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Asymmetric Synthesis of Tryptophan Driviatives and Its Application to Streamlined Synthesis of Tryprosatain A and B

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ASYMMETRIC SYNTHESIS OF TRYPTOPHAN DRIVIATIVES AND ITS APPLICATION TO STREAMLINED SYNTHESIS OF TRYPROSATAIN A AND B.

by

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ABSRACT

ASYMMETRIC SYNTHESIS OF TRYPTOPHAN DRIVIATIVES AND ITS APPLICATION TO STREAMLINED SYNTHESIS OF TRYPROSATAIN A AND B.

BY

Matthew Marcus Huisman

The University of Wisconsin-Milwaukee, 2015
Under the Supervision of Professor Mahmun M. Hossain

Tryprostatins have been shown to be potential antitumor antimitotic agents. Tryprostatins have been isolated from the fermentation broth of marine fungal strain *Aspergillus fumigatus* in trace amounts. Our lab has developed a phase-transfer-catalyzed asymmetric alkylation reaction to produce protected tryptophans (Trp) with high enantioselectivity (90-95% ee) as synthetic precursors to Tryprostatins. Studies of Tryprostatins indicate that manipulation of ring-A may cause enhanced activity. We propose a general synthetic route to several new tryprostatins that may be tolerant to ring-A analogues of gramine utilizing achiral reactants. The synthesis of Tryprostatin B has been completed with 20% overall yield in 7 steps. In the future our group will hopefully be able to utilize this chemistry to develop a large number of Tryprostatin analogs. We hope that one of these derivatives will be selective against cancer cells, with therapeutic concentrations in the nanomolar region.

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To

My family

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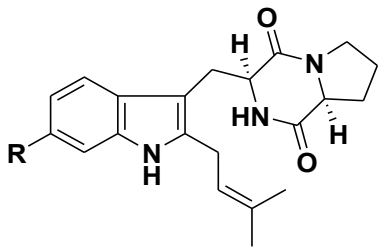
1. INTRODUCTION

1.1. Tryprostatin A and B and their Biological Activity.



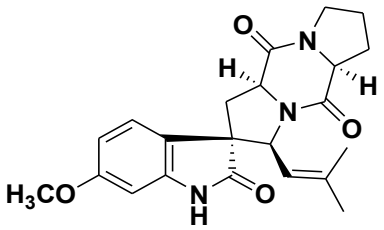
R= OMe, Tryprostatin A (1)

R=H, Tryprostatin B (2)

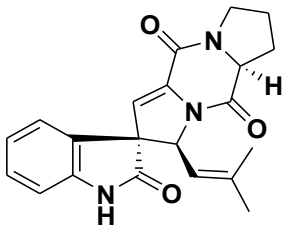
Figure 1. Tryprostatin A and B

Tryprostatins A **1** and B **2** (Figure 1) are natural products with therapeutic activity against breast cancer. The natural source for these compounds is a marine fungus, specifically, strain BM939 of *Aspergillus fumigates*. Tryprostatins A and B were isolated as secondary metabolites from fermentation broth. Tryprostatins A **1** and B **2** were found to have high activity in tsFT210 cells with inhibitory concentrations of 50 µg/ml of **1** and 12.5 µg/ml of **2**, respectively. These molecules function to completely arrest microtubule formation during the G2/M phase, thus inhibiting cell cycle progression.[1] Tryprostatins A **1** and B **2** contain a 2-isoprenyl tryptophan moiety and a proline residue, the latter of which was located in the diketopiperazine unit.

In addition to tryprostatin A **1** and B **2**, spirotryprostatins A **3** and B **4** (Figure 2) and cyclotryprostatins A-D[5] (Figure 3) were isolated from the same species[4] by Osada *et al.* Spirotryprostatins have the same biological function, arresting the cell cycle at the G2/M, but were less potent than Tryprostatins: IC_{50} values of 197.5 (**3**) and 14.0 µM (**4**).[4] On the other hand, cyclotryprostatins A-D[5], which belong to the family of Fumitregorins (Figure 4),[6-11] showed good potency with IC_{50} values of 5.6µM, 19.5µM, 23.4µM, and 25.3µM, respectively. Like Tryprostatins, these function medicinally to inhibit tsFT210 cell cycle progression during the G2/M phase.[2]

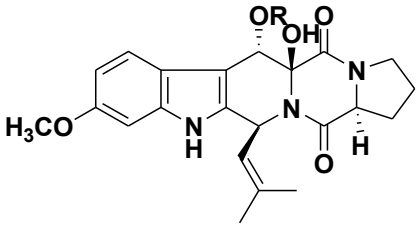


3 Spirotryprostatin A

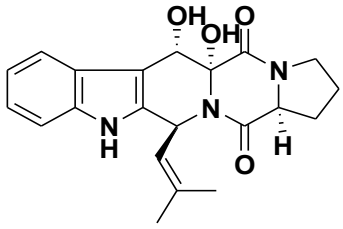


4 Spirotryprostatin B

Figure 2. Spirotryprostatins

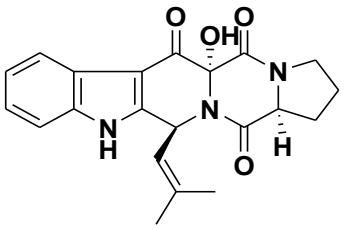


R=H Cyclotryprostatin A



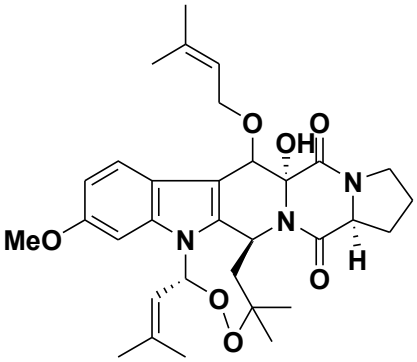
Cyclotryprostatin C

R=CH_3 Cyclotryprostatin B.

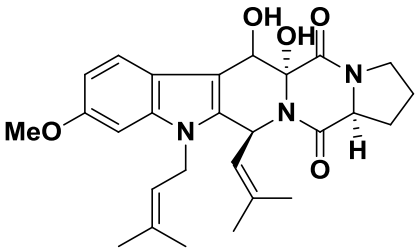


Cyclotryprostatin D

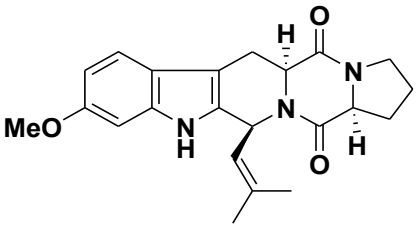
Figure 3. Cyclotryprostatins



Fumitremorgin A



Fumitremorgin B



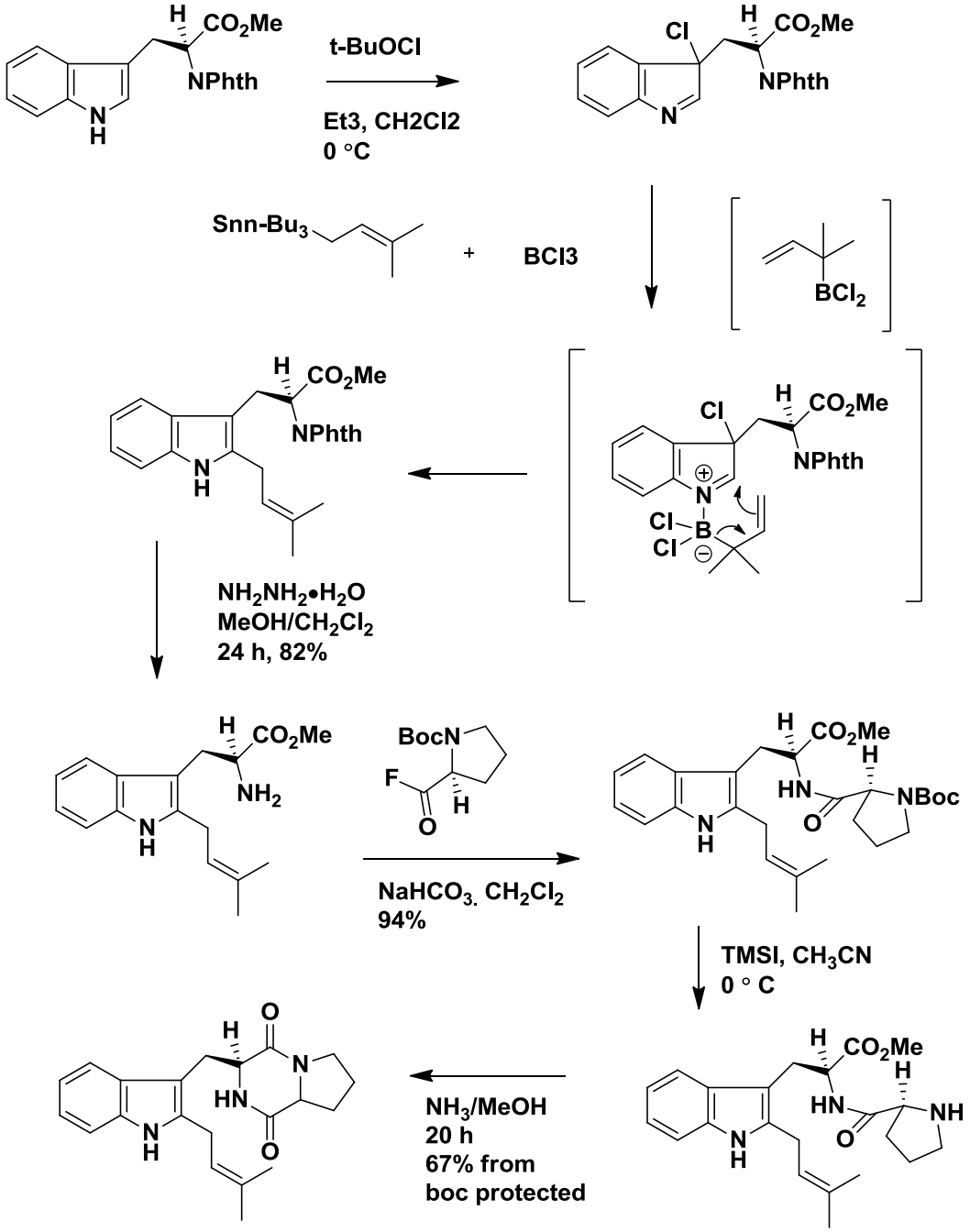
Fumitremorgin C

Figure 4. Fumitremorgins

The interesting biological activity of this family of alkaloids has piqued curiosity in their total synthesis. The first total synthesis of the parent, tryprostatin B was reported by Danishefsky *et al.* [3] Via the chloroindolenine/borane approach, illustrated by the scheme below. The N-phthaloyl-L-tryptophan methyl ester was treated with *tert*-butyl hypochlorite to generate the

chloroindolenine intermediate at 0˚C. This intermediate was then treated with prenyl stannane and followed by rapid addition of boron trichloride (two equivalents) to provide the desired 2-isoprenyl tryptophan derivative. It is thought that the reaction of prenyl stannane with boron trichloride generated a nucleophilic prenylation species *in situ*. This species is believed to react with the chloroindolenine to provide the "ate" like structure complexed to the indolenine N_a-nitrogen atom. This step was followed by intramolecular delivery of the isoprenyl moiety to the indole C(2) position. Smooth removal of the N-phthaloyl protecting group generated the required L-2-isoprenyltryptophan methyl ester. The coupling reaction between the 2-isoprenyl tryptophan unit and the N-Boc-protected L-prolinyl acid fluoride furnished dipeptide as illustrated. The Boc protecting group was removed on treatment of material with trimethylsilyl iodide to afford the free amine. The free amine was stirred in a solution of ammonia/methanol for 24 h, the formation of the diketopiperazine unit resulted in tryprostatin B identical to the natural material. In 1996 Danishefsky's group was able to accomplish the synthesis of Tryprostatin B in eight steps with 46% over all yield.

Scheme 1. Danishefsky's 1996 synthesis of tryprostatin B

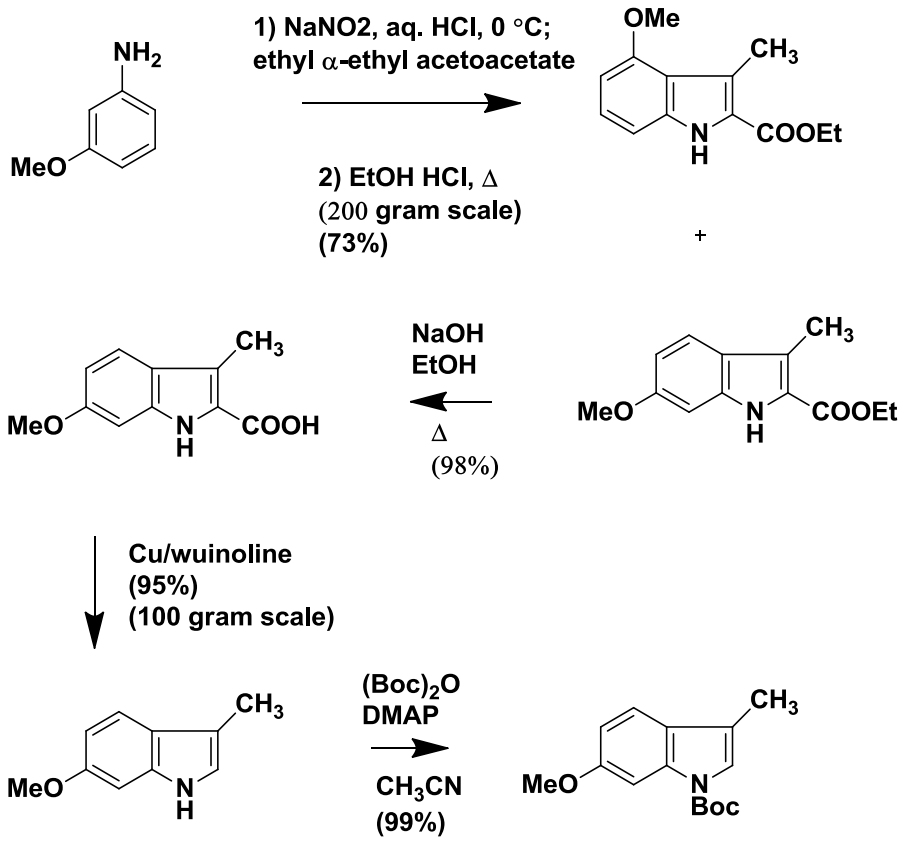


Microtubules have important roles in cell growth and division, making them promising targets for cancer therapeutics. Tryprostatins target and inhibit microtubule growth and therefore have considerable value as potential drugs for treating cancer.[2]

1.2. Development of this work by the Cook Group

Synthesis of the starting material, Boc protected 6-methoxy-3-methylindole is shown below with a 67% yield.

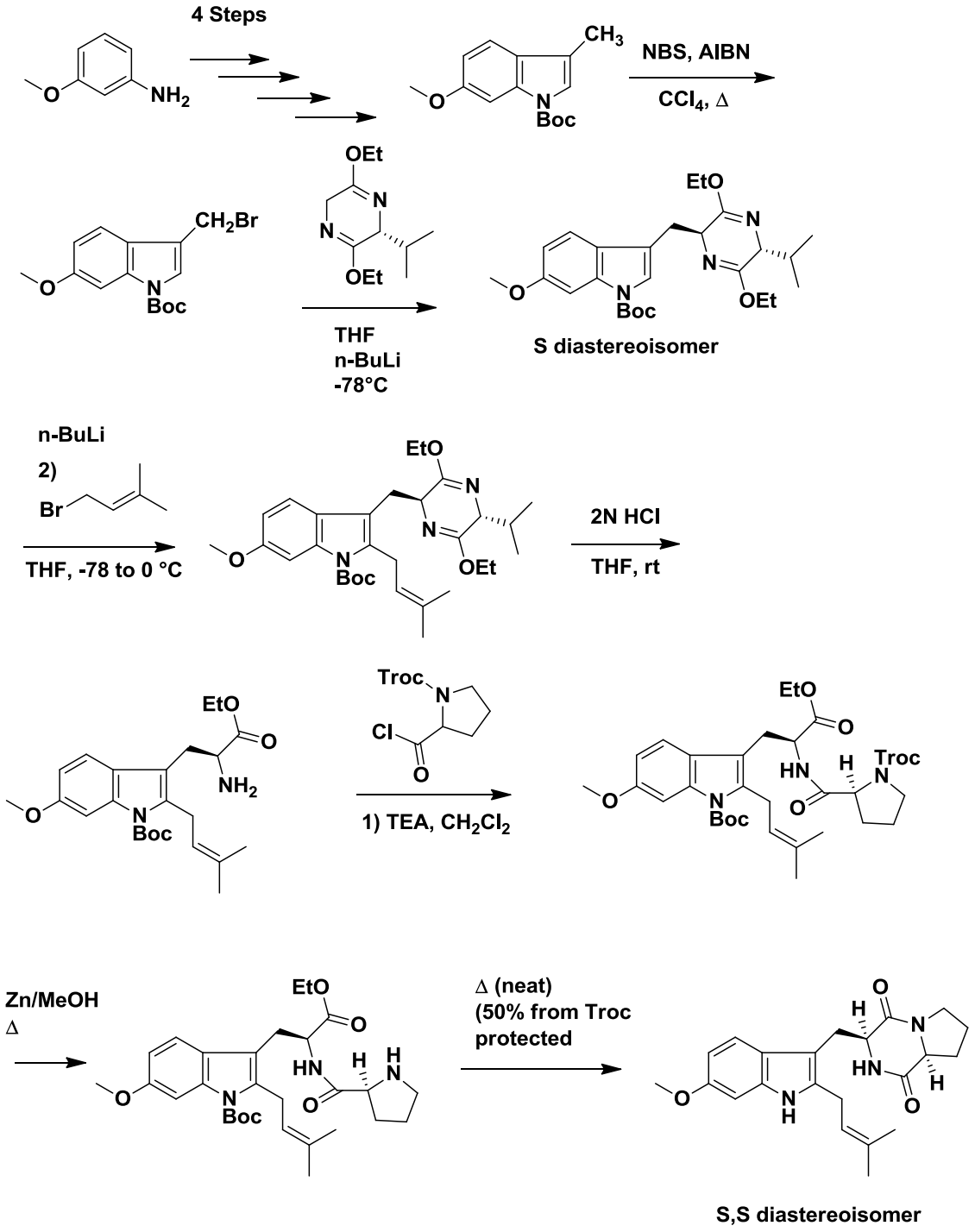
Scheme 2. Synthesis of starting material for Cook's synthesis



In 1997 the first total synthesis of tryprostatin A was completed by Gan in Cook's group *via* a regiospecific bromination process coupled with the Schöllkopf chiral auxiliary.[4] This approach provided the 2-bromo-6-methoxytryptophan as a key intermediate in good yield. 1-*tert*-

butyloxycarbonyl-3-methylindole was prepared in four steps from m-anisidine *via* a Japp-Klingemann/Fischer Indole protocol. The azobisisobutyronitrile (AIBN) initiated regiospecific bromination of 3-methylindole at the allylic position was accomplished using N-bromosuccinimide (NBS) as the brominating agent *via* a radical process. The coupling reaction between the benzylic bromide which resulted and the anion of the Schöllkopf chiral auxiliary provided the stable dihydropyrazine with diastereoselectivity. Electrophilic, regiospecific brominating of pyrazine with NBS at the indole C(2)-position generated under conditions of electrophilic substitution. Using lithium-halogen exchange followed by addition of isoprenyl bromide, the desired C2-functionality was achieved.[1d] Hydrolysis of the pyrazine unit in acidic conditions (THF, aq 2 NHCl) provided the ethyl ester. Treatment of the tryptophan derivative with N-Troc-L-prolinyl chloride afforded the desired dipeptide after reductive cleavage of the protecting Troc group.[4] Cyclization to form the diketopiperazine and removal of the Boc protecting group generated tryprostatin A in a one pot process. The optical rotation and spectral data of synthetic tryprostatin A were in agreement with those of the natural product. In 1997 Cook's group was able to synthesis tryprostatin A in an enantiospecific fashion using sixteen steps 9.15 % yield.

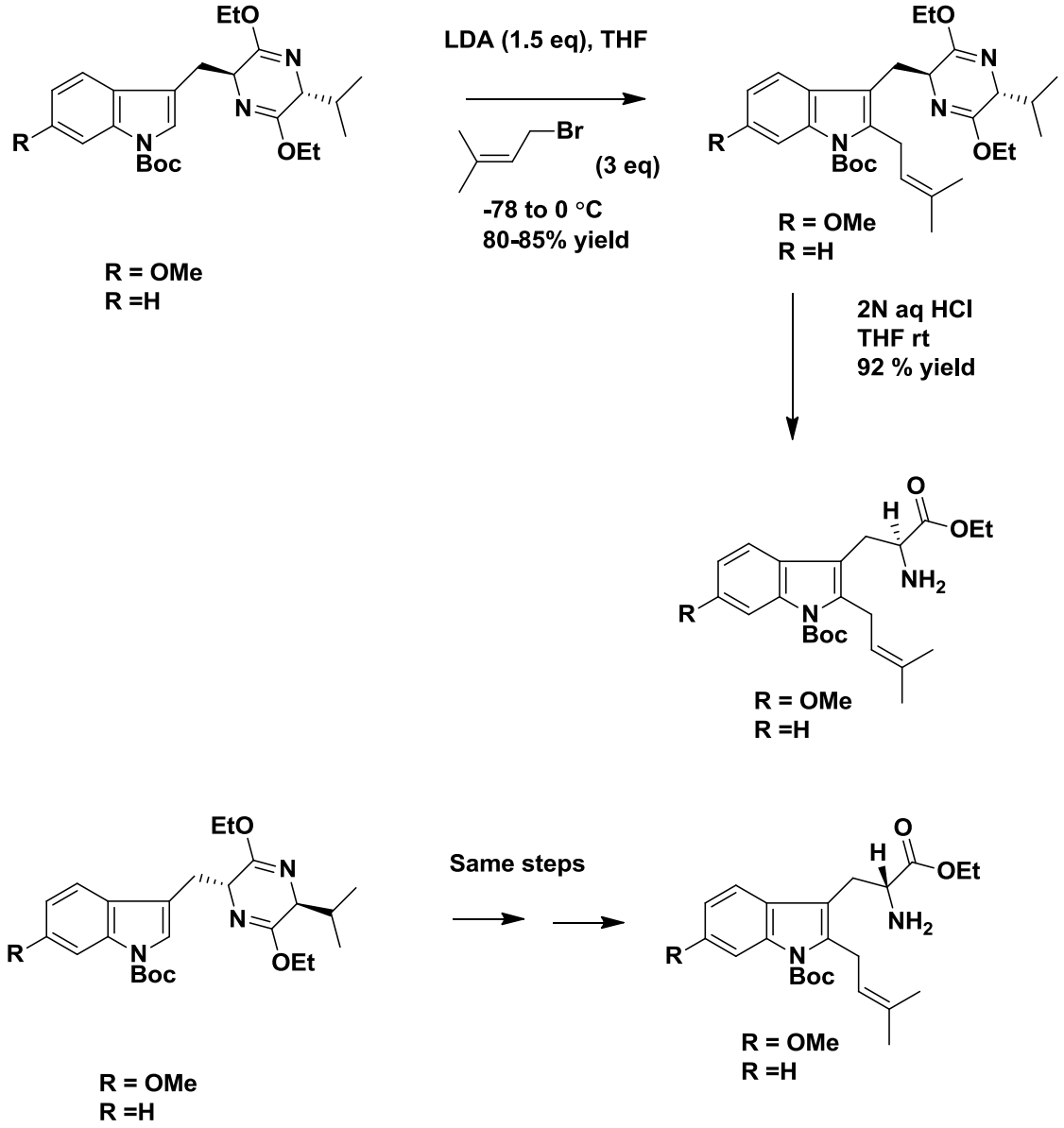
Scheme 3. Cook's tryprostatin synthesis



In 2008, the synthesis of Tryprostatin A and B as well as their enantiomers was developed by Zhao in Cook's group.[2] In order to introduce the isoprenyl group at the indole C(2) position of and decrease the number of steps earlier reported by Gan *et al.*[4] LDA was employed to form the

anion at C(2). The indole was stirred with LDA at -78 ˚C followed by the addition of dry, pure isoprenyl bromide to furnish 2-isoprenylpyrazine. This was an improvement in the synthesis of 2-isoprenylpyrazine, this procedure was used for tryprostatin B. Since the Schöllkopf chiral auxiliary can tolerate strongly alkaline conditions, it served well as a protecting group for the amino acid functional group and prevented racemization. The pyrazine moiety was removed under acidic conditions (aq HCl, THF) in 92% yield to provide L-valine ethyl ester and 2-isoprenyl tryptophan. Using the same conditions, the enantiomeric 2-isoprenyl tryptophans and were synthesized and employed for the enantiospecific synthesis of tryprostatins A and B.[1d] In 2008 Cook's group was able to synthesize tryprostatin B in 40 % yield as well as the enantiomers and diastereomers in through the same procedure using the corresponding amino acids.

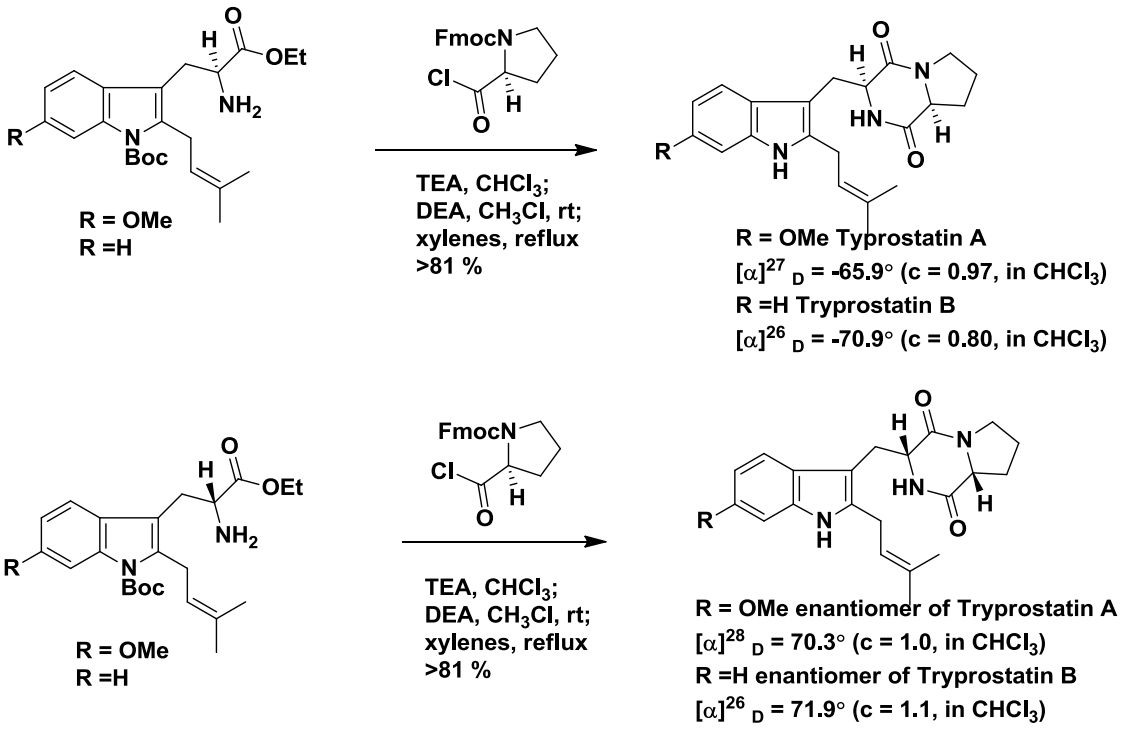
Scheme 4. Cook's synthesis of diastereomers



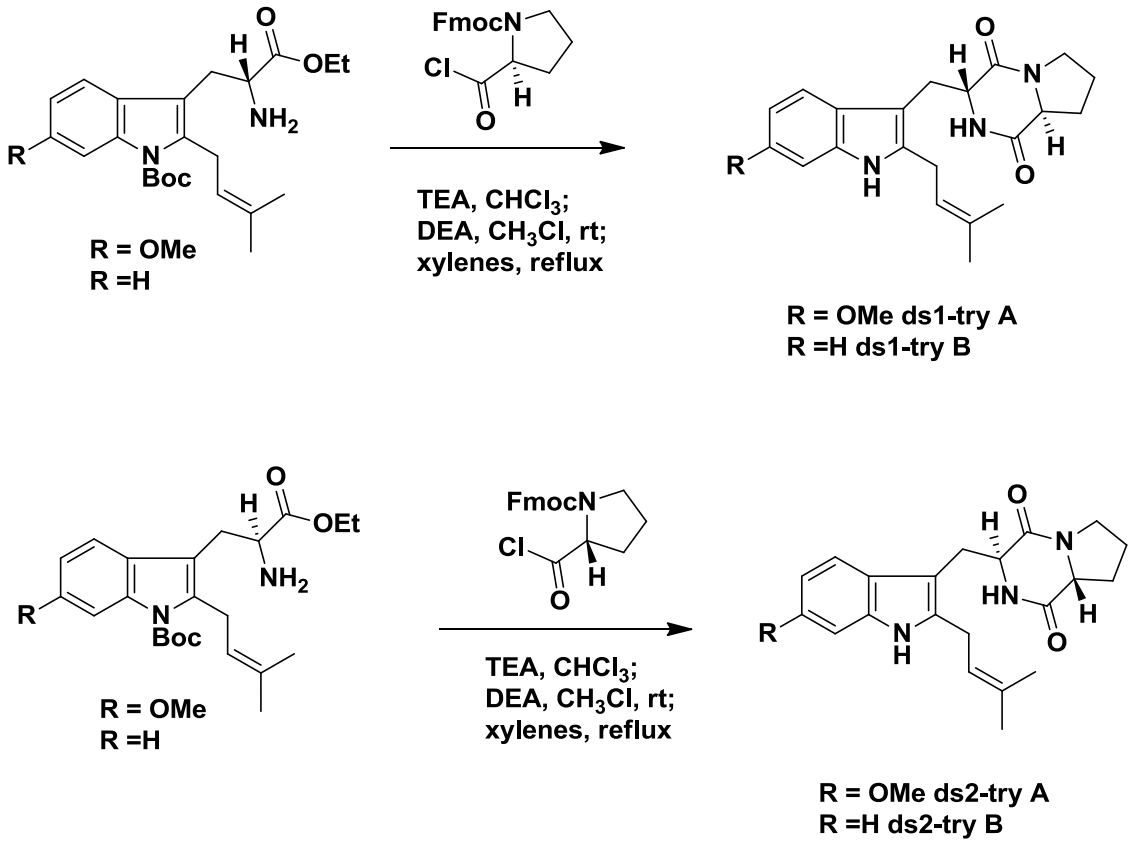
With the key 2-isoprenyltryptophan derivatives in hand, the diketopiperazine unit was built on as illustrated. The various 2-isoprenyl-tryptophans were stirred with N-Fmoc-L-proline chloride in the presence of triethylamine in chloroform at room temperature this was followed by removal of the solvent. The Fmoc protecting group was then removed by addition of diethylamine (DEA) in acetonitrile. Solvents were removed under reduced pressure. Formation of the diketopiperazine as well as the removal of the Boc protecting group from the indole N(H)

were achieved by heating in refluxing xylenes in high dilution. A stereospecific, enantiospecific total synthesis of Tryprostatin A and B was accomplished *via* alkylation of the corresponding 2-lithioindole derivatives. This procedure was also applied to the enantiomers of tryprostatin A and tryprostatin B. The optical rotations of the natural products and the enantiomers were in agreement with those reported by Osada *et al.* for the natural products.[1a-c] This route was used for the total synthesis of the mismatched pairs of Tryprostatin A and B for biological screening.

Scheme 5. Cook's synthesis of enantiomers of tryprostatin



Scheme 6. Cook's synthesis of diastereomers of tryprostatin

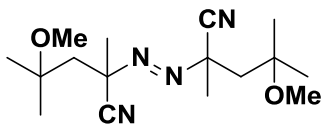


1.3. Fukuyama's synthesis

In 2010 Fukuyama's group decide to pursue the synthesis of Tryprostatins, interest from this sprung from the history of exploring radical mediated cyclization in the formation of the 2, 3 substituted indole rings.[5] They further speculated that they may be able use that chemistry to make natural products Tryprostatin A and Tryprostatin B.

Fukuyama and co-workers began by making an aromatic iodide that they would be able to use a Sonoashira coupling, a palladium-mediated coupling to attach the aromatic ring to the desired alkyne. From this intermediate they were able to make the ortho-alkenyl isocyanide in two steps.

Next the Fukuyama group proceeded to demonstrate their expertise over radical mediated cycliation of indoles by using a unique radical initiator to close the between the newly formed 2 and 3 carbons. This step also utilized tri butyl tin as a leaving group for the isoprenyl group undergoing addition at the indole C2 position.

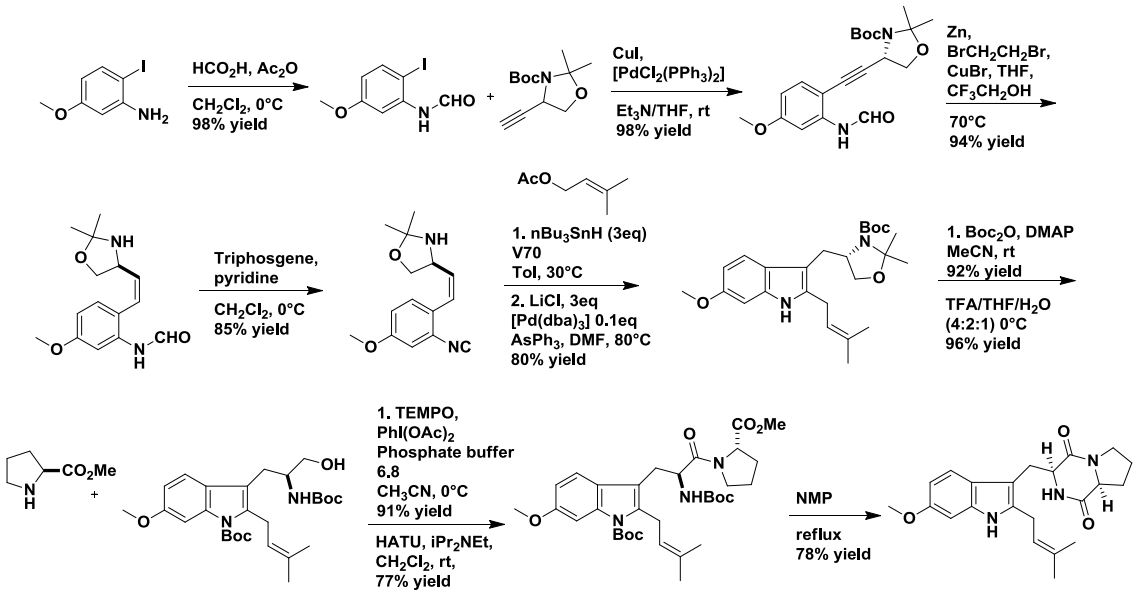


V-70

Figure 5. V-70 radical initiator

Next steps include a ring opening, oxidation and peptide coupling. This was followed by reflux in high boiling solvent N-methyl-2-pyrrolidone (NMP) to close the diketopiperzine ring. The synthesis was achieved in ten steps in 39 percent yield.

Scheme 7. Fukuyama's synthesis of tryprostatins



1.4. Cell Cycle and Anticancer Drugs

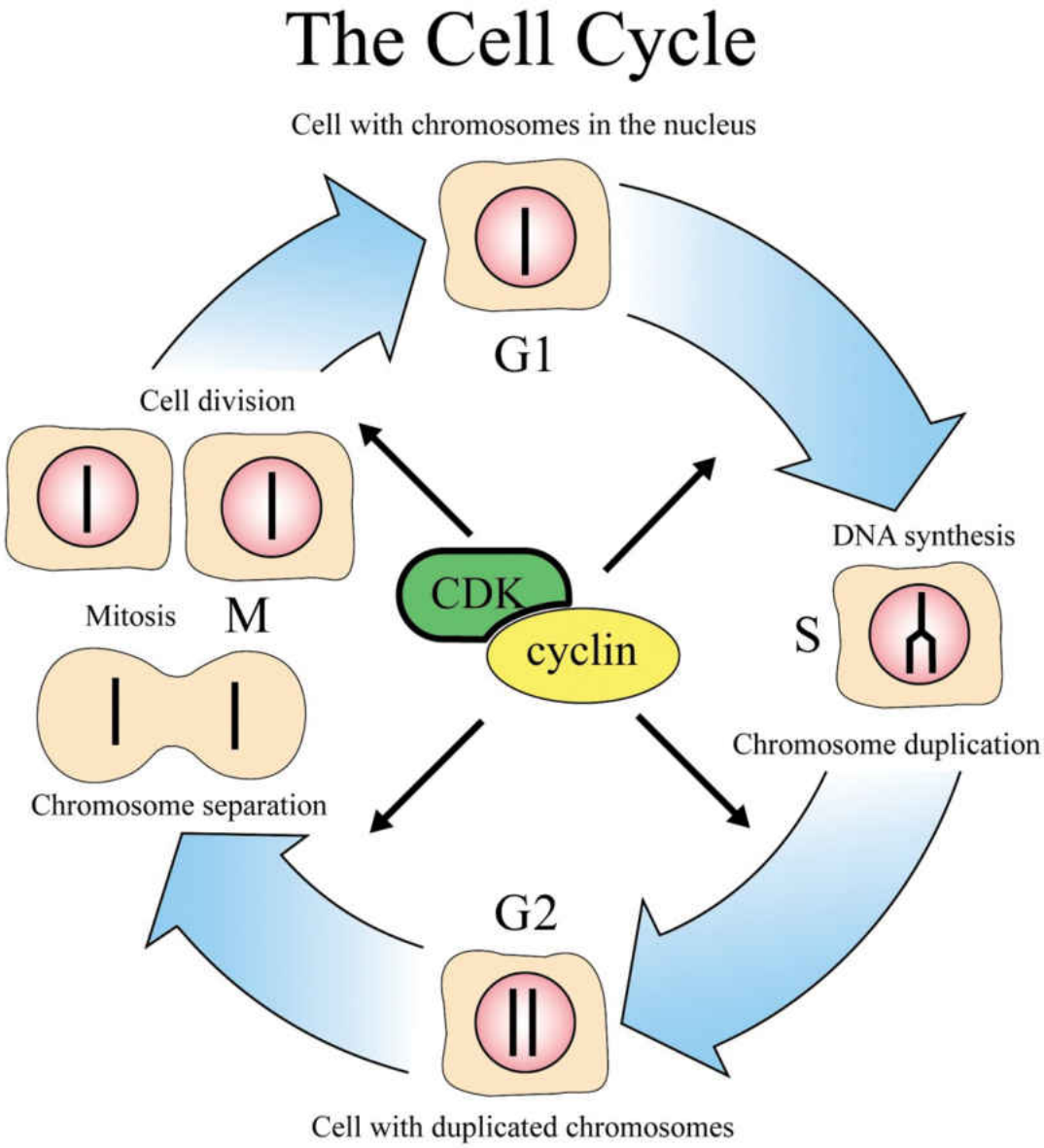


Figure 6. The cell cycle

The cell cycle is the process of one cell turning into two daughter cells. This process is often broken into four parts consisting of G1, S, G2 and M.[6] Interphase is often times used to describe the combination of G1, S, and G2. During interphase the cell grows and prepares for cell division by duplicating its DNA. The remaining mitotic (M) phase splits the cell into two daughter

cells. M phase is followed by cyctokinesis where the cell completely divides. See figure 6.

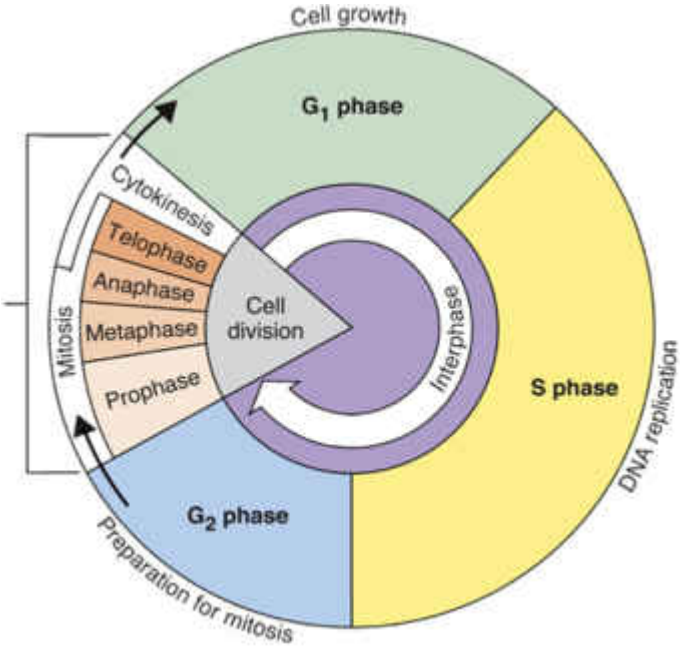


Figure 7. Cell cycle representing G1, S, and G2 phases.

Gap 0 (G_0) Resting phase cell is not dividing. Gap 1 (G_1) Cells increase in size. G_1 check point ensures that the DNA is ready for replication. Synthesis (S) DNA replication occurs. Gap 2 (G_2) the cell grows G_2 checkpoint ensures that cell is ready to enter the M phase and divide. Mitosis (M) Cell growth stops cell divides into two daughter cells, Metaphase checkpoint ensures that the cell is ready to complete cell division. See figure 7.

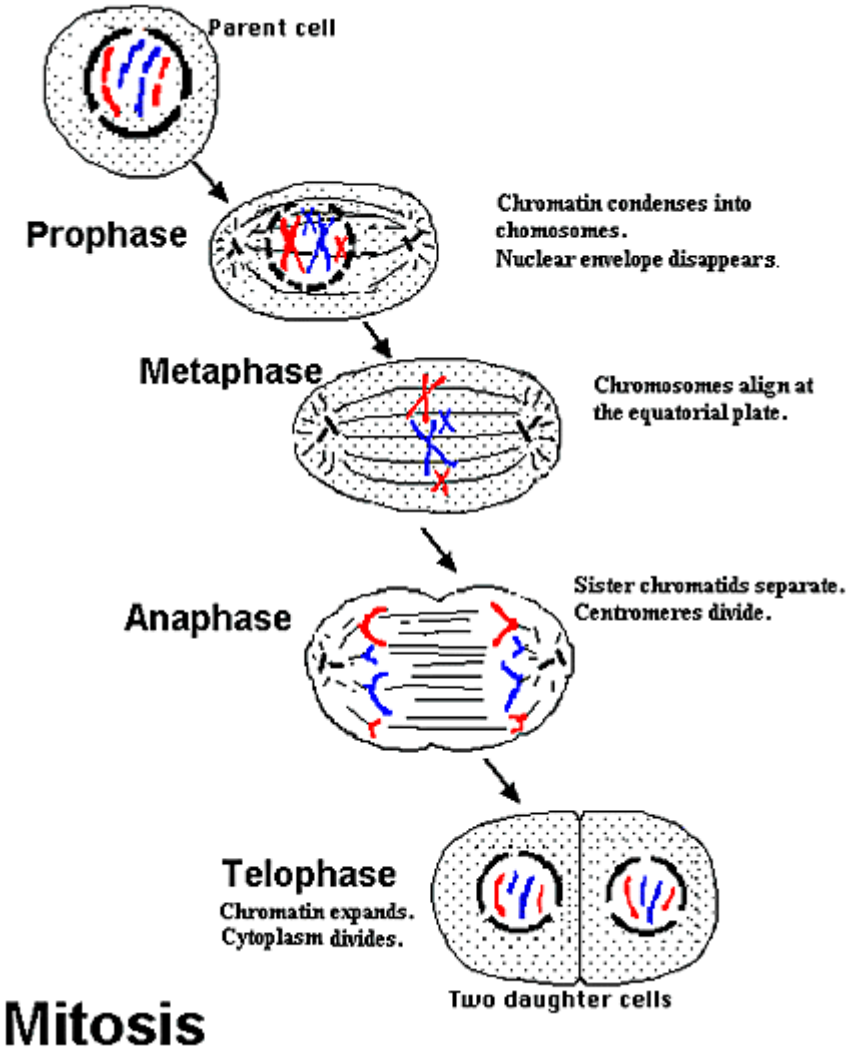


Figure 8. Cell cycle showing mitosis.

Furthermore, the Mitotic phase is broken down into five phases. Furthermore, the Mitotic phase is broken down into five phases demonstrated by the acronym IPMAT. Interphase as discussed earlier is the phase leading up to cell division. During this resting phase gaining nutrients and the cell is not dividing. During prophase the chromatin condenses and membrane surrounding the nucleus disappears. During metaphase telomeres appear and the

chromosomes line up on the equatorial plane. In anaphase the chromosomes divide and separate to opposite sides of the cell. During Telophase the cell divides into two different cells.

Cyclin-dependent kinases (CDKs) are a family of protein kinases, which regulate the cell. [6a, 6b] They are present in all eukaryotes and their regulatory function in the cell cycle has been conserved over time. They bind cyclin and without cyclin CDK has little kinase activity. CDKs phosphorylate their substrates on serines and threonines. Animal cells contain at least nine CDKs four of which are directly involved in cell cycle regulation.[6c]

1.5. Inhibitors of Chromatin Function

Topoisomerase inhibitors

Topoisomerase inhibitors interfere with the enzymes that control the changes in DNA structure via a mechanism that involves catalyzing the breaking and rejoining of the phosphodiester backbone of DNA strands during the cell cycle.[7]

Microtubule Inhibitors

Microtubules are a structural part of the cytoskeleton and are found in the cytoplasm.[8] Microtubules are formed by the combination of alpha and beta tubulin protein segments. The formation and deconstruction of the microtubules are very rapid. The purpose of microtubules is to maintain the structure of the cell. Microtubules provide platforms for intracellular transport, by forming the structures on which motor proteins, dynein and kinesin move. They act as the metaphorical train track and train cars of the cell. Lastly the microtubules are used in cell division connecting to the mitotic spindles to the telomers on which the motor proteins pull the chromosomes apart. Microtubules are long hollow tubes which polymerize end to end.[9] In order for polymerization to occur dimers must be present above an established concentration.

Microtubules have polarity, the end with the alpha subunit exposed is (-) while the beta subunit is the (+). Elongation only occurs from the (+) end.

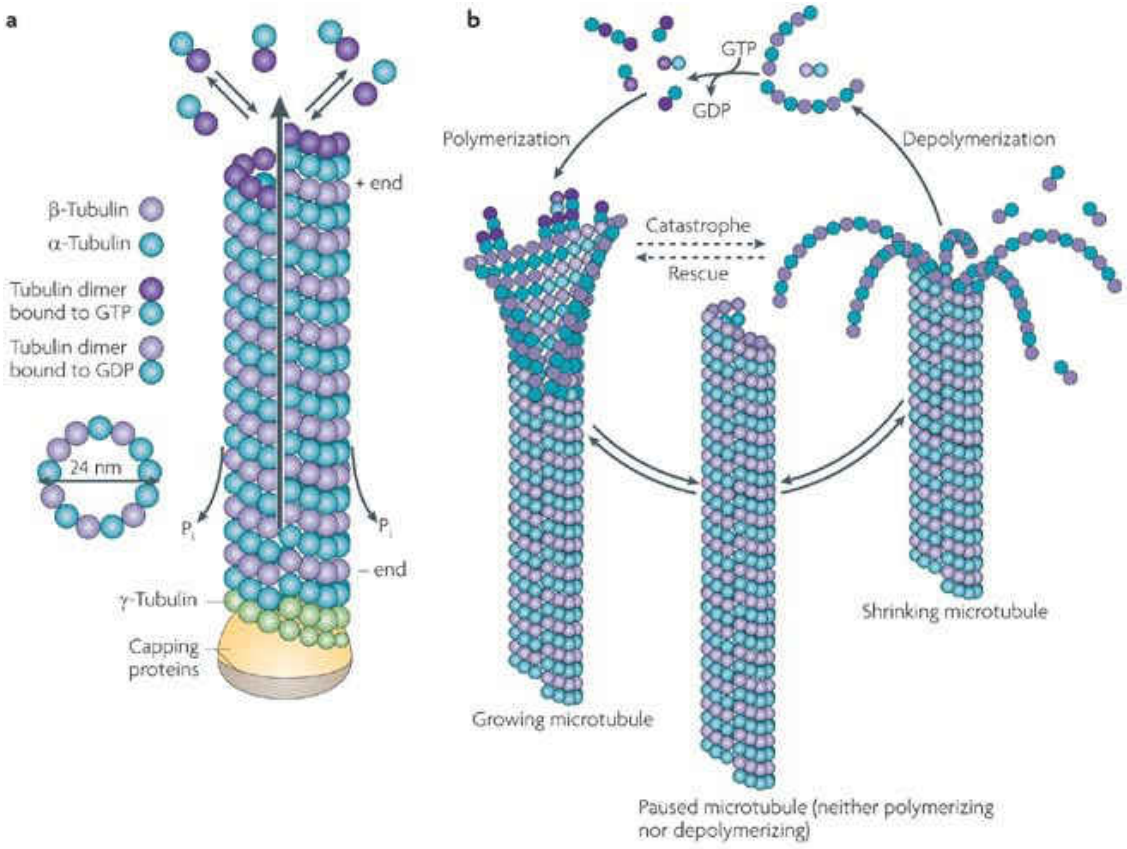


Figure 9. Microtubules roll in cell division

Mitotic spindles also called spindle apparatus are present in all eukaryotes. The job of the microtubules is to separate the cell's sister chromosomes during anaphase in the process of cell division. This spindle microtubules, microtubule-associated proteins (MAPS) and the microtubule organization center (MTOC) are all involved in this dynamic process.

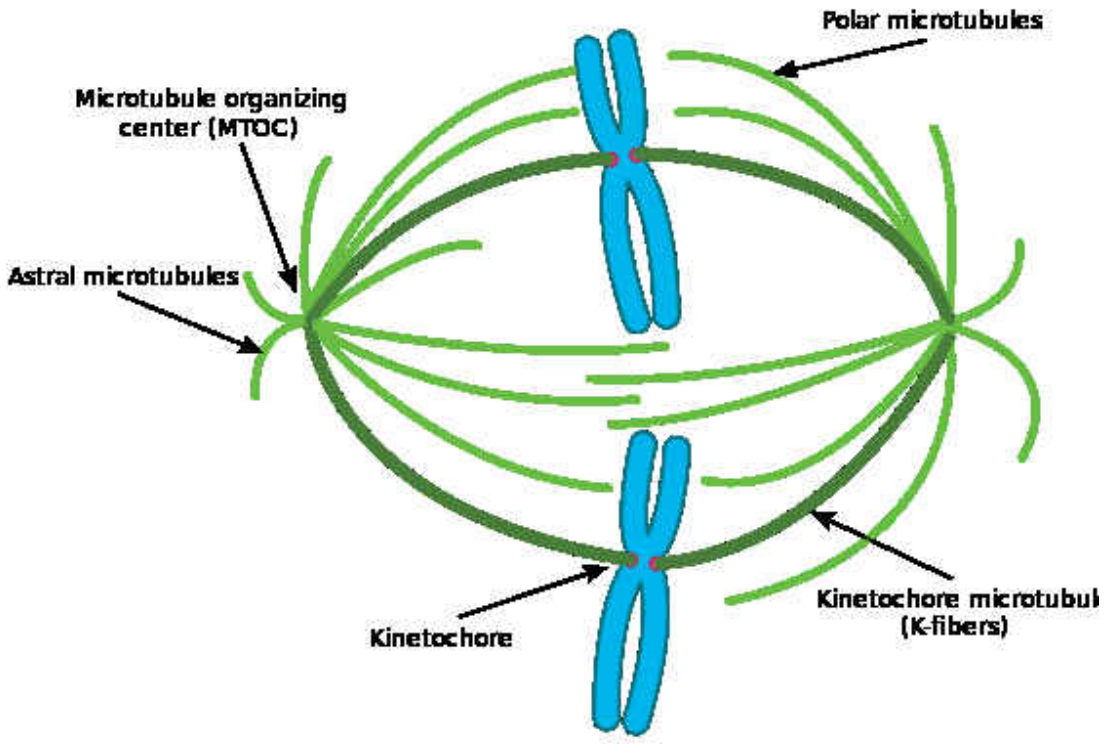


Figure 10. Chromosome division along microtubules

Cytoskeletal drugs are molecules that interact with actin or tubulin.[10] Some such as taxol stabilize the microtubules, while others prevent polymerization. Cytochalasin D binds to actin monomers and prevents polymerization of actin filaments; this is an example of a destabilizing agent.

1.5.1 Microtubules as drugable targets

Many drugs have been able to bind to tubulin by modifying its activation site, the effect of this is that the microtubule dynamics are manipulated.[11] This interference can prevent a cell from going into a cell cycle and can lead to programmed cell death or apoptosis.[12] Both microtubule stabilizer and destabilizers can suppress microtubule dynamics. The Taxane family of anti-cancer drugs, which contains Taxol, is a well-known example of a member of this family. These

compounds work by stabilizing the GDP bound tubulin, stopping depolymerization. Vincristine and Colchicine have the opposite effect, blocking the polymerization of tubulin to microtubules.

1.6. Inhibitors of Breast Cancer Resistance Protein

Multidrug resistance (MDR) has been shown to be one of the most difficult obstacles to over-come in treating cancer. This resistance is often due to membrane bound proteins, driven by ATP push anticancer therapeutics out of the cell. Breast Cancer Resistance Protein (BCRP) an ATP-binding cassette transporter has been shown to be one of these types of "problematic proteins."[13] Inhibition of this class of proteins has been shown to increase the intracellular drug accumulation and reverses BCRP-mediated multidrug resistance. [14] A better understanding of molecules that interact with multidrug resistance proteins such as BCRP or better understanding of the binding site is critical in the advancement of designing more effective therapeutic strategies. BCRP was initially discovered as a placenta-specific adenosine triphosphate ATP) binding cassette transporter (ABCP), but was later found in an assortment of tumor types. Since BCRP is involved in exporting substrates from the cell, the pharmacological efficacy of drugs that are substrates of BCRP are compromised.[15] BCRP has an ability to remove a wide variety of molecules from the cells. Multidrug resistance protein is considered one of the major transporters causing drug resistance in mammalian cells.[16]

1.7. Benzophenone Imine Glycine Schiff Base

Catalytic asymmetric synthetic reactions are attractive because they don't use the often times more expensive chiral control reagent in more than the standard twenty mole percent. Chiral phase-transfer catalysis (PTC) are often preferred by organic chemists due to their mild conditions, simple reaction procedures, safe and inexpensive reagents and solvents, furthermore the PTC reactions have been shown to be tolerant to scale up, making them

incredibly useful for production of products on gram to kilo gram scale.[17] O'Donnell's laboratory originally developed benzophenone imines of glycine alkyl esters in 1978 as an alternative method to obtain diethylacetamidomalonate, which is the starting material for the classical 1903 Sörensen method for the synthesis of racemic α-amino acids."[18]

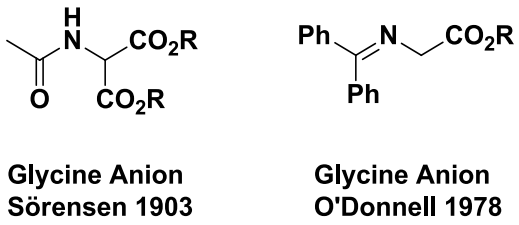


Figure 11. Comparison of Sörensen's Glycine Anion and O'Donnell's Glycine Anion

Since its development the O'Donnell Schiff base or the benzophenone imine glycine ester has found applications in both chiral and racemic amino acids.[17] A critical characteristic of the O'Donnell Schiff base it the selective monoalkylation of the substrate in base, due to the difference in acidity of the α-carbon's proton in the starting material and the monoalkylated product. This change in acidity [pKa (DMSO)] is essential for the stereoselectie addition of an alkyl group, without causing base induced racimization or making the dialkylated product.[19]

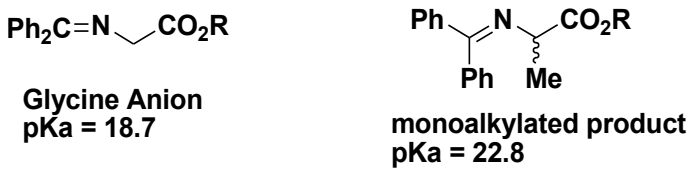


Figure 12. Comparison of acidity of protons on alpha carbon when not alkylated and when mono alkylated.

1.7.1 Entry into Eantioselective PTC utilizing *Cinchona* Alkaloids

The *Cinchona* alkaloids have played a major role in the development of phase-transfer catalysis. Cinchonine and cinchonidine derived catalysts have been used commonly in chiral PTC due to the parent alkaloids being inexpensive and easily converted into effective phase-transfer catalysts.[17]

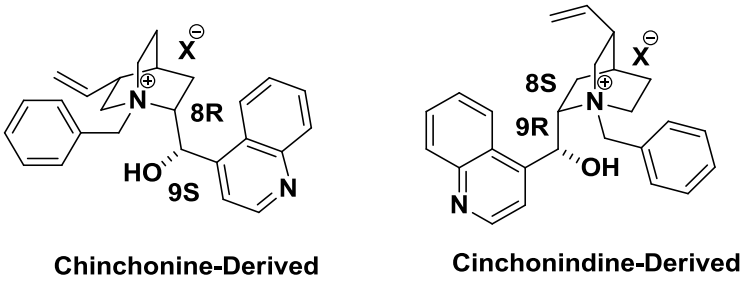


Figure 13. First Generation Cinchona Alkaloids

The first generation of Cinchona alkaloids gave enantioslective alkylations on the order of 66%ee using the O'Donnell Schiff base and Sodium Hydroxide. The O'Donnell group made a large improvement to enantioselectivity by suggesting the O-alkylation of the Cinchona quaternary ammonium salt was the active catalyst. From this idea came the second generation of Chinchona-Derived Catalysts.

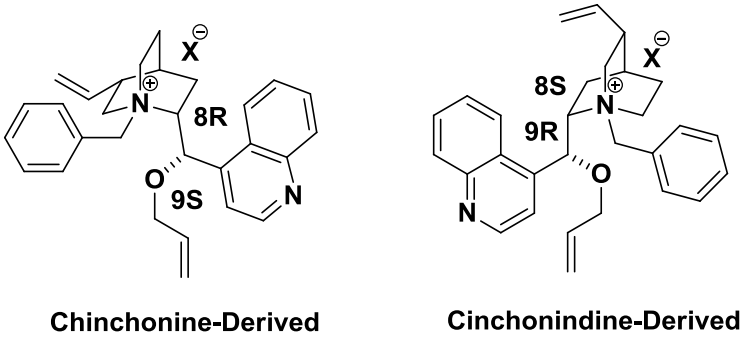


Figure 14. Second Generation Cinchona Alkaloids

The highest reported enantioselectivity for this generation of catalyst was 81%ee. Solvent mixtures for the second generation of catalyst have been reported using toluene:dichloromethane in ratios of (7:3). This solvent ratio has not changed much from this generation into the future.

A number of groups have tried to further change the catalyst, these groups include Lygo,[20] Corey, who were simultaneously reported the third generation of catalyst which employed a larger and therefore more sterically locked aromatic ring as the quaternary portion of the ammonium salt, for this they used N-9-anthracenylmenthyl.

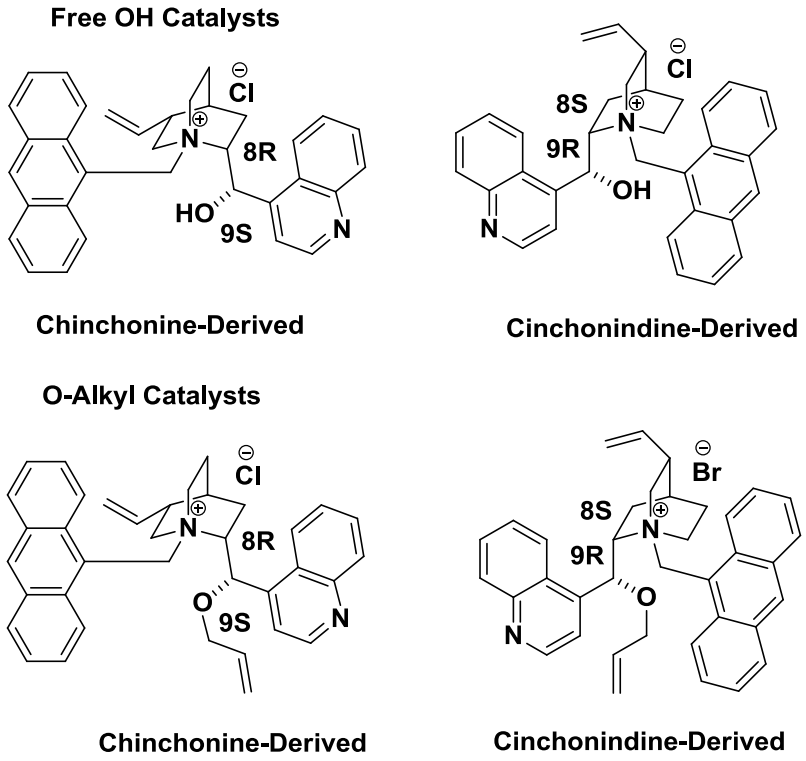


Figure 15. Free OH compared with alkylated oxygen on the phase transfer catalyst

Corey et al. suggested the enantioselectivity may be due to the key ion pair between enolate and catalyst, where the alkyl halide approaches the ion pair from the dashed arrow leading to

the S product in the case of the Cinchonindine catalyst. Depicted on the top is the anion of the Schiff Base and on the bottom is the cation of the 3rd generation catalyst.

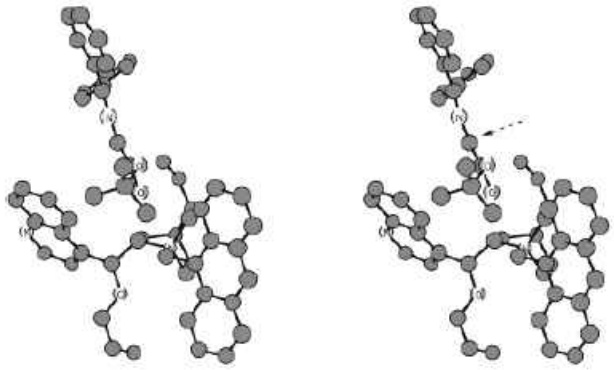


Figure 16. Stereoview of the ion pair between the enolate of the O'Donnell Schiff base and the phase transfer catalyst

Phase-transfer catalysis has been proven to be a powerful tool in synthetic organic chemistry because of its simplicity mild conditions, and suitability for scale up. This field of highly enantioselective alkylations has become a promising area of green sustainable chemistry. Asymmetric transformations catalyzed by chiral onium salts and crown ethers have been used to synthesis an array of compounds from amino acids to natural product to synthetic drugs.[21]

1.8. Asymetric Phase-Transfer Catalysis Utilizing Chiral Quaternary Ammonium Salts: Asymmetric

Phase-transfer catalysts (PTCs) by definition help transfer a substrate molecule or ion from the aqueous phase to the organic phase. The decrease in the amount of energy required to cross the phase barrier

Due to the shuttling of the PTC greatly increases the rate of the reaction. Quaternary ammounium salts are the most commonly utilized PTC. This reaction often proceeds due to the formation of an anion (Y^-) in the organic phase due to the hydrophobic nature of organic phases in comparison to aqueous solutions the anion. Substrate (Y^-) is more reactive in the organic phase when ion paired to Q^+Y^- due to the ion pairs greater charge separation and lower hydration, this effect causes greatly increased reaction rates when compared to not using the PTC.[22]

The controlled delivery of anion to the substrate causes greater selectivity when compared to PTCs alternative homogeneous reactions. The reaction conditions are tolerant to most water-immiscible organic solvents. Although many times there are more reagents added to phase-transfer catalyzed reactions, the reactions are often times easy to purify due to the two phase nature of the reactions, organic products into organic layer and ionic salts and other water-miscible materials in the water layer. Lastly the catalysts are usually cheap and environmentally benign.

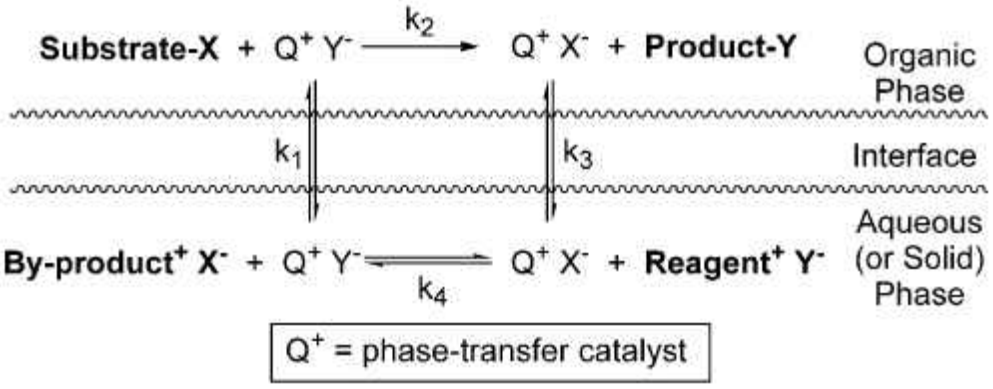


Figure 17. Phase-transfer catalyst diagram of ion exchange

1.8.1 Further development of Glycine Imines

O'Donnell reported the first asymmetric alkylation of the glycine imine ester utilizing phase-transfer catalysis in 1989.[19] These studies using the first generation N-benzyl *Cinchona* alkaloids were used, resulting in enantioselectivities ranging from 42-66% ee. These studies led to the conformation that *tert*-butyl ester imine was the best substrate in terms of enantioselectivity and that the diastereoisomeric catalyst of cinchonine and cinchonidine were enantio-complimntarey meaning they lead to the opposite chirality in the products.

The phase-transfer catalysts, stemming from the *Cinchona* alkaloids, were found to undergo O-benzylation under the reaction conditions. This led to the development of prealkylated salts which gave similar enantioselectivity. Ion-pair arrangement A accounts for the enantioselectivities obtained using *Cinchona* based phase-transfer catalysts. "In this arrangement the Re-face of the enolate carbon is blocked by the quinolone ring of the quaternary ammonium salt, so preferential reaction via the Si-face would be expected. Alternate ion pair's inspection of structure in ion-pair B suggested it should be less favored due to the increased charge separation required to accommodate the *tert*-butyl group in the "groove" between the quinolone and anthracene rings."[22]

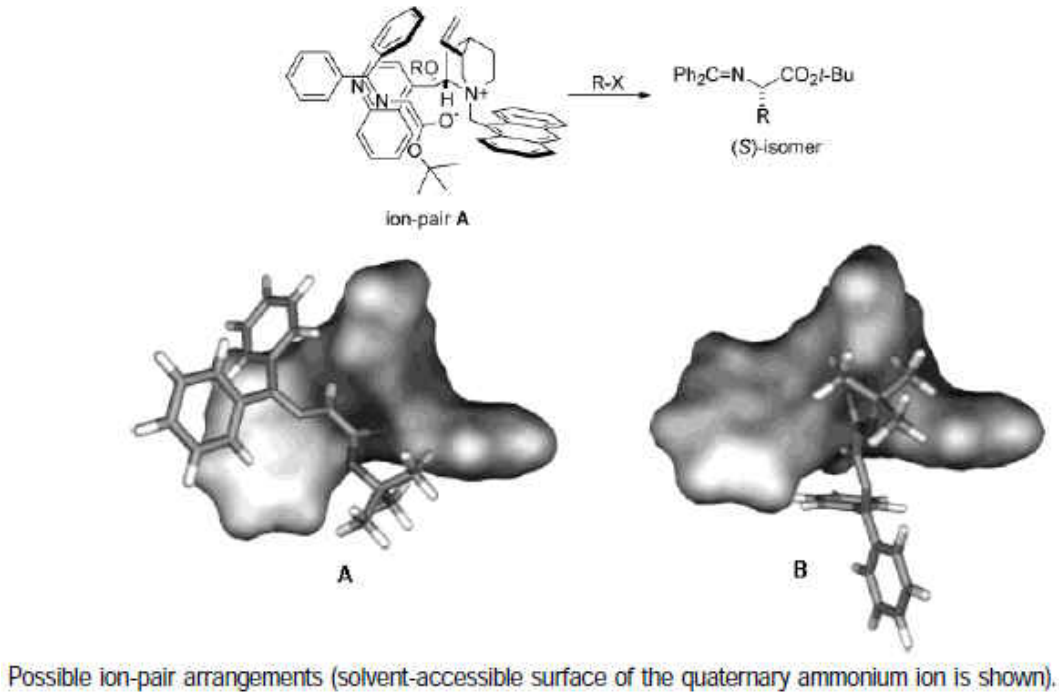


Figure 18. Ion pair between phase-transfer catalysit and O'Donnell Schiff base

The drive to develop asymmetric phase-transfer alkylation reactions as a "green" alternative to its homogeneous counter parts and the reactions tolerance to non-chlorinated solvents, ambient temperature and aqueous base makes them environmentally benign in comparison. Good yields and above 90 percent enantioselectivity have been reported for the synthesis of a wide assortment of amino acids.[23] Often times these products can be crystallized to reach high levels of enantiomericly pure products.

1.9. Diketopiperizine rings and their significance

2,5-Diketopiperazines (2,5-DKPs) are formed by closing a six membered ring made up of the backbones of two amino acids. These cyclodipeptides are prevalent in nature. The combination of these two things have allowed DKPs to become a unique class of naturally occurring privileged structures, allowing significant diversity due the readily available number and wide

range of characteristics including functionality, hydrophobicity and charge at physiological pH throughout amino acids. This family of compounds can bind to a wide assortment of biological receptors; this six membered cyclic dipeptide is constrained four, of the positions can be manipulated in terms of stereochemistry. Due to the manipulation potential in terms of stereochemistry DKP are quite easy to chirally enrich using only amino acids in the synthesis.[24]

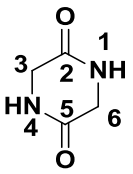


Figure 19. Numbering system of 2,5-DKPs

2,5-DKPs have been extensively examined using crystal and molecular structures. Some of the DKP's ability to bind to enzymes and receptors is due to the two H-bond acceptor and two H-bond donor sites they contain that stem from the two *cis*-amide bonds. 2,5-DKPs exist as flat and slightly puckered boat isomers, separated by only a few kcal/mol because of it slightly rigid yet flexible conformation.[24] All of the known active Typrostatins,Fumitremorgins and Spirotryprostatins contain both an indole ring and a diketopiperizine ring.

Dipeptides can spontaneously cyclize to form a 2,5-DKP, although this requires an amine at one terminus and an ester at the other. Coupling a nitrogen-protected α-amino acid and an α-amino acid ester the most commonly used synthetic procedure.[24]

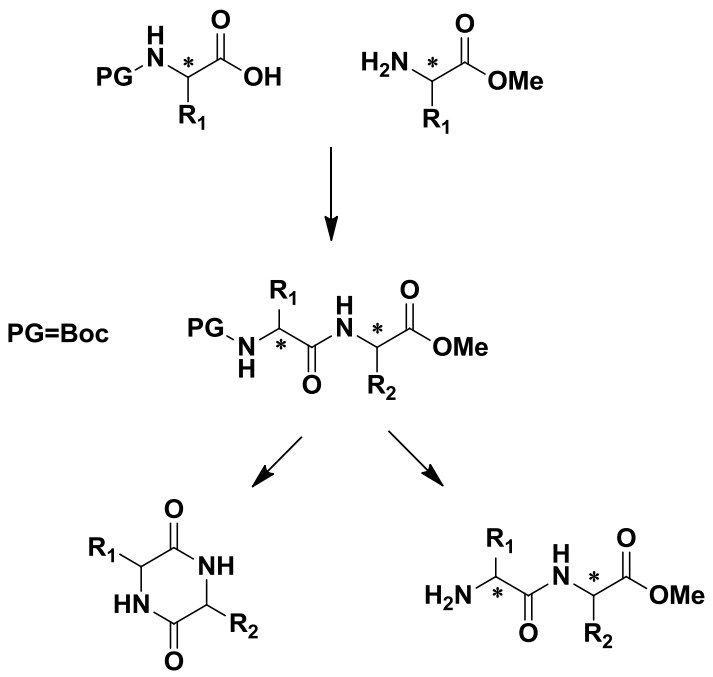


Figure 20. Most common synthetic procedure for closing 2,5-DKP

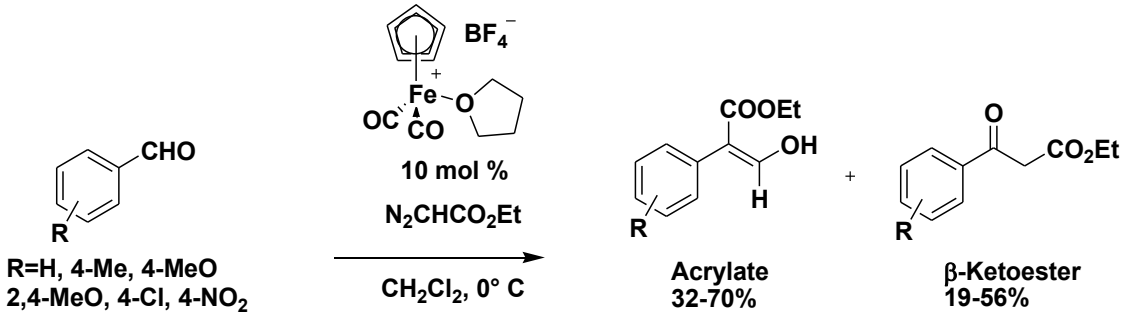
Deprotection of the nitrogen protecting group yields the dipeptide ester, now the nitrogen can act as a nucleophile and attack the carbonyl carbon displacing the ester *insitu* to make an amide. *Cis*-orientation of the amide bond is required for the closing of the six membered ring. Ring closing is difficult if the cis-orientation is prevented due to steric or electronic effects.[24-25] Other strategies that have been used to close dipeptide rings have involved refluxing in high boiling solvents for extended amounts of time. An example of this was reported by Cooks group to close the DKP ring on Tryprostatin, conditions include refluxing in Xylenes for 48 hours.[1d, 26] As of 2006 Tullberg's group has demonstrated DKP ring closures utilizing microwave heating. This procedure utilizes water as a solvent and has shown no epimerization and most importantly is tolerant to amino acid sequence.[27] Due to the Boc protecting groups thermally labiality, Boc nitrogen protected dipeptides became standard in this synthetic procedure, leading to the deprotection and cyclization in one step.

2. BACKGROUND

2.1. Development of the Acrylates

The Hossain group has been working on acrylate chemistry since 1998 when they published a paper on the topic of catalytic iron Lewis acid catalyst activation of benzaldehydes to form acrylates.[28] The products of this reaction are in competition with the minor product the beta keto ester. [28]

Scheme 8. Synthetic equation using iron Lewis acid catalysis to make acrylates



In 2004, the group screened the reaction against other Lewis acids to evaluate the efficiency of the iron Lewis acid. $HBF_4{\cdot}OEt_2$ is used in the production of the iron Lewis acid, so it was questioned whether residual $HBF_4{\cdot}OEt_2$ in the iron Lewis acid could be catalyzing this reaction.[29]

Scheme 9. Comparison between Iron Lewis acid and HBF_4 to make acrylate

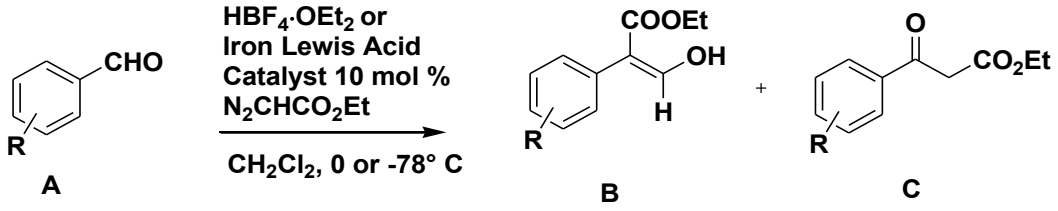


Table 1. Comparison of acrylate and beta-keto ester using Iron Lewis catalyst vs HBF_4

A R^1	Cat	˚C	Yield B	Yield C
H	HBF_4	rt	42	21
	HBF_4	-78	74	0
	$Fp^+BF_4^-$	rt	58	25
	$Fp^+BF_4^-$	0	70	19
	$Fp^+BF_4^-$	-78	68	19
4-MeO	HBF_4	0	75	15
	HBF_4	-78	90	0
	$Fp^+BF_4^-$	0	60	20
	$Fp^+BF_4^-$	-78	4	0
2-Me	HBF_4	0	60	35
	$Fp^+BF_4^-$	0	74	15
4-Br	HBF_4	0	55	34
	$Fp^+BF_4^-$	0	62	17

Letters in table correspond to the letter assignment of the molecules in the synthetic scheme.

This reaction is expected to proceed in favor of the acrylate over the beta-keto ester due to the lowest energy Newman projection, compared to the second lowest energy projection, the temperature depression favors the lowest energy Newman projection in turn leading to higher acrylate yields. This reaction procedes through a unique 1,2-aryl shift instead of by a hydride

migration which has been shown in 1998 in the Hossain's group. The changes of in fine tuning of ratios of observed B and C were varing electron donating group which supports 1,2-aryl migration over hydride migration.

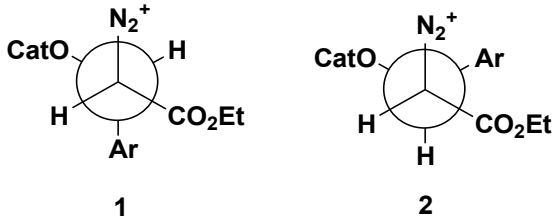


Figure 21. Newman projection of possible transition states between acrylate and beta-keto ester

2.2. Acrylates to 3-ethylesterindoles

In 2006, after identifying that $HBF_4 \cdot OEt_2$ is the best catalyst for the reaction, and that -78°C is the optimum temperature for regioselectivity of the acrylate over the beta-keto ester, the Hossain group began work on making the ortho-nitro-acrylates into 3-ethylesterindoles.[30]

Scheme 10. 2-nitrobenzaldehyde through aldehyde to 3-ethylesterindole

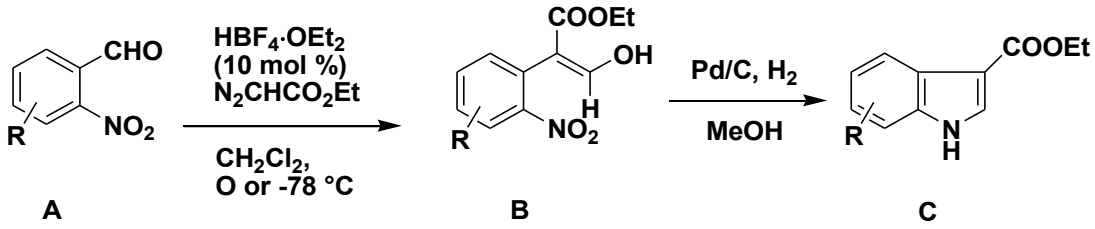


Table 2. Yield of benzaldehyde to acrylate

A R	Catalyst	˚C	Yield B
H	$Fp^+BF_4^-$	0	73
	HBF_4	0	73
	HBF_4	-78	75
5-OCH_3	$Fp^+BF_4^-$	0	68
	HBF_4	0	68
	HBF_4	-78	75
4,5-OCH_3	HBF_4	-78	76
4-OCH_2O-5	HBF_4	-78	86
5-Cl	$Fp^+BF_4^-$	0	35
	HBF_4	0	45
	HBF_4	-78	50

Letters in table correspond to the substrate in the synthetic diagram.

Table 3. Yields of acrylates to 3-ethylesterindole

B	Yield C
H	90
5-OCH_3	62
4,5-OCH_3	76
4-OCH_2O-5	86
5-Cl	66

It is proposed that this reaction proceeded via the following mechanism.

