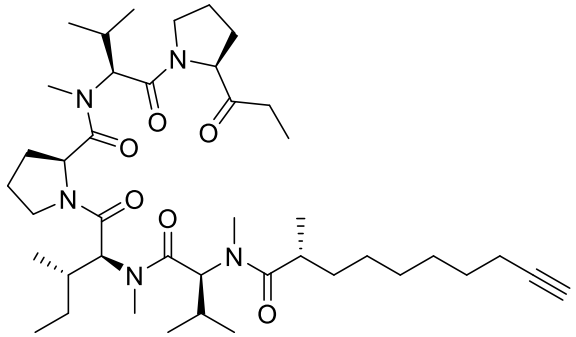
(*R*)-2-methyl-10-(trimethylsilyl)dec-9-ynoic acid (29): A solution of compound **48** (1.64 g, 4.9 mmol) in HCl (2 M, 40 mL) and 1,4-dioxane (10 mL) was refluxed for 4 h, and then the reaction mixture was extracted with EtOAc (50 mL×3). The organic layer was washed with brine (50 mL×2), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/Hexane, 1:3 v/v) to give compound **29** (0.57 g, 3.14 mmol, 65% yield) as a colorless oil: $[\alpha]_D^{29}$ -10.7 (*c* 3.53, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 2.45 (m, 1H), 2.17 (td, *J* = 6.8, 2.8 Hz, 2H), 1.93 (t, *J* = 2.8 Hz, 1H), 1.68 (m, 1H), 1.55-1.48 (m, 2H), 1.45-1.29 (m, 7H), 1.17 (d, *J* = 6.8 Hz, 3H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz) δ 183.5, 84.6, 68.1, 39.3, 33.4, 28.9, 28.5, 28.3, 27.0, 18.3, 16.8; IR (neat) 3306, 2936, 2174, 2118, 1705, 1465 cm^{-1}; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{11}H_{18}O_2Na$ 205.1204; found, 205.1195



25

Odookeanyne A (**25**): A solution of compound **27** (1.02 g, 1.5 mmol) in CH_2Cl_2 (10 mL) and trifluoroacetic acid (5 mL) was stirred at room temperature for 1 h and concentrated, giving the corresponding crude amine (TFA salt). To a solution of the above amine in dry DMF (1.2 mL) were added compound **28** (0.59 g, 3 mmol), HATU (1.20 g, 3.2 mmol), and DIPEA (1.57 mL, 9 mmol) cooled at 0 ˚C. The reaction mixture was then stirred at room temperature overnight, and diluted with EtOAc (50 mL). The whole mixture was washed successively with 10% aq. citric acid (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine (50 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (MeOH/$CHCl_3$, 1:50 v/v) to give odookeanyne A (**25**) (0.90 g, 1.19 mmol, 80% yield) as a colorless oil: $[\alpha]_D^{29}$ -189.2 (*c* 4.91, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 5.21 (d, *J* = 11.2 Hz, 1H), 5.09 (d, *J* = 11.2 Hz, 1H), 5.00 (d, *J* = 10.8 Hz, 1H), 4.79 (dd, *J* = 4.8, 8.0 Hz, 1H), 4.49 (dd, *J* = 5.6, 8.8 Hz, 1H), 3.89 (m, 1H), 3.76-3.69 (m, 2H), 3.64 (m, 1H), 3.12 (s, 3H),

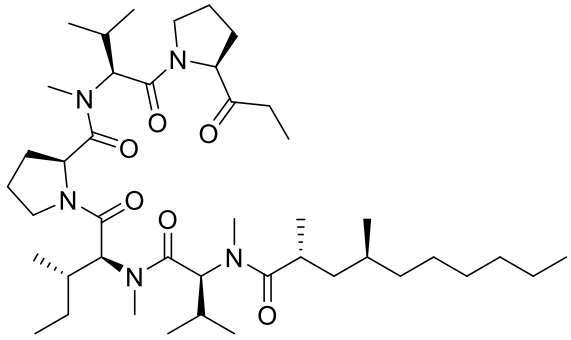
3.05 (s, 3H), 2.98 (s, 3H), 2.76 (m, 1H), 2.57 (dq, *J* = 7.2, 18.0 Hz, 1H), 2.47 (dq, *J* = 7.2, 18.0 Hz, 1H), 2.29 (m, 1H), 2.20 (m, 1H), 2.14 (dt, *J* = 2.8, 6.8 Hz, 2H), 2.11-2.01 (m, 4H), 1.95 (m, 1H), 1.90 (t, *J* = 2.4 Hz, 1H), 1.85-1.71 (m, 4H), 1.57 (m, 1H), 1.49-1.34 (m, 4H), 1.30-1.22 (m, 3H), 1.18-1.10 (m, 2H), 1.04 (t, *J* = 7.2 Hz, 3H), 1.01 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 6.4 Hz, 3H), 0.92 (m, 1H), 0.88 (d, *J* = 6.8 Hz, 3H), 0.85 (d, *J* = 6.8 Hz, 3H), 0.83 (d, *J* = 6.4 Hz, 3H), 0.83 (d, *J* = 6.4 Hz, 3H), 0.80 (d, *J* = 7.2 Hz, 3H), 0.78 (t, *J* = 7.2 Hz, 3H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz) δ 208.7, 177.1, 173.0, 171.3, 169.4, 168.3, 84.4, 68.1, 64.2, 59.2, 57.8, 57.6, 56.4, 47.6, 47.5, 41.1, 36.1, 33.6, 33.4, 33.1, 30.7, 30.5, 30.4, 30.1, 28.9, 28.7, 28.3, 27.4, 27.1, 25.9, 24.92, 24.85, 23.9, 19.8, 19.4, 19.0, 18.8, 18.3, 17.6, 15.0, 10.5, 7.3; IR (neat) 2964, 2935, 2875, 1727, 1634 cm^{-1}; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{43}H_{73}N_5O_6Na$ 778.5459; found, 778.5450.



26

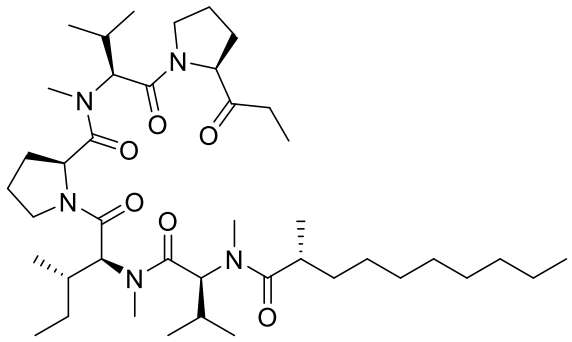
Odookeanyne B (**26**): A solution of compound **27** (0.68 g, 1.0 mmol) in CH_2Cl_2 (10 mL) and trifluoroacetic acid (5 mL) was stirred at room temperature for 1 h and concentrated, giving the corresponding crude amine (TFA salt). To a solution of the above amine in dry DMF (0.8 mL) were added compound **29** (0.36 g, 2 mmol), HATU (0.80 g, 2.1 mmol), and DIPEA (1.05 mL, 6 mmol) cooled at 0 ˚C. The reaction mixture was then stirred at room temperature overnight, and diluted with EtOAc (50 mL). The whole mixture was washed successively with 10% aq. citric acid (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine (50 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (MeOH/$CHCl_3$, 1:50 v/v) to give odookeanyne B (**26**) (0.55 g, 0.75 mmol, 75% yield) as a colorless oil: $[\alpha]_D^{29}$ -197.4 (*c* 3.19, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 5.23 (d, *J* = 10.4 Hz, 1H), 5.10 (d, *J* = 11.6 Hz, 1H), 5.02 (d, *J* = 11.2 Hz, 1H), 4.80 (dd, *J* = 4.4, 7.6 Hz, 1H), 4.51 (dd, *J* = 6.0, 8.8 Hz, 1H), 3.90 (m, 1H), 3.76-3.71 (m, 2H), 3.66 (m, 1H), 3.13 (s, 3H), 3.06 (s, 3H), 2.97 (s, 3H), 2.67 (m, 1H), 2.58 (dq, *J* = 7.6, 18.4 Hz, 1H), 2.49 (dq, *J* = 7.2, 18.4 Hz, 1H), 2.30 (m, 1H), 2.21 (m, 1H), 2.14 (dt, *J* = 2.4, 6.8 Hz, 2H), 2.10-2.02 (m, 4H), 1.96 (m, 1H), 1.90 (t, *J* = 2.8 Hz, 1H), 1.88-1.68 (m, 4H), 1.51-1.44 (m, 2H),

1.39-1.31 (m, 3H), 1.30-1.21 (m, 4H), 1.15 (m, 1H), 1.05 (t, *J* = 7.2 Hz, 3H), 1.04 (d, *J* = 6.8 Hz, 3H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.92 (m, 1H), 0.89 (d, *J* = 6.8 Hz, 3H), 0.86 (d, *J* = 6.4 Hz, 3H), 0.84 (d, *J* = 6.4 Hz, 3H), 0.81 (d, *J* = 6.4 Hz, 3H), 0.78 (t, *J* = 7.2 Hz, 3H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz) δ 208.7, 177.0, 173.0, 171.2, 169.4, 168.3, 84.6, 68.1, 64.3, 59.2, 57.9, 57.7, 56.4, 47.6, 47.5, 36.2, 33.8, 33.4, 33.2, 30.7, 30.4, 30.1, 29.1, 29.0, 28.5, 28.3, 27.5, 27.4, 27.0, 24.94, 24.87, 28.9, 19.4, 19.0, 18.8, 18.3, 17.8, 15.1, 10.5, 7.4; IR (neat) 2965, 2935, 2875, 1727, 1634 cm^{-1}; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{42}H_{71}N_5O_6Na$ 764.5302; found, 764.5314.



49

A suspension of odookeanyne A (**25**) (0.10 g, 132 μmol) and 5% palladium on activated charcoal (0.1 g) in MeOH (30 mL) was stirred under an atmosphere of hydrogen at room temperature overnight. The mixture was filtered to remove the catalyst, and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel ($MeOH/CHCl_3$, 1:50 v/v) to give odookeanane A (**49**) (65.5 mg, 86 μmol, 65% yield) as a colorless oil: $[\alpha]_D^{26}$ -176.8 (*c* 6.55, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.20 (d, *J* = 10.8 Hz, 1H), 5.09 (d, *J* = 11.6 Hz, 1H), 5.00 (d, *J* = 11.2 Hz, 1H), 4.78 (dd, *J* = 4.4, 7.6 Hz, 1H), 4.49 (dd, *J* = 6.0, 8.8 Hz, 1H), 3.88 (m, 1H), 3.75-3.69 (m, 2H), 3.64 (m, 1H), 3.12 (s, 3H), 3.05 (s, 3H), 2.97 (s, 3H), 2.76 (m, 1H), 2.56 (dq, *J* = 18.4, 7.2 Hz, 1H), 2.47 (dq, *J* = 18.4, 7.2 Hz, 1H), 2.29 (m, 1H), 2.20 (m, 1H), 2.15-2.00 (m, 6H), 1.93 (m, 1H), 1.85-1.69 (m, 4H), 1.55 (m, 1H), 1.35 (m, 1H), 1.04 (t, *J* = 6.8 Hz, 3H), 1.00 (d, *J* = 6.8 Hz, 3H), 0.95 (d, *J* = 6.4 Hz, 3H), 0.92 (m, 1H), 0.88 (d, *J* = 6.8 Hz, 3H), 0.85 (d, *J* = 7.2 Hz, 3H), 0.83 (d, *J* = 5.6Hz, 3H), 0.83 (d, *J* = 5.6 Hz, 3H), 0.83 (t, *J* = 6.4 Hz, 3H), 0.80 (d, *J* = 8.4 Hz, 3H), 0.78 (t, *J* = 7.6 Hz, 3H); $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz) δ 208.7, 177.2, 173.0, 171.3, 169.4, 168.3, 64.2, 59.2, 57.8, 57.6, 56.4, 47.54, 47.50, 41.2, 36.7, 33.6, 33.4, 33.1, 31.8, 30.7, 30.5, 30.4, 30.1, 29.6, 28.9, 28.3, 27.4, 27.1, 26.7, 24.9, 24.8, 23.9, 22.6, 19.9, 19.4, 19.0, 18.8, 18.3, 17.6, 15.0, 14.0, 10.5, 7.3; IR (neat) 2963, 2929, 2875, 2462, 1728, 1634 cm^{-1}; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{43}H_{77}N_5O_6Na$ 782.5772; found, 782.5770.



50

A suspension of odookeanyne B (**26**) (60 mg, 81 μmol) and 5% palladium on activated charcoal (0.1 g) in MeOH (30 mL) was stirred under an atmosphere of hydrogen at room temperature overnight. The mixture was filtered to remove the catalyst, and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (MeOH/$CHCl_3$, 1:50 v/v) to give odookeanane B (**50**) (45.8 mg, 61 μmol, 76% yield) as a colorless oil: $[\alpha]_D^{26}$ -192.3 (*c* 4.58, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 5.22 (d, *J* = 10.8 Hz, 1H), 5.09 (d, *J* = 11.2 Hz, 1H), 5.00 (d, *J* = 11.2 Hz, 1H), 4.79 (dd, *J* = 5.2, 8.0 Hz, 1H), 4.49 (dd, *J* = 5.2, 8.4 Hz, 1H), 3.89 (m, 1H), 3.75-3.69 (m, 2H), 3.64 (m, 1H), 3.12 (s, 3H), 3.05 (s, 3H), 2.96 (s, 3H), 2.65 (m, 1H), 2.56 (dq, *J* = 18.0, 7.2 Hz, 1H), 2.48 (dq, *J* = 18.0, 7.2 Hz, 1H), 2.29 (m, 1H), 2.20 (m, 1H), 2.16-2.01 (m, 6H), 1.93 (m, 1H), 1.87-1.66 (m, 4H), 1.20 (br s, 12H), 1.04 (t, *J* = 7.2 Hz, 3H), 1.02 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 6.4 Hz, 3H), 0.92 (m, 1H), 0.88 (d, *J* = 6.8 Hz, 3H), 0.85 (d, *J* = 6.4 Hz, 3H), 0.83 (d, *J* = 5.6 Hz, 3H), 0.83 (t, *J* = 6.4 Hz, 3H), 0.80 (d, *J* = 6.4 Hz, 3H), 0.78 (t, *J* = 7.2 Hz, 3H); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz) δ 208.7, 177.1, 173.0, 171.2, 169.4, 168.3, 64.2, 59.2, 57.9, 57.7, 56.4, 47.6, 47.5, 36.2, 33.9, 33.4, 33.1, 31.8, 30.7, 30.4, 30.1, 29.6, 29.4, 29.2, 29.0, 28.3, 27.6, 27.4, 27.0, 24.93, 24.85, 23.9, 22.6, 19.4, 19.0, 18.8, 18.3, 17.8, 15.0, 14.0, 10.5, 7.4; IR (neat) 2962, 2929, 2874, 2462, 1727, 1637 cm^{-1}; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{42}H_{75}N_5O_6Na$ 768.5615; found, 768.5619.

Biological Methods

***In Vitro* Antitrypansomal Assay**

The bloodstream form parasites of *Trypanosoma brucei rhodesiense* IL-1501 strain cultured at 37 ˚C under a humidified 5% CO_2 atmosphere in HMI-9 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS). For *in vitro* studies, compounds were dissolved in DMSO and diluted in culture medium prior to being assayed. The maximum DMSO concentration in the *in vitro* assays was 1%.

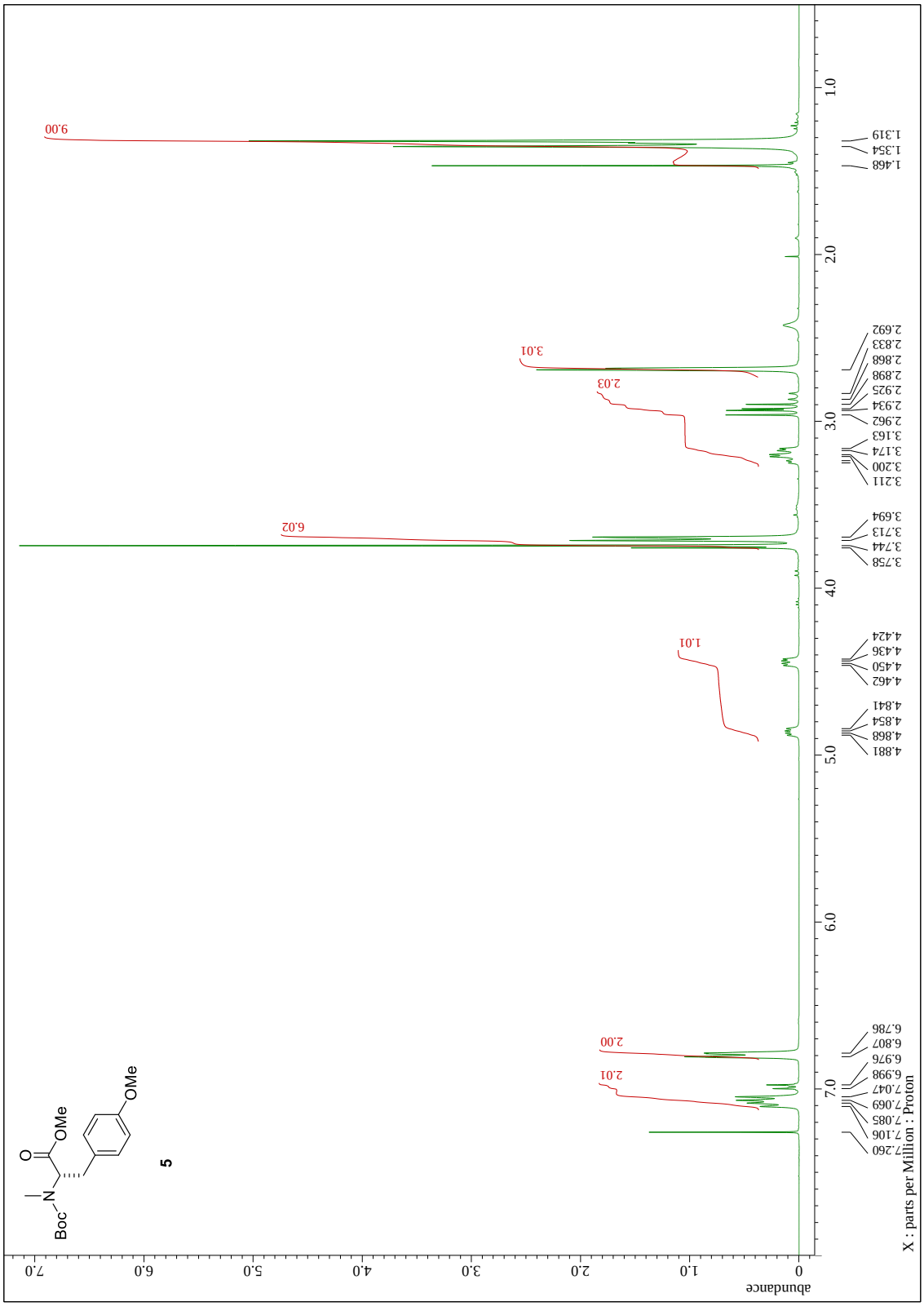
The compounds were tested in an AlamarBlue serial drug dilution assay to determine the 50% inhibitory concentrations (IC_{50}). Serial drug dilutions were prepared in 96-well microtiter plates containing the culture medium, and wells were inoculated with 50 μL of 4.0×10^4 cells/ml parasites. Cultures were incubated for 69 h at 37 ˚C under a humidified 5% CO_2 atmosphere. After this time, 10 μL of resazurin (12.5 mg resazurin [Sigma] dissolved in 100 ml phosphate-buffered saline) was added to each well. The plates were incubated for an additional 3 h. The fluorescence signal of the plates were measured in a SpectraMax Gemini XS microplate fluorescence scanner (Molecular Devices) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm.

***Plasmodium falciparum* Growth Inhibition Assay**

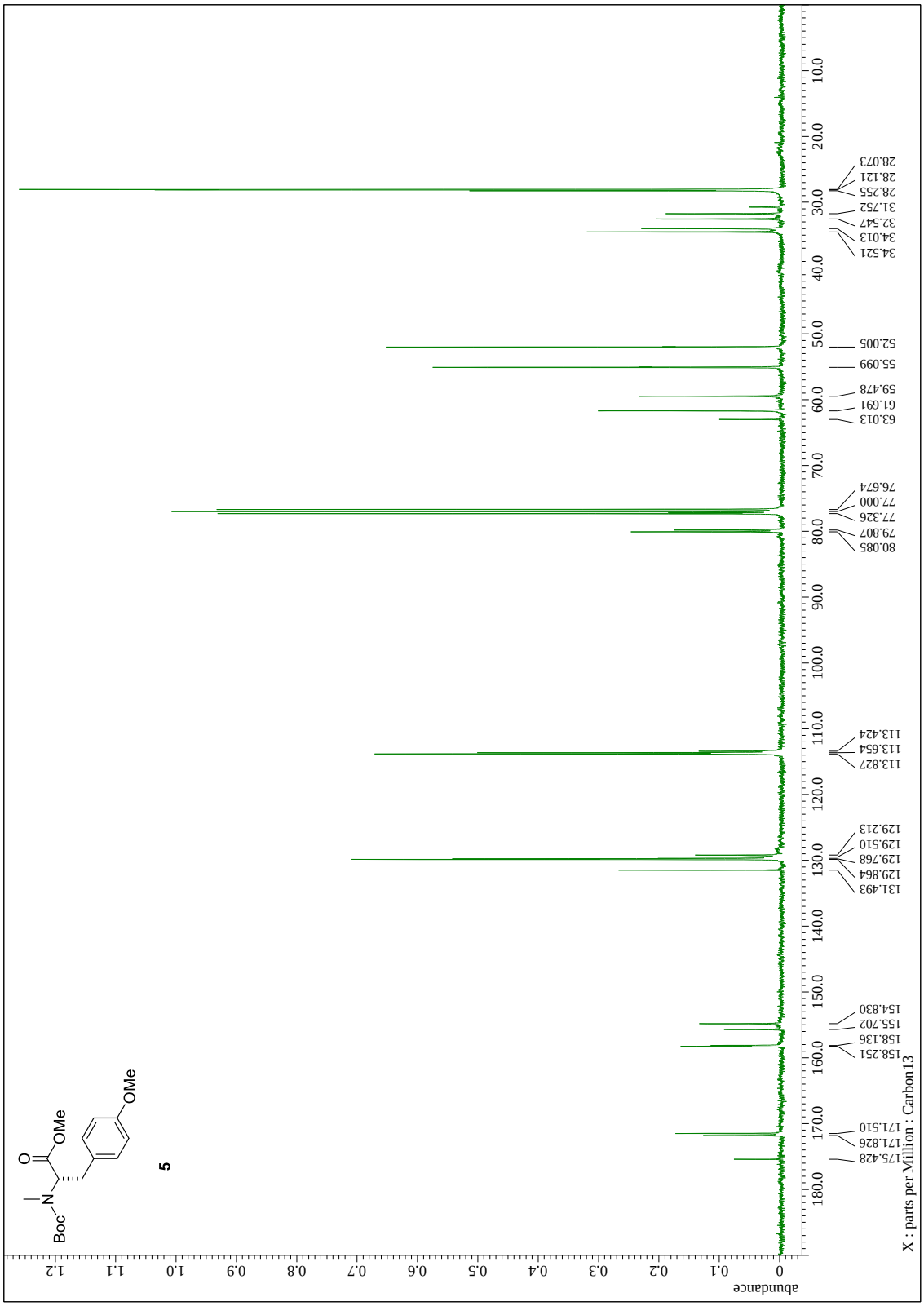
Asynchronous asexual parasites were synchronized using 5% (w/v) D-sorbitol. The level of parasitemia was determined by light microscopy. The stock culture was diluted with complete RPMI medium and 50% RBC (red blood cells) to a starting 2% hematocrit and 0.3% parasitemia. Compounds and drug controls were dissolved in DMSO prior to being assayed. One hundred microliter cultures containing different concentration of the compounds were dispensed into 96-well plates. The maximum concentration of DMSO in each well was 0.5%. The assay plates were then incubated at 37 ˚C for 72 h in a 5% CO_2, 90% N_2, and 5% O_2 atmosphere. After 72 h plates were frozen at −30 ˚C overnight to lyse the red blood cells. Plates were thawed at rt for at least 1 h before starting the assay. The LDH assay was performed as previously described with some modification. Briefly, a solution to assess parasites' LDH activity containing 50 mM sodium L-lactate, 0.25% Triton X-100, 100 mM Tris-HCl (pH 8.0), 50 μM APAD, 240 μM NBT, and 1 U/mL diaphorase was prepared. Immediately, 150 μL of the solution was dispensed into the plates. and plates were shaken to ensure mixing. After 30 min of incubation at rt in the dark, absorbance was measured at 650 nm using a SpectraMax Paradigm multimode microplate reader (Molecular Devices).

Copies of ^{1}H and ^{13}C NMR Charts

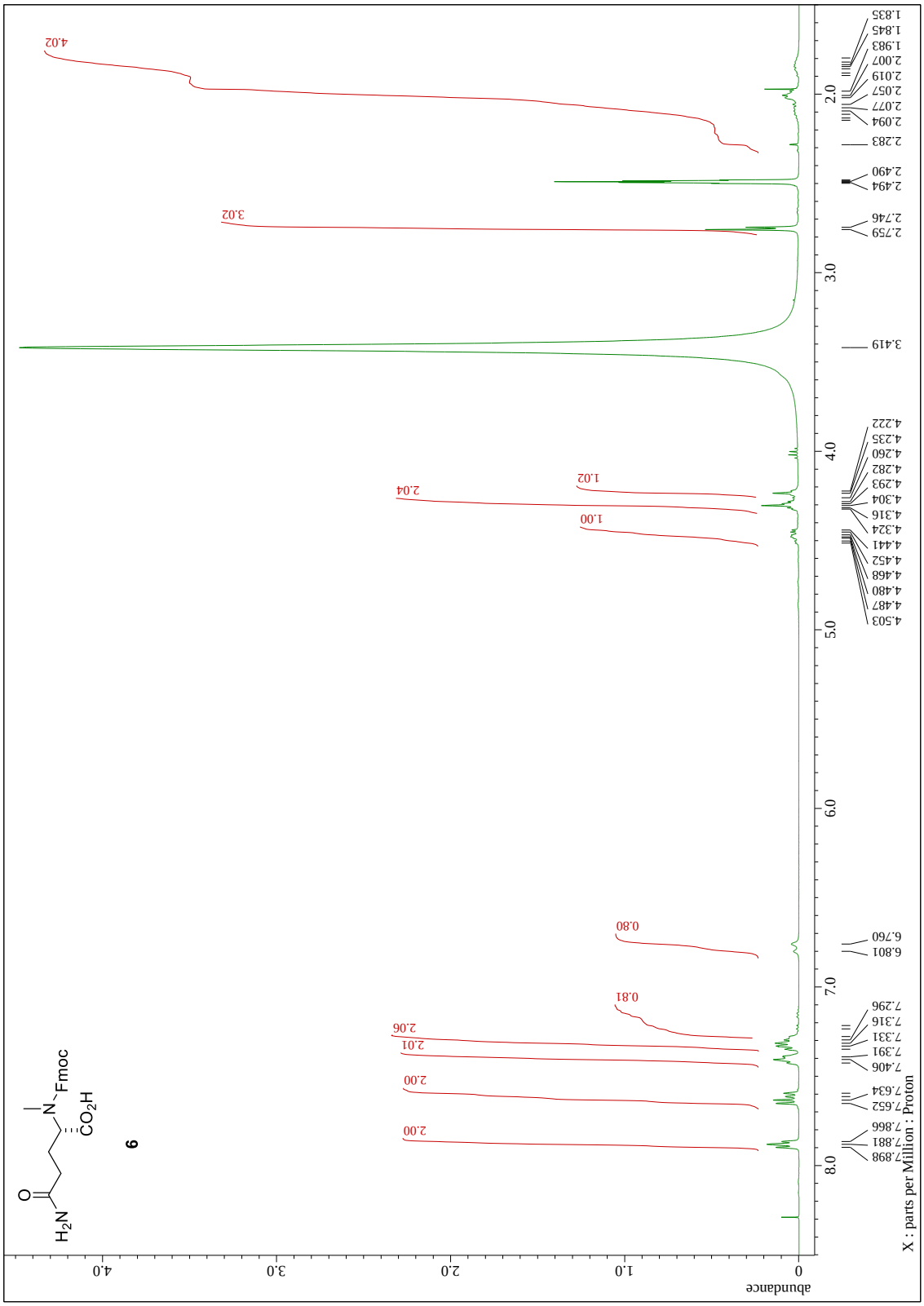
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **5**



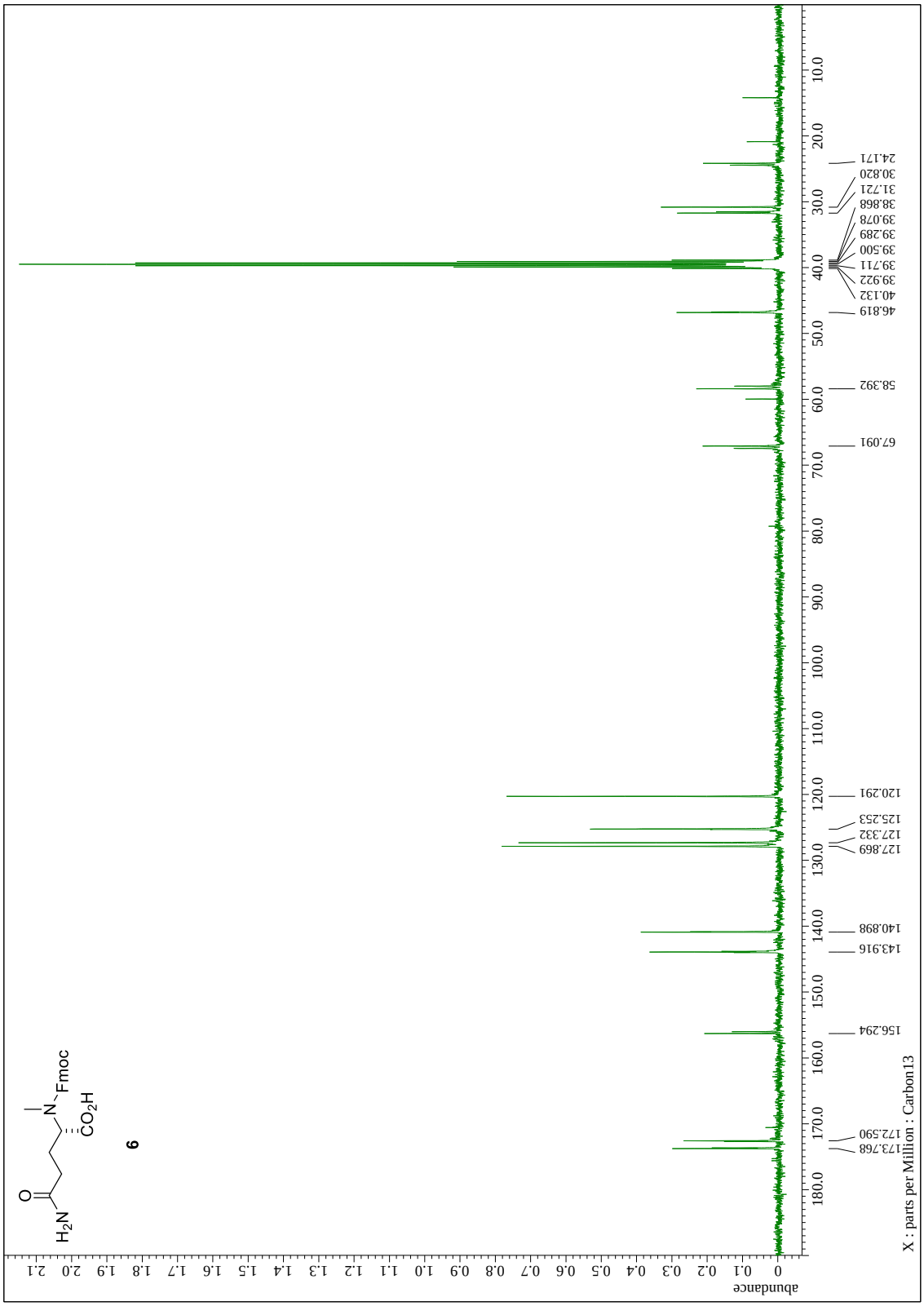
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **5**



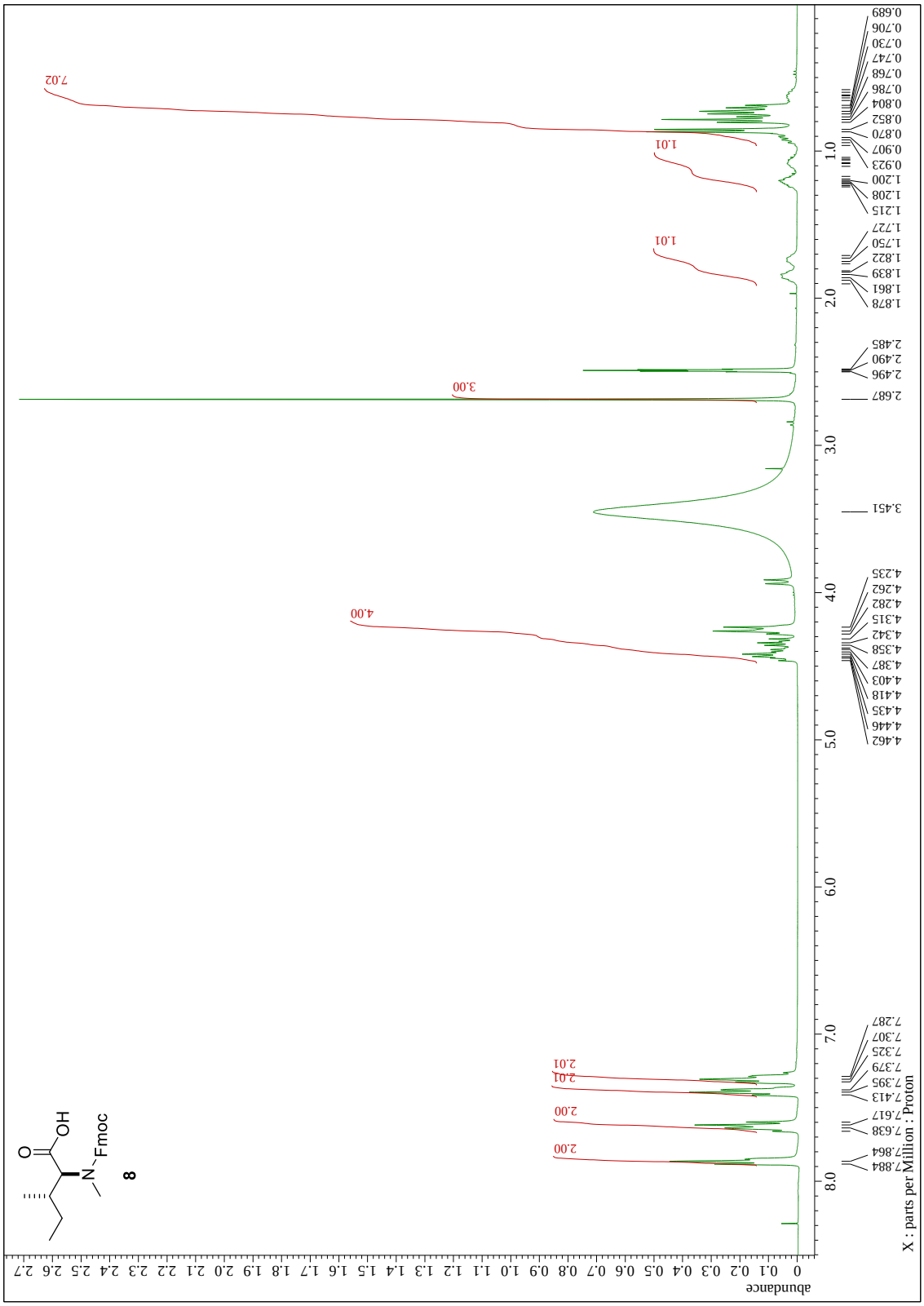
^{1}H NMR (400 MHz in d_6-DMSO) spectrum of **6**



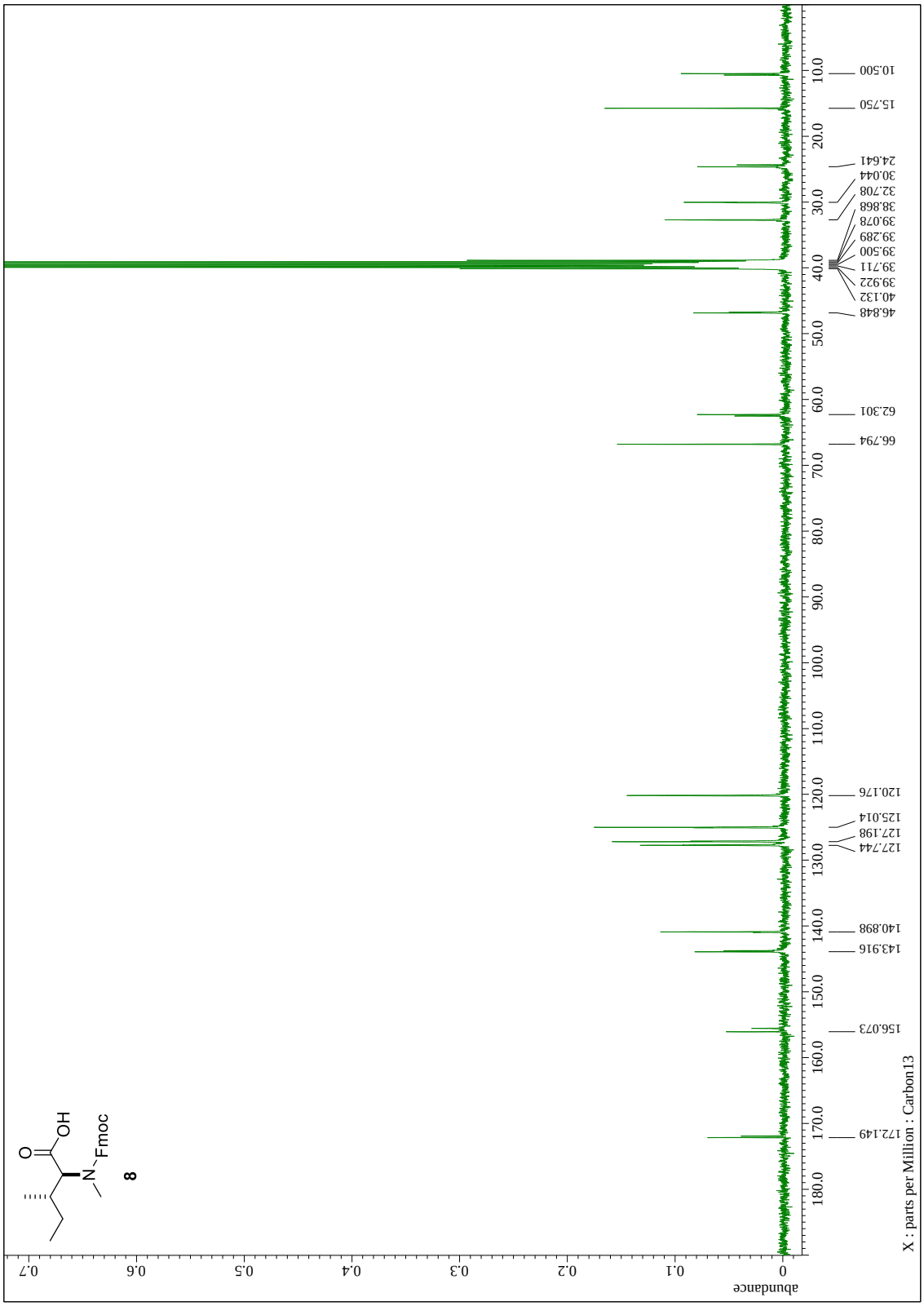
$^{13}C\{^{1}H\}$ NMR (100 MHz in d_6-DMSO) spectrum of **6**



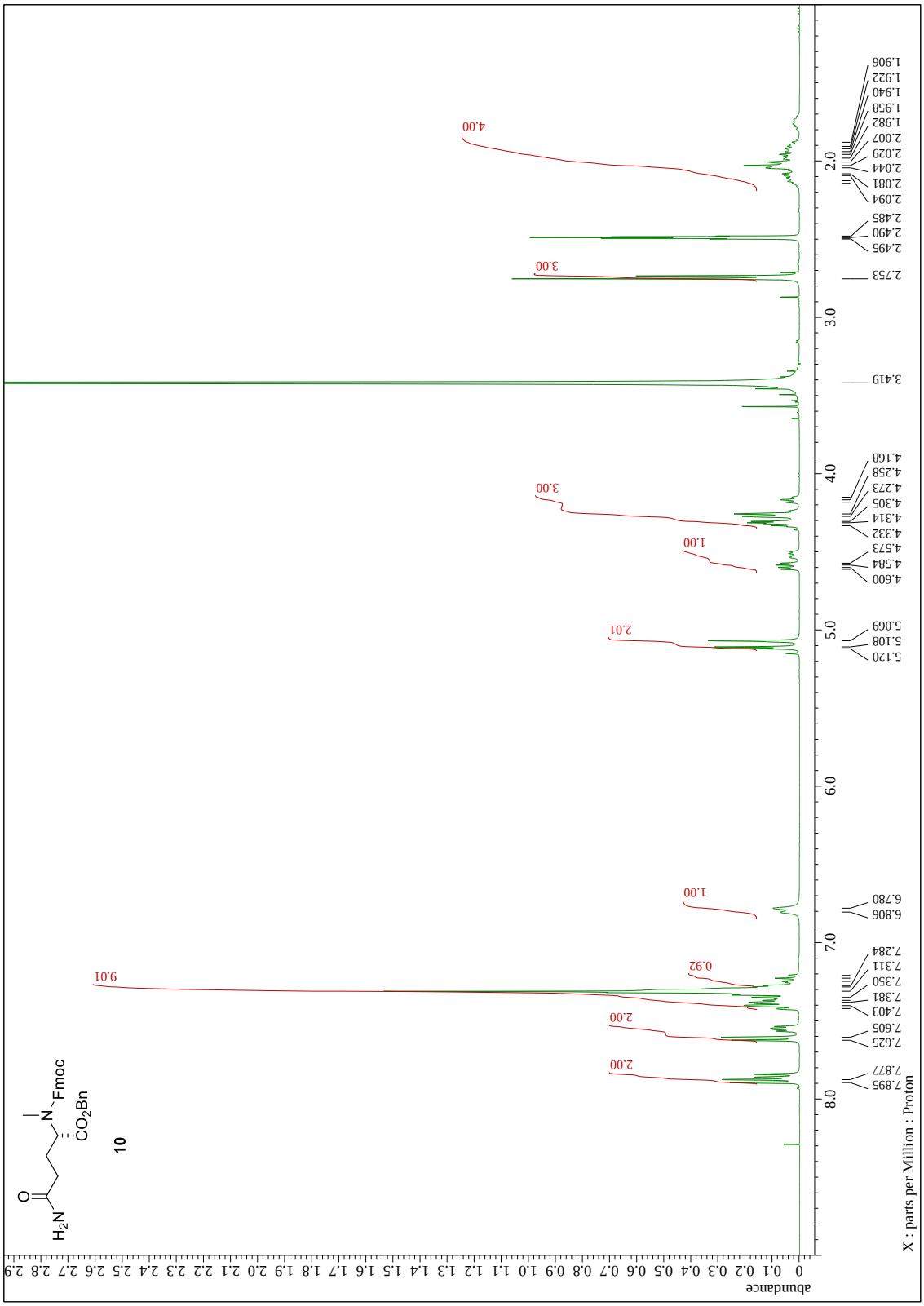
^{1}H NMR (400 MHz in d_6-DMSO) spectrum of **8**



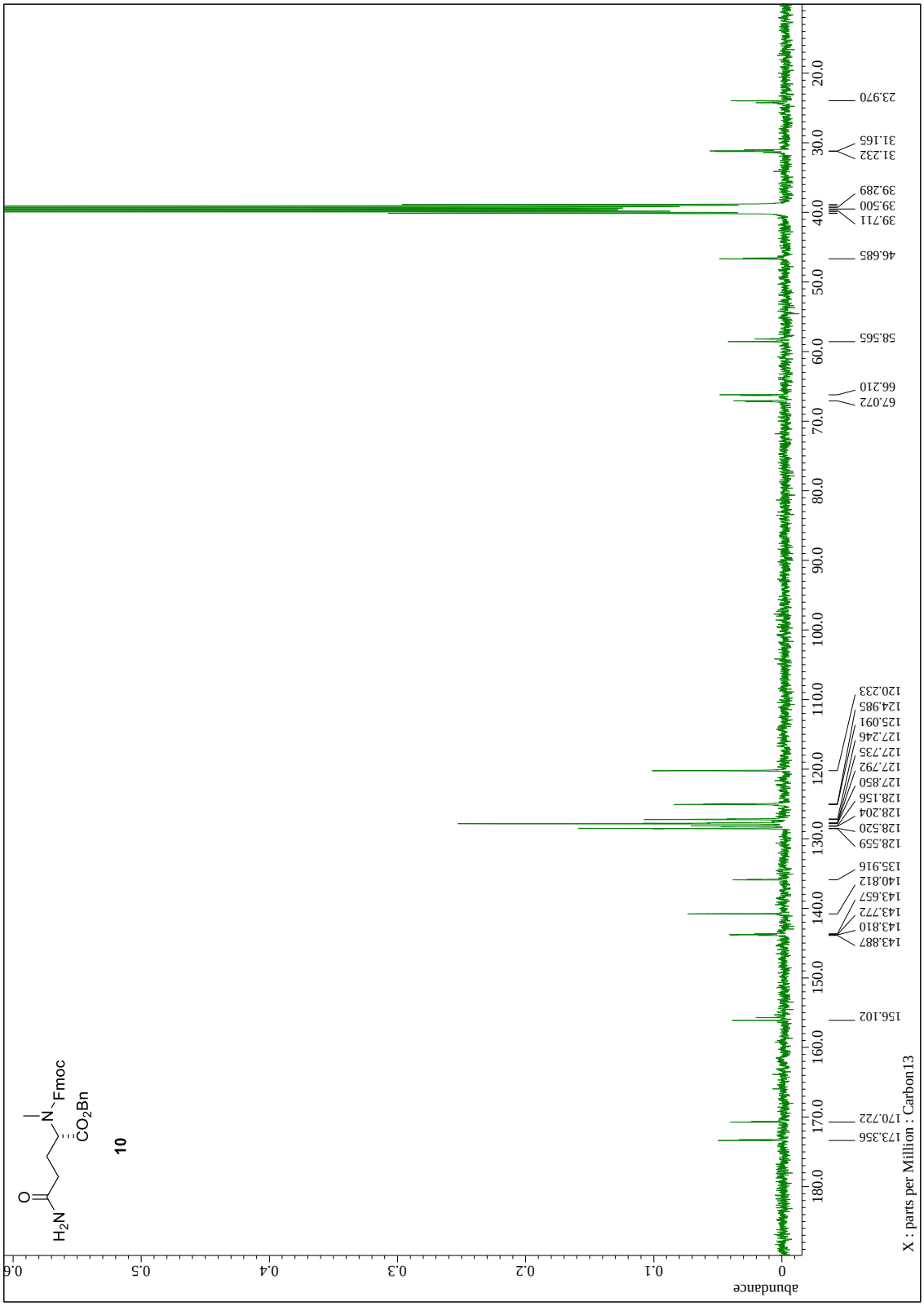
$^{13}C\{^{1}H\}$ NMR (100 MHz in d_6-DMSO) spectrum of **8**



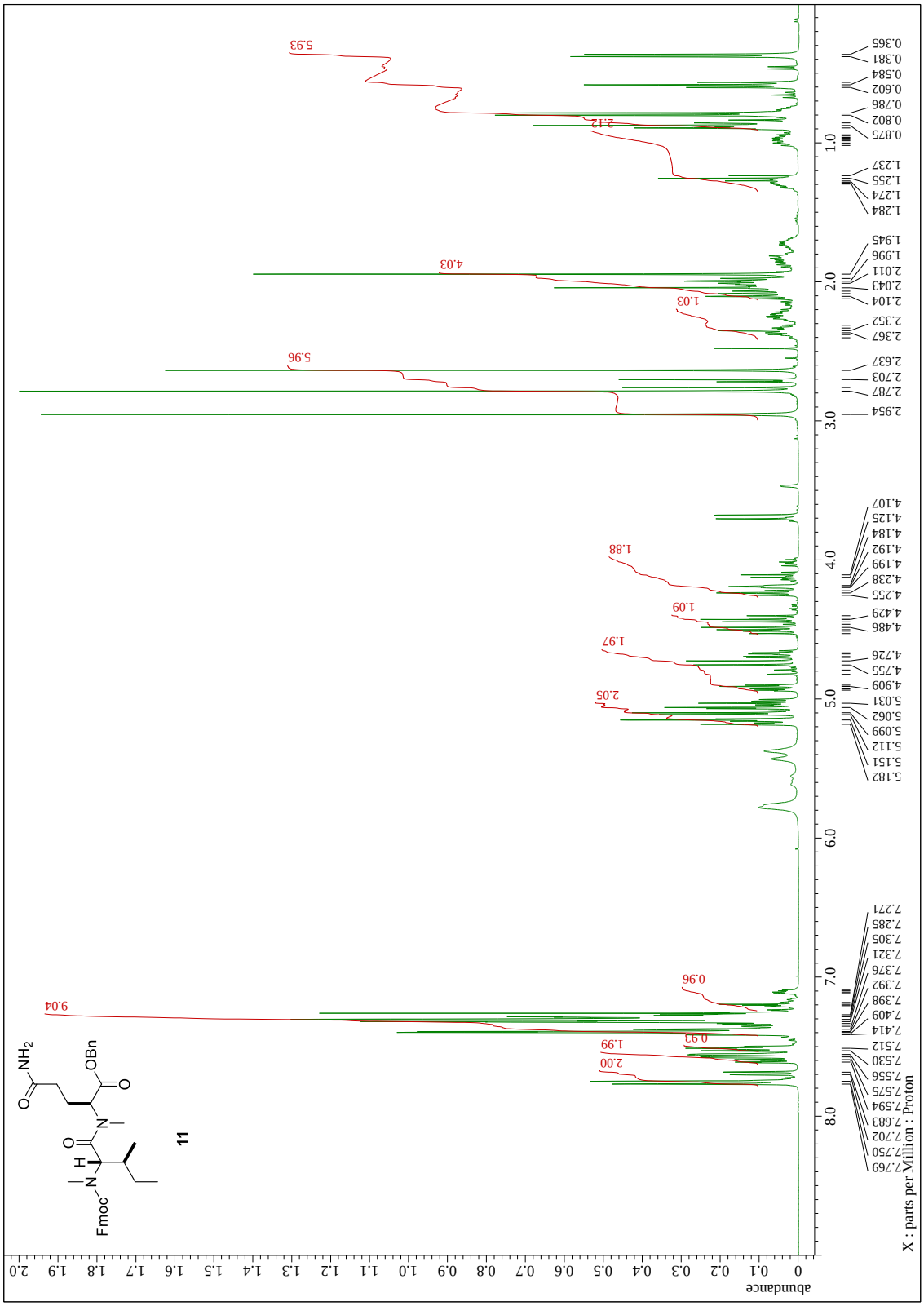
^{1}H NMR (400 MHz in d_6-DMSO) spectrum of **10**



