NOVEL APPLICATIONS OF MICROWAVE CHEMISTRY AND MODERN ANALYTICAL METHODS TOWARD THE DEVELOPMENT OF VERSATILE UNKNOWNS-BASED EXPERIMENTS IN THE SOPHOMORE ORGANIC CHEMISTRY LABORATORY

by

DOUGLAS MICHAEL JACKSON

(Under the Direction of Richard W. Morrison)

ABSTRACT

Developing laboratory experiments that effectively engage, instruct, and assess student performance is a critical challenge for the undergraduate organic chemistry laboratory. The limitations of traditional "cookbook" reactions have been well discussed in the literature and include but are certainly not limited to: "foolproof recipes", limited engagement, and lack of problem solving. Additionally, given the breadth of Internet resources and student access to old lab reports, novel experiments are needed that are versatile enough to remain pedagogically relevant.

Major approaches to combat this nontrivial issue include integration of elements of "discovery" or problem solving, student driven inquiry methods, and experimental unknowns. Modern analytical techniques notably lacking in traditional educational experiments including IR spectroscopy, GC, and NMR spectroscopy are employed in order to directly assess student performance and comprehension. A strict commitment to increased experimental variability and unknown elements has been employed in the development of each experiment.

Increased individual assessment and the possibility of multiple experimental outcomes require students to more carefully plan and conduct experiments and analyze their own data rather than copy from an old report. Careful experimental design is required in order to adhere to the 3-hour lab period employed by most universities. Addressing this issue, the present work makes use of time saving microwave chemistry and PicoSpin™ bench-top NMRs.

Several educational experiments will be highlighted that have been researched, developed, and implemented in the University of Georgia sophomore organic chemistry laboratory program. A newly developed synthetic method for the decarboxylation of amino acids has been integrated into an educational experiment featuring use of both microwave chemistry and the PicoSpin™ NMRs. A novel method for stereoselective "tuning" allows instructors to vary student outcomes of a stereoselective Luche reduction. A new discovery experiment emphasizing critical thinking skills has been developed wherein students combine their experimental data to discover a trend in epoxide ring-opening reactions. Additionally, a microwave Fischer esterification protocol has also been outfitted for inclusion of PicoSpin™ NMR analysis. This work constitutes a significant contribution to the organic chemistry educational literature.

INDEX WORDS: Sophomore organic chemistry, Chemical education, Microwave chemistry, Decarboxylation of amino acids, Luche reduction, Stereoselective tuning, Discovery experiments, Unknowns-based experiments, Epoxide opening, Fischer esterification, Bench-top NMR

NOVEL APPLICATIONS OF MICROWAVE CHEMISTRY AND MODERN ANALYTICAL METHODS TOWARD THE DEVELOPMENT OF VERSATILE UNKNOWNS-BASED EXPERIMENTS IN THE SOPHOMORE ORGANIC CHEMISTRY LABORATORY

by

DOUGLAS MICHAEL JACKSON

B.S. CHEM, The University of Georgia, 2007

M.S., The University of Georgia, 2007

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2014

NOVEL APPLICATIONS OF MICROWAVE CHEMISTRY AND MODERN ANALYTICAL METHODS TOWARD THE DEVELOPMENT OF VERSATILE UNKNOWNS-BASED EXPERIMENTS IN THE SOPHOMORE ORGANIC CHEMISTRY LABORATORY

by

DOUGLAS MICHAEL JACKSON

Major Professor: Richard Morrison

Committee: Jason Locklin
Norbert Pienta

Electronic Version Approved:

Julie Coffield
Interim Dean of the Graduate School
The University of Georgia
December 2014

DEDICATION

To my mema Mary Lois Jackson who always drove me to school.

ACKNOWLEDGEMENTS

I would like to acknowledge my family, friends, mentors, and coworkers for their help and support. I appreciate the support of my wife Renée for her love and patience with me through this process. Among family members, specifically I would like to thank my mema, papa, and dad for providing stability and freedom in my early education and my aunt Pete for always encouraging me to finish. Among friends, I would like to thank Kyle Sears, Zach Evans, and Tom Irvin in particular for support and necessary distractions through the grind. Many thanks to my major professor Dr. Richard Morrison for his love of discovery and refreshingly positive attitude; thanks to Dr. Nigel Adams my first research advisor for the leadership and input in my early undergraduate and graduate career. My coworkers Rupa Gokal and Kasey Darley have shown profound kindness in assisting with the completion of this work and are gratefully acknowledged. I would also like to thank Dr. Richard Hubbard for his assistance in implementing this work into the main organic chemistry sequence. Finally of great importance, I would like to thank the undergraduate researchers who helped make these methods work without (much) complaint in the face of many failures. In order of their time spent with us, many thanks to Daniel Morrison, Hayden Evans, Jessica Fazio, Katie Davis, Callan Brownfield, Robby Ashley, and Joey Sharp.

TABLE OF CONTENTS

Page

LIST OF ABBREVIATIONS

AADC	L-aromatic amino acid decarboxylase
Ala	alanine
Arg	arginine
Asn	asparagine
Asp	aspartic acid
BBB	blood brain barrier
^{13}C NMR	carbon-13 NMR
Cys	cysteine
GC	gas chromatography
D_2O	deuterium oxide
DMSO-d_6	deuterated dimethyl sulfoxide
FT	Fourrier transform
GC/MS	gas chromatography/mass spectrometry
Glu	glutamic acid
Gln	glutamine
Gly	glycine
His	histamine
HDC	histidine decarboxylase
H_n	a histamine receptor
^{1}H NMR	proton NMR

HP	Hewlett Packard
HPLC	high performance liquid chromatography
Ile	isoleucine
IR	infrared
IP	internet protocol
KO	“knockout”
L-DOPA	L-3,4-dihydroxyphenylalanine
Leu	leucine
Lys	lysine
MAC	media access control
Met	methionine
MHz	megahertz
MW	microwave
NMR	nuclear magnetic resonance
Phe	phenylalanine
Pro	proline
Ser	serine
Thr	threonine
TLC	thin layer chromatography
Trp	tryptophan
Tyr	tyrosine
Val	valine
v/v	volume to volume

CHAPTER 1

INTRODUCTION

Developing laboratory experiments that effectively engage, instruct, and monitor student performance is a critical challenge for organic chemistry laboratory coordinators.[1] The limitations of "cookbook" reactions have been well discussed in the literature[1b, 2] and include but are certainly not limited to: "foolproof recipes", limited engagement, and lack of problem solving. Not only can these types of experiments be accomplished with limited student engagement, but they also provide the instructor with unreliable assessments of student learning given the outcomes are already known by virtually every student. Consequently it is difficult to guide students through meaningful instructional exercises using traditional laboratory experiments.

Several major approaches in the chemical education literature have been described to address this nontrivial issue for the sophomore organic chemistry instructional laboratory. One central requirement is the inclusion of modern analytical techniques such as GC,[3] GC/MS,[4] IR spectroscopy,[5] and NMR spectroscopy[5e, 6] in the place of chemical or "eyeball" tests. The use of IR and ^{1}H NMR are particularly useful for rapidly acquiring information of molecular structure within instructional laboratory time constraints. Additionally, a movement to "discovery"[1b, 6k, 7] or puzzle-based experiments has become increasingly significant in recent years. "Discovery" experiments provide students with procedural

information and sufficient background yet allow students to discover the answer to a puzzle proposed by the instructor or to discover some chemical trend or topic before its presentation in lecture. Alternatively "open" or "guided" inquiry[6k, 8] experiments require students themselves to design all or part of the experiment.

All of these approaches have their merits, and components of each should be incorporated into any complete curriculum; however, in order to ensure the highest educational value, the ability of students to cheat on lab preparation and assessments must be actively discouraged through experimental design. While student access to the "fraternity file" of old lab reports has always been a problem at large universities, the advancement of both the speed and breadth of communications technology and internet resources readily provides any student with expected results and multiple student reports on any traditional experiment regularly performed as part of an instructional curriculum. Research[9] and personal experience both confirm that a significant percentage of students are willing to falsify data to get the "right" answer obviating the need for students to draw conclusions from their own experimental data. The ease of data fabrication is only encouraged by experimental protocols persisting in many university curricula[10] that rely upon product analysis via unverifiable chemical tests or melting point identification;[1a] however, after just one set of graded reports is returned the correct analysis of even an NMR spectrum may be uploaded to the cloud for all to see. Moreover, after just one semester a tenable procedure for an "inquiry" lab or the puzzle element of a "discovery" lab will be freely available for any student who cares to probe. It should also be noted that experimental procedures and expected

outcomes for an experiment performed at any given university can be subsequently found on the Internet.

In this light, experiments must be continually modified for the undergraduate laboratory and must be versatile enough to combat the problem of the fraternity file. These experiments should be in keeping with the other reform movements and incorporate modern technology along with elements of discovery and/or inquiry; additionally, the inclusion of experimental variations or experimental "unknowns" is an essential component necessary to ensure the integrity of student results from one lab period to the next as well as from semester to semester. In an unknowns-based approach, student experiments are varied by at least one "unknown" element. Each unknown yields a unique result; thereby, requiring each student to obtain and analyze his or her own data. The use of modern analytical methods is necessary to verify students' abilities to perform the reaction, workup, and purification on-site before students have the chance to alter data. Student engagement is fostered by the elements of discovery and inquiry, and the continued efficacy of these experiments is ensured by the unknown element regardless of whether the experimental protocol is cookbook, discovery, or inquiry in nature.

A quick look at the educational literature shows that an unknowns approach is not unprecedented for organic chemistry experiments. For example a common approach in the organic chemistry educational literature is to identify an unknown via analytical spectroscopy. This approach, however, does not provide the opportunity for students to investigate chemical reactivity.[11] The primary reason for the study of organic chemistry is the exploration of various reaction

transformations, yet unknowns-based experiments where the students identify their own reaction products[12] are less commonly reported.

For those experiments that do incorporate unknowns into the reaction procedure, a common technique is to vary the primary reactant thereby changing the product. This approach is not universally possible to implement for the following reasons: different organic reagents often have easily differentiable physical properties (solid vs. liquid or the characteristic vanilla smell of vanillin, etc); they may have differing safety concerns that should be known to the student; they often require significantly different reaction conditions. The combination of these factors can undermine the generality of a planned experiment and inhibit cohesive implementation. In order to incorporate unknowns-based laboratory experiments, the benefits of "cookbook" experiments such as high yields and economical efficiency must be expanded to entire classes of reactions, ensuring that a procedure that works for one unknown also works for many analogous unknown elements (an exception being perhaps a discovery/inquiry experiment of a poor yielding process to teach yield optimization). Furthermore, if spectroscopy is to be efficaciously included, new experiments must be time efficient given the limitations of the common 3-hour lab period adopted by most universities.

The present work utilizes several strategies for the implementation of novel unknowns-based experiments in the sophomore organic undergraduate laboratory curriculum. These experiments have been designed to accomplish one or more of the following:

1. to fill a gap in the literature for a particular class of reaction;

2. to incorporate unknowns or an element of discovery to an existing or similar experiment;
3. to increase the versatility and thus longevity of the educational impact of a novel or existing experiment combatting the problem of the "fraternity file";
4. to incorporate an element of spectroscopy in an experiment not previously described or available in large laboratory programs.

A list of newly developed and implemented experiments may be viewed in Table 1 with unknown elements and integrated analytical techniques highlighted. Unknown elements invoked by these experiments include the reagents used, catalyst concentration, acidic vs. basic conditions, reaction solvent, or analyte concentration. Two of these experiments are made possible via the use of microwave chemistry, a valuable tool for making the reaction occur more quickly and more uniformly within the constraints of the 3-hour lab period. Three experiments make use of NMR spectroscopy including the incorporation of new economical PicoSpin™ bench-top 45 MHz instruments. Others incorporate the use of GC or polarimetry to determine the variable outcome of each experiment. Learning outcomes include the introduction of synthetic and analytical techniques, reaction breadth, key lecture concepts such as stereochemistry, and critical thinking skills such as data organization and trend recognition.

A Note on Microwave Synthesis in the Sophomore Organic Laboratory

Since its first uses in the mid 1980s, microwave chemistry has become commonplace in organic synthesis[13] for the acceleration of chemical reactions.

Table 1. Novel unknowns-based sophomore organic chemistry experiments

Experiment	Unknown Element	Analytical Techniques	Description
Microwave Amino Acid Decarboxylation	Reactant	PicoSpin™ ^{1}H NMR	Students identify product amine hydrochloride salts via NMR analysis
Stereoselective Tuning of the Luche Reduction of Menthone	Catalyst Concentration	TLC IR GC Polarimetry ^{1}H NMR	Students determine stereochemical outcome of a reduction procedure
Acidic and Basic Epoxide Ring Opening	Reagent/ Solvent	GC IR	Students determine a trend in regioselectivity by pooling class data.
PicoSpin™ Microwave Fischer Esterification	Reactant	PicoSpin™ ^{1}H NMR IR	Students identify product esters given unknown alcohol and/or acid reagents

Reviews of the use of microwave-assisted procedures have been published in many areas with recent research focusing on the scale-up of the procedures to industrial scale[14]. Recent work[15] concludes that the specific effects of microwave heating are the ability to carefully control reactions under solvent superheated conditions, selective heating of strongly microwave absorbing species in a microwave transparent medium, the formation of microscopic molecular "hotspots" within the bulk medium, and the ability to heat from the "inside out" eliminating the need to wait for thermal equilibrium to be reached with the surroundings. The so-called "nonthermal" or photochemical effect arising from the direct absorption of the radiation has been largely dismissed in recent work.[15]

With the growing popularity of microwave chemistry several commercial instruments have become available making the technique safe and effective for use in the instructional laboratory. Modern instruments allow for the precise control of temperature over time via user-specified programs. Educational instruments come in two varieties, so-called "mono" and "multi" mode instruments. The mono-mode instruments run a single reaction that is monitored for temperature control; whereas, multi-mode instruments run several vessels (as few as 2 to more than 20) simultaneously. Multi-mode instruments are highly preferable for running student reactions in an instructional lab, but the method of temperature control is important when running a library of non-identical reactions. Some commercially available multi-mode instruments monitor a single reaction vessel via a fiber optic temperature probe. While this method most accurately measures the internal temperature through immersion, hot and cold spots in other vessels not directly monitored have been reported[16] in the use of these instruments yielding inconsistent reaction completion times among identical student reactions. The Milestone START™ system (Figure 1) used in this work employs a fixed infrared eye located on the wall of the oven that monitors all vessels in the rotor as they pass. As seen in Figure 2, the instrument attempts to reach and maintain the average temperature (bold red line) on the set temperature time line (T1 faded red line) maintaining all vessels within a certain deviation from the mean. A black line represents the wattage of the instrument in real time controlled by the temperature feedback mechanism. No issues with hot and cold spots have been encountered using the START™ system.

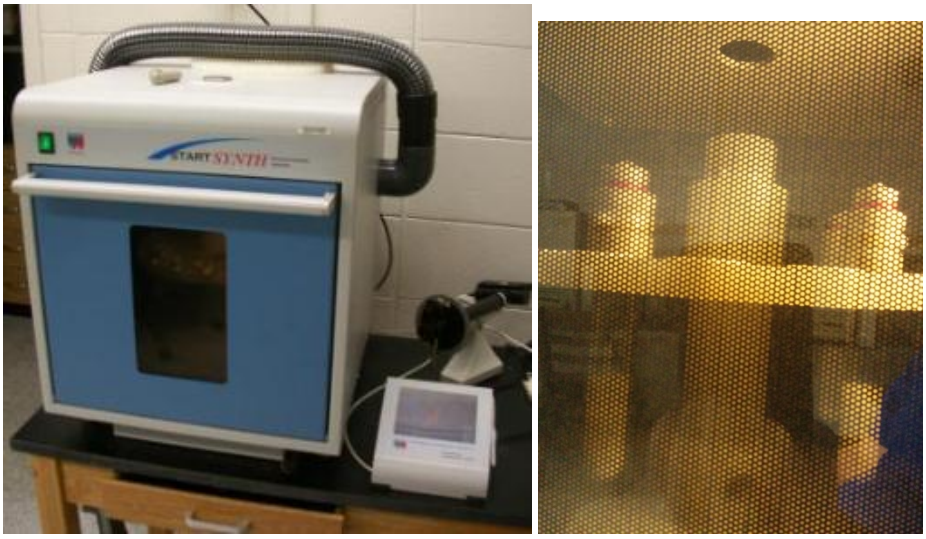


Figure 1. The Milestone START™ multi-mode microwave synthesizer

Figure 2. Control screen of the START™ synthesizer

Two major pedagogical advantages make the use of microwave reflux a valuable tool in the educational laboratory. Microwave reflux gives instructors the ability to incorporate reactions into the curriculum that would otherwise be

inaccessible due to time constraints. Perhaps more importantly, microwave reflux gives the ability to more expediently accomplish reactions so that the majority of lab time may be devoted to product analysis. Many recent examples in the literature emphasize these advantages.[7c, 13d, 16-17] The present work makes use of both of these advantages.

PicoSpin™ 45 MHz ^{1}H NMR Spectrometers in the Undergraduate Laboratory

In the past the major limiting factors for the use of ^{1}H NMR analysis in the instructional laboratory have been expense and time considerations. The difficulty of inserting spectral analyses into the 3-hour lab period has been aided by the amenability of many reactions to microwave reflux; however, laboratory coordinators of large enrollment courses are unable to run hundreds of ^{1}H NMR samples using departmental instruments that are devoted mainly to research. Also, placing traditional low field permanent magnet instruments in every instructional laboratory has been prohibitively expensive.

The present work illustrates the use of 45 MHz PicoSpin™ ^{1}H NMR (Figure 3) analysis as a method of placing high throughput bench-top instruments in every laboratory section. The instruments pictured in Figure 3 are lunchbox-sized due to the reduction in magnet size enabled by patented microsampling technology. Student samples are injected through an HPLC type capillary tube that runs through the bore of the miniature magnet (Figure 4). The uniformity of the magnetic field is improved by this small sample region in addition to control of the Celsius temperature to the fourth decimal place. The instrument is operated via Firefox® browser control so any computer need only an internet connection, the IP address,

and MAC address of the instrument in order to operate the pulse program of the PicoSpin™.

Figure 3. PicoSpin™ 45 MHz NMR Spectrometer

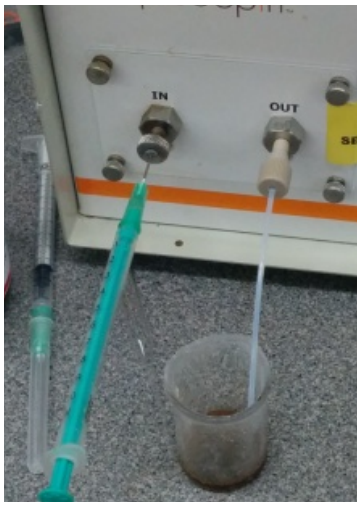


Figure 4. Syringe injection into capillary sampling region

The instrument itself can be placed anywhere with a wired Internet connection or be adapted with a wireless adapter. The syringe injection port makes changing

between student samples simple. A flush with deuterated solvent, followed by injection pushes the sample into the capillary region. The pulse program is readily initiated, and the data are saved to the instrument memory. A quick flush of clean solvent removes the sample into the waste catcher, and the next sample may be run. Total run times are less than 2 minutes for samples of sufficient concentration. For non-viscous liquids, samples may be run neat for maximum concentration. PicoSpin™ instruments do not require a dedicated NMR technician, high voltage connection, or compressed air supply making them ultraportable and ultra convenient for any instructional laboratory.

Summary

In summary, this dissertation highlights several novel experiments that have been researched, developed, and implemented in the University of Georgia sophomore organic chemistry instructional laboratory program. The methods and experiments described here incorporate modern analytical techniques, foster engagement through elements of discovery, and discourage cheating via the unknown elements of the experiments and versatility in implementation. Newly developed synthetic methods such as the "Microwave Amino Acid Decarboxylation"[18] and the "Stereoselective Tuning of the Luche Reduction" are discussed in separate chapters from their respective educational laboratory experiments to highlight their broader methodological impact. The educational experiments "Acidic and Basic Epoxide Ring Opening" and the implementation of PicoSpin™ NMR analysis to the "Microwave Fischer Esterification" are also addressed in detail in separate chapters. Supporting information for each chapter

including additional spectra and student handouts accompanying each experiment are included for reference in the Appendices. In total, a significant contribution is made to the educational literature describing versatile methods for the effective teaching of aspects of sophomore organic chemistry laboratory techniques and principles of chemical reactivity.

CHAPTER 2

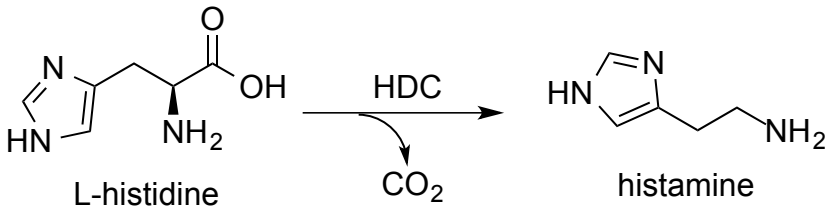
RAPID CONVENTIONAL AND MICROWAVE-ASSISTED DECARBOXYLATION OF L-HISTIDINE AND OTHER AMINO ACIDS VIA ORGANOCATALYSIS WITH R-CARVONE UNDER SUPERHEATED CONDITIONS

The decarboxylation of amino acids is an important synthetic route to biogenic amines. Traditional methods of amino acid decarboxylation are time consuming, taking up to several days in the case of L-histidine, narrow in scope, and make use of toxic catalysts. This research reports a new methodology (US20140275569 A1 patent pending) taking advantage of superheated chemistry using either microwave or conventional heating for the facile decarboxylation of many amino acids. Decarboxylations of amino acids including L-histidine occur in just minutes using the food additive R-carvone as a recoverable organocatalyst. Yields are comparable to previous methods, and purification of product ammonium chloride salts is aided by an isomerization reaction of residual catalyst to phenolic carvacrol. The method has also shown to be effective for the decarboxylations of synthetic and protected amino acids.

Importance of Biogenic Amines

Biogenic amines are ubiquitous in the body regulating a variety of functions ranging from neurotransmission to cellular signaling to various physiological responses.[19] Many of these amines are derived from their respective α-amino acids via a variety of decarboxylase enzymes. For example as shown in Scheme 1,

histamine is formed from the enzymatic decarboxylation of L-histidine by histidine decarboxylase (HDC).[20]



Scheme 1. Enzymatic decarboxylation of L-histidine to histamine

L-aromatic amino acid decarboxylase (AADC)[21], originally called L-DOPA decarboxylase (DDC) upon its discovery in 1939[22], catalyzes the conversion of L-DOPA, tryptophan, tyrosine, phenylalanine, and other aromatic amino acids to their respective amines. An overview of important biogenic amines (structures shown in Figure 5) derived from decarboxylase-catalyzed processes is shown in Table 2.

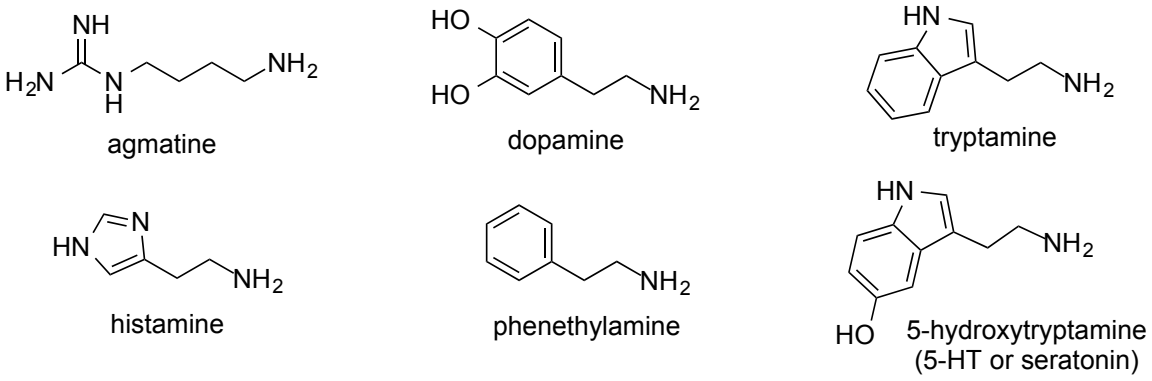


Figure 5. The structures of some important biogenic amines derived directly from enzymatic decarboxylation of amino acid precursors.

Table 2. Some Important Biogenic Amines, Their Amino Acid Precursor, Decarboxylase Enzyme, and Biological Function

Biogenic Amine	Amino Acid Precursor	Decarboxylase Enzyme	Biological Function
agmatine[23]	arginine	arginine decarboxylase	mediator in synthesis of polyamines, possible neurotransmitter
dopamine[21-22]	L-DOPA	AADC	neurotransmitter
histamine[24]	histidine	HDC	allergic response, immune response, gastric acid secretion, neurotransmitter
phenethylamine[21b, 25]	phenylalanine	AADC	neurotransmitter
seratonin[21b, 26]	5-hydroxytryptophan	AADC	neurotransmitter
tryptamine[21b]	tryptophan	AADC	neurotransmitter
tyramine[21b, 27]	tyrosine	AADC	neurotransmitter, vasoconstrictor

Given the physiological sensitivity to biogenic amines, the ability to synthesize these compounds or unnatural derivatives for pharmaceutical purposes is attractive. In fact therapeutic uses for several biogenic amines have been reported including the use of increased dopamine levels to treat Parkinson's disease[28]. The dopamine therapy is delivered as L-DOPA in order to successfully permeate the blood-brain

barrier (BBB); however, new developments in the area of drug delivery[29], may allow for drug targets once cast aside due to poor BBB permeability such as biogenic amine or amino acid derivatives to be reevaluated. Amines that are active in other areas of the body such as histamine may be delivered directly in amine form.[30]

Strategies for laboratory synthesis of biogenic amines generally mimic nature and invoke the decarboxylation of amino acids obtained from the chiral pool. Early methods for the decarboxylation of amino acids rely on either fermentation with a decarboxylase enzyme[31] or via high temperature reflux with an appropriate small molecule catalyst.[32] These methods are plagued with lack of generality, long reaction times, high boiling solvents, toxic catalysts and complicated workup procedures. An improved decarboxylation procedure is desirable for synthesis of pharmaceutically pure amines.

Importance of Histamine

Of the biogenic amines, histamine is one of the most widely dispersed in the body and is known for its importance in the regulation of many bodily processes.[33] The first study[34] of histamine function in the bodies of several animals was published in 1910 noting significant effects to respiration, cardiac function, and muscle contraction. Over the years it has been determined that histamine's action is caused by interaction with four G-protein-coupled receptors. The H_1 receptor has been shown to regulate the acute inflammatory response[24b] associated with allergic reactions. The H_2 receptor regulates gastric acid receptors[24a] and may be inhibited in the treatment of acid reflux disease. The H_3 receptor inhibits histamine release in the neurons,[24c] and the other potential roles for these receptors and the role of the

H_4 receptor represent an area of intense research[24d-f]. In fact Scifinder Scholar™ search for "histamine receptors" accounts for 435 total hits from 2013-2014 and 1256 since 2010.

Evidence is mounting that in certain situations histamine may be used as a pharmaceutical agent. Study of L-histidine decarboxylase knockout (HDC KO) mice has given much insight into the effects of histamine in the body.[33, 35] These mice lack the ability to produce histamine since their HDC encoding gene has been "knocked out" or removed during the mouse cloning process. Studies involving both HDC KO mice and H_4 receptor KO mice show that deficiencies in natural killer T cell production were restored by restoration of histamine to normal levels.[36] Moreover, wound healing rates in HDC KO mice were not only restored to normal levels after administration of therapeutic histamine but could even be accelerated if additional doses of histamine were administered.[30] Other pharmaceutical uses of histamine related to the activation of dendritic cells via the H_4 receptor and subsequent immune response[37] have been reported as well as uses involving the activation or deactivation of the other histamine receptors[38] making it clear that an efficient production of high purity histamine is immediately important.

Review of Current Decarboxylation Procedures

Current methods for histamine production rely on the decarboxylation of L-histidine via either fermentation with L-histamine decarboxylase enzyme or via high temperature reflux with a small molecule ketone catalyst. Enzymatic decarboxylation of amino acids occurs in many organisms and has long been reported as a synthetic option for the decarboxylation of the amino acid L-histidine.

In the first high yielding synthesis of histamine, Ackerman[31a] fermented L-histidine with *B. coli* putrefaction organisms whose decarboxylase enzyme effected the conversion. Histamine was isolated from the reaction mixture as its dipicrate salt. Galat and Friedman[31b] published an improved method of isolation as the disulfonate salt with o-dichloro-benzenesulfonic acid requiring much less solvent and effecting more facile conversion to other forms such as the dihydrochloride salt and freebase.

Methods for decarboxylation of histidine via reflux with a ketone catalyst have also been reported eliminating the need to prepare cultures of decarboxylating organisms. In 1957 Dose reported the decarboxylation of several amino acids via reflux in nitrobenzene in the presence of 4-(dimethylamino)benzaldehyde reporting evolution of CO_2 from the histidine reaction mixture from 195-205 ^{o}C. Isolation of the amines was not achieved from these mixtures, and amine content was inferred via photometric comparison to standards.

In 1986 Hashimoto et al. reported a procedure for the decarboxylation of amino acids including histidine by refluxing the amino acid in cyclohexanol (b.p. 160 ^{o}C) in the presence of 0.1-1 mol % cyclohex-2-en-1-one. Decarboxylation of histidine is reported to require 26 hours with 95% yield of the histamine dihydrochloride salt. The authors noted that increased catalytic load contributed to decreased yield of histamine. Efforts to reproduce the Hashimoto procedure have failed due to lack of conversion at low mol % of catalyst and the presence of overwhelming impurities from competing processes at higher catalyst load (up to 2 mol equivalents).[39]

In a modification of the Hashimoto procedure, Yeh et al. (2002)[39] have described a similar decarboxylation method using 20 mol % acetophenone in cyclohexanol as catalyst. Optimized conditions accomplish the decarboxylation in 54 hours and yield histamine dihydrochloride salt at 94% purity. In all variations 2 major unidentified impurities required a multiday purification process including suspensions in various solvents followed by 3-5 recrystallizations and washes of the filter cake to improve the purity to pharmaceutical grade (<2% impurity).

In the last 6 years two processes for the removal of product free amines by distillation from a high boiling solvent such as polyethylene glycol have been reported in the patent literature.[40] Both reported the use of 1 equivalent of cyclohex-2-en-1-one as catalyst. These methods assist with the problem of solvent removal for lower boiling amines; however, the reaction times were still quite long (~72 hrs) and the success of difficult decarboxylations such as histidine are unsubstantiated and cannot be presently reproduced despite the broad claims of the authors. Especially for free amines that exhibit high boiling points, an alternative method of isolation is needed to prevent thermal degradation.

Solvent Optimization

Addressing the problem of long reaction times in the decarboxylations of many amino acids such as histidine, it was envisioned that chemistry at temperatures above the reflux temperature of cyclohexanol (~160 ^{o}C) may provide a solution. However, as previously noted, a significant amount of effort must be devoted to the removal of high boiling solvents at the expense of yield and efficiency. One advantage to using cylcohexanol or polyethylene glycol reported by

all previous researchers was the solubility of the amine product and insolubility of amino acids allowing visual determination of reaction completion.

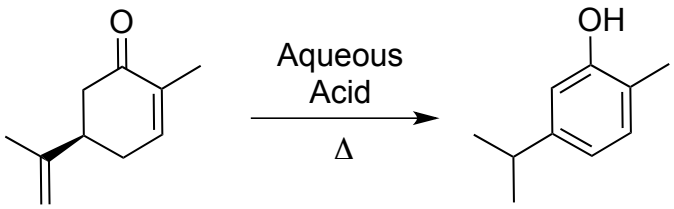
Rather than employing an even higher boiling solvent system, which would involve the same difficulties as previous methods, the possibility of a pressurized reaction system using a solvent with a lower normal boiling point was investigated. Both microwave promoted and hot oil bath systems were employed using a sealed 15 bar maximum pressure reaction vessel. While many solvents satisfy the criterion of greater volatility, the search was narrowed to a series of short chain alcohol solvents in order to promote microwave absorption. Among the short chain alcohol solvents n-butanol, n-pentanol, and isopropanol proved to absorb microwaves insufficiently while ethanol, methanol, and water dissolve the reactant amino acid at the optimum reaction temperatures of > 185 ^{o}C hindering visual determination of reaction completion and failing to facilitate decarboxylation of histidine. n-Propanol was determined to be the optimum solvent for visual inspection of reaction completion that could reach the maximum safe temperature and be easily removed after reaction completion. This solvent achieves a maximum temperature in the instrument (1200W) of 190 ^{o}C (calibrated ±2 ^{o}C) with a vapor pressure of 15 bar as determined by the Clausius-Claperyon equation. It should also be noted that reactions performed neat resulted in poor to no yield and aprotic solvents failed to promote decarboxylation even upon heating to 190^{o}C in an oil bath.

Catalyst Optimization

Another factor affecting the reaction rate and overall ease of purification of the product mixture is the identity and load of the catalyst. Previously 1% v/v of

cyclohex-2-en-1-one has been reported for histidine decarboxylation.[41] Significant amounts of impurities were observed in the resulting reaction mixture in attempts to reproduce the experiments. Alternatively, it was reported that when acetophenone was used at 20 mol % for decarboxylation of histidine, modest success was observed after >54 hrs. In catalyst optimization experiments, cyclohex-2-en-1-one proved to provide a greater catalytic effect at 20 mol % than acetophenone. It was postulated that the enone functionality of cyclohex-2-en-1-one provides some advantage over the benzyl ketone.

It was envisioned that the enone R-carvone, the natural product spearmint oil, may retain the catalytic advantage over acetophenone while providing an alternative method of removal of the catalyst based on the known isomerization reaction to phenolic carvacrol[42] (Scheme 2).



Scheme 2. Isomerization of R-carvone to phenolic carvacrol.

R-carvone provides the added advantage of replacing cyclohex-2-en-1-one, a relatively expensive catalyst having acute human toxicity.[43] It was observed during preliminary experiments that the rate of reaction significantly increased at higher catalyst load than the 1 mol % load previously reported by Hashimoto. As a general rule, the catalytic effect becomes appreciable at about 0.1 mole equivalents for both

cyclohex-2-en-1-one and R-carvone and peaks at about 2 mole equivalents for the amino acids decarboxylation in this study. The reaction times in minutes of a series of microwave-assisted decarboxylations of phenylalanine in n-propanol at 190 °C with varying load of R-carvone are given in Figure 6.