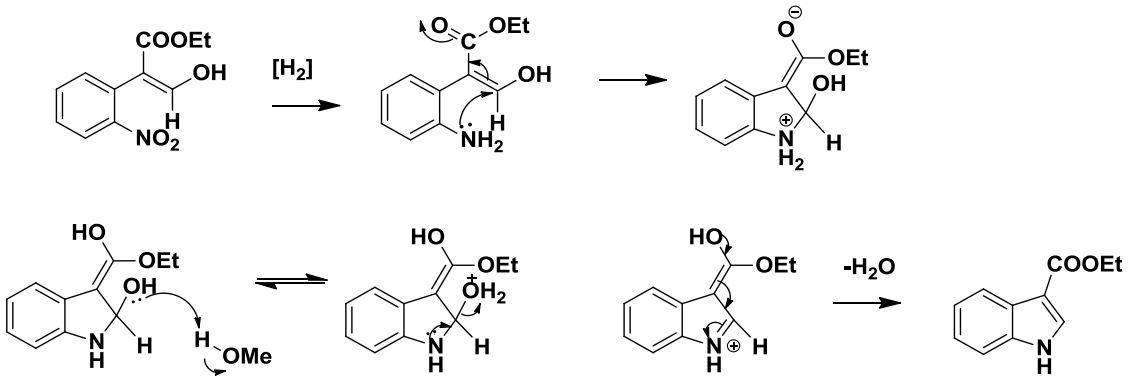


Figure 22. Mechanism demonstrating reduction of acrylate and ring closing of indole

2.3. 3-ethylesterindoles to gramines

In 2009 the group published a procedure to convert the protected 3-ethylesterindole into a 3-carboxamide using an amidoaluminum mediated mechanism. From 3-ethylesterindole using DIBAL-H it was possible to convert the carboxamide to gramine.[31]

Scheme 11. 3-ethylester indole to gramine

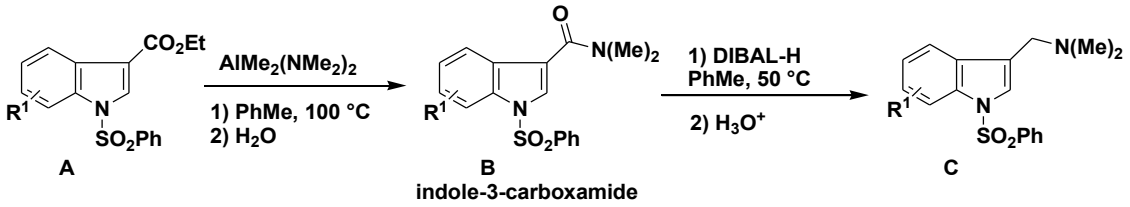
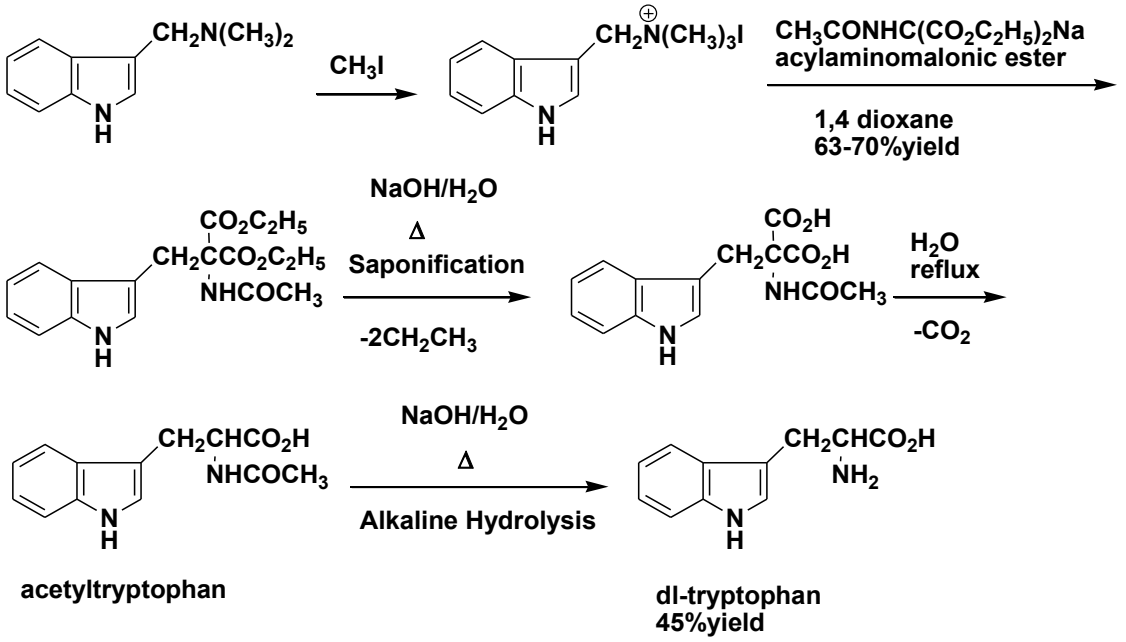


Table 4. Yield of 3-ethylesterindoles to gramines with ring A substitutions

A R^1	Yield B	Yield C
H	77	94
5-MeO	73	87
6-MeO	64	90
5-Br	61	65

This leads us to investigation of what has been done previously with gramines. In the mid-1940s various groups were researching the conversion of gramine to racemic tryptophan.[32]

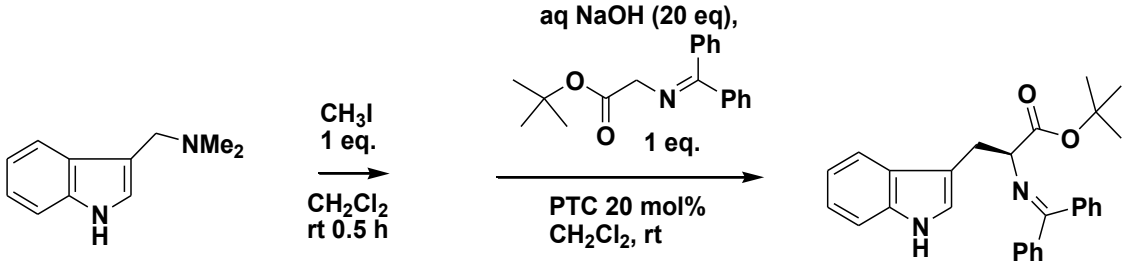
Scheme 12. Synthesis of quaternary ammonium salt to racemic tryptophan



We wondered if it would be possible to make optically-pure tryptophan through a chiral phase transfer catalyst reaction using organo-catalyst. We thought this would be interesting chemistry and would be likely to find industrial use, as tryptophans are important building blocks for

indoles a novel class of compounds. The Hossain group developed the following reaction to make tryptophan.

Scheme 13. Initial screening to make optically active protected tryptophan



At this point we began screening for a catalyst that would give high enantiomeric excess (%ee). The catalysts that were screened are shown below. All of the catalysts are derived from the 3rd generation of cinchonidine catalysts.[31]

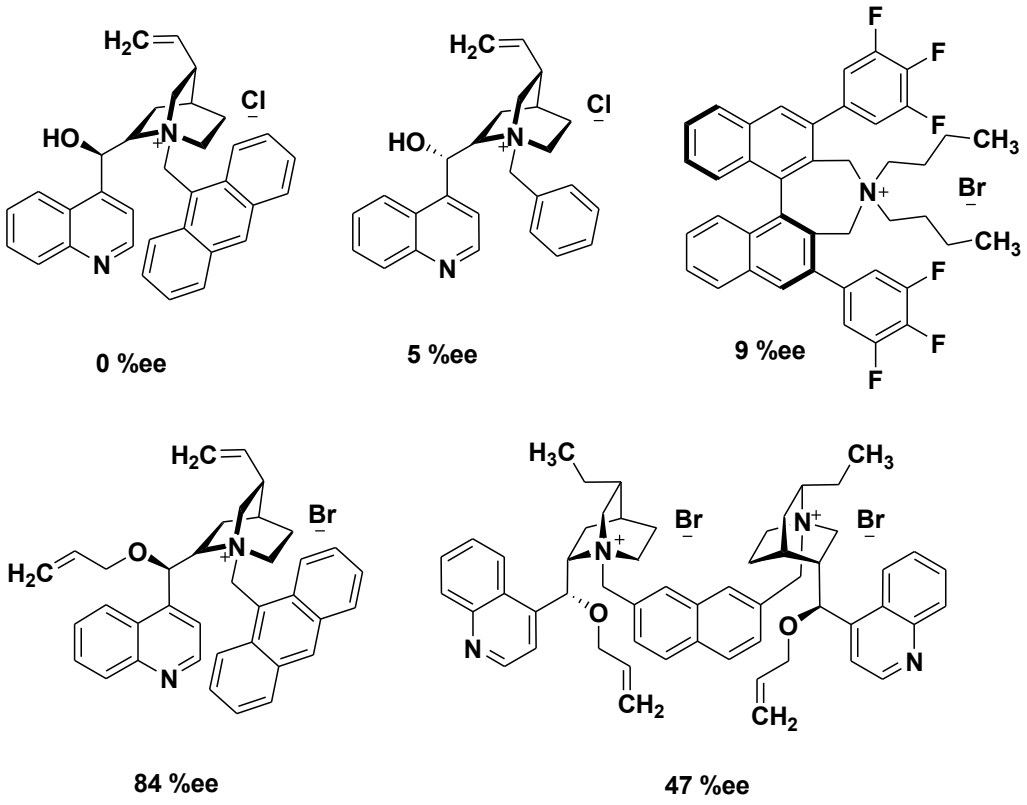


Figure 23. Phase-transfer catalysts that were screened

From this screening process found that O-Allyl-N-Anthrcenyl- bromide gave the highest % ee. The next thing that needed to be screened was the quaternization reagents. Quaternization reagents screened are shown below. Note that it is important for the reaction that the substrate is as soluble in the organic layer as possible; this forces the reaction to proceed *via* the phase transfer catalyst.[33]

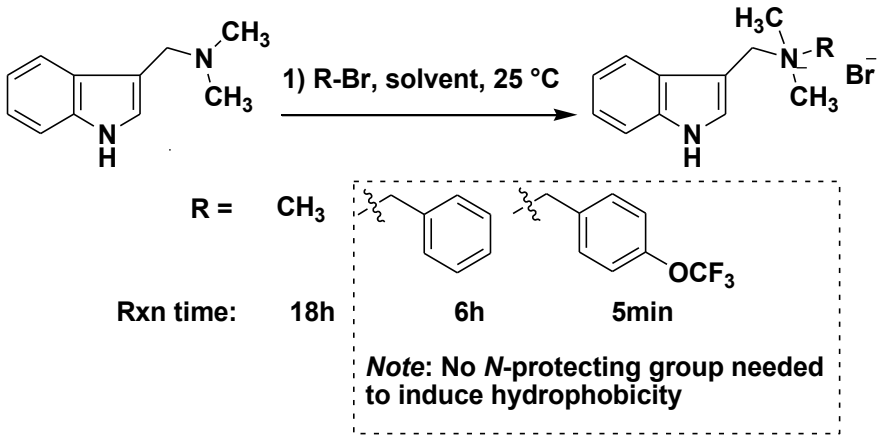


Figure 24. Screening of quaternization reagents

Next the amount of base in the aqueous layer was varied and the results monitored. Results are shown in the table below.

Scheme 14. Synthesis of optically active tryptophan screening various bases and concentration of bases

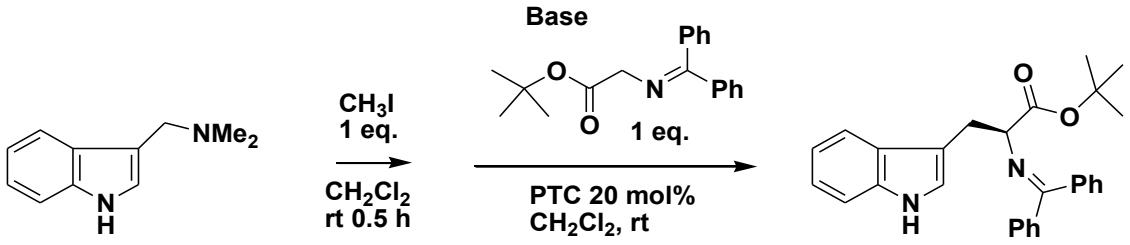


Table 5. Screening of optically active tryptophan with bases at varying concentrations

Run	Conc(**1a**)[c]	% Base (% aq)	Time (h)	% Yield[d]	% ee[e]
1	0.1	10% NaOH	> 24	18	50
2	0.1	50% NaOH	8	47	75
3[b]	0.01	50% NaOH	16	42	71
4	0.1	10% KOH	5	65	65
5	0.1	45% KOH	2	>95	84
6	0.1	10% KOH	2	97	80
7	0.1	10% CsOH	3	18	59
8	0.1	10% $Ba(OH)_2$	13	16	46
9	0.1	25% K_2CO_3	N.R.[f]	0	0

Next, the catalyst loading was varied and monitored as shown in the reaction below; the results are shown in the table.[33]

Scheme 15. Screening of amount of phase transfer catalyst that was used

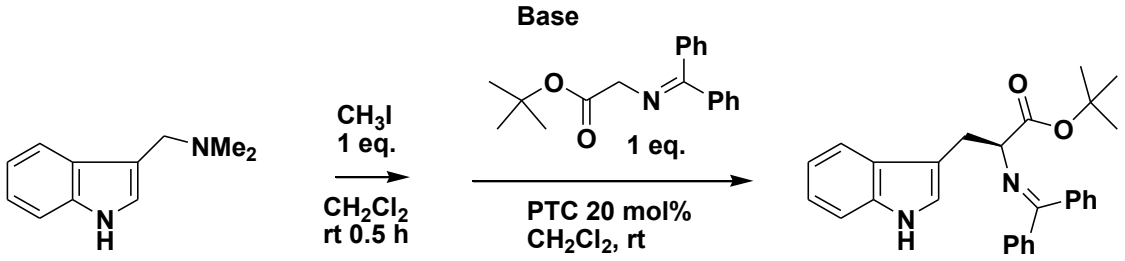


Table 6. Screening of various amounts of phase-transfer catalyst loading

Entry	Substrate Conc.	Base	Catalyst Loading	Reaction Time (h)	% Yield[a]	% ee[b]
1	0.1	NaOH	0.2	11	18	59
2			0.6	4	47	81
3		KOH	0.2	22	9	59
4			0.6	2	97	80
5	0.5		0.6	1	99	75
6	0.1	$CsOH•H_2O$	0.2	3	39	53
7			0.6	3	81	71
8		$Ba(OH)_2•8H_2O$	0.2	13	16	49
9			0.6	3	36	67
10	0.25	K_2CO_3	0.6	2	0	0

Following the same screening process we screened various organic solvents.

Scheme 16. Solvent screening to make optically active tryptophans

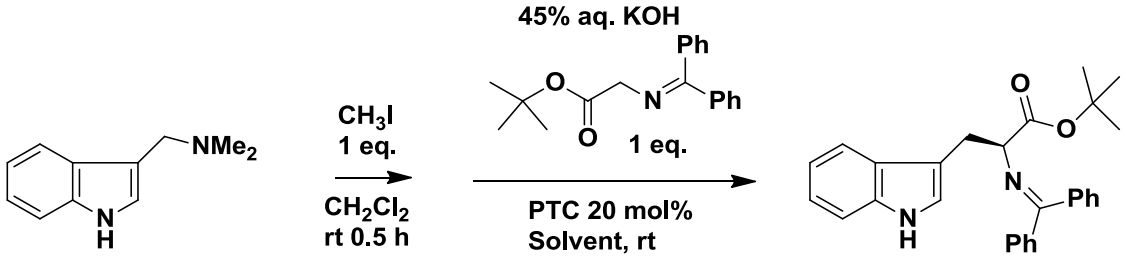


Table 7. Solvent screening to make optically active tryptophan

Entry	Solvent	Time (h)	% Yield[b]	% ee[c]
1	CH_2Cl_2	1	99	75
2	1,4-Dioxane	2	95	84
3	THF	4	85	6
4	$PhCH_3$	3	62	71

The next thing we wanted to monitor was the effect of temperature on the reaction.

Scheme 17. Monitoring the effect of temperature when making optically active tryptophan

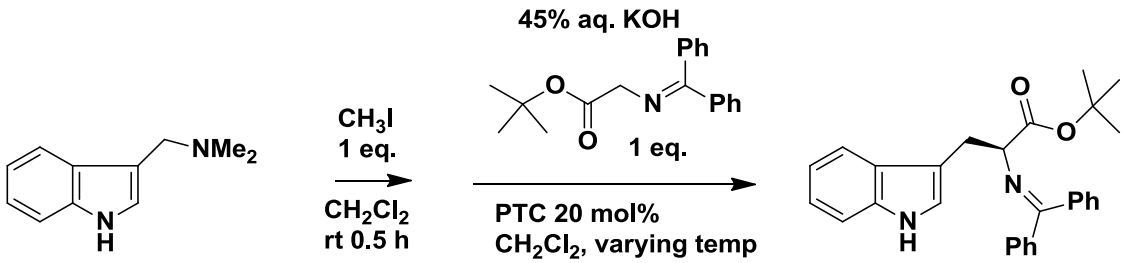


Table 8. Monitoring the effect of temperature depression on the synthesis of optically active tryptophan

Entry	Temp C	Rxn time (hrs)	% Yield	% ee
1	25	1	86	75
2	-30	8	80	84
3	-78	15	81	83

Next we wanted to monitor the effect the number of equivalents of water had on the synthesis of optically active tryptophan.

Scheme 18. Monitoring the effect of number of equivalents of water on the synthesis of optically active tryptophan

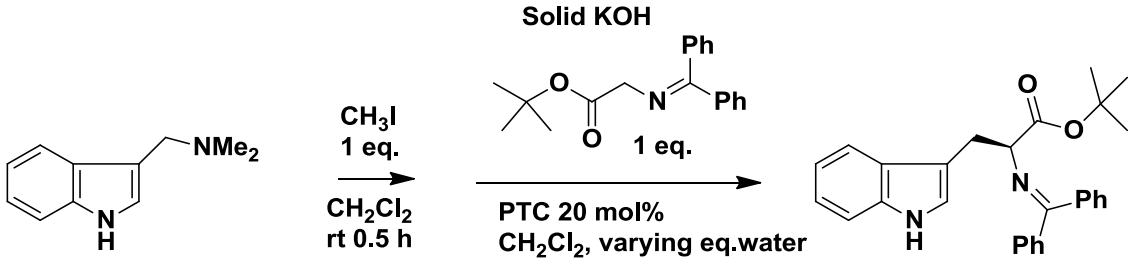


Table 9. Monitoring the effect of number of equivalents of water on the synthesis of optically active tryptophan

Entry	Water (equiv)	Time (h)	% Yield[a]	% ee[b]
1	100	8	80	85
2	6	18	80	92
3	3	19	>95	83

Summary of the screening shows that O-allyl-N-anthrcenyl-cinchonadinium bromide is the best catalyst, 4-trifluoromethoxybenzyl bromide is the best quaternarization reagent, KOH best yielding base, a minimum 6 eq. water for optimal %ee, dioxane is the best solvent in regards to %ee, and dichloromethane is the best solvent in terms of yield.[33]

3. OBJECTIVE

3.1. Optically active tryptophan derivatives

The primary goal was to develop a method to asymmetrically synthesize tryptophan from gramines in a fashion that was tolerant to variations on the 4, 5, 6 and 7 position of the indole ring. To make this objective an accomplished goal, we applied this procedure to 5-bromo, 5-methoxy and 6-methoxy gramines to make the corresponding tryptophan derivatives.

3.2. Using optically active tryptophan to synthesize natural product tryprostatin A and B

After this goal was accomplished, our next goal was to develop a method that could utilize this asymmetric reaction yielding enantiopure tryptophans into a total synthesis of a natural product. We decided our targets would be tryprostatin A and B. These natural products have low natural abundance, lengthy synthesis, and the synthesis utilizes a protected L-tryptophan as starting material.

3.3. Utilizing enantio-enriched tryptophan and tryprostatin synthesis to make derivatives of tryprostatin

In the big picture we set out to develop a method that would asymmetrically synthesize tryptophan and derivatives of tryptophan. We then utilized this newly developed method to streamline the synthesis of tryprostatin B and tryprostatin A. We believe we have developed a procedure that is tolerant to ring-A gramine analogs and reaches far beyond the scope of previous syntheses because of this tolerance to ring-A substitution possibilities. Lastly, although it was not a goal we initially set out to accomplish, we have also developed synthesis that very possibly has the potential to give an entry way into making C2-derivatized tryptophan.

4. RESULTS

4.1. Synthesis of optically active tryptophan and three analogs

We wanted to evaluate how tolerant the phase transfer catalyzed asymmetric reaction was to various substitution patterns on the indole ring. HPLC was used to determine enantiomeric excess mobile phase was 6% Isopropyl alcohol 94 % Hexane at a flow rate of 1 mL/min using a Chiralcel OD column.[34]

Scheme 19. Synthesis of 5 and 6 indole ring position tryptophan analogs

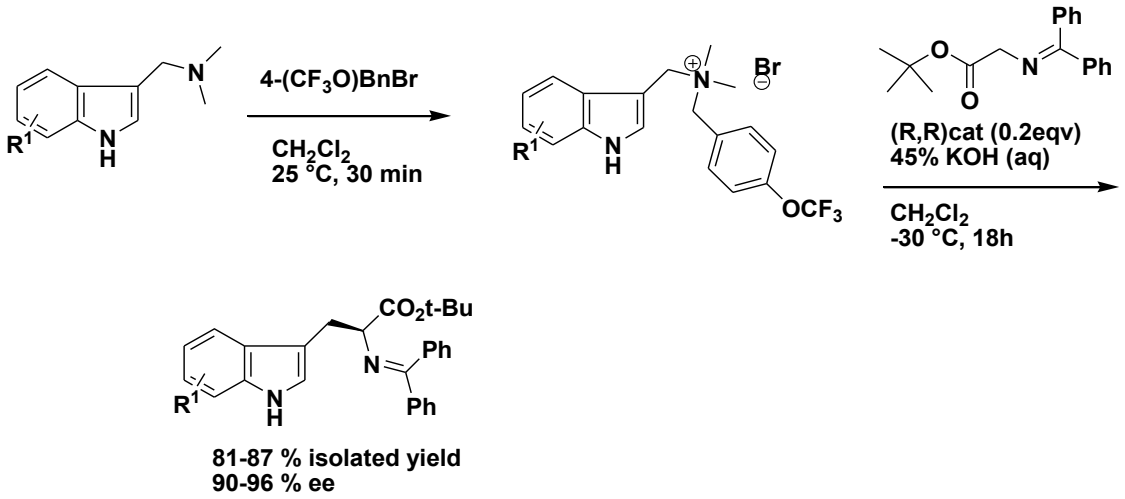


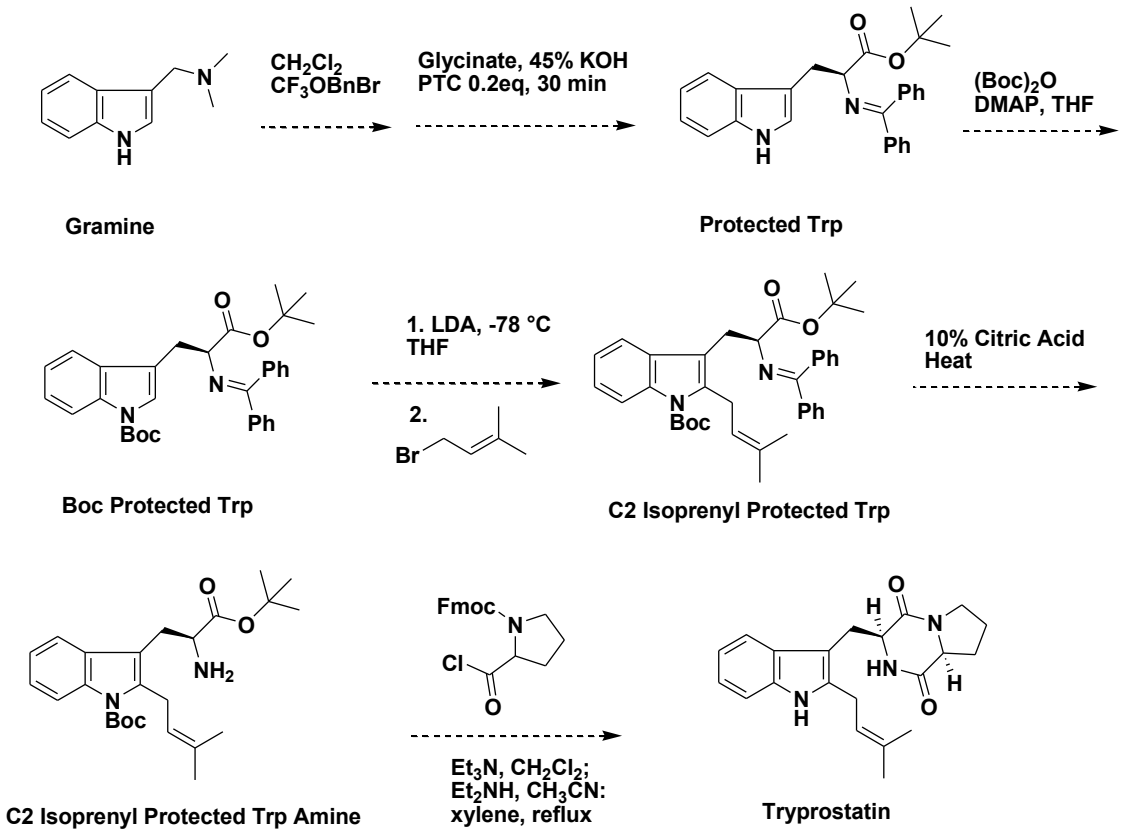
Table 10. Synthesis of 5 and 6 indole ring position tryptophan analogs

Tryptophan %ee		
R^1	Yield	%ee
H	80	92
5-MeO	75	91
6-MeO	65	95
5-Br	73	90

4.2. Utilization of optically active tryptophan to synthesis natural product tryprostatin B

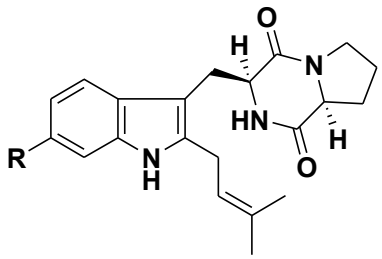
From here we looked for an application of our newly developed chiral phase transfer catalyst reaction. We proposed that we use this new reaction and apply it to the synthesis of tryprostatin and analogs of the parent structure. Shown below is our proposed synthesis.

Scheme 20. Initial proposed synthesis of tryprostatin B



At this point we would have the shortest most concise synthesis of Tryprostatin B. Our objective at this point was to shorten the synthesis making it viable for commercial applications and improve on the anticancer activity by making analogs. The whole time we had to keep in mind that the reaction scheme has to be analog tolerant.

Like other anti-cancer microtubule inhibitors such as the *vinka* alkaloids including the vinblastine family, Tryprostatin has very low abundance in nature. We chose to target the tryprostatin family of compounds due to their simpler synthesis when compared to the Vinblastine family of compounds.



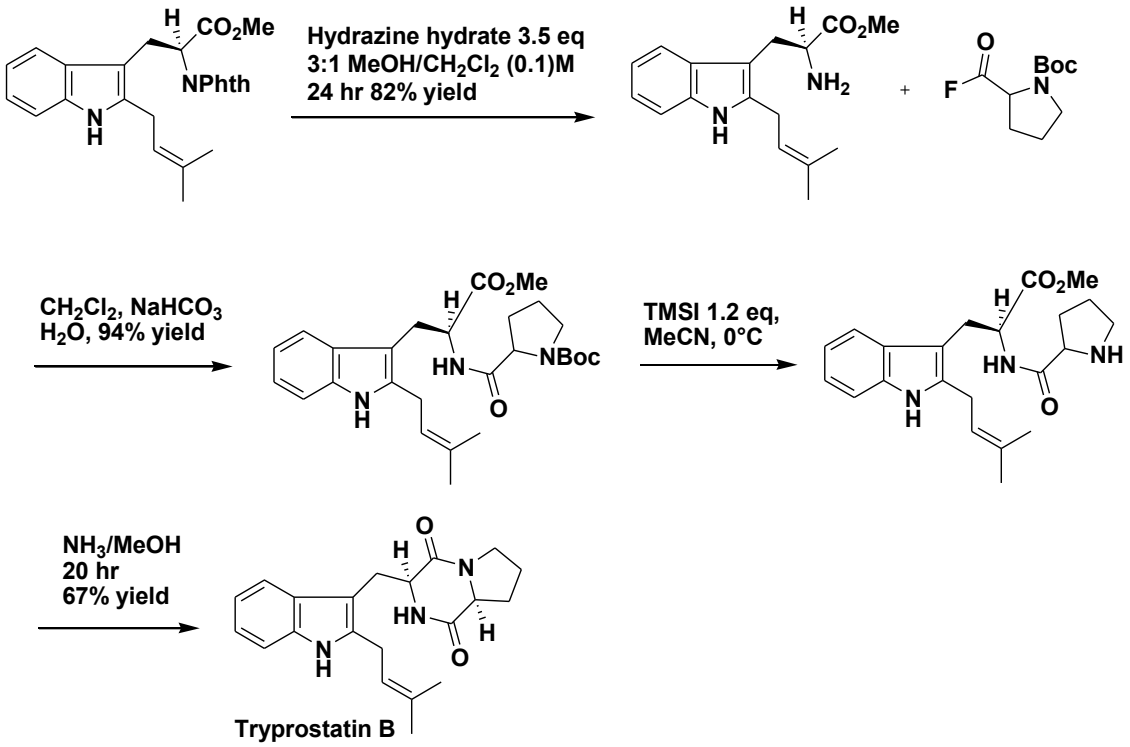
R=MeO Tryprostatin A
R=H Tryprostatin B

Figure 25. Tryprostatin A and B

4.3. Previous Tryprostatin syntheses

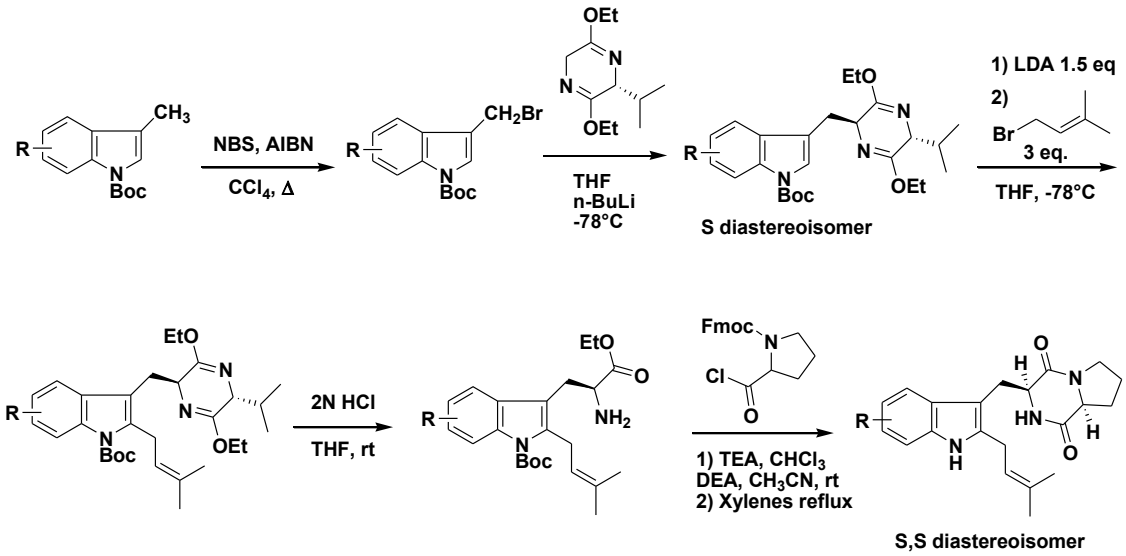
The first synthesis of Tryprostatin B was completed by the Danishefsky's group in 1996 using the following procedure.[3a]

Scheme 21. Danishefsky's 1996 Tryprostatin B synthesis



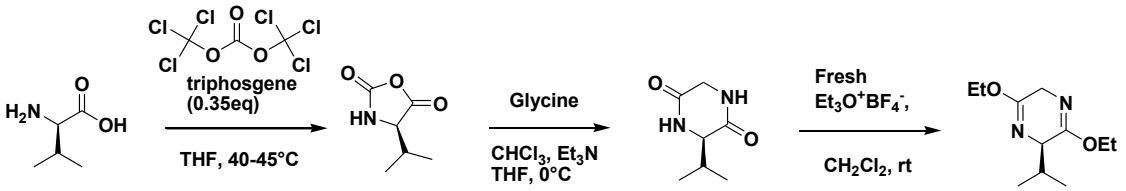
Five years later the Cook group synthesized Tryprostatin A in 2002 and followed that by synthesizing a number of enantiomers, diastereomers and other substituted analogs in 2008 using very similar procedures developed in 2002. In 2008 they substituted in unnatural amino acids or other substituted starting materials. They achieved the synthesis of these new compounds using the reaction scheme shown below. [1d, 2]

Scheme 22. Cook's 2002 Tryprostatin B synthesis. Further developed in 2008 to include diastereomers, enantiomers and a number of derivatives



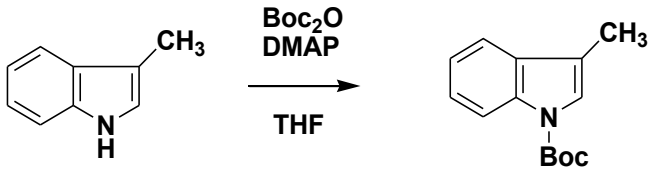
One of the drawbacks to Cook's synthesis is that it uses triphosgene to make the Schöllkopf Chiral Auxiliary. Triphosgene is a chemical that is not preferred to be used by most chemists due to its decomposition to phosgene, which gained infamy due to its use as a chemical weapon during World War 1. Therefore we desired to skip the use of triphosgene altogether.

Scheme 23. Synthesis of Schöllkopf Chiral Auxiliary



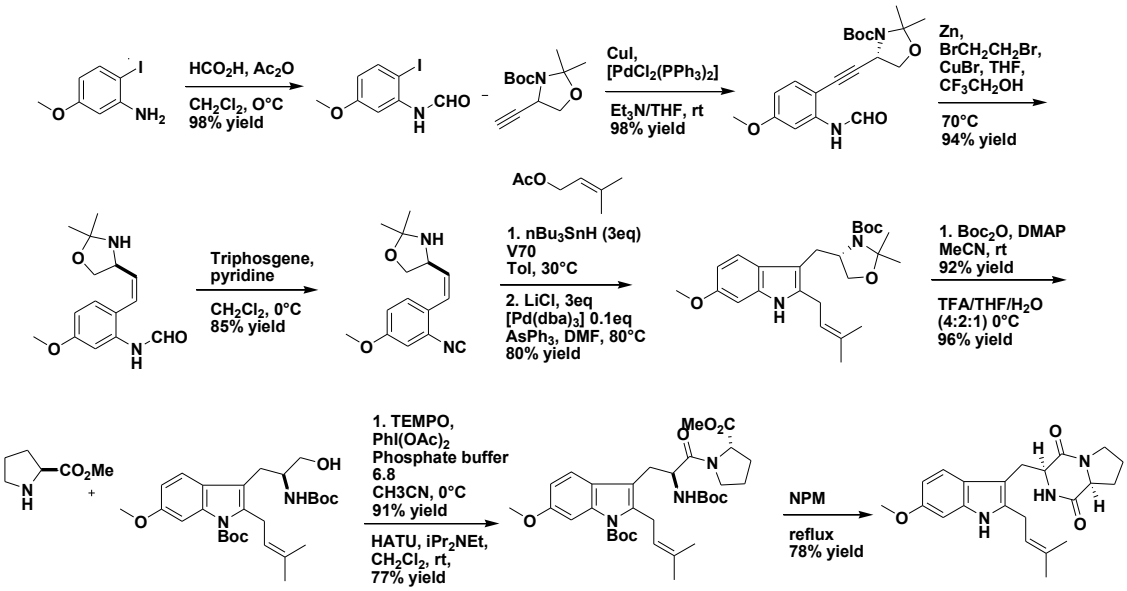
In the synthesis they also have to Boc protect skatole shown below which is not reported as a step in the synthesis. There are very few substituted skatoles commercially available to make Tryprostatin derivatives with.

Scheme 24. Boc protection of skatole



One of the more current syntheses from 2010 is shown in the synthetic scheme shown below.[5]

Scheme 25. Fukuyama's 2010 Tryprostatin A synthesis

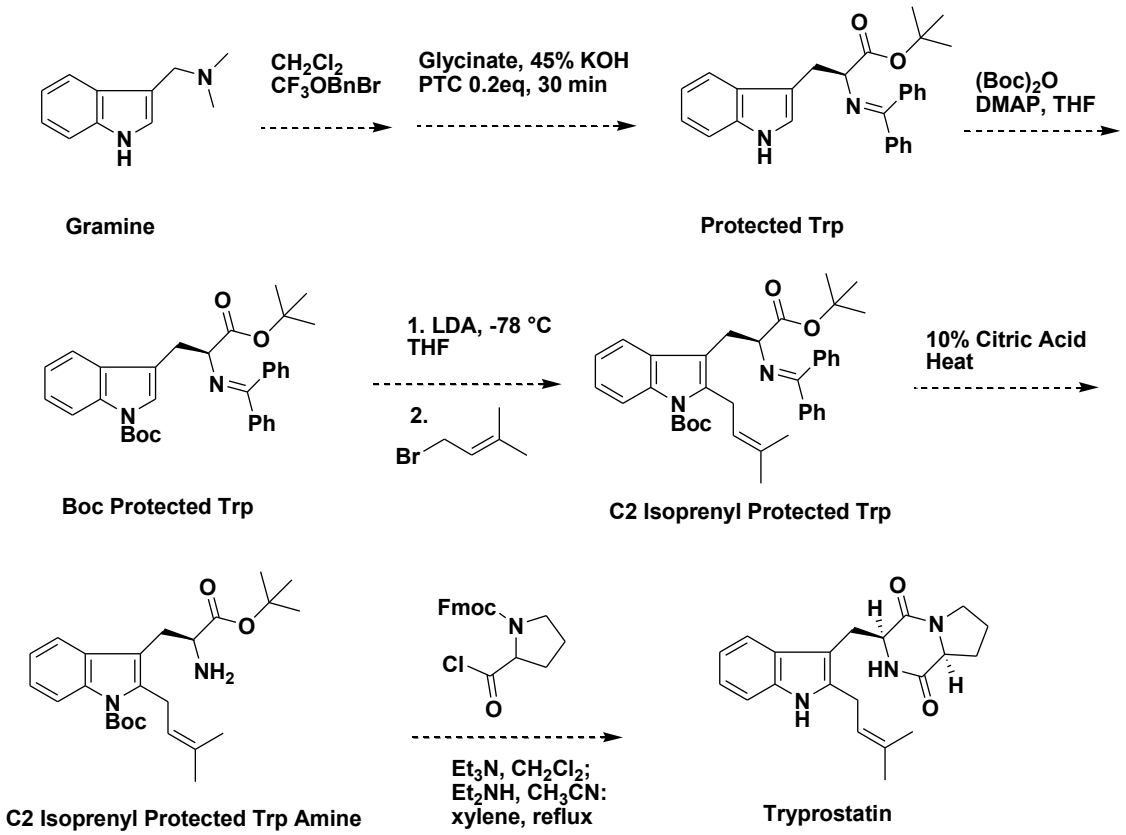


This synthesis has 11 steps a 30% yield and utilizes a toxic tin coupling reagent and triphosgene.

4.4. Our proposed synthesis

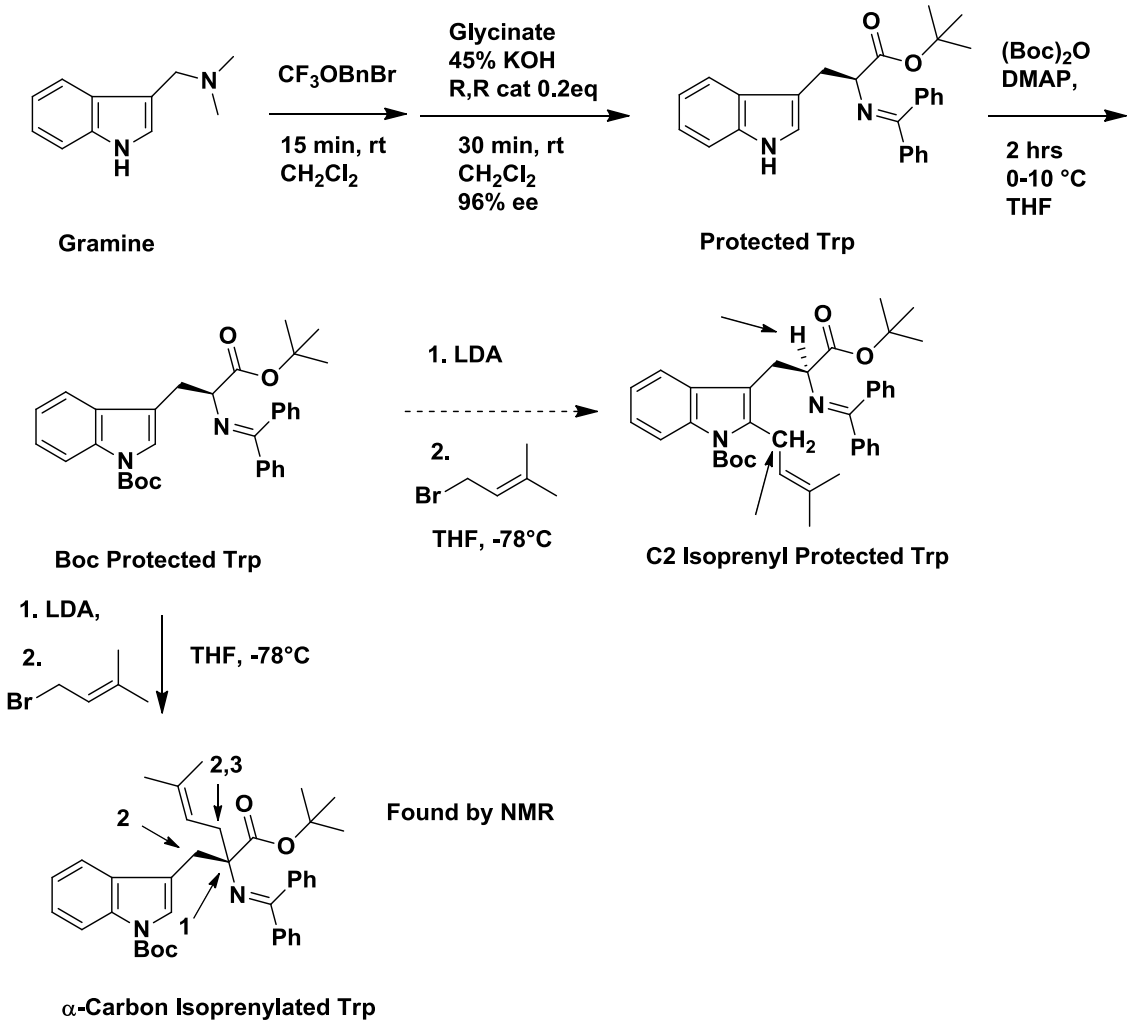
We proceeded with our proposed procedure which is complementary to the Cook group synthesis.

Scheme 26. Our proposed stream-lined tryprostatin B synthesis



This procedure was proceeding smoothly until the lithaiation isoprenyl bromination reaction, at this point it appeared that we isolated a compound that had the isoprenylation on the amino acids α-carbon.

Scheme 27. Isoprenylation at the alpha carbon position instead of the C-2 position



Two compounds could have been expected. We desired to identify proton NMR peaks for the C2 isoprenylated compound include the α-carbon's proton at for one proton at 4.35 ppm (Figure shown above indicated as H), and the carbon at 14 ppm(indicated by CH_2). Unfortunately we found the α-carbon had been isoprenylated, this was determined by three signatures that indicated α-carbon isoprenylation: 1) lack of the α-carbon's proton, 2) two sets of diastereotopic protons and 3) a carbon peak at about 36 ppm. Points are indicated by 1 2 and 3 in the diagram.

This alkylation is consistent with O'Donnell's work making unnatural amino acids. [19, 35] This α-carbon isoprenylation was not seen in Cook's procedure due to the difference in acidity of the proton in the Schöllkopf Chiral Auxiliary protected indole, compared to our very differently protected indole. We think that resonance stability and migration of electrons to stabilizes the anion shown below.

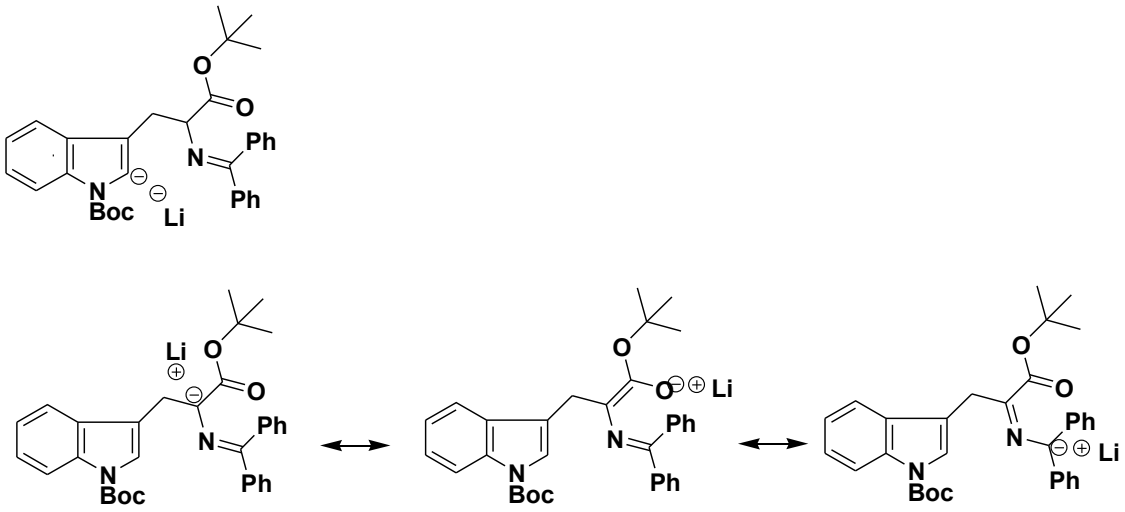


Figure 26. Comparison of resonance stabilization between two proposed transition states

Compared to Cook's compound which cannot undergo this type of resonance stabilization due difference in the chosen protecting group. Cook's compound is shown in Figure 27.

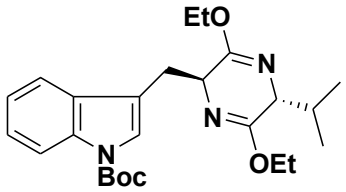
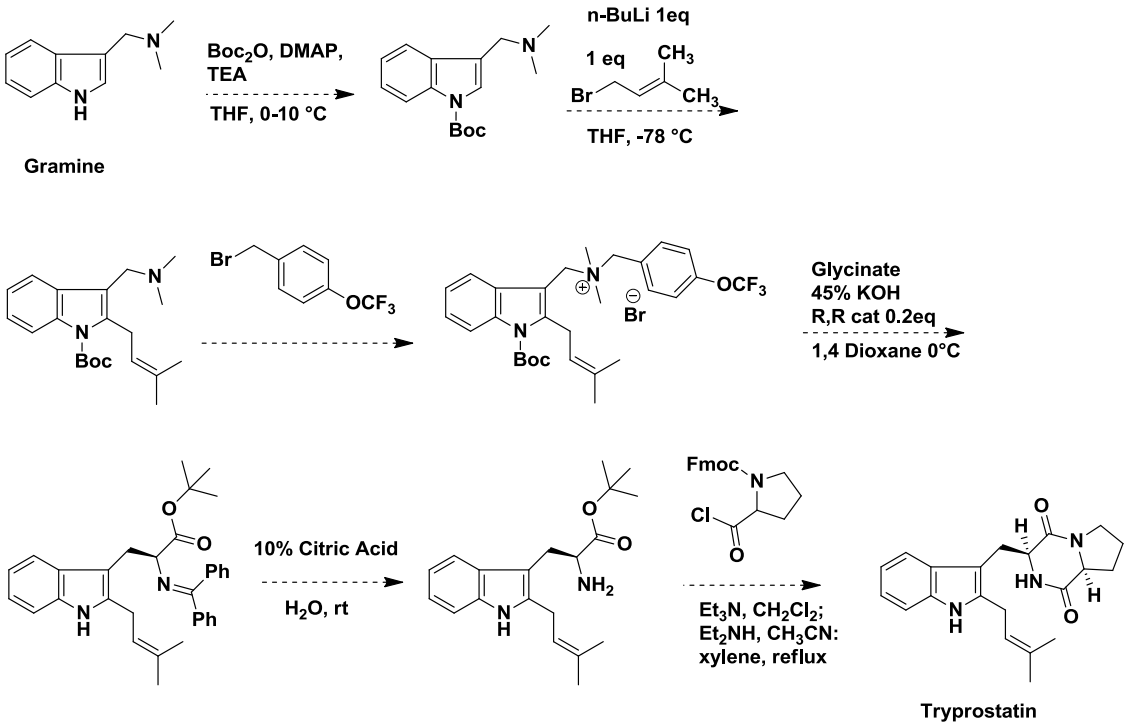


Figure 27. Cook's Schöllkopf Chiral Auxiliary protected indole

4.5. Alternative proposed synthesis

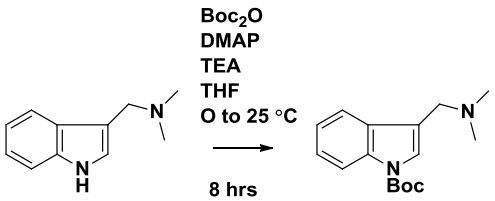
After this disappointing finding, we decided that we must take a new approach to adding the isoprenyl group to the C-2 position of the indole ring. We decided we would attempt to put the isoprenyl group on before the phase transfer reaction following the outlined procedure below.

Scheme 28. Alternative proposed synthesis of tryprostatin B



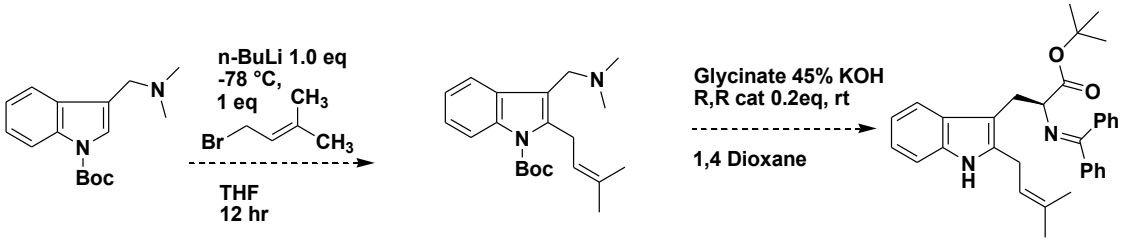
This procedure starts with the boc protection of gramine to protect the indolic nitrogen from the lithiating agent. This reaction went as expected in over 90% yield.

Scheme 29. Boc protection of gramine



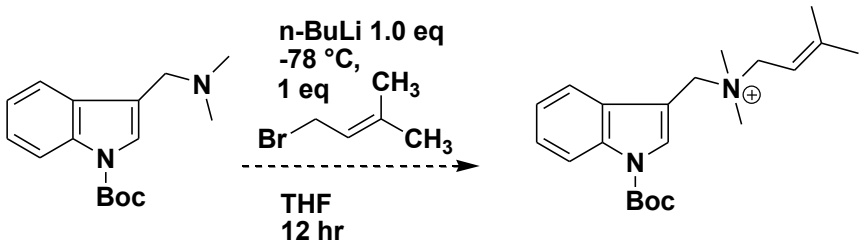
The next reaction had some challenges; we proposed the reaction would work as depicted in equation 30.

Scheme 30. Proposed C-2 isoprenylation



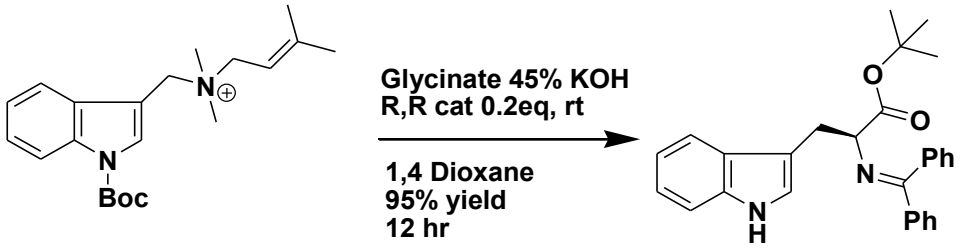
What we had actually saw was that the isoprenyl bromide added to the graminic nitrogen instead of the indolic C-2 position as expected, this is depicted below.

Scheme 31. Actual isoprenylation to make isoprenyl quaternary ammonium salt



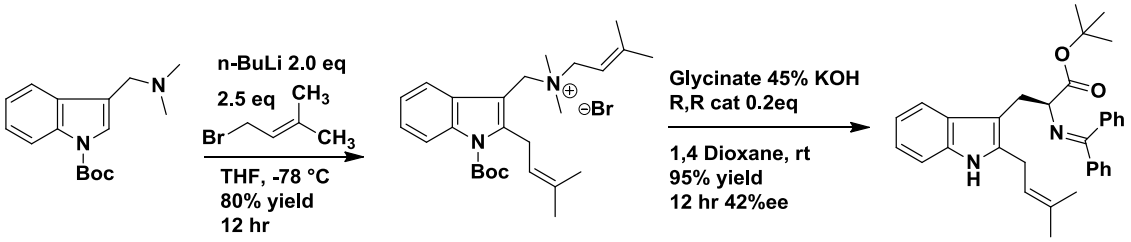
After identifying this compound we wondered if it would be possible to use the isoprenyled nitrogen salt as our substrate for the phase transfer catalysis reaction. This idea is shown in equation 32.

Scheme 32. Utilization of isoprenyl quaternary ammonium salt to synthesis protected tryptophan



The identification of this protected tryptophan was exciting for us because it showed that we could use the isoprenyled nitrogen salt to carry out the phase transfer catalysis reaction. At this point we decided that we should try to put two isoprenyl groups on the substrate and then try the phase transfer reaction as indicated below. Important to point out at this we now had a procedure that used three steps to get to the "key" 2-isoprenyimineprtotectedt-butylestertryptophan.

Scheme 33. Synthesis of C-2 isoprenylated and isoprenyl quaternary ammonium salt, which was able to undergo the phase-transfer catalyst reaction to make C-2 isoprenylated protected tryptophan



Due to the magnitude of our entry to the most important intermediate in our pathway we later obtained a crystal structure of the diisopropyl boc protected gramine salt.

Crystal Structure data
CCDC 922382

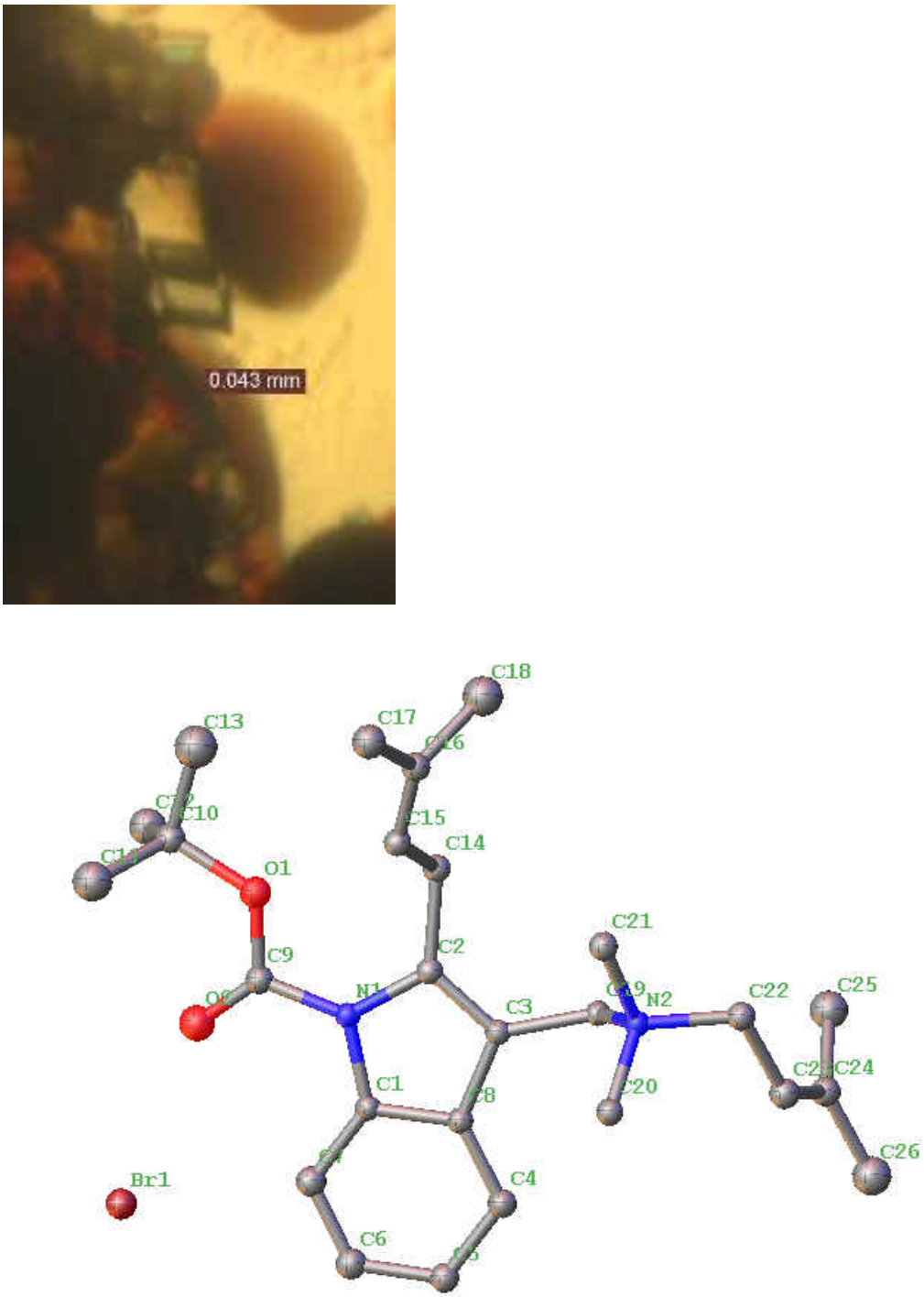


Figure 28. Crystal structure of C-2 isoprenylated isoprenyl quaternary ammonium salt

Blocks grown using slow diffusion method: Ethyl Acetate/Hexane

Analyzed by Xray diffraction at UCSD with Arnie Rheingold

Unit Cell Dimensions: a=8.5784(2); b=12.9668(3); c=13.5267(3)Å
α=109.266(2)° β=103.084(2)° γ=107.596(2)°

Triclinic lattice, P1 space group, Z = 2 molecules per unit cell. R1 = 4.39%

Contact: Matthew Huisman, mhuisman@uwm.edu
Authors: Matthew M. Huisman, Sarah Oehm M. Mahmun Hossain, Arnold L. Rheingold

Table 1 Crystal data and structure refinement for Hossain01_0m

Identification code	Hossain01_0m
Empirical formula	C26H39N2O2Br
Formula weight	491.50
Temperature/K	273.15
Crystal system	triclinic
Space group	P1
a/Å	8.5784(2)
b/Å	12.9668(3)
c/Å	13.5267(3)
α/°	109.266(2)
β/°	103.084(2)
γ/°	107.596(2)
Volume/Å3	1261.86(5)
Z	2
ρcalcmg/mm3	1.294
m/mm 1	1.653
F(000)	520.0
Crystal size/mm3	0.3 × 0.24 × 0.18
2Θ range for data collection	3.42 to 63.92°
Index ranges	-12 ≤ h ≤ 12, -19 ≤ k ≤ 19, -20 ≤ l ≤ 20
Reflections collected	23123
Independent reflections	16463[R(int) = 0.0238]
Data/restraints/parameters	16463/3/577
Goodness-of-fit on F2	0.917
Final R indexes [I>=2σ (I)]	R1 = 0.0440, wR2 = 0.1103
Final R indexes [all data]	R1 = 0.0729, wR2 = 0.1451
Largest diff. peak/hole / e Å-3	0.94/-0.52
Flack parameter	0.21(2)

chemical_name_systematic : N-((1-(tert-butoxycarbonyl)-2-(3-methylbut-2-en-1-yl)-1H-indol-3-yl)methyl)-N,N,3-trimethylbut-e-en-1 aminium bromide

_chemical_name_common Compound synonym: boc protected 2 isoprenyl N isoprenyl gramine salt

