Expanding Non-Natural Chemical Space Through Synthetic Diversification of Natural Product Extracts

by

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B. Sc., Wilfrid Laurier University, 2017

Thesis Submitted in Partial Fulfillment of the
Requirements for the Degree of
Master of Science

in the
Department of Chemistry
Faculty of Science

SIMON FRASER UNIVERSITY
Summer 2019

Approval

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Degree:	**Master of Science (Chemistry)**
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Date Defended/Approved:	August 7th, 2019

Abstract

Natural products are structurally diverse compounds that often possess biological activity, making them the most prominent source for the discovery and development of drugs. Existing discovery methods often lead to the re-discovery of known compounds, causing the pharmaceutical industry to deprioritize natural products as a source of drug leads. Examination of natural products chemical space indicates that there are gaps that have yet to be filled in, suggesting that new methods are needed expand chemical space to access novel chemistry. This work expands chemical space by using synthetic transformations to derivatize natural product extracts. A library of 540 prefractionated derivatized natural product extracts was generated and screened against bacterial pathogens and cancer cells. A change in the antimicrobial and anticancer activity was observed as a result of the derivatizations. Derivatization of extract via a strain release reaction produced a novel staurosporine derivative with increased activity against human osteosarcoma cells.

Keywords: natural product derivatization; chemical space; metabolomics; high-throughput screening.

Acknowledgements

I would like to express my sincere gratitude to my supervisor Dr. Roger Linington for his guidance and mentorship over the course of my master's degree. His enthusiasm, knowledge, encouragement, and belief in my abilities allowed me to succeed at tasks that previously seemed insurmountable. I could not have hoped for a better supervisor.

I would also like to thank the other members of my supervisory committee: Dr. Robert Britton and Dr. Tim Storr for their insight and support. I am incredibly grateful to my supervisor and committee for not only their contributions to this project, but also for their support and aid in advancing my career as a chemist. I would not be afforded the opportunities that I have without them.

My sincere thanks also extends to all of the members of the Linington lab past and present: Cat, Jake, Dennis, Darryl, Joe, Mirelle, Trevor, Fausto, Sanghoon, Claire, Mercia, Dasha, Julienne, and Steve for teaching me nearly everything I know about analytical chemistry, and for providing such a constructive, supportive, and fun place to work. I would also like to thank Johannes in the Britton lab for all that he has taught me, including how to find the silver lining in anything. Many thanks to Mat in the Britton lab for his contribution to this project and the positive energy that he brought into the lab. Finally, I would also like to express my appreciation to Sandra, Christina, and Dennis for their kindness, professionalism, and knowledge of screening principles.

Additionally, I am thankful for all the staff in the Department of Chemistry and Science Stores for all the tangible and intangible ways that they supported my studies and this project.

I would not be where I am today without the love and support of my family. I wish to express my sincere gratitude to my mother for her unwavering faith in my abilities, and for our lunchtime chats. I would also like to thank my father for his encouragement over the last two years, his excitement over my research, and his constant offers to help me with my work. I am so fortunate to have the love and support of my two incredible stepparents as well: Karen and Mike, who have always been there for me when I needed them. Also, I would like to thank my brother Brent and my stepbrothers Brock

and Parker for teaching me how to be tough, always knowing how to make me laugh, and for always having my back.

Finally, I would like to express my deepest gratitude to Joseph for all his love and encouragement over the past two years. Thank you for motivating me to work hard, for teaching me how to step back and look at the bigger picture, and for reminding me that there is a lot more to life than science. I could not have dreamed up a better person for me than you.

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List of Schemes

List of Acronyms

ACN	Acetonitrile
ADC	Antibody drug conjugate
ADMET	Absorption, distribution, metabolism, excretionm toxicity
B1	Reaction using HDDA benzyne precursor
B2	Reaction using 2-(trimethylsilyl)phenyl trifluoromethanesulfonate benzyne precursor
CG	Chemical glycosylation
CP	Dichlorocyclopropanation
CryoEM	Cryo-electron microscopy
Cy5	Indodicarbocyanine
DAPI	4',6-diamino-2-phenylindole
DCE	Dichloroethane
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
DOS	Diversity oriented synthesis
ESI	Electrospray ionization
EtOAc	Ethyl acetate
FITC	Fluorescein isothiocyanate
GNPS	Global Natural Products Social Molecular Networking
HDDA	Hexadehydro Diels-Alder
HPLC	High performance liquid chromatography
HRMS	High-resolution mass spectrometry
IMS	Ion mobility separator
m/z	Mass to charge ratio
microED	Micro-electron diffraction
MMAE	Monomethyl auristatin E
MOA	Mode of action
MR	Morpholine ring formation reaction
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
NMR	Nuclear magnetic resonance spectroscopy
NP	Natural product
PC	Principal component
PCA	Principal component analysis
RF	Reductive amination using 3,5-bis(trifluoromethyl)benzaldehyde
ROV	Remotely operated vehicle
RP	Reductive amination using 3-pyridinecarboxaldehyde
RP	Reverse phase
SAR	Structure activity relationships
SCUBA	Self-contained underwater breathing apparatus
SR	Strain release amination
SYP	Starch yeast peptone broth
TBAC	Tetrabutylammonium chloride
THF	Tetrahydrofuran
TPSA	Topological polar surface area
TRITC	Tetramethylrhodamine

Attribution

The work reported herein was completed as a result of collaborative efforts with Dr. Johannes Lehmann and Mathew Sutherland in Dr. Robert Britton's lab, as well as with Dr. Sandra Keerthisinghe, Dr. Christina Gros, and Dennis Liu in the Center for High-Throughput Chemical Biology.

Chapter 1.

Introduction

1.1. Natural Products Discovery

1.1.1. Definition and Implications on Drug Discovery

Natural products (NP) are compounds produced through the secondary metabolic pathways of organisms. These metabolic pathways evolved to perform specific biological functions in response to environmental stressors. As a result, natural products often possess biological activities and complex structures that can be applied to problems in human health and biotechnology.[1]

Resultingly, natural products are the most common starting point for the discovery and development of pharmaceuticals. The application of natural products in medicine dates to ancient times, when traditional remedies often contained biologically active compounds. The earliest recorded use of natural products was the application of oils from *Cupressus sempervirens* and *Commiphora* species documented on stone Tablets in Mesopotamia around 2600 BC as a remedy for colds and inflammation.[2] The first use of natural products in modern medicine occurred in the 1940's when Dr. Selman Waksman isolated the antibiotic actinomycin from Actinomyces antibioticus.[3] Later, in 1952, Dr. Waksman would earn a Nobel prize for the discovery of streptomycin from Streptomyces griseus using an approach called bioassay-guided fractionation. Bioassay-guided fractionation is the separation of mixtures based on observed biological activity.[4] Nearly 80 years later, this approach is still the most prominent method for the discovery and isolation of new compounds from natural sources.

An assortment of antifungal, antibacterial, antiviral, anticancer, anti-inflammatory, antiprotozoal, and other medications have been discovered from natural products. These therapeutics were sourced from biological macromolecules, botanical drugs, unaltered natural products, natural product derivatives, synthetic drugs with natural product pharmacophores, and

mimics of natural products. Of all drugs produced from 1981 to 2014 between 50 and 70% were inspired by natural products as summarized in Table 1.1.[1]

Table 1.1 Number of Natural Product Derived Therapeutics

Condition treated	Number of NP derived therapeutics
Circulatory disorders	174
Cancer	147
Bacterial infections	84
Viruses	64
Gastrointestinal disorders	52
Diabetes	48
Respiratory disorders	41
Mental health	40
Immune disorders	38
Neurological disorders	37
Hormonal disorders	37
Reproductive and sexual health	36
Arthritis	35
Other	185

This Table was produced using data collected by Newman and Cragg for their article "Natural Products as Sources for New Drugs from 1981 to 2014".[1] Included in this Table of approved natural product therapeutics are biological macromolecules, unaltered natural products, botanical drugs, natural product derivatives, synthetic drugs with natural product pharmacophores, and mimics of natural products.

There are many different biological sources that natural products can be isolated from including plants, bacteria, and fungi. The Linington lab studies marine microbial natural products. Oceans cover over 70% of the earths surface, making the marine environment the richest biosphere on earth. It is estimated that between 50 and 80% of all life on earth is found in the oceans, and until the advent of SCUBA (self-contained underwater breathing apparatus) and ROV (remotely operated vehicle) in the 1940's and 1950's the marine environment was largely unexplored.[2,5] To date more than 30,000 compounds have been isolated from marine organisms. These compounds represent a unique section of chemical space, since approximately 71% of marine scaffolds are not present in terrestrial compounds. [6] The number of approved marine therapeutics has increased steadily from four in 2009 to seven in 2018 (Figure 1.1 and Table 1.2). There are a further six marine pharmaceuticals in phase III, ten in phase II, and nine in phase I clinical trials.[7]

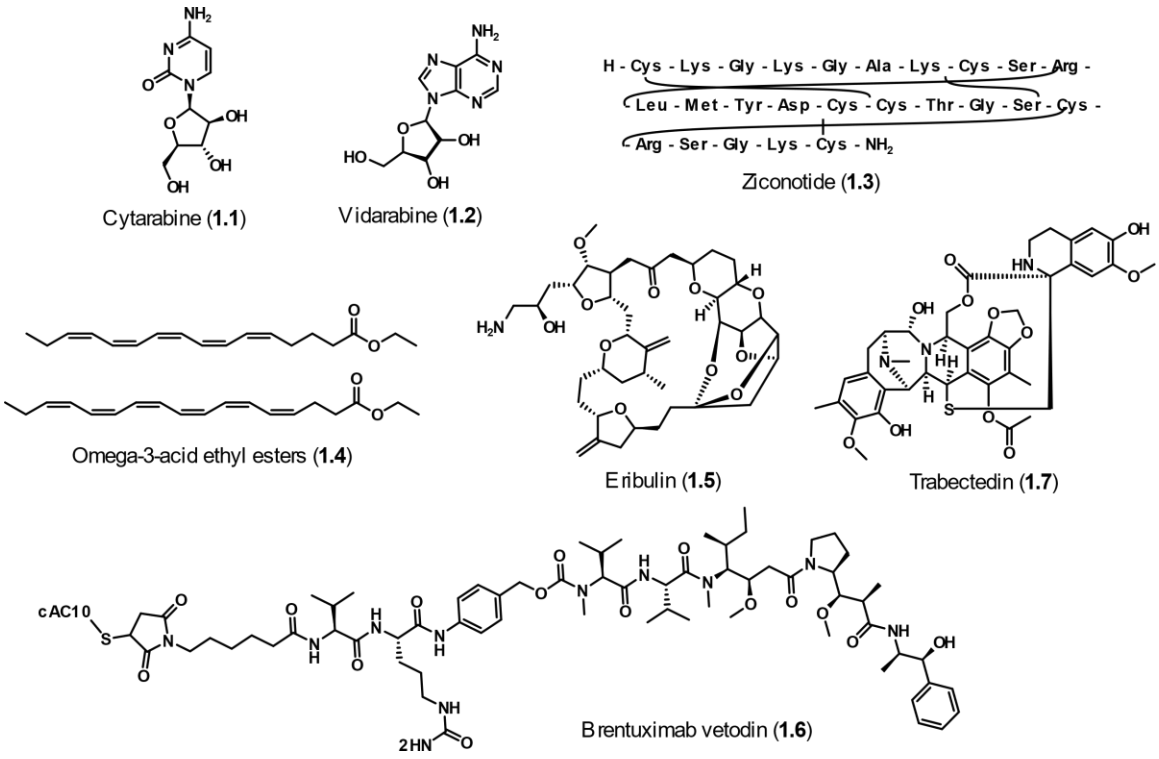


Figure 1.1: Structures of approved marine natural product therapeutics.

Table 1.2 Approved marine pharmaceuticals

Compound Name	Trademark and Year Approved	Company	Marine Organism	Chemical Class	Disease Area
Trabectedin (ET-743)8 (1.7)	Yondelis 2015	Pharmamar	Tunicate	Alkaloid	Cancer
Brentuximab vedotin (SGN-35)9 (1.6)	Adcetris 2011	Seattle Genetics	Mollusk/ cyanobacterium	ADC(MMAE)	Cancer
Eribulin Mesylate (E7389)10 (1.5)	Halaven 2010	Eisai Inc.	Sponge	Macrolide	Cancer
Omega-3-acid ethyl esters11 (1.4)	Lovaza 2004	GlaxoSmithKline	Fish	Omega-3-fatty acids	Hyper-triglyceridemia
Ziconotide12 (1.3)	Prialt 2004	Jazz Pharmaceuticals	Cone snail	Peptide	Pain
Vidarabine (Ara-A)13 (1.2)	Vira-A 1976	Mochida Pharmaceutical Co.	Sponge	Nucleoside	Antiviral
Cytarabine (Ara-C)14 (1.1)	Cytosar-U 1969	Pfizer	Sponge	Nucleoside	Cancer

This Table was reproduced from "The Global Marine Pharmaceuticals Pipeline" database curated by Dr. A. M. S. Mayer at Midwestern University.[7]

The first approved marine pharmaceutical was Cytarabine (**1.1**). Cytarabine was isolated from the Caribbean sponge *Cryptotethia* sp. and was marketed by Pfizer in 1969 under the trademark Cytosar-U.[7,14,15] This medication is prescribed as a cytotoxic chemotherapeutic agent that treats certain types of leukemia. Cytarabine works by blocking the progression of cells from the G1 phase of the cell cycle by inhibition of DNA polymerase, and has also been shown to cross-link and alkylate with DNA.[16] The drug Vidarabine (**1.2**) was also discovered from the same Caribbean sponge species and has a similar structure to Cytarabine. However, it has an entirely different medicinal application as a nucleoside antibiotic. Vidarabine was approved in 1976 and marketed under the trademark Vira-A by Mochida Pharmaceutical Co.[7,13,15] Vidarabine works by competitive inhibition of viral DNA polymerase to inhibit replication of viral DNA. It is used to treat a variety of viral infections such as varicella, herpes zoster, and herpes simplex.[16] The next marine pharmaceutical to enter the market was Ziconotide (**1.3**), which was approved in 2004 under the trademark Prialt and sold by Jazz Pharmaceuticals.[7,12] This marine natural product is a synthetic form of a ω-conotoxin peptide isolated from *Conus magus*. Ziconotide is

an analgesic that selectively blocks N-type voltage gated calcium channels, thus stopping the release of pronociceptive neurochemicals.[16] In the same year Omega-3-acid ethyl esters (**1.4**) (eicosapentaenoic acid ethyl ester, and docosahexaenoic acid ethyl ester) were approved and marketed by GlaxoSmithKline under the trademark Lovaza.[7,11] These Omega-3-acid ethyl esters are isolated from fish oil and are used to treat severe hypertriglyceridemia.[16] In 2010, another marine natural product, halichondrin B was semi-synthetically modified to create Eribulin mesylate (**1.5**). Halichondrin B was isolated from the marine sponge *Halichondria okadai*, was approved and marketed by Eisai Inc. under the trademark Halaven.[7,16,17] Eribulin is a chemotherapeutic agent used to treat metastatic breast cancer by disrupting the microtubule network in cells to inhibit cell division.[18] Since then, the antibody drug conjugate (ADC), Brentuximab vedotin (**1.6**), was approved in 2011 under the trademark Adcetris and is produced by Seattle Genetics.[7,19] This ADC combines the anti-CD30 antibody with monomethyl auristatin E (MMAE), which is a synthetic analogue of the natural product dolastatin 10 isolated from the sea hare *Dolabella auricularia*.[20] Brentuximab vedotin is an anti-neoplastic agent that is used to treat certain types of lymphomas by binding to CD30 expressing cells and subsequently releasing MMAE which binds to tubulin and induces cell cycle arrest.[16] The newest marine natural product derived pharmaceutical is Trabectedin (**1.7**) which was approved in 2015 and marketed under the trademark of Yondelis by Pharmamar.[7,8] This natural product was isolated from the Carrbbean tunicate *Ecteinascidia turbinata* Trabectedin is an antitumor agent that is used to treat soft tissue sarcoma and ovarian cancer by binding to the minor groove of DNA to block progression of the cell cycle at the G2 phase.[16]

Most of these marine derived natural product pharmaceuticals were isolated from macro organisms such as sponges (**1.1**, **1.2**, **1.4**), cone snails (**1.3**), fish (**1.5**), and tunicates (**1.7**). However, examination of the biosynthesis of these compounds indicates that six out of seven of these compounds were actually produced by microorganisms, with five of seven being produced by marine bacteria (**1.1**, **1.2**, **1.4**, **1.6**, **1.7**). The biosynthesis of the remaining two compounds indicates that they are produced by microalgae (**1.5**)**,** and by mollusks (**1.3**).[21] This demonstrates that marine microbes are an exceptional source of novel natural products with complex structures and activities, making them a promising area for natural products research.

1.1.2. Outlook on Natural Products

The rate of discovery of new natural products is still rising annually. For the last two decades there have been over 1,500 new microbial and marine natural products discovered every year. However, the rate of discovery of structurally unique natural products is steadily declining, and made up less than 20% of the new natural products discovered in this timeframe.[22] Because of this, and inefficiencies surrounding natural products discovery and isolation, pharmaceutical companies have deprioritized natural products as a starting point for the discovery and development of drugs.[22,23]

One issue that has plagued the isolation and structure elucidation of biologically active compounds from natural product extracts is the limited amount of material that can be isolated form a given natural source. For example, 12.5 mg of the anticancer agent Halichondrin B was isolated from 600 kg of the sea sponge *Halichondria okadai.*[24] Technological advances such as cryoprobe nuclear magnetic resonance (NMR), high-performance liquid chromatography (HPLC), and more sensitive mass spectrometers have all but solved this problem.[25–29] In November of last year, a new cryo-electron microscopy (cryoEM) method, micro-electron diffraction (microED), was published. Using microED the structures of pure compounds and heterogeneous powders were solved with a resolution of 1 Å or less in under 30 minutes.[30] Currently, structure elucidation is accomplished using NMR spectra and mass spectrometry data which requires significantly more material than microED. Isolation of natural products today requires hundreds of dollars in high grade solvents and days to weeks to complete. Once enough material is isolated, NMR spectra and mass spectra must be obtained to elucidate the structure of the natural product, which can also require days of analysis and instrument time. As microED requires a fraction of the amount of material it will cut down on the labour and cost of isolation. Additionally, as the microED data can be acquired and solved in under an hour the amount of time, effort, and cost required to solve the structure of the natural product will decrease exponentially. MicroED will revolutionize the natural products community as it will enable natural products to be isolated and identified within a fraction of the time it currently takes, and at significantly less cost.

High-throughput screening allows for many compounds to be assayed in rapid succession to detect biological activity. It is commonplace to screen libraries of compounds in 384-well plate format, and the newest technologies now support screening in 1536-well

plates.[31,32] This higher density, lower volume approach makes high throughput screening very cost effective. In general, compounds can either be screened in whole-cell assays or against specific protein targets. Whole cell screens are advantageous because there are many different targets available for the compound to interact with. The downside of whole cell assays is that in order to determine the mode of action of an active compound more orthogonal screening must be done.[33] The development of image-based whole cell screens allows the cells phenotypes to be observed, which provides much more information on the mode of action of a compound than conventional live-dead assays.[34] Screening against specific protein targets is useful for finding compounds with a specific mode of action. However, these screens often result in many false positives, which can be time consuming and costly. Natural products are usually screened as crude mixtures in the form of prefractionated extracts. This is because it is nearly impossible to separate an extract into its individual components. The problem with this approach is that screening the mixtures often leads to masking or compounding biological activity. New screening methods that link mass spectrometry (MS) features with screening data help dereplicate some of the compounds responsible for biological activity and allow natural products chemists to better prioritize hits for re-screening and isolation.[35]

The most outstanding detriment to natural products discovery efforts has been the high rate of rediscovery of known compounds. Bioassay-guided fractionation does not provide any information about the identity of the active compound until it has been purified from the extract. Isolation of natural products is time consuming and costly, requiring many days or months of NMR, MS, HPLC, and screening efforts. Unfortunately, it is now more common to re-isolate known compounds than to discover new ones using bioassay-guided fractionation. The inefficiency and corresponding costliness have caused pharmaceutical companies to deprioritize natural products as a source of drug leads.[23]

In order to reintroduce natural products as a prominent starting point for the development of drug leads new natural products discovery methods are needed to catch up to available technologies. To do this, we must create techniques that expand natural product chemical space.[36–41]

1.2. Natural Product Chemical Space

1.2.1. Introduction to Chemical Space

Chemical space is the macrocosm of all synthetically feasible molecules. It is finite, but so large that it is not yet possible to chart every region.[39] Since natural products evolved to interact with biological targets, focus is being put on exploring the smaller, but still immense, area of natural products chemical space in the quest for new biologically active molecules.[2] Natural product chemical space overlaps heavily with the chemical space occupied by approved therapeutics.[37]

Chemical space is mapped using an assortment of statistical techniques, all of which focus on the features of a compound's structural scaffold.[42] These maps are created by assigning dimensions to a series of molecular descriptors and using these values as positional coordinates. One of the most common ways of displaying this data is using principal component analysis (PCA). PCA uses orthogonal transformations to project data onto a vector called a principal component (PC). The PC intersects the data in a way that maximizes the variance of the projected points, which reduces the data into it's most basic and descriptive PCs. The next PCs are plotted so that there is maximum variance in the projected points without overlapping with previous PCs. A PCA plot can then be generated where each axis represents a different PC.[43] An alternative to using PCA is assigning molecules descriptor value vectors using self-organizing maps. This results in the most similar molecules being close together in chemical space.[42]

Table 1.3 Databases Containing Natural Products and Small Molecule Approved Drugs

Database	Number of Compounds
ZINC[44]	230,000,000
PubChem[45]	97,177,104
ChemSpider[46]	71,000,000
ChemDB[47]	4,100,000
ChEMBL[48]	1,828,820
BindingDB[49]	652,068
SuperNatural[50]	325,509
TOXNET[51]	300,000
NCI Open[52]	250,250
HMDB[53]	114,100
ChEBI[54]	55,453
MarinLit[55]	32,929*
The Natural Products Atlas[56]	20,051
DrugBank16	11,927

The number of compounds in the database is based on information available up to 2019-01-20.
* MarinLit has 32,929 articles published, not compounds, as of 2019-01-20.

There are a variety of databases that each contain a library of compounds that represent their own unique portion of natural product and biologically active small molecule chemical space. Databases containing more than 10,000 compounds are displayed in Table 1.3.

Despite the millions of known natural products there are still voids in natural products chemical space. For example, there should theoretically be 41,110 different tetracyclic peptides produced in nature if only proteinogenic amino acids are considered and rotational symmetry is accounted for, but we only know of 65 in our marine natural products database.[22] There are many possible explanations for this that can only be investigated by developing methods to expand chemical space.

1.2.2. Expansion of Chemical Space Through Examination and Manipulation of Biosynthesis

In recent years the study of biosynthetic pathways has been a prominent method through which new natural products are discovered. Biosynthesis is the multi-step enzyme catalyzed conversion of simple compounds into complex products.[57] Related biosynthetic gene clusters can be linked to the production of similar compounds, with each class of natural products being produced through specific biosynthetic pathways. Study of an organism's genome can reveal the number and type of natural products it can produce.[58]

Cultivation of novel organisms provides access to unstudied biosynthetic gene clusters.[59] Previously cultivated organisms represent a very narrow sampling of biodiversity. It is approximated that under 1% of all bacterial species and less than 5% of all fungal species have been studied to date.[60] Most of these species were obtained from easily accessible environmental sources. In order to discover new natural products more extreme areas must be sampled. Unfortunately, it is often difficult to access and consequently costly to collect samples from extreme environments.[59,61] Extremophiles have yielded complex biologically relevant compounds. For example, Mixirins A-C isolated from a *Bacillus* sp. found in sea mud near the Arctic pole showed cytotoxic activity.[62] Another expedition that studied samples from the Marianas Trench isolated the cytotoxic Dermacozines A-G from a *Dermacoccus abyssi* sp.1.1 and 1.2 by dredging the sea floor at a depth of 10,898 m.[61,63] Once collected, these samples can usually be cultivated using standard laboratory procedures and studied using conventional bioassay-guided fractionation.[59]

Cultivation of organisms that do not grow under standard laboratory conditions is a significant source of new biosynthetic potential, as 99% of all bacterial species in external environments are uncultured.[64] Altering conditions such as pH, salinity, temperature, adding competing species, and adding small molecule epigenetic agents can lead to the growth of previously uncultured bacteria and the production of new natural products. For example, the Piel group demonstrated that a multitude of natural products isolated from a marine sponge, *Theonella swinhoei*, were actually produced by a previously uncultured marine bacterial symbiont.[65] Usually, these newly cultured bacteria are cultivated from environmental samples taken from existing libraries, so the difficulty of obtaining samples is often eliminated. However,

it is difficult to anticipate how biosynthetic pathways are regulated, and how changing growth conditions will impact gene expression.[59]

Another strategy allows gene expression to be controlled through manipulation of genes and proteins. This can be done by regulation, deletion, or introduction of regulatory genes, through heterologous expression, or combinatorial biosynthesis.[66–69] For example, Zhou et. al. stimulated the biosynthesis of the natural product chaetoglobsin Z by over expression of the global regulator LaeA in *Chaetomium globosum*.[69] In order to effectively implement this approach one needs an in-depth understanding of the complicated regulatory networks involved in the expression of the target gene.[59]

Natural product chemical space can be further expanded through diversification of natural products using enzymatic and microbial transformations. Functional enzymes are responsible for the production of many different classes of natural products and the introduction of an assortment of functional groups. In vitro utilization of enzymes can lead to the stereoselective and mild modification of natural products in an extract.[70–72] An example of this is the enzyme catalyzed [4+2] cycloaddition critical to the biosynthesis of spinosyn A derived from *Saccharopolyspora spinosa*.[71] The issue with this approach is that these enzymatic transformations can be costly and low yielding.[59] A way to further expand on this approach is by adding non-natural precursor compounds to introduce novel functionalities on to natural products. Additionally, mutation of the biosynthetic enzymes can further expand the building blocks that can be incorporated onto the natural product scaffolds and thus produce even more natural product derivatives.[59,73,74]

Further implementation and development of approaches such as cultivation of novel organisms, regulation of biosynthetic gene expression, and the utilization of enzymatic transformations will open avenues for the discovery of novel natural products and further expansion into natural products chemical space.

1.2.3. Expansion of Chemical Space Using Synthetic Chemistry

Another approach to the expansion of natural product chemical space is through synthetic chemistry. The field of synthetic chemistry continues to evolve and rise to the challenge of synthesizing natural products, which have many stereogenic centres, polycyclic rings, and diverse functionalities. In fact, most natural product derived pharmaceuticals are synthetic analogues of natural products.[1] This highlights the critical role that synthetic chemistry has in maintaining human health.

Total synthesis is the synthesis of complex natural products with interesting biological activities. This method has been prominent in natural products research for decades and is an essential way to produce large quantities of biologically relevant compounds.[75,76] Total synthesis is an ever-evolving field that enables the production of increasingly structurally complex compounds. It began with the synthesis of urea from inorganic starting materials in 1828 and is now used to produce compounds with many stereogenic centres, complex ring systems, and functional groups. For example, the Nicolaou group along with other collaborators is attempting to synthesize the second largest metabolite isolated to date, the marine neurotoxin maitotoxin (from *Gambierdiscus toxicus*). This compound has a molecular weight of 3,422 g/mol and the formula $C_{164}H_{256}O_{68}S_2Na_2$ and contains 32 rings and 98 stereocenters. The development of total syntheses provides fundamental and tangible advancements to the field of natural products chemistry. Fundamentally, each total synthesis demands new methods and ways of thinking that have implications on future research. Practically, total synthesis provides continuous, dependable, and cost-effective access to natural product drug candidates.[76]

Medicinal chemistry and diversity-oriented synthesis (DOS) use synthetic chemistry techniques to modify natural products for drug discovery. In medicinal chemistry, structure activity relationships (SAR) are analyzed, and drug leads are modified to improve their biological activity. Natural products account for well over half of these biologically active drug leads.[1,77] For an example of this look no further than the antibiotic penicillin (isolated from *Penicillium chrysogenum*). Medicinal chemistry approaches have produced an assortment of beta-lactam antibiotics based on the structure and mode of action of penicillin.[78] DOS is another way of modifying drug leads to further improve their biological activity. However, where medicinal chemistry relies on careful analysis of SAR, DOS relies on sheer number of differently assembled small molecules. Using synthetic chemistry the functional groups, stereochemistry,

and structural skeleton of libraries of known small molecules are modified.[79] To date, DOS has produced a variety of compounds with implications on human health. For example, the compound robotnikinin was produced through DOS on a commercial and natural small molecule library. Robotnikinin is the first compound reported to prevent signalling in the Sonic hedgehog (Shh) pathway, which has a key role in the embryonic development of limbs and digits, as well as in organization of the brain.[80] The diversification of natural products using DOS and medicinal chemistry expands the chemical space of natural products and biologically active compounds.[59]

Other efforts have focused on the diversification of natural product extracts using synthetic chemistry, which are related to the work presented in this thesis. The Furlan group pioneered this area of study. This research was inspired by the discovery of natural products modified by solvent artifacts, and by the discovery of other natural product derivatives produced by treatment of extracts with acid.[81–84] The idea behind this approach is that changing the structure of a natural product will have some influence on the biological activity of said natural product. Therefore, the aim of their work was to diversify as many compounds as possible present in their plant natural product extract library to have the best chance of generating new clinically relevant biological activity. The reactions chosen by the Furlan group include bromination, fluorination, treatment with hydrazine, treatment with hydroxylamine, ethanolysis, and sulfonation. These transformations were all shown to expand chemical space through the production of new natural product-like compounds, many of which displayed interesting biological activity. Bromination of a natural product extract produced compound **1.8**, an acetylcholinesterase inhibitor and compound **1.9**, an xanthine oxidase inhibitor.[85,86] Fluorination of an extract using Selectfluor produced a tyrosinase inhibitor, compound **1.10**.[87] Compounds **1.11- 1.15** were produced by treating an extract with hydrazine. Compound **1.15** was found to have antifungal properties, while compounds **1.11 – 1.14**, isolated by a different research group, were shown to inhibit amyloid beta (Aβ) aggregation which is a critical target for Alzheimer's research.[88,89] Treatment of natural product extracts with hydroxylamine yielded compounds **1.16** and **1.17,** both of which inhibited the bromodomain of *Trypanosoma cruzi,* the parasite that causes Chagas disease.[90] Sulfonation of natural product extracts produced the β glucosidase inhibitor, compound **1.18**.[91] Two other β glucosidase inhibitors, compounds**1.19** and **1.20**, were produced by performing ethanolysis.[92] The structures of these natural product derivatives can be seen in Figure 1.2.

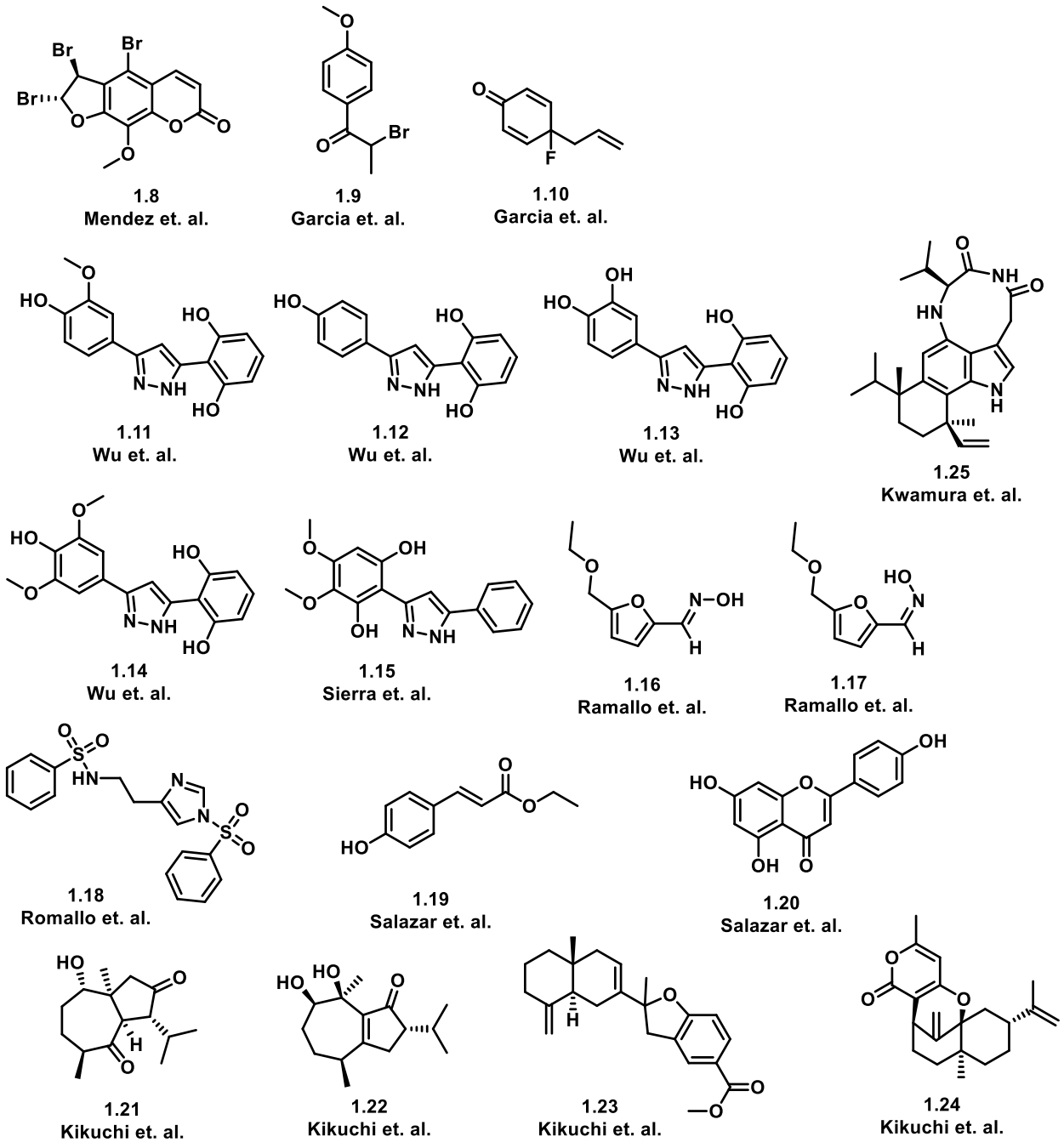


Figure 1.2 Biologically active natural product-like compounds produced through derivitization of a natural product extract.

Other researchers have also expanded the chemical space of natural products by performing other transformations on natural product extracts from different environmental sources. By performing an epoxidation followed by a ring opening reaction using a Lewis acid Kikuchi et. al. were able to isolate a series of new natural product-like compounds. Two of these

compounds, compounds **1.21** and **1.22** were found to inhibit immune response in Drosophila S2.[93] The same researchers then focused on expanding natural products chemical space by synthesizing a series of meroterpenoids via a three-step synthesis on extracts. First, the extracts were reduced using diisobutylaluminum hydride (DIBAL), then either methyl-4-hydroxy-3-iodobenzoate or 4-hydroxy-3-iodo-6-methyl-2-pyrone was added to the extract and a Mitsonobu reaction was performed. Finally, a Mizoroki-Heck reaction was done to generate the meroterpenoid-like compounds. Using this approach, they were able to isolate 25 meroterpenoid-like compounds. Of the meroterpenoid-like compounds, compound **1.23** demonstrated anti-osteoporosis effects, and compound **1.24** showed biological activity against lymphoma and leukemia cells. They were also able to demonstrate that the use of this transformation expanded into chemical space and produced new structurally unique compounds as measured by a Tanimoto score.[94] Other work done by Kawamura derivatized natural product extracts via oxidation with Dess-Martin periodinane, epoxidation with meta-chloroperoxybenzoic acid (mCPBA), and reduction using sodium cyanoborohydride. Oxidation of one extract derivatized telocidin B to produce the XIAP inhibitor compound **1.25**.[95] The structures of all of these compounds are shown in Figure 1.2.

1.3. Summary and Proposal

There is a critical and continual need for the development of new pharmaceuticals to treat a myriad of ailments. Natural products have evolved to fulfill biologically important roles, and as such they occupy and area of chemical space that is rich with biologically active molecules. These biologically active natural products are the most prominent source for the discovery and development of new pharmaceuticals. It is very important that methods of expanding natural products chemical space are continually developed in the search for new biologically active compounds.

Some researchers have expanded natural product chemical space by manipulating and exploring the biosynthetic potential of organisms. Others have used synthesis to build upon existing natural products scaffolds to optimize biological activity. The work reported in this thesis aims to expand the chemical space of natural products produced by marine actinomycetes under standard laboratory conditions by performing a series of transformations on natural product extracts to derivatize the natural products present in the extracts. A series of eight transformations were developed that target key natural products functional groups to expand natural products chemical space via the introduction of biologically relevant functionalities. Derivatization of natural product extracts generated a library of 540 prefractionated derivatized natural product extracts that acted as a source for the discovery of new natural product derivatives with potential applications to problems in human health and biotechnology.

Chapter 2.

Transformation of Natural Product Extracts

2.1. Transformations used to Derivatization of Natural Product Extracts

2.1.1. Rationale

This project represents the first large-scale chemical diversification of natural product extracts. The generation of a library of derivatized natural products will in principal provide a new pool of natural product and drug-like molecules that can be examined for clinically relevant biological activity. Diversification of natural product extracts will be accomplished by performing reactions that are high yielding, have high functional group tolerance, react with functional groups common to natural products, and introduce functionalities that are common to natural products and drugs. It is anticipated that these modifications will impact the biological activities of the derivatized natural product.

The functional groups to be targeted by the transformations were determined by analyzing the prominence of different functional groups in the Natural Products Atlas, a microbial and fungal natural products database maintained by the Linington Lab.[56] The search tool provided by the Atlas was used to identify the most prevalent functional groups. The results of this search were then compared to the structures of all the natural products recognized by Reaxys, a search tool for all published chemical substances and reactions, and were found to be reasonably similar.[96] A separate study published by Ertl et. al. in April 2019 analyzed the prevalence of different functional groups in natural products using an in-house algorithm developped at Novartis.[97,98] The results of this study were supportive of the findings from the Atlas and Reaxys.

When all three databases were compared it was observed that the prevalence of each functional group varied by as much as 20% from one database to the next. This can be attributed to the algorithms that each tool uses to filter the search results, and the area of chemical space that each database covers. The Natural Products Atlas was the best representation of the chemical space that the Linington Lab's microbial natural product library

occupies and thus was given the most weight in this analysis. Reaxys on the other hand covers more of the chemical space occupied by clinically relevant biologically active natural products and natural product-like therapeutics. The study done by Ertl et. al. covered the chemical space of natural products isolated from animals, plants, bacteria, fungi, and those produced through synthesis. Analysis of these datasets determined that alkenes are present in 21% of the compounds in the Atlas, 22% in Reaxys, and 40% in the study done by Ertl et. al. and thus were a promising target for derivatization. Phenols are another functional group of interest as they are present in 18% of the compounds in the Atlas, 34% of the compounds in Reaxys, and 28% of the compounds in the study done by Ertl et al. Amines were also of interest, as they are present in 14% of compounds in the Atlas, 12% in Reaxys, and 10% in the study by Ertl et. al. Finally, 1,3-amino alcohols were found to be present in 7% of the natural products recorded in the Atlas, 3% in Reaxys, and were not covered in the other study. These findings are displayed in Figure 2.1.[56,96,97]

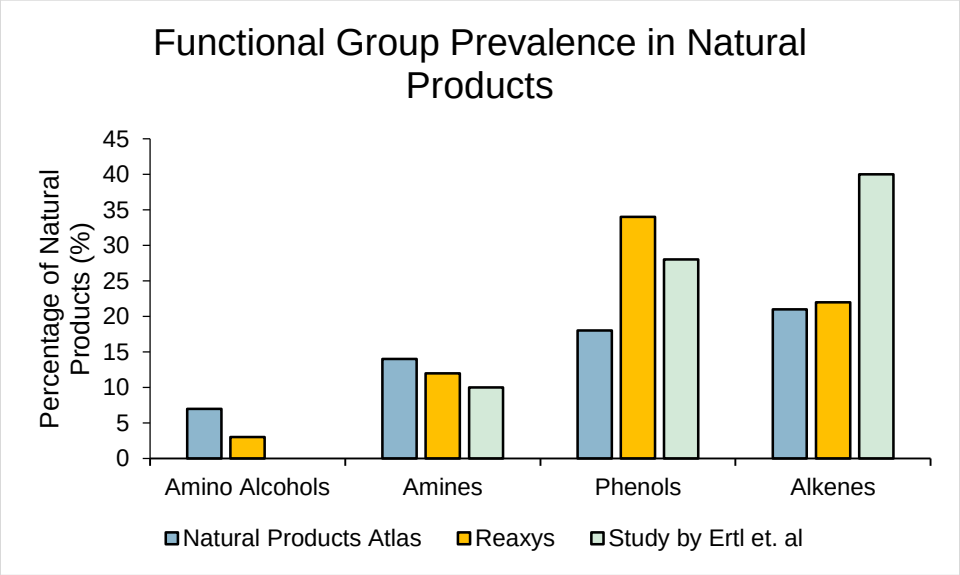


Figure 2.1: Prevalence of functional groups across natural products.

Prevalence of functional groups in natural products was determined by a structure search in the Natural Products Atlas, Reaxys, and recently supported by a study done by Ertl et. al.[56,96,97]

The selection of candidate transformations was dependant upon the reactions meeting specific criteria. Natural product extracts are complex mixtures of a multitude of natural products. It is impossible to say how many different compounds are present in an extract, what these compounds are, and in what abundance they are present as it varies from organism to

organism. Therefore, the transformations used to derivatize the extracts must be selective, have high functional group tolerance, and proceed under relatively mild conditions.

In the search for transformations that would derivatize natural products in a meaningful way a total of 14 different transformations were attempted. These transformations included reductive amination using Pd/C, reductive amination with sodium cyanoborohydride, trifluoromethylation, fluorination using DAST, dichlorocyclopropanations, three strain release reactions, two reactions with benzynes, thionation, mitsonobu reaction, morpholine ring formation reaction, and glycosylations. Each reaction was attempter under different conditions and on many natural products. Ultimately due to low reaction yields and incompatible reagents eight transformations were chosen to diversify the extracts: two reactions with benzynes (B1 and B2), a chemical glycosylation (CG), a dichlorocyclopropanation (CP), a morpholine ring formation reaction (MR), two reductive aminations (RF and RP), and a strain release amination (SR).

An important consideration for these transformations is the impact they may have on biologically and chemically relevant properties. The most well-known predictor of success for pharmaceutical candidates is Lipinski's Rule of Five, otherwise known as Pfizer's Rule of Five. These rules describe properties that are impact the absorption, distribution, metabolism, excretion, and toxicity (ADMET) of a drug in the human body. These rules state that a compound should not have more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, have a molecular weight of 500 g/mol or less, and have an octanol-water partition coefficient (logP) of less than 5.[99,100] However, natural products are often exceptions to Lipinski's rule of five, so these rules should be regarded as guidelines rather than requirements.[101] An example of this is the antibiotic Vancomycin (isolated from *Amycolatopsis orientalis),* which is on the World Health Organization's List of Essential Medicines.[102,103] Vancomycin is a perfect example of a drug that violates the Rule of Five, having a molecular weight of 1449.29 g/mol, a logP of -1.14, 19 hydrogen bond donors, and 33 hydrogen bond acceptors.[104]

Other properties of interest in drug development include solubility, stability, permeability, first-pass effect, clearance rate, biological half life, protein binding properties, and volume of distribution. However, while important, these properties are often optimized in down stream drug development and therefore are not crucial to the success of an initial candidate.[105] Additionally, as it is nearly impossible to predict the natural product(s) that will be derivatized in the extract it

is impossible to anticipate how most of these properties will be impacted by these transformations. The impact of these transformations on the lipophilicity (logP) and topological polar surface area (TPSA) of the original natural product was broadly predicted and is summarized in Table 2.1 These transformations will be discussed in more detail in sections 2.1.2 to 2.1.7.

