SYNTHESIS OF NOVEL ANALOGS OF BELACTOSIN A AND CARFILZOMIB

by

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ABSTRACT

Belactosin A, a naturally occurring proteasome inhibitor with potent anti-tumor activity, was discovered in the late 1980's. The key structural features of the natural product include a cyclopropane ring with a terminal β-lactone "serine trap". Activity is conferred by acylation of the β-lactone with a threonine of the proteasome. Carfilzomib is a tetrapeptide epoxyketone, and an analog of epoxomicin with improved properties. In phase I and phase II clinical trials, carfilzomib exhibited higher selectivity, equal potency and less peripheral neuropathy than bortezomib and salinosporamide. Its activity is due to the formation of a highly stable six membered morpholine ring by the interaction between epoxyketone, free hydroxy and α-amino groups of the threonine.

Several syntheses of belactosin A and carfilzomib have been reported, however only a handful of analogs have been reported. An approach to the synthesis of novel hybrid analogs is reported here, with the synthesis of phenylalanine, leucine and valine cyclopropyl analogs of belactosin A with the epoxyketone of carfilzomib. Key steps in the synthetic route include a cyclopropanation and formation of an enone followed by epoxidation.

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CHAPTER I: INTRODUCTION

Proteasome structure and function

The traditional methods for treatment of cancers are surgery, radiation and chemotherapy. However, many treatments cause side effects, and not all are effective. A novel treatment for cancer chemotherapy is by proteasome inhibition.[1] The 26S proteasome is an ATP-dependent multi-subunit proteolytic complex expressed in the nucleus and cytoplasm of all eukaryotic cells.[2] It is responsible for regulation of the mutant, damaged and misfolded proteins by the ubiquitin proteasome pathway (UPP), and is a subject of research in anticancer therapy.[3] The proteasome consists of two 19S regulatory particles (RP) and a 20S catalytic core particle (CP). The 20S CP is barrel-shaped and is composed of four stacked rings (α_7, β_7, β_7, α_7) with 28 subunits (**Figure 1**). Two outer rings on either side are composed of seven different α-subunits while the central two rings are composed of seven different β-subunits (β1, β2, β3, β4, β5, β6, β7) with six active sites; two of each chymotrypsin-like (CT-L), trypsin-like (T-L) and caspase-like (C-L)[2,4,5]. The 19S RP binds polyubiquitined proteins and, due to its ATP activities, polyubiquitined proteins begin to unfold and insert the protein into the 20S CP, where the protein breaks into smaller peptides having 3-22 amino acids in length.

The protein to be degraded is flagged by polyubiquitination.[6] This process is catalyzed by the enzyme E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugation enzyme) and E3 (ubiquitin ligase) which activate free ubiquitin and attach it to the target protein.

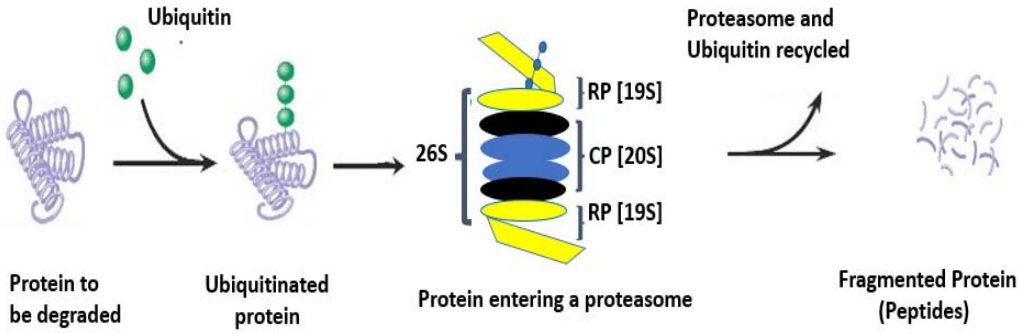


Figure 1: The simplified scheme of the ubiquitin-proteasome pathway

The process starts by the formation of a thioester bond between E1 and ubiquitin in the presence of ATP. This activated ubiquitin then transfers to the E2 enzyme and E2 presents ubiquitin to E3 which brings ubiquitin. This process is repeated several times, creating a polyubiquitin chain that tags the protein for degradation by the proteasome.

Proteasome's catalytic mechanism

The proteasome plays a vital role to control protein quality, antigen processing, signal transduction, cell differentiation, cell-cycle progression and apoptosis.[7] Proteasomes contain a new class of proteolytic enzyme called threonine (Thr) proteases,[8] which are activated by the catalytic triad having acidic (Asp or Gly), a basic histidine (His) and the nucleophilic (Thr) residue.[9] The N-terminal threonine of β-subunits are utilized by all the proteolytic sites present in proteasomes. According to X-ray crystallography, in presence of a water molecule the nucleophilic (Thr-O) attack is activated by N-terminal Thr1 to add to the carbonyl carbon of a protein substrate to form an intermediate tetrahedral oxyanion (**Figure 2**), which forms an intramolecular hydrogen bond with the Gly 47-N (oxyanion hole).

A water molecule is activated by the deprotonated Thr1 amino group to hydrolyze the acyl-enzyme to regenerate the Thr1-OH (free proteasomes) and second product (**Figure 2**).[7]

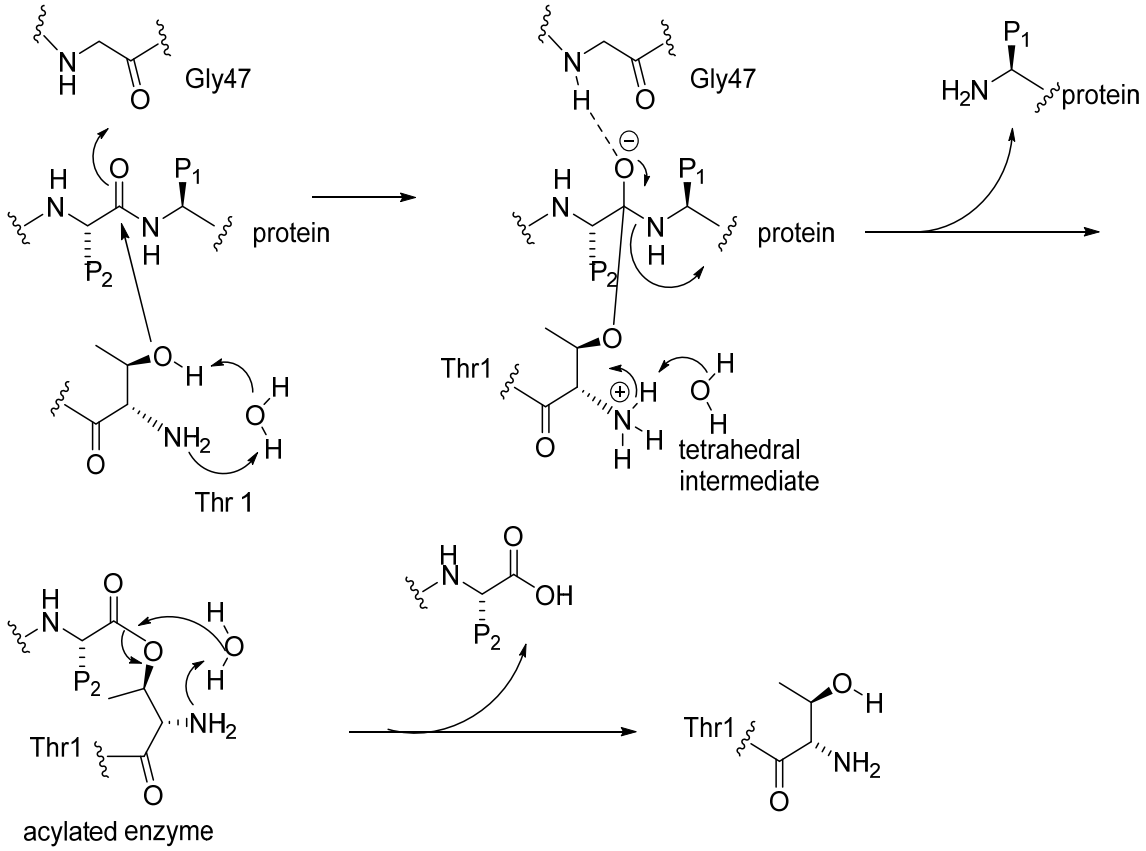


Figure 2: General proposed mechanism for the proteasome-catalyzed hydrolysis of peptides

Proteasome inhibitors

The proteasome crucial role was discovered in the late 1980s.[8] The cell cycle depends on degradation of protein, and proteasome inhibition mechanism is a new way to fight against non-small cell lung carcinoma (NSCLC),[10] chemo resistant multiple myeloma

(MM)[11], and several other types of cancers. According to one model, proteasome inhibitors caused apoptosis mostly in transformed cancer cells.[12] Common features of inhibitors include being peptide-like and having a reactive group "serine trap" to form a covalent bond (**Figure 3**) with the nucleophilic threonine.[8]

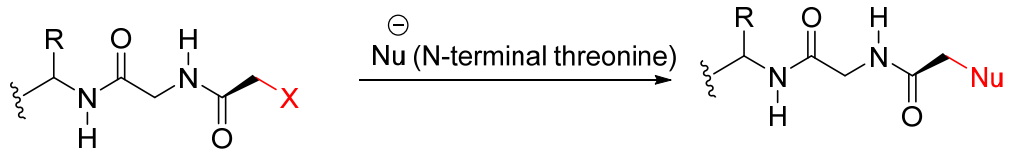


Figure 3: Inhibitors showing active site (a serine trap X)

Proteasome inhibitors are basically two types on the basis of sources: chemically synthesized small molecules, and derivatives of natural products.[13] All inhibitors act on CT-L activity, but also have a weaker effect on T-L and C-L sites. Synthetic inhibitors are classified as peptide aldehydes, peptide boronates, peptide lactones and peptide epoxyketones, and will be discussed further.

1.1 Peptide aldehyde

Peptide aldehydes were the first reported reversible proteasome inhibitors. Aldehyde inhibitors have slow binding activity. These also block other serine and cysteine proteases leading to off-target effects. Because of fast dissociation rates, these are quickly metabolized into inactive acids by the oxidation process and taken out of the cell through the multi-drug resistance (MDR) system carrier.[7,8,13] The electrophilic carbon atom of the carbonyl group forms a covalent bond with the nucleophile, which is semi-reversible (**Figure 4**).

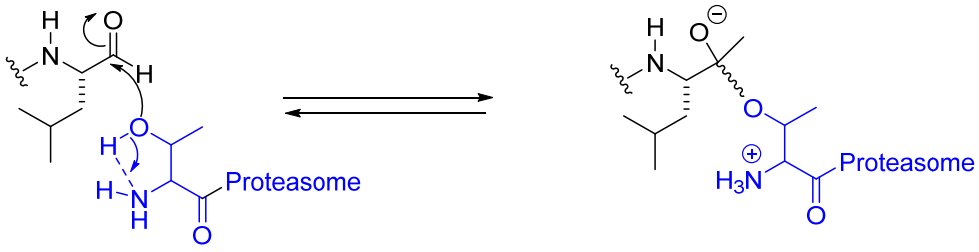


Figure 4: General mechanism of aldehyde inhibitors

Only a few aldehyde inhibitors, like MG132 (Z-Leu-Leu-Leu-al) in **Figure 5**, are commercially available, and can inhibit CT-L activity of proteasomes. None are approved as drugs but are used as research tools to study the proteasome.

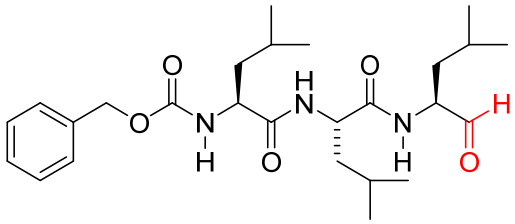


Figure 5: Structure of MG132 (Z-Leu-Leu-Leu-al) (a serine trap in red)

1.2 Peptide boronates

Peptide boronates are formed by replacing the aldehyde functionality of aldehyde inhibitors by a boronic acid moiety and are much improved over aldehyde inhibitors.[14] Even low nanomolar concentrations inhibit CT-L activity and have no effect on cysteine proteases.[7] Because of much slower dissociation rate of the boronate-proteasome adducts with respect to the aldehyde-proteasome adducts, these are practically irreversible, despite being reversible inhibitors. A covalent tetrahedral boron adduct is formed between the electrophilic boron atom and the active site Thr1-O (**Figure 6**).

Figure 6: General mechanism of boronate inhibitors

Boronate inhibitors are found to be more specific than aldehyde inhibitors and show very poor inhibitory effect on cysteine proteases due to the weak interaction between boron and sulfur. Bortezomib (**Figure 7**) is a dipeptide boronate inhibitor, marketed as Velcade. It was approved by the FDA to be used clinically for the treatment of multiple myeloma and mantle cell lymphoma in 2003 and 2006, respectively.[15] The drug is currently given with the various chemotherapeutic agents (doxorubicin, thalidomide, melphalan) in several active clinical trials for the treatment of several types of cancers, but has severe side effects like nausea, diarrhea, and peripheral neuropathy.[7,16] Ixazomib (**Figure 7**) is also known as MLN9708. Ixazomib is the first oral compound and second-generation proteasome inhibitor approved by the US FDA for the treatment of multiple myeloma (MM). Ixazomib has better pharmacokinetic and pharmacodynamic terms with respect to bortezomib. By the use of ixazomib severe peripheral neuropathy was hardly noticed, but side effects like thrombocytopenia, nausea, vomiting, diarrhea, fatigue, and rash were very common.[17,18]

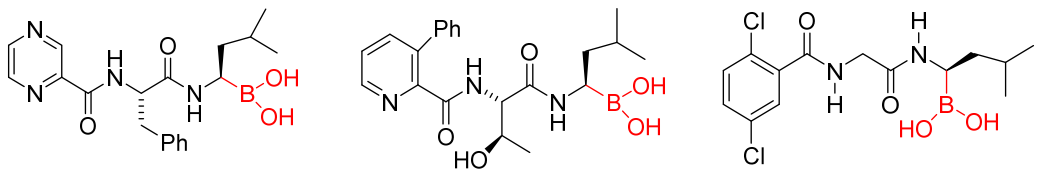


Figure 7: Structure of bortezomib, CEP-18770 and ixazomib (a serine trap in red)

CEP-18770 (**Figure 7**) is found to be far better than bortezomib in terms of various biological activities like enzymatic, cellular and antiproliferative and pharmacological properties,[7] but is not yet approved.

1.3 β-lactones and lactacystin inhibitors

Lactacystin (Figure 8) was discovered by Omura and co-workers, and later synthesized by Corey and co-workers.[8] This is the first natural non-peptidic proteasome inhibitor and was isolated from a strain of *Streptomyces* which produces omuralide or lactacystin- β-lactone.

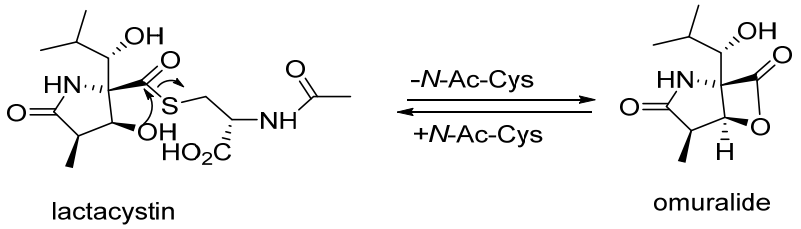


Figure 8: Lactacystin interconversion to omuralide

Omuralide's low nanomolar concentrations can inhibit the CT-L activity of the proteasome (**Figure 8**) without inhibiting most of the serine and cysteine proteases. The β-lactone forms an adduct with proteasome, which is slowly ($t_{1/2} \approx$ 20 hr) hydrolyzed by water, though it is regarded as an irreversible inhibitor and more selective than aldehyde inhibitors.[7,8,14,19]

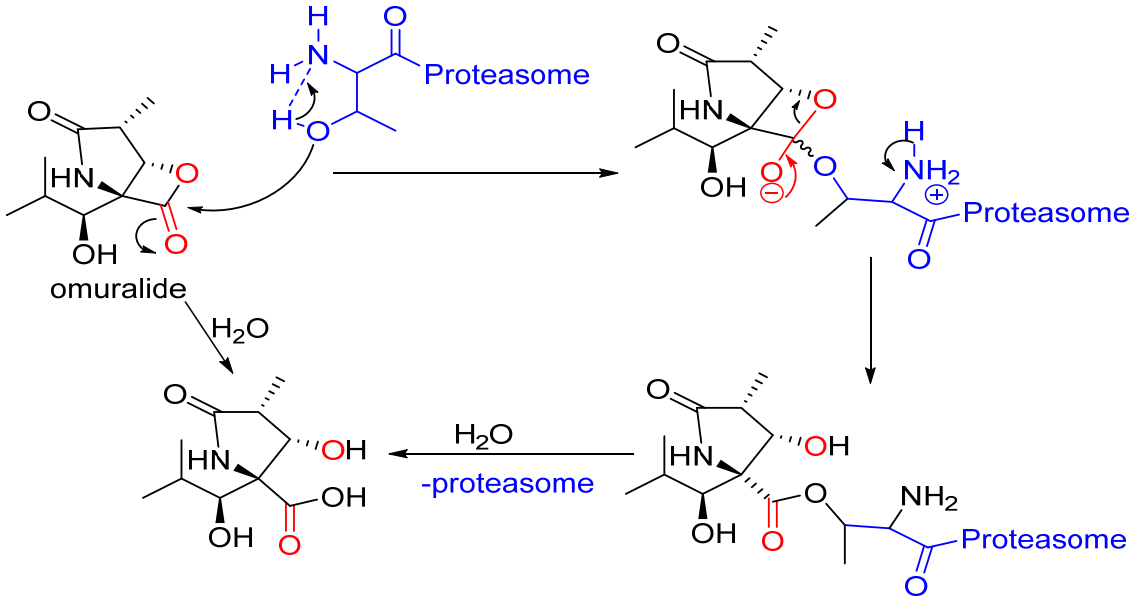


Figure 9: General mechanism of β-lactones and lactacystin inhibitors

Salinosporamide A (**Figure 10**) was isolated from the marine actinomycete *Salinispora tropica* by Fenical *et al*. in 2003.[14] It is very important γ-lactam-β-lactone.

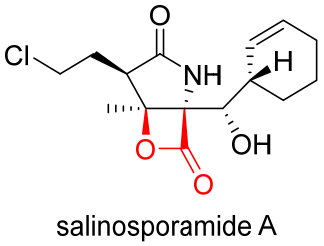


Figure 10: Salinosporamide A (a serine trap in red)

Salinosporamide A has a unique structure, that is a fused γ-lactam-β-lactone bicyclic ring structure similar to lactacystin. According to X-ray crystallography structure, Groll *et al*. proposed it as an irreversible proteasome inhibitor. The N-terminal Thr-O group is acylated through opening of the β-lactone ring (**Figure 11**), and the C6 hydroxy group displaces the chloride group to form the tetrahydrofuran intermediate.[14]

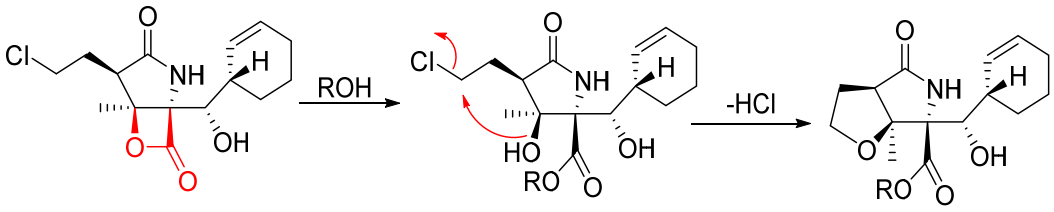


Figure 11: Mechanism of the irreversible inhibition of Salinosporamide A

It stops the cell proliferation of various tumor cells lines (IC_{50} < 10 nM) as well as bortezomib-resistant myeloma cell lines. It is 35 times more potent than omuralide, which irreversibly inhibits 20S proteasome CT-L activity with IC_{50s} of 2.5 to 4.3 nM.

1.4 Belactosin and derivatives

Belactosin A (**Figure 12**) is a tripeptide natural product. Asai and co-worker discovered and isolated it from *Streptomyces sp.* in 2000.[19] It is a reversible proteasome inhibitor with antitumor activity. From X-ray crystallographic analysis, it is found that β-lactone ring opening inhibits CT-L activity of proteasome by acylating the active-site Thr reside.[20,21]

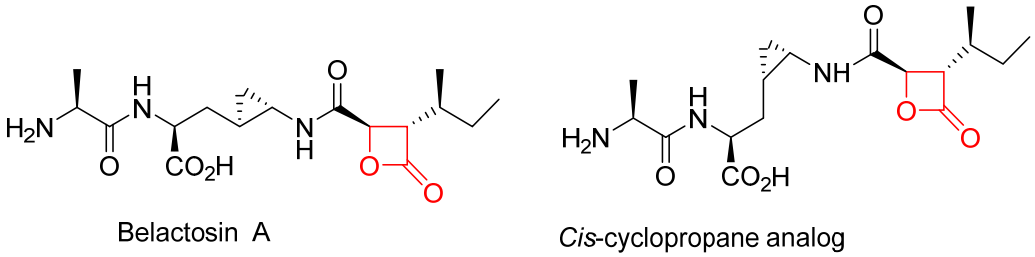


Figure 12: Structure of belactosin A and *cis*-cyclopropane analog

The studies by Shuto *et al.* show that the natural belactosin A with the *trans*-cyclopropane structure is less potent than the unnatural *cis* isomer **(Figure 12).**[22] While investigating the synthesis of cyclopropane derivatives with a desired stereochemistry, Shuto *et al.* studied

systematic structure-activity relationship (SAR) of belactosin A and found compounds **1-3 (Figure 13**) were highly effective proteasome inhibitors.[23] In addition to finding that the trans-cyclopropane stereochemistry is not required, they also found that replacing the carboxylic acid with a hydrophobic group such as phenylethyl improved potency.[24] Also, protection of the primary amine as a carbobenzoxy (cbz) group improved potency (**compound 1**).

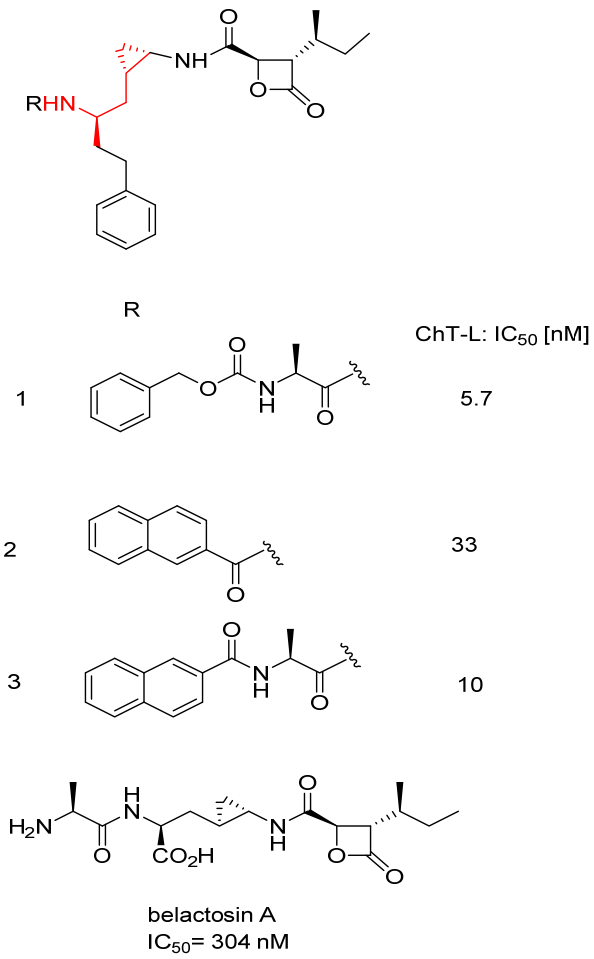


Figure 13: Development of potent proteasome inhibitors using belactosin A analog, compounds assayed for inhibition of ChT-L activity of proteasome and for cancer cell growth.

1.5 Peptide epoxyketones

Epoxyketones are very specific, effective irreversible proteasome inhibitors. They have a different mechanism to act on proteasome active sites. A very stable six membered morpholine adduct (**Figure 14**) is formed by reacting both Thr1 hydroxy and amino group of the proteasome with the epoxyketone site.[8,25]

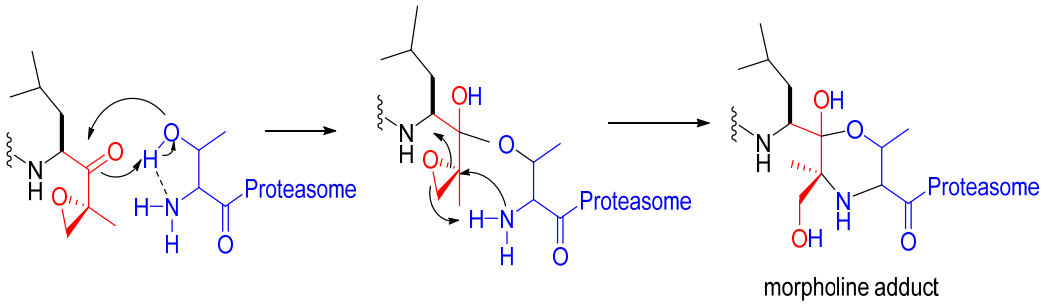


Figure 14: General mechanism of peptide epoxyketone proteasome inhibition.

The first epoxyketone reported as a proteasome inhibitor was epoxomicin (Figure 15), which was isolated from the actinomycete strain Q996-17. It is a α,β-epoxyketone peptide and primarily inhibits CT-L activity irreversibly. It has shown high in vivo antitumor activity against solid B16 melanoma tumors.[7,8,14]

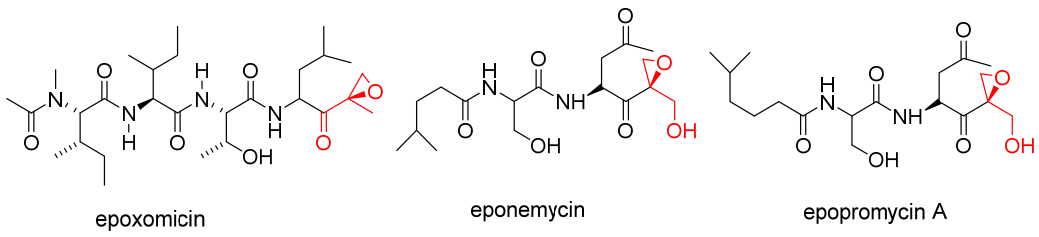


Figure 15: Naturally occurring epoxyketone epoxomicin (a serine trap in red)

Other natural epoxyketone derivatives like eponemycin and epopromycins A (**Figure 15**) have *in vivo* antitumor activity.[8,14] After the clinical success of bortezomib and to enhance

effect of epoxomicin, a second-generation proteasome inhibitor carfilzomib (**Figure 16**) was developed. It is a tetrapeptide epoxyketone and an analog of epoxomicin.

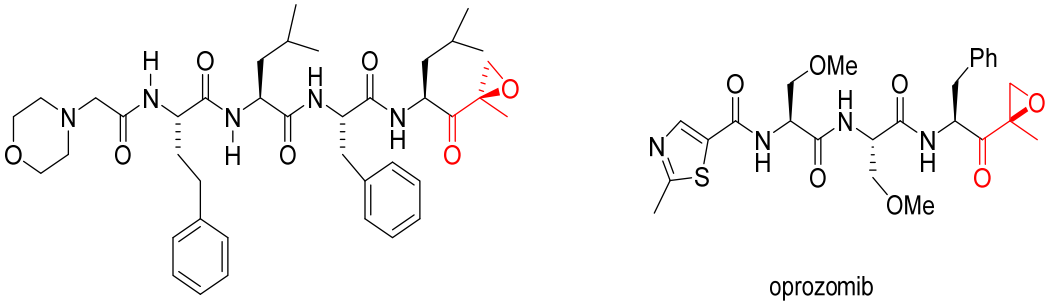


Figure 16: Carfilzomib and oprozomib (a serine trap in red)

Carfilzomib was approved by FDA in 2012 for the treatment of multiple myeloma, non-Hodgkin's lymphoma and solid tumors. In phase I and phase II clinical trials, carfilzomib exhibited higher selectivity (IC_{50}= 6 nM, 3600 nM, 2400 nM for CT-L, T-L, PA activity respectively), equal potency and less peripheral neuropathy with respect to bortezomib and salinosporamide.[7,27,28]

To make treatment easier, and to remove adverse effects of intravenously administered agents like carfilzomib and bortezomib, recently the next generation oprozomib (**Figure 16**) was developed.[29] Oprozomib is orally bioactive and irreversible proteasome inhibitor, and selectively inhibits the CT-L activity.

Since the discovery of the first proteasome inhibitors, much effort has been put into developing these compounds into drugs. While a number of proteasome inhibitors have been developed, there are still only a few on the market.

Goals of project

The primary goal of this project is to synthesize a hybrid analog of belactosin A and carfilzomib. This would combine the semi-rigid cyclopropyl backbone of belactosin A with the epoxyketone serine trap of carfilzomib. The first objective is to synthesize the cyclopropyl backbone of belactosin A from the protected amino acids phenylalanine, leucine and valine, which will give three different hydrophobic replacements for the acid. The second objective is to incorporate the epoxyketone of carfilzomib in the analogs. The final objective is to study biological activity assays on proteasome inhibition and on cancer cells. The retrosynthetic **(Figure 17)** approach to synthesize the final product is a seven-step process.

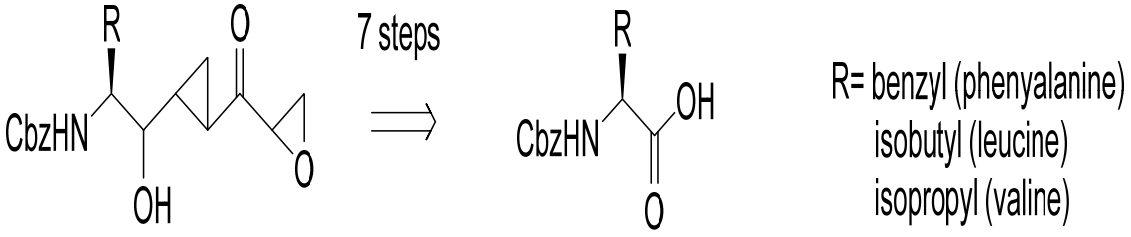


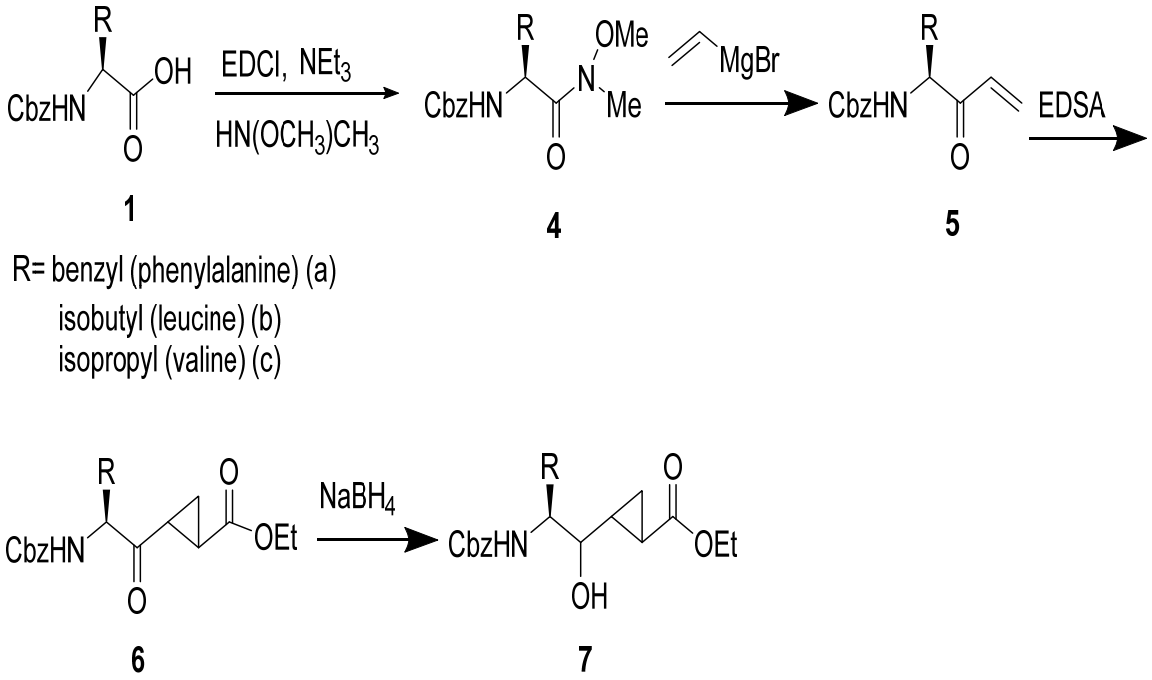
Figure 17: Retro-synthetic approach to proposed analogs

Synthesis of cyclopropyl esters

Dr. Dunlap's group has developed a new route to the cyclopropyl backbone which is outlined in **Scheme 1**. The synthesis starts with protected amino acids peptidomimetic and affords cyclopropyl esters. The protected amino acids are converted to Weinreb

intermediate enones **(5** in **Scheme 1)**. The enones, on treatment with EDSA (ethyl (dimethylsulfuranylidene) acetate, a sulfonium ylide afford cyclopropyl esters, which are then reduced to the corresponding alcohols **(7** in **Scheme 1**).[30,31,32]

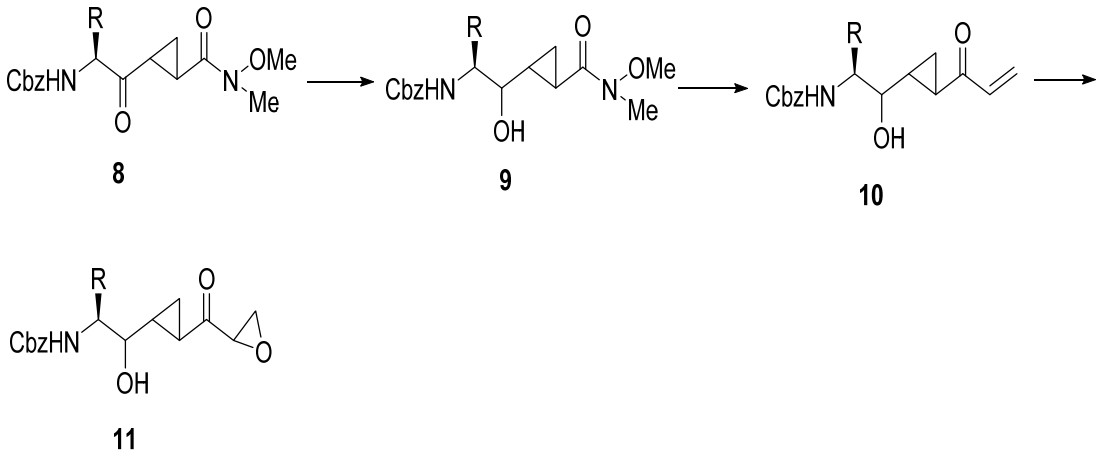
Scheme 1: Synthesis of cyclopropyl esters



Proposed synthesis of Epoxides

The goal is to adapt this synthesis to give cyclopropyl epoxides as hybrid analogs. The synthesis will be modified to give a cyclopropyl Weinreb amide **8**, which will be reduced to the alcohol **9,** as outlined in **Scheme 2** .[33,34]

Scheme 2: Proposed Synthesis of epoxyketone hybrids.



Addition of vinylmangnesium bromide to either **9** or a protected alcohol derived from **9** should give the cyclopropyl enone **10**. Finally, epoxidation of the enone will give the hybrid cyclopropyl epoxyketone **11**.

CHAPTER II: MATERIALS AND METHODS

Instruments, materials, and reagents

The NMR data are obtained using a 500 MHz FT-NMR model ECA-500 JEOL (Peabody, MA) purchased with funding provided by National Science Foundation through the NSF-RUI program (#0321211) and a 300 MHz FT-NMR model ECA-300 JOEl (Peabody, MA). The Chemical shifts are reported in parts per million with respect to tetramethylsilane (TMS). The splitting pattern are represented by the following: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets), ddd (doublet of double doublets), dt (doublet of triplets) and br (broad signal). Coupling constant (J values) are recorded in Hz. High-resolution electrospray ionization-mass spectrometry (ESI-MS) was performed at Notre Dame University, Notre Dame, Indiana.

Thin layer chromatography (TLC) was performed on glass plates coated with silica get and UV active backing purchased from Fisher Scientific, Pittsburg, PA. The TLC plates were analyzed with a short wavelength (254 nm) UV light and followed by staining with either phosphomolybdic acid (PMA), ninhydrin, or anisaldehyde (reagent grade, Aldrich, Milwaukee, WI). Flash column chromatography was performed with silica gel, 32-63-micron ASTM (reagent grade, Fisher Scientific, Pittsburg, PA), and flash chromatography was performed on an ISCO CombiFlash R_f 200 (Teledyne ISCO, Lincoln, NE) using a Teledyne ISCO cartridge preloaded with 5g of normal phase silica and a Teledyne ISCO preloaded 12g flash column.

Dichloromethane (DCM), methanol (MeOH), acetone, ethyl acetate (EA), ethanol (EtOH), and hexanes (Hex) were purchased from Fisher Scientific, Pittsburg, PA. Chloroform was purchased reagent grade form Acros Organic, New Jersey, USA. Anhydrous tetrahydrofuran (THF) was obtained using a Pure Solv solvent purification system (Model PS-MD-3, Innovative Technology, Amesbury, MA). deutero-chloroform ($CDCl_3$), deutero-methanol (Methanol-d_4), and Deutero-acetone (Acetone-d_6) were purchased from Aldrich, Milwaukee, WI. Solvent extractions were performed using EA or DCM where indicated and washed with either distilled water, 1M hydrochloric acid (HCl), saturated sodium bicarbonate ($NaHCO_3$), and brine (reagent grade, Fisher Scientific, Pittsburg, PA). The organic layer was dried with magnesium sulfate (Fisher Scientific, Pittsburgh, PA) and filtered. Evaporation of solvents was achieved using a Heidolph rotary evaporator (Model G3, Schwabach, Germany).

Triethylamine (NEt_3) was obtained from Fisher Scientific, Pittsburgh, PA. Anhydrous reagent grade vinylmagnesium bromide and tetrahydrofuran (THF) were purchased from Aldrich, Milwaukee, WI. Other reagents including vinylmagnesium bromide solution (1M), N-carbobenzyloxy)-L-phenylalanine, Z-L-phenylalanine, N-(3-dimethylaminopropyl)-N^1-ethylcarbodimide hydrochloride, bromoacetyl bromide, dimethyl sulphide, carbobenzyloxy-valine, *tert*-butylhydroperoxide solution, potassium tert-butoxide, trifluoroacetic anhydride (TFAA), N,O-dimethyl hydroxylamine hydrochloride, N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDCI), sodium borohydride, and ammonium formate were obtained from Aldrich, Milwaukee, WI.

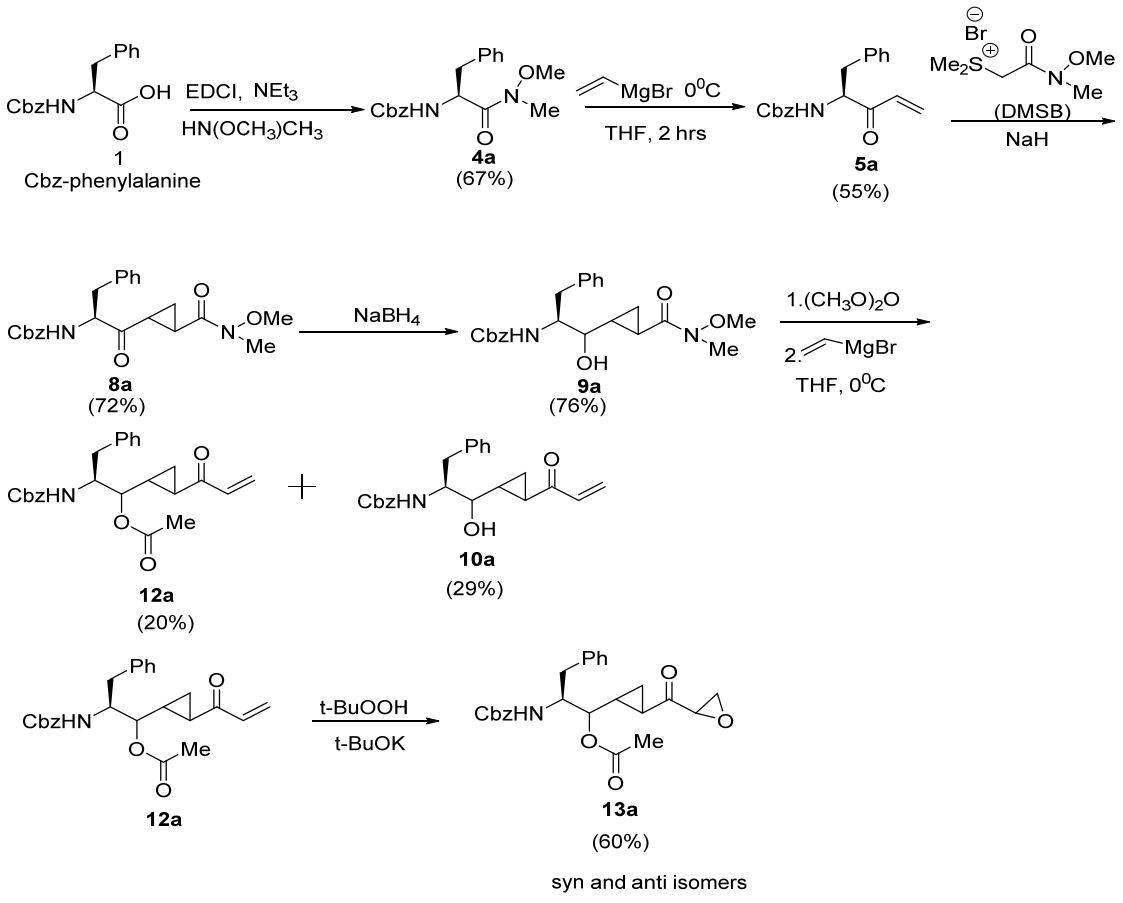
Synthetic methods

The specific synthesis of three of the analogs is described here.