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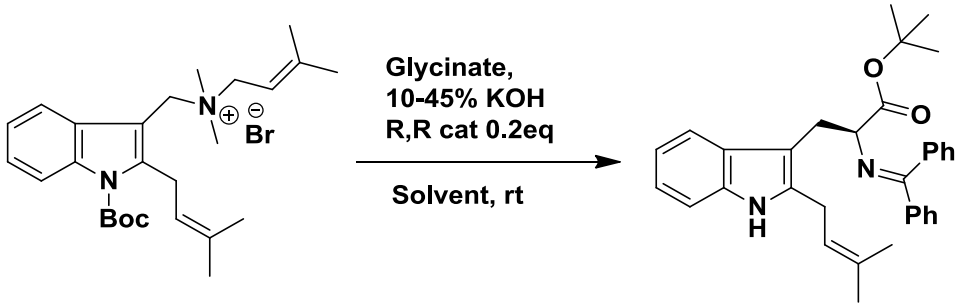
data_hossain1
```

4.6. Monitoring the effectiveness of the C-2 isoprenyl quaternary ammonium bromide salt

We questioned the effectiveness of the phase transfer reaction with an altered substrate. We compared our reaction to similar reactions we have done in the past screening dichloromethane against 1,4 dioxane using different concentrations of KOH to push this reaction.

Scheme 34. Comparison between dichloromethane and dioxane in 10 and 45% aq. KOH



Results indicated in table 11.

Table 11. Comparison between dichloromethane and dioxane in 10 and 45% aq. KOH

Screening comparison between CH_2Cl_2 and 1,4 dioxane					
Rxn #	% KOH in water	Solvent	Time in hours	% Conversion	% ee
1	10%	CH_2Cl_2	18	Trace	ND
2	10%	1,4 Dioxane	18	Trace	ND
3	45%	CH_2Cl_2	18	5	44
4	45%	1,4 Dioxane	18	95	42

Percent conversion was determined by NMR monitoring the disappearance of the α-carbons protons on the Glycinate at 4.1 ppm and comparing it to the formation of the α-carbons proton

at 4.3 ppm. Percent ee was determined by HPLC using Chiralcel OD and Hexane/IPA mobile phase.

These results indicated that diluted solutions of KOH yielded little product, but when using high concentrations of KOH in water the reaction's percent conversion was greatly improved.

Next we wanted to observe the effect of solvent on the percent conversion and percent ee, this led us to solvent screening of the reaction.

Scheme 35. Solvent screening of phase-transfer catalyst

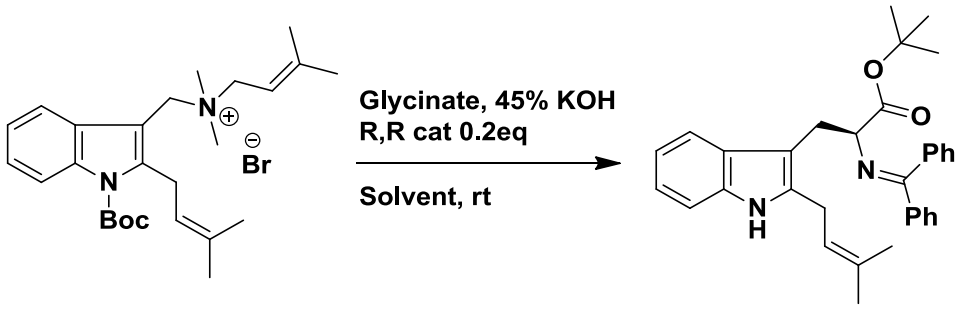


Table 12. Solvent screening of phase-transfer catalyst

Solvent Screening					
Rxn #	% KOH in water	Solvent	Time in hours	% Conversion	% ee
1	45%	THF	18	95	3
2	45%	Ether	18	7	30
3	45%	Toluene	18	80	63
4	45%	EtOAc	18	10	37

Percent conversion determined by NMR, percent ee determined by HPLC using Chiralcel OD and Hexane/IPA mobile phase.

We then screened the equivalents of water that were used in the reaction.

Scheme 36. Screening the effect of equivalents of water

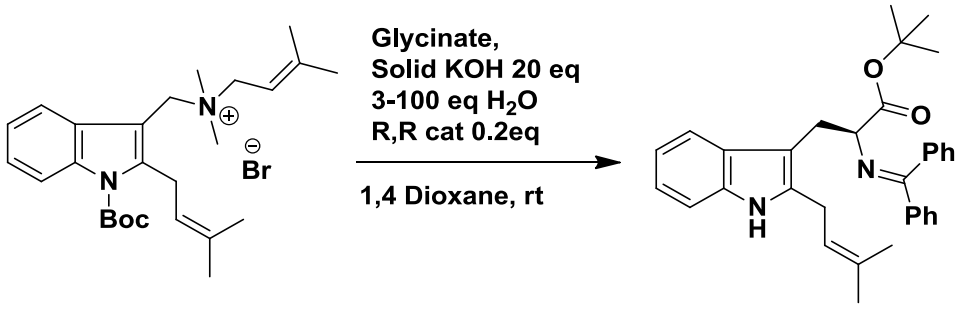


Table 13. Screening the effect of equivalents of water

Equivalents of Water				
Rxn #	Water Equiv	Time (hr)	% Conversion	% ee
1	3	22	79	29
2	6	22	51	26
3	25	22	55	72
4	100	22	54	62

Next we wanted to observe the effect on percent yield and percent ee varying catalyst loading and temperature would have.

Scheme 37. Screening temperature and catalyst loading

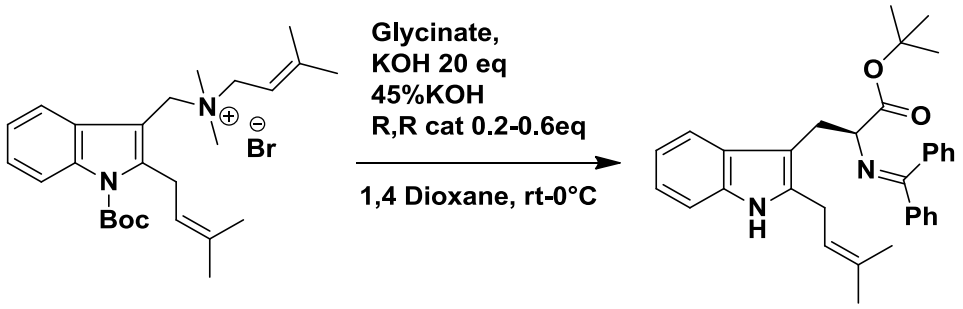


Table 14. Screening temperature and catalyst loading

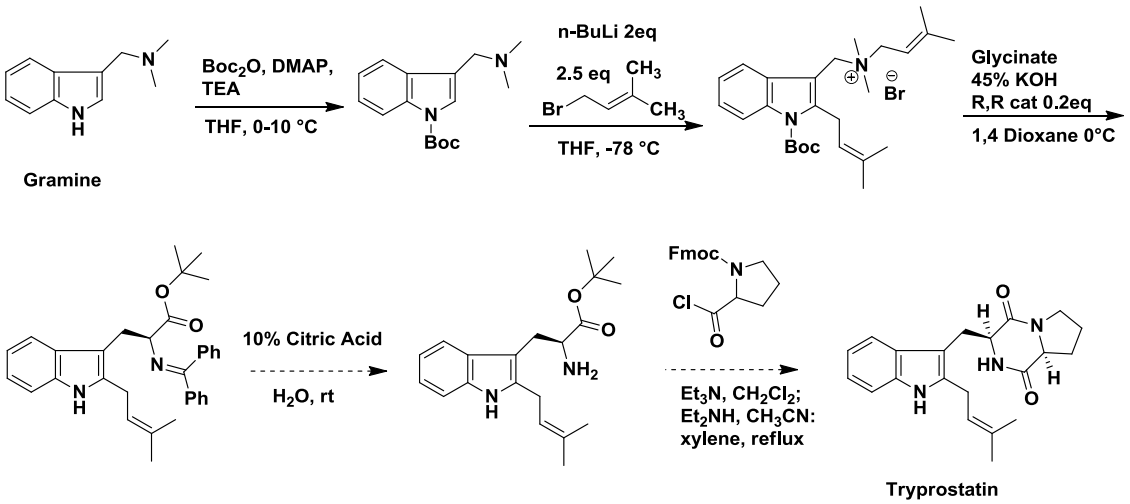
Temperature Screening and Catalyst Loading				
Rxn #	C	eq. cat	% Conversion	% ee
1	rt	0.2	100	42
2	rt	0.6	100	24
3	0	0.2	100	31
4	0	0.6	81	52
5	rt	0.2	100	34
6	rt	0.1	100	41
7	rt	0.05	100	79
8	rt	0.025	100	47
*9	rt	0.2	90	18

*Solid KOH

4.7. Attempts to synthesis Tryprostatin B

We then began pursuing the synthesis of tryprostatin using the procedure below.

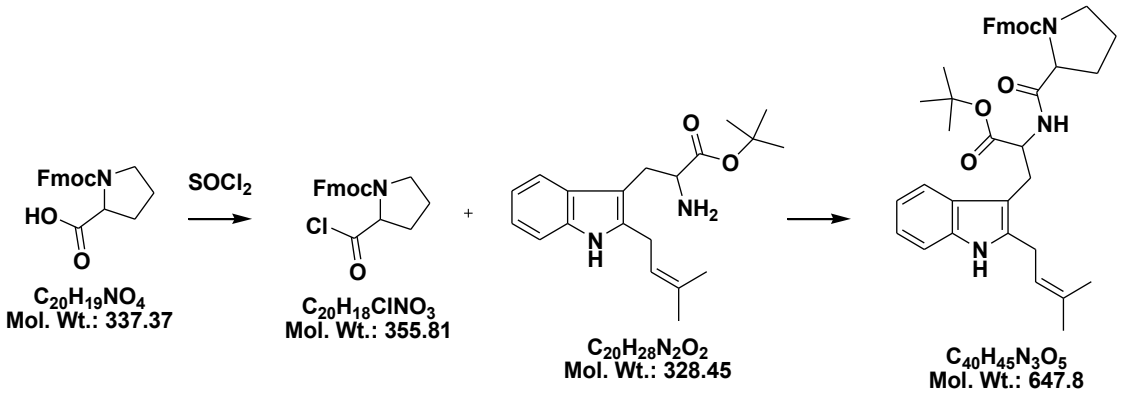
Scheme 38. Proposed synthesis of tryprostatin B



We desired to accomplish three goals in our synthesis: 1) to make the synthesis of tryprostatin highly stream-lined 2) make the synthesis derivative tolerant and 3) make the synthesis more environmentally benign.

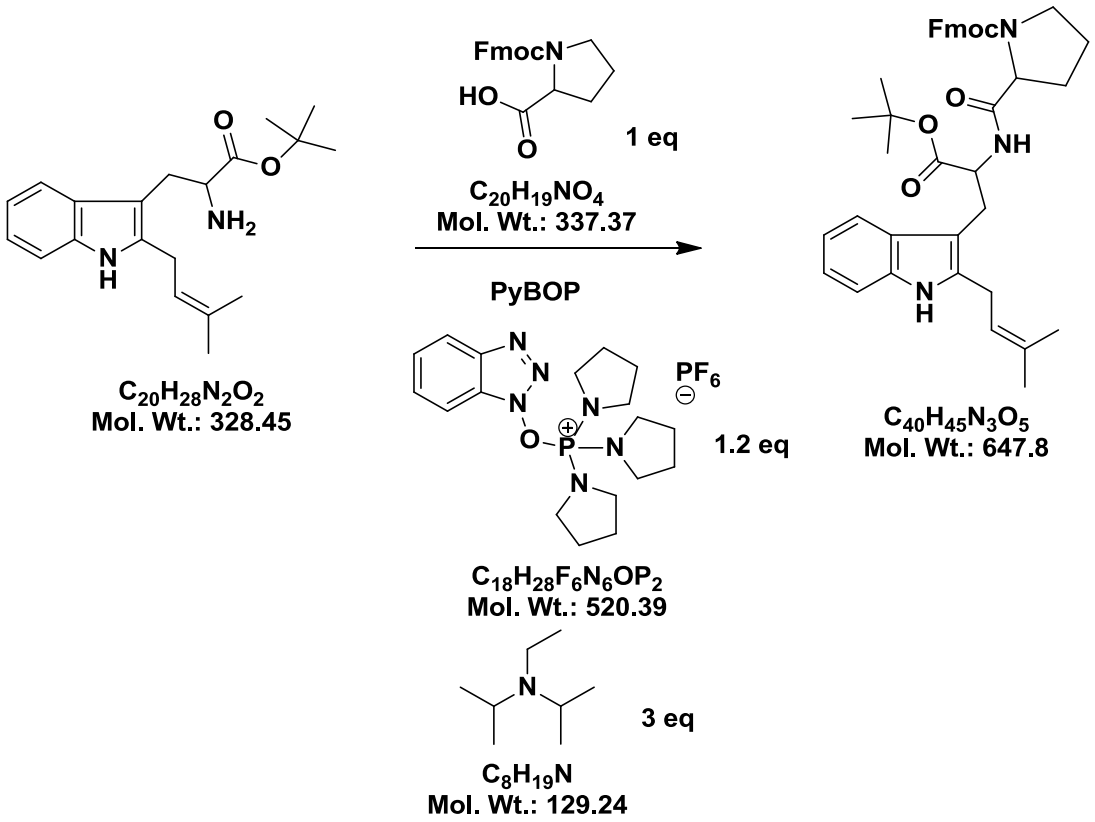
After producing the free amine we approached a variety of methods to couple the Fmoc-L-Pro to the Trp amine. One procedure we decided to pursue was Cook's procedure using the Fmoc-LPro-Cl.[36] Shown in Equation 39.

Scheme 39. Dipeptide synthesis using proline acid chloride



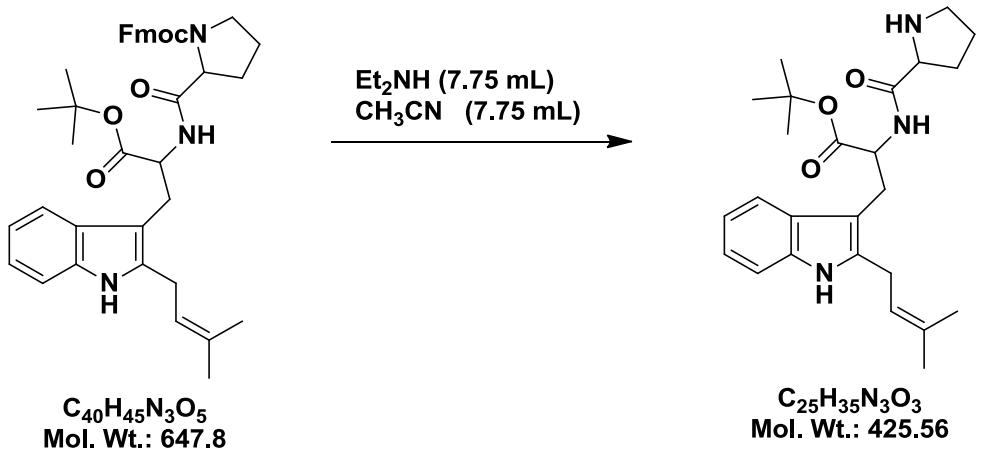
Another possible synthetic route to the desired product was to use a peptide coupling reagent such as PyBOP and couple the peptides together. We found that the peptide coupling reaction was our preferred method due to thionyl chlorides aggressive nature towards the equipment, especially the Tygon tubing.

Scheme 40. Dipeptide synthesis using peptide coupling reagent



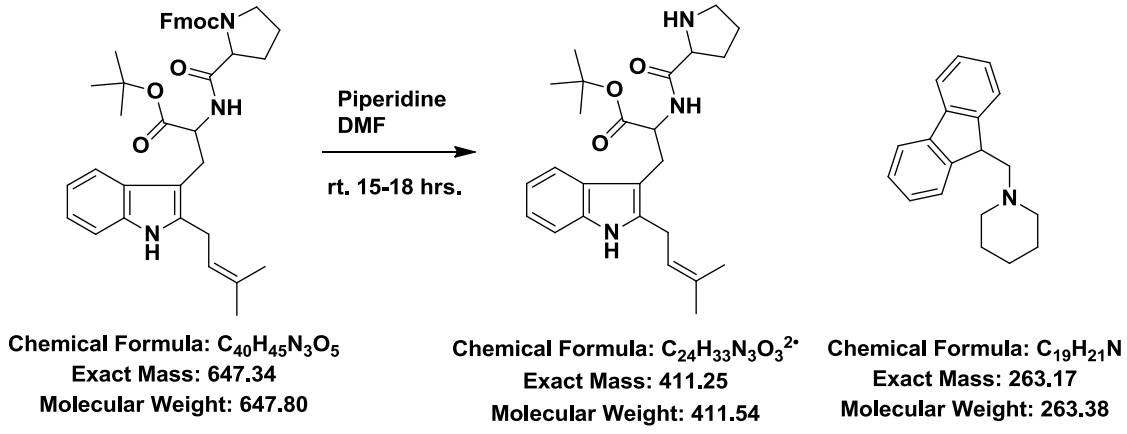
After synthesis of the protected 2-isoprenyltryptophan fmoc proline was completed, it was deprotected using a procedure modeled after the one described in [1d] for the deprotection of fmoc.

Scheme 41. Deprotection of Fmoc from dipeptide



This was successful in and the shown product was purified using column chromatography. This was reattempted using the procedure for fmoc deprotection utilizing piperidine instead of diethyl amine. This procedure formed the fmoc deprotection product as a solid, making it easier to purify, via filtration.[37]

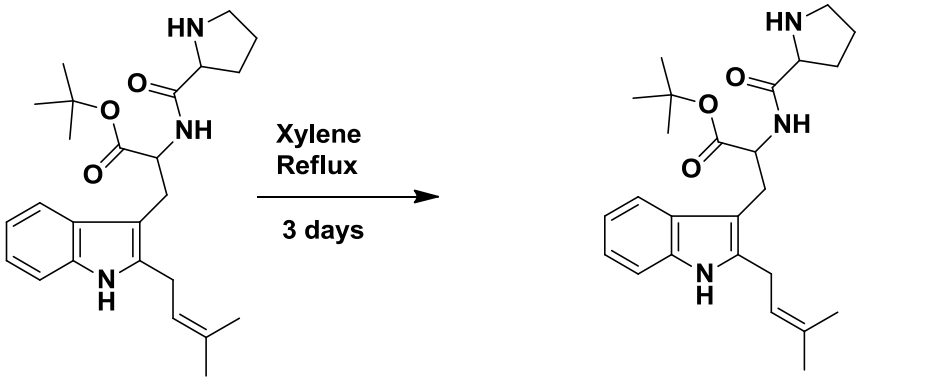
Scheme 42. Alternative method of Fmoc deprotection



After this synthesis of the tbutylester2-isoprenyltryptophanproline dipeptide was completed we evaluated a number of ways to close the diketopiperizine (DKP) ring. Initially we attempted the

ring closing diketopiperzine formation following the method used by the Cook[1d] group which had successfully closed the ethyl ester of this compound. This reaction returned a burn charred material, without any promising NMR peaks to indicate the closing of the ring, such as the loss of the t-butyl protecting group in the proton or the change of the ester to the amide in the carbon NMR.

Scheme 43. First attempt to close DKP ring



Chemical Formula: $C_{24}H_{33}N_3O_3^{2\bullet}$
Exact Mass: 411.25
Molecular Weight: 411.54

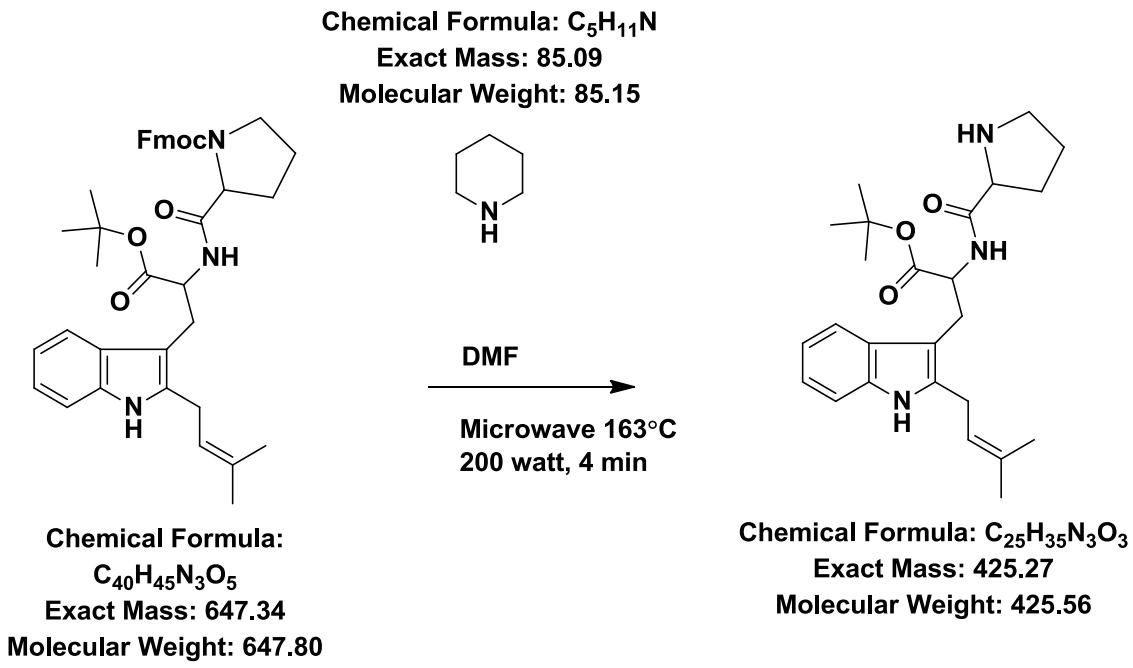
Chemical Formula: $C_{24}H_{33}N_3O_3^{2\bullet}$
Exact Mass: 411.25
Molecular Weight: 411.54

Next we tried a slightly altered version using a more polar dimethylformide (DMF) solvent [37] utilizing the polarity of the solvent to help stabilize the partial positive carbonyl carbon, in turn making it more susceptible to nucleophilic attack. Unfortunately this returned the starting material after the DMF was laboriously removed.

At this point we became concerned that the *tert*-butyl group may require much more energy to overcome the larger activation energy and thus act as a leaving group.

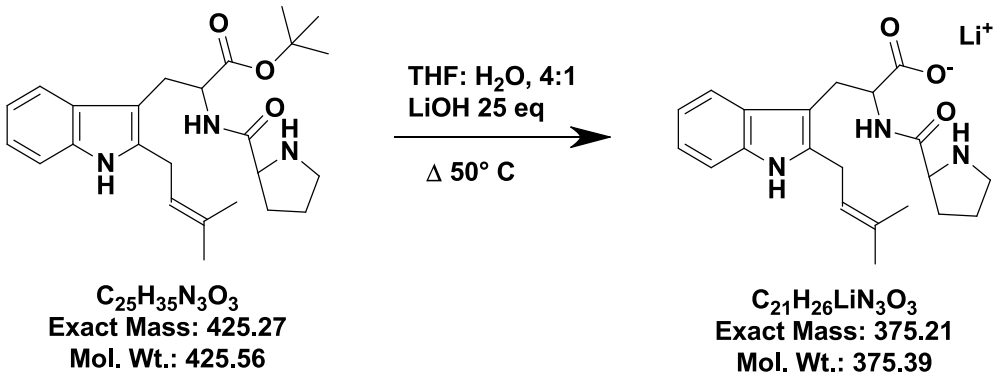
Next we tried using microwave synthesis to get the ring to close in a 20% piperidine/DMF mixture in the microwave.[37] Reaction deprotected the fmoc protecting group but unfortunately did not close the DKP ring.

Scheme 44. Failed DKP ring closure without deprotecting Fmoc dipeptide



We then made an effort to try and remove the t-butyl group using lithium hydroxide using a similar procedure that had been reported to work for different ester deprotection reactions. This reaction either did not yield well or the material got stuck in the water layer. Acidification of this material did not result in it being organic soluble. Water was removed and the material did not appear to be there either.

Scheme 45. Attempt to remove t-butyl using lithium hydroxide

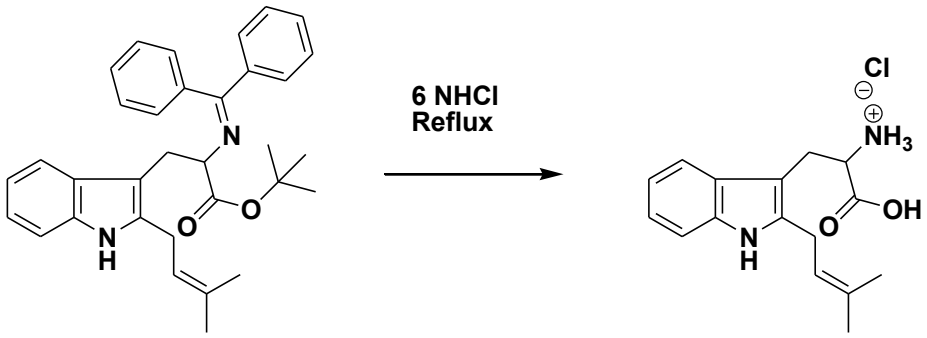


$C_{25}H_{35}N_3O_3$
Exact Mass: 425.27
Mol. Wt.: 425.56

$C_{21}H_{26}LiN_3O_3$
Exact Mass: 375.21
Mol. Wt.: 375.39

After this attempt we decided to pursue the deprotected t-butyl material using a different approach.[38] This procedure used a refluxing 6 N hydrochloric acid (HCl) solution to remove the t-butyl group. This resulted in what appeared to be an acid charred material. This reaction mixture showed little promise via proton NMR due to a strong t-butyl peak.

Scheme 46. Attempt to remove t-butyl group using 6N HCl at reflux

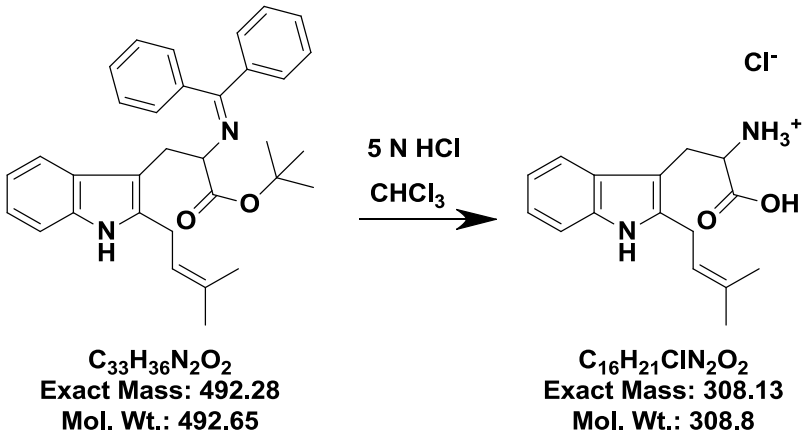


$C_{33}H_{36}N_2O_2$
Exact Mass: 492.28
Mol. Wt.: 492.65

$C_{16}H_{21}ClN_2O_2$
Exact Mass: 308.13
Mol. Wt.: 308.8

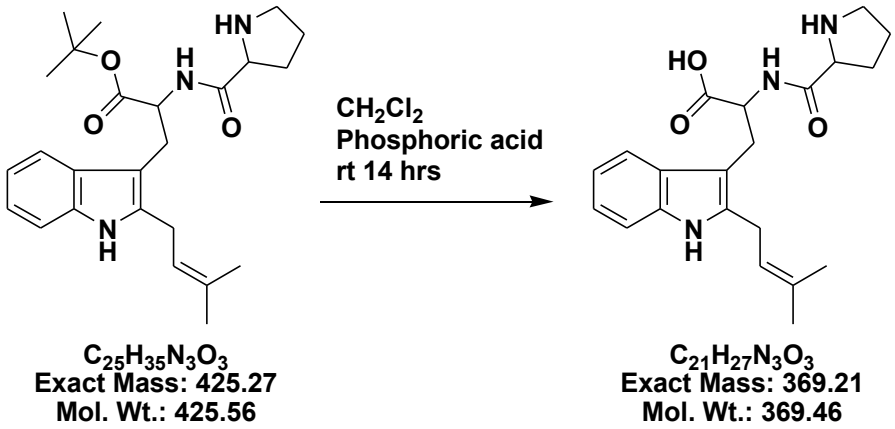
Next we attempted to deprotect the t-butyl peak using a 5N HCl solution at room temperature in chloroform.[39] This crude reaction mixture also showed a strong t-butyl ester presence.

Scheme 47. Attempt to remove t-butyl using 5N HCl at room temp



Next we tried to deprotect the t-butyl group by using phosphoric acid in dichloromethane.[30] The proton NMR spectrum of this material indicated that the stubborn t-butyl group was still present.

Scheme 48. Attempt to remove t-butyl group using phosphoric acid at room temp

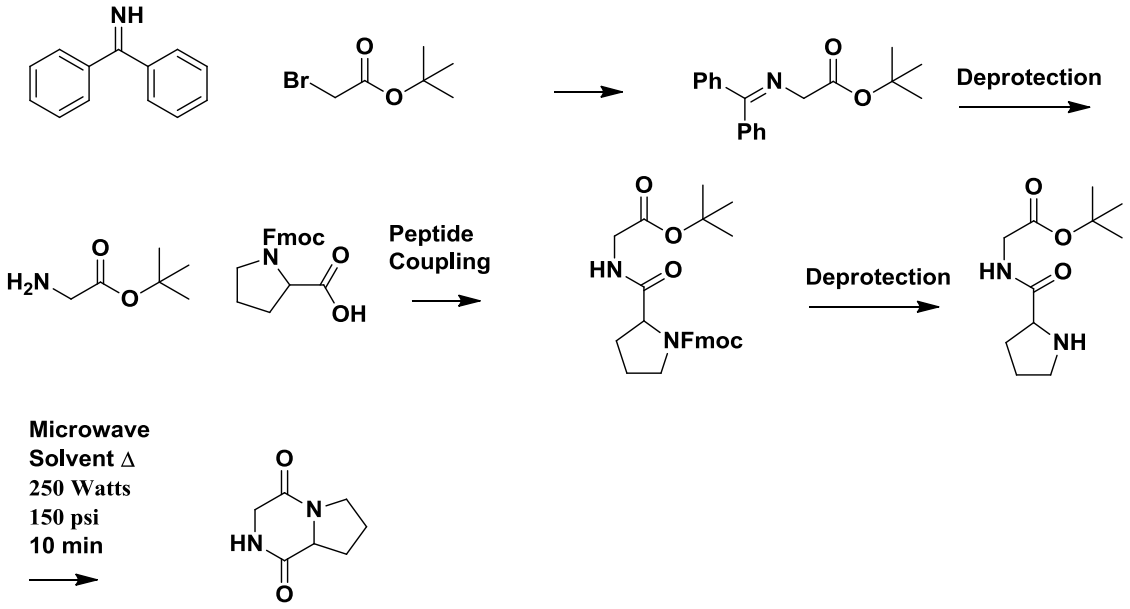


Convinced deprotection of the t-butyl group was not going be an effective process to achieve the DKP cyclization. We began pursuing other routes to close the diketopiperizing ring. After many frustrating failures at attempts to close the DKP ring we decided that it may be advantageous to use a model reaction to find a procedure that was capable of cyclizing the DKP ring. This route was pursued using a model reaction of the glycine Schiff base and Fmoc protected proline.

4.8. DKP ring closure by microwave in water

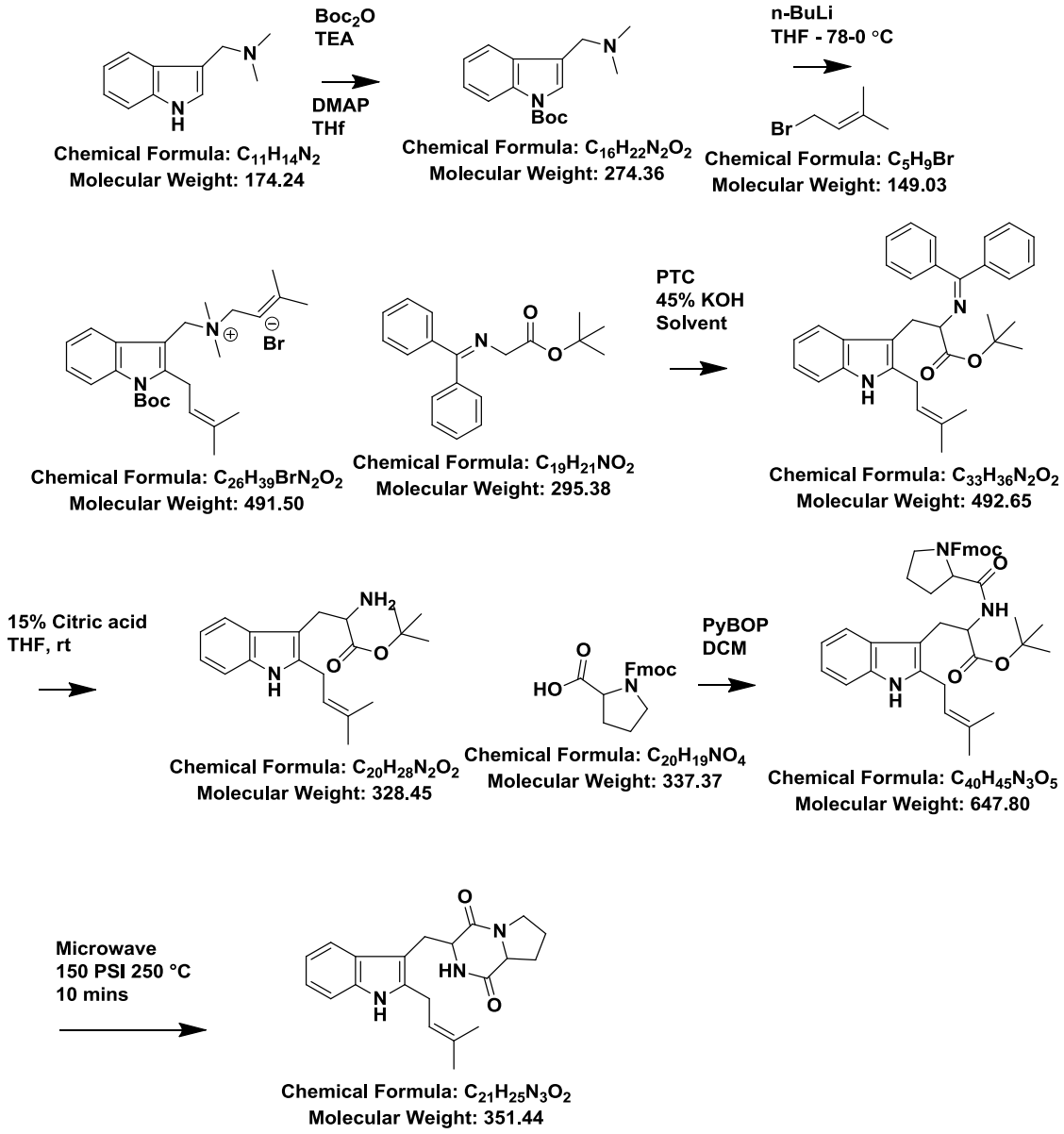
To begin this we synthesized the glycine Schiff on multigram scale.[40]

Scheme 49. Model for DKP ring closure



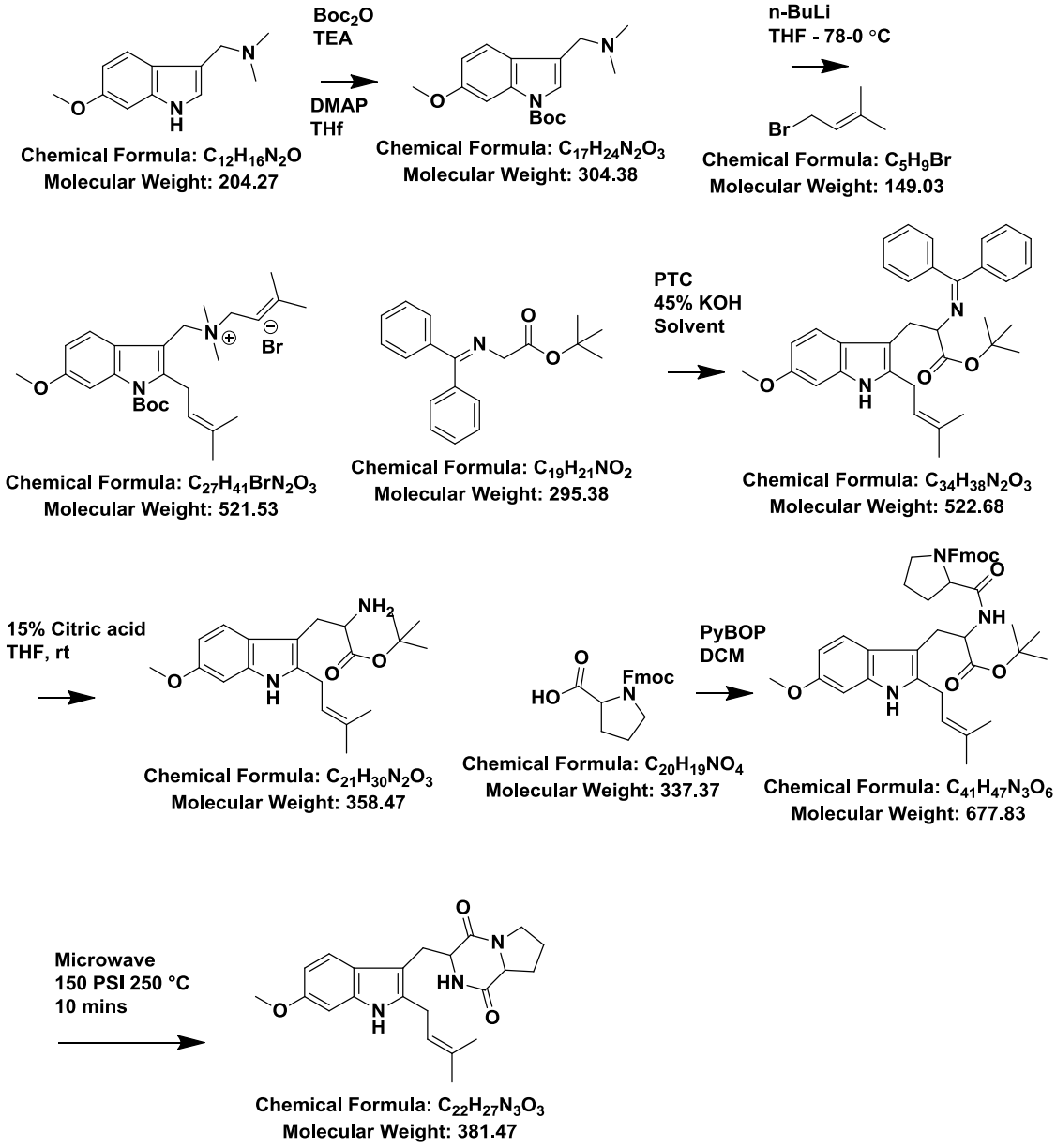
After we found a procedure that was capable of closing the DKP ring we successfully applied this procedure to the synthesis of tryprostatin B.

Scheme 50. Total synthesis tryprostatin B



After completion of screening, synthesis of TPS A was pursued via the analogous scheme.

Scheme 51. Total synthesis tryprostatin A



Now that we have shown the synthesis of tryprostatins is possible through our unpresidented procedure we would like to compare their activity against the past IC50 screenings to insure that

our synthesized compounds behave as the naturally isolated material. In the past evaluation of tryprostatin has been done using percent cell survival compared to the phase of the cells that survived.[1e] Turbimetric assays have been used to determine the stabilization or destabilization of microtubules. Cell titer 96 AQueous from Promega has been used by the Cook group to measure the survival of cells in a solution of tryprostatin. [1d] This microtiter plate has a MTS like compound in each of the wells this MTS is reduced by living cells to Formazan which has a purple/violet color to it; this color is used to determine the amount of living cells and lack of color to determine the amount of dead cells.

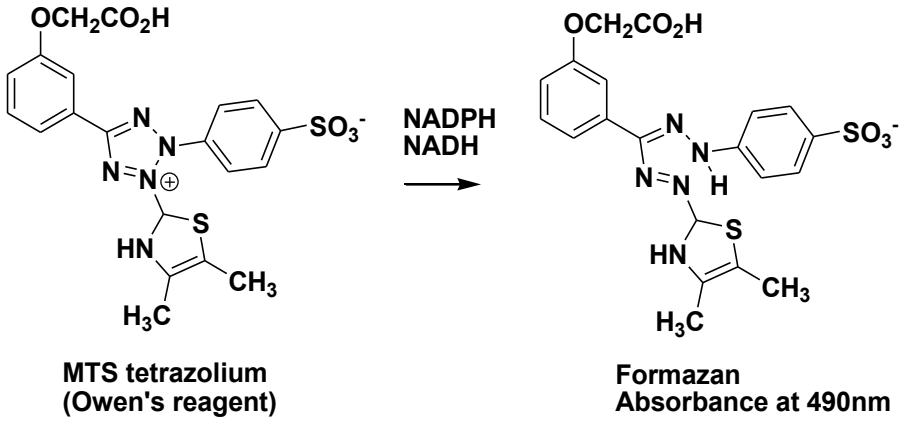


Figure 29. Reducing agents in live cells change Owen's reagent into purple dye

Osada's group presented data comparing a control, various compounds and tryprostatin A and B at 25 and 50 µM concentrations to observe the number of living cells after they were incubated and grown for 24 hours. They also observed the amount of DNA in the cells to predict what phase of the cell cycle the cells were in.[1a 1b, 1c]

Table 15. Shows chromosome content and percent cell survival, indicating tryprostatin A inhibits cell progression after chromosomes double in cell division

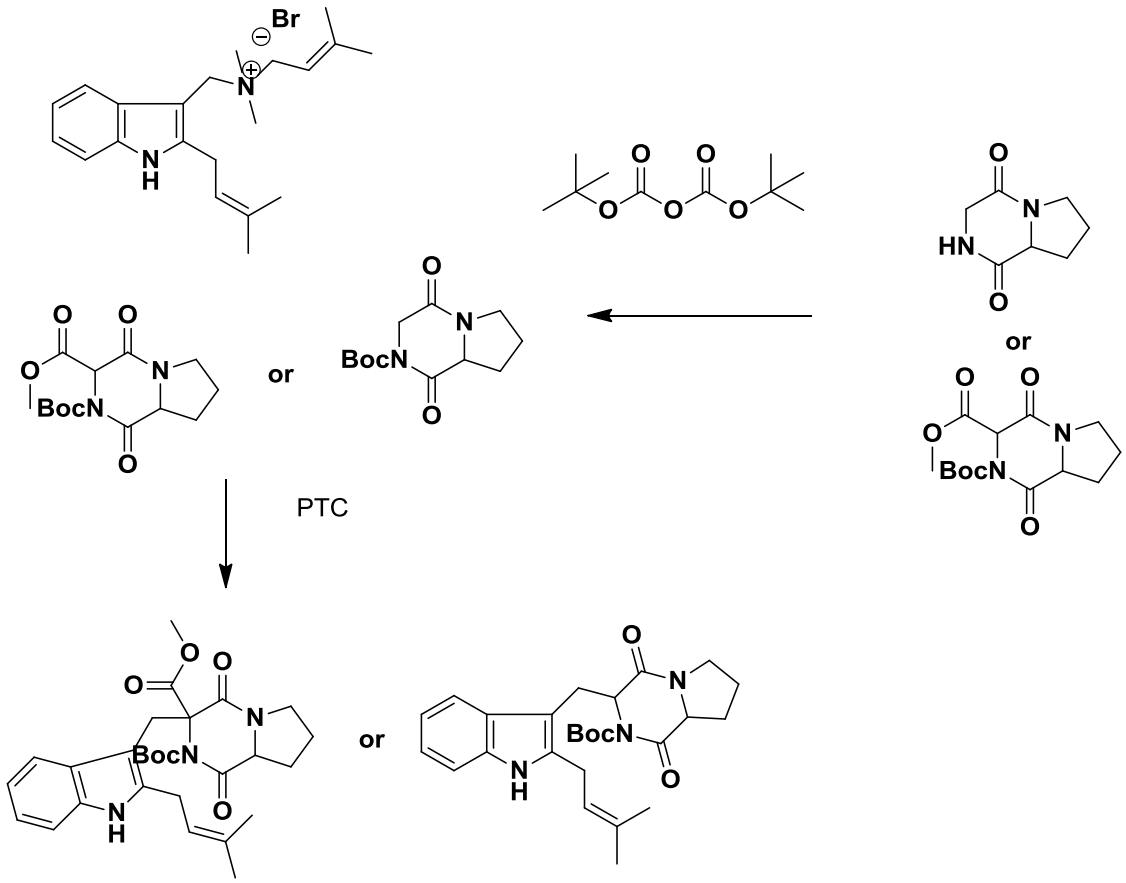
Distribution of DNA content in asynchronous culture of 3Y1 cells treated with various drugs					
		DNA content			
Drug	Concentration (microM)	2C	2C-4C	4C	Cell number (%)
Control	0	65.4	12.3	22.3	161.8
Stauroporine	0.02	76.9	8.5	11.1	143.1
Colchicine	1	9.4	11.5	65.9	69.4
TPS A	25	29.2	16.2	49.4	89.6
	50	9	15.2	70.5	68.7
TPS B	25	28.6	19.1	24.8	57.4
	50	31.8	20.4	15.2	57.8

Exponentially growing 3Y1 cells were treated with various compounds for 24 h and the distribution of DNA content and relative cell numbers were determined. The cell number is the ratio of the number of cells at 24 h to that at 0 h expressed as a percentage.We plan to measure cell toxicity using a similar screening process.

We are interested to see if it would be possible to make the diisoprenylgramine salt apply the phase-transfer procedure to a closed DKP ring. Concerns with that procedure include the

glycine's α-carbons proton may have reduced acidity than the starting material. The acidity may be increased by adding an ester group as shown below.

Scheme 52. Proposed alternative method to tryprostatins



5. CONCLUSION

5.1. Importance of tryptophan and asymmetric synthesis in medicinal chemistry

Tryptophan is a natural amino acid, famous for making people tired after thanksgiving, although that is myth. One of the most interesting things about tryptophan is its unique side chain. Like all amino acids, tryptophan has a carboxylic acid connected to an alpha carbon connected to an amine. From the twenty natural amino acids organisms can make a wide assortment of peptides and proteins.

The nitrogen-containing heterocycle that makes up the tryptophan side chain attached to its alpha carbon is called an indole. Indoles make up a large percent of neurotransmitters, prescription and recreational drugs. Due to unique characteristics of indoles, tryptophan derivatives have been at the center of extensive research for the last century.

Furthermore in the area of medicinal chemistry, the discovery that in many drugs one enantiomer shows high biological activity compared to the low biological activity or even harmful effects of its mirror image has sparked a great deal of interest in asymmetric synthesis. A classic example of the importance of enantiomeric selectivity is demonstrated in thalidomide. The R-isomer is active against morning sickness, while its S-enantiomer causes severe birth defects. The unfortunate administration of its racemic form caused tragic limb malformation in over ten thousand babies before it was pulled from the market. Later it was discovered that the R-isomer racemizes upon metabolism and was not suitable during pregnancy.[41] Similarly, naproxen, a common pain reliever and anti-inflammatory is sold commercially as the optically pure s-enantiomer, as its

mirror image causes liver damage.[42] The interest to synthesize enantio-pure tryptophan building blocks is a well-established concern for discovery of new medicines.

5.2. Our initial goals:

We set out to develop an asymmetric procedure that could make optically active tryptophans.

We were able to synthesize four ring-A substituted tryptophan derivatives in more than 90%ee.

Next we desired to find an application of our new synthesis. The 2-isoprenyl tryptophan moiety is an essential intermediate for the formation of tryprostatins. This intermediate has been present as an intermediate in every known synthesis of tryprostatins to date. The protecting groups vary from synthesis to synthesis, but the 2-isoprenyl tryptophan remains the most crucial synthetic intermediate.

In the first synthesis of tryprostatin[3a] Danishefsky's group used tributyl tin to couple boron dichloride-3-methyl-1-butene with an amino protected tryptophan to the indolic nitrogen, which then relied on a rearrangement to form the protected 2-isoprenyl tryptophan.

Alternatively in Cook's group synthesis[4] the isoprenyl group was added by using the Schöllkopf chiral auxiliary followed by LDA in THF and adding isoprenyl bromide, resulting in a protected 2-isoprenyl tryptophan.

Fukuyama's group became interested in the synthesis due to their history of using radical chemistry to make indole rings.[5] Fukuyama's group built the indole ring and then

utilized the resulting protected tryptophan to make tryprostatin. The synthesis was achieved in ten steps in 39 percent yield.

Our next and most challenging goal was removing steps in the already known procedures of the established syntheses. We were able to reduce number of steps to six compared to the most current synthesis which is ten steps.

We attempted to achieve the 2-isoprenyl tryptophan using a similar approach to the Cook group approach, by making the protected tryptophan and then attempting the alkylation using a lithiating reagent.

The major isolated product had the isoprenyl group on the alpha carbon instead of the 2-position of the indole ring. To overcome this hurdle, we attempted to put the isoprenyl group on the C2-position using n-butyl lithium and isoprenyl bromide before doing the phase-transfer reaction to make tryptophan. When attempting this synthesis with one equivalent of isoprenyl bromide only the graminic isoprenyl ammonium bromide was formed. After this attempt we tried this with 2.25 eq of n-butyl lithium and an excess of isoprenyl bromide, we were able to isolate the 2-isoprenyl quaternary ammonium graminic nitrogen bromide salt. This molecule was able to proceed through the phase-transfer reaction as expected to make C2-isoprenyl tryptophan but with low enantiomeric excess.

Our procedure is one step shorter than Danishefsky's, one step shorter than Cook's (without including the synthesis of Schöllkopf chiral auxiliary which is three steps) and three steps shorter than Fukuyama's ten step synthesis which utilizes V-70 as a radical initiator. Danishefsky's procedure is seven steps and has not been shown to work for the 6-methoxy indole, which leads to tryprostatin A.

This synthesis also starts from L-tryptophan. It is well known that 6-methoxy tryptophan is difficult to obtain and very expensive. Economics is likely the reason this procedure has not been used to make tryprostatin A. To further support this claim, all the known tryprostatin A syntheses start from smaller building blocks than tryptophan. Although Danishefsky's synthesis is elegant in its simplicity, what it lacks is tolerance to ring-A substitution, which seriously limits its synthetic scope.

5.3. Utilization asymmetric synthesis to make natural products tryprostatin A and B

We then moved on and attempted to synthesize two known natural products, tryprostatins A and B. These goals were also accomplished. At this point we are able to obtain enatiomeric excesses in the 50-60 % range. Although this is not as high as we have reported for the phase-transfer catalyzed reactions without the isoprenyl group on the 2 position of the indole ring, it does leave quite a bit of room for improvement. The problem may be that the 2-isoprenyl group acts as a steric blocker and limits the amount of enantioselective alkylation. It has been reported that sterically congested substrates yield lower %ee than their uncongested counterparts. Our unprecedented PTC still may have the potential to yield high enantiomeric excess with further screening during the crucial chiral center forming step.

5.4. The future plans of this project:

In the future, the Hossain group is expected to publish the shortest known synthesis of tryprostatins A and B. Furthermore, they are expected to use the method developed in our lab to make a variety of tryprostatins with ring-A substitutions and screen them in MTT assays to test cell viability. If any of the newly synthesized compounds have higher cell toxicity than the previously known tryprostatins, the compounds will be tested further, along the lines of mechanism of action, target protein interaction, concentration of toxicity and selectivity for cancer cells. Through Milwaukee Institute Drug Discovery (MIDD) and Open Innovation Drug Design (OIDD) Lilly has expressed interest in screening the new tryprostatin targets.

We have shown that we can make ring-A substituted tryprostatins using our unprecedented shortest known synthesis. We have demonstrated that this synthesis is shorter and more tolerant than any of its predecessors.

It would be an effective use of time and energy to synthesize the material in a non-asymmetric fashion then separate each diastereomer and measure its biological activity.

Another interesting idea would be to make the 2-isoprenyl salt and see if it would be possible to couple the diketopiperizine ring directly.

Lastly, it is important to identify the problem we are having with the asymmetry of this reaction. One plausible reason that the 2-isoprenyl gramine salt may not undergo the phase-transfer reaction as well as the 4-trifluoromethoxybenzyl salt may be caused simply by the added steric hindrance of the C2-isoprenyl on the indole ring.

A good way to clarify if the 2-isoprenyl group were acting as a steric blocker would be to make the same isoprenyl ammonium salt without the isoprenyl indole C2-position and see if the phase-transfer reaction yields a high enantiomers excess.

6. EXPERIMENTAL SECTION

General Procedure

All procedures were performed under a dry nitrogen atmosphere using standard Schlenk techniques unless otherwise noted all reaction vessels were flame dried under vacuum and filled with nitrogen prior to use. Reagents were purchased from Aldrich Chemicals and used as is. Flash chromatography was performed using EM Science F_{254} silica gel 60. N-(diphenylmethylene) glycine tert-butyl ester, sodium hydroxide, phase transfer catalysts and anhydrous sodium sulfate were purchased from Aldrich. The chemical shifts (δ) are expressed in ppm relative to tetramethylsilane. $CDCl_3$ was used as the solvent. Previously ^1H NMR or GC identified reported compounds. All new compounds were additionally characterized by ^1H NMR, ^{13}C NMR and GCMS.

Hexanes a mixture of isomers was purchased Aldrich in 200 L drums this solvent was similar to petroleum ether in Purification of Laboratory Chemicals 2nd Edition Perrin page 375. Hexane 4 L was stirred over 75 mL of conc. H_2SO_4 for 24 to 48 hours. 500 mL of this was put into a 1 L separatory funnel along with 50 mL of 10 % H_2SO_4 (10 mL H_2SO_4 90 mL water) and 50 mL of 1% $KnMnO_4$ (1 g $KMnO_4$ in 99 mL of water 0.0063 M) and shaken. (To remove unsaturated, including aromatic, hydrocarbons) until permanganate color persists. Wash with water (50 mL), aq. Na_2CO_3 (sat. 50 mL) and again with water (50 mL). Dried over Na_2SO_4, and distilled over phosphorus pentoxide.

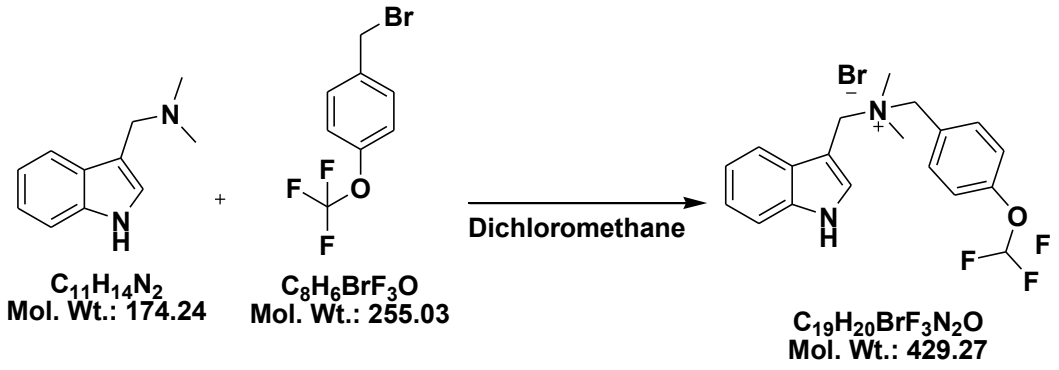
5.2. Instrumentation

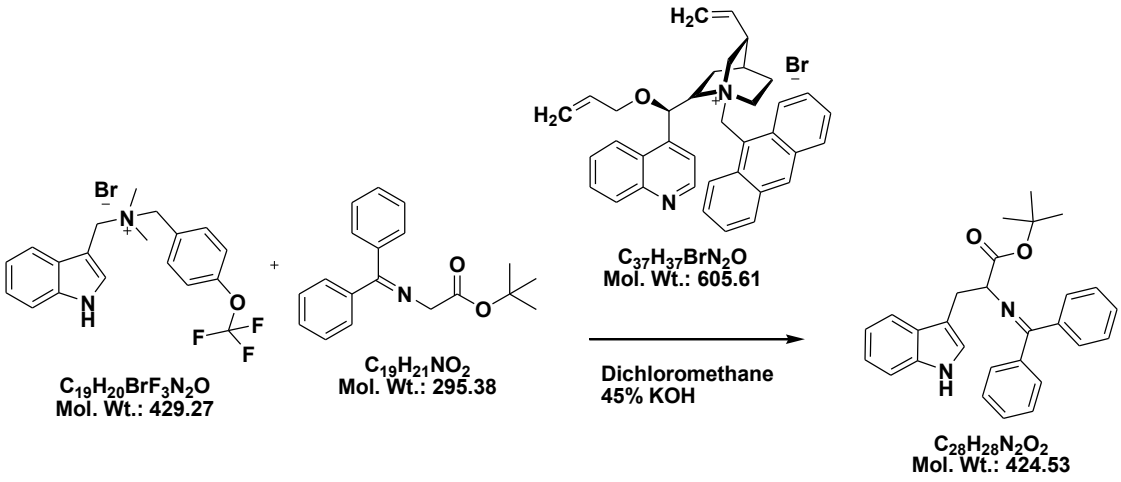
All ^1H (300 MHz), and ^{13}C (75.5 MHz) NMRs were performed with a Burker 300 and samples dissolve in $CDCl_3$ unless otherwise noted. Enantioselectivity was obtained via chiral HPLC using a

Waters setup including an Inline Degasser AF, 2998 Photodiode Array Detector, 1525 Binary HPLC Pump equipped with Breeze Software. This was equipped with a Chiralcel OD (column no. OD00CE-FF071) column using hexane and isopropanol at 254nm and a broad range channel from 200-600nm column temperature was room temperature flow rate was 1 mL/min unless otherwise stated. HPLC grade solvents were used in all HPLC analysis.

Synthetic Procedure for Amine Ester Protected Tryptophan

Scheme 53. Synthesis of protected tryptophan



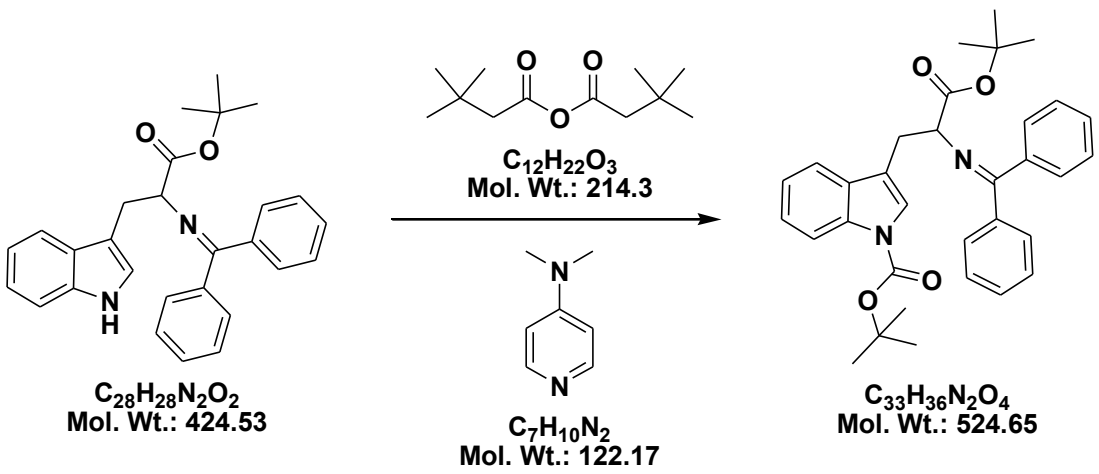


Gramine (.15 g 0.862 mmol 1 eq) was dissolved in a solution of dichloromethane (6 mL) 4-(Triflоromethoxy)-benzyl bromide (0.15 mL 0.938 mmol 1.1 eq) was added forming a solid

precipitate. To this mixture O-Allyl-N-(9-anthracenyl-methyl)cinchonidinium bromide(0.100 g 0.165 mmol 0.193 eq), N-(Diphenylmethylene)-glycine tert-butyl ester(0.265 g 0.897 mmol 1.05 eq), and 45% potassium hydroxide in water (2 mL) was added to the mixture and allowed to stir until the solution became clear and two layers could be seen. Layers were separated and the organic layer was washed three times with water then dried over sodium sulfate. Then compound was purified by column chromatography using 10% ethyl acetate and 90% pentane.

Synthetic Procedure for N Boc Protected Tryptophan

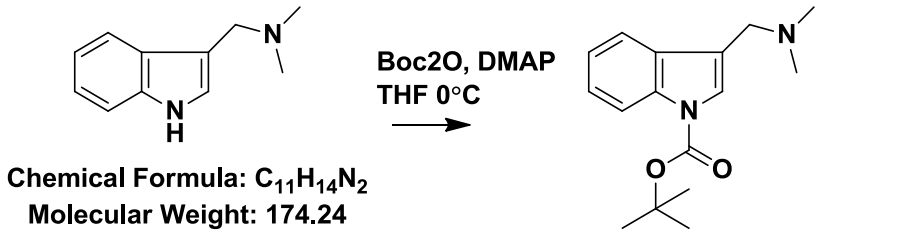
Scheme 54. Boc protection of tryptophan



Tryptophan N-diphenylmethylamine t-buytyl ester (0.745g 1.755 mmol 1 eq) was dissolved in acetonitrile a catalytic amount of DMAP (0.043 g 0.35 mmol 0.2 eq) was added along with di tert-butyl dicarbonate (0.575g 02.63 mmol 1.5 eq) and stirred for 24 hours. Product was purified using column chromatography with 10% ethyl acetate and 90% pentane.

Synthetic procedure for N Boc Gramine

Scheme 55. Boc protection of gramine



Chemical Formula: $C_{11}H_{14}N_2$
Molecular Weight: 174.24

Chemical Formula: $C_{16}H_{22}N_2O_2$
Molecular Weight: 274.36

This reaction was modeled after a very similar reaction discussed in Tetrahedron 55 (1999) 10989-11000, compound 8 to 9. A solution of gramine (3.7 g 21 mmol 1 eq.) in THF (90 mL) was made. This solution was put into an addition funnel on a 250 mL three necked reaction vessel in an ice water cooling bath and added dropwise to a stirred solution of di-*t*-butyl dicarbonate (5.50 g 25 mmol 1.2 eq.), 4-(dimethylamino)pyridine (257 mg, 2.1 mmol 0.1 eq.), triethylamine (3.5 mL, 2.5 mmol, 0.12 eq.) in THF (50 mL). After stirring for 1.5 hours at room temperature, water (50) mL was added to the reaction mixture and the solvent was removed via roto vap. The organic layer was separated and the aqueous layer was extracted twice with ether (50 mL). The combined extract was washed three times with water and then with brine solution and dried over sodium sulfate, and evaporated. The residue was chromatographed over silica gel using (could probably use ethyl acetate alone as mobile phase) hexane:ethyl acetate (1:2) as an eluent to give 5.18 g of Boc Gramine product in 90.0% yield. Theoretical yield is 5.75 g. Apparatus set up shown below.