Table 2.1 Transformations Chosen to Derivatize Natural Product Extracts

Transformation	Code	Functional Group Modified	Impact on cLogP	Impact on TPSA
Hexadehydro Diels-Alder	B1	Most nucleophiles	Increase	Increase
Benzene addition via benzyne	B2	Most nucleophiles	Increase	Decrease
Chemical glycosylation	CG	Phenols	Decrease	Increase
Dichlorocyclopropanation	CP	Alkenes	Increase	No change
Morpholine ring formation	MR	Amino alcohol	Increase	Decrease
Reductive amination: 3,5-bis(trifluoromethyl)benzaldehyde	RF	Amines	Increase	Decrease
Reductive amination: nicotinaldehyde	RP	Amines	Increase	Increase
Strain release amination	SR	Amines	Increase	Increase

The impact on logP and topological surface area was approximately determined using Molinspiration.[106]

The lipophilicity of a compound is measured by the affinity of a compound for the water and octanol phases (octanol-water partition coefficient, or logP). A compound with a logP of 0 has equal affinity for both phases, while hydrophilic compounds will have a logP of less than 0, and hydrophobic compounds will have a logP of greater than 0.[107] Representative natural product derivatives were submitted to the free online molecular property calculator, Molinspiration, to obtain an approximation of each transformations impact on key properties.[106] As expected, the chemical glycosylation, which adds hydrophilic groups to the natural product decreases the logP of the natural product. Conversely, as all other transformations are expected to introduce hydrophobic functional groups the logP was anticipated to generally increase. LogP is an important consideration as most pharmaceuticals must pass through biological membranes such as the lipid bilayer of intestinal epithelial cells or the blood brain barrier. This requires an optimal log P that is hydrophobic enough to partition into the membrane, but not so hydrophobic that it can not partition out of the membrane or impedes metabolism.[108,109]

The topological polar surface area (TPSA) is another contributing factor to the membrane permeability of compounds. This property is a measure of the sum the surface area over all polar atoms. Compounds with a TPSA greater than 140 Å are usually too hydrophobic to successfully permeate membranes. As with logP, Molinspiration was used to approximate the topological surface area of a sample set of derivatized natural products (shown in chapter 2).

Predictably, the reactions that introduced more polar groups increased the TPSA, while those that did not decreased the TPSA.[110]

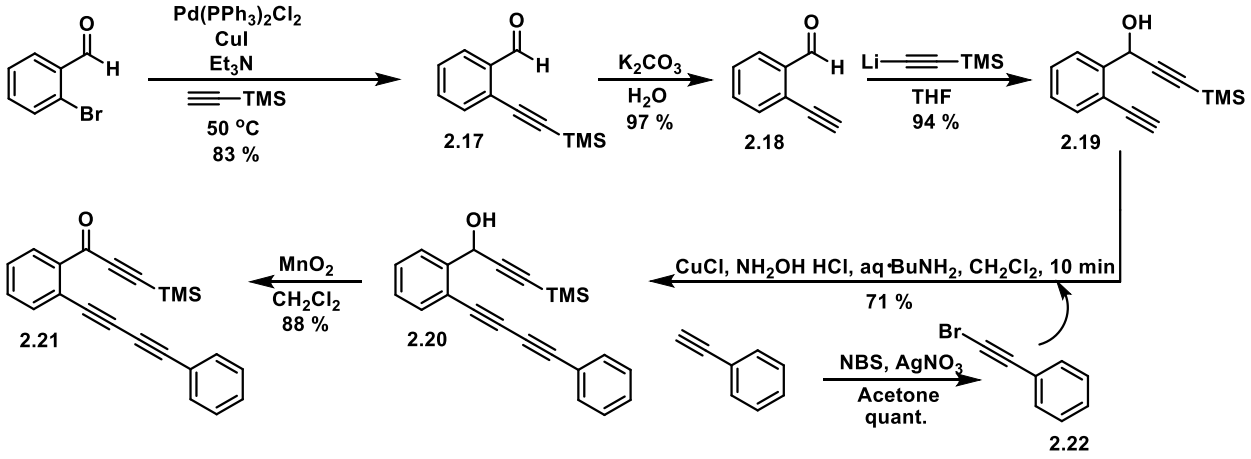
2.1.2. B1 & B2: Reactions with Benzynes

Benzynes are commonly used in synthetic chemistry as a reactive intermediate for nucleophilic aromatic substitution and cycloaddition reactions. These reactions are effective methods through which the aromaticity of a compound can be increased. The addition of aromatic groups to pre-existing natural products is of interest because of the prevalence of aromatic rings in drugs and drug candidates. Over 99% of all compounds present in a database containing 3,500 drug candidates from Pfizer, AstraZeneca, and GlaxoSmithKlein contain at least one aromatic ring.[111] The incorporation of aromatic rings on to natural products provided a quick and simple way through which the structure and intermolecular forces of the natural product were altered. Aromatic rings participate in π - π, cation – π, and CH – π interactions that are essential to the binding of many drugs to biological targets.

Benzynes are commonly formed as a result of elimination reactions of substituted aromatic compounds.[112] In this work, the first benzyne used was generated via a Diels-Alder reaction, and the second was produced by an elimination reaction on 2-(trimethylsilyl)phenyl trifluoromethanesulfonate.[113–116] These reactions were given the code B1 and B2 respectively for ease in naming the samples produced for the library.

Transformation B1 was inspired by the hexadehydro-Diels-Alder (HDDA) reaction reported by Hoye *et. al*.[115] This reaction is an intramolecular thermal cycloaddition reaction between 1,3-diyne and an alkyne dienophile that generates a benzyne *in situ*. Once produced, the benzyne can be trapped by a variety of nucleophiles including phenolics, carboxamaide, alcohols, amines, carboxylic acids, and furanics. A subsequent paper by the Hoye group showed that this reaction works very well on complex polyfunctional natural products. They demonstrated the reactions effectiveness by derivatizing natural products and natural product-like compounds including vitamin E, estradiol, tropinone, scopolamine, sinomenine, brucine, reserpine, colchine, limonin, quinine and quinidine using multiple benzyne precursors. It was demonstrated that the reaction proceeds with very good selectivity despite having many potential reaction sites on a given natural product. [116]

Scheme 2.1: Synthesis of Hexadehydro Diels-Alder reagent



The synthesis outlined above was based on the synthesis reported by Hoye *et. al*. The experimental details and characterization of new compounds can be found in the experimental section 2.4.4.[115]

For the purposes of this work a representative benzyne precursor, 1-(2-(phenylbuta-1,3-diyn-1-yl)phenyl)-3-(trimethylsilyl)prop-2-yn-1-one (**2.21**), was chosen as the HDDA reagent. This reagent is similar to 1-(2-(penta-1,3-diyne-1-yl)phenyl)-3-(trimethylsilyl)prop-2-yne-1-one, which was the highest yielding triyne reported by Hoye et al.[115] The HDDA reagent (**2.21**) was produced in a six-step synthesis shown in Scheme 2.1 performed by Mathew Sutherland, a graduate student in the Britton lab. The synthesis of the reagent began with a Sonogashira cross coupling reaction between 2-bromobenzaldehyde and trimethylsilylacetylene to produce **2.17**. The 2-((trimethylsilyl)ethiynyl)benzaldehyde was then then deprotected with potassium carbonate to produce compound **2.18**. This compound was then reactedwith ((trimethylilyl)ethynyl)lithium to produce the target diyne, compound **2.19.** Next, A Cadiot-Chodkiewicz cross coupling reaction with 1-(2-ethynylphenyl)-3-(trimethylsilyl)prop-2-yn-1-ol (**2.19**) and (bromoethynyl)benzaldehyde (**2.22**) produced **2.20.** (Bromoethynyl)benzaldehyde (**2.22**) was prepared by a bromination of phenyl acetylene. The product of the Cadiot-Chodkiewicz was then oxidized to produce the target hexadehydro-Diels-Alder reagent, compound **2.21**.

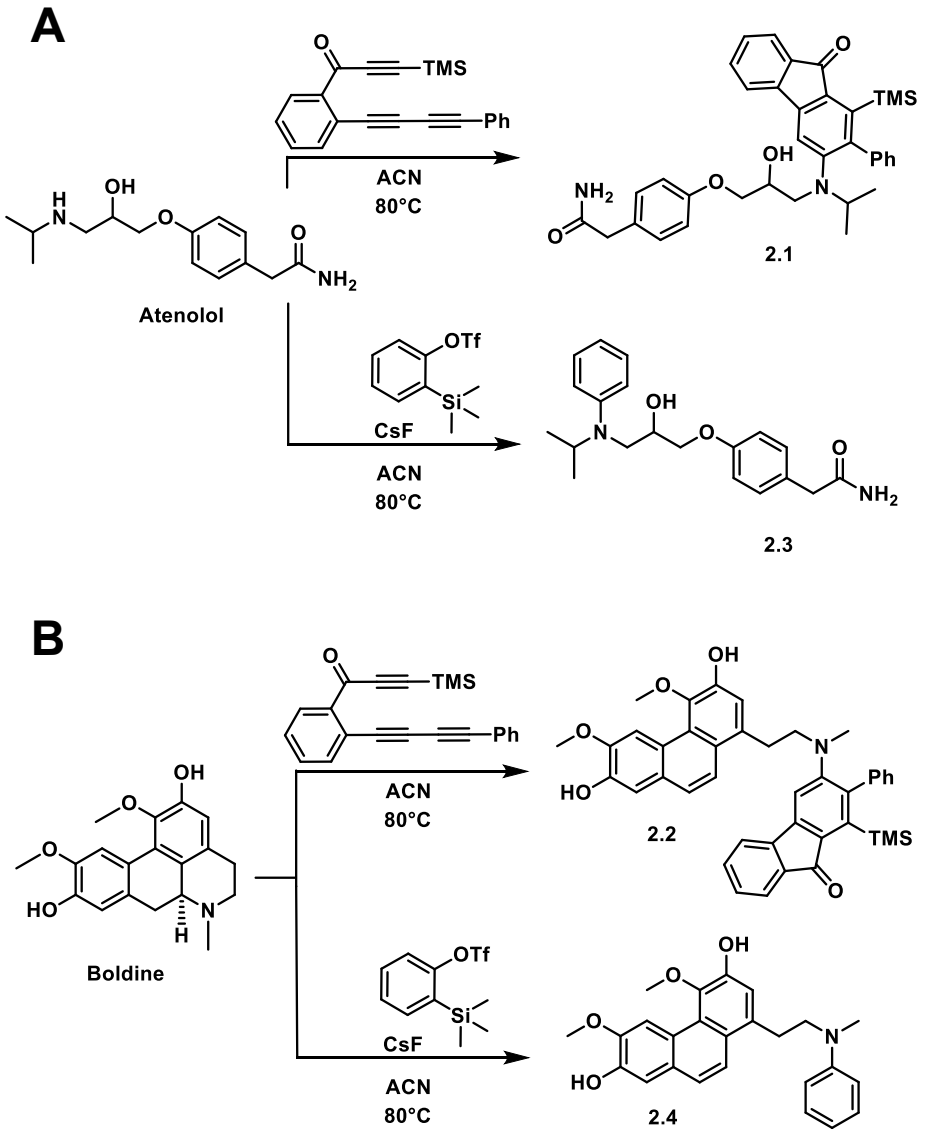
This reaction was then tested on the natural product standards atenolol and boldine to produce compounds **2.1** and **2.2** (Scheme 2.2 A and B). These test reactions supported the findings by the Hoye group that this transformation is selective for the most nucleophilic functional group and proceeds in good yield. Using the solvent acetonitrile (ACN) instead of toluene, as was reported, ensured that most natural products were soluble in the reaction

conditions. These conditions were also mild and found to be amenable to diversification of an extract.

As a second example of this kind of transformation being performed on extracts a simpler compound, benzyne (1,2-didehydrobenzene), was chosen as the B2 reagent. Benzynes can be produced by employing one of four reaction conditions on commercially available precursors. Generation of benzynes from halobenzenes or o-dihalobenzenes can be accomplished via deprotonation using organolithium or metal-halogen exchange using magnesium reagents. Alternatively, thermolysis of benzenediazonium and diphenylidonium can also generate benzynes. A third approach is the generation of benzynes via oxidation of 1-aminobenzotriazole. The fourth approach, and the one that was used in this work, was the mildest condition of reacting a silylated benzene with a fluoride source.[113] In this case, 2-(trimethylsilyl)phenyl trifluoromethanesulfonate was reacted with cesium fluoride (CsF) in acetonitrile at room temperature.[114] This reaction was preferential as all other methods of generating a benzyne were incompatible with a natural product extract due the use of strong acids or bases, high temperatures, and strong oxidizing agents.

1,2-didehydrobenzene was first tested on two natural product standards to optimize the conditions of the reaction and confirm its effectiveness at derivatizing the natural products. The natural product standards used to test this reaction were atenolol and boldine, which yielded compounds **2.3** and **2.4** respectively (Scheme 2.2 A and B). It was demonstrated that this simpler benzyne (B2) showcases the same selectivity for the most nucleophilic group on the natural product as B1, and proceeds under mild conditions that are ideal for derivatization of natural products in an extract.

Scheme 2.2: Synthesis of compounds 2.2 - 2.4 via the B1 and B2 transformations



The synthesis of compound **2.1** from atenolol and compound **2.3** from boldine via **B1** (A). The synthesis of compounds **2.2** from atenolol and **2.4** from boldine via **B2** (B).[114–116]

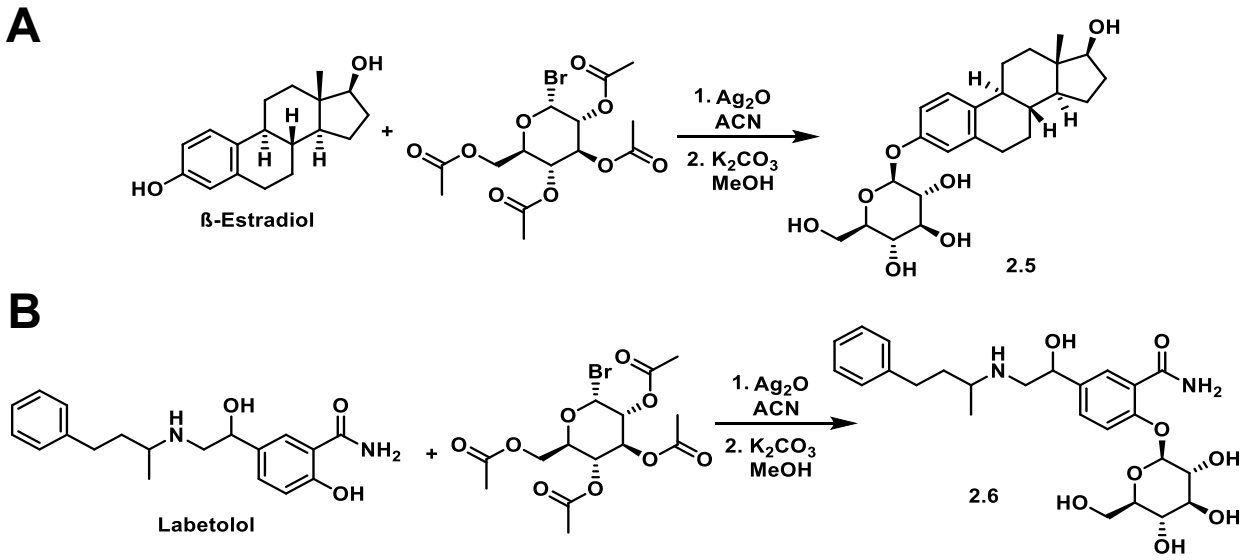
2.1.3. CG: Chemical Glycosylation

Glycosylated natural products are very prevalent, with over 21% of natural products across many chemical classes containing one or more sugar moieties.[117] Introduction of glycosyl groups onto natural products has been proven to increase the pharmacological activity by improving penetration through biological membranes, providing a site for receptor binding, and increasing the metabolic stability of a compound.[118] As such, glycosylated small molecule drugs and natural products have proven to be very successful pharmaceuticals.[118]

Enzymatic transformations are responsible for the glycosylation of compounds in natural systems. Glycosylation is a post-translational modification that covalently attaches a glycosyl group via a N or O–linked glycosidic bond.[119] In this work a synthetic approach for the chemical glycosylation of natural products was used. Ultimately it was determined that a Koenigs-Knorr reaction would be used to derivatize the extracts.[120] This reaction employs Ag_2O or Ag_2CO_3 to react with a glycosyl bromide, in our case acetobromo-α-D-glucose, to produce an oxocarbenium ion that then undergoes an intermolecular cyclization via a nucleophilic attack of the carbonyl oxygen on the oxocarbenium ion. This produces a dioxolaniumnium ion, which is attacked by a phenol to produce an O-linked glycosylated natural product. This results in an inversion of stereochemistry at the site of nucleophilic attack. Subsequently, the glycosyl group was deacetylated by the addition of K_2CO_3.[121]

In order to test the reactions effectiveness on poly-functional natural products the chemical glycosylation (CG) was performed on β-estradiol and labetolol to produce compounds **2.5** and **2.6** (Scheme 2.3). As expected, the reaction was found to be very effective at glycosylating natural products. The conditions of the reaction are very mild, the reagents are easily removed, the reaction is selective for phenols, and proceeds with stereoselectivity. All these considerations make the chemical glycosylation reaction ideal for derivatization of natural product extracts.

Scheme 2.3: Synthesis of compounds 2.5 and 2.6 via CG



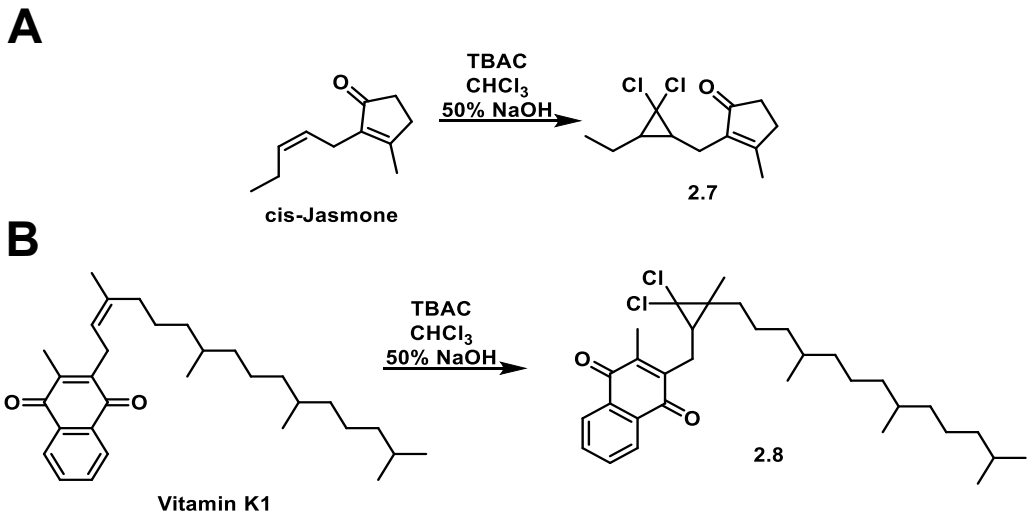
The synthesis of compound **2.5** from β-estradiol (A) and the synthesis of compound **2.6** from labetolol (B) via the chemical glycosylation reaction (CG).[121]

2.1.4. CP: Dichlorocyclopropanation

In order to take advantage of one of the most prominent functionalities present in natural products a transformation that would modify alkenes was selected. The dichlorocyclopropanation (CP) reaction converts an alkene to a dichlorocyclopropane. 45% of pesticides and 13% of pharmaceuticals are chlorinated. Chlorination provides a site for covalent attachment of a pharmaceutical and prevents metabolic hydroxylation.[122] Cyclopropanes are found in natural products, such as the insecticides pyrethrins.[123] They are also found in many pharmaceuticals including a variety of enzymatic inhibitors and opioid antagonists. The cyclopropane moiety is known to increase metabolic stability and the potency of drugs.[124]

The dichlorocyclopropane is formed by reacting an alkene with a dichlorocarbene. The dichlorocarbene is commonly generated by treating chloroform with a strong base, in this case NaOH. Mixing of NaOH and $CHCl_3$ forms a biphasic solution, so a phase transfer catalyst (tetrabutylammonium chloride, TBAC) was added.[125] This transformation was tested on *cis*-jasmone and vitamin k1 to produce compounds **2.7** and **2.8** respectively (Scheme 2.4 A and B). This reaction proved to be very efficient at transforming natural products as it quantitatively converted *cis*-jasmone to compound **2.7** and produced compound **2.8** from vitamin k1 with high yield, making this transformation an excellent choice for the derivatization of natural product extracts.

Scheme 2.4: Synthesis of compounds 2.7 and 2.8 via CP.



The synthesis of compound **2.7** from cis-jasmone (A) and **2.8** from vitamin k1 (B) via the dichlorocyclopropanation reaction (CP). [125]

2.1.5. MR: Morpholine Ring Formation

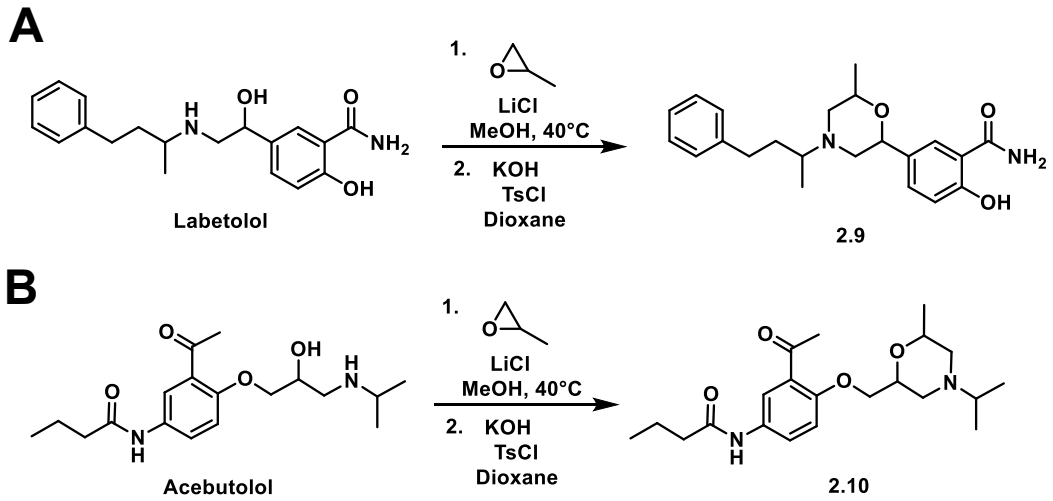
The morpholine ring formation reaction (MR) converts a 1,3-amino alcohol to a morpholine ring. Morpholines are very common to an assortment of natural products and drugs, especially those with fungicidal activity. Many biologically active natural products contain morpholines, including the antifungals fenpropimorph and tridemorph. These compounds work by inhibiting ergosterol biosynthesis which prevents cell wall growth in plants and fungi. Morpholines are also found in anticancer, antidepressant, appetite suppressants, and an assortment of other medications.[126,127]

Morpholines are typically produced through dehydration of amino diols. However, this reaction requires strong acids and high temperatures to proceed, which are incompatible with the natural product extracts as it is highly likely that many natural products would decompose under these conditions.[127] So, a different synthetic route to incorporate a morpholine on to natural products was developed.

The first step of this transformation involved converting the 1,3-amino alcohol into an amino diol. This was done via a ring opening of propylene oxide via nucleophilic attack of the amine. This reaction proceeded with high yields at 40°C in methanol, ideal conditions for extract derivatization.[128] Conversion of the amino diol to the morpholine was done by adding toluene sulfonyl chloride (TsCl) to a solution of the intermediate with KOH in dioxane at 0°C. Tosylation of an alcohol created an excellent leaving group, which was susceptible to nucleophilic attack by the other alcohol to generate the morpholine.[129]

This reaction was tested on acebutolol and labetalol to produce compounds **2.9** and **2.10** respectively (Scheme 2.5 A and B). It was demonstrated that this reaction has high functional group tolerance, and the conditions are mild enough to be carried out on natural product extracts.

Scheme 2.5: Synthesis of compounds 2.9 and 2.10 via MR



Synthesis of **2.9** from labetolol (A) and **2.10** from acebutolol (B) via the morpholine ring formation reaction (MR). [128,129]

2.1.6. RF & RP: Reductive Amination

The reductive amination reacts amines with aldehydes through an imine intermediate to create *N*-substituted derivatives. It was decided that commercially available aldehydes would be reacted with amines since amines are much more prevalent in natural products than aldehydes (14% vs 2% respectively in the Natural Products Atlas)[56]. The aldehydes chosen to derivatize the natural product extracts were 3,5-(bistrifluoromethyl)benzaldehyde for RF, and nicotinaldehyde for RP.

The first aldehyde, 3,5-(bistrifluoromethyl)benzaldehyde was chosen because it contains an aromatic ring which makes the natural product more drug-like. Additionally, the trifluoromethyl groups are functionalities that are linked to increased potency of compounds. Fluorination of natural products and drugs is linked to increased metabolic stability by prevention of oxidation and increasing the binding affinity of drugs. Fluorine is like hydrogen in size, but much more electronegative, which provides the unique opportunity to significantly alter the compounds electronic properties without also having a large impact on the steric interactions between the compound and biological receptors.[130–132]

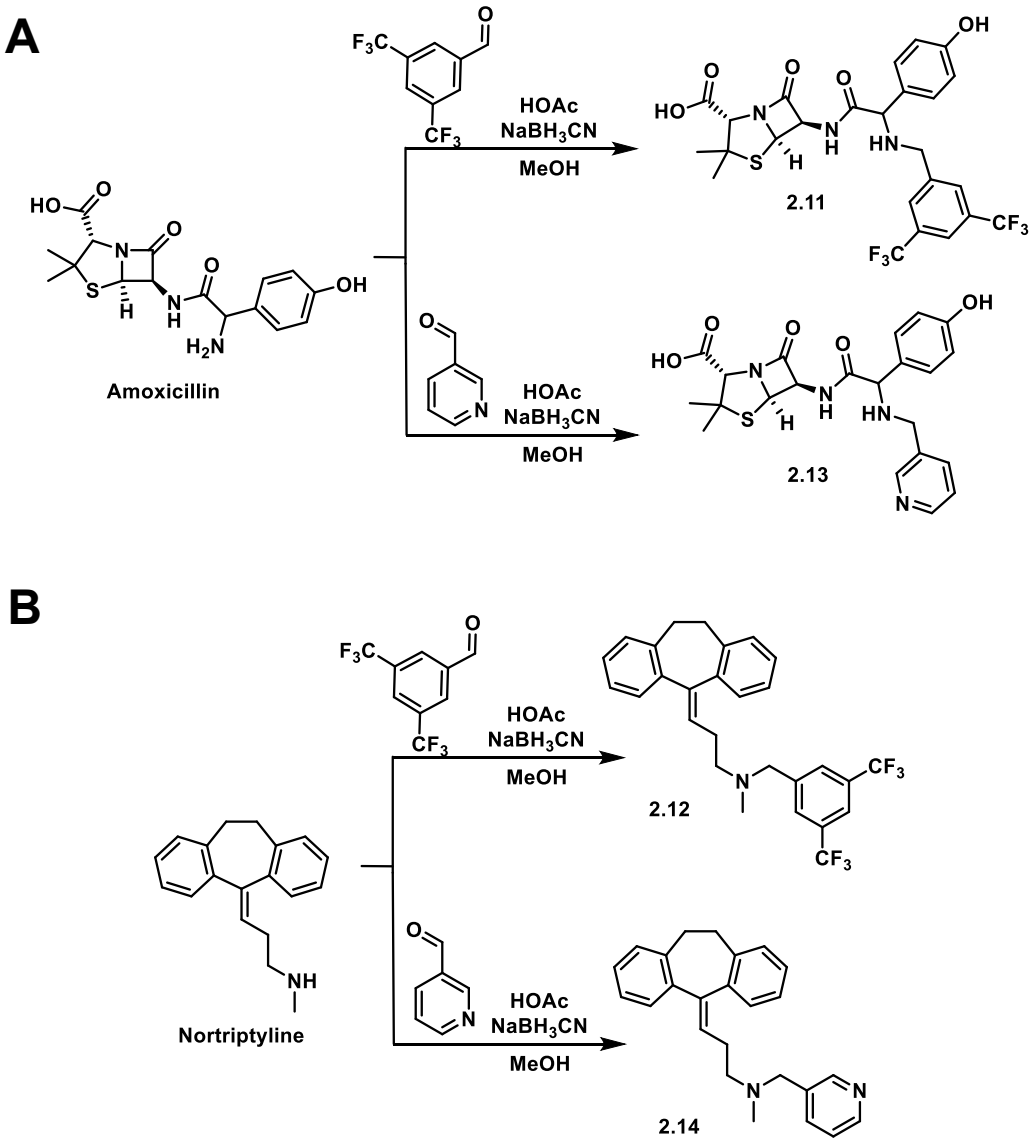
The second aldehyde, nicotinaldehyde, was chosen because it adds a pyridine ring on to the derivatized natural product. Pyridines are very common to many antimicrobial, antifungal, anti-inflammatory, analgesic, and enzyme inhibition agents.[133,134] This transformation also increases the aromaticity and the amount of nitrogen on the derivatized compound. On average natural products typically have a third of the number of nitrogen atoms that drugs do.[77,101] Addition of the pyridine ring increases the number of nitrogen atoms on the natural product. Increased nitrogenation and aromaticity generated by this transformation makes the derivatized natural product more drug-like.

There are a few ways in which the reductive amination can be performed. In general, it begins with a nucleophilic attack of the amine on the aldehyde under acidic conditions, which forms an iminium ion. The next step requires the reduction of the iminium ion to the corresponding amine with a hydride donor. There are three common choices of hydride donors: sodium trisacetoxyborohydride ($NaHB(OAc)_3$), sodium borohydride ($NaBH_4$), and sodium cyanoborohydride ($NaBH_3CN$). $NaHB(OAc)_3$ is sensitive to water and MeOH, and therefore must be carried out in polar aprotic solvents such as THF (tetrahydrofuran) or DCE (dichloroethane). $NaBH_4$ on the other hand is compatible with polar protic solvents such as MeOH but is a much

stronger reducing agent than $NaHB(OAc)_3$ or $NaBH_3CN$, and will reduce aldehydes and ketones into their corresponding alcohols producing unwanted side products. Therefore, the reducing agent $NaBH_3CN$ was chosen to carry out the reductive aminations on the natural product extracts as it is compatible with ideal solvents like MeOH and will not reduce aldehydes or ketones.[135–137]

This reaction was tested with both aldehydes on amoxicillin and nortriptyline to produce compounds **2.11** and **2.12** respectively for RF, and **2.13** and **2.14** for RP (Scheme 2.6 A and B). As anticipated, the reaction proceeded with excellent yield and under mild conditions with good tolerance of other functional groups.

Scheme 2.6: Synthesis of compounds 2.11-2.14 via RF and RP



The synthesis of compound **2.11** from amoxicillin and **2.13** from nortriptyline via RF (A) and compound **2.12** from amoxicillin and **2.14** from nortriptyline via RP (B).[135]

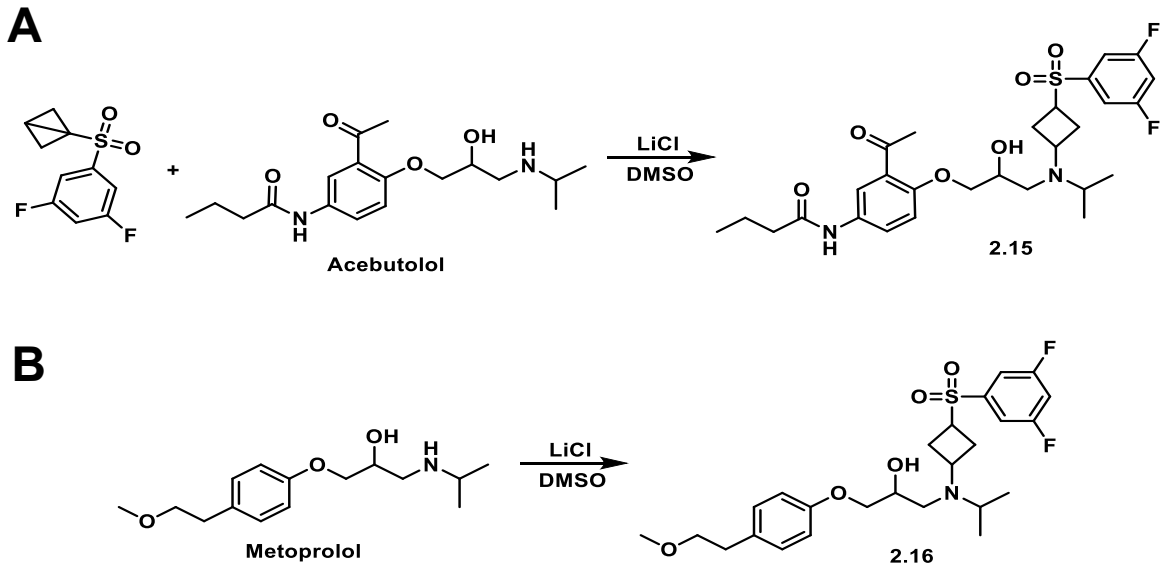
2.1.7. SR: Strain Release Amination

The strain release amination (SR) is a transformation that was first reported by Gianatassio *et al.* in response to the need for more direct routes to adding unconventional structural motifs onto drug candidates.[138,139] Prior to this work, cyclobutanes were synthesized through multistep pathways that often employed harsh conditions and yielded unsTable intermediates.[140–143] This new strain release agent is a bench stable reagent and provides a way to effectively add phenylsulfonylated cyclobutanes onto amines. There were a series of strain release agents reported by Giantassio *et. al*. that varied by the election withdrawing group on the phenyl group. Attempts were made to synthesize a series of these reagents but were ultimately met with with low yields consistent with the published yields. Therefore, this research employed the commercially available 1-((3,5-difluorophenyl)sulfonyl)bicyclo[1.1.0]butane as the strain release agent.[138,139]

Cyclobutanes are present on a series of compounds that act a potent NK1 selective agonists, as well as on compounds that possess antitumor, antibiotic, antimicrobial, antifungal, and immunosuppressive properties.[142,144,145] Incorporation of a strained ring onto a natural product can prevent metabolism and thus improve the potency and stability of a drug candidate.[144,145] Additionally, aryl sulfones are a very common motif to both natural products and drugs and are found in sulfonamide antibiotics and other biologically relevant compounds.[146–148] The fluorine substituents on the phenyl ring also play a key role in increasing drug potency and metabolic stability as discussed previously in section 2.1.6.

The strain release amination occurs via nucleophilic attack of a nitrogen atom on the bicyclobutane. The reaction occurs at room temperature in DMSO in the presence of LiCl. This reaction was tested on acebutolol and metoprolol to produce compounds **2.15** and **2.16** respectively (Scheme 2.7 A and B). This very high yielding, functional group tolerant, and selective transformation is an ideal way to derivatize natural products in an extract and rounds off this series of eight transformations nicely.

Scheme 2.7: Synthesis of compounds 2.15 and 2.16 via SR



Synthesis of compound **2.15** from acebutolol (A) and compound **2.16** from metoprolol (B) via the strain release amination (SR).[138,139]

2.1.8. Reaction Optimization

The candidate transformations were shown to effectively derivatize natural product standards as discussed in sections 2.1.2 to 2.1.7. The next step was to prove that the reactions worked just as effectively when derivatizing natural products in an extract. The main hurdle with this step was determining the amount of reagent to be added in order to get maximum conversion of the extracts. Due to the highly variable nature of the extracts it was impossible to precisely calculate the equivalents of reagents to use.

However, by making a few key assumptions the approximate stoichiometric values for the amount of reagent to be added were determined. The first assumption made was that the average molecular weight of any natural product in the extract is 500 g/mol. The next assumption was that a single natural product makes up approximately 1.5% of a crude extracts total mass (or 1/66). This was based on the observation that 1 L of bacterial culture produces between 0.5 g to 1 g of crude material. The final assumption was that approximately 10 mg of a pure natural product can usually be isolated from this mixture.

With these approximations in hand the effectiveness of the reaction at 10, 30, 100, 300, and 1000 equivalents of reagent was tested. This was done by adding a natural product standard to an extract and tracking the conversion of the natural product to its derivative using high-resolution mass spectrometry (HRMS). Each transformation was optimized in the natural product extracts using one standard. The condition that demonstrated the greatest conversion was then re-tested using a different natural product standard. The results of this trial can be seen in Figure 2.2.

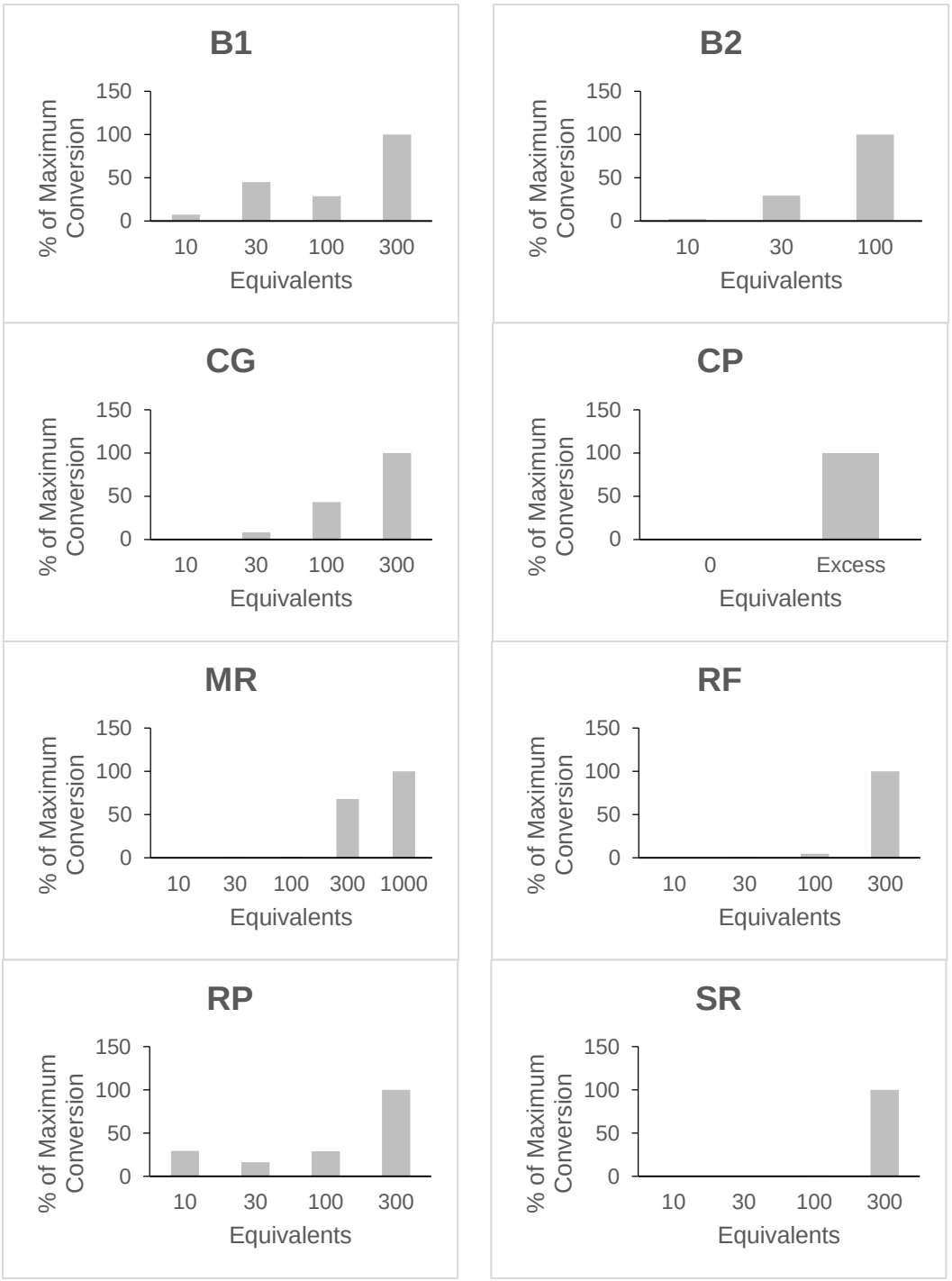


Figure 2.2 Optimization of reaction conditions.

The percentage of maximum conversion was determined by comparing the ratio of the product to the starting material (natural product) on a sample by sample basis. This was done to limit any inaccuracies in the data that would arise from differences in sample concentrations. The conditions where the greatest conversion of starting material to product were seen was deemed the maximum conversion, and the other values were normalized to this number. Using this data, the optimal equivalents of reagent to be added were determined to be 100 for B2,300 for B1, CG, RF, RP, and SR, and 1000 for MR. It was impossible to optimize the amount of reagent for CP as the reagent employed in this reaction is the solvent.

Due to the cost of reagents and the time required to prepare the natural product extracts it was impossible to perform the reactions on a large enough scale to isolate the products from extracts. So, the products used to test these conditions were purified from crude reaction mixtures (without any extract added) and characterized by ^{1}H NMR, ^{13}C NMR, HRMS, and ^{19}F NMR where applicable. The compounds used as standards to test the reactions in the extracts were the same ones that were used in the initial reaction test. The characterization data for these compounds can be seen in the appendix.

2.2. Library Preparation

2.2.1. Extract Preparation

Once the reaction conditions were optimized the next step was to perform the transformations on natural product extracts to produce the library of derivatized natural products. It was determined that ten extracts would be selected for the generation of the derivatized natural product library.

These extracts were prepared from ten different strains of marine actinomycetes collected on numerous scuba diving expeditions to American Samoa, Papua New Guinea, and the United States (California, Maine, Oregon, and Washington). Technicians and previous graduate students were responsible for the isolation of the actinomycetes from the samples of marine sediment. Once isolated the bacteria were stored in a 1:1 glycerol to water mix in a -80°C freezer for future growth and analysis.

The marine actinomycetes used in this work were cultured from these library stocks. This was done by first plating the actinomycetes on SYP and culturing them until enough growth was observed, which usually occurred in one to two weeks. A colony was then picked from the plate and suspended in a 20 mL test tube containing 6 mL of SYP broth and 3 glass beads. This small-scale culture was then placed on a shaker for approximately three days. Then, 3 mL of the bacterial culture was transferred into 60 mL of SYP in a 250 mL Erlenmeyer flask containing a spring and agitated on the shaker for another three to five days. Finally, 40 mL of the bacterial culture was transferred into a 4 L Erlenmeyer flask containing 1 L of SYP, resin, and a spring to be shaken for a further week to 10 days.

