Synthesis of Cyclopropyl Peptidomimetics as BACE Inhibitors

by
Mahmuda Akter

A thesis presented to the Honors College of Middle Tennessee State University in partial fulfillment of the requirements for graduation from the University Honors College

Fall 2017

Synthesis of Cyclopropyl Peptidomimetics as BACE Inhibitors

by
Mahmuda Akter

APPROVED:

_____
Dr. Norma Dunlap
Chemistry

_____
Dr. Kevin Bicker
Chemistry
Honors Thesis Committee Member

_____
Dr. Paul Van Patten
Chairperson, Chemistry

_____
Dr. John Vile
Dean, University Honors College

ACKNOWLEDGEMENTS

This was an amazing journey in so many ways and that was only possible for all the support and encouragement I have received during the course of this project from the people around me. I would like to thank my advisor, Dr. Norma Dunlap, whose support, wisdom and dedication made this work possible. Her counseling and guidance have been incredibly helpful and much needed over the course of my academic experience in MTSU. I would like to thank Dr. Kevin Bicker for being in my committee and for his instructions and advices. I would like to thank Dr. Rebecca Seipelt-Thiemann for introducing me to the field of research. I would also like to give a special thanks to Ms. Judy Albakry, my honors college advisor, who has always been there to answer all of my inquiries with enthusiasm, helping me keep on track with all the official procedures.

I'm extremely grateful to the Almighty for granting me such an amazing life full of adventures and opportunities. Finally, I want to thank my sister, Mahbuba, my "Mejho", for being there and listening to me when I was struggling, motivating me when I was lost, loving me unconditionally, and for all her sacrifices only to see me succeed, for being not only my elder sister, but my best friend and the greatest support system of all. Thanking her for a thousand years would not be enough.

Abstract

Since the development of the amyloid hypothesis, correlating the production of amyloid plaques to the pathology of the Alzheimer's disease, numerous research groups have worked on developing an inhibitor for the BACE1 protease which is considered to be primarily responsible for the cleavage of Amyloid-Precursor Protein leading to the formation of amyloid-beta plaques. Our laboratory has attempted to synthesize peptidomimetic inhibitors with a cyclopropyl core. While an effective route has been developed for a series of amides, there were difficulties in synthesizing a particular series of compounds with a reverse amide. Hence, we have used synthetic routes developed in this lab to design a new scheme in order to synthesize analogs of cyclopropyl peptidomimetic with the isophthalamide side chain and a reverse amide.

Table of Contents

List of Figures:

Chapter I: Introduction

Alzheimer's disease (AD) has been a dreadful chapter of our society for a very long time due to its devastating effects on the patients' and their loved ones' lives. Moreover, the lack of treatment options and advances in pharmaceuticals have turned it into a nightmare. It chronically ravages the cognitive capabilities of the sufferer, resulting in gradual decline in cognition, memory and changes in behavior, which inevitably leads to death. With the demographic shift towards an aging population, resulting in an increased number of patients, this disease is fast becoming a serious economic and social burden.[1]

In 2013, official death certificates recorded 84,767 deaths from Alzheimer's disease, making it the sixth leading cause of death in the United States and the fifth leading cause of death in Americans age ≥ 65 years. Between 2000 and 2013, deaths resulting from stroke, heart disease, and prostate cancer decreased 23%, 14%, and 11%, respectively, whereas deaths from Alzheimer's disease increased 71%.[2] According to a WHO report, it is the most common form of dementia. The report also mentions that treating and caring for people with dementia currently costs the world more than US $604 billion per year.[3] Putting these findings into perspective, it is clear that the attempts in finding a cure for AD has been a priority in the research field. One of the most dominant theories for explaining the pathogenesis of AD is the amyloid cascade hypothesis, which is based on the idea that the plaques and neurofibrillary tangles created by the accumulation of the amyloid-β peptide in the brain is a central event in Alzheimer's

disease pathology.[4] Hence, research conducted in academia and the pharmaceutical industry for the past twenty years have been dominated by the aspects of this hypothesis.

Amyloid Hypothesis: In 1907, Alois Alzheimer, a German psychiatrist and neuro-pathologist, observed the connection between amyloid plaque formation and symptoms like memory loss and cognitive dysfunctions as he performed postmortem studies on a 51-year-old-female patient who had suffered from a progressive pre-senile dementia.[5,6] The identification of *β*-amyloid (A*β*) in senile plaques, leading to the accumulation of A*β* and early-onset familial dementia resulted in the formulation of the "Amyloid Cascade Hypothesis."[7-9] In 1992, John Hardy proposed the amyloid cascade hypothesis, which states that deposition of amyloid-beta (Aβ) protein, the main component of the plaques, is the causative agent of Alzheimer's pathology and that the neurofibrillary tangles, cell loss, vascular damage, and dementia follow as a direct result of this deposition.[10]

The Aβ peptide is generated by the endoproteolysis of APP (Amyloid- Precursor Protein), a type I membrane protein of 770 amino acids in length, between positions 671–672, and anywhere between positions 710–715, to give rise to 39–43 amino acid Aβ.[9-13] APP is cleaved by two proteases, the *β*- and *γ*-secretases. The *β*-secretase initiates the process by cutting APP to generate the N terminus of A*β* peptide, thus producing a membrane bound C-terminal fragment called C99. Then, *γ*-secretase cleaves C99 fragment to release the mature A*β* peptide.[14] This process is pictured in Figure 1. Two isoforms of β-secretase proteases have been identified and are known as BACE1 (β-site APP cleaving enzyme) and BACE2.[13] However, BACE2 was found to be expressed at a

very low level in the brain and to have almost no effect on the production of Aβ.[15] Whereas, BACE1 mRNA is high in brain and BACE1 deficient mice have shown abolished Aβ peptides, establishing that BACE1 is the principal β- secretase in the neurons.[16] In addition to that, studies found that there are no apparent adverse effects associated with BACE1 deficiency in mice suggesting that therapeutic inhibition of BACE1 for the treatment of AD could be possible.[17]

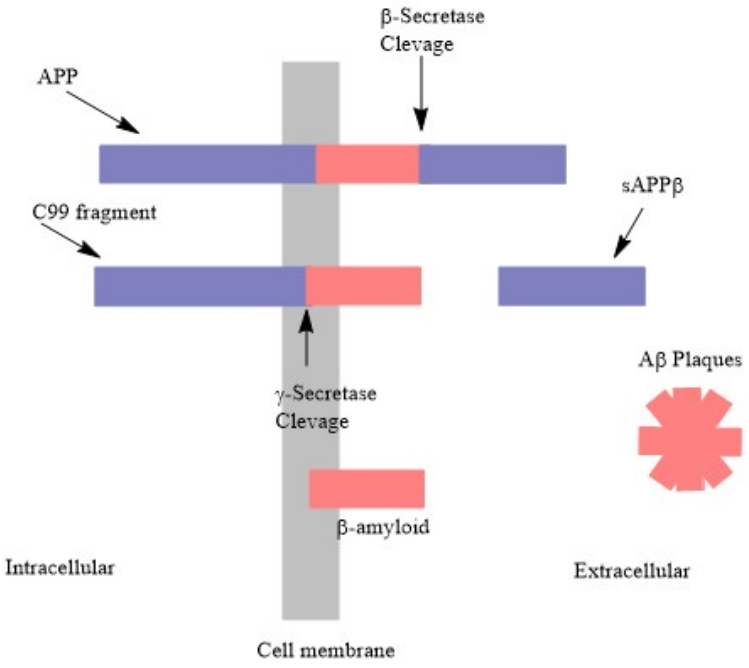


Figure 1. Formation of Aβ from cleavage of APP. β-secretase cleaves APP to give sAPPβ and C99 fragment; C99 fragment is cleaved by γ-secretase to give Aβ, which then forms plaques.

BACE-1 (also known as β-secretase or memapsin-2) is a novel 501 amino acid type 1 transmembrane aspartic protease related to the pepsin and retroviral aspartic protease families.[9] The highest expression levels of BACE1 are found in the pancreas and in neurons of the brain, and significantly lower in most other tissues, as expected.[9, 14]

The active site of aspartic proteases is characteristically covered over by flaps.[19] The BACE1 active site is covered by a flexible antiparallel β-hairpin, acting as the flap, which regulates access to the active site.[18] Two conserved water molecules along with the catalytic aspartates (Asp32/Asp228) control the opening and closing of the flap along with the peptide bond cleavage by an acid-base mechanism, as seen in Figure 2.[19,20]

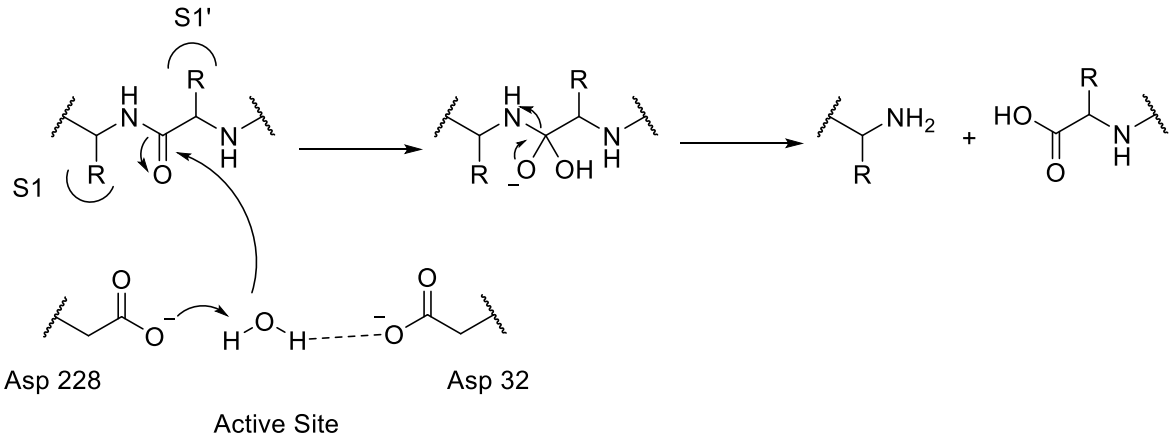


Figure 2. BACE 1 catalytic activity mechanism. Asp228 removes a hydrogen from water, which then adds to the amide carbonyl. The tetrahedral intermediate collapses and picks up a hydrogen from Asp32 to give the two fragments.

Potential Therapeutic strategies: The therapeutic strategies proposed based on the amyloid cascade hypothesis include: inhibition of BACE1 or *γ*-secretase with potent small molecule inhibitors, attempts to prevent the oligomerization of Aβ and enhance its

clearance from the cerebral cortex, and also an anti-inflammatory approach is a possibility.[12,21] Among these, inhibition of BACE1 has been an attractive strategy for drug development. In designing an inhibitor, the features that needed to be included were:

- The compound is effectively selective towards BACE1 over other human aspartyl proteases like BACE2, renin, and cathepsin D.
- It must be large enough to fit the active site of the protease.[22]
- An effective BACE-1 inhibitor must have sufficient hydrophobicity to pass through the blood-brain barrier and yet be sufficiently water-soluble to reach the aqueous acidic compartment where BACE-1 is active.[23]

Historically, brain penetrating and central nervous system (CNS) active aspartyl protease inhibitors have been difficult to obtain.[23] Previously, inhibitors of aspartyl proteases, most notably for HIV protease and renin have been synthesized and marketed successfully, and examples are shown in Figure 3. This has provided researchers with resources and data to work on an inhibitor for the BACE1 protease.

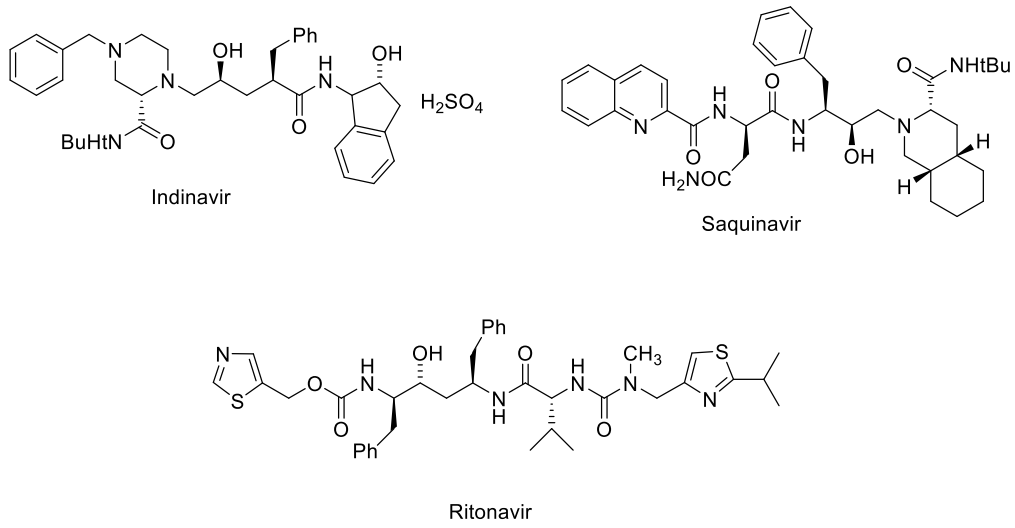


Figure 3. HIV protease inhibitors. The chemical structures of the clinically approved HIV protease inhibitors indinavir, saquinavir, and ritonavir.

Peptidomimetics: The known aspartic protease inhibitors are peptidomimetics. These are compounds that resemble the peptide substrate but don't have the cleavable amide. The very first inhibitor for BACE1, OM99-2 was a peptidomimetic and showed potency against the enzyme.[23] However, it lacked selectivity for BACE1. Morever, its large size and peptide-like properties made it unsuitable to be developed further.[24] Other early BACE inhibitors were all designed using knowledge of the peptide substrates and usually contained some variation of a few well-known transition-state isosteres, while these showed potency, as OM99-2, they proved to be too large, polar and had no CNS availability.[25]

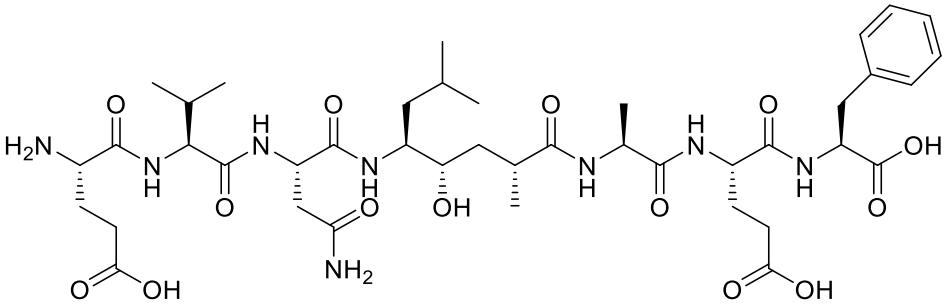


Figure 4. OM99-2, first generation peptidomimetic for BACE.

Due to the fact that peptide derived inhibitors suffered from lack of CNS availability because of the prevalence of polar amide bonds, many research groups sought alternative scaffolds to replace the peptide backbone of the first- generation inhibitors. A common strategy in inhibitor design is replacement of the substrate scissile amide bond with a tetrahedral intermediate isostere, typically a secondary alcohol. Four common hydroxyl-containing inhibitor scaffolds include the hydroxyethylene (HE), hydroxyethylamine (HEA), statine, and hydroxymethylcarbonyl (HMC) motifs.[26]

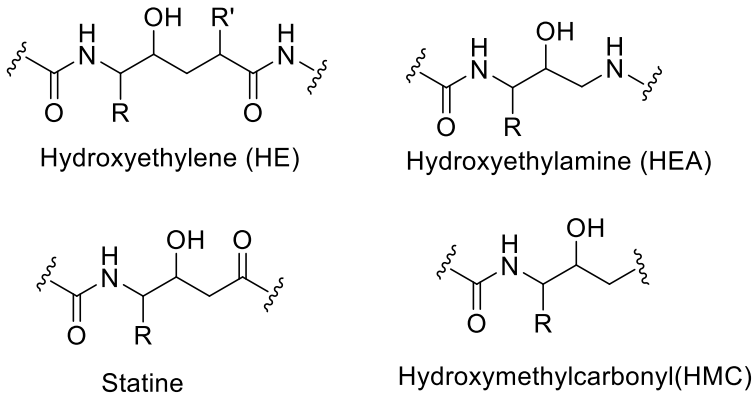


Figure 5. Core motifs for aspartyl protease inhibitors.

