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Santa Barbara

Regiochemistry of Nitroso Hetero Diels-Alder Reactions, Catechol Siderophore Analogues for Surface Wet Adhesion, and an Inquiry-Based Synthetic Organic Laboratory Course

A dissertation submitted in partial satisfaction of the requirements for

the degree Doctor of Philosophy in Chemistry

by

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January 2018

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Acknowledgements

When I decided to pursue my PhD in chemistry, I had no idea how much of a monumental undertaking it would be. It has been a long and difficult journey, and this dissertation only gives a brief glimpse of what went into the process. Nothing contained in this dissertation would possible without the continued support of all my family, friends, and co-workers.

First and foremost, I want to thank my advisors and mentors. Javier Read de Alaniz, you gave me my first home at UCSB. You were always patient and caring, and you fostered a wonderful group environment, both in and out of the lab. Alison Butler, you gave me my second home at UCSB. Your unbridled enthusiasm for science is infectious and I appreciate that you allowed me to explore my interest in education by taking a few quarters to teach a lecture course. Morgan Gainer, you have been instrumental in helping me grow as an instructor. You have been a great mentor over the years and I cherished all our talks about education, chemistry labs, and fantasy books. Thank you to all my advisors for your support, dedication, and encouragement.

I would also like to thank those who helped guide me to this point. Thank you to my committee member Dan Little, who let me pursue my interests and write about chemical education for my research proposal. Thank you to Tim Hoag for inspiring my interest in chemistry. I do not know where life would have taken me if I didn't have you to inspire such a great interest in chemistry and to be an excellent example of a caring instructor. Thank you to Yitzak Tor for providing me with an undergraduate research experience and fostering my love of organic chemistry.

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Thank you to my parents, Brad and Cathy, and my siblings, Amanda and Matthew. All of you have supported me my whole life. You have all helped me become the person I am today. You guys never once doubted that I could do this, no matter how often I doubted it. Your unwavering belief that I had the capacity to succeed undoubtedly got me through some tough times. I love you all more than I know how to say.

Lastly, I have to thank Samantha. You are my sunshine. I can always count on you to brighten up my day, to keep me entertained, and to provide unwavering support. I couldn't have possibly finished graduate school without you. I am constantly impressed by your tenacity and determination, and I look forward to repaying all you've done for me in the years to come. You also brought Stella and Harley into my life, along with the rest of your family, so thank you for that. I should also acknowledge Grammy for keeping me fed throughout that last few years, thank you Elanor!

Curriculum Vitae of Robert Bradley Lewis

EDUCATION

University of California, Santa Barbara (UCSB) 01/2018
Ph.D., Chemistry
Certificate in College and University Teaching

University of California, San Diego (UCSD) 06/2011
B.S., Chemistry
GPA 3.75, Graduated Cum Laude, Provost Honors 3 quarters.

Moorpark Community College 06/2009
General course work; transferred to UCSD
GPA 3.71, Dean's List 4 semesters

TEACHING CERTIFICATES

Certificate in College and University Teaching, UCSB 06/2017
This certificate is awarded to doctoral students who have demonstrated the ability to plan and conduct sections using a variety of instructional strategies to promote student learning and evaluate student performance, to use instructional technologies to support instruction, and to discuss cogently and evaluate the efficacy of instructional technique.

ISEE Certificate in Inclusive Inquiry STEM Education, UCSC 10/2014
Certificate was earned for completing the Institute for Science and Engineering Educators (ISEE) Professional Development Program (PDP). The Certificate acknowledges the successful design and teaching of an inquiry laboratory experience that was designed to engage and support learners from diverse backgrounds in an equitable and inclusive environment.

AWARDS

Nominated for GSA Excellence in Teaching Award, UCSB 2016 to 2017
Graduate Student Association (GSA) Excellence in Teaching Award is a campus wide award that is presented yearly.

Outstanding Teaching Assistant Award, UCSB 2014 to 2015
The Department of Chemistry and Biochemistry at UCSB honor teaching assistants with this faculty nominated award each year.

Outstanding Service to the Department Award, UCSB 2013 to 2014
Awarded by the Department of Chemistry and Biochemistry at UCSB for exceptional leadership and outstanding commitment to the mission of the Department.

Outstanding Service to the Department Award, UCSB 2012 to 2013
Awarded by the Department of Chemistry and Biochemistry at UCSB for exceptional leadership and outstanding commitment to the mission of the Department.

UNIVERSITY TEACHING EXPERIENCE

Teaching Associate, UCSB Summer 2016 & 2017
Instructor of record for Organic Chemistry. Developed course material for large lectures for 40 or more students, built course website on university platform, implemented instructional technology to

increase student participation, and held regular office hours where I worked with students from a variety of backgrounds, majors, and ages.

Lead Teaching Assistant, Organic Chemistry, UCSB 09/2015 to 06/2017
Updated and developed new course material for organic chemistry labs. Assessed teaching assistant performance and mentored teaching assistants in teaching pedagogy.

Teaching Assistant, UCSB 08/2011 to 06/2016
Teaching assistant for several laboratory courses, including General Chemistry Labs A and B, and Organic Chemistry Lab A, B, and C. Prepared and led biweekly lectures, review sessions, and experiments. Graded quizzes, reports, and exams, providing feedback to students.

Design and Implementation of Advanced Lab Course, UCSB Spring 2013 to Fall 2017
Designed and implemented an upper division, inquiry-based organic chemistry lab course that introduces students to academic research and encourages the development of skills vital for STEM majors. Resulted in a publication in the Journal of Chemical Education.

TEACHING TRAINING

Professional Development Program, UCSC 04/2014 to 11/2014
The Institute for Scientist and Engineer Educators (ISEE) Professional Development Program (PDP) is a suite of teaching pedagogy workshops at UC Santa Cruz. Themes include inquiry, diversity and equity, and assessment.

Summer Institute for Teaching Associates, UCSB 05/2015 to 08/2015
The Summer Institute for Teaching Associates (STIA) provides student instructors guidance for course planning, syllabus design, class and lecture planning, active learning, designing learning outcomes and assessments, and giving and receiving feedback.

Lead Teaching Assistant Institute, UCSB 08/2015
The Lead prepares students to take on the multifaceted role of Lead TA by developing skills important for conflict management, the mentoring of novice TAs in pedagogy, and development of orientation material.

COMMUNITY TEACHING EXPERIENCE

Lead Mentor at CAMP, UCSB Summer 2015
Lead mentor to a group of 15 multidiscipline students from historically underrepresented groups through the California Alliance for Minority Participation (CAMP), a NSF funded program, in the Materials Research Laboratory. Designed and led workshops meant to develop skills in scientific communication, provided student feedback, and offered conflict resolution for student-mentor relationships.

Facilitator at School for Scientific Thought (SST), UCSB 09/2014 to 10/2014
Designed and implemented a 5-week course taught to high schoolers through the SST program in the Center for Science and Engineering Partnerships at UCSB. The program exposes students to scientific concepts that extend beyond the typical high school science class.

Facilitator for Campus wide TA orientation, UCSB Summer 2015
Designed and implemented a workshop titled “How to effectively lead a lab section” that was given during the campus wide TA orientation. Worked with graduate students from a variety of STEM fields.

Organic Chemistry Tutor, UCSB 2015 to 2017
Offered individual tutoring to a variety of students for organic chemistry lecture and lab courses.

Mentor for High School Science Fair, UCSB 10/2014 and 10/2015
Offered guidance for high school students in designing and carrying out experiments for their science fair projects.

SCIENCE OUTREACH AND VOLUNTEERING

SCOPE Volunteer, UC Santa Barbara 2014 to 2018
Science as a Career Outreach Project Experiment (SCOPE) sends graduate students to local high schools to encourage interest in higher education, especially in STEM fields.

5th Grade Chemistry Outreach Volunteer, UC Santa Barbara 2011 to 2014
Graduate Student Coordinator for outreach program designed to nourish natural curiosity in science and to stimulate an interest in pursuing a higher education in science.

SciTrek Volunteer, UC Santa Barbara 11/2014
Volunteers are sent to local classrooms (grades 2-8) to conduct inquiry driven modules that align with grade level standards and provide hands-on experience with rigorous scientific method.

RESEARCH EXPERIENCE

Graduate Student Researcher, UCSB 2011 to 2018
Department of Chemistry and Biochemistry
Advisors: Professors Javier Read de Alaniz and Alison Butler

Undergraduate Research Assistant, UCSD 08/2010 to 06/2011
Department of Chemistry and Biochemistry
Advisor: Professor Yitzhak Tor

PUBLICATIONS

Weaver, M.G.; Samoshin, A.V.; **Lewis, R.B.**; Gainer, M.J. Developing Students' Critical Thinking, Problem Solving, and Analysis Skills in an Inquiry-Based Synthetic Organic Laboratory Course. *J. Chem. Educ.* **2016**, *93*, 847-851.

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Abstract

Regiochemistry of Nitroso Hetero Diels-Alder Reactions, Catechol Siderophore Analogues for Surface Wet Adhesion, and an Inquiry-Based Synthetic Organic Laboratory Course

by

Robert B. Lewis

Carbon-nitrogen bonds are prevalent in a wide range of natural products and biologically active molecules. The availability of efficient methods for the construction of these bonds and means of controlling their regio- and stereochemistry is therefore of great interest to the synthetic community. The nitroso hetero Diels-Alder reactions offers a way to simultaneously generate carbon-nitrogen and carbon-oxygen bonds with high regio- and stereoselectivity. However, there is little experimental evidence to support computational models for the prediction of regioselectivity in the reaction of 2-substituted dienes with nitrosocarbonyl compounds.

A study of the reactivity of 2-substituted 1,3-butadienes with nitrosocarbonyl compounds in the 4+2 cycloaddition has been carried out showing that the regioselectivity involves a delicate balance of steric and electronic effects. 2-Aryl 1,3-butadienes favor the distal isomer with the magnitude of preference ranging from 4:1 to 15:1 depending on the electronic nature of the aryl substituent and the nitrosocarbonyl group. However, sterics can override the observed electronic preference and form the proximal isomer preferentially. The results

obtained, together with previous theoretical calculations and experimental data, provide further data to aid in synthetic planning.

Small molecule and polymeric adhesives offer a wide range of potential applications, especially in the biomedical field. In this and other application areas, the hydration layers that form at surfaces in saline solutions severely limit the ability of adhesives to form strong surface interactions that lead to adhesion. However, marine mussels regularly adhere to surfaces in wet saline conditions with a suite of specialized mussel foot proteins (mfps). These proteins rely on the intramolecular catechol-cation synergy of amino acid residues to evict the hydration layer and bond to the underlying surface. A recent theory speculates that the molecular mechanics of detachment also contribute to the synergy enhanced adhesion.

This work focuses on the use of small molecule siderophore-based mfp-mimics to further probe the observed intramolecular catechol-cation synergy. Systematically varying the spacing between the catechol and cation groups in the adhesive molecules reveals that the synergy between catechol and lysine remains effective in promoting adhesion even when the moieties are separated by one or two glycine amino acids. The results also provide evidence that the detachment sequence of catechol and lysine in mussel-inspired adhesives do not contribute significantly to the observed synergy between these moieties. This research clarifies the understanding of mussel adhesion and provides information that will inform the design of synthetic mussel-inspired adhesives.

Laboratory activities have the potential for a large impact on student learning and the development of skills vital to becoming a competent scientist. Traditional or expository teaching laboratories have received their fair share of criticism for not meeting this potential fully. There is a growing call for the departure from traditional laboratory activities and the adoption of more inquiry based instruction. In this work, a course is described where

students are engaged in an inquiry-based quarter-long research project to synthesize a known pharmaceutical target. Students use literature search engines, such as Reaxys and SciFinder, and the primary chemical literature as resources to plan and perform the synthesis of their pharmaceutical target. This course provides students with a simulated research experience in which they develop critical thinking, problem solving, and data analysis skills, along with a greater appreciation of the processes of science.

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List of Abbreviations

2,3-DHBA – 2,3-dihydroxybenzoic acid

3,4-DHBA – 3,4-dihydroxybenzoic acid

9,10-DMA – 9,10-dimethylanthracene

Ac – acetyl

AFM – atomic force microscope

Arg – arginine

Bn - benzyl

Boc – tert-butyloxycarbonyl

CAC – critical adsorption concentration

Cbz – carboxybenzyl

CTC – cyclic trichrysobactin

DCC – dicyclohexylcarbodiimide

DCHU – dicyclohexylurea

DMSO – dimethylsulfoxide

Dopa – 3,4-dihydroxy-L-phenylalanine

D_T – hardwall thickness

ESI-MS – electrospray ionization mass spectrometry

Et_3N – triethylamine

Equiv – equivalent

EtOx – 2-ethyl-2-oxazoline

Fmoc – fluorenylmethyloxycarbonyl

HDA – hetero Diels-Alder

HMBC – heteronuclear multiple bond correlation

hrs - hours

HSQC – heteronuclear single quantum correlation

hν – light

IR – infrared spectroscopy

Lys – lysine

min - minutes

Mfp – mussel foot protein

NDA – nitroso Diels-Alder

NHS – N-hydroxysuccinimide

NMO – *N*-methylmorpholine-*N*-oxide

NMR – nuclear magnetic resonance

Pd/C – palladium on carbon

PIDA – phenyliodoso diacetate

Pyr - pyridine

preCOL – prepolymerized collagen

RP-HPLC – reverse phase high performance liquid chromatography

rt – room temperature

Ser - serine

SFA – surface forces apparatus

STEM – science, technology, engineering, and mathematics

TAC – Tren-Arg-Cam

TBS – *tert*-butyldimethylsilyl

TC – Tren-Cam

TDC – Tren-Dab-Cam

TEBMA – 2,4,6-triethyl-1,3,5-benzenetrimethylamine

TEMPO – 2,2,6,6-tetramethylpiperidine-1-oxy radical

TFA – trifluoroacetic acid

TGGGC – Tren-Gly-Gly-Gly-Cam

TGGLB – Tren-Gly-Gly-Lys-Bam

TGGLC – Tren-Gly-Gly-Lys-Cam

TGLGC – Tren-Gly-Lys-Gly-Cam

THF - tetrahydrofuran

$TL^{Ac}C$ – Tren-Lys^{Ac}-Cam

TLB – Tren-Lys-Bam

TLC – Tren-Lys-Cam

TLC – thin layer chromatography

TLGGC – Tren-Lys-Gly-Gly-Cam

TLLC – Tren-Lys-Lys-Cam

TLP – Tren-Lys-Pam

TMP – Thread matrix protein

TMS – trimethylsilyl

TREN – tris(2-aminoethyl)amine

1. Nitrosocarbonyl Compounds: History and Generation

1.1 Discovery of C-Nitroso Compounds

Carbon–nitrogen bonds are prevalent in a wide range of natural products and biologically active molecules. The availability of efficient methods for the construction of these bonds and means of controlling their regio- and stereochemistry is therefore of great interest to the synthetic community. As such, C-nitroso compounds have long been a focus of organic chemists since they are reactive, ambident nitrogen electrophiles. These reactive compounds can trace their history back to 1874, when Bayer first synthesized nitrosobenzene.[1] Since then, the field has grown to encompass a variety of different C-nitroso species with varying reactivities and stabilities, including alkyl nitroso **1**,[2] vinyl nitroso **2**,[3-4] heteroaryl nitroso **3**,[5-6] α-chloro[7-9] and α-acetoxynitroso[10] **4**, and nitrosocarbonyl compounds (Figure 1.1).[11-17]

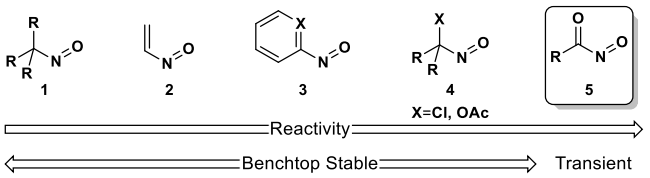

Figure 1.1 - Relative reactivities and stabilities of C-nitroso compounds

While a comparative study of the reactivity of different C-nitroso compounds hasn't been conducted, reactivity can be generalized by the electronic nature of the N-substituent. Generally, the more electron withdrawing the substituent, the more reactive the nitroso compound. Despite the exceptional reactivity of nitrosocarbonyl compounds **5**, their chemistry remained largely underdeveloped since their discovery while less reactive arylnitroso compounds were studied more thoroughly. This is largely due to the bench top

stability of arylnitroso species and the transient nature of nitrosocarbonyls. For the sake of this dissertation, the focus of the introduction will be on nitrosocarbonyl compounds.

1.1.1 History of Nitrosocarbonyl Compounds

Nitrosocarbonyl compounds are highly reactive, short-lived C-nitroso species. They were first proposed as the transient intermediate in the oxidation of hydroxamic acids.[17] However, the only early evidence for their existence were the products of nucleophilic attack at the carbonyl group to give carboxylic acids **7**, amides **8**, and O-acylhydroxamates **9**, as well as the [4+2] cycloaddition product **6** (Figure 1.2). [17-21] These transient species were first detected several decades later in the gas phase by neutralization-reionization mass spectrometry[22] then in solution by time-resolved infrared spectroscopy.[23] Toscano and co-workers estimated the lifetime of an acylnitroso species at infinite dilution in an organic solution to be on the order of 1 ms.[23] As such, nitrosocarbonyl compounds must be generated *in situ*, a sharp contrast to other benchtop stable C-nitroso compounds such as arylnitroso compounds.

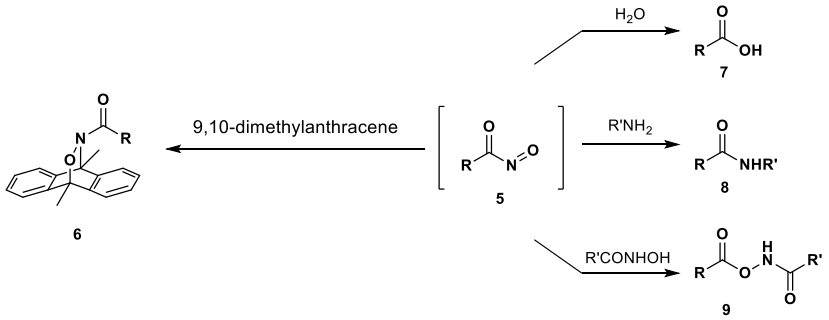

Figure 1.2 – Products from the reaction of a nitrosocarbonyl compound with various nucleophiles. Early evidence for existence of the transient nitrosocarbonyl species.

Kirby was the first to highlight the powerful dienophilic activity and synthetic potential of nitrosocarbonyl compounds.[17-18] However, the requirement for *in situ* generation and the lack of mild methods to generate this transient species caused the field of nitrosocarbonyl

chemistry to remain largely underdeveloped. In recent years, there has been an acceleration in the field with many new synthetic applications being reported as novel and mild methods of nitrosocarbonyl generation are discovered.[14-15, 24-26] Methods for the generation of nitrosocarbonyl species will be discussed below.

1.2 Nitrosocarbonyl Reactivity and Generation

Nitrosocarbonyl compounds are highly reactive and transient species. Their ambident reactivity gives these compounds exciting potential as electrophilic synthons for making carbon-nitrogen and carbon-oxygen bonds. The earliest use of a nitrosocarbonyl in a synthetic reaction was Kirby's 1973 report where thebaine **12** was used to trap the nitroso intermediate **11** as a Diels-Alder adduct **13** (Figure 1.3A).[17] It was also shown that heating the Diels-Alder adduct **14** caused a thermo-retro-cleavage which regenerated the nitroso species **11** and transferred it to another diene (Figure 1.3B).[17] This pioneering work laid the foundation for nitrosocarbonyl research for years to come.

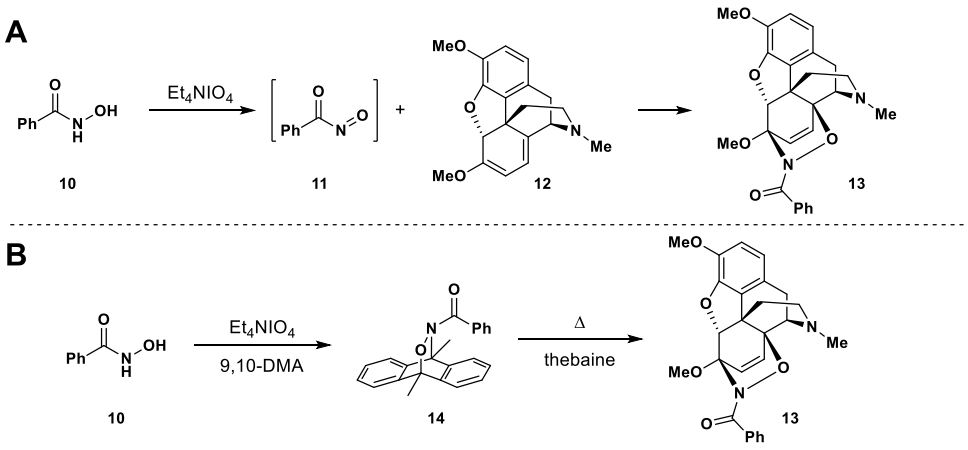

Figure 1.3 – First examples of hetero Diels-Alder with nitrosocarbonyl compounds.

The nitroso hetero Diels-Alder reaction remains the most utilized transformation involving nitrosocarbonyl species because it allows for the simultaneous regio- and

stereospecific introduction of carbon-nitrogen and carbon-oxygen bonds in one synthetic step, and provides direct access to 3,6-dihydro-1,2-oxazines. The rich chemistry of these cycloadducts has led to the use of the nitrosocarbonyl hetero Diels-Alder reaction is many natural product syntheses.[12, 14-15, 24, 27-30]

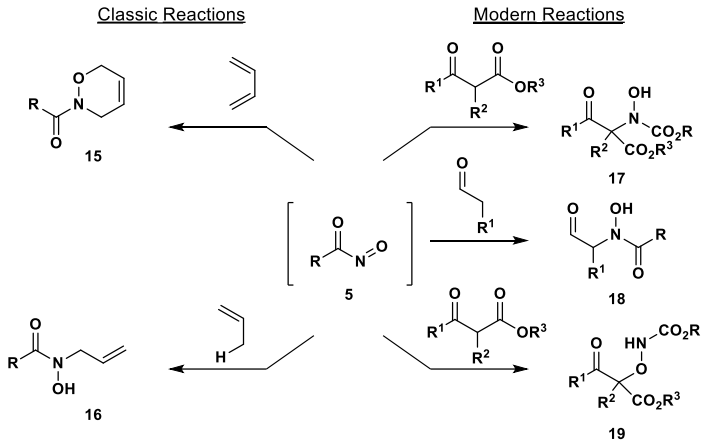

Figure 1.4 – Select examples of reactions of nitrosocarbonyl compounds.

Prior to 2012, applications of nitrosocarbonyl chemistry were limited to the hetero Diels-Alder and ene reactions. Despite the fact that Keck reported the nitrosocarbonyl ene reaction in 1981, it remains much less explored. [31-33] Nitrosocarbonyl compounds have increased in popularity in recent decades, leading to an expansion in the range of applications of these useful compounds (Figure 1.4). Many recent advances in the field of nitrosocarbonyl chemistry have been in α-hydroxylation and α-amination reactions (Figure 1.4).[34-39] Efforts to expand the field have also seen the synthesis of α-ketoamides and α-keto oximes through nitroso Henry reactions,[40] the formation of useful benzo[c]isoxazol-3(1H)-ones through a hydroamination of aryl C-H bonds,[41] and access to pyrroloindoline derivatives through a dearomatization reaction involving nitrosoformates.[42] All these novel synthetic uses are made possible by the discovery of new methods to generate nitrosocarbonyl compounds. Additionally, much of the synthetic utility of nitroso reactions lies in the ability to carry out

facile modification and post-functionalization of the resulting product. Here, nitrosocarbonyl compounds, especially nitrosoformates, offer an advantage over their other C-nitroso counterparts in that they are highly reactive, readily available from easily synthesized starting materials, and are easy to manipulate after the reaction.[15, 24]

1.3 Generating Nitrosocarbonyl Compounds

For many years, the harsh and cumbersome methods used to generate nitrosocarbonyl compounds hindered the growth of the field beyond the initially disclosed reactions. The development of novel methods to access these reactive species has led to a resurgence in the field of nitrosocarbonyl chemistry. Generally, both classical and modern methods of generation rely on the *in situ* oxidation of hydroxamic acids to access nitrosocarbonyl compounds. This route is highly attractive as hydroxamic acids are operationally simple to obtain and are largely benchtop stable.[24] However, oxidation is not the only route used to obtain a nitrosocarbonyl compound. There are also photochemical and thermal methods of generation, as well as a few unique methods. This section will highlight some of the classical and modern methods used to access nitrosocarbonyl compounds, with a focus on the oxidative routes.

1.3.1 Classical Methods of Generation

Kirby first reported the oxidation of a hydroxamic acid to the reactive nitroso species and subsequent trapping with a diene in 1973 (Figure 1.3A).[17] In this report, ammonium periodate salts were used to carry out the oxidation. Subsequently, Kirby and Keck independently demonstrated the *in situ* generation of nitrosocarbonyl species through the thermo-retro-cleavage of Diels-Alder adducts previously generated through periodate oxidation (Figure 1.3B).[17, 21, 43-44] The nitroso species is initially trapped as a Diels-Alder

adduct with a sacrificial diene, such as 9,10-dimethylanthracene, and purified to remove oxidants. This adduct can then be heated in the presence of an excess of either another diene or olefin to effectively transfer the nitroso moiety (Figure 1.5). This particular method for the generation of nitroso species is useful for more sensitive reactions, such as the nitrosocarbonyl ene, where the reaction product is susceptible to oxidation and decomposition by the harsh periodate oxidants.[43, 45-46] However, this 2-step method is cumbersome and not atom economical as it requires the use of stoichiometric oxidants, a sacrificial diene, and the use of a large excess of olefin or diene to outcompete the reverse reaction. Despite the limitations of periodate oxidation and thermo-retro-cleavage methodologies, they remained the most popular methods for several decades and were used in the synthesis of many natural products.[11, 43, 47-50]

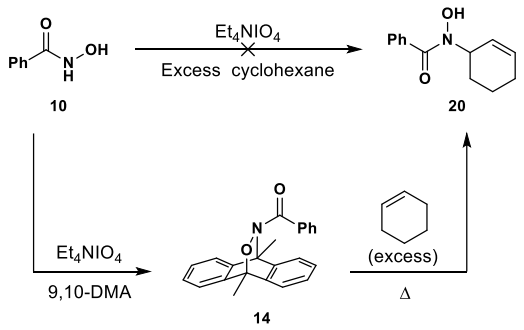

Figure 1.5 - 2-step procedure for the generation of nitrosocarbonyls.

1.3.2 Modern Methods of Nitrosocarbonyl Generation

It wasn't until the late 1990's that alternative methods for accessing nitrosocarbonyls started to surface. Caramella and co-workers reported oxidative[51-52] and photochemical[53] methods to generate nitrosocarbonyl compounds from hydroximoyl halides and 1,2,4-oxadiazole-4-oxides, respectively (Figure 1.6). These methods proved mild enough to carry

out one-pot, single step nitrosocarbonyl ene reactions, something that was inaccessible when periodate salts were used for generation.

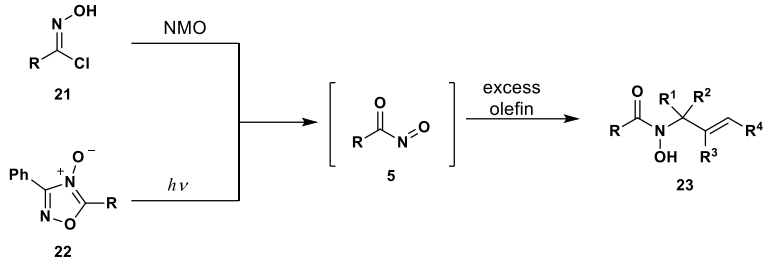

Figure 1.6 - Caramella's methods for generation of nitrosocarbonyl species.

While Caramella's work was an important step forward, a method for the mild oxidation of readily accessible hydroxamic acids was still desired. Adam and co-workers were the first to detail a non-periodate based oxidation of hydroxamic acids for use in a single-pot ene reaction.[54] Their methodology utilized phenyliodoso diacetate (PIDA) as a stoichiometric oxidant. While this methodology is high yielding for simple olefins with a variety of hydroxamic acids, yields suffered when more sensitive substrates were used (Figure 1.7).[54] A methodology using Dess-Martin periodinane for the *in situ* generation nitrosocarbonyls for the hetero Diels-Alder reaction was also reported, but low to modest yields make it an unsustainable method.[55]

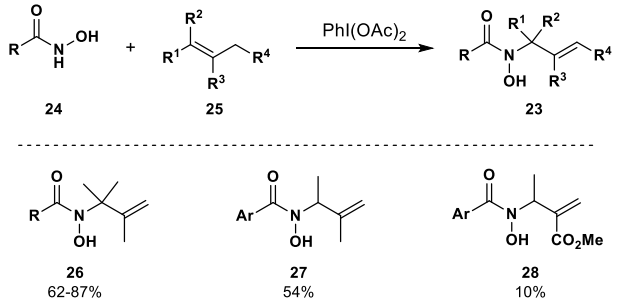

Figure 1.7 - Adam's single-pot nitrosocarbonyl ene reaction under oxidative conditions

The renaissance of methods for the *in situ* mild oxidation of hydroxamic acids really started with the work of Iwasa and co-workers.[32, 56] In their first report, a ruthenium(II)

catalyst **27** was used to carry out the *in situ* oxidation of hydroxamic acids for use in a hetero Diels-Alder reaction with respectable yields (Figure 1.8A).[56] When this methodology was extended to the nitrosocarbonyl ene reaction, conditions proved too harsh to achieve anything but modest yields.[32] However, a screening of several transition metals identified CuI as proficient catalyst for the single-pot nitrosocarbonyl ene reaction as products were obtained in high yields (Figure 1.8B).[32] Several other research groups have reported examples of the catalytic oxidation of hydroxamic acids with transition metals and stoichiometric peroxides for both the Diels–Alder[57-61] and ene reaction.[62-63]

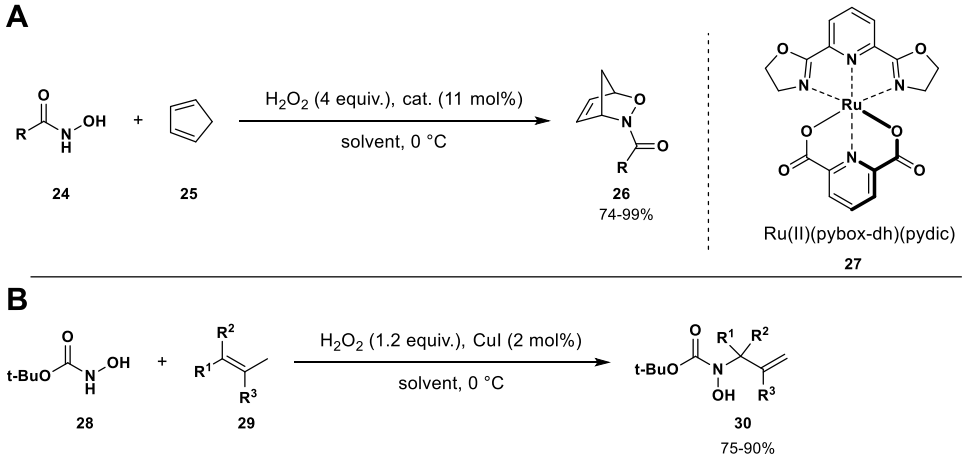

Figure 1.8 - Earliest example of transition metal catalyzed oxidation of hydroxamic acids from Iwasa.

While improved from the classic Kirby and Keck method, all these previously discussed methods suffer from many drawbacks including a limited scope, substrate dependence, low yields, the necessity for a large excess of olefin or diene, or harsh conditions that preclude them from use in the nitroso ene reaction.[26, 64] Recently, both the Whiting group[65] and the Read de Alaniz group[66] have independently developed general and mild aerobic oxidations, catalyzed by Cu(II) and Cu(I) respectively, to generate nitrosocarbonyl intermediates from hydroxamic acids. Both methods generate environmentally benign byproducts as they use

inexpensive and readily available air as the terminal oxidant. The Read de Alaniz group demonstrated the versatility of this general methodology and used it to access a variety of nitroso HDA adducts[67] and nitrosocarbonyl ene products[66] with diverse of substrates, with good functional group tolerance, in good yields, and with stoichiometric amounts of olefin (Figure 1.9). Using Oppolzer's chiral auxiliary, they were able to carry out the first intermolecular asymmetric nitrosocarbonyl ene reaction (compound **33**).[66]

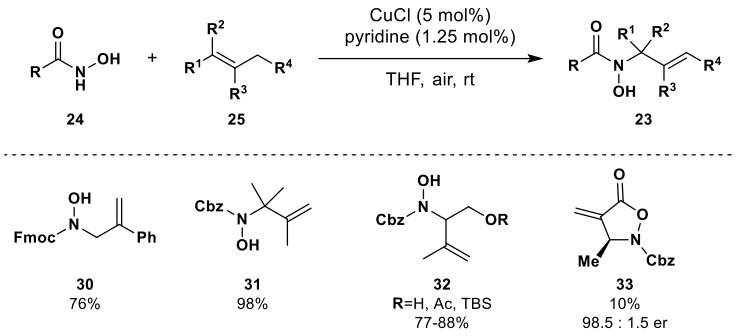

Figure 1.9 - Read de Alaniz catalytic aerobic oxidation of hydroxamic acids in the nitrosocarbonyl ene reaction.

Since the reporting of mild, copper catalyzed aerobic oxidation methodology in 2011, there has been a deluge of different mild oxidation methods reported in literature.[30] This includes, but is not limited to, Yamamoto's use of MnO_2,[34] the metal-free oxidations using TEMPO and BPO developed by Maruoka and co-workers,[68] and the various photoredox methodologies using rose Bengal[69], $Ru(bpy)_3Cl_2$,[70] or metal-organic frameworks (MOFs).[71] Recently, Toscano and co-workers reported the novel formation of nitrosocarbonyl intermediates through the elimination of a pyrazolone leaving group, though the synthetic utility of this methodology has not been investigated.[72-73] All these new methodologies aid in the study of nitrosocarbonyl reactions and help develop a deeper understanding of how to control the regio- and stereochemistry of the products.

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2. Nitrosocarbonyl Hetero-Diels-Alder Cycloaddition with 2-Substituted 1,3-Butadienes

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2.1 Introduction

Transformations that simultaneously construct carbon–nitrogen and carbon–oxygen bonds are essential for streamlining the synthesis of natural products and pharmaceutically relevant agents.[1-6] In this regard, hetero-Diels-Alder reactions between nitroso compounds and dienes have played an important role in organic chemistry since their discovery in 1947.[7] The oxazine scaffold, which results from the [4+2] cycloaddition, serves as a strategic intermediate for the synthesis of a wide-range of natural products.[8-13] Much of the utility of the nitroso hetero-Diels-Alder results from the rich chemistry of the oxazine cycloaddition product. For example, cleavage of the N–O oxazine bond results in a skeleton with a 1,4-relationship between the alcohol and amine substituents, which are valuable for further elaboration.[1-6,8-13,14-18] However, like many heterocycloadditions, the use of unsymmetrical dienes, such as 2-substituted 1,3-butadiene, necessitates regiocontrol for this transformation to be truly synthetically useful because the nitroso [4+2]-cycloaddition can provide two regioisomers. Figure 2.1 shows the regiochemical outcome for 2-substituted dienes. When the 2-substituent is close to the oxygen heteroatom of the oxazine adduct it is referred to as the proximal isomer and when the 2-substituent is close to the nitrogen heteroatom of the oxazine adduct it is referred to as the distal isomer.

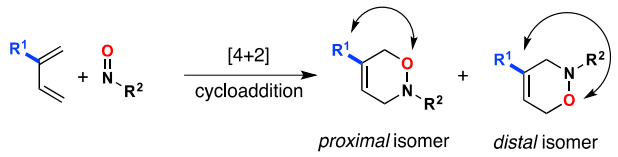


Figure 2.1 - Regioselectivity of nitroso-Diels-Alder reactions with 2-substituted dienes.

Several groups have independently reported on the regiocontrol of the nitroso-Diels-Alder (NDA) reaction using both computational and experimental studies.[19-27] As can be seen by Figure 2.2, the regioselectivity for 2-substituted dienes can depend on a combination of both steric and electronic effects on both the nitroso and the diene partners. Using FMO and DFT calculations, as well as experimental data accumulated from the literature, Houk and co-workers proposed a general rational for regiochemical preference for various monosubsubstituted dienes, including 2-substituted dienes, and nitroso sources (Figure 2.2a).[22, 28-29] They showed that the principle interaction is between the HOMO(diene) and LUMO(nitroso heterodienophile) and that there is a strong preference for an endo transition state. In general, for 2-substituted dienes the HOMO should have its largest coefficient at the C1-position and hence, the distal isomer is typically expected (Figure 2.2b). However, as seen by Figure 2.2c using an electron-rich diene in combination with an electron-poor aryl nitroso compound favors the proximal isomer.[20] Furthermore, Whitting and co-workers recently reported that the magnitude of preference for the proximal vs. distal isomer can also depends on the nature of the nitroso group.[26] For example, the reaction of nitrosoformate (Figure 2.2d) and nitrosoformamide (Figure 2.2e), generated in situ through a copper-catalyzed aerobic oxidation, with 2-methyl-1,3-butadiene gave distal and proximal isomers with a preference for the distal form that varied from ~1.7:1 to ~4.6:1 depending on the nature of the nitroso compound. Finally, Boger and co-workers showed that steric effects are also important in governing regioselectivity (Figure 2.2f).[21] Because there is a significant

unfavorable interaction between the nitrogen lone pair with the π electrons of the electro-rich diene, the endo transition state is favored.[22] This exo lone pair effect causes the diene to be in close proximity to the nitrogen substituent and if the steric interaction is large the proximal isomer can be favored. It is clear that the regiochemical preference for the NDA reaction can vary from substrate to substrate and more studies are necessary to help facilitate predictability, specifically for the nitrosocarbonyl Diels-Alder reaction. In this chapter, we report our studies with a range of both nitrosocarbonyl compounds and 2-substituted dienes.

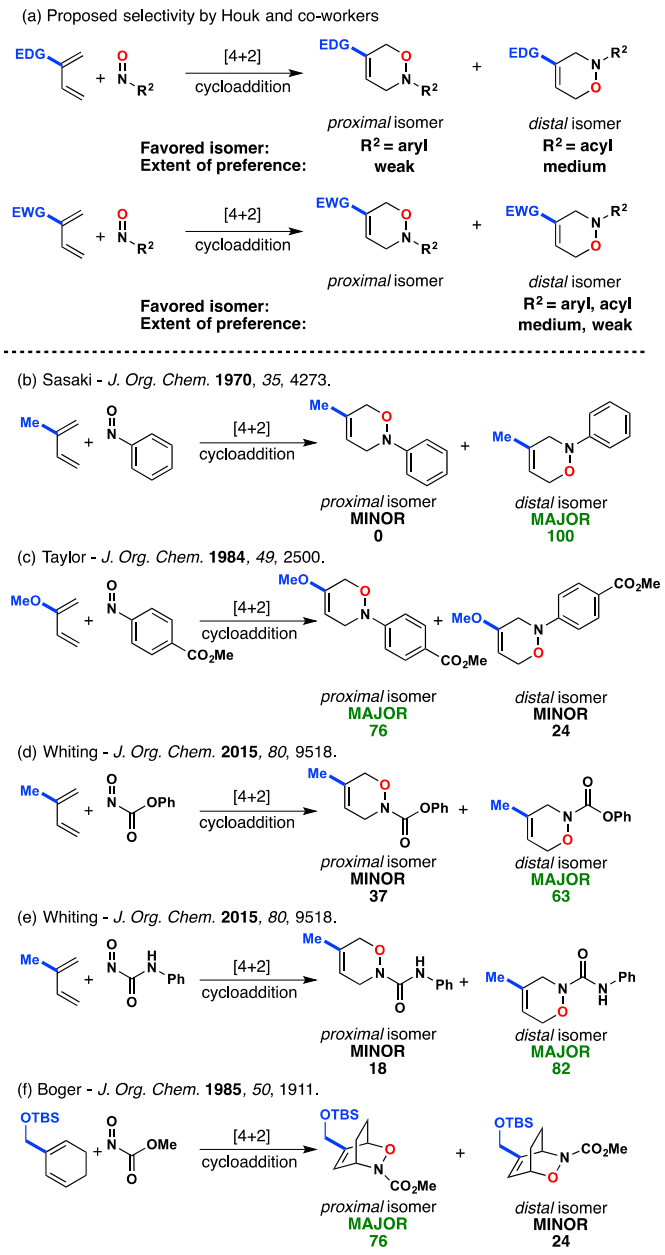


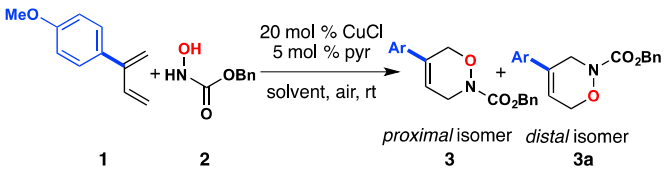
Figure 2.2 – Literature examples of regiochemical studies for the nitroso-Diels-Alder reaction with 2-substituted dienes.

2.2 Results and Discussion

We chose to study 2-aryl-1,3-butadienes initially as they avoid the potentially competing nitrosocarbonyl ene reaction due to their lack of allylic protons.[30-36] To generate the nitrosocarbonyl compound in situ we employed our previously developed copper-catalyzed

aerobic oxidation conditions (20 mol% CuCl, 5 mol% pyridine).[35, 37-40] Initially a solvent and ligand screen were conducted using 2-aryl-1,3-butadiene **1** and *N*-hydroxycarbamate **2** (Table 2.1) and in all cases we observed a preference for the distal isomer. The regioselectivity was determined with the aid of HSQC and ^{15}N HMBC. The reaction solvent and ligand for copper showed a modest influence on the overall yield of the NDA reaction, but had little effect on the observed regioselectivity. This is consistent with previous results and supports a transition state with little polar character.[21-22]

Table 2.1 - Optimization screen for NDA reaction.



proximal isomer *distal* isomer

1 **2** **3** **3a**

Ar = *p-OMePh*

Entry	Solvent	Ligand	% Yield	Proximal : Distal
1	toluene	pyridine	61	1 : 14
2	MeOH	pyridine	61	1 : 15
3	2-Me THF	pyridine	71	1 : 15
4	THF	pyridine	85	1 : 15
5	THF	ethyl oxazoline	78	1 : 15
6	THF	ethyl nicotinate	58	1 : 14
7	THF	bipyridyl	60	1 : 15

We next evaluated the electronic effects of the 2-substituent of the 1,3-butadiene on regioselectivity using *N*-hydroxycarbamate **2** as the nitroso precursor (Table 2.2, only major regioisomer shown). Based on Houk's model one would predict that the distal isomer would be preferred as the group at the 2-position increases in electron density because the interaction between the HOMO(diene) and LUMO(nitroso heterodienophile) is maximized.[22] This trend was observed in our experimental results. Electron donating aryl groups give the highest preference for the distal regioisomer with selectivities up to 15:1, while electron withdrawing aryl groups show a lower preference for the distal regioisomer

with selectivities dropping to 4:1. Interestingly, the *o*-methoxy aryl group (**11**) resulted in a modest 4:1 selectivity despite being electronically similar to the *p*-methoxy aryl group (**7**). Presumably, the reduced selectivity results from allylic strain that causes the aryl ring to rotate out of conjugation with the diene in the reactive *s*-cis conformation thus reducing electron density on the alkene and minimizing the electronic preference for the distal isomer. Consistent with the literature, 2-methyl-1,3-butadiene gave only a slight preference for the distal isomer (2:1, distal:proximal) and nitrosocarbonyl ene adduct was also observed in 38% yield.[26] However, in the case of a bulky 2-substituent the proximal isomer was formed preferentially (Figure 2.3). Of note, no nitrosocarbonyl ene reaction was observed for this example. This highlights that sterics can override the electronic preference for the distal isomer. Presumably, the reversal in selectivity is due to unfavorable interactions between the bulky group of the diene and the nitrosocarbonyl in the preferred endo transition state. While the exo transition state would lead to the distal isomer, this is unlikely due to the exo lone pair effect and hence the proximal isomer is favored in this case.

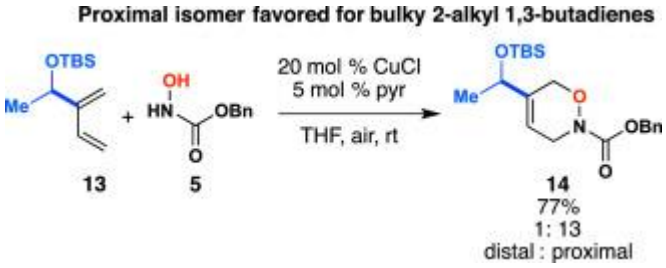
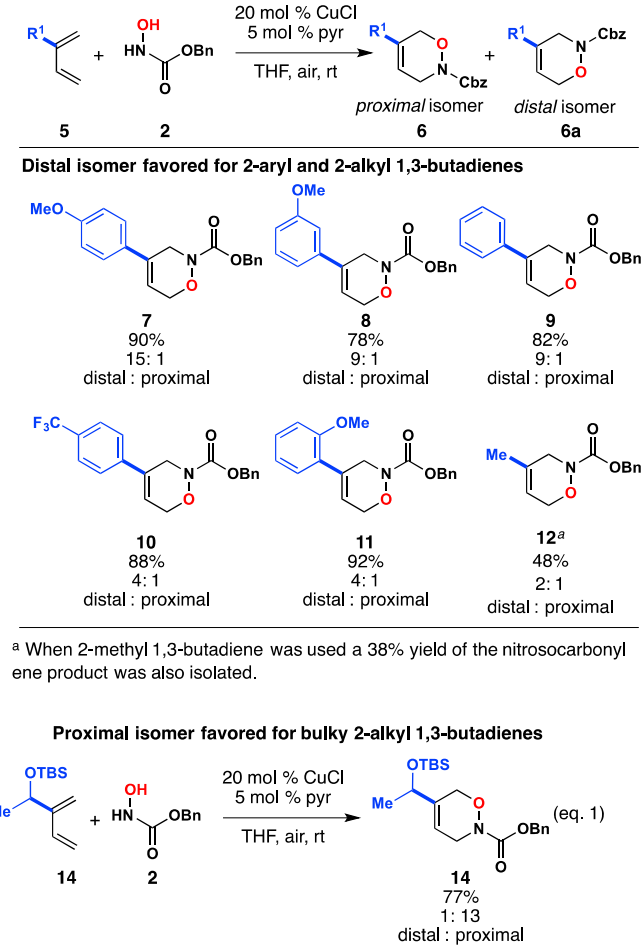


Figure 2.3 - Reaction with bulky 2-alkyl 1,3-butadiene.

Table 2.2 - Scope of nitrosoformate hetero-Diels-Alder reaction with a range of 2-substituted 1,3-butadienes.

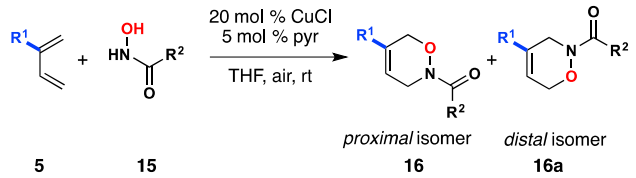


[a] When 2-methyl 1,3-butadiene was used a 38% yield of the nitrosocarbonyl ene product was also isolated.

To further study the regiochemical outcome of the NDA reaction, we next evaluated the effects of the N-substituent of the nitrosocarbonyl compound on the reaction (Table 2.3). Initially, using neutral 2-phenyl-1,3-butadiene, which gave average regioselectivity for the distal isomer (9:1), we evaluated three different formate groups (Cbz, Troc, and Boc) on the nitrogen substituent of the nitrosocarbonyl (Table 2.3, **10**, **19**, and **20**). While N-Troc protected hydroxylamine gave comparable degrees of regioselectivity to N-Cbz (8:1 and 9:1, respectively), switching to the bulky N-Boc group caused a significant drop in selectivity from 9:1 to 3:1. These results show that steric effects of the nitrosocarbonyl compound

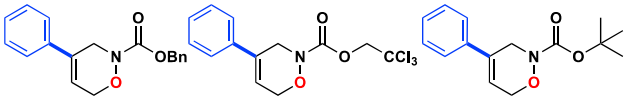
influence the regioselectivity and should be considered at the design stage. In this case, we speculate that the drop in selectivity is due to unfavorable interactions of the O-*tert*-butyl group of the nitrosocarbonyl and the phenyl substituent of the diene in the preferred endo transition state. Next, we investigated the use of nitrosoformamide derivatives, which provided the highest selectivity for Whiting and co-workers with 2-methyl-1,3-butadiene (Figure 2.2e vs. Figure 2.2d).[26] In the presence of electron rich *p*-methoxy aryl group, mixed results were observed with the preference for the distal isomer varying from 20:1 (**19**), which was an improvement compared to Cbz-nitrosoformate (**7**, 15:1), to 9:1 (**20**). Switching the methoxy group on the aryl ring to the *meta*-position decreased the selectivity from 9:1 to as low as 4:1 compared to the analogous nitrosoformate (**8** vs. **21**–**23**). However, with electron deficient *p*-CF_3-aryl group a notable increase in selectivity was observed with the formation of **24**, while adduct **25** was isolated in with a similar distal preference as the corresponding N-Cbz adduct **10**. When 2-methyl 1,3-butadiene was employed improved regioselectivity was observed (**26** and **27**), which is consistent with Whiting and co-workers results. Finally, the use of bulky 2-substituted 1,3-dienes were studied with Cbz, Troc, and Boc nitrogen protected nitrosocarbonyl compounds (**14**, **28**, and **29**). Similar to our previous results, these reactions were highly selective for the proximal isomer with selectivities up to 1:17. However, unlike the 2-aryl substituted dienes, the selectivity was improved when bulky N-Boc group was used.

Table 2.3 - Scope of nitrosocarbonyl hetero-Diels-Alder reaction with a range of 2-substituted 1,3-butadienes.



5 15 16 16a

Distal isomer favored for 2-aryl and 2-alkyl 1,3-butadienes

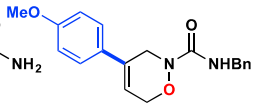


9
82%
9 : 1
distal : proximal

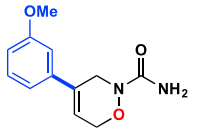
17
81%
8 : 1
distal : proximal

18
77%
3 : 1
distal : proximal

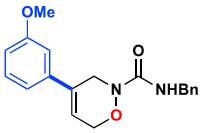
19
86%
20 : 1
distal : proximal



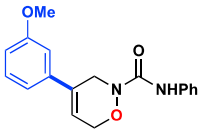
20
59%
9 : 1
distal : proximal



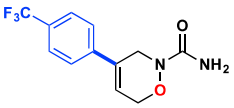
21
82%
4 : 1
distal : proximal



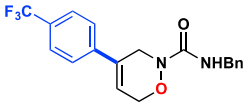
22
77%
7 : 1
distal : proximal



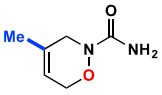
23
64%
4 : 1
distal : proximal



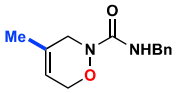
24
72%
15 : 1
distal : proximal



25
58%
4 : 1
distal : proximal

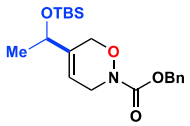


26[a]
20%
4 : 1
distal : proximal

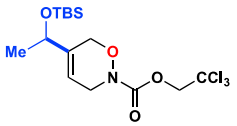


27[a]
33%
5 : 1
distal : proximal

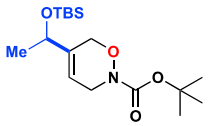
Proximal isomer favored for bulky 2-alkyl 1,3-butadienes



14
77%
1: 13
distal : proximal



28
88%
1: 13
distal : proximal



29
81%
1: 17
distal : proximal

[a] When 2-methyl 1,3-butadiene was used the ene adduct was also isolated (**26** = 10% and **27** = 12%, respectively).

2.3 Conclusion

In conclusion, this study revealed that the hetero-Diels-Alder cycloaddition of nitrosocarbonyl compounds and 2-substituted 1,3-butadienes can afford either the distal or proximal isomer preferentially. The factors governing the regioselectivity depend on the both the steric and electronic effects of both the diene and nitroso reagent. With aryl groups at the 2-position, the distal isomer is always favored but the ratio can vary depending on the sterics of the formate group appended to the nitrosocarbonyl. In this case, N-Boc gave the lowest selectivity. While nitrosoformamides consistently afforded oxazine adducts with a preference for the distal isomer, the factors governing the selectivity was less predictable. This work also showed that the proximal isomer is favored when a bulky 2-substituted 1,3-butadiene is used. In general, the results can be rationalized using Houk's model where there is a strong preference for the endo path and the regioselectivities of 2-substituted dienes involves the delicate balance of stereoelectronic effects. The results presented will help aid in synthetic planning and provide further examples to strengthen the predicted outcome of the nitrosocarbonyl hetero-Diels-Alder reaction.