Once the bacteria were cultured the natural products were then extracted. This was done by first filtering off the cells and resin. Then, the natural products were extracted by stirring the resin and cells in 250 mL of a mixture of 1:1 DCM to MeOH per liter of extract. The cell debris and resin were then filtered off and the filtrate was concentrated and adsorbed onto celite. The celite was then loaded into a RediSep Solid Load Cartridge and washed with water over a RediSep Rf C18 Column on the Combiflash. The water wash was discarded, and the remaining extract was eluted off the column and concentrated. The bacterial strains used to produce each extract are summarized in Table 2.2.

Table 2.2: Bacterial Strains and Associated Extracts

Strain	Extract
RLUS-09-068-SCS-B	RLUS_1398
RLUS-07-006-SNF-C	RLUS_1403
RLUS-09-060-IMF-A	RLUS_1431
RLUS-10-253-NTS-A	RLUS_1498
RLUS-10-342-HVF-B	RLUS_1565
RLUS-10-348-HVF-A	RLUS_1728
RLUS-09-241-NTF-B	RLUS_1775
RLUS-12-045-HVS-A	RLUS_2031
RLUS-12-180-NTF-B	RLUS_2041
RLUS-12-067-NTF-D	RLUS_2173

2.2.2. Extract Derivatization

The next step was to derivatize all the extracts. This was done by first weighing 80 mg of each extract into nine vials, providing one vial per transformation and one vial of the underivatized extract to carry through as a control. In total there were 90 vials of extract to derivatize as shown in Figure 2.3. Each derivatization was performed by adding reagents as a solution in the reaction solvent to the extracts. The detailed procedure for each transformation is described in the experimental section 2.4.3.

Figure 2.3 Natural product extracts prepared for derivatization.

Work-up of the derivatized and control extracts was performed as consistently as possible across reactions. The reactions were worked up by adding 12 mL of water and extracting the solution three times with 4 mL of EtOAc. The organic layer was then dried with $MgSO_4$, filtered, and concentrated to yield the crude derivatized extracts and controls.

Next, the extracts were fractionated as the final step towards the library generation. The extracts were fractionated in order to deconvolute the mixtures, which helps with screening and metabolomics studies. In screening, fractionation reduces masking or compounding of biological activity, which leads to false negatives and positives. Additionally, when compounds are present in more than one fraction it provides validation of the biological activity of that compound since the activity should be observed in all fractions that the compound is present in. Fractionation is equally important to metabolomics studies of the extracts. When analyzing extracts using HPLC-MS or MS/MS the more simplistic the mixture, the easier it is to identify and isolate the individual components.

The fractions were prepared by first adding 1.5 mL of the fraction solvent to the crude extract. For the wash fraction, or fraction W, this was 100 % H_2O. Fraction A was prepared with

75% H_2O and 25% MeOH, B was 50% H_2O and 50% MeOH, C was 25% H_2O and 75% MeOH, D was 100% MeOH, and E was 100% EtOAc. The vials containing the extract and extract solvent were sonicated to dissolve as much of the extract as possible. The solution was then transferred to an Eppendorf tube and centrifuged. The supernatant was then pipetted onto a Supelco 96-well plate. This was repeated 90 times until the first wash fraction of each extract was prepared. The Supelco plate is a deep well 96-well plate that contains a C18 cartridge and funnel at the base of each well. The Supelco plate was then stacked on top of a 96 deep well plate and centrifuged to run the sample over the C18 cartridge to collect the fraction in the bottom plate. The subsequent fraction was prepared by again adding the fraction solvent to the vial and sonicating the mixture. Then the mixture was transferred to the same Eppendorf tube that contained that extracts previous fraction and sonicating the solution before again centrifuging the Eppendorf tube. The supernatant was then pipetted into the same well on the Supelco plate and the fraction was collected in a new 96 deep well plate after centrifugation. This procedure was repeated an additional four times to produce all six fractions of the 90 extracts. Thus, producing a library of 540 fractionated extracts.

The extracts were then dried and reformatted into seven 96-well plates. They were reformatted so that the first and last well of every row were available for screening controls. The extracts were then stored in the -80°C freezer until needed. From this library stock two 384 well plates were prepared and used for screening analysis. Additionally, two more 384 well plates were prepared in a 1:400 dilution from the original library stock to be analyzed using an IMS-qTOF mass spectrometer. The dilution plates were prepared in triplicate to enable replicate comparison of the data.

2.3. Summary

A series of synthetic transformations were selected and developed to derivatize crude natural product extracts. These transformations were selective, high-yielding, targeted functional groups common to natural products, and introduced functionalities common to drugs or other natural products. The careful selection of these transformations provided the best chance of diversifying compounds present in the extracts to generate new natural product derivatives with clinically relevant biological activity.

The transformations include reactions that increase the aromaticity of the derivatized natural product via reactions with benzynes (B1 & B2), a chemical glycosylation (CG), a dichlorocyclopropanation (CP), a reaction to convert 1,3-amino alcohols to morpholine rings (MR), reductive aminations (RF & RP), and a strain release amination (SR). Each reaction was first tested on pure natural product standards before being optimized for derivatization of natural product extracts.

All transformations were then employed to convert extracts from 10 actinomycetes into a library of 540 prefractionated natural product extracts. These derivatized extracts were reformatted into 384-well plates for screening and metabolomics analysis, which ultimately lead to the identification of derivatives and characterization of their biological activity.

2.4. Experimental

2.4.1. General Considerations

All reactions were carried out using commercial reagents and solvents that were used as received. Reagents and solvents were purchased from Sigma Aldrich, Fisher Scientific, LC Laboratories, and/or Alfa Aesra. Flash chromatography was carried out with Geduran Si60 silica gel (Merck). HPLC was performed on Agilent 1200 series HPLC and HPLC-MS (ESI) instruments using Genimi RP prep column, Synergi Fusion-RP analytical and semi-prep columns, and a Kinetix C18 analytical column (Phenomenex). UPLC-MS experiments were performed using an Acquity UPLC equipped with the Synergi-G2Si IMS-QTOF mass spectrometer (Waters). NMR spectra were recorded using methanol-d_4 (MeOD), or chloroform-*d* ($CDCl_3$). Signal positions (δ) are given as parts per million (ppm) NMR spectra were recorded on a Bruker Avance 500 (500 MHz) or a Bruker Avance 600 (600 MHz) equipped with a QNP.

2.4.2. Reactions on Natural Product Standards

Preparation of Compounds 2.1 & 2.2 (B1)

General Procedure[116]

1-(2-(phenylbuta-1,3-diyn-1-yl)phenyl)-3-(trimethylsilyl)prop-2-yn-1-one (**2.21**) (10 mg, 0.03 mmol) was dissolved in dry ACN (1 mL) along with a natural product (0.05 mmol, 12 mg of atenolol for compound **2.1**, and 16 mg of boldine for compound **2.2**) and stirred for 20 hours at 78°C. The reaction was then filtered and concentrated to yield the crude product.

Isolation of Compound 2.1

Compound **2.1** was separated by RP-HPLC (Phenomenex Gemini 5µ C18, 110 Å, ϕ 50 x 30 mm, 15 mL/min) with a gradient elution from 2% ACN + 0.1% TFA to 100% ACN + 0.1% TFA to afford **2.1** as a mixture of 4 stereoisomers. HRMS *m/z* $[M+H]^{+}$ = 593.2802 (calcd for $C_{36}H_{41}N_2O_4Si^{+}$, 593.2830).

^{1}H NMR: (600 MHz, $CDCl_3$) δ 7.59 (d, J = 7.2 Hz, 1H), 7.47 (m, 3 H), 7.41 (td, J = 7.2, 1H), 7.36 (m, 2H), 7.30 (dt, J = 7.2, 1H), 7.23 (d, J = 7.2, 1H), 7.20 (d, J = 7.2,1H), 6.85 (d, J = 8.2, 2H), 5.48 (s, 1H), 5.34 (s, 1H), 3.94 (s, 2H), 3.49 (s, 2H), 3.33 (d, 1H), 3.09 (p, J = 6.5, 1H), 3.01 (s, 1H), 2.65 (s, 1H), 0.77 (s, 3H), 0.64 (S, 3H), -0.08 (s, 9H).
See Figure 5.1 in Appendix

^{13}C NMR: (150 MHz, $CDCl_3$) δ 194.3, 174.0, 158.1, 154.6, 147.1, 145.4, 144.7, 143.4, 142.0, 135.6, 134.7, 134.2, 131.0, 130.6, 130.4, 129.2, 128.4, 128.3, 127.5, 127.3, 123.9, 119.7, 115.3, 115.1, 69.9, 67.4, 55.7, 46.5, 42.5, 29.9, 22.9, 19.2, 17.8, 1.31, 1.13, 0.97.
See Figure 5.2 in Appendix

Isolation of Compound 2.2

Compound **2.2** was separated by RP-HPLC (Phenomenex Gemini 5µ C18, 110 Å, ϕ 50 x 30 mm, 15 mL/min) with a gradient elution from 2% ACN + 0.1% TFA to 100% ACN + 0.1% TFA to afford **2.2**. HRMS *m/z* $[M+H]^{+}$ = 654.2665 (calcd for $C_{41}H_{39}NO_5Si^{+}$, 654.2670).

^{1}H NMR: (600 MHz, MeOD) δ 7.83 (d, J = 7.8 Hz, 1H), 7.78 (s, 1H), 7.39 (m, 5H), 7.28 (t, J = 7.6 Hz, 1H), 7.13 (t, 7.4 Hz, 1H), 7.01 (d, J = 7.4 Hz, 1H), 6.93 (d, J = 7.4 Hz, 1H), 6.70 (s, 1H), 6.66 (s, 1H), 6.25 (s, 1H), 3.66 (s, 3H) ,3.54 (s, 3H), 3.03 (m, 2H), 2.83 (m, 1H), 2.46 (d, J = 13.7, 1 H), 2.30 (m, 1 H), 2.20 (s, 3H), 2.10 (d, J = 13.9 Hz, 1H), -0.21 (s, 9H).
See Figure 5.3 in Appendix

^{13}C NMR: (150 MHz, MeOD) δ 196.7, 151.7, 151.2, 151.0, 147.4, 146.5, 145.4, 145.1, 144.7, 144.4, 143.1, 139.5, 135.7, 135.5, 135.1, 133.4, 131.5, 130.7, 130.3, 129.7, 129.1, 128.5, 127.6, 127.2, 125.2, 124.5, 124.4, 120.1, 115.9, 115.9, 112.4, 67.0, 60.5, 56.5, 47.0, 36.8, 35.5, 22.6, 1.9, 1.7, 1.4
See Figure 5.4 in Appendix

Preparation of Compounds 2.3 & 2.4 (B2)

General Procedure[114]

2-(trimethylsilyl)phenyl trofluoromethanesulfonate (50 mg, 0.17 mmol) was weighed into a pressure vial and dry ACN (5.6 mL) was added. Then a natural product (0.2 mmol) was added to the solution (54 mg of atenolol for compound **2.3**, and 65 mg of boldine for compound **2.4**) followed by CsF (28 mg, 0.18 mmol). The reaction was then heated to 78°C for 20 hours. The solution was then filtered and concentrated to yield the crude product.

Isolation of Compound 2.3:

Compound **2.3** was separated by RP-HPLC (Phenomenex Gemini 5µ C18, 110 Å, ϕ 50 x 30 mm, 15 mL/min) with a gradient elution from 2% ACN + 0.1% TFA to 100% ACN + 0.1% TFA to afford **2.3** as a mixture of 4 stereoisomers. HRMS *m/z* $[M+H]^+$ 343.2014 (calcd for $C_{20}H_{27}N_2O_3^+$, 343.2016).

^{1}H NMR: (600 MHz, MeOD) δ 7.62 (d, J = 4.2 Hz, 4H), 7.58 (m, 1H), 7.20 (dt, J = 8.6, 3.2, 2.1 Hz, 2H), 6.85 (dt, J = 8.7, 3.1, 2.1 Hz, 2H), 4.05 (hept, J = 6.5 Hz, 1H), 3.91 (m, 3H), 3.82 (dd, J = 12.9, 10.1 Hz, 1H), 3.64 (s, 1H), 3.43 (s, 1H), 1.48 (d, J = 6.5 MHz, 3H), 1.21 (s, 3H).
See Figure 5.5 in Appendix

^{13}C NMR: (150 MHz, MeOD) δ 177.2, 158.8, 138.5, 131.4, 131.4, 131.3, 129.7, 124.2, 115.7, 70.9, 65.6, 63.6, 57.8, 42.5, 18.9, 18.7.
See Figure 5.6 in Appendix

Isolation of Compound 2.4

Compound **2.4** was separated by RP HPLC (Phenomenex Gemini 5µ C18, 110 Å, ϕ 50 x 30 mm, 15 mL/min) with a gradient elution from 2% ACN + 0.1% TFA to 100% ACN + 0.1% TFA to afford **2.4**. HRMS *m/z*$[M+H]^+$ = 404.1835 (calcd for for $C_{25}H_{26}NO_4^+$, 404.1856).

^{1}H NMR: (600 MHz, ACN) δ 9.04 (S, 1H), 7.75 (d, J = 9.1 Hz, 1H), 7.31 (td, J = 8.5, 7.4, 1.5 Hz, 2H), 7.27 (s, 1H), 7.08 (s, 1H), 7.00 (d, J = 8.1 Hz, 2H), 6.89 (t, J = 7.3, 1H), 4.06 (s, 3H), 3.81 (s, 3H), 3.36 (m, 2H), 3.23 (m, 2H), 2.96 (s, 3H).
See Figure 5.7 in Appendix

^{13}C NMR: (150 MHz, ACN) δ 148.8, 148.4, 147.2, 146.3, 143.3, 133.0, 130.7, 129.6, 125.8, 125.2, 125.0, 124.1, 123.5, 121.6, 118.6, 117.6, 112.0, 109.1, 60.6, 57.0, 56.5, 42.2, 30.3.
See Figure 5.8 in Appendix

Preparation of Compounds 2.5 & 2.6 (CG)

General Procedure[121]

Ag_2O (226 mg, 2.25 mmol) was added to a solution of acetobromo-*α*-D-glucose (411 mg, 0.55 mmol) in ACN (800 µL). A natural product (0.5 mmol) was then added to the solution (272 mg of β-estradiol for compound **2.5**, and 328 mg of labetalol for compound **2.6**). The reaction was stirred at room temperature for 16 hours. The reaction mixture was then filtered over celite and the filtrate was concentrated. The solid intermediate was then suspended in 5 mL of MeOH and K_2CO_3 (344 mg, 2.5 mmol) was added. The reaction was then stirred for 16 hours. Water was then added, and the mixture was extracted with EtOAc. The organic layer was the dried with $MgSO_4$ and concentrated to yield the crude product.

Isolation of Compound 2.5

Compound **2.5** was separated by RP-HPLC (Phenomenex Synergi 10µ C18, 80 Å, ϕ 10 x 250 mm, 4 mL/min) with a gradient elution from 40% MeOH + 0.02% formic acid to 80% MeOH + 0.02% formic acid to afford **2.5**. HRMS *m/z* $[M+H]^+$ = 435.2381 (calcd for $C_{24}H_{35}O_7^+$, 435.2377).

^{1}H NMR: (600 MHz, MeOD) δ 7.15 (d, J = 8.6 Hz, 1H), 6.82 (dd, J = 8.5, 2.6 Hz, 1H), 6.76, (d, J = 2.4 Hz, 1H), 4.80 (d, J = 7.5 Hz, 1H), 3.65 (dd, J = 6.4, 5.5 Hz, 1H), 3.63 (t, J = 6.0 Hz, 1H), 3.37 (m, 4H), 2.79 (m, 2H), 2.30 (m, 1H), 2.14 (td, J = 11.2, 3.7 Hz, 2H), 2.00 (dtd, J = 13.3, 9.4, 5.8 Hz, 1H), 1.93 (dt, J = 12.5, 3.3 Hz, 1H), 1.85 (m, 1H), 1.67 (m, 1H), 1.13 (m, 7H), 0.74 (s, 3H).
See Figure 5.9 in Appendix

^{13}C NMR: (150 MHz, MeOD) δ 156.9, 139.0, 127.2, 117.8, 115.2, 102.5, 82.5, 78.1, 78.0, 75.0, 71.4, 62.5, 51.3, 45.5, 44.4, 40.4, 38.0, 30.8, 30.7, 28.5, 27.6, 24.0, 11.7.
See Figure 5.10 in Appendix

Isolation of Compound 2.6

Compound **2.6** was separated by by RP-HPLC (Phenomenex Synergi 10µ C18, 80 Å, ϕ 10 x 250 mm, 4 mL/min) with gradient elution from 70% MeOH + 0.02% formic acid to 50% MeOH + 0.02% formic acid to afford **2.6** as a mixture of 4 stereoisomers. HRMS *m/z* $[M+H]^+$ = 369.2175 (calcd for $C_{25}H_{35}N_2O_8^+$, 369.2173).

^{1}H NMR: (600 MHz, MeOD) δ 8.52 (s, 1H), 7.92, (m, 1H), 7.54 (m, 1H) 7.40 (d, 8.6 Hz, 1H), 7.26 (t, 7.5 Hz, 2H), 7.20 (m, 2H), 7.16 (t, J = 7.3 Hz, 1H), 4.97 (d, J = 7.7 Hz, 1H), 3.88 (d, J = 12.1 Hz, 1 H), 3.68 (dd, J = 12.1, 2.2 Hz, 1H), 3.27 (m, 6H), 3.04 (m, 4H), 2.76 (m, 1H), 2,62 (m, 1H), 2.07 (m, 1H), 1.77 (m, 1H), 1.33 (d, J = 6.4 Hz, 3H).
See Figure 5.11 in Appendix

^{13}C NMR: (150 MHz, MeOD) δ 169.7, 169.7, 157.3, 157.3, 141.9, 137.3, 132.0, 131.9, 129.8, 129.7, 129.7, 129.4, 129.4, 127.4, 127.4, 124.4, 124.3, 118.4, 103.8, 103.7, 78.7, 78.1, 74.8, 71.2, 62.5, 55.1, 55.1, 52.3, 43.9, 36.5, 35.9, 32.8, 32.7, 32.6, 17.0, 16.3.
See Figure 5.12 in Appendix

Preparation of Compounds 2.7 & 2.8 (CP)

General Procedure[125]

TBAC (6 mg, 0.05 mmol) was added to a solution of a natural product (0.5 mmol, 87 µL of jasmone for compound **2.7**, and 230 µL of vitamin K1 for compound **2.8**) in $CHCl_3$ (400 µL). Then, 50% NaOH (120 µL) was added and the mixture was stirred vigorously for 18 hours. Water was then added, and the mixture was extracted with DCM. The organic layer was the dried with $MgSO_4$ and concentrated to yield the crude product.

Isolation of Compound 2.7

Compound **2.7** was separated by RP-HPLC HPLC (Phenomenex Synergi 10µ C18, 80 Å, ϕ 4.6 x 250 mm, 2 mL/min) with gradient elution from 30% MeOH + 0.02% formic acid to 100% MeOH + 0.02% formic acid to afford **2.17** as a mixture of enantiomers. HRMS *m/z* $[M+H]^+$ = 247.0652 (calcd for $C_{12}H_{17}Cl_2O^+$, 247.0651).

^{1}H NMR: (500 MHz, $CDCl_3$) δ 2.54 (m, 2H), 2.40 (q, J = 4.7 Hz, 2H), 2.29 (qd, J = 14.8, 6.9 Hz, 2H), 1.48 (m, 3H), 1.05 (m, 3H).
See Figure 5.13 in Appendix

^{13}C NMR: (125 MHz, $CDCl_3$) δ 209.5, 171.5, 138.0, 37.9, 34.6, 34.4, 32.0, 31.1, 19.0, 18.6, 17.9, 13.3.
See Figure 5.14 in Appendix

Isolation of Compound 2.8

Compound **2.8** was separated on a normal phase coloumn packed with Geduran Si60 silica gel with isocratic elution of 98% hexanes and 2% EtOAc to afford **2.8** as a mixture of enantiomers. HRMS *m/z* $[M+H]^+$ = 533.2943 (calcd for $C_{32}H_{47}Cl_2O_2^+$, 533.2948).

^{1}H NMR: (500 MHz, $CDCl_3$) δ 8.16 (m, 2H), 7.78 (m, 2H), 1.87 (dd, J = 15.3, 4.2 Hz, 1H), 1.83 (s, 3H), 1.30 (m, 25H), 0.87 (m, 12H), 0.76 (dd, J = 11.5, 6.4 Hz).
See Figure 5.15 in Appendix

^{13}C NMR: (125 MHz, $CDCl_3$) δ 189.6, 188.9, 134.8, 134.7, 134.6, 134.6, 133.8, 133.4, 127.0, 126.9, 126.7, 126.7, 71.7, 69.7, 48.1, 47.9, 44.1, 43.8, 39.4, 38.4, 37.4, 37.4, 37.3, 37.3, 37.3, 37.3, 37.3, 37.2, 37.1, 37.0, 36.9, 35.7, 35.6, 33.1, 33.1, 33.08, 33.05, 32.81, 32.64, 32.56, 32.54, 29.7, 28.0. 24.8, 24.8, 24.6, 24.4, 24.3, 23.9, 23.9, 22.7, 22.6, 19.8, 19.7, 19.6, 19.5, 19.5, 19.5, 14.8, 14.8, 14.8, 14.0, 14.0.
See Figure 5.16 in Appendix

Preparation of Compounds 2.9 & 2.10 (MR)[128,129]

General Procedure[128,129]

LiCl (42 mg, 1.2 mmol) and propylene oxide (58 mg, 1.2 mmol) were added to a solution of natural product (1 mmol, 336 mg of acebutolol for compound **2.9**, and 328 mg of labetalol for compound **2.10**) in MeOH (2 mL). Propylene oxide (84 µL, 1 mmol) was then added and the reaction was stirred at 40°C for 16 hours. The methanol was then evaporated off and the intermediate was resuspended in dioxane (5 mL) and cooled to 0°C. KOH (1.12 g, 20 mmol) was then added followed by TsCl (393 mg, 2mmol). The reaction was stirred and allowed to warm to room temperature for 18 hours. Water was then added, and the mixture was extracted with EtOAc, dried with $MgSO_4$ and concentrated to yield the crude product.

Isolation of Compound 2.9

Compound **2.9** was separated by RP-HPLC (Phenomenex Kinetex 5µ C18, 100 Å, ϕ 4.6 x 250 mm, 1 mL/min) with gradient elution from 30% MeOH + 0.02% formic acid to 80% MeOH + 0.02% formic acid to afford **2.9** as a mixture of enantiomers. HRMS *m/z* $[M+H]^+$ = 377.2438 (calcd for $C_{21}H_{33}N_2O_4^+$, 377.2435).

^{1}H NMR: (600 MHz, MeOD) δ 7.75 (m, 2H), 7.09 (m, 1H), 4.23 (m, 1H), 4.14 (m, 1H), 3.98 (m, 1H), 3.64 (m, 1H), 3.44 (m, 2H), 3.31 (m, 1H), 3.18 (m, 1H), 2.62 (s, 3H), 2.02 (m, 1H), 2.29 (t, J = 7.5 Hz, 2H), 1.68 (h, J = 7.4, 1 Hz, 2H), 1.13 (m, 3H), 1.11 (m, 6H), 0.96 (t, J = 7.2 Hz, 3H).
See Figure 5.17 in Appendix

^{13}C NMR: (150 MHz, MeOD) δ 174.5, 133.9, 129.9, 129.2, 127.3, 123.0, 116.4, 115.5, 114.5, 112.1, 75.2, 71.4, 59.8, 57.6, 39.7, 37.7, 36.9, 32.2, 20.8, 20.3, 20.0, 18.6, 18.4, 17.2, 14.1, 14.0.
See Figure 5.18 in Appendix

Isolation of Compound 2.10

Compound **2.10** was separated by RP-HPLC (Phenomenex Kinetex 5µ C18, 100 Å, ϕ 4.6 x 250 mm, 1.5 mL/min) with gradient elution from 5% ACN + 0.02% formic acid to 55% ACN + 0.02% formic acid. HRMS *m/z* $[M+H]^+$ = 369.2175 (calcd for $C_{22}H_{29}N_2O_3^+$, 369.2173).

^{1}H NMR: (600 MHz, MeOD) δ 12.45 (s, 1H), 8.76 (s, 2H), 7.85 (d, J = 8.4, 2H), 7.45 (m, 1H), 7.34 (m, 2H), 7.20 (m, 2H), 7.01 (m, 1H), 4.94 (s, 1H), 3.48 (m, 2H), 2.69 (m, 2H), 2.46 (s, 3H), 2.34 (m, 1H), 1.81 (m, 2H), 1.53 (m, 3H), 1.32 (m, 3H).
See Figure 5.19 in Appendix

^{13}C NMR: (150 MHz, MeOD) δ 165.2, 162.4, 143.8, 131.8, 129.9, 129.2, 128.9, 128.7, 128.5, 128.4, 128.4, 126.7, 126.7, 126.6, 126.3, 125.9, 125.6, 119.0, 118.6, 75.0, 52.9, 32.9, 32.9, 32.6, 29.9, 21.7, 19.0, 17.9, 14.1, 13.8, 13.6, 12.9.
See Figure 5.20 in Appendix

Preparation of Compounds 2.11 & 2.12 (RF)

General Procedure[135]

Acetic acid (29 µL, 0.5 mmol) was added to a solution of 3,5-bis(trifluoromethyl)benzaldehyde (90 µL, 0.55 mmol) and a natural product (0.5 mmol, 182 mg of amoxicillin for compound **2.11**, and 132 mg of nortriptyline for compound **2.12**) in MeOH (5 mL). The reaction was stirred for 30 minutes before $NaBH_4CN$ (63 mg, 1 mmol) was added. The reaction proceeded for 18 hours before water was added and the mixture was extracted with ethyl acetate. The organic layer was dried with $MgSO_4$, filtered, and concentrated to yield the crude product.

Isolation of Compound 2.11

Compound **2.11** was separated by RP-HPLC (Phenomenex Synergi 10µ C18, 80 Å, ϕ 10 x 250 mm, 4 mL/min) with gradient elution from 60% MeOH + 0.02% formic acid to 84% MeOH + 0.02% formic acid to afford **2.11**. HRMS *m/z* $[M+H]^+$ = 592.1342 (calcd for $C_{25}H_{24}F_6N_3O_5S^+$, 592.1335).

^{1}H NMR: (600 MHz, MeOD) δ 8.02 (s, 2H), 7.96 (s, 1H), 7.26 (m, 2H), 6.79 (m, 2H), 5.55 (d, J = 4.1 Hz, 1H), 5.45 (d, J = 4.0 Hz, 1H), 4.69 (s, 1H), 4.25 (s, 1H), 2.58 (dd, J = 11.1, 14.0 Hz, 2 H), 1.50 (s, 3H), 1.45 (s, 3H).
See Figure 5.21 in Appendix

^{13}C NMR: (150 MHz, MeOD) δ 172.9, 170.3, 169.4, 158.6,132.0, 137.5, 131.7, 131.5, 131.3, 130.2, 130.1, 129.9, 129.7, 124.2, 122.0, 115.7, 115.5, 72.7, 71.8, 71.1, 67.0, 65.2, 64.0, 58.5, 58.2, 49.2, 29.9, 26.0, 25.6, 25.1.
See Figure 5.22 in Appendix

^{19}F NMR: (565 MHz, MeOD) δ -65.8
See Figure 5.23 in Appendix

Isolation of Compound 2.12

Compound **2.12** was separated by RP-HPLC HPLC (Phenomenex Kinetex 5µ C18, 100 Å, ϕ 4.6 x 250 mm, 1 mL/min) with gradient elution from 30% MeOH + 0.02% formic acid to 100% MeOH + 0.02% formic acid to afford **2.12** as a mixture of enantiomers. HRMS *m/z* $[M+H]^+$ = 490.1966v(calcd for $C_{28}H_{26}F_6^+$, 490.1964).

^{1}H NMR: (600 MHz, MeOD) δ 8.02 (s, 2H), 7.96 (s, 1H), 7.26 (m, 2H), 6.79 (m, 2H), 5.55 (d, J = 4.1 Hz, 1H), 5.45 (d, J = 4.0 Hz, 1H), 4.69 (s, 1H), 4.25 (s, 1H), 2.58 (dd, J = 11.1, 14.0 Hz, 2 H), 1.50 (s, 3H), 1.45 (s, 3H).
See Figure 5.24 in Appendix

^{13}C NMR: (150 MHz, MeOD) δ 172.9, 170.3, 169.4, 158.6,132.0, 137.5, 131.7, 131.5, 131.3, 130.2, 130.1, 129.9, 129.7, 124.2, 122.0, 115.7, 115.5, 72.7, 71.8, 71.1, 67.0, 65.2, 64.0, 58.5, 58.2, 49.2, 29.9, 26.0, 25.6, 25.1.
See Figure 5.25 in Appendix

^{19}F NMR: (565 MHz, MeOD) δ -64.3
See Figure 5.26 in Appendix

Preparation of Compounds 2.13 & 2.14 (RP)

General Procedure[135]

Acetic acid (29 µL, 0.5 mmol) was added to a solution of 3-pyridinecarboxaldehyde (38 µL, 0.55 mmol) and a natural product (0.5 mmol, 182 mg of amoxicillin for compound **2.13**, and 132 mg of nortriptyline for compound **2.14**) in MeOH (5 mL). The reaction was stirred for 30 minutes before $NaBH_4CN$ (63 mg, 1 mmol) was added. The reaction proceeded for 18 hours before water was added and the mixture was extracted with ethyl acetate. The organic layer was dried with $MgSO_4$, filtered, and concentrated to yield the crude product.

Isolation of Compound 2.13

Compound **2.13** was separated by RP-HPLC HPLC (Phenomenex Synergi 10µ C18, 80 Å, ϕ 4.6 x 250 mm, 2 mL/min) with gradient elution from 10% ACN + 0.02% formic acid to 60% ACN + 0.02% formic acid to afford **2.13**. HRMS *m/z* $[M+H]^+$ = 457.1542 (calcd for $C_{22}H_{25}N_4O_5S^+$, 457.1540).

^{1}H NMR: (600 MHz, MeOD) δ 8.61 (s, 1H), 8.56 (d, J = 5.0 Hz, 1H), 7.94 (m, 1H), 7.51 (m, 1H), 7.30 (m, 2H), 6.85 (m, 2H), 5.58 (d, J = 4.0 Hz, 1H), 5.50 (d, J = 4.0 Hz, 1H), 4.61 (m, 1H), 4.28 (s, 1H), 4.02 (m, 2H), 1.59 (s, 3H), 1.54 (s, 3H).
See Figure 5.27 in Appendix

^{13}C NMR: (150 MHz, MeOD) δ.172.0, 171.9, 170.1, 159.2, 154.5, 153.8, 150.7, 150.4, 149.5, 144.8, 139.3, 135.4, 130.7, 125.4, 116.7, 75.5, 75.4, 66.7, 65.5, 60.2, 59.0, 27.4, 27.0.
See Figure 5.28 in Appendix

Isolation of Compound 2.14

Compound **2.14** was separated by RP-HPLC HPLC (Phenomenex Synergi 10µ C18, 80 Å, ϕ 4.6 x 250 mm, 2 mL/min) with gradient elution from 5% ACN + 0.02% formic acid to 100% ACN + 0.02% formic acid to afford **2.14**. HRMS *m/z* $[M+H]^+$ = 355.2171 (calcd for $C_{25}H_{27}N_2^+$, 355.2169).

^{1}H NMR: (600 MHz, MeOD) δ 8.49 (dd, J = 4.9, 1.9 Hz, 1H), 8.46 (d, J = 2.2 Hz, 1H), 8.27 (s, 1H), 7.75 (dt, J = 7.9, 1.9 Hz, 1H), 7.40 (m, 1H), 7.20 (m, 3H), 7.15 (td, J = 7.1, 2.2 Hz, 1H), 7.08 (m, 3H), 7.00 (dd, J = 7.3, 1.7 Hz), 5.78 (t, J = 7.4 Hz, 1H), 3.82 (s, 2H), 2.72 (m, 4H), 2.41 (q, J = 7.6 Hz, 2H), 2.35 (s, 3H).
See Figure 5.29 in Appendix

^{13}C NMR: (150 MHz, MeOD) δ.167.5, 151.5, 150.1, 146.8, 142.0, 141.0, 140.7, 139.9, 138.2, 132.5, 131.1, 129.4, 129.2, 128.9, 128.9, 127.7, 127.1, 127.0, 125.4, 59.0, 57.0, 41.3, 34.8, 33.0, 27.1.
See Figure 5.30 in Appendix

Preparation of Compounds 2.15 & 2.16 (SR)

General Procedure[138]

1-((3,5-Difluorophenyl)sulfonyl)bicyclo[1.1.0]butane (126 mg, 0.55 mmol) was added to a solution of LiCl (42 mg, 1.5 mmol) and a natural product (0.5 mmol, 168 mg acebutolol for compound **2.15**, and 134 mg of metoprolol for compound **2.16**) in DMSO (1 mL). The reaction was stirred for 20 hours at room temperature before water was added and the mixture was extracted with EtOAc. The organic layer was dried with $MgSO_4$, filtered, and concentrated to yield crude product.

Isolation of Compound 2.15

Compound **2.15** was separated by RP-HPLC (Phenomenex Synergi 10µ C18, 80 Å, ϕ 10 x 250 mm, 2 mL/min) with a gradient elution from 5% MeOH + 0.02% formic acid to 64% MeOH + 0.02% formic acid to afford **2.15** as a mixture of 8 stereoisomers. HRMS *m/z* $[M+H]^+$ = 557.2339 (calcd for $C_{28}H_{37}F_2N_2O_6S^+$,557.2335).

^{1}H NMR: (600 MHz, MeOD) δ 8.29 (s, 1H), 7.84 (dd, J + 18.9, 2.7 Hz, 1H), 7.68 (ddd, J = 16.4, 8.9, 2.7 Hz, 1H), 7.57 (m, 1H), 7.43 (m, 1H), 7.37 (m, 1H), 7.02 (d, J = 14.4, 9.0 Hz, 1H), 4.10 (m, 4H), 3.95 (tt, J = 10.0, 3.1 Hz, 1H), 3.74 (m, 1H), 3.51 (m, 1H), 3.13 (m, 1H), 3.04 (m, 1H), 2.86 (m, 1H), 2.72 (m, 2H), 2.60 (s, 1H), 2.56 (s, 1H), 2.27 (t, J = 7.2 Hz, 2H), 1.66 (q, J = 7.4 Hz, 2H), 1.22 (dd, J = 17.5, 5.6 Hz, 3H), 1.18 (dd, J = 24.0, 6.9 Hz, 3H), 0.94 (t, J = 7.4 Hz, 3H).
See Figure 5.31

^{13}C NMR: (150 MHz, MeOD) δ.201.4, 201.3, 174.5, 165.4, 165.4, 165.3, 165.3, 163.7, 163.7, 163.6, 163.6, 155.5, 155.4, 142.9, 142.8, 142.8, 142.3, 142.2, 142.2, 133.5, 133.5, 129.0, 128.8, 127.2, 127.1, 123.4, 123.3, 114.6, 114.6, 113.4, 113.3, 113.2, 113.2, 113.0, 112.9, 112.8, 112.8, 111.0, 110.9, 110.8, 110.7, 110.7, 110.6, 72.0, 67.2, 66.9, 56.2, 55.3, 55.0, 53.1, 52.6, 51.9, 51.5, 50.8, 39.7, 31.8, 31.7, 30.4, 30.1, 29.3, 28.9, 20.3, 17.9, 17.6, 17.3, 17.1, 14.0.
See Figure 5.32 in Appendix

^{19}F NMR: (565 MHz, MeOD) δ –107.1, -107.2, -107.2, -107.2
See Figure 5.33 in Appendix

Isolation of Compound 2.16

Compound **2.16** was separated by RP-HPLC (Phenomenex Synergi 10µ C18, 80 Å, ϕ 10 x 250 mm, 2 mL/min) with a gradient elution from 40% MeOH + 0.02% formic acid to 70% MeOH + 0.02% formic acid to afford **2.16** as a mixture of 8 stereoisomers. HRMS *m/z* $[M+H]^+$ = 498.2129 (calcd for $C_{25}H_{34}F_2NO_5S^+$, 498.2120).

^{1}H NMR: (600 MHz, MeOD) δ 8.39 (s, 1H), 7.65 (m, 1H), 7.54 (m, 1H), 7.45 (m, 1H), 7.17 (m, 2H), 6.90 (m, 2H), 4.04 (m, 4H), 3.58 (m, 2H), 3.35 (s, 3H), 3.12 (m, 1H), 2.93 (m, 1H), 2.79 (m, 5H), 2.45 (m, 1H), 1.24 (dd, J = 16.7, 6.7 Hz, 3H), 1.19 (dd, 18.8, 6.6 Hz, 3H).
See Figure 5.34 in Appendix

^{13}C NMR: (150 MHz, MeOD) δ 167.6, 165.4, 165.3, 165.3, 163.7, 163.7, 163.7, 163.6, 158.6, 158.5, 143.1, 142.5, 142.5, 142.4, 132.9, 132.8, 130.9, 115.5, 115.5, 113.3, 113.3, 113.2, 113.1, 113.0, 112.9, 112.8, 112.8, 110.9, 110.8, 110.8, 110.7, 110.6, 110.5, 75.0, 74.9, 71.0, 70.8, 68.3, 67.8, 58.8, 55.4, 54.4, 54.0, 53.3, 51.8, 51.1, 51.0, 50.5, 49.6, 36.1, 36.1, 30.7, 30.4, 29.7, 29.3, 18.3, 18.0, 17.6.
See Figure 5.35

^{19}F NMR: (565 MHz, MeOD) δ –107.4, -107.4, -107.5, -107.5
See Figure 5.36 in Appendix

2.4.3. Reactions on Natural Product Extracts

B1

Natural product extract (80 mg) was dissolved in ACN (8.25 mL) and the HDDA reagent (1-(2-(phenylbuta-1,3-diyn-1-yl)phenyl)-3-(trimethylsilyl)prop-2-yn-1-one (**2.21**), 237 mg, 0.73 mmol) was added. The reaction was heated to 78°C for 24 hours. The reaction was then removed from heat and cooled to room temperature. Water (12 mL) was then added and then extracted with EtOAc (3 x 6 mL), dried, filtered, and concentrated to yield the crude derivatized extract. The extract was then lyophilized and stored at -80°C until fractionation.

B2

Natural product extract (80 mg) was dissolved in ACN (8.25 mL) and 2-(trimethylsilyl)phenyl trofluoromethanesulfonate (141 mg, 2.42 mmol) was added along with CsF (103 mg, 2.42 mmol) and the reaction was heated to 78°C for 24 hours. The reaction was then removed from heat and cooled to room temperature. Water (12 mL) was then added and then extracted with EtOAc (3 x 6 mL), dried, filtered, and concentrated to yield the crude derivatized extract. The extract was then lyophilized and stored at -80°C until fractionation.

CG

Acetobromo-*α*-D-glucose (299 mg, 0.73 mmol) was combined with Ag_2O (169 mg, 0.73) in ACN (4 mL). A natural product extract (80 mg) was then added and the reaction was stirred at room temperature for 18 hours. The reaction mixture was then filtered over celite to remove the precipitate. Then the ACN was evaporated and the intermediate was re-dissolved in MeOH (5 mL). K_2CO_3 (101 mg, 0.73 mmol) was then added and the reaction was stirred at room temperature for 20 hours. Water (12 mL) was then added and then extracted with EtOAc (3 x 6 mL), dried, filtered, and concentrated to yield the crude derivatized extract. The extract was then lyophilized and stored at -80°C until fractionation.

CP

Natural product extract (80 mg) was dissolved in chloroform (2 mL). TBAC (40 mg, 0.18 mmol) was then added followed by 50% NaOH (600 µL). The reaction was stirred at room temperature for 20 hours. Water (12 mL) was then added and then extracted with DCM (6 mL) followed by EtOAc (3 x 6 mL), dried, filtered, and concentrated to yield the crude derivatized extract. The extract was then lyophilized and stored at -80°C until fractionation.

MR

Natural product extract (80 mg), propylene oxide (170 µL, 2.42 mmol), and LiCl (103 mg, 2.42 mmol) were dissolved in MeOH (660 µL) and heated to 40°C for 24 hours. The reaction was cooled to room temperature and the MeOH was evaporated off. The intermediate was then resuspended in dioxane (2 mL) and cooled to 0°C. KOH (136 mg, 2.42 mmol) was then added followed by TsCl (477 mg, 2.42 mmol) and the reaction was stirred for 24 hours and allowed to warm to room temperature. Water (12 mL) was then added and then extracted with EtOAc (3 x 6 mL), dried, filtered, and concentrated to yield the crude derivatized extract. The extract was then lyophilized and stored at -80°C until fractionation.

RF

Natural product extract (80 mg), 3,5-bis(triflluoromethyl)benzaldehyde (120 µL, 0.73 mmol), and HOAc (40 µL, 0.73 mmol) were combined in MeOH and stirred at room temperature for 30 minutes. Then $NaBH_3CN$ (46 mg, 0.73 mmol) was added and the reaction continued to stir at room temperature for an additional 20 hours. Water (12 mL) was then added and then extracted with EtOAc (3 x 6 mL), dried, filtered, and concentrated to yield the crude derivatized extract. The extract was then lyophilized and stored at -80°C until fractionation.

RP

Natural product extract (80 mg), 3-pyridinecarboxaldehyde (78 µL, 0.73 mmol), and HOAc (40 µL, 0.73 mmol) were combined in MeOH and stirred at room temperature for 30 minutes. Then $NaBH_3CN$ (46 mg, 0.73 mmol) was added and the reaction continued to stir at room temperature for an additional 20 hours. Water (12 mL) was then added and then extracted with EtOAc (3 x 6 mL), dried, filtered, and concentrated to yield the crude derivatized extract. The extract was then lyophilized and stored at -80°C until fractionation.

SR

Natural product extract (80 mg), 1-((3,5-Difluorophenyl)sulfonyl)bicyclo[1.1.0]butane (167 mg, 0.73 mmol), and LiCl (92 mg, 2.18 mmol) were combined in DMSO (900 µL) and stirred for 22 hours. Water (12 mL) was then added and then extracted with EtOAc (3 x 6 mL), dried, filtered, and concentrated to yield the crude derivatized extract. The extract was then lyophilized and stored at -80°C until fractionation.

2.4.4. Synthesis of Hexadehydro-Diels-Alder Reagent

Compounds **2.17** to **2.19**, and **2.22** were synthesized according to literature procedures (Scheme 2.1).[115] The synthesis and characterization of compounds **2.20** and **2.21** are described below. These procedures were performed by Mat Sutherland, a graduate student in the Britton lab.

Compound 2.20

Compound **2.22** (130 mL, 56.9 mmol) added to a solution of compound **2.19** (8.656 g, 37.9 mmol) in dry DCM (54.1 mL) which was then added dropwise to a solution of 30% aqueous n-$BuNH_2$ (52.6 mL) that was charged with CuCl (560.9 mg, 5.66 mmol) and $NH_4OH{\bullet}HCl$ (1.22g, 18.95 mmol) at 0°C for two hours. Saturated NH_4Cl was then added and the reaction was warmed to room temperature. This was then extracted with EtOAc (3 x 300 mL), dried, filtered, and concentrated.

^{1}H NMR: (600 MHz, $CDCl_3$) δ 7.73 (m, 1H), 7.55 (m, 3H), 7.43 (td, J = 7.6, 1.4 Hz, 1H), 7.36 (m, 3H), 7.32 (m, 1H), 5.87 (d, J = 5.5 Hz, 1H), 2.51 (d, J = 5.8 Hz, 1H), 0.22 (s, 9H).
See Figure 5.37 in Appendix

Compound 2.21

Compound **2.20** (4.00 g, 12.20 mmol) was added to dry DCM (12.2 mL). This solution was then added to MnO_2 (5.29 g, 60.9 mmol) and stirred at room temperature for 15 hours. The solution was then filtered over celite, dried, filtered, and concentrated.