Diverse high-affinity inhibitors evolved from a common chemotype based on an isophthalamide.[27] Ghosh et al. synthesized a series of inhibitors containing Leu-Ala hydroxyethylene isostere and isophthalamide derivatives. They found a potential compound with potency against BACE1 and selectivity over BACE2 and Cathepsin D.[29] The isophthalamide based ligand, shown in Figure 6, had interactions with various residues at the S2 and S3 region of the enzymes active site. [29]

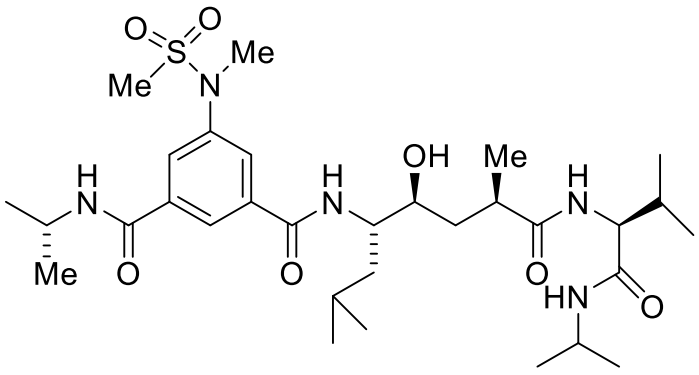


Figure 6. Peptidomimetic with isophthalamide derivative.

Note: When a peptide bond is broken, the amino acid residues on the amino terminal is indicated as P1, P2 and so forth and the residues on the carboxyl side are identified as P1', P2' and so on. The corresponding binding sites on the enzyme for the amino acids on either side of the scissile bonds are hence referred as: S1, S1', S2, S2' and so forth as illustrated in Figure 7.

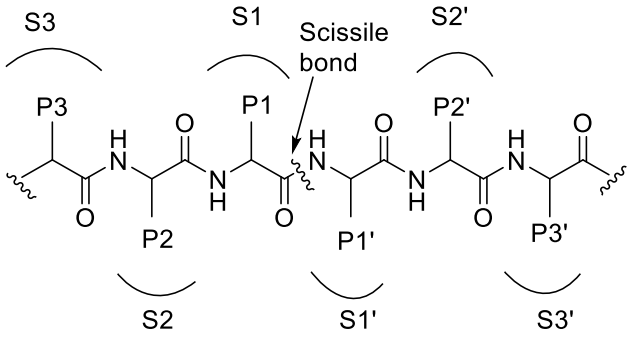


Figure 7. Designation of amino acids and their corresponding enzyme site on either side of the scissile bond.

Along with academic research foundations, many pharmaceutical companies such as Merck, Pfizer and Schering-Plough discovered potential BACE1 inhibitors with isophthalamide derivatives.

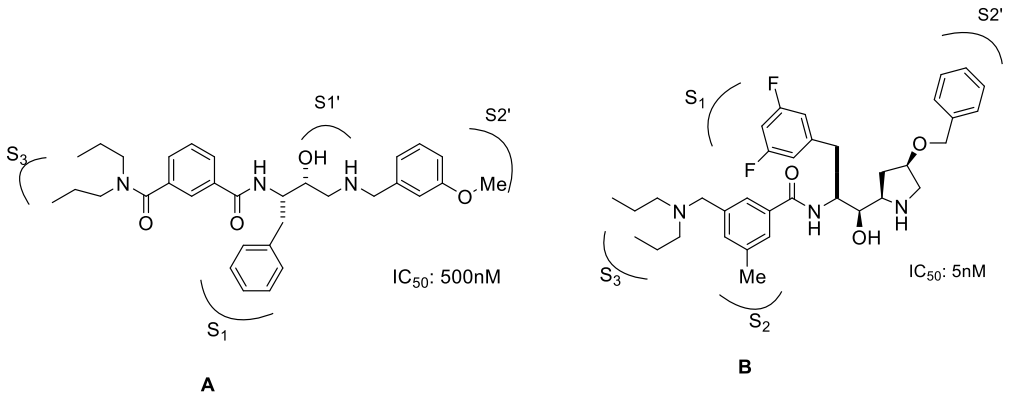


Figure 8. Potential BACE inhibitors with isophthalamide side chain.

Compound A was synthesized by Pfizer, and showed high potency in isolated enzyme assays. The isophthalamide binds in the S3 region and the hydroxyethylamine

isostere interacts with the S1' region of BACE1. They improved the potency and selectivity of this compound by modifying the aryl substituent. Schering-Plough made modifications to the S2' binding region by cyclizing the benzylamino group to afford B which showed high *in vitro* potency but lacked selectivity over other aspartyl proteases. Moreover, most of the compounds also showed a high P-glycoprotein susceptibility, which effluxes compound out of the cell and leads to poor CNS distribution.[28] Hence, while the isophthalamide peptidomimetics show promise, there is still room for improvement.

Synthesis of cyclopropyl core: Addition of cyclopropyl ring into the backbone of peptidomimetics has been shown to increase stability toward degradation, as well as decreasing conformational flexibility. Examples include Martin's protease inhibitors, Wipf's cyclopropyl tripeptide isoteres, and the natural product proteasome inhibitor belactosin A, shown in Figure 9.[31-33]

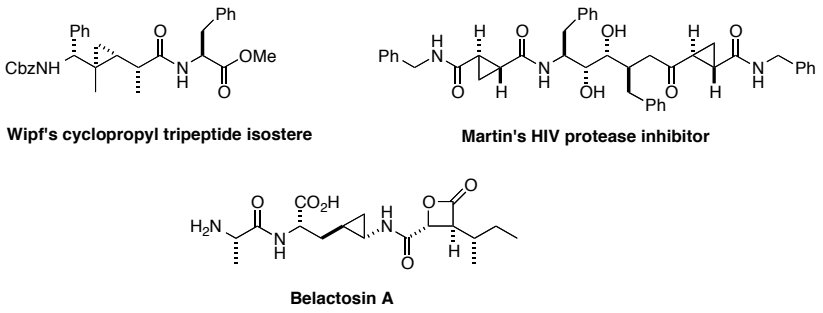


Figure 9. Peptidomimetics and natural products with cyclopropyl core.

Theoretically, constrained analogs should show reduced entropy effects, leading to improved binding in target proteins. Although there is some evidence that these effects may be minimal, the improved metabolic stability still makes cyclopropyl peptidomimetics attractive targets for drug design. The strategy for BACE inhibition is to couple an isophthalamide side chain (BACE side chain) with the nitrocyclopropyl core to synthesize amide analogs. A four-step synthetic process developed in the Dunlap laboratory is going to be followed in synthesizing the nitrocyclopropyl core, as shown in Figure 10.[30]

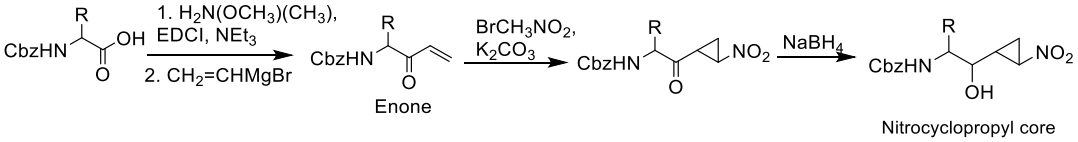


Figure 10. Four-step synthesis of the nitrocyclopropyl core. Protected amino acids are converted to Weinreb amides and then to enones. Cyclopropanation of the enone is followed by reduction to give the core structure.

Prior work in this lab had made several amide analogs from the analogous ester cyclopropyl core. Several of these compounds have shown inhibitory activity against BACE 1 in an enzymatic assay. In an attempt to observe if the orientation of the amide bond had effects on the activity of the inhibitor, synthesis of "reverse amide" analogs were proposed as shown in Figure 11.

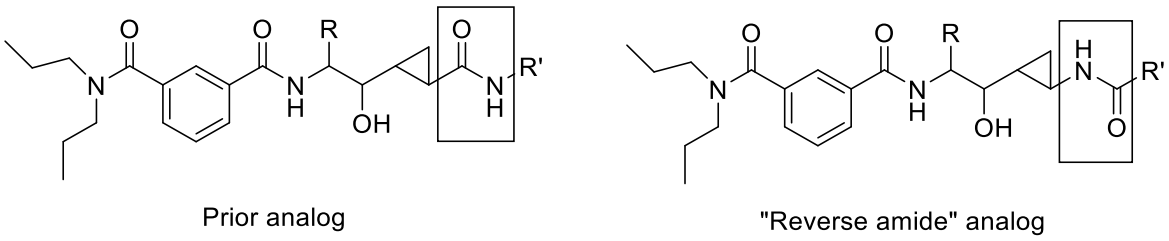


Figure 11. Prior amide analog and "reverse" amide analog.

Previous attempts to prepare the reverse amides included nitrocyclopropanation to produce the core, which was then coupled with the isophthalamide side chain. These earlier schemes were designed in three parts: synthesis of the isophthalamide side chain, synthesis of the cyclopropyl core, and removal of the Cbz protecting group to couple the nitrocyclopropyl to the isophthalamide chain. The final sequence was nitro reduction and amide formation. However, the routes followed proved to have certain difficulties. One of the issues was the opening of the cyclopropyl ring under certain conditions, as illustrated in Figure 12. Another issue was the lack of selectivity while reducing the nitro group. This resulted in removal of the Cbz protecting group and formation of a di-amine, which is shown in Figure 12.

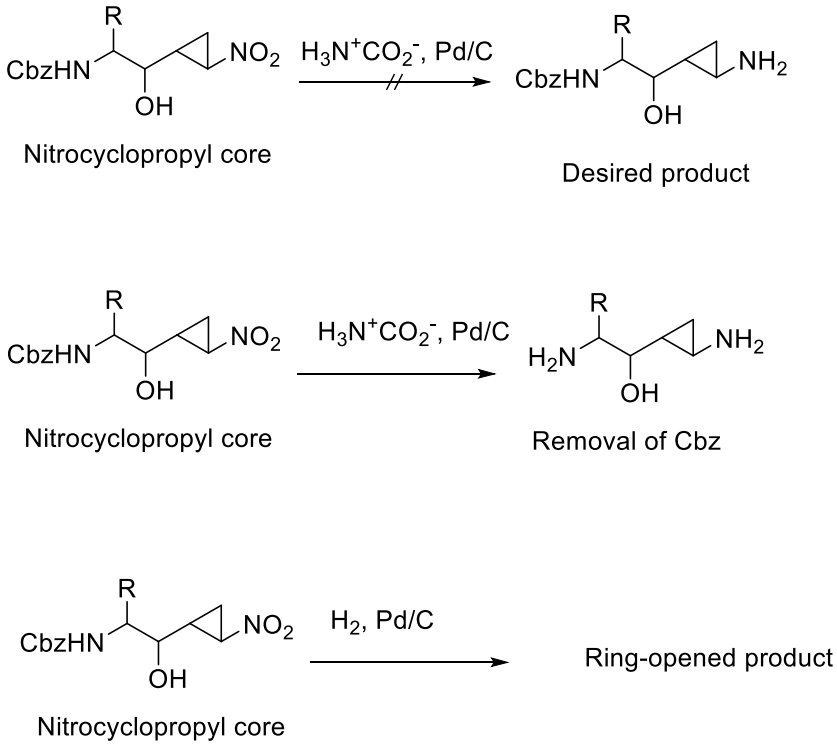


Figure 12. Prior approach to amide BACE inhibitors. The only effective nitro reduction also removes the Cbz protecting group or cleaved the cyclopropyl ring.

Objective: A new route is the subject of this research. It avoids the problem regarding the reduction of the nitro group and removal of the Cbz protecting group and includes synthesis of the isophthalamide side chain and its coupling with a de-protected Weinreb amide, which then goes through a four step synthesis of the nitrocyclopropyl core. The resulting nitrocyclopropyl core with the isophthalamide side chain attached is reduced and then coupled with different acids in the final step to obtain different amide analogs of the compound. By adding the isophthalamide side chain at the beginning of the synthesis, problems with competing reduction of the nitro and Cbz group are avoided. Two proposed analogs are shown in Figure 13.

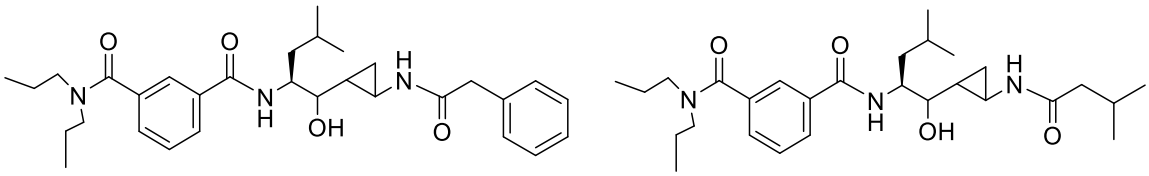


Figure 13. Proposed amide analogs from nitrocyclopropyl core.

Chapter II: Materials and Methods

Materials:

The NMR data were obtained on a 500 MHz FT-NMR model ECA-500 JEOL (Peabody, MA) purchased with funding provided by the National Science Foundation through the NSF-RUI program (# 0321211) and where indicated a 300MHz FT-NMR model ECA-300 JOEL (Peabody, MA). Chemical shifts are reported in parts per million using tetramethylsilane (TMS) as an internal reference. Splitting patterns are designated by the following: s (singlet), d (doublet), m (mutiplet), and dd (doublet of doublets). Coupling constants (J values) are recorded in hertz (Hz). Most signal assignments are based on COSY, HMQC and DEPT. Polarimetry was performed using an Autopol III polarimeter (Rudolph Research, Fairfield, NJ). High resolution ESI-MS (electrospray ionization-mass spectrometry) was performed at Notre Dame University, Notre Dame, IL.

Analytical thin layer chromatography (TLC) was performed on glass plates coated with silica gel and UV active backing purchased from Fisher Scientific, Pittsburgh, PA. The TLC plates were analyzed with a short wavelength (254 nm) UV light and subsequently stained with ninhydrin or phosphomolybdic acid (reagent grade, Aldrich, Milwaukee, WI) prepared as a 10% solution in ethanol. Column chromatography was performed with silica gel, 60 Å 230-400 mesh ASTM (reagent grade, Fisher Scientific, Pittsburgh, PA). Flash column chromatography was performed on an ISCO CombiFlashRf 200 (Teledyne ISCO, Lincoln, NE).