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3. Wet Adhesion

3.1 Hydration layers at wet solid surfaces

Adhesives must form an intimate interaction with a surface to display their characteristic sticky properties. Most synthetic adhesives available are incapable of forming the necessary strong interactions when they are faced with wet surfaces. This lack of adhesion is due to a hydration layer that forms at wet surfaces, impeding adhesive interactions. Disruption or eviction of the hydration layer must occur for an adhesive molecule to interact with a surface.

Additional repulsive forces occur when hydrated cations adsorb to a surface, such as mica, making a thin hydrated salt layer. This repulsive hydration force keeps hydrophilic surfaces and macromolecules, such as adhesive proteins or polymers, well separated in aqueous solutions, thus preventing intimate interactions from forming.[1] Mica, a hydrophilic and negatively charged aluminosilicate mineral, has been used extensively to demonstrate that repulsive hydration forces arise due to hydrated cations, such as K^+ or Na^+, adsorbing to the negatively charged sites of the mineral surface.[1-5] Understanding how a wet adhesive can overcome these hydration forces to form a strong adhesive bond is important for the development of functional synthetic adhesives. We can look to Nature for inspiration in solving this problem as many marine organisms have found ways to adhere to surfaces in wet, saline environments. Mica is a well-studied mineral that allows for molecular-level insight into adhesive mechanisms and serves as an ideal model substrate for the mineral surfaces to which many marine organisms commonly attach.

3.2 Wet adhesion by marine mussels

Many marine organisms have developed methods to adhere to solid surfaces in their marine environments. Mussels in particular have been a large focus of research for several decades. These sessile marine mollusks are often found in the intertidal zone, an environment of harsh extremes with varying salinity, temperatures, and physical forces from crashing waves.[6-7] Despite these conditions, mussels have found a way to strongly adhere to surfaces.

3.2.1 Mussel Byssus

Mussels use a set of radially distributed proteinaceous threads, known as the byssus, to anchor themselves to a surface. At the distal end of these threads are specialized plaques that provide an interface between the byssus and a solid surface. The proximal end of the threads remains attached to the mussel via the stem and root, which allows for the mollusk to control thread tension with a set of muscles.[8] Each thread of the byssus is produced in a method reminiscent of injection molding, where the proteins that make up the plaque and thread are secreted by the mussel foot into the distal depression (Figure 3.1).[9] The average mussel has 50 to 100 threads to maintain its hold to a surface.[10]

While all portions of the byssus are important for secure attachment, the plaque is responsible for surface adhesion. Though there is some differentiation between species, most mussel byssus contain 25-30 different proteins. About 7-8 of these proteins are found in the plaque, while 5 are unique to the plaque. These unique mussel foot proteins (mfps) are mfp-2, mfp-3, mfp-4, mfp-5, and mfp-6.[11] All of these proteins contain 3,4-dihydroxyphenyl-L-alanine (Dopa), a posttranslationally modified amino acid, in varying concentration.

However, they all have widely varying sequences and are specialized for their individual roles, which gives the byssus a surprising degree of hierarchal structure (Figure 3.1).[10]

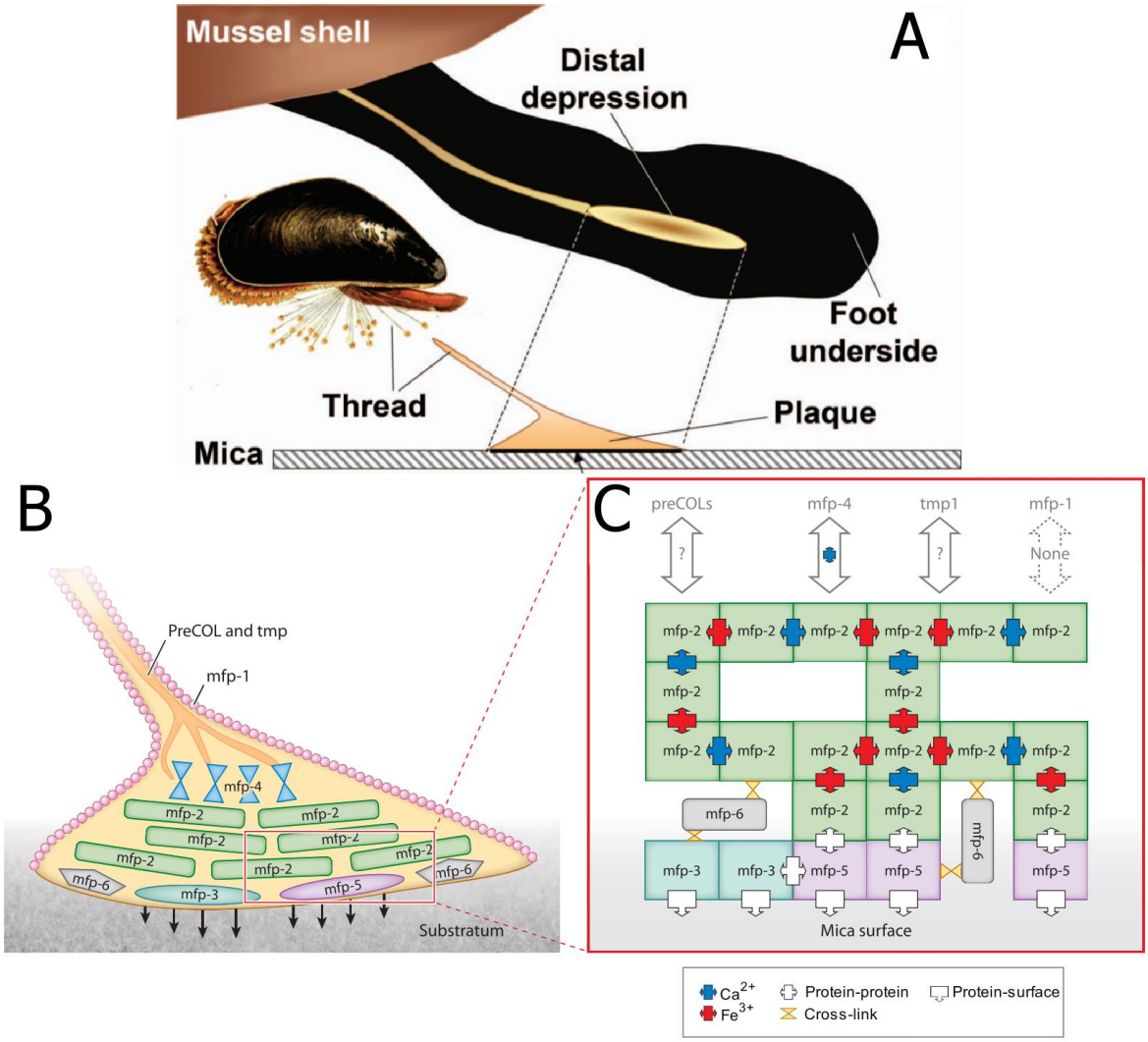

Figure 3.1 - Schematic view of mussel byssus and mussel foot proteins (mfps) of *Mytilus*. (a) Each plaque is made by the mussel (inset) in a few minutes in the distal depression of the foot. (b) Molecular model of the plaque. Approximate distribution of known plaque proteins is shown in relation to the substratum. Mfp-3 variants and mfp-5 are thought to be the adhesives (arrows). The red boxed region is enlarged in panel c. (c) Schematic view of all known mfp interactions as determined by the surface forces apparatus (SFA). Interactions denoted by a question mark remain to be determined. Tmp denotes thread matrix protein and preCOL denotes prepolymerized collagen. Adapted from references [10, 12].

3.2.2 Mussel Foot Proteins

Mfp-1, though not isolated to the plaque, also contains Dopa. This particular mfp is the major component of the cuticle coating of byssal threads.[13] The cuticle displays remarkable mechanical properties in that it has high extensibility while still being stiff. These properties can be attributed to the fact that two variants of mfp-1 exist within the cuticle and they differ in their concentration of Dopa (10 mol% vs 20 mol%).[14] Each variant can complex Fe^{3+} to a different extent, based on their Dopa concentrations, which leads to an interesting fine structure. Hard granules of mfp-1 with high Fe-Dopa crosslink density are surrounded by a matrix of mfp-1 with lower Fe-Dopa crosslink density, giving the cuticle its impressive simultaneous extensibility and stiffness.[14-16] More on the interesting physical and biological properties of mfp-1 and its variants can be found in the literature.[13-17]

The most abundant plaque protein, mfp-2, accounts for 25 wt% of the plaque and contains <5 mol% Dopa.[9-10, 18] This protein provides much of the cohesive strength of the plaque through limited cross-linking and cooperative complexation of Fe^{3+} and Ca^{2+} with other mfp-2s, mfp-3, and mfp-4 (**Error! Reference source not found.**).[10, 19] Though it has a low Dopa concentration (2 mol%), mfp-4 cooperatively binds Ca^{2+} to interact with mfp-2. Mfp-4 is a histidine rich protein that also strongly binds copper, which allows it to link the fibrous collagen core of the thread, the preCOL, and provide a strong bridge between the thread and plaque.[20]

Mfp-3 and mfp-5 are some of the first proteins deposited by the mussel foot and are found primarily at the plaque-surface interface. These so-called vanguard proteins contain the highest levels of Dopa of any mfp and are surface priming proteins that are primarily responsible for adhesion of mussel plaques to surfaces.[21-22] Mfp-3 has a large number of

variants, often grouped as either fast or slow, a classification determined by movement in electrophoresis.[23-24] The slow isoforms contain a greater number of hydrophobic residues (15 mol%) and fewer cationic residues (9 mol%), which lends the isoforms increased resistance to oxidation and provides an alternative means of plaque cohesion through inter- and intramolecular hydrophobic interactions.[25] Both mfp-3 fast and mfp-5 contain high levels of Dopa (20 and 30 mol%) and cationic amino acid residues (24.5 and 22.6 mol%). However, mfp-3 fast contains a large amount of arginine where mfp-5's cationic residues are almost exclusively lysine.[26-27] These cationic residues potentially play a dual role by first helping to prime the surface by evicting hydrated salt ions and then by forming adhesive electrostatic interactions with the negatively charged surface.[28-33]

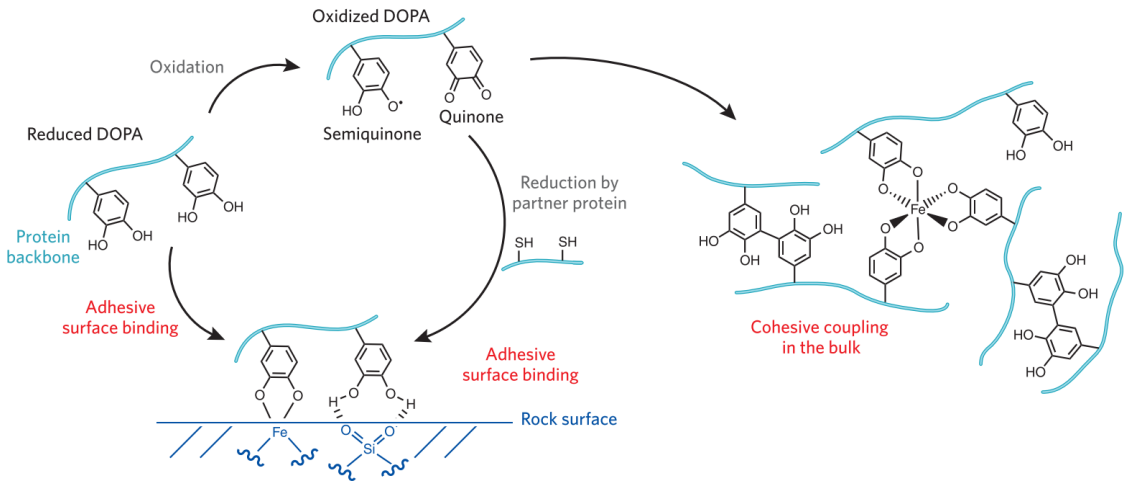

Figure 3.2 - Role of Dopa is adhesive and cohesive interactions. The reduced catechol form of Dopa binds surfaces directly for adhesive bonding (left). The oxidized states of dopa, the semiquinone and quinone, may be reduced by a thiol-rich partner protein (mfp-6) to regain surface-binding ability. Cohesion within the bulk material (right) can be brought about by metal ion complexation and oxidation chemistry, including radical-radical coupling. Reproduced from reference [34]

The prevalence of Dopa in all mfps suggests its importance in mussel byssus formation and function. Indeed, Dopa has been shown to contribute to plaque adhesion

through interfacial surface interactions[19, 35-41] and byssus cohesion through metal coordination and covalent crosslinking (

Figure 3.2).[14, 19, 37, 42-46] This posttranslationally modified amino acid owes its remarkable versatility to the catechol moiety of the sidechain. Catechols form strong, noncovalent bonds with metal ions, metal oxides, and silicon dioxide found on mineral surfaces.[47-48] The catechol of Dopa-containing proteins can also form strong hydrogen bonds with other protein sidechains as well as with hydrogen bond donor sites of surfaces, lending bulk cohesion and surface adhesion, respectively.[47-48] Catechol is also a readily oxidized reducing agent susceptible pH-dependent to one- and two-electron oxidations to generate semiquinone and *o*-quinone respectively.[22, 49-50]

Oxidation of Dopa to the semiquinone or *o*-quinone can lead to covalent cross-linking of proteins and increased bulk cohesion.[12, 37, 51-52] However, this oxidation significantly reduces the ability of Dopa to strongly adhere to a surface.[38, 53] A balance must be maintained to allow for interprotein interactions such as covalent crosslinking and cation complexation, as well as significant surface interactions. Since Dopa can readily oxidize at neutral pH, and sea water is typically at pH ~8, a method to retard the oxidation of Dopa would be beneficial for mussel plaque formation.[30, 49] Mfp-6 is secreted along with the surface priming proteins mfp-3 and -5, but shows only weak adhesive properties.[53] Mfp-6 has been shown to rescue the adhesive ability of oxidized mfp's by reducing the *o*-quinone back to Dopa.[53] This protein contains high levels of cysteines that maintain a redox balance during plaque formation as well as allow it to form Dopa-cysteinyl cross-links to add to the cohesive strength of the plaque.[27]

3.3 Synergy of catechol and cationic residues

To achieve robust surface adhesion in wet environments, the surface must be primed by removing the hydration layer so that interfacial interactions between the adhesive molecule and surface can form. The two surface priming proteins, mfp-3 and mfp-5, contain a high concentration of Dopa (20-30 mol%). Dopa has been extensively studied over the past few decades and its contributions to surface adhesion and bulk cohesion of the adhesive plaque have been elucidated. Studies using a surface forces apparatus (SFA) have shown that mfps can displace the hydration layer on a mica surface, allowing Dopa to form strong bridging interactions between the mica surfaces.[21, 25, 39, 41] However, the mechanism of how mussels remove the hydration layer to affect adhesion was not fully understood for some time. Mfp-3 and mfp-5 are also rich in cationic residues, primarily lysine (Lys) and arginine (Arg), often in adjacent positions and stoichiometric amounts to Dopa.[23, 26-27, 54] This abundancy and proximity of the two groups hint at a possible synergistic relationship. However, studying contributions towards surface drying and adhesion of individual amino acids in these intrinsically disordered proteins is not possible. An analogous small molecule system can provide a more tractable approach to investigating these effects.

3.3.1 Catechol Siderophores

Most bacteria, whether terrestrial or marine, pathogenic or non-pathogenic, require iron for fundamental biological processes. However, in most environments, the solubility of Fe^{3+} limits its availability, putting it at concentrations many orders of magnitude below necessary levels.[55] Bacteria have developed several iron acquisition pathways in order to acquire this essential nutrient.[55] Many bacteria produce small molecule iron chelators known as siderophores. Siderophores have a high affinity for iron, with some iron-siderophore

complexes like Fe(enterobactin) having a proton independent stability constant of 10^{49}.[56] These compounds are excreted into the environment or anchored in the outer membrane where they can strongly bind iron, increase its solubility, and deliver the iron complex to the cell.[55] Uptake of the Fe(III)-siderophore complex occurs through outer membrane receptor proteins that recognize and transport the complex across the membrane for further processing and subsequent use. Siderophores contain several different functional groups, including hydroxamic acids, α-hydroxycarboxylic acids, and catechols that are used to bind and sequester iron for use by the microbe. Evidence suggests catechol siderophores, like enterobactin and pyoverdines, can play another role by forming complexes with metal surfaces and aid in biofilm formation by anchoring cells to the metal surface.[57-58]

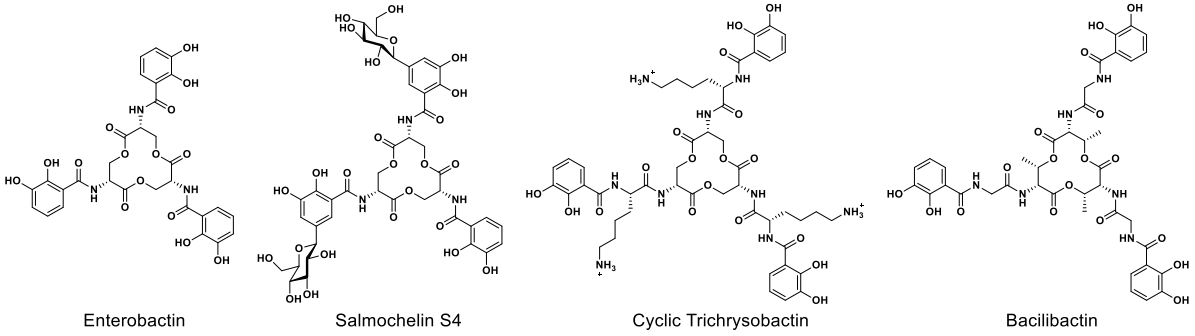

Figure 3.3 – Examples of tris-catechol siderophores. Structures of enterobactin, salmochelin S4, cyclic trichrysobactin, and bacillibactin

Tris-catechol siderophores are some of the most thoroughly studied siderophores. Enterobactin, produced by many enteric and pathogenic bacteria, including *E. coli*, is a cyclic trimer of 2,3-dihydroxybenzoyl-L-serine (2,3-DHBA-L-Ser). Other tris-catechol siderophores include bacilibactin, salmochelin S4, and cyclic trichrysobactin (Figure 3.3). Cyclic trichrysobactin (CTC) is a cyclic trimer of 2,3-DHBA-D-Lys-L-Ser produced by plant pathogen *Dickeya chrysanthemi*.[59] This siderophore contains adjacent lysine and catechol

groups in a similar mol% to the strongest adhesive protein, mfp-5 (Figure 3.4). Cyclic trichrysobactin is an excellent small molecule analogue to the more complex adhesive protein mfp-5, which allows for the straightforward study of cation effects on adhesion and surface drying

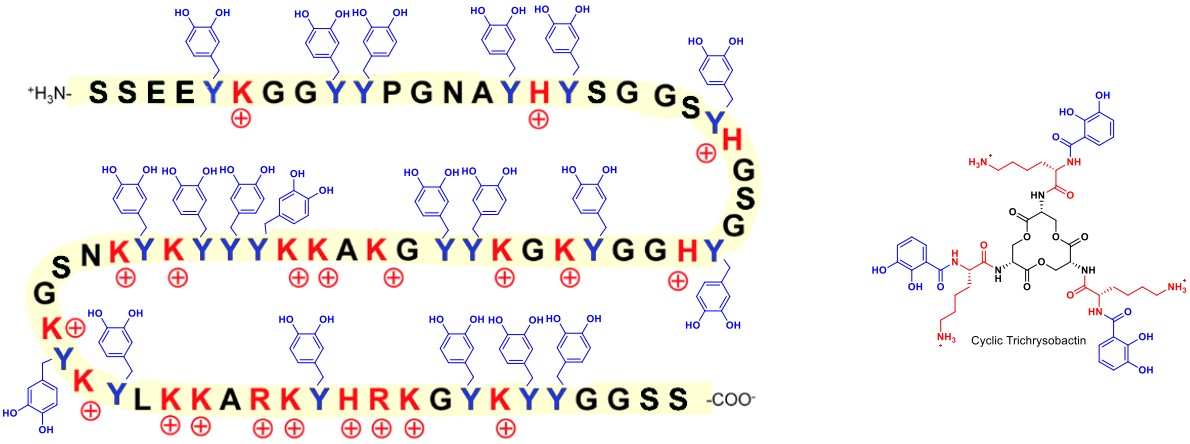

Figure 3.4 - Sequence of mfp-5 and structure of cyclic trichrysobactin. Red residues are positively charged, and blue residues are Dopa modifications (Y) depicted with catecholic sidechains. Figure Adapted from [21].

The catechol functional group found in most catechol siderophores, including cyclic trichrysobactin, is a 2,3-dihydroxybenzoyl group rather than the 3,4-dihydroxyphenyl configuration of Dopa found in mfps. The 2,3-dihydroxybenzoyl configuration offers the advantage of being less readily oxidized due to the intramolecular hydrogen bonding and electron withdrawing effects of the carbonyl group (Figure 3.5).[30, 50] While catechol oxidation leads to beneficial protein crosslinking in Dopa-rich mfps, oxidation resistance is advantageous for microbial siderophores because it allows them retain their metal binding abilities over a wider range of pH. Employing this oxidation resistant 2,3-dihydroxybenzoyl configuration in siderophore analogues allows them to be studied over a wide range of pH

without compromising their ability to adhere to a surface or introducing potential cross-linking side reactions that can obfuscate adhesion data.

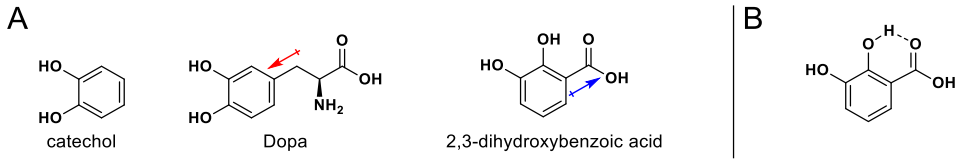

Figure 3.5 – (a) Structures catechol and catechol derivatives. Arrows indicate the electron-donating or electron-withdrawing effects of the substituents. (b) Intramolecular hydrogen bonding of 2,3-dihydroxybenzoyl moieties.

3.3.2 Wet Adhesion of Tren-Based Siderophore Analogues

Surface priming adhesive proteins, mfp-3 and mfp-5, can displace hydration layers and adhere to a variety of surfaces. In SFA experiments, they show a maximum adhesion at pH 2.6 to 3.3, while the measured adhesion drops significantly at pH 5.5 and is completely abolished above pH 7.5.[21-22, 25, 41] Cyclic trichrysobactin was able to displace a layer of hydrated salt ions from a mica surface and showed remarkable adhesion at pH 6.7.[30] However, the lactone core of CTC is susceptible to hydrolysis at the acidic pH associated with mussel plaque formation (pH 2.2–3.3).[60] Raymond and co-workers have synthesized various tris-catechol siderophore analogues using *tris*(2-aminoethyl)amine (Tren) in place of the tris-serine or tris-threonine macrolactone core.[61-63] This Tren scaffold for siderophore analogues provides an easily synthesized and modified molecule that is more stable over a wide range of pHs.

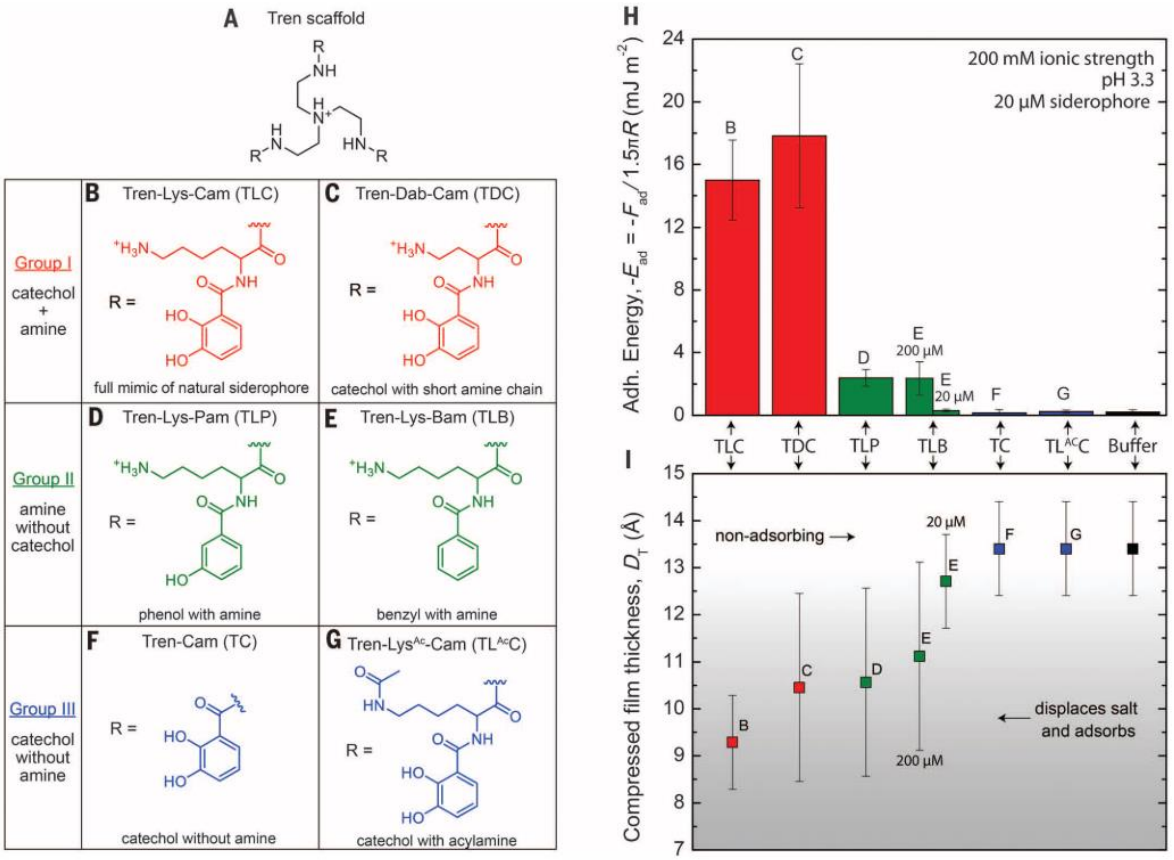

Figure 3.6 – The synergy of catechol and lysine in siderophore analogues. (A) Structure of the Tren scaffold. (B to G) The R groups appended to Tren. (H) The average adhesion energy required to separate two mica surfaces adsorbed with 1 nmole (20 mM, except where indicated at 200 mM) of the homolog in buffer (50mMacetate + 150mMKNO3) at pH3.3 after 10min of contact. (I) D_T of the siderophore monolayer between two mica surfaces at 10mN/m of compressive load. The film thicknesses correspond with the adhesion energy displayed in (H). A decreased film thickness (<12Å) indicates that homologs B, C, D, and E (200 mM) adsorb, displace hydrated salt at the mica surface, and mediate adhesion between two mica surfaces. Reproduced from [30].

A suite of siderophore analogues based on the Tren backbone was synthesized by Maier et al. to probe the influence of individual amino acids to surface adhesion and investigate any synergistic relationships with catechol.[30-31] Specifically, the effects of cationic residues such as lysine and arginine were investigated. Tren-Lys-Cam (TLC), a synthetic analogue of CTC, was shown to mediate strong adhesion between two mica surfaces from pH 3.3 to 7.5, with nearly identical behavior to CTC.[30] Other Tren-based homologues with variations in the

aromatic group and cationic amine residue were used to determine specific contributions of each group to surface drying and adhesion (Figure 3.6). Removing one or both hydroxyl groups from the catechol group while maintaining the cationic amine significantly reduces overall adhesion of the molecule. However, the molecules still show an ability to displace the hydrated salt layer and adsorb to the mica surface as shown by the decrease in film thickness (<12 Å) when the mica surfaces were compressed (Figure 3.6). Conversely, when the lysine is either acetylated or removed completely, the homologues can no longer displace the hydrated salt layer to adsorb to the surface and show no subsequent adhesion. Ultimately, these results demonstrate that there is a synergistic effect that occurs between catechol and adjacent cationic groups. The cationic group is required to evict the hydrated salt layer and expose the underlying mineral surface to allow for catechol to form strong adhesive interactions (Figure 3.7). These observations provide a rationale for the design of mfp-5, with its stoichiometric and often adjacent Dopa and lysine residues, and explains its exceptional ability to dry surfaces and adhere strongly.

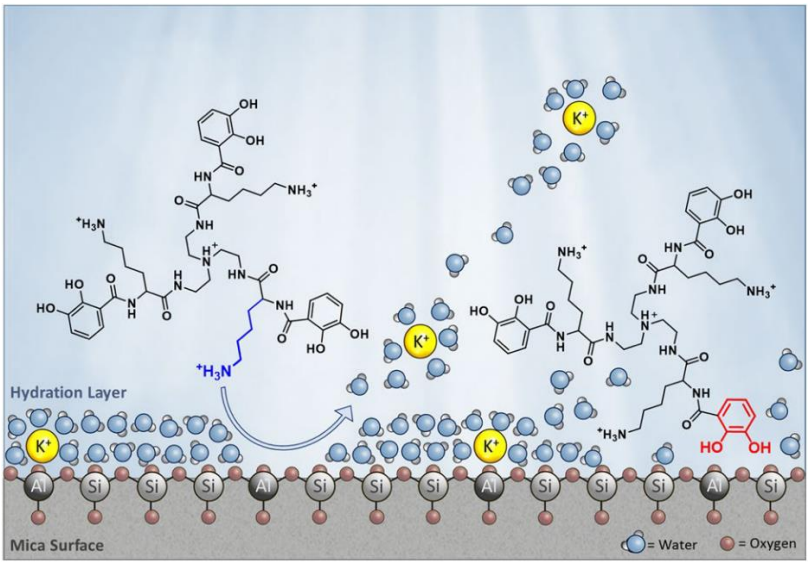

Figure 3.7 - Catechol-cation synergy in siderophore analogues. Reproduced from [32].

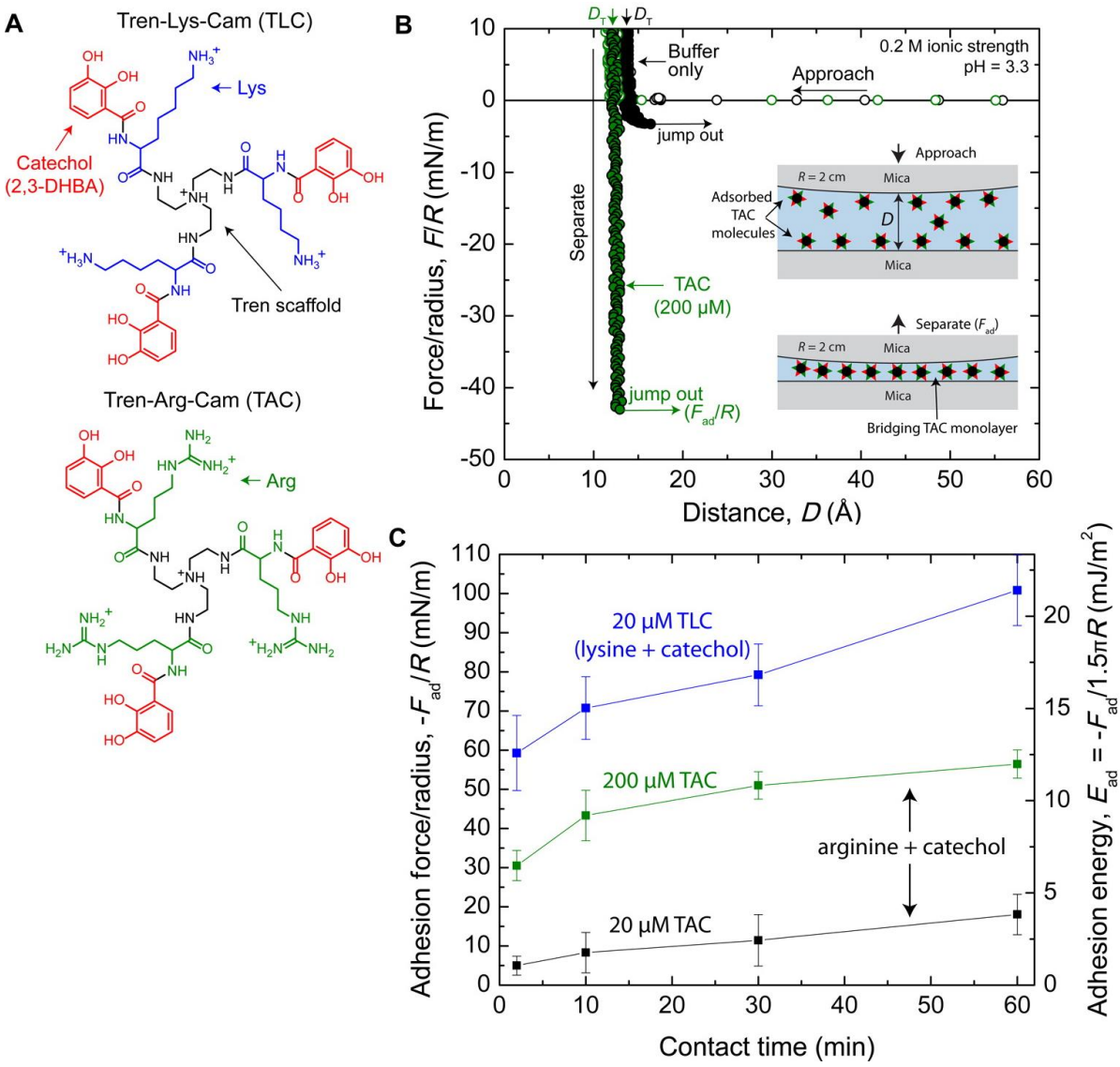

Figure 3.8 - (A) Structures of the siderophore analogues TLC and TAC. (B) SFA force−distance interactions for two mica surfaces in aqueous buffer (150 mM KNO3 + 50 mM acetate, pH 3.3) (black circles) and in 200 μM TAC (green circles). Open circles represent measurements during the approach of the two surfaces, while solid circles represent measurements during separation. The inset depicts the surfaces as they interact throughout the measurement. (C) TLC- and TAC-mediated adhesion forces, F_{ad}, and energies, E_{ad}, required to separate two mica surfaces in aqueous solution, as functions of the time the surfaces were left in adhesive contact. Error bars represent one standard deviation. Reproduced from [31].

Arginine is another common cationic residue found in mfp-5 and, to a much greater extent, in mfp-3 fast.[24-25] A Tren-based siderophore analogue incorporating arginine, Tren-

Arg-Cam (TAC) , can also displace the hydrated salt layer from a mica surface and promote adhesion, although there are some differences from the lysine containing homologue.[31] TAC requires a concentration approximately 10 times higher than TLC before the molecules adsorb to the surface and it adheres at 50-60% the strength (Figure 3.8). This increased critical adsorption concentration is due to the greater electrostatic charge density of lysine which allows it to form stronger interactions with the negatively charged sites on mica, as compared with the delocalized charge of the larger arginine moiety.[31, 64]

Interestingly, comixtures of a siderophore homologue with only catechol (Tren-Cam) and various cationic amines do not show the same synergistic effect of the intramolecular configuration in SFA studies.[31] Intramolecular adjacency of catechol and cationic residues leads to a significant synergistic effect and greater adhesion. Small changes in the separation distance between these two groups do not significantly alter the adhesive strength, but some cutoff separation should exist beyond which the synergy is not observed.[31]

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4. Catechol-Lysine Spacing and its Effect on Adhesion Synergy

4.1 Introduction

Adhesives use van der Waals interactions, hydrogen bonding, coordinate bonding, or covalent bonding to form adhesive interactions with a surface. However, many factors inhibit the effectiveness of adhesives in wet environments. In aqueous solutions, adhesives must displace a water layer bound to the substrate to make adhesive interactions with the surface, and the high dielectric constant of water reduces the strength of the van der Waals attractions. Furthermore, biologically relevant environments contain oxidants which degrade adhesive materials, and dissolved salts which screen electrostatic interactions. Charged surfaces in electrolyte solutions develop a surface layer of adsorbed, hydrated cations that act as a substantial barrier to adsorption of adhesives.[1-2] Despite these challenges, marine mussels strongly adhere to a variety of surfaces under water.[3] Mussels achieve this impressive feat through the use of a holdfast known as the byssus. The byssus is a proteinaceous thread with an adhesive plaque at the distal end. The plaque contains a suite of specialized mussel foot proteins (mfp) with an unusual abundance of 3,4-dihydroxyphenylalanine, a catecholic amino acid commonly referred to as Dopa, which has been shown to adhere to a variety of substrates.[4-6] The adhesive nature of the catechol moiety of Dopa make it an attractive functional group for use in synthetic adhesives.[7-9]

Despite Dopa being an attractive candidate for incorporation into synthetic adhesives, the mechanism by which Dopa adheres to charged, wet surfaces is not fully understood. Maier and Rapp et al. have proposed that cationic residues neighboring Dopa in protein adhesives evict surface bound hydrated cations, facilitating bidentate hydrogen bonding or

coordination bonding between Dopa and the substrate.[10-11] Additionally, Li et al. have proposed that molecular mechanics of detachment contribute to the synergy between Dopa and cationic residues.[12] They invoke a theory of adhesion proposed by George Bell[13] to argue that paired Dopa and lysine residues have a larger bond energy with substrates than Dopa alone. Therefore, detachment of the residues simultaneously requires a larger force than sequential detachment.[12]

4.2 Chapter Objectives

To determine whether the detachment order of catechol and lysine influence the adhesion energy, siderophore inspired adhesive molecules were synthesized. Siderophores are small molecule iron chelators produced by microorganisms. They use catechol, among other functional groups, to sequester iron for cellular uptake. Synthetic analogues of siderophores containing catechol and amine groups were shown by Maier et al. to adhere strongly to mica, with the authors attributing the observed synergy between catechol and cation to salt eviction.[10-11] Further variations of Maier's siderophore analogues were synthesized for this work. These adhesive molecules incorporate three arms branching from a central *tris*(2-aminoethyl)amine (Tren) scaffold. Three siderophore analogues have been synthesized, in which the catechol in each arm is separated from the amine by either zero, one, and two glycine residues (Figure 4.1). Systematically varying the spacing between the catechol and cation groups in the adhesive molecules allowed a direct test of the proposed mechanism based on the Bell Theory. It was hypothesized that if the Bell Theory mechanism contributed to catechol-cation synergy, the addition of glycine spacers between catechol and cation would lower the interfacial energy between a siderophore analog film and a mica substrate. Therefore, the force required to separate two mica surfaces with an intervening siderophore

analog film would be reduced for the siderophore analogues with one or two glycine spacers (**2** and **3** in Figure 4.1, respectively) relative to the siderophore analogue with no glycine spacer (**1**, Figure 4.1).

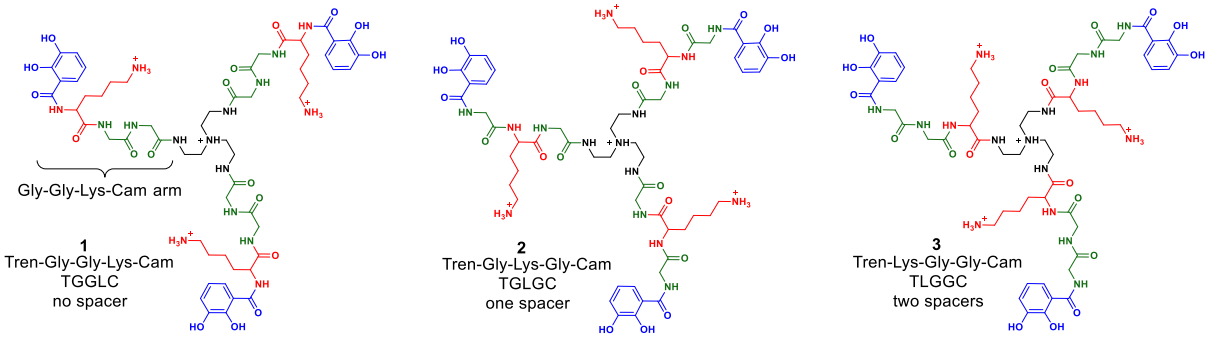


Figure 4.1 - Structures of siderophore analogues

4.3 Experimental

4.*3.1 Materials*

2,3-dihydroxybenzoic acid (2,3-DHBA) and tris(2-aminoethyl)amine (TREN) were purchased from Sigma Aldrich. THF, acetic acid, triethylamine (NEt_3), glycine, N-hydroxysuccinimide (NHS), and potassium hydroxide were purchased from Fisher. Glycine trimer (H-Gly-Gly-Gly-OH) was purchased from Chem Impex International. Benzyl bromide and N,N'-dicyclohexylcarbodiimide were purchased from Alfa Aesar. H-Lys(Z)-OH was purchased from Bachem. Palladium on carbon (10 wt %) was purchased from Acros. All commercially obtained reagents were used as received. Unless stated otherwise, reactions were performed at room temperature (rt, approximately 23 ˚C). Thin-layer chromatography (TLC) was conducted with E. Merck silica gel 60 F254 pre-coated plates, (0.25 mm) and visualized by exposure to UV light (254 nm) or stained with potassium permanganate. Flash

column chromatography was performed using normal phase silica gel (60 Å, 230-240 mesh, Geduran®).

4.3.2 Siderophore Analogue Synthesis

Siderophore analogues Tren-Gly-Gly-Lys-Cam (TGGLC), Tren-Gly-Lys-Gly-Cam (TGLGC), Tren-Lys-Gly-Gly-Cam (TGGLC), and control molecules Tren-Gly-Gly-Gly-Cam (TGGGC) and Tren-Gly-Gly-Lys-Bam (TGGLB) were synthesized according to previously published methods with minor variations.[10, 14-17] O,O'-dibenzyl-2,3-dihydroxybenzoic acid (Bn-2,3-DHBA) was synthesized from known procedure.[6] An example synthesis of TGGLC can be seen in Figure 4.2 and the general procedures for the syntheses and purifications are outlined below.

4.3.2.1 General Procedures for Synthesis and Purification

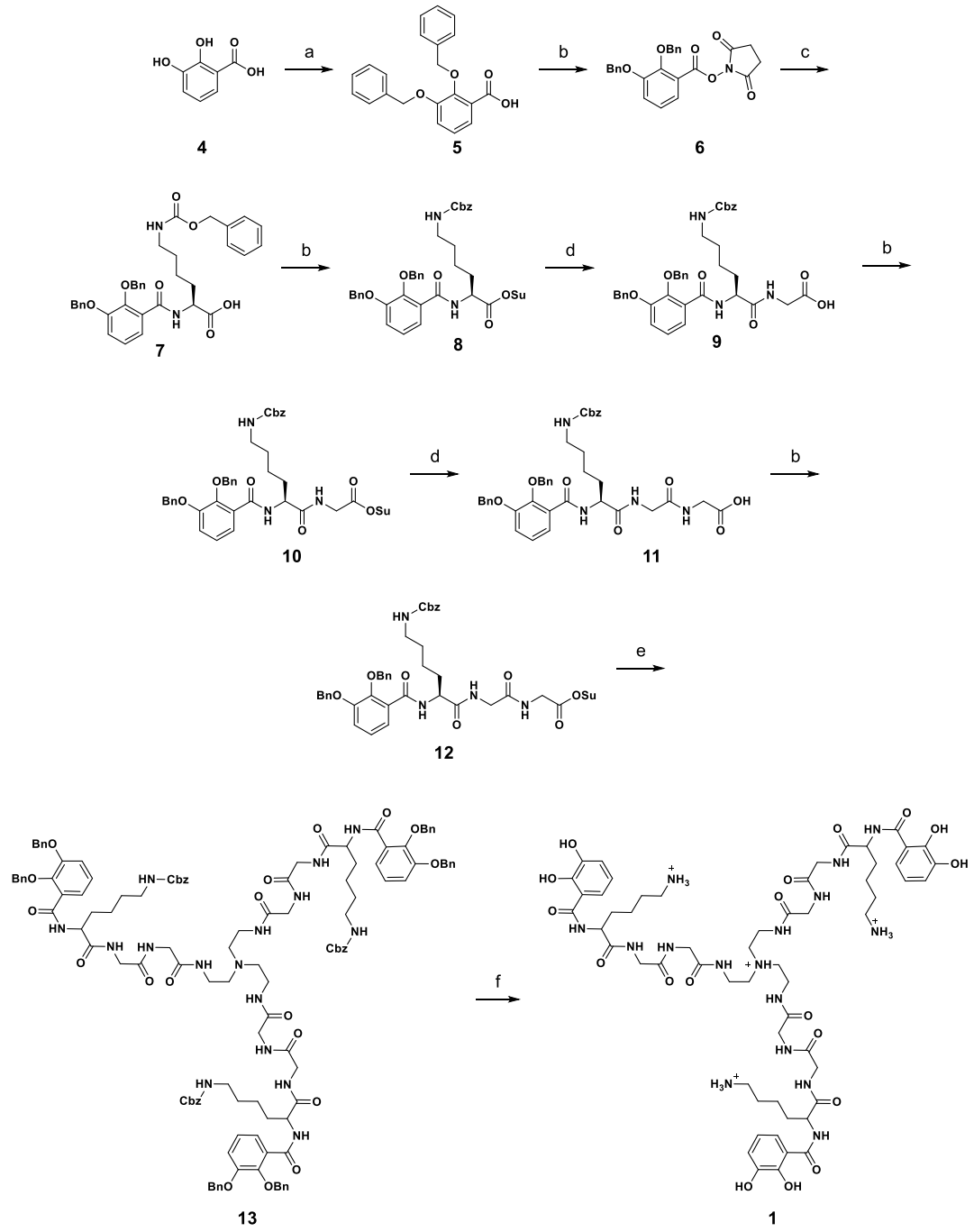


Figure 4.2 – Representative synthetic scheme. Synthesis for Tren-Gly-Gly-Lys-Cam following the general procedure of all siderophore analogues. Reaction conditions: (a) KOH, DMSO, benzyl bromide, 4 h. (b) NHS, DCC, anhydrous THF under argon, overnight. (c) THF, H_2O, Et_3N, H-Lys(Z)-OH, overnight. (d) THF, H_2O, Et_3N, glycine, overnight. (e) Et_3N, Tren, anhydrous THF under argon, overnight. (f) THF, H_2O, 3% AcOH, Pd/C, 3 h.

Step a, Synthesis of O,O'-dibenzyl-2,3-dihydroxybenzoic acid: To a mixture of 2,3-dihydroxybenzoic acid (1 equiv.) and powdered KOH (13 equiv.) was added DMSO (0.3 M). Benzyl bromide (5 equiv.) was added, and the reaction mixture was stirred for 4 h. Aqueous work-up and extraction with EtOAc yielded a crude product that was purified by recrystallization from CH_2Cl_2/hexanes or flash chromatography with EtOAc/hexanes.

Step b, NHS ester activation: In a flame-dried flask under argon, the carboxylic acid (1 equiv.) and N-hydroxysuccinimide (NHS, 1.1 equiv.) were dissolved with sonication in anhydrous THF. The contents of this flask were added via syringe to a second flame-dried flask, containing N,N'-dicyclohexylcarbodiimide (DCC, 1.1 equiv.) dissolved in anhydrous THF. The reaction was allowed to stir overnight at room temperature. The following day, the reaction was concentrated *in vacuo* then resuspended in ethyl acetate. The suspension was filtered to remove the insoluble N,N'-dicyclohexylurea by product. The filtrate was then concentrated *in vacuo* to give a crude, white to yellowish white product. This product was used in subsequent coupling reactions without further purification.

Step c and d, coupling reaction: Crude NHS-activated product (1 equiv.) was dissolved with sonication in THF. This solution was added via syringe to a second flask containing the desired amino acid (e.g. glycine or H-Lys(Z)-OH, 1.1 equiv.) and triethylamine (4 equiv.) dissolved in 40% water in THF. The reaction was allowed to stir overnight at room temperature. The following day, THF was removed *in vacuo* and the resulting aqueous solution was acidified with 1 M HCl then extracted three times with ethyl acetate. The combined organic layers were dried over $MgSO_4$ and concentrated to obtain crude product. The product was purified by recrystallization from 2-propanol/hexanes or H_2O/ethanol to obtain white crystals.

Step e, attachment to Tren core: In a flame-dried flask under argon, the NHS-activated arms (3.3 equiv.) was dissolved with sonication in anhydrous THF. This solution was added via syringe to a second flame-dried flask containing tris(2-aminoethyl)amine (TREN, 1 equiv.) and triethylamine (6 equiv.) dissolved in anhydrous THF. The reaction was allowed to stir overnight at room temperature. The following day, the reaction was concentrated *in vacuo* to give the protected siderophore mimics as crude crystals. This product was used in subsequent deprotection reactions without further purification.

Step f, global deprotection: In a triple washed flask (conc. HCl-H_2O-MeOH), the protected siderophore mimic (1 equiv.) was dissolved in 25% water in THF with 3% acetic acid. The flask was then alternatively purged and flushed with N_2 five times before palladium on carbon (10 wt %, 0.25 equiv.) was added. The reaction was then alternatively purged and flushed with H_2 five times. The reaction was allowed to stir at room temperature for three hours under H_2. Once complete, the reaction was filtered through a grade 5 Whatman filter to remove the palladium on carbon. The filtrate was concentrated *in vacuo* to give a white to yellowish-white powder. Product was purified via reverse phase HPLC.

Purification: Siderophore analogues were purified via RP-HPLC using a preparative C_4 column (22 mm i.d. x 250 mm, Vydac). Compounds were eluted with a linear gradient of 10% methanol (with 0.05% TFA) in H_2O (nanopure with 0.05% TFA) to 30% methanol in H_2O over 38 minutes. The column was exchanged back to 10% methanol over 12 minutes. The eluent was monitored at 215 nm and each prominent peak was manually collected and analyzed by ESI-MS until the desired siderophore analog was identified. The desired fractions were concentrated *in vacuo* then lyophilized to obtain a product. Products were stored under argon at -20 ˚C.

Characterization: ^{1}H NMR spectra were recorded on a Varian Unity Inova 600 MHz spectrometer and are reported relative to deuterated solvent signals. Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz) and integration. ^{13}C NMR spectra were recorded on a Varian Inova 500 MHz spectrometer. Data for ^{13}C NMR spectra are reported in terms of chemical shift (δ ppm). ^{1}H-^{13}C HMBC NMR spectra were recorded on a Varian Unity Inova 600 MHz spectrometer. ESI-MS was performed on a Xevo G2-XS TOF mass spectrometer, part of a shared facilities of the UCSB MRSEC (NSF DMR 1702056).

4.3.2.2 Synthesis and purification of Tren-Gly-Gly-Lys-Cam

The synthesis of TGGLC is shown in Figure 4.1 and it follows the general procedure outlined above without any variation. TGGLC was purified using RP-HPLC using the general procedure outlined above without any variations. The final product after lyophilization was a hygroscopic white powder, which was stored under argon at -20 ˚C.
TGGLC: white powder; ^{1}H, ^{13}C, 2D NMR data; ESI-MS *m/z* 1281.72 $[M + H]^{+}$.

4.3.2.3 Synthesis and purification of Tren-Gly- Lys- Gly-Cam

The synthesis of TGLGC follows the general procedure outlined above without any variation. TGLGC was purified using RP-HPLC using the general procedure outlined above without any variations. The final product after lyophilization was a hygroscopic white powder, which was stored under argon at -20 ˚C.
TGLGC: white powder; ^{1}H, ^{13}C, 2D NMR data; ESI-MS *m/z* 1281.58 $[M + H]^{+}$.

4.3.2.4 Synthesis and purification of Tren-Lys-Gly-Gly-Cam

The synthesis of TLGGC follows the general procedure outlined above with minor variation. The arm prior to attachment, Bn-2,3-DHBA-Gly-Gly-Lys-OH, proved difficult to recrystallize. As such, it was used without further purification. TLGGC was purified using RP-HPLC using the general procedure outlined above without any variations. The final product after lyophilization was a hygroscopic white powder, which was stored under argon at -20 ˚C.

TLGGC: white powder; ^{1}H, ^{13}C, 2D NMR data; ESI-MS *m/z* 1281.61 $[M + H]^{+}$.

4.3.2.4 Synthesis and purification of Tren-Gly-Gly-Lys-Bam

The synthesis of TGGLB follows the same procedure as the synthesis of TGGLC with the exception of using benzoic acid in place of 2,3-DHBA, which obviates step a and allows the synthesis to begin directly with step b (Figure 4.1). Additionally, the intermediates to this compound proved difficult to purify by recrystallization and were therefore used without further purification. After collecting the appropriate peak from the general RP-HPLC purification method, TGGLB was further purified via RP-HPLC using a preparative C_4 column (22 mm i.d. x 250 mm, Vydac). Compounds were eluted with a linear gradient of 10% methanol (with 0.05% TFA) in H_2O (nanopure with 0.05% TFA) to 30% methanol in H_2O over 45 minutes. The column was exchanged back to 10% methanol over 10 minutes. The final product after lyophilization was a hygroscopic white powder, which was stored under argon at -20 ˚C.