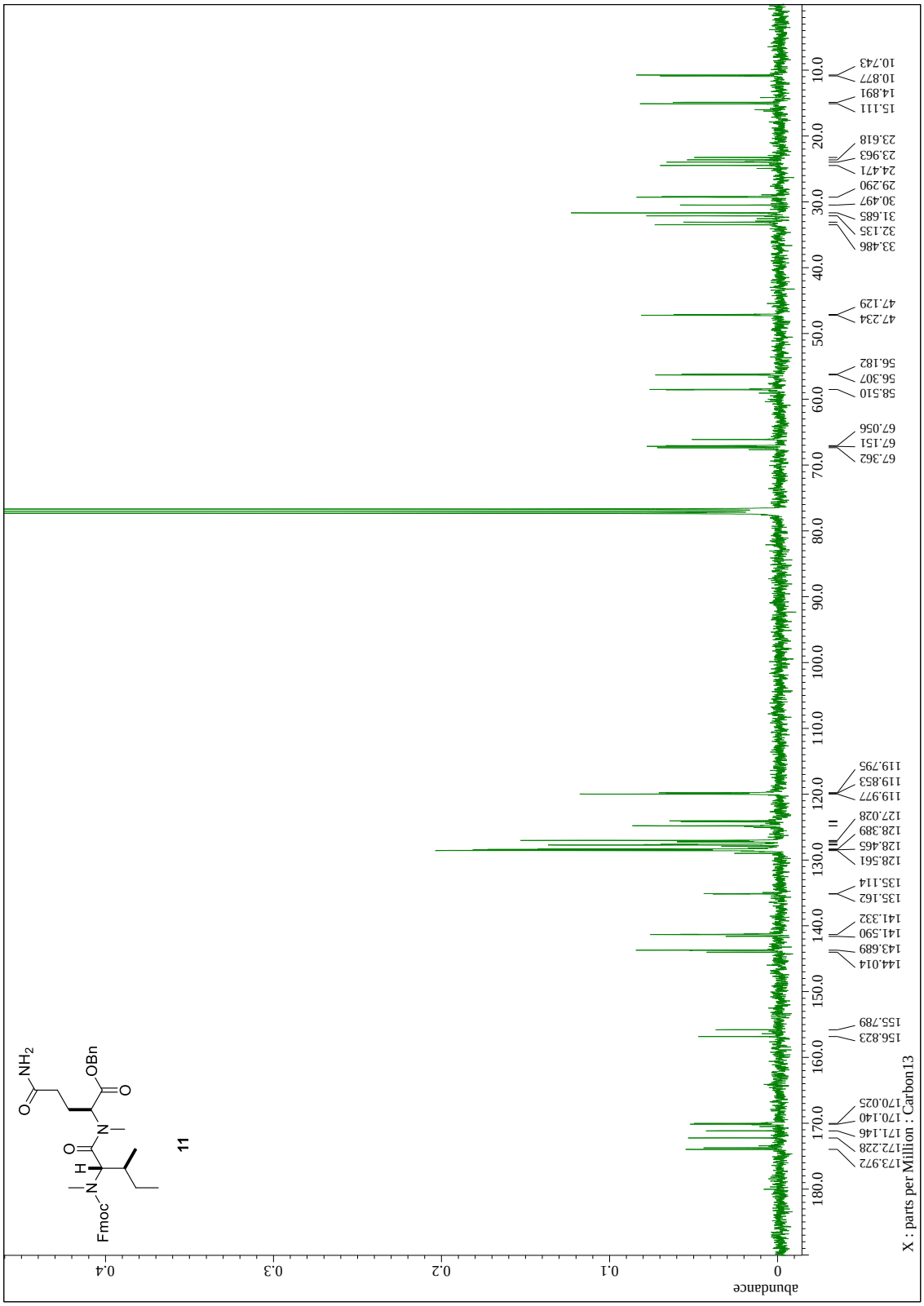
$^{13}C\{^{1}H\}$ NMR (100 MHz in d_6-DMSO) spectrum of **10**



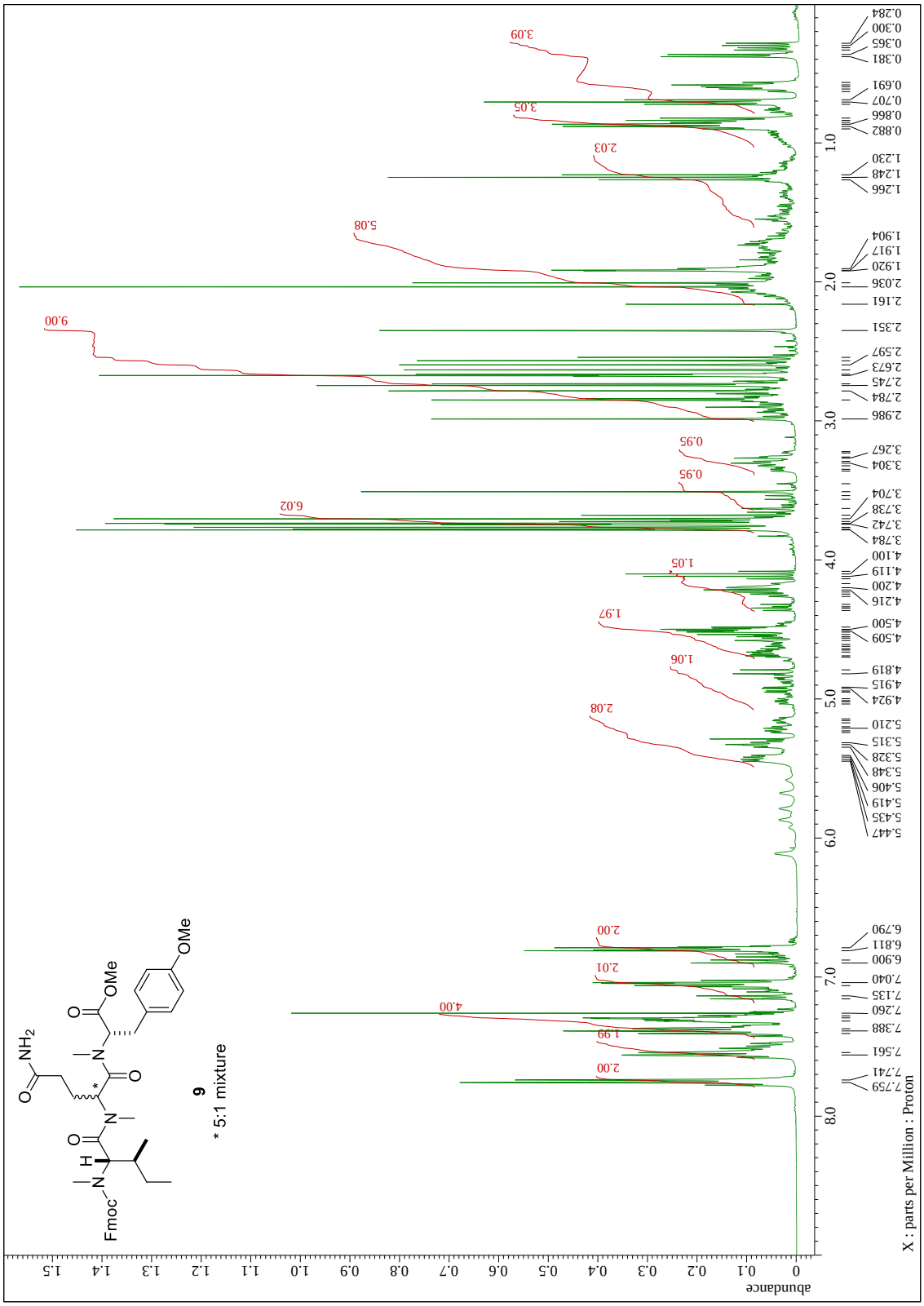
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **11**



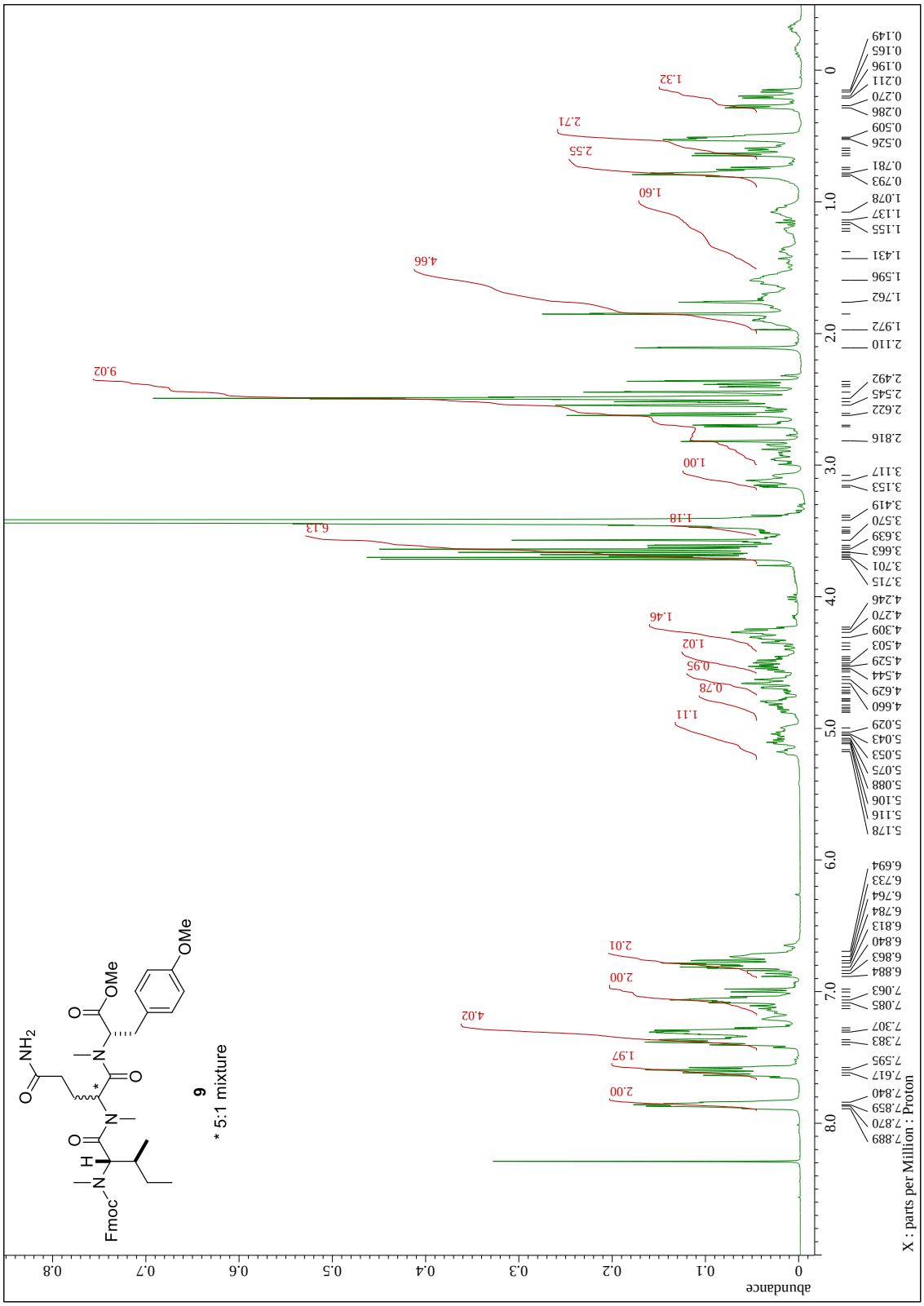
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **11**



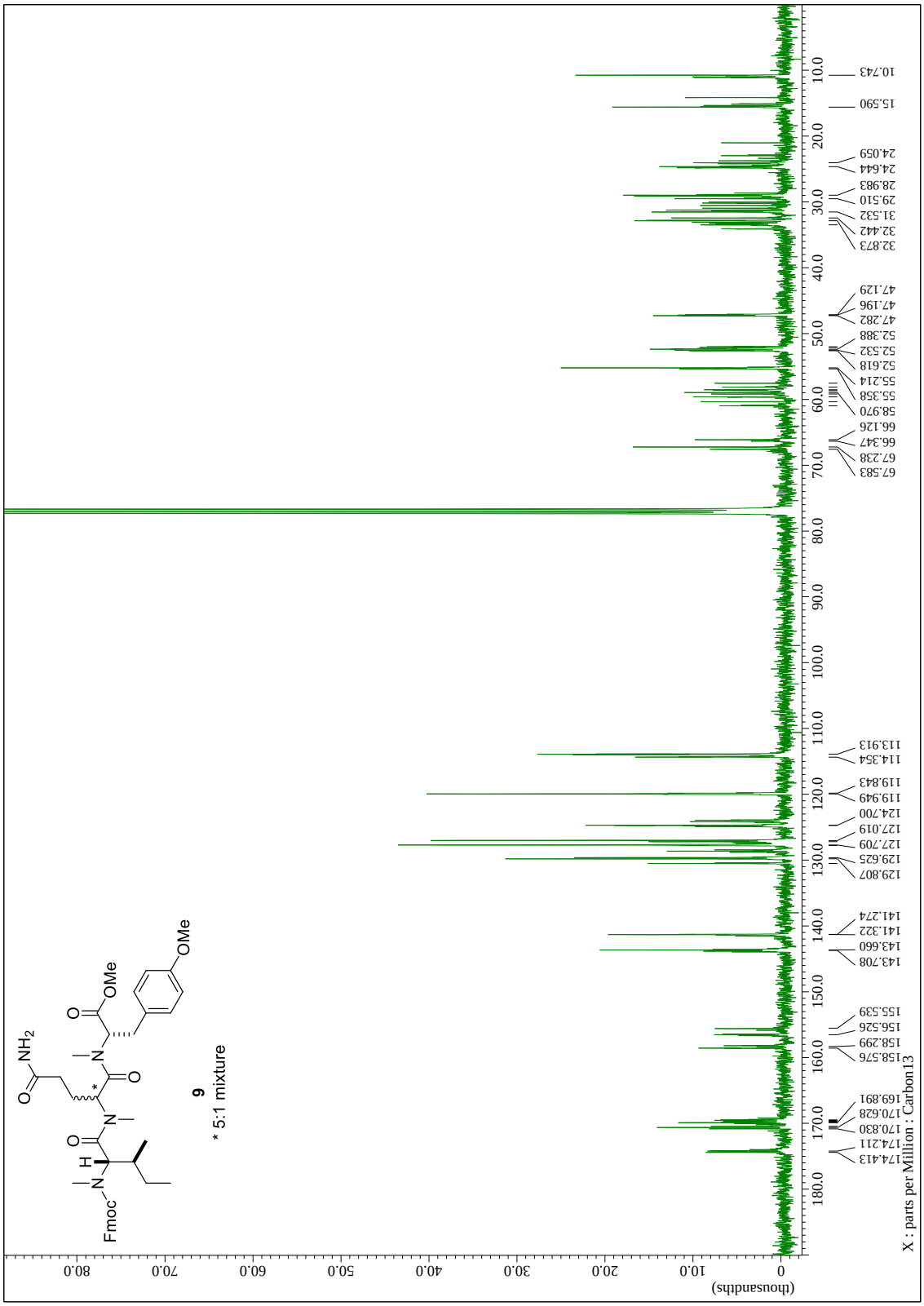
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **9**



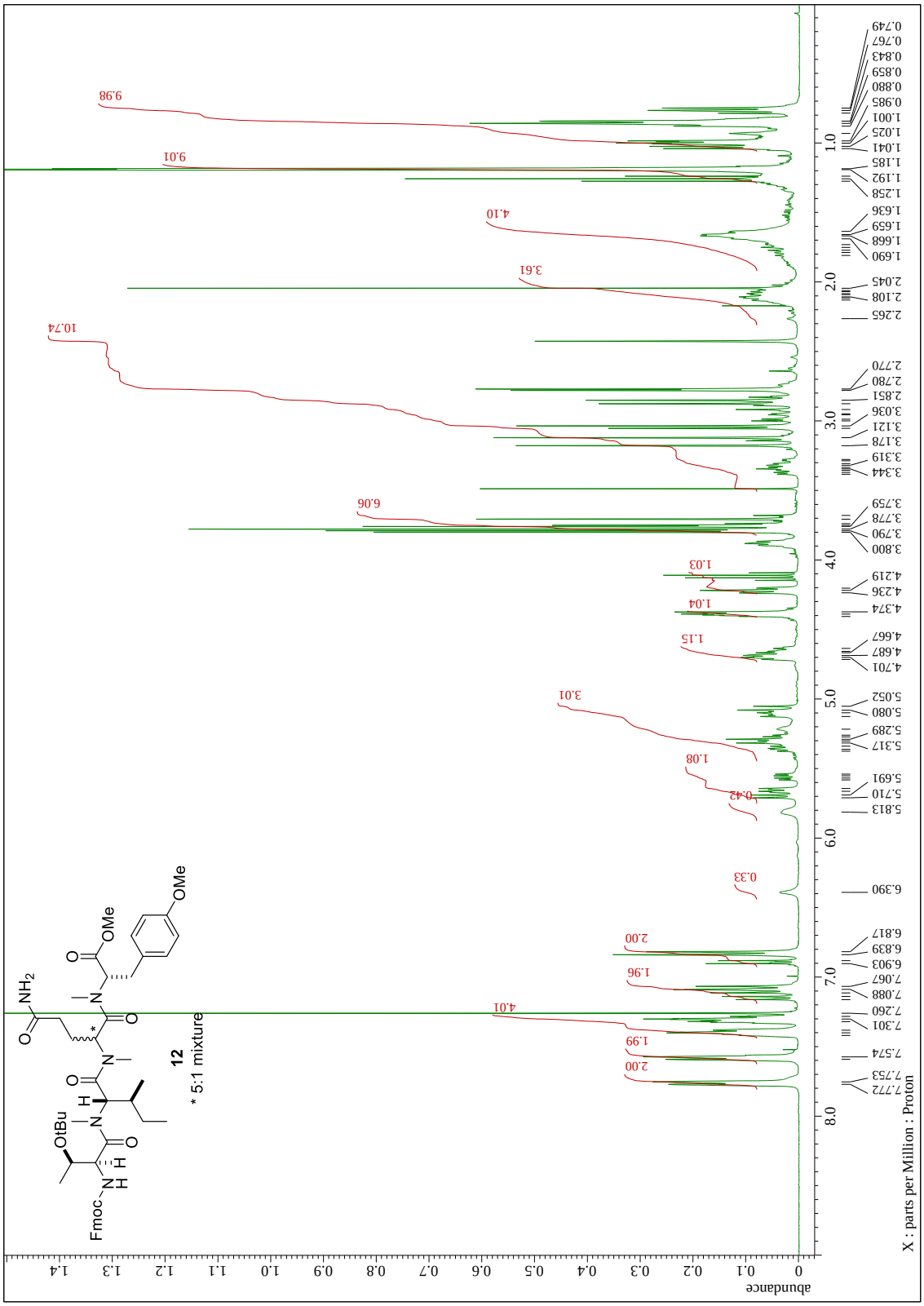
^{1}H NMR (400 MHz in d_6-DMSO) spectrum of **9**



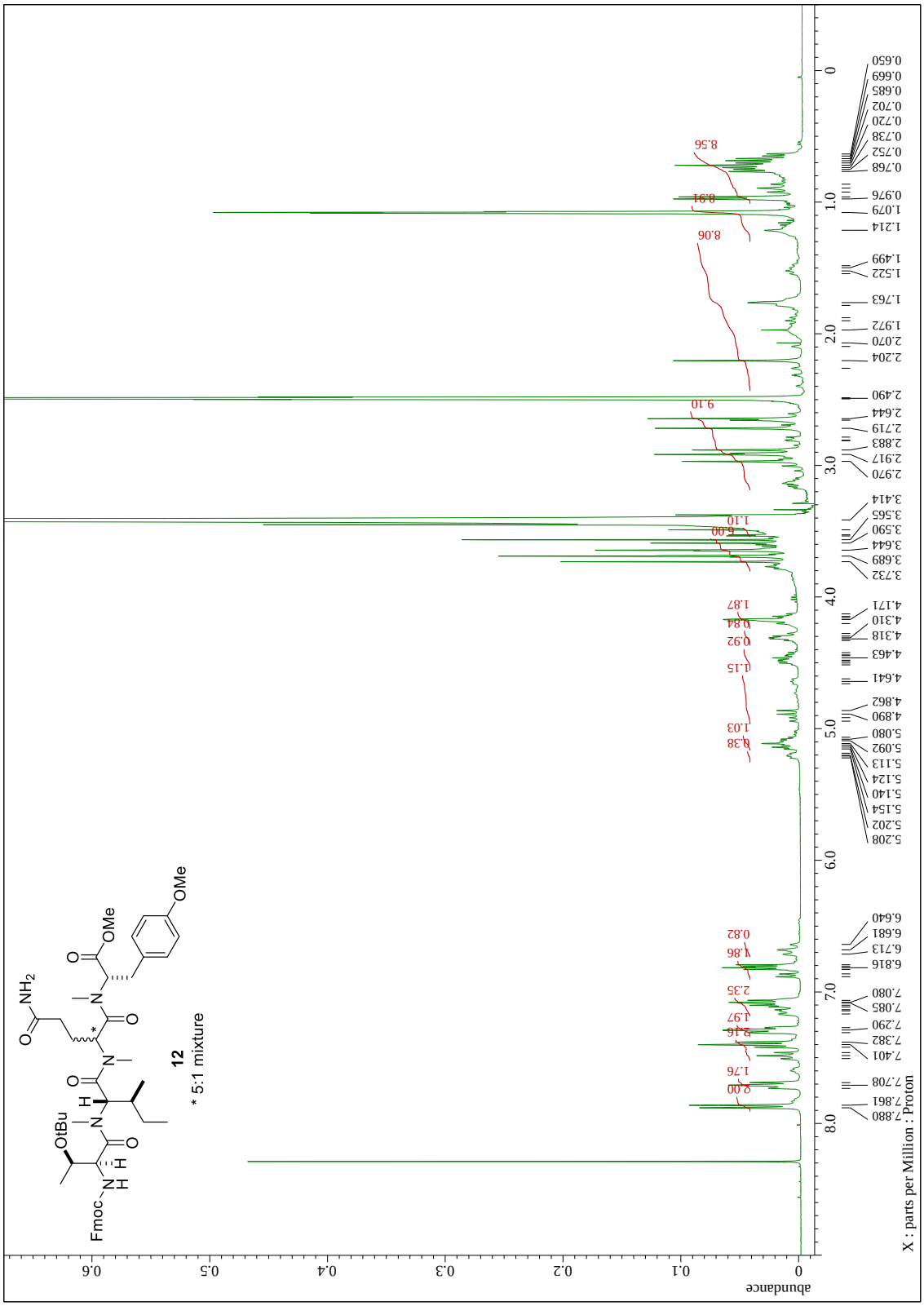
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **9**



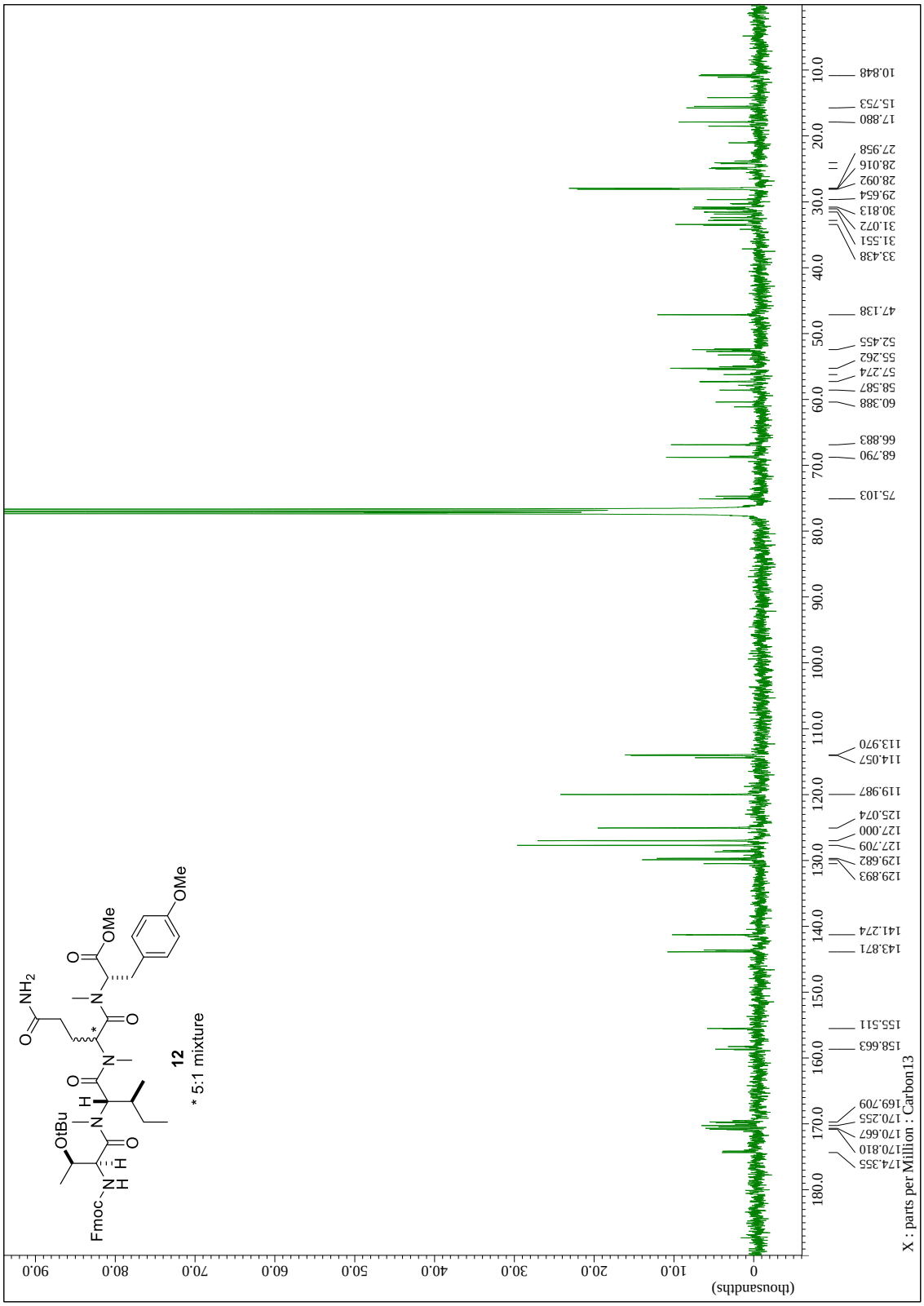
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **12**



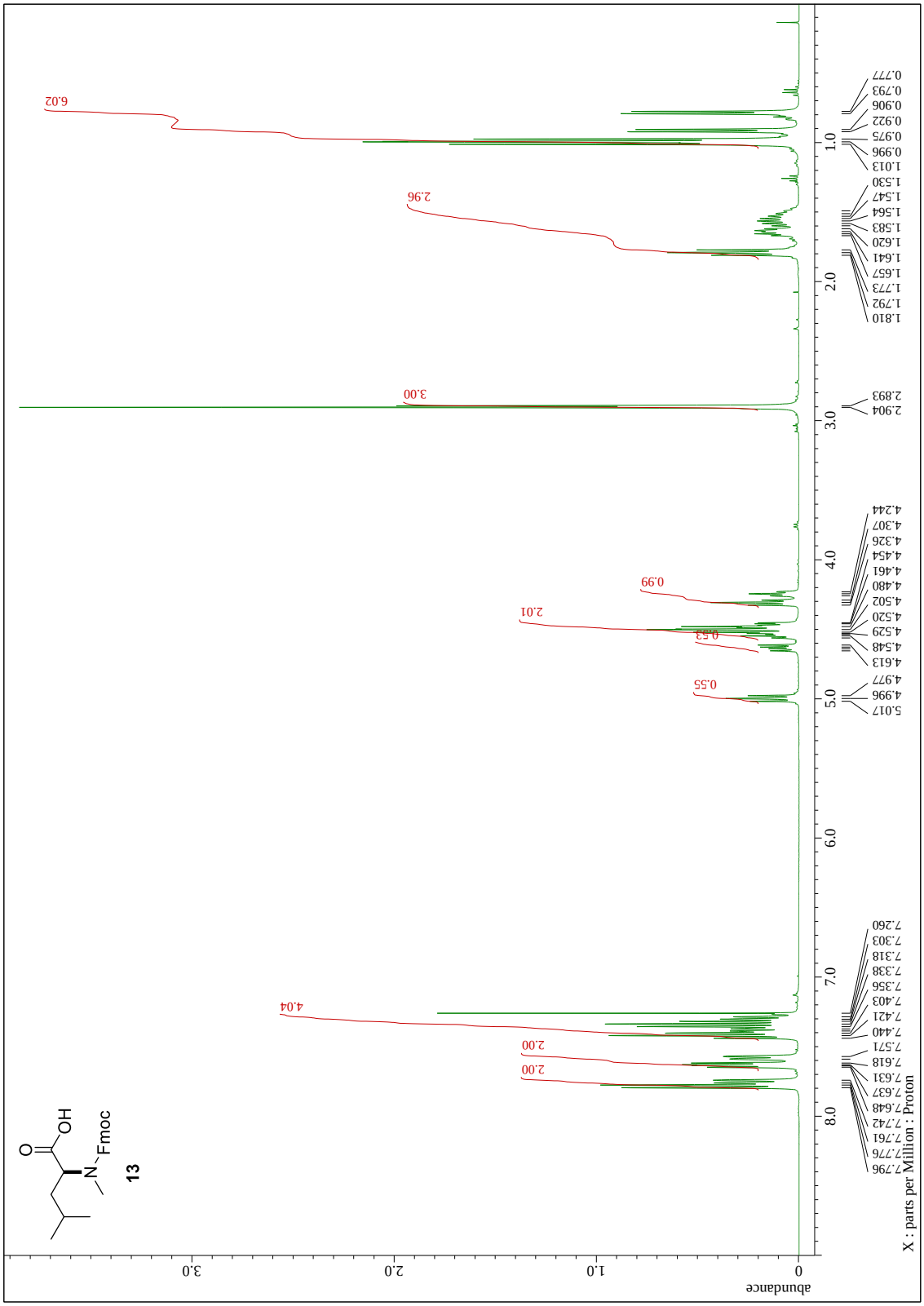
^{1}H NMR (400 MHz in d_6-DMSO) spectrum of **12**



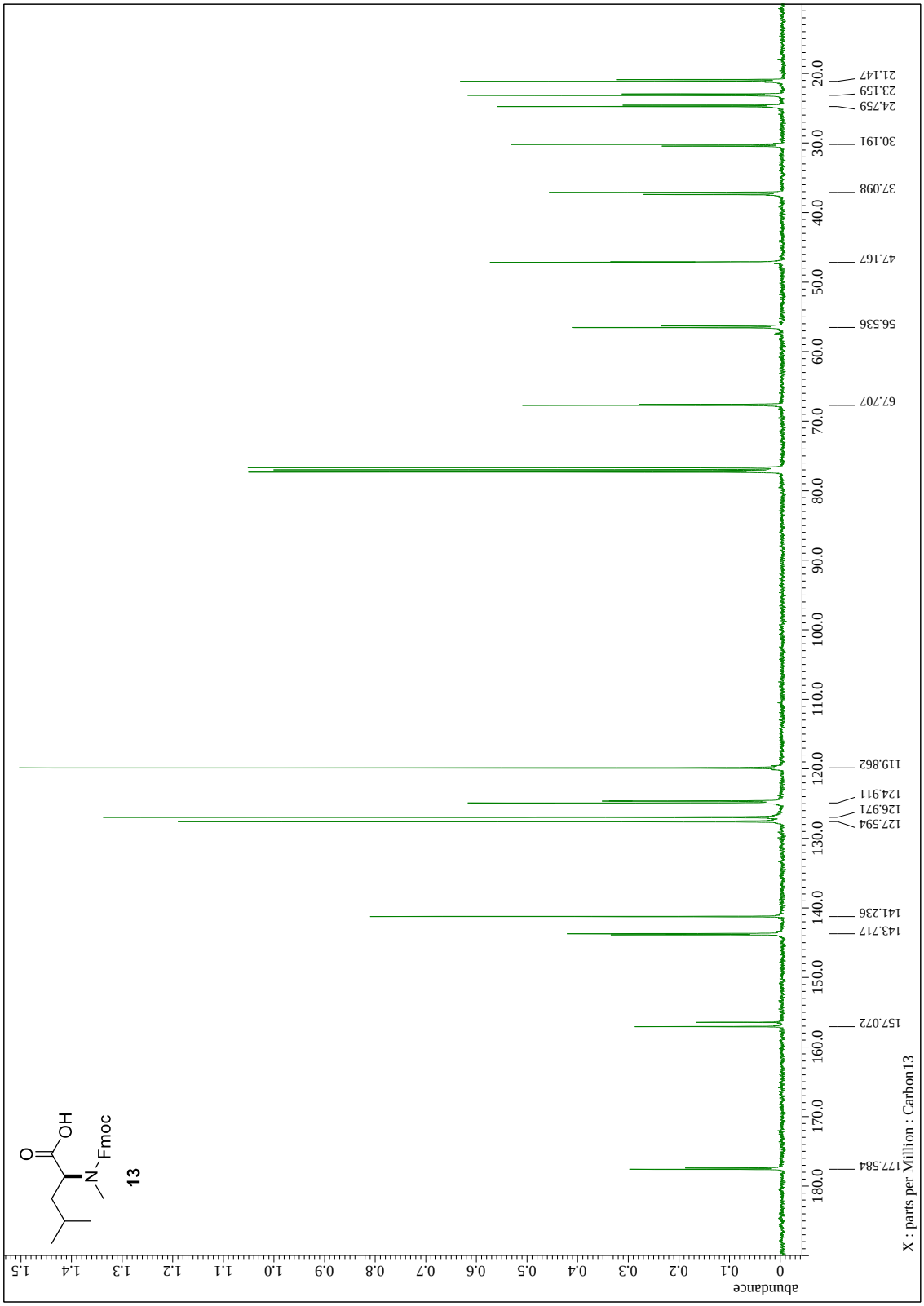
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **12**



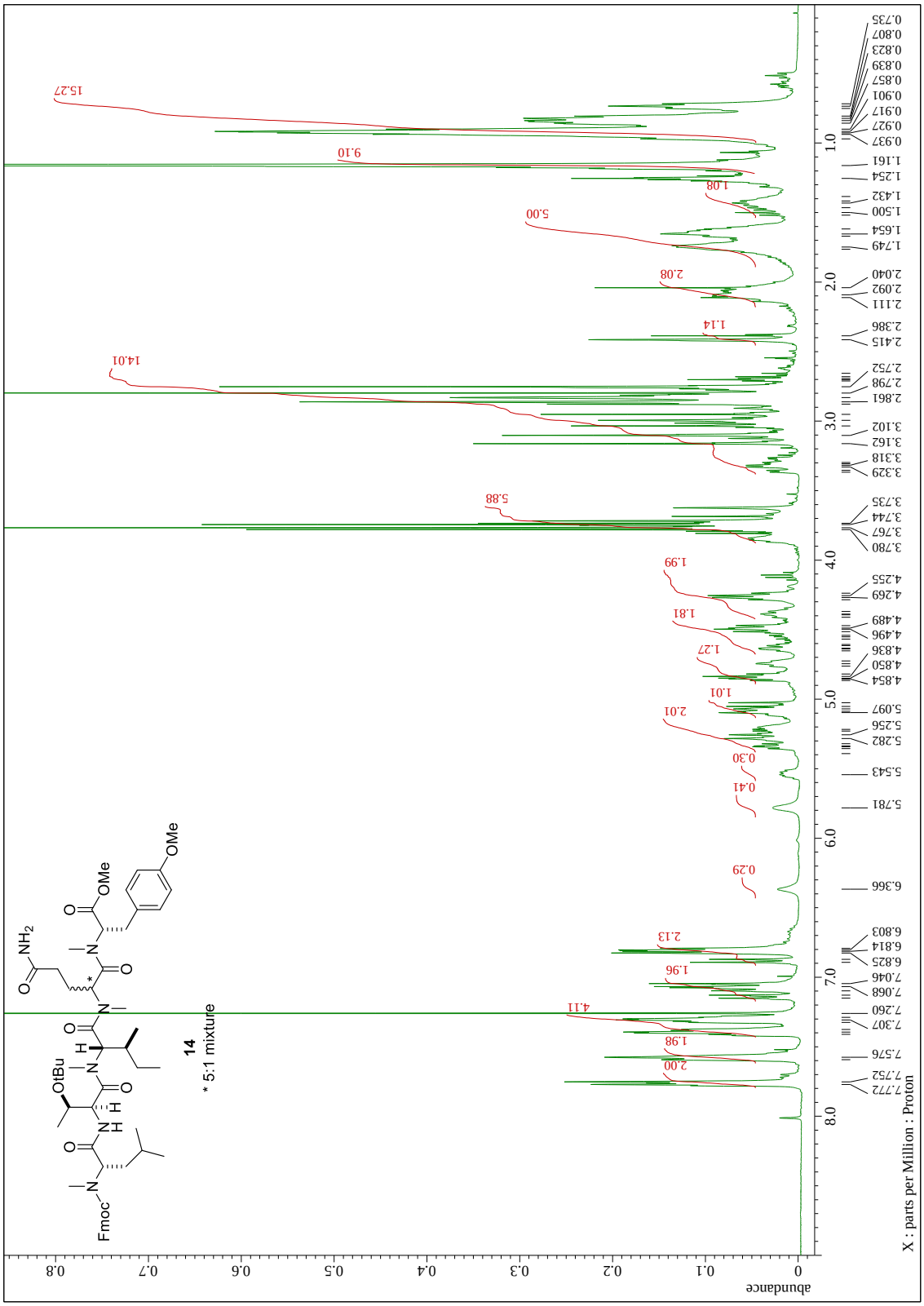
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **13**



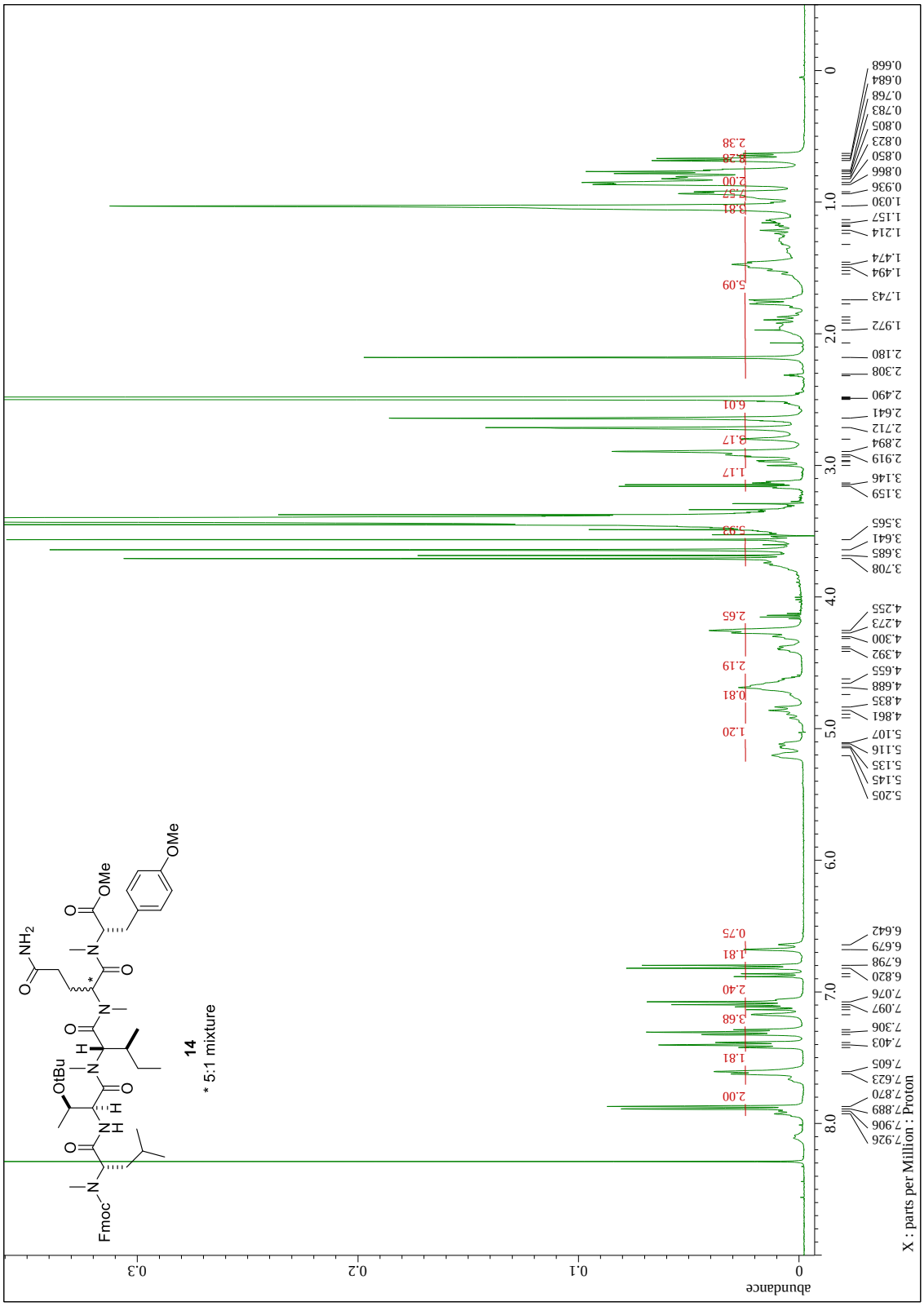
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **13**



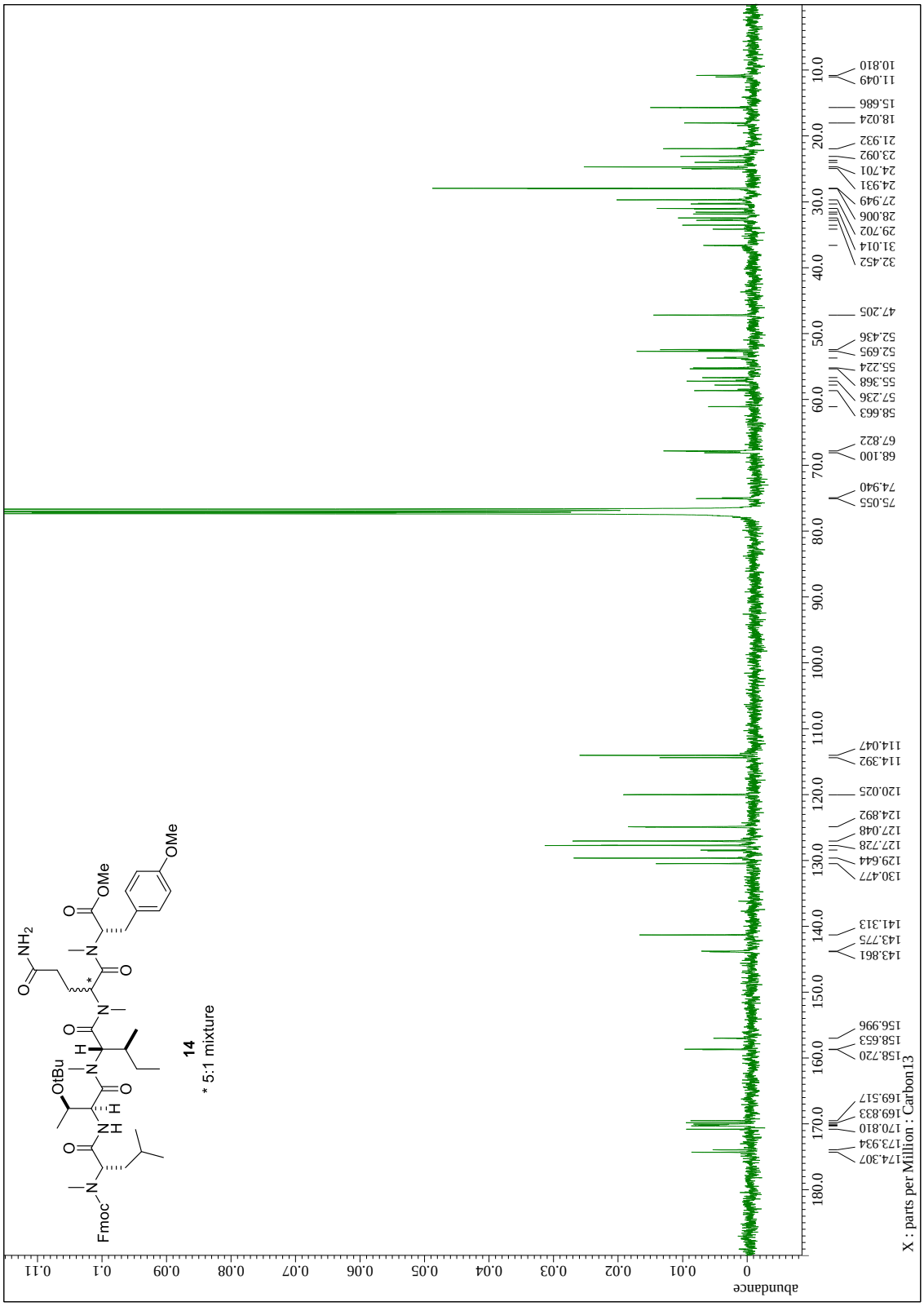
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **14**



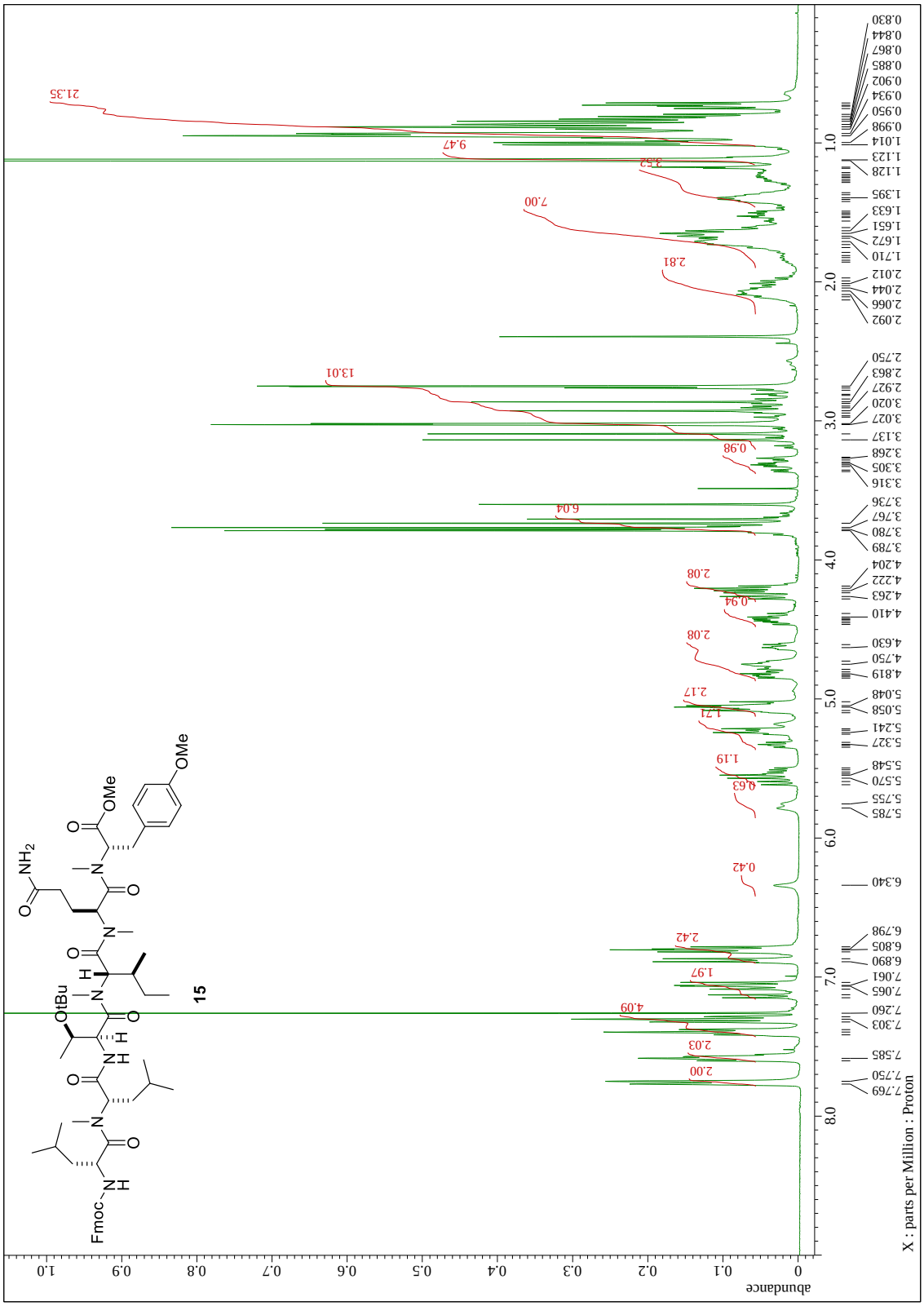
^{1}H NMR (400 MHz in d_6-DMSO) spectrum of **14**