^{1}H NMR: (600 MHz, Benzene-d_6) δ 8.02 (m, 1H), 7.31 (m, 3H), 6.88 (m, 3H), 6.78 (m, 2H), 0.11 (s, 9H).
See Figure 5.38 in Appendix

Chapter 3.

Identification and Isolation of a Biologically Active Natural Product Derivative

3.1. Using BioMAP to Identify Derivatives with Antimicrobial Activity

3.1.1. Background

Identification of natural products with biological activity against clinically relevant targets is typically done through high-throughput screening. This is the process of treating whole cells or specific protein targets with one or more compounds to illicit a response. In this work whole cell assays were used to detect biologically active compounds. Whole cell assays have broader biological coverages because the compounds can in principle impact any biochemical process in the cell. Thus, the untargeted nature of these assays makes them ideal for screening the derivatized natural product library.[32,33]

The first screen that the derivatized natural product library was screened against was a small section of the antibiotic mode of action profile (BioMAP) screen. BioMAP screens compounds against 20 clinically relevant bacterial pathogens and compares their activity profiles to those of known compounds to identify a potential mode of action. The bacterial strains used in this screen consist of nine Gram-positive pathogens (BSL1: *Bacillus subtilis* (ATCC 23857), *Staphylococcus epidermis* (ATCC 14990), *Listeria ivanovii* (ATCC BAA-139), BSL2: *Enterococcus faecium* (ATCC 6569), *Staphylococcus aureus* (Methicillin-Sensitive) (ATCC 29123), *Staphylococcus aureus* (Methicillin-Resistant) (ATTC BAA-44), *Haemophilus influenzae* (ATCC 49766), *Enterococcus faecalis* (ATCC 29121), *Streptococcus pneumoniae* (ATCC 49619)) and eleven Gram-negative pathogens (BSL1: *Escherichia coli* (K12 MG1655), *Klebsiella aerogenes* (ATCC 35029), *Ochrobactrum anthropi* (ATCC 49687), *Providencia alcalifaciens* (ATCC 9886), BSL2: *Yersinia pseudotuberculosis* (ATCC 6904), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enterica* (ATCC 13311), *Vibrio cholerae* (ATTCC 14033), *Actineobacter baumannii* (ATCC 19606), *Klebsiella pneumoniae* (ATCC 700603), *Shigella sonnei* (ATCC 25931)).[149]

The discovery of new antibiotics with activity against Gram-negative pathogens is of specific interest.[150,151] Bacteria are divided into two broad categories based on their cell wall according to the results of the Gram stain test. This test stains cells using Crystal Violet, which is retained in the thick peptidoglycan cell wall of Gram-positive bacteria. Gram-negative bacteria have an outer cell membrane that prevents staining of their much thinner peptidoglycan cell wall. Gram-negative bacteria are much less receptive to antibiotics because of this outer membrane, especially antibiotics that target the cell wall. The identification of novel compounds with antimicrobial activity is of increased interest due to the need for new antibiotics to target pathogens that have developed resistance to the currently available antibiotics.[151–153]

Due to the current availability of the strains at the Centre for High Throughput Chemical Biology the derivatized natural product library was screened against a small subset of these pathogens: *Bacillus subtilis* (ATCC 23857), *Staphylococcus aureus* (Methicillin-Sensitive) (ATCC 29123), and *Escherichia coli* (K12 MG1655). Future work will involve screening the library against all the BioMAP pathogens.

Bacillus subtilis is a Gram-positive, rod-shaped, catalase positive, aerobic bacteria found in the gastrointestinal tract of humans and ruminants as well as in soil. This bacterium is generally considered non-pathogenic but has been implicated in some cases of food poisoning. It is one of the safest *Bacillus* sp. and as a result is often used to screen for antimicrobials that may have activity against some of the more hazardous *Bacillus* species.[154] *Staphylococcus aureus* (Methicillin-Sensitive) is another Gram-positive, rod-shaped, catalase positive, facultative anaerobic bacteria frequently found in the upper respiratory tract and on the skin of humans. These bacteria are usually commensal of the human microbiota; however, they are also opportunistic pathogens. *Staphylococcus aureus* has been implicated in food poisoning and respiratory infections. The strain of *Staphylococcus aureus* used in this work was methicillin sensitive. Related methicillin-resistant strains are of great clinical relevance as these strains are exceptionally difficult to treat and have a 13% in hospital fatality rate in the United States.[155–157] The third bacteria that the library was screened against was *Escherichia coli*. This is a Gram-negative, facultative anaerobic, rod-shaped, catalase positive bacteria found in the lower intestines of endotherms. Most strains of *Escherichia coli* are non-pathogenic; however, some can cause serious food poisoning. The strain used in this screen (K1) has been modified so that it is unable to survive in human intestines, and is used to identify compounds that may have activity against pathogenic strains of *Escherichia coli*.[158]

3.1.2. Analysis

Each strain was cultivated for approximately 24 hours before being dispensed into sterile, clear, polypropylene 384 well plates. A Tecan Evo 100 was used to pin the derivatized natural product extracts from the compound plate into the wells containing the strains. Three technical replicates were prepared of the bacteria. Once the compounds were pinned into the screening plates a measurement of the optical density at 600nm was taken of each of the wells using a SpectraMa i3x plate reader. The samples were then incubated at 40°C for 20 hours in a Thermo Cytomat 42C. The optical density at 600 nm was then measured using the SpectraMax i3x and the plates were disposed of using standard biohazard disposal protocols.

The optical density of the cells at t_0 was subtracted from the optical density at t_{20} to determine cell growth over the 20-hour period, which were normalized against controls to get relative growth values. These values were used to generate a heat map as shown in Figure 3.1 A. The heat map represents the activity of the derivatized extract against the bacteria. Little to no change in cell density over the 20-hour period indicates that the growth of the cells was inhibited by an extract with antimicrobial activity (bright blue). Contrastingly, an increase in optical density of 0.35 or more is indicative of healthy cell growth and therefore the extract had no antimicrobial activity (black).

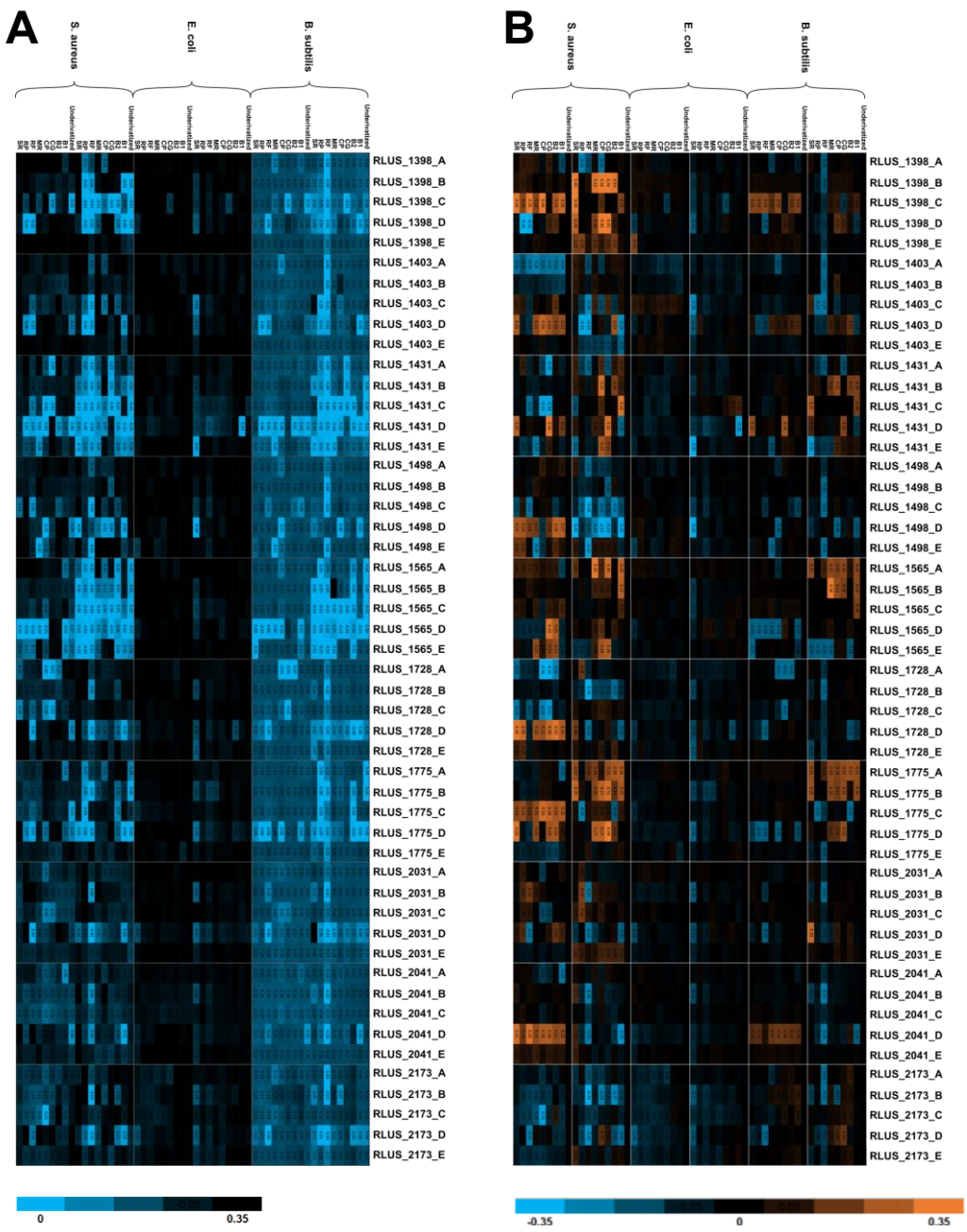


Figure 3.1 Heatmap of cell growth after treatment with the derivatized natural product extract library.

Antimicrobial activity of the derivatized natural product library against *S. aureus, E. coli, and B. subtilis* measured by the change in the optical density of the treated cells at 600 nm. Bright blue indicates no change in the optical density over 20 hours and thus the derivatized extracts were biologically active. Black on the other hand indicates that there was a change in the cell growth consistent with healthy cells and therefore there was no antimicrobial activity in the extracts.

A second way of analyzing this data is by subtracting the change in optical density of the cells treated with the derivatized extracts from the optical density of the cells treated with the underivatized natural product extracts. When this data is used to generate a heat map as seen in Figure 3.1 B. It is very evident that some transformations increased the biological activity of the extracts (bright blue) while others decreased the activity (orange). This view, while insightful requires further consideration along with metabolomics analysis before it can be conclusively used as evidence to support the finding that derivatization has an impact on biological activity.

3.2. Cell Painting Screen

3.2.1. Background

The second screen that was used to identify any clinically relevant biological activity present in the extracts was Cell Painting. This assay used five fluorescent dyes to highlight different cellular components to illuminate morphological changes to the cells as a result of treatment with an extract. Automated image analysis of different cell markers including size, shape, and texture identifies about 150 different cellular features to produce profiles. These profiles can be compared to the profiles of known biologically active compounds to indicate a potential mode of action for the newly identified compound.[159]

The cells that the derivatized natural product library were screened against were human Osteosarcoma Cells from the U2OS cell line. This cell line was first derived in 1964 by Jan Ponten and Eero Saksela from the tibia of a 15-year-old girl.[160] The growth protocol for these cells is elaborate and is described in a Nature Protocol.[159] Essentially, the cells are grown to near confluence before being stained with the fluorescent dyes. They are then washed to remove excess dye. Cell imaging was performed using a Molecular Devices Image Xpress Screening microscope. Compounds that have an impact on the morphology of these osteosarcoma cells have the potential to become leads for anticancer drugs.

The dyes used to stain the cells include 4',6-diamidino-2-phenylindole (DAPI), indodicarbocyanine (Cy5), sulfur rhodamine 101 acid chloride (Texas Red), tetramethylrhodamine (TRITC), and fluorescein isothiocyanate (FITC). The first of these dyes, DAPI, binds to adenine-thymine rich segments of DNA to image the nucleus. The excitation maximum of DAPI is at 358 nm (UV) and the emission maximum is 461 nm (blue).[161] The second dye, Cy5, stains the mitochondria of the cells to determine morphological changes that

arise from treatment with the extract. This dye is excited at 650 nm (red) and emits light at 670 nm (red).[162] Texas Red is excited and emits light at a similar wavelength to Cy5, with an excitation maximum of 596 nm (orange) and an emission maximum of 615 nm (orange). Texas Red stains the filamentous actin present in the cells.[163] TRITC stains the Golgi of cells and is excited at 557 nm (yellow) and emits light at 576 nm (yellow).[164] Finally, FITC is excited at 490 nm (blue) and emits light at 525 nm (green).[165] FITC stains the DNA present in the nucleus and the endoplasmic reticulum. Using dyes that emit light across the visual spectrum is critical in obtaining clear images of the different cellular components to identify morphological changes indicative of biological activity. An example of an overlay of these images can be seen in Figure 3.2, where A is an overlaid image of the cells treated with DMSO (negative control) and B is an overlaid image of the cells treated with latrunculin B, an actin inhibitor (positive control).[166]

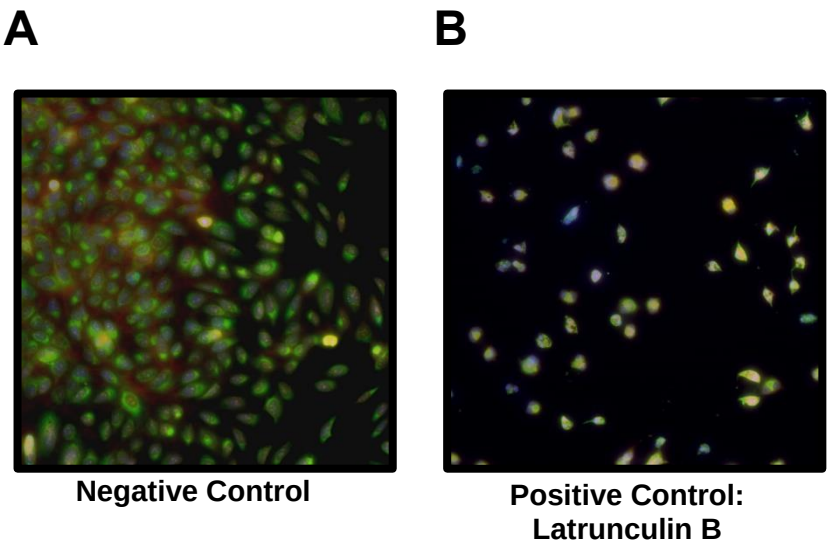


Figure 3.2 Images of Controls for Cell Painting Assay.

Images of negative (A) and positive (B) controls in the Cell Painting assay. The negative control shown here are osteosarcoma cells treated with DMSO. The positive control is treated with Latrunculin B, an actin inhibitor.

3.2.2. Analysis

In some circumstances it is easy to identify derivatized natural product extracts that demonstrate activity against the human osteosarcoma cells. Biological activity is particularly apparent when the cells drastically change size and shape, or when the extract is particularly cytotoxic and there are very few cells alive to be imaged. In order to generate morphological profiles of the cells each image must be computationally analyzed on a cell by cell basis and then again on a well by well basis. This enables characterization of morphological changes that are not apparent to the naked or untrained eye. Unfortunately, this analysis falls outside the scope of this work, but will be implemented in the future to identify more derivatized natural product extracts that demonstrate biological activity against cancer cells. In preliminary analysis it was apparent that there were 157 instances of cells treated with the derivatized extracts that demonstrated changes in morphology noticeable to the naked eye. Of these 157, at least 85 were likely due to reagent toxicity or activity that was present in the underivatized extract and therefore not of immediate interest.

While these screening results demonstrate an exciting example of biological activity being created through these derivatizations one must be careful when analyzing the biological activity of the extracts based on this data. This is because there are instances of biological activity appearing consistently across different extracts derivatized in the same way and in the same fraction(s). This indicates that there is some sort of reagent or by-product of the reaction that is causing the biological activity and should resultingly be deprioritized for analysis. Additionally, as the fractionation was performed after the derivatization, and the transformations are predicted to alter the polarity of the compound, the derivatized compounds may appear in a different fraction from the underivatized compounds. Therefore, the results of the screening data should be compared to metabolomics data before any conclusions can be drawn about the biological activity created or destroyed by the transformations.

3.3. Metabolomics Analysis of the Derivatized Natural Product Library

3.3.1. Acquisition and Analysis of Mass Spectrometry Data

In order to gain insight into the identity of the natural products present in the underivatized and derivatized extracts a metabolomics study was needed. This was done by acquiring MS data on the library of 540 prefractionated derivatized natural product extracts on an IMS QToF Synapt G2Si mass spectrometer in triplicate.

The MS data was acquired on a UPLC-IMS system consisting of Waters Acquity UPLC and Waters Synapt G2Si (IMS QToF) spectrometer in ion-mobility spectrometry (IMS) mode. Each 1:400 dilution of extract was injected onto the UPLC and separated on a 7.6 minute gradient from 5% MeOH + 0.01% formic acid to 100% MeOH + 0.01% formic acid on a RP-UPLC (Waters HSS T3 1.8µ C18, ϕ 2.1 x 100 mm, 0.5 mL/min). Once this stage of chromatographic separation was complete the compounds were then ionized using electrospray ionization (ESI). In the IMS-QToF the ions first entered the Stepwave ion guide which eliminated neutrally charged contaminants before being transferred to the quadrupole which separated the ions based on their *m/z*. Ion Mobility Separation (IMS) then separated the ions based on their size, charge, and shape which enabled the separation of compounds with the same exact mass. This UPLC-IMS analysis enables analytes to be detected at concentrations as low as 100 ng/mL and with a ppm error of less than one. It also provides fragmentation data on every component of the mixture to provides insight about the structure of the analytes.[167]

Once the mass spectrometry data was collected in triplicate for all 540 derivatized natural product extracts the raw data was processed using MSeXpress 2.0. This program generated a consensus list of all the analytes it identified to be present in the samples. This list was then uploaded to the Global Natural Products Social Molecular Networking site (GNPS) to enable the data to be visualized in a more accessible way. GNPS analyzes the MS2 spectra associated with any given MS1 feature. Comparison across features in the MS1 spectra identifies MS1 features that have related fragmentation patterns in the MS2 spectra and clusters these features together. Simultaneously these spectra are compared to libraries of known compounds to annotate features when possible. Ultimately this program generates a molecular network where nodes (features in the MS1 spectra) are connected to other nodes based on related MS2 features. These related nodes are grouped together to form clusters of related

compounds. The molecular network generated from the derivatized natural product library can be seen in Figure 3.3. This molecular network contains 3255 nodes after removal of blanks and single unconnected nodes.

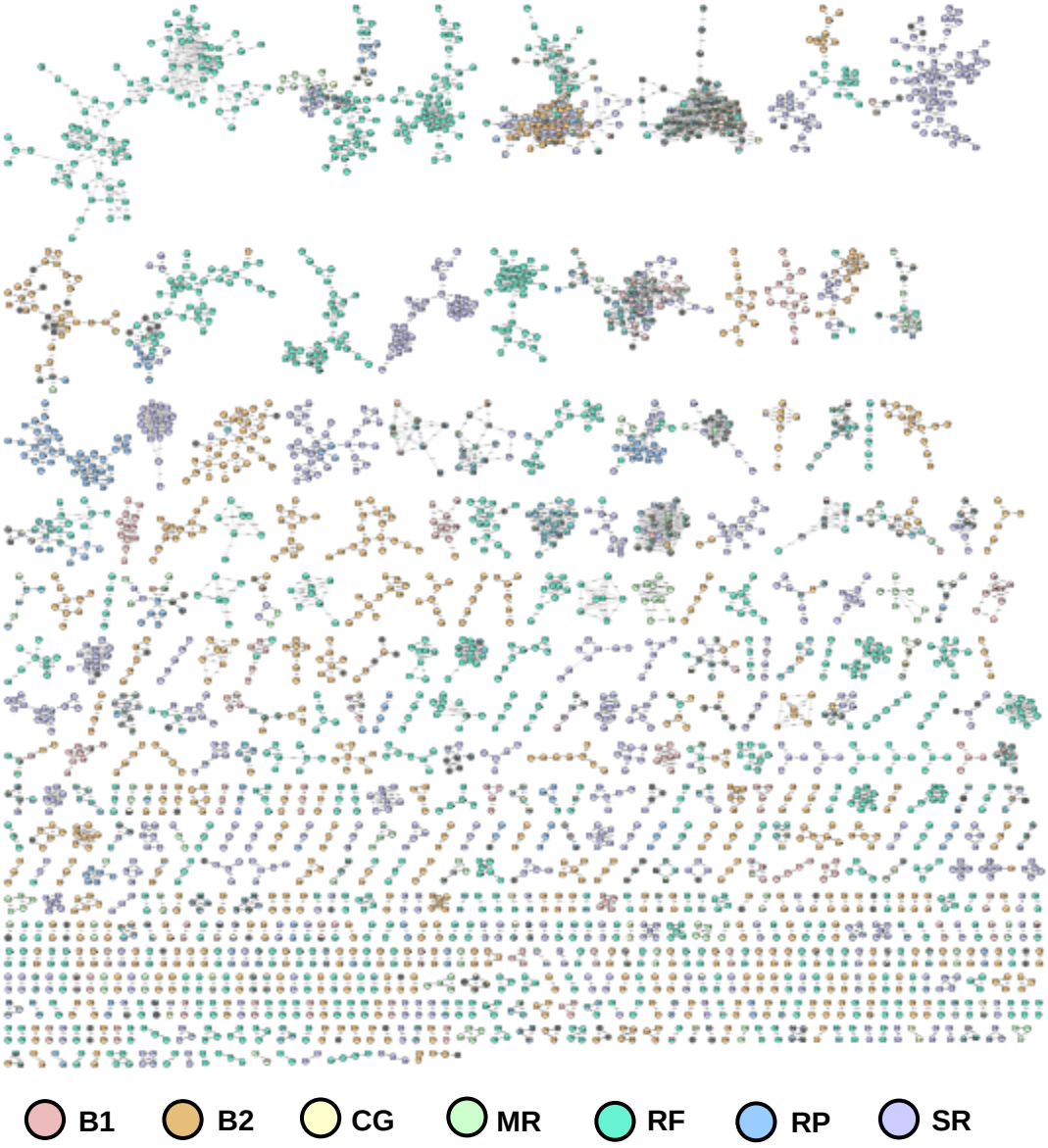


Figure 3.3 Molecular network generated by GNPS of all features in the derivatized natural product extract library.

Molecular networks generated by GNPS clustered related compounds together based on shared MS2 features.[168] Each cluster contains related nodes (MS1 features) that are colour coded based on the transformation performed on the extract that the feature was detected in. Pink is B1, orange is B2, yellow is CG, green in MR, teal is RF, blue is RP, and purple is SR. This molecular network contains 3255 nodes after the removal of blanks and single nodes.

The way in which GNPS produces its molecular network is key in the identification of natural product derivatives. Derivatized natural products either linked directly to their underivatized parent compound or were present within the same cluster due to their similar MS2 fragmentation patterns. These derivatized compounds were identified by mass differences characteristic to the transformations. These mass differences are recorded in Table 3.1. This does not mean that new nodes with no direct link to an identifiable underivatized natural product are not still of interest for analysis, but they were deprioritized.

Table 3.1: Mass Differences Characteristic of Each Transformation

Transformation	Mass Difference
B1	336.12
B2	76.03
CG	162.05
CP	81.94
MR	40.04
RF	226.02
RP	91.04
SR	230.02

Each node was converted into a pie chart that indicates the derivatization that was performed on the extract in which that MS1 feature was identified: Pink for B1, orange for B2, yellow for CG, green for MR, teal for RF, blue for RP, purple for SR, and grey for the underivatized extracts. Therefore, natural products that were present in the underivatized extract will appear as a multicoloured pie chart, since they were present in the same fraction of the extract across all derivatizations. Nodes that are one colour indicate a feature that was generated by a transformation. An example of this can be seen in Figure 3.4, where the node with a mass to charge ratio (*m/z*) of 467.209, present in the underivatized extract, was derivatized by RF, RP, and SR to produce compounds with a *m/z* of 693.232, 558.257, and 697.230 respectively along with other nodes that appear as a result of the transformations.

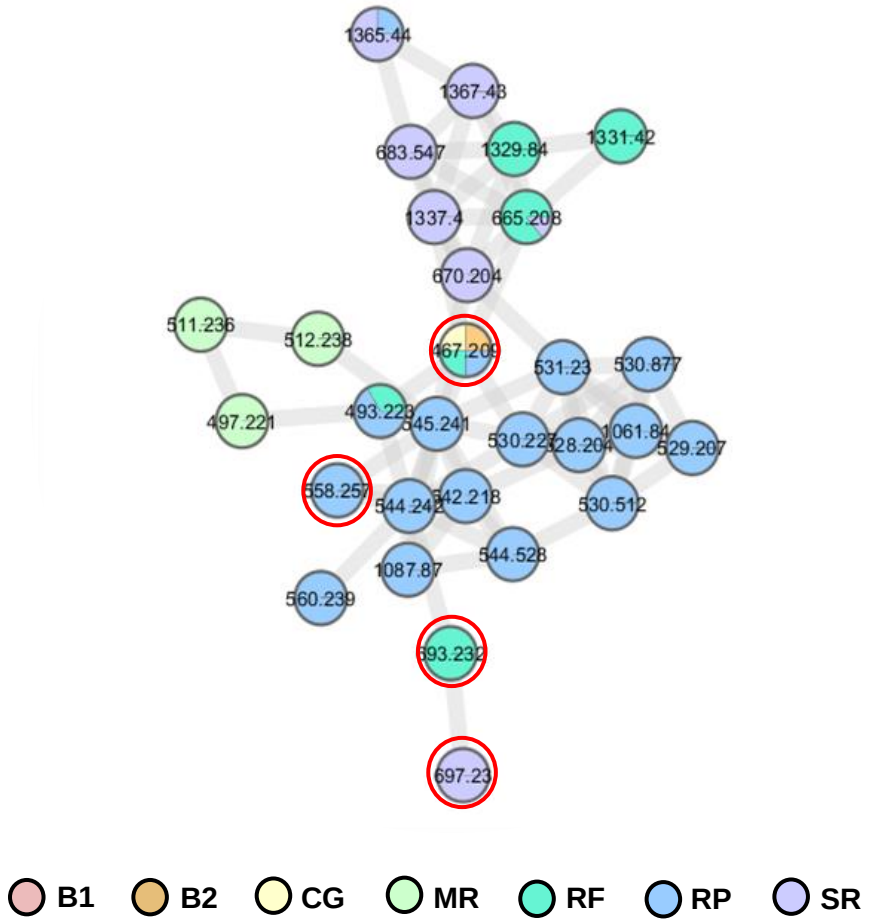


Figure 3.4 Cluster containing a natural product and its derivatives.

Cluster generated by GNPS containing a natural product (*m/z* 467.209) and three of its derivatives identified by characteristic differences in mass between the underivatized natural product and other nodes within the cluster. The mass differences of the transformations of interest in this node are as follows

Of interest is the node with a *m/z* of 697.23, corresponding to the natural product with a *m/z* = 467.209 being derivatized by the SR. The extract containing the underivatized natural product was RLUS_1398_D, and the extract containing the derivatized natural product was RLUS_1398_SR_D. Analysis of the images obtained in the cell painting assay of the osteosarcoma cells treated with these extracts shows an increase in the anticancer activity of the derivatized extract relative to the underivatized extract. Figure 3.5 shows the images of the cells treated with the underivatized extract RLUS_1398_D (A) and cells treated with the derivatized extract RLUS_1398_SR_D (B).

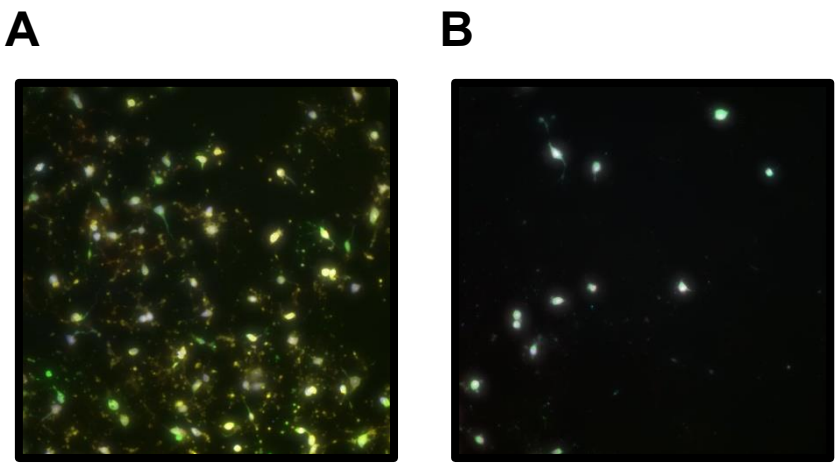


Figure 3.5 Osteosarcoma cells treated with underivatized RLUS_1398_D and derivatized RLUS_1398_SR_D extract.

Cell painting images of osteosarcoma cells treated with the underivatized (A) and derivatized (B) extract. As evidenced by the morphology and decreased number of cells that the derivatized extract (RLUS_1398_SR_D) is a more potent anticancer agent than the underivatized extract (RLUS_1398_D).

An additional 12 instances of natural products being derivatized were detected via GNPS analysis, which are listed in Table 3.2. These derivatizations were detected by looking for differences in mass characteristic of the transformations. However, it is very likely that some side reactions may have occurred, or the reactions may have produced a product that is no longer recognizable as related to the starting material in GNPS. In the latter situation this node would be in an unrelated cluster or removed during filtering. Instances such as these will be deprioritized for identification and isolation of the novel natural product derivatives.

Table 3.2: Identities and Locations of Derivatized Natural Products

Extract	*m/z* of Underivatized Natural Product	Extract	*m/z* of Derivatized Natural Product
RLUS_1775_D	461.327	RLUS_1775_B2_A-E	537.358
RLUS_1565_A-E	613.242	RLUS_1565_CG_A-C	789.310
RLUS_1398_D	467.209	RLUS_1398_RF_D	693.232
RLUS_1398_D	467.209	RLUS_1398_RP_C-D	558.257
RLUS_1398_D	467.209	RLUS_1398_SR_D	693.230
RLUS_1431_C-D	373.164	RLUS_1431_RF_D-E	613.167
RLUS_1431_A-C	349.190	RLUS_1431_RF_C	575.214
RLUS_1565_C-E	570.237	RLUS_1565_SR_B-E	800.258
RLUS_1431_B-E	361.164	RLUS_1431_SR_C-E	591.188
RLUS_1775_A-D	472.306	RLUS_1775_SR_D	702.329
RLUS_1775_A-D	454.294	RLUS_1775_SR_D-E	684.318
RLUS_1775_A-E	461.327	RLUS_1775_SR_D-E	691.353
RLUS_1431_C-D	373.164	RLUS_1431_SR_A-C	603.186

3.3.2. Isolation and Identification of the Biologically Active Natural Product and Natural Product Derivative.

GNPS identified the derivatized natural product with *m/z* 467.20 as staurosporine. Staurosporine is an alkaloid natural product that was first isolated in 1977 by Omura et. al. from a *Streptomyces sp*.[169] Since its isolation staurosporine has proven to be potent inhibitor of many kinases especially protein kinase C causing apoptosis and cell death. This natural product has been previously reported to demonstrate anticancer activity, which is consistent with the results observed in Cell Painting.[170–172] However, the derivatized staurosporine found in the derivatized natural product library may prove to be a more potent anticancer agent than its parent compound. Isolation of this new derivative and quantification of its biological activity will prove whether this is the case. However, re-screening of the pure staurosporine derivative falls outside of the scope of the research presented in this thesis.

In order to confirm that the compound identified in the extract was staurosporine a commercially available staurosporine standard was purchased. The structure of the standard was confirmed using ^{1}H and ^{13}C. The identity of the compound in the extract RLUS_1398_SR_D was then confirmed to be staurosporine by a co-injection on a UPLC-MS. First the extract RLUS_1398_D and staurosporine were independently analyzed on the same gradient on the UPLC-MS and shown to have the same retention time on the UPLC (2.58 mins) and the same exact mass (*m/z* = 467.2086). Then a mixture of the staurosporine standard and RLUS_1398_D was prepared and analyzed again using the same gradient on the UPLC-MS. The EIC for *m/z*

467.20 (calculated mass of staurosporine) showed only one peak at the expected retention time of 2.57 mins that had a *m/z* of 467.2086, which is consistent with staurosporine. This proved that the compound in the extract was indeed staurosporine as shown in Figure 3.6.

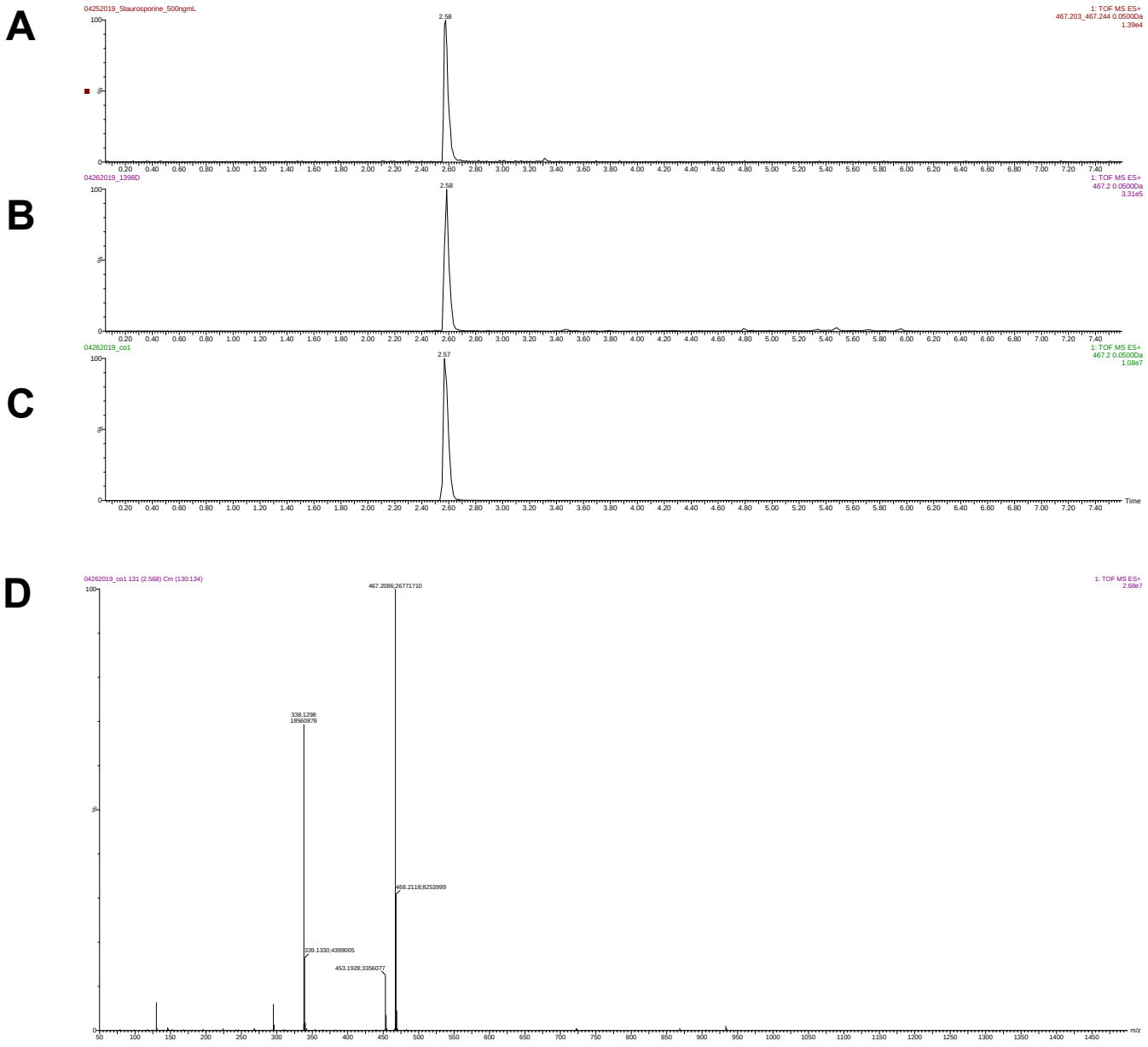


Figure 3.6 Co-injection of staurosporine and the compound of interest in extract RLUS_1398_D.

EIC (*m/z* = 467.20) of the Co-injection (C) of staurosporine (A) and extract RLUS_1398_D (B) and the mass spectrum of the co-injection at 2.58 minutes (D) demonstrating that the compound of interest in the extract has the same retention time (2.58 min) and exact mass (*m/z* = 467.2086) as staurosporine.

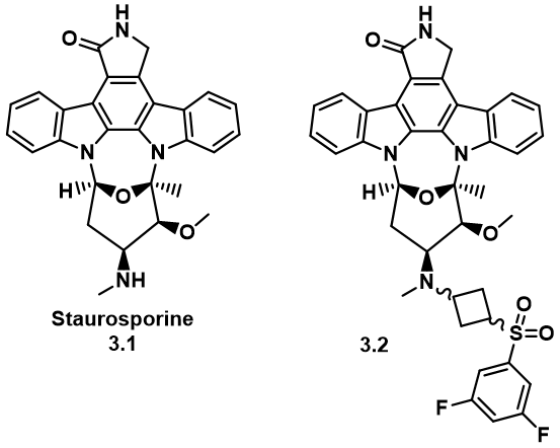


Figure 3.7 Staurosporine and the natrual product derivatized via SR.

Next the staurosporine standard was derivatized by the strain release amination. There is only one secondary amine present on staurosporine, which is the functional group targeted by SR. Therefore, the structure of the staurosporine derivative identified in extract RLUS_1398_SR_D was predicted to be compound **3.2** (Figure 3.7), which is synthesized as a mixture of four isomers. This mixture of isomers was able to be separated into its diastereomers by HPLC (cis and trans on the cyclobutane ring) and characterized by ^{1}H and ^{13}C NMR and high-resolution mass spectrometry (HRMS). UPLC-MS characterization of the synthesized compounds showed a retention time of 4.00 and 4.05 minutes for compound A and B respectively and both were found to have a similar *m/z* of 697.2298 and 697.2297 (0.1 ppm error). The extract RLUS_1398_SR_D was also run on the same UPLC method and after EIC of 697.22 (calculated $[M+H]^{+}$ of the derivatized staurosporine) it was observed that there were two peaks at 4.00 and 4.05 minutes with the same *m/z* of 697.2293, which is consistent with the synthesized staurosporine derivatives. Co-injection of the standards with extract RLUS_1398_SR_D again showed that there were two peaks again at 4.00 and 4.05 minutes with the same *m/z* of 697.2293. This proved that the compound identified in RLUS_1398_SR_D were the anticipated staurosporine derivatives as shown in Figure 3.8.

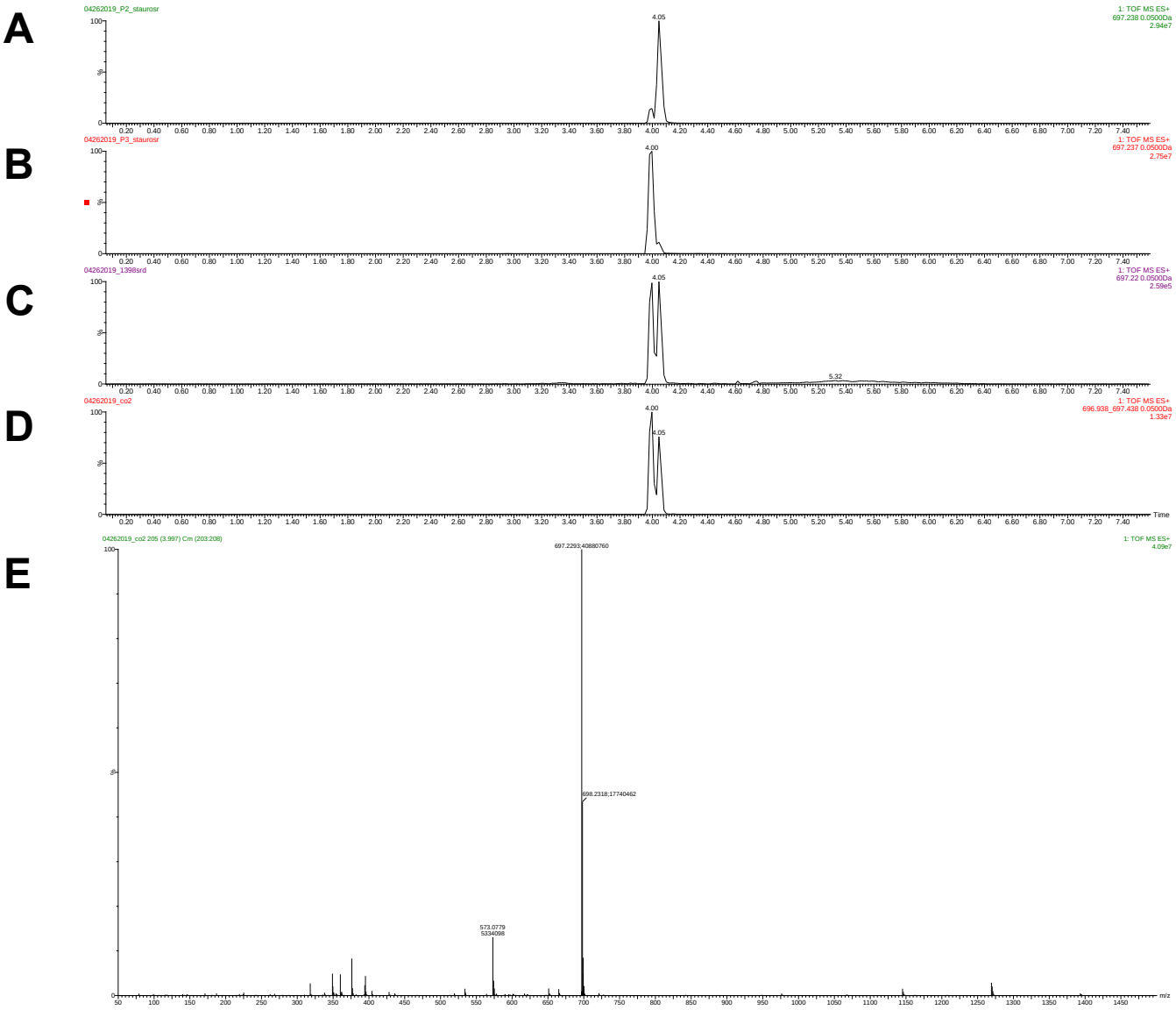


Figure 3.8 Co-injection of staurosporine derivatives and the compound of interest in extract RLUS_1398_SR_D

EIC (*m/z* = 697.22) of the Co-injection (D) of staurosporine derivatives **3.2** (A and B), and extract RLUS_1398_SR_D (C) the mass spectrum of the co-injection from 4.00 to 4.05 minutes (E) demonstrating that the compounds of interest in the extract have the same retention time (4.00 and 4.05 min) and exact mass (*m/z* = 697.2293) as the anticipated staurosporine derivatives.

3.4. Summary

High-throughput screening of the 540 samples in the derivatized natural product extract library was performed against clinically relevant bacterial pathogens (BioMAP) and in a cell-based fluorescent imaging assay Cell Painting. The results from these screens indicate that there was an assortment of new biologically active compounds generated by these transformations.