TGGLB: white powder; ^{1}H, ^{13}C, 2D NMR data; ESI-MS *m/z* 1186.73 $[M + H]^{+}$.

4.3.2.5 Synthesis and Purification of Tren-Gly-Gly-Gly-Cam

The synthesis of TGGGC follows the same general procedure outlined above outline above with the exception of using a glycine trimer (H-Gly-Gly-Gly-OH) to couple with NHS-activated Bn-2,3-DHBA. The arm prior to attachment, Bn-2,3-DHBA-Gly-Gly-Gly-OH, proved difficult to recrystallize. As such, it was used without further purification. TGGGC was purified using RP-HPLC using a preparative C_4 column (22 mm i.d. x 250 mm, Vydac). Compounds were eluted with a linear gradient of 10% methanol (with 0.05% TFA) in H_2O (nanopure with 0.05% TFA) to 37% methanol in H_2O over 17 minutes. The column was flushed with 100% methanol for 10 minutes, then exchanged back to 10% methanol over 10 minutes. The final product after lyophilization was a hygroscopic white powder, which was stored under argon at -20 ˚C.

TGGGC: white powder; ^{1}H, ^{13}C, 2D NMR data; ESI-MS *m/z* 1068.35 $[M + H]^+$.

4.3.3 Surface Forces Apparatus Technique and Measurements

Surface forces apparatus measurements were performed by George Degen in the research lab of Professor Jacob Israelachvili at UC Santa Barbara. All figures describing experimental results from the SFA were supplied by George Degen. Details on the SFA instrument and experimental methods have been published elsewhere.[10-11, 18] Siderophore analogs were dissolved at 1 mM in a solution of 50 mM acetic acid, 150 mM KNO_3, pH 3.3. These solution conditions match the solution conditions of the siderophore analog studies of Maier et al. which were chosen to be similar to the pH and ionic strength imposed by the mussel during plaque deposition.[19]

For each experiment, molecularly smooth mica surfaces were prepared in air, arranged in a crossed cylinder geometry. Forces recorded during experiments are normalized by the

radius of these cylinders to account for small differences in mica-mica contact area between experiments. Then 50 µL of the acetic acid and potassium nitrate solution described above, without dissolved siderophore analogs, was injected between the surfaces, trapped in a capillary meniscus. The surfaces were then made to undergo cycles of approach and separation while measuring the surface separation and normal force between the surfaces. These cycles allow the generation of a force-distance plot, which includes the maximum force needed to separate the contacting surfaces, or adhesive force.

Subsequently, 10 µL of 1 mM siderophore analog solution containing one of the three siderophore analogs synthesized was injected into the buffer meniscus between the surfaces. The system was allowed to equilibrate for at least 30 minutes, resulting in deposition of a film of siderophore analog on each surface. Then, approach and separation cycles were performed while varying approach and separation velocities and time spent at maximum compression. Each experiment consisted of at least 3 approach and separation cycles, followed by translation of one of the surfaces to introduce uncompressed siderophore analog film into the contact area.

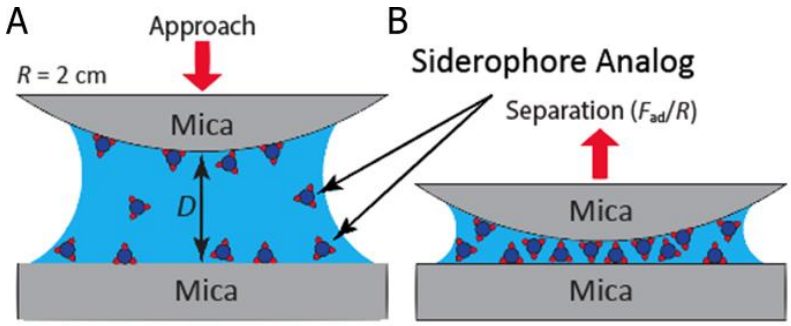


Figure 4.3 - SFA experimental procedure. (A) Prior to the approach of the mica surfaces, a siderophore analogue was allowed to adsorb to the mica surfaces. (B) The adsorbed molecules were compressed into a monolayer or a multilayer and held under force for a certain amount of time before the surfaces are separated.

4.4 Results

4.4.1 Siderophore Analogue Purification

A total of 5 siderophore analogues were synthesized. TGGLC (Figure 4.4), TGLGC (Figure 4.5), and TLGGC (Figure 4.6) retain the same molecular weight and ratio of catechol to lysine, but vary the spacing between the two groups by zero, one, or two glycine residues. TGGLB (Figure 4.7) retains the lysine residue but lacks the hydroxyl groups of catechol responsible for strong adhesion. TGGGC (Figure 4.8) retains the catechol moiety but lacks cationic lysine residues.

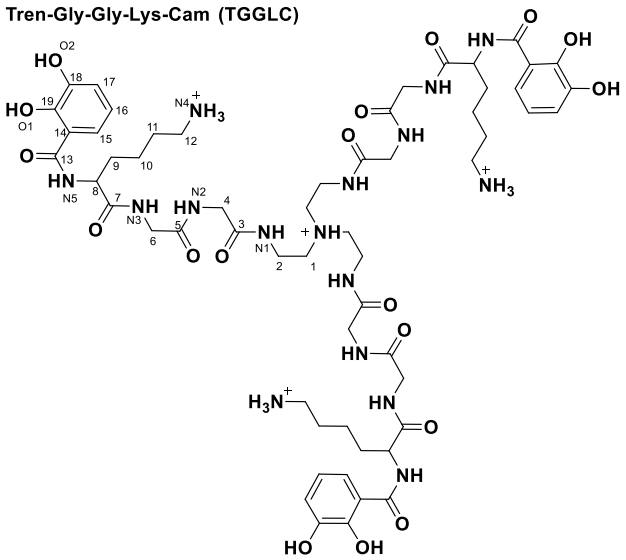


Figure 4.4 - Structure of TGGLC. Numbered carbons correspond to NMR data in Table 4.1.

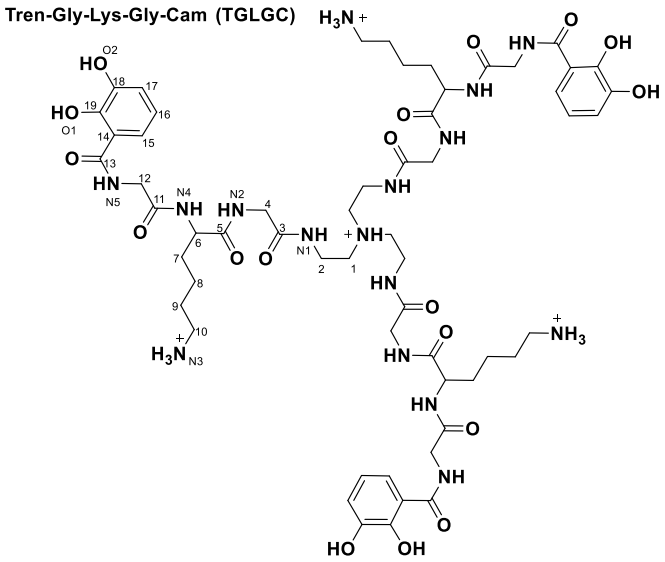


Figure 4.5 - Structure of TGLGC. Numbered carbons correspond to NMR data in Table 4.1.

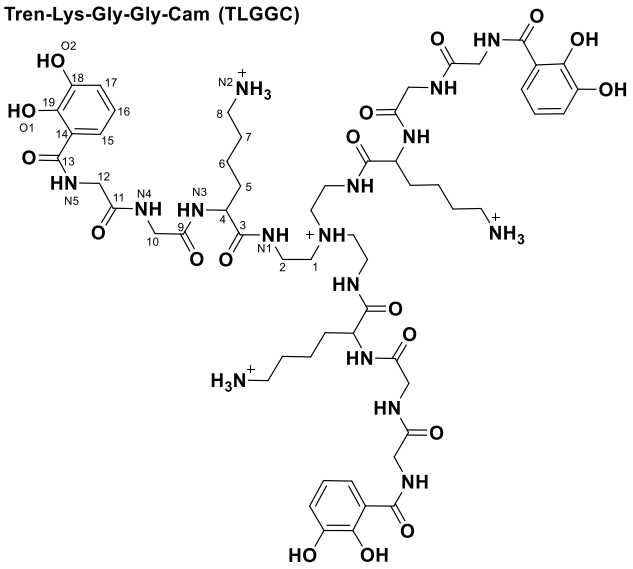


Figure 4.6 - Structure of TLGGC. Numbered carbons correspond to NMR data in Table 4.1.

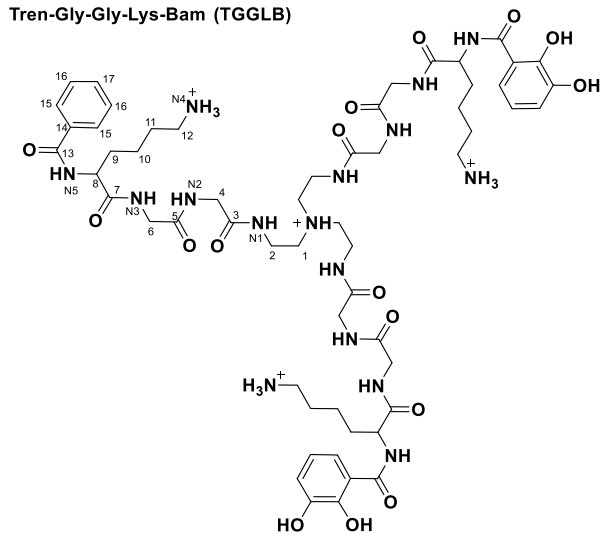


Figure 4.7 - Structure of TGGLB. Numbered carbons correspond to NMR data in Table **4.2**.

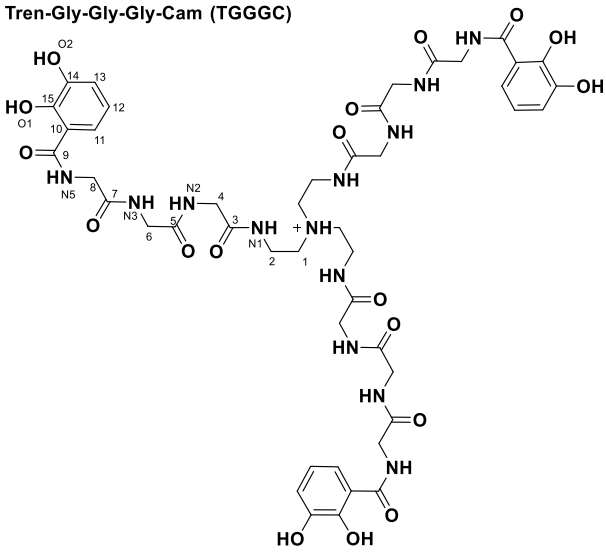


Figure 4.8 - Structure of TGGGC. Numbered carbons correspond to NMR data in Table **4.2**.

Siderophore analogues were purified by RP-HPLC as discussed above in 4.3.2 Siderophore Analogue Synthesis. Purified siderophore analogues were checked for purity via RP-HPLC (Figure 4.9) and ESI-MS (Figure 4.10) prior to characterization via NMR. An asymmetric homologue of TGGLB, Tren(Gly-Gly-Lys-Bam)$_2$(Gly-Lys-Bam), co-elutes with the desired compound under all attempted RP-HPLC conditions and can be seen in the ESI-MS (Figure 4.13).

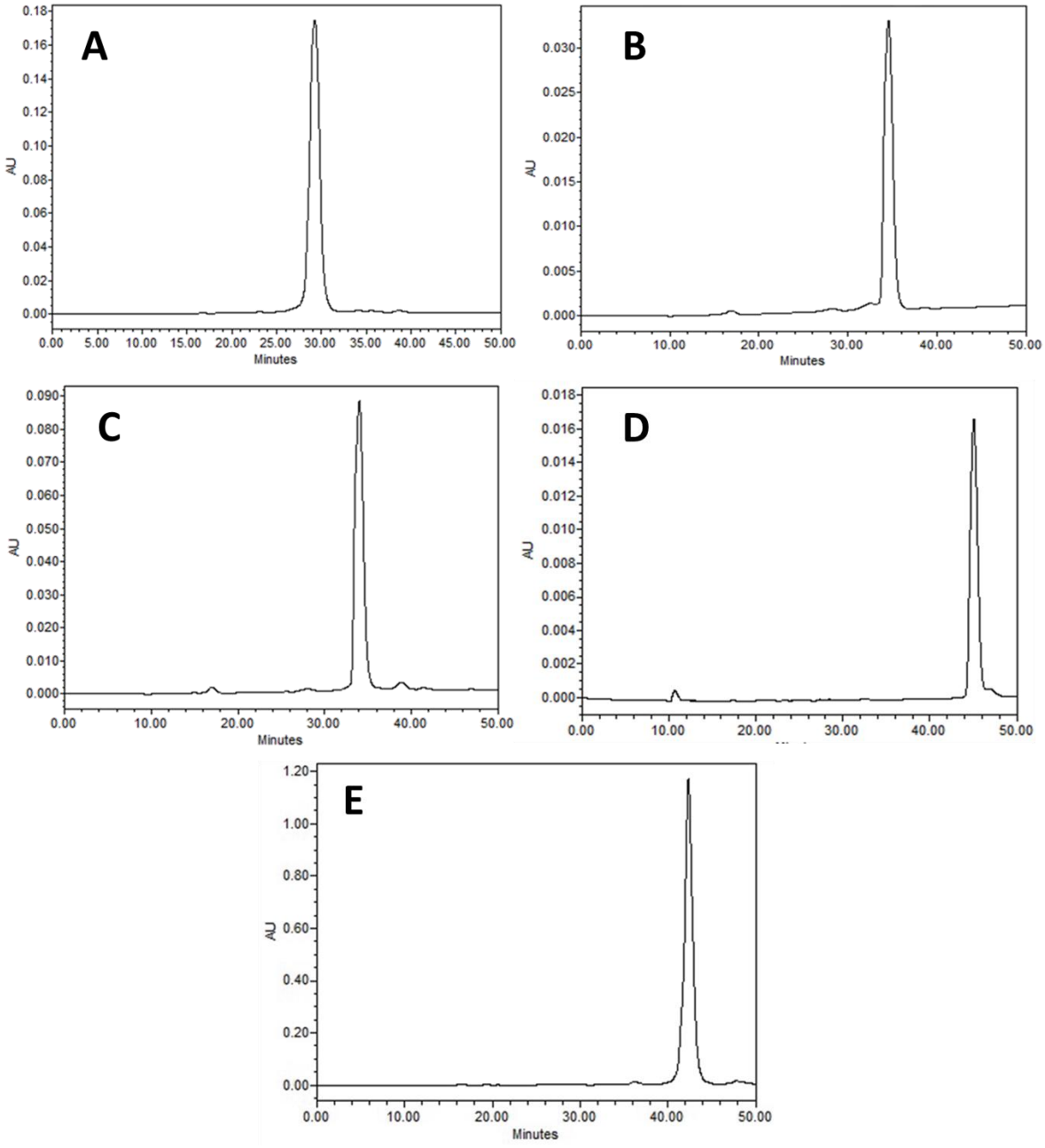
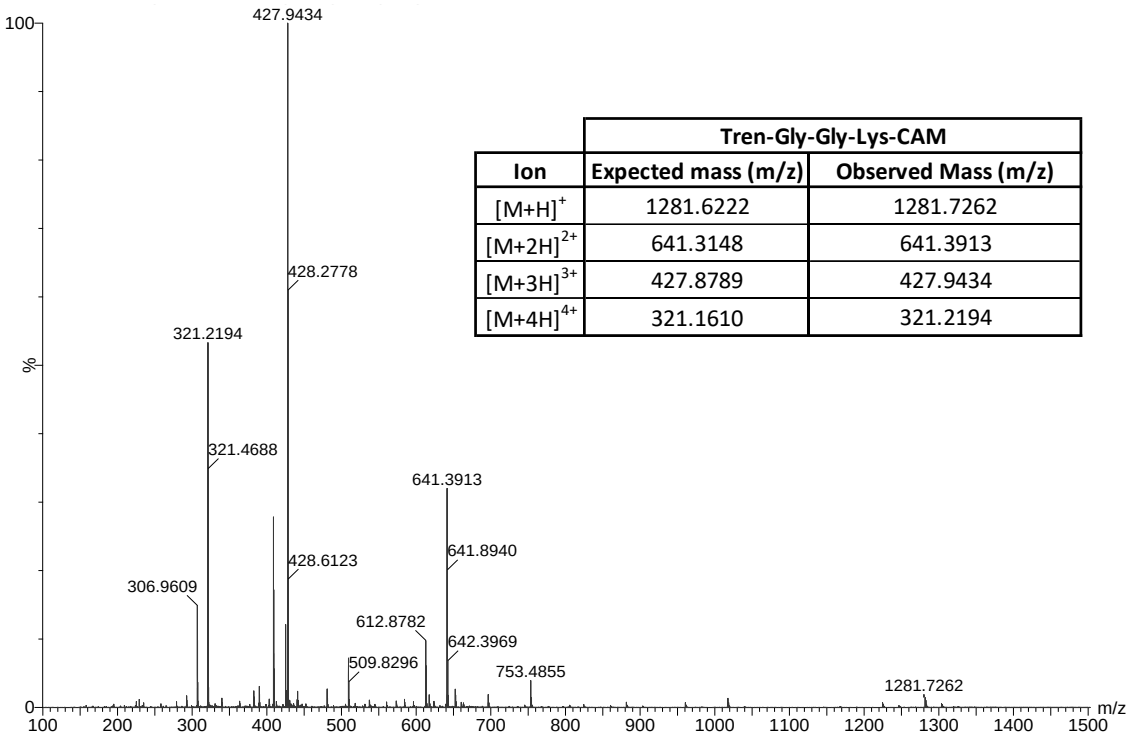


Figure 4.9 - Reverse Phase HPLC traces of purified siderophore analogues. RP-HPLC was carried out on a C4 preparative column (22 mm i.d. x 250 mm, Vydac). Compounds were eluted with a linear gradient of 10% methanol (with 0.05% trifluoroacetic acid (TFA)) in H_2O (nanopure with 0.05% TFA) to 30% methanol in H_2O over 38 minutes. The column was exchanged back to 10% methanol over 12 minutes. The samples were detected at 215. (A) Tren-Gly-Gly-Lys-Cam. (B) Tren-Gly-Lys-Gly-Cam. (C) Tren-Lys-Gly-Gly-Cam. (D) Tren-Gly-Gly-Lys-Bam. (E) Tren-Gly-Gly-Gly-Cam.

4.4.2 Siderophore Analogue Characterization

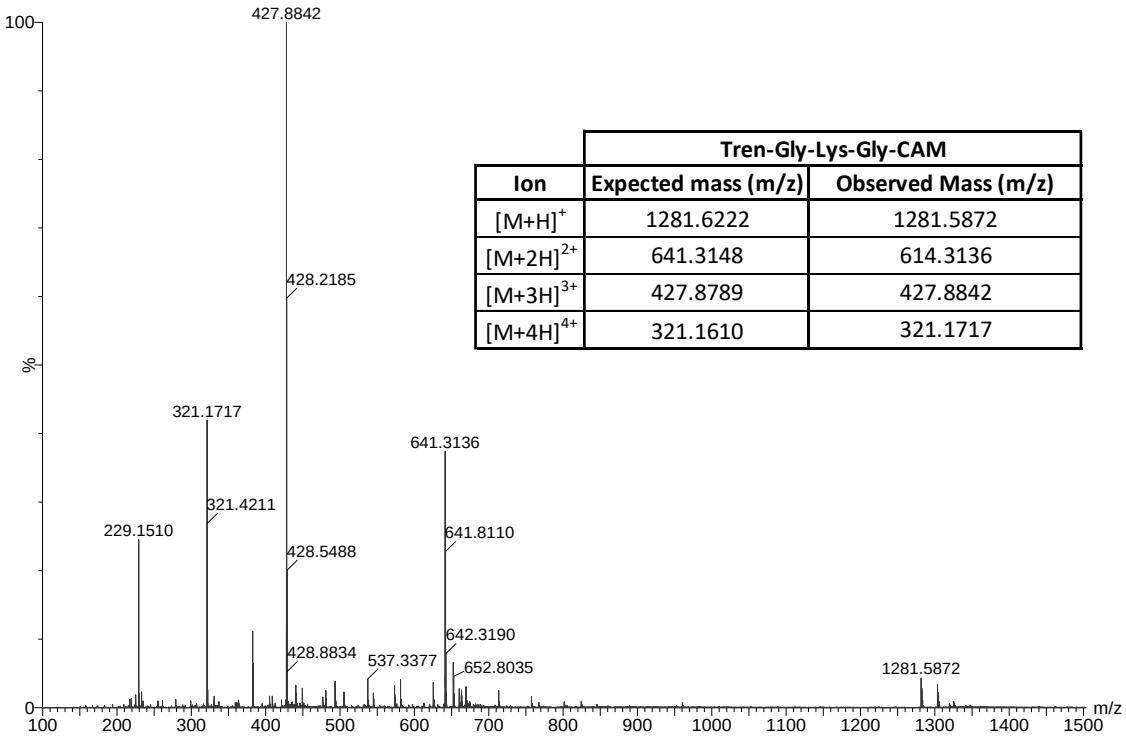
4.4.2.1 Siderophore Analogue Mass Spectrometry

Siderophore analogues structures were characterized with ESI-MS (Figure 4.10). Due to the presence of 5 possible cationic amine groups, masses corresponding to $[M+H]^{+}$, $[M+2H]^{+2}$, $[M+3H]^{+3}$, and $[M+4H]^{+4}$ were observed for most compounds, with the exception of TGGGC as this compound lacks cationic lysine residues (Figure 4.14).



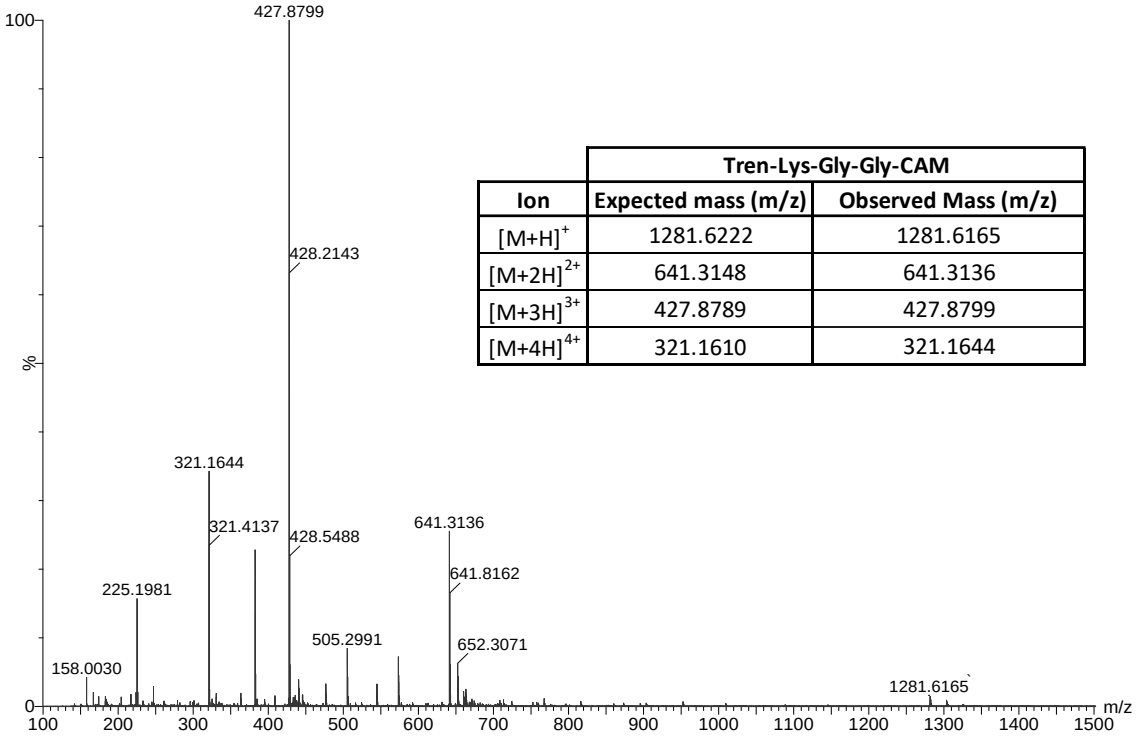
	Tren-Gly-Gly-Lys-CAM	
Ion	**Expected mass (m/z)**	**Observed Mass (m/z)**
$[M+H]^{+}$	1281.6222	1281.7262
$[M+2H]^{2+}$	641.3148	641.3913
$[M+3H]^{3+}$	427.8789	427.9434
$[M+4H]^{4+}$	321.1610	321.2194

Figure 4.10 – ESI-MS of TGGLC.



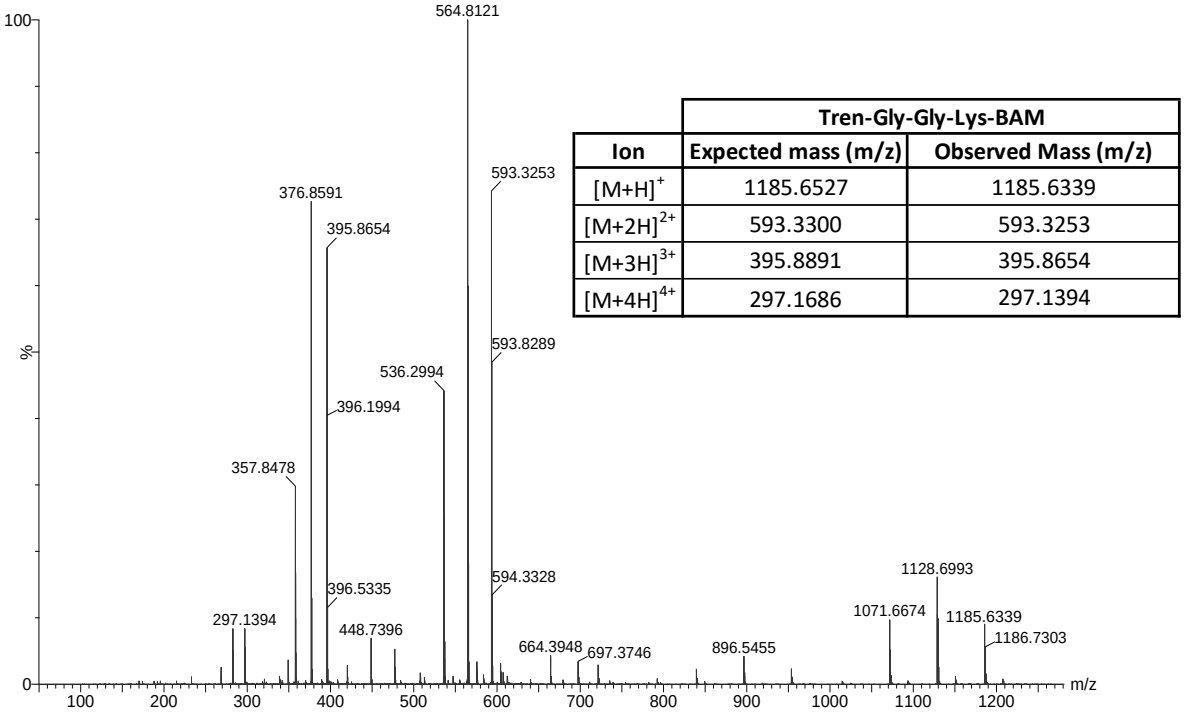
	Tren-Gly-Lys-Gly-CAM	
Ion	**Expected mass (m/z)**	**Observed Mass (m/z)**
$[M+H]^{+}$	1281.6222	1281.5872
$[M+2H]^{2+}$	641.3148	614.3136
$[M+3H]^{3+}$	427.8789	427.8842
$[M+4H]^{4+}$	321.1610	321.1717

Figure 4.11 – ESI-MS of TGLGC.



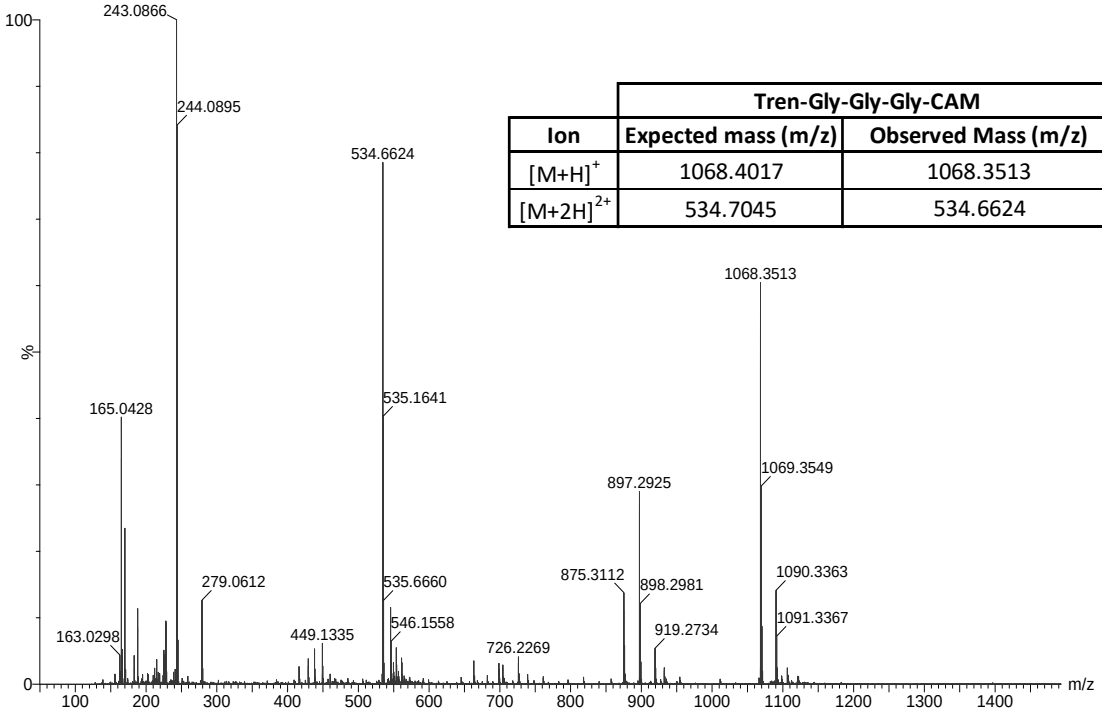
	Tren-Lys-Gly-Gly-CAM	
Ion	**Expected mass (m/z)**	**Observed Mass (m/z)**
$[M+H]^{+}$	1281.6222	1281.6165
$[M+2H]^{2+}$	641.3148	641.3136
$[M+3H]^{3+}$	427.8789	427.8799
$[M+4H]^{4+}$	321.1610	321.1644

Figure 4.12 – ESI-MS of TLGGC.



	Tren-Gly-Gly-Lys-BAM	
Ion	**Expected mass (m/z)**	**Observed Mass (m/z)**
$[M+H]^{+}$	1185.6527	1185.6339
$[M+2H]^{2+}$	593.3300	593.3253
$[M+3H]^{3+}$	395.8891	395.8654
$[M+4H]^{4+}$	297.1686	297.1394

Figure 4.13 - ESI-MS of TGGLB.



	Tren-Gly-Gly-Gly-CAM	
Ion	**Expected mass (m/z)**	**Observed Mass (m/z)**
$[M+H]^{+}$	1068.4017	1068.3513
$[M+2H]^{2+}$	534.7045	534.6624

Figure 4.14 - ESI-MS of TGGGC.

4.4.2.2 Siderophore Analogue NMR Characterization

^{1}H, ^{13}C, and ^{1}H-^{13}C HMBC NMR confirmed the desired molecular structures of each siderophore analogue. ^{1}H and ^{13}C results are summarized in Table 4.1 and

Table 4.2. ^{1}H NMR results for TGGLC show peaks characteristic of the 2,3-DHBA moiety at δ_H 6.70 (t, J = 7.9 Hz, 1H), 6.94 (d, J = 7.7 Hz, 1H), and 7.40 (d, J = 7.7 Hz, 1H). There are two sets diastereotopic protons centered at δ_H 1.78 (m, 2H) and 1.37 (m, 2H), labeled 9 and 10 in Figure 4.15, which reside near the chiral α-carbon of the lysine residue. The protons on carbon 11, seen at δ_H 1.54 (m, 2H), also appear to be diasterotopic in the ^{1}H-^{13}C HMBC spectrum, though the coupling constant appears to be small enough that the peaks aren't resolved in the ^{1}H NMR spectra. The methine α-carbon give a signal at δ_H 4.52 (m, 1H). The remaining protons of the lysine residue were seen at δ_H 2.77 (m, 2H). While water obscured the protons of the Tren scaffold in other analogues, the protons can be seen in the spectrum of TGGLC at δ_H 3.21 (s, 1H) and 3.40 (s, 1H), though they appear as singlets rather than well defined multiplets. Assignments of the glycine residue protons was aided by ^{1}H-^{13}C HMBC (Figure 4.16) and were seen at δ_H 3.73 (m, 2H) and 3.79 (m, 2H). Protons attached to catechol oxygens (δ_H 11.91, 9.38) and amide nitrogens (δ_H 8.18, 8.36, 7.71, and 8.79) were assigned through correlation with carbonyl carbons and other adjacent groups in the ^{1}H-^{13}C HMBC spectrum.

In the ^{13}C NMR results for TGGLC, the carbonyl group of the 2,3-DHBA moiety and the lysine group can be seen at δ_C 168.86 and 171.75, respectively. The carbonyl groups of the two glycine residues were resolved with the use of ^{1}H-^{13}C HMBC and are seen at δ_C 169.38 and 169.23. DMSO obscures the methylene carbons of the Tren scaffold in the ^{13}C Spectra. The methylene carbons of the lysine residue are observed at δ_C 31.07, 22.48, 26.62, and

38.65, and the methine lysine carbon at δ_C 52.79. The two methylene carbons of the glycine spacers were resolved by ^{1}H-^{13}C HMBC and are seen at δ_C 41.94 and 42.10.

The ^{1}H and ^{13}C NMR spectra for TGLGC and TLGGC closely resemble that of TGGLC. The characteristic ^{1}H NMR peaks for the 2,3-DHBA and the diastereotopic peaks of the lysine residue are present in all spectra. Key differences between the siderophore analogues are seen in the different correlations in the ^{1}H-^{13}C HMBC spectra. Correlation between the α-carbon of the lysine residue and adjacent groups confirms the sequence of the arm in each siderophore analogue. The ^{1}H spectra of TGGLB (Figure 4.21A, Table) shows the characteristic splitting pattern and integration of a singly substituted phenyl group and retains the peaks corresponding to a lysine residue. TGGGC is easily identified due to the absence of all signals associated with the lysine residue present in the other siderophore analogues.

Table 4.1 - NMR data for siderophore analogues. ^{1}H NMR was obtained on a Varian Unity Inova 600 MHz spectrometer and ^{13}C on a Varian Unity Inova 500 MHz spectrometer. NMR data was taken in deuterated DMSO. See Figure 4.15-4.20 for NMR spectra.

	Tren-Lys-Gly-Gly-CAM		Tren-Gly-Lys-Gly-CAM		Tren-Gly-Gly-Lys-CAM	
Position	δ_c (ppm)	δ_H (ppm)	δ_c (ppm)	δ_H (ppm)	δ_c (ppm)	δ_H (ppm)
1	Obscured by DMSO	Obscured by water	Obscured by DMSO	3.19 (s, 2H)	Obscured by DMSO	3.21 (s, 2H)
2	Obscured by DMSO	Obscured by water	Obscured by DMSO	3.39 (s, 2H)	Obscured by DMSO	3.40 (s, 2H)
3	172.06	-	169.59	-	169.38	-
4	52.56	4.17 (td, J = 8.5, 4.7 Hz, 1H)	42.22	3.72 (m, 2H)	41.94	3.73 (m, 2H)
5	30.92	1.68 (m, 1H)	171.91	-	169.23	-
6	22.34	1.28 (ddt, J = 34.6, 17.7, 8.1 Hz, 2H)	52.50	4.28 (td, J = 8.5, 5.0 Hz, 1H)	42.10	3.79 (m, 2H)
7	26.58	1.49 (m, 3H) (H7 and H5' overlap)	31.22	1.71 (m, 1H)	171.75	-
8	38.69	2.73 (m, 2H)	22.23	1.33 (m, 2H)	52.79	4.52 (m, 1H)
9	168.94	-	26.63	1.52 (m, 3H) (H9 and H7' overlap)	31.07	1.78 (m, 2H)
10	42.07	3.93 (d, J = 5.7 Hz, 2H)	38.74	2.75 (m, 2H)	22.48	1.37 (m, 2H)
11	168.58	-	168.82		26.62	1.54 (m, 2H)
12	42.4	3.77 (d, J = 5.7 Hz, 2H)	42.00	3.97 (d, J = 5.6 Hz, 2H)	38.65	2.77 (m, 2H)
13	169.62	-	169.30	-	168.86	-
14	115.36	-	115.60	-	115.73	-
15	118.02	7.29 (d, J = 8.0 Hz, 1H)	118.22	7.30 (d, J = 7.7 Hz, 1H)	118.38	7.40 (d, J = 7.7 Hz, 1H)
16	117.81	6.69 (t, J = 7.9 Hz, 1H)	117.87	6.70 (t, J = 7.9 Hz, 1H)	118.11	6.70 (t, J = 7.9 Hz, 1H)
17	118.84	6.93 (d, J = 7.7 Hz, 1H)	118.86	6.94 (d, J = 7.7 Hz, 1H)	118.8	6.94 (d, J = 7.7 Hz, 1H)
18	146.12	-	146.15	-	146.02	-
19	148.97	-	148.86	-	148.61	-
N1	-	-	-	-	-	-
N2	-	7.69 (s, 3H)	-	9.01 (t, J = 5.8 Hz, 1H)	-	8.18 (t, J = 6.1 Hz, 2H)
N3	-	-	-	7.75 (m, 3H)	-	8.36 (t, J = 5.9 Hz, 1H)
N4	-	8.28 (t, J = 5.5 Hz, 1H)	-	8.26 (d, J = 7.8 Hz, 2H)	-	7.71 (m, 3H)
N5	-	9.07 (t, J = 5.6 Hz, 1H)	-	-	-	8.79 (d, J = 7.5 Hz, 1H)
O1	-	12.11 (s, 1H)	-	12.07 (s, 1H)	-	11.91 (s, 1H)
O2	-	9.31 (s, 1H)	-	9.36 (s, 1H)	-	9.38 (s, 1H)

Table 4.2 – NMR data for remaining siderophore analogues. ^{1}H NMR was obtained on a Varian Unity Inova 600 MHz spectrometer and ^{13}C on a Varian Unity Inova 500 MHz spectrometer. NMR data was taken in deuterated DMSO. See Figure 4.21-4.24 for NMR spectra

	Tren-Gly-Gly-Lys-Bam		Tren-Gly-Gly-Gly-Cam	
Position	**δ_c (ppm)**	**δ_H (ppm)**	**δ_c (ppm)**	**δ_H (ppm)**
1	Obscured by DMSO	3.19 (s, 2H)	Obscured by DMSO	3.24 (s, 2H)
2	Obscured by DMSO	3.38 (s, 2H)	Obscured by DMSO	Obscured by H_2O
3	168.51	-	169.39	-
4	42.01	3.73 (m, 2H)	42.15	3.79 (d, J = 5.6 Hz, 2H)
5	169.23	-	169.09	-
6	42.12	3.77 (d, *J* = 5.8 Hz, 1H)	42.10	3.73 (d, J = 5.8 Hz, 2H)
7	172.31	-	170.30	-
8	53.43	4.43 (m, 1H)	42.35	3.96 (d, J = 5.6 Hz, 2H)
9	30.71	1.76 (m, 2H)	169.56	-
10	22.69	1.38 (m, 2H)	115.34	-
11	26.67	1.55 (m, 2H)	118.11	7.31 (dd, J = 8.3, 1.5 Hz, 1H)
12	38.72	2.77 (m, 2H)	117.79	6.70 (t, J = 7.9 Hz, 1H)
13	166.91	-	118.86	6.93 (dd, J = 7.7, 1.5 Hz, 1H)
14	133.9	-	146.12	-
15	128.19	7.90 (d, *J* = 6.9 Hz, 2H)	149.07	-
16	127.56	7.47 (t, *J* = 7.6 Hz, 2H)	-	-
17	131.44	7.55 (t, *J* = 7.4 Hz, 1H)	-	-
N1	-	-	-	-
N2	-	8.18 (m, 1H)	-	8.21 (t, J = 5.8 Hz, 1H)
N3	-	8.28 (m, 1H)	-	8.32 (t, J = 5.7 Hz, 1H)
N4	-	7.73 (br, 3H)	-	9.05 (t, J = 5.8 Hz, 1H)
N5	-	8.56 (d, *J* = 7.6 Hz, 1H)	-	-
O1	-	-	-	12.18 (s, 1H)
O2	-	-	-	9.27 (s, 1H)

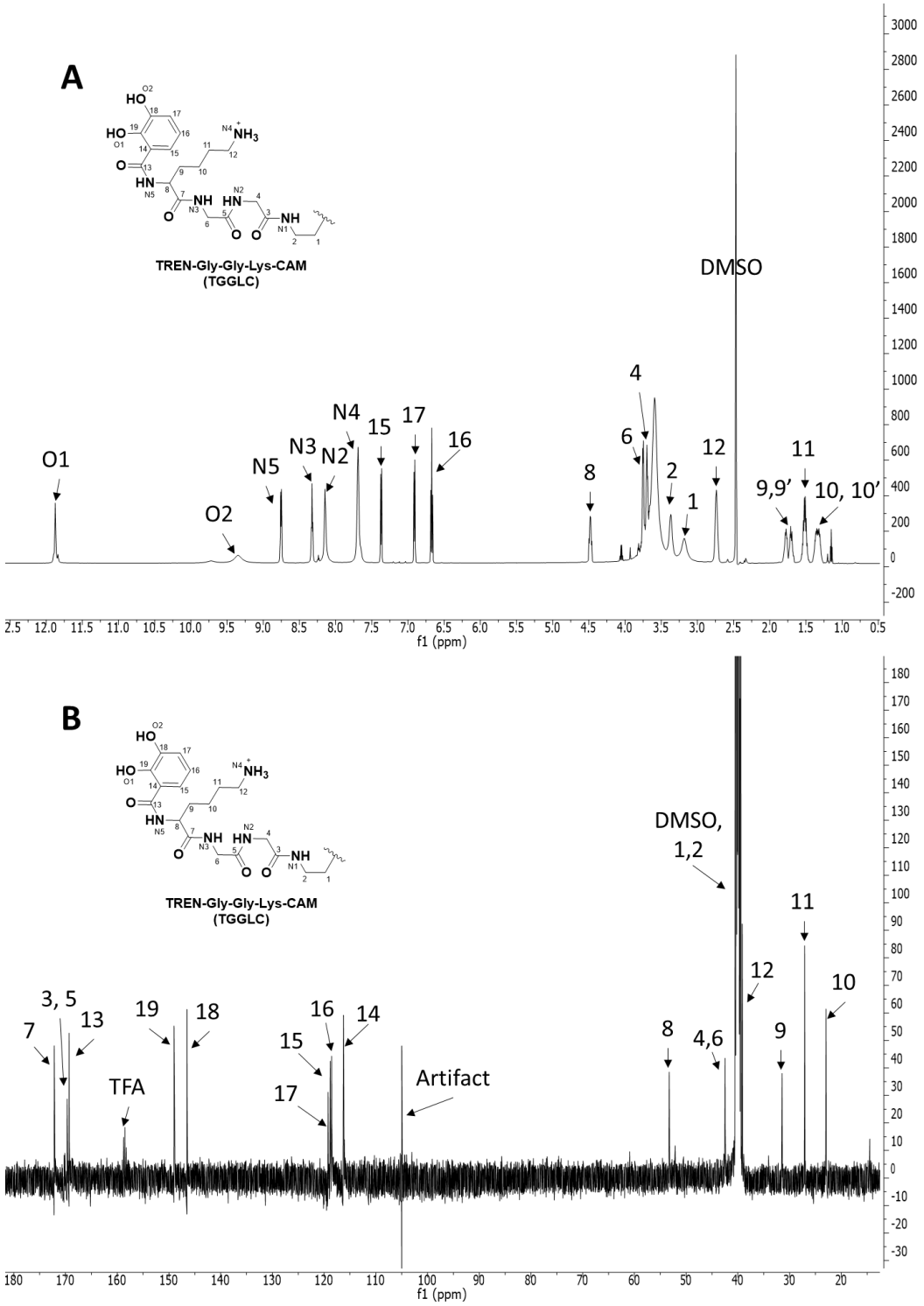


Figure 4.15 - NMR data for Tren-Gly-Gly-Lys-Cam. A.^{1}H NMR data for Tren-Gly-Gly-Lys-Cam. NMR (600 MHz) in DMSO. B. ^{13}C NMR data for Tren-Gly-Gly-Lys-Cam. NMR (500 MHz) in DMSO. Trifluoroacetic acid (TFA) originates from RP-HPLC purification. Assignments were confirmed with HMBC, Figure 4.16. The artifact present in the ^{13}C NMR is from a damaged receiver.

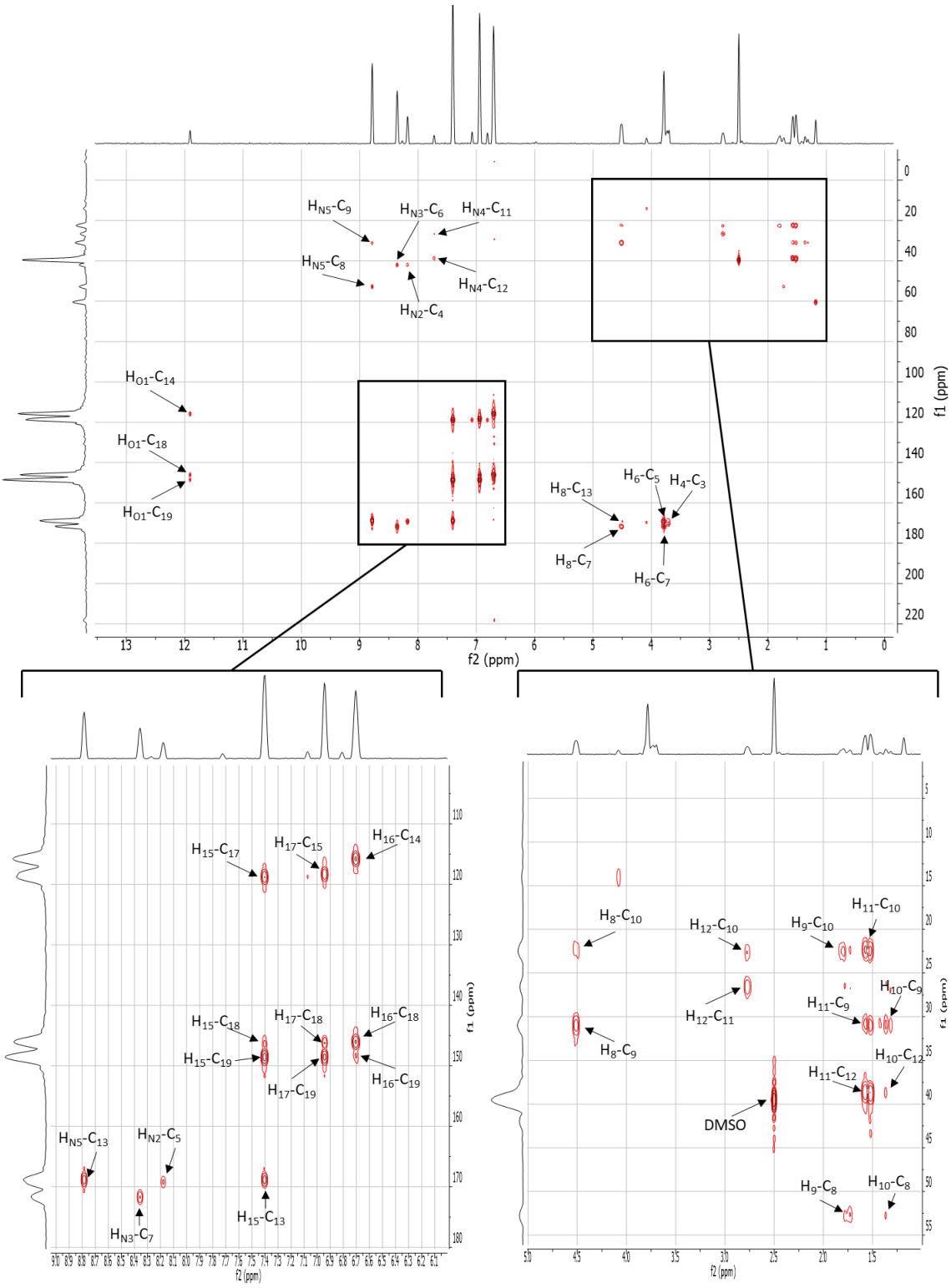


Figure 4.16 - ^{1}H-^{13}C HMBC NMR for Tren-Gly-Gly-Lys-Cam. NMR (600 MHz) in DMSO. Enlarged regions of the ^{1}H-^{13}C HMBC NMR spectrum are in the bottom panels. The spectrum is annotated with the correlations between specific hydrogens and carbons.

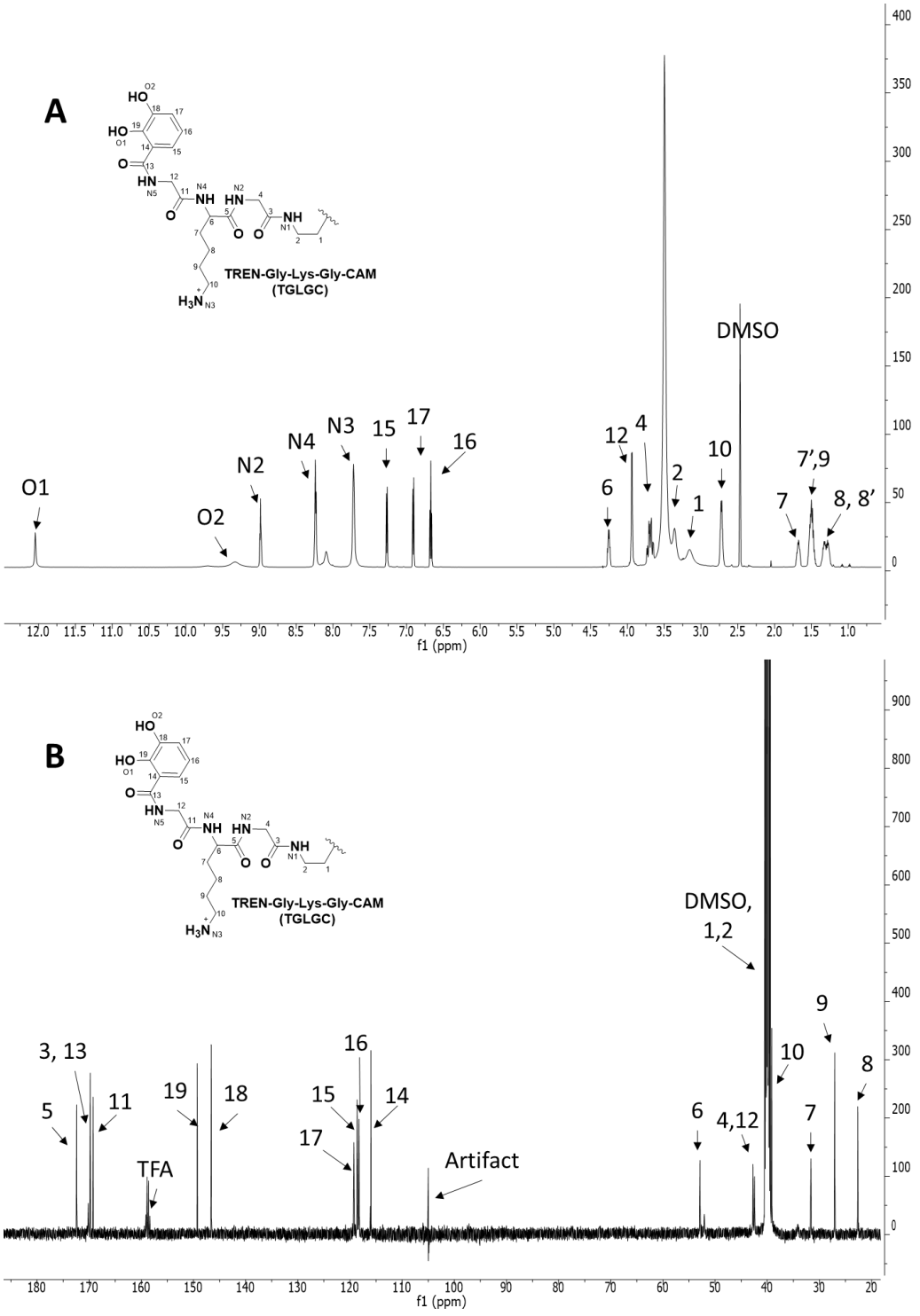


Figure 4.17 - NMR data for Tren-Gly-Lys-Gly-Cam. A.[1]H NMR data for Tren-Gly-Lys-Gly-Cam. NMR (600 MHz) in DMSO. B. [13]C NMR data for Tren-Gly-Lys-Gly-Cam. NMR (500 MHz) in DMSO. Trifluoroacetic acid (TFA) originates from RP-HPLC purification. Assignments were confirmed with HMBC, Figure 4.18. The artifact present in the [13]C NMR is from a damaged receiver.