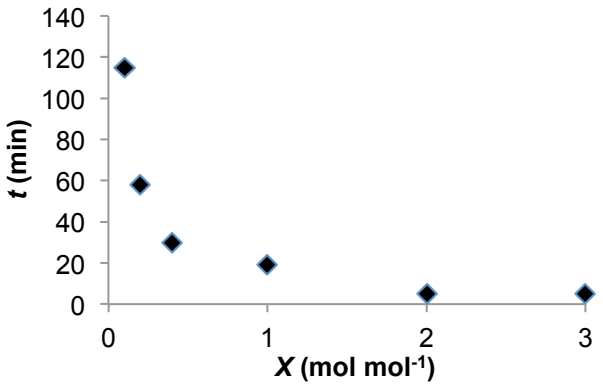


Figure 6. Decarboxylation times of phenylalanine with variable R-carvone load.

Decarboxylations of a series of amino acids were performed at the 2 equivalent catalyst load comparing the catalytic abilities of cyclohex-2-en-1-one, acetophenone, both carvone enantiomers, and the simple ketone acetone under solvent optimized conditions. Five minutes were allowed for the microwave to achieve maximum reaction temperature of 195 °C. Additional reflux time at the maximum temperature was allowed if necessary. Reaction completion was determined by the transformation of amino acid slurry to clear solution as shown in Figure 7. A summary of catalyst optimization experiments may be viewed in Table 3.

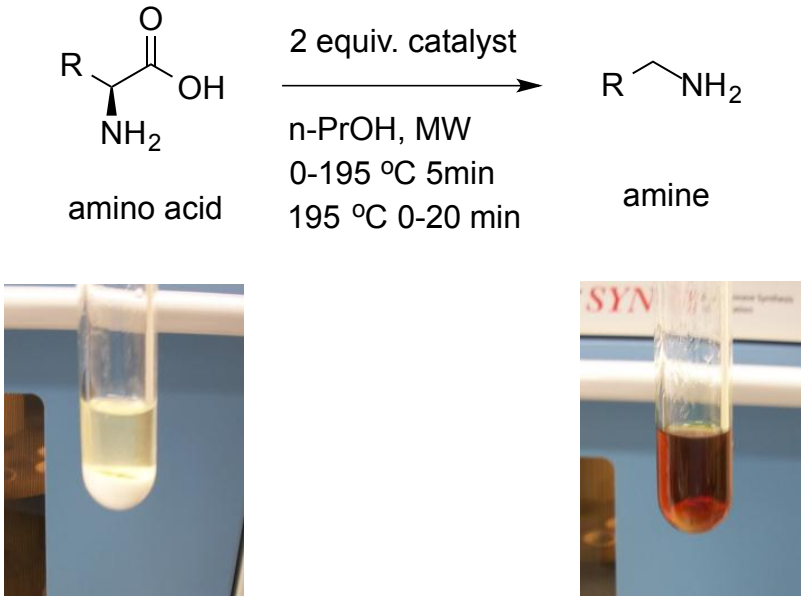
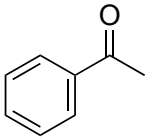
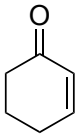
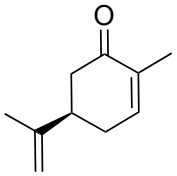
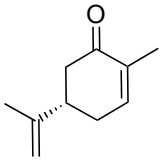


Figure 6. Generic decarboxylation for determination of catalytic effect.

Table 3. Summary of Catalyst Optimization. Reaction Times Given in Minutes

Amino Acid			R-carvone 	S-carvone 	
Phe	5	5	5	7	5
His	25	25	25	50	[a]
Trp	5	5	5	5	7
Tyr	10	6	20	16	40

[a] completed in ~72 hrs in an oil bath with decomposition of products

In these reactions R-carvone showed similar reactivity to previous catalysts. Decarboxylations with S-carvone as catalyst were also effective though decarboxylation of L-histamine required 50 min reflux as opposed to 25 min. Given these results, decarboxylations of D-amino acids with R-carvone as catalyst may also be performed as desired. With catalytic effect confirmed it remained to be seen whether the decarboxylations occur cleanly with minimal byproducts. Isolation of products using the other catalysts under the higher temperature conditions and catalyst load proved impossible due to decomposition of products.

Catalyst Removal and Purification

Given that the reaction is thought to occur through an imine intermediate[32a, 32c] and the observed rise in impurities as a result of increasing the catalyst load following the Hashimoto procedure, it became apparent that the fate of the decarboxylated imine should be investigated. GC-MS analysis of the product mixture after the decarboxylation of phenylalanine shows all decarboxylated imine, no free amine, and minimal volatile impurities. The reaction mixture was then diluted with aqueous hydrochloric acid, excess R-carvone was removed via ether wash, and then the free-based product was partitioned to the organic phase via aqueous sodium hydroxide wash. A significant degree of hydrolysis was expected, however, in GC/MS analysis of the organic extract the imine of the decarboxylated product is quite stable and persists as shown by the chromatogram and mass spectrum in Figure 7.

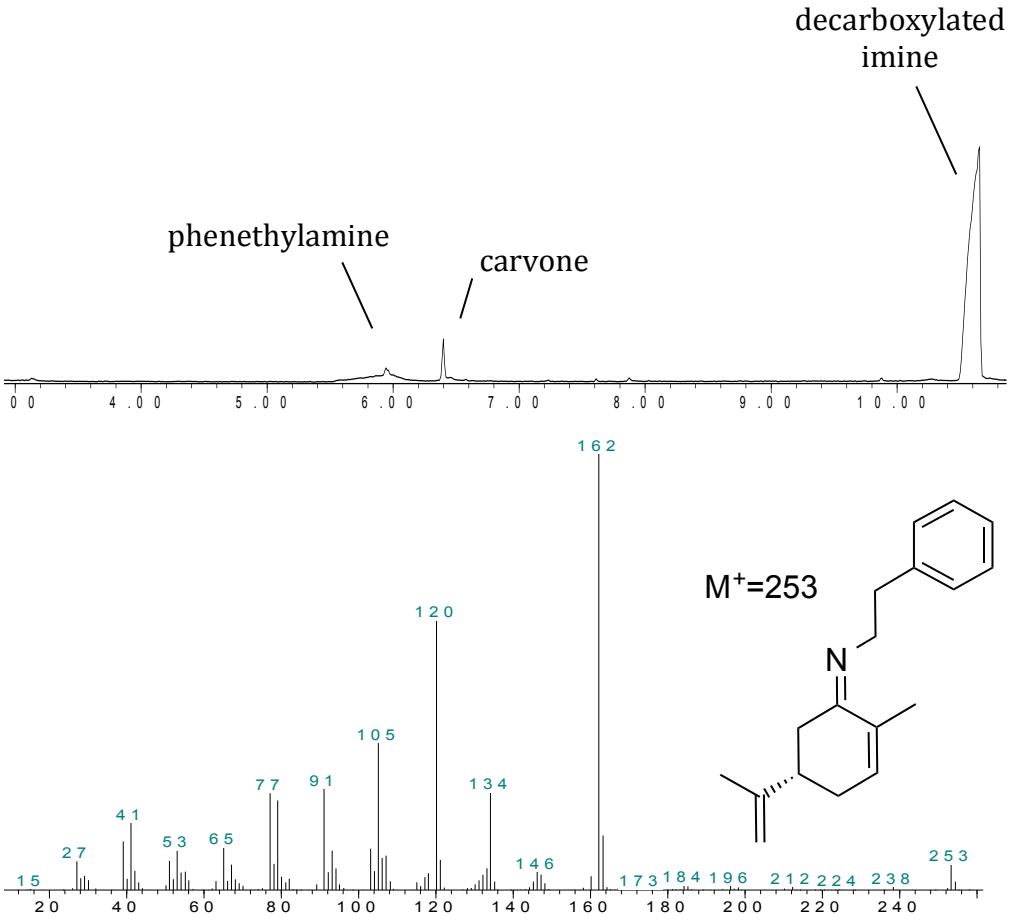


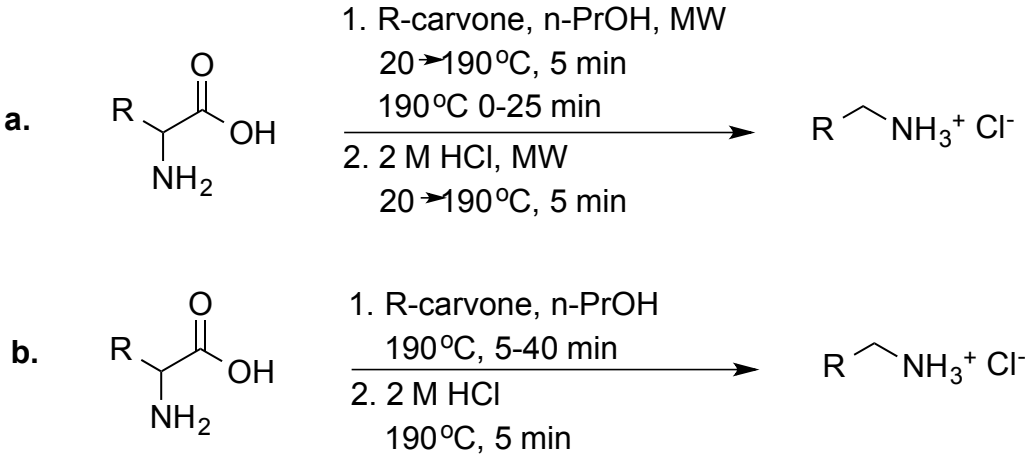
Figure 7. GC/MS analysis of product mixture after acidic workup followed by free-basing

It was observed that the desired imine hydrolysis occurs readily only after heating in aqueous acid at >50 °C. Even after treatment of the reaction mixture with many times the reaction volume of 2.0 M HCl, it proved difficult to adequately remove all traces of the imine at system equilibrium. However, a 5 min reflux at 190 °C in 2 M HCl accomplished both hydrolysis of the imine and isomerization of R-carvone to carvacrol. Carvacrol is then easily removed via ether extraction. It should be noted that gentle reflux at 80 °C allows the imine to hydrolyze in

equilibrium and ~80% of R-carvone may be recovered via three sequential refluxes and extractions with ether or via soxhlet extraction with warm toluene. If R-carvone is recovered via the three quick extractions, a final high temperature reflux should be performed to isomerize residual R-carvone to carvacrol. All methods of amino acid decarboxylation in the literature fails to account for the quantity and reactivity of imine that may remain thus lowering the yield and purity of the crude product and necessitating further purification. Product amines are isolated as hydrochloride salts by rotary evaporation of water under reduced pressure and further drying overnight in a vacuum oven.

Results and Scope

A summary of both microwave and conventional procedures is highlighted in Scheme 3.



Scheme 3. Summary of microwave (**a**) and conventional heating (**b**) one-pot decarboxylation procedures

The reaction times for the decarboxylation of selected amino acids of interest are reported in Table 4 for both microwave and conventional heating. Isolated yields of the amine hydrochloride or dihydrochloride salts are also given in Table 4 for the optimized reaction conditions highlighted in reaction Scheme 3. Decarboxylation using this procedure was ineffective for the natural amino acids Arg, Asp, L-DOPA, Glu, Ser, Asn, Gln, Cys, and Met.

Table 4. Optimized decarboxylations of natural amino acids

Amino Acid	MW	Reaction Time (min)[a]	% Yield	Oil Bath	Reaction Time (min)	% Yield
Ala		5	60		38	74
Gly		13	86		40	59
His		25	87		12	92
Ile		9	69		12	76
Leu		5	72		5	69
Lys		12	73		17	93
Phe		5	76		5	78
Pro		5	80		5	48
Thr[b]		5	59		12	41
Trp		20	53		9	72
Tyr		20	53		40	67
Val		5	79		9	55

[a] reaction times represent total programmed time including 5 min temperature initial heating period
[b] requires 80 ^{o}C hydrolysis with soxhlet extraction

The slight differences in the overall reaction times reported between MW heating and oil bath heating are the result of the necessary differences in experimental protocols. In the microwave reactor initial heating occurs over a 5 min period and temperature is computer controlled by an infrared thermometer in a continuous feedback system to ±2 °C. Conventional heating was performed in a preheated bath with observed temperature oscillations of no more than ±5 °C.

Figure 8 shows a representative ^{1}H NMR for the product amine hydrochloride salts in D_2O. The solvent peak arising from acidic proton exchange was suppressed. Note that no organic impurities are observed in ^{1}H NMR of the hydrochloride salts using DMSO-d_6 as solvent.

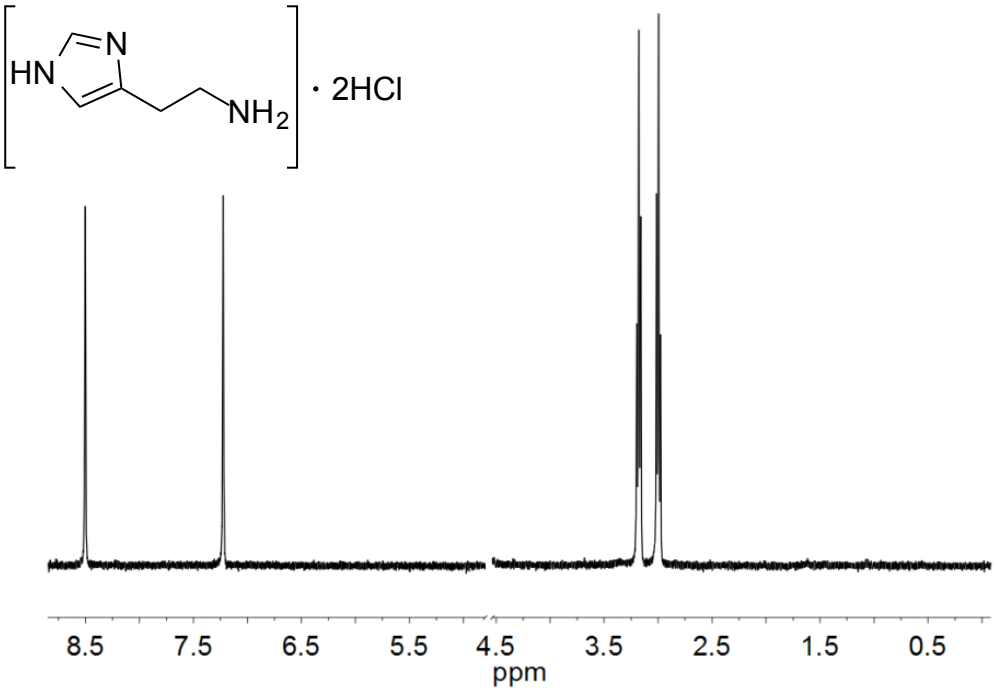


Figure 8. ^{1}H NMR of histamine dihydrochloride with solvent D_2O suppressed

Further decarboxylations were investigated with unnatural amino acids and protected amino acids. Results are summarized in Table 5. Note that the procedure allows for the simultaneous decarboxylation and deprotection of the protected amino acids investigated. Decarboxylated imines with protecting groups are present in GC/MS chromatograms after the initial step.

Table 5. MW decarboxylations of selected unnatural amino acids

Amino Acid	Reaction Time (min)[a]	% Yield
4-amino-Phe	8	99
4-bromo-Phe	5	47
4-methyl-Phe	5	99
4-nitro-Phe	5	74
3,5-dibromo-Tyr	5	41
3-iodo-Tyr	5	77
cycloleucine	16	40
N-trityl-His[a]	5	86
N-formyl-Trp[b]	5	74
O-t-butyl-Tyr[c]	5	81
O-acetyl-Tyr[c]	5	76
O-2,6-diclorobenzyl-Tyr[c]	5	64

[a] isolated product is histamine dihydrochloride
[b] isolated product is tryptamine hydrochloride
[c] isolated product is tyramine hydrochloride

Summary

Decarboxylation of L-histidine and other L-amino acids has been accomplished via organocatalysis with R-carvone and subsequent one-pot hydrolysis under solvent superheated conditions using both conventional heating and microwave irradiation. Decarboxylation is more rapid than previous methods as the vessels are heated to 190 ^{o}C over 5 min. Approximately 80% of the R-carvone catalyst can be recovered via extraction with diethyl ether if the hydrolysis is conducted at 80 ^{o}C; however, to obtain highest purity it is necessary to conduct a high temperature hydrolysis to isomerize residual R-carvone to carvacrol. Isolated yields of amine hydrochloride salts are comparable or improved over previous methods ranging from 60-90% with purity of hydrochloride salts estimated to be >99 % by ^{1}H NMR. This process promises to provide a more versatile and efficient option in the synthesis of biologically active amines from amino acids given the demonstrated versatility with not only natural amino acids but protected and synthetic derivatives as well.

Experimental

5 mmol scale microwave experiments were performed in Milestone 25 mL 15 bar glass pressure reactors inside the Milestone StartSYNTH™ microwave reactor with external infrared temperature control. Conventional heating experiments were performed in silicone oil in identical reaction vessels. Solvents and reagents were purchased from Sigma-Aldrich or Chem-Impex International and used without additional purification. FT NMR experiments were recorded at 400 MHz in D_2O solvent.

General decarboxylation procedure

A magnetic stir bar, 3 mL of n-PrOH, 10 mmol of R-carvone, and 5 mmol of amino acid were charged to a pressure vessel. The vessel was heated from room temperature to 190 ^{o}C over 5 min with stirring. If necessary the reaction vessel was maintained at 190 ^{o}C for additional time until the slurry became clear. The vessel was allowed to cool to below the solvent boiling point, carefully vented to release evolved CO_2, and analyzed via HP 5970 GC/MS to verify the presence of decarboxylated imine. 10 mL of 2M HCl was then added in "one-pot" fashion, and, in all cases but threonine, the reaction vessel was heated to 190 ^{o}C over 5 min with stirring and allowed to cool. In the case of the temperature sensitive threonine, soxhlet extraction with toluene was performed at 80 ^{o}C to remove R-carvone from the reaction mixture. The aqueous reaction mixture was washed three times with 25 mL of ether, and then water solvent was distilled off from the hydrochloride salt. The hydrochloride salt was transferred to a vacuum oven and dried overnight at 150 ^{o}C and 10 Torr. The hydrochloride salt was then weighed and analyzed via NMR. Additional spectra and experimental data may be viewed in Appendix A.

CHAPTER 3

ORGANOCATALYZED MICROWAVE DECARBOXYLATION OF ALPHA AMINO ACIDS: AN UNKNOWNS–BASED SOPHOMORE ORGANIC CHEMISTRY EXPERIMENT WITH PICOSPIN™ NMR ANALYSIS

A sophomore organic chemistry experiment is reported here taking advantage of a new methodology using microwave heating for the facile decarboxylation of many amino acids. Traditional methods of amino acid decarboxylation are unsuitable for the undergraduate laboratory in that they are time consuming, taking up to several days in the case of L-histidine, narrow in scope, and make use of toxic catalysts. Under microwave conditions decarboxylations of amino acids including L-histidine occur in just minutes using the food additive R-carvone as a recoverable organocatalyst. Yields are comparable to previous methods, and purification of product ammonium chloride salts is aided by an isomerization reaction of residual catalyst to phenolic carvacrol. Applications to the sophomore organic educational laboratory have been demonstrated and highlight instructional use of Milestone START™ microwave synthesizers and 45 MHz PicoSpin™ bench-top NMR spectrometers.

Undergraduate Experiment Overview

When developing the improved decarboxylation procedure for α-amino acids (Scheme 3), it became evident that the same features of the process that are attractive for research or industrial synthesis of bioactive amines are attractive for

use in instructional labs. The process replaces the toxic catalyst cyclohex-2-en-1-one with spearmint oil, R-carvone, giving an entry into a discussion of green chemistry. The reaction times are reduced from very prohibitive to at most 20 min not only allowing for the reaction to be performed but also ample time for spectral analysis within the 3-hour lab period. Additionally, the product HCl salts are easily dissolved in D_2O for NMR analysis yielding clear ^1H NMR spectra suitable for students learning introductory NMR analysis.

An experiment, which mimics the ubiquitous processes by which amino acids are decarboxylated in the body to form biogenic amines, is both interesting and pedagogically important for students of organic chemistry. A search of the chemical education literature yields only 2 experiments[44] dealing with the synthetic topic of decarboxylation; one[44a] addressing kinetic isotope effects in the 3rd year organic chemistry course, the other[44b] investigating the decarboxylation of an intermediate involving the synthesis of a triazole compound with no comment on the generality of the process or incorporation of an unknown element.

The present experiment tasks the student to identify the product amine and thus starting unknown amino acid in a microwave decarboxylation procedure. Learning outcomes of the experiment include:

1. the mechanism of decarboxylation catalytic cycle, which addresses equilibrium and imine chemistry
2. acid-base extraction and the isolation of amines as HCl salts
3. Simple ^1H NMR analysis including chemical shift, integration, and multiplicity

Given that the amino acid is unknown, it is essential that the students obtain clean reaction products and properly analyze the ^{1}H NMR as each unknown sample is uniquely labeled for each student.

Detailed Experimental Procedure

Students measured 0.900 grams of assigned unknown amino acid into a microwave vessel. 2.75 grams of (R)-carvone was added to the vessel along with 5 mL of n-propyl alcohol. This catalyst load was selected to ensure that at least 2 equivalents of R-carvone are used regardless of unknown amino acid identity. Only 10 minutes were allowed by the instructor for reaction preparation to encourage efficiency. Note that the scale of the experiment was chosen so that as few as 3 vessels could be run in the multi-mode microwave instrument and still meet the minimum 15 mL microwave absorbent solvent required for proper instrument operation. Experiment scale may be reduced at the discretion of the instructor if more vessels are loaded in the carousel.

The microwave vessels were sealed and loaded into the carousel. The reaction vessels were heated in the microwave oven to 190 ^{O}C over a period of five minutes and held at 190 ^{O}C for an additional 15 minutes with continuous stirring. Reaction completion is determined as the cloudy slurries become clear (Figure 6). This clearing will occur at different times for different amino acids; however, no adverse effect was observed for the faster decarboxylations subject to additional heating time. The instructor allowed time for the vessels to cool below 60 ^{o}C and returned the vessels to the students. Students opened the vessels carefully as the vessels are slightly pressurized and added 10 mL of 2M HCl to the mixture. The

vessels were resealed and returned to the carousel for another reflux from room temperature to 190 ^{0}C over 5 minutes with stirring. During this time the imine formed between the product amines and R-carvone is hydrolyzed and the R-carvone is isomerized to carvacrol as shown in Figure 9.

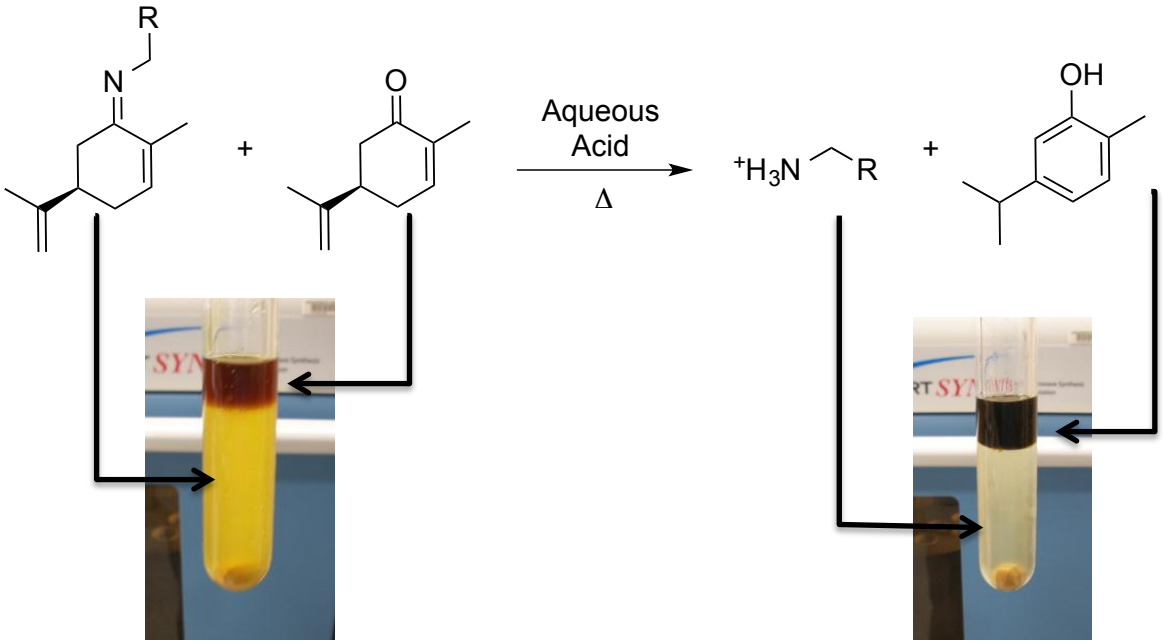


Figure 9. Isomerization reaction of R-carvone to carvacrol

The vessels were returned to the students after cooling and reopened. The students washed the aqueous reaction mixture twice with 25 mL portions of diethyl ether to remove organic impurities. Water was removed from the aqueous solutions by heating to dryness on a hot plate under snorkel ventilation (Figure 10). Care was taken not to burn the product as the solution dried. Product weights were obtained along with IR spectra. The remaining product was dissolved in a minimal amount of D_2O and ^{1}H NMR spectra were obtained on the PicoSpin™ instruments.

Exceptional care was taken to ensure the entire sample was dissolved, as any particulate can clog the internal NMR capillary.

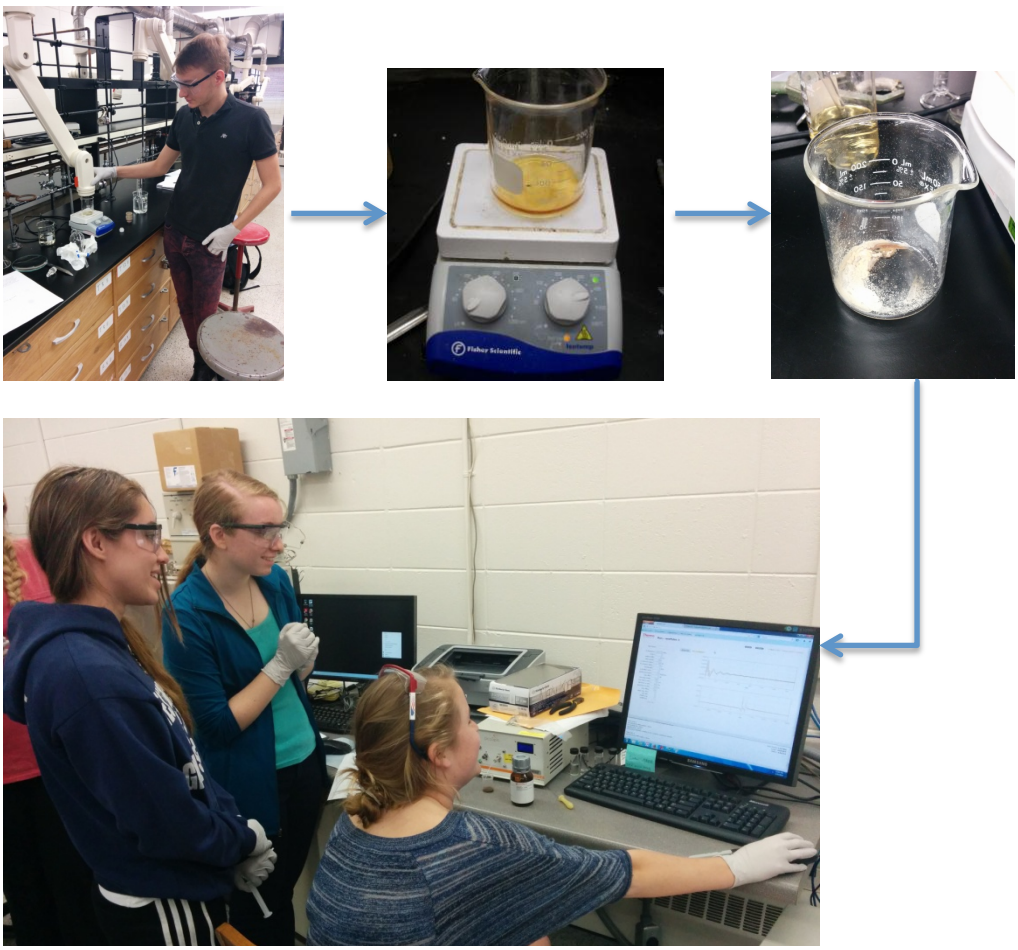


Figure 10. Isolation of hydrochloride salt and PicoSpin™ analysis

Hazards

Care should be taken by students and instructors when handling contents under pressure. Only microwave instruments rated for chemical use have the proper safety features for performing this experiment. Vessels should never be moved from microwave until temperature reading drops well below the boiling

point of the reaction mixture. As an extra precaution, vessels should be opened only in a fume hood. Note that evaporation of the aqueous layer will result in the evolution of a small amount of $HCl_{(g)}$. This evaporation should be performed under ventilation.

Results and Discussion

The experiment was completed by 50 lab groups in the honors/chemistry majors course spanning 6 lab sections over two days. An FT IR and 45 MHz PicoSpin™ ^1H NMR spectrum were obtained by all lab groups within the 3 hr lab period with minimal teaching assistant input. Student FT IR spectra may be viewed in Appendix B. Each shows a broad signal from about 3200-2500 cm^{-1} representative of the protonated amine, but the FT IR spectra were not very discriminating of student unknowns in the functional group region. Student analyses relied predominantly upon the NMR spectra. Figure 11 shows a student 45 MHz PicoSpin™ proton spectrum of the decarboxylation product of the amino acid valine sampled and obtained by a student during the lab period along with a 400 MHz proton NMR spectrum obtained by the TA of the very same student sample. The samples were very pure, and resolution at 45 MHz was sufficient for student analysis. Students did NOT receive 400 MHz spectra. Note that the presence of the observed water peak is expected, as the purity of the D_2O solvent was only 99.9 atomic percent deuterium and the rapid acidic proton exchange of the hydrochloride salts. Additional spectra for comparison may be viewed in Appendix B.

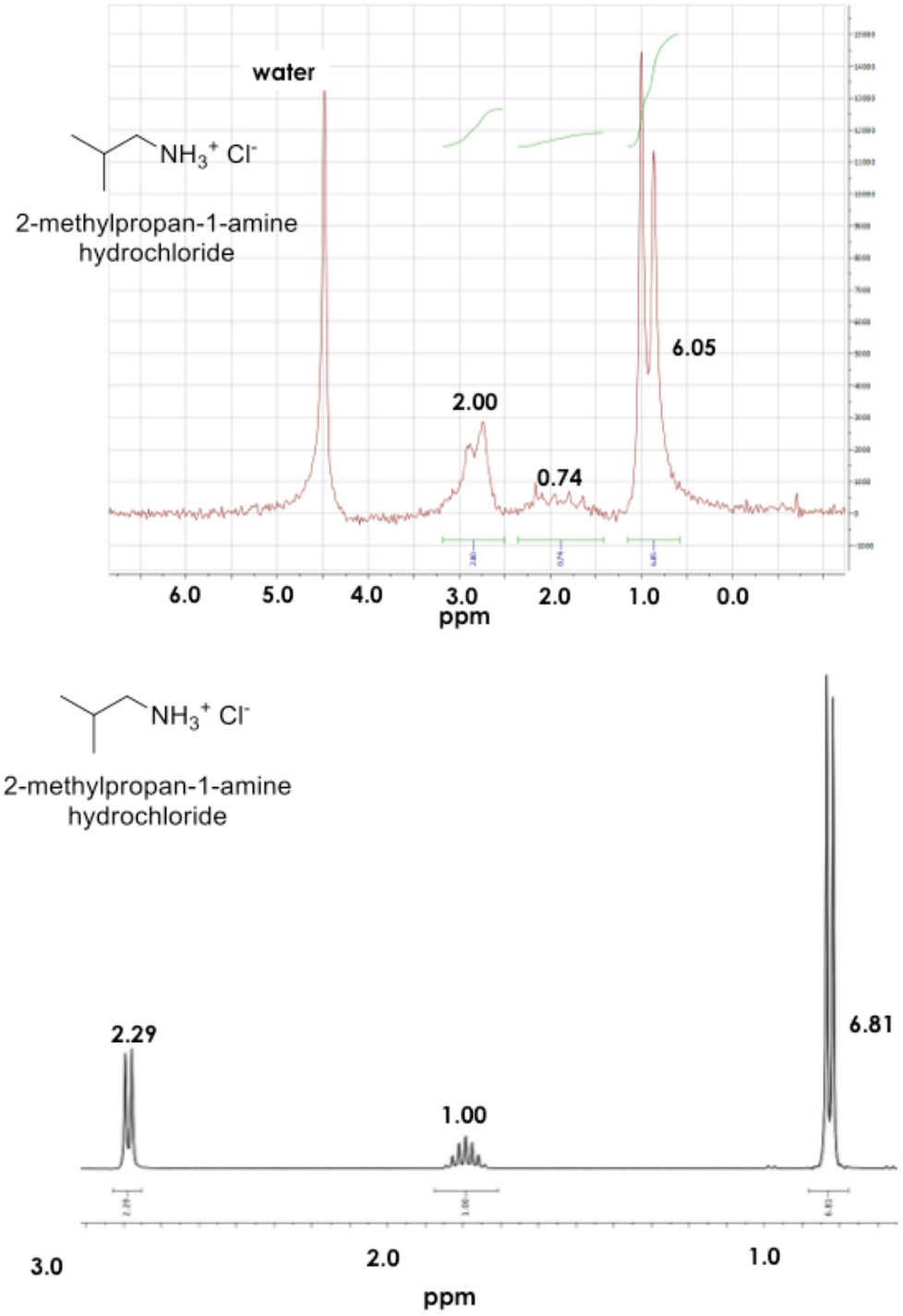


Figure 11. ^{1}H NMR comparison of 2-methylpropan-1-amine hydrochloride

Student unknown identification results were obtained from student lab reports by the three course teaching assistants. The results are summarized in Table 6. The teaching assistants were asked to comment on whether the spectra were "solvable" based on spectral quality and to tabulate the number of correct assignments. Impressively, the PicoSpin™ instruments yielded a 100% solvable spectrum rate. The students achieved an 84% composite identification rate given 6 amino acid possibilities: the three unknowns, valine, isoleucine, and phenylalanine and distracters leucine, tyrosine, and proline.