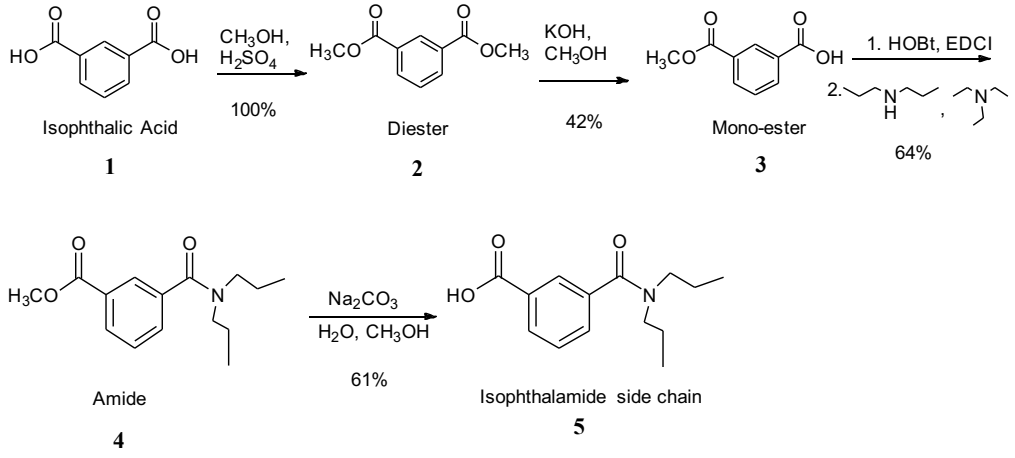
Chloroform was purchased reagent grade from Acros Organic, New Jersey, USA. Ethyl acetate and hexane were purchased HPLC grade from Acros Organic, New Jersey, USA. All other solvents including methylene: chloride, acetone, methanol, ethanol, ethyl acetate, and hexanes were also obtained from Fisher Scientific, Pittsburgh, PA. Deutero-chloroform ($CDCl_3$) was purchased from Aldrich, Milwaukee, WI. Solvent extractions were performed using methylene chloride or ethyl acetate as the extracting solvents when appropriate. Evaporation of solvents was conducted using a Buchi rotary evaporator (Model RII, Buchi, Switzerland).

Vinylmagnesium bromide and tetrahydrofuran (THF) were purchased anhydrous reagent grade from Aldrich, Milwaukee, WI. Reagents such as N-(carbobenzyloxy)-L-Leucine, N,O-dimethyl hydroxylamine hydrochloride, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDCI), ammonium formate, and 10% palladium on charcoal were obtained from Aldrich, Milwaukee, WI. Other reagents including triethylamine (NEt_3) and sodium hydroxide were purchased from Fisher Scientific, Pittsburgh, PA.

Synthetic Methods:

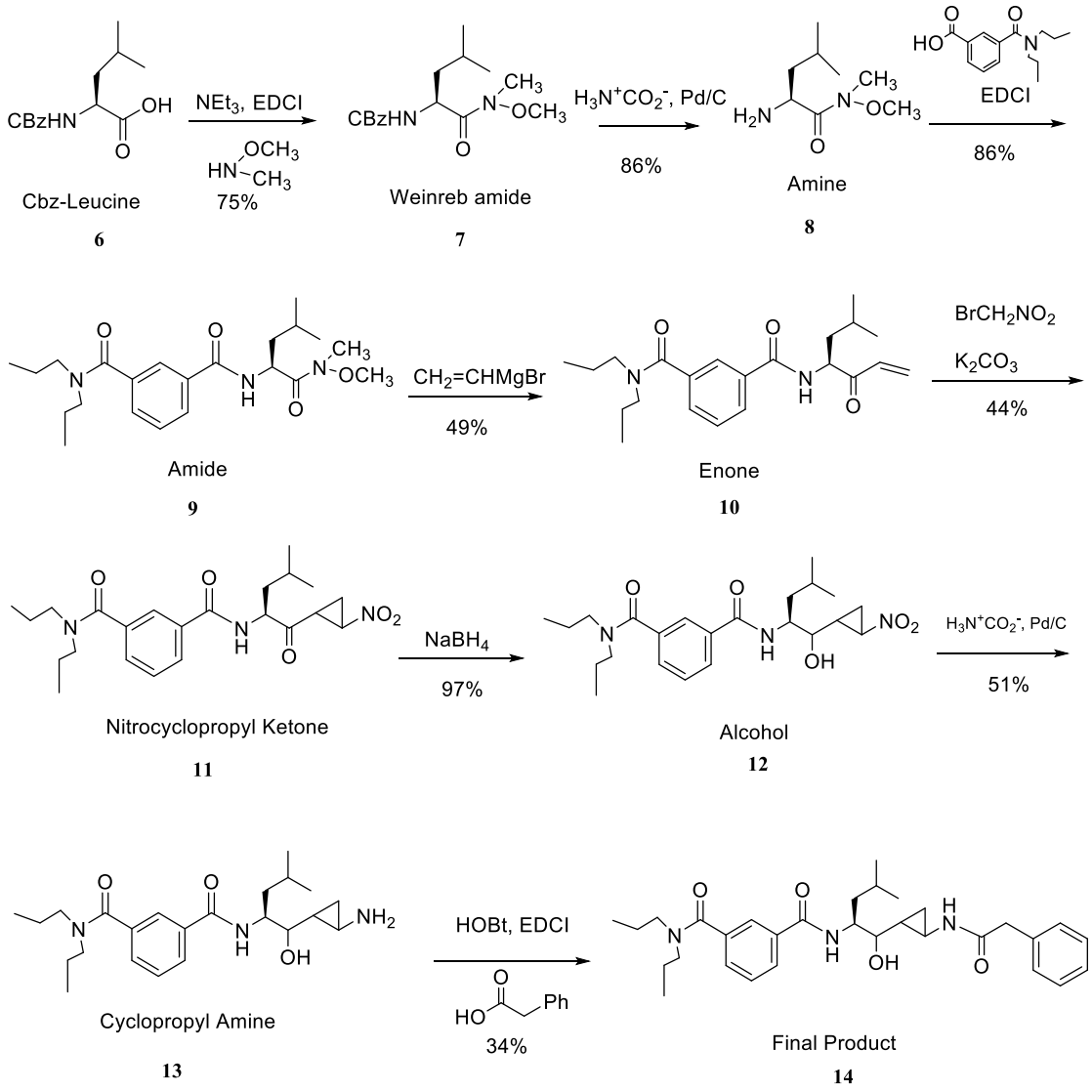
The synthesis consists of three main parts: the synthesis of the isophthalamide side chain, and the synthesis of the nitro-cyclopropyl core from a Weinreb amide with the isophthalamide side chain attached to it and finally, coupling the core to phenylacetic acid to give the final product. The isophthalamide side chain is an N-dipropyl amide of isophthalic acid. The synthesis of the isophthalamide side chain began by esterification of isophthalic acid **1** to give a di-ester **2**. One of the esters was then hydrolyzed to provide a mono ester **3**. The acid of **3** was then coupled with dipropylamine to give **4**. The remaining methyl ester was then hydrolyzed to yield **5** with the dipropyl amine and an acid for further coupling as shown in Scheme 1.

Scheme 1. Synthesis of the isophthalamide side chain.



Scheme 2 illustrates the next phase, which was to prepare the nitrocyclopropyl core. Protected L-leucine **6** was converted to a Weinreb amide **7**. The protecting Cbz group was removed by hydrogenation to produce an amine **8**. The amine was then coupled with the isophthalamide side chain to give the amide **9**. The conversion of the amide to the nitrocyclopropyl core began with Grignard reaction of **9** to afford an enone **10**. The resulting enone was treated with bromonitromethane and potassium carbonate to produce the nitrocyclopropyl **11**. The next step was the reduction of the ketone in **11** to provide an alcohol **12**. Reduction of the nitro group by hydrogenation of **12** produced an amine **13**. This was then coupled to phenylacetic acid to give the final product **14**. All new compounds were identified by NMR spectroscopy and confirmed with high resolution mass spectrometry.

Scheme 2. Synthesis of the nitrocyclopropyl- leucine Series.



Experimentals:

Isophthalamide Side Chain

Diester: dimethyl isophthalate (2): Isophthalic acid **1** (5.0 g, 30.22 mmol) was dissolved in 50 mL of methanol followed by addition of 4 drops of HCl. The solution mixture was heated to reflux and left to react overnight. It was cooled to room temperature and the methanol evaporated to afford 5.0 g (100%) of the crude product **2**. ^{1}H-NMR (500 MHz, $CDCl_3$): δ8.45 (m, 1H, aryl), 7.99 (m, 2H, aryl), 7.40 (m, 1H, aryl), 3.06 (s, 6H, $OC\underline{H}_3$, $OC\underline{H}_3$').

Monoester: 3-(Methoxycarbonyl)benzoic acid (3): Dimethyl isphthalate **2** was dissolved in 80 mL of methanol followed by addition of potassium hydroxide (KOH) (1.29 g,0.77 mmol) and left to stir overnight at room temperature. The methanol was evaporated and the remaining mixture was poured in water and extracted with dichloromethane. HCl was added to the resulting aqueous phase to reach a pH of 3.0. The resulting precipitate was filtered and dried in a vacuum dessicator to afford 2.27 g (42%) of the monoester **3**. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.77 (s, 1H, aryl), 8.76 (d, 2H, aryl), 7.59 (m, 1H, aryl), 3.96 (s, 3H, $OC\underline{H}_3$).

Monoester/ Amide: methyl 3-(dipropylcarbamoyl)benzoate (4): 3-(Methoxycarbonyl)benzoic acid **3** (2.27 g, 12.6 mmol) was dissolved in 22.6 mL of dichloromethane followed by addition of EDCI (2.41 g,12.6 mmol) and HOBt (2.04 g, 15.1 mmol) and left to stir for 5 minutes. Dipropyl amine (2.1 mL, 15.1 mmol) and

triethylamine (2.11 mL, 12.6 mmol) was added to the reaction solution and left to stir overnight at room temperature. The solution was then diluted with 10 mL of dichloromethane and washed with 1 M HCl, 0.1 M NaOH, and brine successively. The organic layer was collected, dried over anhydrous magnesium sulfate, filtered, and the solvent evaporated to afford 2.29 g (64%) of the amide/ester **4**. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.99 (m, 2H, aryl), 7.51 (t, 1H, aryl), 7.44 (s, 1H, aryl), 3.88 (s, 3H, OCH_3); 3.42 (t, 2H, NCH_2), 3.13 (t, 2H, NCH_2'), 1.65 (t, 2H, NCH_2CH_2), 1.47 (t, 2H, NCH_2CH_2'), 0.94 (t, 3H, CH_2CH_3), 0.72 (t, 3H, CH_2CH_3').

Amide/ Acid: 3-(dipropylcarbamoyl)benzoic acid (4): Methyl 3-(dipropylcarbamoyl)benzoate **4** (0.88 g, 3.09 mmol) was dissolved in 12.4 mL of methanol and 8.3 mL of H_2O followed by addition of sodium carbonate (1.64 g, 15.0 mmol) and the solution was left to stir overnight at room temperature. The methanol was evaporated and the solution was diluted with 30 mL of H_2O. The aqueous solution was extracted twice with dichloromethane. The resulting aqueous phase was then brought to a pH of 3.0 by adding concentrated HCl in order to protonate the acid. The aqueous layer was then extracted three time with chloroform and the organic layer collected was dried over anhydrous magnesium sulfate, filtered, and the solvent evaporated to yield 512 mg (61%) of the amide/acid **5**. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.12-7.49 (m, 4H, aryl), 3.47 (t, 2H, NCH_2), 3.15 (t, 2H, NCH_2'), 1.70 (m, 2H, CH_2CH_3), 1.53 (m, 2H, CH_2CH_3), 0.99 (t, 3H, CH_2CH_3), 0.67 (t, 3H, CH_2CH_3).

Leucine Series

(S)-Benzyl (1-(methoxy(methyl)amino)-4-methyl-1-oxopentan-2- yl) carbamate (7): N-(carbobenzyloxy)-L-leucine **6** (2.0 g, 7.5 mmol) was dissolved in 20 mL of dichloromethane followed by addition of EDCI (0.73 g, 7.5 mmol), dimethylhydroxylamine (1.05 g, 7.5 mmol), and triethylamine (1.4 g, 7.5 mmol). The mixture was left to stir at room temperature for 24 hours. After 24 hours the reaction mixture was poured into 1 M HCl and extracted twice with dichloromethane. The organic layer was washed with sodium bicarbonate and dried over anhydrous magnesium sulfate, filtered, and the solvent evaporated. The crude product was purified on the Combiflash with ethyl acetate and hexane with a gradual increase of 0 to 100% of ethyl acetate to afford 1.73 g of Weinreb amide **7** (75%). ^{1}H-NMR: (300 MHz, $CDCl_3$): δ 7.34-7.25 (m, 5H, aryl) 5.2 (d, 1H, N$\underline{H}$) 5.09 (dd, 2H, C$\underline{H}_2$O) 4.79 (m, 1H, C$\underline{H}$N) 3.79 (s, 3H, NC$\underline{H}_3$) 3.19 (s, 3H, OC$\underline{H}_3$) 1.71 (m, 1H, C$\underline{H}$(CH_3)$_2$) 1.46 (dd, 2H, CHC$\underline{H}_2$CH) 0.95 (dd, 6H, (C$\underline{H}_3$)$_2$).

(*R*)-2-(methoxy(methyl)amino)-4-methylpentanamide (8): The amide **7** (0.5 g, 1.62 mmol) was dissolved in 30 mL of methanol. Ammonium formate (1.53 g, 24.3 mmol) was added followed by 10% Pd on carbon (0.5 g). The mixture was left to stir at room temperature. After 45 minutes, the reaction mixture was filtered with celite under vacuum. The flask was washed with methanol and filtered through celite and the methanol was evaporated to reduce the volume to 5 mL. The mixture was then poured into 10 mL of sodium bicarbonate ($NaHCO_3$) and extracted four times with 20 mL of

ethyl acetate each time, dried over anhydrous magnesium sulfate, filtered and the solvent evaporated to afford 244 mg (86%) of the amine **8**.

***N*^{1}-(*N*-methoxy-*N*-methyl-*D*-leucyl)-*N*^{3},*N*^{3}-dipropylisophthalamide (9):** 3-(Dipropylcarbamoyl)benzoic acid **5** (0.19 g, 0.78 mmol) was dissolved in 3.5 mL of dichloromethane (CH_2Cl_2) and EDCI (0.15 g, 0.78 mmol) was added, followed by addition of the amine **8** and triethylamine. The reaction mixture was left to stir overnight at room temperature. The reaction mixture was then poured in sodium bicarbonate and extracted three times with ethyl acetate, and then washed with 1 M HCl. The collected organic layer was dried over anhydrous magnesium sulfate, filtered, and evaporated to yield 270 mg (86%) of the coupled amide **9**. ^{1}H-NMR(500 MHz, $CDCl_3$): δ 7.8(m, 2H, aryl), 7.41 (m,2H, aryl), 7.15(d, 1H, J=8.59, N$\underline{H}$), 5.2(s,1H, NHC$\underline{H}$), 3.81 (s, 3H, OC$\underline{H}_3$), 3.40(m, 2H, NC$\underline{H}_2$), 3.17 (s, 3H, NC$\underline{H}_3$), 3.06 (3H, NC$\underline{H}_2$'), 1. 69 (m, 5H, $NCH_2C\underline{H}_2$, $NCH_2C\underline{H}_2$', C$\underline{H}(CH_3)_2$), 1.46(m, 3H, $NCH_2CH_2C\underline{H}_3$), 1.19 (m, 2H, CHC$\underline{H}_2$), 0.95- 0.88 (dd, 6H, J=6.87, $CH(CH_3)_2$), 0.68 (t, J=6.87, 6H, $CH(C\underline{H}_3)_2$), 0.65 (t, 3H, $NCH_2CH_2C\underline{H}_3$'); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 174.0 (C=O), 170.5 (C=O), 167.5 (C=O), 138.0- 123.7 (aryl C), 60.1 (OCH_3), 50.5 (NCH_2), 48.7 (NCH_2'), 45.0 (NHCH), 42.5 (NCH_2CH2), 32.7 (NCH_3), 25.0 (NCH_2CH2'), 24.0-27.7 ($CH_2(CH_3)_2$), 21.0-22.5(NCH_2CH_2, NCH_2CH_2'); mass spectrum (ESI-MS) *m/z* ($C_{21}H_{34}N_3O_4$) calcd for 392.2543. Found 392.2543.

