UNIVERSITY OF CALIFORNIA SAN DIEGO

Total Synthesis and Anti-Cancer Activity of Micrococcin P1 and Synthetic Fragments

A dissertation submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy

in

Chemistry

By

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The Dissertation of Mitchell Patrick Christy is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California San Diego

2019

DEDICATION

To my family who has been so supportive, especially when it was hard. Without them none of this is possible.

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LIST OF ABBREVIATIONS

ACN	Acetonitrile
AcOH	Acetic Acid
ADME	Absorption, distribution, metabolism, and excretion
Ag_2CO_3	Silver Carbonate
$BrCCl_3$	Bromotrichloromethane
Boc	*tert*-butyloxycarbonyl
^{13}C NMR	Carbon Nuclear Magnetic Resonance
CsF	Cesium Fluoride
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DIBAL	Diisobutylaluminum Hydride
DIPEA	Diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DPPA	Diphenylphosphoryl Azide
EA	Ethyl Acetate

EDC	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
EtOH	Ethanol
HATU	1-[Bis(dimethylamino)methylene]-1H 1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate
HCl	Hydrochloric Acid
Hex	Hexanes
^{1}H NMR	Proton Nuclear Magnetic Resonance
HOBt	Hydroxybenzotriazole
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
IPA	2-Propanol
i-PrMgCl•LiC	Isopropylmagnesium chloride lithium chloride complex
IR	Infrared Spectroscopy
K_2CO_3	Potassium Carbonate
LiCl	Lithium Chloride

MeOH	Methanol
MIC	Minimum Inhibitory Concentration
MnO_2	Manganese (IV) oxide
NaOH	Sodium Hydroxide
NH_4OH	Ammonium Hydroxide
NOE	Nuclear Overhauser Effect
P_2O_5	Phosphorous pentoxide
$PdCl_2(dppf)$	[1,1'-Bis(diphenylphosphino)ferrocene] palladium (II) dichloride
$Pd_2(dba)_3$	Tris(dibenzylideneacetone)dipalladium (0)
$Pd(P(t\text{-}Bu)_3)_2$	Bis(tri-*tert*-butylphosphine)palladium
$Pd(PPh_3)_4$	Tetrakis(triphenylphosphine)palladium(0)
PPTS	Pyridinium *p*-toluenesulfonate
PyAOP	((7-Azabenzotriazol-1-yloxy)tripyrrolidino phosphonium hexafluorophosphate)
r.t.	Room Temperature
Sn_2Me_6	Hexamethylditin
$SnCl_2$	Tin (II) Chloride
TBAB	Tetrabutylammonium bromide

TBS	*tert*-butyldimethylsilyl
t-BuOH	*tert*-butyl alcohol
TBSCl	*tert*-butyldimethylsilyl chloride
TCEP	(tris(2-carboxyethyl)phosphine)
tetrakis	Tetrakis(triphenylphosphine)palladium(0)
TFA	Trifluoroacetic Acid
TEA	Triethylamine
TLC	Thin Layer Chromatography
TMSCN	Cyanotrimethylsilane

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PUBLICATIONS

1. Mitchell P. Christy, Trevor Johnson, Clare D. McNerlin, John Woodard, Andrew T. Nelson, Bryant Lim, Tiffany L. Hamilton, Kaitlyn M. Freiberg, Dionicio Siegel, Chemical Synthesis of Micrococcin P1 Through Scalable Thiazole Forming Reactions of Cysteine Derivatives and Nitriles, *J. Am. Chem. Soc.* **2019**, *submitted*.

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2. Robert A. Quinn, Alexy V. Melnik, Alison Vrbanac, Kathryn A. Patras, Mitchell Christy, Zsolt Bodai, Alexander Aksenov, Anupriya Tripathi, Lawton Chung, Greg Humphrey, Morgan Panitchpakdi, Ricardo da Silva, Julian Avila-Pacheco, Clary Clish, Sena Bae, Himel Mallick, Eric A. Franzosa, Jason Lloyd-Price, Robert Bussell, Taren Thron, Andrew T. Nelson, Mingxun Wang, Fernando Vargas, Julia M. Gauglitz, Michael J. Meehan, Emily Gentry, Timothy D. Arthur, Michael Downes, Ting Fu, Alexis C. Komor, Orit Poulsen, Brigid S. Boland, John T. Chang, William J. Sandborn, Meerana Lim, Neha Garg, Julie C. Lumeng, Ramnik J. Xavier, Barbara I. Kazmierczak, Ruchi Jain, Marie Egan, Kyung E. Rhee, Ron M. Evans, Manuela Raffatellu, Hera Vlamakis, Gabriel G. Haddad, Dionicio Siegel, Curtis Huttenhower, Sarkis K. Mazmanian, Victor Nizet, Rob Knight, Pieter C. Dorrestein, Global Chemical Impacts of the Microbiomen Include Unique Bile Acid Conjugates that Stimulate FXR, *Nature* **2019**, *manuscript in review*

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ABSTRACT OF THE DISSERTATION

Total Synthesis and Anti-Cancer Activity of Micrococcin P1 and Synthetic Fragments

by

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Doctor of Philosophy in Chemistry

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Professor Dionicio Siegel, Chair

Thiopeptides are a versatile group of natural products with a wealth of therapeutic potential. To expand the methods of syntheses of these compounds and more closely study their *in vitro* activity, we report the total synthesis of micrococcin P1 enabled by robust thiazole forming reactions. Never before used in the synthesis of thiopeptides, we were able to access decagram quantities of several thiazole containing fragments through the condensation of nitriles and aminothiols. These thiazole forming reactions are robustly scalable and we were able to produce nearly 200 mg of the natural product. Micrococcin and other synthetic precursors were used to probe structure-activity relationships in *in vivo* anti-cancer assays where micrococcin was not found to have significant anti-cancer activity,

though several smaller fragments were found to have low micromolar to high nanomolar potency. Previously, only series B thiopeptides and their complex central piperidine fragments were known to have this activity. This finding shows that not only series B thiopeptides like thiostrepton and siomycin A have anti-cancer activity. That fragments of micrococcin P1, a series D thiopeptide, show comparable activity indicates a broader scope of activity in the family. Importantly, we have established a minimum scaffold for activity, and these fragments are more easily accessible with our shorter, higher yielding synthesis than that of previous fragments from thiostrepton. Following these discoveries, medicinal chemistry efforts to increase pharmacokinetic properties and potency of these fragments can be explored.

Chaper 1: Total Synthesis of Micrococcin P1

Globally, cancer is one of the leading causes of morbidity and mortality, with millions of new cases and deaths every year.[1] In 2018 it is estimated that there was 18.1 million new cases and 9.6 million deaths from the disease and because of an aging global populace, these numbers are only expected to rise. An estimated 1 in 8 men and 1 in 10 women will be affected in one form or another in their lifetime. The need for effective treatments is very apparent across the board for all forms of the disease.

Chemotherapy in medicine arose as a concept in the early 20th century and was realized first in the 1940s in the form of nitrogen mustard.[1] Treatment with this wartime chemical agent was shown to reduce the size of a tumor. Before this breakthrough discovery the primary treatment for the disease was surgery, known to be an ineffective cure. The exploration and evolution of cancer therapy in the following years were tumultuous yet occasionally bore fruit. This approach to cancer treatment was initially met with heavy skepticism and opposition, though in time would prove to be one of the most effective. Chemotherapy has several major advantages with respect to other forms of treatment including radiation therapy, as it can be much more targeted and is able to treat metastatic forms of the disease. The initial discovery spurred research into screening other compounds that might act as anti-cancer agents and researchers developed analogues of known compounds such as 5-fluorouracil **1** and 6-mercaptopurine **2** (Figure 1.1). And thus began the era of cytotoxic agents.

Early therapeutics were inhibitors of DNA synthesis and replication like the compounds mentioned above, where many of them were designed to mimic natural machinery and compete with the natural substrate. Antimetabolites such as 5-fluorouracil or 6-mercaptopurine act as inhibitors of natural metabolite synthesis or action, usually

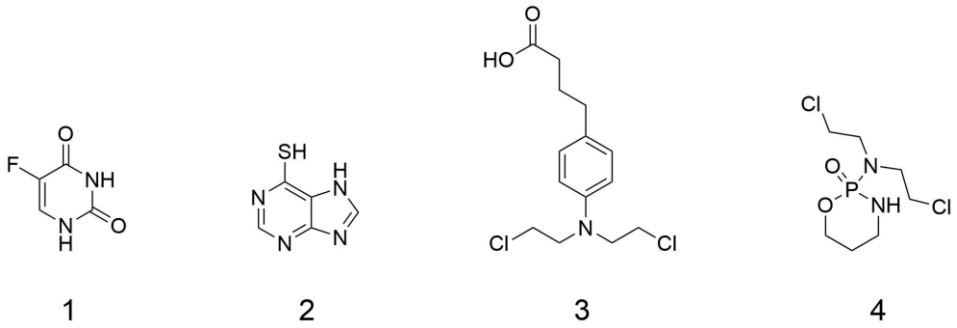


Figure 1.1. Early anti-cancer agents. 1) 5-fluorouracil 2) 6-mercaptopurine 3) chlorambucil 4) cyclophosphamide

resulting in halt of cell growth and division. Others like nitrogen mustard, chlorambucil **3**, and cyclophosphamide **4** are known to be alkylating agents that damage or cross link DNA, also preventing cell division. These compounds interfere with RNA synthesis and induce an accumulation of cytosolic p53 and lead to apoptotic cell death. One of the first effective treatments was antimetabolite methotrexate **5**, a methylated derivative of the B vitamin folic acid **6** (Figure 1.2). From the observation that addition of folate stimulated proliferation of acute lymphoblastic leukemia, pathologist Sydney Farber tested a series of analogues that led to the discovery that blocking the function of folate requiring enzymes resulted in successful remission.[1,2] Though this treatment was ultimately unsuccessful because of a short remission and eventual relapse, this proved that chemotherapy could be a viable alternative. Following this discovery there were efforts to test different synthetic compounds to treat

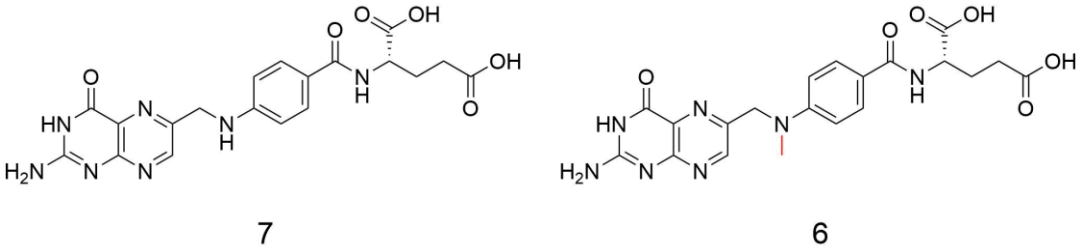


Figure 1.2. Folic acid 6 and methotrexate 5.

various cancers. This began with the anti-folates like methotrexate and led to the discovery of the anti-mitotic vinca alkaloids **7** and DNA replication dysregulators such as 5-fluorouracil and 6-mercaptopurine.

As effective as some early treatments were, there were still significant problems with side effects, toxicity, and relapse. In the mid 1960's there arose a logical solution to some of these problems in the form of combination therapy. Because of detrimental side effects and compromised immune response, researchers began to use multiple drugs to target several pathways or enzymes. Incredibly, this approach helped to reduce the rate of relapse and increase chances of survival. The combination of drugs proved to be the most effective way to prevent proliferation of drug resistant tumor cells and induce long-term remission. A further improvement came later with the arrival of adjuvant therapy in which chemotherapy was

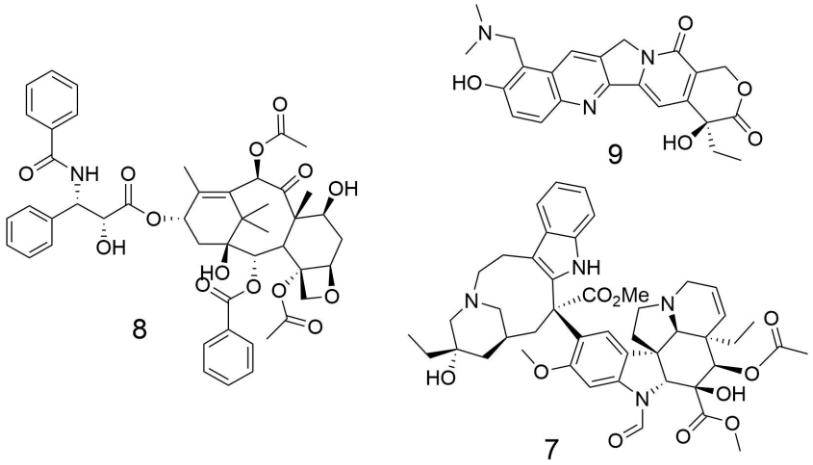


Figure 1.3. Natural products in cancer therapy. Vinca alkaloid vincristine 7, taxanes represented by taxol 8, and camptethecin analogue topotecan 9.

applied after surgery to remove a cancerous tumor. This was found to significantly extend survival, especially to those whose cancer had spread to auxiliary lymph nodes.

In the search for new and improved chemotherapy drugs, some researchers turned to natural products for inspiration. In the 1960's, C. Gordon Zubrod and other scientists at the National Cancer Institute built up a sizeable program of collecting and testing plant and marine natural products that led to the discovery of the taxanes **8** in 1964 and the camptothecins **9** later in 1966 (Figure 1.3).[1] These compounds had novel mechanisms of action. The taxanes were found to be inhibitors of proper microtubule function which resulted

in a halt of mitosis, whereas the camptothecins are topoisomerase inhibitors. Both classes of compounds are still used to this day in cancer therapy. Though the discovery of these natural products was useful, the initial development was troubled because of undesirable physical properties that prevented their use to an extent including poor aqueous solubility and complex structure. Because of this, medicinal chemistry efforts were made to improve solubility and other properties that resulted in novel analogues that greatly outperformed the natural products in key aspects. The model of using natural products as therapeutics or inspiration for others has been a constant theme of drug discovery and development. Approximately 49% of all drugs approved for treatment of cancers since the 1940's were either natural products or derived from natural products.[3] Though early drug candidates met with trouble because of poor solubility or stability at physiological pH, synthetic chemistry is used to supplement the creations of nature to help craft a gem out of a coarse stone.

Many drugs since the initial discovery of chemotherapeutic agents have been developed, but despite the advent of supportive measures to mitigate side effects & infection, the potential harm including long term effects on major organs remain formidable barriers to effective treatment. This leaves a lot of room for improvement that can be addressed in different ways including targeted therapy and medicinal chemistry. Many early drug candidates discovered in screening are not optimized for the target nor are their properties particularly attractive when it comes to their pharmacokinetic (ADME) properties. According to Chabner and Roberts, the characteristics of a model anti-cancer drug include 1) metabolic stability with a long half-life and slow metabolism by the cytochrome P-450 family. 2) Good absorption after oral administration (not typical of drugs prior to 1980). 3) Favorable toxicity profile at biologically effective doses.[1] These properties, not always initially present in drug screening hits, can be incorporated with synthesis. It can be a powerful tool that can provide unique contributions to drug development in that minor or major changes can be made to the

initial structure to assess structure activity relationships, improve aqueous solubility and absorption, prevent fast metabolism, and lower toxicity. Synthesis also establishes a route to provide material for further screening and eventual drug trials that isolation from natural sources cannot reasonably provide. Taxol, for instance, was isolated from the pacific yew tree.[4] For years the material used for testing was gathered by extraction of the tree bark that quickly became unsustainable due to limited supply. After years of effort in development, the first total syntheses were published.[5-7] Because of the ubiquitous nature of natural products in pharmaceuticals, total synthesis has and will continue to be a useful tool for development.

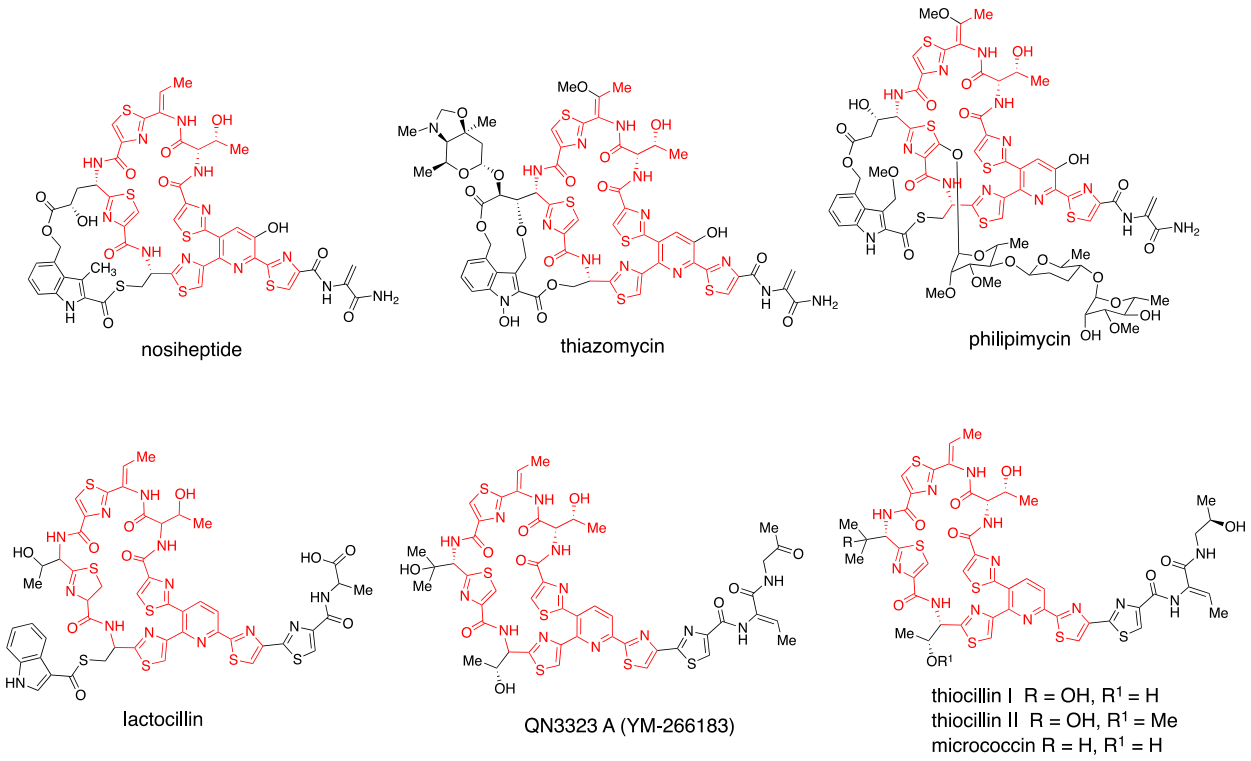


Figure 1.4. Various series D and E thiopeptides highlighting the common macrocycle.

Thiopeptides are a family of natural products with many faces and facets in structure and activity that are characterized by a large macrocycle, an array of heterocycles, and a central, 6-membered pyridine or piperidine (Figure 1.4).[8,9] They are highly modified sulfur-rich peptides of ribosomal origin with over 100 members currently known. They all share a

series of common motifs that differentiate them from other peptides including the central nitrogen-containing six-membered ring that is found in many oxidation states. This central ring provides the scaffold for the large macrocycle, or multiple in some cases, and a tail consisting of several amino acid residues. Throughout the structure there are diverse functionalities and heterocycles including various dehydroamino acids and azoles. These

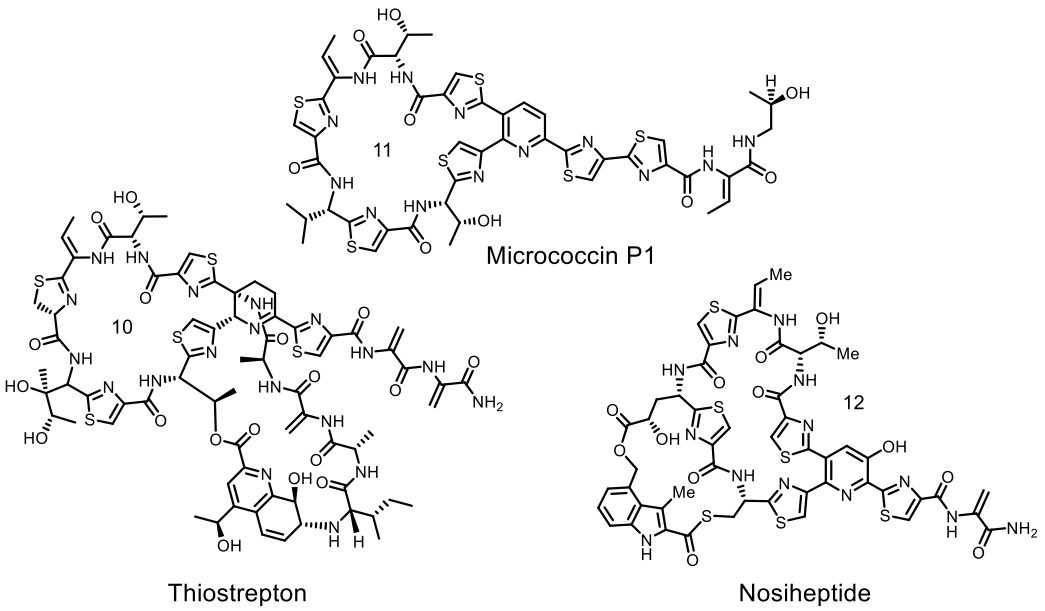


Figure 1.5. Examples of thiopeptide natural products of different series & macrocycle size.

common motifs are derived from several amino acids including cysteine, serine, and threonine *via* dehydration or cyclization between adjacent peptide residues. There are several subsets of this family defined as series A through E described by the oxidation state of the central ring. Series A thiopeptides have a piperidine heterocycle and series E has a fully oxidized hydroxypyridine. All have at least one large macrocycle, and some two, tethered to the central ring. Representative examples are Thiostrepton **10**, Micrococcin **11**, and Nosiheptide **12** of series B, D, and E respectively (Figure 1.6). Throughout the thiopeptide family there are several common features that define their niche and predict their activity including the oxidation state of the central ring as well as the size of the main macrocycle.

There are 3 categories that all thiopeptides fall into including 26-membered, 29-membered, and 35-membered macrocycles. The size depends on the number of peptide residues in the precursor peptide that fall between the several residues that participate in the cyclization to form the macrocycle (*vide infra*). All these features, large and small, contribute to their activity in different contexts.

Thiopeptides have been known for many years. First discovered in the sewers of Cambridge in 1948, dozens have since been disclosed from a variety of sources around the globe including terrestrial and marine samples.[8,10] Soil bacteria, however are the primary source of these compounds. Structural assignment was initially very challenging, especially with the complex nature of the macrocycles and heterocycles. Structural elucidation was initially addressed by degradation studies and characterization of fragments.[11] X-ray diffraction would eventually be the catch all solution to structural connectivity and stereochemistry.[12,13] Though the characterization through these means proved fruitful, a high degree of uncertainty remained until further efforts in synthesis were published to confirm these proposals. The impossibility to obtain crystals for the vast majority of them prompted the assignment of their structure without a clear evidence of their stereochemistry. The story of micrococcin P1 details the essence of this problem. Initially described shortly after its discovery in Cambridge, it was not until after many total synthesis efforts to confirm its structure that it was resolved in 2009.[14] For complex products like these, synthesis can be the ultimate answer to structural ambiguities.

Thiopeptide biosynthesis has been investigated for some time, and it was recently revealed that these products are in fact of ribosomal origin. A precursor peptide is translated first that includes a structural portion of between 12-17 residues rich in serine, cysteine, and threonine at the C-terminus that contains the amino acids that will constitute the thiopeptide itself. This is preceded by a leader peptide sequence of 34-55 residues at the N-terminus

which is cleaved during the biosynthetic process.[15-19] The translated linear precursor is modified by a series of tailoring enzymes that first form the multitude of heterocycles and finally facilitate the macrocyclization with concurrent cleavage of the leader peptide. The gene clusters for several of these products have been identified including the precursor peptide that will eventually become the cyclic thiopeptide.

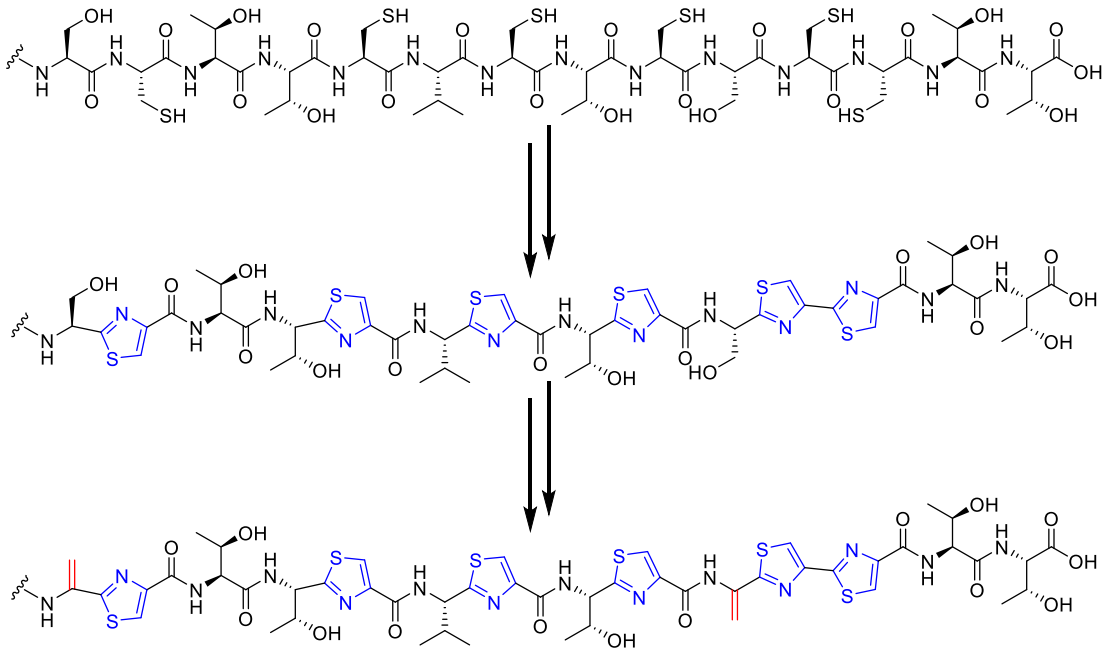


Figure 1.6. Posttranslational modifications of Micrococcin after translation.

In this cluster is also included the set of tailoring enzymes including cyclodehydratases and dehydrogenases that modify the precursor peptide after ribosomal transcription (Figure 1.7). This biosynthetic space has been explored in a number of ways to probe the extent of diversity and potential number of products in the family.[20-23] This approach is useful for the discovery and extrapolation of the current field of products known, but is prone to limitations such as producing precursor peptides that do not mature into cyclic thiopeptides though to be accessible. And though a multitude of new products have been discovered in this way, the finer details and properties of the products aren't greatly different than those already known.

Thiopeptides have been the focus of academic and industrial study for decades for drug development in search of new modes of action against bacterial pathogens, especially with regard to resistant strains.[24-29] Though these natural products are known to be potent inhibitors of bacterial protein synthesis, their activity is wide ranging. In addition to their antibiotic activity, they also show inhibitory activity against the malaria parasite *Plasmodium falciparum*[30-33] and cytotoxicity against a variety of cancer cell lines.[34-38] The modes of action vary widely depending on the organism or disease. In an antibiotic context, the major macrocycle size is what determines the cellular target. Though the family of products are all know to be bactericidal through inhibition of protein biosynthesis, the 26, 29, and 35-membered macrocycles have different roles in doing so. The 26-membered thiopeptides such as thiostrepton, nosiheptide, and micrococcin are known to selectively target the 50S ribosomal subunit and disrupt interactions between the 23S rRNA and ribosomal protein L11.[39-41] The peptides bind within a cleft located between the L11 subunit and the adjacent 23S rRNA and inhibit binding of the elongation factor G (EF-G). This GTPase enzyme is responsible for peptide extension by catalyzing the translocation of transfer RNA (tRNA) and messenger RNA (mRNA) through the ribosome where preventing it's binding effectively halts translation. It is notable that the thiopeptides inhibit bacterial protein biosynthesis through disruption of protein-protein interactions rather than inhibition of individual enzyme activity. The 29-membered thiopeptides act by binding to elongation factor Tu (EF-Tu), blocking its tRNA/amino acyl complex binding site. As a result, the complex isn't delivered into the ribosome and peptide elongation stops.[42,43] The molecular target of the 35-memebered thiopeptides has not yet been identified, though they also maintain potent activity. It has been demonstrated that antibiotic activity is dependent most on the existence of the large macrocycle, though it is not the only factor. Without the

macrocycle present, none of the thiopeptides tested retain activity. Even so, some of the them don't have activity even with the macrocycle whether they be any size or series. Within the family there are some regions of conserved residues but this doesn't always correlate to activity. These components are thought to contribute to stabilization of the active conformation *via* transannular H-bonds.[44]

Another interesting activity of the thiopeptides is their ability to selectively kill a broad range of cancer cells without showing any cytotoxicity against normal cells. This activity has been linked to the selective binding of transcription factor forkhead box M1 (FOXM1).[45-48] FOX M1 in normal cells plays a role in cell cycle progression and regulates expression of a wide array of G2/M-specific genes and plays an important role in maintenance of chromosomal segregation and genomic stability.[49] Its overexpression is associated with the development and progression of cancer in that it leads to unregulated cell growth and suppressed differentiation. 26-membered thiopeptides have been found to interact directly with FOX M1 and downregulate its expression as well as induce apoptosis in cancer cells. These finding established FOXM1 is one of the primary cellular targets of thiostrepton and other thiopeptides, and that transcription factors, once thought to be undruggable, can be targeted for potential cancer therapy.

With therapeutic potential, synthesis of this group of natural products has been explored previously with products like thiostrepton[50-52], GE2270 A[53-55], and micrococcin P1.[56,57] These pioneering syntheses outlined some general strategies toward these products that we use for guidance in this work. We focus our attention on micrococcin P1, a member of the D series of thiopeptides.[8] The D series is characterized by a central trisubstituted pyridine as the cornerstone of the large, 26-membered macrocycle. Micrococcin contains 6 thiazoles, 2 olefins, and an extended "tail", all with unique challenges. We focus on scalability and simplification of the synthesis by uniform construction of the thiazoles and other

fragments with little or no chromatography. A major advantage is our use of chiral building blocks to set the stereocenters throughout the natural product in a chiral pool approach in which each thiazole is derived from a proteinogenic amino acid with only a few manipulations.

Micrococcin P1 is the earliest known member of the thiopeptide family and its characterization has a storied history. A 26-membered, D series thiopeptide, its exact structure was a topic of debate for many years. Early studies on its structure by hydrolysis of the extract gave most of the information needed to identify most moieties present, however there was no clear evidence of its connectivity[58-60] Subsequent NMR and synthetic studies proposed structures of the natural product that were similar, but none that were made were identical to the natural isolate.[61,62] The leading proposal for decades was that of Bycroft and Gowland which differs from the natural product only at the valine stereocenter. [63] Because of a previous report of an R stereocenter and therefore a thiazole derived from D-valine, the structure proposed was incorrect.[64] It was not until 1999 that the product matching this proposed structure was synthesized and the authors found it did not match the natural product spectroscopically.[65] Unabated, Ciufolini and Lefranc pursued the synthesis of alternative structures and found in 2009 the true structure to be consistent with a combination of several past reports.[66] Though more than 50 years passed between its discovery in Cambridge until the confirmation in 2009, persistent synthetic efforts were able to reveal the true structure of the natural product.

The reason for our interest in micrococcin is two-fold. We observed from previous reports that some thiopeptides have activity against cancer cell lines, and even some synthetic fragments containing the central 6-membered ring.[67] Also, in our survey of the literature we came across a seldom used method that could significantly improve upon the current routes to these products by reducing instances of chromatography and use of

hazardous intermediates, conditions, or reagents. There have been several approaches to the thiopeptide family of natural products. Some construct the central ring from scratch,[50,57,68-70] and others choose to manipulate an already existing structure.[53-55,71] The macrocyclization has been achieved in ways including peptide coupling[50,56,57,68,69,72] and cross-coupling.[54,55,73]

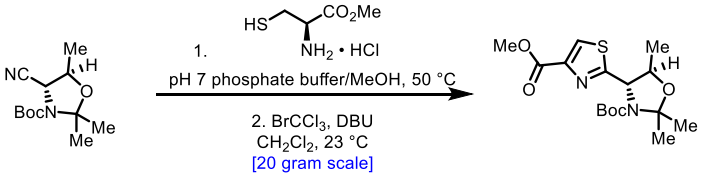


Figure 1.7. Example of a cysteine-nitrile condensation / oxidation reaction sequence.

Our approach to micrococcin is focused around the uniform, robust, and scalable synthesis of the many thiazoles throughout the structure and the macrocyclization to form the characteristic ring unique to this family of natural products. A secondary goal for this project was to reduce the instances of chromatography wherever possible to further simplify the synthesis of this complex product. Heterocycles are ubiquitous in the thiopeptides and classically, many are made through the Hantzsch synthesis[50,54,68-70,72-74] that, though fairly reliable, utilizes the undesirable Lawesson's reagent. In addition to the unpleasant smell, the byproducts of the reaction are also challenging to remove by chromatography. This is something that we avoided in this synthesis. To this end, we modified an underutilized thiazole forming method to our advantage: A condensation / cyclization reaction between an organic nitrile and cysteine that can be run under very mild conditions and with inexpensive and readily available reagents (Figure 1.8).[75] This method proved to be a robust way to form the precursor thiazolines derived from several amino acids that can be oxidized to thiazoles without intervening purification. We were able to employ this method to make 5 of the 6 thiazoles in micrococcin.

The total syntheses of several thiopeptides have been accomplished over the past few decades. In general, the different peptide scaffolds of these natural products are made using standard peptide chemistry and Hantzsch thiazole syntheses from natural amino acids. The challenge when synthesizing these molecules is in assembly of the heterocyclic cores and macrocyle(s). Strategies have involved either stepwise elaboration of simple pyridine or piperidine starting materials or pyridine synthesis from acyclic precursors using modified Chichibabin syntheses or hetero Diels-Alder reactions. There have been 2 reported syntheses of micrococcin P1 to date.[57,71] These incorporate many of the features of past syntheses including the Hantszch thiazole synthesis. The first described earlier was completed by Ciufolini in 2009. The authors propose constructing the central pyridine *via* a Hantzsch

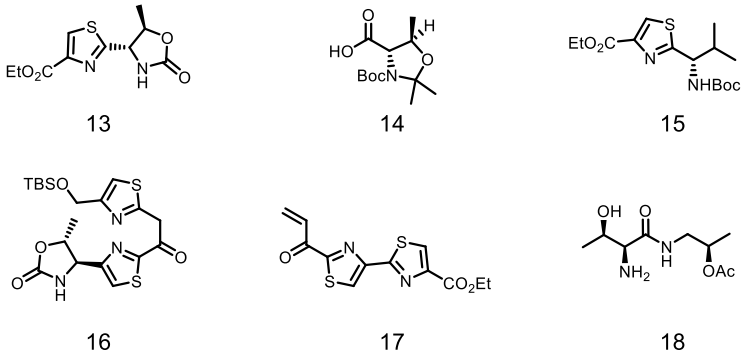
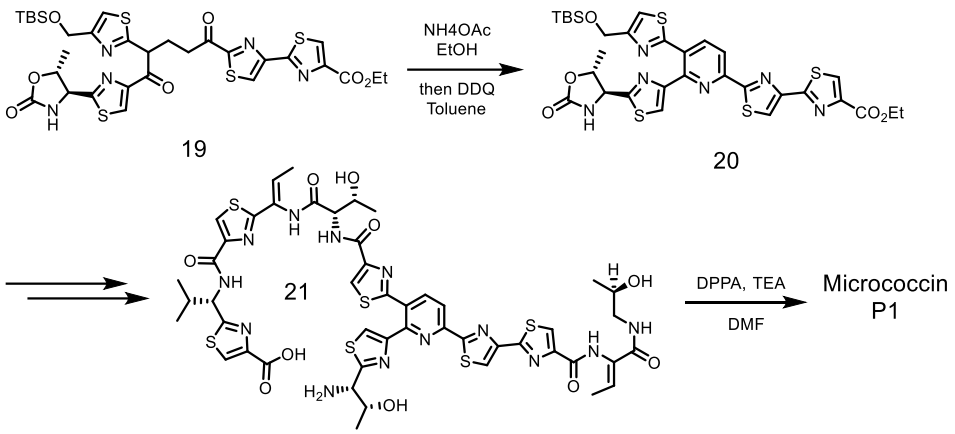


Figure 1.8. Fragments for Ciufolini's synthesis of Micrococcin P1.

pyridine synthesis and the thiazoles mostly through the Hantzsch thiazole synthesis. The backbone is constructed through a series of peptide coupling reactions to make the completed fragments with the synthesis concluding with ring closure to make the 26-membered



Scheme 1.1. Ciufolini's synthesis of Micrococcin P1.

macrocycle. They began by constructing fragments **13-18** (Figure 1.9), some of which are known from previous reports. Conjugate addition of **16** to **17** gives intermediate **19** that is cyclized under Hantzsch conditions to give pyridine **20** (Scheme 1.1). After some peptide coupling and functional group manipulations, intermediate **21** is cyclized using diphenylphosphoryl azide (DPPA) to give micrococcin P1 that is isolated by HPLC.

Most recently, a synthesis was reported in 2018 by Siddhartha Akasapu et al. As in previous syntheses they mostly rely on the Hantzsch method for construction of the thiazoles,

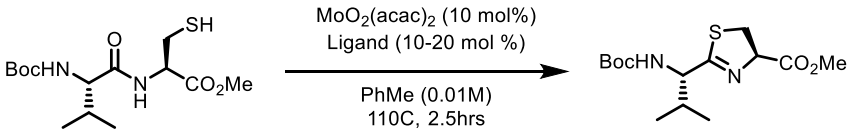
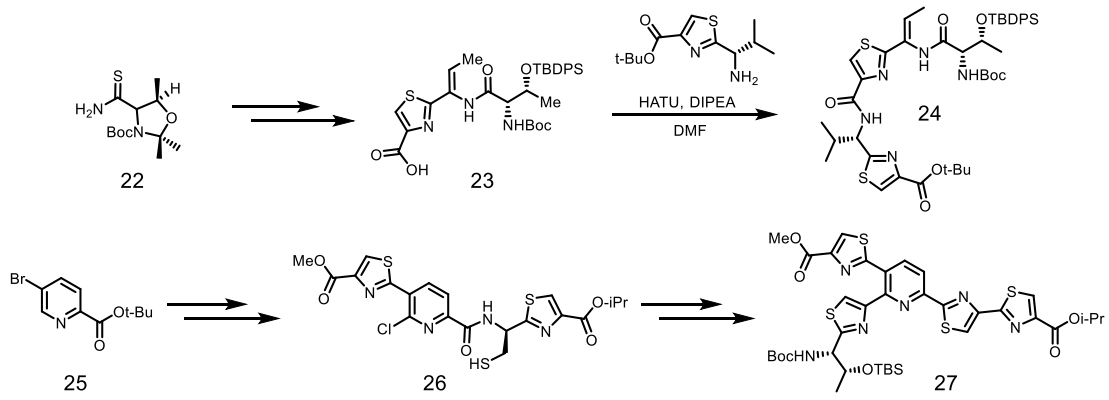


Figure 1.9. Akasapu's thiazoline formation *via* molybdenum catalyzed cyclodehydration.

however, they introduce a new method as well. After screening ligands with a molybdenum catalyst, they found that they could affect cyclization between adjacent residues in a dipeptide to give thiazolines in good yield (Figure 1.10). These thiazolines are oxidized with established chemistry using bromotrichloromethane ($BrCCl_3$) and DBU to thiazoles. Their synthesis of micrococcin is completed in 15 steps (longest linear sequence). From known thioamide **22**, Hantzsch synthesis, dehydration, and peptide coupling gave **23** (Scheme 1.2).

The valine derived thiazole appended next is made *via* the molybdenum catalyzed cyclodehy-



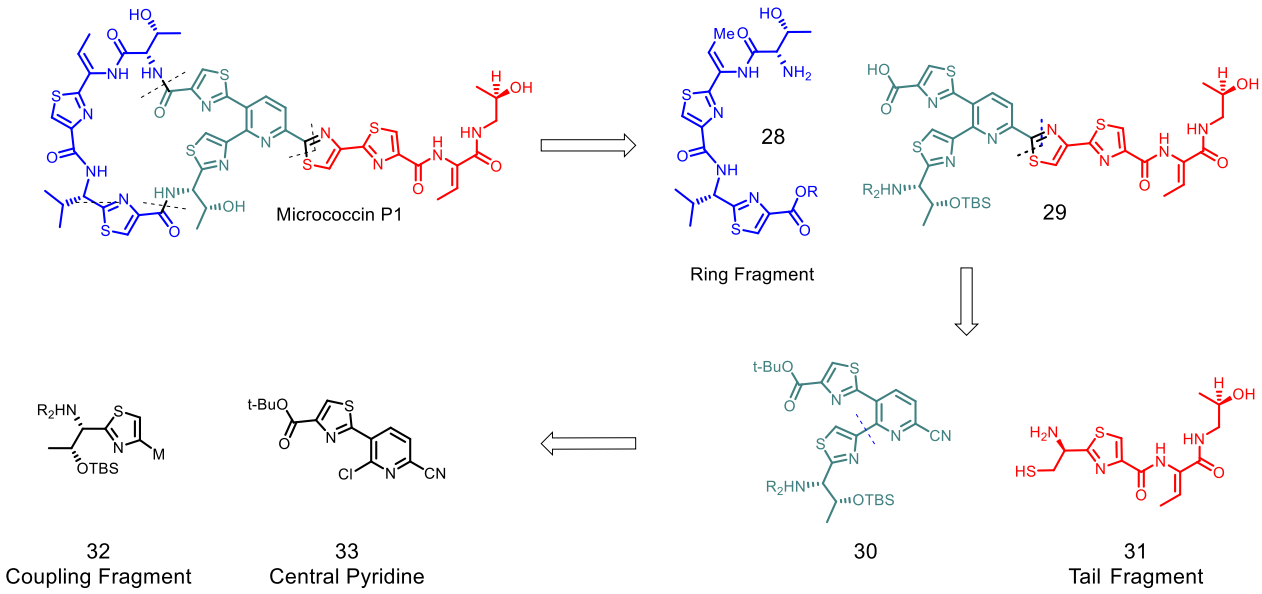
Scheme 1.2. Akasapu synthesis of Micrococcin P1.

dration and gives fully assembled ring fragment **24**. The other fragment is made from bromopyridine **25**. Cross coupling to place the first thiazole is followed by chlorination and peptide coupling to give **26**. To construct the thiazole-thiazole linkage found micrococcin's tail, the authors used their molybdenum methodology followed by heterogeneous oxidation with MnO_2. Stille coupling followed by another Hantzsch thiazole synthesis gives trithiazolylpyridine **27**. During fragment assembly, the tail is completed by coupling to the chiral aminoalcohol and dehydrating to form the olefin. Finally, the macrocyclization is completed by peptide coupling with PyAOP and DIPEA to give 18mg of micrococcin P1 that is further purified by HPLC.

The key challenges in synthesizing micrococcin include macrocyclization, construction of the thiazoles including the thiazole-thiazole connection in the tail, and manipulation of the central pyridine. Heterocycles such as thiazoles, thiazolines, oxazoles, and oxazolines are found throughout the thiopeptide family and a strategy to construct such motifs is critical for the successful synthesis of these natural products. In micrococcin, there are 6 thiazoles scattered throughout the structure. Previously, these heterocycles were made through a combination of the Hantzsch or aza-Wittig reaction.[76] Here we utilize a robust method for the

synthesis of 5 of 6 thiazoles in micrococcin in the form of a cycloaddition / condensation reaction using easily accessible nitriles and cysteine. The initial condensation reaction gives a thiazoline that can be oxidized crude using either a combination of $BrCCl_3$ and DBU or activated MnO_2 (Figure 1.8). This method gave the thiazoles in moderate to good yields without chromatography. These thiazoles can be derived from nitriles of several amino acids including cysteine, threonine, and valine in a few synthetic steps. We expect that this can be extrapolated to include the rest of the proteinogenic amino acids as well, and beyond. Generally speaking, the amino acids are first protected at the amine and side chain, transformed from acid to primary amide, and dehydrated to give the necessary nitriles for condensation. The condensation reaction occurs in a mixture of alcoholic solvent (either MeOH or IPA) and phosphate buffer and proceeds cleanly and completely. Afterward, the crude thiazoline can be oxidized directly following a short work up. This methodology was used to make 5 of the 6 thiazoles found in micrococcin. The last is derived from commercially available 2,4-thiazolidinedione via a dehydration / bromination reaction to give 2,4-dibromo-thiazole for the synthesis of a chiral aminothiazole and eventual cross coupling.

In the retrosynthetic sense, we disassemble micrococcin at two peptide bonds to give a large ring fragment **28** and the trithiazolyl pyridine **29** (Scheme 1.3). The large ring fragment, though not immediately apparent, can be made solely from L-threonine, L-cysteine, and L-valine with some functional group manipulations. The central pyridine is deconstructed at the 6-thiazole *via* cysteine-nitrile condensation that gives two fragments **30** and **31**. The pyridyl nitrile can be made by cross coupling at the 2 position, giving chiral thiazole fragment **32** and 2-chloropyridine **33**. The tail fragment **31** can be synthesized from L-threonine, L-cysteine, and the commercial R-(-)-1-amino-2-propanol by peptide coupling and thiazole formation. Pyridine **33** is derived from readily available 2-chloronicotinonitrile

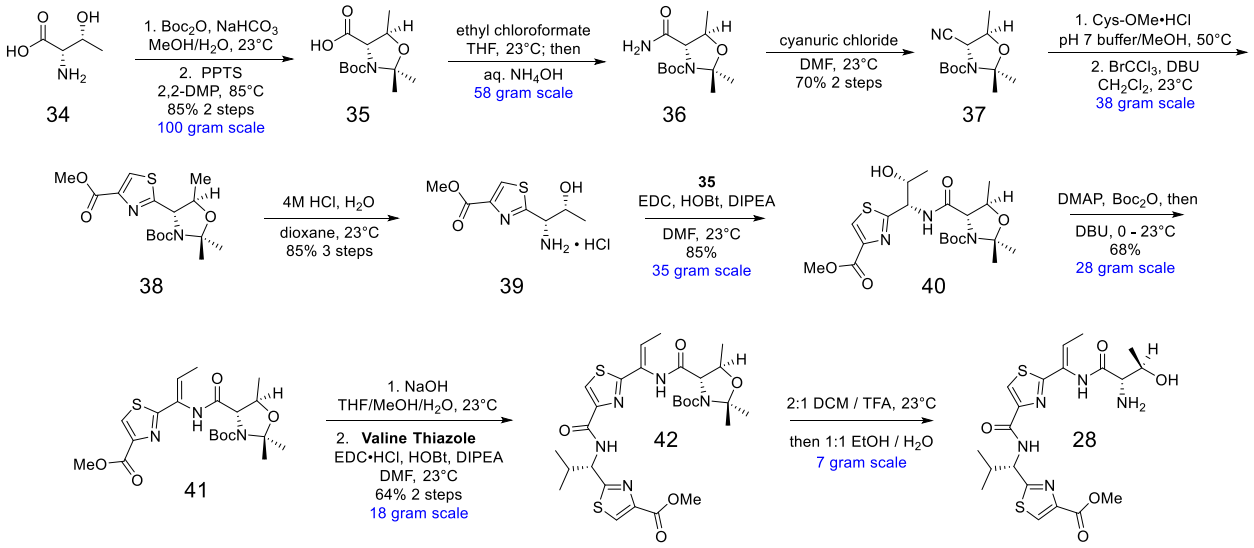


Scheme 1.3. Retrosynthetic deconstruction of micrococcin P1.

in several steps with some routine and robust transformations, whereas coupling fragment **32** is constructed by Grignard addition into a chiral imine (derived from methyl D-lactate).

This synthetic route takes a unique approach to this family of natural products and can be applied to many other members of this group. The methods we chose to use in this endeavor also greatly reduce the instances of chromatography required, simplifying the synthesis in an unconventional way. We have 4 distinct fragments to make in parallel toward the synthesis of micrococcin. They are the large ring fragment, the cross-coupling fragment, the central pyridine, and the tail. I will address each of these individually, then describe the assembly and finally the cyclization to complete the synthesis. These fragments are mostly derived from commercial proteinogenic amino acids L-threonine, L-cysteine, and L-valine as well as readily available methyl D-lactate, 2,4-thiazolidinedione, and 2-chloronicotinonitrile. The synthesis is designed to be scalable, utilize a common and robust methodology for multiple aspects of the scaffold, and thus provide a substantial amount of material with ease for probing all aspects of its activity.

The synthesis begins with the large ring fragment **28** (Scheme 1.4) that consists solely of residues derived from L-threonine, L-cysteine, and L-Valine. From previous reports, we protect threonine with Boc anhydride and sodium bicarbonate followed by formation of acetonide **35** with PPTS in refluxing 2,2-dimethoxypropane[77-79]. This intermediate is used several times in the synthesis of micrococcin and can be prepared on multi-hundred-gram scale without chromatography. The free acid is then transformed to the primary amide **36** by activation with ethyl chloroformate followed by addition of ammonium hydroxide. The amide is subjected to dehydration with cyanuric chloride, a reagent that consistently avoids epimerization. Precipitation of the resulting nitrile **37** from DMF into water gives an analytically pure solid that requires no further purification. The nitrile is subjected to our



Scheme 1.4. Synthesis of the large ring fragment 28.

optimized cyclization conditions with excess cysteine methyl ester hydrochloride in a mixture of methanol and pH 7 phosphate buffer with gentle heating to 50°C to give crude thiazoline. The thiazoline is oxidized using bromotrichloromethane and DBU, and the Boc group and acetonide are removed with 4M HCl in dioxane and 10% v/v water. The free amine **39** is prepared in 7 steps without chromatography. This amine is coupled to protected threonine

intermediate **35** with EDC, HOBt, and DIPEA in DMF in good yield, followed by dehydration of the unprotected threonine side chain to give exclusively the Z olefin **41.** This is completed by activation of the secondary alcohol with Boc anhydride and DMAP, then elimination with DBU. The final piece of the ring fragment we assemble is a valine derived thiazole that we make through the cysteine-nitrile condensation. However, I encountered significant issues using the standard conditions including sluggish reactions that rarely went to completion and significant loss of optical purity. These we had to address separately and came to a solution by screening conditions to optimize for yield and optical purity.

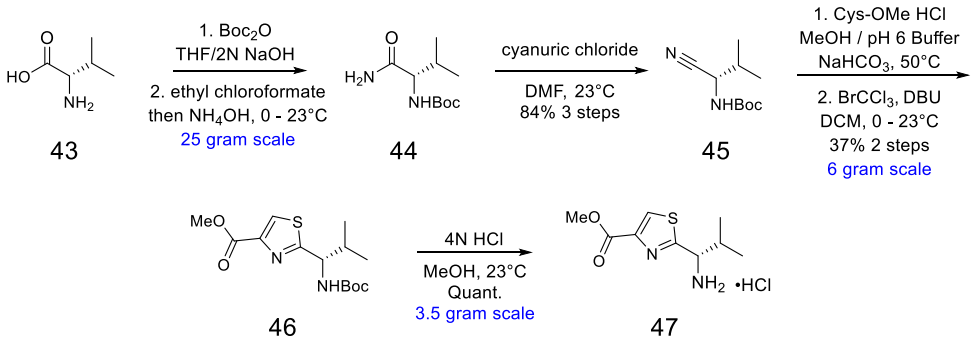
Throughout the synthesis of micrococcin we used our optimized conditions that were robust and consistent for the synthesis of thiazoles except for valine. We found that under our standard conditions (1.5:1 IPA / pH 7 phosphate buffer at 50°C and 1.5 eq. of cysteine methyl ester hydrochloride) the reaction was sluggish and never went to completion with low conversion and we saw a significant turnover to a byproduct not seen previously. In an attempt to improve conversion and yield, we screened conditions including altering reaction temperature, buffer concentration, buffer pH, equivalents of cysteine, and solvent. When solvent, temperature, or quantity of cysteine alone was manipulated, little to no useful change occured. Ultimately, we discovered that increasing buffer pH and / or concentration drove the reaction to completion (data not shown). Although with these results came another problem; the product thiazoline e.e. was found to be significantly diminished, indicating an epimerization occuring at the side chain. We verified this erosion by derivitization and NMR analysis with the Mosher acid as well as optical rotation measurements of the thiazole after oxidation. This issue has been encountered before in the literature when forming thiazoles using the Hantzsch method.[57,80,81] Other methods and modifications were published addressing this problem.[82] Using these and other reports as a starting point, another set of screening conditions was executed, this time making sure to keep buffer concentration low

and at neutral or slightly acidic pH. These conditions gave a variety of results from low to high erosion of e.e., only one providing complete retention of enantiopurity. The screening conditions are summarized in Table 1.1. Interestingly, epimerization increased from MeOH to IPA to *t*-BuOH with buffer. TEA and other weak bases did not appear to prevent epimerization in several solvents. The amount of cysteine used had no bearing on the resulting e.e. measurements, and the temperature had only a small effect. After extensive testing, we found that pH6 buffer and methanol with sodium bicarbonate as an additive gave the desired result with no detectable epimerization by NMR (after derivatization). With these conditions in hand, we were confident to continue without further problems, as the oxidation is not known to cause epimerization when using DBU and $BrCCl_3$.

Table 1.1. Screening conditions for optimization of valine thiazole synthesis with retention of e.e. *1.5:1 ratio of alcoholic solvent to phosphate buffer. a) 2 eq. Cys-OMe HCl. b) 3 eq. Cys-OMe HCl. c) 1 eq. Cys-OMe HCl. d) based on optical rotation of oxidized thiazole.

Rxn	Solvent*	Temp. (°C)	Buffer pH	Buffer conc. (M)	Reaction Time (hrs)	Additives	e.e. (%)[d]
1[a]	MeOH / Buffer	50	6.0	0.2	24	-	<50
2[a]	MeOH / Buffer	65	6.0	0.2	48	2 eq. $NaHCO_3$	>99
3[b]	MeOH / Buffer	60	7.0	0.2	3	3 eq. TBAC	94
4[b]	IPA / Buffer	60	7.0	0.2	3	3 eq. TBAC	81
5[b]	t-BuOH / Buffer	60	7.0	0.2	3	3 eq. TBAC	56
6[b]	IPA / Buffer	23	7.0	0.2	6	3 eq. TBAC	86
7[b]	IPA / Buffer	40	7.0	0.2	6	3 eq. TBAC	85
8[b]	IPA / Buffer	60	7.0	0.2	6	3 eq. TBAC	78
9[c]	IPA / Buffer	60	7.0	0.2	3	1 eq. TBAC	74
10[a]	MeOH / Buffer	60	6.0	0.2	3	2 eq. $NaHCO_3$	>99
11[a]	MeOH / Buffer	60	6.0	0.2	6	2 eq. $NaHCO_3$	>99
12[a]	MeOH / Buffer	60	6.0	0.2	16	2 eq. $NaHCO_3$	>99

The sole residue of the large ring fragment that isn't derived from L-threonine or L-cysteine is derived from valine. L-valine is protected with Boc anhydride in a mixture of THF and 2N NaOH to give Boc-valine (Scheme 1.5). The free acid is converted to primary amide **31** with activation *via* ethyl chloroformate followed by addition of ammonium hydroxide and dehydrated with cyanuric chloride. The crude material is precipitated from DMF into ice

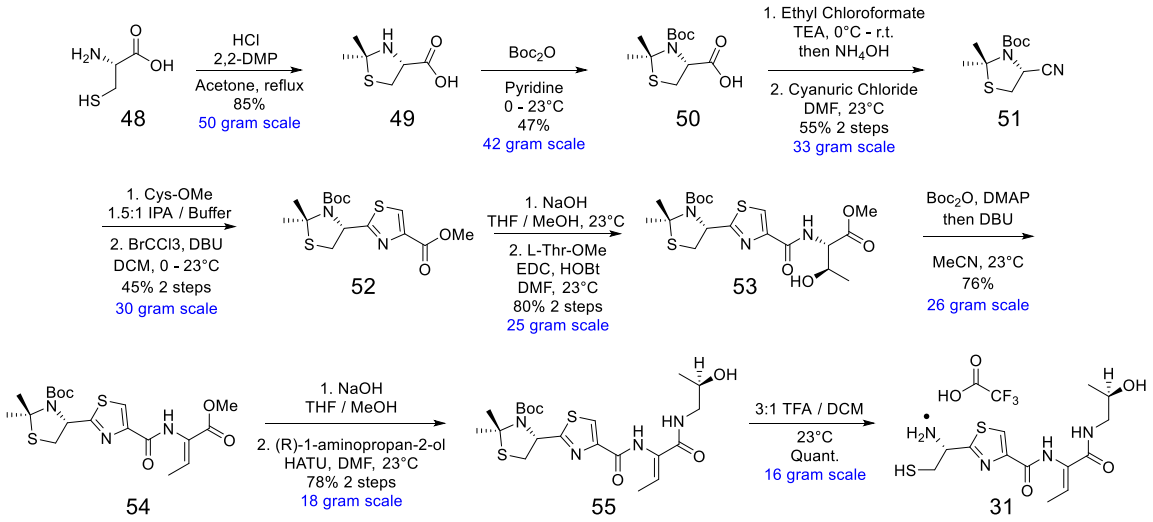


Scheme 1.5. Synthesis of the valine thiazole using modified cysteine-nitrile conditions.

water to give analytically pure nitrile **45** after filtration and drying in 3 steps without chromatography. The nitrile is cyclized with cysteine methyl ester hydrochloride in MeOH / pH 6 buffer with sodium bicarbonate followed by oxidation of the crude thiazoline using $BrCCl_3$ with DBU to give thiazole **46** in good yield. Boc-valine thiazole is deprotected with HCl in dioxane and precipitated after work up with diethyl ether (sonication gives the best result; a fine white powder) to give HCl salt **47**.

Once the valine thiazole has been prepared, the methyl ester intermediate **41** is saponified with NaOH in THF / MeOH and coupled with valine thiazole **47** using EDC & HOBt, giving the fully assembled ring fragment **48** in good yield (Scheme 1.4). Finally, the Boc-acetonide protecting group is removed with 1:3 TFA in DCM followed by concentration from 1:1 ethanol / water 3 times to ensure complete removal of the acetonide. After removing excess acid and water *via* azeotrope with benzene or toluene, the large ring fragment **28** is ready for coupling to the central pyridine fragment.