In order to characterize and identify some components of these extracts a metabolomics study was performed. HRMS data was acquired in triplicate for the whole extract library, processed using MSeXpress 2.0, and uploaded to GNPS. The output of this analysis linked compounds together based on shared structural characteristics. Analysis of the clusters identified 13 instances of the derivatizations generating natural product-like compounds within the extracts.

One such instance, the derivatization of staurosporine via SR, appears to have increased anti-cancer activity relative to the extract containing staurosporine.

3.5. Experimental

3.5.1. General Considerations

All reactions were carried out using commercial reagents and solvents that were used as received. Reagents and solvents were purchased from Sigma Aldrich, Fisher Scientific, LC Laboratories, and/or Alfa Aesra. Flash chromatography was carried out with Geduran Si60 silica gel (Merck). HPLC was performed on Agilent 1200 series HPLC and HPLC-MS (ESI) instruments using Synergi Fusion-RP analytical and semi-prep columns, and a Kinetix C18 analytical column (Phenomenex). UPLC-MS experiments were performed using an Acquity UPLC equipped with the Synergi-G2Si IMS-QTOF mass spectrometer (Waters). NMR spectra were recorded using methanol-d_4 (MeOD), or chloroform-*d* ($CDCl_3$). Signal positions (δ) are given as parts per million (ppm) NMR spectra were recorded on a Bruker Avance 600 (600MHz) equipped with a QNP.

3.5.2. Preparation, Isolation, and Characterization of Staurosporine Derivatives

General Procedure

1-((3,5-Difluorophenyl)sulfonyl)bicyclo[1.1.0]butane (3 mg, 0.014 mmol) was added to a solution of LiCl (2 mg, 0.039 mmol) and staurosporine (6 mg, 0.013 mmol) in DMSO (40 µL). The reaction was stirred for 20 hours at room temperature before water was added and the mixture was extracted with EtOAc. The organic layer was dried with $MgSO_4$, filtered, and concentrated to yield crude product. HRMS *m/z* $[M+H]^+$ 697.2293 (calcd for $C_{38}H_{35}F_2N_4O_5S^+$,697.2291).

The staurosporine derivatives (**3.2**) were separated by RP-HPLC (Phenomenex Kinetex 5µ C18, 100 Å, ϕ 4.6 x 250 mm, 1 mL/min) with isocratic elution from 90% MeOH + 0.02% formic acid to afford **3.2** as a mixture of 4 stereoisomers. HRMS *m/z* $[M+H]^+$ 697.2293 (calcd for $C_{38}H_{35}F_2N_4O_5S^+$,697.2291).

Derivative A:

^{1}H NMR: (500 MHz, DMSO-d_6) δ 9.28 (d, J = 8.0 Hz, 1H), 8.05 (d, J = 7.8 Hz, 1H), 8.03 (d, J = 8.5 Hz, 1H), 7.76 (m, 1H), 7.65 (m, 2H), 7.56 (d, J = 8.2 Hz, 1H), 7.47 (m, 2H), 7.34 (t, J = 7.4 Hz, 1H), 7.28 (t, J = 7.9 Hz, 1H), 6.85 (dd, J = 9.3, 4.1 Hz, 1H), 4.98 (s, 2H), 4.16 (s, 1H), 3.90 (m, 1H), 3.28 (m, 2H), 2.73 (m, 1H), 2.53 (m, 2H), 2.39 (s, 3H), 2.34 (s, 3H), 2.22 (m, 4H), 2.03 (s, 3H).
See Figure 5.39 in Appendix

^{13}C NMR: (150 MHz, DMSO-d_6) δ 172.0, 138.1, 136.3, 132.3, 129.9, 126.0, 125.7, 125.2, 125.1, 123.8, 122.6, 120.3, 119.4, 114.7, 113.9, 111.9, 109.1, 94.9, 82.8, 82.6, 58.8, 53.7, 51.1, 48.7, 45.4, 32.5, 28.2, 28.1, 27.2.
See Figure 5.40 in Appendix

^{19}F NMR: (565 MHz, DMSO-d_6) δ -105.7
See Figure 5.41 in Appendix

Derivative B:

^{1}H NMR: (500 MHz, DMSO-d_6) δ 9.28 (d, J = 7.9 Hz, 1H), 8.04 (m, 2H), 7.71 (m, 1H), 7.66 (m, 2H), 7.58 (d, J = 8.1 Hz, 1H), 7.48 (m, 2H), 7.35 (t, J = 7.0 Hz, 1H), 7.29 (t, J = 7.8 Hz, 1H), 6.85

(dd, J = 9.0, 4.1 Hz, 1H), 4.97 (s, 2H), 4.13 (s, 1H), 4.04 (m, 1H), 3.55 (m, 1H), 3.25 (m, 1H), 2.70 (m, 2H), 2.53 (m, 2H), 2.42 (s, 3H), 2.38 (s, 3H), 2.28 (m, 4H), 1.98 (s, 3H).
See Figure 5.42 in Appendix

^{13}C NMR: (150 MHz, DMSO-d_6) δ 172.0, 138.3, 136.3, 132.3, 129.9, 126.0, 125.7, 125.2, 125.1, 123.8, 122.6, 121.5, 120.3, 119.4, 119.3, 114.7, 113.9, 113.2, 112.1,111.9, 109.9, 94.8, 83.0, 82.7, 58.9, 53.6, 53.4, 51.3, 45.4, 32.6, 28.2, 27.7, 27.0, 26.9.
See Figure 5.43 in Appendix

^{19}F NMR: (565 MHz, DMSO-d_6) δ 105.8
See Figure 5.44 in Appendix

Chapter 4.

Conclusion and Outlook

To address the critical need for new methods that expand natural product chemical space to identify novel compounds with biological applications a new advancement to existing synthetic methods of producing natural product-like compounds from natural product extracts was developed. A series of high yielding synthetic derivatizations with high functional group tolerance were employed to transform functional groups common to natural products into functionalities common to other natural products or drugs. These transformations include reactions that increase the aromaticity of the derivatized compounds through reactions between nucleophiles and benzynes (B1, B2), a chemical glycosylation (CG), a dichlorocyclopropanation (CP), morpholine ring formation (MR), reductive aminations (RF, RP), and a strain release amination (SR).

These transformations were used to derivatize 10 natural product extracts obtained from marine actinomycetes, which after fractionation lead to the generation of a library of 540 derivatized natural product extracts. These extracts were screened against a panel of clinically relevant bacterial pathogens in BioMAP and against osteosarcoma cells in the Cell Painting assay.[149,159] Screening results indicate that there is a marked difference in the antimicrobial activity of the extracts after derivatization. Additionally, there were many instances of the derivatized extracts displaying anticancer activity against the osteosarcoma cells imaged in cell painting.

In order to prioritize extracts for further analysis a metabolomics study of all the compounds present in the extracts was performed. This was done by acquiring HRMS data on all samples in triplicate on an IMS QToF mass spectrometer. The data was processed with MSeXpress 2.0 and GNPS to generate a molecular network of all the features present across the MS1 spectrum. These features were clustered together based on having related structures, which was determined by features in the MS2 spectrum. Searching for characteristic mass differences within the clusters identified natural products and their derivatives.

Comparison of the metabolomics data and high throughput screening data allowed for the identification of a staurosporine derivative that had increased activity against osteosarcoma

cells compared to the activity of the underivatized staurosporine. Co-injection of the staurosporine with the underivatized extract RLUS_1398_D along with HRMS, ^{1}H NMR, and ^{13}C NMR data proved that the identity of the underivatized natural product was staurosporine. Subsequent derivatization and purification yielded expected staurosporine derivative along with co-injection of the compound with the derivatized extract RLUS_1398_SR_D, HRMS, ^{1}H NMR, and ^{13}C NMR data proved the identity of the natural product derivative that is believed to be responsible for the increased anticancer activity observed in the cell painting screen.

Ultimately, further screening efforts and isolation of natural product derivatives will enable the identification of more derivatized natural products that can be used as a starting point for the development of new drug leads. Additionally, this platform will enable the generation of a much larger library of natural product derivatives that represent an even larger expansion of natural product chemical space. These derivatized natural product libraries will enable the identification of numerous natural product-like compounds with biological activity that can be applied to problems in human health and biotechnology.