(*S*)-*N*^{1}-(2-isobutyl-3-oxopent-4-enoyl)-*N*^{3}, *N*^{3}-dipropylisophthalamide (10): The coupled amide **9** (0.27 g, 0.67 mmol) was dissolved in 1.8 mL of tetrahydrofuran (THF) and cooled to 0 °C under argon. Vinylmagnesium bromide in 1 M THF (2.13 mL,

2.13 mmol) was added to the solution and left to stir to room temperature for four hours. The reaction mixture was then poured into 1 M HCl and extracted three times with ethyl acetate. The organic layer was washed with sodium bicarbonate, dried over magnesium sulfate, filtered and evaporated. The crude product collected was purified on the Combiflash with ethyl acetate and hexane with a gradual increase of ethyl acetate to 100% to afford 0.123 g (49%) of the enone **10**. $[\alpha]_D^{25}$= +30.05° (*c* 0.03, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.77-7.45 (m, 4H, aryl), 7.15(d, 1H, J=8.59, NH), 6.49 (M, 2H, CH=CH_2), 5.9 (m, 1H, CH=CH_2), 5.2(s,1H, NHCH), 3.43(t, J=7.45, 2H, NCH_2), 3.11 (t, J=6.87, 2H, NCH_2'), 1.68 (m, 2H, NCH_2CH_2), 1.53 (m, 2H, NCH_2CH_2'), 0.93 (m, 3H, $NCH_2CH_2CH_3$), 1.19 (m, 2H, $CHCH_2$), 1.02-0.9 (dd, 6H, J=6.30, $CH(CH_3)_2$, $NCH_2CH_2CH_3$), 0.72 (t, 3H, $NCH_2CH_2CH_3$'); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 198.5 (C=O),171.0 (C=O), 168.5 (C=O), 138.0- 123.7 (aryl C), 133.0 (CH=CH_2), 131.2 (CH=CH_2), 60.1 (OCH_3), 57.5 (NCH_2), 51.7 (NCH_2'), 46.8 (NHCH), 42.5 (NCH_2CH2), 25.0 (NCH_2CH2'), 24.0-27.7 ($CH_2(CH_3)_2$), 21.0-22.5(NCH_2CH_2, NCH_2CH_2'), 12.0 ($NCH_2CH_2CH_3$); mass spectrum (ESI-MS) *m/z* ($C_{22}H_{33}N_2O_3$) calcd for 373.2485. Found 373.2499.

N^1-((2*S*)-4-methyl-2-(2-nitrocyclopropane-1-carbonyl)pentanoyl)-N^3,N^3-dipropylisophthalamide (11): The enone **10** (0.123 g, 0.33 mmol) was dissolved in 3 mL of acetonitrile (CH_3CN). Potassium carbonate (0.045 g, 0.33 mmol) was crushed in a mortar and pestle, and added to the solution, followed by addition of bromonitromethane (0.046 g, 0.33 mmol). The mixture was stirred at 0° C for the first hour after which another equivalent of bromonitromethane was added and the ice-bath removed to allow

the reaction solution to warm up to room temperature. Half an hour after the ice bath was removed and one more equivalent of bromonitomethane was added to the solution. After three hours the reaction mixture was extracted twice with ethyl acetate, washed with brine, dried over anhydrous magnesium sulfate, filtered and evaporated. The collected crude product was purified on Combiflash with an increasing gradient of ethyl acetate reaching 100% to get 63 mg (44%) of the nitrocyclopropyl **11**. ^{1}H-NMR: (500 MHz, $CDCl_3$): δ 7.77-7.45 (m, 4H, aryl), 7.15(d, 1H, J=8.59, NH), 4.9 (m, 1H, NHCH) , 4.57 (m, 1H, cyclopropylC$\underline{H}NO_2$), 3.42 (m, 3H, NC$\underline{H}_2$'), 3.14(m, 4H, NC$\underline{H}_2$, CHC=OC$\underline{H}$), 2.08 (m, 2H, cyclopropylC$\underline{H}_2$), 1.75-1.52 (m, 2H, $NCH_2C\underline{H}2$), $NCH_2C\underline{H}2$',), 1.25 (m, 2H, CHC$\underline{H}_2$), 1.27-0.9 (dd, J=6.30, 9H, $CH(C\underline{H}_3)_2$, $NCH_2CH_2C\underline{H}_3$), 0.72 (t, 3H, $NCH_2CH_2C\underline{H}_3$'); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 198.5 ($\underline{C}$=O), 171.0 ($\underline{C}$=O), 168.5 ($\underline{C}$=O), 138.0- 123.7 (aryl C), 60.6 ($\underline{C}HNO_2$), 58.4 (NH$\underline{C}$H), 50.75(N$\underline{C}H_2$), 46.5 (N$\underline{C}H_2$'), 28.9 (cyclopropyl $\underline{C}$H), 19.8 (cyclopropyl $\underline{C}H_2$).

***N*^{1}-((2*S*)-2-(hydroxy(2-nitrocyclopropyl)methyl)-4-methylpentanoyl)-*N*^{3},*N*^{3}-dipropylisophthalamide (12):** The nitrocyclopropyl ketone **11** (.063 g, 0.15 mmol) was dissolved in 4 mL of methanol and $NaBH_4$ (0.048 g, 1.28 mmol) was added. The reaction mixture was left to stir at room temperature. After 3 hours the reaction mixture was extracted twice with ethyl acetate, washed with brine, dried over anhydrous magnesium sulfate, filtered and evaporated to afford 63 mg of the alcohol **12** (97% yield). ^{1}H-NMR: (500 MHz, $CDCl_3$): δ 7.77-7.45 (m, 4H, aryl), 6.90(d, 1H, NH), 4.5 (s, 1H, OH), 4.37-δ4.27 (m, 4H, NHC$\underline{H}$, cyclopropylC$\underline{H}NO_2$), 3.84 (m, 1H, CHOH), 3.6 (m, 1H, C$\underline{H}$OH) 3.48 (m, 2H, NC$\underline{H}_2$, cyclopropylC$\underline{H}$), 3.12(m, 2H, NC$\underline{H}_2$), 2.04, 1.63 (m, 2H, cyclopropylC$\underline{H}_2$), 1.75-1.28 (m, 2H, $NCH_2C\underline{H}_2$, $NCH_2C\underline{H}_2$'), 1.25 (m, 2H, CHC$\underline{H}_2$),

0.97-0.90 (dd, 10H, $CH(C\underline{H}_3)_2$, $NCH_2CH_2C\underline{H}_3$, $C\underline{H}CH_2$), 0.74 (t, 3H, $NCH_2CH_2C\underline{H}_3$'); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 171.0 (C=O), 168.5 ($\underline{C}$=O), 138.0- 123.7 (aryl C), 70.2 ($\underline{C}$HOH), 70.1 ($\underline{C}HNO_2$), 58.4 (NH$\underline{C}$H), 50.75(NCH_2), 46.5 (N$\underline{C}H_2$'), δ28.9 (cyclopropyl CH), 15.8 (cyclopropyl $\underline{C}H_2$).

***N*^{1}-((2*S*)-2-((2-aminocyclopropyl)(hydroxy)methyl)-4-methylpentanoyl)-*N*^{3},*N*^{3}-dipropylisophthalamide (13):** The nitrocyclopropyl **12** (0.06 g, 0.14 mmol) was dissolved in 5 mL of methanol followed by addition of ammonium formate (0.130 g, 2.07 mmol) and 10% palladium on charcoal (0.60 g) and left to stir at room temperature. After an hour the reaction solution was filtered with celite under vacuum. The flask was washed with methanol and filtered through celite, and the methanol solution was evaporated to reduce the volume to 5 mL. The mixture was then poured into aqueous sodium bicarbonate ($NaHCO_3$) and extracted 4 times with ethyl acetate, dried over anhydrous magnesium sulfate, filtered and the solvent evaporated to afford 29.8 mg (51%) of the amine **13**. ^{1}H-NMR: (500 MHz, $CDCl_3$): δ 7.74-7.30 (m, 4H, aryl), 6.77 (d, 1H, N$\underline{H}$), 4.66 (m, 1H, OH), 4.37 (m, 1H, NHC$\underline{H}$), 4.0 (cyclopropylC$\underline{H}NH_2$), 3.84 (m, 1H, CHOH), 3.43 (m, 2H, NC$\underline{H}_2$), 3.12(m, 2H, NC$\underline{H}_2$'), 1.68, 1.24 (m, 2H, cyclopropylC$\underline{H}_2$), 1.65-1.06 (m, $NCH_2C\underline{H}_2$, $NCH_2C\underline{H}$2', 2H, $CHC\underline{H}_2$), 1.67 (m, $C\underline{H}CH_2$) 0.97-0.90 (dd, $CH(C\underline{H}_3)_2$, $NCH_2CH_2C\underline{H}_3$), 0.74 (t, 3H, $NCH_2CH_2C\underline{H}_3$'); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 171.0 (C=O), 168.5 ($\underline{C}$=O), 140.0- 128.0 (aryl C), 54.9 ($\underline{C}$HOH), 50.9 ($\underline{C}HNH_2$), 42.0 (N$\underline{C}H_2$), 50.9 (NH$\underline{C}$H), 48.7 (N$\underline{C}H_2$'), 38.0 (cyclopropyl $\underline{C}$H), 20.7 (cyclopropyl $\underline{C}H_2$), 11.56 ($CH(\underline{C}H_3)_2$).

N^1-((2*S*)-2-((2-aminocyclopropyl)(hydroxy)methyl)-4-methylpentanoyl)-N^3,N^3-dipropylisophthalamide (14): Phenyl acetic acid (0.009 g, 0.07 mmol), EDCI (0.013 g, 0.07 mmol), and HOBT (0.009 g, 0.07 mmol) was dissolved in 1.5 mL CH_2Cl_2 and the misture was left to stir at room temperature. After an hour the amine **13** (0.029 g, 0.07 mmol) dissolved in 1.5 mL of CH_2Cl_2 was added to the mixture and left to stir overnight. The reaction mixture was then poured in sodium bicarbonate and extracted 3 times with ethyl acetate, and washed with 1 M HCl. The collected organic layer was dried over anhydrous magnesium sulfate, filtered, and evaporated to yield 55.6 mg crude product which was purified by eluting a silica gel column with 1:4, 1:2, 2:1, 4:1, 6:1, and 1:0 ethyl acetate/hexane to afford 12.4 mg (34%) of the purified amide **14**. ^{1}H-NMR: (500 MHz, $CDCl_3$): δ 7.90-7.05 (m, 7H, aryl), 6.77 (1H, N<u>H</u>), 6.05 (1H, NHC<u>H</u>), 5.85(m, 1H, NH'), 4.62 (m, 1H, OH), 4.37 (m, 1H, NHC<u>H</u>), 4.0 (cyclopropylC<u>H</u>NH_2), 3.58 (m, 2H, NC<u>H_2</u>), 3.52 (m, 2H, NC<u>H_2</u>'), 3.03 (m, 1H, C<u>H</u>OH), 1.68, 1.40 (m, 2H, cyclopropylC<u>H_2</u>), 1.69 (C<u>H_2</u>Ph), 1.40-1.06 (m, $NCH_2C\underline{H}_2$, $NCH_2C\underline{H}$2', 2H, $CHC\underline{H}_2$), 0.97-0.79 (m, $CH(C\underline{H}_3)_2$, $NCH_2CH_2C\underline{H}_3$), 0.74 (t, 3H, $NCH_2CH_2C\underline{H}_3$'); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 171.0 (C=O), 168.5 (<u>C</u>=O), 140.0- 128.0 (aryl C), 66.9 (<u>C</u>HOH), 61.6 (<u>C</u>HNH_2), 42.0 (N<u>C</u>H_2), 55.7 (NH<u>C</u>H), 49.6 (N<u>C</u>H_2'), 29.78 (<u>C</u>H_2PH), 31.9 (cyclopropyl <u>C</u>H), 19.4 (cyclopropyl <u>C</u>H_2), 14.2 ($CH(\underline{C}H_3)_2$); mass spectrum (ESI-MS) *m/z* ($C_{31}H_{44}N_3O_4$) calcd for 522.3326. Found 522.3330.

Chapter III: Results and Discussion

The overall goal of this project was the successful synthesis of the proposed "reverse" amide analogs. As mentioned earlier, the previous attempts in working with the nitrocyclopropyl has proven to be difficult and it hasn't been possible to synthesize these reverse amides. Initially, the same synthetic route was followed, which included the synthesis of the nitrocyclopropyl core. Several attempts were made to find suitable conditions for the selective reduction of the nitro group. However, that did not work out and a new route was explored by re-arranging the steps to avoid the reduction of the nitro group while reducing the Cbz protecting group. Hence, the new route started with the synthesis of a Weinreb amide **7** and the Cbz protecting group was removed by transfer hydrogenation. The resulting amine **8** was then coupled with the isophthalamide side chain. The question then was whether the resulting compound **9** could be used to carry out the rest of the steps.

The enone **10** was successfully synthesized, which was the first step in the four-step synthesis of the nitrocyclopropyl core. The next two steps, cyclopropanation and ketone reduction, were carried out to successfully synthesize the core **12**. As the Cbz protecting group had already been removed, the issue with non-selective reduction of the nitro group was avoided. The resulting amine **13** was then coupled with phenyl acetic acid and the synthesis of one of the proposed amide analogs **14** was successfully completed following the new scheme. While previous attempts at getting to the product were unsuccessful, this project focused on developing an alternative route and it proved

to be successful. Removing the protecting Cbz group to form the amine and coupling it to the isophthalamide side chain before the synthesis of the nitrocyclopropyl core eliminates the issue of reducing the the nitrocyclopropyl while also removing the Cbz group.

Previous compounds synthesized in this lab based on the ester cyclopropyl series have shown activity against BACE1 in an enzymatic assay. This is the very first "reverse" amide analog that has been successfully synthesized following the nitrocyclopropyl route. The next step would be to optimize the low yield steps. These include reduction of the nitro group to give the amine and the final coupling of the amine to the phenyl acetic acid. There is also scope for optimization of the route to get an overall high yield. Also upon synthesis of several analogs, an enzymatic assay of these reverse amides inhibitors on BACE1 activity will give insight into its effectiveness as an inhibitor and show whether the orientation of the amide group effects the activity of these BACE inhibitors.