The acyclic tail fragment at the pyridyl 6 position is constructed in a manner similar to the large ring fragment in its formation of heterocycles. A 2,4'-thiazole-thiazole linkage is present here providing an interesting challenge that we resolved by using a cysteine based thiazole in a cyclization reaction with the central pyridine. Starting with facile protection of cysteine first with 2,2-DMP in acetone gives **49** followed by Boc protection in pyridine to give

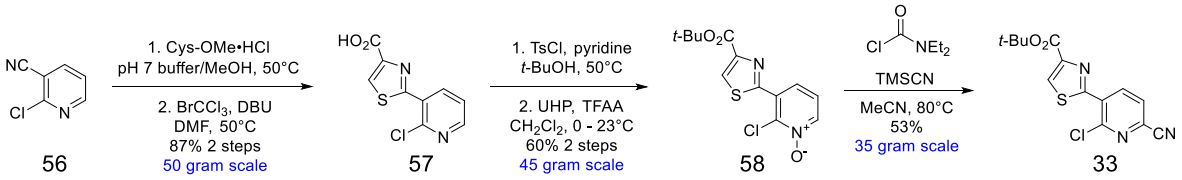


Scheme 1.6. Synthesis of the tail fragment from cysteine and threonine.

50, the free acid intermediate is transformed as before to a primary amide using ethyl chloroformate and ammonium hydroxide (Scheme 1.6). Although we found reports that used cysteine hydrochloride monohydrate in the initial step, in our hands using dry HCl in dioxane to form the cysteine acetonide instead gave a more consistent, higher yielding result.[79] Recrystallization of the Boc-acetonide in hexanes provides pure **50**. It is essential that the pyridine solvent used in the Boc protection is fully removed in the work up for effective recrystallization. The next steps are significantly hindered otherwise. The amide is dehydrated with cyanuric chloride in DMF and precipitated by pouring slowly into ice cold water (~10:1 H_2O / DMF) with vigorous stirring to give the nitrile **51** after filtration & drying. Condensation with cysteine methyl ester hydrochloride in IPA / buffer followed by oxidation with $BrCCl_3$ and DBU gives intermediate **52**. Saponification of the methyl ester and coupling to commercially available L-threonine methyl ester hydrochloride yields **53**. Akin to the dehydration in the large ring fragment sequence, the threonine residue is dehydrated here using Boc anhydride and catalytic DMAP followed by DBU at ambient temperature. Saponification with NaOH in MeOH and THF reveals the carboxylic acid that is used directly in a peptide coupling reaction with EDC, HOBt, and (R)-1-amino-2-propanol. This coupling

provides the fully assembled tail fragment **55**, that is then deprotected with TFA in DCM followed by concentration thrice from 1:1 EtOH and H_2O. Thorough azeotroping with 5:1 benzene and MeOH (2-3x) gives TFA salt **31** (found to be very hygroscopic) that is used to construct the thiazole-thiazole linkage in the natural product with the cysteine-nitrile condensation-oxidation methodology.

The central fragment of micrococcin contains a trisubstituted pyridine where there have been a wide range of approaches undertaken to construct throughout the thiopeptide family including via aza-Diels-Alder reaction[50,72], Bohlmann-Rahtz[68,70] pyridine synthesis, or individual manipulations of an existing pyridine.[53-55,71,73] We took the latter approach, preparing this intermediate in 6 synthetic steps with only 2 chromatographic purifications necessary. The major transformations include a thiazole formation in the usual manner, introduction of a nitrile at the 6 position, and Stille coupling with a chiral thiazolyl stannane.

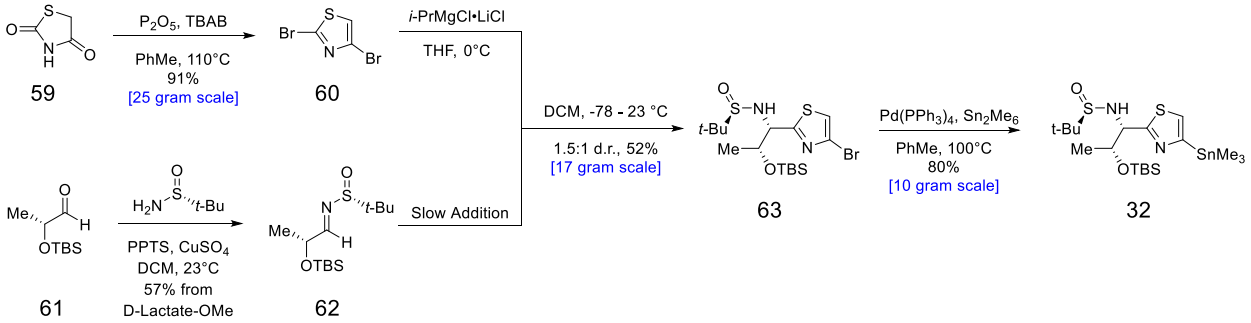


Scheme 1.7. Synthesis of the central pyridine without chromatography.

We begin the synthesis of the central pyridine with commercial 2-chloronicotinonitrile **56** in a cyclization with cysteine under slightly modified conditions (0.5M phosphate buffer rather than 0.2M). The higher buffer concentration increases the reaction rate, and there was no for caution in this context as only cysteine was used rather than the methyl ester. The crude thiazoline is oxidized with $BrCCl_3$ & DBU in DMF at 50°C and precipitated into cold 2M HCl to give free acid thiazole **57** in 87% over 2 steps. This oxidation is very exothermic on large scale and the DBU should be added dropwise while immersed in a water bath to avoid an exotherm. This intermediate is protected as a *t*-Bu ester with *p*-tosyl chloride in

pyridine and *t*-butanol, followed by N-oxidation of the pyridine with urea-hydrogen peroxide adduct and trifluoroacetic anhydride to give pyridine *N*-oxide **58** in 75% over 2 steps. The crude *N*-oxide is transformed in a rearrangement using diethylcarbamyl chloride and TMS-cyanide in refluxing acetonitrile to give pyridine-6-nitrile **33** chemoselectively in 90% yield after precipitation and drying (Scheme 1.7).

Meanwhile, the coupling fragment is prepared and contains two stereocenters. One derived from the chiral pool, while the other is set using a chiral auxiliary. First, as reported previously,[83] we protect commercially available D-lactic acid methyl ester with TBSCl and imidazole in DCM in near quantitative yield (Scheme 1.8). This is followed by reduction to aldehyde **61** with DIBAL in DCM at -78C and condensation with the Ellman chiral auxiliary (R)-*tert*-butanesulfinamide[84] using PPTS and anhydrous copper sulfate to give imine **62**. This reaction was initially very sluggish in the absence of PPTS and never reached completion

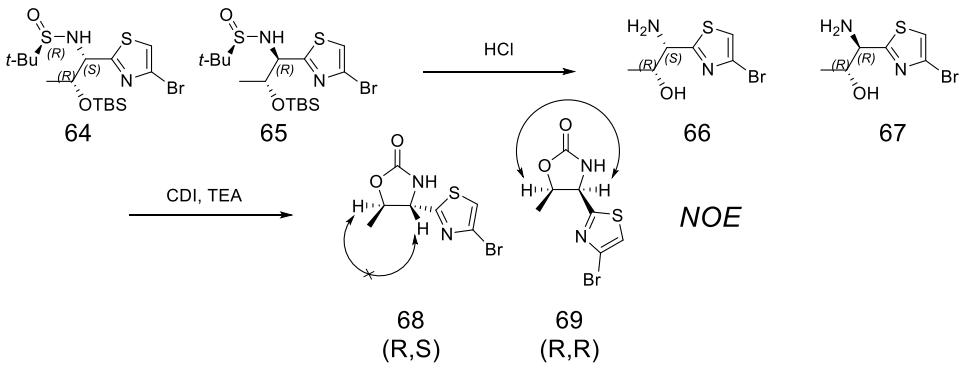


Scheme 1.8. Synthesis of the thiazolyl stannane cross-coupling fragment.

even after several days and additional $CuSO_4$. Other additives including TEA or DMAP were tried as well with no success. Addition of catalytic PPTS (20-25%) accelerated the reaction significantly and also saw it to completion. The imine is purified before Grignard addition and should be used shortly after its synthesis, as it is not particularly stable and will degrade after several weeks on the benchtop. Although it can be stored indefinitely under inert gas in the freezer.

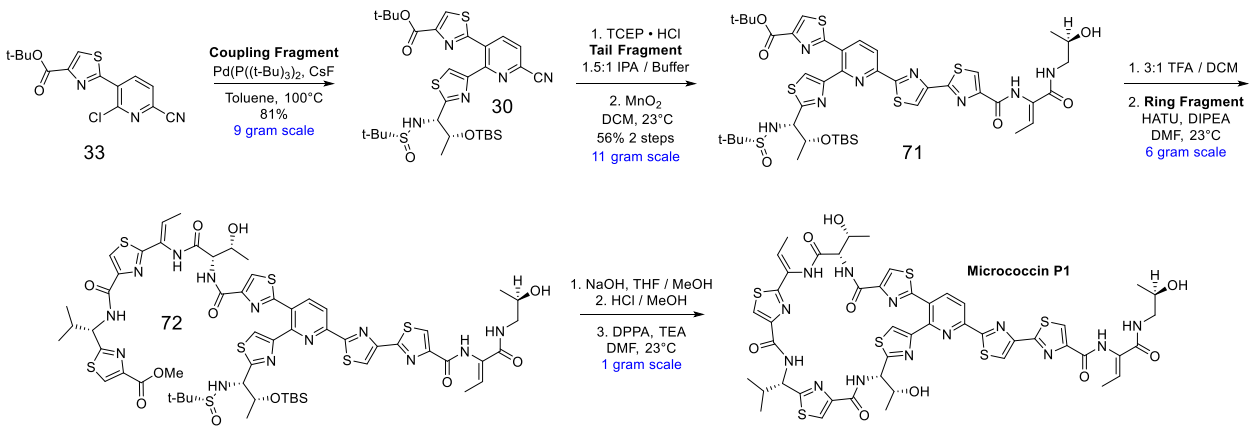
The sole thiazole that isn't made *via* the cysteine-nitrile condensation methodology comes from commercially available 2,4-thiazolidinedione **59**. The material is brominated with deoxygenation with P_2O_5 and tetrabutylammonium bromide in refluxing toluene to give dibromothiazole **60** in 91% yield.[85] Vigorous stirring and thorough mixing of the biphasic mixture are critical for the success of this reaction, and to this end the TBAB should be added to starting material and P_2O_5 in toluene while hot. The dibromothiazole can be purified by sublimation with gentle heating under vacuum with a cold trap equipped. Grignard reagent is generated *via* chemoselective magnesium-halogen exchange at the 2-bromide with "turbo Grignard" *i*-PrMgCl•LiCl[86] at 0°C and promptly added dropwise to the chiral imine **62** in DCM at -78°C over an hour. This reaction gave a mixture of diastereomers separable by chromatography whose absolute stereochemistry was confirmed by NMR after derivatization. Both *R* and *S* Ellman auxiliaries were tried, where the *S* enantiomer gave a ~6:1 diastereomeric ratio of which the major diastereomer is the undesired one (*R*,*R*), whereas the *R* enantiomer gave a ~1.5:1 d.r. where the major diastereomer is the desired **63** (*R*,*S*). We confirmed this result by deprotection of the chiral auxiliary and cyclization with carbonyldiimidazole and TEA to give a 5 membered oxazolidinone. We analyzed these two diastereomers by NMR and observed a strong NOE between the two ring H's on the same face in the undesired (*R*,*R*) configuration whereas only a very weak signal from the H's on opposing faces in the (*R*,*S*) configuration[87] (Scheme 1.9).

The cross coupling was a significant challenge in this synthesis for several reasons. First, we found that any attempt to form an aryl boronate at the 2 position of the pyridine in **57** or other variations of this fragment were unsuccessful, and coupling to this position hashistorically been unusually problematic.[88] We were able to borylate the bromothiazole **64**, but any attempt at Suzuki coupling to the chloropyridine failed. Because of this we chose to explore other coupling conditions and found that previous syntheses by Bach and others



Scheme 1.9. Confirmation of absolute stereochemistry *via* NOE experiment.

showed several successful Stille couplings in pursuit of this thiopeptide family.[55,71] Stannylation of a bromothiazole with palladium tetrakis and hexamethylditin and reportedly gives the stannane in good yield after chromatography. Coupling to a 2-chloropyridine follows in moderate yield according to the authors. With this in hand, stannylation with palladium tetrakistriphenylphosphine and hexamethylditin in toluene gives stannane **32** in good yield after chromatography with 1% TEA to prevent decomposition. The two coupling partners **32** and **33** are assembled to give the completed fragment **30** (Scheme 1.10). Stille coupling with palladium tetrakis gave a fair amount of the coupled product, and after some optimization the reaction was improved significantly. We screened palladium catalysts including tetrakis, $Pd_2(dba)_3$ with various phosphine ligands, $PdCl_2(dppf)$, and $Pd(P(t\text{-}Bu)_3)_2$ and all provided some of the desired product by NMR. Coupling with $Pd(P(t\text{-}Bu)_3)_2$ and CsF in toluene at 100°C consistently gave the best yields and a more facile workup and purification. We observed during our studies that the Stille coupling resulted in greater yield with increasing aryl substitution of the pyridine, with a 2-chloro-3,6-dithiazolyl pyridine from an earlier model study coupling nearly quantitatively. Because of solubility issues with the corresponding intermediate in which the tail is already installed, we were unable to successfully couple here. This led us back to a previous intermediate **33**, where coupling went smoothly and in



Scheme 1.10. Assembly of the fragments and macrocyclization.

81% isolated yield on multi-gram scale.

With all fragments in hand, the assembly and cyclization was straightforward. The thiazole-thiazole linkage at the pyridine 6 position is made by cyclization of the tail fragment **31** and pyridyl nitrile **30** to give an intermediate thiazoline that is oxidized to the thiazole **71** with activated MnO_2 in DCM. Conversion is complete and nearly quantitative for this oxidation and only filtration through a pad of celite or silica is required depending on MnO_2 mesh size. The ring fragment **28** is installed next by chemoselective deprotection of the *t*-Bu ester of 71 with TFA in DCM followed by peptide coupling with HATU and DIPEA in DMF. Both deprotection of the *t*-Bu ester as well as the Ellman auxiliary were tried separately under various conditions, however no set of conditions we tried were selective for one over the other aside from TFA. Even under conditions that would regenerate the t-Bu ester if deprotected, it did not survive the reaction fully intact. Although we initially planned to couple the acid of the valine nitrile portion of the ring fragment first and cyclize at the threonine residue to complete the synthesis, we expected that we could also complete the synthesis in the reverse order. With all fragments assembled, there were two routes we could potentially take. The first is an acidic deprotection of the Ellman auxiliary and TBS group followed by saponification of the methyl ester of the ring fragment, or vice versa, before

macrocyclization to give the natural product. We found that the first deprotection, whether it be under acidic or basic conditions, was successful. However, only the saponification followed by acidic deprotection gave a significant amount of isolated product micrococcin P1 after cyclization. We attempted cyclization in several different ways including traditional peptide coupling reagents EDC & HATU but found these to be unsuccessful in our hands. We eventually found that diphenylphosphoryl azide enabled the cyclization to give the desired natural product micrococcin P1. In a single batch, we were able to produce 200 milligrams of micrococcin where previous synthesis of the thiopeptides yielded no more than 5-10mg. The robust and scalable methods we used, especially the cysteine-nitrile condensation, enabled this 20-fold increase in production. This material has been validated by NMR, mass spectroscopy, and an MIC assay of gram-positive bacterial pathogens.

This synthetic micrococcin P1 was tested against several gram-positive and gram-negative pathogens to validate the structure as well as the previously reported activity. The antibiotic activity matched what was previously reported, with MIC values against several gram-positive strains in the low micromolar range though it had no activity against gram positive strains (Table 1.2). The activity of micrococcin and its synthetic intermediates and analogues were evaluated in several different contexts that will be discussed in detail in chapter 2.

Table 1.2. Minimum inhibitory concentration (MIC) values for Micrococcin P1 against various gram positive and negative pathogens.

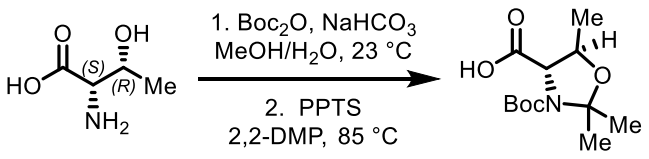
		MIC (μg/mL)
Isolate	Gram Stain	Micrococcin P1
S. aureus	+	0.5
MRSA	+	0.5
VRE	+	1.0
A. baumannii	-	>128
Klebsiella	-	>128

Experimental Section

General Information

All reactions were performed in flame dried round bottom or 20mL scintillation vials fitted with rubber septa unless otherwise indicated. Air and moisture sensitive liquids and solutions were transferred via syringe or cannula without the use of an air-free glove box. Diethyl ether (Et_2O), DCM (CH_2Cl_2), tetrahydrofuran (THF) and toluene were purified using a Pure-Solv MD-5 Solvent Purification System (Innovative Technology). All other reagents were used directly from the supplier without further purification. Analytical thin-layer chromatography (TLC) was carried out using 0.2 mm commercial silica gel plates (silica gel 60, F254, EMD chemical) and visualized using a UV lamp and/or aqueous ceric ammonium molybdate (CAM) or aqueous potassium permanganate (KMnO4) stain. High-resolution mass spectra (HRMS) were recorded on a Karatos MS9 and are reported as m/z (relative intensity). Accurate masses are reported for the molecular ion $[M+Na]^+$, $[M+H]^+$, [M] or $[M-H]^-$. Nuclear magnetic resonance spectra (^{1}H-NMR and ^{13}C-NMR) were recorded with a Varian Gemini [(400 MHz, ^{1}H at 400 MHz, ^{13}C at 100 MHz), (500 MHz, ^{1}H at 500 MHz, ^{13}C at 125 MHz), (600 MHz, ^{1}H at 600 MHz, ^{13}C at 150 MHz)]. For $CDCl_3$ solutions the chemical shifts are reported as parts per million (ppm) referenced to residual proton or carbon of the solvent; $CHCl_3$ δ H (7.26 ppm) and $CDCl_3$ δ D (77.0 ppm). For DMSO-d6 solutions the chemical shifts are reported as parts per million (ppm) referenced to residual proton or carbon of the solvents; DMSO-d6 δ H (2.50 ppm) or DMSO-d6 δ C (39.5 ppm). For CD_3OD solutions the chemical shifts are reported as parts per million (ppm) referenced to residual proton or carbon of the solvents; CHD_2OD δ H (3.31 ppm) or CD_3OD δ C (49.0 ppm). Coupling constants are reported in Hertz (Hz). Data for 1H-NMR spectra are reported as follows: chemical shift (ppm, referenced to proton; s = singlet, d = doublet, t = triplet, q= quartet, dd = doublet of

doublets, td = triplet of doublets, bs = broad singlet, bd = broad doublet, m = multiplet, coupling constant (Hz), and integration). Melting points were measured on a MEL-TEMP apparatus without corrections.

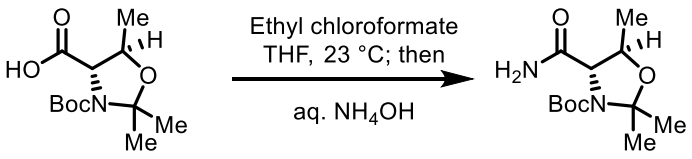


Compound **35:** This compound was prepared from a previously reported method.[89] L-threonine (100 g, 839 mmol, 1 eq.) was dissolved in 1.7:1 THF / 2 M NaOH and cooled in an ice bath. Boc anhydride was added (220 g, 1.01 Mol, 1.2 eq.) in portions over 10 minutes and stirred cold for one hour. The reaction was removed from ice and stirred at 23 °C for 48 hours. THF was removed under vacuum and taken up in 2 M HCl (2 L) and extracted with ethyl acetate (3 x 1 L). Wash the combined organic layers with water (750 mL), with brine (750 mL), dried over sodium sulfate and concentrated to a colorless oil. Crude material was used directly in the next reaction without further purification. 175.4 g of crude product recovered after work up.

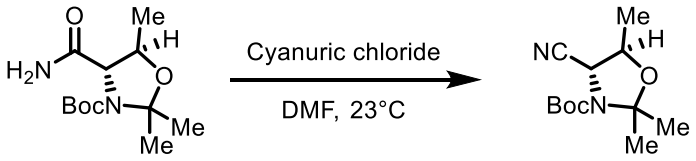
Crude Boc-L-threonine was dissolved in 2,2-dimethoxypropane (1.03 L) and PPTS (recrystallized from acetone) was added (60.3 g, 0.3 eq.) and the reaction was heated to reflux for 14 hours. The reaction was concentrated and taken up in 2 L of ethyl acetate, washed with water and brine (500 mL/ea), dried over sodium sulfate and concentrated. Recrystallized from hexanes to give 113.1 g from first recrystallization and an additional 58 g collected from a 2nd as a white solid (85% over 2 steps).

^{1}H NMR (600 MHz, MeOD) [Rotamers] δ 4.21 – 4.13 (m, 1H), 3.90 – 3.80 (m, 1H), 1.58 (br s, 3H), 1.54 (br s, 3H), 1.45 (br d, 9H), 1.37 (d, J = 6.1 Hz, 3H).

.

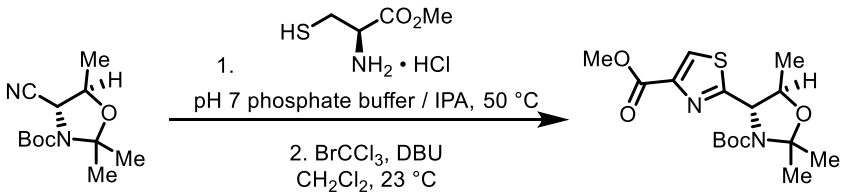


Compound **36:** This compound was prepared from a previously reported method.[89] Starting material (58 g, 224 mmol, 1 eq.) was dissolved in THF (447 mL, 0.5 M) and cooled in an ice bath. Ethyl chloroformate (25.8 mL, 269 mmol, 1.2 eq.) was added dropwise with vigorous stirring over 20 minutes to ensure that stirring was not hindered. After addition the reaction was warmed to 23 °C and stirred for 4 hours until the consumption of starting material was observed by TLC (ninhydrin). The reaction was cooled again and 25% aq. NH_4OH (48.8 mL, 1.4 eq.) was added in a single portion and stirred as the ice bath melts for 14 hours. THF was removed under reduced pressure and the residue was taken up in 1 L ethyl acetate, washed with water (2 x 350 mL), brine (350 mL), dried over sodium sulfate and concentrated to an amber oil. This crude material was used directly in the next reaction without further purification. 48.8 g recovered after work up.



Compound **37:** Crude amide starting material was dissolved in DMF (189 mL, 1.0 M) and immersed in a water bath. Solid cyanuric chloride (17.4 g, 112 mmol, 0.5 eq.) was added in several portions over 15 minutes and the reaction was stirred at 23 °C for 2 hours until no amide remains (TLC, ninhydrin) and a white precipitate has appeared. After completion the reaction was slowly poured into ice cold water (1 L) with vigorous stirring to precipitate the nitrile. The solids were collected *via* Buchner funnel, washed with additional water (3 x 200 mL) and air dried to give 37.7 g of analytically pure nitrile product as a white solid (157 mmol, 70% over 2 steps).

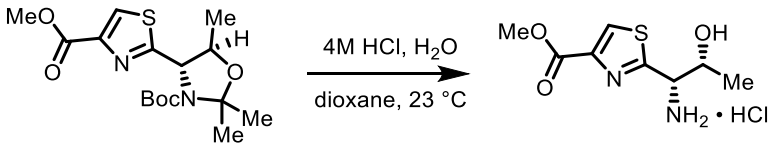
^{1}H NMR (600 MHz, CDCl3): δ 4.40 (p, *J* = 6.2 Hz, 1H), 3.99 (m, 1H), 1.59 (br s, 3H), 1.52 (br s, 4H), 1.48 (s, 9H), 1.40 (d, *J* = 6.1 Hz, 3H); **^{13}C NMR** (150 MHz, CDCl3): δ 150.4, 117.1, 95.7, 82.0, 74.1, 52.9, 28.1, 26.4, 24.4, 18.2; **IR** (film, cm^{-1}): 2358, 1715; **HRMS** (ESI): calc. for $C_{12}H_{20}N_2O_3$ $[M+Na]^+$: 263.1366, obs. 263.1366.



Compound **38:** Starting material (37.7 g, 157 mmol, 1 eq.) was dissolved in a 1.5:1 mixture of IPA / pH 7 phosphate buffer (785 mL, 0.2 M buffer, 0.1 M reaction) and cysteine methyl ester hydrochloride was added (40.4 g, 236 mmol, 1.5 eq.). The reaction was stirred & heated to 50 °C for 14 hours. After completion, IPA was removed under reduced pressure and the residue was partitioned between water (1 L) and ethyl acetate (500 mL) and the aqueous layer was extracted with ethyl acetate (3 x 250 mL). The combined organic layers were dried over sodium sulfate and concentrated. The crude product was used directly in the next reaction without further purification. 50.9 g was collected crude after work up as a colorless oil that solidifies upon standing.

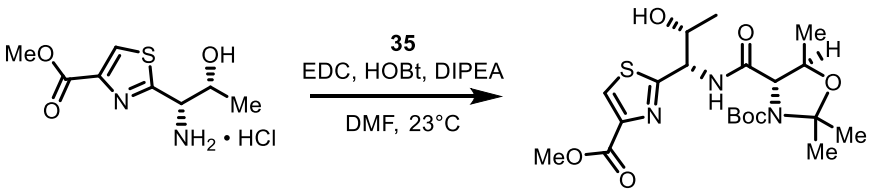
Crude thiazoline was dissolved in DCM (475 mL, 0.3 M) and cooled in an ice bath. Bromotrichloromethane was added (21 mL, 188 mmol, 1.2 eq.) followed by DBU (25.4 mL, 188 mmol, 1.2 eq.) dropwise over 10 minutes. The reaction was allowed to warm to 23 °C as the ice bath melts. The reaction was poured into 1 M HCl (500 mL) and extracted with additional DCM (3 x 200 mL). The combined organic layers were washed with water and brine (250 mL / ea), dried over sodium sulfate and concentrated. Crude thiazole was used directly in the next reaction without further purification. 47.8 g was recovered after work up as an off white solid (134 mmol, 85% over 2 steps).

^{1}H NMR (600 MHz, CDCl3): δ 8.17 (br s, 1H), 4.78 (m, 1H), 4.16 (m, 1H), 3.94 (s, 3H), 1.69 (br s, 6H), 1.42 (br s, 9H), 1.18 (br s, 3H)



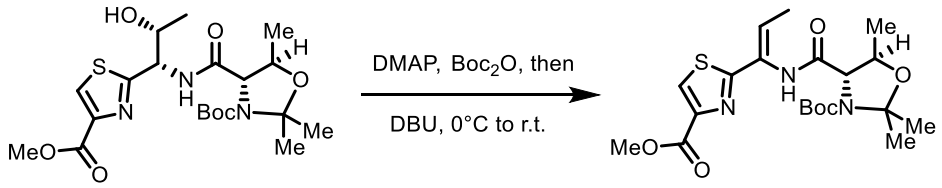
Compound **39:** Dioxane (1 mL/g) was added to starting material (30 g, 84 mmol, 1 eq.) to solubilize it. 4 M HCl in dioxane was added (168 mL, 5 eq.) followed by dropwise addition of DI water (17 mL, 10% v/v). The reaction was stirred at 23 °C for 2 hours. An oil or solid may appear to precipitate from the reaction that was the desired HCl salt. The reaction was concentrated from benzene (3 x 100 mL) and the crude solid HCl salt was used directly in the next reaction without further purification (>99% yield).

^{1}H NMR (600 MHz, MeOD): δ 8.54 (s, 1H), 4.88 (s, 1H), 4.76 (d, J = 6.6 Hz, 1H), 4.32 – 4.26 (m, 1H), 3.92 (s, 3H), 1.23 (d, J = 6.4 Hz, 3H)



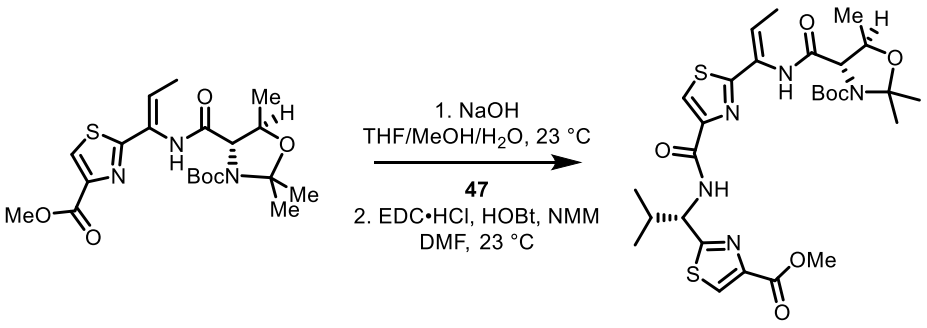
Compound **40:** Starting material (35.3 g, 140 mmol, 1 eq.) was dissolved in DMF (280 mL, 0.5 M) along with protected threonine (39.9 g, 1.1 eq.), EDC hydrochloride (32.2 g, 1.2 eq.), and HOBt (22.7 g, 1.2 eq., 80% w/w). DIPEA was added dropwise over 10 minutes (73 mL, 3 eq.) and the reaction was stirred at 23 °C for 14 hours. The reaction was diluted in 2 L water and extracted with ethyl acetate (3 x 500 mL). The combined organic layers were washed with 3 M LiCl (3 x 300 mL), dried over sodium sulfate, and concentrated. Crude material was purified by column chromatography with 60 --> 80% EA/Hex on a short length column. 54.48 g collected pure after chromatography (119 mmol, 85% yield).

^{1}H NMR (600 MHz, CDCl3): δ 7.98 (s, 1H), 5.15 (bs, 1H), 4.44 (m, 1H), 4.13 (bs, 1H), 3.82 (d, J = 7.4 Hz, 1H), 3.76 (s, 3H), 1.48 (s, 3H), 1.46 (s, 3H), 1.26 (bs, 9H), 1.15 (d, J = 6.5, 3H); **^{13}C NMR** (150 MHz, CDCl3) δ 171.1, 170.1, 161.2, 151.9, 145.9, 127.6, 94.5, 80.7, 73.7, 68.7, 67.1, 55.8, 52.0, 28.0, 27.4, 25.1, 19.3, 18.7; **IR** (film, cm^{-1}): 3125, 1691; **HRMS** (ESI): calc. for $C_{20}H_{31}N_3O_7S$ $[M+Na]^+$: 480.1775, obs. 480.1778.



Compound **41:** Starting material (28.1 g, 61.4 Mmol, 1 eq.) was dissolved in acetonitrile (205 mL, 0.3 M). Boc anhydride (1.78 g, 1.2 eq.) was added in a single portion followed by DMAP (1.5 g, 0.2 eq.) and the reaction was stirred until all starting material was consumed. After full conversion of starting material by TLC, DBU (45.8 mL, 5 eq.) was added dropwise at 23 °C and the reaction was stirred for 14 hours or until the carbonate intermediate was consumed. The reaction was diluted in ethyl acetate (500 mL) and washed with 1 M HCl, water, brine, (200 mL / ea) dried over sodium sulfate and concentrated. Crude material was purified by column chromatography with 15 --> 35% EA/Hex on a medium length column. 18.4 g recovered pure after chromatography as a colorless oil (41.8 mmol, 68.1% yield).

^{1}H NMR (600 MHz, CDCl3): δ 7.99 (s, 1H), 7.96 (bs, 1H), 6.54 (bs, 1H), 4.32 (bs, 1H), 3.97 (d, J = 7.7 Hz, 1H), 3.85 (s, 3H), 1.82 (d, J = 6.6 Hz, 3H), 1.61 (bs, 6H), 1.44 (d, J = 6.1 Hz, 3H), 1.40 (s, 9H); **^{13}C NMR** (150 MHz, CDCl3): δ 168.4, 167.3, 161.7, 152.3, 146.7, 127.9, 127.1, 95.1, 81.1, 74.2, 67.8, 52.3, 28.3, 27.7, 25.5, 19.0, 14.4; **IR** (film, cm^{-1}): 3125, 2982, 2250, 1693; **HRMS** (ESI)$^+$: calc. for $C_{20}H_{29}N_3O_6S$ $[M+Na]^+$: 462.1669, obs. 462.1665.

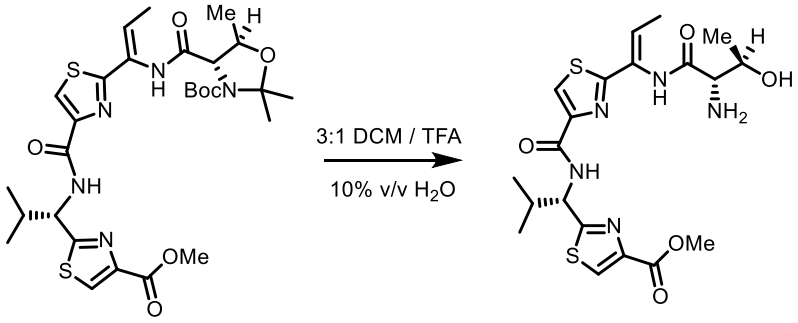


Compound **42:** Starting material (18.38 g, 41.8 mmol, 1 eq.) was dissolved in a mixture of THF and MeOH (3:1, 209 mL, 0.2 M) and 10% aq. NaOH was added (50.2 g, 3 eq.). The reaction was stirred for 1 hour and poured into 1 L of 2 M HCl to acidify, extracted with DCM (3 x 350 mL), the combined organic layers were dried over sodium sulfate and concentrated. Crude acid was used directly in the next reaction without further purification (>99% yield).

Crude acid (5.15 g, 12.11 mmol, 1.1 eq.) was dissolved in DMF (44 mL, 0.25 M) and amine hydrochloride **47** was added (2.36 g, 1 eq.) followed by HATU (5.02 g, 1.2 eq.). DIPEA was added dropwise (7.7 mL, 4 eq.) and the reaction was stirred for 14 hours. The reaction was poured into 500 mL brine and extracted with ethyl acetate (3 x 200 mL). The combined organic layers were washed with 3 M LiCl (3 x 200 mL), dried over sodium sulfate and concentrated. The crude material was purified by column chromatography with 40 --> 60% EA/Hex on a medium length column. 3.16 g was recovered pure after chromatography as a white solid (5.08 mmol, 64% yield).

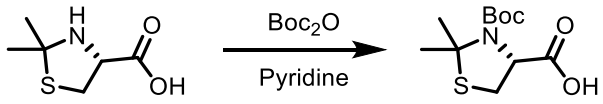
^{1}H NMR (500 MHz, CDCl3)[Rotamers] δ 8.10 (s, 1H), 8.01 (s, 1H), 7.92 (d, J = 9.1 Hz, 1H), 7.75 (s, 1H), 6.63 (br s, 1H), 5.32 (dd, J = 8.9, 7.3 Hz, 1H), 4.40 (br s, 1H), 4.05 (d, J = 7.2 Hz, 1H), 3.94 (s, 3H), 2.67 – 2.56 (m, J = 6.7 Hz, 1H), 1.88 (d, J = 7.2 Hz, 3H), 1.79 (br s, 3H), 1.66 (br s, 3H), 1.64 – 1.38 (m, 12H), 1.03 (d, J = 6.7 Hz, 3H), 0.99 (d, J = 6.7 Hz, 3H). **^{13}C NMR**

(126 MHz, *several C signals were not observed due to peak broadening, CDCl3) δ 168.53, 161.87, 160.82, 149.43, 147.08, 127.77, 127.43, 123.75, 81.39, 67.69, 60.48, 56.71, 52.51, 33.08, 28.39, 25.91, 19.77, 19.47, 18.19, 14.27. **IR** (film, cm^{-1}): 2980, 2360, 1692, 1367, 1213, 726; **HRMS** (ESI): calc. for $C_{28}H_{39}N_5O_7S_2Na$ $[M+Na]^+$: 644.2183, obs. 644.2176.



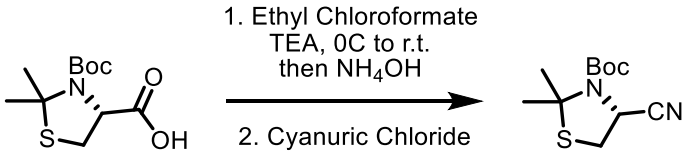
Compound **28:** Starting material (7.34 g, 11.8 mmol, 1 eq.) was dissolved in DCM and TFA was added in a single portion (29 mL, 1:3 v/v, 0.1 M) followed by DI water (3 mL, 10% v/v). The reaction was stirred for 4 hours, then concentrated from benzene (3 x 125 mL). The crude material was taken up in saturated sodium bicarbonate solution (100 mL) and extracted with ethyl acetate (3 x 50 mL), dried and concentrated. The crude product was used directly in the next reaction without further purification.

^{1}H NMR (600 MHz, MeOD) δ 8.35 (s, 1H), 8.15 (s, 1H), 6.76 (q, J = 7.1 Hz, 1H), 5.24 (d, J = 7.9 Hz, 1H), 4.42 – 4.35 (m, 1H), 3.99 (d, J = 5.4 Hz, 1H), 3.92 (s, 3H), 2.53 – 2.44 (m, 1H), 1.88 (d, J = 7.1 Hz, 3H), 1.46 (d, J = 6.4 Hz, 3H), 1.07 (d, J = 6.7 Hz, 3H), 0.96 (d, J = 6.7 Hz, 3H).



Compound **50**: This procedure is adapted from a previous method by Kazuo et al. with minor modifications.[90] Starting material **49** (42.61g, 264mmol, 1 eq.) is dissolved in pyridine (203mL, 1.3M) and cooled to 0°C in an ice bath. Boc anhydride (63.4g, 1.1 eq.) is added and the reaction is stirred overnight to completion. The reaction is diluted with EA (1L) and washed with 1M HCl (3 x 250mL), brine (2 x 250mL), dried over sodium sulfate and concentrated to a viscous oil that crystallizes upon standing. After the crude material crystallizes, hexanes are added, and the solids are pulverized and filtered with a Buchner funnel to give pure desired product as a white solid (32.76g, 125mmol, 47.4%).

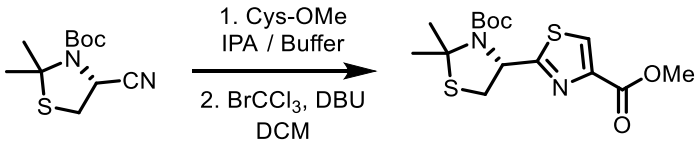
^{1}H NMR (600 MHz, MeOD) [Rotamers] δ 4.90 – 4.76 (m, 1H), 3.39 – 3.32 (m, 1H), 3.12 (dd, *J* = 12.1, 3.0 Hz, 1H), 1.84 – 1.75 (m, 6H), 1.46 (br d, 9H).



Compound **51:** This procedure was adapted from a previous method by Kazuo et al. with minor modifications.[90] Starting material (32.76 g, 125 mmol, 1 eq.) was dissolved in THF (251 mL, 0.5 M) and cooled in an ice bath. Ethyl chloroformate (14.5 mL, 163 mmol, 1.3 eq.) and TEA (21 mL, 163 mmol, 1.3 eq.) were added slowly to ensure continued stirring despite formation of a precipitate. The reaction was brought to 23 °C and stirred vigorously until starting material was consumed (TLC, ninhydrin). After full conversion of starting material, the reaction was cooled again and NH_4OH (25% w/w, 18.58 mL, 1.4 eq.) was added and the reaction was stirred, warming to 23 °C in the ice bath as it melts. After full conversion by TLC the reaction was concentrated to remove THF, diluted in ethyl acetate (500 mL), washed with water (2 x 150 mL) and brine (150 mL), dried over sodium sulfate, and concentrated to a white solid. The crude material was used directly in the next reaction with no further purification.

The crude amide was dissolved in DMF (1.0 M) and cyanuric chloride (0.5 eq.) was added. The reaction was stirred at 23 °C for one hour. The reaction was slowly added to vigorously stirring, ice cold DI water. The solids were collected by filtration and washed with additional cold water and dried to give 16.6 g of analytically pure nitrile as a white solid (68.5 mmol, 55% yield).

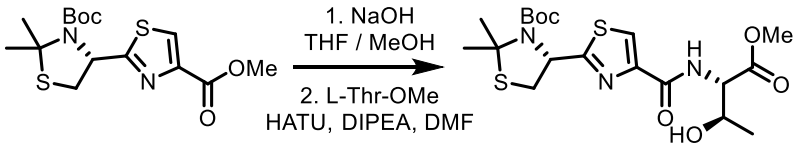
^{1}H NMR (600 MHz, $CDCl_3$): δ 5.13 (bs, 1H), 3.28 (dd, J = 5.9, 12.3 Hz, 1H), 3.05 (d, J = 12.3 Hz, 1H), 1.82 (bs, 3H), 1.73 (bs, 3H), 1.48 (s, 9H); **^{13}C NMR** (150 MHz, $CDCl_3$): δ 150.7, 118.1, 82.3, 54.3, 31.2, 30.0, 29.0, 28.2; **IR** (film, cm^{-1}): 3132, 2980, 2936, 2360, 1705; **HRMS** (ESI): calc. for $C_{11}H_{18}N_2O_2S$ $[M+Na]^+$: 265.0981, obs. 265.0983.



Compound **52:** Starting material (30.2 g, 125 Mmol, 1 eq.) was dissolved in a mixture of 1.5:1 IPA / pH 7 phosphate buffer (623 mL, 0.2 M) and Cys-OMe HCl (32.1 g, 188 mmol, 1.5 eq.) was added. The reaction was heated to 50 °C and stirred for 24 hours. The reaction was concentrated to remove IPA, diluted in water (500 mL), extracted with EA (3 x 200 mL), dried over sodium sulfate, and concentrated to give 40.6 g of desired product (113 mmol, 90% yield). Crude material was used directly in the next reaction without further purification.

Crude thiazoline was dissolved in DCM (751 mL, 0.15 M) and cooled in an ice bath. Bromotrichloromethane was added (13.33 mL, 150 mmol, 1.2 eq.) followed by dropwise addition of DBU (20.2 mL, 150 mL, 1.2 eq.). The reaction was stirred as the ice bath melts for 14 hours. After completion the reaction poured into 1 M HCl (1 L) and the aq. layer was extracted an additional with DCM (2 x 200 mL) and the combined organic layers were washed with water & brine (250 mL / ea), dried over sodium sulfate and concentrated. Crude thiazole was used directly in the next reaction without further purification (17.9 g, 50.0 mmol, 44.5% over 2 steps).

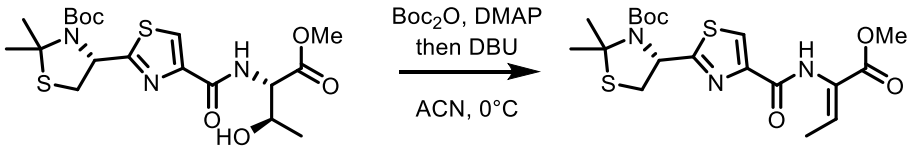
^{1}H NMR (600 MHz, $CDCl_3$): δ 8.10 (s, 1H), 5.66 (m, 1H), 3.92 (s, 3H), 3.51 (dd, J = 6.5, 12.4 Hz, 1H), 3.15 (m, 1H), 1.94 (m, 3H), 1.78 (s, 3H), 1.37 (m, 9H)



Compound **53:** Starting material (25.2 g, 70.4 mmol, 1 eq.) was dissolved in a mixture of 3:1 THF / Methanol (290 mL, 0.25 M) and 10% aq. NaOH (8.45 g, 352 mmol, 5 eq.) and stirred at 23 °C for 1 hour. After full conversion of starting material, the reaction was poured into 1 M HCl (750 mL) and extracted with DCM (3 x 200 mL). The combined organic layers were dried over sodium sulfate and concentrated. Crude product was used directly in the next reaction without further purification.

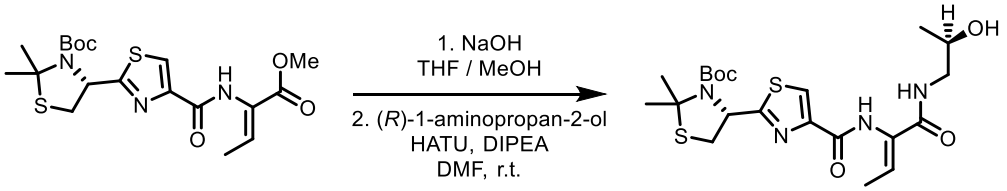
Crude carboxylic acid and L-threonine methyl ester hydrochloride (13.1 g, 77.4 mmol, 1.1 eq.) were dissolved in DMF (352 mL, 0.2 M) and HATU (29.4 g, 77.4 mmol, 1.1 eq.) was added followed by dropwise addition of DIPEA (36.8 mL, 211 mmol, 3 eq.) at 23 °C and the reaction was stirred for 14 hours. The reaction was diluted with water (1 L) and extracted with EA (3 x 200 mL). The combined organic layers were washed with 3 M LiCl (3 x 200 mL), dried over sodium sulfate and concentrated. Crude material was purified by column chromatography with 30 --> 55% EA/Hex on a medium length column. 26.0 g was collected pure after chromatography (56.6 mmol, 80% yield).

^{1}H NMR (600 MHz, $CDCl_3$)[Rotamers] δ 8.06 (br s, 1H), 7.94 (br s, 1H), 5.77 – 5.54 (m, 1H), 4.76 (dd, *J* = 9.1, 2.5 Hz, 1H), 4.49 – 4.39 (m, 1H), 3.78 (d, 3H), 3.53 (dd, *J* = 12.3, 6.5 Hz, 1H), 3.14 (br s, 1H), 2.53 (br s, 1H), 1.93 (br d, 3H), 1.82 (br s, 3H), 1.58-1.27 (m, 12H). **^{13}C NMR** (126 MHz, ***** several C signals were not observed due to peak broadening, MeOD) δ 172.37, 163.51, 125.44, 68.46, 61.56, 59.09, 52.99, 28.52, 20.87, 20.51, 20.49, 15.92, 15.51, 14.46. **IR** (film, cm^{-1}): 3399, 2975, 2360, 2341, 1660, 1345; **HRMS** (ESI): calc. for $C_{19}H_{30}N_3O_6S_2$ $[M+H]^+$: 460.1571, obs. 460.1566



Compound **54:** Starting material (26.01 g, 56.6 mmol, 1 eq.) was dissolved in acetonitrile (283 mL, 0.2 M) and immersed in a 23 °C water bath. Boc anhydride (14.82 g, 1.2 eq.) was added in a single portion followed by DMAP (1.73 g, 14.2 mmol, 0.25 eq.) and the reaction was brought to 23 °C immediately and stirred until all starting material was consumed. After full conversion of starting material, DBU (42.2 mL, 283 mmol, 5 eq.) was added dropwise at 23 °C and the reaction was stirred for 14 hours or until carbonate intermediate was consumed. The reaction was concentrated, and the residue taken up in 1 L ethyl acetate. The solution was washed with 1 M HCl, water, brine (200 mL / ea), dried over sodium sulfate and concentrated. The crude material was purified by column chromatography with 15 --> 35% EA / Hex on a medium length column to give 18.9 g of pure olefin product as a white solid (42.8 mmol, 76% yield).

^{1}H NMR (599 MHz, $CDCl_3$)[*Rotamers*] δ 8.61 (br s, 1H), 8.08 (br s, 1H), 6.89 (q, J = 7.2 Hz, 1H), 5.68 (br d, 1H), 3.78 (s, 3H), 3.54 (dd, J = 12.3, 6.4 Hz, 1H), 3.17 (br s, 1H), 1.94 (br d, 3H), 1.86 (d, J = 7.2 Hz, 3H), 1.83 (br s, 3H), 1.41 (br d, 9H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 165.03, 158.96, 140.82, 134.40, 125.79, 124.32, 99.36, 60.53, 57.90, 57.14, 52.53, 34.13, 33.65, 29.03, 28.50, 28.31, 15.15. **IR** (film, cm^{-1}): 3371, 2976, 2358, 1686, 1343; **HRMS** (ESI): calc. for $C_{19}H_{28}N_3O_5S_2$ $[M+H]^+$: 442.1465, obs. 442.1462.

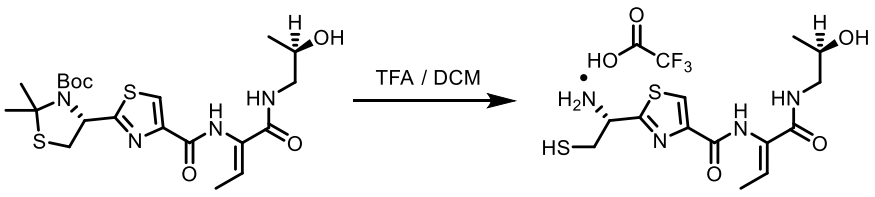


Compound **55:** Starting material (18.28 g, 42.8 mmol, 1 eq.) was dissolved in a mixture of 3:1 THF / Methanol (0.2 M) and 10% aq. NaOH (5 eq.) and stirred at 23 °C for 1 hour. After full conversion of starting material by TLC, the reaction was poured into 1 M HCl (100 mL) and extracted with ethyl acetate (3 x 30 mL). The combined organic layers were dried over sodium sulfate and concentrated. Crude product was used directly in the next reaction without further purification.

Crude carboxylic acid, (R)-1-aminopropan-2-ol (4.04 mL, 1.1 eq.), and HATU (17.9 g, 1.1eq.), were dissolved in DMF (214 mL, 0.2 M). DIPEA (22.34 mL, 3eq.) was added dropwise with stirring over 10 minutes at 23 °C and the reaction was stirred for 12 hours. The reaction was then diluted with water (250 mL) and extracted with ethyl acetate (3 x 200 mL). The combined organic layers were washed with 3 M LiCl (3 x 200 mL), dried over sodium sulfate and concentrated to give a colorless oil. The crude material was purified by column chromatography with 70 --> 100% EA / Hex to give 16.2 g of desired product as a yellow foam (33.4 mmol, 78% over 2 steps).

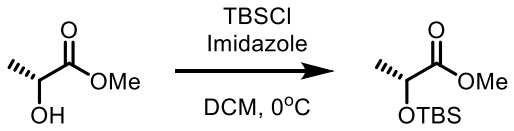
^{1}H NMR (600 MHz, MeOD; peak broadening due to rotamers) d 8.23 (br s, 1H), 6.68 (br s, 1H), 5.74 (br d, 1H), 3.92 - 3.84 (m, 1H), 3.67 (s, 1H), 3.29 - 3.15 (m, 4H), 1.95 (br d, 3H), 1.86 (s, 3H), 1.79 (d, J = 7.0 Hz, 3H), 1.6-1.35 (9H), 1.15 (d, J = 6.3 Hz, 3H). **^{13}C NMR** (126 MHz, MeOD; rotamers) δ 176.68, 175.93, 167.30, 162.37, 154.53, 153.33, 149.81, 132.13, 131.64, 131.15, 131.04, 125.94, 83.10, 82.28, 73.19, 72.04, 67.54, 67.31, 66.87, 48.05, 34.67, 34.10,

30.30, 29.30, 28.95, 28.63, 28.46, 28.33, 20.84, 13.78. **IR** (film, cm^{-1}): 3375, 2975, 2359, 1660, 1342, 1162; **HRMS** (ESI): calc. for $C_{21}H_{33}N_4O_5S_2$ $[M+H]^+$: 485.1887, obs. 485.1882



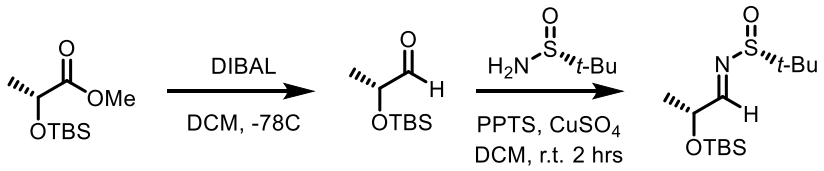
Compound **31:** Starting material (16.2 g, 33.4 mmol, 1 eq.) was dissolved in DCM and TFA was added (3:1 v/v, 0.2 M). The reaction was stirred at 23 °C for 2 hours and concentrated under reduced pressure. The residue was taken up in 1:1 EtOH / H_2O and concentrated repeatedly (3 x 125 mL) at 60 °C to facilitate removal of the acetonide. This was followed by azeotroping (2 x 125 mL) with 4:1 benzene / MeOH to give the amino thiol TFA salt as a yellow solid that was used directly in the next reaction without further purification. This material is **very** hygroscopic (>99% yield).

^{1}H NMR (599 MHz, MeOD) δ 8.43 (s, 1H), 6.59 (q, J = 7.0 Hz, 1H), 4.98 (t, J = 6.4 Hz, 1H), 3.93 – 3.86 (m, 1H), 3.31 (dd, 1H), 3.28 – 3.18 (m, 3H), 1.80 (d, J = 7.0 Hz, 3H), 1.16 (d, J = 6.4 Hz, 4H).



Compound **61**: This compound is prepared from a previously reported method with minor modifications.[91] (R)-Lactate methyl ester (50 g, 480 mmol, 1 eq.) was dissolved in DCM (480 mL, 1.0 M) and imidazole was added (45.8 g, 672 mmol, 1.4 eq.) and the mixture was cooled in an ice bath. TBSCl (80 g, 528 mmol, 1.1eq.) was added in a single portion and the reaction was stirred cold for 10 minutes and then brought to 23 °C to stir for 1 hour. The reaction was terminated by diluting with additional DCM (1 L) and washing with water (500 mL) and brine (2 x 500 mL). The organic layer was then dried over sodium sulfate and concentrated to give the TBS protected product as a colorless oil (116 g, >99% yield). Crude material was used directly in the next reaction without further purification.

^{1}H NMR (600 MHz, $CDCl_3$) δ 4.33 (q, J = 6.7 Hz, 1H), 3.72 (s, 3H), 1.40 (d, J = 6.8 Hz, 3H), 0.90 (s, 9H), 0.10 (s, 3H), 0.07 (s, 3H).



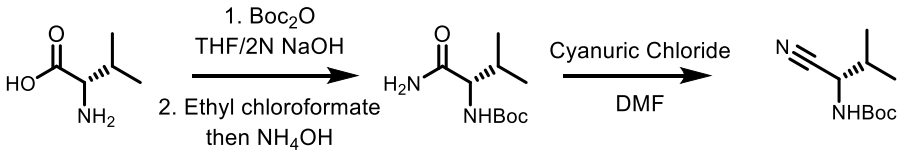
Compound **61** and **62**: [4] Methyl ester (25 g, 114 mmol, 1 eq.) was dissolved in dry DCM (450 mL, 0.25 M) and cooled to -78 °C. DIBAL was added (100 mL, 1.2 M in toluene, 1.05 eq.) dropwise *via* a flame-dried addition funnel over approximately 1 hour. After addition the reaction was stirred for 6 hours at -78 °C, The reaction was quenched with a saturated solution of potassium sodium tartrate (250 mL) and stirred for 30 minutes at 23 °C. The mixture was diluted with 1 L water and extracted with DCM (3 x 250 mL). The combined organic layers were washed with 250 mL brine, dried over sodium sulfate and concentrated to give the crude aldehyde as a colorless oil. Crude material was used in the next reaction without further purification.

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.63 (d, J = 1.2 Hz, 1H), 4.10 (q, J = 6.8, 1.2 Hz, 1H), 1.29 (d, J = 6.8 Hz, 3H), 0.93 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H).

Aldehyde **61** (18.75 g, 100 mmol, 1 eq.) and (R)-tert-butanesulfinamide (13.0 g, 110 mmol, 1.1 eq.) were dissolved in DCM (400 mL, 0.25 M) and anhydrous $CuSO_4$ was added (31.8 g, 200 mmol, 2.0 eq.) followed by PPTS (6.28 g, 25 mmol, 0.25 eq.) and the reaction was stirred at 23 °C for 2 hours. The reaction was filtered through celite, washing with additional DCM, and concentrated. The crude material was then purified by column chromatography with 5 -> 15% EA / Hex on a medium length column to give 19.0 g of pure imine product (65.3 mmol, 57% over 3 steps) as a colorless oil that solidifies upon standing.

^{1}H NMR (599 MHz, $CDCl_3$) δ 7.96 (d, J = 4.0 Hz, 1H), 4.57 (qd, J = 6.6, 4.0 Hz, 1H), 1.36 (d, J = 6.6 Hz, 3H), 1.20 (s, 9H), 0.90 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$)

δ 171.61, 70.62, 56.85, 25.95, 25.78, 22.44, 21.58, -4.61, -4.77. **IR** (film, cm^{-1}): 2929, 2359, 1088, 832 **HRMS** (ESI): calc. for $C_{13}H_{30}NO_2SSi$ $[M+H]^+$: 292.1761, obs. 292.1760



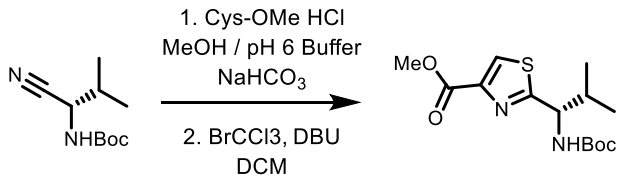
Compounds **44** and **45:** L-valine (25 g, 213 mmol, 1 eq.) was dissolved in a mixture of THF and 2N NaOH (2:1, 0.675 M) and cooled in an ice bath. Boc anhydride (55.9 g, 256 mmol, 1.2 eq.) was added in 3 portions over 15 minutes and the reaction was stirred at 0 °C for 0.5 hours, then brought to 23 °C to stir for 24 hours. The reaction was concentrated to remove THF, taken up in ethyl acetate (1.5 L) and acidified with 2 M HCl to pH~2. The organic layer was separated and washed with water and brine (500 mL /ea), dried over sodium sulfate and concentrated to a colorless, viscous oil. This crude material was used directly in the next reaction without further purification.

Crude Boc-Valine was dissolved in THF (426 mL, 0.5 M) and cooled in an ice bath. Ethyl chloroformate (24.6 mL, 256 mmol, 1.2 eq.) was added followed by triethylamine (35.6 mL, 256 mmol, 1.2 eq.) dropwise over 15 minutes. The reaction was then brought to 23 °C to stir for several hours until conversion to mixed anhydride intermediate was complete by TLC (ninhydrin). The reaction was cooled once again and 25% w/w ammonium hydroxide was added (46.4 mL, 289 mmol, 1.4 eq.). The reaction was allowed to warm to 23 °C as the ice bath melts for 14 hours. The reaction was concentrated to remove THF, then taken up in ethyl acetate (1.5 L) and washed with 1 M HCl, water, brine (500 mL /ea), dried over sodium sulfate and concentrated to a white solid. The crude material was used directly in the next reaction without further purification.