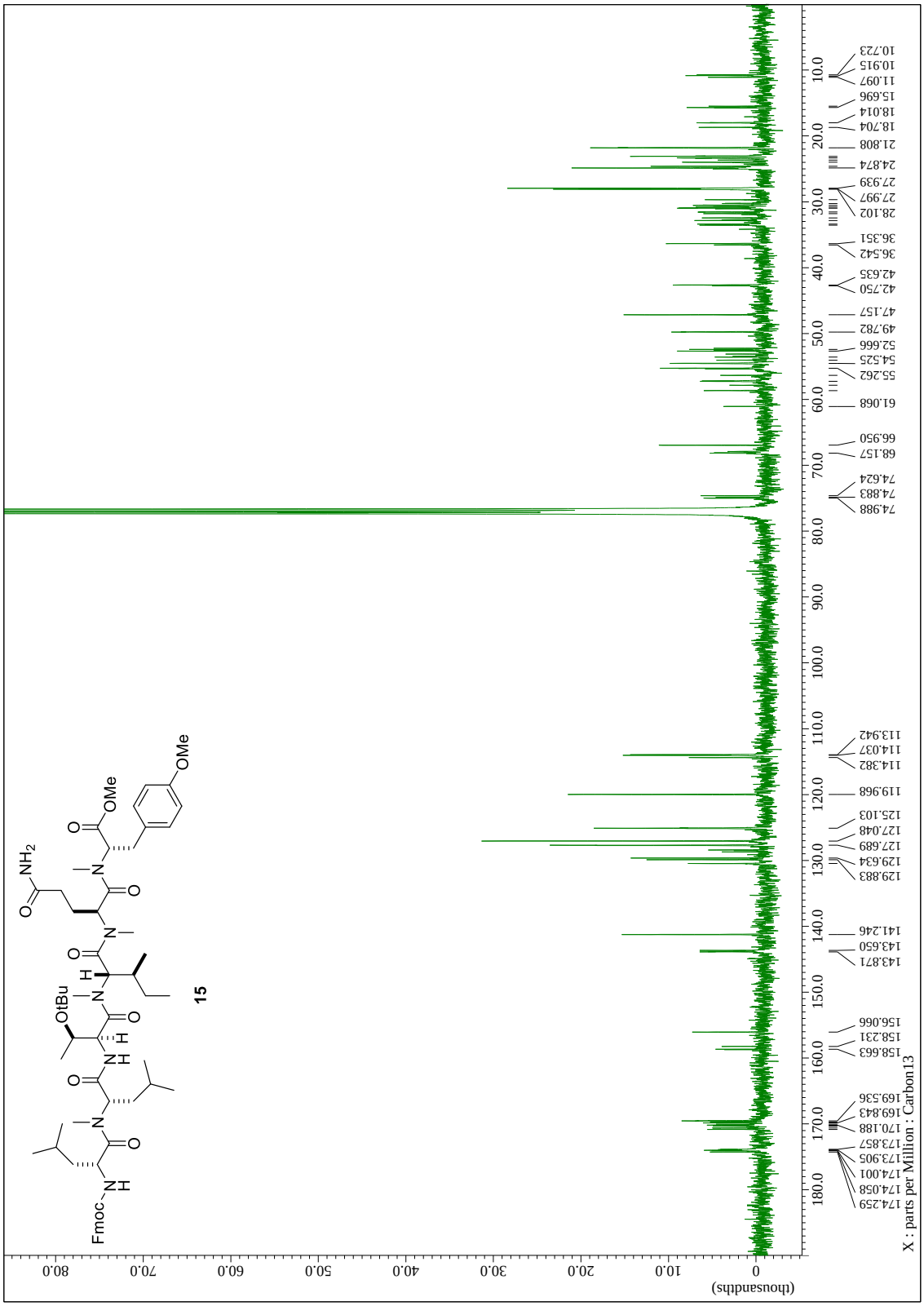
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **14**



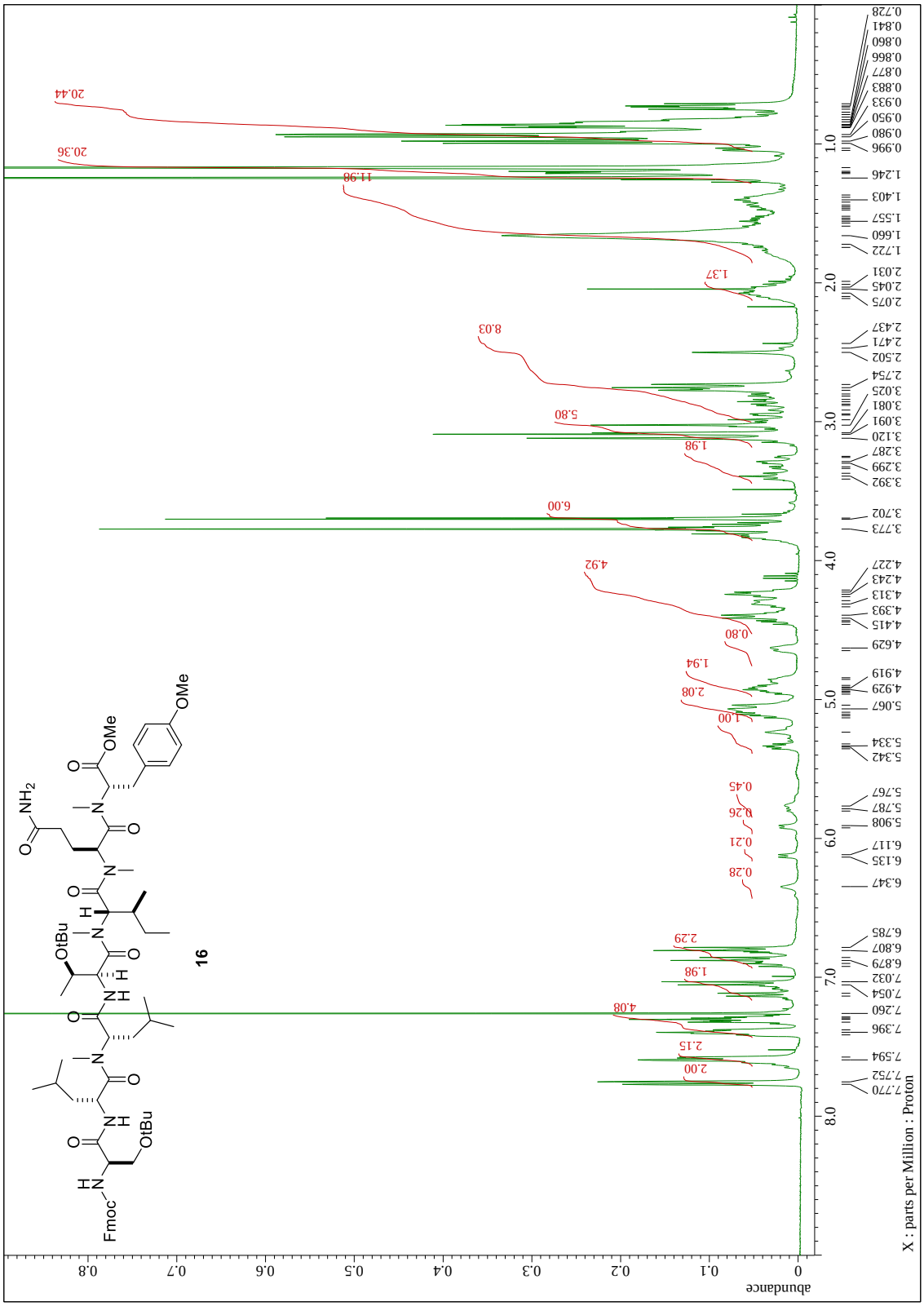
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **15**



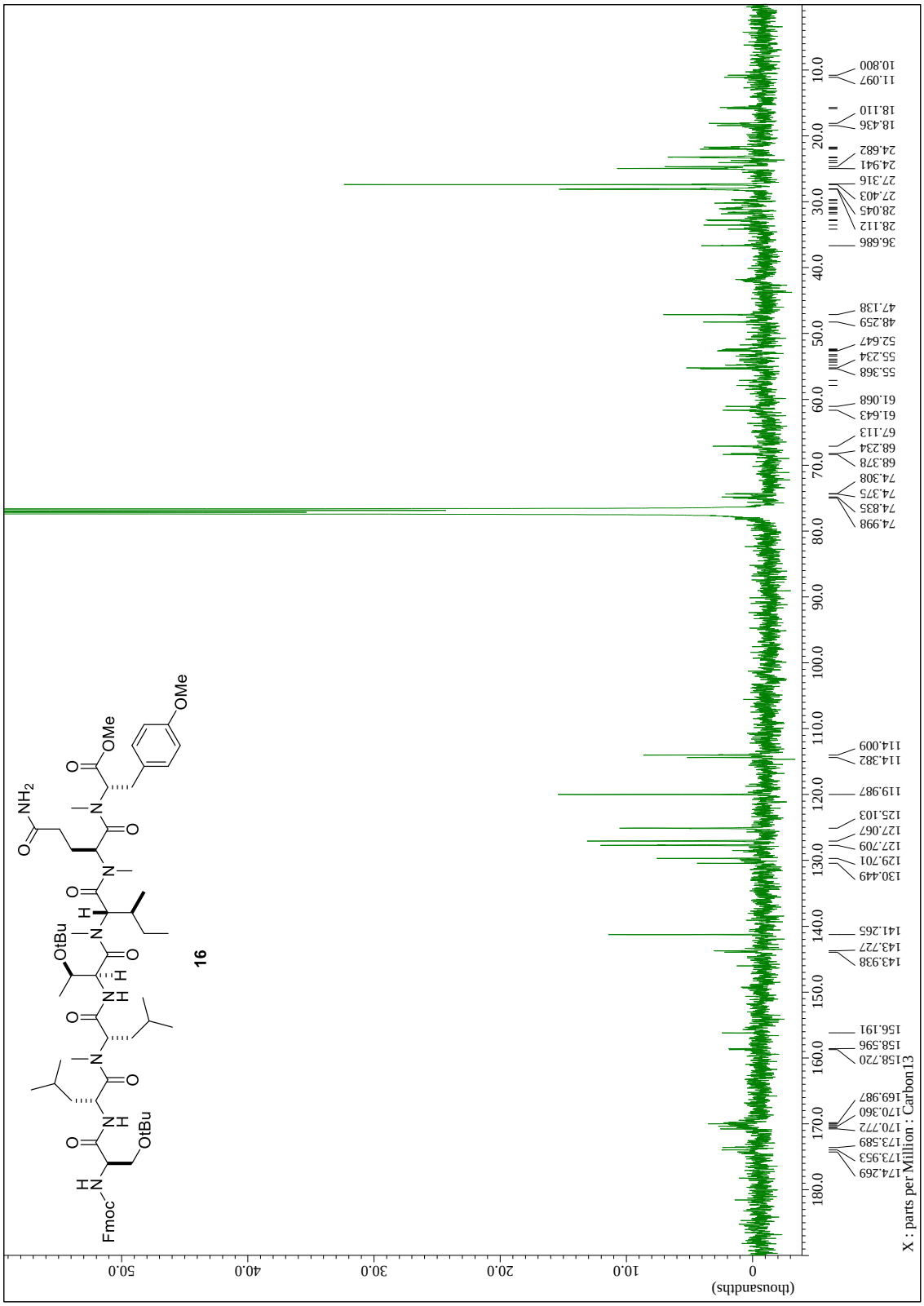
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **15**



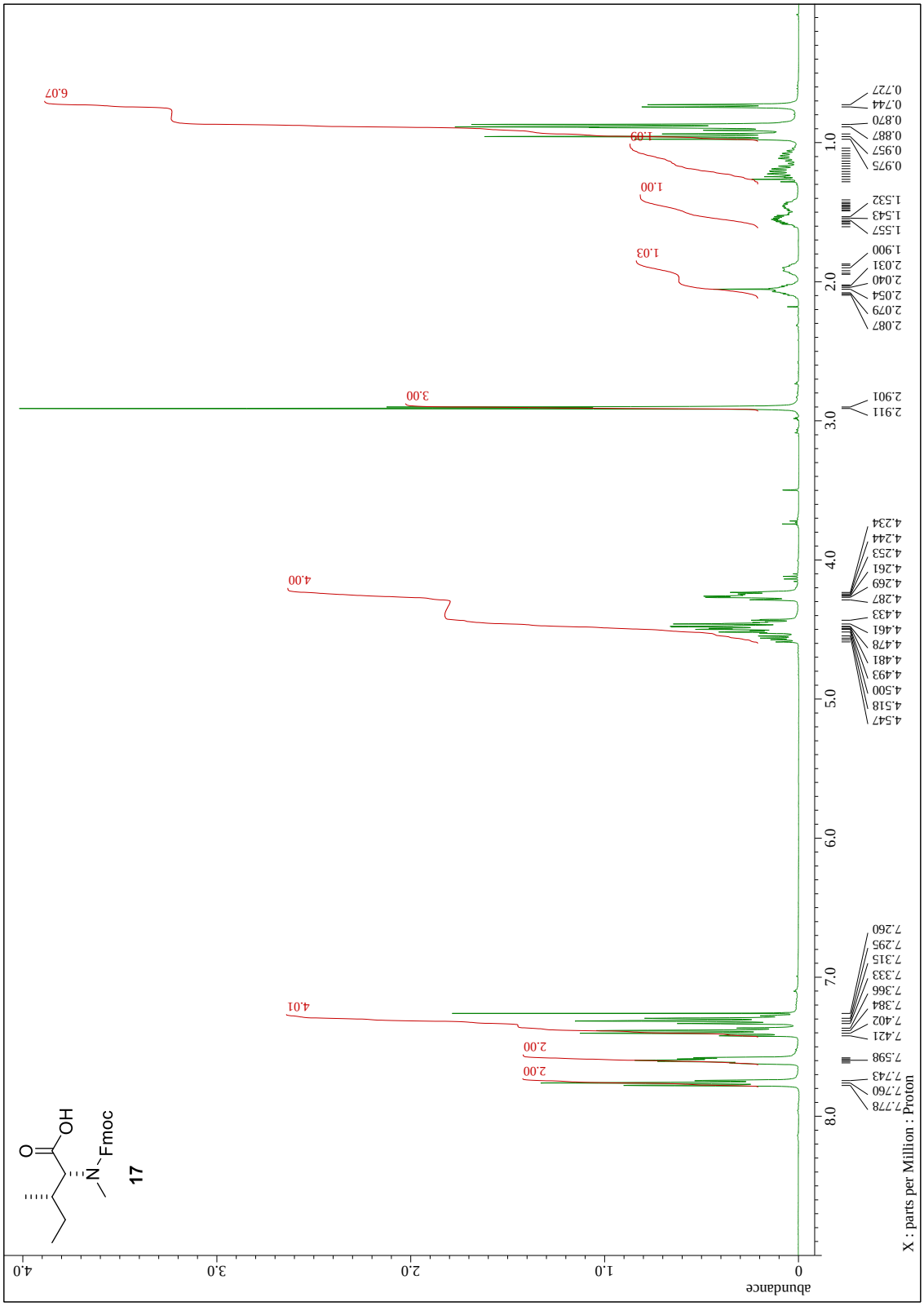
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **16**



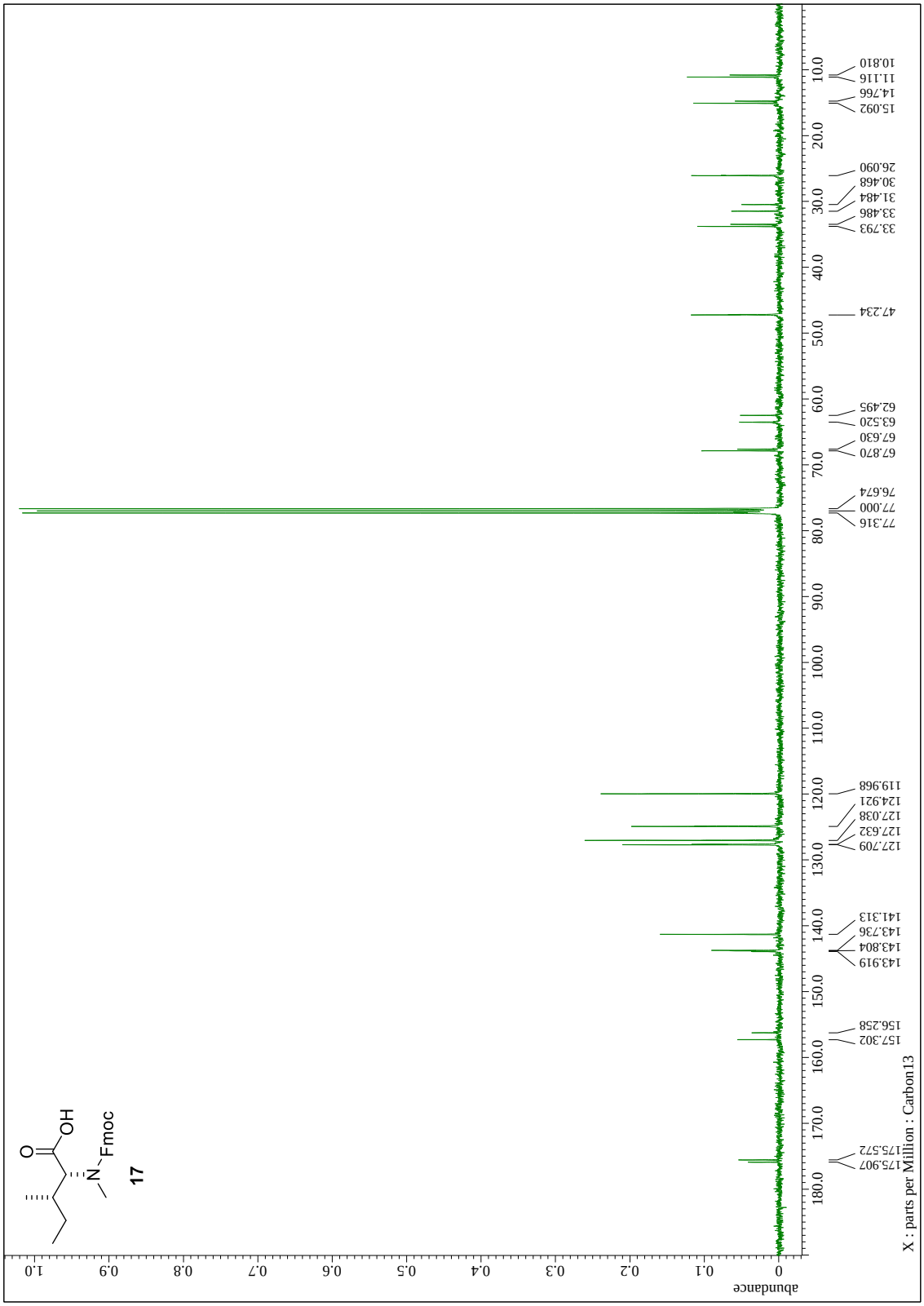
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **16**



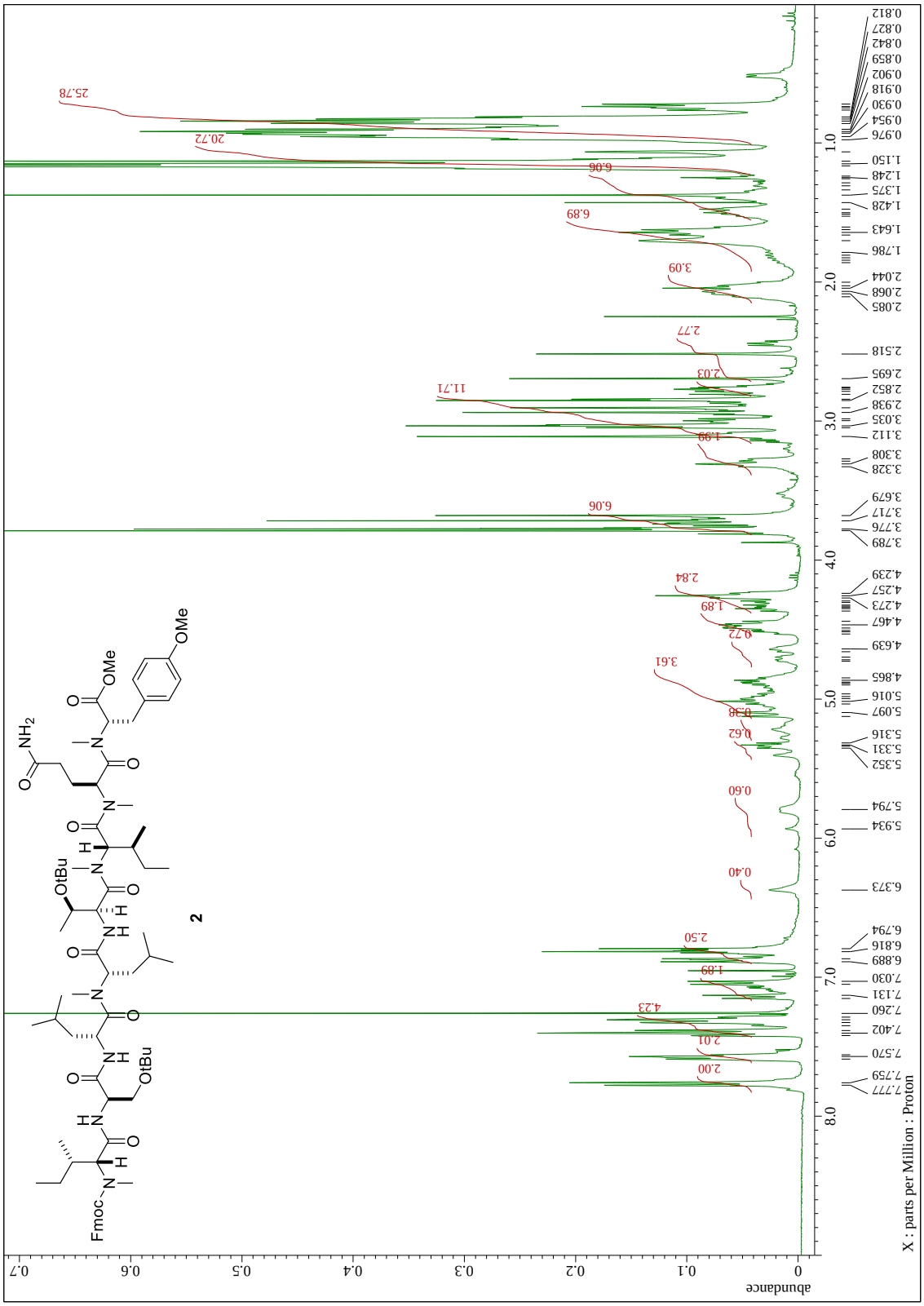
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **17**



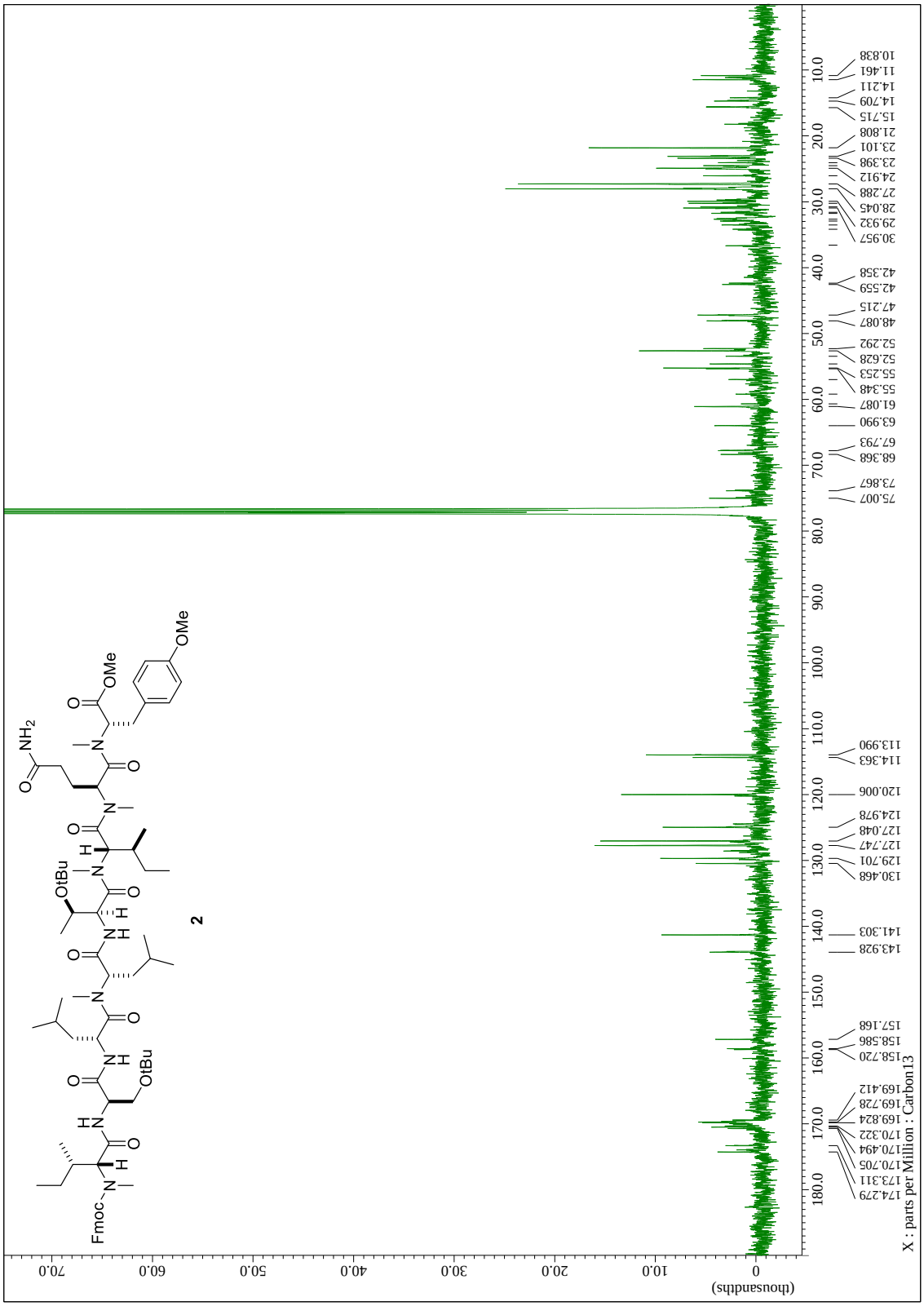
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **17**



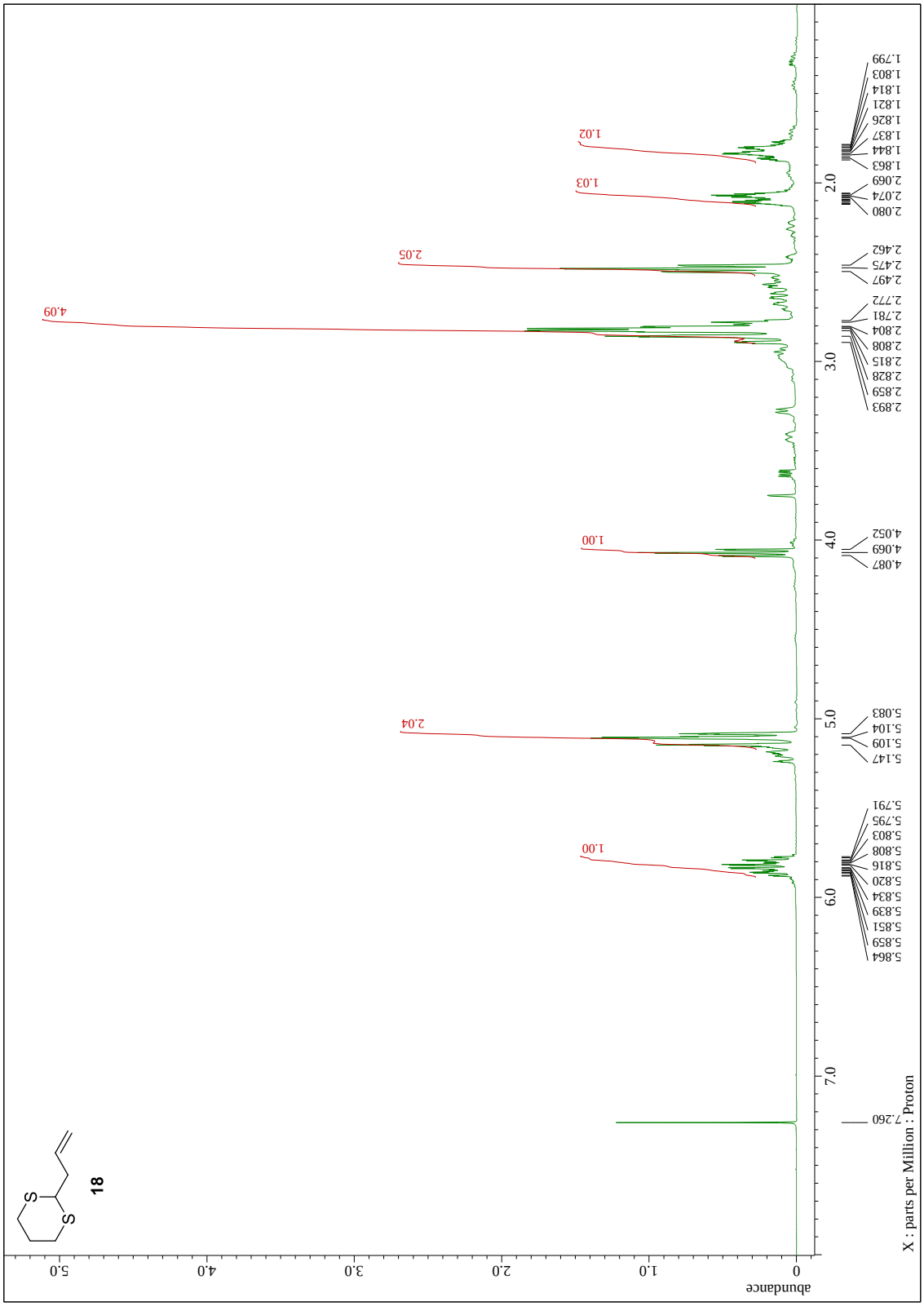
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **2**



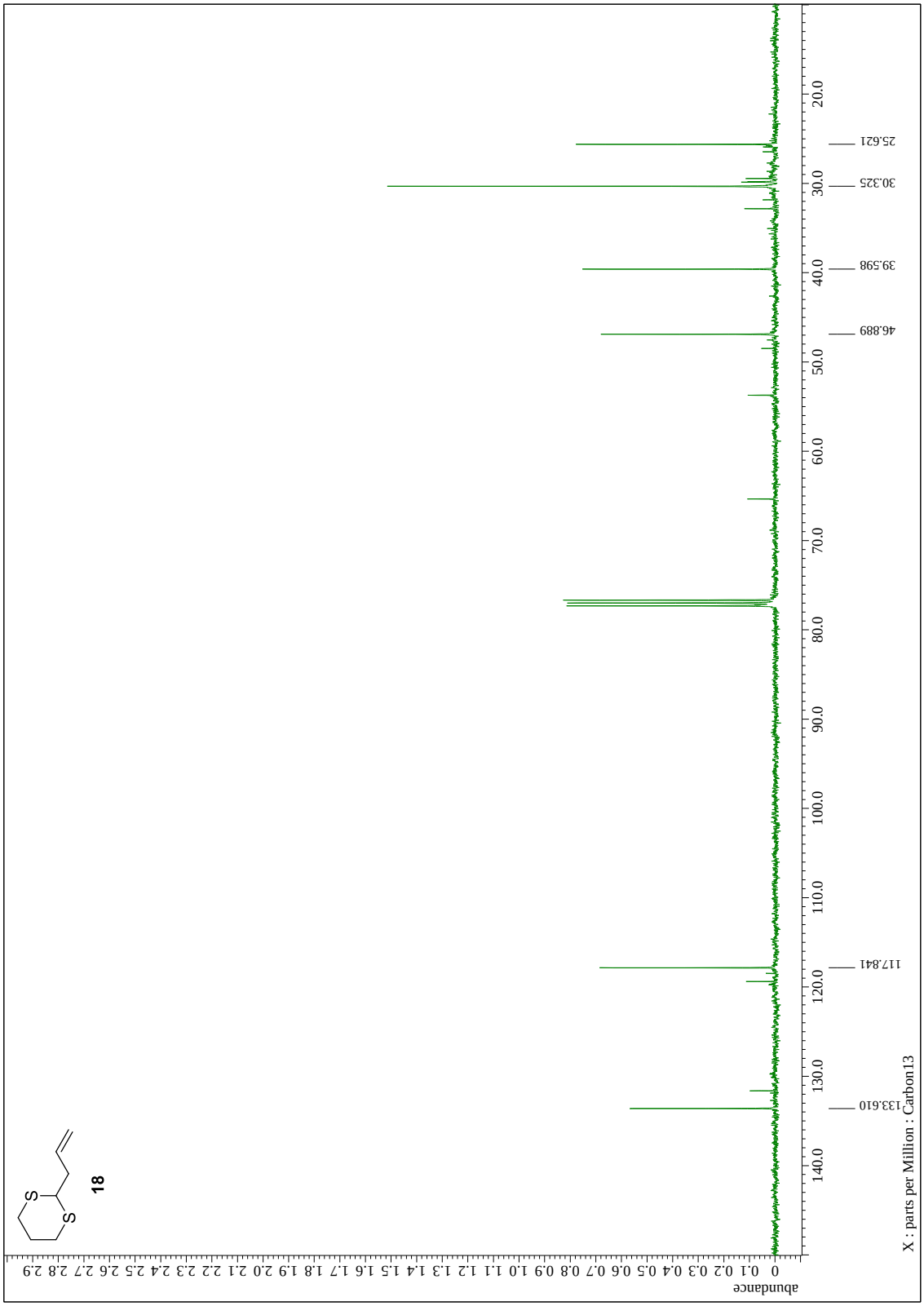
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **2**



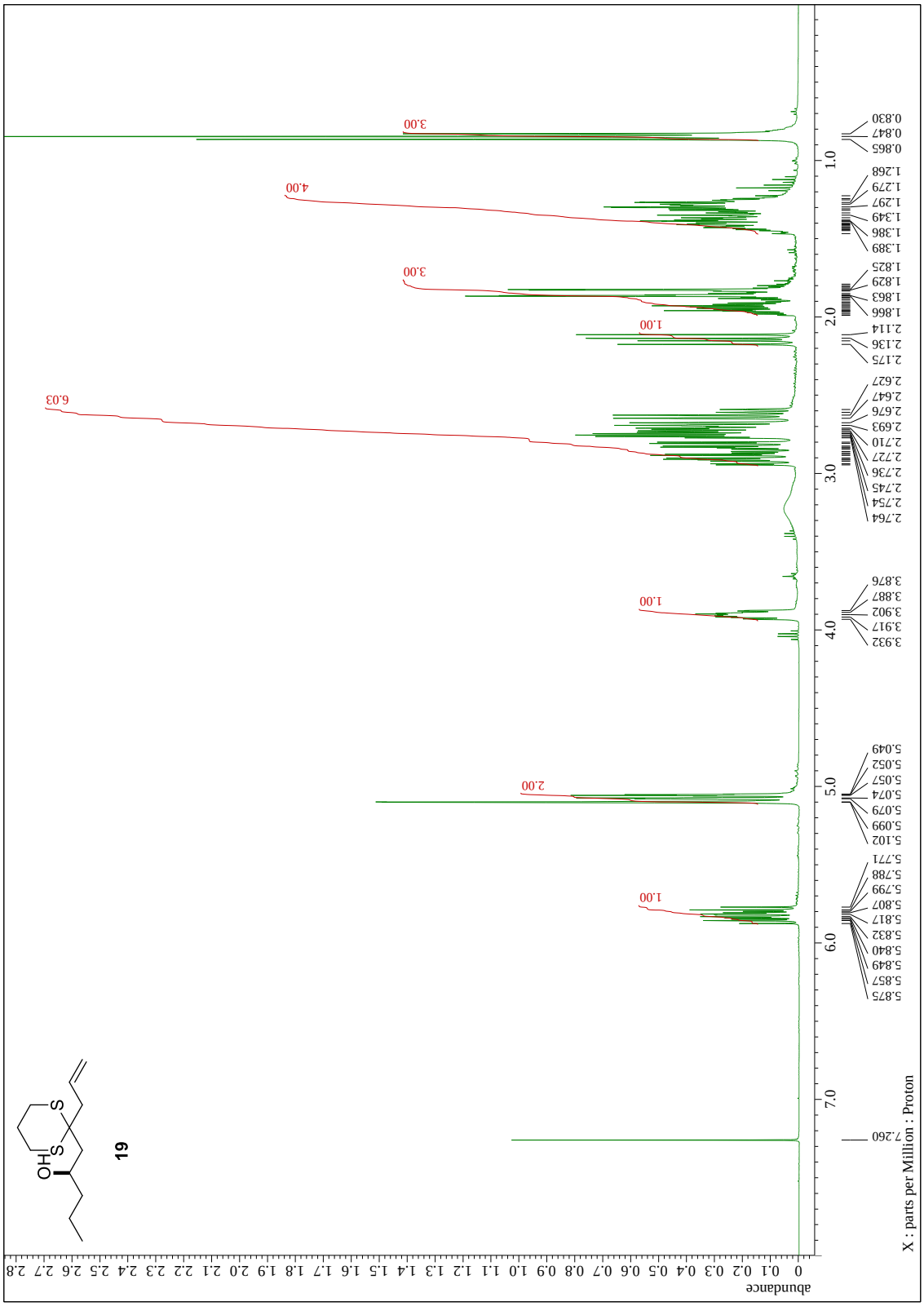
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **18**



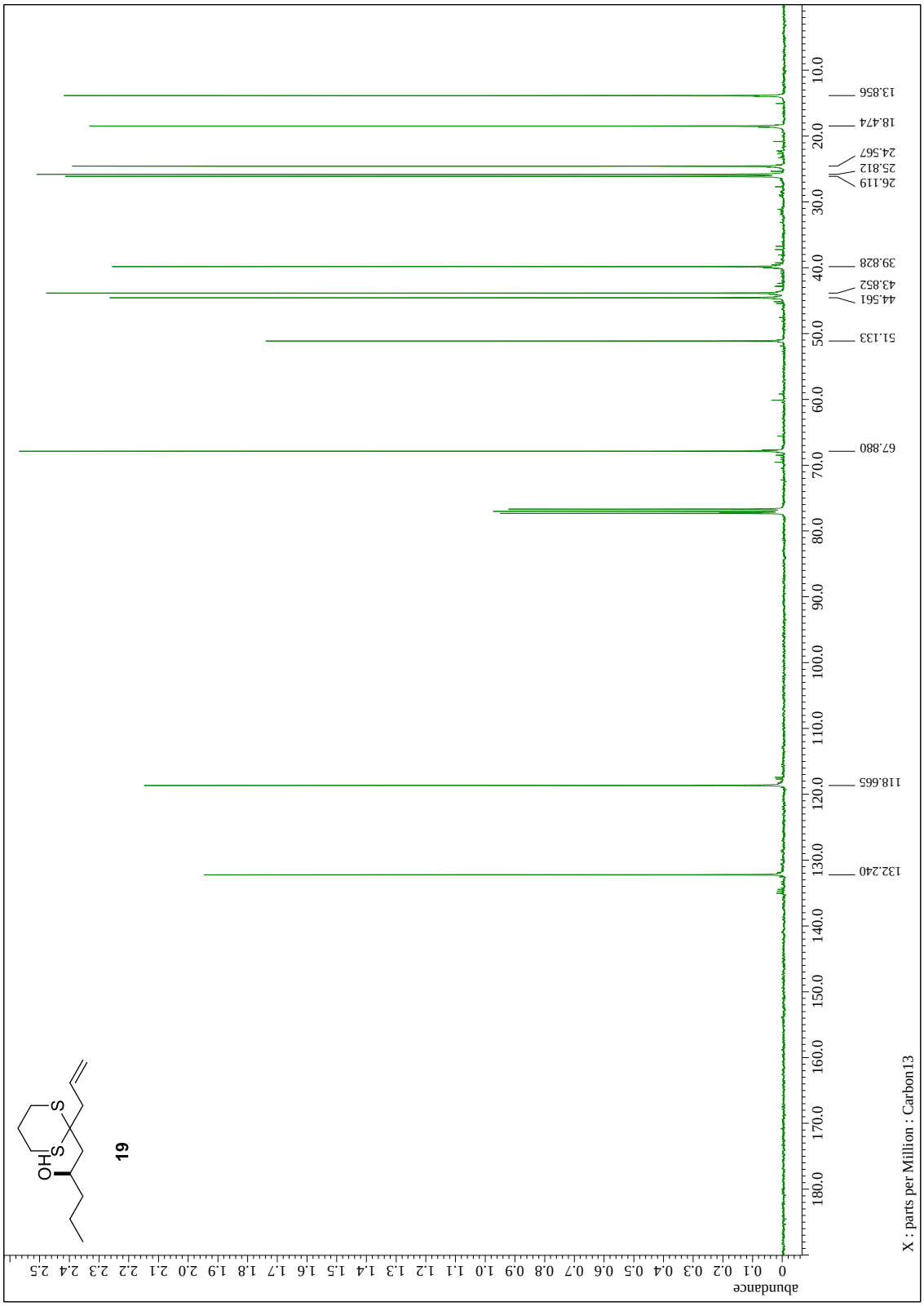
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **18**



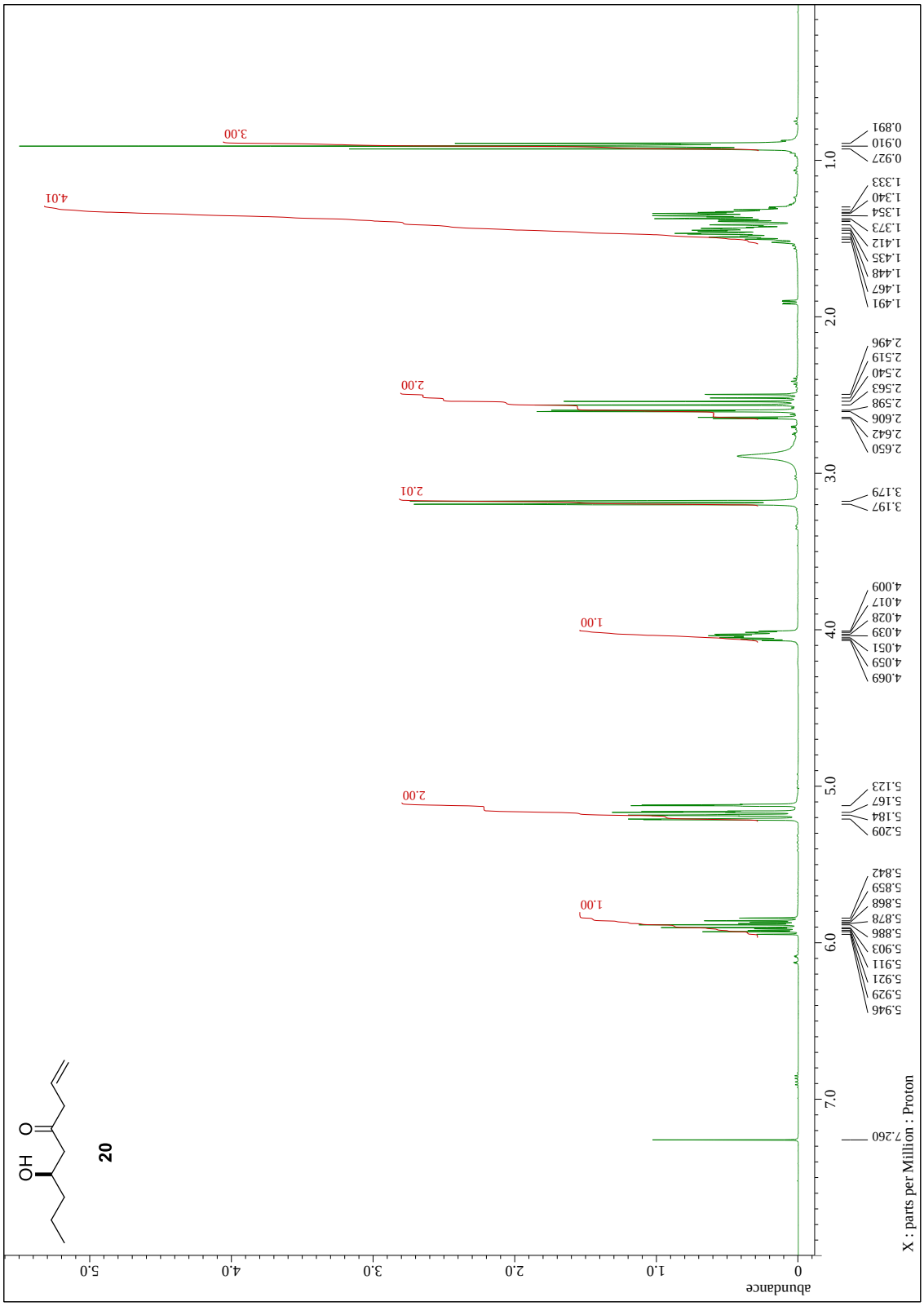
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **19**



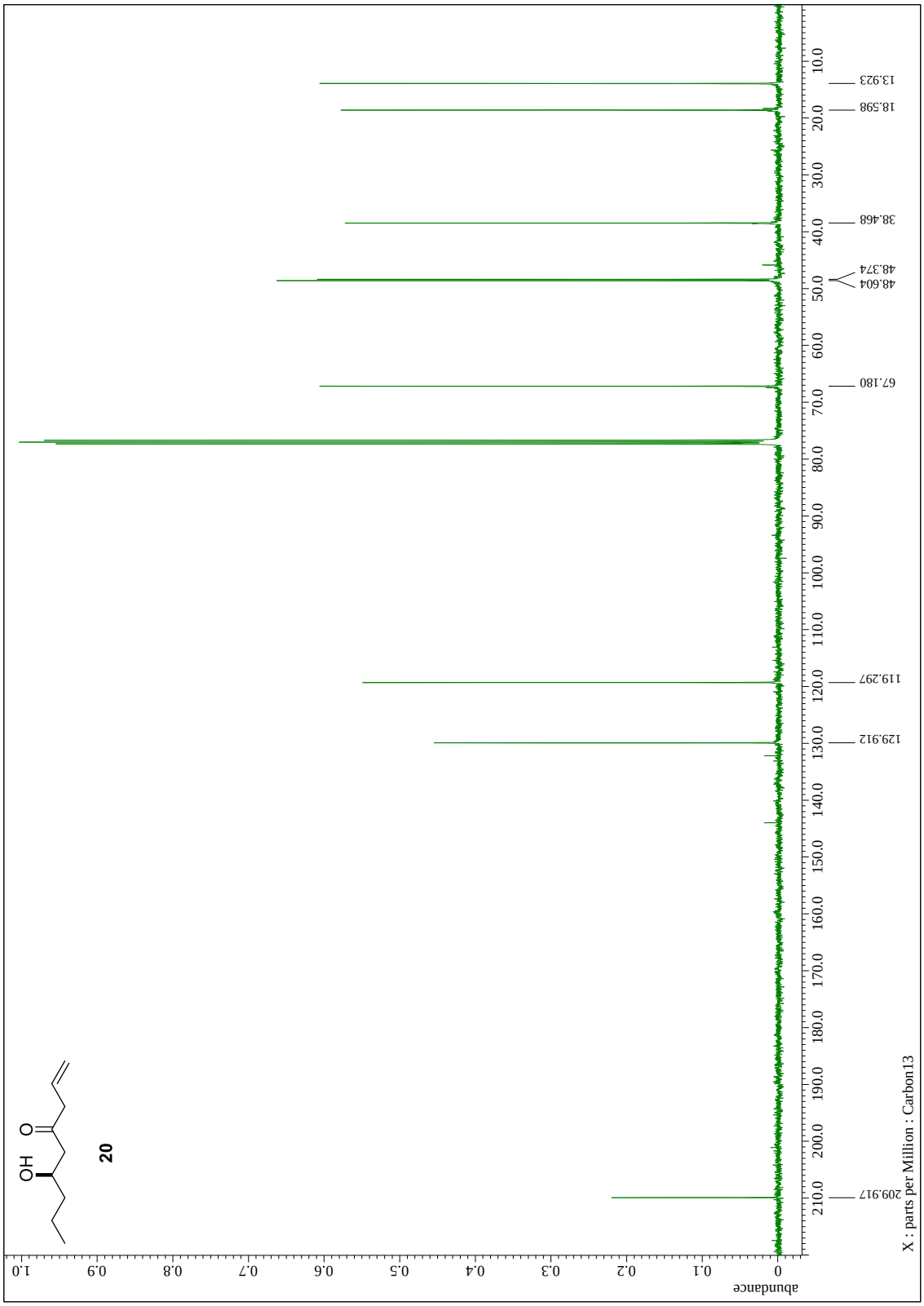
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **19**



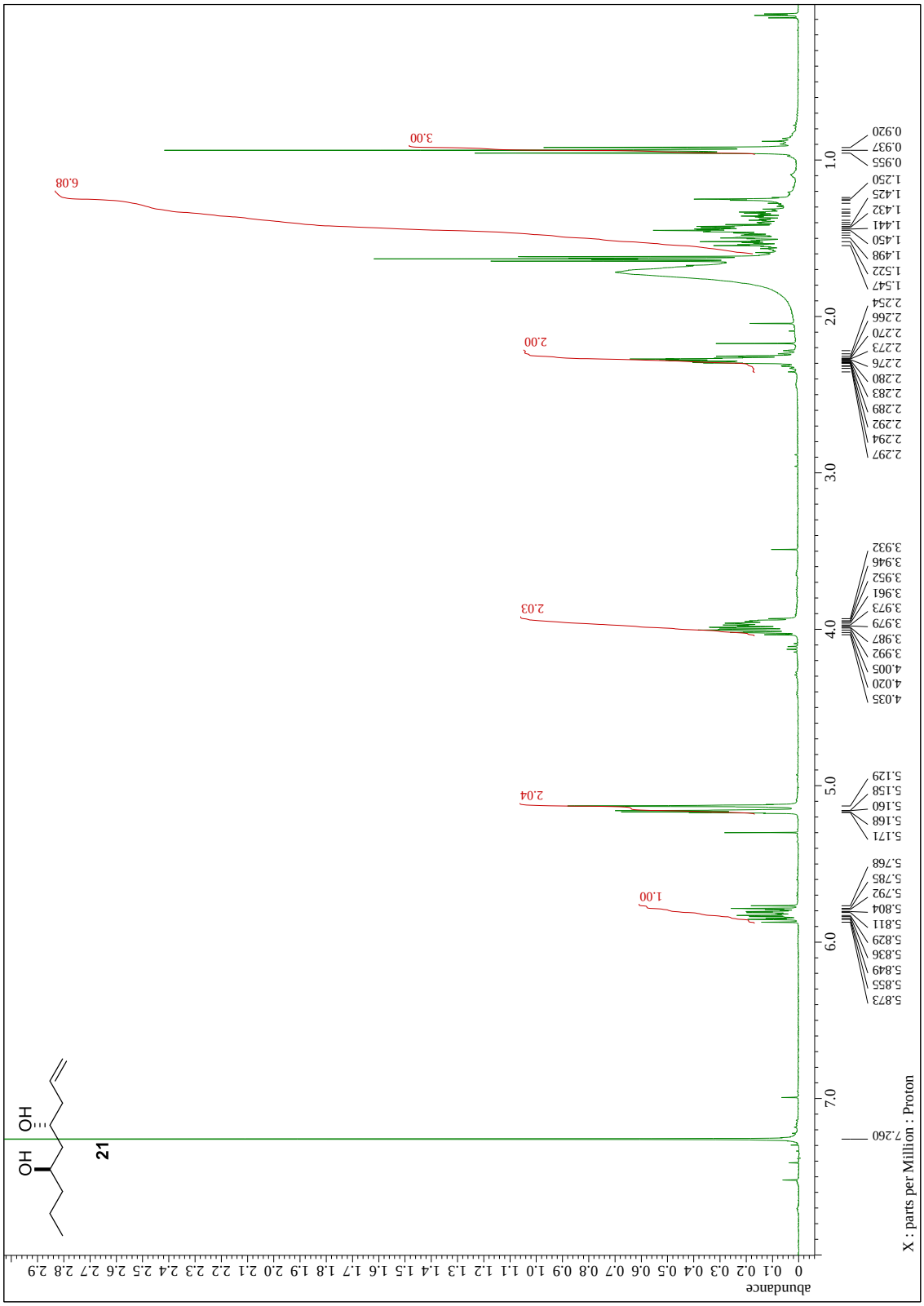
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **20**



