TOTAL SYNTHESIS OF TUBIASHINOL

by

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The genus *Vibrio* harbors over 100 species and some of them are well-known (e.g. *Vibrio anguillarum and Vibrio cholerae.*) They are Gram-negative and comma-shaped bacteria which are present within many diverse marine organisms and habitats, such as mollusks, shrimps, fishes, and corals. They have an array of extracellular products some of which cause toxicity. (*1*)

Another species, *Vibrio tubiashii*, is found in Pacific Ocean and has shown to be a prevalent pathogen of oyster larvae. It increasingly affects oyster populations as well as being a problem for oyster hatcheries, nurseries and farms. (*2*) With implementing genome mining, our group has identified a gene cluster which is responsible for a bio-pathway of couple metabolites. Isolated and characterized authentic samples were used in a collaborative study and one of the metabolites showed toxicity towards oyster larvae and zebra fish embryo. After this observation, we become interested in the synthesis of this toxic marine natural product and named it tubiashinol.

The coupling of the first 2 building blocks was achieved after testing few different agents and the diastereomerically pure dipeptide (N-terminus) was obtained. However, synthesis of (3*R*,4*S*)-4-amino-6-methylheptane-1,3-diol (C-terminus) was challenging and we had to try number of strategies to attain the desired amino-diol. In the process, we also obtained the 3-*epi* diastereomer and the absolute stereochemistry of its precursor was determined by X-Ray which fortified our characterizations. This diastereomer was, then, used to synthesize 3-*epi* tubiashinol which was compared to tubiashinol later.

In this study, we reported the first *Vibrio tubiashii* metabolite which is toxic to oyster larvae. We successfully synthesized and characterized tubiashinol and 3-*epi* tubiashinol and compared them to determine the absolute stereochemistry of tubiashinol in proton (^{1}H) NMR studies.

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PREFACE

I would like to thank my advisor, Dr. Xinyu Liu for the immense guidance and support that he has provided me throughout this study. I am very grateful for his patience during our weekly meetings which has nurtured me to the researcher that I am today. It is a great honor to have him as my mentor.

I thank my committee members Dr. Alexander Deiters and Dr. Kabirul Islam for their valuable counsel during the course of this project.

I would also like to thank my wife, Tugba for the continuous emotional support that she has given me. Thank you for being by my side during this arduous journey. Moreover, if it wasn't for the constant moral and financial support that my family has provided me over the years I could not have chased my goals and wouldn't be where I am at in my career today, so I would like to give them special thanks. Thank you for being there unconditionally.

It is a pleasure being a member of the Liu research laboratory. I want to thank the undergraduate student, John Hong who worked with me and helped in some parts of the project. I also want to thank all former and current members; Dr. Aniruddha Sasmal, Dr. Qin Zhu, Thomas Tugwell, Yelissa Sosa, and Xiaole Yang for their support.

1.0 INTRODUCTION

Metabolites obtained from various marine microorganisms have been investigated and synthesized over the past century. Some of these natural products were summarized and highlighted by Garson, Gerwick, and Moore. (*3*) (*4*) (*5*)

With improvements in laboratory techniques and instrumentation, obtaining marine natural products and elucidating their structures have been possible even though determining the structure of modern and more complex small molecules remain challenging. (*6*) Nonetheless, advancements in nuclear magnetic resonance (NMR) field in recent years has enabled researchers to battle and overcome these challenges. Moreover, improvements in pulse sequences and increased NMR instrument sensitivity has streamlined assignments of natural product structures through elucidation of stereoisomers. (*7*) Today, developments in NMR instrument designs open new venues for scientists and empower isolation and characterization of marine bacterial metabolites, even in very small quantities which wasn't possible previously. (*8*)

Natural products from marine organisms are rarely abundant, restricting rapid analysis of a particular metabolite or resulting in extended waiting period – days often months – to obtain required scale of a molecule to carry on scientific research. Therefore, the most definitive and clear answer to the structure of a natural product relies on synthesizing it stereochemically on a laboratory bench and comparing the authentic and synthetic molecules side-by-side using NMR and other characterization techniques. Additionally, holding the ability at hand to produce a natural

product of interest allow researchers to propel their research by derivatizing their structure rapidly and studying various applications simultaneously without having to wait for their microorganism to generate enough natural product to continue their research. The following dissertation will report and characterize the first marine bacterial natural product from a vibrio species which is responsible for toxicity towards oyster larvae and zebra fish embryo. Subsequently, derivatization of this bacterial metabolite and future study of interest will also be discussed.

1.1 BACKGROUND

Natural products (NPs) are present in variety of settings and can be isolated from terrestrial and marine plants and/or organisms. They exhibit different effects and can be toxin, antibiotic, therapeutic and more.

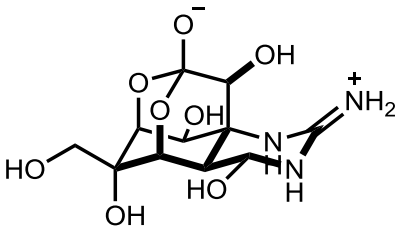


Figure 1.1. Tetrodotoxin

One of the early examples of a toxin, tetrodotoxin, **Figure 1.1** was found in puffer fish by Dr Tahara in 1909. (*9*) The structure elucidation was performed by Woodward in 1964 and later confirmed in an X-ray crystallography experiment by Kishi in 1970. The first asymmetric total synthesis was completed by Isobe and Du Bois in 2003.

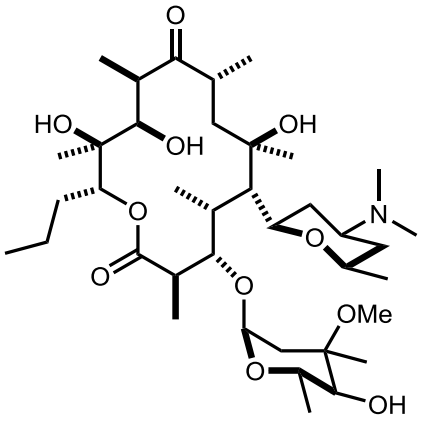


Figure 1.2. Erythromycin

A well-known example of an antibiotic is erythromycin **Figure 1.2** which was isolated from a gram-positive bacteria, *Saccharopolyspora erythraea* by McGuire in 1952. (*10*) Its asymmetric synthesis was accomplished by Woodward laboratory in 1981 and it has been used against respiratory, skin, and chlamydia infections. (*11*)

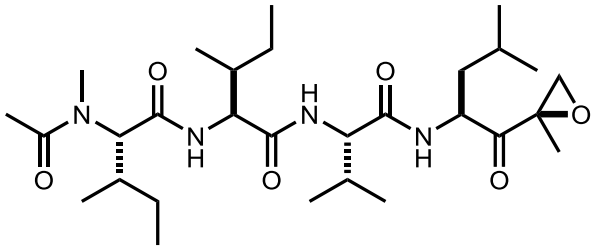


Figure 1.3. Epoxomicin

There are number of examples of NPs which were turned into successful and best-selling drugs. Epoxomicin **Figure 1.3** was obtained from *Actinomycetes* strain Q996-14 by Hanada in 1992. (*12*) After the total synthesis was achieved by Crews groups in 1999 (*13*), they derivatized epoxomicin with a biotin tag (Different types of tags for probe design will be discussed in the next chapter.) and developed a probe **Figure 1.4** to study its target protein. (*14*) Further research found

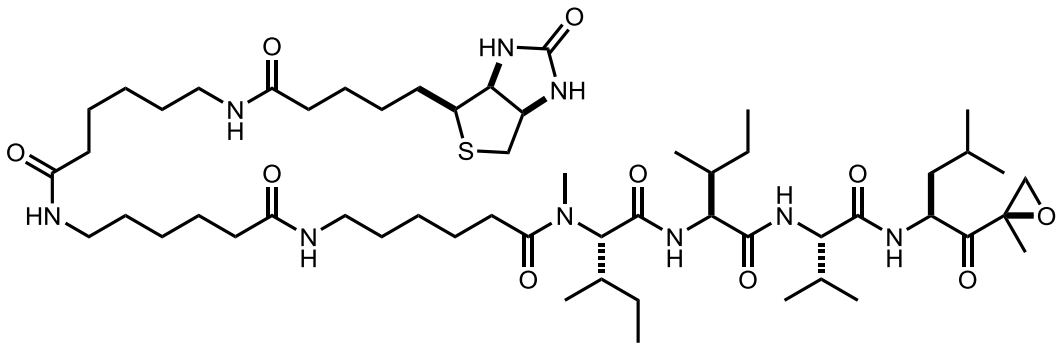


Figure 1.4. Epoxomicin-biotin probe

epoxomicin to be a lead compound as a proteasome inhibitor and it carried value due to its anti-inflammatory property. The findings from this research helped Crews laboratory to turn epoxomicin into an antitumor drug, Carfilzomid **Figure 1.4** which generated an annual revenue of $512 million in 2015. (*15*)

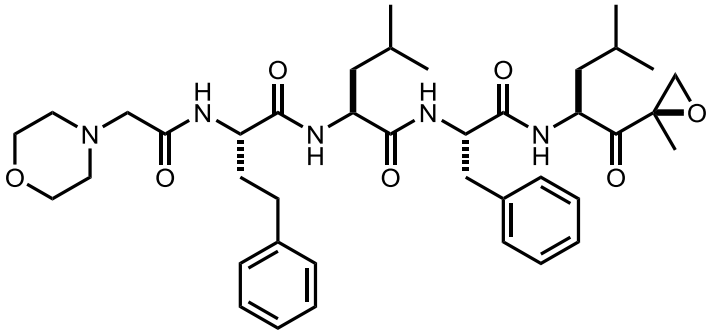


Figure 1.5. Carfilzomid

Even these few examples of NPs is suffice to show scientists that NPs hold great potential and value for future research and drug discovery. This motivation will continue to lead natural product and synthetic chemists into new studies which may yield new tools to investigate complex biological systems and eventually unveil new techniques and therapeutics.

1.2 *VIBRIO TUBIASHII*

The genus *Vibrio* harbors over 100 species and some of them are well-studied (e.g. *Vibrio anguillarum and Vibrio cholerae.*) They are Gram-negative and comma-shaped bacteria which are omnipresent within many diverse marine habitats and organisms, such as mollusks, shrimps, and fishes throughout the world. They have an array of extracellular products, some of which are antibiotics and reported in the literature before. (*16*) (*17*) (*18*) Yet, there are other secondary metabolites, which are toxigenic, inducing mortality by vibriosis at larval and juvenile stages of marine organisms; but these natural products aren't identified and their structures are unknown. (*1*)

One of the vibrio species, *Vibrio tubiashii (V. tubiashii)*, was first isolated from larval and juvenile bivalves of a hard clam and Eastern oyster and reported by Tubiash et al. (*19*) It was further studied and named by Hada et al. (*20*) The genome of *V. tubiashii* was sequenced by Temperton et al. (*2*) and some of structural genes were identified and shown to be related to the toxicity towards Pacific oyster larvae. In recent years, subsequent papers described vibriosis caused by *V. tubiashii*. (*21*) (*22*)

1.2.1 Natural Products from *V. Tubiashii*

While utilizing genome mining, researchers can shed light on and gain insight about various bacterial metabolites and their biological pathways. (*23*) Following this approach, our research group isolated, characterized and named two natural products from *V. tubiashii.*

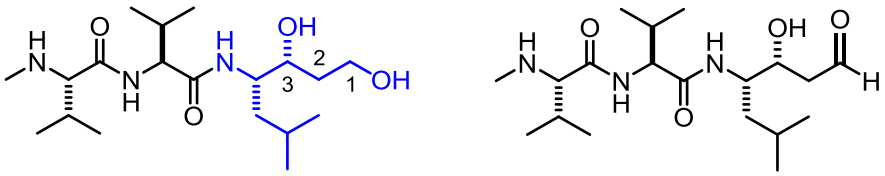


Figure 1.6. Tubiashinol (on the left) and Tubiashinal (on the right)

Tubiashinol **Figure 1.2** was the major secondary metabolite and drew our attention due to its toxicity towards oyster larvae and zebra fish embryo which will be discussed in the next subchapter. It is a tripeptide derivative with two valine and one leucine residues. More specifically, it contains a leucine-derived 1,3–diol motif which resembles statine **Figure 1.3**.

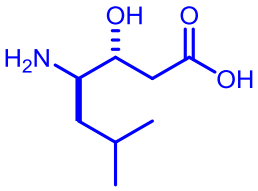


Figure 1.7. Statine, (3*S*,4*S*)-4-amino-3-hydoxy-6-methylheptanoic acid

Statine contains a leucine-derived *γ*-amino-*β*-hydroxy acid moiety and was first reported in 1970. (*24*) It was a structure found in the natural product pepstatin **Figure 1.4** which inhibits pepsin, a protease. (*25*)

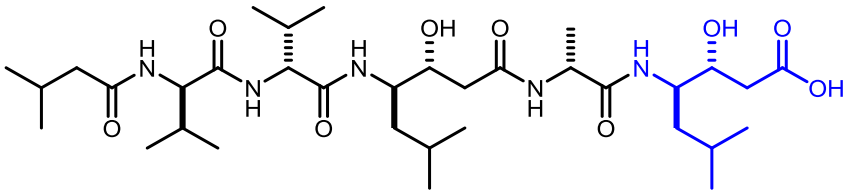


Figure 1.8. Pepstatin

In the research investigating the mode of inhibition of pepstatin against number of proteases, the removal of statine halted the inhibition process and, therefore, statine unit was found

to be the main cause of inhibitory activity. (*26*) (*27*) It operates by emulating the tetrahedral transition state of a peptide bond hydrolysis and uniquely inhibits aspartyl proteases. (*28*) (*29*) There are number of other reported protease inhibitors derived from statine; Tamandarins A-B, miraziridine A, ahpatinins, YF-044P-D, symplocin A, and more. (*30*) Natural products which are more prominent for this research are listed in **Table 1.1**.

Entry	Natural Product	Bioactivity
1	Pepstatin	Aspartyl protease inhibitor
2	Grassystatin A	Cathepsin D and E protease inhibitor
3	Thalassospiramide A	Calpain 1 protease inhibitor

Table 1.1. Natural Products Containing γ-amino-β-hydroxy Acid Derivatives

Luesch found Grassystatin A **Figure 1.5** in marine cyanobacteria and screened against 59 proteases. It was found to inhibit Cathepsins D and E with IC_{50} of 26.5 nM and 889 pM, respectively. (*31*)

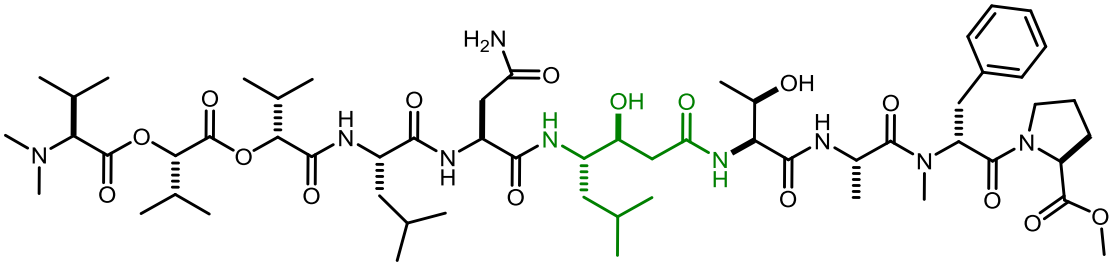


Figure 1.9. Grassystatin A

Another natural product with a serine derivative of statine was described by Fenical et al. This new cyclic peptide was isolated from the marine α-proteobacterium *Thalassospira*. (*32*) In a

later study, it is concluded that Thalassospiramide A **Figure 1.6** is a human calpain 1 protease inhibitor with a IC_{50} of 56 nM. (*33*)

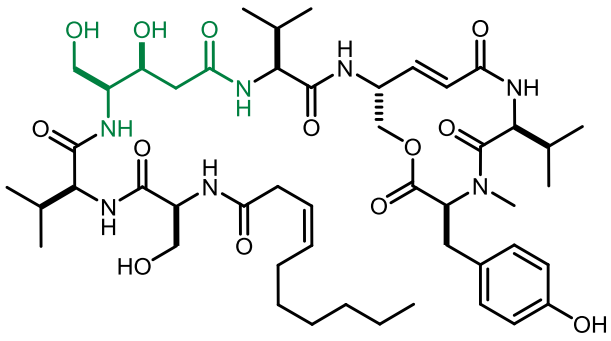
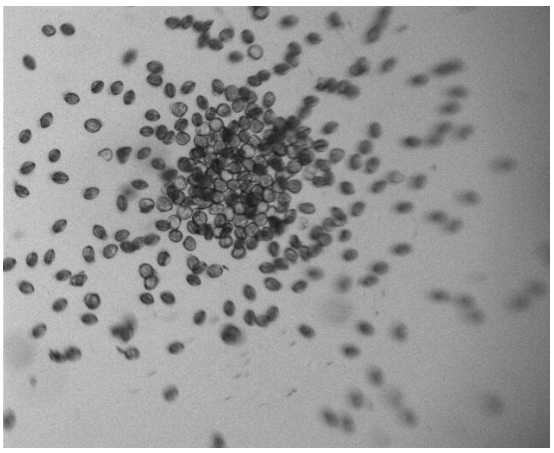


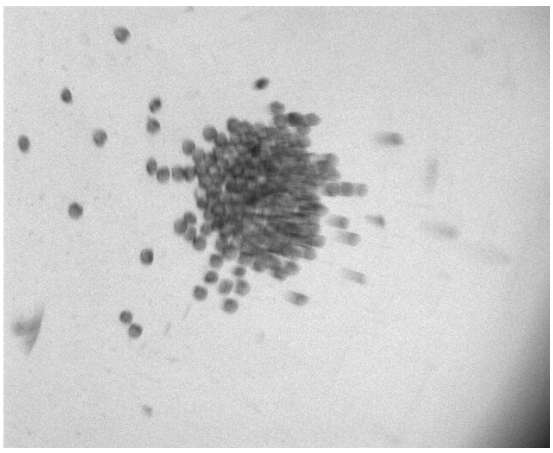
Figure 1.10. Thalassospiramide A

1.2.2 Toxicity Assays with Tubiashinol

In our collaboration with Tsang laboratory, only tubiashinol showed toxicity towards oyster larvae **Figure 1.7** and zebra fish embryo **Figure 1.8** at their early stage of development. It is the first toxigenic bacterial marine natural product from *V. tubiashii* and has become the focus of this research.



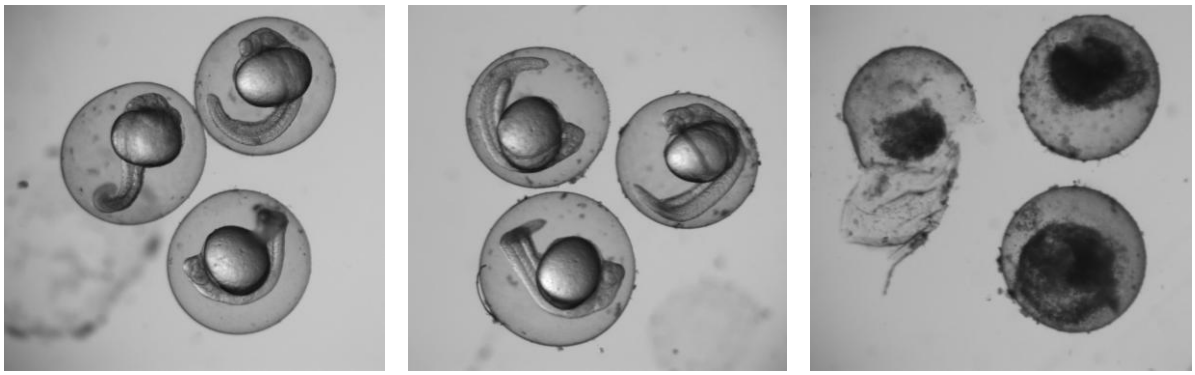
Treated with 2% DMSO



Treated with 100 µM Tubiashinol in 2% DMSO

Figure 1.11. Toxicity Assay with Oyster Larvae after 18 Hours of Incubation at 28°C (Dr Michael Tsang)

V. tubiashii increasingly affects oyster populations as well as being a problem for oyster hatcheries, nurseries and farms both on the west and east coasts of North America. (*34*) Any discovery in this regard will be valuable and find fruitful applications both in the marine research and industry.



DMSO control **50 µm Tubiashinol** **500 µm Tubiashinol**

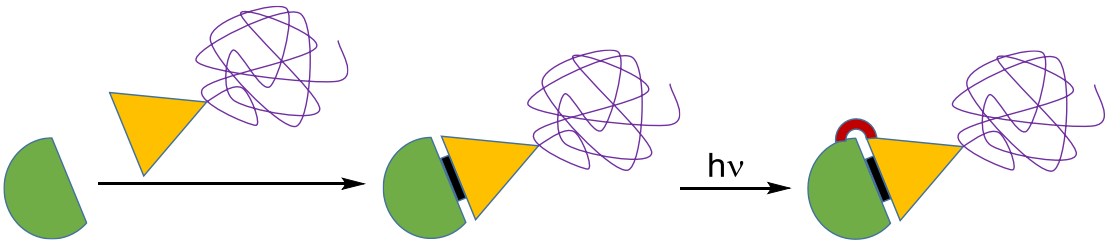
Figure 1.12. Toxicity Assay with Zebra Fish Embryo after 3 Hours of Incubation at 28ºC (Dr Michael Tsang)

1.3 CHEMICAL PROBES AND PHOTOAFFINITY LABELING

Chemical probes are natural organic molecule analogs which can be utilized to explore and investigate a certain biological pathway in a cell or organism, for example, a protein's function. Using chemical probes is a powerful strategy as they are interrelated with biological and genetic approaches, bringing scientists of varying backgrounds together and creating an interdisciplinary research.

While studying with chemical probes it's important to be able to monitor these agents as they travel within a biological system. This need gave birth to photoreactive molecules. Among these are photoaffinity compounds introduced by Westheimer in 1962. (*35*) In addition to this

valuable contribution, he also defined photoafinity labeling (PAL). In PAL, a bioactive ligand is derivatized with a photoreactive group. When the new synthetic compound is introduced into a biological system, it forms a complex with a target. Following irradiation, a reactive species such as nitrene, carbonyl, or carbine would be generated and it would covalently bind to the target **Scheme 1.7**. (*36*) Then, this complex can be separated from the cocktail of natural molecules, purified and characterized by spectroscopic techniques.



Scheme 1.1. Process of PAL

Over the past 55 years, photoreactive probes helped researchers to study ligand-receptor (protein), protein-protein, protein-nucleic acid, and protein-cofactor interactions in various biological systems. (*37*) Even though there are different examples of photoaffinity probes (PAP) in the literature, most of them are far from being ideal. (*38*) An ideal PAP should (i) be stable in storage, under light and at different pH ranges, (ii) possess similar features to that of those the natural molecule – groups attached by derivatization need to be small and not cause any steric interactions with the target, it needs to have similar affinity towards the target and form a stable complex which can undergo purification and characterization, (iii) have a fitting photoaffinity label which can be activated at longer wavelengths to avoid any damage to the biological target and which can also generate a notably reactive species with a short lifetime, and (iv) contain a reporting group or removable handle (for instance, a fluorophore or tag) to track down the PAP in a biological system. (*39*)

Since they have been discovered, different classes of photoreactive molecules have been studied; aryl azides, (*40*) benzophenones, (*41*) diazirines, (*42*) diazonium salts, (*43*) and nitrobenzenes. (*44*) Although these different group of molecules were explored in the early days of PAL, the first three classes have found more applications in medicinal and biological chemistry and depicted in **Figure 1.14**.