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Appendix

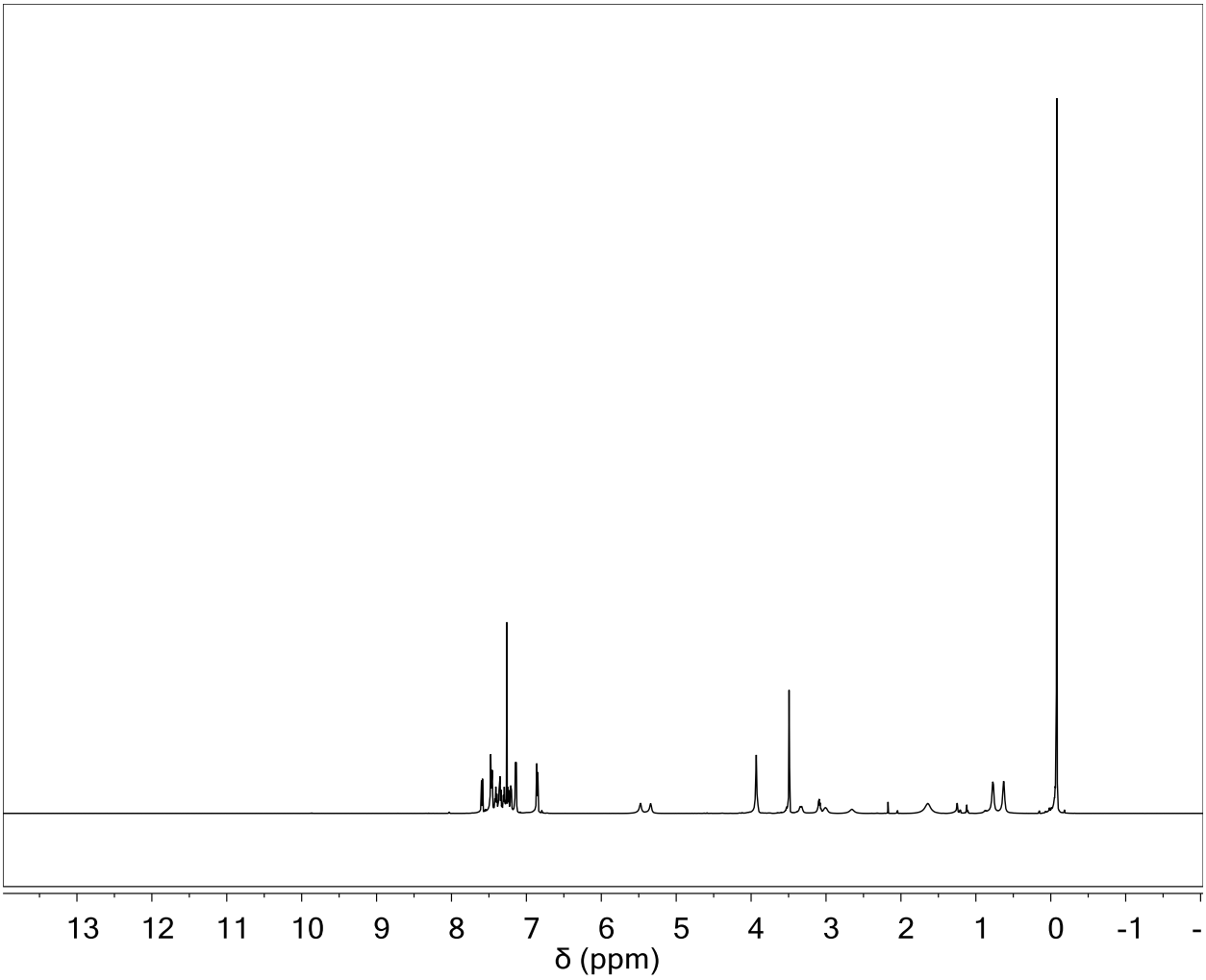


Figure 5.1 ^{1}H NMR of compound **2.1**

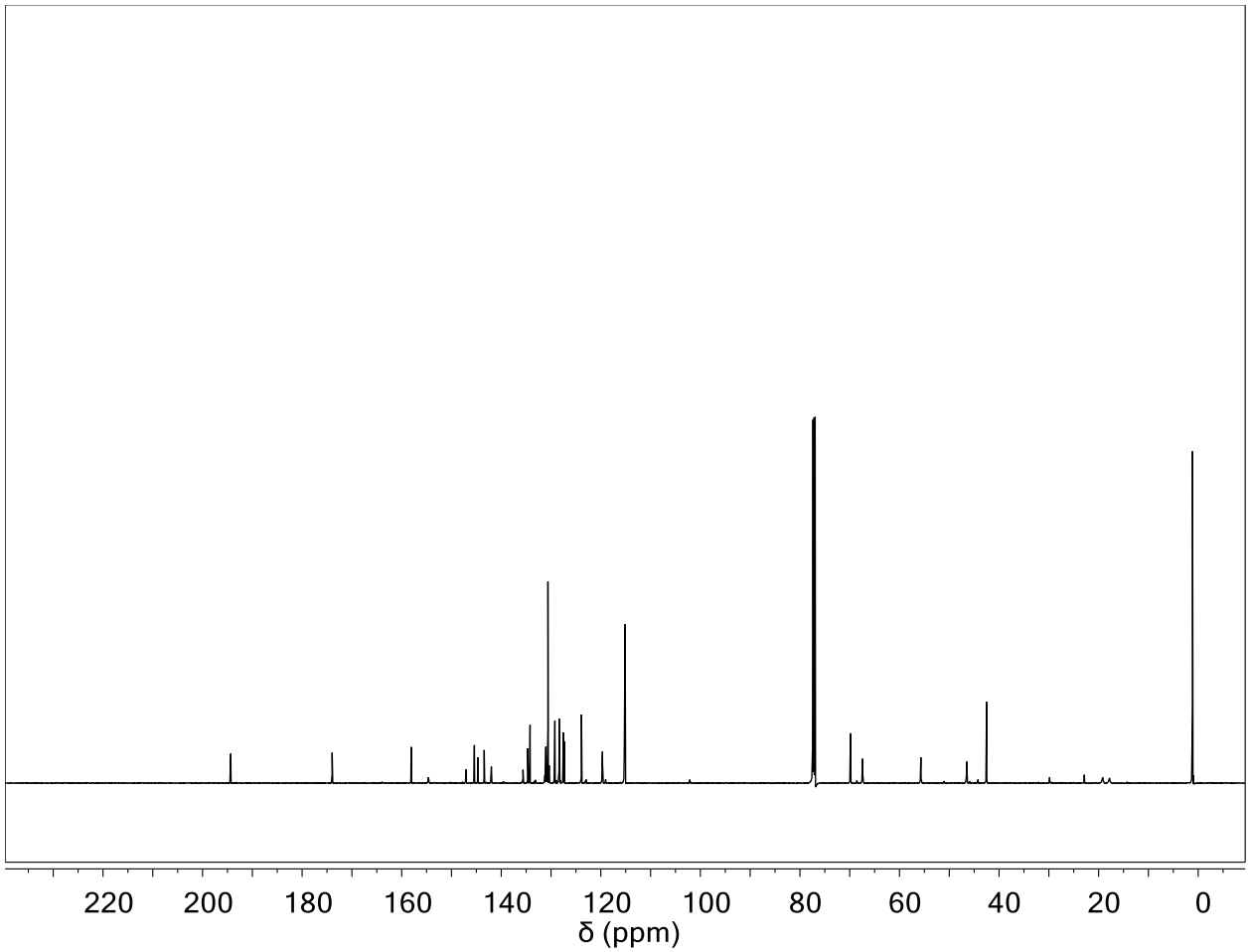


Figure 5.2 ^{13}C NMR of compound **2.1**

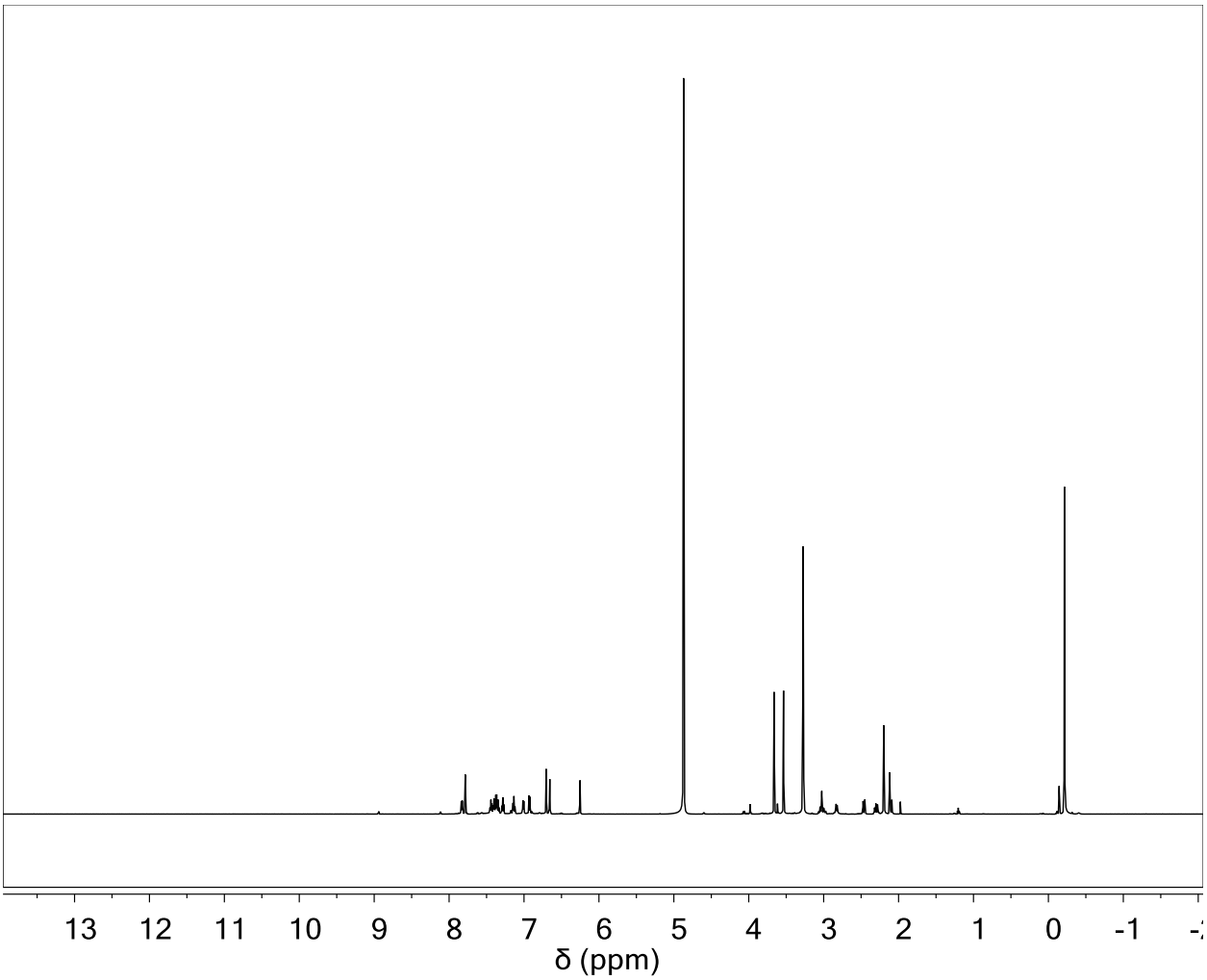


Figure 5.3 ^{1}H NMR of compound **2.2**

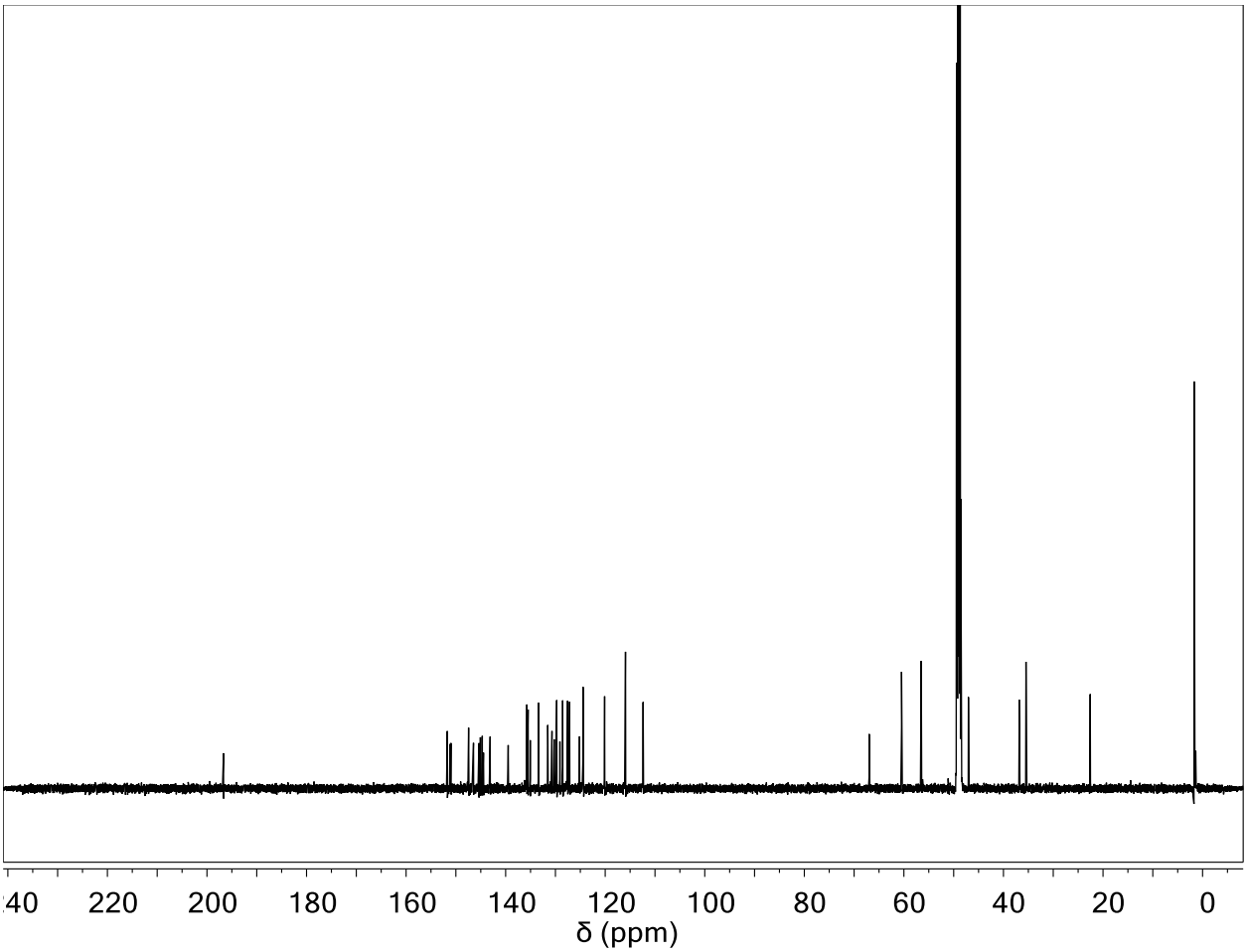


Figure 5.4 ^{13}C NMR of compound **2.2**

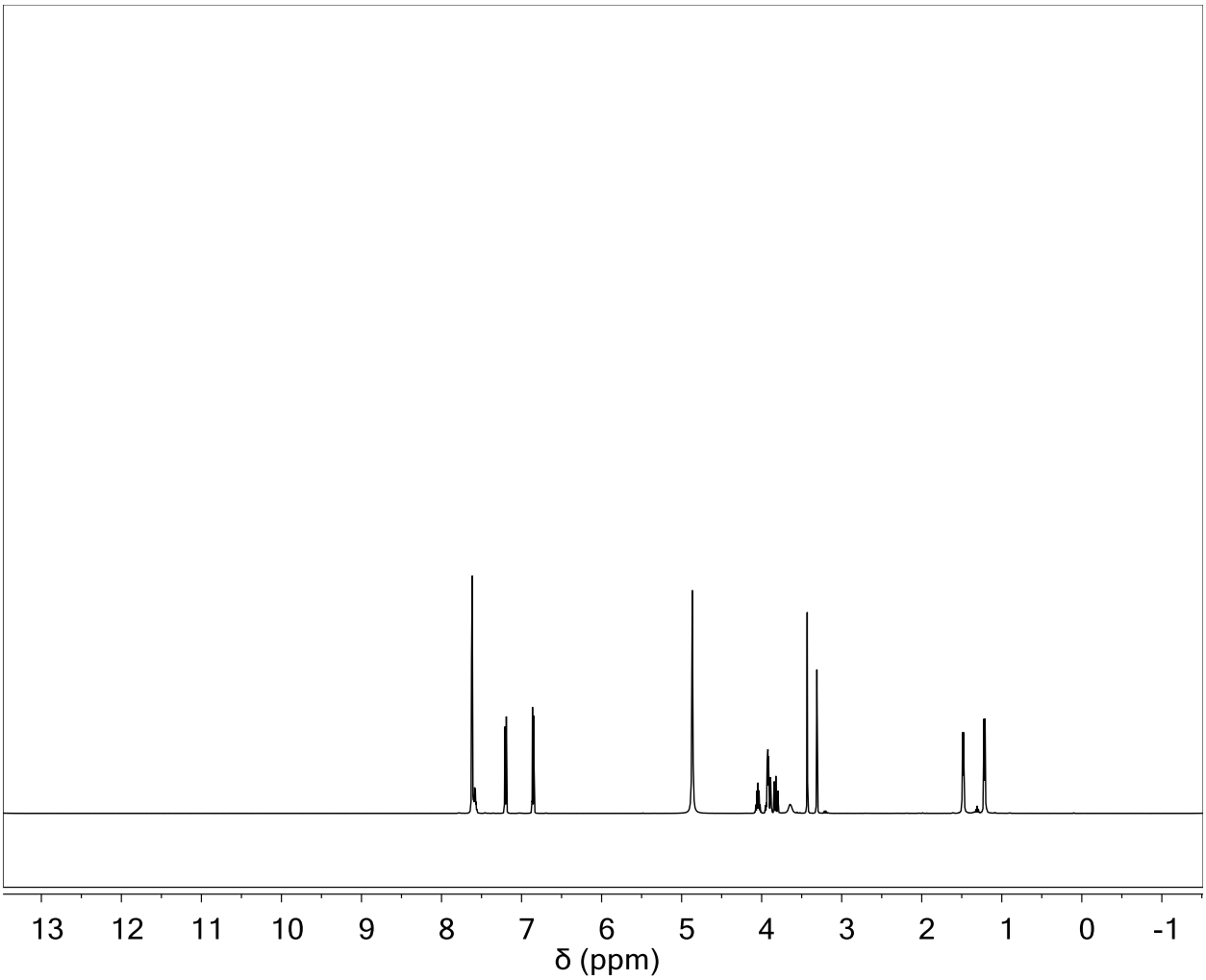


Figure 5.5 1H NMR of compound **2.3**

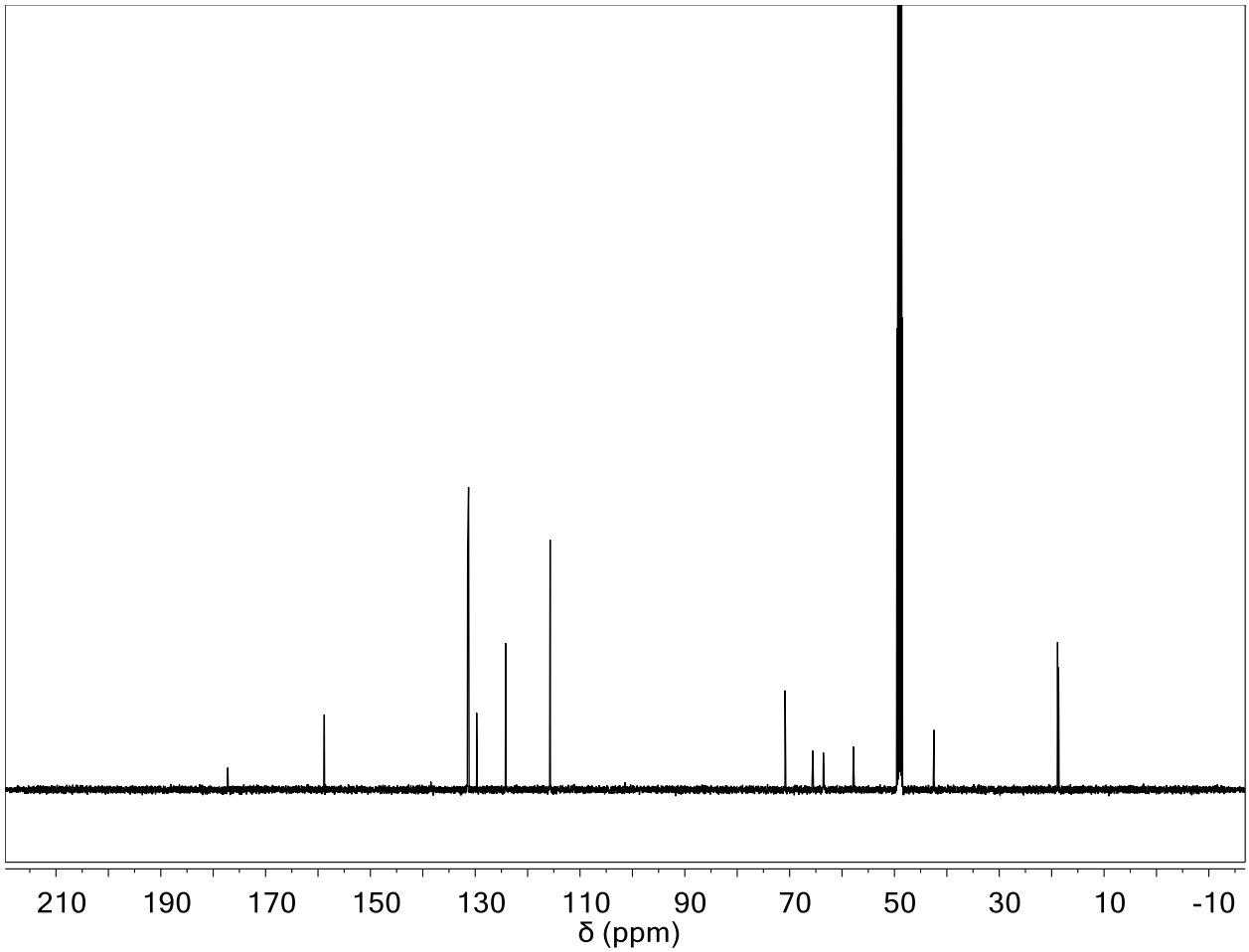


Figure 5.6 13C NMR of compound **2.3**

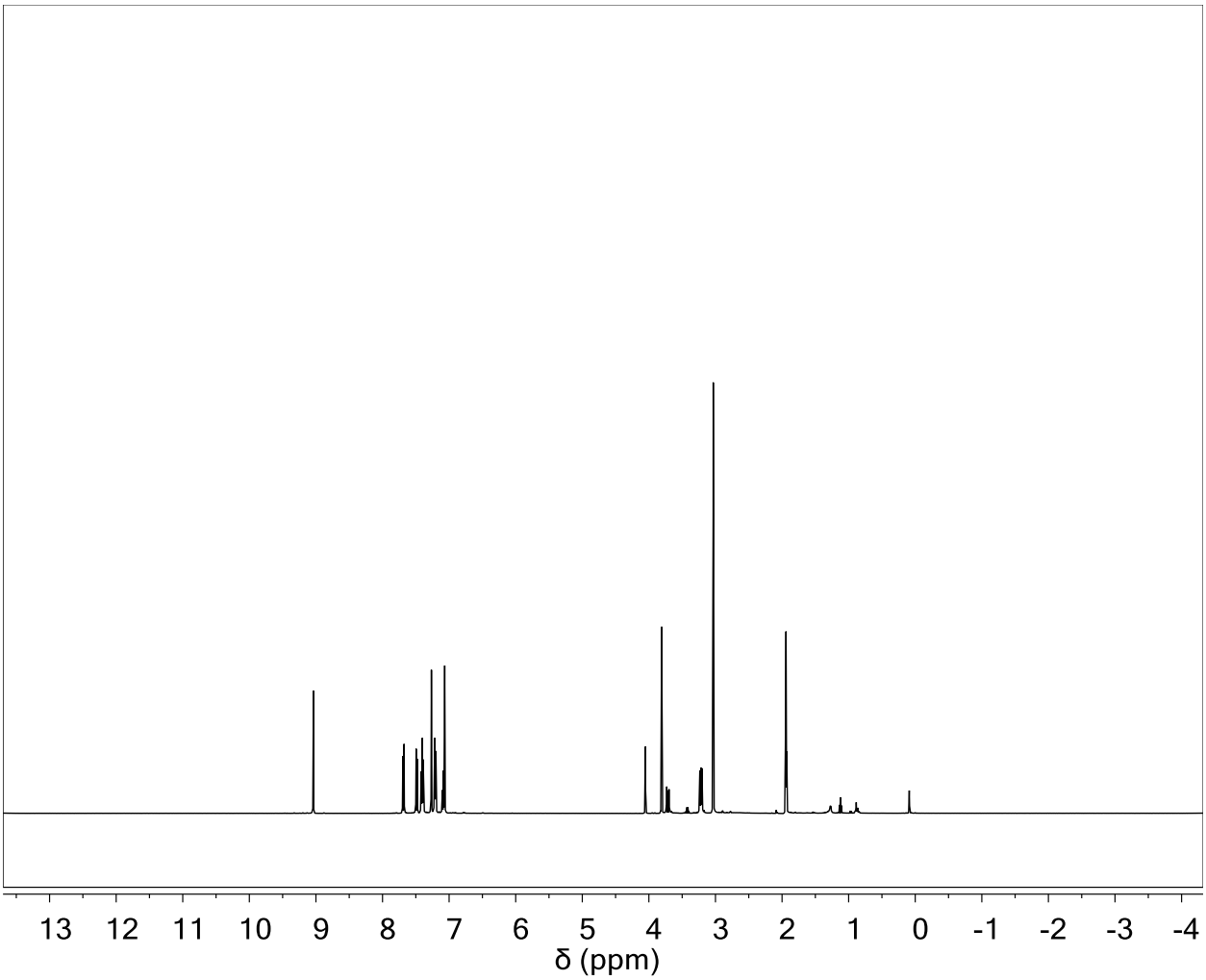


Figure 5.7 ^{1}H NMR of compound **2.4**

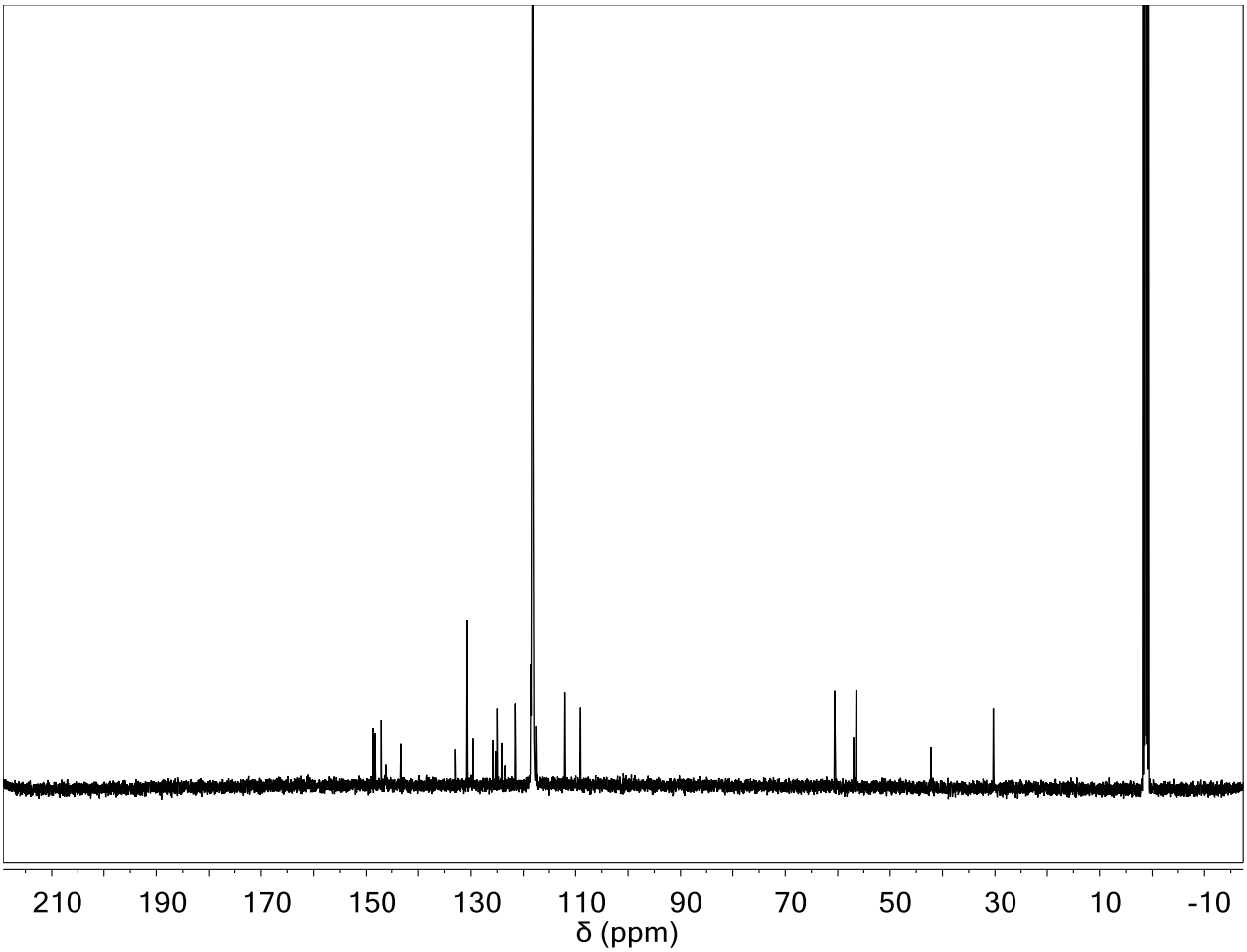


Figure 5.8 ^{13}C NMR of compound **2.4**

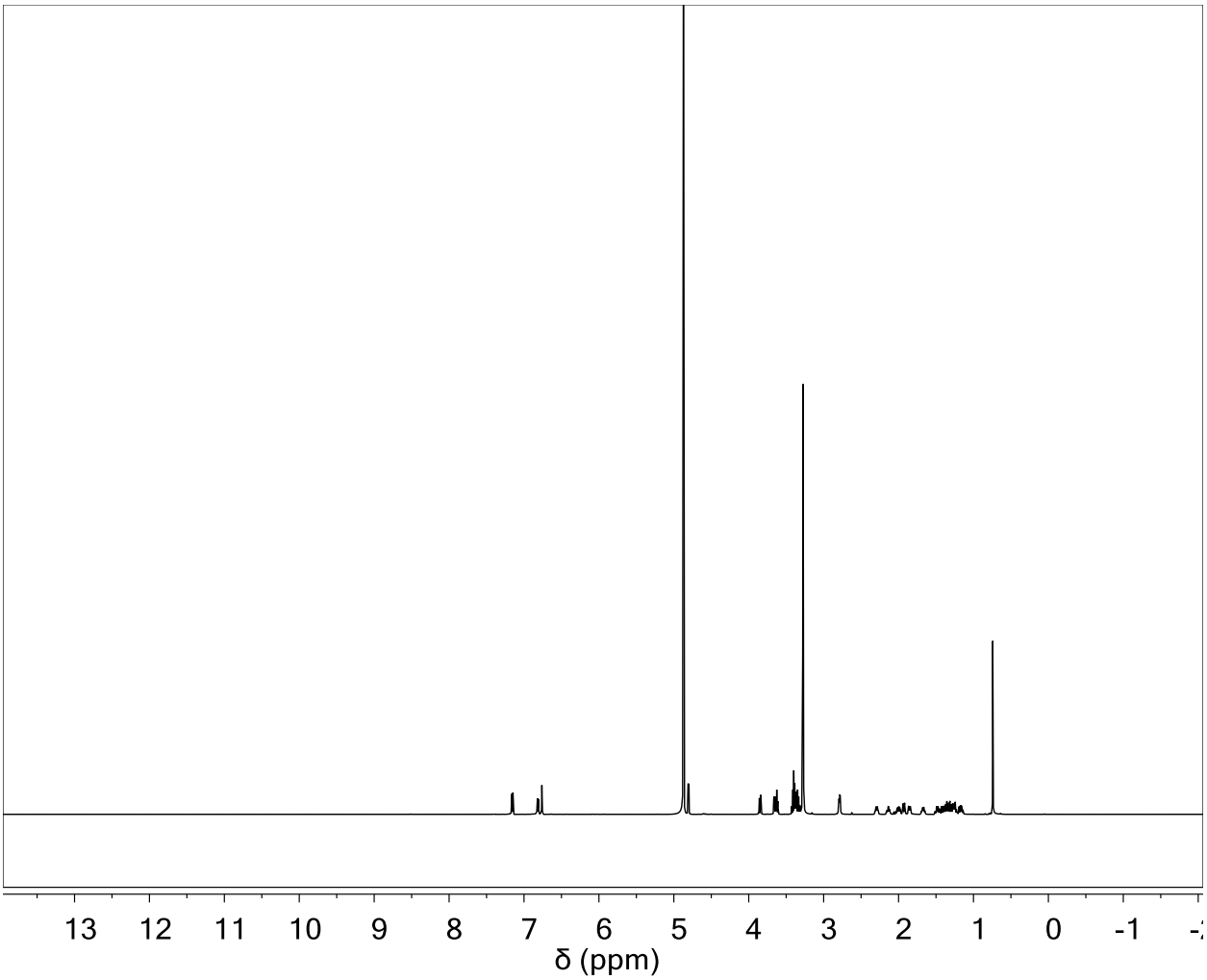


Figure 5.9 ^{1}H NMR of compound **2.5**

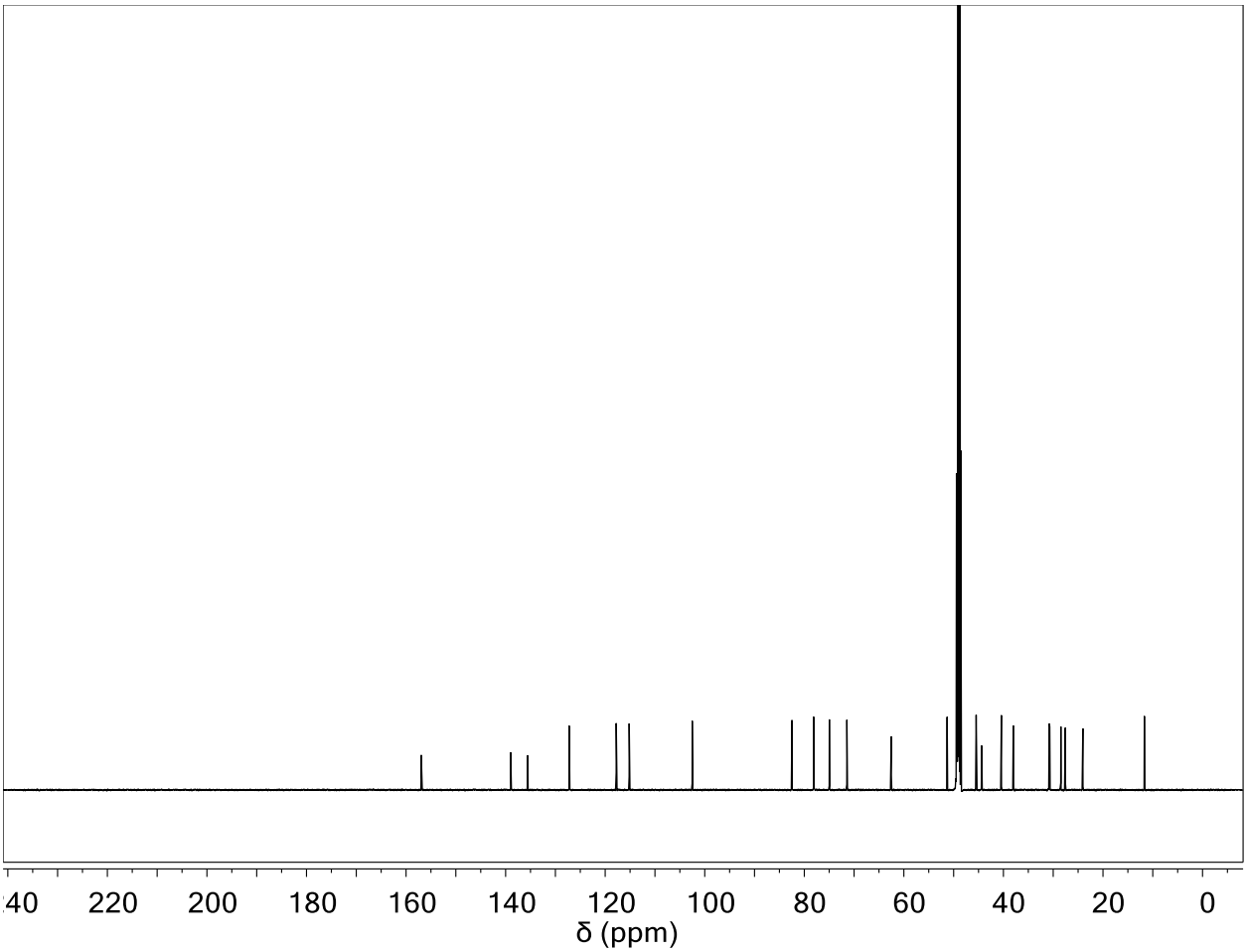


Figure 5.10 ^{13}C NMR of compound **2.5**

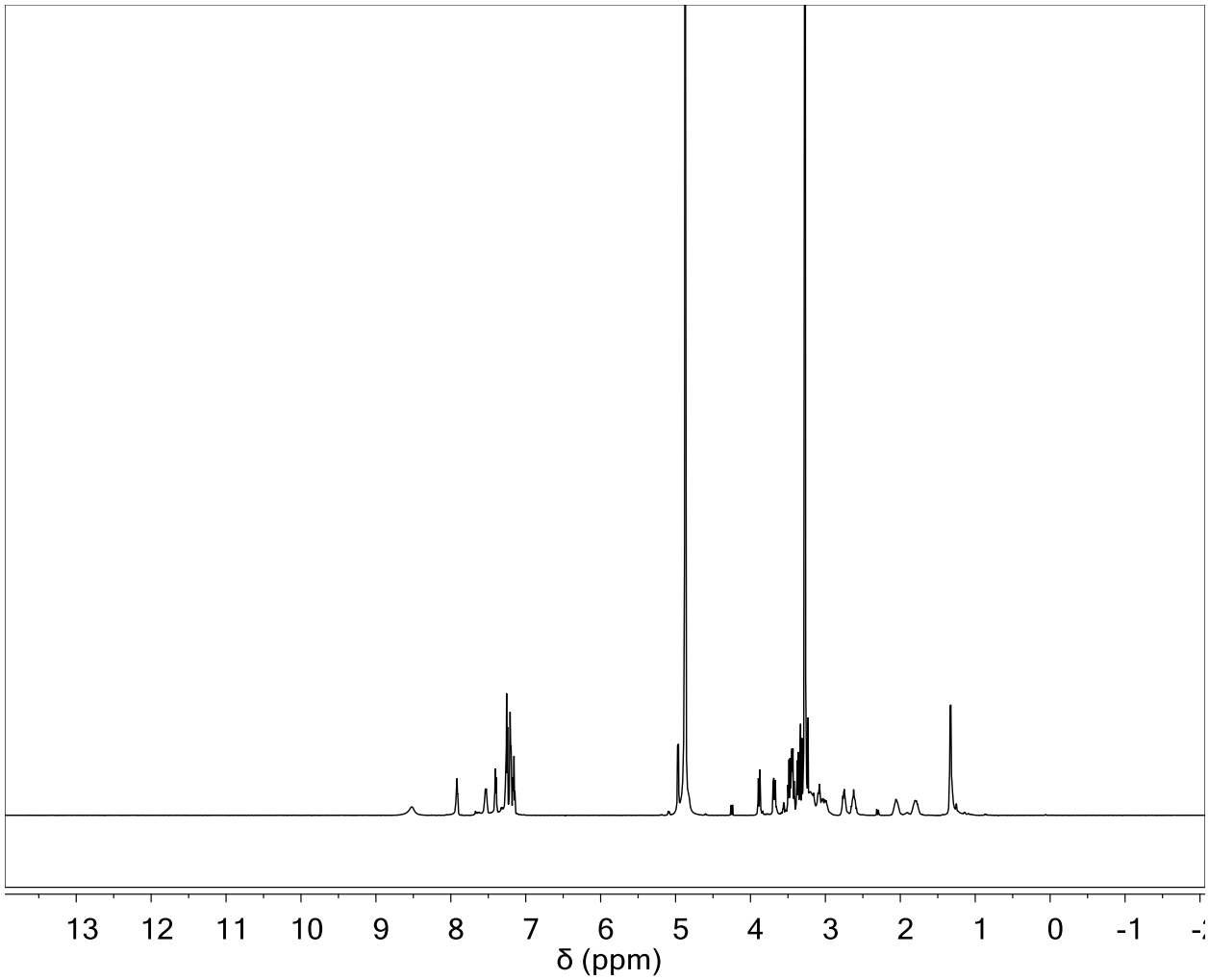


Figure 5.11 ^{1}H NMR of compound **2.6**

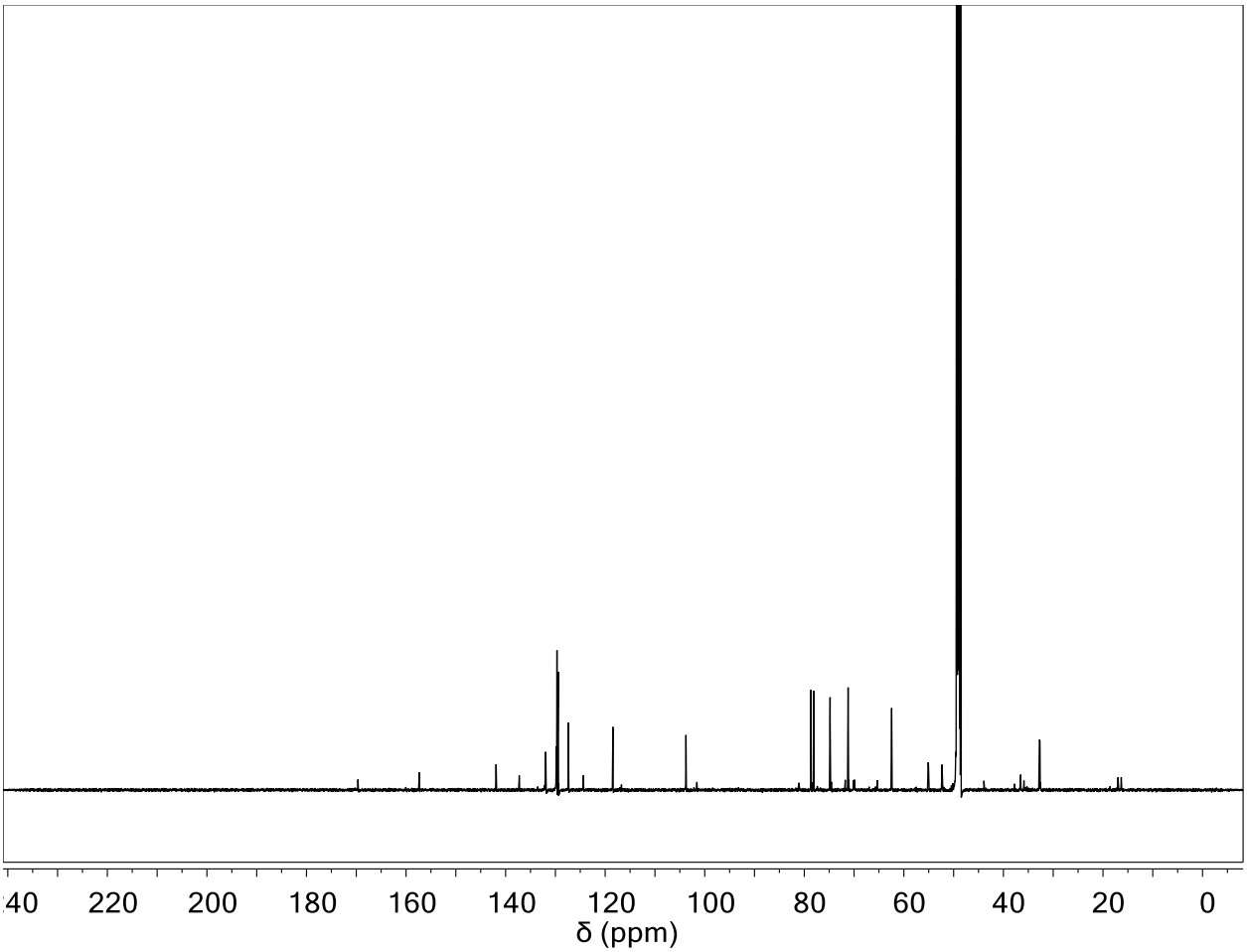


Figure 5.12 ^{13}C NMR of compound **2.6**

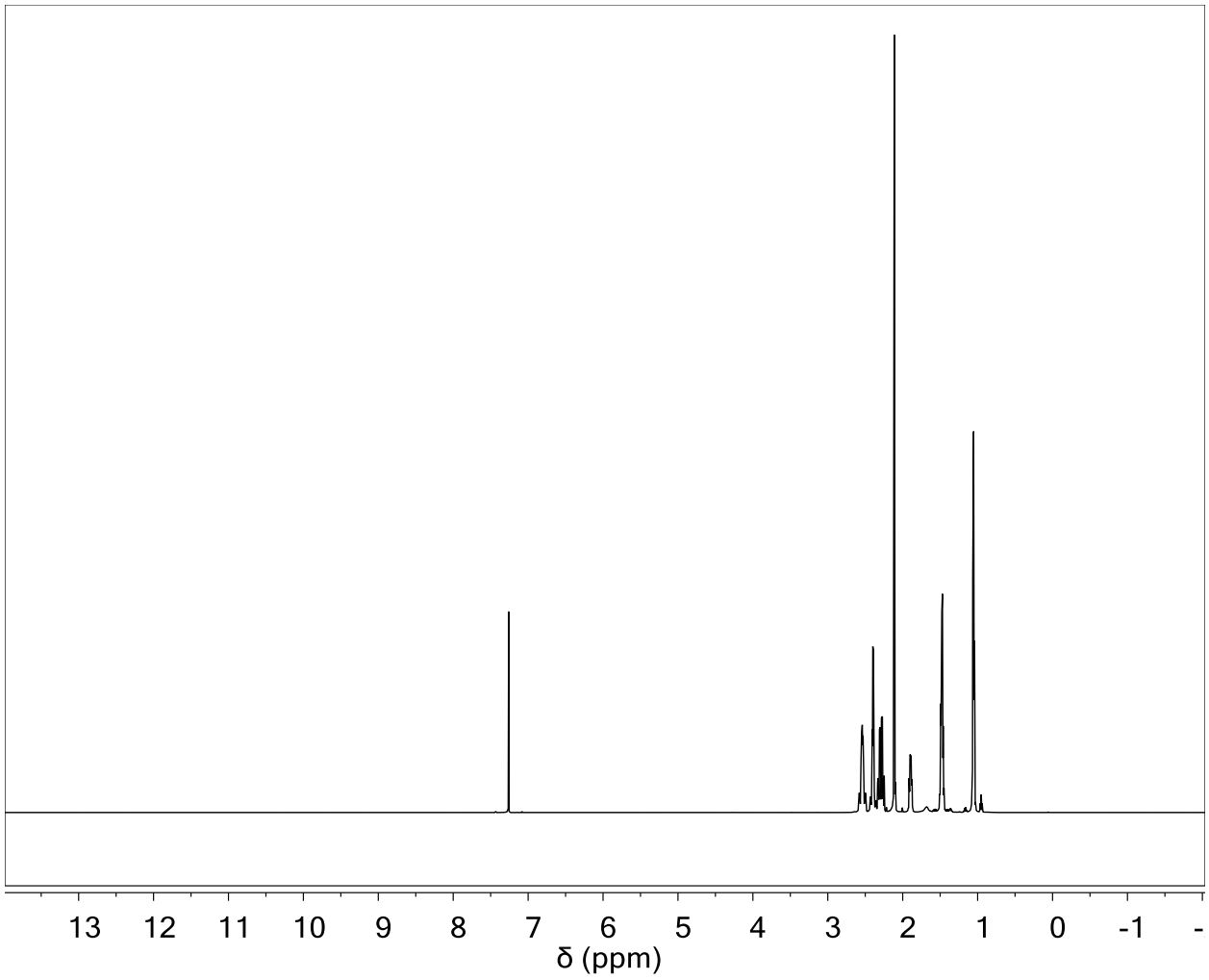


Figure 5.13 ^{1}H NMR of compound **2.7**

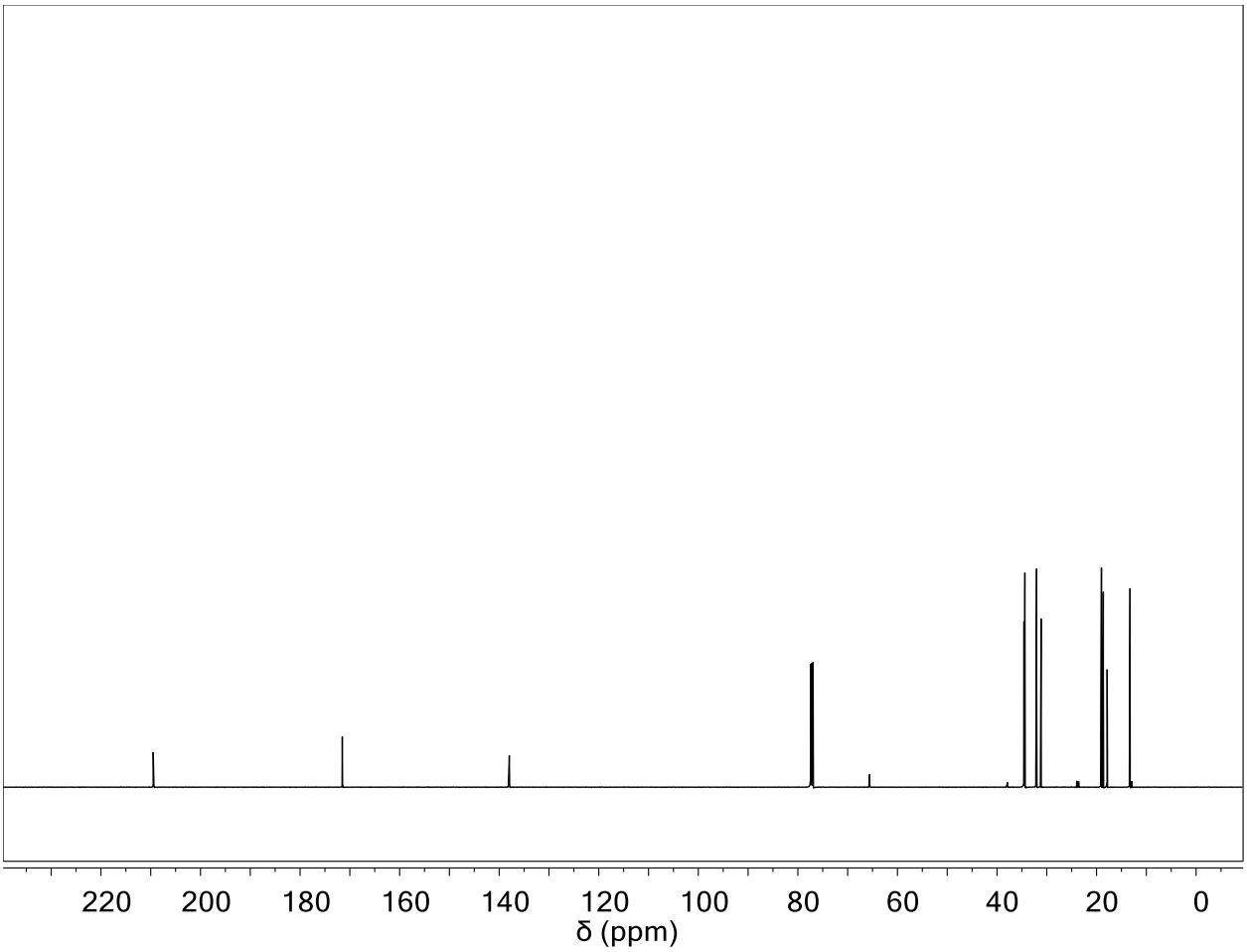


Figure 5.14 ^{13}C NMR of compound **2.7**