Crude amide was dissolved in DMF (213 mL, 1 M) and cyanuric chloride (19.64 g, 107 mmol, 0.5 eq.) was added and the reaction was stirred at 23 °C for 1.5 hours with appearance of a white precipitate. After TLC showed complete conversion, the reaction was slowly poured

into ice cold, vigorously stirring DI water (1.5 L) and stirred for 15 minutes. The precipitate was filtered and washed with cold water (3 x 200 mL) and the solids were thoroughly dried to give 35.3 g of the pure nitrile (178 mmol, 84% over 3 steps) as a white solid.

^{1}H NMR (600 MHz, CDCl3) δ 4.87 (br s, 1H), 4.46 (br s, 1H), 2.02 (dq, J = 13.4, 6.7 Hz, 1H), 1.46 (s, 9H), 1.08 (d, 3H), 1.06 (d, 3H). **^{13}C NMR** (126 MHz,$CDCl_3$) δ 154.60, 118.14, 81.16, 48.54, 31.90, 28.29, 18.61, 18.01. **IR** (film, cm^{-1}): 3339, 2975, 1698, 1157, 729 **HRMS** (ESI): calc. for $C_{10}H_{18}N_2O_2Na$ $[M+Na]^+$: 221.1260, obs. 221.1263

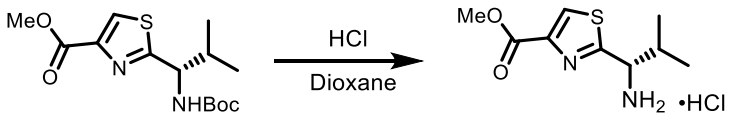


Compound **46:** Nitrile **45** (6.13 g, 30.9 mmol, 1 eq.) was dissolved in a 1.5:1 mixture of MeOH and pH 6 phosphate buffer (310mL, 0.2 M buffer, 0.1 M rxn). Sodium bicarbonate was added (4.67 g, 55.6 mmol, 1.8eq.) followed by cysteine methyl ester hydrochloride (10.61 g, 61.8 mmol, 2 eq.) and the reaction was warmed to 60 °C for 6 hours and promptly concentrated to remove methanol. The residue was diluted with water (500 mL) and extracted with ethyl acetate (3 x 200 mL). The combined organic layers were washed with water and brine (250 mL / ea), dried over sodium sulfate and concentrated. The crude mixture was used directly in the next reaction without further purification.

Crude thiazoline was dissolved in DCM (309 mL, 0.1 M) and cooled in an ice bath. Bromotrichloromethane was added (3.65 mL, 1.2 eq.) followed by DBU (5.53 mL, 1.2 eq.) dropwise over 10 minutes. The reaction was stirred for one hour cold and the reaction was poured into 1 M HCl (500 mL) and extracted with additional DCM (3 x 200 mL). The combined organic layers were washed with water and brine (250 mL / ea), dried over sodium sulfate and concentrated. The crude material was purified by column chromatography with 15 --> 35% EA/Hex on a medium length column to give 3.59 g of the thiazole product as a white solid (11.42 mmol, 37% over 2 steps)

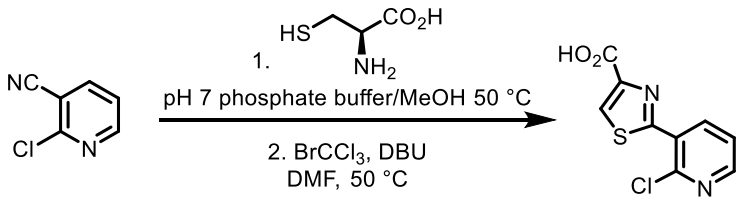
^{1}H NMR (600 MHz, $CDCl_3$) δ 8.09 (s, 1H), 5.27 (d, J = 8.4 Hz, 1H), 4.90 (dd, J = 8.3, 5.7 Hz, 1H), 3.94 (s, 3H), 2.50 – 2.39 (m, 1H), 1.44 (s, 9H), 0.98 (d, J = 6.7 Hz, 3H), 0.89 (d, J = 6.8 Hz, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 173.65, 161.96, 155.50, 147.10, 127.18, 80.22, 52.53,

33.31, 28.48, 28.28, 19.55, 17.30. **IR** (film, cm^{-1}): 3347, 2965, 2359, 1702, 1161 **HRMS** (ESI): calc. for $C_{14}H_{22}N_2O_4SNa$ $[M+Na]^+$: 337.1192, obs. 337.1189.



Compound **47:** Starting material (3.59 g, 11.4 mmol, 1 eq.) was dissolved in a solution of 4N HCl in dioxane (14.3 mL, 57.0 mmol, 5 eq.) and the reaction was stirred at 23 °C. The reaction was concentrated from benzene (2 x 50 mL). Ether was added to the residue, and sonication was used to precipitate the product as a fine white solid that was collected by filtration and washed with additional ether. Crude salt was used directly in the next reaction without further purification (>99% yield).

^{1}H NMR (599 MHz, MeOD) δ 8.51 (s, 1H), 4.64 (d, J = 6.7 Hz, 1H), 3.93 (s, 3H), 2.41 – 2.33 (m, 1H), 1.11 (d, J = 6.9 Hz, 3H), 0.99 (d, J = 6.8 Hz, 3H).

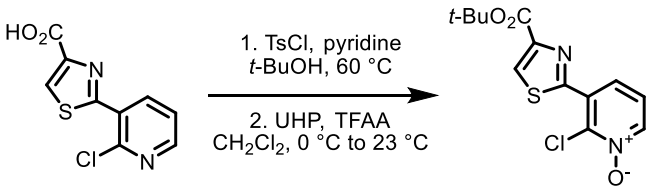


Compound **57:** Starting material (50 g, 361 mmol, 1 eq.) was dissolved in 1.5:1 IPA / pH 7 phosphate buffer (0.5 M) and L-cysteine (65.6 g, 1.5 eq.) was added. The reaction was heated to 50 °C and stirred for 14 hours. The reaction was terminated by removing IPA under vacuum and diluted with 2 M HCl until the solution was acidic (pH>2) and the mixture was extracted with ethyl acetate (3 x 400 mL). The combined organic layers were dried over sodium sulfate and concentrated to give the thiazoline intermediate as a yellow solid (76.9 g, 88% yield).

^{1}H NMR (600 MHz, MeOD) δ 8.49 (dd, *J*= 4.9, 1.8 Hz, 1H), 8.10 (dd, *J*= 7.7, 1.8 Hz, 1H), 7.49 (dd, *J*= 7.7, 4.9 Hz, 1H), 5.36 (t, *J*= 9.2 Hz, 1H), 3.87 – 3.80 (m, 2H).

Crude thiazoline (76.9 g, 317 mmol, 1 eq.) and $BrCCl_3$ (46.9 mL, 476 mmol, 1.5 eq.) were dissolved in DMF (1 M) and cooled in an ice bath. DBU (99 mL, 666 mmol, 2.1 eq.) was added dropwise *via* addition funnel over 20 minutes. On large scale this addition was significantly exothermic. The reaction was then brought to 50 °C and stirred for four hours, then the brown solution slowly poured into vigorously stirring 1 M HCl solution at 0 °C to ensure a uniform precipitate. The mixture stirred for 15 minutes and the fine brown precipitate was filtered and dried under vacuum at 50 °C for 18 hours to give 76 g of crude thiazole product as a grey-brown solid. The crude precipitate was used directly in the next reaction without further purification.

^{1}H NMR (600 MHz, MeOD) δ 8.76 (dd, *J*= 7.9, 1.8 Hz, 1H), 8.58 (s, 1H), 8.50 (dd, *J*= 4.7, 1.9 Hz, 1H), 7.58 (dd, *J*= 7.9, 4.7 Hz, 1H).

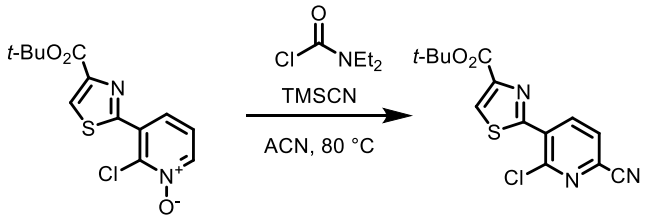


Compound **58:** Starting material (45.4 g, 189 mmol, 1 eq.) was dissolved in 3:1 t-BuOH / Pyridine (0.25 M) in portions to prevent clumping. Tosyl chloride (71.9 g, 378 mmol, 2 eq.) was added slowly at 23 °C and the reaction was then stirred at 60 °C for 4 hours. The reaction was slowly poured into vigorously stirring DI water and stirred for 15 minutes. The precipitate was filtered, washed with cold DI water (2 x 200 mL), and air-dried for 2 hours to give analytically pure *t*-Bu ester as a brown solid (51.83 g, 93% yield).

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.80 (dd, *J* = 7.8, 1.6 Hz, 1H), 8.46 (dd, *J* = 4.6, 1.6 Hz, 1H), 8.21 (s, 1H), 7.40 (dd, *J* = 7.8, 4.6 Hz, 1H), 1.63 (s, 9H).

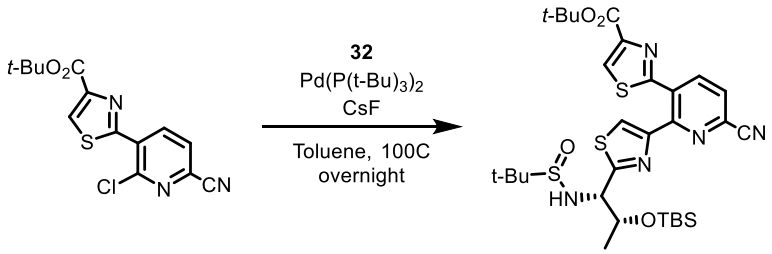
Crude t-Bu ester and urea-hydrogen peroxide complex (33.88 g, 378 mmol, 2 eq.) were dissolved in dry DCM (630 mL, 0.3 M) in a flame-dried flask equipped with a stir bar. TFAA (49.1 mL, 387 mmol, 2 eq.) was added dropwise via a flame-dried addition funnel at 0 °C. The reaction was then warmed to 23 °C as the ice bath melts and stirred for 14 hours. The reaction was diluted with additional DCM (370 mL) and 10% K_2CO_3 solution (500 mL). The organic layer was washed with water (2 x 500 mL), saturated sodium thiosulfate (2 x 500 mL) and brine (500 mL), dried over sodium sulfate, and concentrated to give crude N-oxide as a yellow solid (35.1 g, 112 mmol, 64.3% yield). Crude material was used directly in the next reaction without further purification.

^{1}H NMR (599 MHz, MeOD) δ 8.61 (dd, *J* = 6.5, 1.4 Hz, 1H), 8.56 (s, 1H), 8.36 (dd, *J* = 8.2, 1.4 Hz, 1H), 7.60 (dd, *J* = 8.2, 6.5 Hz, 1H), 1.63 (s, 9H).



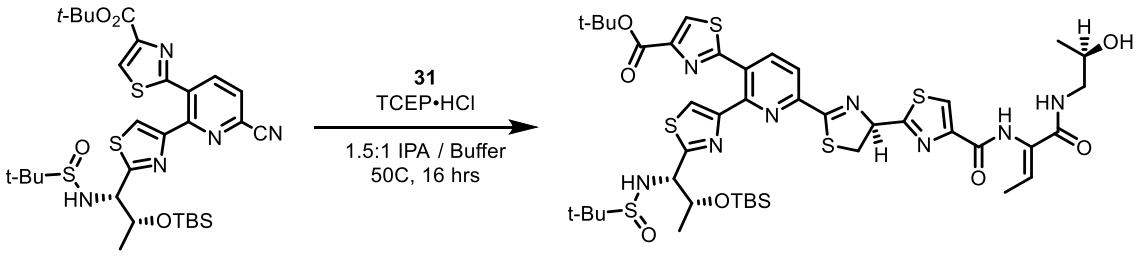
Compound **33:** Starting material N-oxide (35.1 g, 112 mmol, 1 eq.) was dissolved in ACN (374 mL, 0.3 M) and TMSCN (35.1 mL, 280 mmol, 2.5 eq.) and diethylcarbamyl chloride (35.6 mL, 280 mmol, 2.5 eq.) were added at 23 °C. The reaction was then brought to reflux and stirred for 14 hours, then slowly poured into a vigorously stirring ice cold solution of 10% K_2CO_3 (1.5 L) and stirred for 15 minutes. The brown precipitate was filtered and washed with additional cold water. The collected solids were dissolved in ethyl acetate (500 mL), washed with brine (300 mL), dried over sodium sulfate, and concentrated. The crude product was recrystallized from Hex / EA to give a crystalline, slightly yellow solid. (19.1 g, 59.5 mmol, 53% yield).

^{1}H NMR (600 MHz, $CDCl_3$): δ 9.01 (d, J = 8.0 Hz, 1H), 8.30 (s, 1H), 7.78 (d, J = 8.0 Hz, 1H), 1.64 (s, 9H); **^{13}C NMR** (150 MHz, $CDCl_3$): δ 160.0, 159.6, 149.2, 148.7, 140.4, 133.0, 131.6, 129.4, 127.2, 115.6, 82.8, 28.2; **IR** (film, cm^{-1}): 2363, 1727; **HRMS** (ESI): calc. for $C_{14}H_{12}ClN_3O_2S$ $[M+Na]^+$: 344.0231, obs. 344.0227.



Compound **30**:[91] Pyridine starting material (5.77 g, 16.3 mmol, 1 eq.) and stannane (8.80 g, 17.1 mmol, 1.05 eq.) were dissolved in toluene (163 mL, 0.1 M) and $Pd(P(t\text{-}Bu)_3)_2$ was added (417 mg, 0.04 eq.) followed by cesium fluoride (5.45 g, 35.9 mmol, 2.2eq.) and the reaction was sparged with nitrogen for 10 minutes. The reaction was then heated to 100 °C for 14 hours, then filtered through celite and concentrated. The crude mixture was purified by column chromatography with 15 --> 40% EA / Hex on a medium length column to give 8.25 g of pure dithiazolyl pyridine product as a yellow solid (13.2 mmol, 81% yield).

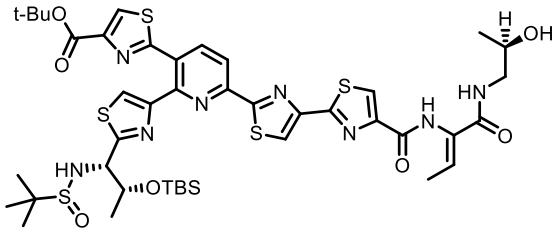
^{1}H NMR (600 MHz, $CDCl_3$) δ 8.32 (d, J = 8.0 Hz, 1H), 8.14 (s, 1H), 7.89 (s, 1H), 7.74 (d, J = 8.0 Hz, 1H), 4.71 (d, J = 5.6 Hz, 1H), 4.36 (dd, J = 5.6, 3.0 Hz, 1H), 4.21 (qd, J = 6.3, 3.0 Hz, 1H), 1.61 (s, 9H), 1.29 (s, 9H), 1.22 (d, J = 6.3 Hz, 3H), 0.79 (s, 9H), -0.02 (s, 3H), -0.29 (s, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 174.39, 163.89, 160.16, 152.64, 152.20, 148.83, 140.33, 134.08, 131.13, 128.82, 126.84, 122.93, 116.85, 82.53, 71.51, 63.24, 56.53, 28.31, 25.81, 22.91, 21.15, 17.92, -4.30, -5.18. **IR** (film, cm^{-1}): 2954, 2359, 1725, 1159, 751; **HRMS** (ESI): calc. for $C_{30}H_{44}N_5O_4S_3Si$ $[M+H]^+$: 662.2319, obs. 662.2315.



Compound **71:** Starting material (10.91 g, 16.5 mmol, 1 eq.) was dissolved in 1.5:1 IPA / pH 7 phosphate buffer (165 mL, 0.2 M buffer, 0.1 M reaction) followed by the tail fragment (8.00 g, 18.2 mmol, 1.1 eq.) and TCEP • HCl (1.18 g, 4.1 mmol, 0.25 eq.). The reaction was heated to 55 °C for 8 hours. IPA was then removed under vacuum and the residue was diluted with 500 mL of water and the mixture was extracted with ethyl acetate (3 x 200 mL). The combined organic layers were washed with 200 mL brine, dried over sodium sulfate and concentrated. The crude product was purified by column chromatography with 3 --> 8% MeOH / DCM on a long column, dry loaded on silica to give 9.08 g of pure thiazoline intermediate as a yellow solid (9.18 mmol, 56 % yield).

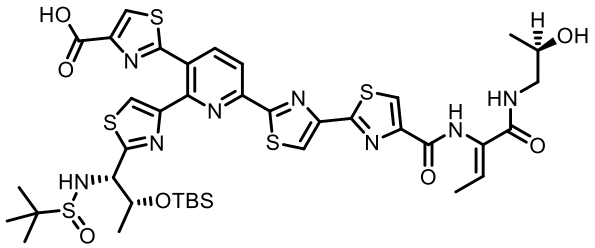
^{1}H NMR (600 MHz, CDCl3) δ 8.66 (s, 1H), 8.30 (d, J = 8.1 Hz, 1H), 8.23 (d, J = 8.1 Hz, 1H), 8.16 (s, 1H), 8.12 (s, 1H), 6.65 – 6.56 (m, 2H), 6.13 (t, J = 8.8 Hz, 1H), 4.72 (d, J = 5.6 Hz, 1H), 4.39 (dd, J = 5.6, 3.0 Hz, 1H), 4.27 – 4.21 (m, J = 6.2, 3.1 Hz, 1H), 4.05 – 3.98 (m, 1H), 3.94 (dd, J = 11.2, 8.8 Hz, 1H), 3.73 (dd, J = 11.2, 8.8 Hz, 1H), 3.54 (ddd, J = 13.9, 6.7, 2.8 Hz, 1H), 3.15 – 3.08 (m, J = 17.1, 8.2, 5.5 Hz, 1H), 1.84 (d, J = 7.1 Hz, 3H), 1.62 (s, 9H), 1.30 (s, 9H), 1.23 (d, J = 6.3 Hz, 3H), 1.19 (d, J = 6.3 Hz, 3H), 0.81 (s, 9H), -0.00 (s, 3H), -0.25 (s, 3H).
^{13}C NMR (151 MHz, MeOD) δ 175.37, 173.92, 173.90, 173.28, 167.42, 167.35, 162.30, 161.76, 154.48, 152.35, 151.88, 150.57, 149.13, 141.30, 132.01, 131.12, 130.75, 130.73, 126.49, 123.49, 121.38, 83.46, 79.63, 72.58, 67.36, 64.70, 64.56, 57.73, 57.72, 48.10, 38.38, 28.48, 26.24, 23.00,

21.50, 20.87, 18.71, 13.83, -4.16, -4.91. **IR** (film, cm^{-1}): 3330, 2930, 2359, 1667, 750; **HRMS** (ESI): calc. for $C_{43}H_{61}N_8O_7S_5Si$ $[M+H]^+$: 989.3031, obs. 989.3030.



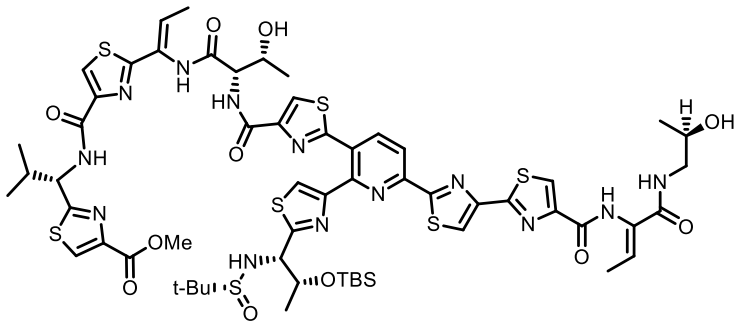
Compound **71**: Thiazoline starting material (9.08 g, 9.18 mmol, 1 eq.) was dissolved in dry DCM (92 mL, 0.1 M) and manganese (IV) oxide (17.53 g, 184 mmol, 20 eq.) was added (Activated, Alfa Aesar, tech. 90%, LOT: W08D050). The reaction was stirred rapidly for 18 hours, then filtered through a pad of silica with methanol to give the desired trithiazolyl pyridine product as a yellow solid (>99% yield).

^{1}H NMR (600 MHz, MeOD) δ 8.45 (s, 1H), 8.42 (s, 1H), 8.35 – 8.28 (m, 3H), 8.19 (s, 1H), 6.72 (q, J = 7.0 Hz, 1H), 4.36 (d, J = 3.3 Hz, 1H), 4.26 – 4.20 (m, 1H), 3.91 (dq, J = 12.8, 6.4 Hz, 1H), 3.31 (dd, 1H) 3.22 (dd, J = 13.5, 7.1 Hz, 1H), 1.84 (d, J = 7.0 Hz, 3H), 1.62 (s, 9H), 1.31 (s, 9H), 1.20 (d, J = 6.3 Hz, 3H), 1.17 (d, J = 6.3 Hz, 3H), 0.84 (s, 9H), 0.04 (s, 3H), -0.20 (s, 3H). **^{13}C NMR** (151 MHz, MeOD) δ 173.98, 170.15, 167.43, 167.38, 164.00, 162.25, 161.79, 154.47, 152.21, 152.20, 151.27, 151.26, 149.11, 141.72, 132.04, 131.17, 130.65, 129.96, 126.58, 123.49, 122.11, 119.50, 83.44, 72.61, 67.39, 64.57, 57.74, 48.13, 28.50, 26.25, 23.03, 21.51, 20.90, 18.72, 13.90, -4.15, -4.88. **IR** (film, cm^{-1}): 2954, 2359, 1663, 1159, 752; **HRMS** (ESI): calc. $C_{43}H_{59}N_8O_7S_5Si$ $[M+H]^+$: 987.2874, obs. 987.2870.



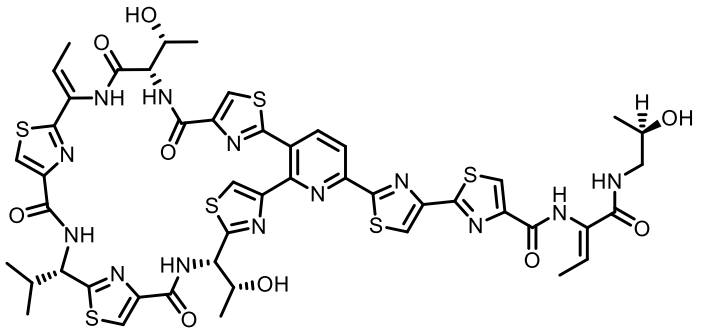
Compound **72:** Starting material (7.00 g, 7.1 mmol, 1 eq.) was dissolved in 3:1 DCM / TFA (71 mL, 0.1 M) and the reaction was stirred for 14 hours at 23 °C. The reaction was then concentrated from 4:1 benzene / MeOH (2 x 100 mL) and the resulting crude residue was used directly in the next reaction without further purification.

^{1}H NMR (600 MHz, MeOD) δ 8.42 (br s, 1H), 8.35 (br s, 1H), 8.25 (m, 3H), 8.11 (br s, 1H), 6.58 (q, J = 7.0 Hz, 1H), 5.24 – 5.17 (m, 1H), 4.31 (d, J = 2.7 Hz, 1H), 4.20 – 4.13 (m, 1H), 3.55 – 3.48 (m, 1H), 3.45 – 3.38 (m, 1H), 1.78 (d, J = 7.0 Hz, 3H), 1.30 (d, J = 6.4 Hz, 3H), 1.24 (s, 9H), 1.14 (d, J = 6.2 Hz, 3H), 0.77 (s, 9H), -0.03 (s, 3H), -0.28 (s, 3H).



Compound **72:** Starting material (1.25 g, 1.34 mmol, 1 eq.) was dissolved in DMF (13.5 mL, 0.1 M) and the large ring fragment **28** was added (786 mg, 1.47 mmol, 1.1 eq.) followed by HATU (561 mg, 1.47 mmol, 1.1 eq.) and DIPEA (0.70 mL, 4.02 mmol, 3 eq.). The reaction was stirred for 14 hours at 23 °C. The reaction was diluted in 100 mL of water and extracted with ethyl acetate (3 x 25 mL). The combined organic layers were washed with 3 M LiCl (3 x 25 mL), dried over sodium sulfate and concentrated. The crude material was dry loaded on silica gel and purified by column chromatography with 4 --> 10% MeOH/DCM + 1% AcOH on a long column to give 1.15 g of pure coupled product as a yellow-orange solid (0.82 mmol, 61% yield).

^{1}H NMR (600 MHz, MeOD) δ 8.58 – 8.10 (m, 8H), 6.81 – 6.71 (m, 2H), 5.17 (d, *J* = 8.4 Hz, 1H), 4.70 (d, *J* = 13.7 Hz, 1H), 4.53 – 4.44 (m, 2H), 4.40 – 4.34 (m, 1H), 3.98 – 3.89 (m, 2H), 3.61 – 3.58 (m, 3H), 3.26 – 3.19 (m, 1H), 2.61 – 2.46 (m, 1H), 1.91 (d, *J* = 7.1 Hz, 3H), 1.84 (d, *J* = 6.9 Hz, 3H), 1.34 – 1.28 (m, 12H), 1.17 (d, *J* = 6.1 Hz, 3H), 1.06 (d, *J* = 6.8 Hz, 3H), 0.93 (d, *J* = 6.6 Hz, 3H), 0.85 (s, 9H), 0.04 (s, 3H), -0.18 (s, 3H). **^{13}C NMR** (151 MHz, *****several C signals were not observed due to peak broadening, MeOD) δ 202.11, 200.21, 198.13, 197.20, 195.81, 193.62, 192.39, 191.57, 191.40, 191.11, 190.86, 189.09, 182.70, 180.71, 180.25, 179.77, 179.65, 178.79, 176.13, 159.63, 158.92, 158.80, 158.05, 157.88, 156.27, 155.25, 153.76, 152.11, 150.62, 148.75, 101.27, 97.76, 95.91, 93.18, 91.06, 88.55, 87.17, 86.40, 62.59, 57.15, 54.91, 54.80, 51.67, 51.60, 50.10, 49.50, 49.35, 48.67, 47.91, 47.34, 43.18.

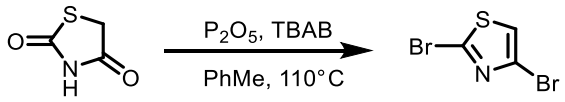


Micrococcin P1: Starting material (970 mg, 0.69 mmol, 1 eq.) was dissolved in 3:1 THF / MeOH (6.88 mL, 0.1 M) and 10% aq. NaOH was added (1.38 g, 3.45 mmol, 5 eq.). The reaction was stirred at 23 °C for 3 hours. The reaction was acidified with DOWEX 50WX8 & stirred for 10 minutes. The mixture was filtered and concentrated to a light yellow solid. The crude material was used directly in the next reaction without further purification.

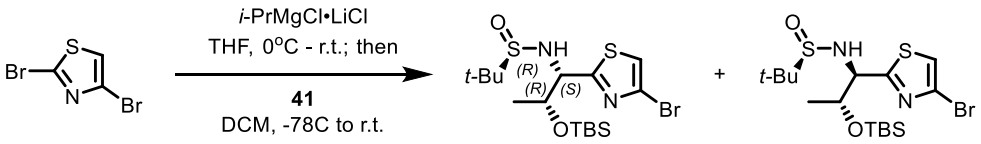
Crude carboxylic acid was dissolved in methanol (5 mL) and 4N HCl in dioxane was added (10 mL, xs). The reaction was stirred for 2 hours at 23 °C and then concentrated from 4:1 benzene / MeOH (2 x 25 mL). The resulting crude solid was used directly in the next reaction without further purification.

Crude, fully deprotected starting material was dissolved in DMF (68.8 mL, 0.01 M) and DPPA was added (0.294 mL, 1.38 mmol, 2eq.) followed by DIPEA (0.479 mL, 2.76 mmol, 4 eq.). The reaction was stirred for 14 hours at 23 °C and then diluted with EA (500 mL) and washed with 3 M LiCl (3 x 200 mL), dried over sodium sulfate and concentrated. The crude residue was purified by column chromatography to give micrococcin P1 (149 mg) as a white solid. Additional mixed material was collected from the column and purified by preparative TLC to give an additional 50 mg of micrococcin P1 (199 mg, 0.26 mmol, 38 % over 3 steps). The product was spectroscopically indistinguishable from previous reports.

$[\alpha]_D$ = +39.7 (T = 23, c = 0.5, 9:1 EtOH / H_2O); **^{1}H NMR** (300 MHz, DMSO) δ 9.52 (s, 2H), 8.59 (s, 1H), 8.46 (s, 1H), 8.44 (s, 1H), 8.41 (s, 1H), 8.38 (s, 2H), 8.35 (s, 1H), 8.32 (s, 1H), 8.30 (s, 1H), 8.25 – 8.20 (m, 2H), 8.12 (s, 1H), 7.95 – 7.84 (m, 3H), 6.55 – 6.42 (m, 2H), 5.44 (d, *J* = 6.7 Hz, 1H), 5.18 – 5.05 (m, 2H), 4.78 (d, *J* = 4.7 Hz, 1H), 4.71 – 4.62 (m, 2H), 4.43 – 4.32 (m, 1H), 4.06 – 3.97 (m, 1H), 3.77 – 3.64 (m, 1H), 3.08 (s, 2H), 1.75 (d, *J* = 6.9 Hz, 3H), 1.70 (d, *J* = 6.9 Hz, 3H), 1.38 (d, *J* = 6.3 Hz, 3H), 1.05 – 0.96 (m, 9H), 0.86 (d, *J* = 6.7 Hz, 3H). **HRMS** (ESI): calc. $C_{48}H_{49}N_{13}O_9S_6$ $[M+Na]^+$: 1144.2173, obs. 1144.2164.

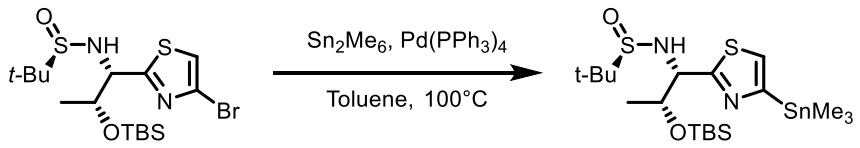


Compound **60:** This compound was prepared from a previously reported method.[4] 2,5-thiazolidinedione (25 g, 213 mmol, 1 eq.) was added to hot toluene (427 mL, 0.5 M) and P_2O_5 (144 g, 1,014 mmol, 4.75 eq.) was added followed by portion-wise addition of TBAB (158 g, 491 mmol, 2.3 eq.) to prevent clumping. On large scale, efficient stirring and mixing was crucial to reaction success. The yellow reaction was stirred vigorously at reflux for 14 hours then concentrated under reduced pressure to remove toluene. The residue was diluted in water (1.5L) and extracted with ether (3 x 350 mL). The combined organic layers were washed with brine (500 mL), dried over sodium sulfate, and concentrated to give analytically pure thizole product (47.13 g, 194 Mmol, 91% yield) as a light brown solid. This crude material may be purified by sublimation under vacuum with gentle heating to give a white solid.



Compound **63:** 2,5-dibromothiazole (28.1 g, 116 mmol, 1.6 eq.) was dissolved in a minimal amount of THF (25 mL, ~1 mL/g) in a flame-dried, nitrogen filled flask and cooled in an ice water bath. Isopropylmagnesium chloride lithium chloride solution (4.8 mL, 1.3 M, 209 mmol, 1.8 eq.) was added dropwise over 10 minutes. The reaction was slowly warmed to 23 °C to stir for 30 minutes until grignard formation was complete by TLC (UV). Meanwhile, imine starting material **41** (16.9 g, 57.9 mmol, 1 eq.) was dissolved in dry DCM (69 mL, 0.05 M) and cooled to -78 °C. The thiazole grignard was added dropwise to the cold imine solution over one hour and, after addition, allowed to warm to 23 °C as the ice bath warms for 14 hours. After completion the reaction was quenched with saturated aqueous ammonium chloride solution and extracted with 1:1 hexanes-ether (3 x 250 mL). The combined organic layers were washed with brine (250 mL), dried over sodium sulfate and concentrated to a brown oil. Crude product was purified by column chromatography (10 --> 30% EA/Hex). *Top diastereomer (R, S, R):* R_f = 0.32 in 20% EA / Hex. 13.72 g was collected pure after chromatography as a light brown oil (30.1 mmol, 52% yield).

Top diastereomer by TLC (R, S, R): R_f = 0.32 in 20% EA/Hex. **^{1}H NMR** (599 MHz, $CDCl_3$): δ 7.14 (s, 1H), 4.74 (d, J = 6.7 Hz, 1H), 4.59 (qd, J = 6.3, 1.6 Hz, 1H), 4.50 (dd, J = 6.8, 1.6 Hz, 1H), 1.38 (d, J = 6.4 Hz, 3H), 1.32 (s, 9H), 0.76 (s, 9H), -0.02 (s, 3H), -0.35 (s, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 176.79, 125.30, 117.27, 71.33, 63.66, 56.72, 25.74, 22.97, 21.38, 17.88, -4.51, -5.63. **IR** (film, cm^{-1}): 2929, 2359, 1473, 1130; **HRMS** (ESI): calc. for $C_{16}H_{32}BrN_2O_2S_2Si$ $[M+H]^+$: 455.0852, obs. 455.0844



Compound **32:** Starting material (10.1 g, 22.6 mmol, 1 eq.) was dissolved in dry Toluene (222 mL, 0.1 M) and Sn_2Me_6 (6.89 mL, 33.9 mmol, 1.5 eq.) and $Pd(PPh_3)_4$ (1.28 g, 1.11 mmol, 0.05 eq.) were added. The reaction was degassed by sparging with nitrogen for 10 minutes then warmed to 100 °C and stirred for one hour. The reaction became dark and after complete conversion by TLC was partially concentrated and loaded directly onto a column with toluene. The crude mixture was purified by column chromatography with 15 --> 30% EA/Hex + 1% TEA to give the desired product as a brown oil (9.5 g, 17.6 mmol, 80% yield).

^{1}H NMR (599 MHz, $CDCl_3$) δ 7.25 (t, 1H), 4.77 (d, J = 6.8 Hz, 1H), 4.64 – 4.59 (dq, 1H), 4.58 (dd, J = 6.9, 1.8 Hz, 1H), 1.38 (d, J = 6.3 Hz, 3H), 1.32 (s, 9H), 0.75 (s, 9H), 0.39 – 0.28 (t, 9H), -0.05 (s, 3H), -0.42 (s, 3H). **^{13}C NMR** (151 MHz, $CDCl_3$) δ 176.17, 160.64, 125.73, 71.69, 64.05, 62.47, 56.56, 25.78, 22.98, 21.34, 17.91, -4.53, -5.63. **IR** (film, cm^{-1}): 2954, 2360, 1082, 767; **HRMS** (ESI): calc. for $C_{19}H_{41}N_2O_2S_2SiSn$ $[M+H]^+$: 541.1395, obs. 541.1390.

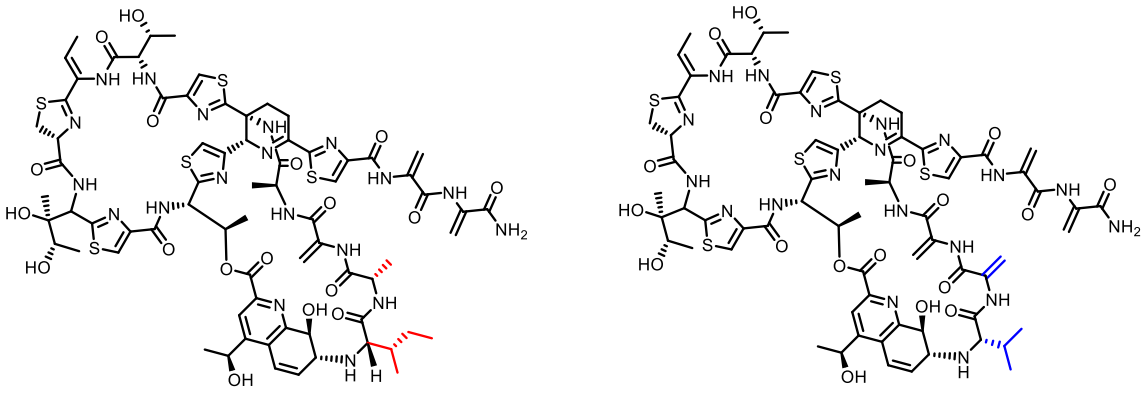
Chapter 1, in part is work in preparation for publication, was contributed to by Clare McNerlin, Katie Freiberg, Trevor Johnson, John Woodard, Bryant Lim, Dionicio Siegel and Tiffany Hamilton by generating large quantities of intermediates for advancing the total synthesis of the natural product. I would also like to thank Andrew T. Nelson for synthetic advice and expertise as well as mentorship and guidance throughout my academic career. Mitchell P. Christy is the primary author of this material.

Chapter 2: Anti-Cancer Activity of Micrococcin P1 and Synthetic Intermediates

The thiopeptide are able to selectively kill a broad range of cancer cells without showing any cytotoxicity against normal cells. This activity has been linked to the binding of transcription factor FOXM1.[35,36,38,45] It is important for the execution of cell division by upregulating several genes implicated in mitosis.[92] FOX M1 in normal cells plays a role in cell cycle progression and regulates expression of a wide array of G2/M-specific genes and is crucial for maintenance of chromosomal segregation and genomic stability.[49] It is expressed in all dividing mammalian cells and tumor-derived cells, though it is turned off in terminally differentiated cells. Its overexpression is associated with the development and progression of cancer in that it leads to unregulated cell growth and suppressed differentiation. Of the classical hallmarks of cancer, dysregulation of FOXM1 directly leads to limitless replicative potential, sustained angiogenesis, self-sufficiency in growth signals, insensitivity to anti-growth signals, and evasion of apoptosis.[93] It is an attractive therapeutic target because it is overexpressed in a majority of human tumors. Certain 26-membered thiopeptides have been found to interact directly with FOX M1 and downregulate its expression as well as induce apoptosis in cancer cells. This finding established FOXM1 as one of the primary cellular targets of thiostrepton and others and that transcription factors, once thought to be undruggable, can be targeted for potential cancer therapy.

Thiostrepton and another series b thiopeptide siomycin A were first found to inhibit FOXM1 activity in 2006 from a high-throughput screen aimed at identifying inhibitors (Figure 2.1).[45] The authors observed that siomycin A was able to downregulate the transcriptional activity as well as the protein and mRNA abundance of FOXM1. Downstream target genes were repressed as a consequence, and the thiopeptides selectively induce apoptosis in transformed but not normal cells of the same origin. Following this discovery,

Kwok et al. showed that FOXM1 is one of the primary cellular targets of thiostrepton, highlighting that these natural products may be novel lead compounds for targeted therapy



Thiostrepton Siomycin A

Figure 2.1. Thiopeptides initially found to inhibit FOXM1 activity.

with no toxicity against normal cells.[46] They demonstrated that thiostrepton selectively induces cell cycle arrest and cell death in breast cancer cells through downregulation of FOXM1 expression in a time- and dose-dependent manner. Several years later, Hedge and co-workers were able to show that FOXM1 is a direct target of thiostrepton, observing inhibition of binding to genomic target sites in MCF-7 cells (human breast cancer).[48]

A study by Nicolaou and Co-workers, through total synthesis efforts to make thiostrepton, demonstrated for the first time that fragments of this natural product retained activity against cancers.[67] Some even showed better IC_{50} values than the natural product itself. This work prompted the question, do other thiopeptides of different series have activity, and what is the minimum scaffold necessary for this activity? We set out to answer these questions by evaluating micrococcin and its synthetic intermediates. If activity is found in other series of thiopeptides, this could greatly simplify the route to new therapeutics and provide a platform for promising medicinal chemistry development.

To date, only series B thiopeptides have been tested for activity against cancer

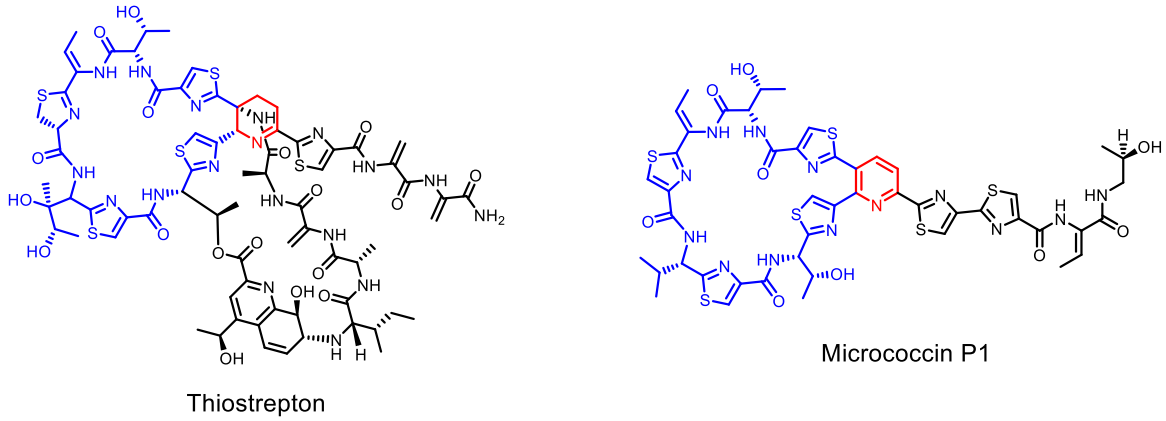


Figure 2.2. Comparative structures of thiostrepton (series B) and micrococcin P1 (series D).

including thiostrepton and siomycin A. The complex nature of these natural products makes it difficult to produce them economically and in a timely fashion. Though total synthesis of these thiopeptides has been accomplished, it has been fruitful to explore subunits for activity to reduce the synthetic burden and trim any unnecessary residues. Micrococcin P1, a D-series thiopeptide, differs from thiostrepton in many ways. Most notably, the central 6-membered ring that provides anchor for the macrocycle(s) and the arrangement of amino acid residues. In thiostrepton the central ring is a chiral, cyclic 6-membered imine whereas in micrococcin it is a fully aromatic pyridine (Figure 2.2). Both are rich in cysteine and serine residues that are post-translationally modified to thiazoles and dehydroamino acids, though their sequence is significantly different. Lastly, both micrococcin and thiostrepton share a common motif in the main 26-membered macrocycle, though micrococcin lacks a 2nd macrocycle that thiostrepton has. Where the activity stems from within thiostrepton has been established, though the study didn't propose a mechanism of action nor directly link the fragment to binding FOXM1 as the connection hadn't been made at the time (Figure 2.3). Whether this structure can be simplified further remained to be seen, and something we established in this work. Though they tested each region of thiostrepton across several cancer cell lines, the

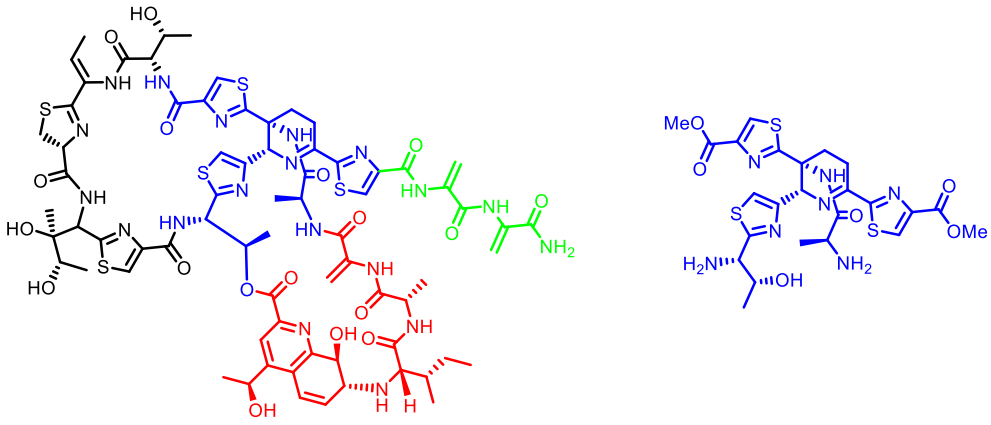


Figure 2.3. Different regions of thiostrepton tested for activity (left) and the central fragment that retains potent activity (right).

Nicolaou lab showed that the activity isn't reliant on the macrocycles nor the tail, but in fact the central trisubstituted tetrahydropyridine. Overall, the activity was lost when each individual fragment was tested except the central fragment which was more potent than the parent natural product by nearly an order of magnitude. That the activity was retained was somewhat surprising due to the very large structural difference and the disappearance of the two macrocycles, at least one of which is absolutely necessary for its antibiotic activity. Nevertheless, this substructure retains activity and as a result we chose to follow up to explore another member of the family.

As the thiopeptides go, thiostrepton and siomycin A are of the most complex. They contain 18 or 16 stereocenters respectively, with 2 quaternary carbons, more than any other thiopeptide. The 2nd macrocycle also adds to the synthetic difficulty. The analogous fragments we wanted to test were various iterations of the central pyridine *en route* to the natural product as seen in Figure 2.4. Some are early intermediates with only 1 thiazole as in **33** and the others have either 2 or 3 thiazoles attached. Because the series D thiopeptides have a pyridine center rather than a cyclic imine with 2 stereocenters, accessing these products is greatly simplified. There are no extraneous diastereomers to separate and the route developed in chapter 1 is more efficient. In fact, Nicolaou and co-workers constructed their

central fragment in 12 steps in less than 1% yield whereas the longest sequence to make our core compound **71** is 13 steps in 2% yield. Chloro-cyanopyridine **33** is made in only 5 steps

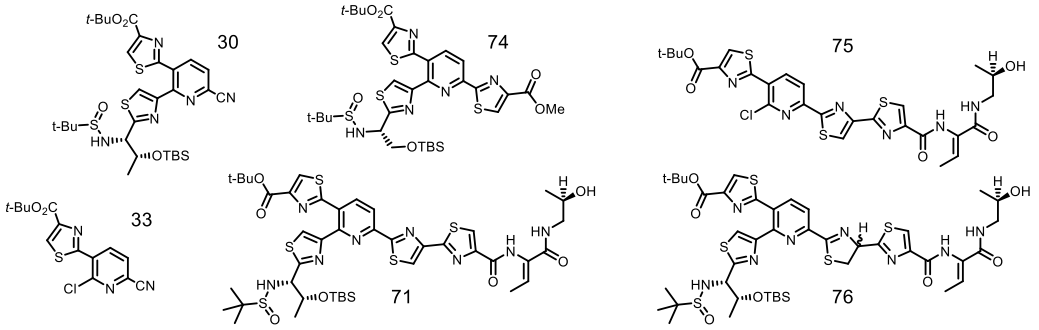
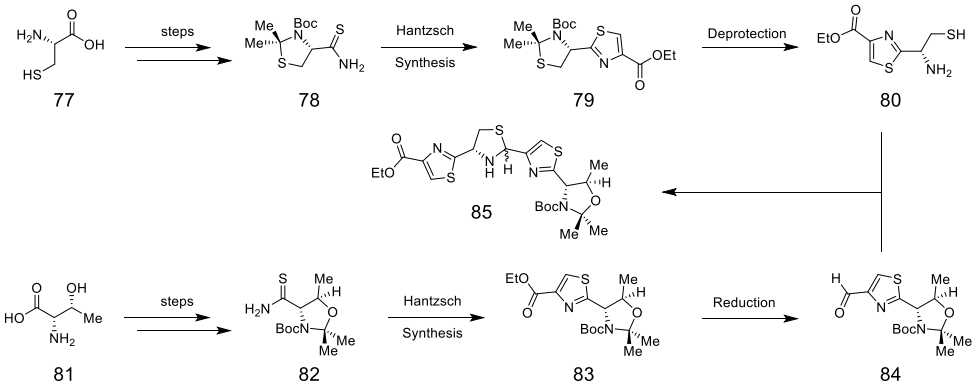


Figure 2.4. Synthetic intermediates of micrococcin P1 analogous to the thiostrepton core.

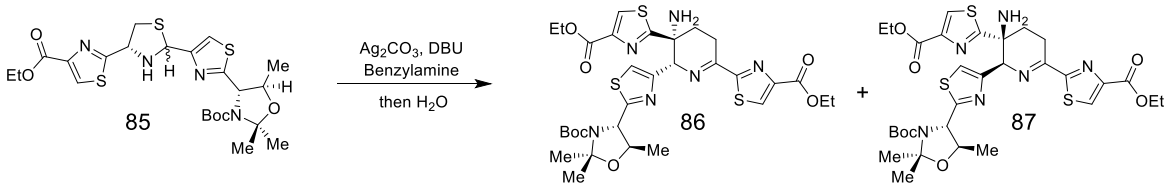
(28% yield) with one instance of chromatography. **30** is made in one additional step in 23% overall yield and the disubstituted **75** in 12 steps in 16% yield. If these compounds have activity, it would lead to further development toward tuning these for better activity and ADME properties. Binding studies should also be done to assess how these fragments interact with FOXM1 in order to understand its precise mechanism of action and how activity might be improved.

The reported synthesis of the thiostrepton fragment utilizes the Hantzsch thiazole synthesis on several occasions and the authors report an aza-Diels-Alder reaction to form the cyclic imine. Intermediate **85** is made in 7 steps from L-cysteine and L-threonine by protecting first as a cyclic acetonide followed by Boc protection, or vice versa (Scheme 2.1). A



Scheme 2.1. Synthesis of thiostrepton central fragment intermediate 85.

thioamide is formed from the carboxylic acid and the 2-step Hantzch thiazole synthesis gives intermediates **79** and **83**. Deprotection of the cysteine-derived acetonide gives free aminothiol **80** and reduction of the ethyl ester of the threonine-derived thiazole gives aldehyde **84**. These two are brought together in a cyclization event providing thiazolidine **85** that is the precursor to the Diels-Alder starting material. This intermediate undergoes dimerization upon *in-situ* decomposition of the thiazolidine in the presence of Ag_2CO_3, DBU, and benzylamine yielding 60% of adduct **86** and **87**. Although the Diels-Alder reaction proceeded both in a regioselective



Scheme 2.2. Endo-selective Diels-Alder reaction to form the thiostrepton core 86 and undesired diastereomer 87.

and endo-selective manner, it showed no facial selectivity and as a result was isolated as a mixture of diastereomers (~1:1) with regards to the relative stereochemistry of the core substituents (Scheme 2.2). After the cyclization, an azido amino-acid chloride is coupled to the free amine and ethyl esters exchanged for methyl esters. To produce the active fragment from Figure 2.3, the azide is reduced with $SnCl_2$ (after which the two diastereomers are finally separable) followed by aqueous acid deprotection of the Boc acetonide. Table 2.1 gives

Table 2.1. Anti-cancer activity of thiostrepton and core fragment (Figure 2.3).[67]

	LC_{50} (μM)[a]				
compound	NCI-H460[b]	HCT-116[c]	SK-OV-3[d]	MCF-7[e]	K-562[f]
doxorubicin	0.042	0.12	0.075	0.36	0.08
Taxol	0.043	0.017	0.042	0.018	0.021
thiostrepton	1.5	1.6	2.8	3.8	1.7
Core Fragment	0.9	0.6	1.2	0.9	0.8

[a] Concentration required to kill 50% of the cell. [b] Human nonsmall cell lung cancer cell line. [c] Human colon cancer cell line. [d] Human ovarian cancer cell line. [e] Human breast cancer cell line. [f] Human chronic myelogenous leukemia cancer cell line.

a summary of thiostrepton's activity as well as the core fragment in addition to controls doxorubicin and Taxol. Overall this route is plagued by inefficiency due to the complex stereochemistry of the natural product and is not very suitable for large scale preparation. This synthesis also requires the use of hazardous reagents such as Lawesson's reagent and an organic azide prone to detonation on occasion. Our chosen fragments and route eliminate the need for such reagents and the mostly achiral nature of micrococcin's core allows for a shorter, higher yielding synthesis.

During the micrococcin synthesis, we characterized and tested many intermediates and derivatives against several cancer cell lines. We found that several of the compounds had activity in the low micromolar range as the natural products showed. To our surprise, micrococcin P1 showed no activity in the tested range. Fragments without the central pyridine showed no activity including the large ring fragment nor the tail fragment. Activity was fairly consistent otherwise, with variations of micrococcin's central fragment testing between 8.5 to 0.26μM IC_{50}. Surprisingly, the smaller fragments had the best activity including **75** and trithiazolyl fragment **74**. A previous study by Novartis, in an attempt to increase aqueous solubility of thiopeptide antibiotic LFF571, showed that changing the structure of the natural product's tail didn't much affect potency in cell extracts. However,

Table 2.2. Micrococcin fragment IC_{50} values compared to thiostrepton control against ovarian carcinoma [A2780], cervical cancer [HeLa], and colon adenocarcinoma [SW480].

	IC50 (μM)		
Compound	A2780	HeLa	SW480
Thiostrepton	0.85	1.7	0.67
Micrococcin	>10	>10	>10
30	6.5	>10	>10
71	8.5	>10	7.5
74	0.26	-	-
75	1.7	2.8	3.9
76	>10	>10	3.5

in vitro activity varied widely suggesting that the discrepancy resulted from a difference in cell penetration.[27] This may also be the case with our fragments, as the activity seems to correlate negatively with the size of the compound. There appears to be a middle ground between simple di- or trisubstituted pyridines and micrococcin where the activity is retained, and cell penetration isn't affected. Though we haven't confirmed this hypothesis, it would not be wise to pursue medicinal chemistry optimization of the larger structures for this reason. The results of the active fragment testing are summarized in Table 2.2. No other fragments had detectable activity below our 10μM threshold against any of the cell lines tested. The collection of inactive compounds tested in this assay is seen in Figure 2.5. Fragments **88**, **89**, **40**, **42**, and **28** represent increasingly larger parts of the macrocycle, though not even the

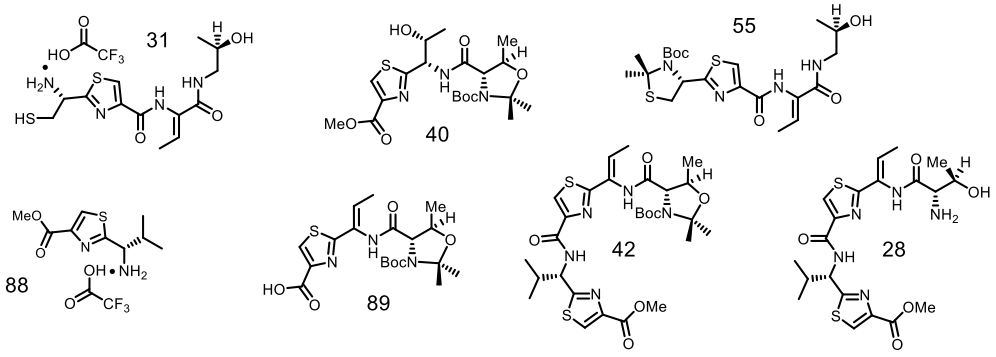


Figure 2.5. Inactive micrococcin fragments in the anti-cancer assay.

largest had any activity in the tested range. Fragments **31** and **55** are two late stage iterations of the tail that also failed to perform. The case of micrococcin is a strange one. Though privileged as a natural product, it showed no activity *in vitro* despite cousin thiostrepton having potent activity. The discrepancy may stem from poor cell penetration, though the mechanism of entry into the cell for these products has not been characterized. The array of structural differences may also be enough to manifest the variation in binding affinity to the putative target FOXM1. Even so, the central piece that ties them together is the common denominator when it comes to activity.

These experiments have established a common scaffold between two series of thiopeptide natural products, B and D, that are active against cancers with no noticeable toxicity to normal cells. Access to the natural products themselves can be prohibitively expensive or take a great deal of time and resources to make. By identifying a minimum scaffold for activity, we can significantly lower the barriers to access it. This work may have implications for other series of thiopeptides as well. Thought series D is the simplest synthetically with regard to stereochemistry and substitution, others may have better activity or ease of access. Between thiostrepton and micrococcin fragments, the latter is a better alternative in that it can be made in fewer synthetic steps in higher yield without many chromatographic separations. Micrococcin's synthesis is also scalable whereas the current synthesis of thiostrepton is not so amenable. Though not all fragments tested were as potent as thiostrepton, several including **75** show promise and with further medicinal chemistry development may provide a platform for a new class of anti-cancer therapeutic. This could finally put to use a family of natural products long known for their diverse activity that have been sidelined for so long because of their physical properties and lack of access.

Experimental Section

Almost all intermediates tested and reported here are described in the previous chapter unless reported below. Thiostrepton was purchased from Sigma-Aldrich and used as received. Compounds were tested in one of three formats: 96-well, 384-well, or clonogenic assays. Protocols are described below, and the data shown in this chapter was collected from clonogenic assays.

96 well viability assays:

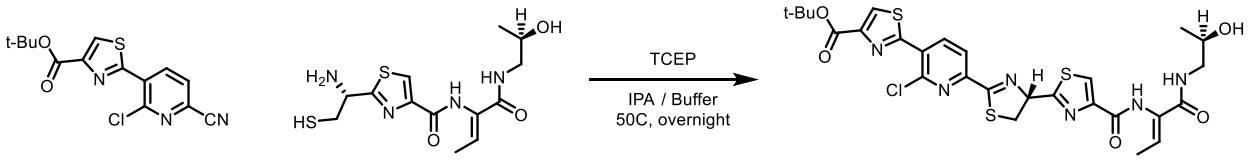
Cancer cells were seeded in clear 96 well plates and allowed to attach overnight. The next day drugs or DMSO solvent control was added and the cells allowed to grow for 72 hours in the presence of drug, and six replicates were performed for each dose. After 72 hours, 20 μL of 10X Resazurin (450 μM) was added to each well and florescence was read on an Infinite F200 plate reader (Tecan) at excitation wavelength 565nM and emission wavelength 590nM. Dose-response curves were fit using 4 parameter non-linear regression in the software package GraphPad Prism (GraphPad Software, Inc).

384 well viability assays:

Cancer cells were seeded in opaque 384 well plates and allowed to attach overnight. The next day drugs or DMSO solvent control was added and the cells allowed to grow for 72 hours in the presence of drug, four replicates were performed for each dose. After 72 hours, cellular viability was assayed using the Cell Titer Glow luminescence protocol (Promega). Luminesce was measured using an EnVision plate reader (PerkinElmer). Dose-response curves were fit using 4 parameter non-linear regression in the software package GraphPad Prism (GraphPad Software, Inc).

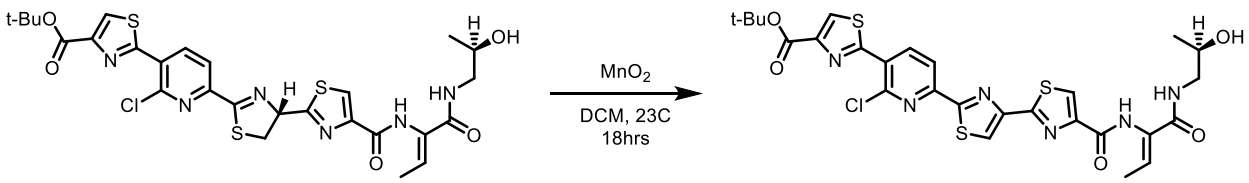
Clonogenic assays:

Clonogenic assays were performed according to standard protocol.[94] Cells were counted using Moxi automated cell counter (Orflo Technologies) and 1000 cells were seeded per plate. Cells were treated with compounds or DMSO solvent control for 9 days. Canon Rebel T3i digital camera was used to create a digital image of each plate, number of colonies larger than cutoff threshold was scored using a custom Matlab script. Number of colonies per plates was normalized to number of colonies on plates treated only with DMSO solvent.