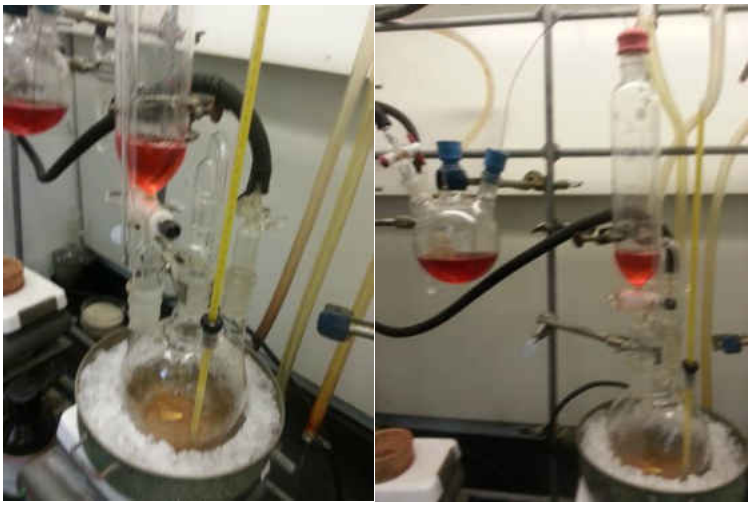
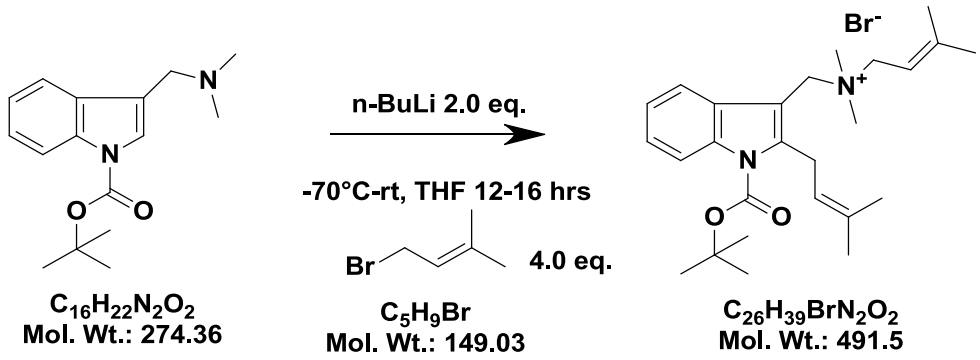


Figure 30. Boc protection of gramine

Synthetic procedure for N+ diisoprenyl bromide salt of gramine

Scheme 56. Diisoprenylation of Boc gramine



5.0 g (18.2 mmol, 1 eq.) Boc Gramine was weighed out in a beaker. 500 mL three-neck with thermometer adapter round bottom flask was oven dried for 3 hours with stir bar inside. It was removed from the oven and clamped. On one neck rubber septum was inserted, in the other nitrogen outlet was inserted, at this point the boc gramine was charged to the flask via a powder funnel, and in the last neck nitrogen inlet was inserted, as quickly as possible. To the reaction

vessel blue distilled THF (245) mL was charged via syringe. The reaction mixture was allowed to stir for 1 hour to insure that all of the starting material was dissolved in the solution. At this point solution is orange/peach in color. Reaction vessel was cooled in a dry ice/acetone bath until reaction was -70 °C. At this time n-butyl lithium (14.58 mL 2.5 M 36.44 mmol 2.0 eq.) was added dropwise to the reaction vessel via a syringe over 1 hour, maintaining a temperature range between -65 and -70 °C. At this point the reaction is bright red/orange. After addition of n-butyl lithium reaction was let stir undisturbed for 1 hour and 30 minutes at -70 °C. Isoprenyl bromide (9.4 mL 81.99 mmol 4.5 eq.) was added to the reaction dropwise. After addition of isoprenyl bromide the color of the reaction mixture is orange. At this point the reaction was left to warm overnight. When returning the next day color of the reaction mixture was clear orange. Deionized water (5 mL) was added to the reaction vessel, no reaction indicated that the n-butyl lithium was quenched. At this point the solvent was removed using the roto vap. After organic solvent was removed the water and residue was poured into a separatory funnel and extracted with dichloromethane three times (50 mL). Organic layer was dried over sodium sulfate. Solvent was removed via roto vap and high vac with cold finger. (Purify immediately or freeze left on bench it turns an undesirable brown/black heat from the roto vap bath may also be the cause) Residue was purified using flash chromatography (10 x 6 cm silica gel) eluent was 5% methanol: 95% dichloromethane to provide a light brown solid 6.14 g in 69% yield. See TLC plate developed in 9:1 Dichloromethane:Methanol observed with short range UV lamp and stained with ninhydrin stain and heated on a hot plate until colored. Spot with Rf of 0.5 is product and has a purple/violet color when the TLC plate is developed in the ninhydrin stain. Recrystallization solvents that have been tried, material is soluble in ethanol, material with water and heat forms a white cloudy solution, attempts at purification by recrystallization has failed.

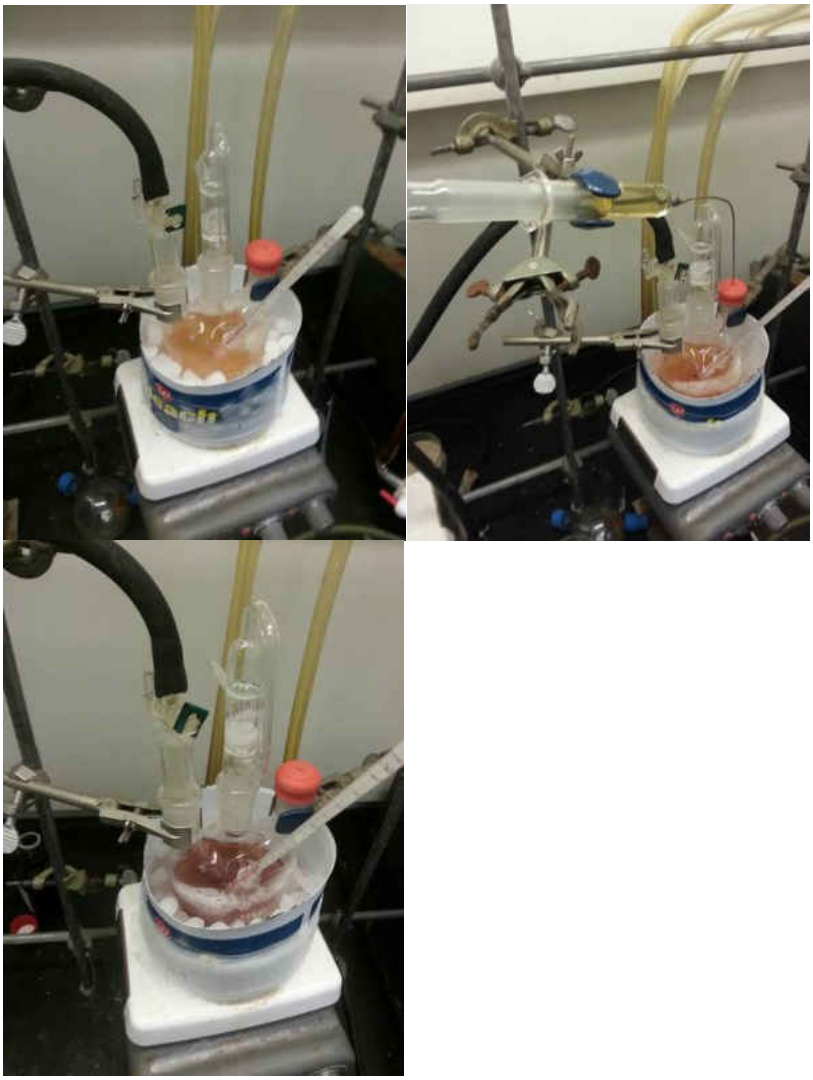


Figure 31. Diisoprenylation of Boc gramine

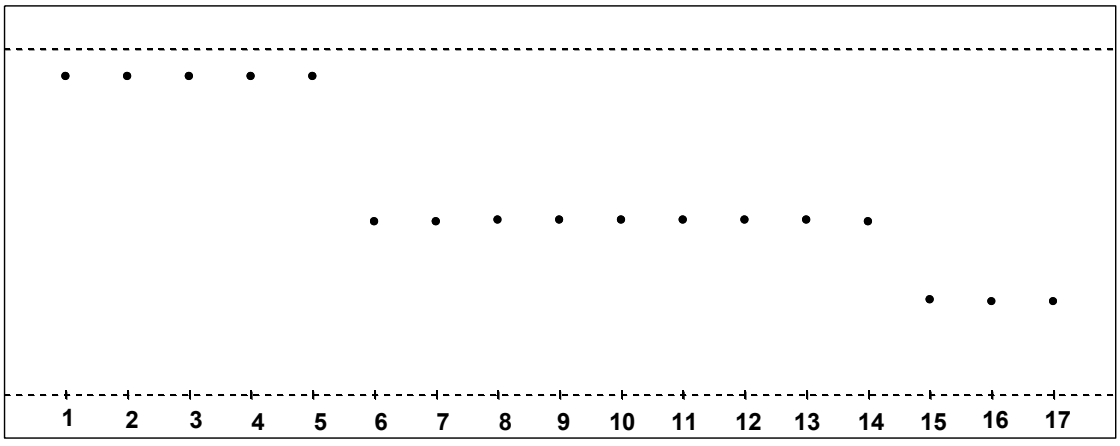
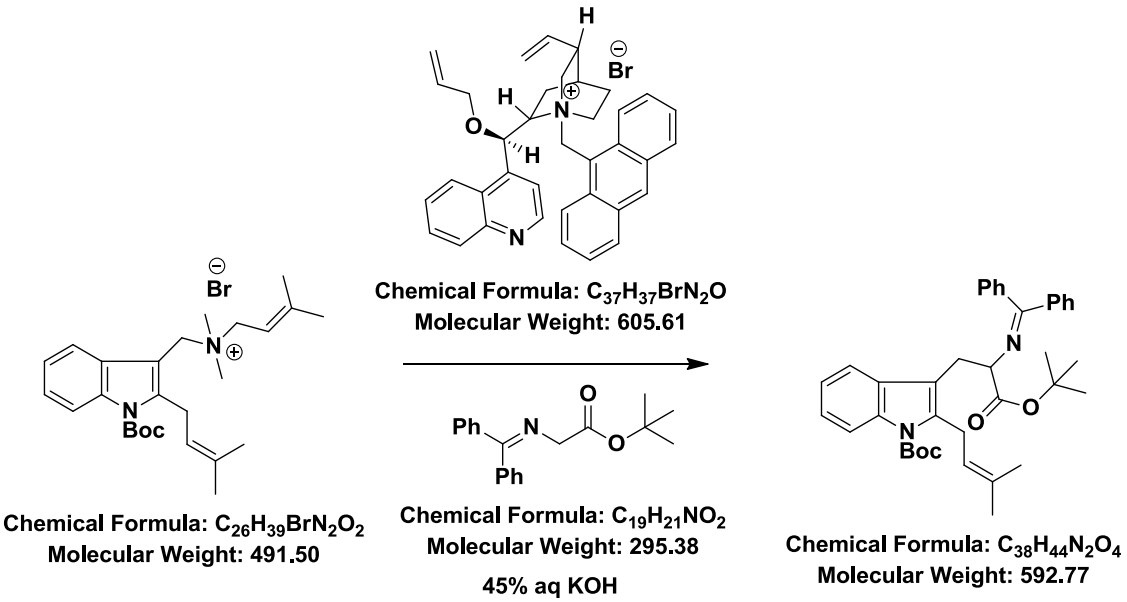


Figure 32. TLC plate of fractions eluted from column chromatography

Synthesis of 2-isoprenyl-N-diphenylmethylene-t-butylestertryptophan

Scheme 57. Phase-transfer catalyst using diisoprenylated quaternary ammonium bromide salt



N-isoprenyl-2-isoprenylbocgramine (2.00 g, 4.069 mmol, 1 eq.) N-(diphenylmethylene) glycine tert-butyl ester (1.202 g, 4.069 mmol) and O-allyl-N-(9-anthracenylmethyl) cinchonidinium bromide (0.4961 g 0.8192 mmol, 0.2 eq) dissolved in acetonitrile (30 mL) in a 250 mL round bottom flask with a stir bar. Reaction mixture was allowed to stir for 30 minutes. At this point the reaction mixture is a dark brown color with a light yellow precipitate that appears to be the phase transfer catalyst. At this point 20 mL 45% KOH solution was added to the reaction mixture and allowed to stir, the aqueous layer was on the bottom and was clear and light yellow and the organic layer was dark brown and on the top. Reaction was allowed to stir for 20 hours; at this point the reaction has a dark layer on top and a clear orange layer on bottom. A small

sample was pulled from the reaction vessel, dissolved in $CDCl_3$ and taken to the 300 NMR where I looked for the singlet at 4.1 representing the CH_2 peak from the glycinate, disappearance of this peak indicates that the reaction has gone to completion. From previous attempts at this experiment if the singlet at 4.1 remains add more KOH and let the reaction continue. After confirmation that the reaction has gone to completion, solvent was removed from the reaction by rotovap leaving water and an orange residue on top of the water. Dichloromethane (3x 50 mL) was added to the reaction vessel this solution was put into a separatory funnel with 50 mL deionized water diluting the water layer enough that the density becomes less than the dichloromethane. Organic layer was collected and dried over sodium sulfate. Solvent was removed weight of this portion is 2.9158 g. Thin layer chromatography (TLC) was used to identify a solvent system for column chromatography, TLC indicated that mobile phase for the column should be 9:1 Hexane:Ethyl Acetate. Colum used was 9 cm tall by 6 cm wide, isolating 1.21 g of product in 60.5 % yield.

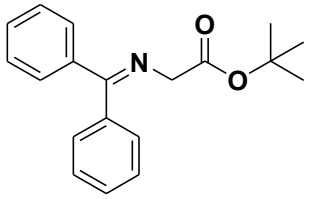


Figure 33. Structure of glycine Schiff base

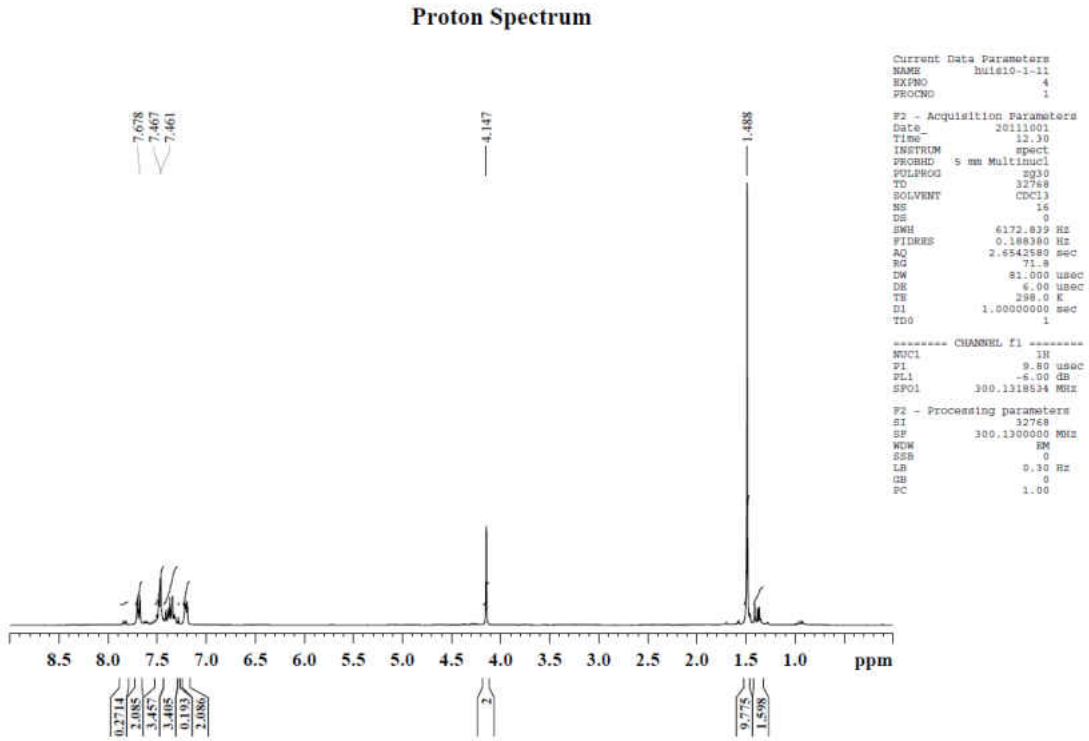


Figure 34. NMR of Glycine Schiff base

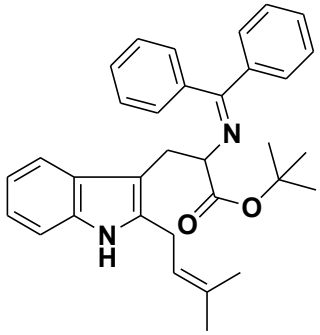


Figure 35. Structure of protected tryptophan

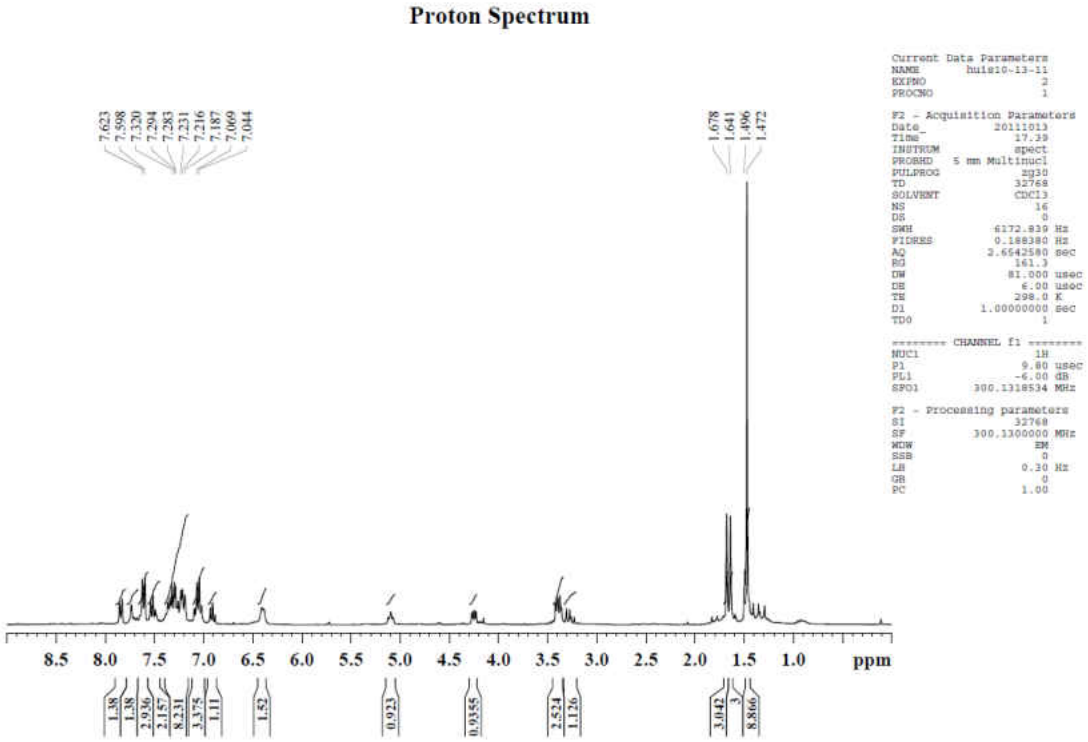
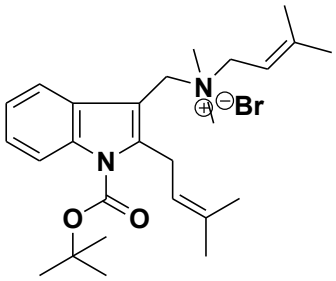


Figure 36. NMR of protected tryptophan



$C_{26}H_{39}N_2O_2^+$
Mol. Wt.: 411.6

Figure 37. Structure of diisoprenylated quaternary ammonium bromide salt

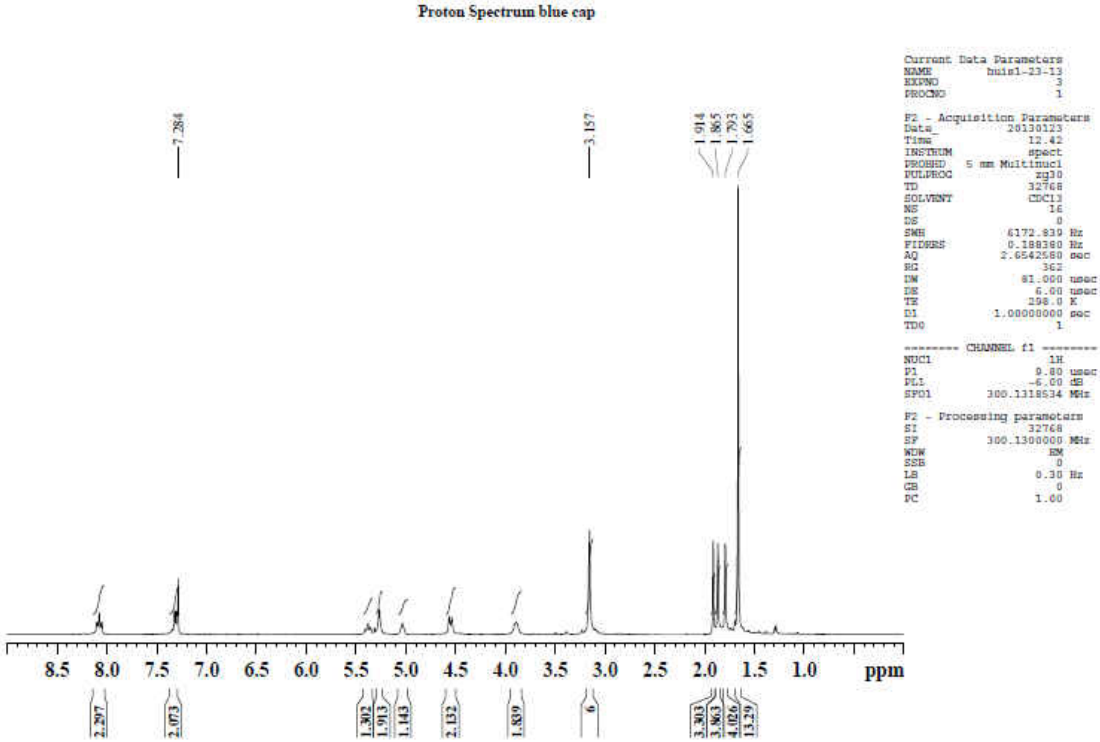
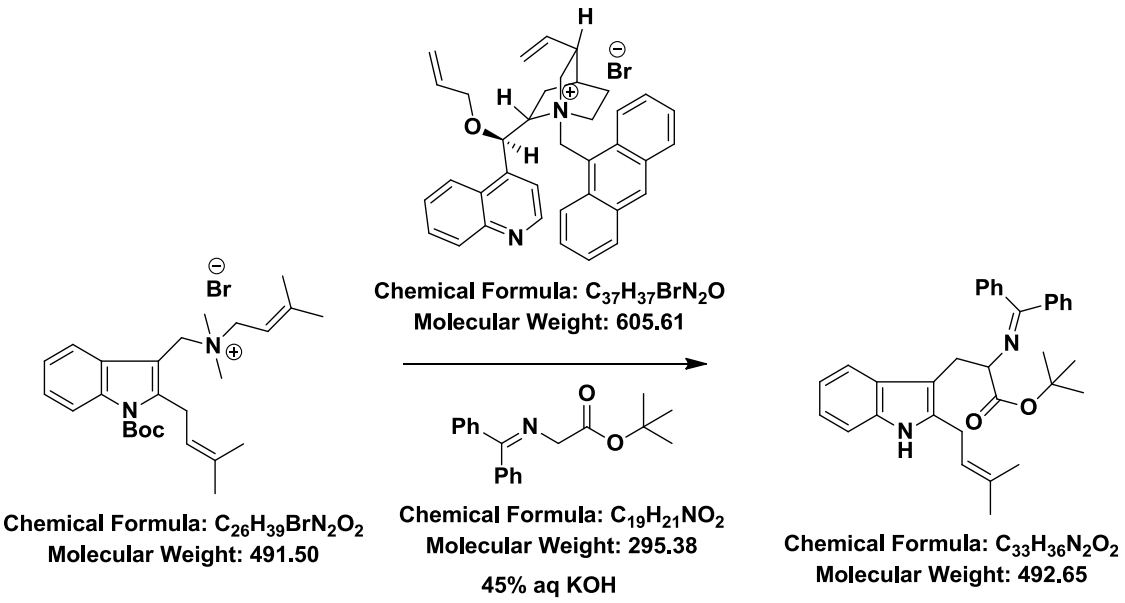


Figure 38. NMR of diisoprenylated quaternary ammonium bromide salt

Screening of %ee N+ isoprenyl salt

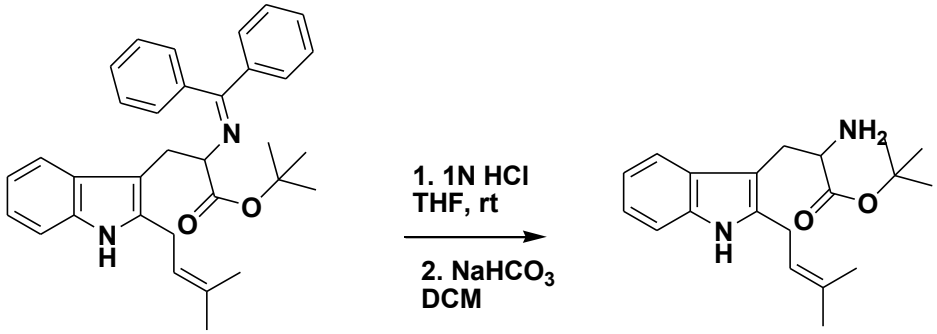
Scheme 58. Phase-transfer catalyst screening procedure



(0.1 g 0.203 mmol 1 eq.) of N+ salt was added to a 7.5 mL vial with a mini stir bar. To this (0.07 g 0.236 mmol 1.16 eq) of Schiff base and (0.03 g 0.04954 mmol 0.2440 eq) of phase transfer catalyst was added. To the reaction mixture 2 mL of solvent was added and reaction mixture was let stir 30 min. 1 mL of 45% KOH was added to the reaction vessel. This was let stir for 18 hours. Crude reaction mixture was run through a short silica plug using 25 mL of 20 % Ethyl Acetate and Hexane. Percent conversion was monitored via proton NMR of organic layer by comparing the integration of the multipet at 4.2 and the singlet at 4.1.

Synthesis of 2-Isoprenylt-N-aminebutylestertryptophan

Scheme 59. *Deprotection of tryptophan amine*



$C_{33}H_{36}N_2O_2$
Mol. Wt.: 492.65

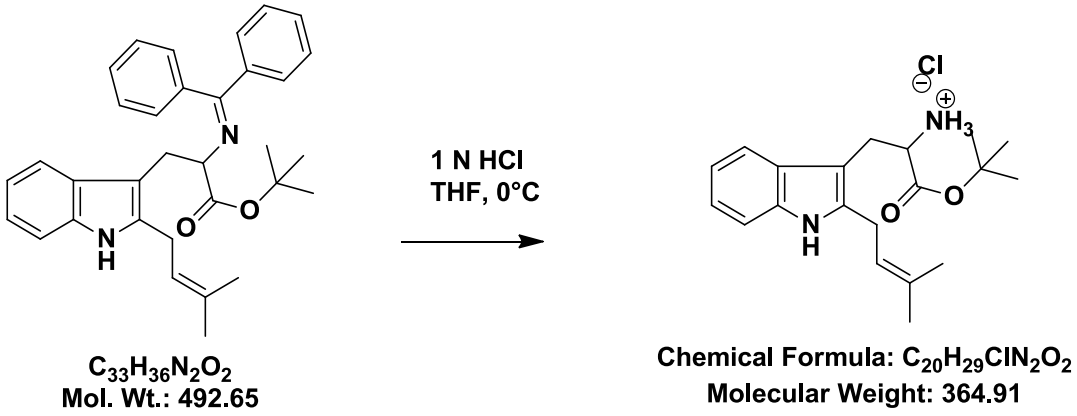
$C_{20}H_{28}N_2O_2$
Mol. Wt.: 328.45

Procedure adapted from Journal of Organic Chemistry Vol. 68, No. 11, 2003. 2-isoprenyl-N-diphenylmethylene-t-butylestertryptophan (1.04 g 2.111 mol) was dissolved in THF (12.66 mL) reaction mixture is clear and orange in color. To the reaction mixture 1 N HCl was added, upon addition of the HCl color changed from clear orange to dark red, and the reaction mixture was allowed to stir 2 hours. The reaction was monitored by TLC (mobile phase 1:2 Hexane:Ethyl Acetate) after 2 hours small amount of starting material in reaction mixture on the TLC indicated the reaction was not complete. Reaction was let stir overnight for 16 hours. TLC at this time indicated no starting material present in the reaction mixture. The resulting mixture was washed with hexanes (2 x 43 mL) and then the aqueous phase was basified with solid sodium bicarbonate and extracted with dichloromethane (4 x 50 mL). Dichloromethane extracts were dried over sodium sulfate and concentrated under reduced pressure (yielding 0.30 g of material, 43.3%). NMR of this product indicated the product was present but contained minor impurities. Reaction mixture was further purified by column chromatography 9:1 Dichloromethane:Methanol.

Also there is similar chemistry in *Organic Letters* 2010 Vol. 12, No. 8 1688-1691 Supporting documents S-13. 2-isoprenyl-N-diphenylmethylene-t-butylestertryptophan simi pure was dissolved in THF (50 mL) and 1 N HCl (50 mL) was added at 0 °C. After stirred for 4 hours, THF was removed under reduced pressure. The resulting solution was washed with ether (3 x 25 mL) and aqueous layer was neutralized with $NaHCO_3$. The mixture was then extracted with CH_2Cl_2 (3 x 50 mL). The ether and dichloromethane layers were dried over anhydrous Na_2SO_4. Ether layer contained benzophenone and dichloromethane layer contained amine. After filtration and concentration under reduced pressure, the product was obtained in % yield after purification by flash column chromatography using mixtures of CH_2Cl_2/MeOH (50:1) as eluent. Also Ethyl Acetate:Hexane 3:7 may be used. Material has an Rf value of 0.4 to 0.5. This is especially useful if the PTC has not been removed previously.

Synthesis of 2-isoprenyl tryptophan t-butyl ester hydrochloride amine

Scheme 60. Attempted isolation of ammonium chloride salt

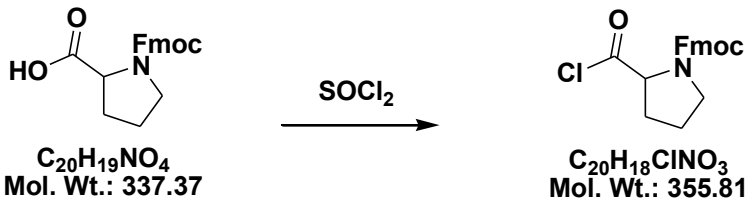


Also there is similar chemistry in *Organic Letters* 2010 Vol. 12, No. 8 1688-1691 Supporting documents S-13. 2-isoprenyl-N-diphenylmethylene-t-butylestertryptophan simi pure (0.61 g

1.24 mmol 1 eq.) was dissolved in THF (20 mL) and 1 N HCl (20 mL) was added at 0 °C. After stirred for 4 hours, THF was removed under reduced pressure. The resulting solution was washed with ether (3 x 50 mL) and aqueous layer roto vaped to dryness. NMR of this aqueous layer was taken in d_2O ether extraction taken in $CDCl_3$. Aqueous layer mass 0.15 g and organic layers mass is 0.67 g. Aqueous layer did not agree with product spectrum, organic layer looked like benzophenone and the amine.

Synthesis of Fmoc Proline Acid Chloride

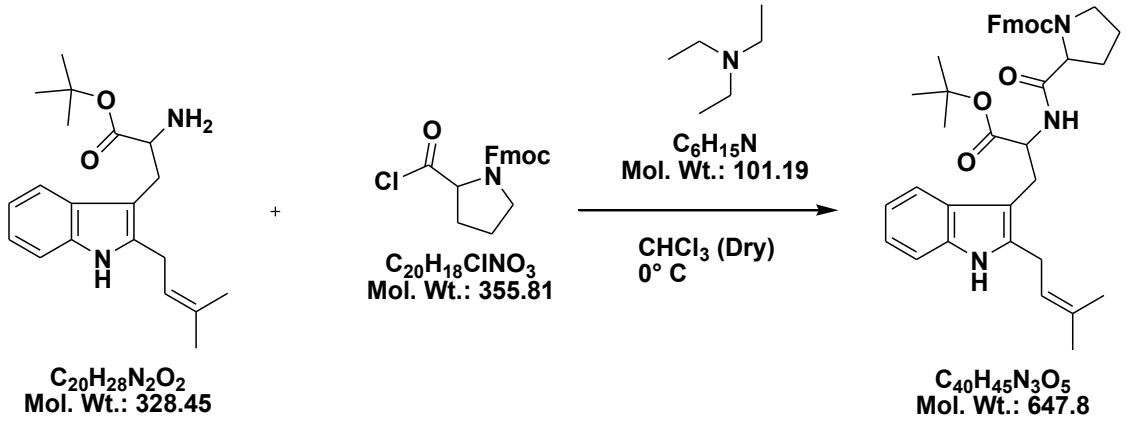
Scheme 61. Synthesis of Fmoc proline acid chloride



Fmoc-L-proline (1.53 g 3.01 mmol 1 eq) was dissolved in thionyl chloride (12.09 mL). The solution which resulted was stirred overnight at rt. Excess thionyl chloride was removed under reduced pressure, yielding 1.70 g of white yellow solid.

Synthesis of 2-isoprenyltryptophan t-butylester fmoc proline

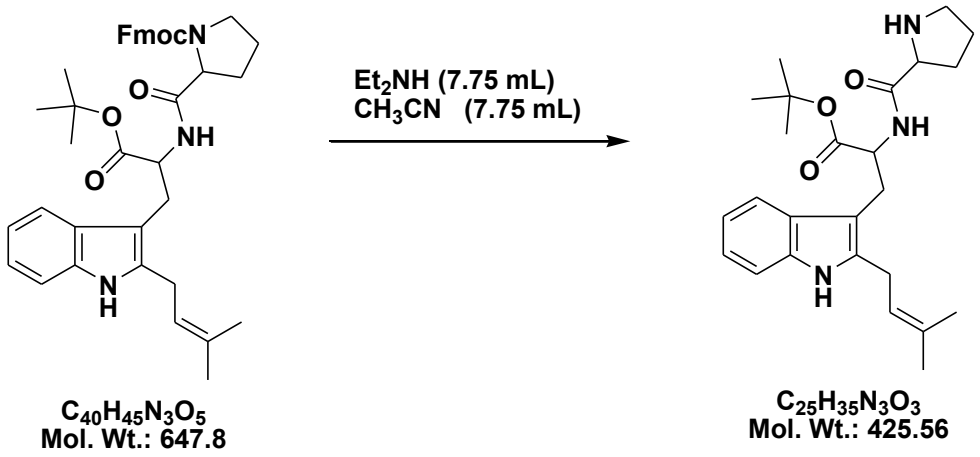
Scheme 62. Synthesis of dipeptide using acid chloride



This procedure was done following the procedure for a similar compound in *H.D. Jain et al Bioorg. Med. Chem. 16 2008 4626-4651*. Fmoc-L-proline chloride (1.70 g 4.78 mol 1.59 eq) which resulted was dissolved in dry $CHCl_3$ (12.09 mL). This solution was added dropwise at 0° C to a solution of 2-isoprenyltryptophant-butylamine (0.99 g 3.01 mmol 1 eq) and triethylamine (0.762 g 0.00726mol 1.05 mL 2.5 eq) in dry $CHCl_3$ (72.5 mL). The mixture that resulted was stirred at 0 °C for 0.5 hr and then at rt overnight. Solvent was remove under rotovap and oil pump to remove solvent producing 3.78 g of orange solid in 194 % yield purity determined by NMR in $CDCl_3$.

Deprotection of the Fmoc protecting group on the 2-isoprenyltryptophan-t-butylester-fmocproline

Scheme 63. Deprotection of Fmoc



This procedure was done following the procedure for a similar compound in *H.D. Jain et al Bioorg. Med. Chem. 16 2008 4626-4651*. Crude solid material from the acid chloride reaction (see above) was dissolved in Acetonitrile (7.75 mL) and stirred via a stir bar until it made a homogeneous solution. To this solution Diethyl amine (7.75 mL) was added dropwise to the reaction flask using an addition funnel. The reaction was let stir overnight and progress was monitored by TLC. Mobile Phase is 9:1 Dichloromethane:Methanol. Fraction 1 is Flourne amine Fmoc deprotection side product Rf = 0.9. Fraction 2 and 3 are undistinguishable by NMR unknowns Rf = 0.55-0.50. Fraction 4 and 5 are product Rf = 0.50. Fraction 6 and 7 are Proline derivatives Rf = 0.35. It is highly suggested to use the smallest collection vessels possible to collect fractions from Rf 0.6-0.4.

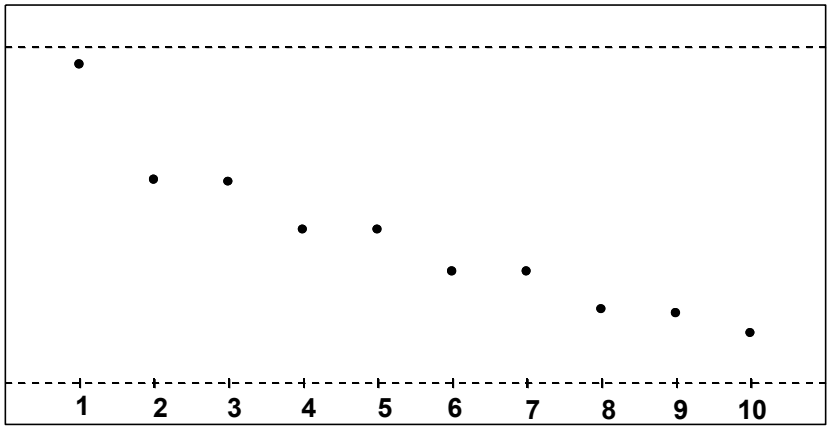
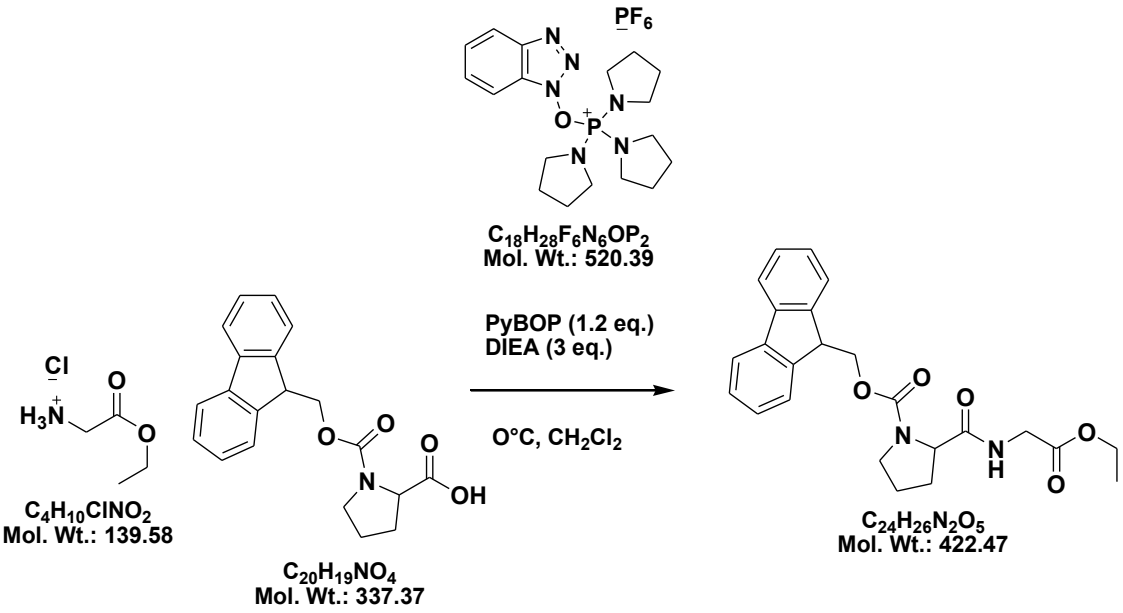


Figure 39. TLC of Fmoc deprotection

Synthesis of Fmoc Proline Gycine ethyl ester

Scheme 64. Peptide coupling of proline and ethyl ester glycine

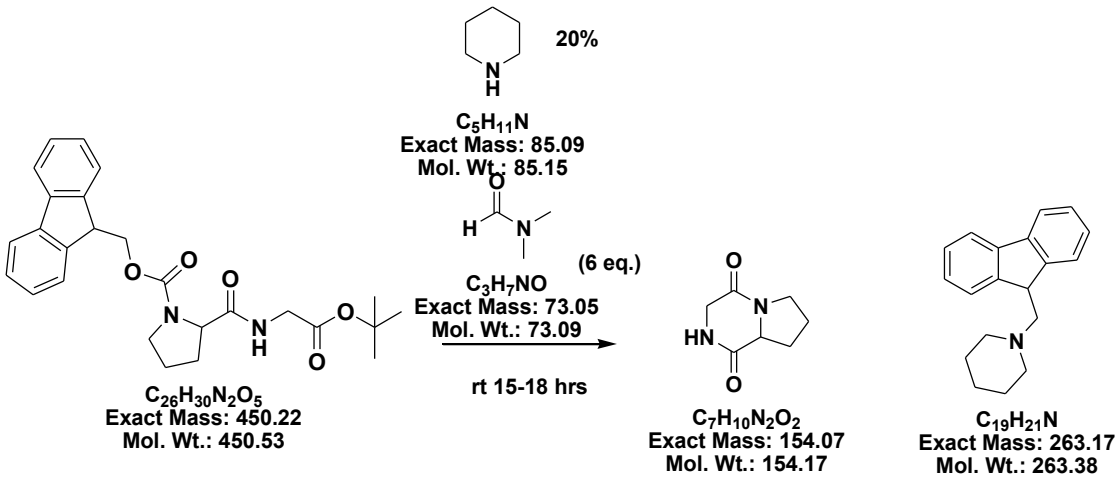


Following procedure found in *V.L. Campo et al. Tetrahedron 65 (2009) 5343*. Also procedure in Org. Lett. 2011 vol. 13, No. 24 6334. To a solution of FmocProline-OH (1.00g 2.96 mmol 1 eq)

in Dichloromethane (20 mL) at room temp, PyBOP (1.85 g 3.55 mole 1.2 eq) and Diisopropyl ethyl amine (DIEA) (1.15 g 8.90 mmol 3 eq) were added. The reaction mixture was stirred for 10 min before the glycineethylester HCl (0 .413 g 2.96 mmol 1 eq) was added to the reaction vessel. The reaction mixture was stirred overnight and concentrated on the rotovap and oil pump. The crude material was purified by column chromatography using Hexane/EtOAc (7:3) to give FmocProlineGlycineethylester. Material eluted with the byproduct of the coupling reagent, this gave an NMR that appeared to have THF like spectra. It was not purified further.

Synthesis of diketopiperazine

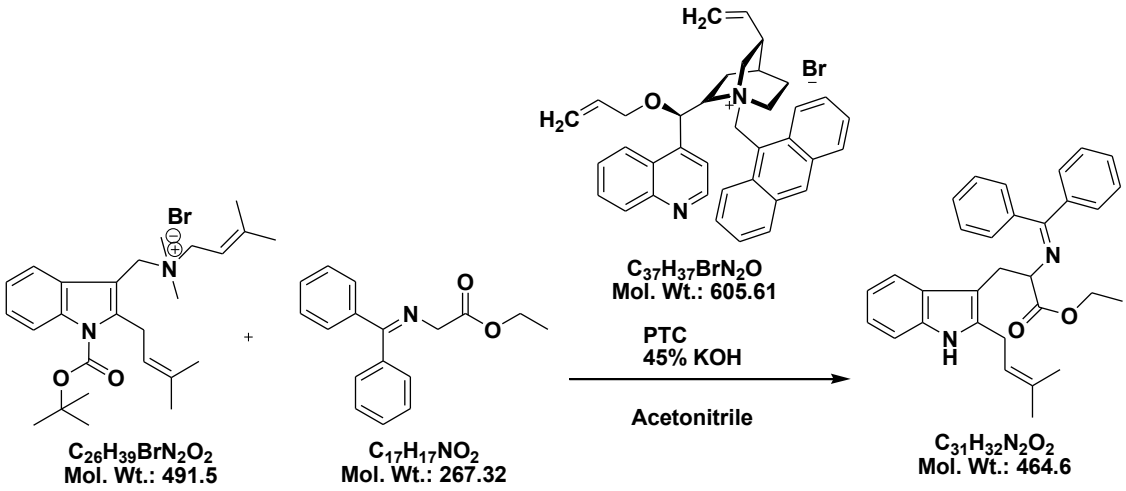
Scheme 65. Attmpted DKP ring closure in DMF



Procedure adapted from V.L. Campo et al. Tetrahedron 65 (2009) 5343-5349. The protected dipeptide (0.25 g 0.555 mmol 1eq.) was treated with 20% piperidine 0.32 mL /DMF 1.28 mL (6 eq.) and allowed to stir at room temperature for 18 hours. After concentration in vacuo, the residue was purified by column chromatography (EtOAc/hexane 1:1v/v, DCM/MeOH 9/1 v/v).

Synthesis of 2-isoprenyl tryptophan ethyl ester benzophenone imine

Scheme 66. Phase-transfer catalyst reaction using ethyl ester glycine



2-isoprenyl Indole N+dimethyl isoprenyl bromide salt (0.1 g 0.2034 mmol 1 eq) N-(diphenylmethylene) glycine ethyl ester (0.05438 g 0.2034 mmol 1 eq) and O-allyl-N-(9-anthracenylmethyl) cinchonidinium bromide (0.02464 g 0.04069 mmol 0.2 eq) was dissolved in solvent (2 mL) in a 5 mL vial round bottom flask with stir bar stirring. Reaction mixture was allowed to stir for 30 minutes. At this point the reaction mixture is a dark brown color with a light yellow precipitate that appears to be the phase transfer catalyst. TLC plate was taken of starters and crude mixture (see bleow). TLCs were exposed to both cerium (IV) ammonium sulfate stain for alkaloids where the N+ salt turned red no effect on the other spots. Ninhydrin stain was also used on TLC plate and two spots were sensitive to it, N+ salt purple, Schiff base is pink, and PTC shows no reaction. At this point 1 mL 45% KOH was added to the reaction mixture and allowed to stir, the aqueous layer was on the bottom and was clear and light yellow and the organic layer was dark brown and on the top. After about 10 minutes the organic layer turned from brown to dark red. After 30 min the KOH layer was removed and replaced and the

reaction was continued to stir. Shortly after this time color of organic layer was red and clear. Reaction was allowed to stir for 20 hours; at this point the reaction has a dark layer on top and a clear orange/yellow layer on bottom. At this point the crude reaction mixture was monitored by TLC. After 20 hours TLC was run Cerium (IV) ammonium sulfate stain indicates presence of indole in lane 4, ninhydrin stain indicated very weak signal for amines in lane 4. NMR was taken of crude reaction mixture, which suggested that the reaction had gone at least to some extent to product. Gradient column was run on the reaction mixture using Hexane:EtOAc 9:1, 8:2, 7:3 in 100 mL portions product appears to be purple on TLC in high concentrations.

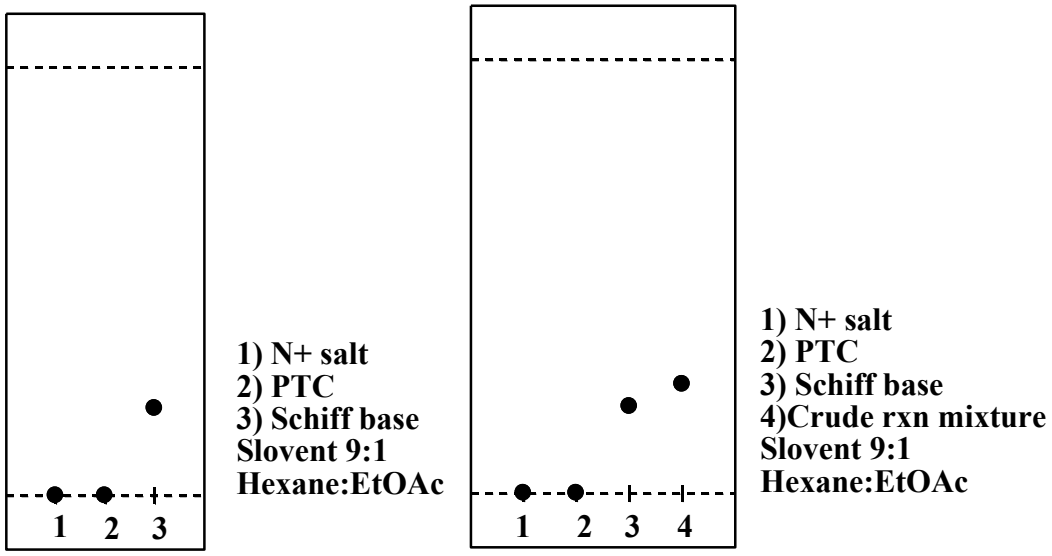
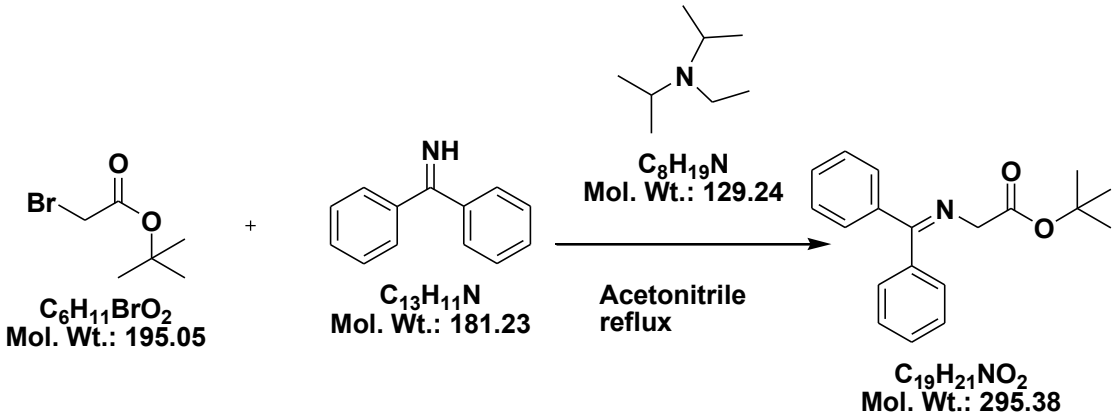


Figure 40. TLC of reagents and crude reaction mixture

Synthesis of tert-butyl N-(Diphenylmethylene)glycinate

Scheme 67. Synthesis of glicine schiff base



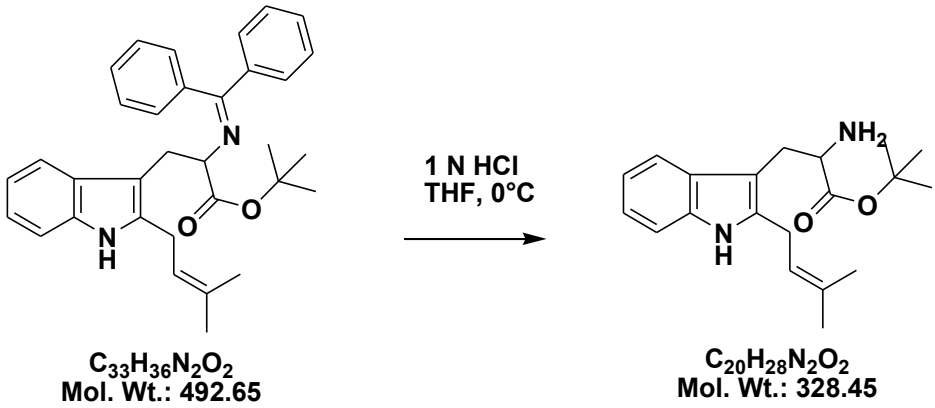
See *Eur. J. Org. Chem.* 2005, 317-325, procedure is taken from there.

A solution of tert-butyl 2-bromoacetate (5.8 mL 7.7 g, 39.5 mmol 1 eq) in acetonitrile (44 mL) was treated with benzophenonimine (6.6 mL 7.1 g, 39.3 mmol 1 eq) and diisopropylethylamine (6.8 mL, 5.0 g, 35.0 mmol 0.90 eq), and the mixture was then heated at reflux for 12 hours. After the system had cooled to room temperature, most of the acetonitrile was removed in vacuo. The residue was partitioned between water (40 mL) and diethyl ether (60 mL) and the phases were separated. The organic layer was dried with Na_2SO_4, filtered and concentrated in vacuo until the mixture became turbid. Crystallization was done using ethanol/petroleum ether (in our case we substituted hexane for petroleum ether) 1:4. The yield was 86 % slightly yellow solid.

Figure 41. Apperatus for synthesis of glycine schiff base

Synthesis of 2-Isoprenylt-N-aminebutylestertryptophan

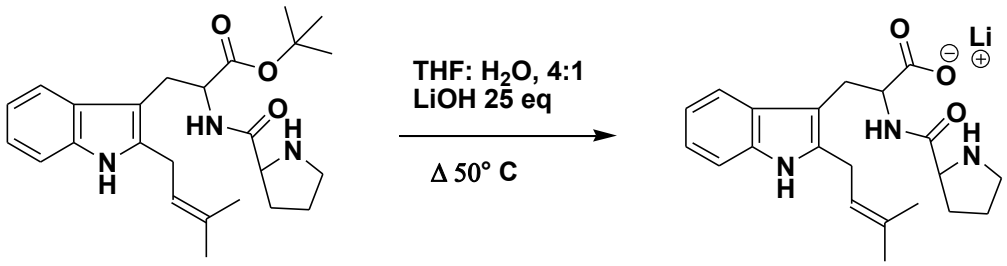
Scheme 68. Deprotection of amine using 1 N HCl



Procedure adapted from Organic Letters 2010 vol. 12, No. 8 1688-1691. Crude reaction mixture forming 2-isoprenyl-N-diphenylmethylene-t-butylestertryptophan (100.0 mg 0.24 mmol) was dissolved in THF (2 mL) and 1 N HCl (2 mL) was added at 0 ° C. After stirred for 4 hours, THF was

removed under reduced pressure. The resulting aqueous solution was washed with ether (3 x 20 mL) and neutralized with $NaHCO_3$. The mixture was then extracted with CH_2CCl_2 (3 x 20 mL). The organic layers were combined and dried over anhydrous Na_2SO_4. After filtration and concentration under reduced pressure, the product was obtained after purification by flash column chromatography using gradient mixtures of CH_2CCl_2/MeOH (98:2 95:5 90:10) as the eluent.

Scheme 69. Attempted deprotection of t-butyl group



$C_{25}H_{35}N_3O_3$
Exact Mass: 425.27
Mol. Wt.: 425.56

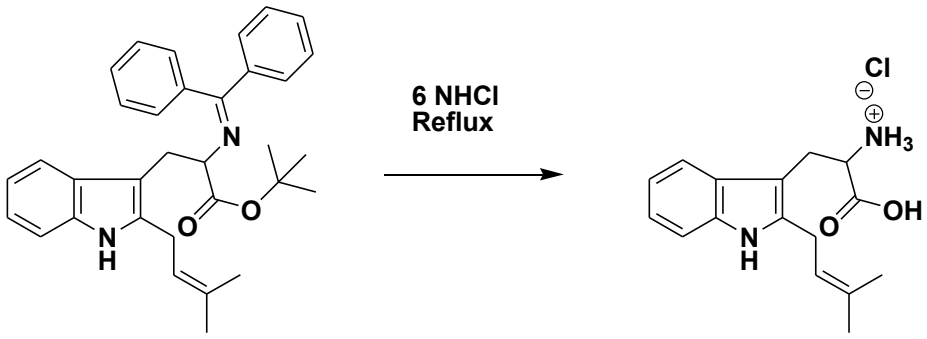
$C_{21}H_{26}LiN_3O_3$
Exact Mass: 375.21
Mol. Wt.: 375.39

tert-butyl 3-(2-(3-methylbut-2-enyl)-1H-indol-3-yl)-2-(pyrrolidine-2-carboxamido)propanoate (0.01 g 0.234 mmol 1 eq) in a round bottom flask. 4 mL of tetrahydrofuran was added to the reaction vessel along with 1 mL of water. To this Lithium hydroxide (0.14 g 5.87 mmol 25 eq) were added as a solid. This reaction was heated to and let stir. Reaction was monitored by TLC and cerium ammounium sulfate (indole stain) using a 9:1 Dichloromethane:Methanol mobile phase until the stained spot changed Rf value from 0.5 to 0.1. At this point the reaction was let cool then it was put on the roto vap to remove tetrahydrofuran and water. To this deionized water was added to the reaction vessel and pH was taken indicating the mixture was strongly basic. $KHSO_4$ was added as a solid until the reaction mixture indicated a pH in between 2 and 3. At this point dichloromethane was added to the reaction mixture, and then the mixture was

poured into a separatory funnel. Organic layer was removed and dried over Na_2SO_4. Solvent was removed via roto vap and high vac on oil pump. NMR was taken of the material in the organic layer this did not appear to be the product.

Synthesis of 2-isoprenyl tryptophan hydrochloride

Scheme 70. Attempted deprotection of t-butyl group



$C_{33}H_{36}N_2O_2$
Exact Mass: 492.28
Mol. Wt.: 492.65

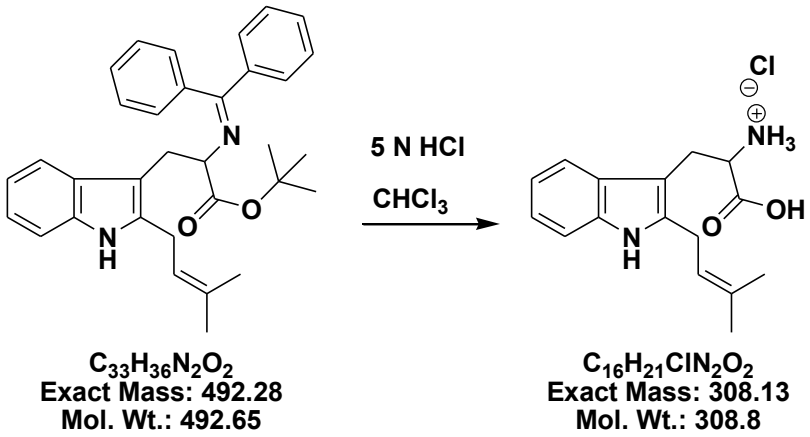
$C_{16}H_{21}ClN_2O_2$
Exact Mass: 308.13
Mol. Wt.: 308.8

Procedure adapted from a similar reaction discussed in in Organic Letters 2010 Vol. 12, No.8 1688-1691 N diphenylmethylene t-butyl ester 2-isoprenyl tryptophan (0.18 g 0.3659 mmol 1 eq.) in a round bottom flask was put into a was heated to reflux with stirring in 6 M HCl (10 mL) under an N_2 atmoshere for 24 hours. After it was cooled, the reaction mixture was washed successively with CH_2Cl_2 (2 x 5 mL) and ether (2 x 5 mL) before concentration to dryness under vacuum and in 5 mL methanol. Organic layer was evaluated with proton NMR this indicated the material was benzophenone. Aqueous layer was evaporated and evaluated via proton NMR which did not appear to be the product.

Figure 42. Charred reaction product

Synthesis of 2-isoprenyl tryptophan hydrochloride

Scheme 71. Attempted deprotection of t-butyl group using 5 N HCl

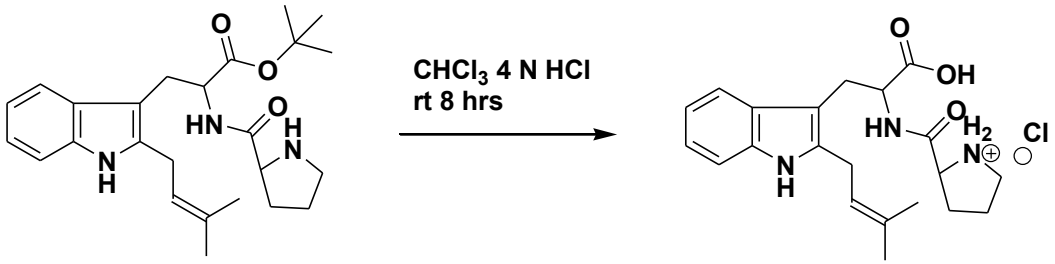


Procedure adapted from a similar reaction in *J. Org. Chem*., Vol. 62, No. 12, 1997. To a round bottom flask 2-isoprenylN-diphenylmethylenet-butylester tryptophan (.05 g 0.0101 mmol 1 eq.),

0.1 mL 5N HCl and 1 mL of $CHCl_3$ was added. The reaction was stirred at room temperature for 4 hours until tlc showed disappearance of starting material. The $CHCl_3$ was then removed, the aqueous layer was washed with $CHCl_3$ (3 x 2.5 mL) and then separated, and the solvent was evaporated to give 2-isoprenylN-diphenylmethylenet-butylester tryptophan. Reaction did not appear to deprotect t-butyl group.

Synthesis of 2-isoprenyl proline tryptophan

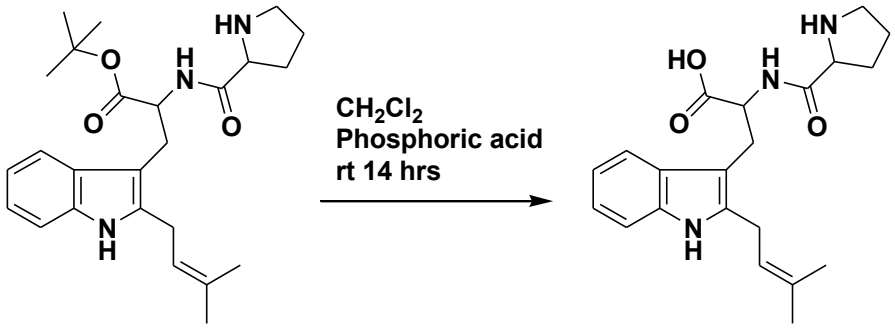
Scheme 72. Attempted synthesis of dipeptide proline salt



Procedure adapted from J. Org. Chem., Vol. 62, No. 12, 1997. 2-isoprenyl t-butyl ester tryptophan proline was dissolved in 4 mL of $CHCl_3$ to this a 0.25 mL of 4 N HCl was added and reaction was stirred for 7 hours TLC plate at this time indicated very little change. Half of this reaction was worked up at this point, remaining reaction mixture was heated to 45 °C for an hour then the reaction mixture was let cool to room temperature and mixture was stirred for 16 hours. Both first half and second half were worked up in the same way organic layer was washed with water and then dried over Na_2SO_4. NMRs were taken of the samples both looked like they contained the t-butyl ester peak.

Synthesis of 2-isoprenyl proline tryptophan

Scheme 73. Attempted deprotection of t-butyl group



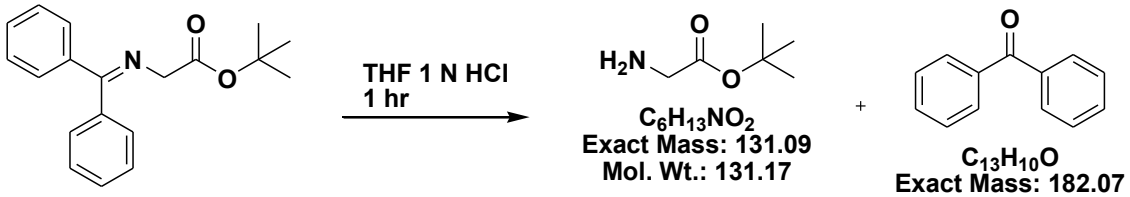
$C_{25}H_{35}N_3O_3$
Exact Mass: 425.27
Mol. Wt.: 425.56

$C_{21}H_{27}N_3O_3$
Exact Mass: 369.21
Mol. Wt.: 369.46

Reaction adapted from J. Org. Chem., Vol. 71, No. 24, 2006 4675. 70 mg of 2-isoprenyl t-butyl ester tryptophan proline dissolved in 1 mL of dichloromethane and stirred. To this 0.1 mL of phosphoric acid 85 wt % was added dropwise vial syringe dropwise. The reaction was stirred for 14 hours. NMR of material indicated t-butyl group was still present.

Synthesis of t–butyl glycinate

Scheme 74. Deprotection of Glycine Schiff base



$C_{19}H_{21}NO_2$
Exact Mass: 295.16
Mol. Wt.: 295.38

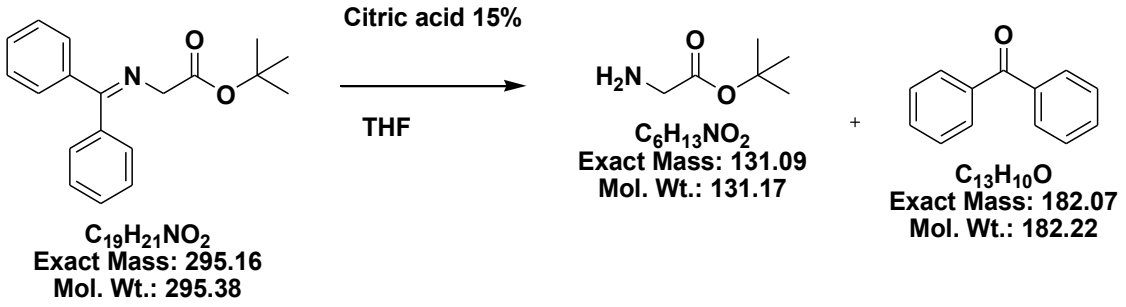
$C_6H_{13}NO_2$
Exact Mass: 131.09
Mol. Wt.: 131.17

$C_{13}H_{10}O$
Exact Mass: 182.07
Mol. Wt.: 182.22

Procedure adapted from Organic Letters 2010 vol. 12, No. 8 1688-1691. N-diphenylmethylene-t-butylestertryptophan (0.5 g 1.69 mmol 1 eq) dissolved in 10.5 mL THF along with 10.5 mL of 1 N HCl solution. Reaction mixture was stirred at room temperature for 1 hour. THF was rotovaped off. Reaction mixture was washed with hexane three times; this layer was dried over Na_2SO_4 and rotovaped to dryness. NMR indicates this is benzophenone. Aqueous layer was basified using sodium bicarbonate, until adding solid gave no more bubbles. Aqueous layer was extracted with dichloromethane three times. Dichloromethane layer was dried over Na_2SO_4 and rotovaped to dryness.

Synthesis of t–butyl glycinate

Scheme 75. Deprotection of glycine Schiff base using 15% citric acid

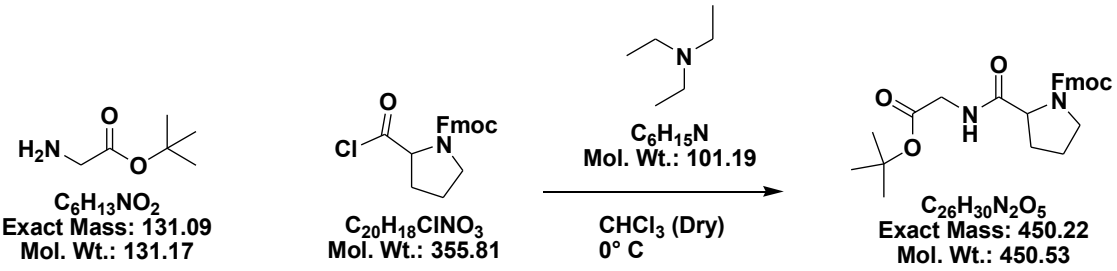


3-26-13 Procedure adapted from Tetrahedron Letters 43 (2002) 6677-6679. Glycine imine (1.4 g 4.7 mmol 1 eq.) was dissolved in (23.3 mL) of tetrahydrofuran and (8.7 mL) of 15% aqueous citric acid. Reaction mixture was stirred vigorously at room temperature for 18 h, and then diluted with 1 M hydrochloric acid (5.83 mL). THF remove via rotovap. The mixture is extracted with diethyl ether (2x11.66 mL) to remove the benzophenone, then the aqueous layer was basified (K_2CO_3) until no more K_2CO_3 would dissolve. Extraction with chloroform (5x17.5 mL) followed by drying of the extracts (Na_2SO_4) and concentration under reduced pressure gives the

crude amino acid tert-butyl ester which can generally be purified by passing through a plug of silica. Amine looked clean. 96 % Yield 0.6 g theoretical was 0.62 g.

Synthesis of Fmoc-Proline glycine t-butylester dipeptide