Table 6. Summary of Student Results

Day	Unknown	# Students	# Solvable IR/HNMR?	# Correct ID	%
Tuesday	Val	6	6	6	100
	Ile	5	5	4	80
	Phe	6	6	6	100
				Composite	**94**
Thursday	Val	16	16	15	94
	Ile	7	7	4	57
	Phe	10	10	7	70
				Composite	**79**

The isoleucine decarboxylation product yielded the lowest identification rate due to misidentification with valine. The spectra have similar splitting patterns in the

low field instrument with overlapping signals in isoleucine, and the necessary integration analysis for correct identification resulted in slightly higher difficulty. There was no observable improvement in unknown identification for lab sections meeting later in the week indicating that any communication between students from day to day was ineffective in aggregate. Ideally this trend will hold from semester to semester as the set of amino acids issued to the students can be cycled.

Summary and Outlook

The microwave decarboxylation of amino acids experiment has been effectively implemented in the second semester sophomore organic chemistry laboratory. The increased reaction rate provided by the organocatalytic microwave procedure allows for student spectral analysis within the 3-hour lab period. Use of the new bench-top PicoSpin™ 45 MHz ^{1}H NMR instruments allows for students to perform NMR analysis rapidly with minimal teaching assistant input. The generality of the experiment allows facile incorporation of unknowns, which allows the instructor to accurately assess student laboratory technique as well as student ability to analyze spectra. Future modifications of this experiment will be to incorporate even more unknowns and include ^{13}C NMR analysis using PicoSpin™ 90 MHz instruments thereby eliminating the need for unknown "options" to be given to the student for positive identification. Identification of the intermediate imine via NMR and GC/MS analysis are also possible variations of the experiment. Additionally, an inquiry experiment where students optimize the reaction catalyst load using combined class data has also been considered given the large number of

possible ketone catalysts. It is clear that this experiment and its variations have the versatility to remain a pedagogically relevant part of the instructional laboratory curriculum. Supporting Information for this experiment may be viewed in Appendix B.

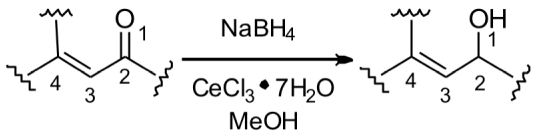
CHAPTER 4

STEREOSELECTIVE TUNING: THE LUCHE REDUCTION OF (-)-MENTHONE AS A MODEL FOR CONTINOUSLY VARIABLE STEREOSELECTIVITY

This work details a novel protocol for the stereoselective "tuning" of the Luche reduction allowing for the variable selectivity of product diastereomer alcohols between two extremes. The product ratio of (-)-menthol to (+)-neomenthol in the Luche reduction of (-)-menthone has been shown to be continuously dependent on the concentration of lanthanide catalyst $CeCl_3 \cdot 7H_2O$ in methanol reaction solvent. The two diastereomeric products varied in concentration from a 48:52 (-)-menthol:(+)-neomenthol mixture when no catalyst was added to a ratio of 75:25 (-)-menthol:(+)-neomenthol when the concentration of catalyst approaches 60.0 mM. The desired product ratio may be accurately predicted to within ±2% as determined by ^{1}H NMR and GC.

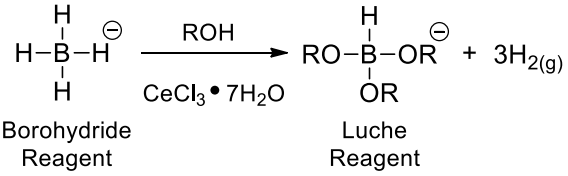
Characteristics of the Luche Reduction

The Luche reduction[45] is a variant of the common sodium borohydride reduction that is historically used for regioselective 1,2 reduction of α,β-unsaturated ketones and aldehydes to the corresponding unsaturated alcohols (Scheme 4). The mechanism of the Luche reduction is believed to involve "solvent activation" via catalytic methanolysis of the reducing agent by a lanthanide catalyst, most commonly $CeCl_3 \cdot 7H_2O$, converting the "soft" borohydride reducing agent to a



Scheme 4. Regioselective Luche reduction of α,β-unsaturated ketones and aldehydes

much "harder" alkoxy borohydride Luche reagent in situ (Scheme 5). The Luche reagent donates hydride directly to the carbonyl with 1,2 regioselectivity in high yield.



Scheme 5. Catalytic methanolysis of borohydride in forming the Luche reagent

The mild conditions and near neutral pH of the Luche reduction make it an excellent alternative to analogous regioselective $LiAlH_4$ reduction in many synthetic endeavors. The Luche reduction is also much safer by avoiding the pyrophoric $LiAlH_4$.

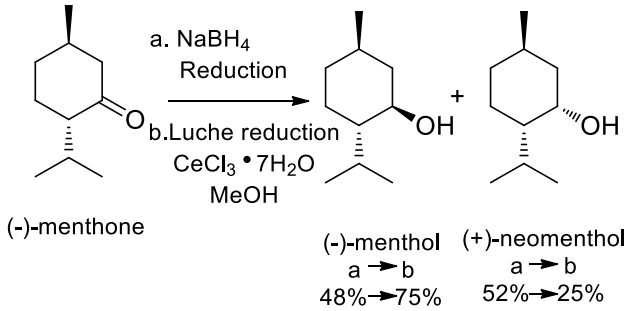
Perhaps most interestingly, it has been reported in the literature that the Luche reduction is inherently stereoselective as well.[45a] Reduction of chiral carbonyl compounds quite often yields significantly different stereoselectivity under Luche conditions compared to standard sodium borohydride reductions.

This differential stereoselectivity, observed but not emphasized in the original paper, has been exploited in recent work.[46] Mechanistically, selectivity may be influenced by the bulkiness of the Luche reagent as well as possible complexation of the lanthanide Lewis acid to the carbonyl.[46b, 46c]

It was envisioned that the selectivity of one reduction process may be emphasized at the expense of the other, effectively allowing selection of a desired product ratio between two extremes: borohydride reduction selectivity vs. Luche reduction selectivity. The rate of formation and reaction of the Luche reagent is quite rapid relative to the rate of reaction of sodium borohydride with carbonyls;[45a] therefore, when adding a reasonable amount of sodium borohydride under Luche conditions (400 mM Ce^{3+}) the sodium borohydride will rapidly convert to Luche reagent as evidenced by rapid hydrogen gas evolution. The effective approach for selecting the desired product ratio proved to be lowering the concentration of catalyst, thus systematically lowering the rate of the Luche reduction until the sodium borohydride reduction becomes competitive and eventually dominant.

Results and Discussion

Scheme 6 illustrates the conversion of (-)-menthone to product diastereomers via sodium borohydride reduction and the Luche reduction. The ratio of (-)-menthol to (+)-neomenthol is 48:52 via sodium borohydride reduction and 75:25 via Luche reduction at 400 mM catalyst load. Preliminary reactions were run to elucidate the nature of the variability of the product percentages as the concentration of $CeCl_3 \cdot 7H_2O$ in MeOH is varied. A ^1H NMR of the product mixture of both diastereomers is shown in Figure 12.



Scheme 6. Comparison of (-)-menthone reductions

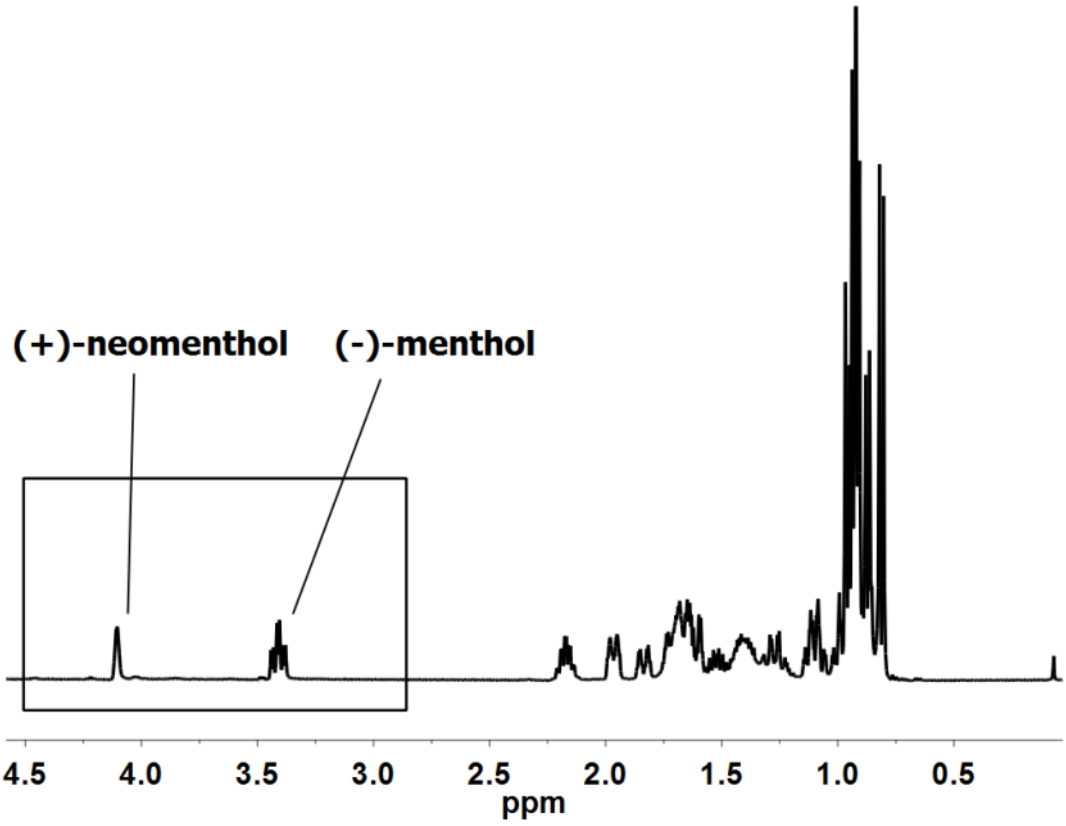


Figure 12. 400 MHz ^{1}H NMR of a typical reduction product mixture

Product percentages are determined by the relative integration of the resolved deshielded signals corresponding to the single proton of the secondary alcohol carbon of each isomer. The product percentages of (-)-menthol as determined by ^{1}H NMR are plotted in Figure 13 with the stereoselectivity of the Luche reduction maximizing at about 60.0 mM catalyst concentration.

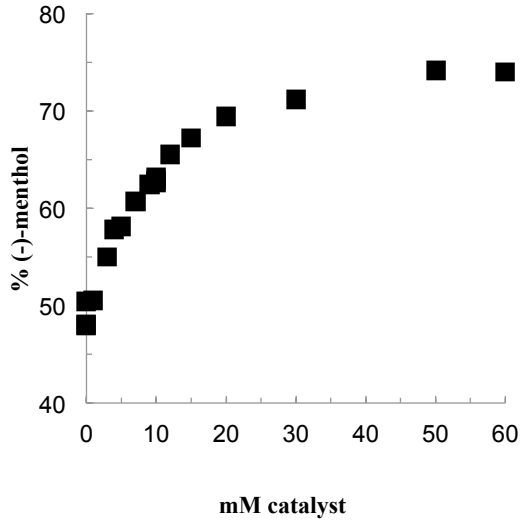


Figure 13. Product % of (-)-menthol as determined by ^{1}H NMR is plotted vs. catalyst concentration.

Each plotted point represents the average calculated (-)-menthol concentration of triplicate experiments. All product percentages of each set of triplicate experiments lie within ±2% of the average value. Solutions were prepared by dissolving a mass of at least 0.100 g of catalyst in an appropriate volumetric flask (rated ±0.1 %). Further dilutions when necessary were accomplished by transfer of

the appropriate volume of concentrated solution to a new volumetric flask using a calibrated pipet (rated ±0.2 %).

Summary and Outlook

An effective strategy for tuning the stereochemical outcome of the Luche reduction has been developed based on the control of $CeCl_3 \cdot 7H_2O$ catalyst concentration. A tunable stereochemical outcome is convenient when a particular ratio of diastereomers or a variety of stereochemical mixtures is desired. (-)-Menthone has proven to be an ideal candidate for demonstration of this tuning process with a dynamic range of 27%. Currently the range of possible ratios is limited by the inherent stereochemical outcomes of the sodium borohydride reduction and the Luche reduction. Given that the basis of control is the competing rates of two separate chemical processes, it is conceivable that similar tuning may be possible with other processes as well. One could conceive controlling the ratio of enantiomeric chiral catalysts to control the ratio of a reaction product. Using these catalysts ranges of 97% enantiomeric excess have been reported[47] for similar borohydride type reductions of prochiral ketones.

CHAPTER 5

THE LUCHE REDUCTION OF (-)-MENTHONE: A SOPHOMORE ORGANIC CHEMISTRY EXPERIMENT WITH CONTINOUSLY VARIABLE STEREOSELECTIVITY

A novel unknowns-based sophomore organic chemistry laboratory protocol has been developed addressing stereoisomerism, optical activity, reaction monitoring, and introductory instrumental analysis. The product ratio of (-)-menthol to (+)-neomenthol in the Luche reduction of (-)-menthone has been shown to be continuously dependent on the concentration of lanthanide catalyst $CeCl_3 \cdot 7H_2O$ in methanol reaction solvent. The ability of the instructor to select a desired product ratio, ±2% by ^1H NMR, is exploited by providing students with solvent samples containing varying catalyst concentrations with which to run the reaction. Reaction completion was readily determined by IR spectroscopy and GC Student product ratio determination was accomplished by polarimetry, GC, or ^1H NMR analysis of the resulting optically active diastereomeric mixture.

Experiment Overview

Equipped with a reliable methodology for controlling or "tuning" the stereochemical outcome of the Luche reduction of (-)-menthone vs. the traditional reduction[48], work was focused on the development of an unknowns-based sophomore organic chemistry experiment addressing stereoisomerism, optical activity, reaction monitoring, and introductory instrumental analysis. The majority of current experiments investigating stereoselective reactions[6j, 48-49] result in a set

outcome in the optically active product mixture. This novel experiment asks the students to determine the stereochemical outcome of the highly variable "tuned" Luche reduction via one of several analytical techniques. Stereoselective tuning by the instructor prevents students from knowing the outcome of the experiment even after several iterations of the experiment from semester to semester. The variability was achieved by providing students with coded vials containing a solvent of specific catalyst concentration that is unknown to the student. Vials were coded more carefully than "unknowns A,B, C, and D" but rather with unique numeric strings as shown in Figure 14.

Figure 14. Unknown reaction solvent samples. Example vial codes for "Thursday, 3:30 pm lab, sample number 9".

Typically 3 or 4 unique solvent concentrations were prepared. For each unknown batch, three sample reactions were run to calibrate the unknown stock solutions to a particular product ratio as determined by ^{1}H NMR. This calibrated value is considered the correct value for the "unknown" concentration. In all cases the standard reactions gave product ratios within ±2% of the average as demonstrated in Table 7.

Table 7. Standardization of stock $CeCl_3 \cdot 7H_2O$ solutions (% (-)-menthol shown)

[$CeCl_3 \cdot 7H_2O$] mM	Trial 1 %	Trial 2 %	Trial 3 %	Avg %	St. Dev.
0	48.0	50.4	48.1	**48.8**	1.36
5	60.4	57.9	57.7	**58.7**	1.50
10	64.3	64.5	63.2	**64.0**	0.70
50	75.3	74.8	75.1	**75.1**	0.25

After assignment of solvent solutions, students then performed the experiment given a simple procedure. Students confirmed reaction completion using TLC, IR, or GC, and then were able to determine the stereochemical outcome by polarimetry, ^{1}H NMR, and/or GC. Note the products are diastereomers and do not require chiral chromatography for separation. There is a high diversity of possible outcomes and methods by which the reaction may be performed. This

highly discourages the recycling of lab reports and necessitating students to carefully plan their experiment and analyze their experimental results.

Detailed Experimental Procedure

Students were individually provided 20 mL of a coded unknown solvent stock solution. Students then dissolved 1g of (-) - menthone in the sample solution and placed the solution in a room temperature water bath with magnetic stirring. To this solution 1.5 mol equivalents of $NaBH_4$ were carefully added in portions over a 30 second period. After 10 minutes, in all variations, the reaction was completed and the students were asked to show this by TLC, IR or GC. The reaction mixture was diluted with 25 mL of distilled water and extracted with 2 x 25 mL portions of diethyl ether. The ether layers were combined and washed with 25 mL of distilled water and 25 mL of brine. The ethereal solution was further dried over $MgSO_{4(s)}$. After decanting, the ether was removed by rotary evaporation at 50 °C and ~60 Torr.

Results

Typical student yields of pure samples ranged from 60-90 % (literature 97%) depending on the level of care taken during workup. Well-prepared students completed the reduction and workup within 1 hr, leaving ample time for analytical determination of product ratios by polarimetry, GC, and/or ^1H NMR. In the standard sections (24 students/section), IR and polarimetry data were obtained. In the honors sections (14 students/section) where more instrumentation was available IR, polarimetry, and either ^1H NMR or GC data were obtained. Student ^1H

NMR, GC, and IR data were recorded electronically and associated with each student by the instructor at the time of acquisition.

Student product ratios as determined by ^{1}H NMR and GC were generally within ±2% of the calibrated value regardless of the quality of student samples as evidenced by impurities present in the student ^{1}H NMR. For polarimetry analysis, properly purified student samples yielded a product ratio within ±5% of the calibrated value; however, impure samples vary more widely from this value.

Reaction Monitoring via IR

All IR spectra for reaction monitoring were taken on computer integrated FT-IR instruments with one dedicated instrument per section. Student IR spectra clearly showed the disappearance of the carbonyl stretch of menthone at ~1710 cm^{-1} in favor of the OH stretch of the product alcohols at ~3380 cm^{-1} as shown in Figure 15.

Reaction Monitoring via TLC

5 cm silica bonded TLC plates from Fischer Scientific in homemade glass jar TLC chambers were developed with a solvent of 3:2 hexane:ethyl acetate. UV lamps, iodine chambers, and a homemade dye of phosphomolybdic acid (1 g per 10 mL ethanol) were used for spot visualization. Menthone is identifiable via all 3 methods; however, the product alcohols are only visible after dipping in phosphomolybic acid dye with subsequent heating on a hotplate. Average R_f's for menthone, (-)-menthol, and (+)-neomenthol were 0.70, 0.58, and 0.82 respectively.

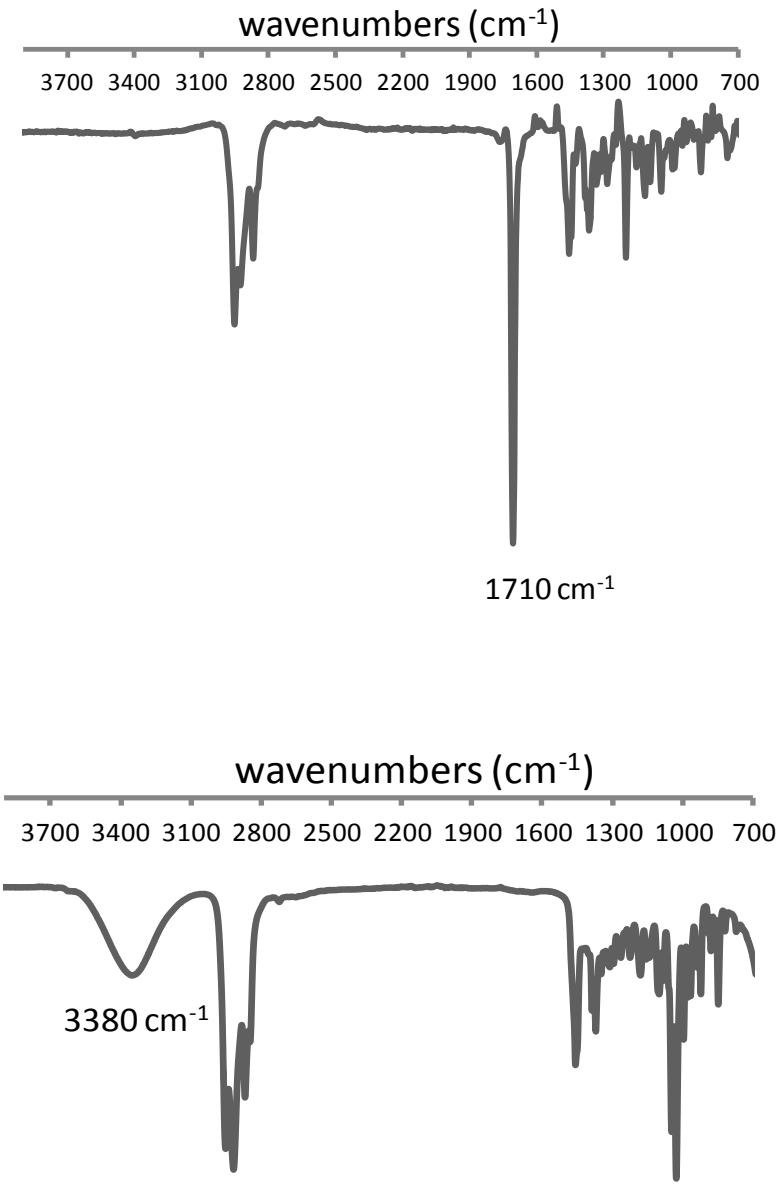


Figure 15. IR spectra of (-)-menthone (top) and a student reduction product mixture (bottom) are shown.

Reaction Monitoring and Product Analysis via GC

GC analysis for reaction monitoring and product ratio determination was performed on a computer integrated capillary column gas chromatograph. Before workup, students obtained a GC chromatogram for 1 µL of reaction mixture. The splitless injector and FID detector were maintained at 230 ˚C. Column temperature was held constant at 45 °C with a He carrier gas flow rate of 50 mL/min. A sample student chromatogram can be seen in Figure 16. Chromatograms were analyzed against a standard spectrum of (-)-menthone for qualitative determination of reaction completion. A new Restek RTX-5 (15m x 0.55mm x 0.25µm) column yielded complete, fully resolved chromatograms in fewer than 5 min. It has been previously shown[50] that these diastereomeric terpenes respond similarly to flame ionization detection and thermal conductivity detection; therefore, peaks were integrated and product ratios were determined using standard GC software with no further calibration.

Product Analysis via ^{1}H NMR

Student product percentages as determined by ^{1}H NMR were within ±2% of the calibrated unknown values regardless of the quality of student sample. Figure 17 displays ^{1}H NMR spectra of a high quality student spectrum along with a student spectrum with diethyl ether present. The single protons on the alcohol carbons of (-)-menthol (3.4 ppm) and (+)-neomenthol (4.1 ppm) are well resolved in chemical shift as shown in Figure 17 and may be readily integrated. Relative integration of the two gave the product ratio. Students prepared ^{1}H NMR samples by dissolving a few mg of their sample in chloroform-d. The instructor then performed the

experiments on a 400 MHz FT NMR instrument after the lab period and provided the students with the data electronically.

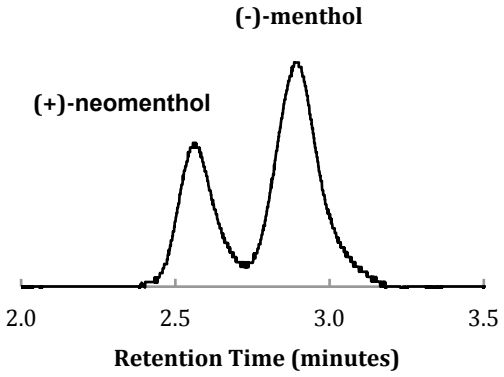


Figure 16. Student gas chromatograph of reduction product mixture is shown. Retention times for (-)-menthol and (+)-neomenthol are 2.6 min and 2.9 min respectively, and calculated product ratio is 65:35 where the expected ^{1}H NMR calibrated value is 64:36.

Product Analysis via Polarimetry

Polarimetric determination of the product ratios was employed for all laboratory sections since it is inexpensive, requires little instrument time, and pedagogically relevant for demonstrating the relationship between stereoisomerism and optical activity. After all other analyses were performed students weighed accurately their remaining sample and dissolved it in an appropriate amount of denatured ethanol (195 proof with isopropanol). Typically at least 15 mg/mL concentration was required for an accurate reading. Knowing the path length, *l*, in dm and the sample concentration, *c*, in g/mL, students

measured the rotation of the unknown sample and calculated the sample's standard rotation, $[\alpha]_{20}^{D}$, using equation "a" in Figure 18.

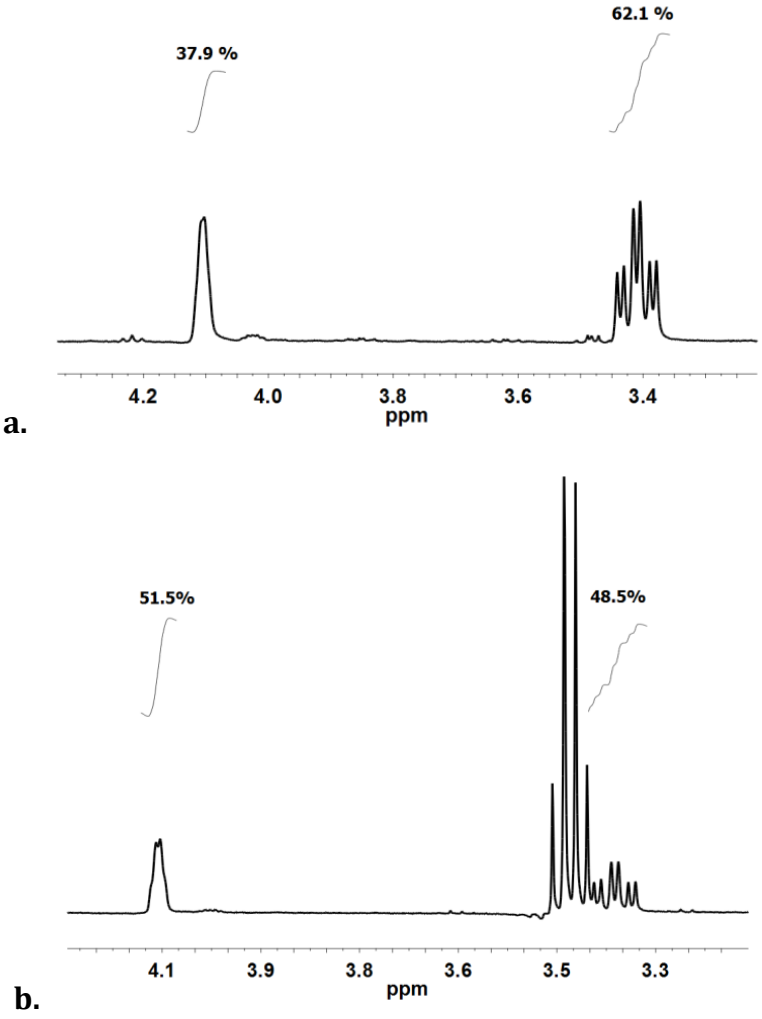


Figure 17. A high quality student ^{1}H NMR of purified product mixture is shown (a) and the analogous region of a spectrum of an impure student sample (b) with diethyl ether present are shown along with the experimental product ratios. The expected ratio of (-)-menthol : (+)-neomenthol for spectrum (a) is 64:36 ±2% and for spectrum (b) is 48:52 ±2% based on standardization results.

Given the standard rotation of (-)-menthol (-50˚) and (+)-neomenthol (+20.7˚) students solved for the percent contribution of each isomer to the total rotation using equation "b" of Figure 18.

a. $\alpha_D^{20} = \frac{\alpha}{l\ x\ c}$

b. $100 * \alpha^o_{product\ mixture} = x(\alpha^o_{menthol}) + (100 - x)(\alpha^o_{neomenthol})$

Figure 18. Equations for calculation of standard rotation (**a**) and product percentages (**b**)

Polarimetry data is highly dependent on the quality of student sample as students will tend to significantly underestimate the percentage of menthol if they do not adequately remove extraction solvents. Measurements were made at 589 nm at 20 ˚C.

Hazards

Upon the addition of $NaBH_4$ to the reaction mixture flammable H_2 gas is evolved from the Luche pathway accompanying the methanolysis of borohydride to the Luche reagent. The more concentrated the catalyst, the more vigorous this gas evolution will be. Keep the vessel open to the air and away from sources of ignition. Students are reminded that (-)-menthol and (+)-neomenthol are irritants and cause a burning sensation if contacted with the skin or eyes.

Summary

The stereoselctively tuned Luche reduction has been an effective recent addition to our laboratory program with over 1000 students having performed the experiment. The protocol replaces a standard cookbook experiment with an unknowns-based experiment highlighting a central problem for the students to solve. Polarimetry has proven to be a most effective technique for gauging student performance because shoddy work-up practices skew the results more dramatically. In our classes, 15% of students fall within ±5% of the expected value, 35% of students within ±10%, and 50% of students obtain values slightly outside the ±10% range. If other methods are used, instructors should obtain digitally tagged copies of student spectra to be investigated for impurities. We have found it most efficient for our large lecture program to utilize polarimetry and IR as the techniques of choice given the volume of instrument time required for 400 MHz ^{1}H NMR. GC analysis is accomplished quickly and can be used as desired in conjunction with polarimetry and IR in larger lab sections. Our honors course has made use of all the techniques in a single lab period (^{1}H NMR were run overnight and emailed to the students). Supporting Information for this experiment may be viewed in Appendix C.

CHAPTER 6

"DISCOVERY" OF AN UNEXPECTED TREND IN EPOXIDE RING-OPENING REACTIONS: AN UNKNOWNS-BASED SOPHOMORE ORGANIC CHEMISTRY EXPERIMENT

A sophomore organic chemistry experiment is presented here for the "discovery" of a trend in regioselectivity for a series of nucleophilic ring-opening reactions of the epoxide 1,2-epoxyhexane. Student lab partners are each assigned an alcohol nucleophile and analogous alkoxide nucleophile and perform the epoxide opening reaction under both acidic and basic conditions. Students pool product distribution data as determined by GC to discover a trend in regioselectivity of nucleophilic epoxide ring-opening reactions before the topic's presentation in lecture. Students are further challenged to analyze the meaning of their collective data when the results do not necessarily obey the rule readily available to them in their textbook and online resources.

Introduction

The reactions of epoxides are a pedagogically important class of reactions for illustrating the interplay of sterics and transition state stability in reaction mechanisms. With a few notable exceptions,[51] many textbooks[52] commonly teach that nucleophiles add to the least hindered carbon under basic/kinetic conditions as addition to this position has the lowest energy transition state. For acidic conditions, textbooks commonly indicate that the added charge build up of the

transition state of the more substituted carbon of the epoxide lowers the energy of the transition state enough to where a Markovnikov type addition is preferred. These textbook rules are summarized in Figure 19.

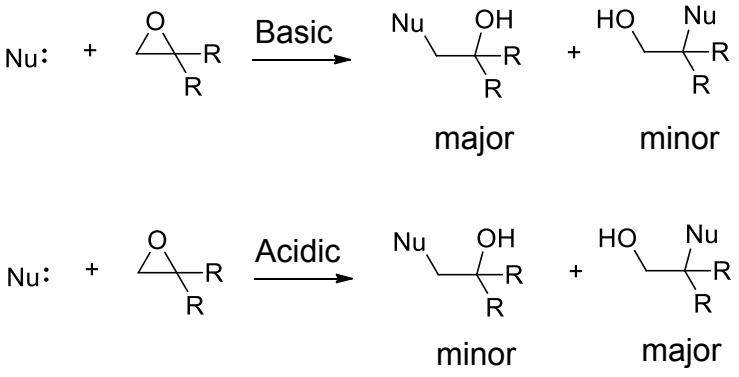


Figure 19. Summary of Epoxide Regioselectivity Presented in Many Textbooks

Surprisingly, the reactions of epoxides are notably underrepresented in the educational laboratory literature.[53] A new "discovery"[54] experiment is presented here that allows students to probe this class of reactions and discover this interplay of transition state effects before presentation of epoxides in lecture. Students as a whole perform a series of experiments with differing conditions and nucleophiles, determine product constitutional isomer ratios via gas chromatography (GC), and pool the data for analysis. As will be seen, the trend students expect may not necessarily be the trend represented by the data.

Detailed Experimental Procedure

Partnered-students were assigned one of four alcohol/alkoxide nucleophile pairs (MeOH/MeO^-, EtOH/EtO^-, n-PrOH/PrO^-, t-BuOH/t-BuO^-) as nucleophiles for the epoxide ring-opening reaction. Students then simultaneously perform the experiment under acidic and basic conditions, isolate the product mixture, and determine the product ratios via GC. It should be noted that students using the higher boiling solvents PrOH and t-BuOH had greater difficulty removing solvent from the reaction mixture, leading to yields of greater than 100%. These overages in reported yields were inconsequential as the analysis of the GC data does not require absolute purity of product.

Acidic Conditions

0.5 g of 1,2-epoxyhexane was placed into a 10 mL round bottom flask. The epoxide was dissolved in 5 mL of alcohol with stirring. 1 drop of concentrated sulfuric acid was added to this solution and allowed to stir for 30 min. In a separatory funnel, the reaction mixture was washed with 5 mL of saturated sodium bicarbonate solution (no layers will form). Then the products were extracted from this wash with 2 x 10 mL portions of diethyl ether. The ether layer was dried with magnesium sulfate and decanted. The organic extract was removed by rotary evaporation in room temperature water until no change in mass was observed. The final mass of the product oil was recorded.

Basic Conditions

1.20 mol equivalents of sodium alkoxide powder was placed into a 10 mL round bottom flask along with 5 mL of alcohol and a spin vane. To this slurry 0.5 g

of 1,2-epoxyhexane was added. The reaction mixture was refluxed for 30 min. In a separatory funnel, the reaction mixture was washed with 5 mL of saturated ammonium chloride solution. The product was extracted from this wash with 2 x 10 mL portions of diethyl ether. The ether layer was dried with magnesium sulfate and decanted. The organic extract was removed by rotary evaporation in room temperature water until no change in mass was observed. The final mass of the product oil was recorded.

GC Analysis

One drop (~0.01g) of product oil was dissolved in ~2 mL of methanol solvent. The chromatogram was recorded after injection of 1 μL of solution. A new Restek RTX-5 (15m x 0.55mm x 0.25μm) column yielded complete, fully resolved chromatograms in fewer than 5 min with injector and detector set at 250 ^{o}C and the column maintained at a constant 50 ^{o}C. The students saved the chromatogram on the lab computer and recorded peak retention times and areas on a community log sheet. A teaching assistant converted the log sheet into electronic form and posted the unorganized raw data on the course webpage.

Results

A student chromatogram is shown in Figure 20 for the acidic reaction in methanol. All other chromatograms are similar in nature having 2 product peaks of varying retention times that increase with increasing molecular weight of the products. Product peaks will be referred to as retention times, R_{t1} or R_{t2}, to indicate whether they are the first or second peak in the chromatogram. All reagents and solvents elute together early in the chromatogram, thus purity of final product

mixture is not inferred from collected data nor is it pertinent to the goals of the experiment. It was noted that a number of students assigned the higher boiling solvents n-propanol and t-butanol report yields greater than 100%. A yield of ~80% was achieved for students using the more volatile solvents methanol and ethanol.