To a solution of nitrile (620 mg, 9.6 mmol, 1.1 eq.) and aminothiol (600 mg, 8.8 mmol, 1 eq.) in 1.5:1 IPA / pH 7 phosphate buffer (17.5 mL, 0.1 M) was added TCEP hydrochloride (85 mg, 2.2 mmol, 0.25 eq.) and the reaction was warmed to 50 C and stirred for 16 hours. The reaction was then concentrated to remove IPA and diluted with 50 mL of water. The mixture was extracted with ethyl acetate (3 x 25 mL), then the combined organic layers were washed with water and brine (25 mL / ea), dried over sodium sulfate and concentrated. The crude material was used directly in the next reaction without further purification. A sample was purified for characterization with 3 --> 7% MeOH/DCM on a medium length column.

^{1}H NMR (600 MHz, MeOD) δ 8.86 (d, *J* = 8.1 Hz, 1H), 8.51 (s, 1H), 8.28 – 8.23 (m, 2H), 6.69 (q, *J* = 7.1 Hz, 1H), 6.21 (t, *J* = 8.8 Hz, 1H), 4.01 (t, *J* = 10.3 Hz, 1H), 3.92 – 3.81 (m, 2H), 3.30 – 3.25 (m, 1H), 3.23 – 3.16 (m, 1H), 1.80 (d, *J* = 7.1 Hz, 2H), 1.63 (s, 9H), 1.15 (d, *J* = 6.3 Hz, 3H). **^{13}C NMR** (151 MHz, MeOD) δ 173.60, 172.98, 167.35, 162.52, 162.26, 161.93, 152.18, 150.54, 149.55, 148.36, 141.54, 131.99, 131.39, 131.11, 131.10, 126.54, 121.91, 83.74, 79.58, 67.36, 48.09, 38.55, 28.41, 20.87, 13.85.



Thiazoline starting material (345mg, 0.53mmol, 1 eq.) is dissolved in dry DCM (10mL, 0.05M) and manganese (IV) oxide (~20 eq.) is added (Activated; Alfa Aesar; tech. 90%; LOT: W08D050). The reaction is stirred rapidly for 18 hours, then filtered through a pad of silica with methanol to give the desired product as a yellow solid (>99% yield).

^{1}H NMR (599 MHz, $CDCl_3$) δ 8.97 (d, *J* = 8.1 Hz, 1H), 8.74 (s, 1H), 8.28 (d, *J* = 8.1 Hz, 1H), 8.24 (s, 1H), 8.23 (s, 1H), 8.19 (s, 1H), 6.71 (t, 1H), 6.63 (q, *J* = 7.1 Hz, 1H), 4.09 – 4.00 (m, 1H), 3.55 (ddd, *J* = 13.8, 6.5, 2.8 Hz, 1H), 3.15 (ddd, *J* = 13.8, 8.2, 5.7 Hz, 1H), 1.85 (d, *J* = 7.1 Hz, 3H), 1.65 (s, 9H), 1.21 (d, *J* = 6.3 Hz, 3H); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 167.25, 165.57, 162.63, 161.22, 160.43, 159.92, 150.81, 150.02, 149.73, 148.82, 147.63, 140.91, 129.72, 129.25, 129.15, 128.71, 125.68, 120.82, 118.88, 99.37, 82.69, 66.96, 57.91, 47.66, 28.32, 20.76, 13.90;

Chapter 2, in part is unpublished work, was contributed to by J.P. Shen, M.D. and Dionicio Siegel by generating data for the anti-cancer assays from compounds made in the lab. I would like to thank our collaborators in the Ideker Lab at UC San Diego for their work in producing data in biological experiments. Mitchell P. Christy is the primary author of this material.

Chapter 3: Conclusions and Prospects

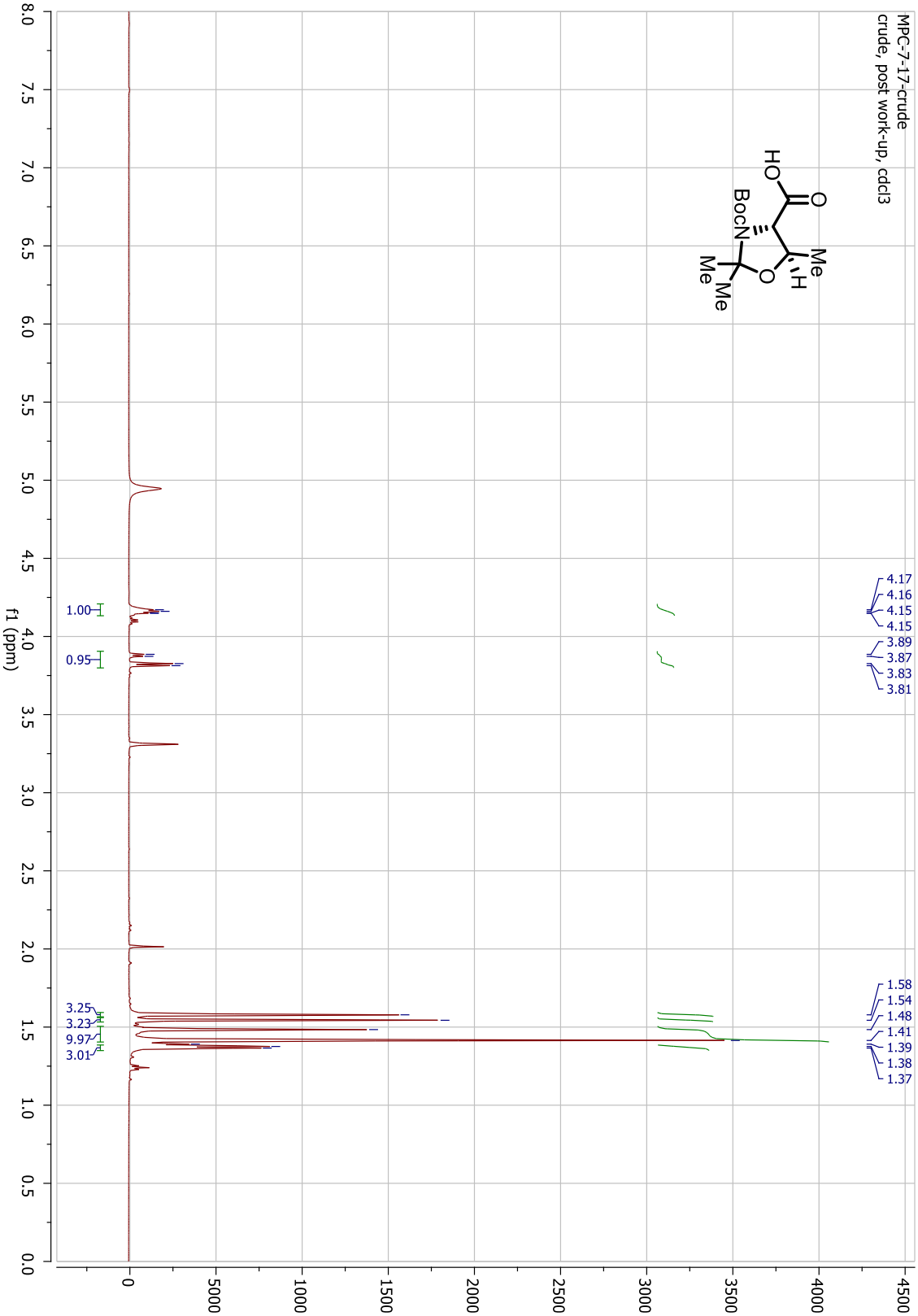
With the synthesis of micrococcin we were able to reveal a minimum scaffold for activity in a simpler form (series D rather than series B core) with comparable activity to that of previous thiopeptide fragments that is more easily accessible. The central pyridine of series D thiopeptides is the essence of these natural products activity in anti-cancer assays, without the complex stereocenters like that of thiostrepton. Several di- and trithiazolyl pyridine fragments showed low micromolar (and one with high nanomolar) activity, though there appears to be a size threshold for activity. Larger trisubstituted fragments including **71** and **76** tended to perform poorly as compared to their smaller precursors **30**, **74**, and **75**. That these fragments retain activity where the natural product micrococcin does not is a promising discovery, where medicinal chemistry development may reveal a more potent analog that is more easily accessible and higher yielding than previously reported fragments of thiostrepton. Previously, only series B thiopeptides and their complex central piperidine fragments were known to have this activity. This finding shows that not only series B thiopeptides like thiostrepton and siomycin A have anti-cancer activity. That fragments of micrococcin P1 show comparable activity indicates a broader scope of activity in the family that may be explored.

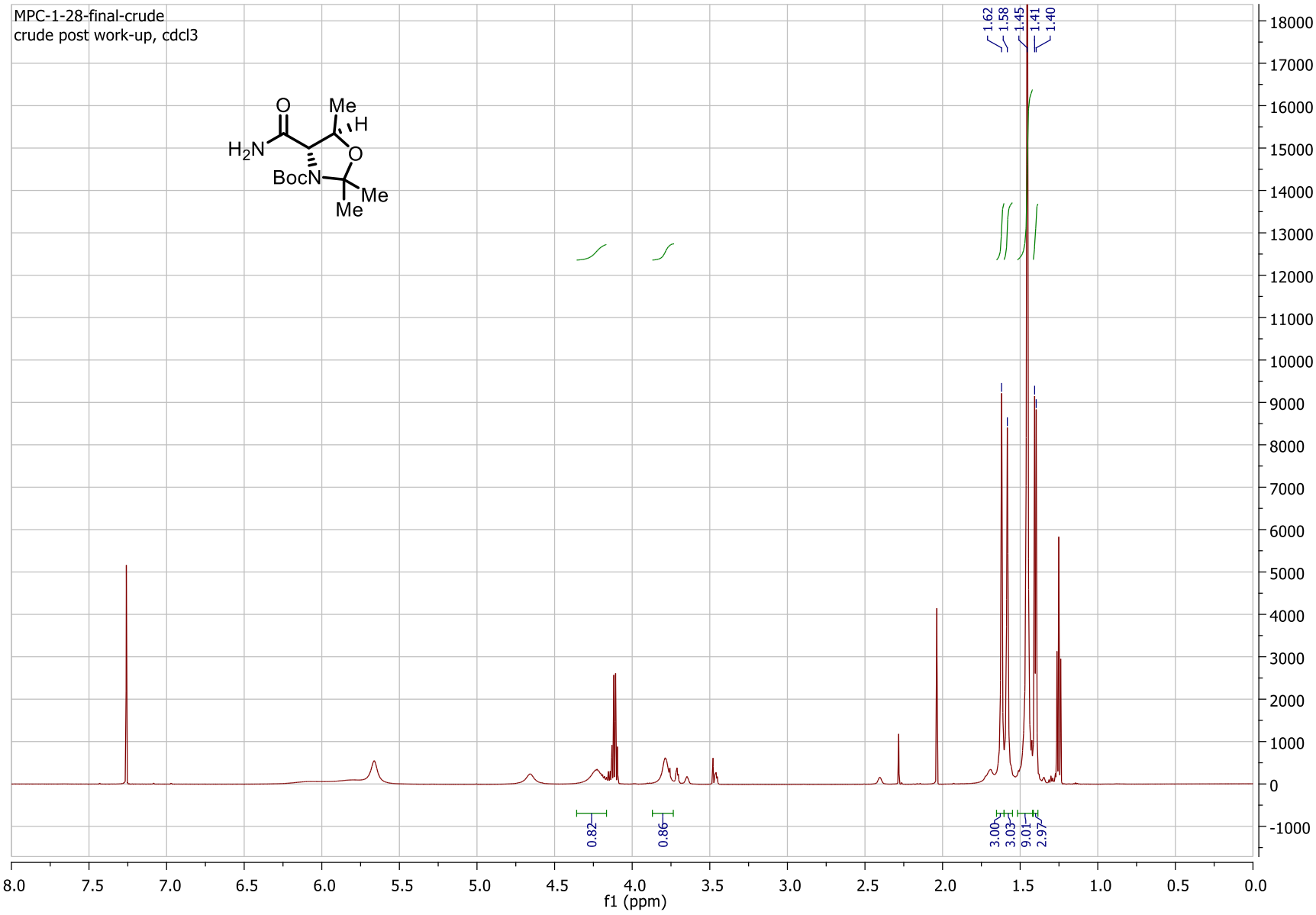
In an effort to expand the methods of syntheses of these compounds and more closely study their *in vitro* activity, we completed the total synthesis of micrococcin P1 enabled by robust thiazole forming reactions. Never before used in the synthesis of thiopeptides, we were able to access decagram quantities of several of the thiazole containing fragments through condensation of nitriles and aminothiols followed by mild oxidation. These thiazole forming reactions are robustly scalable and we were able to produce nearly 200 mg of the natural product, with 5 of the 6 thiazoles in micrococcin formed using this methodology. The synthesis

of this natural product was especially trim with very little chromatographic purification necessary, where techniques such as precipitation and recrystallization were used to provide pure material instead.

Thiopeptides are a versatile group of natural products with a wealth of therapeutic potential. Because of their wide scope of activity in a number of different contexts, it is not difficult to see why they have been studied so extensively. As it has been from the beginning of pharmaceutical research, natural products have been the inspiration for many drugs and therapeutics. Thiopeptides, with all they have to offer, have the potential to advance treatments for cancer, provide as antibiotics against multi-drug resistant bacteria, and more. Even now we have only scratched the surface, and as this project aims to push for further development, we hope to enable future discovery by providing access to these compounds.

APPENDIX: CATALOG OF SPECTRA

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H_2N
O
BocN
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Me
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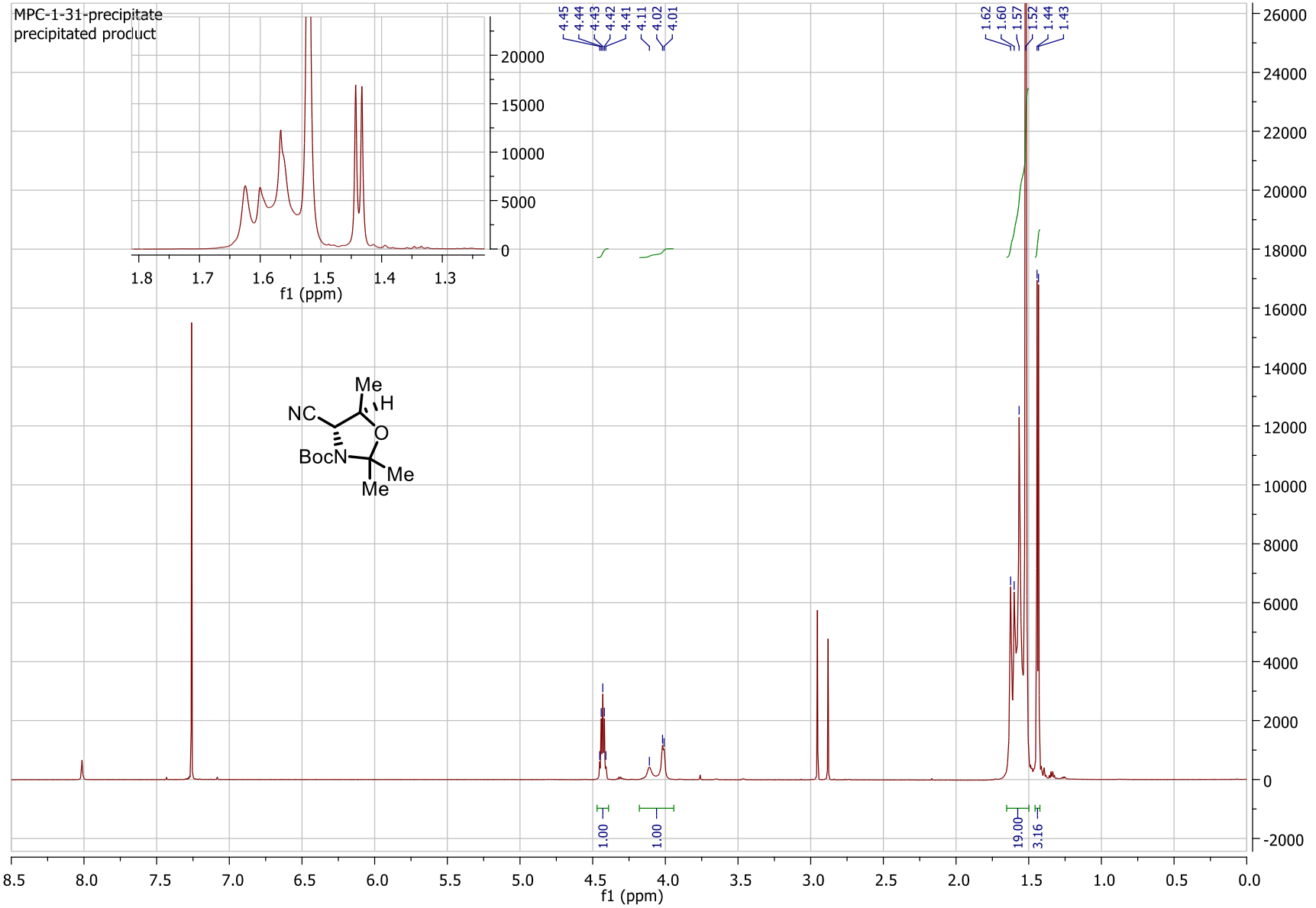
3.00
3.03
9.01
2.97
f1 (ppm)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4.41
4.11
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4.01
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1.57
1.52
1.44
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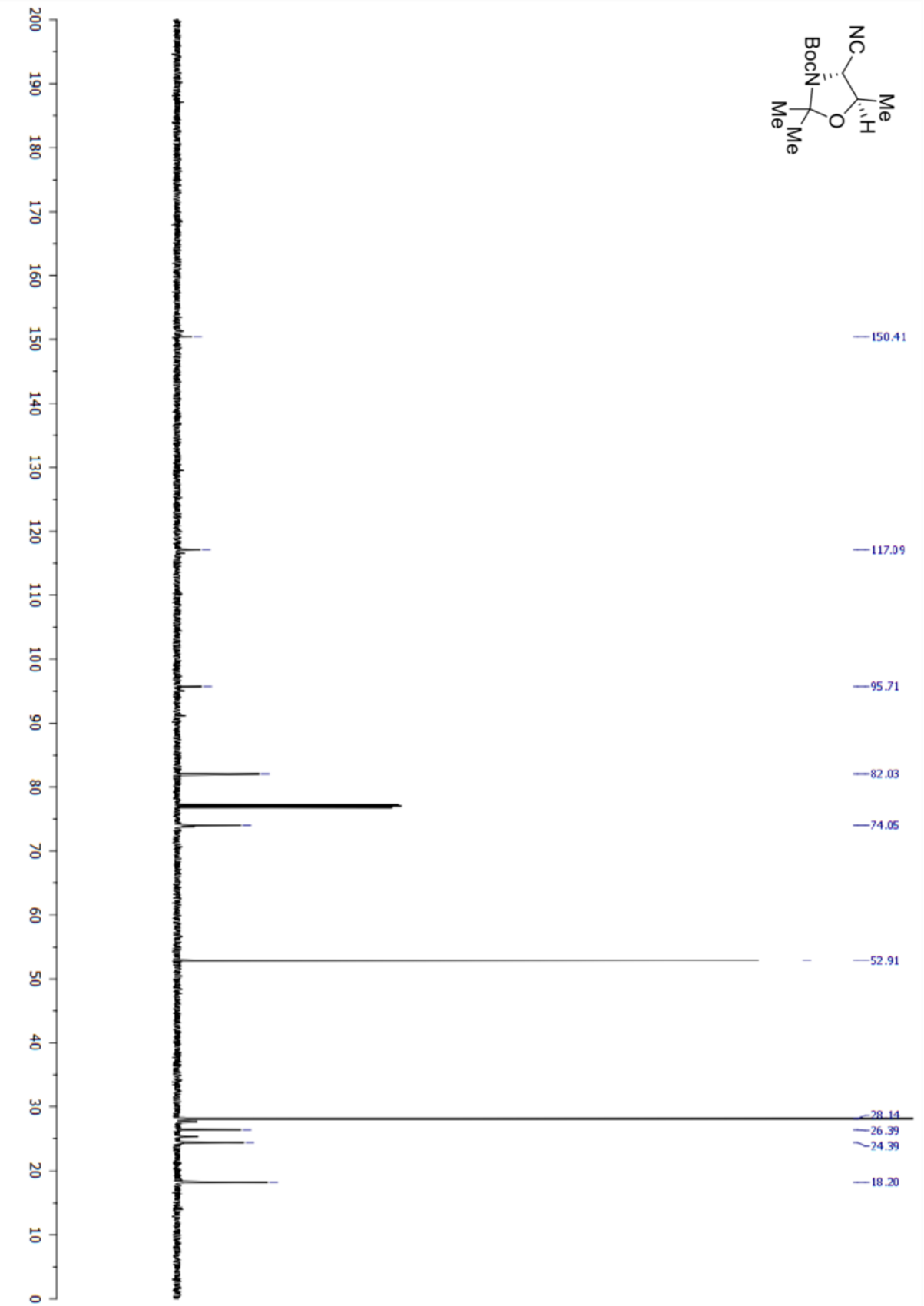
f1 (ppm)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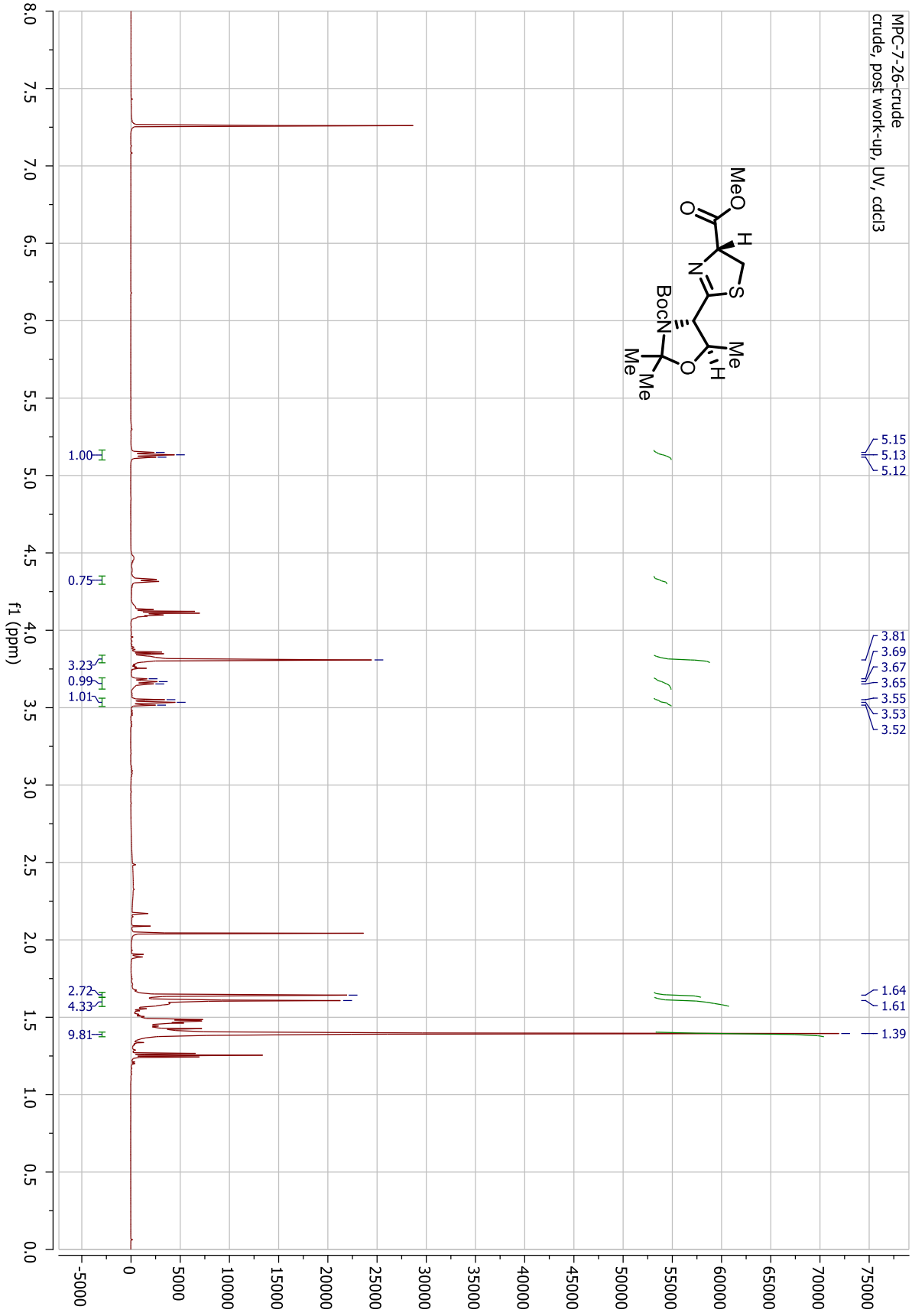
18.20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3.69
3.67
3.65
3.55
3.53
3.52
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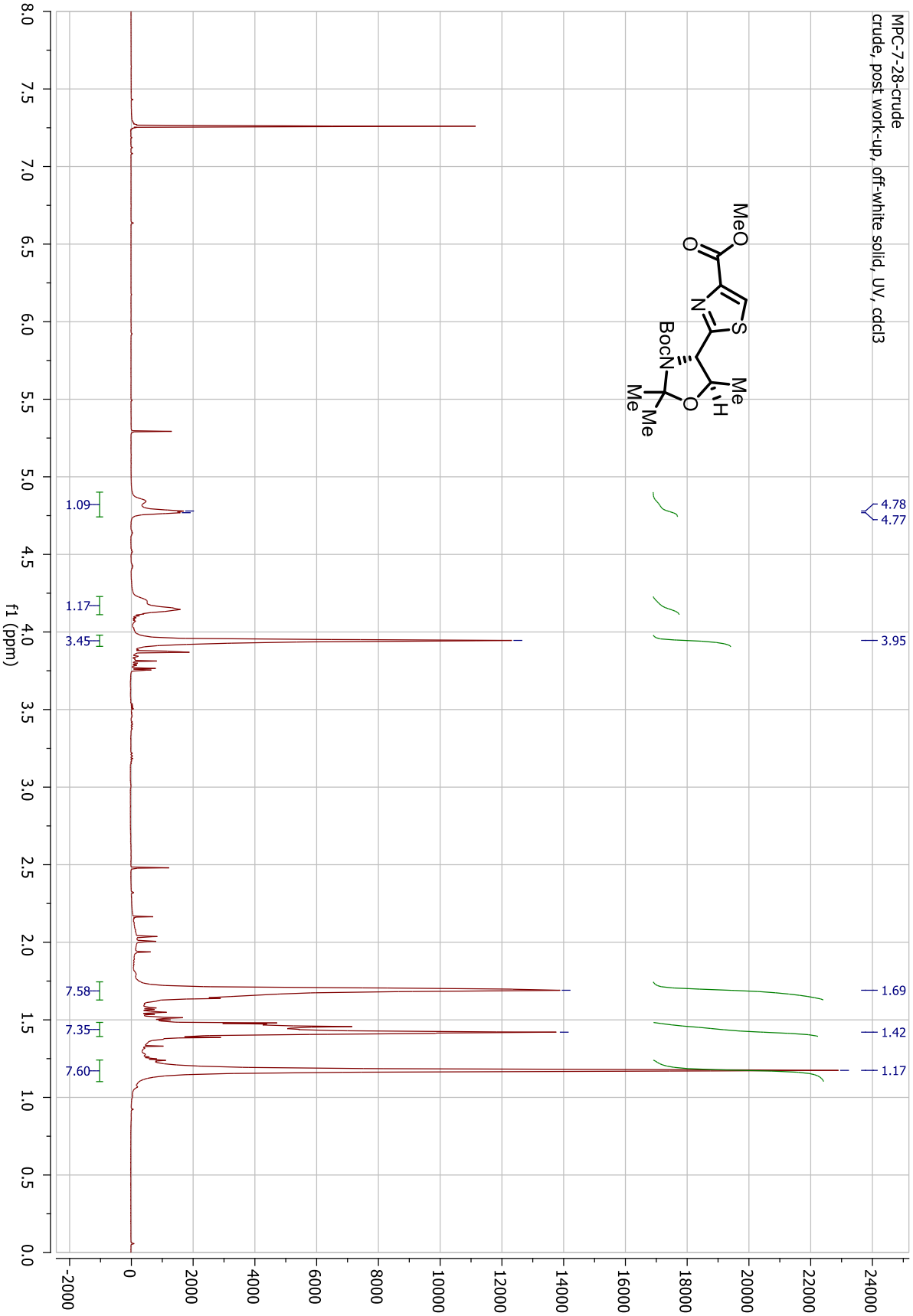
f1 (ppm)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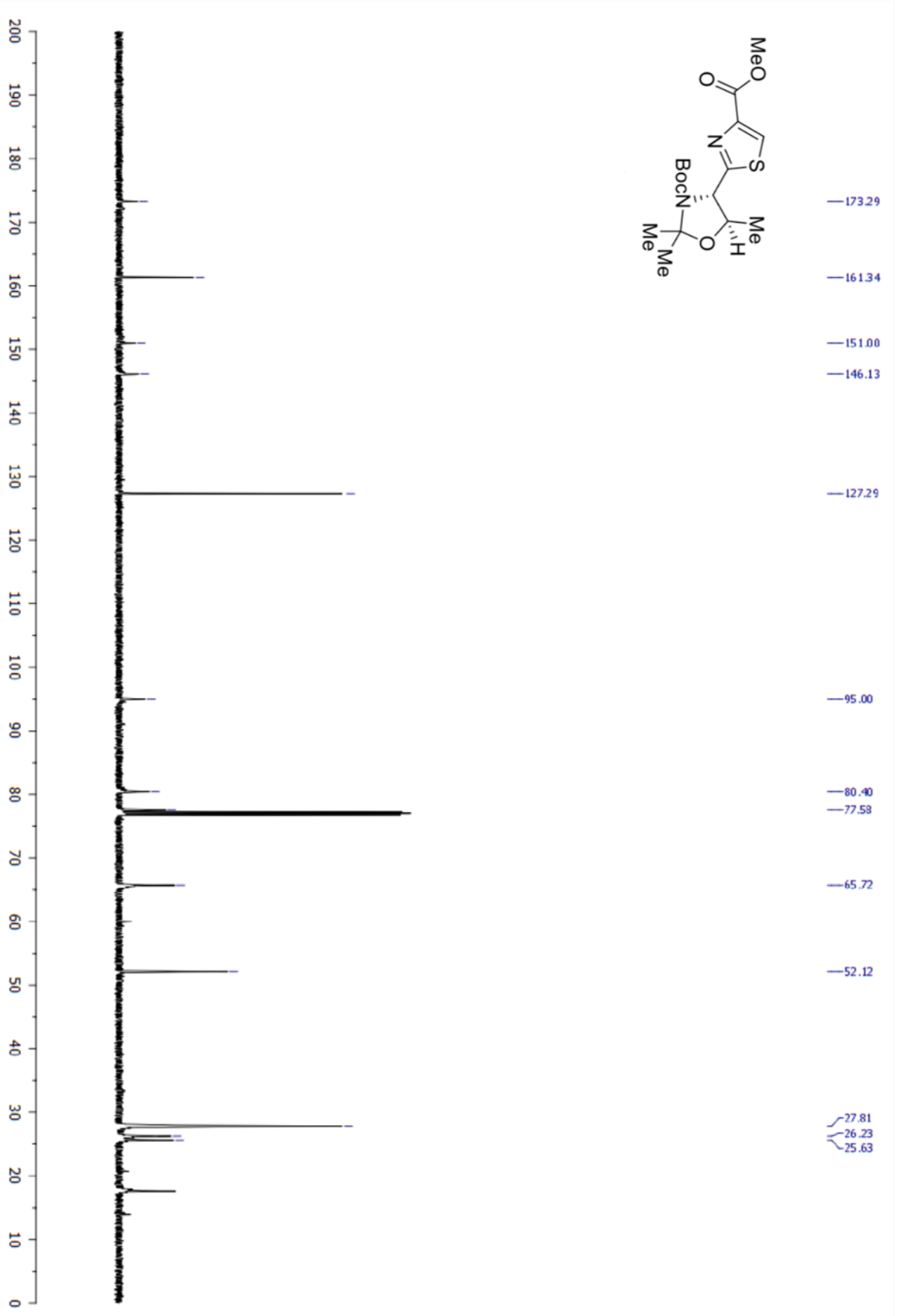
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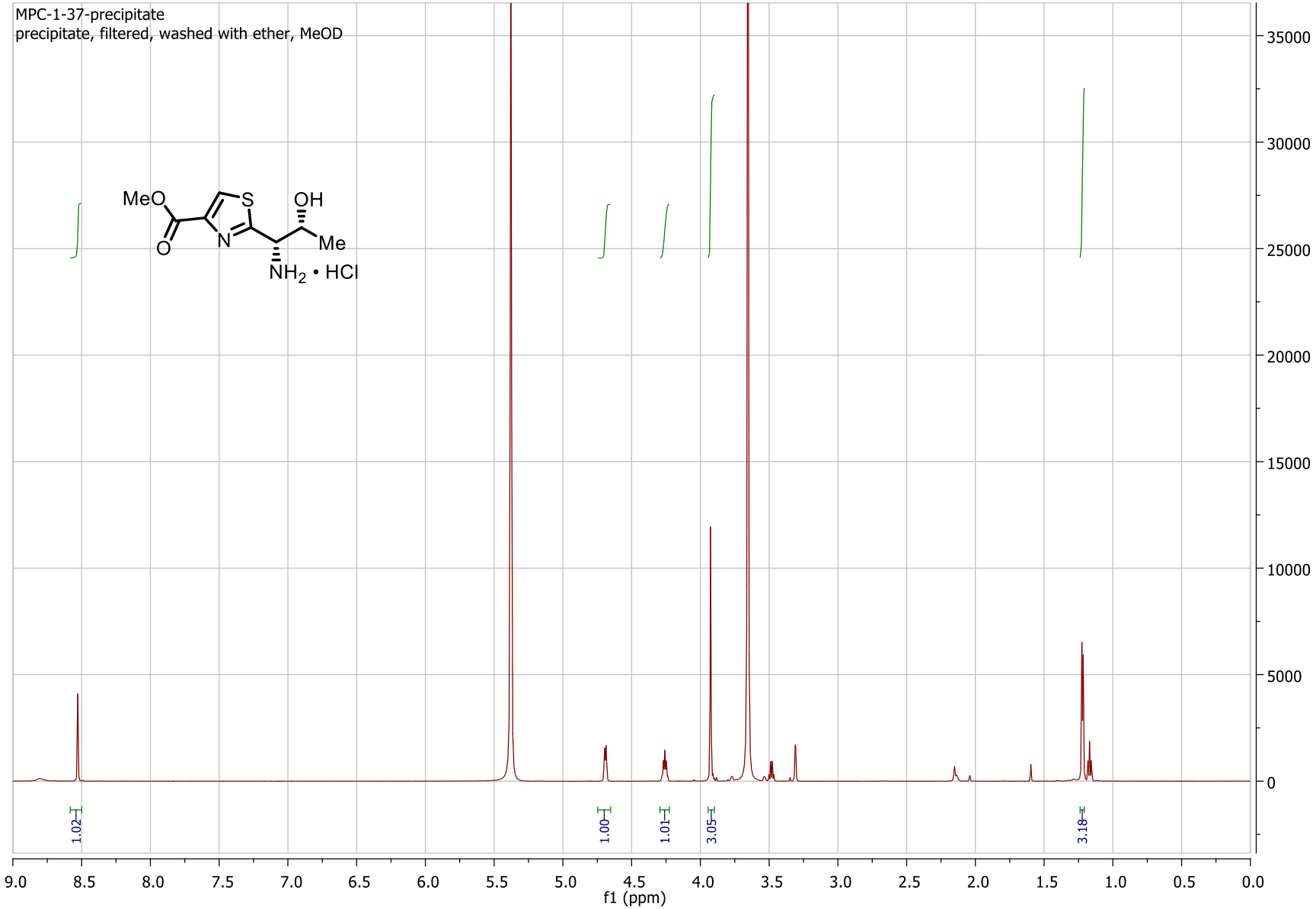
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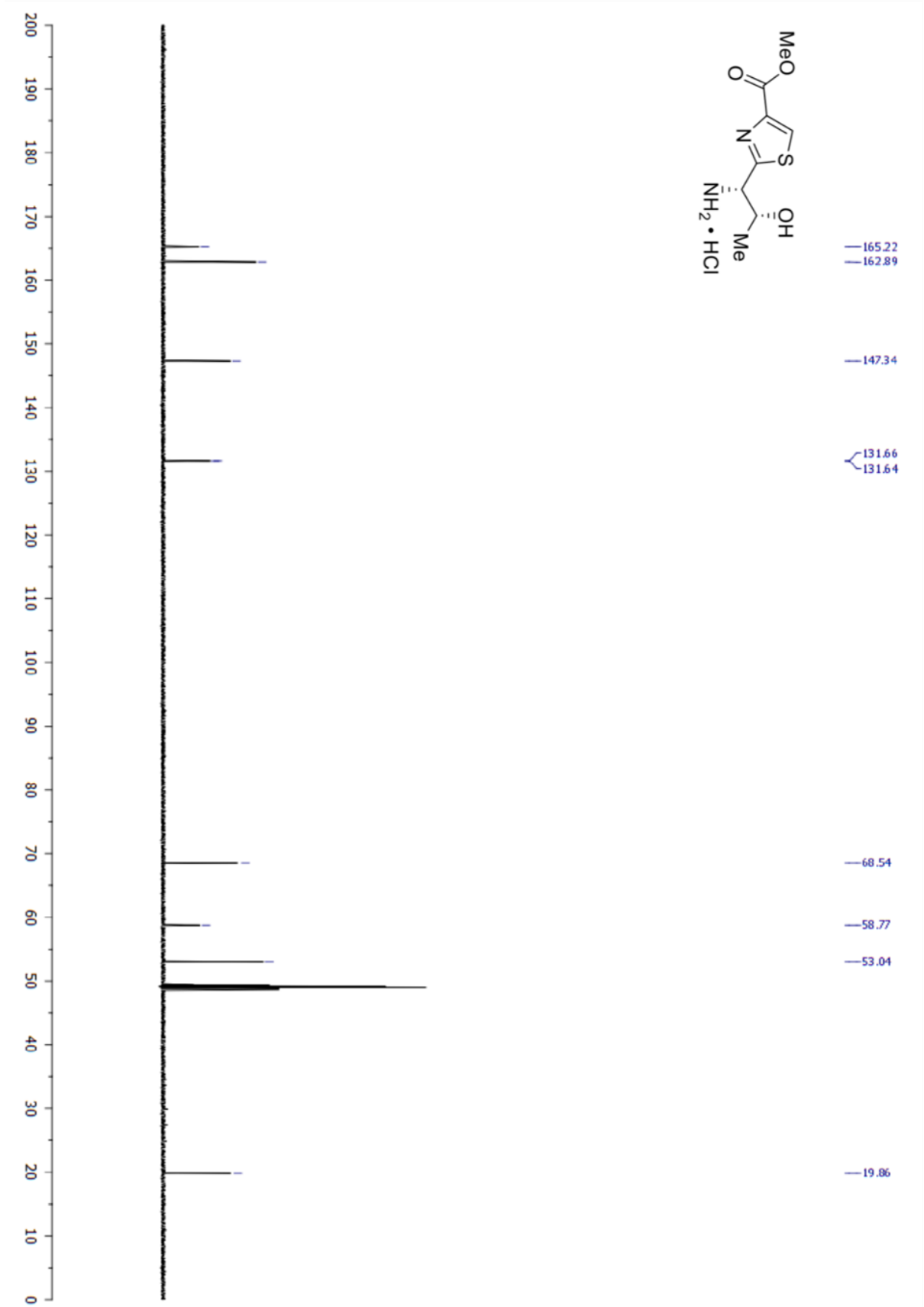
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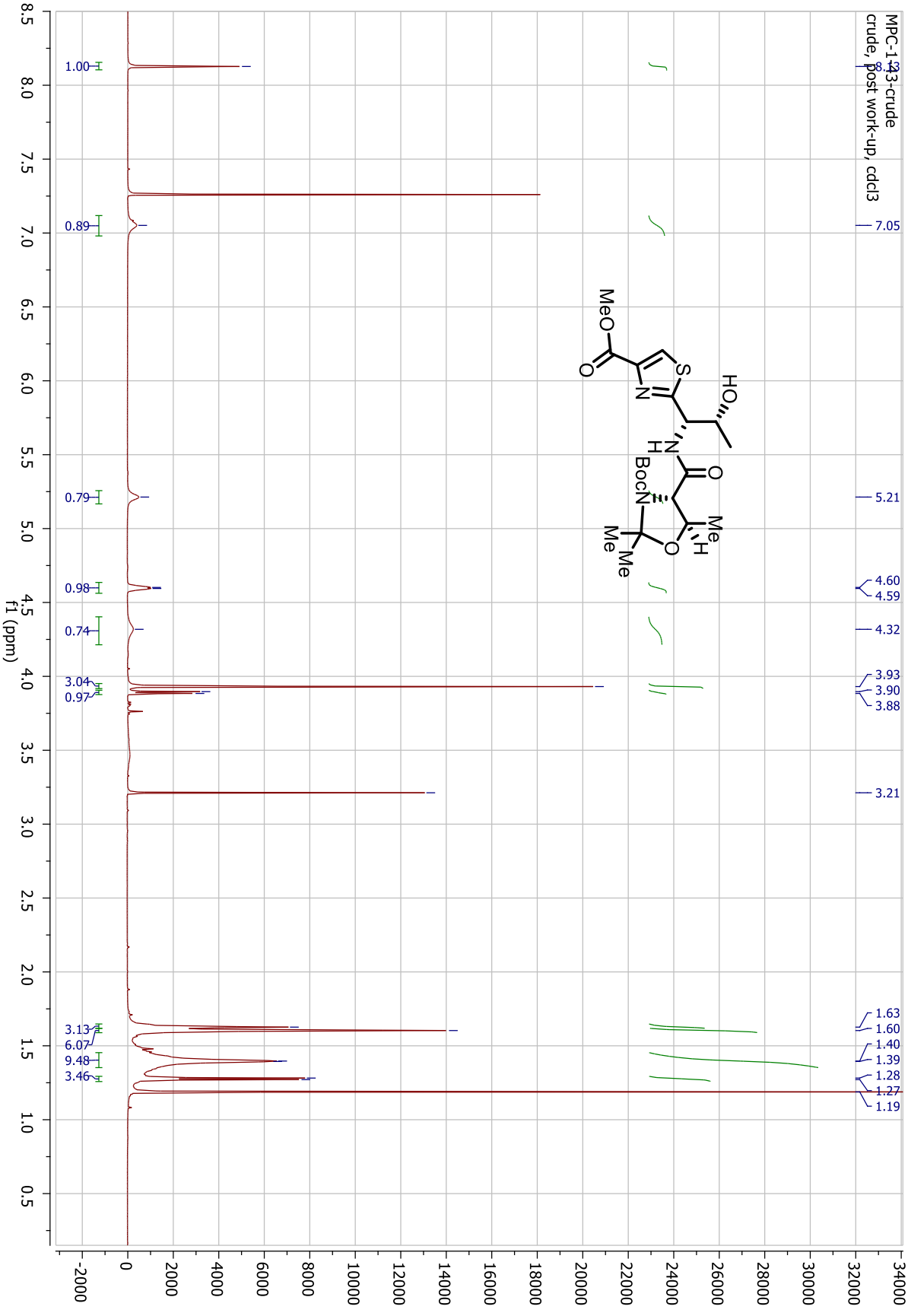
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crude, post work-up, cdcl3
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4.60
4.59
4.32
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3.90
3.88
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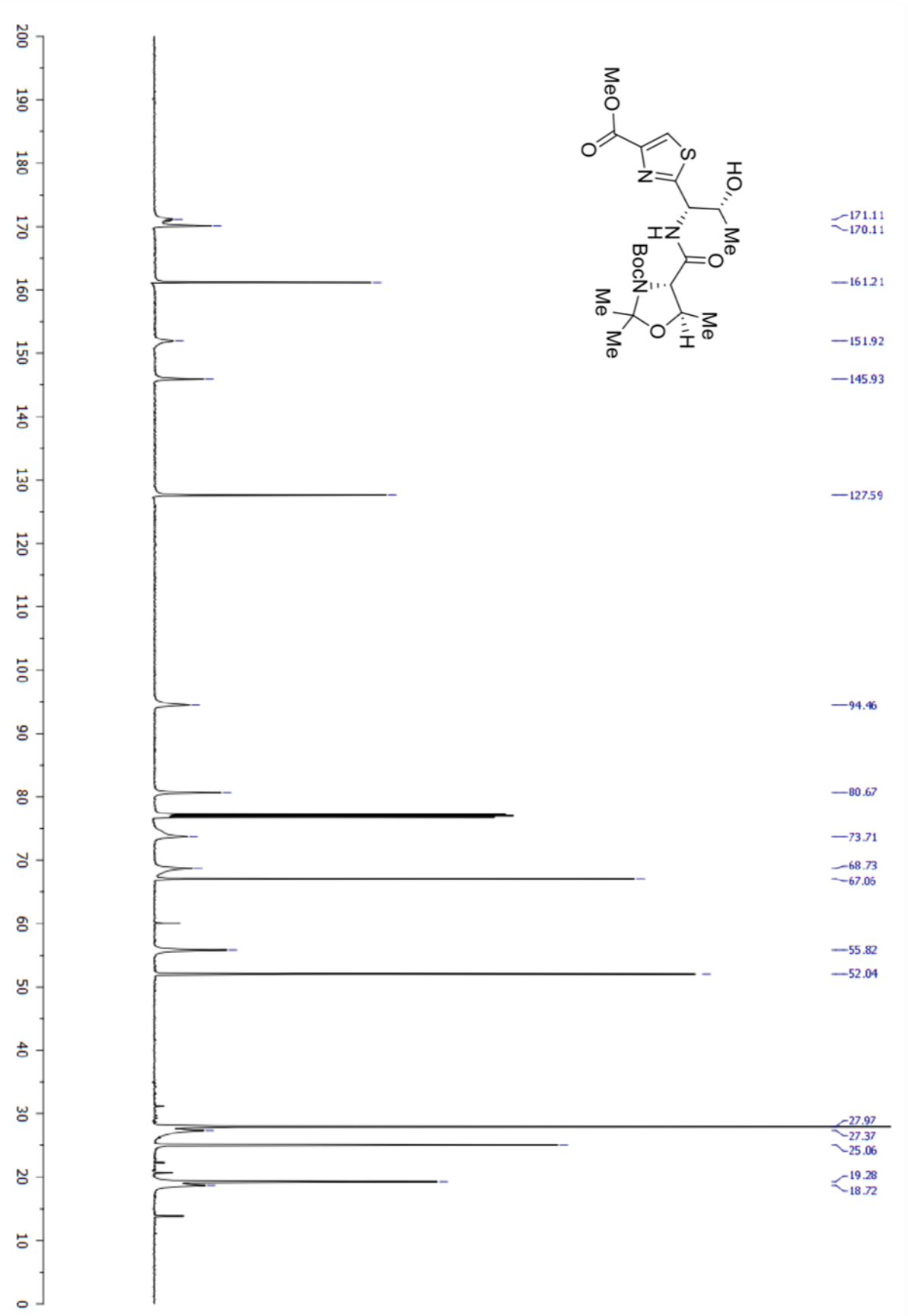
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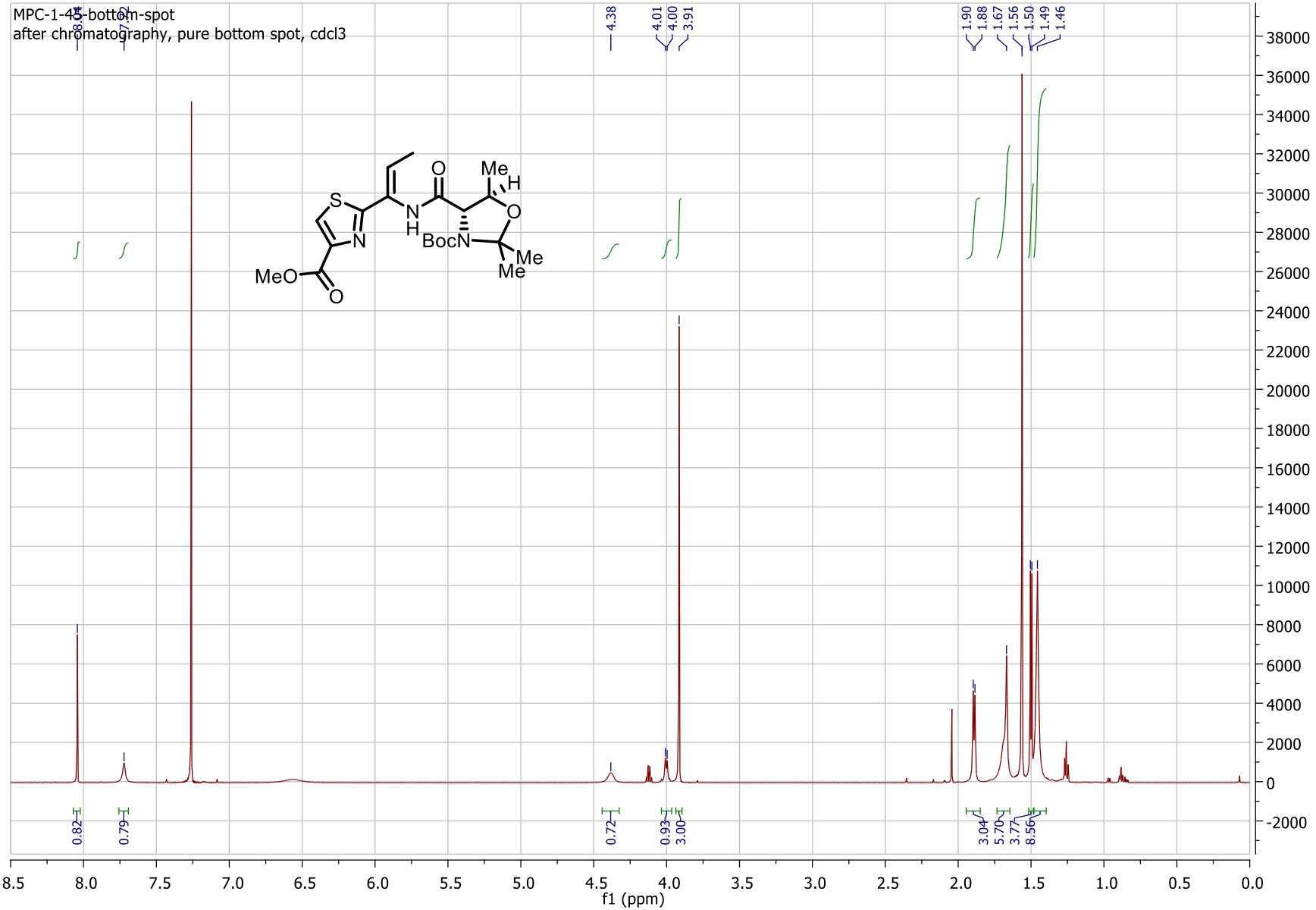
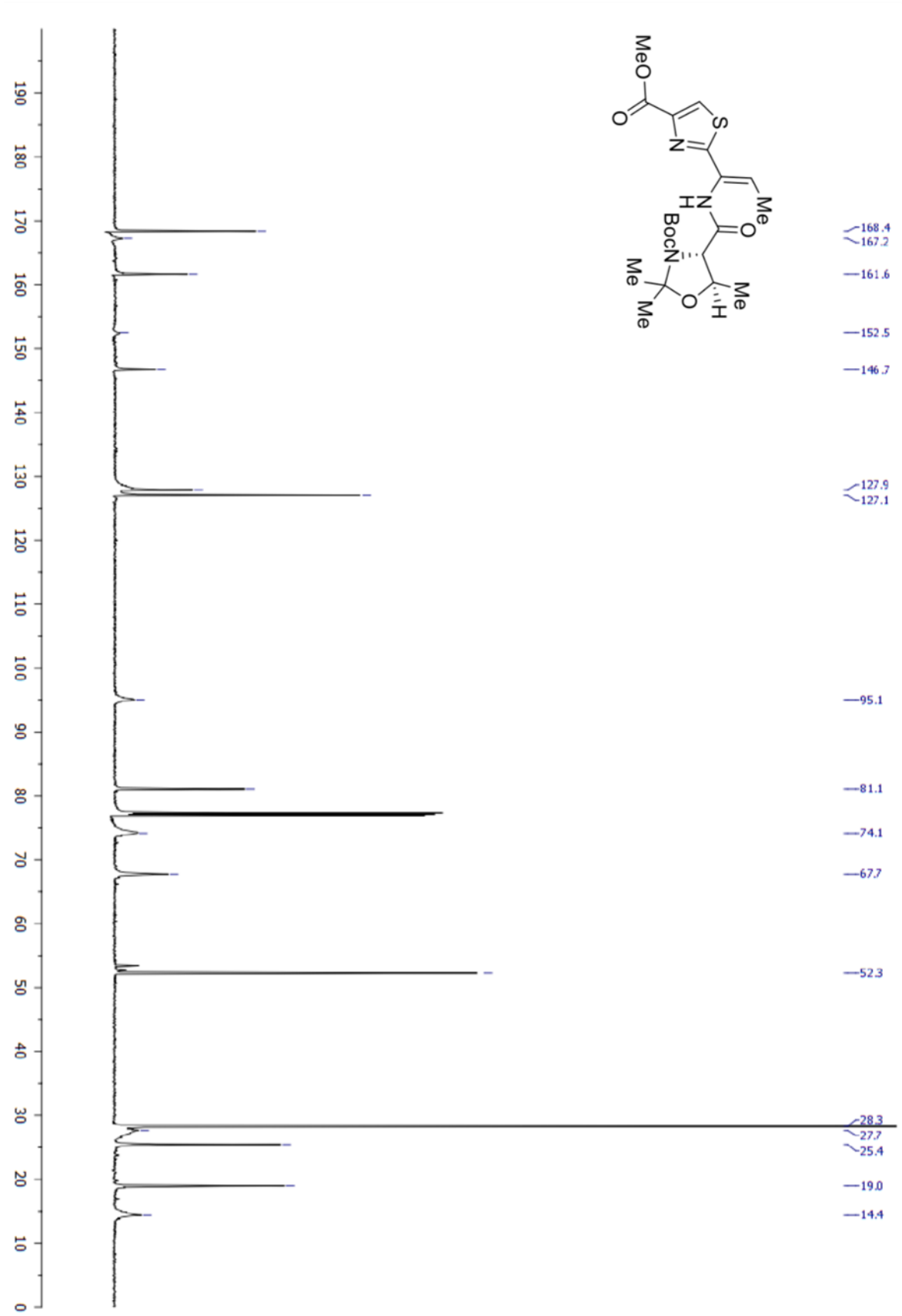
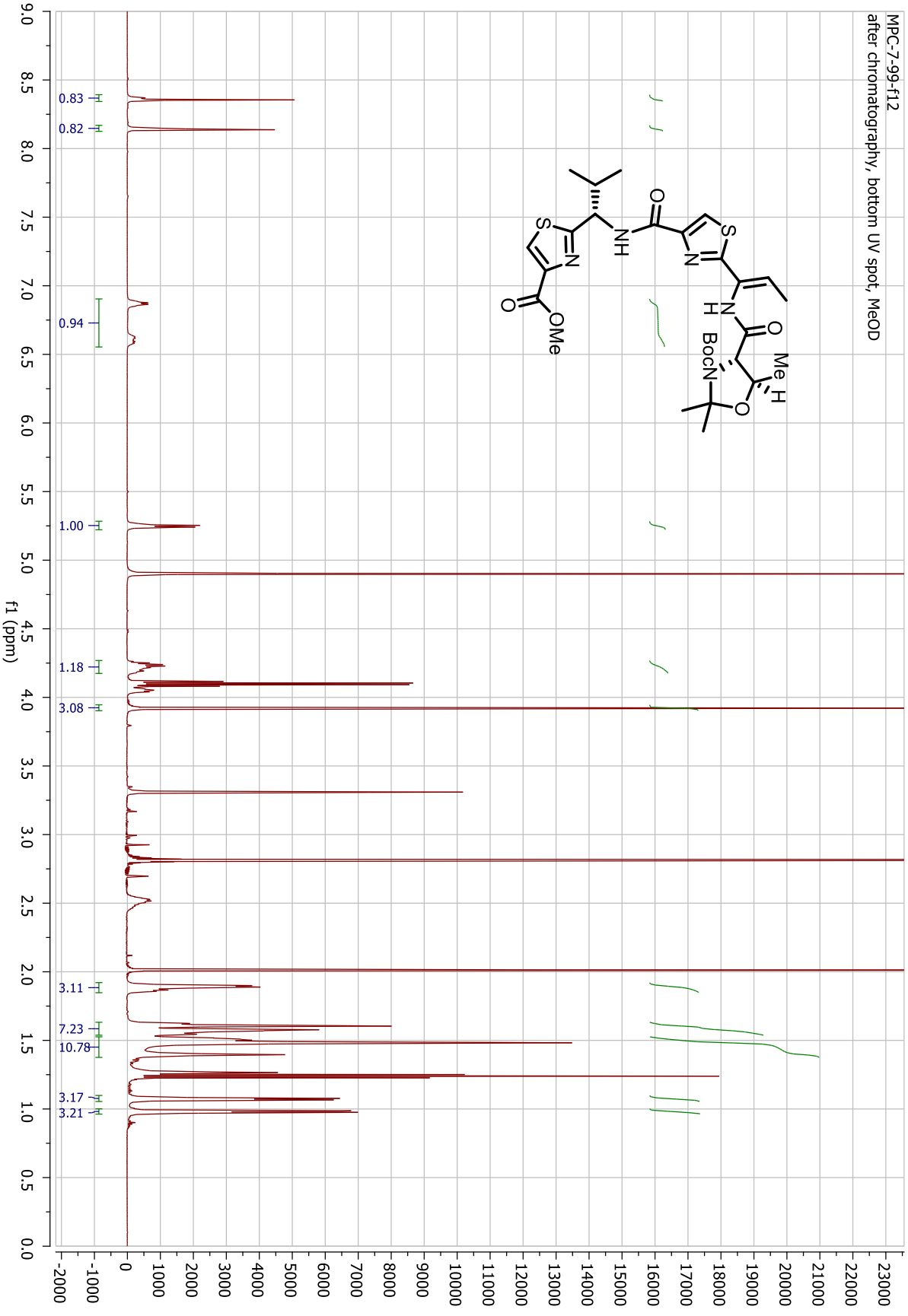
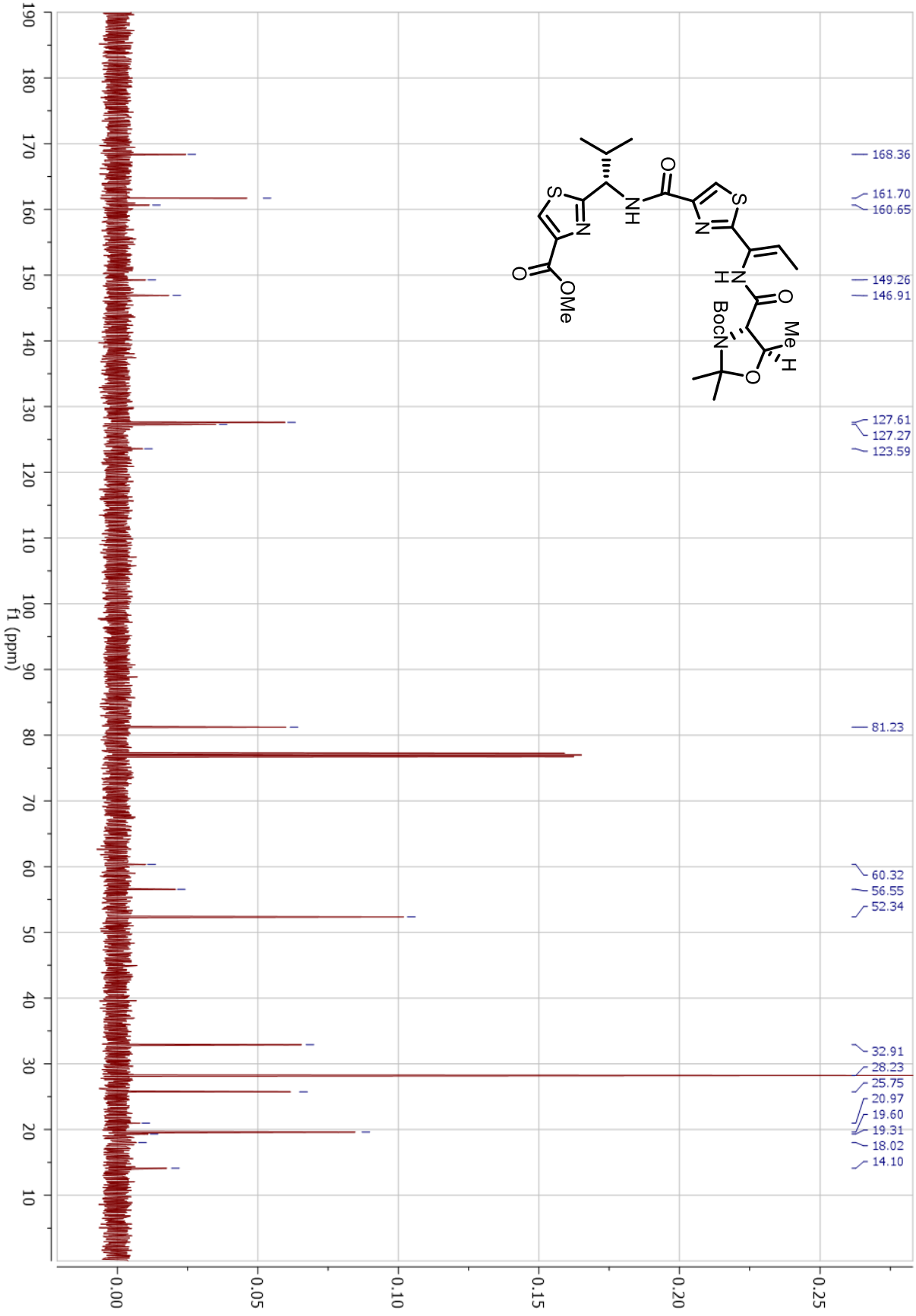
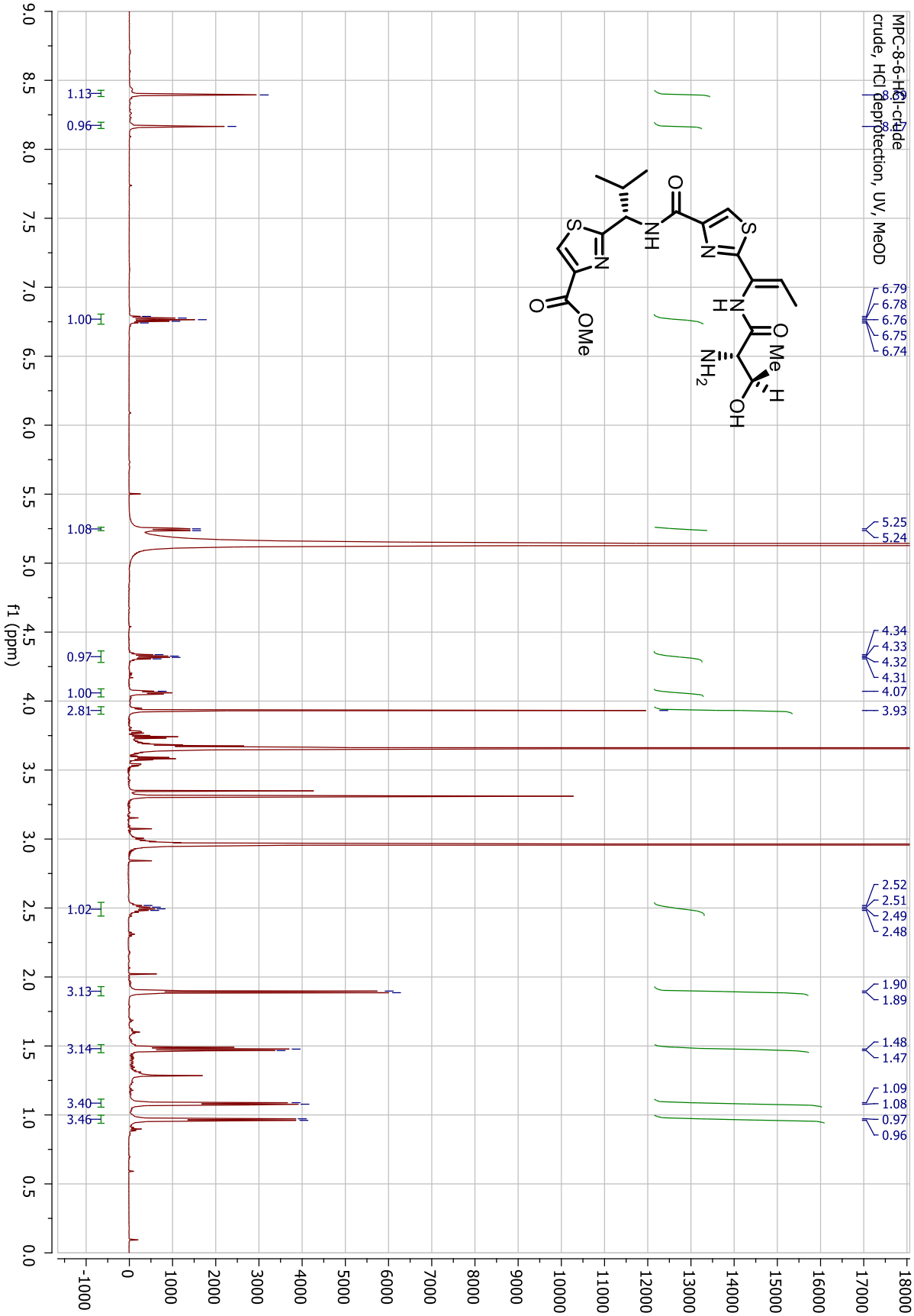
40
30
20
10
0