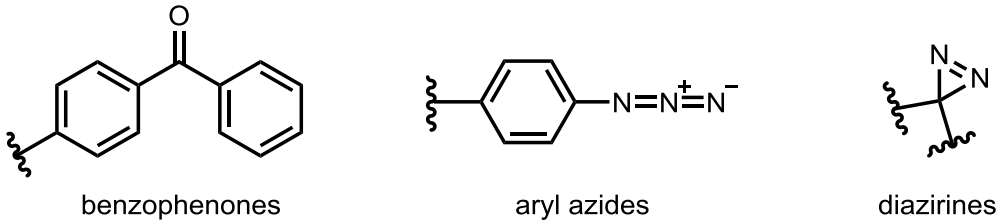
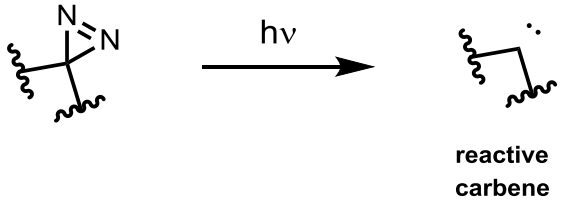


Figure 1.13. Three Major Classes of Photoreactive Groups

Benzophenone photoreactive probes present number of advantages over the other classes. They are stable under light and can be handled conveniently. Moreover, they are inert to many organic solvents and can withstand a wide range of pH. They can be irradiated at longer wavelengths of UV light (330-360 nm) which avoids protein degradation. Finally, they generate adducts which are very reactive even in water. On the other hand, they are bulky and hydrophobic groups which may be problematic for certain small ligands. They also require long irradiation times which may result in non-specific binding. (*45*) (*46*)

Aryl azides are smaller in size and have been typically used in PAL until recently. They can be easily prepared and store well. Upon irradiation, nitrenes are propagated and they are short-lived; however, they are activated in short wavelengths (254-265 nm) which can disintegrate biopolymers. (*47*) In addition, nitrenes can suffer from side reactions (dimerization to form azobenzenes or reduction with thiols to aryl amines) which lower photolabeling yield. (*48*) (*49*)

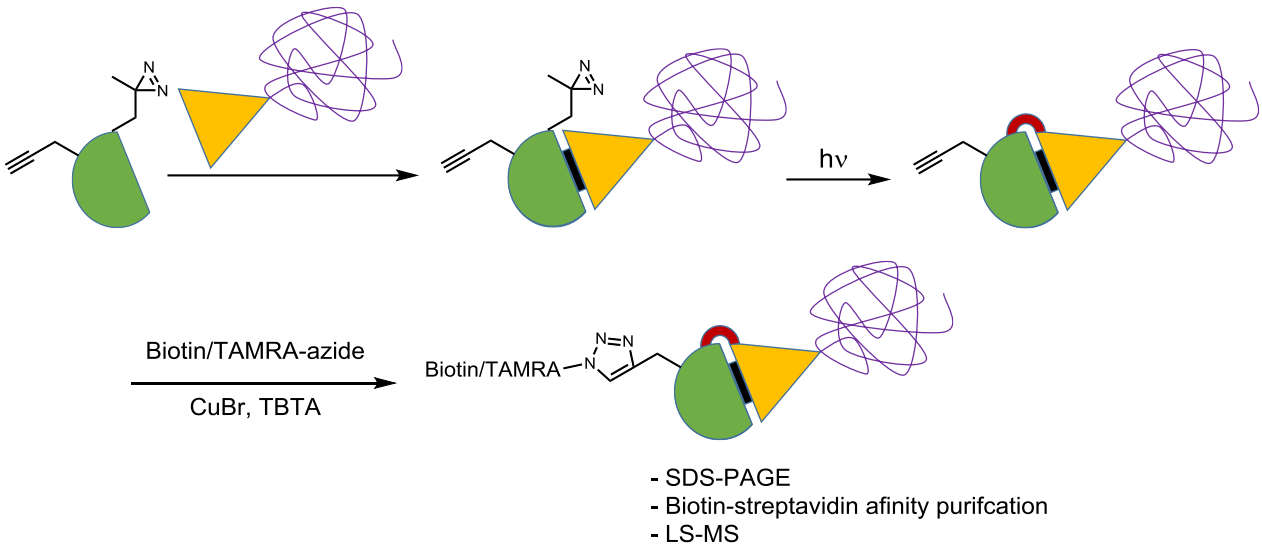
Diazirines are the smallest photoaffinity groups. Because of their small size, diazirine derivatized ligands are widely used as PAPs. They are stable at room temperature, under acidic and basic conditions. They are also relatively stable against nucleophiles, such as thiols. In different studies, diazirines were found to be more stable than aryl azides. (*50*) (*51*) Due to their long irradiation wavelenths (350-380 nm), diazirines don't damage biological macromolecules immensely, but generate extremely reactive carbene species **Scheme 1.8** which rapidly form covalent bonds with closest functional groups as a result of C-C, C-H, O-H and H-X (X = heteroatom) insertions. PAL with carbenes usually results in low yields because carbenes quickly get quenched in the presensce of water. Nevertheless, this short lifetime becomes an advantage rather than a drawback by reducing nonspecific labeling – only tightly bound photoreactive ligands would establish covalent bonds with their targets, other diazirines would get quenched by water before they could bind nonspecifically. (*52*)



Scheme 1.2. Photoactivation of Diazirines

PAPs usually include a reporting group to validate and monitor photolabeling. Some of the common reporters are radiolabels, fluorophores, and biotin. These reporting tags are generally introduced into PAPs during their syntheses. A PAL process can be validated by different methods, based on reporter types. For example, in-gel fluorescence detection can be used to monitor fluorophore reporters. (*52*)

For isolation and analysis of target-bound PAPs, various strategies have been promoted in recent years. (*53*) (*54*) One of the strategies involve stitching a terminal alkyne or azide functional group on a PAP during its synthesis which can be employed in a Hüisgen 1,3-dipolar cycloaddition or click chemistry at a later step. The reagents for this later step usually contain a fluorophore or biotin tag with a complementary alkyne or azide functionality. After target interaction and irradiation of a PAP to form a covalently bond with its target, the new complex can undergo a click reaction with the previously designed complementary tag and consequently analyzed with a relevant method **Scheme 1.9**. (*55*) (*56*)



Scheme 1.3. PAL Using A Diazirine and Alkyne PAP and Analysis

A trifluorophenyldiazirine-based PAP relevant to this research was reported by Yao in 2005. It was successfully used to probe aspartic proteases with high selectivity. The diazirine photoreactive group was installed to a statine derivative and the structure is shown in **Figure 1.15**. (*57*)

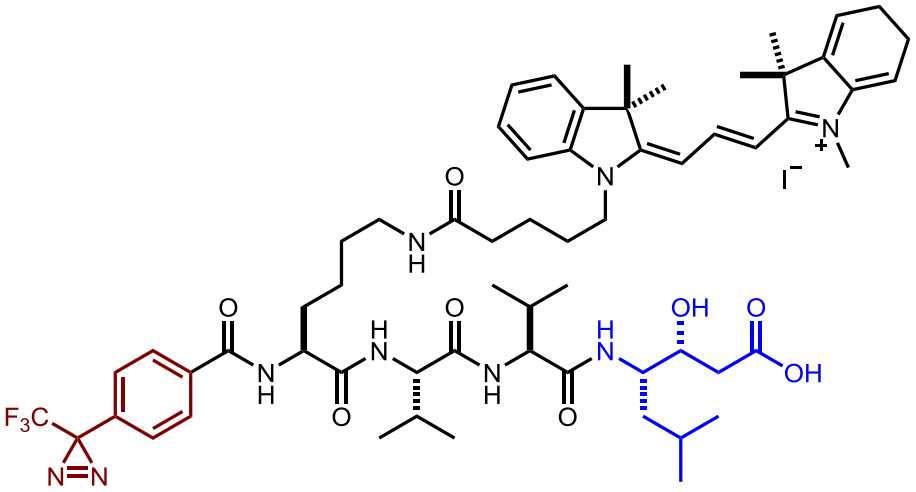


Figure 1.14. A Trifluorophenyldiazirine-based Aspartic Protease PAP

We mentioned earlier that the statine moiety of pepstatin was responsible for the inhibitory activity towards aspartyl proteases. We hypothesize that having a statine alcohol derivative at its C-terminal, tubiashinol can also be a protease inhibitor found in *V. tubiashii*. We are planning on developing affinity probes as in **Figure 1.15** and therefore need a modular total synthetic pathway to obtain tubiashinol derivatives rapidly while keeping the C-terminal intact.

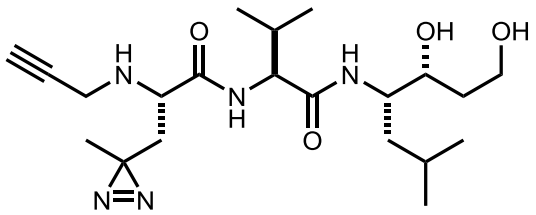
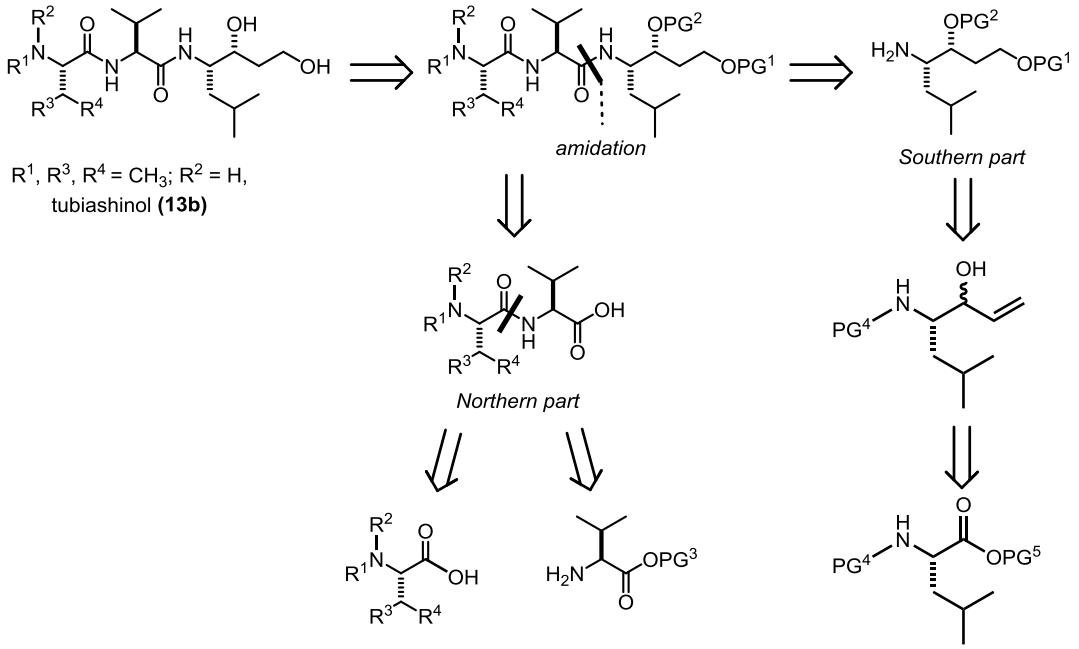


Figure 1.15. A Prospective Tubiashinol PAP

2.0 TOTAL SYNTHESIS OF TUBIASHINOL

At the beginning of our research, our initial objective was to synthesize tubiashinol so that we can validate the structural conformation of our authentic compound. Provided we obtained this marine natural product, we also considered carrying on our research to further investigate the biological pathway causing mortality to oyster larvae and zebra fish embryo which is a financial burden on the Pacific aquaculture industry. In order to accomplish this notion, we planned a modular total synthesis which would allow us to easily derivatize our first building block at the N-terminal. Subsequently, the assembly of our dipeptide and its amidation by 4-amino-1,3-diol would help us build a small library of molecules which can be utilized in photoaffinity probe applications.

2.1 RETROSYNTHESIS



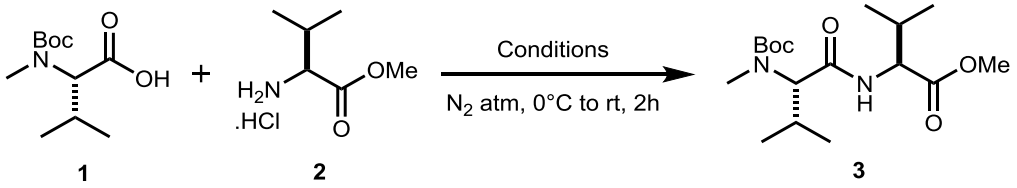
Scheme 2.1. Retrosynthesis of Tubiashinol

As it is summarized in **Scheme 2.1**, the total synthesis of tubiashinol starts with a coupling reaction of the first two amino acids and subsequent deprotection of the dipeptide methyl ester to generate the dipeptide (Northern part).

To obtain 4-amino-1,3-diol compound (Southern part), *N*-methyl-L-leucine methyl ester (*N*-Me-L-Leu-OMe) is protected, using a *tert*-butoxycarbonyl (Boc) group. The next reaction reduces the Boc-protected ester to the corresponding aldehyde. A Grignard reaction on the aldehyde generates a vinyl alcohol which goes through a hydroboration reaction to yield the diol compound. The diol is, then, protected to separate the diastereomers. The following reaction cleaves the Boc protecting group. Finally, the Northern part is coupled to the Southern part and a consequent global deprotection gives out tubiashinol or its derivatives.

2.2 SYNTHESIS OF NORTHERN PART

The synthesis of the Northern part **4** started with the coupling of Boc-*N*-methyl-L-valine **1** [*tert*-butoxycarbonyl (Boc)] and L-leucine methyl ester **2** which were commercially available.

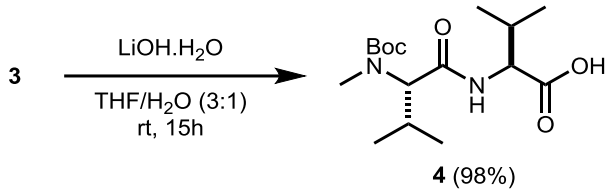


1 **2** **3**

Entry	Conditions	% Yield
1	TSTU, HOSu, $CuCl_2$, DIEA, DMF (*58*)	10
2	EDC.HCl, HOBt, DIEA, DMF (*59*)	51
3	HATU, DIEA, DMF (*59*)	90

Table 2.1. Screening of Coupling Conditions for Boc-*N*-Me-L-Val-L-Val-OMe

Three different coupling conditions were tested for the peptide coupling when all three entries from **Table 2.1** were run side-by-side for two hours. HATU coupling condition was superior to TSTU/HOSu/$CuCl_2$ and EDC/HOBt conditions. It didn't require a racemization suppressing additive, nor did it cause an epimerization.



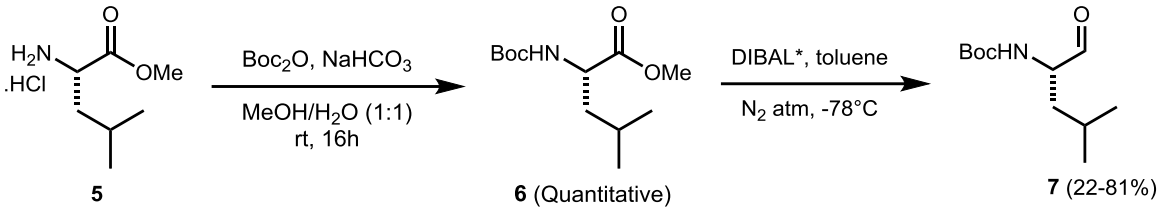
Scheme 2.2. Synthesis of Boc-*N*-Me-L-Val-L-Val-OH

Hydrolysis of the dipeptide methyl ester **3** under basic conditions provided the dipeptide Boc-*N*-methyl-L-valine-L-valine (Boc-*N*-Me-L-Val-L-Val-OH) **4** with an excellent yield. (*60*)

2.3 SYNTHESIS OF SOUTHERN PART

L-Leu-OMe **5** was purchased commercially and protected according to the procedure by Ley. (*61*) A quantitative yield was attained.

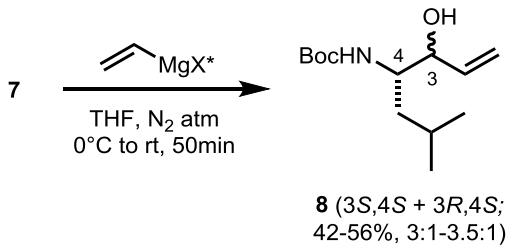
5 was reduced to the corresponding aldehyde **7** in a DIBAL reaction. (*62*) As opposed to the literature procedure, 49 minute was found to be the optimum reaction time **Table 2.2**.



*Entry	DIBAL (eq.)	Time	% Yield
1	1.1	35 min	22
2	2.5	25 min	51
3	2.5	49 min	81

Table 2.2. Synthesis of Boc-L-Leu-Al

7 was run into a Grignard reaction following the literature procedure. (*63*) Vinyl magnesium bromide gave a better yield for similar reaction conditions **Table 2.3**. Both conditions suffered from low to moderate yields due to nucleophile labile Boc protecting group.



*Entry	VinylMgX	Eq.	Time	% Yield
1	Cl	2.2	60 min	42
2	Br	2.2	50 min	56

Table 2.3. Synthesis of Boc-L-Leu-OH-Vinyl

In an attempt to separate the diastereomeric mixture of **8**, two different strategies were employed; hydroxyl protection and ring formation **Figure 2.1**. Acetyl and silyl ether protections (*64*) weren't successful in separating the diastereomers. Introducing rigidity to the molecule and ring formation of 2- oxazolidone (*65*) and *N*-acetonide (*66*) didn't help separate the diastereomers, either.

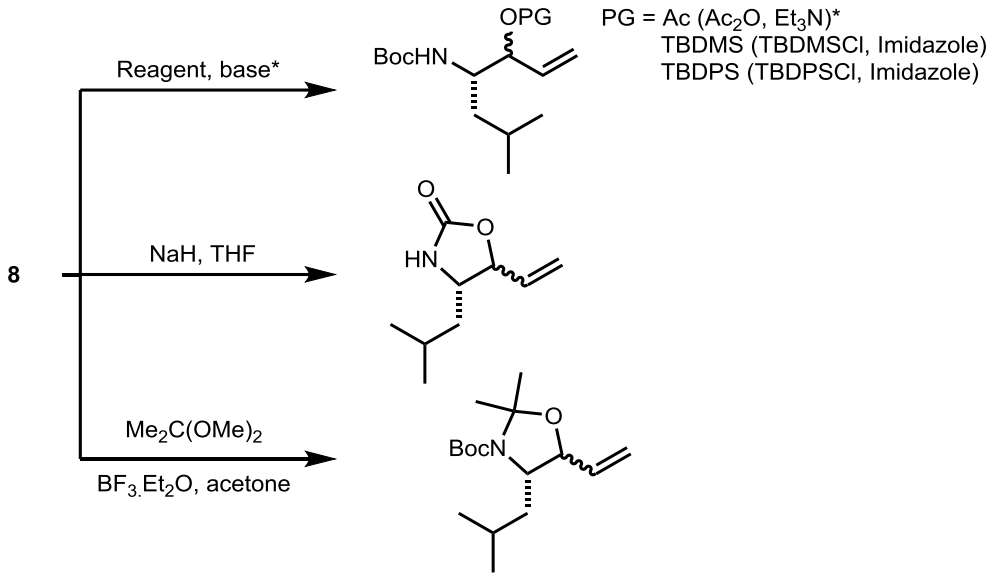
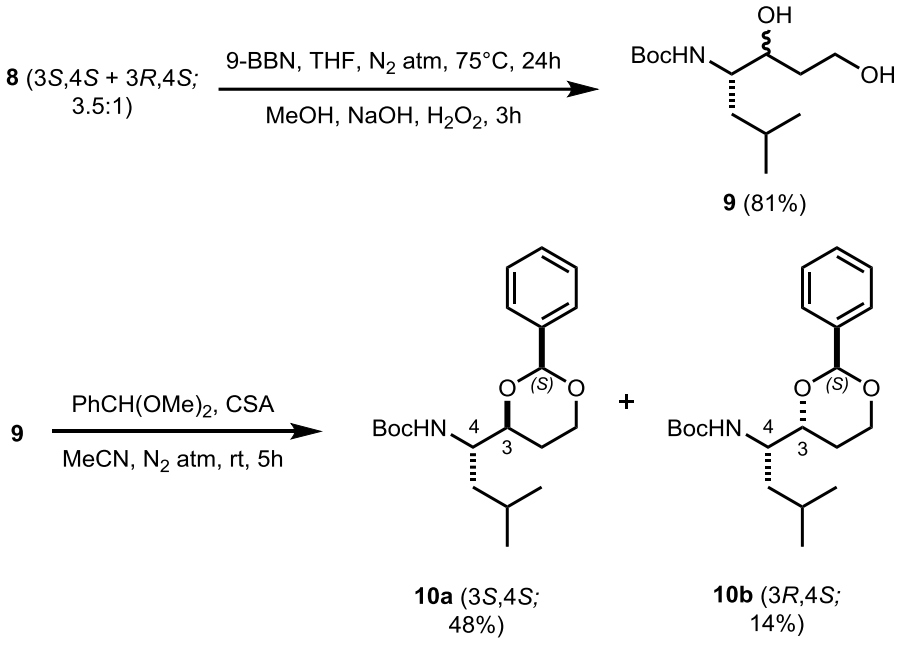


Figure 2.1. Trials for Analog Syntheses to Separate Diastereomers

After failed attempts to separate the diastereomeric mixture, **8** was set up for a hydroboration reaction with 9-Borabicyclo[3.3.1]nonane (9-BBN). (*67*) Even though 9-BBN hydroboration reactions are generally sluggish, good yields were observed after running the reaction at reflux for 24 hours and subsequently oxidizing it at 50°C for few hours **Scheme 2.3**.

After a column purification, the diol **9** as a diastereomeric mixture was treated with (dimethoxymethyl)benzene in the presence of catalytic amount of camphorsulfonic acid to form

benzylidene acetals **Scheme 2.3**. (*68*) The newly introduced molecular rigidity enabled a partial separation of the diastereomers. After number of consecutive column chromatography experiments, diastereomers were separately characterized by NMR spectroscopy for the first time. Furthermore, **10a** was submitted for X-ray crystallography study and its crystal structure was determined, verifying the orientation of the benzylidene ring at the equatorial position **Figure 2.2**.



Scheme 2.3. Synthesis of Boc-L-Leu-Benzylidene Acetal and Separation of Diasteromers

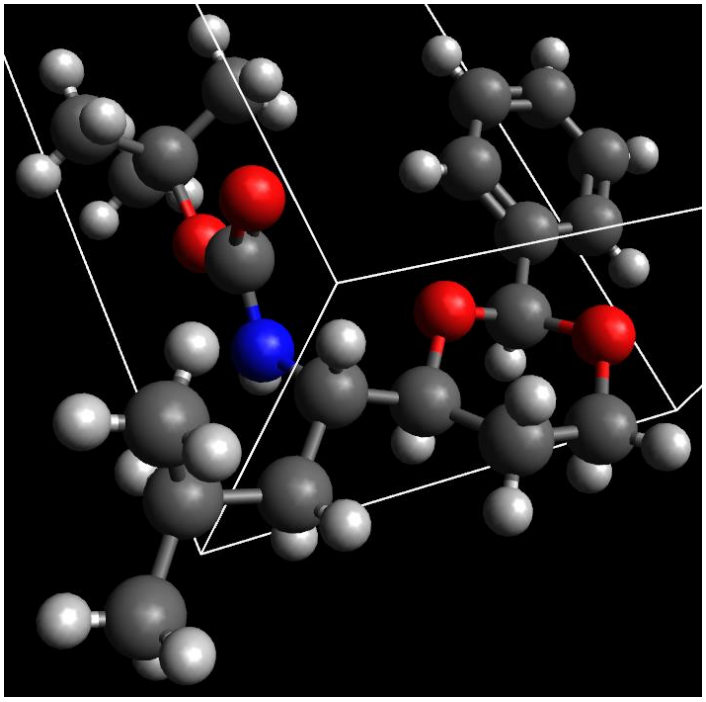
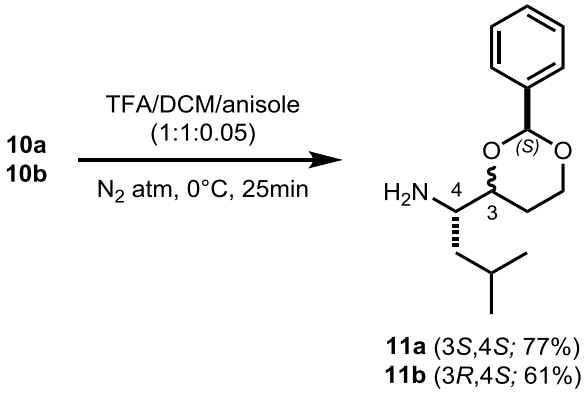


Figure 2.2. Crystal structure of (3*S*,4*S*)-Boc-L-Leu-Benzylidene Acetal, 10a

After the successful separation of the diastereomers, they were treated with TFA/DCM to cleave the Boc protecting group. (*69*) Even though the reaction was performed under anhydrous conditions to prevent hydrolysis of benzylidene acetal, fully deprotected 4-amino-1,3-diol was observed as a by-product.

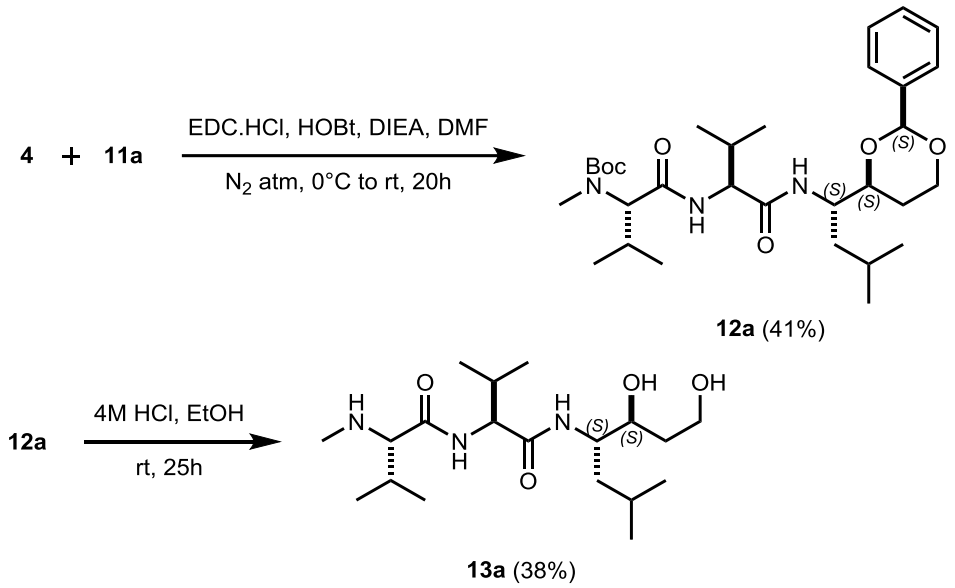


11a (3*S*,4*S;* 77%)
11b (3*R*,4*S;* 61%)

Scheme 2.4. Synthesis of L-Leu-Benzylidene Acetals

2.4 UNION OF NORTHERN AND SOUTHERN PARTS

The final coupling to unite Northern and Southern parts **Scheme 2.5** initially followed the same condition as **3**; however, HATU reagent didn't offer a good reaction this time. Pilot coupling reactions of **4** and **11a** with sterically less hindered substrates suggested that the reaction yield was reduced due to the sterically hindered **11a**. Even though another coupling method, triphenylphosphine/iodine (PPh_3/I_2), (*70*) was tested and promising for a pilot reaction, it wasn't suitable with **11a** and wasn't implemented. EDC/HOBt coupling condition proved to be the ideal condition for the final coupling even though the yield was low due to steric hindrance of **11a** **Scheme 2.5**.

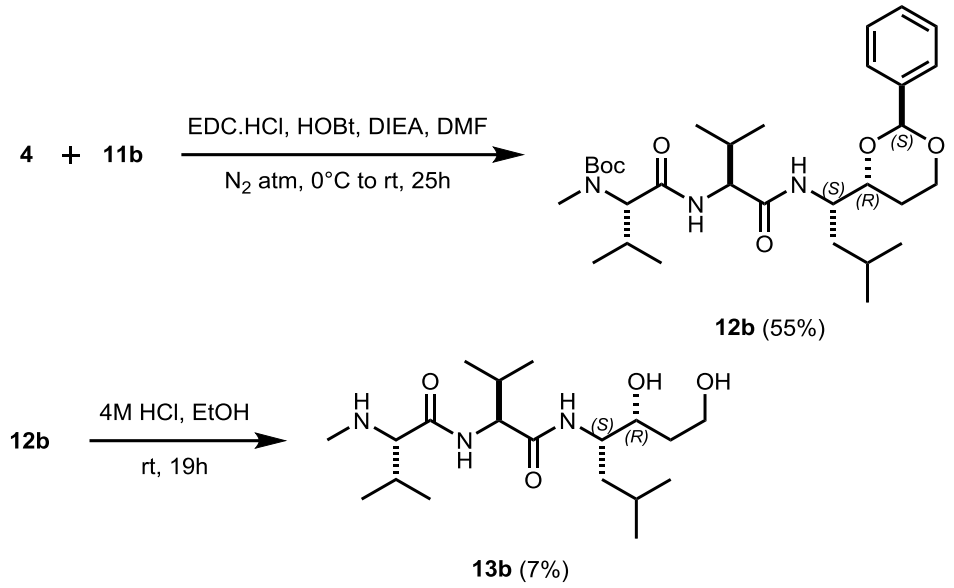


Scheme 2.5. Synthesis of 3-*epi*-Tubiashinol

TFA/DCM pair was suboptimal for the global deprotection of **12a** because even after 20 hours, the reaction mixture contained partially hydrolized benzylidene acetal and other adducts.

On the other hand, applying HCl/EtOH deprotection (*71*) provided a better conversion and yield compared to TFA/DCM for 3-*epi*-tubiashinol, **13a**.