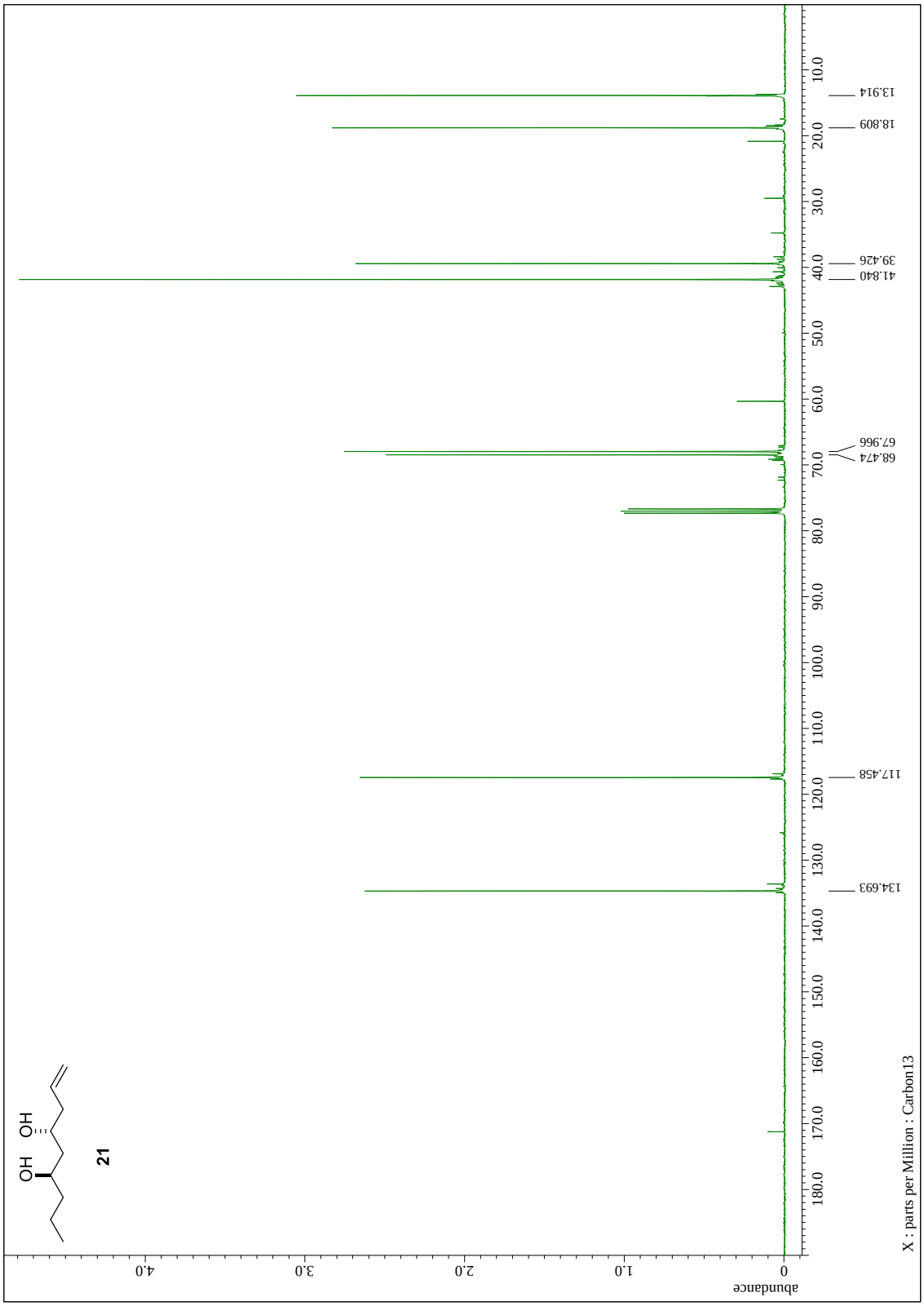
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **20**



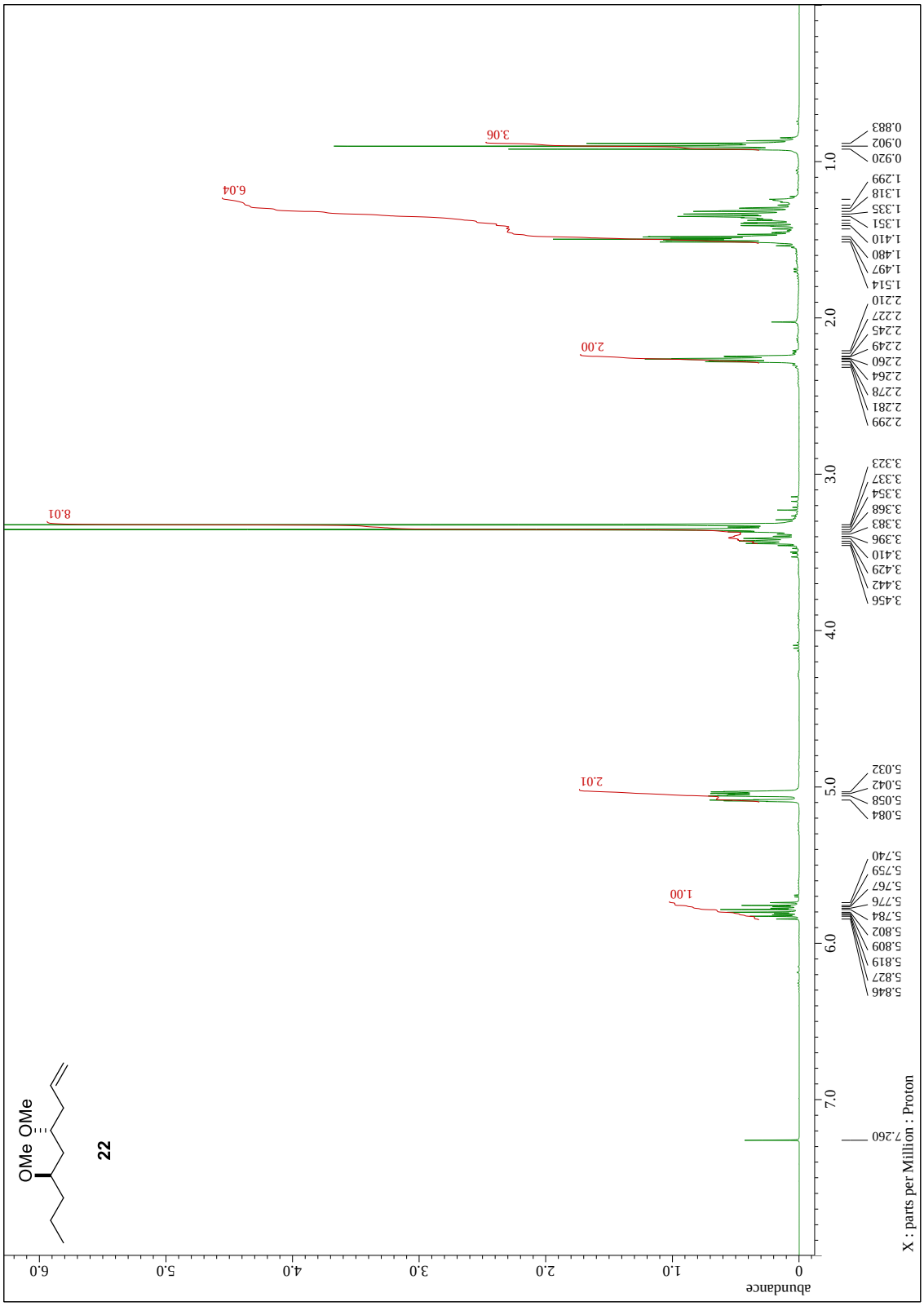
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **21**



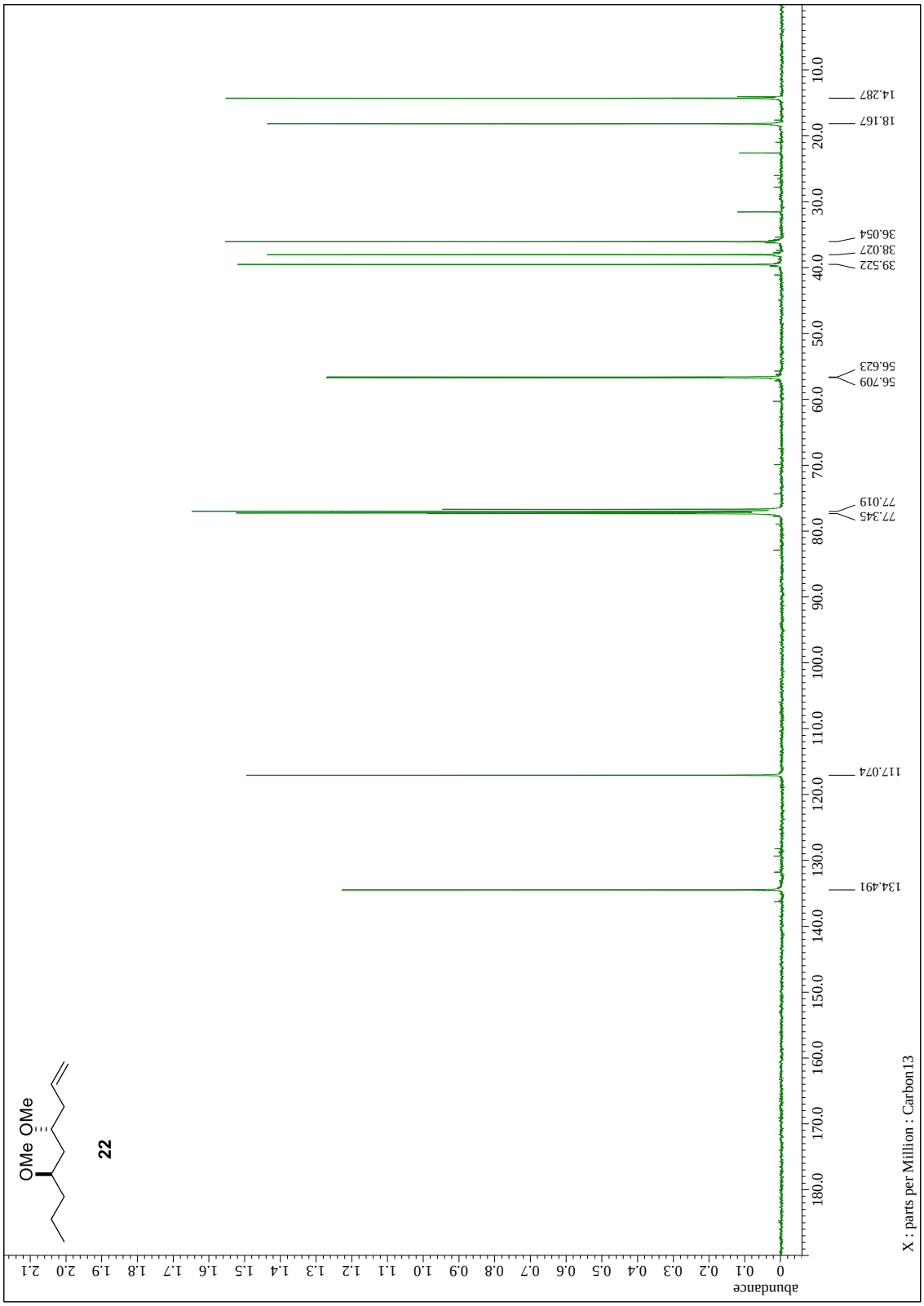
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **21**



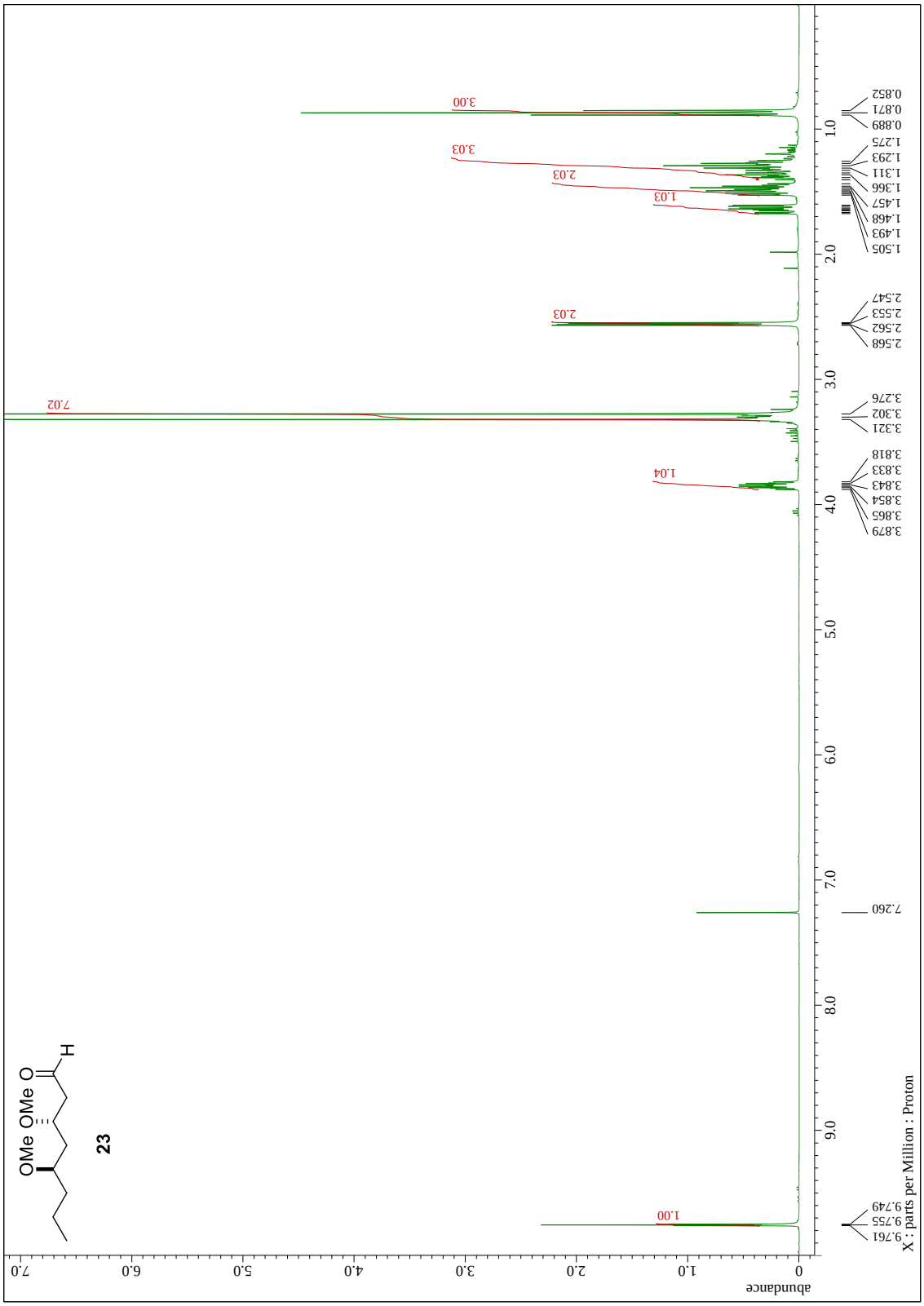
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **22**



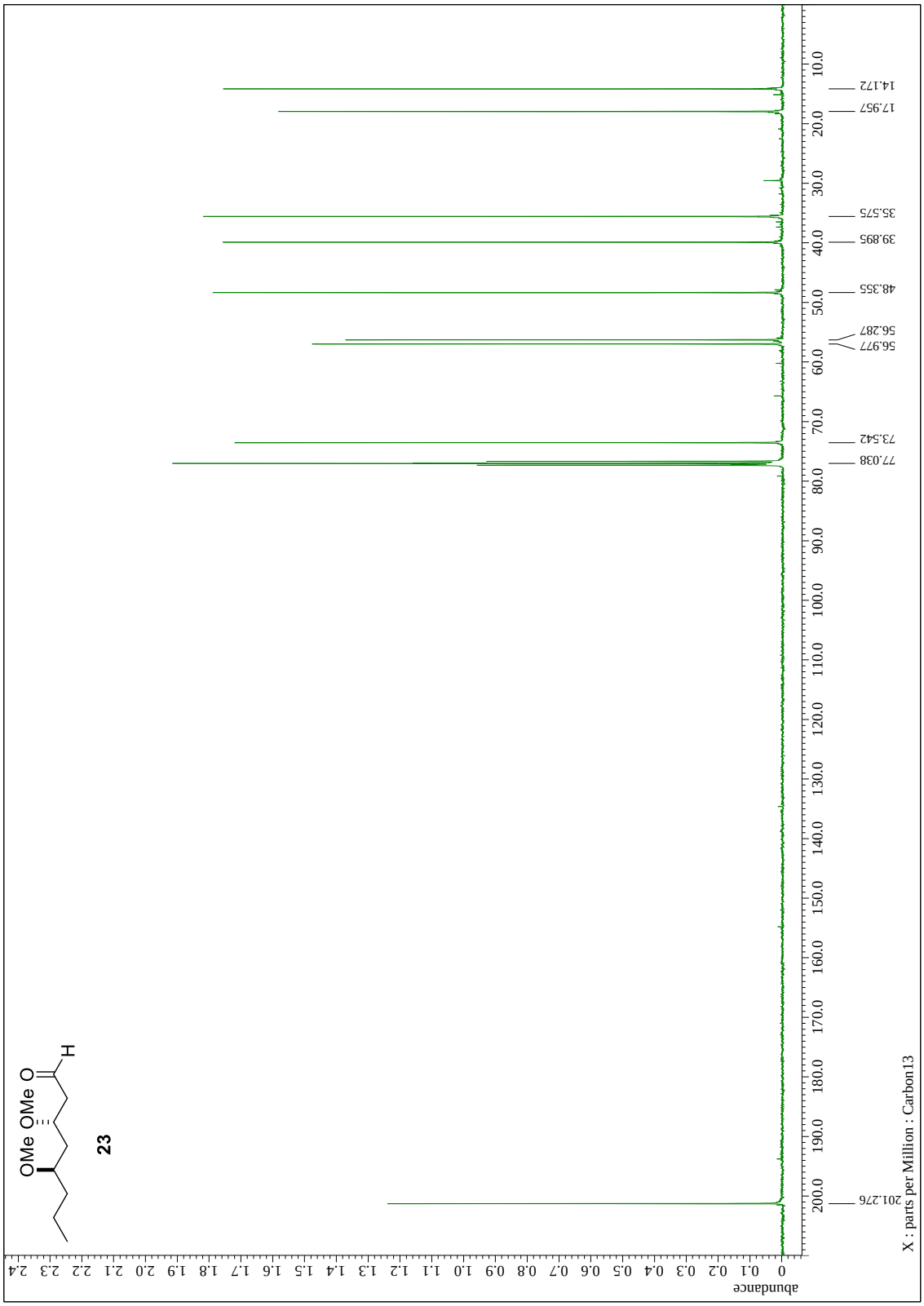
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **22**



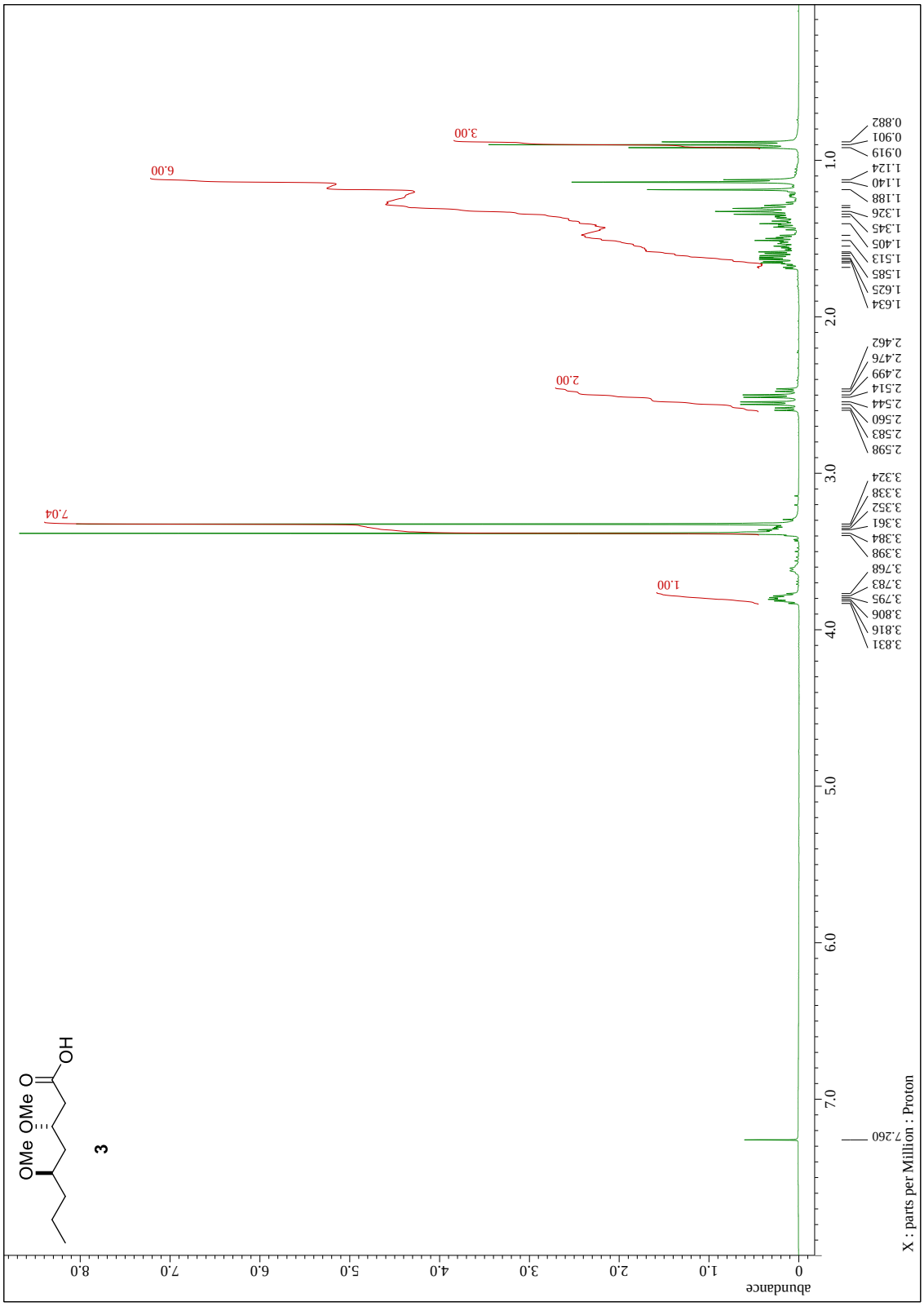
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **23**



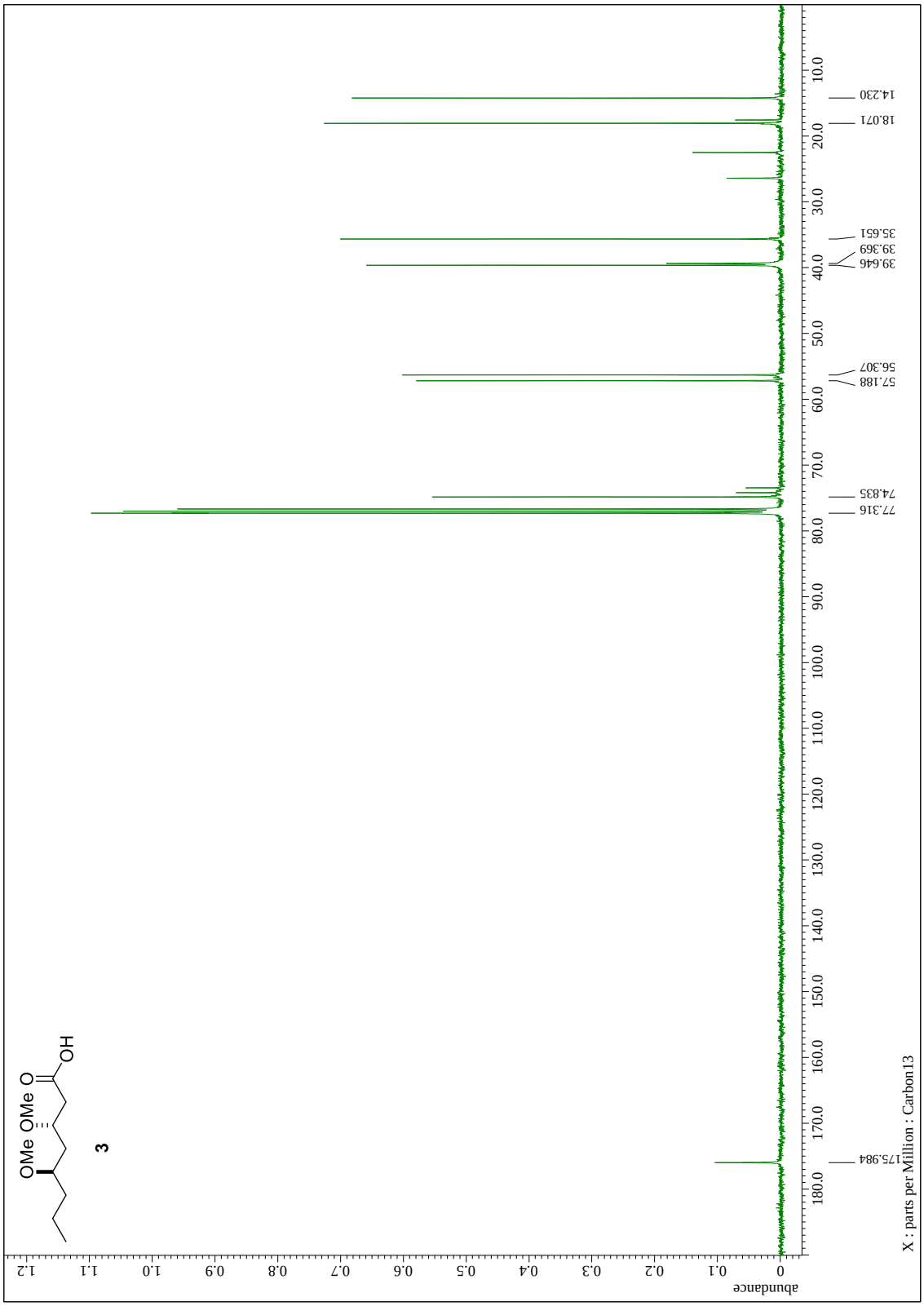
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **23**



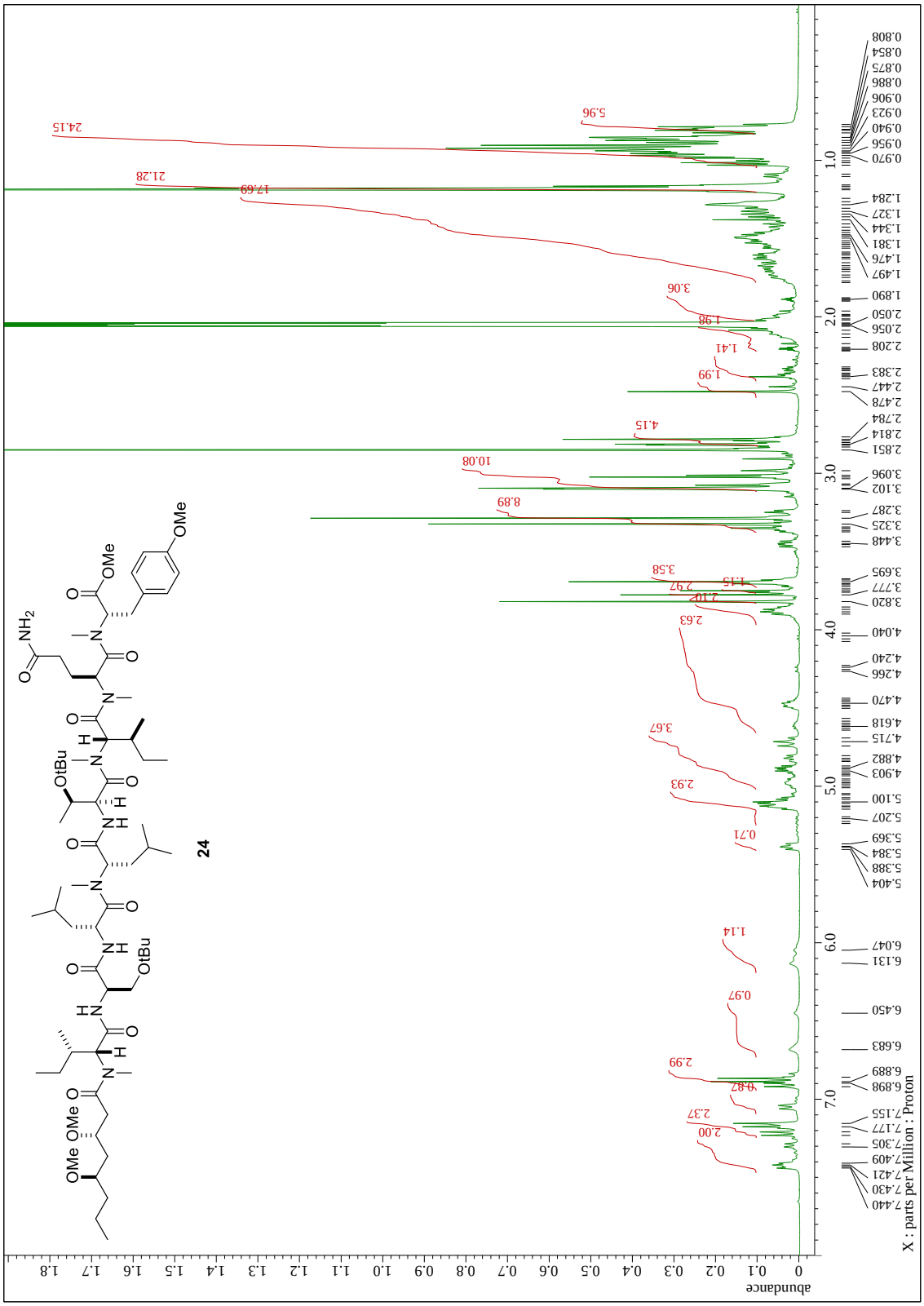
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **3**



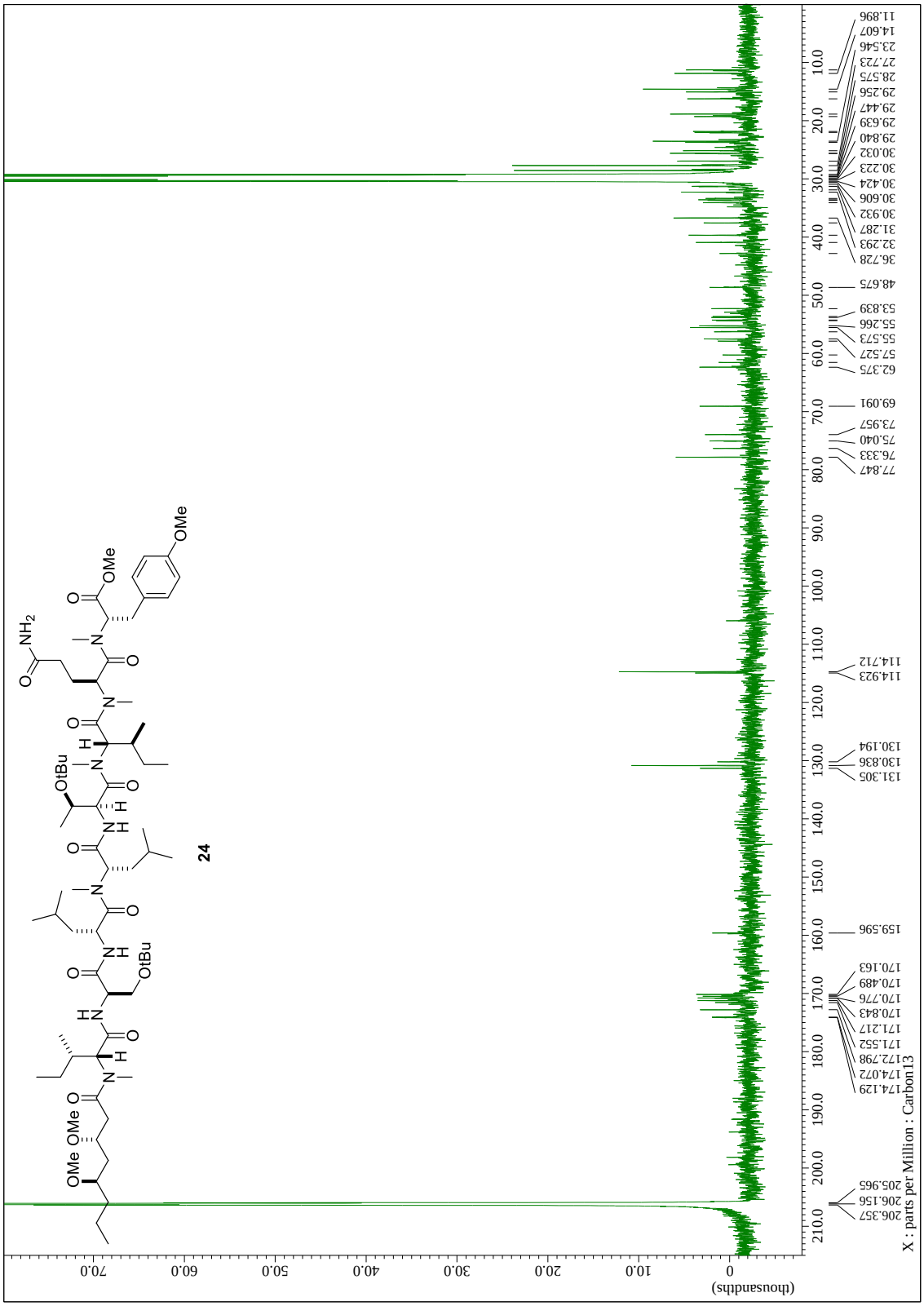
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **3**



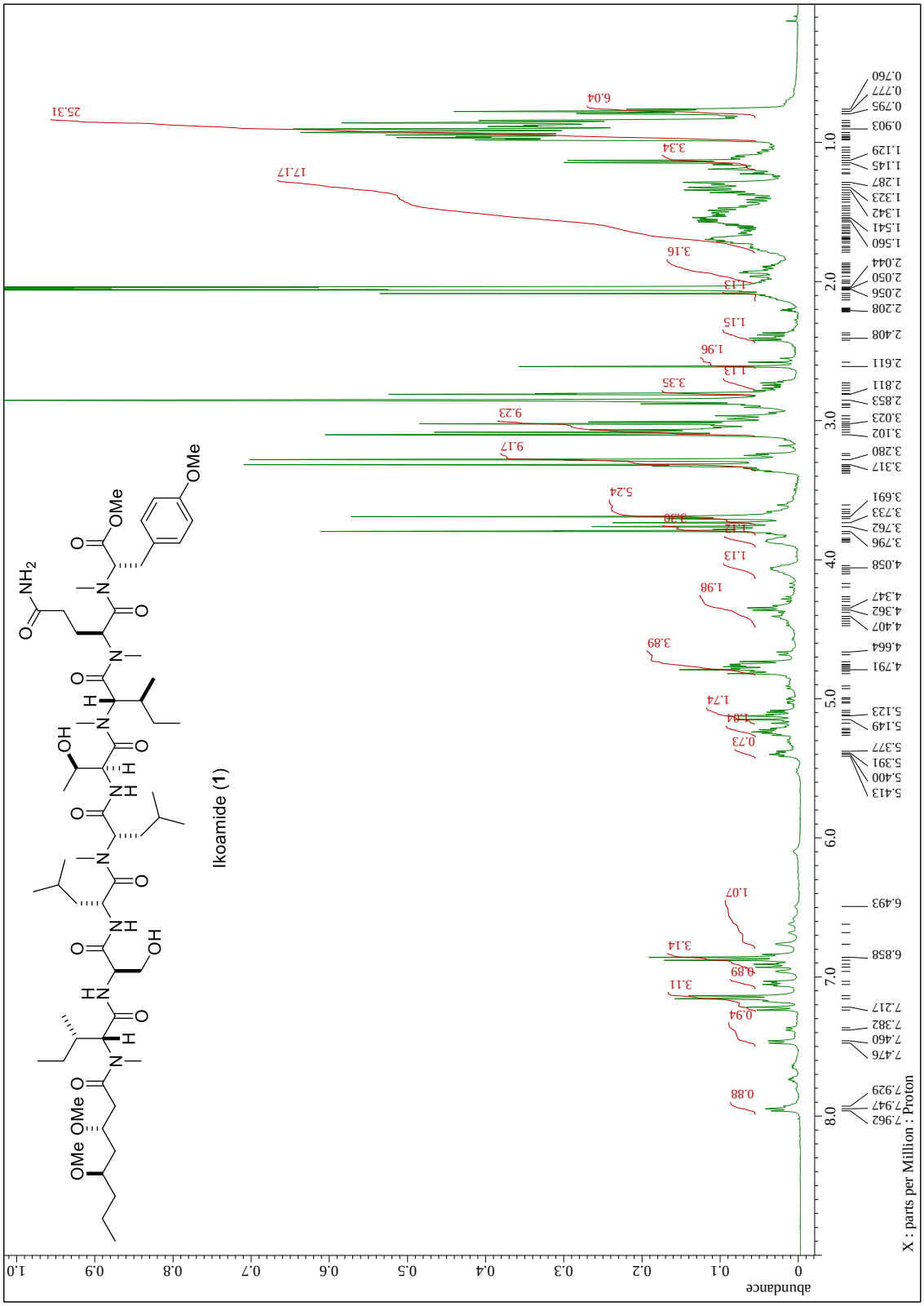
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **24**



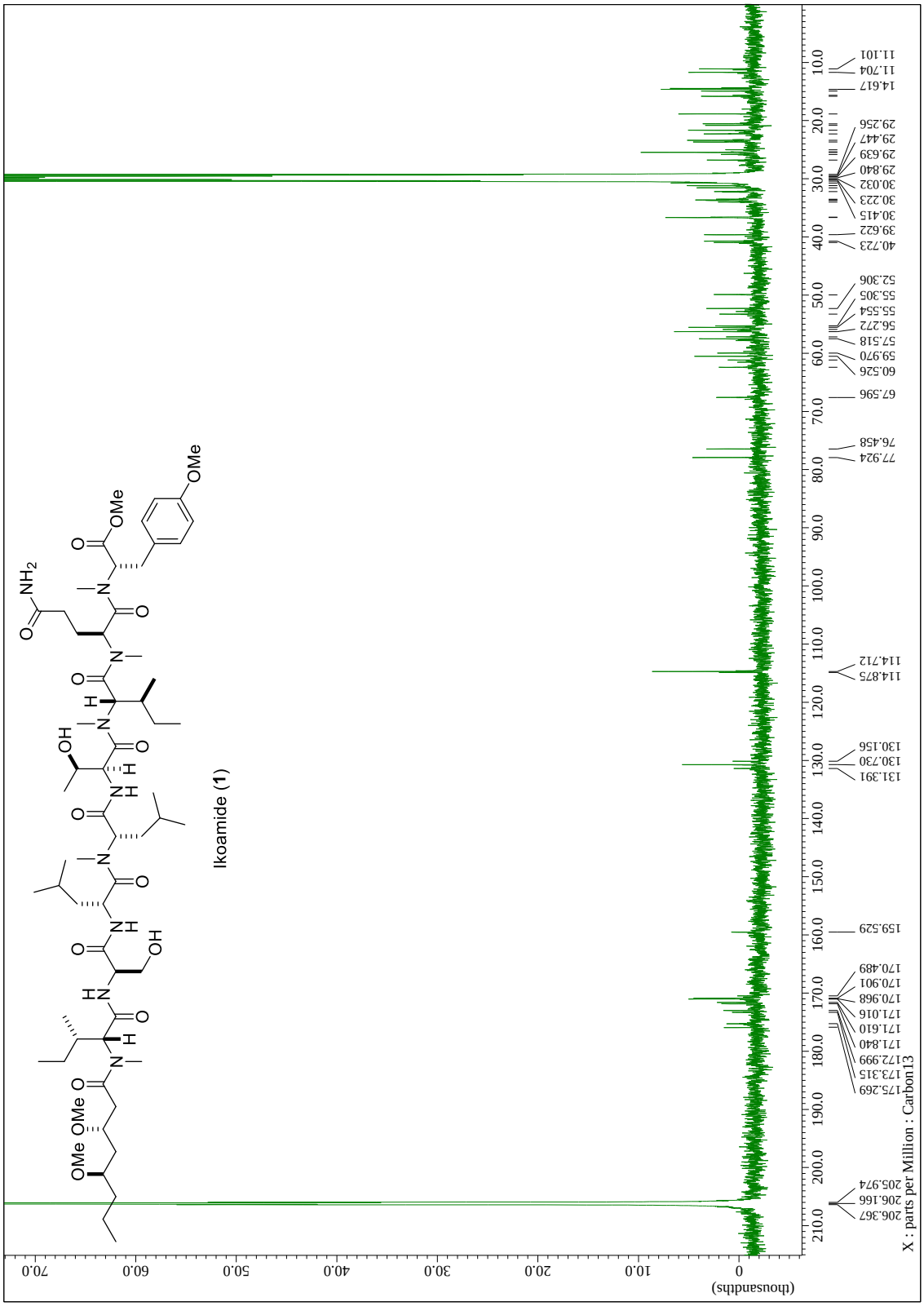
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **24**



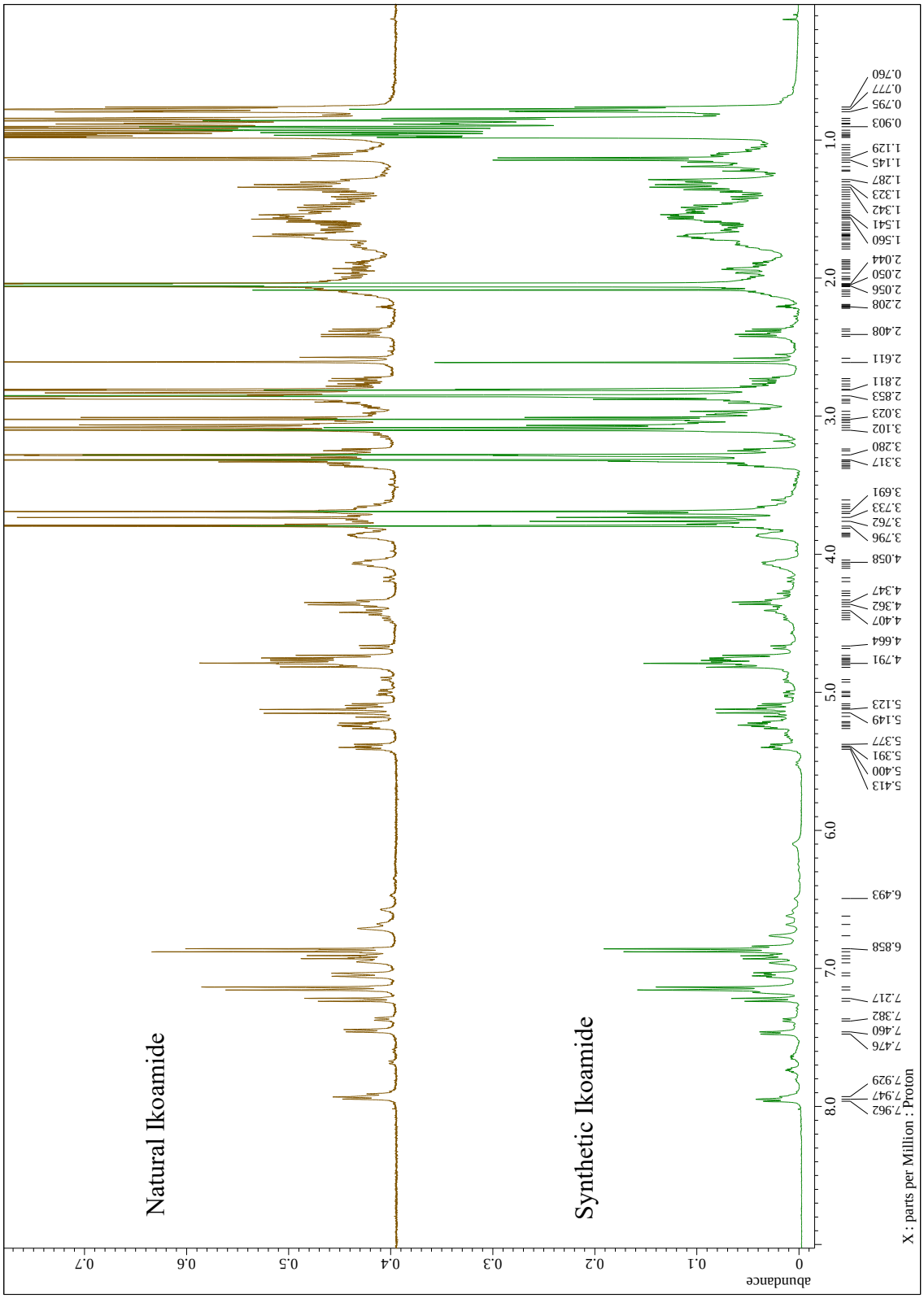
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **1**



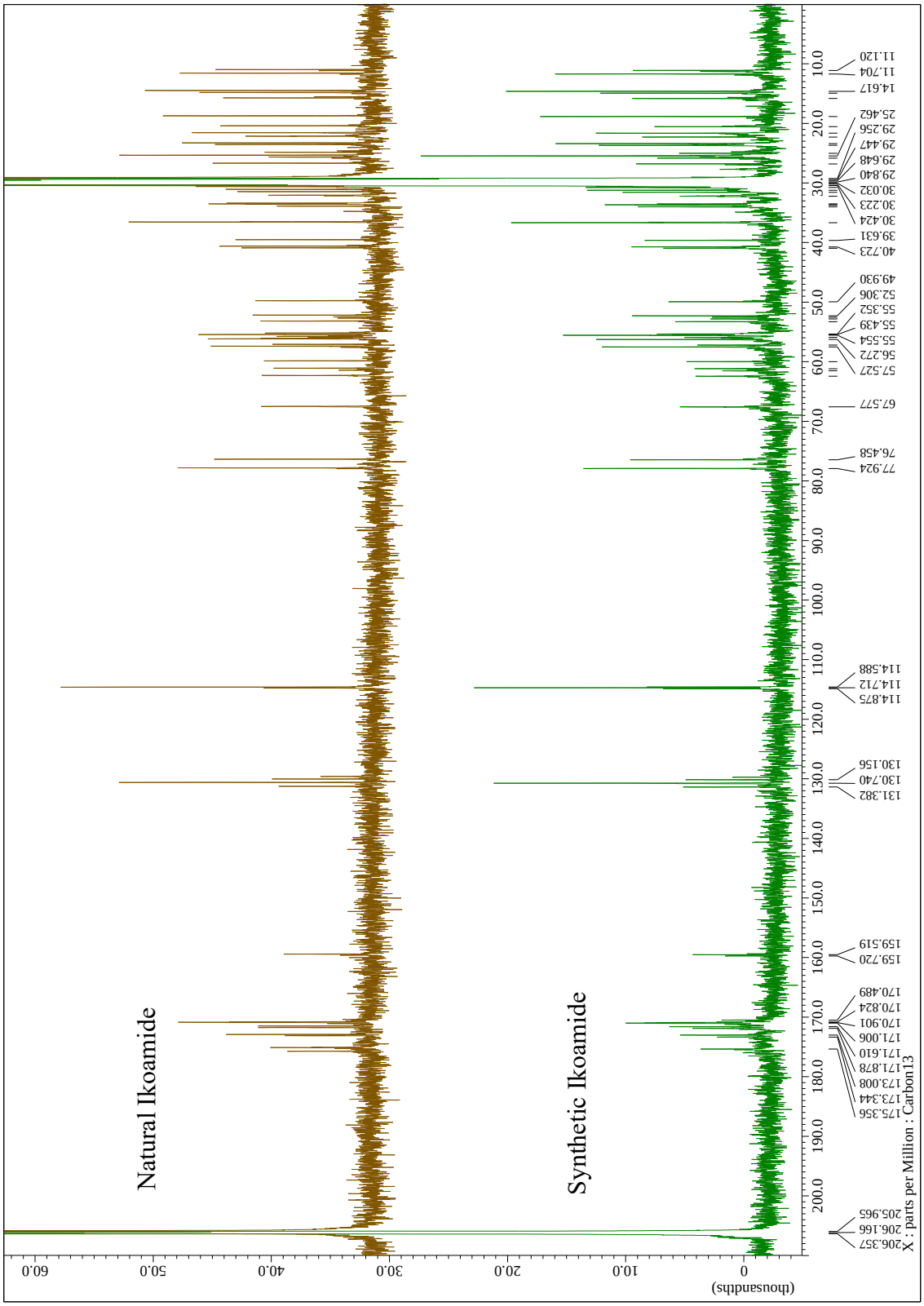
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **1**