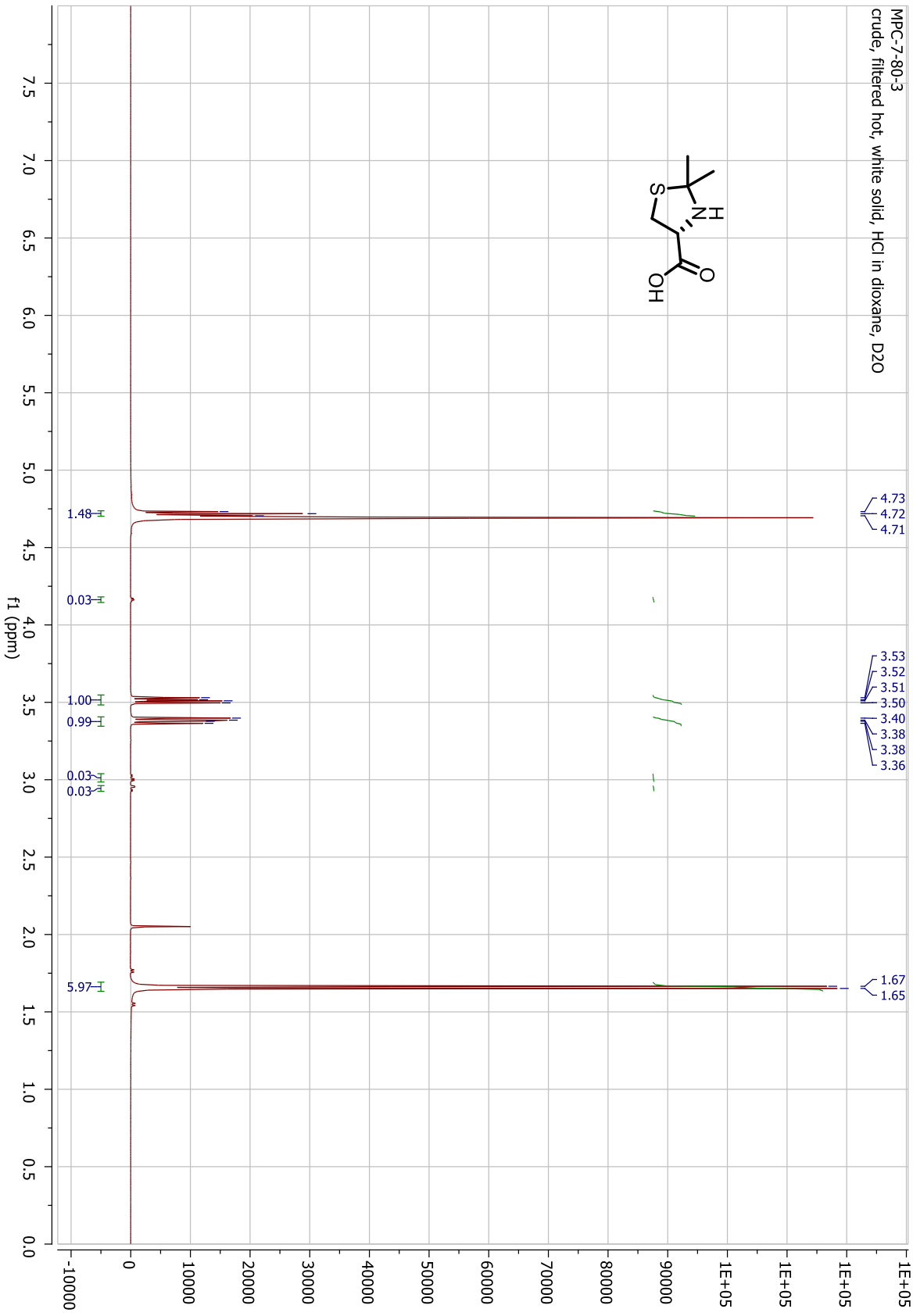

MPC-1-45-bottom-spot
after chromatography, pure bottom spot, cdcl3
8.04
7.72
4.38
4.01
4.00
3.91
1.90
1.88
1.67
1.56
1.50
1.49
1.46
0.82
0.79
0.72
0.93
3.00
3.04
5.70
3.77
8.56
f1 (ppm)

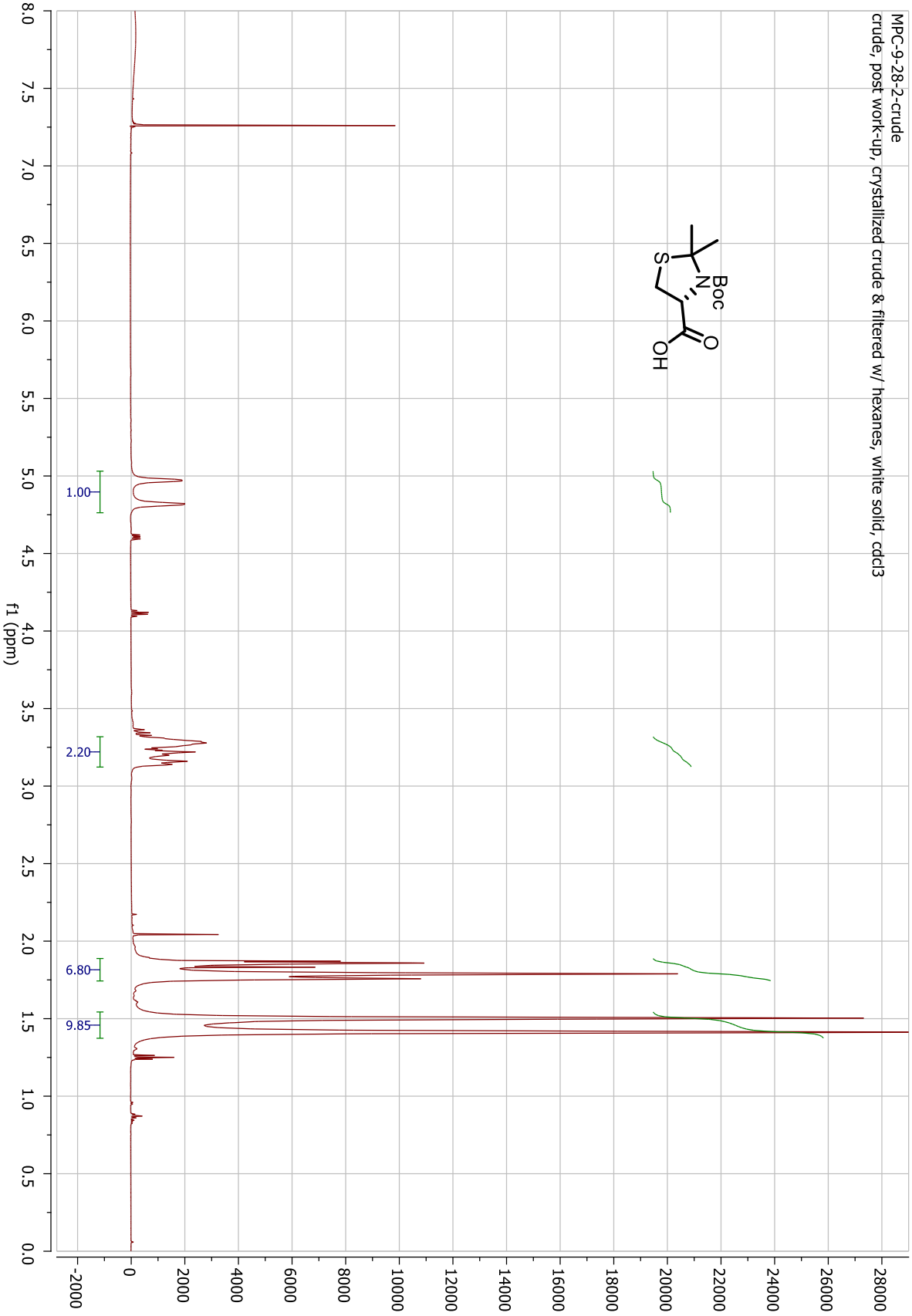

MeO
O
S
N
N
H
Me
O
BocN
Me
Me
Me
O
H
168.4
167.2
161.6
152.5
146.7
127.9
127.1
95.1
81.1
74.1
67.7
52.3
28.3
27.7
25.4
19.0
14.4
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

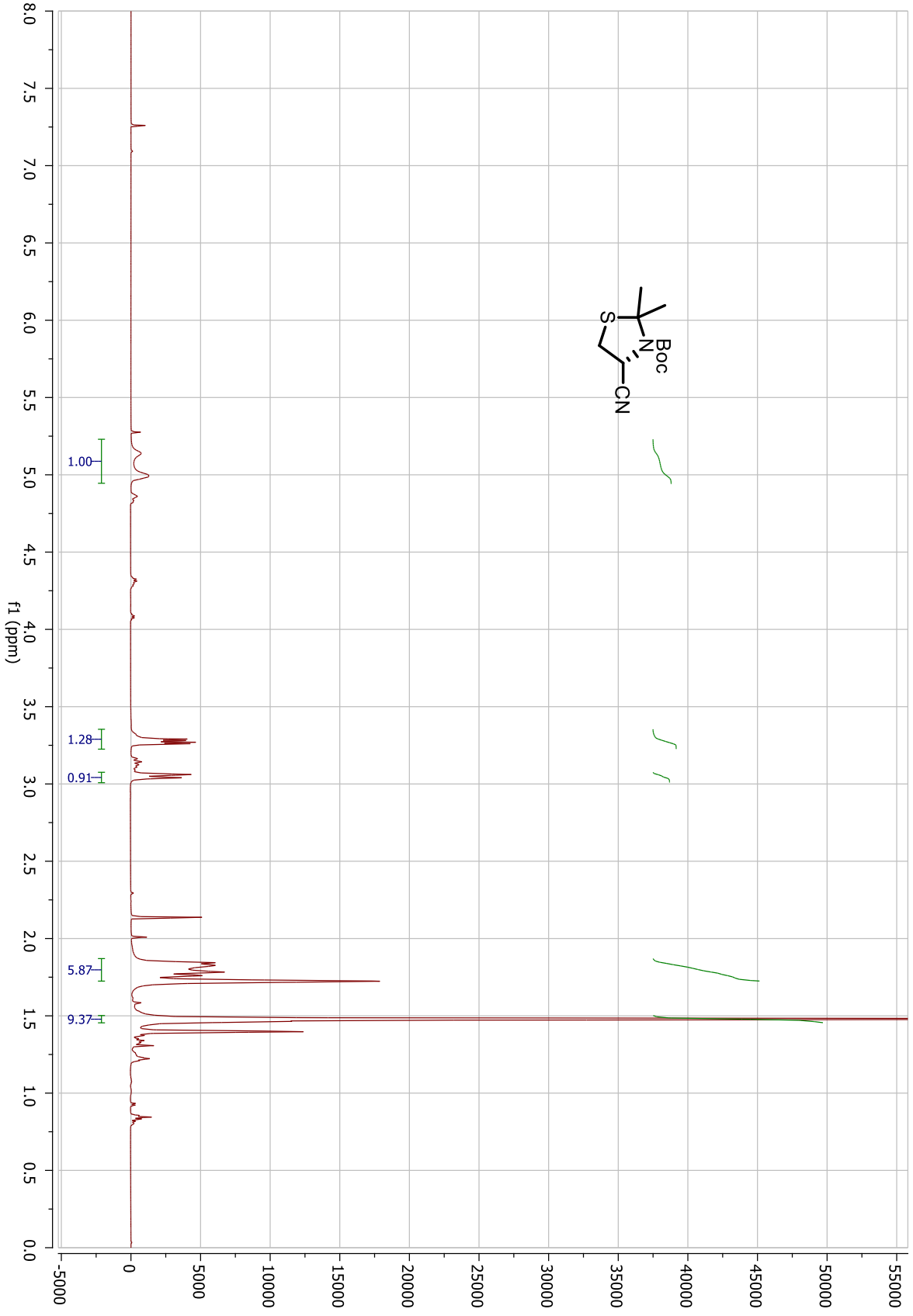

MPC-7-99-f12
after chromatography, bottom UV spot, MeOD
0.83
0.82
0.94
1.00
1.18
3.08
3.11
7.23
10.78
3.17
3.21
f1 (ppm)
OMe
NH
BocN
Me H

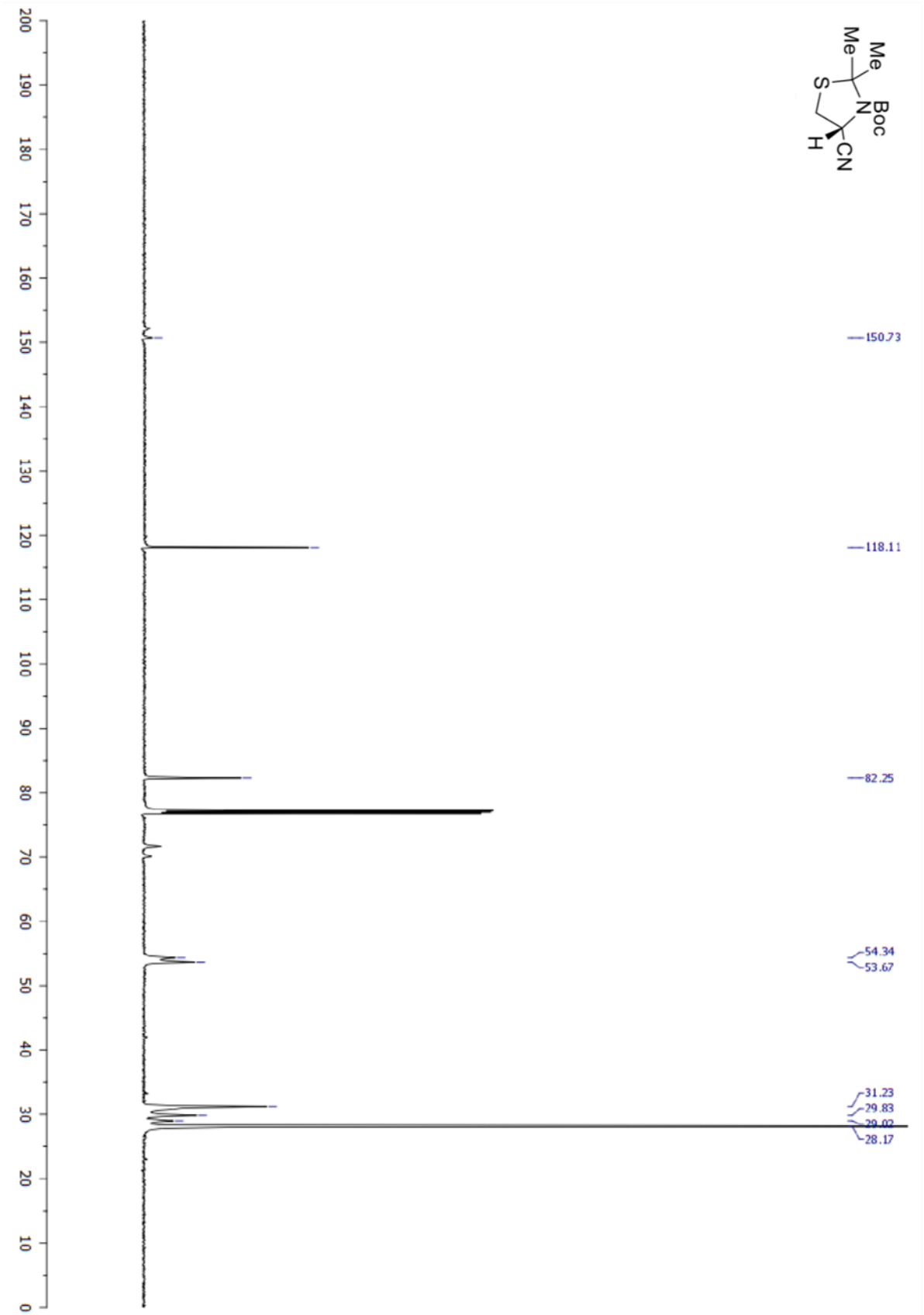

168.36
161.70
160.65
149.26
146.91
127.61
127.27
123.59
81.23
60.32
56.55
52.34
32.91
28.23
25.75
20.97
19.60
19.31
18.02
14.10
f1 (ppm)
OMe
BocN
Me
H

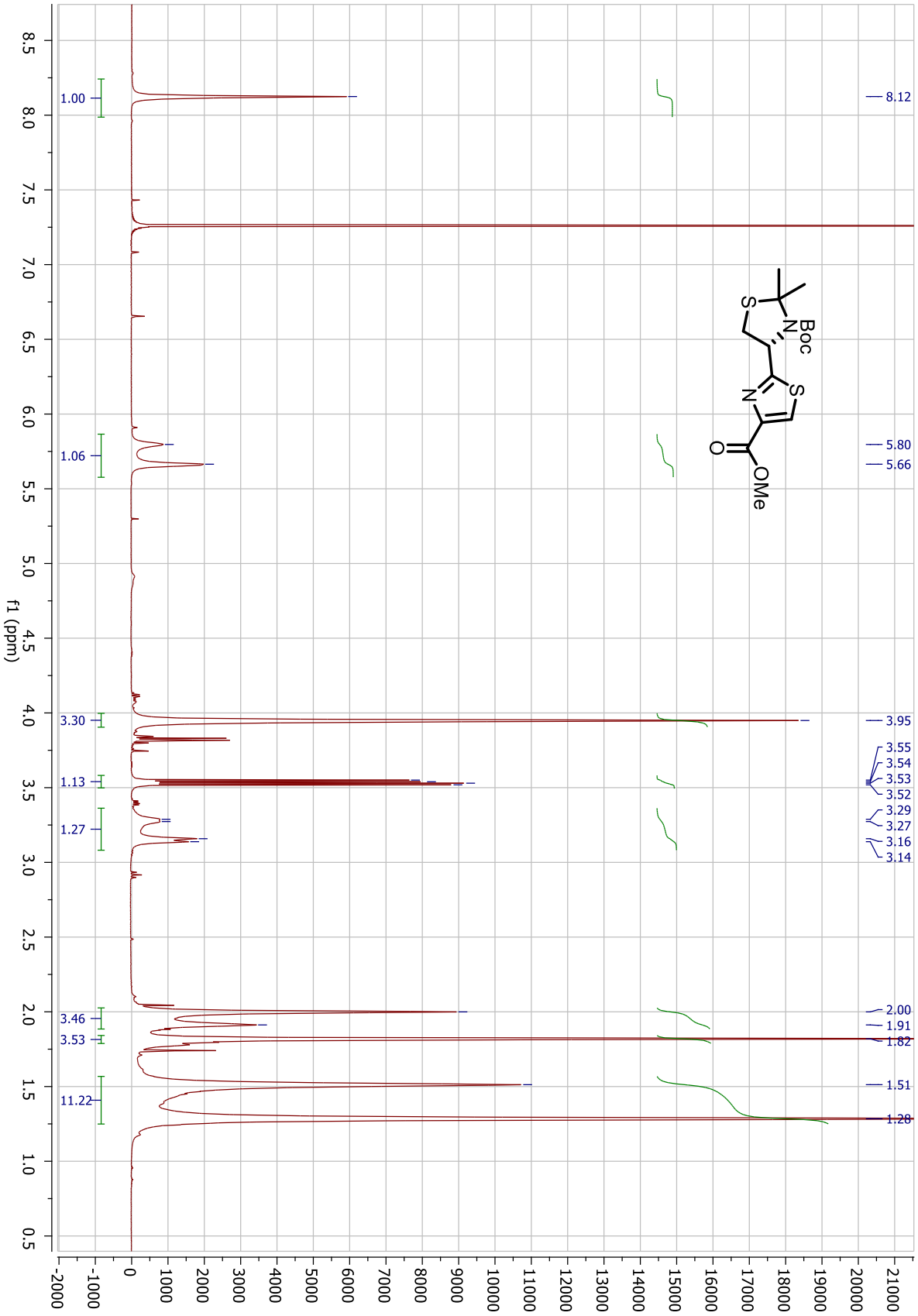

MPC-8-6-HCl-crude
crude, HCl deprotection, UV, MeOD
f1 (ppm)
1.13
0.96
1.00
1.08
0.97
1.00
2.81
1.02
3.13
3.14
3.40
3.46
8.79
8.77
6.79
6.78
6.76
6.75
6.74
5.25
5.24
4.34
4.33
4.32
4.31
4.07
3.93
2.52
2.51
2.49
2.48
1.90
1.89
1.48
1.47
1.09
1.08
0.97
0.96
OMe
NH
NH2
OH
Me
H

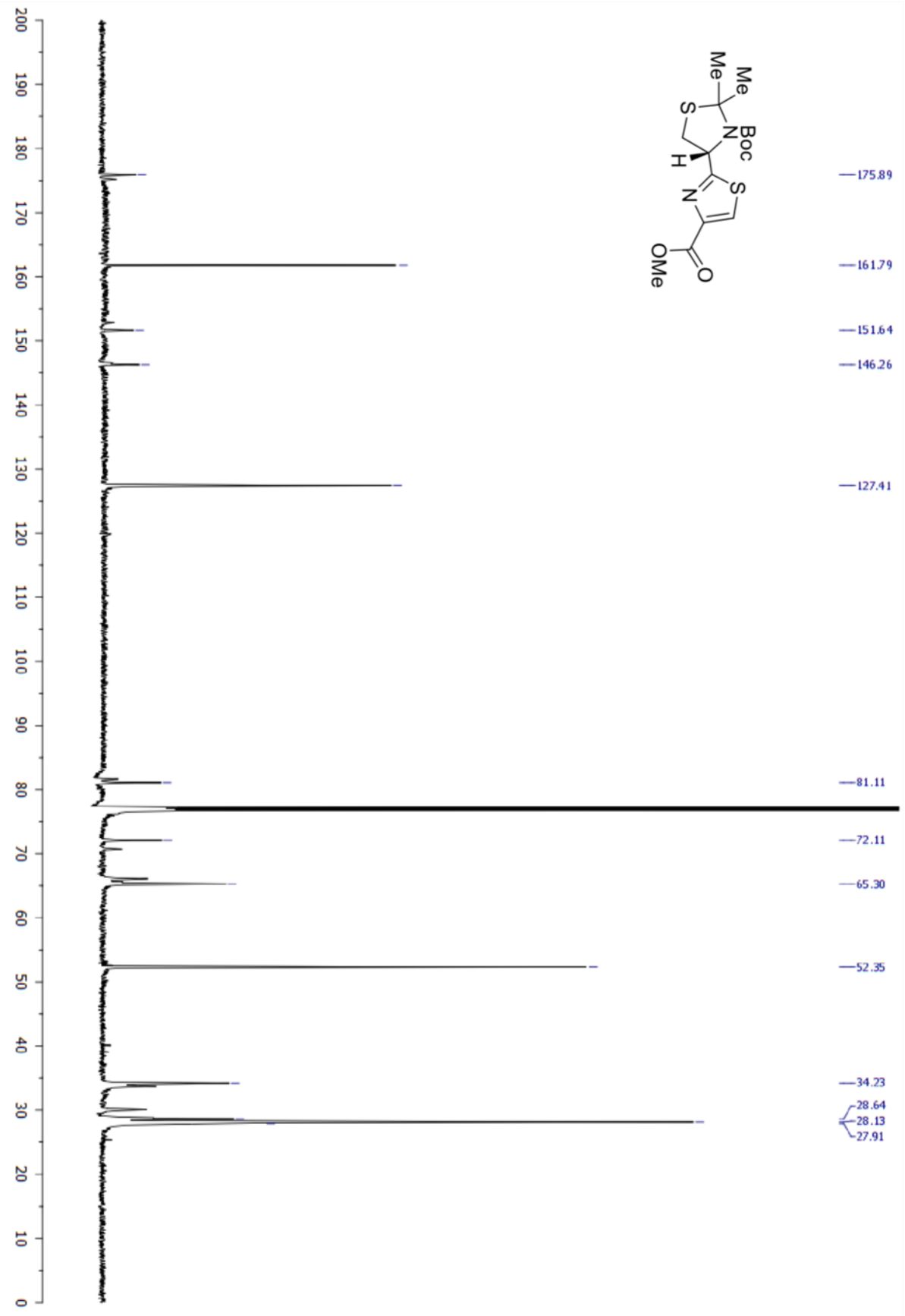

MPC-7-80-3
crude, filtered hot, white solid, HCl in dioxane, D2O
4.73
4.72
4.71
3.53
3.52
3.51
3.50
3.40
3.38
3.38
3.36
1.67
1.65
1.48
0.03
1.00
0.99
0.03
0.03
5.97
f1 (ppm)

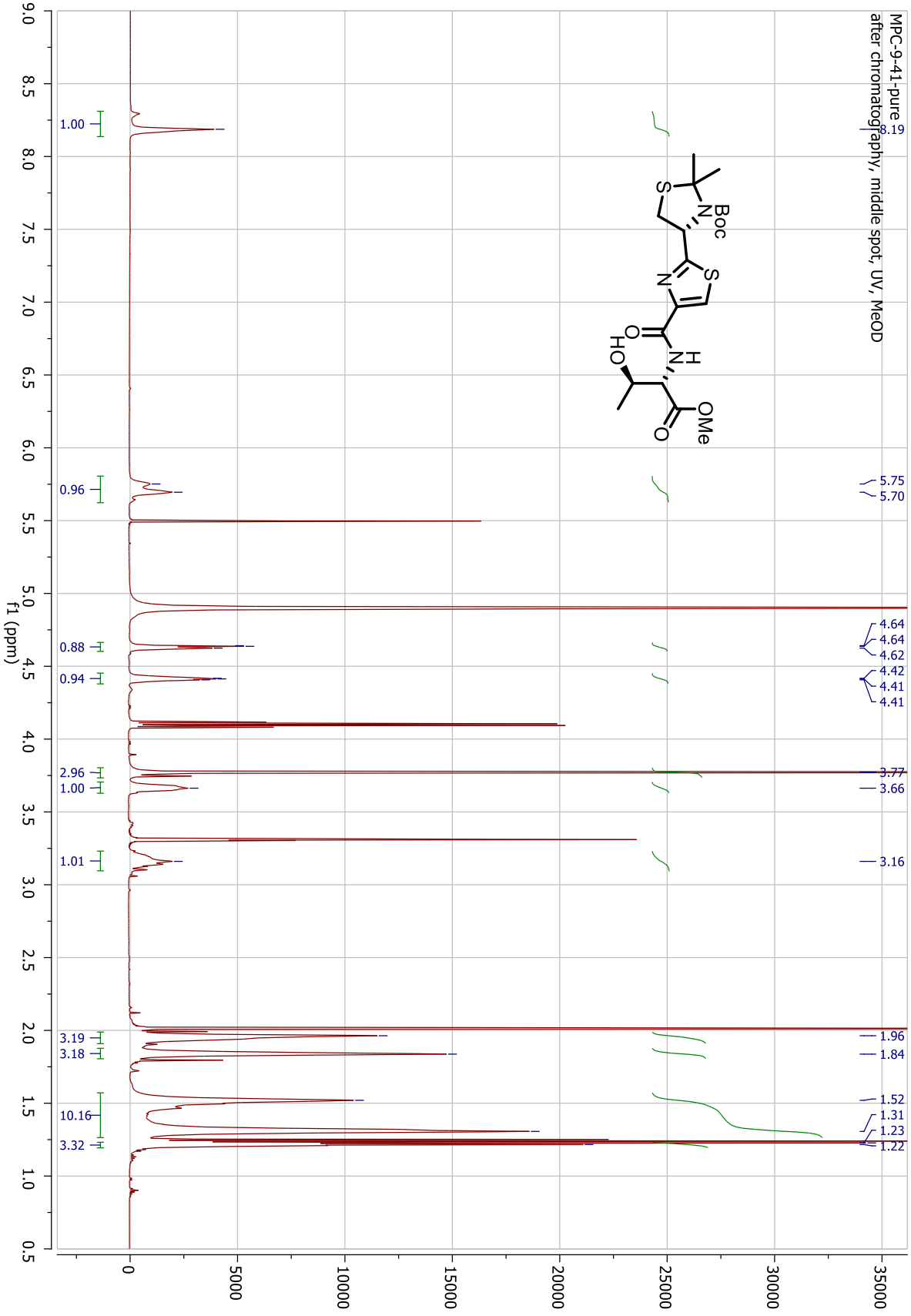

MPC-9-28-2-crude
crude, post work-up, crystallized crude & filtered w/ hexanes, white solid, cdcl3
Boc
N
S
O
OH
1.00
2.20
6.80
9.85
f1 (ppm)

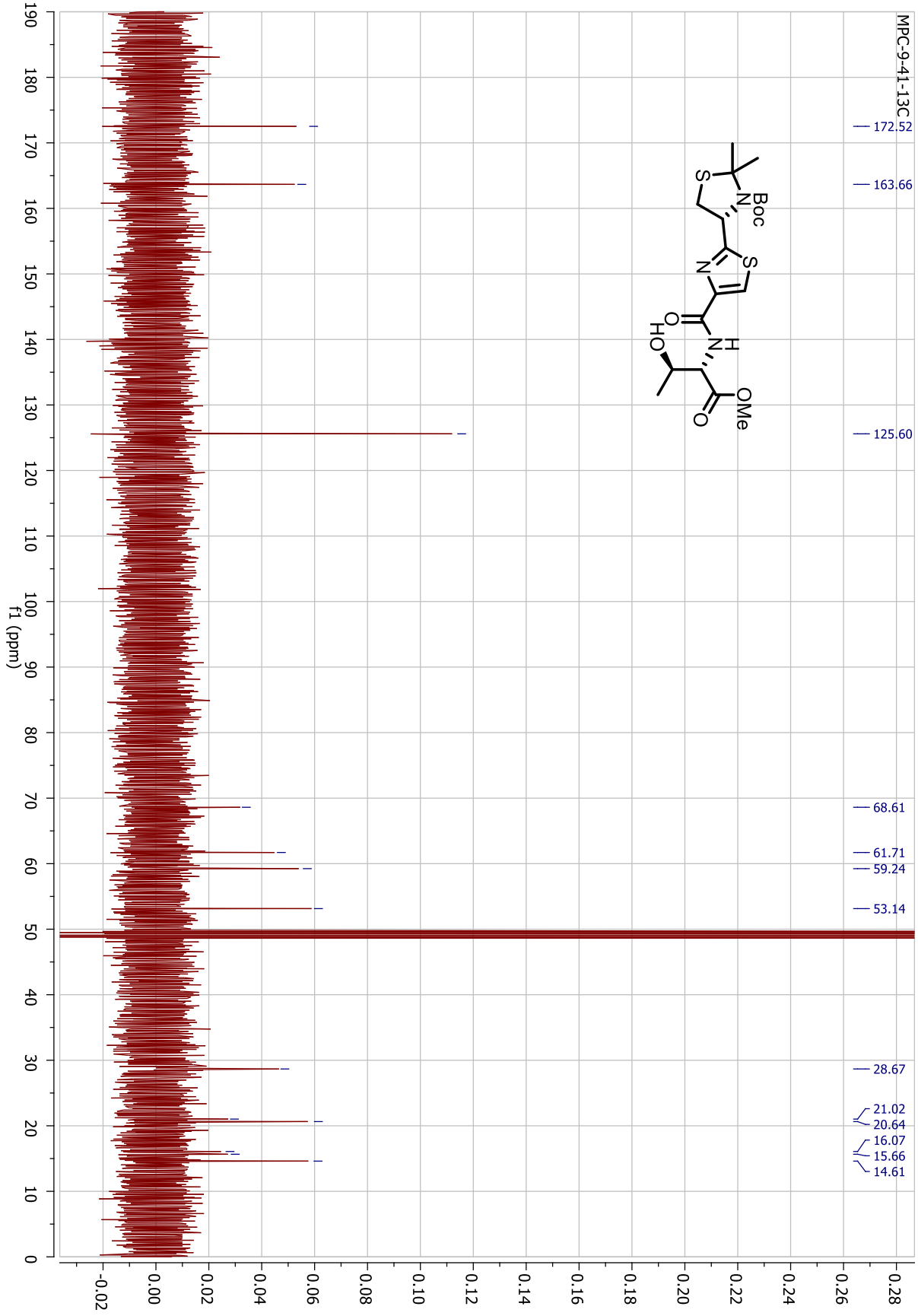

f1 (ppm)
1.00
1.28
0.91
5.87
9.37
Boc
N
S
CN

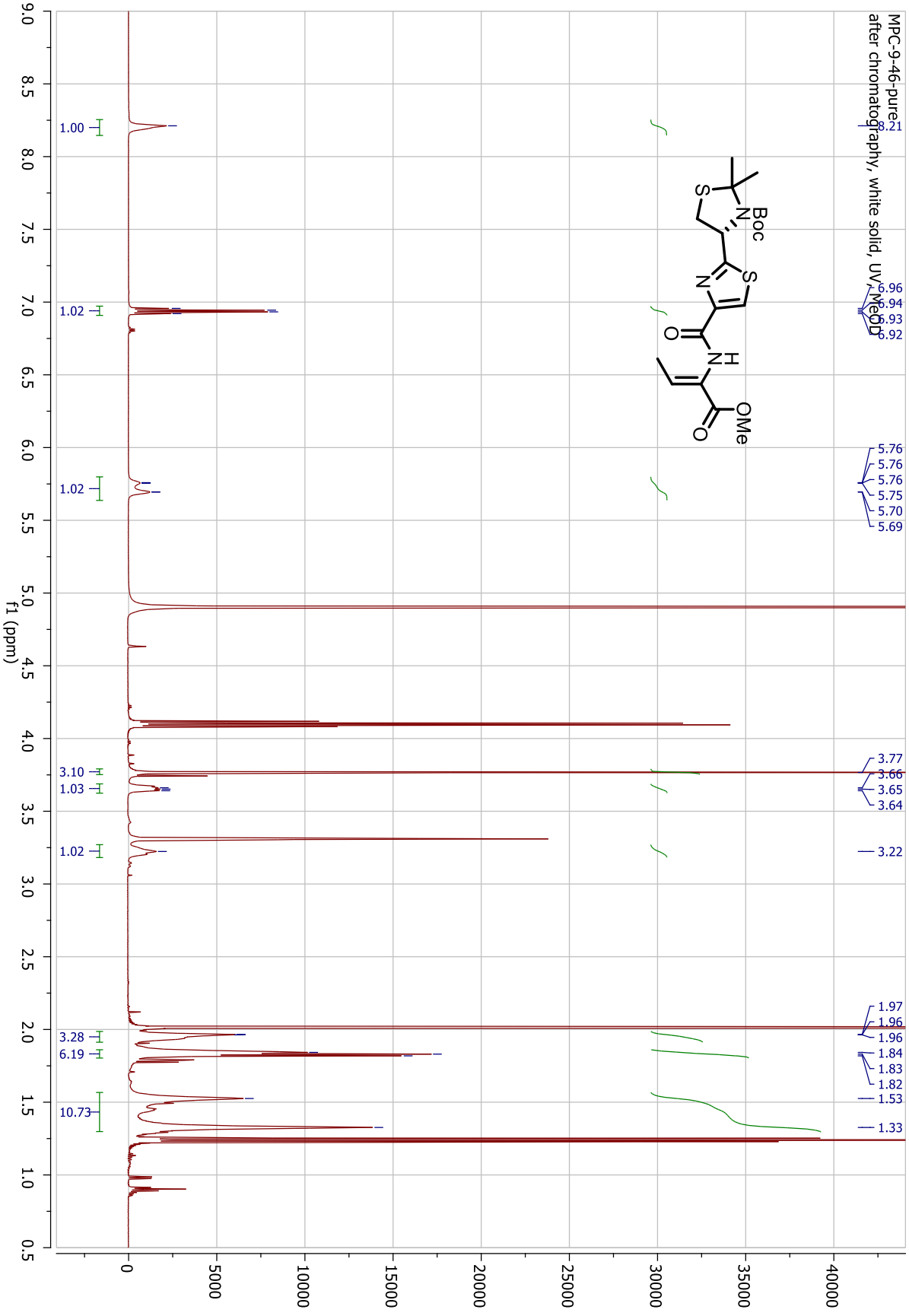

Me
Me
S
N
Boc
CN
H
150.73
118.11
82.25
54.34
53.67
31.23
29.83
29.02
28.17
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

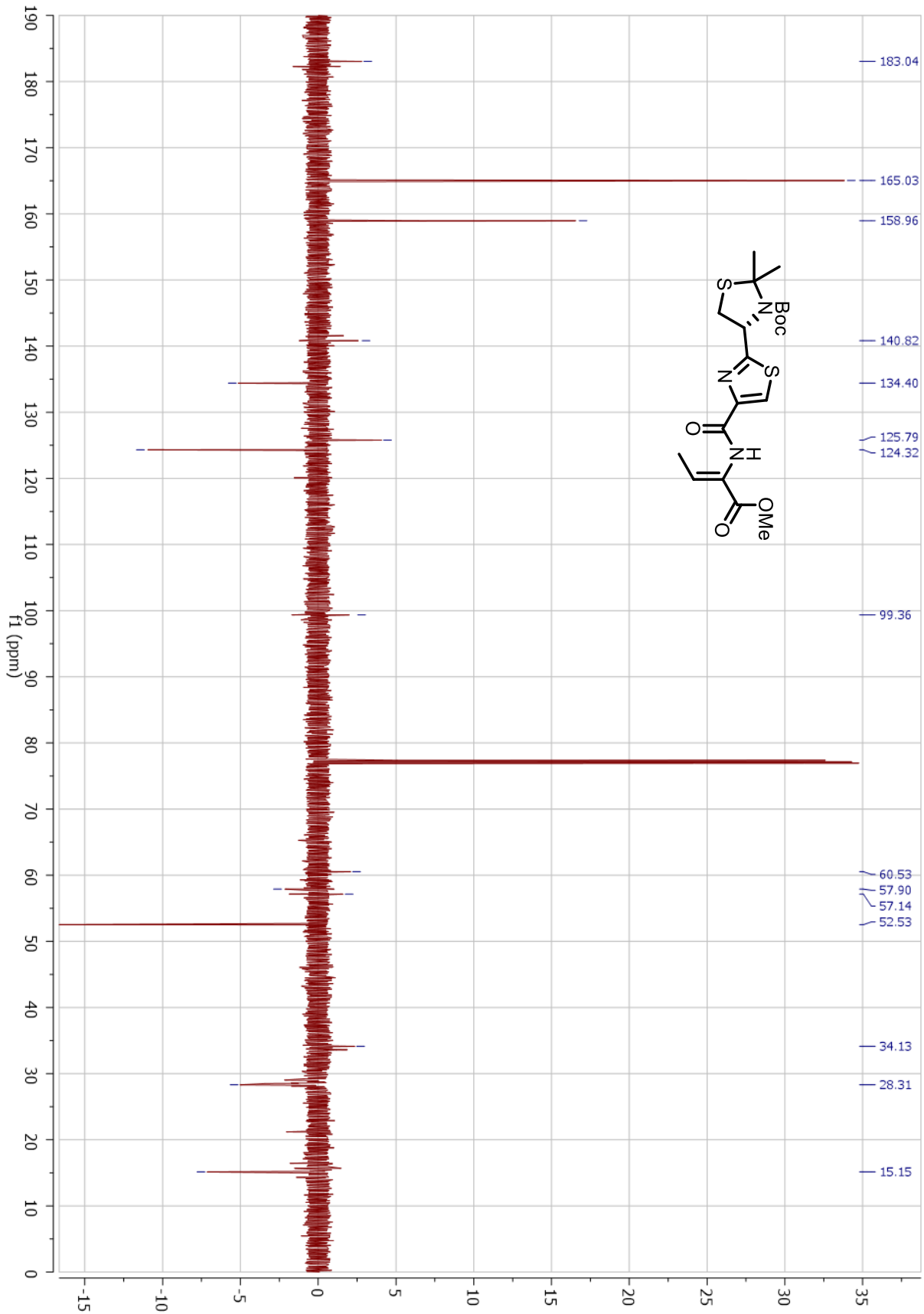

f1 (ppm)
1.00
1.06
3.30
1.13
1.27
3.46
3.53
11.22
8.12
5.80
5.66
3.95
3.55
3.54
3.53
3.52
3.29
3.27
3.16
3.14
2.00
1.91
1.82
1.51
1.28
Boc
N
S
S
N
O
OMe

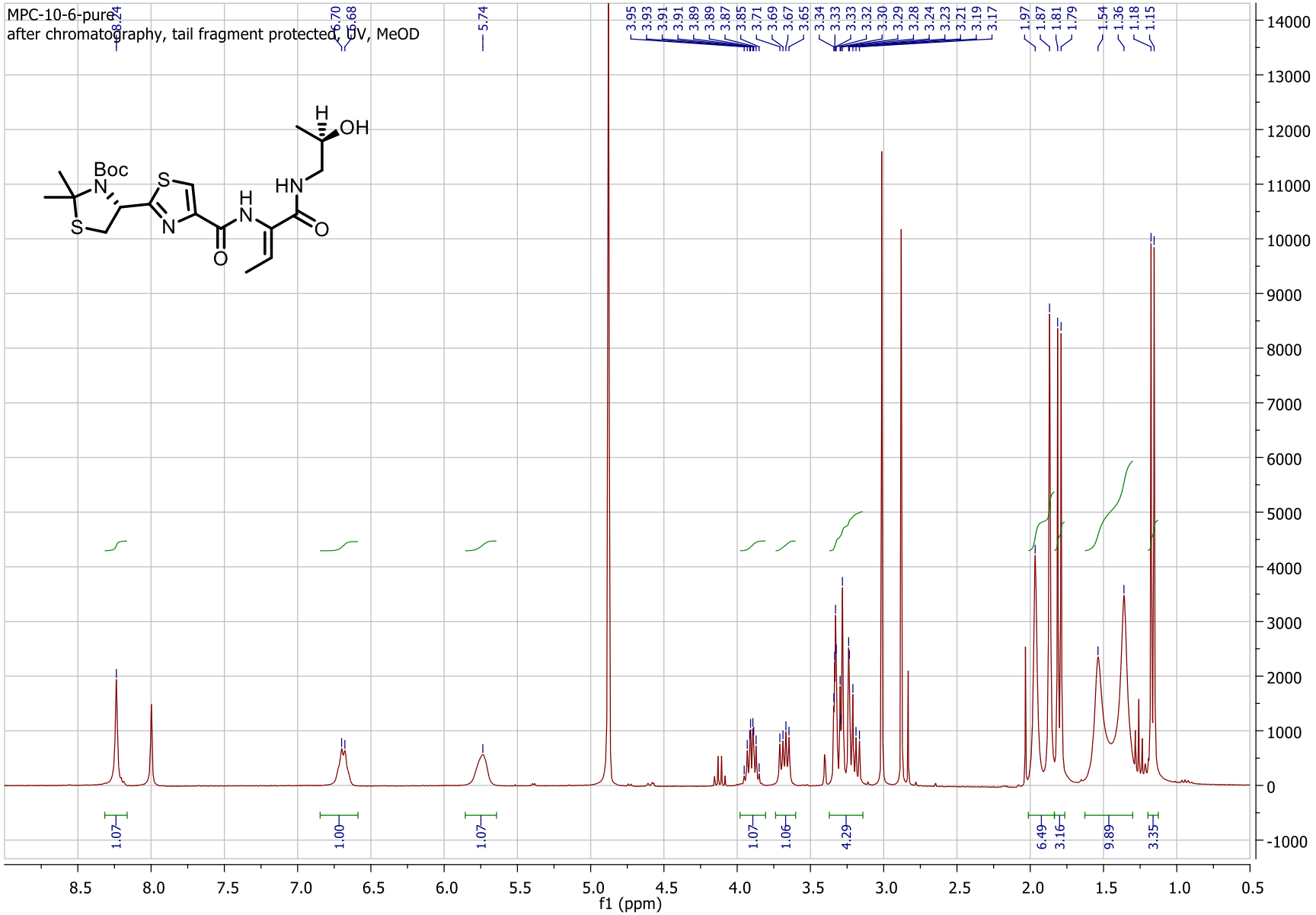

175.89
161.79
151.64
146.26
127.41
81.11
72.11
65.30
52.35
34.23
28.64
28.13
27.91
Me
Me
S
N
Boc
H
N
S
OMe
O

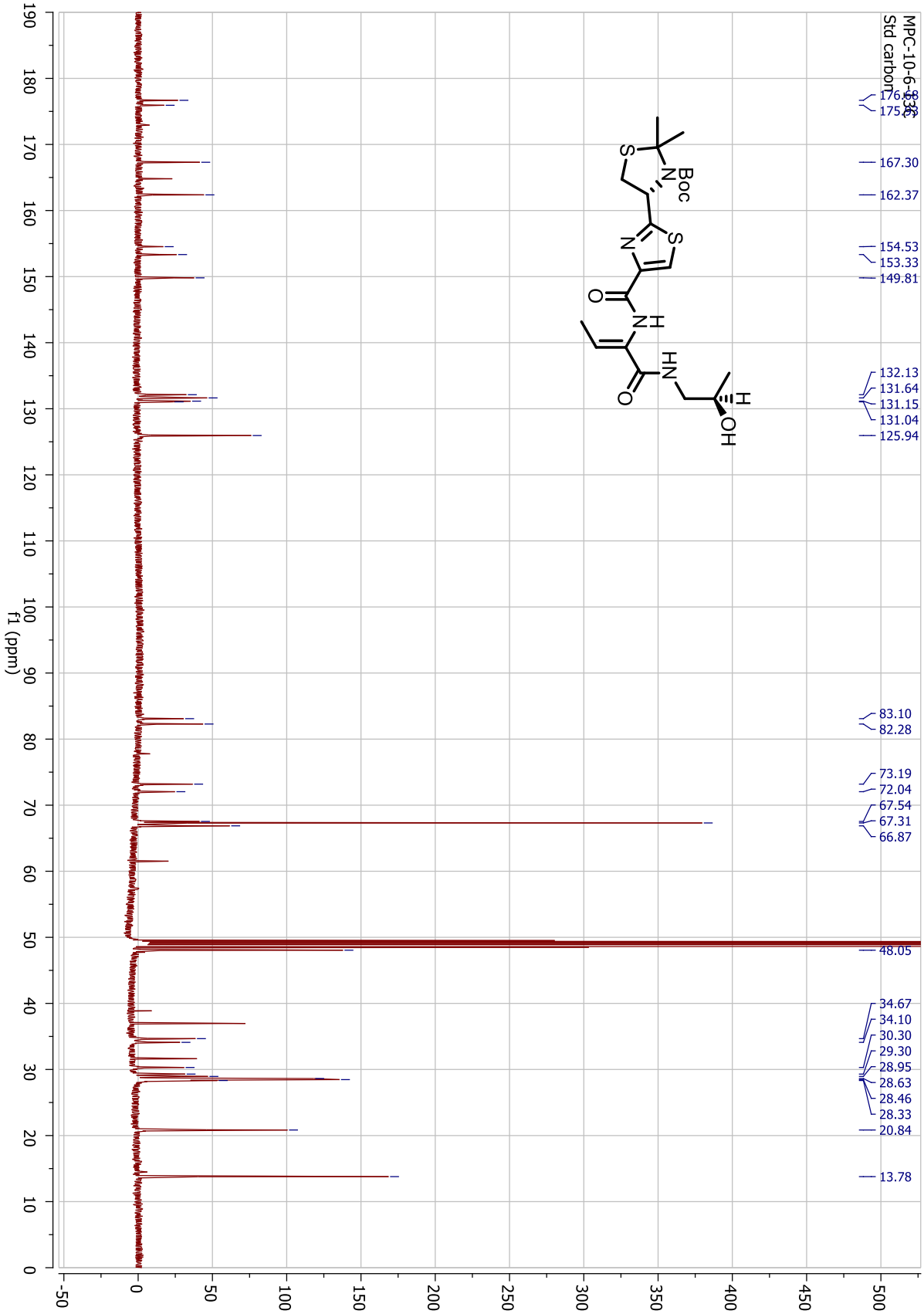

MPC-9-41-pure
after chromatography, middle spot, UV, MeOD
f1 (ppm)
1.00
0.96
0.88
0.94
2.96
1.00
1.01
3.19
3.18
10.16
3.32
8.19
5.75
5.70
4.64
4.64
4.62
4.42
4.41
4.41
3.77
3.66
3.16
1.96
1.84
1.52
1.31
1.23
1.22
Boc
OMe

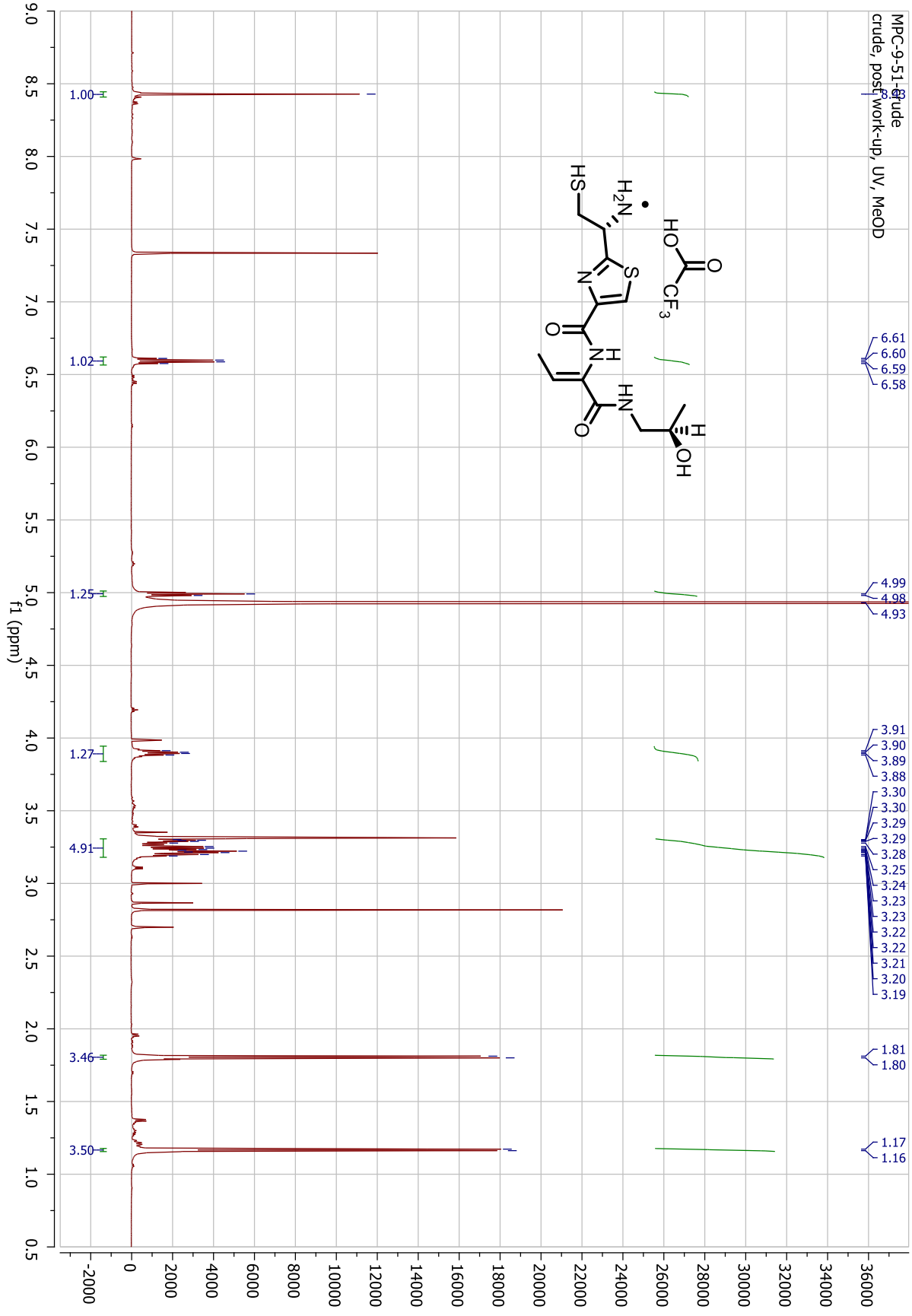

MPC-9-41-13C
172.52
163.66
125.60
68.61
61.71
59.24
53.14
28.67
21.02
20.64
16.07
15.66
14.61
Boc
OMe
f1 (ppm)