2.1 Synthesis of hybrid analog from Cbz-phenylalanine

The Cbz protected phenylalanine was converted to a Weinreb amide **4a**, followed by treatment with vinylmagnesium bromide to afford an enone **5a**. The enone was treated with DMSB salt and a base to form mixture of cyclopropyl ketone isomers **8a**. There were reduced by sodium borohydride to form mixture of cyclopropyl alcohols **9a.**

Scheme 3: Synthesis of hybrid analog of belactosin A and carfilzomib from Cbz-phenylalanine

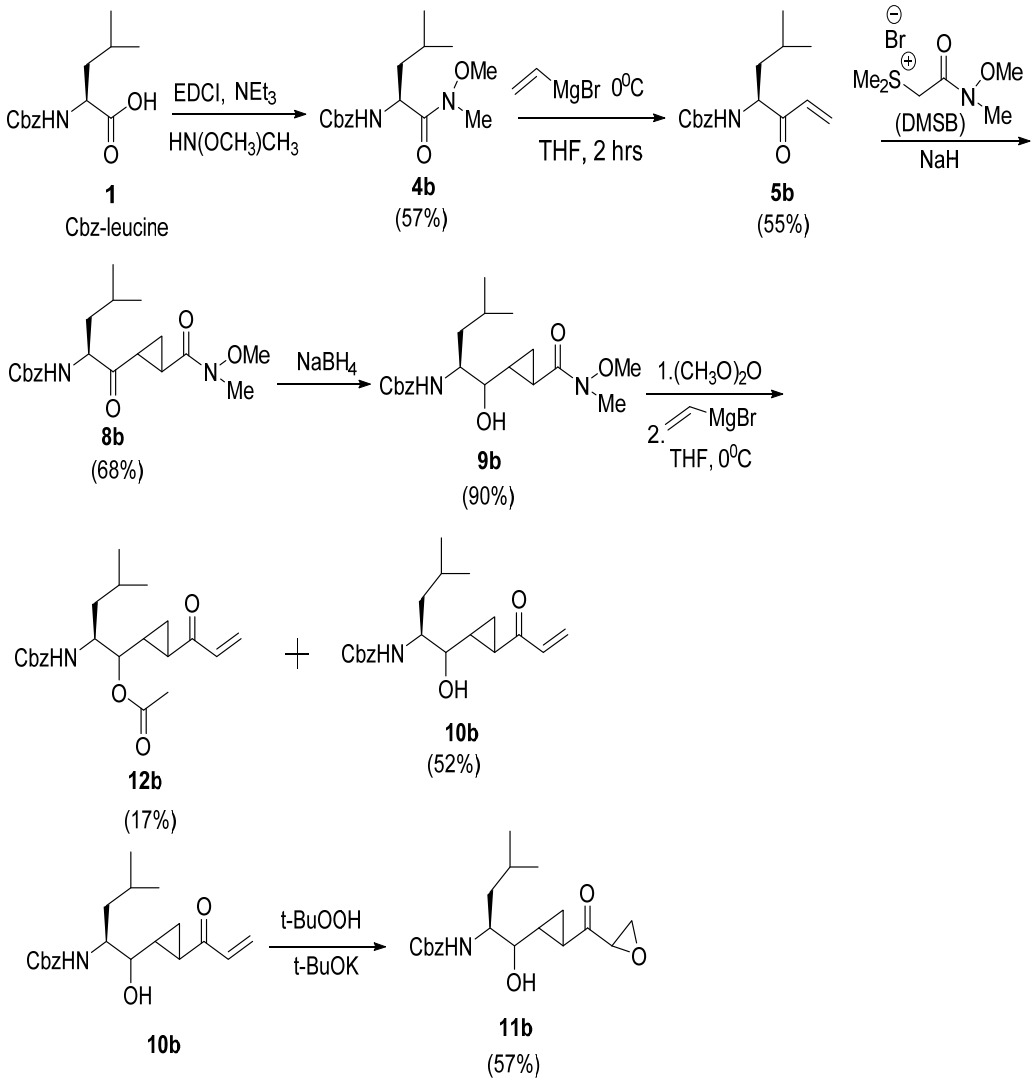


The alcohol group was protected by acylation then treated with vinylmagnesium bromide to afford enone **12a,** as well as deacylated alcohol **10a**. The enone was treated with t-butylperoxide in presence of base toafford a hybrid analog of belactosin A and carfilozomib **13a**. The route is outlined in **Scheme 3.**

2.2 Synthesis of hybrid analog from Cbz-leucine

The Cbz protected leucine was converted to a Weinreb amide **4b**, followed by treatment with vinylmagnesium bromide to afford an enone **5b**. The enone was treated with DMSB salt and a base to form mixture of cyclopropyl ketone isomers **8b**. There were reduced by sodium borohydride to form mixture of cyclopropyl alcohols **9b**. The alcohol group was protected by acylation then treated with vinylmagnesium bromide to afford enone **12b**, as well as deacylated alcohol **10b.** The enone was treated with t-butylperoxide in presence of base to afford a hybrid analog of belactosin A and carfilozomib **11b.** The route is outlined in **Scheme 4**.

Scheme 4: Synthesis of hybrid analog of belactosin A and carfilzomib from Cbz-leucine

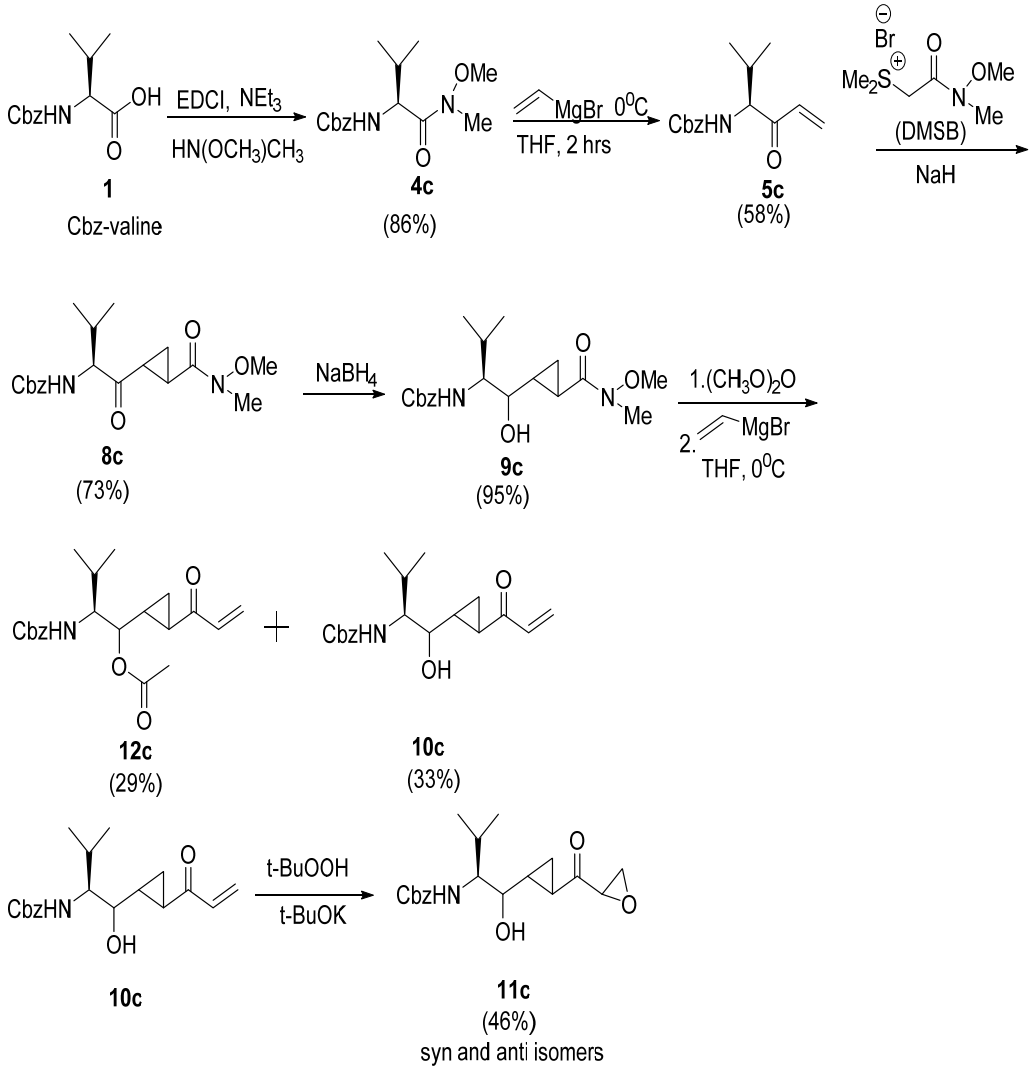


2.3 Synthesis of hybrid analog from Cbz-valine

The Cbz protected phenylalanine was converted to a Weinreb amide **4c**, followed by treatment with vinylmagnesium bromide to afford an enone **5c**. The enone was treated with DMSB salt and a base to form mixture of cyclopropyl ketone isomers **8c**. There were reduced by sodium borohydride to form mixture of cyclopropyl alcohols **9c**. The alcohol group was protected by acylation then treated with vinylmagnesium bromide to afford

enone **12c**, as well as deacylated alcohol **10c.** The enone was treated with t-butylperoxide in presence of base to afford a hybrid analog of belactosin A and carfilozomib **11c** The route is outlined in **Scheme 5**.

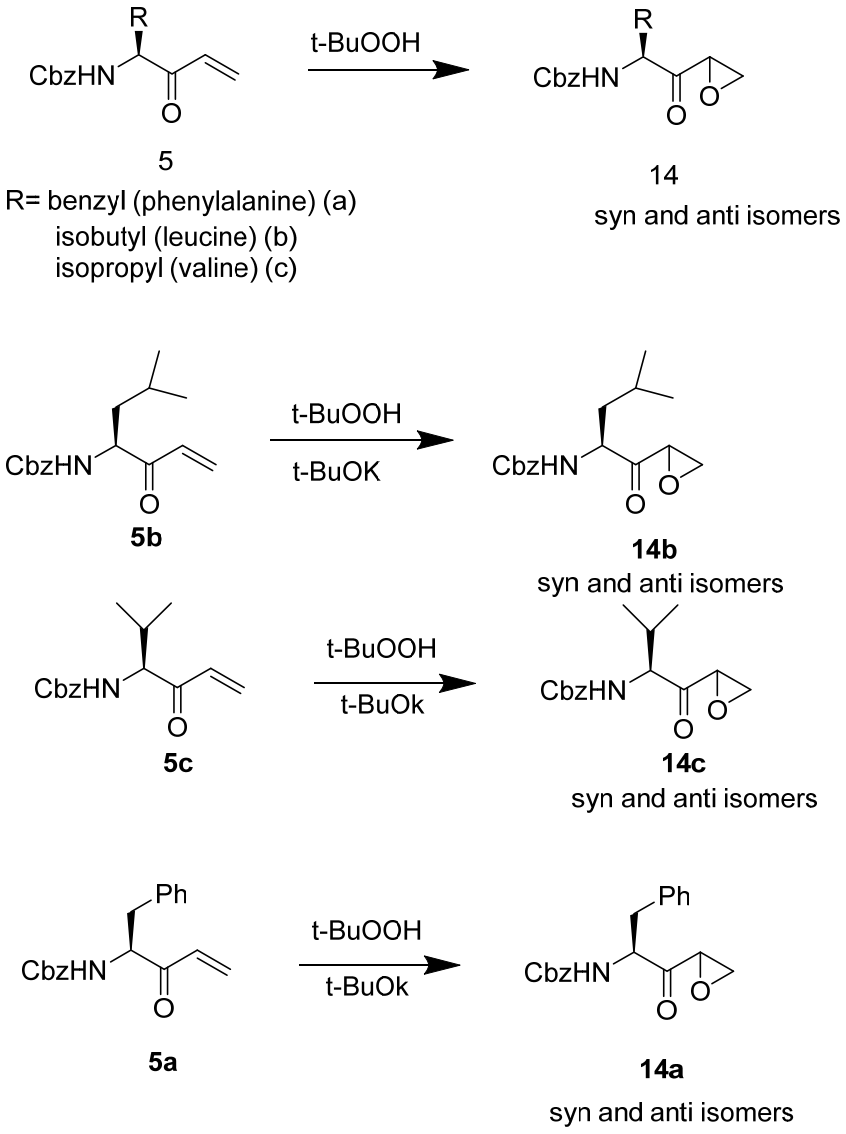
Scheme 5: Synthesis of hybrid analog of belactosin A and carfilzomib from Cbz- valine



Simple epoxides

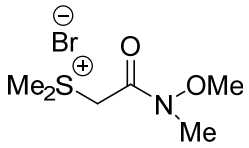
The synthesis of of simple epoxides was accomplished by treating Cbz-protected enones **5a-c** with t-butyl hydrogen peroxide in the presence of the strong base potassium

tert-butoxide, These were prepared as model systems for the cyclopropyl epoxyketones hybrid analogs.



Experimental procedures

(2-(methoxy(methyl)amino)-2-oxoethyl)dimethylsulfonium bromide (DMSB) (3):

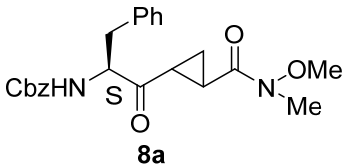


Formation of 2-bromo-N-methoxy-N-methylamide: To a stirred suspension of N,O-dimethylhydroxylamine hydrochloride (2.1 g, 21.5 mmol), and K_2CO_3 (14.86 g, 107.70 mmol) in MeCN (80 mL), bromoacetyl bromide (4.32 mL, 49.54 mmol) was added dropwise at room temperature. After 80 minutes, the reaction mixture was poured into water and extracted twice with CH_2Cl_2. The organic layer was dried over anhydrous magnesium sulfate, filtered and the solvent was evaporated to afford 3.2 g (81.75 %) of the Weinreb amide. H-NMR (500 MHz, $CDCl_3$): δ 3.70 (2H, s), 3.44 (3H, s), 2.86 (3H, s)

To a solution of bromo-N-methoxy-N-methylamide (3.2 g, 17.6 mmol) in acetone, dimethyl sulfide (1.93 mL, 26.41 mmol) was added, and stirred for 18 hours. The mixture was filtered and washed with acetone, the solid was dried under vacuum for 20 minutes to afford 3.22 g (75.01%) (**3**), mp 107-109 ˚C.

2.4 Cbz-phenylalanine series

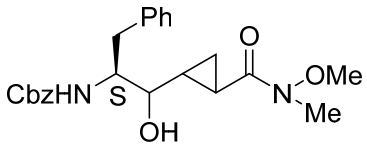
Benzyl(1-(2-(methoxy(methyl)carbamoyl)cyclopropyl)-1-oxo-3-Phenylpropan-2-yl)carbamate (8a):



8a

To a suspension of NaH (60%, in mineral oil, 370.0 mg, 9.32 mmol) in DMSO (11 mL), (2-(methoxy(methyl)amino)-2-oxoethyl) dimethylsulfonium bromide (2.27 g, 9.32 mmol) was added at room temperature, and was stirred for 25 minutes. The solution was cooled to 0˚C, a solution of the (S)-enone **5a (**1.31 g, 4.23 mmol) in THF (11 ml) was added, and the mixture was stirred for 10 minutes. The creamy semisolid was dissolved in methylene chloride, and then poured into 1M HCl and extracted with methylene chloride thrice. The organic layer was washed with brine, dried with anhydrous magnesium sulfate and the solvent was evaporated. The crude product was purified by CombiFlash and evaporated to afford 1.25 g (72.1%) (**8a**) as a mixture of syn and antiisomers. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.34-7.08 (m, 10H, aryl), 5.71, 5.68 (2d, J=7.45, 7.45 Hz, 1H, NH), 5.06 (m, 2H, $CbzCH_2$), 4.81 (m, 1H, CHN), 3.69, 3.67 (s,3H, OCH_3), 3.18, 3.17 (s, 3H, NCH_3), 3.07 (m, 2H, CH_2Ph), 2.8-2.69 (d, 1H, CHcpyl), 2.58 (m, 1H, CHcpyl), 1.5, 1.41 (m, 1H, CH_2cpyl); ^{13}C NMR (125 MHz, $CDCl_3$): δ 206.04, 205.91 (C=O, ketones), 171.0 (C=O, amide), 156.73, 155.65 ($CbzCH_2$), 136.5, 135.7(4˚aryls), 129.51, 129.46, 128.62, 128.27, 128.16, 127.25 (10 aryl), 66.98 ($CbzCH_2$), 61.92 (OCH_3), 61.70, 61.58 (CHN'S), 37.35, 37.25 (CH_2Ph), 32.63 (NCH_3), 27.34, 27.19 (CHcpyl), 23.05, 22.90 (CHcpyl), 18.48, 18.03 (CH_2cpyl); mass spectrum (ESI-MS) m/z ($C_{23}H_{27}N_2O_5$) calculated for (M+H) 411.1917, found 411.1914.

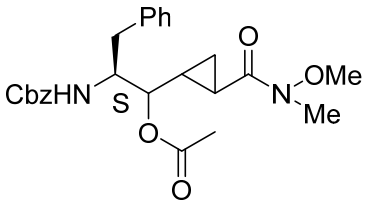
Benzyl(1-hydroxy-1-(2-methoxy(methyl)carbamoyl)cyclopropyl)-3-phenylpropan-an-2-yl)carbamate (9a):



9a

To a solution of the ketone (**8a**) (1.18 g, 2.87 mmol) in methanol, $NaBH_4$ (200 mg, 5.23 mmol) was added and the mixture was stirred for 170 minutes at room temperature. The solution was poured in to 1M HCl and extracted with methylene chloride twice. The solution was washed with $NaHCO_3$ and brine, and was dried with anhydrous magnesium sulfate, filtered and the solvent evaporated. The crude was purified on CombiFlash and evaporated to afford 904.9 mg (76%) (**9a**) as a mixture of isomers. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.3-7.18 (m, 10H, aryl), 5.83, 5.75, 5.71 (3d, J= 9.15, 9.15, 8.6 Hz, 1H, NH), 5.42, 5.38 (d, J=9.74 Hz, NH), 4.99 (m, 2H, CH_2Cbz), 4.1, 3.9 (m, 1H, CHN), 3.69, 3.65 (s, 3H, OCH_3), 3.52, 3.35 (m, 1H, CHO), 3.16, 3.14, 3.12 (s, 1H, NCH_3), 2.99, 2.94, 2.79 (m, 2H, CH_2Ph), 2.25, 2.15 (brs, 1H, OH), 1.64, 1.25, 1.17 (m, 1H, CHcpyl), 1.03, 0.9, 0.65 (m, 2H, CH_2cpyl); ^{13}C (125 MHz, $CDCl_3$): δ 172.82 (C=O, amide), 155.99, 155.86, 155.67 (C=O, Cbz), 137.44, 137.3, 135.62, 135.52 (4˚ aryl C's), 128.18, 127.46, 126.99, 126.89, 126.83, 125.37 (aryl C's), 74.18, 73.23, 72.38, 71.48 (CHO), 65.62 (CH_2Cbz), 60.70 (OCH_3), 56.43, 56.15 (CHN), 36.90, 36.71, 34.82, 34.55 (CH_2Ph), 31.56 (NCH_3), 24.27, 23.92, 23.12, 22.56 (Ccpyl), 14.82, 14.12, 13.55 (Ccpyl), 11.37, 11.04 (CH_2cpyl); Mass spectrum (ESI-MS) m/z ($C_{23}H_{29}N_2O_5$) calculated for (M+H) 413.2071, found 413.2071.

Formation of (2S)-2-(((benzyloxy)carbonyl)amino)-1-(2-(methoxy(methyl)carbamoyl)cyclopropyl)-3-phenylpropyl acetate (15a):

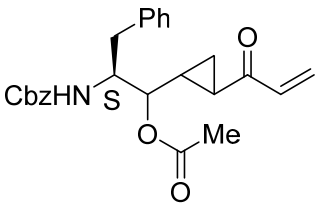


15a

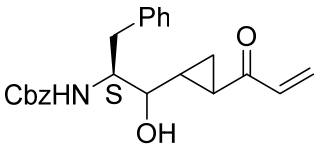
To a solution of (**9a**) (254 mg, 0.614 mmol) in 5 mL CH_2Cl_2, acetic anhydride (0.48 mL, 4.30 mmol) and pyridine (0.35 mL, 4.3 mmol) were added. The resulting solution was stirred for 36 hours, and then poured into 1M HCl and extracted with ethyl acetate twice. The organic layer was washed with aqueous $NaHCO_3$ and dried over magnesium sulfate, filtered and the solvent was evaporated. The crude product was purified on flash column chromatography on silica gel eluting with 1:3, 1:1, and 2:1 ethyl acetate-hexane to afford 248.6 mg (88.38%) (**15a**) as a mixture. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.28-7.17 (m, 10H, aryl), 5.64, 5.42, 5.32, 5.29 (4d, J= 9.15, 9.7, 10.3, 10.3 Hz, 18.3 Hz, 12.6Hz,1H, NH), 5.06-4.83 (m, 2H, CH_2Cbz), 4.67, 4.5, 4.34 (m, 1H, CHO), 4.28, 4.19 (m, 1H, CHN), 3.69, 3.65, 3.62 (s, 3H, OCH_3), 3.15, 3.14, 3.11, 3.1 (s, 3H, NCH_3), 2.96, 2.75 (m, 2H, CH_2Ph), 2.28-2.2 (d, 1H, CHcpyl), 2.01, 1.99 (s, 3H, CH_3), 1.72 (m, 1H, CHcpyl), 1.29, 1.2, 1.01, 0.91, 0.71 (m, 2H, CH_2cpyl); ^{13}C NMR (125Hz, $CDCl_3$): δ 173.19, 172.95 (C=O, ester), 170.71, 170.68, 170.31 (C=O, amide), 156.29, 156.11, 156.06 (C=O, CbZ), 137.60, 137.4, 137.22, 136.73, 136.54 (4˚ aryl), 129.27, 129.09, 128.54, 128.19, 127.99, 127.91, 127.81, 126.75, 126.62 (6 aryl C's), 76.7, 76.41, 75.54 (CHO), 66.85, 66.49 (CH_2Cbz), 61.79, 61.73, 61.65 (OCH_3), 55.55, 55.41, 54.74, 54.46 (CHN), 38.71, 38.39, 36.44, 36.20

(CH_2Ph), 32.56 (NCH_3), 23.34, 22.85, 21.74, 21.11 (CHcpyl), 21.05 (CH_3CO), 15.79, 15.58 (CHcpyl), 12.92, 12.66, 12.59 (CH_2cpyl) Mass spectrum (ESI-MS) m/z ($C_{25}H_{31}N_2O_6$) calculated for (M+H) 455.2178, found 455.2177.

Formation of benzyl ((2S)-1-(2-acryloylcyclopropyl)-1-hydroxy-3-phenylpropan-2-yl) carbamate (10a) and ((2S)-1-(2-acryloylcyclopropyl)-2-(((benzyloxy)carbonyl)amino)-3-phenylpropyl acetate, (12a):



12a



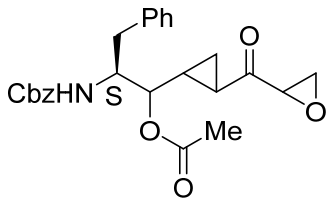
10a

To a solution of (**15a**) (110 mg, 0.24 mmol) in THF (1.5 mL), at 0˚C under argon, vinylmagnesium bromide (0.61 ml of 1M solution) was added, and stirred for 1 hour, and again the same amount of vinylmagnesium bromide was added. After 3 hours, the resulting solution was poured into 1M HCl, and extracted with ethyl acetate thrice. The organic layer was washed with $NaHCO_3$, dried over anhydrous magnesium sulfate, filtered and the solvent evaporated. The crude product was purified by flash column chromatography on silica gel eluting with 1:10, 1:5, 1:3, and 1:2 ethyl acetate-hexane to afford 31.49 mg (28.5%) (**10a**) and 19.2 mg (19.2%) (**12a**) as a mixture of isomers.

COMPOUND (**10a**): ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.33-7.18 (m, 10H, aryl), 6.44 (m, 1H, CH alkene), 6.3 (m, 2H, CH_2 trans alkene), 5.85 (m, 2H, CH_2 cis alkene), 5.00 (m, 3H, NH and Cbz CH_2) alkene), 4.03 (s, 1H, CHN), 3.63, 3.45 (s, 1H, CHO), 3.18, 3.09 (s, 1H, OH), 2.96, 2.81 (m, 2H, CH_2Ph), 2.34 (m, 1H, CHcpyl), 1.68 (m, 1H, CHcpyl), 1.28, 1.12, 1.02, 0.88 (m, 2H, CH_2cpyl); ^{13}C (125 Mz, $CDCl_3$): δ 200.41 (C=O, ketone), 157.23 (C=O,Cbz), 137.66, 136.65, 136.15 (CH alkene and 4˚ aryl C's), 129.12, 128.11, 128.5 (CH alkene and aryl C's), 74.90, 74.4 (CHO), 66.86 (CH_2Cbz), 57.83, 57.32 (CHN), 35.74, 29.71 (CH_2Ph), 27.09, 26.7 (CHcpyl), 23.19, 23.18 (CHcpyl), 15.16, 14.65 (CH_2cpyl); Mass spectrum (ESI-MS) m/z ($C_{23}H_{26}NO_4$) calculated for (M+H) 380.1849, found 380.1856.

COMPOUND (12a): ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.34-7.14 (m, 10H, aryl), 6.42 (m, 1H, CH alkene), 6.24 (m, 2H, CH_2 trans alkene), 5.8 (m, 2H, CH_2 cis alkene) 5.43, 5.3 (m, 1H, NH), 4.98 (m, 2H, CH_2Cbz), 4.66, 4.53, 4.35 (m, 1H, CHO), 4.26, 4.19 (m, 1H, CHN), 2.95, 2.71 (m, 2H, CH_2Ph), 2.42, 2.25 (m, 1H, CHcpyl), 2.02, 1.99 (s, 3H, CH_3), 1.76 (m, 1H, CHcpyl), 1.25, 1.1, 1.02, 0.88, 0.81 (m, 2H, CH_2cpyl); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 198.91, 198.69, 198.27, 198.15 (C=O, ketone), 170.35, 170.30, 169.98, 169.88 (C=O, ester), 155.67 (C=O, Cbz), 136.99, 136.51, 136.07 (CH alkene, 4˚aryl C's), 128.8, 128.67, 128.31, 128.23, 127.73, 127.62, 127.48, 127.42, 126.38, 126.26 (CH_2 alkene, 6 aryl C's), 76.26, 75.83, 75.24, 74.97, 73.78, 72.1 (CHO), 66.46, 66.4, 66.13 (CH_2Cbz), 54.93, 54.35, 54.15 (CHN), 38.29, 37.95, 35.84, 35.78 (CH_2Ph), 32.53, 31.30, 30.53, 29.93, 29.33 (CHcpyl), 26.45, 25.46, 25.29, 24.38, 23.8 (CHcpyl), 23.05, 22.83, 20.68, 20.54 (acetate CH_3), 15.63, 15.40, 15.56, 15.56 (CH_2cpyl); Mass spectrum (ESI-MS) m/z ($C_{25}H_{28}NO_5$) calculated for (M+H) 422.1950, found 422.1962.

Formation of (2S)-2-(((benzyloxy)carbonyl)amino)-1-(2-(oxirane-2-carbonyl)cyclpropyl)-3-phenylpropyl acetate (13a):



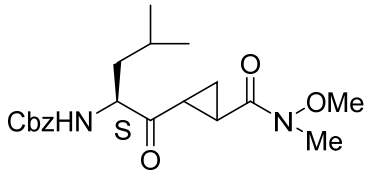
13a

A solution of potassium tert-butoxide in tetrahydrofuran (1.0 M, 0.73 µL, O.806 mmol) was added to a stirred solution of (**12a**) (50 mg, 0.12 mmol) and tert-butyl hydroperoxide in hexane (26.46 µL, 0.237 mmol) in tetrahydrofuran (1.64 mL) at 0˚C. To the reacting mixture the same amount of tert-butyl hydroperoxide was added after one and three hours, and the same amount of potassium tert-butoxide was added every 1 hour over 8 h. The reacting mixture was maintained at 0˚C for another 13 h, and solid then sodium sulfite (75 mg, 0.6 mmol) was added. The resulting mixture was stirred at room temperature for an hour. The crude product was dissolved in ethyl acetate (15 mL), and filtered, evaporated, then purified by flash column chromatography on silica gel eluting with 1:10, 1:5 and 1:3 ethyl acetate-hexane to afford 31 mg (59.61%) **(13a)** as a mixture of isomers. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.36-7.20 (m, 10H, aryl) 5.06-4.96 (m, 2H, $CbzCH_2$), 4.88, 4.78 (2d, J= 10, 10 Hz, 1H, NH), 4.71, 4.57, 4.35 (m, 1H, CHO), 4.26, 4.16, 4.21 (m, 1H,CHN), 3.41 (m, CH epoxide), 3.01 (m, 2H, CH_2 epoxide), 2.75 (m, 2H, CH_2Ph), 2.07, 2.06, 2.05, 2.04 (s, 3H, acetate CH_3), 1.88, 1.74, 1.66, 1.25 (m, 1H, CHcpyl), 0.86 (m, 2H, CH_2cpyl); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 204.91, 204.73, 204.57 (C=O, ketone), 169.52, 169.12 (C=O, ester), 154.93, 154.79 (C=O, Cbz), 135.96, 135.4, 135.29 (4˚aryl C's), 128.06, 127.68,

127.57, 127.14, 126.3 (aryl C's), 75.36, 75.07, 74.43, 74.32 (CHO), 66.04, 65.85 (CH_2Cbz), 54.3, 54.1, 53.83, 53.32,52.78, 52.66 (CH epoxide, CHN), 44.87, 44.63 (CH_2 epoxide), 37.9, 37.47, 35.36, 35.22 (CH_2Ph), 26.12, 25.57, 25.25, 24.94, 24.78, 24.35, 23.99 (CHcpyl), 20.79, 19.98, 19.8, 19.26, 18.75, 18.03 (CHcpyl and acetate CH_3), 16.01, 15.08, 14.92, 14.24, 13.88 (CH_2cpyl); mass spectrum (ESI-MS) m/z ($C_{25}H_{28}NO_6$) calculated for (M+H) 438.1907, found 438.1911.

2.5 Cbz-leucine series

Formation of benzyl ((2S)-1-(2-(methoxy(methyl)carbamoyl)cyclopropyl)-4-methyl-1-oxopentan-2-yl) carbamate (8b):

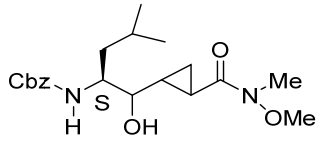


8b

To a suspension of NaH (60% in mineral oil, 52.0 mg, 1.28 mmol) in DMSO (1.5 mL), DMSB (310.0 mg, 1.27 mmol) was added at room temperature, and was stirred for 25 minutes. The solution was cooled to 0˚C, a solution of (**5b)** (160 mg, 0.58 mmol) in THF (1.6 ml) was added, and the mixture was stirred for 20 minutes. The creamy semisolid was dissolved in methylene chloride, and then poured in to 1M HCl and extracted with methylene chloride thrice. The organic layer was washed with brine, dried with anhydrous magnesium sulfate and the solvent was evaporated. The crude product was purified by CombiFlash and evaporated to afford 148 mg (67.62%) (**8b)** as a mixture of syn and anti-isomers**.** ^{1}H-NMR (500 Mhz, $CDCl_3$): δ 7.37-7.35 (m, 5H, aryl isomers), 5.29 (d, J=8 Hz,

1H, NH isomers), 5.1, 5.09 (s, 2H, CbzCH$_2$ isomers), 4.61 (d, J=14.9 Hz, 1H, CHN isomers), 3.72 (s, 3H, OCH$_3$ isomers), 3.21 (s, 3H, CH$_3$ isomers), 2.56, 2.50 (m, 1H, CHcpyl isomers), 1.76-1.72 (m, 2H, isobutyl CH and one H of isobutyl CH$_2$ isomers), 1.54, 1.50 (m, 2H, CHcpyl and one H of CH$_2$cpyl isomers), 1.43-1.39 (m, 2H, one H of isobutyl CH$_2$ and one H of CH$_2$ cpyl), 1.01 (t, J=4.6 Hz, 3H, isobutyl CH$_3$), 0.93, 0.92 (d, J=6.3, 6.85 Hz, 3H, isobutyl CH$_3$); ^{13}C-NMR (125 MHz, CDCl$_3$): δ 207.48 (C=O, ketone), 171.12 (C=O, amide), 156.13, 155.6 (C=O, Cbz), 136.40, 136.17 (4˚aryl C), 128.57, 128.18, 128.09 (aryl C's), 66.95 (CH$_2$Cbz), 61.87, 61.82 (OCH$_3$), 59.49, 59.32 (CHN), 40.61, 40.51 (isobutyl CH$_2$), 32.57 (NCH$_3$), 26.94, 26.55 (CHcpyl), 24.95, 24.91 (isobutyl CH), 23.42, 23.35 (CH$_3$), 22.42 (CHcpyl), 21.75 (CH$_3$), 17.84, 17.72 (CH$_2$ cpyl); mass spectrum (ESI-MS) m/z (C$_{20}$H$_{29}$N$_2$O$_5$) calculated for (M+H) 377.2053, found 377.2071.

Formation of benzyl ((2S)-1-hydroxy-1-(-2-(methoxy(methyl)carbamoyl) cyclopropyl)-4-methylpentan-2-yl)carbamate (9b):

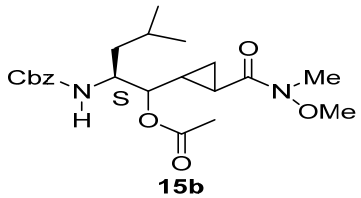


9b

To a solution of (**8b**) (129 mg, 0.34 mmol) in methanol (5 mL), NaBH$_4$ (26 mg, 0.68 mmol) added and the mixture was stirred for 120 minutes at room temperature. The solution was poured in to 1M HCl and extracted with ethyl acetate twice. The solution was washed with NaHCO$_3$ and brine, and was dried with anhydrous magnesium sulfate, filtered and the solvent evaporated. The crude was purified on CombiFlash and the solvent evaporated to afford 117.4 mg (90%) (**9b**) as a mixture of isomers. ^{1}H-NMR (500 MHz, CDCl$_3$): ^{1}H-

NMR (500 MHz, $CDCl_3$): δ 7.34-7.27 (m, 5H, aryl isomers), 5.33, 5.28, 5.24 (d, J=8.6, 4.6, 12.95 Hz, 1H, NH isomers), 5.12-5.4 (m, 2H, Cbz CH_2 isomers), 3.89 (m, 1H, CHN isomers), 3.74, 3.72 (d, J=3.6, 12.05 Hz, 3H, OCH_3 isomers), 3.45, 3.29 (d, J=4.0, 5.15 Hz, 1H, CHO isomers), 3.17, 3.16 (s, 3H, NCH_3 isomers), 2.20, 2.15 (br s, 1H, OH isomers), 1.65-1.63 (m, 1H, isobutyl CH isomers), 1.5-1.43 (m, 2H, CHcpyl and one H of isobutyl CH_2), 1.34-1.29 (m, 1H, isobutyl CH isomers), 1.2-1.18 (m, 2H, CH_2cpyl isomers), 1.18-1.14 (m, 1H, CHcpyl isomers), 0.9, 0.86 (s, 6H, isobutyl CH_3 isomers); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 173.88 (C=O, amide), 157.36, 157.31 (C=O, Cbz), 136.6, 136.44 (4˚aryl C's), 128.58, 128.21, 128.08 (aryl C's), 76.93, 75.26 (CHO), 66.98, 66.94 (CH_2Cbz), 61.67, (OCH_3), 54.45, 54.36, 53.92 (CHN), 39.98, 38.67 (isobutyl CH_2), 32.05 (CH_3N), 25.28, 24.83, 24.67 (isobutyl CH), 23.69, 23.61, 23.30, 23.13 (CHcpyl), 22.13, 21.75, 21.65 (CH_3), 14.26, 14.17 (CHcpyl), 11.86, 11.56 (CH_2cpyl); mass spectrum (ESI-MS) m/z ($C_{20}H_{31}N_2O_5$) calculated for (M+H) 379.2233, found 379.2227.

Formation of (2S)-2-((benzyloxy)carbonyl)amino)-(2(methoxy(methyl) carbamoyl)cyclopropyl)-4-methylpentyl acetate (15b):

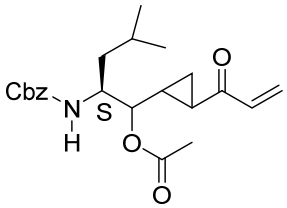


15b

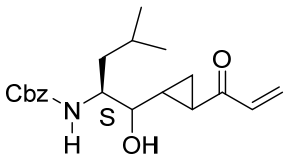
To a solution of (**9b**) (104 mg, 0.275 mmol) in 3 mL CH_2Cl_2, acetic anhydride (0.182 mL, 1.92 mmol) and pyridine (0.166 mL, 2.061 mmol) were added. The resulting solution was stirred for 48 hours at room temperature, and then poured into 1M HCl and extracted with ethyl acetate twice. The organic layer was washed with aqueous $NaHCO_3$ and dried over

magnesium sulfate, filtered and the solvent was evaporated. The crude product was purified on flash column chromatography on silica gel eluting with 1:20, 1:10, 1:5, 1:2, 1:1, 2:1 and 3:1 ethyl acetate-hexane to afford 248.6 mg (94.89%) (**15b**). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.35-7.33 (m, 5H, aryl isomers), 5.10-5.06 (m, 2H, Cbz CH_2), 4.95, 4.89 (d, J=9.7, 10.0 Hz, 1H, NH isomers), 4.55, 4.49, 4.44, 4.36 (dd, J=11.45 Hz, 1H, CHO isomers), 4.06-4.02 (m, 1H, CHN isomers), 3.75, 3.68 (s, 3H, OCH_3 isomers), 3.18 (t, J=15.45 Hz, 3H, NCH_3 isomers), 2.03, 2.00 (s, 3H, acetate CH_3 isomers), 1.68-1.64 (m, 1H, isobutyl CH isomers), 1.61-1.57 (m, 1H, CHcpyl isomers), 1.41-136 (m, 2H, isobutyl CH_2 isomers), 1.26, 1.19 (m, 1H, CHcpyl isomers), 1.01 (m, 2H, CH_2cpyl isomers), 0.9 (m, 6H, isobutyl CH_3 isomers): ^{13}C-NMR (125 MHz, $CDCl_3$): δ 173.24, 173.09 (C=O, ester), 170.71, 170.65, 170.5, 170.41 (C=O, amide), 156.16 (C=O, Cbz), 136.64, 136.55 (4˚aryl C's), 128.6, 128.54, 128.27, 128.22, 128.1, 128.98 (aryl C's), 76.96, 76.27 (CHO), 66.89, 66.71 (CH_2Cbz), 61.78, 61.62 (OCH_3), 52.19, 51.87, 55.77 (CHN), 41.52, 39.13, 39.02 (isopropyl CH_2), 32.58 (CH_3N), 24.76, 24.64 (CHcpyl), 23.58, 23.54 (CH_3), 23.21, 22.39 (CHcpyl), 21.63, 20.89 (acetate CH_3), 21.54, 21.09 (CHcpyl) 15.45 (isobutyl CH), 12.73, 12.33 (CH_2cpyl); mass spectrum (ESI-MS) m/z ($C_{22}H_{32}N_2O_6$) calculated for (M) 420.2260.

Formation of (2S)-1-(2-acryloylcyclopropyl)-2-(((benzyloxy)carbonyl)amino)-4-methylpentyl acetate (12b) and benzyl ((2S)-1-(2-acryloylcyclopropyl)-1-hydroxy-4-methylpentan-2-yl) carbamate (10b):



12b



10b

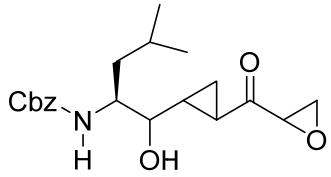
To a solution of (**15b**) (151 mg, 0.36 mmol) in THF (2.0 mL), at 0˚C under argon, vinylmagnesium bromide (0.9 mL of 1M solution) was added, and stirred for 1 hour and gradually warmed to RT and again same amount of vinylmagnesium bromide was added after 2 and 4.0 hrs. After 5 hours, the resulting solution was poured into 1M HCl, and extracted twice with ethyl acetate. The organic layer was washed with $NaHCO_3$, dried over anhydrous magnesium sulfate, filtered and the solvent evaporated. The crude product was purified by flash column chromatography on silica gel eluting with 1:10, 1:5, 1:2, and 1:1 ethyl acetate-hexane to afford and 64.3 mg (51.85%) (**10b**) and 24 mg (17.15%) (**12b**) as a mixture of isomers.

Compound (10b) (alcohol) : ^{1}H-NMR (500 MHz, CD_3OD): δ 7.33-7.26 (m, 5H, aryl isomers), 6.47-6.4 (m, 1H, vinyl CH isomers), 6.35, 6.34, 6.30 (d, J=1.15 Hz, 1H, vinyl CH_2 trans isomers), 5.88-5.82 (m, 1H, vinyl CH_2 trans isomers), 5.08-4.94 (m, 2H, Cbz CH_2 isomers and 1H, NH isomers), 3.68-3.65 (m, 1H, CHN isomers), 3.06-3.04 (m, 1H, CHO isomers), 2.35-2.31 (m, 1H, CHcpyl isomers), 1.66-1.58 (m, 2H, CHcpyl isomers and isobutyl CH isomers), 1.45-1.38 (m, 2H, isobutyl CH_2 isomers), 1.2-1.17 (m, 2H, CH_2 cpyl isomers), 1.05-1.02 (m, 2H,CH_2cpyl isomers), 0.9-0.82 (m, 6H, isobutyl CH_3 isomers); ^{13}C-NMR (125 MHz, CD_3OD): δ 200.45, 199.87 (C=O, ketone), 156.86, 156.81 (C=O, Cbz), 135.95, (alkene, CH), 135.85 (4˚aryl C), 127.55, 126.79 (aryl C's), 126.91

(alkene CH_2), 75.30, 75.18 (CHO), 65.44 (CH_2Cbz), 53.56 (CHN), 38.47, 38.39 (isobutane CH_2), 28.32, 27.75 (isobutyl CH), 24.03, 24.01 (CHcpyl), 22.74, 23.37, 22.28 (CHcpyl), 19.96 (CH_3), 14.06, 13.88 (CH_2cpyl); mass spectrum (ESI-MS) m/z ($C_{20}H_{28}NO_4$) calculated for (M+H) 346.2009, found 346.2013.

Compound (12b): ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.37-7.33 (m, 5H, aryl isomers), 6.48-6.34 (m, 1H, CH alkene isomers), 6.30-6.24 (m, 2H, CH_2 alkene trans isomers), 5.85 (m, 2H, CH_2 alkene cis isomers), 5.07, 4.70 (m, 3H, one H of NH and two H of Cbz isomers), 4.56, 4.53, 4.38 (dd, J= 3.45, 2.85 Hz, 1H, CHO isomers), 4.0 (m, 1H, CHN isomers), 2.44, 2.25, 2.16 (m, 1H, CHcpyl isomers), 2.03 (m, 3H, acetate CH_3 isomers), 1.72, 1.64 (m, 2H, one H of isobutyl CH and one of H of CHcpyl isomers), 1.29 (m, 2H, isobutyl CH_2), 1.16, 1.12 (m, 2H, CH_2 cpyl), 0.9-0.85 (m, 6H, isobutyl CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 197.72, 197.38, 197.19 (C=O, ketone), 169.39, 169.16 (C=O, ester), 154.83, 154.78 (C=O, amide), 135.33 (CH alkene), 135.14 (4˚aryl), 127.30 (CH_2 alkene), 126.99, 126.95, 126.83, 126.76 (aryl C's), 77.02, 76.50 (CHO), 65.63, 65.58 (CH_2 Cbz), 50.87, 50.60 (CHN), 40.31, 39.96, 37.59 (CH_2 isobutyl), 28.45 (CH isobutyl), 25.38, 24.39, 24.11, 23.3, 22.64, 22.26, 21.77 (CHcpyl), 20.77, 20.24 (CH_3 acetate), 19.80, 19.60 (CH_3 isobutyl), 14.52, 14.39 (CH_2 cpyl); mass spectrum (ESI-MS) m/z ($C_{22}H_{29}NO_5$) calculated for (M) 387.2046.

Formation of benzyl ((2S)-1-hydroxy-4-methyl-1-(2-(oxirane-2-carbonyl)cyclopropyl pentan-2-yl)carbamate (11b):



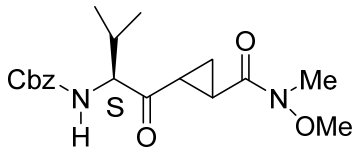
11b

A solution of potassium tert-butoxide in tetrahydrofuran (1.0 M, 65 µL, 0.52 mmol) was added to a stirred solution of (**10b)** (60 mg, 0.17 mmol) and tert-butyl hydroperoxide in hexane (67 µL, 0.87 mmol) in tetrahydrofuran (2.0 mL) at 0˚C. To the reacting mixture,

the same amount of tert-butyl hydroperoxide and the same amount of potassium tert-butoxide were added in every 1 hr for another 6 hrs, and the reacting mixture was maintained at 0˚C for another 14 hrs. The resulting mixture was stirred at room temperature by adding solid sodium sulfite (220 mg, 1.74 mmol) for 30 minutes. The crude product was dissolved in ethyl acetate (15 mL), and filtered, evaporated, then purified by flash column chromatography on silica gel eluting with 1:10, 1:5, 1:4, and 1:2 ethyl acetate-hexane to afford 35.8 mg (57.02%) **(11b)** as a mixture of isomers. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.36-7.34 (m, 5H, aryl isomers), 5.10-5.08 (m, 2H, Cbz CH_2 isomers), 4.89 (t apparently (real dd, J=8.6, 8.0 Hz), 1H, NH isomers), 3.87-3.85 (m, 1H, CHN isomers), 3.48 (m, 1H, CHO isomers), 3.47-3.45 (m, 1H, CH epoxide isomers), 3.02, 2.96 (m, 2H, CH_2 epoxide isomers), 2.15, 2.14 (1H, OH), 2.09-2.02 (m, 1H, isobutyl CH isomers), 1.65, 1.59 (m, 2H, CHcpyl isomers), 1.40 (m, 2H, one H of isobutyl CH_2 and one H of CH_2cpyl isomers), 1.28 (m, 2H, one H of isobutyl CH_2 and one H of CH_2cpyl isomers), 0.97-0.84 (m, 6H, isobutyl CH_3 isomers); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 206.87, 206.42 (C=O, ketone), 157.53, 157.37 (C=O, Cbz), 136.52, 136.22 (4˚aryl C), 128.64, 128.36, 128.16 (aryl C's), 74.58, 74.13 (CHO), 67.24, 67.08 (CH_2Cbz), 54.66, 54.52, 54.18 (CHN), 53.85, 53.68 (CHepoxide) 46.13, 45.92 (CH_2epoxide), 39.18, 38.73 (isobutyl CH_2), 24.93 (CHcpyl), 23.54 (CHcpyl), 21.64 (CH_3), 19.79, 18.90 (isobutyl CH), 15.03, 14.20 (CH_2cpyl); mass spectrum (ESI-MS) m/z ($C_{..}H_{..}N_2O_5$) calculated for (M) 403.1995.