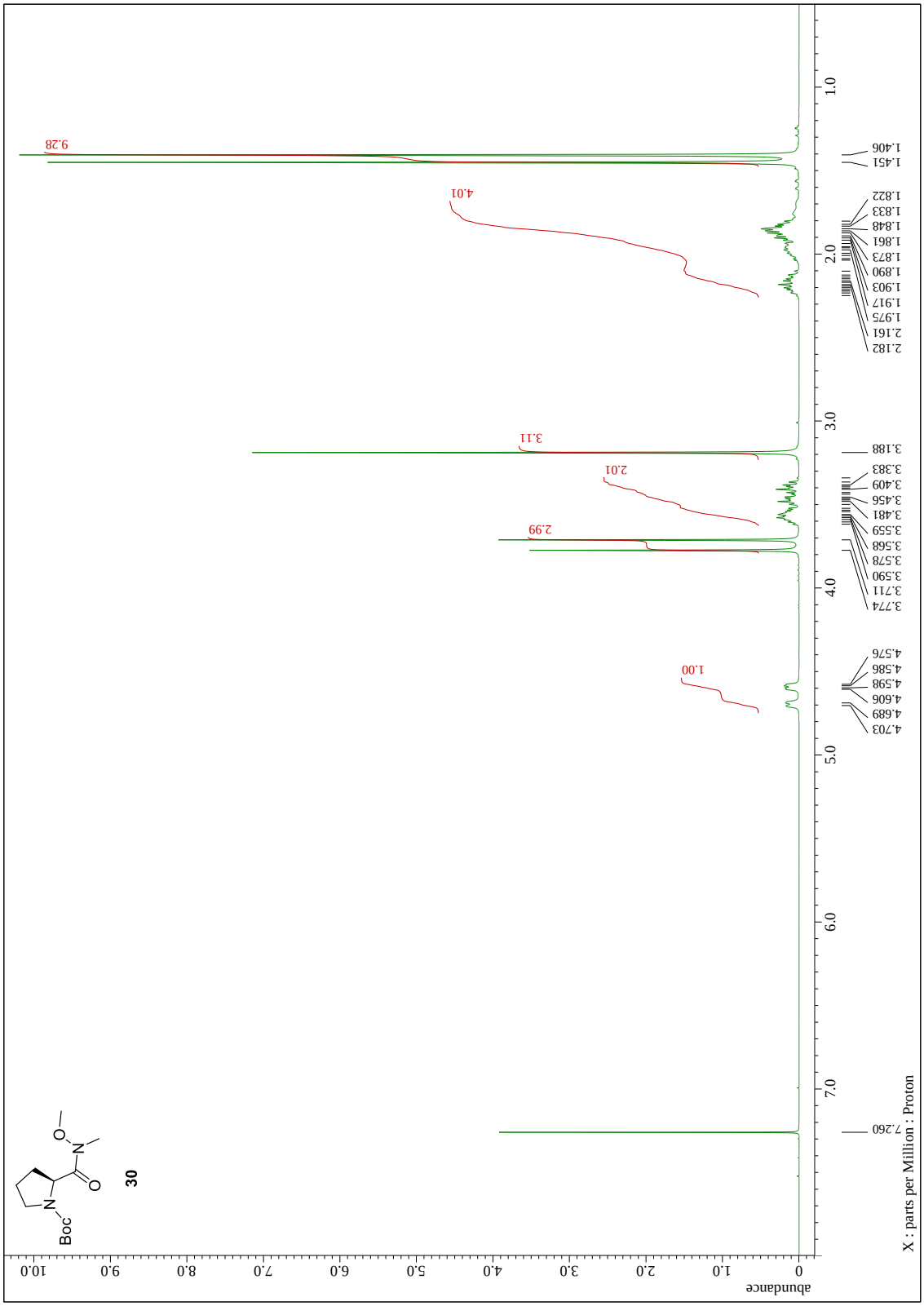
Comparison of ^{1}H NMR (400 MHz, d_6-Acetone) spectra between natural and synthetic **1**



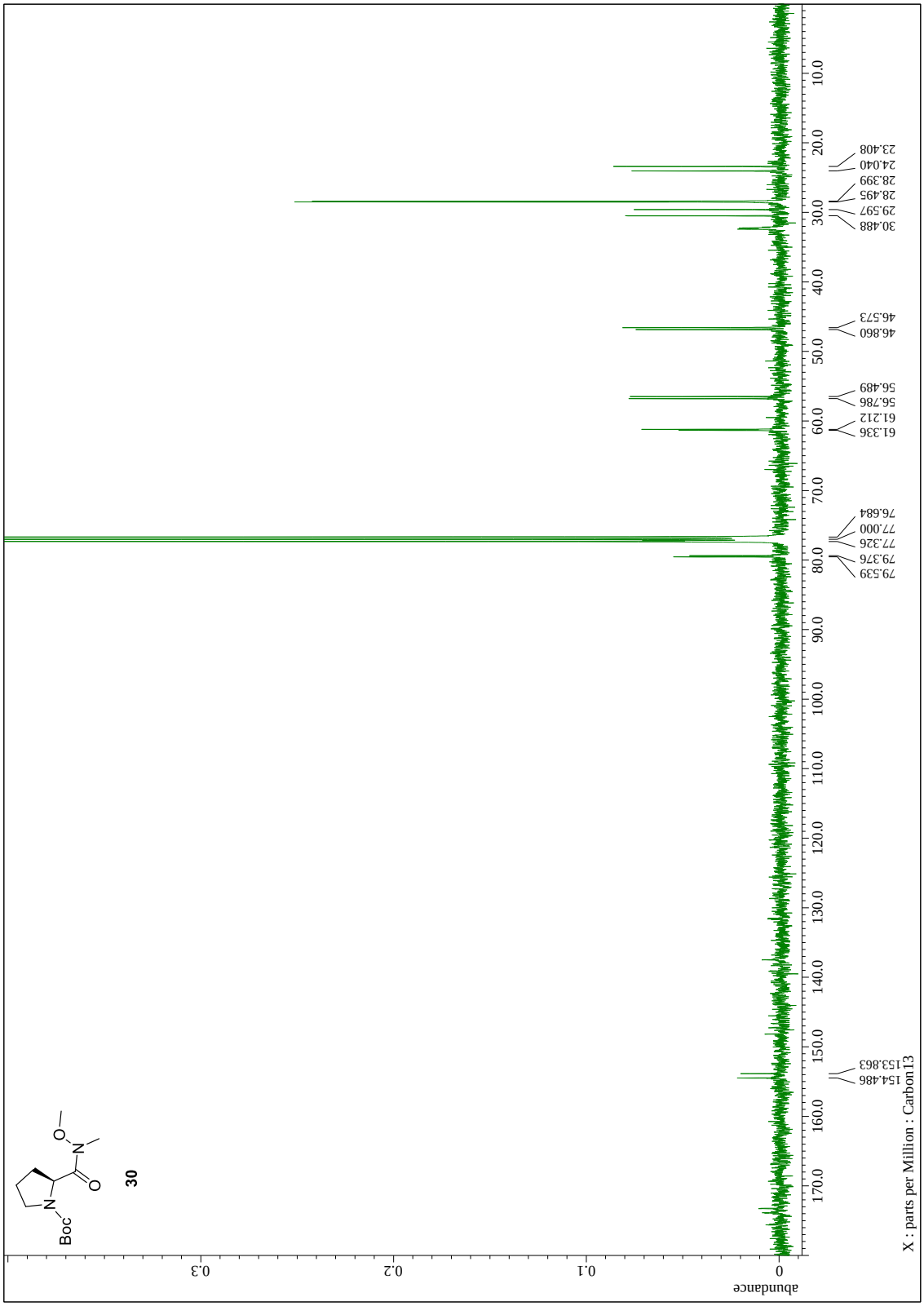
Comparison of $^{13}C\{^{1}H\}$ NMR (100 MHz, d_6-Acetone) spectra between natural and synthetic **1**



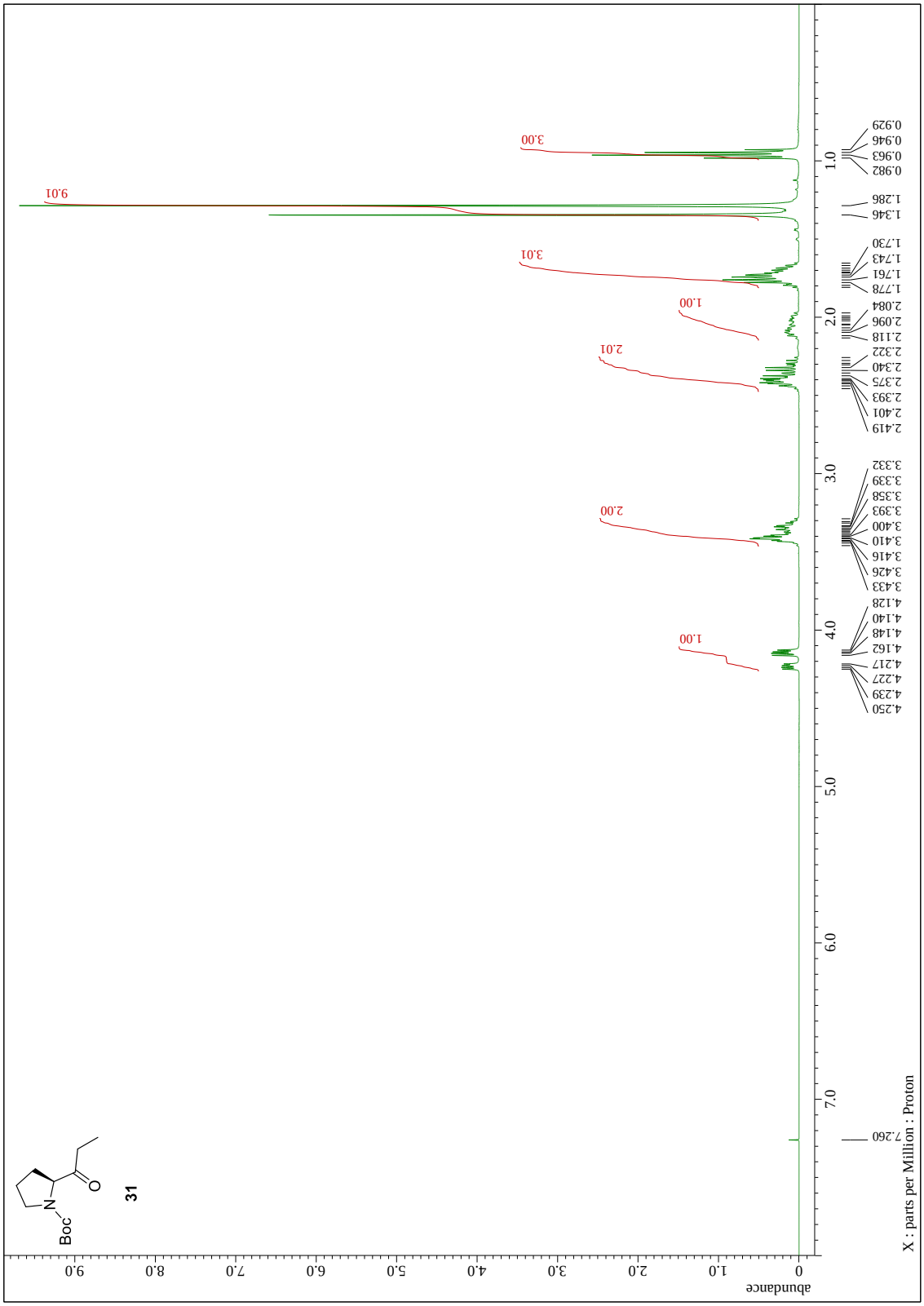
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **30**



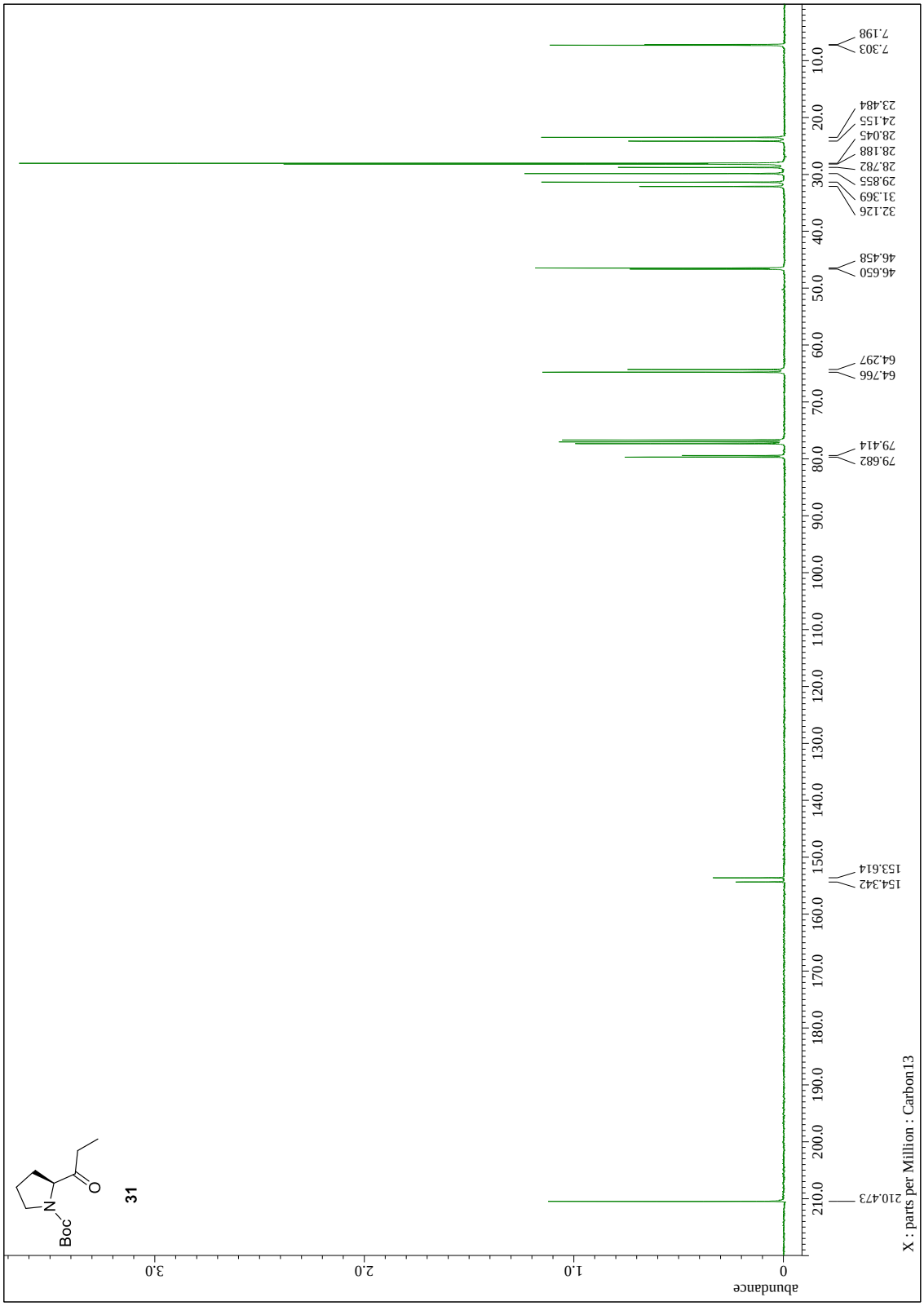
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **30**



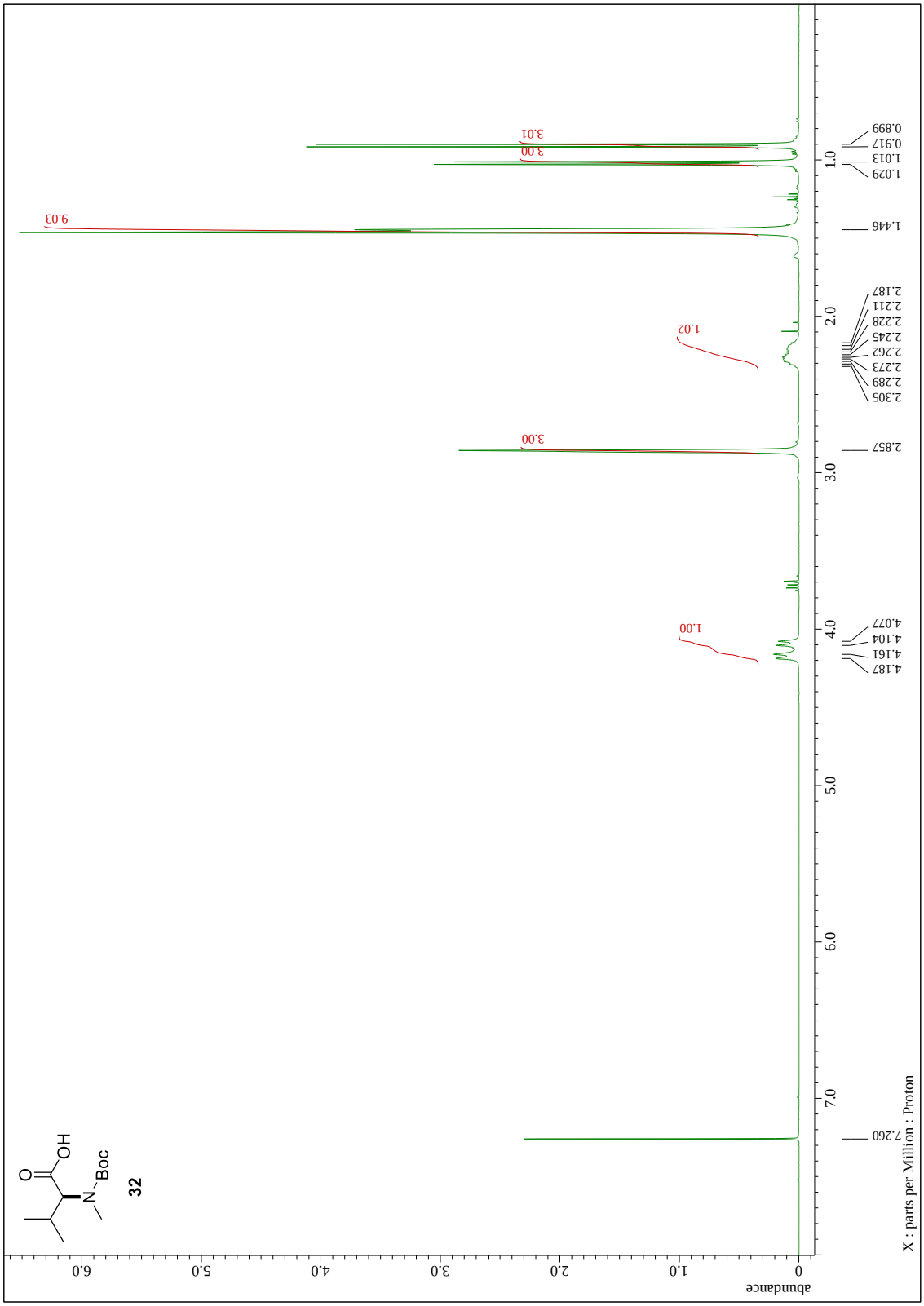
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **31**



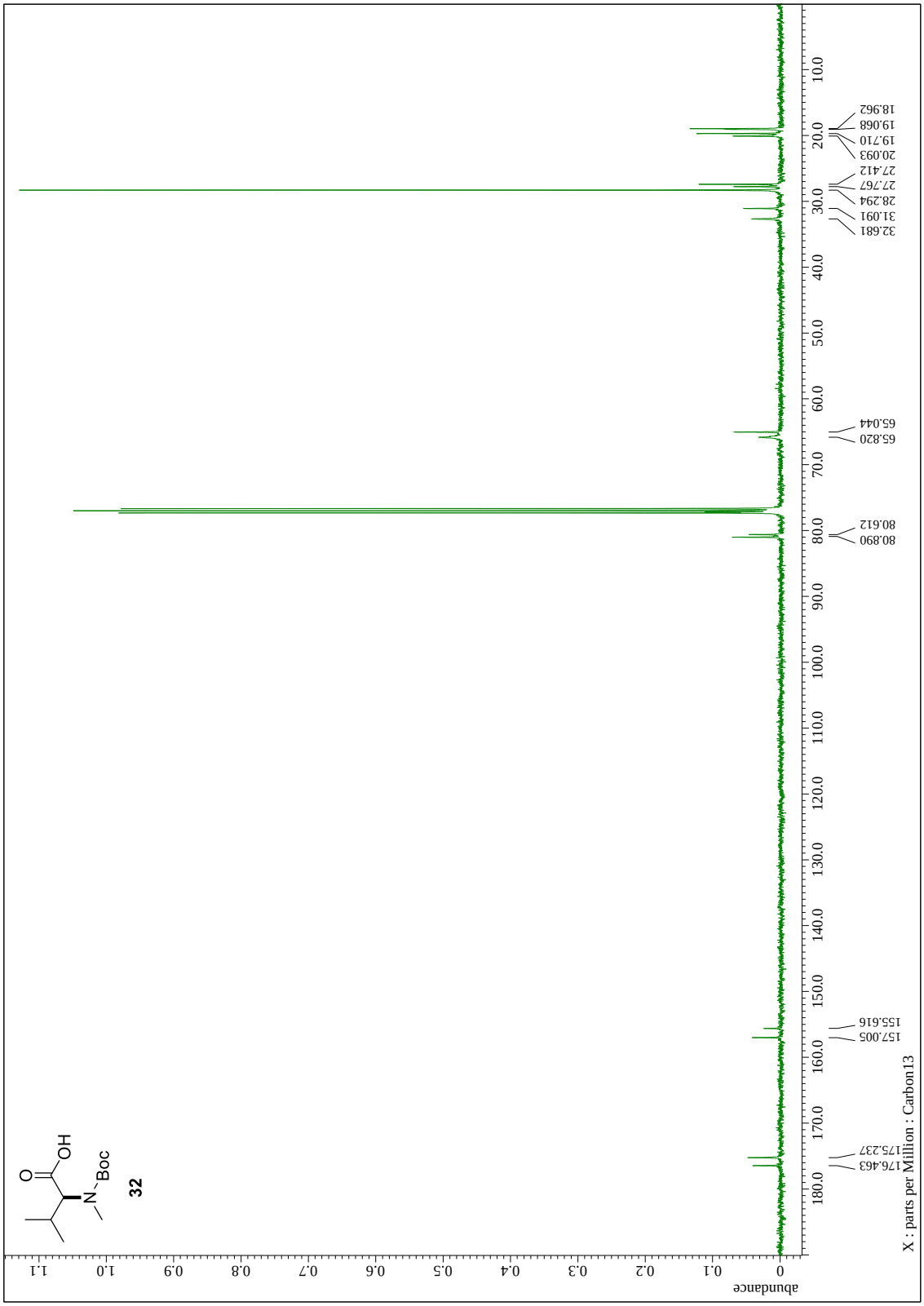
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **31**



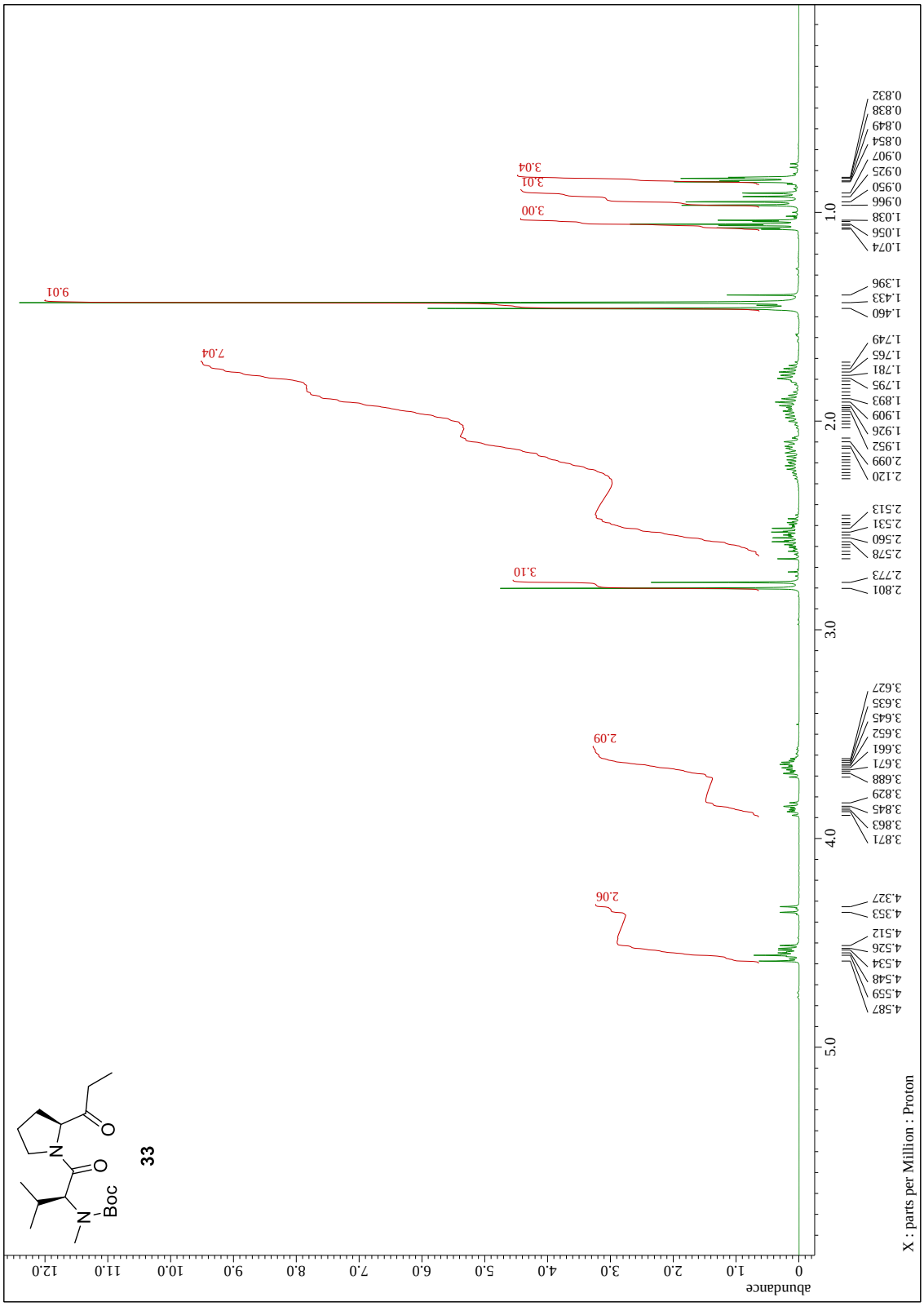
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **32**



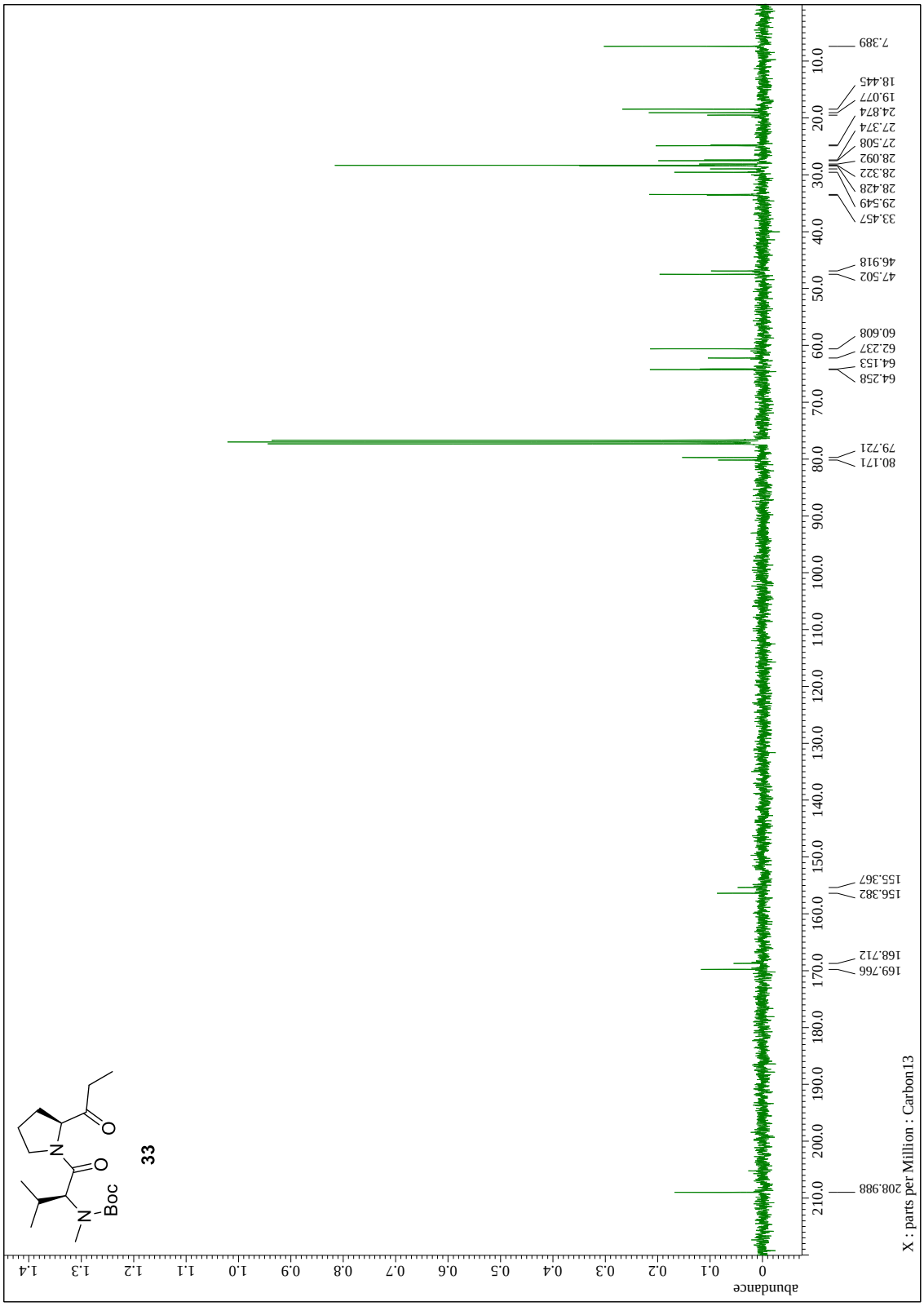
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **32**



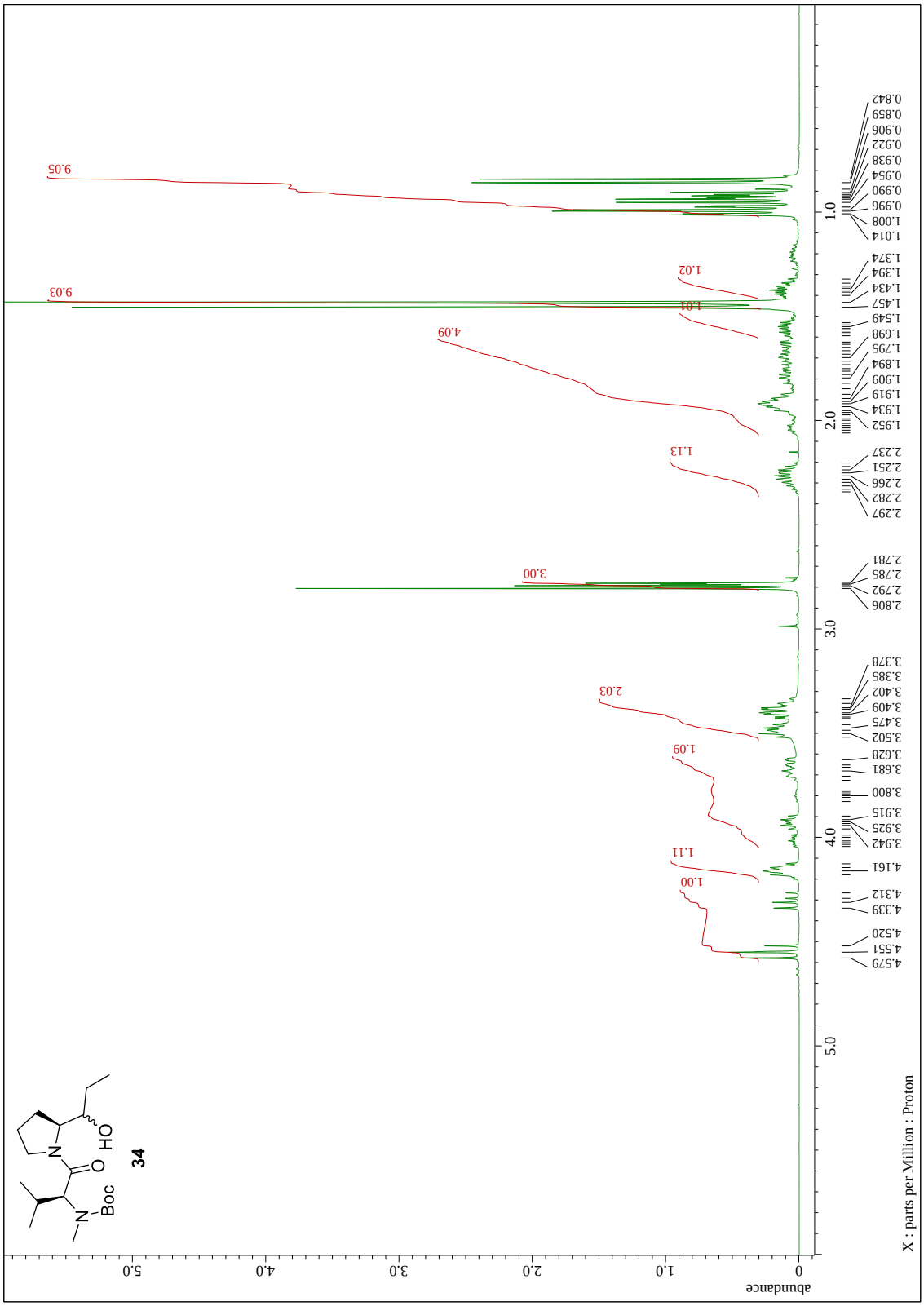
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **33**



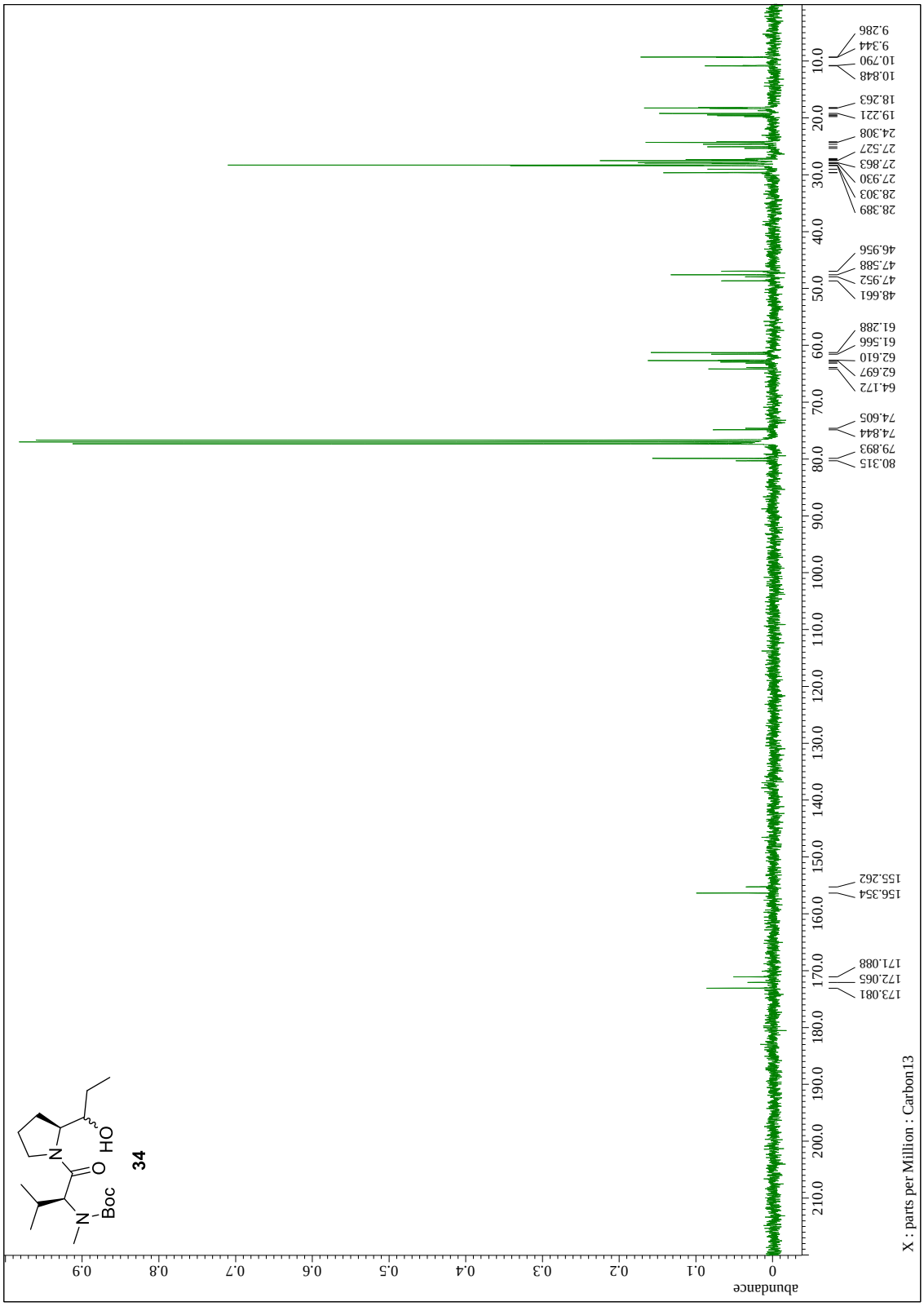
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **33**



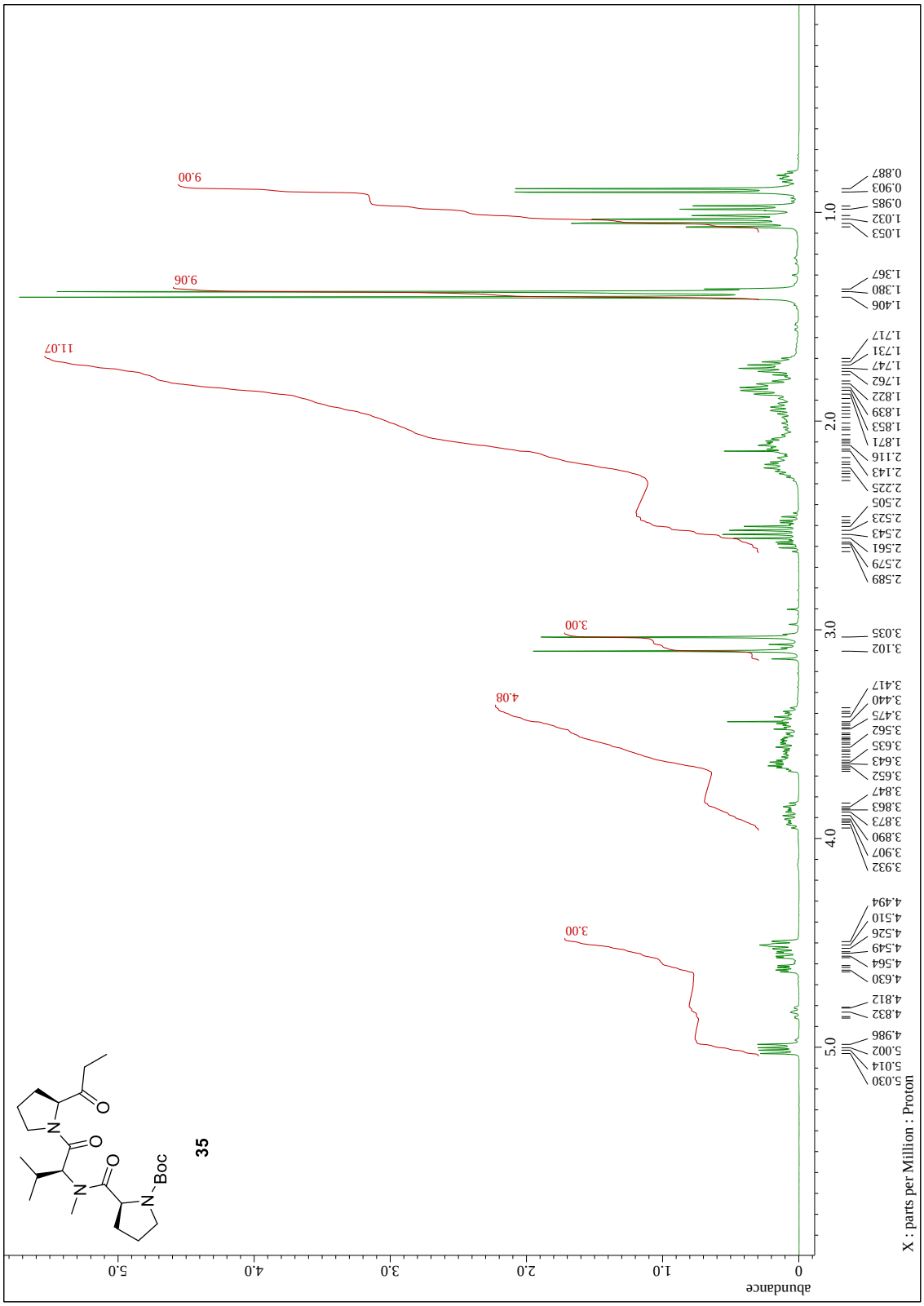
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **34**



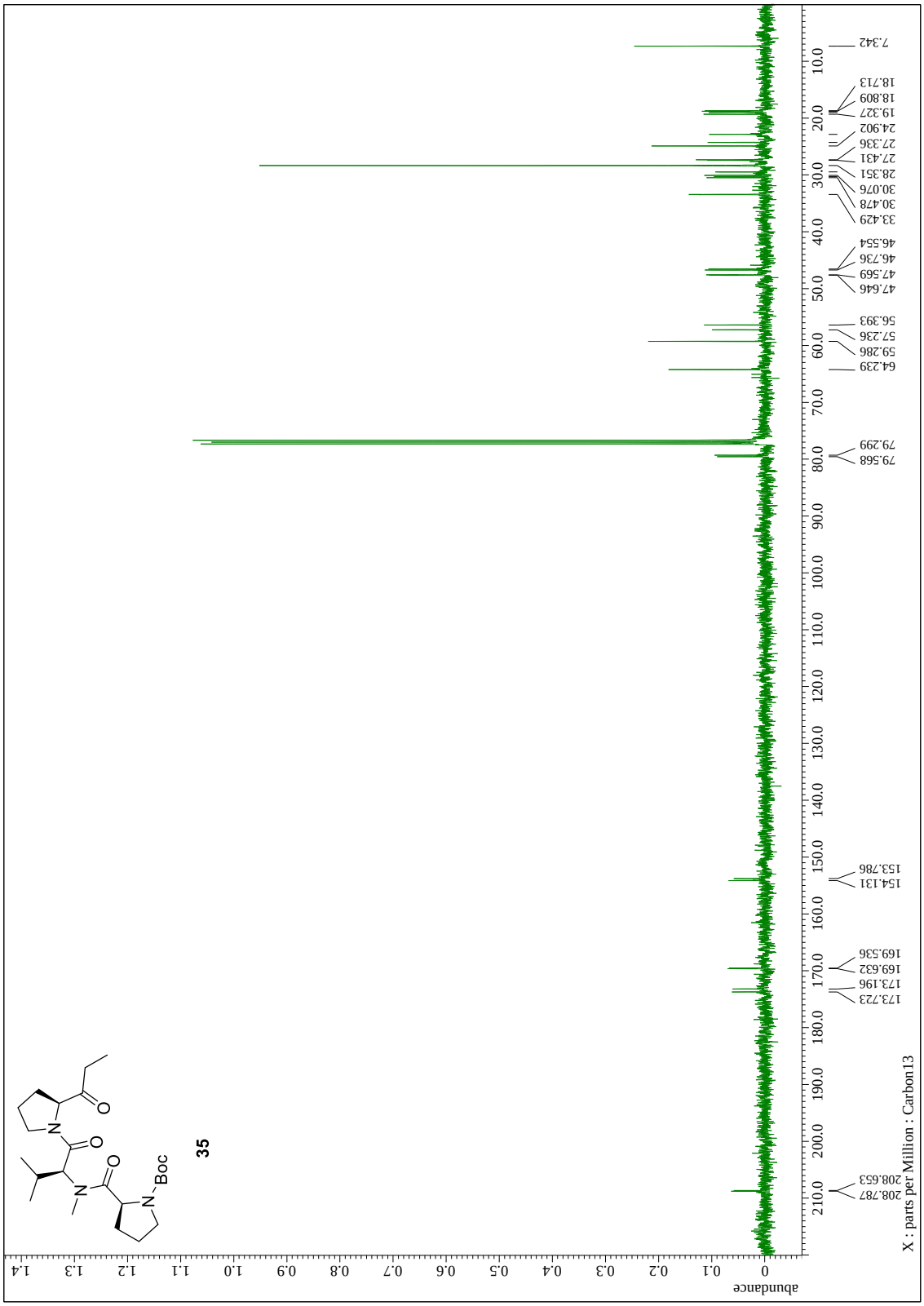
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **34**



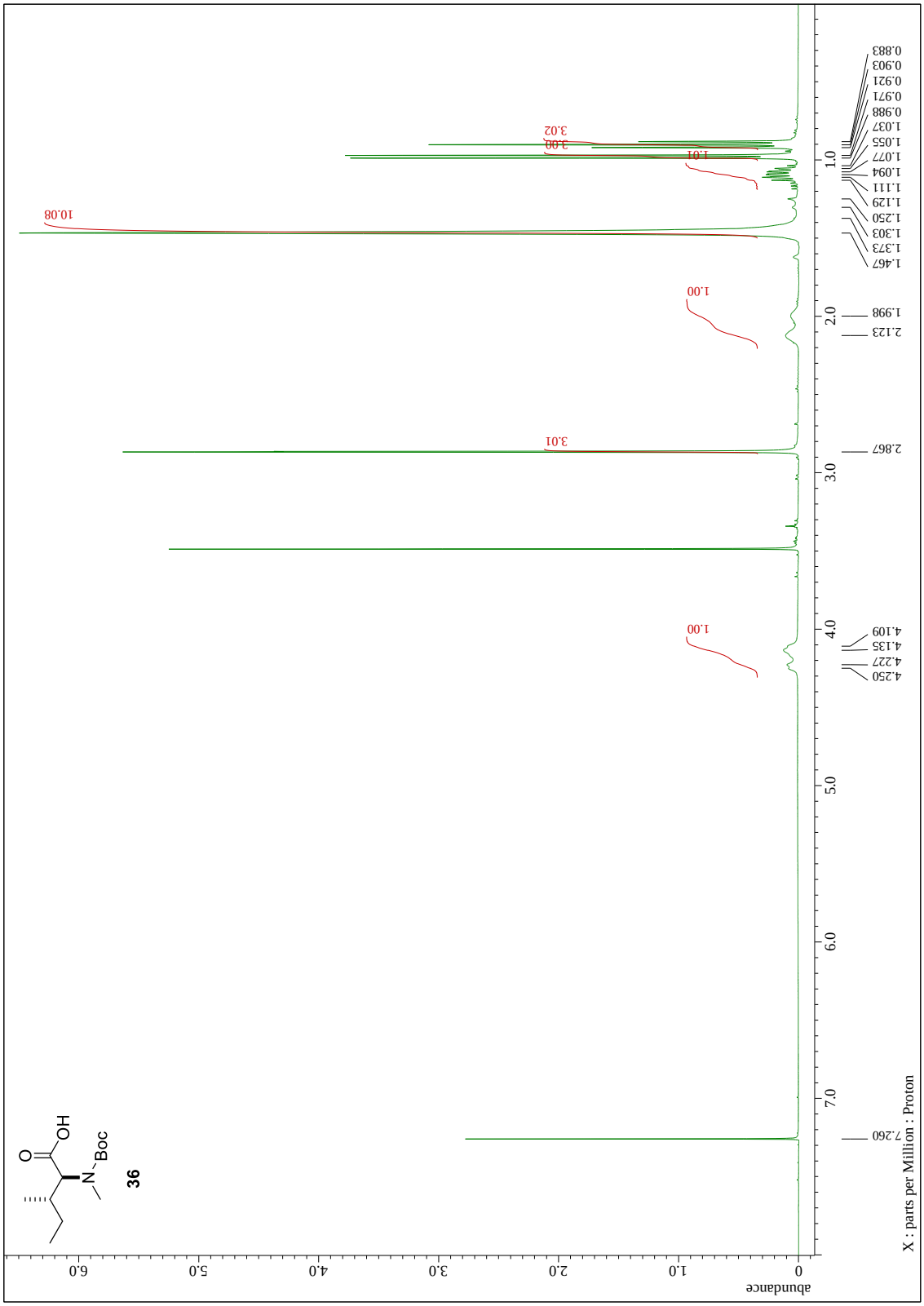
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **35**



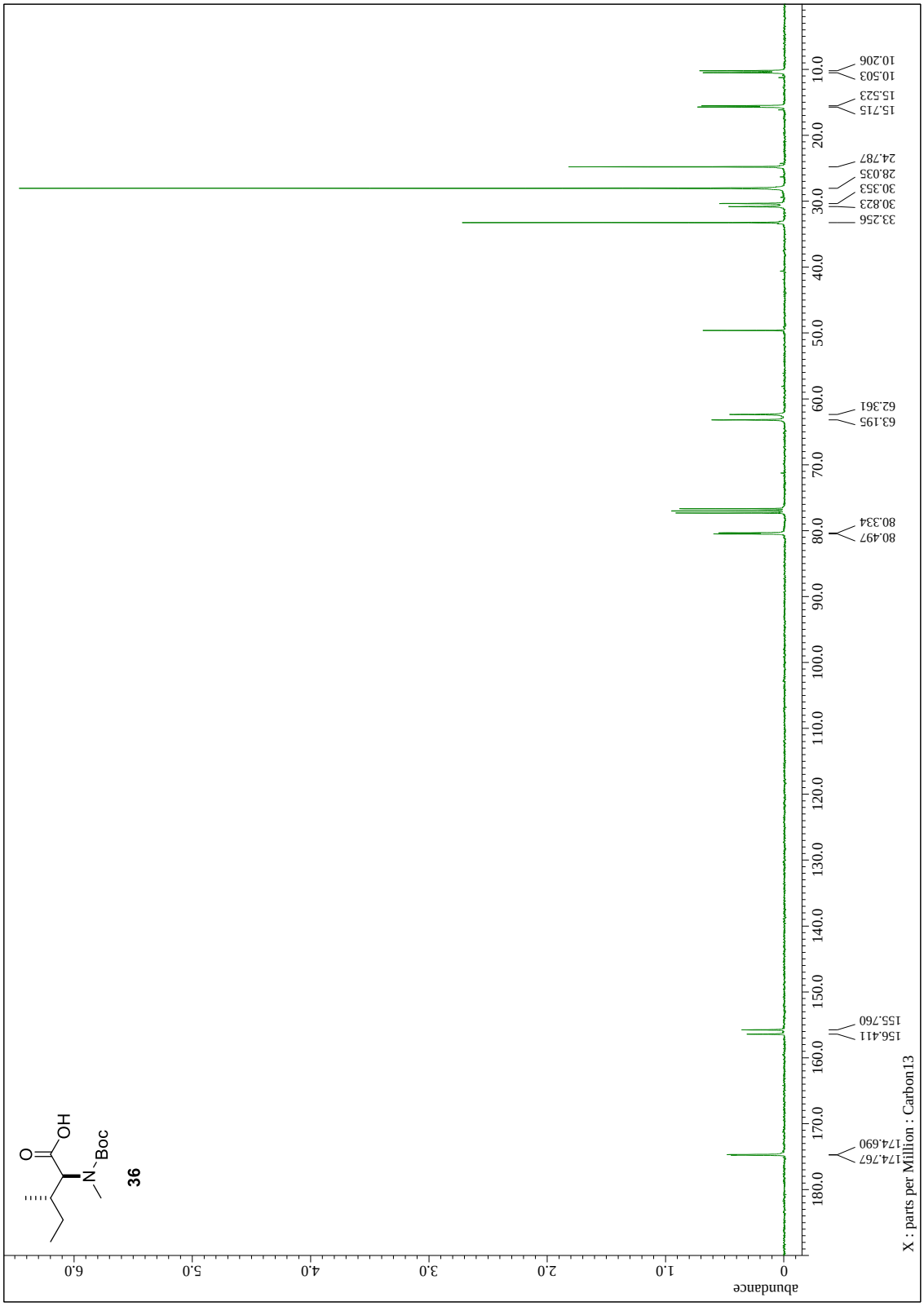
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **35**