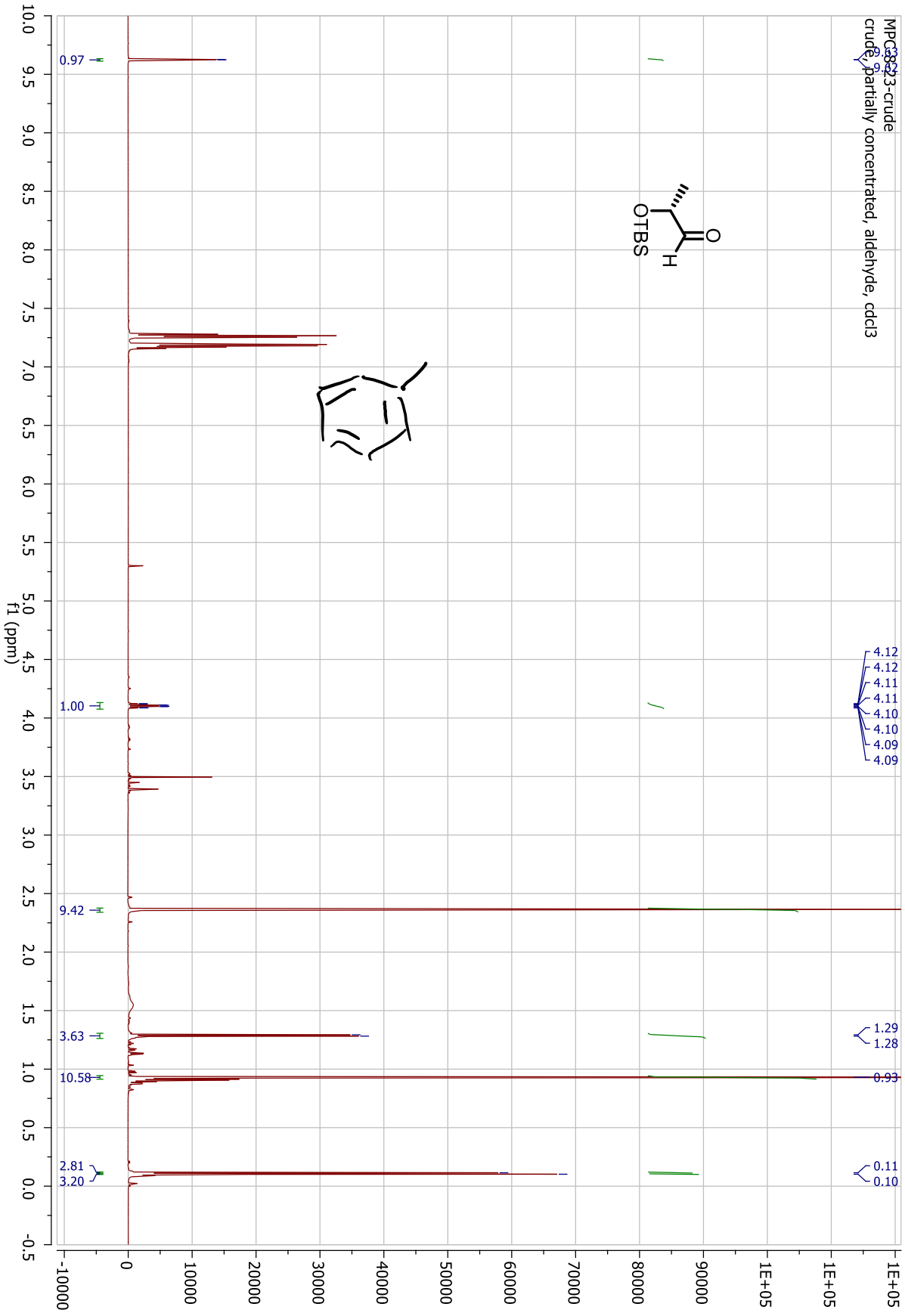

MPC-9-46-pure
after chromatography, white solid, UV, MeOD
Boc
OMe
f1 (ppm)
1.00
1.02
1.02
3.10
1.03
1.02
3.28
6.19
10.73
8.21
6.96
6.94
6.93
6.92
5.76
5.76
5.76
5.75
5.70
5.69
3.77
3.66
3.65
3.64
3.22
1.97
1.96
1.96
1.84
1.83
1.82
1.53
1.33

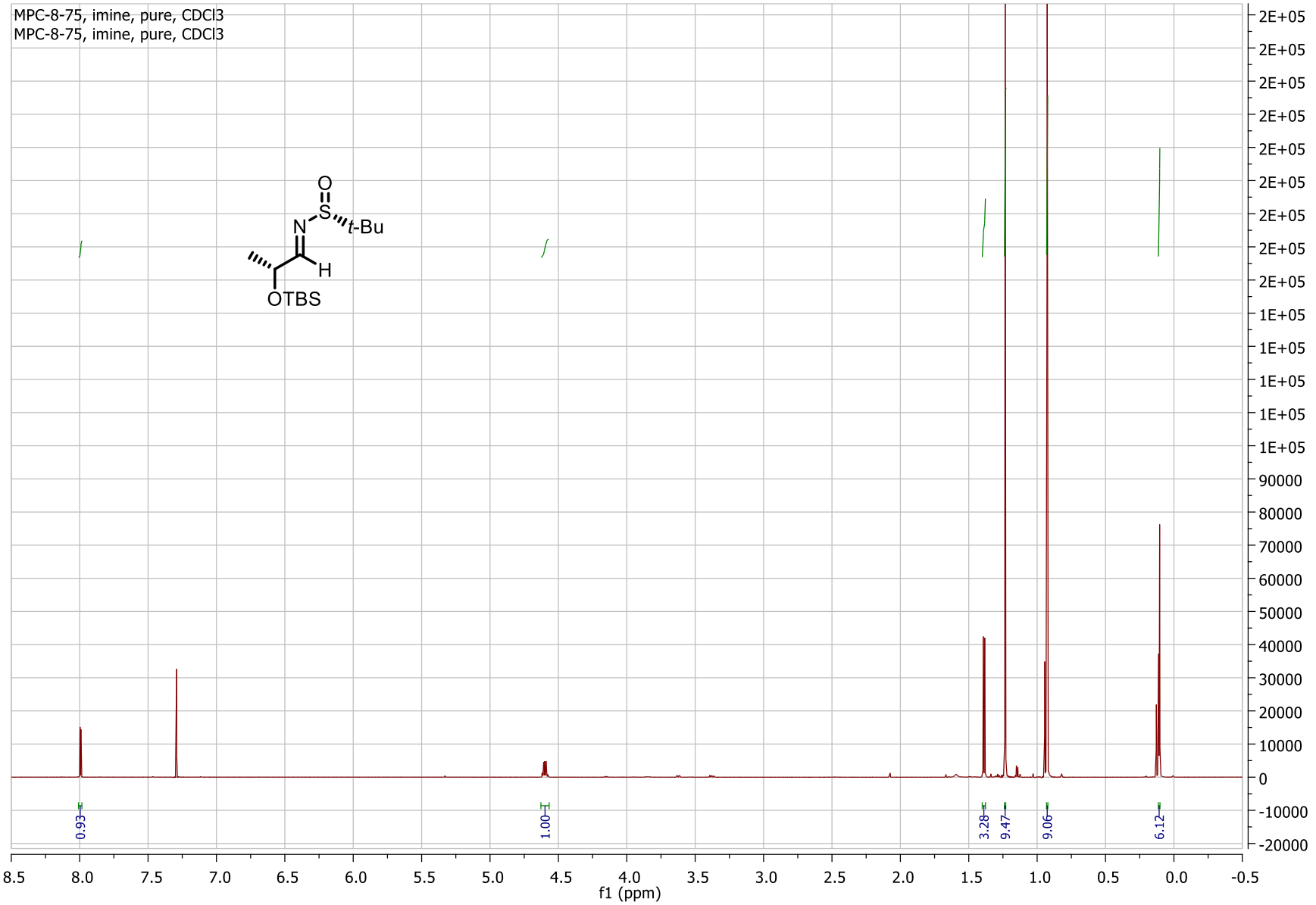

183.04
165.03
158.96
140.82
134.40
125.79
124.32
99.36
60.53
57.90
57.14
52.53
34.13
28.31
15.15
Boc
OMe
f1 (ppm)

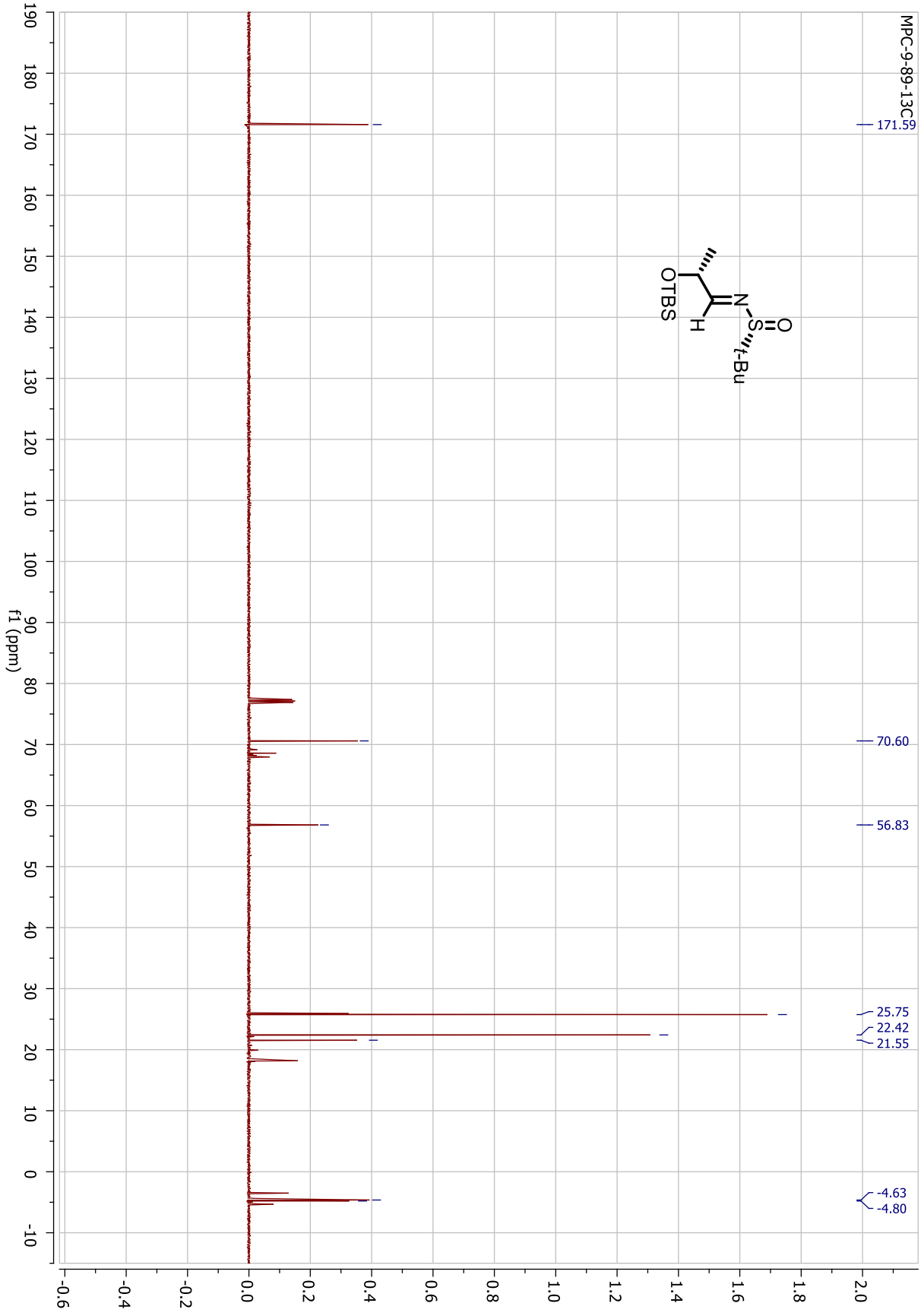

MPC-10-6-pure
after chromatography, tail fragment protected, UV, MeOD
8.24
6.70
6.68
5.74
3.95
3.93
3.91
3.91
3.89
3.89
3.87
3.85
3.71
3.69
3.67
3.65
3.34
3.33
3.33
3.32
3.30
3.29
3.28
3.24
3.23
3.21
3.19
3.17
1.97
1.87
1.81
1.79
1.54
1.36
1.18
1.15
Boc
N
S
S
N
H
N
O
HN
O
H
OH
1.07
1.00
1.07
1.07
1.06
4.29
6.49
3.16
9.89
3.35
f1 (ppm)

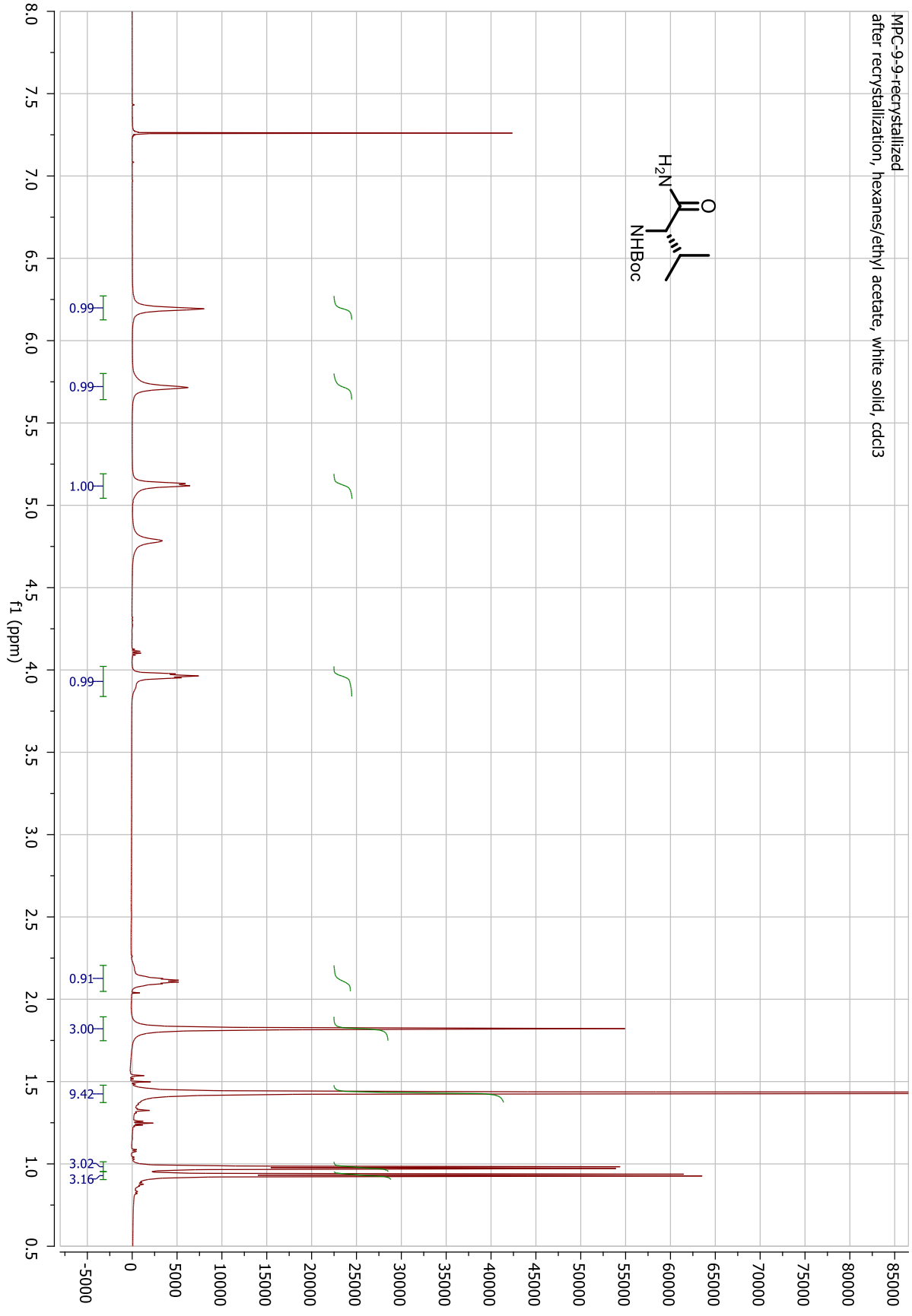

MPC-10-6-33C
Std carbon
176.68
175.43
167.30
162.37
154.53
153.33
149.81
132.13
131.64
131.15
131.04
125.94
83.10
82.28
73.19
72.04
67.54
67.31
66.87
48.05
34.67
34.10
30.30
29.30
28.95
28.63
28.46
28.33
20.84
13.78
Boc
f1 (ppm)

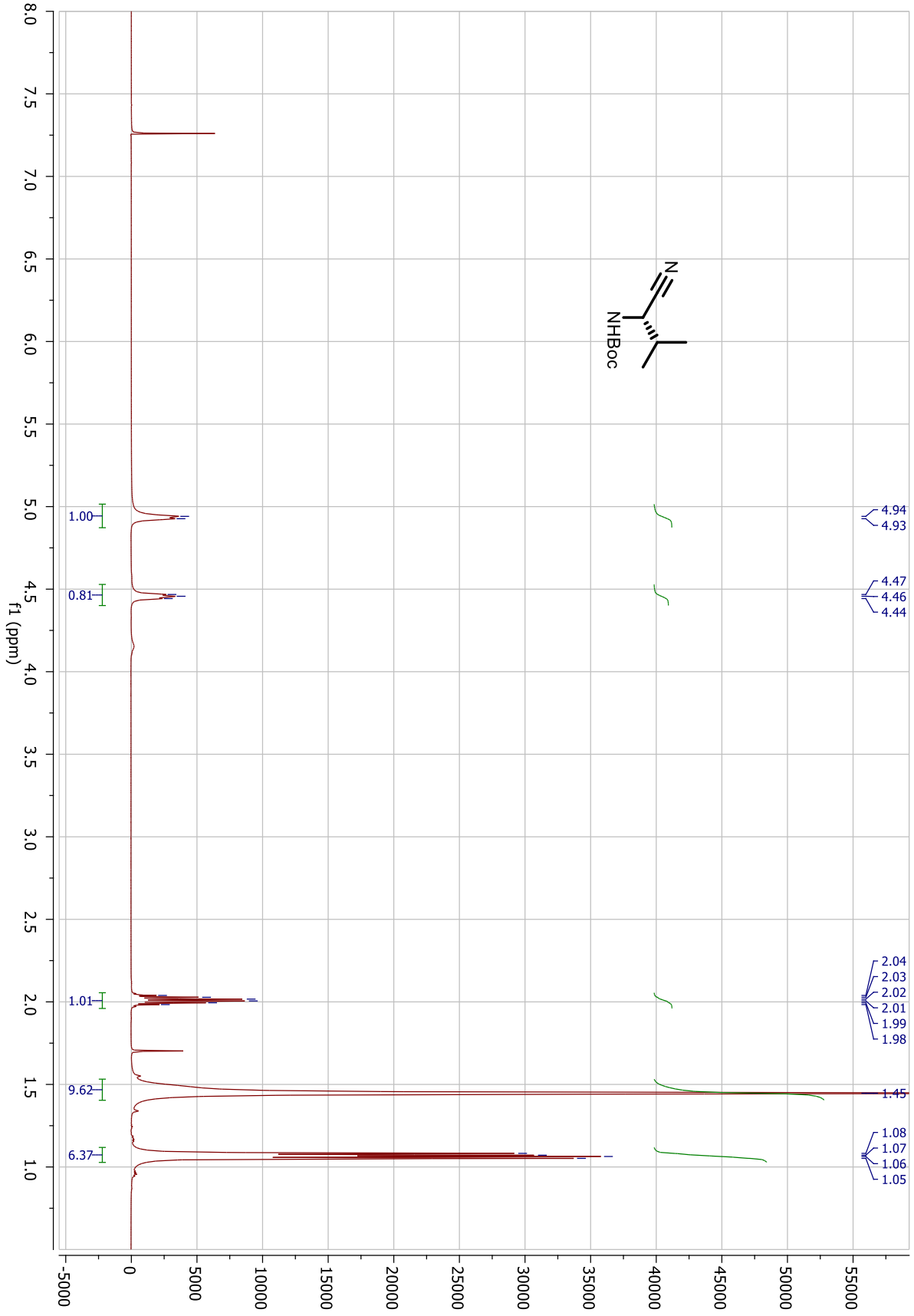

MPC-9-51-crude
crude, post work-up, UV, MeOD
f1 (ppm)
1.00
1.02
1.25
1.27
4.91
3.46
3.50
8.43
6.61
6.60
6.59
6.58
4.99
4.98
4.93
3.91
3.90
3.89
3.88
3.30
3.30
3.29
3.29
3.28
3.25
3.24
3.23
3.23
3.22
3.22
3.21
3.20
3.19
1.81
1.80
1.17
1.16

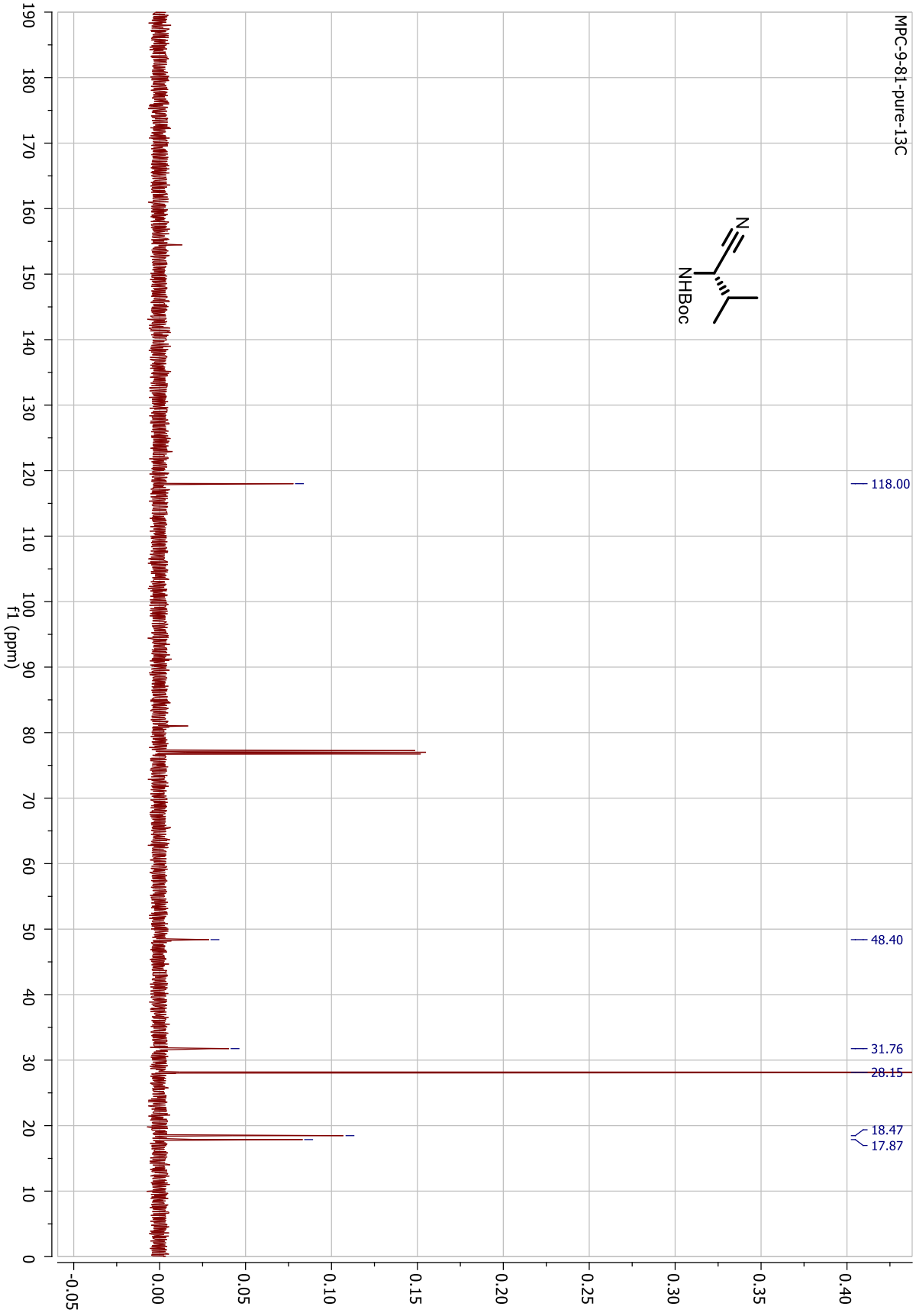

MPC3223-crude
crude, partially concentrated, aldehyde, cdcl3
OTBS
O
H
0.97
1.00
9.42
3.63
10.58
2.81
3.20
4.12
4.12
4.11
4.11
4.10
4.10
4.09
4.09
1.29
1.28
0.93
0.11
0.10
f1 (ppm)

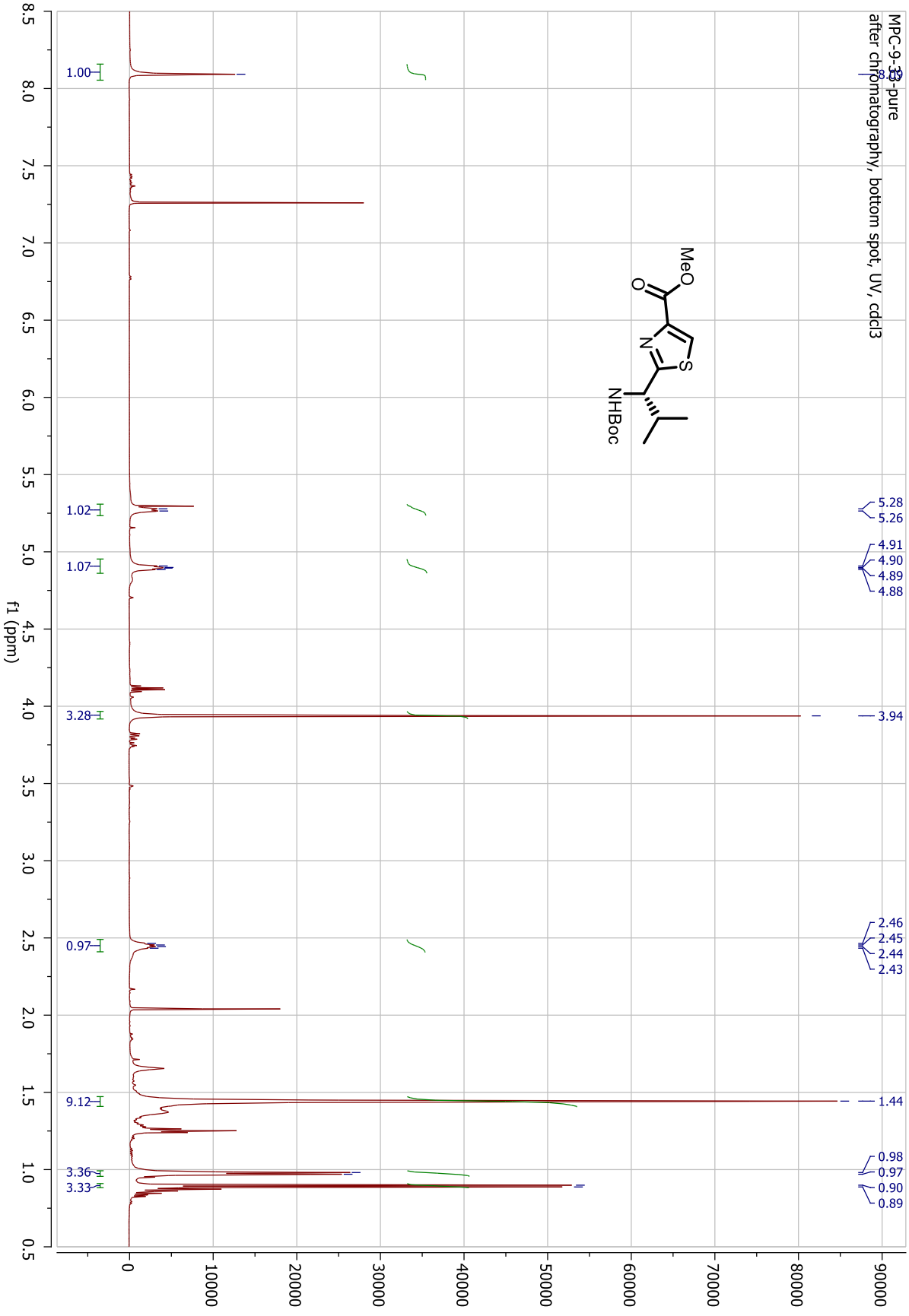

MPC-8-75, imine, pure, CDCl3
MPC-8-75, imine, pure, CDCl3
O
S
N
t-Bu
H
OTBS
0.93
1.00
3.28
9.47
9.06
6.12
f1 (ppm)

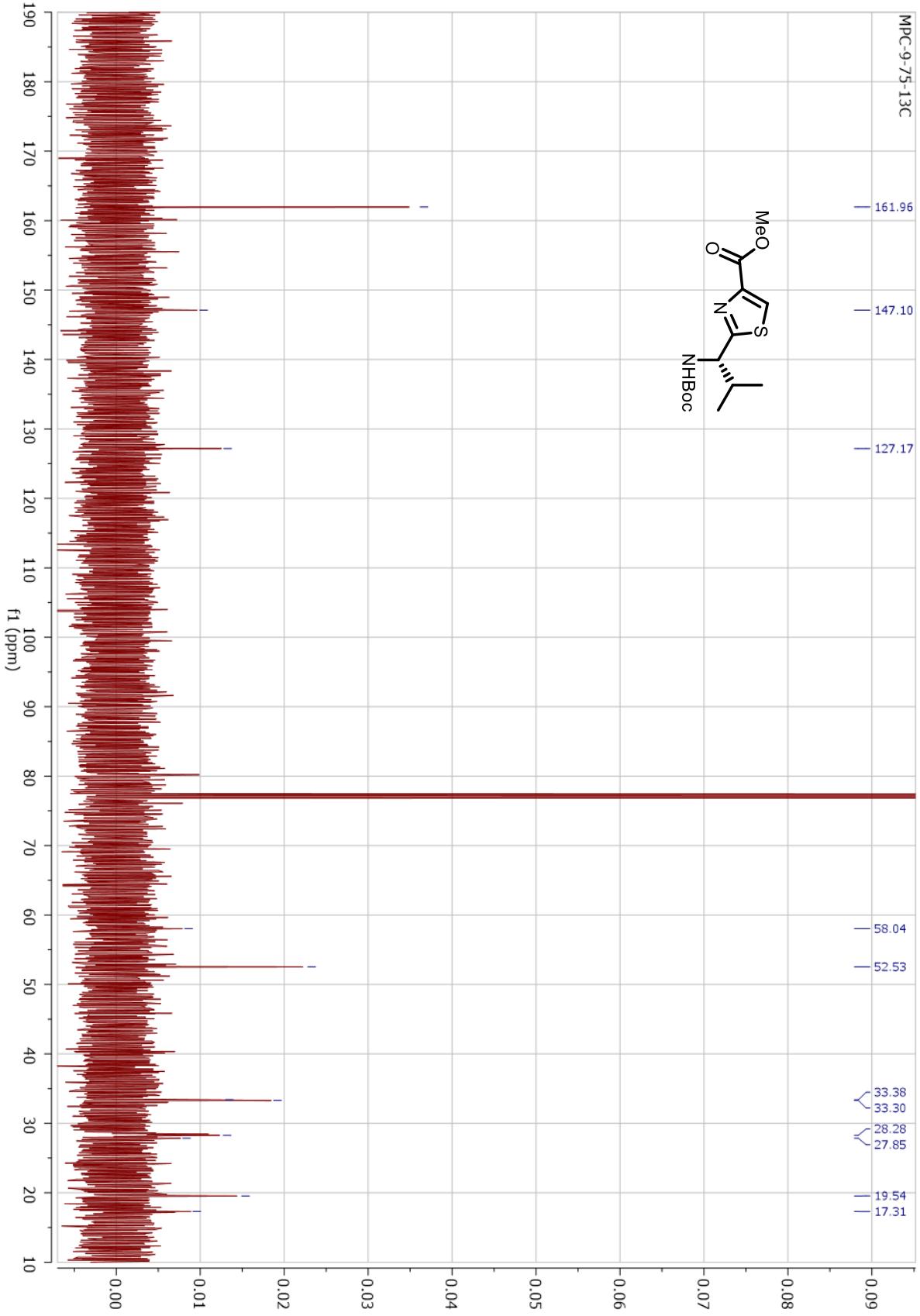

MPC-9-89-13C
171.59
70.60
56.83
25.75
22.42
21.55
-4.63
-4.80
OTBS
N
H
S
O
t-Bu
f1 (ppm)

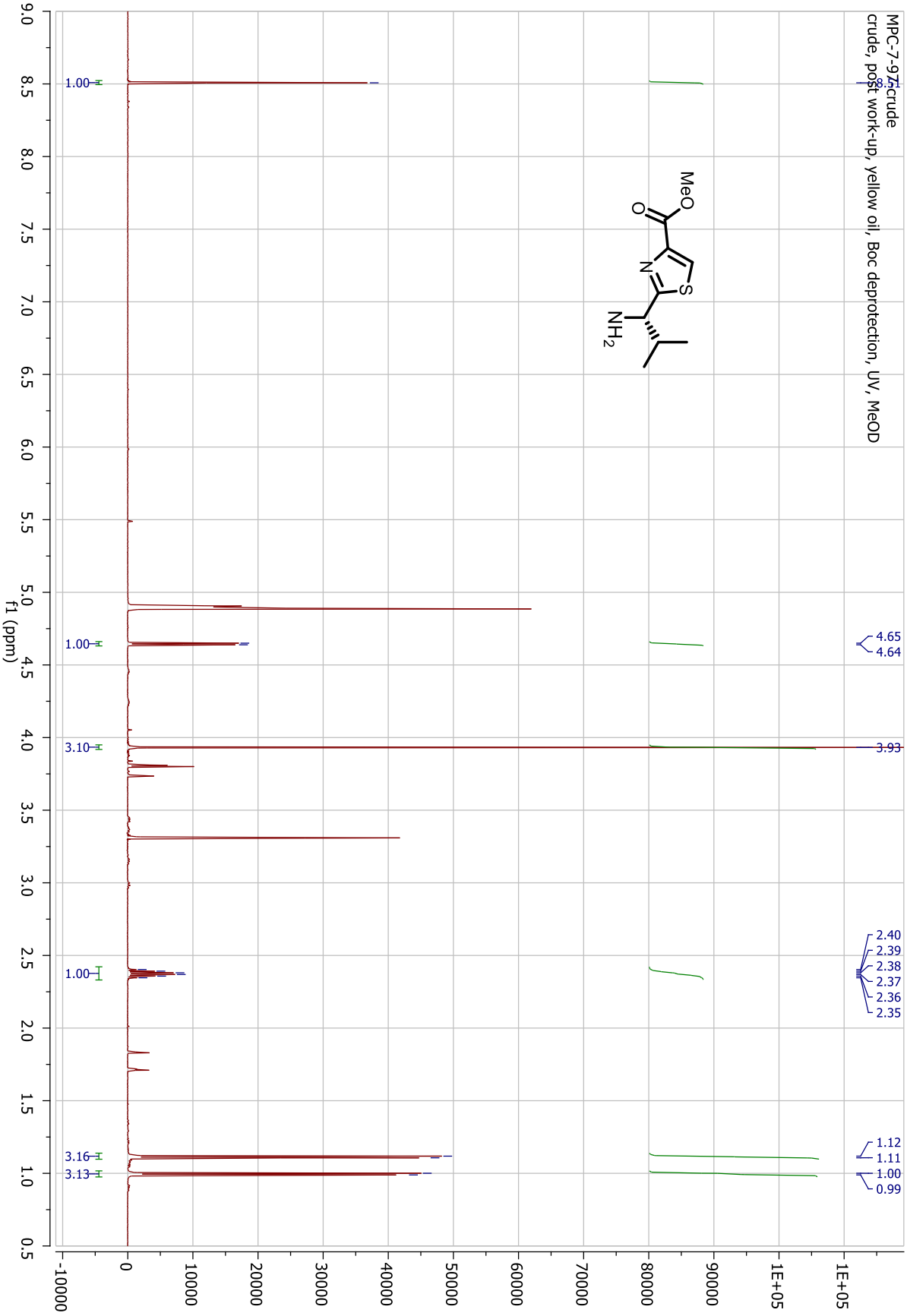

MPC-9-9-recrystallized
after recrystallization, hexanes/ethyl acetate, white solid, cdcl3
H2N
O
NHBoc
0.99
0.99
1.00
0.99
0.91
3.00
9.42
3.02
3.16
f1 (ppm)

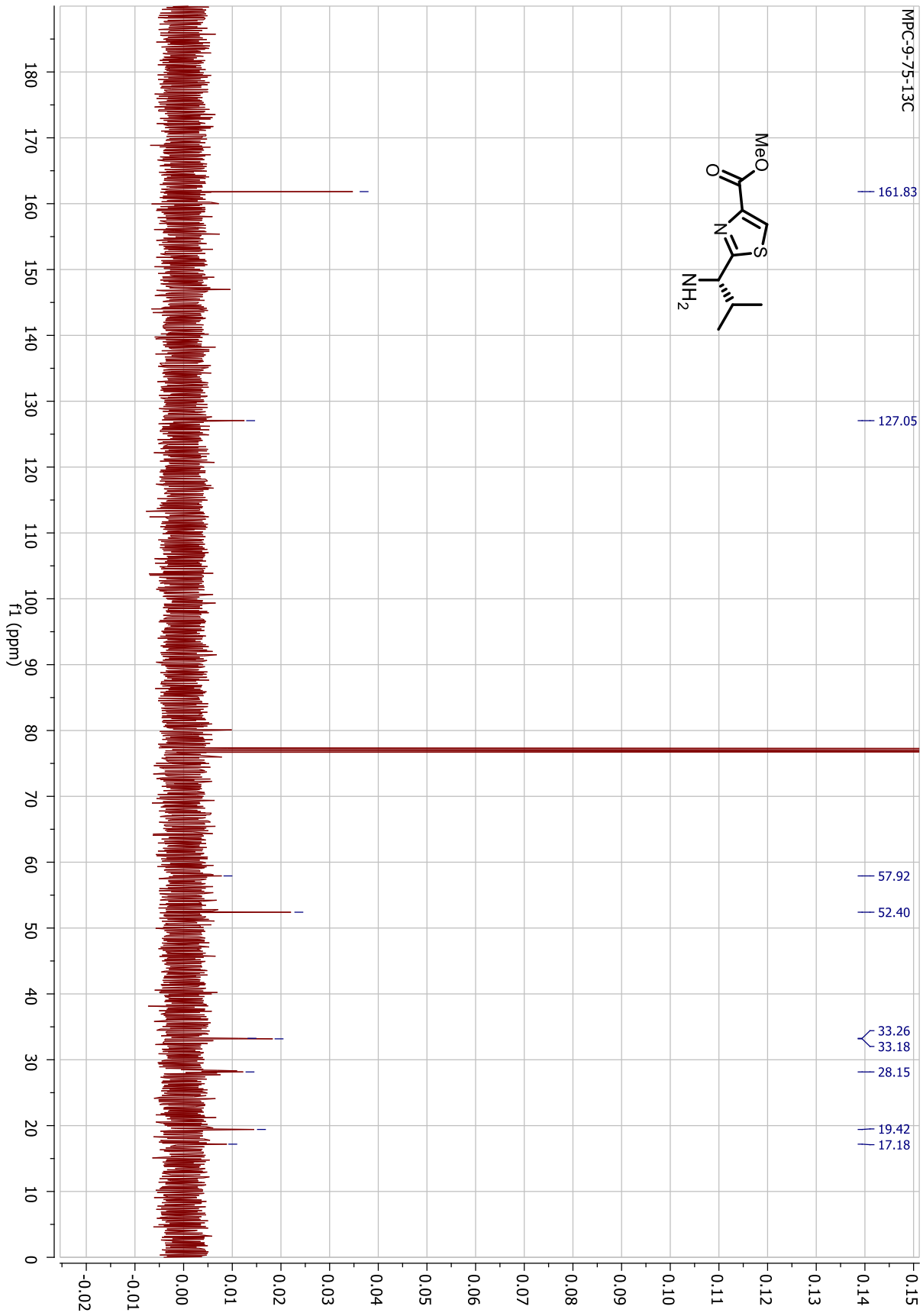

NHBoc
N
f1 (ppm)
1.00
0.81
1.01
9.62
6.37
4.94
4.93
4.47
4.46
4.44
2.04
2.03
2.02
2.01
1.99
1.98
1.45
1.08
1.07
1.06
1.05

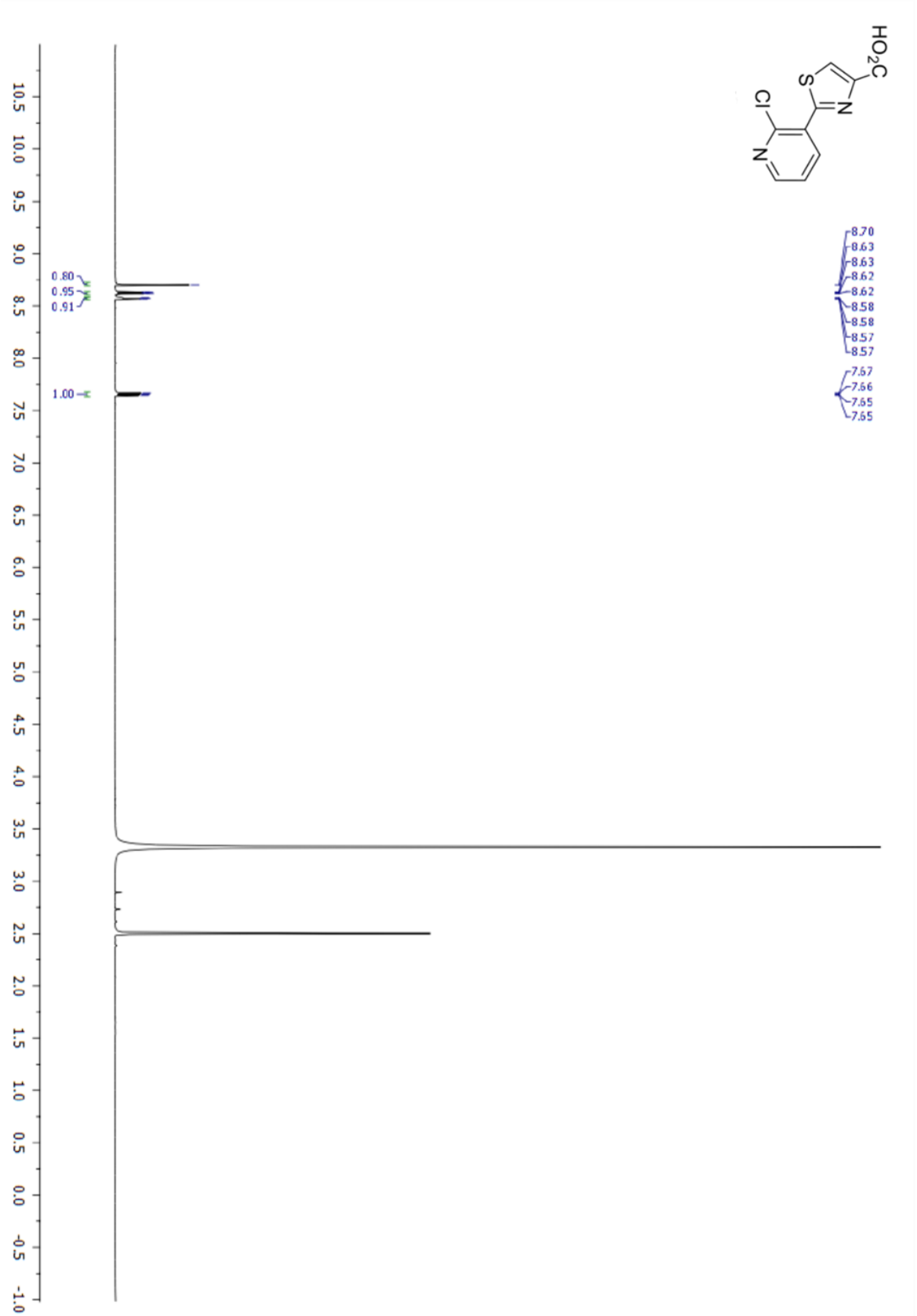

MPC-9-81-pure-13C
NHBoc
N
118.00
48.40
31.76
28.15
18.47
17.87
f1 (ppm)

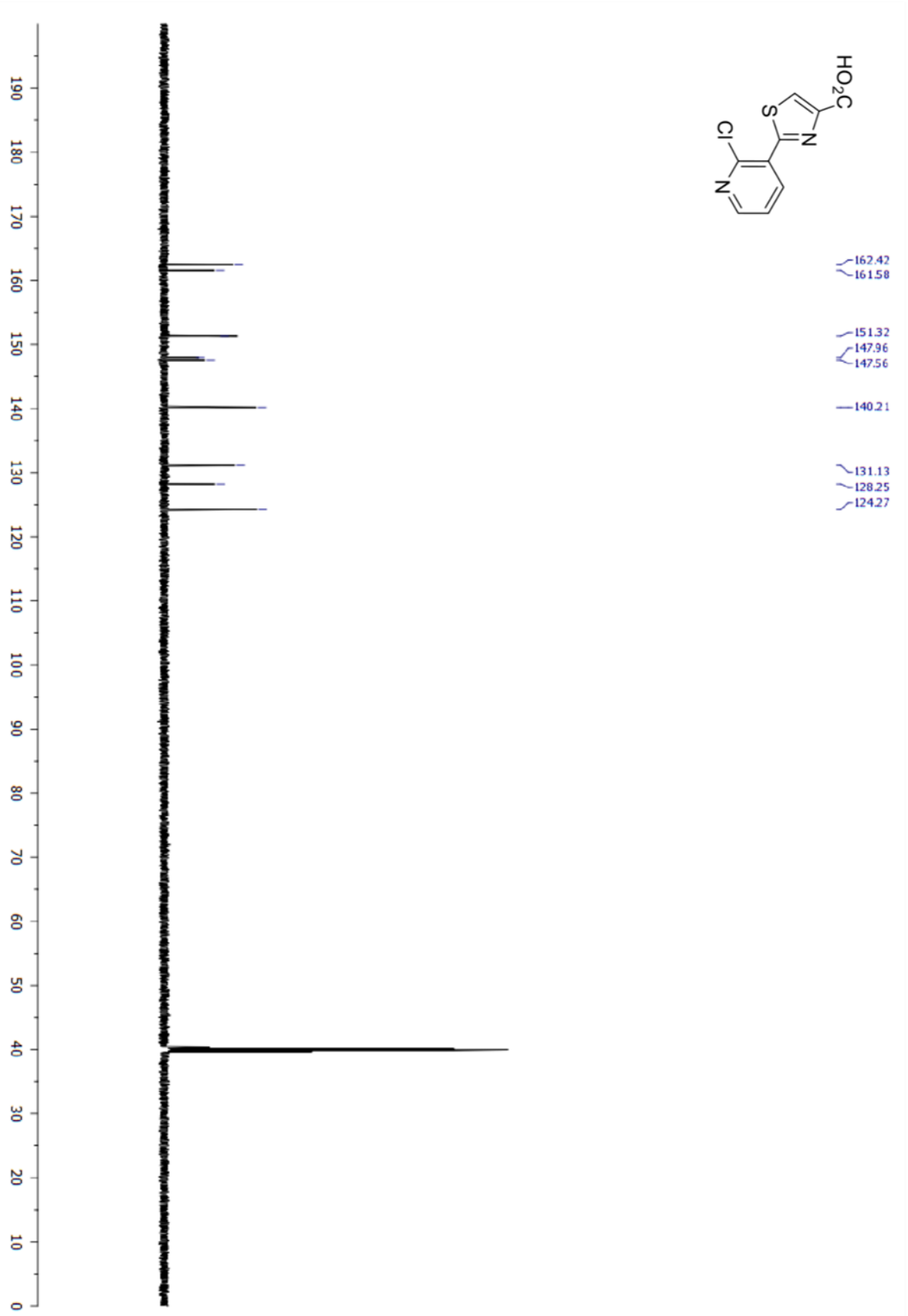

MPC-9-23-pure
after chromatography, bottom spot, UV, cdcl3
MeO
O
N
S
NHBoc
f1 (ppm)
1.00
1.02
1.07
3.28
0.97
9.12
3.36
3.33
5.28
5.26
4.91
4.90
4.89
4.88
3.94
2.46
2.45
2.44
2.43
1.44
0.98
0.97
0.90
0.89

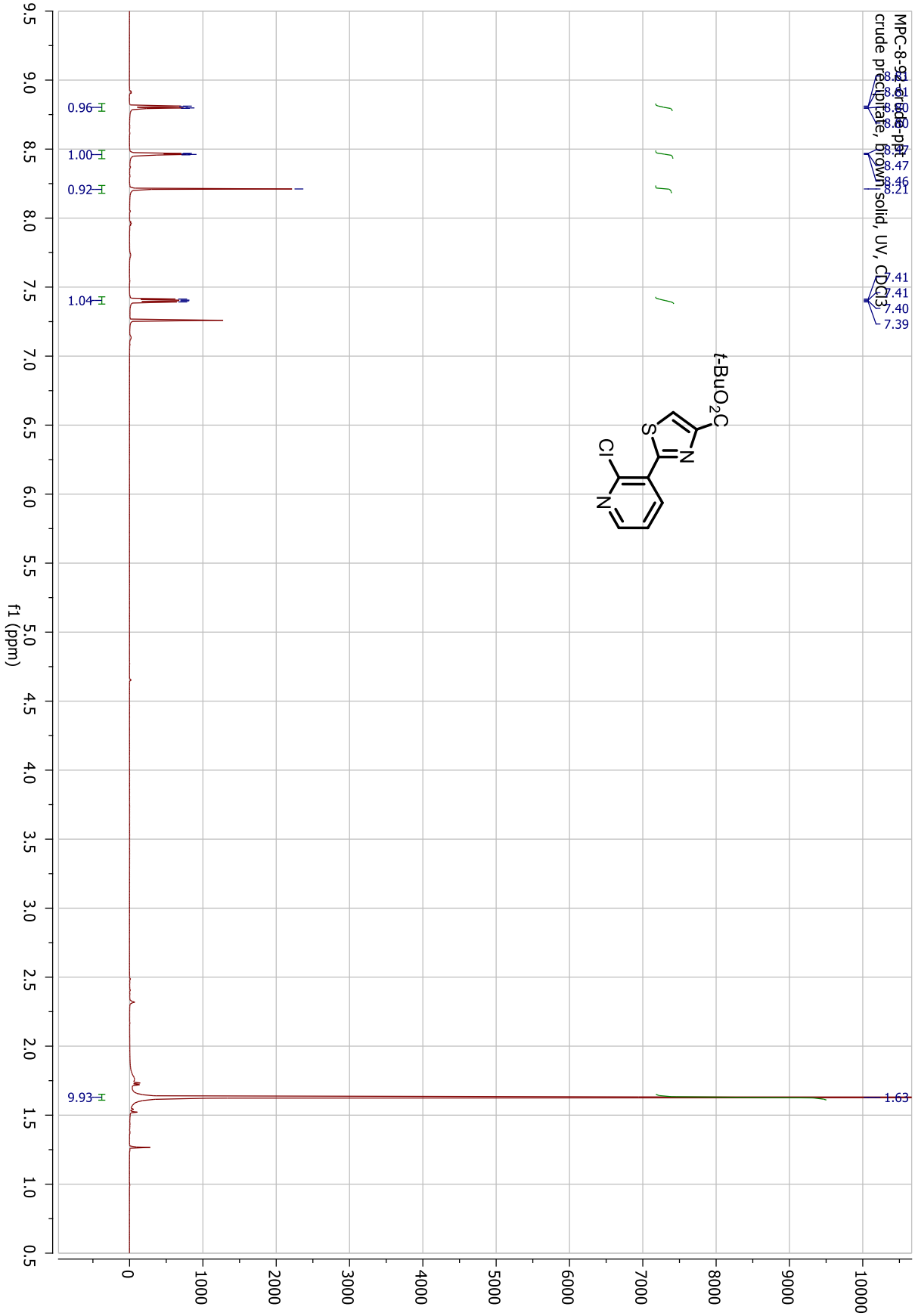

MPC-9-75-13C
MeO
O
N
S
NHBoc
161.96
147.10
127.17
58.04
52.53
33.38
33.30
28.28
27.85
19.54
17.31
f1 (ppm)

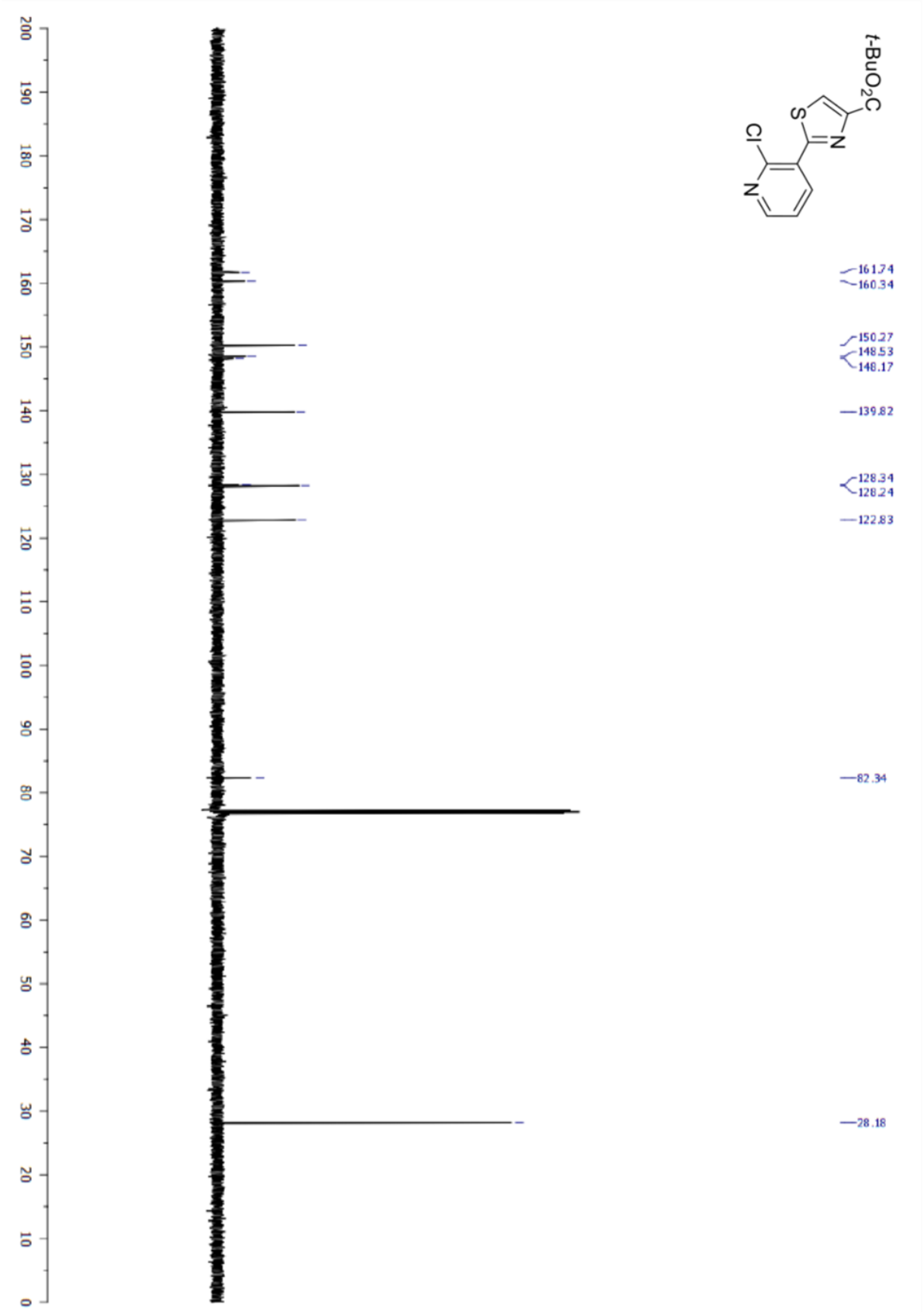

MPC-7-97-crude
crude, post work-up, yellow oil, Boc deprotection, UV, MeOD
MeO
O
N
S
NH2
f1 (ppm)
1.00
1.00
3.10
1.00
3.16
3.13
8.71
4.65
4.64
3.93
2.40
2.39
2.38
2.37
2.36
2.35
1.12
1.11
1.00
0.99