2.6 Cbz-valine series

Formation of benzyl ((2S)-1-(2-(methoxy(methyl)carbamoyl)cyclopropyl)-3-methyl-1-oxobutan-2-yl)carbamate (8c):

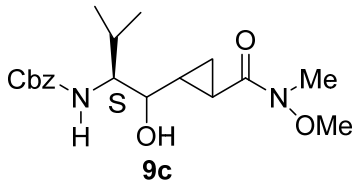


8c

To a suspension of NaH (60% in mineral oil, 157.0 mg, 3.92 mmol) in DMSO (5.0 mL), DMSB (957.0 mg, 3.92 mmol) was added at room temperature, and was stirred for 25 minutes. The solution was cooled to 0˚C, a solution of (**5c**) (465.9 mg, 1.78 mmol) in THF (5.0 ml) was added, and the mixture was stirred for 15 minutes. The creamy semisolid was dissolved in ethyl acetate and pure into 1M HCl and extracted with ethyl acetate. The organic layer was washed with $NaHCO_3$, dried with anhydrous magnesium sulfate and the solvent was evaporated. The crude product was purified by CombiFlash and the solvent evaporated to afford 472.6 mg (73.16%) (**8c**). ^{1}H-NMR (500 MHz, $CDCL_3$): δ 7.37-7.29 (m, 5H, aryl isomers), 5.54-5.50 (t apparently (real d , J= 8.6, 8.6 Hz), 1H, NH), 5.09 (s, 2H, $CbzCH_2$), 4.59, 4.58 (dd, J= 4.0, 4.0 Hz, 1H, CHN isomers), 3.70, 3.69 (s, 3H, OCH_3 isomers), 3.2 (s, 3H, NCH_3 isomers), 2.59-2.54 (m, 1H, CHcpyl isomers), 2.39, 2.33 (m, 1H, isopropyl CH isomers), 1.50 (m, 1H, CHcpyl), 1.40 (m, 2H, CH_2cpyl isomers), 1.04 (t,J= 14.35 Hz, 3H, isopropyl CH_3), 0.78 (t, J= 16.5, 3H, isopropyl CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 206.87 (C=O, ketone), 171.02 (C=O, amide), 156.50, 155.8 (C=O, Cbz), 136.49 (4˚aryl C), 128.53, 128.09 (aryl C's), 66.84, 66.41 (CH_2Cbz), 65.74, 65.63 (CHN), 61.74 (OCH_3), 31.49 (NCH_3), 30.01 (isopropyl CH), 27.52, 27.18 (CHcpyl), 22.66, 22.22

(CHcpyl), 19.8 (CH_3), 17.6, 17.27 (CH_2 cpyl), 16.9 (CH_3);mass spectrum (ESI-MS) m/z ($C_{19}H_{27}N_2O_5$) calculated for (M+H) 363.1906, found 363.1914.

Formation of benzyl ((2S)-1-hydroxy-1-(2-(methoxy(methyl)carbamoyl)cyclopropyl)-3-methylbutan-2-yl)-3-methylbutan-2-yl)carbamate (9c):

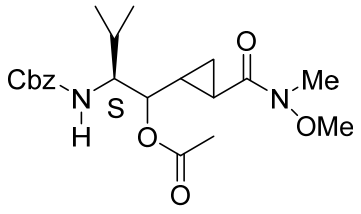


9c

To a solution of (**8c**) (391 mg, 1.08 mmol) in methanol (13 mL), $NaBH_4$ (75 mg, 0.075 mmol) added and the mixture was stirred for 2 hrs at room temperature. The solution was poured in to 1M HCl and extracted twice with ethyl acetate. The organic layer was washed with $NaHCO_3$ and brine, and was dried with anhydrous magnesium sulfate, filtered and the solvent evaporated. The crude was purified on CombiFlash and the solvent evaporated to afford 374.1 mg (95.19 %) (**9c**) as a mixture of isomers. ^{1}H-NMR (500 MHz, $CDCL_3$): δ 7.25-7.19 (m, 5H, aryl isomers), 5.51 (t (apparently), real 2d, J= 9.15, 9.15 Hz 1H, NH), 5.03-4.94 (m, 2H, CH_2Cbz isomers), 3.61 (s, 3H, CH_3 isomers), 3.57- 3.52 (m, 1H, CHN and CHO isomers), 3.38 (m, 1H, CHO isomers), 3.06, 3.04 (s, 3H, NCH_3 isomers), 2.16, 2.01 (br s, 1H, OH), 1.91 (m, 1H, isopropyl CH isomers), 1.49, 1.44 (m, 1H, CHcpyl isomers), 1.08, 1.02 (m, 1H, CHcpyl isomers), 0.94 (m, CH_2cpyl isomers), 0.87 (d, J= 6. 85 Hz, 3H, isopropyl CH_3), 0.84 (t, J= 10.9 Hz, 3H, isopropyl CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 174.02, 173.38, 171.24 (C=O, amide), 157.6, 157.19, 157.15 (C=O, Cbz), 136.82, 136.64 (4˚aryl C's), 128.48, 128.06, 127.97, 127.85 (aryl C's), 72.54, 72.04 (CHO), 66.75 (CH_2Cbz), 61.58, 60.42 (OCH_3), 61.39 (CHN), 32.52 (CH_3N), 28.66

(isopropyl CH), 23.94 (CHcpyl), 23.14 (CHcpyl), 20.69, 20.55 (CH_3), 18.49, 18.38 (CH_3), 14.20 (CHcpyl), 11.66, 11.34 (CH_2cpyl); mass spectrum (ESI-MS) m/z ($C_{19}H_{28}N_2O_5$) calculated for (M) 364.1998.

Formation of (2S)-2-(((benzyloxy)carbonyl)amino)-1-(2-(methoxy(methyl)carbamoyl) cyclopropyl)-3-methylbutyl acetate (15c):

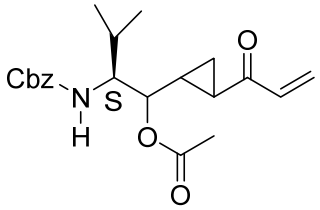


15c

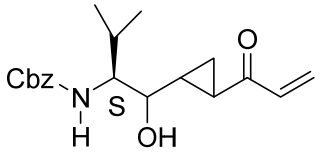
To a solution of (**9c**) (332.1 mg, 0.91 mmol) in 5.0 mL CH_2Cl_2, acetic anhydride (0.6 mL, 6.38 mmol) and pyridine (0.52 mL, 6.38 mmol) were added. The resulting solution was stirred for 52 hours at room temperature, and then poured into 1M HCl and extracted twice with ethyl acetate. The organic layer was washed with aqueous $NaHCO_3$ and dried over magnesium sulfate, filtered and the solvent was evaporated. The crude product was purified on CombiFlash ethyl acetate-hexane (55:45) to afford 326.9 mg (88.35%) (**15c**) as a mixture of isomers. H-NMR (500 MHZ, $CDCl_3$): δ 7.30-7.24 (m, 5H, aryl isomers), 5.07, 5.05 (d, J= 6.85, 5.15 Hz, 1H, NH isomers), 5.03 (m, 2H, CH_2 Cbz isomers), 4.69, 4.61 (dd, J= 5.2, 5.15 Hz, 1H, CHO isomers), 3.76 (m, 1H, CHN isomers), 3.7, 3.64, 3.62 (s, 3H, OCH_3 isomers), 3.11, 3.1 (s, 3H, NCH_3 isomers), 1.98, 1.96 (s, 3H, acetate CH_3 isomers), 1.83 (m, 1H, isopropyl CH isomers), 1.67, 1.6, 1.54 (m, 1H, CHcpyl isomers), 1.12 (m, 2H, CH_2cpyl isomers), 0.95 (d, J= 6.85 Hz, 3H, isopropyl CH_3 isomers), 0.85 (d, J= 6.85 Hz, 3H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 173.28, 172.96 (C=O, ester), 170.55, 170.46 (C=O, amide), 156.62, 156.59 (C=O, Cbz), 136.72 (4˚aryl C's), 128.53,

128.12, 128.08, 127.98 (aryl C's), 75.05, 74.58 (CHO), 66.75 (CH_2Cbz), 61.68, 61.61, 60.42 (OCH_3), 58.67 (CHN), 32.56 (CH_3N), 28.85, 28.77 (isopropyl CH), 21.66, 21.08, 20.89 (CHcpyl), 20.39, 20.34(acetate CH_3) 18.22 (CH_3), 15.45, 14.25 (CHcpyl), 12.09, 11.89 (CH_2cpyl); mass spectrum (ESI-MS) m/z ($C_{21}H_{30}N_2O_6$) calculated for (M) 406.2104.

Formation of (2S)-1-(2-acryloylcyclopropyl)-2-(((benzyloxy)carbonyl)amino)-methylbutyl acetate (12c) and benzyl ((2S)-1-(2-acryloylcyclopropyl)-1-hydroxy-3-methylbutan-2-yl) carbamate (10c):



12c



10c

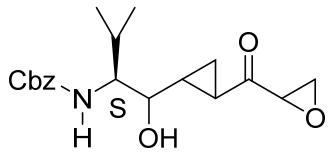
To a solution of (**15c**) (151.3 mg, 0.36 mmol) in THF (2.0 mL), at 0˚C under argon, vinylmagnesium bromide (0.93 ml of 1M solution) was added, and stirred for 1 hour on melting ice bath, and again same amount of vinylmagnesium bromide was added in every 1 hour for two more time at 0˚C under argon. After 4 hours, the resulting solution was poured into 1M HCl, and extracted twice with ethyl acetate. The organic layer was washed with $NaHCO_3$, dried over anhydrous magnesium sulfate, filtered and the solvent evaporated. The crude product was purified by flash column chromatography on silica gel eluting with 1:10, 1:5, and 1:2 ethyl acetate-hexane to afford and 41.1 mg (33.31%) (**10c**) and 39.8 mg (28.63%) (**12c**) as a mixture of isomers.

Compound 10c (alcohol group):^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.35-7.31 (m, 5H, aryl isomers), 6.47-6.39 (m, 1H, vinyl CH isomers), 6.28 (d, J= 17.75 Hz, 2H, vinyl CH_2 trans isomers), 5.81, 5.77 (dd, J= 10.9, 10.85 Hz, 2H, vinyl CH_2 cis isomers), 5.11-5.05 (m, 2H,

CH_2Cbz), 5.0 (m, 1H, NH isomers), 3.69 (m, 1H, CHO isomers), 3.63 (m, 1H, CHN isomers), 3.48 (m, 1H, CHO isomers), 3.01, 2.96 (1H, OH isomers), 2.32, 2.26 (m, 1H, CHcpyl isomers), 1.94 (m, 1H, isopropyl CH isomers), 1.61 (m, 1H, CHcpyl isomers), 1.24, 1.12 (m, 2H, CH_2cpyl isomers), 0.97 (t, J= 12.6 Hz, 3H, isobutyl CH_3 isomers), 0.92 (t, J=12.6 Hz, 3H, isobutyl CH_3 isomers); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 201.06, 200.05 (C=O, ketone), 157.73, 157.65 (C=O, Cbz), 136.77, 136.73 (alkene, CH), 136.32 (4˚aryl C), 128.63, 128.3, 128.14 (aryl C's), 128.22 (alkene CH_2), 73.32, 71.86 (CHO), 67.17 (CH_2Cbz), 61.75, 61.6 (CHN), 28.89, 28.65 (isopropyl CH), 27.33, 26.32 (CHcpyl), 22.46, 22.12 (CHcpyl), 20.59, 20.51, 18.97, 18.49 (CH_3), 15.00, 14.22 (CH_2cpyl); mass spectrum (ESI-MS) m/z ($C_{19}H_{26}N_2O_4$) calculated for (M+H) 332.1857, found 332.1856.

Compound (12c): ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.38-7.2 (m, 5H, aryl isomers), 6.43 (m, 1H, vinyl CH isomers), 6.28, 6.25 (dd, J= 6.85, 6.85 Hz, 2H, vinyl CH_2 trans isomers), 5.84 (m, 2H, vinyl CH_2 cis isomers), 5.07 (m, 2H, CH_2 Cbz isomers), 4.79 (m, 1H, NH isomers), 4.70, 4.55 (m, 1H, CHO isomers), 3.82, 3.66 (m, 1H, CHN isomers), 2.49, 2.39, 2.20 (m, 1H, CHcpyl isomers), 2.06, 2.03, 2.02, 1.99 (s, 3H, acetate CH_3 isomers), 1.86 (m, 1H, isopropyl CH isomers), 1.74, 1.69 (m, 1H, CHcpyl isomers), 1.39, 1.35, 1.28, 1.12 (m, 2H, CH_2cpyl isomers), 0.99, 0.93, 0.89 (ddd, J=2.85, 1.7, 4.6 Hz, 6H isopropyl CH_3 isomers); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 199.05, 198.65 (C=O, ketone), 170.68, 170.61 (C=O, ester), 156.62, 156.53 (C=O, Cbz), 137.03 (4˚aryl C), 136.57, 136.48 (alkene, CH), 128.64, 128.42, 128.27, 128.23, 128.15 (aryl C's), 128.62 (alkene CH_2), 75.78, 74.59 (CHO), 67.03, 66.98 (CH_2Cbz), 58.59 (CHN), 28.61 (isopropyl CH), 25.80 (CHcpyl), 23.53 (CHcpyl), 21.16, 20.5 (acetate CH_3), 17.79 (CH_3), 15.25, 14.94 (CH_2cpyl); mass spectrum (ESI-MS) m/z ($C_{21}H_{27}NO_5$) calculated for (M) 373.1889.

Formation of benzyl ((2S)-1-hydroxyl-3-methyl-1-(2-(oxirane-2-carbonyl)cyclopropyl) butan-2-yl)carbamate (11c):

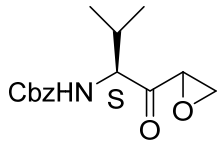


11c

A solution of potassium tert-butoxide in tetrahydrofuran (1.0 M, 43 µL, 0.348 mmol) was added to a stirred solution of (**10c**) (38.5 mg, 0.116 mmol) and tert-butyl hydroperoxide in hexane (65 µL, 0.58 mmol) in tetrahydrofuran (1.0 mL) at 0˚C. To the reacting mixture, the same amount of tert-butyl hydroperoxide and the same amount of potassium tert-butoxide were added in every 1 hr for another 5 hrs, and the reacting mixture was maintained at 0˚C for another 17 hrs. The resulting mixture was stirred at room temperature by adding solid sodium sulfite (146 mg, 1.16 mmol) for 30 minutes. The crude product was dissolved in ethyl acetate (15 mL), and filtered, the solvent evaporated. The crude product was purified by flash column chromatography on silica gel eluting with 1:10, 1:5 and 1:2 ethyl acetate-hexane to afford 18.6 mg (46.1%) (**11c**) as a mixture of isomers. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.36-7.31 (m, 5H, aryl isomers), 5.10, 5.06 (dd, J=13.75, 3.4 Hz, 2H, CH_2 Cbz isomers), 4.84 (d, J= 8.05 Hz, 1H, NH), 3.7 (m, 1H, CHO isomers), 3.61 (m, 1H, CHN isomers), 3.46, 3.43 (q, J=6.85Hz, 1H, CHepoxide isomers), 3.35 (m, 1H, CHO isomers), 3.12, 2.96, 2.90 (dd, J= 10.0, 10.5, 8.6 Hz, CH_2epoxide isomers), 2.78 (m, 1H, CHcpyl isomers), 2.35, 2.31 (1H, OH isomers), 2.14, 2.09, 1.93, 1.87 (m, 1H, isopropyl CH isomers), 1.30, 1.21 (m, 1H, CH_2cpyl isomers), 0.98-0.9 (m, 6H, isopropyl CH_3 isomers); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 205.66, 205.5 (C=O, ketone), 156.44, 156.27

(C=O, Cbz), 134.94 (4˚aryl C), 127.40, 127.12, 126.96, 126.80 (aryl C's), 72.48, 70.91, 70.5, 70.35, 70.07 (CHO), 66.03 (CH_2Cbz), 60.44, 60.27 (CHN), 52.67, 52.36 (CHepoxide) 44.88, 44.72 (CH_2epoxide), 28.5, 27.69, 27.54, 27.24, 27.14 (CHisopropyl), 25.52 (CHcpyl), 19.26, 19.11 (CHcpyl), 17.75, 17.52 (CH_3), 13.34 (CH_2cpyl); mass spectrum (ESI-MS) m/z ($C_{19}H_{26}N_2O_5$) calculated for (M+H) 348.1802, found 348.1805.

benzyl ((2S)-3-methyl-1-(oxiran-2-yl)-1-oxobutan-2-yl)carbamate (**14c**):

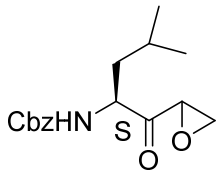


14c

A solution of potassium tert-butoxide in THF (1.0 M, 1.3 µL, 0.096 mmol) was added to a stirred solution of (**5c**) (50 mg, 0.19 mmol) and tert-butyl hydroperoxide in hexane (43 µL, 0.38 mmol) in THF (1.5 mL) at 0˚C and stirred. In every one-hour same amount of tert-butyl hydroperoxide and potassium tert-butoxide were added over 4 h, then the resulting solution was kept at 0˚C for another 13 h, then solid sodium sulfite (121 mg, 0.96 mmol) was added and stirred at room temperature for 1 h. The crude compound was dissolved in ethyl acetate (15 mL) and filtered, the solvent evaporated, and then purified by flash column chromatography on silica gel eluting with 1:20, 1:10 and 1:6 ethyl acetate-hexane to afford 25.1 mg (47.36%) (**14c**) as a mixture of syn and anti-isomers. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.36 (m, 5H, aryl), 5.38 (d, J=8.6 Hz, 1H, NH), 5.1 (m, 2H, CH_2Cbz), 4.59 (m, 1H, CHN), 3.67 (s, 1H, CH epoxide),$_2$ 2.99, 2.92 (d, 2H, CH_2 epoxide), 2.28 (m, 1H, $CH(CH_3)_2$,1.06, 0.88 (s, 3H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 204.84 (C=O, ketone), 156.39 (C=O, Cbz), 136.17 (4˚ aryl C's), 128.67, 128.37, 128.22 (3 aryl C's),

67.29 (CH_2Cbz), 63.37 (CHN), 51.12 (CH epoxide), 48.02 (CH_2 epoxide), 30.19 ($CH(CH_3)_2$, 19.85, 17.01 (CH_3); Mass spectrum (ESI-MS) m/z ($C_{15}H_{20}NO_4$) calculated for (M+H) 278.1370, found 278.1387.

benzyl ((2S)-4-methyl-1-(oxiran-2-yl)-1-oxopentan-2-yl)carbamate (14b):



14b

A solution of potassium tert-butoxide in THF (1.0 M, 1.3 µL, 0.011 mmol) was added to a stirred solution of (**5b**) (60 mg, 0.21 mmol) and tert-butyl hydroperoxide in hexane (48.64 µL, 0.44 mmol) in THF (2.0 mL) at 0˚C and stirred. In every 1 h, the same amount of tert-butyl hydroperoxide and the same amount of potassium tert-butoxide were added over 3 h, then the resulting solution was kept at 0˚C for another 12 h, then solid sodium sulfite (137 mg, 1.1 mmol) was added and stirred at room temperature for 1 h. The crude compound was dissolved in ethyl acetate (20 mL) and filtered, the solvent evaporated, and then purified by flash column chromatography on silica gel eluting with 1:20, 1:10 and 1:6 ethyl acetate-hexane to afford 34.6 mg (56.72%) (**14b**) as a mixture of syn and anti-isomers. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.36-7.33 (m, 5H, aryl), 5.16 (d, j=8.6, 1H, NH), 5.07 (m, 2H, CH_2Cbz), 4.38 (m, 1H, CHN), 3.51 (s, 1H, CH epoxide), 3.14, 3.06 (d, 2H, CH_2 epoxide), 1.73 (m, 1H, $CH(CH_3)_2$), 1.57, 1.28 (m, 2H, CH_2), 0.97, 0.94 (s, 3H, CH_3); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 207.23 (C=O, ketone), 156.19 (C=O, CH_2Cbz), 136.17 (4˚ aryl C's), 128.63, 128.3, 128.01 (3 aryl C's), 67.11 (CH_2Cbz), 52.73 (CHN), 52.61 (CH epoxide),

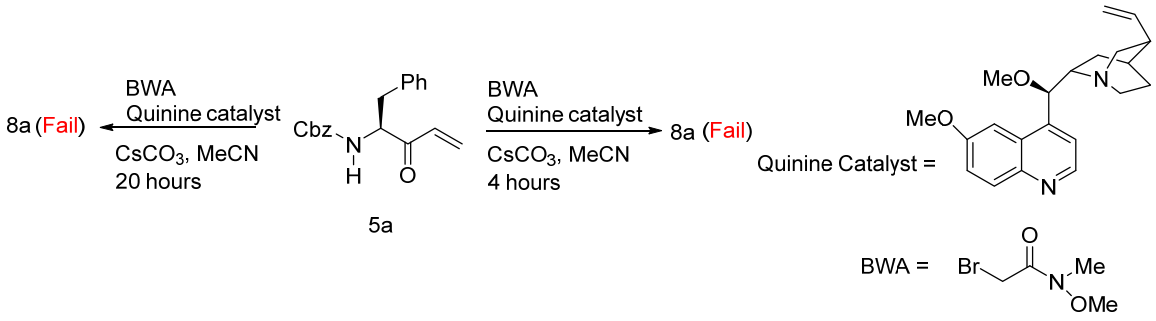
46.39 (CH_2 epoxide), 40.26 (CH_2), 25.04 ($CH(CH_3)_2$), 23.42, 21.41 (CH_3); mass spectrum (ESI-MS) m/z ($C_{16}H_{22}NO_4$) calculated for (M+H) 292.1529, found 292.1543.

CHAPTER III: RESULTS AND DISCUSSION

Synthesis of cyclopropyl Weinreb amide

In order to synthesize the proposed cyclopropyl epoxy ketones, the starting point would be our existing synthesis of cyclopropyl esters. The amino acid-derived enones need to be converted to cyclopropyl Weinreb amides. These could then be converted to enones which could be epoxidized to give the epoxyketones. Hoping to get a stereoselective cyclopropyl Weinreb amide as reported by Gaunt, the bromo Weinreb amide of bromoacetic acid (BWA) was treated with **5a** in presence of the quinine catalyst and $CsCO_3$ as seen in **Scheme 6**.[35] But, it was found that the starting compound **5a** was decomposed at the end of the reaction despite trying different reaction times.

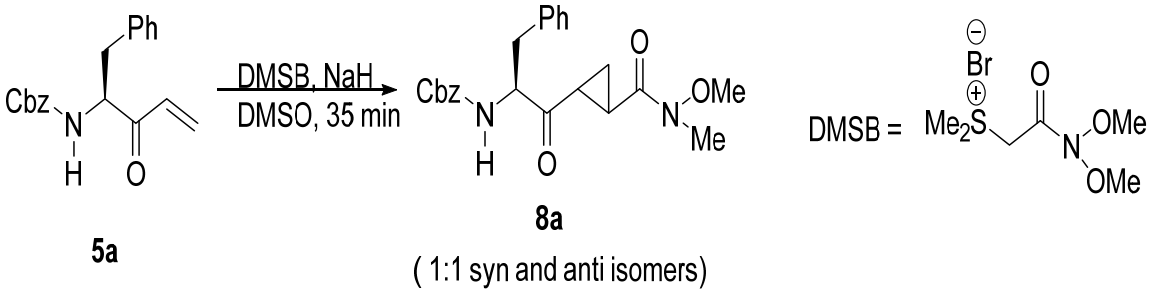
Scheme 6: Attempted synthesis of cyclopropyl Weinreb amide



Although Gaunt had reported successful stereoselective cyclopropranations of other enones using BWA, this did not work with our substrate. After the failure with BWA, a salt, (2-(methoxy(amino)-2-oxoethyl)dimethylsulfonium bromide (DMSB), was synthesized by displacing the bromide of BWA with dimethyl sulfide. This salt was a precursor to the sulfonium ylide, an analog of EDSA, used in the earlier synthesis. The

salt was treated with NaH in DMSO followed by **5a** to afford the cyclopropyl Weinreb amide **8a,** as a 1:1 mixture of diastereomers (Scheme **7**). During this reaction, it was observed that the reaction time was very crucial, that is, the reactants were decomposed completely when they were allowed to react for 1 hour.

Scheme 7: Successful synthesis of cyclopropyl Weinreb amide



5a **8a**

(1:1 syn and anti isomers)

The general mechanism for Michael induced ring closure to form a cyclopropyl ester or Weinreb amide is shown in **Figure 18**. The strong base NaH removes an acidic hydrogen from BWA or DMSB *in situ* to form an ylide that can react with **5a** to form an intermediate enolate, which displaces the leaving group and forms a cyclopropane.

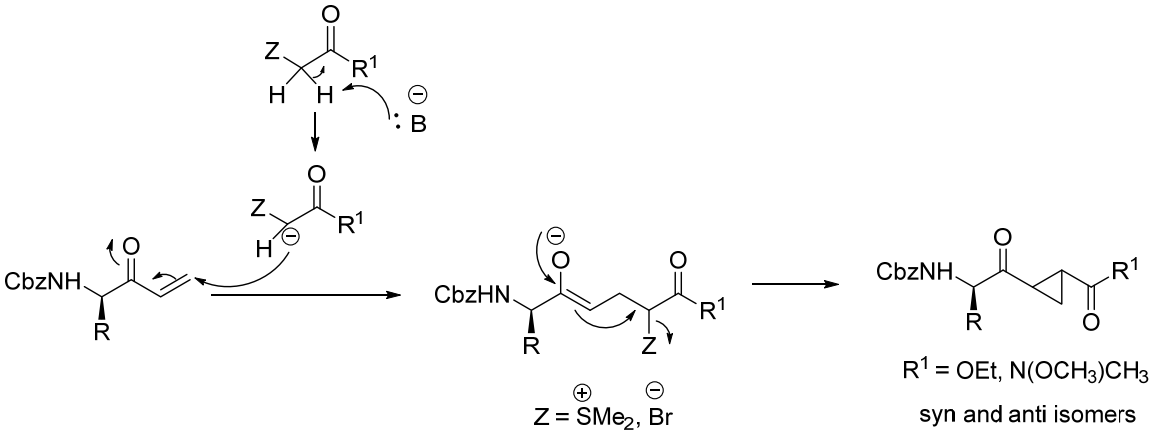
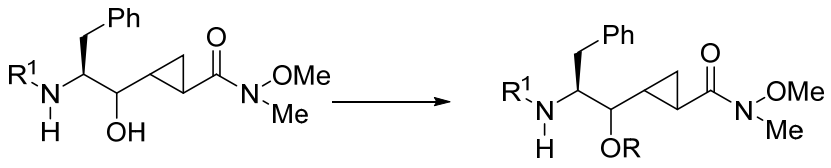


Figure 18: General cyclopropanation mechanism

Protection of alcohol

The ketone of compound **8a** was reduced to the alcohol **9a** with $NaBH_4$ without difficulty, but with the alcohol group, a vinylmagnesim bromide addition to the Weinreb amide did not work in presence of the acidic hydrogen.[30,31] So, several protecting groups were investigated to protect this hindered alcohol.[36] The first attempts were to add silyl protecting groups using either t-BDMSCl or TMSCl. These were attempted several times using different conditions, but they gave only the starting materials. Also, addition of a benzyl ether protecting group failed with the hindered alcohol.

Table 1: Attempts at protection of the alcohol **9a**



9a **15a**

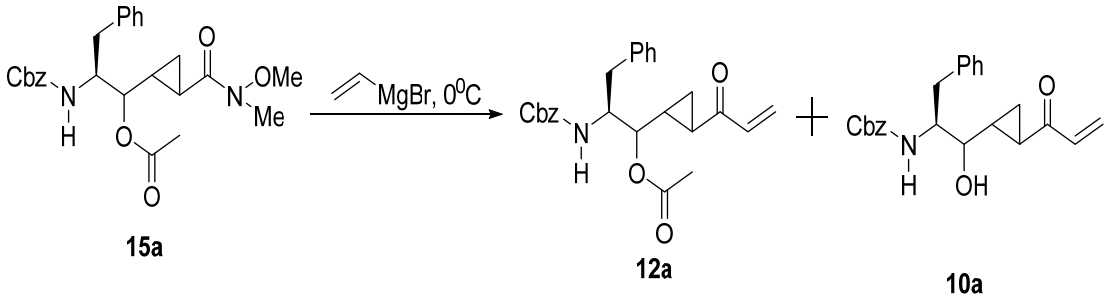
9a ⟶ **15a**			
R	R’	Conditions	Results
t-Bu$(CH_3)_2$Si	Cbz	t-Bu$(CH_3)_2$SiCl , Et_3N, CH_2Cl_2, RT, 24 hrs	No reaction
$(CH_3)_3$Si	Cbz	$(Me_3)_3$SiCl , NEt_3, CH_2Cl_2, RT, 8 hrs	No reaction
Bn	Cbz	BnBr, NaH, THF, 24 hrs	decomposition
Bn	$PhCH_2CO$	BnBr, NaH, THF	decomposition
Ac	Cbz	$(CH_3O)_2O$, Pyridine, CH_2Cl_2, 3 days	88% yield

These protecting groups did not work because of their bulky size towards the hindered alcohol. In this case, with both the Cbz analog **9a** or its corresponding amide, we saw decomposition. So, the final attempt was to protect the alcohol group as the acetate, using acetic anhydride, which successfully gave the acetate **15a**.[33] The protection results are summarized in Table **1**.

Synthesis of the cyclopropyl enone

After the successful protection of the hindered alcohol as an acetate group, compound **15a** was treated with vinylmagnesium bromide to convert it into the enone **12a.** During the reaction, we also observed a cleavage of the acetate, affording the alcohol **10a** as a byproduct (**Scheme 9**)[30,31]. This was an acceptable result since both series could be tested for bioactivity.

Scheme 8: Synthesis of the cyclopropyl enone

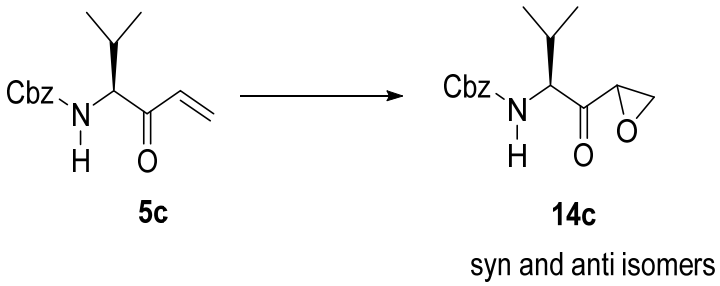


15a **12a** **10a**

Synthesis of the epoxide

Formation of the epoxide proved to be another challenging task. Three different procedures were attempted multiple times. Reactions were investigated first using the simple enone **5c**.[34,37,38] The first attempts used mCPBA However no reaction was observed even after 6 days. A second attempt with oxone also gave no reaction. We concluded that this electron- deficient alkene needed a different approach. Finally, the Weitz-Scheffer epoxidation in basic conditions was the successful route to synthesize **14c** from **5c** (**Table 2**).

Table 2: Summary of the attempted synthesis of the simple epoxides



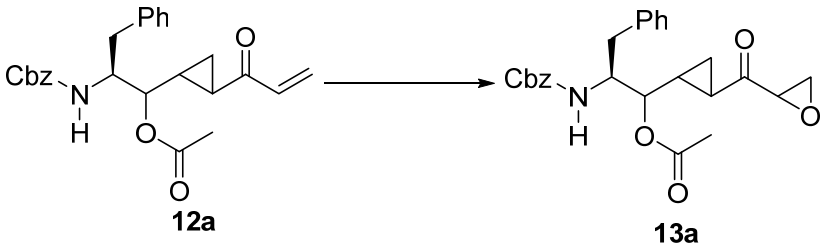
5c **14c**

syn and anti isomers

5c ⟶ **14c**		
Reagents	Conditions	Results
mCPBA	CH_2Cl_2, RT, 3 days	No reaction
mCPBA	CH_2Cl_2, 0˚C, 19 hrs	No reaction
mCPBA	CH_2Cl_2, 0˚C, 6 days	No reaction
Oxone, $NaHCO_3$	acetone, 4 hrs	No reaction
t-BuOOH, t-BuOK	THF,0˚C, 4 hrs	47 yield%

After the success of the synthesis of simple epoxides by Weitz-Scheffer epoxidation, it was implemented to synthesize the cyclopropyl epoxyketone **13a** from **12a** in presence of peroxide (t-BuOOH) and a strong base (t-BuOK) at 0˚C (**Table 3**).

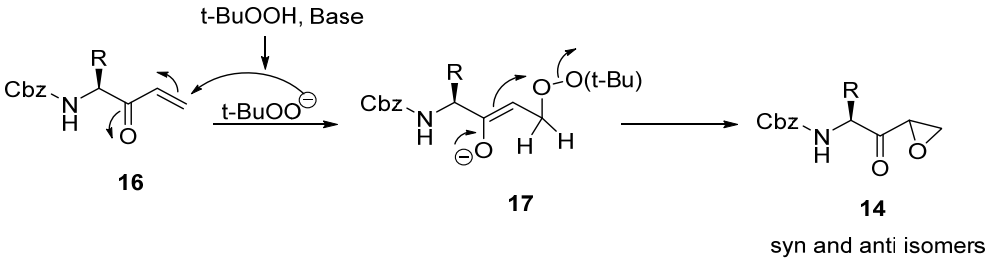
Table 3: Summary of the attempted synthesis of the cyclopropyl epoxyketone



12a ⟶ **13a**		
Reagents	Conditions	Results
mCPBA	CH_2Cl_2, RT,4 hrs	No reaction
mCPBA	CH_2Cl_2, RT,18 hrs	No reaction
t-BuOOH, t-BuOK	THF,0˚C, 13 hrs	60%

The peroxyanion formed from alkyl hydroperoxide *in situ* under basic conditions undergoes conjugate addition at the beta-position of an α, β-unsaturated ketone **(16)** to afford an intermediate β-peroxyenolate (**17**). This further undergoes intramolecular nucleophile substitution by the cleavage of O-O bond with the removal of alkoxide (RO^-) to afford (**14**). The mechanism of the Weitz-Scheffer epoxidation is shown in **Scheme 9**.

Scheme 9: The two-step mechanism of the Weitz-Scheffer epoxidation



Results of testing in phenotypic assay

Several of the simple epoxides and also compound 13a were tested in a phenotypic assay in the nematode C. *elegans* as seen in Figure 19.[39] Simple epoxides **14b** and **14c** show similar in potency to bortezomib at 20 and 200 µM and hybrid analog **13a** less potent than **14b** and **14c** at 20 and 200 µM.

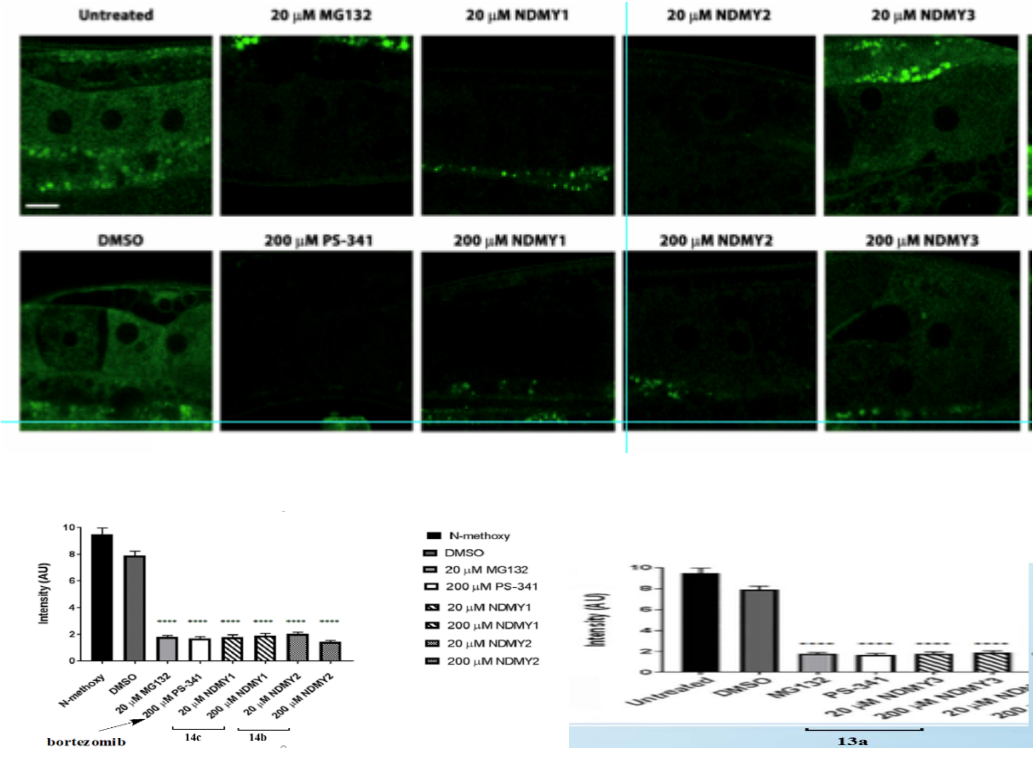


Figure 19: Phenotypic assay for simple epoxides and analog

Conclusions

Belactosin A is a naturally occurring proteasome inhibitor with potent anti- tumor activity that has inhibitory effects on the 20s core particle of the proteasome. Several analogs have been proposed and synthesized. Carfilzomib is a tetrapeptide epoxyketone, and an analog of epoxomicin with improved properties. Carfilzomib was approved by FDA for the treatments of multiple myeloma, non-Hodgkin's lymphoma and solid tumors. The primary goal of this project was to synthesize hybrid analogs of belactosin A and carfilzomib, with the cyclopropyl backbone and epoxyketone serine trap.

Several problems were encountered during the synthesis. The first one was a protection of a hindered alcohol. While silyl ethers and benzyl ether were not obtained, it was finally protected as an acetate. Another issue was encountered during epoxidation of the cyclopropyl enone. Traditional epoxidation with electrophilic reagents was not successful. However a Weitz-Scheffer epoxidation led to two different series of epoxyketones.

In summary a successful synthesis of the hybrid analogs of belactosin A and carfilozomib has been completed. Starting from phenylalanine, leucine and valine, three series, giving both the acetate analogs **13a-c** and alcohol analogs **11a-c** have been made. In addition, the "simple" epoxyketones **14a-c** have been prepared. Initial testing of several epoxyketones in a phenotypic assay indicates that both the simple epoxides and hybrid analogs are effective proteasome inhibitors.[39]

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Tripeptide Proteasome inhibitor Belactosin A and Its Highly Potent Cis-cyclopropane Stereoisomer" *Organic Letters*, **2008**, 10, 3571-3574.