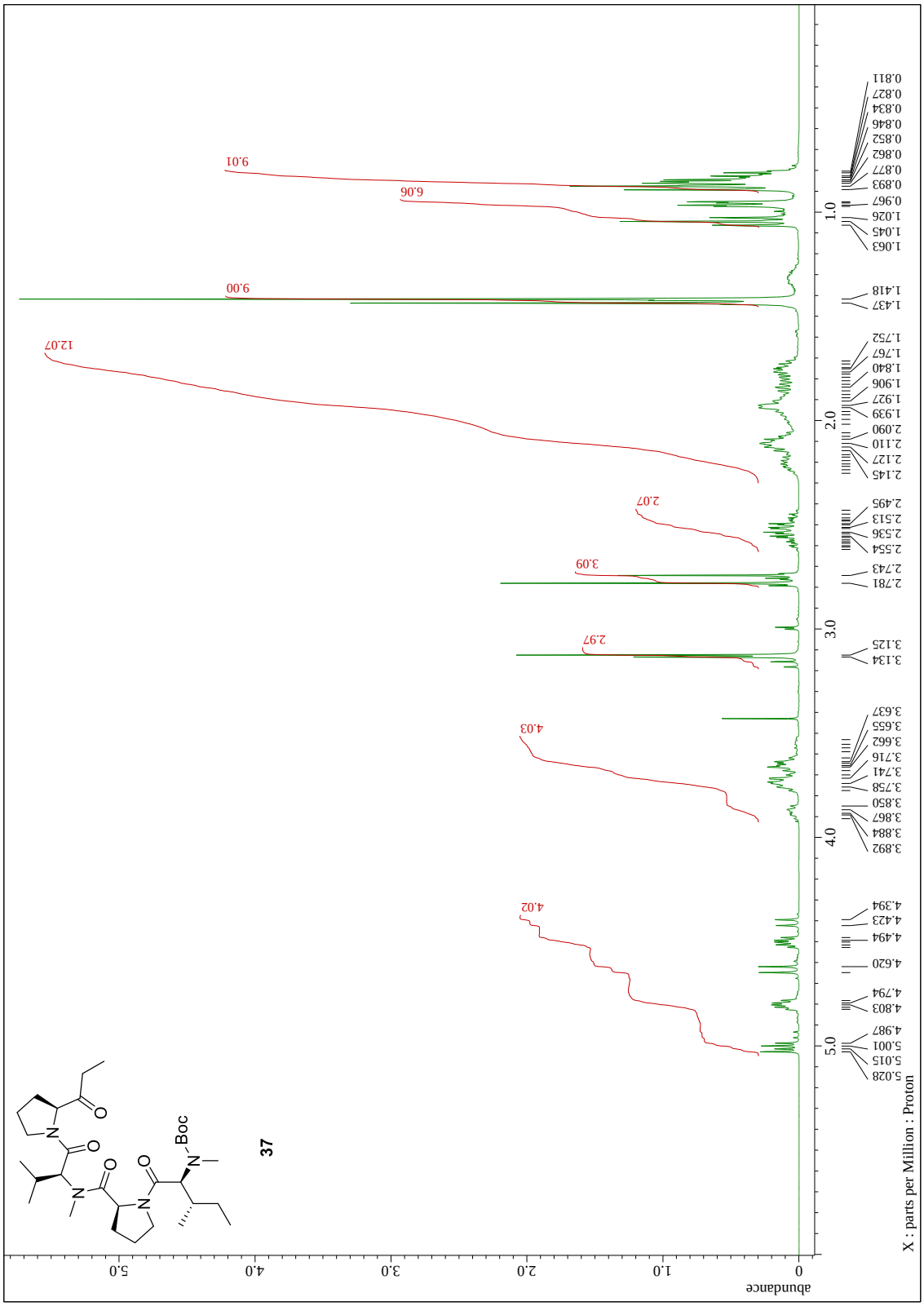
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **36**



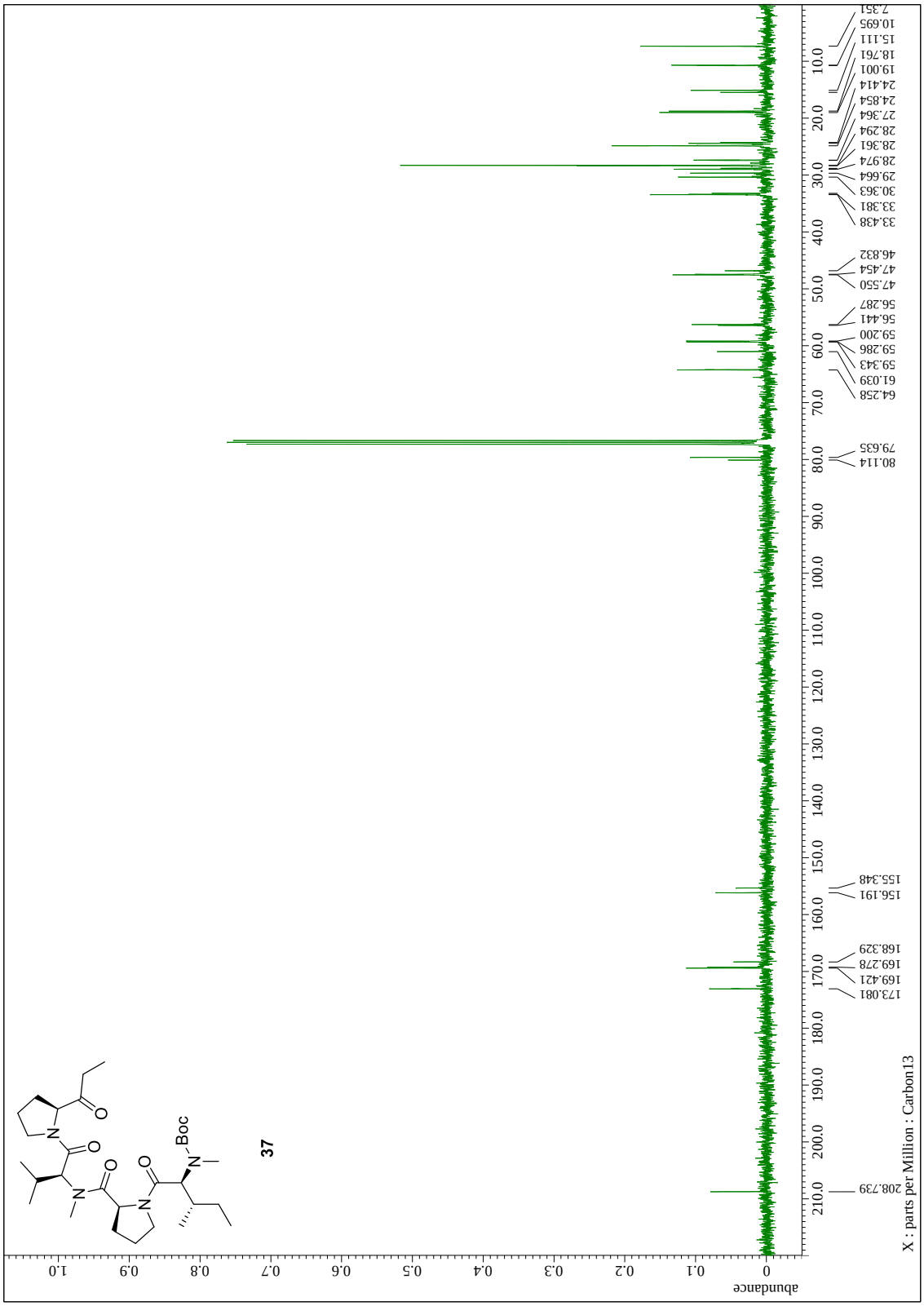
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **36**



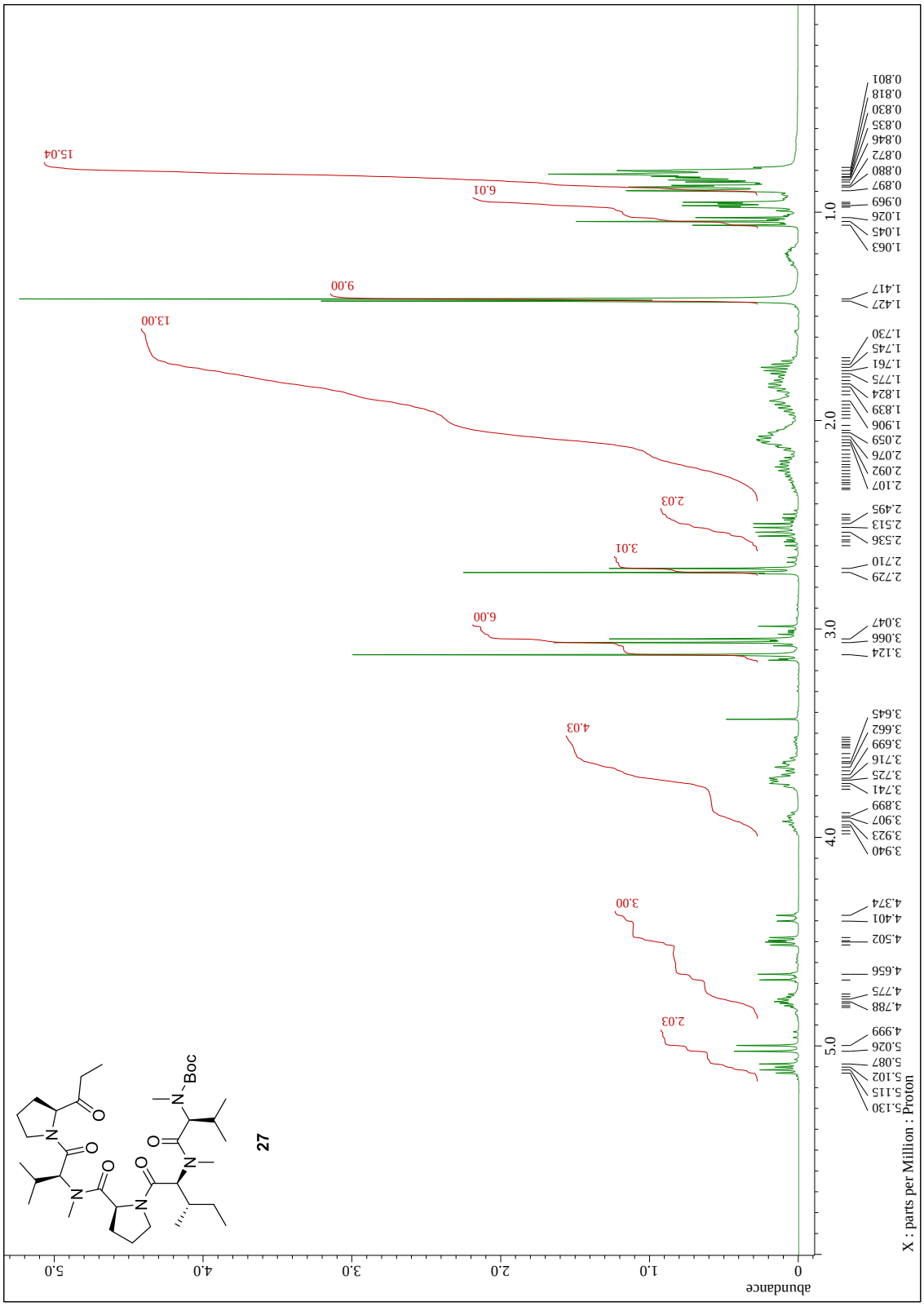
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **37**



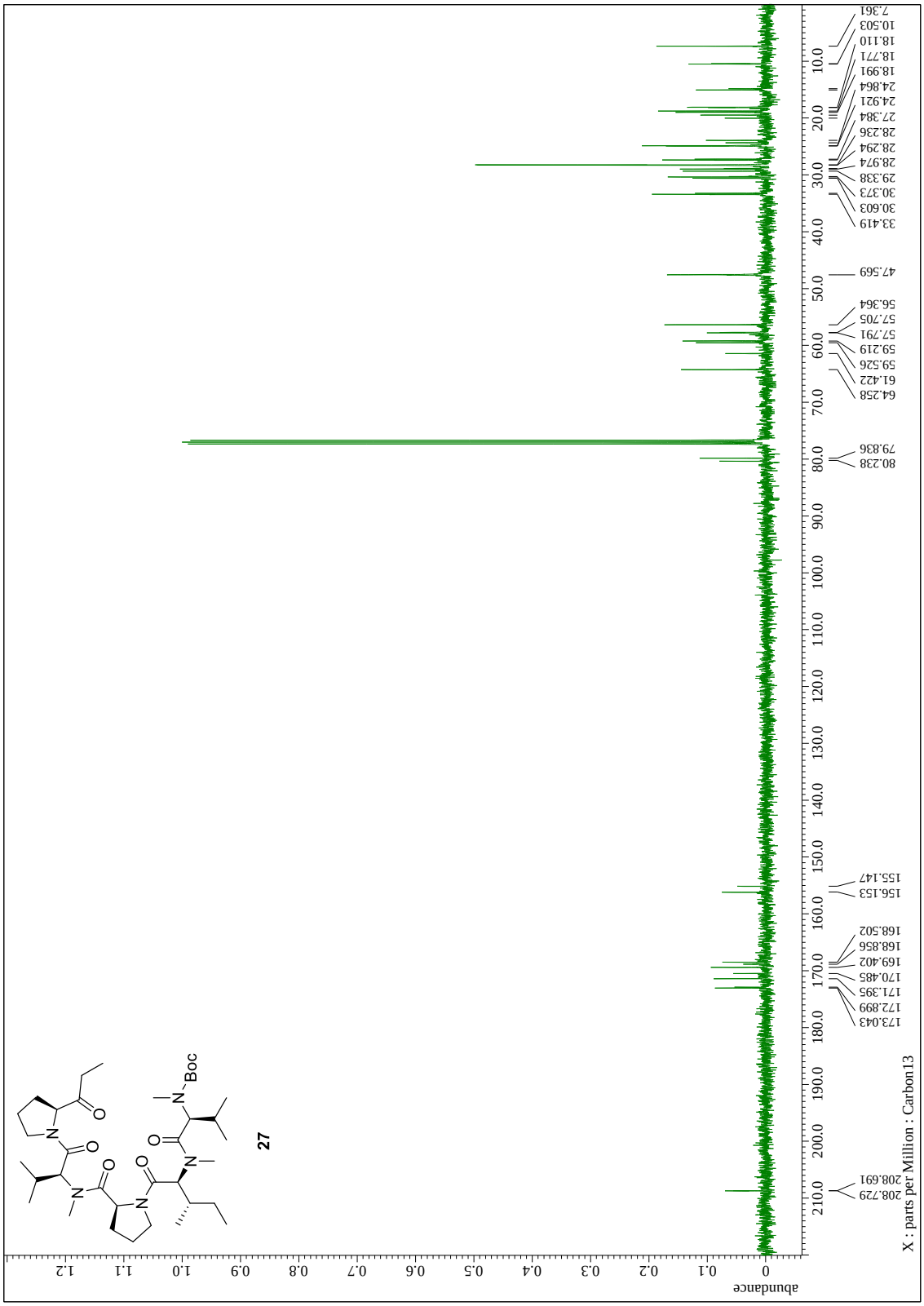
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **37**



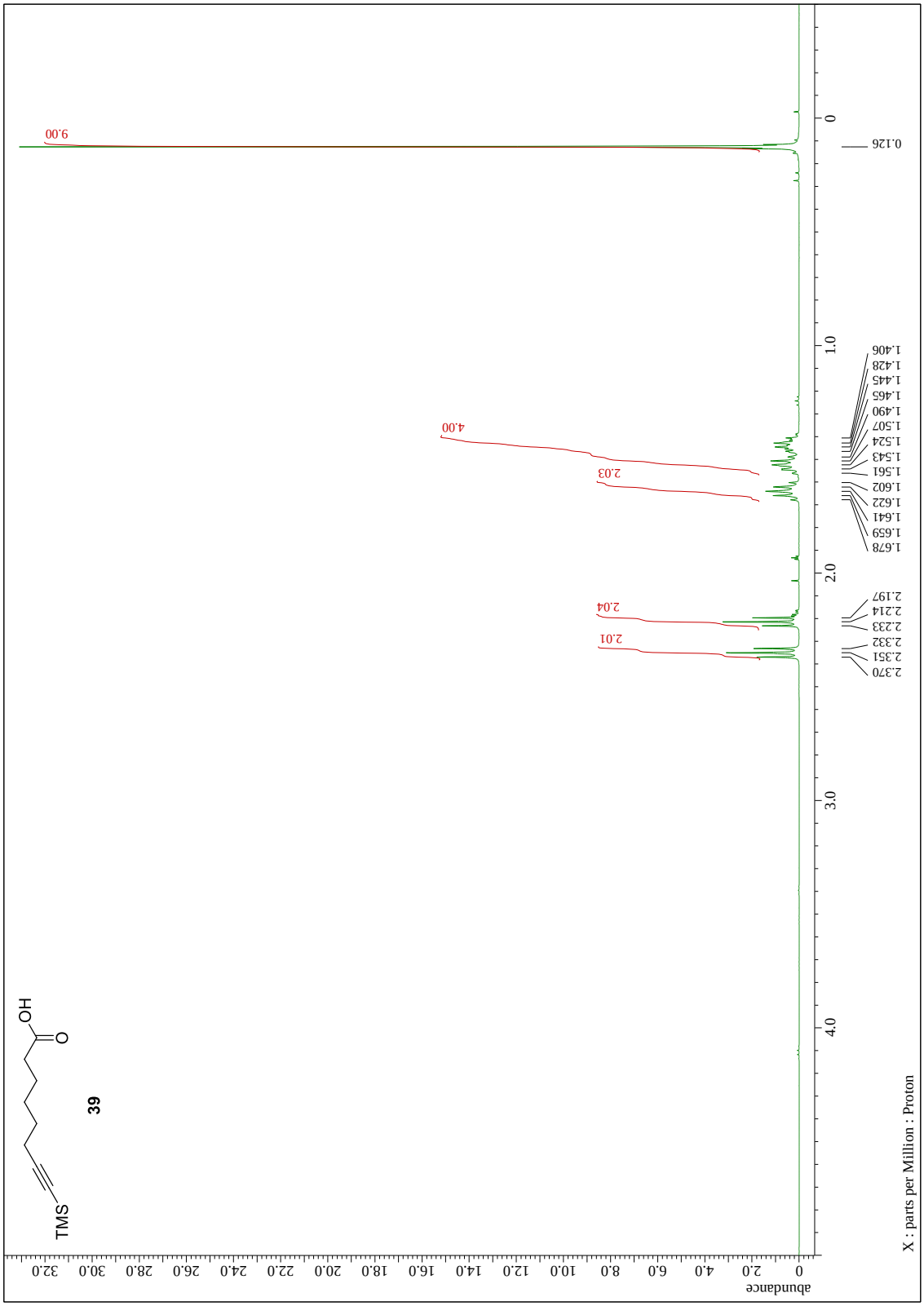
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **27**



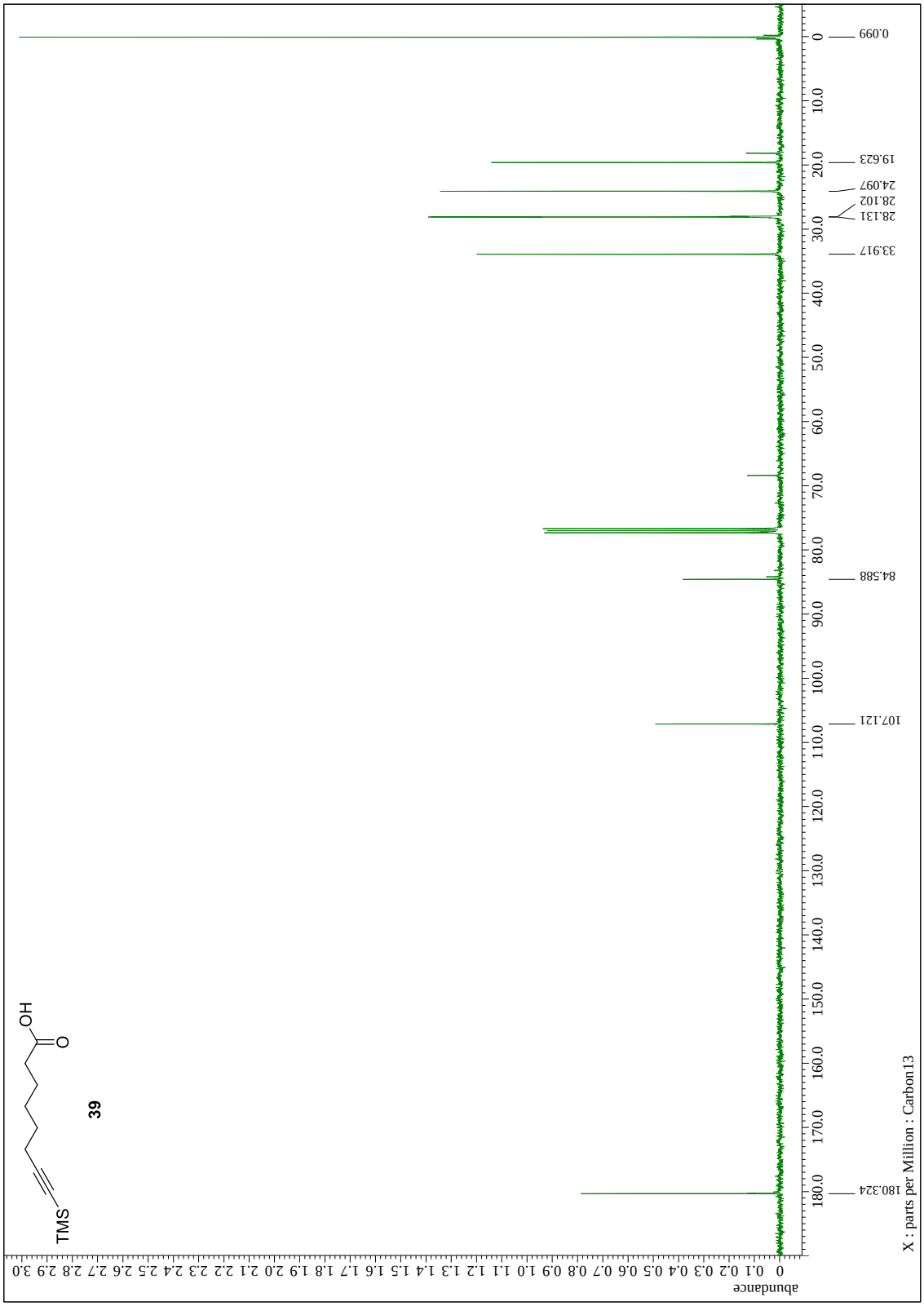
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **27**



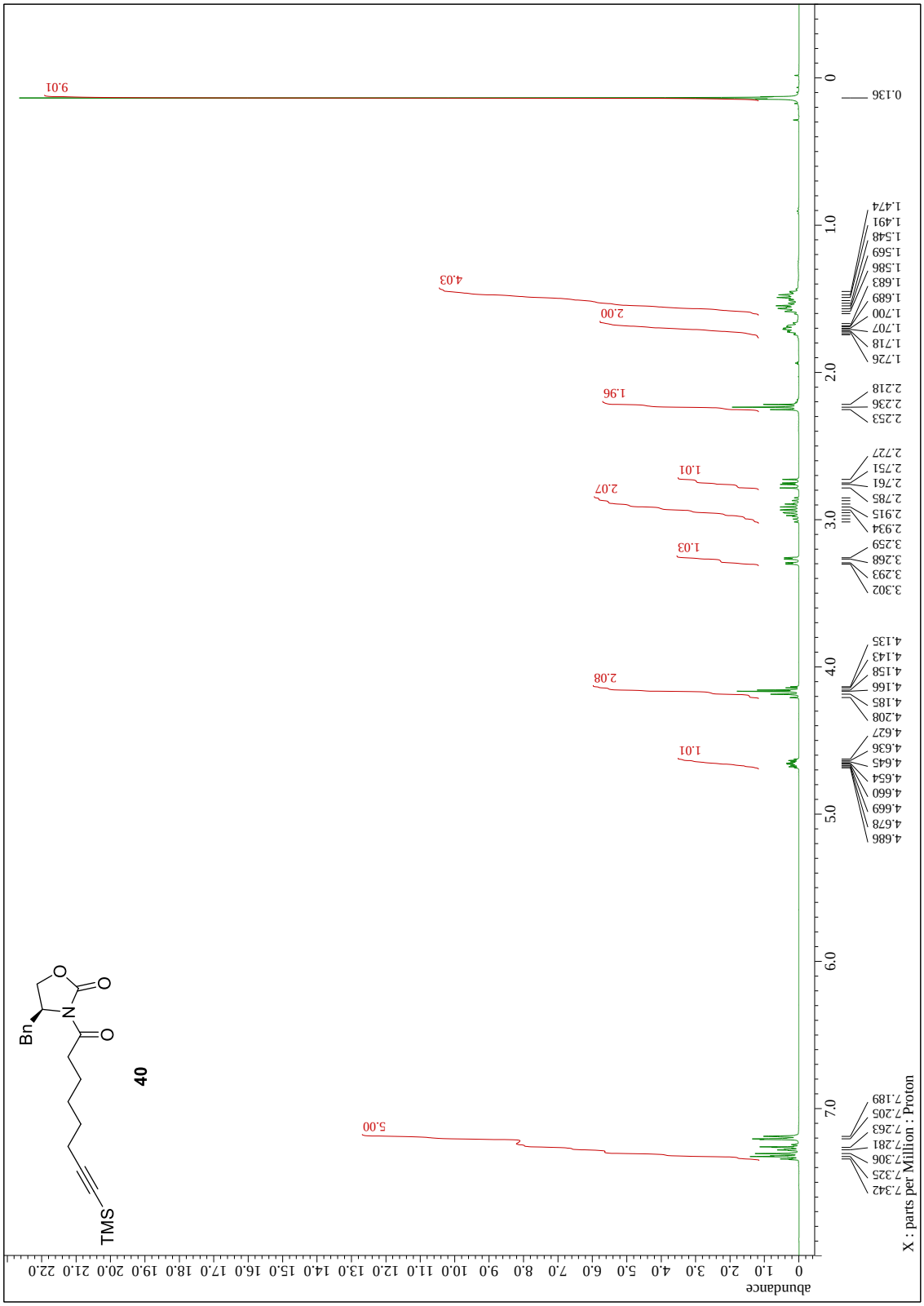
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **39**



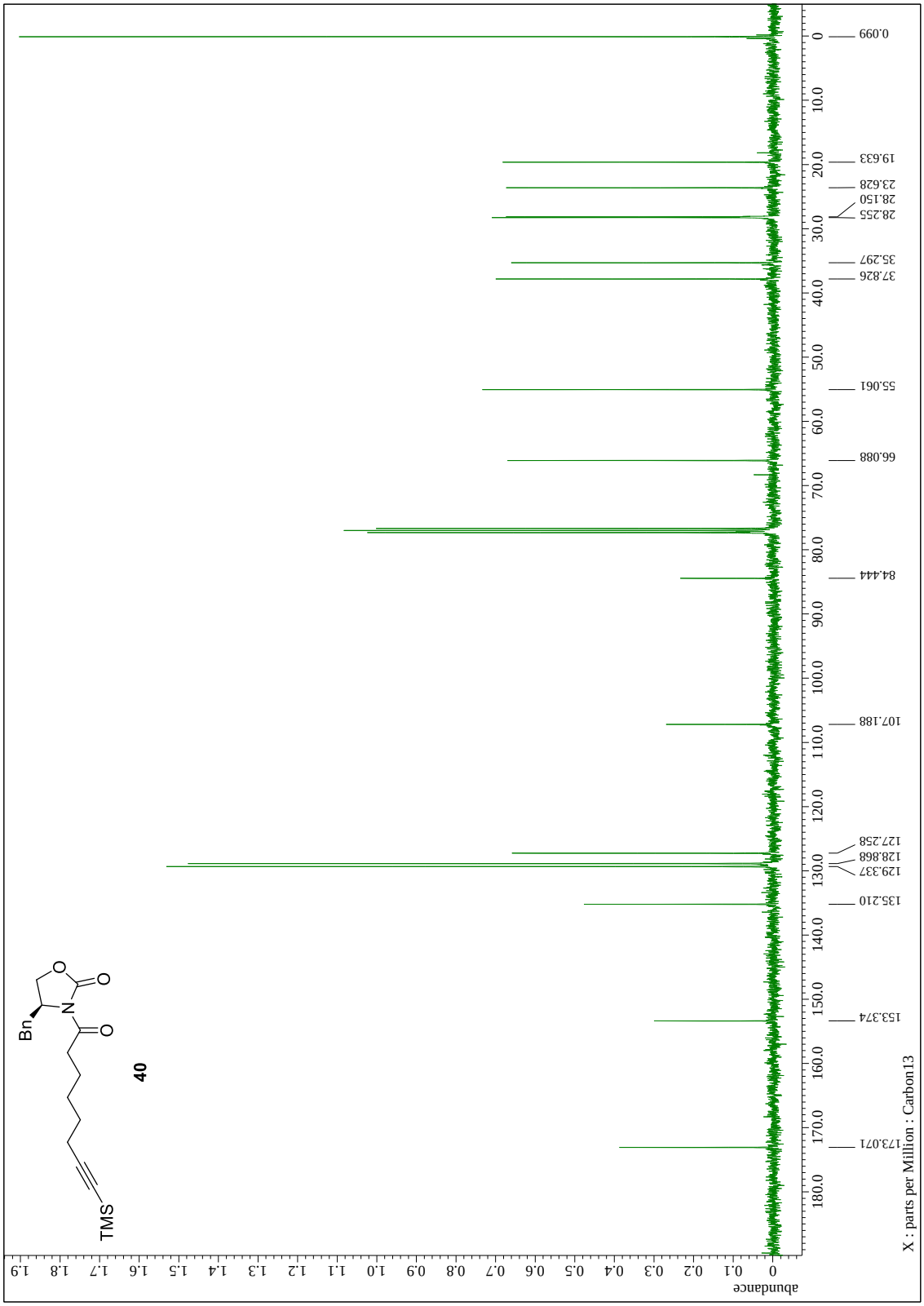
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **39**



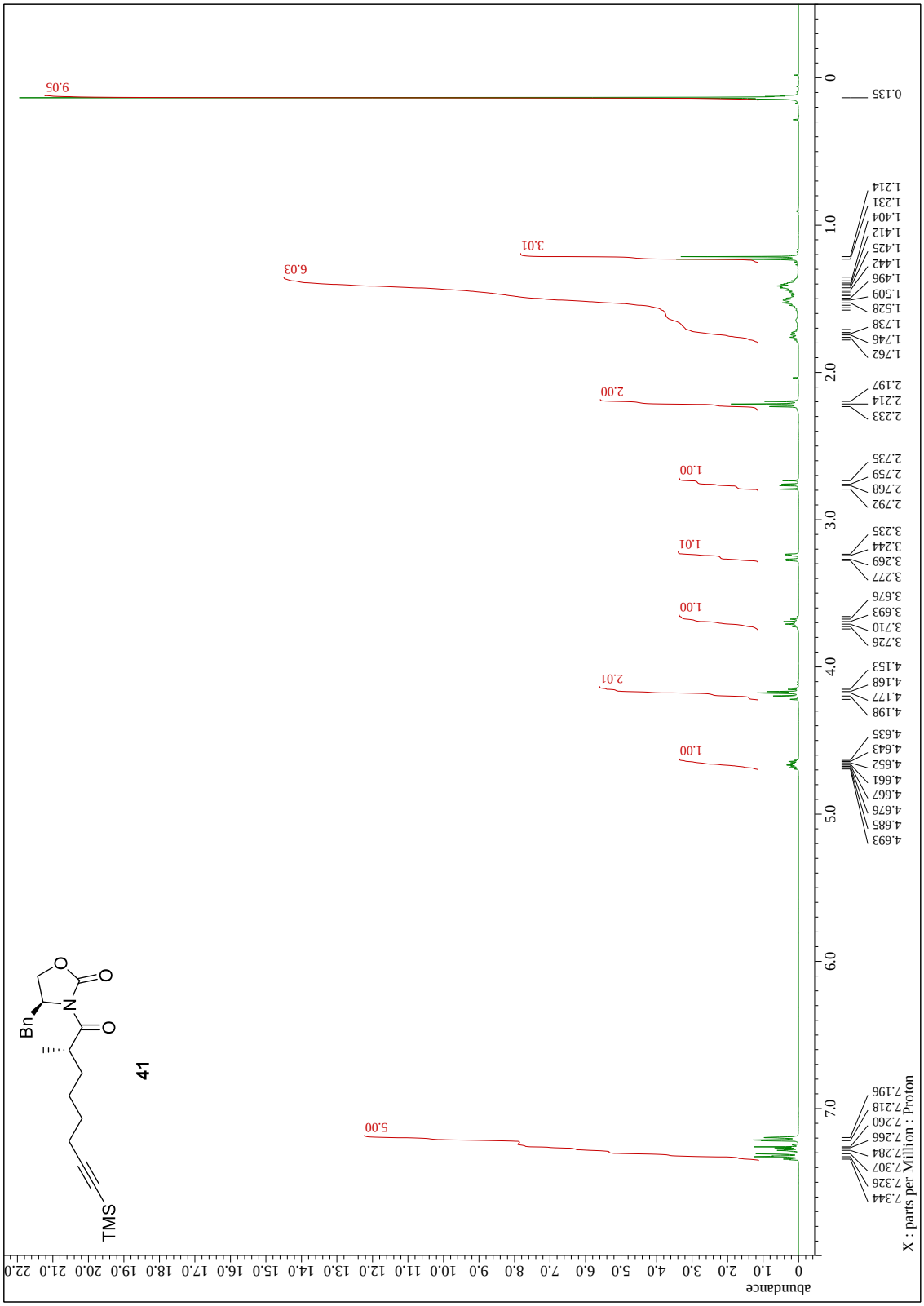
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **40**



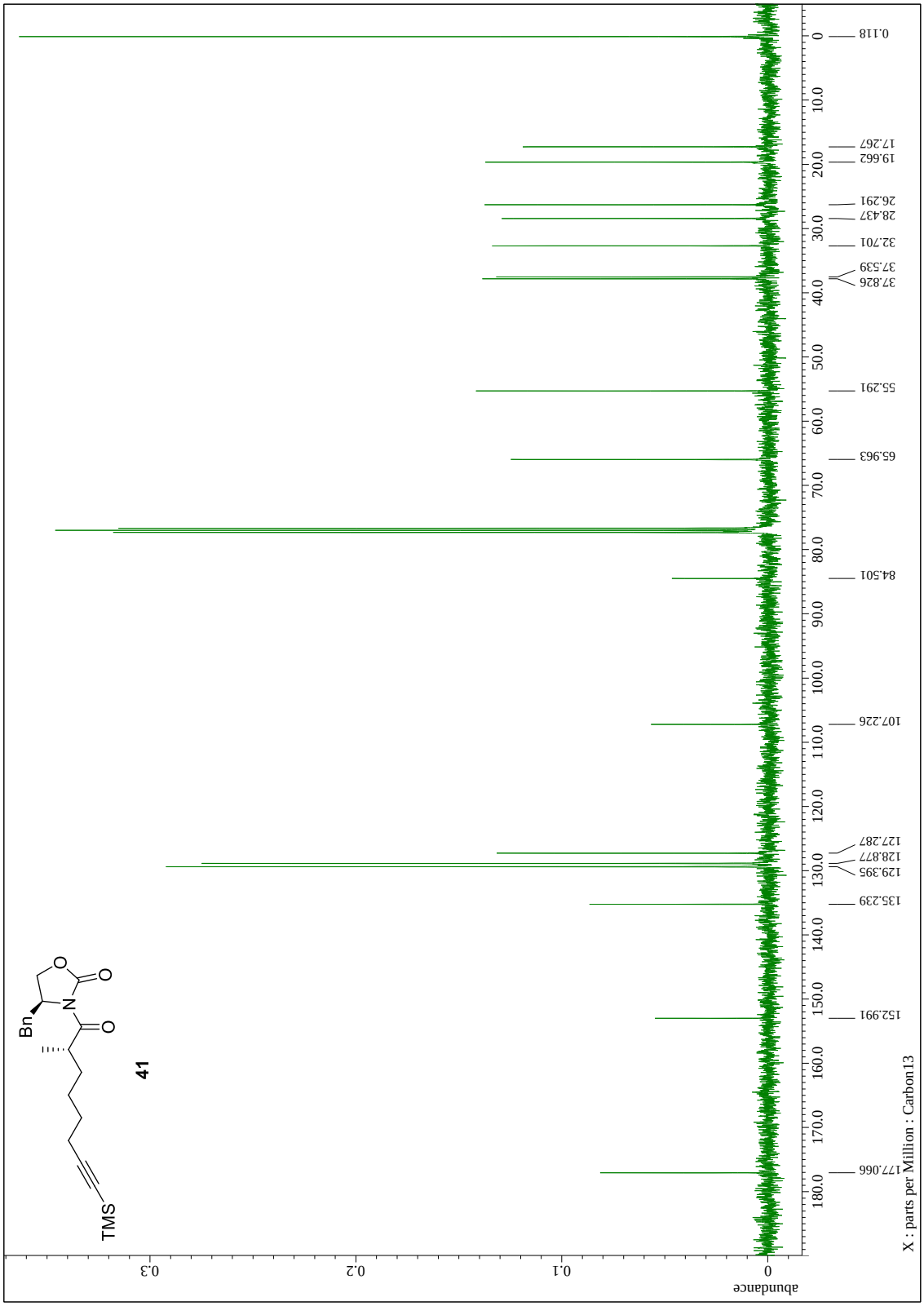
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **40**



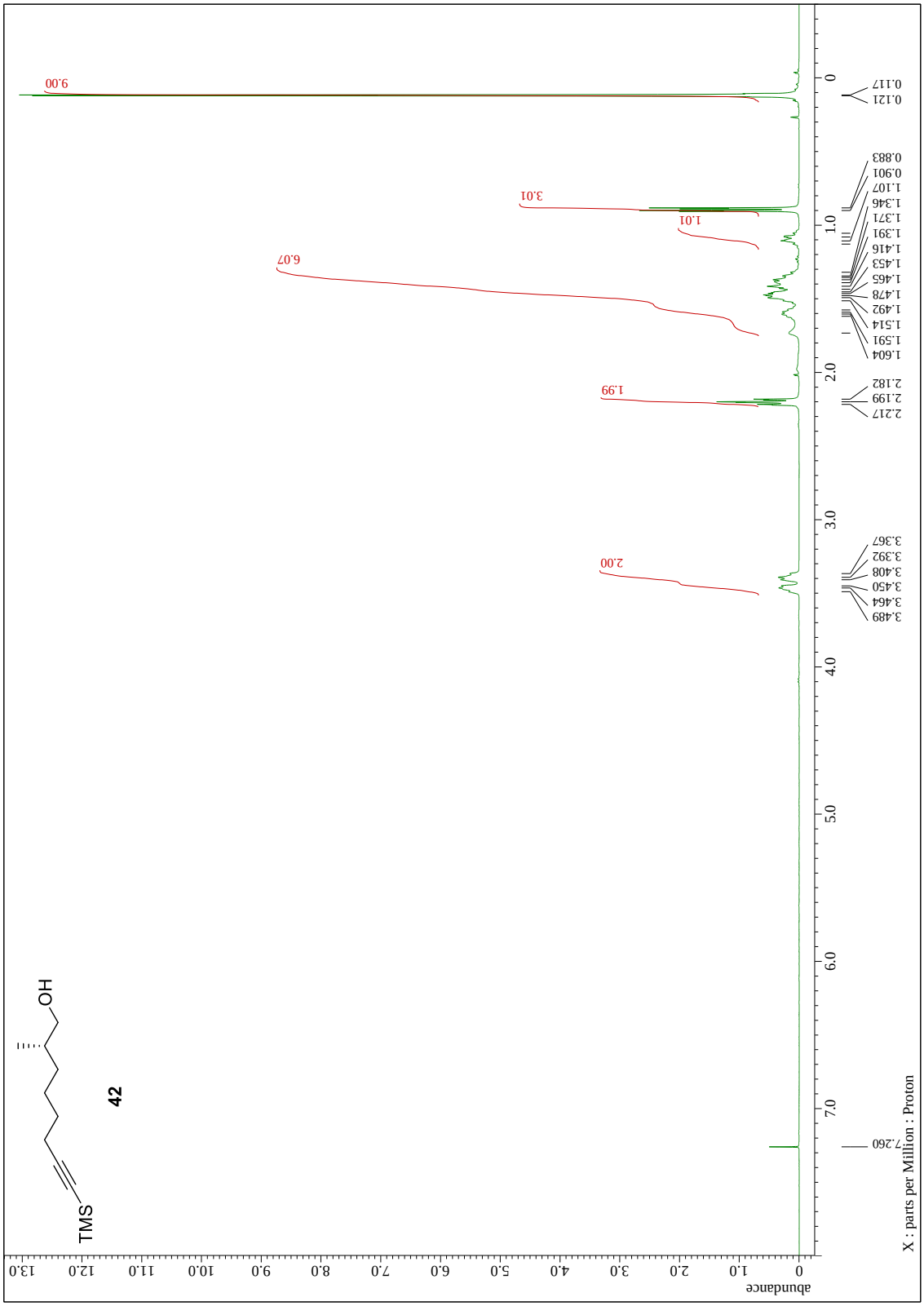
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **41**



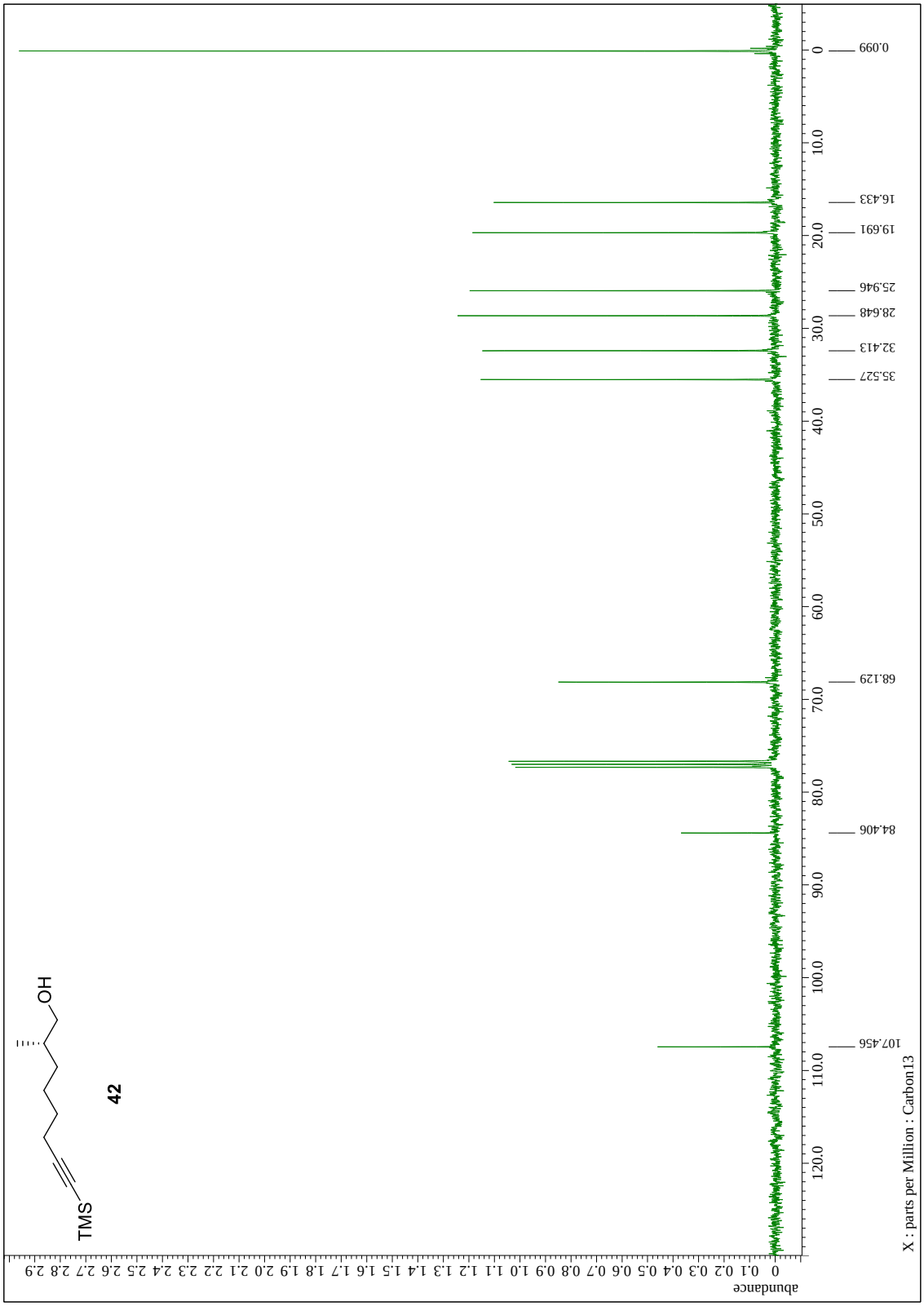
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **41**



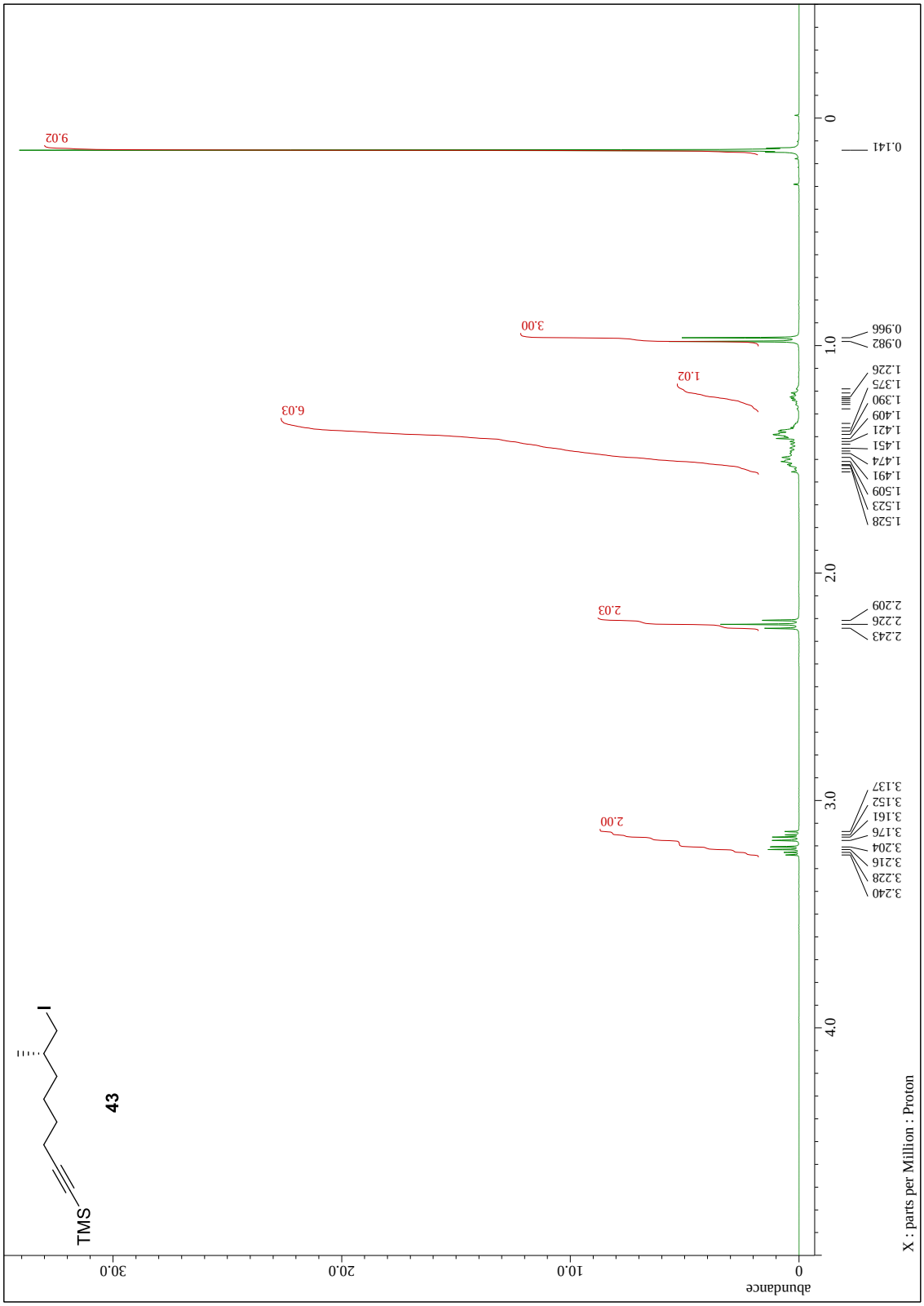
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **42**