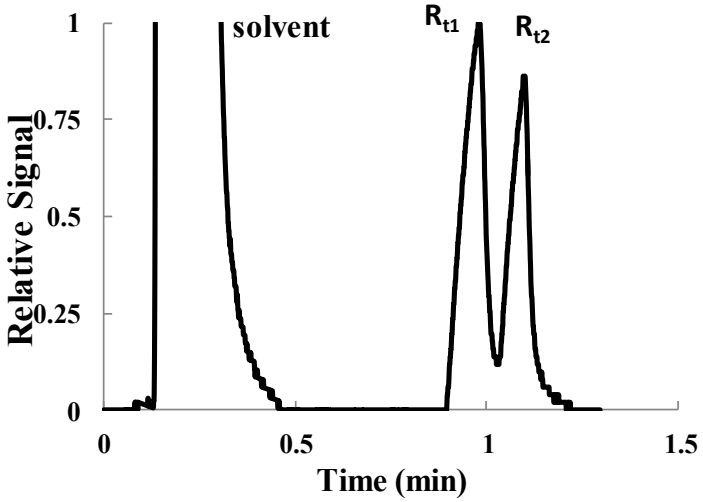


Figure 20. A student chromatogram for the reaction of 1,2-epoxyhexane in acidic methanol is shown. All other chromatograms are similar.

Table 8 shows the composite results of 64 students organized by reaction type (acidic or basic) and nucleophile. The percentages of the product giving rise to the peaks at R_{t1} and R_{t2} are averaged for each of the possible reaction variations.

Discussion

Students were tasked in the lab handout and prelab lecture to make sense of the raw pooled data of the class of 64 students. It was expected that the students

have some familiarity with using an electronic spreadsheet for formation of charts and execution of calculations pertinent to data analysis. This material was reviewed in the prelab lecture.

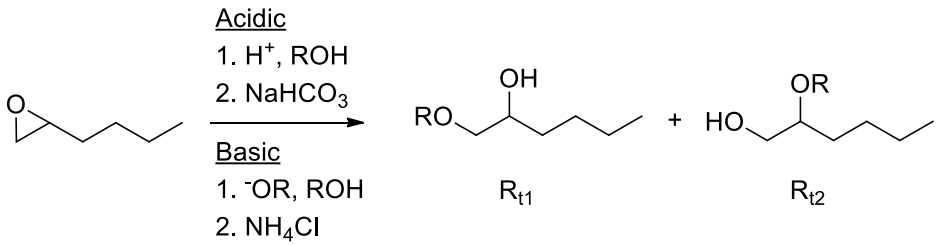
Table 8. Composite Student Reaction Results

Conditions	#	Nucleophile	R_{t1} (min)	R_{t2} (min)	% R_{t1}	% R_{t1}	St. Dev.
Acidic	9	MeOH	0.58	0.71	52.2	47.8	2.8
	8	EtOH	0.92	1.01	50.6	49.4	4.5
	9	n-PrOH	1.78	1.92	54.5	45.5	3.1
	7	t-BuOH	1.83	1.92	75.8	24.2	1.8
Basic	8	MeO^-	0.6	0.74	99.5	0.46	1.4
	8	EtO^-	0.92	-	100	0	0
	9	n-PrO^-	1.78	-	100	0	0
	6	t-BuO^-	1.86	-	100	0	0

Upon collection of the lab reports it was interesting to note the conclusions drawn by the students regarding the data represented in Table 8. In grading and analyzing student "results and discussion" sections a series of metrics was devised that would ideally be included in an excellent analysis of the data:

1. Did the student make a table similar if not identical to Table 8?

2. Did the student recognize there was a significant difference in regioselectivity of the ring-opening reaction under acidic and basic conditions?
3. Did the student research the topic to find that under basic conditions the regioselectivity favors addition to the least substituted carbon of the epoxide and correctly identify the peak which corresponds to each product (Scheme 7)? Note in Table 8 the product giving rise to R_{t1} is clearly the primary product of all basic reactions.



Scheme 7. Many students recognize that the major or exclusive product of the basic reactions results from addition to the less substituted position of the epoxide. This product gives rise to R_{t1} in each chromatogram.

4. Did the student notice that the trend is affected by steric bulk of the nucleophile significantly favoring the attack of the more accessible carbon for addition of t-BuOH in acidic conditions?
5. Did the student notice that under acidic conditions the reaction does NOT favor addition to the more substituted product, or did they parrot a rule found in many textbooks that attack at the more substituted carbon

is favored in acidic conditions or did they notice that the trend in acid promoted opening in their data does not follow this rule especially for the attack of the bulky t-BuOH?

A summary of student responses is given in Table 9 as the percentages of students meeting the grading criteria rounded to the nearest ±5%. It is interesting to note that while not every student organized the data into an efficient table, all students noticed there were distinctly different trends for the acidic and basic epoxide opening reactions. 60% noticed that steric bulk played a part in the regioselectivity but only about half of the students correctly matched the identities of the products to the GC signals. Perhaps the most telling statistic of all is that 20% of the students (all of which made a table similar to Table 8) stated in their conclusions section that the acid promoted reaction strongly favors addition at the more substituted carbon even in the face of the data which indicated otherwise.

Table 9. Summary of Student Responses

Response Metrics	% Student Responses
Made Similar Table	90
Recognized Acid/Base Trend	100
Matched Isomers to GC R_t 's	50
Recognized Steric Trend	60
Parroted Book Trend	20

Hazards

1,2-epoxyhexane is flammable liquid with a mildly unpleasant odor that is harmful if ingested. The solvents used in this experiment are also flammable and toxic if ingested. Perform the experiment in a well-ventilated room or hood away from sources of ignition. Gloves should be worn especially when handling the alcoxide salts and sulfuric acid.

Summary

This discovery experiment of the epoxide ring-opening reactions of 1,2-epoxyhexane is a valuable component of the University of Georgia organic chemistry laboratory curriculum. It is important to note that the lab is performed prior to the presentation of epoxide ring-openings in lecture. As a result, students have the opportunity to discover the trends in regioselectivity. The reactions themselves are reliable and quick allowing facile variation of the experiment and inclusion of many experimental combinations in one class of 20-25 students. Reagents are inexpensive, the reaction is performed on a microscale, and chromatograph collection is rapid requiring only 1 instrument per 20 student lab section (2.5 min x 20 samples = 50 min). Given the summary of student responses in Table 9, this experiment clearly identifies students who were able to organize the data, recognize trends, and summarize their findings accordingly as well as those who were not. Minor variations of this experiment such as varying the epoxide electrophile will present different data perhaps necessitating different conclusions. This experiment readily identifies students using old reports and allows instructors

to stay one step ahead of the “fraternity file”. Supporting Information for this experiment may be viewed in Appendix D.

CHAPTER 7

PICOSPIN™ ^{1}H NMR ANALYSIS AND MICROWAVE REFLUX IN THE FISCHER ESTERIFICATION SOPHOMORE ORGANIC CHEMISTRY EXPERIMENT

An unknowns-based sophomore organic chemistry experiment has been developed for the Fischer Esterification incorporating the use of microwave reflux and bench-top PicoSpin™ ^{1}H NMR analysis. Students are charged with identifying the product ester of the reaction of an unknown carboxylic acid and an unknown alcohol. Microwave reflux accelerates the esterification process allowing for additional analysis time by the students. Students perform IR and ^{1}H NMR analyses using bench-top instruments gaining experience in sample prep, instrument operation, and digital spectra manipulation. Equipped with the spectral data students identify the unknown ester products.

Introduction

The Fischer esterification is an important reaction in organic chemistry and has long been incorporated into the organic chemistry laboratory curriculum. Pedagogical elements introduced by the Fischer esterification include the concept of chemical equilibrium, reactivity of carboxylic acids, and acid catalysis with many published variations.[7c, 55] Early experiments have relied upon boiling point analysis of the product mixtures to identify the product esters. Experience has shown that unknown identification by boiling point analysis yields poor accuracy in many student experiments. One problem includes the similarity of the boiling points of

many esters. Another problem is the difficulty of achieving thermal equilibrium with the thermometer before exhausting the product ester when running the experiment in the microscale.

Because of these difficulties, ^{1}H NMR analysis was introduced in order to more accurately verify reaction products. Simple esters yield ideal ^{1}H NMR spectra even on low field instruments allowing for simple analysis of integration, chemical shift, and multiplicity. In the past the major limiting factors for the use of ^{1}H NMR analysis in the undergraduate laboratory have been expense and time considerations. The difficulty of squeezing spectroscopy into the 3-hour lab period has been aided by the amenability of the Fischer esterification to microwave reflux. Unknowns and even microwave chemistry have been previously[7c, 16] introduced in Fischer esterification undergraduate experiments due to the uniformity of the process where many acids and alcohols may be reacted to form esters using the same experimental protocol. It is clearly noted in these reports that students were NOT able to perform the NMR experiments themselves and relied on TA provided spectra of their samples. The present work illustrates the use of 45 MHz PicoSpin™ ^{1}H NMR analysis as a method of placing high throughput bench-top instruments in every lab section thus allowing students to perform their own ^{1}H NMR experiments within the 3-hour lab period.

Detailed Experimental Procedure

Students obtained 5 mL of unknown carboxylic acid (acetic, propionic, or butyric) and 4 mL of an unknown alcohol (n-PrOH, n-BuOH, or isopentanol) and added these to a microwave vessel along with a small Teflon coated stir bar. These

reagent choices were known to the students via the table of reagents in the student handout. At least 15-20 mL total of microwave absorbing solvent are necessary to prevent heating of internal microwave components and damage to the magnetron. Students carefully added 1 mL of concentrated sulfuric acid under ventilation, then sealed the MW vessels. The students recorded their vessel number and placed their vessel in the MW carousel of the Milestone START® system. The reaction was carried out over 10 min at 125 ^{o}C compared to 1 hr by conventional reflux.

The vessels were allowed to cool, and the contents were poured into a separatory funnel along with 10 mL of water for removal of acidic reagents. The product esters partitioned from the aqueous layer. The aqueous wash was duplicated followed by a wash with 10 mL portions of 5% sodium bicarbonate to neutralize any residual acid. Neutralization was confirmed with a pH indicator strip. Finally, the ester was washed with 5 mL of brine solution to remove residual water. The ester was weighed and transferred neat into a vial for PicoSpin™ analysis. Students determined the identity of the product esters and unknown reagent alcohols via ^{1}H NMR analysis.

Hazards

Only microwave instruments rated for chemical use have the proper safety features for performing this experiment. Care should be taken by students and instructors when handling contents under pressure. Vessels should never be moved from the microwave until temperature reading drops well below the boiling point of the reaction mixture. As an extra precaution, vessels should be opened only in a fume hood. CO_2 gas forms during neutralization so care must be taken to

avoid excess pressure buildup within the separatory funnel. The procedure makes use of concentrated acids emitting irritating fumes. Gloves, goggles and ventilation are essential.

Results and Discussion

Experiments were performed in 18 lab sections spanning three days with 10 different teaching assistants who were not involved with experimental development. Each TA received brief training in using the MW and NMR instruments before conducting the laboratory. The experiment was performed in three different lab rooms each equipped with a unique PicoSpin™ instrument. TA's were asked to review student reports and tabulate the number of correctly identified ester products by unknown alcohol identity, lab date, and time. Initially, experiments were limited to only acetic acid as the unknown carboxylic acid. Table 10 organizes student results by unknown identity, lab day, and lab time of day. Table 11 organizes student data by TA as well. A typical student ^{1}H NMR spectrum is shown in Figure 21.

Student spectra were of sufficient quality for analysis as demonstrated by the ^{1}H NMR of propyl ethanoate in Figure 21. It was apparent that the ester resulting from the reaction of n-butanol proved slightly more difficult for the students to analyze. This is presumably due to the need for a careful integration analysis of overlapping signals arising from interior methylene units to distinguish the product from propyl ethanoate. The doublet methyl of isopentyl ethanoate was identified by the students at the same ~90% frequency as propyl ethanoate.

Table 10. Student Results by Unknown, Day, and Time

Unknown	n	% Correct	Day	n	% Correct	Time	n	% Correct
1-propanol	115	92	T	56	86	8:00	53	75
1-butanol	62	79	W	32	91	12:20	115	97
isopentanol	83	94	Th	172	91	3:30	92	89
Total	**260**	**89**						

Table 11. Student results by time and teaching assistant

TA	Day	Time	n	% Correct
A	T	3:30	16	94
	Th	3:30	16	88
B	T	3:30	12	83
C	T	3:30	14	71*
	Th	8:00	17	59*
D	T	3:30	14	93
	W	12:20	16	88
E	Th	12:20	17	94
	Th	3:30	14	100
F	Th	8:00	14	93
	Th	12:20	14	100
G	Th	8:00	9	78*
	Th	12:20	19	100
H	W	12:20	10	90
	W	3:30	6	100
I	Th	12:20	20	100
J	Th	8:00	13	77*
	Th	12:20	19	100
Total			**260**	**90**

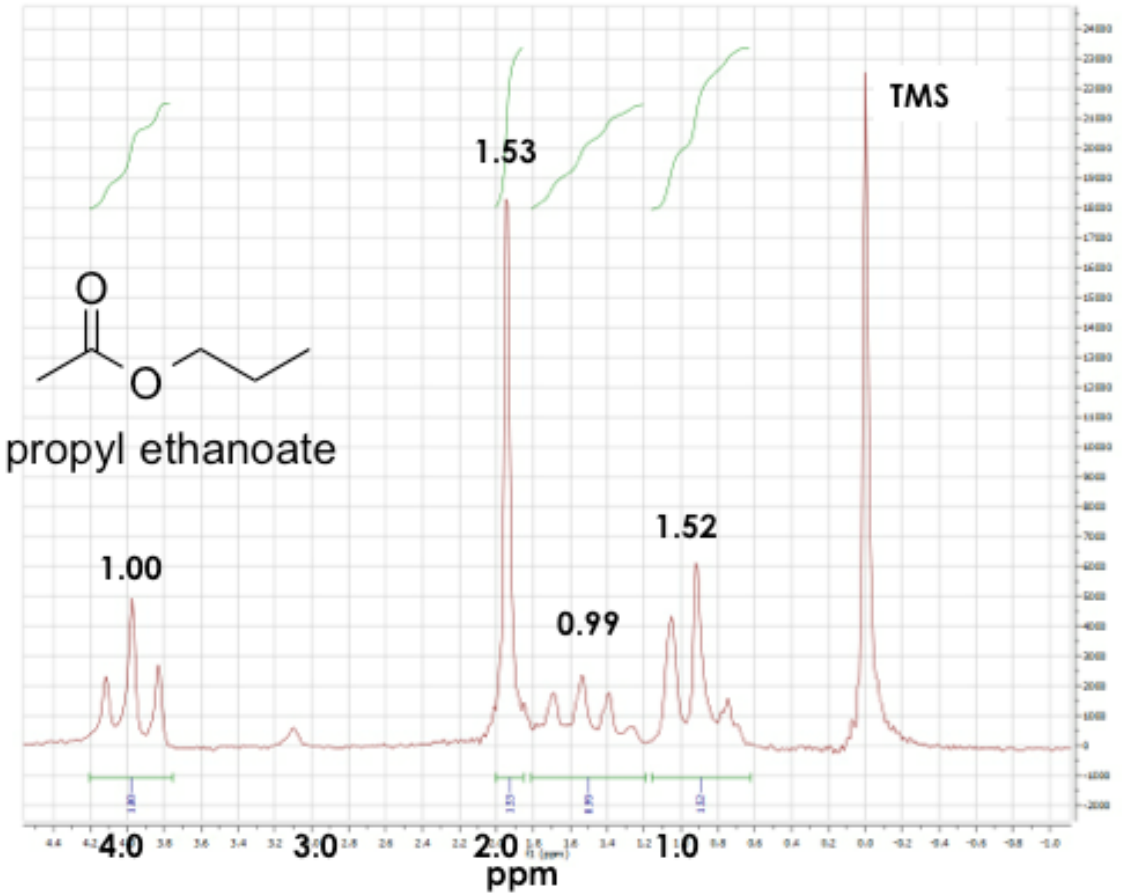


Figure 21. Typical student PicoSpin™ ^{1}H NMR

There was no observable improvement in unknown identification for lab sections meeting later in the week or later in the day indicating that any communication between students was ineffective in aggregate. Ideally this trend will hold from semester to semester as the unknown identities can be cycled.

Summary and Outlook

The new PicoSpin™ NMR-adapted microwave Fischer esterification experiment has been effectively implemented in the second semester sophomore organic chemistry laboratory. The increased reaction rate provided by the microwave procedure combined with the use of the new bench-top PicoSpin™ 45

MHz ^{1}H NMR instruments allows for student spectral analysis within the 3-hour lab period with minimal teaching assistant input. The generality of the experiment provides for facile incorporation of unknowns, which allows the instructor to accurately assess student laboratory technique as well as student ability to analyze spectra. Future modifications of this experiment will be to incorporate even more unknowns through the use of higher field PicoSpin™ 90 MHz instruments. Based on the increased student involvement in spectral acquisition even in large student sections, with up to 20 ^{1}H NMR experiments run by students within the 3-hour lab period, it is clear that use of PicoSpin™ NMR analysis can and should be expanded in the curriculum. Supporting Information for this experiment may be viewed in Appendix E.

CHAPTER 8

CONCLUSIONS AND OUTLOOK

Successful student engagement and effective assessment of student learning in the educational laboratory are evolving challenges that require dynamic solutions. Challenges including lack of problem solving, limited experimental variability, and student access to copious Internet resources have been addressed. The educational laboratory experiments presented here facilitate student engagement through problem solving, integration of instrumental analysis, and inclusion of unknown elements while also allowing assessment of student learning. These experiments are in stark contrast to traditional laboratory experiments relying upon single or predictable outcomes that all but assume students enter the laboratory with no prior knowledge of the experiment and are intellectually isolated when performing post lab analysis.

The "Decarboxylation of Amino Acids" experiment introduces a new class of reactions underrepresented in traditional experiments. It allows the instructor to introduce microwave and imine chemistry, chemical equilibria, and the common practice for the purification of amines as their hydrochloride salts. Instructors are able to directly assess the ability of individual students to properly perform the reaction workup and purification procedures via the inspection of the ^{1}H NMR spectrum for impurities. The ability of students to operate the IR and NMR instruments is essential as sharing of spectra is made impractical by the TA log

sheet. Correct student ^{1}H NMR interpretation is assessed through the inclusion of unknowns.

The "Luche Reduction" experiment is a versatile experiment for the instructor to assess a variety of learning outcomes. The inclusion of a useful synthetic reaction forming stereoisomers encourages a discussion of chemical reactivity in stereoisomerism over traditional experiments analyzing unknown stereoisomer mixtures. Students' execution of the experimental protocol is assessed more reliably given that incomplete removal of solvent under reduced pressure leads to poor polarimetry values or a large solvent signal in the ^{1}H NMR. The highly variable unknown product ratio of (-)-menthol:(+)-neomenthol ensures that experimental procedures and product analysis are performed precisely and accurately. The versatility that accompanies the use of GC, ^{1}H NMR, or polarimetry for quantitative analysis of product ratios and the IR, GC, or TLC for the analysis of reaction completion gives the instructor a high degree of variability in the experimental protocol. Future development of other stereoselective tuning procedures introduces the potential to expand the ability of instructors to select multiple stereochemical outcomes in many other experiments.

The "Epoxide Ring-Opening" experiment contains both an unknowns-based protocol and an element of discovery. Students are given an unknown alcohol or alkoxide nucleophile and are required to identify reaction products via comparison to GC standards. This experimental approach allows instructors to assess student ability to interpret chromatograms and perform quantitative analysis. The ability of students to organize and manipulate bulk data using a spreadsheet is also

assessed. The log sheets of student retention times and corresponding peak areas are uploaded to the course webpage for student interpretation. Furthermore, student abilities to draw conclusions based on the aggregate data are also assessed; however, future variations of this experiment will need to be implemented to ensure that the trend in the data remains unknown to the students as well.

As highlighted in the "Decarboxylation" and "Fischer Esterification" experiments, the use of microwave chemistry and PicoSpin™ NMR analysis has also been shown to be an effective combination for providing an extensive hands-on spectroscopy experience. Both of these experiments use microwave chemistry to drastically shorten reflux times, thereby allowing for spectroscopic analysis including IR and ^{1}H NMR. Use of the PicoSpin™ instruments allows for the placement of a bench-top instrument in every lab section that is robust enough for students to perform their own ^{1}H NMR spectroscopy rather than the disconnected experience of handing over the sample to the TA. Operating the PicoSpin™ and interface only differs from the experience of using traditional instruments in the means of sampling, injection vs. use of a tube and spinner.

These experiments have been effective additions to the University of Georgia educational laboratory curriculum for multiple semesters. Furthermore, they have not been developed to be a part of a static curriculum, but rather to add to the growing library of possible experiments that may be incorporated into an effective sophomore organic chemistry laboratory program. The key elements of unknowns and experimental variability are what allow laboratory coordinators to continually encourage critical thinking and engagement inside the laboratory rather than

promoting Internet searches for expected laboratory results. Thus, ongoing work in the area of experimental development is a necessity for maintaining meaningful and engaging instruction.

REFERENCES

1. (a) Mohrig, J. R., The problem with organic chemistry labs. *J. Chem. Educ.* **2004,** *81*, 1083-1084; (b) Horowitz, G. J., The state of organic teaching laboratories. *J. Chem. Educ.* **2007,** *84*, 346; (c) Bopegedera, A. M. R. P., Putting the laboratory at the center of teaching chemistry. *J. Chem. Educ.* **2004,** *88*, 443-448.

2. Wade, L. G., Chemistry without a cookbook: an effective alternative. *J. Chem. Educ.* **1979,** *56*, 825-826.

3. (a) Lee, S.-H.; Mukherjee, S.; Brewer, B.; Ryan, R.; Yu, H.; Gangoda, M., A laboratory experiment to measure Henry's Law constants of volatile organic compounds with a bubble column and a gas chromatography flame ionization detector. *J. Chem. Educ.* **2013,** *90*, 495-499; (b) Wharry, D. L., The Study of Substitution and Elimination Reactions Using Gas Chromatography: An Examination of the Effects of Alkane and Base Structure on Product Distributions. *J. Chem. Educ.* **2011,** *88*, 1720-1723; (c) Csizmar, C. M.; Force, D. A.; Warner, D. L., Implementation of Gas Chromatography and Microscale Distillation into the General Chemistry Laboratory Curriculum as Vehicles for Examining Intermolecular Forces. *J. Chem. Educ.* **2011,** *88*, 966-969.

4. (a) Kusch, P., Identification of synthetic polymers and copolymers by analytical pyrolysis-gas chromatography mass spectrometry. *J. Chem. Educ.* **2014,** *91*, 1725-1728; (b) Azman, A. M.; Barrett, J. A.; Darragh, M.; Esteb, J. J.; McNulty, L. M.; Morgan, P. M.; O'Reilly, S. A.; Wilson, A. M., Adding gas chromatography mass

spectrometry to a melting point and thin-layer chromatography laboratory. *J. Chem. Educ.* **2013,** *90*, 140-141; (c) Giarikos, D. G.; Patel, S.; Lister, A.; Razeghifard, R., Incorporation of Gas Chromatography–Mass Spectrometry into the Undergraduate Organic Chemistry Laboratory Curriculum. *J. Chem. Educ.* **2013,** *90*, 106-109; (d) Johnson, B. O.; Burke, F. M.; Harrison, R.; Burdette, S., Quantitative Analysis of Bisphenol A Leached from Household Plastics by Solid–Phase Microextraction and Gas Chromatography–Mass Spectrometry (SPME–GC–MS). *J. Chem. Educ.* **2012,** *89*, 1555-1560; (e) Clennan, M. M.; Clennan, E. L., Dehydration of Methylcyclohexanol Isomers in the Undergraduate Organic Laboratory and Product Analysis by Gas Chromatography−Mass Spectroscopy (GC−MS). *J. Chem. Educ.* **2011,** *88*, 646-648.

5. (a) McEvoy, J. P., Characterizing Carbonyls with Infrared Spectroscopy: An Introductory Chemistry Experiment in a Molecular Bioscience Program. *J. Chem. Educ.* **2014,** *91*, 726-729; (b) Leong, J.; Ackroyd, N. C.; Ho, K., Collaborative Student Laboratory Exercise Using FT-IR Spectroscopy for the Kinetics Study of a Biotin Analogue. *J. Chem. Educ.* **2014,** *91*, 1073-1076; (c) Conklin, A.; Goldcamp, M. J.; Barrett, J., Determination of Ethanol in Gasoline by FT-IR Spectroscopy. *J. Chem. Educ.* **2014,** *91*, 889-891; (d) Wan, H.; Djokic, N.; Brown, B. A.; Kwon, Y., Using Whiskey-Flavoring Compounds To Teach Distillation and IR Spectroscopy to First-Semester Organic Chemistry Students. *J. Chem. Educ.* **2014,** *91*, 123-125; (e) Pavel, J. T.; Hyde, E. C.; Bruch, M. D., Structure Determination of Unknown Organic Liquids Using NMR and IR Spectroscopy: A General Chemistry Laboratory. *J. Chem. Educ.* **2012,** *89*, 1450-1453; (f) Livengood, K.; Lewallen, D. W.; Leatherman, J.; Maxwell, J. L., The Use and Evaluation of Scaffolding, Student Centered-Learning, Behaviorism,

and Constructivism To Teach Nuclear Magnetic Resonance and IR Spectroscopy in a Two-Semester Organic Chemistry Course. *J. Chem. Educ.* **2012,** *89*, 1001-1006.

6. (a) Paciliio, J. E.; Tokarski, J. T.; Quinones, R.; Iuliucci, R. J., High-Resolution Solid-State NMR Spectroscopy: Characterization of Polymorphism in Cimetidine, a Pharmaceutical Compound. *J. Chem. Educ.* **2014,** *91*, 1236-1239; (b) Chamberlain, P. H., Identification of an Alcohol with 13C NMR Spectroscopy. *J. Chem. Educ.* **2013,** *90*, 1365-1367; (c) Wagner, C. E.; Marshall, P. A.; Cahill, T. M.; Mohamed, Z., Visually Following the Hydrogenation of Curcumin to Tetrahydrocurcumin in a Natural Product Experiment That Enhances Student Understanding of NMR Spectroscopy. *J. Chem. Educ.* **2013,** *90*, 930-933; (d) Manbeck, K. A.; Boaz, N. C.; BAir, N. C.; Sanders, A. M. S.; Marsh, A. L., Substituent Effects on Keto–Enol Equilibria Using NMR Spectroscopy. *J. Chem. Educ.* **2011,** *88*, 1444-1445; (e) Li, W.; Kagan, G.; Hopson, R.; Williard, P. G., Measurement of Solution Viscosity via Diffusion-Ordered NMR Spectroscopy (DOSY). *J. Chem. Educ.* **2011,** *88*, 1331-1335; (f) Izunobi, J. U.; Higginbotham, C. L., Polymer Molecular Weight Analysis by 1H NMR Spectroscopy. *J. Chem. Educ.* **2011,** *88*, 1098-1104; (g) Fenton, O. S.; Sculimbrene, B. R., A Wet-Lab Approach to Stereochemistry Using 31P NMR Spectroscopy. *J. Chem. Educ.* **2011,** *88*, 662-664; (h) Davis, C. M., Identifying the Isomers of Octahedral Complexes with 119Sn and 207Pb NMR Spectroscopy: A Computational Exercise. *J. Chem. Educ.* **2011,** *88*, 306-308; (i) Linenberger, K.; Bretz, S. L.; Crowder, M. W.; McCarrick, R.; Lorigan, G. A.; Tierny, D. L., What Is the True Color of Fresh Meat? A Biophysical Undergraduate Laboratory Experiment Investigating the Effects of Ligand Binding on Myoglobin Using Optical, EPR, and NMR Spectroscopy. *J. Chem. Educ.* **2011,** *88*,

223-225; (j) Kehlbeck, J. D.; Slack, C. C.; Turnbull, M. T.; Kohler, S. J., Exploring the Hydrolysis of Sucrose by Invertase Using Nuclear Magnetic Resonance Spectroscopy: A Flexible Package of Kinetic Experiments. *J. Chem. Educ.* **2014,** *91*, 734-738; (k) Pelter, M. W.; Walker, N. M., A Discovery-Based Hydrochlorination of Carvone Utilizing a Guided-Inquiry Approach To Determine the Product Structure from 13C NMR Spectra. *J. Chem. Educ.* **2012,** *89*, 1183-1185.

7. (a) Ricci, R. W.; Ditzler, M. A., Discovery chemistry: a laboratory-centered approach to teaching general chemistry. *J. Chem. Educ.* **1991,** *68*, 228-231; (b) Ricci, R. W.; Ditzler, M. A.; Jarret, R.; McMaster, P.; Herrick, R., The Holy Cross Discovery Chemsitry Program. *J. Chem. Educ.* **1994,** *71*, 404-405; (c) Reilly, M. K.; King, R. P.; Wagner, A. J.; King, S. M., Microwave-assisted esterification: a discover-based microscale laboratory experiment. *J. Chem. Educ.* **2014,** *91*, 1706-1709; (d) Bockman, M. R.; Miedema, C. J.; Brennan, B. B., A Discovery-Oriented Approach to Solid-Phase Peptide Synthesis. *J. Chem. Educ.* **2012,** *89*, 1470-1473; (e) Wilczek-Vera, G.; Salin, E. D., Understanding fluorescence measurements through a guided-inquiry and discovery experiment in advanced analytical laboratory. *J. Chem. Educ.* **2011,** *88*, 216-219; (f) Polito, V.; Hamann, C. S.; Rhile, I. J., Carbocation rearrangement in an electrophilic aromatic substitution discovery laboratory. *J. Chem. Educ.* **2010,** *87*, 969-970; (g) Christensen, J. E.; Huddle, M. G.; Rogers, J. L.; Yung, H.; Mohan, R. S., The discovery-oriented apporach to organic chemistry. 7: rearrangement of trans-stilbene oxide with bismuth trifluoromethanesulfonate and other metal triflates. A microscale green organic chemistry laboratory experiment. *J. Chem. Educ.* **2008,** *85*, 1274-1275; (h) Moroz, J. S.; Pellino, J. L.; Field, K. W., A

series of small-scale, discover-based organic laboratory experiments illustrating the concepts of addition, substitution, and rearrangement. *J. Chem. Educ.* **2003,** *80*, 1319-1321; (i) Horowitz, G., A discovery approach to thee organic laboratory techniques: extraction, recrystallization, distillation. *J. Chem. Educ.* **2003,** *80*, 1039-1041.

8. (a) Allen, J. B.; Barker, L. N.; Ramsden, J. H., Guided inquiry laboratory. *J. Chem. Educ.* **1986,** *63*, 533-534; (b) Chatterjee, S.; Williamson, V. M.; McCann, K.; Peck, M. L., Surveying student's attitudes and perceptions toward guided-inquiry and open-inquiry laboratories. *J. Chem. Educ.* **2009,** *86*, 1427-1432; (c) Briggs, M.; Long, G.; Owens, K., Qualitative assessment of inquiry-based teaching methods. *J. Chem. Educ.* **2011,** *88*, 1034-1040; (d) Deters, K. M., Student opinions regarding inquiry-based labs. *J. Chem. Educ.* **2005,** *82*, 1178-1180; (e) Mohrig, J. R.; Hammond, C. N.; Colby, D. A., On the successful use of inquiry-driven experiments in the organic chemistry laboratory. *J. Chem. Educ.* **2007,** *84*, 992-998; (f) Mackay, J. A.; Wetzel, N. R., Exploring the Wittig Reaction: A Collaborative Guided-Inquiry Experiment for the Organic Chemistry Laboratory. *J. Chem. Educ.* **2014,** *91*, 722-725; (g) Xu, H.; Talanquer, V., Effect of the Level of Inquiry on Student Interactions in Chemistry Laboratories. *J. Chem. Educ.* **2013,** *90*, 29-36; (h) Long, R. D., Using Group-Inquiry To Study Differing Reaction Conditions in the E2 Elimination of Cyclohexyl Halides. *J. Chem. Educ.* **2012,** *89*, 672-674; (i) Schepmann, H. G.; Mynderse, M., Ring-Closing Metathesis: An Advanced Guided-Inquiry Experiment for the Organic Laboratory. *J. Chem. Educ.* **2010,** *87*, 721-723; (j) Mohrig, J. R.;

Hammond, C. N.; Schatz, P. F., *Techniques in Organic Chemistry: Miniscale, Standard Taper Microscale, and Williamson Microscale, 3d ed.* W. H. Freeman: 2010.

9. (a) Carlo, D. D.; Mazzaro, D.; Page, S., High school student's perceptions of their laboratory classroom and the copying of laboratory work. *J. Chem. Educ.* **2006,** *83*, 1362-1367; (b) Charlesworth, P., Student's perspectives of the influence of web-enhanced coursework on incidences of cheating. *J. Chem. Educ.* **2006,** *83*, 1368-1375.

10. Pavia, D. L.; Lampman, G. M.; Kriz, G. S.; Engel, R. G., *A Microscale Approach to Organic Laboratory Techniques, Fifth Ed.* Brooks/Cole: Belmont, CA, 2012.

11. (a) Feist, P. L., The separation and identification of two uknown solid organic compounds: an experiment for the sophomore organic chemistry laboratory. *J. Chem. Educ.* **2004,** *81*, 109-110; (b) Chapman, O. L.; Russell, A. A., Structure, chirality, and FT-NMR in sophomore organic chemistry. A modern approach to teaching. *J. Chem. Educ.* **1992,** *69*, 779-782; (c) Shine, T. D.; Glagovich, N. M., Organic spectroscopy laboratory: utilizing IR and NMR in the identification of an unknown substance. *J. Chem. Educ.* **2005,** *82*, 1382-1384; (d) Wietstock, S. M.; Peterson, K. A.; Lashua, D. M. G.; Miller, D. A.; Johnson, J. F., NMR spectrscopy in the undergraduate curriculum at the University of Notre Dame. In *ACS Symposium Series*, American Chemical Society: Washington DC, 2013; Vol. 1128, pp 275-289.

12. (a) McElveen, S. R.; Gavardinas, K.; Stamberger, J. A.; Mohan, R. S., The discovery-oriented approach to organic chemistry. 1. Nitration of unknown organic compounds. An exercise in 1H NMR and 13C NMR spectroscopy for sophomore organic laboratories. *J. Chem. Educ.* **1999,** *76*, 535-536; (b) Callam, C. S.; Lowary, T.