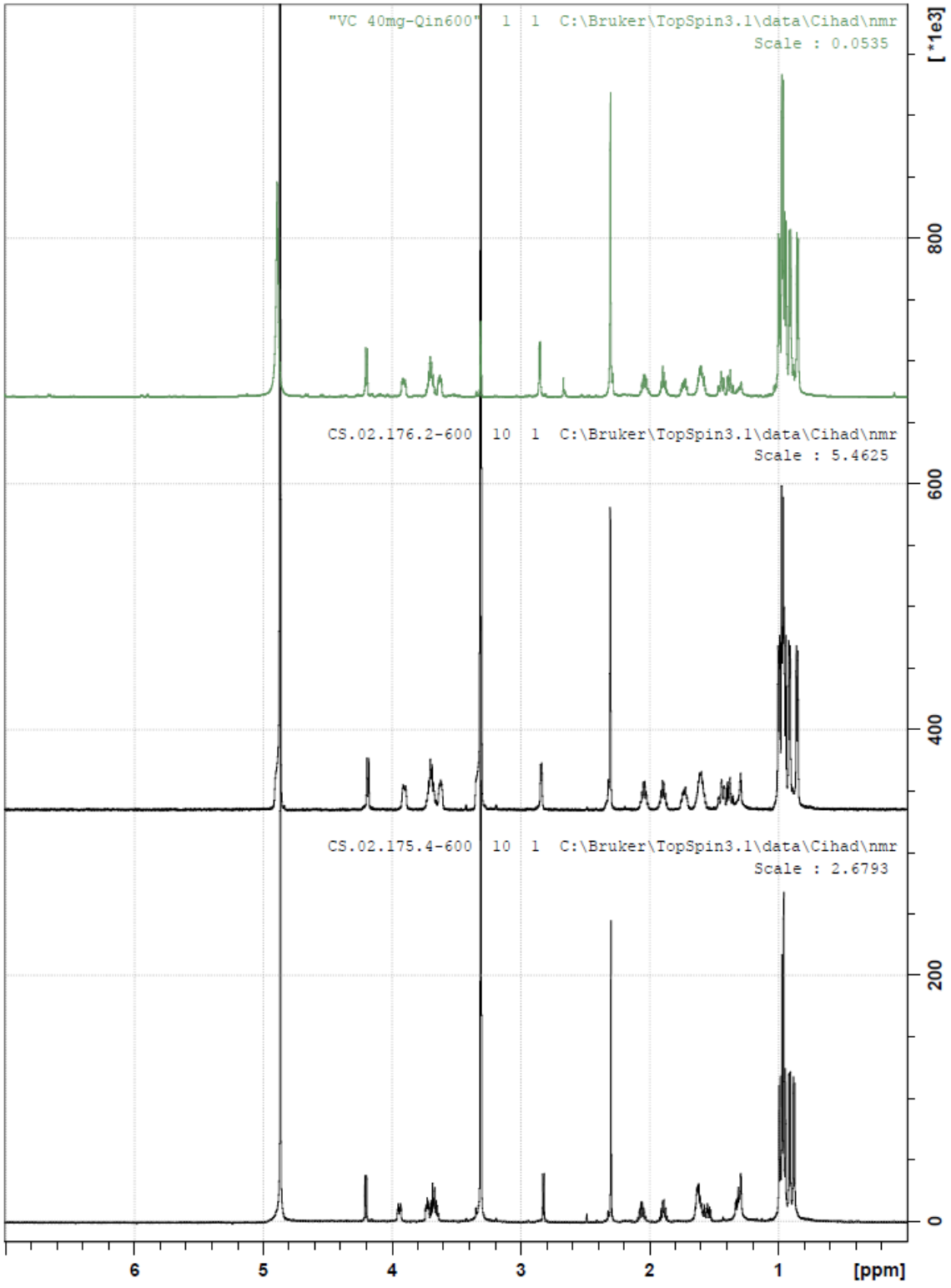
Tubiashinol, **13b**, was synthesized similarly to **13a**, using the optimized reaction conditions.



Scheme 2.6. Synthesis of Tubiashinol

Figure 2.3 compares ^{1}H NMR data of authentic tubiashinol, synthetic tubiashinol and 3-*epi*-tubiashinol, respectively from top to bottom. Also, the ^{1}H and carbon (^{13}C) NMR data of authentic and synthetic tubiashinol is tabulated in **Figure 2.4** and **2.5**, respectively. It is to our joy that authentic and synthetic tubiashinol NMR data match and indicate the success of our total synthesis and structure verification.

a)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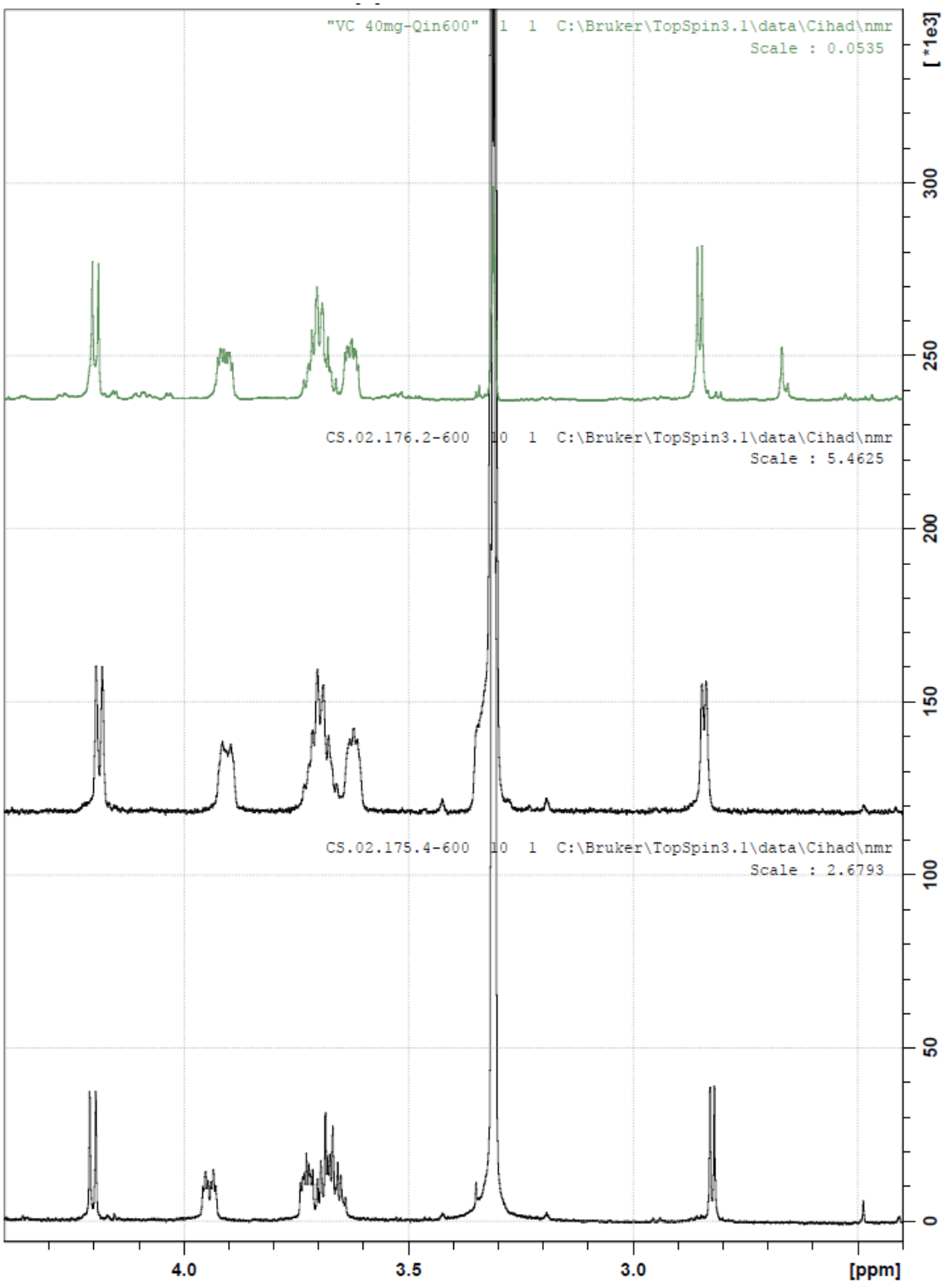
Scale : 0.0535
CS.02.176.2-600 10 1 C:\Bruker\TopSpin3.1\data\Cihad\nmr
Scale : 5.4625
CS.02.175.4-600 10 1 C:\Bruker\TopSpin3.1\data\Cihad\nmr
Scale : 2.6793
[*1e3]
800
600
400
200
0
6
5
4
3
2
1
[ppm]

b)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Scale : 0.0535
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CS.02.175.4-600 10 1 C:\Bruker\TopSpin3.1\data\Cihad\nmr
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4.0
3.5
3.0
[ppm]

c)

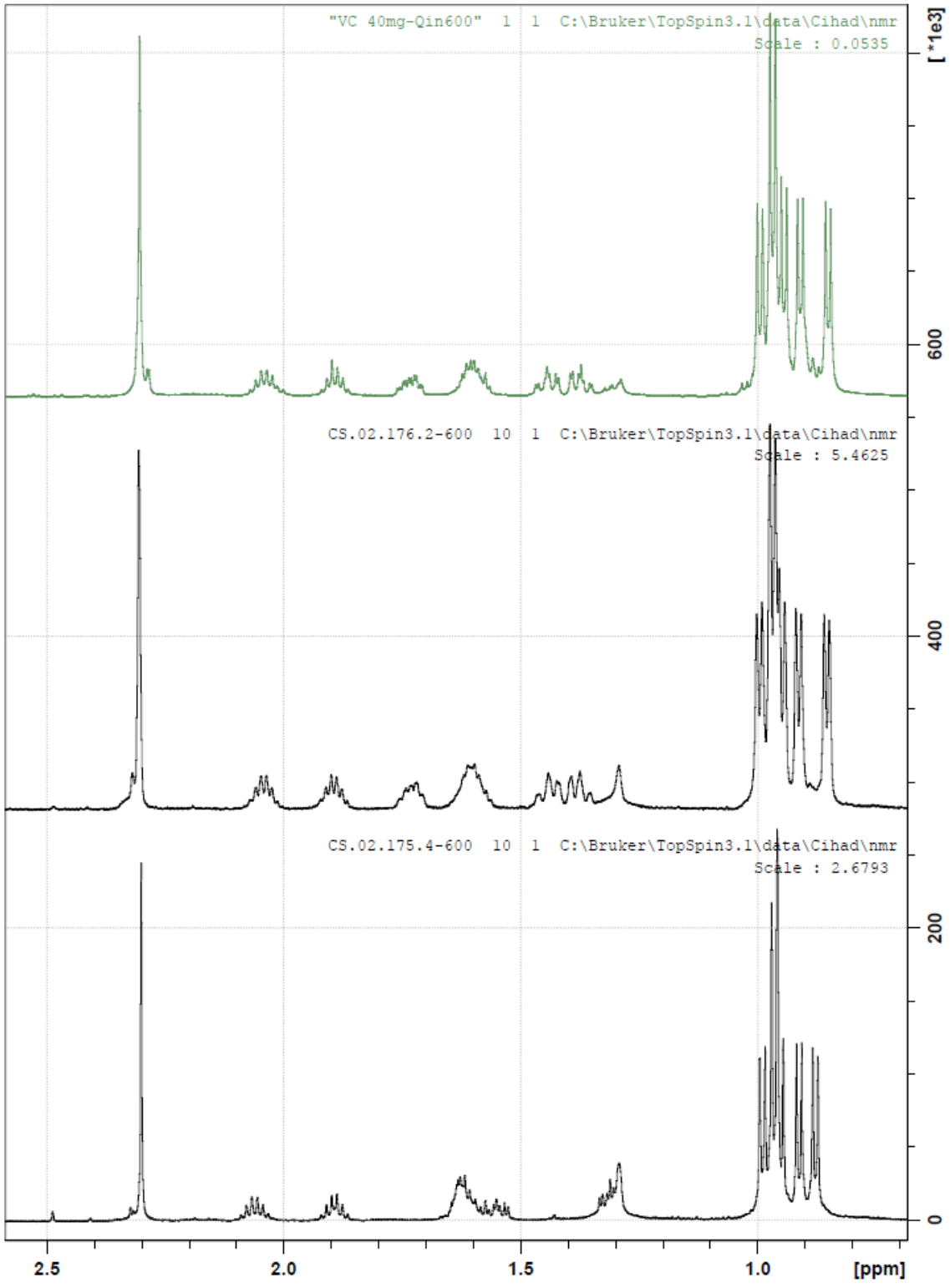


Figure 2.3. Overlay of ^{1}H NMR Data

(From top to bottom: Authentic Tubiashinol, Synthetic Tubiashinol, Synthetic 3-epi-Tubiashinol)

a) 0-7 ppm b) 2.4-4.4 ppm c) 0.7-2.6 ppm range

		Authentic tubiashinol*	Synthetic tubiashinol
NMe-Val	NMe	2.30 (s, 3H)	2.30 (s, 3H)
	α	2.85 (d, *J*=6 Hz, 1H)	2.84 (d, *J*=6 Hz, 1H)
	β	1.92-1.86 (m, 1H)	1.92-1.86 (m, 1H)
	γ	0.94 (d, *J*=7 Hz, 3H)	0.95 (d, *J*=7 Hz, 3H)
	γ	0.97 (d, *J*=7 Hz, 3H)	0.97 (d, *J*=7 Hz, 3H)
Val	α	4.20 (d, *J*=8 Hz, 1H)	4.19 (d, *J*=8 Hz, 1H)
	β	2.07-2.00 (m, 1H)	2.07-2.01 (m, 1H)
	γ	0.99 (d, *J*=7 Hz, 3H)	1.00 (d, *J*=7 Hz, 3H)
	γ	0.97 (d, *J*=7 Hz, 3H)	0.97 (d, *J*=7 Hz, 3H)
Leu	α	3.64-3.61 (m, 1H)	3.62 (app t, *J*=5 Hz, 1H)
	β	1.63-1.56 (m, 1H); 1.76-1.71 (m, 1H)	1.64-1.57 (m, 1H); 1.75-1.71 (m, 1H)
	γ	1.63-1.56 (m, 1H)	1.64-1.57 (m, 1H)
	δ	0.91 (d, *J*=7 Hz, 3H)	0.91 (d, *J*=7 Hz, 3H)
	δ	0.85 (d, *J*=7 Hz, 3H)	0.85 (d, *J*=7 Hz, 3H)
	α'	1.44 (ddd, *J*=14, 11, 4 Hz, 1H) 1.37 (ddd, *J*=14, 11, 3 Hz, 1H)	1.44 (app t, *J*=11 Hz, 1H), 1.37 (app t, *J*=11 Hz, 1H)
	β'	3.73-3.66 (m, 2H)	3.73-3.66 (m, 2H)
	C-OH	3.92-3.89 (m, 1H)	3.90 (app d, *J*=11 Hz, 1H)

* Unpublished data from our group

Table 2.4. ^{1}H NMR Data Comparison of Authentic and Synthetic Tubiashinol

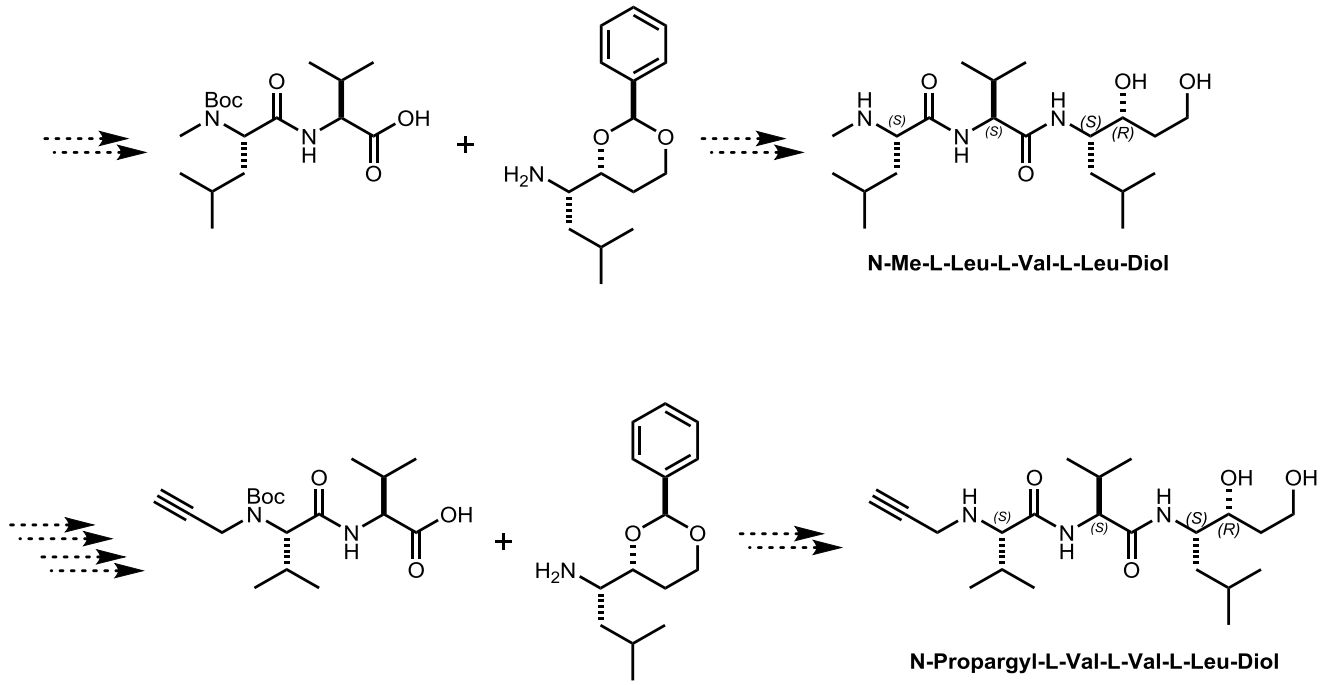
		Authentic tubiashinol	Synthetic tubiashinol
N-Me-Val	NMe	35.1	35.3
	α	71.2	71.4
	β	32.7	32.8
	γ	19.6	19.6
	γ	19.4	19.3
	C=O	175.2	175.7
Val	α	60.2	60.2
	β	31.9	31.9
	γ	20.1	20.0
	γ	19.2	19.0
	C=O	173.2	173.2
Leu	α	72.6	72.7
	β	37.2	37.2
	γ	25.6	25.7
	δ	24.3	24.3
	δ	21.8	21.8
	α'	39.6	39.6
	β'	60.2	60.2
	C-OH	53.4	53.5

* Unpublished data from our group

Table 2.5. ^{13}C NMR Data Comparison of Authentic and Synthetic Tubiashinol

2.5 FUTURE PLANS FOR PROBE DESIGN

After successfully verifying the structure of our native tubiashinol by synthesis, now, our plan is to derivatize the N-terminal of tubiashinol and synthesize *N*-Me-L-Leu-L-Val-L-Leu-Diol and *N*-Propargyl-L-Val-L-Val-L-Leu-Diol to investigate these molecules for structure activity relationship before deciding on our prospective PAP.



Scheme 2.7. Synthesis of *N*-Me-L-Leu-L-Val-L-Leu-Diol and *N*-Propargyl-L-Val-L-Val-L-Leu-Diol

3.0 CONCLUSION

Total synthesis of tubiashinol and 3-*epi*-tubiashinol was successfully completed. We verified the structure and stereochemistry of the authentic molecule by total synthesis. In the ^{1}H NMR overlay, while the synthetic tubiashinol data is slightly different than that of synthetic 3-*epi*-tubiashinol, it matches perfectly with the native compound. There aren't any reported marine natural products from *V. tubiashii* defined by their toxicity towards oyster larvae and zebra fish embryo. Statine derivatives are known for their ability to inhibit proteases. Because tubiashinol contains a statine derivative at its C-terminal and showed positive results in toxicity assays, we suspect that tubiashinol can be the first protease inhibitor isolated from *V. tubiashii.*

During this study, we utilized a 1,3-diol protecting group, benzylidene acetal and validated that diastereomers can be manipulated and separated by introducing rigidity to their structures. We also observed that, in some cases, HATU may not be a suitable coupling reagent for bulky amino acids.

After successfully synthesizing and characterizing tubiashinol, our future focus is going to be in the biological mechanism that cause mortality in oyster larvae and zebra fish embryo. We will be designing a small library of affinity probes to be used in in vitro studies.

4.0 EXPERIMENTAL

All the chemicals and solvents were purchased from commercial suppliers; Sigma-Aldrich, Acros, Alfa Aesar, and Chem Impex. They were used as received, excluding the following. Acetonitrile, dichloromethane, and *N*,*N*-diisopropylethylamine were freshly distilled from calcium hydride before being used in reactions. Methanol, tetrahydrofuran and toluene were dried over 3Å molecular sieves for at least 72 h prior to usage and kept under nitrogen atmosphere. (*72*)

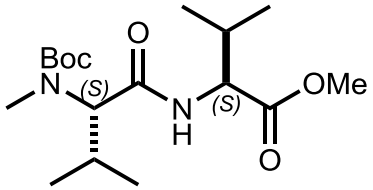
All reactions were performed under nitrogen atmosphere unless stated otherwise and monitored by TLC (TLC silica gel 60 F_{254} plates by Merck), Mass and NMR Spectroscopy. Two UV lamps at 254 nm and 365 nm were used for visualization of TLC plates. Furthermore, the plates were stained with ninhydrin and/or potassium permanganate. Products were purified by flash column chromatography with silica gel 60 (230–400 mesh) purchased from Silicycle or by Biotage Isolera™ One.

Two high performance liquid chromatography instruments were used to record low and high resolution mass data, respectively; Shimadzu UFLC XR using SPD-20A prominence diode array detector and LCMS-2020 quadrupole mass detector; and Thermo Fisher UltiMate™ 3000 equipped with a diode array detector and Q-Exactive orbitrap mass detector. Both instruments were run at a flow rate of 0.2 mL/min and used acetonitrile and water with 0.1% formic acid as mobile phase.

^{1}H and 2D NMR experiments were performed on Bruker Ultrashield™ Plus 400, Bruker Ultrashield™ Plus 500, and Bruker Ultrashield™ 600 insttruments at 400, 500, and 600 MHz, respectively. ^{13}C NMR experiments were also performed on the same aforementioned instruments, but at 100, 125, and 150 MHz, respectively. The chemical shifts in spectra were measured in parts

per million (ppm) on the delta (δ) scale relative to the resonance of the solvent peak ($CDCl_3$: ^1H= 7.26 ppm, ^{13}C= 77.2 ppm, MeOD: ^1H= 3.31 ppm, ^{13}C= 49.0 ppm.) NMR spectra were obtained at 298 K. Couplings in the spectra were described with the following abbreviations: s = singlet, d = doublet; t = triplet, m = multiplet, br = broad, app = apparent.

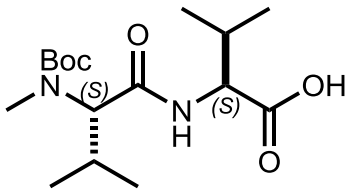
Optical rotations were recorded at 19˚C using the sodium D line (589 nm) on a Perkin Elmer 241 polarimeter. The X-ray intensity data was measured on a Bruker Apex II CCD system equipped with a IMuS Cu K/a Bruker X8 Prospector Ultra (λ = 1.54178 Å) and recorded by Dr. Steven Geib.



Boc-*N*-Me-L-Val-L-Val-OMe (3).

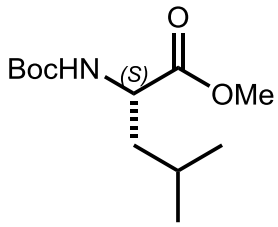
Boc-*N*-Me-L-Val-OH (1.0 g, 4.3 mmol), L-Val-OMe.HCl (0.8 g, 4.5 mmol) and HATU (1.8 g, 4.7 mmol) was placed in a 50 mL flask. The flask was placed under vacuum, back-filled with N_2 gas 3 times and stirred at 0 ˚C. 18 mL dry DMF and freshly-distilled DIEA (1.6 mL, 9.1 mmol) were added successively. The reaction was warmed up and stirred at room temperature. After 2.5 h, the crude was taken into 200 mL EtOAc and washed with 150 mL 1 M aq HCl, 75 mL water and 75 mL brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo before purifying with flash chromatography which gave 1.4 g (90% yield) product as a colorless oil. TLC: 3:1, Hexanes/EtOAc; R_f = 0.39; ^1H NMR ($CDCl_3$, 400 MHz, δ): 6.57 (br d, *J*=6 Hz, 1H), 4.52 (dd, *J*=9, 6 Hz, 1H), 4.06 (app d, *J*=11 Hz, 1H), 3.72 (s, 3H), 2.78 (s, 3H), 2.33–2.20 (m, 1H), 2.20–2.09 (m, 1H), 1.46 (s, 9H), 0.93 (d, *J*=7 Hz, 3H), 0.88 (d, *J*=7 Hz, 3H), 0.86 (d, *J*=7 Hz, 3H), 0.85 (d,

J=7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 172.2, 170.6, 157.1, 80.4, 56.8, 31.1, 28.5, 25.9, 19.8, 19.1, 18.7, 17.5; HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{17}H_{33}O_5N_2$, 345.23840; found, 345.23862.



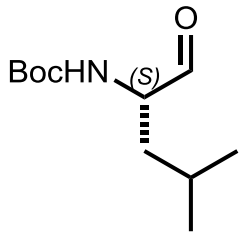
Boc-*N*-Me-L-Val-L-Val-OH (4).

Into a vial with a stir bar, Boc-N-Me-L-Val-L-Val-OMe (1.1 g, 3.3 mmol) in 6.6 mL THF/H_2O (3:1, v/v) and LiOH.H_2O (0.4 g, 10 mmol) were charged and stirred at ambient atmosphere. After 15 h, the reaction mixture was diluted with 15 mL cold water and the pH was set about 4 while adding 1 M aq HCl. The aqueous layer was extracted with 60 mL EtOAc. The organic layers were collected together, washed with 20 mL brine, dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography gave a white solid (1.1 g, 98% yield.) TLC: 9:1, DCM/MeOH; R_f = 0.31; ^{1}H NMR ($CDCl_3$, 600 MHz, δ): 10.90 (br s, 1H), 6.98 (br d, *J*=7 Hz, 1H), 4.53–4.50 (m, 1H), 4.12 (app d, *J*=11 Hz, 1H), 2.79 (s, 3H), 2.27–2.16 (m, 2H), 1.43 (s, 9H), 0.89 (d, *J*=7 Hz, 6H), 0.87 (d, *J*=7 Hz, 3H), 0.82 (d, *J*=7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz, δ): 174.4, 170.9, 157.1, 80.8, 56.8, 30.9, 28.4, 26.2, 19.6, 19.0, 18.7, 17.4; HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{16}H_{31}O_5N_2$, 331.22275; found, 331.22253.



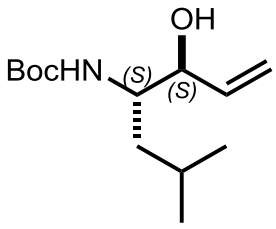
Methyl (*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-methylpentanoate (6).

To a solution of L-leucine methyl ester hydrochloride (1.8g, 10 mmol) in 20 mL H_2O at room temperature was added $NaHCO_3$ (1.3 g, 15 mmol). After 15 min, 20 mL MeOH and Boc_2O (3.4 mL, 15 mmol) were added and the reaction was stirred for 16 h. The pH was adjusted to 4 by careful addition of 1 M aq HCl. The suspension was extracted with 30 mL EtOAc three times. The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, and concentrated under reduced pressure to afford a clear oil. Flash chromatography gave 2.4 g (97% yield) of a colorless oil. TLC: 9:1, Hexanes/EtOAc; R_f = 0.26; ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 4.87 (d, *J*=8 Hz, 1H), 4.31 (m, 1H), 3.73 (s, 3H), 1.69 (m, 1H), 1.62–1.56 (m, 2H), 1.51–1.46 (m, 2H), 1.44 (s, 9H), 0.94 (d, *J*=6.5 Hz, 3H), 0.94 (d, *J*=6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 174.2, 155.6, 80.0, 52.3, 42.0, 28.5, 24.9, 23.0, 22.1; HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{12}H_{24}O_4N$, 246.16998; found, 246.16921.



(*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-methylpentanal (7).