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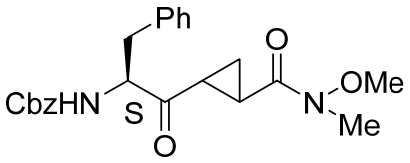
APPENDICES

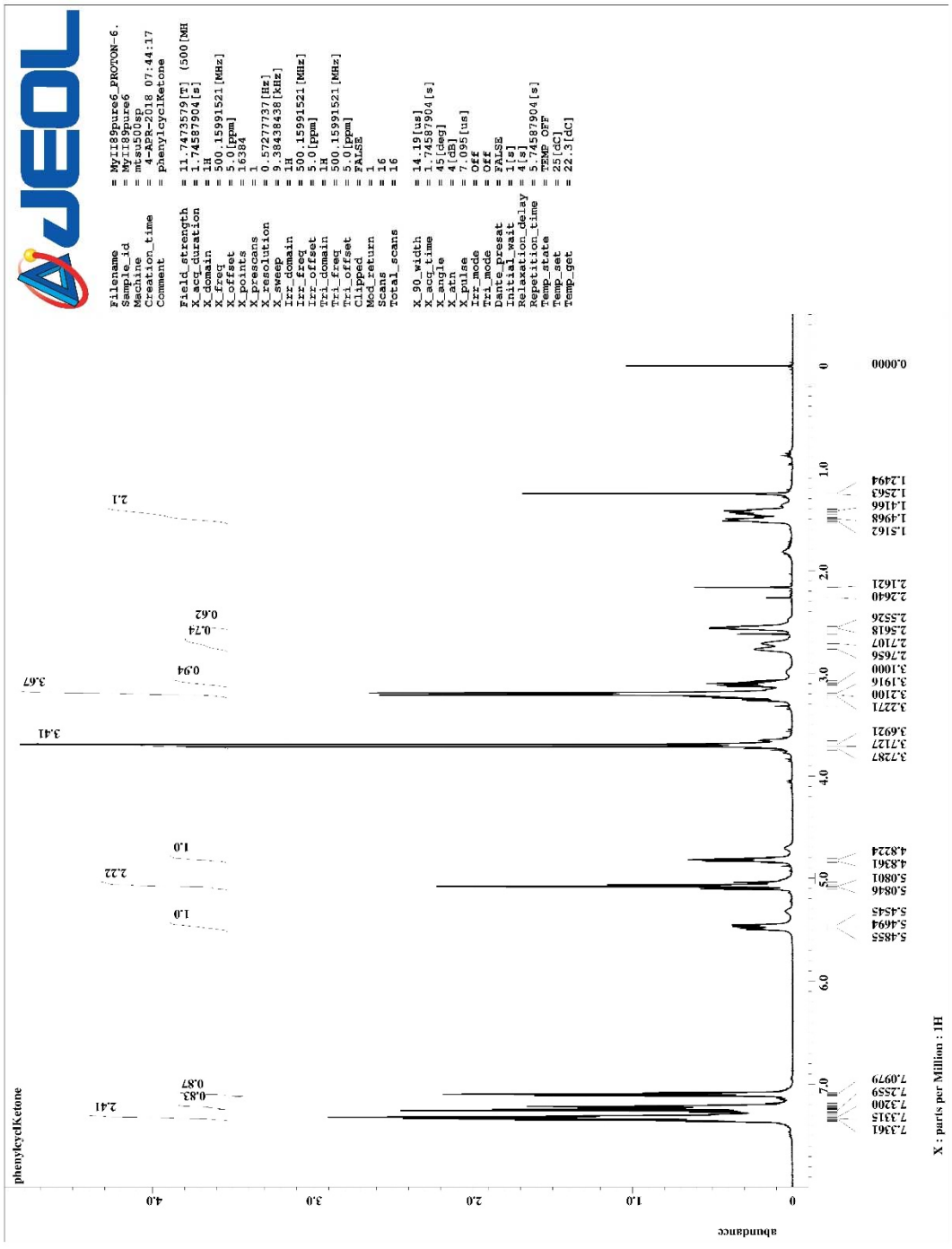
APPENDIX A: NMR SPECTRA



^{1} H-NMR (500 MHz, $CDCl_3$)

^{13}C-NMR (125 MHz, $CDCl_3$)

HRMS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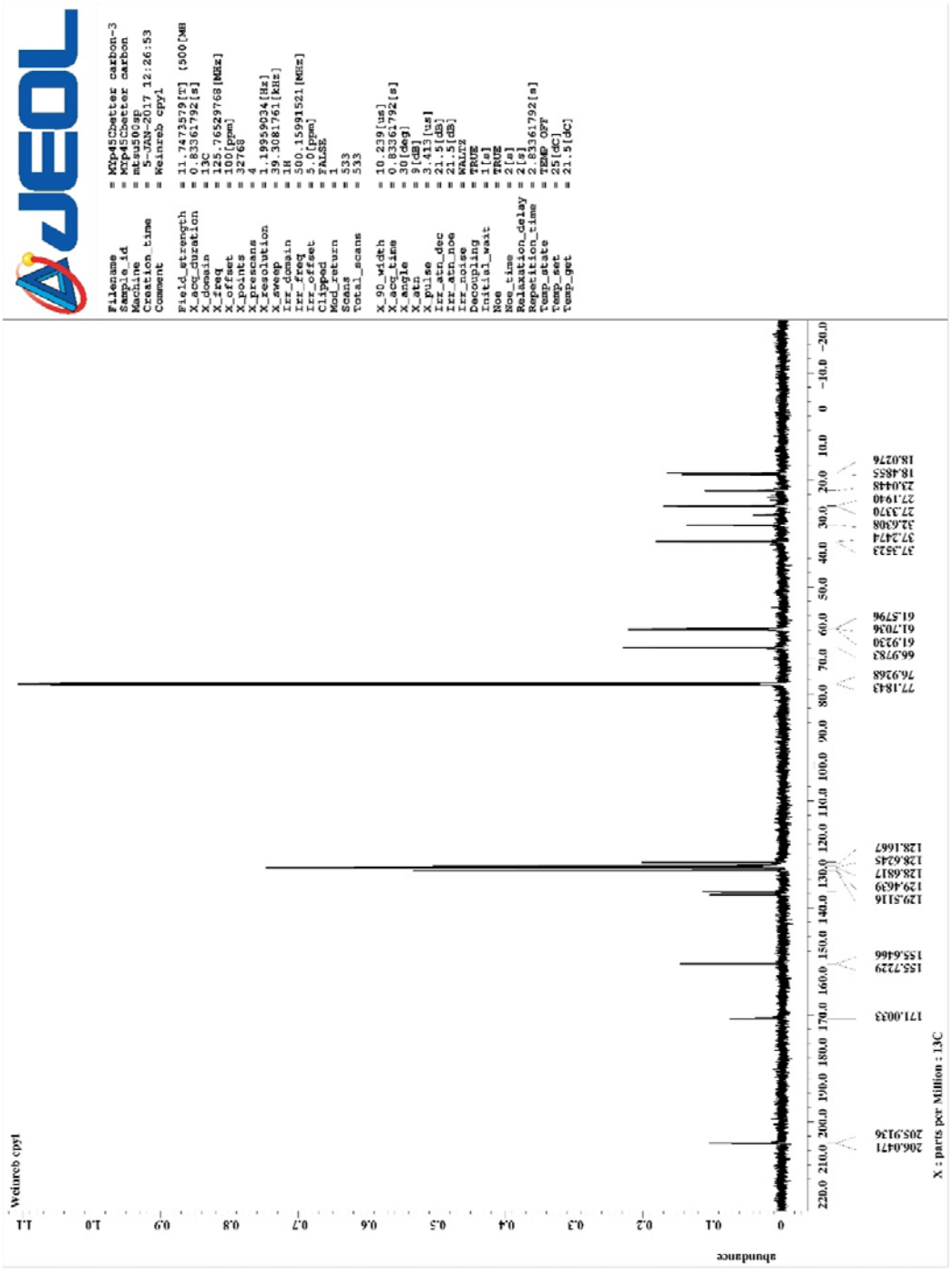
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 16
Total_scans = 16
X_90_width = 14.19[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 7.095[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 22.3[dC]
phenylcyclKetone
abundance
X : parts per Million : 1H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Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.3081761[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 533
Total_scans = 533
X_90_width = 10.239[us]
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X_angle = 30[deg]
X_atn = 9[dB]
X_pulse = 3.413[us]
Irr_atn_dec = 21.5[dB]
Irr_atn_noe = 21.5[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe_time = TRUE
Relaxation_delay = 2[s]
Repetition_time = 2.83361792[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 21.5[dC]
Weinreb cpy1
abundance
X : parts per Million : 13C
206.0471
205.9136
171.0033
155.7229
155.6466
129.5116
129.4639
128.6817
128.6245
128.1667
77.1843
76.9268
66.9783
61.9230
61.7036
61.5796
37.3523
37.2474
32.6308
27.3370
27.1940
23.0448
18.4855
18.0276

Mass Spectrometry & Proteomics Facility

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\0202\67825-4.MYp51 .d	Acquisition Date	2/2/2017 2:50:53 PM
Method	050916_ns_tune_low_pos.m	Operator	BDAL@DE
Sample Name	4.MYp51	Instrument / Ser#	micrOTOF II 8213750.10314
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
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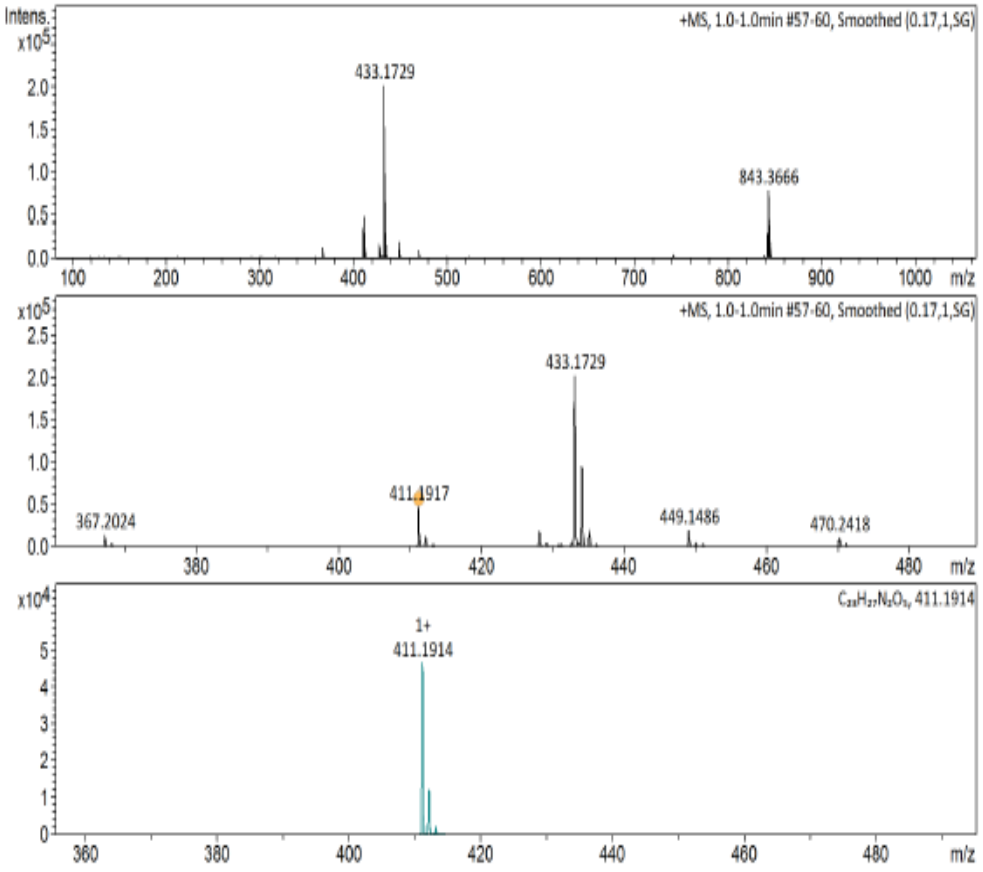
Scan End	1650 m/z	n/a	n/a	Set Divert Valve	Source



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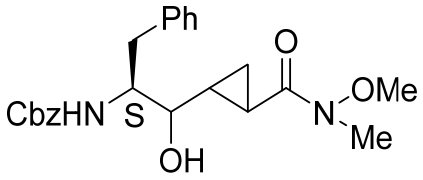
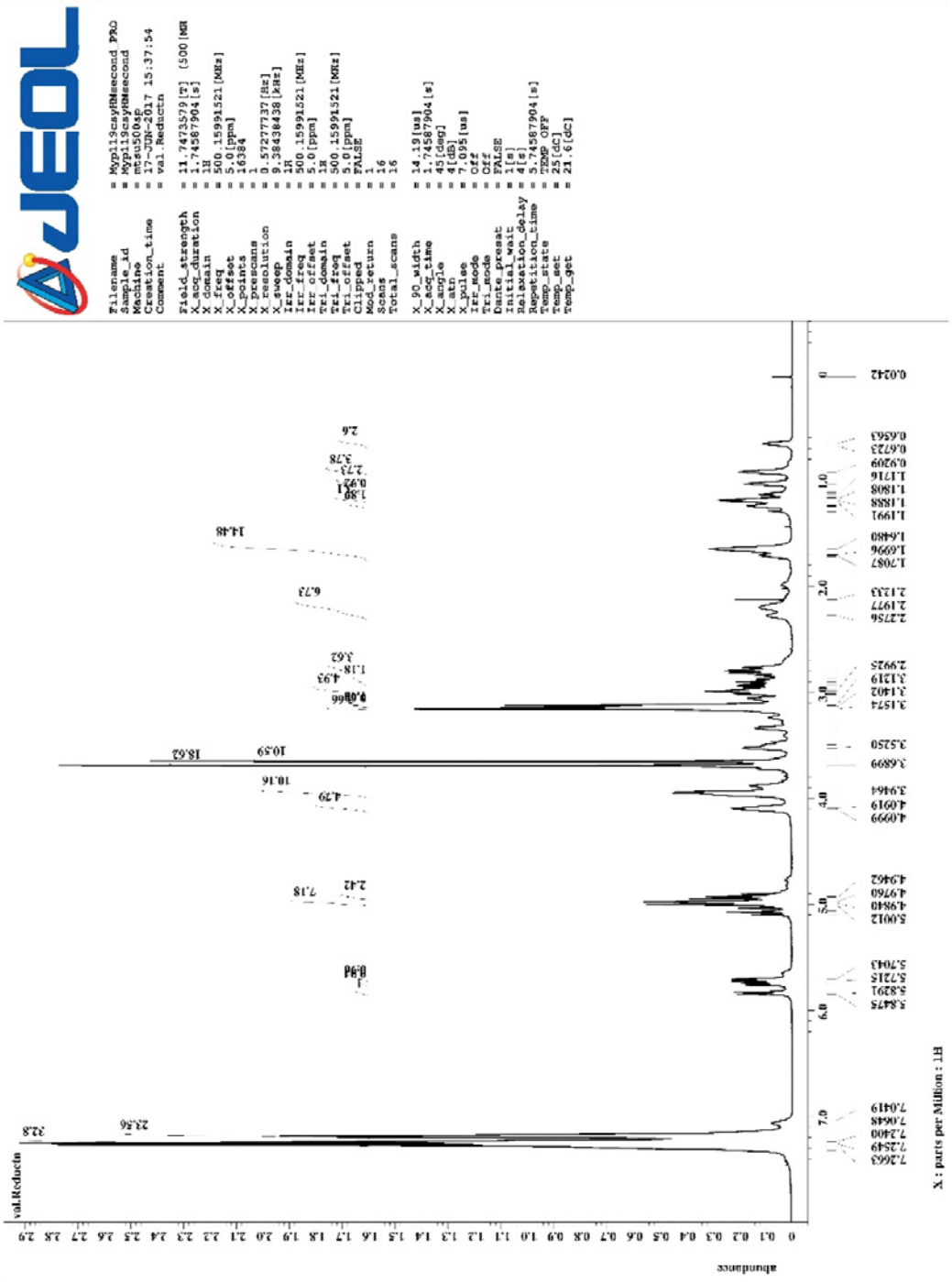


^{1}H-NMR (500 MHz, $CDCl_3$)

^{13}C-NMR (125 MHz, $CDCl_3$)

HRMS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Irr_offset = 5.0[ppm]
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Tri_freq = 500.15991521[MHz]
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Dante_presat = FALSE
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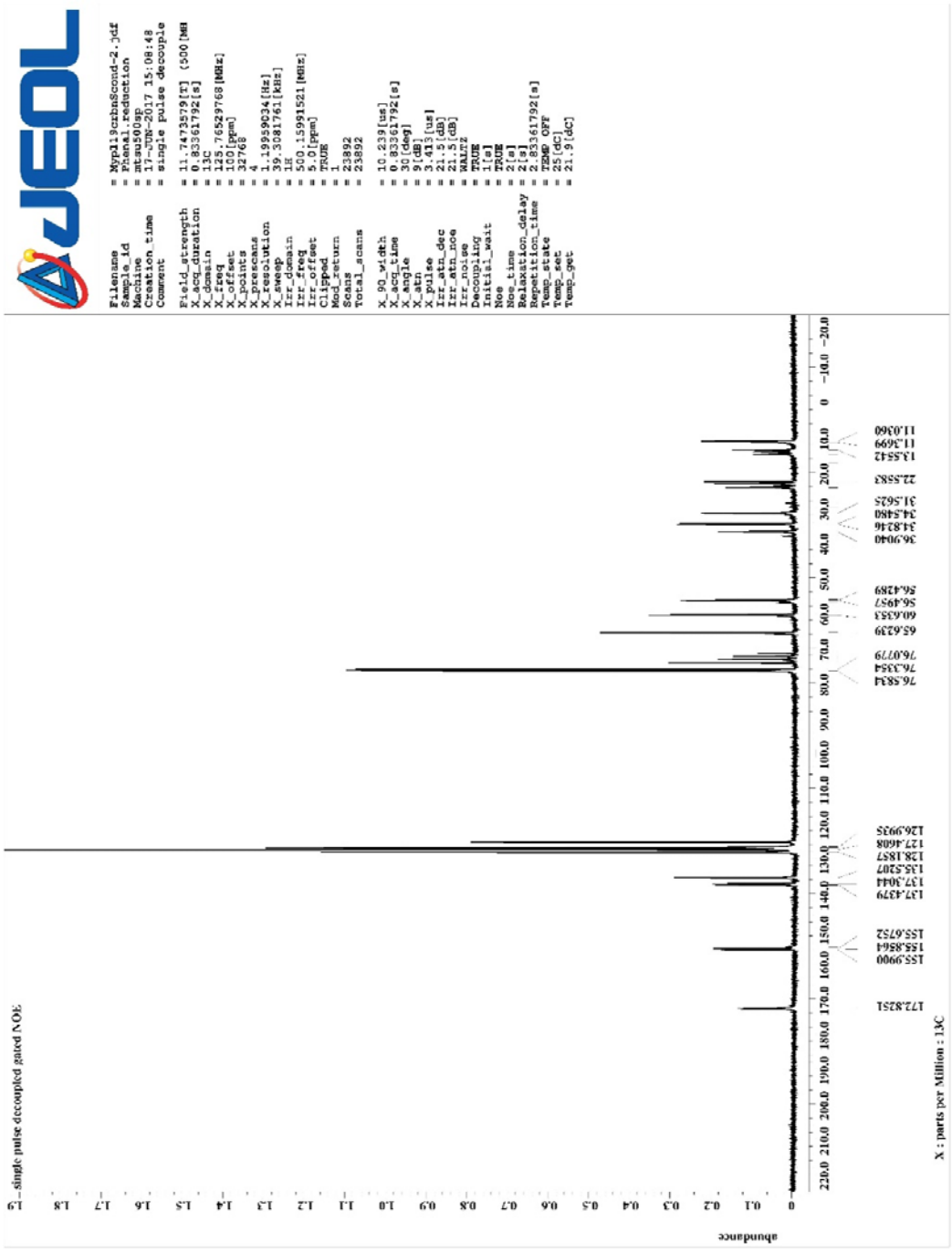
val.Reductn
abundance
X : parts per Million : 1H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155.9900
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155.6752
137.4379
137.3044
135.5207
128.1857
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76.3354
76.0779
65.6239
60.6353
56.4957
56.4289
36.9040
34.8246
34.5480
31.5625
22.5583
13.5542
13.3699
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single pulse decoupled gated NOE
abundance
X : parts per Million : 13C

Mass Spectrometry & Proteomics Facility

Mass Spectrum SmartFormula Report

Analysis Info

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Sample Name	3.NYp53	Instrument / Ser#	micrOTOF II 8213750.10314
Comment			

Acquisition Parameter

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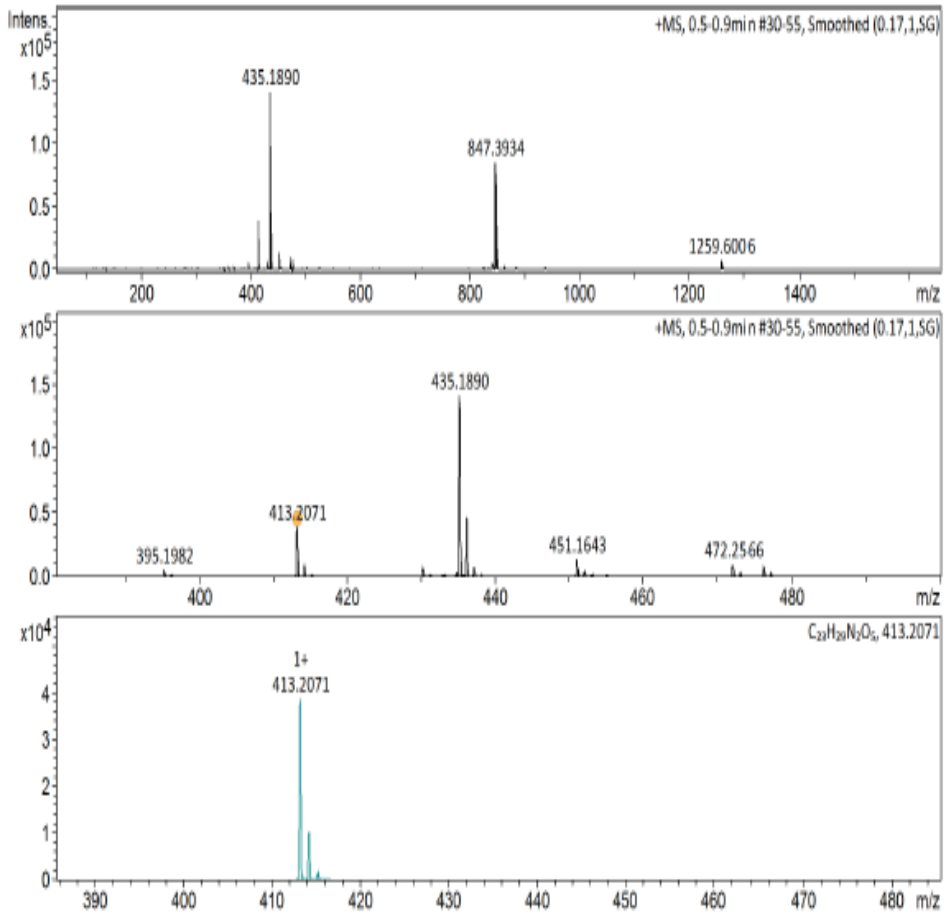
Scan End	1650 m/z	n/a	n/a	Set Divert Valve	Source



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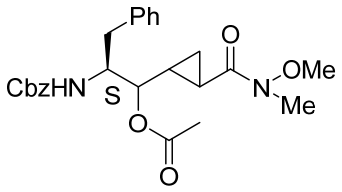

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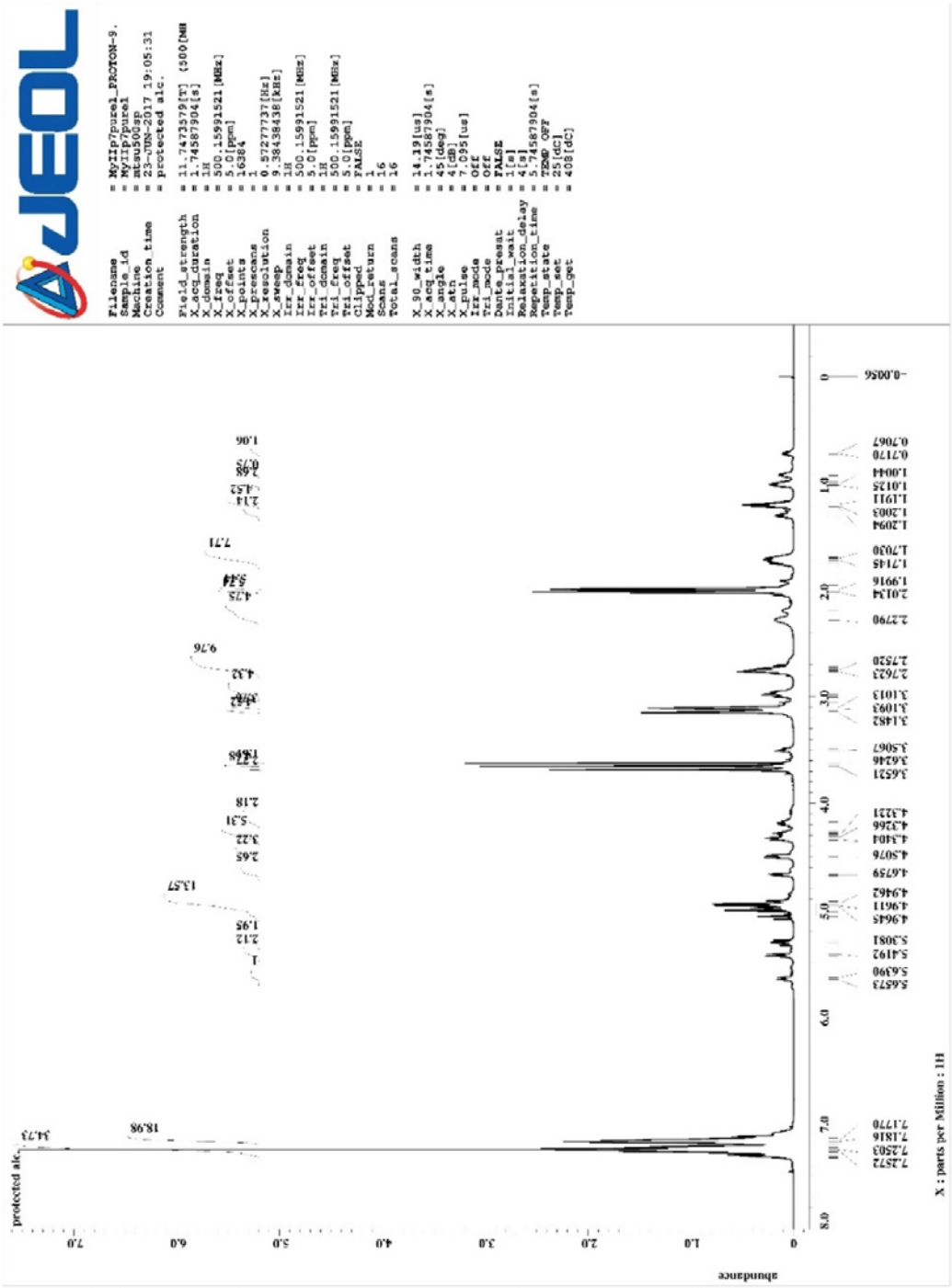
HRMS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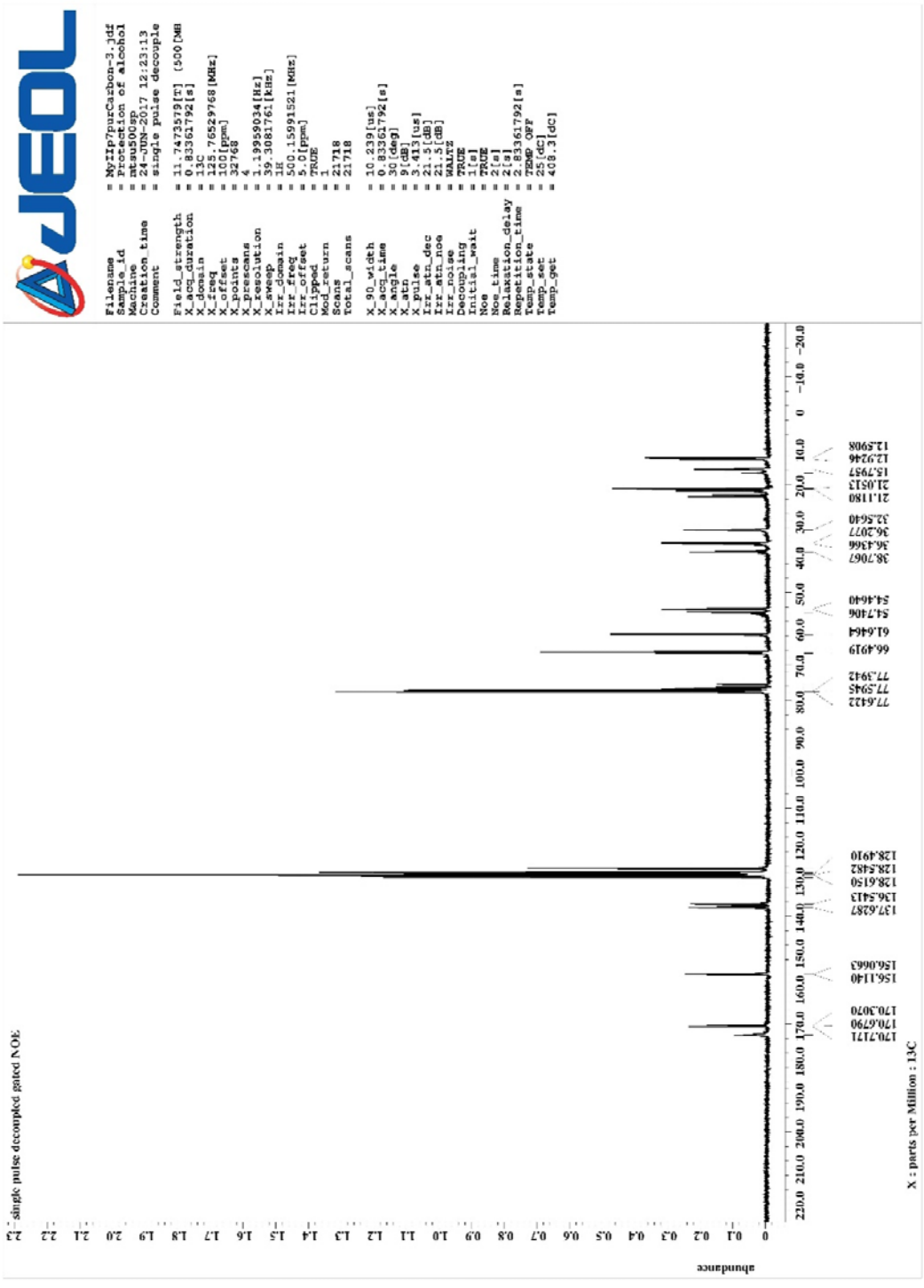
protected alc.
34.73
18.98
13.57
9.76
7.71
1.06
abundance
X : parts per Million : 1H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Noe_time = 2[s]
Relaxation_delay = 2[s]
Repetition_time = 2.83361792[s]
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Temp_set = 25[dC]
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abundance
single pulse decoupled gated NOE
X : parts per Million : 13C
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170.6790
170.3070
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77.5945
77.3942
66.4919
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54.4640
38.7067
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36.3077
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12.9246
12.5908

Mass Spectrometry & Proteomics Facility

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\0412\71447-2.MYp95.d	Acquisition Date	4/12/2017 10:44:52 AM
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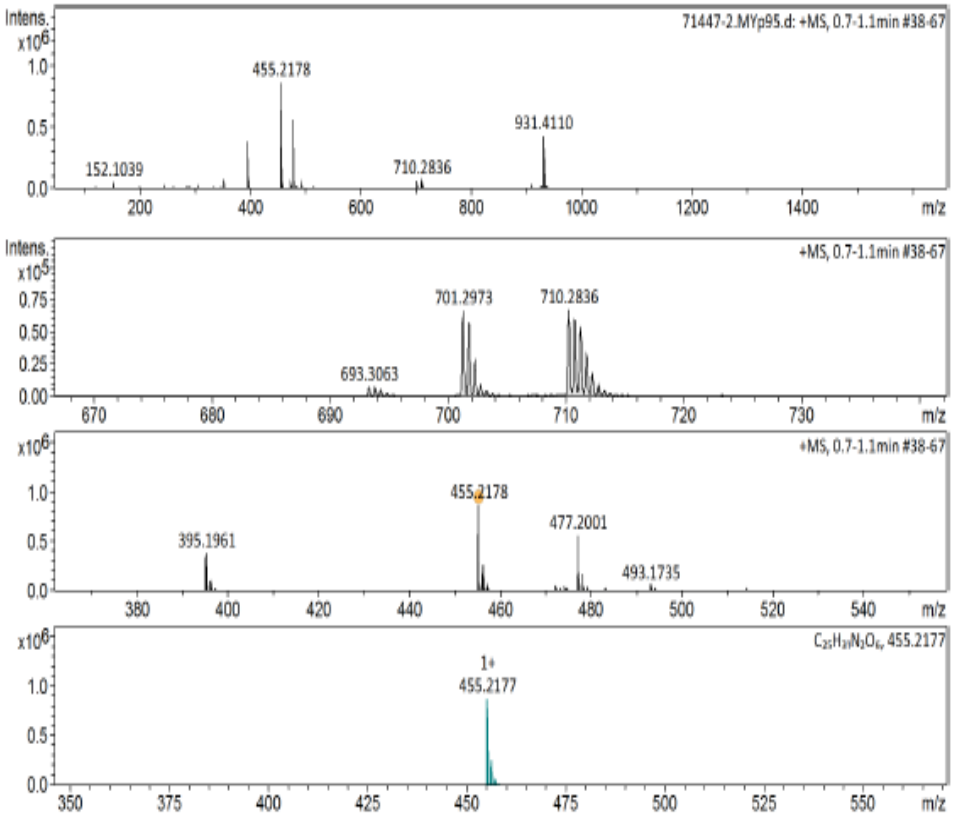
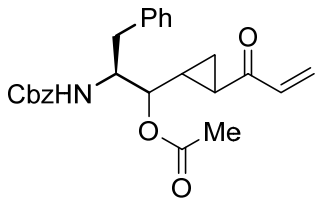
Sample Name	2.MYp95	Instrument / Ser#	micrOTOF II 8213750.10314
Comment	direct infusion		

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1650 m/z	n/a	n/a	Set Divert Valve	Source



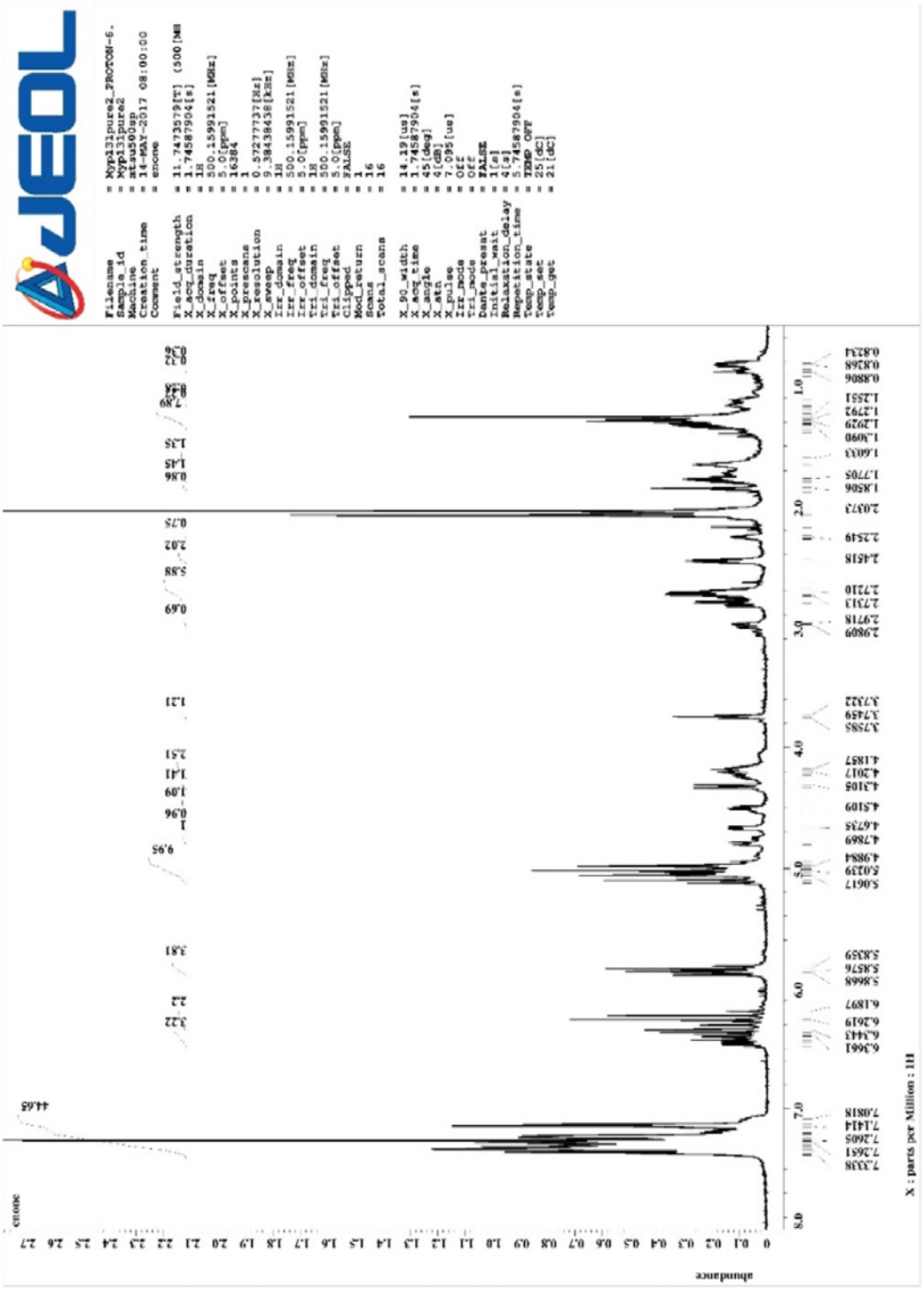
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf
455.217818	1	C25H31N2O6	455.217663	-0.3	1.2	11.5	ok	even

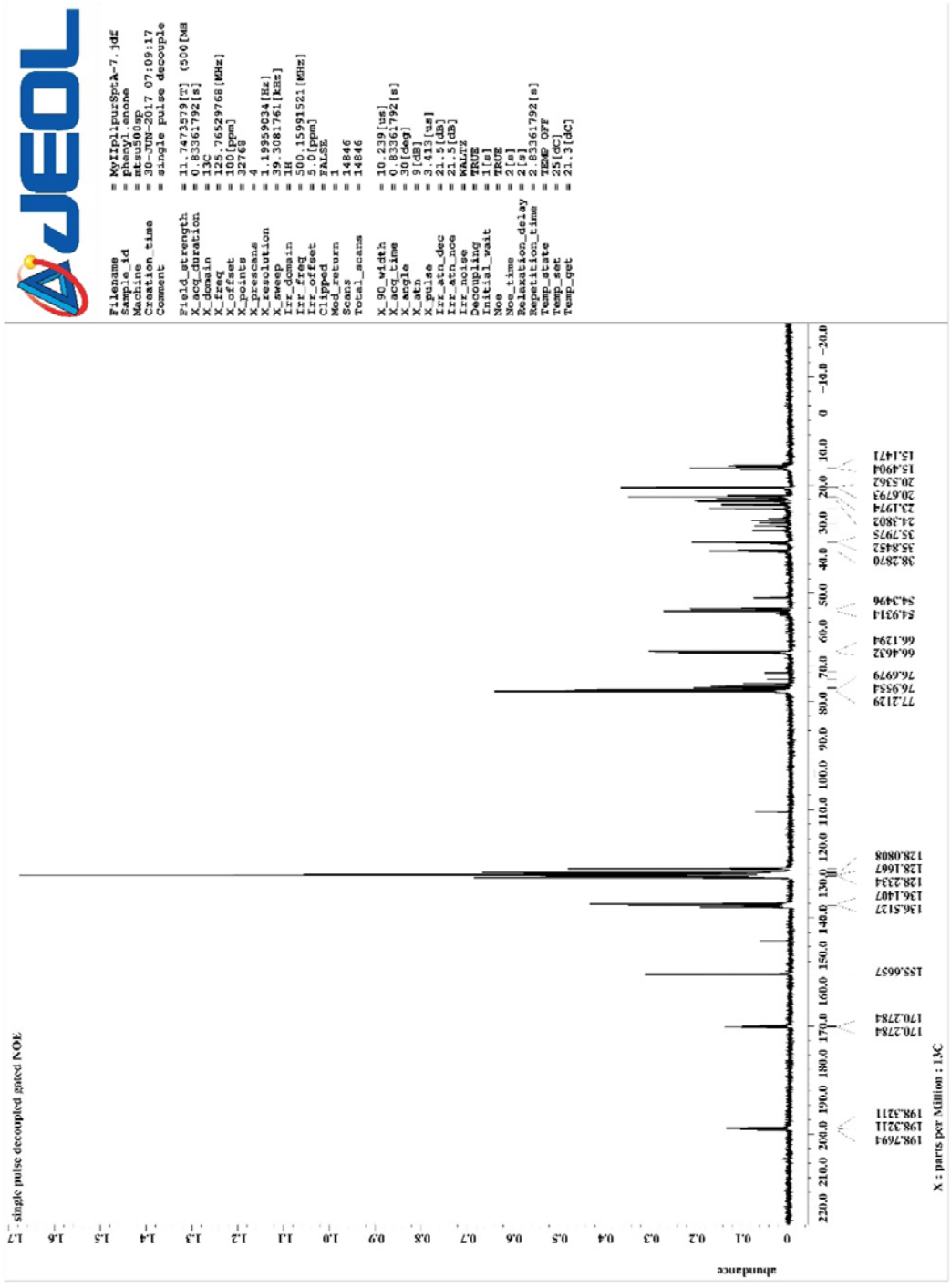


^{1}H-NMR (500 MHz, $CDCl_3$)

^{13}C-NMR (125 MHz, $CDCl_3$)

HRMS


JEOL
Filename = Nyp131pure2_PROTON-6-
Sample_id = Nyp131pure2
Machine = ntsu500sp
Creation_time = 14-MAY-2017 08:00:00
Comment = enome
Field_strength = 11.7473579[T] (500.[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 16
Total_scans = 16
X_90_width = 14.19[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_atn = 6[dB]
X_pulse = 7.095[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 21[dC]
abundance
X : parts per Million : 1H


JEOL
Filename = KyIIplpurSptA-7.jdf
Sample_id = phenyl.enone
Machine = mtsu500gp
Creation_time = 30-JUN-2017 07:09:17
Comment = single pulse decouple
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959634[Hz]
X_sweep = 39.3081761[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 14846
Total_scans = 14846
X_90_width = 10.239[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 9[dB]
X_pulse = 3.413[us]
Irr_atn_dec = 21.5[dB]
Irr_atn_noe = 21.5[dB]
Irr_noise = WALTZ2
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 2[s]
Relaxation_delay = 2[s]
Repetition_time = 2.83361792[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 21.3[dC]
single pulse decoupled gated NOE
abundance
X : parts per Million : 13C
198.7694
198.3211
198.3211
170.2784
170.2784
155.6657
136.5127
136.1407
128.2334
128.1667
128.0808
77.2129
76.9554
76.6979
66.4632
66.1294
54.9314
54.3496
38.2870
35.8452
35.7975
24.3802
23.1974
20.6793
20.5362
15.4904
15.1471

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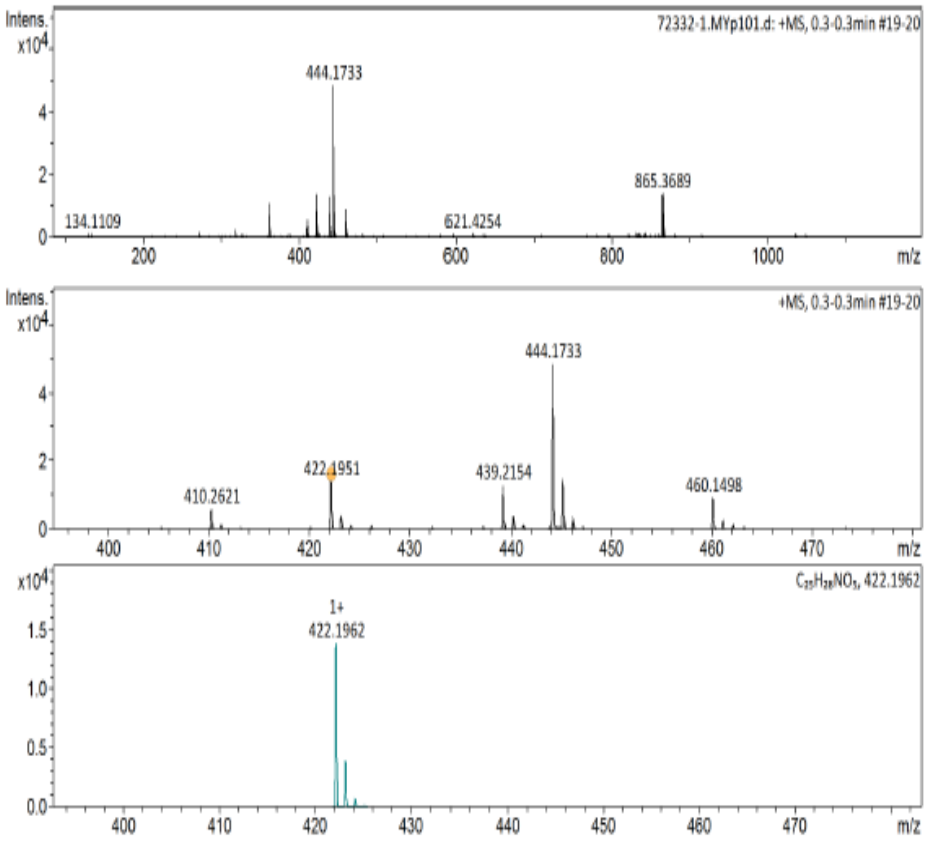
Mass Spectrum SmartFormula Report

Analysis Info

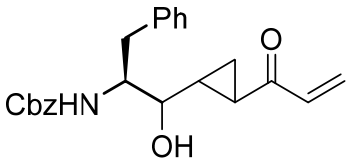
Analysis Name	D:\Data\0502\72332-1.MYp101.d	Acquisition Date	5/2/2017 4:17:55 PM
Method	050916_ns_tune_low_pos.m	Operator	BDAL@DE
Sample Name	1.MYp101	Instrument / Ser#	micrOTOF II 8213750.10314
Comment	direct infusion		

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1650 m/z	n/a	n/a	Set Divert Valve	Source



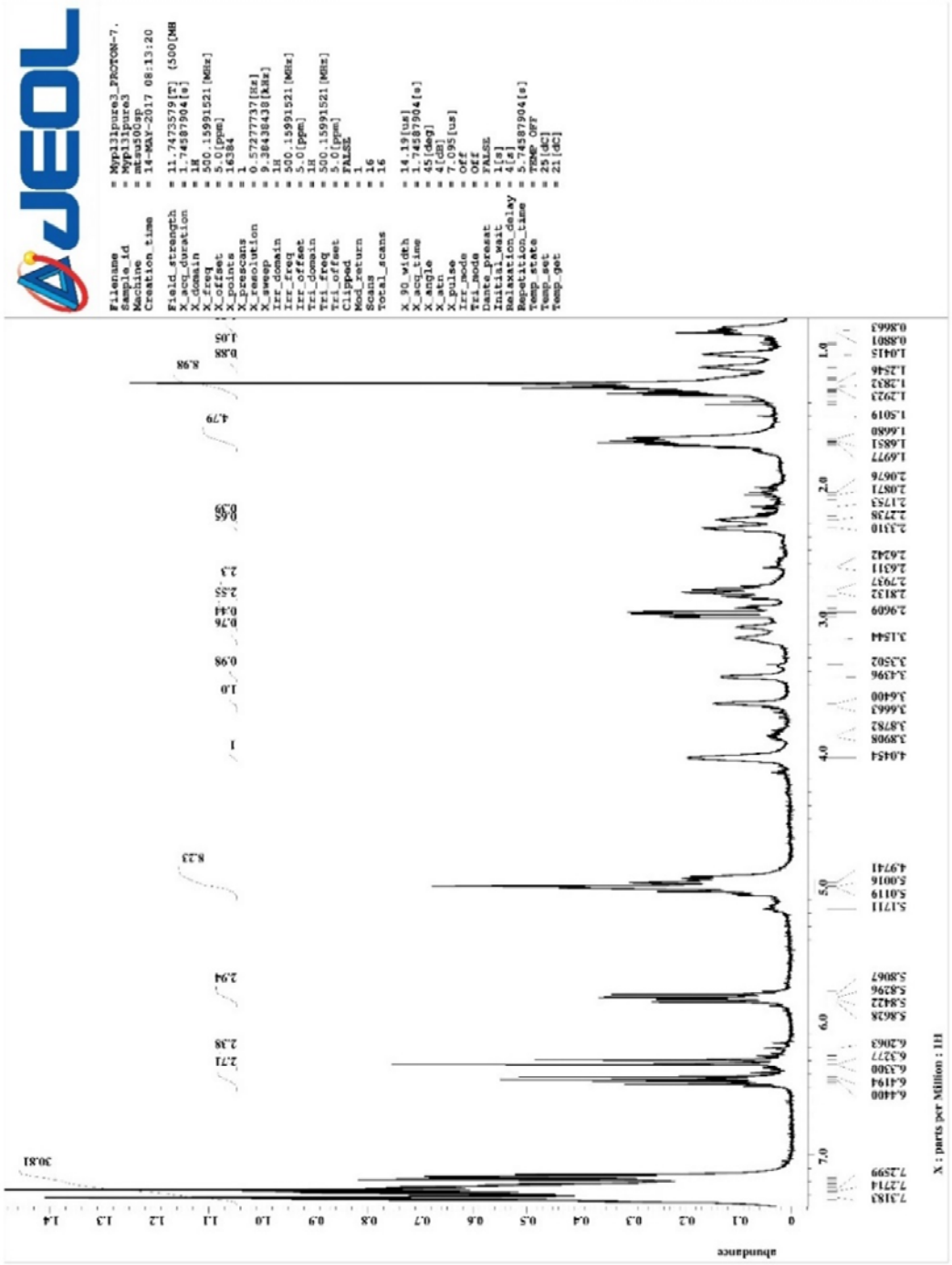
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf
422.195075	1	C25H28NO5	422.196199	2.7	4.5	12.5	ok	even