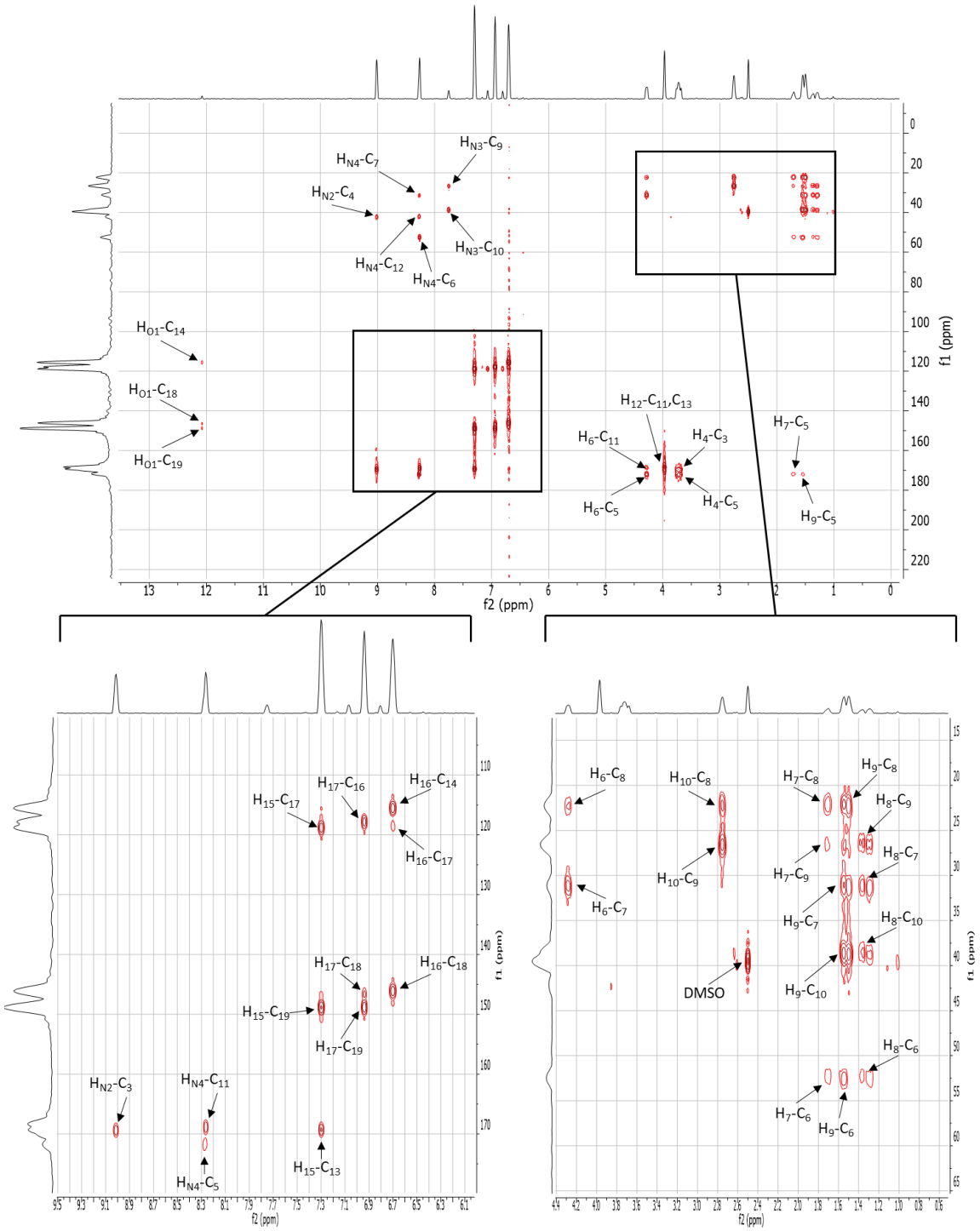


Figure 4.18 - ^{1}H-^{13}C HMBC NMR for Tren-Gly-Lys-Gly-Cam. NMR (600 MHz) in DMSO. Enlarged regions of the ^{1}H-^{13}C HMBC NMR spectrum are in the bottom panels. The spectrum is annotated with the correlations between specific hydrogens and carbons.

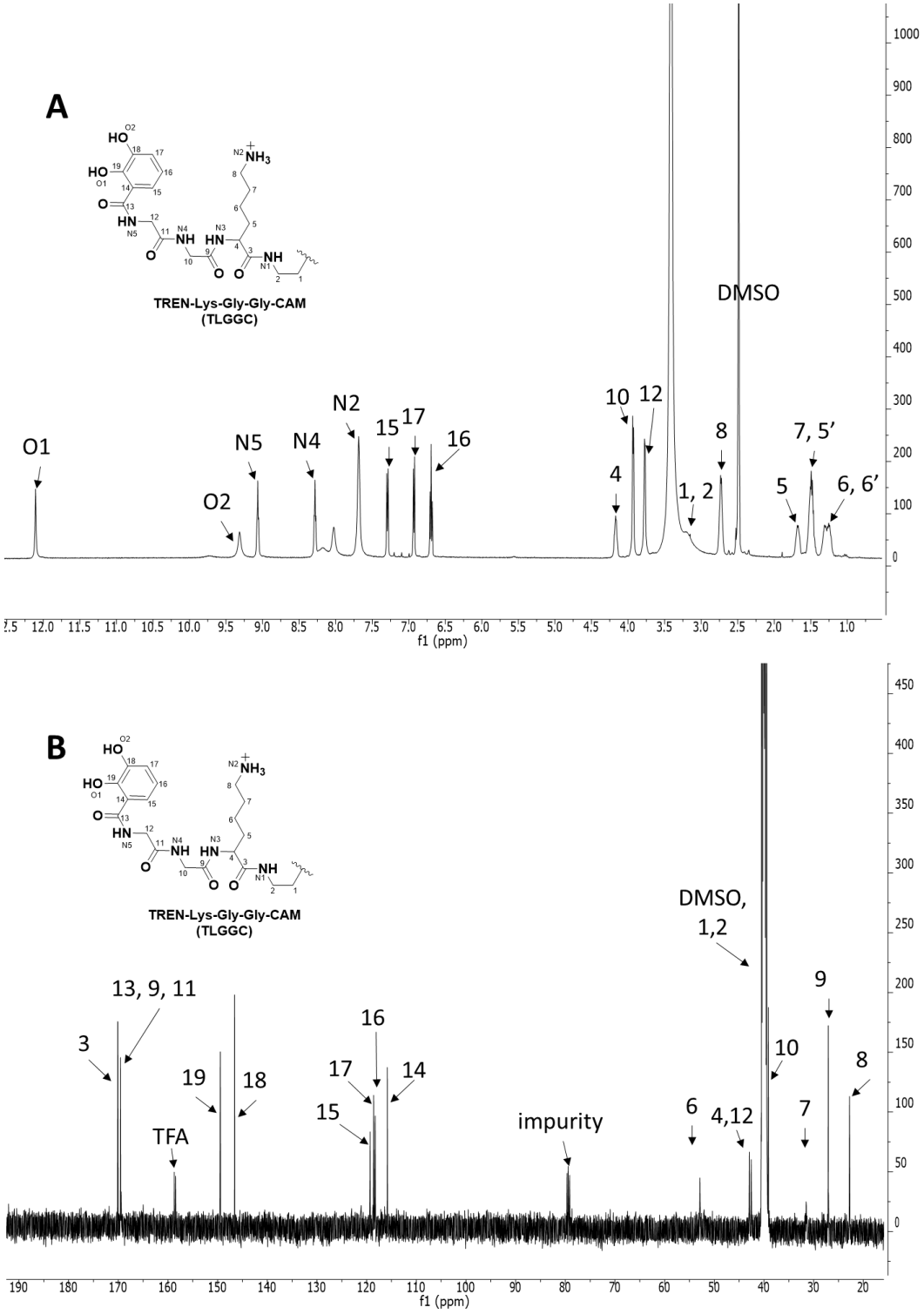


Figure 4.19 - NMR data for Tren-Lys-Gly-Gly-Cam. A.[1]H NMR data for Tren-Lys-Gly-Gly-Cam. NMR (600 MHz) in DMSO. B. [13]C NMR data for Tren-Lys-Gly-Gly-Cam. NMR (500 MHz) in DMSO. Trifluoroacetic acid (TFA) originates from RP-HPLC purification. Assignments were confirmed with HMBC, Figure 4.20.

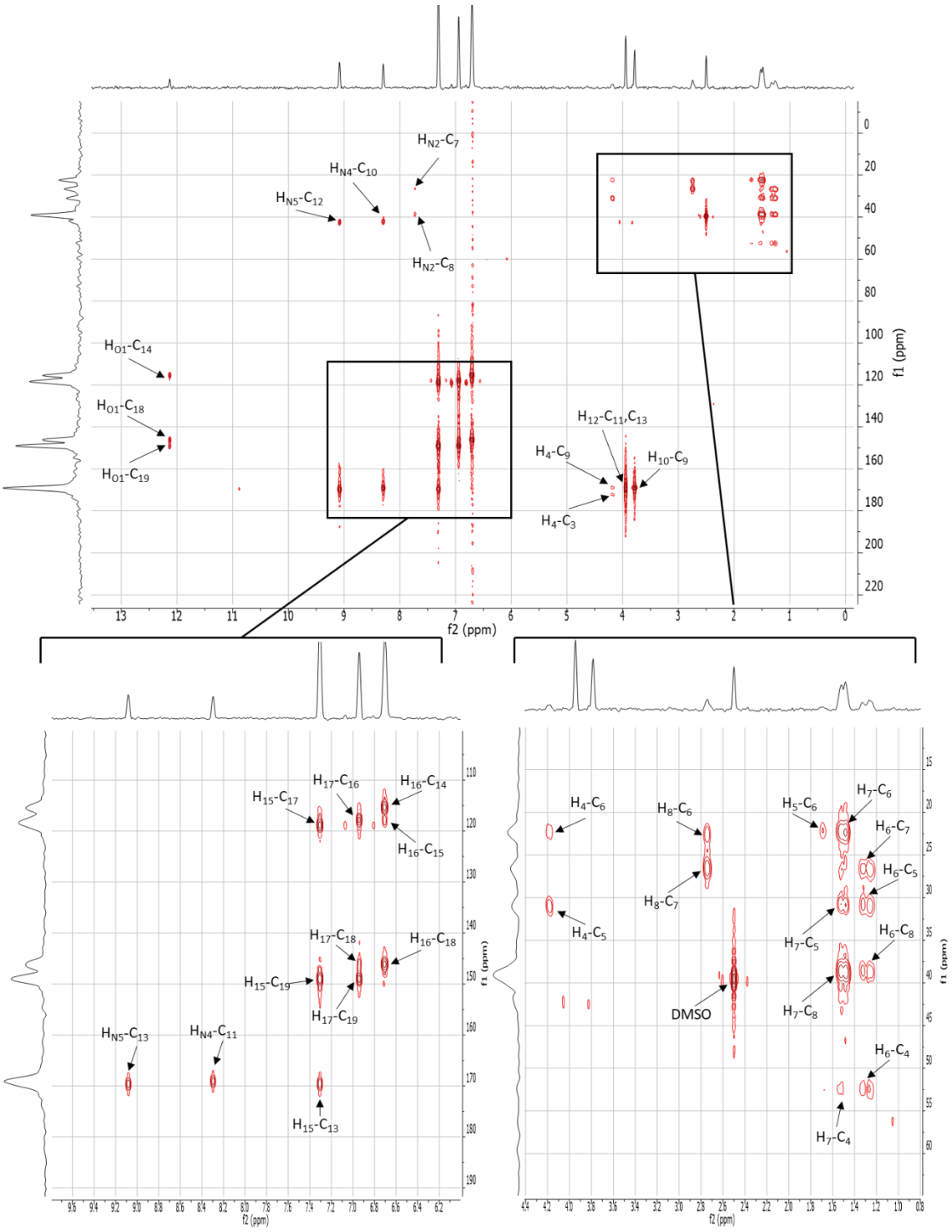


Figure 4.20 - ^{1}H-^{13}C HMBC NMR for Tren-Lys-Gly-Gly-Cam. NMR (600 MHz) in DMSO. Enlarged regions of the ^{1}H-^{13}C HMBC NMR spectrum are in the bottom panels. The spectrum is annotated with the correlations between specific hydrogens and carbons.

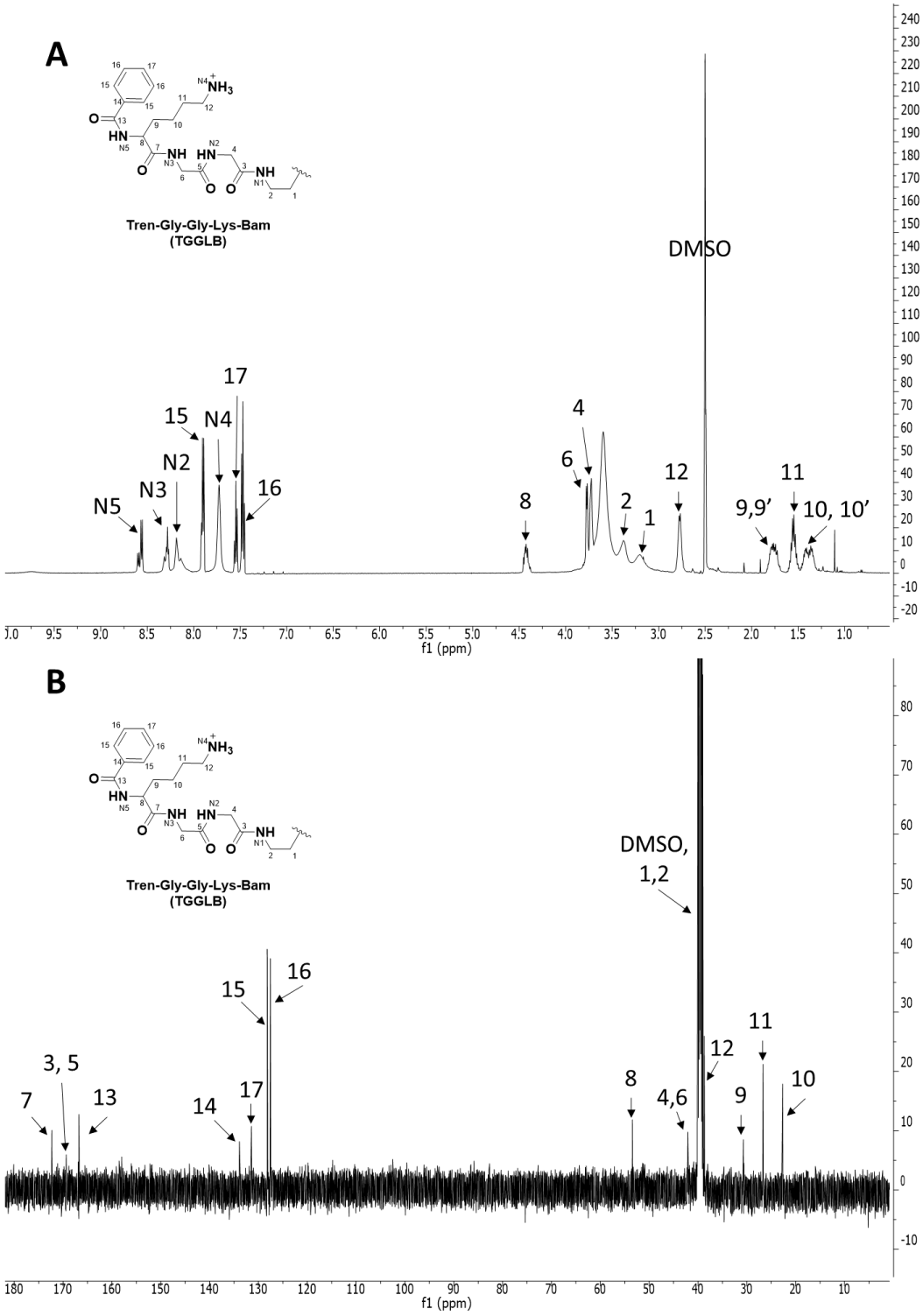


Figure 4.21 – NMR data for Tren-Gly-Gly-Lys-Bam. A.^{1}H NMR data for Tren-Gly-Gly-Lys-Bam. NMR (600 MHz) in DMSO. B. ^{13}C NMR data for Tren-Gly-Gly-Lys-Bam. NMR (500 MHz) in DMSO. Assignments were confirmed with HMBC, Figure 4.22.

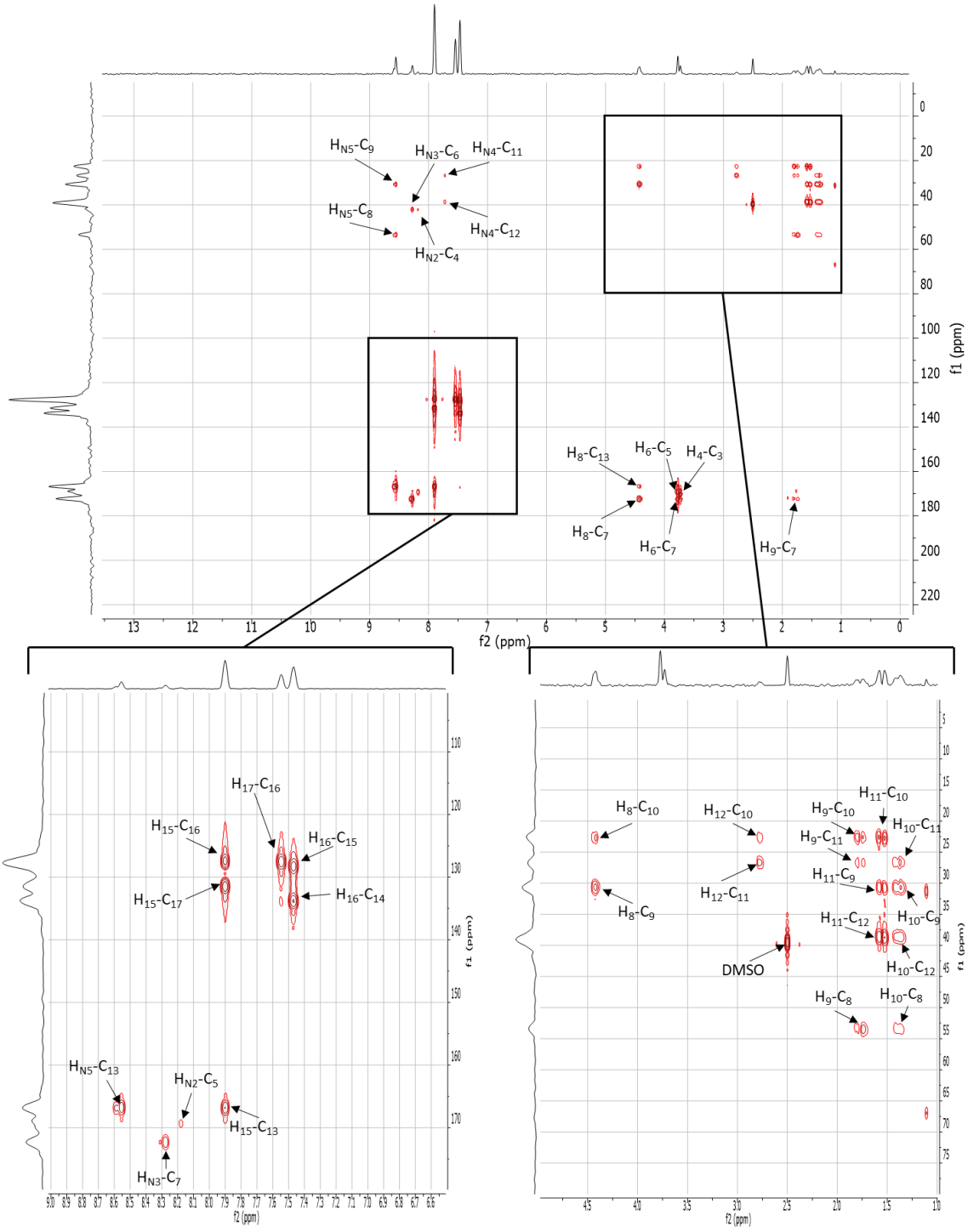


Figure 4.22 – ^{1}H-^{13}C HMBC NMR for Tren-Gly-Gly-Lys-Bam. NMR (600 MHz) in DMSO. Enlarged regions of the ^{1}H-^{13}C HMBC NMR spectrum are in the bottom panels. The spectrum is annotated with the correlations between specific hydrogens and carbons.

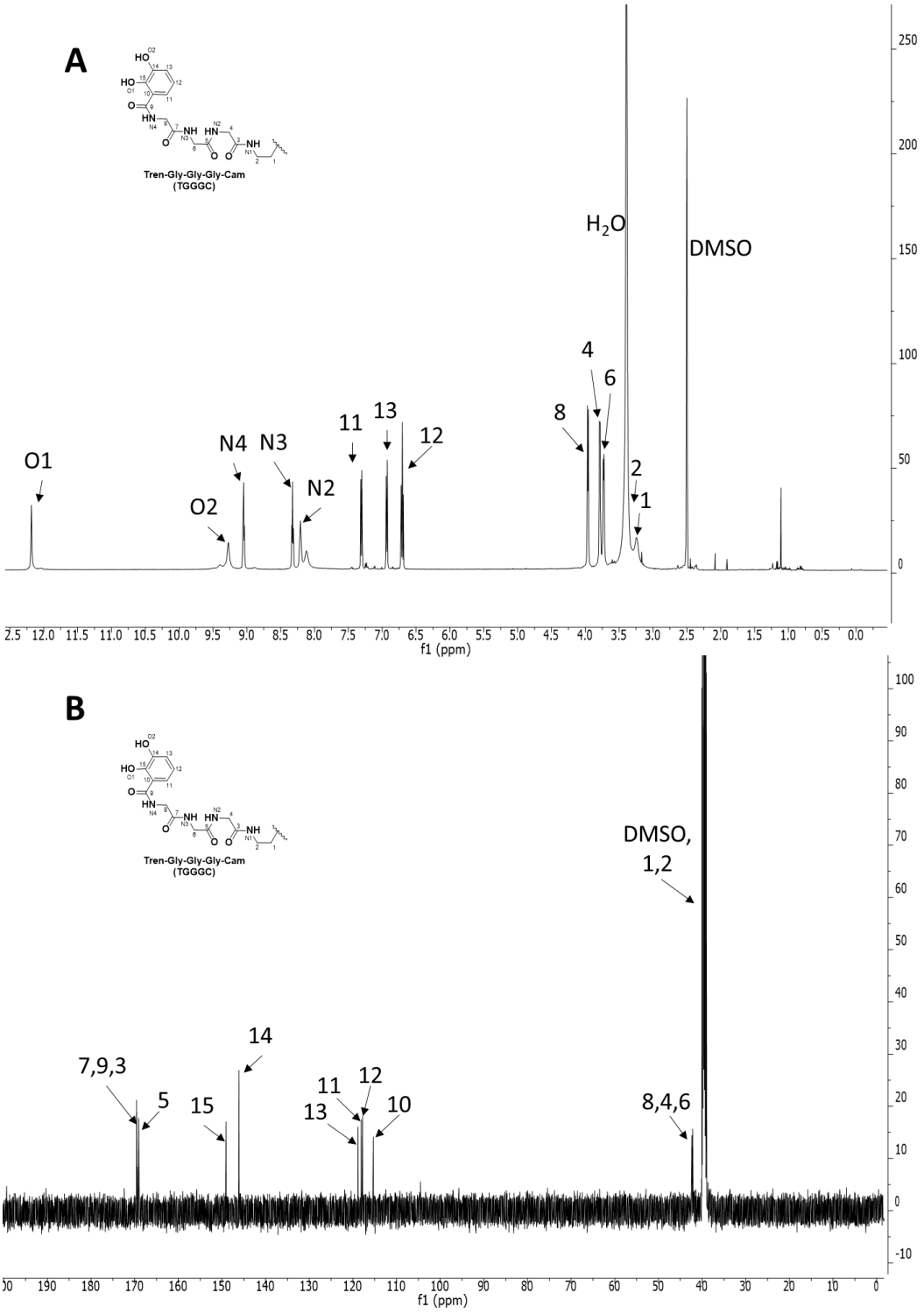


Figure 4.23 – NMR data for Tren-Gly-Gly-Gly-Cam. A.^{1}H NMR data for Tren-Gly-Gly-Gly-Cam. NMR (600 MHz) in DMSO. B. ^{13}C NMR data for Tren-Gly-Gly-Gly-Cam. NMR (500 MHz) in DMSO. Assignments were confirmed with HMBC, Figure 4.24.

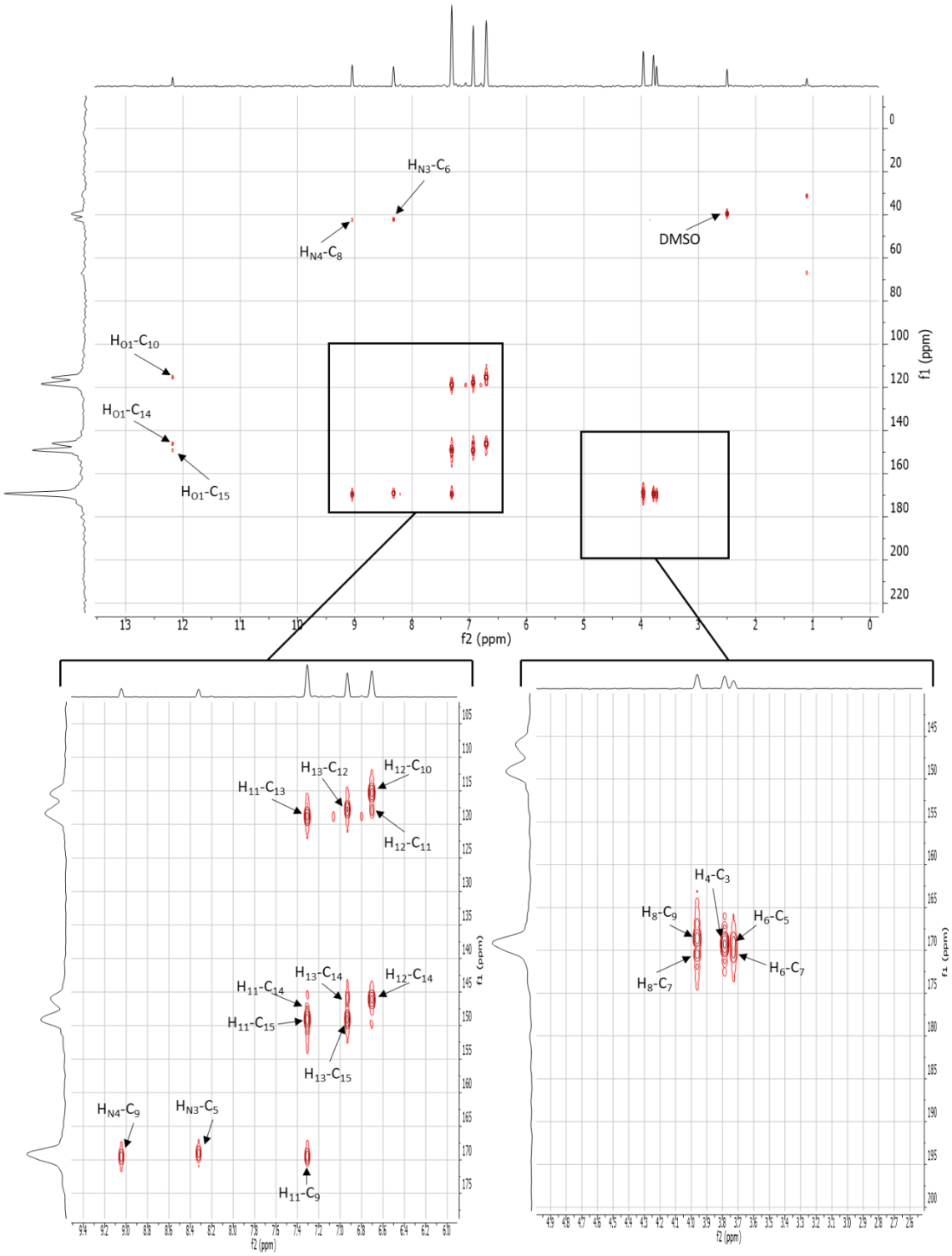


Figure 4.24 – ^{1}H-^{13}C HMBC NMR for Tren-Gly-Gly-Gly-Cam. NMR (600 MHz) in DMSO. Enlarged regions of the ^{1}H-^{13}C HMBC NMR spectrum are in the bottom panels. The spectrum is annotated with the correlations between specific hydrogens and carbons.

4.4.3 Surface Forces Apparatus Adhesion Measurements

Force-distance measurements were taken for all siderophore analogues following the general procedure outlined above. All experiments were performed in 50 mM acetate buffer at pH 3.3 with 150 mM KNO_3. Optimal deposition conditions, 10 µL of 1 mM siderophore analogue solution injected into the 50 µL salt solution droplet between the surfaces, was determined by progressive injection of siderophore analogue solution. 10 µL was the concentration at which the adhesive force and the compressed film thickness reached a maximum, typically 1-2 nm. These film thicknesses are slightly larger than the thicknesses reported by Maier et al. (~1-1.3 nm),[10-11] which is consistent with the larger molecular size and weight of the siderophore analogues used in this work. Further injection of siderophore analogue typically resulted in no significant changes in adhesion force and compressed thickness up to 50 µL of total siderophore analogue solution injected.

In force-distance measurement data, positive values represent repulsive forces while negative values are attractive forces by convention. Data points are recorded as open circles as the two surfaces are brought into contact and solid circles as the surfaces are pulled apart. The minimum force/radius value is the maximum adhesion recorded for the adhesive material being tested (Figure 4.25 – Figure 4.28).[18] Force-distance plots showed slight long-range repulsion on approach of the surfaces followed by a jump into adhesive contact. Further compression yielded a constant value for compressed film thickness, known as the hardwall thickness (D_T). This pattern of weak, long-range repulsion followed by a persistent shift in the point of closest approach of the mica surfaces is consistent with a monolayer of siderophore analogs adsorbed to each surface with a weakly bound diffuse multilayer extending into solution. Thus, on approach of the surfaces, overlap of the diffuse multilayers

yields weak repulsion until the adhesive groups on either monolayer can contact the opposing surface and the surface jump into adhesive contact.

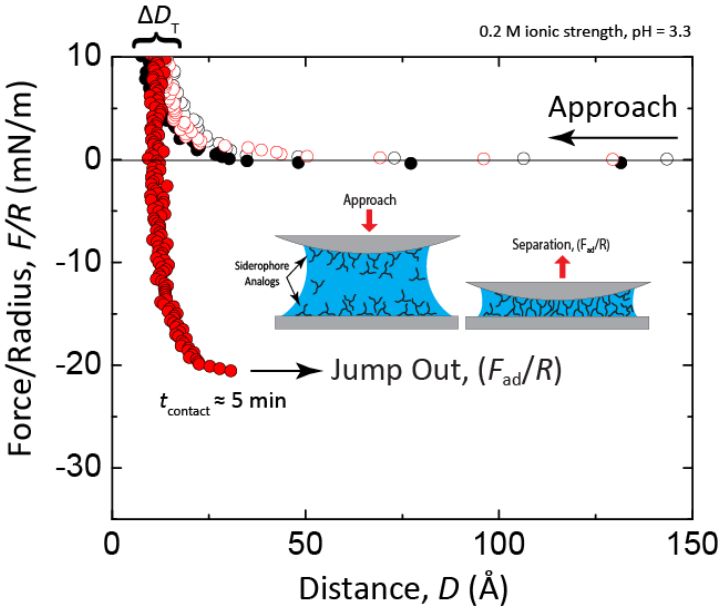


Figure 4.25 - Adhesion of TGGLC. Force-distance measurements were performed in 50 mM acetate buffer with 150 mM KNO_3 at pH 3.3. The surfaces were left in contact for 5 min before separation. Black and red data points represent measurements of the buffer and siderophore analogue, respectively. The open and solid circles are for data measured on approach and separation, respectively, of the mica surfaces. The inset displays a schematic of the interacting surfaces during SFA experiments.

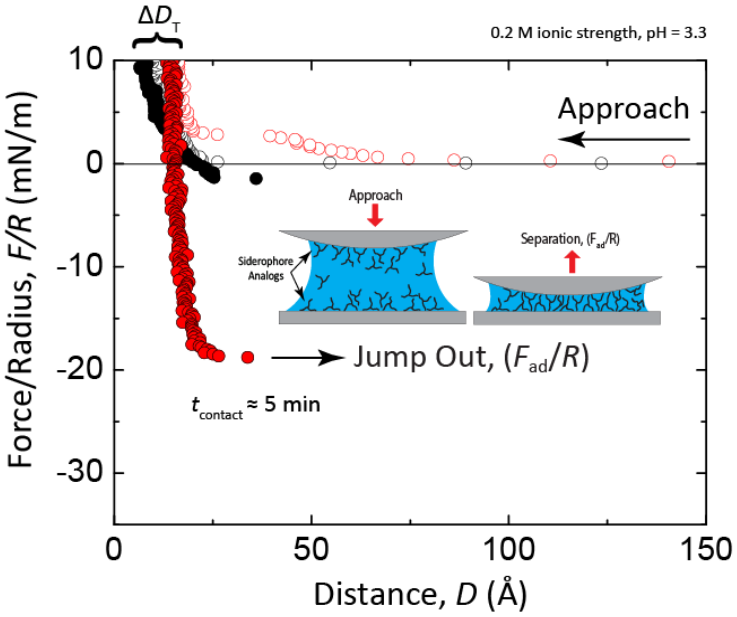


Figure 4.26 - Adhesion of TGLGC. Force-distance measurements were performed in 50 mM acetate buffer with 150 mM KNO_3 at pH 3.3. The surfaces were left in contact for 5 min before separation. Black and red data points represent measurements of the buffer and siderophore analogue, respectively. The open and solid circles are for data measured on approach and separation, respectively, of the mica surfaces. The inset displays a schematic of the interacting surfaces during SFA experiments.

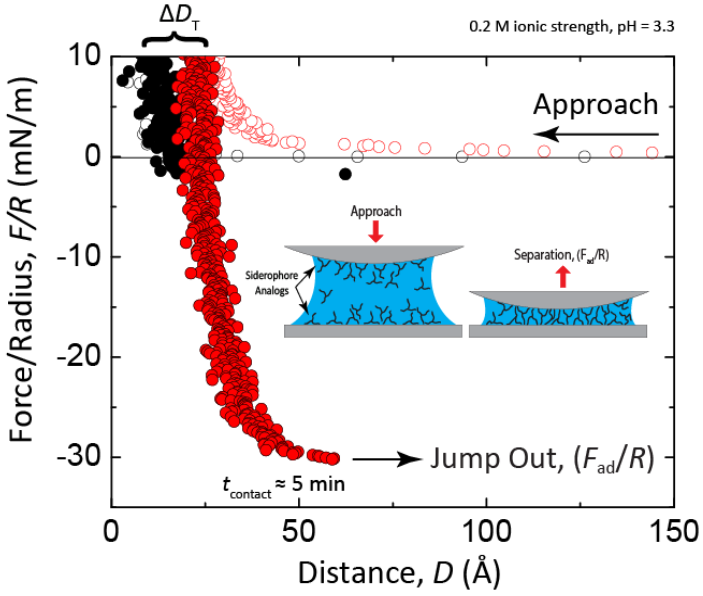


Figure 4.27 - Adhesion of TLGGC. Force-distance measurements were performed in 50 mM acetate buffer with 150 mM KNO_3 at pH 3.3. The surfaces were left in contact for 5 min before separation. The open and solid circles are for data measured on approach and separation, respectively, of the mica surfaces. Black and red data points represent measurements of the buffer and siderophore analogue, respectively. The inset displays a schematic of the interacting surfaces throughout the SFA experiments.

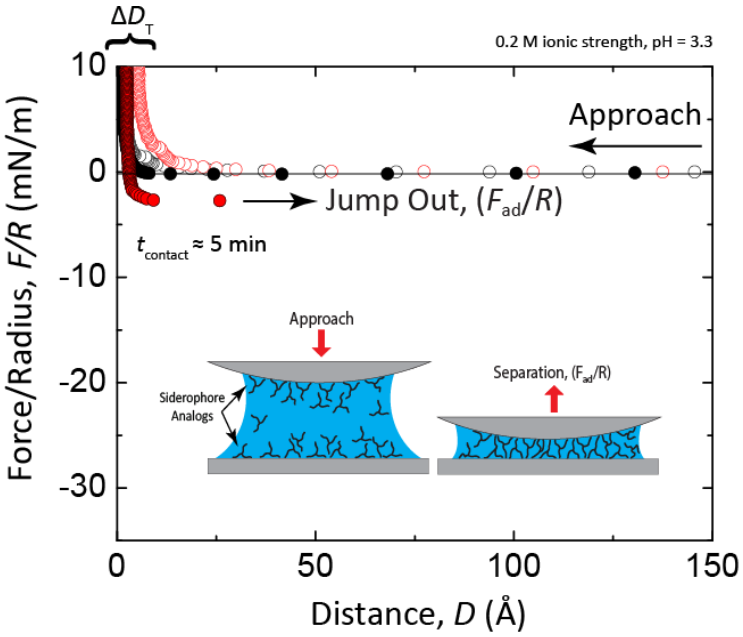


Figure 4.28 - Adhesion of TGGGC. Force-distance measurements were performed in 50 mM acetate buffer with 150 mM KNO_3 at pH 3.3. The surfaces were left in contact for 5 min before separation. Black and red data points represent measurements of the buffer and siderophore analogue, respectively. The open and solid circles are for data measured on approach and separation, respectively, of the mica surfaces. The inset displays a schematic of the interacting surfaces during SFA experiments.

4.5 Discussion

In SFA experiments with the siderophore analogues (TGGLC, TGLGC, and TLGGC), significant change in adhesive forces were not observed, within error, when catechol-cation spacing was varied (Figure 4.29). Rapp et. al speculated that while the intramolecular adjacency of catechol and cationic residues leads to a significant synergistic effect and greater adhesion, some cutoff separation should exist beyond which synergy would be weakened or abolished.[11] This prediction was supported by the weak adhesion forces measured upon co-injection of two different molecules, i.e., those containing only catechols and those containing only cationic amines.[11] Although the synergy between catechol and lysine was observed upon spacing of the groups by up to two glycine residues, it is likely that there exists some intramolecular catechol-cation spacing beyond which the synergy

would no longer operate. Future work is necessary to determine this distance; nevertheless, the current results are useful for informing the design of new adhesive compounds.

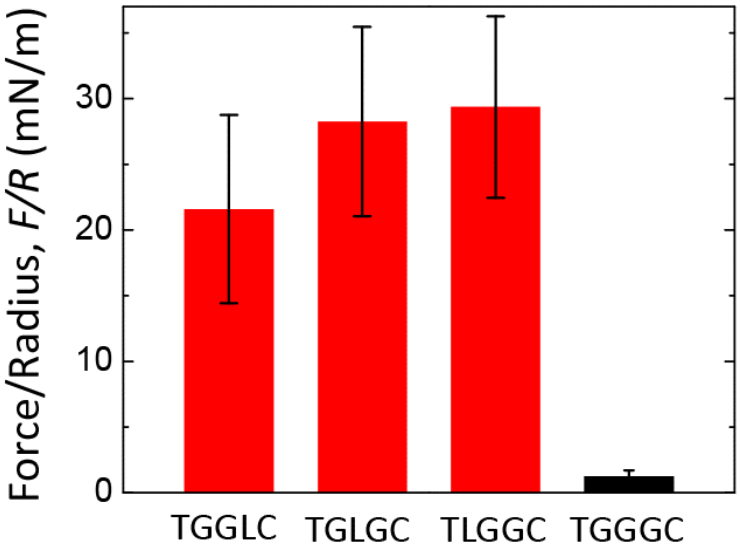


Figure 4.29 – The average measured adhesion force, normalized by radii of curvature of mica surfaces, to separate two mica surfaces adsorbed with 1 nmole of the analogue in buffer (50 mM acetate + 150 mM KNO_3) at pH 3.3 after 5 min of contact.

The magnitudes of the adhesion forces for TGGLC, TGLGC, and TLGGC (~30 mN/m) were less than the TLC siderophore analogs used in the work of Maier et al. (~60 mN/m) under similar experimental conditions.[10] This decrease in adhesion force is consistent with the increased size of the siderophore analogues used in this study. The larger molecular size leads to an increased area at the surface, which decreases the density of adhesive catechol and cationic groups per unit area in an adsorbed film.

The siderophore analogues used in this work also showed a similar dependence of adhesion force on contact time, with increased time spent at maximum compression of the surfaces resulting in increased adhesion force. Time spent in contact also resulted in a thinning of the siderophore analogue film. The increase in adhesion force and decrease in

film thickness is consistent with the following description of the film behavior (Figure 4.30). Upon injection of siderophore analogue molecules into solution between the surfaces, the molecules adsorb, forming a monolayer on each surface. Upon compression of the surfaces, free catechol and cation groups on each surface penetrate through the apposing monolayer and make adhesive contact with the apposing mica surface, therefore bridging the two mica surfaces. Increased time spent in contact allows the compressed film to rearrange, resulting in an increase in the number of bridging interactions and a decrease in the film thickness. Future experiments will measure the adhesive forces for each siderophore analogue (TGGLC, TGLGC, and TLGGC) at increased contact times to allow for comparison of the maximum adhesive forces.

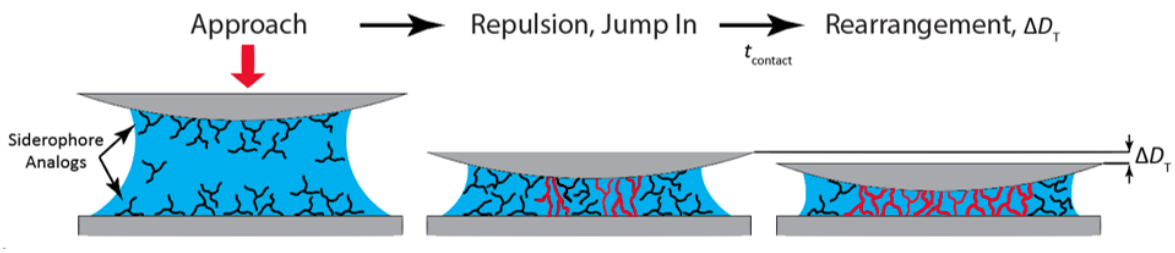


Figure 4.30 – Film behavior in SFA experiments. Siderophore analogue molecules highlighted in red represent those that are interacting with both surfaces creating a bridging interaction. Increased contact time ($t_{contact}$) leads to a decrease in film thickness (D_T) and more bridging interactions.

Two control molecules were synthesized: a siderophore analogue containing lysine and an aromatic group without the hydroxyl groups present in catechols (TGGLB), and an analogue containing only catechol, without the cationic lysine (TGGGC). Preliminary force measurements of films of the TGGGC molecule show low adhesion (~1 mN/m, Figure 4.29), consistent with the results of Maier et al. for siderophore analogues containing only catechol.[10-11] Future experiments will test the adhesion of films of the TGGLB molecules. It

is expected that the adhesion of the TGGLB analogue will be less than the adhesion of the siderophore analogue molecules containing both catechol and cation tested in this work.

These experimental results show that the synergy between catechol and lysine in adhesion to mica remains effective in promoting adhesion even when the moieties are separated by two glycine amino acids. The results also provide evidence that the detachment sequence of catechol and lysine in mussel-inspired adhesives does not majorly contribute to the observed synergy between these moieties as proposed by Li et al.[12] This research adds to the understanding of mussel adhesion and provides information that will inform the design of synthetic mussel-inspired adhesives.

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5. Pedagogy in the Chemistry Laboratory

5.1 Background

Laboratory instruction has held an important role in chemistry education for well over a century and its central role can be traced back to the German chemist, Jutus von Liebig (1803-1873). Liebig helped to expand and institutionalize the teaching of chemistry by emphasizing a practical laboratory approach. His popularity with students, both foreign and domestic, ensured the spread of his pedagogical methods.[1] By the 1920s, laboratory instruction and activities were largely used to verify information learned in lectures or textbooks, a change from student-centered activities.[2] Verification style laboratory activities have become commonplace and are now considered the "traditional" mode of instruction.

While laboratory instruction has the potential to enhance learning and encourage the development of important scientific skills (e.g. problem-solving, communication, and data interpretation), research has shown it rarely obtains this full potential.[2-3] Some researchers cite ineffectual promotion of conceptual change,[4] unrealistic portrayal of scientific experimentation,[5] and little requirement for critical thinking[6] as reasons for the apparent shortcomings of traditional laboratory teaching methods. This has led to an ongoing discussion of the value of the traditional laboratory, which gives little consideration to experimental planning and interpretation.[2-3, 5, 7-9]

It wasn't until the 1960s that we saw some of the first efforts to depart from traditional verification or "cookbook" style laboratory activities and a greater emphasis on the process of science and the development of higher cognitive skills through inquiry-based activities.[2] However, instructors still lacked an objective evaluation of what the best kinds of

experiences were and how to best implement them.[10] Educational goals for the laboratory were identified and classified by Shulman and Tamir[11] then further elaborated by Anderson.[12] However, these goals were synonymous with those defined for science courses in general. Hofstein and Lunetta[2] suggested an organization of goals specific to laboratory work into the three domains of Bloom's taxonomy:

Cognitive Domain:

1. To foster creative thinking.
2. To develop problem-solving skills
3. To increase understanding of the process of science and the scientific method.
4. To foster intellectual development.
5. To aid in the learning of scientific concepts.

Practical and Psychomotor Domain:

1. To develop skills in communication
2. To develop skills in performing laboratory activities.
3. To develop skills in observation.
4. To develop skills in analyzing experimental data.

Affective Domain:

1. To develop favorable attitudes toward science.
2. To foster creativity.
3. To promote a positive learning environment.

Hofstein and Lunetta called upon instructors and researchers to carry out a responsible dialogue on laboratory activities by examining the goals of science teaching and learning with care to identify optimal activities and experiences from all modes of instruction that

will best facilitate these goals.[2] The increased scrutiny of the traditional laboratory, especially through the lens of the above goals, and the dialogue regarding effective pedagogical methods has inspired a significant amount of work regarding effective laboratory activities. Today, there is little debate over the value of traditional methods that reduce the organic chemistry laboratory to "a rote exercise designed to consume minimal resources whether these be time, space, equipment, or personnel."[13] In fact, the 2015 ACS guidelines for undergraduate professional education in chemistry states:

> An approved program should use effective pedagogies in classroom and laboratory course work… Examples [for laboratory work] include… inquiry-driven and open-ended investigations that promote independent thinking, critical thinking and reasoning, and a perspective of chemistry as a scientific process of discovery.[14]

There is a growing consensus in the field that the instructive potential of laboratory activities can be more fully realized by abandoning traditional "cookbook" style labs in favor of active or inquiry-based learning. The President's Council of Advisors on Science and Technology (PCAST),[15] the National Center for Education Statistics (NCES),[16] and the National Research Council (NRC)[17] are just a few of the organizations that are joining the call for more inquiry-based instruction. Many instructors and education researchers have begun to move away from the verification style labs by incorporating nontraditional laboratory activities into the curriculum, such as guided inquiry (discovery), open inquiry, and project- or problem-based experiments.[18-22] It is believed that these types of laboratory activities promote the development of skills such as critical thinking,[5-6] problem-solving,[23] searching the chemical literature,[20] and communication,[5] as well as higher-order cognitive

skills.[24-25] The remainder of this chapter will briefly discuss the different types of laboratory activities.

5.2 Types of Laboratory Activities

Laboratory activities can take on many forms, but most can be classified into categories that lie on a continuum.[26] On one end of the continuum lies the traditional, highly structured and "cookbook" verification style experiments and at the other end lies investigative or inquiry activities where students are generating their own questions and experiments.[26] The categories used to differentiate laboratory activities were identified by Domin as: expository, discovery or guided inquiry, open inquiry, and project- or problem-based inquiry.[27] These styles can be differentiated by three dichotomic descriptors: outcome, approach, and procedure (Table 5.1).[27]

Table 5.1 - Descriptors of the laboratory instruction styles.

Laboratory style	Procedure	Approach	Outcome
Expository	Given	Deductive	Predetermined
Guided inquiry	Given	Inductive	Predetermined
Open inquiry	Student Generated	Inductive	Undetermined
Problem-based	Student Generated	Deductive	Undetermined

The procedure to be followed in a laboratory activity can either be designed by the student or provided to the student, either by the instructor or laboratory manual. The intended outcome of an activity can either be known by the student and instructor (predetermined) or known only by the instructor (undetermined). The dichotomy also exists in how the activity is approached. In the deductive approach, students apply a general principal towards understanding a specific phenomenon. In contrast, the inductive approach requires students to derive the general principal through observation of a phenomenon.

5.2.1 Expository Style Laboratory Activities

The expository style, also known as traditional or verification style, is the most commonly used and heavily criticized method for laboratory instruction. With this style, the instructor provides the topic of the investigation, connects the experiment to previous work, and directs how students approach the investigation.[23, 27] The procedure followed is unambiguous and guides the student to a predetermined outcome. Results obtained are often only used to compare to expected outcomes. The level of learning is limited, and the students are unclear of the aims of the exercise and unsure of what the results mean or how they are applied to the theory provided in lecture.[28] Wade states that "students can successfully complete the course by following the manipulative instructions without acquiring a fundamental understanding of the rationale behind any given manipulation."[29] Pickering takes this further with his view of the expository style in the organic chemistry teaching laboratory:

> Organic labs have degenerated to cooking… Organic chemistry is an inquiry into a specific aspect of Nature… Who would guess that from our teaching labs? Our labs show what organic chemists do with their fingers, but not what they do with their brains… There is some sort of cosmic futility in most organic labs. Make a white powder, prove that it is what you expect, donate it to chemical waste, again, and again, and again, if not to the last syllable of recorded time, at least until the end of the semester.[30]

Since the cognitive demand of expository laboratory activities tends to be low, they do not allow for the development of students' scientific thinking skills.[25, 31] However, there is some literature that suggests that expository activities can develop students' observational

skills, manual dexterity, competency with laboratory equipment, and accuracy and precision of measurements.[32] Young also recognized that expository instruction provides clarity in teaching of principles and techniques, clearly shows how the procedure fits the experiment, and increases student confidence.[33] The expository style of laboratory teaching gained so much popularity because it addresses some complications with laboratory instruction; namely, it allows for a large number of students to participate simultaneously with minimal instructor involvement, the costs associated with running the laboratory are minimized, and the time required for the entire process is generally only a two to three hour span.[13]

5.2.2 Guided Inquiry or Discovery Style Laboratory Activities

Guided inquiry or discovery laboratory teaching originated in the 20th century with Henry Armstrong, who taught with a heuristic method where students generated their own questions for investigation.[34-35] Students received minimal guidance and, as such, were placed in the role of discoverer. Piaget's descriptions of how children learn through unstructured and self-directed observations and experimentation helped make this and other inquiry-based laboratory techniques popular in the 1960s, where they became the basis for science education reform.[27, 35-36] Schwab was a proponent of the discovery methodology and he argued that the laboratory manual should "cease to be a volume which tells the student what to do and what to expect" and be "replaced by permissive and open materials which point to areas in which problems can be found."[37]

In the discovery approach, students are given general procedures to investigate a specific phenomenon and told what data might be useful to collect, all without any theoretical introduction to the topic. The role of the instructor is to guide students in developing their

investigations and to aid in the development of theories/principles from their observations.[38] This approach is inductive; students develop a general understanding of an underlying principle by studying specific examples of a phenomenon. Inductive activities are meant to give students an ownership of their learning process, making the knowledge obtained more personal, meaningful, and easier to retain.[39] This guided inquiry, however, "confuses learning science with doing science" by claiming to be student-driven and open-ended while demanding a particular outcome.[35]

Discovery learning has rightfully received a large amount of criticism since it was popularized in the 1960s.[35, 40-43] The process tends to be very time consuming and, as Dearden puts it, "a teaching method which genuinely leaves things open for discovery also necessarily leaves open the opportunity for not discovering them."[44] This can lead to many experiments failing to give the results required to learn the intended concepts. Hodson describes discovery learning as "philosophically unsound (because it is based on a false model of science)" and "pedagogically unworkable."[35] He goes on to say:

> What had started out as a psychological justification of learning by discovery had slipped over into an epistemological one. Unfortunately, the view of science promoted by discovery learning was a highly distorted one, based on a number of mistaken assumptions about the priority and security of observations.[35]

While this style of laboratory teaching has largely been abandoned in favor of other inquiry-based techniques, some still advocate for its use.[45-50]

5.2.3 Open Inquiry Style Laboratory Activities

Like discovery or guided inquiry, open inquiry can trace its beginning back to the 1960s, where it was also a basis for laboratory education reform. Open inquiry based activities are also inductive and require students to generate their own procedure.[34] However, unlike guided inquiry and expository methods, the outcome of open inquiry is unknown by both the student and the instructor. Students are given a vague assignment and asked to formulate the problems they investigate, to relate the investigation to previous work, to state the purposes of the investigation; and the students actually identify the problem, state the purpose, predict the results, identify the procedures, and perform the investigation.[23] Inquiry activities encourage higher-order cognitive processes such as hypothesizing, explaining, criticizing, analyzing, judging evidence, inventing, and evaluating arguments.[6] However, since students receive less guidance from the instructor in open inquiry than guided inquiry, they are better able to develop these skills. Like other inductive methods, open inquiry gives students ownership of the learning process, which results in improved student attitudes toward science.[5-6, 51-52]

Despite the benefits of open inquiry instruction, it has not been adopted extensively.[6] There was a strong movement in the 1960s to incorporate open inquiry into curriculum, but analysis shows the attempts largely failed to achieve their anticipated goals.[27] Reasons range from the technique being too taxing on student's short term memory[53] to placing too much focus on the scientific process and not enough on science content.[54] Open inquiry does share some of the same criticisms associated with discovery based activities in that it is often time consuming, meaning less time can be spent learning new concepts and principles, and that the openness of the activity can lead to a failure to adequately teach concepts. Additionally,

there is common debate as to whether developing the techniques of scientific inquiry are more important than the acquisition of particular concepts.[35]

5.2.4 Project- or Problem-Based Laboratory Activities

Project- or problem-based activities are becoming a popular alternative to the previously discussed techniques.[5, 18-19, 55-61] This method of instruction can also trace its origin back to the early 20th century where Smith and Hall described a method of laboratory instruction where the instructor played an active role by posing problems to the students, providing the necessary materials and equipment, and carefully moving the students towards a successful solution to the problem based on their conceptual understanding.[62]

Problem-based learning was also used in the 1960s, but to a much lesser extent.[27] Young used a procedure that discarded the lab manual and had students create their own procedures to solve a problem and, after results were obtained and conclusions reached, submit a written lab report.[63] His method put emphasis on the development of testable hypotheses rather than obtaining correct results.[63] Battino similarly designed a method that lacked a lab manual.[64] Instead, students participated in a discussion to design the experiment.