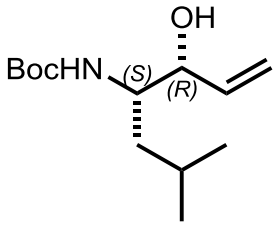
An oven-dried flask under N_2 atmosphere was charged with **1** (12.4 g, 51 mmol) in 176 mL dry toluene. The solution was stirred at -78 ˚C while adding a hexane solution of diisobutylaluminum hydride (126 mmol) over a period of 30 min. After stirring for 19 min, the reaction was quenched with 10 mL MeOH. 170 mL 1 M aq potassium sodium tartrate and 175 mL Et_2O were added successively and the mixture was warmed up to room temperature. After stirring for 45 min, layers were separated and aqueous layer was extracted with 175 mL Et_2O three times. Pooled organic layers were dried over Na_2SO_4 and concentrated in vacuo. Purification by flash chromatography gave a clear oil (8.8 g, 81% yield). TLC: 4:1, Hexanes/EtOAc; R_f= 0.38; ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 9.59 (s, 1H), 4.91 (br s, 1H), 4.25 (m, 1H), 1.81–1.72 (m, 1H), 1.68–1.62 (m, 1H), 1.45 (s, 9H), 1.41–1.36 (m, 1H), 0.97 (d, *J*=6.5 Hz, 3H), 0.97 (d, *J*=6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 200.5, 80.2, 58.6, 38.3, 28.4, 24.8, 23.2, 22.1; HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{11}H_{22}O_3N$, 216.15942; found, 216.15826.



(*3S,4S*)-4-[(*tert*-butoxycarbonyl)amino]-6-methyl-1-hepten-3-ol (8a).

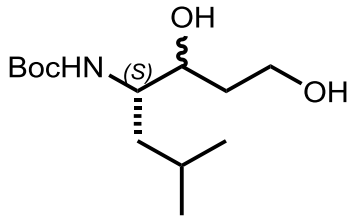
A THF solution of vinylmagnesium bromide (26 mmol) and 20 mL dry THF was added into an oven-dried flask under N_2 atmosphere and stirred at 0 ˚C. A solution of **2** (2.5 g, 12 mmol) in 10 mL dry THF was then added over 10 min. The reaction had let to warm up to room temperature and stirred for 50 min before it was quenched with 25 mL 15% (w/v) aq NH_4Cl. The mixture was taken into a separatory funnel and extracted with 30 mL EtOAc four times. Combined organic

layers were dried over Na_2SO_4 and concentrated under reduced pressure before purifying with flash chromatography which gave 1.6 g (55% yield) product as a clear oil mixture of **9a** and **9b** in a ratio of 3:1. TLC: 4:1, Hexanes/EtOAc; R_f = 0.26; ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 5.90 (ddd, *J*=17, 11, 6 Hz, 1H), 5.31 (app t, *J*=17 Hz, 1H), 5.21 (dd, *J*=11 Hz, 1H), 4.56 (br d, 1H), 4.06 (br s, 1H), 3.69–3.63 (m, 1H), 1.73–1.62 (m, 1H), 1.43 (s, 9H), 1.49–1.36 (m, 2H), 0.93 (d, *J*=6.5 Hz , 3H), 0.91 (d, *J*=6.5 Hz , 3H); ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 156.6, 138.4, 116.3, 75.5, 53.1, 40.9, 28.5, 25.0, 23.5, 22.1; HRMS-ESI (*m/z*): [M+H]+ calcd for $C_{13}H_{26}O_3N$, 244.19072; found, 244.18991.



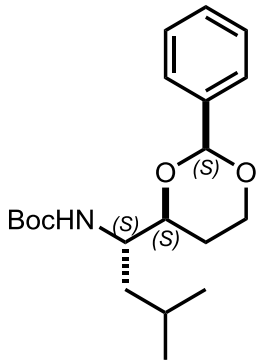
(*3R,4S*)-4-[(*tert*-butoxycarbonyl)amino]-6-methyl-1-hepten-3-ol (8b).

^{1}H NMR ($CDCl_3$, 500 MHz, δ):5.83 (ddd, *J*=17, 11, 6 Hz, 1H), 5.31 (app t, *J*=17 Hz, 1H), 5.21 (dd, *J*=11 Hz, 1H), 4.50 (br d, 1H), 4.18 (br s, 1H), 3.85–3.79 (m, 1H), 1.73–1.62 (m, 1H), 1.44 (s, 9H), 1.28–1.22 (m, 2H), 0.93 (d, *J*=6.5 Hz , 3H), 0.91 (d, *J*=6.5 Hz , 3H). ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 156.6, 136.9, 116.7, 76.1, 53.8, 39.4, 28.4, 25.0, 23.6, 21.9



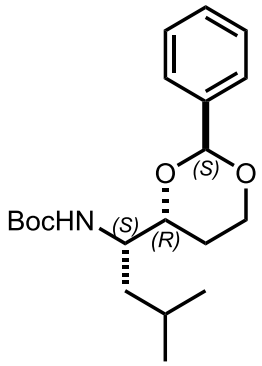
(3*S*,4*S*)-4-[(*tert*-butoxycarbonyl)amino]-6-methyl-1,3-heptandiol (9a) and (3*R*,4*S*)-4-[(*tert*-butoxycarbonyl)amino]-6-methyl-1,3-heptandiol (9b).

To a solution of **3a** and **3b** (4.1 g, 17 mmol) in 56 mL dry THF under N_2 atmosphere at room temperature was added 9-borabicyclo [3.3.1]nonane (42 mmol) in two portions. The reaction was stirred at 70 ˚C for 18 h and quenched with 30 mL EtOH before adding 20 mL 6 M aq NaOH and 11 mL 30% H_2O_2 at 0 ˚C. The mixture was warmed up and stirred at 50 ˚C for 16 h, then extracted with 40 mL EtOAc three times. Organic layers were washed with 20 mL H_2O and brine, dried over Na_2SO_4 and concentrated. The flash chromatography afforded 3.8 g (86% yield) yellow oil as a mixture of **10a** and **10b** in a ratio of 3.5:1. TLC: 2:1, Hexanes/EtOAc; R_f = 0.11; ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 4.68–4.56 (br s, 1H), 3.90–3.85 (m, 2H), 3.83–3.78 (m, 1H), 3.70–3.60 (br s, 1H), 1.83–1.76 (m, 1H), 1.72–1.60 (m, 1H), 1.72–1.60 (m, 1H), 1.44 (s, 9H), 1.50–1.41 (m, 1H), 1.34–1.26 (m, 1H), 0.94 (d, *J*=6.5 Hz, 3H), 0.92 (d, *J*=6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 156.6, 79.4, 73.8, 61.4, 53.1, 41.4, 35.7, 28.5, 24.9, 23.4, 22.2; HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{13}H_{28}O_4N$, 262.20128; found, 262.20140.



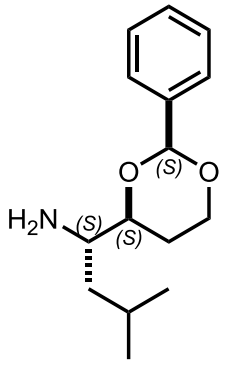
***tert*-Butyl (*S*)-1-[(*4S*)-2-phenyl-1,3-dioxan-4-yl]-3-methylbutylcarbamate (10a).**

A mixture of **10a** and **10b** (59 g, 0.22 mmol) and camphorsulfonic acid (5 mg, 0.02 mmol) were charged into an oven-dried flask under N_2 atmosphere, then 2 mL dry MeCN and benzaldehyde dimethyl acetal (0.05 mL, 0.34 mmol) were added successively. The mixture was stirred at room temperature for 5 h, quenched with 1 mL 10% Et_3N in toluene, and coevaporated with toluene. The crude oil was purified by sequential flash chromatography several times to separate the diastereomers, **11a** and **11b**, as pale yellow oils (49 mg, 62% yield.) TLC: 3:1, Hexanes/EtOAc; R_f = 0.44; $[\alpha]^{19}_D$ -52.3˚ (c 0.4, CDCl3); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 7.48 (dd, *J*=8, 2 Hz, 2H), 7.40–7.32 (m, 3H), 5.51 (s, 1H), 4.73 (d, *J*=10 Hz, 1H), 4.29 (dd, *J*=11, 4 Hz, 1H), 3.98 (td, *J*=12, 2 Hz, 1H), 3.88 (app d, *J*=12 Hz, 1H), 3.80–3.73 (m, 1H), 2.04 (ddd, *J*=12, 12, 5 Hz, 1H), 1.75–1.63 (m, 1H), 1.62–1.55 (m, 1H), 1.47–1.39 (m, 1H), 1.44 (s, 9H), 1.41–1.34 (m, 1H), 0.95 (d, *J*=6 Hz, 3H), 0.94 (d, *J*=6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 156.1, 138.8, 128.8, 128.3, 126.0, 101.0, 78.8, 67.0, 51.7, 41.7, 28.5, 27.8, 24.9, 23.3, 22.4; HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{32}O_4N$, 350.23258; found, 350.23259.



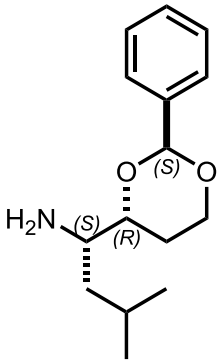
***tert*-Butyl (*S*)-1-[(*4R*)-2-phenyl-1,3-dioxan-4-yl]-3-methylbutylcarbamate (10b).**

TLC: 3:1, Hexanes/EtOAc; R_f = 0.36; $[\alpha]^{19}_{D}$ -66.1˚ (c 0.4, CDCl3); ^{1}H NMR ($CDCl_3$, 500 MHz, δ): 7.48 (dd, *J*=8, 2 Hz, 2H), 7.39–7.32 (m, 3H), 5.49 (s, 1H), 4.65 (d, *J*=10 Hz, 1H), 4.28 (dd, *J*=11, 4 Hz, 1H), 3.94 (td, *J*=12, 2 Hz, 1H), 3.88 (app d, *J*=12 Hz, 1H), 3.77–3.73 (m, 1H), 1.93 (ddd, *J*=12, 12, 5 Hz, 1H), 1.73–1.65 (m, 1H), 1.52–1.40 (m, 3H), 1.44 (s, 9H), 0.94 (d, *J*=6.5 Hz, 3H), 0.93 (d, *J*=6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz, δ): 155.8, 138.8, 128.9, 128.3, 126.3, 101.4, 80.3, 67.1, 52.2, 38.7, 28.5, 27.8, 24.9, 24.0, 21.7; HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{32}O_4N$, 350.23258; found, 350.23269.



(*S*)-3-methyl-1-[(*4S*)-2-phenyl-1,3-dioxan-4-yl]-1-butanamine (11a).

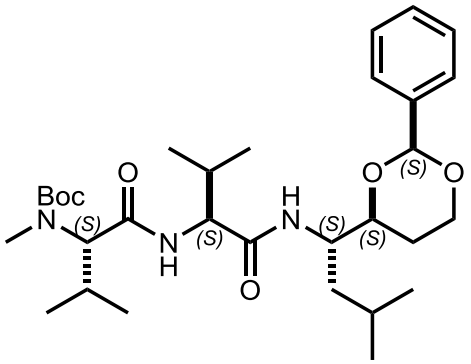
11a (0.5g, 1.3 mmol) in 3.5 mL dry DCM was added into an oven-dried flask under N_2 atmosphere. The solution was stirred at 0 ˚C before addition of anisole (0.2 mL, 1.8 mmol) and dropwise addition of 3 mL TFA over a period of 5 min. After 21 min, the reaction was immediately poured onto 25 mL toluene and co-evaporated. The flash chromatography gave 0.2 g (61%) of a pale yellow oil. TLC: 9:1, DCM/MeOH; R_f = 0.38; $[\alpha]^{19}_D$ -6.2˚ (c 0.4, CDCl3); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 7.46 (dd, *J*=8, 2 Hz, 2H), 7.38–7.31 (m, 3H), 5.87 (br s, 2H), 5.50 (s, 1H), 4.28 (dd, *J*=12, 5 Hz, 1H), 3.93 (app t, *J*=12 Hz, 1H), 3.87 (app t, *J*=10 Hz, 1H), 3.05 (td, *J*=10, 4 Hz, 1H), 1.79 (ddd, *J*=12, 5, 5 Hz, 2H), 1.59 (app d, *J*=12 Hz, 1H), 1.52 (ddd, *J*=10, 5, 5 Hz, 1H), 1.29 (ddd, *J*=10, 4, 4 Hz, 1H), 0.92 (d, *J*=7 Hz, 3H), 0.89 (d, *J*=7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 137.9, 129.3, 128.5, 126.4, 101.6, 66.5, 53.9, 38.6, 27.9, 24.3, 23.5, 21.4; HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{15}H_{24}O_2N$, 250.18016; found, 250.18062.



(*S*)-3-methyl-1-[(*4R*)-2-phenyl-1,3-dioxan-4-yl]-1-butanamine (11b).

TLC: 9:1, DCM/MeOH; R_f = 0.37; $[\alpha]^{19}_D$ -12.0˚ (c 0.4, CDCl3); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 7.90 (br s, 2H), 7.46 (dd, *J*=8, 2 Hz, 2H), 7.38–7.32 (m, 3H), 5.49 (s, 1H), 4.28 (dd, *J*=12, 4 Hz, 1H), 4.09 (app t, *J*=12 Hz, 1H), 3.89 (app t, *J*=10 Hz, 1H), 3.37 (b s, 1H), 2.00 (ddd, *J*=12, 4, 4 Hz, 1H), 1.74–1.64 (m, 1H), 1.64–1.55 (m, 1H), 1.50–1.41 (m, 1H), 0.93 (d, *J*=6 Hz, 3H), 0.90 (d,

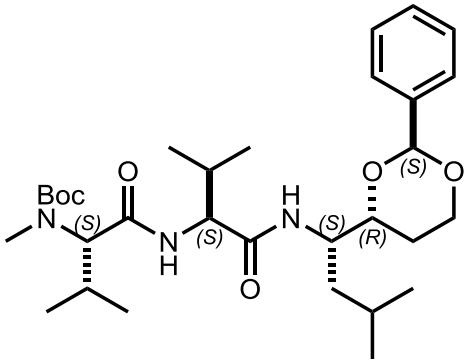
J=6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 137.7, 129.3, 128.4, 126.3, 101.5, 66.5, 52.8, 36.8, 25.0, 24.3, 22.7, 22.0; HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{15}H_{24}O_2N$, 250.18016; found, 250.18125.



***N*-methyl-L-valyl-*N*-[(1*S*)-1-[(*4S*)-2-phenyl-1,3-dioxan-4-yl]-3-methylbutyl]-L-valinamide (12a).**

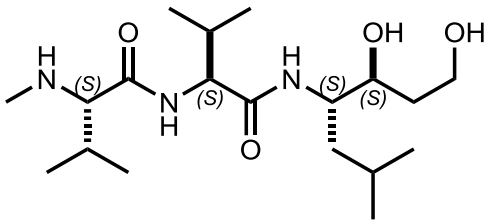
Boc-N-Me-Val-Val-OH (25 mg, 0.08 mmol), EDC.HCl (15 mg, 0.08 mmol) and HOBt (11 mg, 0.08 mmol) were placed in an oven-dried 10 mL pear-shaped flask under N_2 atmosphere. 1.5 mL dry DMF was added into the flask on an ice-bath. After 5 min, freshly-distilled DIEA (0.04 mL, 0.22 mmol) and **6a** (18 mg, 0.07 mmol) in 0.5 mL dry DMF were added successively. The mixture was allowed to warm up to room temperature and stirred for 24 h before the solvent was removed under high vacuum. 22 mg (55%) colorless oil was obtained by flash chromatography. TLC: 7:3, Hexanes/EtOAc; R_f = 0.26; $[\alpha]^{19}_D$ -111.3˚ (c 1.0, CDCl3); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 7.44 (dd, *J*=8, 2 Hz, 2H), 7.40–7.31 (app d, 3H), 6.54 (d, *J*=8 Hz, 1H), 6.05 (d, *J*=9 Hz, 1H), 5.48 (s, 1H), 4.27–4.18 (m, 2H), 4.17– 4.07 (m, 1H), 4.03 (d, *J*=11 Hz, 1H), 3.99–3.85 (m, 2H), 2.74 (s, 3H), 2.29–2.18 (m, 2H), 1.90 (ddd, *J*=12, 5, 5 Hz, 1H), 1.67–1.54 (m, 2H), 1.46 (s, 9H), 1.43–1.33

(m, 2H), 0.92 (d, *J*=6 Hz, 3H), 0.90 (d, *J*=6 Hz, 6H), 0.89– 0.81 (m, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 170.9, 157.1, 138.6, 129.2, 128.9, 128.4, 128.3, 126.1, 101.3, 80.5, 66.9, 64.7, 58.6, 50.2, 41.4, 30, 29.8, 28.5, 27.9, 25.8, 24.8, 23.3, 22.2, 19.8, 19.6, 18.6, 17.5; HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{31}H_{52}O_6N_3$, 562.38506; found, 562.38525.



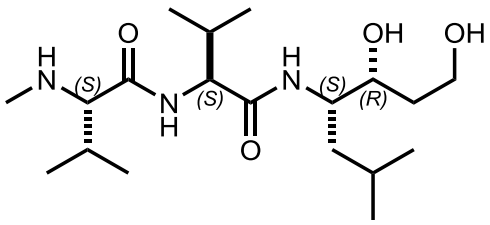
***N*-methyl-L-valyl-*N*-[(1*S*)-1-[(*4S*)-2-phenyl-1,3-dioxan-4-yl]-3-methylbutyl]-L-valinamide (12b).**

TLC: 7:3, Hexanes/EtOAc; R_f = 0.25; $[\alpha]^{19}_D$ -147.7˚ (c 0.9, CDCl3); ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 7.44 (dd, *J*=8, 2 Hz, 2H), 7.40–7.30 (app d, 3H), 6.57 (d, *J*=8 Hz, 1H), 6.03 (d, *J*=9 Hz, 1H), 5.43 (s, 1H), 4.27 (dd, *J*=11, 4 Hz, 1H), 4.21– 4.08 (m, 2H), 4.03 (d, *J*=11 Hz, 1H), 3.97–3.80 (m, 2H), 2.74 (s, 3H), 2.32–2.14 (m, 2H), 1.93 (ddd, *J*=12, 5, 5 Hz, 1H), 1.59–1.53 (m, 1H), 1.51–1.39 (m, 2H), 1.46 (s, 9H), 0.91 (d, *J*=7 Hz, 3H), 0.88 (d, *J*=6 Hz, 6H), 0.86 (d, *J*=7 Hz, 3H), 0.83 (d, *J*=7 Hz, 3H), 0.79 (d, *J*=6 Hz, 3H)); ^{13}C NMR ($CDCl_3$, 100 MHz, δ): 170.6, 157.2, 138.6, 129.0, 128.3, 126.2, 101.5, 80.6, 67.0, 58.7, 50.6, 38.1, 30.1, 30.0, 28.5, 27.7, 25.8, 24.8, 23.9, 21.5, 19.7, 19.4, 18.6, 17.6; HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{31}H_{52}O_6N_3$, 562.38506; found, 562.38467.



***N*-methyl-L-valyl-*N*-[(*1S, 2S*)-1-(2-methylpropyl)-2,4-dihydroxybuthyl]-L-valinamide (13a).**

A 10 mL round-bottom flask with a stir bar was charged with **7a** (20 mg, 0.04 mol) in 1.5 mL EtOH. 3 mL 1M aq HCl was added and the reaction was stirred at ambient atmosphere. After 4 h, reaction was heated to 45 ˚C and stirred for 11 h. The mixture was concentrated under reduced pressure and purified by flash chromatography to give a white hydrochloric acid salt of **8a** (11 mg, 85% yield.) TLC: 5:1, DCM/MeOH; R_f = 0.36; $[\alpha]^{19}_{D}$ -13.9˚ (c 0.2, MeOD); ^{1}H NMR (MeOD, 600 MHz, δ): 4.20 (d, 1H, *J*=8 Hz), 3.94 (dt, 1H, *J*=11, 4 Hz), 3.74–3.71 (m, 1H), 3.70–3.64 (m, 2H), 2.82 (d, 1H, *J*=6 Hz), 2.30 (s, 3H), 2.08–2.03 (m, 1H), 1.92–1.86 (m, 1H), 1.64–1.59 (m, 2H), 1.58–1.52 (m, 1H), 1.33–1.29 (m, 2H), 0.99 (d, 3H, *J*=7 Hz), 0.96 (d, 6H, *J*=7 Hz), 0.95 (d, 3H, *J*=7 Hz), 0.91 (d, 3H, *J*=7 Hz), 0.88 (d, 3H, *J*=7 Hz); ^{13}C NMR (MeOD, 150 MHz, δ): 176.0, 173.3, 71.5, 71.3, 60.2, 60.2, 52.9, 41.4, 37.5, 35.4, 32.8, 31.8, 25.8, 23.9, 22.2, 20.0, 19.7, 19.3, 18.9; HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{40}O_4N_3$, 374.30133; found, 374.30265.

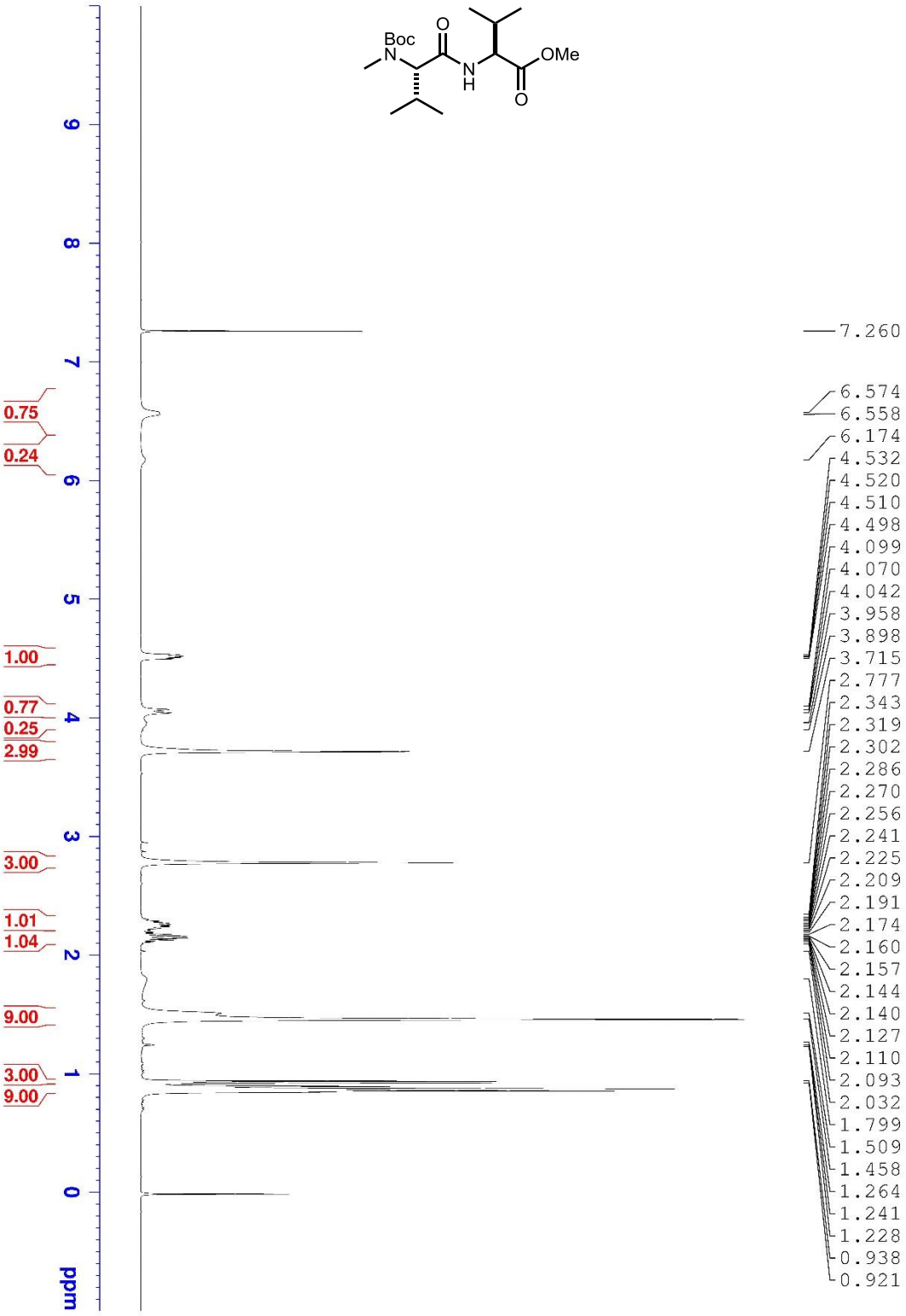


***N*-methyl-L-valyl-*N*-[(*1S, 2R*)-1-(2-methylpropyl)-2,4-dihydroxybuthyl]-L-valinamide (13b).**

TLC: 5:1, DCM/MeOH; R_f = 0.31; $[\alpha]^{19}_D$ -38.9˚ (c 0.2, CDCl3); ^{1}H NMR (MeOD, 600 MHz, δ): 4.19 (d, 1H, *J*=8 Hz), 3.90 (app d, 1H, *J*=11 Hz), 3.73–3.66 (m, 2H), 3.62(app t, 1H, *J*=5 Hz), 2.84 (d, 1H, *J*=5 Hz), 2.30 (s, 3H), 2.07–2.01 (m, 1H), 1.92–1.87 (m, 1H), 1.75–1.71 (m, 1H), 1.65–1.56 (m, 2H), 1.44 (app t, 1H, *J*=11 Hz), 1.37 (app t, 1H, *J*=11 Hz), 1.00 (d, 3H, *J*=7 Hz), 0.97 (d, 6H, *J*=7 Hz), 0.95 (d, 3H, *J*=7 Hz), 0.91 (d, 3H, *J*=7 Hz), 0.85 (d, 3H, *J*=7 Hz); ^{13}C NMR (MeOD, 150 MHz, δ): 175.7, 173.2, 72.7, 71.4, 60.2, 60.2, 53.5, 39.6, 37.2, 35.3, 32.8, 31.9, 25.7, 24.3, 21.8, 20.0, 19.6, 19.3, 19.0; HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{40}O_4N_3$, 374.30133; found, 374.30309.