References

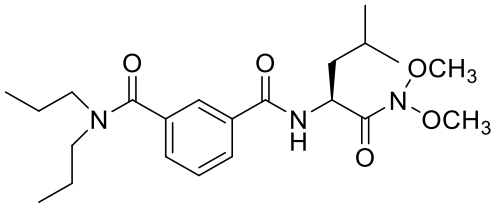
1. Oehlrich, D., Prokopcova, H., & Gijsen, H. M. The evolution of amidine-based brain penetrant BACE1 inhibitors. *Bioorganic & Medicinal Chemistry Letters*, 2014. *24*(9), 2033-2045. doi:10.1016/j.bmcl.2014.03.025
2. Gaugler, J., James, B., Johnson, T., Scholz, K., & Weuve, J. (2016). 2016 Alzheimer's disease facts and figures. *Alzheimer's And Dementia*, *12*(4), 459-509. doi:10.1016/j.jalz.2016.03.001
3. Europe's health priorities for the world. *Lancet*. 1912, Nov. 27, 2004.
4. Karran, E., Mercken, M., & Strooper, B. D. The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. *Nature Reviews Drug Discovery*, 2011. *10*(9), 698-712. doi:10.1038/nrd3505.
5. Alzheimer, Alois. A Characteristic Disease of the Cerebral Cortex. Medizin. 1907, 44, 146-148.
6. Small, D. H., & Cappai, R. Alois Alzheimer and Alzheimer's disease: a centennial perspective. *Journal Of Neurochemistry*, 2006. *99*(3), 708-710.
7. Glenner G.G, Wong C.W.Alzheimer's disease and Down's syndrome: Sharing of a unique cerebrovascular amyloid fibril protein. *Biochem. And Biophys. Research Commu.* August 16, 122(3):1131-5.
8. Giasson, B. I., Lee, V. M., & Trojanowski, J. QInteractions of amyloidogenic proteins. *Neuromolecular Medicine*, 2003. 4(1-2), 49-58.
9. Roggo, S. (2002). Inhibition of BACE, a promising approach to Alzheimer's disease therapy. *Current Topics In Medicinal Chemistry*, 2(4). Apr. 1, 2002, 359-370.
10. Hardy, John A., and Gerald A. Higgins. "Alzheimer's disease: the amyloid cascade hypothesis." *Science*, vol. 256, no. 5054, 1992, p. 184+.
11. Vassar, R., & Citron, M. Aβ-generating enzymes: Recent advances in β- and γ-secretase research. *Neuron*. 2000, *27*(3), 419-422.
12. Selkoe, D. J. Alzheimer's disease: genes, proteins, and therapy. *Physiological Reviews*. 2011, *81*(2), 741-766.
13. Johnston, J. A., Liu, W. W., Todd, S. A., Coulson, D. R., Murphy, S., Irvine, G. B., & Passmore, A. P. Expression and activity of beta-site amyloid precursor protein cleaving enzyme in Alzheimer's disease. *Biochemical Society Transactions*, 2005, *33*(Pt 5), 1096-1100.
14. Vassar, R., Kovacs, D. M., Yan, R., & Wong, P. C. The β-secretase enzyme BACE in health and Alzheimer's disease: Regulation, cell biology, function, and therapeutic potential. *The Journal Of Neuroscience*, 2009. *29*(41), 12787-12794. doi:10.1523/JNEUROSCI.3657-09.2009
15. Bennett, B. D., Babu-Khan, S., Loeloff, R., Louis, J. C., Curran, E., Citron, M., & Vassar, R. Expression analysis of BACE2 in brain and peripheral tissues. *The Journal Of Biological Chemistry*, 2000. *275*(27), 20647-20651.
16. Cai, H., Wang, Y., McCarthy, D., Wen, H., Borchelt, D. R., Price, D. L., & Wong, P. C. (2001). BACE1 is the major β-secretase for generation of Aβ peptides by neurons. *Nature Neuroscience*, *4*(3), 233.
17. Luo, Y., Bolon, B., Kahn, S., Bennett, B. D., Babu-Khan, S., Denis, P., & ...

Vassar, R. Mice deficient in BACE1, the Alzheimer's β-secretase, have normal phenotype and abolished β-amyloid generation. *Nature Neuroscience*, 2001. *4*(3), 231.
18. Shimizu, H., Tosaki, A., Kaneko, K., Hisano, T., Sakurai, T., & Nukina, N. Crystal Structure of an Active Form of BACE1, an Enzyme Responsible for Amyloid β Protein Production. *Molecular & Cellular Biology*, 2008. *28*(11), 3663-3671. doi:10.1128/MCB.02185-07.
19. Hong, L., & Tang, J. Flap Position of Free Memapsin 2 (β-Secretase), a Model for Flap Opening in Aspartic Protease Catalysis. *Biochemistry*, 2004. *43*(16), 4689-4695.
20. Gorfe, A. A., & Caflisch, A. Functional Plasticity in the Substrate Binding Site of β-Secretase. *Structure*, 2005. *13*(10), 1487-1498. doi:10.1016/j.str.2005.06.015.
21. Hardy, J., & Selkoe, D. J. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science (New York, N.Y.)*, 2002, *297*(5580), 353-356.
22. Patick, A. K., & Potts, K. E. Protease inhibitors as antiviral agents. *Clinical Microbiology Reviews*, 1998. *11*(4), 614-627.
23. Zhu, Z., Sun, Z., Ye, Y., Voigt, J., Strickland, C., Smith, E. M., & ... Hunter, J. C. Discovery of cyclic acylguanidines as highly potent and selective beta-site amyloid cleaving enzyme (BACE) inhibitors: Part I--inhibitor design and validation. *Journal Of Medicinal Chemistry*, 2010. *53*(3), 951-965. doi:10.1021/jm901408p
24. Hong, L., Koelsch, G., & Lin, X. Structure of the protease domain of memapsin 2 (β-secretase) complexed with inhibitor. *Science*, 2000. *290*(5489), 150-153
25. Stachel, S. J. Progress toward the Development of a Viable BACE-1 Inhibitor. Merck Research Laboratories. Department of Medicinal Chemistry. DRUG DEVELOPMENT RESEARCH 70 : 101–110 (2009).
26. Hong, L., Koelsch, G., & Lin, X. Structure of the protease domain of memapsin 2 (β-secretase) complexed with inhibitor. *Science*, 2000. *290*(5489), 150-153.
27. Yang, W., Lu, W., Lu, Y., Zhong, M., Sun, J., Thomas, A. E., & ... Ballinger, M. D. Aminoethylenes: a tetrahedral intermediate isostere yielding potent inhibitors of the aspartyl protease BACE-1. *Journal Of Medicinal Chemistry*, 2006. *49*(3), 839-842.
28. Albert, J. S. Progress in the development of beta-secretase inhibitors for Alzheimer's disease. *Progress In Medicinal Chemistry*, 2009. *48*133-161.
29. Ghosh, A. K., Kumaragurubaran, N., Hong, L., Kulkarni, S. S., Xu, X., Chang, W., & ... Tang, J. Design, synthesis, and X-ray structure of potent memapsin 2 (beta-secretase) inhibitors with isophthalamide derivatives as the P2-P3-ligands. *Journal Of Medicinal Chemistry*, 2007. *50*(10), 2399-2407.
30. Dunlap, N., Lankford, K. R., Pathiranage, A. L., Taylor, J., Reddy, N., Gouger, D., & ... Reibenspies, J. Three-step synthesis of Cyclopropyl Peptidomimetics. *Organic Letters*, 2011. *13*(18), 4879-4881. doi:10.1021/ol201828u
31. Martin, S. F., Dorsey, G. O., Gane, T., Hillier, M. C., Kessler, H., Baur, M., & ... Topol, I. A. Cyclopropane-derived peptidomimetics. Design, synthesis, evaluation, and structure of novel HIV-1 protease inhibitors. *Journal Of*

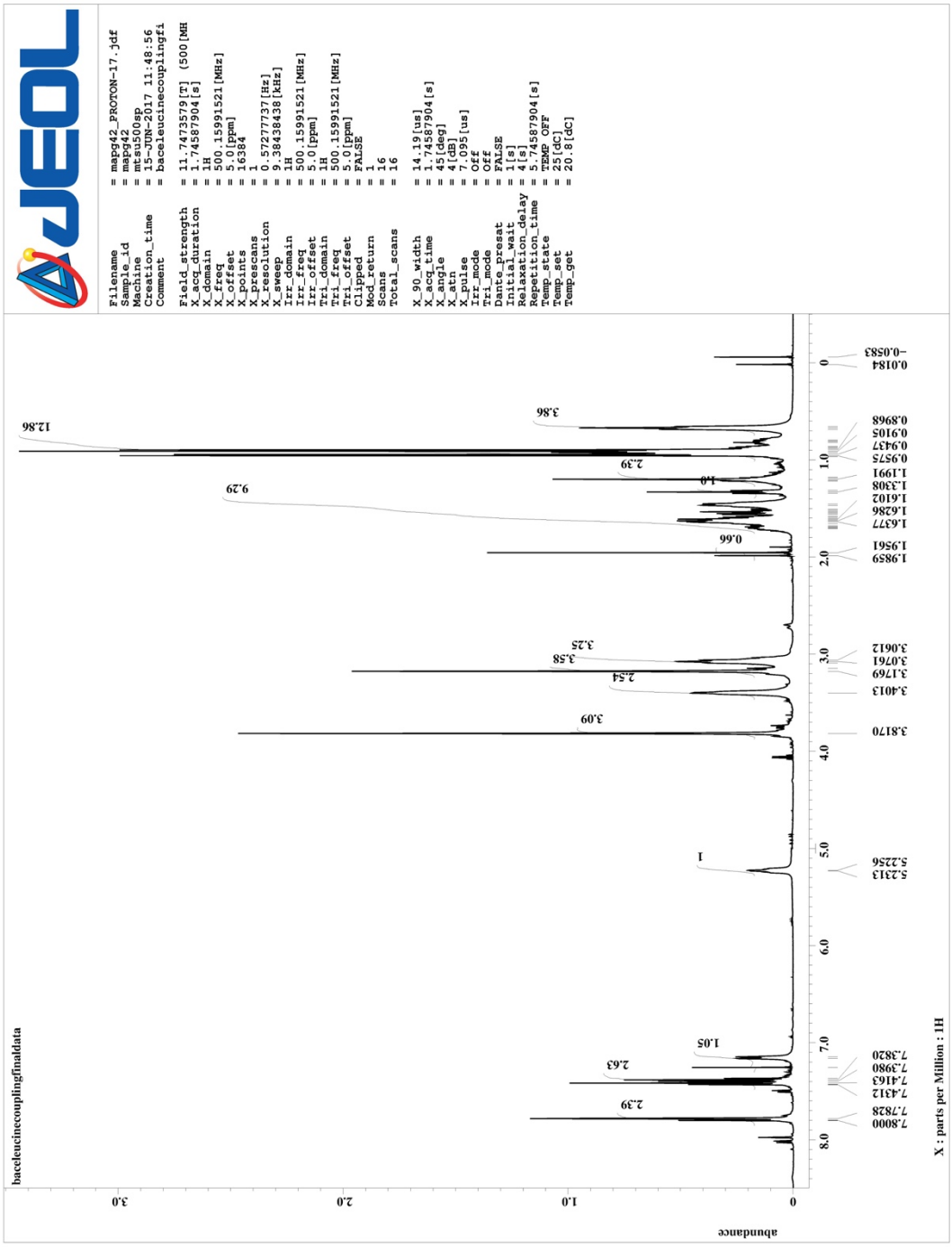
Medicinal Chemistry, 1998. *41*(10), 1581-1597.
32. Hillier, M. C., Davidson, J. P., & Martin, S. F. Cyclopropane-derived peptidomimetics. design, synthesis, and evaluation of novel Ras farnesyltransferase inhibitors. *The Journal Of Organic Chemistry*, 2011. *66*(5), 1657-1671.
33. Wipf, P., & Xiao, J. Convergent approach to (E)-alkene and cyclopropane peptide isosteres. *Organic Letters*, 2005. *7*(1), 103-106.

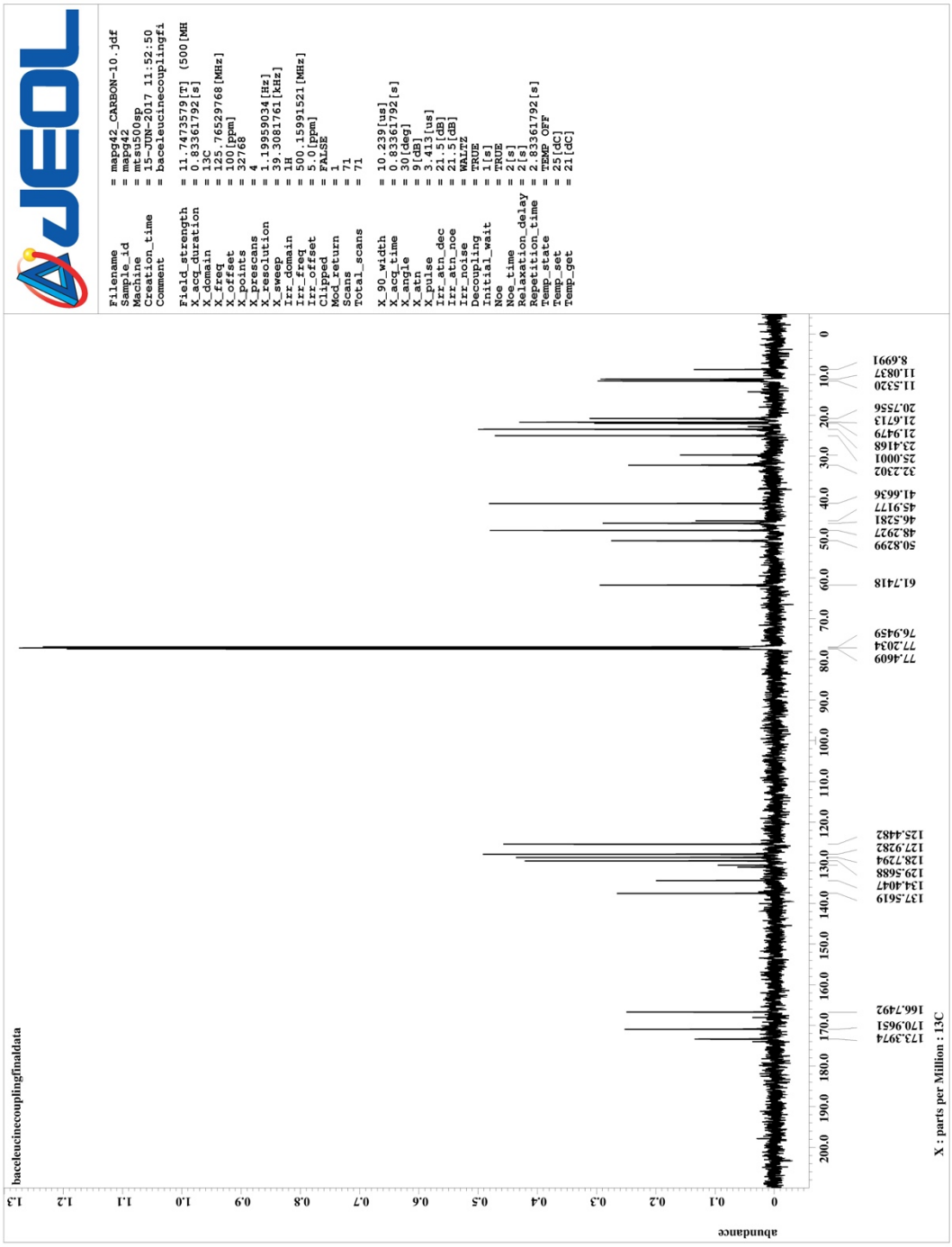
Appendix: NMR Spectroscopy Data

N^1-(*N*-methoxy-*N*-methyl-*D*-leucyl)-N^3,N^3-dipropylisophthalamide (9):

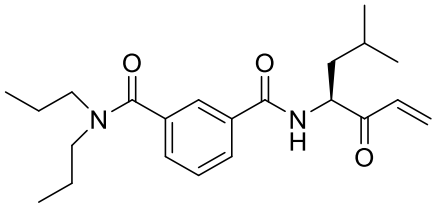


- ^{1}H NMR (500 MHz, $CDCl_3$)
- ^{13}C NMR (125 MHz, $CDCl_3$)