L., Suzuki cross-coupling reactions: synthesis of unsymmetrical biaryls in the organic laboratory. *J. Chem. Educ.* **2001,** *78*, 947-948; (c) Treadwell, E. M.; Lin, T.-Y., A more challenging interpretative nitration experiment employing substituted benzoic acids and acetanilides. *J. Chem. Educ.* **2008,** *85*, 1541-1543; (d) Gandhari, R.; Maddukuri, P. P.; Vinod, T. K., Oxidation of aromatic aldehydes using oxone. *J. Chem. Educ.* **2007,** *84*, 852-854; (e) Demchenko, A. V.; Pornsuriyasak, P.; Meo, C. D., Acetal Protecting Groups in the Organic Laboratory: Synthesis of Methyl 4,6-O-Benzylidene-α-D-Glucopyranoside. *J. Chem. Educ.* **2006,** *83*, 782-783; (f) Mayo, D.; Pike, R.; Forbes, D., *Microscale Organic Laboratory with Multistep and Multiscale Syntheses, 6th ed.* Wiley: Hoboken, NJ, 2013.

13. (a) Lidstrom, P.; Tierney, J.; Wathey, B.; Westman, J., Microwave assisted organic synthesis-a review. *Tetrahedron* **2001,** *57*, 9225-9283; (b) Baig, R. B. N.; Varma, R. S., Alternative energy input: mechanochemical, microwave and ultrasound-assisted organic synthesis. *Chem. Soc. Rev.* **2012,** *41*, 1559-1584; (c) Polshettiwar, V.; Varma, R. S., Aqueous microwave chemistry. A clean and green synthetic tool for rapid drug discovery. *Chem. Soc. Rev.* **2008,** *37*, 1546-1557; (d) Dallinger, D.; Kappe, C. O., Microwave-assisted synthesis in water as solvent. *Chem. Rev.* **2007,** *107*, 2563-2591; (e) Kappe, C. O., Controlled microwave heating in organic synthesis. *Angew. Chem. Int. Ed.* **2004,** *43*, 6250-6284; (f) Kempe, K.; Becer, C. R.; Schubert, U. S., Microwave-assisted polymerizations: recent status and future perspectives. *Macromolecules* **2011,** *44*, 5825-5842; (g) Zhu, Y.-J.; Chen, F., Microwave-assisted preparation of inorganic nanostructures in liquid phase. *Chem. Rev.* **2014,** *114*, 6462-6555.

14. (a) Dressen, M. H. C. L.; van-de-Kruijs, B. H. P.; Meuldijk, J.; Vekemans, J. A. J. M.; Hulshof, L. A., Flow processing of microwave-assisted (heterogeneous) organic reactions. *Org. Process Res. Dev.* **2010,** *14*, 351-361; (b) Dallinger, D.; Lehmann, H.; Moseley, J. D.; Stadler, A.; Kappe, C. O., Scale-up of microwave-assisted reactions in a multimode bench-top reactor. *Org. Process Res. Dev.* **2011,** *15*, 841-854; (c) Patil, N. G.; Benaskar, F.; Rebrov, E. V.; Meuldijk, J.; Hulshof, L. A.; Hessel, V.; Schouten, J. C., Scale-up of microwave assisted flow synthesis by transient processing through monomode cavities in series. *Org. Process Res. Dev.* **2014**, ASAP.

15. Kappe, C. O.; Pieber, B.; Dallinger, D., Microwave effects in organic synthesis: myth or reality? *Angew. Chem. Int. Ed.* **2013,** *52*, 1088-1094.

16. Baar, M. R.; Gammerdinger, W.; Leap, J.; Morales, E.; Shikora, J.; Weber, M. H., Pedagogical comparison of five reactions performed under micrwave heating in multi-mode versus mono-mode ovens: Diels-Alder cycloaddition, Wittig salt formation, E2 dehydrohalogenation to form an alkyne, Williamson ether synthesis, and Fischer esterification. *J. Chem. Educ.* **2014,** *91*, 1720-1724.

17. (a) Tian, J.; Yan, L.; Sang, A.; Yuan, H.; Zheng, B.; Xiao, D., Microwave-Assisted Synthesis of Red-Light Emitting Au Nanoclusters with the Use of Egg White. *J. Chem. Educ.* **2014,** *91*, 1715-1719; (b) Dziedzic, R. M.; Gillian-Daniel, A. L.; Petersen, G. M.; Martinez-Hernandez, K. J., Microwave Synthesis of Zinc Hydroxy Sulfate Nanoplates and Zinc Oxide Nanorods in the Classroom. *J. Chem. Educ.* **2014,** *91*, 1710-1714; (c) Damkaci, F.; Dallas, M.; Wagner, M., A Microwave-Assisted Friedel–Crafts Acylation of Toluene with Anhydrides. *J. Chem. Educ.* **2013,** *90*, 390-392; (d) Shell, T. A.; Shell, J. R.; Poole, K. A.; Guetzloff, T. F., Microwave-Assisted Synthesis of N-

Phenylsuccinimide. *J. Chem. Educ.* **2011,** *88*, 1439-1441; (e) Pohl, N. L. B.; Kirshenbaum, K.; Yoo, B.; Schulz, N.; Zea, C. J.; Streff, J. M.; Schwarz, K. L., Student-Driven Design of Peptide Mimetics: Microwave-Assisted Synthesis of Peptoid Oligomers. *J. Chem. Educ.* **2011,** *88*, 999-1001; (f) Horta, J. E., Simple Microwave-Assisted Claisen and Dieckmann Condensation Experiments for the Undergraduate Organic Chemistry Laboratory. *J. Chem. Educ.* **2011,** *88*, 1014-1015; (g) Coursindel, T.; Martinez, J.; Parrot, I., Microwave-Assisted Hydantoins Synthesis on Solid Support. *J. Chem. Educ.* **2010,** *87*, 640-642; (h) Baar, M. R.; Falcone, D.; Gordon, C., Microwave-Enhanced Organic Syntheses for the Undergraduate Laboratory: Diels–Alder Cycloaddition, Wittig Reaction, and Williamson Ether Synthesis. *J. Chem. Educ.* **2010,** *87*, 84-86.

18. Morrison, R. W.; Jackson, D. M. Method for Decarboxylation of Amino Acids via Imine formation. 2014.

19. (a) Sanchez-Jimenez, F.; Ruiz-Perez, M. V.; Urdiales, J. L.; Medina, M. A., Pharmacological potential of biogenic amine-polyamine interactions beyond neurotransmission. *Brit. J. Pharmacol.* **2013,** *170*, 4-16; (b) Nuutinen, S.; Panula, P., Histamine in neurotransmission and brain diseases. *Adv. Exp. Med. Biol.* **2010,** *709*, 95-107; (c) Lodge, D. J.; Grace, A. A., Hippocampal dysregulation of dopamine system function and the pathophysiology of schizophrenia. *Trends Pharmacol. Sci.* **2011,** *32*, 507-513; (d) Tiligada, E.; Kyriakidis, K.; Chazot, P. L.; Passani, M. B., Histamine pharmacology and new CNS drug targets. *CNS Neurosci. Ther.* **2011,** *17*, 620-628.

20. Dere, E.; Ambree, O.; Zlomuzica, A., Histidine decarboxylase. In *Amino Acids in Human Nutrition and Health*, D'Mello, J. P. F., Ed. 2012; pp 89-102.

21. (a) Lovenberg, W.; Weissbach, H.; Undenfriend, S., Aromatic L-amino acid decarboxylase. *J. Biol. Chem.* **1962,** *237*, 89-93; (b) Zhu, M.-Y.; Juorio, A. V., Aromatic L-amino acid decarboxylase: biological characterization and functional role. *Gen. Pharmacol.* **1995,** *26*, 681-696.

22. Blaschko, H., The specific action of l-dopa decarboxylase. *J. Physiol. Lond.* **1939,** *96*, 50P-51P.

23. (a) S. M. Morris, J., Arginine metabolism: boundaries of our knowledge. *J. Nutr.* **2007,** *137*, 1602S-1609S; (b) Regunathan, S.; Reis, D. J., Characterization of arginine decarboxylase in rat brain and liver: Distinction from ornithine decarboxylase. *J. Neurochem.* **2002,** *74*, 2201-2208; (c) Uzbay, T. I., The pharmacological importance of agmatine in the brain. *Neurosci. Biobehav. Rev.* **2012,** *36*, 502-519.

24. (a) Gantz, I.; Munzert, G.; Tashiro, T., Molecular cloning of the human histamine H2 receptor. *Biochem Biophys Res Commun* **1991,** *178*, 1386-1392; (b) Fukui, H.; Fujimoto, K.; Mizuguchi, H., Molecular cloning of the human histamine H1 receptor gene. *Biochem Biophys Res Commun* **1994,** *201*, 894-901; (c) Lovenberg, T. W.; Roland, B. L.; Wilson, S. J., Cloning and functional expression of the human histamine H3 receptor. *Mol. Pharmacol.* **1999,** *55*, 1101-1107; (d) Oda, T.; Morikawa, N.; Saito, Y., Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. *J. Biol. Chem.* **2000,** *275*, 36781-36786; (e) Jemima, E. A.; Prema, A.; Thangam, E. B., Functional

characterization of histamine H4 receptor on human mast cells. *Mol. Immunol.* **2014,** *62*, 19-28; (f) Vinet, R.; Cortes, M. P.; Alvarez, R.; Depiano, M. A., Bradykinin and histamine-induced cytosolic calcium increase in capillary endothelial cells of bovine adrenal medulla. *Cell Biol. Int.* **2014,** *38*, 1023-1031.

25. Saavedra, J. M., Enzymatic isotopic assay for the presence of B-phenethylamine in brain. *J. Neurochem.* **1974,** *22*, 211-216.

26. Brodie, B. B.; Kuntzman, R.; Hirch, C. W.; Costa, E., Effects of decarboxylase inhibition on the biosynthesis of brain monoamines. *Life Sci.* **1962,** *1*, 81-84.

27. Boulton, A. A.; Juorio, A. V., Cerebral decarboxylation of meta- and para-tyrosine. *Eperientia* **1983,** *39*, 130-134.

28. (a) Ehringer, H.; Hornykiewicz, O., Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in dieseases of the extrapyramidal system. *Klin. Wochenschr.* **1960,** *38*, 1236-1239; (b) Dorszewska, J.; Prendecki, M.; Lianeri, M.; Kozubski, W., Molecular effects of L-dopa therapy in Parkinson's disease. *Current Genomics* **2014,** *15*, 11-17.

29. (a) Masserini, M., Nanoparticles for Brain Drug Delivery. *ISRN Biochem.* **2013**, 1-18; (b) Pardeshi, C. V.; Belgamwar, V. S., Direct nose to brain drug delivery via integrated nerve pathways bypassing the blood-brain barrier: an excellent platform for brain targeting. *Exp. Opin. on Drug Del.* **2013,** *10*, 957-972; (c) Gayam, S. R.; Wu, S.-P., Redox responsive Pd(II) templated rotaxane nanovalve capped mesoporous silica nanoparticles: a folic acid mediated biocompatible cancer-targeted drug delivery system. *J. Mater. Chem. B.* **2014**, Ahead of Print.

30. Numata, Y.; Terui, T.; Okuyama, R., The accelerating effect of histamine on the cutaneous wound-healing process through the action of basic fibroblast growth factor. *J. Invest. Dermatol.* **2006,** *126*, 1403-1409.

31. (a) Ackermann, Z., *Physiol. Chem.* **1910,** *65*, 505; (b) Galat, A.; Friedman, H. L., A new method for the isolation of histamine. *J. Am. Chem. Soc.* **1949,** *71*, 3976-3977.

32. (a) Dose, K., A new method for the preparation of amino acids from amino acid carboxylic acids. *Chemische Berichte* **1957,** *90*, 1251-1258; (b) Curtius; Lederer, *Ber.* **1886,** *19*, 2462; (c) Dose, K., Catalytic decarboxylation of alpha-amino acids. *Nature* **1957,** *179*, 734-735.

33. Ohtsu, H., Histamine Synthesis and Lessons Learned from Histidine Decarboxylase Deficient Mice. In *Histamine in Inflammation*, Thurmond, R. L., Ed. Landes Bioscience and Springer Science: 2010; pp 21-31.

34. Dale, H. H.; Laidlaw, P. P., The physiological action of Beta-iminazolylethylamine *J. Physiol.* **1910,** *41*, 318-344.

35. Neumann, D.; Schneider, E. H.; Seifert, R., Analysis of histamine receptor knockout mice in models of imflammation. *J. Pharmacol. Exp. Therap.* **2014,** *348*, 2-11.

36. Leite-de-Morales, M. C.; Diem, S.; Michel, M. L., Histamine receptor H4 activation positively regulates in vivo IL-4 and IFN-gamma production by invariant NKT cells. *J. Immunol.* **2009,** *182*, 1233-1236.

37. (a) Gerssende, C.; Pascale, J.; Bonnefoy, J.-Y. Polarization of Dendritic Cells using Histamine, EPO WO02078698 (A2). 2002; (b) Hellstrand, K.; Hermodsson, S. Preparation for activation of natural killer cells (NK-cells) said preparation

containing interferon-alpha and histamine, serotonin, amines or substances with corresponding receptor activity, USPTO 5,728,378. 1998.

38. (a) Arnou, C. Methods for Restoration of Histamine Balance, WO 2014/088641 A1. 2014; (b) Pinsker, J. S., Use of Histamine as a Drug Delivery Enhancing Compound for Use in Transmucosal or Transdermal Delivery, US2003143195 A1. **2003**; (c) Hellstrand, K.; Hermodsson, S., Use of Histamine for Elevating Blood Histamine Levels, WO 9925341 A1. **1999**.

39. Yeh, W.-L.; Antxczak, C.; McGolrick, J. D.; Roth, M. J.; Wrona, M. Synthesis of Histamine Dihydrochloride. 2002.

40. (a) Omeis, M.; Koehler, G.; Neumann, M.; Kuebelbaeck, T. Continuous process for decarboxylating carboxylic acids. 2008; (b) Yaegashi, K.; Mikami, M. Process for preparing amines. 2009.

41. Hashimoto, M.; Eda, Y.; Osanai, Y.; Iwai, T.; Aoki, S., A novel decarboxylation of a-amino acids. A facile method of decarboxylation by the use of 2-cyclohexen-1-one as a catalyst. *Chem. Lett.* **1986,** *6*, 893-896.

42. Buchi, G.; Erickson, R. E., Terpenes. II. The Reactions of Carvone and Dihydrocarvone with Methanol in the

Presence of Sulfuric Acid *J. Am. Chem. Soc.* **1954,** *76*, 3493-3495.

43. https://http://www.sigmaaldrich.com/united-states.html.

44. (a) Mundle, S. O. C.; Opinska, L. G.; Kluger, R.; Dicks, A. P., Investigating the mechanism of heteroaromatic decarboxylation using solvent kinetic isotope effects and eyring transition-state theory. *J. Chem. Educ.* **2011,** *88*, 1004-1006; (b) Menders, D. E.; Schoffstall, A. M., Citrus peel additives for one-pot triazole formation

by decarboxylation, nucleophilic substitution, and azide-alkyne cycloaddition reactions. *J. Chem. Educ.* **2011,** *88*, 1582-1585.

45. (a) Gemal, A. L.; Luche, J.-L., Lanthanoids in Organic Synthesis. 6. The Reduction of α-enones by Sodium Borohydride in the Presence of Lanthanoid Chlorides: Synthetic and Mechanistic Aspects. *J. Am. Chem. Soc.* **1981,** *103*, 5456-5459; (b) Kelly, L. F.; Deeble, G. J., Selectivity in Organic Synthesis: Chemo- and Regiospecific Reductions of Carvone. *J. Chem. Educ.* **1986,** *63*, 1107-1108.

46. (a) Marchart, S.; Gromov, A.; Mulzer, J., Total Synthesis of the Antibiotic Branimycin. *Angew. Chem., Int. Ed.* **2010,** *49* (11), 2050-2053; (b) Constantino, M. G.; Matias, L. G. d. O.; Silva, G. V. J. d.; Barbieri, E.; Gambardella, M. T. d. P., Stereoselective Sodium Borohydride Reductions of Cyclopentanones: Influence of Ceric Chloride on the Stereochemistry of Reaction . *Quim. Nova* **1998,** *21* (6), 719-721; (c) Schulz, J.; Beaton, M. W.; Gani, D., Synthesis of (+)-(1R,2R,4R,6S)-1,6-epoxy-4-benzyloxycyclohexan-2-ol, a precursor to inositol monophosphetase inhibitors, from (-)-quinic acid. *J. Chem. Soc. Perkin Trans.* **2000,** *1*, 943-954.

47. Kim, J.; Suri, J. T.; Cordes, D. B.; Singaram, B., Asymmetric Reductions Involving Borohydrides: A Practical Asymmetric Reduction of Ketones Mediated by (L)-TarB-NO2: A Chiral Lewis Acid. *Org. Process Res. Dev.* **2006,** *10*, 949-958.

48. Barry, J., Determination of the relative and absolute configurations of (-)-menthol and (+)neomenthol. An introductory stereochemistry experiment. *J. Chem. Educ.* **1973,** *50*, 292.

49. (a) Wade, E. O.; Walsh, K. E., A Multistep Organocatalysis Experiment for the Undergraduate Organic Laboratory: An Enantioselective Aldol Reaction Catalyzed by Methyl Prolinamide. *J. Chem. Educ.* **2011,** *88*, 1152-1154; (b) Wong, T. C.; Sultana, C. M.; Vosburg, D. A., A Green, Enantioselective Synthesis of Warfarin for the Undergraduate Organic Laboratory. *J. Chem. Educ.* **2010,** *87*, 194-195; (c) Cuccia, L. A.; Koby, L.; Ningappa, J. B.; Dakessian, M., Chiral Crystallization of Ethylenediamine Sulfate. *J. Chem. Educ.* **2005,** *82*, 1043-1045; (d) Burke, A.; Dillon, P.; Martin, K.; Hanks, T. W., Catalytic Asymmetric Epoxidation Using W a Fructose-Derived Catalyst. *J. Chem. Educ.* **2000,** *77*, 271-272; (e) Ginzburg, A. L.; Baca, N. A.; Hampton, P. D., The Isomerization of (−)-Menthone to (+)-Isomenthone Catalyzed by an Ion-Exchange Resin. *J. Chem. Educ.* **2014,** *91*, 1748-1750.

50. Gemal, A. L.; Luche, J.-L., Lanthanoids in Organic Synthesis. 6. The Reduction of α-enones by Sodium Borohydride in the Presence of Lanthanoid Chlorides: Synthetic and Mechanistic Aspects. *J. Am. Chem. Soc.* **1981,** *103*, 5456-5459.

51. (a) Klein, D., *Organic Chemistsry*. 2nd ed.; Wiley: Hoboken, NJ, 2015; p 654-661; (b) McMurry, J., *Organic Chemistry*. 7th ed.; Thompson Learning: Belmont, CA, 2008; p 662-665.

52. (a) Bruice, P. Y., *Organic Chemistry*. 7th ed.; Pearson: Boston, MA, 2014; (b) Wade, L. G., *Organic Chemistry*. 8th ed.; Pearson: Boston, MA, 2013; (c) Hornback, J. M., *Organic Chemistry*. 2nd ed.; Thompson Learning, Inc.: Belmont, CA, 2006; (d)

Jones, M.; Fleming, S. A., *Organic Chemistry*. 5th ed.; W. W. Norton and Co.: New York, NY, 2014; (e) Karty, J., *Organic Chemistry: Principles and Mechanisms*. W. W. Norton and Co.: New York, NY, 2014; p 547-553; (f) Carey, F. A.; Giuliano, R. M., *Organic Chemistry*. 8th ed.; McGraw Hill: New York, NY, 2011; p 700-705.

53. (a) Ciaccio, J. A.; Volpi, S.; Clarke, R., Grignard Reaction of an Epoxide: A Mechanistic Study. *J. Chem. Educ.* **1996,** *73*, 1196-1198; (b) Krishnamurty, H. G.; Jain, N.; Samby, K., Epoxide Chemistry: Guided Inquiry Experiment W Emphasizing Structure Determination and Mechanism. *J. Chem. Educ.* **2000,** *77*, 511-513.

54. (a) Ricci, R. W.; Ditzler, M. A., A Laboratory-Centered Approach to Teaching General Chemistry. *J. Chem. Educ.* **1991,** *68*, 228-231; (b) Ricci, R. W.; Ditzler, M. A.; Jarret, R.; McMaster, P.; Herrick, R., The Holy Cross Chemistry Program. *J. Chem. Educ.* **1994,** *71*, 404-405.

55. (a) Janssens, N.; Wee, L. H.; Martens, J. A., Esterification Reaction Utilizing Sense of Smell and Eyesight for Conversion and Catalyst Recovery Monitoring. *J. Chem. Educ.* **2014,** *91*, 876-879; (b) Clausen, T. P., Combining a Standard Fischer Esterification Experiment with Stereochemical and Molecular-Modeling Concepts. *J. Chem. Educ.* **2011,** *88*, 1007-1009; (c) Smith, J. B.; Byrd, H.; O'Donnell, S. E.; Davis, W., Hammett Parameter and Molecular-Modeling Correlations of Substituent Effects on Esterification Kinetics. *J. Chem. Educ.* **2010,** *87*, 845-847; (d) Broomfield-Lee, D. C.; Oliver-Hoyo, M. T., An Esterification Kinetics Experiment That Relies on the Sense of Smell. *J. Chem. Educ.* **2009,** *86*, 82-84; (e) Gallaher, T. N.; Gaul, D. A.; Schreiner, S., The Esterification of Trifluoroacetic Acid: A Variable Temperature

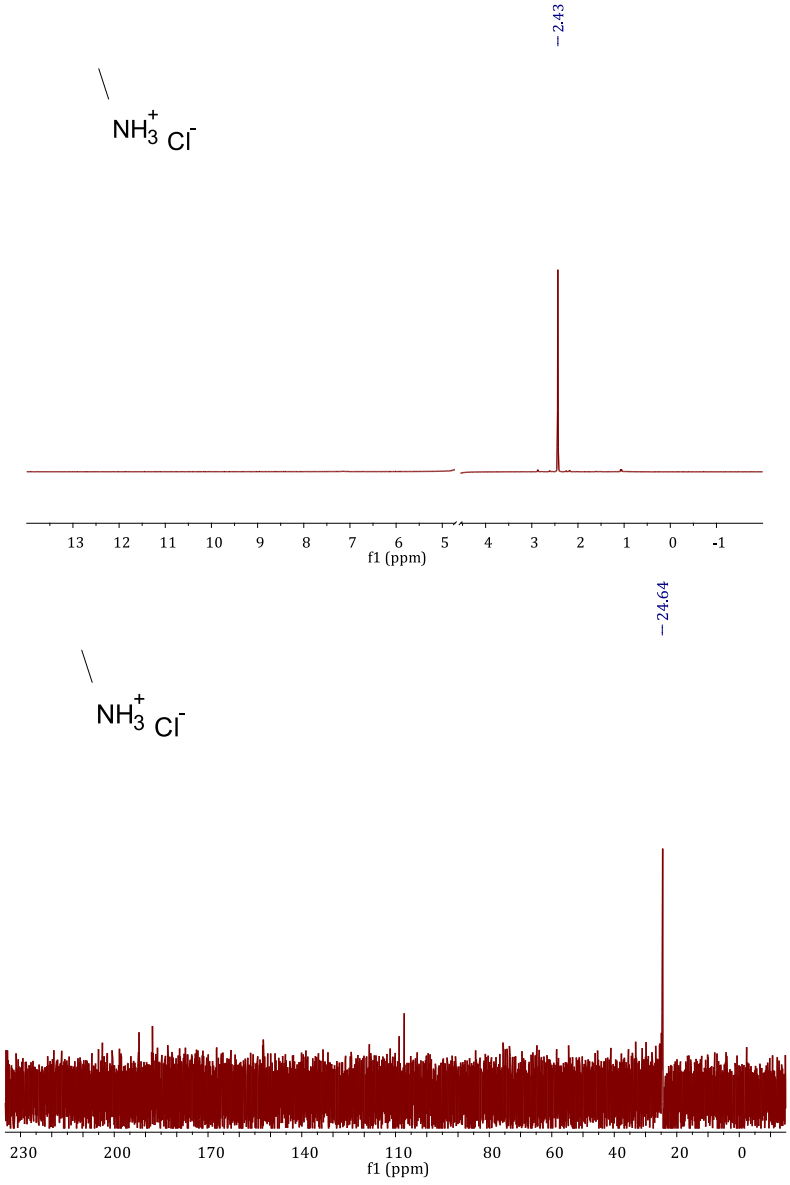
NMR Kinetics Study. *J. Chem. Educ.* **1996,** *73*, 465-467; (f) Sarlo, E.; Svoronos, P., Calculation of Equilibrium Constant in Esterification Reactions. *J. Chem. Educ.* **1990,** *67*, 796; (g) Puterbaugh, W. H.; Vanselow, C. H.; Nelson, K.; Shrawder, E. J., Esterification for the introductory organic laboratory course: A modified Dean-Stark trap. *J. Chem. Educ.* **1963,** *40*, 349.

APPENDIX A

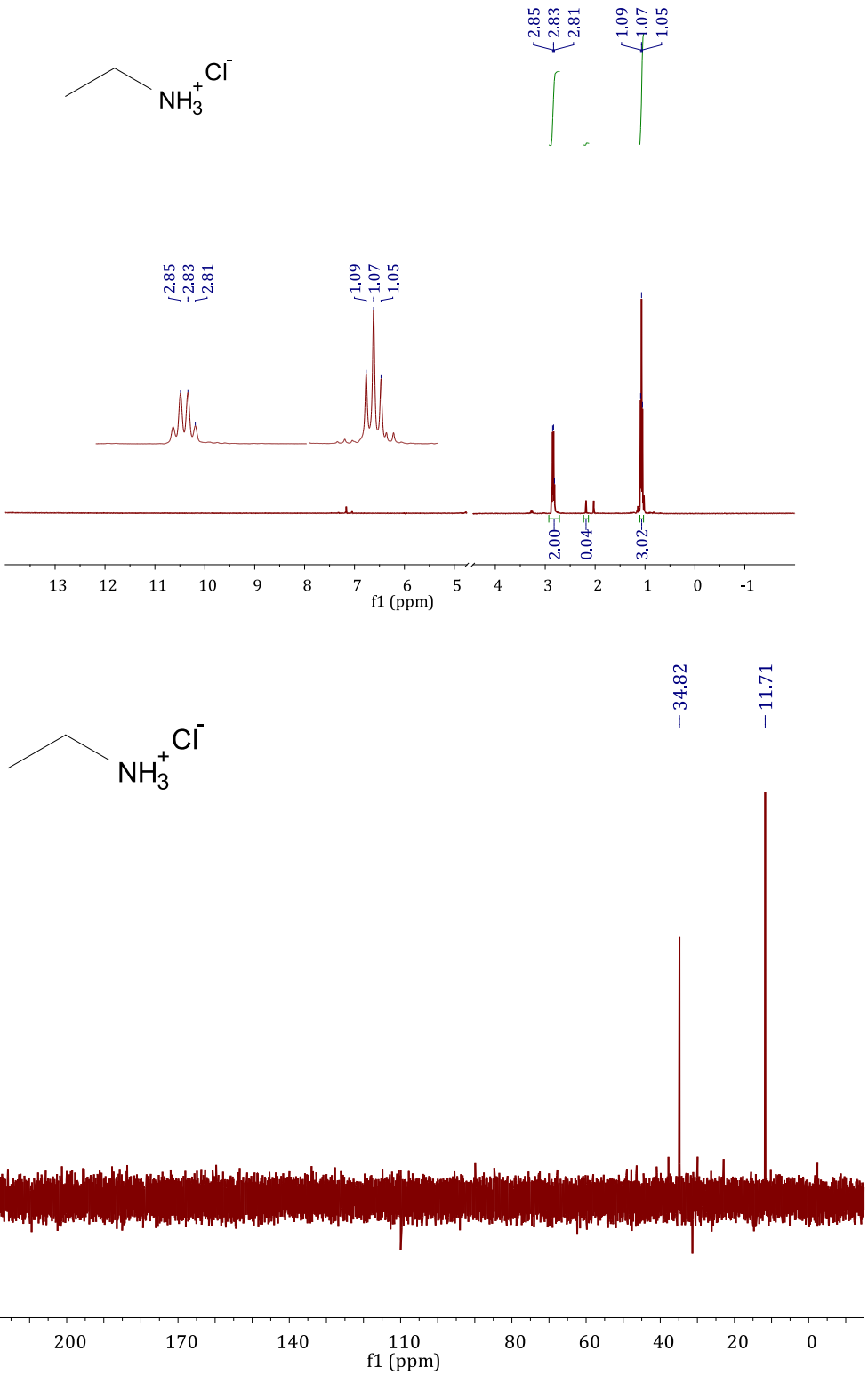
SUPPORTING INFORMATION: CHAPTER 2

Note: D_2O solvent signal is suppressed in all proton spectra for clarity.

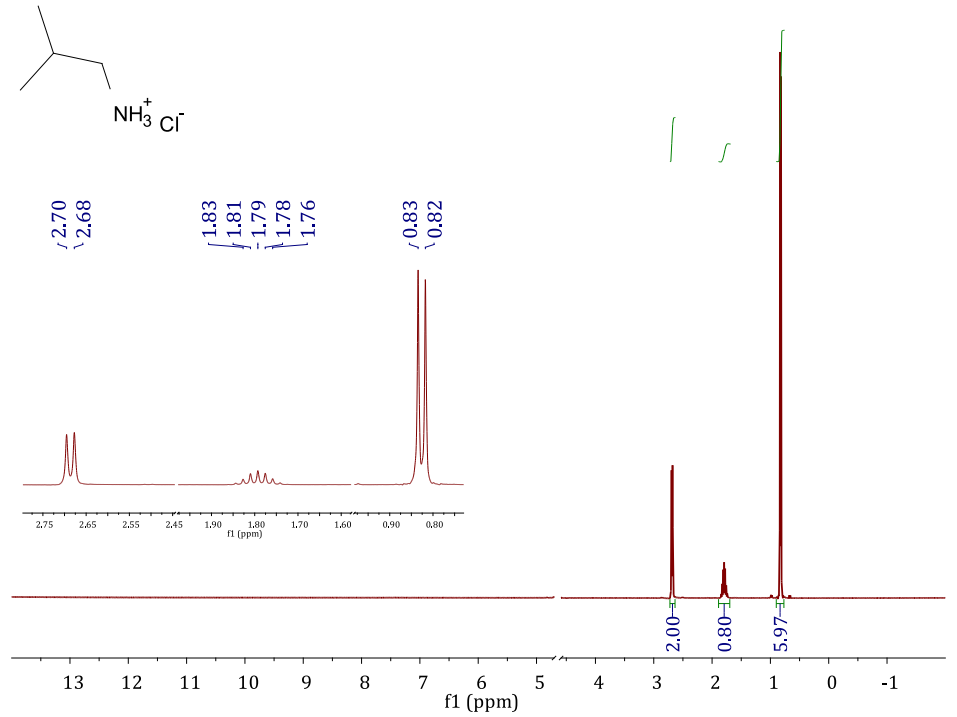
methylamine hydrochloride, 235-237 °C; white powder from PrOH; δH 2.43 (s); δ_C 24.64

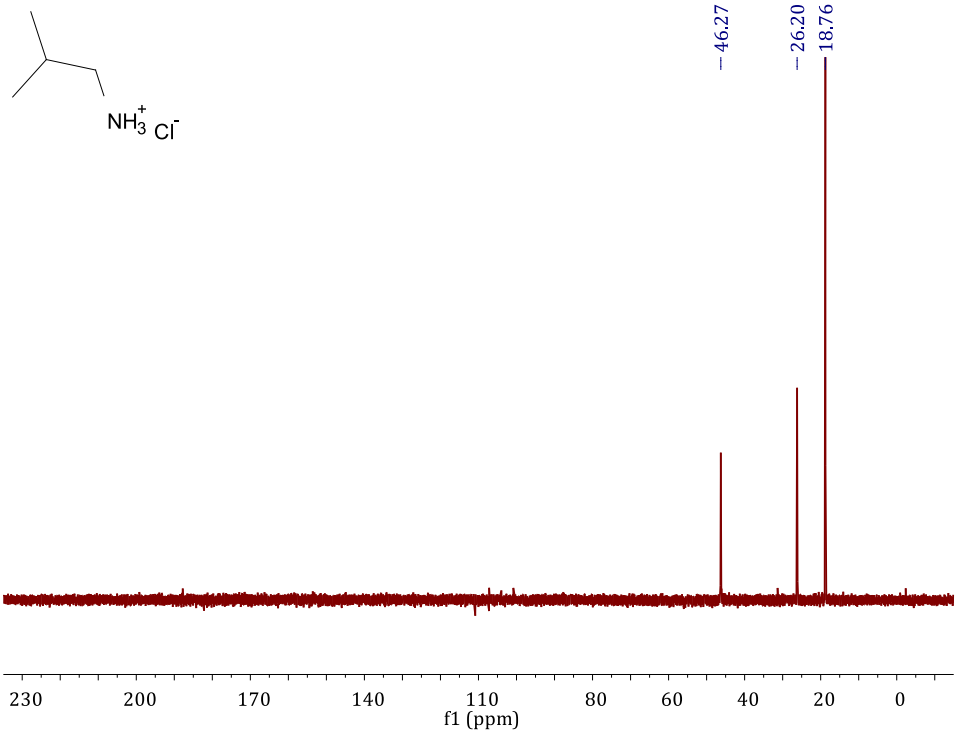


ethylamine hydrochloride, 108-109 °C; white plates from PrOH; δ_H 1.07 (3H t J=8), 2.83 (2H q J=8); δ_C 11.71, 34.82