APPENDIX A

NMR DATA OF COMPOUNDS


Boc
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H
OMe
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8
7
6
5
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2
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ppm
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1.00
0.77
0.25
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3.00
1.01
1.04
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3.00
9.00
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2.209
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2.140
2.127
2.110
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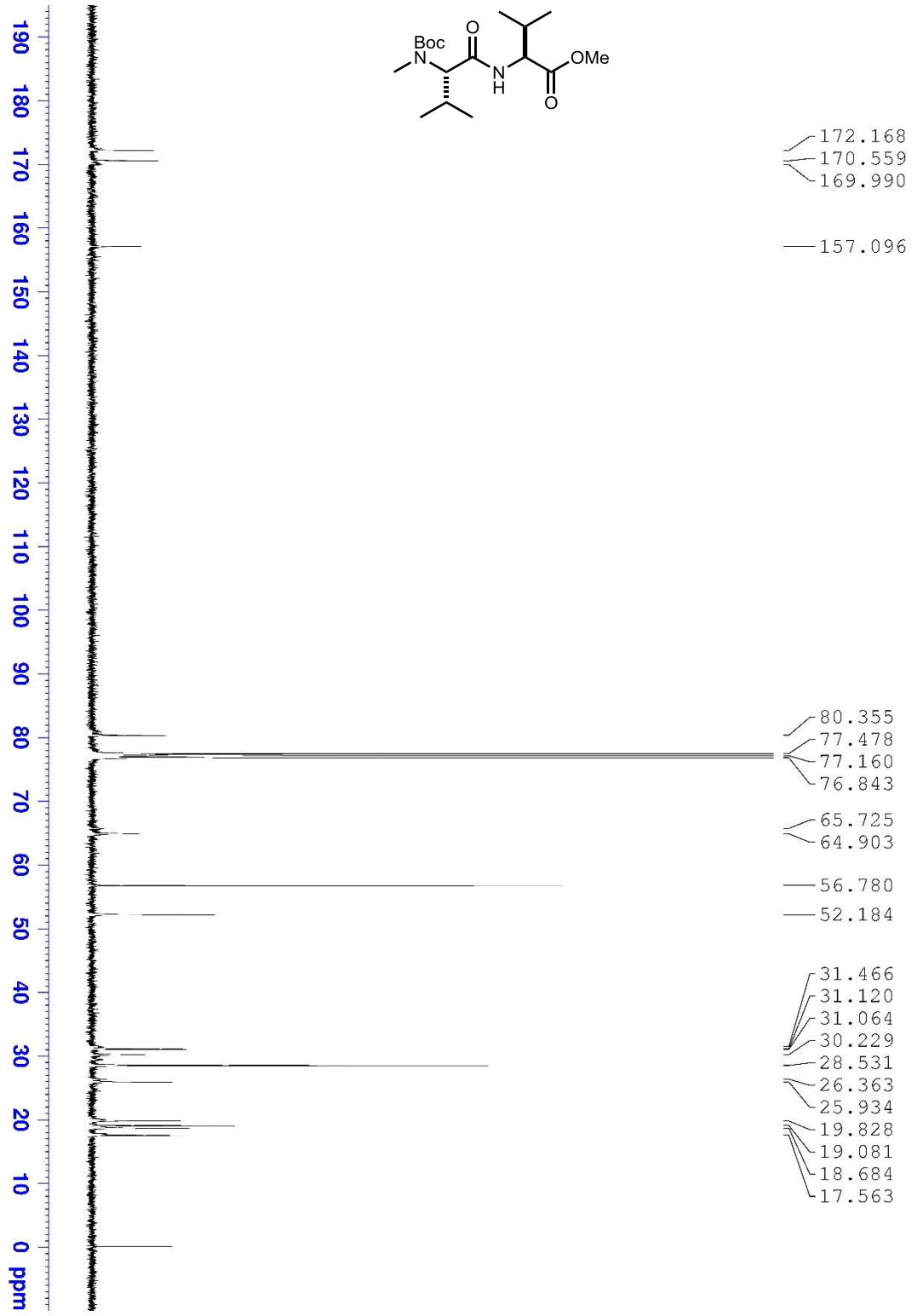
1.799
1.509
1.458
1.264
1.241
1.228
0.938
0.921


Boc
N
O
N
H
OMe
O
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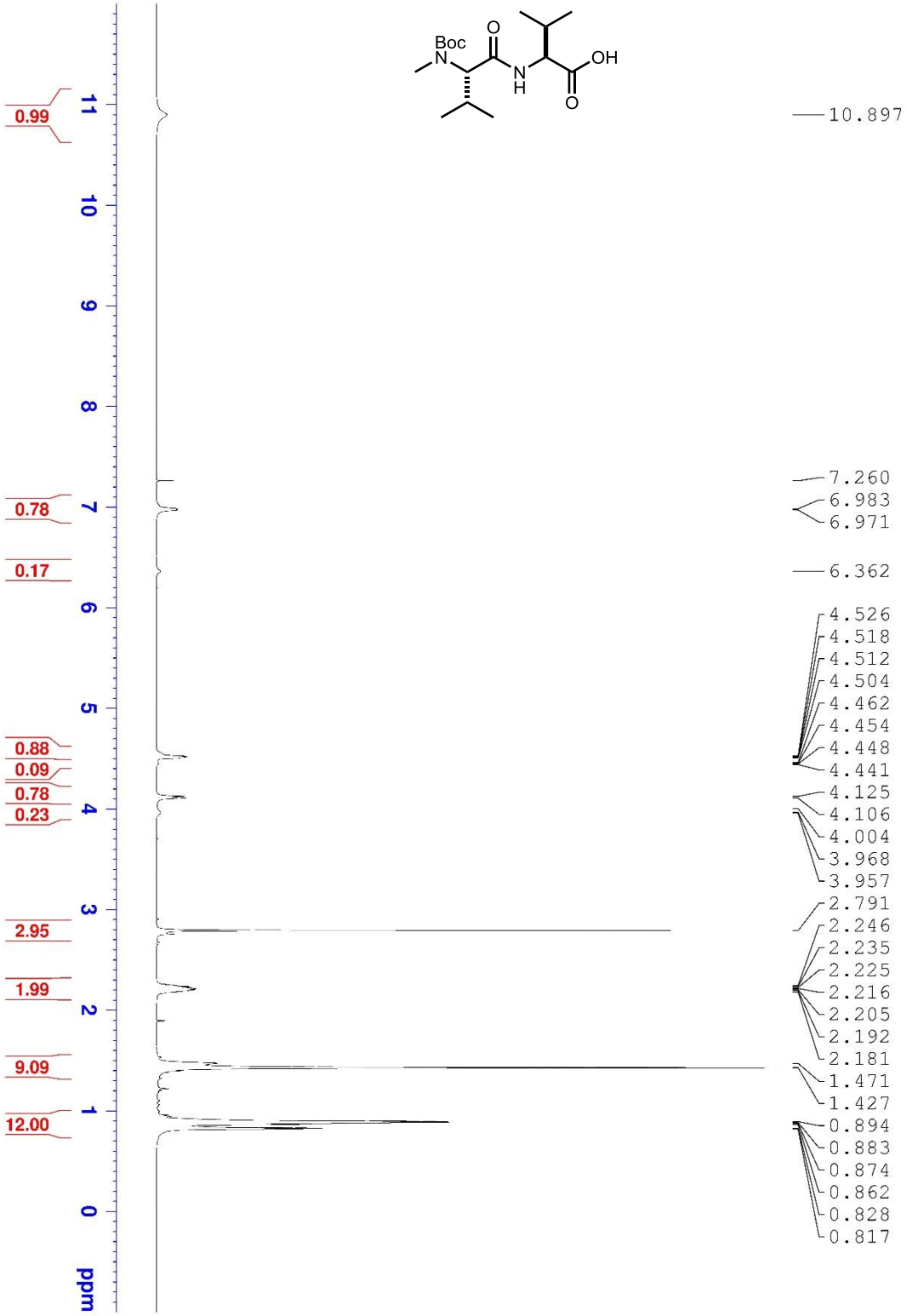
170.559
169.990
157.096
80.355
77.478
77.160
76.843
65.725
64.903
56.780
52.184
31.466
31.120
31.064
30.229
28.531
26.363
25.934
19.828
19.081
18.684
17.563
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0 ppm


Boc
N
O
N
H
OH
O
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0.78
0.17
0.88
0.09
0.78
0.23
2.95
1.99
9.09
12.00
11
10
9
8
7
6
5
4
3
2
1
0
ppm
10.897
7.260
6.983
6.971
6.362
4.526
4.518
4.512
4.504
4.462
4.454
4.448
4.441
4.125
4.106
4.004
3.968
3.957
2.791
2.246
2.235
2.225
2.216
2.205
2.192
2.181
1.471
1.427
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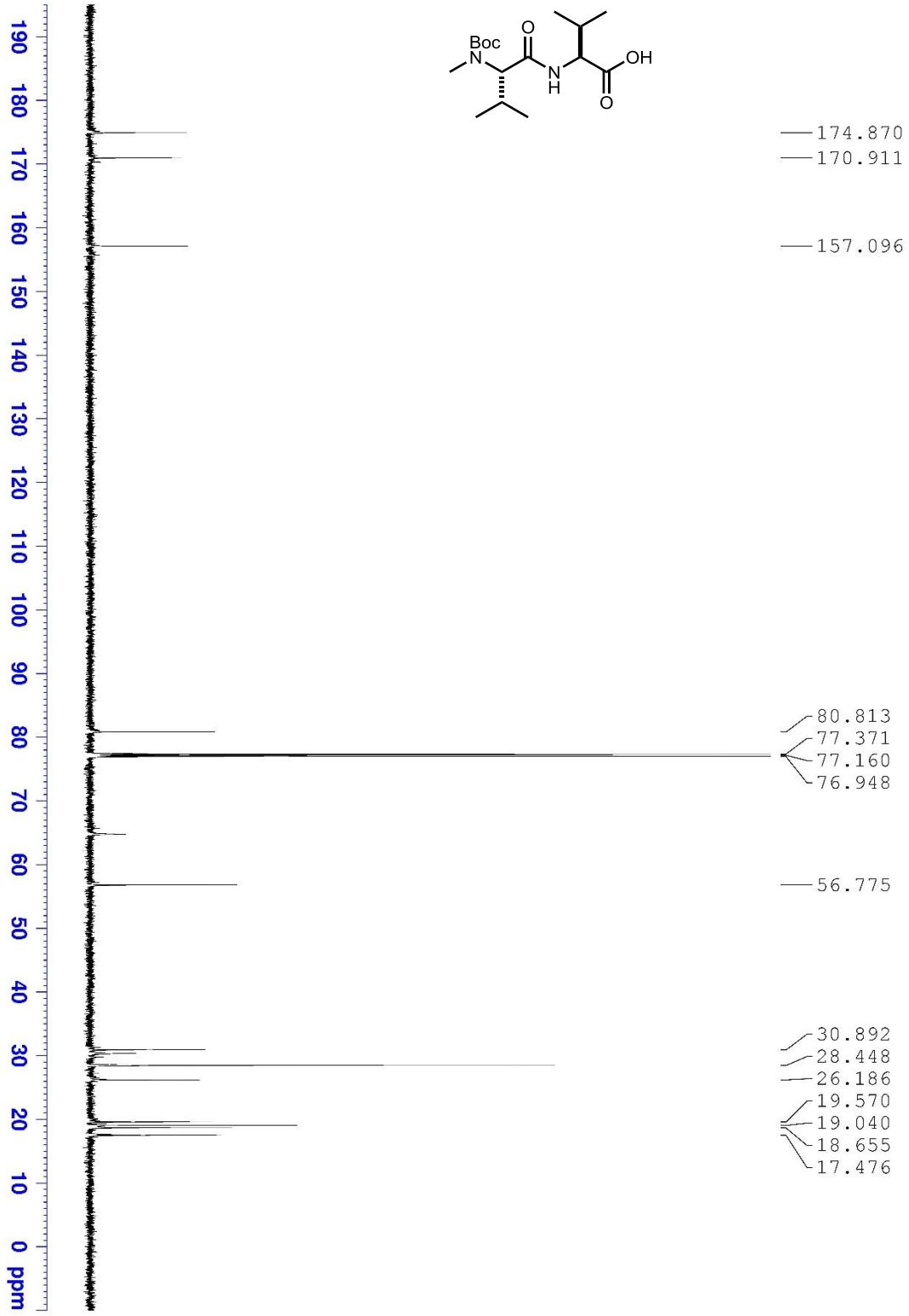
0.883
0.874
0.862
0.828
0.817


Boc
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O
N
H
OH
O
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157.096
80.813
77.371
77.160
76.948
56.775
30.892
28.448
26.186
19.570
19.040
18.655
17.476
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
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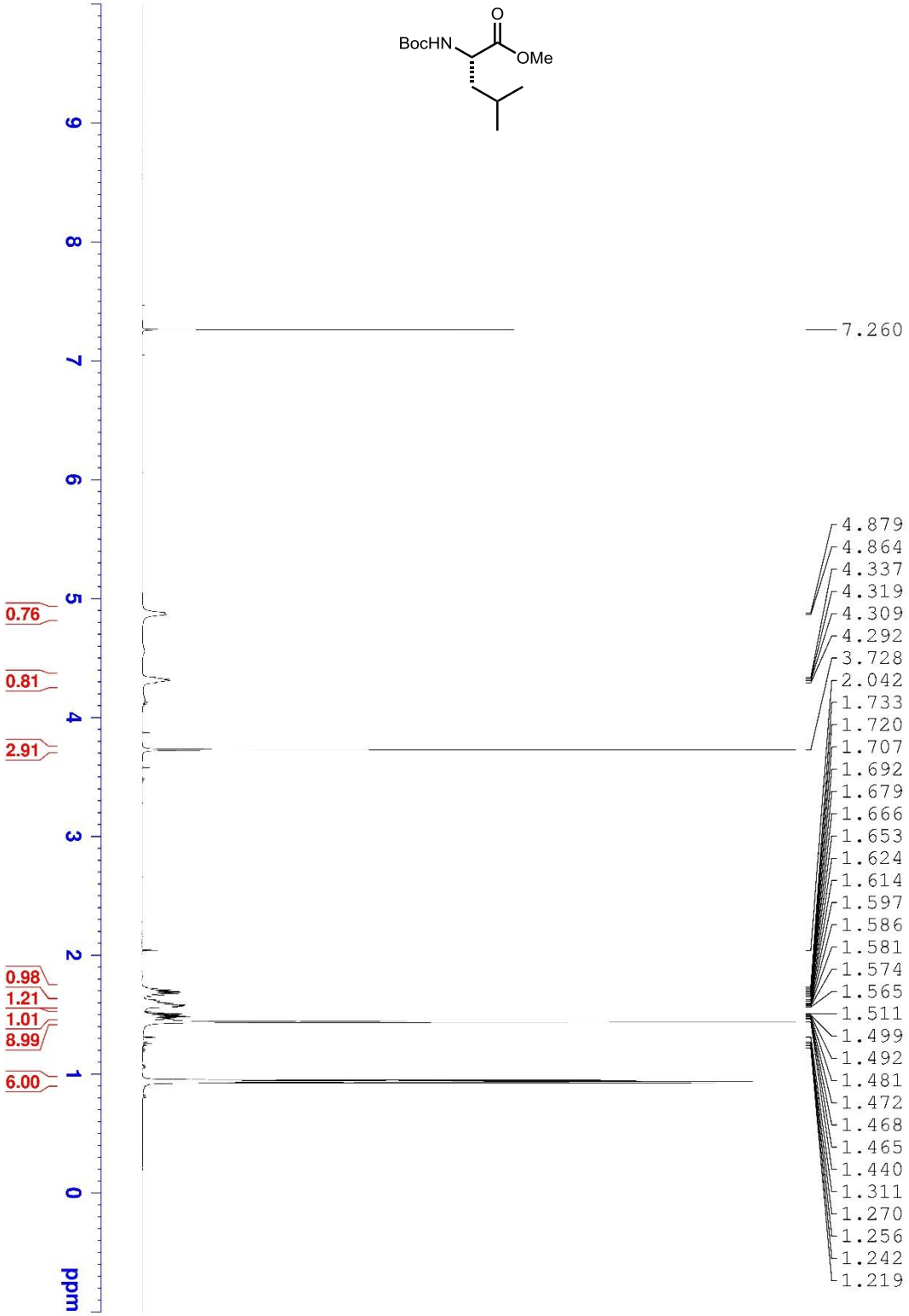
BocHN
O
OMe
7.260
4.879
4.864
4.337
4.319
4.309
4.292
3.728
2.042
1.733
1.720
1.707
1.692
1.679
1.666
1.653
1.624
1.614
1.597
1.586
1.581
1.574
1.565
1.511
1.499
1.492
1.481
1.472
1.468
1.465
1.440
1.311
1.270
1.256
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1.219
0.76
0.81
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1.21
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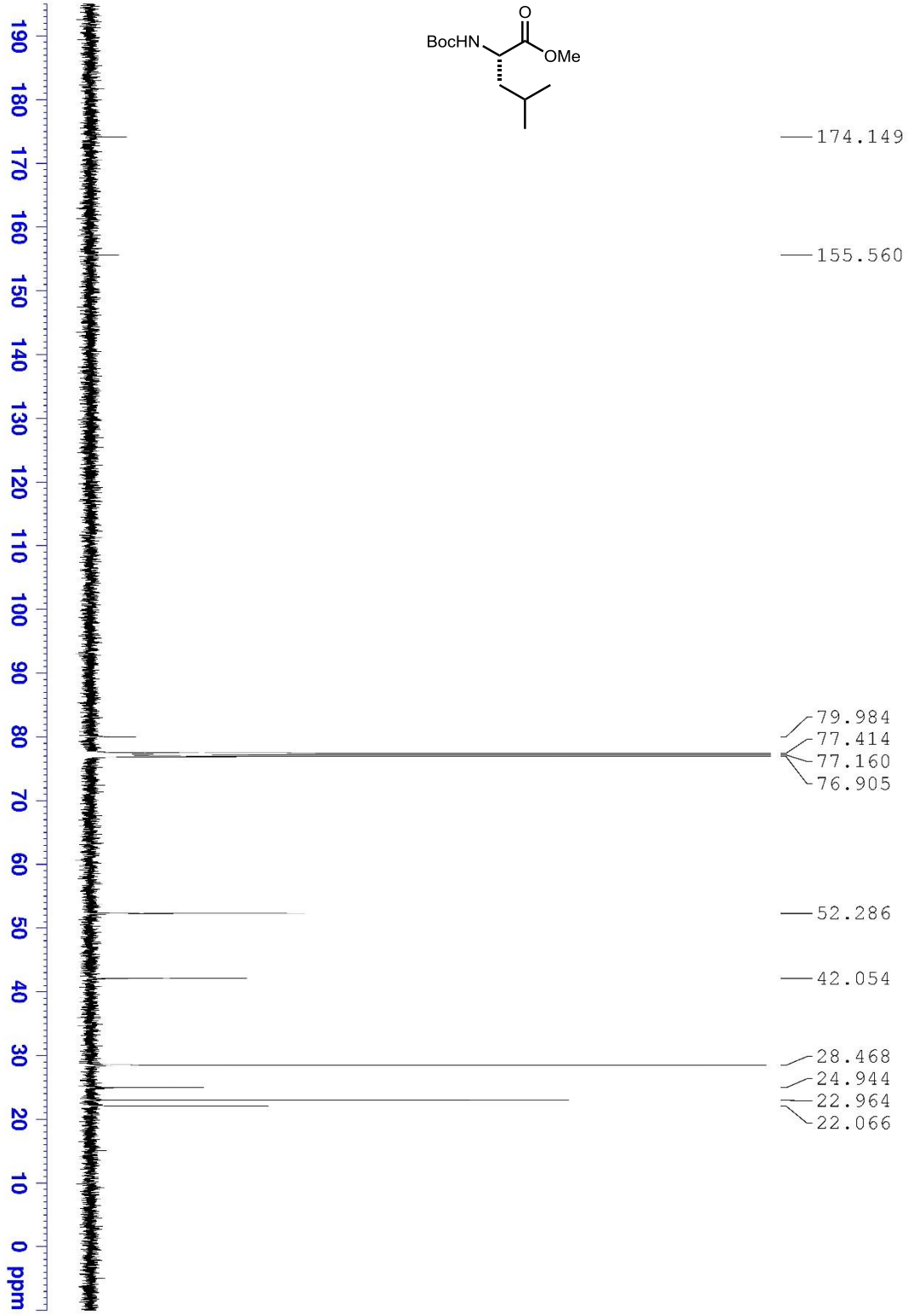
8.99
6.00
ppm


BocHN
O
OMe
174.149
155.560
79.984
77.414
77.160
76.905
52.286
42.054
28.468
24.944
22.964
22.066
190
180
170
160
150
140
130
120
110
100
90
80
70
60
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40
30
20
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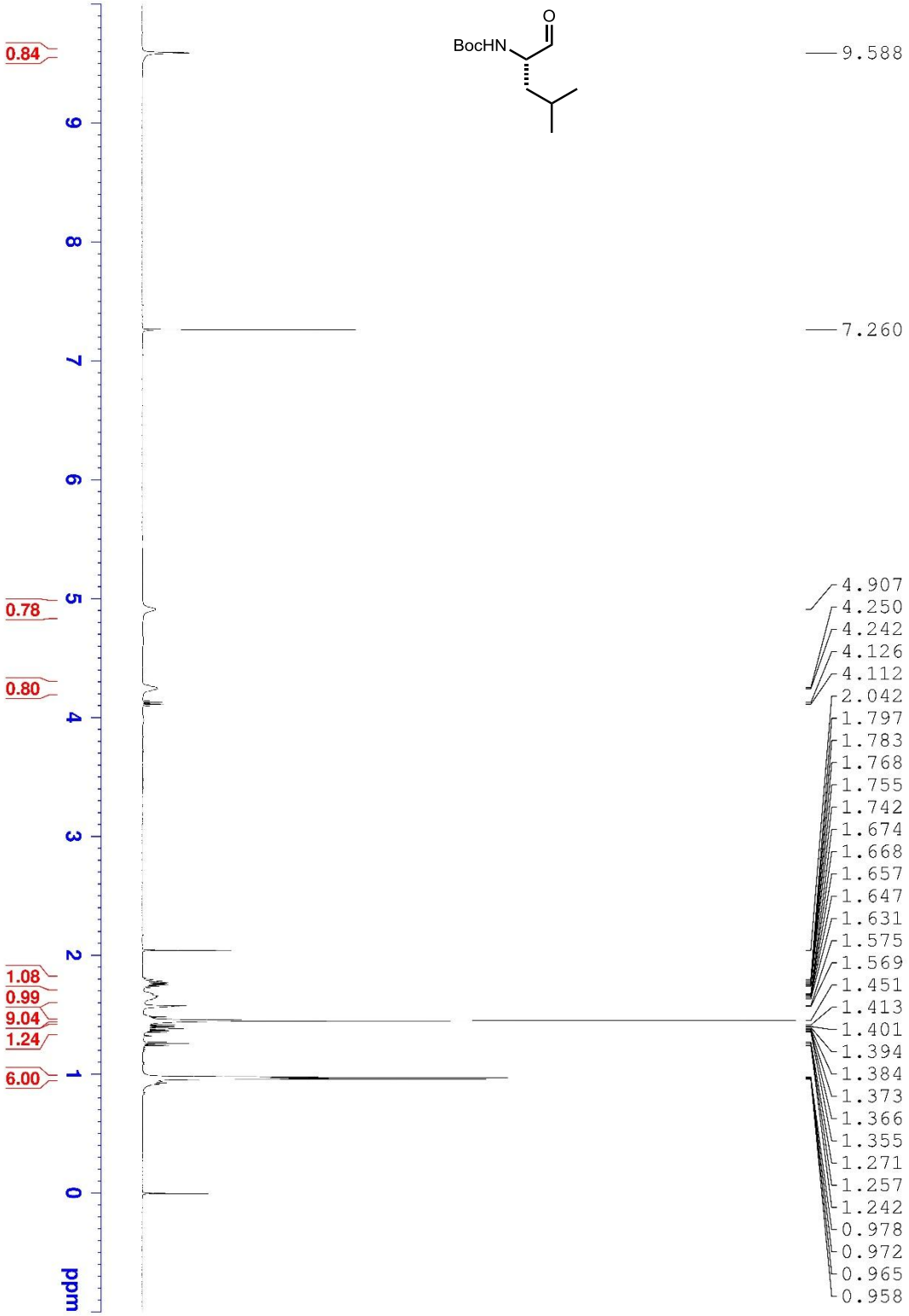
BocHN
O
9.588
7.260
4.907
4.250
4.242
4.126
4.112
2.042
1.797
1.783
1.768
1.755
1.742
1.674
1.668
1.657
1.647
1.631
1.575
1.569
1.451
1.413
1.401
1.394
1.384
1.373
1.366
1.355
1.271
1.257
1.242
0.978
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0.958
0.84
0.78
0.80
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0.99
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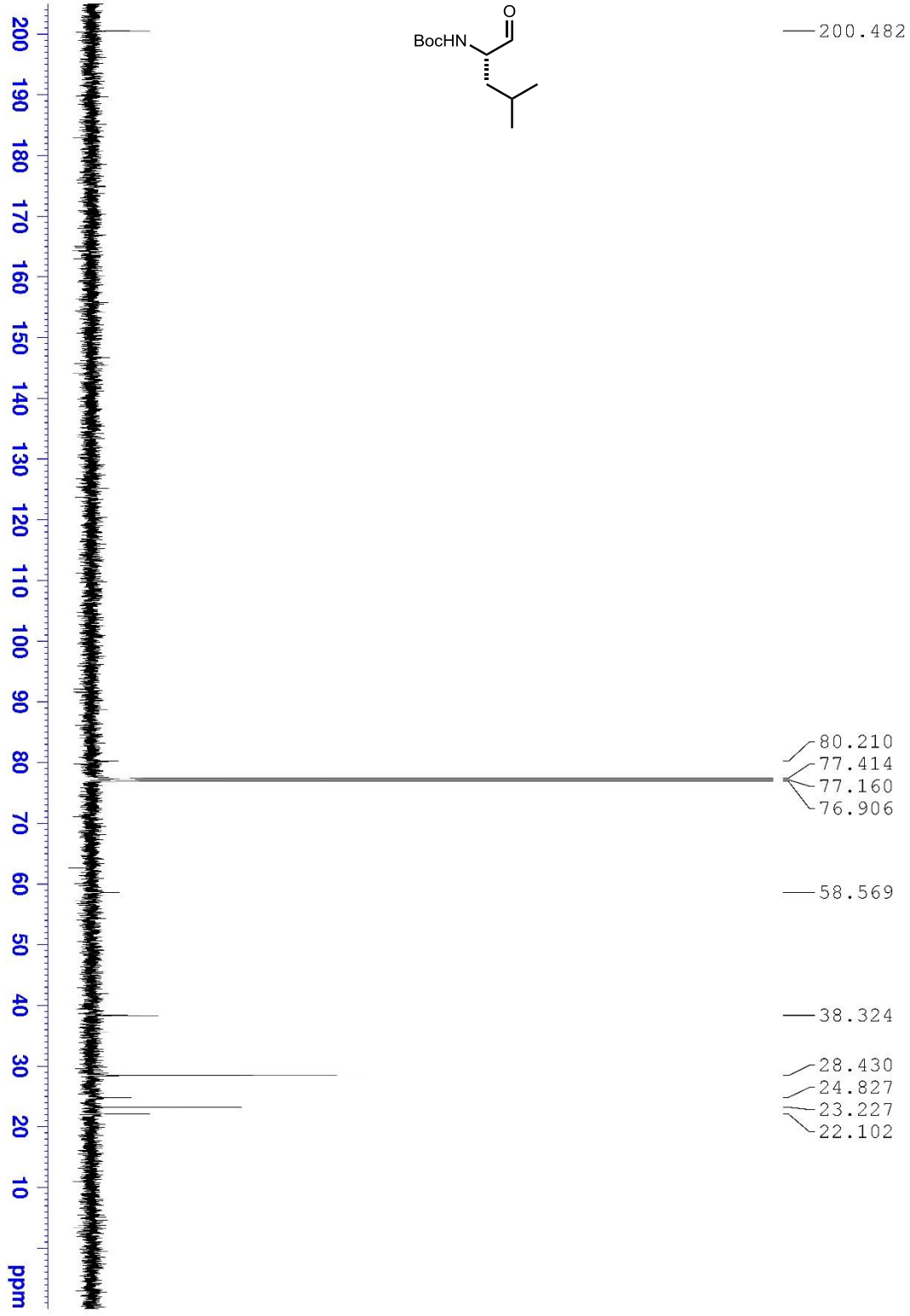
1.24
6.00
ppm