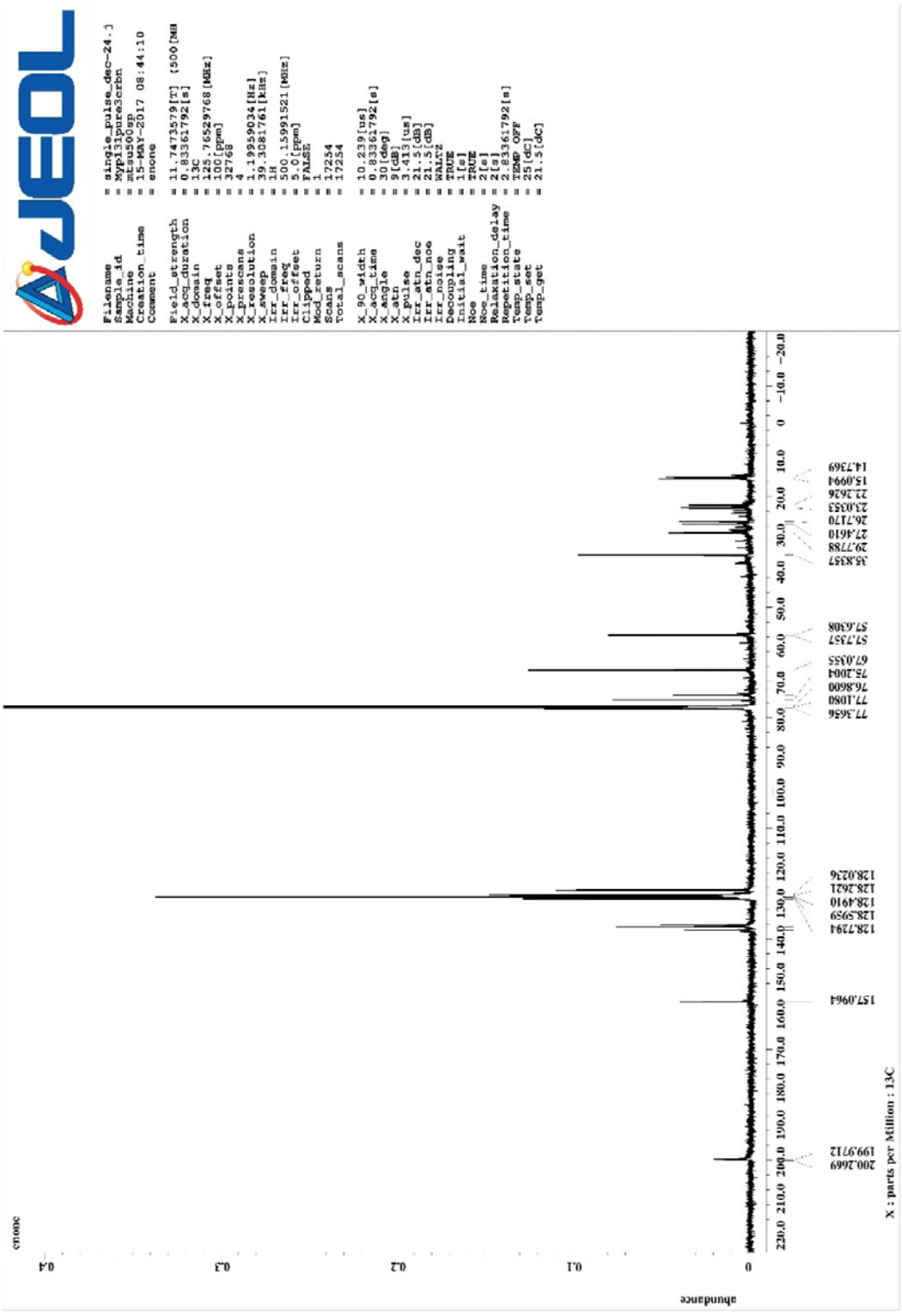


^{1}H-NMR (500 MHz, $CDCl_3$)

^{13}C-NMR (125 MHz, $CDCl_3$)

HRMS


JEOL
Filename = Myp131pure3_PROTON-7.
Sample_id = Myp131pure3
Machine = mtsu500qp
Creation_time = 14-MAY-2017 08:13:20
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438430[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 16
Total_scans = 16
X_90_width = 14.19[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 7.095[us]
Irr_mode = Off
Tri_mode = Off
Decouple = FALSE
Delta1_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 21[dC]
30.81
8.98
8.23
4.79
2.94
2.71
2.38
2.5
2.55
2.44
2.3
1.05
1.0
0.98
0.88
0.76
0.65
0.39
1
7.3183
7.2714
7.2599
6.4400
6.4194
6.3300
6.3277
6.2063
5.8638
5.8422
5.8296
5.8067
5.1711
5.0119
5.0016
4.9741
4.0454
3.8908
3.8782
3.6663
3.6400
3.4396
3.3502
3.1544
2.9609
2.8132
2.7937
2.6311
2.6242
2.3310
2.2738
2.1753
2.0871
2.0676
1.6977
1.6851
1.6680
1.5019
1.2923
1.2832
1.2546
1.0415
0.8801
0.8663
abundance
X : parts per Million : 1H


JEOL
Filename = single_pulse_dec-24.j
Sample_id = Myp131pure3crbn
Machine = mtsu500sp
Creation_time = 15-MAY-2017 08:44:10
Comment = enone
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100.0[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.3081761[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 17254
total_scans = 17254
X_90_width = 10.239[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 9[dB]
X_pulse = 3.413[us]
Irr_atn_dec = 21.5[dB]
Irr_atn_noe = 21.5[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 2[s]
Relaxation_delay = 2.13361792[s]
Relaxation_time = 2.13361792[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 21.9[dC]
enone
abundance
X : parts per Million : 13C
200.2669
199.9712
157.0964
128.7794
128.5959
128.4910
128.2621
128.0236
77.3656
77.1080
76.8600
76.2004
67.0355
57.7357
57.6308
35.8357
29.7788
27.4610
26.7170
23.0353
22.3626
15.0994
14.7369

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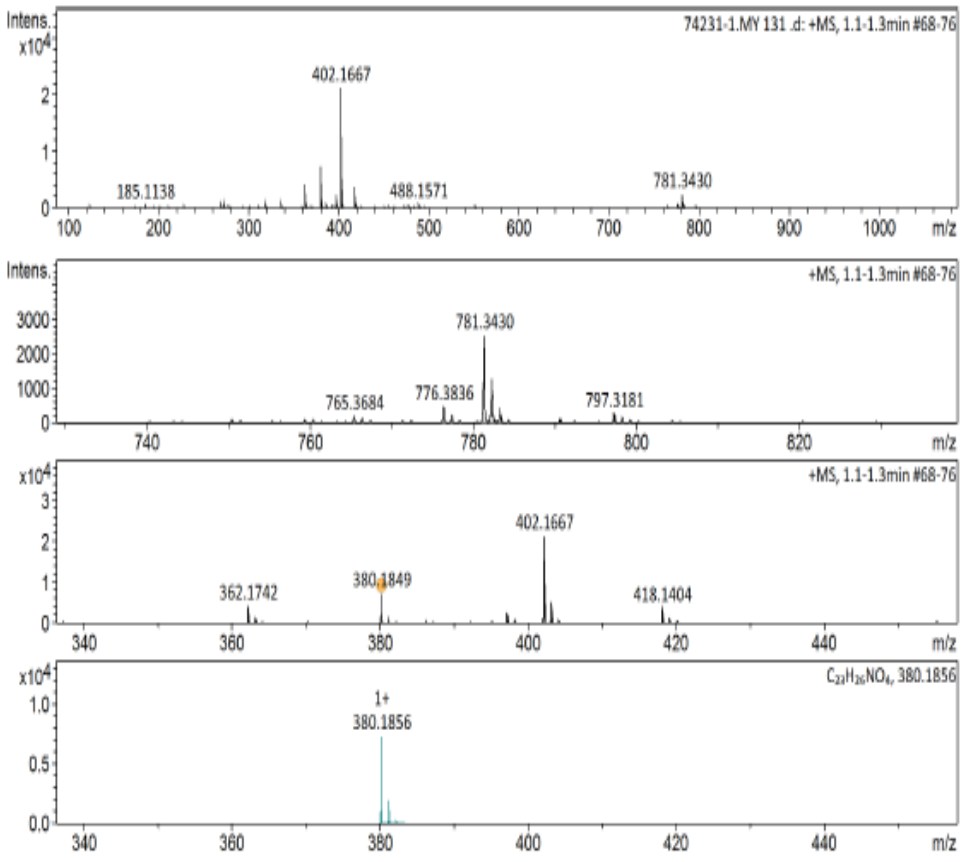
Mass Spectrum SmartFormula Report

Analysis Info

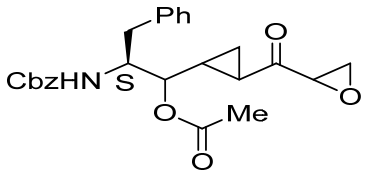
Analysis Name	D:\Data\0609\74231-1.MY 131 .d	Acquisition Date	6/9/2017 3:37:57 PM
Method	050916_ns_tune_low_pos.m	Operator	BDAL@DE
Sample Name	1.MY 131	Instrument / Ser#	micrOTOF II 8213750.10314
Comment	direct infusion		

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	1.5 l/min
Scan End	1650 m/z	n/a	n/a	Set Divert Valve	Source



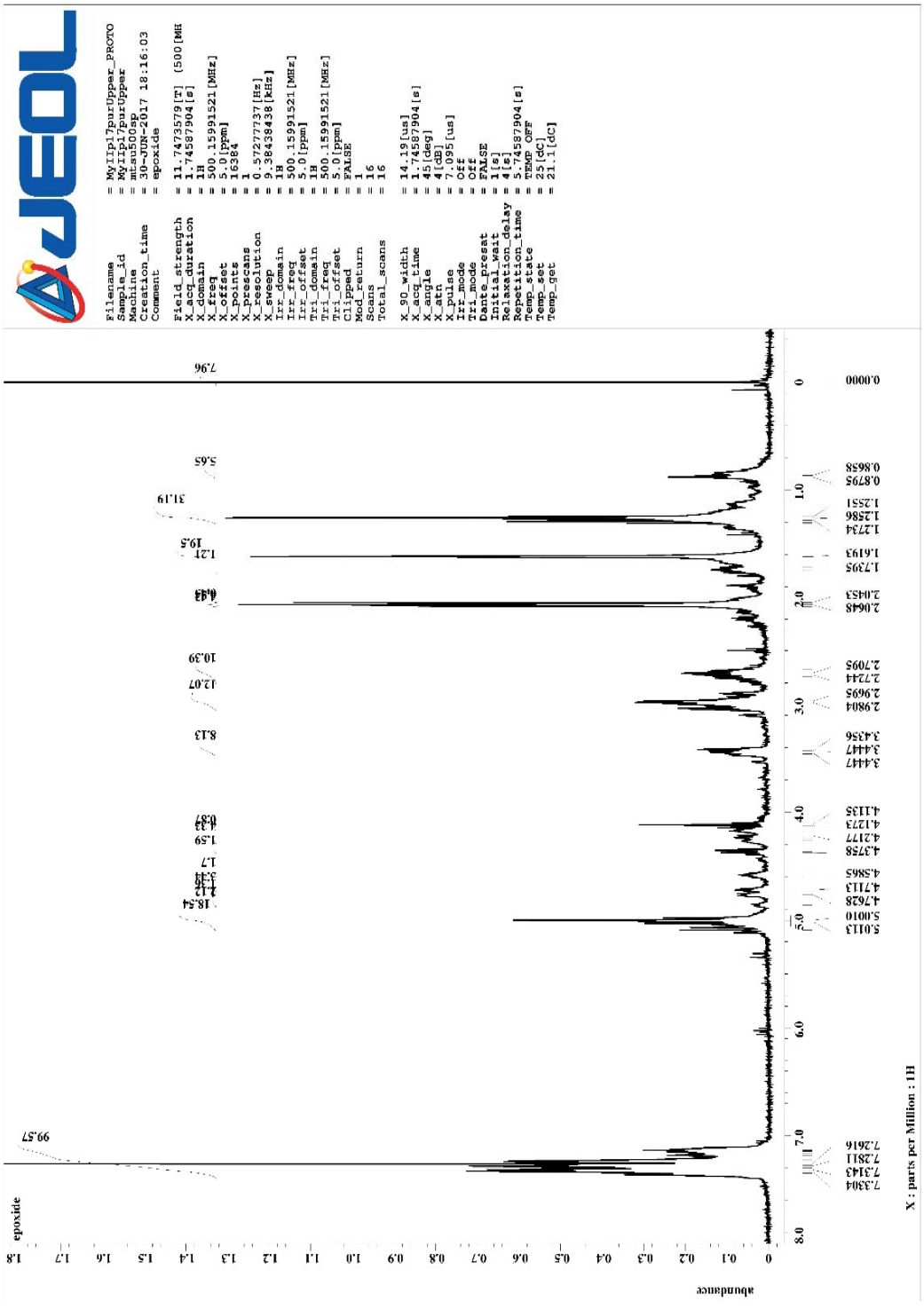
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf
380.184935	1	C23H26NO4	380.185635	1.8	2.6	11.5	ok	even

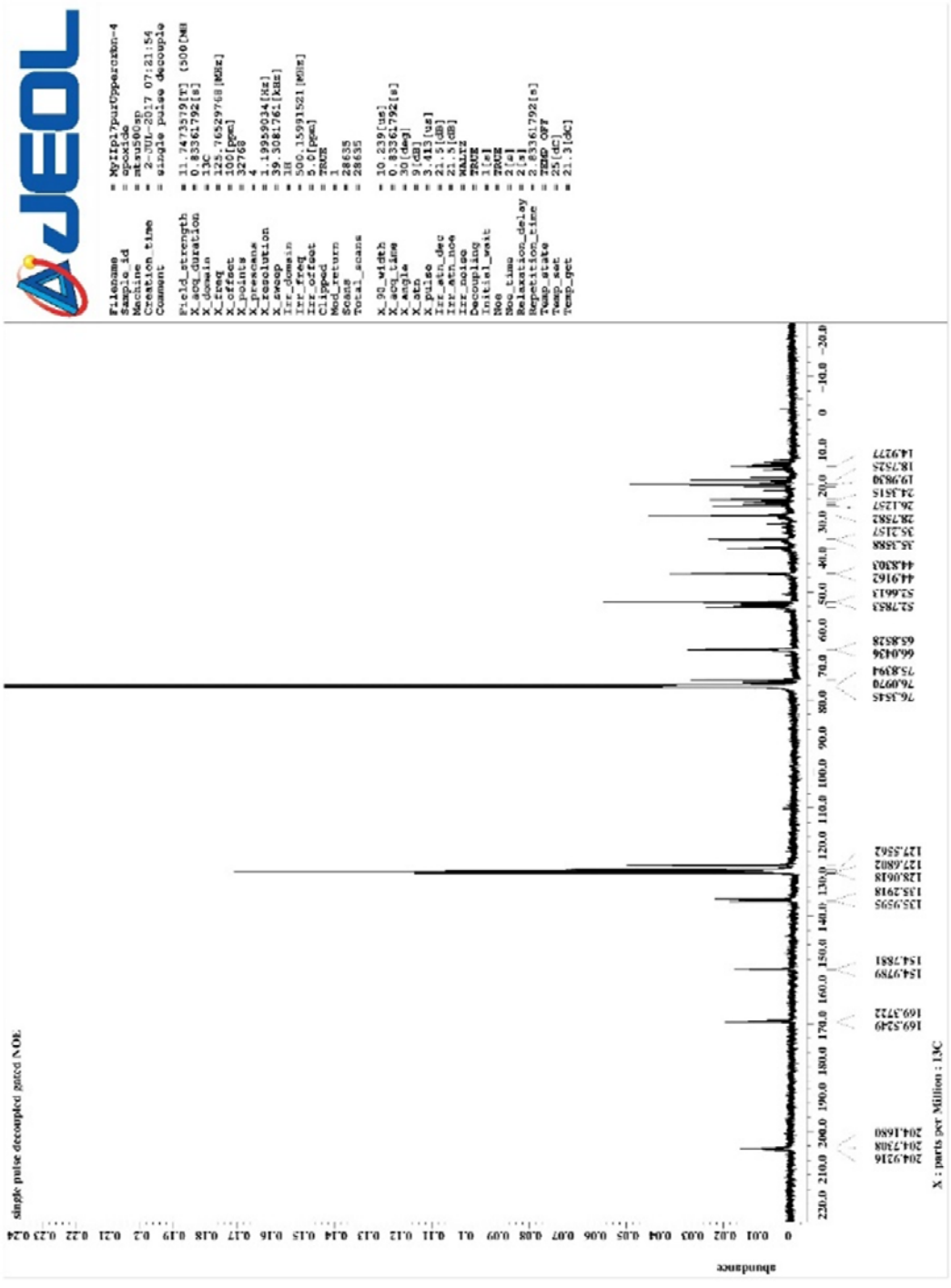


^{1}H-NMR (500 MHz, $CDCl_3$)

^{13}C-NMR (125 MHz, $CDCl_3$)

HRMS


JEOL
Filename = MyIIp17purUpper_PROTO
Sample_id = MyIIp17purUpper
Machine = mtsu500sp
Creation_time = 30-JUN-2017 18:16:03
Comment = epoxide
Field_strength = 11.74735579[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 16
Total_scans = 16
X_90_width = 14.19[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 7.095[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 21.1[dC]
epoxide
99.57
18.54
8.13
12.07
10.39
6.83
12.27
19.5
31.19
5.65
7.96
abundance
X : parts per Million : 1H


JEOL
Filename = MyIp17purOpperation-4
Sample_id = apomide
Machine = ntsu500sp
Creation_time = 2-JUL-2017 07:21:54
Comment = single pulse decouple
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.765297[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.3081761[kHz]
Irr_domain = 1H
Irr_freq = 500.35991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = TRUE
Mod_return = 16
Scans = 28535
Total_scans = 28535
X_90_width = 10.239[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 9[dB]
X_pulse = 3.413[us]
Irr_atn_dec = 21.5[dB]
Irr_atn_noe = 21.5[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 2[s]
Relaxation_delay = 2[s]
Repetition_time = 2.83361792[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 21.3[dC]
single pulse decoupled gated NOE
abundance
X : parts per Million : 13C
204.9216
204.7308
204.1680
169.5249
169.3722
154.9789
154.7881
135.9595
135.2918
128.0618
127.6802
127.5562
76.3545
76.0970
75.8394
66.0436
65.8528
52.7853
52.6613
44.9162
44.8303
35.3588
35.3157
28.7582
26.1257
24.3515
19.9824
18.7525
14.9277

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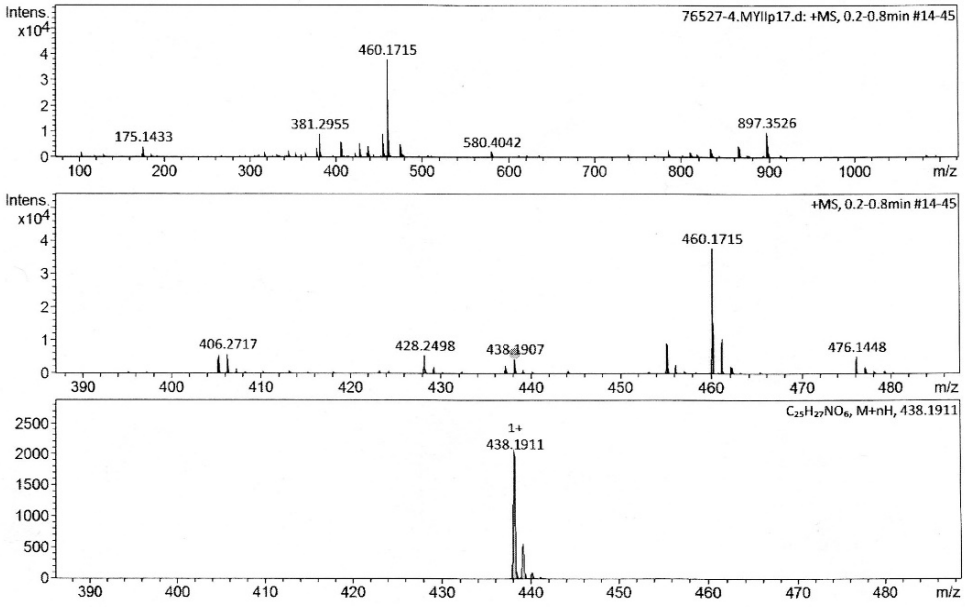
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\0726\76527-4.MYIIp17.d	Acquisition Date	7/26/2017 3:07:10 PM
Method	061517_tune_low_pos_NS.m	Operator	BDAL@DE
Sample Name	4.MYIIp17	Instrument / Ser#	micrOTOF II 8213750.10314
Comment	direct ifusion		

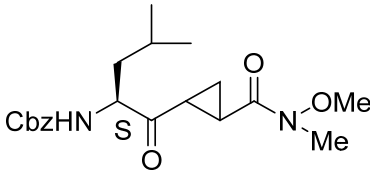
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1650 m/z	n/a	n/a	Set Divert Valve	Waste



Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf
438.190724	1	C25H28NO6	438.191114	0.9	1130.7	12.5	ok	even

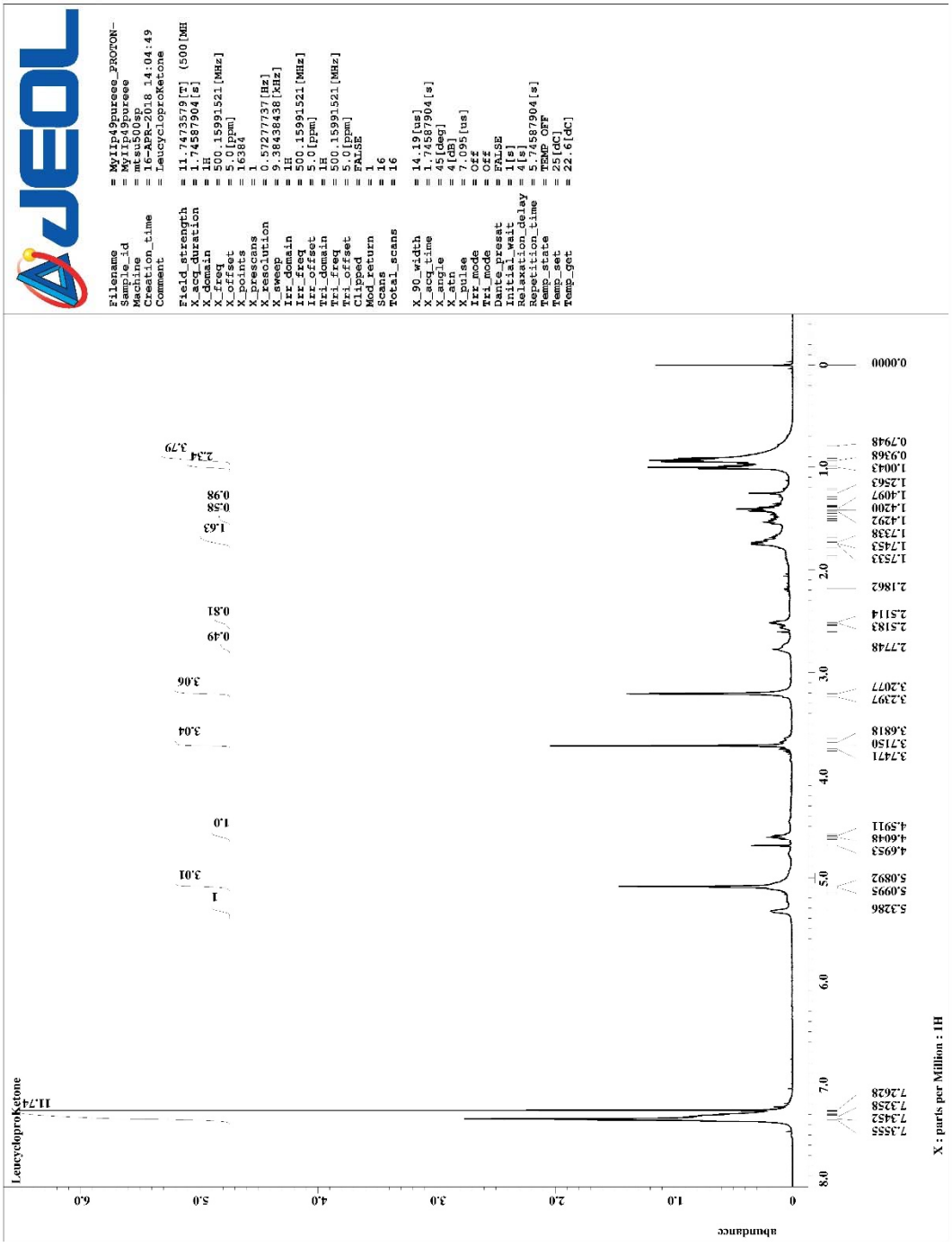
This material is based upon work supported by the National Science Foundation under CHE-0741793

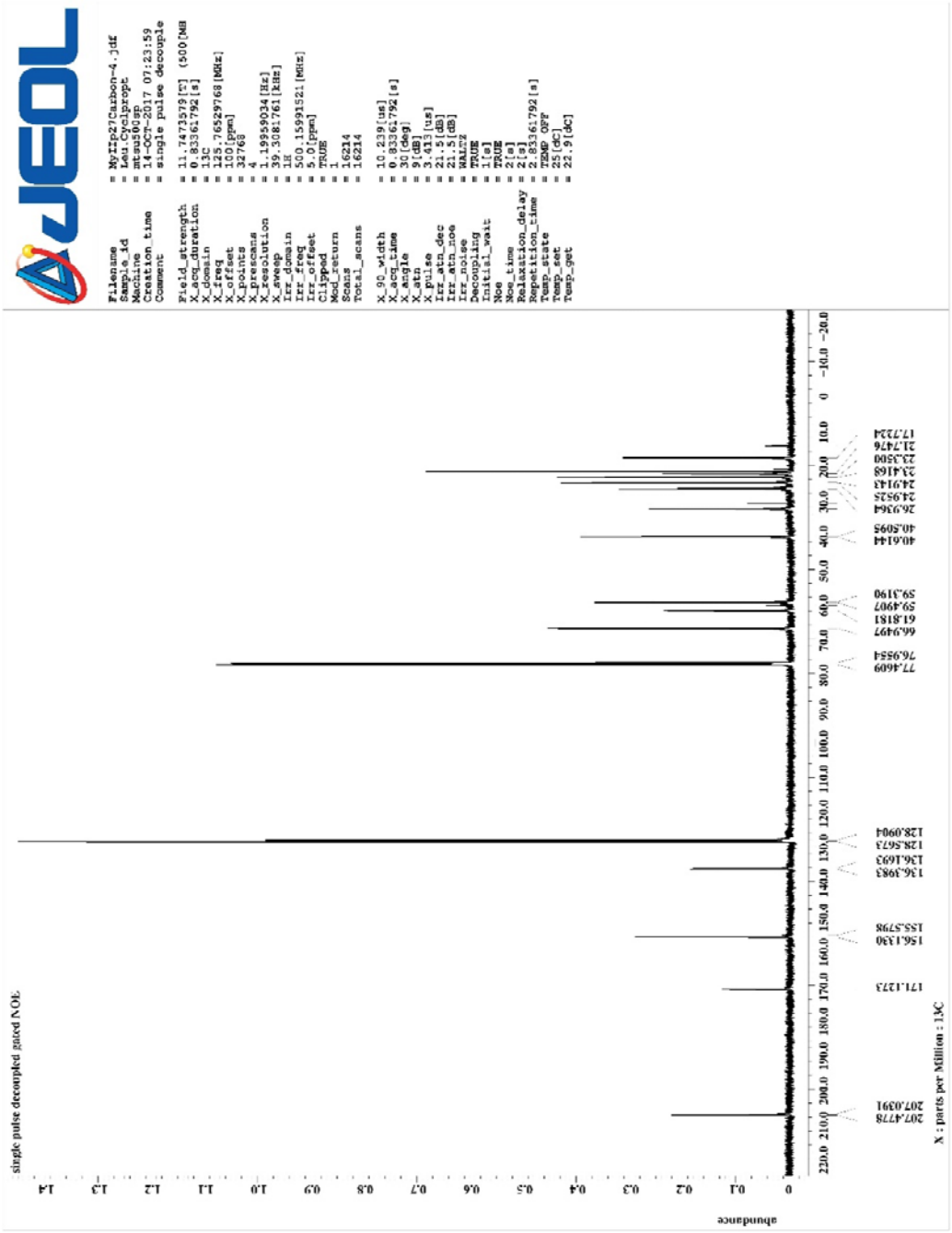


^{1}H-NMR (500 MHz, $CDCl_3$)

^{13}C-NMR (125 MHz, $CDCl_3$)

HRMS


JEOL
Filename = MyIIp449puresee_PROTON-
Sample_id = MyIIp449puresee
Machine = mtsu500sp
Creation_time = 16-APR-2018 14:04:49
Comment = LeucycloproKetone
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438436[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 16
Total_scans = 16
X_90_width = 14.19[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 7.095[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 22.6[dC]
LeucycloproKetone
11.74
3.01
1.0
3.04
3.06
0.49
0.81
1.63
0.58
0.98
2.34
3.79
abundance
X : parts per Million : 1H
7.3555
7.3452
7.3258
7.2628
5.3286
5.0995
5.0892
4.6953
4.6048
4.5911
3.7471
3.7150
3.6818
3.2397
3.2077
2.7748
2.5183
2.5114
2.1862
1.7533
1.7453
1.7338
1.4292
1.4200
1.4097
1.2563
1.0043
0.9368
0.7948
0.0000


JEOL
Filename = MyITp27Carbon-4.jdf
Sample_id = Leu.Cyclpropt
Machine = mtsu500gp
Creation_time = 14-OCT-2017 07:23:59
Comment = single pulse decouple
Field_strength = 11.747357[T] (500[MH
X_corr_duration = 0.8336792[s]
X_domain = 13C
X_freq = 125.765297681[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19559034[Hz]
X_sweep = 39.3081761[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clip_mod = TRUE
Mod_return = 1
Scans = 16214
Total_scans = 16214
X_90_width = 10.239[us]
X_acq_time = 0.8336792[s]
X_angle = 30[deg]
X_atn = 9[dB]
X_pulse = 3.413[us]
Irr_atn_dec = 21.5[dB]
Irr_atn_noe = 21.5[dB]
Irr_noise = WALTZ2
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 2[s]
Relaxation_delay = 2[s]
Repetition_time = 2.8336792[s]
Temp_state = ZERO OFF
Temp_set = 23[deg]
Temp_get = 22.9[deg]
single pulse decoupled gated NOE
abundance
X : parts per Million : 13C
207.4778
207.0391
171.1273
156.1330
155.5798
136.3983
136.1693
128.5673
128.0904
77.4609
76.9554
66.9497
61.8181
59.4907
59.3190
40.6144
40.5095
26.9364
24.9525
24.9143
23.4168
23.3500
21.7476
17.7224

Mass Spectrometry & Proteomics Facility

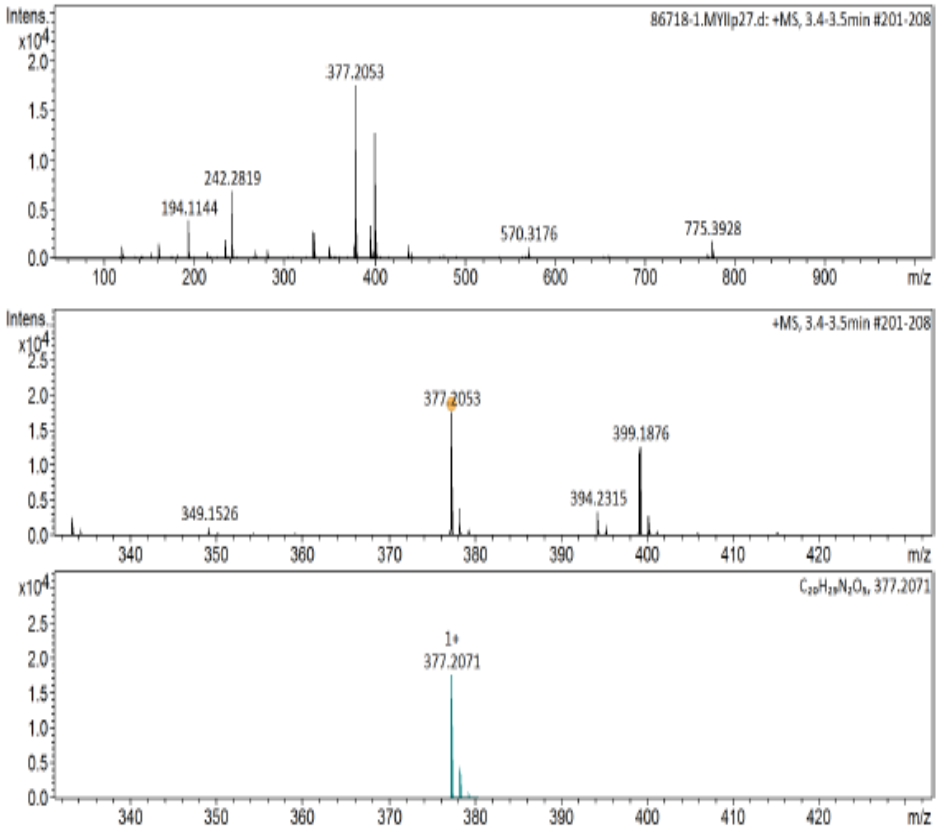
Mass Spectrum SmartFormula Report

Analysis Info

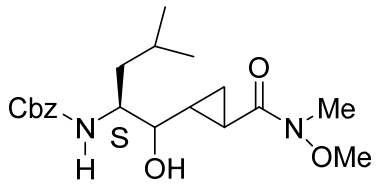
Analysis Name	D:\Data\0206\86718-1.MYIIp27.d	Acquisition Date	2/6/2018 2:23:15 PM
Method	0118_tune_low_pos_NS.m	Operator	BDAL@DE
Sample Name	1.MYIIp27	Instrument / Ser#	micrOTOF II 8213750.10314
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1650 m/z	n/a	n/a	Set Divert Valve	Source



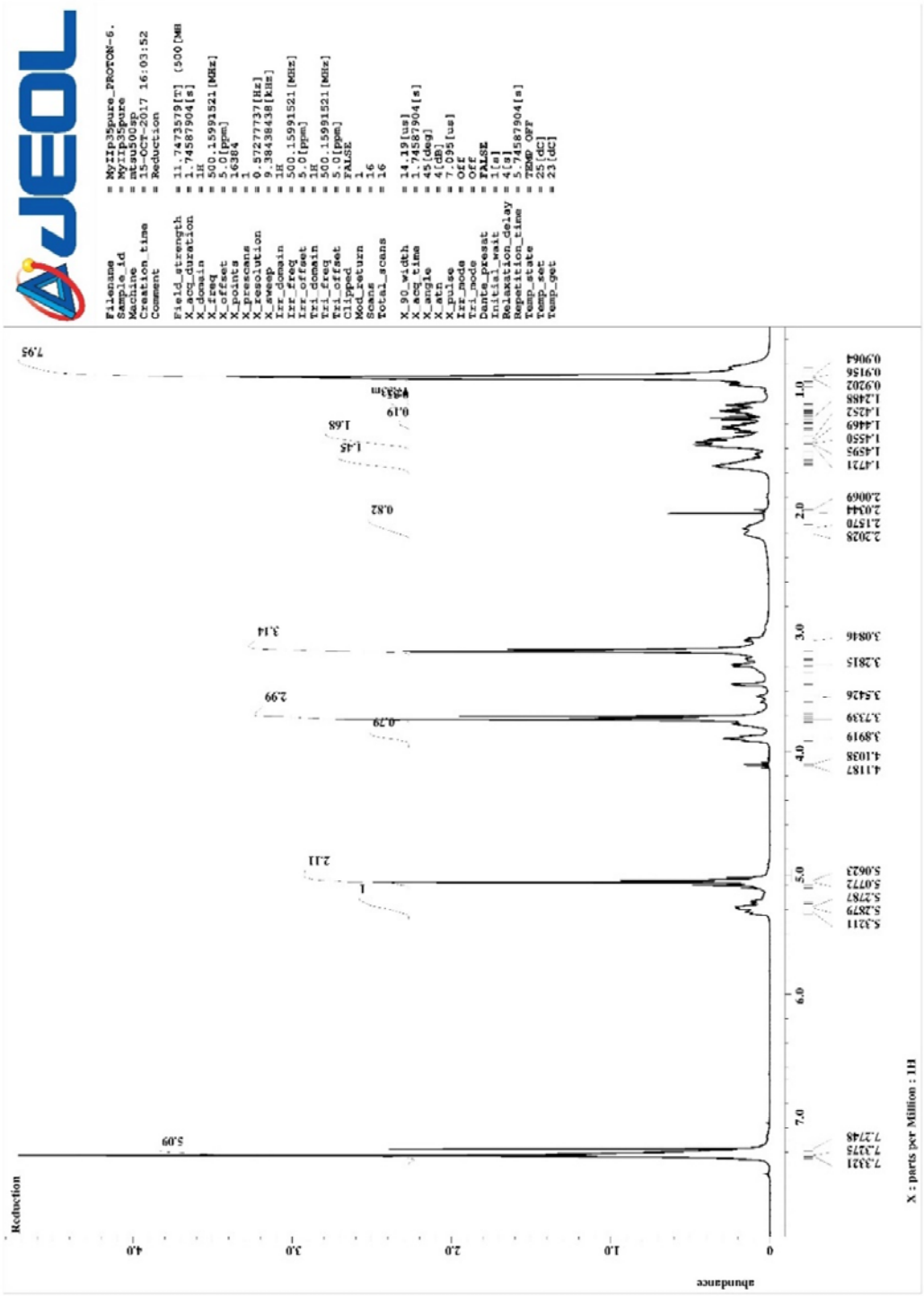
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf
377.205315	1	C20H29N2O5	377.207098	4.7	4.8	7.5	ok	even

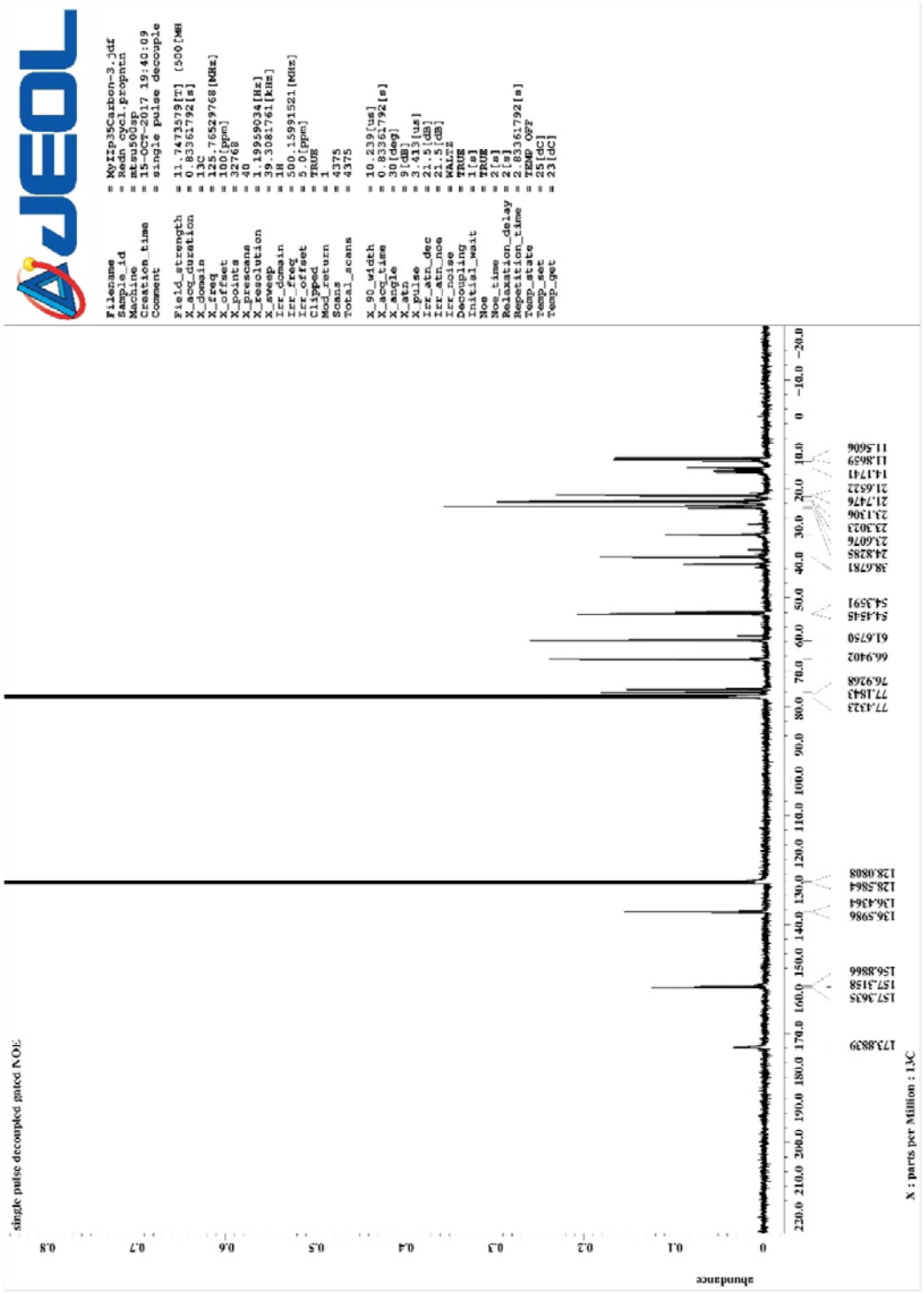


^{1}H-NMR (500 MHz, $CDCl_3$)

^{13}C-NMR (125 MHz, $CDCl_3$)

HRMS


JEOL
Filename = MyIp35pure_PROTON-6.
Sample_id = MyIp35pure
Machine = mtsu500sp
Creation_time = 15-Oct-2017 16:03:52
Comment = Reduction
Field_strength = 11.74735797[T] (500[MH
Acq._duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 16
Total_scans = 16
X_90_width = 14.19[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 7.095[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = ZMO OFF
Temp_set = 25[dC]
Temp_get = 23[dC]
7.95
5.09
3.14
2.99
2.11
1.68
1.45
0.82
0.79
0.19
1
Reduction
abundance
X : parts per Million : 1H
7.3321
7.3275
7.2748
5.3211
5.2879
5.2787
5.0772
5.0623
4.1187
4.1038
3.8919
3.7339
3.5426
3.3815
3.0846
2.3028
2.1570
2.0344
2.0069
1.4721
1.4595
1.4550
1.4469
1.4252
1.2488
0.9202
0.9156
0.9064


JEOL
Filename = MyIrp35Carbon-3.jdf
Sample_id = Redn cycl.propnrn
Machine = mtsu500sp
Creation_time = 15-OCT-2017 19:40:09
Comment = single pulse decouple
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.765297 68[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 40
X_resolution = 1.19959034[Hz]
X_sweep = 39.3081761[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = TRUE
Mod_return = 1
Scans = 4375
Total_scans = 4375
X_90_width = 10.239[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 9[dB]
X_pulse = 3.413[us]
Irr_atn_dec = 21.5[dB]
Irr_atn_noe = 21.5[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = TRUE
Noe = TRUE
Noe_time = 2[s]
Relaxation_delay = 2[s]
Repetition_time = 2.83361792[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 23[dC]
single pulse decoupled gated NOE
abundance
X : parts per Million : 13C
173.8839
157.3635
157.3158
156.8866
136.5986
136.4364
128.5864
128.0808
77.4323
77.1843
76.9268
66.9402
61.6750
54.4545
54.3591
38.6781
24.8285
23.6076
23.3023
23.1306
21.7476
31.6522
14.1741
11.8659
11.5606