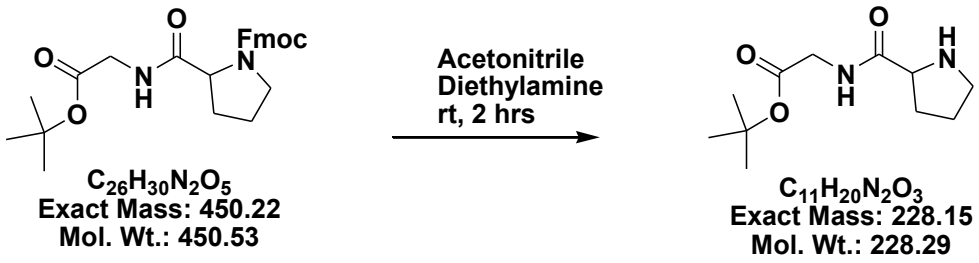
Scheme 76. Synthesis of glycine proline using acid chloride



This procedure was done following the procedure for a similar compound in *H.D. Jain et al Bioorg. Med. Chem. 16 2008 4626-4651*. Fmoc-L-proline chloride (1.71 g 0.00481 mol 1.85 eq) which resulted was dissolved in dry $CHCl_3$ (12.09 mL). This solution was added dropwise at 0° C to a solution of t-butyl ester glycine amine (0.34 g 0.00259 mol 1eq) and triethylamine (0.0.655 g 0.00655 mol 0.90 mL 2.5 eq) in dry $CHCl_3$ (72.5 mL). The mixture that resulted was stirred at 0 °C for 0.5 hr and then at rt overnight. Solvent was remove under rotovap and oil pump to remove solvent producing 2.54 g of solid in 218% yield purity determined by NMR in $CDCl_3$. Theoretical yield was 1.16 g. NMR showed two large peaks at ca. 3.0 and 1.5 ppm.

Synthesis of t-butyl glycine proline

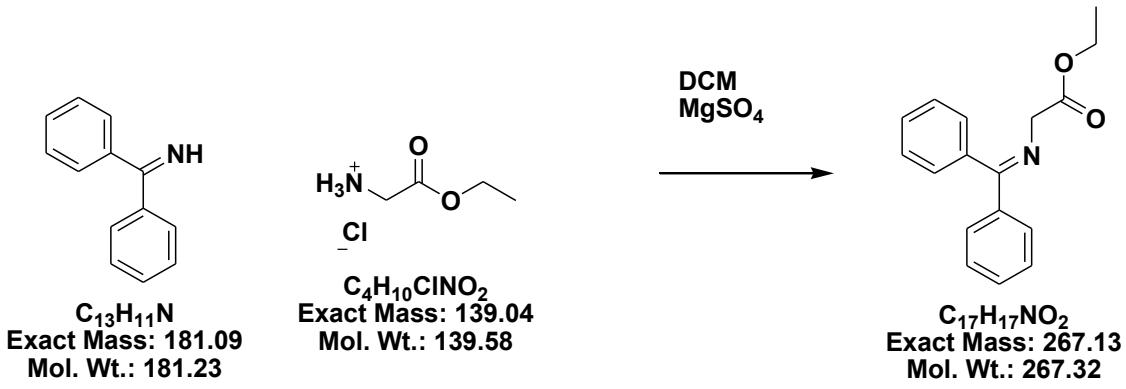
Scheme 77. Deprotection of dipeptide



Reaction was modeled after H. D. Jain Bioorg. Med. Chem. 16 2008 4626-4651. 0.5 g of the t-butyl glycine fmoc proline was dissolved in acetonitrile (10 mL) and diethylamine (10 mL). The reaction mixture was stirred for two hours at room temperature.

Synthesis of benzophenone imine glycine ethyl ester

Scheme 78. Synthesis of ethyl ester glycine Schiff base

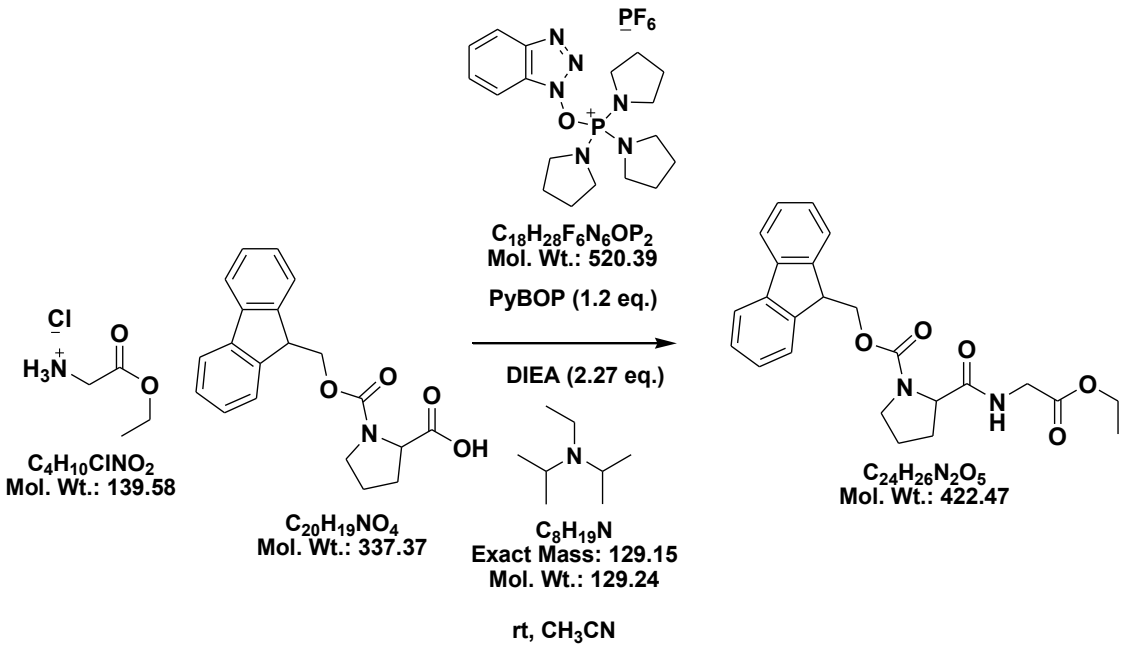


Procedure from Chem. Eur. J. 2010, 16, 1153-1157. A mixture of the corresponding benzophenone NH-imine (0.181 g 0.167 mL mmol 1 eq. 1.08 g/mL), amino acid ester hydrochloride (0.153 g 1.1 mmol 1.1 eq) and $MgSO_4$ (0.181 g 1.5 mmol 1.5 eq) were stirred in

dichloromethane (10 mL) at room temperature for 24 hours. The reaction was filtered and the filtrate was washed with water and brine, and dried over $MgSO_4$. Filtration and solvent removal afford a-ketiminoesters which were used without further purification. Theoretical yield 0.267 g.

Synthesis of Fmoc proline glycine ethyl ester

Scheme 79. Peptide coupling of proline and glycine ethyl ester

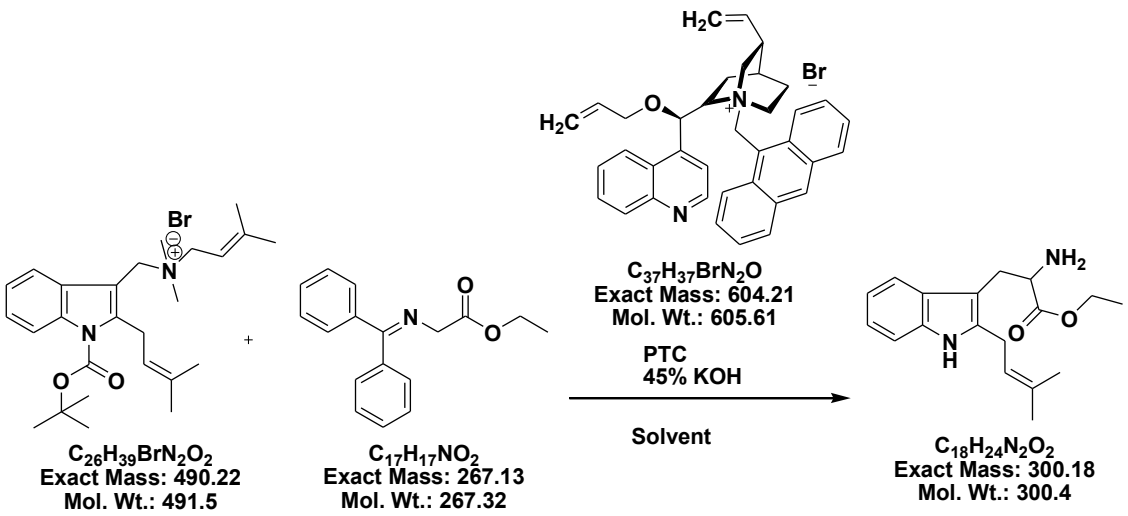


4-10-13 Procedure adapted from Org. Lett. Vol. 15 No. 1, 2013 22-25. Paper uses HATU as peptide coupling reagent I choose to use PyBOP because we had it as a peptide coupling reagent. PyBOB (2.09 g 4.02 mmol 1.36 eq) and i-Pr_2Net (0.8682 g 1.17 mL 6.72 mmol Density 0.742 g/mL 2.27 eq) were added to a solution of proline (1.36 g 3.93 mmol 1.33 eq) and ethyl ester glycine (0.412 g 2.95 mmol 1 eq) in CH_3CN (30 mL) and the reaction was stirred at room temperature for 4 hours. The reaction was concentrated under reduced pressure, and the residue partitioned between CH_2Cl_2 (100 mL) and 1 M HCl (100 mL). The layers were separated,

and the aqueous phase was extracted with CH_2Cl_2 (2 x 25 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with 60 % EtOAc/Hexane to give 1.18 g (95 % yield) of dipeptide as clear viscous oil.

Synthesis of 2-isoprenyl tryptophan ethyl ester

Scheme 80. Phase-transfer catalyst with glycine ethyl ester

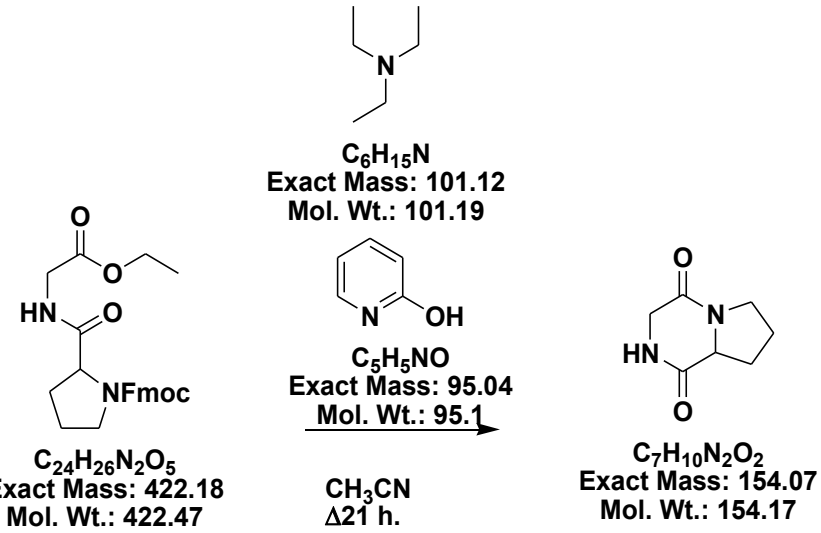


4-10-13 2-isoprenyl Indole N+dimethyl isoprenyl bromide salt (1.0 g 0.002034 mol 1 eq) N-(diphenylmethylene) glycine ethyl ester (0.5438 g 0.002034 mol 1 eq) and O-allyl-N-(9-anthracenylmethyl) cinchonidinium bromide (0.2464 g 0.4069 mmol 0.2 eq) was dissolved in 1,4 dioxane (20 mL) in a round bottom flask with stir bar stirring. Reaction mixture was allowed to stir for 30 minutes. At this point the reaction mixture is a dark brown color with a light yellow precipitate that appears to be the phase transfer catalyst. At this point 20 mL 45% KOH was added to the reaction mixture and allowed to stir, the aqueous layer was on the bottom and

was clear and light yellow and the organic layer was dark brown and on the top. After about 10 minutes the organic layer turned from brown to dark red. Shortly after this time color of organic layer was red and clear. Reaction was allowed to stir for 20 hours; at this point the reaction has a dark layer on top and a clear orange/yellow layer on bottom. At this point the crude reaction mixture was monitored by TLC. After 20 hours TLC was run Cerium (IV) ammonium sulfate stain indicates presence of indole in lane 4, ninhydrin stain indicated very weak signal for amines in lane 4. NMR was taken of crude reaction mixture, which suggested that the reaction had gone at least to some extent to product. Gradient column was run on the reaction mixture using Hexane:EtOAc 9:1, 8:2, 7:3 in 100 mL portions product appears to be purple on TLC in high concentrations.

Synthesis of diketopiperizine

Scheme 81. Attempted DKP ring closure

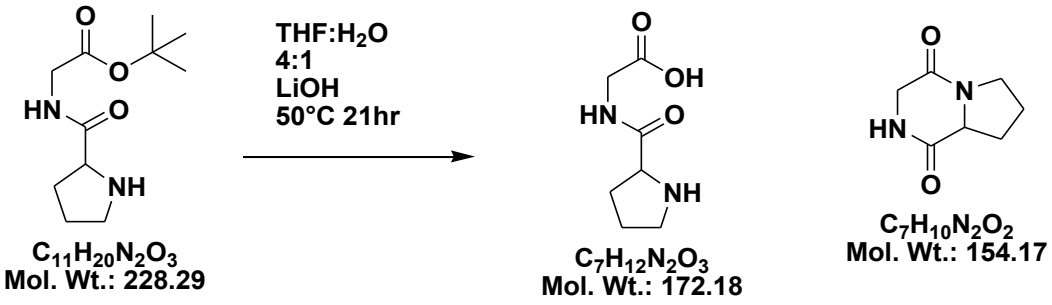


Procedure adapted from Org. Lett., Vol. 15 No.1, 2013 pg. 22-25 Procedure on S21 of supporting documents. 1.34 times scale. Et_3N (2.80 g 3.86 mL 0.7255 g/mL 10 eq.) and 2-hydroxypyridine

(0.058 g 0.615 mmol 0.22 eq.) were added to a solution of dipeptide (1.18 g 2.79 mmol 1 eq.) in CH_3CN (53.6 mL) and the reaction was heated under reflux for 21 hrs. The reaction was cooled to room temperature and then concentrated to reduced pressure. The residue was partitioned between 1 M HCl (67 mL) and CH_2Cl_2 (134 mL). The organic phase was removed, and the aqueous phase was extracted with CH_2Cl_2 (2 x 33.5 mL). The organics were combined, washed with saturated aqueous NaCl (134 mL), then dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with MeOH/$CHCl_3$ to give no promising looking material by NMR.

Synthesis of Diketopiperzine

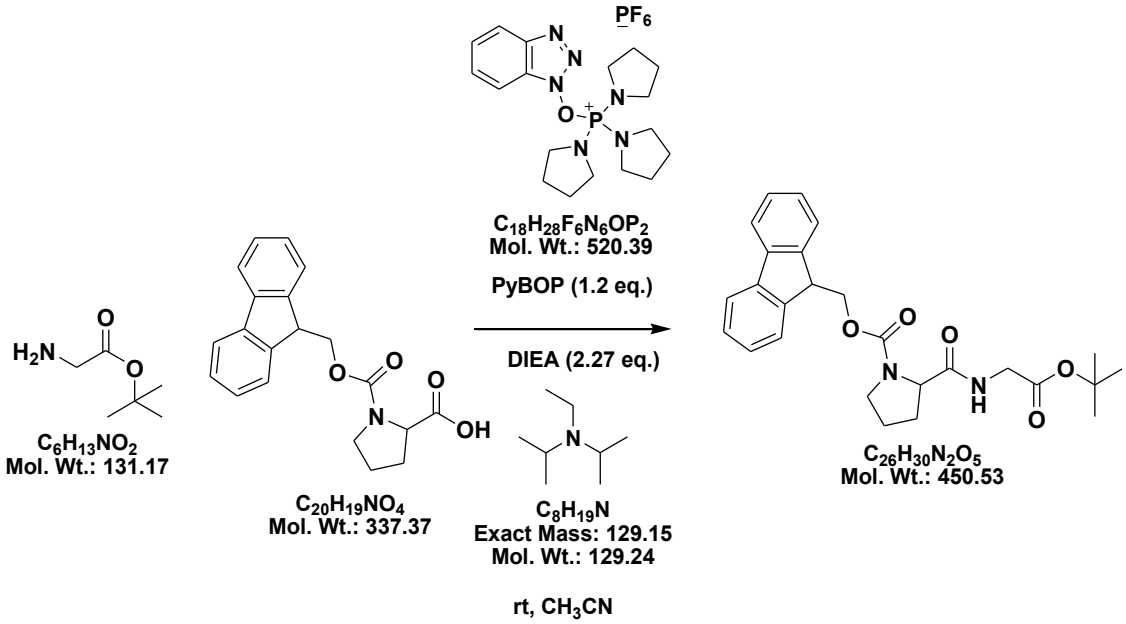
Scheme 82. Attempted DKP ring closure



T-butyl glycine Fmoc proline was dissolved in a 4:1 ratio of THF/water (2.5 mL). Next Lithium hydroxide was added to the reaction vessel (25 eq.) The solution was heated at 50 ^oC for 15 hours. The reaction was diluted with water (10 mL) then was acidified to a pH of 5 with $KHKSO_4$. The aqueous layer was extracted with ethyl acetate (10 mL) four times. The organic layers were combined and washed with water, brine, and dried over Na_2SO_4. Material was concentrated in vacuo. Organic layer contained no material. Aqueous layer was evaporated and NMR was taken in d_2O. HMBC indicated that the material was the open dipeptide, due to the carbonyl carbon

not seeing the apha carbons proton or the other protons on the other side of the proline nitrogen.

Scheme 83. Peptide coupling between t-butyl glycine and proline



5-8-13 Procedure adapted from Org. Lett. Vol. 15 No. 1, 2013 22-25. Paper uses HATU as peptide coupling reagent I choose to use PyBOP because we had it as a peptide coupling reagent. PyBOB (2.49 g 4.78 mmol 1.36 eq) and i-Pr_2NEt (1.03 g 1.39 mL 7.97 mmol Density 0.742 g/mL 2.27 eq) were added to a solution of proline (1.61 g 4.77 mmol 1.33 eq) and t-butyl ester glycine (0.63 g 4.80 mmol 1 eq) in CH_3CN (35.7 mL) and the reaction was stirred at room temperature for 4 hours. The reaction was concentrated under reduced pressure, and the residue partitioned between CH_2Cl_2 (100 mL) and 1 M HCl (100 mL). The layers were separated, and the aqueous phase was extracted with CH_2Cl_2 (2 x 25 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was

purified by flash chromatography eluting with 60 % EtOAc/Hexane to give dipeptide as clear viscous oil. Dipeptide is the spot at about 0.5 Rf in 1:2 Hex:EtOAc.

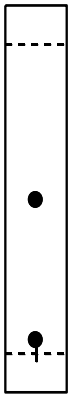
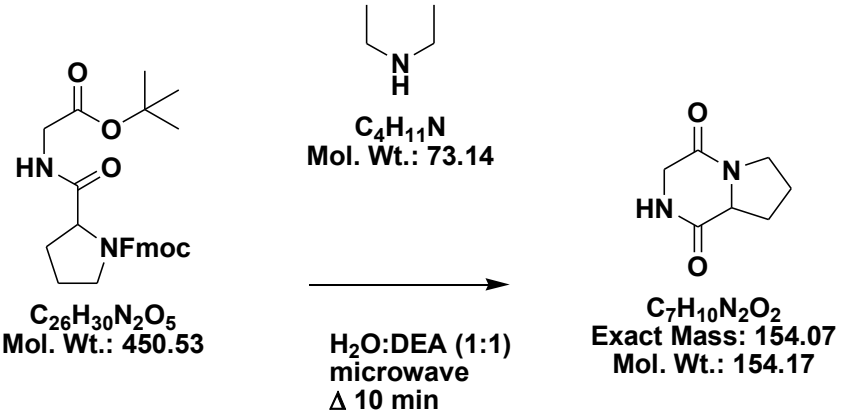


Figure 43. TLC plate of crude reaction mixture

Synthesis of Diketopiperazine Ring

Scheme 84. Attempted DKP ring closure

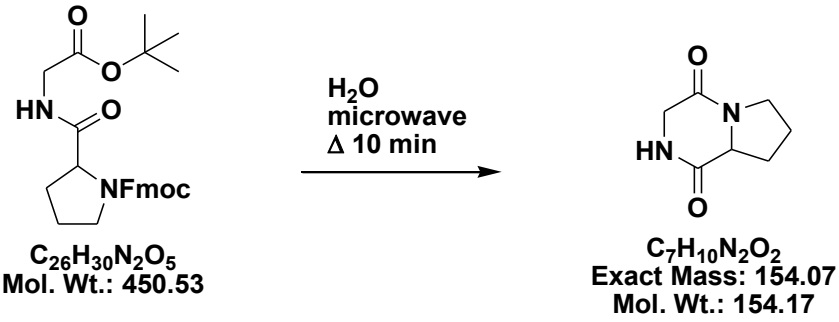


Reaction was modeled after Molecules 2009, 14, 2836-2849. Each dipeptitidyl ester (0.25 mmol 0.113 g 1 eq.) was suspended in water:diethylamine (1 mL) and heated for 10 minutes at 250 ^{o}C and 150 psi, using a CEM Discover microwave apparatus at 250 W. The resulting suspension was

filtered through a Hirsch funnel and washed with water (5 mL); the solid was dried under high vacuum and analyzed without further purification by NMR. Reaction did not work as well as the reaction in just water.

Synthisis of Diketopiperazine Ring

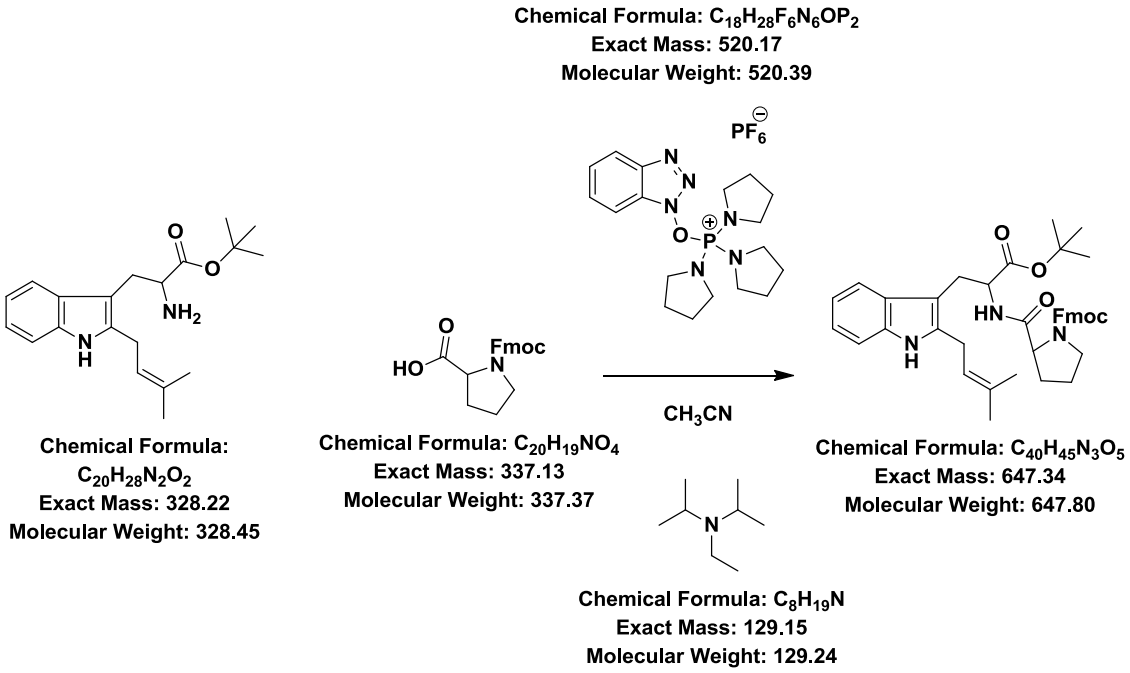
Scheme 85. Attempted DKP ring closure



5-13-13 Reaction was modeled after Molecules 2009, 14, 2836-2849. Each dipeptitidyl ester (0.25 mmol 0.113 g 1 eq.) was suspended in water (1 mL) and heated for 10 minutes at 250 ^{o}C and 150 psi, using a CEM Discover microwave apparatus at 250 W. The resulting suspension was filtered through a Hirsch funnel and washed with water (5 mL); the solid was dried under high vacuum and analyzed without further purification by NMR. The aqueous layer was dissolved in deuterated MeOH**,** the proton NMR was consistent with proton spec reported in J. Braz. Chem. Soc. Vol. 16, No. 6B, 1448-1453, 2005. The carbon showed signature peaks of the amide carbons at 165.3, and 170.8 ppm, for carbon 1 and 7 respectively. What appear to be Fmoc fragments were attempted to be removed by washing with hexane.

Synthesis of 2-isoprenyltryptophant-butyl ester fmoc proline

Scheme 86. Peptide coupling of isoprenyl tryptophan and proline

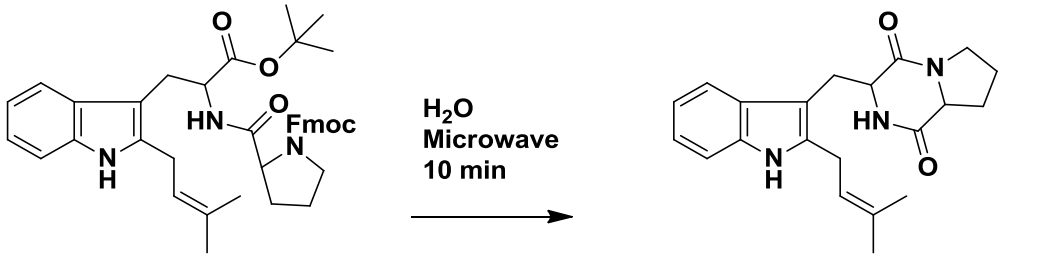


5-21-13 Procedure adapted from Org. Lett. Vol. 15 No. 1, 2013 22-25. Paper uses HATU as peptide coupling reagent I choose to use PyBOP because we had it as a peptide coupling reagent. PyBOB (0.642 g 1.24 mmol 1.36 eq) and i-Pr_2NEt (0.270 g 0.361 mL 2.07 mmol Density 0.742 g/mL 2.27 eq) were added to a solution of proline (0.410 g 1.21 mmol 1.33 eq) and 2-isoprenyl tryptophan t-butyl ester (0.30 g 0.913 mmol 1 eq) in CH_3CN (9 mL) and the reaction was stirred at room temperature for 4 hours. The reaction was concentrated under reduced pressure, and the residue partitioned between CH_2Cl_2 (30 mL) and 1 M HCl (30 mL). The layers were separated, and the aqueous phase was extracted with CH_2Cl_2 (2 x 7.5 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The

residue was purified by flash chromatography eluting with 1:1 EtOAc:Hexane to give 0.39 g 66 % yield of dipeptide as yellow oil. Dipeptide is the spot at about 0.5 Rf in 1:1 Hex:EtOAc.

Synthesis of Tryprostatin B

Scheme 87. Attempted synthesis of tryprostatin B



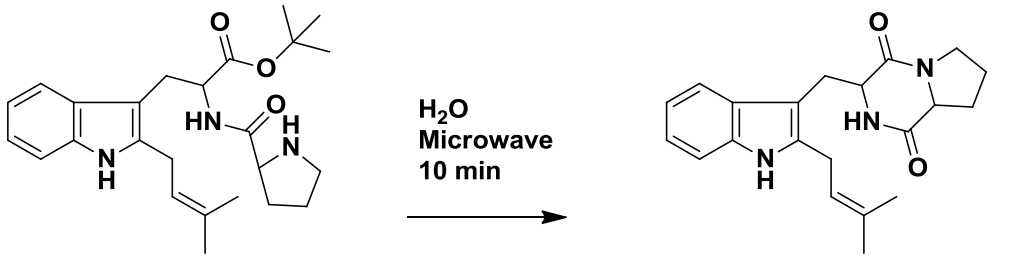
Chemical Formula: $C_{40}H_{45}N_3O_5$
Exact Mass: 647.34
Molecular Weight: 647.80

Chemical Formula: $C_{21}H_{25}N_3O_2$
Exact Mass: 351.19
Molecular Weight: 351.44

Reaction was modeled after Molecules 2009, 14, 2836-2849. 2-isoprenyltryptophanproline fmoc t-butyl ester (0.162 g 0.25 mmol 1 eq.) was suspended in water (1 mL) and heated for 10 minutes at 250 ^{o}C and 150 psi, using a CEM Discover microwave apparatus at 250 W. The resulting suspension was filtered through a Hirsch funnel and washed with water (5 mL); the solid was dried under high vacuum and analyzed without further purification by NMR. This material was purified by column chromatography material that was recovered did not look like Tryprostatin it looked more like the dipeptide with no t- butyl group.

Synthesis of Tryprostatin B

Scheme 88. Synthesis of tryprostatin B



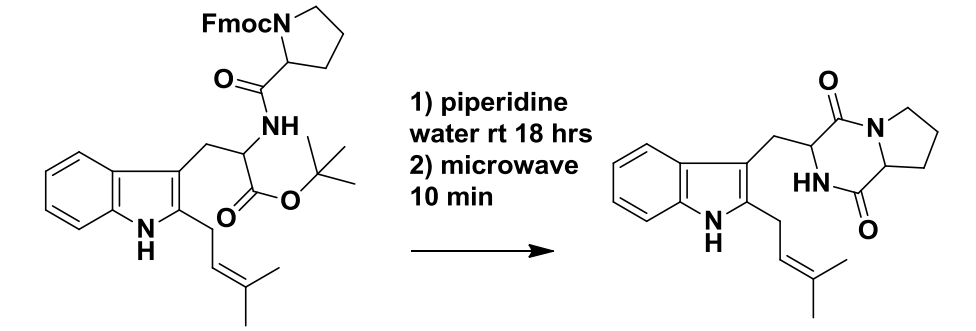
Chemical Formula: $C_{25}H_{35}N_3O_3$
Exact Mass: 425.27
Molecular Weight: 425.56

Chemical Formula: $C_{21}H_{25}N_3O_2$
Exact Mass: 351.19
Molecular Weight: 351.44

5-24-13 Reaction was modeled after Molecules 2009, 14, 2836-2849. 2-isoprenyltryptophanproline t-butyl ester (0.097 g 0.27 mmol 1 eq.) was suspended in water (1 mL) and heated for 10 minutes at 250 ^{o}C and 150 psi, using a CEM Discover microwave apparatus at 250 W. The resulting suspension was filtered through a Hirsch funnel and washed with water (5 mL); the solid was dried under high vacuum and analyzed without further purification by NMR. This material contained no t-butyl peak at 1.3 ppm in the proton and also contained 169.5 and 165.8 in the carbon NMR which is very close to the reported values of the amides for Tryprostatin B. The starting material for this reaction has diasteriomeric esters and amides which come at 175.1 174.8 and 171.7 171.3 respectively. This material was dry loaded onto a column and a gradient column was run on it using methanol:dichloromethane solutions from 0:100 to 15:85. Material eluted with about 2% methanol:dichloromethane. Theoretical yield 0.08016g. Diastereomers isolated 0.05/0.08 = 62.5% ca. 63% of desired products Tryprostatin B (0.01 g) 13 % yield diastereomers of Tryprostatin B (0.04 g) in 38% yield.

Synthesis of Tryprostatin B

Scheme 89. Attmepted synthesis of tryprostatin B



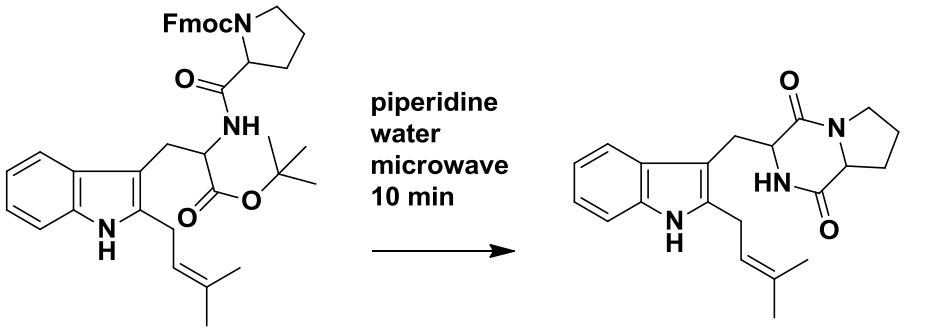
Chemical Formula: $C_{40}H_{45}N_3O_5$
Exact Mass: 647.34
Molecular Weight: 647.80

Chemical Formula: $C_{21}H_{25}N_3O_2$
Exact Mass: 351.19
Molecular Weight: 351.44

Reaction was modeled after Molecules 2009, 14, 2836-2849 and V.L. Campo et al. Tetrahedron 65 (2009) 5343-5349. 2-isoprenyltryptophanproline fmoc t-butyl ester (0.162 g 0.25 mmol 1 eq.) was suspended in water (1 mL) and piperidine (0.25 mL) this was let stir at room temp for 18 hours. This material was then heated for 10 minutes at 250 ^{o}C and 150 psi, using a CEM Discover microwave apparatus at 250 W. The resulting suspension was filtered through a Hirsch funnel and washed with water (5 mL); the solid was dried under high vacuum and analyzed without further purification by NMR. This material was purified by column chromatography material that was recovered did not look like Tryprostatin it looked more like the dipeptide with not butyl group.

Synthesis of Tryprostatin B

Scheme 90. Attempted synthesis of tryprostatin B



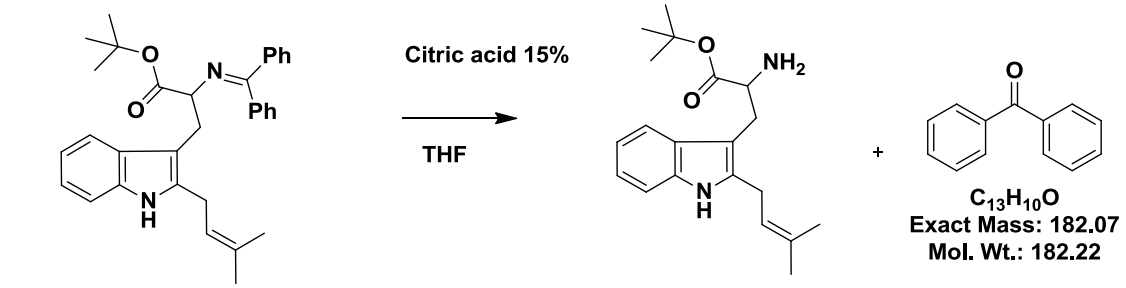
Chemical Formula: $C_{40}H_{45}N_3O_5$
Exact Mass: 647.34
Molecular Weight: 647.80

Chemical Formula: $C_{21}H_{25}N_3O_2$
Exact Mass: 351.19
Molecular Weight: 351.44

Reaction was modeled after Molecules 2009, 14, 2836-2849 and V.L. Campo et al. Tetrahedron 65 (2009) 5343-5349. 2-isoprenyltryptophanproline fmoc t-butyl ester (0.162 g 0.25 mmol 1 eq.) was suspended in water (1 mL) and piperidine (0.25 mL). This material was then heated for 10 minutes at 250 ^{o}C and 150 psi, using a CEM Discover microwave apparatus at 250 W. The resulting suspension was filtered through a Hirsch funnel and washed with water (5 mL); the solid was dried under high vacuum and analyzed without further purification by NMR. This material was purified by column chromatography material that was recovered did not look like Tryprostatin it looked more like the dipeptide with no t-butyl group.

Synthesis of 2-isoprenyl tryptophan t–butyl ester amine

Scheme 91. Synthesis of tryptophan amine



Chemical Formula: $C_{33}H_{36}N_2O_2$
Exact Mass: 492.28
Molecular Weight: 492.65

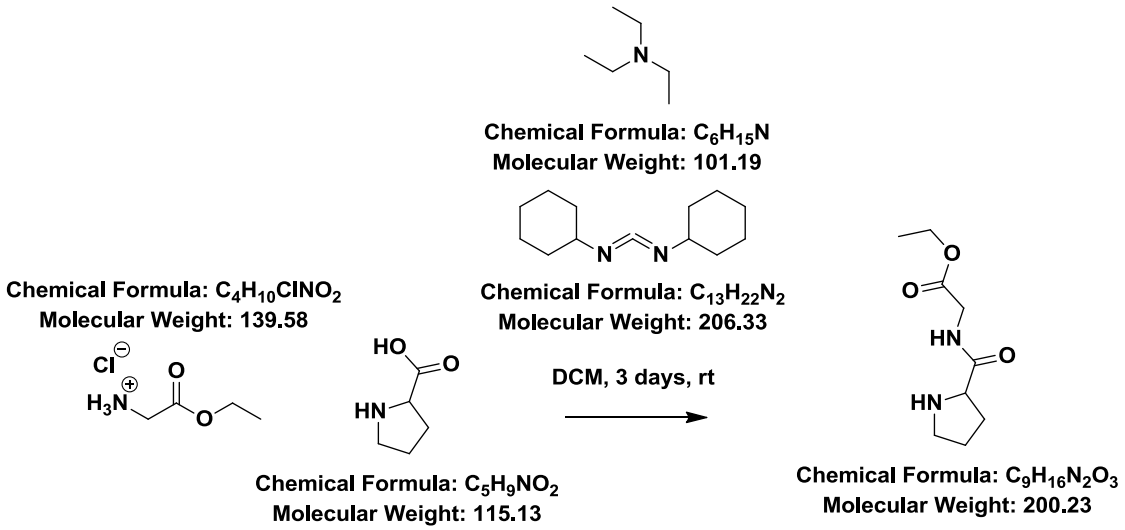
Chemical Formula: $C_{20}H_{28}N_2O_2$
Exact Mass: 328.22
Molecular Weight: 328.45

$C_{13}H_{10}O$
Exact Mass: 182.07
Mol. Wt.: 182.22

Procedure adapted from Tetrahedron Letters 43 (2002) 6677-6679. 2-isoprenyltryptophan-t-butyl ester diphenyl imine (g mmol 1 eq.) was dissolved in (mL) of tetrahydrofuran and (mL) of 15% aqueous citric acid. Reaction mixture was stirred vigorously at room temperature for 18 h, and then diluted with 1 M hydrochloric acid (mL). The mixture is extracted with diethyl ether (2x mL) to remove the benzophenone, then the aqueous layer was basified (K_2CO_3) until no more K_2CO_3 would dissolve. Extraction with chloroform (5x mL) followed by drying of the extracts (Na_2SO_4) and concentration under reduced pressure gives the crude amino acid tert-butyl ester which can generally be purified by passing through a plug of silica. Amine looked clean. 97 % yield 0.6 g theoretical was 0.62 g.

Synthesis of ethyl ester glycine proline

Scheme 92. Attmpted peptide coupling with DCC



7-12-13 Procedure adapted from Eur. J. Org. Chem. 2009, 5717. 32 times scale. DIC we did not have so we substituted DCC (1.97 g 9.55 mmol 1.1 eq.) and triethylamine (0.879 g 0.726 g/mL 1.211 mL 1 eq.) and glycine ethyl ester hydrochloride (1.21 g 8.69 mmol 1 eq.) in DCM (50 mL) were successively added at room temperature to a stirred solution of L-Proline (1.0 g 8.69 mmol 1 eq.) in DCM (100 mL). Reaction mixture was stirred for 3 days and then diluted with DCM (mL) and HCl (0.1 N mL) the layers were separated the aqueous phase was extracted with DCM (3x mL) and the combined chlorinated extracts were washed with water, dried with $MgSO_4$, filtered and concentrated under reduced pressure. Crude residue was purified by flash chromatography hexane:EtOAc 80:20.

N+ salt to amine

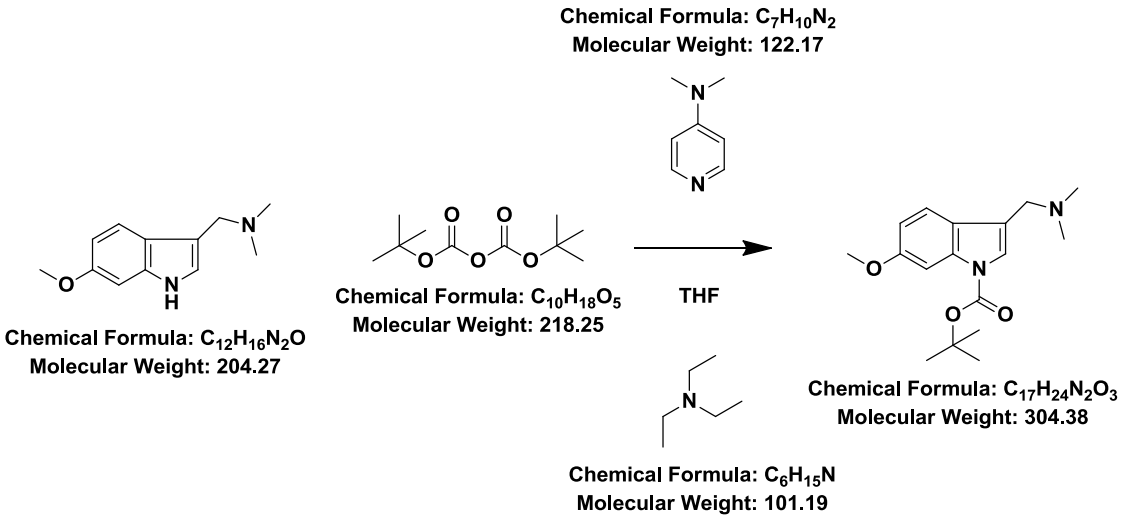
Procedure to attempt skipping the isolation step of the 2isopropyltryptopahn Schiff base was tried using HCl and 15% citric acid. Phase transfer catalyst reaction was done on a 2.0 g scale. Crude reaction material weighed 2.75 g, about 10% of the material was used for each of these screenings. Theoretically this should yield about 0.2 of material.

Reaction 1) Followed procedure for Tett Lett 43 (2002) 6677-6679. 0.275 g crude reaction material from the N+ salt, PTC, Schiff base, 45% KOH reaction. Material was dissolved in THF (2 mL) and (0.75 mL) of 15% citric acid, reaction vessel was allowed to stir for 18 hours. Then it was diluted with 1 M HCl (0.5 mL). Mixture was extracted with diethyl ether (2 x 1.5 mL) to remove benzophenone then basified with K_2CO_3. This was extracted with dichloromethane (5 x 1.5 mL) and then dried over Na_2SO_4 and concentrated via rotovap. NMR of material did not show any tryptophan amine.

Reaction 2) Reaction was modeled after Org. Lett. 2010 vol 12 No. 8 pg. 1688. 0.275 g crude material reaction material from the N+ salt, PTC, Schiff base, 45% KOH reaction. Material was dissolved in THF (0.5 mL) and 1 N HCl (0.5 mL) at 0° C. After the reaction was stirred for 4 hours the THF was removed under reduced pressure. The resulting aqueous layer was washed with ether (3 x 5 mL) and neutralized with $NaHCO_3$. Mixture was then extracted with dichloromethane (3x 5 mL) organic layers dried over anhydrous $MgSO_4$. The NMR of this material indicated that the product was there, but contained impurities mass of this material was 0.10 g. This was attempted to be purified by running through a pipet column, mobile phase 9:1 DCM:MeOH.

Synthesis of 6-Methoxybocgramine

Scheme 93. Synthesis of 6-methoxygramine

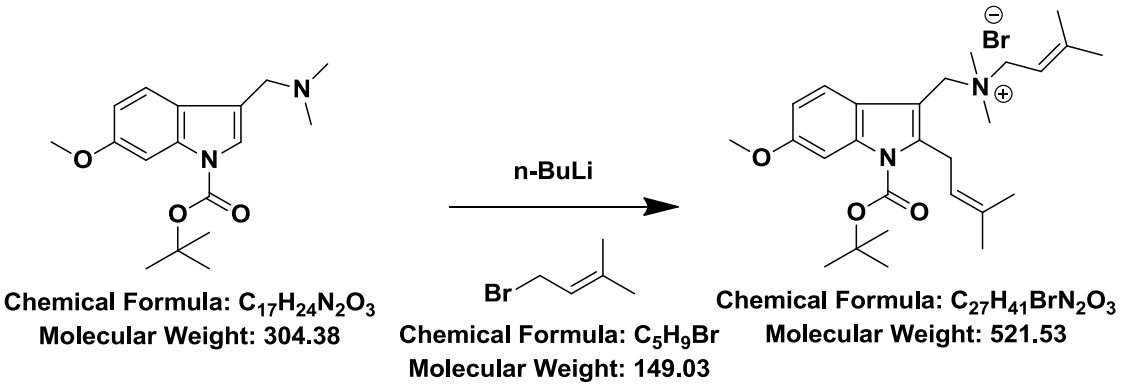


9-3-13 All glassware was oven dried for 4 hours previous to use. A solution of 6-methoxygramine (0.37 g 1.8 mmol 1 eq) was made in an addition funnel using THF (9 mL). This addition funnel was put in top of a 100 mL three neck round bottom flask which was placed in an ice water cooling bath and added dropwise to a solution of ditert-butyldicarbonate (0.47 g 2.2 mmol 1.2 eq) 4-Dimethylaminopyridine (22 mg 0.18 mmol 0.1 eq) and triethylamine (0.30 mL 0.25 mmol 0.12 eq 0.726 g/mL) in THF (5 mL). About half way through the addition the reaction mixture changed from clear to cloudy. After stirring for one and a half hours the reaction water was added to the reaction mixture. Solvent was removed via roto vap and the material was extracted with ether three times. The extract was washed with brine and dried over sodium sulfate. The mixture was run through a cotton plugged funnel to remove sodium sulfate and the solvent was removed via roto vap. 9-3-13 80% yield.

12-12-13 All glassware was oven dried for 4 hours previous to use. A solution of 6-methoxygramine (2.27 g 11.1 mmol 1 eq) was made in an addition funnel using THF (55 mL). This addition funnel was put in top of a 500 mL three neck round bottom flask which was placed in an ice water cooling bath and added dropwise to a solution of ditert-butyldicarbonate (2.91 g 13.3 mmol 1.2 eq) 4-Dimethylaminopyridine (0.136 g 11.1 mmol 0.1 eq) and triethylamine (0.135 g 0.186 mL 1.33 mmol 0.12 eq 0.726 g/mL) in THF (30 mL). About half way through the addition the reaction mixture changed from clear to cloudy. After stirring for 16 hours water (25 mL) was added to the reaction mixture. Solvent was removed via roto vap and the material was extracted with ether two times. The extract was washed with brine and dried over sodium sulfate. The mixture was run through a cotton plugged funnel to remove sodium sulfate and the solvent was removed via roto vap. 12-12-13 3.28 g/3.38 g = 97% yield.

Synthesis of N+ isoprenyl-6-methoxy-2-isoprenylbocgramine

Scheme 94. Synthesis on diisoprenyl 6-methoxygramine salt



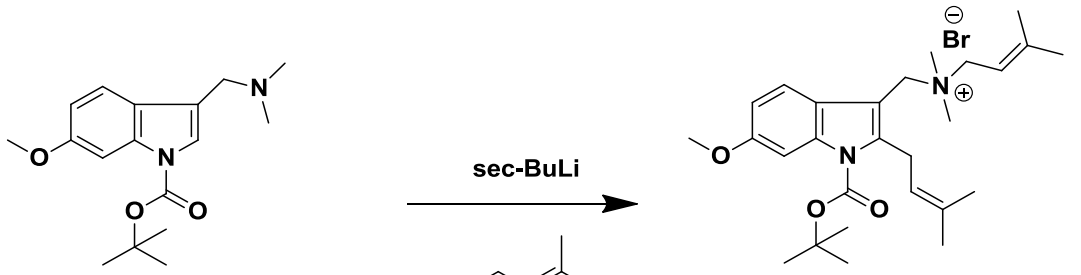
6-methoxy boc gramine (0.20 g 0.657mmol 1 eq.) was weighed out. A 100 mL 3 neck flask was oven dried for 3 hours with a stir bar inside. It was removed from the oven and clamped. On two necks rubber septums were inserted, in the other a nitrogen inlet under oil bubbler was inserted boc gramine was charged to the flask using a powder funnel, under positive nitrogen flow. To the reaction vessel blue distilled THF (9 mL) was charged. The reaction mixture was allowed to stir for one hour to insure that all the starting material was dissolved in the solution. The color of the mixture at this point is clear with a brown tint. Reaction vessel was cooled in a dry ice acetone bath until the reaction mixture was at -70˚C. n-Butyl Lithium (~~1.05~~ 0.5256 mL 2.5 M 1.314 mmol 2.0 eq) was added dropwise over an hour maintaining a temperature range between -65 and -70˚C to the reaction mixture leaving the reaction mixture a bright red orange. After the addition of n-Butyl Lithium the reaction was let stir undisturbed for one and a half hours at -70˚C. Isoprenyl bromide (0.314 mL 0.399 g 2.6 mmol 4 eq. 1.27 g/mL) was added dropwise to the reaction vessel. At this point the reaction mixture was orange. The reaction was then let warm to room temp overnight. When returning the next day the color of the reaction mixture was clear orange. Deionized water (5 mL) was added to the reaction mixture, no reaction from this indicated that the b-butyl lithium was quenched. The solvent was removed using the rotovap. After the solvent was removed the water and residue was poured into a separatory funnel and extracted with dichloromethane three times (10 mL). Organic layer was dried over sodium sulfate. Solvent was removed via rotovap and oil pump. NMR was taken to see if material was present. Material was purified with a pipet column using neutral alumina gel as stationary phase and DCM as mobile phase. 0.15 g of product was isolated in 40 % yield.

Scale up

4-24-14 6-Methoxybocgramine (2.24 g 7.36 mmol 1 eq.) weighted out. A 500 mL three neck round bottom flask was oven dried over night with stir bar inside was removed from oven and clamped. To this vessel 6-Methoxybocgramine was charged via a powder funnel. Nitrogen inlet, oil bubbler, and rubber septum were used to fill necks in round bottom flask. Tetrahydrofuran 100 mL (dry blue distilled) was used to solvate the material. This was let stir for thirty minutes. Reaction vessel was submerged in an acetone/dry ice bath for one half to an hour until temperature was constant. To the reaction mixture n–butyllitium (5.9 mL 2.5 M 14.75 mmol 2.00 eq.) was added in a drop wise fashion using a syringe through the septum. Reaction mixture was orange in color. Reaction was let stir for one hour. Isoprenyl Bromide (3.45 mL 4.38 g 29.40 mmol 4.00 eq 1.27 g/mL) was added to the reaction mixture in a drop wise fashion. Reaction mixture was yellow/orange in color and was left to stir overnight for 16 hours. Upon returning the next day reaction mixture was orange in color. Reaction mixture was poured into a single neck round bottom flask and solvent was removed via roto vap and oil pump. Column diameter was 6 cm x 9 cm tall. Column was run on the material by slurry loading the silica gel on to the column and dry loading the crude sample. Column was run using 9:1 Dichloromethane:Methanol mobile phase void volume collected in beakers and fractions were collected in test tubes until they were half way full. Fractions 2-5 mass 0.61 g, Frac 6-8 1.02 g, Frac 9-10 0.86 g, and Frac 11-12 0.83 g. Totaling 3.32 g actual yield / 3.84 g theoretical yield = 86% yield.

Synthesis of N+ isoprenyl-6-methoxy-2-isoprenylbocgramine

Scheme 95. Attempted synthesis using sec-BuLi



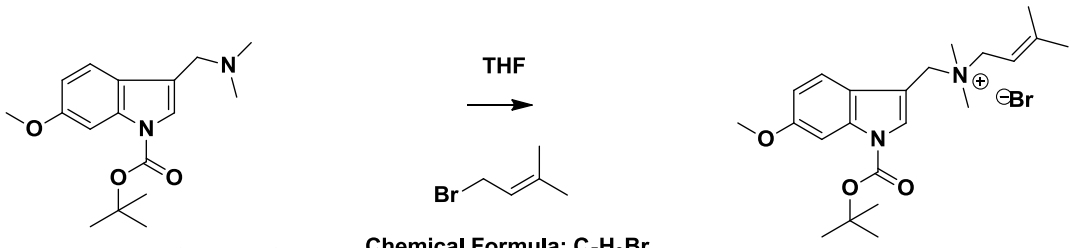
Chemical Formula: $C_{17}H_{24}N_2O_3$
Molecular Weight: 304.38

Chemical Formula: $C_{27}H_{41}BrN_2O_3$
Molecular Weight: 521.53