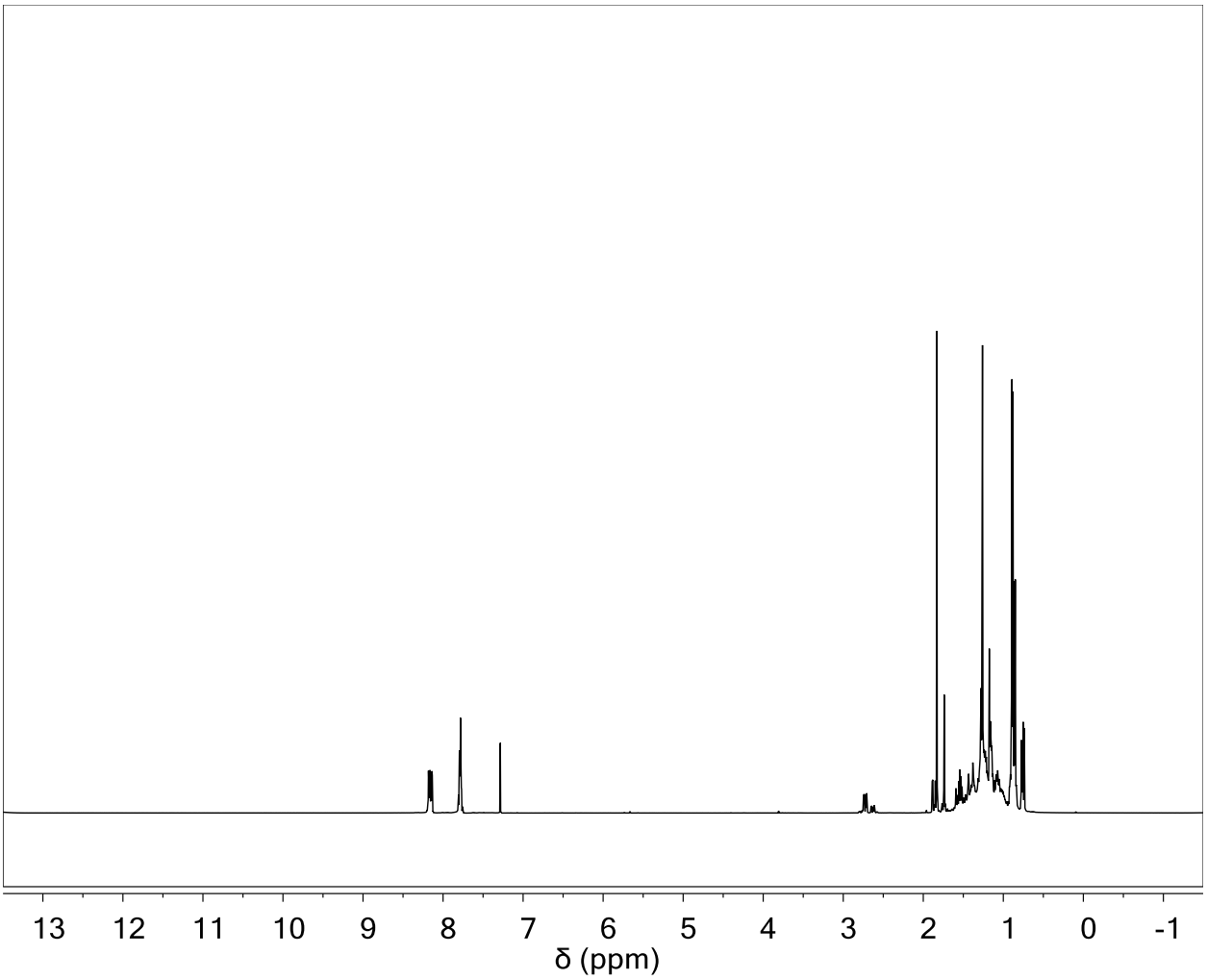


Figure 5.15 ^{1}H NMR of compound **2.8**

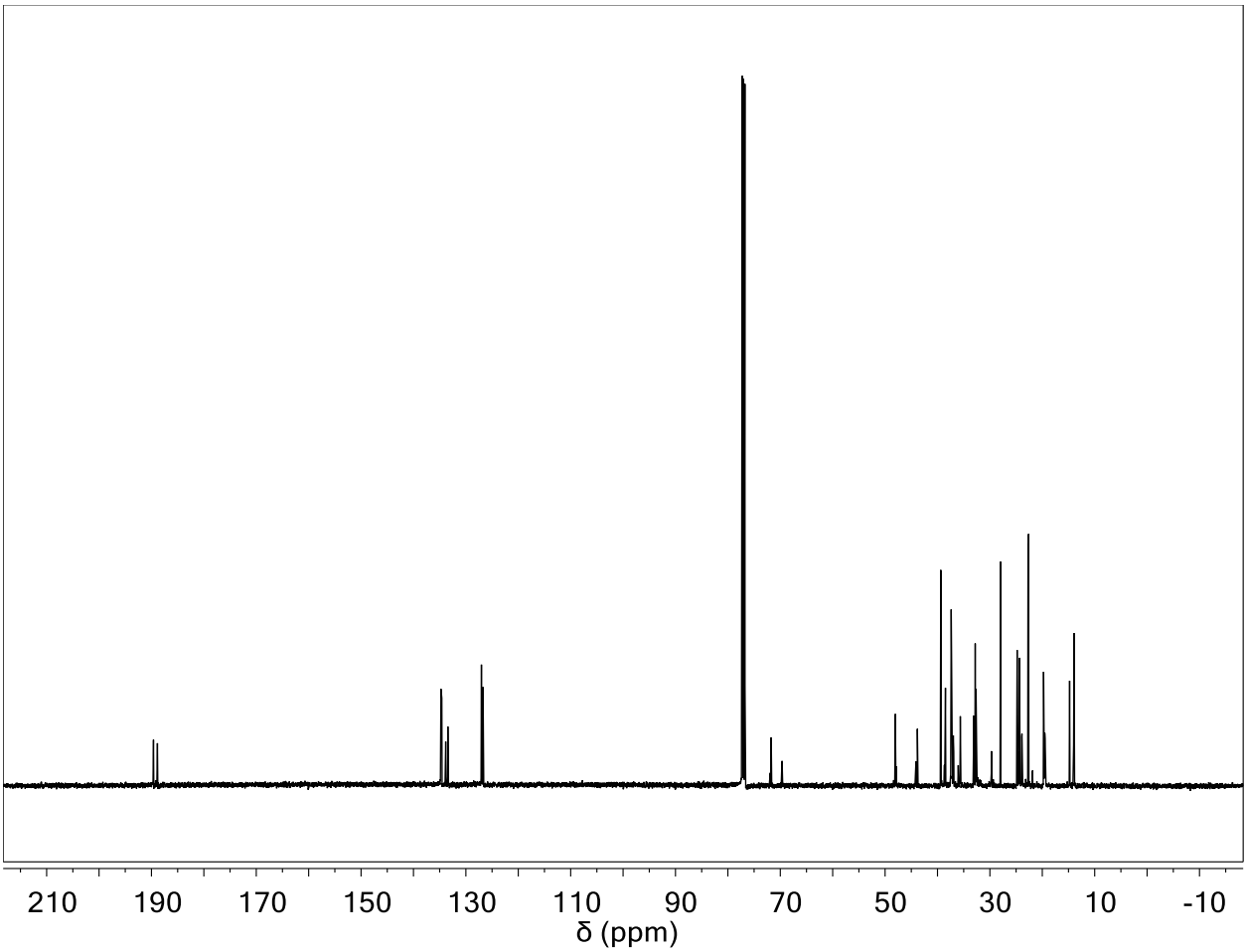


Figure 5.16 ^{13}C NMR of compound **2.8**

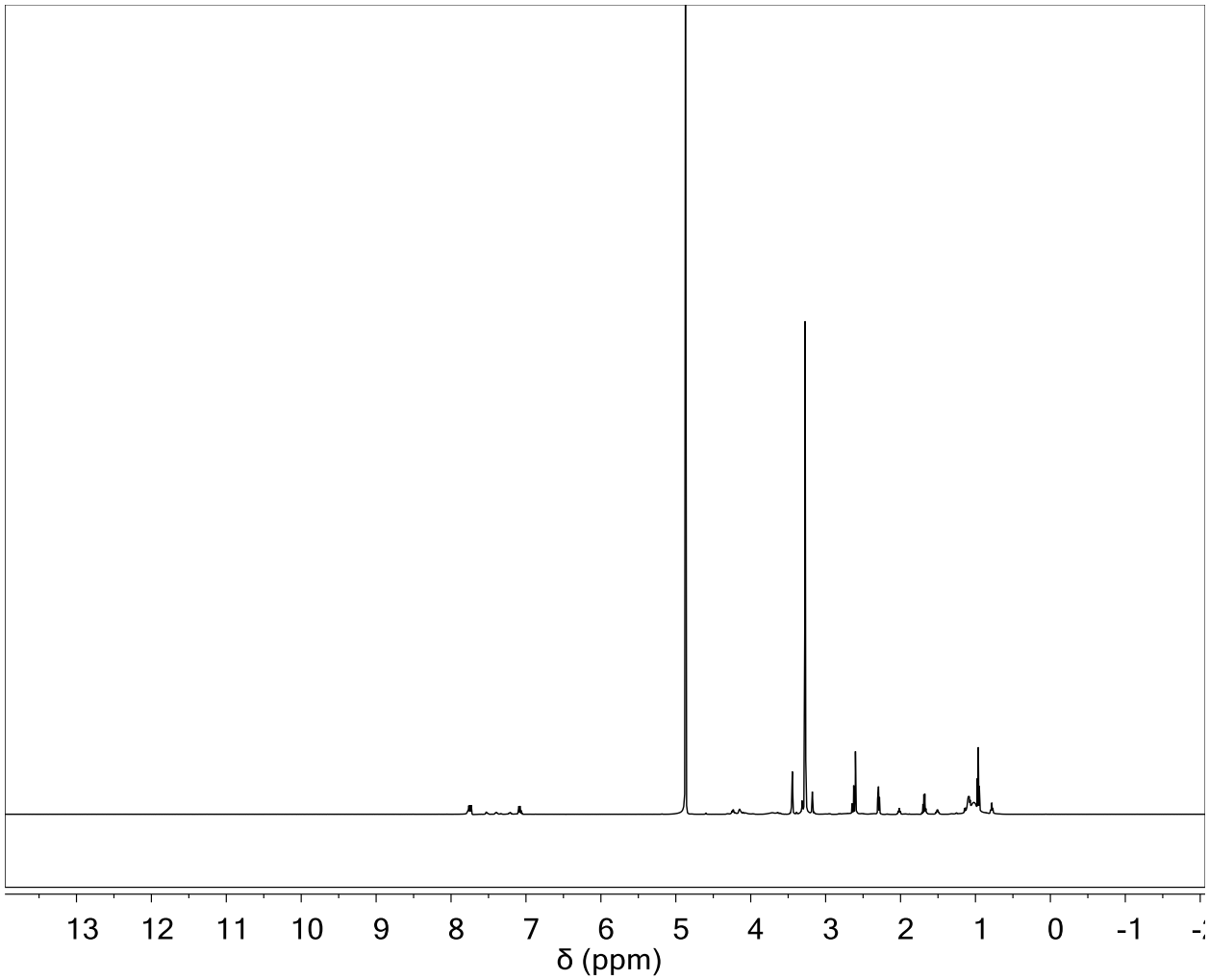


Figure 5.17 ^{1}H NMR of compound **2.9**

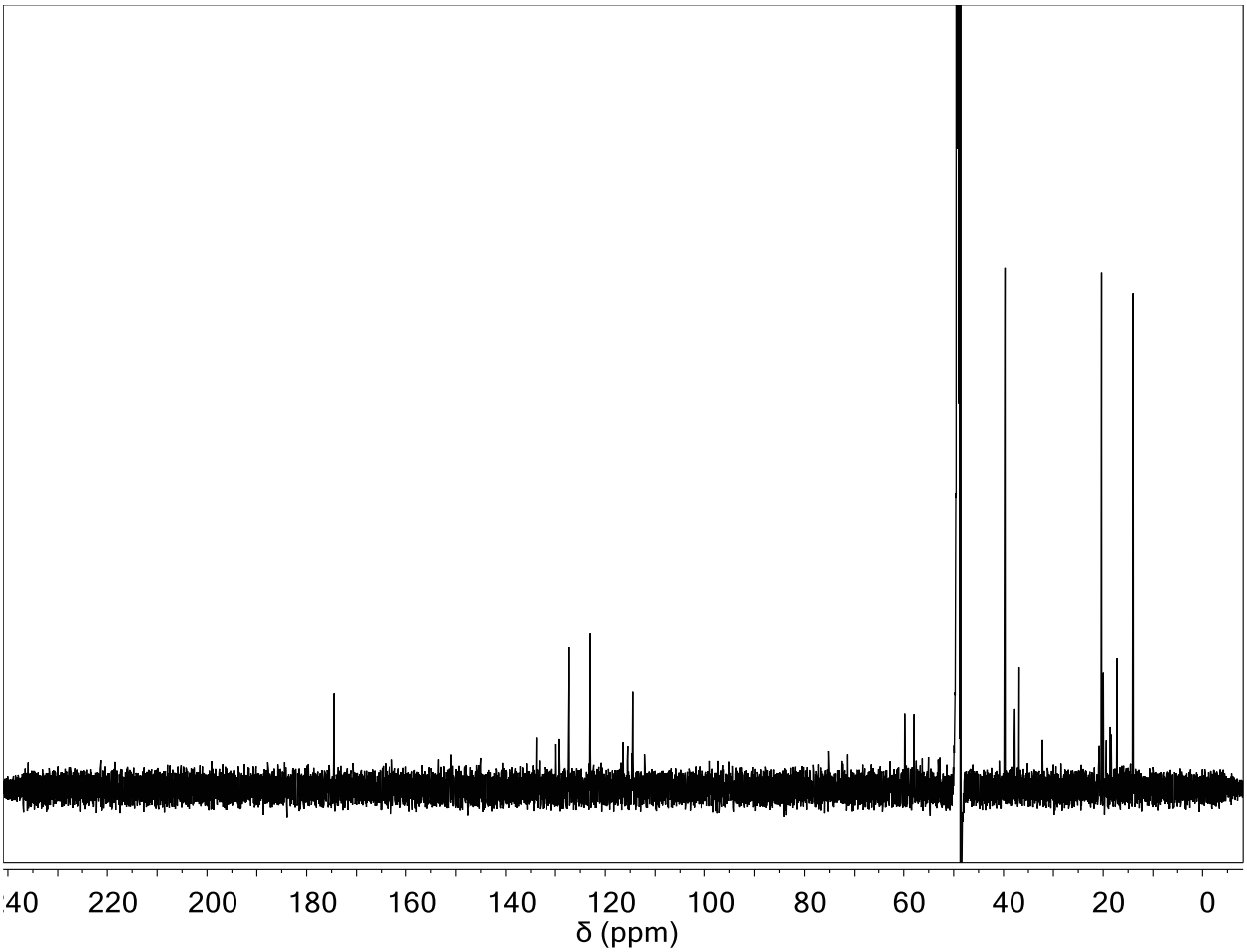


Figure 5.18 ^{13}C NMR of compound **2.9**

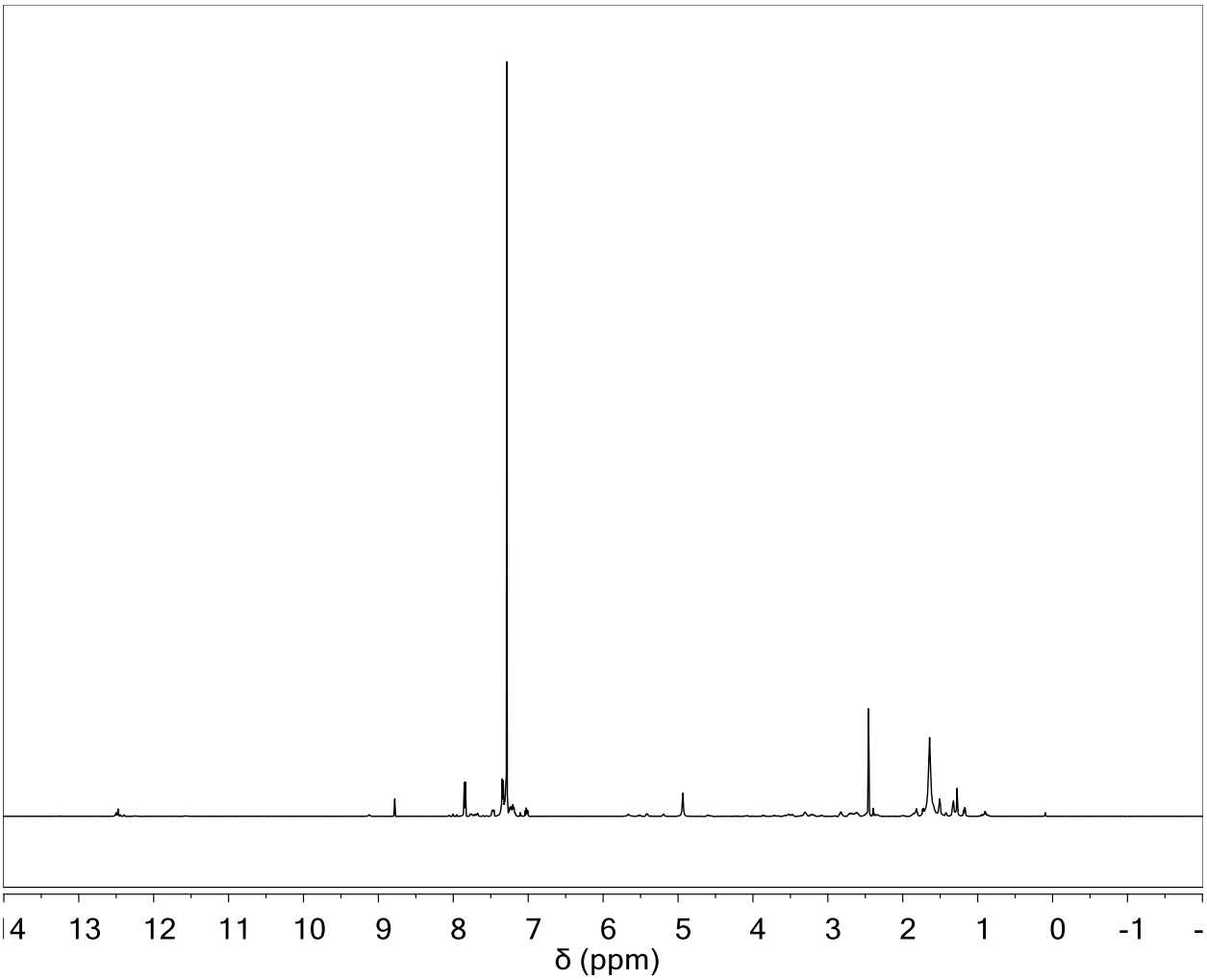


Figure 5.19 ^{1}H NMR of compound **2.10**

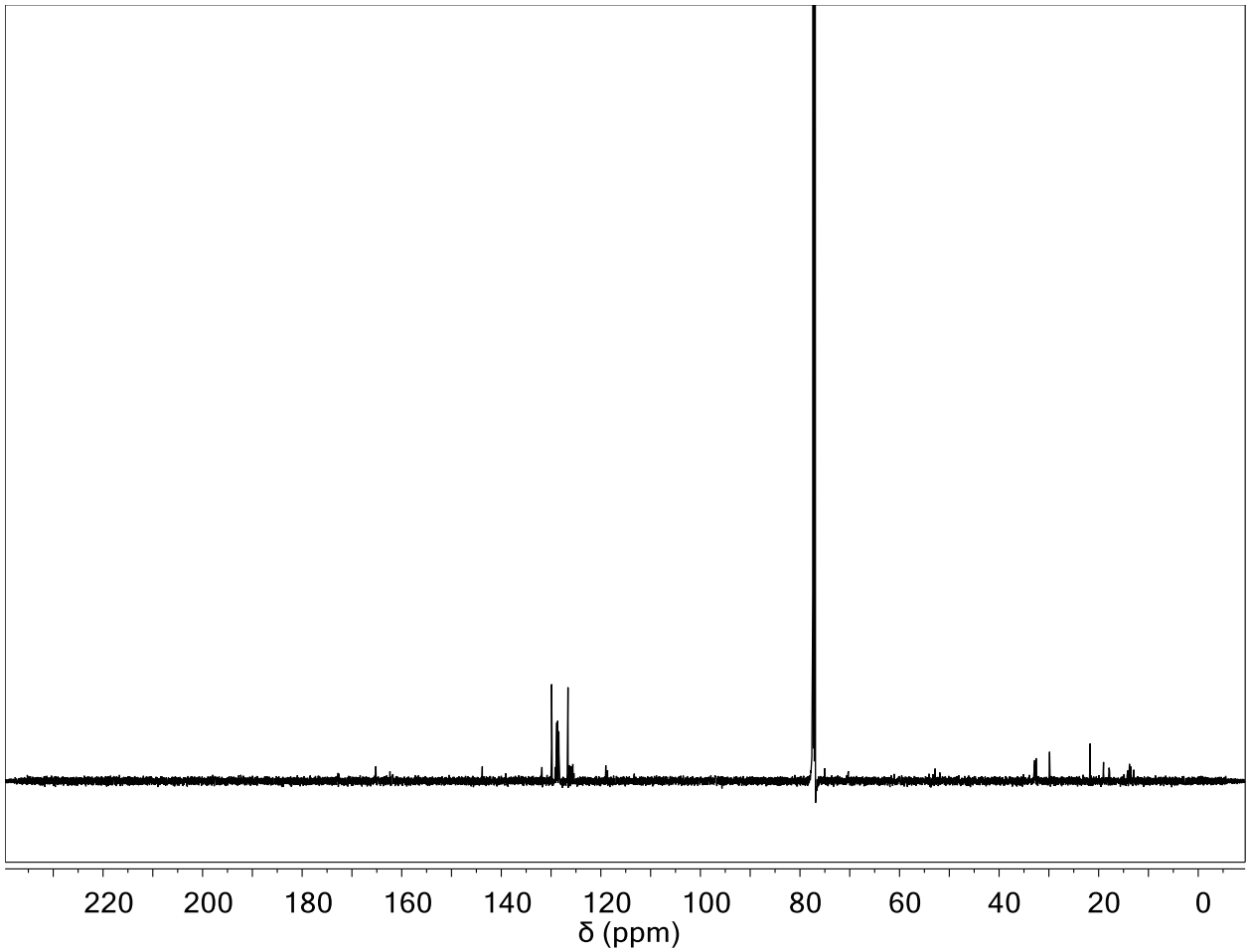


Figure 5.20 ^{13}C NMR of compound **2.10**

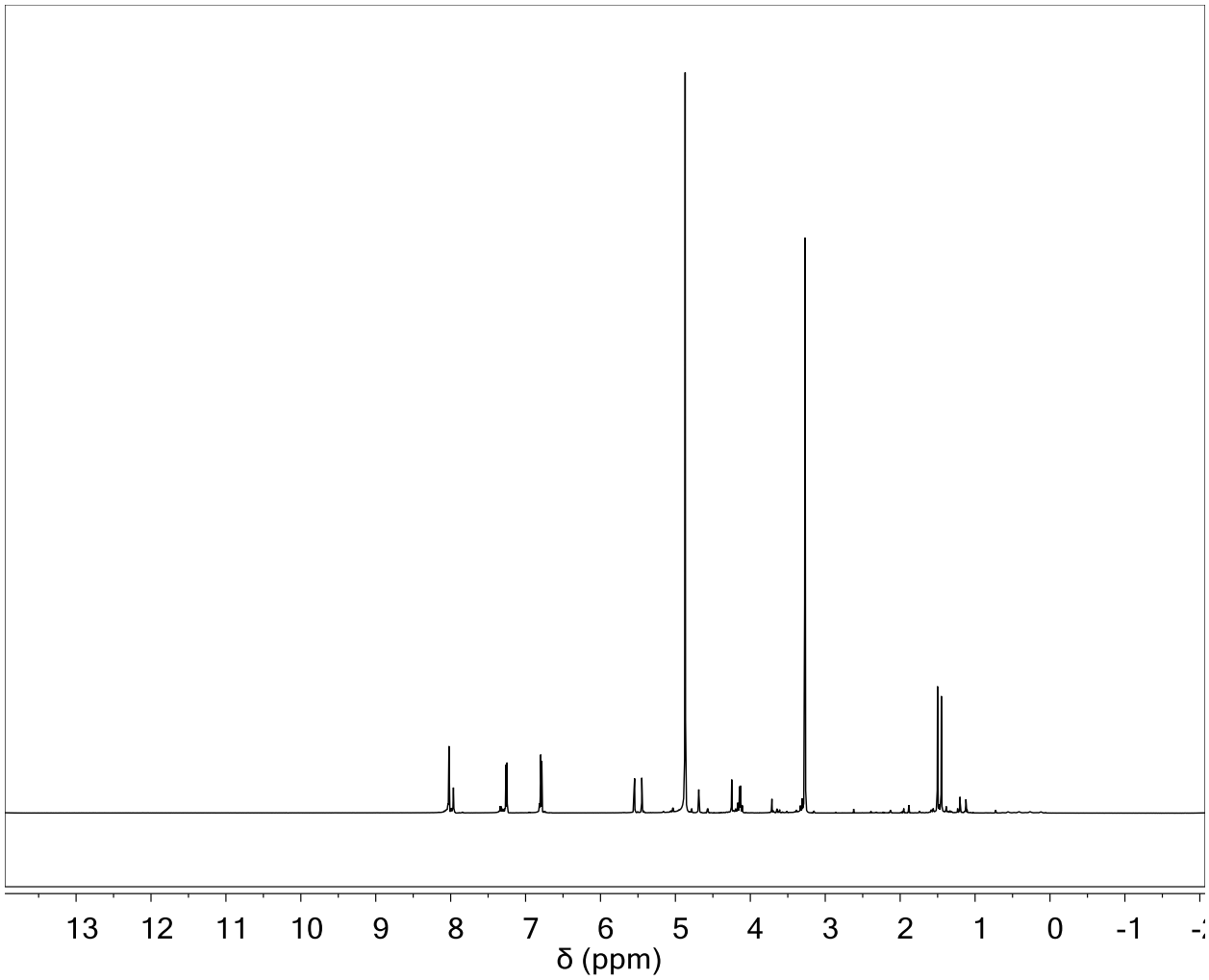


Figure 5.21 ^{1}H NMR of compound **2.11**

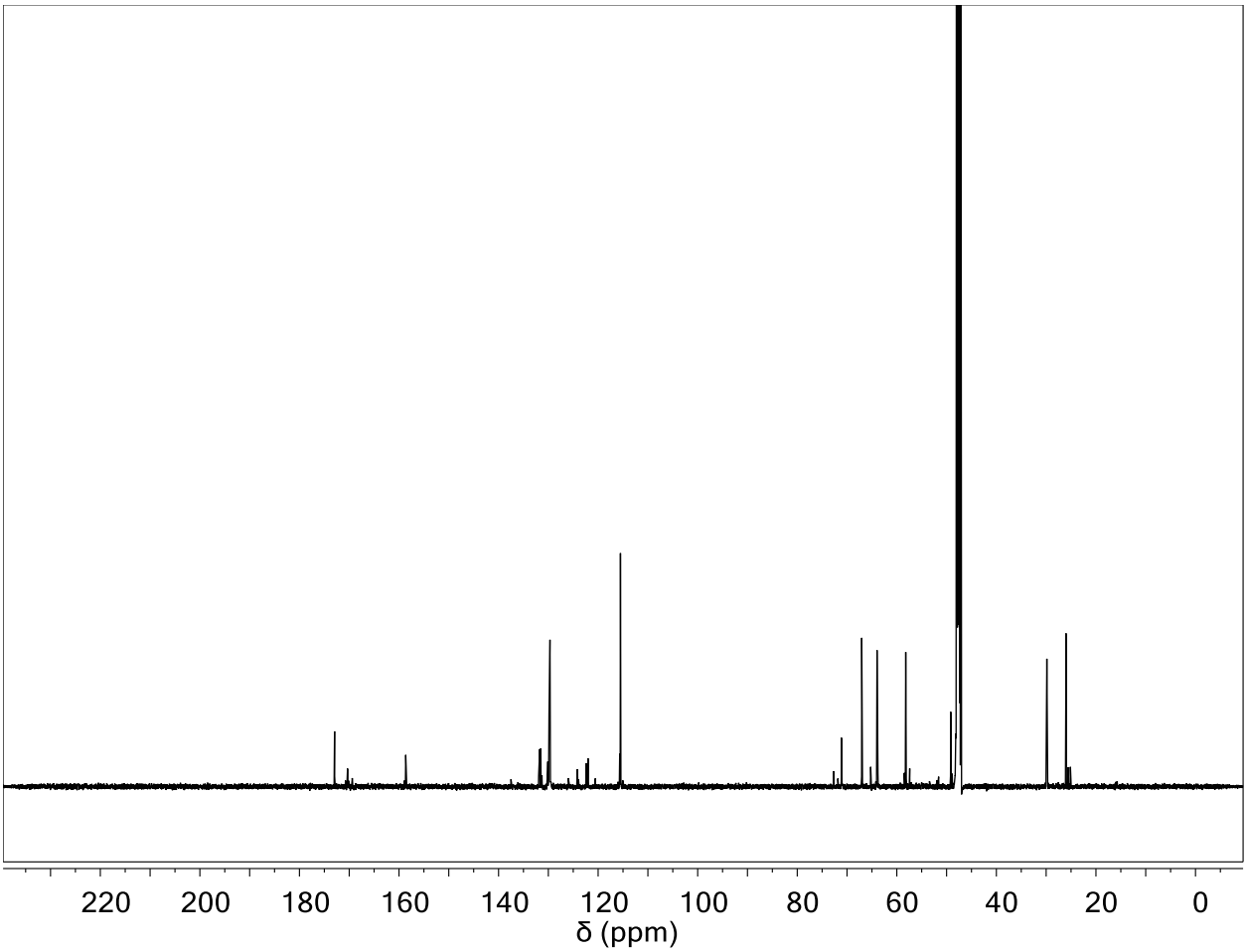
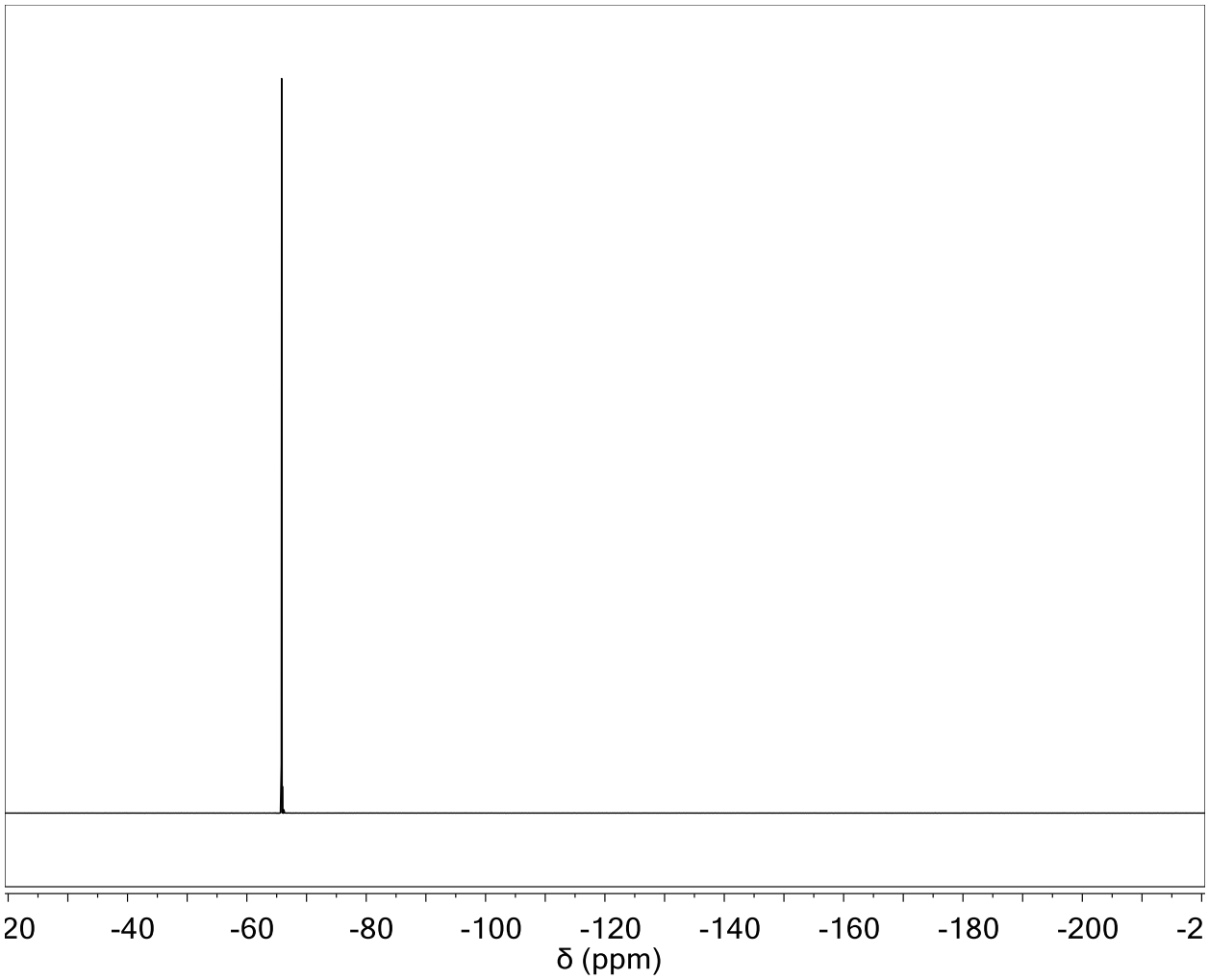


Figure 5.22 ^{13}C NMR of compound **2.11**



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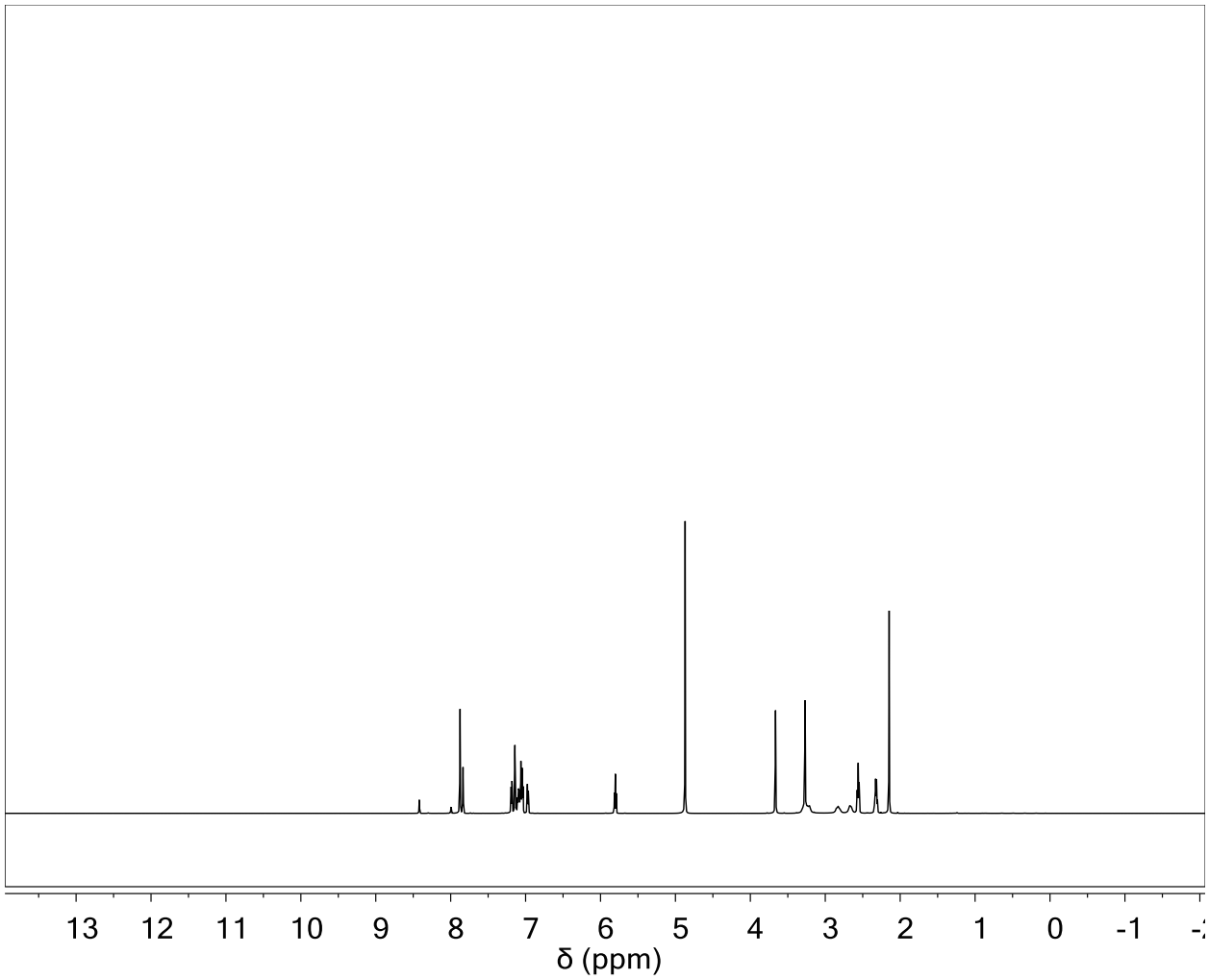


Figure 5.24 ^{1}H NMR of compound **2.12**

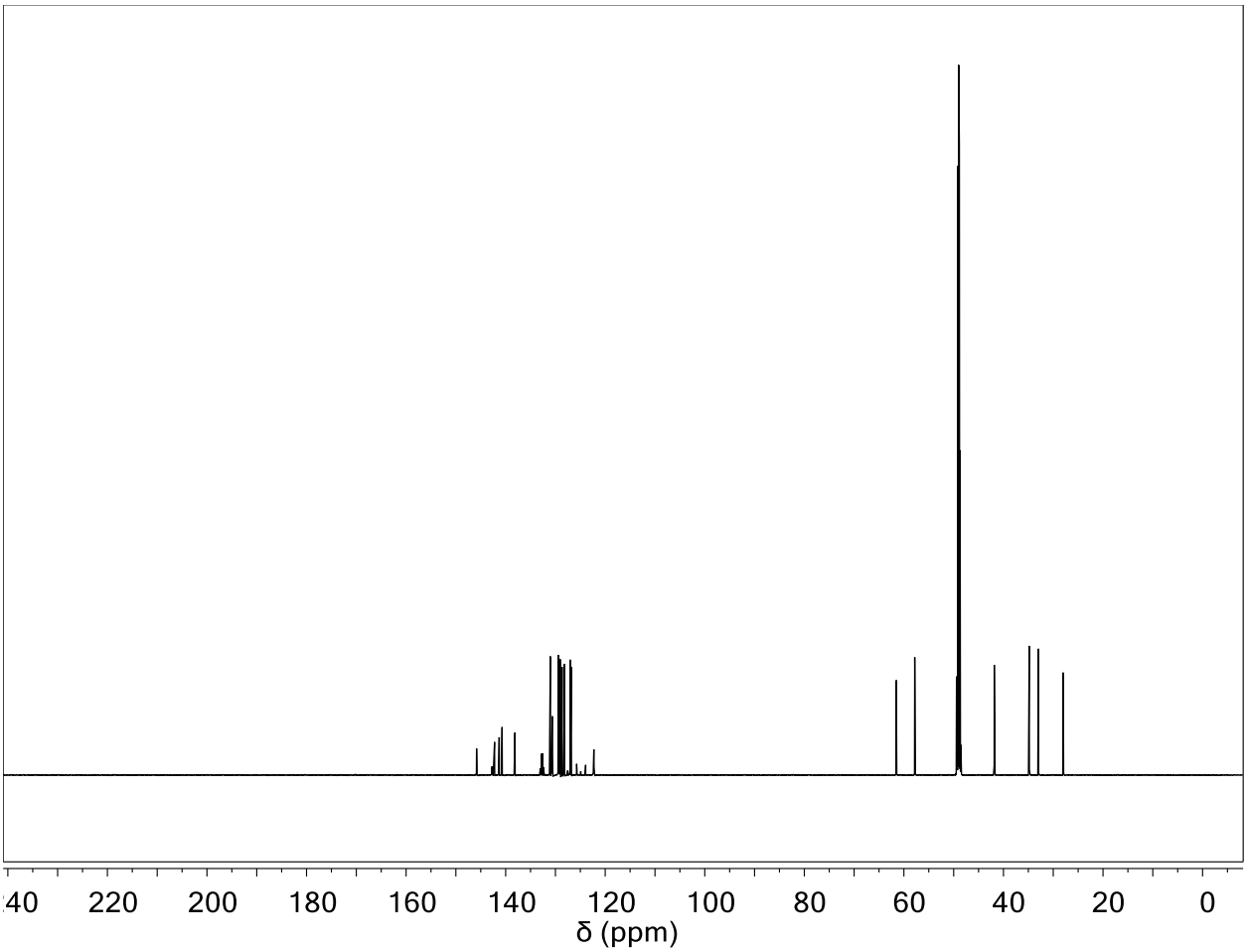


Figure 5.25 ^{13}C NMR of compound **2.12**

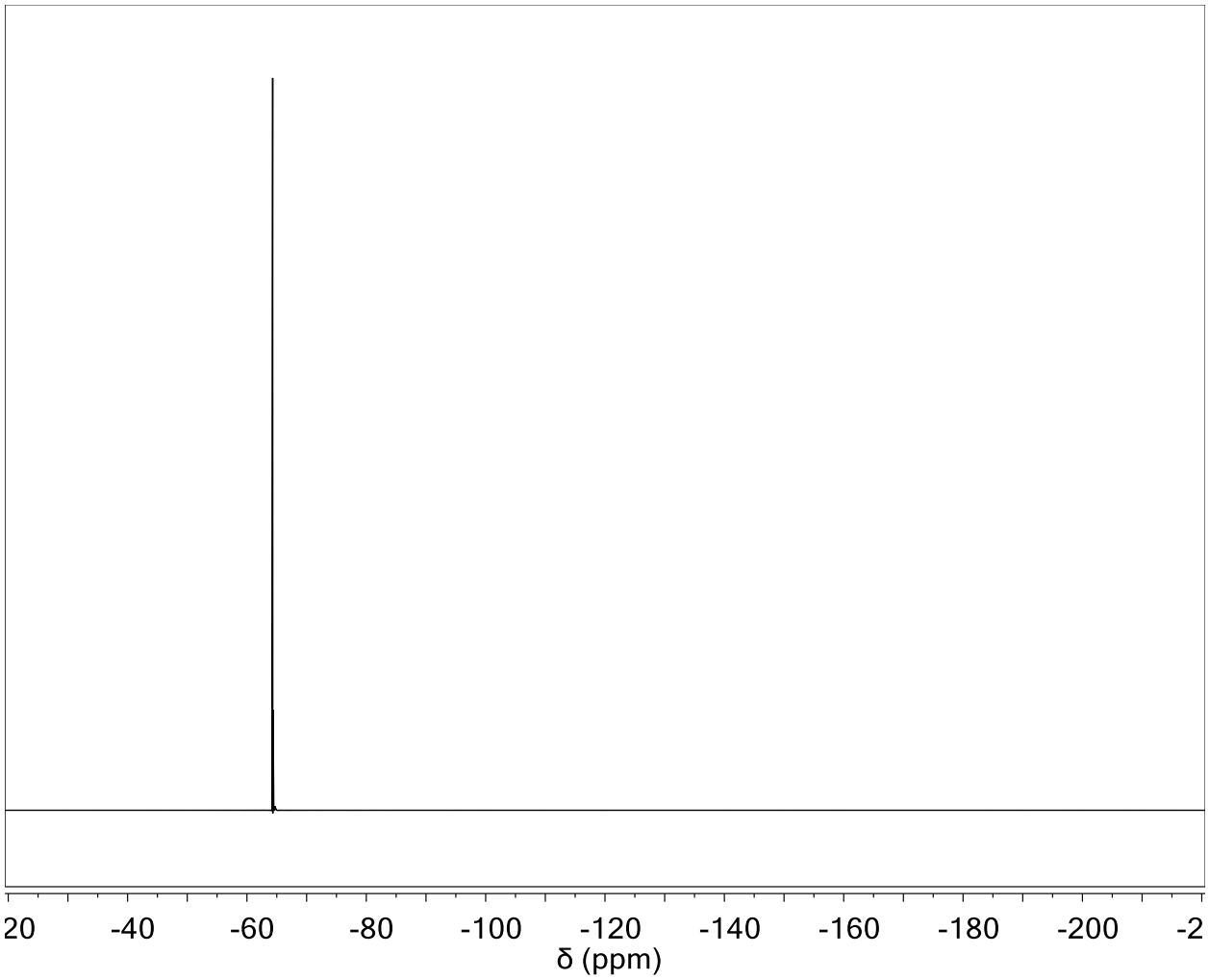
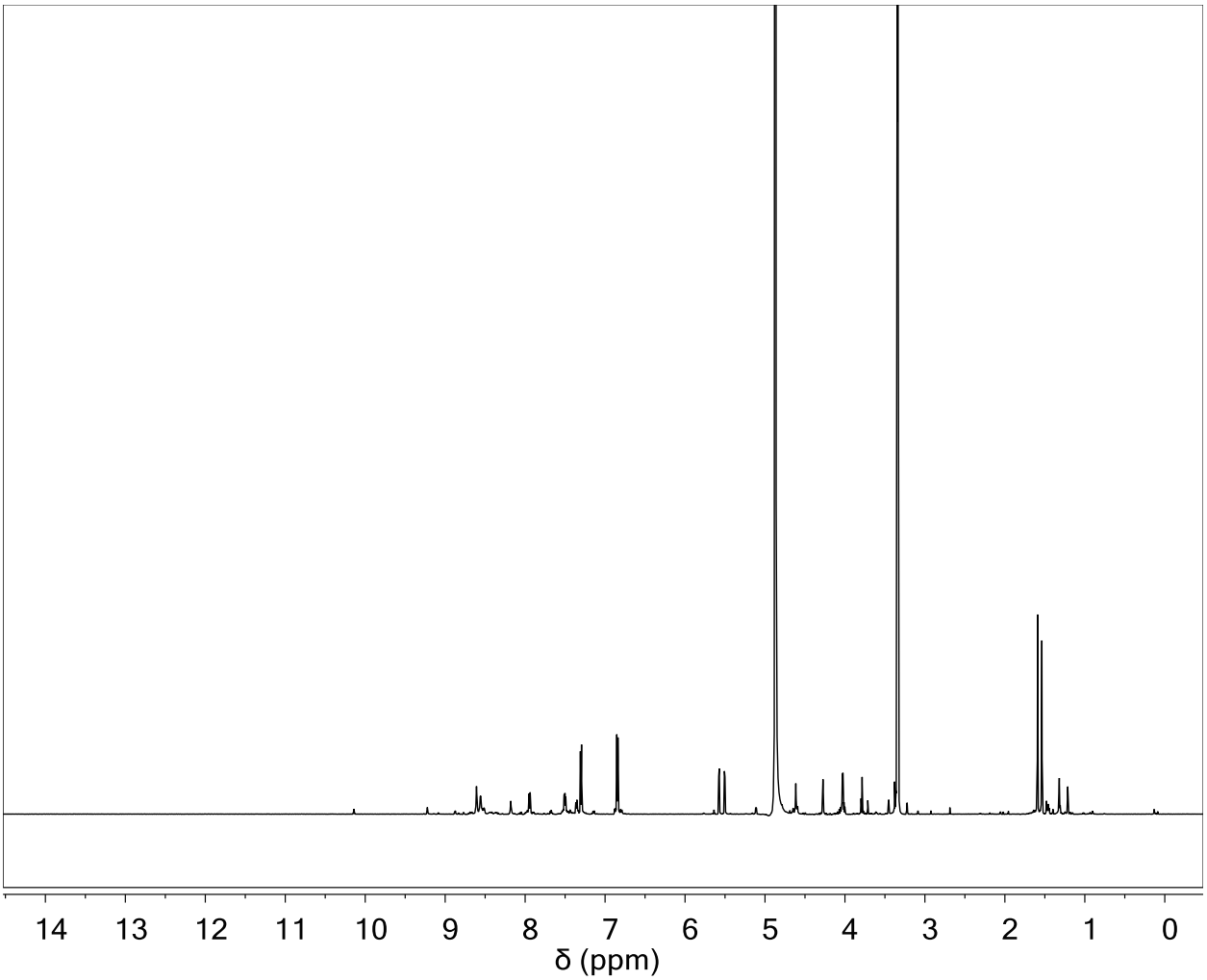


Figure 5.26 ^{19}F of compound **2.12**



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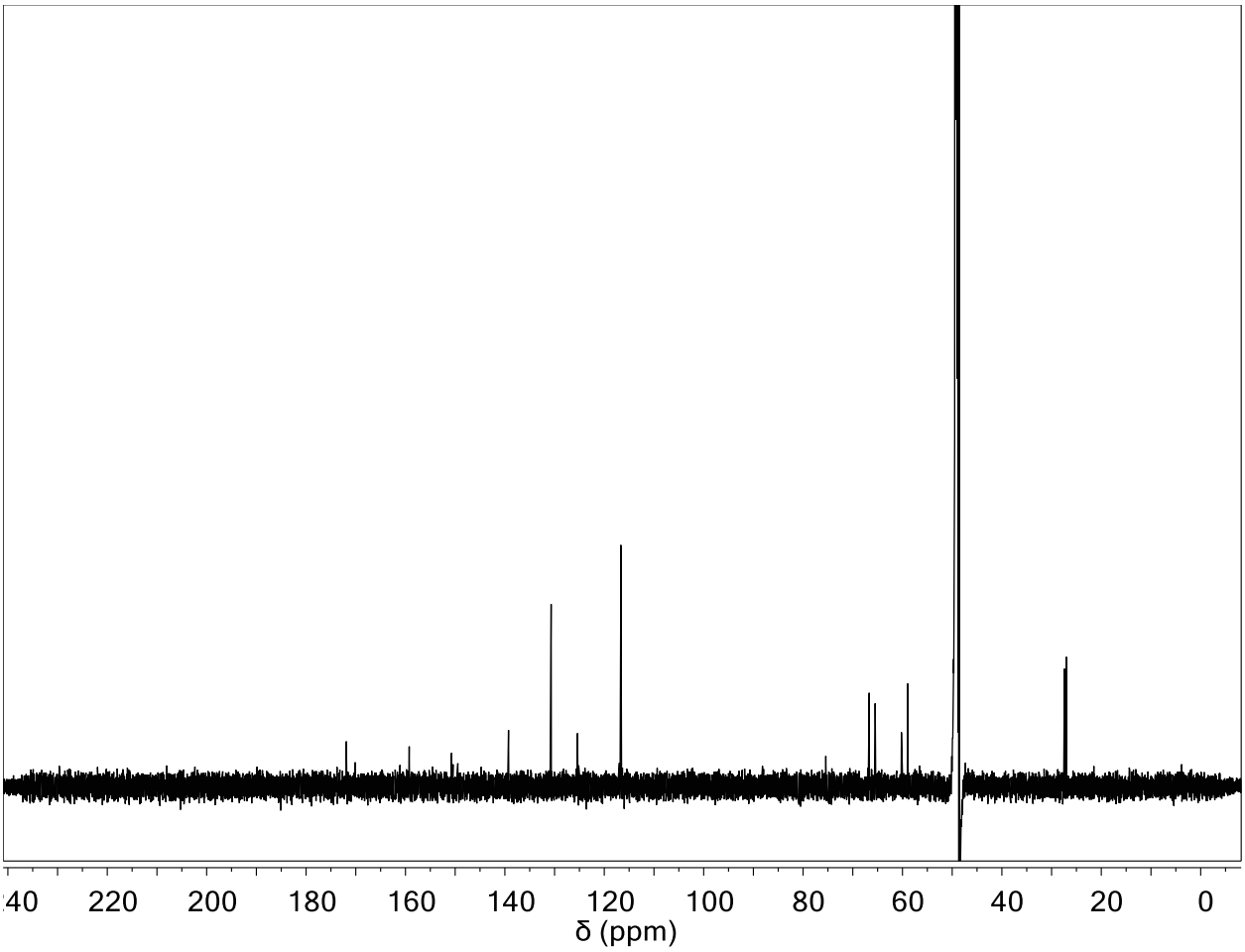

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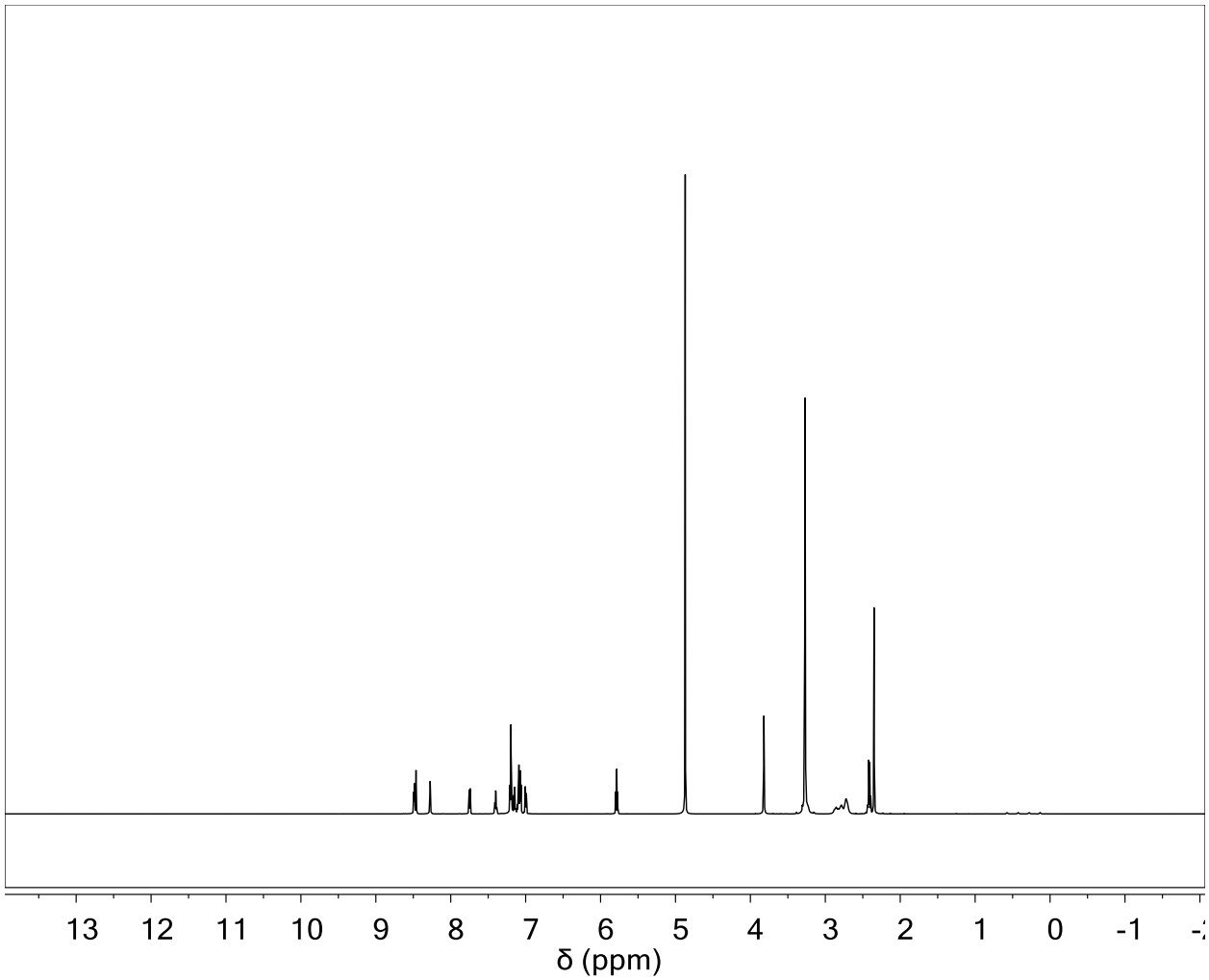