BocHN
O
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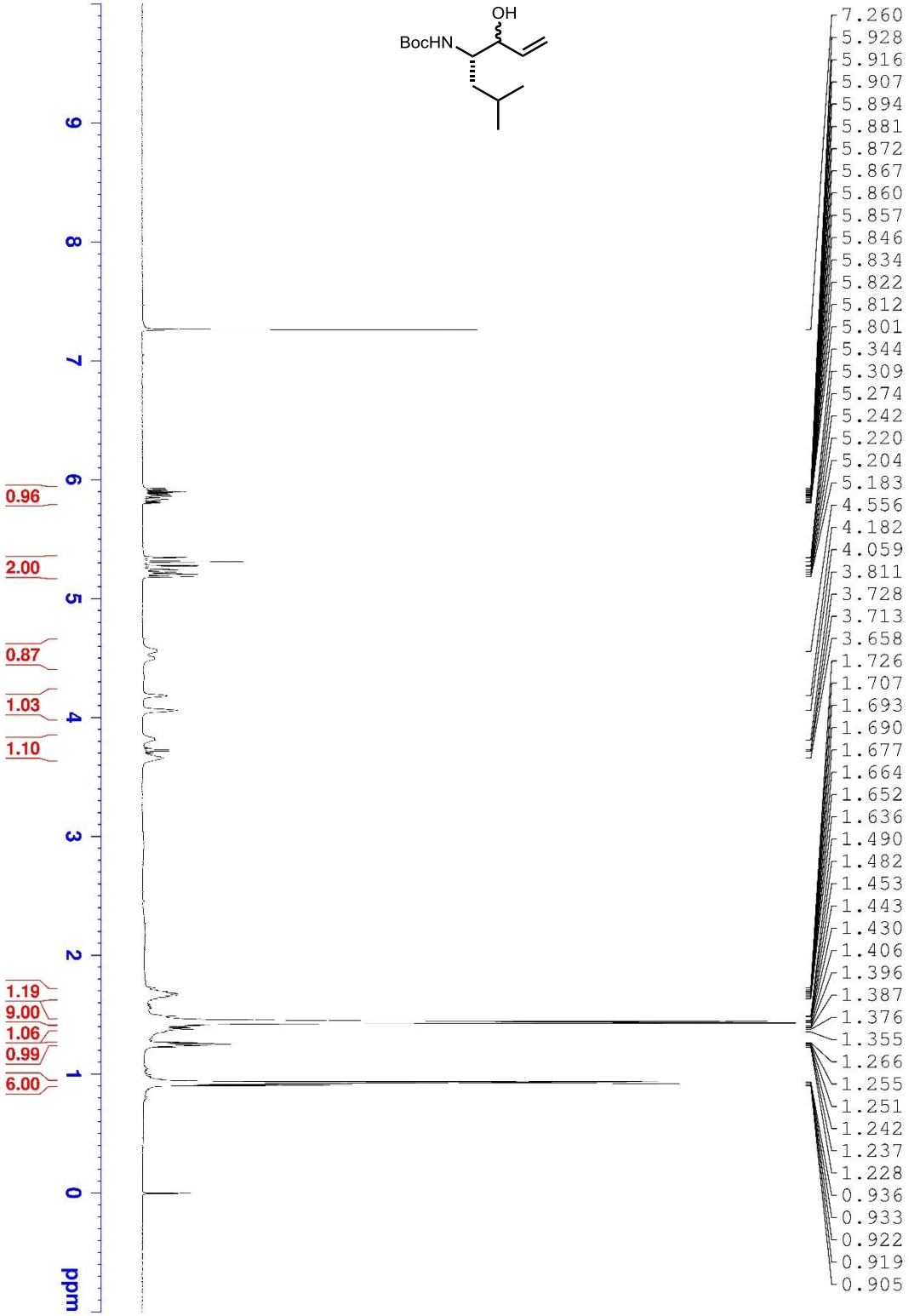
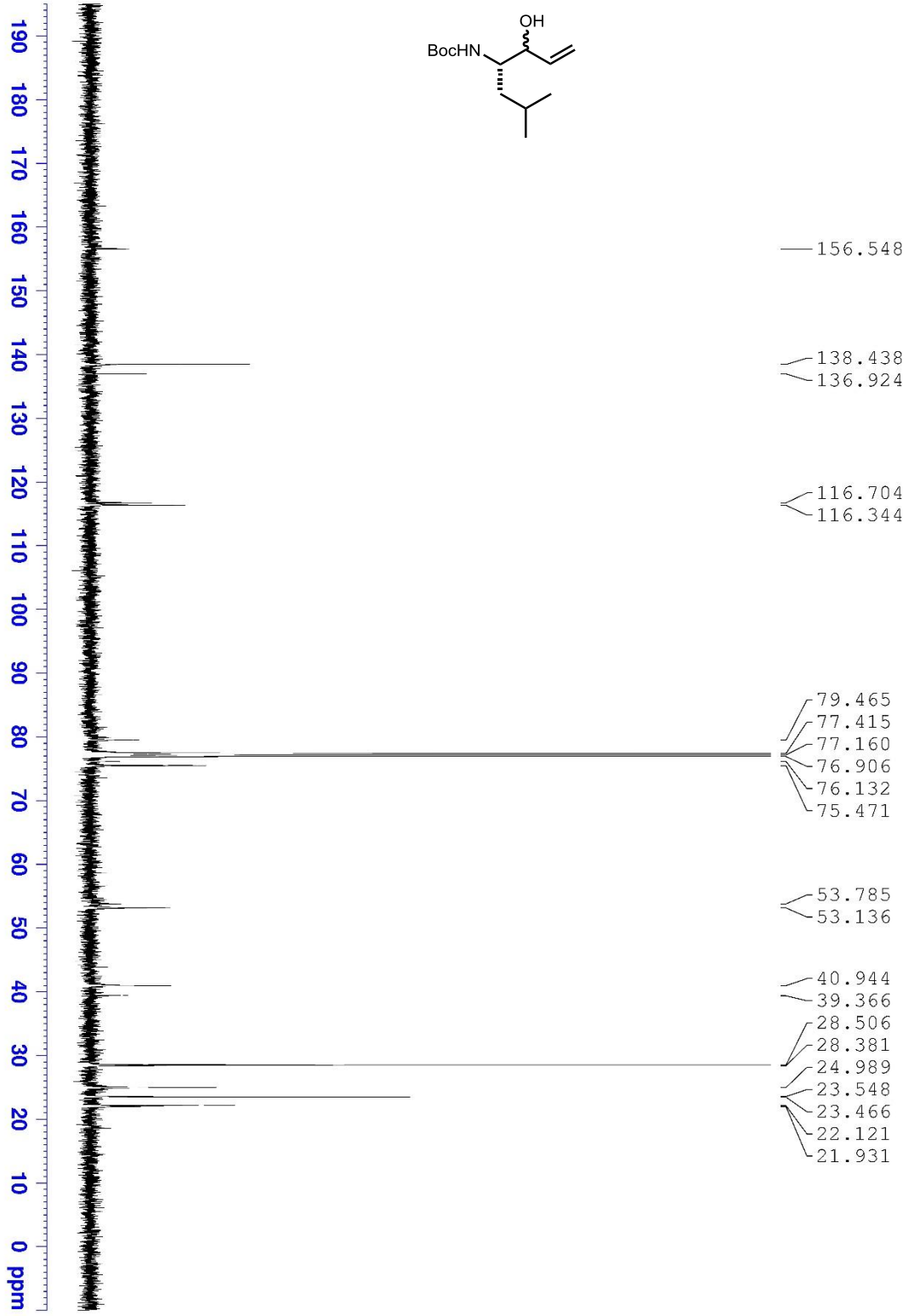
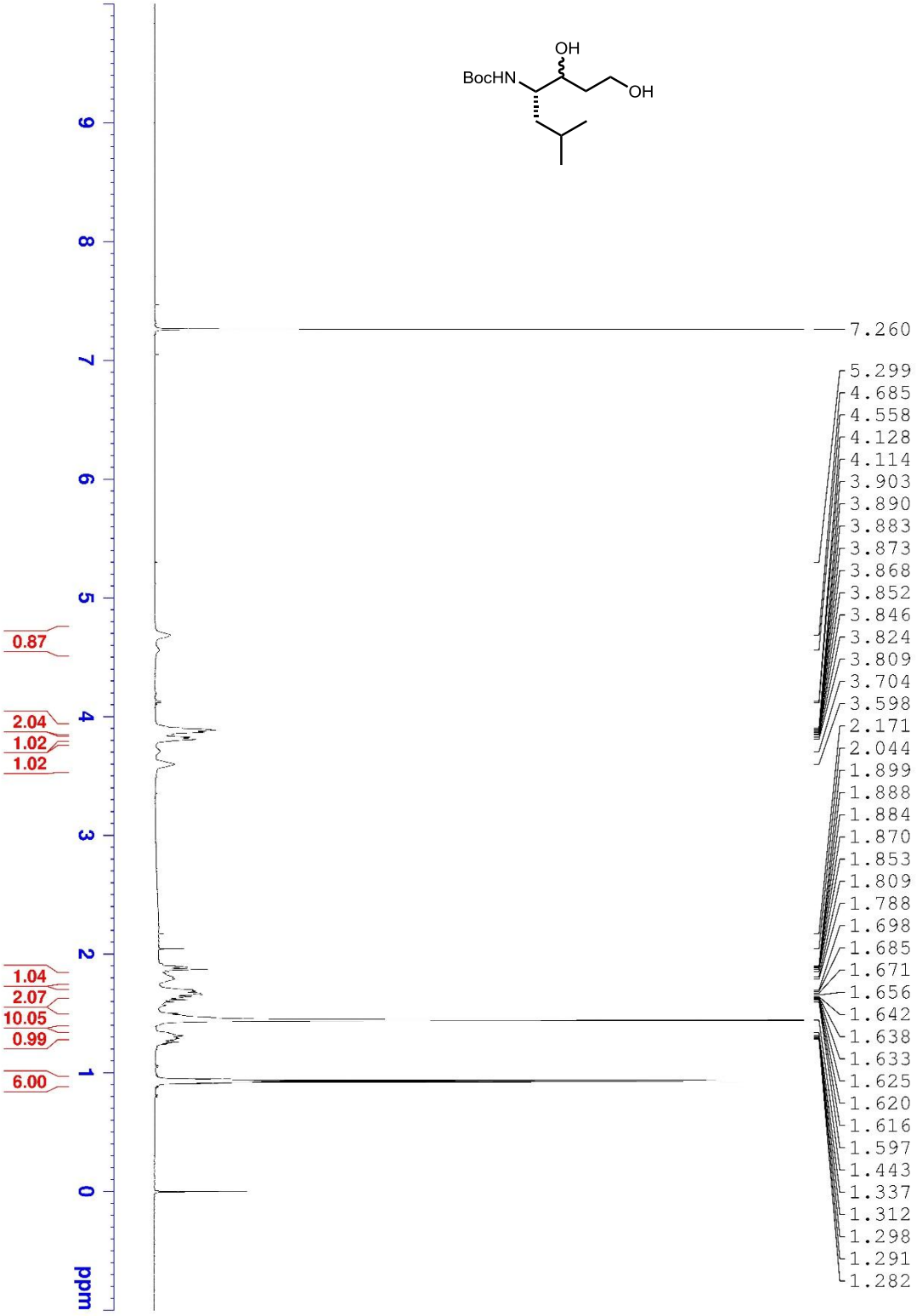
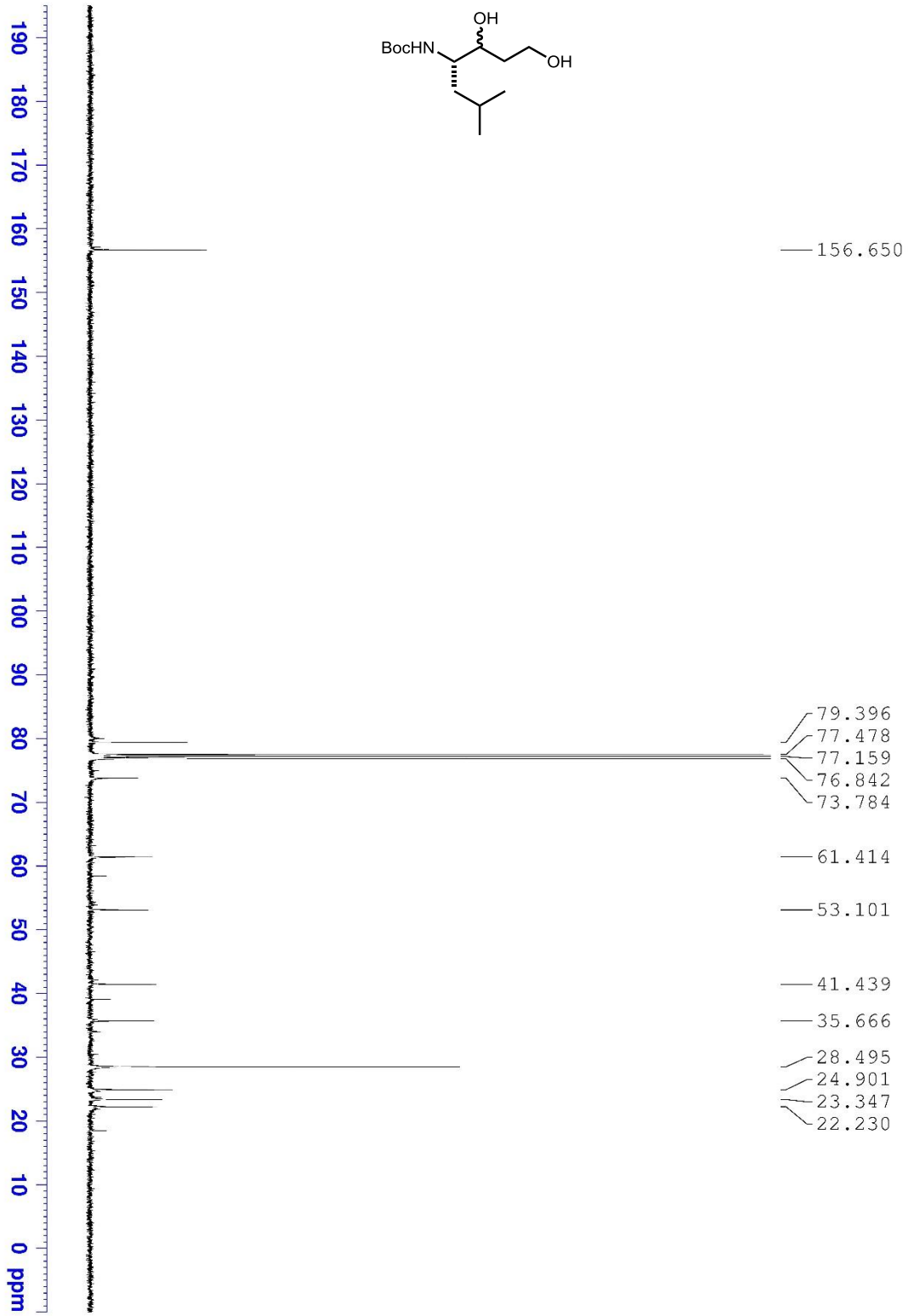
80.210
77.414
77.160
76.906
58.569
38.324
28.430
24.827
23.227
22.102
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


OH
BocHN
7.260
5.928
5.916
5.907
5.894
5.881
5.872
5.867
5.860
5.857
5.846
5.834
5.822
5.812
5.801
5.344
5.309
5.274
5.242
5.220
5.204
5.183
4.556
4.182
4.059
3.811
3.728
3.713
3.658
1.726
1.707
1.693
1.690
1.677
1.664
1.652
1.636
1.490
1.482
1.453
1.443
1.430
1.406
1.396
1.387
1.376
1.355
1.266
1.255
1.251
1.242
1.237
1.228
0.936
0.933
0.922
0.919
0.905
0.96
2.00
0.87
1.03
1.10
1.19
9.00
1.06
0.99
6.00
ppm


OH
BocHN
156.548
138.438
136.924
116.704
116.344
79.465
77.415
77.160
76.906
76.132
75.471
53.785
53.136
40.944
39.366
28.506
28.381
24.989
23.548
23.466
22.121
21.931
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


OH
BocHN
OH
7.260
5.299
4.685
4.558
4.128
4.114
3.903
3.890
3.883
3.873
3.868
3.852
3.846
3.824
3.809
3.704
3.598
2.171
2.044
1.899
1.888
1.884
1.870
1.853
1.809
1.788
1.698
1.685
1.671
1.656
1.642
1.638
1.633
1.625
1.620
1.616
1.597
1.443
1.337
1.312
1.298
1.291
1.282
0.87
2.04
1.02
1.02
1.04
2.07
10.05
0.99
6.00
ppm


OH
BocHN
OH
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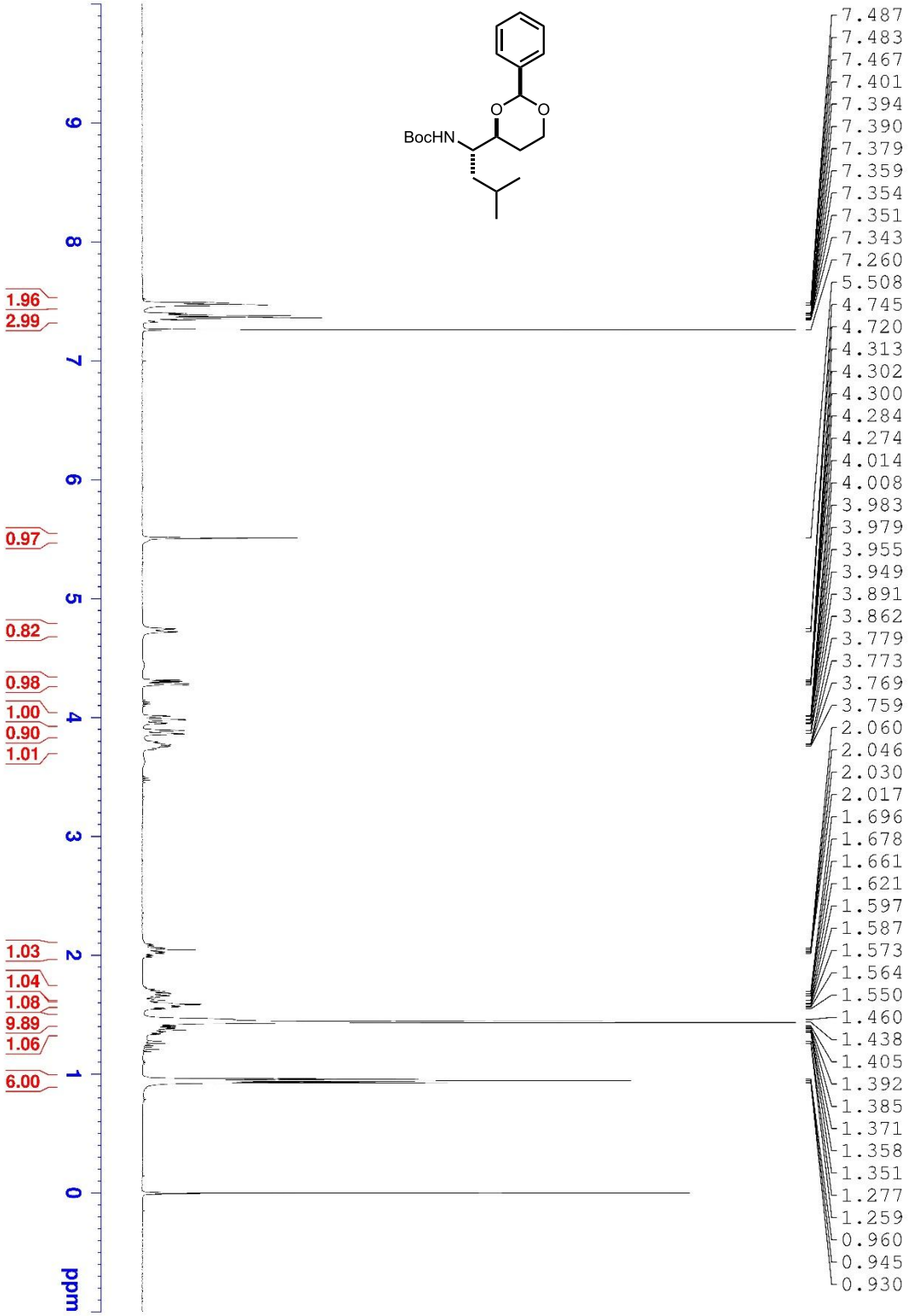
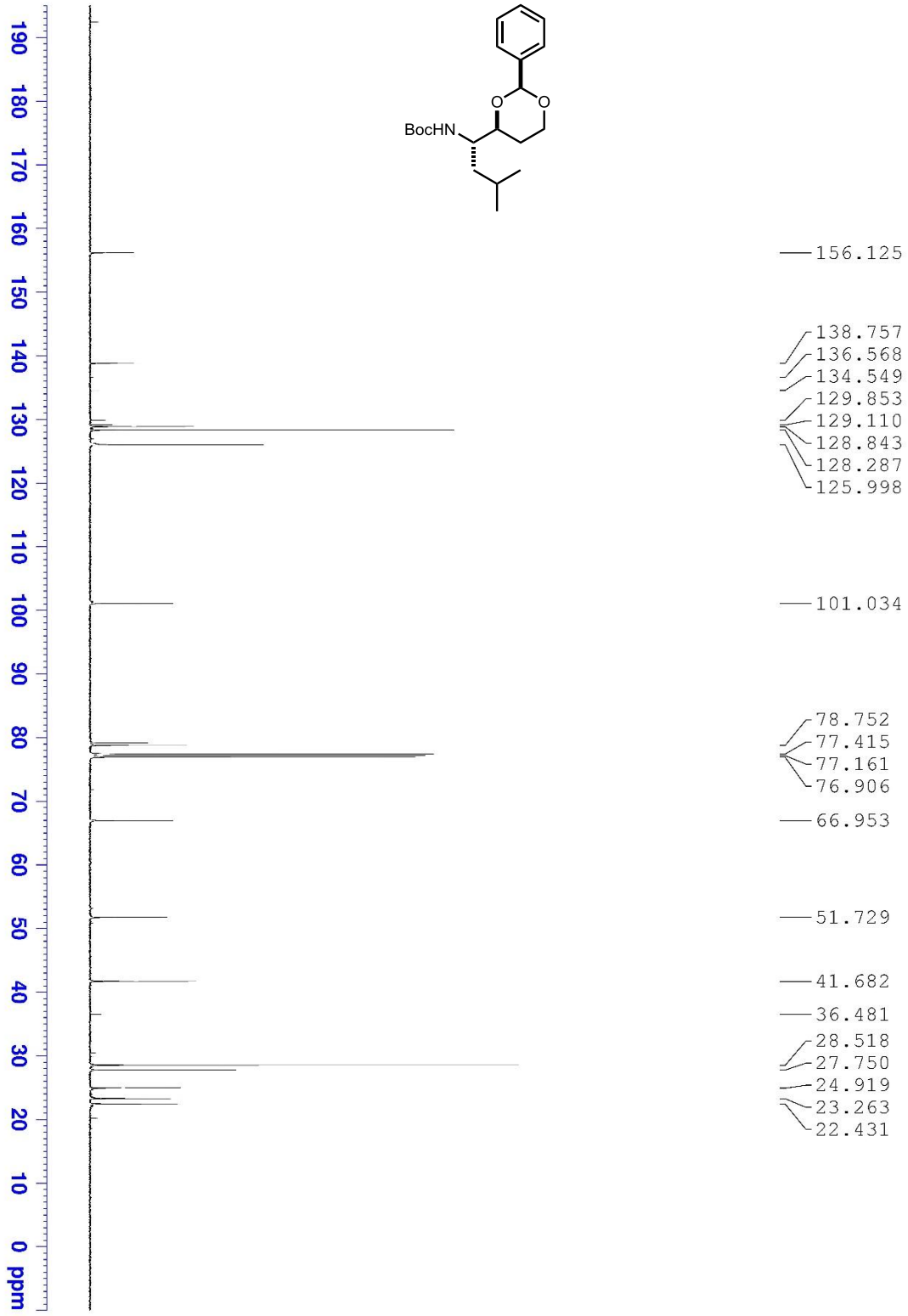
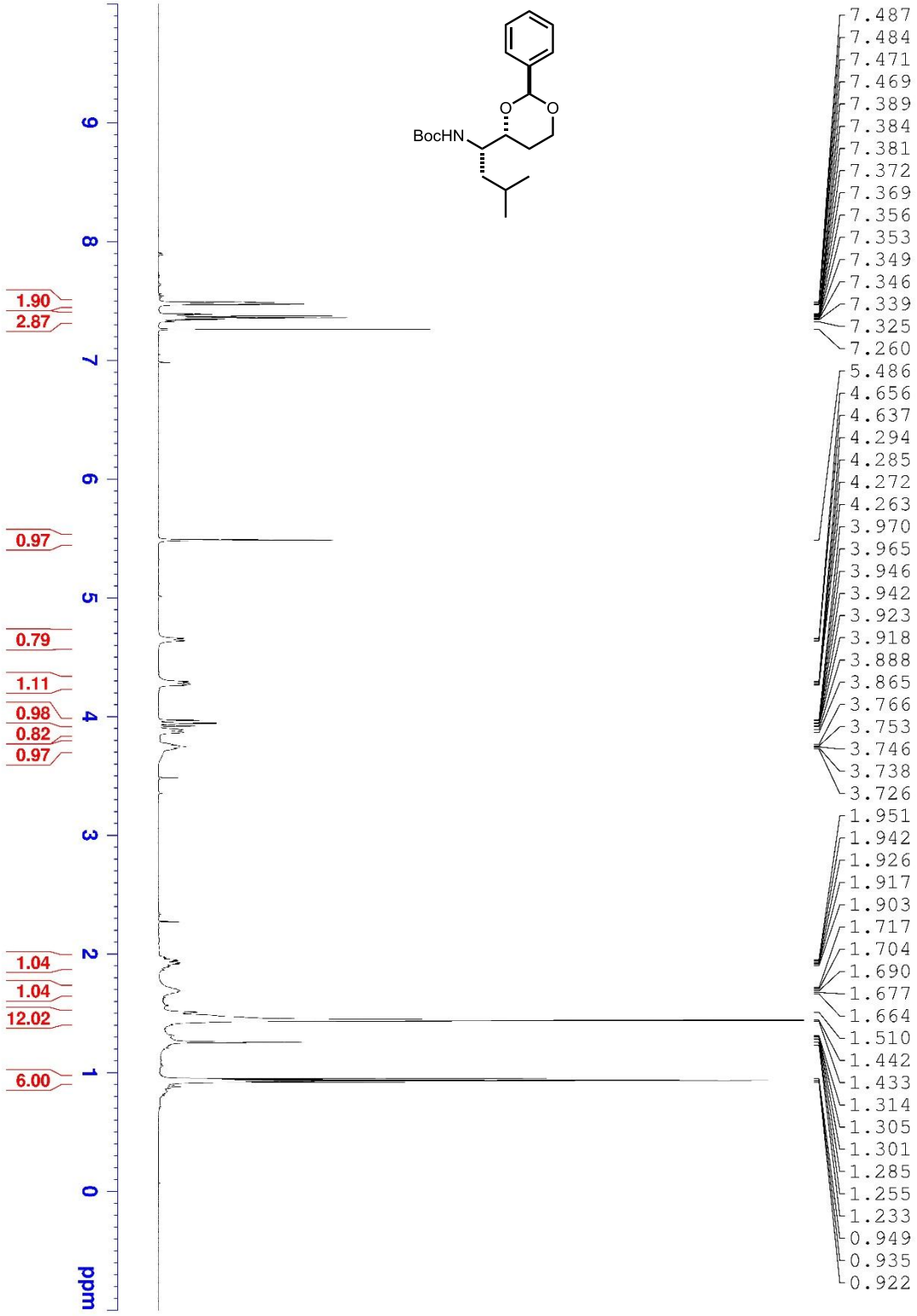
79.396
77.478
77.159
76.842
73.784
61.414
53.101
41.439
35.666
28.495
24.901
23.347
22.230
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