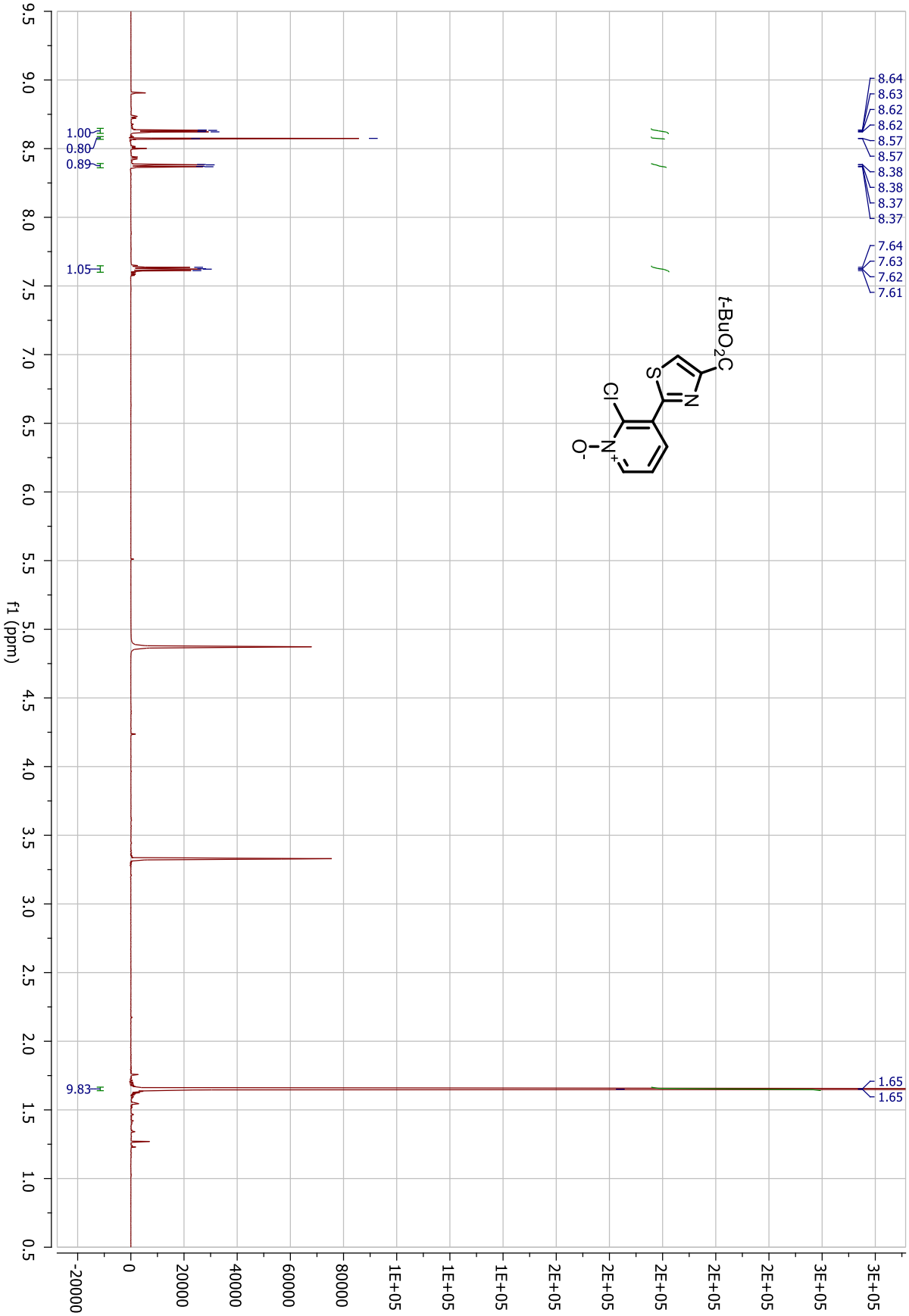

MPC-9-75-13C
MeO
O
N
S
NH2
161.83
127.05
57.92
52.40
33.26
33.18
28.15
19.42
17.18
f1 (ppm)

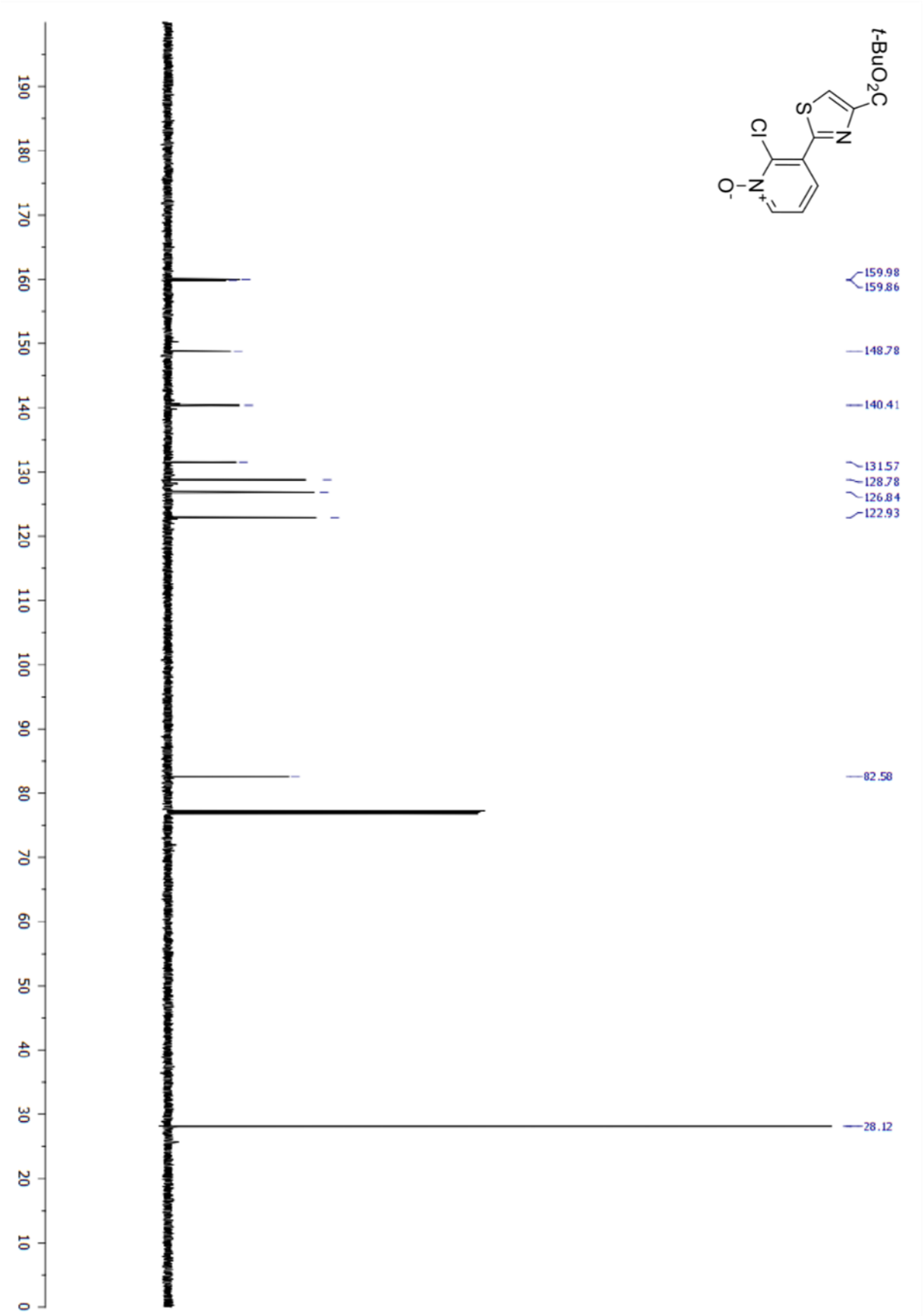

HO2C
S
N
Cl
N
8.70
8.63
8.63
8.62
8.62
8.58
8.58
8.57
8.57
7.67
7.66
7.65
7.65
0.80
0.95
0.91
1.00
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0

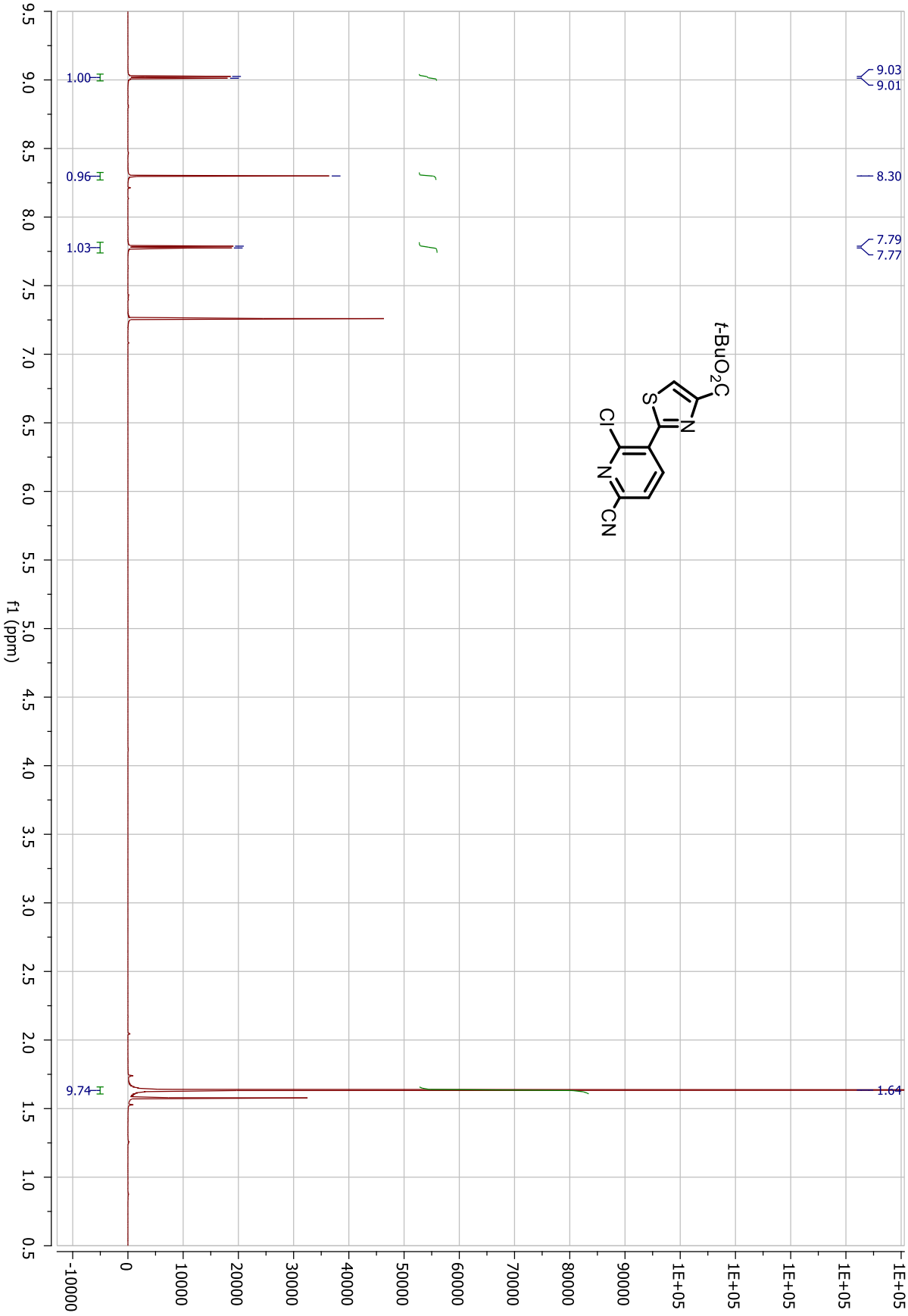

HO2C
S
N
Cl
N
162.42
161.58
151.32
147.96
147.56
140.21
131.13
128.25
124.27
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

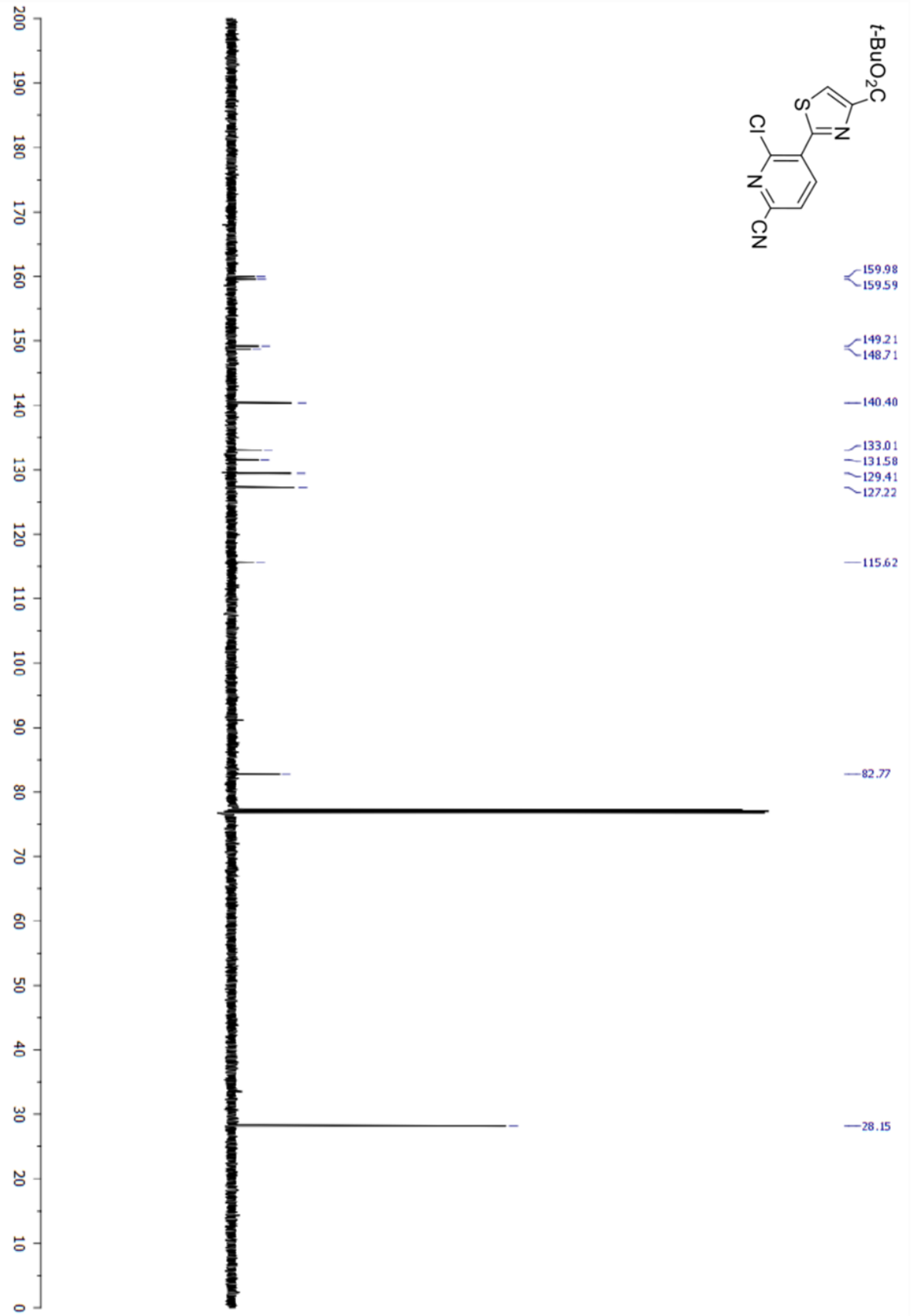

MPC-8-92 crude ppt
crude precipitate, brown solid, UV, CDCl3
8.48
8.47
8.46
8.21
7.41
7.41
7.40
7.39
t-BuO2C
S
N
Cl
N
0.96
1.00
0.92
1.04
9.93
1.63
f1 (ppm)

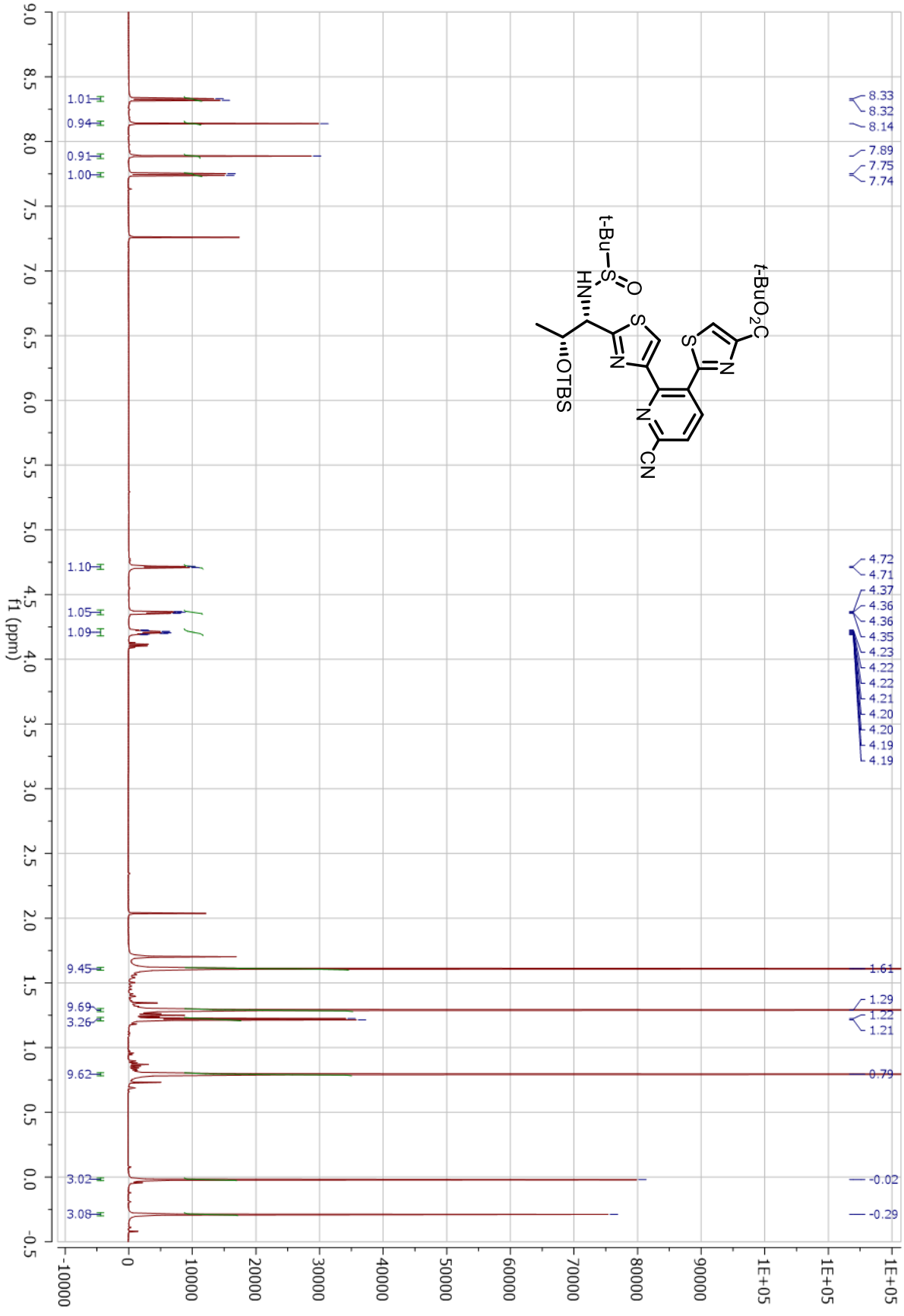

t-BuO2C
S
N
Cl
N
161.74
160.34
150.27
148.53
146.17
139.82
128.34
128.24
122.83
82.34
28.18

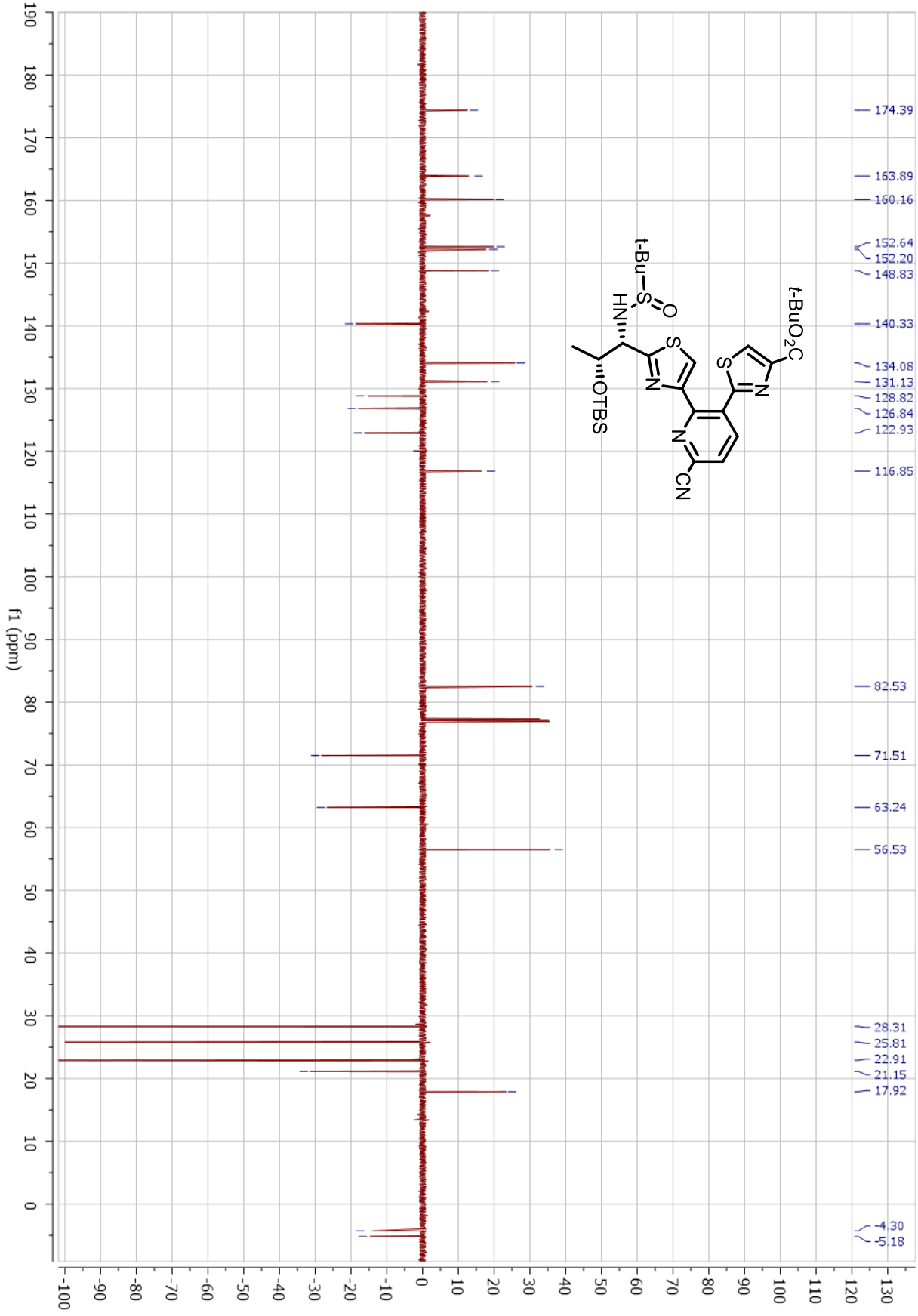

8.64
8.63
8.62
8.62
8.57
8.57
8.38
8.38
8.37
8.37
7.64
7.63
7.62
7.61
1.65
1.65
1.00
0.80
0.89
1.05
9.83
t-BuO2C
S
N
Cl
O-
N+
f1 (ppm)

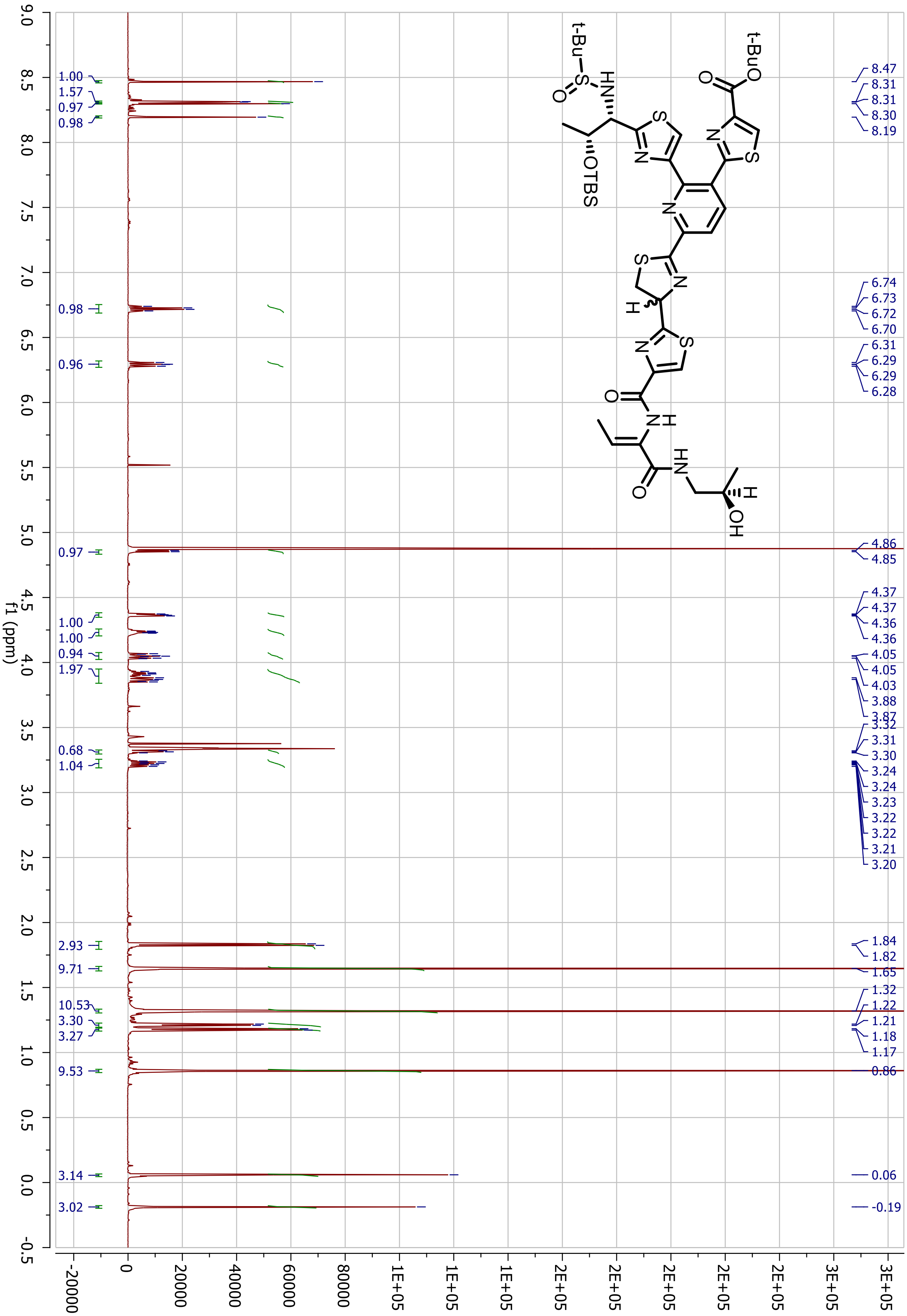

t-BuO_2C
Cl
S
N
O^-
N^+
159.98
159.86
148.78
140.41
131.57
128.78
126.84
122.93
82.58
28.12

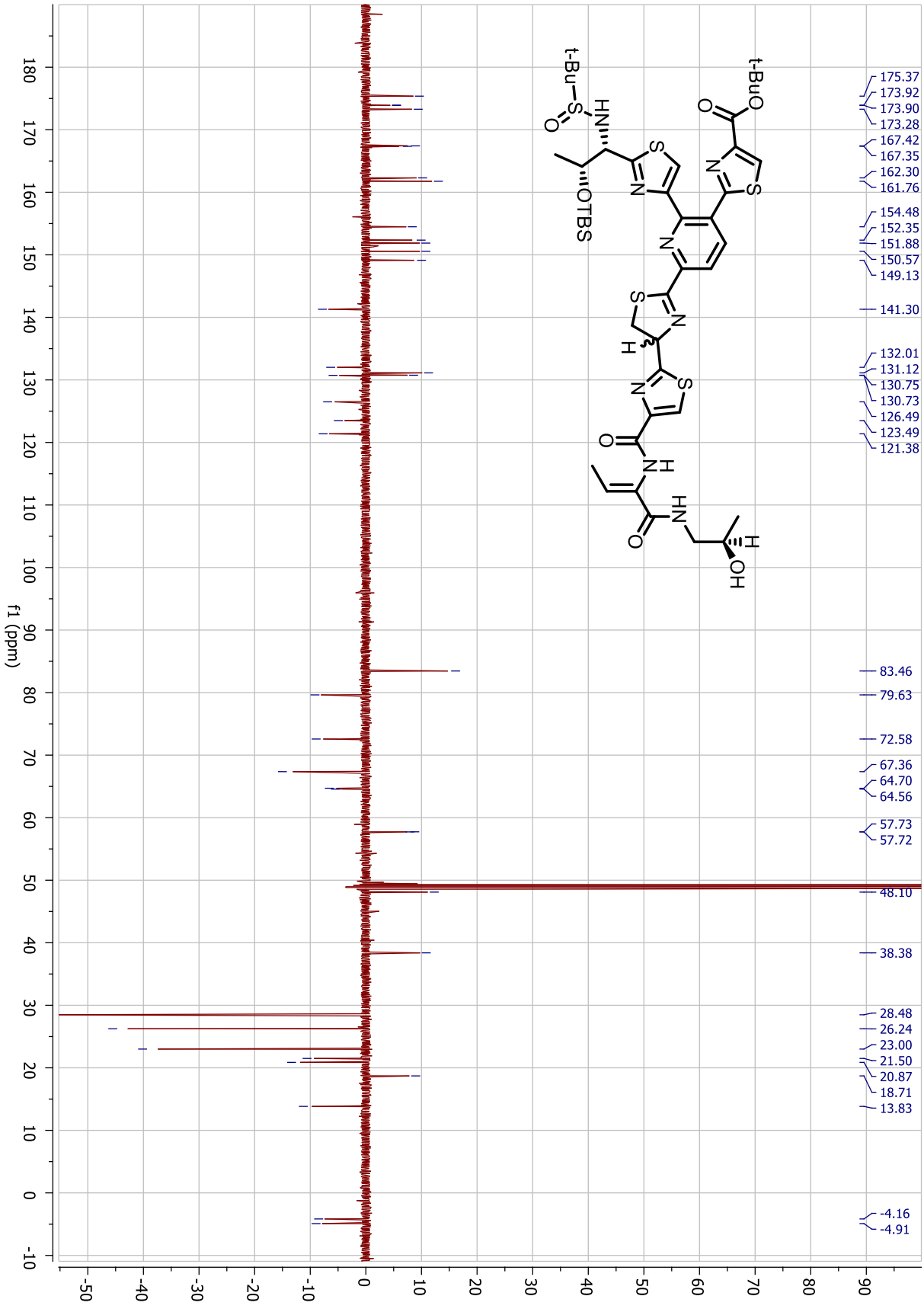

f1 (ppm)
1.00
0.96
1.03
9.74
9.03
9.01
8.30
7.79
7.77
1.64
t-BuO2C
S
N
Cl
N
CN

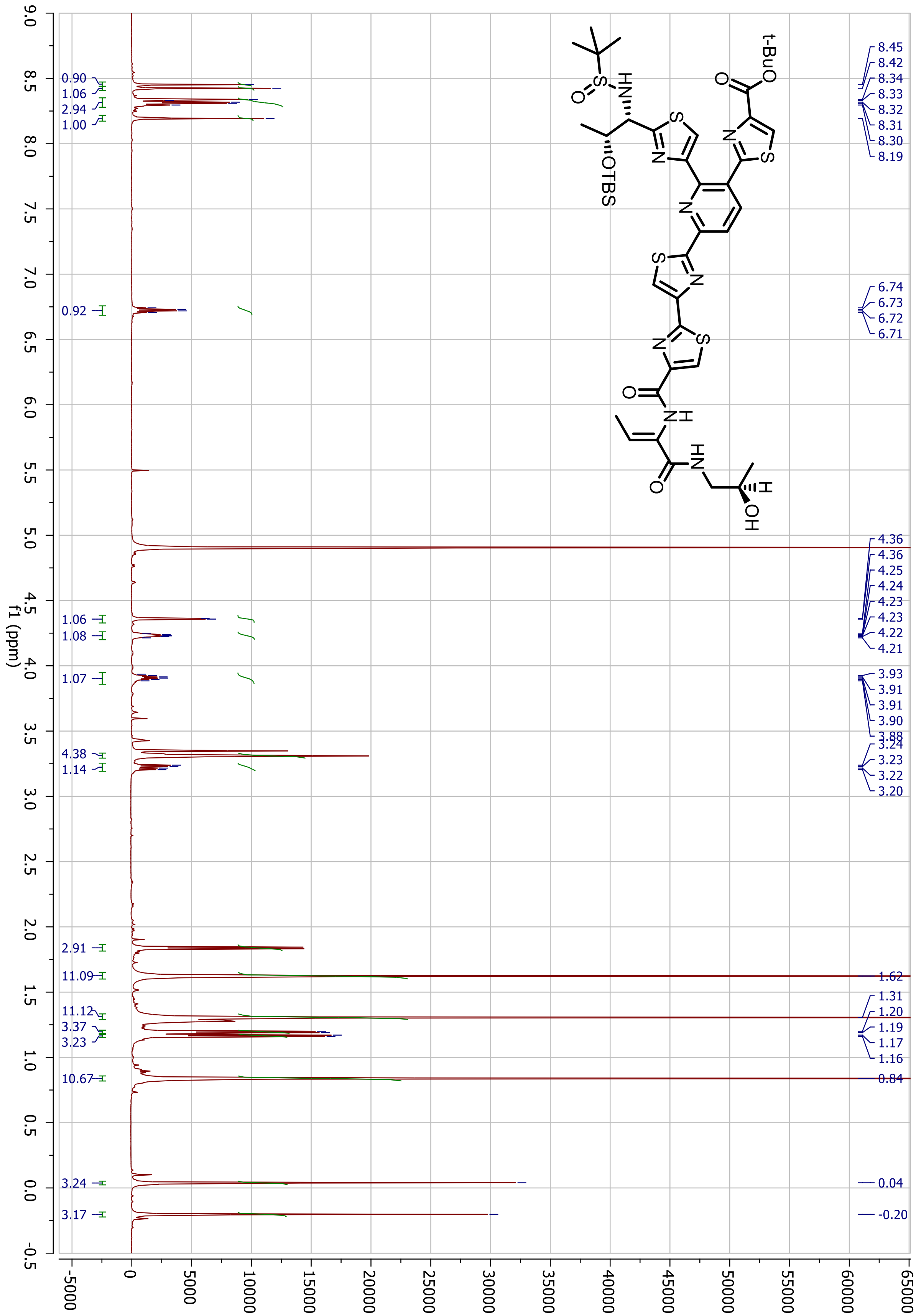

t-BuO2C
S
N
Cl
N
CN
159.98
159.59
149.21
148.71
140.40
133.01
131.58
129.41
127.22
115.62
82.77
28.15
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

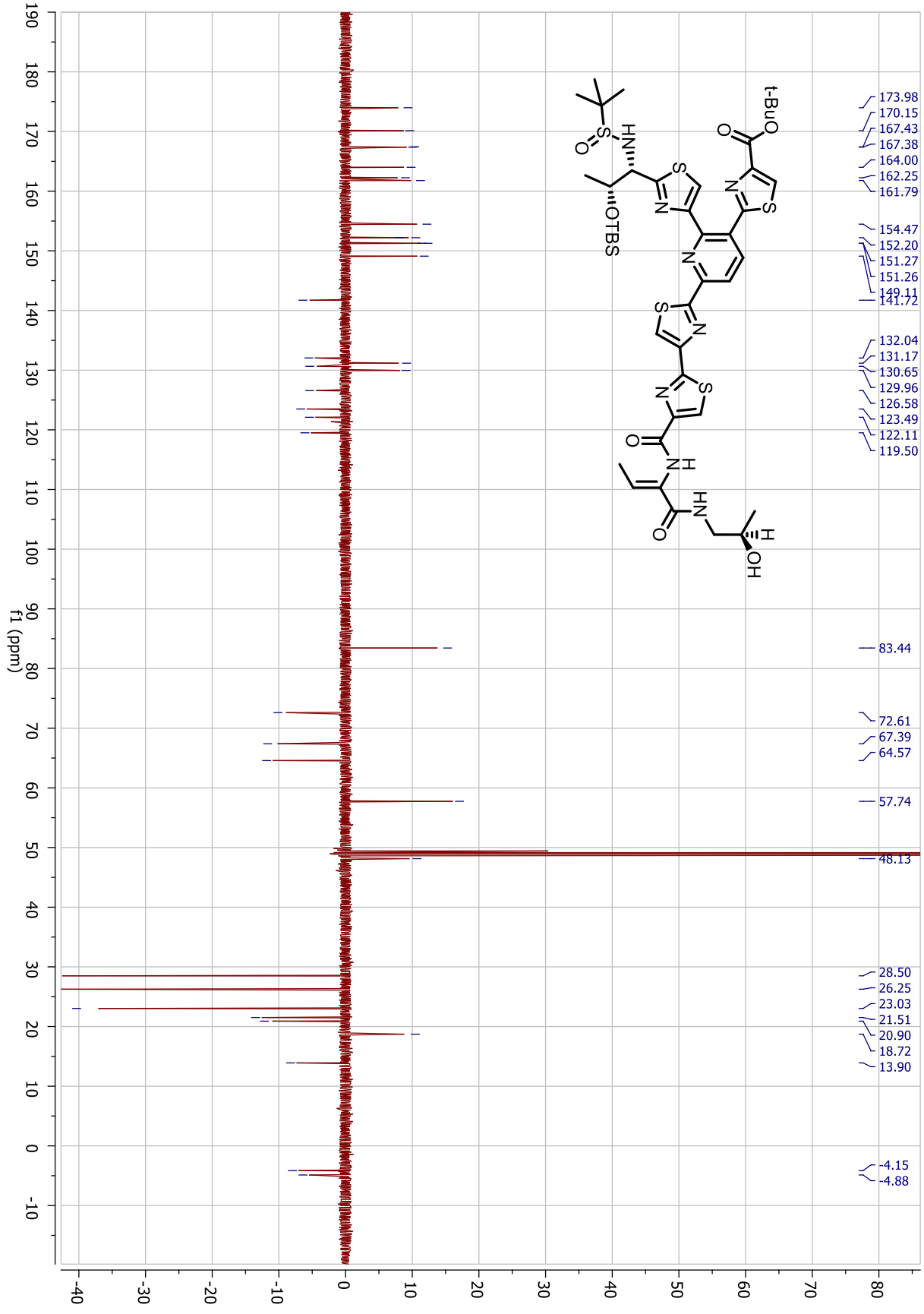

f1 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-10000
0
10000
20000
30000
40000
50000
60000
70000
80000
90000
1E+05
1E+05
1E+05
1.01
0.94
0.91
1.00
1.10
1.05
1.09
9.45
9.69
3.26
9.62
3.02
3.08
8.33
8.32
8.14
7.89
7.75
7.74
4.72
4.71
4.37
4.36
4.36
4.35
4.23
4.22
4.22
4.21
4.20
4.20
4.19
4.19
1.61
1.29
1.22
1.21
0.79
-0.02
-0.29
t-Bu
S
O
HN
OTBS
S
N
S
N
N
CN
t-BuO2C

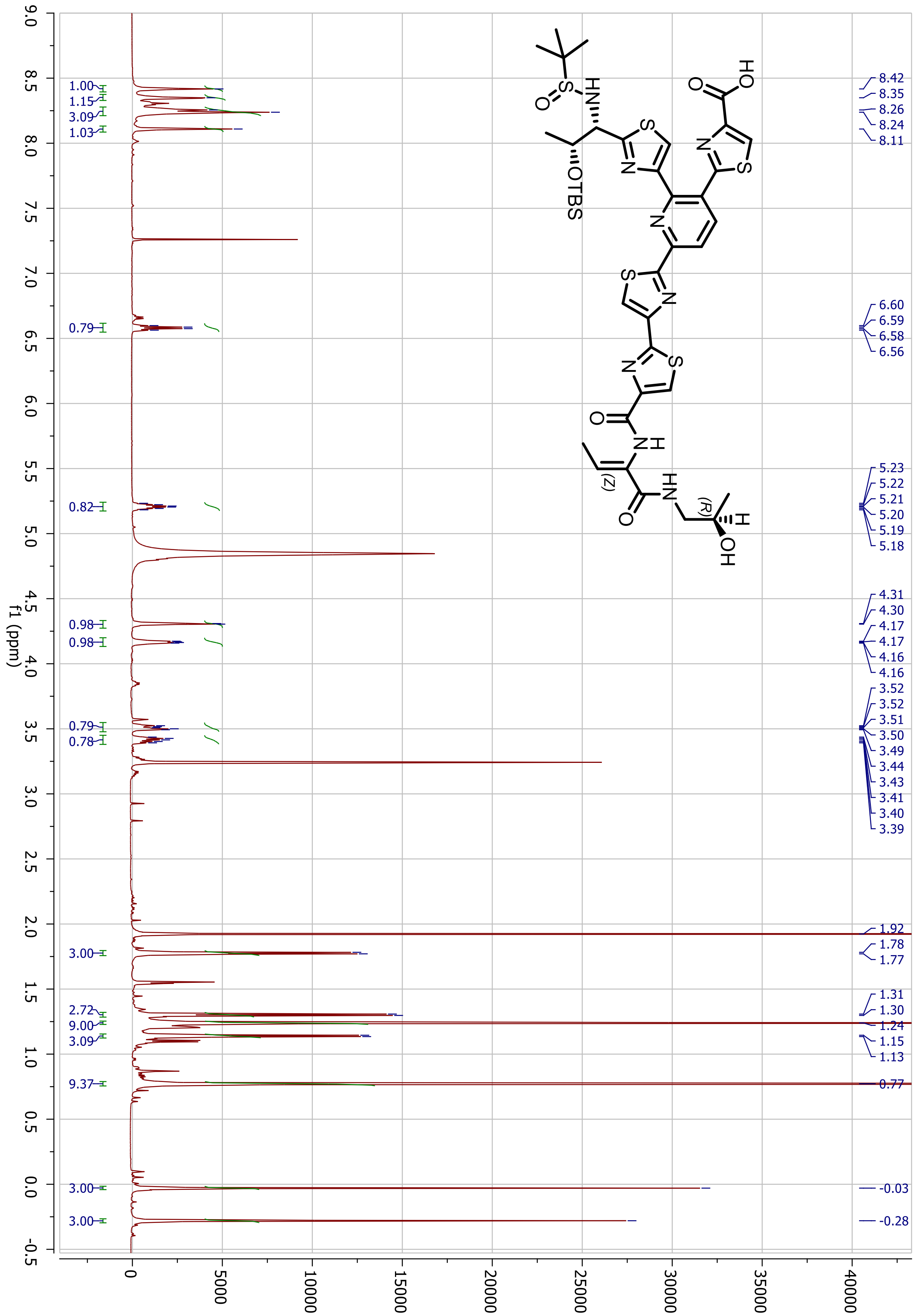

174.39
163.89
160.16
152.64
152.20
148.83
140.33
134.08
131.13
128.82
126.84
122.93
116.85
82.53
71.51
63.24
56.53
28.31
25.81
22.91
21.15
17.92
-4.30
-5.18
f1 (ppm)

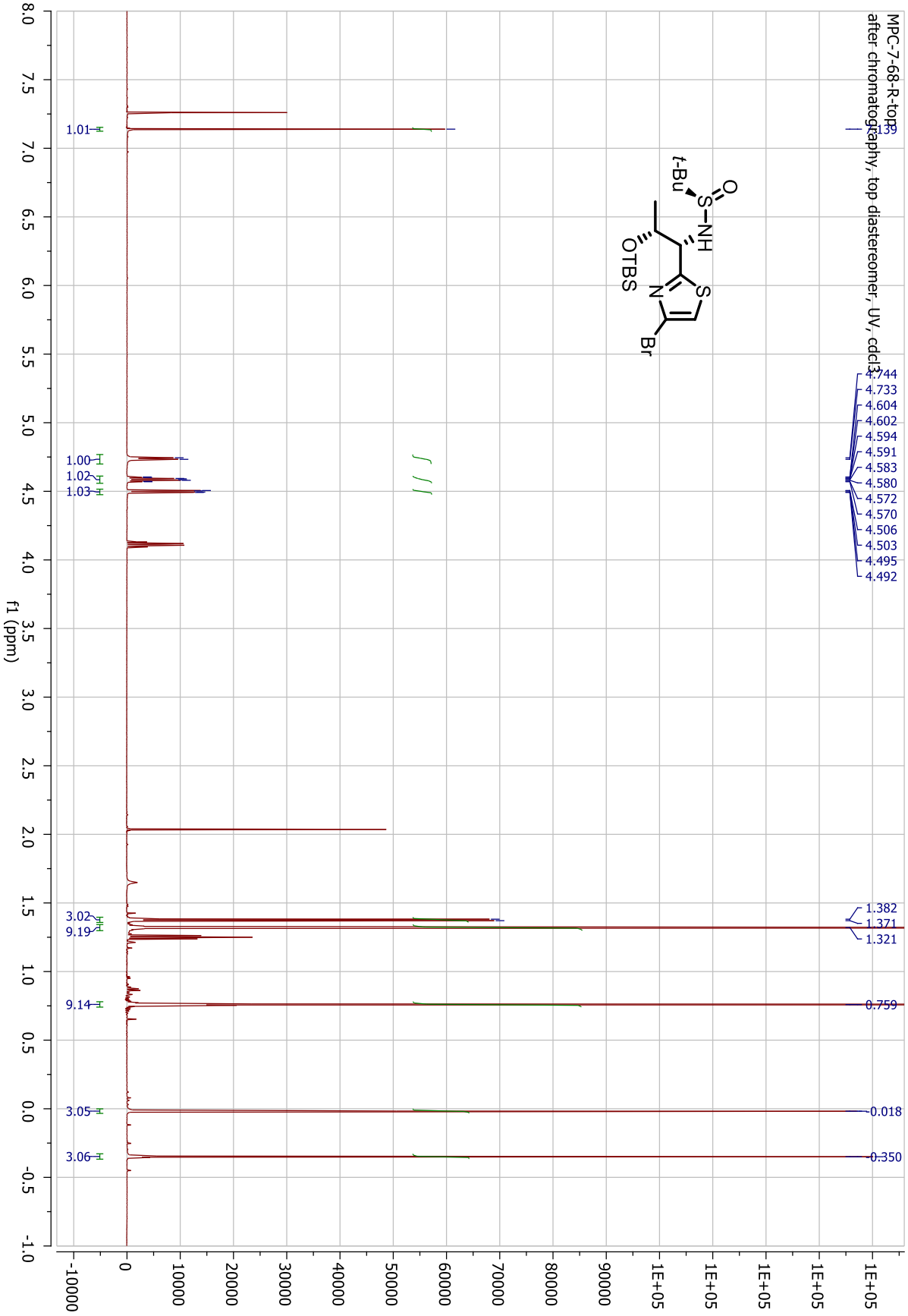

f1 (ppm)
8.47
8.31
8.31
8.30
8.19
6.74
6.73
6.72
6.70
6.31
6.29
6.29
6.28
4.86
4.85
4.37
4.37
4.36
4.36
4.05
4.05
4.03
3.88
3.87
3.32
3.31
3.30
3.24
3.24
3.23
3.22
3.22
3.21
3.20
1.84
1.82
1.65
1.32
1.22
1.21
1.18
1.17
0.86
0.06
-0.19
t-BuO
t-Bu
OTBS
OH

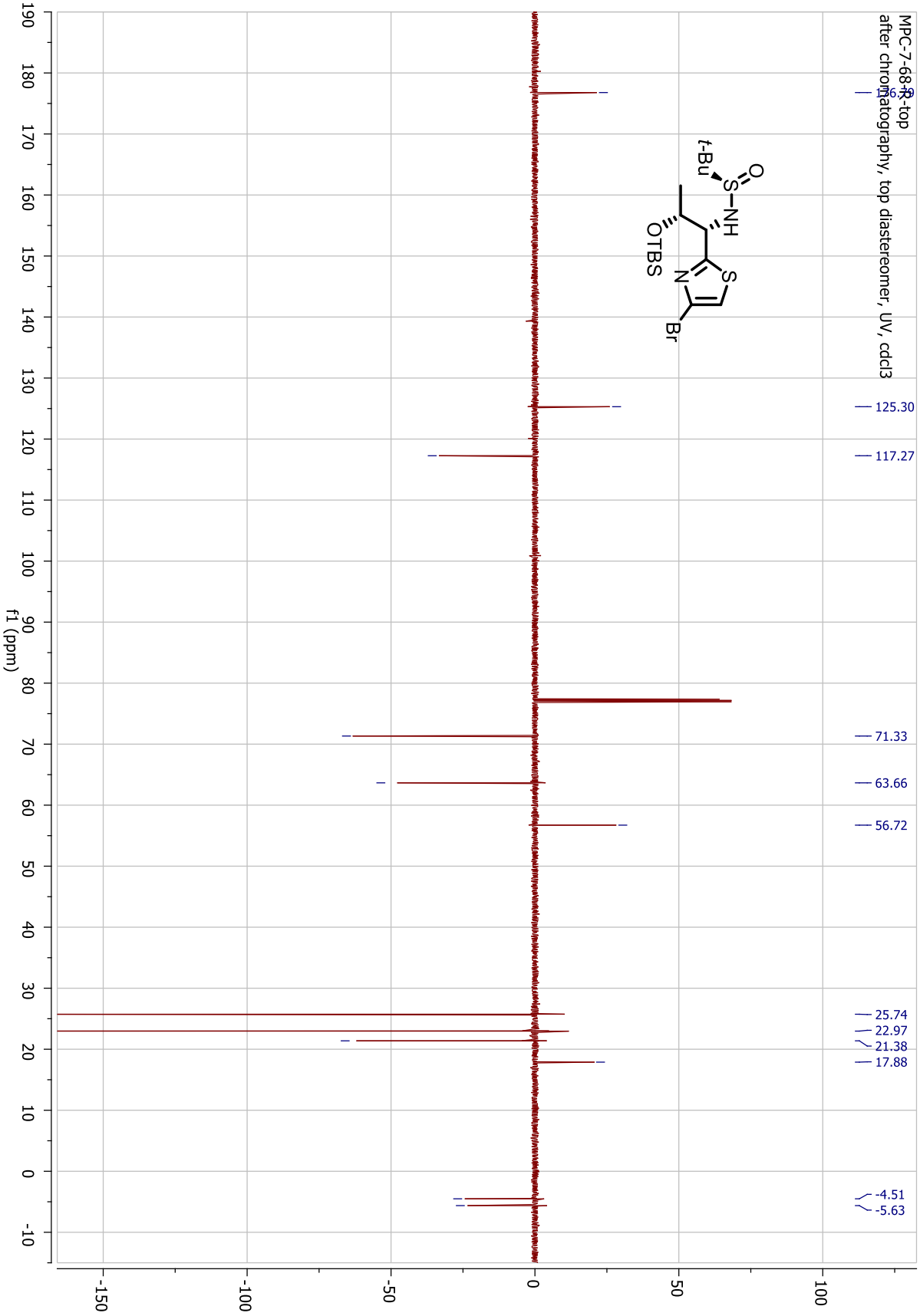

175.37
173.92
173.90
173.28
167.42
167.35
162.30
161.76
154.48
152.35
151.88
150.57
149.13
141.30
132.01
131.12
130.75
130.73
126.49
123.49
121.38
83.46
79.63
72.58
67.36
64.70
64.56
57.73
57.72
48.10
38.38
28.48
26.24
23.00
21.50
20.87
18.71
13.83
-4.16
-4.91
f1 (ppm)

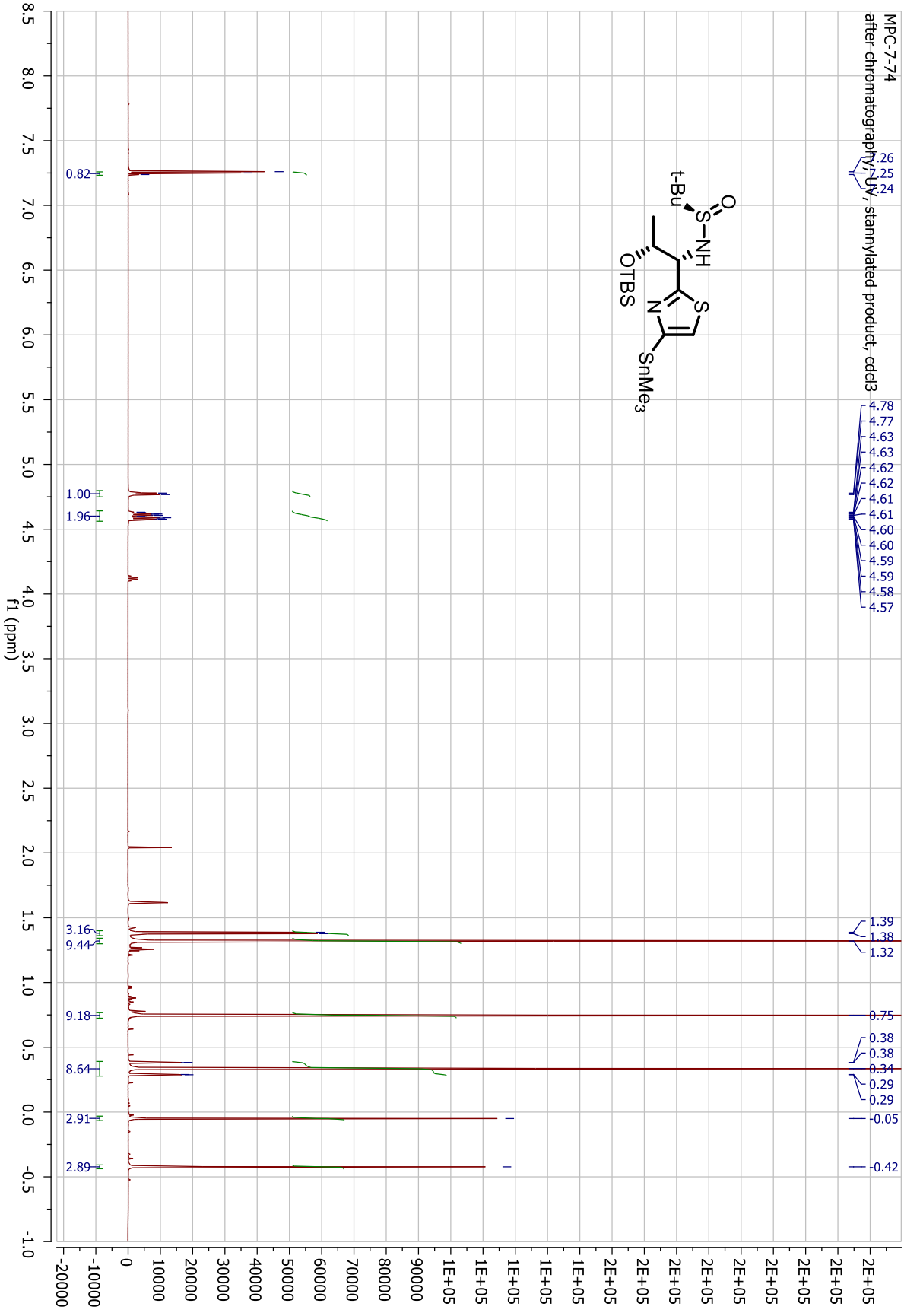

f1 (ppm)
t-BuO
OTBS
8.45
8.42
8.34
8.33
8.32
8.31
8.30
8.19
6.74
6.73
6.72
6.71
4.36
4.36
4.25
4.24
4.23
4.23
4.22
4.21
3.93
3.91
3.91
3.90
3.88
3.24
3.23
3.22
3.20
1.62
1.31
1.20
1.19
1.17
1.16
0.84
0.04
-0.20

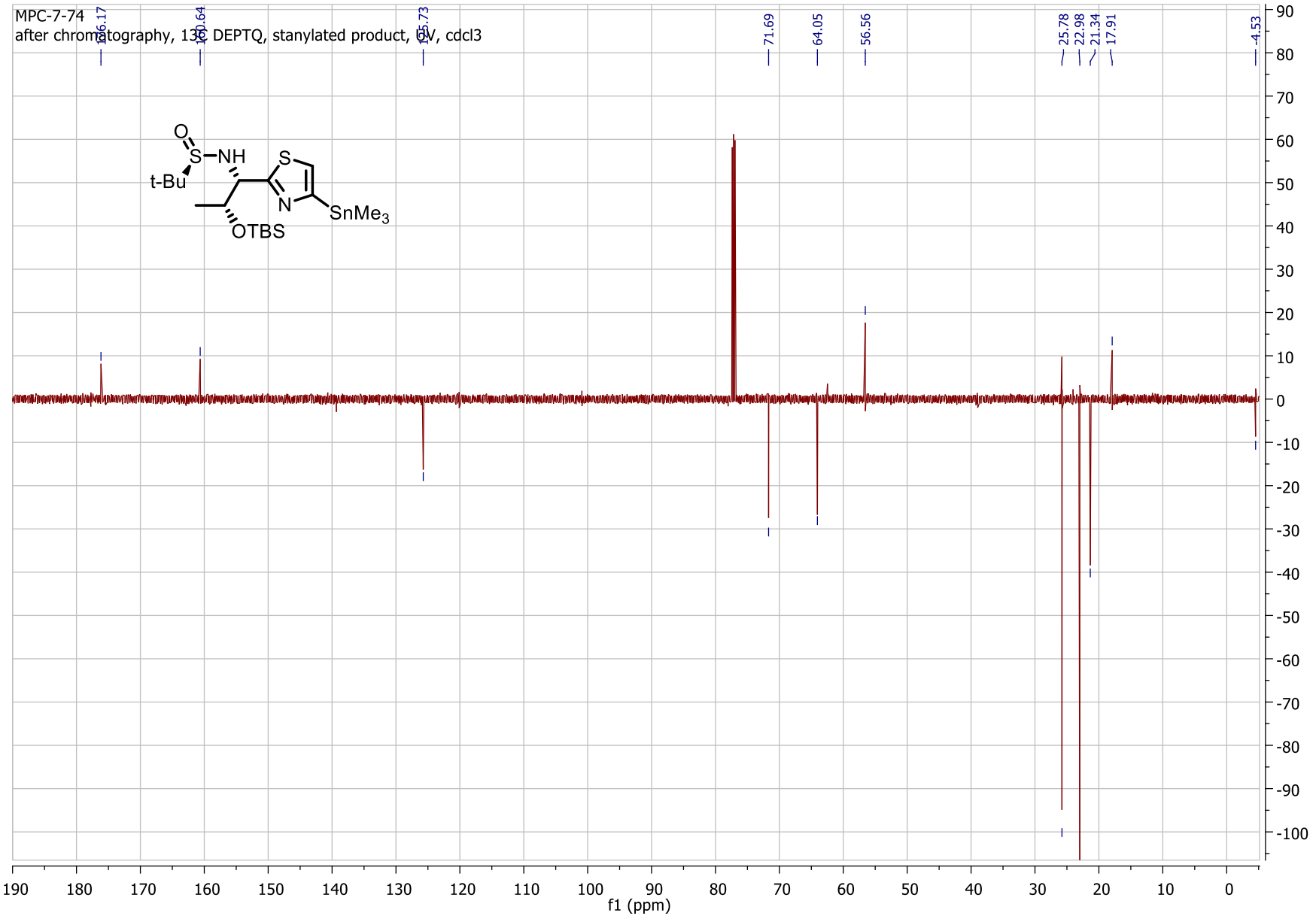

f1 (ppm)
173.98
170.15
167.43
167.38
164.00
162.25
161.79
154.47
152.20
151.27
151.26
149.11
141.72
132.04
131.17
130.65
129.96
126.58
123.49
122.11
119.50
83.44
72.61
67.39
64.57
57.74
48.13
28.50
26.25
23.03
21.51
20.90
18.72
13.90
-4.15
-4.88
t-BuO
OTBS
HN
OH