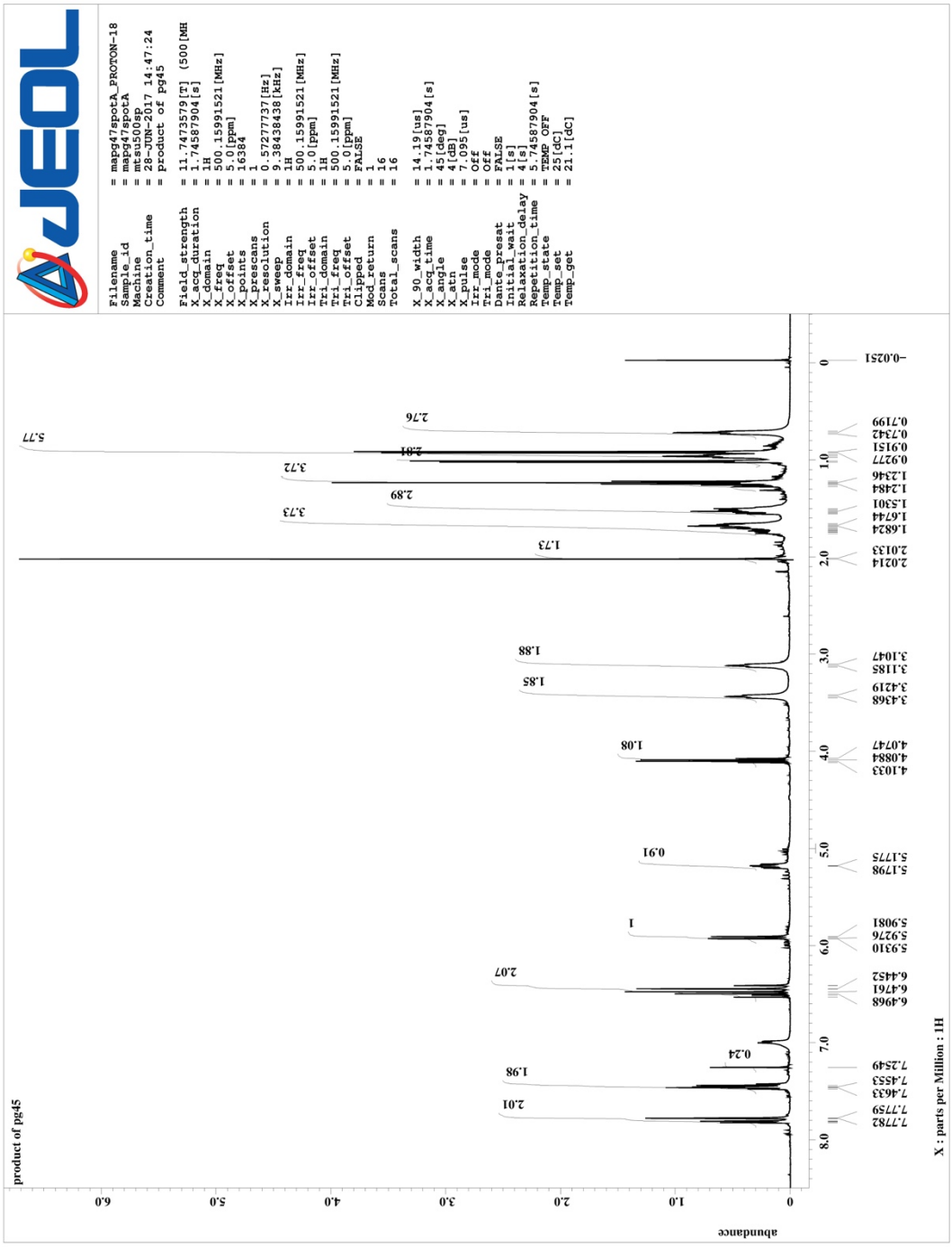

JEOL
Filename = mapg42_PROTON-17.jdf
Sample_id = mapg42
Machine = ms500sp
Creation_time = 15-JUN-2017 11:48:56
Comment = baceleucinecouplingfi
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[kHz]
X_window = 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 = 20.8[dc]
baceleucinecouplingfinaldata
abundance
X : parts per Million : 1H

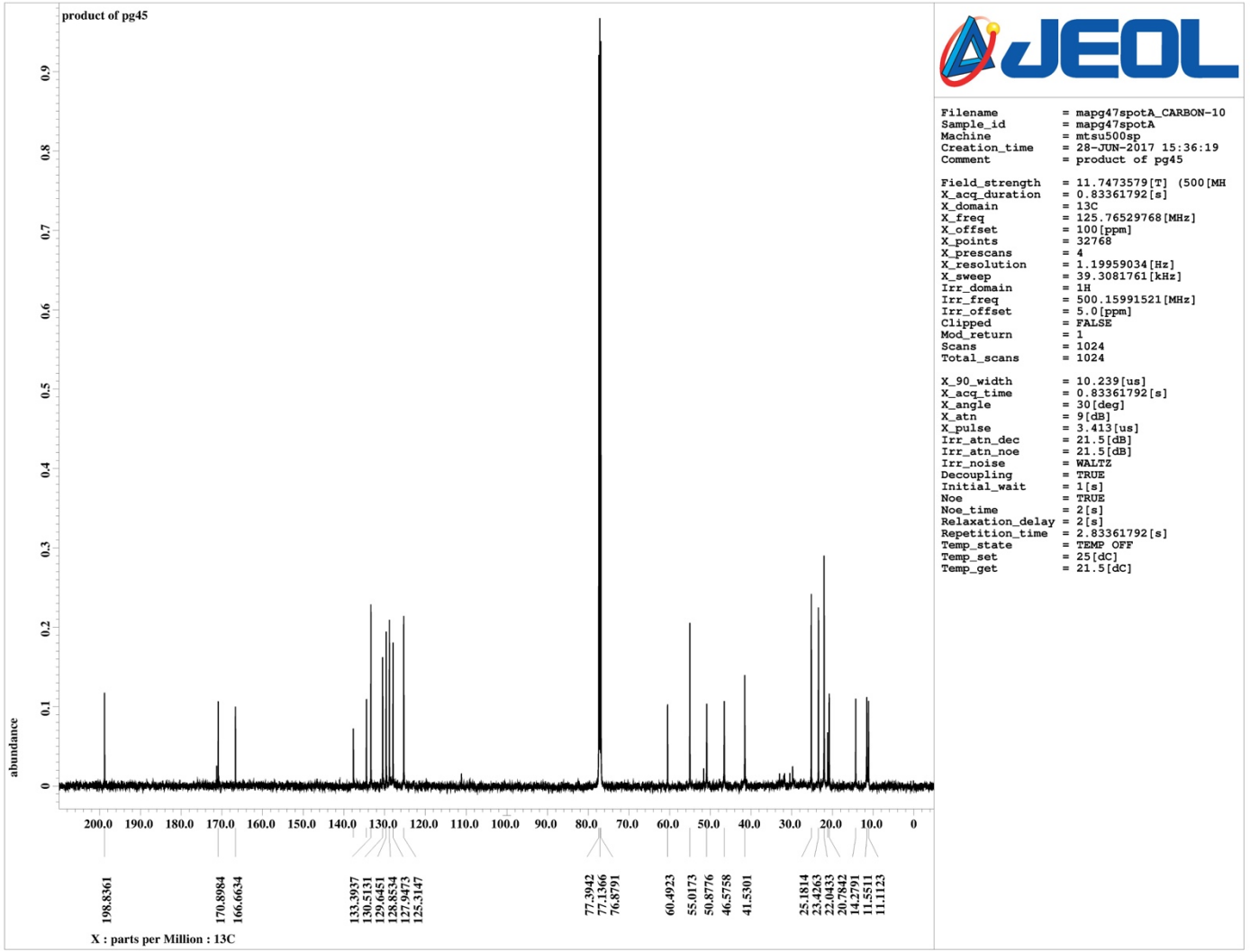

JEOL
Filename = mapg42_CARBON-10.jdf
Sample_id = mapg42
Machine = ms500sp
Creation_time = 15-JUN-2017 11:52:50
Comment = baceleucinecouplingfi
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.30817761[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 71
Total_scans = 71
X_90_width = 10.239[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 9[dB]
X_pulse = 3.41[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[dC]
baceleucinecouplingfinaldata
abundance
X : parts per Million : 13C
173.3974
170.9651
166.7492
137.5619
134.4047
129.5688
128.7294
127.9282
125.4482
77.4609
77.2034
76.9459
61.7418
50.8299
48.2927
46.5281
45.9177
41.6636
32.2302
25.0001
23.4168
21.9479
21.6713
20.7556
11.5320
11.0837
8.6991

(*S*)-N^1-(2-isobutyl-3-oxopent-4-enoyl)-N^3,N^3-dipropylisophthalamide (10):



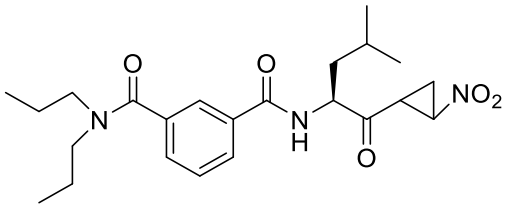
- ^{1}H NMR (500 MHz, $CDCl_3$)
- ^{13}C NMR (125 MHz, $CDCl_3$)


JEOL
Filename = mapg47spotA_PROTON-18
Sample_id = mapg47spotA
Machine = ms500pp
Creation_time = 28-JUN-2017 14:47:24
Comment = product of pg45
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]
X_window = 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]
product of pg45
abundance
X : parts per Million : 1H
7.7782
7.7759
7.4633
7.4553
7.2549
6.4968
6.4761
6.4452
5.9310
5.9276
5.9081
5.1798
5.1775
4.1033
4.0884
4.0747
3.4368
3.4219
3.1185
3.1047
2.0214
2.0133
1.6824
1.6744
1.5301
1.2484
1.2346
0.9277
0.9151
0.7342
0.7199
-0.0251
2.01
1.98
0.24
2.07
1
0.91
1.08
1.85
1.88
1.73
3.73
2.89
3.72
2.81
2.76
5.77

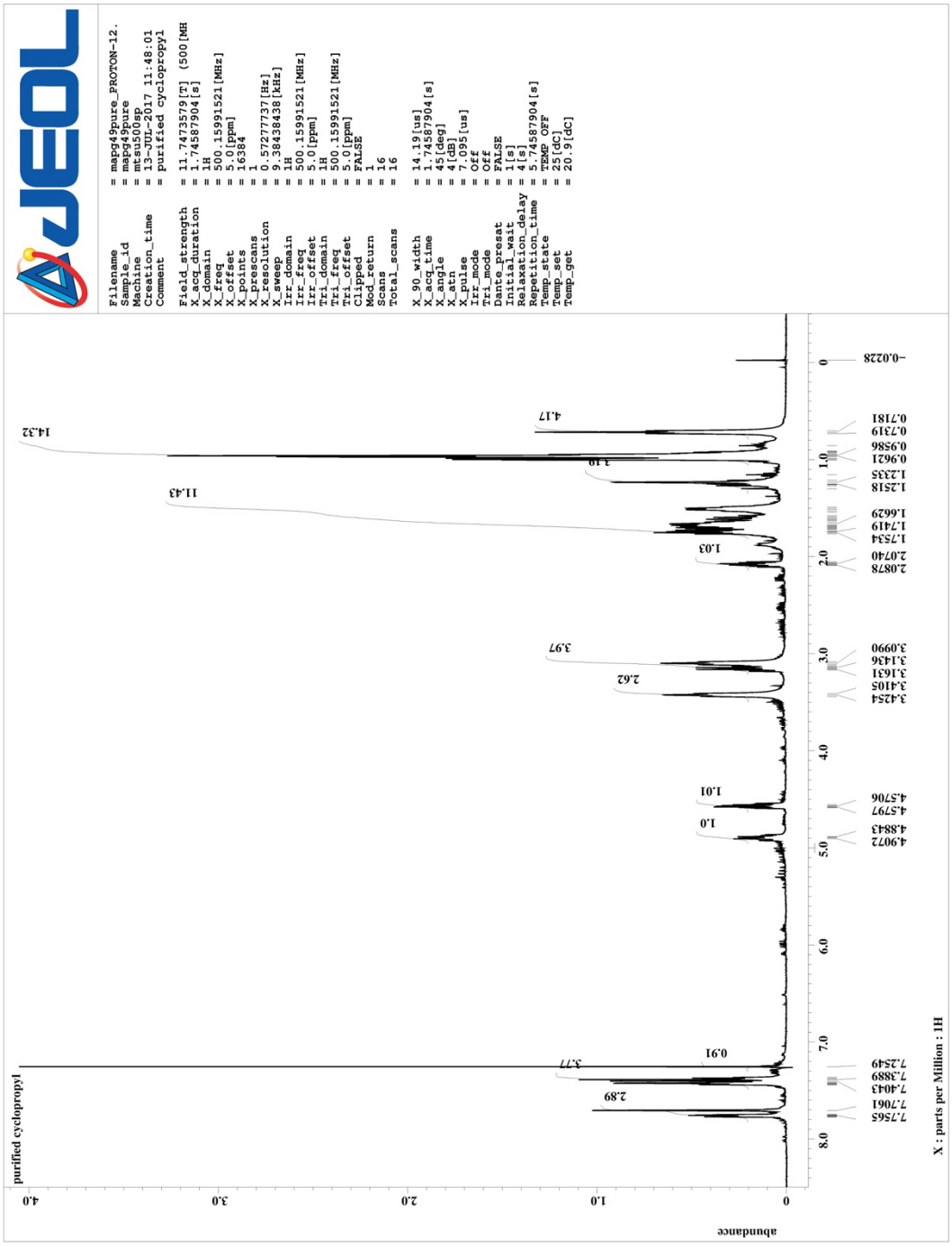


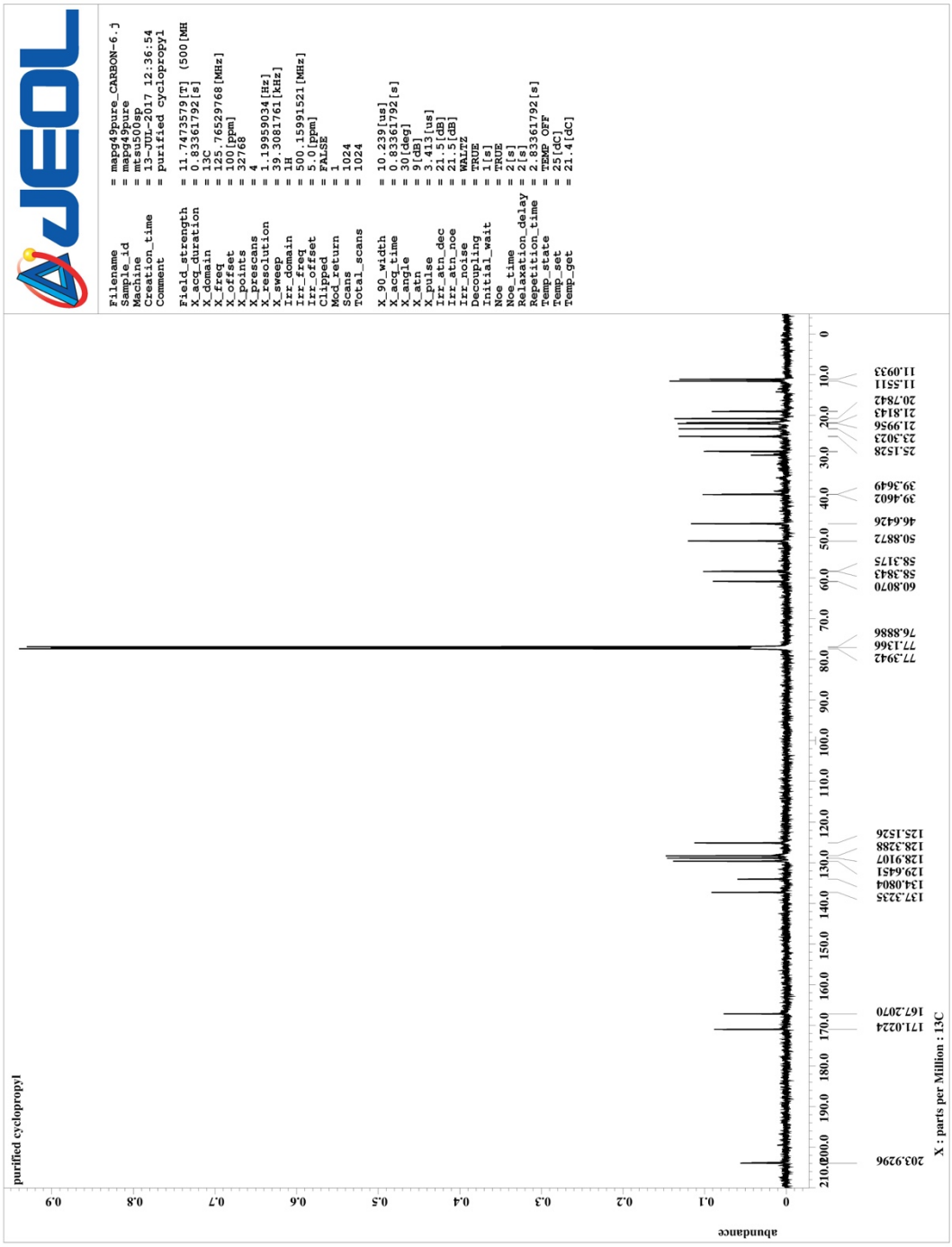

```
Filename          = mapg47spotA_CARBON-10
Sample_id         = mapg47spotA
Machine           = mtsu500sp
Creation_time     = 28-JUN-2017 15:36:19
Comment           = product of pg45

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             = 1024
Total_scans       = 1024

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          = 21.5[dC]
```