6-methoxy boc gramine (0.20 g 0.657mmol 1 eq.) was weighed out. A 100 mL 3 neck flask was oven dried for 3 hours with a stir bar inside. It was removed from the oven and clamped. On two necks rubber septums were inserted, in the other a nitrogen inlet under oil bubbler was inserted boc gramine was charged to the flask using a powder funnel, under positive nitrogen flow. To the reaction vessel blue distilled THF (9 mL) was charged. The reaction mixture was allowed to stir for one hour to insure that all the starting material was dissolved in the solution. The color of the mixture at this point is clear with a brown tint. Reaction vessel was cooled in a dry ice acetone bath until the reaction mixture was at -70° C. Sec Butyl Lithium (~~4.26~~ 0.938 mL 1.4 M 1.314 mmol 2.0 eq.) was added dropwise to the reaction mixture was orange and clear. After one hour of stirring isoprynyl bromide (0.3084 mL 0.3917 g 2.6284 mmol 4.0 eq) was added to the reaction mixture in a dropwise fashion this gives off a white gas reaction mixture at this point is light yellow. This reaction mixture was left to warm overnight. Upon returning the next day the reaction color was red orange brown and clear. Reaction was let stir overnight. Upon returning in the morning water was added (5 mL) the reaction was put on the rotovap to remove the THF. Organic layer was extracted with DCM (3 x 10 mL) dried over sodium sulfate filtered using cotton plug and concentrated via roto vap, NMR indicated that the single isoprenylation salt was found.

Isoprenylated boc gramine salt

Scheme 96. Single isoprenylation



Chemical Formula: $C_{17}H_{24}N_2O_3$
Molecular Weight: 304.38

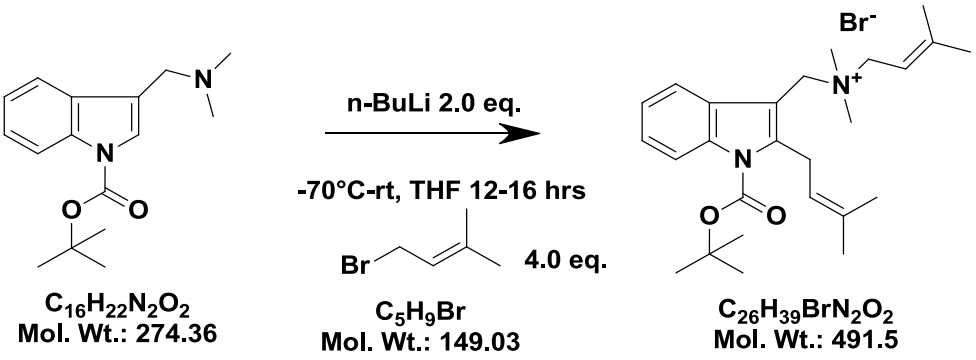
Chemical Formula: C_5H_9Br
Molecular Weight: 149.03

Chemical Formula: $C_{22}H_{33}BrN_2O_3$
Molecular Weight: 453.41

6-methoxy boc gramine (0.20 g 0.657mmol 1 eq.) was weighed out. A 100 mL 3 neck flask was oven dried for 3 hours with a stir bar inside. It was removed from the oven and clamped. On two necks rubber septums were inserted, in the other a nitrogen inlet under oil bubbler was inserted boc gramine was charged to the flask using a powder funnel, under positive nitrogen flow. To the reaction vessel blue distilled THF (9 mL) was charged. The reaction mixture was allowed to stir for one hour to insure that all the starting material was dissolved in the solution. The color of the mixture at this point is clear. After stirring for one hour isoprenyl bromide (0.3084 mL, 0.3917 g 2.6284 mmol 4 eq.) was added in a dropwise fashion. Reaction was let stir overnight.

Synthesis of diisoprenylated boc gramine

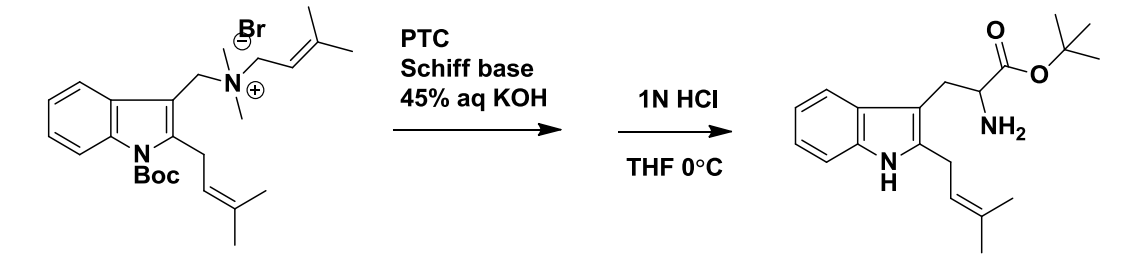
Scheme 97. Synthesis of diisoprenyl quaternary ammonium salt



Reaction is being done to test if this reaction is not working because of the electronics of the 6meogramine or if it is the procedure. Boc gramine (0.20 g 0.7290 mmol 1 eq) was weighed out. A 100 mL 3 neck flask was oven dried for 3 hours with a stir bar inside. It was removed from the oven and clamped. On two necks rubber septums were inserted, in the other a nitrogen inlet under oil bubbler was inserted boc gramine was charged to the flask using a powder funnel, under positive nitrogen flow. To the reaction vessel blue distilled THF (9 mL) was charged. The reaction mixture was allowed to stir for one hour to insure that all the starting material was dissolved in the solution. The color of the mixture at this point is light yellow and clear. Reaction vessel was cooled in a dry ice acetone bath until the reaction mixture was at -70˚C. n-Butyl Lithium (0.583 mL 2.5 M 1.458 mmol 2.0 eq) was added dropwise over an hour maintaining a temperature range between -65 and -70˚C to the reaction mixture leaving the reaction mixture a bright red orange. After the addition of n-Butyl Lithium the reaction was let stir undisturbed for one and a half hours at -70˚C. Isoprenyl bromide (0.314 mL 0.399 g 2.6 mmol 4 eq. 1.27g/mL) was added dropwise to the reaction vessel.

Synthesis of 2-IsoprenylN-amine t-butylestertryptophan

Scheme 98. PTC and deprotection steps combined



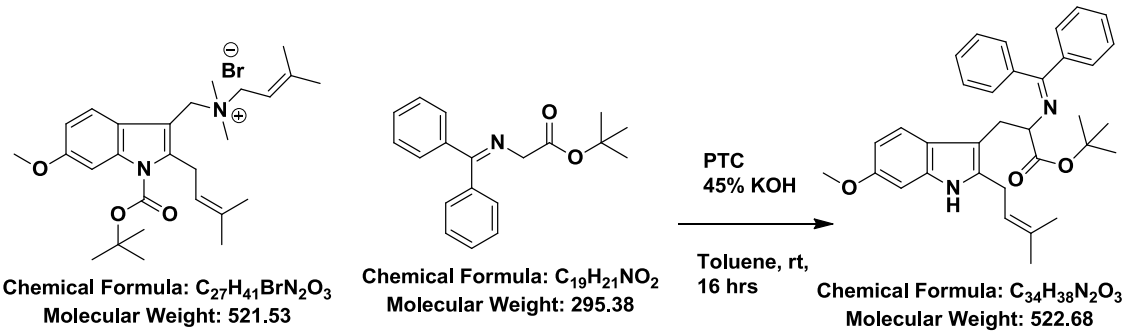
Chemical Formula: $C_{26}H_{39}BrN_2O_2$
Molecular Weight: 491.50

Chemical Formula: $C_{20}H_{28}N_2O_2$
Molecular Weight: 328.45

Procedure adapted from Organic Letters 2010 vol. 12, No. 8 1688-1691. Crude reaction mixture starting with N-isoprenyl-2-isoprenylboc gramine (100.0 mg 0.203 mmol) was dissolved in THF (2 mL) and 1 N HCl (2 mL) was added at 0 ° C. After stirred for 4 hours, THF was removed under reduced pressure. The resulting aqueous solution was washed with ether (3 x 20 mL) and neutralized with $NaHCO_3$. The mixture was then extracted with CH_2CCl_2 (3 x 20 mL). The organic layers were combined and dried over anhydrous Na_2SO_4. After filtration and concentration under reduced pressure, the product was obtained after purification by flash column chromatography using gradient mixtures of CH_2CCl_2/MeOH (98:2 95:5 90:10) as the eluent.

Synthesis of 6-MeO-2-isoprenyl t-buesterdiphenyl amine tryptophan

Scheme 99. Synthesis of 6-MeOtryptophan



Chemical Formula: $C_{27}H_{41}BrN_2O_3$
Molecular Weight: 521.53

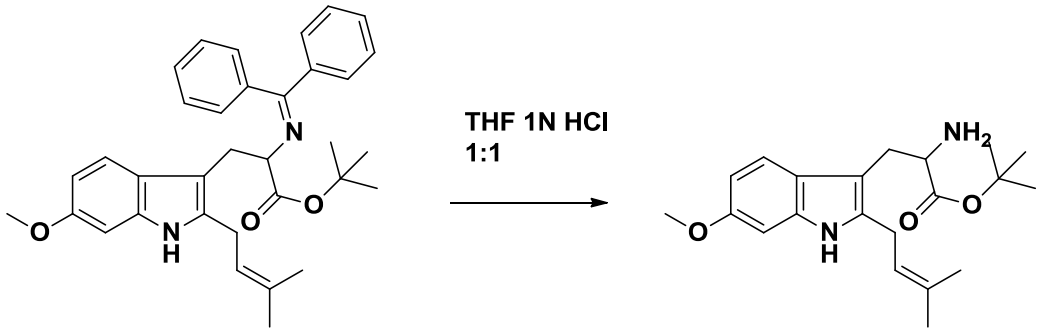
Chemical Formula: $C_{19}H_{21}NO_2$
Molecular Weight: 295.38

Chemical Formula: $C_{34}H_{38}N_2O_3$
Molecular Weight: 522.68

6-MeON+ salt (0.1 g 0.192 mmol 1 eq.) was added to a 7.5 mL vial with a mini stir bar. To this (0.07 g 0.237 mmol 1.23 eq) of Schiff base and (0.03 g 0.04954 mmol 0.2580 eq) of phase transfer catalyst was added. To the reaction mixture 2 mL of toluene was added and reaction mixture was let stir 30 min. 1 mL of 45% KOH was added to the reaction vessel. Crude reaction mixture was run through a short neutral alumina plug using 3:1 Hexane:Ethyl Acetate and gradient to higher polarity. Percent conversion was monitored via proton NMR of organic layer by comparing the integration of the multipet at 4.2 and the singlet at 4.1.

Synthesis of 6-Methoxy-2-isoprenyl-t-butylestertryptophanamine

Scheme 100. Attempted deprotection of benzophenone imine



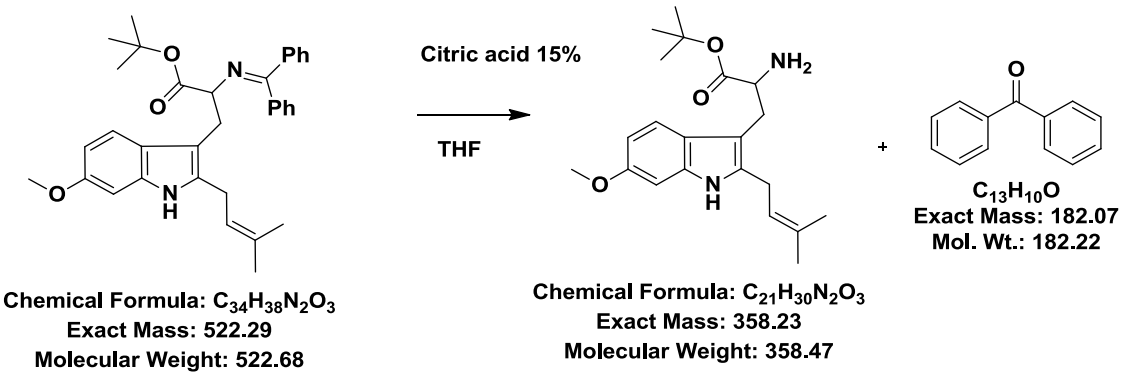
Chemical Formula: $C_{34}H_{38}N_2O_3$
Molecular Weight: 522.68

Chemical Formula: $C_{21}H_{30}N_2O_3$
Molecular Weight: 358.47

Procedure adapted from Organic Letters 2010 vol. 12, No. 8 1688-1691 (S-13). 6-Methoxy-2-isoprenyl-t-butylestertryptophandiphenylmethylene (0.03 g 0.00574 mmol 1 eq) was dissolved in 1 mL THF along with 1 mL of 1 N HCl solution. Reaction mixture was stirred at 0°C for 4 hours. THF was rotovaped off. Reaction mixture was washed with hexane three times; this layer was dried over Na_2SO_4 and rotovaped to dryness. NMR indicates this is benzophenone. Aqueous layer was basified using sodium bicarbonate, until adding solid gave no more bubbles. Aqueous layer was extracted with dichloromethane three times. Dichloromethane layer was dried over Na_2SO_4 and rotovaped to dryness.

Synthesis of 6Methoxy-2-isoprenyltryptophan t–butyl ester amine

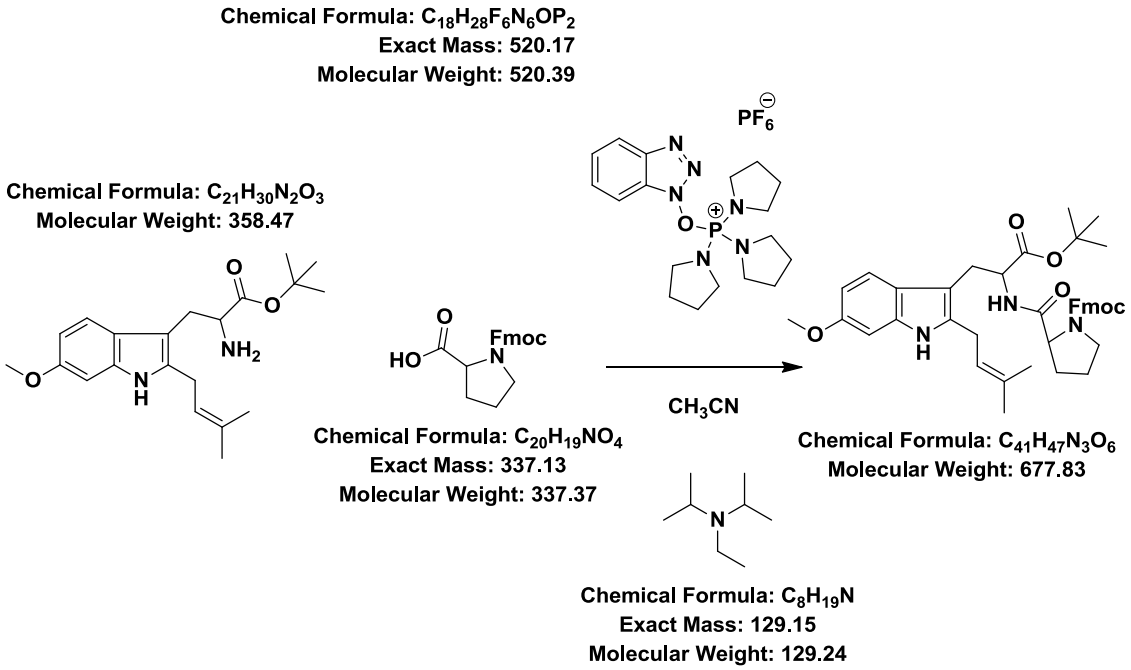
Scheme 101. Deprotection of benzophenone imine using citric acid



5-19-14 Procedure adapted from Tetrahedron Letters Vol 43 Iss 37(2002) 6677-6679. 6-Methoxy-2-isoprenyltryptophan-t-butyl ester diphenyl imine partially purified most of which is benzophenone (1.22 g mmol 1 eq.) was dissolved in tetrahydrofuran (4 mL) and of 15% aqueous citric acid (1.5 mL) added. Reaction mixture was stirred vigorously at room temperature for 18 h, and then diluted with 1 M hydrochloric acid (1 mL). The mixture is extracted with diethyl ether (3 x 5 mL) to remove the benzophenone, then the aqueous layer was basified (K_2CO_3) until no more K_2CO_3 would dissolve. Extraction with dichloromethane (5 x 5 mL) followed by drying of the extracts (Na_2SO_4) and concentration under reduced pressure gives the crude amino acid tert-butyl ester which can generally be purified by passing through a plug of silica or alumina. Gradient column was run using Hexane:Ethyl acetate 9:1, 7:3, 1:1 Dichloromethane, Dichloromethane:Methanol 9:1. Appeared to come out with 1:1 Hex:EtOAc or DCM, but make sure to check earlier fractions.

Synthesis of 6Methoxy-2-isoprenyltbutylestertryptophanfmocprolinedipeptide

Scheme 102. Peptide coupling of 6MeOtryptophan and proline

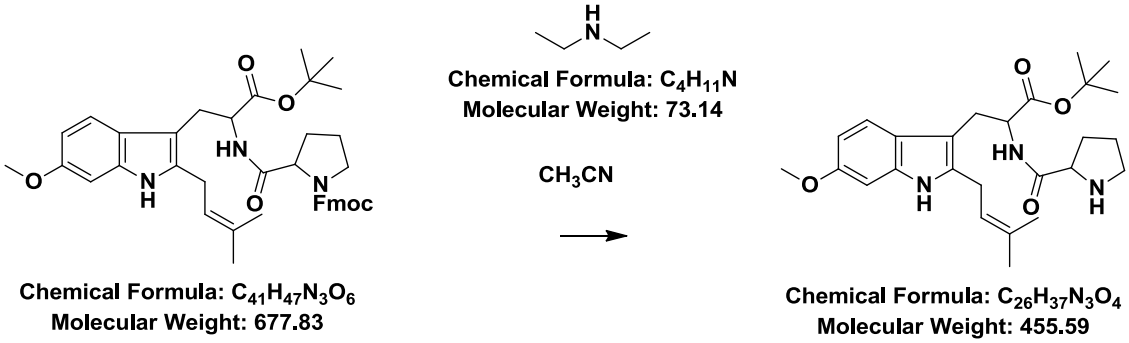


7-2-14 Procedure adapted from Org. Lett. Vol. 15 No. 1, 2013 22-25 Supporting docs S20 Dipeptide 13. Paper uses HATU as peptide coupling reagent I choose to use PyBOP because we had it as a peptide coupling reagent. PyBOB (0.197 g 0.379 mmol 1.36 eq) and i-Pr_2NEt (0.0818 g 0.110 mL 0.633 mmol Density 0.742 g/mL 2.27 eq) were added to a solution of Fmoc proline (0.125 g 0.371 mmol 1.33 eq) and 6-Methoxy2-isoprenyl tryptophan t-butyl ester amine (0.1 g 0.279 mmol 1 eq) in CH_3CN (3 mL) and the reaction was stirred at room temperature for 4 hours. The reaction was concentrated under reduced pressure, and the residue partitioned between CH_2Cl_2 (10 mL) and 1 M HCl (10 mL). The layers were separated, and the aqueous phase was extracted with CH_2Cl_2 (2 x 5 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography eluting

with 1:1 EtOAc:Hexane to give 0.39 g 66 % yield of dipeptide as yellow oil. Dipeptide is the spot at about 0.5 Rf in 1:1 Hex:EtOAc.

Synthesis of 6-Methoxy-2-isoprenyltryptophanprolinetbutylester

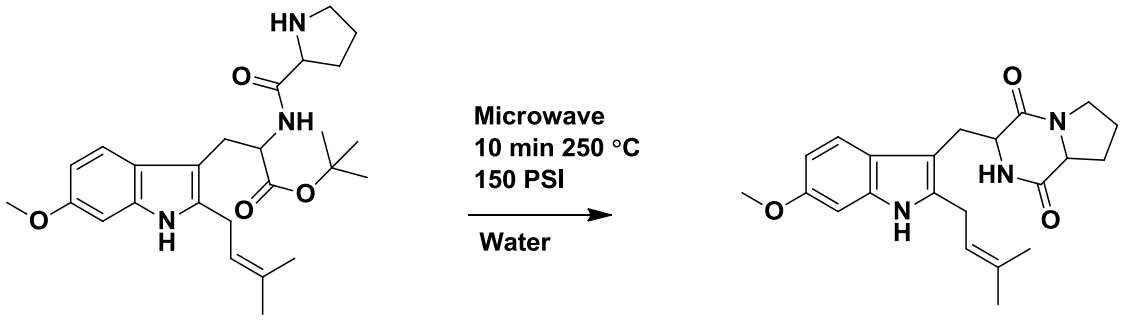
Scheme 103. Deprotection of Fmoc



7-7-14Following H.D Jai *et al.* Bioorg. Med. Chem. 16 (2008) 4626-4651. Crude reaction mixture was dissolved in acetonitrile (3 ml) and to this diethylamine (3 mL). The reaction mixture was stirred overnight. The next day the mixture was TLCed and roto vaped to dryness.

Synthesis of Tryprostatin A

Scheme 104. Synthesis of tryprostatin A



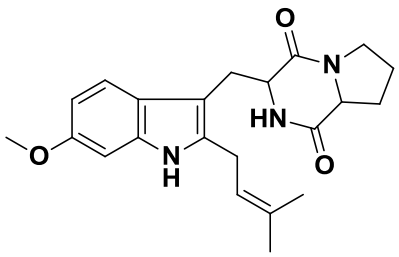
Chemical Formula: $C_{26}H_{37}N_3O_4$
Molecular Weight: 455.59

Chemical Formula: $C_{22}H_{27}N_3O_3$
Molecular Weight: 381.47

Procedure was modeled after a similar procedure in Molecules 2009, 14, 2836-2849. Material was transferred from previous vessel and evaporated to dryness using roto vap in microwave reaction vessel. Water (1 mL) was then added to this reaction vessel, at this point the material made an orange milky suspension. Microwave maximums were set to 250 ˚C and 150 PSI. Actual values were about 195 ˚C and 140 PSI. After the reaction was complete the material was a dark brown oil. This was submitted for mass spec to determine if product was present. It may have been in a very small amount. Mass spec provided evidence that tryprostatin A was present.

7. DATA

Tryprostatain A



Chemical Formula: $C_{22}H_{27}N_3O_3$
Molecular Weight: 381.47

==== Shimadzu LabSolutions Data Report ====

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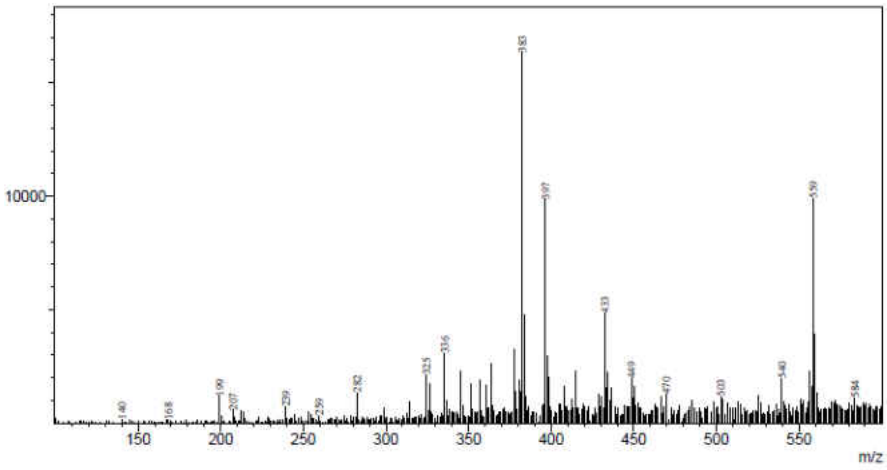
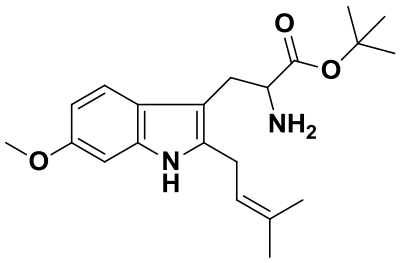


6-Methoxy-2-isoprenylt-butylestertryptophanamine



Chemical Formula: $C_{21}H_{30}N_2O_3$
Molecular Weight: 358.47

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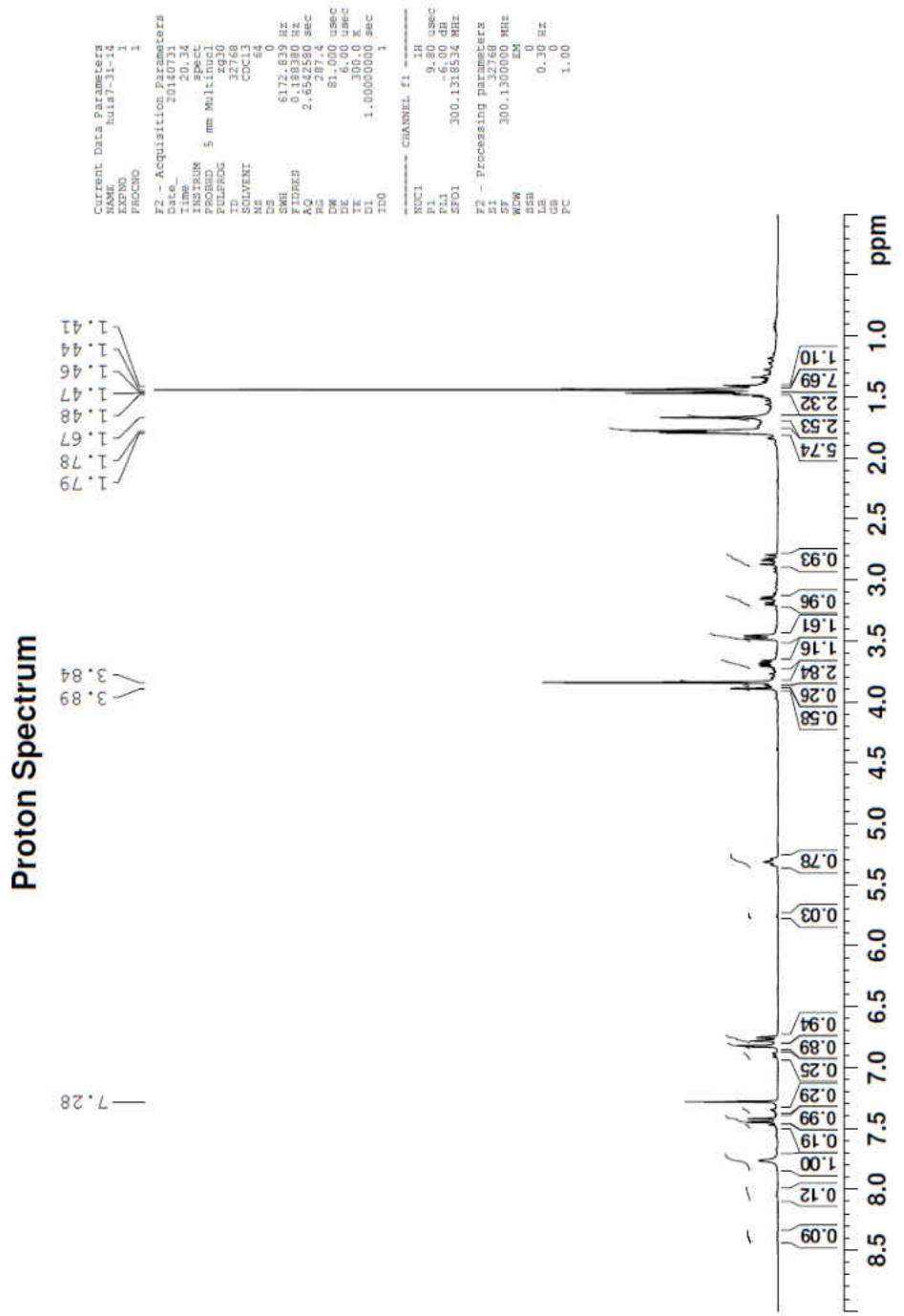
^{13}C Name: huis7-31-14 Expno: 3 Procno: 1

HSQC Name: huis7-31-14 Expno: 2 Procno: 1

HRMS Name: Huis7-31-14 Date: 8-5-2014 Time 11:31:00 PM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PROCNO 1
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Time 20.34
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FIDRES 0.188380 Hz
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RG 287.4
DW 81.000 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec
TD0 1
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P1 9.80 usec
PL1 -6.00 dB
SFO1 300.1318534 MHz
F2 - Processing parameters
SI 32768
SF 300.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
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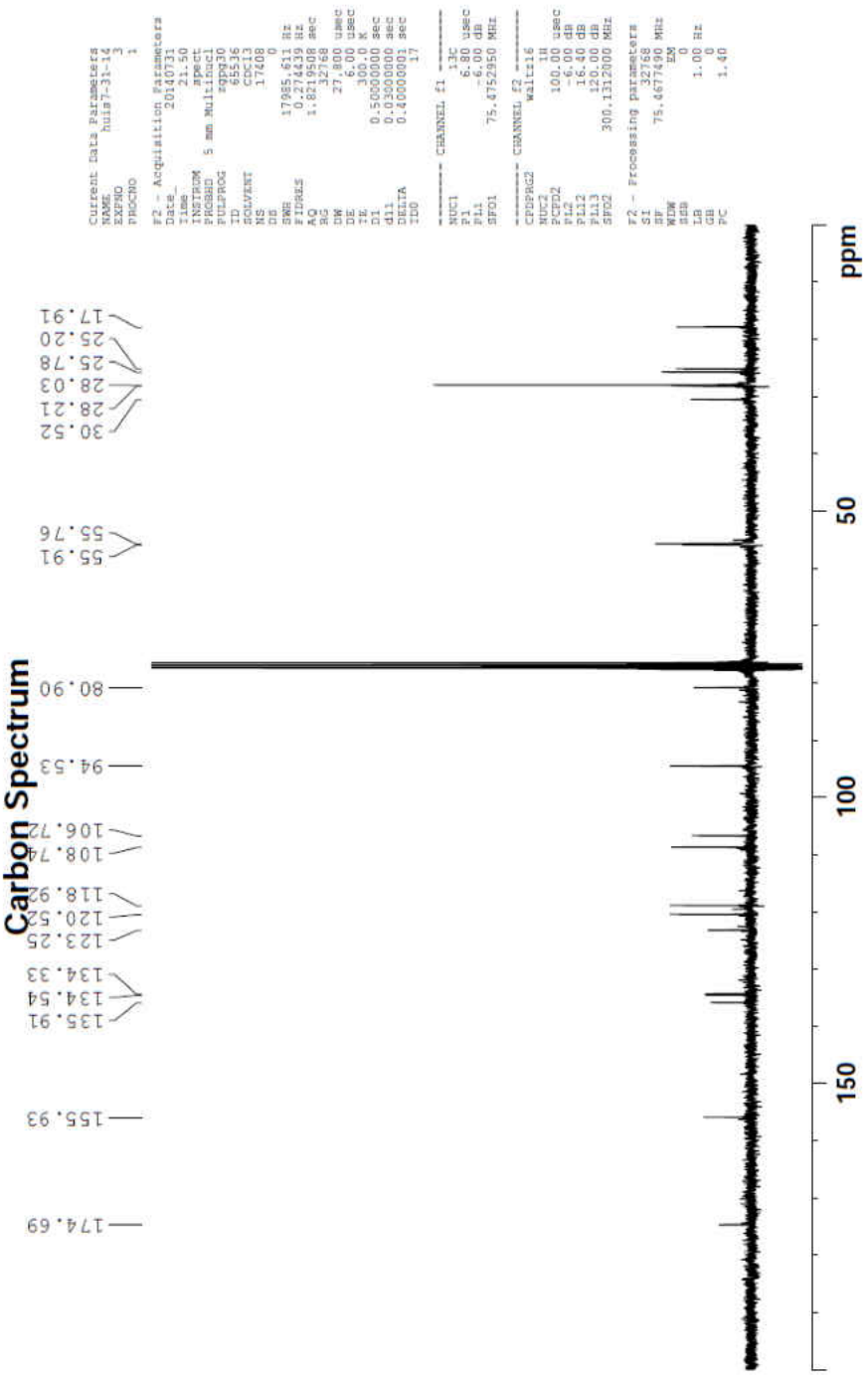
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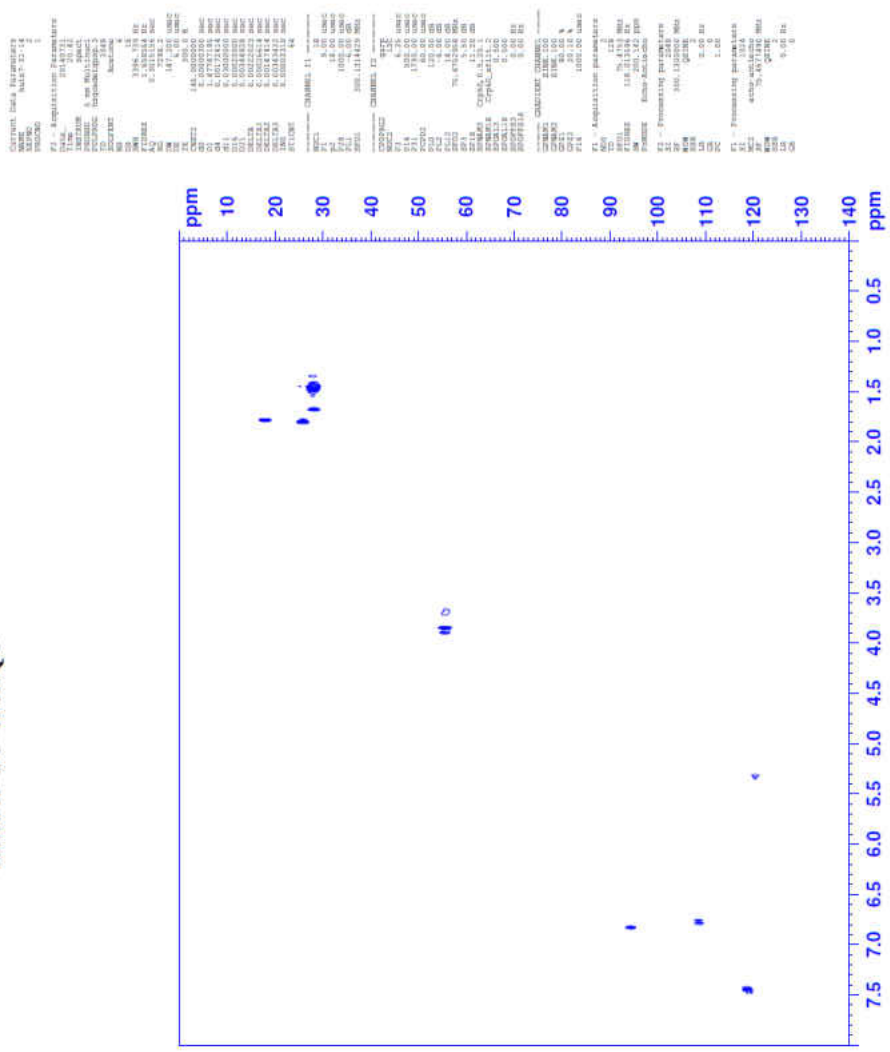
edited H-1/C-13 HSQC



Shimadzu LCMS-IT-TOF Analysis Report
Aug 05, 2014

Acquired by	: Mark Wang, Department of Chemistry and Biochemistry, UW-Milwaukee
Sample Name	: Huis 7-31-14
Sample ID	: Huis 7-31-14
Vail #	: 2
Injection Volume	: 5 uL
Data File Name	: Huis 7-31-014.lcd
Method File Name	: MW Manual (MS-MS, +, -).lcm
Batch File Name	:
Report File Name	: DefaultLCMS.lcr
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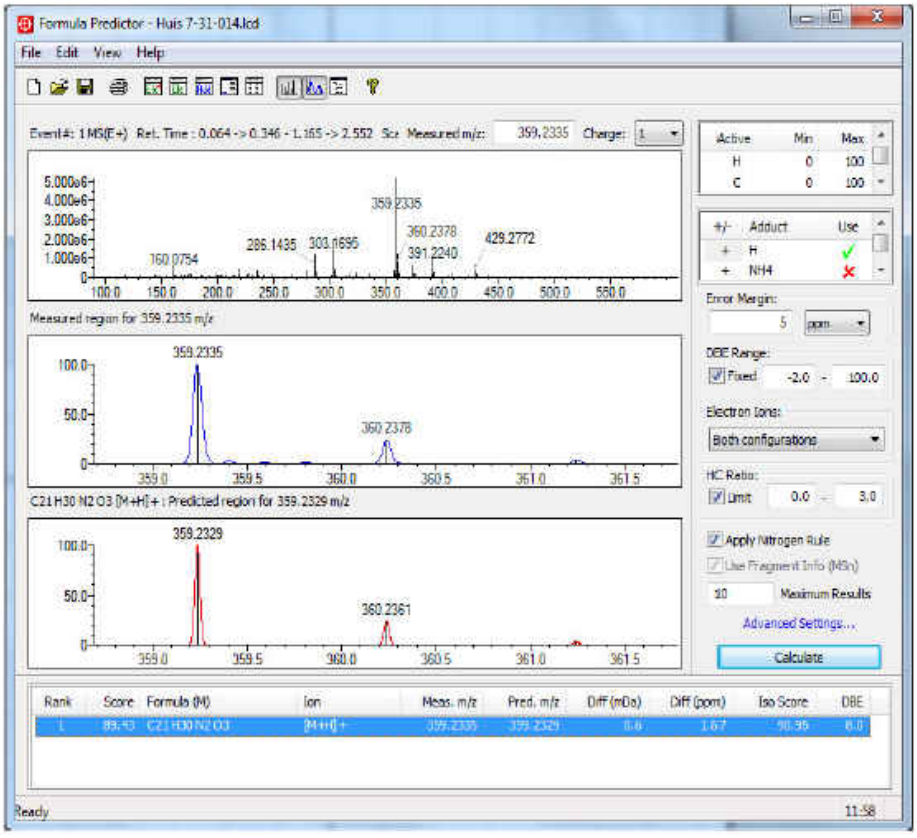
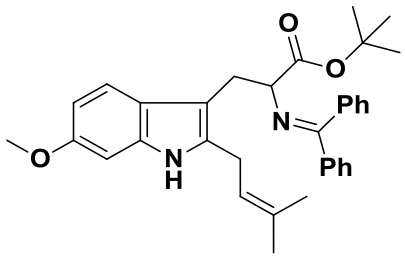
Formula Prediction Results



6-Methoxy-2-isoprenylt-butylesterdephenylmethylenetryptopan



Chemical Formula: $C_{34}H_{38}N_2O_3$
Molecular Weight: 522.68

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^{13}C Name: huis3-8-14 Expno: 3 Procno: 1

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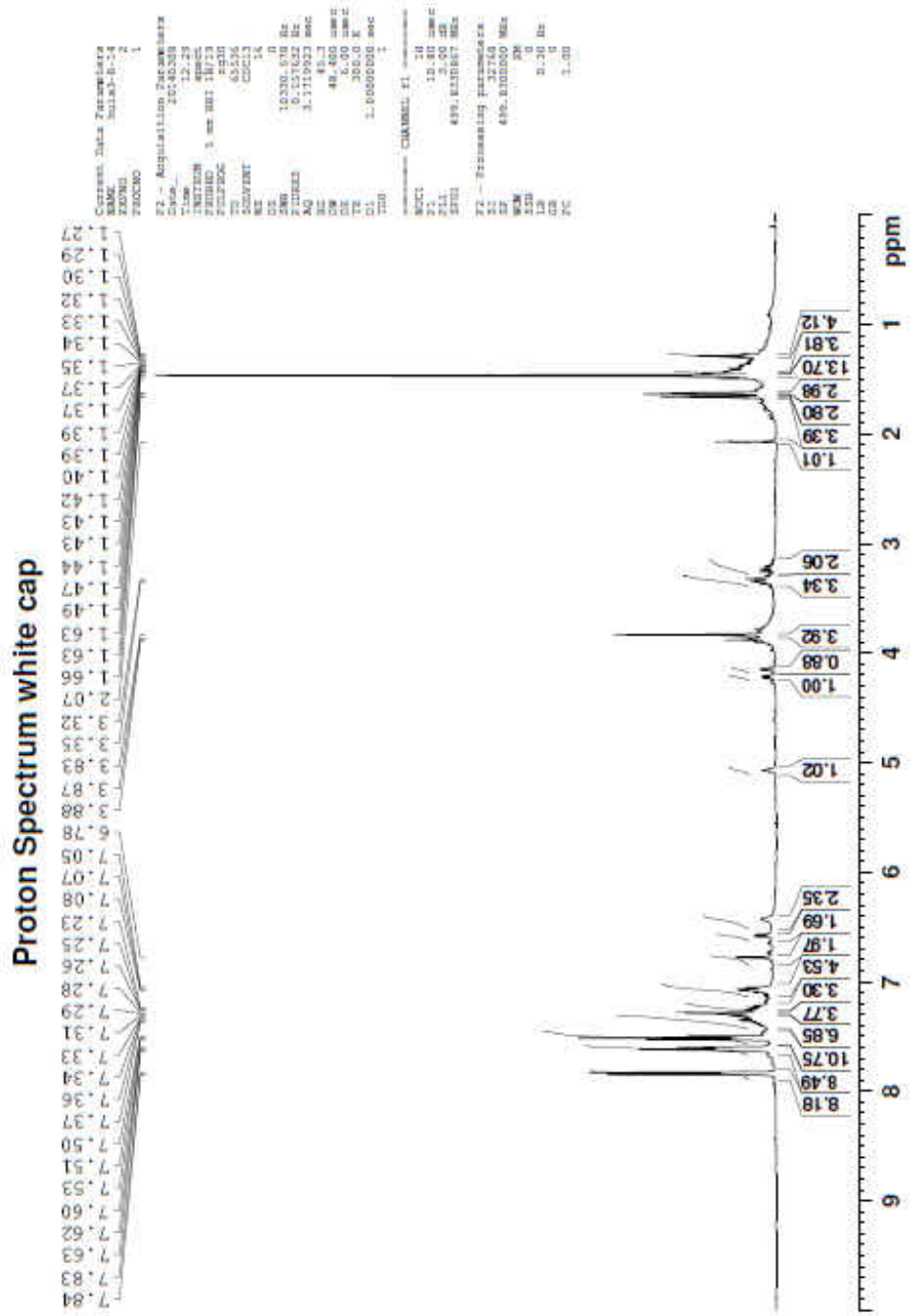
HRMS Name: Huis 8-11-14 (3)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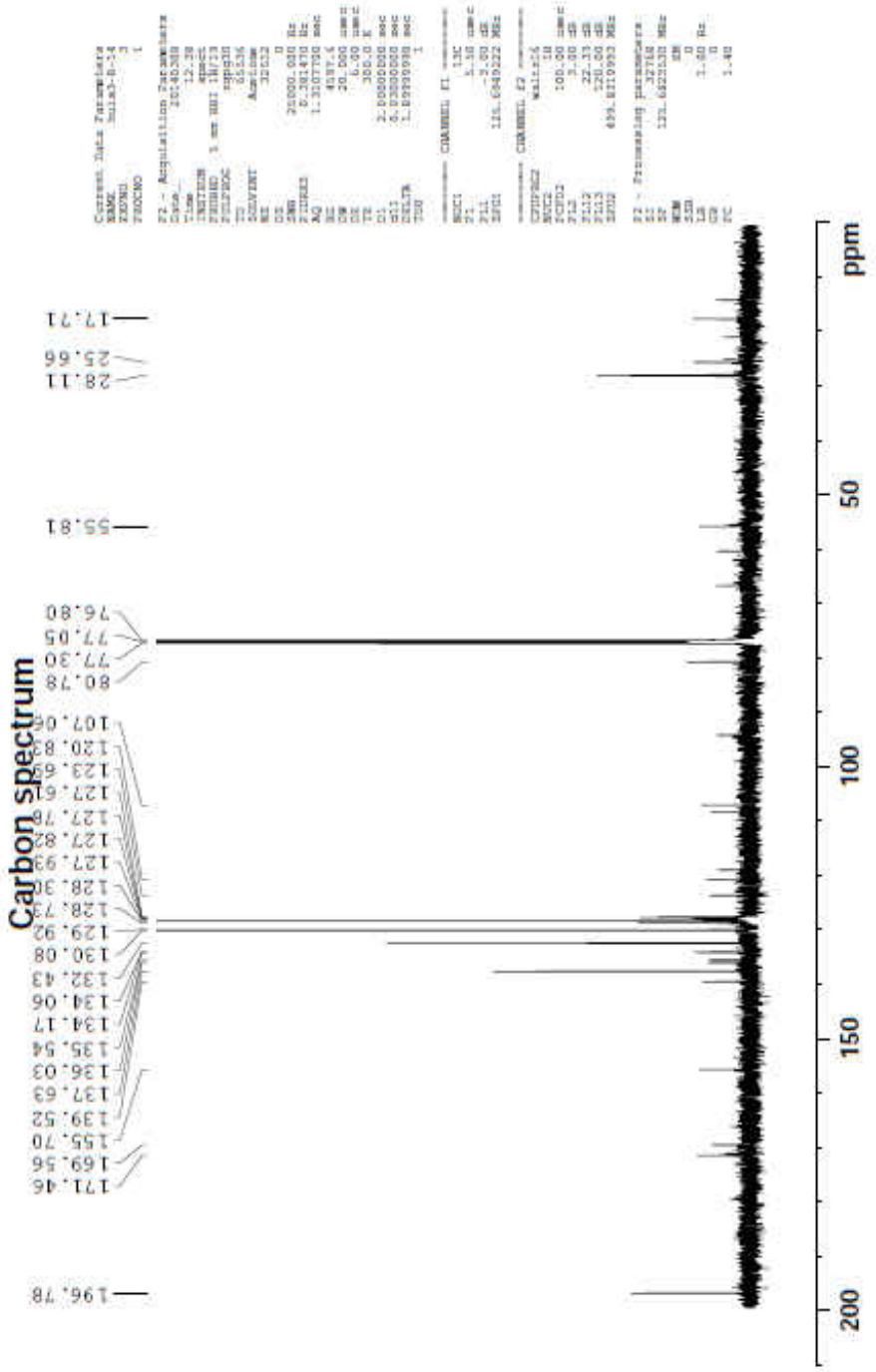
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==== Shimadzu LabSolutions Data Report ====

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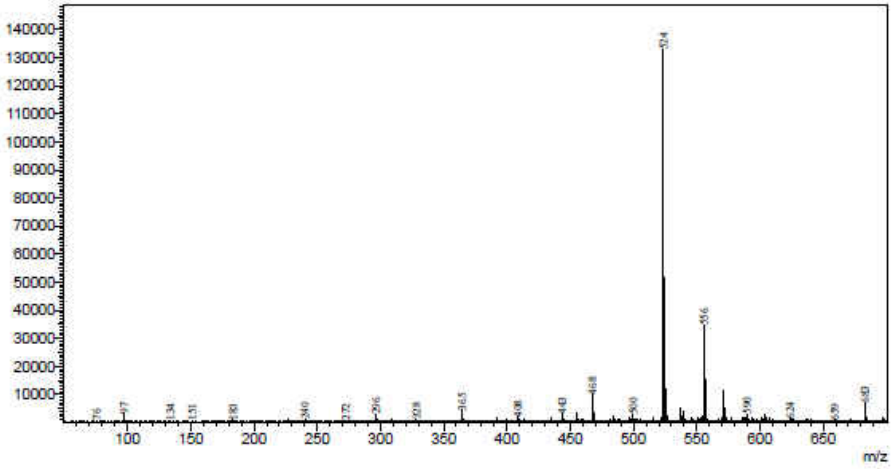

Shimadzu LCMS-IT-TOF Analysis Report
Aug 12, 2014

Acquired by	: Mark Wang, Department of Chemistry and Biochemistry, UW-Milwaukee
Sample Name	: Huis 8-11-14(3)
Sample ID	: Huis 8-11-14(3)
Vail #	: 4
Injection Volume	: 5 uL
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Batch File Name	:
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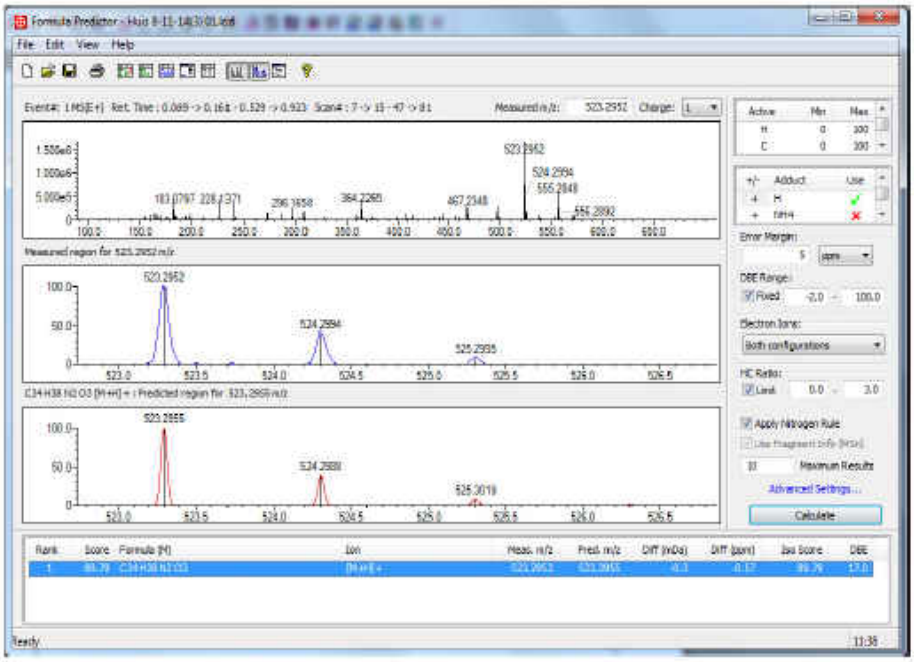
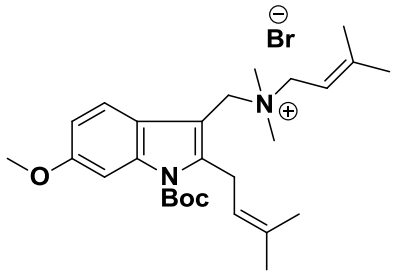


6-Methoxydiisoprenylbocgramine



Chemical Formula: $C_{27}H_{41}BrN_2O_3$
Molecular Weight: 521.53

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^{13}C Name: huis11-4-13 Expno: 8 Procno: 1

HSQC Name: huis11-4-13 Expno: 9 Procno: 1

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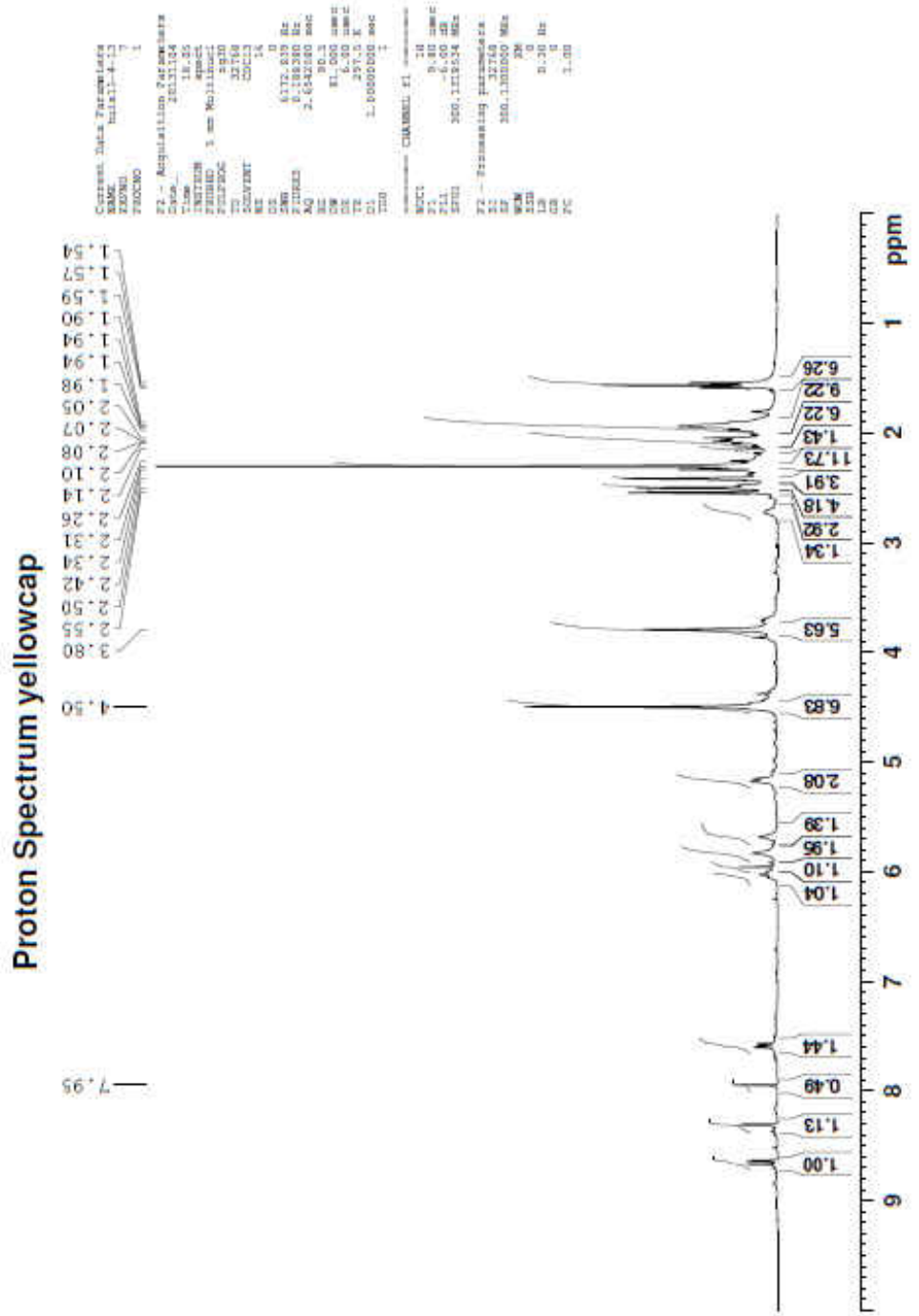
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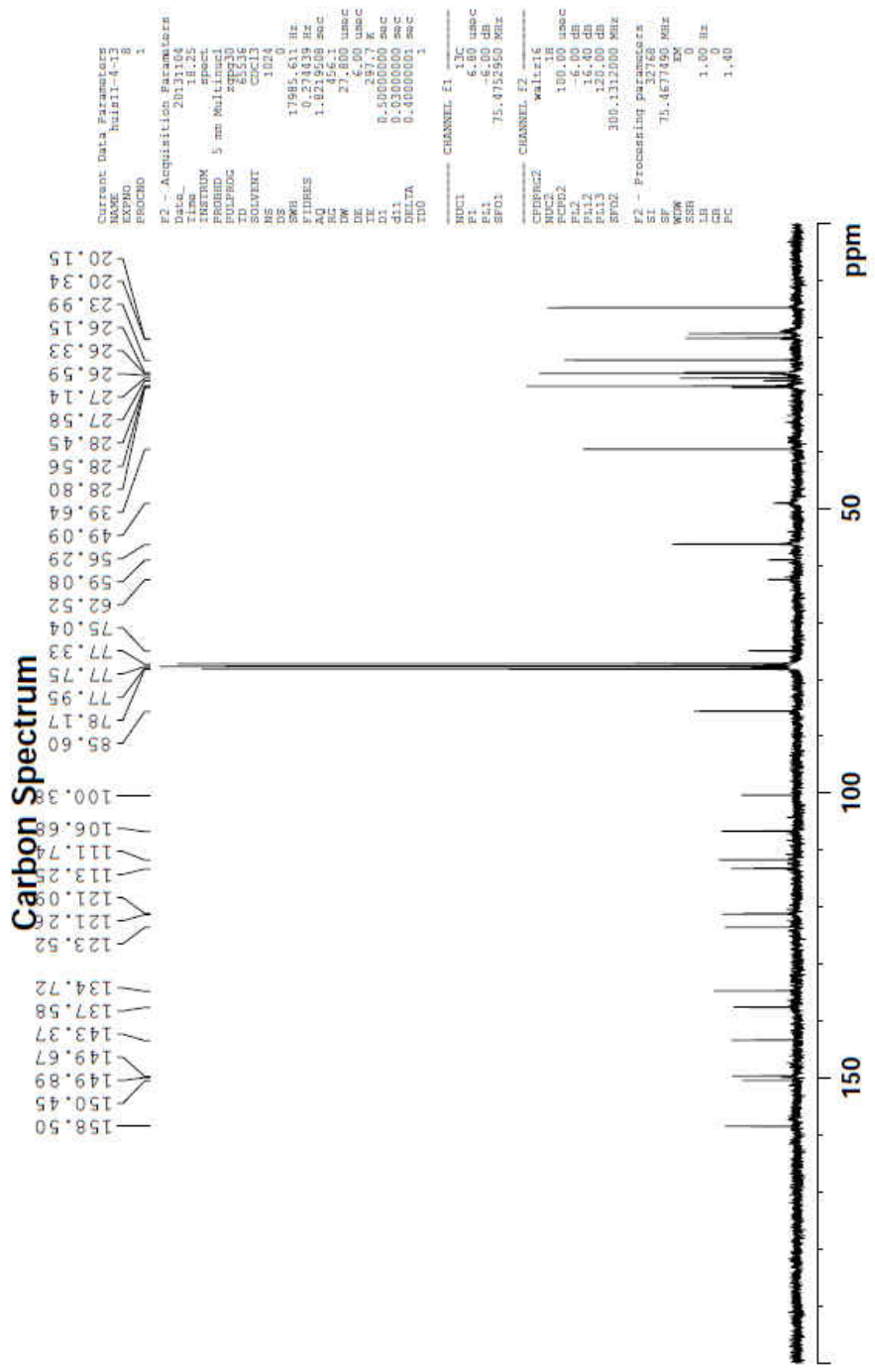
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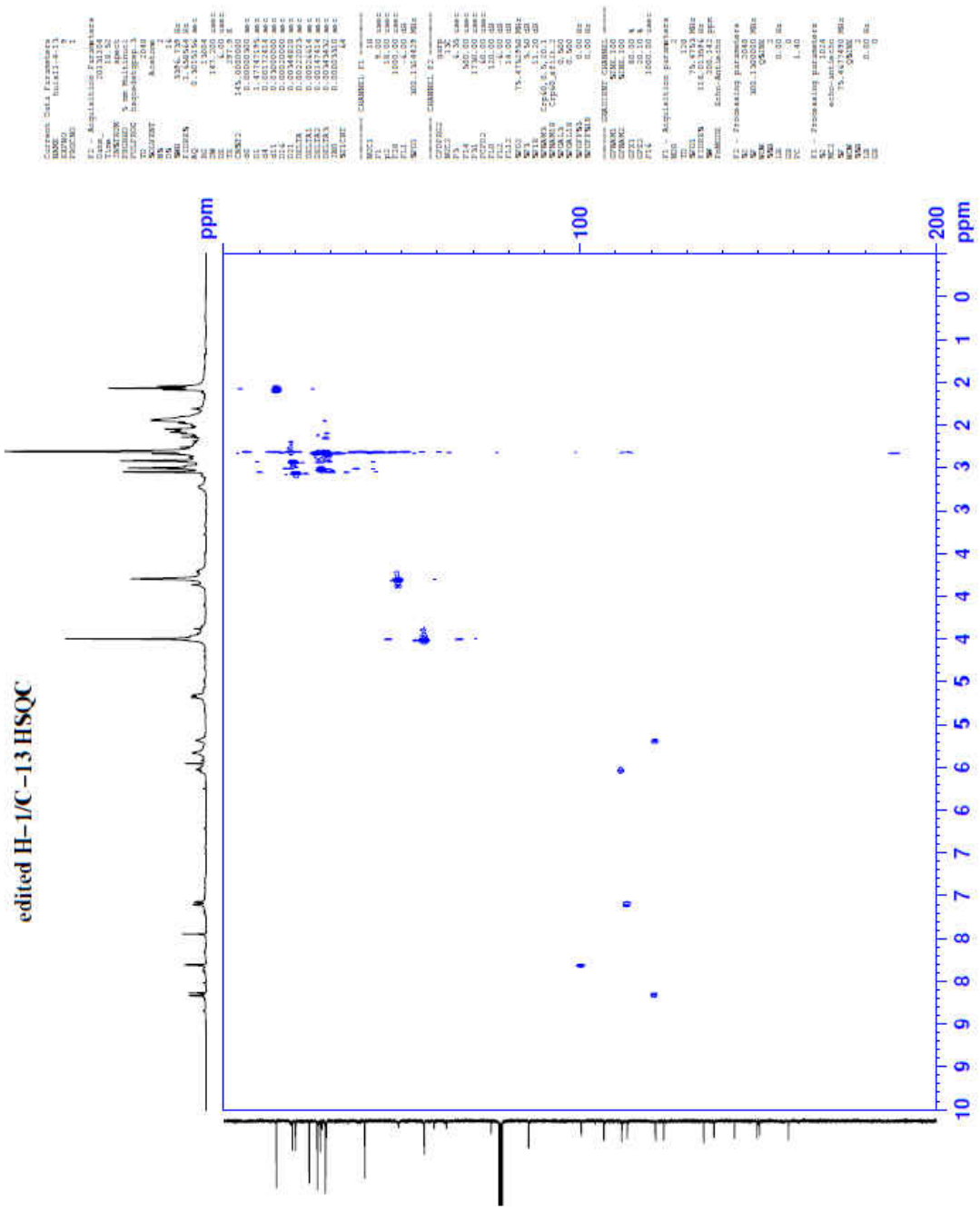
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==== Shimadzu LabSolutions Data Report ====

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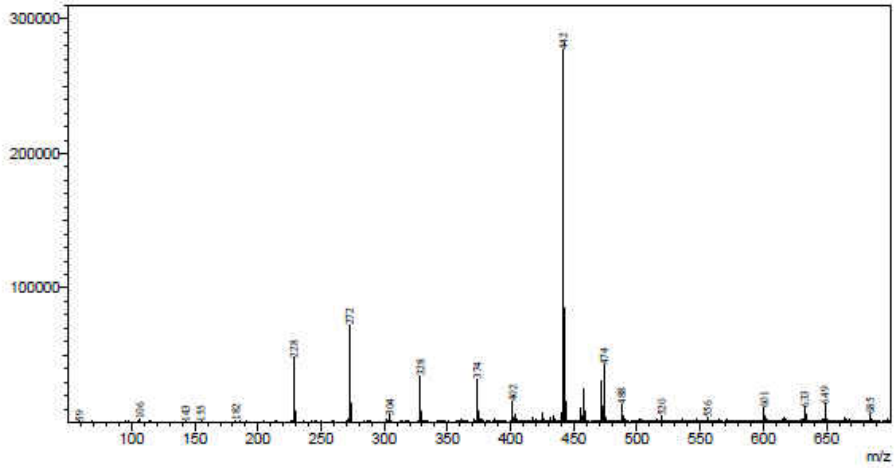

Shimadzu LCMS-IT-TOF Analysis Report
Aug 12, 2014

Acquired by	: Mark Wang, Department of Chemistry and Biochemistry, UW-Milwaukee
Sample Name	: Huis 8-11-14(2)
Sample ID	: Huis 8-11-14(2)
Vail #	: 3
Injection Volume	: 5 uL
Data File Name	: Huis 8-11-14(2) 01.lcd
Method File Name	: MW Manual (MS-MS, +, -).lcm
Batch File Name	:
Report File Name	: DefaultLCMS.lcr
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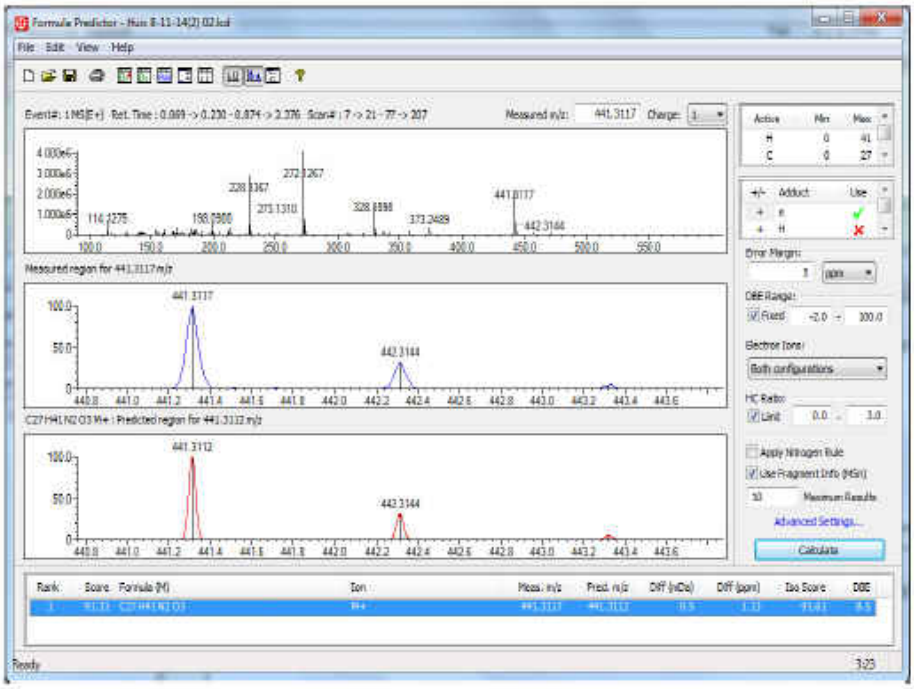
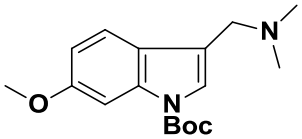
Data Processed	: 8/12/2014 11:29:14 AM

Formula Prediction Results



6-Methoxybocgramine



Chemical Formula: $C_{17}H_{24}N_2O_3$
Molecular Weight: 304.38

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^{13}C Name: huis9-5-13 Expno: 3 Procno: 1

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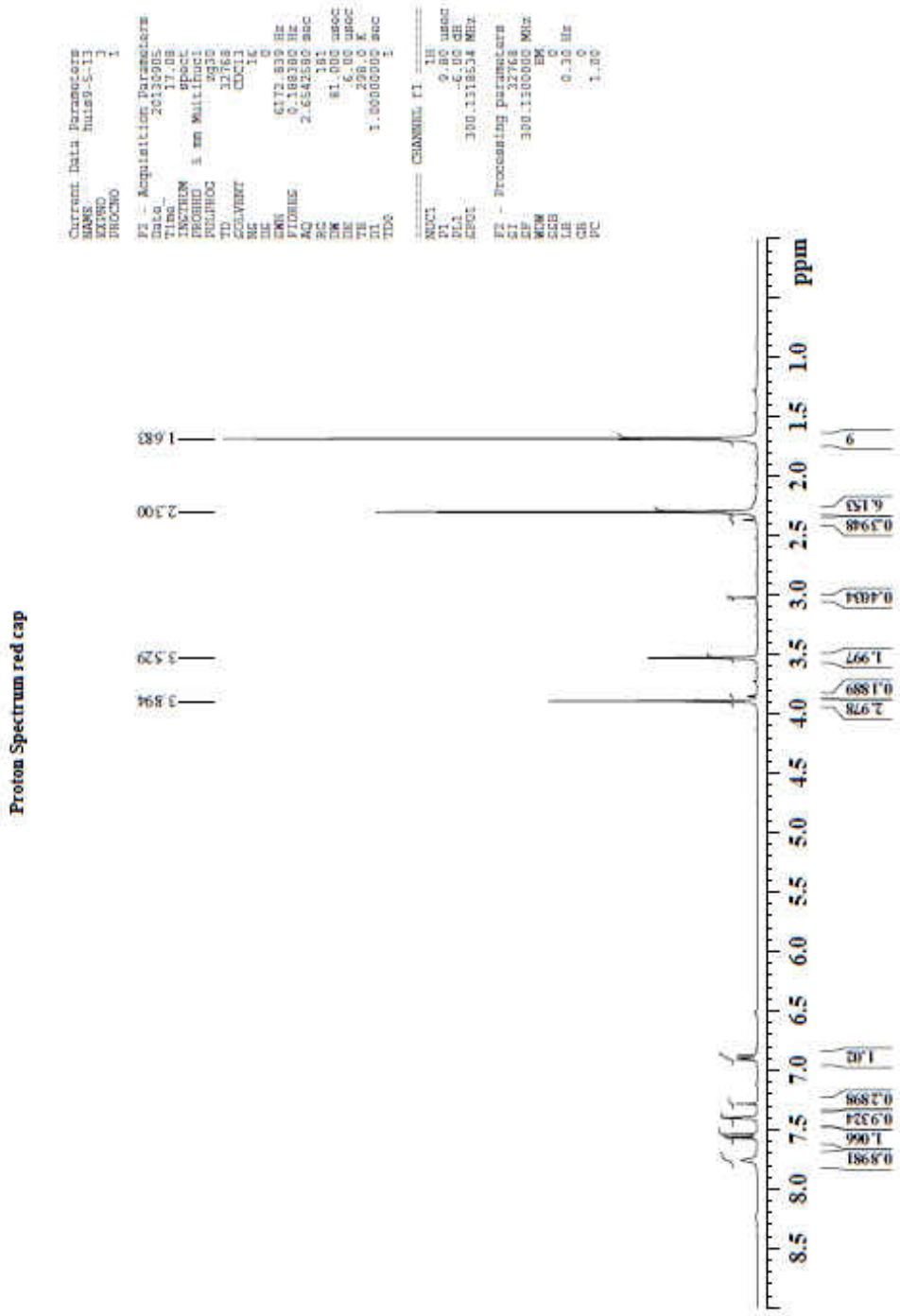
HRMS Name: Huis 8-11-14 (1)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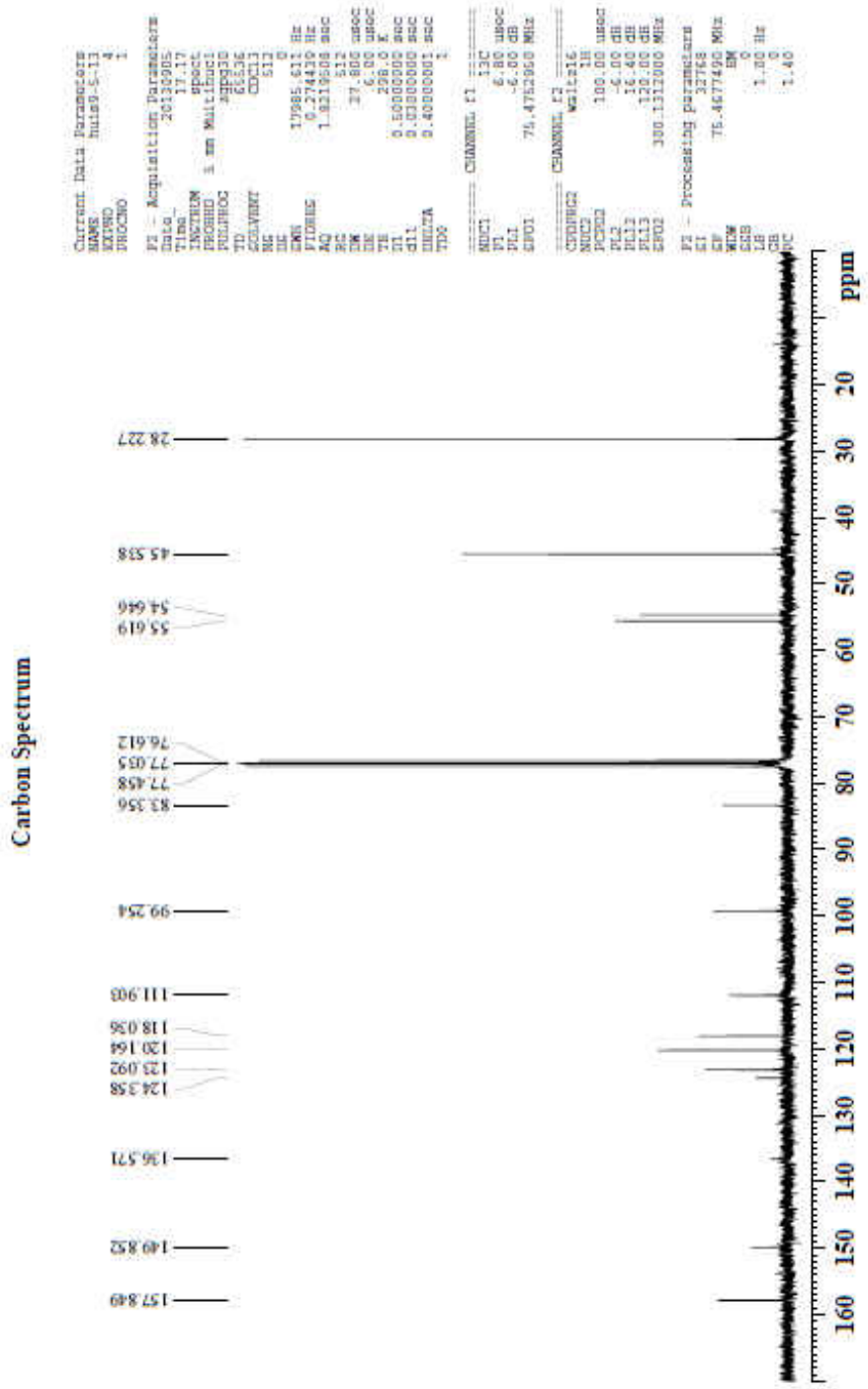
NAME huisg-5-13
EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130905
Time 17.08
INSTRUM spect
PROBHD 5 mm Multinucl
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 16
DS 0
SWH 6172.839 Hz
FIDRES 0.188380 Hz
AQ 2.6542580 sec
RG 161
DW 81.000 usec
DE 6.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
CHANNEL f1
NUC1 1H
P1 9.80 usec
PL1 -6.00 dB
SFO1 300.1318534 MHz
F2 - Processing parameters
SI 32768
SF 300.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
1.683
2.100
3.529
3.894
9
6.153
0.3988
0.4654
1.997
0.1889
2.978
1.00
0.2898
0.9324
1.066
0.8981
ppm


Carbon Spectrum
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54.646
55.619
76.612
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99.254
111.901
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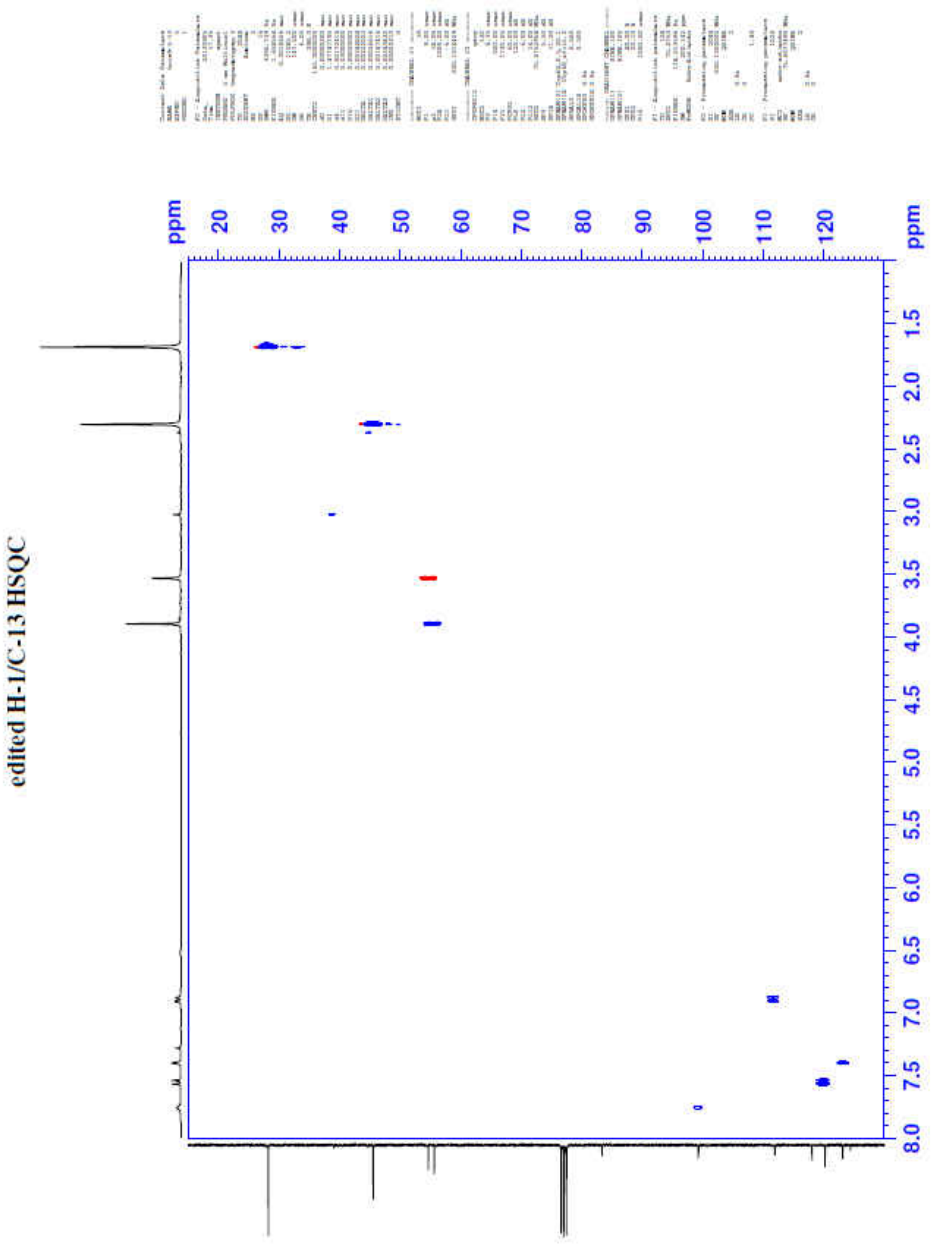
123.092
124.358
136.571
149.852
157.849

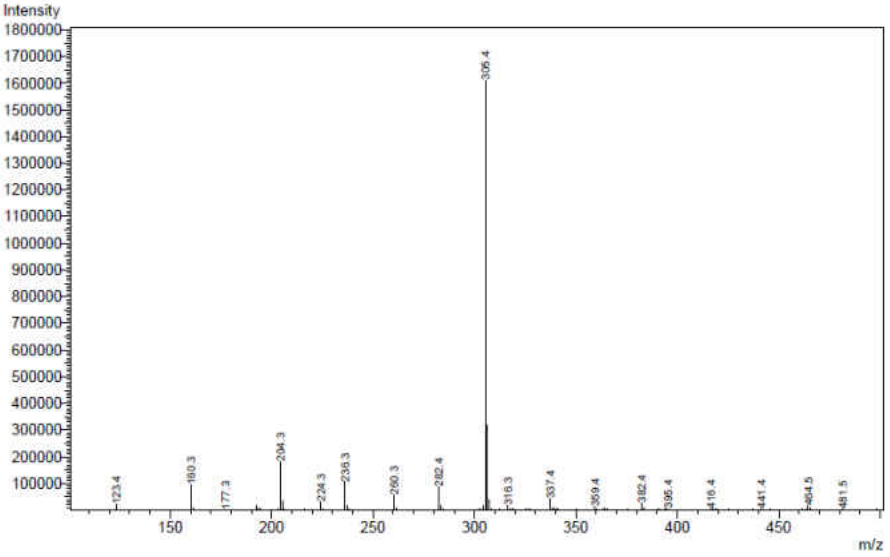

edited H-1/C-13 HSQC
ppm
20
30
40
50
60
70
80
90
100
110
120
ppm
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0

Shimadzu LCMS-2020 Data Report

Mass Spectrum for Sample:
Matt-Frac-3

Operator: Mark Wang

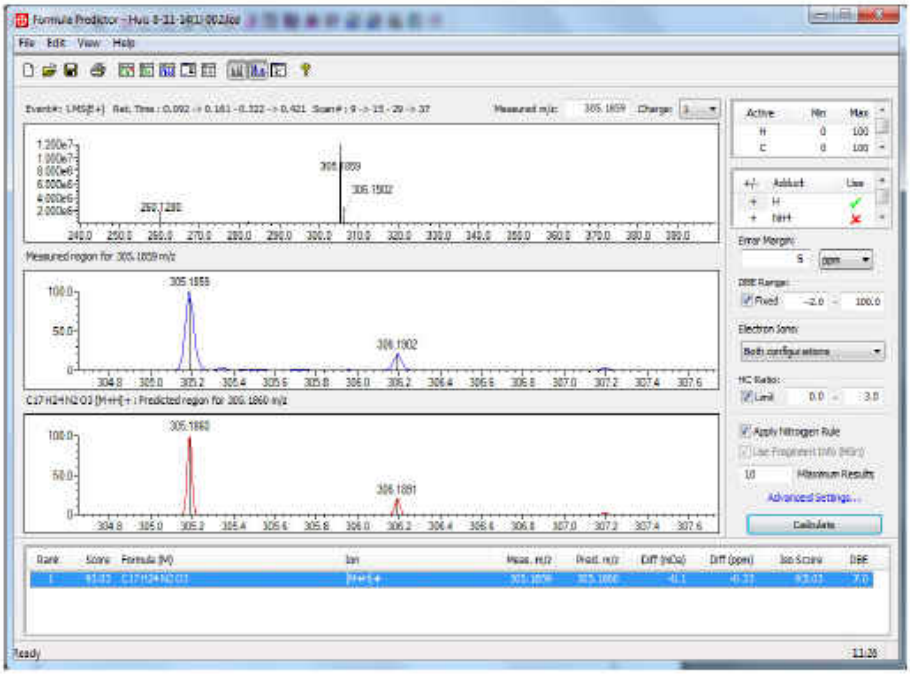
Data filename: C:\LabSolutions\Data\Hossain Mahmun\Matt-Frac-3.lcd
Spectrum Mode: Single
Retention Time: 0.133 min.
Interface Type (ESI, APCI, DUIS): DUIS
Aquisition Mode (Scan, SIM, Profile): Scan
Polarity (+,-): +

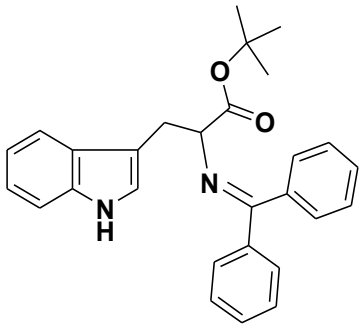


Shimadzu LCMS-IT-TOF Analysis Report
Aug 12, 2014

Acquired by	: Mark Wang, Department of Chemistry and Biochemistry, UW-Milwaukee
Sample Name	: Huis 8-11-14(1)
Sample ID	: Huis 8-11-14(1)
Vail #	: 2
Injection Volume	: 5 uL
Data File Name	: Huis 8-11-14(1) 002.lcd
Method File Name	: MW Manual (MS-MS, +, -).lcm
Batch File Name	:
Report File Name	: DefaultLCMS.lcr
Data Acquired	: 8/12/2014 11:21:10 AM
Data Processed	: 8/12/2014 11:24:10 AM

Formula Prediction Results





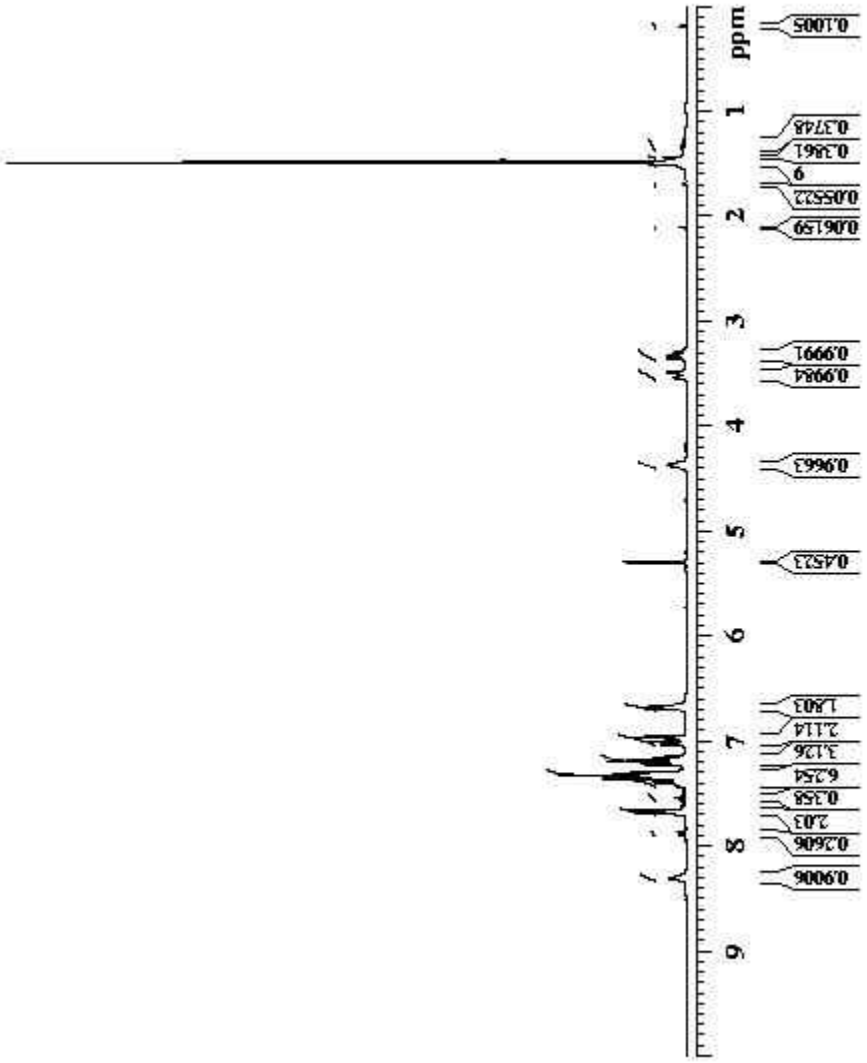
$C_{28}H_{28}N_2O_2$
Mol. Wt.: 424.53

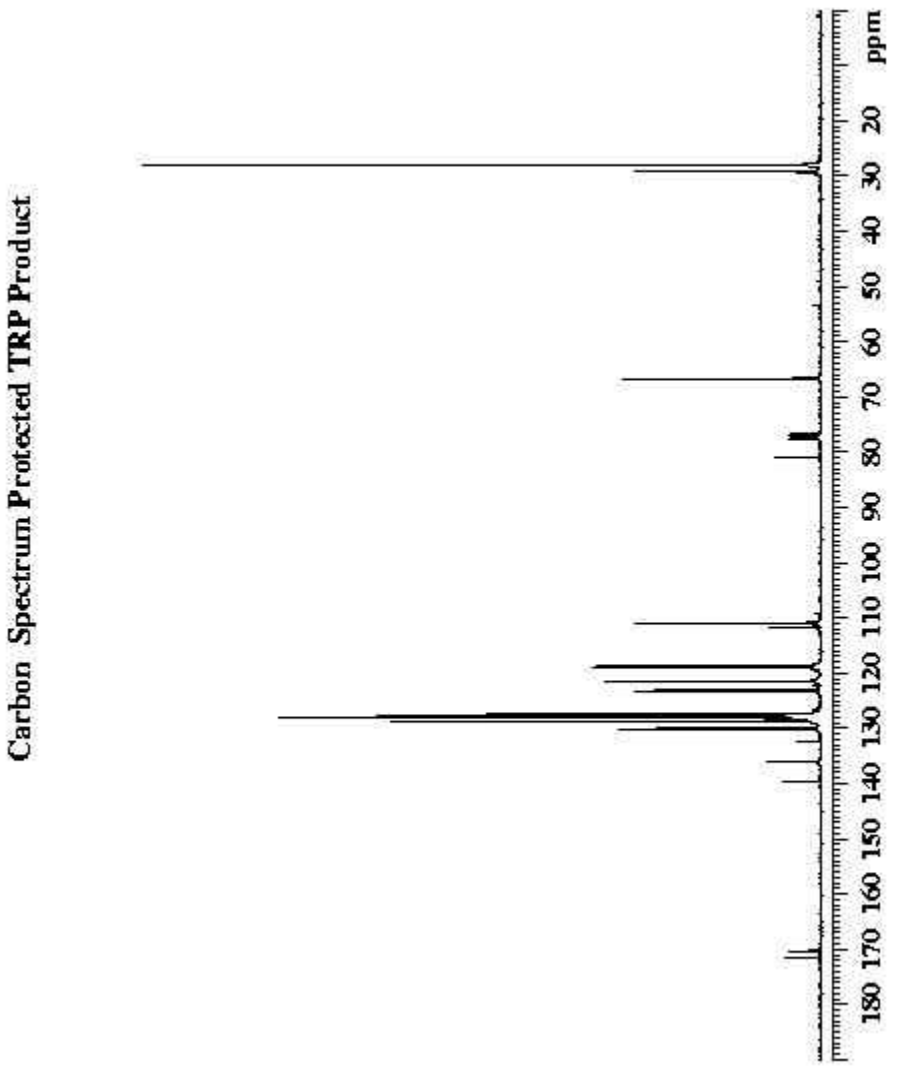
^{1}H Name: huis Expno:1 Procno: 1 Date: 20091117

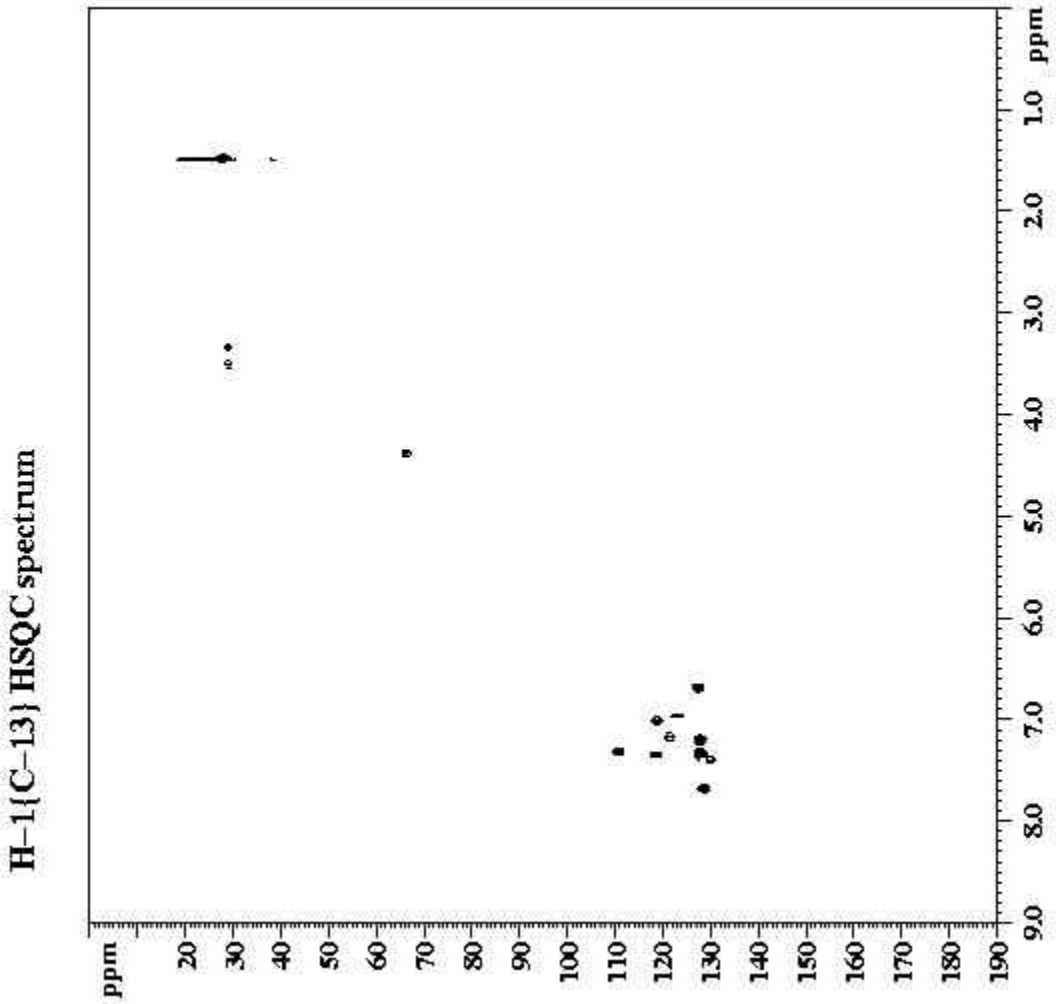
^{13}C Name: huis Expno: 3 Procno: 1 Date: 20091117

^{13}C HSQC Name: huis Expno: 2 Procno: 1 Date: 20091117

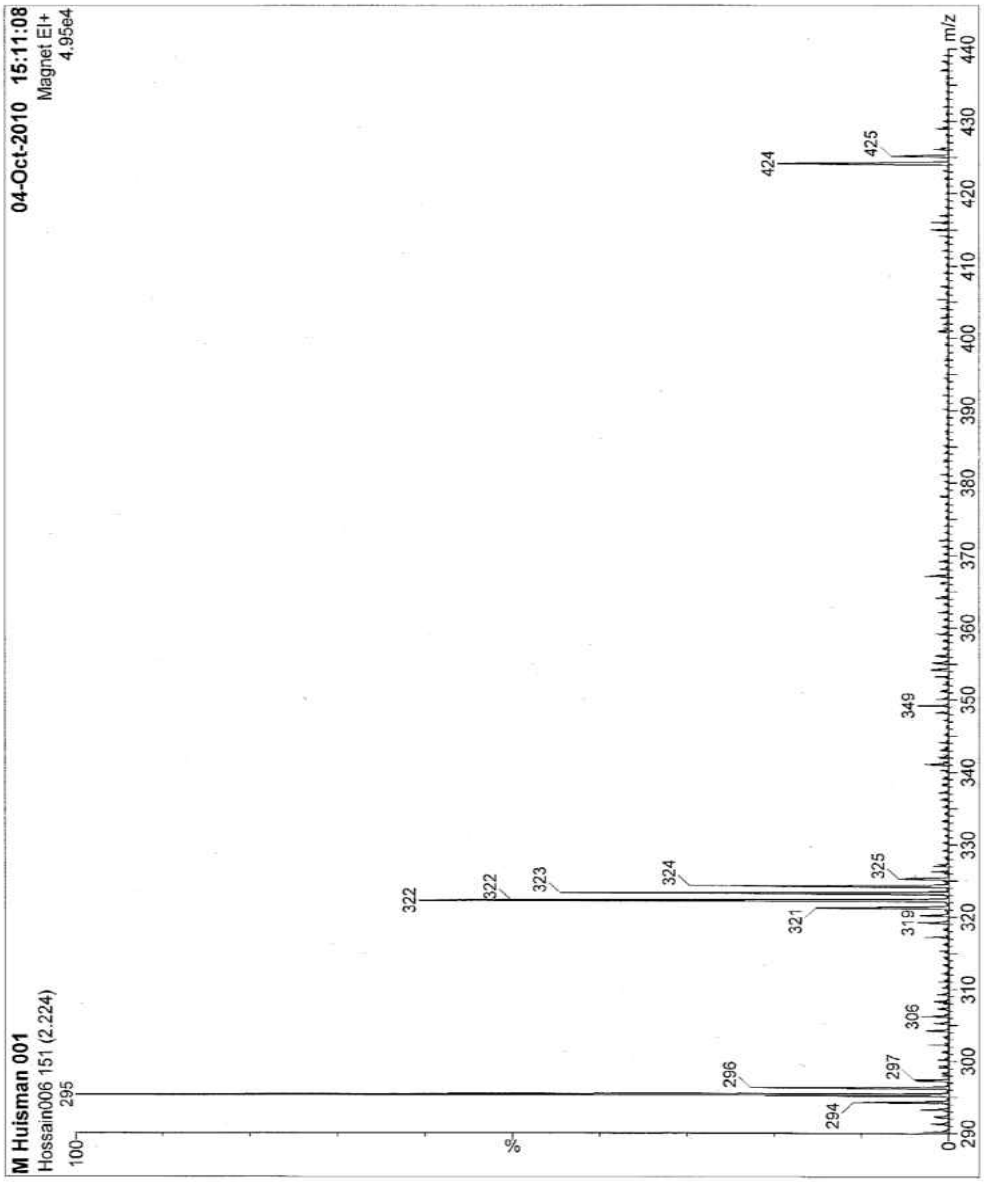
Proton Spectrum Protected Trp product




Carbon Spectrum Protected TRP Product
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
ppm


H-1{C-13} HSQC spectrum
ppm
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm

HRMS


M Huisman 001
Hossain006 151 (2.224)
04-Oct-2010 15:11:08
Magnet EI+
4.95e4
100
%
0
295
296
297
294
306
322
322
323
324
321
319
325
349
424
425
m/z
290
300
310
320
330
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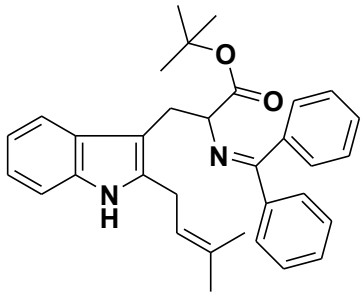
350
360
370
380
390
400
410
420
430
440



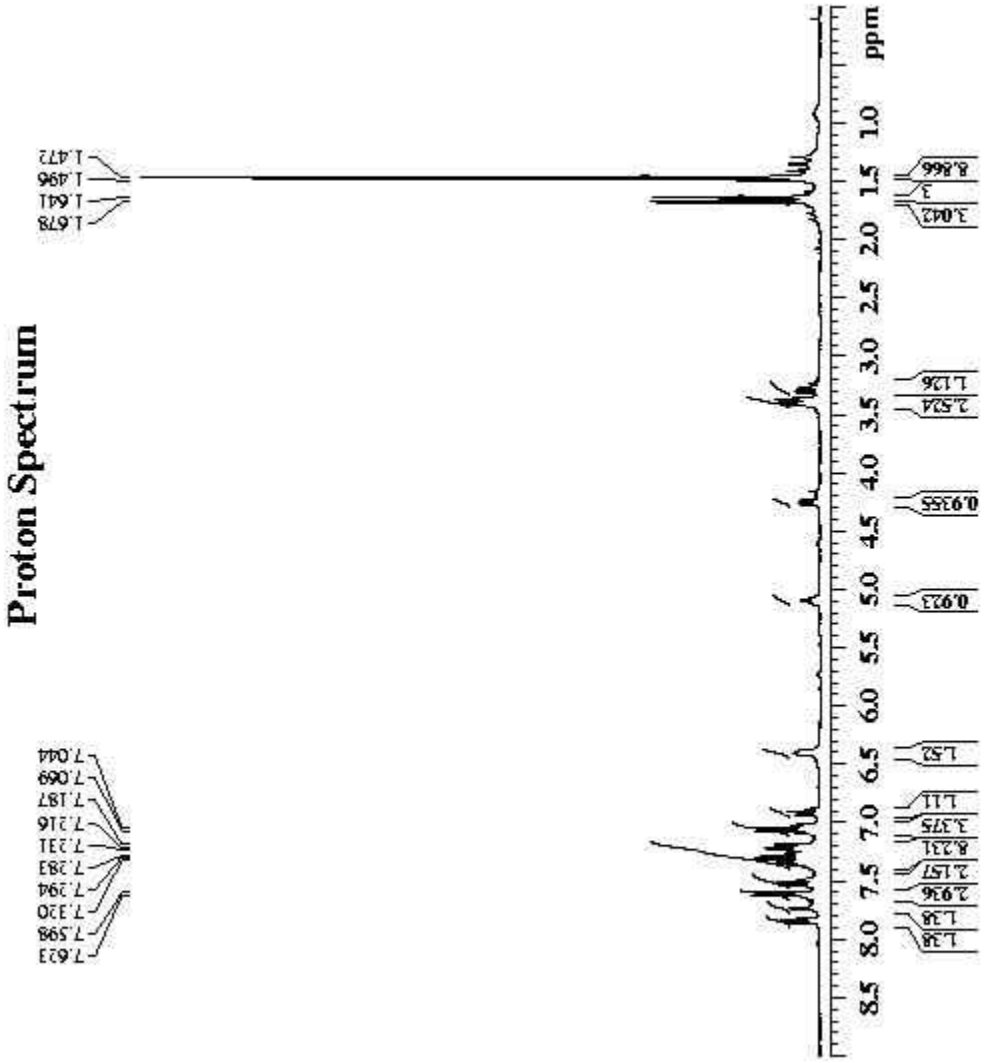
$C_{33}H_{36}N_2O_2$
Mol. Wt.: 492.65

^{1}H Name: huis10-13-11 Expno: 1 Procno: 1

^{13}C Name: huis10-13-11 Expno: 3 Procno: 1

LRMS

HRMS


Proton Spectrum
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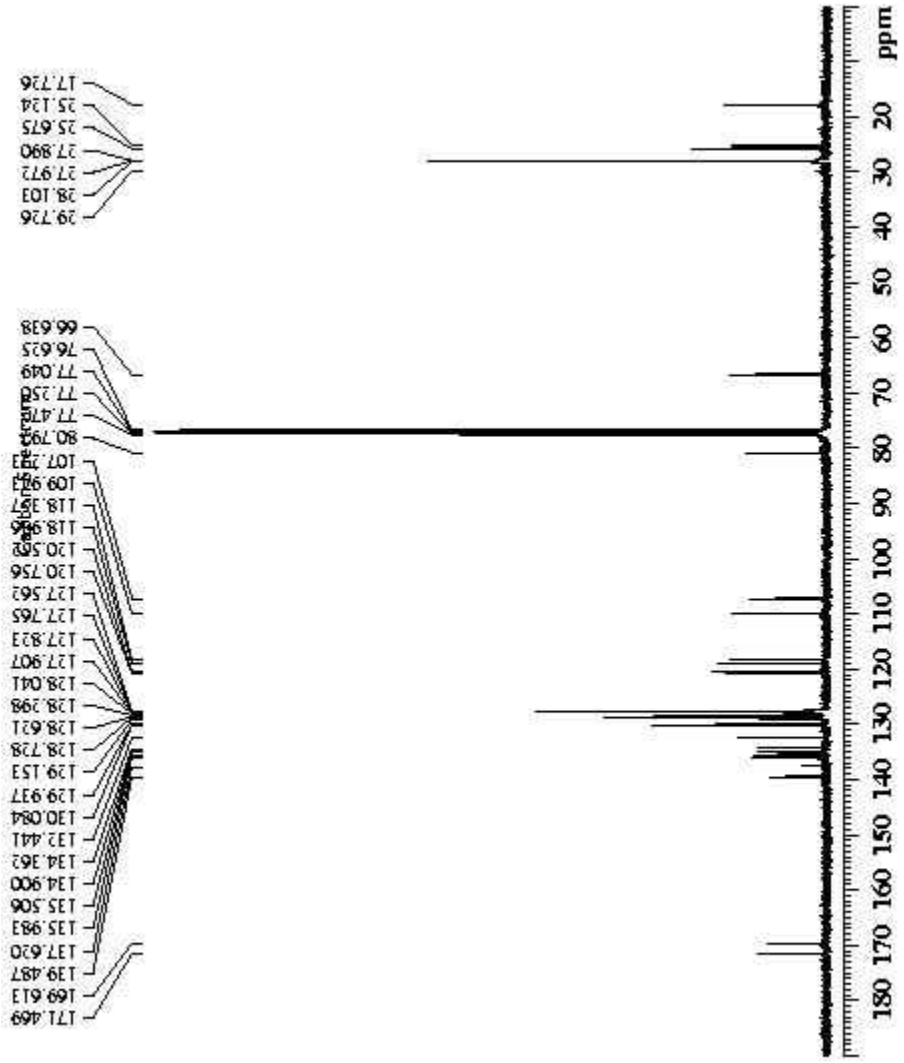
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
7.623
7.598
7.320
7.294
7.283
7.231
7.216
7.187
7.069
7.044
1.678
1.641
1.496
1.472
1.38
1.38
2.936
2.157
8.231
3.375
1.11
1.52
0.923
0.9355
2.524
1.126
3.042
3
8.866


17.726
25.134
25.675
27.890
27.972
28.103
29.726
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77.049
77.250
77.473
80.792
107.793
109.923
118.357
118.986
120.569
120.756
127.562
127.765
127.823
127.907
128.041
128.298
128.621
128.728
129.153
129.937
130.084
132.441
134.362
134.900
135.506
135.983
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169.613
171.469
ppm
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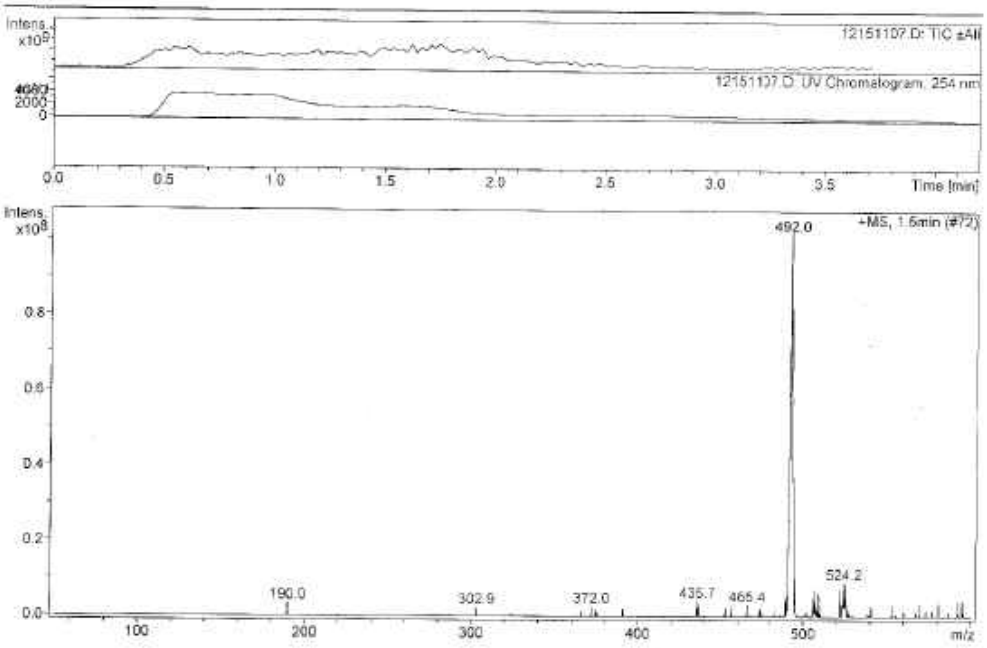
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180

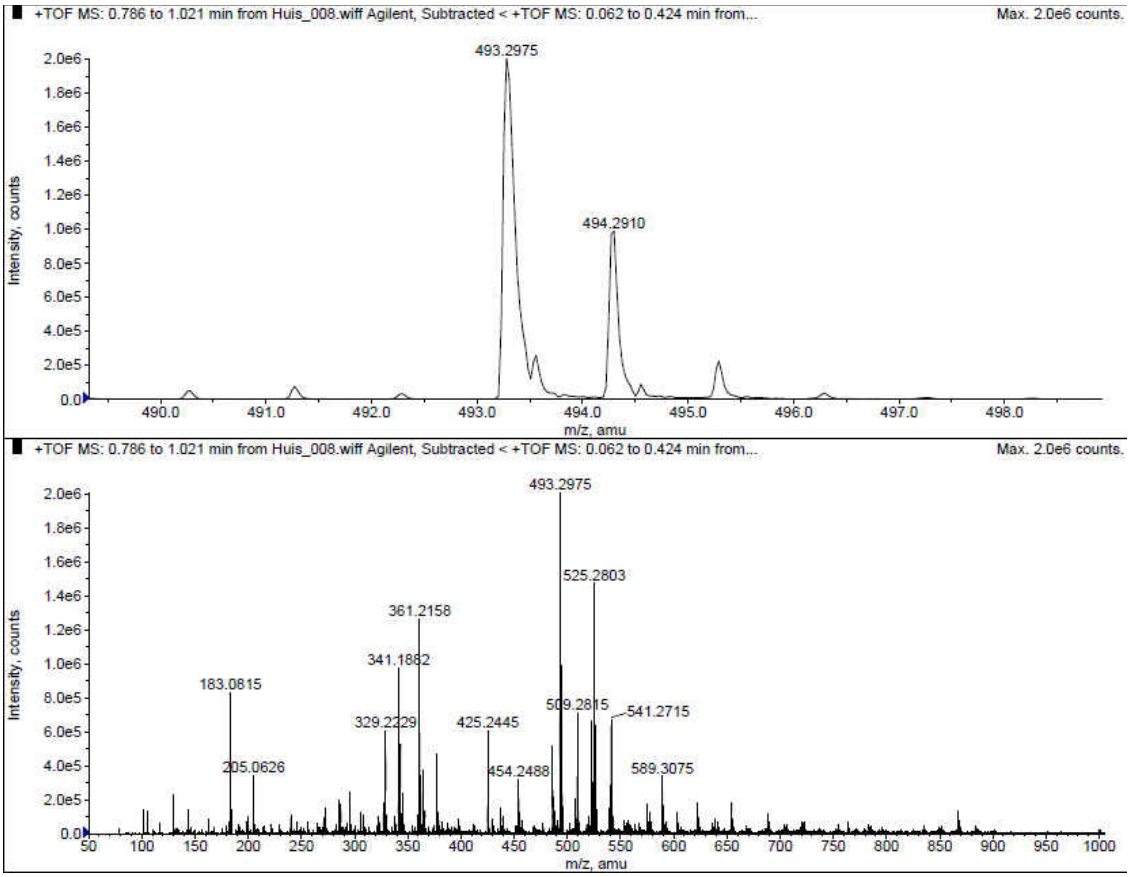
Display Report - All Windows Selected Analy

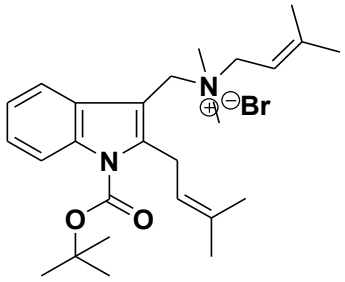
Analysis Name: 12151107.D
Method: Copy of MARK02.M
Sample Na M Huisman
Analysis Inf

Instrume LC-MSD-Trap-SL
Operator: Mark Wang

Print Date: 12/15/11 15:14:55
Acq. Date 12/15/11 15:06:47





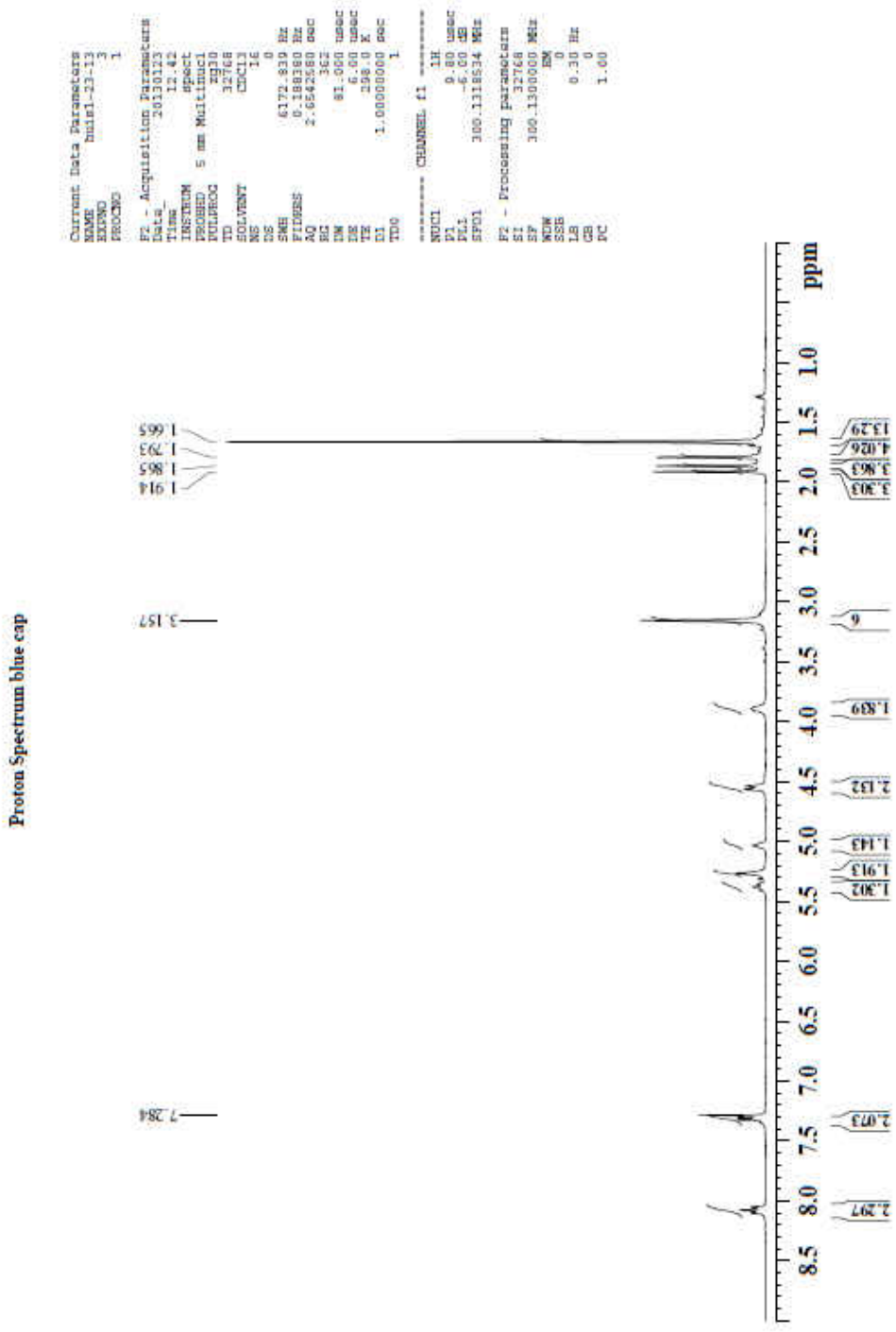


$C_{26}H_{39}N_2O_2^+$
Mol. Wt.: 411.6

^{1}H Name: huis1-23-13 Expno: 3 Procno: 1

^{13}C Name: huis1-23-13 Expno: 5 Procno: 1

^{13}C Dept135 Name: huis1-23-13 Expno: 6 Procno: 1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PROCNO 1
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Time 12.42
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PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 16
DS 0
SWH 6172.839 Hz
FIDRES 0.188380 Hz
AQ 2.6542560 sec
RG 362
DW 81.000 usec
DE 6.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 9.80 usec
PL1 -6.00 dB
SFO1 300.1318534 MHz
F2 - Processing parameters
SI 32768
SF 300.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.60
7.284
3.157
1.914
1.865
1.793
1.665
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2.297
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1.913
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6
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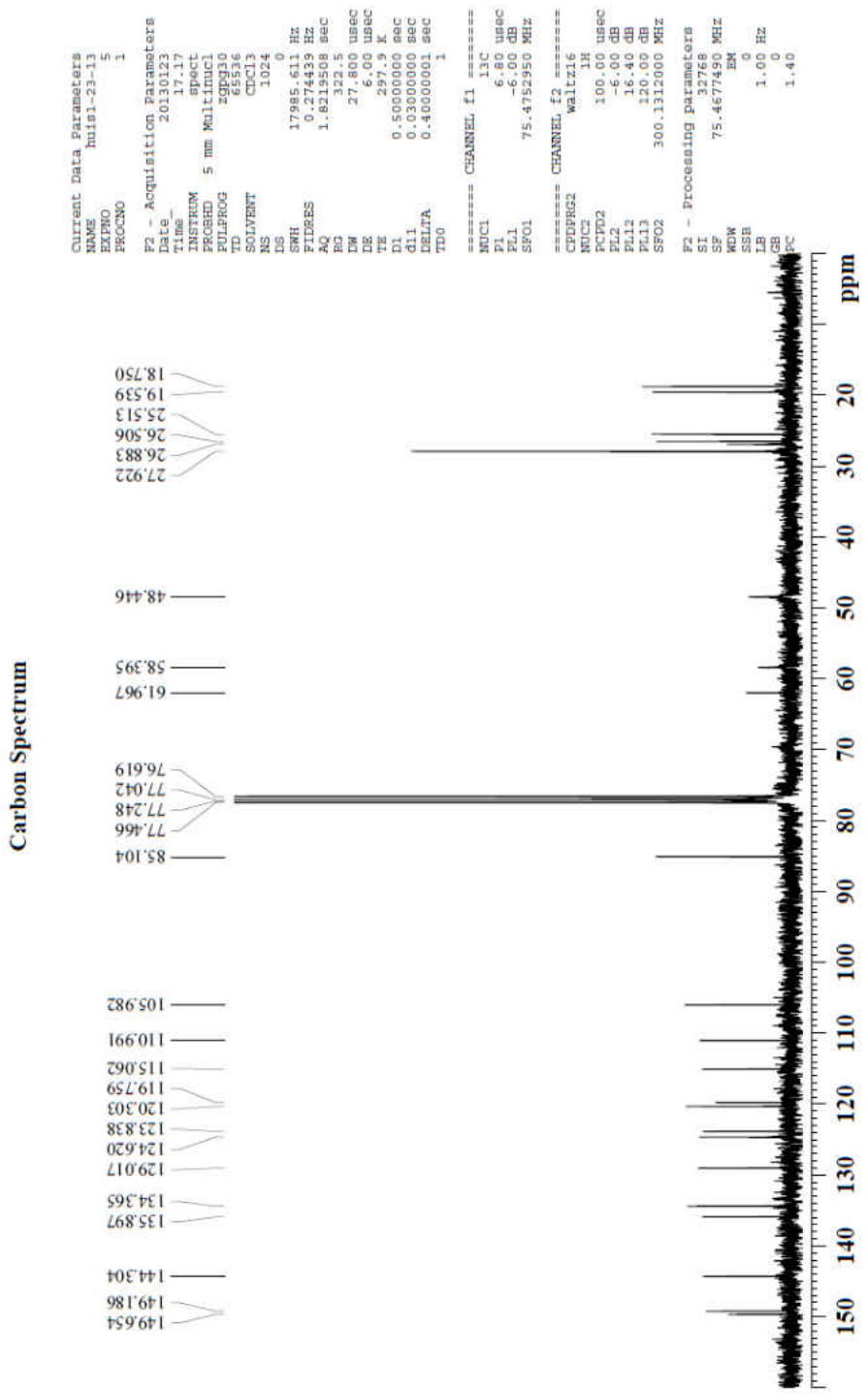
ppm


Carbon Spectrum
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NAME huisi-23-13
EXPNO 5
PROCNO 1
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Time 17.17
INSTRUM spect
PROBHD 5 mm Multinuc1
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 1024
DS 0
SWH 17985.611 Hz
FIDRES 0.274439 Hz
AQ 1.8219508 sec
RG 322.5
DW 27.800 usec
DE 6.00 usec
TE 297.9 K
D1 0.50000000 sec
d11 0.03000000 sec
DELTA 0.40000001 sec
TD0 1
========== CHANNEL f1 ==========
NUC1 13C
P1 6.80 usec
PL1 -6.00 dB
SFO1 75.4752950 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 -6.00 dB
PL12 16.40 dB
PL13 120.00 dB
SFO2 300.1312000 MHz
F2 - Processing parameters
SI 32768
SF 75.4677490 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
27.922
26.883
26.506
25.513
19.539
18.750
48.446
61.967
58.395
85.104
77.466
77.248
77.042
76.619
105.982
110.991
115.062
119.759
120.303
123.838
124.620
129.017
134.365
135.897
144.304
149.186
149.654
ppm
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140
130
120
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100
90
80
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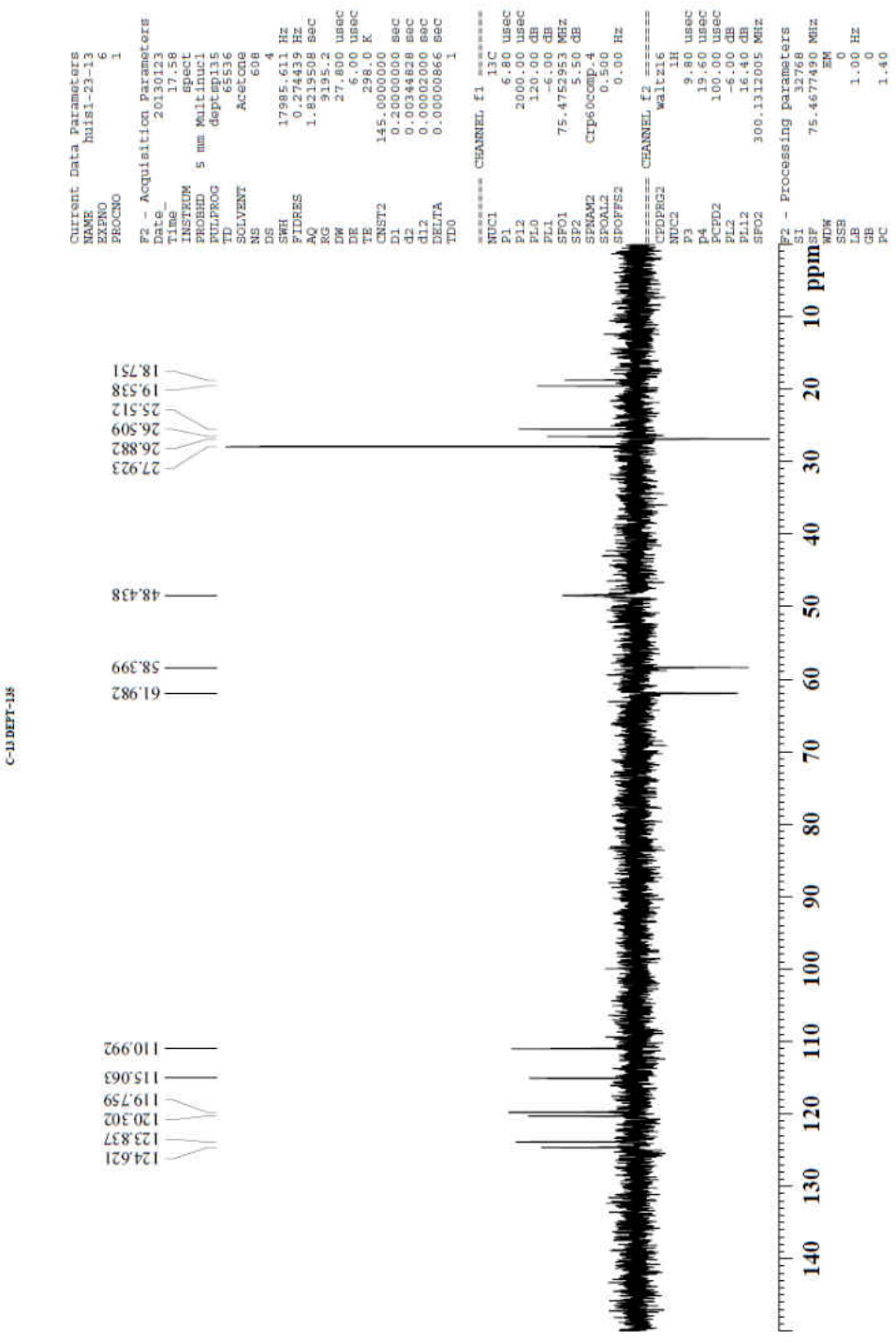
60
50
40
30
20


C-13 DEPT-135
Current Data Parameters
NAME huisi-23-13
EXPNO 6
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130123
Time 17.58
INSTRUM spect
PROBHD 5 mm Multinuc1
PULPROG deptsp135
TD 65536
SOLVENT Acetone
NS 608
DS 4
SWH 17985.611 Hz
FIDRES 0.274439 Hz
AQ 1.8219508 sec
RG 3195.2
DW 27.800 usec
DE 6.00 usec
TE 298.0 K
CNST2 145.0000000
D1 0.20000000 sec
d2 0.00344828 sec
d12 0.00002000 sec
DELTA 0.00000866 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 6.80 usec
P12 2000.00 usec
PL0 120.00 dB
PL1 -6.00 dB
SFO1 75.4752953 MHz
SP2 3.50 dB
SPNAM2 Crp60comp.4
SPOAL2 0.500
SPOFFS2 0.00 Hz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
P3 9.80 usec
P4 19.60 usec
PCPD2 100.00 usec
PL2 -6.00 dB
PL12 16.40 dB
SFO2 300.1312005 MHz
F2 - Processing parameters
SI 32768
SF 75.4677490 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
18.751
19.538
25.512
26.509
26.882
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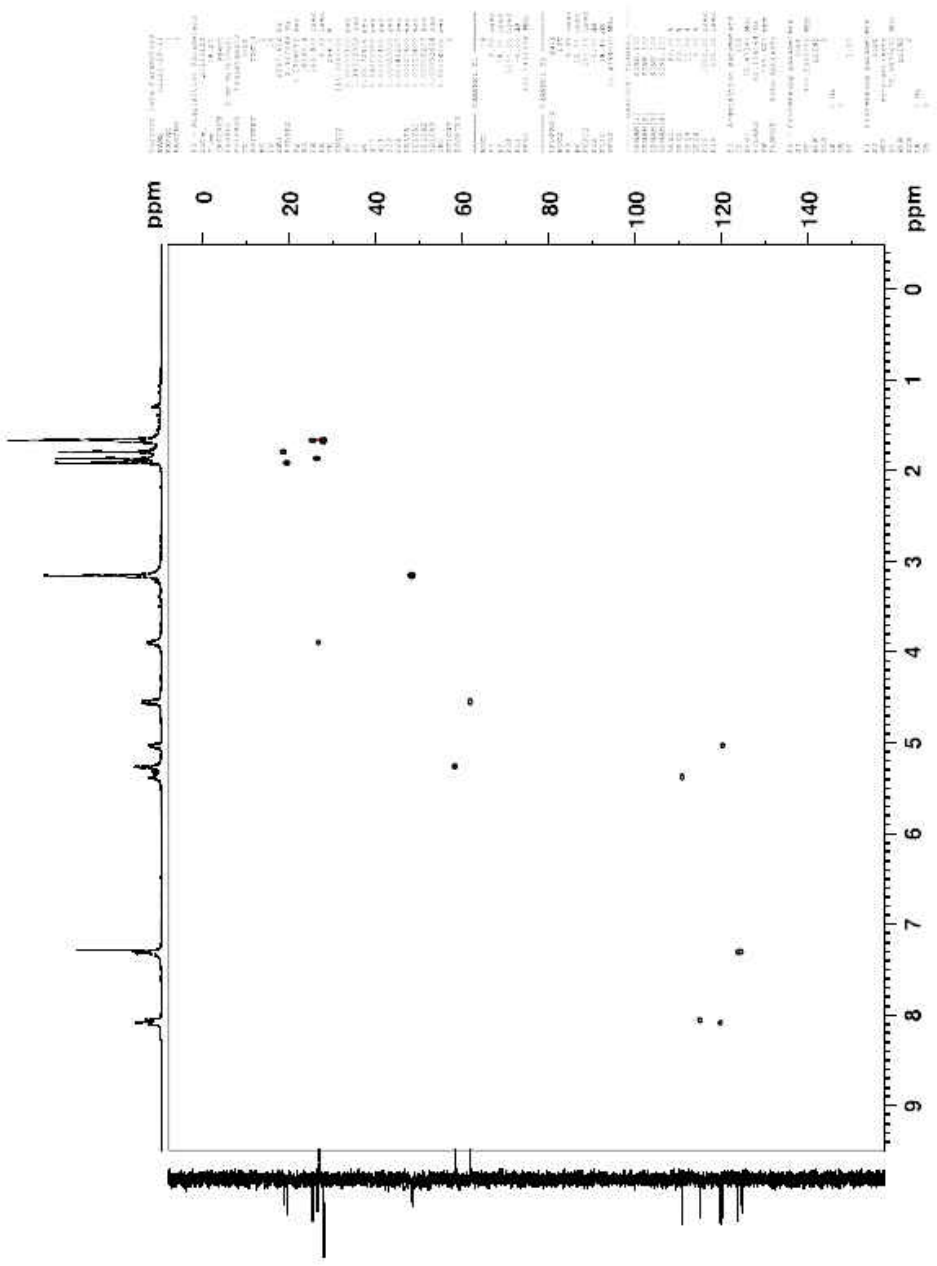
ppm

Proton Spectrum



UNIVERSITY OF WISCONSIN-MILWAUKEE
CHEMISTRY DEPARTMENT

ELEMENTAL ANALYSIS REQUEST (C. H. N.)

1. Sample size for a single analysis is approximately 2 mg. Multiple runs on the same sample can be done. Request it in the comments section. Ash weight is not available.
2. Solids and non-volatile liquids are analyzed in the same manner. Volatile liquids are analyzed differently and may require prior arrangements.
3. Normal error tolerance is ± 0.3%
4. **The sample ID is limited to 13 characters and no extensions**. ID characters may be any combination of letters, numbers, and the underscore character. Do not use Roman numerals.
5. Do not reuse ID codes, as a new result may overwrite an older one.
6. Combustion conditions are; pure oxygen at 30 psi, and 1800° C.

FILL IN THE ITEMS BELOW

Faculty name (print) Dr. Mahmun Hossain

Student name (print) Md. Shanit Asad

Sample ID Sa – 061711

Date submitted 06/20/11

Molecular formula $C_{26}H_{29}BrN_2O_2$

Calculated N% by wt. 5.70(with Br) / 6.81(without Br) 5.7210

Calculated C% by wt. 63.54 (") / 75.87 (") 63.3038

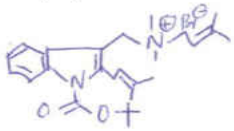
Calculated H% by wt. 8.00 (") / 9.55 (") 8.3621

Comments:

Good Job !

Mark 06/22/2011

A separate page will be returned showing the results.



C.H.N Elemental Analysis

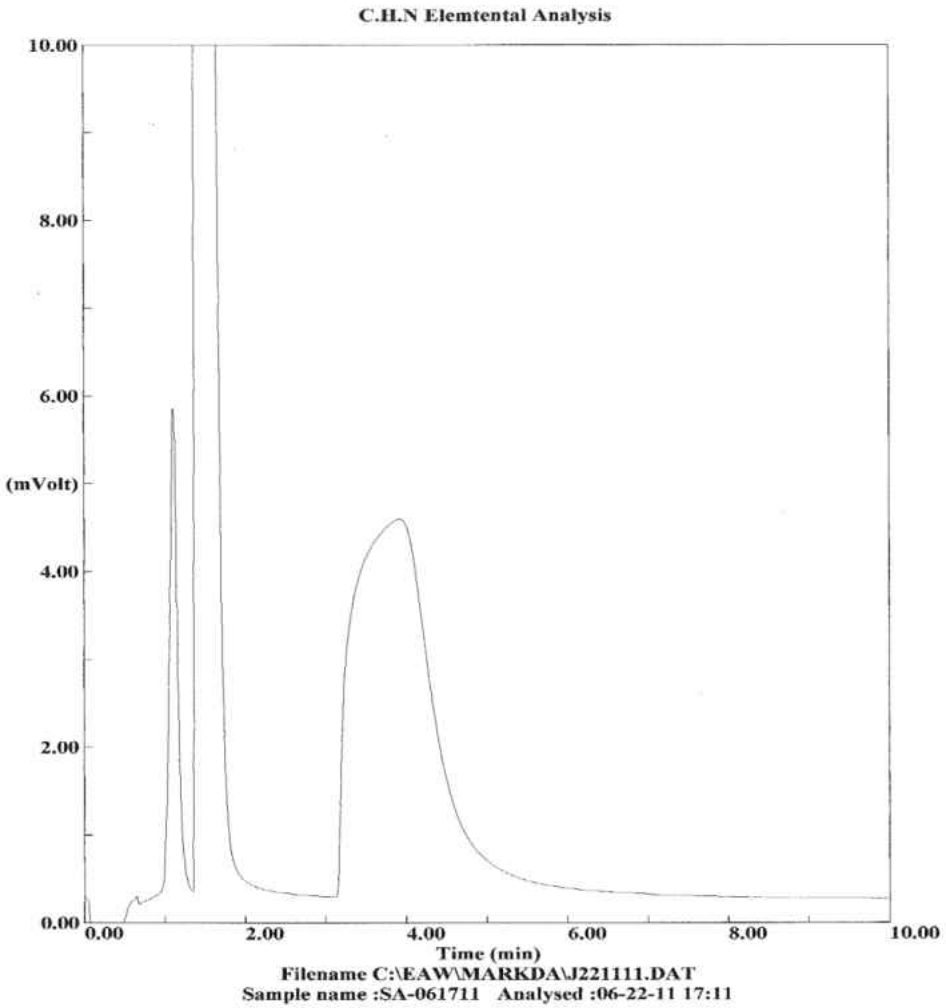
Page: 1 Sample: SA-061711 (J221111)

S/W version : 1.06
erator ID : Mark Wang Company Name : UWM Chemistry
thod Name : Minute8 Method File : M8M0111.MTH
Analysed : 06-22-11 17:11 Printed : 6/22/2011 17:21

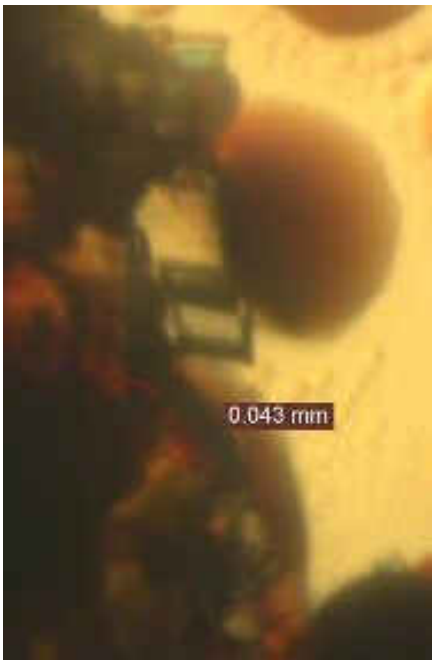
Sample ID : SA-061711 (# 11) Channel : E.A. Channel A
Analysis Type : UnkNown (Area) Sample weight : 2.326
Chromatogram : C:\EAW\MARKDA\J221111.DAT

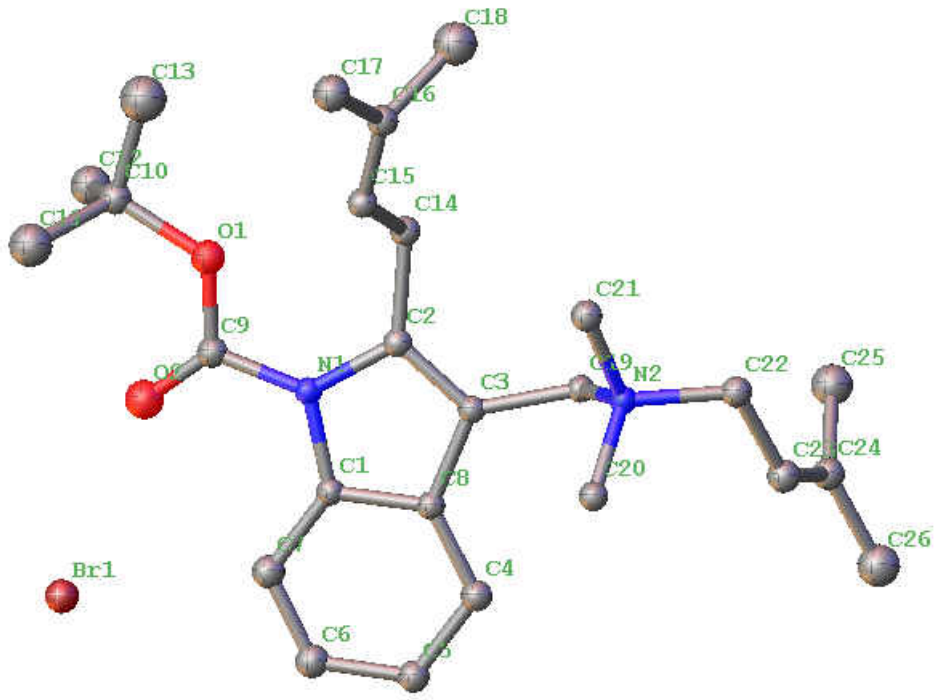
Calib. method : using 'K Factors'

Element Name	Element %	Ret.Time	Area	BC	Area ratio	K factor
Nitrogen	5.7210	1.11	414285	FU	20.073450	.311330E+07
Carbon	63.3038	1.41	8316138	FU	1.000000	.564379E+07
Hydrogen	8.3621	3.92	2895544	RS	2.872047	.148870E+08
Totals	77.3868		11625970			



Crystal Structure data
CCDC 922382





Blocks grown using slow diffusion method: Ethyl Acetate/Hexane

Analyzed by Xray diffraction at UCSD with Arnie Rheingold

Unit Cell Dimensions: a=8.5784(2); b=12.9668(3); c=13.5267(3)Å