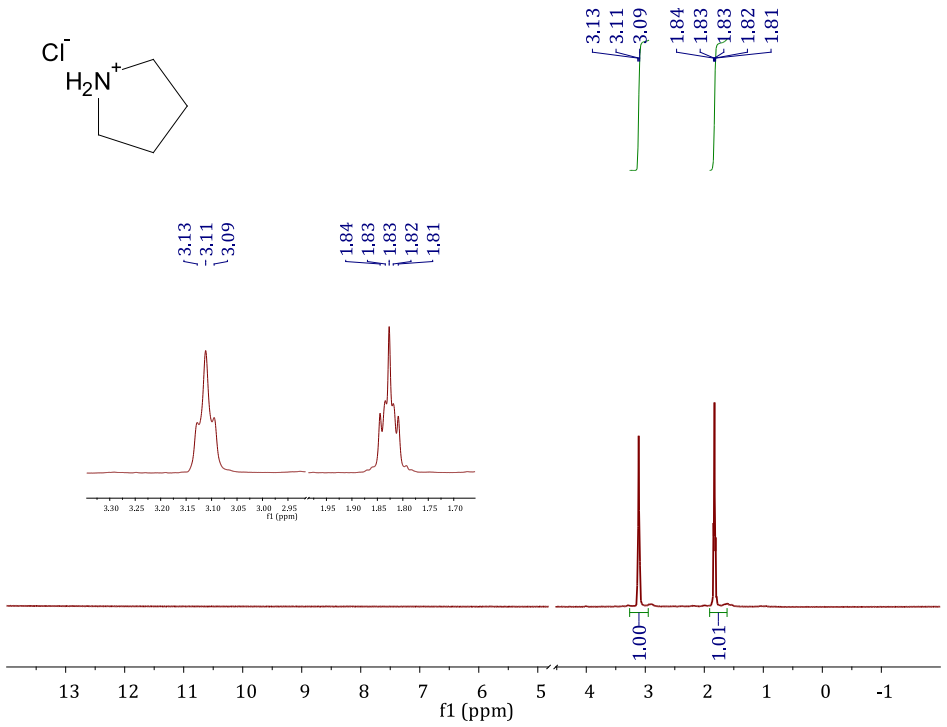


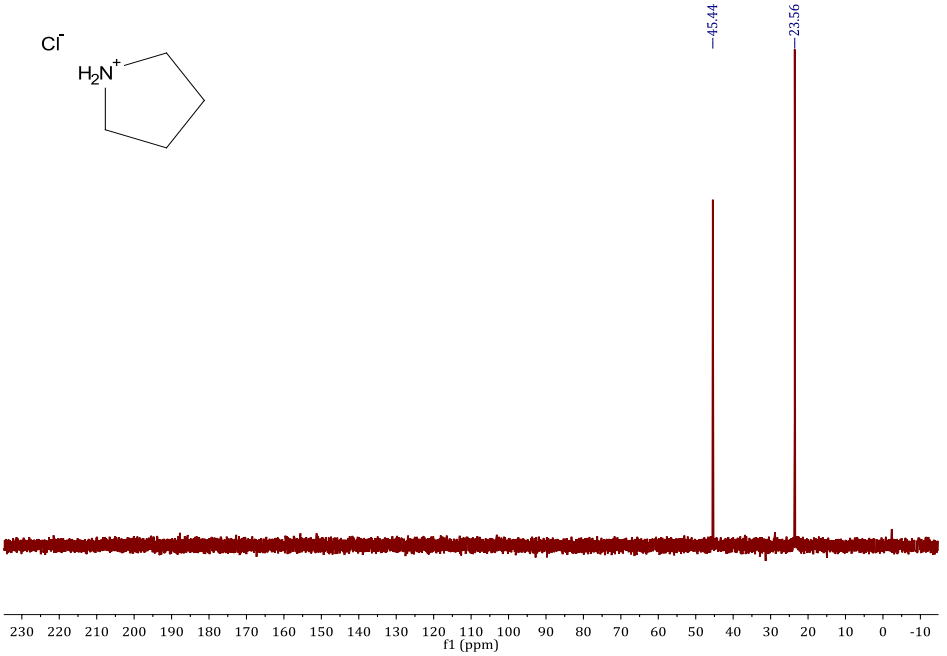
2-methylpropan-1-amine hydrochloride, 178-182 ^{o}C; light brown powder from PrOH; δ_H 0.83 (6H d J=4), 1.79 (1H m J=8), 2.69 (2H d J=8); δ_C 18.76, 26.20, 46.27



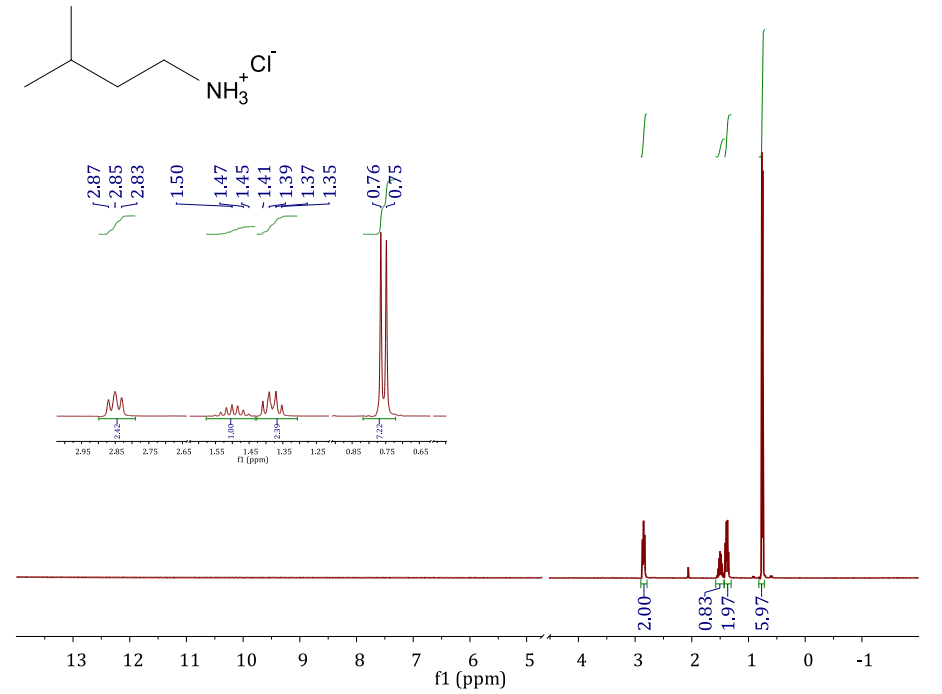


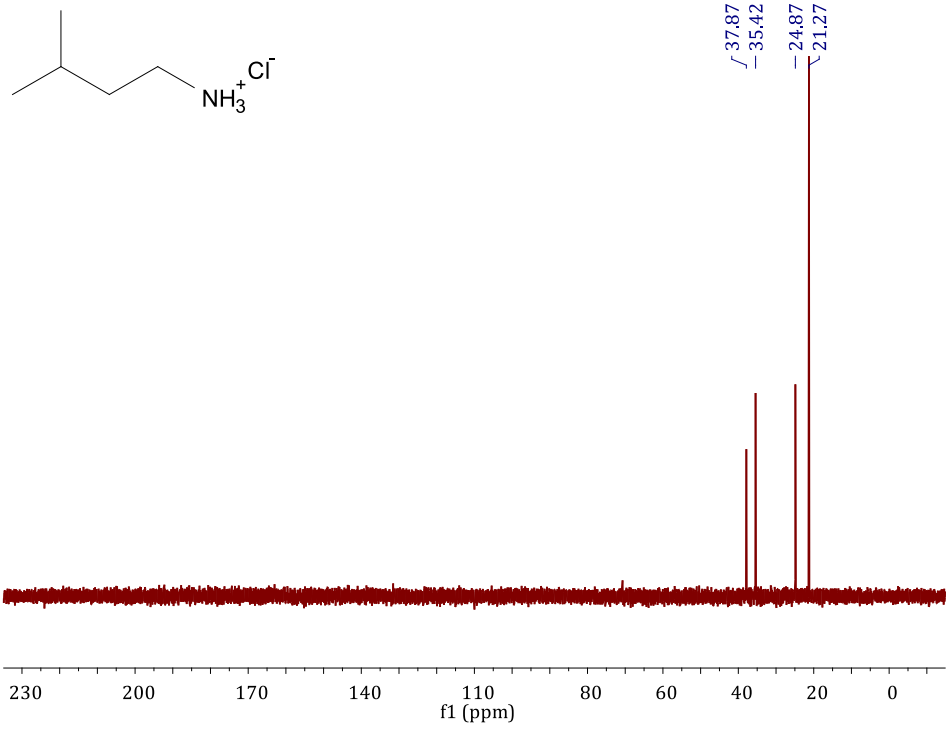
pyrrolidine hydrochloride, δ_H 1.83 (4H t J=4), 3.11 (4H t J=4); δ_C 23.56, 45.44



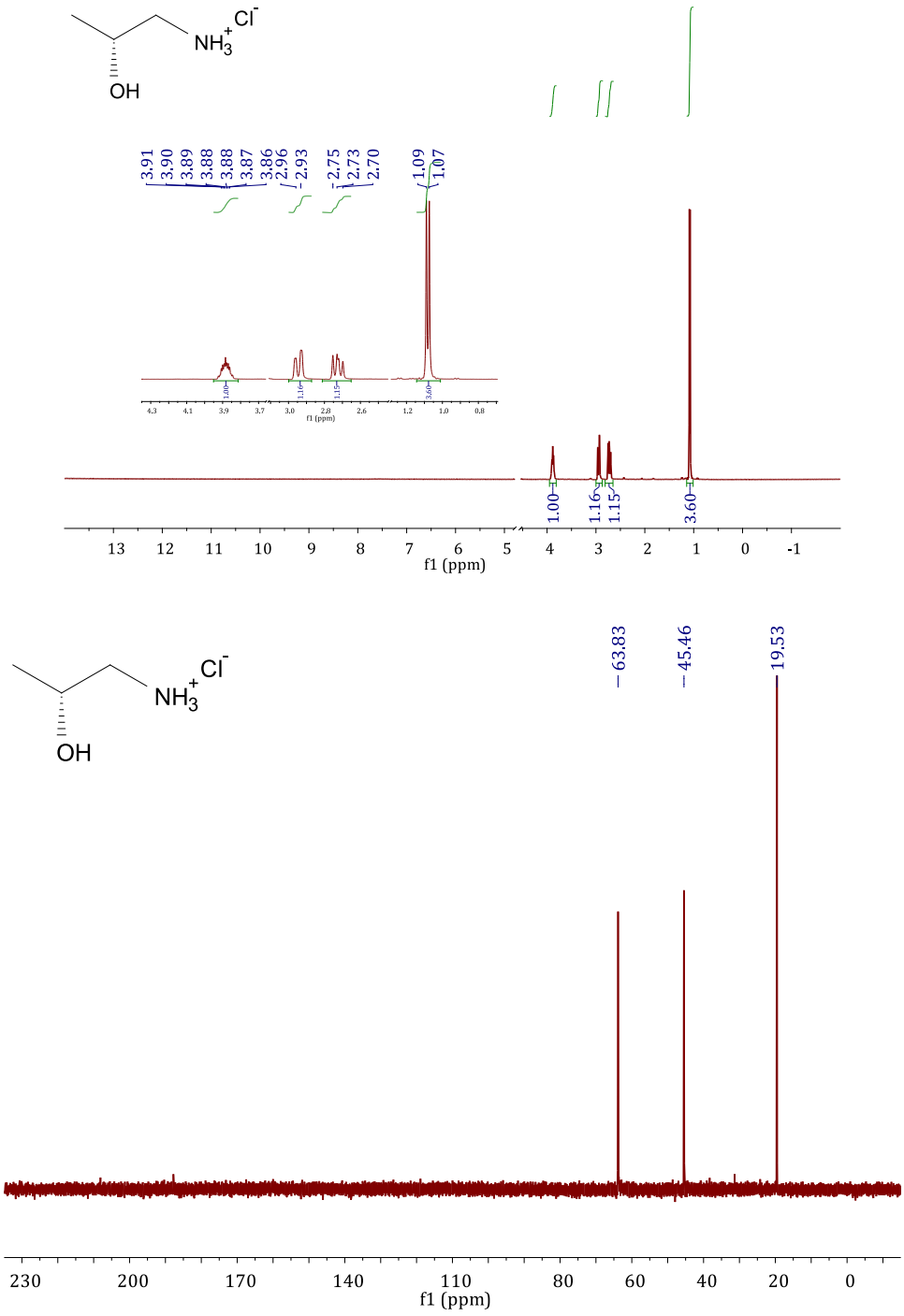


3-methylbutan-1-amine hydrochloride, 213-215 ^{o}C; white powder from hexane; δ_H 0.76 (6H d J=4), 1.38 (2H q J=8), 1.48 (1H m J=8), 2.85 (2H t J=8); δ_C 21.27, 24.87, 35.42, 37.87

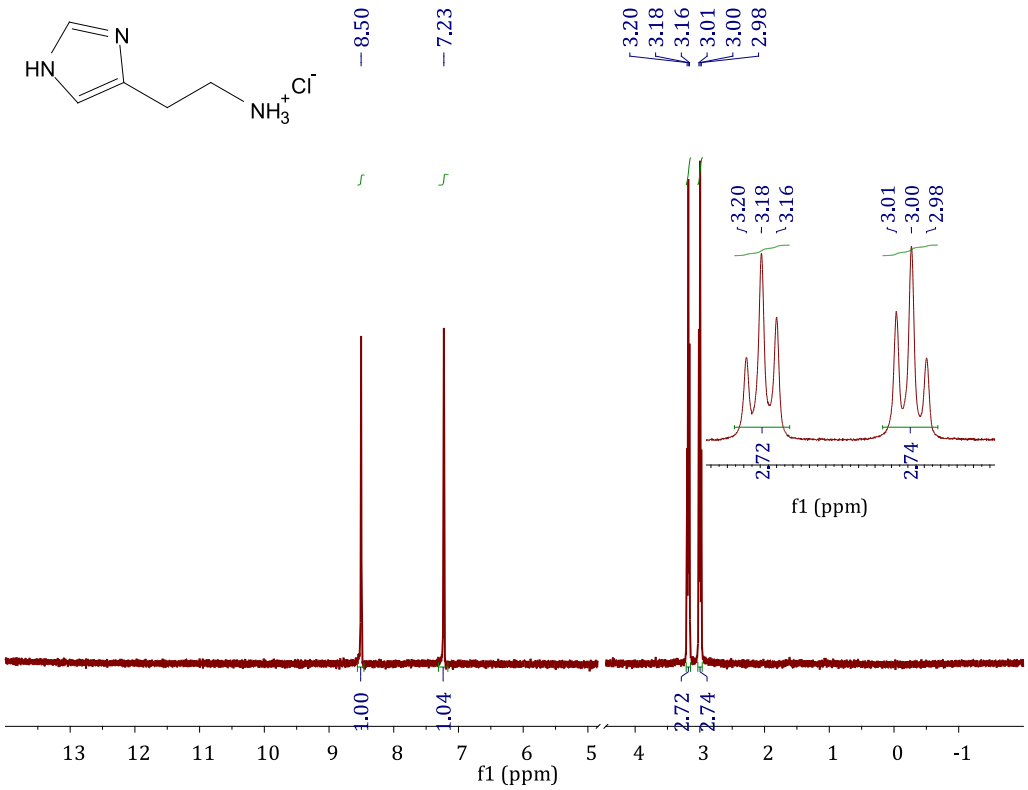


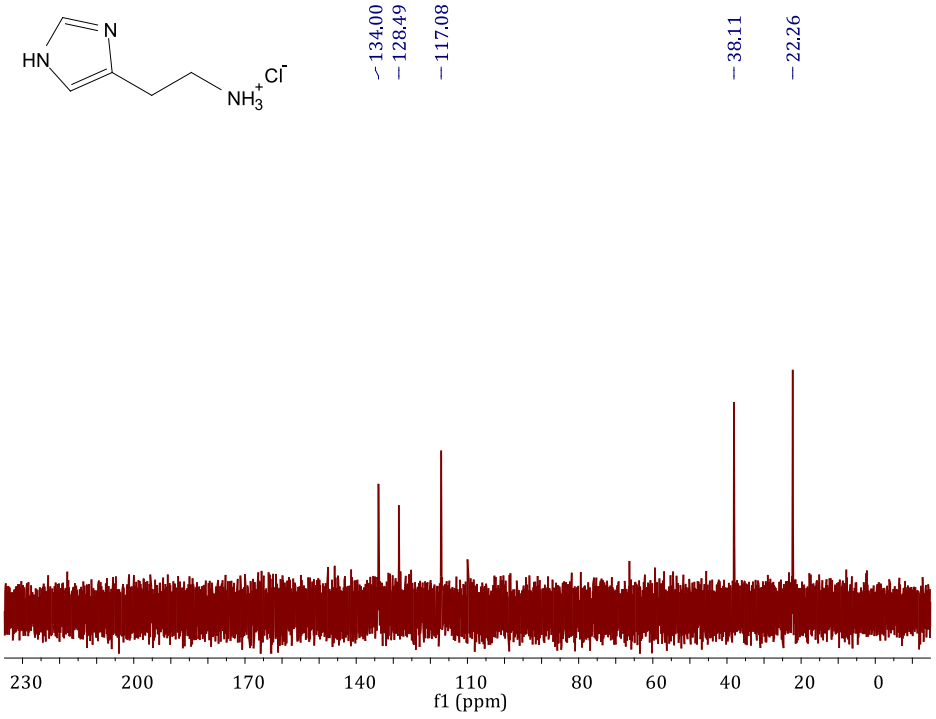


(R)-1-aminopropan-2-ol hydrochloride, 75-80 °C; white powder from EtOH; δ_H 1.08 (3H d J=4), 2.73 (1H dd J=8), 2.95 (1H dd J=12), 3.88 (1H m J=4); δ_C 19.53, 45.46, 63.83

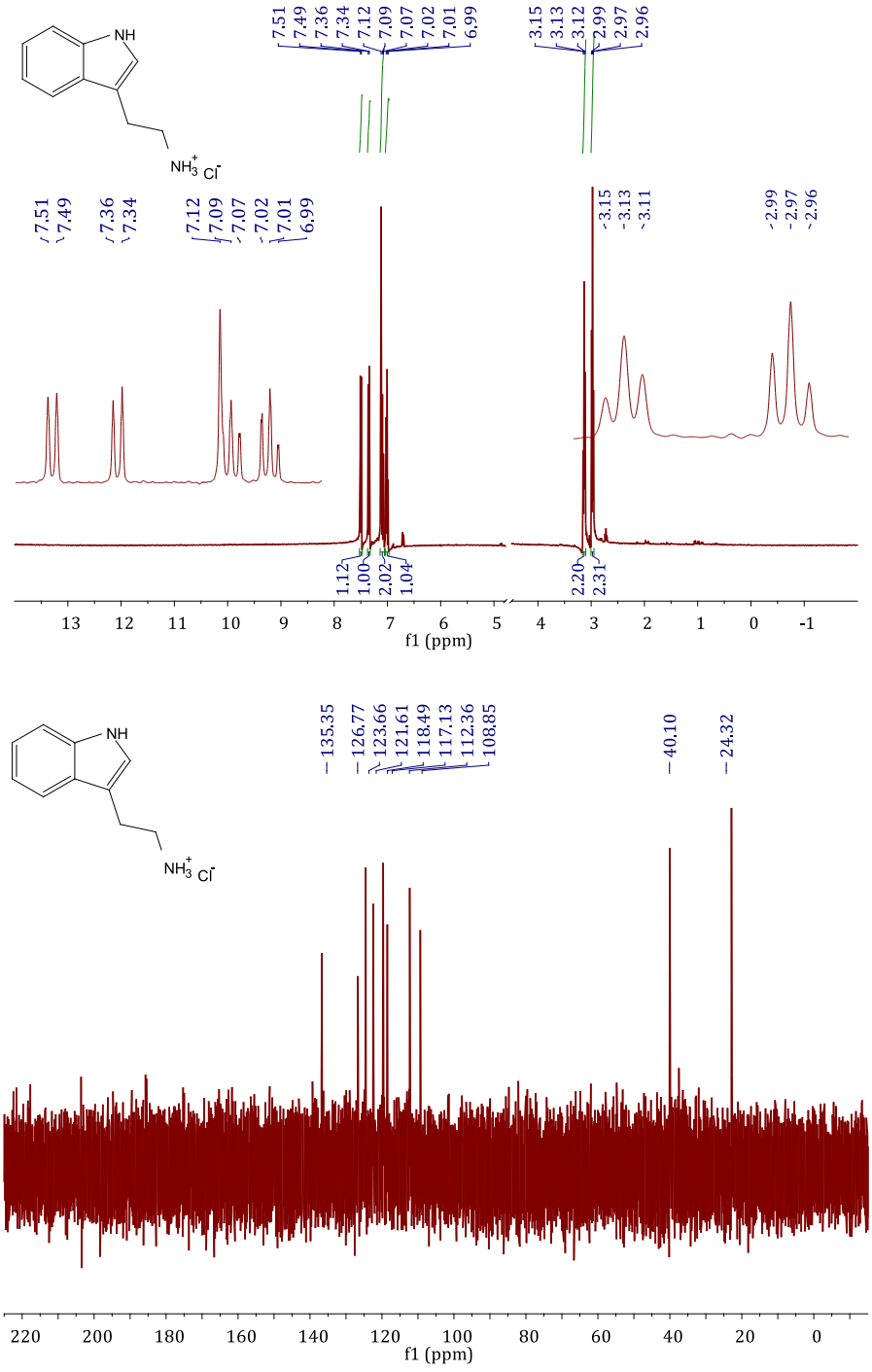


histamine dihydrochloride, 239-244 °C; white powder from EtOH; δ_H 3.00 (2H t J=8), 3.18 (2H t J=8), 7.23 (1H s), 8.50 (1H s); δ_C 22.26, 38.11, 117.08, 128.49, 134.00

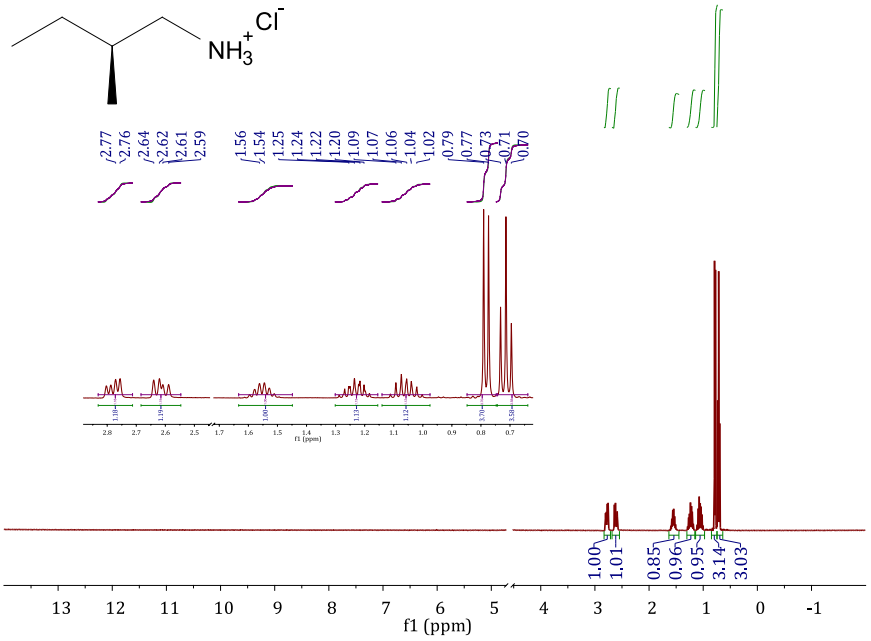


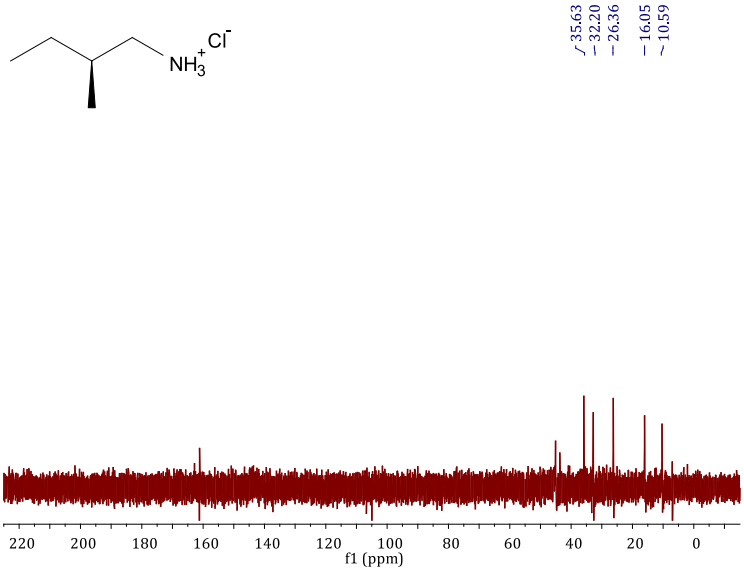


tryptamine hydrochloride, 245-248 °C; brown powder from EtOH; δ_H 2.97 (2H t J=9), 3.13 (2H t J=8), 7.01 (1H t J=4), 7.07-7.12 (2H m), 7.34 (1H d J=4), 7.49 (1H d J=4); δ_C 22.88, 40.03, 108.85, 112.36, 117.13, 118.49, 121.61, 123.66, 126.77, 135.35

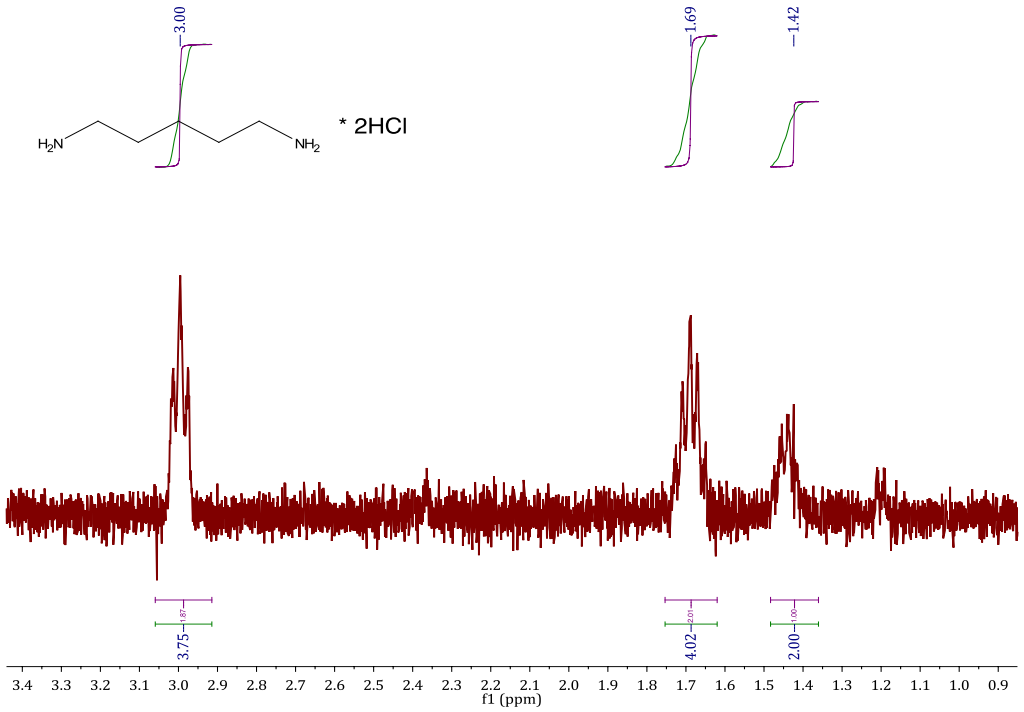


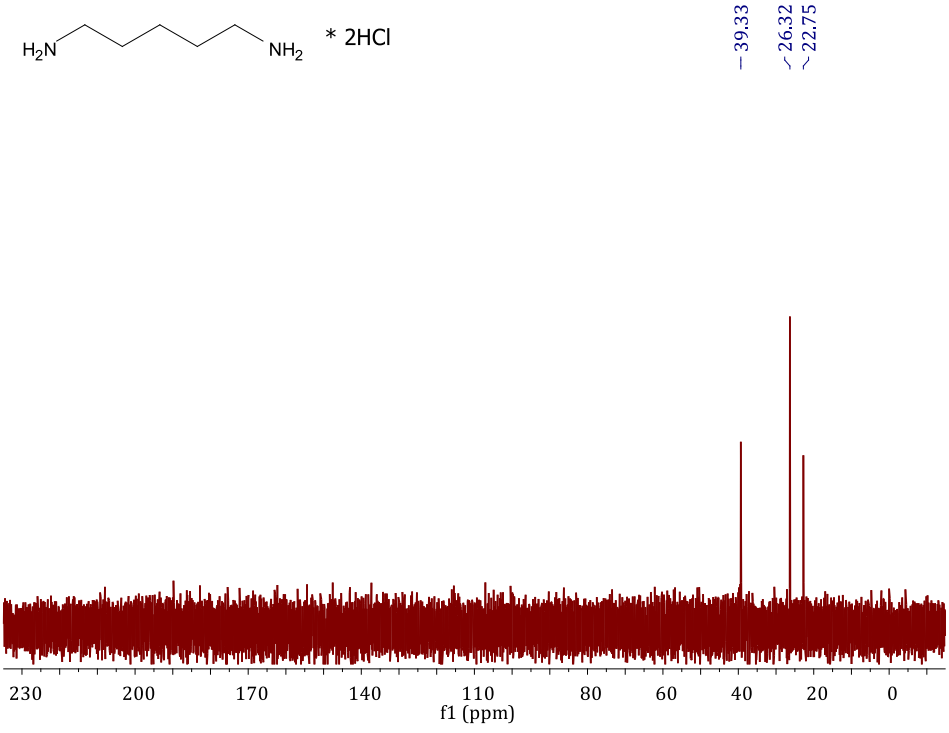
S-2-methyl-1-butylamine hydrochloride 163-167 °C; light brown powder from hexane; δ_H 0.71 (3H t J=8), 0.78 (3H d J=8), 1.02 (1H septet J=8), 1.22 (1H septet J=8), 1.55 (1H o J=8), 2.59-2.64 (1H m), 2.76-2.80 (1H m); δ_C 10.07, 15.75, 25.94, 32.48, 44.75



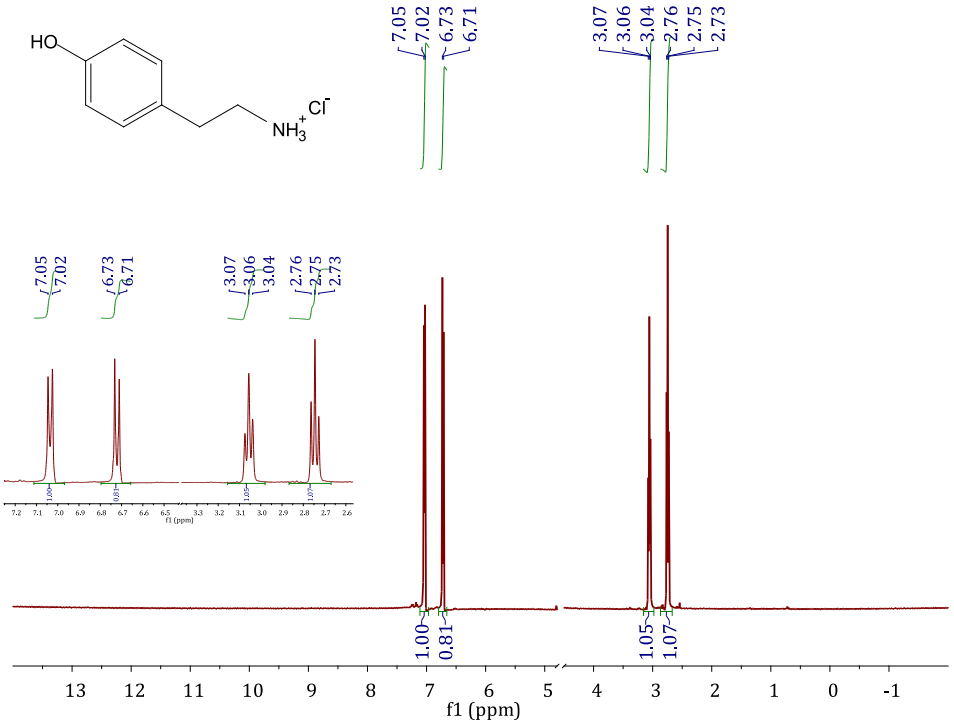


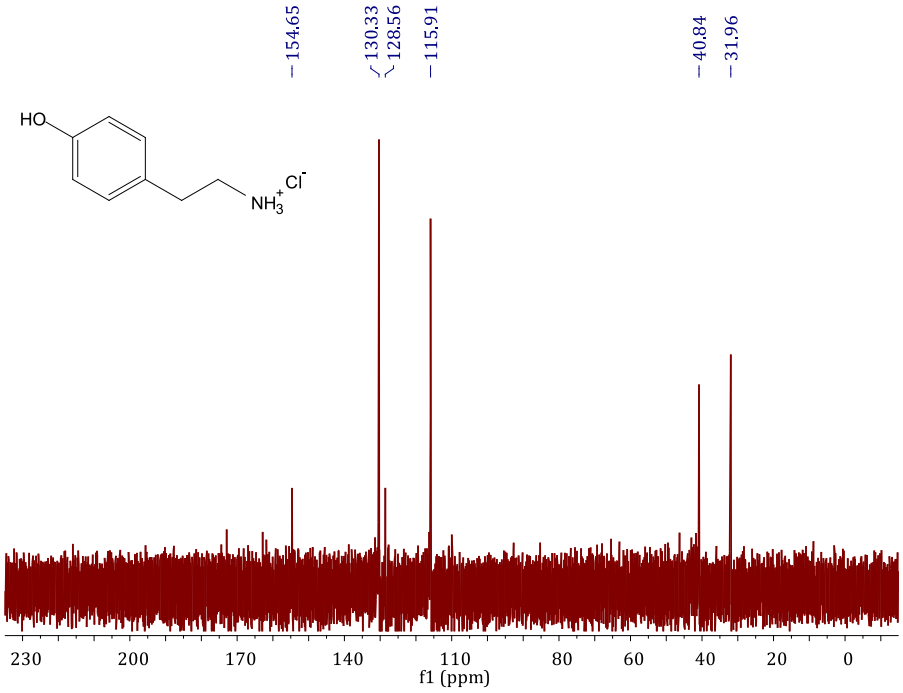
cadaverine dihydrochloride 249-256 °C; white powder from PrOH; δ_H 1.42 (2H p J=8), 1.69 (4H p J=8), 3.00 (4H t J=8); δ_C 22.75, 26.32, 39.33