N^1-((2*S*)-4-methyl-2-(2-nitrocyclopropane-1-carbonyl)pentanoyl)-N^3,N^3-dipropylisophthalamide (11):

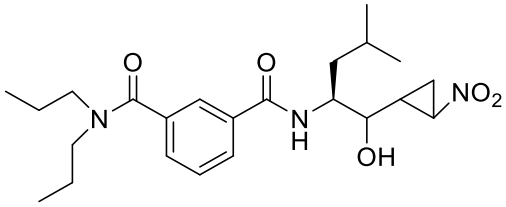


- ^{1}H NMR (500 MHz, $CDCl_3$)
- ^{13}C NMR (125 MHz, $CDCl_3$)

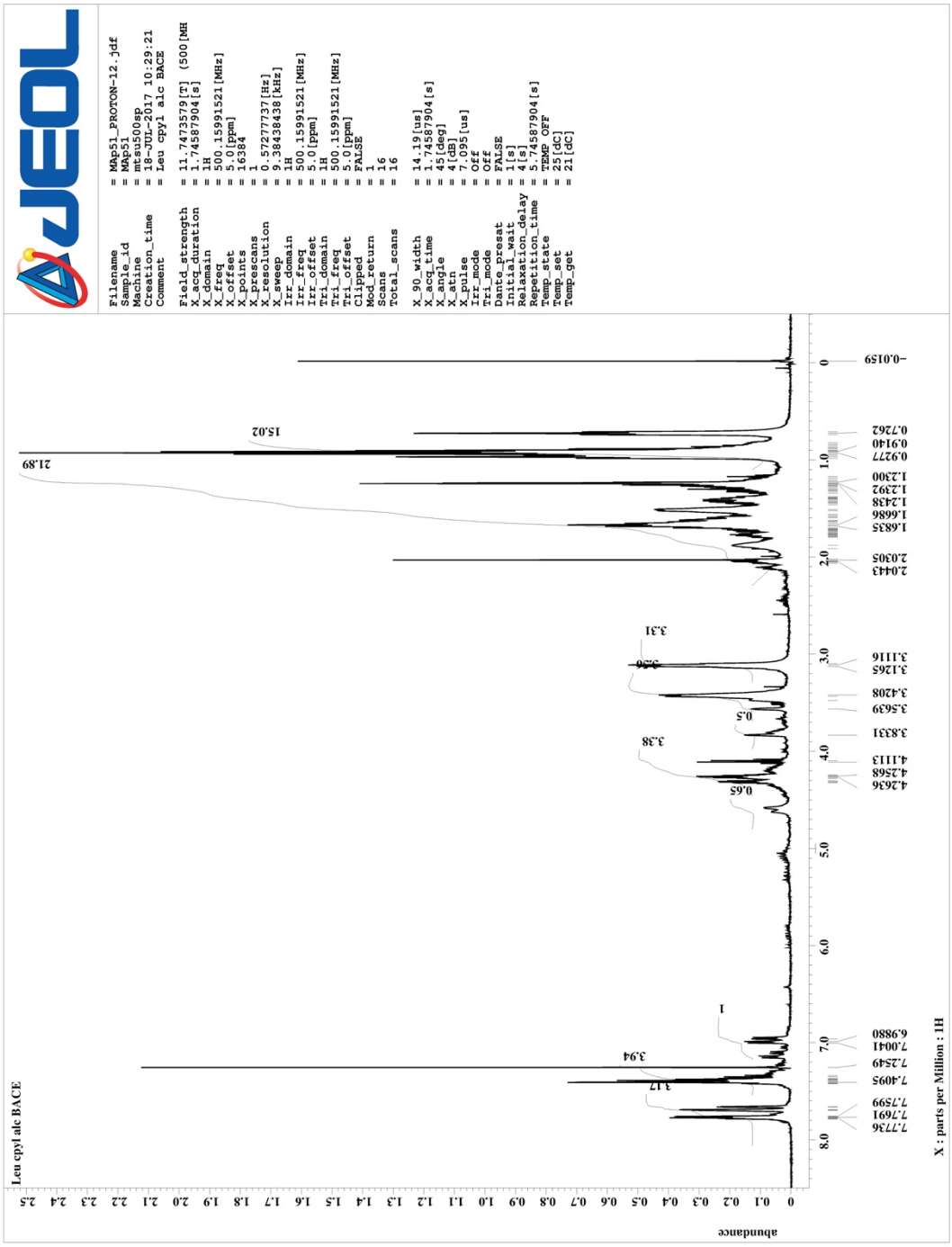

JEOL
Filename = mapg49pure_PROTON-12.
Sample_id = mapg49pure
Machine = ms-500sp
Creation_time = 13-JUL-2017 11:48:01
Comment = purified cyclopropyl
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[kHz]
X_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 = 20.9[dc]
purified cyclopropyl
abundance
X : parts per Million : 1H
14.32
11.43
4.17
3.19
3.97
2.62
1.03
1.01
1.0
3.77
2.89
0.91
7.7565
7.7061
7.4043
7.3889
7.2549
4.9072
4.8843
4.5797
4.5706
3.4254
3.4105
3.1631
3.1436
3.0990
2.0878
2.0740
1.7534
1.7419
1.6629
1.2518
1.2335
0.9621
0.9586
0.7319
0.7181
-0.0228

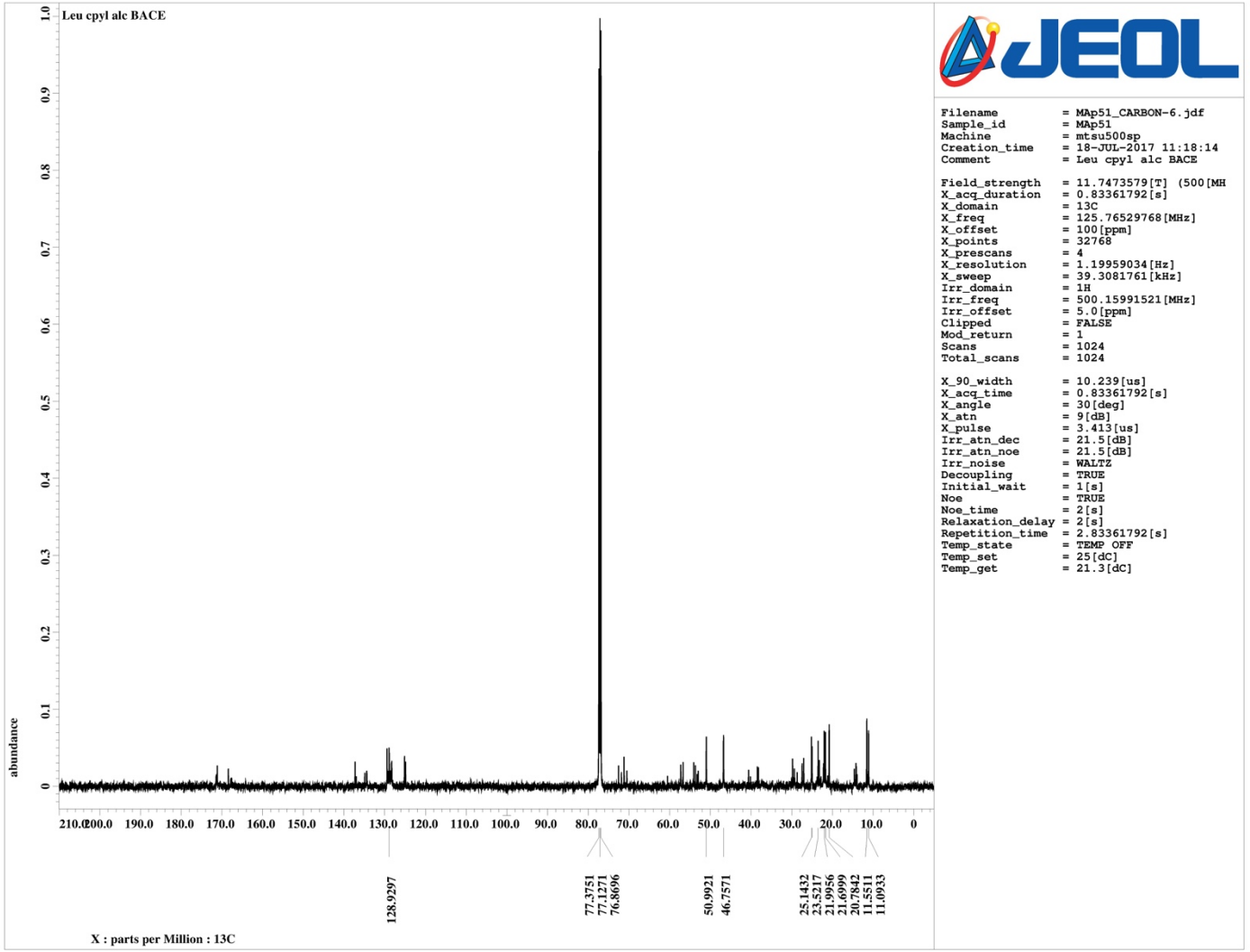

JEOL
Filename = mapg49pure_CARBON-6.j
Sample_id = mapg49pure
Machine = ms-500sp
Creation_time = 13-JUL-2017 12:36:54
Comment = purified cyclopropyl
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.33081761[kHz]
X_window = EM
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = FALSE
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 = 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 = 21.4[dC]
purified cyclopropyl
abundance
X : parts per Million : 13C
203.9296
171.0224
167.2070
137.3235
134.0804
129.6451
128.9107
128.3288
125.1526
77.3942
77.1366
76.8886
60.8070
58.3843
58.3175
50.8872
46.6426
39.4602
39.3649
25.1528
23.3023
21.9956
21.8143
20.7842
11.5511
11.0933

N^1-((2*S*)-2-(hydroxy(2-nitrocyclopropyl)methyl)-4-methylpentanoyl)-N^3,N^3-dipropylisophthalamide (12):

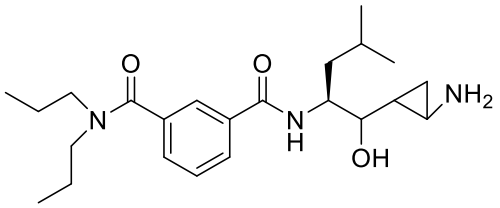


- ^{1}H NMR (500 MHz, $CDCl_3$)
- ^{13}C NMR (125 MHz, $CDCl_3$)

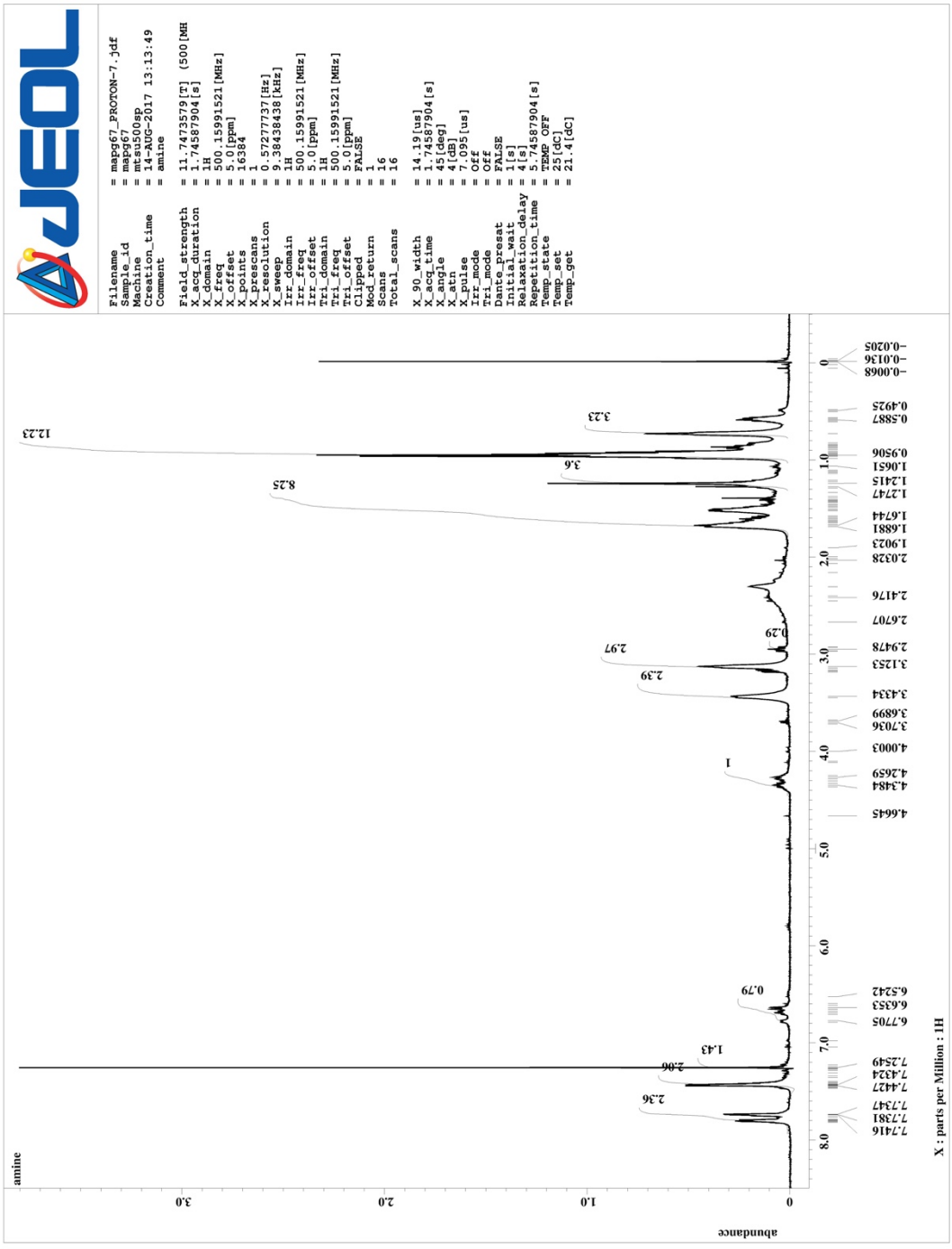

Filename = MAp51_PROTON-12.jdf
Sample_id = MAp51
Machine = mt-su500sp
Creation_time = 18-JUL-2017 10:29:21
Comment = Leu cpyl alc BACE
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[kHz]
X_window = 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[dC]
Leu cpyl alc BACE
abundance
X : parts per Million : 1H
21.89
15.02
3.94
3.17
3.38
3.31
3.30
1
0.65
0.5
7.7736
7.7691
7.7599
7.4095
7.2549
7.0041
6.9880
4.2636
4.2568
4.1113
3.8331
3.5639
3.4208
3.1265
3.1116
2.0443
2.0305
1.6835
1.6686
1.2438
1.2392
1.2300
0.9277
0.9140
0.7262
-0.0159