Figure 5.29 ^{1}H NMR of compound **2.14**

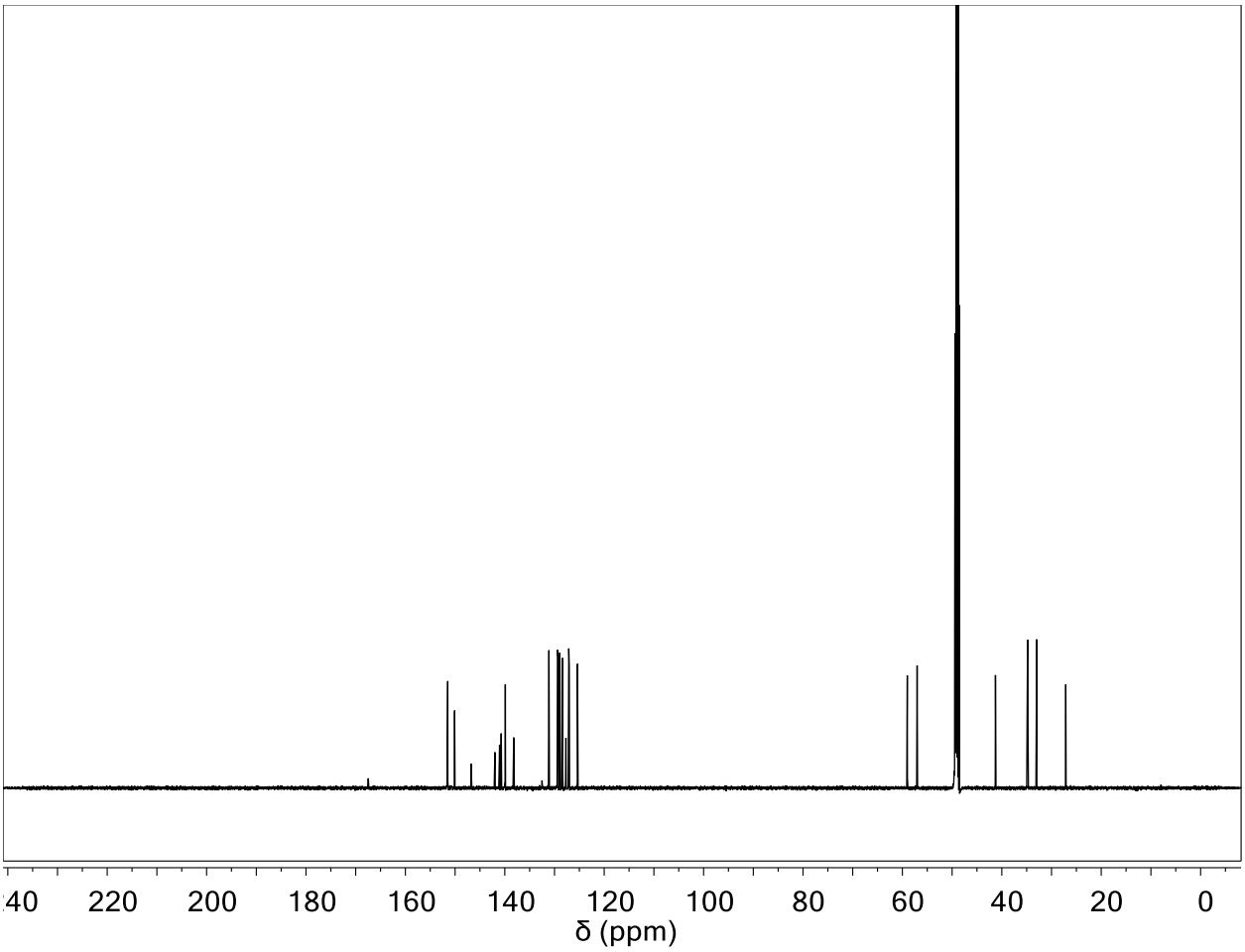

Figure 5.30 ^{13}C NMR of compound **2.14**

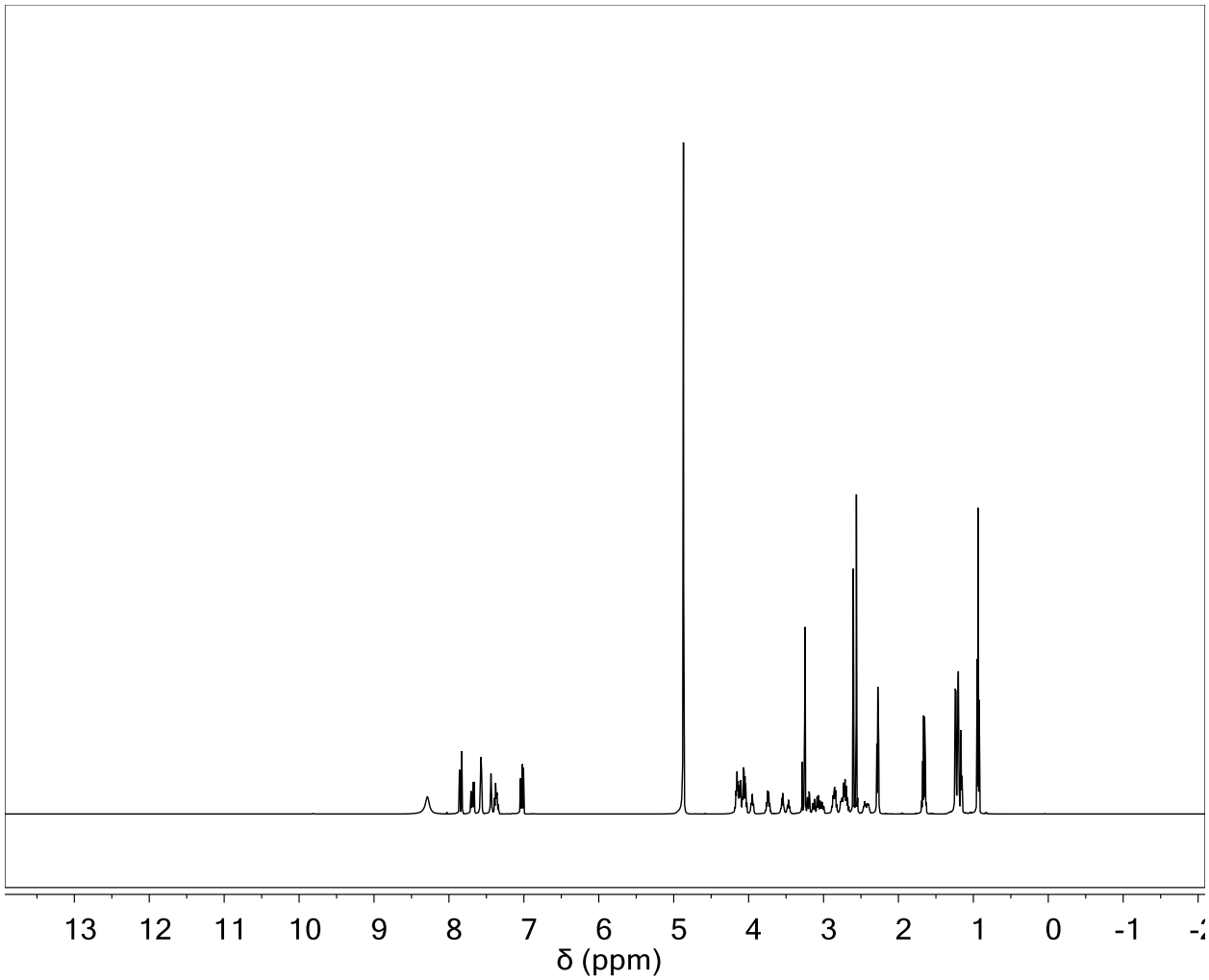

Figure 5.31 ^{1}H NMR of compound **2.15**

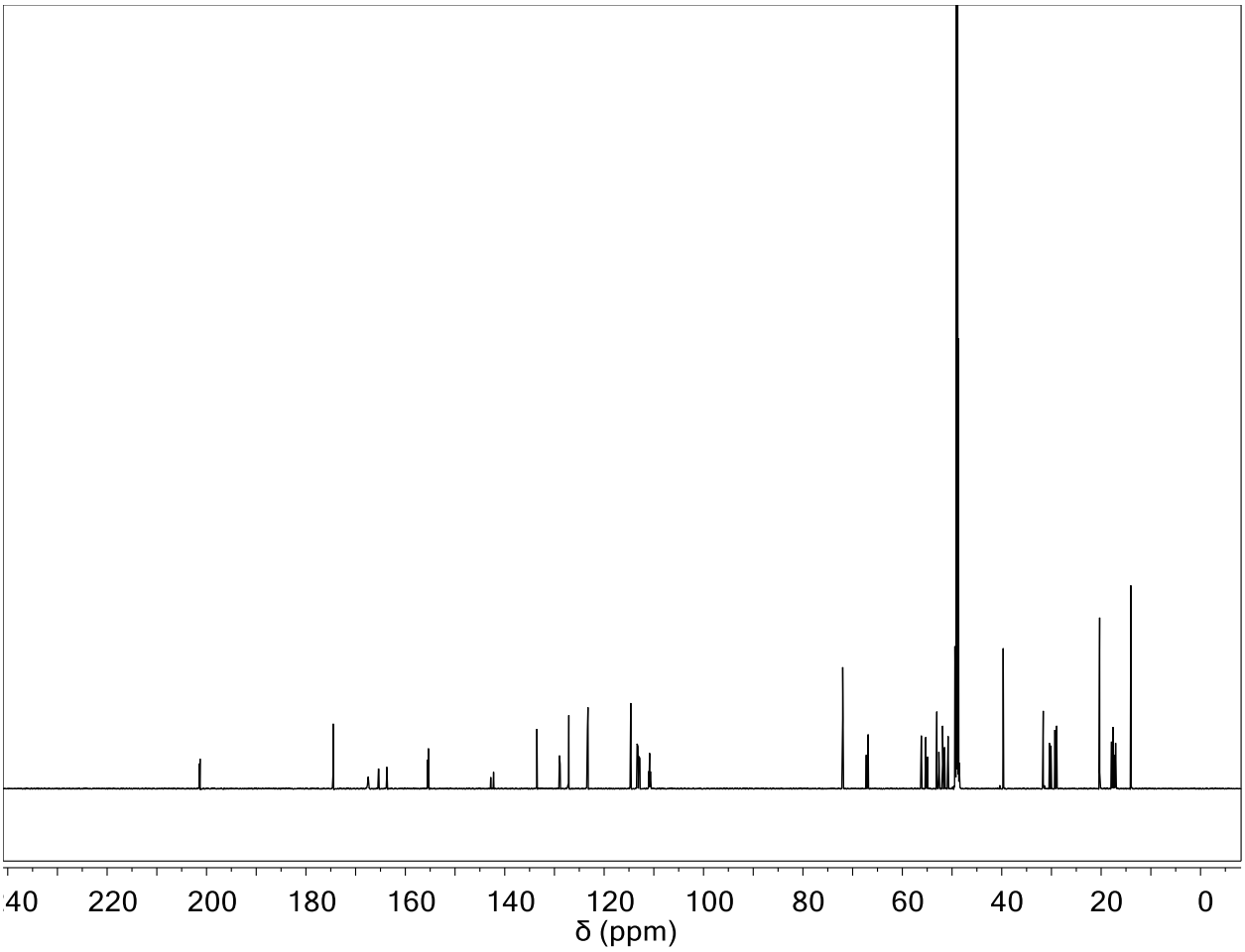

Figure 5.32 ^{13}C NMR of compound **2.15**

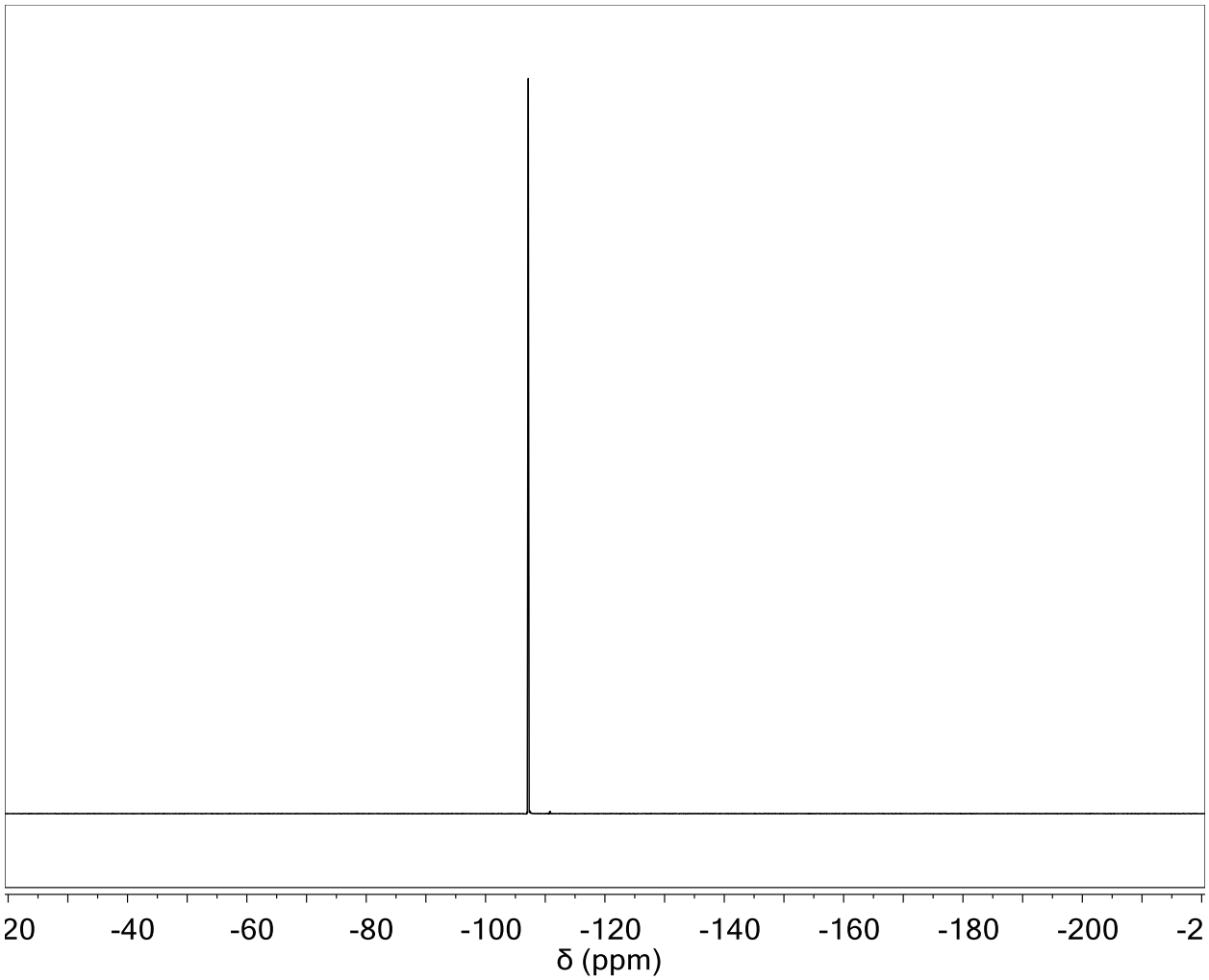

Figure 5.33 ^{19}F NMR of compound **2.15**

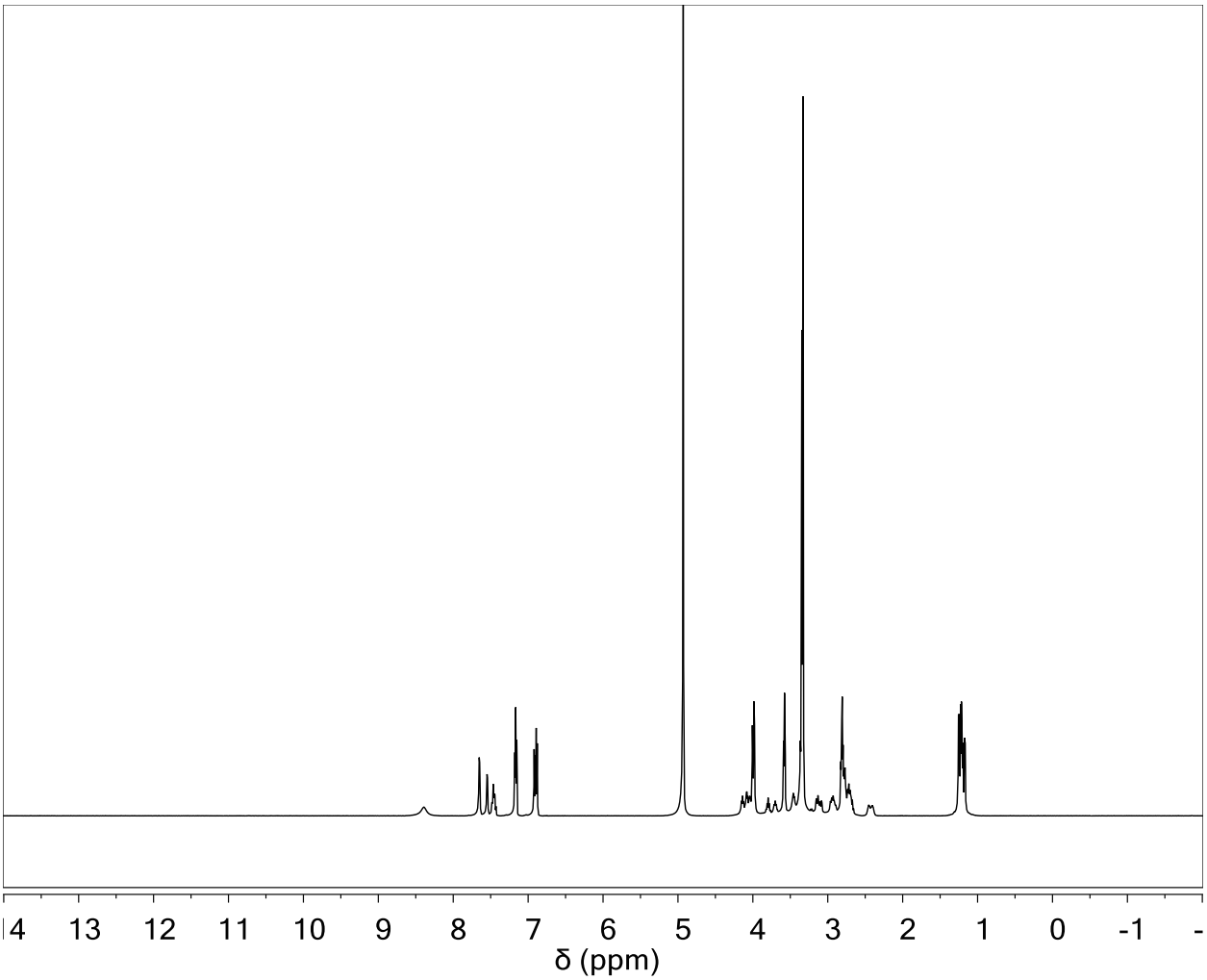

Figure 5.34 ^{1}H NMR of compound **2.16**

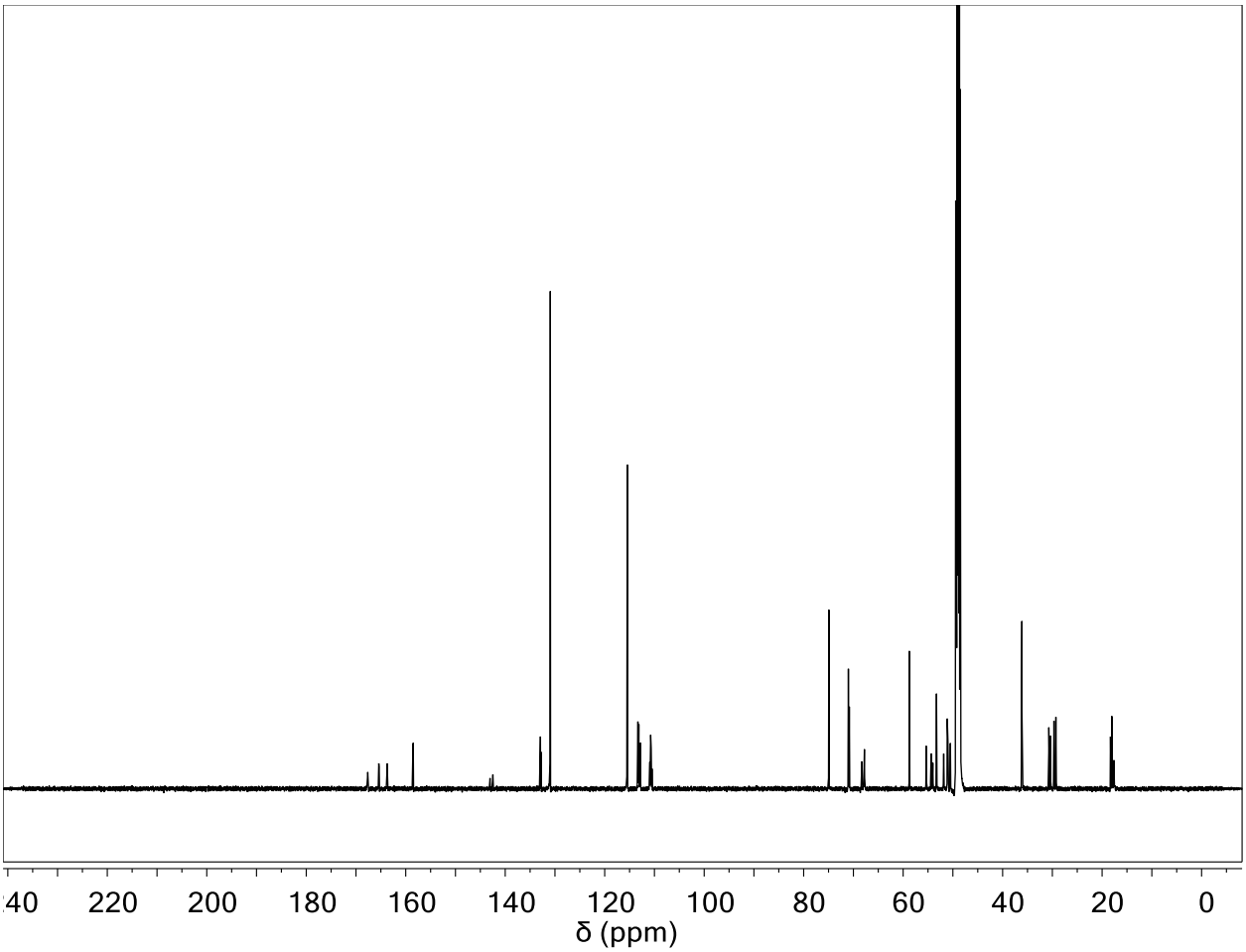

Figure 5.35 ^{13}C NMR of compound **2.16**

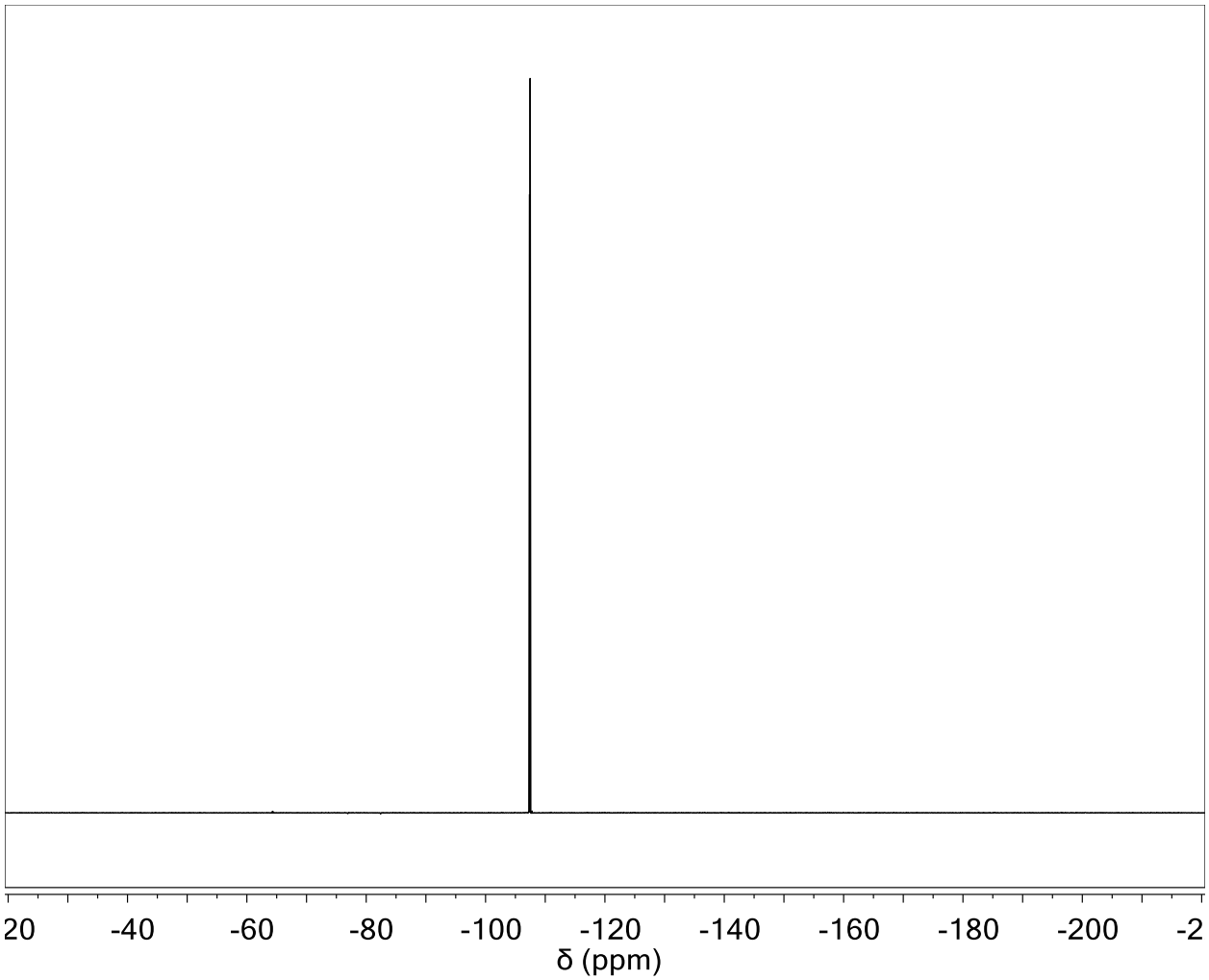

Figure 5.36 ^{19}F NMR of compound **2.16**

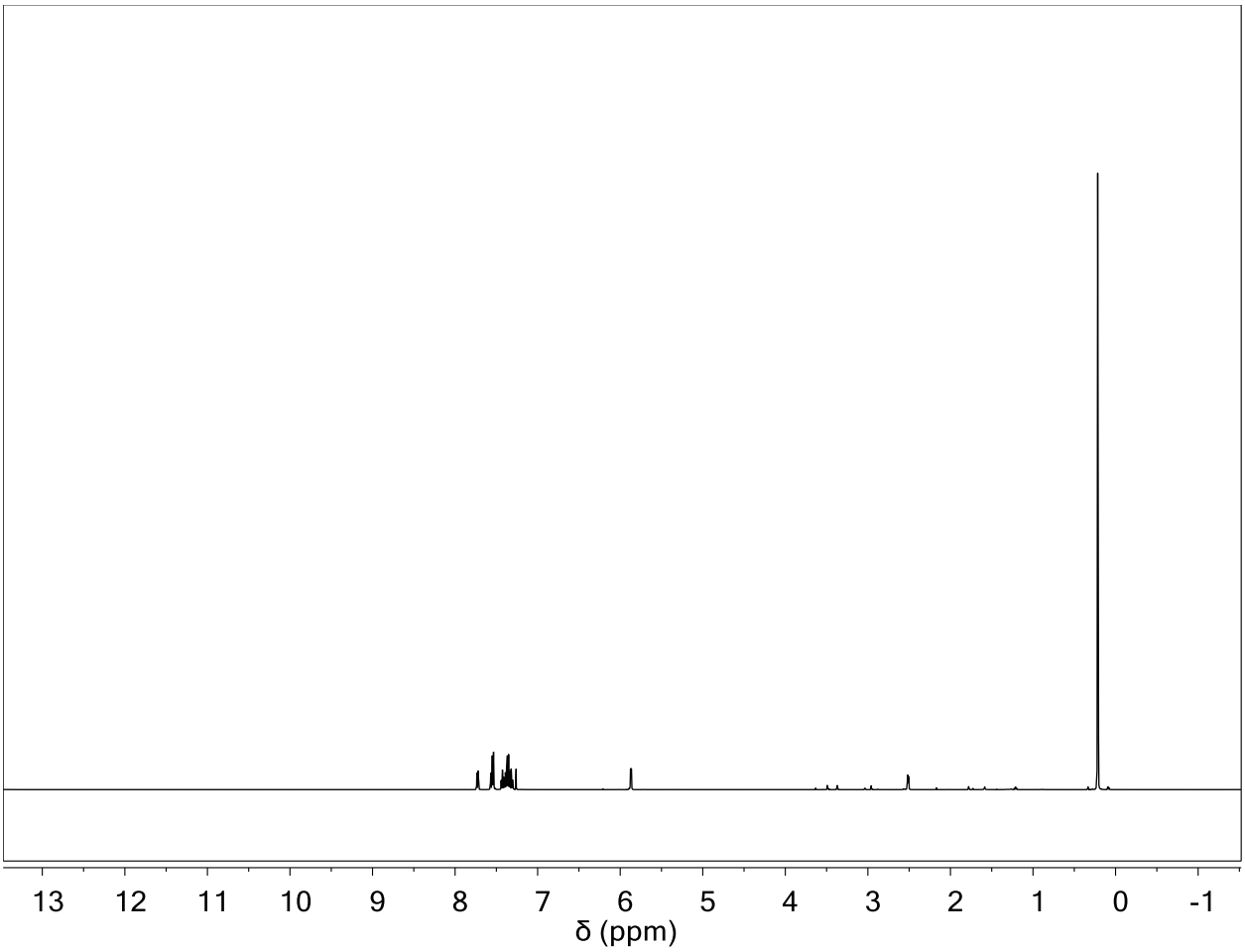

Figure 5.37 ^{1}H NMR of compound **2.20**

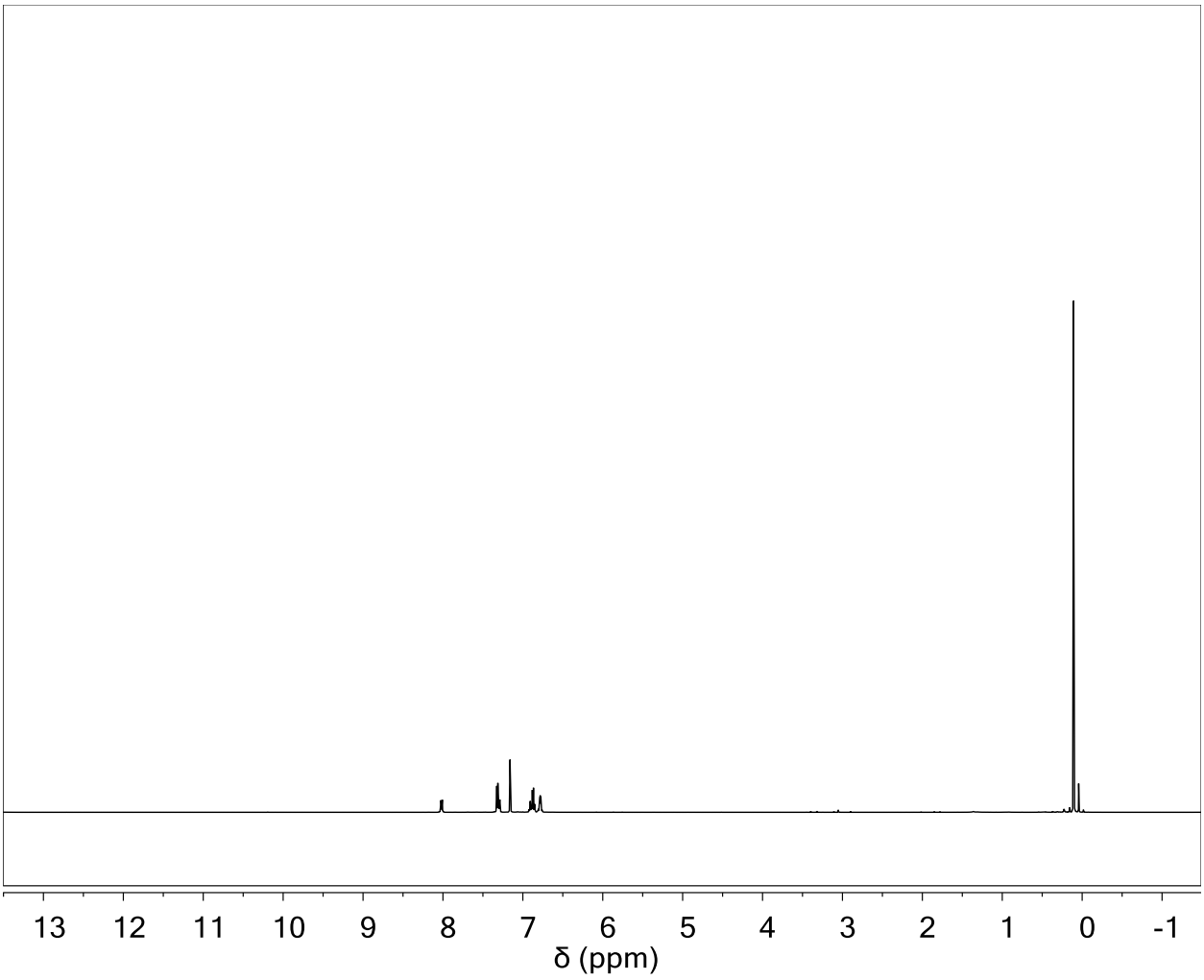

Figure 5.38 ^{1}H NMR of compound **2.21**

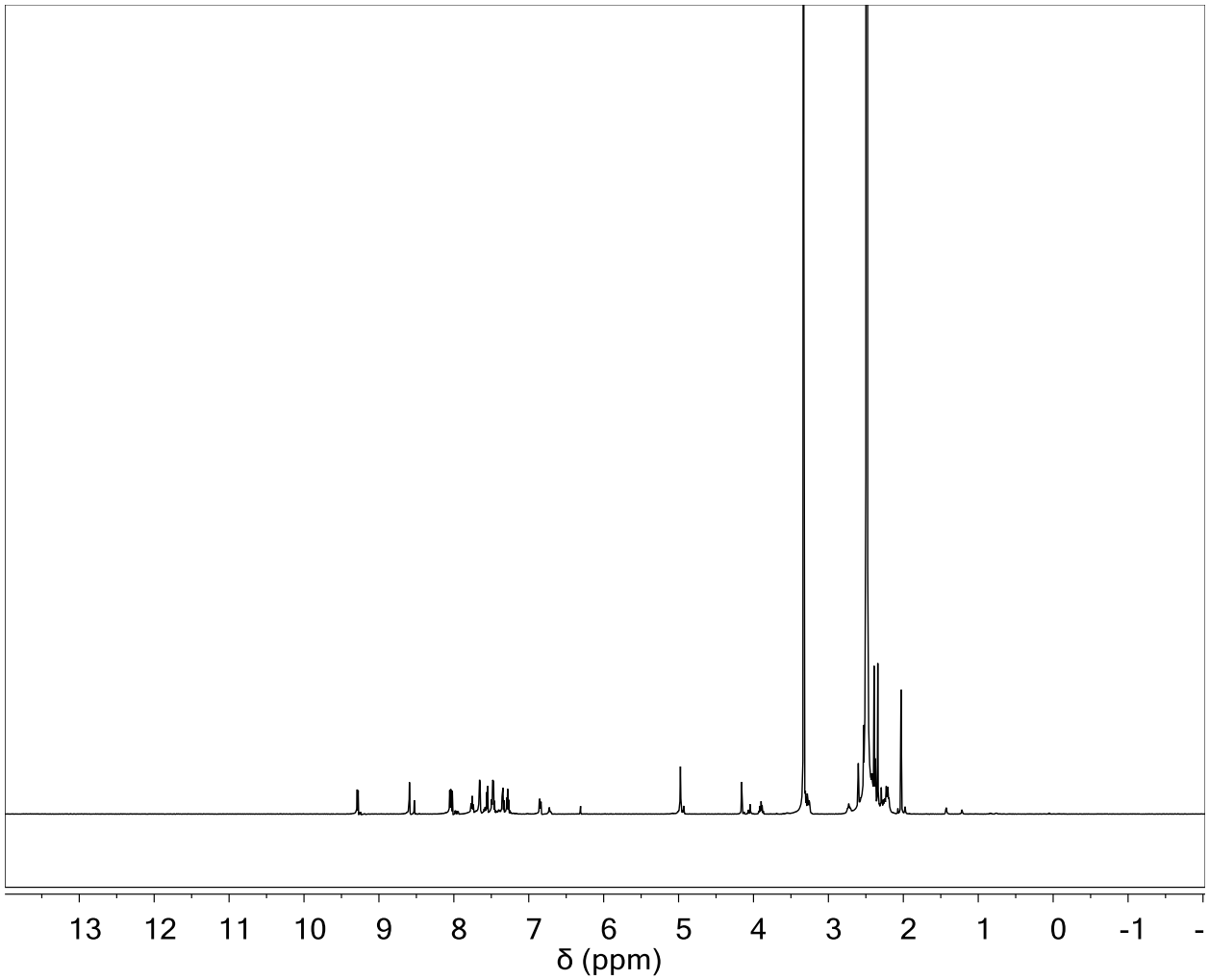

Figure 5.39 ^{1}H NMR of compound **3.2 A**

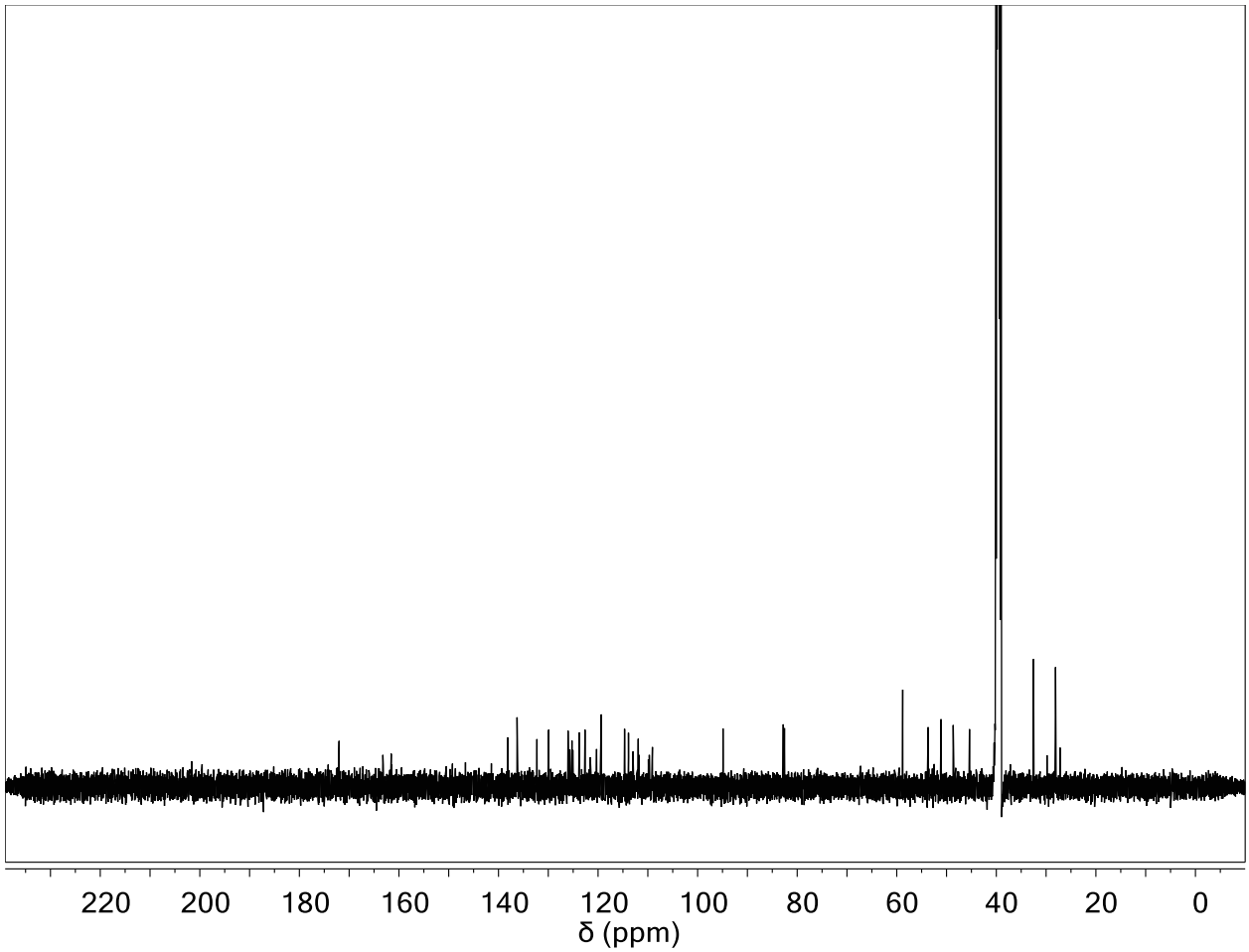

Figure 5.40 13C NMR of compound **3.2 A**

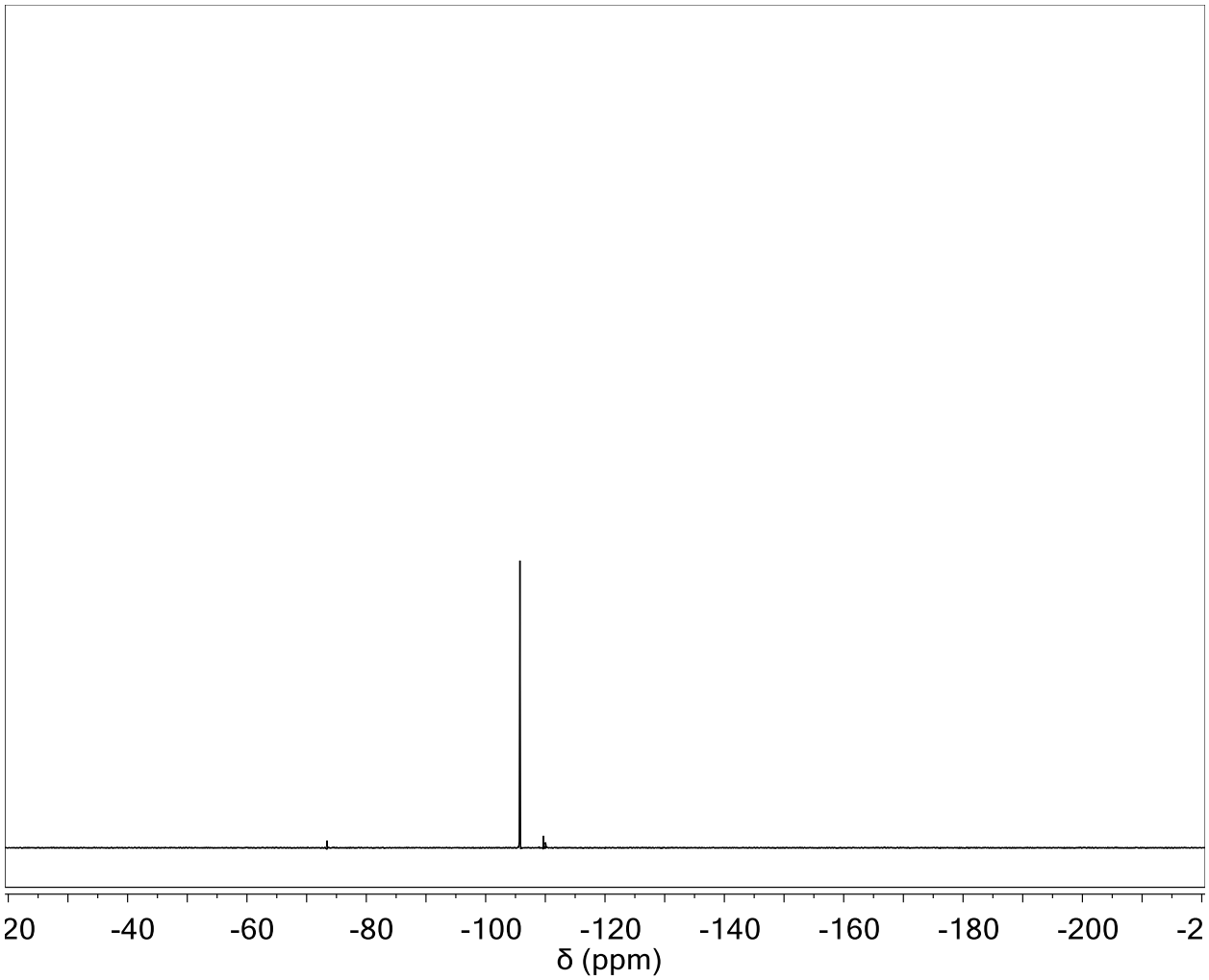

Figure 5.41 ^{19}F NMR of compound **3.2 A**

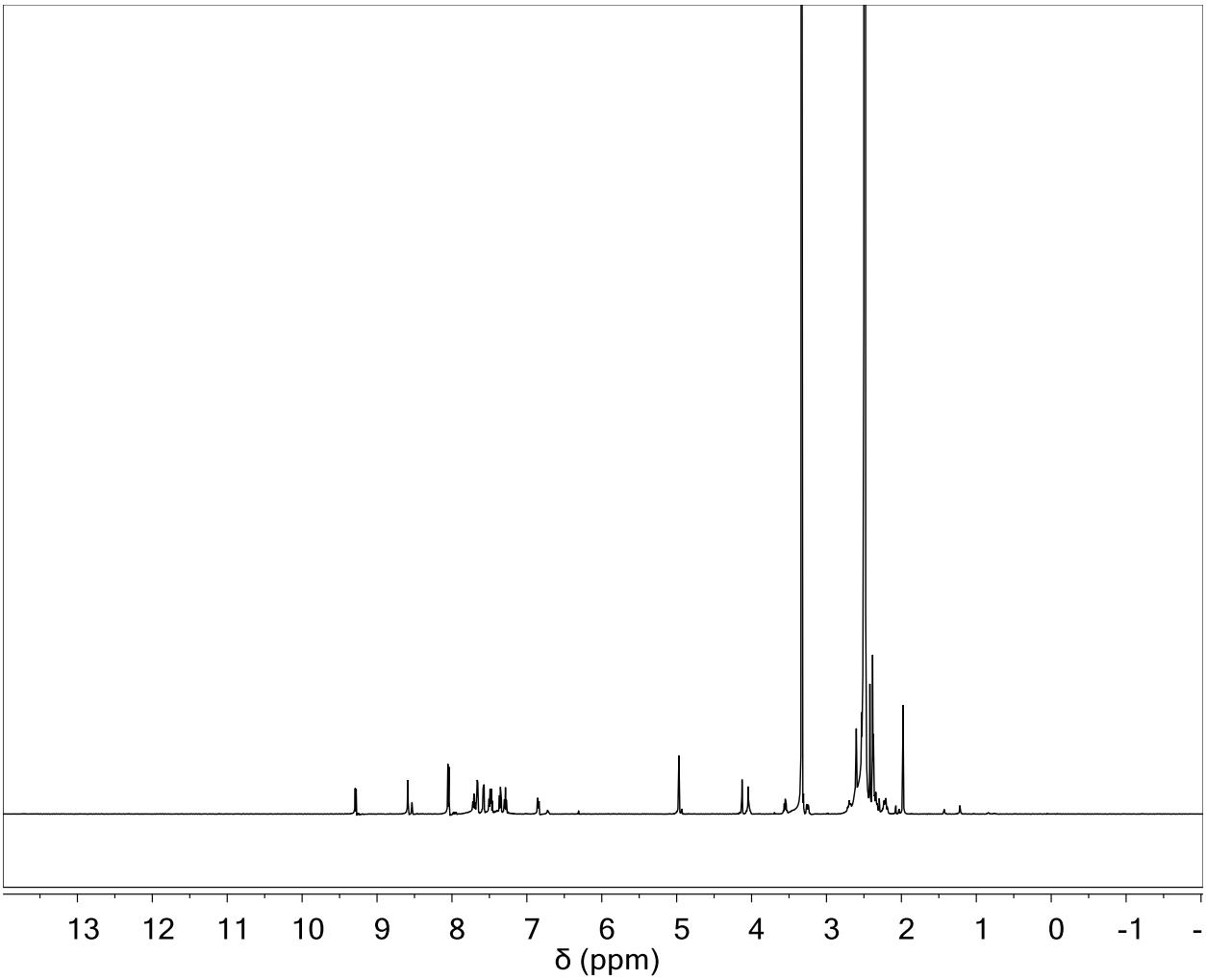

Figure 5.42 ^{1}H NMR of compound **3.2 B**

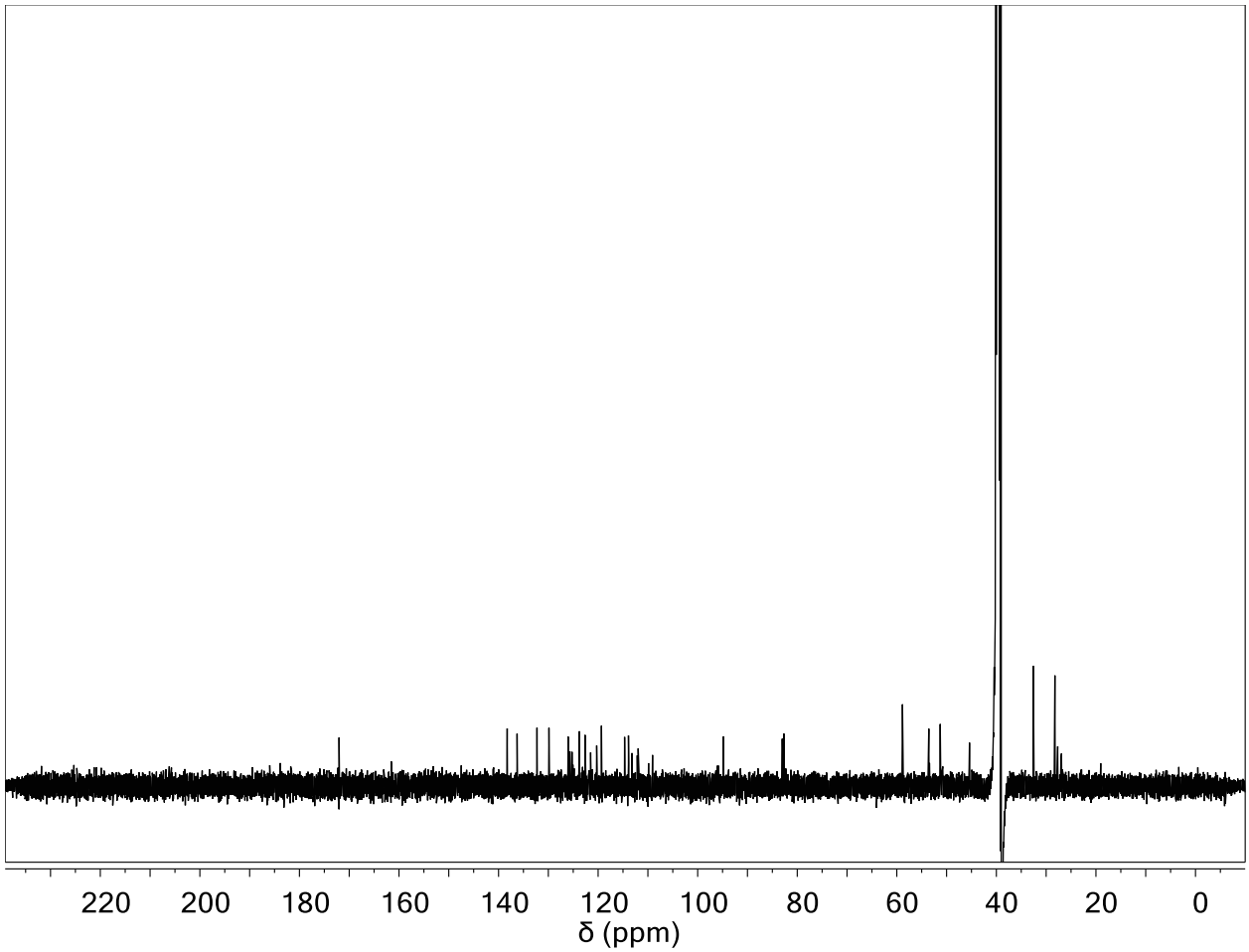

Figure 5.43 13C NMR of compound 3.2 B

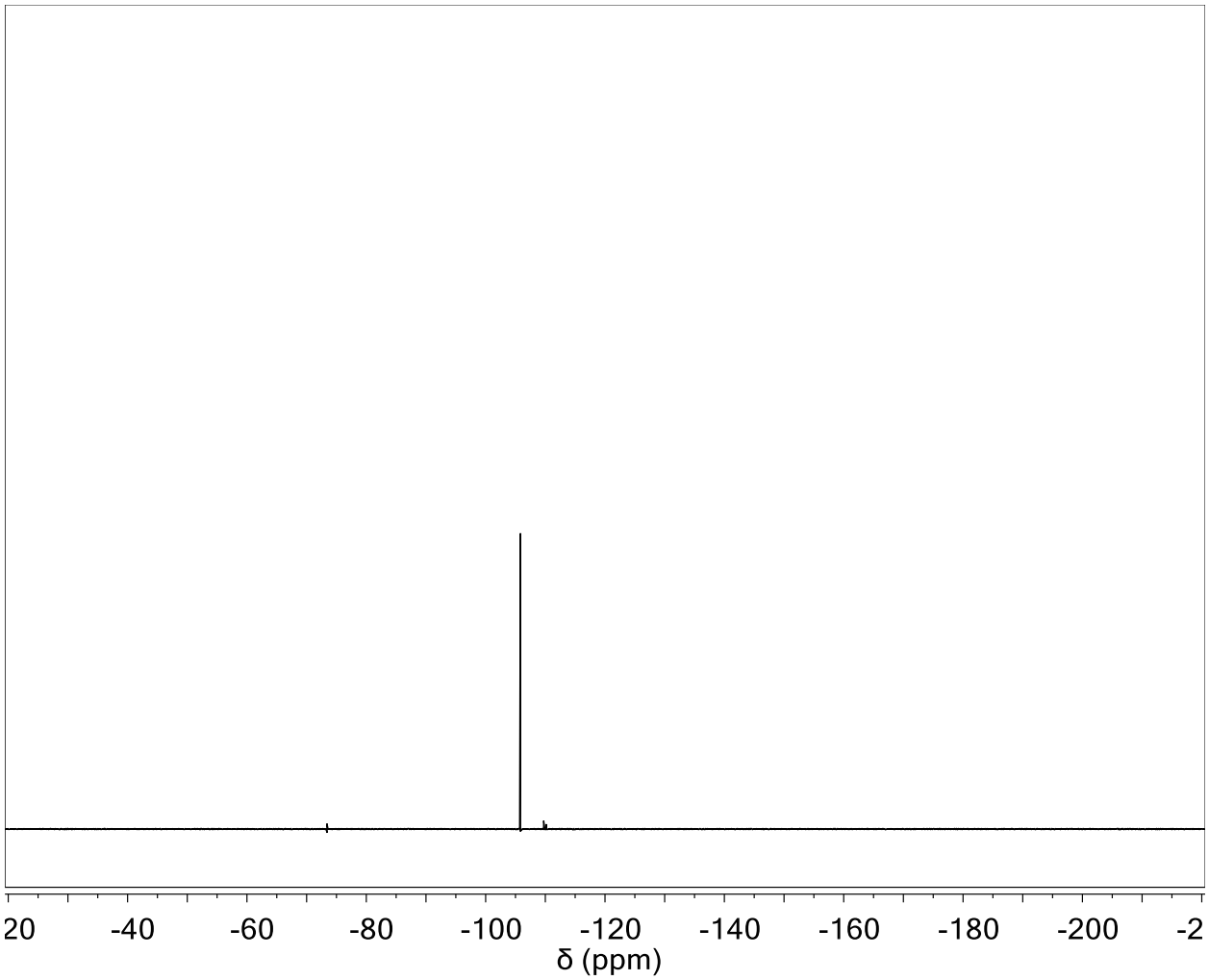

Figure 5.44 ^{19}F NMR of compound **3.2 B**