α=109.266(2)° β=103.084(2)° γ=107.596(2)°

Triclinic lattice, P1 space group, Z = 2 molecules per unit cell. R1 = 4.39%

Contact: Matthew Huisman, mhuisman@uwm.edu
Authors: Matthew M. Huisman, Sarah Oehm M. Mahmun Hossain, Arnold L. Rheingold
Table 1 Crystal data and structure refinement for Hossain01_0m

Identification code	Hossain01_0m
Empirical formula	C26H39N2O2Br
Formula weight	491.50
Temperature/K	273.15
Crystal system	triclinic
Space group	P1
a/Å	8.5784(2)
b/Å	12.9668(3)
c/Å	13.5267(3)
α/°	109.266(2)
β/°	103.084(2)
γ/°	107.596(2)
Volume/Å3	1261.86(5)
Z	2
ρcalcmg/mm3	1.294
m/mm 1	1.653
F(000)	520.0
Crystal size/mm3	0.3 × 0.24 × 0.18
2Θ range for data collection	3.42 to 63.92°
Index ranges	-12 ≤ h ≤ 12, -19 ≤ k ≤ 19, -20 ≤ l ≤ 20
Reflections collected	23123
Independent reflections	16463[R(int) = 0.0238]
Data/restraints/parameters	16463/3/577
Goodness-of-fit on F2	0.917
Final R indexes [I>=2σ (I)]	R1 = 0.0440, wR2 = 0.1103
Final R indexes [all data]	R1 = 0.0729, wR2 = 0.1451
Largest diff. peak/hole / e Å-3	0.94/-0.52
Flack parameter	0.21(2)

chemical_name_systematic : N-((1-(tert-butoxycarbonyl)-2-(3-methylbut-2-en-1-yl)-1H-indol-3-yl)methyl)-N,N,3-trimethylbut-e-en-1 aminium bromide

_chemical_name_common Compound synonym: boc protected 2 isoprenyl N isoprenyl gramine salt

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          data_hossain1

_audit_creation_method              SHELXL-97
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 'C26 H39 Br N2 O2'
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 'H'  'H'   0.0000   0.0000
 'International Tables Vol C Tables 4.2.6.8 and 6.1.1.4'
 'N'  'N'   0.0061   0.0033
 'International Tables Vol C Tables 4.2.6.8 and 6.1.1.4'
 'O'  'O'   0.0106   0.0060
 'International Tables Vol C Tables 4.2.6.8 and 6.1.1.4'
 'Br'  'Br'  -0.2901   2.4595
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 'x, y, z'
 '-x, -y, -z'

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_cell_length_c                    13.5267(3)
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_cell_angle_beta                  103.084(2)
_cell_angle_gamma                 107.596(2)
_cell_volume                      1261.86(5)
_cell_formula_units_Z             2
_cell_measurement_temperature     100(2)
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_computing_molecular_graphics     ?
_computing_publication_material   ?

_refine_special_details
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 Refinement of F^2^ against ALL reflections.  The weighted R-factor
wR and
 goodness of fit S are based on F^2^, conventional R-factors R are
based
 on F, with F set to zero for negative F^2^. The threshold
expression of
```

```
 F^2^ > 2sigma(F^2^) is used only for calculating R-factors(gt) etc.
and is
 not relevant to the choice of reflections for refinement.  R-
factors based
 on F^2^ are statistically about twice as large as those based on F,
and R-
 factors based on ALL data will be even larger.
;

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 'calc w=1/[\s^2^(Fo^2^)+(0.0514P)^2^+0.0082P] where
P=(Fo^2^+2Fc^2^)/3'
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_atom_sites_solution_hydrogens    geom
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_refine_ls_extinction_method      none
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O1 O 0.09717(16) 0.64626(11) 0.35352(10) 0.0225(2) Uani 1 1 d . . .
O2 O -0.14268(17) 0.66066(13) 0.26027(10) 0.0300(3) Uani 1 1 d . . .
N1 N -0.00475(17) 0.77056(11) 0.44977(11) 0.0160(2) Uani 1 1 d . . .
N2 N 0.21275(16) 0.96019(11) 0.83787(11) 0.0154(2) Uani 1 1 d . . .
C1 C -0.1248(2) 0.82304(14) 0.46308(13) 0.0162(3) Uani 1 1 d . . .
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C2 C -0.2837(2) 0.80211(15) 0.38790(14) 0.0198(3) Uani 1 1 d . . .
H2A H -0.3311 0.7422 0.3128 0.024 Uiso 1 1 calc R . .
C3 C -0.3704(2) 0.87209(15) 0.42681(14) 0.0213(3) Uani 1 1 d . . .
H3A H -0.4788 0.8600 0.3770 0.026 Uiso 1 1 calc R . .
C4 C -0.3024(2) 0.95980(15) 0.53715(14) 0.0208(3) Uani 1 1 d . . .
H4A H -0.3648 1.0065 0.5610 0.025 Uiso 1 1 calc R . .
C5 C -0.1464(2) 0.97930(15) 0.61168(14) 0.0183(3) Uani 1 1 d . . .
H5A H -0.1004 1.0388 0.6869 0.022 Uiso 1 1 calc R . .
C6 C -0.05673(19) 0.90973(13) 0.57441(13) 0.0153(3) Uani 1 1 d . . .
C7 C 0.10789(19) 0.90980(13) 0.62936(12) 0.0148(3) Uani 1 1 d . . .
C8 C 0.13886(19) 0.82623(13) 0.55342(13) 0.0153(3) Uani 1 1 d . . .
C9 C 0.2969(2) 0.79779(14) 0.57135(13) 0.0177(3) Uani 1 1 d . . .
H9A H 0.3909 0.8621 0.6422 0.021 Uiso 1 1 calc R . .
H9B H 0.3399 0.7975 0.5093 0.021 Uiso 1 1 calc R . .
C10 C 0.2635(2) 0.67882(15) 0.57709(14) 0.0195(3) Uani 1 1 d . . .
H10A H 0.1449 0.6222 0.5468 0.023 Uiso 1 1 calc R . .
C11 C 0.3849(2) 0.64669(15) 0.62055(15) 0.0226(3) Uani 1 1 d . . .
C12 C 0.3380(3) 0.52332(17) 0.61499(18) 0.0306(4) Uani 1 1 d . . .
H12A H 0.2114 0.4761 0.5749 0.046 Uiso 1 1 calc R . .
H12B H 0.3704 0.5286 0.6913 0.046 Uiso 1 1 calc R . .
H12C H 0.4015 0.4846 0.5751 0.046 Uiso 1 1 calc R . .
C13 C 0.5772(2) 0.72660(18) 0.6740(2) 0.0356(5) Uani 1 1 d . . .
H13A H 0.5940 0.8090 0.6878 0.053 Uiso 1 1 calc R . .
H13B H 0.6370 0.6993 0.6237 0.053 Uiso 1 1 calc R . .
H13C H 0.6263 0.7238 0.7454 0.053 Uiso 1 1 calc R . .
C14 C 0.2305(2) 0.99903(14) 0.74480(12) 0.0159(3) Uani 1 1 d . . .
H14A H 0.2119 1.0737 0.7611 0.019 Uiso 1 1 calc R . .
H14B H 0.3522 1.0179 0.7466 0.019 Uiso 1 1 calc R . .
C15 C 0.0274(2) 0.91355(16) 0.83170(14) 0.0210(3) Uani 1 1 d . . .
H15A H -0.0457 0.8448 0.7589 0.031 Uiso 1 1 calc R . .
H15B H -0.0157 0.9767 0.8400 0.031 Uiso 1 1 calc R . .
H15C H 0.0218 0.8885 0.8922 0.031 Uiso 1 1 calc R . .
C16 C 0.2782(2) 0.86466(14) 0.83030(14) 0.0198(3) Uani 1 1 d . . .
H16A H 0.2072 0.7948 0.7580 0.030 Uiso 1 1 calc R . .
H16B H 0.2693 0.8416 0.8916 0.030 Uiso 1 1 calc R . .
H16C H 0.4010 0.8948 0.8364 0.030 Uiso 1 1 calc R . .
C17 C 0.3288(2) 1.06740(14) 0.95033(13) 0.0182(3) Uani 1 1 d . . .
H17A H 0.3337 1.0402 1.0108 0.022 Uiso 1 1 calc R . .
H17B H 0.4495 1.0995 0.9507 0.022 Uiso 1 1 calc R . .
C18 C 0.2668(2) 1.16523(15) 0.97513(14) 0.0216(3) Uani 1 1 d . . .
H18A H 0.1619 1.1499 0.9910 0.026 Uiso 1 1 calc R . .
C19 C 0.3441(2) 1.27206(15) 0.97723(14) 0.0226(3) Uani 1 1 d . . .
C20 C 0.2701(3) 1.36419(18) 1.00961(17) 0.0341(4) Uani 1 1 d . . .
H20A H 0.1613 1.3294 1.0214 0.051 Uiso 1 1 calc R . .
H20B H 0.2454 1.3897 0.9494 0.051 Uiso 1 1 calc R . .
H20C H 0.3554 1.4335 1.0791 0.051 Uiso 1 1 calc R . .
C21 C 0.5024(3) 1.31118(17) 0.94698(17) 0.0314(4) Uani 1 1 d . . .
H21A H 0.5436 1.2470 0.9283 0.047 Uiso 1 1 calc R . .
H21B H 0.5955 1.3832 1.0108 0.047 Uiso 1 1 calc R . .
H21C H 0.4717 1.3289 0.8820 0.047 Uiso 1 1 calc R . .
C22 C -0.0258(2) 0.68790(14) 0.34429(13) 0.0178(3) Uani 1 1 d . . .
C23 C 0.1041(2) 0.55761(15) 0.25343(14) 0.0217(3) Uani 1 1 d . . .
C24 C 0.2593(3) 0.5350(2) 0.30555(17) 0.0400(5) Uani 1 1 d . . .
H24A H 0.3653 0.6100 0.3427 0.060 Uiso 1 1 calc R . .
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H24B H 0.2757 0.4755 0.2467 0.060 Uiso 1 1 calc R . .
H24C H 0.2371 0.5050 0.3609 0.060 Uiso 1 1 calc R . .
C25 C -0.0627(3) 0.44514(18) 0.1996(2) 0.0422(5) Uani 1 1 d . . .
H25A H -0.1615 0.4623 0.1683 0.063 Uiso 1 1 calc R . .
H25B H -0.0828 0.4166 0.2562 0.063 Uiso 1 1 calc R . .
H25C H -0.0522 0.3834 0.1393 0.063 Uiso 1 1 calc R . .
C26 C 0.1391(3) 0.61289(19) 0.17451(17) 0.0333(4) Uani 1 1 d . . .
H26A H 0.0393 0.6299 0.1447 0.050 Uiso 1 1 calc R . .
H26B H 0.1552 0.5572 0.1124 0.050 Uiso 1 1 calc R . .
H26C H 0.2455 0.6876 0.2151 0.050 Uiso 1 1 calc R . .

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0.01419(7)
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O2 0.0271(6) 0.0448(8) 0.0162(6) 0.0063(5) 0.0055(5) 0.0216(6)
N1 0.0161(6) 0.0158(6) 0.0153(6) 0.0056(5) 0.0055(5) 0.0067(5)
N2 0.0134(6) 0.0171(6) 0.0161(6) 0.0079(5) 0.0057(5) 0.0056(5)
C1 0.0153(7) 0.0169(7) 0.0191(7) 0.0093(6) 0.0081(6) 0.0068(6)
C2 0.0182(7) 0.0213(8) 0.0189(7) 0.0087(6) 0.0055(6) 0.0078(6)
C3 0.0160(7) 0.0259(8) 0.0250(8) 0.0142(7) 0.0065(6) 0.0096(7)
C4 0.0198(7) 0.0236(8) 0.0264(8) 0.0140(7) 0.0115(6) 0.0124(7)
C5 0.0196(7) 0.0200(8) 0.0205(8) 0.0110(6) 0.0099(6) 0.0104(6)
C6 0.0155(7) 0.0163(7) 0.0173(7) 0.0094(6) 0.0076(6) 0.0071(6)
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C8 0.0137(6) 0.0154(7) 0.0172(7) 0.0080(6) 0.0062(5) 0.0050(6)
C9 0.0143(7) 0.0198(8) 0.0194(7) 0.0080(6) 0.0073(6) 0.0071(6)
C10 0.0146(7) 0.0205(8) 0.0215(8) 0.0075(6) 0.0066(6) 0.0063(6)
C11 0.0199(8) 0.0238(8) 0.0260(8) 0.0113(7) 0.0099(7) 0.0096(7)
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C16 0.0219(8) 0.0184(8) 0.0207(8) 0.0094(6) 0.0077(6) 0.0093(6)
C17 0.0172(7) 0.0188(7) 0.0156(7) 0.0066(6) 0.0045(6) 0.0054(6)
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C19 0.0286(9) 0.0215(8) 0.0149(7) 0.0052(6) 0.0070(6) 0.0103(7)
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C21 0.0311(10) 0.0230(9) 0.0340(10) 0.0109(8) 0.0116(8) 0.0048(8)
C22 0.0171(7) 0.0187(7) 0.0193(7) 0.0090(6) 0.0086(6) 0.0071(6)
C23 0.0255(8) 0.0209(8) 0.0191(8) 0.0048(6) 0.0098(6) 0.0128(7)
C24 0.0516(13) 0.0498(13) 0.0276(10) 0.0100(9) 0.0125(9) 0.0407(11)
C25 0.0417(12) 0.0240(10) 0.0461(13) -0.0002(9) 0.0241(10) 0.0057(9)
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planes)
 are estimated using the full covariance matrix.  The cell esds are
taken
 into account individually in the estimation of esds in distances,
angles
 and torsion angles; correlations between esds in cell parameters
are only
 used when they are defined by crystal symmetry.  An approximate
(isotropic)
 treatment of cell esds is used for estimating esds involving l.s.
planes.
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O1 C23 1.4860(19) . ?
O2 C22 1.195(2) . ?
N1 C22 1.405(2) . ?
N1 C1 1.4084(19) . ?
N1 C8 1.4202(19) . ?
N2 C16 1.4909(19) . ?
N2 C15 1.4923(19) . ?
N2 C14 1.5234(19) . ?
N2 C17 1.523(2) . ?
C1 C2 1.390(2) . ?
C1 C6 1.400(2) . ?
C2 C3 1.386(2) . ?
C2 H2A 0.9500 . ?
C3 C4 1.395(2) . ?
C3 H3A 0.9500 . ?
C4 C5 1.374(2) . ?
C4 H4A 0.9500 . ?
C5 C6 1.398(2) . ?
C5 H5A 0.9500 . ?
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C7 C8 1.365(2) . ?
C7 C14 1.487(2) . ?
C8 C9 1.499(2) . ?
C9 C10 1.513(2) . ?
C9 H9A 0.9900 . ?
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C10 C11 1.323(2) . ?
C10 H10A 0.9500 . ?
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C11 C13 1.500(3) . ?
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C12 H12B 0.9800 . ?
C12 H12C 0.9800 . ?
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C17 H17B 0.9900 . ?
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C18 H18A 0.9500 . ?
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C21 H21C 0.9800 . ?
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C16 N2 C15 108.57(12) . . ?
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C15 N2 C14 112.22(12) . . ?
C16 N2 C17 107.51(12) . . ?
C15 N2 C17 110.41(12) . . ?
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C14 N2 C17 108.15(11) . . ?
C2 C1 C6 121.28(14) . . ?
2 C1 N1 131.41(14) . . ?
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C3 C2 C1 117.41(15) . . ?
C3 C2 H2A 121.3 . . ?
C1 C2 H2A 121.3 . . ?
C2 C3 C4 121.74(15) . . ?
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C4 C3 H3A 119.1 . . ?
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C4 C5 H5A 120.7 . . ?
C6 C5 H5A 120.7 . . ?
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C5 C6 C7 132.10(15) . . ?
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C7 C8 N1 108.08(13) . . ?
C7 C8 C9 127.56(14) . . ?
N1 C8 C9 124.34(13) . . ?
C8 C9 C10 114.05(12) . . ?
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C10 C9 H9A 108.7 . . ?
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C10 C9 H9B 108.7 . . ?
H9A C9 H9B 107.6 . . ?
C11 C10 C9 125.73(15) . . ?
C11 C10 H10A 117.1 . . ?
C9 C10 H10A 117.1 . . ?
C10 C11 C12 121.22(16) . . ?
C10 C11 C13 123.70(16) . . ?
C12 C11 C13 115.03(15) . . ?
C11 C12 H12A 109.5 . . ?
C11 C12 H12B 109.5 . . ?
H12A C12 H12B 109.5 . . ?
C11 C12 H12C 109.5 . . ?
H12A C12 H12C 109.5 . . ?
H12B C12 H12C 109.5 . . ?
C11 C13 H13A 109.5 . . ?
C11 C13 H13B 109.5 . . ?
H13A C13 H13B 109.5 . . ?
C11 C13 H13C 109.5 . . ?
H13A C13 H13C 109.5 . . ?
H13B C13 H13C 109.5 . . ?
C7 C14 N2 115.32(12) . . ?
C7 C14 H14A 108.4 . . ?
N2 C14 H14A 108.4 . . ?
C7 C14 H14B 108.4 . . ?
N2 C14 H14B 108.4 . . ?
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H14A C14 H14B 107.5 . . ?
N2 C15 H15A 109.5 . . ?
N2 C15 H15B 109.5 . . ?
H15A C15 H15B 109.5 . . ?
N2 C15 H15C 109.5 . . ?
H15A C15 H15C 109.5 . . ?
H15B C15 H15C 109.5 . . ?
N2 C16 H16A 109.5 . . ?
N2 C16 H16B 109.5 . . ?
H16A C16 H16B 109.5 . . ?
N2 C16 H16C 109.5 . . ?
H16A C16 H16C 109.5 . . ?
H16B C16 H16C 109.5 . . ?
C18 C17 N2 113.48(13) . . ?
C18 C17 H17A 108.9 . . ?
N2 C17 H17A 108.9 . . ?
C18 C17 H17B 108.9 . . ?
N2 C17 H17B 108.9 . . ?
H17A C17 H17B 107.7 . . ?
C19 C18 C17 126.26(16) . . ?
C19 C18 H18A 116.9 . . ?
C17 C18 H18A 116.9 . . ?
C18 C19 C21 125.49(16) . . ?
C18 C19 C20 119.98(17) . . ?
C21 C19 C20 114.51(16) . . ?
C19 C20 H20A 109.5 . . ?
C19 C20 H20B 109.5 . . ?
H20A C20 H20B 109.5 . . ?
C19 C20 H20C 109.5 . . ?
H20A C20 H20C 109.5 . . ?
H20B C20 H20C 109.5 . . ?
C19 C21 H21A 109.5 . . ?
C19 C21 H21B 109.5 . . ?
H21A C21 H21B 109.5 . . ?
C19 C21 H21C 109.5 . . ?
H21A C21 H21C 109.5 . . ?
H21B C21 H21C 109.5 . . ?
O2 C22 O1 126.88(15) . . ?
O2 C22 N1 122.37(14) . . ?
O1 C22 N1 110.75(13) . . ?
O1 C23 C26 109.67(14) . . ?
O1 C23 C25 109.57(14) . . ?
C26 C23 C25 113.22(17) . . ?
O1 C23 C24 101.88(13) . . ?
C26 C23 C24 111.06(16) . . ?
C25 C23 C24 110.85(17) . . ?
C23 C24 H24A 109.5 . . ?
C23 C24 H24B 109.5 . . ?
H24A C24 H24B 109.5 . . ?
C23 C24 H24C 109.5 . . ?
H24A C24 H24C 109.5 . . ?
H24B C24 H24C 109.5 . . ?
C23 C25 H25A 109.5 . . ?
C23 C25 H25B 109.5 . . ?
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H25A C25 H25B 109.5 . . ?
C23 C25 H25C 109.5 . . ?
H25A C25 H25C 109.5 . . ?
H25B C25 H25C 109.5 . . ?
C23 C26 H26A 109.5 . . ?
C23 C26 H26B 109.5 . . ?
H26A C26 H26B 109.5 . . ?
C23 C26 H26C 109.5 . . ?
H26A C26 H26C 109.5 . . ?
H26B C26 H26C 109.5 . . ?

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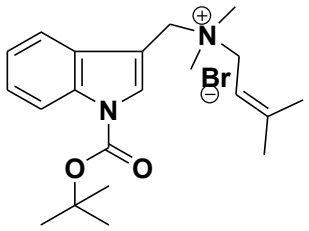
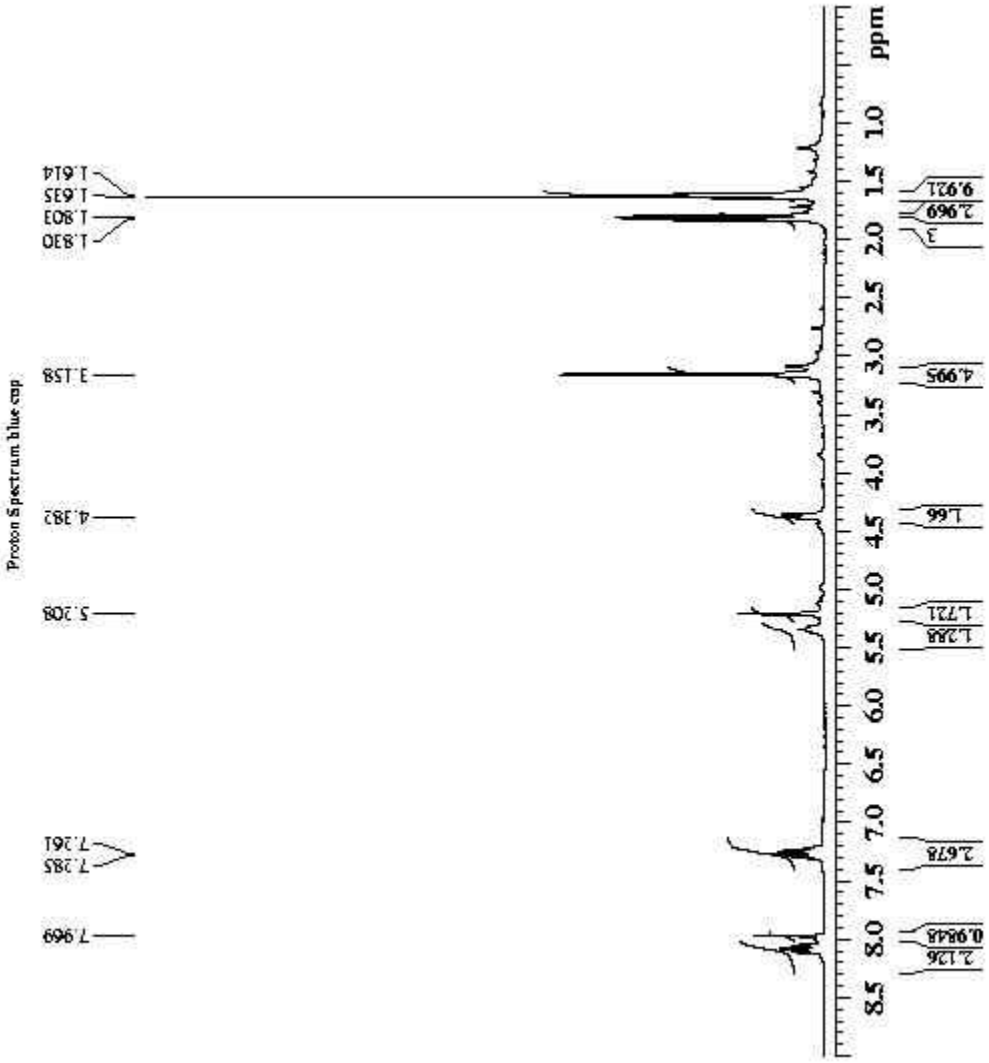
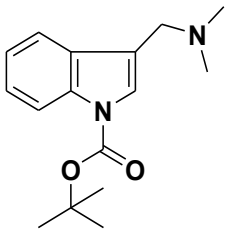


$C_{21}H_{31}BrN_2O_2$
Mol. Wt.: 423.39

^{1}H Name: huis4-25-11 Expno: 4 Procno: 1





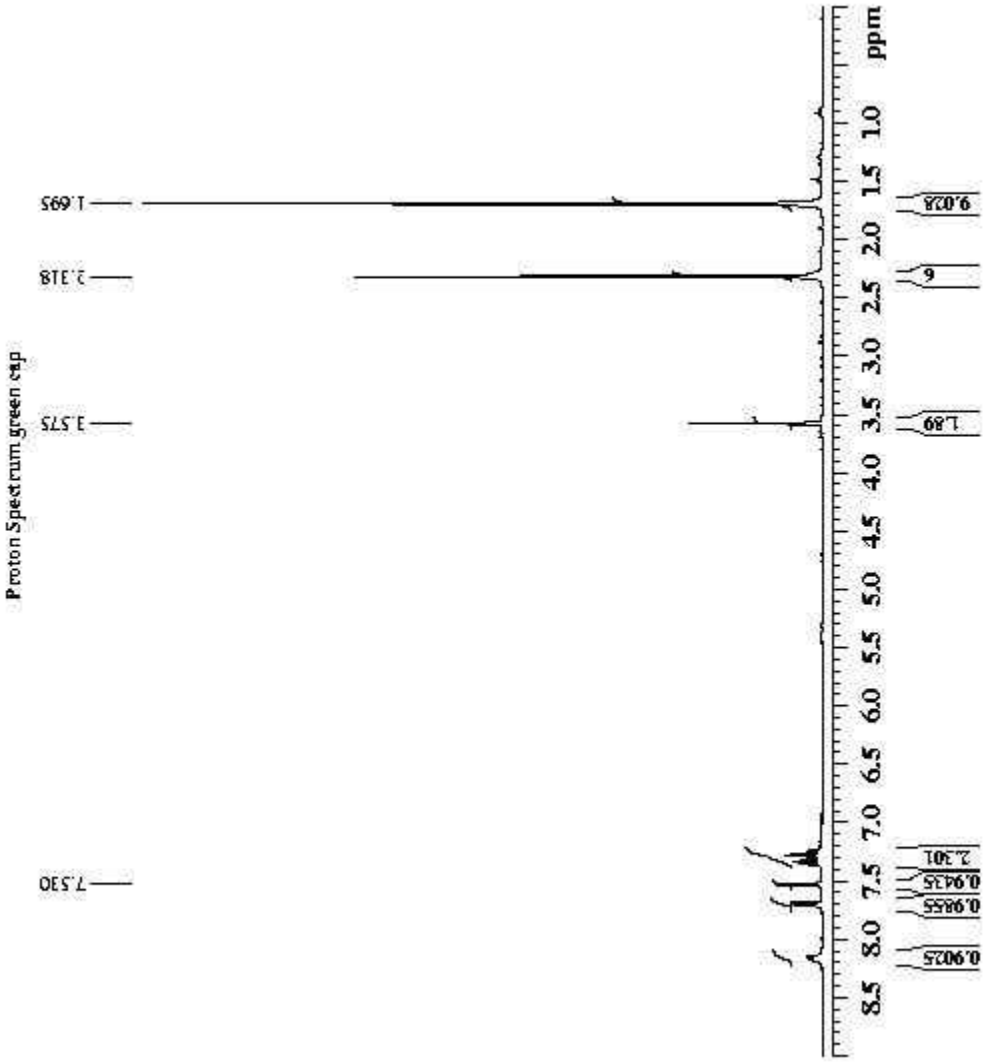
$C_{16}H_{22}N_2O_2$
Mol. Wt.: 274.36

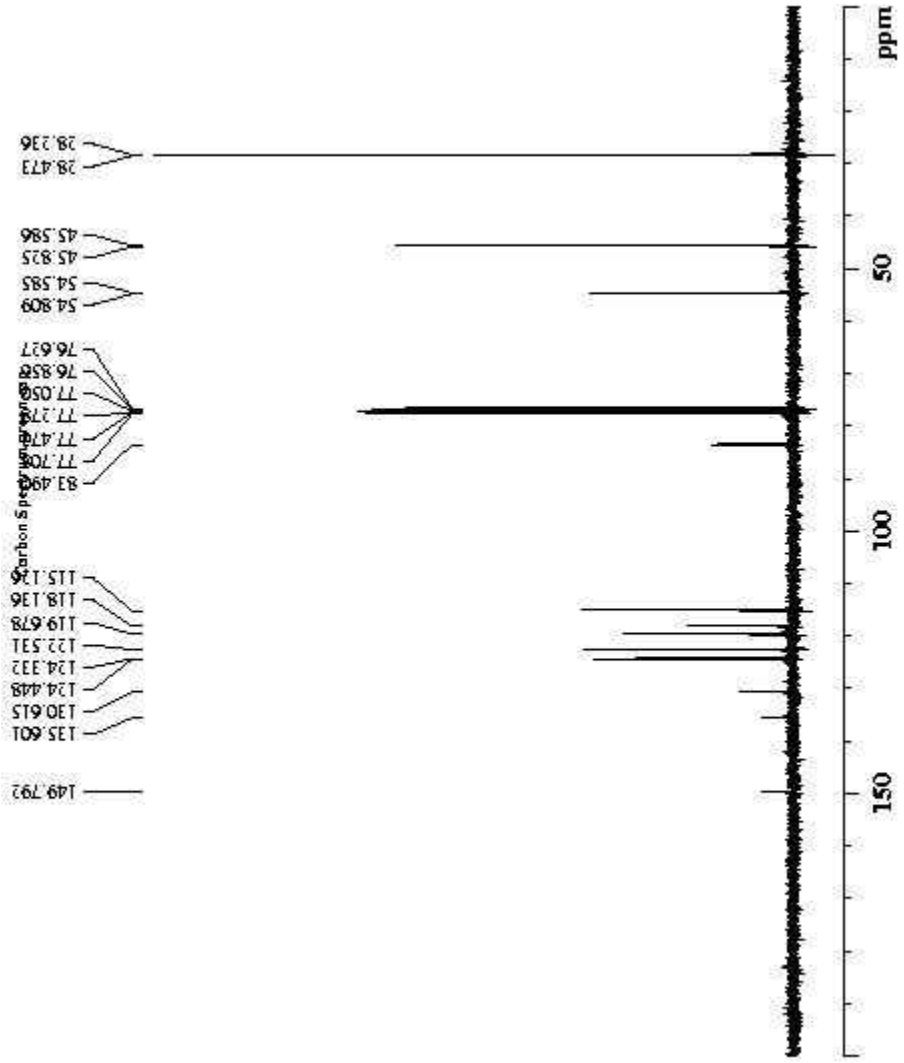
^{1}H Name: huis5-15-12 Expno: 2 Procno: 1

^{13}C Name: huis5-15-12 Expno: 3 Procno: 1

^{13}C HSQC Name: huis5-15-12 Expno: 4 Procno: 1

HRMS

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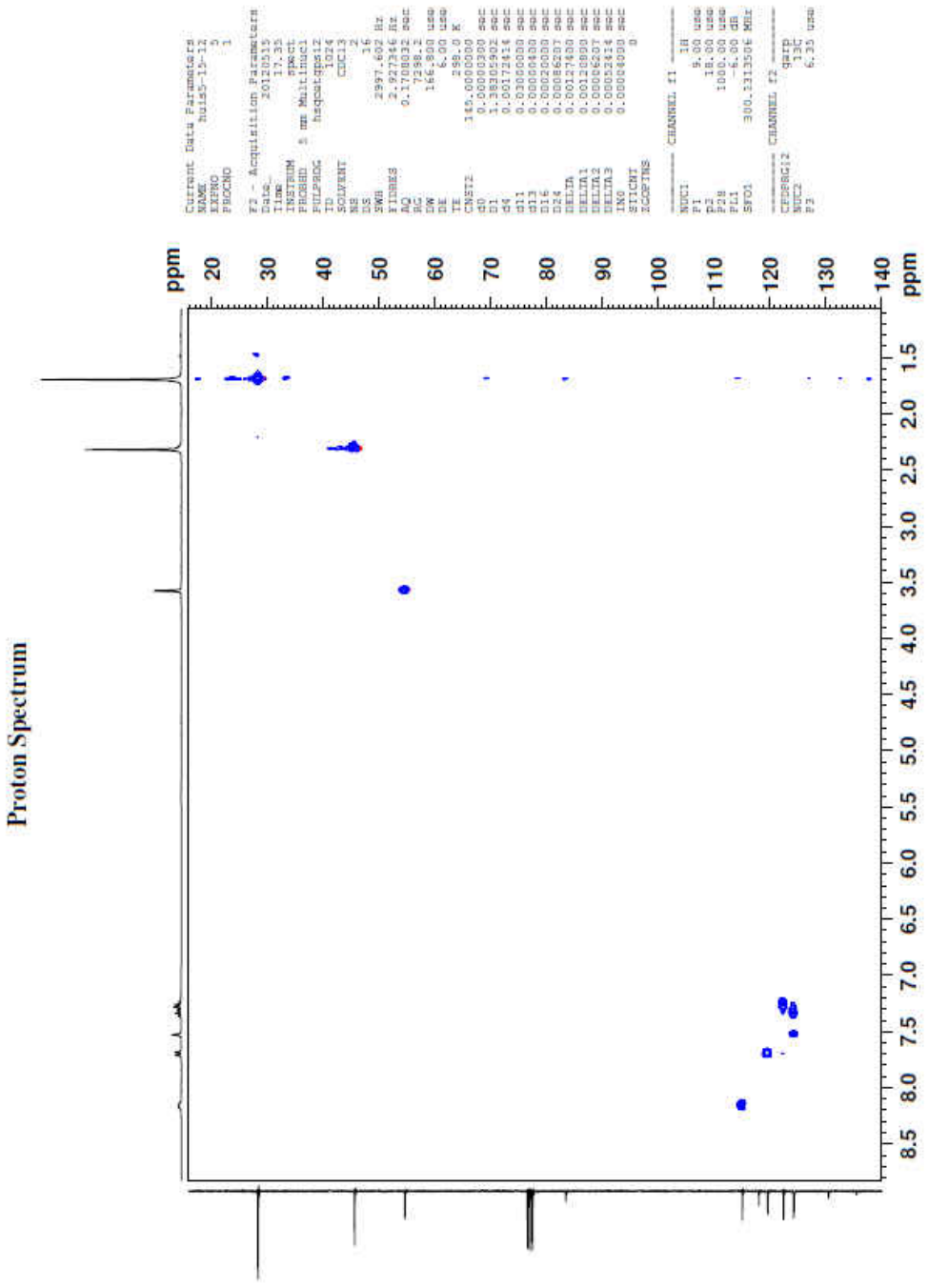
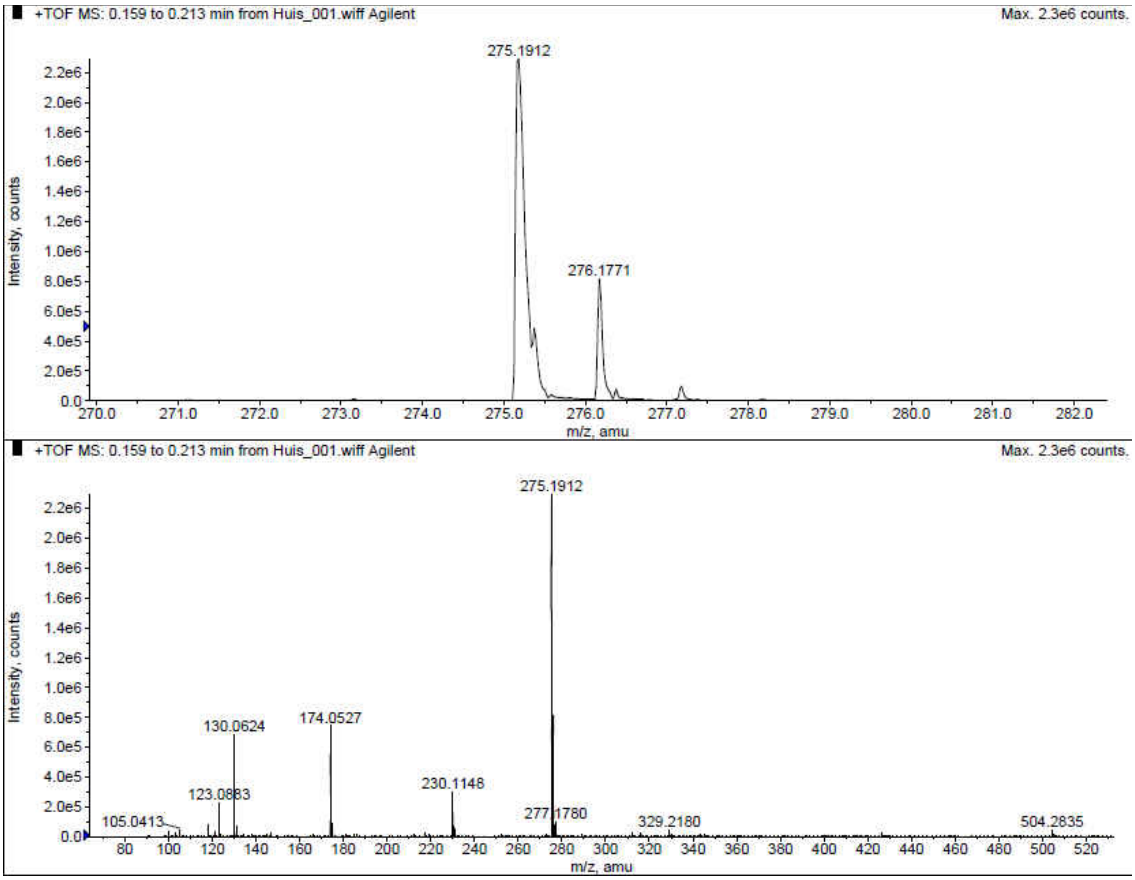

Proton Spectrum
ppm
ppm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Max. 2.3e6 counts.
275.1912
276.1771
Intensity, counts
m/z, amu
+TOF MS: 0.159 to 0.213 min from Huis_001.wiff Agilent
Max. 2.3e6 counts.
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174.0527
230.1148
123.0883
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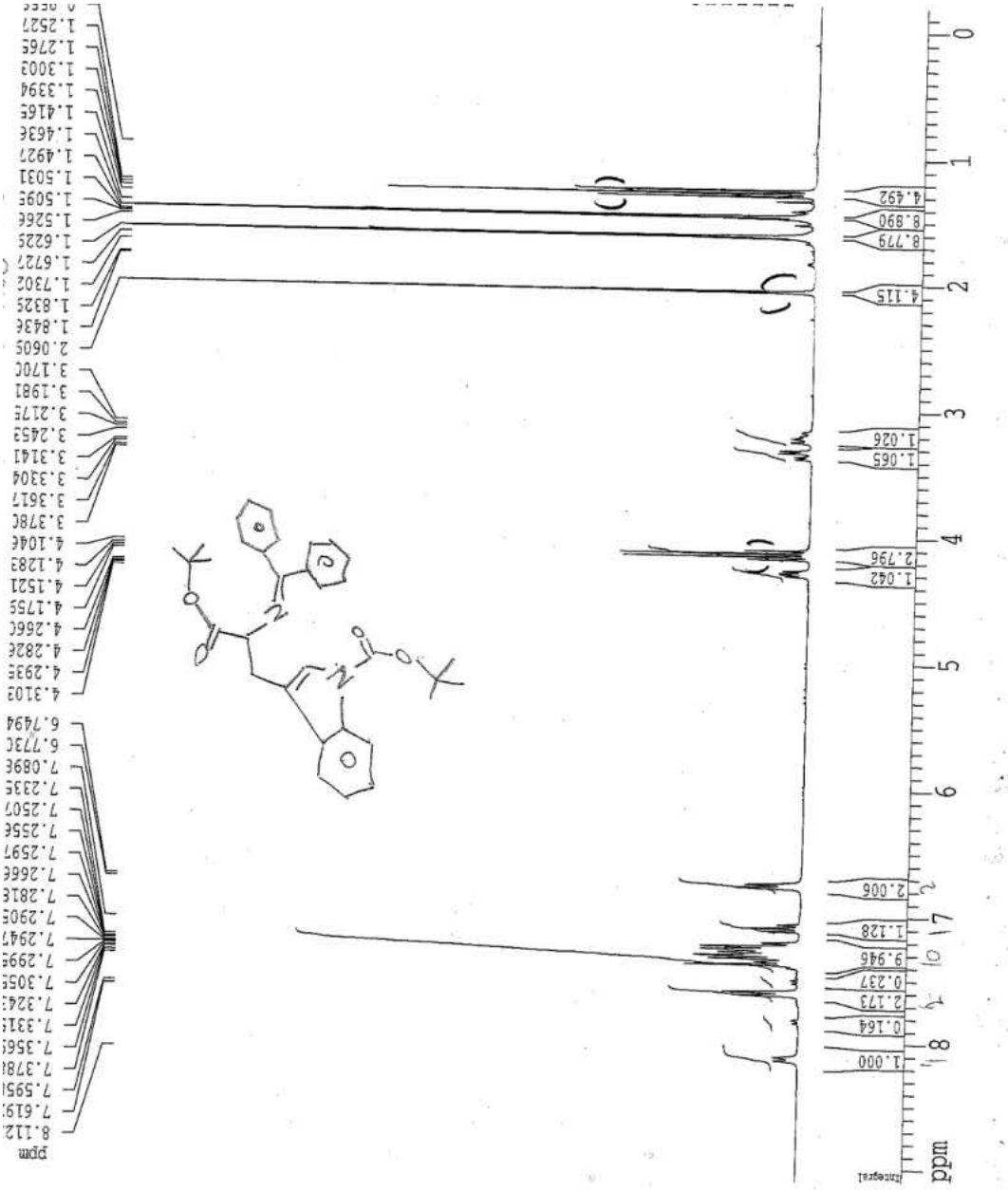
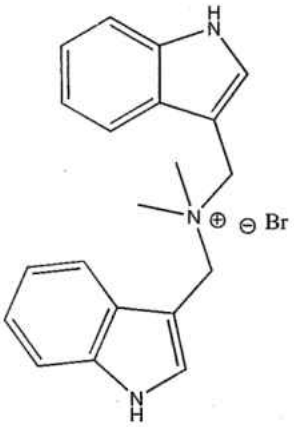
329.2180
504.2835
Intensity, counts
m/z, amu

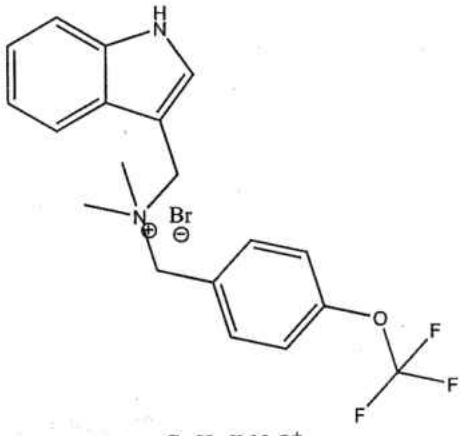
^{1}H NMR of Boc protected Tryptophan N-diphenyl methylene t-butyl ester



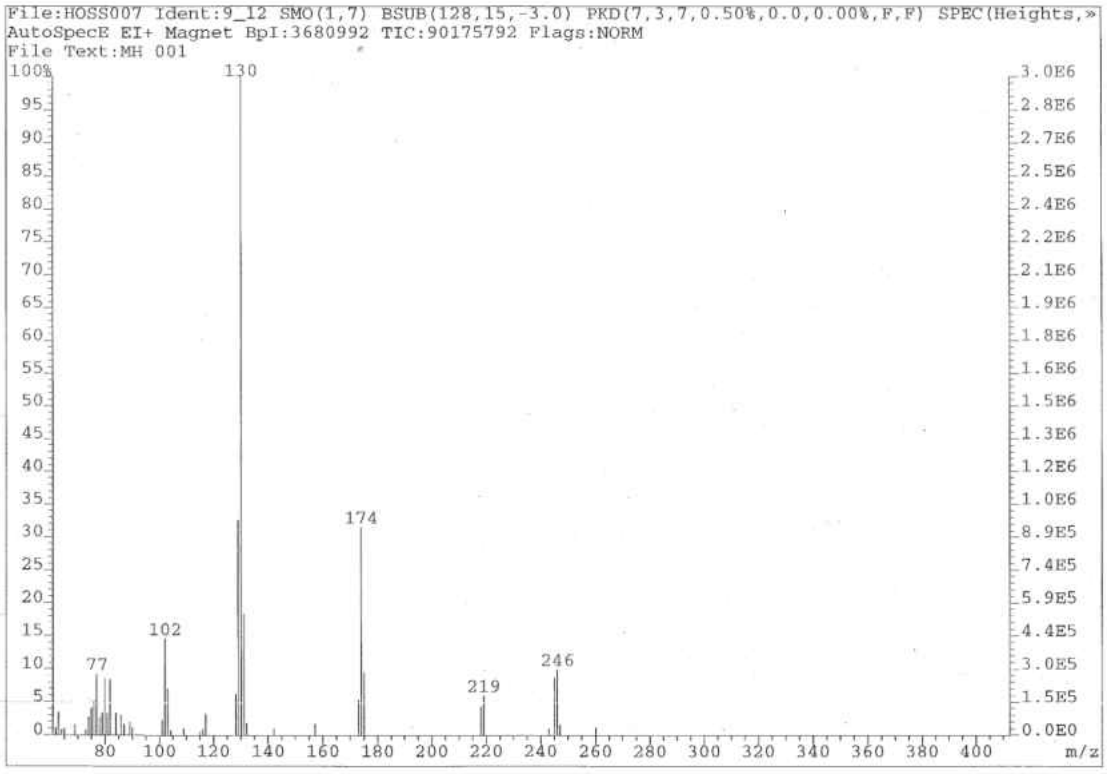
N+ salt charicteration



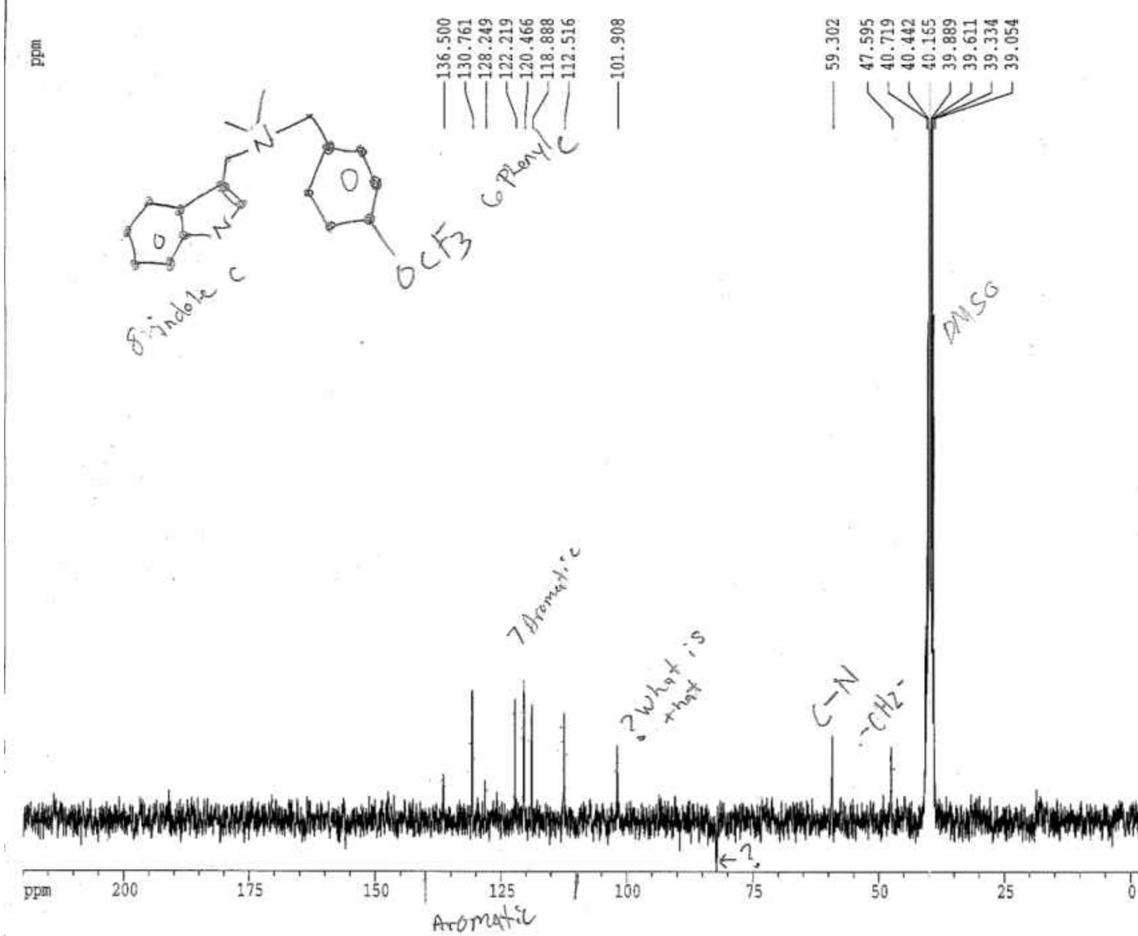
$C_{20}H_{22}BrN_3$
Exact Mass: 383.1
Mol. Wt.: 384.31
m/e: 383.10 (100.0%), 385.10 (97.5%), 384.10 (22.7%), 386.10 (21.3%), 387.10 (2.4%), 385.11 (2.3%), 386.09 (1.1%)
C, 62.50; H, 5.77; Br, 20.79; N, 10.93



$C_{19}H_{20}F_3N_2O^+$
Exact Mass: 349.15
Mol. Wt.: 349.37
m/e: 349.15 (100.0%), 350.16 (20.8%), 351.16 (2.3%)
C, 65.32; H, 5.77; F, 16.31; N, 8.02; O, 4.58



Carbon Spectrum
N+ salt rinsedCH2Cl2 and filtered over vac in d6 DMSO



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EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
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PULPROG zgdc
TD 65536
SOLVENT CDCl3
NS 500
DS 0
SWH 18115.941 Hz
FIDRES 0.276427 Hz
AQ 1.8088436 sec
RG 724.1
DW 27.600 usec
DE 6.00 usec
TE 300.0 K
D1 0.10000000 sec
d11 0.03000000 sec

============ CHANNEL f1 ======
NUC1 13C
P1 6.80 usec
PL1 -6.00 dB
SFO1 75.4758695 MHz

============ CHANNEL f2 ======
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 -6.00 dB
PL12 16.60 dB
SFO2 300.1312005 MHz

F2 - Processing parameters
SI 32768
SF 75.4677567 MHz
WDW EM
SSB 0
LB 3.00 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 22.00 cm
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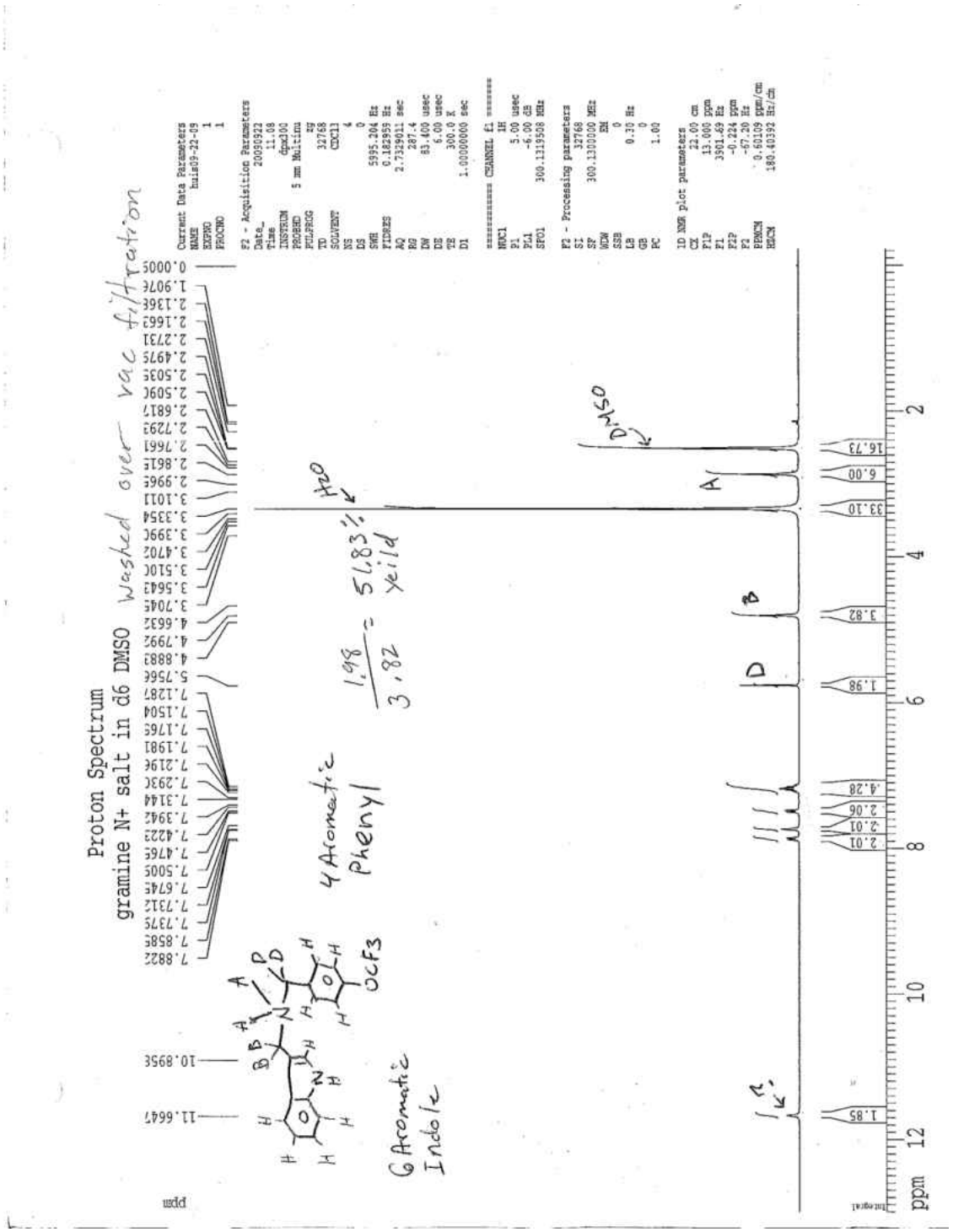
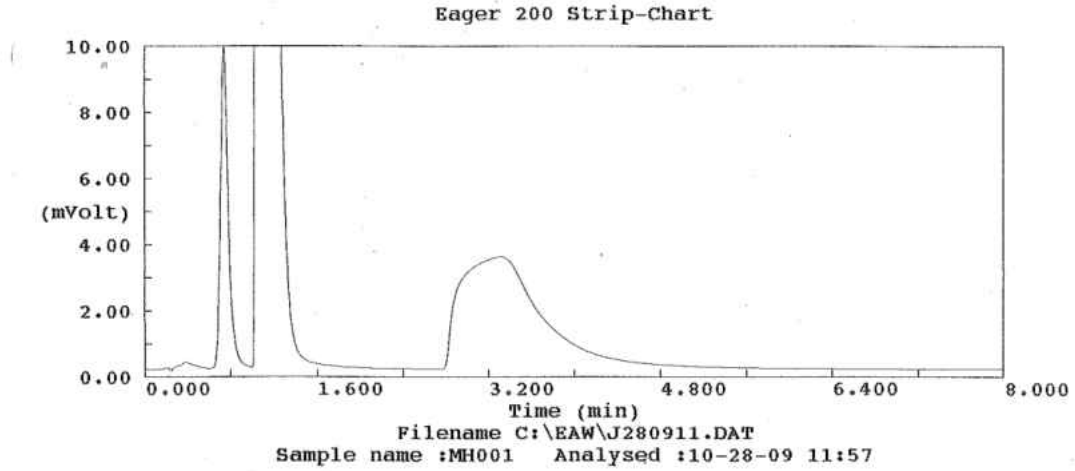
F2P -5.000 ppm
F2 -377.34 Hz
PPMCM 10.22727 ppm/cm
HZCM 771.82935 Hz/cm


Proton Spectrum
gramine N+ salt in d6 DMSO
Washed over vac filtration
Current Data Parameters
NAME
EXPNO
PROCNO
F2 - Acquisition Parameters
Date_
Time
INSTRUM
PROBHD
PULPROG
TD
SOLVENT
NS
DS
SWH
FIDRES
AQ
RG
DW
DE
TE
D1
F2 - Processing parameters
SI
SF
WDW
SSB
LB
GB
PC
1D NMR plot parameters
CX
F1P
F1
F2P
F2
PPMCM
HZCM
H2O
DMSO
4 Aromatic
Phenyl
1,98 = 51,83% yeild
3,82
6 Aromatic
Indole
ppm



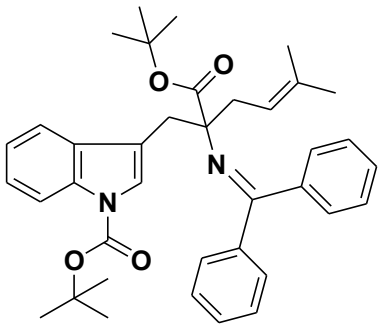
Eager 200 Report

S/. version : 1.06
Operator ID : LAIB
Company Name : UWM Chemistry
Method Name : Minute8
Method File : MINUTE8.MTH
Analysed : 10-28-09 11:57
Printed : 10/28/2009 12:06

Sample ID : MH001 (# 11)
Channel : E.A. Channel A
Analysis Type : UnkNown (Area)
Sample weight : 2.006
Chromatogram : C:\EAW\J280911.DAT

Calib. method : using 'K Factors'

Element Name	Element %	Ret.Time	Area	BC	Area ratio	K factor
Nitrogen	10.0081	0.74	498618	FU	13.387840	.247099E+07
Carbon	59.4638	1.06	6675412	FU	1.000000	.559125E+07
Hydrogen	5.6060	3.31	1841161	RS	3.625653	.163442E+08

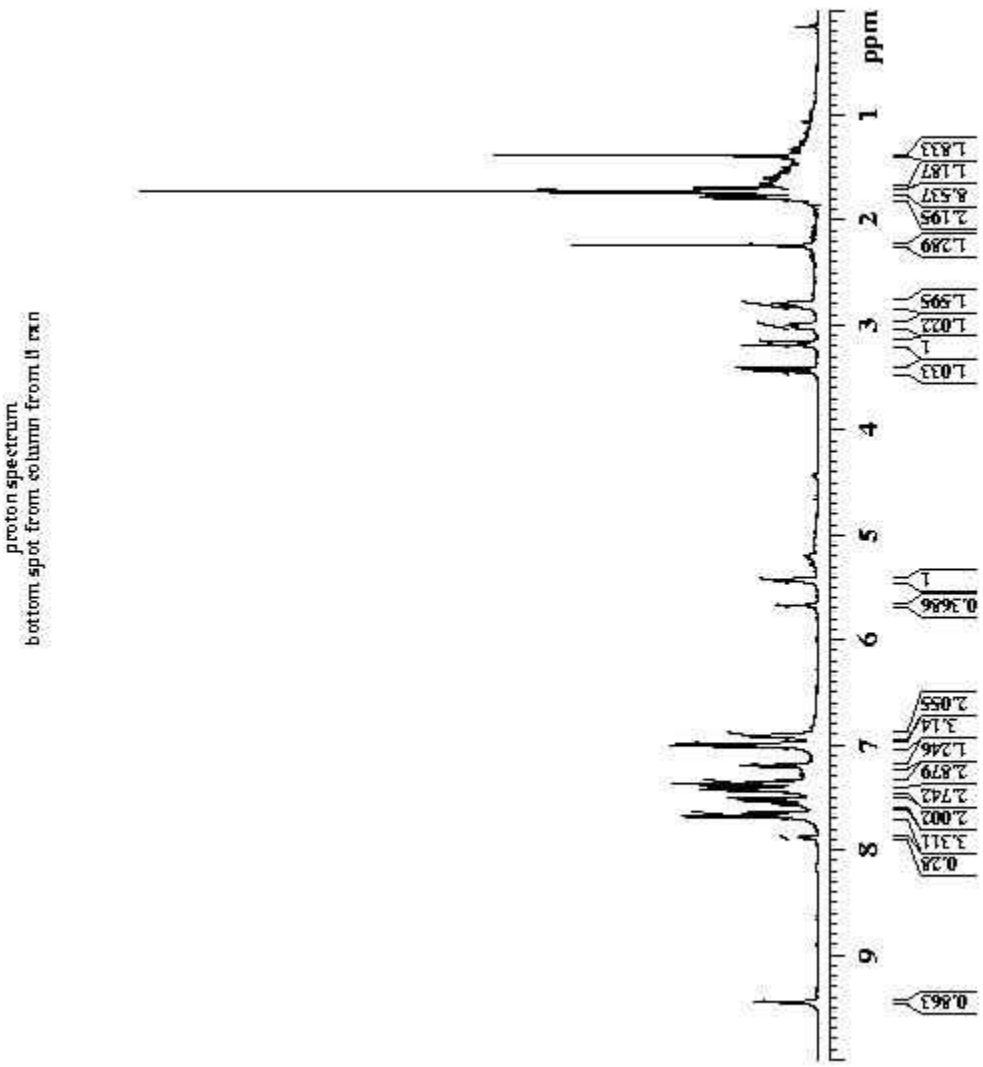


$C_{38}H_{44}N_2O_4$
Mol. Wt.: 592.77

^{1}H Name: huis7-20-10 Expno: 6 Procno: 1

^{13}C Name: huis7-20-10 Expno: 7 Procno: 1

^{13}C HSQC Name: huis7-20-10 Expno: 8 Procno: 1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bottom spot from column from 1H run
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2
3
4
5
6
7
8
9
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2.002
2.742
2.879
1.246
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1
1.022
1.595
1.289
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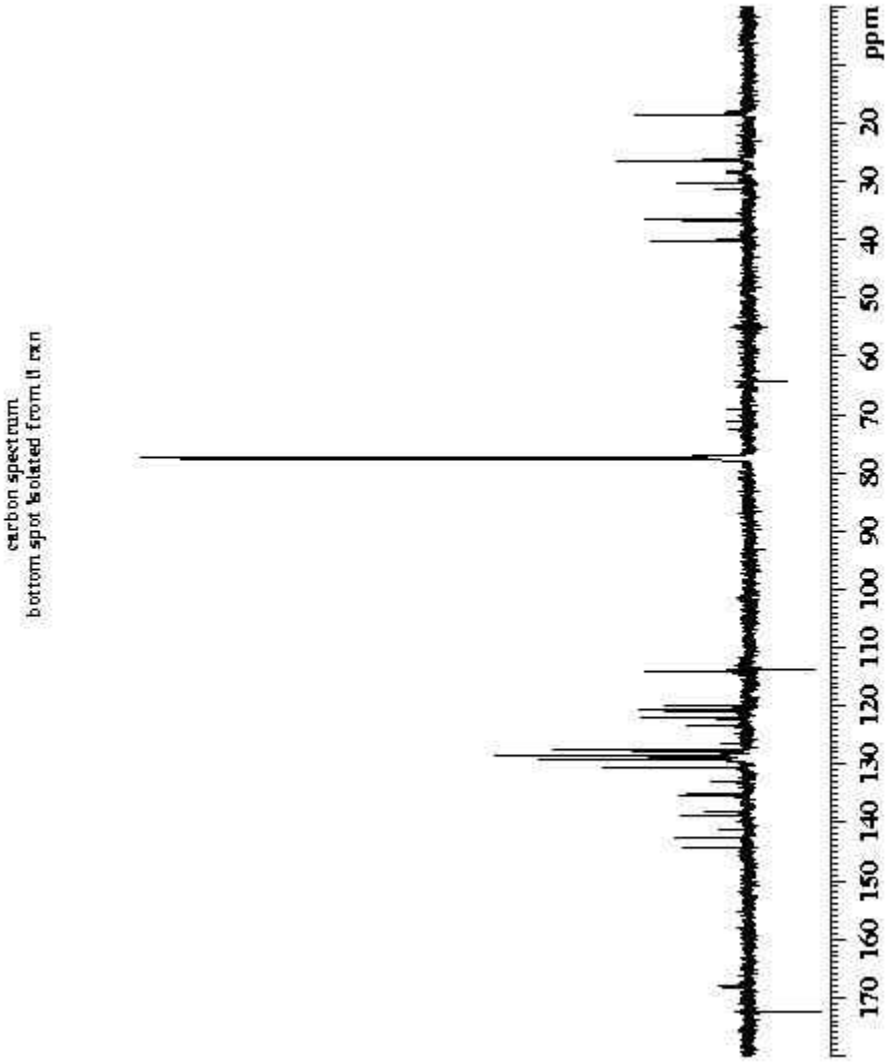
carbon spectrum
bottom spot isolated from tlc run
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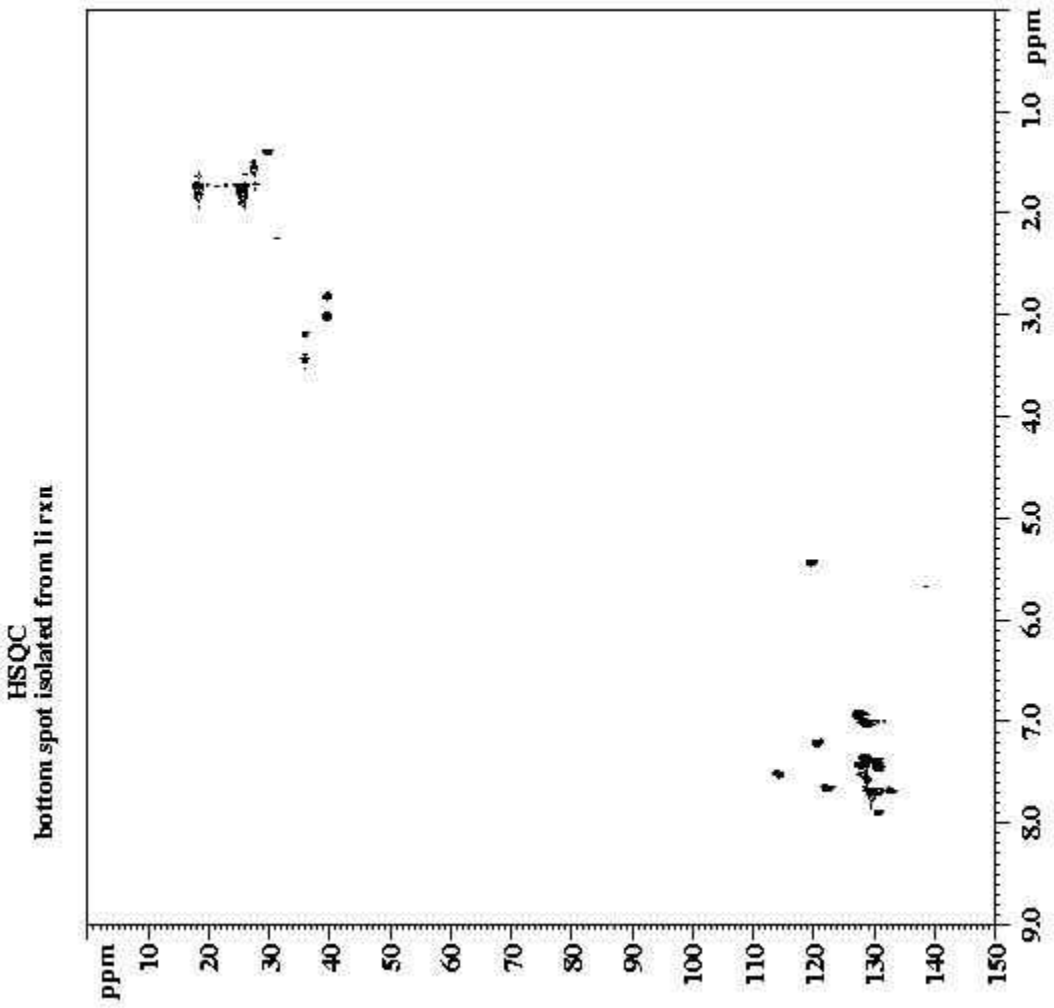
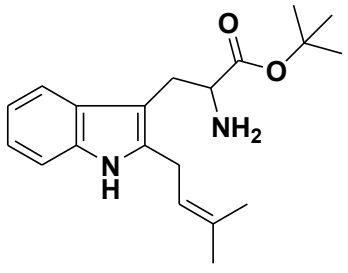
160
170





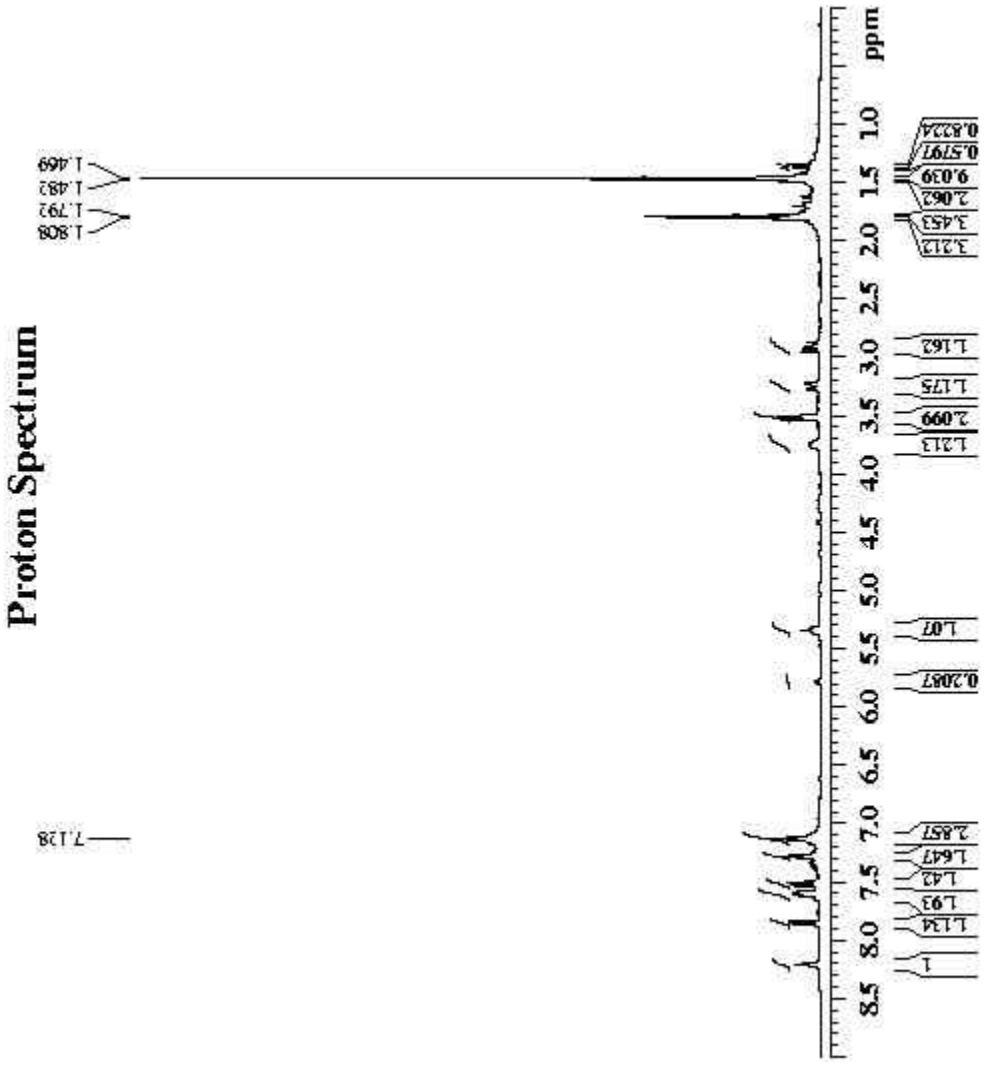
$C_{20}H_{28}N_2O_2$
Mol. Wt.: 328.45

^{1}H Name: huis2-20-12 Expno: 1 Procno: 1

^{13}C Name: huis2-20-12 Expno: 2 Procno: 1

HSQC Name: huis2-20-12 Expt: 3 Procno: 1

HRMS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1.5
1.0
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1.808
7.128
1
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1.193
1.423
1.647
2.857
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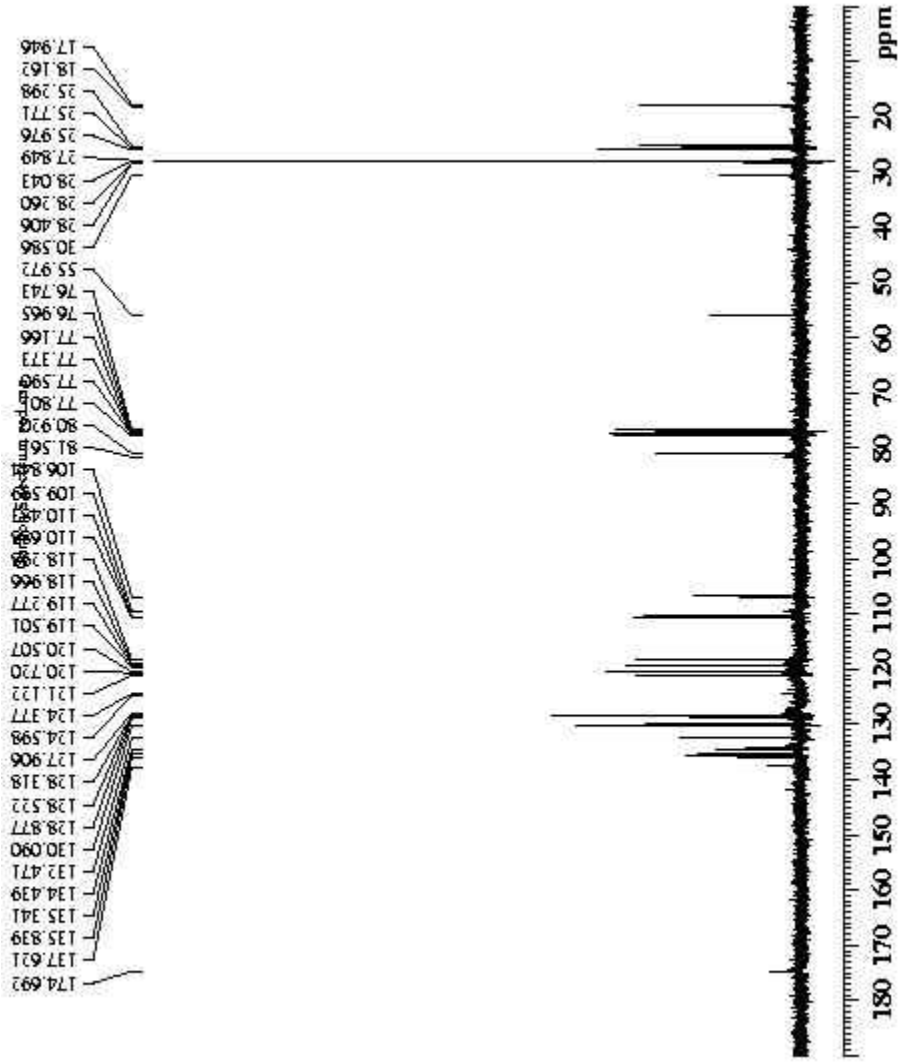
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77.373
77.590
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80.928
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109.589
110.463
110.698
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118.966
119.277
119.501
120.507
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121.122
124.377
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135.839
137.621
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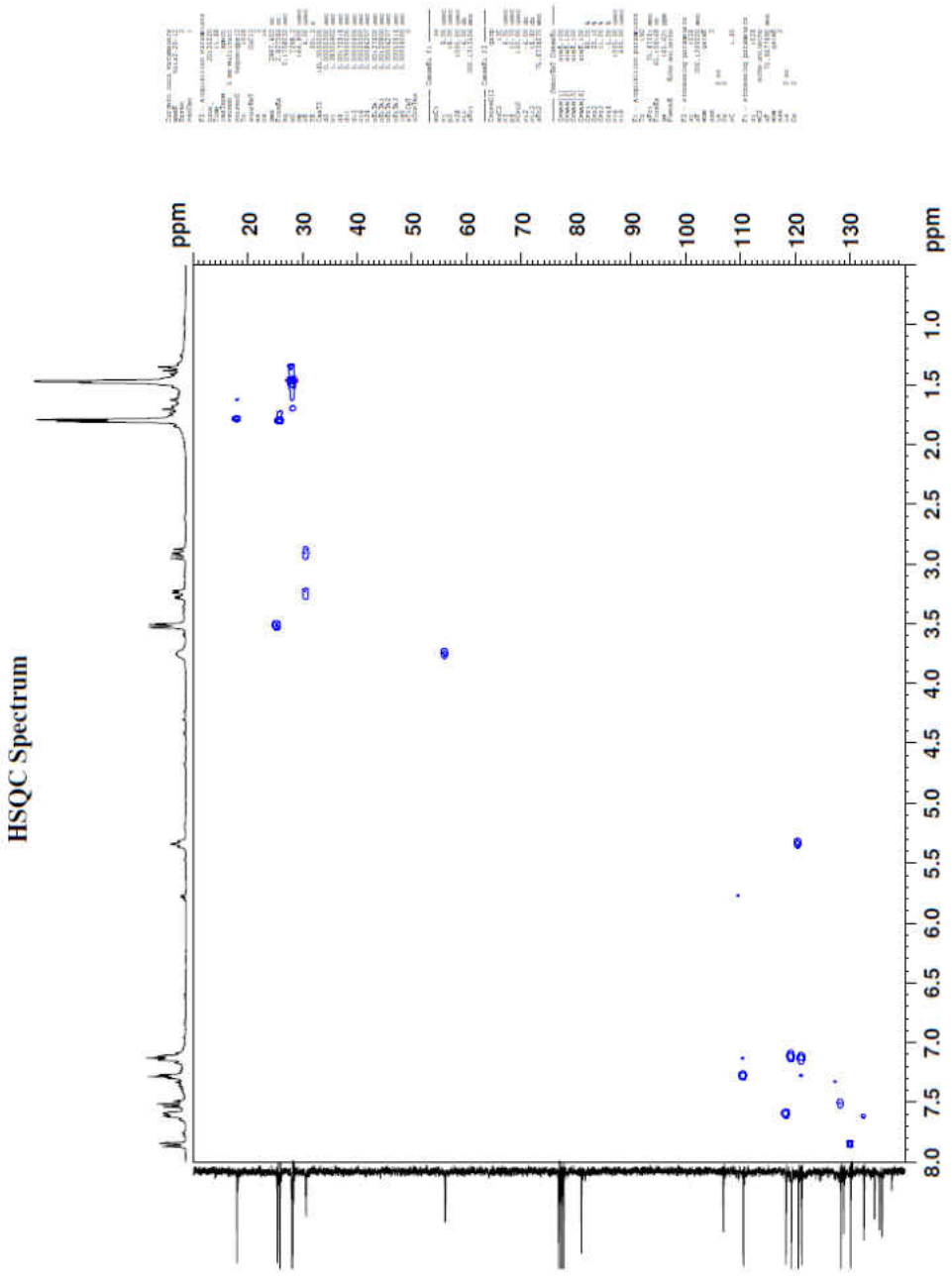
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ppm
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2.0
2.5
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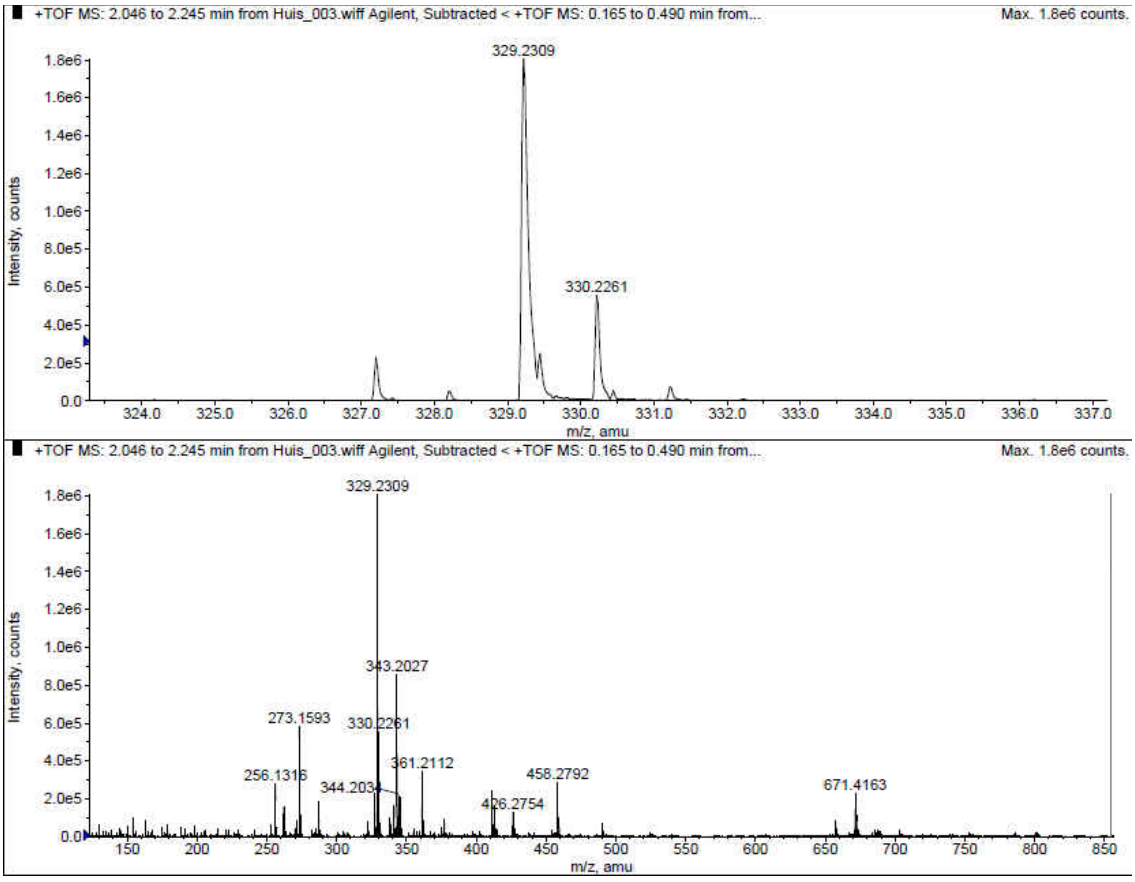
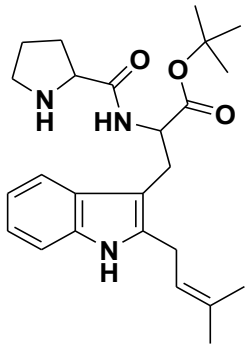
8.0





$C_{25}H_{35}N_3O_3$
Mol. Wt.: 425.56

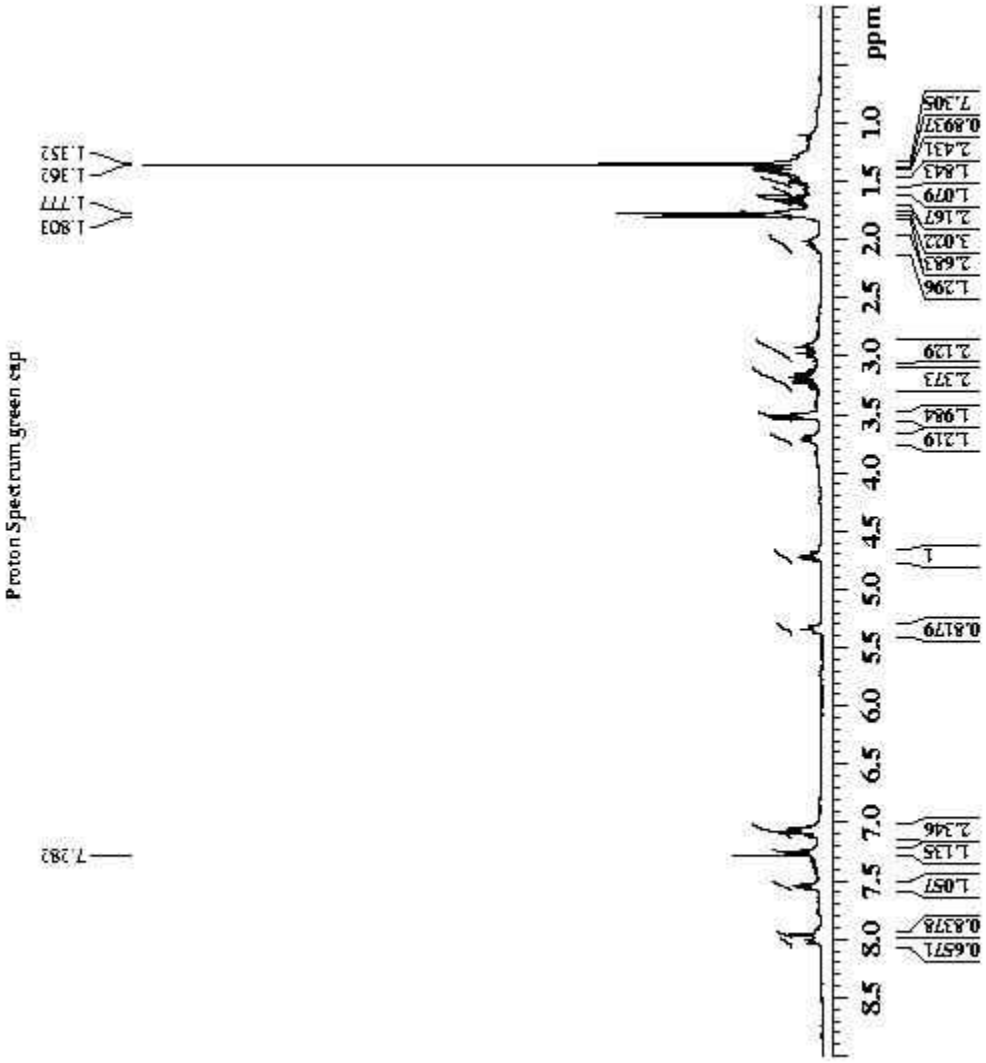
^{1}H Name: huis4-9-12 Expno: 3 Procno: 1

^{13}C Name: huis4-9-12 Expno: 4 Procno: 1

^{13}C HSQC Name: huis4-9-12 Expno: 5 Procno: 1

^{13}C Dept135 Name: huis4-9-12 Expno: 6 Procno: 1

HRMS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7.5
7.0
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6.0
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5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
7.282
1.803
1.777
1.362
1.352
0.6571
0.8378
1.057
1.135
2.346
0.8179
1
1.219
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1.843
2.431
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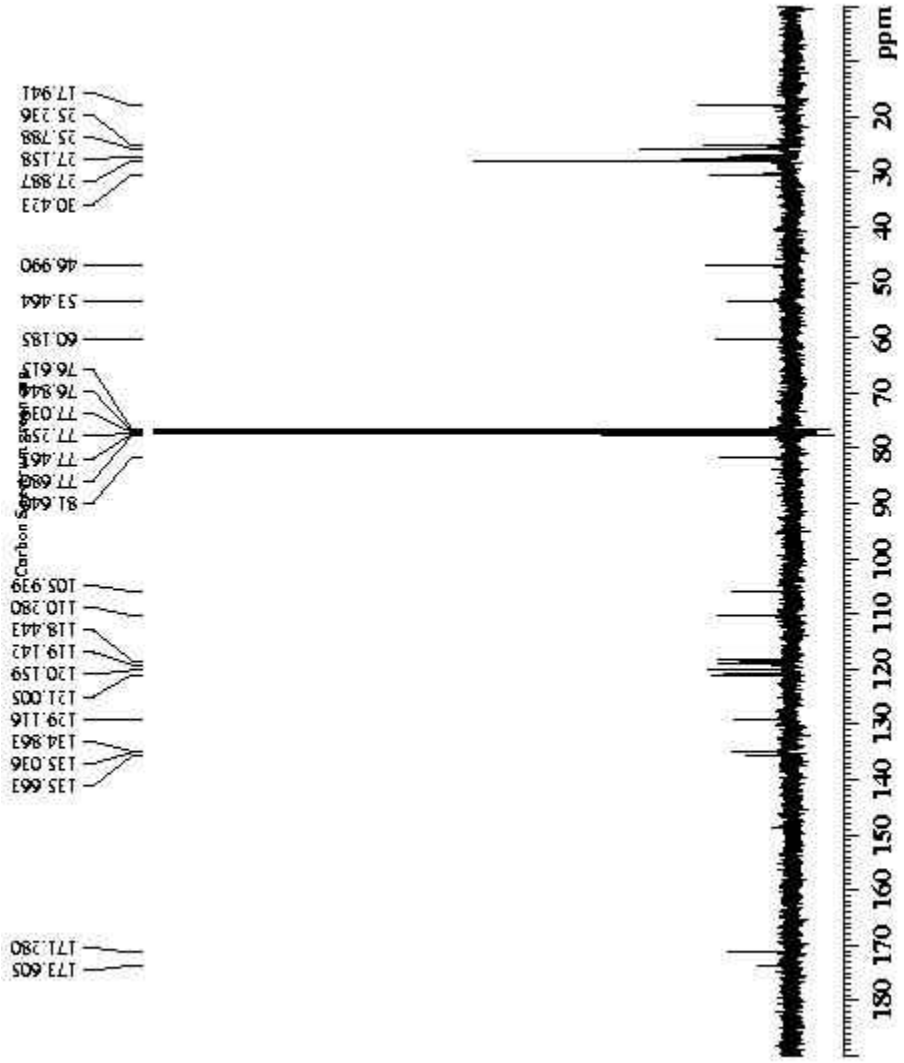
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60
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120
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140
150
160
170
180
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27.158
27.887
30.423
46.990
53.464
60.185
76.615
76.844
77.039
77.259
77.465
77.686
81.646
Carbon Spectrum
105.939
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120.159
121.005
129.116
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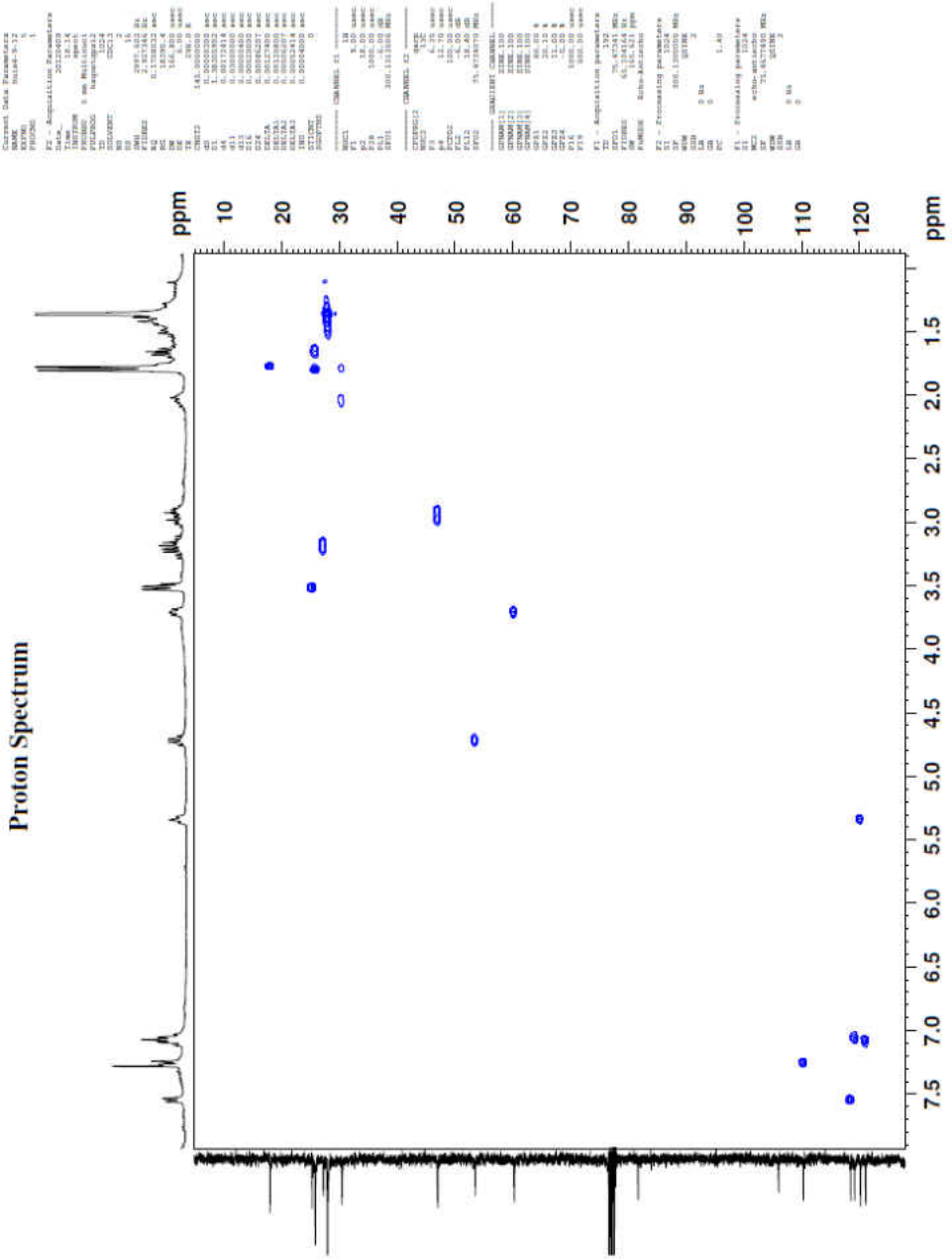
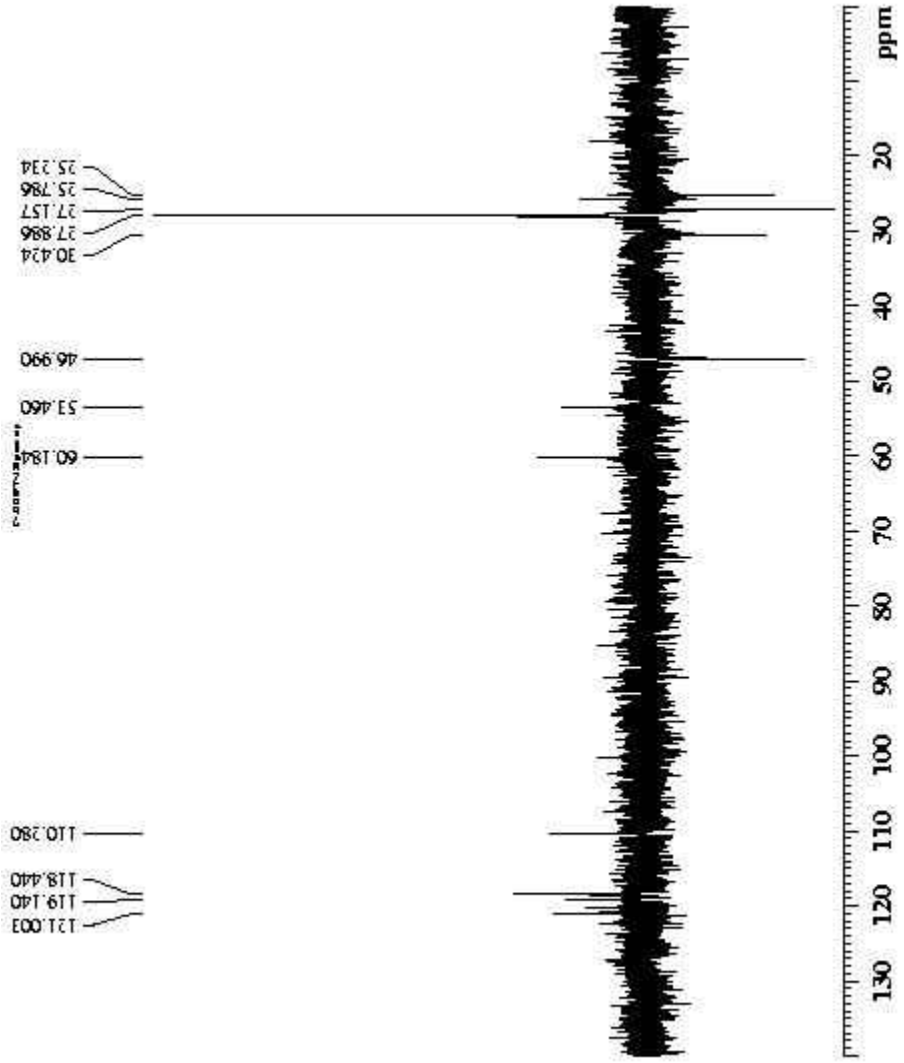
171.280
173.605


Proton Spectrum
ppm
ppm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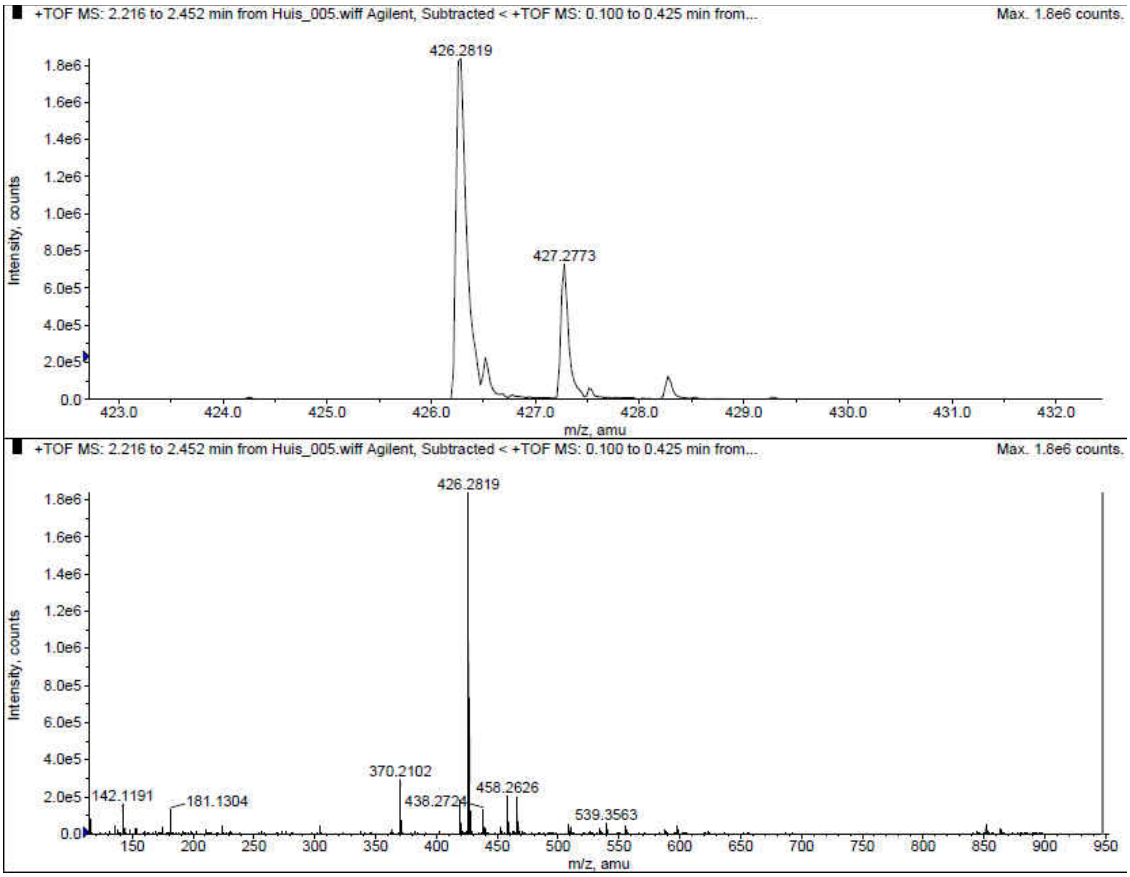
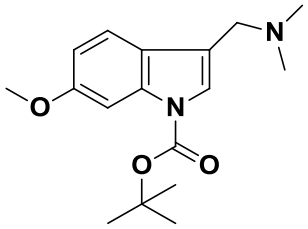




^{1}H Name: huis--13 Expno: 3 Procno: 1

^{13}C Name: huis--13 Expno: 4 Procno: 1

^{13}C HSQC Name: huis--13 Expno: 5 Procno: 1

HPLC Racemic 9-28-2010 11 27 33

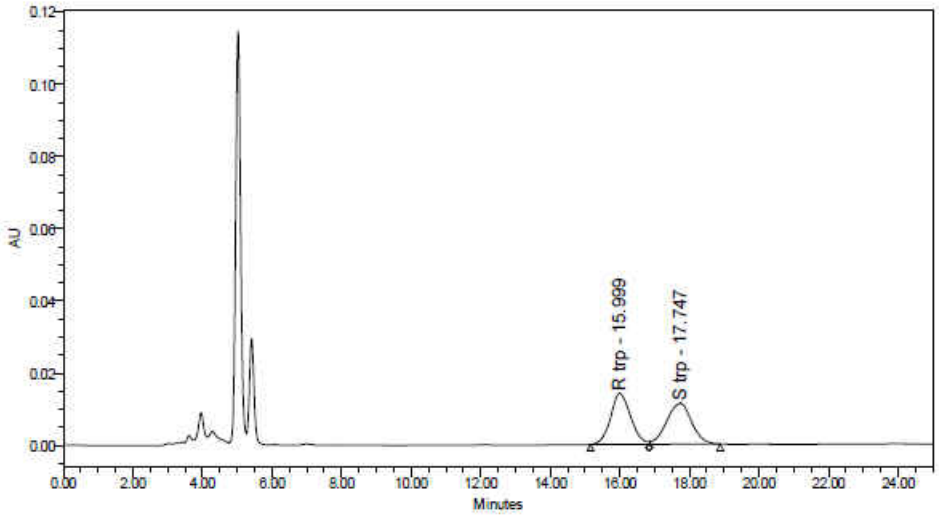
UW - Milwaukee
Project Name M Huisman
Reported by User: Breeze user (Breeze)

Breeze 2 HPLC System

SAMPLE INFORMATION

Sample Name:	Unk	Acquired By:	Breeze
Sample Type:	Unknown	Date Acquired:	9/28/2010 10:30:22 AM CDT
Vial:	1	Acq. Method:	Chiralcel OD 09022010
Injection #:	1	Processed By:	Breeze/Breeze
Injection Volume:	5.00 ul	Date Processed:	9/28/2010 11:27:33 AM CDT
Run Time:	25.00 Minutes	Channel Name:	2998 Ch1 254nm@1.2nm
Sampling Rate:	10.00 per sec	Channel Desc.:	2998 Ch1 254nm@1.2nm
		Sample Set Name	racemic trp

Sample Values Used in Calculation: Injection Volume = 5.00 SampleWeight = 1.00000 Dilution = 1.00000



	Peak Name	RT (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Integration Type	Response	Peak Codes
1	R trp	15.999	Found	566447	50.15	14127	55.70	BV	5.664e+005	Q20
2	S trp	17.747	Found	563089	49.85	11235	44.30	VB	5.631e+005	Q20

UW - Milwaukee

Project Name M Huisman

Reported by User: Breeze user (Breeze)

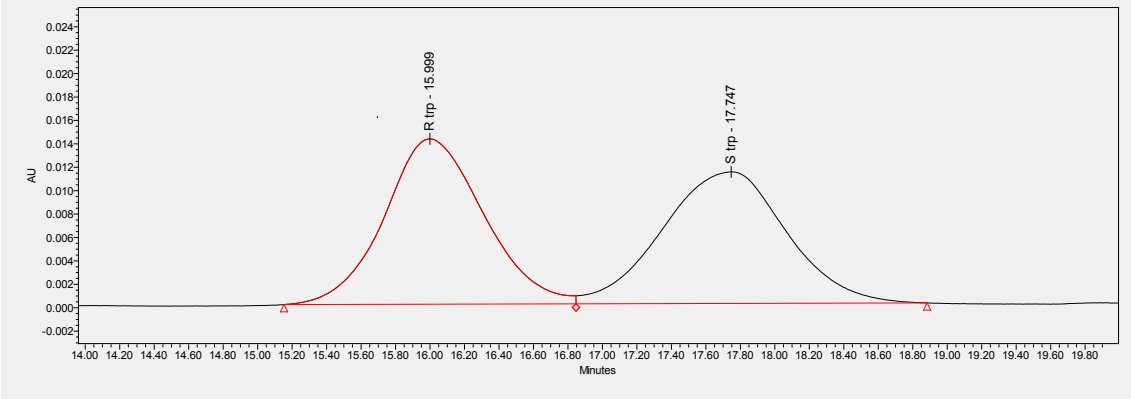
Breeze 2 HPLC System

	Points Across Peak	Start Time (min)	End Time (min)	Baseline Start (min)	Baseline End (min)	Slope (µV/sec)	Offset (µV)
1	1016	15.153	16.847	15.153	18.883	3.831099e-005	-3.126393e-004
2	1222	16.847	18.883	15.153	18.883	3.831099e-005	-3.126393e-004

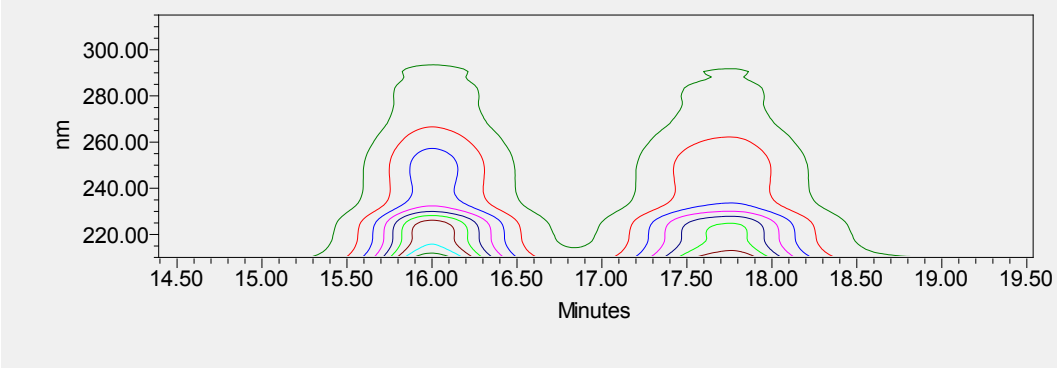
Acquisition Log

Acquired By	Breeze
Injection	1
Date Acquired	9/28/2010 10:30:22 AM CDT
Run Time	25.00(Minutes)
Acq Method Set	Chiralcel OD 09022010
Injection Volume	5.00(uL)
Injection Id	1685
Instrument Method Name	Chiralcel OD
Superseded	No

Racemic Trp



	Name	Retention Time	Area	% Area	Height	Int Type	Amount	Units	Peak Type	Peak Codes
1	R trp	15.999	566447	50.15	14127	BV			Found	Q20
2	S trp	17.747	563089	49.85	11235	VB			Found	Q20



HPLC of chiral trp 9-21-2010 time 3 32 18

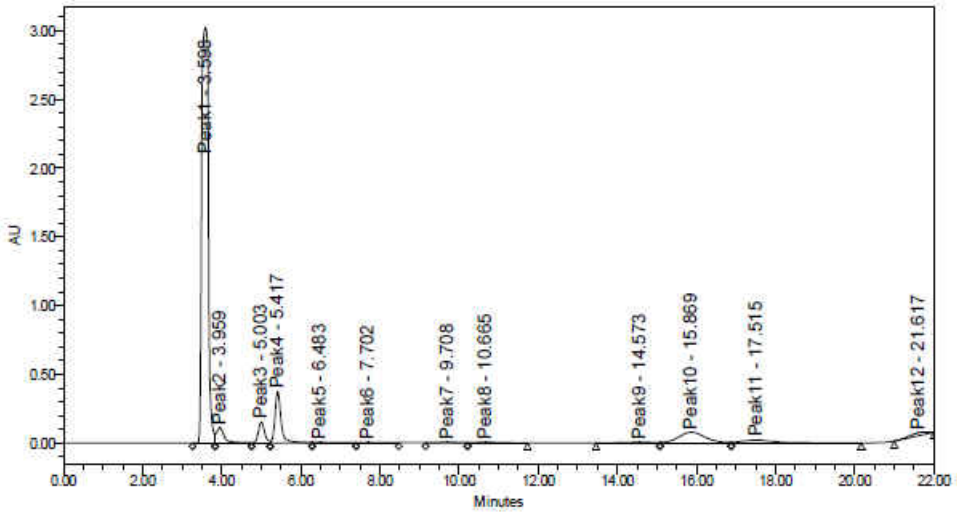
UW - Milwaukee
Project Name M Huisman
Reported by User: Breeze user (Breeze)

Breeze 2 HPLC System

SAMPLE INFORMATION

Sample Name:	Chiral Trp 20x in mp	Acquired By:	Breeze
Sample Type:	Unknown	Date Acquired:	9/21/2010 2:43:16 PM CDT
Vial:	1	Acq. Method:	Chiralcel OD 09212010
Injection #:	1	Processed By:	Breeze/Breeze
Injection Volume:	20.00 ul	Date Processed:	9/21/2010 3:32:18 PM CDT
Run Time:	22.00 Minutes	Channel Name:	2998 Ch1 254nm@1.2nm
Sampling Rate:	10.00 per sec	Channel Desc.:	2998 Ch1 254nm@1.2nm
		Sample Set Name	Chiralcel OD 09212010

Sample Values Used in Calculation: Injection Volume = 20.00 SampleWeight = 1.00000 Dilution = 1.00000



	Peak Name	RT (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Integration Type	Response
1	Peak1	3.598	Found	35279369	72.13	3018113	79.42	VV	3.528e+007
2	Peak2	3.959	Found	1568257	3.21	110235	2.90	VV	1.568e+006
3	Peak3	5.003	Found	1673874	3.42	154911	4.08	VV	1.674e+006
4	Peak4	5.417	Found	4071960	8.33	374097	9.84	VV	4.072e+006

UW - Milwaukee

Project Name M Huisman

Reported by User: Breeze user (Breeze)

Breeze 2 HPLC System