In a problem-based activity, students must apply their understanding of a concept to devise a solution, requiring them to think about what they are doing and why they are doing it.[27] Students are typically presented with a problem that has a clear goal, but many feasible paths toward a solution. Wright described the problems as:

> …conceptually simple; but there are enough details and enough unanticipated problems arise during the projects to provide students with many opportunities for creativity and practice in problem solving. In addition, the students struggle with course concepts in the context of a realistic problem,

and this opportunity provides much greater insight into the course material. It also gives better insights into the way that science is actually practiced.[18]

Like guided and open inquiry, problem-based learning can be time consuming and is much more demanding of both the instructor and student.[27] However, it also fosters independence, higher-order cognitive skills, and student ownership of the learning process.[65] The problem-based approach differs from open or guided inquiry in that the approach is deductive. Students are applying knowledge of concepts and principles already gained to carry out their experiment and demonstrate an understanding of the relevant concepts. Increased student independence, participation, morale, and positive perception of science of science have been observed and confirmed by feedback when project-based laboratory activities are implemented.[58, 61, 66-69]

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6. A Project-Based Synthetic Organic Chemistry Laboratory Course

6.1 Background

How students experience and perceive the laboratory is an important factor to consider when designing the laboratory curriculum.[1] Each student has their own image of science and these views influence how students approach and practice science.[2] Ryder et al. describe the important images as "students' views about the relationship between knowledge claims and data in science; the existence of lines of scientific inquiry and how these are sustained; and the role of social interactions in the scientific community."[2] These views guide the student in making many important decisions often faced by scientists, including decisions about the validity and reliability of data, about proper experimental design, and about how and where to search for scientific knowledge.[2-3]

In traditional laboratory activities, many students are not challenged to think independently. There is a consensus in the chemical education literature that traditional organic laboratory experiments do not provide students with an authentic research experience and deprive them of the necessary skills to be successful scientists.[4-9] These traditional labs place little emphasis on critical thinking, planning, interpreting results, or discussing conclusions.[4-8, 10-11] Students are often only trying to find the correct answer,

trying to avoid mistakes in the lab, and have the major goal of finishing lab early, all of which leads to a disconnect between the lab tasks and concepts, and a failure to achieve meaningful learning.[12-13]

To address the laboratory curriculum concerns mentioned previously, many authors advocate for a project-based approach.[1, 4, 14-22] The project-based laboratory provides an excellent alternative to traditional laboratory exercises as they are an opportunity to develop students' images of science,[2] to think about the procedures and the chemical principles being investigated more deeply,[1] to be involved in an activity that better reflects real-life problem-solving situations,[23] and to provide an introduction to the chemical literature.[15] In this environment, students are encouraged to think independently about a problem while the instructor is readily available for consultation.[24]

While the research thus far is limited, there are some studies on students' perspectives and experiences in project-based labs and their effects on learning. In one study, students in a traditional laboratory environment had a more passive view of what the lab was about while those in a project-based lab viewed the lab as a place to make mistakes and engage in experimentation.[25] Students who participate in research-based laboratory activities, which are closely related to project-based labs, were better able to explain what they did in the lab, had a greater sense of accomplishment, and understood the application of the laboratory to their lives.[26]

Undergraduate students, especially those underrepresented in STEM fields, benefit greatly from research experiences.[27-28] Limited research opportunities for undergraduates leaves many students unable to participate in authentic research and forces them to look toward undergraduate teaching labs to satisfy their interest. Research-based laboratory courses have been shown to mimic some of the true research experiences and similarly

improve inquiry skills (developing hypotheses, designing experiments, and interpreting data) and self-efficacy.[29] Linn states that much of the benefit associated from research comes from "discussion with mentors, participation in group meetings where current research is discussed, guided opportunities to explore relevant research literature, reflection on observations in weekly journals, and synthesis of their insights by creating research proposals, reports, or posters."[30] A Project-based laboratory courses can employ many of the same activities to bring the benefits of authentic research to the undergraduate teaching labs.

To provide students an opportunity to participate in a more research-like setting and to develop their data interpretation and analysis skills, a project-based laboratory course was developed. This course is ideally taken at the end of their second year or beginning of their third year, after two quarters of organic laboratory. In this project-based course, students have the opportunity to learn to navigate the primary chemical literature and to develop critical-thinking skills and advanced research and laboratory techniques.[15, 31-32] Students are assigned to synthesize a pharmaceutical target through a process modeled after academic research with three phases: (1) literature-based proposal, (2) experimentation and analysis, and (3) communication of research findings via an oral presentation and written report. This course was designed to minimize the demand placed on the undergraduate stockroom while providing students a simulated research experience. The course was implemented at the University of California, Santa Barbara.

6.2 Course Goals

To foster a research environment, our goal is to encourage the mentoring relationship between each student and their teaching assistant and to encourage the investment of each student in their project. The teaching assistant's role in the course is to be a mentor,

encouraging students to use the scientific method, ask questions about their results, overcome problems when necessary, and make valid conclusions about their work. This helps the students become engaged in their project. The teaching assistant is also a resource for students when they do not understand an idea or a new technique. When needed, the teaching assistant can intervene to ensure that each student manages their time efficiently and stays motivated throughout their synthesis project. The element of the "right or wrong answer" is attenuated in the inquiry-based approach. Rather, the teaching assistant helps students realize that there are generally many methods to reach their synthesis goals, but one might produce better results, be safer, cheaper, or more efficient.

The successful student realizes that there are a variety of aspects to consider when approaching their project and any problems that arise. For example, the student must use their previous knowledge of organic chemistry combined with their new search tools (Reaxys and SciFinder) as a means to build their knowledge and formulate successful laboratory protocols. The students must also reassess their synthetic procedures and adapt their methods when unexpected results are encountered. As a result, students are directly engaged in developing their own learning process and in gaining a sense of responsibility for their project and their education. Additionally, students often become interested in other students' projects and engage in discussion between groups about the larger goal of their synthesis and the differing synthetic procedures and concepts. We have found that when students have an invested interest in their project, they often come to the instructor with proposals, asking for guidance rather than instruction. This learning transformation is important as they begin their transition to becoming scientifically minded individuals.

6.3 Pharmaceutical Research Project

Seven distinct targets (Figure 6.1) have been implemented thus far.[33-57] The chosen targets provide a tangible, real-world application of organic synthesis as opposed to the abstract syntheses performed in traditional labs. However, the potential syntheses are not limited to those in this manuscript. This enables the projects to be continually rotated. Each quarter, the aim is to have distinct targets for each group in each section.

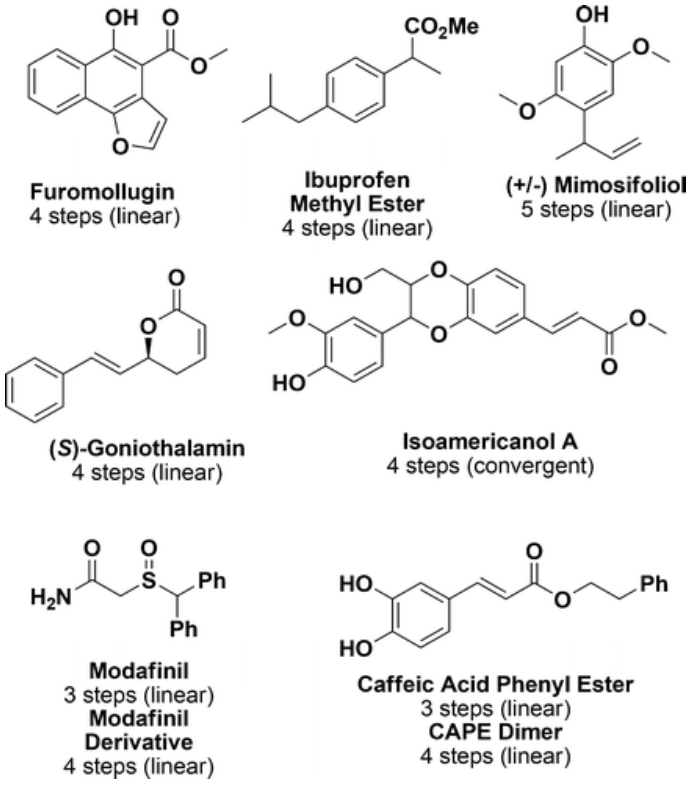

Figure 6.1 - Pharmaceutical targets

6.4 Course Implementation

The course design and curriculum described was taught to 90 students in ten unique laboratory sections, spanning four quarters, and instructed by seven different teaching assistants. Each section contained up to twelve students and met for two sessions that lasted

4 hours each per week over a ten week quarter. Requirements pertaining to the laboratory space and project materials are detailed in the Supporting Information[58] for both the instructor and the person(s) responsible for setting up the laboratory.

Students work in pairs; each pair is assigned to synthesize one of the target structures. Students are then guided through the three phases outlined below.

6.4.1 Phase 1: Literature-Based Proposal

The first phase of the course is dedicated to developing the project using Web-based resources. Students begin with a retrosynthesis assignment, guided by the teaching assistant, and learn the importance of their assigned target molecule.[59] Next, they delve into the primary literature with the aid of Reaxys and SciFinder to find a viable synthetic pathway.[32, 60-61] Most of the target molecules have more than one known synthetic route; a worksheet is provided to guide their planning toward a feasible, safe, and cost-effective known route that has been identified for each pharmaceutical target so the stockroom can plan ahead of time what reagents each group will use. However, the students are given the freedom to try other routes/reagents if they can find sufficient literature precedent. Students are required to discuss their synthesis plan with the instructor and make necessary adjustments before proceeding to the laboratory. The background research, retrosynthetic analysis, and synthesis plan are then used to write a "grant-like proposal" that ultimately serves as a starting point for writing their written report (Phase 3).[62]

6.4.2 Phase 2: Experimentation and Analysis

During the second phase, students complete the synthesis of their pharmaceutical target; each synthetic route is 3–5 steps in length and uses readily available starting material. Each group is given 4 g of starting material and is encouraged to begin on a smaller scale (~100

mg), then scale-up when the reaction conditions are validated. Due to the diverse nature of the projects, students are required to complete reaction sheets that detail the reaction scheme, intended experimental setup and procedure, workup, and characterization predictions. Students are also asked to include any relevant physical and safety data about the reagents they will use. As students work through their procedures, they use characterization techniques such as TLC and NMR spectroscopy to analyze their results.[63] Although most of the procedures are reported in the literature, students are required to adapt protocols for their project within the constraints of their laboratory setting. Instructors continually assess student progress and provide recommendations. An instructor guide for each synthesis is included in the Supporting Information.[58]

6.4.3 Phase 3: Communication of Research

In the third and final phase of this course, each group presents their research findings and each student writes a manuscript, using the *Organic Letters* template, to communicate their progress. Approximately 2 weeks before the conclusion of the course, students submit a draft of their initial manuscript and slides that will be used in their oral presentation. The instructor and student peers (if desired) provide feedback to the students before they submit the final manuscript and present their work orally. Emphasis is placed upon writing and effective presentation styles. The final oral presentation is delivered to their lab mates, teaching assistant, and the class instructor on the last day of instruction. Classmates are required to ask questions and provide written peer review commentary and scores to the instructor. After a peer-led discussion, the instructors ask additional questions and provide constructive criticisms on presentation content and style. We have found that for many students this is their first opportunity to present scientific findings.

6.5 Hazards and Safety

Some of the reagents used in this course are hazardous to health or dangerous when used improperly. These hazards are outlined in the Supporting Information[58] in the instructor guides. To be certain students used these reagents properly, Standard Operating Procedures (SOPs) were developed and kept in the laboratory for reference. Students signed the SOPs pertaining to their project after reading them in order to ensure understanding. Instructors made certain to be mindful of students using these reagents and closely supervised their work. Proper disposal containers were provided for organic, aqueous, and solid waste. Students also completed a safety video and a brief online quiz through the on-campus Environmental Health and Safety Web site. Proper attire was required at all times. This included lab coats, goggles, and gloves.

6.6 Student Feedback

Student feedback was positive and indicated that they had valuable pedagogical relationships with both their teaching assistant and their project. They commented on learning about synthesis and the problem-solving aspects that are necessary to complete a research project. Students realized that it was necessary to think about the *what*, *why*, and *how* questions through analysis of results. Students often mentioned that the independent nature of the research project was unexpected, but the ability to see a complex project from beginning to end made it more interesting than previous laboratory courses. With this ownership of the learning process, teaching assistants noticed that the students had a greater desire to learn and a greater appreciation for what is learned. Since this course has many different projects running concurrently, it encouraged students to think more independently and help each other understand conceptual points and knowledge rather than just check with

their lab mates to be sure they were doing the experiments "correctly". Although most students still found organic chemistry difficult, they realized that synthesis requires more than just following a recipe. Select student quotes are included below. All student feedback collected is found in the Supporting Information.[58]

- "I actually feel like I did real chemistry for the first time."
- "It taught you to be organized and effective in your planning."
- "Nothing is necessarily set in stone, use common sense and apply it to the basic principles given. [This course] allowed for critical thinking besides just following instructions."
- "I liked how it was one big experiment as opposed to several small ones."

6.7 Conclusions and Possible Adaptations

The Supporting Information[58] includes reported literature yields and we have found that while many students were able to achieve these yields, or even higher, others struggled. However, most students that were diligent were able to synthesize their final product in the time allotted. This course was implemented into a 10-week quarter system, but can easily be adapted to the semester system in a variety of ways. In a semester system, we suggest implementing this course as an advanced organic laboratory course or as part of the second semester organic chemistry laboratory. If the semester is divided into two parts, the first part can be dedicated to other wet-lab experiments and Phase I of the pharmaceutical research project to allow for more investigative time and instructor feedback in the planning stages. The second half can then be used for Phase II and III of the project. An alternate way to implement this course in the semester system is to expand the curriculum to go in depth on related topics including the following:

1) A presentation on grant proposal writing with more opportunities for feedback.

2) A more in-depth assignment or group-work to introduce retrosynthesis of more complex molecules.

3) Hands-on demonstrations of standard laboratory techniques (this could include time to implement reagent titration or purification for the projects).

4) An iterative review process for writing the final report manuscript.

5) A discussion on how to formulate figures, schemes, and tables for publication.

6) Demo presentation given by the teaching assistant, professor, or guest speaker to address presentation skills and style.

7) A discussion on green chemistry and analyses of how the students can make their synthetic routes “greener”.

If Internet resources are limited and students do not have access to Reaxys, SciFinder, or the primary literature articles, the necessary articles for the corresponding project can be provided to the students as a literature packet or they could be available to check out upon request. This approach does not allow students to explore the chemical literature in the most authentic way, but nonetheless, it provides students with the opportunity to read the primary literature, to adapt the experimental procedures, and to learn about the process of chemical research in a pedagogical laboratory setting.

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7. Supporting Information

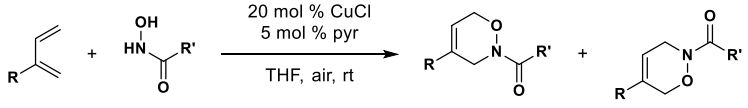
7.1 Chapter 2 Supporting Information

7.1.1 Materials and Methods

Unless stated otherwise, reactions were conducted in flame-dried glassware open to air using reagent grade solvents. All commercially obtained reagents were used as received. Reaction temperatures were controlled using a Heidolph temperature modulator, and unless stated otherwise, reactions were performed at room temperature (rt, approximately 23 ˚C). Thin-layer chromatography (TLC) was conducted with E. Merck silica gel 60 F254 pre-coated plates, (0.25 mm) and visualized by exposure to UV light (254 nm) or stained with potassium permanganate. Flash column chromatography was performed using normal phase silica gel (60 Å, 230-240 mesh, Geduran®). Regioisomers were isolated as mixtures and data is reported as follows: mass isolate (mg), percent yield, regioisomeric ratio (distal:proximal). ^{1}H NMR spectra were recorded on Varian Spectrometers (at 400, 500 and 600 MHz) and are reported relative to deuterated solvent signals. Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz) and integration. Unless stated otherwise, reported ^{1}HNMR spectra are of the major regioisomer. ^{13}C NMR spectra were recorded on Varian Spectrometers (125 and 150 MHz). Data for ^{13}C NMR spectra are reported in terms of chemical shift. Unless stated otherwise, reported ^{13}CNMR spectra are of the major regioisomer. IR spectra were recorded on a Perkin Elmer Spectrum 100 FT/IR and are reported in terms of frequency of absorption (cm^{-1}). Low and high resolution mass spectra were obtained from the UC Santa Barbara Mass Spectrometry Facility.

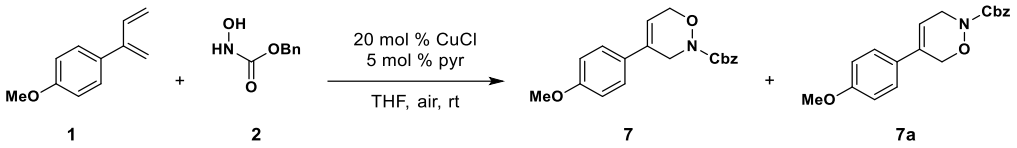
N-hydroxycarbamates **2**, **S-7**, and **S-8** were prepared from the corresponding chloroformate or anhydride according to literature precedent.[1] N-hydroxyureas **S-10** and **S-11** were prepared from the corresponding amine according to literature precedent.[2] Hydroxyurea (**S-9**) and isoprene (**S-5**) were purchased from a commercial source and used as received. Starting 2-aryl 1,3-butadienes (**1**, **S-1**, **S-2**, **S-3**, **S-4)** were prepared from the corresponding aryl alkyne according to literature precedent.[3] Bulky 2-alkyl 1,3-butadiene (**13**) was prepared according to the same procedure from commercially available 3-butyn-2-ol following a TBS protection.

7.1.2 General Procedure



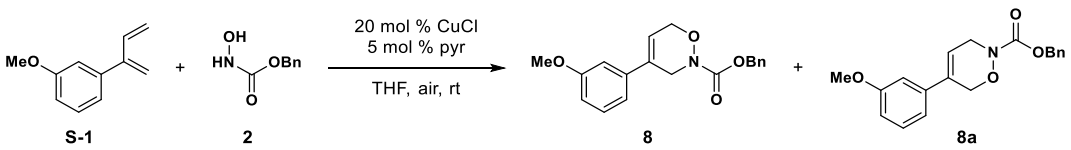
To a stirred solution of hydroxamic acid (1 equiv) and 2-substituted-1,3-butadiene (1.2 equiv) in THF (0.1 M) was added 20 mol % CuCl and 5 mol % pyridine. The reaction was stirred at room temperature open to the air until complete by TLC. Upon completion, the reaction was quenched with EDTA (0.5 M, pH 7.0), diluted with ethyl acetate and stirred until color no longer persisted in organic layer (approx. 30 min). The reaction was extracted with ethyl acetate three times and the combined organic layers were dried over MgSO4. The product was filtered and then concentrated *in vacuo*. The residue was purified by column chromatography to afford the corresponding oxazine product.

7.1.3 Syntheses and Compound Characterization



1 2 7 7a

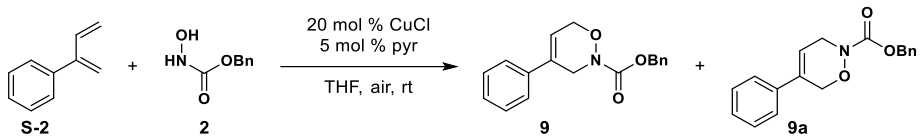
benzyl 4-(4-methoxyphenyl)-3,6-dihydro-2H-1,2-oxazine-2-carboxylate (7): According to the general procedure, oxazine products **7** and **7a** were isolated as a mixture (28 mg, 90%, 15:1). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.42 – 7.25 (m, 7H), 6.91 – 6.84 (m, 2H), 6.09 – 6.04 (m, 1H), 5.24 (s, 2H), 4.61 – 4.56 (m, 2H), 4.51 – 4.46 (m, 2H), 3.80 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.6, 155.6, 135.9, 132.3, 129.5, 128.5, 128.3, 128.2, 126.0, 118.1, 114.0, 68.8, 67.8, 55.3, 46.3; IR (thin film) 3041, 2933, 1706, 1608, 1515, 1455, 1346, 1283, 1246, 1182, 1095, 1032 cm^{-1}; MS (ESI) *m/z* 348.13 (348.12 calculated for $C_{19}H_{19}NO_4Na$ $[M+Na]^+$)



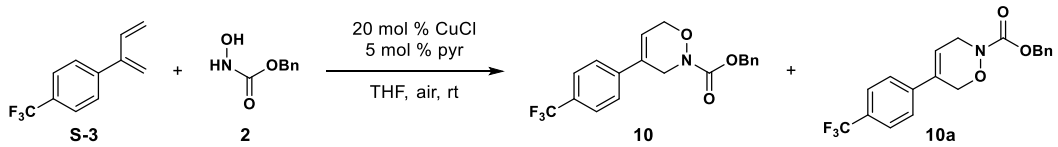
S-1 2 8 8a

benzyl 4-(3-methoxyphenyl)-3,6-dihydro-2H-1,2-oxazine-2-carboxylate (8):

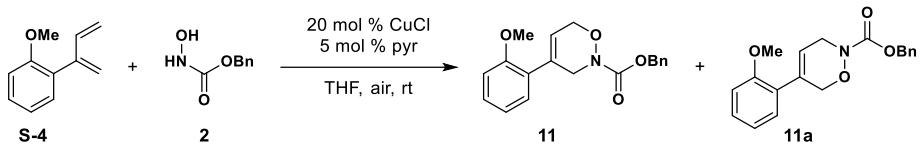
According to the general procedure, oxazine products **8** and **8a** were isolated as a mixture (24 mg, 78%, 9:1). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 – 7.22 (m, 6H), 6.96 – 6.91 (m, 1H), 6.89 – 6.82 (m, 2H), 6.19 – 6.11 (m, 1H), 5.24 (s, 2H), 4.62 – 4.57 (m, 2H), 4.52 – 4.48 (m, 2H), 3.80 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.8, 155.6, 138.4, 135.9, 132.9, 129.7, 128.6, 128.3, 128.2, 120.2, 117.3, 113.4, 110.9, 68.7, 67.8, 55.2, 46.3; IR (thin film) 3064, 2941, 2839, 1706, 1600, 1580, 1430, 1346, 1289, 1211, 1171, 1098, 1050 cm^{-1}; HRMS (ESI) *m/z* 348.1248 (348.1212 calculated for $C_{19}H_{19}NO_4Na$ $[M+Na]^+$)



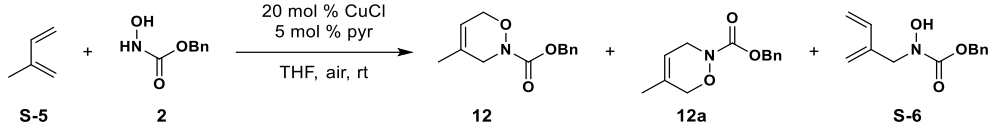
benzyl 4-phenyl-3,6-dihydro-2H-1,2-oxazine-2-carboxylate (9): According to the general procedure, oxazine products **9** and **9a** were isolated as a mixture (25 mg, 82%, 9:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 – 7.25 (m, 10H), 6.23 – 6.18 (m, 1H), 5.27 (s, 2H), 4.65 – 4.60 (m, 2H), 4.57 – 4.52 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.6, 136.9, 135.9, 133.0, 128.7, 128.6, 128.3, 128.2, 128.2, 124.9, 119.9, 68.8, 67.9, 46.3; IR (thin film) 3033, 2906, 2849, 1704, 1497, 1408, 1347, 1221, 1096 cm^{-1}; MS (ESI) *m/z* 318.12 (318.11 calculated for $C_{18}H_{17}NO_3Na$ $[M+Na]^+$)



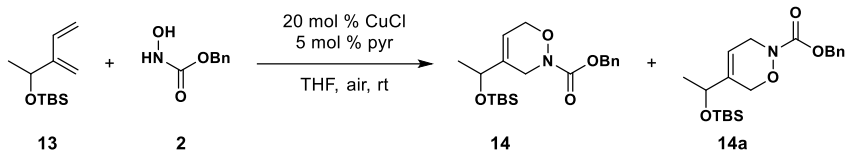
benzyl 4-(4-(trifluoromethyl)phenyl)-3,6-dihydro-2H-1,2-oxazine-2-carboxylate (10): According to the general procedure, oxazine products **10** and **10a** were isolated as a mixture (30 mg, 88%, 4:1). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.61 (d, J = 8.0 Hz, 2H), 7.45 (d, J = 8.0 Hz, 2H), 7.42 – 7.30 (m, 5H), 6.30 – 6.26 (m, 1H), 5.25 (s, 2H), 4.64 – 4.60 (m, 2H), 4.54 – 4.50 (m, 2H); Mixture ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.5, 155.5, 140.3, 139.9, 135.8, 133.8, 132.1, 130.1 (q, *J* = 32.7 Hz), 128.6, 128.4, 128.2, 128.2, 125.6 (q, *J* = 3.7 Hz), 125.1, 123.9 (q, *J* = 272.2 Hz), 122.2, 120.5, 69.6, 68.6, 68.0, 68.0, 46.1, 45.1; IR (thin film) 3035, 2922, 2850, 1708, 1616, 1414, 1327, 1167, 1117, 1071 cm^{-1}; HRMS (ESI) *m/z* 386.1095 (100%), 387.1143 (21%), 388.1161 (3%) (386.0980, 387.1014, 388.1047 calculated for $C_{19}H_{16}F_3NO_3Na$ $[M+Na]^+$)



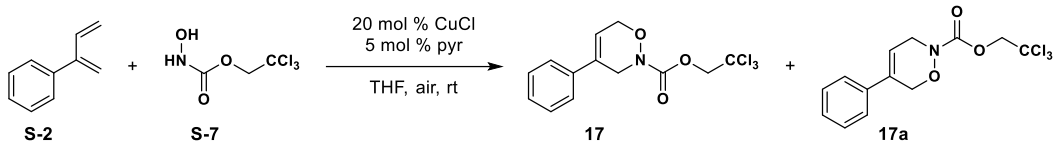
benzyl 4-(2-methoxyphenyl)-3,6-dihydro-2H-1,2-oxazine-2-carboxylate (11): According to the general procedure, oxazine products **11** and **11a** were isolated as a mixture (29 mg, 92%, 4:1). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43 – 7.25 (m, 6H), 7.19 – 7.14 (m, 1H), 6.96 – 6.84 (m, 2H), 5.95 – 5.91 (m, 1H), 5.23 (s, 2H), 4.63 – 4.58 (m, 2H), 4.55 – 4.51 (m, 2H), 3.77 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 156.9, 155.6, 136.1, 133.8, 129.5, 129.4, 128.6, 128.3, 128.2, 121.9, 120.9, 110.9, 77.2, 68.8, 67.7, 55.4, 47.5; IR (thin film) 3064, 3033, 2943, 2838, 1727, 1706, 1597, 1490, 1455, 1343, 1215, 1094 cm^{-1}; HRMS (ESI) *m/z* 348.1326 (348.1212 calculated for $C_{19}H_{19}NO_4Na$ $[M+Na]^+$)



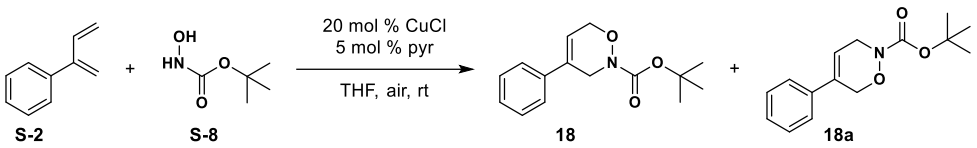
benzyl 4-methyl-3,6-dihydro-2H-1,2-oxazine-2-carboxylate (12) and **benzyl hydroxy(2-methylenebut-3-en-1-yl)carbamate (S-6):** According to the general procedure, oxazine products **12** and **12a** were isolated as a mixture (62 mg, 48%, 2:1) separate from carbamate product **S-6** (49 mg, 38%). **(12):** ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40 – 7.27 (m, 5H), 5.54 – 5.47 (m, 1H), 5.20 (s, 2H), 4.40 – 4.34 (m, 2H), 4.02 – 3.98 (m, 2H), 1.72 (s, 3H); **(12a):** ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40 – 7.27 (m, 5H), 5.54 – 5.47 (m, 1H), 5.20 (s, 2H), 4.28 – 4.24 (m, 2H), 4.12 – 4.06 (m, 2H), 1.64 (s, 3H); Mixture: ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.6, 155.5, 136.0, 136.0, 131.5, 130.1, 128.5, 128.5, 128.2, 128.1, 128.1, 118.0, 116.2, 71.6, 68.5, 67.7, 67.7, 48.5, 44.7, 19.7, 18.3.



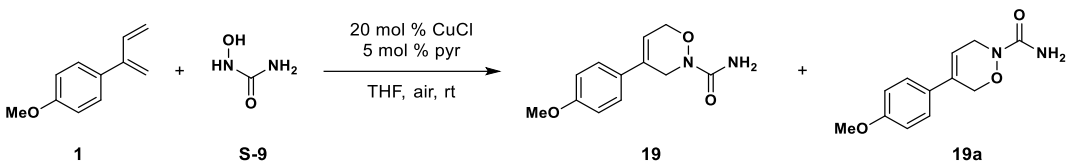
benzyl 4-(1-((*tert*-butyldimethylsilyl)oxy)ethyl)-3,6-dihydro-2H-1,2-oxazine-2-carboxylate (14): According to the general procedure, oxazine products **14** and **14a** were isolated as a mixture (95 mg, 77%, 1:13). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.27 (m, 5H), 5.67 – 5.62 (m, 1H), 5.20 (s, 2H), 4.46 – 4.39 (m, 2H), 4.25 (q, *J* = 6.6 Hz, 1H), 4.14 – 4.10 (m, 2H), 1.19 (d, *J* = 6.5 Hz, 3H), 0.86 (s, 9H), 0.03 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.5, 139.8, 136.0, 128.5, 128.2, 128.1, 115.0, 69.3, 68.4, 67.7, 44.6, 25.7, 23.2, 18.1, -4.8, -4.9; IR (thin film) 3066, 2955, 2856, 1712, 1343, 1214, 1088 cm^{-1}; MS (ESI) *m/z* 400.21 (400.19 calculated for $C_{20}H_{31}NO_4SiNa$ $[M+Na]^+$)



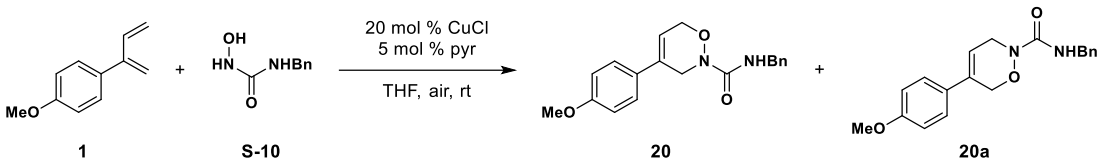
2,2,2-trichloroethyl 4-phenyl-3,6-dihydro-2H-1,2-oxazine-2-carboxylate (17): According to the general procedure, oxazine products **17** and **17a** were isolated as a mixture (94 mg, 81%, 8:1). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38 – 7.28 (m, 5H), 6.22 – 6.18 (m, 1H), 4.84 (s, 2H), 4.69 – 4.65 (m, 2H), 4.61 – 4.57 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.6, 136.7, 132.8, 128.8, 128.3, 124.9, 119.7, 95.1, 75.1, 68.9, 46.2; IR (thin film) 3059, 2955, 2851, 1745, 1720, 1496, 1436, 1347, 1229, 1114 cm^{-1}; MS (ESI) *m/z* 357.99 (100%), 359.99 (95%), 361.98 (33%) ((357.98, 359.98, 361.97 calculated for $C_{13}H_{12}Cl_3NO_3Na$ $[M+Na]^+$)



***tert*-butyl 4-phenyl-3,6-dihydro-2H-1,2-oxazine-2-carboxylate (18):** According to the general procedure, oxazine products **18** and **18a** were isolated as a mixture (70 mg, 77%, 3:1). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38 – 7.23 (m, 5H), 6.19 – 6.15 (m, 1H), 4.60 – 4.55 (m, 2H), 4.46 – 4.42 (m, 2H), 1.51 (s, 9H); Mixture ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.0, 155.0, 137.2, 136.6, 134.6, 133.2, 128.7, 128.6, 128.0, 124.8, 124.8, 120.1, 118.5, 81.8, 81.8, 69.3, 68.2, 46.4, 45.2, 28.3, 28.3, 27.6; IR (thin film) 3058, 2978, 2849, 1702, 1496, 1367, 1235, 1164, 1099 cm^{-1}; MS (ESI) *m/z* 284.13 (284.13 calculated for $C_{15}H_{19}NO_3Na$ $[M+Na]^+$)

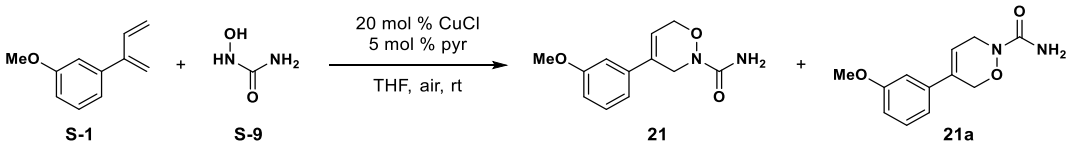


4-(4-methoxyphenyl)-3,6-dihydro-2H-1,2-oxazine-2-carboxamide (19): According to the general procedure, oxazine products **19** and **19a** were isolated as a mixture (25 mg, 86%, 20:1). ^{1}H NMR (600 MHz, CD_3OD) δ 7.39 – 7.33 (m, 2H), 6.93 – 6.87 (m, 2H), 6.19 – 6.14 (m, 1H), 4.60 – 4.55 (m, 2H), 4.37 – 4.33 (m, 2H), 3.78 (s, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 161.8, 161.1, 133.8, 131.0, 127.0, 119.3, 115.1, 70.1, 55.7, 46.1; IR (thin film) 3453, 3221, 2905, 1677, 1607, 1518, 1446, 1281, 1241, 1031 cm^{-1}; HRMS (ESI) *m/z* 257.0966 (257.0902 calculated for $C_{12}H_{14}N_2O_3Na$ $[M+Na]^+$)

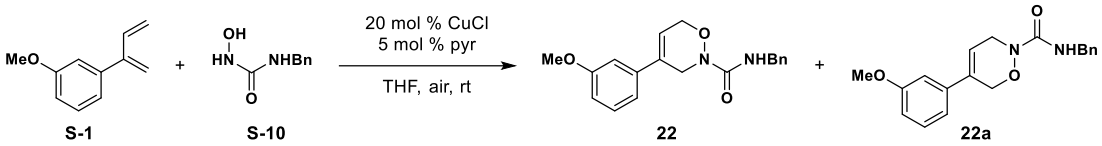


N-benzyl-4-(4-methoxyphenyl)-3,6-dihydro-2H-1,2-oxazine-2-carboxamide (20):

According to the general procedure, oxazine products **20** and **20a** were isolated as a mixture (20 mg, 59%, 9:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 – 7.19 (m, 7H), 6.93 – 6.85 (m, 2H), 6.18 – 6.13 (br, 1H), 6.08 – 6.05 (m, 1H), 4.60 – 4.53 (m, 2H), 4.53 – 4.48 (m, 2H), 4.47 – 4.44 (m, 2H), 3.82 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.5, 158.6, 138.8, 133.2, 129.7, 128.6, 127.7, 127.4, 126.1, 117.8, 114.0, 69.4, 55.3, 46.2, 43.9; IR (thin film) 3424, 3346, 3032, 2934, 1673, 1514, 1454, 1247, 1182, 1032 cm^{-1}.

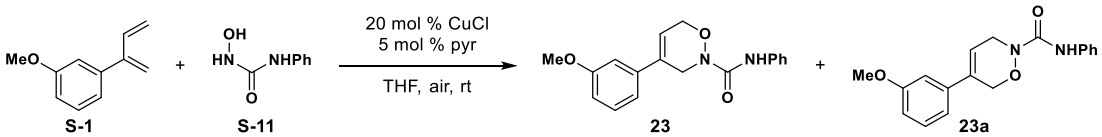


4-(3-methoxyphenyl)-3,6-dihydro-2H-1,2-oxazine-2-carboxamide (21): According to the general procedure, oxazine products **21** and **21a** were isolated as a mixture (24 mg, 82%, 4:1). ^{1}H NMR (600 MHz, CD_3OD) δ 7.25 (t, *J* = 8.0 Hz, 1H), 7.01 – 6.82 (m, 3H), 6.29 – 6.26 (m, 1H), 4.61 – 4.56 (m, 2H), 4.38 – 4.34 (m, 2H), 3.79 (s, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 160.3, 160.0, 138.6, 132.9, 129.3, 120.1, 116.8, 113.1, 110.2, 68.6, 54.2, 44.7; IR (thin film) 3469, 3331, 2924, 1676, 1580, 1430, 1288, 1210, 1050 cm^{-1}; HRMS (ESI) *m/z* 257.0966 (257.0902 calculated for $C_{12}H_{14}N_2O_3Na$ $[M+Na]^+$)



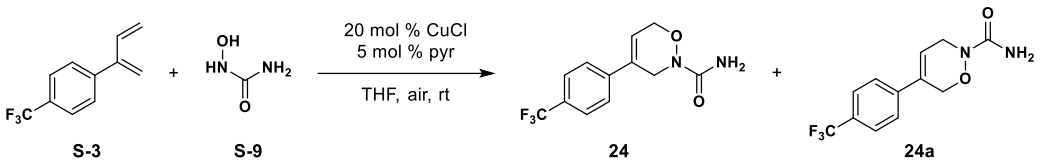
N-benzyl-4-(3-methoxyphenyl)-3,6-dihydro-2H-1,2-oxazine-2-carboxamide (22):

According to the general procedure, oxazine products **22** and **22a** were isolated as a mixture (31 mg, 77%, 7:1). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38 – 7.23 (m, 6H), 7.03 – 6.82 (m, 3H), 6.19 – 6.15 (m, 1H), 4.59 – 4.56 (m, 2H), 4.49 (d, *J* = 5.8 Hz, 2H), 4.47 – 4.44 (m, 2H), 3.81 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.8, 158.6, 138.7, 138.7, 133.8, 129.6, 128.7, 127.7, 127.4, 119.9, 117.5, 113.5, 110.8, 69.3, 55.3, 46.3, 44.0; IR (thin film) 3341, 3033, 2933, 2837, 1670, 1523, 1431, 1288, 1209, 1049 cm^{-1}; HRMS (ESI) *m/z* 347.1475 (347.1372 calculated for $C_{19}H_{20}N_2O_3Na$ $[M+Na]^+$)



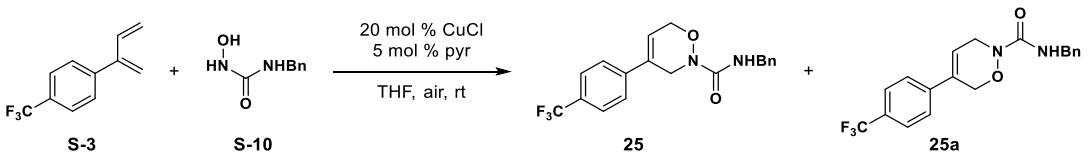
4-(3-methoxyphenyl)-N-phenyl-3,6-dihydro-2H-1,2-oxazine-2-carboxamide (23):

According to the general procedure, oxazine products **23** and **23a** were isolated as a mixture (25 mg, 64%, 4:1). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.55 – 7.26 (m, 5H), 7.11 – 6.81 (m, 4H), 6.22 – 6.19 (m, 1H), 4.72 – 4.67 (m, 2H), 4.53 – 4.49 (m, 2H), 3.82 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 160.0, 155.7, 138.7, 138.0, 133.9, 129.9, 129.8, 129.2, 123.7, 119.8, 119.5, 117.7, 113.8, 111.0, 77.2, 69.9, 55.5, 45.9; IR (thin film) 3321, 3063, 2920, 2837, 1675, 1531, 1446, 1288, 1208, 1050 cm^{-1}; HRMS (ESI) *m/z* 333.1364 (333.1215 calculated for $C_{18}H_{18}N_2O_3Na$ $[M+Na]^+$)

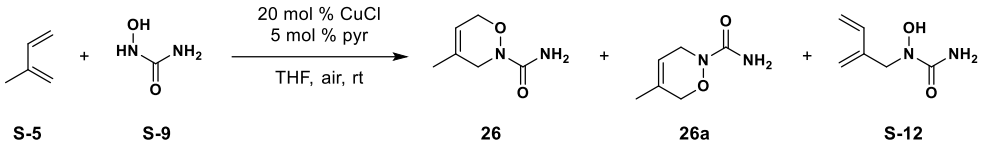


4-(4-(trifluoromethyl)phenyl)-3,6-dihydro-2H-1,2-oxazine-2-carboxamide (24):

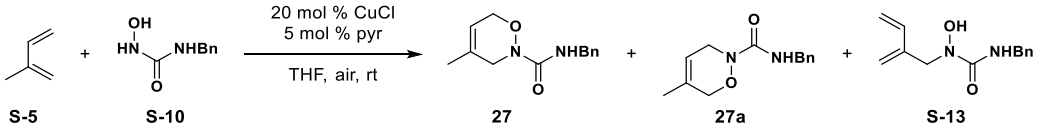
According to the general procedure, oxazine products **24** and **24a** were isolated as a mixture (109 mg, 72%, 15:1). ^{1}H NMR (600 MHz, CD_3OD) δ 7.67 (d, *J* = 8.5 Hz, 2H), 7.64 (d, *J* = 8.5 Hz, 2H), 6.50 – 6.46 (m, 1H), 4.67 – 4.62 (m, 2H), 4.46 – 4.41 (m, 2H); ^{13}C NMR (151 MHz, CD_3OD) δ 160.3, 140.9, 131.9, 129.45 (q, *J* = 32.3 Hz), 125.20 (q, *J* = 3.8 Hz), 125.0, 122.6, 121.0, 68.6, 44.5; IR (thin film) 3420, 3192, 2959, 2846, 1638, 1592, 1326, 1171, 1128, 1070 cm^{-1}; MS (ESI) *m/z* 295.06 (100%), 296.07 (25%), 297.07 (6%) (295.07, 296.07, 297.08 calculated for $C_{12}H_{11}F_3N_2O_2Na$ $[M+Na]^+$)



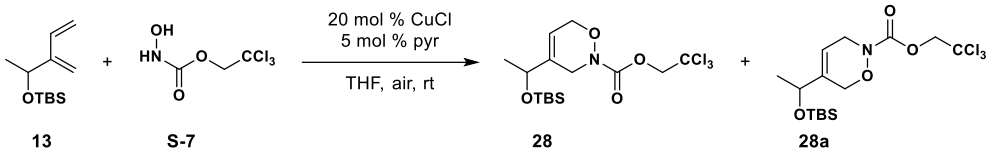
N-benzyl-4-(4-(trifluoromethyl)phenyl)-3,6-dihydro-2H-1,2-oxazine-2-carboxamide (25): According to the general procedure, oxazine products **25** and **25a** were isolated as a mixture (118 mg, 58%, 4:1). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.61 (d, *J* = 8.2 Hz, 2H), 7.49 (d, *J* = 8.1 Hz, 2H), 7.40 – 7.25 (m, 5H), 6.28 – 6.24 (m, 1H), 6.18 – 6.13 (br, 1H), 4.62 – 4.56 (m, 2H), 4.51 – 4.48 (m, 2H), 4.47 – 4.44 (m, 2H); ^{13}C NMR (151 MHz, CD_3OD) δ 159.4, 140.9, 139.4, 131.9, 129.34 (q, *J* = 32.2 Hz), 128.0, 126.9, 126.7, 125.19 (q, *J* = 3.7 Hz), 125.0, 122.6, 68.7, 45.3, 43.0; IR (thin film) 3435, 3342, 3032, 2891, 2844, 1674, 1525, 1326, 1166, 1116, 1071 cm^{-1}; MS (ESI) *m/z* 386.11 (100%), 387.12 (24%), 388.12 (7%) (385.11, 386.12, 387.12 calculated for $C_{19}H_{17}F_3N_2O_2Na$ $[M+Na]^+$)



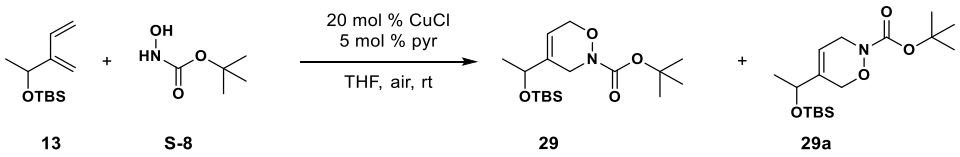
4-methyl-3,6-dihydro-2H-1,2-oxazine-2-carboxamide (26) and **1-hydroxy-1-(2-methylenebut-3-en-1-yl)urea (S-12):** According to the general procedure, oxazine products **26** and **26a** were isolated as a mixture (34 mg, 20%, 4:1) separate from urea product **S-12** (20 mg, 10%). ^{1}H NMR (600 MHz, CD_3OD) δ 5.60 – 5.53 (m, 1H), 4.39 – 4.33 (m, 2H), 3.89 – 3.85 (m, 2H), 1.74 (s, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 160.2, 130.2, 117.7, 68.2, 46.8, 18.4; IR (thin film) 3335, 2853, 1668, 1584, 1440, 1102 cm^{-1}; MS (ESI) *m/z* 165.06 (165.06 calculated for $C_6H_{10}N_2O_2Na$ $[M+Na]^+$)



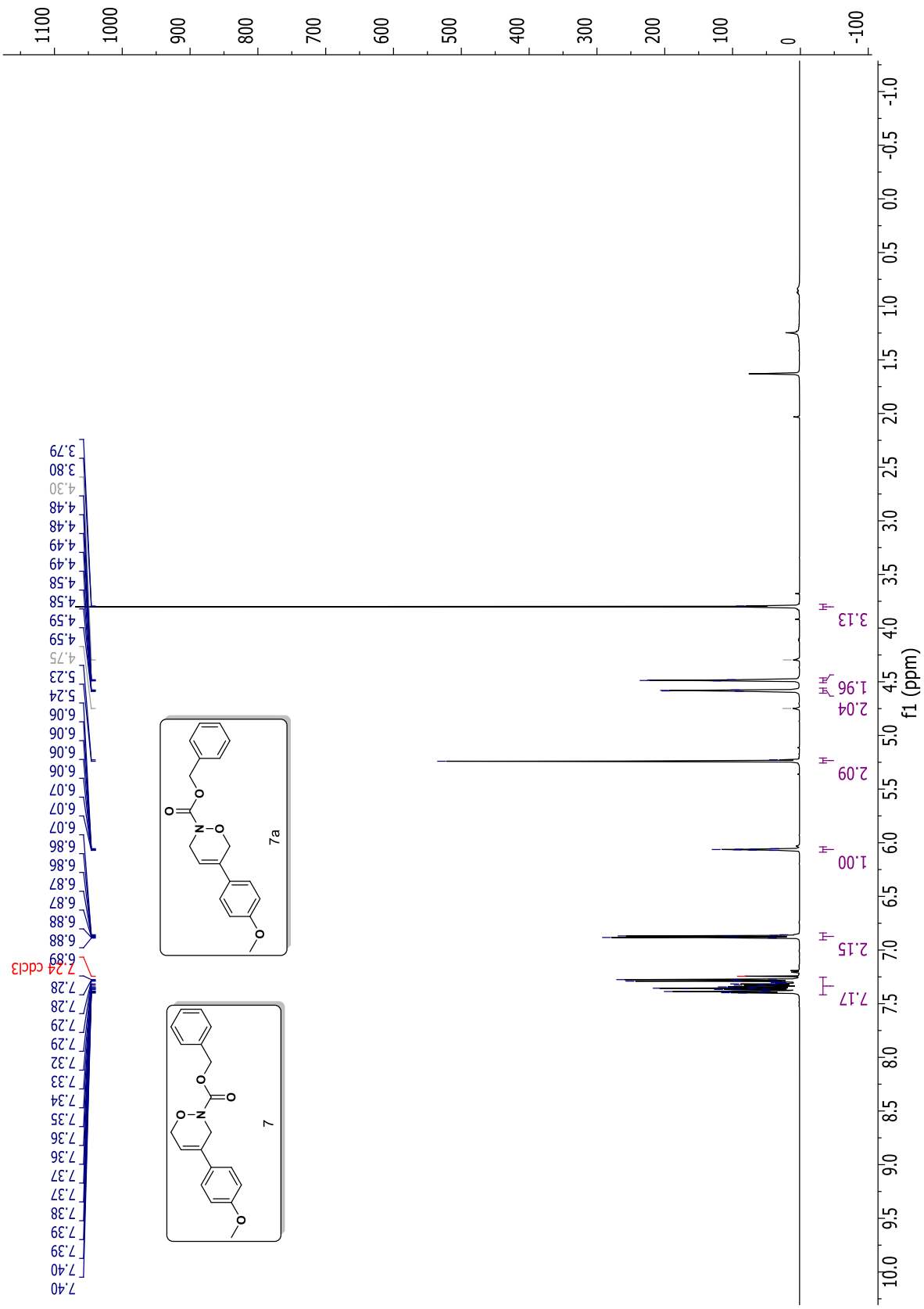
N-benzyl-4-methyl-3,6-dihydro-2H-1,2-oxazine-2-carboxamide (27) and **3-benzyl-1-hydroxy-1-(2-methylenebut-3-en-1-yl)urea (S-13):** According to the general procedure, oxazine products **27** and **27a** were isolated as a mixture (46 mg, 33%, 5:1) separate from urea product **S-13** (16 mg, 12%). **(27):** ^{1}H NMR (600 MHz, CD_3OD) δ 7.61 – 7.56 (br, 1H), 7.31 – 7.24 (m, 5H), 5.58 – 5.52 (m, 1H), 4.35 (s, 2H), 4.35 – 4.33 (m, 2H), 3.89 – 3.85 (m, 2H), 1.73 (s, 3H); **(27a):** ^{1}H NMR (600 MHz, CD_3OD) δ 7.61 – 7.56 (m, 1H), 7.24 – 7.16 (m, 5H), 5.58 – 5.52 (m, 1H), 4.36 (s, 2H), 4.30 – 4.23 (m, 2H), 3.95 – 3.90 (m, 2H), 1.65 (s, 3H); Mixture: ^{13}C NMR (151 MHz, CD_3OD) δ 159.4, 159.3, 139.6, 139.5, 131.6, 130.3, 128.0, 126.8, 126.8, 126.6, 126.6, 117.8, 116.3, 71.5, 68.3, 47.6, 43.9, 43.0, 42.9, 18.5, 16.8; IR (thin film) 3444, 3030, 2914, 1658, 1524, 1496, 1452, 1206, 1105, 1059, 1027 cm^{-1}; MS (ESI) *m/z* 255.11 (255.11 calculated for $C_{13}H_{16}N_2O_2Na$ $[M+Na]^+$)