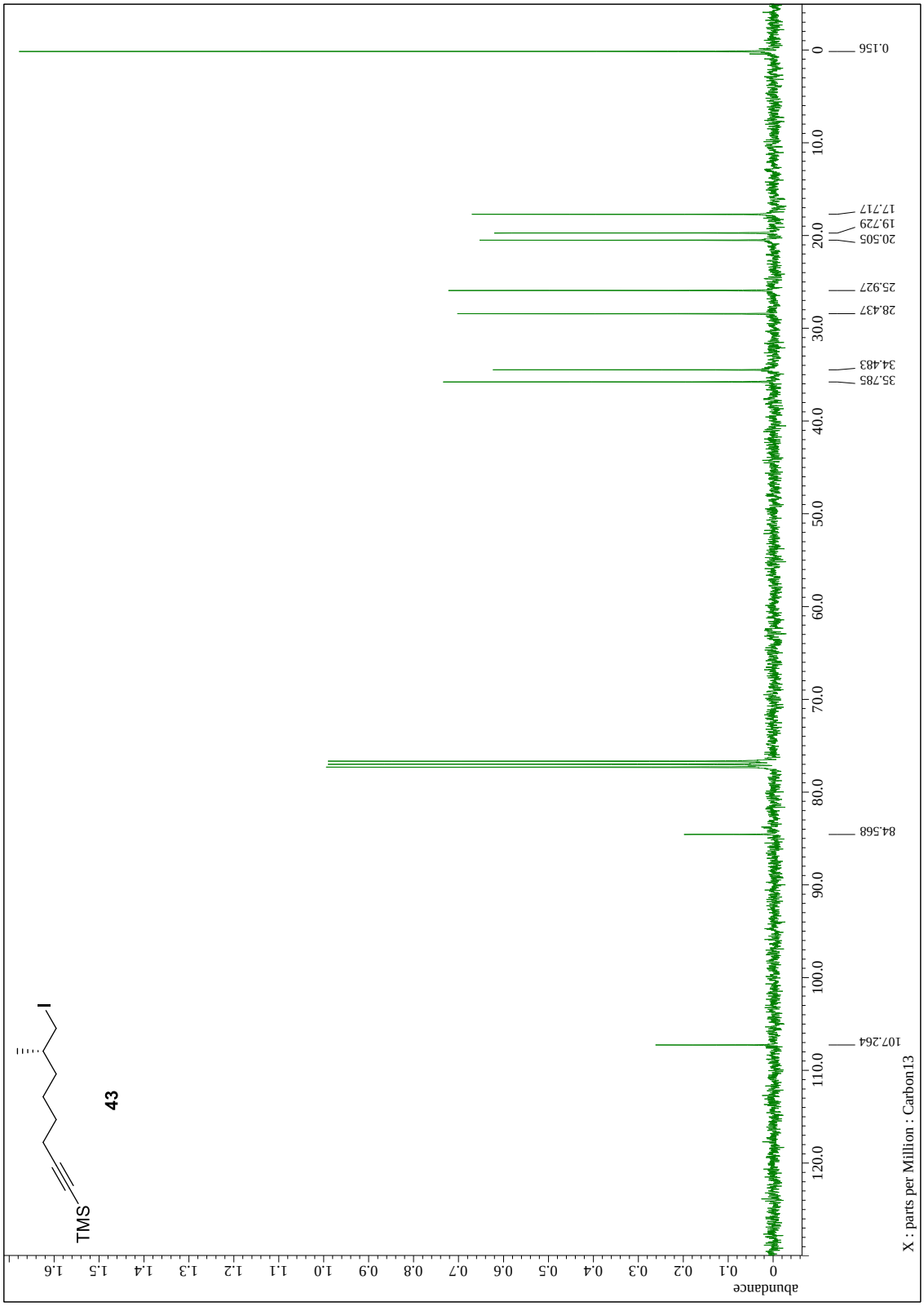
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **42**



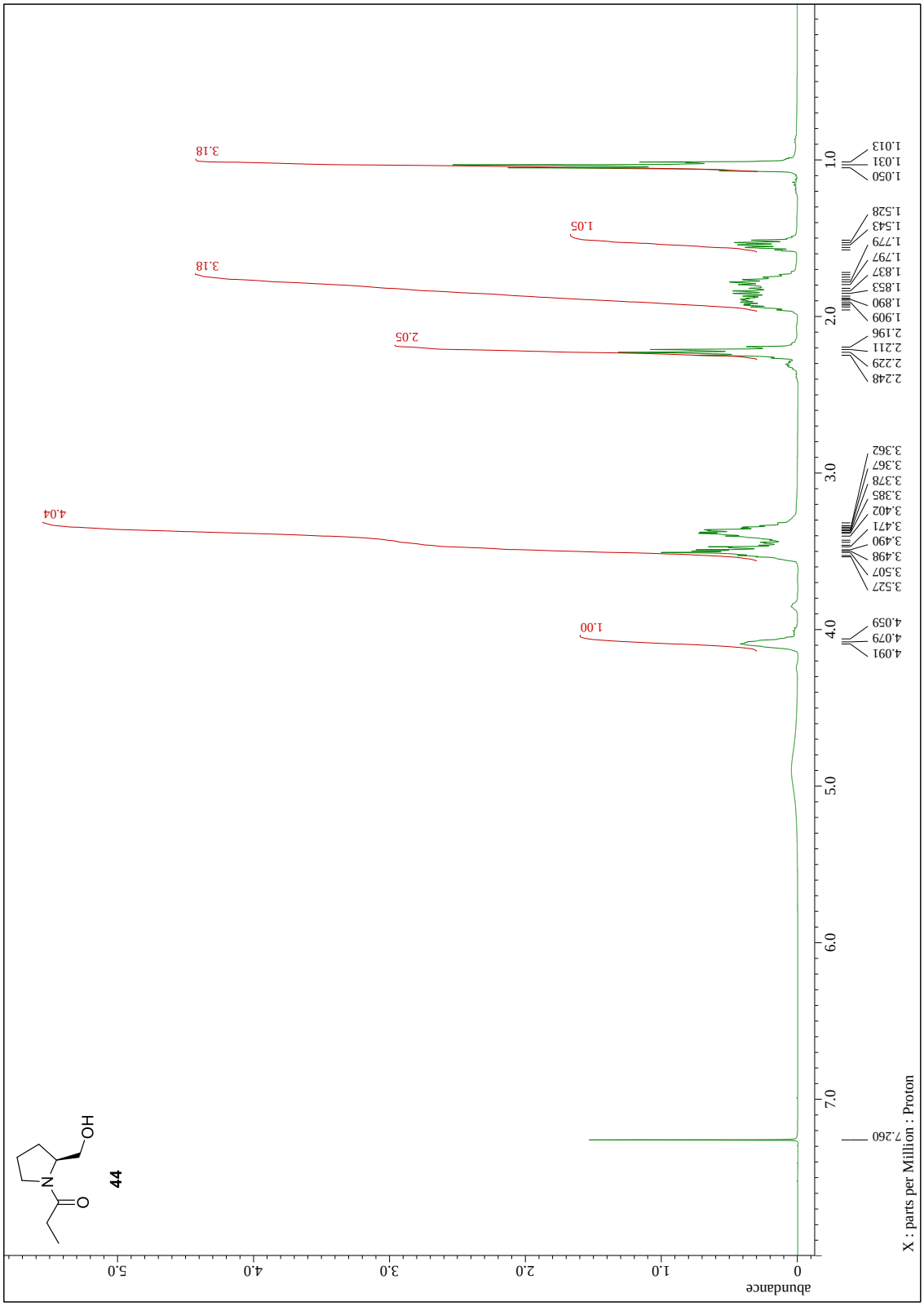
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **43**



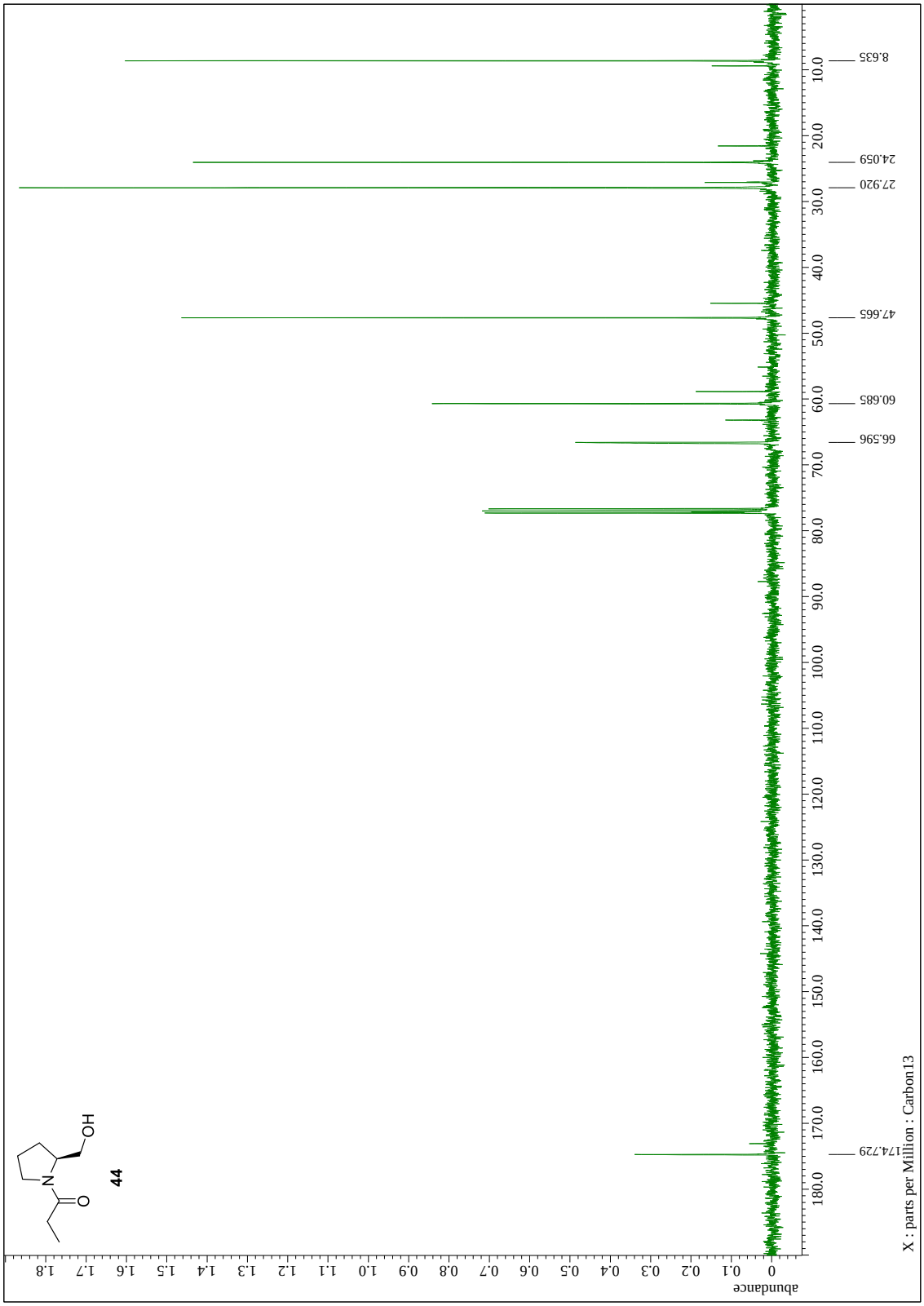
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **43**



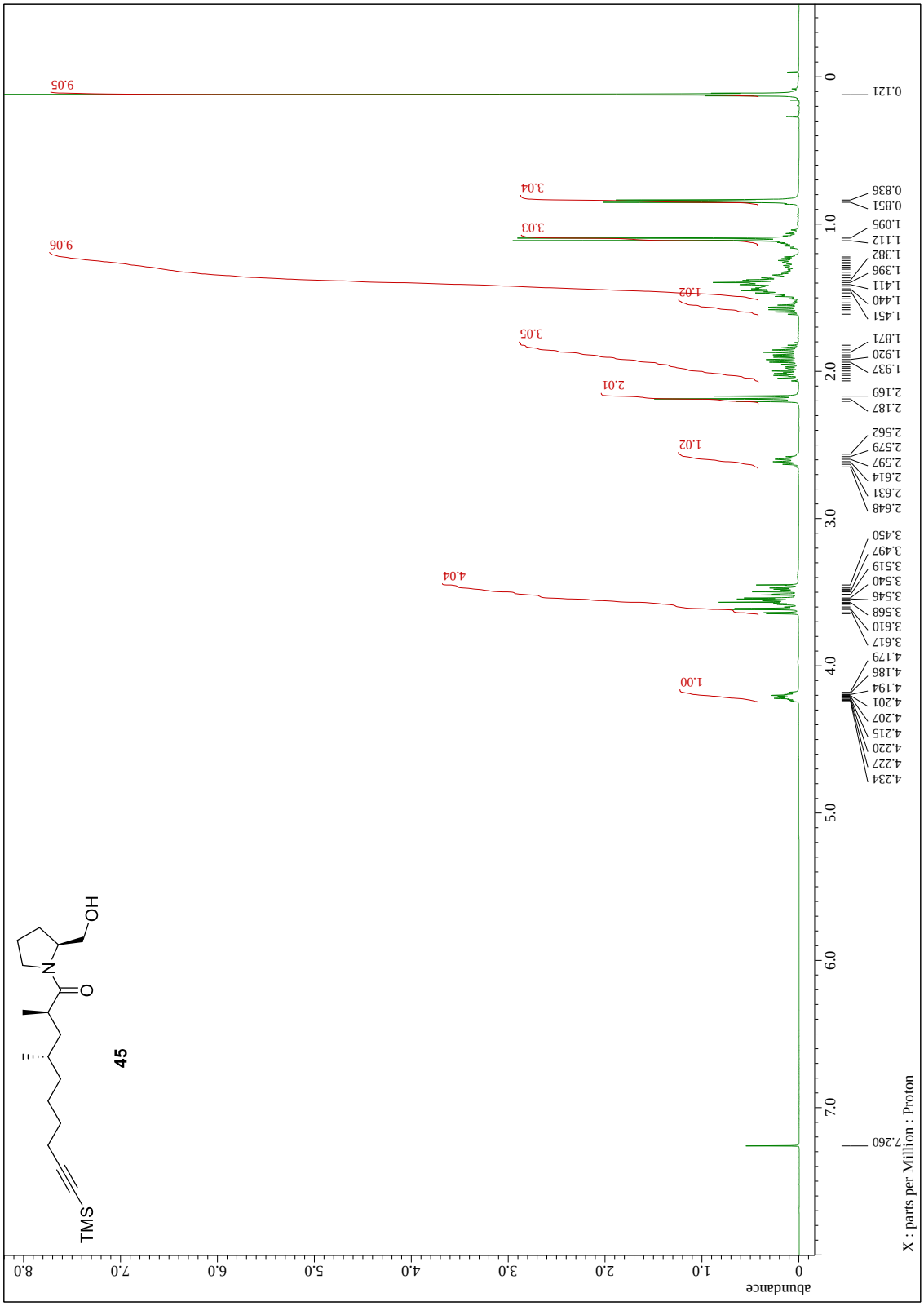
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **44**



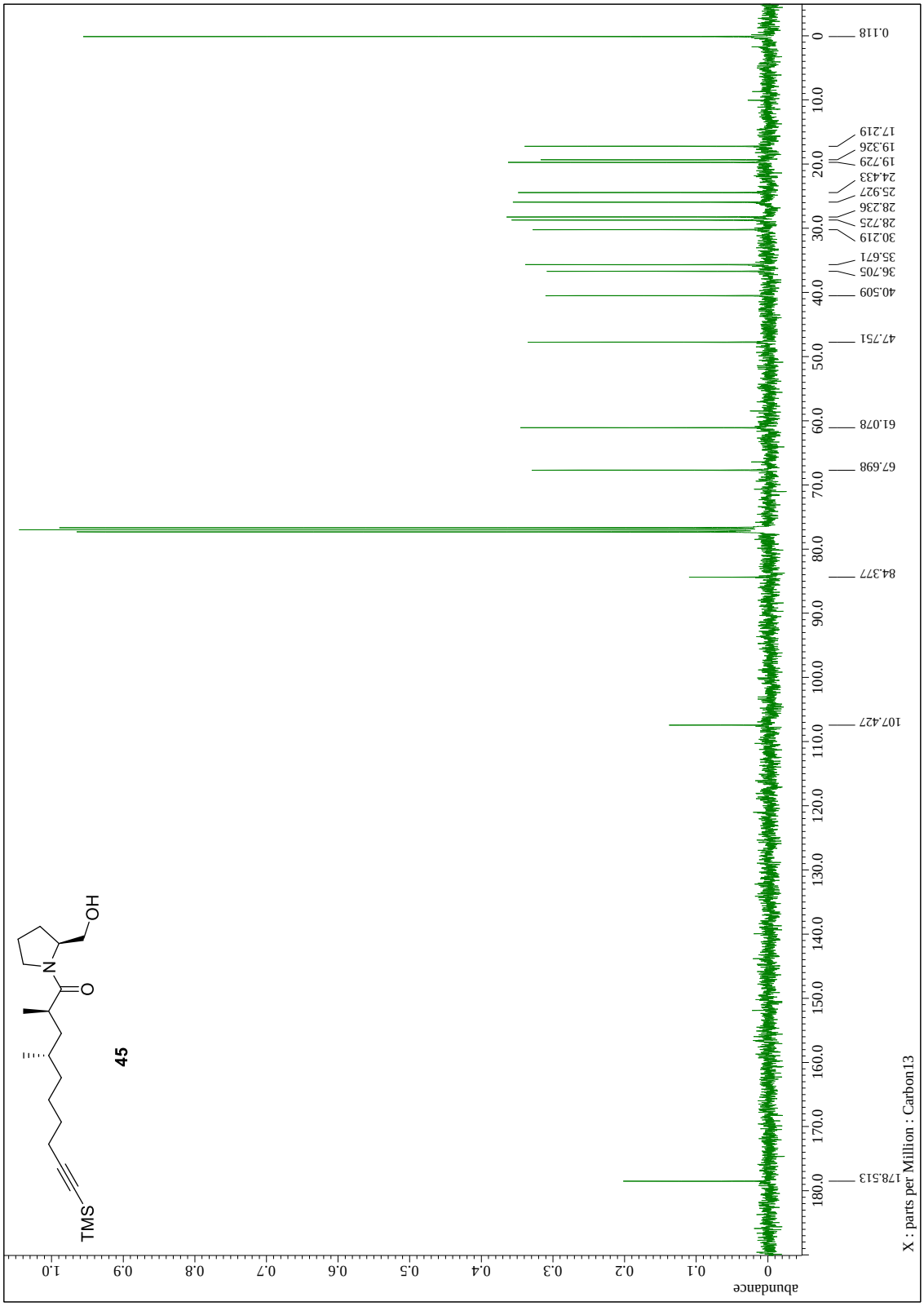
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **44**



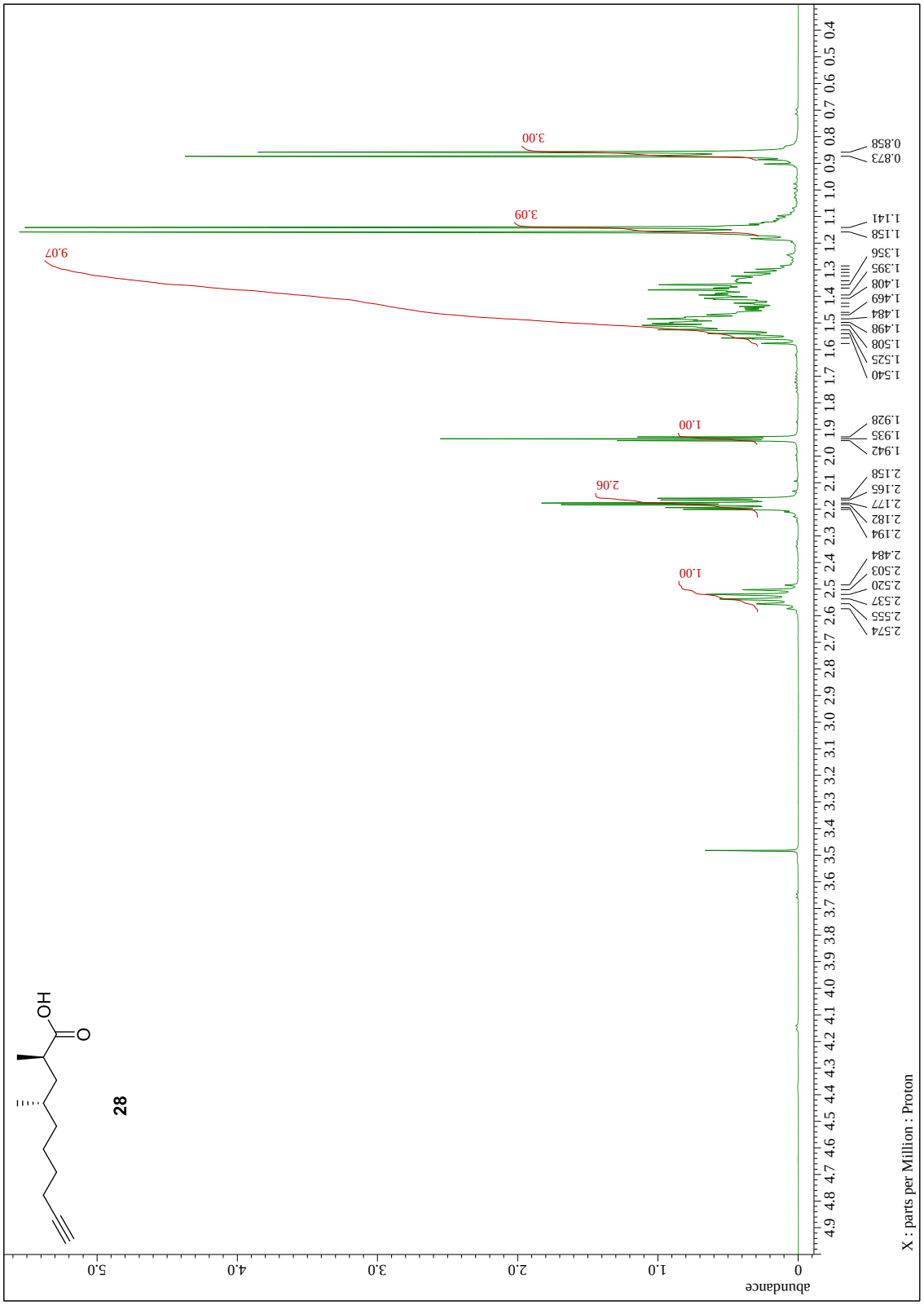
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **45**



$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **45**



^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **28**



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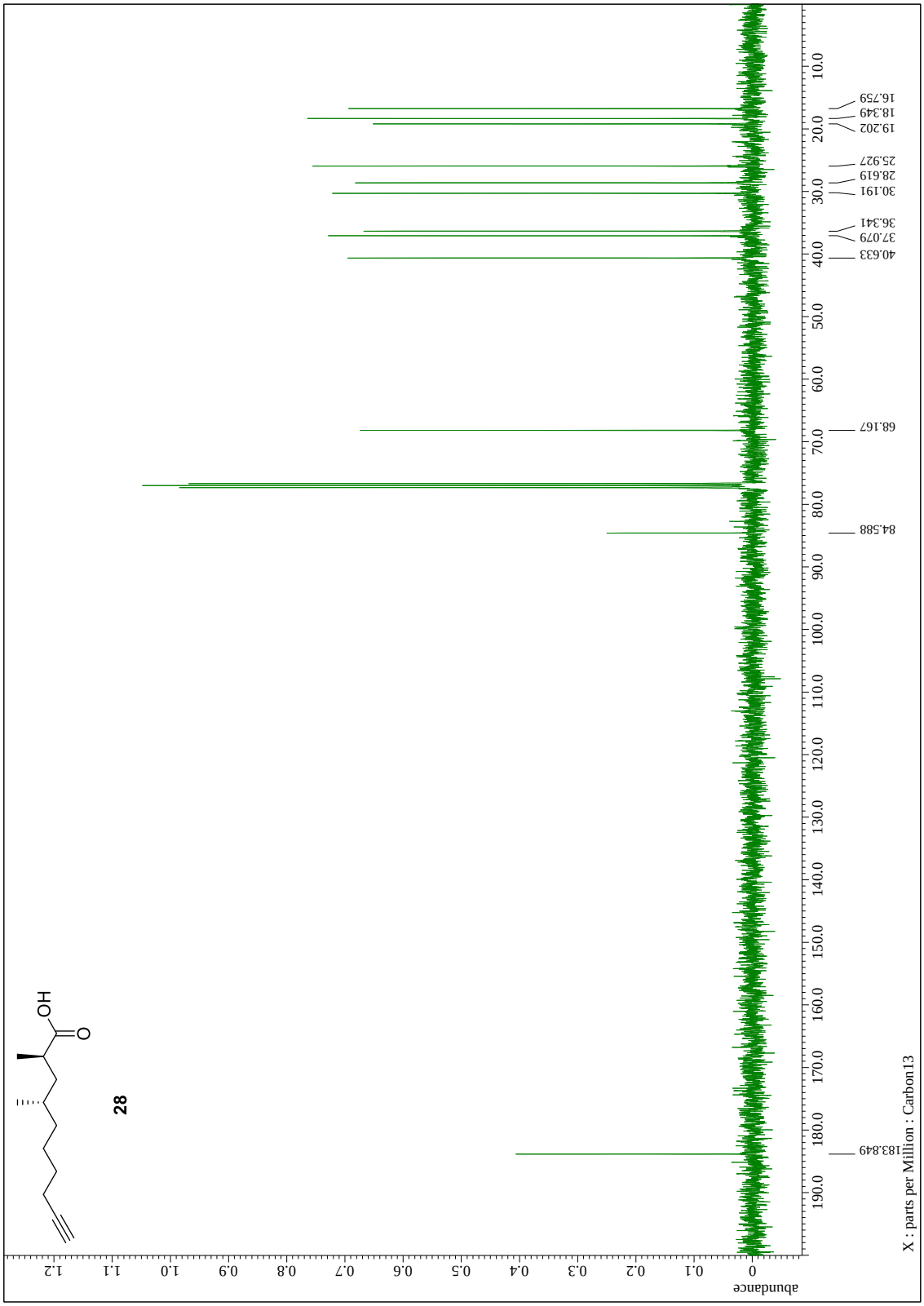

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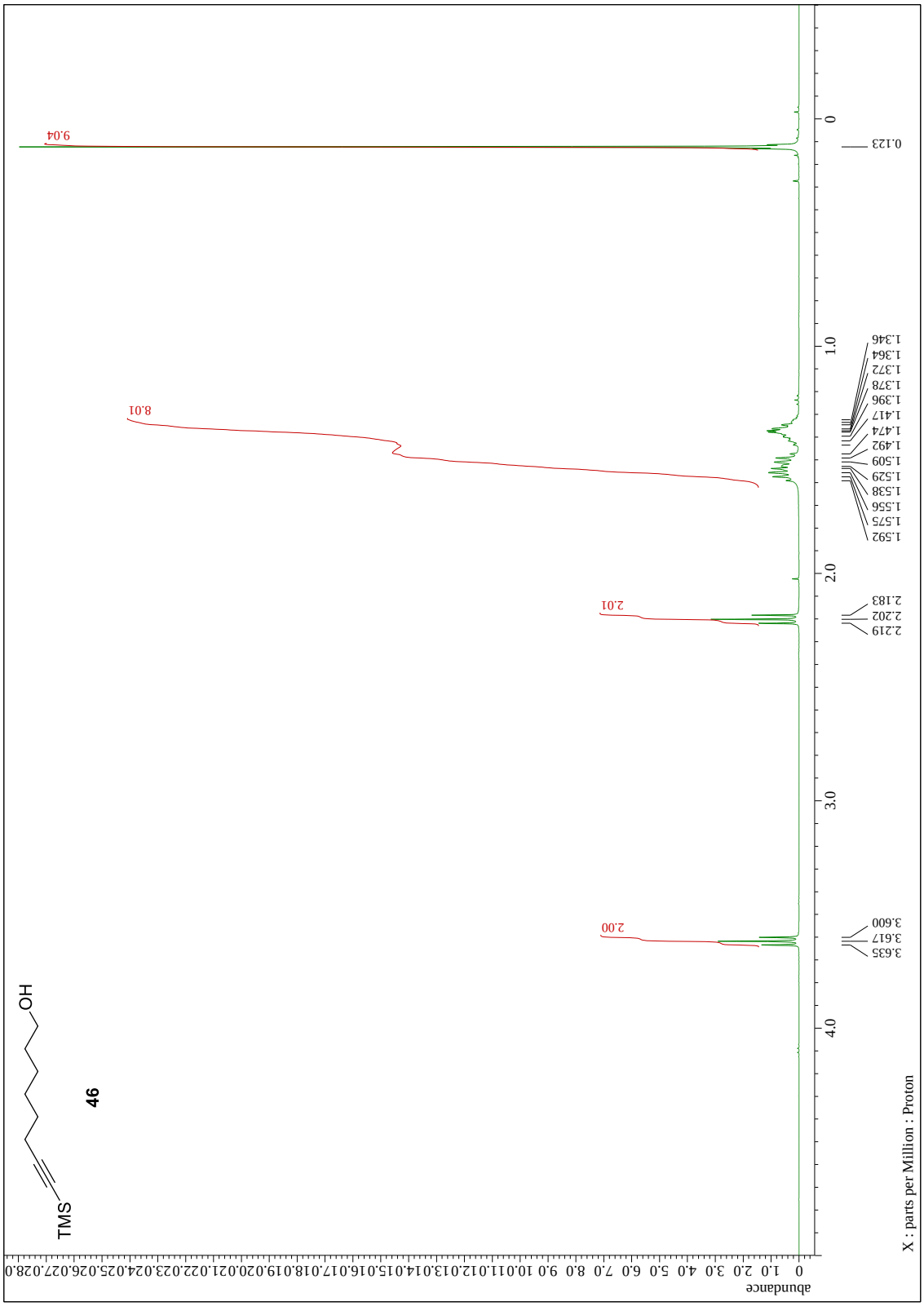

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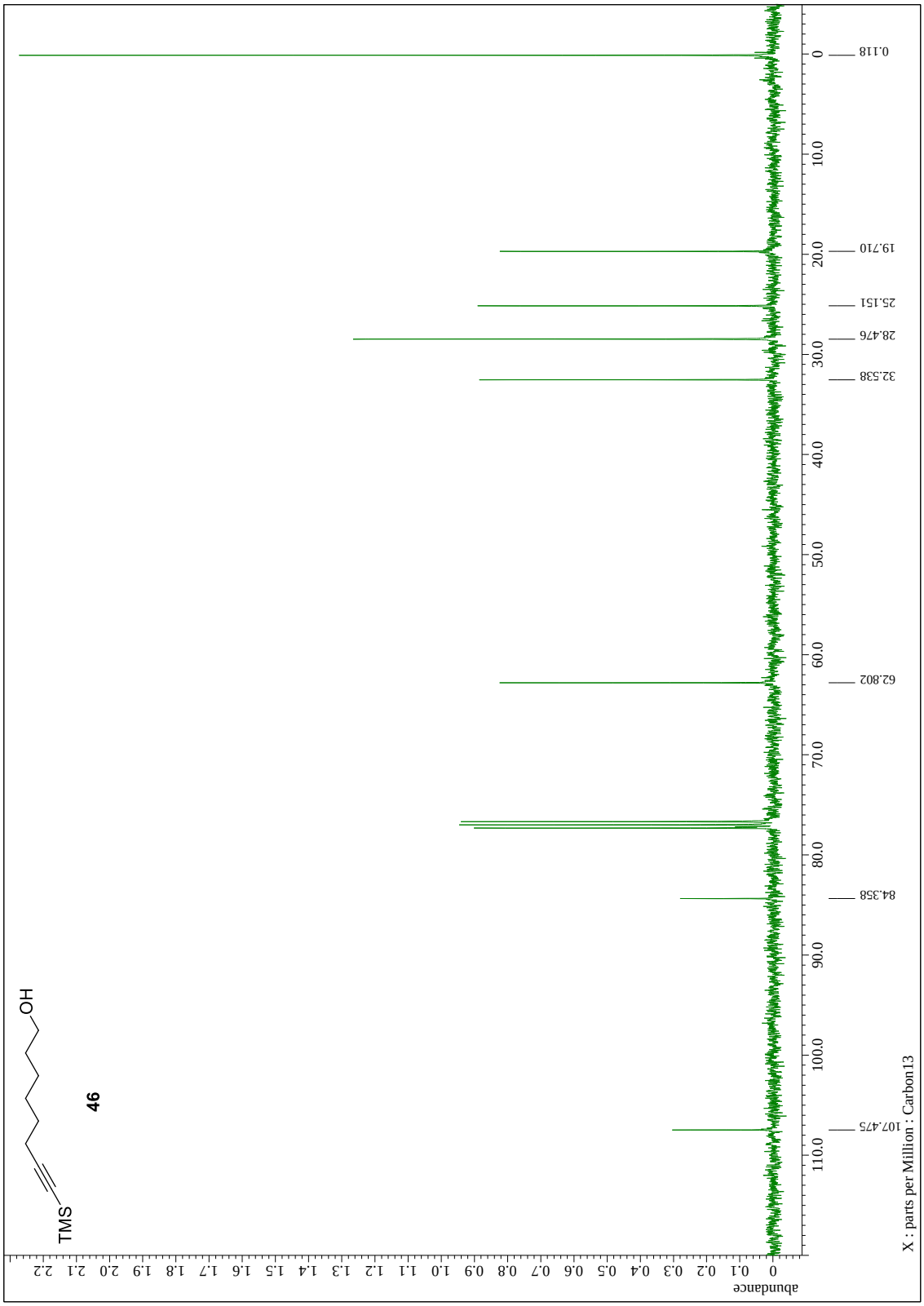

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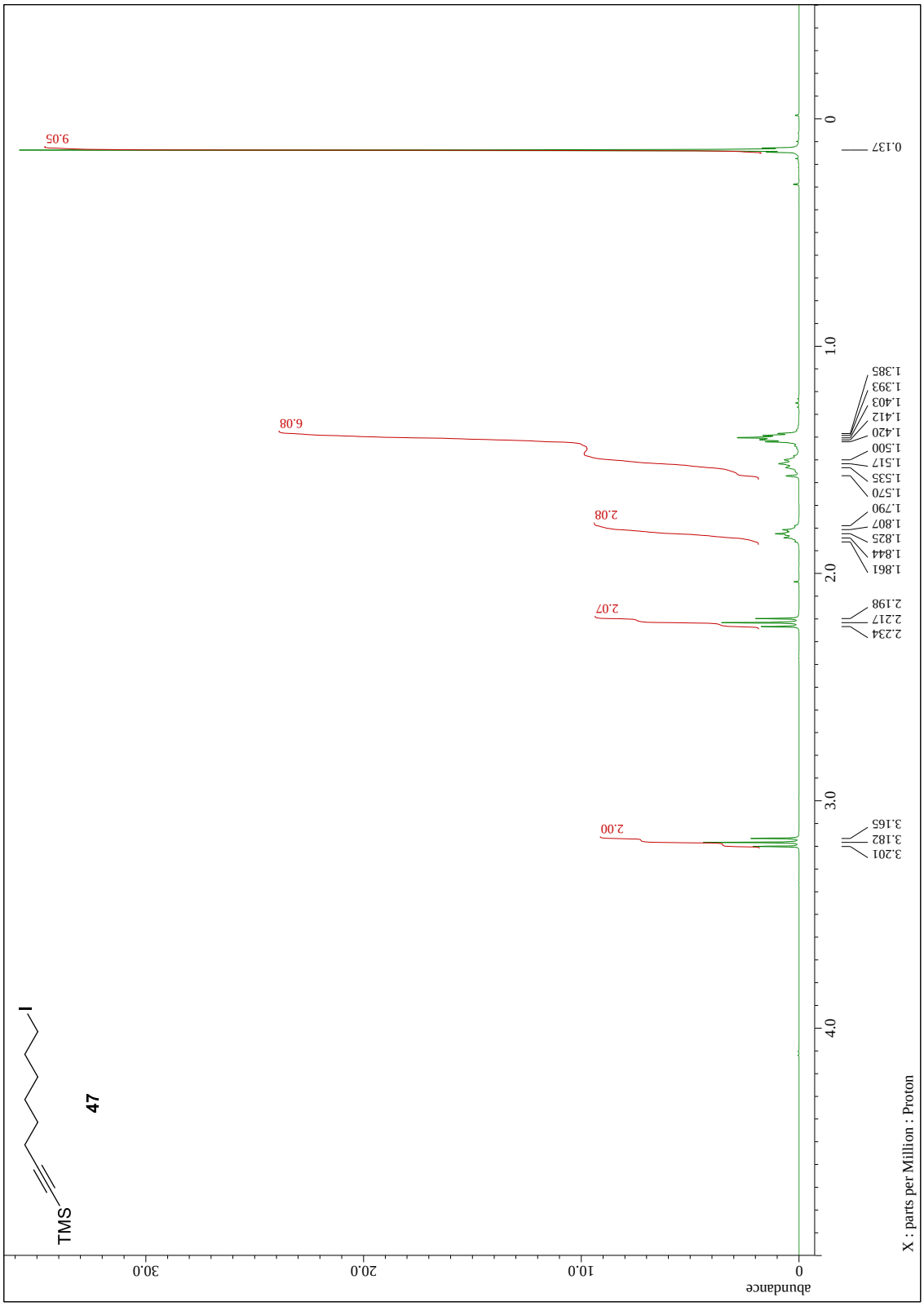

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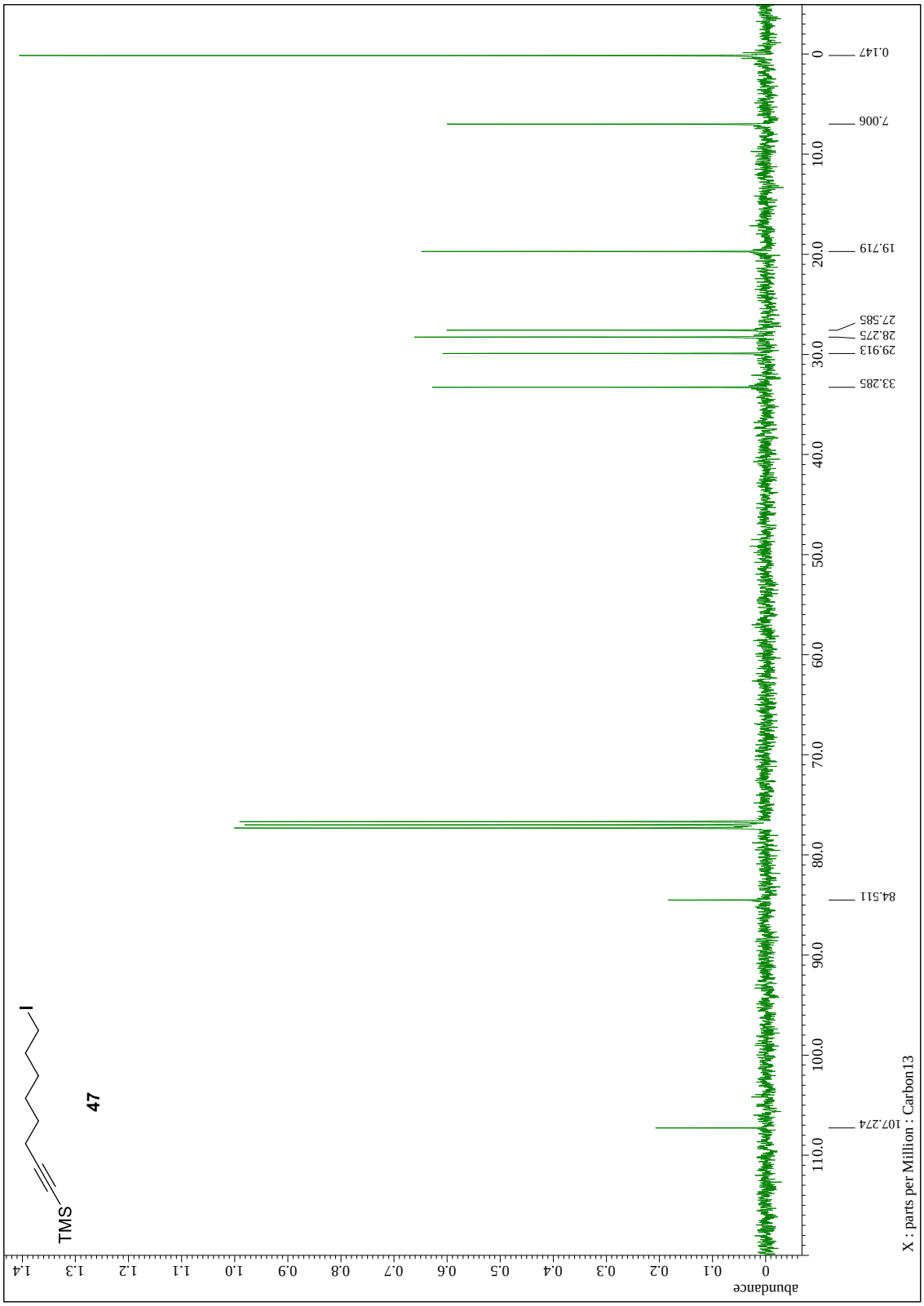
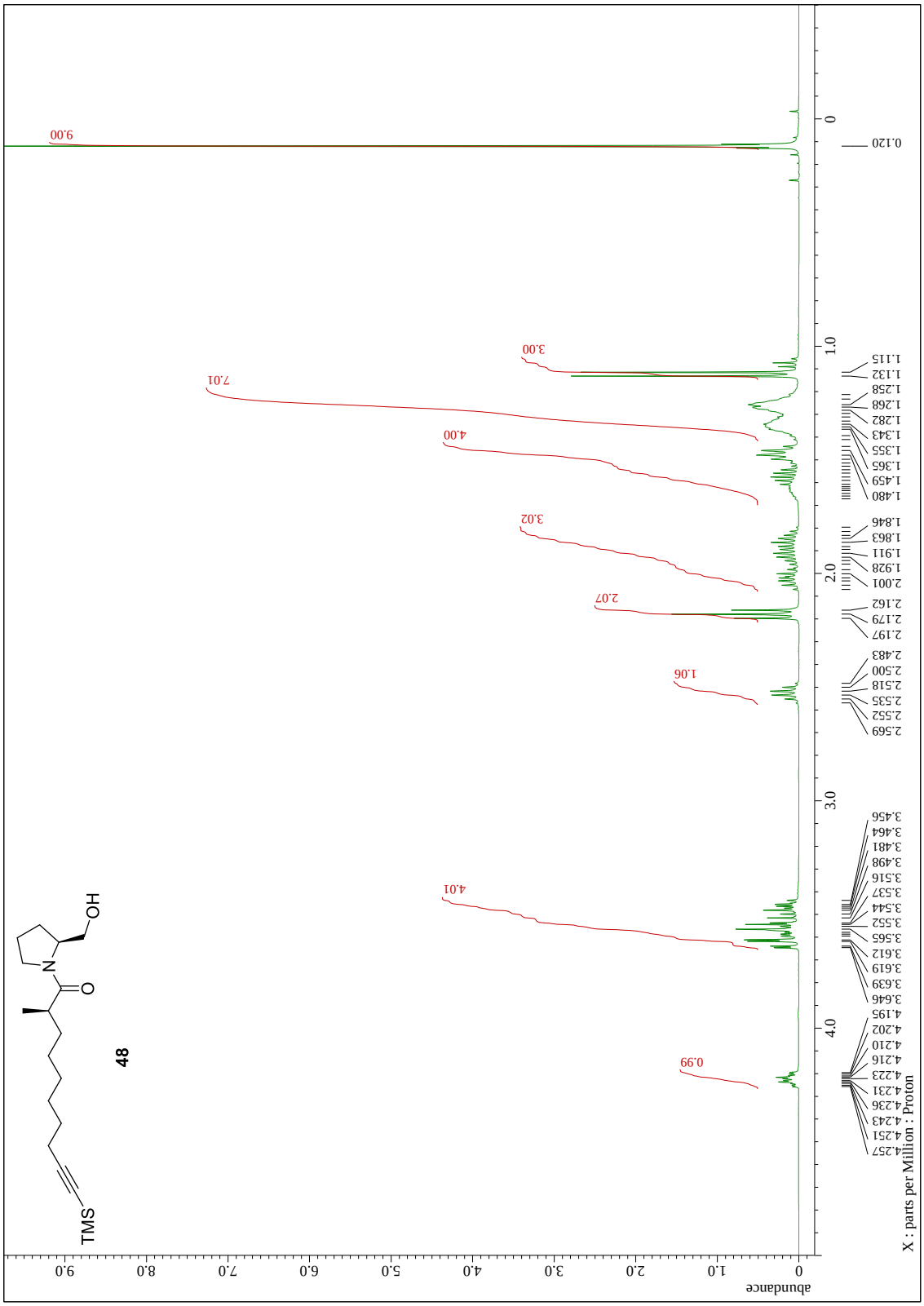


^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **48**



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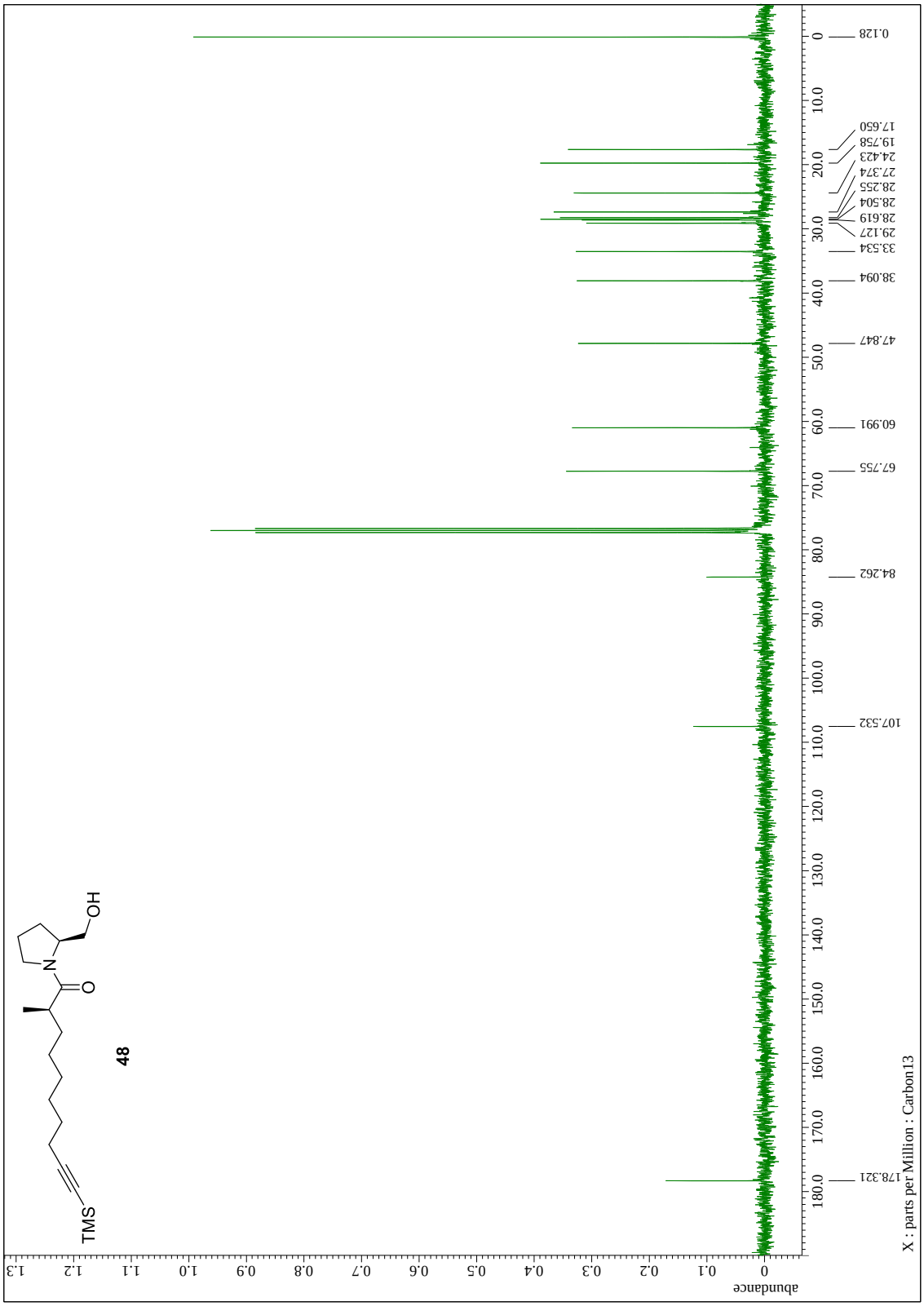