Mass Spectrometry & Proteomics Facility

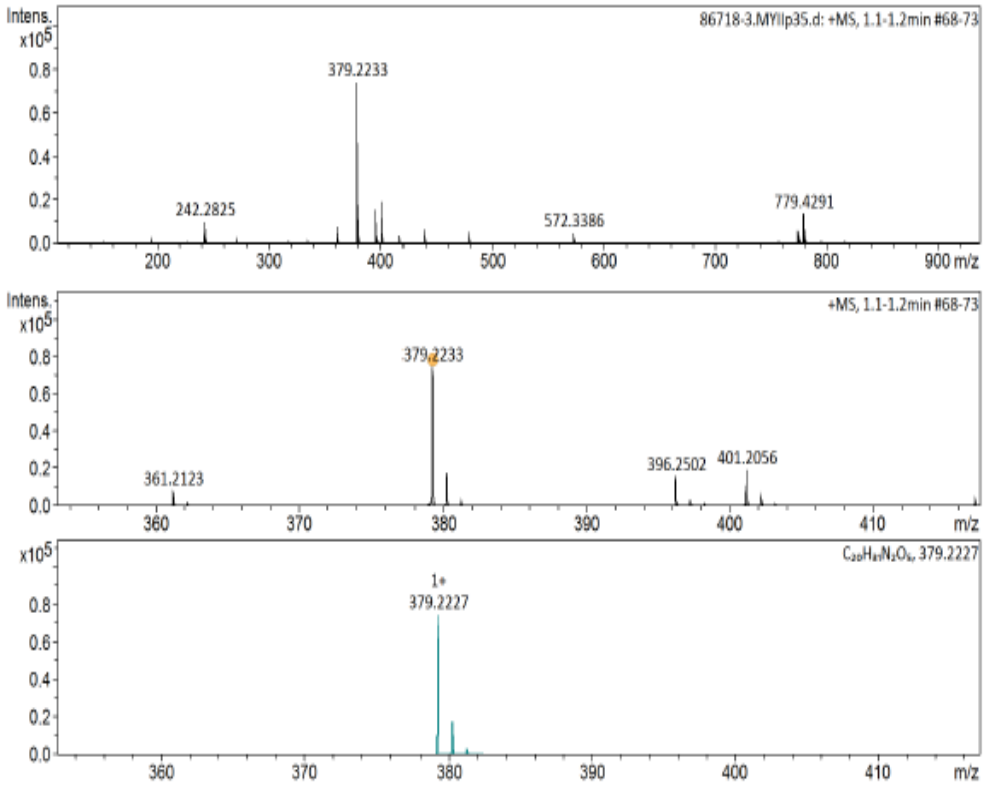
Mass Spectrum SmartFormula Report

Analysis Info

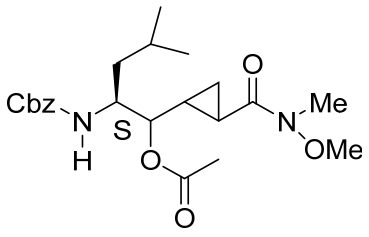
Analysis Name	D:\Data\0206\86718-3.MYIIp35.d	Acquisition Date	2/6/2018 2:37:08 PM
Method	0118_tune_low_pos_NS.m	Operator	BDAL@DE
Sample Name	3.MYIIp35	Instrument / Ser#	micrOTOF II 8213750.10314
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1650 m/z	n/a	n/a	Set Divert Valve	Source

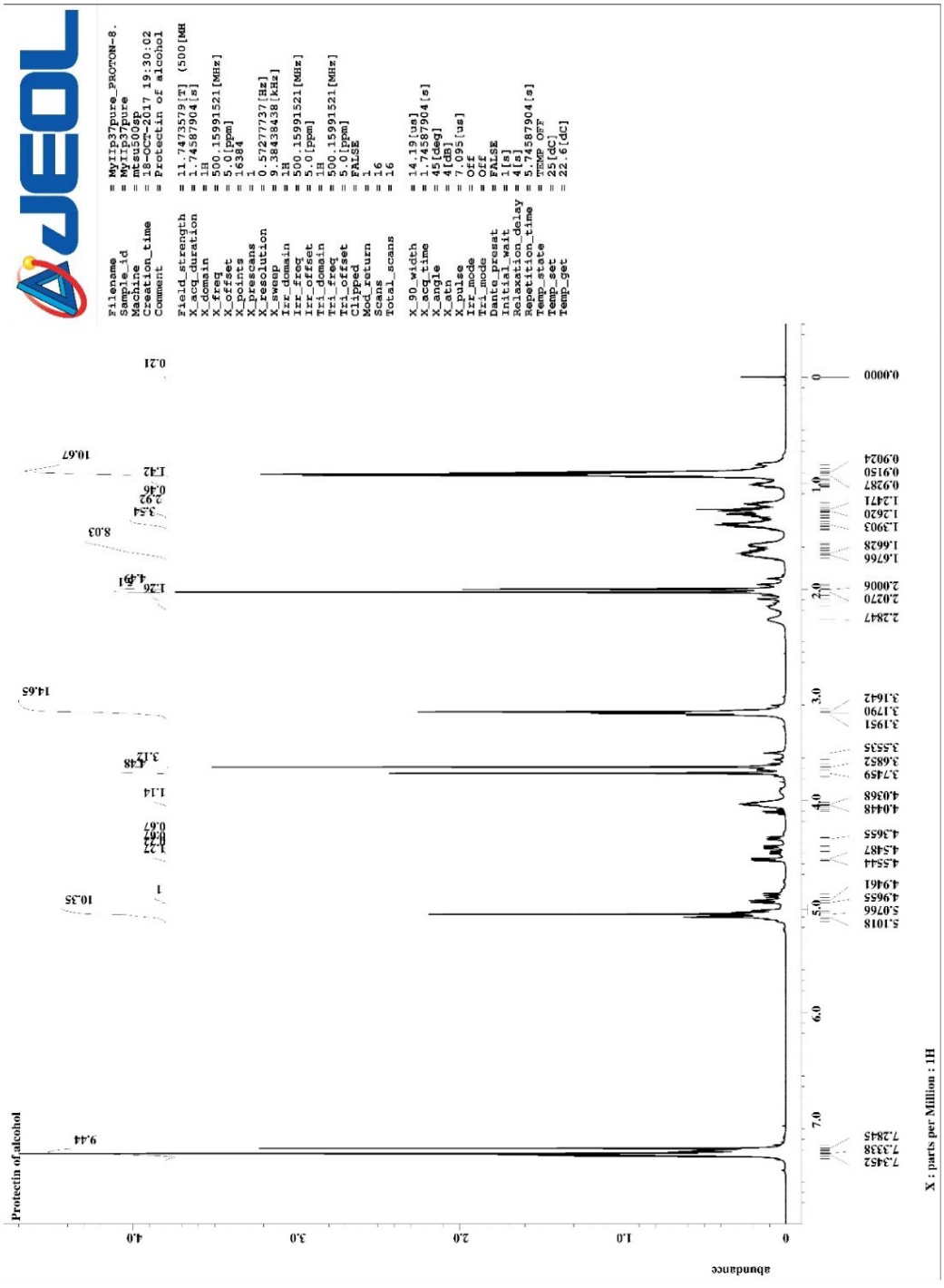


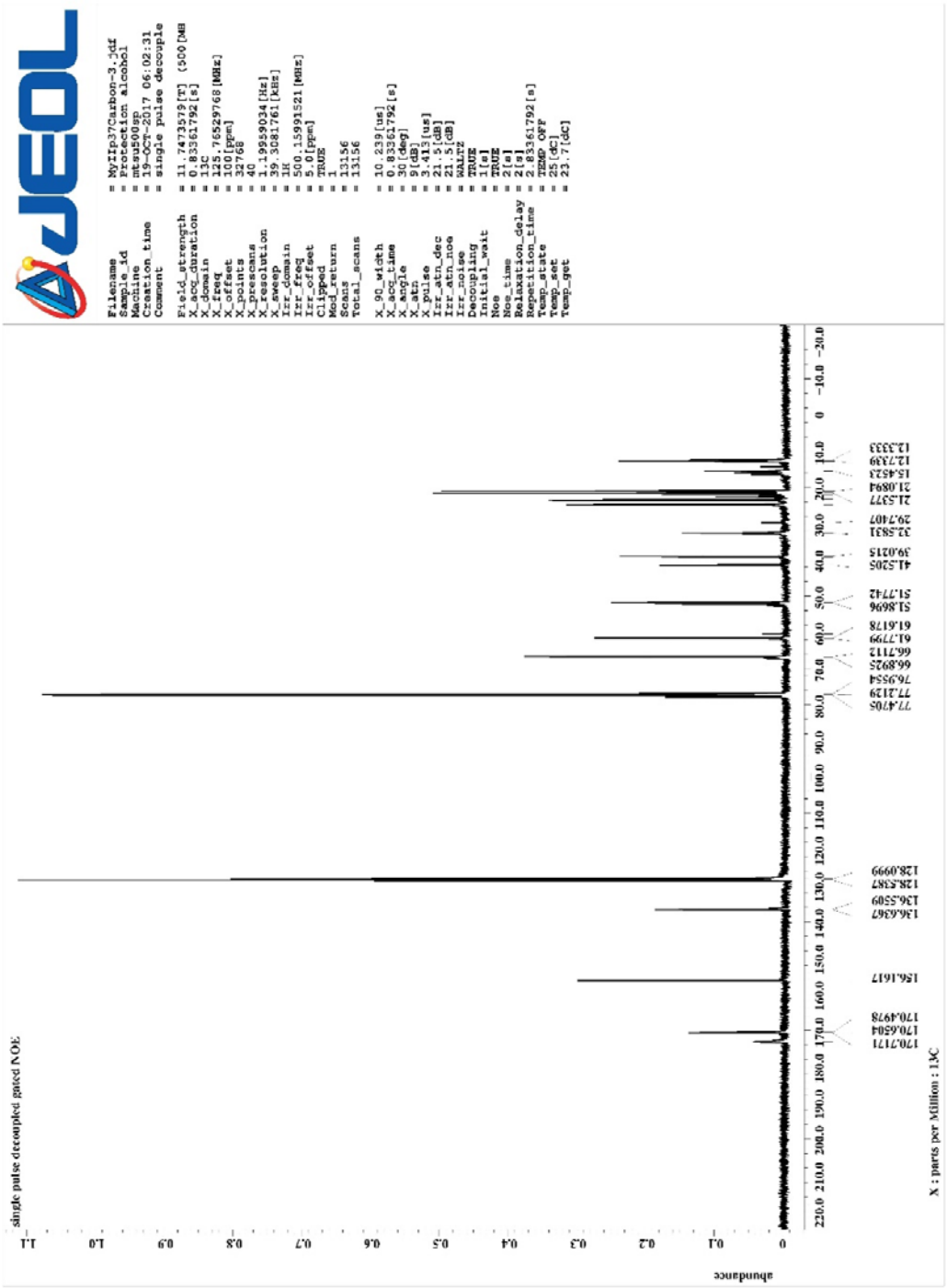
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf
379.223273	1	C20H31N2O5	379.222749	-1.4	-0.7	6.5	ok	even

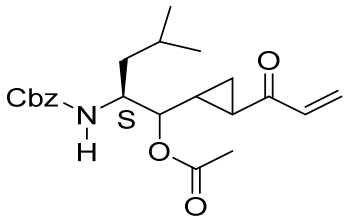


^{1}H-NMR (500 MHz, $CDCl_3$)

^{13}C-NMR (125 MHz, $CDCl_3$)

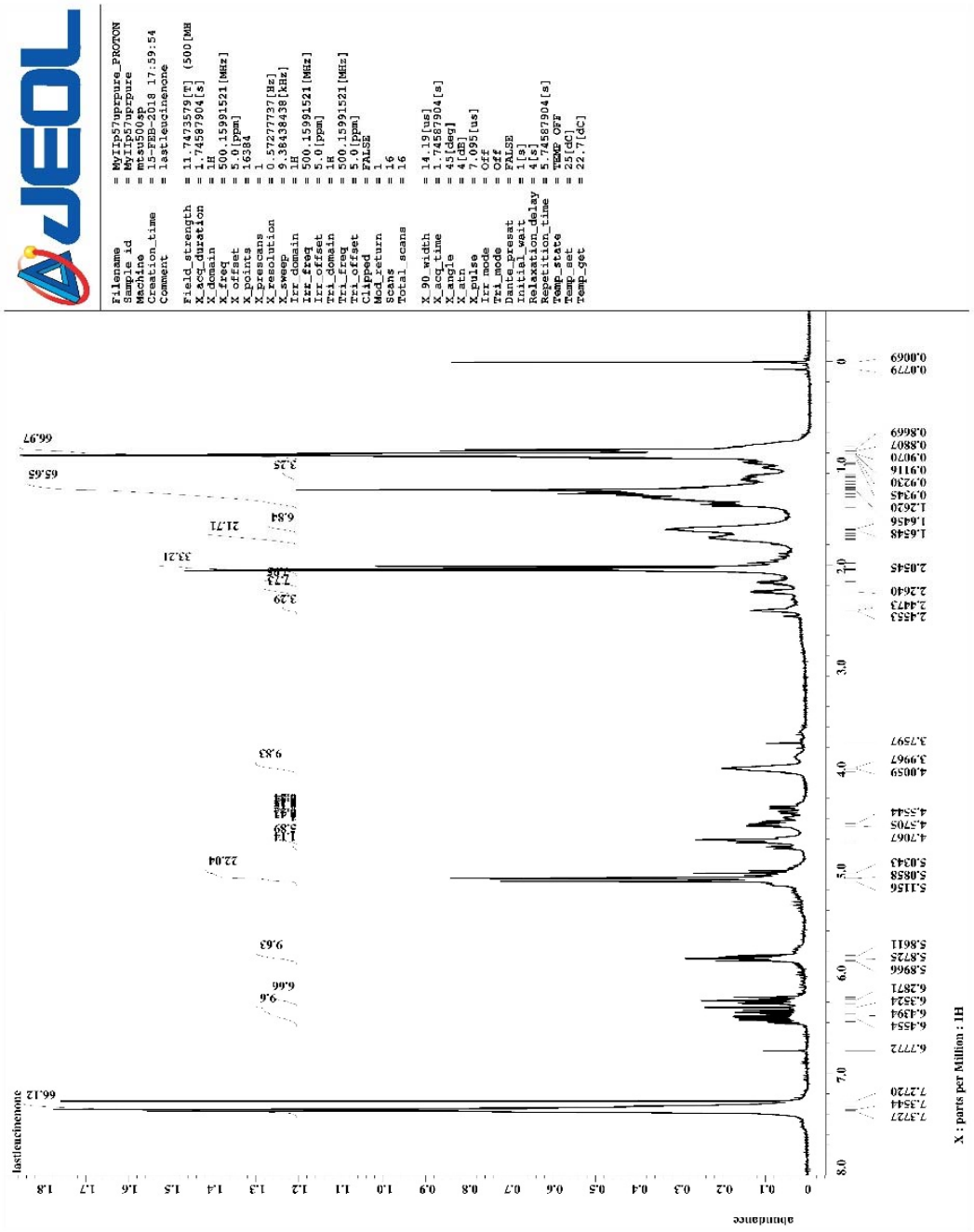

JEOL
Filename = MyIIp37pure_PROTON-8.
Sample_id = MyIIp37pure
Machine = mEsu500sp
Creation_time = 18-OCT-2017 19:30:02
Comment = Protectin of alcohol
Field_strength = 11.74735579[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 16
Total_scans = 16
X_90_width = 14.19[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_ath = 4[dB]
X_pulse = 7.095[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_set = 25[dc]
Temp_get = 22.6[dc]
Protectin of alcohol
abundance
X : parts per Million : 1H

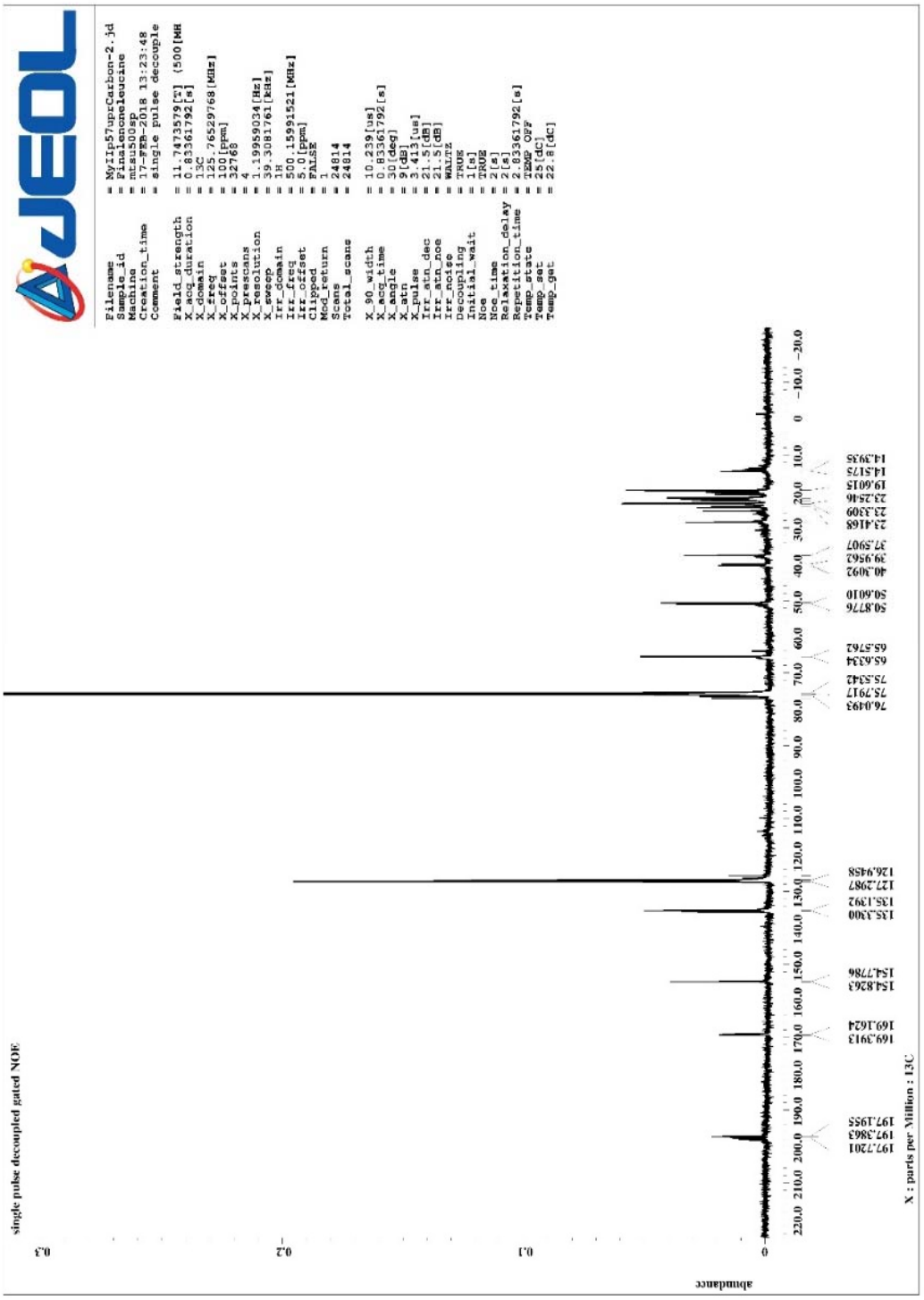

JEOL
Filename = MyIp37Carbon-3.jdf
Sample_id = Protection alcohol
Machine = mtsu500gp
Creation_time = 19-Oct-2017 06:02:31
Comment = single pulse decouple
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 40
X_resolution = 1.19959034[Hz]
X_sweep = 39.3081761[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = TRUE
Mod_return = 1
Scans = 13156
Total_scans = 13156
X_90_width = 10.239[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 9[dB]
X_pulse = 4.43[us]
Irr_atn_dec = 21.5[dB]
Irr_atn_noe = 21.5[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Nos = TRUE
Nos_time = 2[s]
Relaxation_delay = 2[s]
Repetition_time = 2.83361792[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 23.7[dC]
single pulse decoupled gated NOE
abundance
X : parts per Million : 13C
170.7171
170.6504
170.4978
156.1617
136.6367
136.5509
128.5387
128.0999
77.4705
77.2129
76.9554
66.8925
66.7112
61.7799
61.6178
51.8696
51.7742
41.5205
39.0215
32.5831
29.7407
21.5377
21.0894
15.4523
12.7339
12.3333

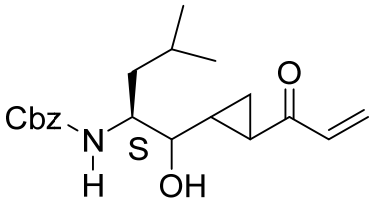


^{1}H-NMR (500 MHz, $CDCl_3$)

^{13}C-NMR (125 MHz, $CDCl_3$)


JEOL
Filename = MyTip57uprpure_PROTON
Sample_id = MyTip57uprpure
Machine = mtsu500sp
Creation_time = 15-FEB-2018 17:59:54
Comment = lastleucinenone
Field_strength = 11.74735579[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
CT_used = 1[s]
Mod_return = PULSE
Scans = 16
Total_scans = 16
X_90_width = 14.19[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 7.095[us]
Irr_mode = Off
Tri_mode = Off
Decpl_preset = FALSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 22.7[dC]
lastleucinenone
abundance
X : parts per Million : 1H

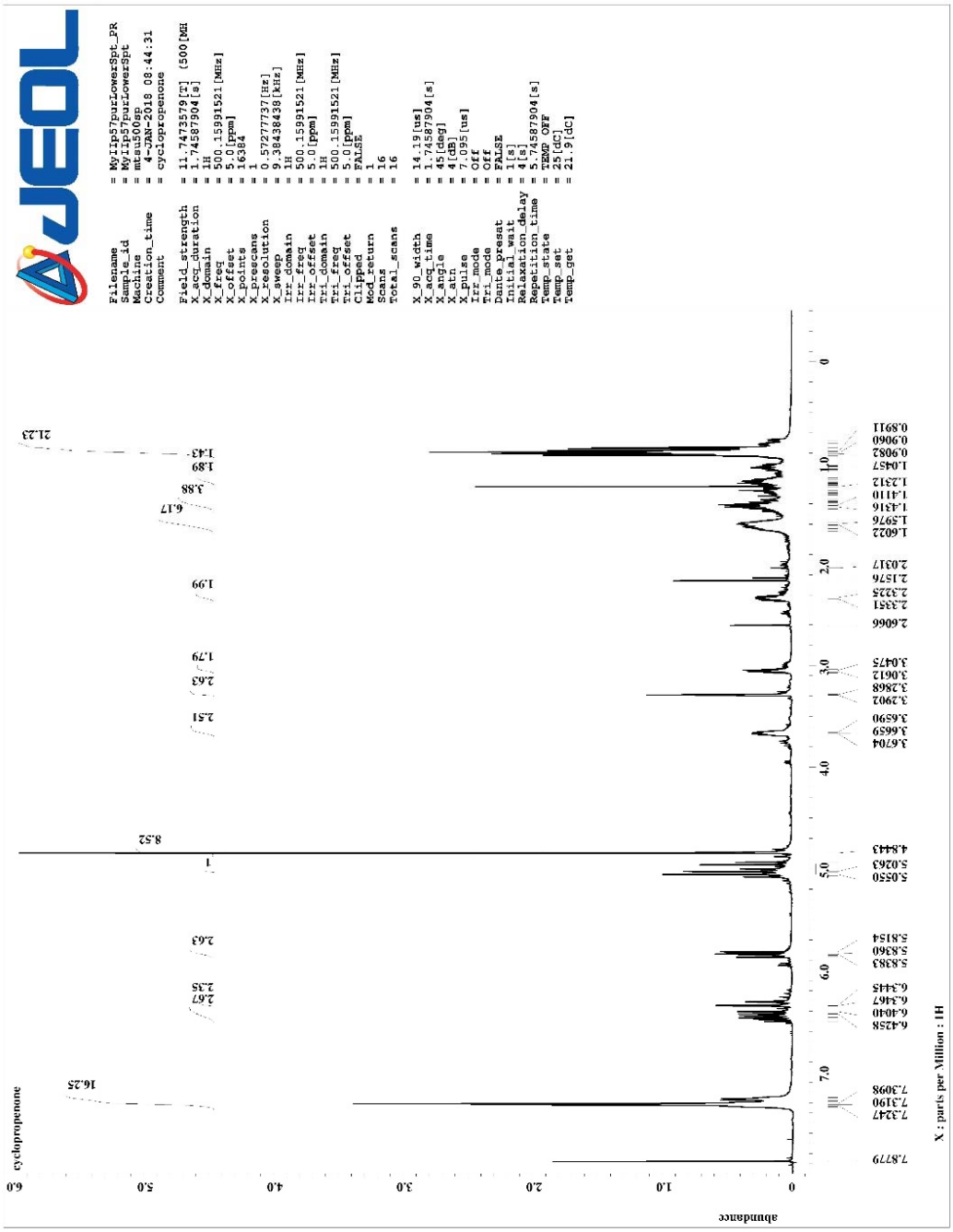

JEOL
Filename = MyIip57uprCarbon-2.jd
Sample_id = Finalenonenaleucine
Machine = mtsu500sp
Creation_time = 17-FEB-2018 13:23:48
Comment = single pulse decouple
Field_strength = 11.7473579[T] (500[MH
X_domain = 0.83361792[s]
X_domain = 13C
X_freq = 125.765299768[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.3081761[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 24814
Total_scans = 24814
X_90_width = 10.239[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 9[dB]
X_pulse = 4.5[us]
Irr_atn_dec = 21.5[dB]
Irr_atn_noe = 21.5[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 2[s]
Relaxation_delay = 2[s]
Repetition_time = 2.83361792[s]
Temp_state = ZERO OFF
Temp_set = 25.0[dC]
Temp_get = 22.8[dC]
single pulse decoupled gated NOE
abundance
X : parts per Million : 13C
197.7201
197.3863
197.1955
169.3913
169.1624
154.8263
154.7786
135.1392
127.2987
126.9458
76.0493
75.7917
75.5342
65.6334
65.5762
50.8776
50.6010
40.3092
39.9562
37.5907
23.4168
23.3309
23.2546
19.6015
14.5175
14.3935

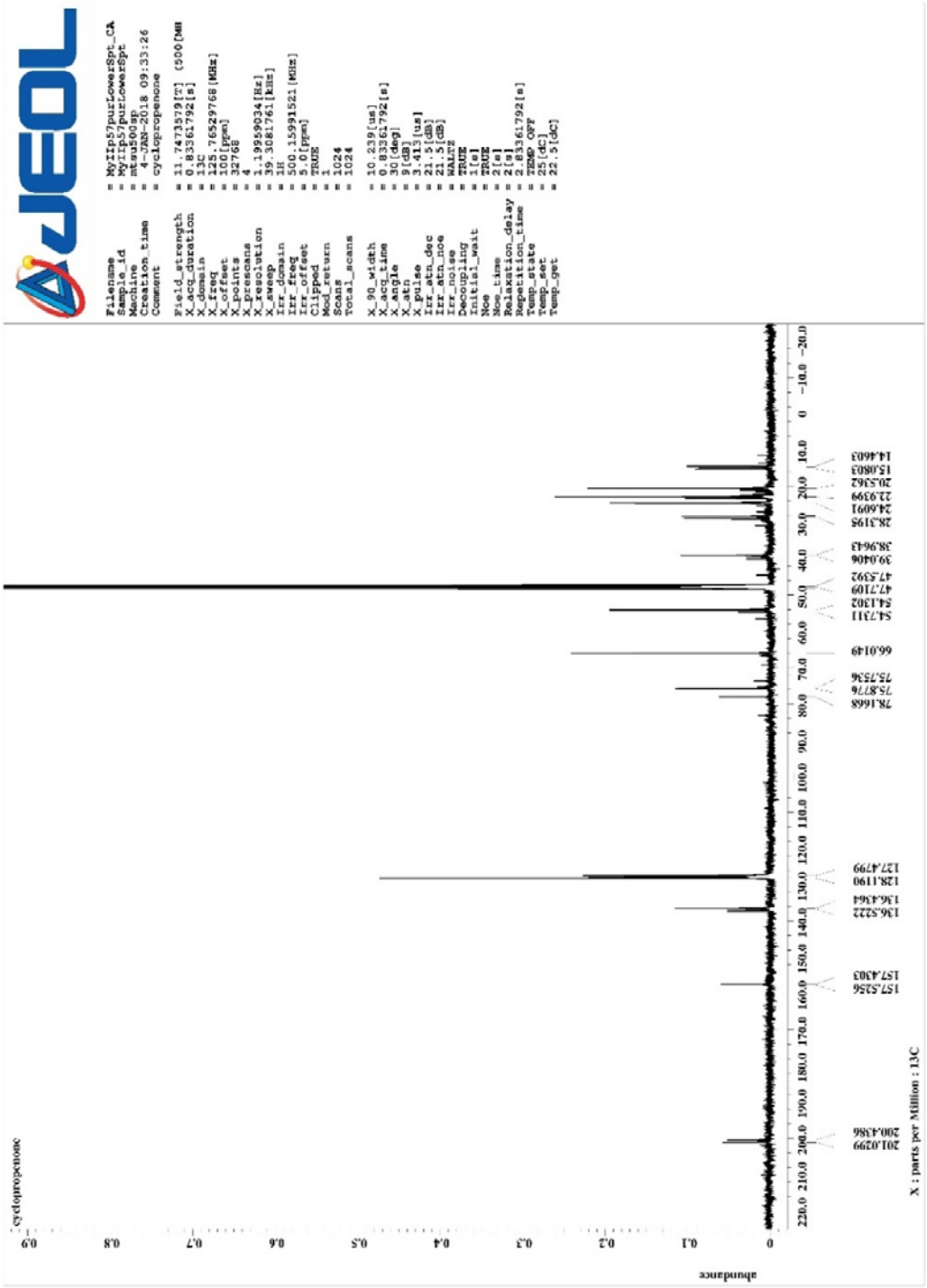


^{1}H-NMR (500 MHz, CD_3OD)

^{13}C-NMR (125 MHz, CD_3OD)

HRMS


JEOL
Filename = MyiIp57purLowerSpt_PR
Sample_id = MyiIp57purLowerSpt
Machine = mtsu500sp
Creation_time = 4-JAN-2018 08:44:31
Comment = cyclopropenone
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 16
Total_scans = 16
X_90_width = 14.19[us]
X_acq_time = 1.74587904[s]
X_angle = 45.0[deg]
X_atten = 4[dB]
X_pulse = 7.095[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 21.9[dC]
cyclopropenone
16.25
8.52
2.63
2.67
2.35
2.63
1
2.51
2.63
1.79
1.99
6.17
3.88
1.89
1.43
21.23
7.8779
7.3247
7.3190
7.3098
6.4258
6.4040
6.3467
6.3415
5.8383
5.8360
5.8154
5.0550
5.0263
4.8443
3.6704
3.6659
3.6590
3.2902
3.2868
3.0612
3.0475
2.6066
2.3351
2.3225
2.1576
2.0317
1.6022
1.5976
1.4316
1.4110
1.2312
1.0457
0.9082
0.9060
0.8911
abundance
X : parts per Million : 1H


JEOL
Filename = MyIIp57purLower8pt_CA
Sample_id = MyIIp57purLower8pt
Machine = ntsu500dp
Creation_time = 4-JAN-2018 09:33:26
Comment = cyclopropenone
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.7652976[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.3081761[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = TRUE
Mod_return = 1
Scans = 1024
Total_scans = 1024
X_90_width = 10.239[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 3[dB]
X_pulse = 3.413[us]
Irr_atn_dec = 21.5[dB]
Irr_atn_noe = 21.5[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 2[s]
Relaxation_delay = 2.0[s]
Repetition_time = 2.83361792[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 23.9[dC]
cyclopropenone
abundance
X : parts per Million : 13C
201.0299
200.4386
157.5256
157.4303
136.5222
136.4364
128.1190
127.4799
78.1668
75.8776
75.7536
66.0149
54.7311
54.1302
47.7109
47.5392
39.0406
38.9643
28.3195
24.4091
22.9399
20.5362
15.0803
14.4603

Mass Spectrometry & Proteomics Facility

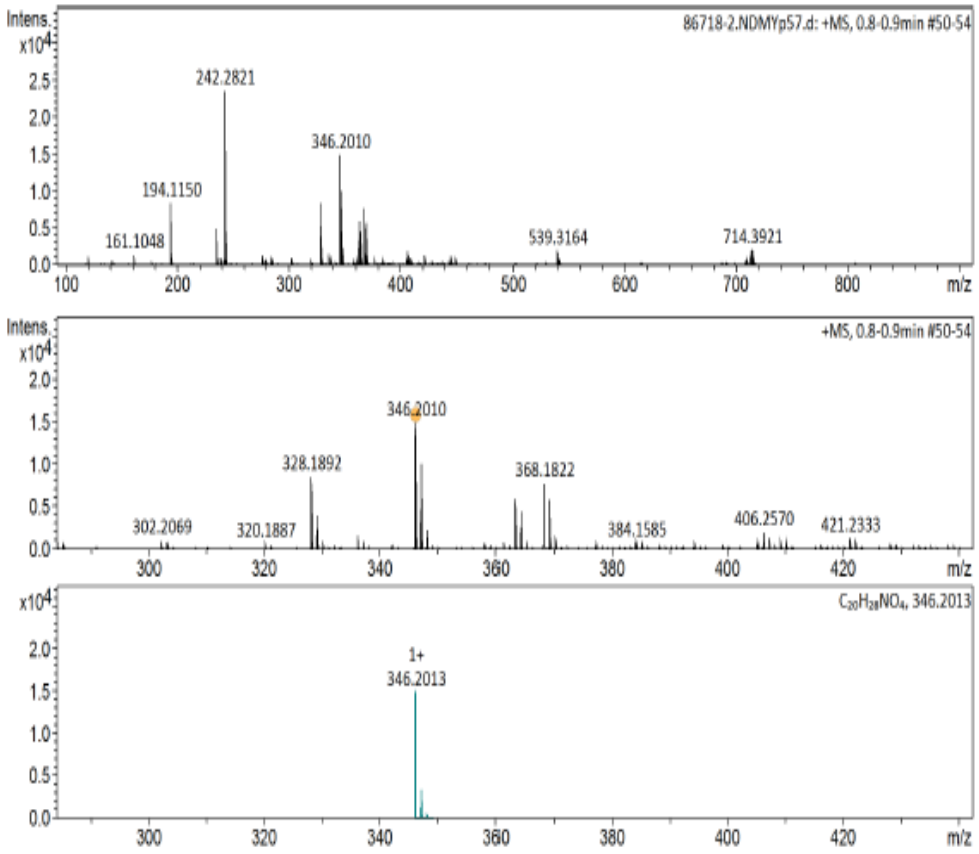
Mass Spectrum SmartFormula Report

Analysis Info

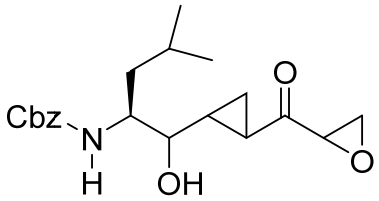
Analysis Name	D:\Data\0206\86718-2.NDMYp57.d	Acquisition Date	2/6/2018 2:31:06 PM
Method	0118_tune_low_pos_NS.m	Operator	BDAL@DE
Sample Name	2.NDMYp57	Instrument / Ser#	micrOTOF II 8213750.10314
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1650 m/z	n/a	n/a	Set Divert Valve	Source

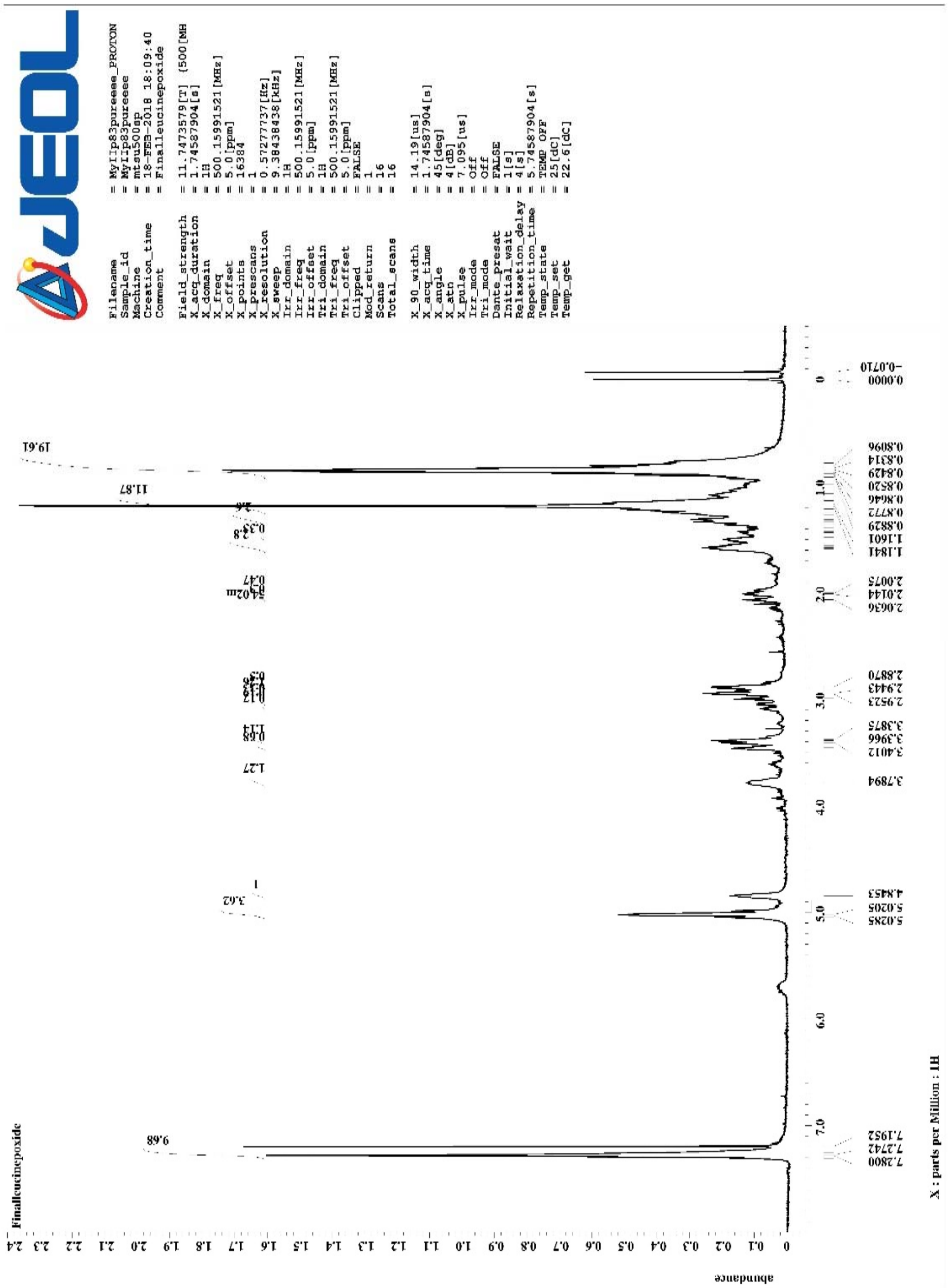


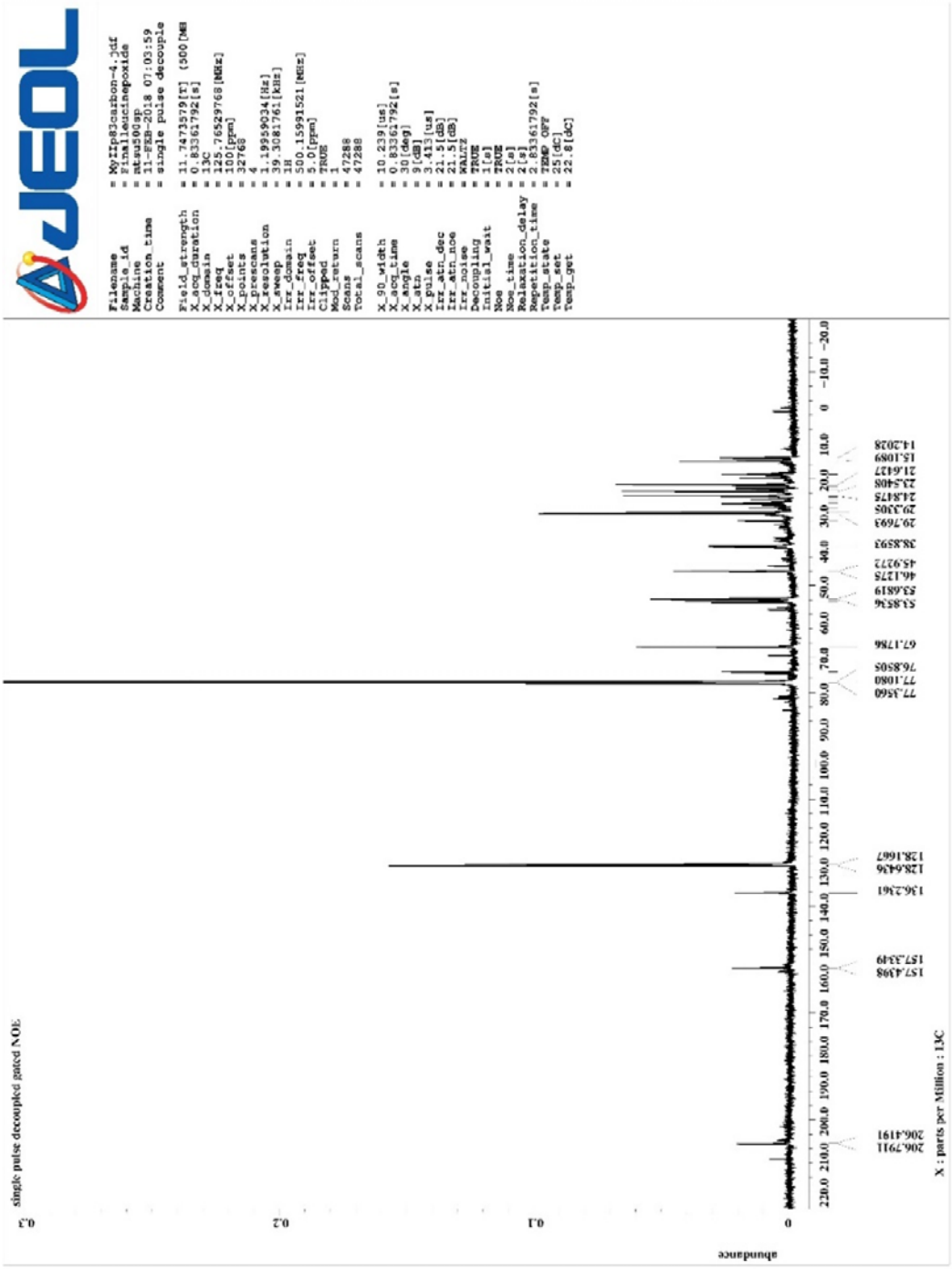
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf
346.200967	1	C20H28NO4	346.201285	0.9	-2.1	7.5	ok	even

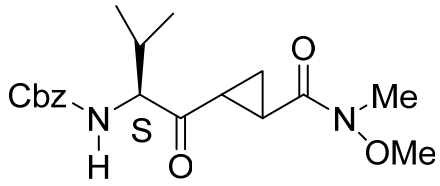


^{1}H-NMR (500 MHz, $CDCl_3$)

^{13}C-NMR (125 MHz, $CDCl_3$)


Filename = MyIp83pureeee_PROTON
Sample_id = MyIp83pureeee
Machine = mtsu500sp
Creation_time = 18-FEB-2018 18:09:40
Comment = Finalleucinepoxide
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 16
Total_scans = 16
X_90_width = 14.19[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 7.095[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 22.6[dC]
Finalleucinepoxide
X : parts per Million : 1H
abundance