2,2,2-trichloroethyl 4-(1-((*tert*-butyldimethylsilyl)oxy)ethyl)-3,6-dihydro-2H-1,2-oxazine-2-carboxylate (28): According to the general procedure, oxazine products **28** and **28a** were isolated as a mixture (69 mg, 88%, 1:13). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.71 – 5.66 (m, 1H), 4.81 (d, *J* = 3.3 Hz, 2H), 4.52 – 4.50 (m, 2H), 4.28 (q, *J* = 6.4 Hz, 1H), 4.23 – 4.18 (m, 2H), 1.22 (d, *J* = 6.4 Hz, 3H), 0.87 (s, 9H), 0.05 (s, 3H), 0.03 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.5, 139.8, 120.5, 114.6, 75.0, 69.3, 68.6, 25.7, 23.2, 18.1, -4.8, -4.9; IR (thin film) 2956, 2857, 1724, 1440, 1213, 1112 cm^{-1}; MS (ESI) *m/z* 440.07 (99%), 442.07 (100%), 444.07 (36%) (440.06, 442.06, 444.05 calculated for $C_{15}H_{26}Cl_3NO_4SiNa$ $[M+Na]^+$)



***tert*-butyl 4-(1-((*tert*-butyldimethylsilyl)oxy)ethyl)-3,6-dihydro-2H-1,2-oxazine-2-carboxylate (29):** According to the general procedure, oxazine products **29** and **29a** were isolated as a mixture (52 mg, 81%, 1:17). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.66 – 5.61 (m, 1H), 4.43 – 4.35 (m, 2H), 4.25 (q, *J* = 6.4 Hz, 1H), 4.05 – 4.00 (m, 2H), 1.47 (s, 9H), 1.19 (d, *J* = 6.5 Hz, 3H), 0.85 (s, 9H), 0.02 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.1, 139.8, 115.3, 81.5, 69.4, 67.7, 44.6, 28.3, 25.8, 25.7, 23.2, 18.1, -4.9, -5.0; IR (thin film) 2930, 2857, 1707, 1473, 1368, 1251, 1169, 1087 cm^{-1}; MS (ESI) *m/z* 366.21 (366.21 calculated for $C_{17}H_{33}NO_4SiNa$ $[M+Na]^+$)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7.37
7.37
7.36
7.36
7.35
7.34
7.33
7.32
7.29
7.29
7.28
7.28
7.24 cdcl3
6.89
6.88
6.88
6.87
6.87
6.86
6.86
6.07
6.07
6.07
6.06
6.06
6.06
5.24
5.23
4.75
4.59
4.59
4.58
4.58
4.49
4.49
4.48
4.48
4.30
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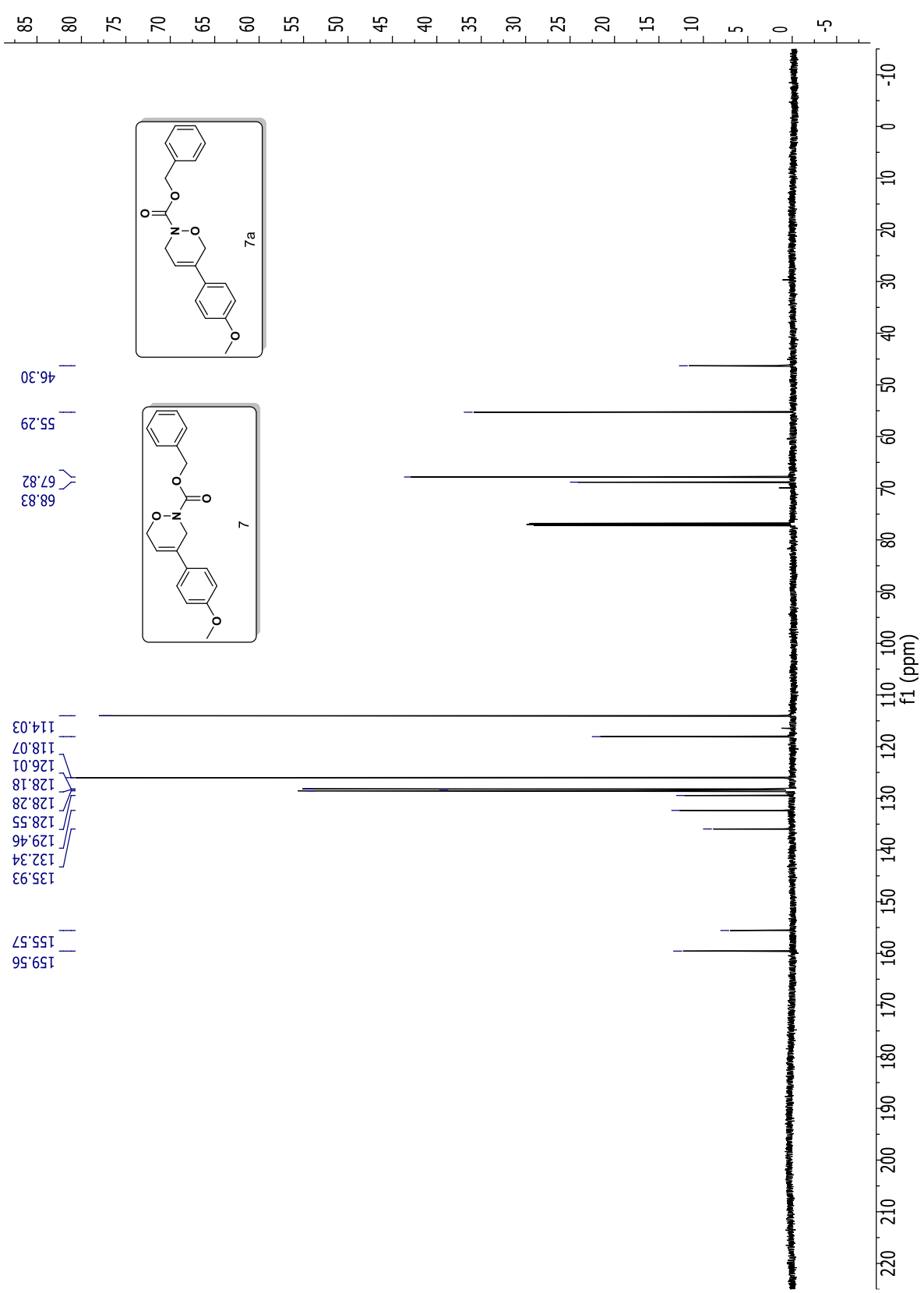
2.09
2.04
1.96
3.13
f1 (ppm)


7a
7
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126.01
118.07
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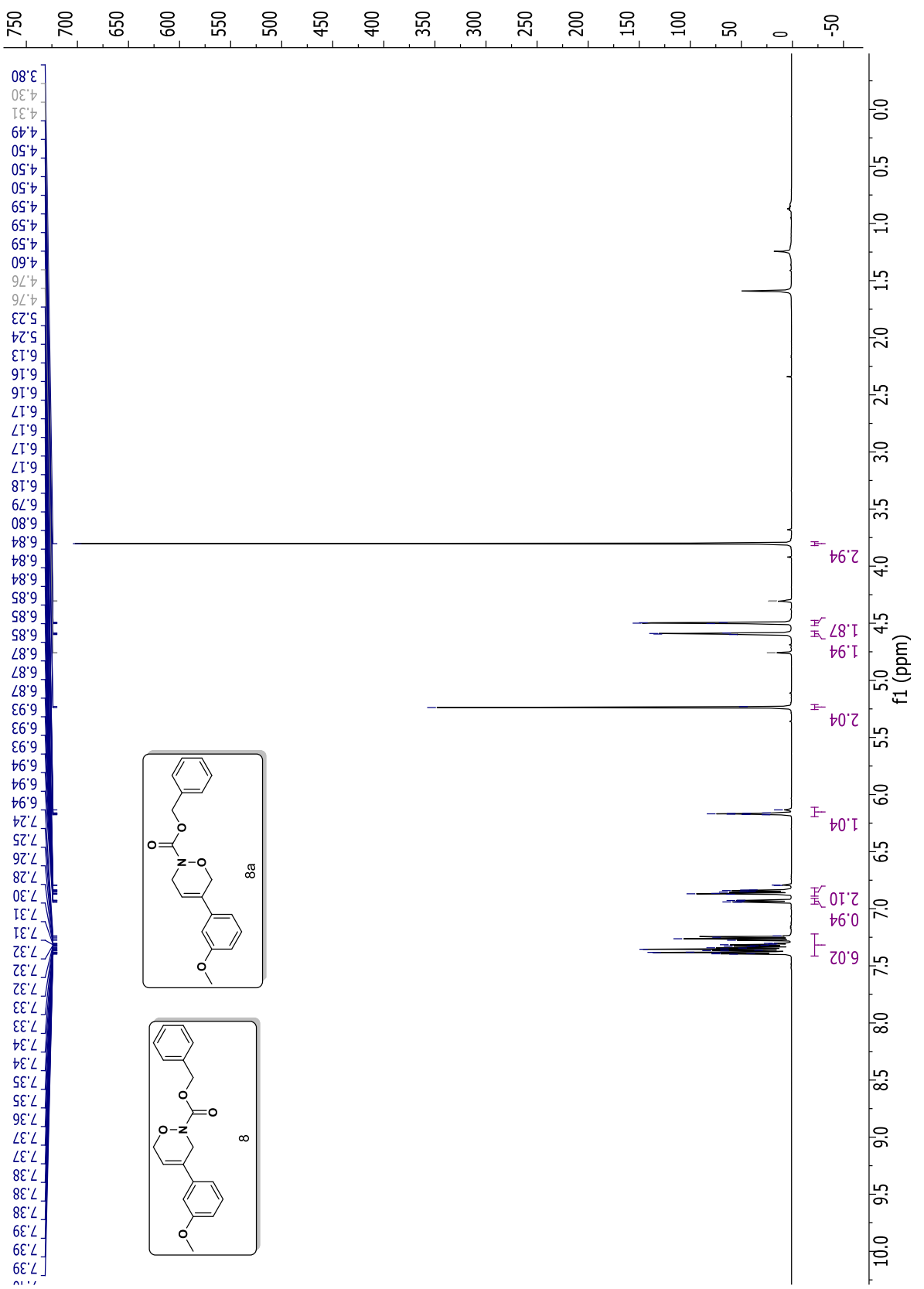
68.83
67.82
55.29
46.30
f1 (ppm)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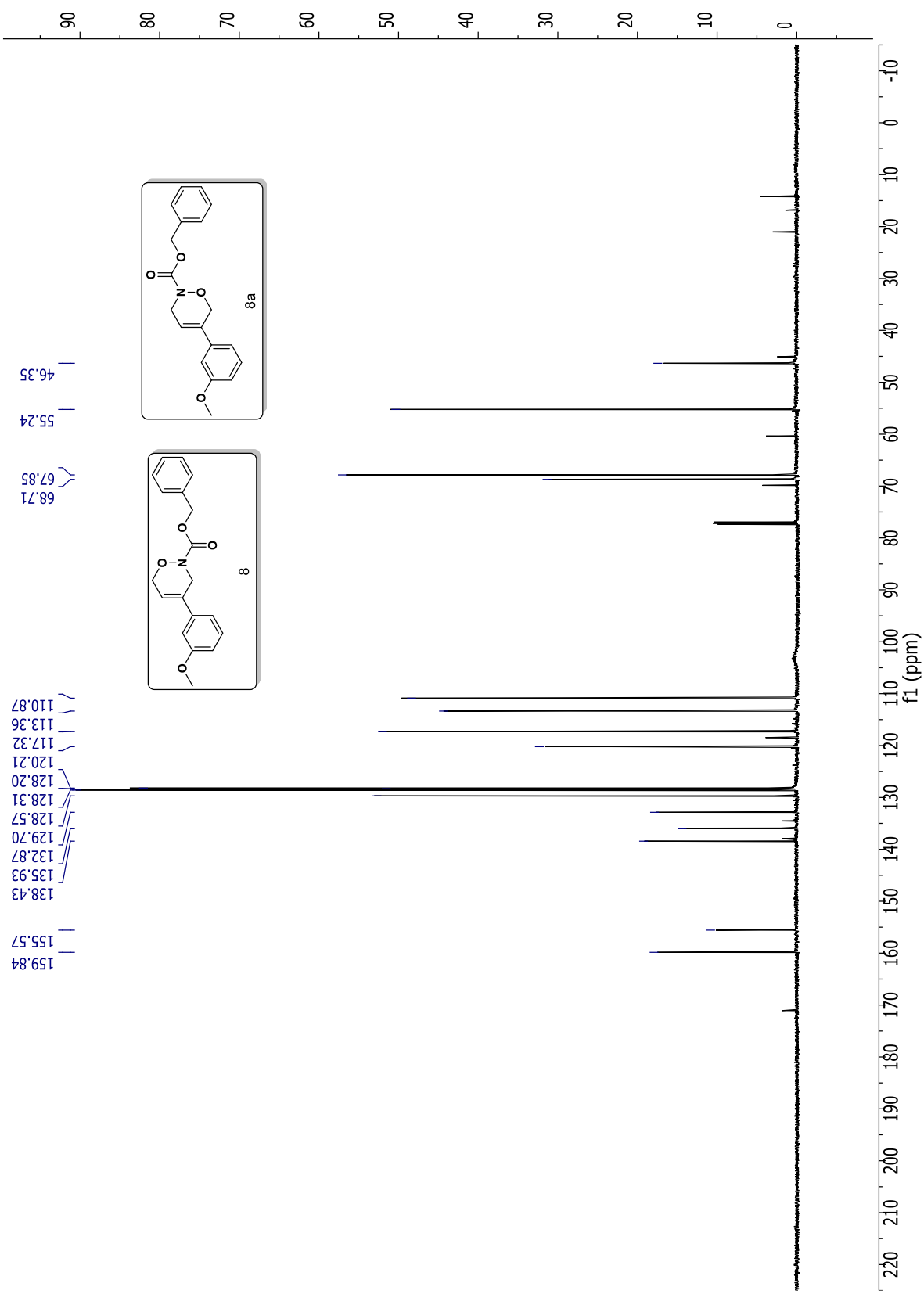
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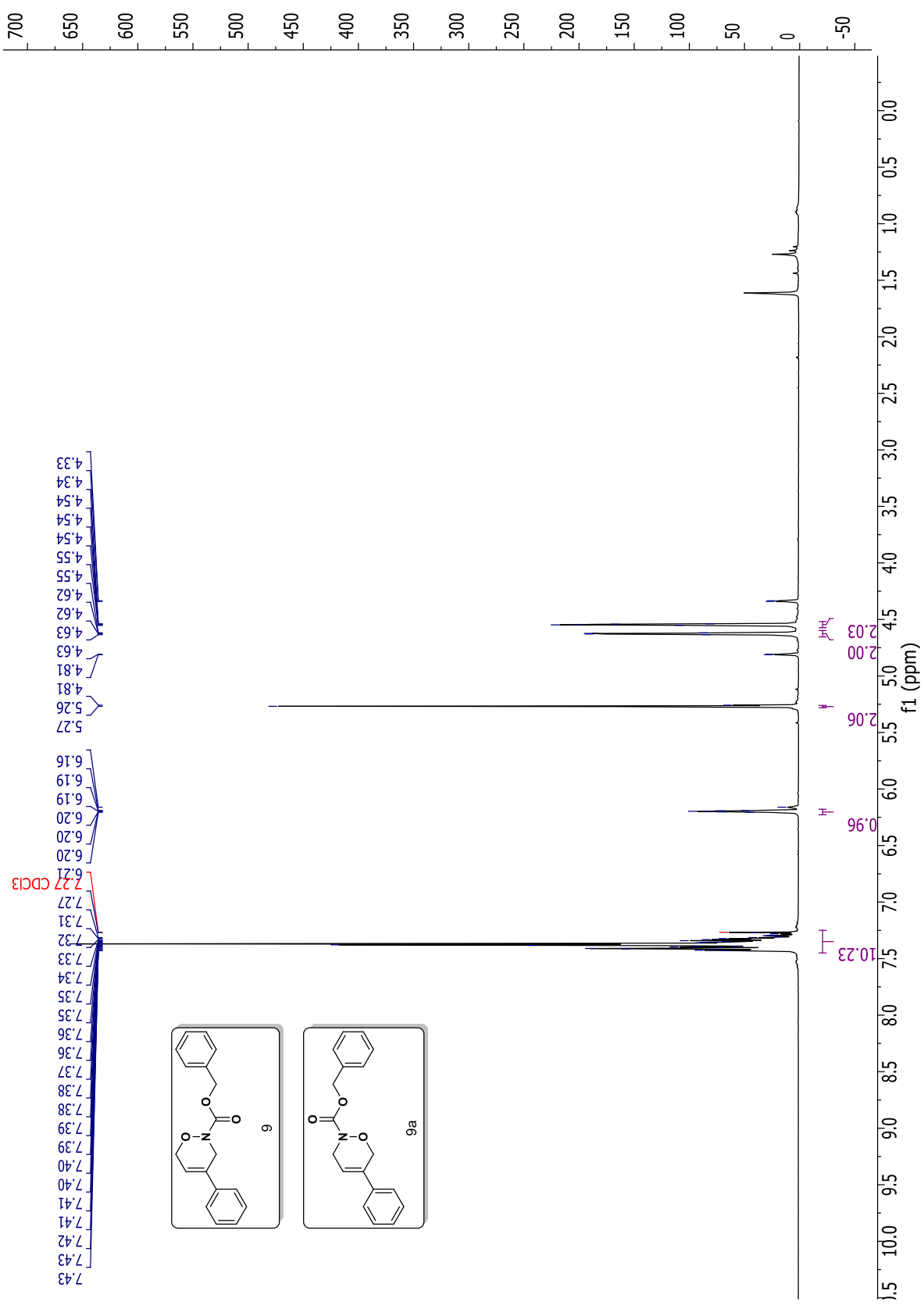
68.71
67.85
55.24
46.35
f1 (ppm)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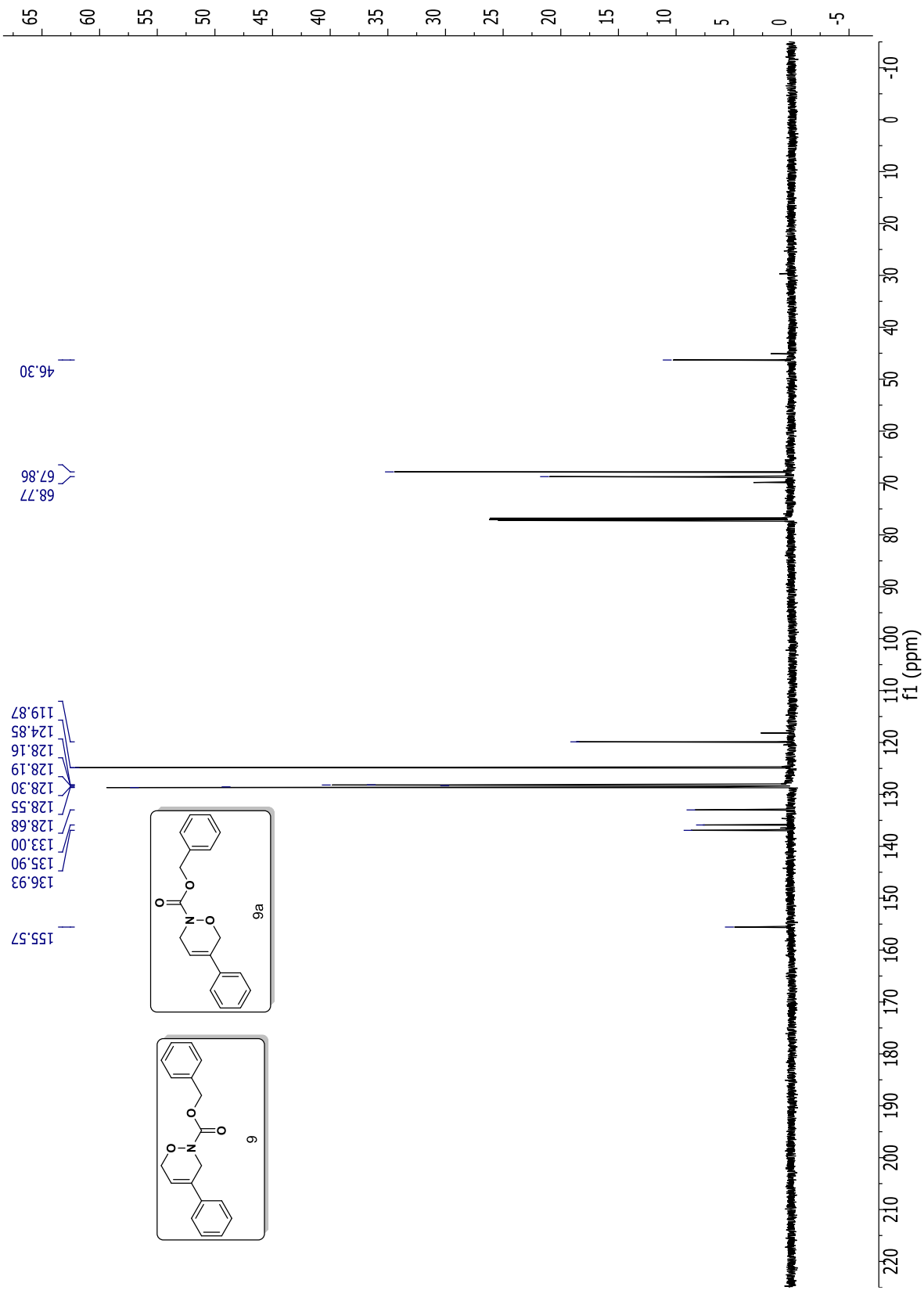
2.00
2.03
f1 (ppm)
7.27 CDCl3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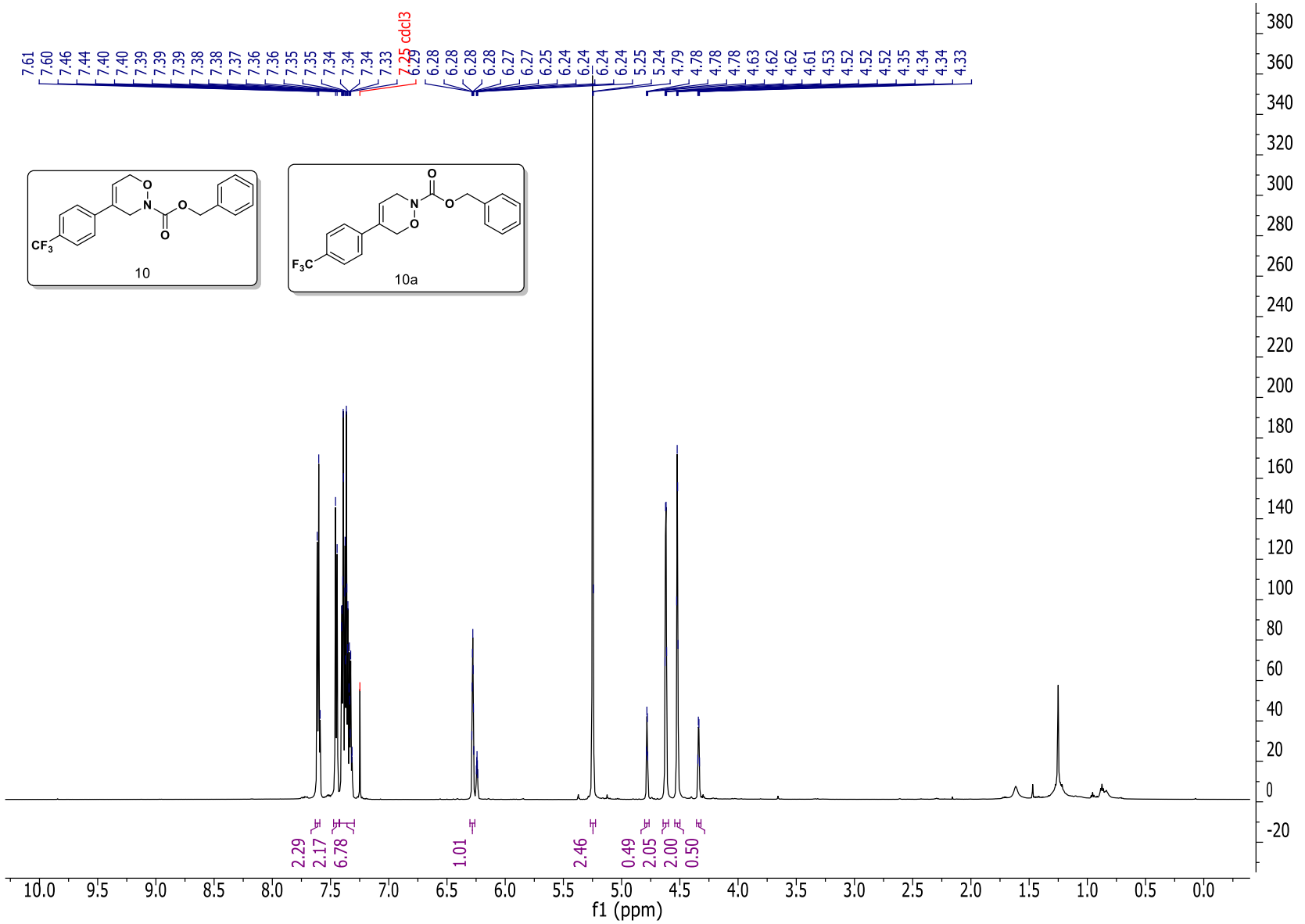
67.86
46.30
9a
9
f1 (ppm)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CF3
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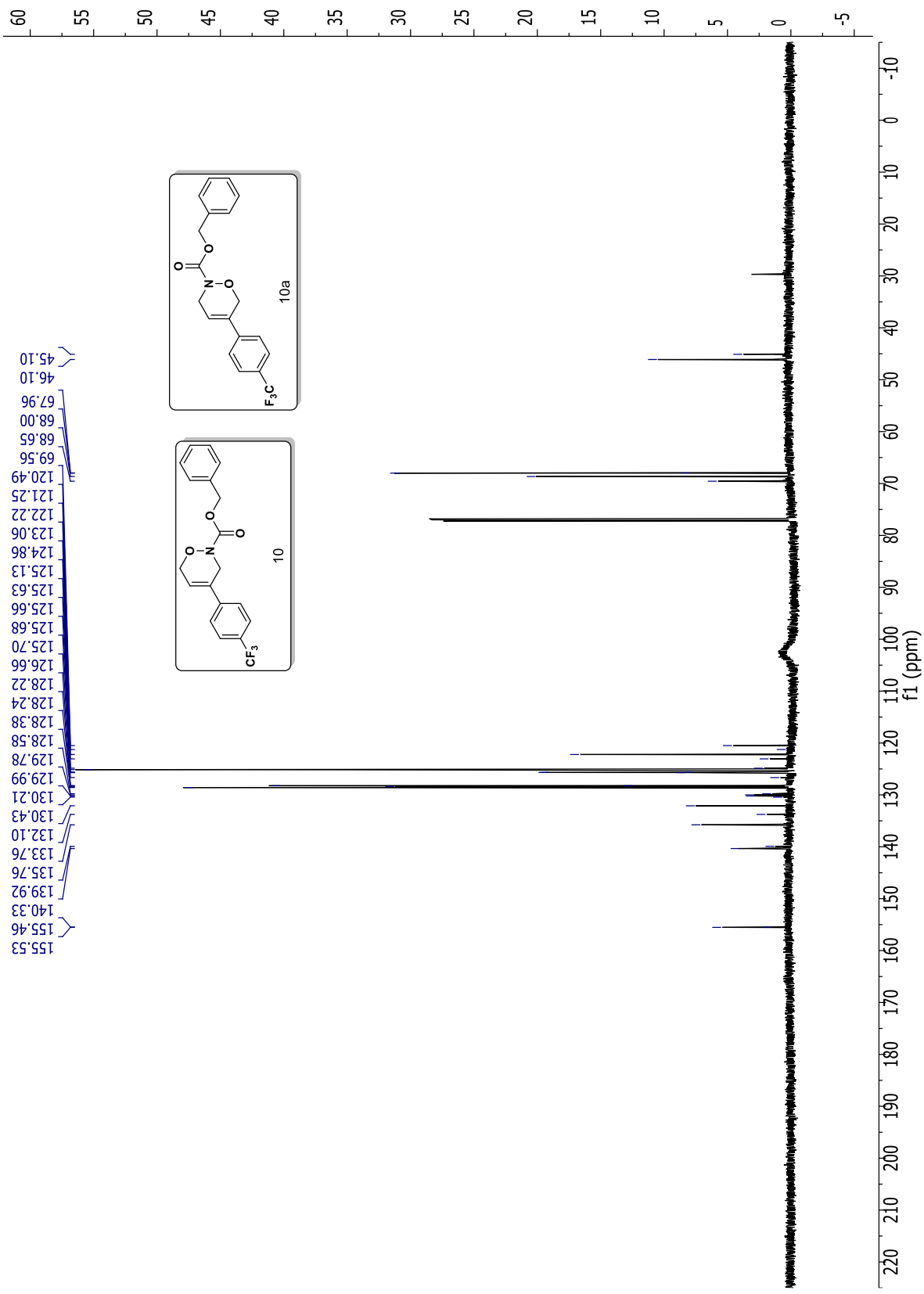
F3C
10a
2.29 2.17 6.78 1.01 2.46 0.49 2.05 2.00 0.50
f1 (ppm)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128.24
128.22
126.66
125.70
125.68
125.66
125.63
125.13
124.86
123.06
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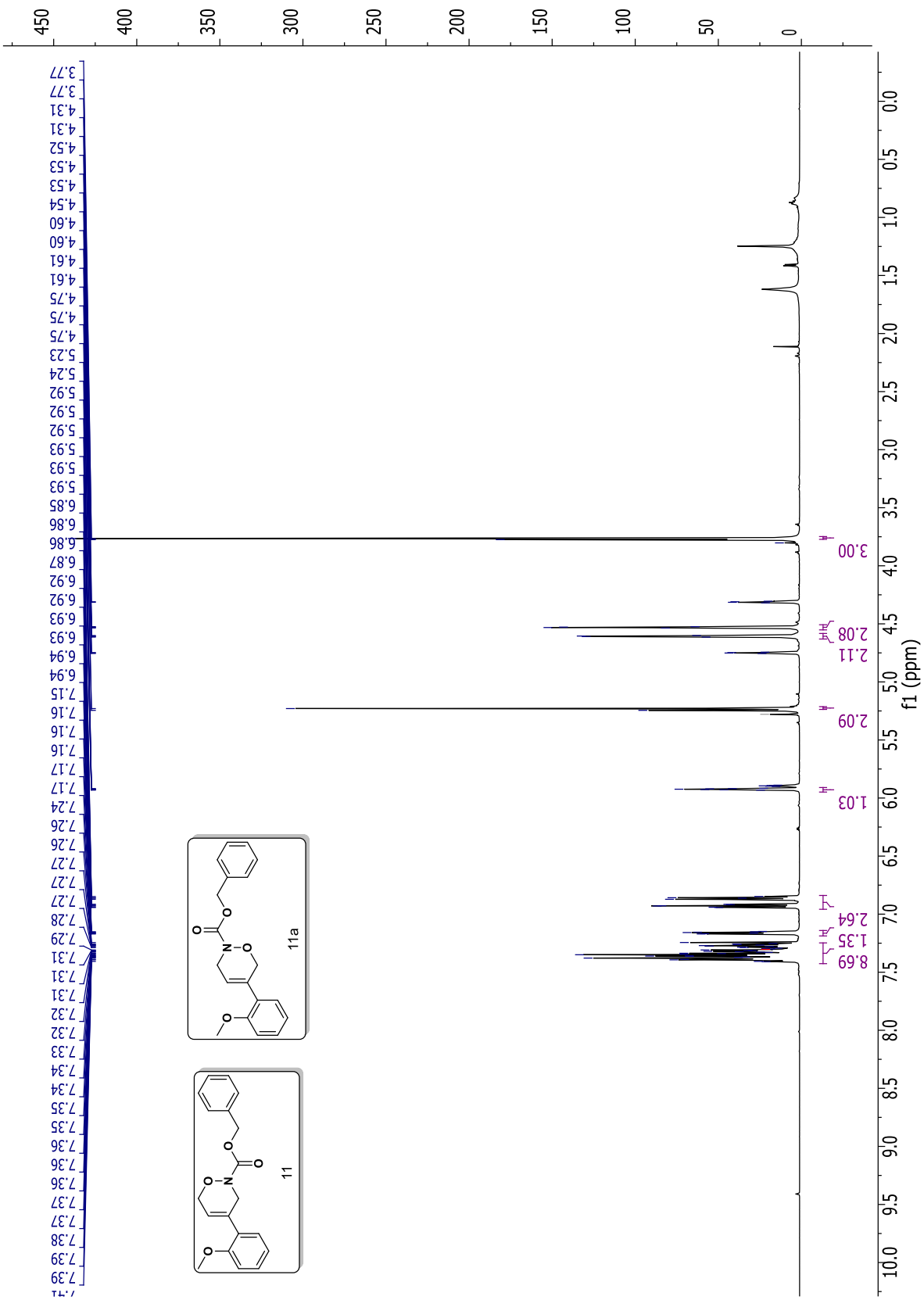
67.96
46.10
45.10
10
10a
f1 (ppm)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2.11
2.09
1.03
2.64
1.35
8.69
f1 (ppm)

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155.64
136.14
133.77
129.54
129.38
128.59
128.28
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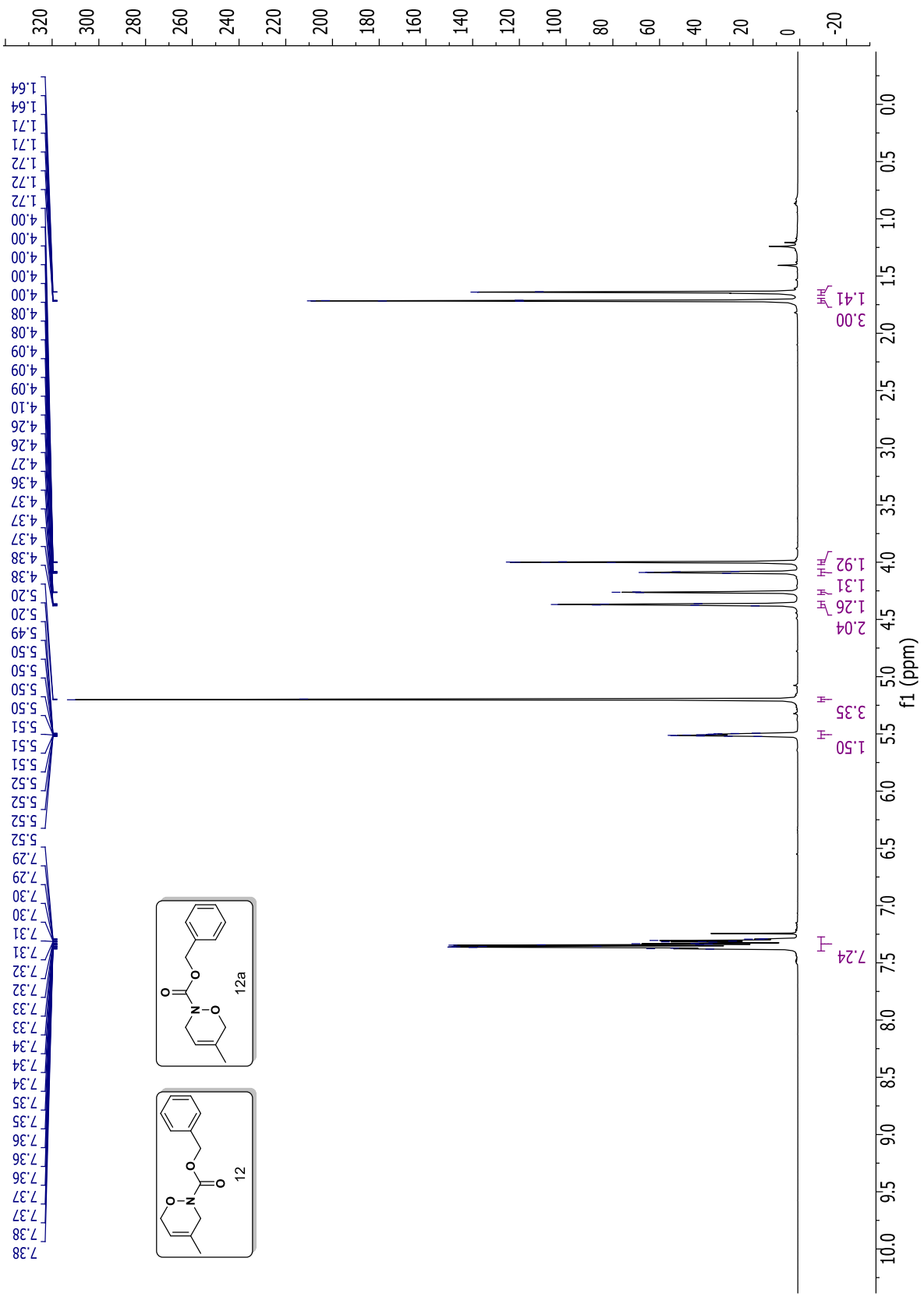
55.37
47.46
11a
11
f1 (ppm)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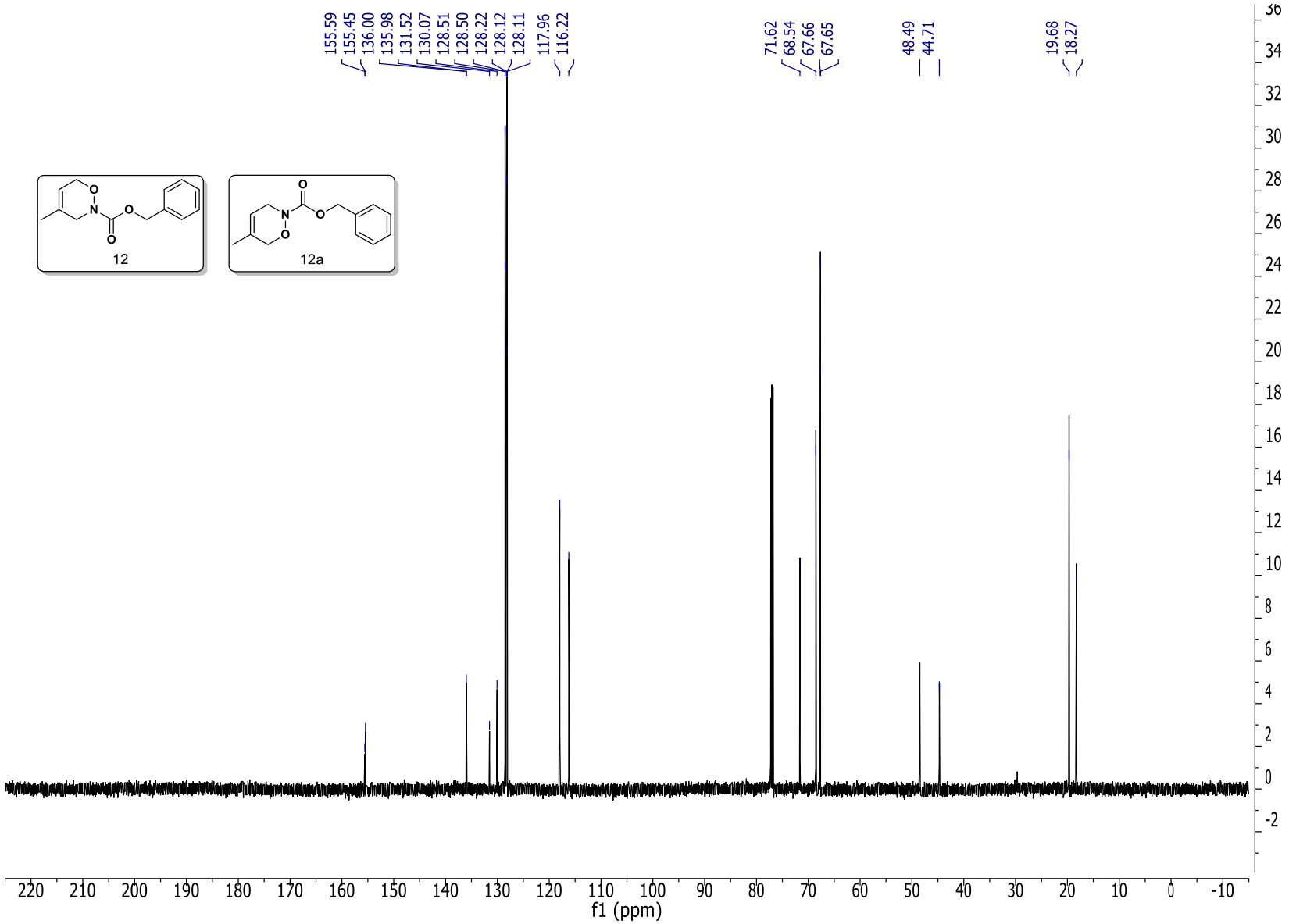
12a
f1 (ppm)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128.11
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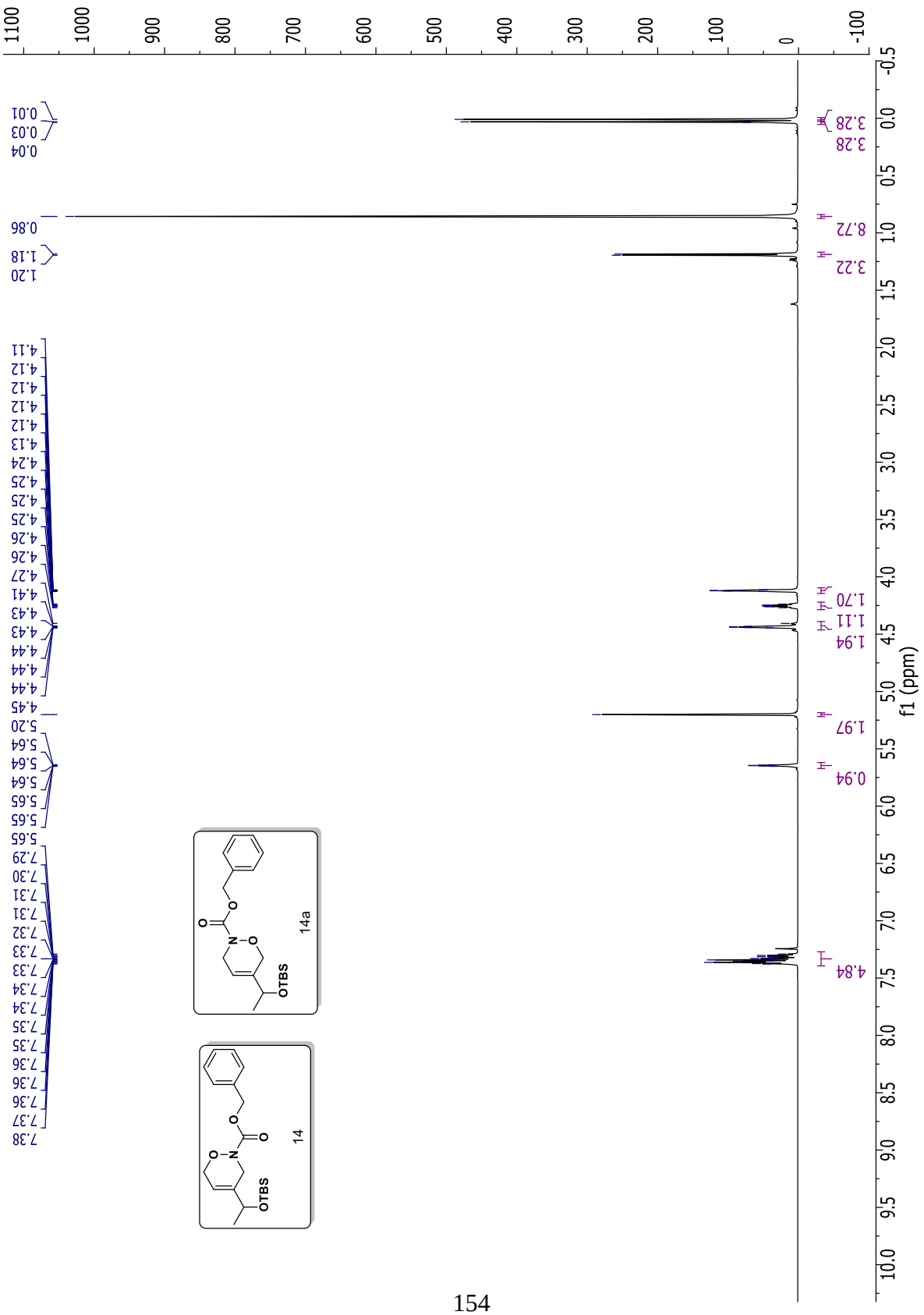
19.68
18.27
12
12a
f1 (ppm)