	Peak Name	RT (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Integration Type	Response
5	Peak5	6.483	Found	240682	0.49	5680	0.15	VV	2.407e+005
6	Peak6	7.702	Found	128651	0.26	2511	0.07	VV	1.287e+005
7	Peak7	9.708	Found	199787	0.41	5727	0.15	VV	1.998e+005
8	Peak8	10.665	Found	201239	0.41	5809	0.15	VB	2.012e+005
9	Peak9	14.573	Found	216485	0.44	5107	0.13	BV	2.165e+005
10	Peak10	15.869	Found	3585419	7.33	77144	2.03	VV	3.585e+006
11	Peak11	17.515	Found	1076641	2.20	19864	0.52	VB	1.077e+006
12	Peak12	21.617	Found	668238	1.37	20832	0.55	BB	6.682e+005

	Peak Codes	Points Across Peak	Start Time (min)	End Time (min)	Baseline Start (min)	Baseline End (min)	Slope (µV/sec)	Offset (µV)
1	Q20	344	3.262	3.835	2.573	11.735	2.794361e-004	-1.081082e-003
2	Q20	552	3.835	4.755	2.573	11.735	2.794361e-004	-1.081082e-003
3	Q20	283	4.755	5.227	2.573	11.735	2.794361e-004	-1.081082e-003
4	Q20	636	5.227	6.287	2.573	11.735	2.794361e-004	-1.081082e-003
5	Q20	667	6.287	7.398	2.573	11.735	2.794361e-004	-1.081082e-003
6	Q20	647	7.398	8.477	2.573	11.735	2.794361e-004	-1.081082e-003
7	Q20	638	9.152	10.215	2.573	11.735	2.794361e-004	-1.081082e-003
8	Q20	912	10.215	11.735	2.573	11.735	2.794361e-004	-1.081082e-003
9	Q20	970	13.460	15.077	13.460	20.165	-1.824012e-005	2.170612e-003
10	Q20	1081	15.077	16.878	13.460	20.165	-1.824012e-005	2.170612e-003
11	Q20	1972	16.878	20.165	13.460	20.165	-1.824012e-005	2.170612e-003
12	I08 Q20	599	21.000	21.998	21.000	21.998	6.419379e-002	-1.333622e+000

Acquisition Log

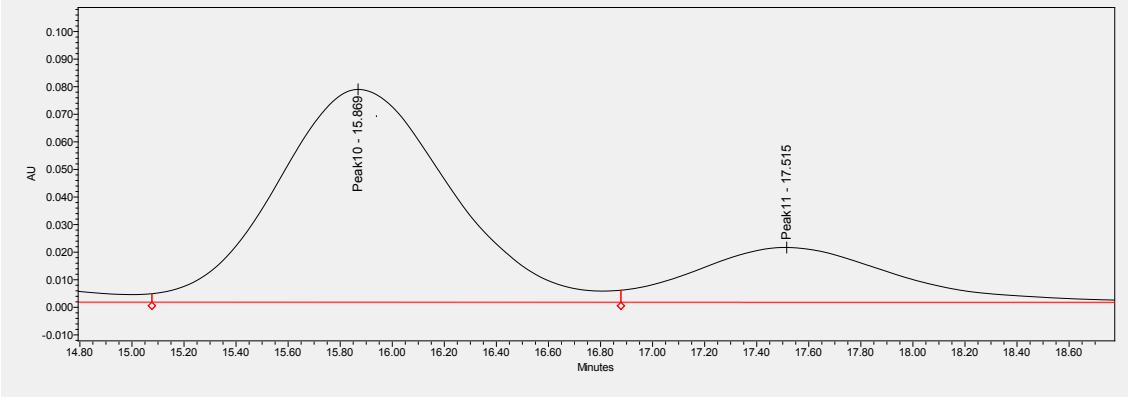
Acquired By	Breeze
Injection	1
Date Acquired	9/21/2010 2:43:16 PM CDT
Run Time	22.00(Minutes)
Acq Method Set	Chiralcel OD 09212010
Injection Volume	20.00(uL)

UW - Milwaukee

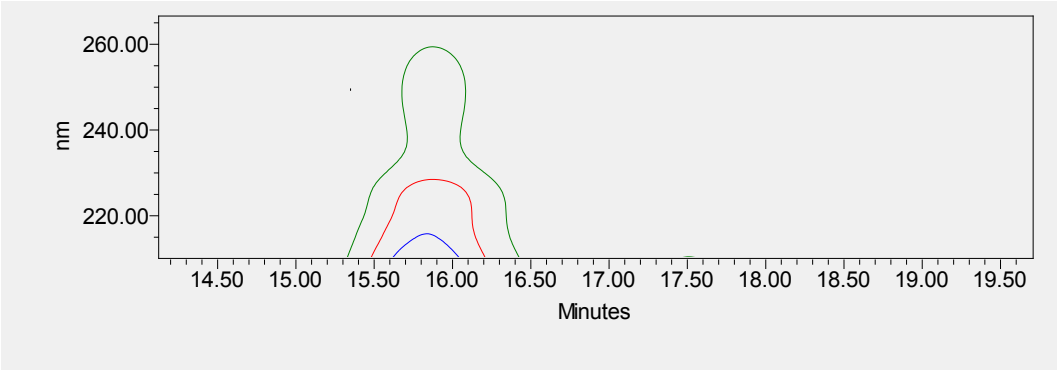
Project Name	M Huisman
Reported by User:	Breeze user (Breeze)
Injection Id	1306
Instrument Method Name	Chiralcel OD 09212010
Superseded	No

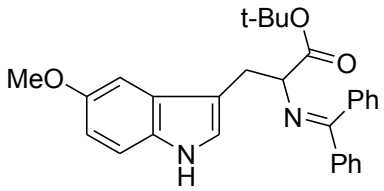
Breeze 2 HPLC System

Chiral Trp

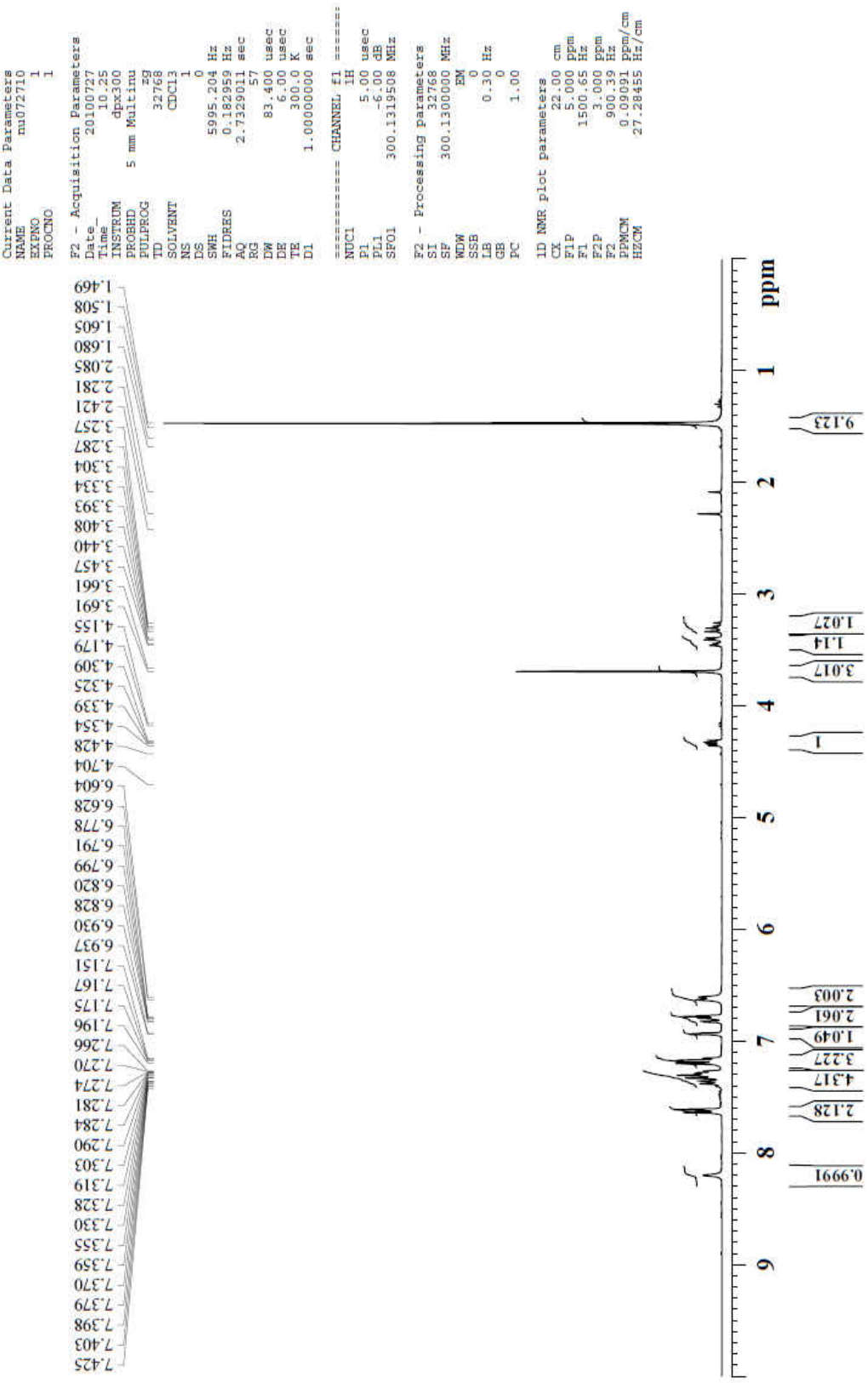


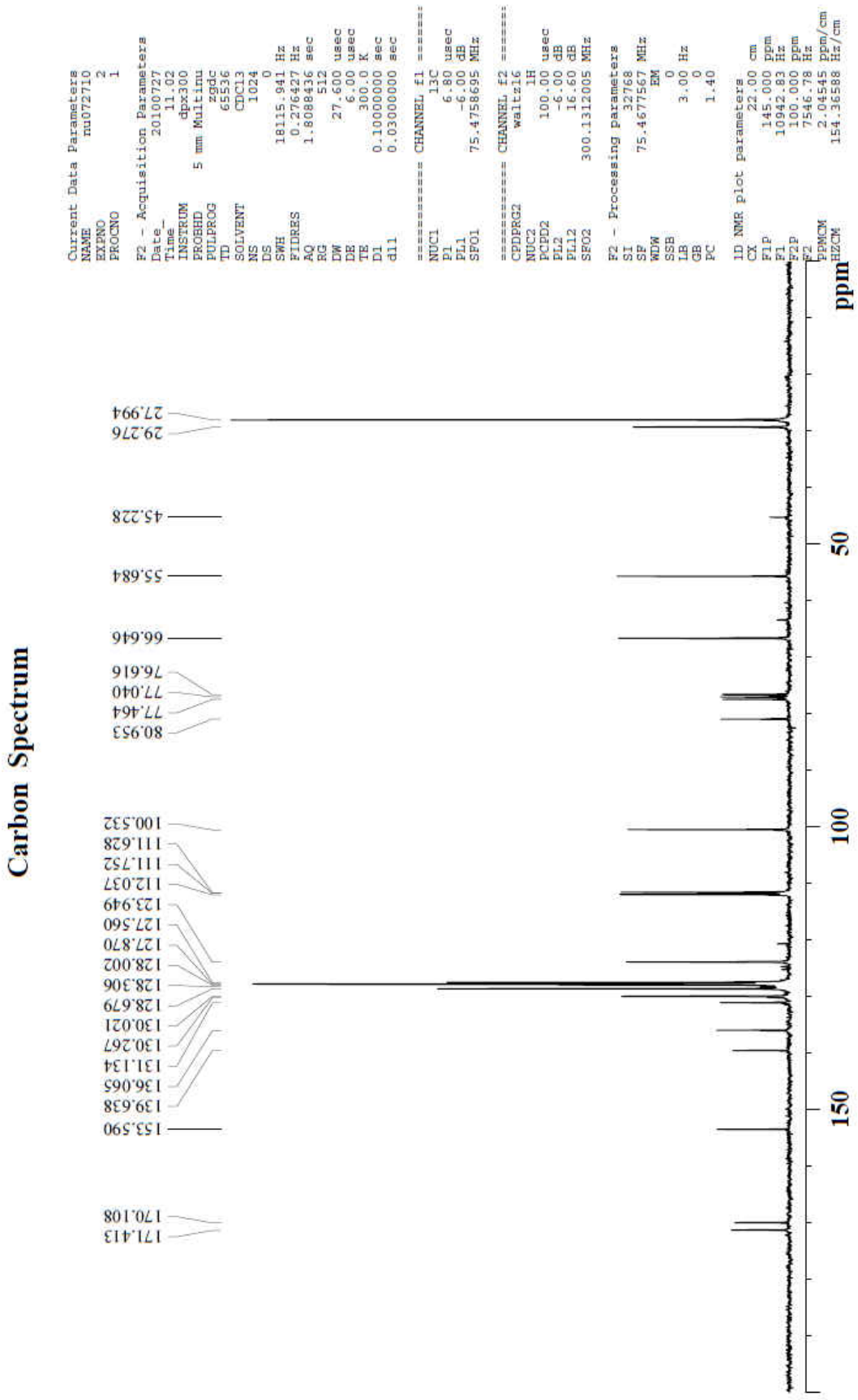
	Name	Retention Time	Area	% Area	Height	Int Type	Amount	Units	Peak Type	Peak Codes
10	Peak10	15.869	3585419	7.33	77144	VV			Found	Q20
11	Peak11	17.515	1076641	2.20	19864	VB			Found	Q20





Proton Spectrum

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EXPNO 2
PROCNO 1
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PULPROG zgdc
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SOLVENT CDCl3
NS 1024
DS 0
SWH 18115.941 Hz
FIDRES 0.276427 Hz
AQ 1.8088436 sec
RG 512
DW 27.600 usec
DE 6.00 usec
TE 300.0 K
D1 0.10000000 sec
dl1 0.03000000 sec
========== CHANNEL f1 ========
NUC1 13C
P1 6.80 usec
PL1 -6.00 dB
SFO1 75.4758695 MHz
========== CHANNEL f2 ========
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NUC2 1H
PCPD2 100.00 usec
PL2 -6.00 dB
PL12 16.60 dB
SFO2 300.1312005 MHz
F2 - Processing parameters
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WDW EM
SSB 0
LB 3.00 Hz
GB 0
PC 1.40
1D NMR plot parameters
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F2P 100.000 ppm
F2 7546.78 Hz
PPMCM 2.04545 ppm/cm
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45.228
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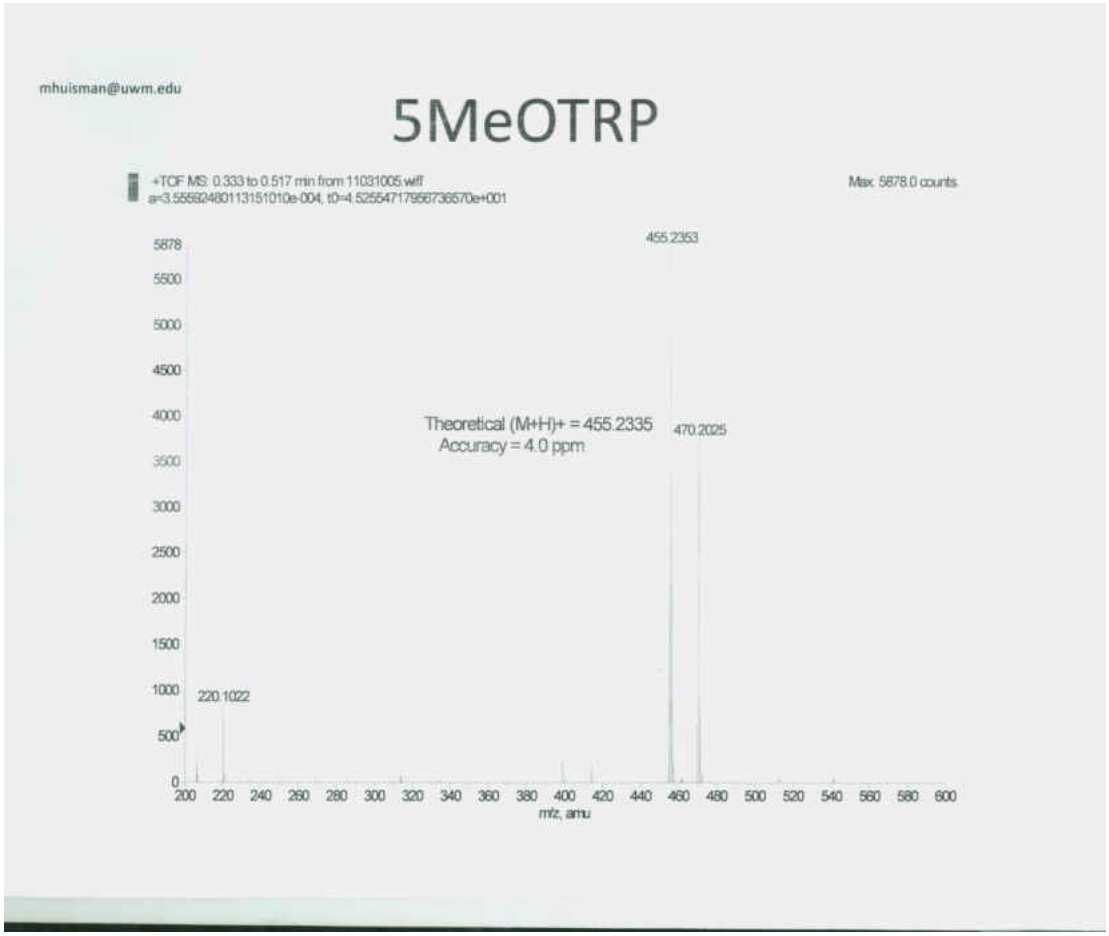
mhuisman@uwm.edu
5MeOTRP
+TOF MS: 0.333 to 0.517 min from 11031005.wiff
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Max. 5878.0 counts.
455.2353
470.2025
Theoretical (M+H)+ = 455.2335
Accuracy = 4.0 ppm
220.1022
5878
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540 560 580 600
m/z, amu

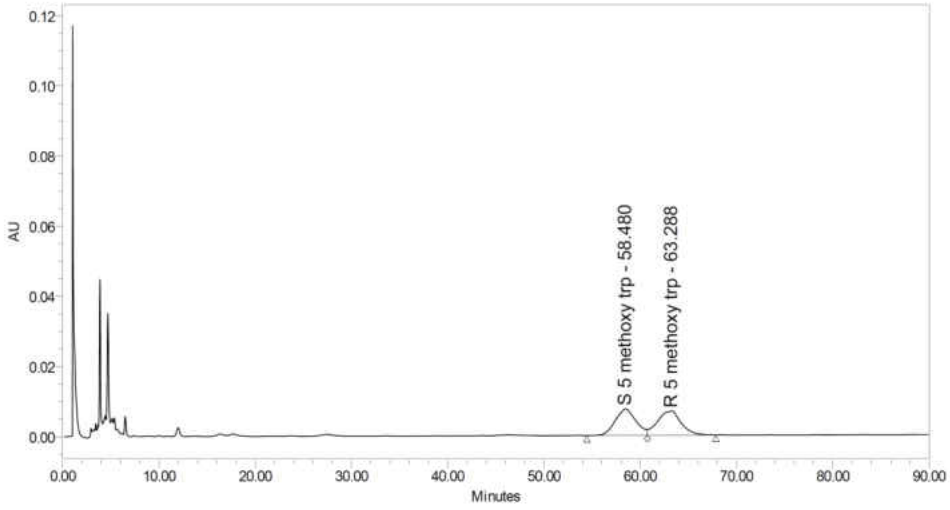
UW - Milwaukee
Project Name M Huisman
Reported by User: Breeze user (Breeze)

Breeze 2 HPLC System

SAMPLE INFORMATION

Sample Name:	Unk	Acquired By:	Breeze
Sample Type:	Unknown	Date Acquired:	10/12/2010 6:33:57 PM CDT
Vial:	1	Acq. Method:	Chiralcel OD
Injection #:	1	Processed By:	Breeze
Injection Volume:	5.00 ul	Date Processed:	11/22/2010 12:24:37 PM CST
Run Time:	90.00 Minutes	Channel Name:	2998 Ch1 254nm@1.2nm
Sampling Rate:	10.00 per sec	Channel Desc.:	2998 Ch1 254nm@1.2nm
		Sample Set Name	5 methoxy trp

Sample Values Used in Calculation: Injection Volume = 5.00 SampleWeight = 1.00000 Dilution = 1.00000



	Peak Name	RT (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Integration Type	Response
1	S 5 methoxy trp	58.480	Found	1131332	49.01	7599	52.28	BV	1.131e+006
2	R 5 methoxy trp	63.288	Found	1176911	50.99	6935	47.72	VB	1.177e+006

UW - Milwaukee

Project Name M Huisman

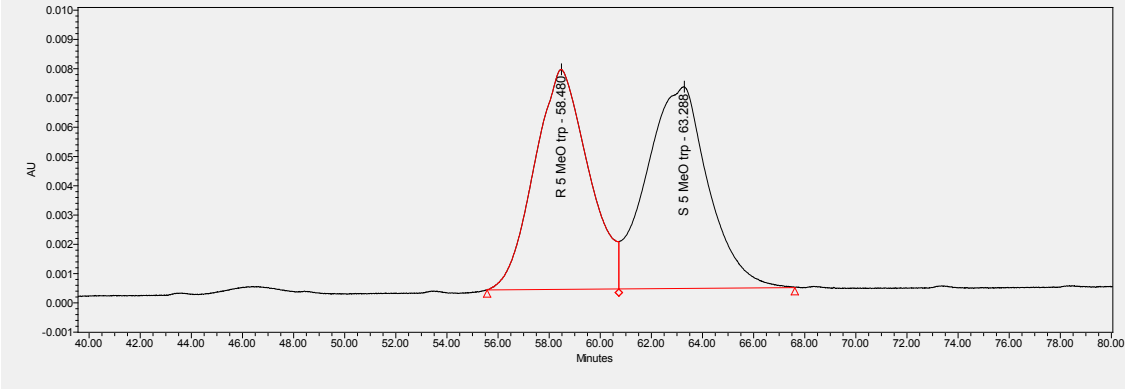
Reported by User: Breeze user (Breeze)

Breeze 2 HPLC System

	Peak Codes	Points Across Peak	Start Time (min)	End Time (min)	Baseline Start (min)	Baseline End (min)	Slope (µV/sec)	Offset (µV)
1	Q20	3762	54.457	60.727	54.457	67.845	1.448276e-005	-4.682829e-004
2	Q20	4271	60.727	67.845	54.457	67.845	1.448276e-005	-4.682829e-004

Acquisition Log

Acquired By	Breeze
Injection	1
Date Acquired	10/12/2010 6:33:57 PM CDT
Run Time	90.00(Minutes)
Acq Method Set	Chiralcel OD
Injection Volume	5.00(uL)
Injection Id	2119
Instrument Method Name	Chiralcel OD
Superseded	No



	Name	Retention Time	Area	% Area	Height	Int Type	Amount	Units	Peak Type	Peak Codes
1	R 5 methoxy trp	58.480	1131332	49.01	7599	BV			Found	Q20
2	S 5 methoxy trp	63.288	1176911	50.99	6935	VB			Found	Q20

Racemic 5-methoxy trp (

Solvent: 2% IPA/Hexane; Flow rate: 1mL/min

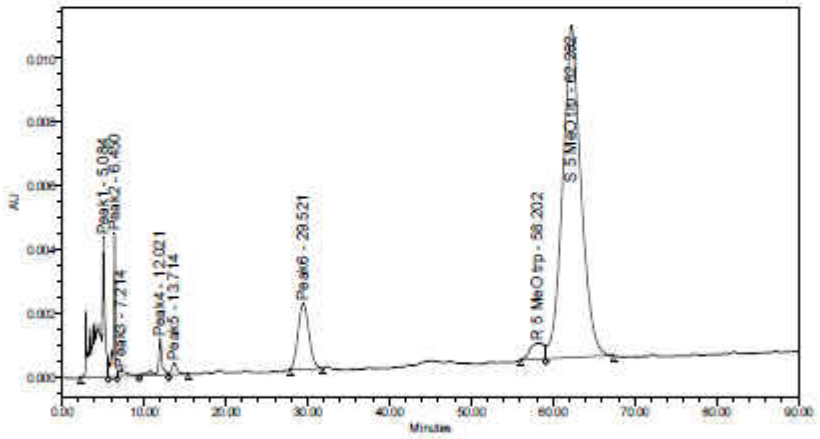
UW - Milwaukee
Project Name M Hulsman
Reported by User: Breeze user (Breeze)

Breeze 2 HPLC System

SAMPLE INFORMATION

Sample Name:	Unk	Acquired By:	Breeze
Sample Type:	Unknown	Date Acquired:	10/12/2010 4:33:54 PM CDT
Vial:	1	Acq. Method:	Chiralcel OD
Injection #:	1	Processed By:	Breeze
Injection Volume:	5.00 ul	Date Processed:	11/16/2011 3:52:03 PM CST
Run Time:	90.00 Minutes	Channel Name:	2998 Ch1 254nm@1.2nm
Sampling Rate:	10.00 per sec	Channel Desc.:	2998 Ch1 254nm@1.2nm
		Sample Set Name	5 methoxy trp

Sample Values Used In Calculation: Injection Volume = 5.00 SampleWeight = 1.00000 Dilution = 1.00000



	Peak Name	RT (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Integration Type	Response
1	Peak1	5.084	Found	221592	9.97	4354	18.54	BV	2.216e+005
2	Peak2	6.450	Found	72004	3.24	4466	19.01	VV	7.200e+004
3	Peak3	7.214	Found	13569	0.61	190	0.81	VV	1.357e+004
4	Peak4	12.021	Found	37427	1.68	1112	4.74	VV	3.743e+004

UW - Milwaukee
Project Name M Hulsman
Reported by User: Breeze user (Breeze)

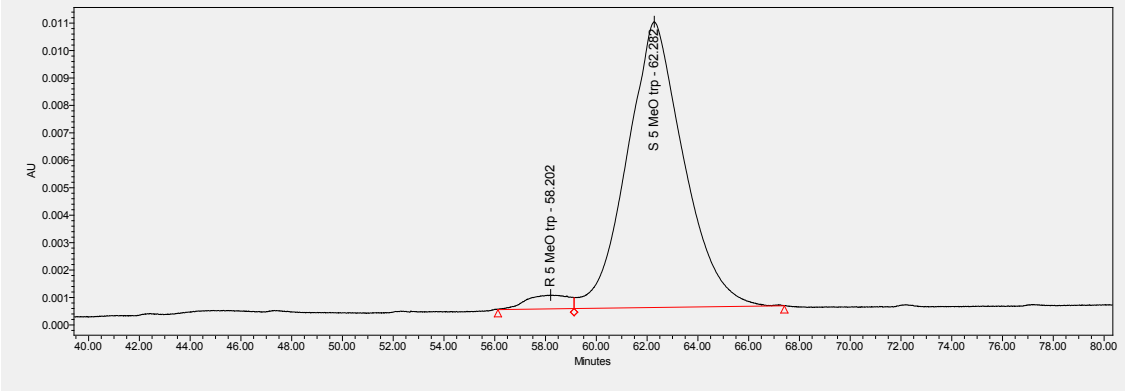
Breeze 2 HPLC System

	Peak Name	RT (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Integration Type	Response
5	Peak5	13.714	Found	16608	0.75	364	1.55	VB	1.661e+004
6	Peak6	29.521	Found	190632	8.58	2080	8.86	BB	1.906e+005
7	R 5 MeO trp	58.202	Found	59332	2.67	507	2.16	BV	5.933e+004
8	S 5 MeO trp	62.282	Found	1610677	72.49	10415	44.34	VB	1.611e+006

	Peak Codes	Points Across Peak	Start Time (min)	End Time (min)	Baseline Start (min)	Baseline End (min)	Slope (µV/sec)	Offset (µV)
1	Q20	2037	2.255	5.650	2.255	15.425	8.321944e-006	-1.356598e-005
2	Q20	670	5.650	6.767	2.255	15.425	8.321944e-006	-1.356598e-005
3	Q20	1608	6.767	9.447	2.255	15.425	8.321944e-006	-1.356598e-005
4	Q20	2151	9.447	13.032	2.255	15.425	8.321944e-006	-1.356598e-005
5	Q20	1436	13.032	15.425	2.255	15.425	8.321944e-006	-1.356598e-005
6	Q20	2396	27.888	31.882	27.888	31.882	9.040070e-006	2.675150e-007
7	Q20	1800	56.123	59.123	56.123	67.407	1.231906e-005	-1.319865e-004
8	Q20	4970	59.123	67.407	56.123	67.407	1.231906e-005	-1.319865e-004

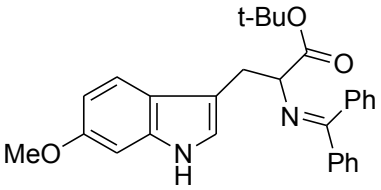
Acquisition Log

Acquired By	Breeze
Injection	1
Date Acquired	10/12/2010 4:33:54 PM CDT
Run Time	90.00(Minutes)
Acq Method Set	Chiralcel OD
Injection Volume	5.00(uL)
Injection Id	2101
Instrument Method Name	Chiralcel OD
Superseded	No

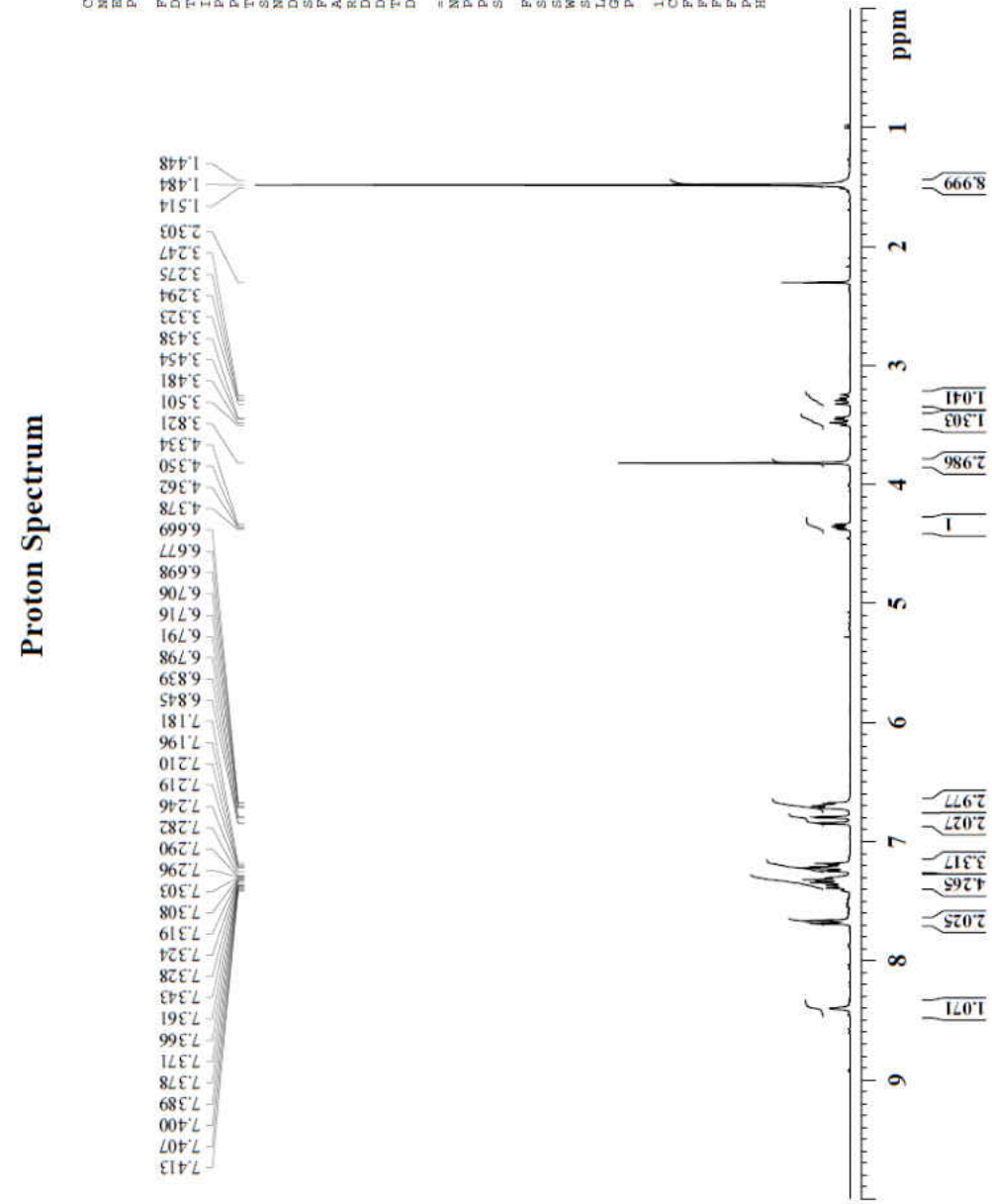


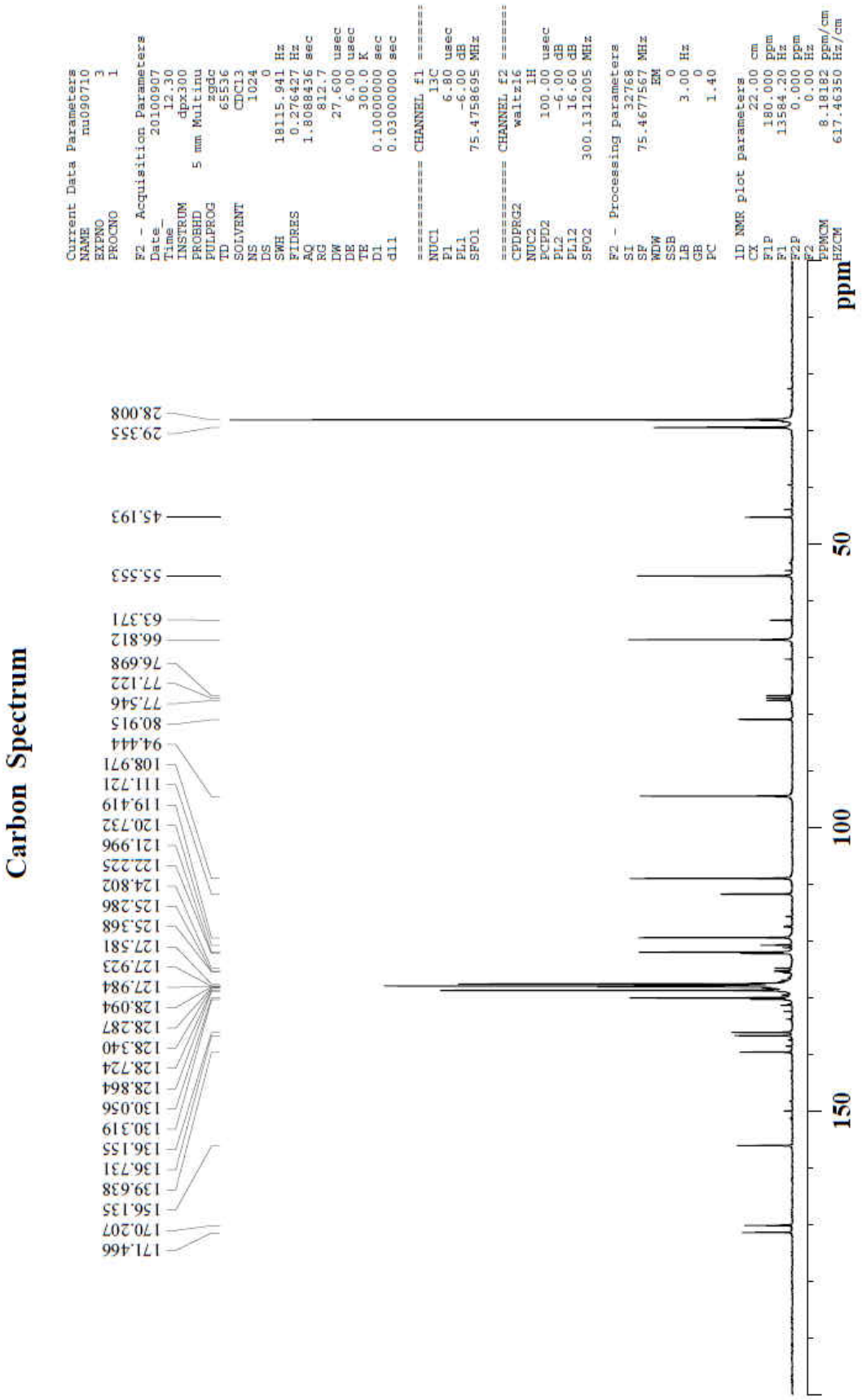
	Name	Retention Time	Area	% Area	Height	Int Type	Amount	Units	Peak Type	Peak Codes
1	R 5 meo trp	58.202	76088	4.41	584	VV			Found	Q20
2	S 5 meo trp	62.282	1650931	95.59	10491	VB			Found	Q20

Compound with 91 % ee



Proton Spectrum

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PROCNO 1
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FIDRES 0.2764427 Hz
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RG 812.7
DW 27.600 usec
DE 6.00 usec
TE 300.0 K
D1 0.10000000 sec
d11 0.03000000 sec
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P1 6.80 usec
PL1 -6.00 dB
SFO1 75.4758695 MHz
========== CHANNEL f2 =======
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PCPD2 100.00 usec
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F2 0.00 Hz
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128.864
130.056
130.319
136.155
136.731
139.638
156.135
170.207
171.466
150
100
50
ppm

mhuisman@uwm.edu

6MeOTRP

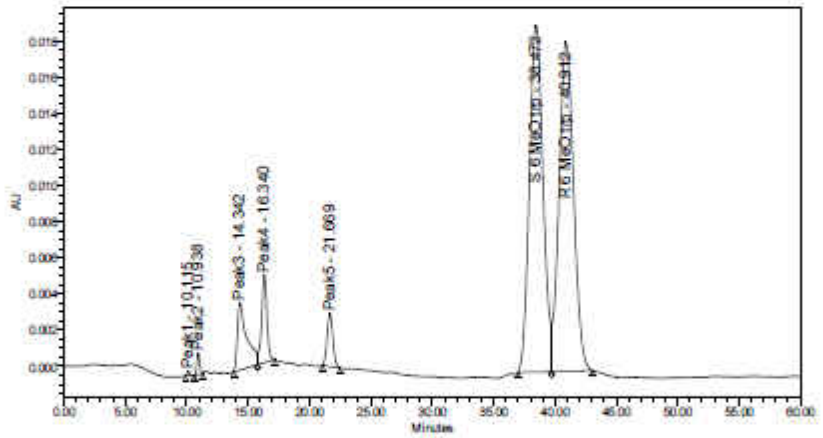
UW - Milwaukee
Project Name M Huisman
Reported by User: Breeze user (Breeze)

Breeze 2 HPLC System

SAMPLE INFORMATION

Sample Name:	Unk	Acquired By:	Breeze
Sample Type:	Unknown	Date Acquired:	11/1/2010 11:32:36 AM CDT
Vial:	1	Acq. Method:	Chiralcel OD
Injection #:	1	Processed By:	Breeze
Injection Volume:	5.00 ul	Date Processed:	11/16/2011 3:48:07 PM CST
Run Time:	60.00 Minutes	Channel Name:	2998 Ch1 254nm@1.2nm
Sampling Rate:	10.00 per sec	Channel Desc.:	2998 Ch1 254nm@1.2nm
		Sample Set Name	6 meo tp racemic

Sample Values Used In Calculation: Injection Volume = 5.00 SampleWeight = 1.00000 Dilution = 1.00000



	Peak Name	RT (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Integration Type	Response
1	Peak1	10.115	Found	3912	0.11	237	0.47	BB	3.912e+003
2	Peak2	10.938	Found	18906	0.55	1193	2.37	BB	1.891e+004
3	Peak3	14.342	Found	194527	5.67	3730	7.40	BV	1.945e+005
4	Peak4	16.340	Found	146872	4.28	4857	9.64	VB	1.469e+005

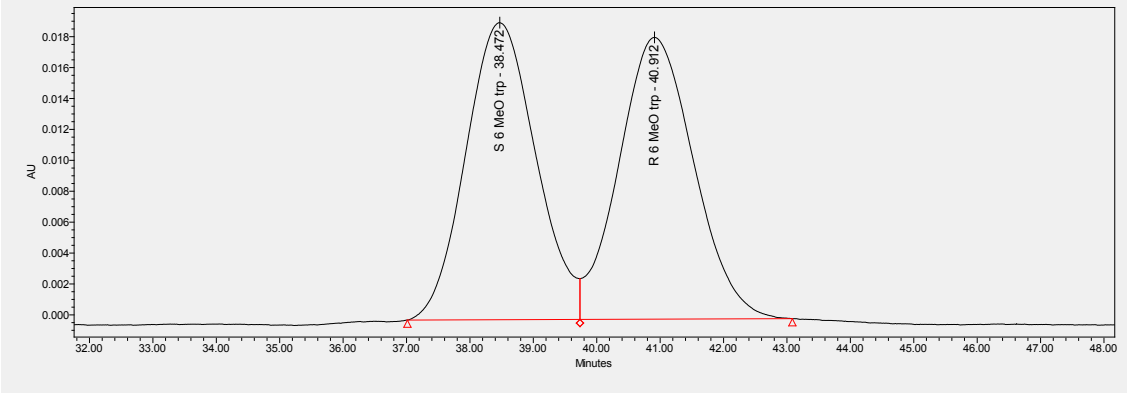
UW - Milwaukee
Project Name M Hulsman
Reported by User: Breeze user (Breeze)

	Peak Name	RT (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Integration Type	Response
5	Peak5	21.669	Found	94079	2.74	2905	5.77	BB	9.408e+004
6	S 6 MeO trp	38.472	Found	1474460	42.96	19221	38.16	BV	1.474e+006
7	R 6 MeO trp	40.912	Found	1499449	43.69	18230	36.19	VB	1.499e+006

	Peak Codes	Points Across Peak	Start Time (min)	End Time (min)	Baseline Start (min)	Baseline End (min)	Slope (µV/sec)	Offset (µV)
1	Q20	399	9.925	10.590	9.925	10.590	-9.774423e-006	-4.238889e-004
2	Q20	423	10.618	11.323	10.618	11.323	1.635461e-004	-2.257487e-003
3	Q20	1158	13.845	15.775	13.845	17.168	2.028084e-004	-3.109383e-003
4	I08 Q20	836	15.775	17.168	13.845	17.168	2.028084e-004	-3.109383e-003
5	Q20	846	21.068	22.498	21.068	22.498	-9.737588e-005	2.101195e-003
6	Q20	1633	37.013	39.735	37.013	43.085	1.576174e-005	-9.203944e-004
7	Q20	2010	39.735	43.085	37.013	43.085	1.576174e-005	-9.203944e-004

Acquisition Log

Acquired By	Breeze
Injection	1
Date Acquired	11/1/2010 11:32:36 AM CDT
Run Time	60.00(Minutes)
Acq Method Set	Chiralcel OD
Injection Volume	5.00(uL)
Injection Id	3002
Instrument Method Name	Chiralcel OD
Superseded	No



	Name	Retention Time	Area	% Area	Height	Int Type	Amount	Units	Peak Type	Peak Codes
1	S 6 meo trp	38.472	1474460	49.58	19221	BV			Found	Q20
2	R 6 meo trp	40.912	1499449	50.42	18230	VB			Found	Q20

Racemic 6-methoxy trp

Solvent: 10% IPA Hexane; Flow rate : 0.3mL/min

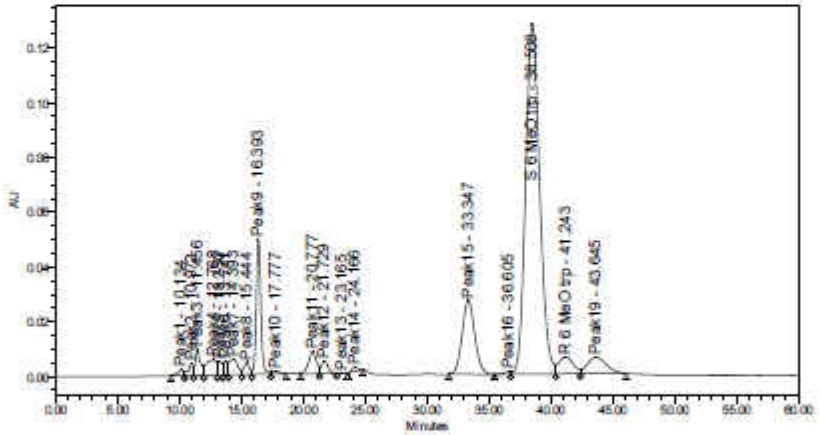
UW - Milwaukee
Project Name M Hulsman
Reported by User: Breeze user (Breeze)

Breeze 2 HPLC System

SAMPLE INFORMATION

Sample Name:	Unk	Acquired By:	Breeze
Sample Type:	Unknown	Date Acquired:	11/1/2010 12:34:48 PM CDT
Vial:	1	Acq. Method:	Chiralcel OD
Injection #:	1	Processed By:	Breeze
Injection Volume:	5.00 ul	Date Processed:	11/16/2011 3:45:46 PM CST
Run Time:	60.00 Minutes	Channel Name:	2998 Ch1 254nm@1.2nm
Sampling Rate:	10.00 per sec	Channel Desc.:	2998 Ch1 254nm@1.2nm
		Sample Set Name	6 meo tp chiral

Sample Values Used In Calculation: Injection Volume = 5.00 SampleWeight = 1.00000 Dilution = 1.00000



	Peak Name	RT (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Integration Type	Response
1	Peak1	10.134	Found	69447	0.42	2232	0.81	BV	6.945e+004
2	Peak2	10.972	Found	111879	0.68	4626	1.68	VV	1.119e+005
3	Peak3	11.456	Found	258807	1.57	9223	3.35	VV	2.588e+005
4	Peak4	12.766	Found	321070	1.95	5569	2.02	VV	3.211e+005

UW - Milwaukee
Project Name M Hulsman
Reported by User: Breeze user (Breeze)

Breeze 2 HPLC System

	Peak Name	RT (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Integration Type	Response
5	Peak5	13.250	Found	123925	0.75	4927	1.79	VV	1.239e+005
6	Peak6	13.741	Found	129256	0.78	5154	1.87	VV	1.293e+005
7	Peak7	14.393	Found	257524	1.56	5501	2.00	VV	2.575e+005
8	Peak8	15.444	Found	134944	0.82	4500	1.64	VV	1.349e+005
9	Peak9	16.393	Found	1271546	7.71	49415	17.96	VV	1.272e+006
10	Peak10	17.777	Found	43606	0.26	1126	0.41	VB	4.361e+004
11	Peak11	20.777	Found	357502	2.17	6135	2.96	BV	3.575e+005
12	Peak12	21.729	Found	172180	1.04	4531	1.65	VV	1.722e+005
13	Peak13	23.165	Found	8292	0.05	320	0.12	VB	8.292e+003
14	Peak14	24.166	Found	48887	0.30	1594	0.58	BB	4.889e+004
15	Peak15	33.347	Found	1909547	11.58	26980	9.81	BB	1.910e+006
16	Peak16	36.605	Found	35852	0.22	856	0.31	BV	3.585e+004
17	S 6 MeO trp	38.508	Found	10099731	61.23	128171	46.59	VV	1.010e+007
18	R 6 MeO trp	41.243	Found	505093	3.06	6235	2.27	VV	5.051e+005
19	Peak19	43.645	Found	634727	3.85	6038	2.19	VB	6.347e+005

	Peak Codes	Points Across Peak	Start Time (min)	End Time (min)	Baseline Start (min)	Baseline End (min)	Slope (µV/sec)	Offset (µV)
1	Q20	643	9.347	10.418	9.347	18.608	7.342090e-005	-3.492407e-004
2	Q20	444	10.418	11.158	9.347	18.608	7.342090e-005	-3.492407e-004
3	Q20	475	11.158	11.950	9.347	18.608	7.342090e-005	-3.492407e-004
4	Q20	672	11.950	13.070	9.347	18.608	7.342090e-005	-3.492407e-004
5	I08 Q20	267	13.070	13.515	9.347	18.608	7.342090e-005	-3.492407e-004
6	Q20	277	13.515	13.977	9.347	18.608	7.342090e-005	-3.492407e-004
7	Q20	646	13.977	15.053	9.347	18.608	7.342090e-005	-3.492407e-004
8	Q20	486	15.053	15.863	9.347	18.608	7.342090e-005	-3.492407e-004
9	Q20	922	15.863	17.400	9.347	18.608	7.342090e-005	-3.492407e-004
10	Q20	725	17.400	18.608	9.347	18.608	7.342090e-005	-3.492407e-004
11	Q20	924	19.788	21.328	19.788	23.565	1.029744e-004	-1.116991e-003
12	Q20	832	21.328	22.715	19.788	23.565	1.029744e-004	-1.116991e-003

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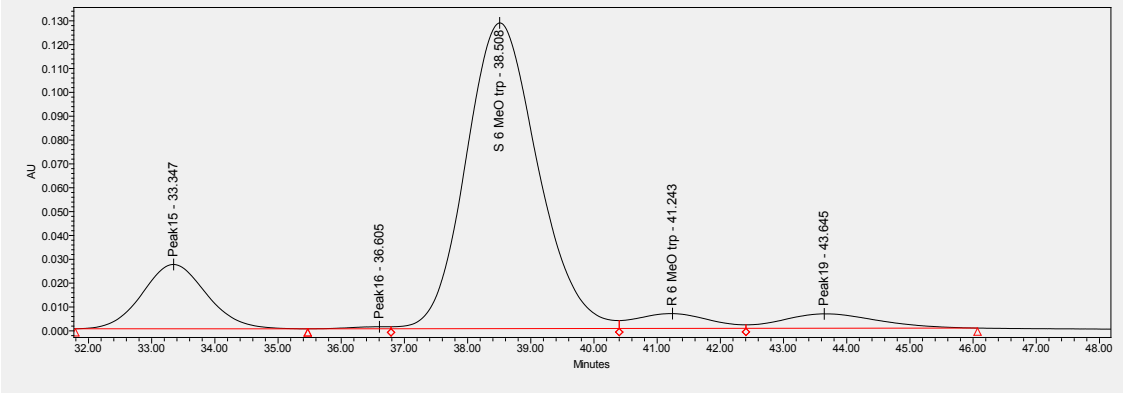
Project Name M Hulsman
Reported by User: Breeze user (Breeze)

	Peak Codes	Points Across Peak	Start Time (min)	End Time (min)	Baseline Start (min)	Baseline End (min)	Slope (µV/sec)	Offset (µV)
13	Q20	510	22.715	23.565	19.788	23.565	1.029744e-004	-1.116991e-003
14	Q20	716	23.628	24.822	23.628	24.822	9.697206e-004	-2.156868e-002
15	Q20	2207	31.792	35.470	31.792	35.470	-4.295425e-006	9.963587e-004
16	Q20	791	35.470	36.788	35.470	46.070	3.036793e-005	-2.331503e-004
17	Q20	2167	36.788	40.400	35.470	46.070	3.036793e-005	-2.331503e-004
18	Q20	1203	40.400	42.405	35.470	46.070	3.036793e-005	-2.331503e-004
19	Q20	2199	42.405	46.070	35.470	46.070	3.036793e-005	-2.331503e-004

Acquisition Log

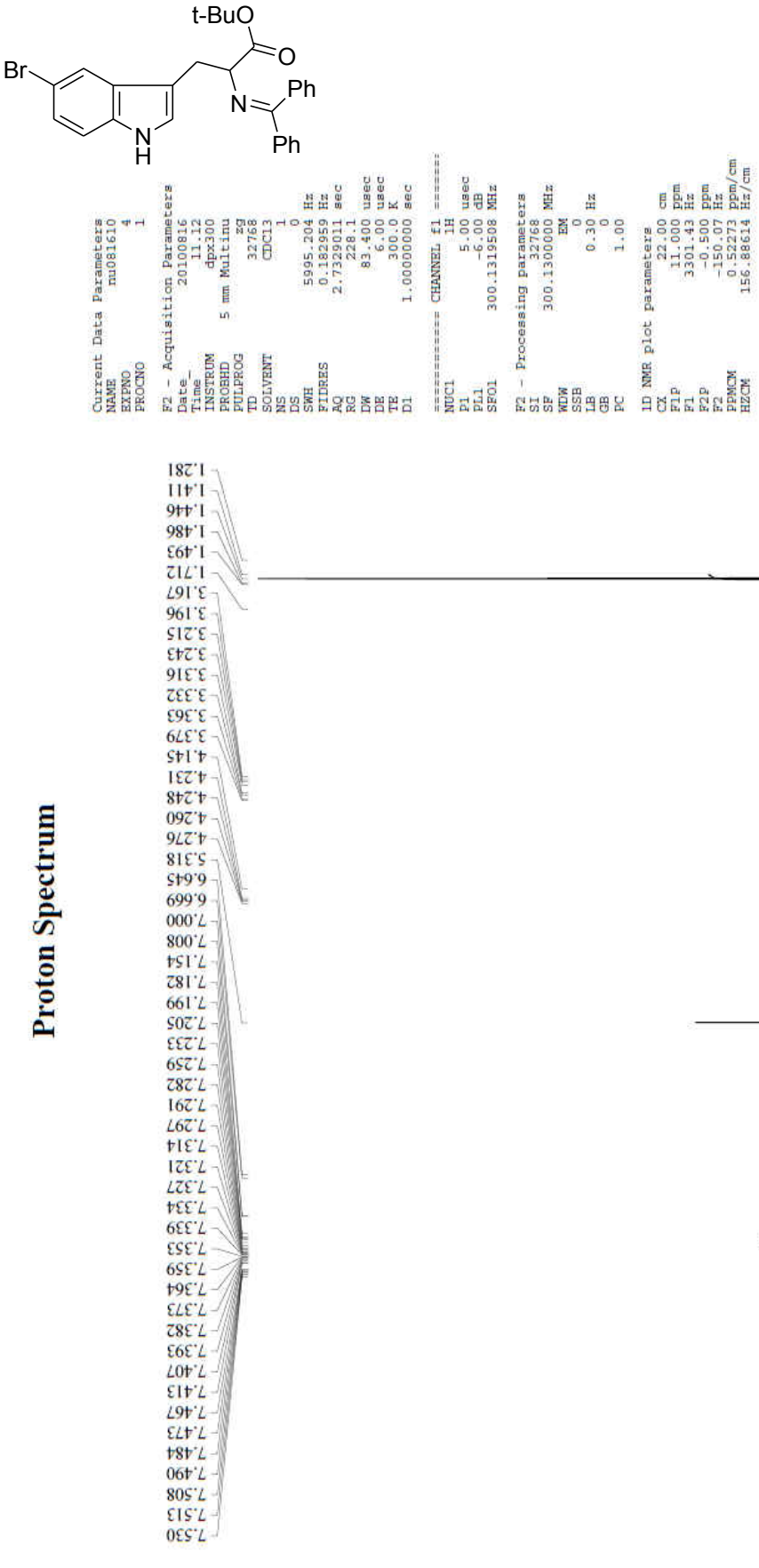
Acquired By	Breeze
Injection	1
Date Acquired	11/1/2010 12:34:48 PM CDT
Run Time	60.00(Minutes)
Acq Method Set	Chiralcel OD
Injection Volume	5.00(uL)
Injection Id	3010
Instrument Method Name	Chiralcel OD
Superseded	No

User stopped flow at 59.45 minutes



	Name	Retention Time	Area	% Area	Height	Int Type	Amount	Units	Peak Type	Peak Codes
3	S 6 meo trp	38.508	10099731	70.04	128171	VV			Found	Q20
4	R 6 meo trp	41.243	505093	3.50	6235	VV			Found	Q20

Compound with 95 %ee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Br
Ph
N
N
H
Ph
Proton Spectrum
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PROCNO 1
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TD 32768
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DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec
======== CHANNEL f1 ======
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SSB 0
LB 0.30 Hz
GB 0
PC 1.00
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F2 -150.07 Hz
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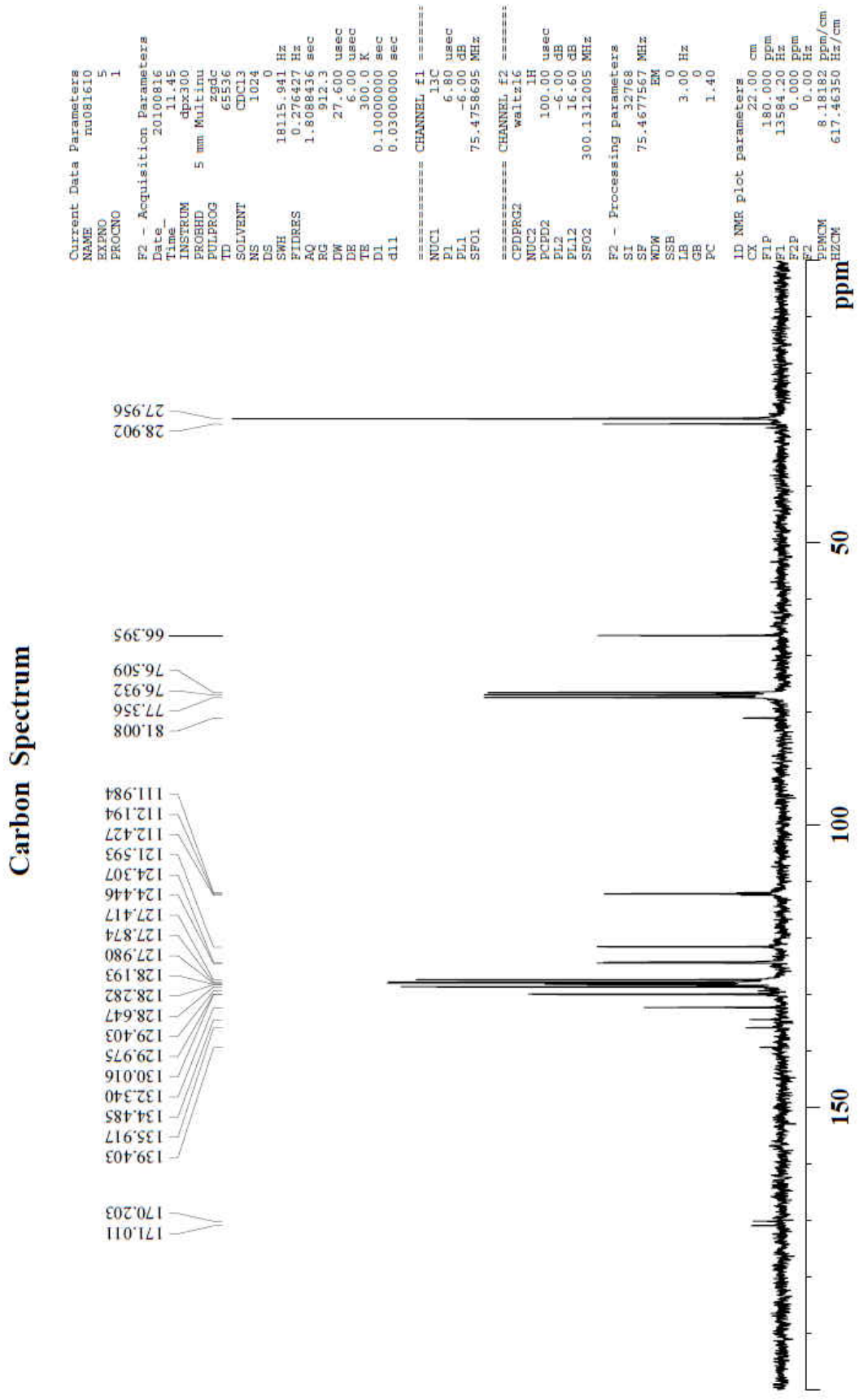
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PROCNO 1
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NS 1024
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FIDRES 0.276427 Hz
AQ 1.8088436 sec
RG 912.3
DW 27.600 usec
DE 6.00 usec
TE 300.0 K
D1 0.10000000 sec
d11 0.03000000 sec
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NUC1 13C
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PL1 -6.00 dB
SFO1 75.4758695 MHz
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NUC2 1H
PCPD2 100.00 usec
PL2 -6.00 dB
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SFO2 300.1312005 MHz
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GB 0
PC 1.40
1D NMR plot parameters
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F1 13584.20 Hz
F2P 0.000 ppm
F2 0.00 Hz
PPMCM 8.18182 ppm/cm
HZCM 617.46350 Hz/cm
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129.975
130.016
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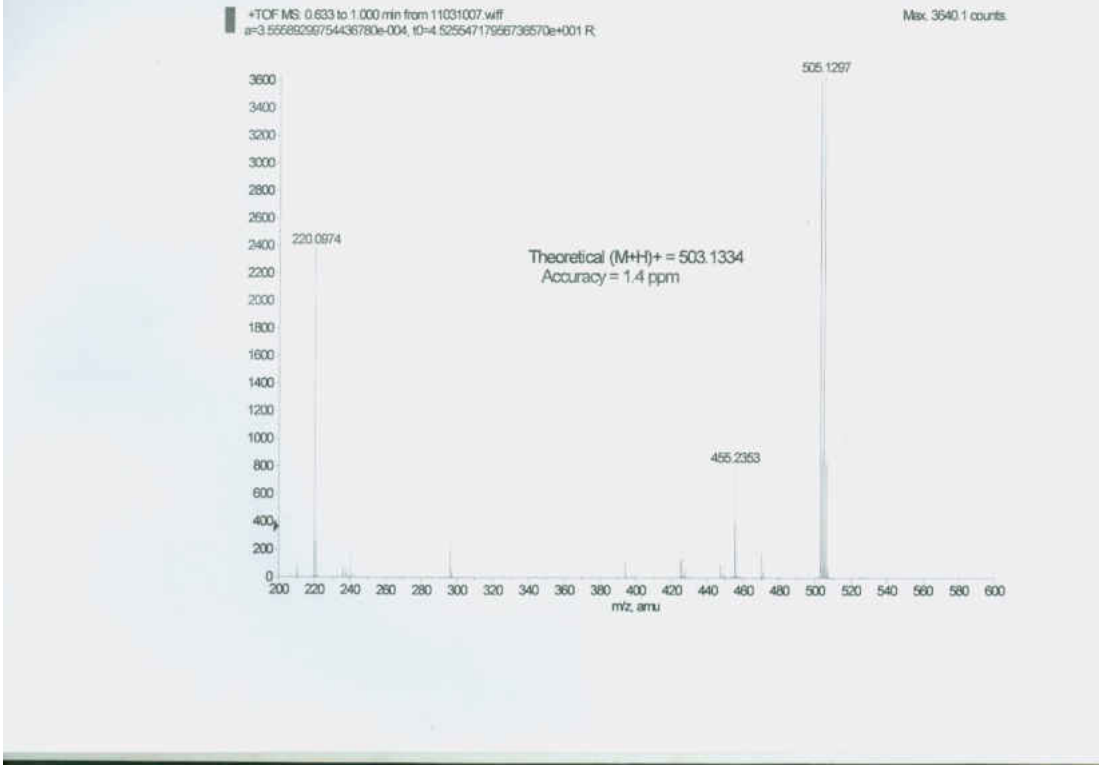
135.917
139.403
170.203
171.011
150
100
50
ppm

mhuisman@uwm.edu

5BrTRP



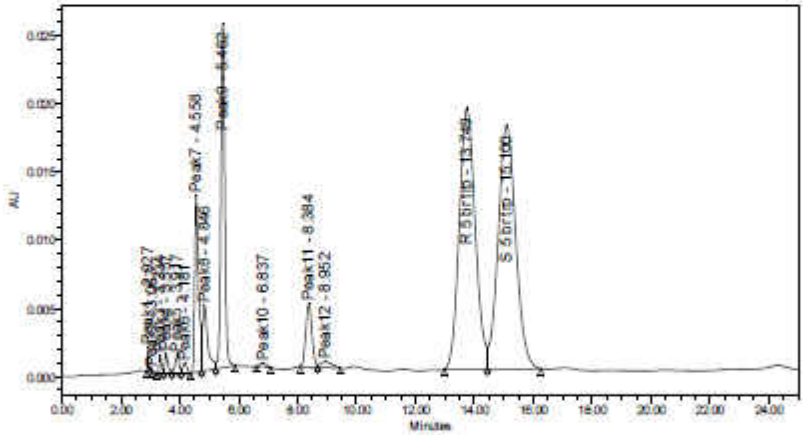
UW - Milwaukee
Project Name M Hulsman
Reported by User: Breeze user (Breeze)

Breeze 2 HPLC System

SAMPLE INFORMATION

Sample Name:	Unk	Acquired By:	Breeze
Sample Type:	Unknown	Date Acquired:	10/27/2010 11:17:14 AM CDT
Vial:	1	Acq. Method:	Chiralcel OD
Injection #:	1	Processed By:	Breeze
Injection Volume:	5.00 ul	Date Processed:	11/16/2011 3:31:17 PM CST
Run Time:	25.00 Minutes	Channel Name:	2998 Ch1 254nm@1.2nm
Sampling Rate:	10.00 per sec	Channel Desc.:	2998 Ch1 254nm@1.2nm
		Sample Set Name	5 br trp racemic

Sample Values Used In Calculation: Injection Volume = 5.00 SampleWeight = 1.00000 Dilution = 1.00000



	Peak Name	RT (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Integration Type	Response	Peak Codes
1	Peak1	2.927	Found	4715	0.24	1773	1.92	BB	4.715e+003	Q20
2	Peak2	3.067	Found	708	0.04	218	0.24	BB	7.085e+002	Q20
3	Peak3	3.334	Found	7053	0.35	1276	1.38	BV	7.053e+003	Q20
4	Peak4	3.537	Found	10523	0.53	1388	1.51	VV	1.052e+004	Q20

UW - Milwaukee
Project Name M Hulsman
Reported by User: Breeze user (Breeze)

Breeze 2 HPLC System

	Peak Name	RT (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Integration Type	Response	Peak Codes
5	Peak5	3.917	Found	12322	0.62	1386	1.50	VV	1.232e+004	Q20
6	Peak6	4.181	Found	7180	0.36	703	0.76	VB	7.180e+003	Q20
7	Peak7	4.558	Found	119943	6.02	13010	14.12	BV	1.199e+005	Q20
8	Peak8	4.846	Found	59473	2.99	4750	5.15	VV	5.947e+004	Q20
9	Peak9	5.462	Found	232109	11.66	25301	27.45	VB	2.321e+005	Q20
10	Peak10	6.837	Found	3969	0.20	269	0.29	BB	3.969e+003	Q20
11	Peak11	8.384	Found	66131	3.32	4622	5.02	BV	6.613e+004	Q20
12	Peak12	8.952	Found	11482	0.58	428	0.46	VB	1.148e+004	Q20
13	R 5 or trp	13.749	Found	719268	36.12	19144	20.77	BV	7.193e+005	Q20
14	S 5 or trp	15.100	Found	736576	36.99	17894	19.42	VB	7.366e+005	Q20

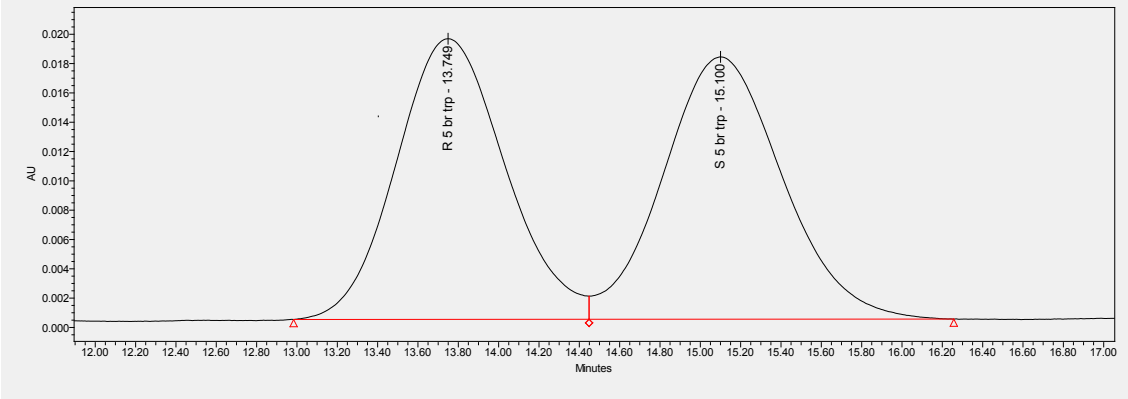
	Points Across Peak	Start Time (min)	End Time (min)	Baseline Start (min)	Baseline End (min)	Slope (µV/sec)	Offset (µV)
1	95	2.678	3.037	2.678	3.037	-5.804211e-004	2.075345e-003
2	104	3.038	3.212	3.038	3.212	-2.688460e-004	1.128944e-003
3	127	3.230	3.442	3.230	4.345	2.331856e-006	2.544661e-004
4	173	3.442	3.730	3.230	4.345	2.331856e-006	2.544661e-004
5	191	3.730	4.048	3.230	4.345	2.331856e-006	2.544661e-004
6	178	4.048	4.345	3.230	4.345	2.331856e-006	2.544661e-004
7	230	4.365	4.748	4.365	5.882	3.471429e-004	-1.248379e-003
8	288	4.748	5.228	4.365	5.882	3.471429e-004	-1.248379e-003
9	392	5.228	5.882	4.365	5.882	3.471429e-004	-1.248379e-003
10	287	6.603	7.082	6.603	7.082	-9.595819e-005	1.380044e-003
11	357	8.085	8.660	8.085	9.452	-7.600000e-005	1.362330e-003
12	463	8.660	9.452	8.085	9.452	-7.600000e-005	1.362330e-003
13	879	12.983	14.448	12.983	16.257	8.095714e-006	4.365907e-004
14	1085	14.448	16.257	12.983	16.257	8.095714e-006	4.365907e-004

Acquisition Log

Acquired By Breeze

UW - Milwaukee

Project Name	M Hulsman
Reported by User:	Breeze user (Breeze)
Injection	1
Date Acquired	10/27/2010 11:17:14 AM CDT
Run Time	25.00(Minutes)
Acq Method Set	Chiralcel OD
Injection Volume	5.00(uL)
Injection Id	2644
Instrument Method Name	Chiralcel OD
Superseded	No



	Name	Retention Time	Area	% Area	Height	Int Type	Amount	Units	Peak Type	Peak Codes
1	R 5 br trp	13.749	719288	49.41	19144	BV			Found	Q20
2	S 5 br trp	15.100	736576	50.59	17894	VB			Found	Q20

Racemic 5-bromo trp

Solvent: 5% IPA Hexane; Flow rate: 1mL/min

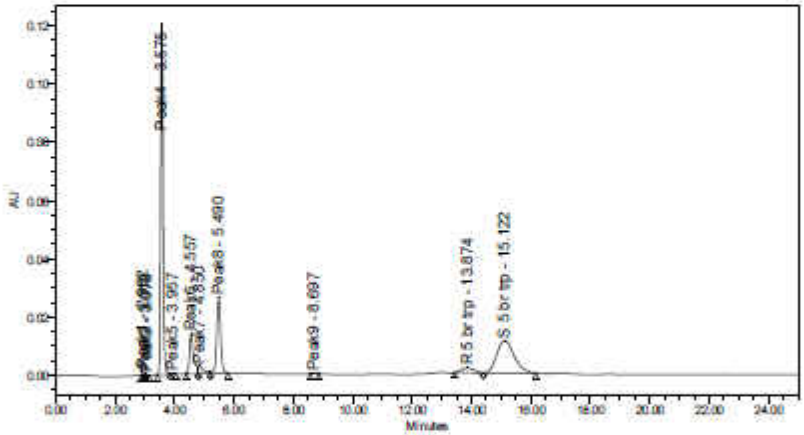
UW - Milwaukee
Project Name M Hulsman
Reported by User: Breeze user (Breeze)

Breeze 2 HPLC System

SAMPLE INFORMATION

Sample Name:	Unk	Acquired By:	Breeze
Sample Type:	Unknown	Date Acquired:	10/27/2010 12:56:33 PM CDT
Vial:	1	Acq. Method:	Chiralcel OD
Injection #:	1	Processed By:	Breeze
Injection Volume:	5.00 ul	Date Processed:	11/16/2011 3:28:28 PM CST
Run Time:	25.00 Minutes	Channel Name:	2998 Ch1 254nm@1.2nm
Sampling Rate:	10.00 per sec	Channel Desc.:	2998 Ch1 254nm@1.2nm
		Sample Set Name	5 br trp chiral

Sample Values Used In Calculation: Injection Volume = 5.00 SampleWeight = 1.00000 Dilution = 1.00000



	Peak Name	RT (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Integration Type	Response	Peak Codes
1	Peak1	2.932	Found	3925	0.25	1750	0.98	BV	3.925e+003	Q20
2	Peak2	3.012	Found	1057	0.07	440	0.25	VV	1.057e+003	Q20
3	Peak3	3.070	Found	697	0.04	250	0.14	VB	6.971e+002	Q20
4	Peak4	3.575	Found	649598	41.44	120650	67.47	BV	6.496e+005	Q20

UW - Milwaukee
Project Name M Hulsman
Reported by User: Breeze user (Breeze)

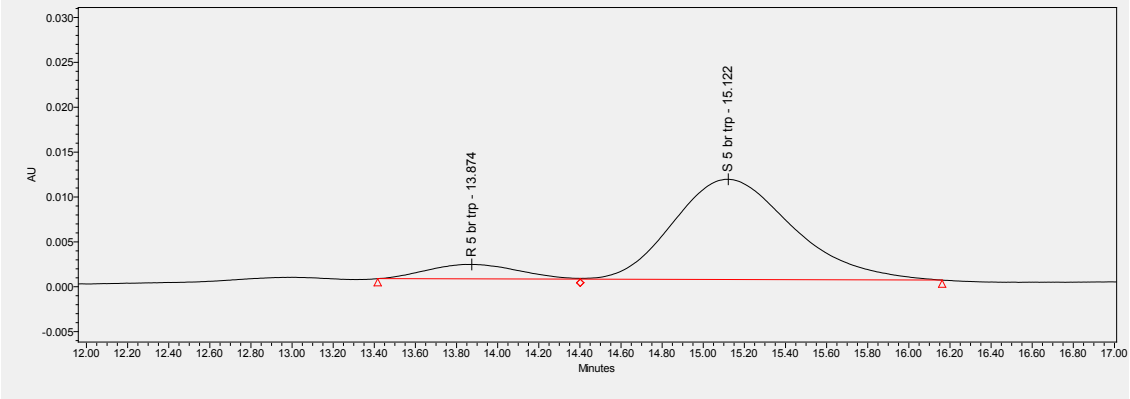
Breeze 2 HPLC System

	Peak Name	RT (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Integration Type	Response	Peak Codes
5	Peak5	3.957	Found	1868	0.12	333	0.19	VB	1.868e+003	Q20
6	Peak6	4.557	Found	135270	8.63	13761	7.70	BV	1.353e+005	Q20
7	Peak7	4.850	Found	32896	2.10	2594	1.45	VV	3.290e+004	Q20
8	Peak8	5.490	Found	239667	15.29	26018	14.55	VB	2.397e+005	Q20
9	Peak9	8.697	Found	2204	0.14	233	0.13	BB	2.204e+003	Q20
10	R 5 br trp	13.874	Found	49506	3.16	1611	0.90	BV	4.951e+004	Q20
11	S 5 br trp	15.122	Found	450865	28.76	11170	6.25	VB	4.509e+005	Q20

	Points Across Peak	Start Time (min)	End Time (min)	Baseline Start (min)	Baseline End (min)	Slope (µV/sec)	Offset (µV)
1	56	2.883	2.977	2.883	3.147	6.531646e-004	-1.941391e-003
2	40	2.977	3.043	2.883	3.147	6.531646e-004	-1.941391e-003
3	62	3.043	3.147	2.883	3.147	6.531646e-004	-1.941391e-003
4	267	3.432	3.877	3.432	4.055	5.355080e-004	-1.642985e-003
5	107	3.877	4.055	3.432	4.055	5.355080e-004	-1.642985e-003
6	252	4.387	4.807	4.387	5.602	3.564665e-004	-1.192899e-003
7	244	4.807	5.213	4.387	5.602	3.564665e-004	-1.192899e-003
8	353	5.213	5.802	4.387	5.602	3.564665e-004	-1.192899e-003
9	167	8.577	8.855	8.577	8.855	-1.282636e-004	1.646374e-003
10	591	13.417	14.402	13.417	16.162	-5.894354e-005	1.695826e-003
11	1056	14.402	16.162	13.417	16.162	-5.894354e-005	1.695826e-003

Acquisition Log

Acquired By	Breeze
Injection	1
Date Acquired	10/27/2010 12:56:33 PM CDT
Run Time	25.00(Minutes)
Acq Method Set	Chiralcel OD
Injection Volume	5.00(uL)
Injection Id	2712
Instrument Method Name	Chiralcel OD
Superseded	No



	Name	Retention Time	Area	% Area	Height	Int Type	Amount	Units	Peak Type	Peak Codes
4	R 5 br trp	13.874	49506	3.25	1611	BV			Found	Q20
5	S 5 br trp	15.122	450865	29.57	11170	VB			Found	Q20

Compound with 90 %ee

8. REFERENCE

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9. VITA

CURRICULIM VITA

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Robert Todd, **Matthew Huisman**, Nazim Uddin, Sarah Oehm, M. Mahmun Hossain. "One-Pot Enatioselective Synthesis of Tryptophan Derivatives via Phase-Transfer Catalytic Alkylation of Glycine Using a Cinchona-Derived Catalyst." Synlett, 23, 2687-2691 (2012)

Teaching Experience

Led discussions for Chemistry (100) Chemical Science, (103) Survey of Biochemistry, (104) General Chemistry and Qualitative Analysis, and (105) General Chemistry for Engineers. Also taught Chemistry 103, 104, 105, 342 Introductory to Organic Chemistry and 344 Organic Chemistry labs.

Research Experience: Knowledgeable about synthetic organic chemistry skills include: synthesis, purification procedures, ^{1}H, ^{13}C and 2D NMR, HPLC, Mass spec, chemdraw, scifinder, word, endnote, powerpoint.

Awards/Honors 2014 Moczynski Outstanding Teaching Assistant Award (Recommended by professors)

First Year Student Success Award 2011-2012 (Named by UWM first year students as person on campus who helped them most in their college success)