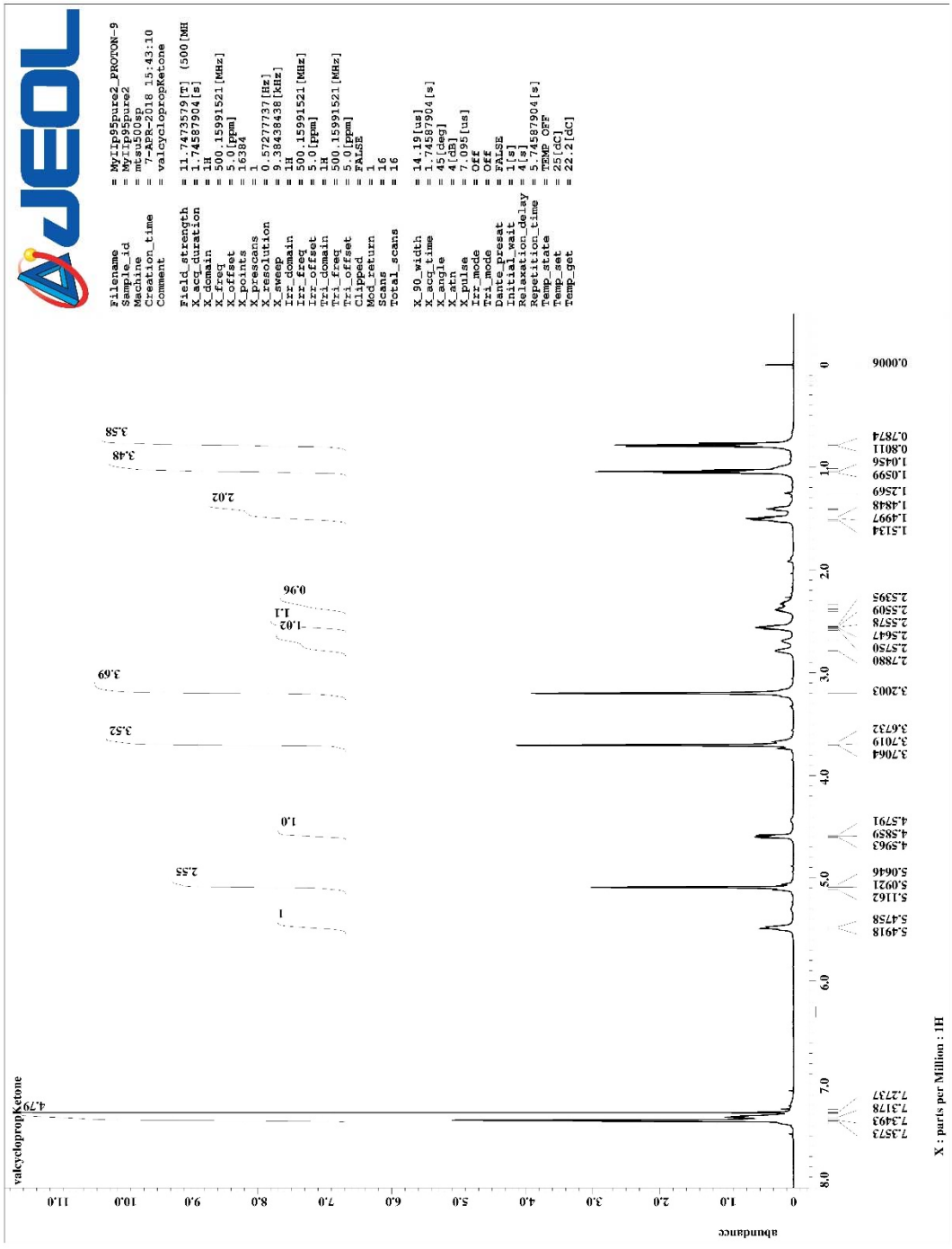

JEOL
Filename = MyIp83carbon-4.jdf
Sample_id = Finalleucinepoxide
Machine = ntsu500gp
Creation_time = 11-FEB-2018 07:03:59
Comment = single pulse decouple
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 1H
X_freq = 125.76529768[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.3081761[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = TRUE
Mod_return = 1
Scans = 47288
total_scans = 47288
X_90_width = 10.239[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 9[dB]
X_pulse = 3.413[us]
Irr_atn_dec = 21.5[dB]
Irr_atn_noe = 21.5[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 2[s]
Noe = TRUE
Noe_time = 2[s]
Relaxation_delay = 2[s]
Repetition_time = 2.83361792[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 22.8[dC]
single pulse decoupled gated NOE
abundance
0.3
0.2
0.1
0
220.0 210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0 -10.0 -20.0
206.7911
206.4191
157.4398
157.3349
136.2361
128.6436
128.1667
77.3560
77.1080
76.8505
67.1786
53.8534
53.6819
46.1275
45.9272
38.8593
29.7693
29.3305
24.8475
23.5408
21.6127
15.1089
14.2028
X : parts per Million : 13C

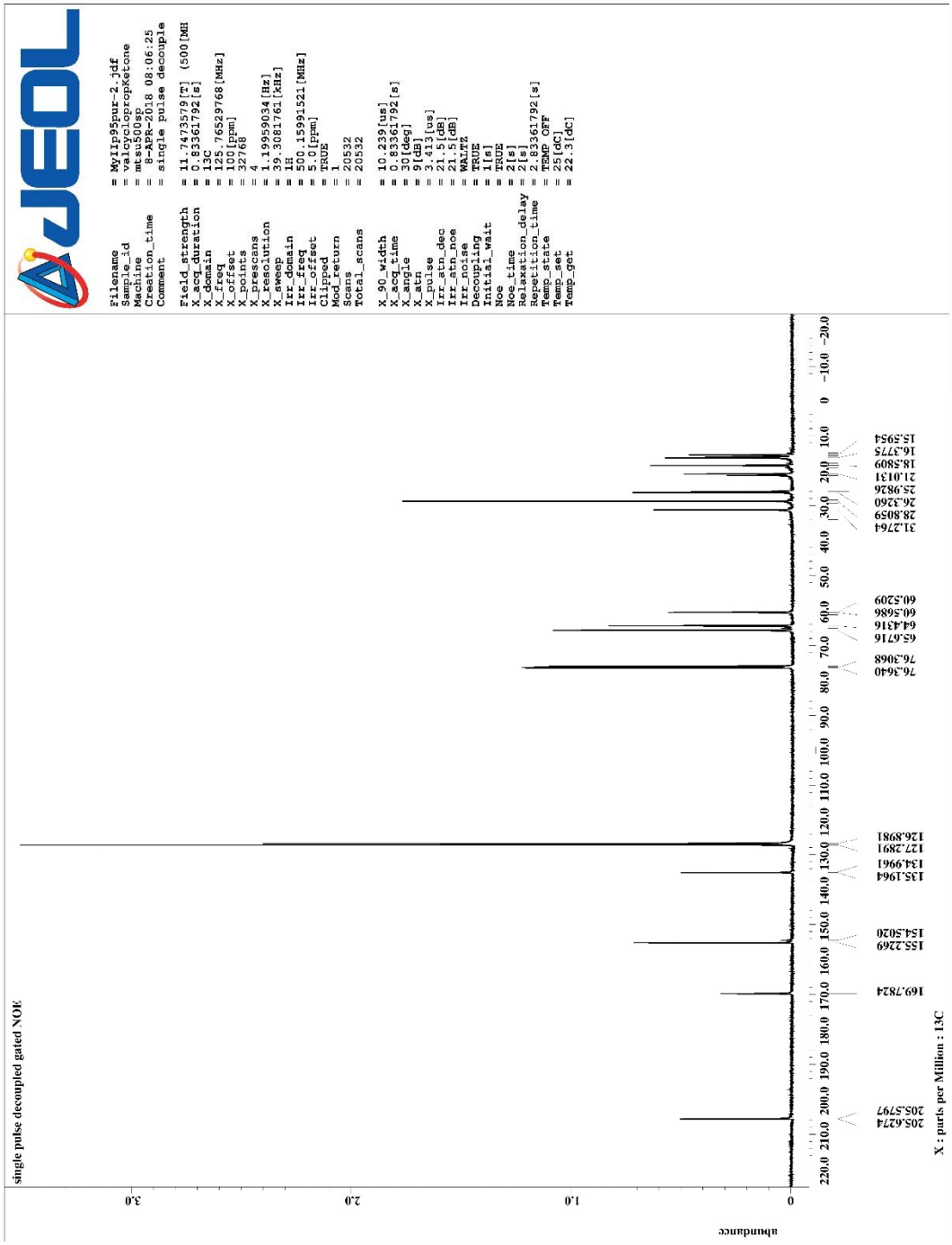


^{1}H-NMR (500 MHz, $CDCl_3$)

^{13}C-NMR (125 MHz, $CDCl_3$)

HRMS


JEOL
Filename = MyTip95pure2_PROTON-9
Sample_id = MyTip95pure2
Machine = mtsu500sp
Creation_time = 7-APR-2018 15:43:10
Comment = valcyclopropKetone
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 16
Total_scans = 16
X_90_width = 14.10[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 7.095[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 22.2[dC]
valcyclopropKetone
4.79
7.2737
7.3178
7.3493
7.3573
5.4758
5.4918
5.0646
5.0921
5.1162
4.5791
4.5859
4.5963
3.7019
3.7064
3.6732
3.5200
3.4800
3.5800
2.5395
2.5509
2.5578
2.5647
2.5750
2.7880
1.5134
1.4997
1.4848
1.2569
1.0599
1.0456
0.8011
0.7874
0.0006
2.55
1.0
1.0
1.1
1.02
0.96
2.02
3.52
3.69
3.48
3.58
abundance
X : parts per Million : 1H


JEOL
Filename = MyIIp95pur-2.jdf
Sample_id = valdpclpropKetone
Machine = mtsu500sp
Creation_time = 8-APR-2018 08:06:25
Comment = single pulse decouple
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.765297 68[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.3081761[kHz]
Irr_domain = 1H
Irr_freq = 500.1599152 1[MHz]
Irr_offset = 5.0[ppm]
Clipped = TRUE
Mod_return = 1
Scans = 20532
Total_scans = 20532
X_90_width = 10.239[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 9[dB]
X_pulse = 3.43[us]
Irr_atn_dec = 21.5[dB]
Irr_atn_noe = 21.5[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 2[s]
Relaxation_delay = 2[s]
Repetition_time = 2.83361792[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 22.3[dC]
single pulse decoupled gated NOE
abundance
X : parts per Million : 13C
205.6274
205.5797
169.7824
155.2269
154.5020
135.1964
134.9961
127.2891
126.8981
76.3640
76.3068
65.6716
64.4316
60.5686
60.5209
31.2764
28.8059
26.3260
25.9826
21.0131
18.5809
16.3775
15.5954

Mass Spectrometry & Proteomics Facility

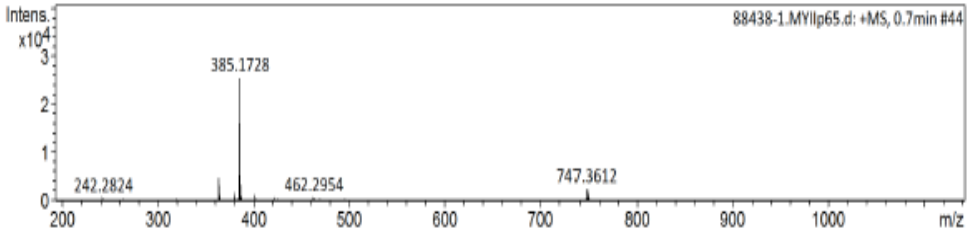
Mass Spectrum SmartFormula Report

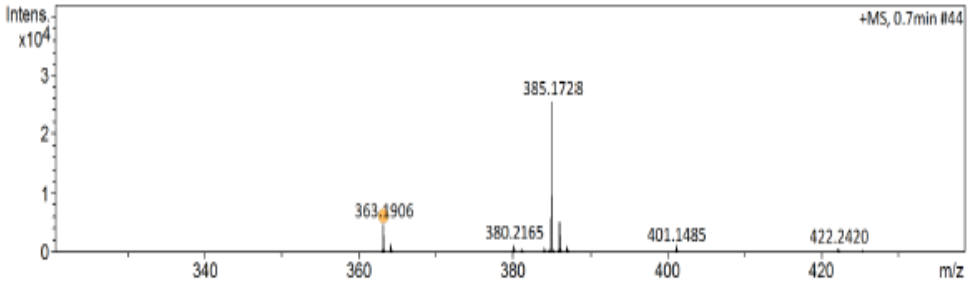
Analysis Info

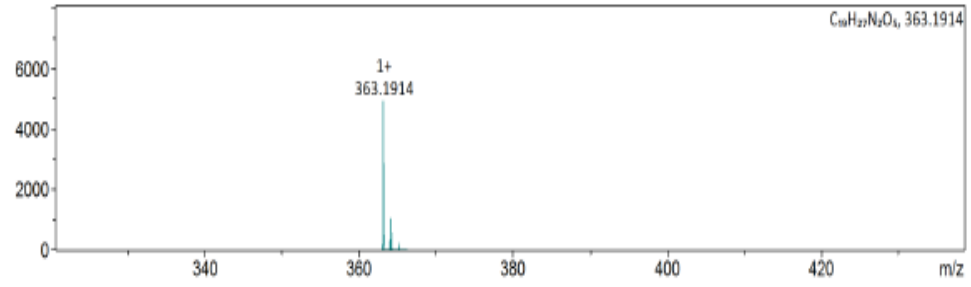
Analysis Name	D:\Data\0302\88438-1.MYIIp65.d	Acquisition Date	3/2/2018 3:11:50 PM
Method	0118_tune_low_pos_NS.m	Operator	BDAL@DE
Sample Name	1.MYIIp65	Instrument / Ser#	micrOTOF II 8213750.10314
Comment			

Acquisition Parameter

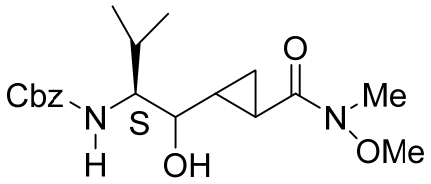
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1650 m/z	n/a	n/a	Set Divert Valve	Source





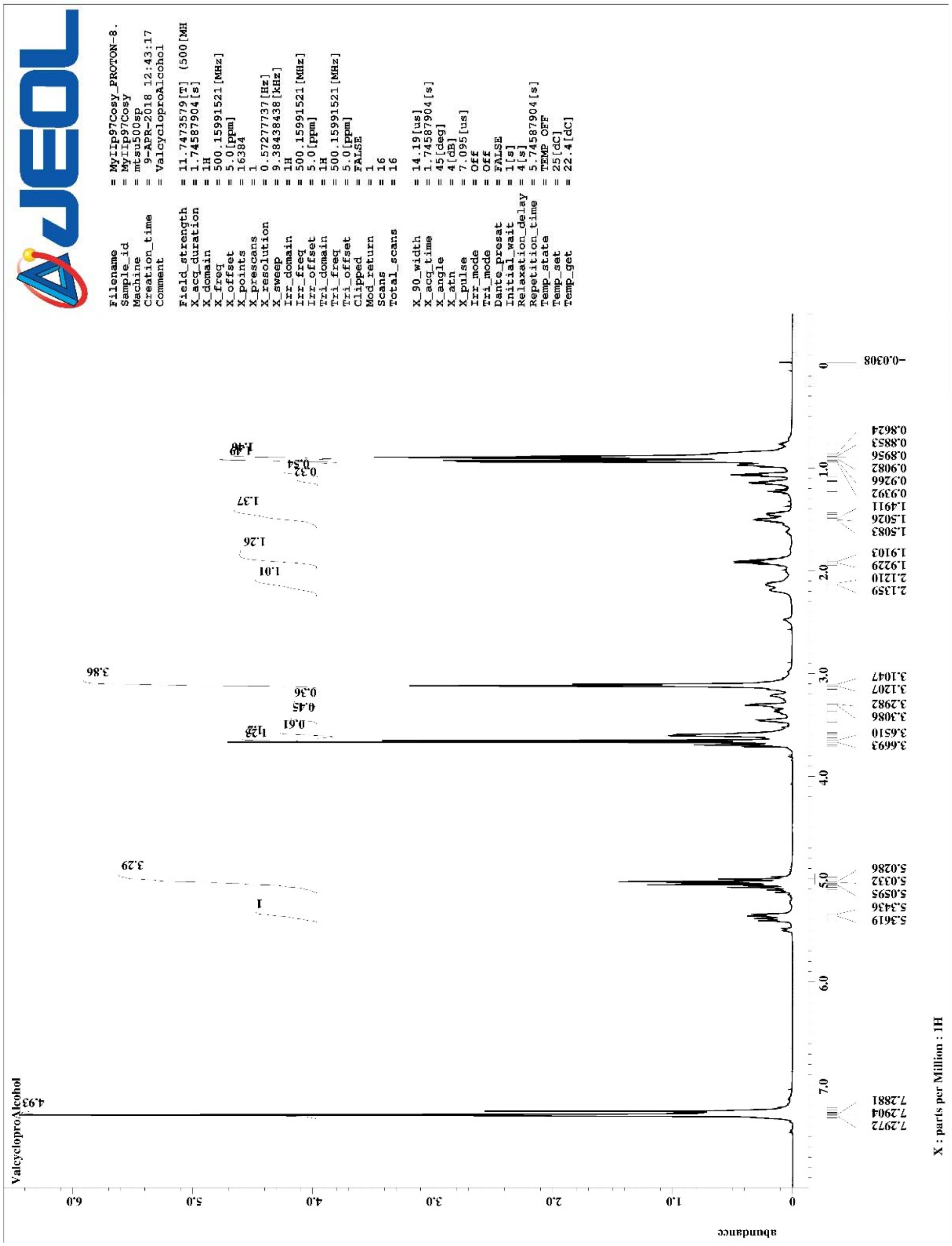


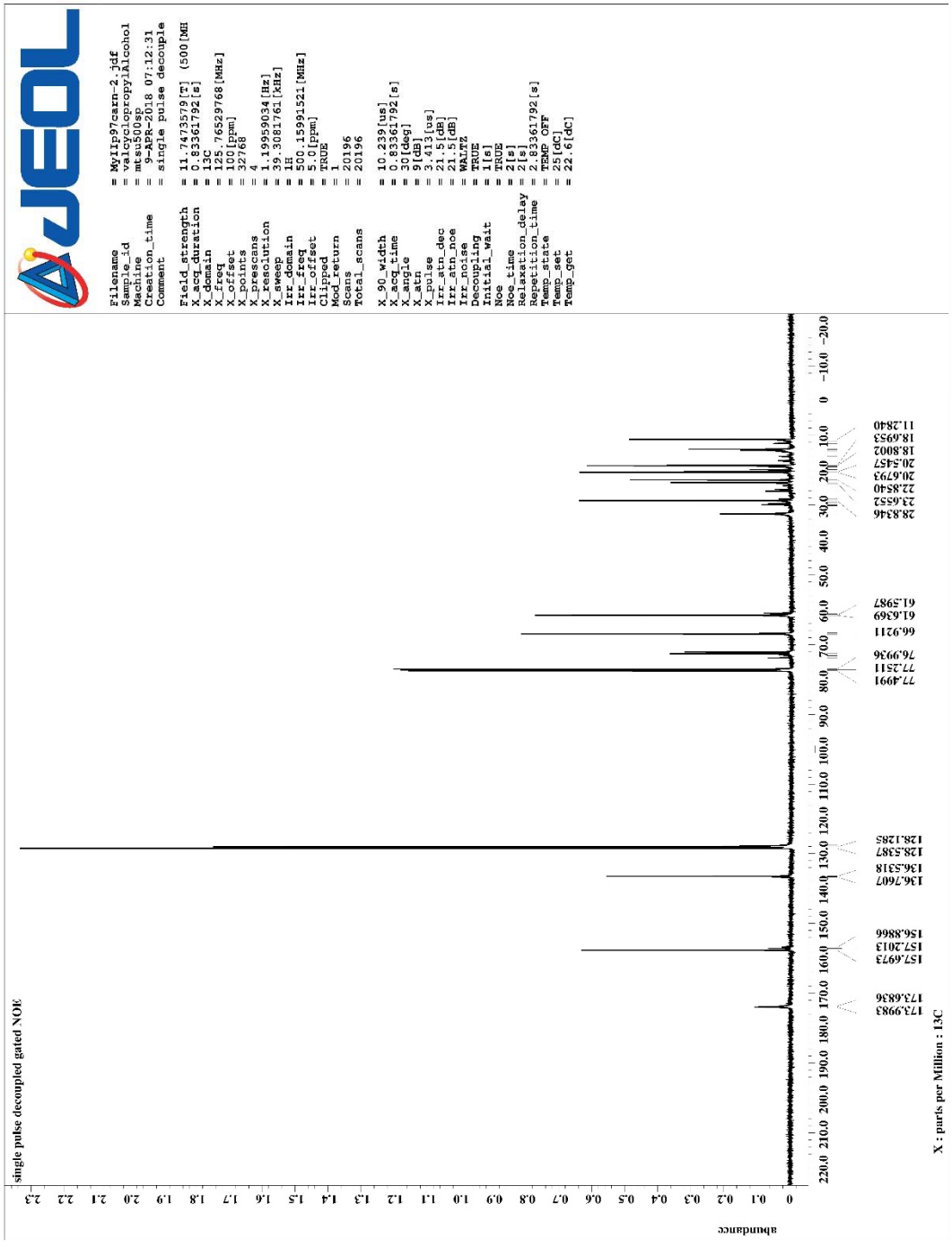
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf
363.190646	1	C19H27N2O5	363.191448	2.2	-1.3	7.5	ok	even

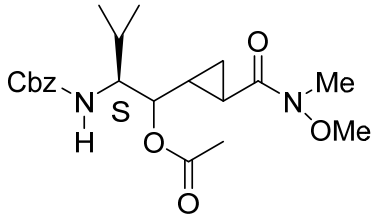


^{1}H-NMR (500 MHz, $CDCl_3$)

^{13}C-NMR (125 MHz, $CDCl_3$)

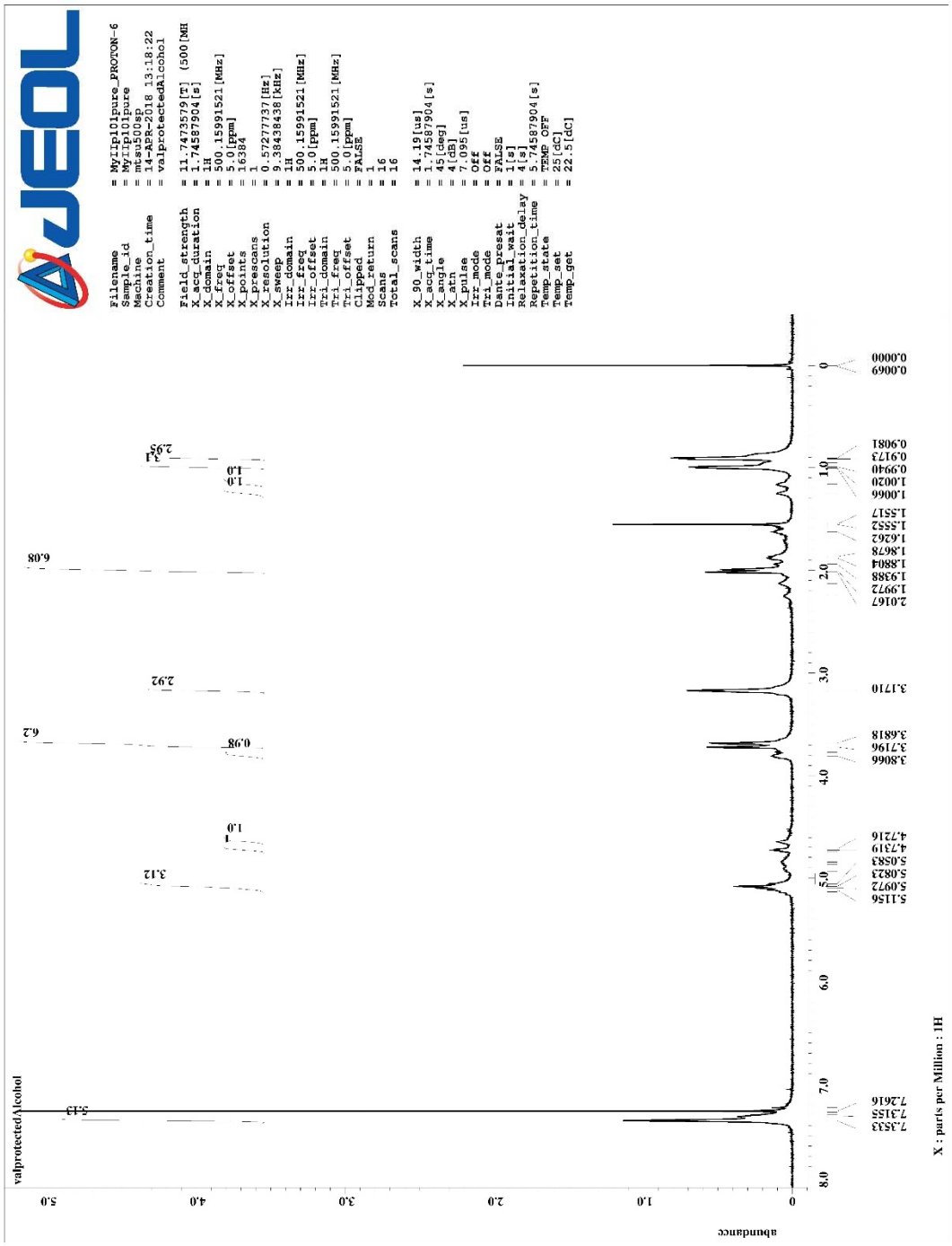

Filename = MyIIp97Cosy_PROTON-8.
Sample_id = MyIIp97Cosy
Machine = mtsu500sp
Creation_time = 9-APR-2018 12:43:17
Comment = ValcycloproAlcohol
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 16
Total_scans = 16
X_90_width = 14.19[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 7.095[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 22.4[dC]
ValcycloproAlcohol
X : parts per Million : 1H
abundance

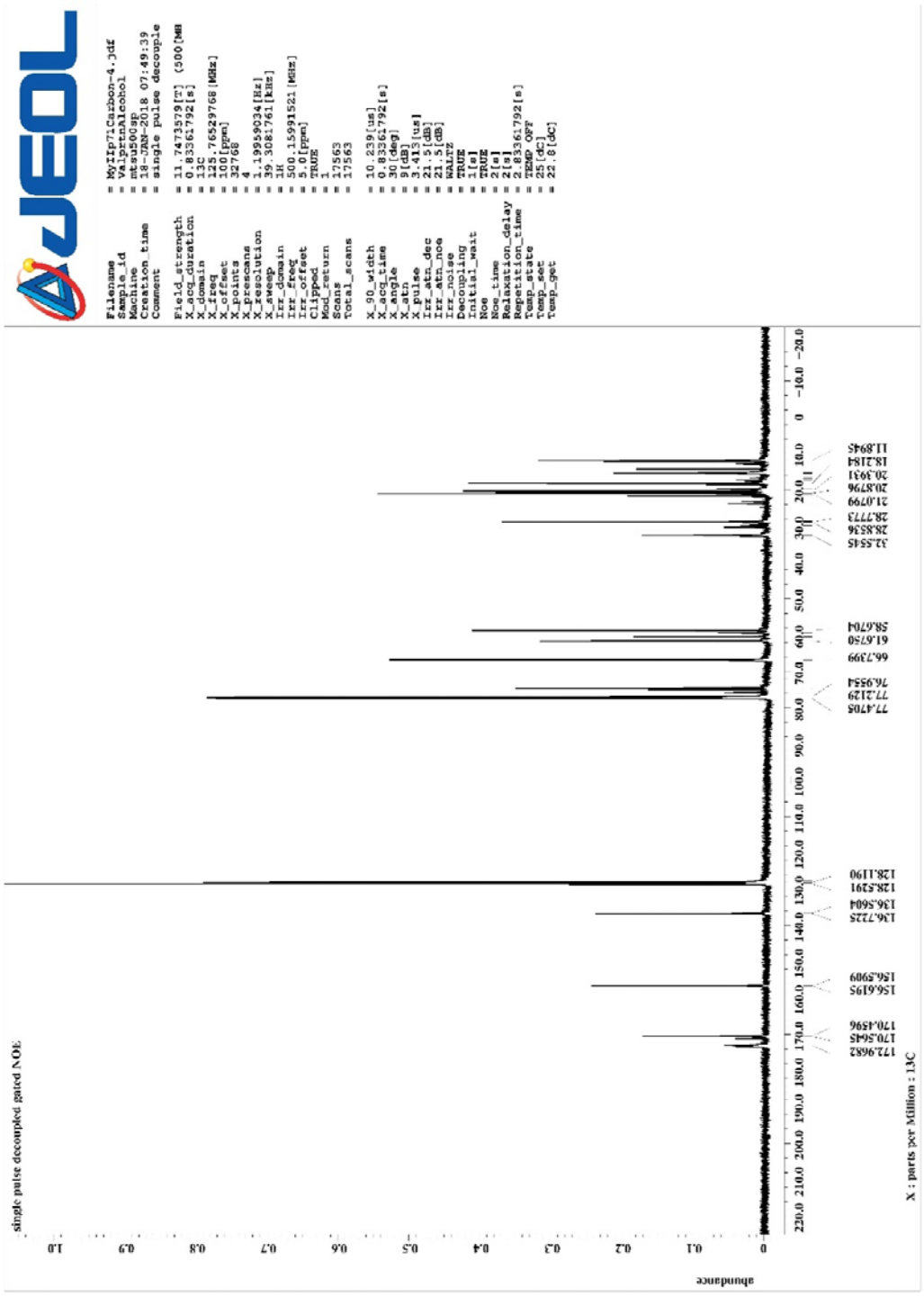

JEOL
Filename = MyIIp97carn-2.jdf
Sample_id = valpyclopropylAlcohol
Machine = mtsu500sp
Creation_time = 9-APR-2018 07:12:31
Comment = single pulse decouple
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.765297 68[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.3081761[kHz]
Irr_domain = 1H
Irr_freq = 500.1599152 1[MHz]
Irr_offset = 5.0[ppm]
Clipped = TRUE
Mod_return = 1
Scans = 20196
Total_scans = 20196
X_90_width = 10.239[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 9[dB]
X_pulse = 3.413[us]
Irr_atn_dec = 21.5[dB]
Irr_atn_noe = 21.5[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 2[s]
Relaxation_delay = 2[s]
Repetition_time = 2.83361792[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 22.6[dC]
single pulse decoupled gated NOE
abundance
X : parts per Million : 13C
173.9983
173.6836
157.6973
157.2013
156.8866
136.7607
136.5318
128.5387
128.1285
77.4991
77.2511
76.9936
66.9211
61.6369
61.5987
28.8346
23.6552
22.8540
20.6793
20.5457
18.8002
18.6953
11.2840

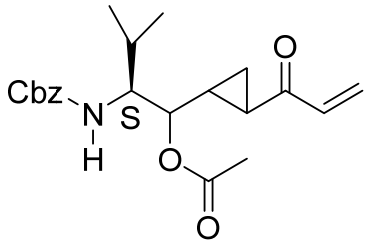


^{1}H-NMR (500 MHz, $CDCl_3$)

^{13}C-NMR (125 MHz, $CDCl_3$)

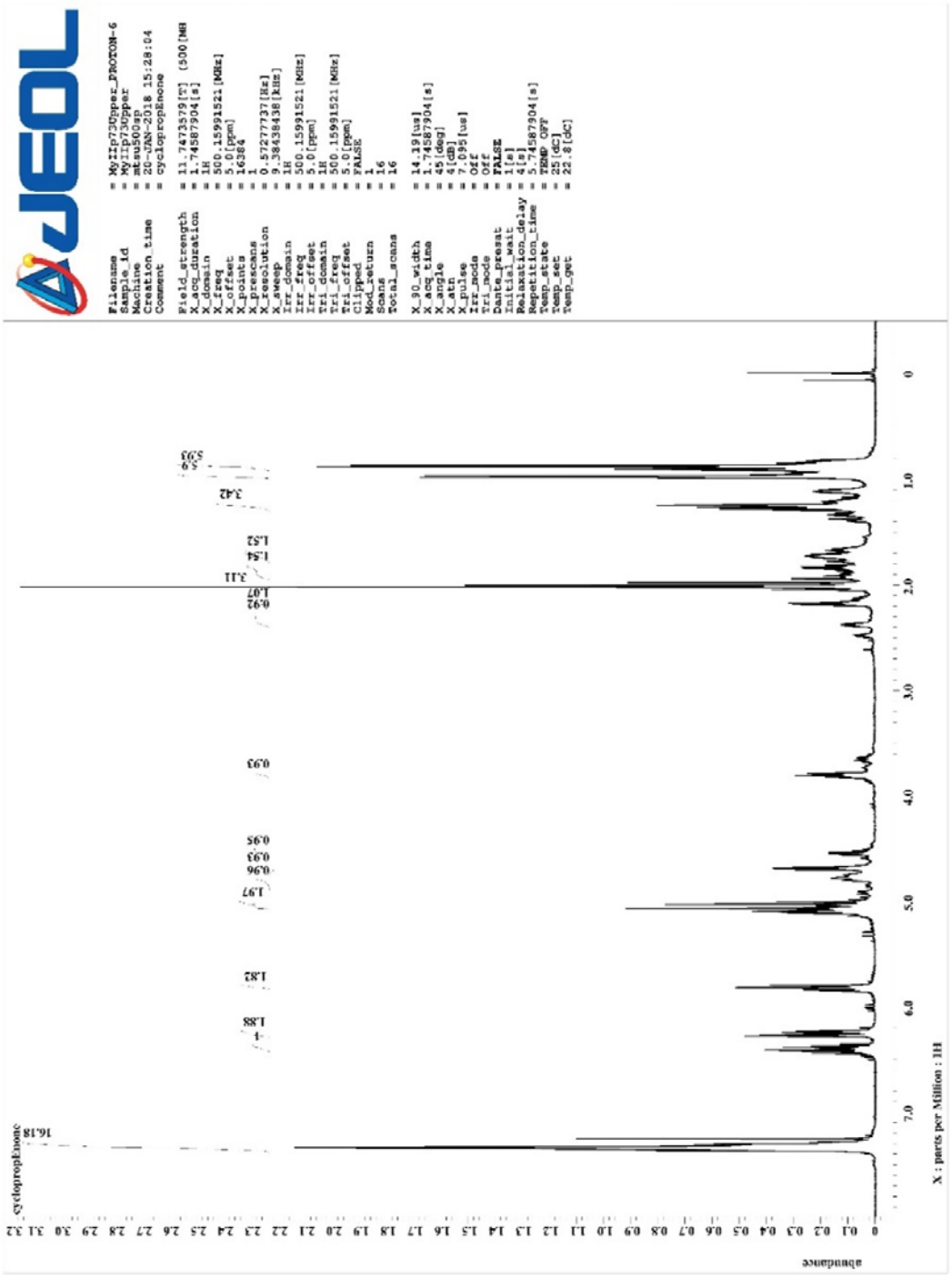

JEOL
Filename = MyIIp101pure_PROTON-6
Sample_id = MyIIp101pure
Machine = mtsu500sp
Creation_time = 14-APR-2018 13:18:22
Comment = valprotectedAlcohol
Field_strength = 11.74735579[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438436[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 16
Total_scans = 16
X_90_width = 14.19[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 7.095[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 22.5[dC]
valprotectedAlcohol
abundance
X : parts per Million : 1H
5.13
6.08
2.92
6.2
0.98
3.12
1.0
2.95
3.1
1.0
1.0
7.3533
7.3155
7.2616
5.1156
5.0972
5.0823
5.0583
4.7319
4.7216
3.8066
3.7196
3.6818
3.1710
2.0167
1.9972
1.9388
1.8804
1.8678
1.6262
1.5552
1.5517
1.0066
1.0020
0.9940
0.9173
0.9081
0.0069
0.0000

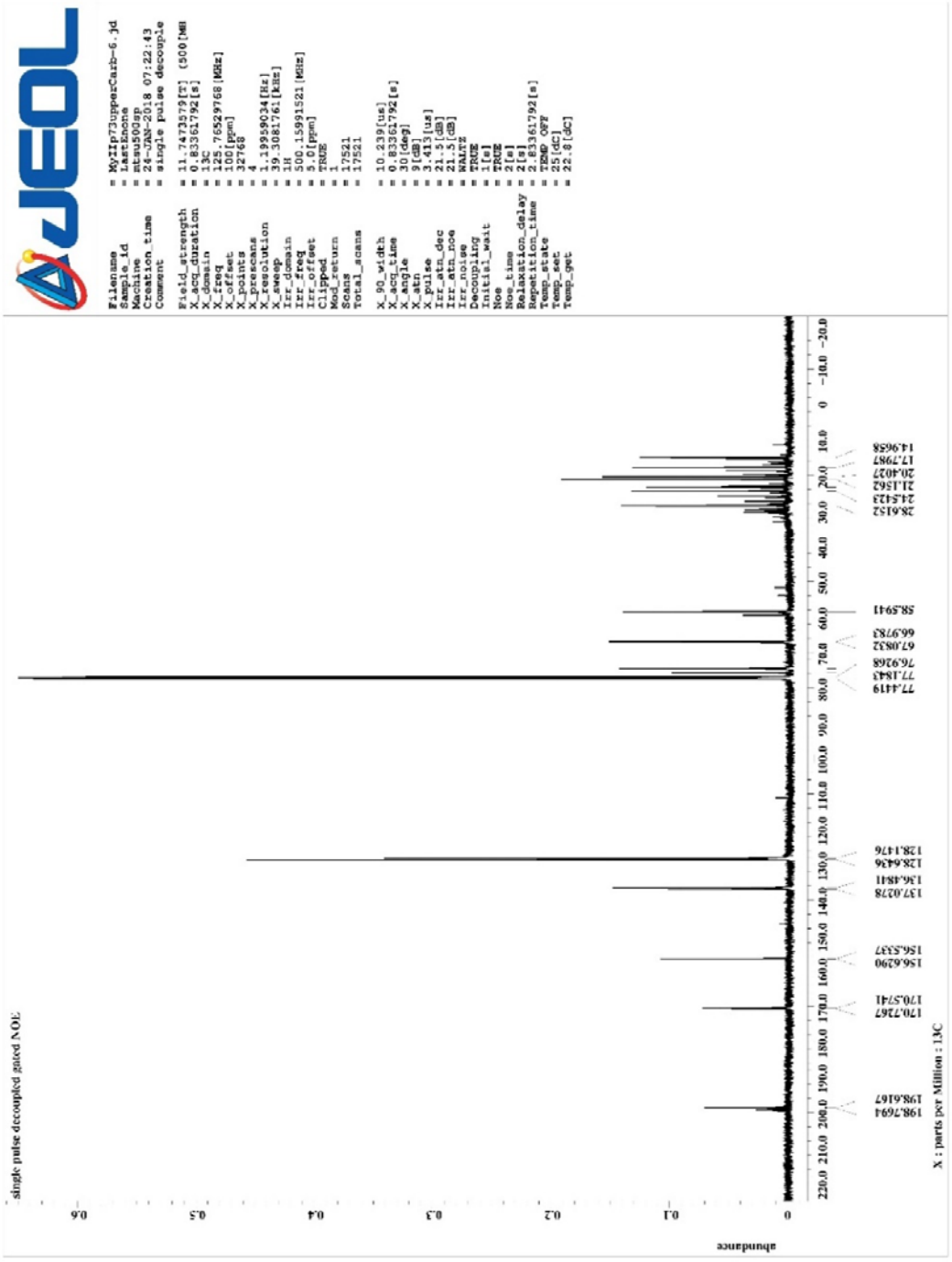

JEOL
Filename = MyIIp71Carbon-4.jdf
Sample_id = ValpertnAlcohol
Machine = mksu500sp
Creation_time = 18-JAN-2018 07:49:39
Comment = single pulse decouple
Field_strength = 11.74735879[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.30817661[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = TRUE
Mod_return = 1
Scans = 17563
Total_scans = 17563
X_90_width = 10.239[us]
X_acq_time = 0.83361792[s]
X_angle = 90.0[deg]
X_atn = 9[dB]
X_pulse = 3.413[us]
Irr_atn_dec = 21.5[dB]
Irr_atn_noe = 21.5[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 2[s]
Relaxation_delay = 1[s]
Repetition_time = 2.83361792[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 22.0[dC]
single pulse decoupled gated NOE
abundance
X : parts per Million : 13C
172.9682
170.5645
170.1496
156.6195
156.5909
136.7225
136.5604
128.5291
128.1190
77.4705
77.2129
76.9554
66.7399
61.6750
58.6704
32.5545
28.8536
28.7773
21.0799
20.8796
20.3931
18.2184
11.8945

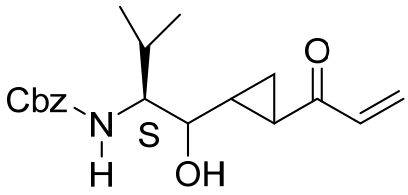


^{1}H-NMR (500 MHz, $CDCl_3$)

^{13}C-NMR (125 MHz, $CDCl_3$)


JEOL
Filename = MyIp730ppes_PROTON-6
Sample_id = MyIp730ppes
Machine = mesu500ap
Creation_time = 20-JAN-2018 15:28:04
Comment = cyclopropEnone
Field_strength = 11.74735679[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_precurn = 1
Scans = 16
Total_scans = 16
X_90_width = 14.19[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 7.095[us]
Irr_mode = Off
Tri_mode = Off
Decoupler_presat = FALSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_sec = 25[dC]
Temp_get = 22.8[dC]
cyclopropEnone
16.18
5.93
5.9
3.42
1.54
1.52
3.11
1.07
0.92
0.93
1.97
0.96
0.93
0.95
1.82
1.88
abundance
X : parts per Million : 1H

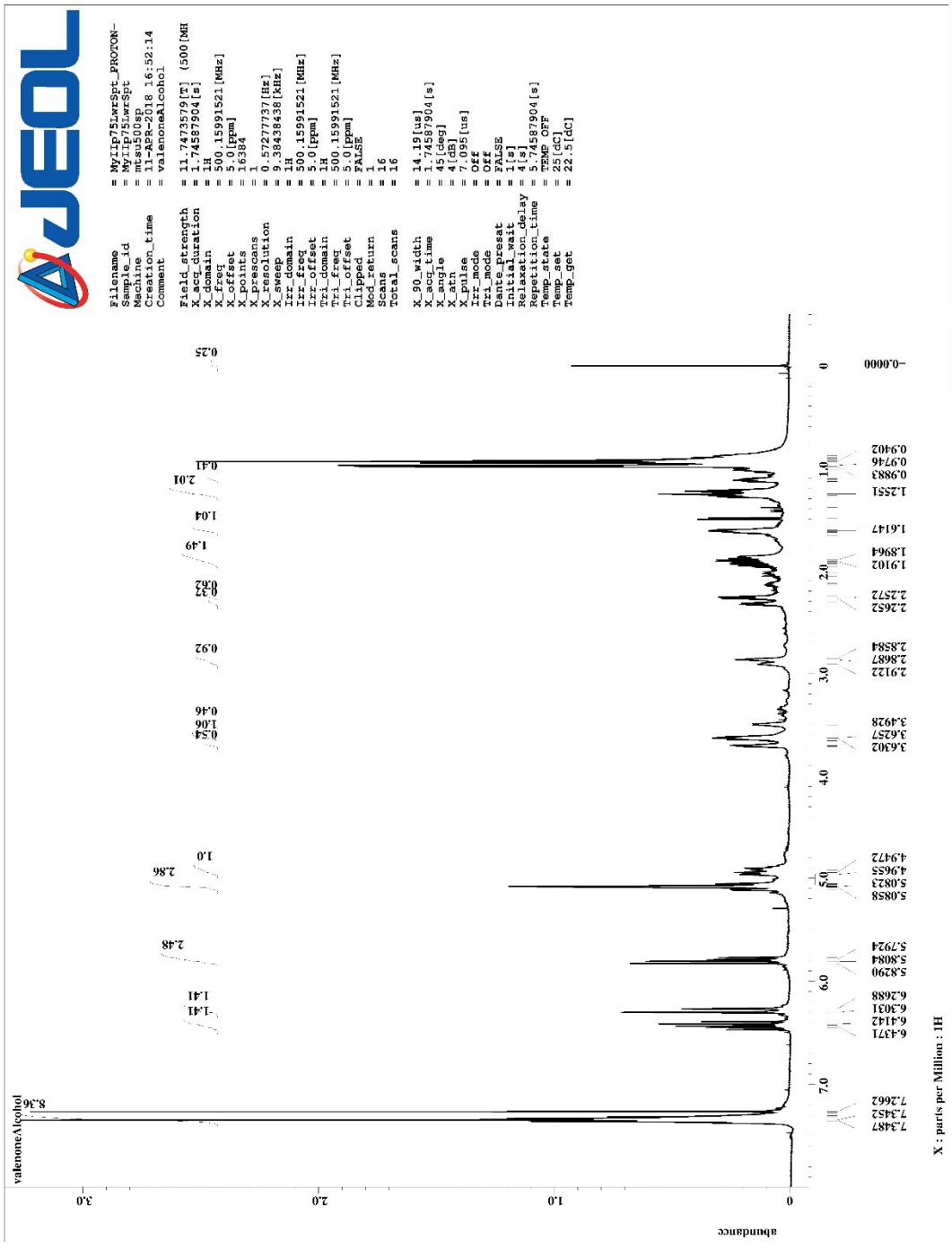

JEOL
Filename = MyZip73upperCarb-6.jd
Sample_id = LastEnone
Machine = ntmu500qp
Creation_time = 24-JAN-2018 07:22:43
Comment = single pulse decouple
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.765529768[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.3081761[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = TRUE
Mod_return = 1
Scans = 17521
Total_scans = 17521
X_90_width = 10.239[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 9[dB]
X_pulse = 3.413[us]
Irr_atn_dec = 21.5[dB]
Irr_atn_noe = 21.5[dB]
Irr_mode = NNER
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 2[s]
Relaxation_delay = 2[s]
Repetition_time = 2.83361792[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 22.8[dC]
simple pulse decoupled gated NOE
abundance
X : parts per Million : 13C
198.7694
198.6167
170.7267
170.5741
156.6290
156.5337
137.0278
136.4841
128.6436
128.1476
77.4419
77.1843
76.9268
67.0832
66.9783
58.5941
28.6152
24.5423
21.1562
20.4027
17.7987
14.9658