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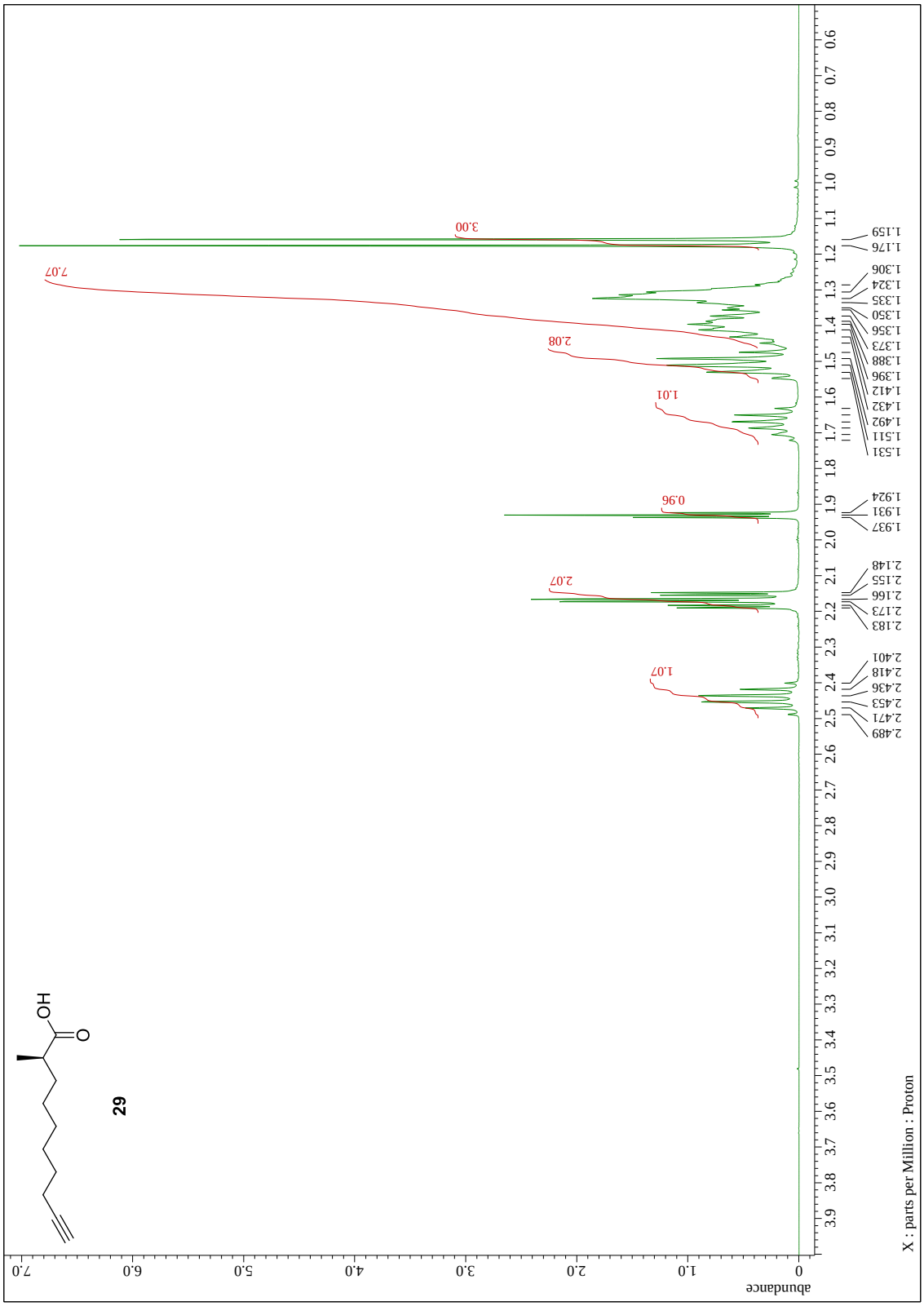
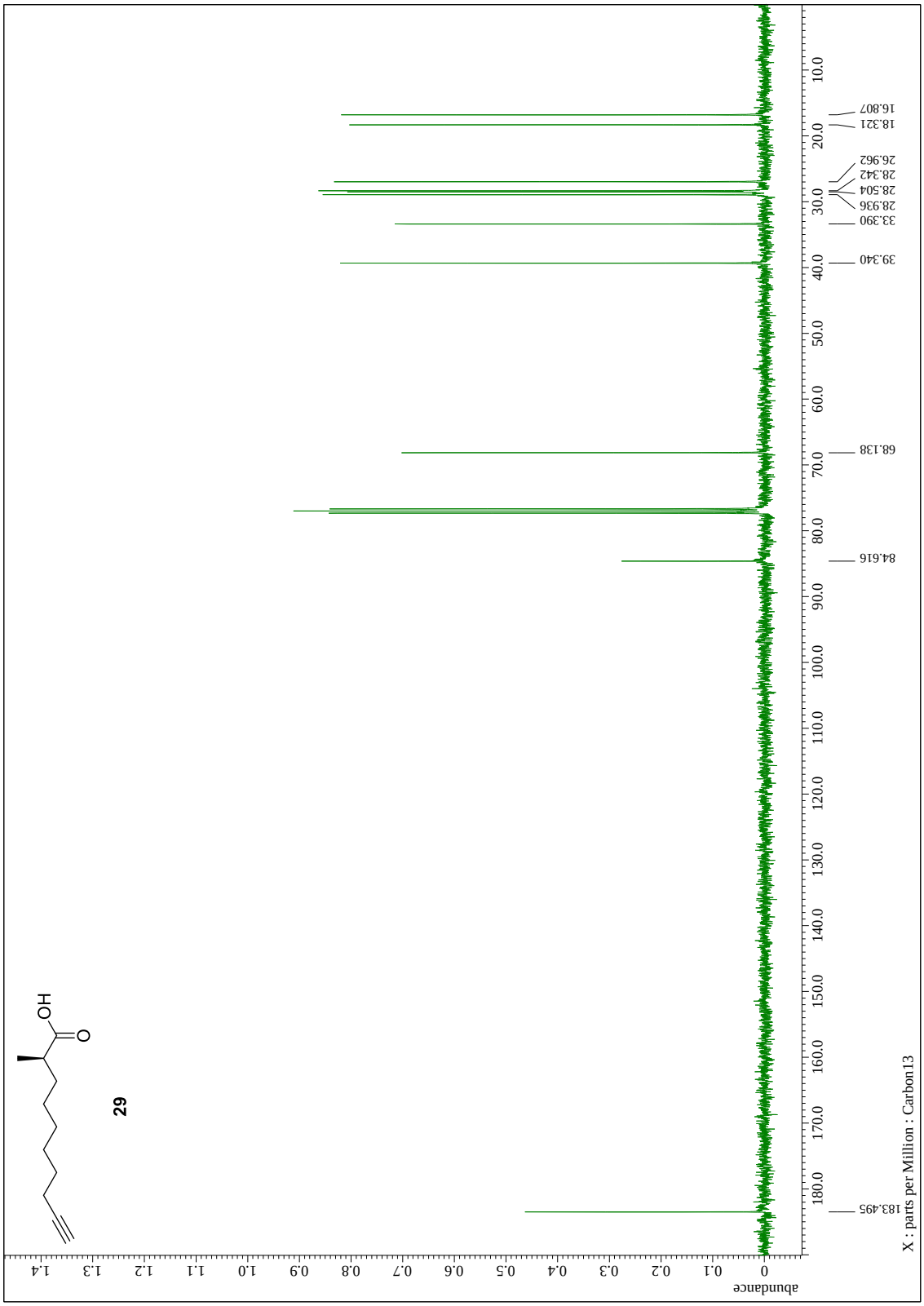
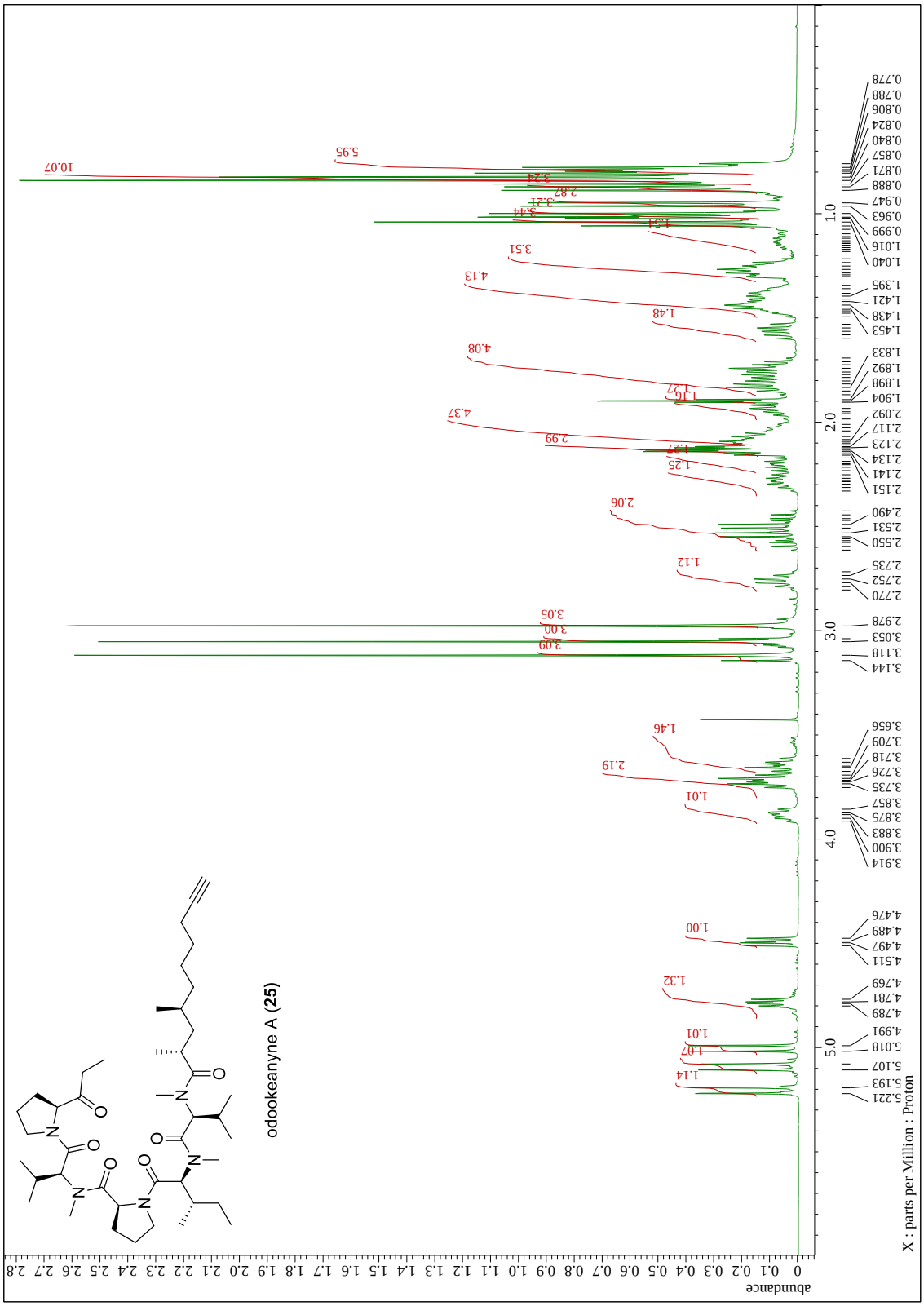


$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **29**



^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **25**



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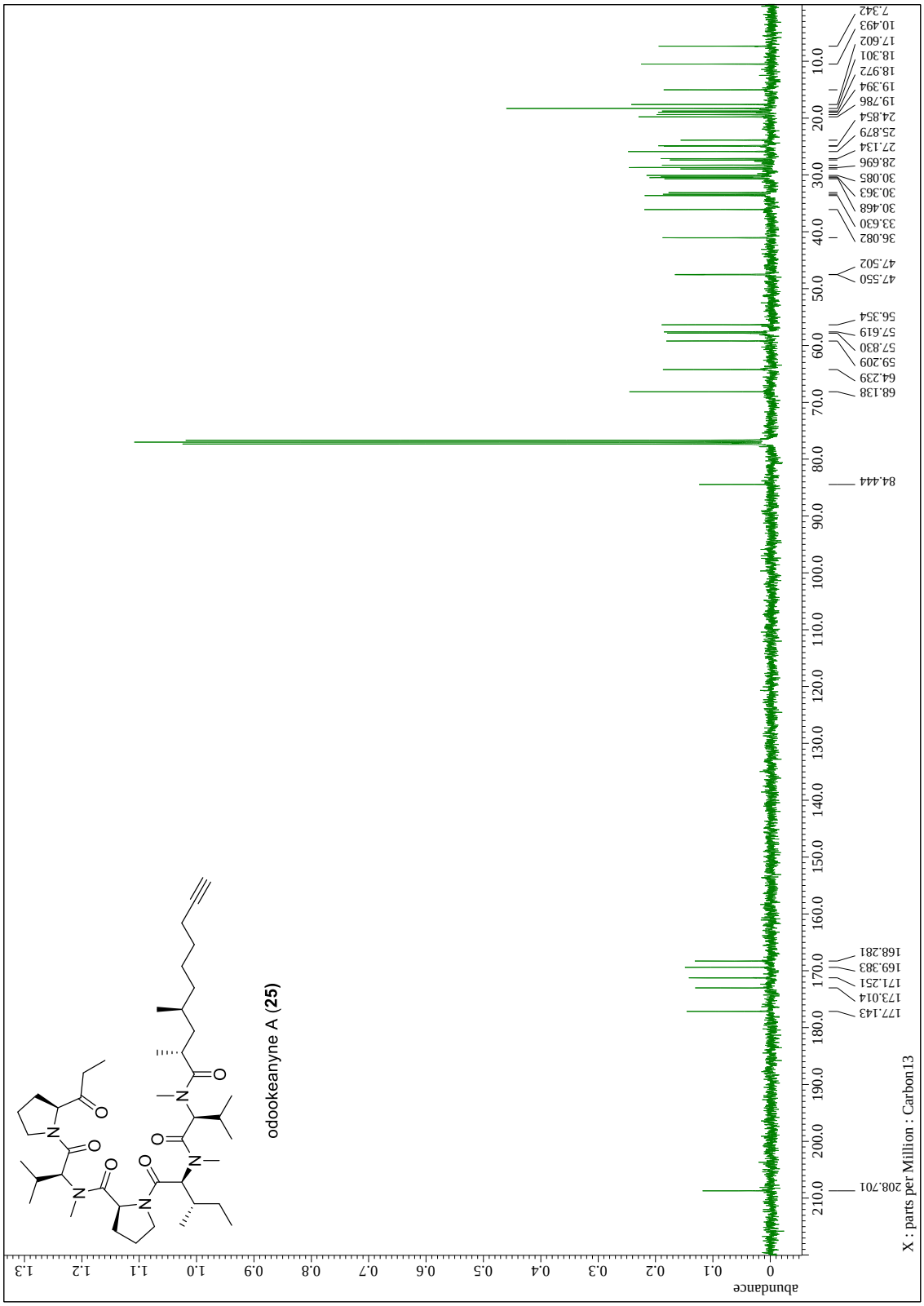
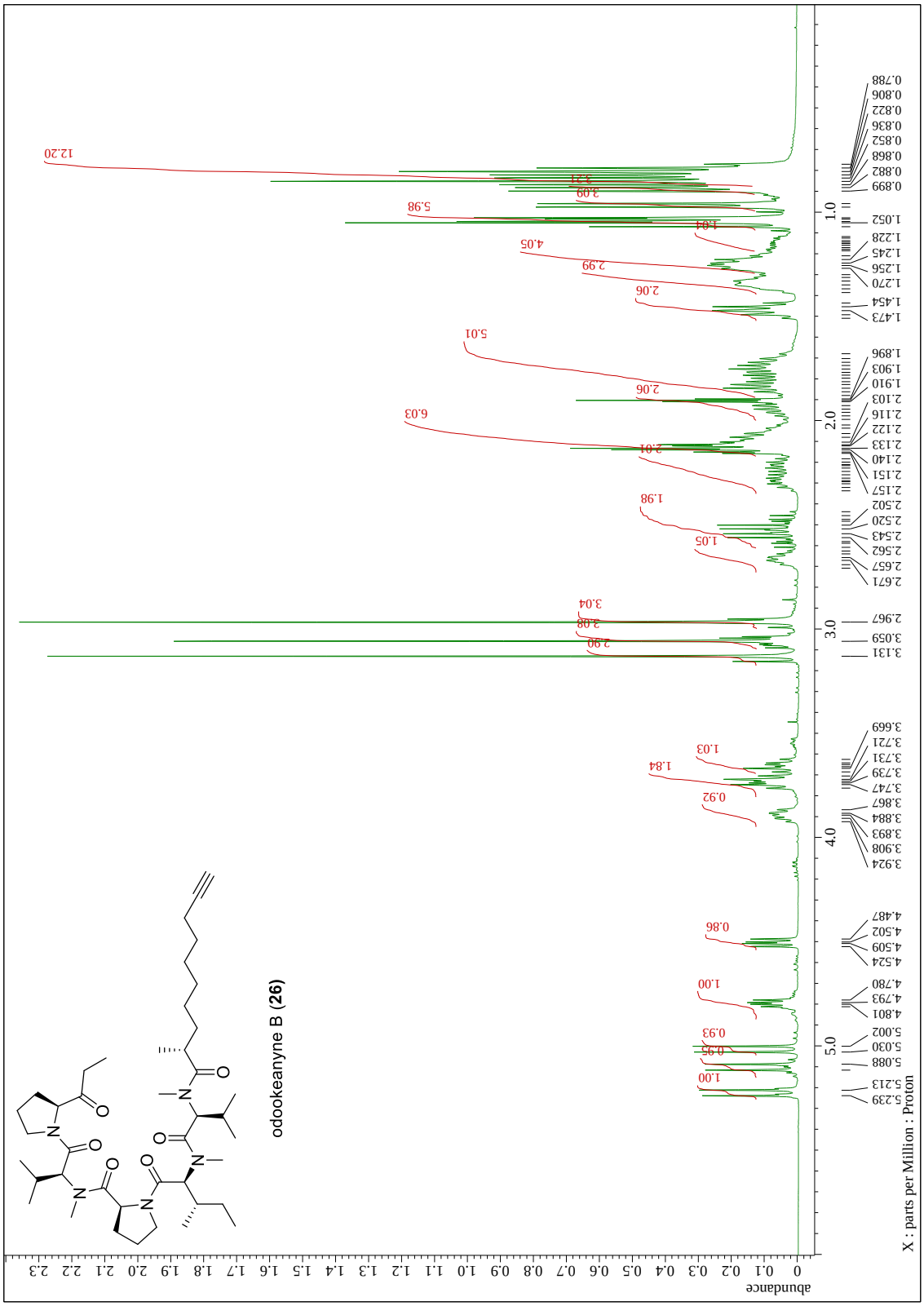
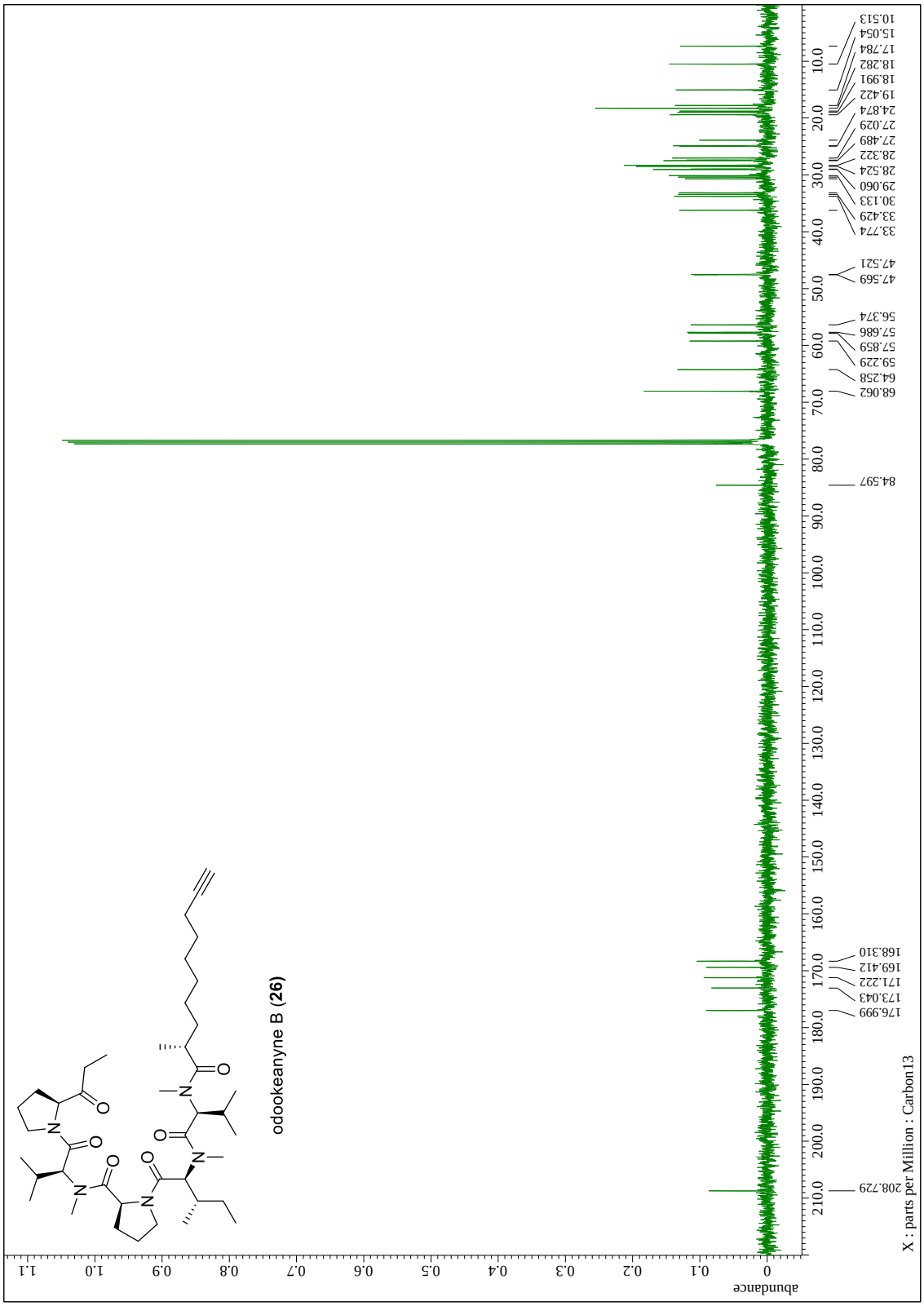


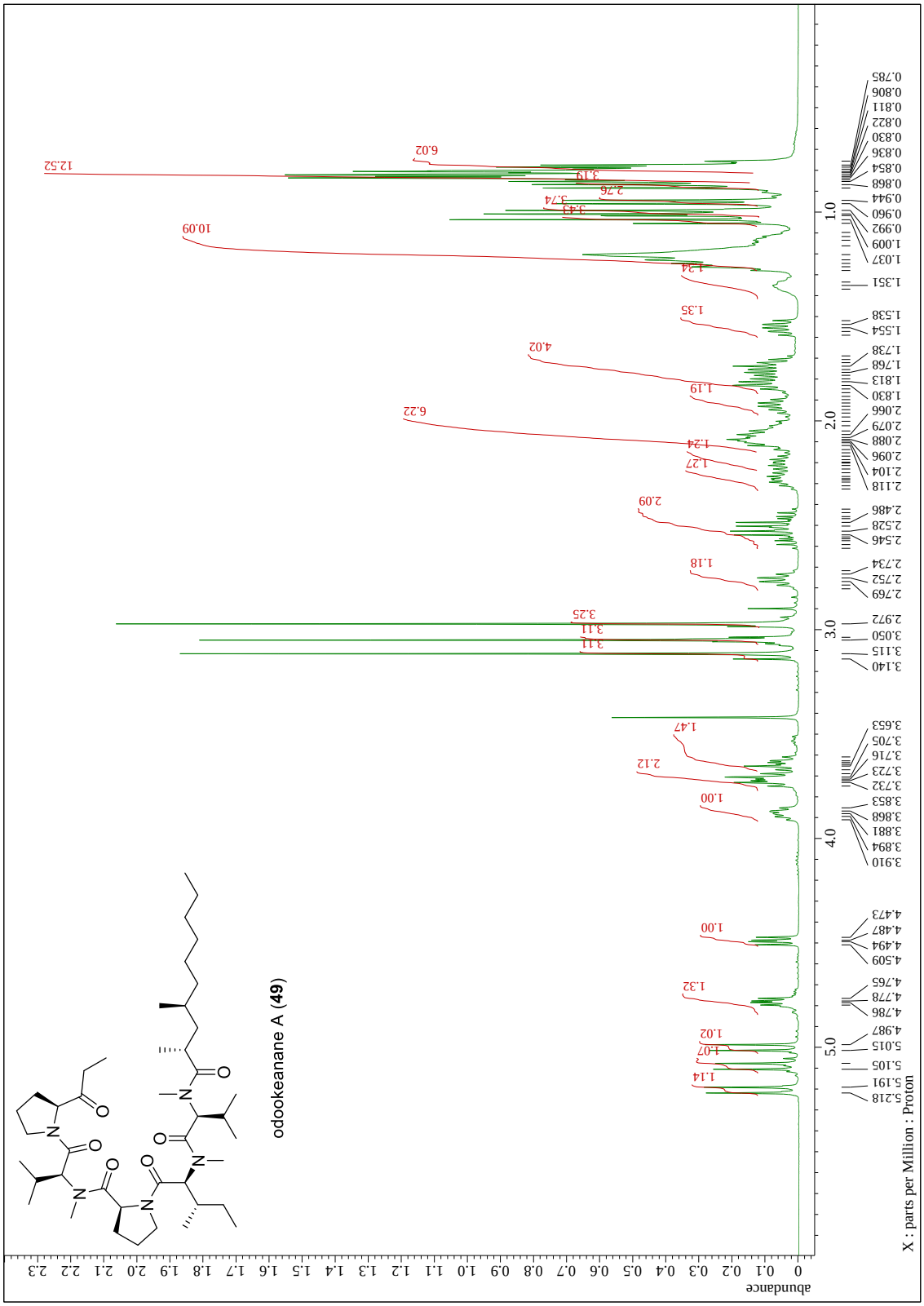
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **26**



$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **26**



^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **49**



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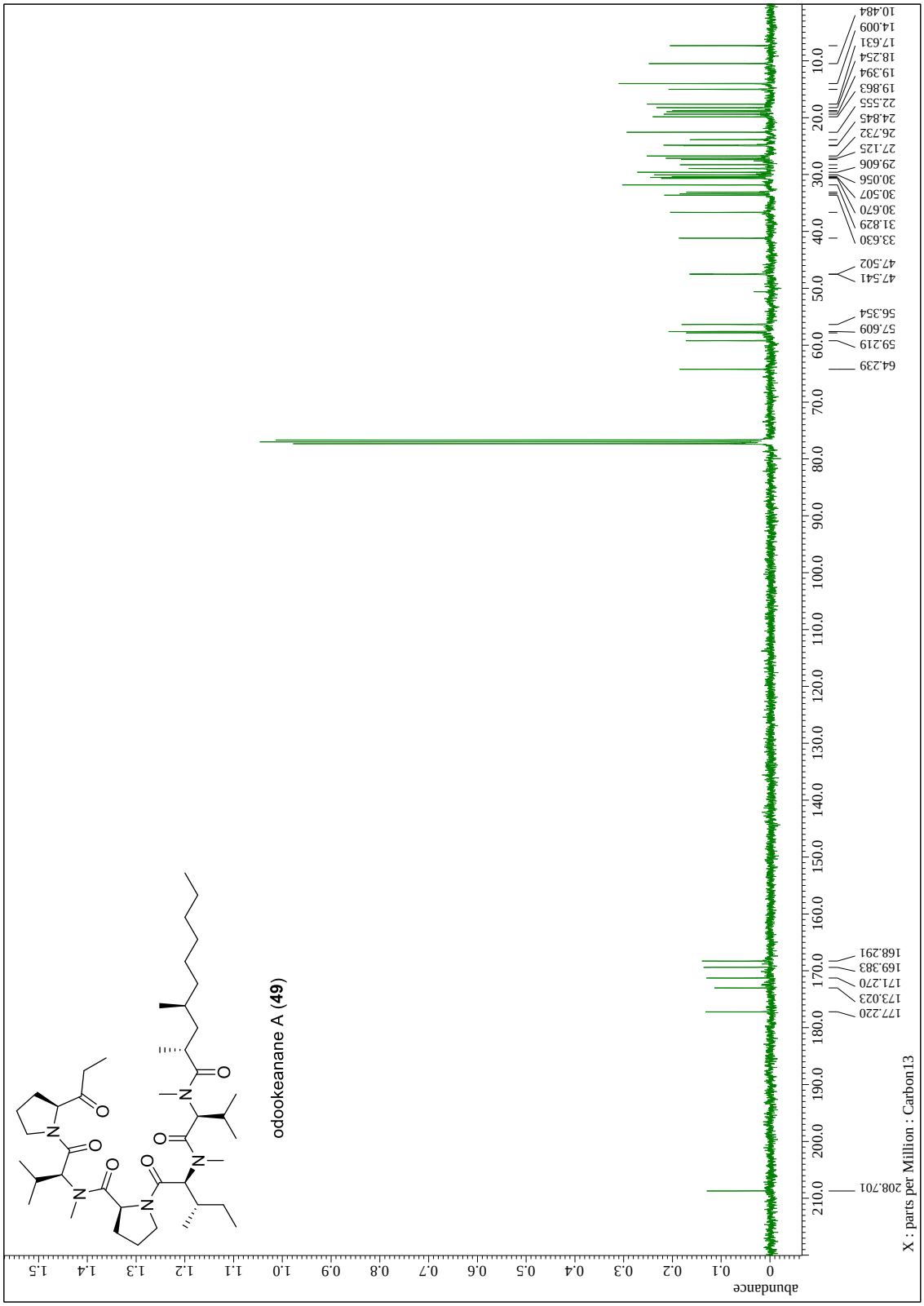
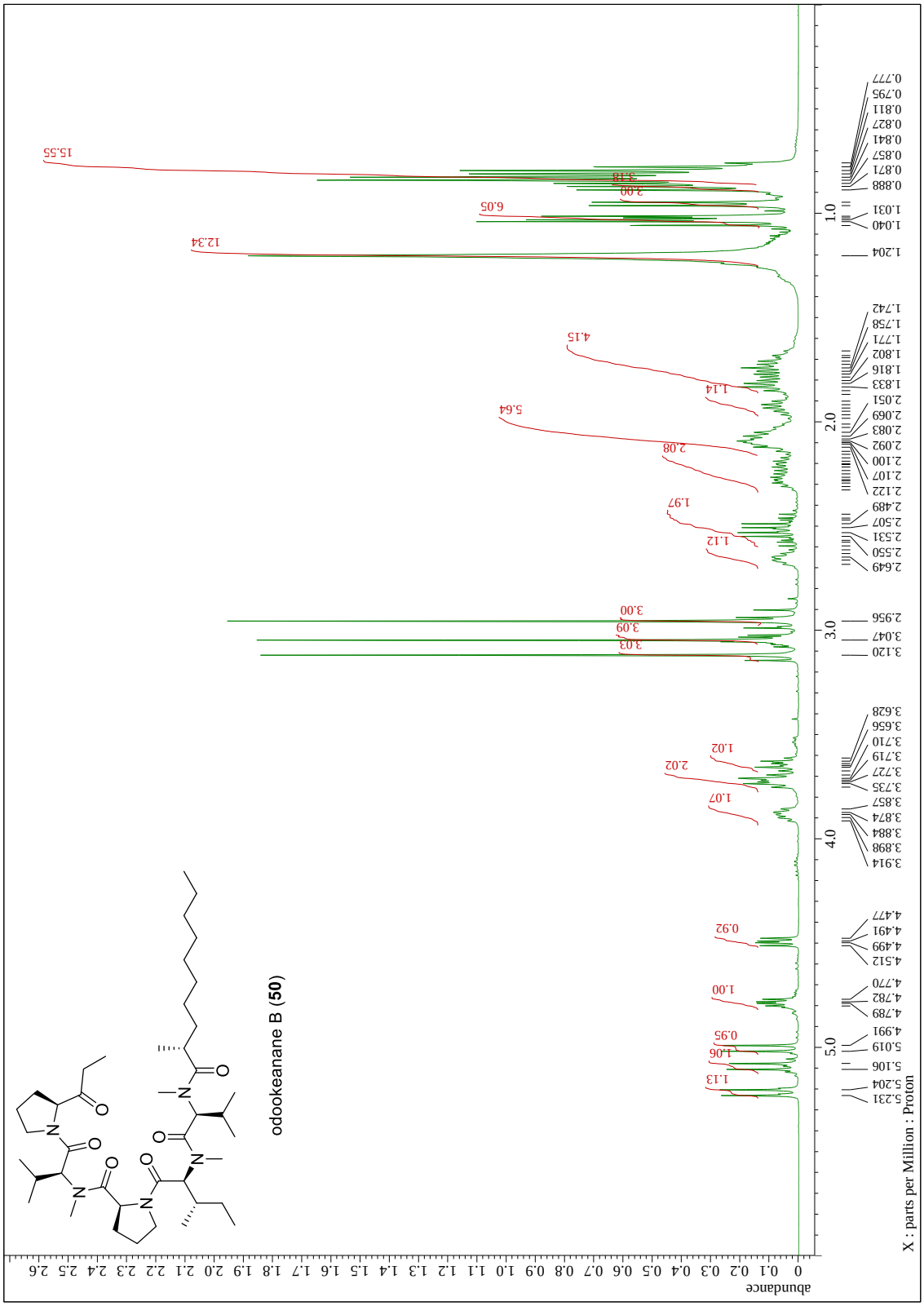
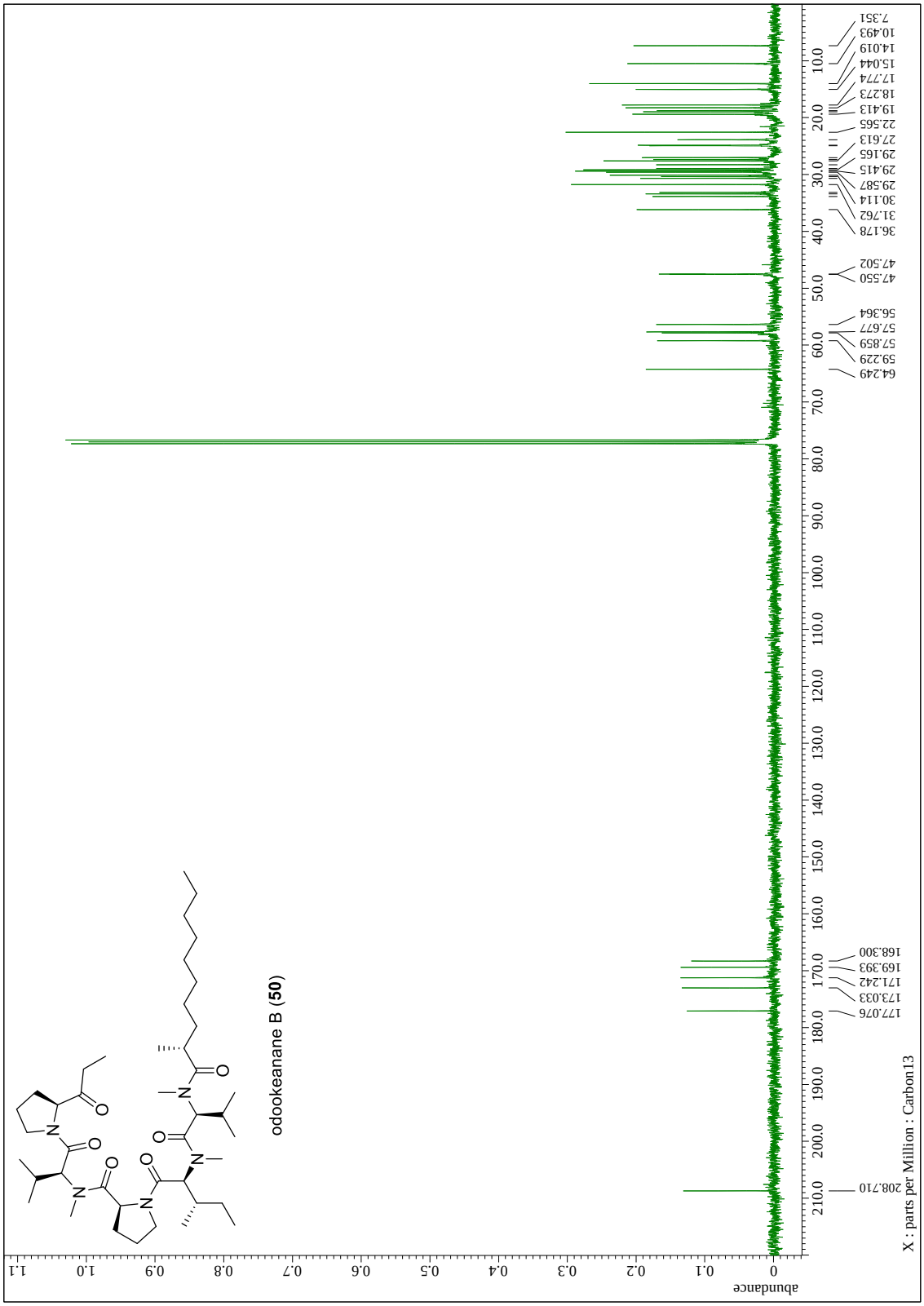


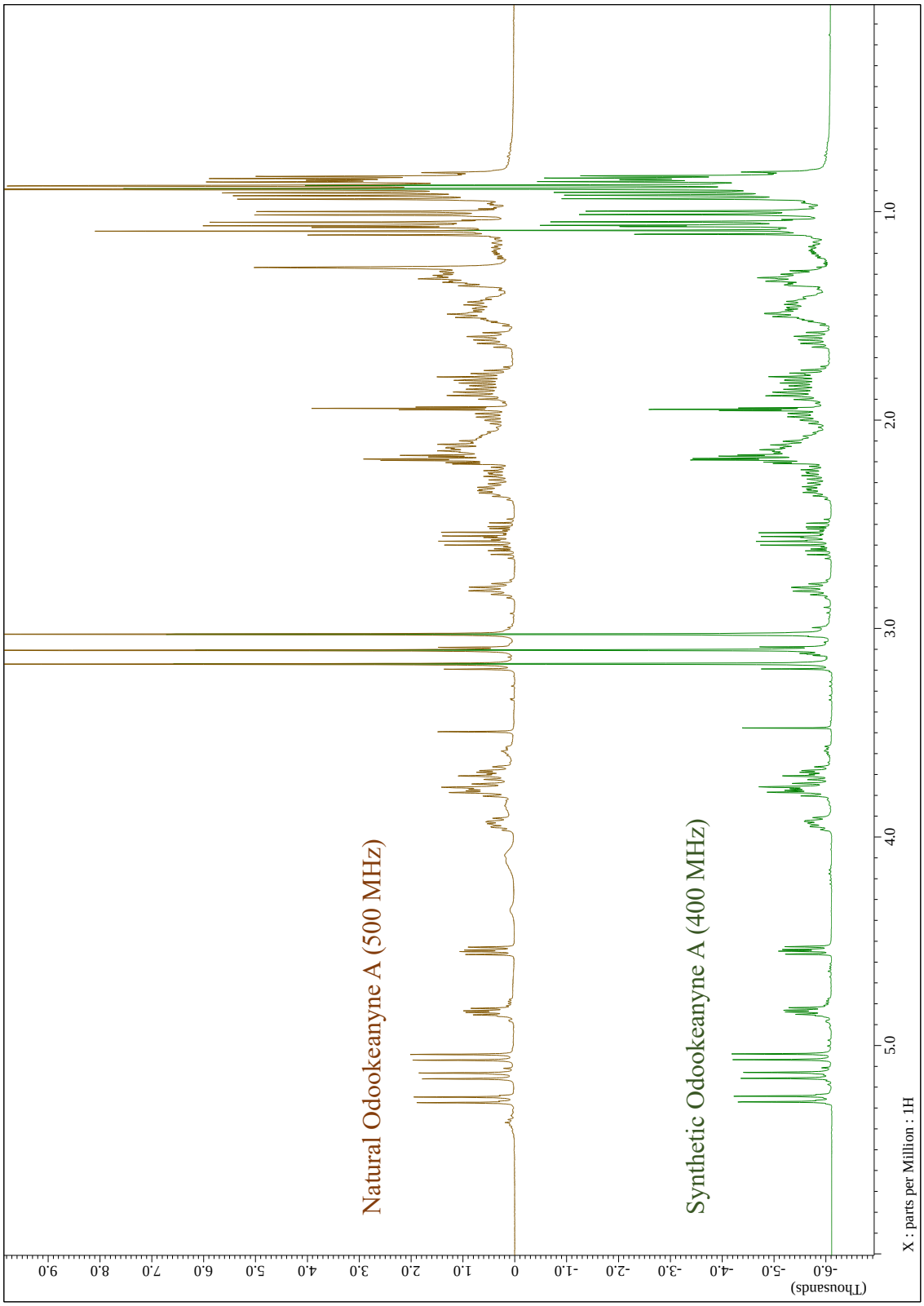
^{1}H NMR (400 MHz in $CDCl_3$) spectrum of **50**



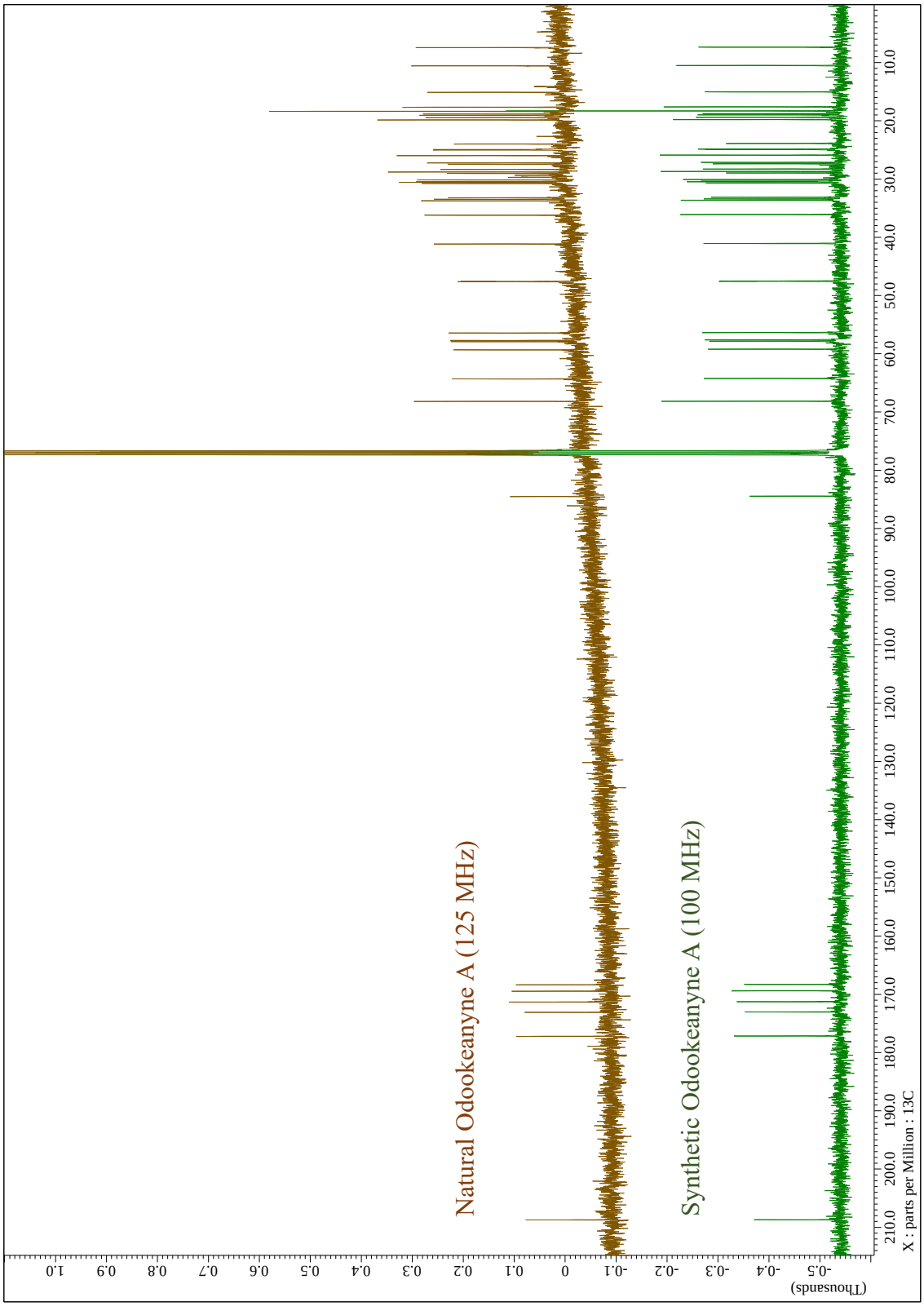
$^{13}C\{^{1}H\}$ NMR (100 MHz in $CDCl_3$) spectrum of **50**



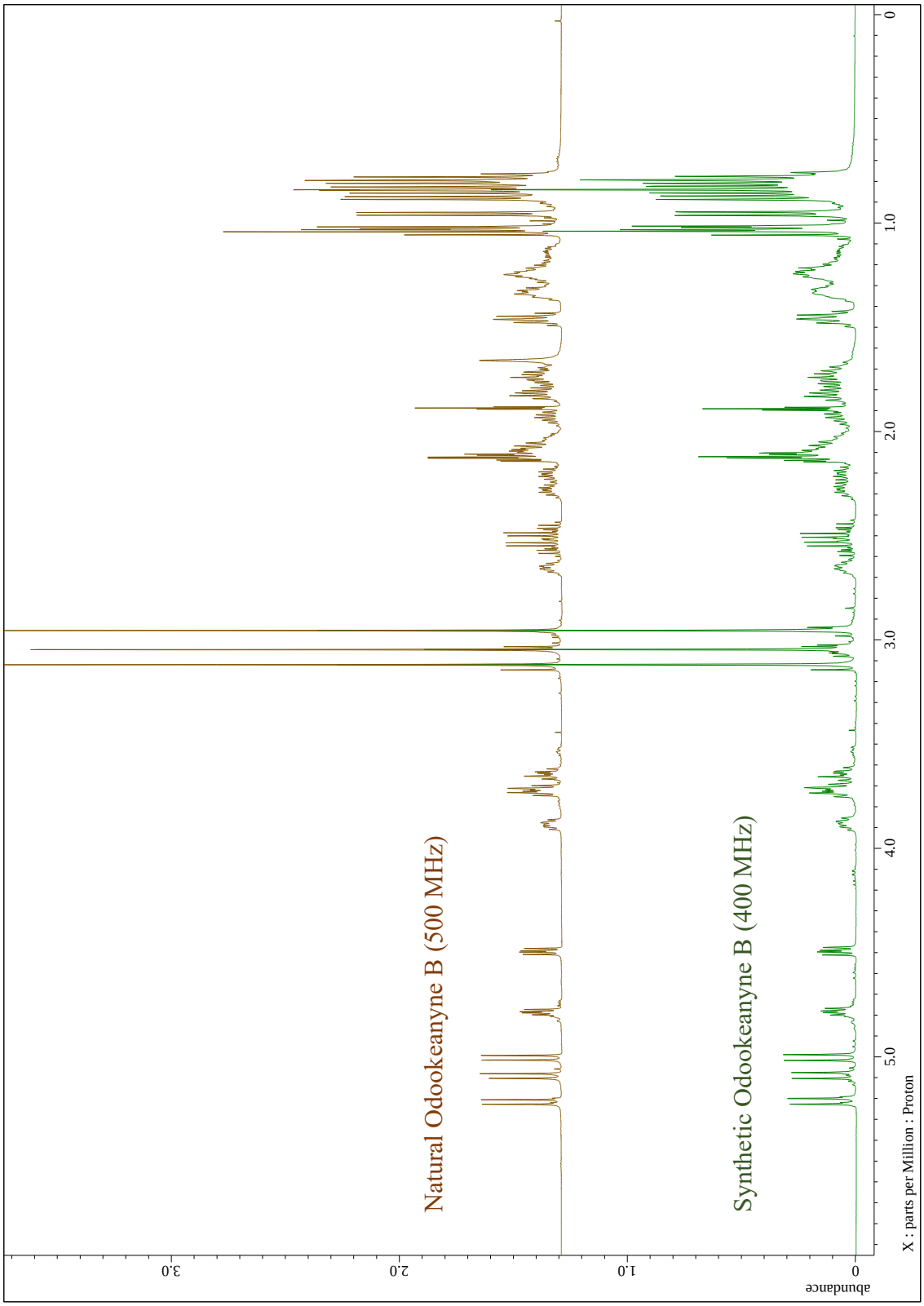
Comparison of ^{1}H NMR ($CDCl_3$) spectra between natural and synthetic odookeanyne A (**25**)



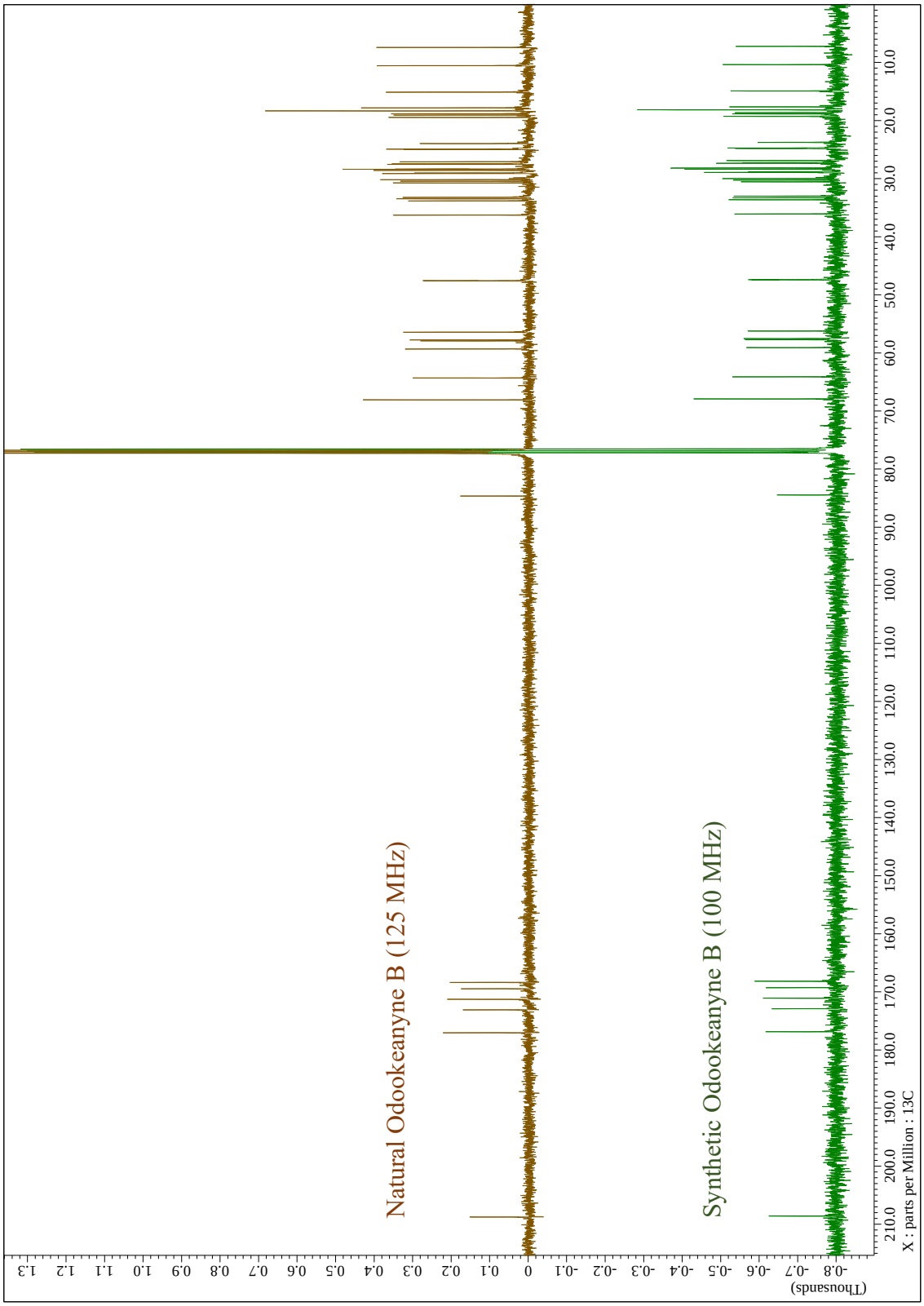
Comparison of $^{13}C\{^{1}H\}$ NMR ($CDCl_3$) spectra between natural and synthetic odookeanyne A (**25**)



Comparison of ^{1}H NMR ($CDCl_3$) spectra between natural and synthetic odookeanyne B (**26**)



Comparison of $^{13}C\{^{1}H\}$ NMR ($CDCl_3$) spectra between natural and synthetic odookenayne B (**26**)



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