BocHN
O
O
7.487
7.483
7.467
7.401
7.394
7.390
7.379
7.359
7.354
7.351
7.343
7.260
5.508
4.745
4.720
4.313
4.302
4.300
4.284
4.274
4.014
4.008
3.983
3.979
3.955
3.949
3.891
3.862
3.779
3.773
3.769
3.759
2.060
2.046
2.030
2.017
1.696
1.678
1.661
1.621
1.597
1.587
1.573
1.564
1.550
1.460
1.438
1.405
1.392
1.385
1.371
1.358
1.351
1.277
1.259
0.960
0.945
0.930
1.96
2.99
0.97
0.82
0.98
1.00
0.90
1.01
1.03
1.04
1.08
9.89
1.06
6.00
ppm


BocHN
O
O
156.125
138.757
136.568
134.549
129.853
129.110
128.843
128.287
125.998
101.034
78.752
77.415
77.161
76.906
66.953
51.729
41.682
36.481
28.518
27.750
24.919
23.263
22.431
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0 ppm


BocHN
O
O
1.90
2.87
0.97
0.79
1.11
0.98
0.82
0.97
1.04
1.04
12.02
6.00
ppm
7.487
7.484
7.471
7.469
7.389
7.384
7.381
7.372
7.369
7.356
7.353
7.349
7.346
7.339
7.325
7.260
5.486
4.656
4.637
4.294
4.285
4.272
4.263
3.970
3.965
3.946
3.942
3.923
3.918
3.888
3.865
3.766
3.753
3.746
3.738
3.726
1.951
1.942
1.926
1.917
1.903
1.717
1.704
1.690
1.677
1.664
1.510
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1.314
1.305
1.301
1.285
1.255
1.233
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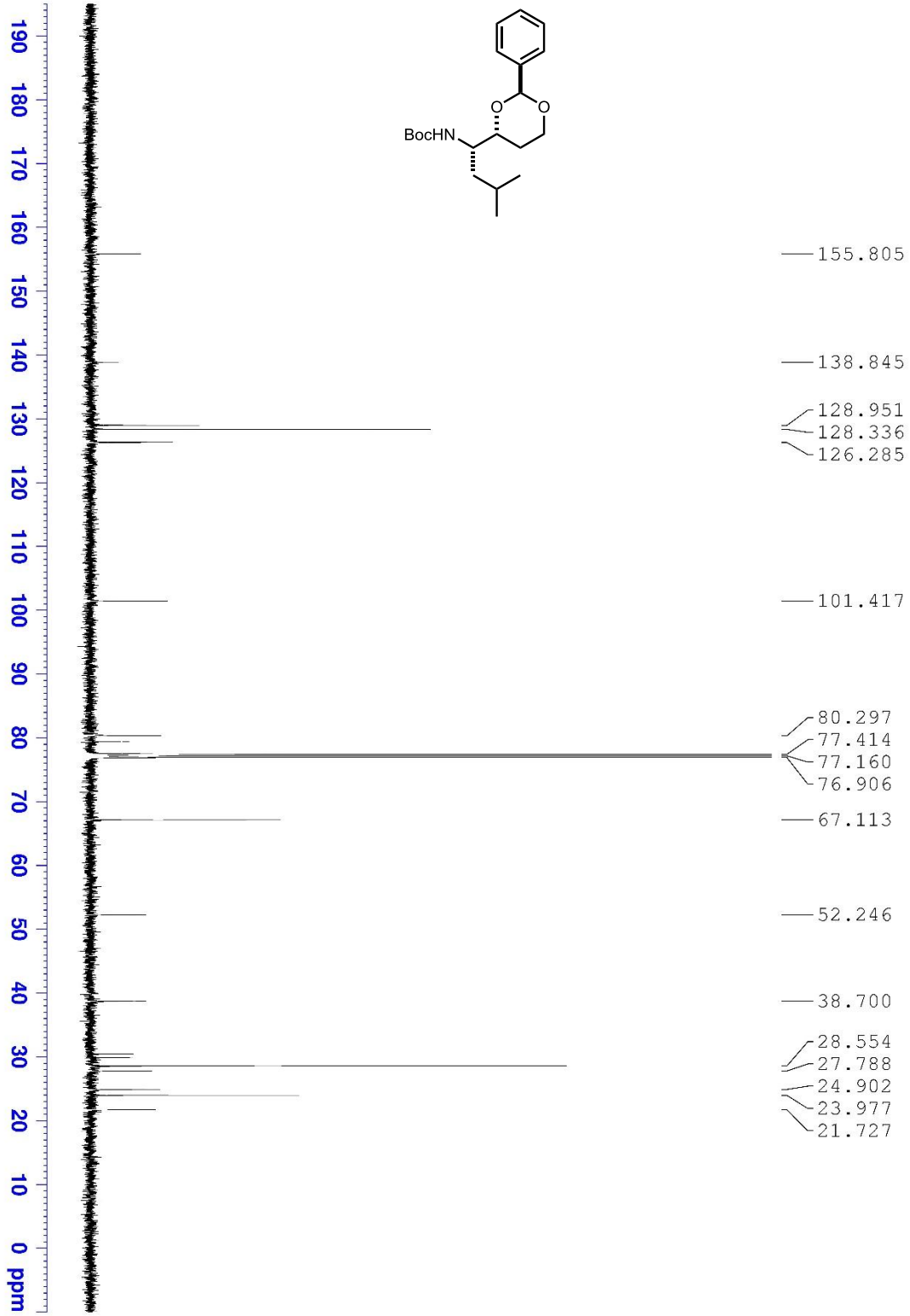
0.935
0.922


BocHN
O
O
155.805
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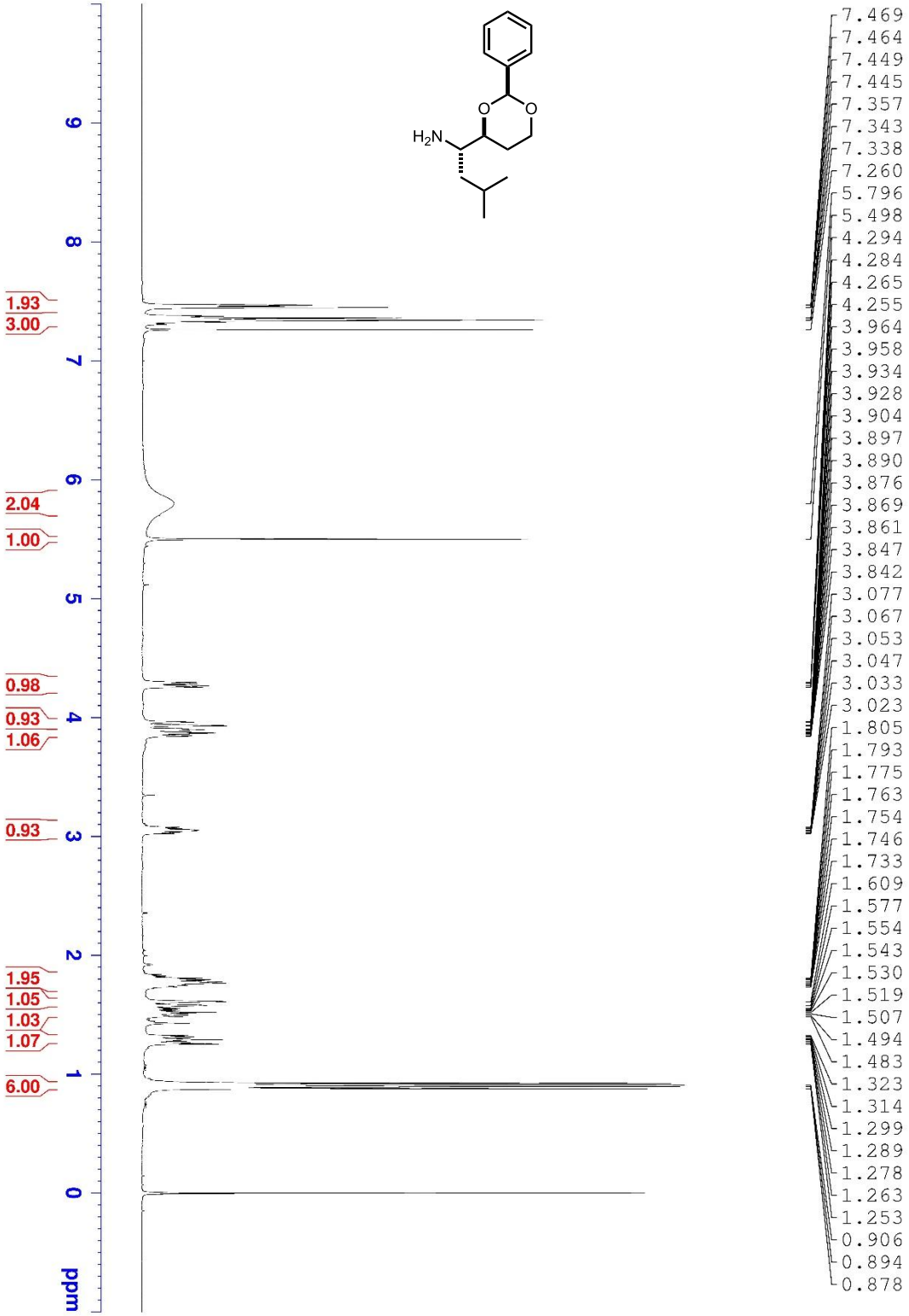
128.336
126.285
101.417
80.297
77.414
77.160
76.906
67.113
52.246
38.700
28.554
27.788
24.902
23.977
21.727
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0 ppm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7.445
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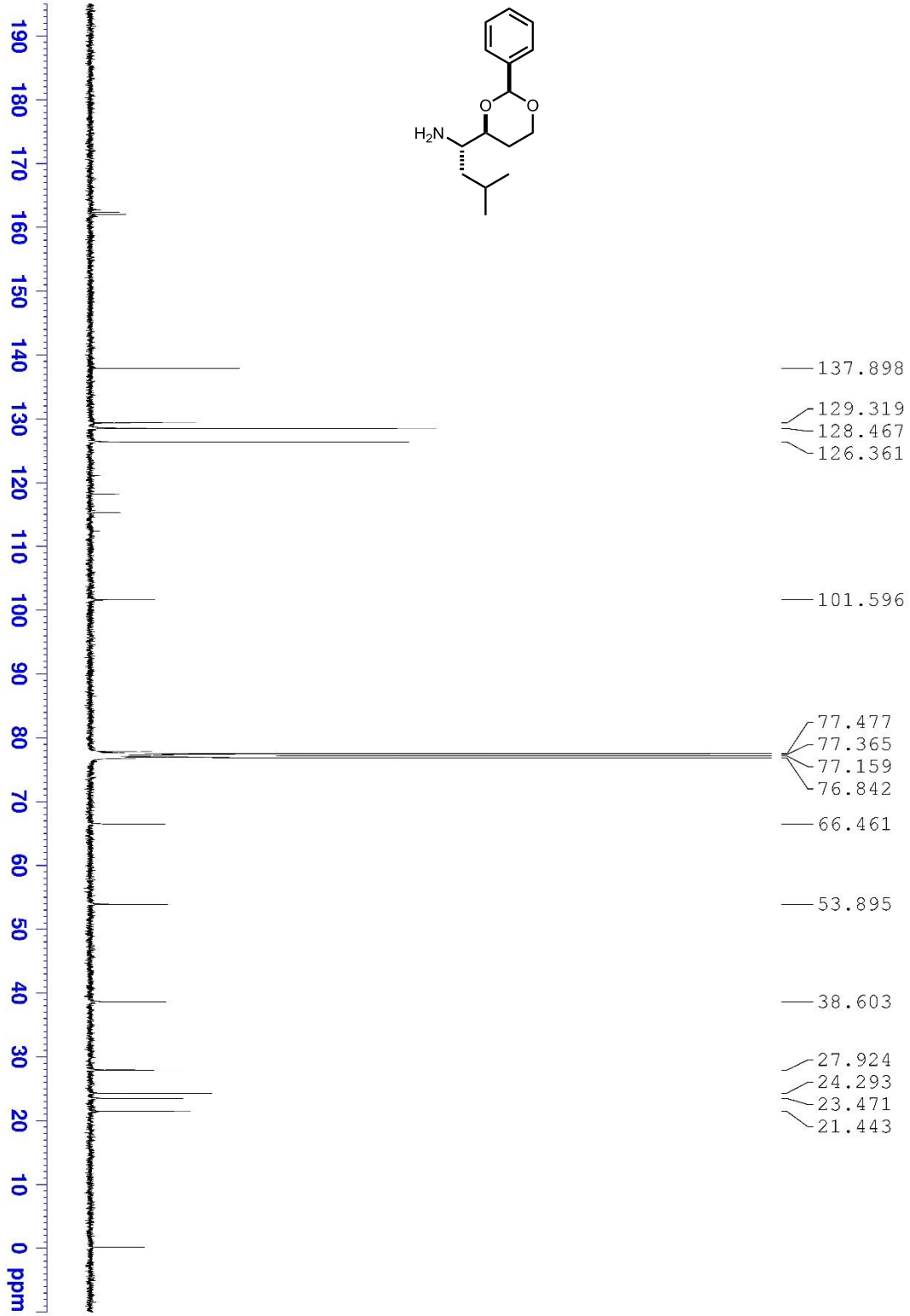
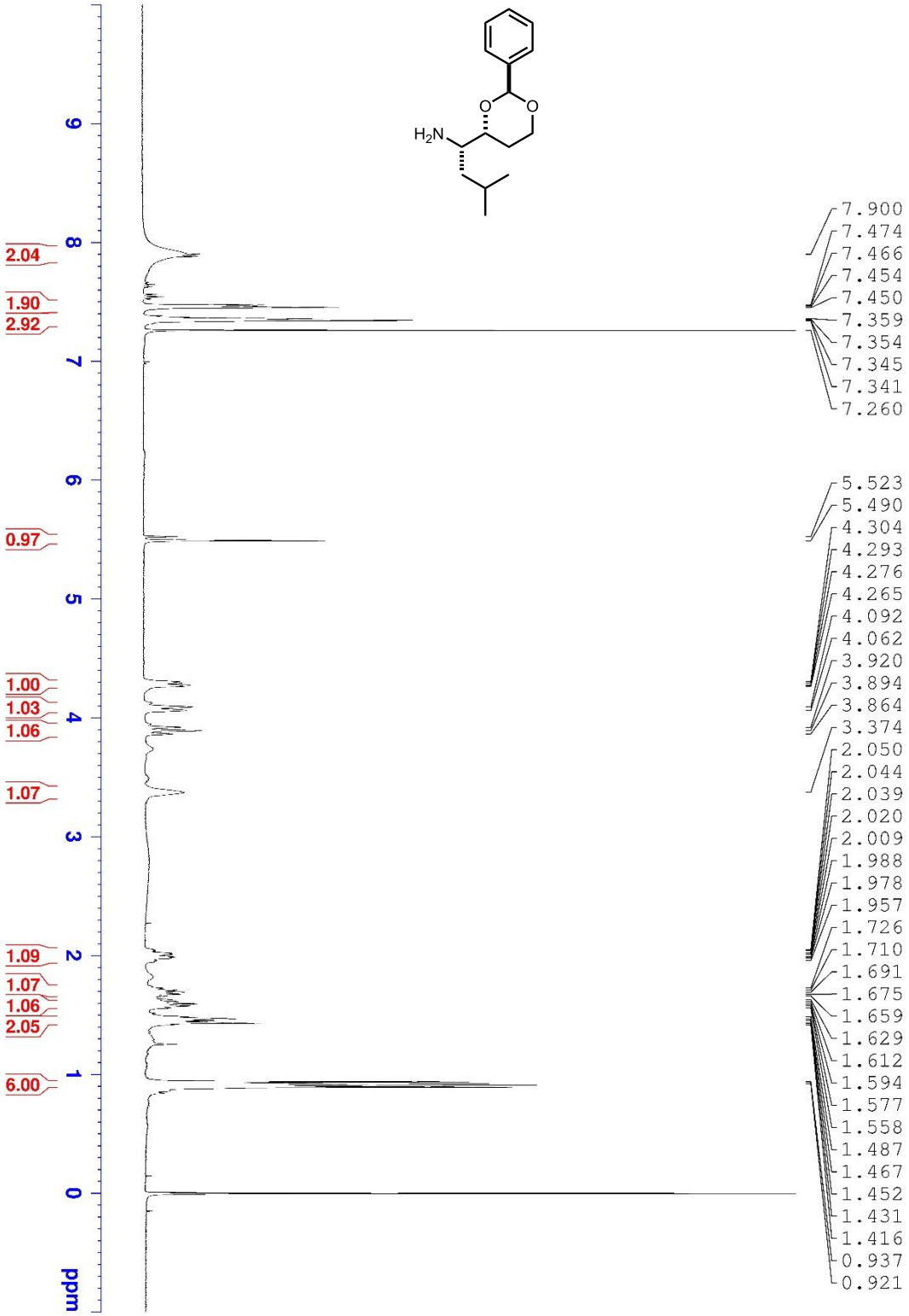
7.343
7.338
7.260
5.796
5.498
4.294
4.284
4.265
4.255
3.964
3.958
3.934
3.928
3.904
3.897
3.890
3.876
3.869
3.861
3.847
3.842
3.077
3.067
3.053
3.047
3.033
3.023
1.805
1.793
1.775
1.763
1.754
1.746
1.733
1.609
1.577
1.554
1.543
1.530
1.519
1.507
1.494
1.483
1.323
1.314
1.299
1.289
1.278
1.263
1.253
0.906
0.894
0.878
1.93
3.00
2.04
1.00
0.98
0.93
1.06
0.93
1.95
1.05
1.03
1.07
6.00
ppm
O
O
H2N


190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0 ppm
H2N
O
O
137.898
129.319
128.467
126.361
101.596
77.477
77.365
77.159
76.842
66.461
53.895
38.603
27.924
24.293
23.471
21.443


H2N
O
O
9
8
7
6
5
4
3
2
1
0
ppm
2.04
1.90
2.92
0.97
1.00
1.03
1.06
1.07
1.09
1.07
1.06
2.05
6.00
7.900
7.474
7.466
7.454
7.450
7.359
7.354
7.345
7.341
7.260
5.523
5.490
4.304
4.293
4.276
4.265
4.092
4.062
3.920
3.894
3.864
3.374
2.050
2.044
2.039
2.020
2.009
1.988
1.978
1.957
1.726
1.710
1.691
1.675
1.659
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1.577
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1.487
1.467
1.452
1.431
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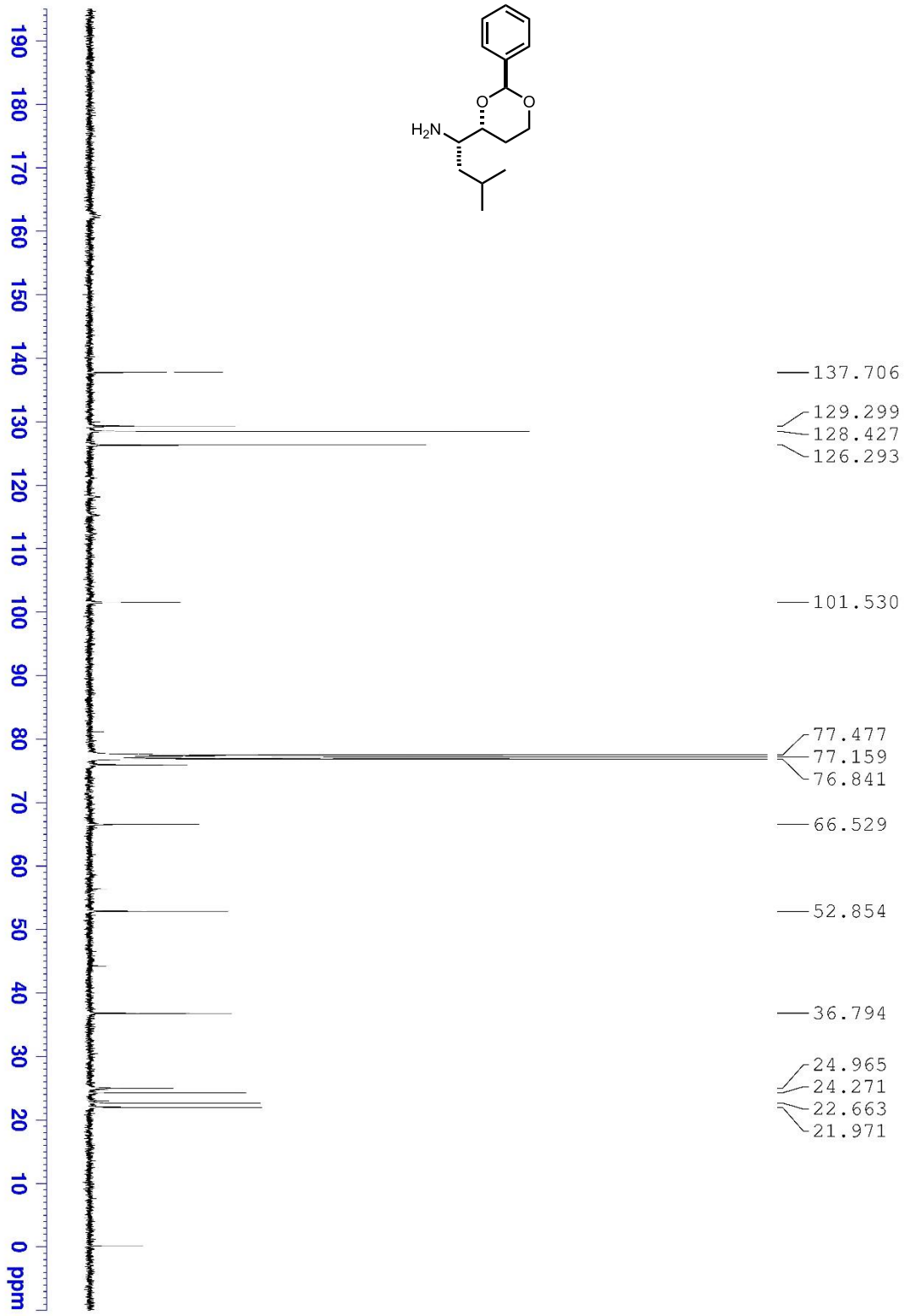
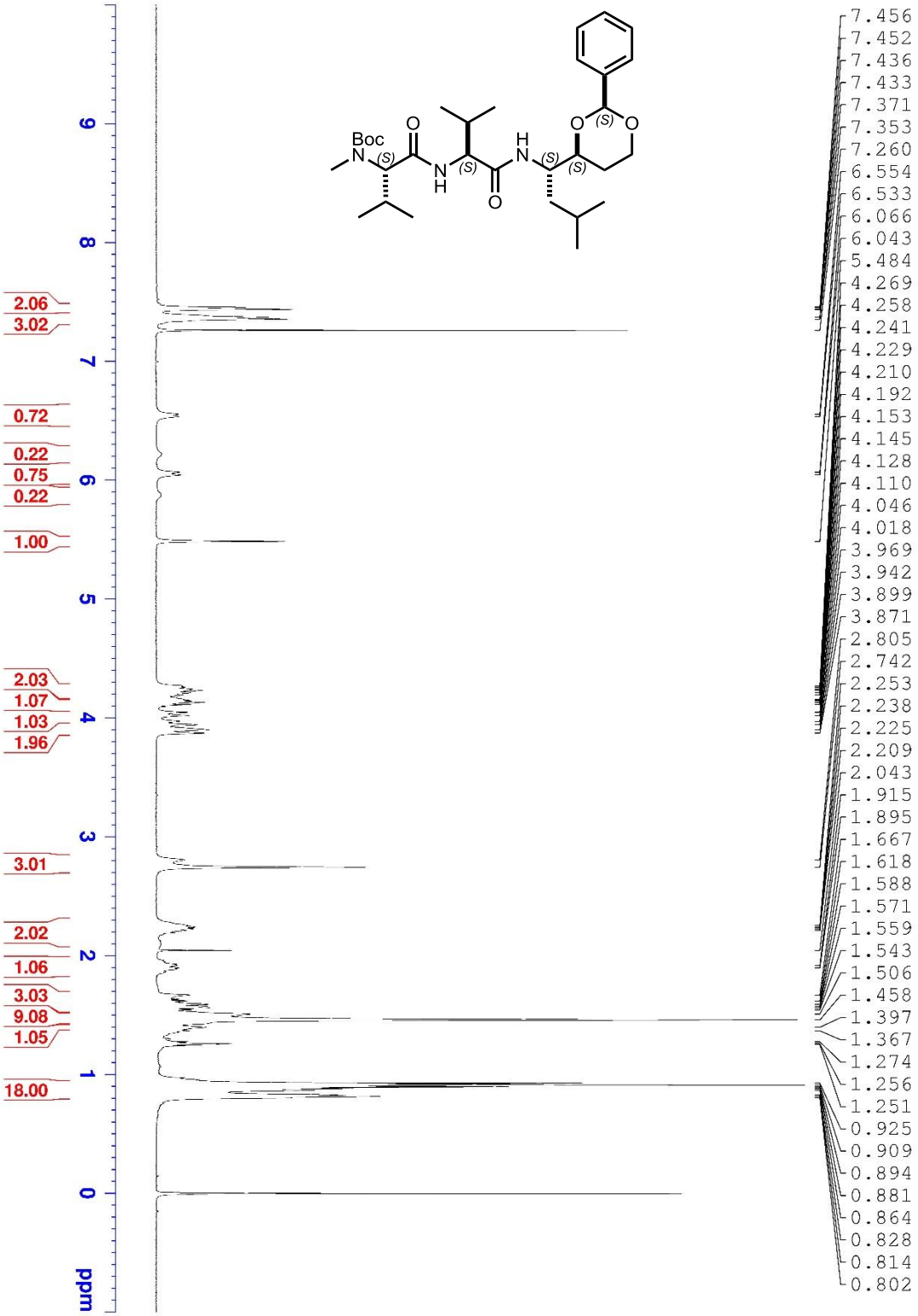
0.921


190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0 ppm
H2N
O
O
137.706
129.299
128.427
126.293
101.530
77.477
77.159
76.841
66.529
52.854
36.794
24.965
24.271
22.663
21.971