f1 (ppm)
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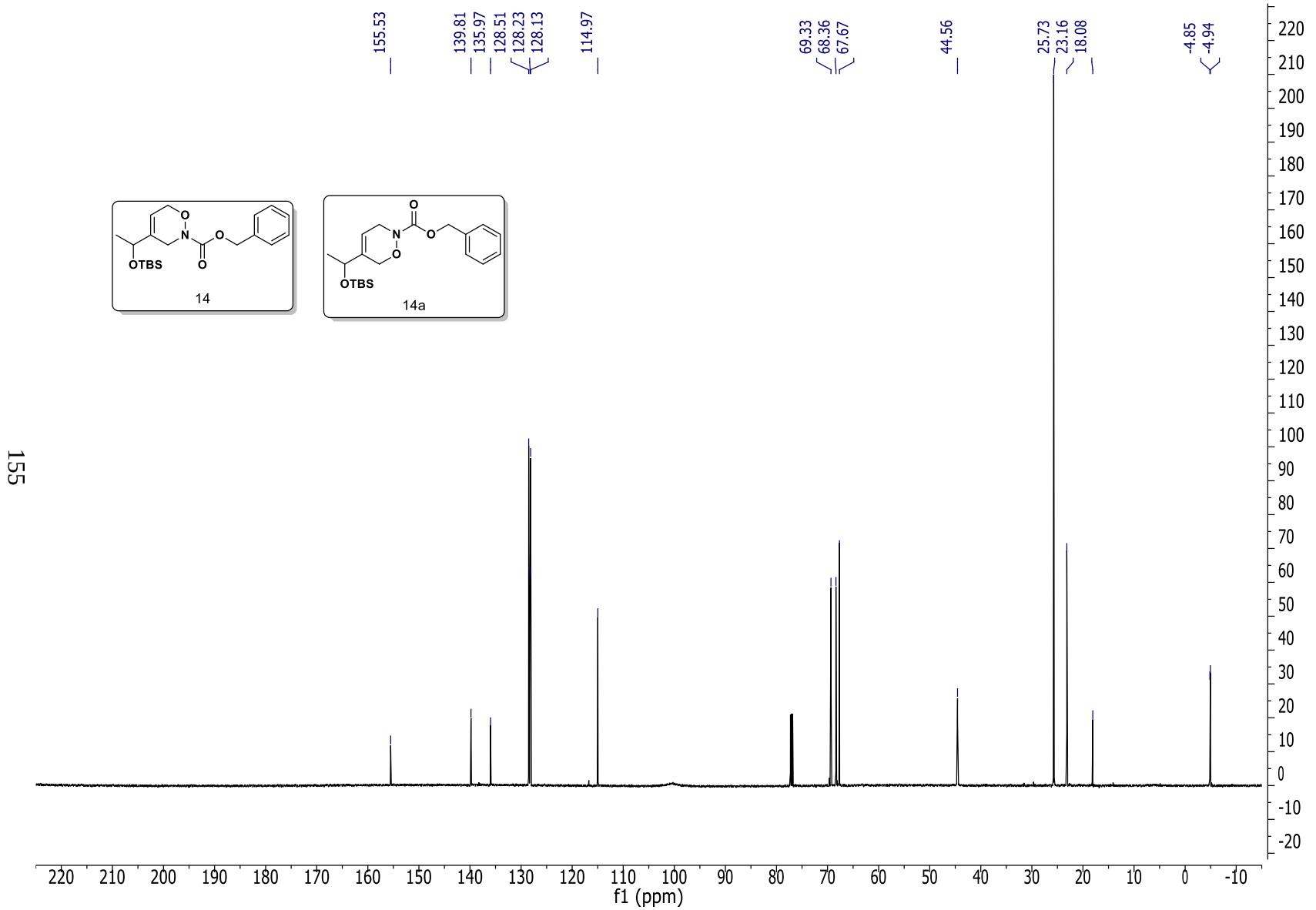
14a
OTBS
OTBS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23.16
18.08
-4.85
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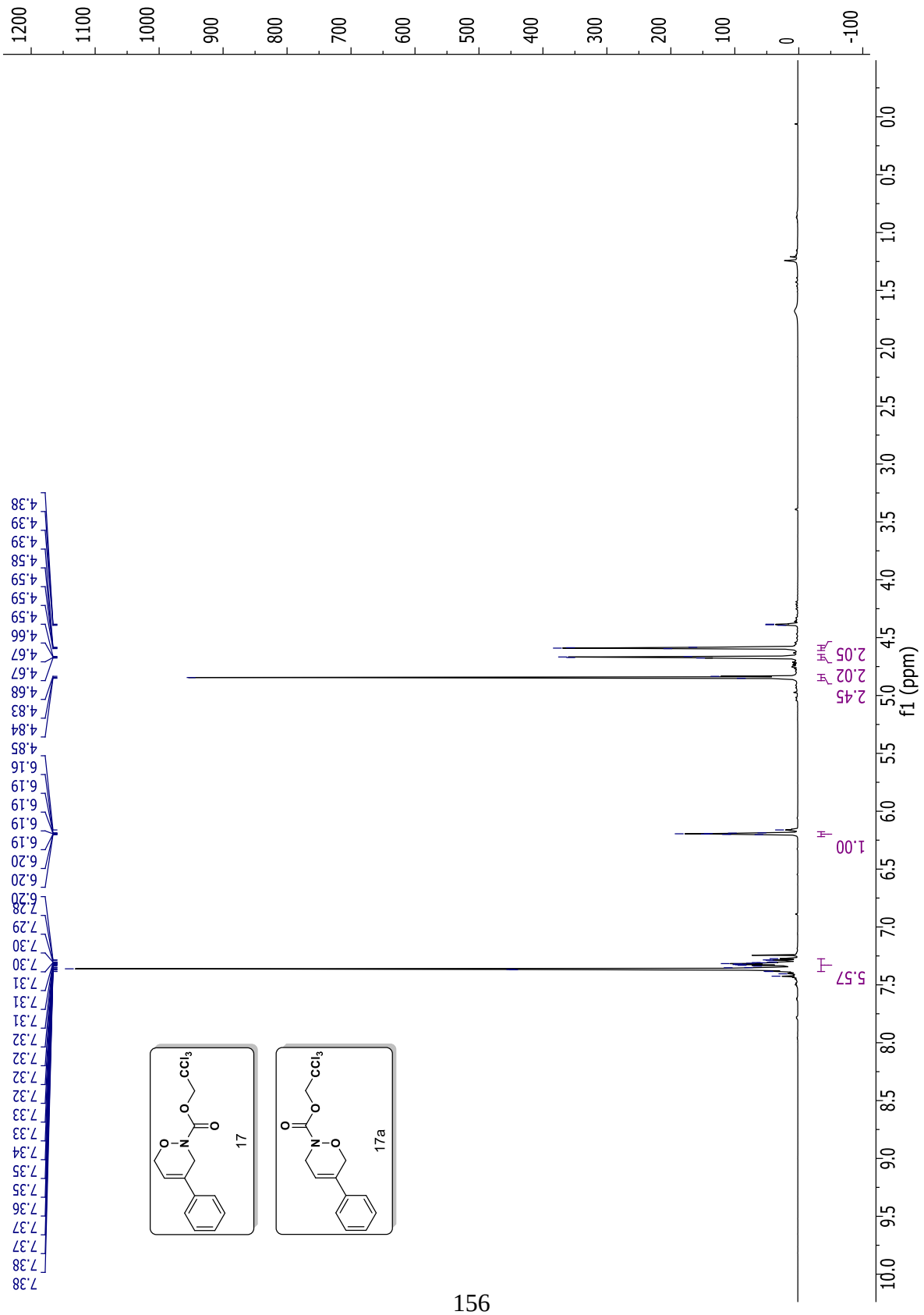
N
O
O
14
OTBS
O
N
O
O
14a
f1 (ppm)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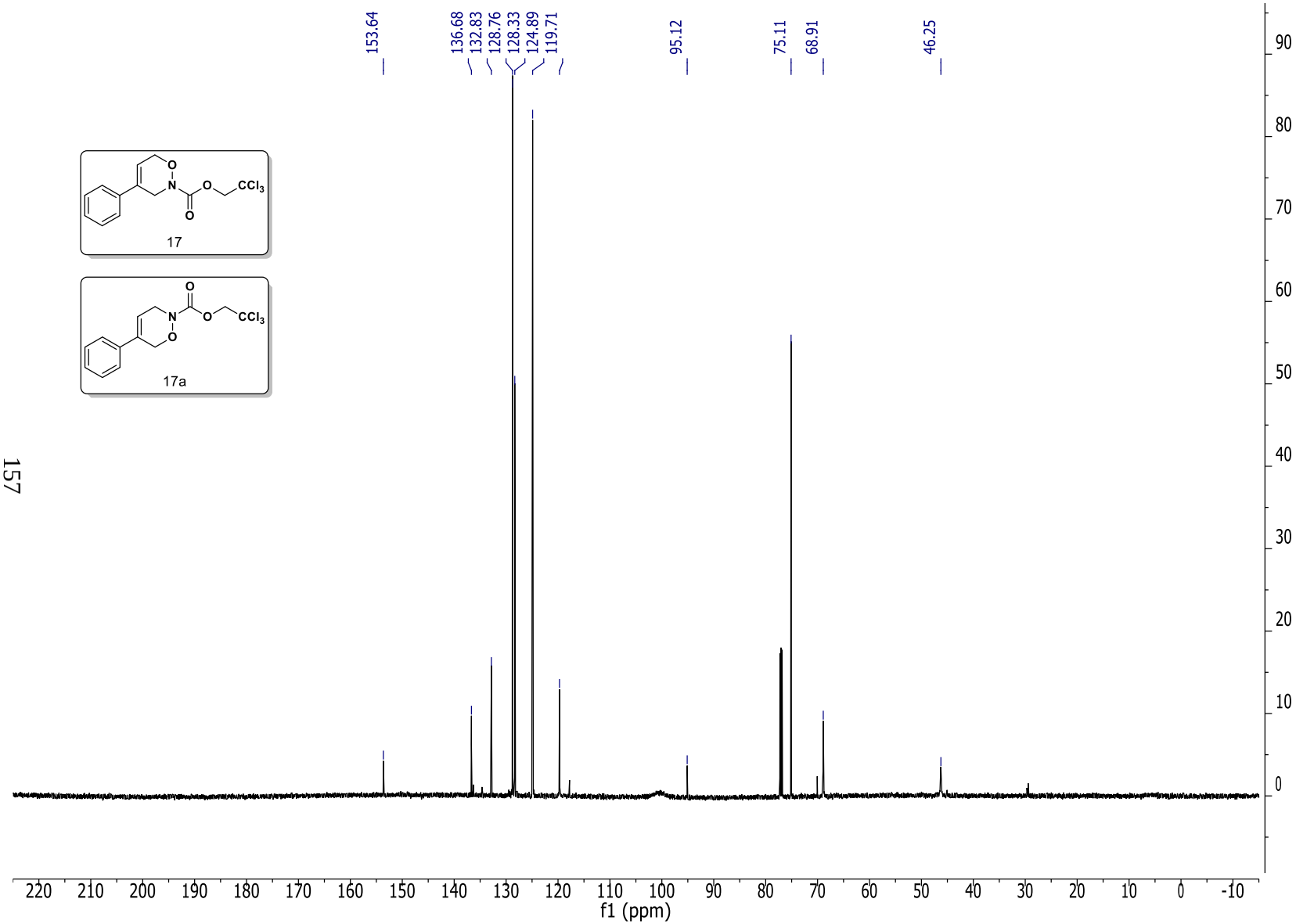
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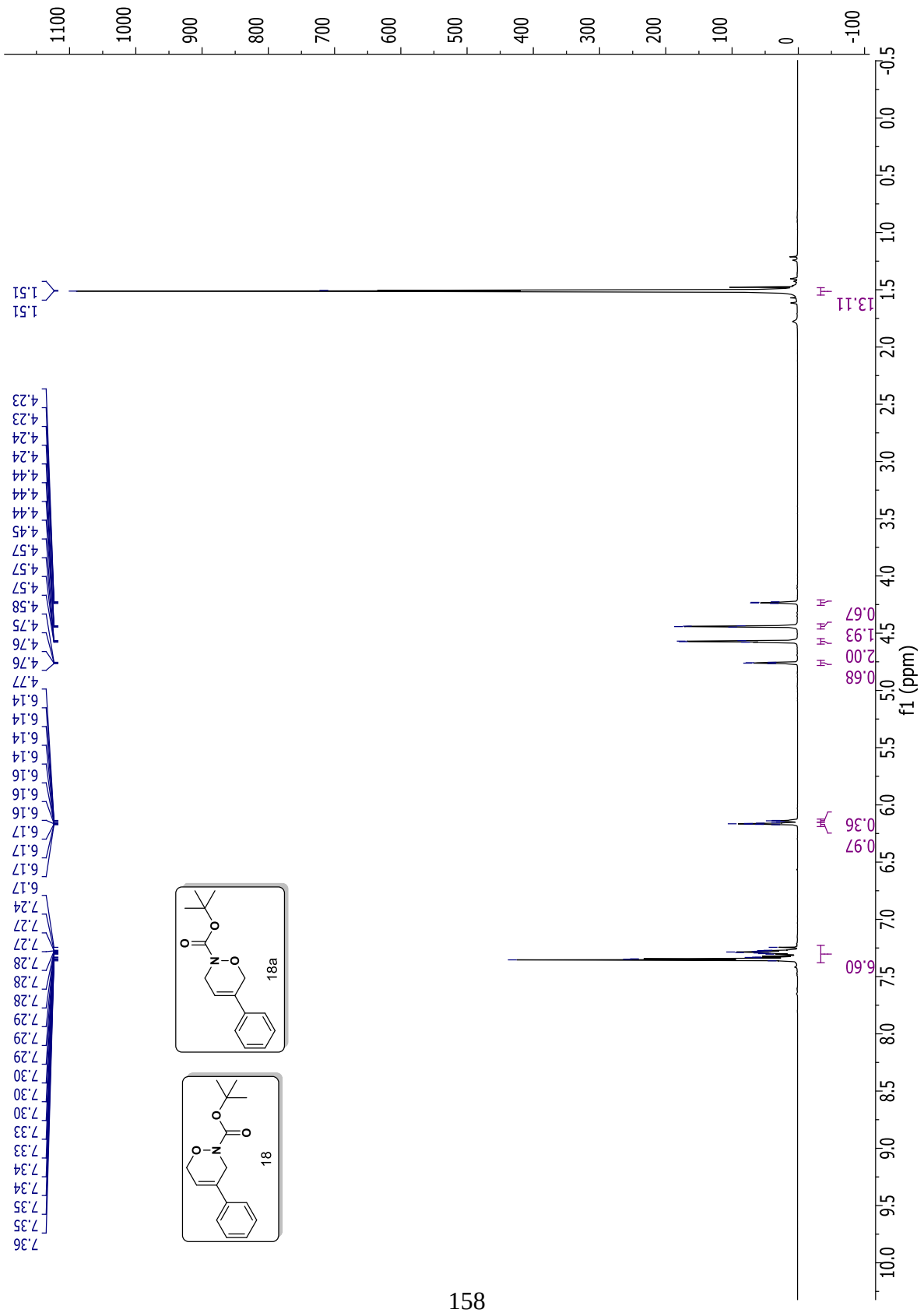
17
17a
f1 (ppm)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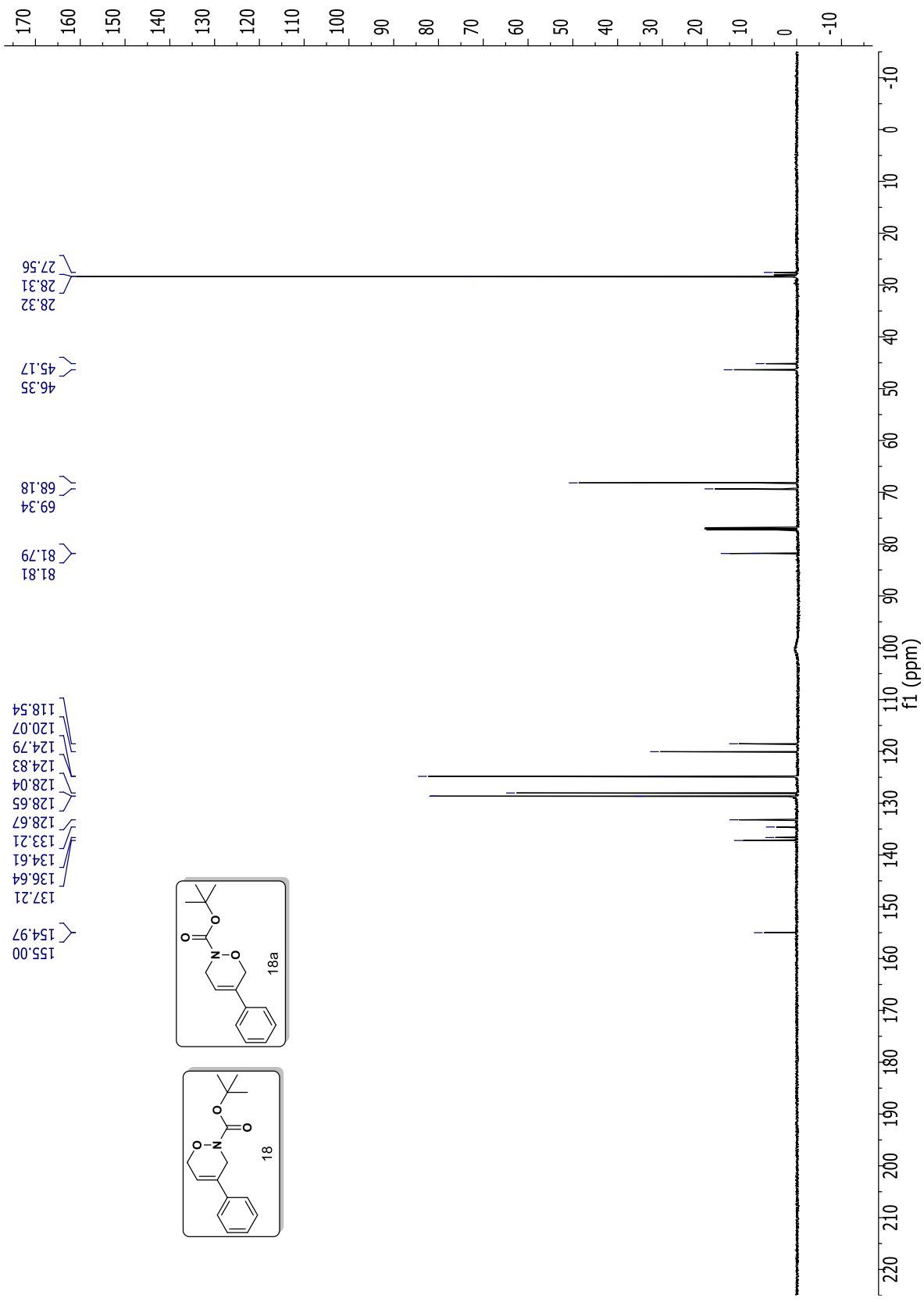
1.51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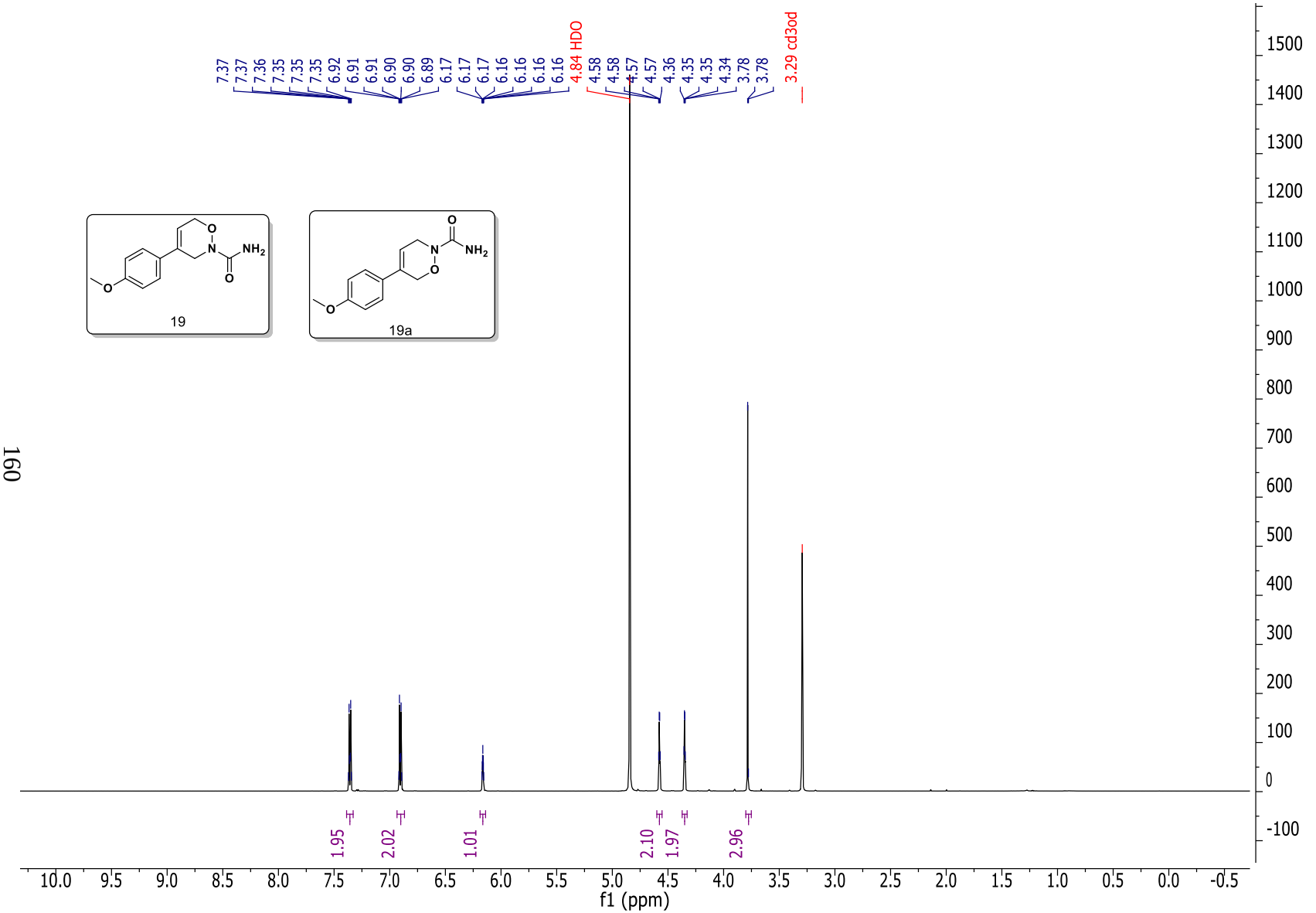
154.97
155.00
18a
18
f1 (ppm)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4.84 HDO
4.58
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4.57
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4.34
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1.01
2.10
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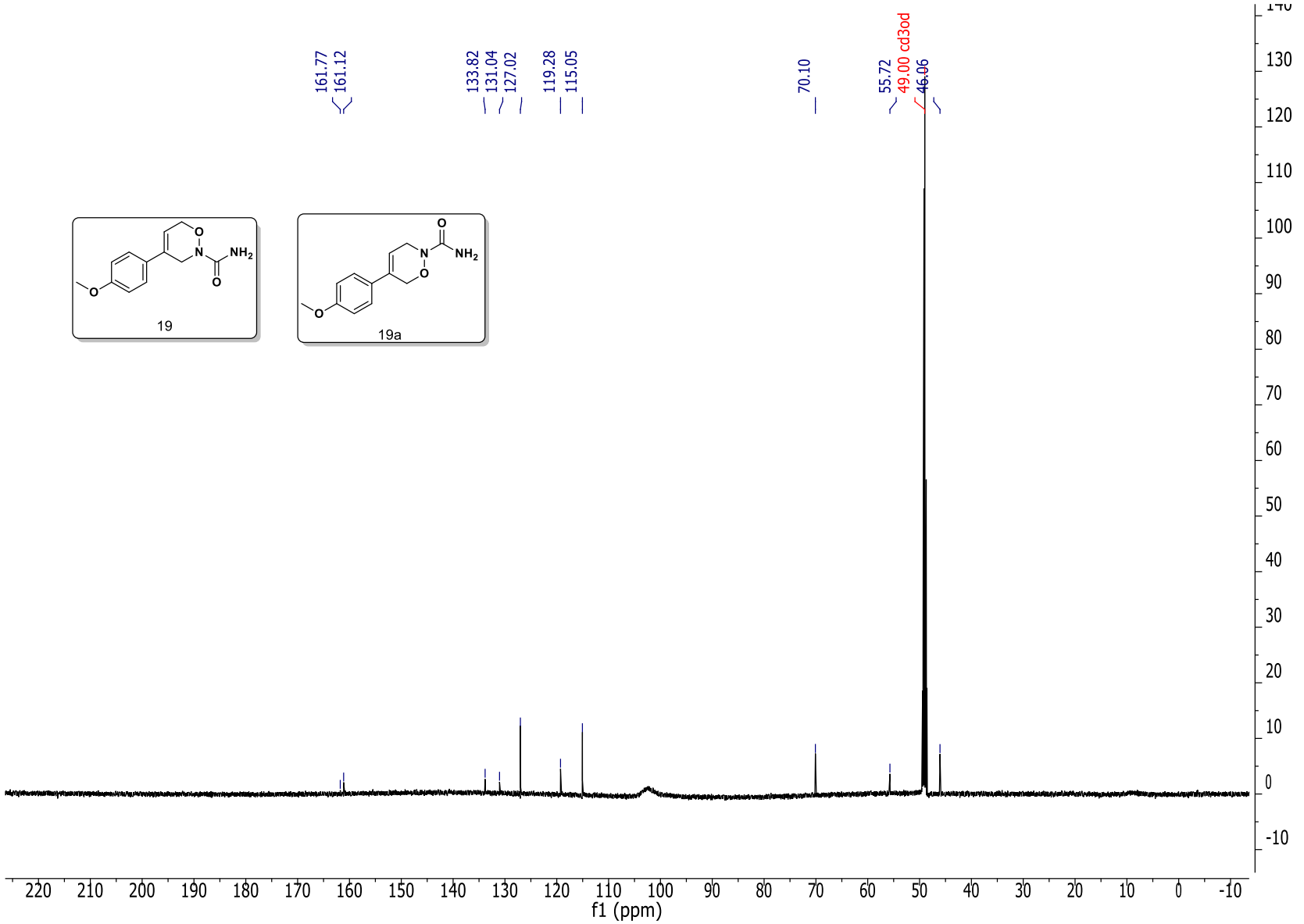
2.96
f1 (ppm)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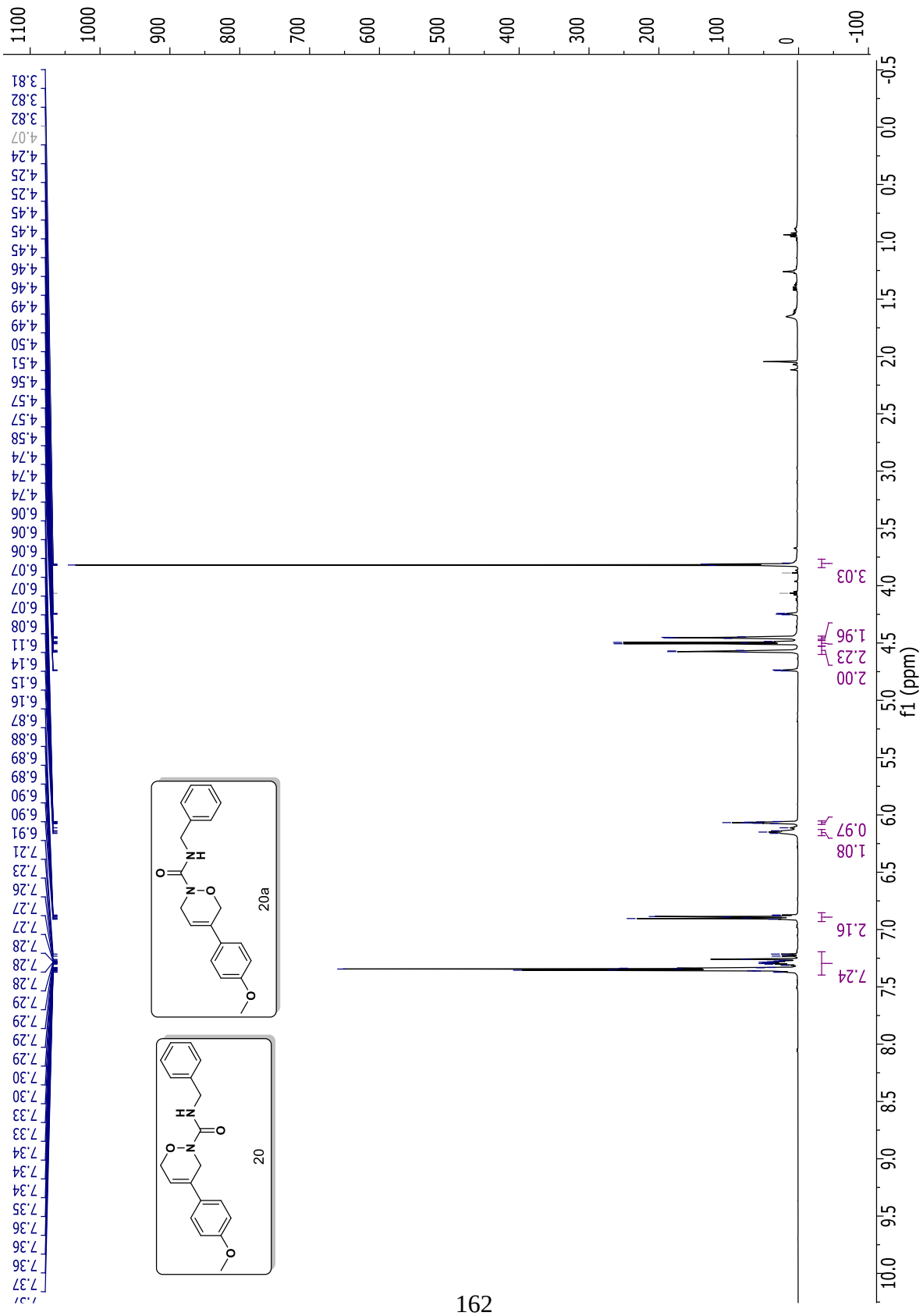
19
19a
f1 (ppm)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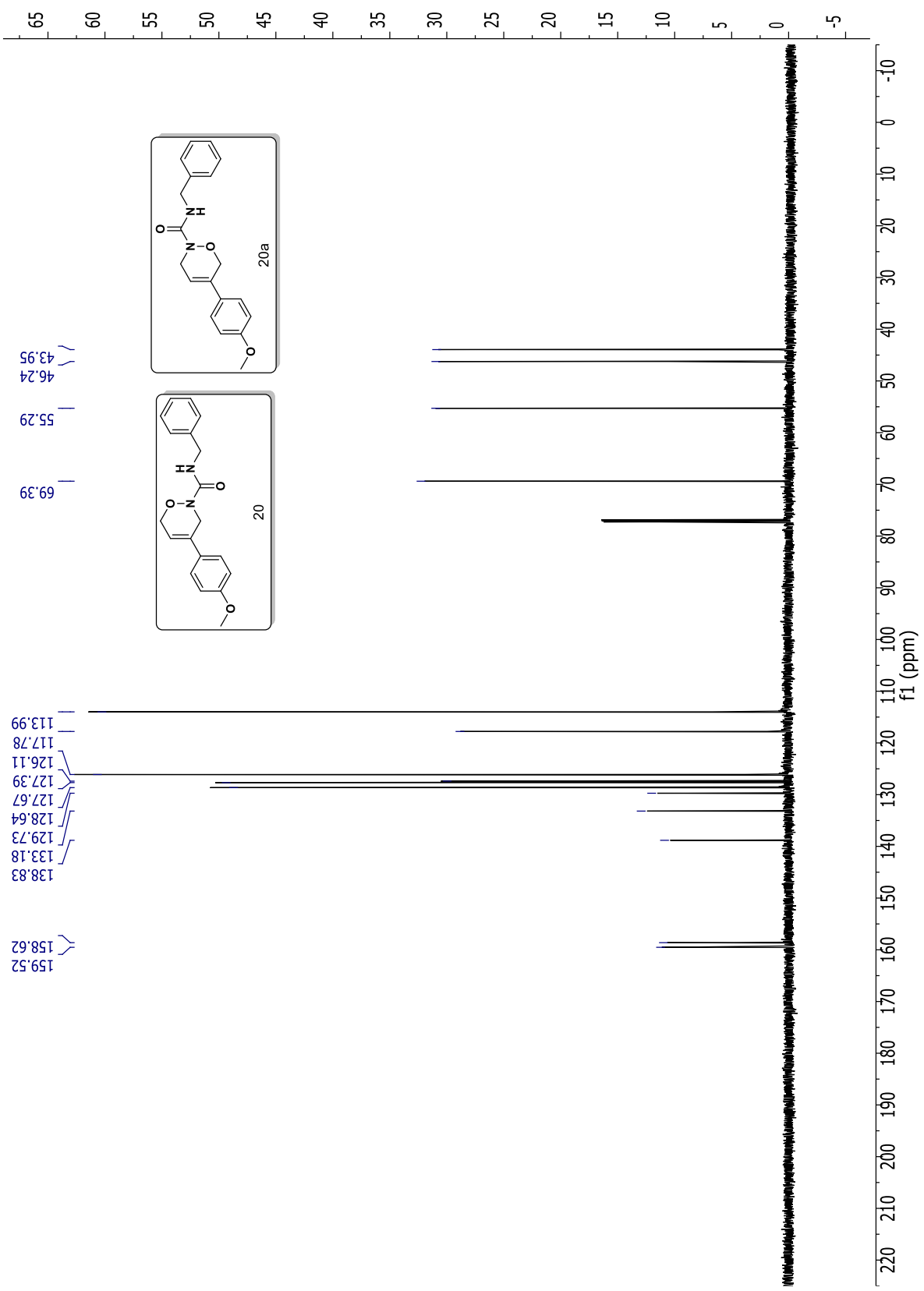
3.03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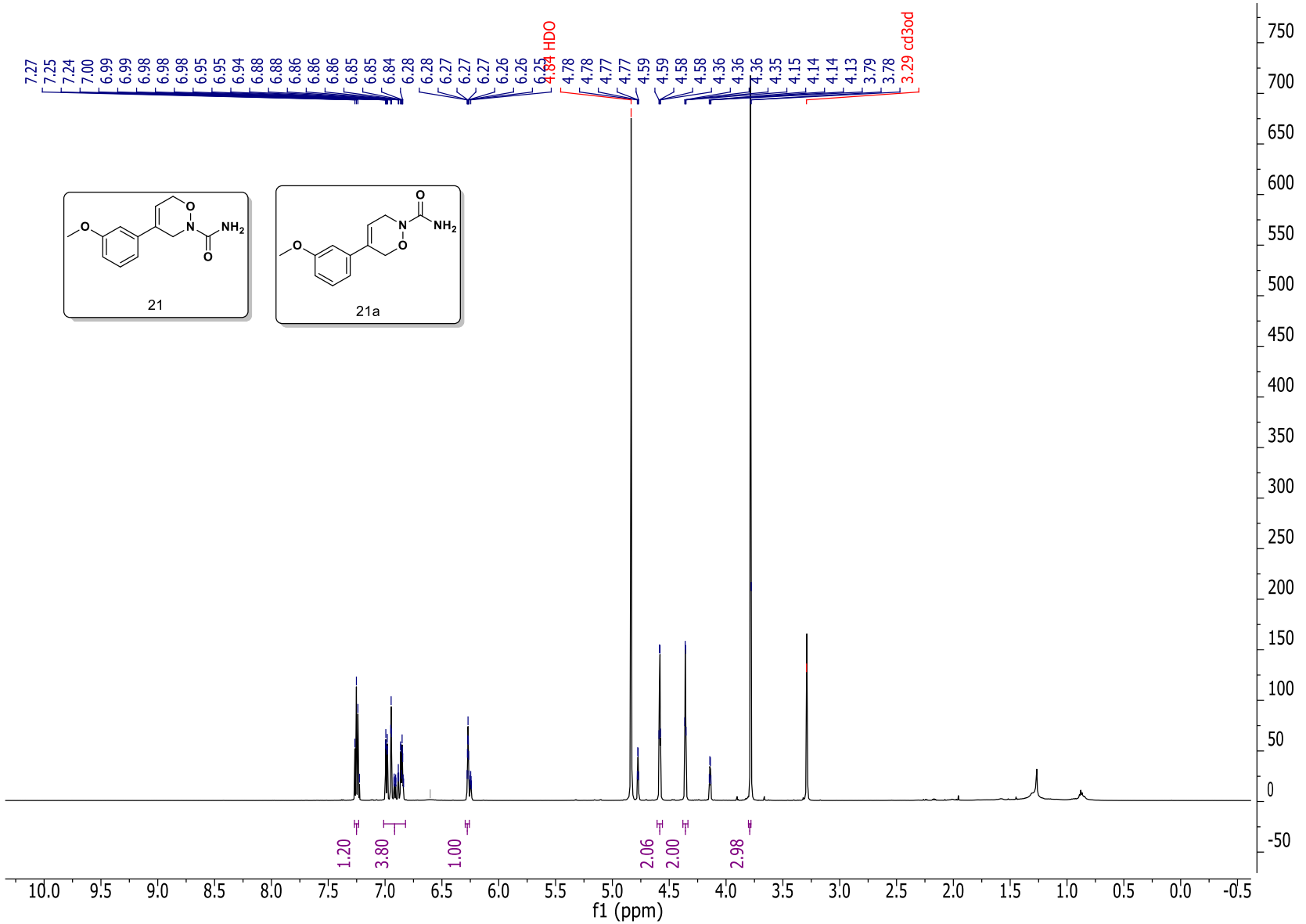
46.24
43.95
20
20a
f1 (ppm)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4.36
4.35
4.15
4.14
4.14
4.13
3.79
3.78
3.29 cd3od
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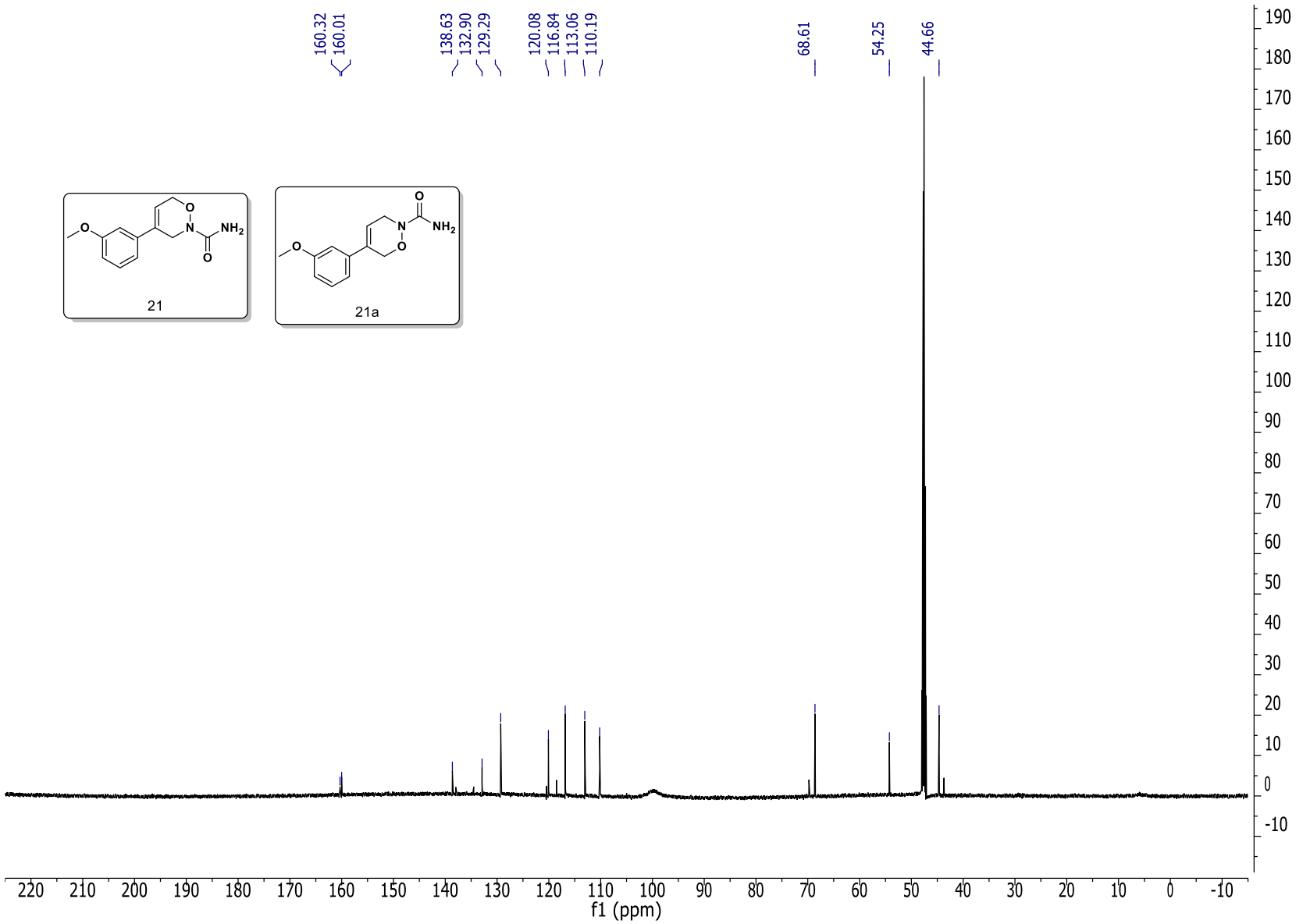
1.00
2.06
2.00
2.98
f1 (ppm)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68.61
54.25
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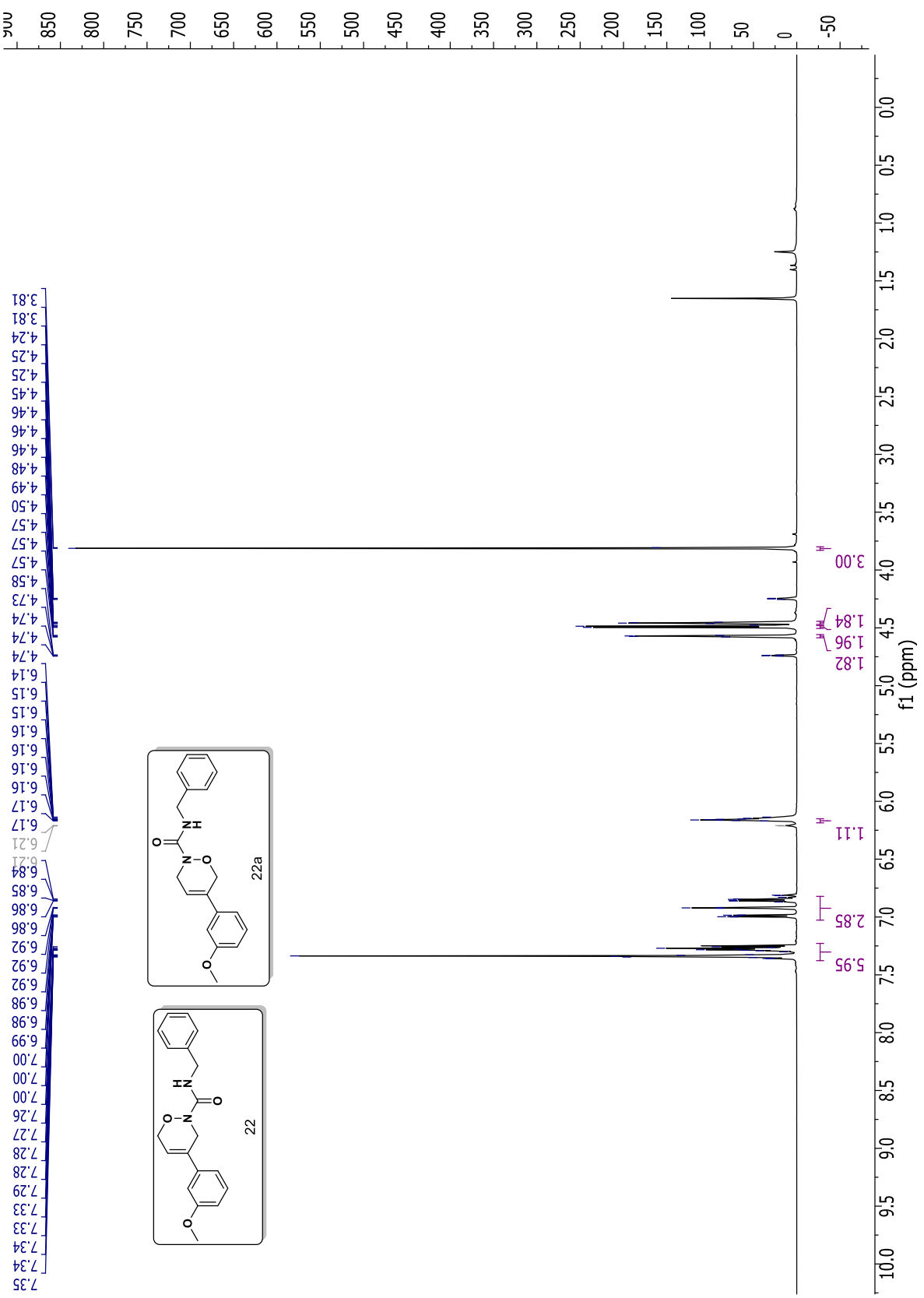
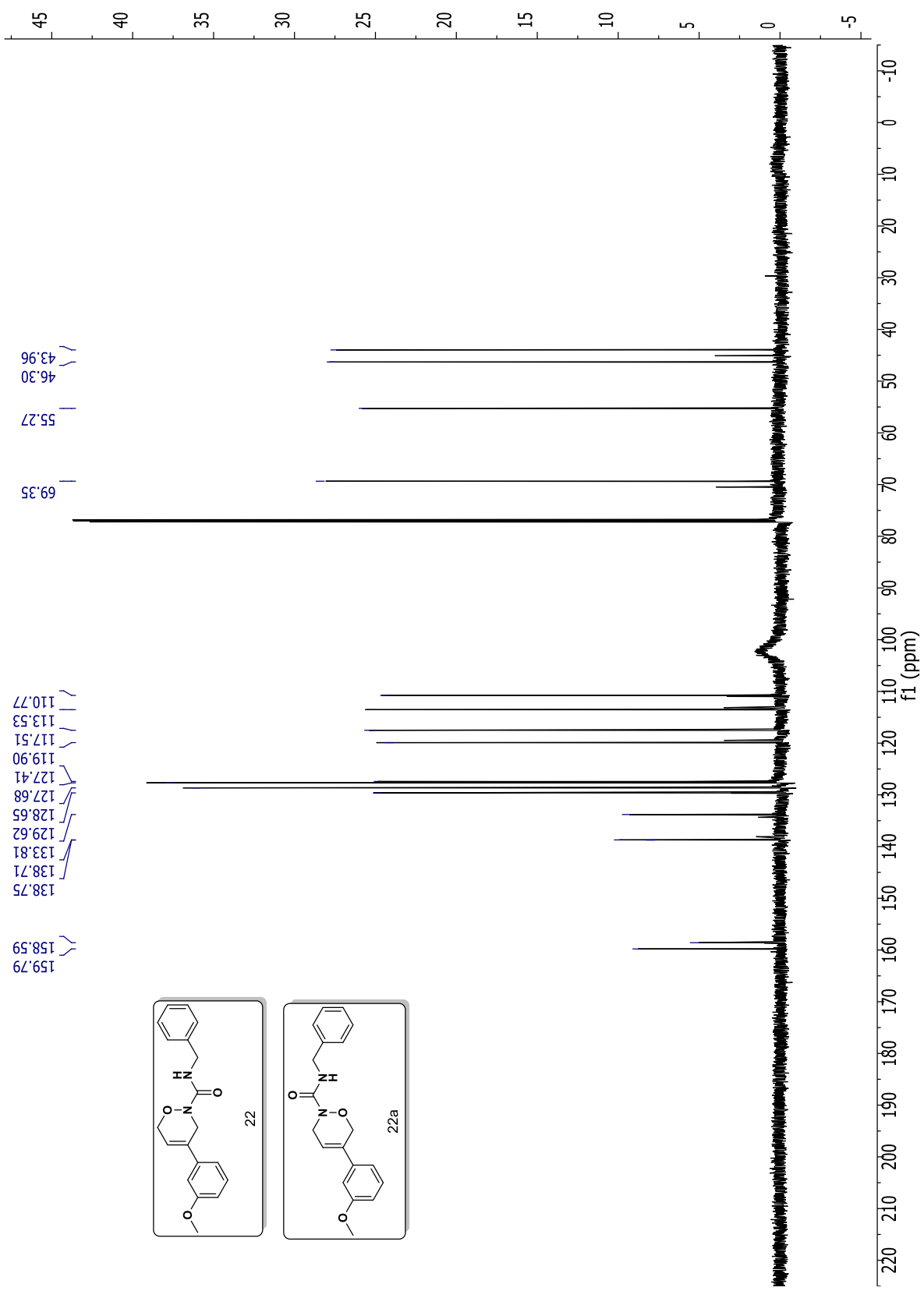
21
21a
f1 (ppm)


22
22a
f1 (ppm)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55.27
46.30
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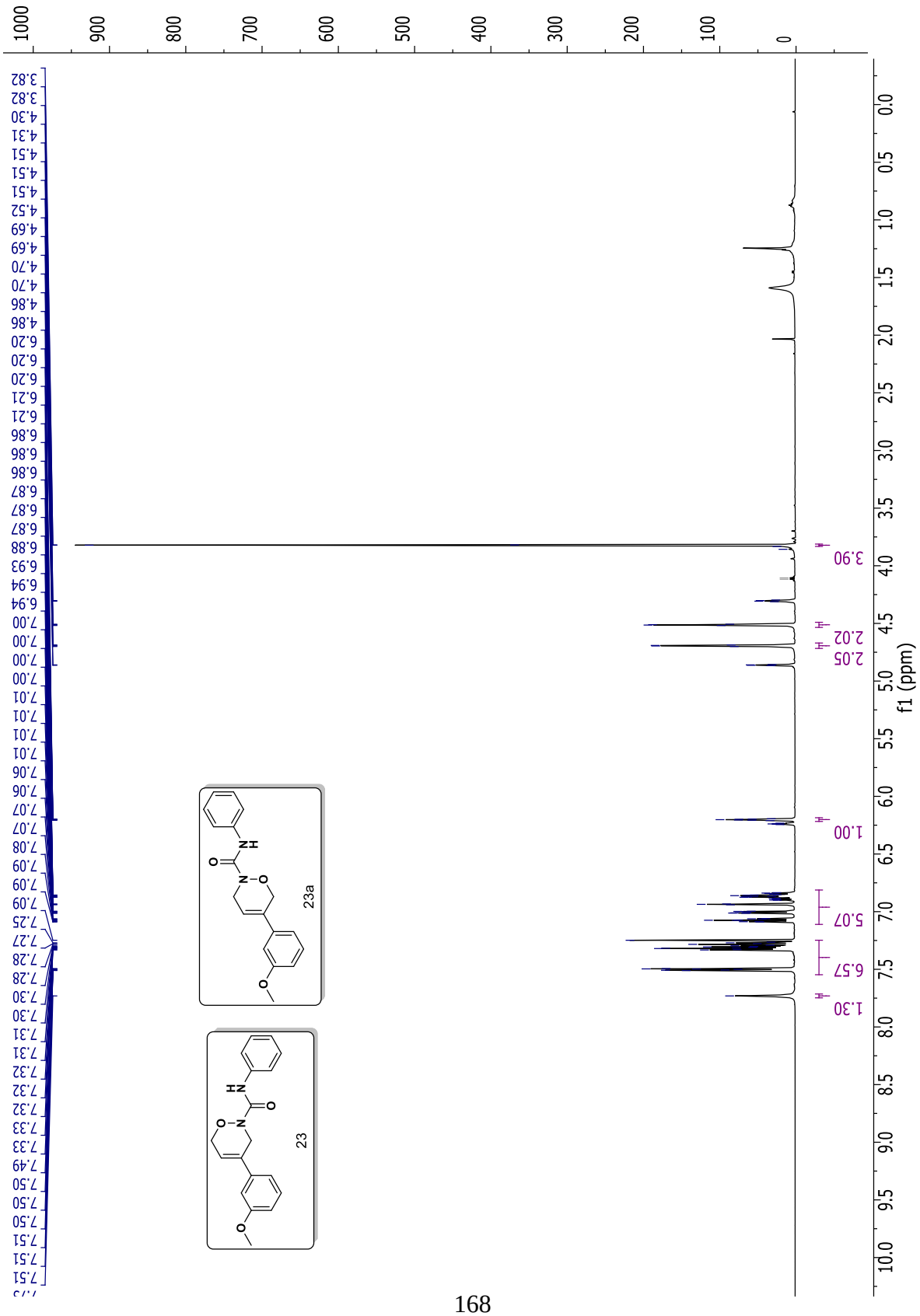
22
22a
f1 (ppm)