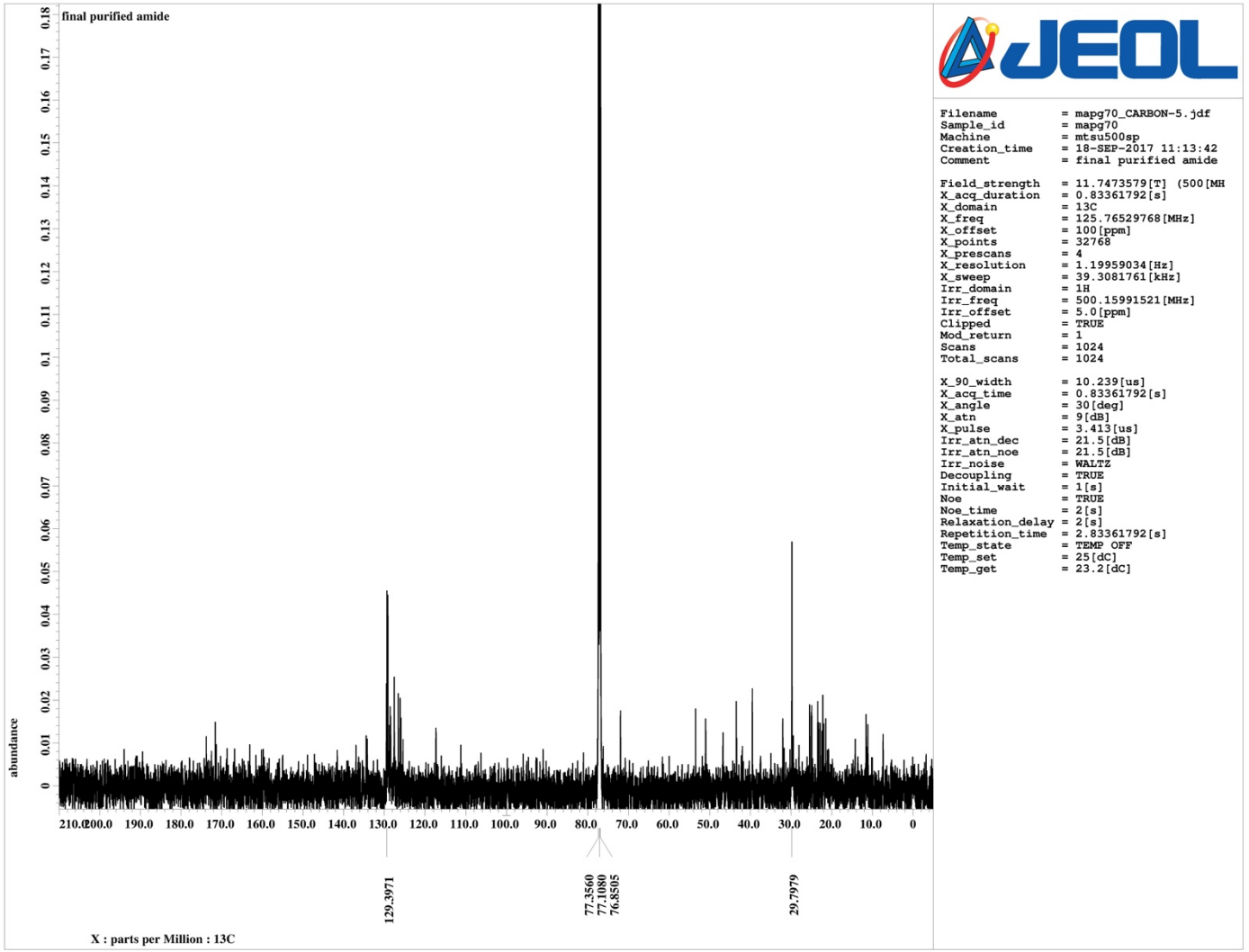

Leu cpyl alc BACE
abundance
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0
128.9297
77.3751
77.1271
76.8696
50.9921
46.7571
25.1432
23.5217
21.9956
21.6999
20.7842
11.5511
11.0933
X : parts per Million : 13C
JEOL
Filename = MAp51_CARBON-6.jdf
Sample_id = MAp51
Machine = mtsu500sp
Creation_time = 18-JUL-2017 11:18:14
Comment = Leu cpyl alc BACE
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 = 1024
Total_scans = 1024
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]

N^1-((2*S*)-2-((2-aminocyclopropyl)(hydroxy)methyl)-4-methylpentanoyl)-N^3,N^3-dipropylisophthalamide (13):

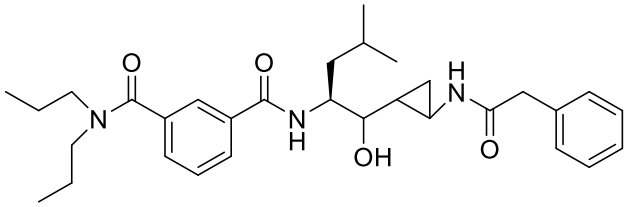


- ^{1}H NMR (500 MHz, $CDCl_3$)
- ^{13}C NMR (125 MHz, $CDCl_3$)

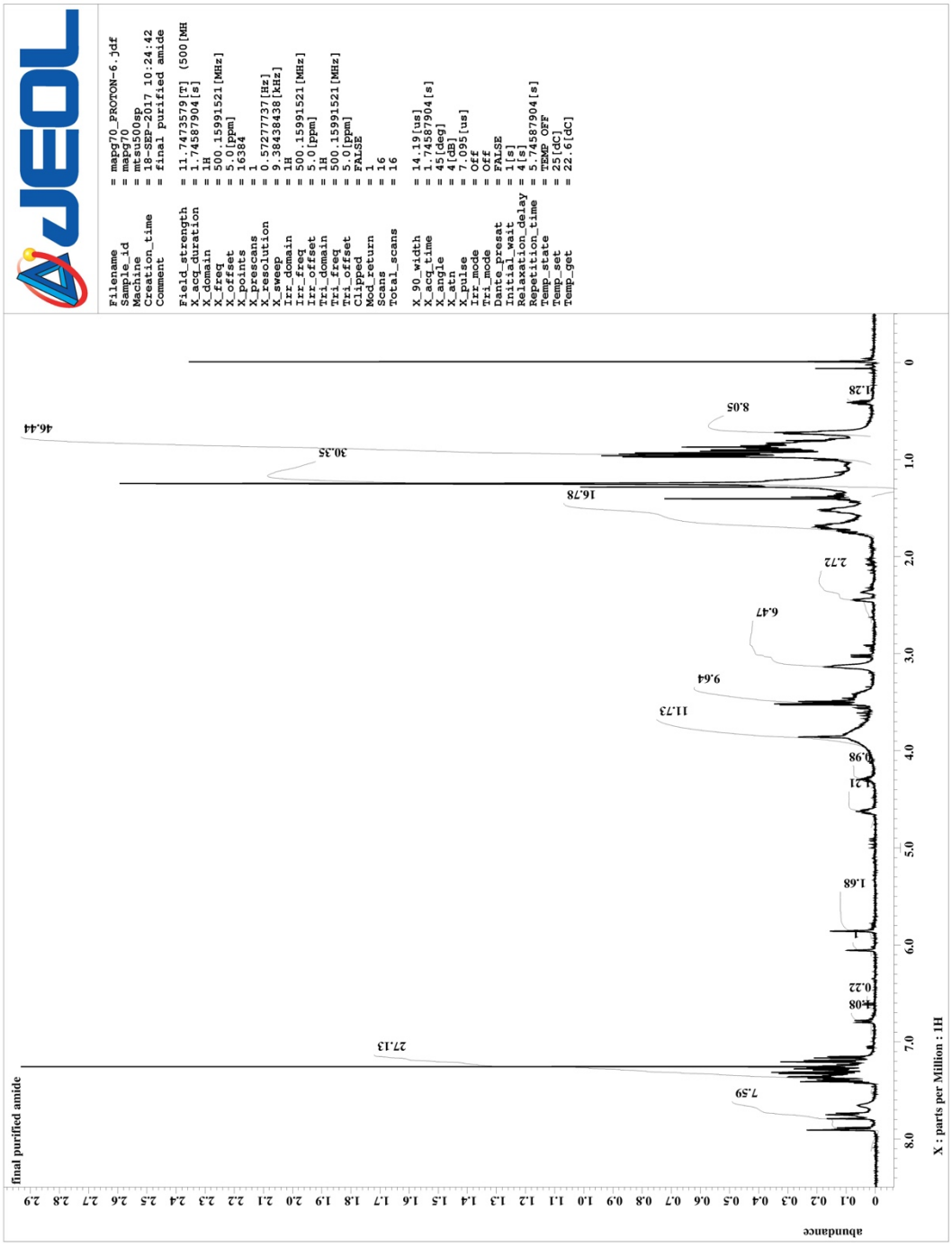

JEOL
Filename = mapg67_PROTON-7.jdf
Sample_id = mapg67
Machine = ms500sp
Creation_time = 14-AUG-2017 13:13:49
Comment = amine
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[kHz]
X_window = 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 = 21.4[dC]
amine
12.23
8.25
3.6
3.23
2.97
2.39
2.29
2.36
2.06
1.43
0.79
I
abundance
X : parts per Million : 1H

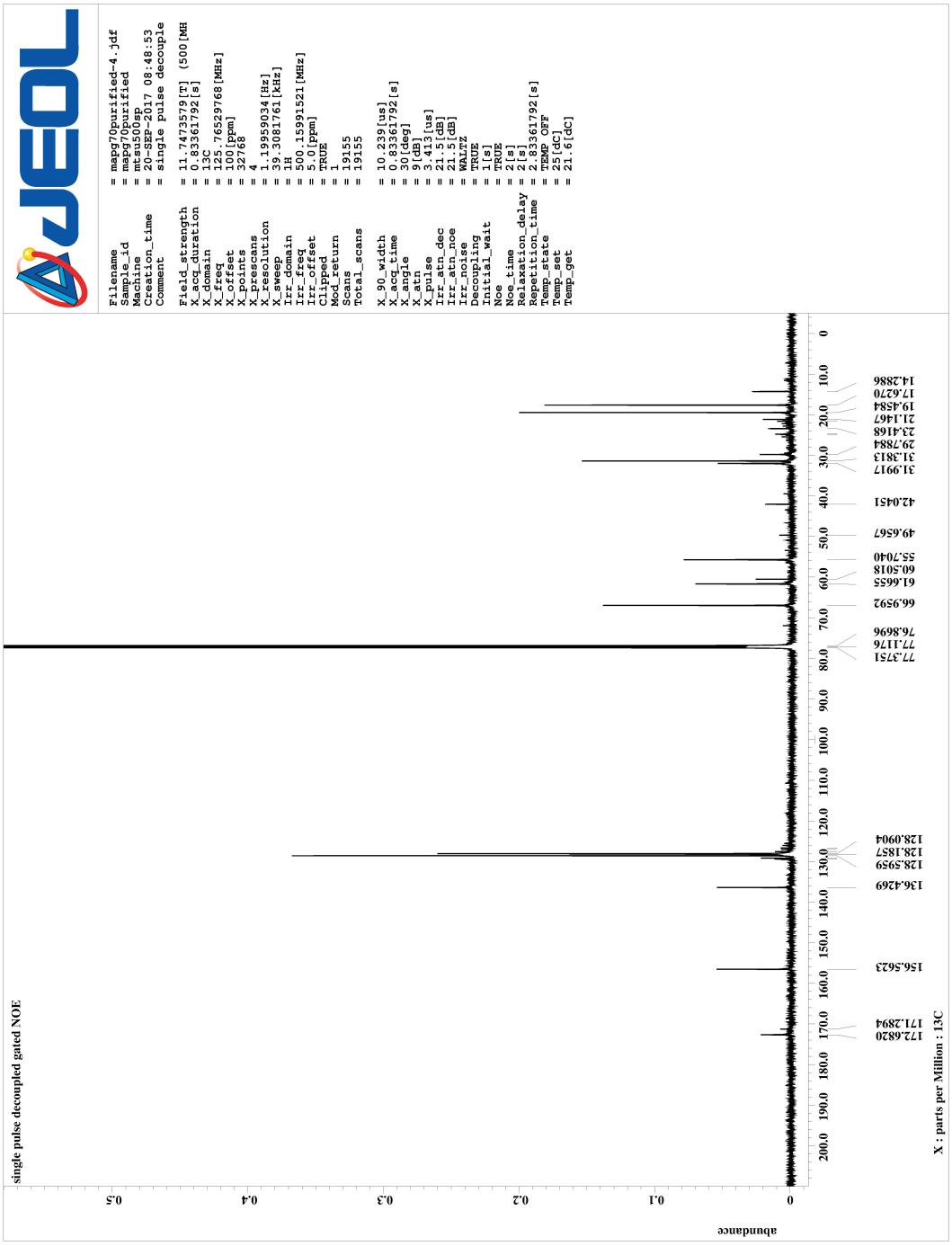

final purified amide
abundance
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.1
0.11
0.12
0.13
0.14
0.15
0.16
0.17
0.18
210.0
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
129.3971
77.3560
77.1080
76.8505
29.7979
X : parts per Million : 13C
JEOL
Filename = mapg70_CARBON-5.jdf
Sample_id = mapg70
Machine = mtsu500sp
Creation_time = 18-SEP-2017 11:13:42
Comment = final purified amide
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 = 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 = 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 = 23.2[dC]

N^1-((2*S*)-2-((2-aminocyclopropyl)(hydroxy)methyl)-4-methylpentanoyl)-N^3,N^3-dipropylisophthalamide (14):



- ^{1}H NMR (500 MHz, $CDCl_3$)
- ^{13}C NMR (125 MHz, $CDCl_3$)


JEOL
Filename = mapg70_PROTON-6.jdf
Sample_id = mapg70
Machine = ms-500sp
Creation_time = 18-SEP-2017 10:24:42
Comment = final purified amide
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]
X_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]
final purified amide
27.13
7.59
4.08
0.22
1.68
2.21
0.98
11.73
9.64
6.47
2.72
16.78
30.35
46.44
8.05
1.28
abundance
X : parts per Million : 1H


JEOL
Filename = mapg70purified-4.jdf
Sample_id = mapg70purified
Machine = ms500sp
Creation_time = 20-SEP-2017 08:48:53
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 = 39.3081761[kHz]
X_window = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = TRUE
Mod_return = 1
Scans = 19155
Total_scans = 19155
X_90_width = 10.239[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 9[dB]
X_pulse = 3.41[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 = 21.6[dC]
single pulse decoupled gated NOE
abundance
X : parts per Million : 13C
172.6820
171.2894
156.5623
136.4269
128.5959
128.1857
128.0904
77.3751
77.1176
76.8696
66.9592
61.6655
60.5018
55.7040
49.6567
42.0451
31.9917
31.3813
29.7884
23.4168
21.1467
19.4584
17.6270
14.2886