Boc
N
(S)
O
N
H
(S)
O
H
N
(S)
O
(S)
O
(S)
O
2.06
3.02
0.72
0.22
0.75
0.22
1.00
2.03
1.07
1.03
1.96
3.01
2.02
1.06
3.03
9.08
1.05
18.00
ppm
7.456
7.452
7.436
7.433
7.371
7.353
7.260
6.554
6.533
6.066
6.043
5.484
4.269
4.258
4.241
4.229
4.210
4.192
4.153
4.145
4.128
4.110
4.046
4.018
3.969
3.942
3.899
3.871
2.805
2.742
2.253
2.238
2.225
2.209
2.043
1.915
1.895
1.667
1.618
1.588
1.571
1.559
1.543
1.506
1.458
1.397
1.367
1.274
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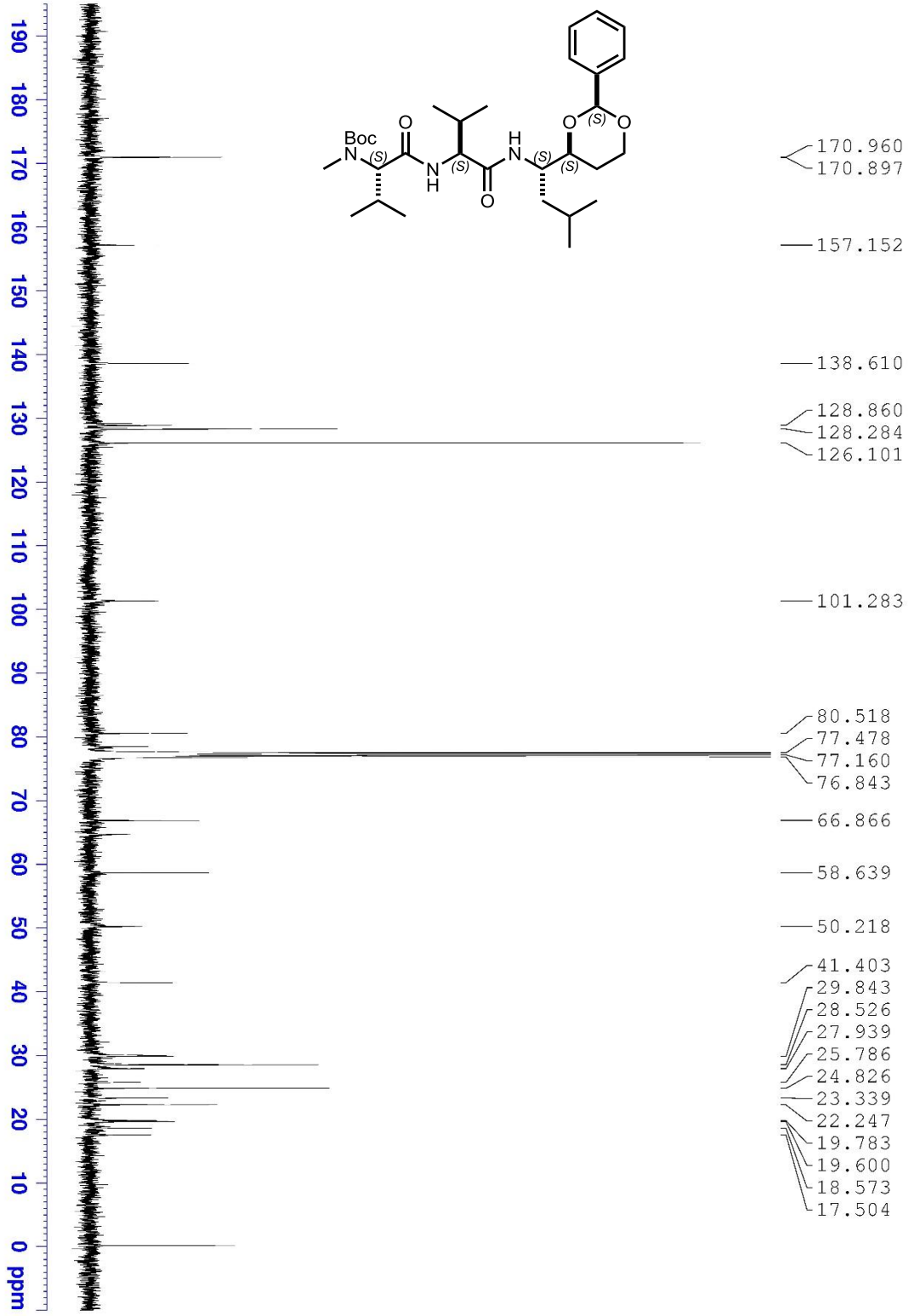
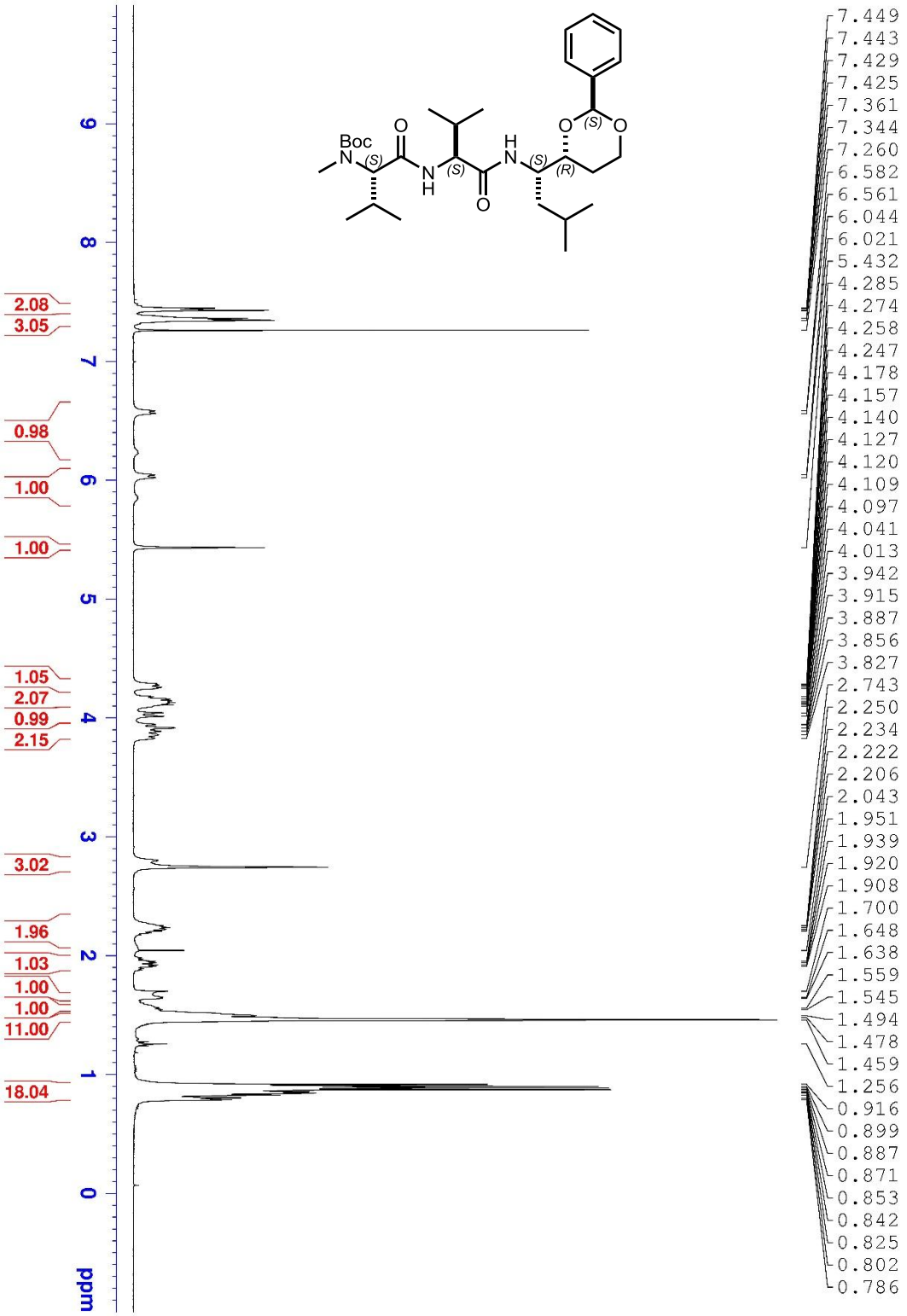
1.251
0.925
0.909
0.894
0.881
0.864
0.828
0.814
0.802


Boc
N
(S)
O
N
H
(S)
O
H
N
(S)
(S)
O
(S)
O
170.960
170.897
157.152
138.610
128.860
128.284
126.101
101.283
80.518
77.478
77.160
76.843
66.866
58.639
50.218
41.403
29.843
28.526
27.939
25.786
24.826
23.339
22.247
19.783
19.600
18.573
17.504
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0 ppm


Boc
N
(S)
O
N
H
(S)
O
H
N
(S)
O
(S)
O
(R)
2.08
3.05
0.98
1.00
1.00
1.05
2.07
0.99
2.15
3.02
1.96
1.03
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1.00
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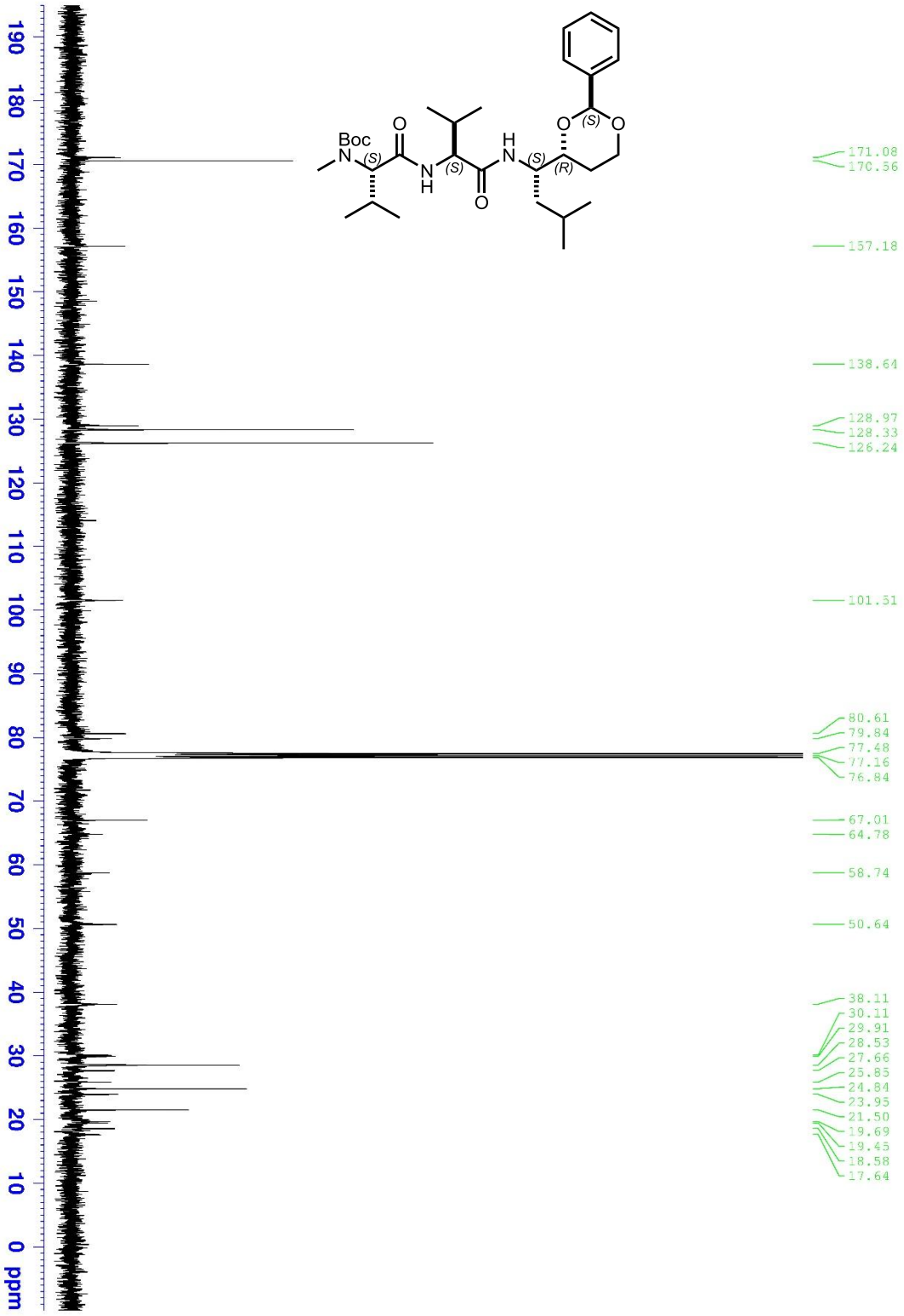
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(S)
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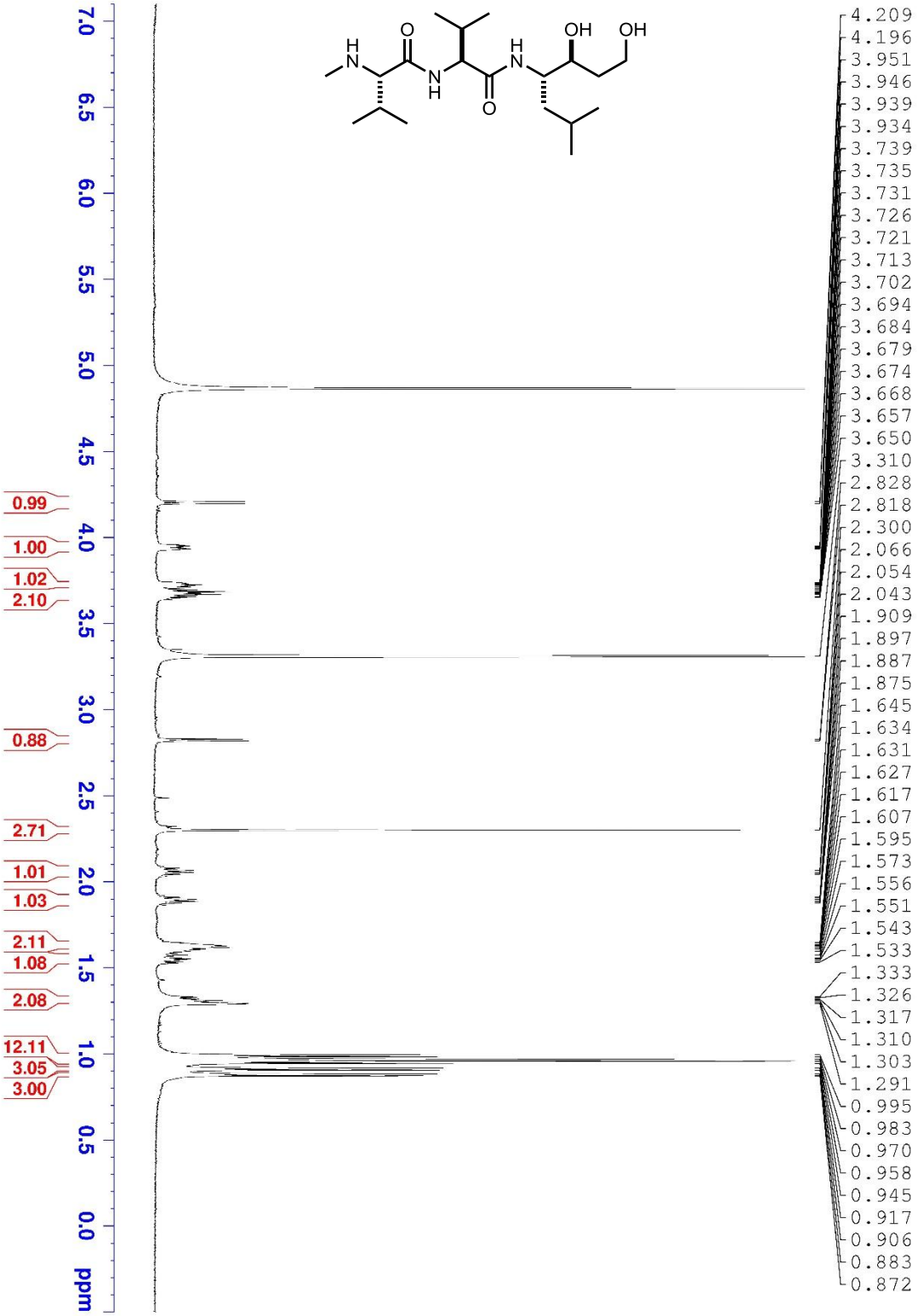
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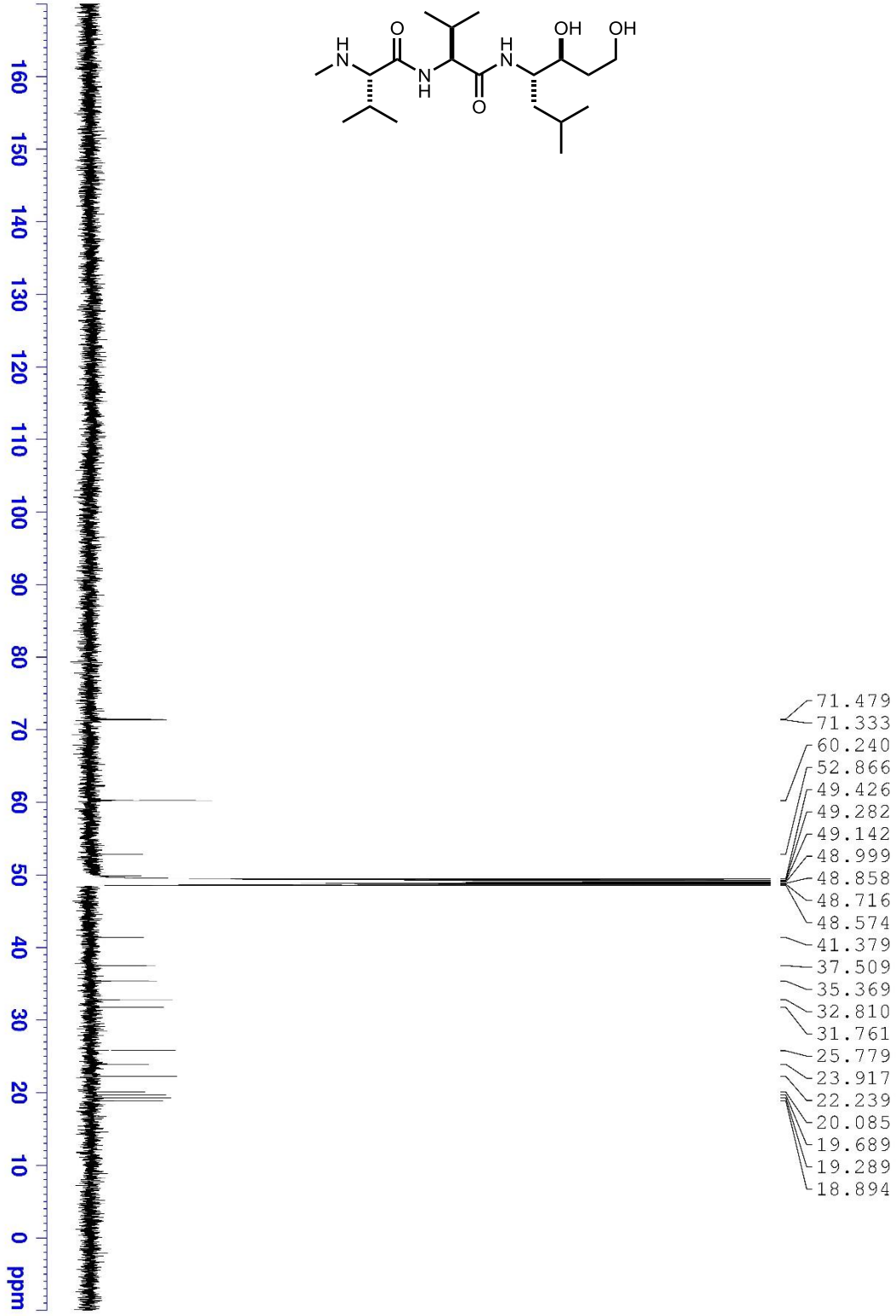
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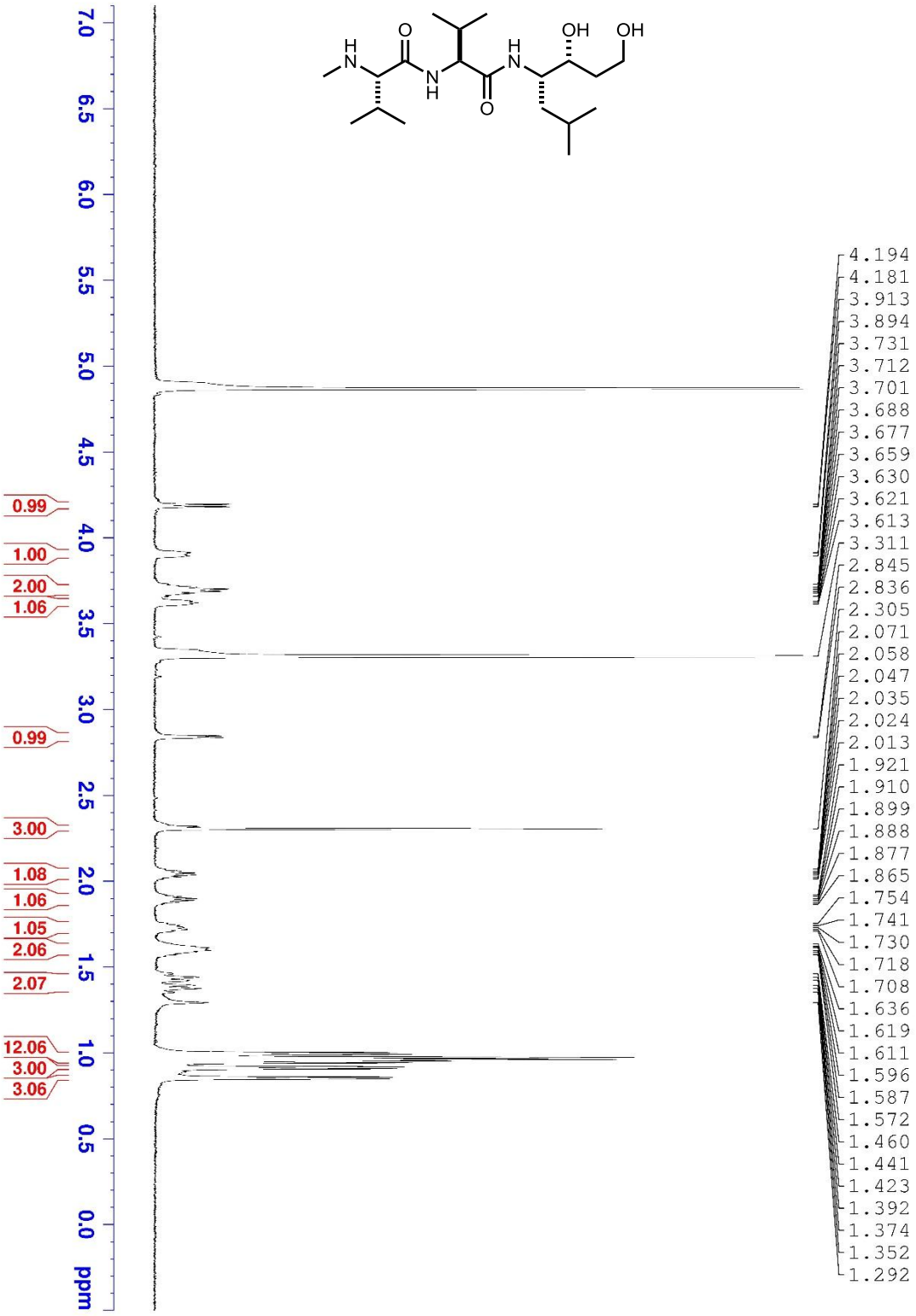
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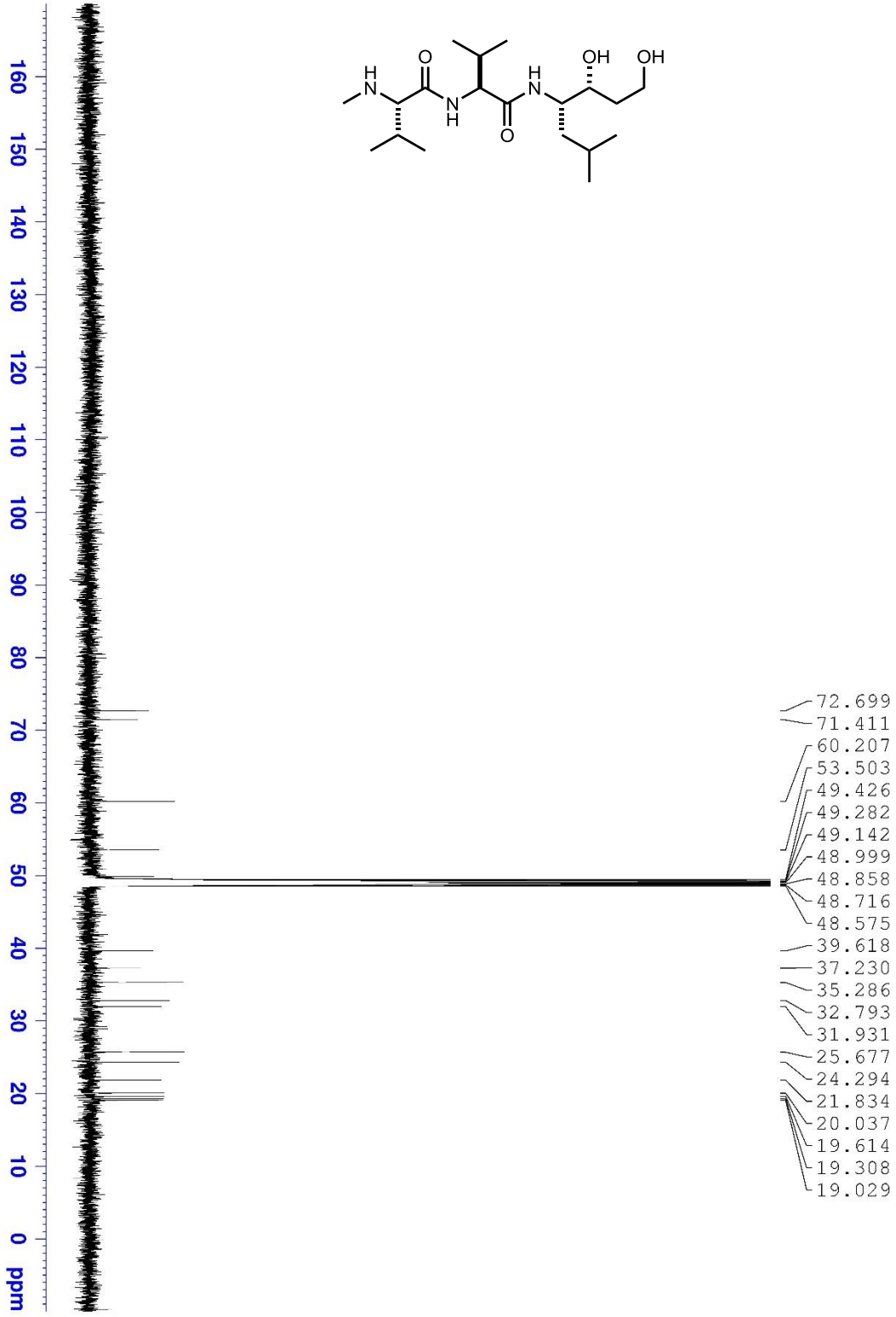
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