23a
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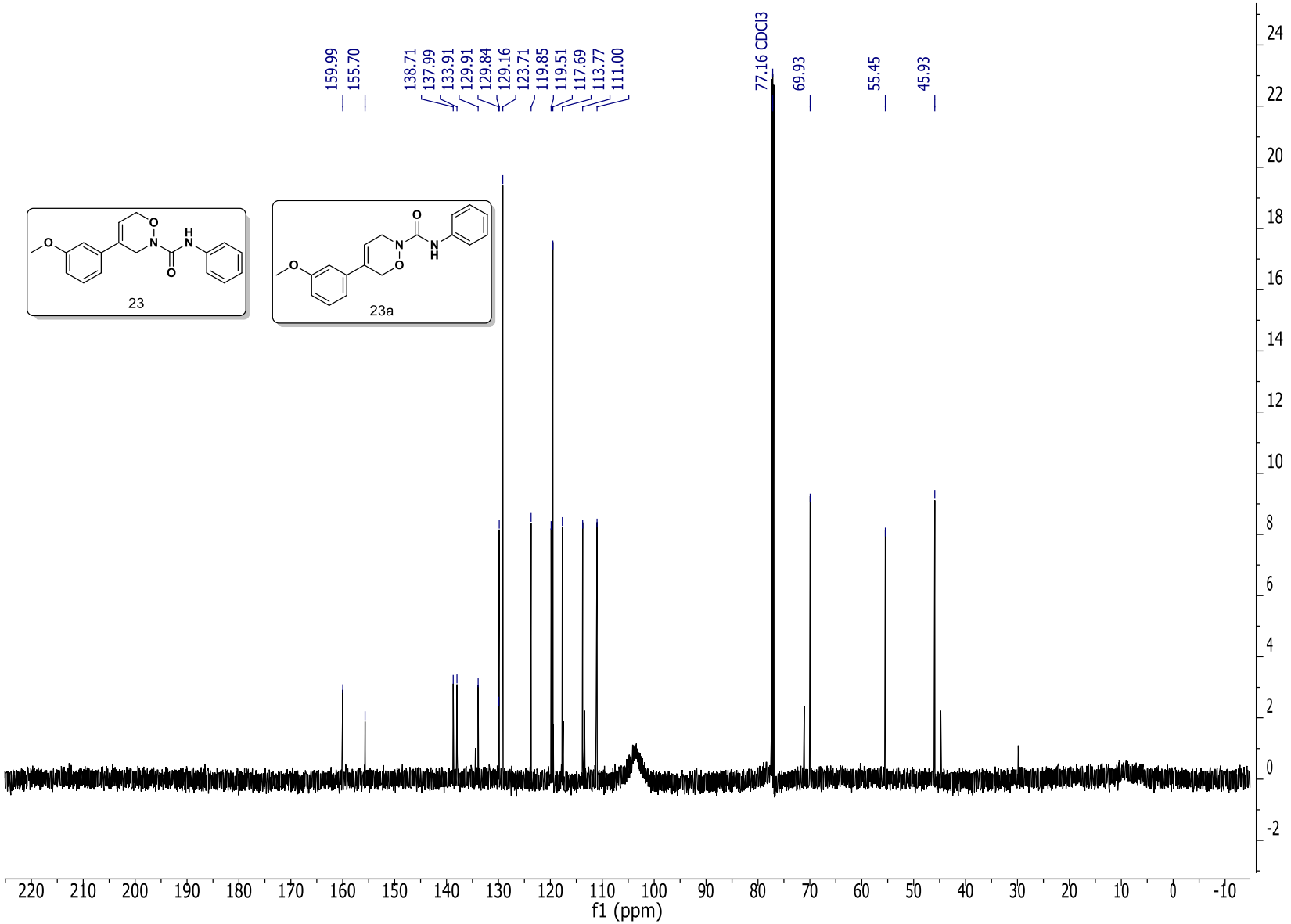
1.00
2.05
2.02
3.90
f1 (ppm)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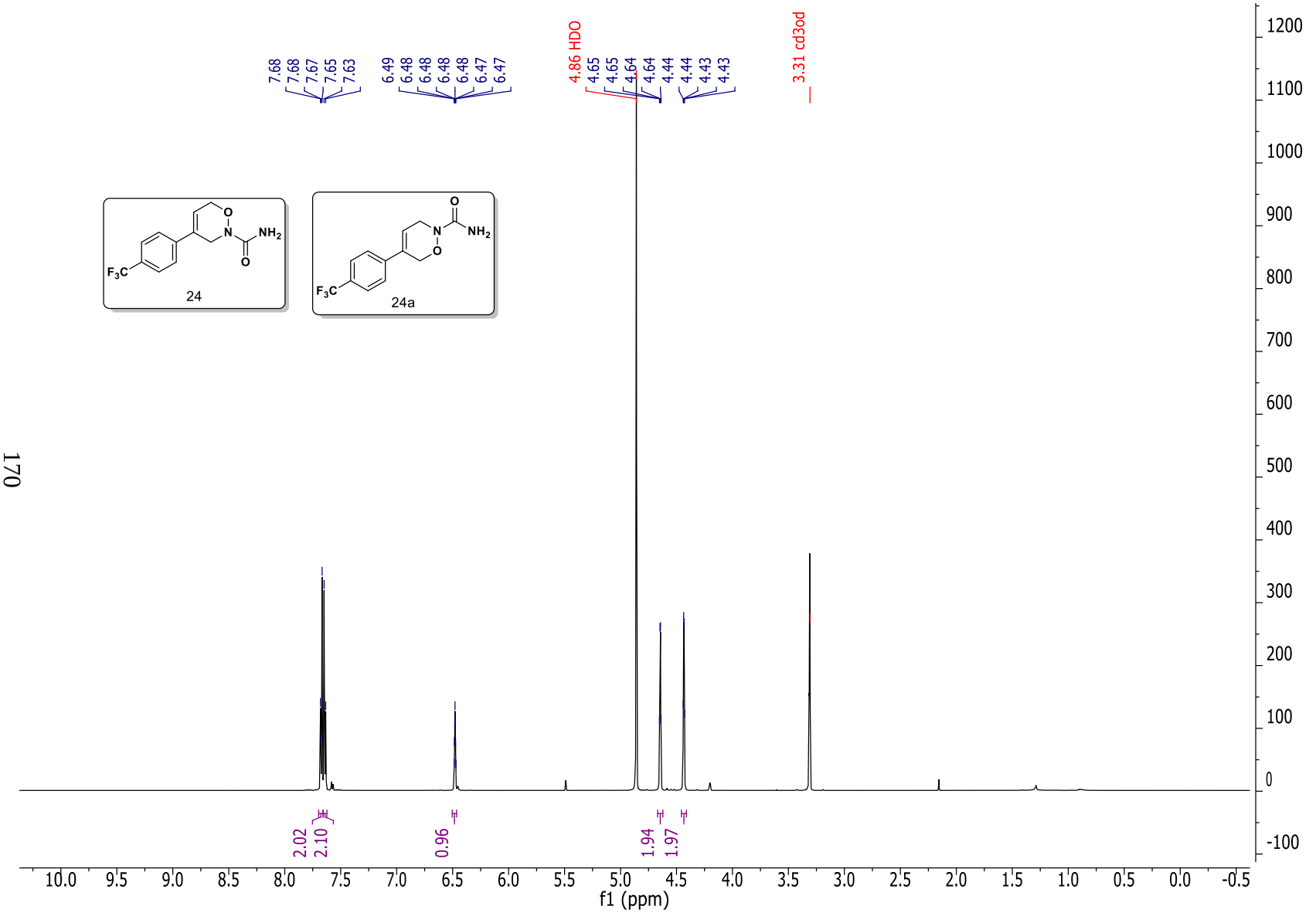
138.71
137.99
133.91
129.91
129.84
129.16
123.71
119.85
119.51
117.69
113.77
111.00
77.16 CDCl3
69.93
55.45
45.93
23
23a
f1 (ppm)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7.68
7.67
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7.63
6.49
6.48
6.48
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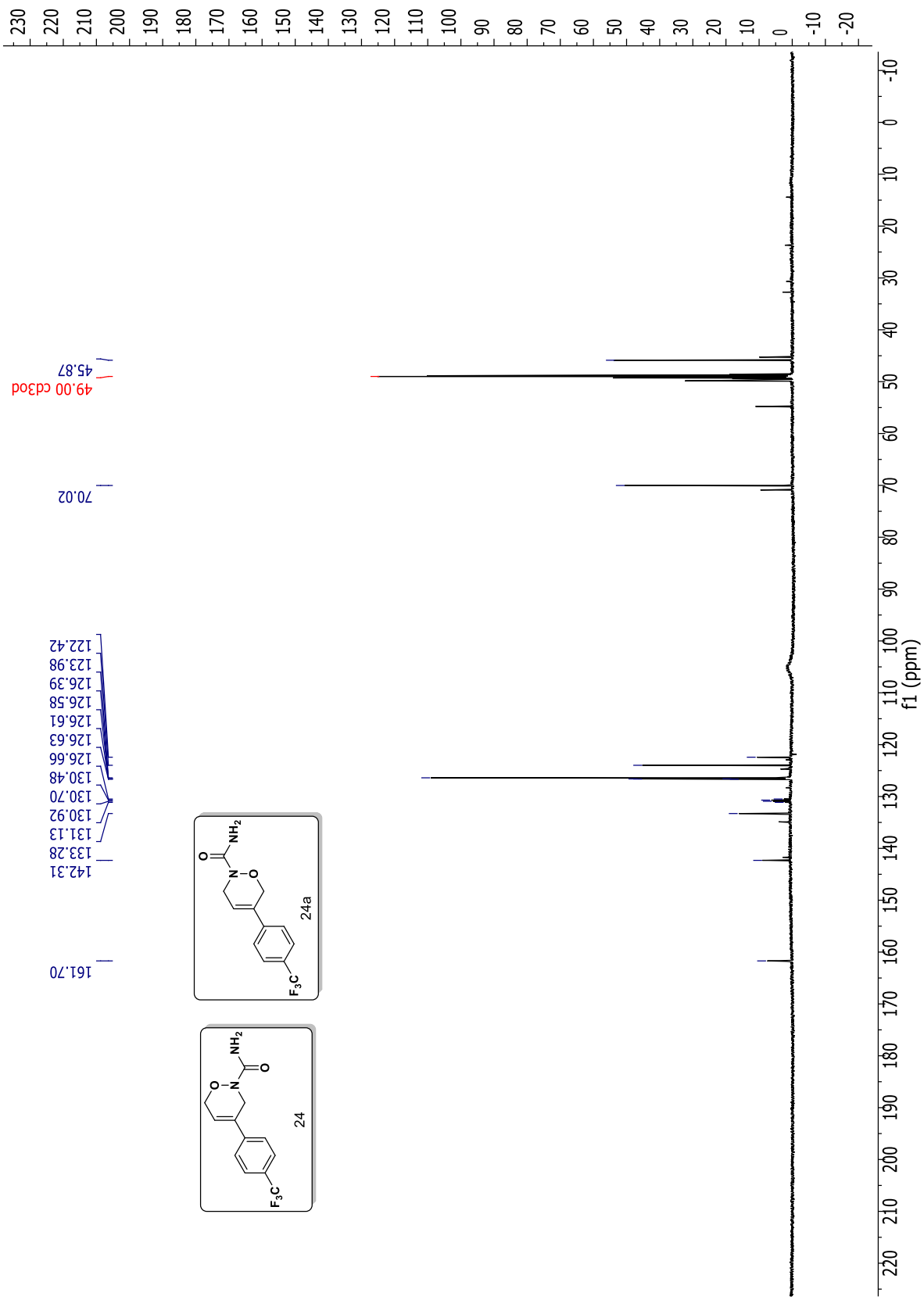
6.47
6.47
4.86 HDO
4.65
4.65
4.64
4.64
4.44
4.44
4.43
4.43
3.31 cd3od
24
24a
2.02
2.10
0.96
1.94
1.97
f1 (ppm)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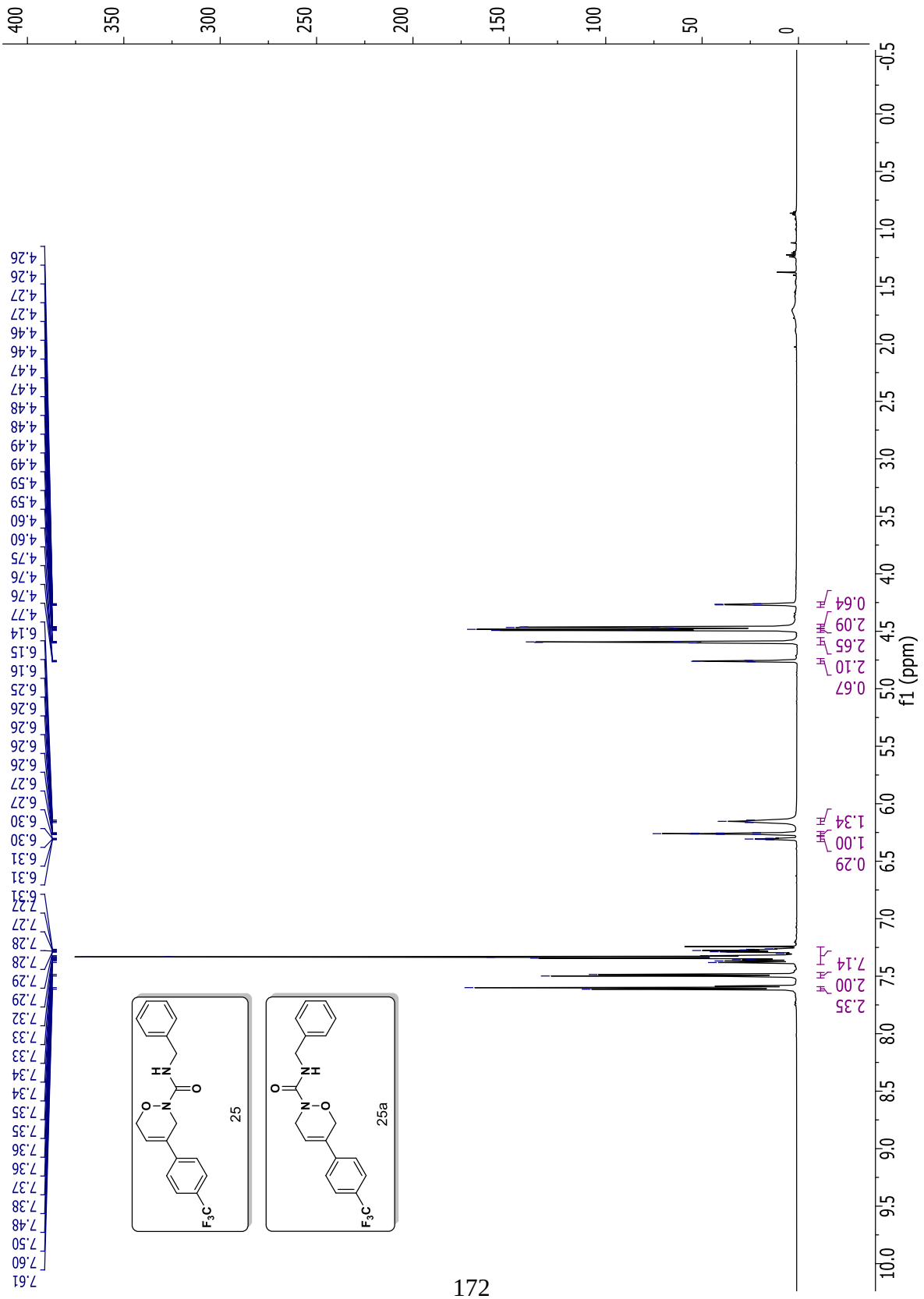
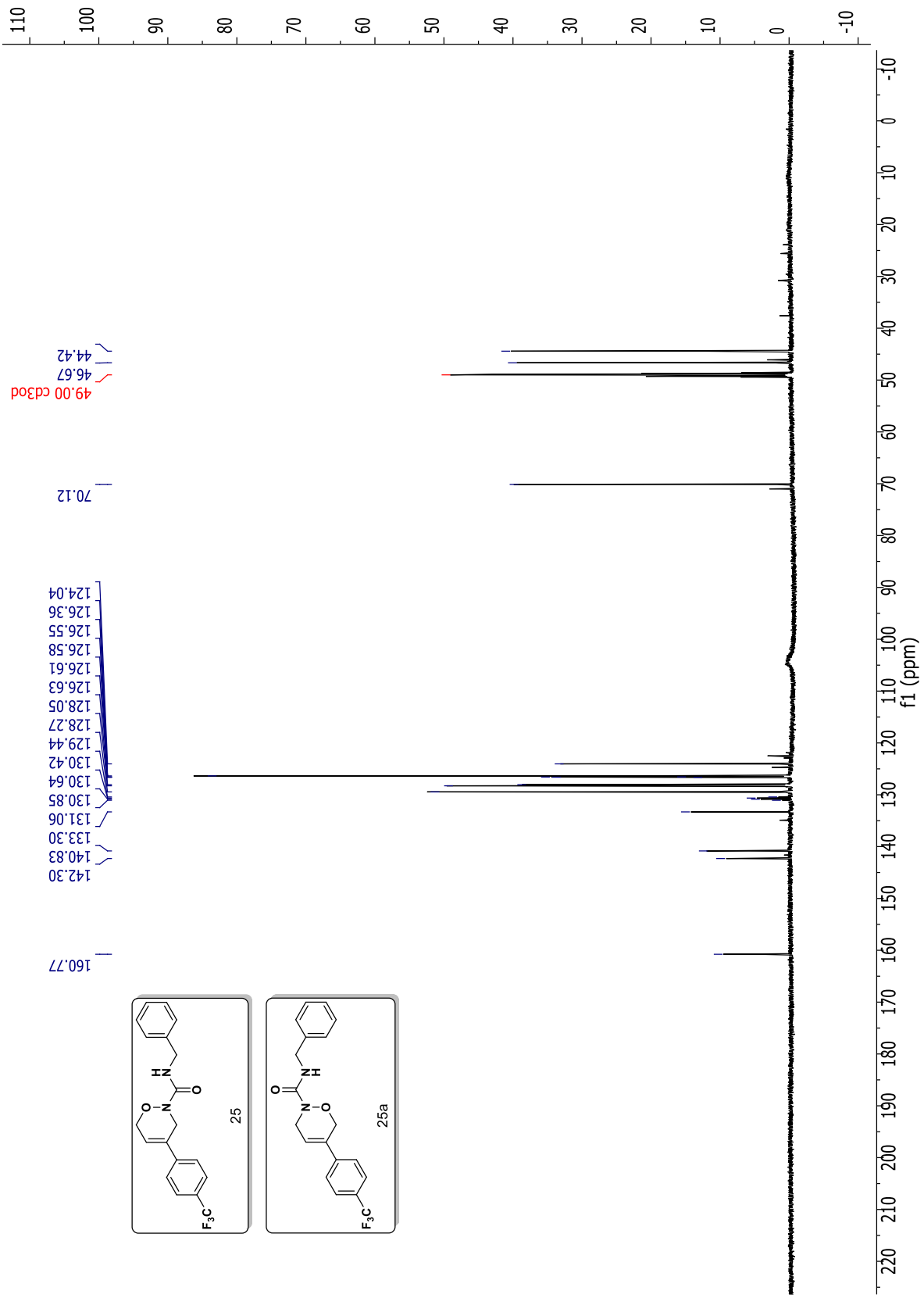
142.31
133.28
131.13
130.92
130.70
130.48
126.66
126.63
126.61
126.58
126.39
123.98
122.42
70.02
49.00 cd3od
45.87
24
24a
f1 (ppm)


25
25a
f1 (ppm)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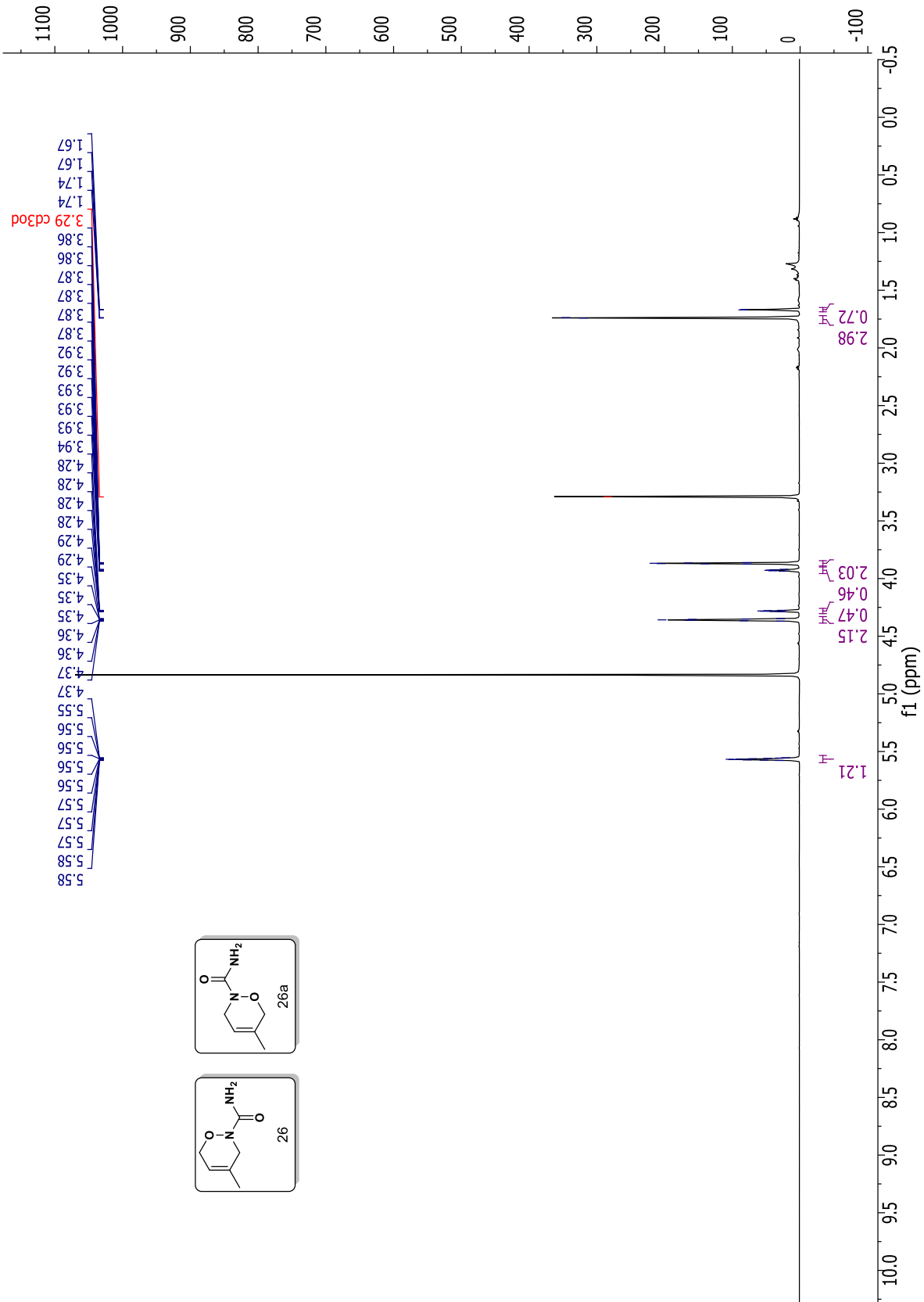
142.30
140.83
133.30
131.06
130.85
130.64
130.42
129.44
128.27
128.05
126.63
126.61
126.58
126.55
126.36
124.04
70.12
49.00 cd3od
46.67
44.42
f1 (ppm)
25
25a


f1 (ppm)
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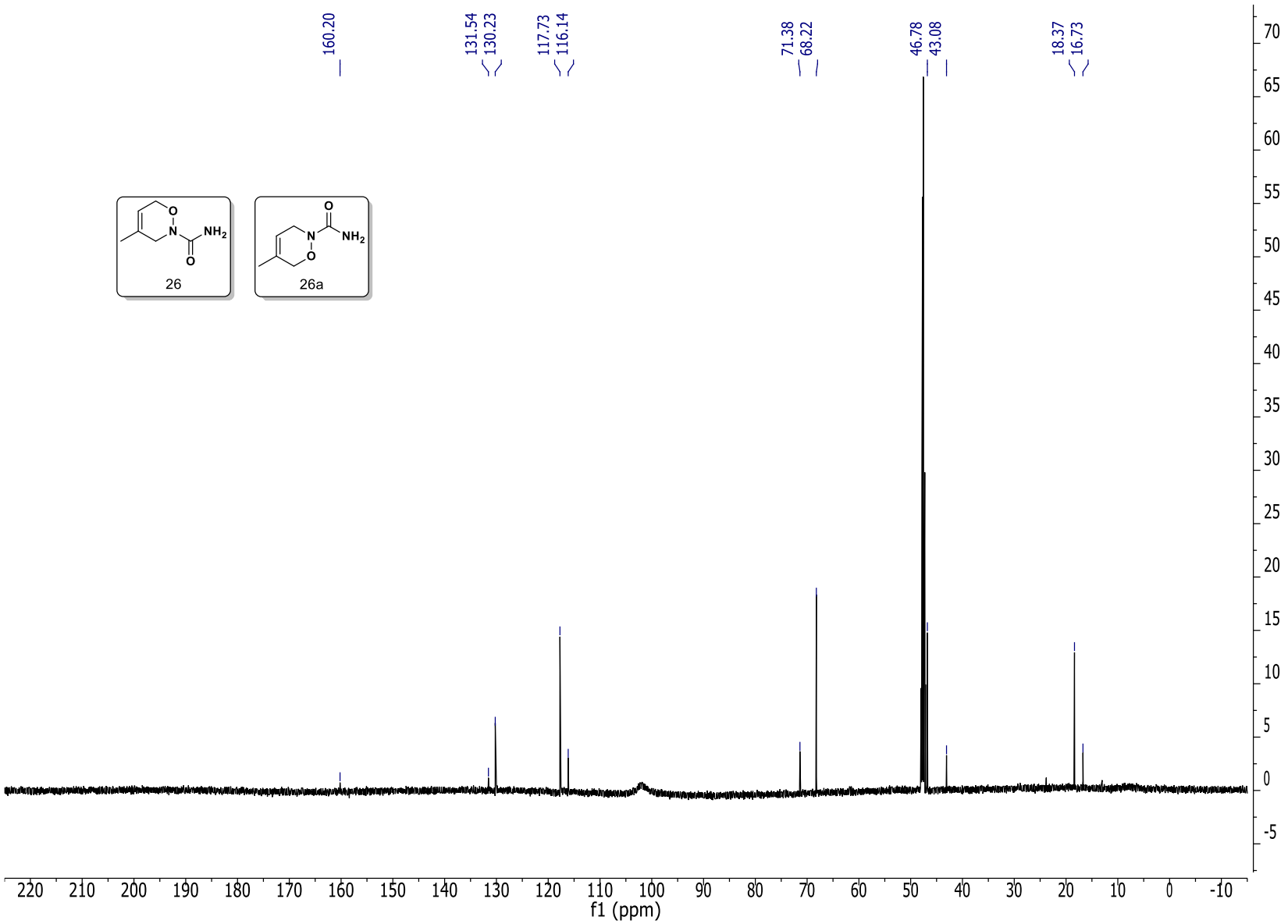
0.47
2.15
26
26a
cd3od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131.54
130.23
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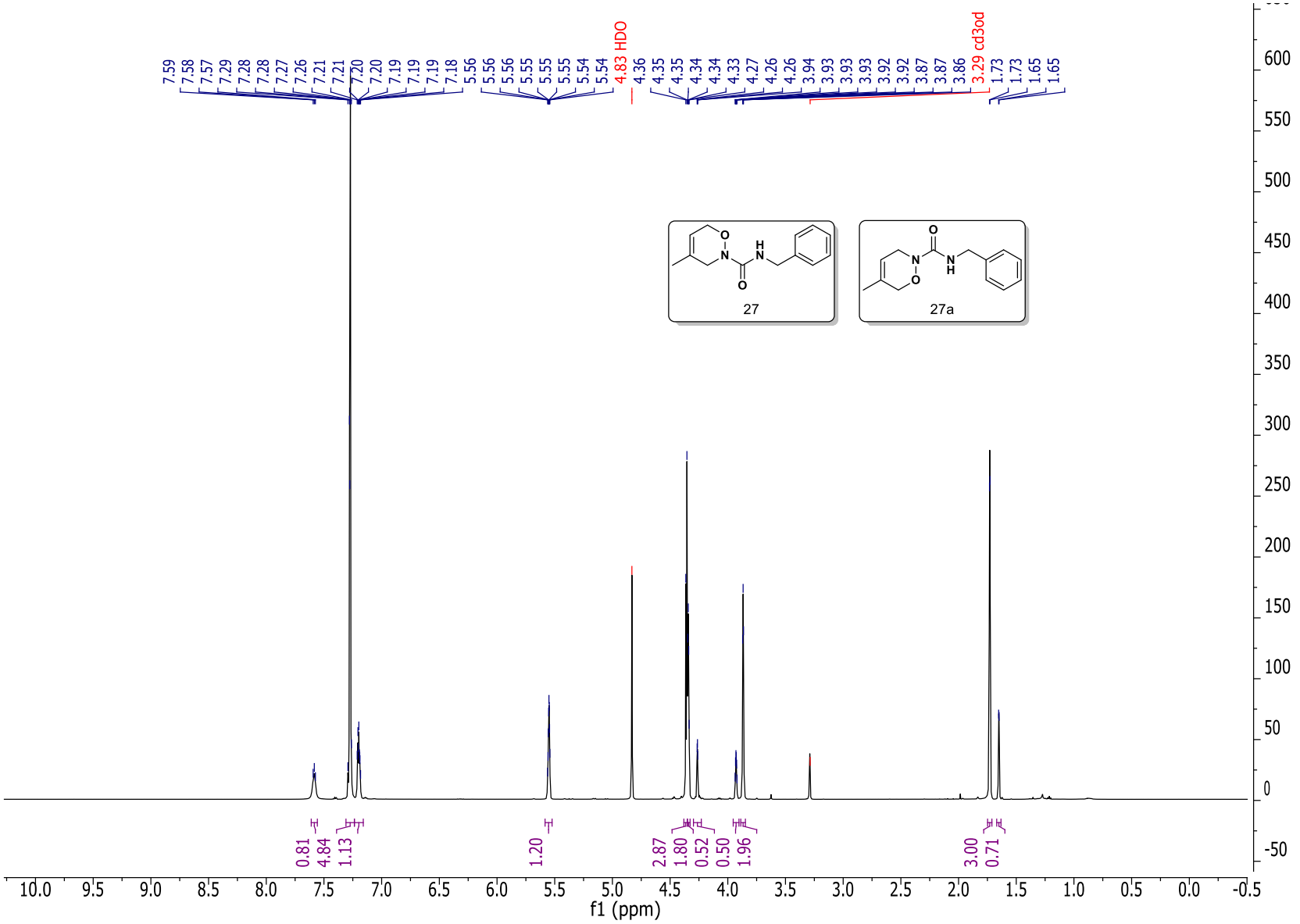
116.14
71.38
68.22
46.78
43.08
18.37
16.73
26
26a
f1 (ppm)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7.20
7.20
7.19
7.19
7.19
7.18
5.56
5.56
5.56
5.55
5.55
5.55
5.55
5.54
5.54
4.83 HDO
4.36
4.35
4.35
4.34
4.34
4.33
4.27
4.26
4.26
3.94
3.93
3.93
3.93
3.92
3.92
3.87
3.87
3.86
3.29 cd3od
1.73
1.73
1.65
1.65
27
27a
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4.84
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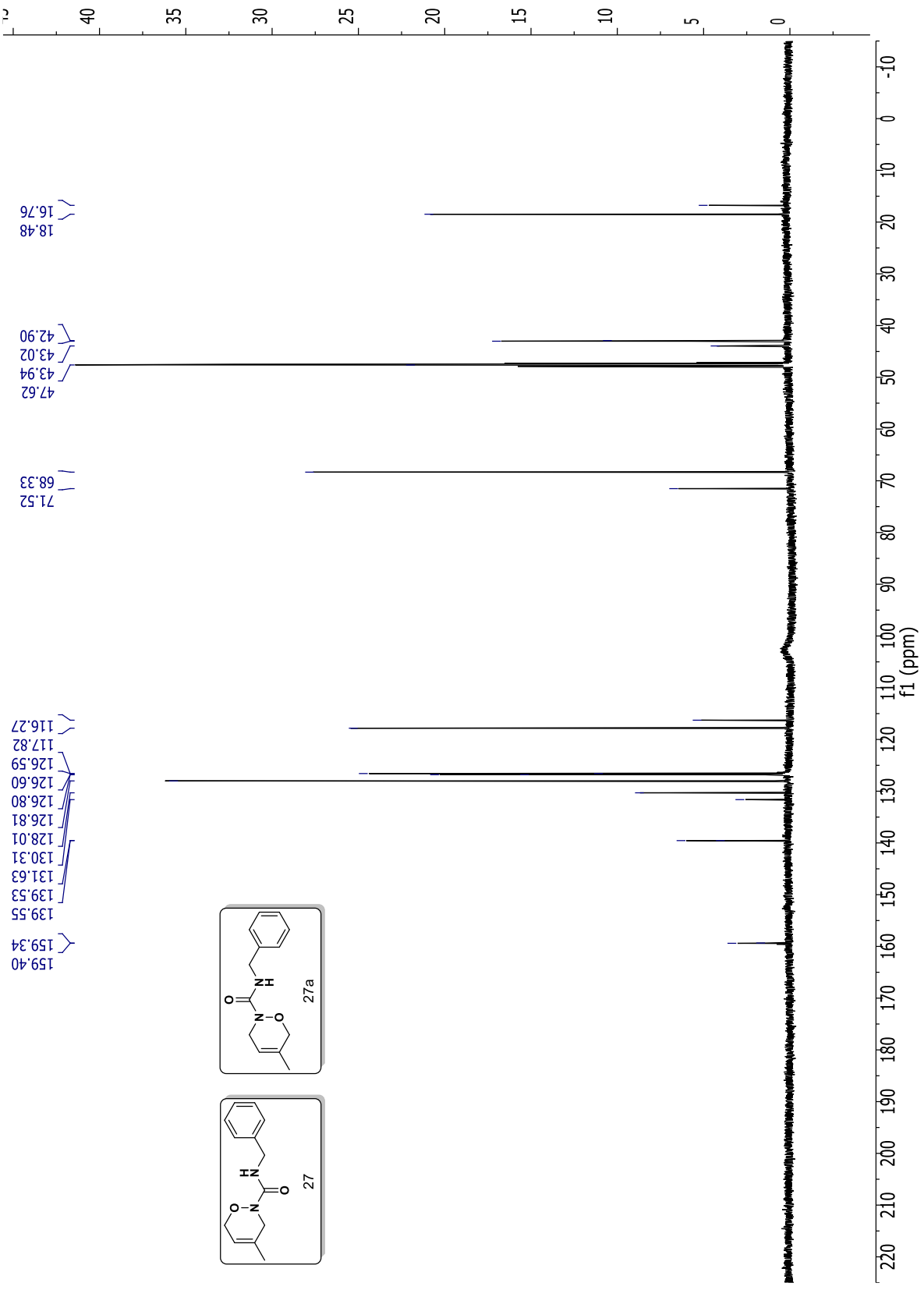
1.20
2.87
1.80
0.52
0.50
1.96
3.00
0.71
f1 (ppm)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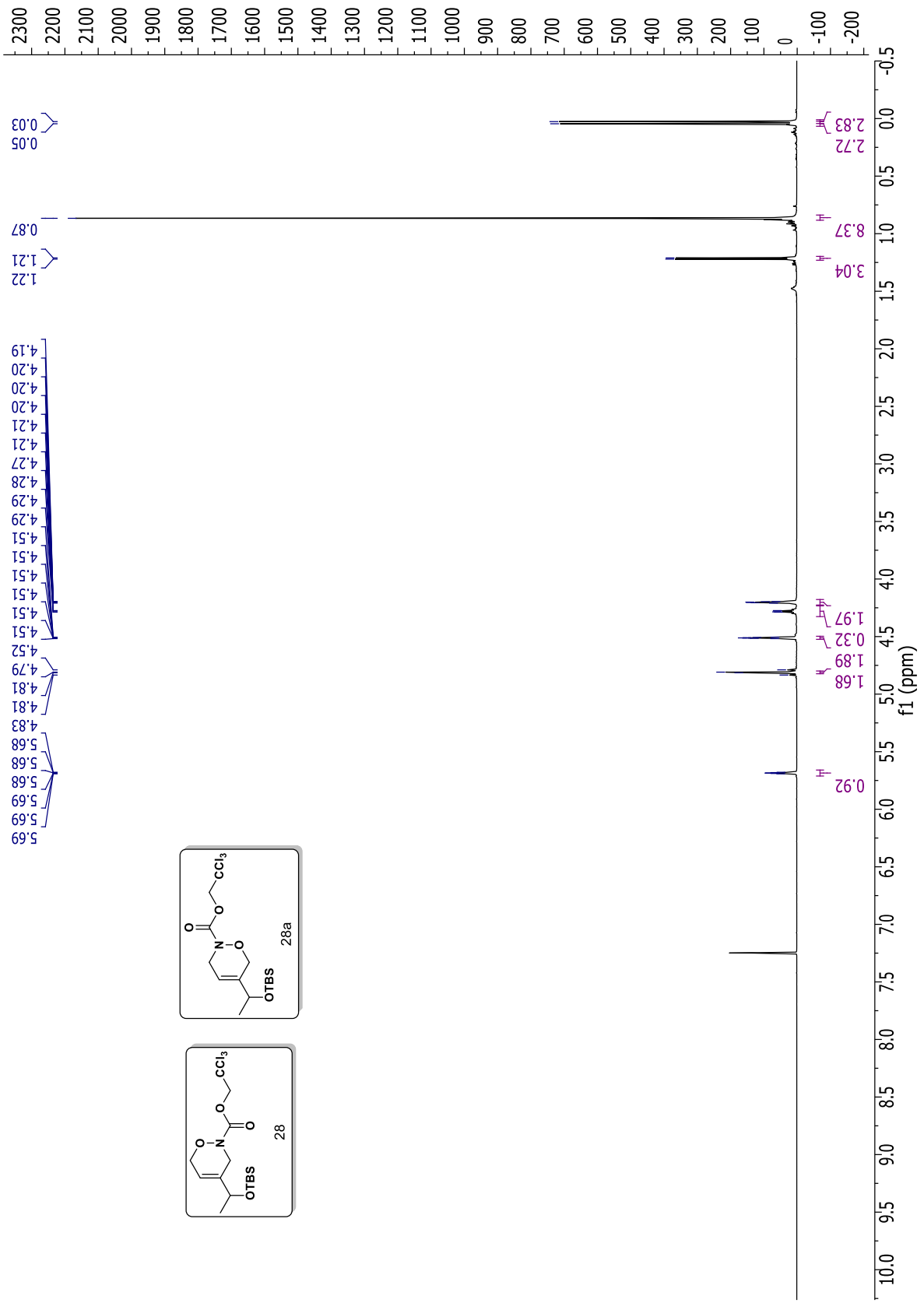
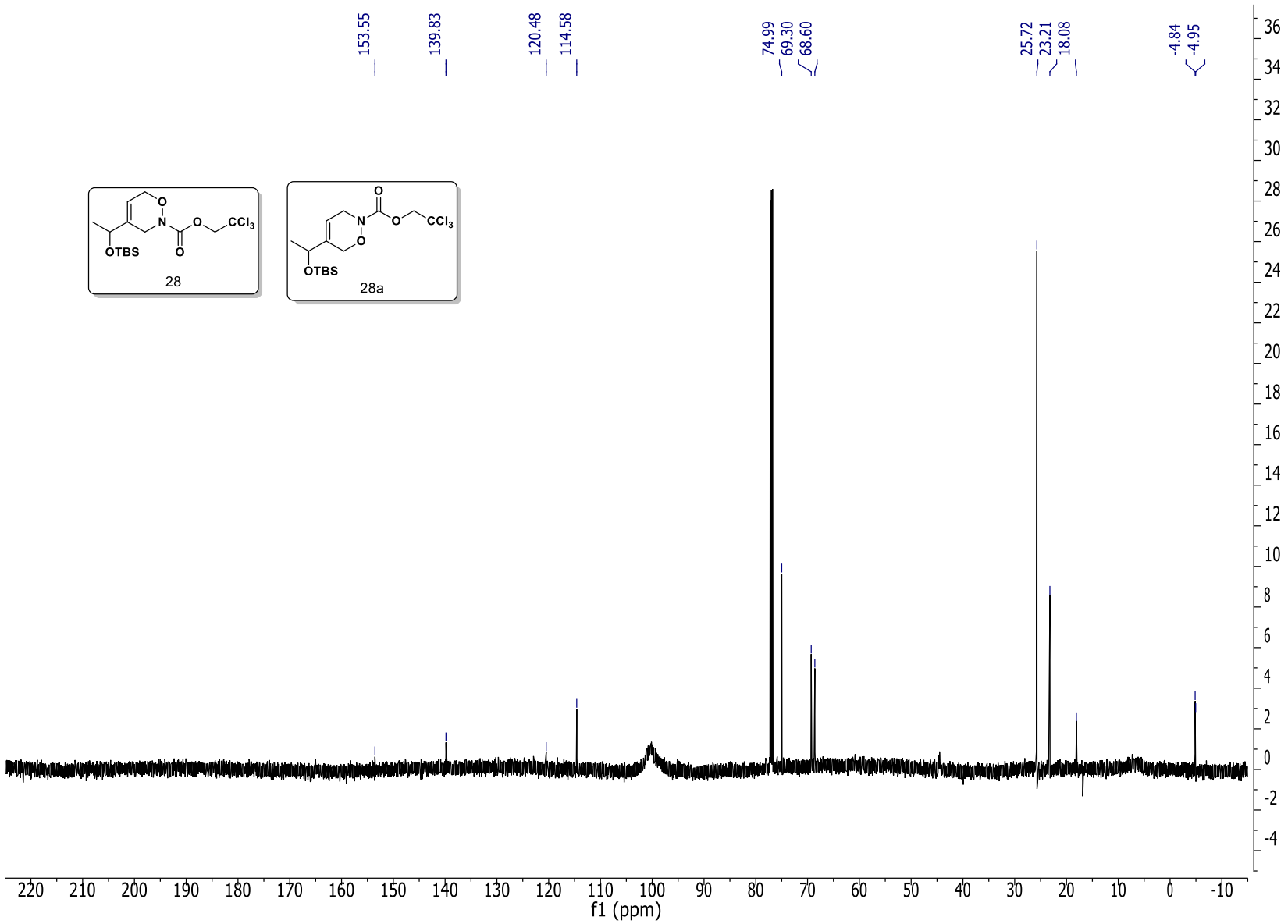
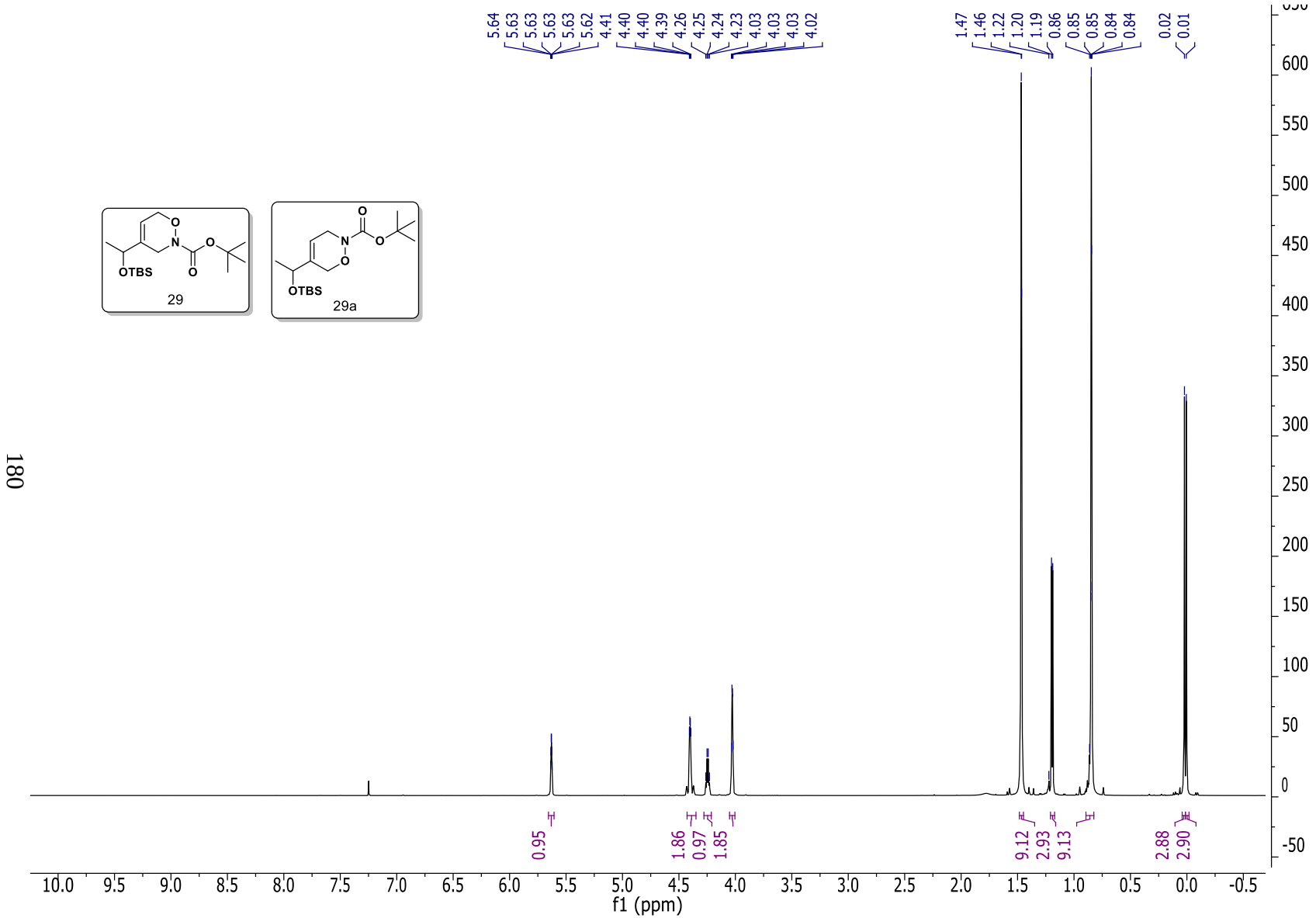
159.34
139.55
139.53
131.63
130.31
128.01
126.81
126.80
126.60
126.59
117.82
116.27
71.52
68.33
47.62
43.94
43.02
42.90
18.48
16.76
27a
27
f1 (ppm)


28
28a
OTBS
CCl3
f1 (ppm)


153.55
139.83
120.48
114.58
74.99
69.30
68.60
25.72
23.21
18.08
-4.84
-4.95
OTBS
28
OTBS
28a
f1 (ppm)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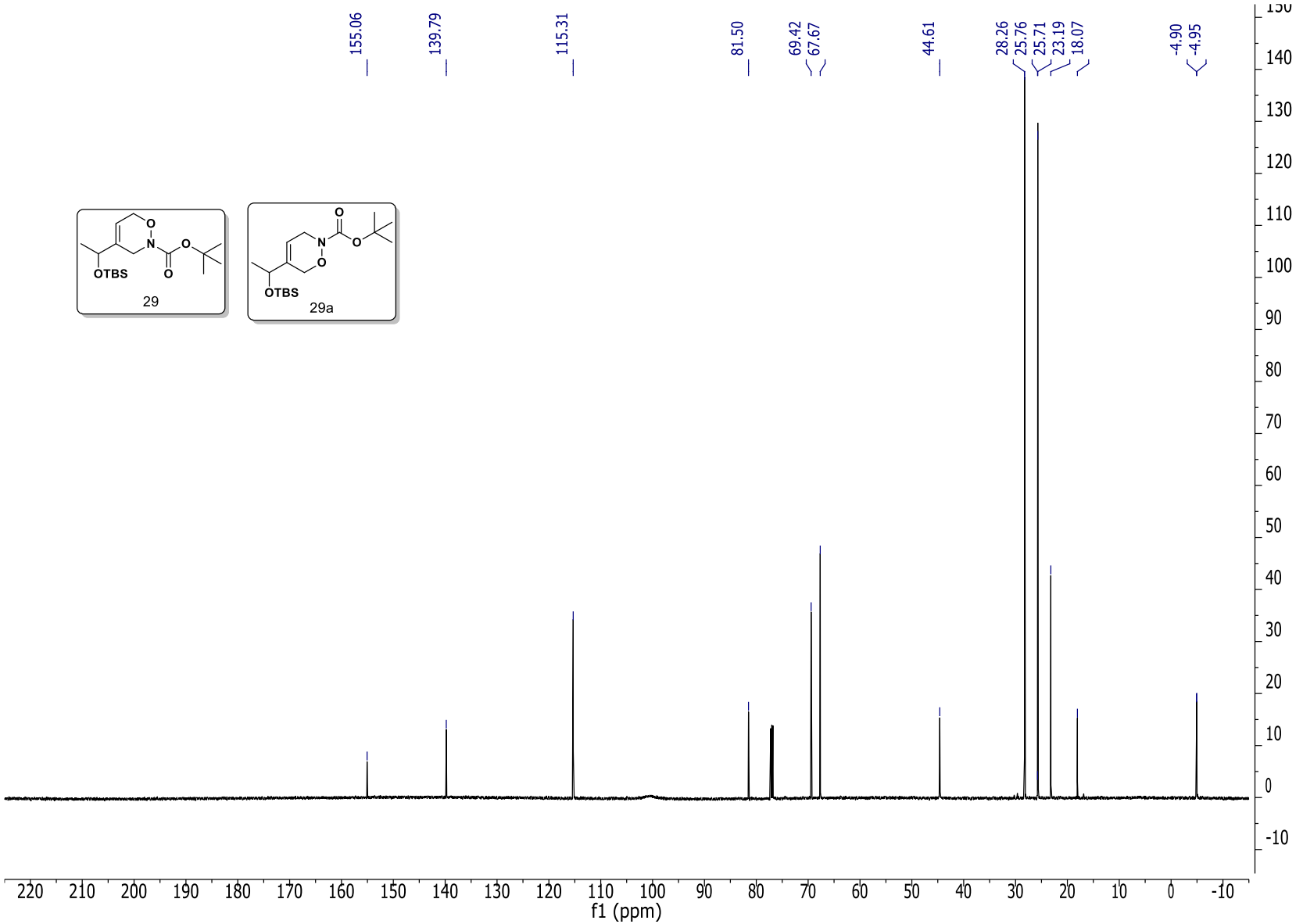
5.63
5.63
5.63
5.63
5.62
4.41
4.40
4.40
4.39
4.26
4.25
4.24
4.23
4.03
4.03
4.03
4.02
1.47
1.46
1.22
1.20
1.19
0.86
0.85
0.85
0.84
0.84
0.02
0.01
OTBS
29
OTBS
29a
0.95
1.86
0.97
1.85
9.12
2.93
9.13
2.88
2.90
f1 (ppm)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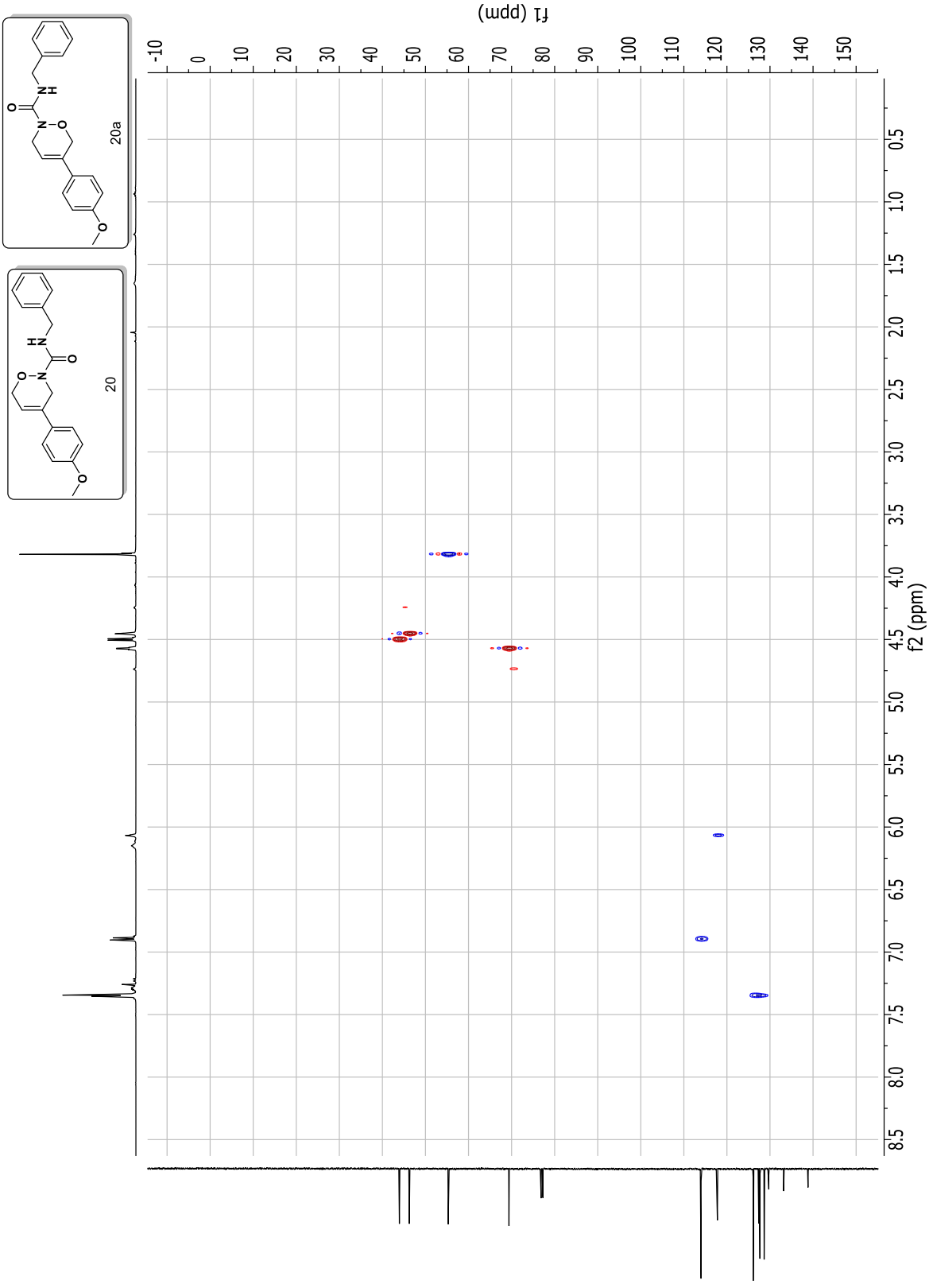
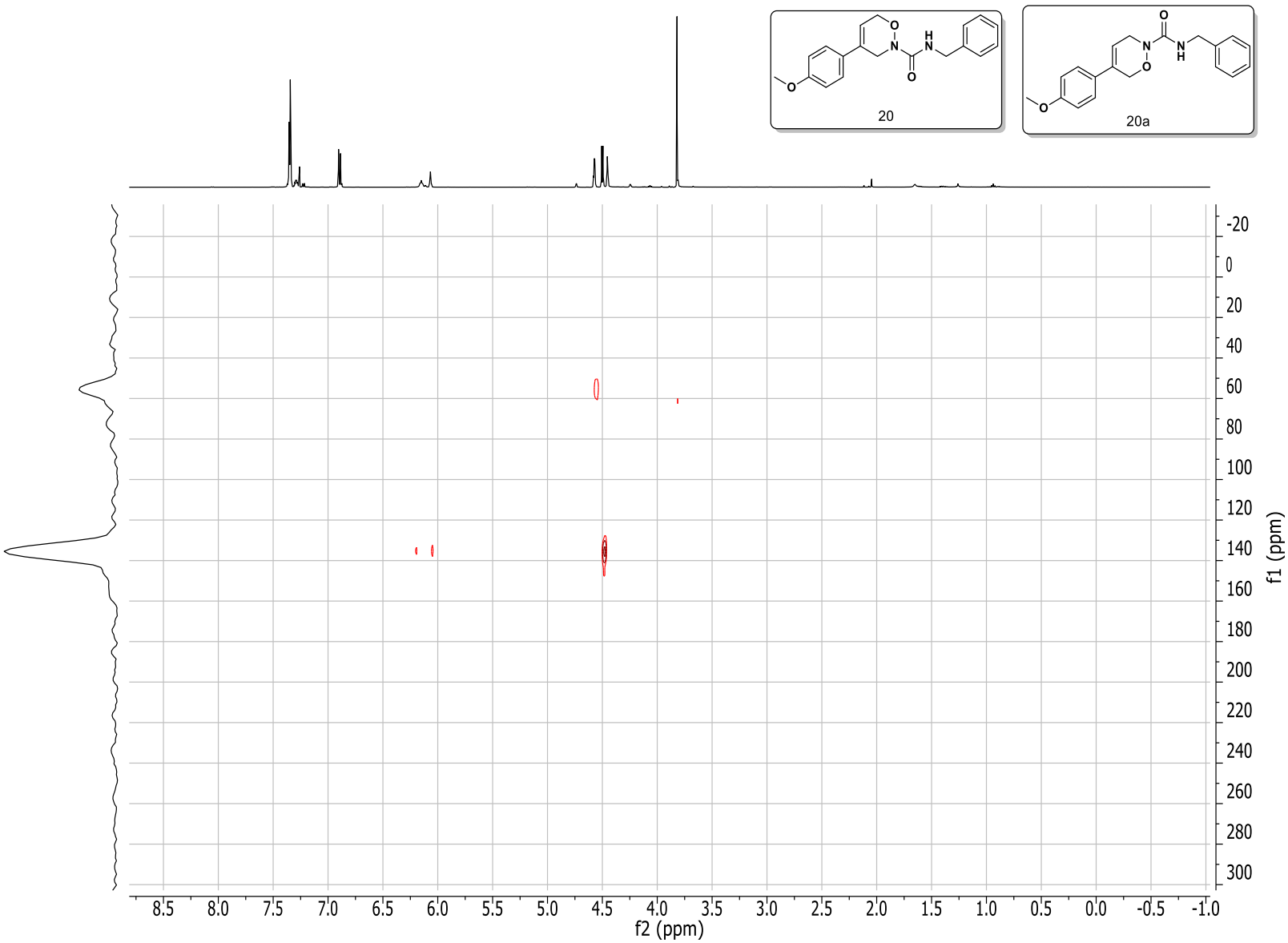
139.79
115.31
81.50
69.42
67.67
44.61
28.26
25.76
25.71
23.19
18.07
-4.90
-4.95
OTBS
29
OTBS
29a
f1 (ppm)


20a
20
f1 (ppm)
f2 (ppm)


20
20a
f2 (ppm)
f1 (ppm)

7.2 References

1. Qin, L.; Zhou, Z.; Wei, J.; Yan, T.; Wen, H., General and Efficient Synthesis of O-Sulfonylhydroxylamine Derivatives. *Synth. Commun.* **2010,** *40* (5), 642-646.

2. Donohoe, T. J.; Chughtai, M. J.; Klauber, D. J.; Griffin, D.; Campbell, A. D., N-Sulfonyloxy Carbamates as Reoxidants for the Tethered Aminohydroxylation Reaction. *J. Am. Chem. Soc.* **2006,** *128* (8), 2514-2515.

3. Tonogaki, K.; Mori, M., An improved 1,3-diene synthesis from alkyne and ethylene using cross-enyne metathesis. *Tetrahedron Lett.* **2002,** *43* (12), 2235-2238.