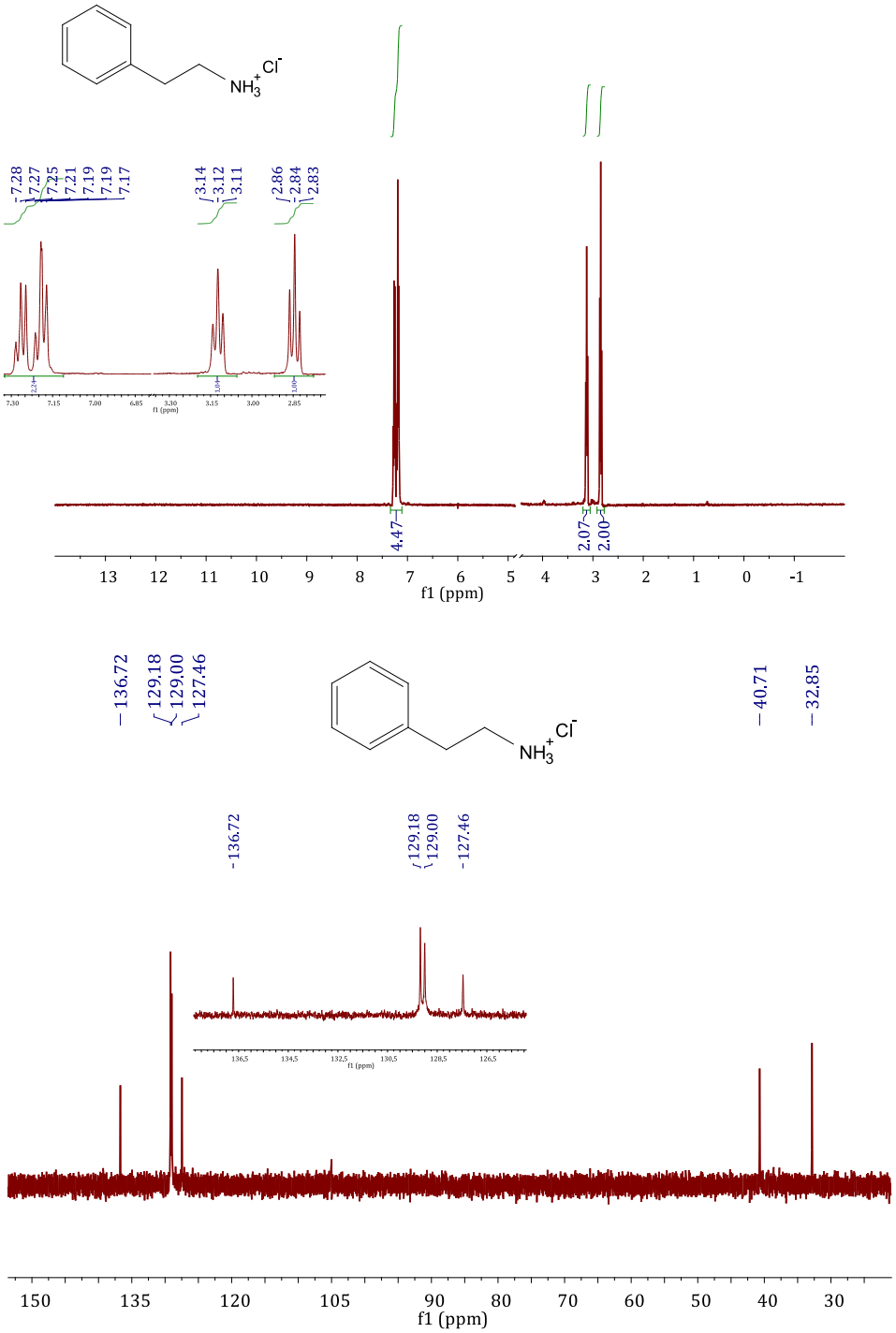


tyramine hydrochloride 270-273 °C; white powder from EtOH; δ_H 2.75 (2H t J=8), 3.06 (2H t J=8), 6.73 (2H d J=8), 7.04 (2H d J=8); δ_C 31.95, 40.83, 115.90, 128.55, 130.32, 154.65

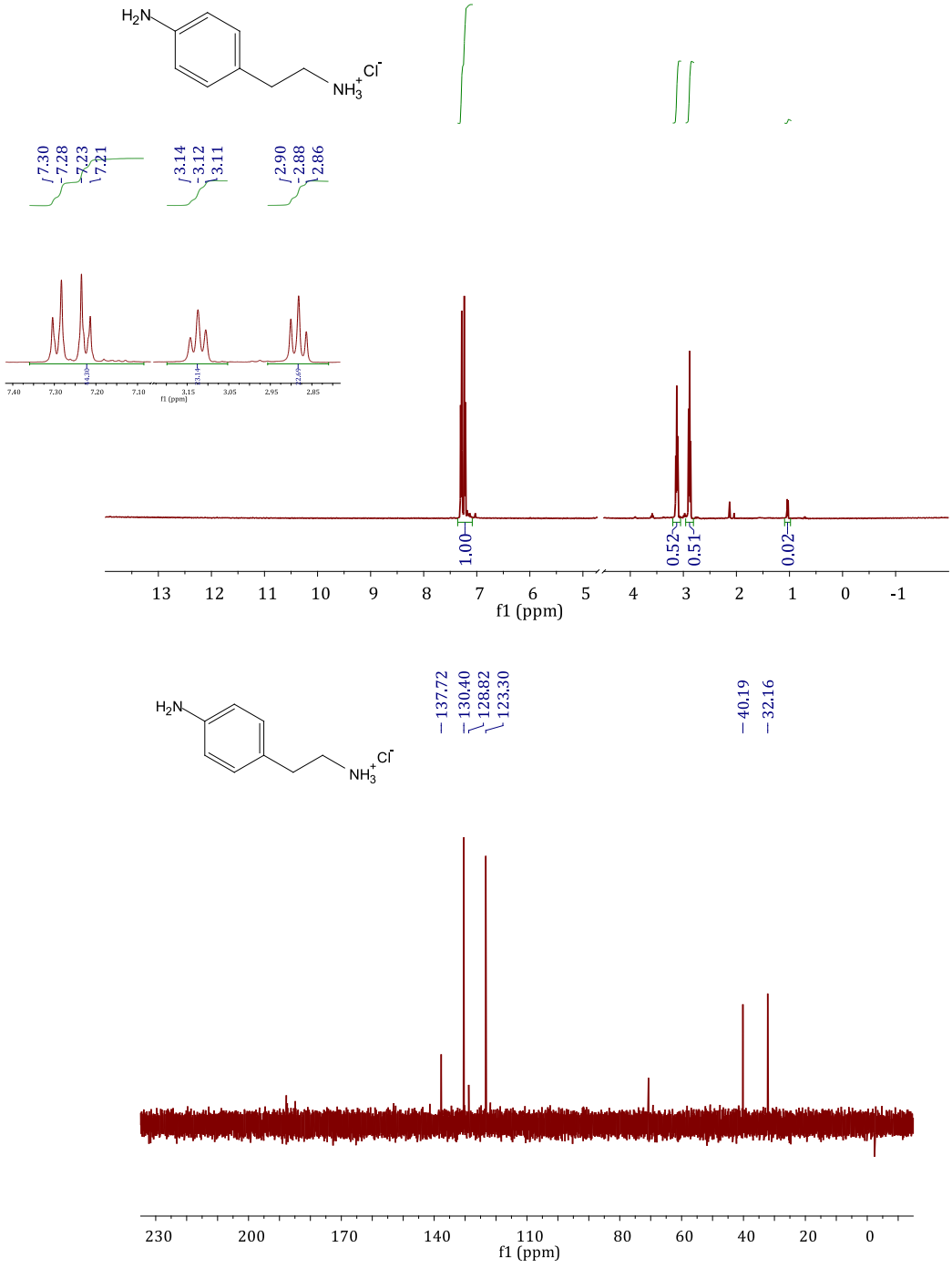




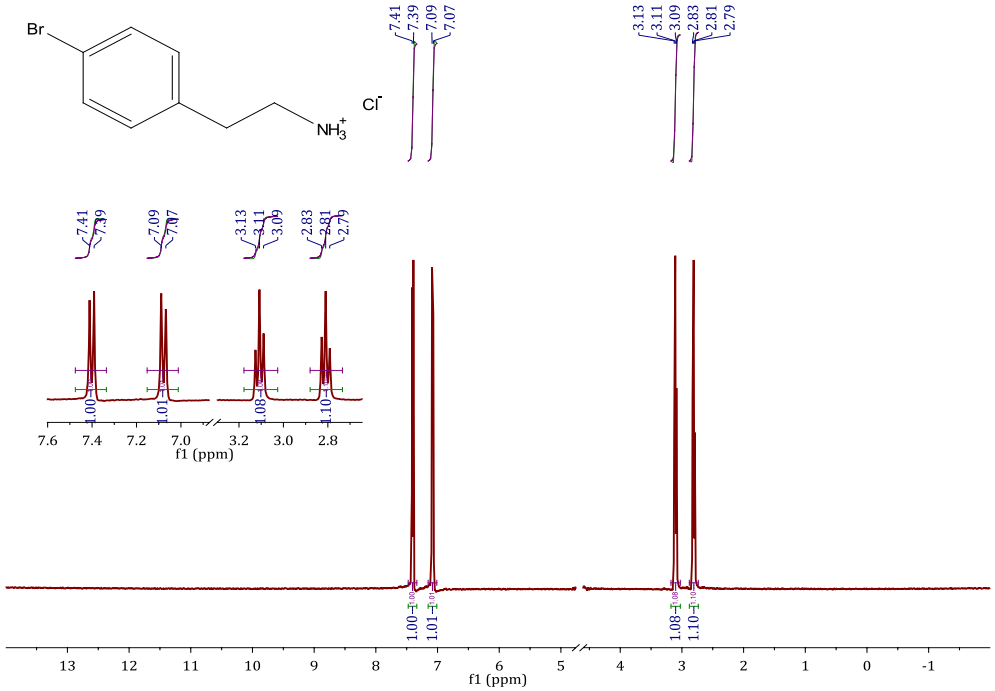
2-phenylethylamine hydrochloride 217-220 °C; white plates from PrOH; δ_H 2.84 (2H t J=8), 3.12 (2H t J=8), 7.17-7.28 (5H m); δ_C 32.85, 40.71, 127.46, 129.00, 129.18, 136.72

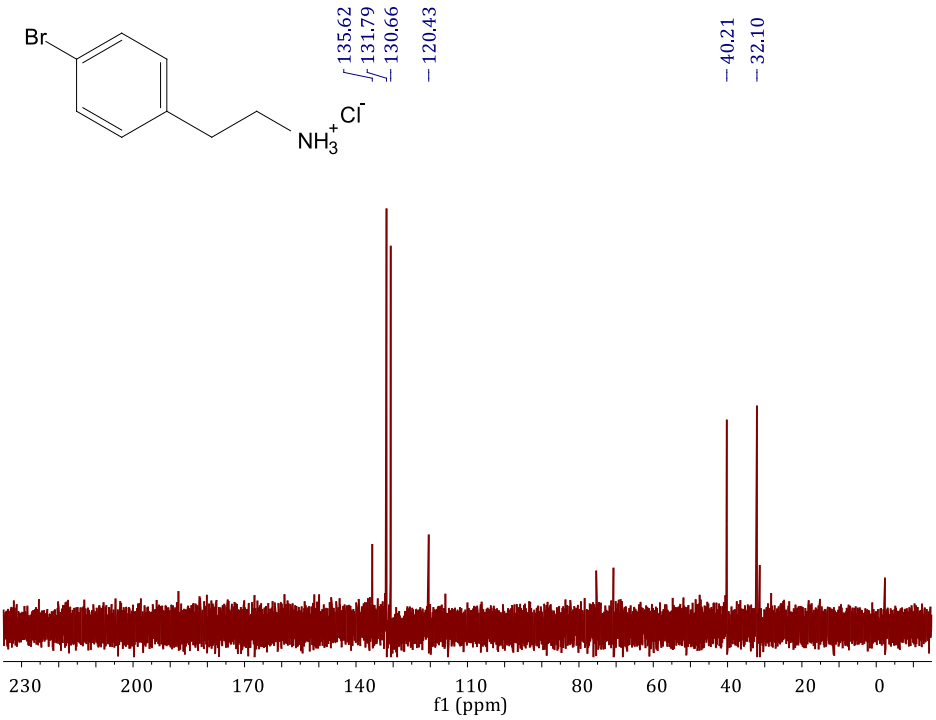


4-(2-aminoethyl)aniline dihydrochloride, 303-306 °C; white powder from EtOH; δ_H 2.88 (2H t J=8), 3.12 (2H t J=8), 7.22 (2H d J=8), 7.29 (2H d J=8); δ_C 32.16, 40.19, 123.30, 128.82, 130.40, 137.72

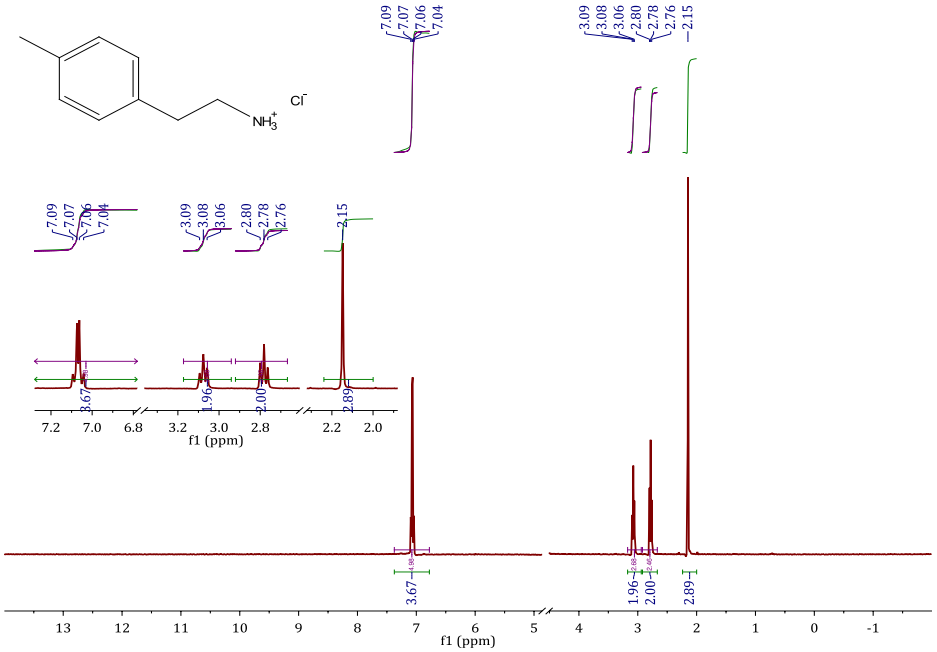


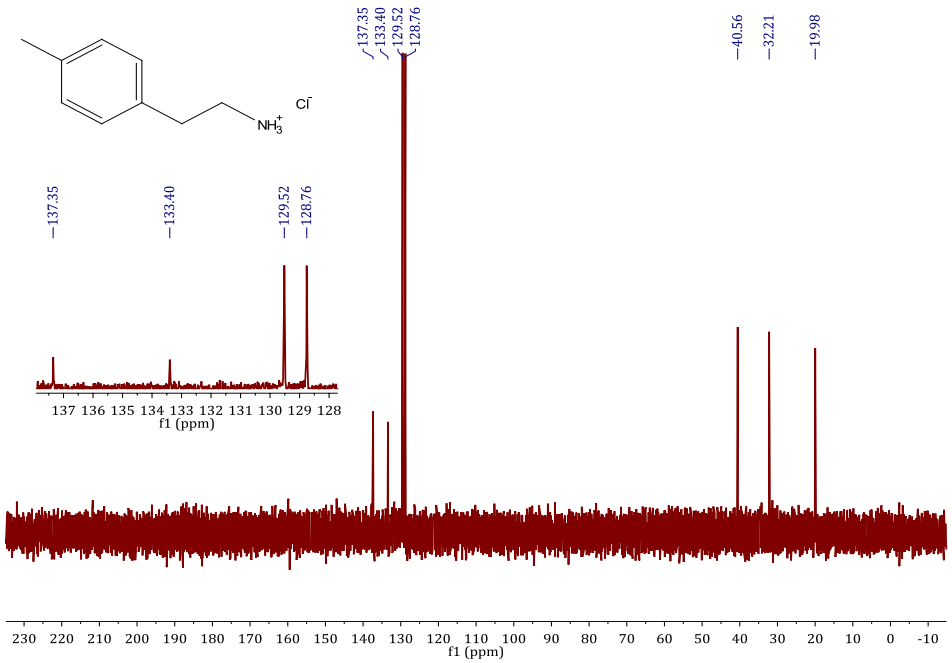
2-(4-bromophenyl)ethanamine hydrochloride, δ_H 2.81 (2H t J=8), 3.11 (2H t J=8), 7.08 (2H d J=8), 7.40 (2H d J=8); δ_C 32.10, 40.21, 120.43, 130.65, 131.78, 135.61



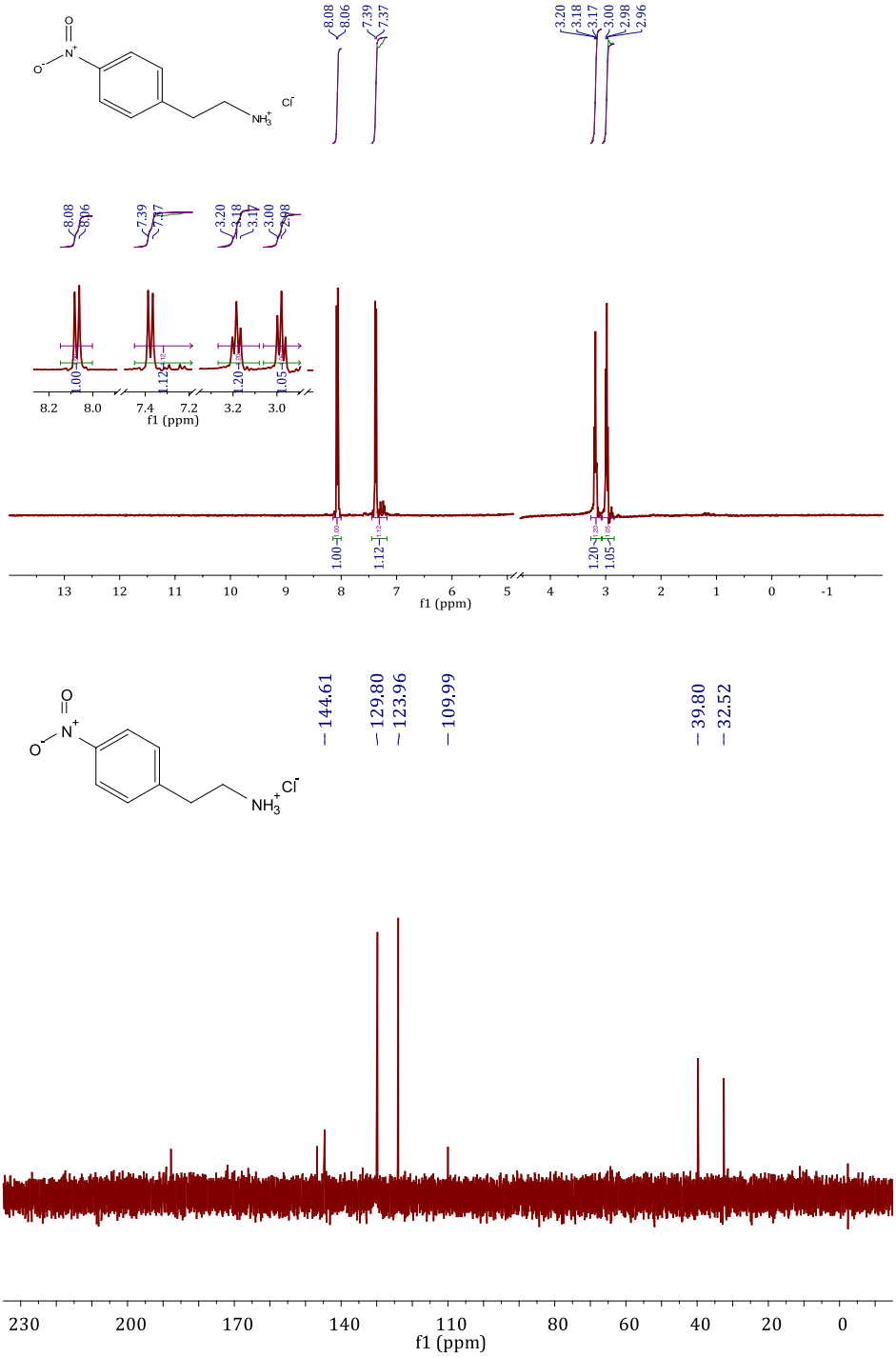


2-(p-tolyl)ethanamine hydrochloride, 219-221 °C; white plates from PrOH; δ_H 2.15 (3H, s), 2.78 (2H t J=8), 3.08 (2H t J=8), 7.06 (4H m); δ_C 19.98, 32.21, 40.56, 128.76, 129.52, 133.40, 137.35

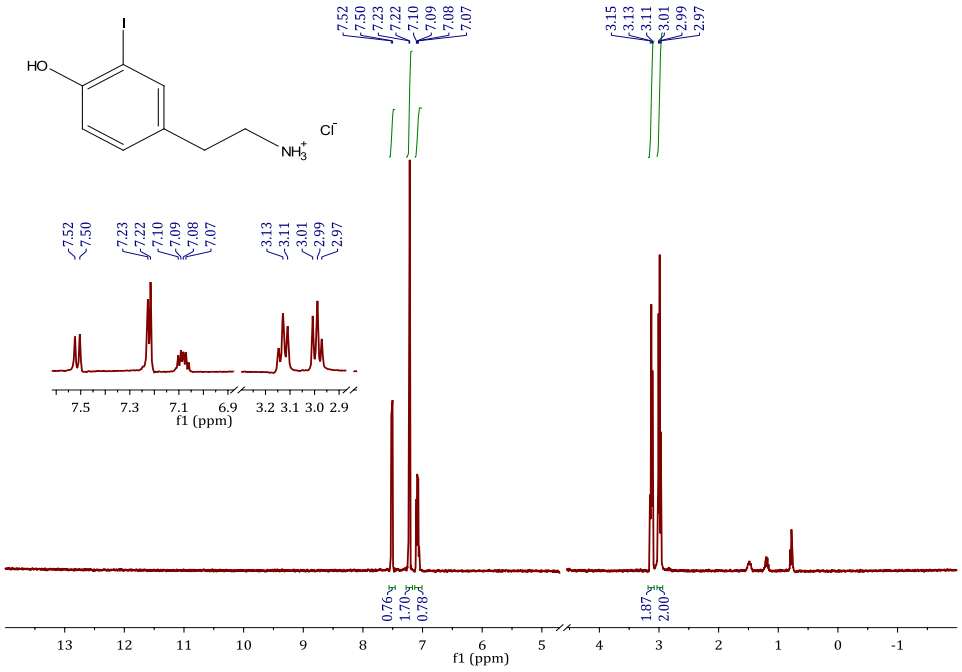


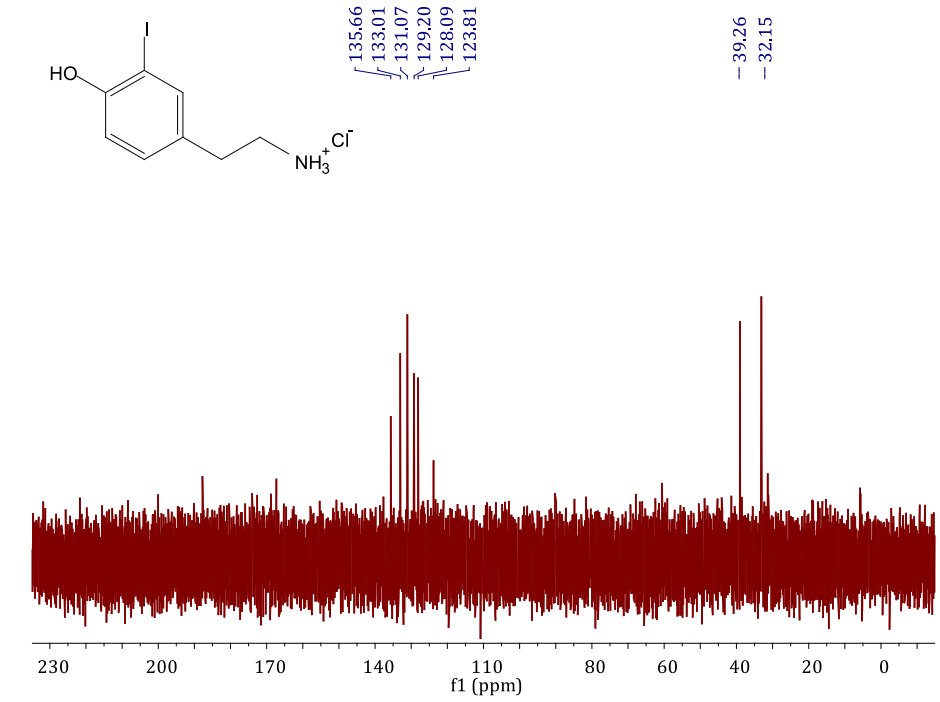


2-(4-nitrophenyl)ethanamine hydrochloride, 208-211 ^{o}C; brown crystals form EtOH; δ_H 2.98 (2H t J=8), 3.18 (2H t J=8), 7.38 (2H d J=8), 8.07 (2H d J=8); δ_C 32.52, 39.81, 110.08, 123.96, 129.80, 144.61

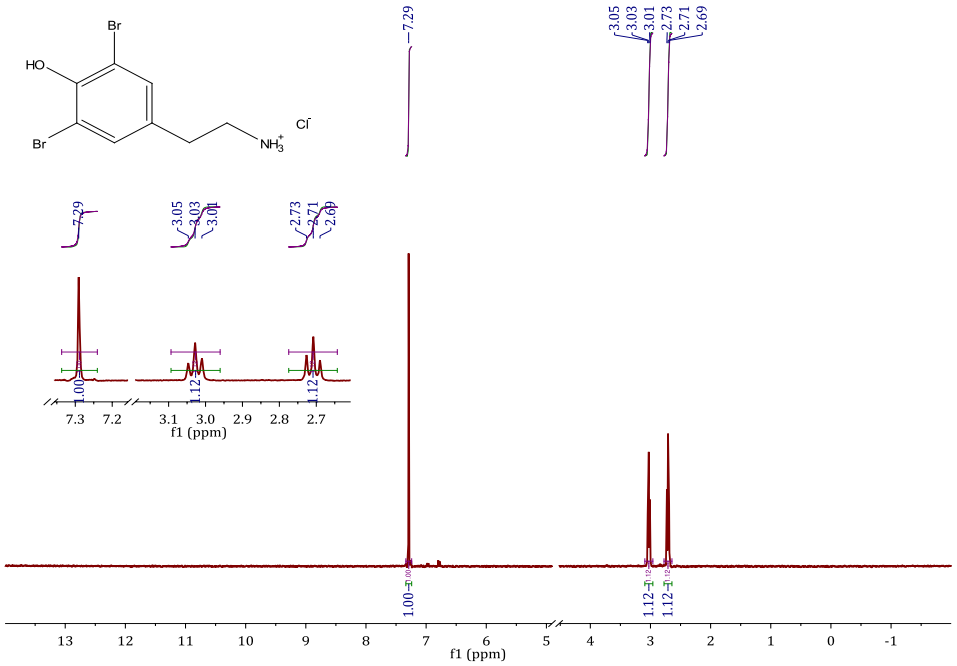


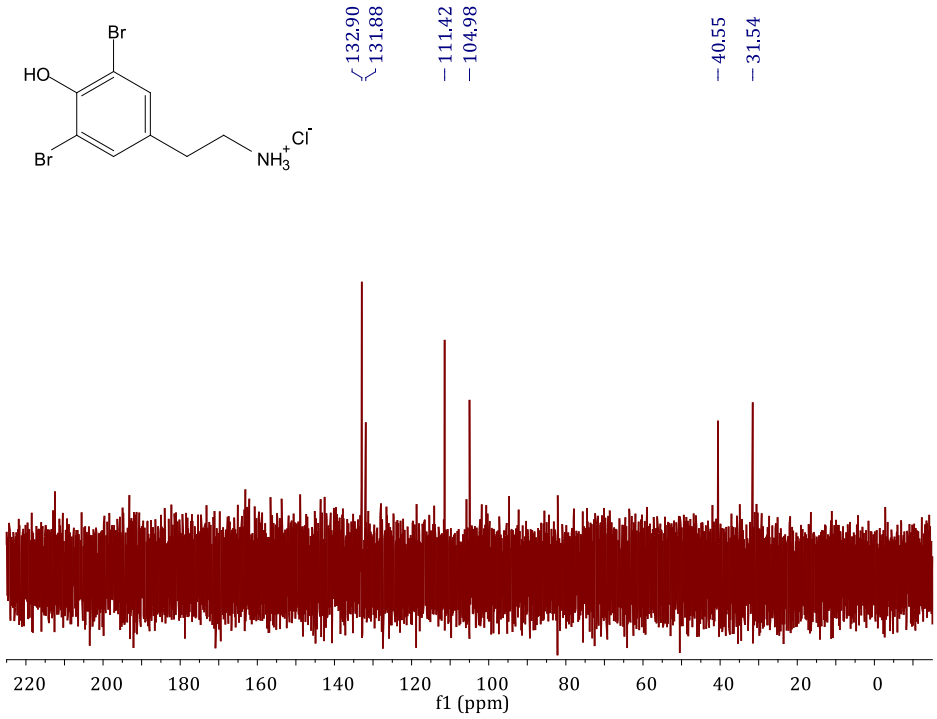
4-(2-aminoethyl)-2-iodophenol hydrochloride, 249-253 °C; light brown powder from PrOH; δ_H 2.99 (2H t J=8), 3.13 (2H t J=8), 7.09 (1H m), 7.23 (2H m), 7.51 (1H m); δ_C 32.15, 29.26, 123.81, 128.09, 129.20, 131.07, 133.01, 135.66



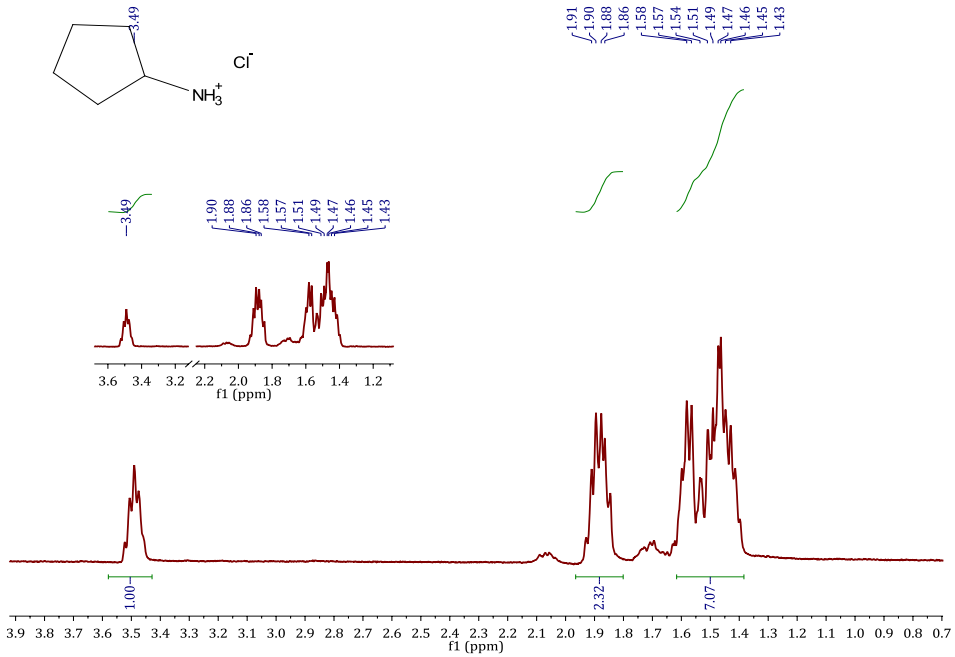


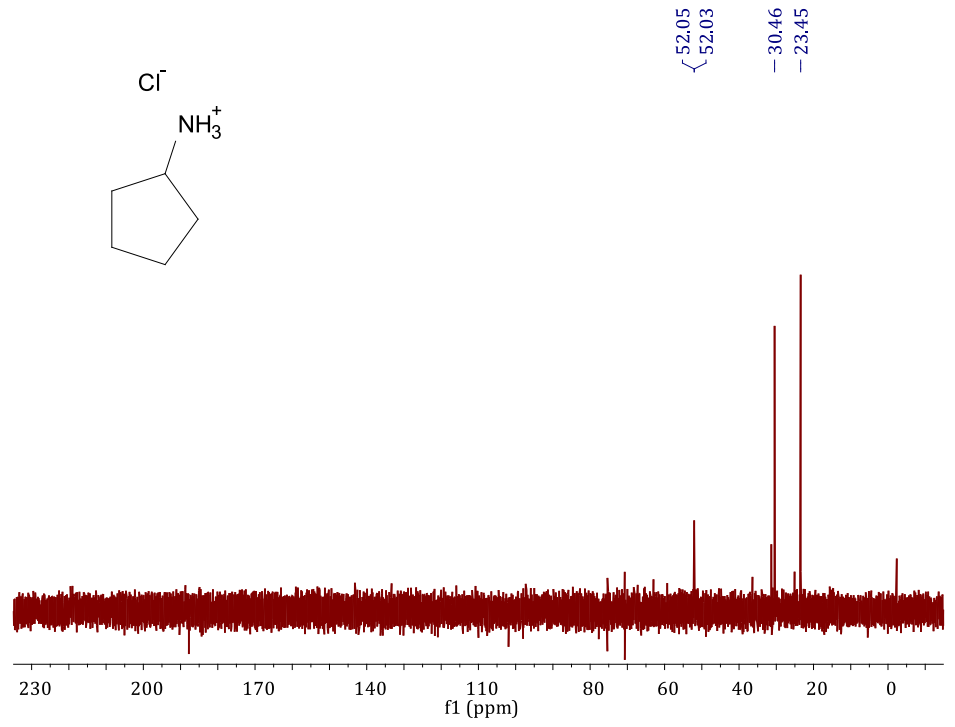
4-(2-aminoethyl)-2,6-dibromophenol hydrochloride, 206-208 °C; tan powder from MeOH; δ_H 2.71 (2H t J=8), 3.03 (2H t J=8), 7.29 (2H s); δ_C 31.54, 40.55, 104.98, 111.42, 131.88, 132.90





cyclopentamine hydrochloride, 198-201 °C; brown powder from BuOH; δ_H 1.53 (6H m), 1.89 (2H m), 3.49 (1H m); δ_C 23.45, 30.46, 52.05 (estimated 15% impurity)