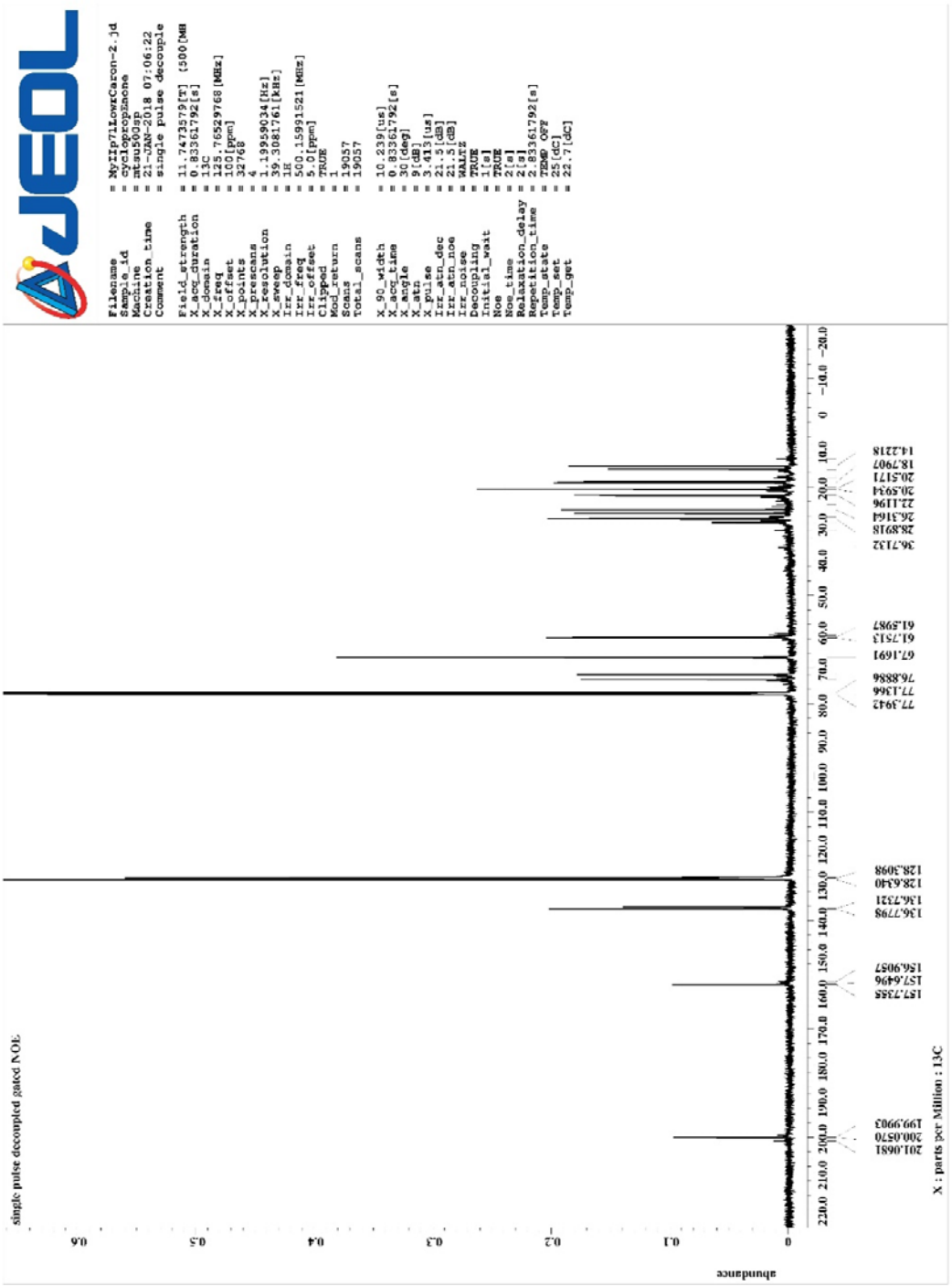


^{1}H-NMR (500 MHz, $CDCl_3$)

^{13}C-NMR (125 MHz, $CDCl_3$)

HRMS


JEOL
Filename = MyID75swrSpt_PROTON-
Sample_id = MyID75swrSpt
Machine = mtsu500sp
Creation_time = 11-APR-2018 16:52:14
Comment = valenoneAlcohol
Field_strength = 11.74735579[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438436[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 16
Total_scans = 16
X_90_width = 14.19[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 7.095[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 22.5[dC]
valenoneAlcohol
abundance
X : parts per Million : 1H


JEOL
Filename = MyIp71owrCaron-2.jd
Sample_id = cyclopropaNone
Machine = mtsu500jp
Creation_time = 21-JAN-2018 07:06:22
Comment = single pulse decouple
Field_strength = 11.74735979[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.30817761[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = TRUE
Mod_return = TRUE
Scans = 19057
Total_scans = 19057
X_90_width = 10.239[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 9[dB]
X_pulse = 3.413[us]
Irr_atn_dec = 21.5[dB]
Irr_atn_noe = 21.5[dB]
Irr_noise = NALTE
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Non_time = 2[s]
Relaxation_delay = 2[s]
Repetition_time = 2.83361792[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 22.7[dC]
single pulse decoupled gated NOE
abundance
X : parts per Million : 13C
201.0681
200.0570
199.9903
157.7385
157.6496
156.9057
136.7798
136.7321
128.6340
128.3098
77.3942
77.1366
76.8886
67.1691
61.7513
61.5987
36.7132
28.8918
26.3164
22.1196
20.5934
20.5171
18.7907
14.2218

Mass Spectrometry & Proteomics Facility

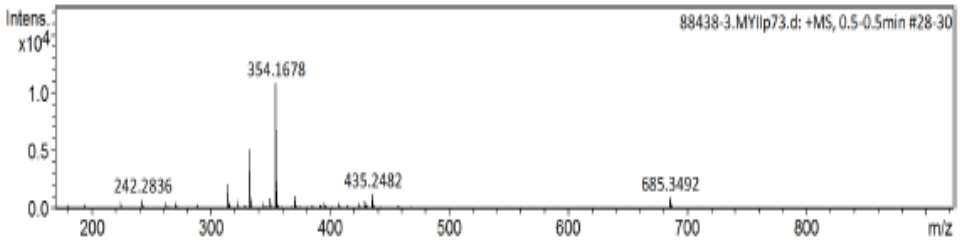
Mass Spectrum SmartFormula Report

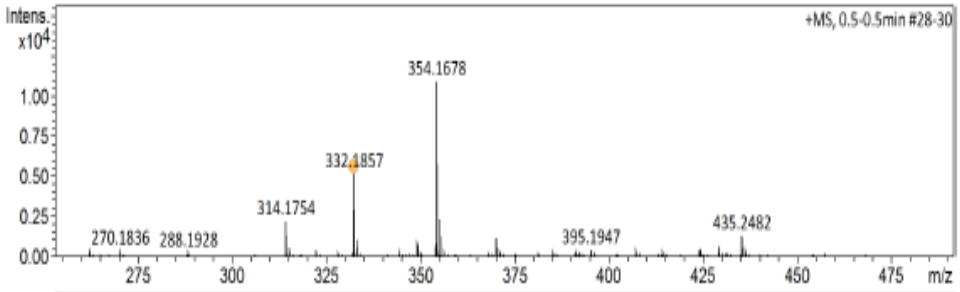
Analysis Info

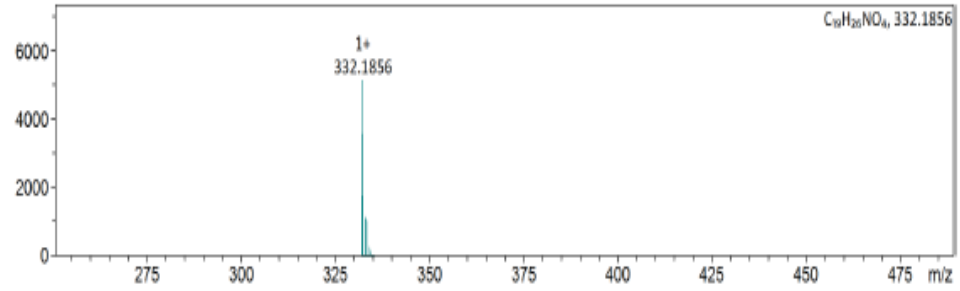
Analysis Name	D:\Data\0302\88438-3.MYIIp73.d	Acquisition Date	3/2/2018 3:19:47 PM
Method	0118_tune_low_pos_NS.m	Operator	BDAL@DE
Sample Name	3.MYIIp73	Instrument / Ser#	micrOTOF II 8213750.10314
Comment			

Acquisition Parameter

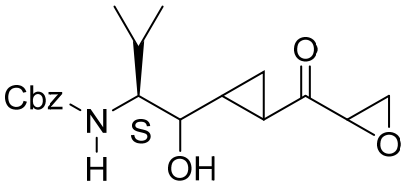
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1650 m/z	n/a	n/a	Set Divert Valve	Source







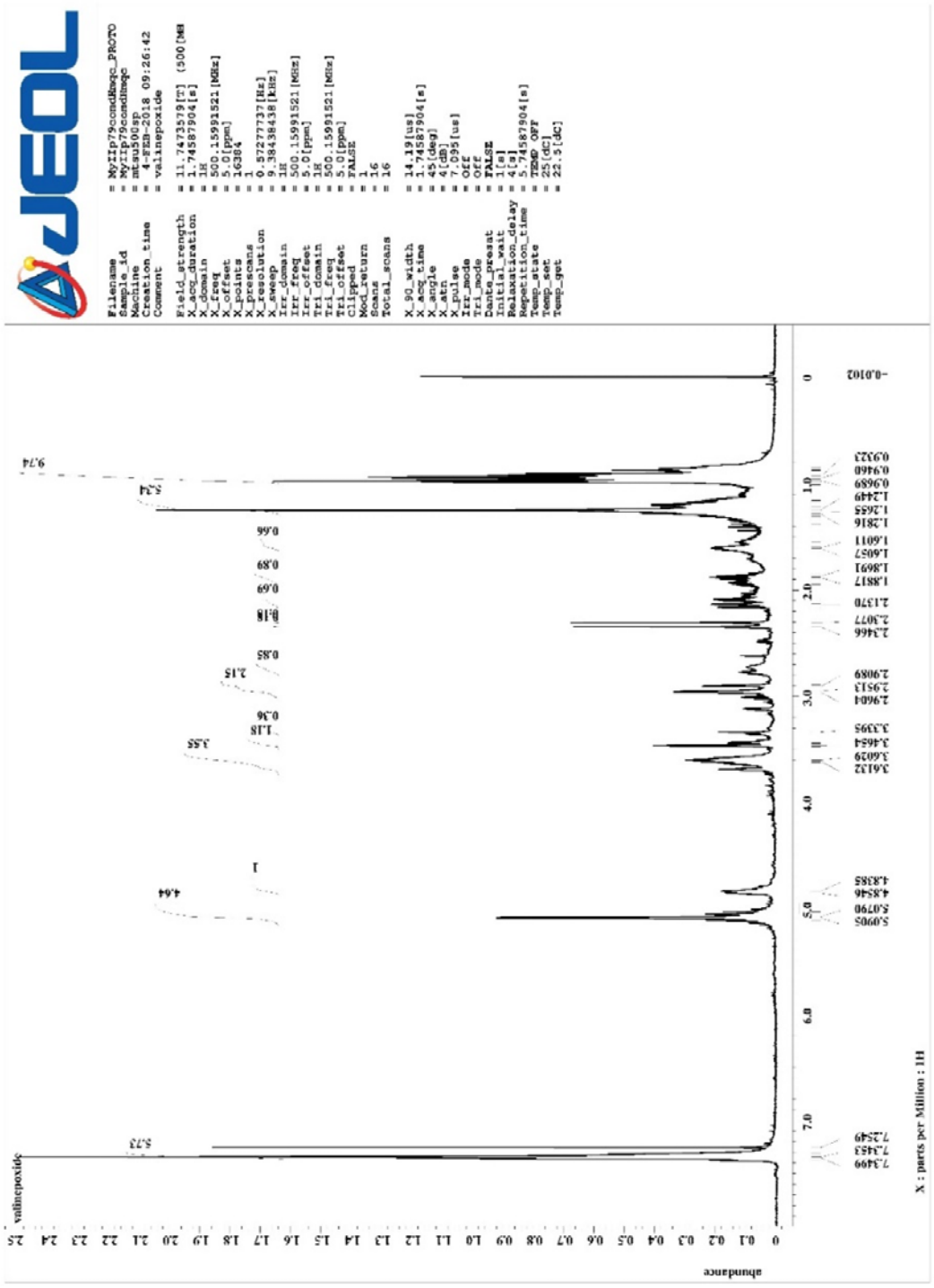
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf
332.185672	1	C19H26NO4	332.185635	-0.1	-2.3	7.5	ok	even

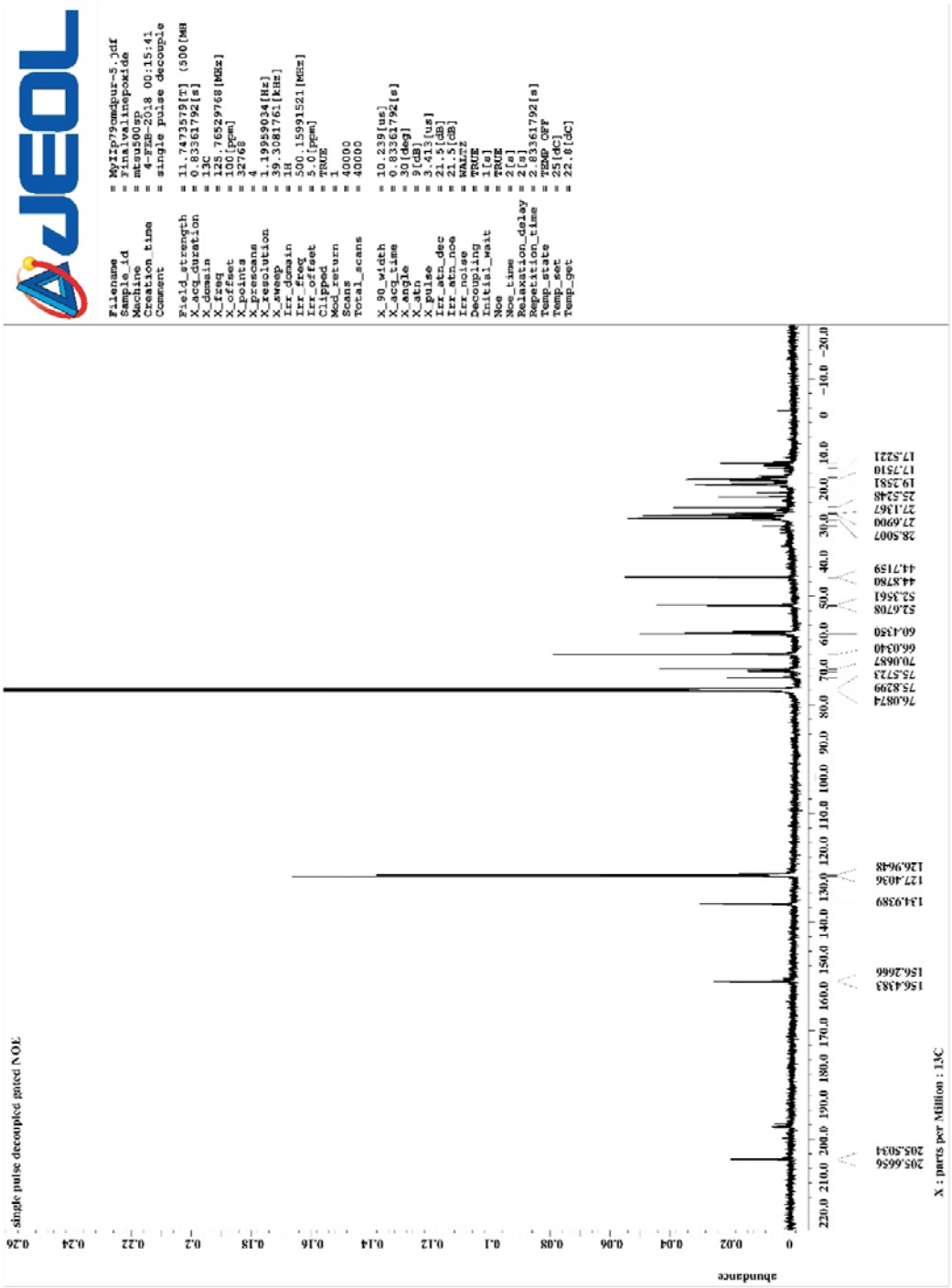


^{1}H-NMR (500 MHz, $CDCl_3$)

^{13}C-NMR (125 MHz, $CDCl_3$)

HRMS


JEOL
Filename = MyIp79comdHmqc_PROTO
Sample_id = MyIp79comdHmqc
Machine = mtsu500sp
Creation_time = 4-FEB-2018 09:26:42
Comment = valinepoxide
Field_strength = 11.74735197[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 16
Total_scans = 16
X_90_width = 14.19[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 7.095[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 22.5[dC]
valinepoxide
abundance
X : parts per Million : 1H


JEOL
Filename = MyIIp79cmdgur-5.jdf
Sample_id = Finalvalinepoxide
Machine = mksu500sp
Creation_time = 4-FEB-2018 00:15:41
Comment = single pulse decouple
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100.[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.30881761[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = TRUE
Mod_return = 1
Scans = 40000
Total_scans = 40000
X_90_width = 10.239[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 9[dB]
X_pulse = 3.413[us]
Irr_atn_dec = 21.5[dB]
Irr_atn_noe = 21.5[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe_time = 2[s]
Relaxation_delay = 2[s]
Repetition_time = 2.83361792[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 22.8[dC]
single pulse decoupled gated NOE
abundance
X : parts per Million : 13C
205.6656
205.5034
156.4383
156.2666
134.9389
127.4036
126.9648
76.0874
75.8299
75.5723
70.0687
66.0340
60.1330
52.6708
52.3561
44.8780
44.7159
28.5007
27.6900
27.1367
25.5248
19.2581
17.7510
17.5221

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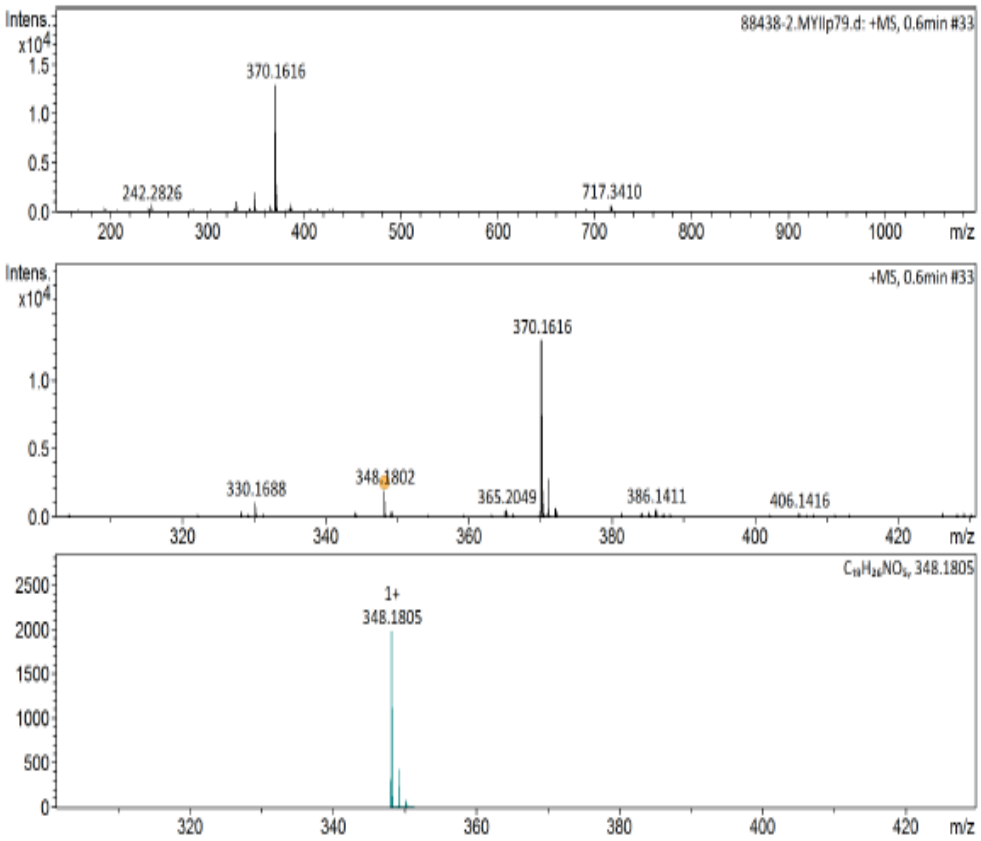
Mass Spectrum SmartFormula Report

Analysis Info

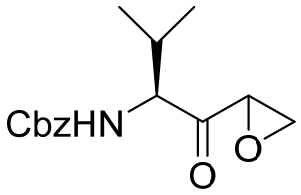
Analysis Name	D:\Data\0302\88438-2.MYIIp79.d		
Method	0118_tune_low_pos_NS.m	Acquisition Date	3/2/2018 3:16:42 PM
Sample Name	2.MYIIp79	Operator	BDAL@DE
Comment		Instrument / Ser#	micrOTOF II 8213750.10314

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1650 m/z	n/a	n/a	Set Divert Valve	Source



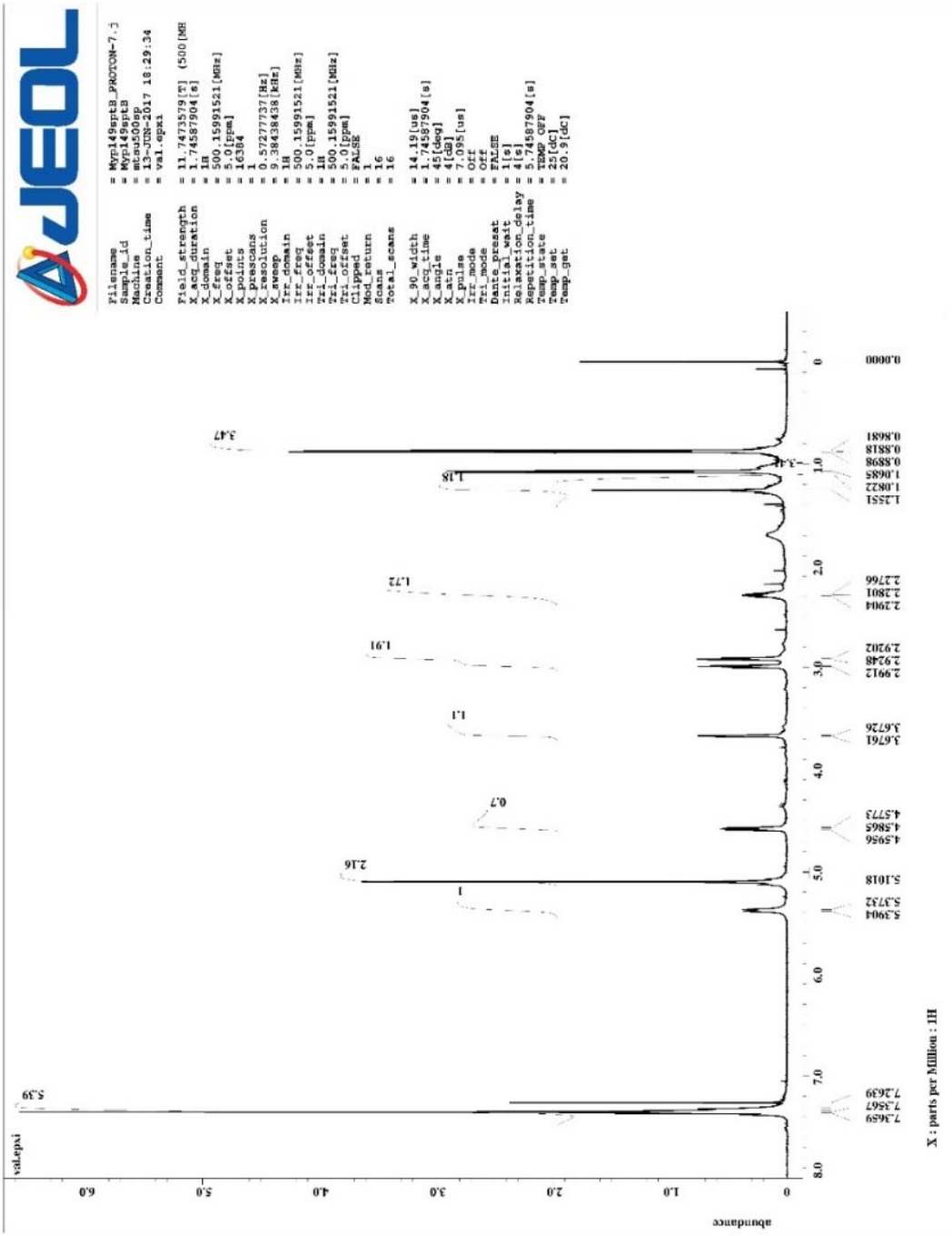
Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf
348.180233	1	C19H26NO5	348.180549	0.9	2.0	7.5	ok	even

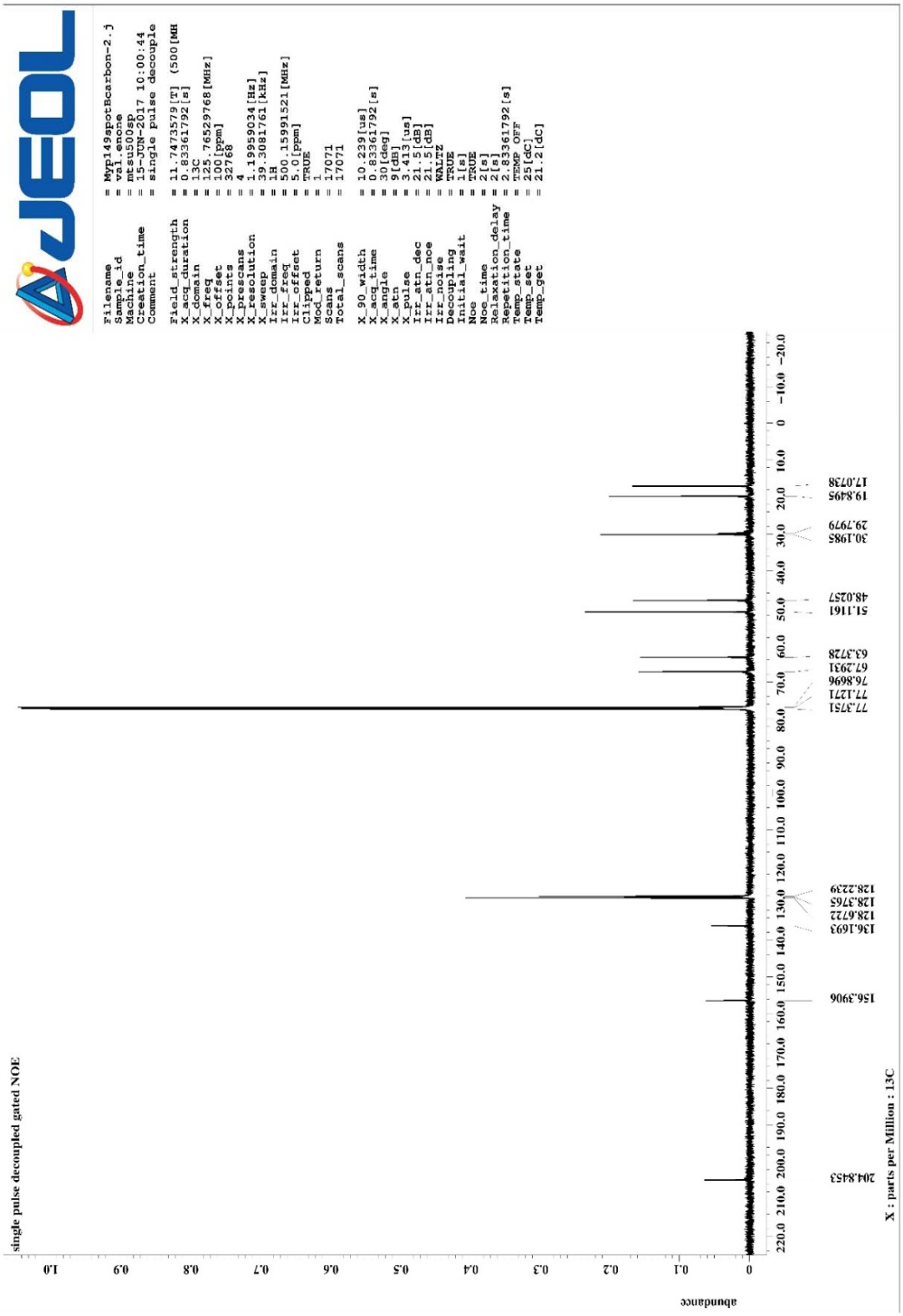


^{1}H-NMR (500 MHz, $CDCl_3$)

^{13}C-NMR (125 MHz, $CDCl_3$)

HRMS


JEOL
Filename = Myp149sptB_PROTON-7.3
Sample_id = Myp149sptB
Machine = ms500spp
Creation_time = 13-JUN-2017 18:29:34
Comment = val.epxi
Field_strength = 11.74735791[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Irr_power = 40
Fil_freq = 500.15991521[MHz]
Fil_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 16
Total_scans = 16
X_90_width = 14.19[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 7.095[us]
Irr_mode = Off
Fil_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 20.9[dC]
valepxi
abundance
X : parts per Million : 1H
5.39
3.47
2.16
1.91
1.72
1.18
1.1
1
0.7
7.3659
7.3567
7.2639
5.3904
5.3732
5.1018
4.5956
4.5865
4.5773
3.6761
3.6726
2.9912
2.9248
2.9202
2.3904
2.3801
2.2766
1.2351
1.0822
1.0685
0.8898
0.8818
0.8681
0.0000


JEOL
Filename = Myp149spotBcarbon-2.j
Sample_id = vai.enone
Machine = mtsu500sp
Creation_time = 15-JUN-2017 10:00:44
Comment = single pulse decouple
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.30817614[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = TRUE
Mod_return = 1
Scans = 17071
Total_scans = 17071
X_90_width = 10.239[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 9[dB]
X_pulse = 3.413[us]
Irr_atn_dec = 21.5[dB]
Irr_atn_noe = 21.5[dB]
Irr_noise = WALTZ2
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 2[s]
Relaxation_delay = 2[s]
Repetition_time = 2.83361792[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 21.2[dC]
single pulse decoupled gated NOE
abundance
X : parts per Million : 13C
204.8453
156.3906
136.1693
128.6722
128.3763
128.2239
77.3751
77.1271
76.8696
67.2931
63.3728
51.1161
48.0257
30.1985
29.7979
19.8495
17.0738

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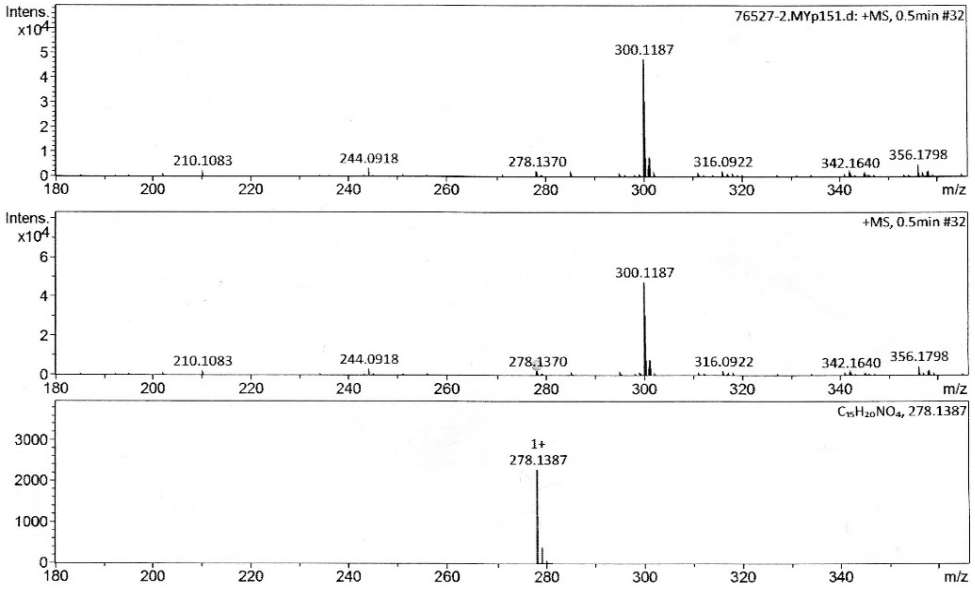
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\0726\76527-2.MYp151.d	Acquisition Date	7/26/2017 2:41:45 PM
Method	061517_tune_low_pos_NS.m	Operator	BDAL@DE
Sample Name	2.MYp151	Instrument / Ser#	micrOTOF II 8213750.10314
Comment	direct ifusion		

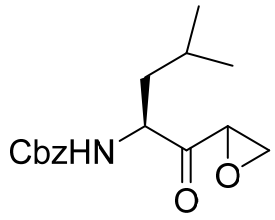
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1650 m/z	n/a	n/a	Set Divert Valve	Source



Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf
278.137027	1	C15H20NO4	278.138685	6.0	1495.6	6.5	ok	even

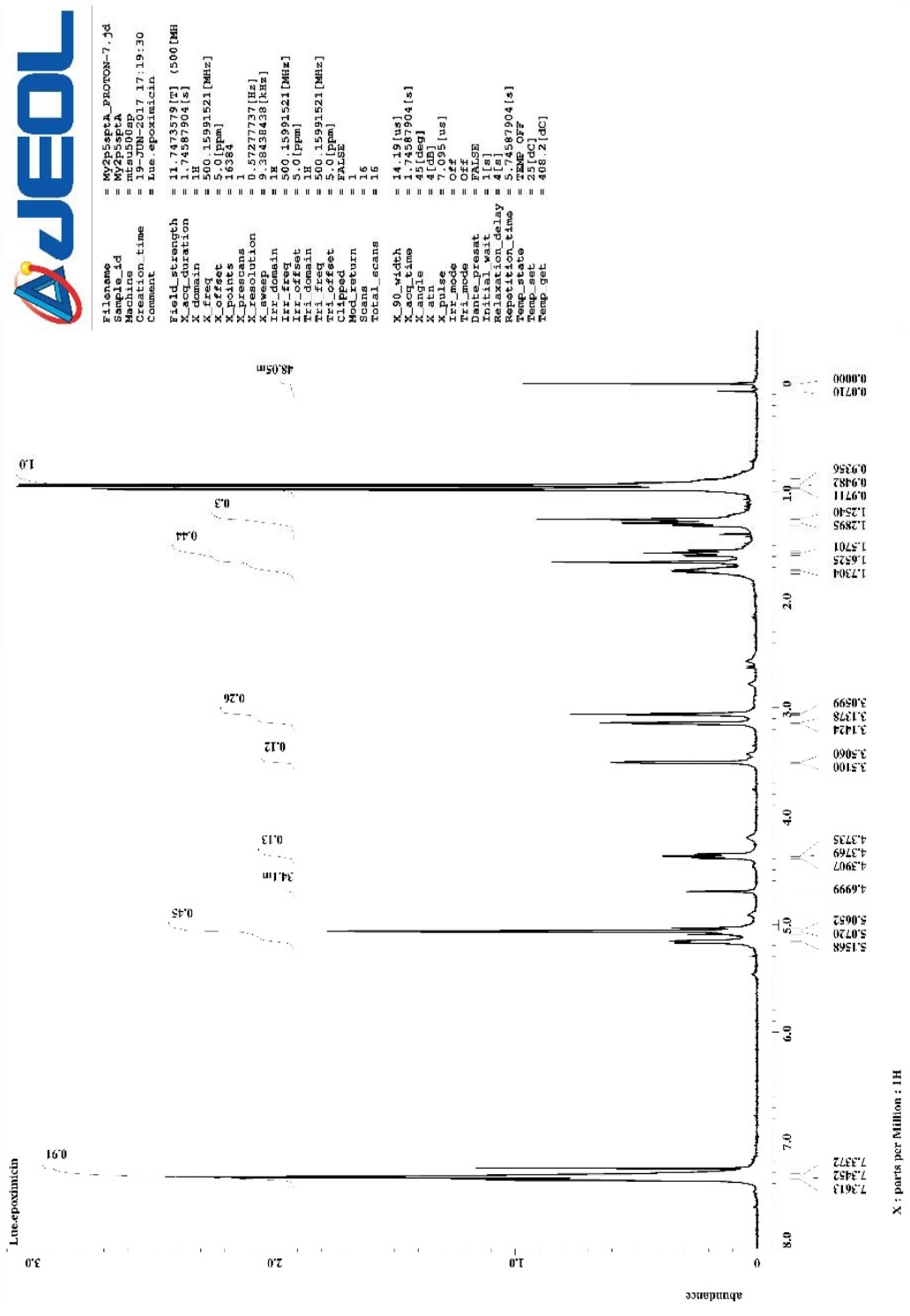
This material is based upon work supported by the National Science Foundation under CHE-0741793

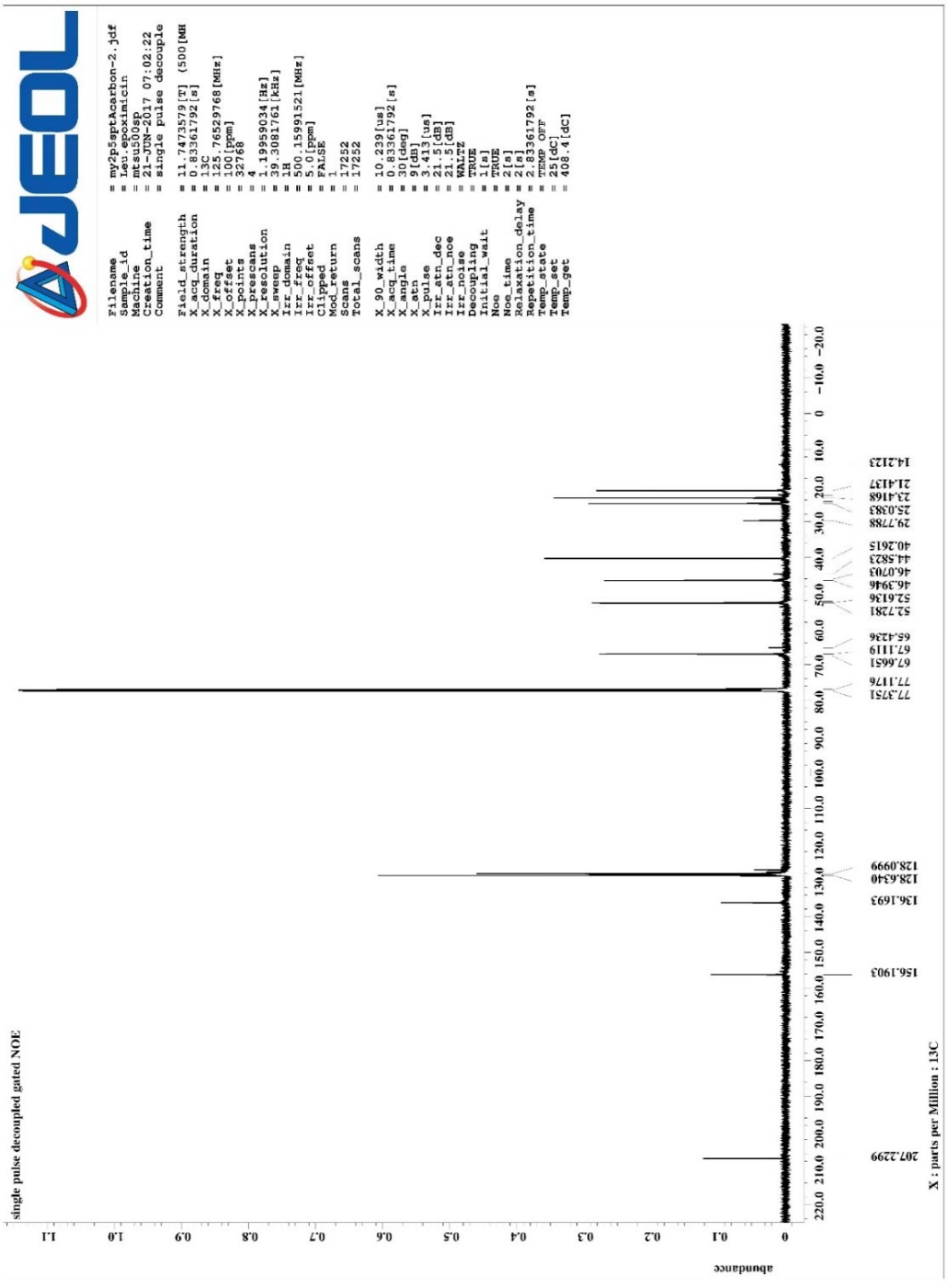


^{1}H-NMR (500 MHz, $CDCl_3$)

^{13}C-NMR (125 MHz, $CDCl_3$)

HRMS


JEOL
Filename = My2p5septA_PROTON-7.jd
Sample_id = My2p5septA
Machine = mtsu500sp
Creation_time = 19-JUN-2017 17:19:30
Comment = Lue.epoxinicin
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 16
Total_scans = 16
X_90_width = 14.19[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 7.095[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 408.2[dC]
Lue.epoxinicin
abundance
X : parts per Million : 1H


JEOL
Filename = my2p5gpttAcarbon-2.jdf
Sample_id = Leu.epoximicin
Machine = mtsu500sp
Creation_time = 21-JUN-2017 07:02:22
Comment = single pulse decouple
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.765297681MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.30817611kHz]
Irr_domain = 1H
Irr_freq = 500.159915211MHz]
Irr_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 17252
Total_scans = 17252
X_90_width = 10.239[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 9[dB]
X_pulse = 3.1[us]
Irr_atn_dec = 21.5[dB]
Irr_atn_noe = 21.5[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 2[s]
Relaxation_delay = 2[s]
Repetition_time = 2.83361792[s]
Temp_control = TEMP_OFF
Temp_set = 25[dC]
Temp_get = 408.4[dC]
single pulse decoupled gated NOE
abundance
X : parts per Million : 13C
207.2299
156.1903
136.1693
128.6340
128.0999
77.3751
77.1176
67.6651
67.1119
65.4236
52.7281
52.6136
46.3946
46.0703
44.5823
40.2615
29.7788
25.0383
23.4168
21.4137
14.2123

Mass Spectrometry & Proteomics Facility

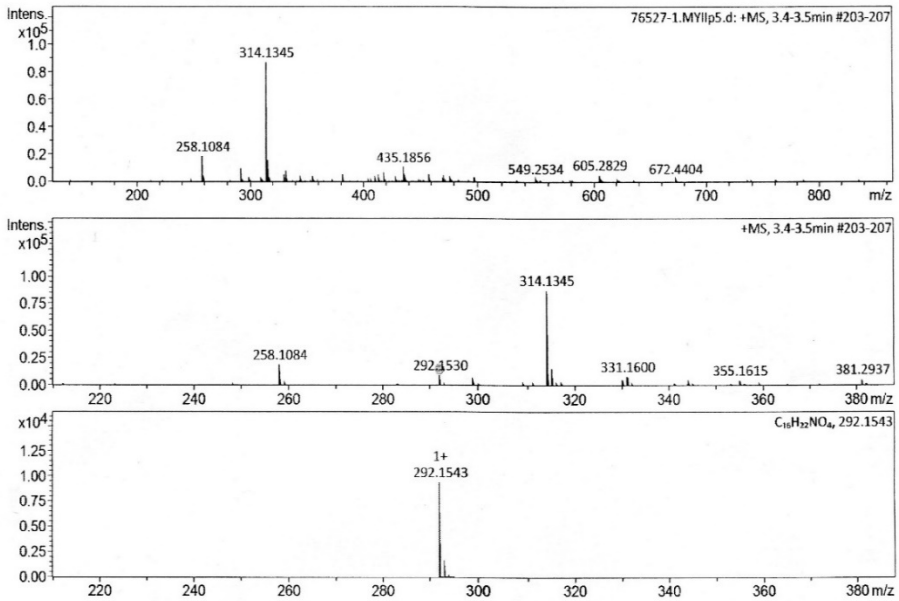
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\0726\76527-1.MYIIp5.d	Acquisition Date	7/26/2017 2:33:24 PM
Method	061517_tune_low_pos_NS.m	Operator	BDAL@DE
Sample Name	1.MYIIp5	Instrument / Ser#	micrOTOF II 8213750.10314
Comment	direct ifusion		

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1650 m/z	n/a	n/a	Set Divert Valve	Source



Meas. m/z	#	Ion Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf
292.152982	1	C16H22NO4	292.154335	4.6	3.0	6.5	ok	even

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