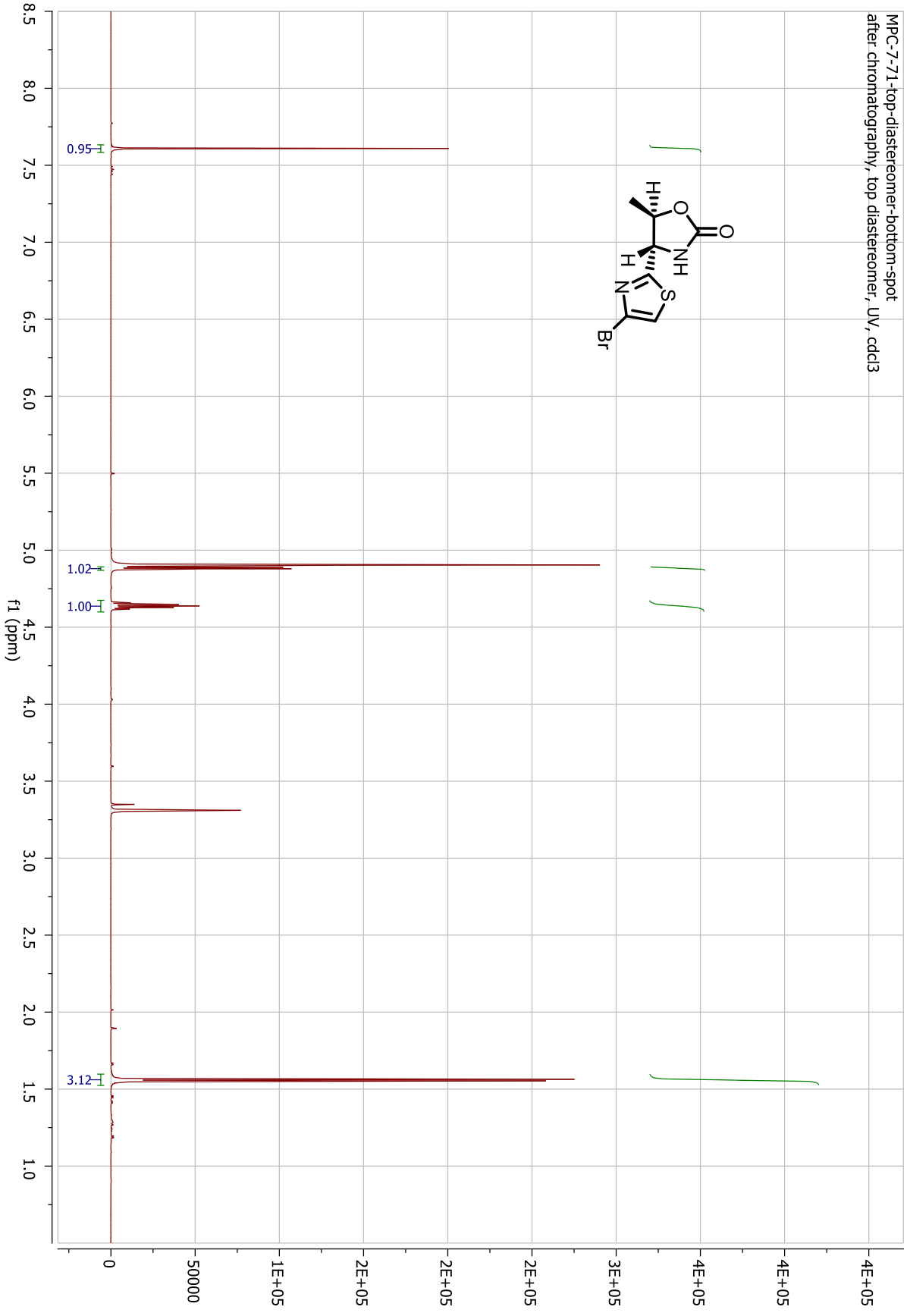

f1 (ppm)
OTBS
(Z)
(R)

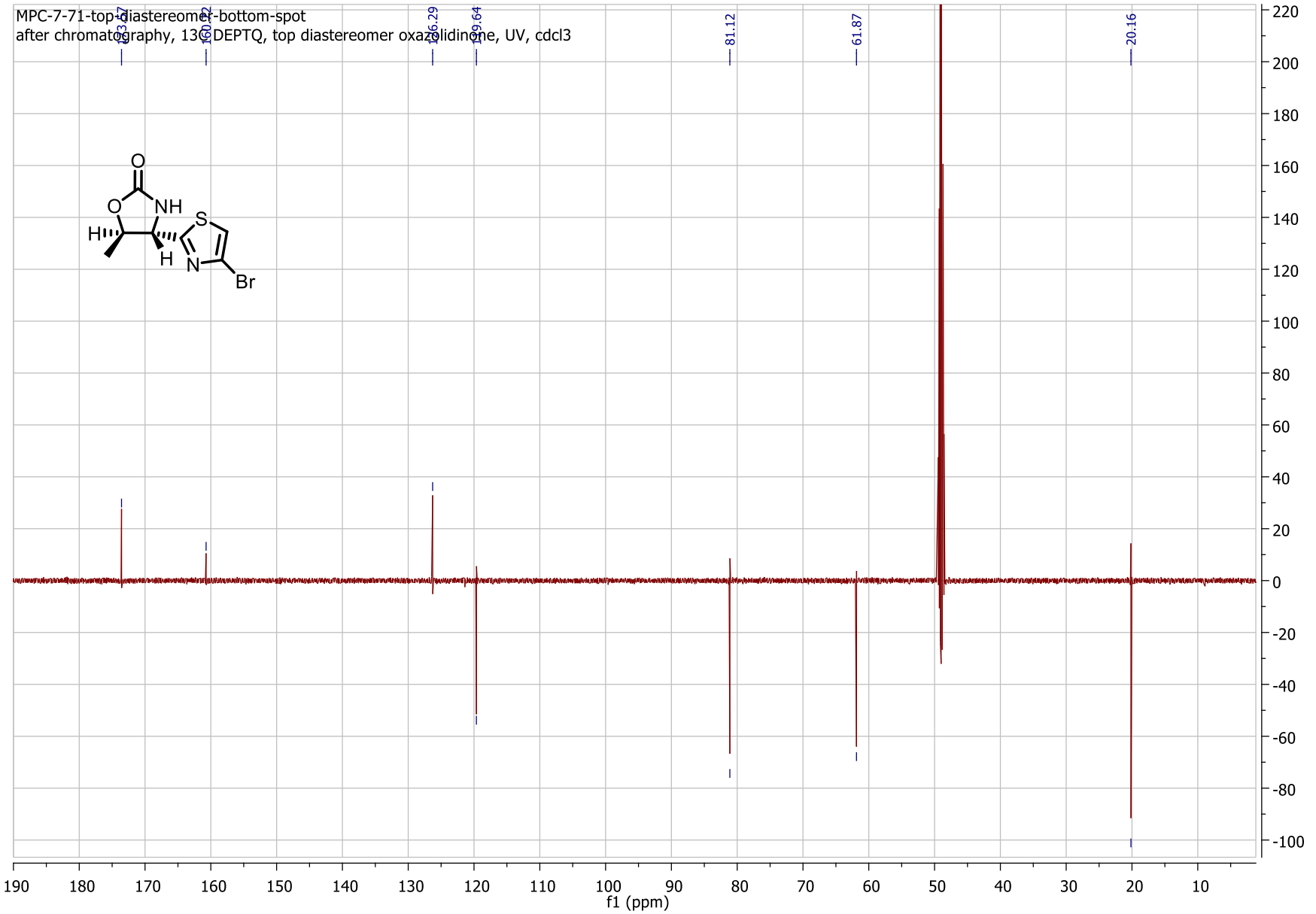

MPC-7-68-R-top
after chromatography, top diastereomer, UV, cdcl3
f1 (ppm)
1.01
1.00
1.02
1.03
3.02
9.19
9.14
3.05
3.06
4.744
4.733
4.604
4.602
4.594
4.591
4.583
4.580
4.572
4.570
4.506
4.503
4.495
4.492
1.382
1.371
1.321
0.759
0.018
0.350
t-Bu
O
S
NH
OTBS
N
S
Br

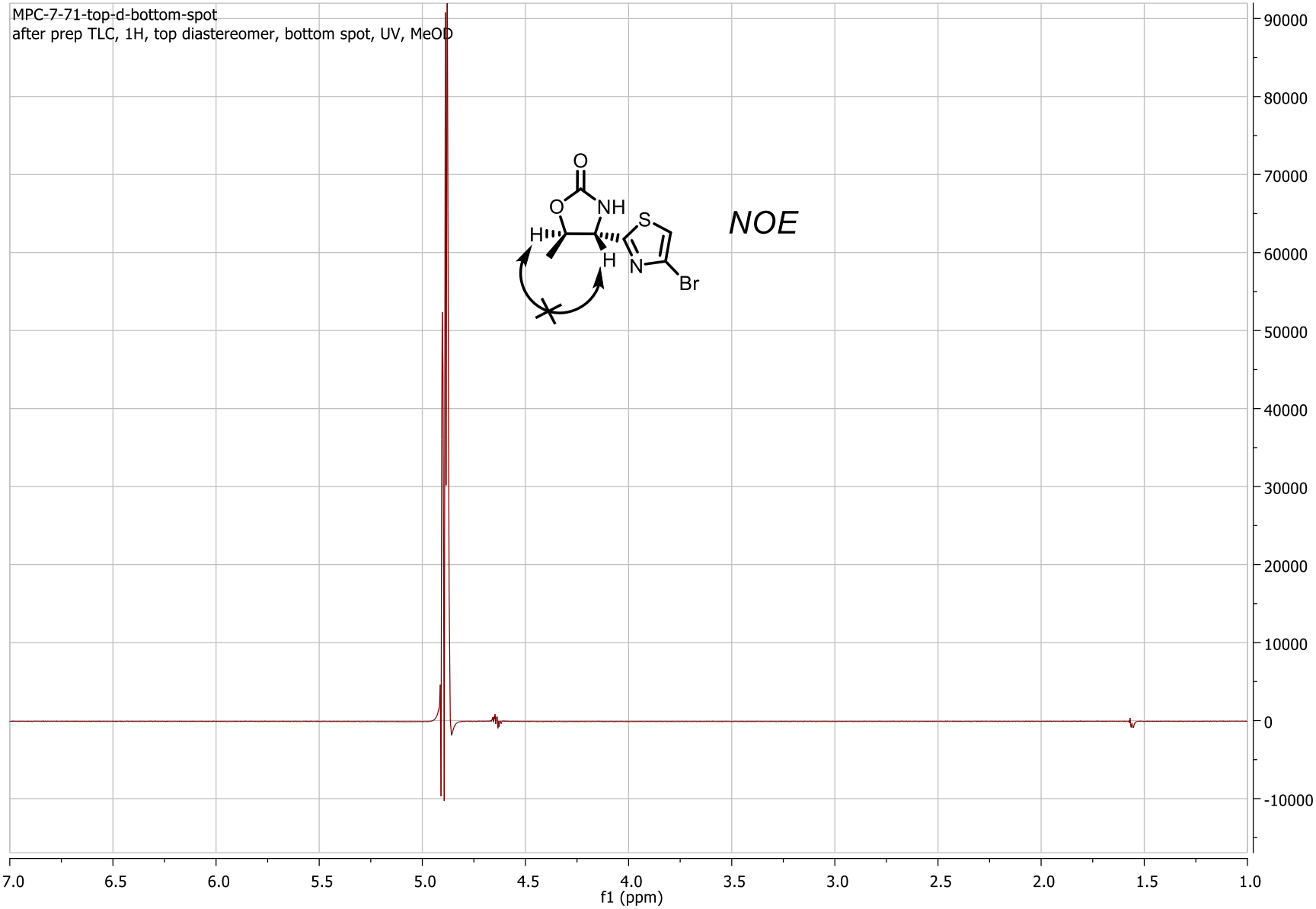

MPC-7-68-R-top
after chromatography, top diastereomer, UV, cdcl3
136.79
125.30
117.27
71.33
63.66
56.72
25.74
22.97
21.38
17.88
-4.51
-5.63
t-Bu
O
S
NH
OTBS
N
S
Br
f1 (ppm)

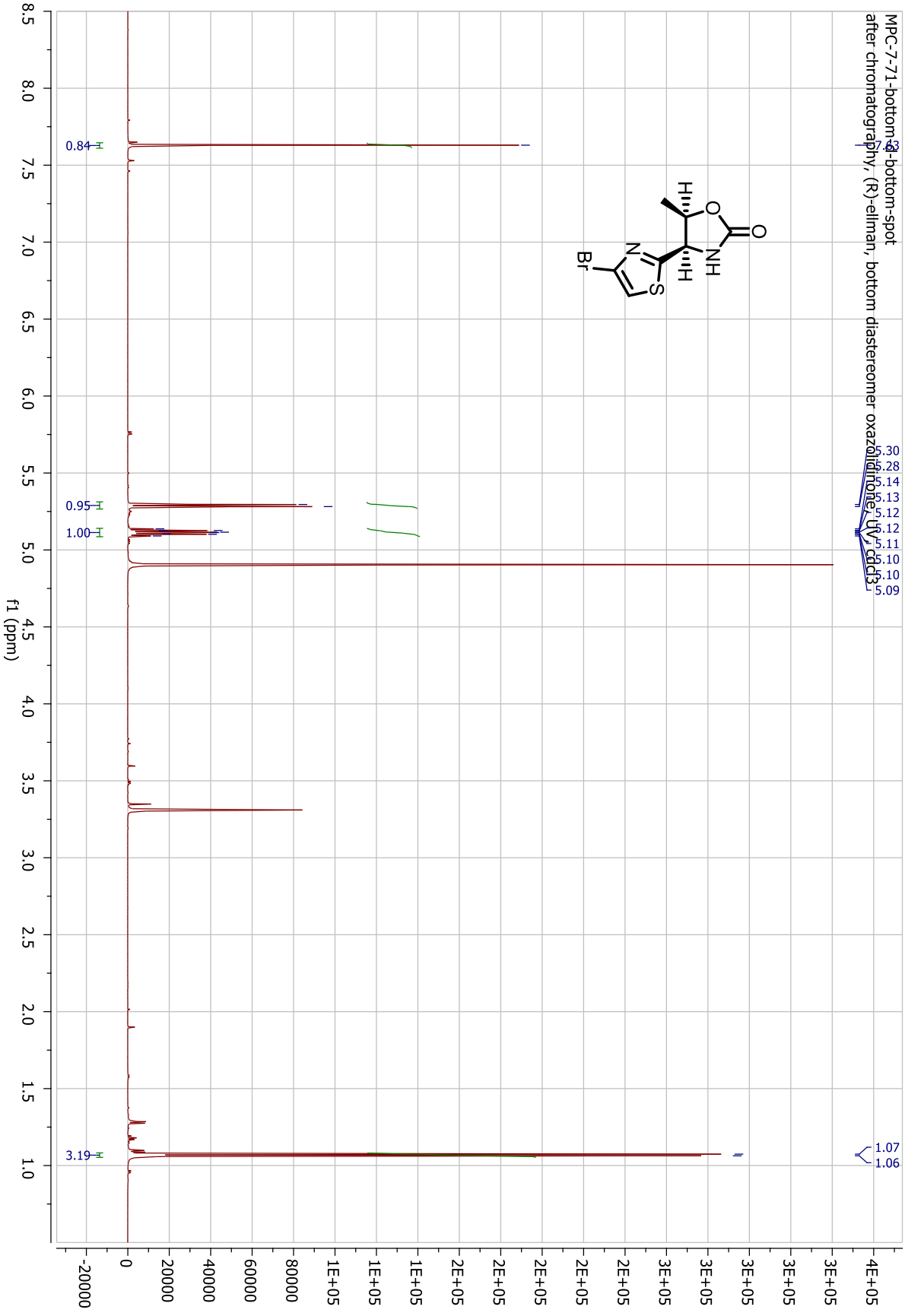

MPC-7-74
after chromatography, UV, stannylated product, cdcl3
t-Bu
S
NH
OTBS
N
S
SnMe3
7.26
7.25
7.24
4.78
4.77
4.63
4.63
4.62
4.62
4.61
4.61
4.60
4.60
4.59
4.59
4.58
4.57
1.39
1.38
1.32
0.75
0.38
0.38
0.34
0.29
0.29
-0.05
-0.42
0.82
1.00
1.96
3.16
9.44
9.18
8.64
2.91
2.89
f1 (ppm)

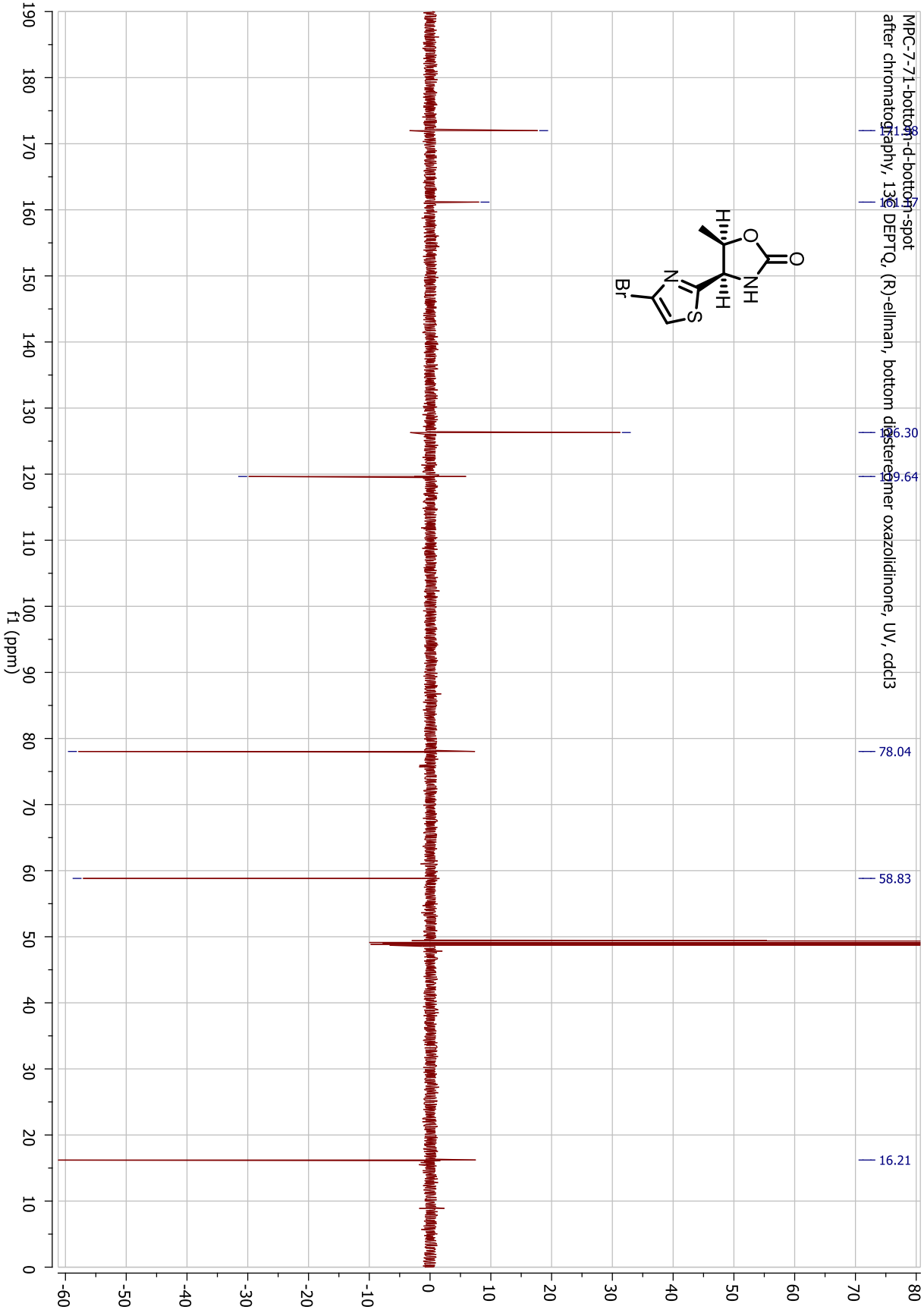

MPC-7-74
after chromatography, 13C DEPTQ, stanylated product, DV, cdcl3
176.17
160.64
135.73
71.69
64.05
56.56
25.78
22.98
21.34
17.91
-4.53
O
S
NH
t-Bu
S
N
SnMe3
OTBS
f1 (ppm)

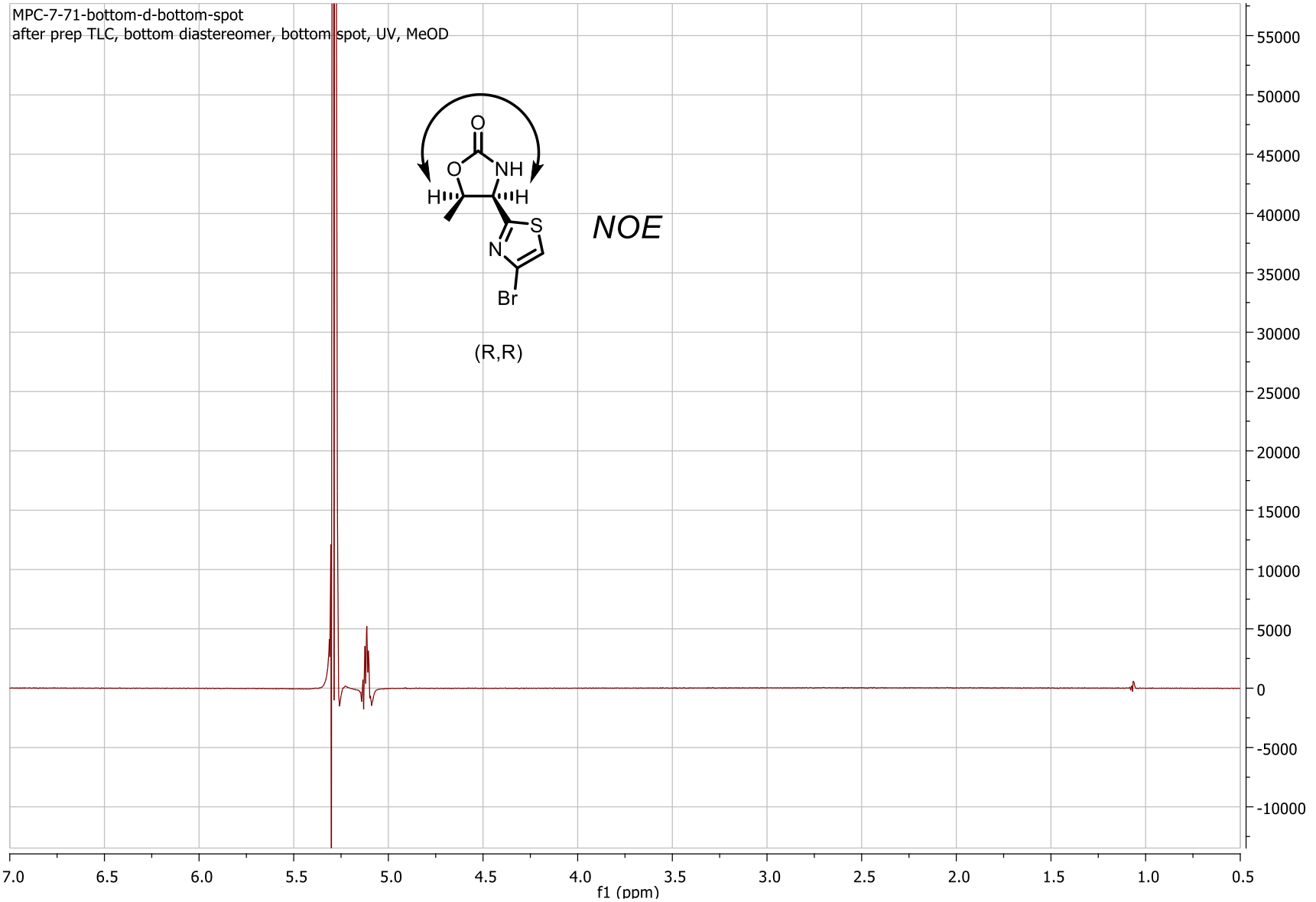

MPC-7-71-top-diastereomer-bottom-spot
after chromatography, top diastereomer, UV, cdcl3
0.95
1.02
1.00
3.12
f1 (ppm)
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0
50000
1E+05
2E+05
2E+05
2E+05
3E+05
4E+05
4E+05
4E+05
H
O
O
NH
H
N
S
Br

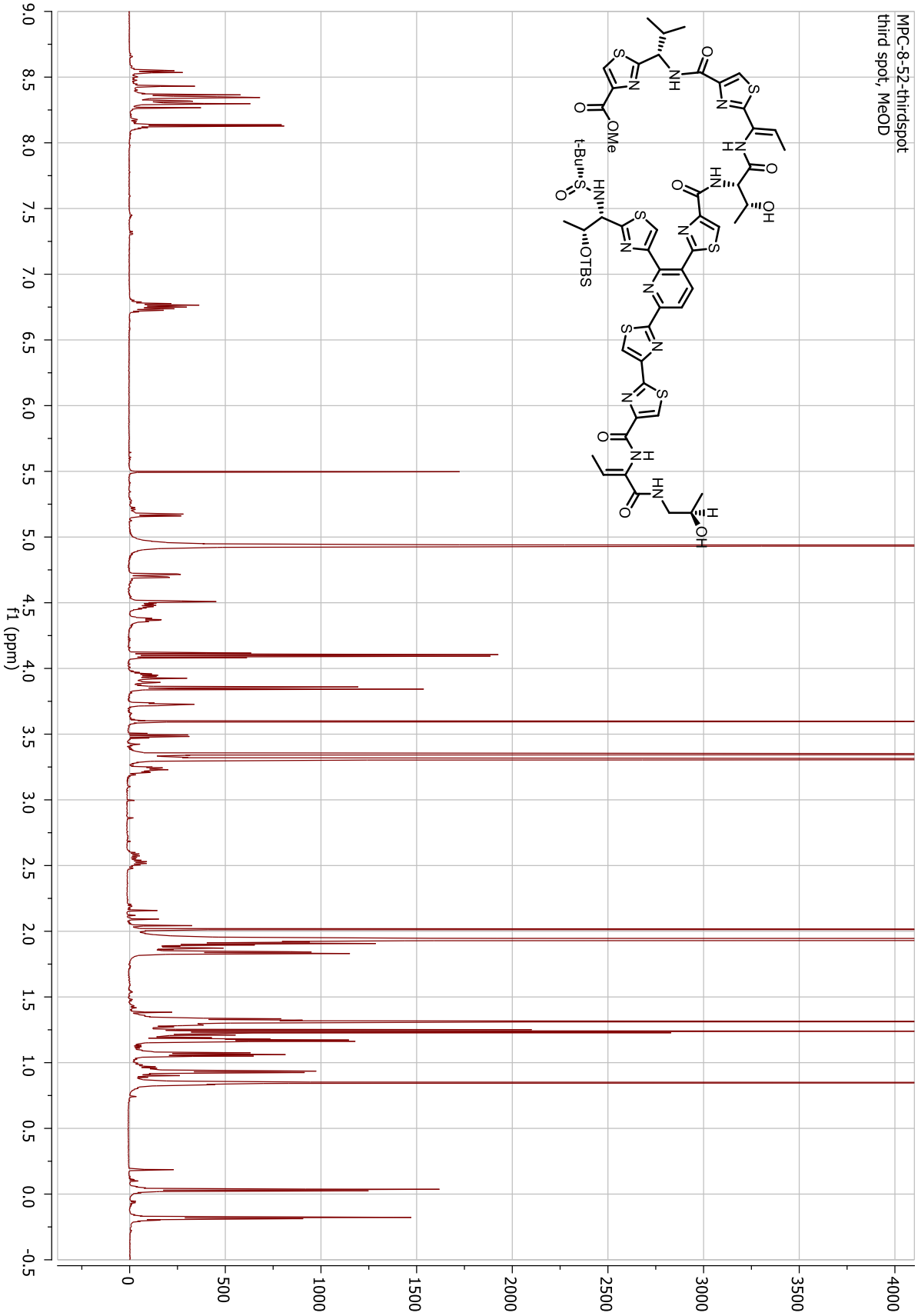

MPC-7-71-top-diastereomer-bottom-spot
after chromatography, 13C DEPTQ, top diastereomer oxazolidinone, UV, cdcl3
173.47
160.82
126.29
119.64
81.12
61.87
20.16
O
NH
O
H
H
S
N
Br
f1 (ppm)

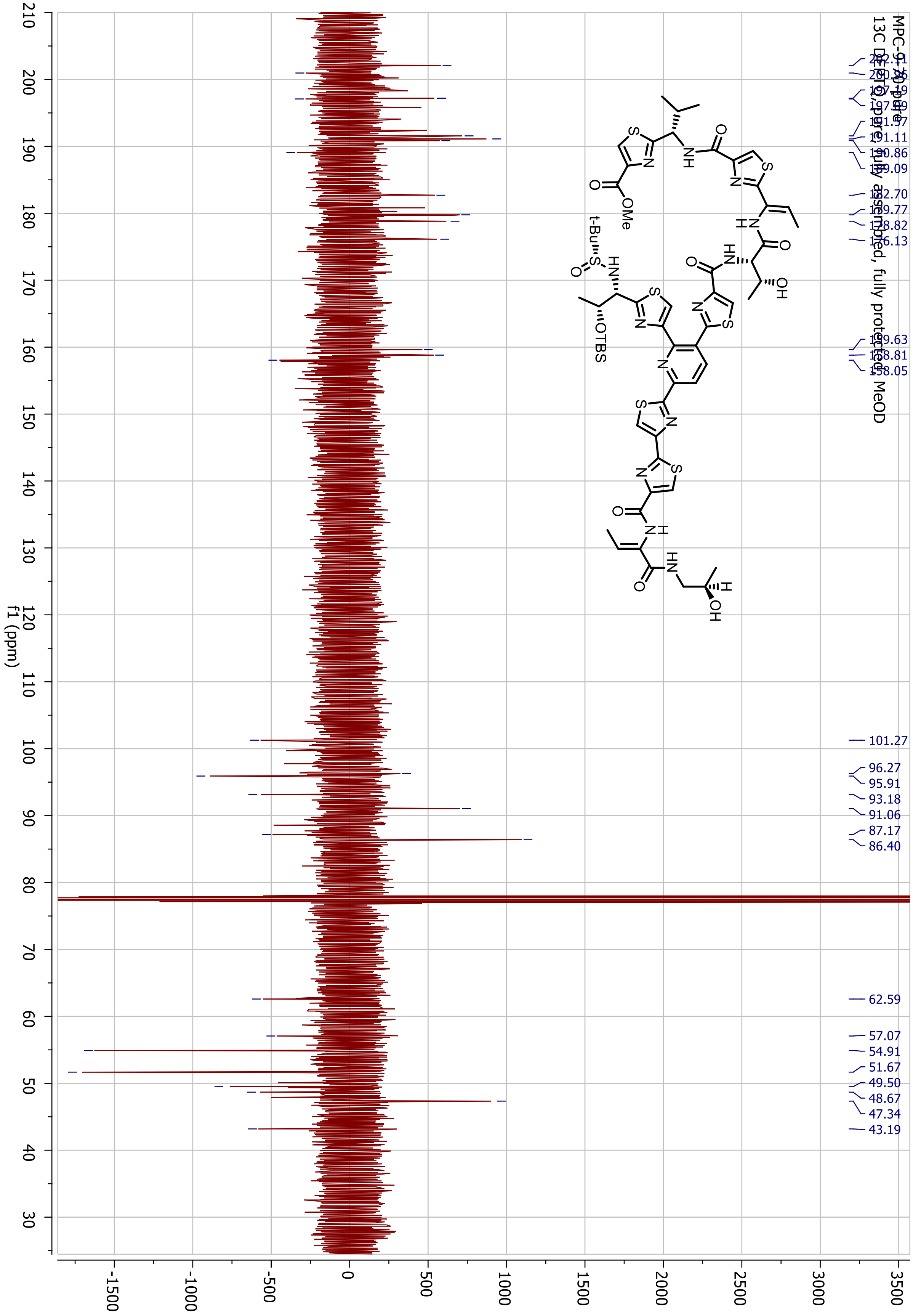

MPC-7-71-top-d-bottom-spot
after prep TLC, 1H, top diastereomer, bottom spot, UV, MeOD
O
NH
O
H
H
S
N
Br
NOE
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
-10000
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)


MPC-7-71-bottom-bottom-spot
after chromatography, (R)-ellman, bottom diastereomer oxazolidinone, 1H, cdcl3
0.84
0.95
1.00
3.19
7.63
5.30
5.28
5.14
5.13
5.12
5.12
5.11
5.10
5.10
5.09
1.07
1.06
Br
N
S
H
O
O
NH
H
f1 (ppm)

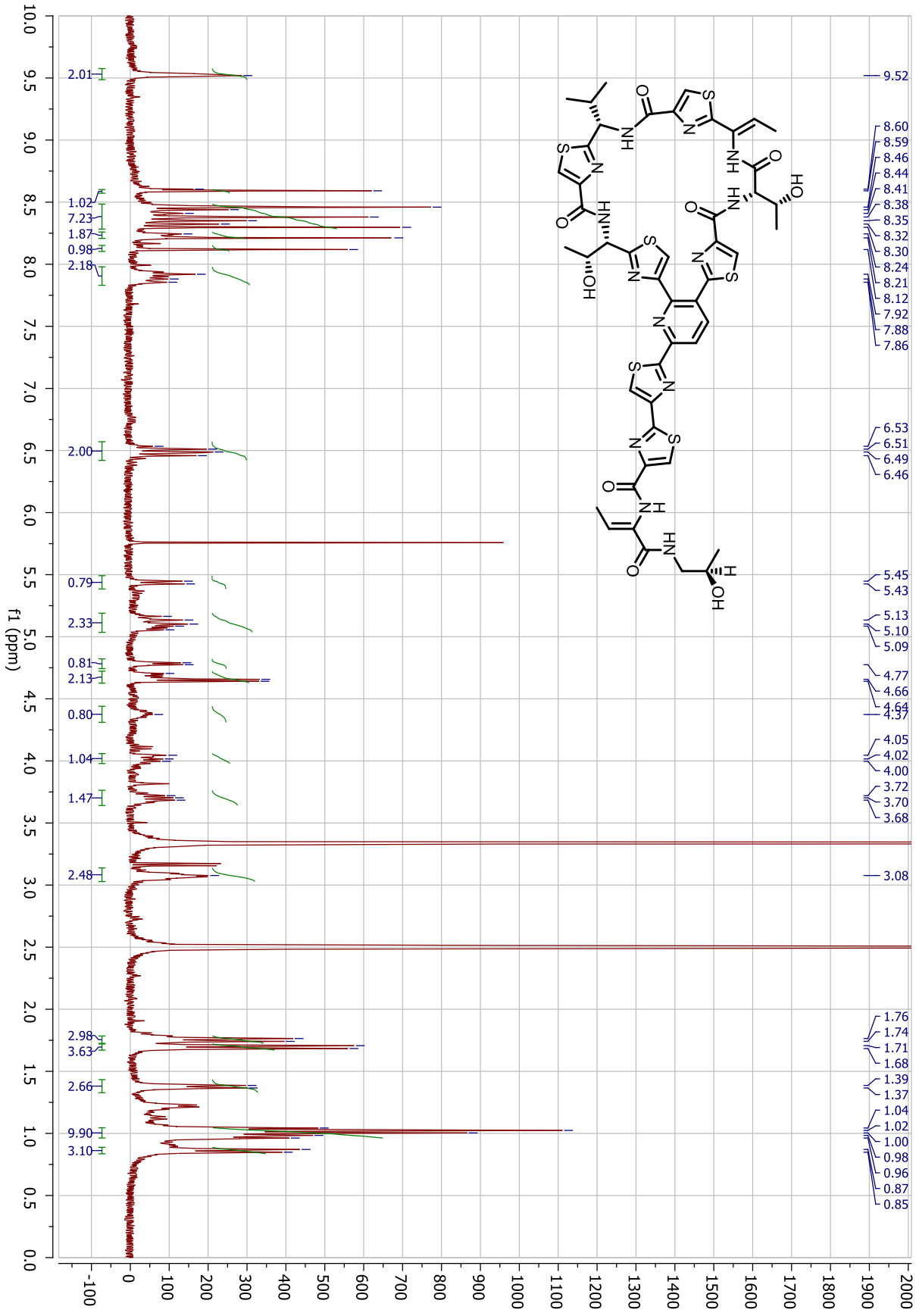

MPC-7-71-bottom-d-bottom-spot
after chromatography, 13C DEPTQ, (R)-ellman, bottom diastereomer oxazolidinone, UV, cdcl3
171.88
161.57
126.30
109.64
78.04
58.83
16.21
f1 (ppm)


MPC-7-71-bottom-d-bottom-spot
after prep TLC, bottom diastereomer, bottom spot, UV, MeOD
O
O
NH
H
H
S
N
Br
NOE
(R,R)
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
-5000
-10000
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

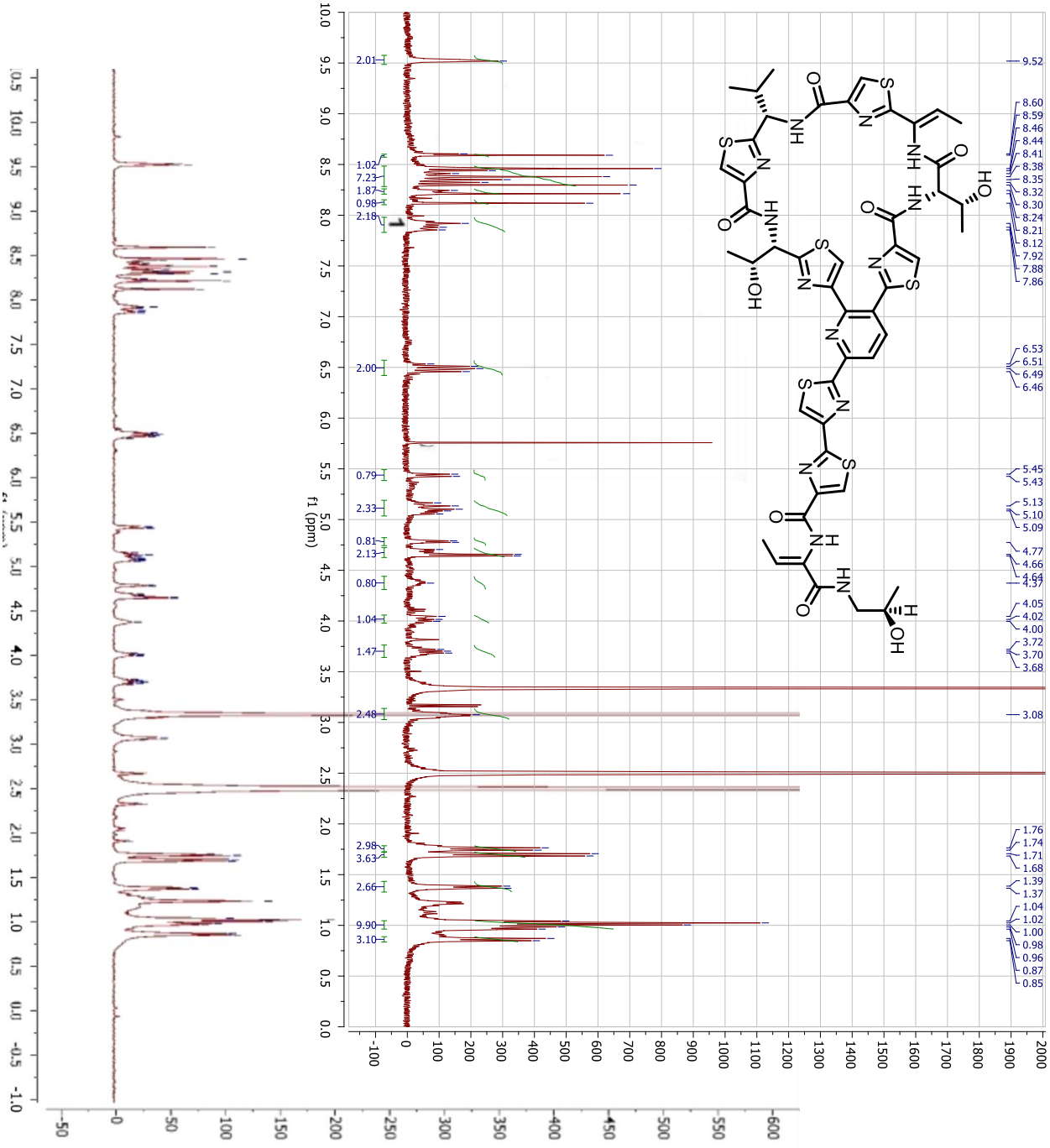

MPC-8-52-thirdspot
third spot, MeOD
f1 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
0
500
1000
1500
2000
2500
3000
3500
4000

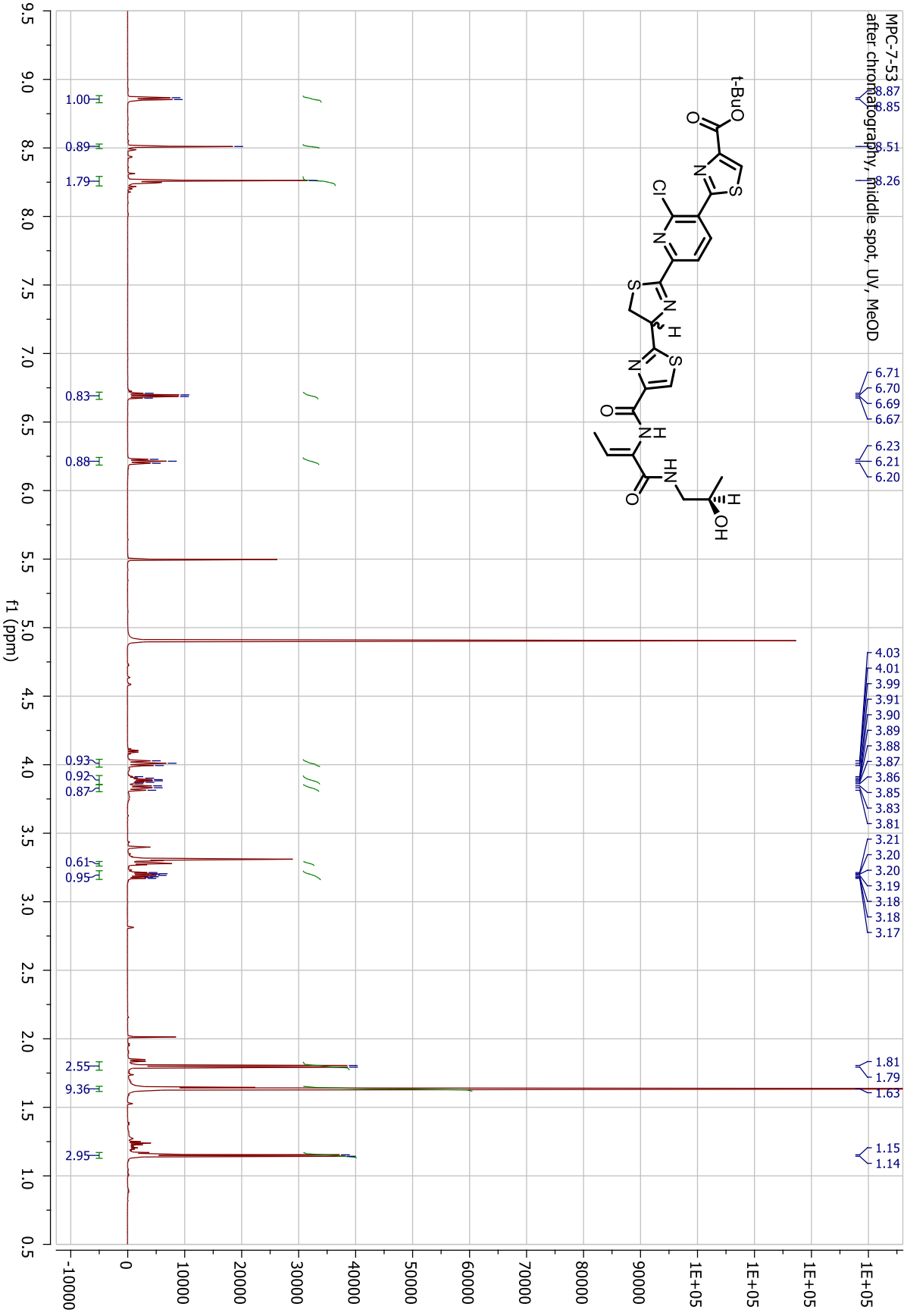


MPC-9-70-pure
13C DEPTQ, pure, fully assembled, fully protected, MeOD
101.27
96.27
95.91
93.18
91.06
87.17
86.40
62.59
57.07
54.91
51.67
49.50
48.67
47.34
43.19
f1 (ppm)
OMe
t-Bu
OTBS


f1 (ppm)
9.52
8.60
8.59
8.46
8.44
8.41
8.38
8.35
8.32
8.30
8.24
8.21
8.12
7.92
7.88
7.86
6.53
6.51
6.49
6.46
5.45
5.43
5.13
5.10
5.09
4.77
4.66
4.64
4.37
4.05
4.02
4.00
3.72
3.70
3.68
3.08
1.76
1.74
1.71
1.68
1.39
1.37
1.04
1.02
1.00
0.98
0.96
0.87
0.85
2.01
1.02
7.23
1.87
0.98
2.18
2.00
0.79
2.33
0.81
2.13
0.80
1.04
1.47
2.48
2.98
3.63
2.66
9.90
3.10

Akasapu micrococcin vs Christy micrococcin ^{1}HNMR (DMSO-d6)




MPC-7-53
after chromatography, middle spot, UV, MeOD
f1 (ppm)
t-BuO
Cl
OH
1.00
0.89
1.79
0.83
0.88
0.93
0.92
0.87
0.61
0.95
2.55
9.36
2.95
8.87
8.85
8.51
8.26
6.71
6.70
6.69
6.67
6.23
6.21
6.20
4.03
4.01
3.99
3.91
3.90
3.89
3.88
3.87
3.86
3.85
3.83
3.81
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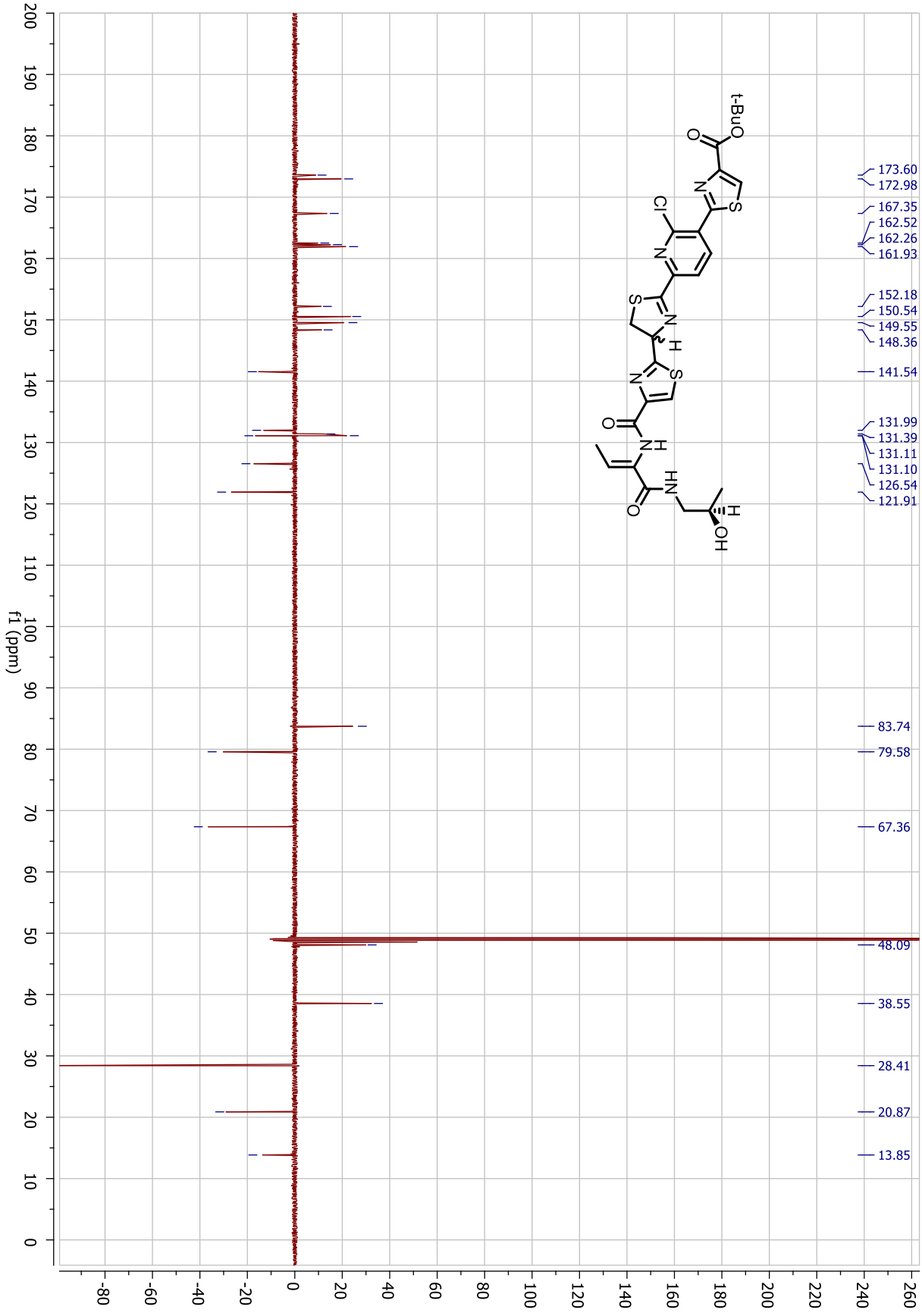
3.19
3.18
3.18
3.17
1.81
1.79
1.63
1.15
1.14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140
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100
90
80
70
60
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30
20
10
0
-80
-60
-40
-20
0
20
40
60
80
100
120
140
160
180
200
220
240
260
173.60
172.98
167.35
162.52
162.26
161.93
152.18
150.54
149.55
148.36
141.54
131.99
131.39
131.11
131.10
126.54
121.91
83.74
79.58
67.36
48.09
38.55
28.41
20.87
13.85
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N
S
Cl
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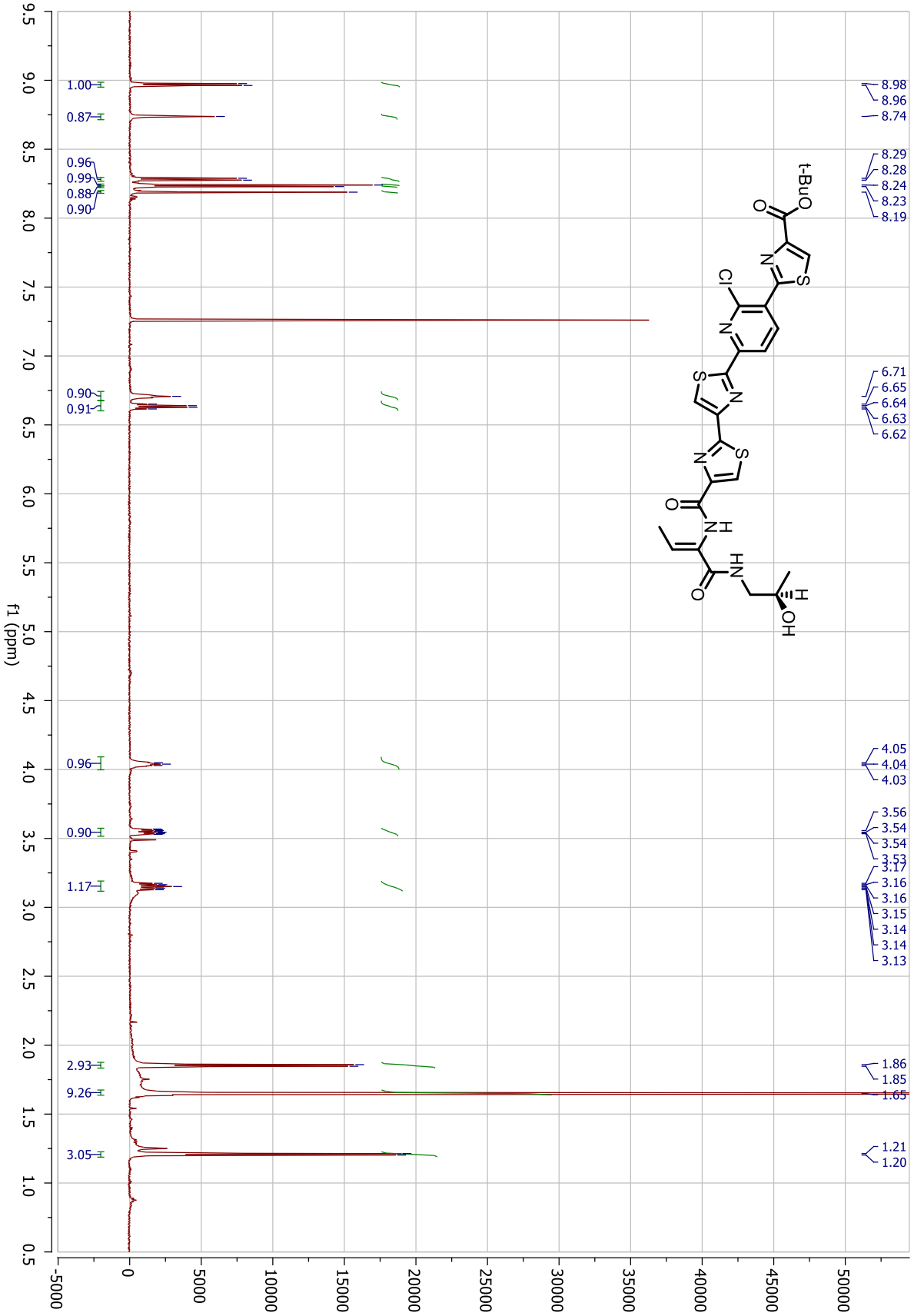
N
O
N
H
HN
O
H
OH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0.90
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0.96
0.90
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2.93
9.26
3.05
8.98
8.96
8.74
8.29
8.28
8.24
8.23
8.19
6.71
6.65
6.64
6.63
6.62
4.05
4.04
4.03
3.56
3.54
3.54
3.53
3.17
3.16
3.16
3.15
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Cl
HN
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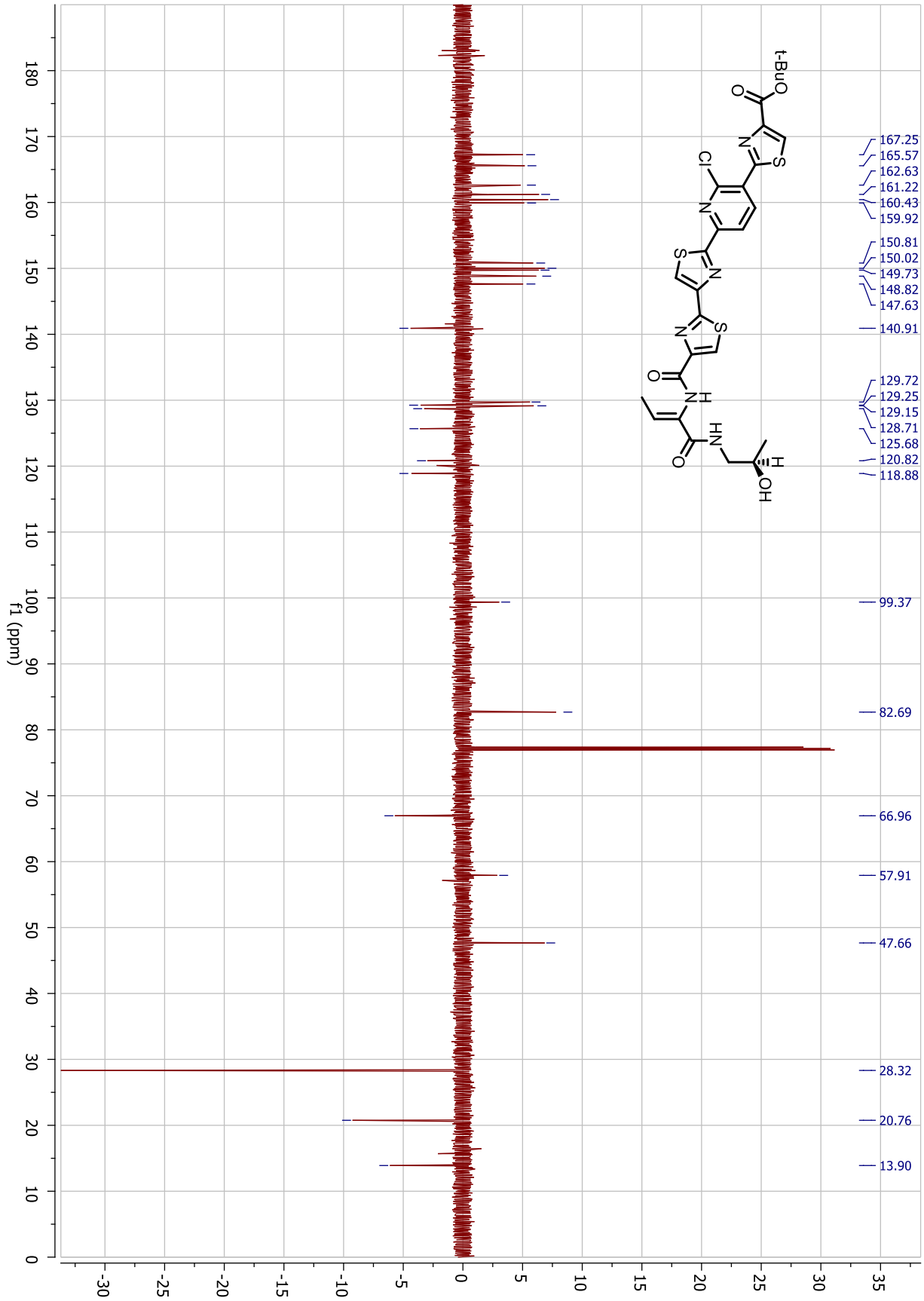
f1 (ppm)
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140.91
129.72
129.25
129.15
128.71
125.68
120.82
118.88
99.37
82.69
66.96
57.91
47.66
28.32
20.76
13.90
t-BuO
Cl
HN
OH

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