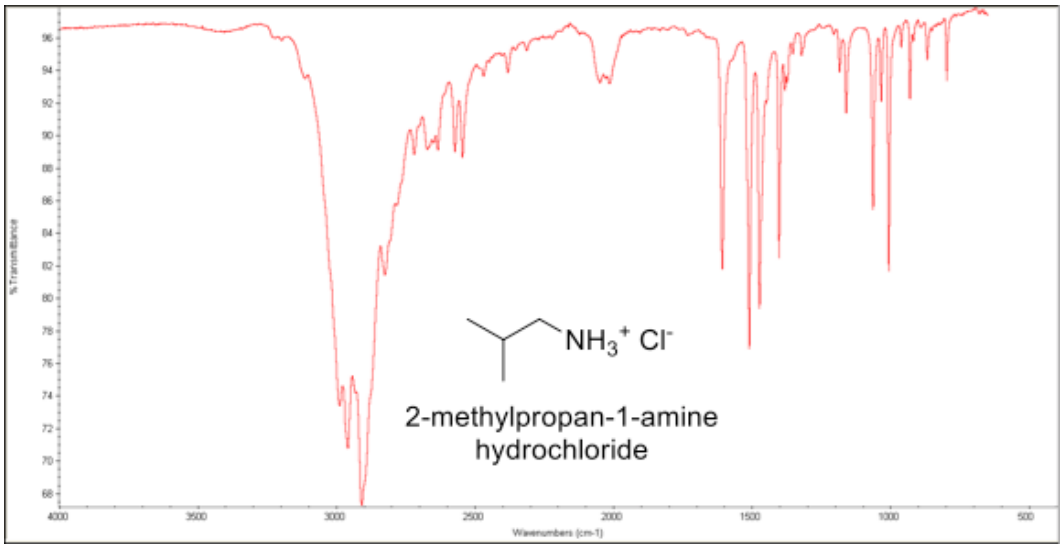


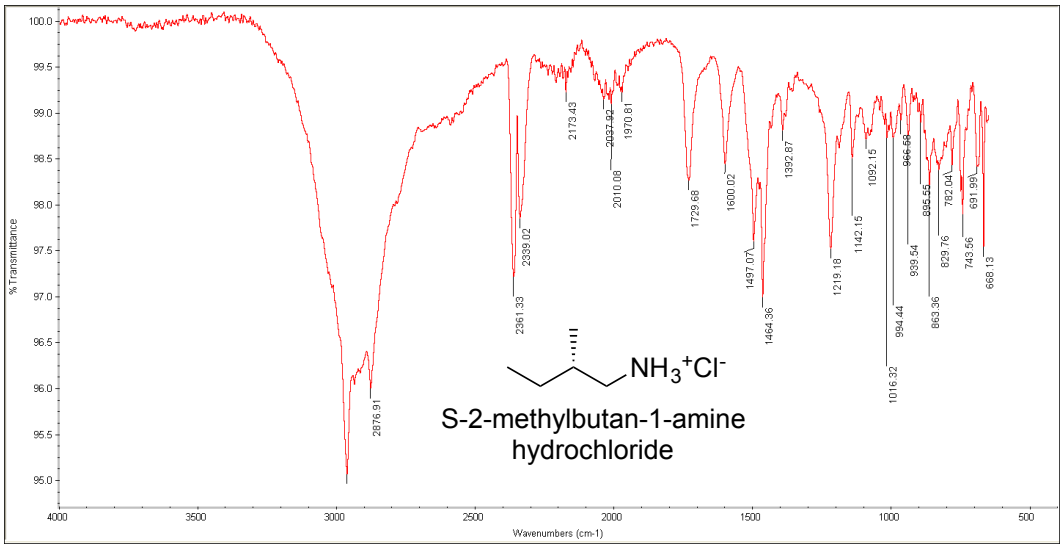
APPENDIX B

SUPPORTING INFORMATION FOR CHAPTER 3

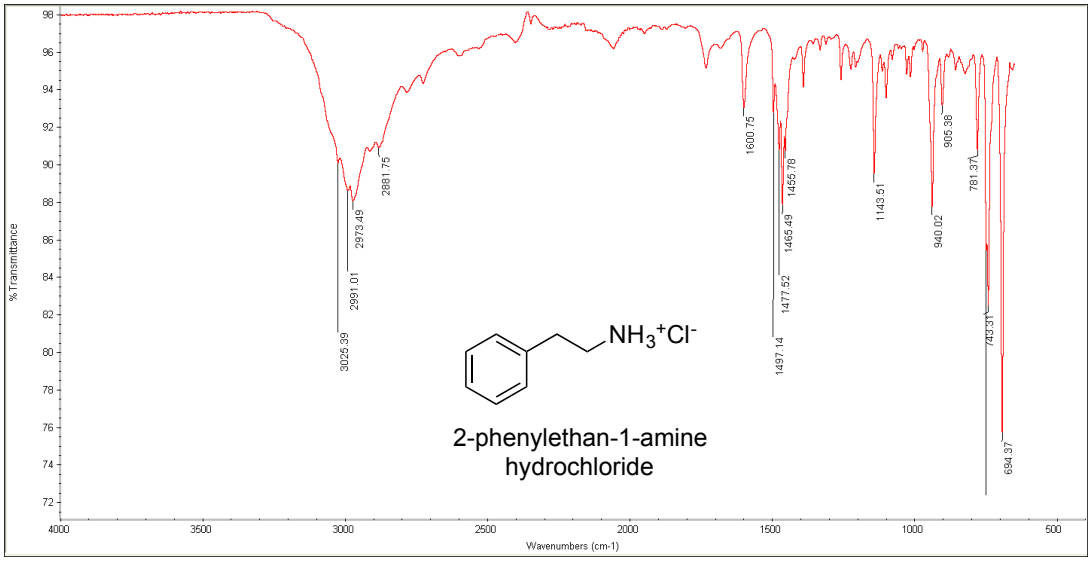
Student Spectra

Infrared Spectra

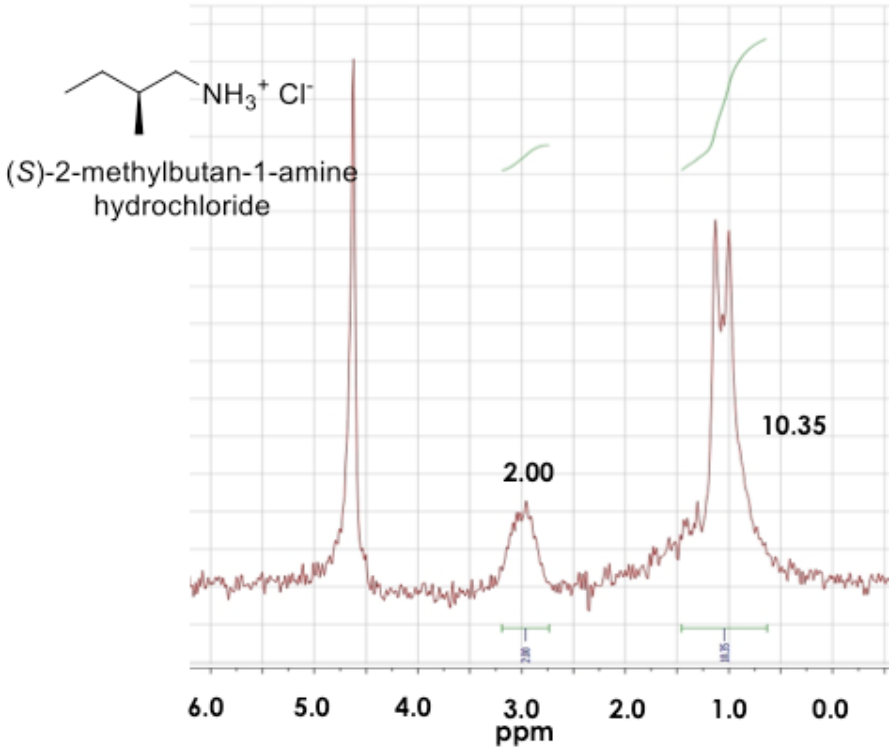


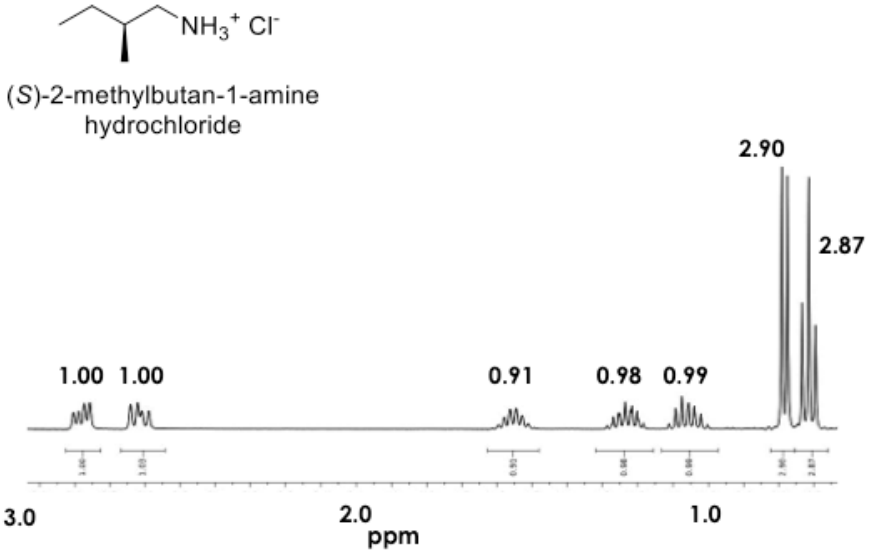


(Peaks at 2361 cm^{-1} and 2338 cm^{-1} are spectral artifacts from instrument)

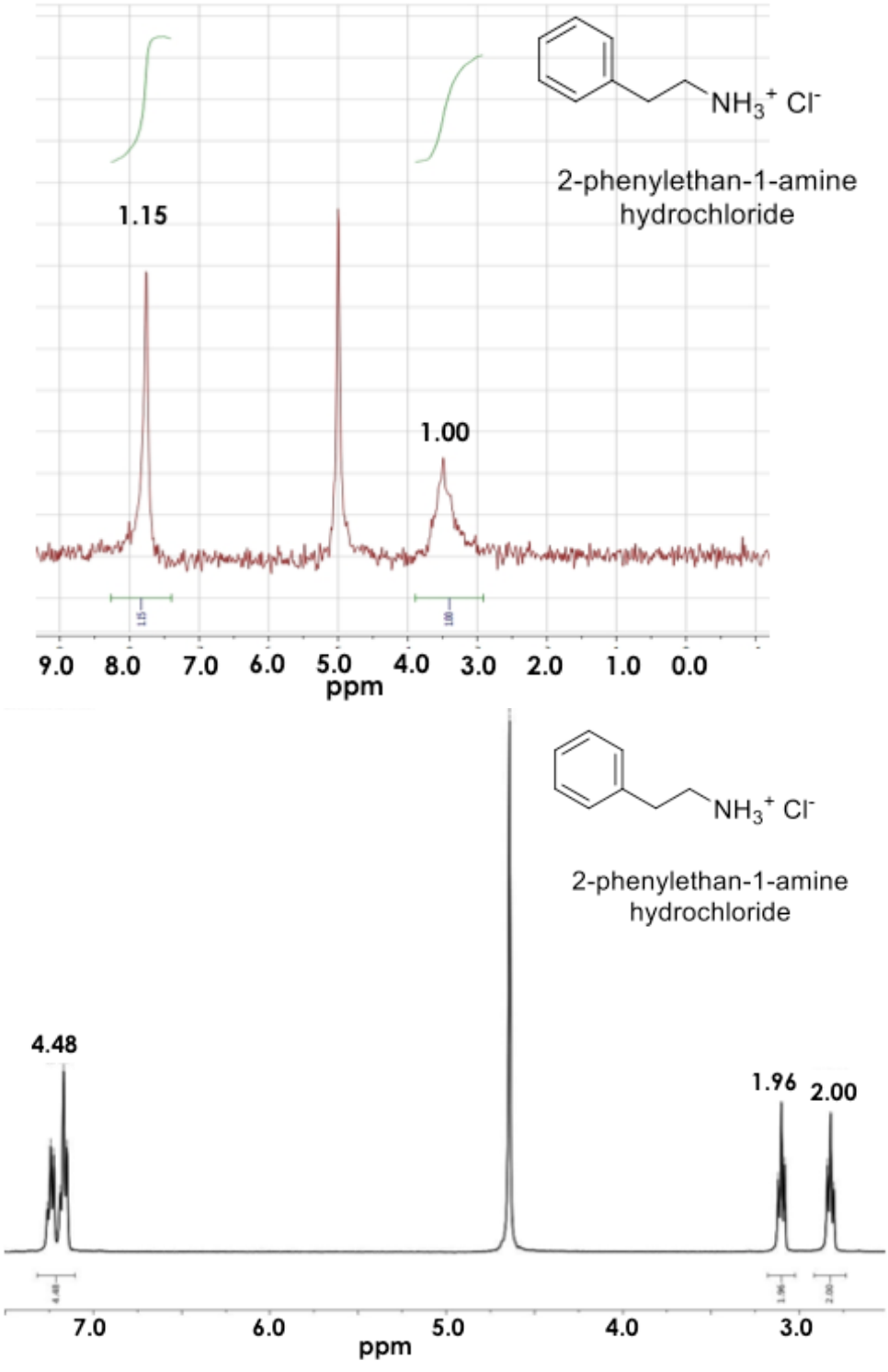

%Transmittance
Wavenumbers (cm-1)
3025.39
2991.01
2973.49
2881.75
1600.75
1497.14
1477.52
1465.49
1455.78
1143.51
940.02
905.38
781.37
743.31
694.37
NH3+Cl-
2-phenylethan-1-amine
hydrochloride

45 MHz PicoSpin™ vs. 400 MHz ^{1}H NMR





^{1}H NMR comparison of (S)-2-methylbutan-1-amine hydrochloride

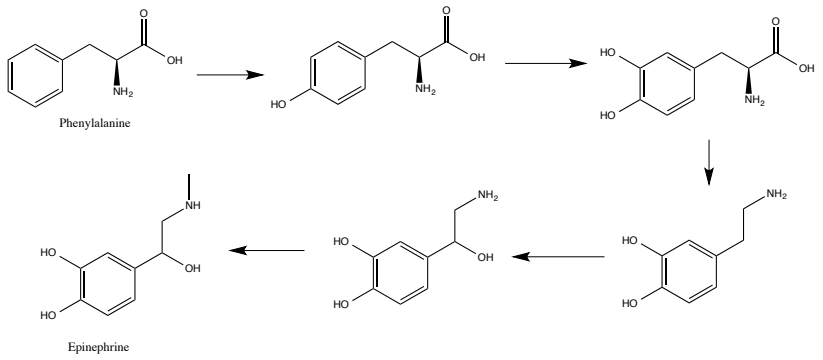


^{1}H NMR comparison of 2-phenylethan-1-amine hydrochloride

Student Handout

Introduction

It is commonly known that amino acids are the building blocks of protein and thus life as we know it. A lesser-known fact is that many amino acids are the progenitors of neurotransmitters and hormones that regulate a diverse set of functions. Adrenaline (epinephrine) enabled our ancestors to escape from bears and is used today to treat allergic reactions and cardiac arrest. Norepinephrine gives you the familiar butterflies in the stomach feeling before organic chemistry exams. Dopamine is responsible for reward-motivated behavior and drugs such as Ritalin® (methylphenidate), Adderall® (amphetamine salts), and cocaine function by mimicking or enhancing its effects. Serotonin regulates appetite and is thought to be responsible for overall happiness and wellbeing. All of these neurotransmitters have one thing in common: they are biologically synthesized by the decarboxylation of amino acids.



In this lab you will decarboxylate an unknown amino acid using a technique newly discovered by the Morrison lab involving microwave chemistry. A microwave reflux enables an organic chemist to carry out a reaction at a temperature much higher than that of the solvent's boiling point. As you all know from general

chemistry, the Arrhenius equation relates rate to temperature. As a general rule of thumb, the rate doubles for every ten-degree increase in temperature.

Once isolated, you will use IR and the state-of-the-art bench-top PicoSpin 45 MHz NMR to determine the structure of your decarboxylated product and thus your amino acid starting material.

Procedure

Measure 0.905 grams of your assigned unknown amino acid into a microwave vessel. Record the letter of your unknown. Add 2.75 grams of (R)-carvone to the vessel along with 5.5 mL of n-propyl alcohol. Seal the microwave vessel and give it to your TA for microwave reflux. *Be sure to record the symbol on the top of the screw-cap for identification.* The reaction vessel will be heated in the microwave oven from room temperature to 190 ^{O}C over a period of five minutes and will remain at 190 ^{O}C for an additional 5 minutes with continuous stirring. Allow time for the vessel to cool to room temperature then *open the vessel slowly and carefully* as gas has evolved. Note any physical changes to the reaction mixture. Add 10 mL of 2M HCl to the mixture. Seal the vessel again and return it to your TA for another round of reflux to 190 ^{O}C over 5 minutes with stirring. Allow to cool and open carefully. Wash the aqueous reaction mixture three times with a 25 mL portion of diethyl ether (remember densities). Do not discard any layers until the product has been confirmed. Heat the aqueous solution on a hot plate to dryness. If available, use a heat gun (if available) to dry the outside of the beaker while heating. Be careful not to burn the product as the solution dries. Weigh your product then obtain an IR spectrum. Dissolve the remaining product in the minimal amount of

D_2O possible. Ensure that the entire sample is dissolved, as any particulate can clog the NMR. Alert your TA before using the Pico Spin™ NMR and obtain your spectrum using the instructions handout. Determine the percent yield after you have analyzed your spectrum and identified the product and reactant. Keep the product in a sealed vessel as it is hydroscopic.

List of Reagents

L-phenylalanine

L-tyrosine

L-valine

L-leucine

(R)-carvone

n-propyl alcohol

Hydrochloric acid

Diethyl ether

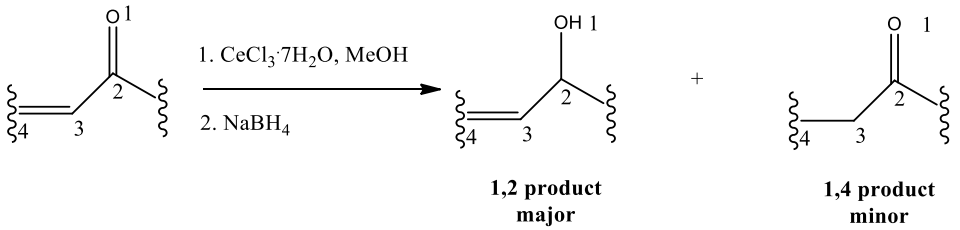
APPENDIX C

SUPPORTING INFORMATION FOR CHAPTER 5

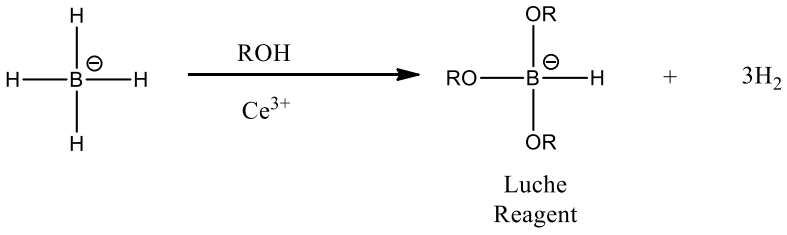
Student Handout

Introduction

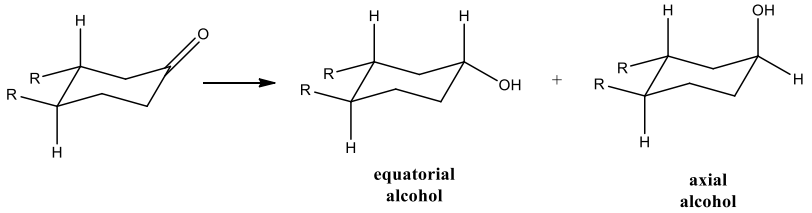
The Luche reduction is a common reaction for the regioselective reduction of α-enones (Fig. 1).



Using a Lanthanide catalyst (most commonly cerium (III) chloride heptahydrate) to activate the alcohol solvent, the Luche Reagent is rapidly formed from reaction of Sodium Borohydride with methanol solvent (Fig. 2). The alkoxyborohydride reagent formed favors reduction in 1,2 fashion more so than would sodium borohydride reduction (Fig 1).



An additional feature of the Luche reduction is the stereoselective preference for the formation of an equatorial alcohol upon reduction of a substituted cyclic ketone. The Luche Reduction in most cases can be used to increase the stereoselectivity of chiral ketone reductions over the Sodium Borohydride reduction. This selectivity has been shown to be dependent on the concentration of catalyst (i.e. the product ratio varies as the concentration of catalyst is varied in solution).



In this experiment (-)-menthone, a naturally occurring cyclic ketone, will be reduced under partial or full Luche conditions to some unknown percentage of product alcohols, (-)-menthol and (+)-neomenthol. The complete conversion of (-)-menthone to product alcohols will be examined by IR spectroscopy. The product ratio will be determined via optical rotation and analysis of the ^{1}H NMR spectrum of the final product mixture.

Product Ratio Determination by Polarimetry

The standard rotation, α_D^{20} or α°, of a given substance at room temperature and the 589 nm sodium D line is given by the following equation:

$$\alpha_D^{20} = \frac{\alpha}{l\ x\ c}$$

Where "α" is the observed rotation in degrees, is the length of the sample tube in decimeters, and is the concentration of the solution in g/mL. Once the standard rotation of your product mixture is determined, it can be represented as a linear combination of the values of (-)-menthol and (+)-neomenthol. The unknown product percentages can then be determined by the following equation: given the standard rotations of menthol (-50.10) and neomenthol (+20.70) in 95% ethanol.

$$100 * \alpha^{o}_{product\ mixture} = x(\alpha^{o}_{menthol}) + (100 - x)(\alpha^{o}_{neomenthol})$$

Product Ratio Determination by ^{1}H NMR

The products will be obtained as a mixture in the form of a homogeneous oil. Signals from both products will appear in the ^{1}H NMR spectrum obtained experimentally with their relative intensities dependent on the relative percentage of each in the sample. It has been shown that the protons on the alcohol carbons of your product alcohols give well-resolved HNMR signals; therefore, the relative integration of these signals may be used to determine the product ratio.

Hazards

Typical laboratory safety procedures may be referenced in the lab manual and will not be included here. Please reference the MSDS data for all chemicals. Flammable gas evolution occurs upon addition of sodium borohydride. Take care to avoid sources of ignition.

Luche Reduction General Procedure

Obtain 1.0 g of (-)-menthone and dissolve in 20 mL of unknown solvent with stirring in a small beaker or flask. (Be sure to record your unknown number!) Place solution in a room temperature water bath. Add 1.5 mole equiv. of $NaBH_4$ in portions. Note H_2 gas bubbles off. A white precipitate may form a suspension in your beaker (this is a byproduct and not the desired product!). Let the reaction mixture stir 5-10 min until H_2 evolution slows.

Dilute the reaction mixture with 25 mL of distilled water and extract with 2 x 25 mL portions of diethyl ether. Combine the ether layers and wash with 25 mL of distilled water and 25 mL of brine. Now dry the ethereal solution over $MgSO_4$(s). The ether may then be removed by distillation, rotary evaporation, or evaporation from a hot plate set to 100˚C as the boiling points of the diastereomer alcohols are approximately 216˚C.

Record an IR spectrum of your product mixture using as little material as possible (Be sure to also obtain an IR spectrum of the starting material for comparison). Weigh your flask one final time and determine the weight of product remaining for polarimetry analysis. Dissolve the sample in approximately 15 mL of 95% ethanol and transfer this solution to the polarimetry tube and fill to the top. 14 mL should just fill the tube. Take a polarimetry reading. Note the degree of rotation and the sign (dextrorotatory or levorotatory). From the polarimetry data, determine the product ratio of menthol to neomenthol.

Pour your polarimetry solution into a round bottom flask and rotovap the solvent away. Once you have recovered your product oil, use the remaining amount

to prepare an ^{1}H NMR sample in chloroform-d for analysis after lab. You will receive your spectrum by email within 48 hrs.

Polarimetry Notes: 0.25 g of product is generally sufficient for an accurate polarimetry reading. Length of Polarimetry Tube = 20 cm

Questions:

1. Propose a mechanism for the formation of the Luche reagent from BH4-.
2. Are the products formed achiral, enantiomers, diastereomers, or meso compounds?
3. How many chiral centers does each product have? What are the IUPAC names of (-)-menthol and (+)- neomenthol?
4. What other methods may be used to determine the product ratios of the alcohols.

APPENDIX D

SUPPORTING INFORMATION FOR CHAPTER 6

Student Handout

Nucleophilic Ring-opening of Epoxides

Epoxides are a unique functional group in which an oxygen atom is bonded to two carbons in a 3-membered ring forming a cyclic ether. Due to the high strain associated with small rings, epoxides are susceptible to nucleophilic attack undergoing facile ring-opening. Upon proper workup this process places a nucleophile alpha (one carbon over) to an alcohol as shown in Figure 1 below.

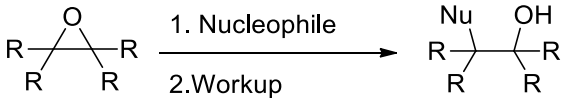


Figure 1

Interesting results are seen when asymmetrical epoxides are subjected to ring opening reactions. Regioisomers are formed when the nucleophile is able to add to either carbon of the epoxide (Figure 2).

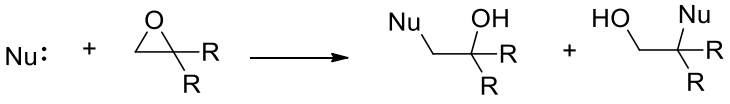


Figure 2

Several factors affect the regioselectivity of nucleophilic epoxide openings including the nature of the nucleophile or electrophile and reaction conditions. In this experiment 1,2-epoxyhexane will be subjected to a nucleophilic ring-opening reaction in both acidic and basic conditions for a series of alcohol/alkoxide nucleophiles. The product percentages of each process will then be determined by Gas Chromatography. The percentage of a product, P, can be calculated from the following equation where "A" represents the area of a given signal in the gas chromatograph.

$$\%P_1 = \frac{A_1}{A_1 + A_2 + \cdots A_n}\ x\ 100$$

Hazards

Please review online MSDS sheets of all chemicals including products. Never seal a reflux apparatus air tight. The experiment will be performed under snorkel ventilation. Gloves and goggles should be worn especially when handling the alkoxide salts and sulfuric acid.

Procedure

This lab for convenience will be performed in pairs. Obtain an alcohol/alkoxide combination from your instructor and perform the reaction under both conditions simultaneously. One partner will use the acidic procedure and one partner will use the basic procedure.

Partner A: Acidic Conditions

Obtain 0.5 g of 1,2-epoxyhexane and place into a microscale round bottom flask. Dissolve the epoxide by pouring in 5 mL of assigned alcohol and stirring with a spin vane. Add 1 drop of concentrated sulfuric acid to this solution and allow the solution to stir for 30 min.

In a separatory funnel, wash your reaction mixture with 5 mL of saturated sodium bicarbonate solution (no layers will form). Then extract your product from this wash with 2 x 10 mL portions of diethyl ether. Dry the ether layer with magnesium sulfate and decant. Rotovap your organic extract in room temperature water until no change in mass is observed. Record the final mass of your product.

Partner B: Basic Conditions

Obtain 1.20 mol equivalents of assigned alkoxide powder and place into a microscale round bottom flask along with 5 mL of assigned alcohol and a spin vane (powder will not dissolve). To this slurry, add 0.5 g of 1,2-epoxyhexane. Reflux this reaction mixture for 30 min.

In a separatory funnel, wash your reaction mixture with 5 mL of saturated ammonium chloride solution (no layers will form). Then extract your product from this wash with 2 x 10 mL portions of diethyl ether. Dry the ether layer with magnesium sulfate and decant. Rotovap your organic extract in room temperature water until no change in mass is observed. Record the final mass of your product.

GC Analysis: Both Partners

Obtain a small vial from your instructor and make a solution of one drop of your product mixture in a 2 mL's of methanol or acetone. Run the GC of your

product mixture noting the retention times and areas of each peak on the log sheet. Save your GC file in the desktop class folder.

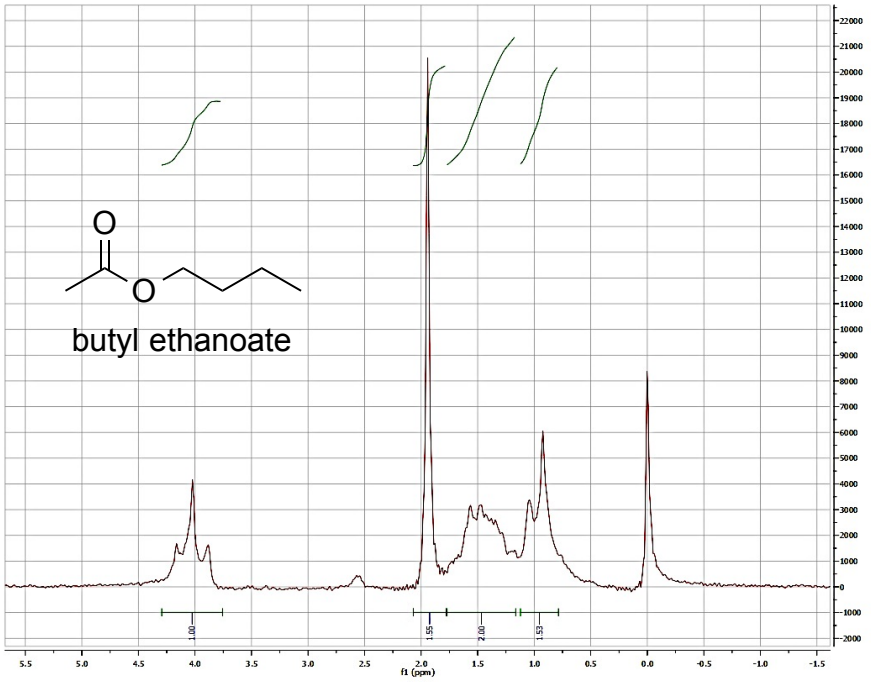
Post Lab Analysis

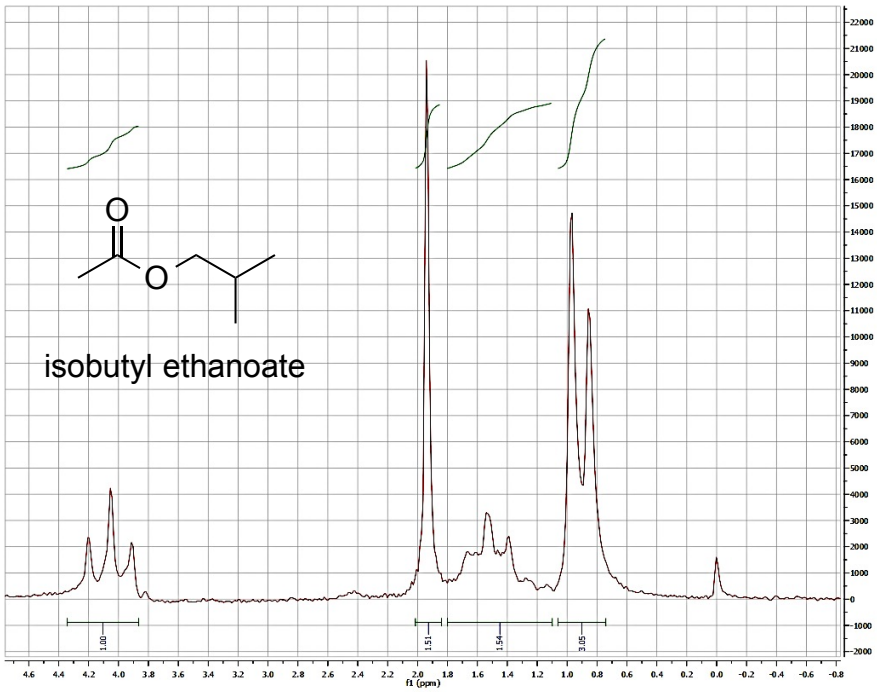
Raw GC data from the log sheets will be posted on the course webpage. Organize the class data into a useful table in your lab report. Compare the spectra to the GC of the starting material and using what you know about epoxide openings, and identify the compound eluting at each retention time. In your report make a statement summarizing any observable trends in this class of epoxide opening reactions.

APPENDIX E

SUPPORTING INFORMATION FOR CHAPTER 7

<u>Student Spectra</u>





Student Handout

Introduction

Esters are carboxylic acid derivatives containing an alkyl chain in place of the acidic proton that can be produced on the industrial scale via a process known as the Fischer Esterification. This reaction is an equilibrium reaction between a carboxylic acid and an alcohol in the presence of sulfuric acid catalyst. A generic reaction is shown below in Figure 1.

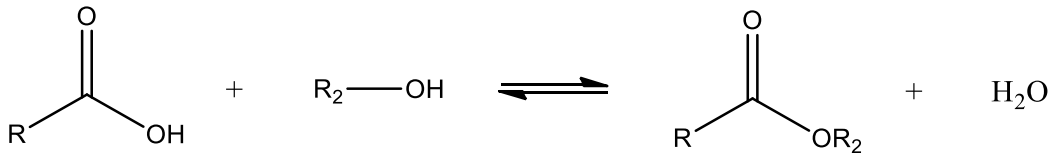


Figure 1

Since this is an equilibrium process, LeChatlier's Principle must be used to drive the reaction to completion. In this reaction adding an excess of acetic acid (which can be easily removed by an acid base extraction) will be used to drive the reaction to completion. The esters of this experiment are quite fragrant and are actually sold commercially. ^{1}H NMR and ^{13}C NMR will be used to characterize the product esters.

Hazards

Alcohols and esters are flammable. Acetic acid and sulfuric acid cause severe burns and should be treated with care. Pressure may buildup when neutralizing sodium bicarbonate due to the release of carbon dioxide gas. Never open MW

vessels outside of a hood or before the temperature has lowered below the boiling point of any contained chemical as pressure can build up.

Procedure

Obtain 5 mL of glacial acetic acid and 4 mL of an unknown alcohol from your TA and add these to a microwave vessel. Carefully under the hood add 1 mL of concentrated sulfuric acid and a small round stir bar (not a spin vane). Seal the MW vessel, write the vessel number in your notebook, and place in the MW carousel. The reaction will be carried out over 10 min (compared to 1 hr by standard reflux). Once the vessel is cool, pour the contents into a separatory funnel, add 10 mL of water and shake gently. The ester will separate from the water. Remove the aqueous layer and wash with an additional 10 mL of water. Wash the ester again with 10 mL portions of 5% sodium bicarbonate until the resulting aqueous wash is basic according to pH paper. Note: CO_2 forms during this step so be careful of pressure buildup in the separatory funnel. Finally, wash the ester with 5 mL of brine solution to remove residual water.

Weigh the ester and transfer the solution neat into a vial for PicoSpin™ ^1H NMR analysis. Obtain your spectrum using the PicoSpin™ operation instructions. You must determine the ID of your ester and unknown alcohol. You must also assign each proton and carbon of your compound with a signal from the 2 NMR's. Label all spectra as you are shown in the pre lab lecture.