DISSERTATION

SYNTHETIC AND BIOSYNTHETIC STUDIES OF OKARAMINE AND HAPALINDOLE NATURAL PRODUCTS

Submitted by

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In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Spring 2018

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ABSTRACT

SYNTHETIC AND BIOSYNTHETIC STUDIES OF OKARAMINE AND HAPALINDOLE NATURAL PRODUCTS

This dissertation is a report on the first total syntheses of hapalindoles C and D, natural products of a few cyanobacteria. Additionally, we report on our collaborative investigations on the biosynthesis of the hapalindole family of natural products. Lastly our efforts toward the total synthesis of okaramines R and B and attempts to synthesize a novel indoloazetidine ring are enclosed.

ACKNOWLEDGEMENTS

I thank my family for the unwavering support and love. My beloved mother who, all her life, was an example of unwavering dedication and love to all around her. No greater Christian example has ever been presented to me. My father, whose seemingly unparalleled intelligence is both frustrating and inspiring. He remains the greatest example of hard work and commitment to family. My siblings who were instrumental in showing me the way in a morally shifting world, and are also eternally my best friends. And to my stepmother, who has been a valuable support in difficult times.

Thank you to my coworkers who were good company and council. Lastly to Professor Robert Williams who gave me room and time to decide what I wanted to do with my career.

DEDICATION

In memory of my mother, Mary.

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Part 1. Studies Toward the Synthesis and Biosynthesis of the Okaramine Family

Chapter 1. Introduction

1.1. Isolation and Activity

While investigating insecticides produced by various fungi, Hayashi et al. found that *Penicillium simplicissimum* AK-40 cultured on okara, the insoluble residue of whole soybean, was toxic to silkworms. Isolation of the various extracts lead to characterization of an indole alkaloid with potent insectidical activity; Okaramines A (**Figure 1.1**).[1] With 30 μg of alkaloid per gram of diet approximately 90% of the silkworm population expired within 24 hours. Further investigation of the extraction from *Penicillium simplicissimum* yielded Okaramine B, which was more than 100 times as potent (only 0.3 μg/g diet killed 100% of the silkworms in 24 hours).[2] Although both are complex natural products, okaramine B is particularly intriguing because it contains a rare azetidine ring. Okaramine B also showed activity against beet armyworm larvae. An additional sixteen members of the family were isolated between 1988 and 2000 from *Penicillium simplicissimum* (C–G, and J–R) and *Aspergillus aculeatus* (H and I), although not all were effective insecticides.[3-9] More recently a third strain yielded okaramines S–U isolated by Cai et al. from *Aspergillus taichungensis*.[10] Okaramines S–U were not isolated based on their activity against silkworms, rather for their cytotoxic activity against HL-60 cells with an IC_{50} of 0.78 μM.

A 2014 investigation into the target of okaramine B showed that the indole alkaloid targets the L-glutamate-gated chloride (GluCl) channel.[11-12] Testing on human chloride channels showed

little to no activity, making okaramine B a potential starting point in the search for new and safe insecticides. A 2017 investigation into how okaramine B affects the GluCl showed that a L319F mutation reduces the enzyme's sensitivity to okaramine B.[13]

Hayashi reasoned in an early okaramine publication that the indoline reverse-prenyl group of okaramine A and the azetidine ring of okaramine B are key to their activity.[3] This was later substantiated when the remaining okaramines were isolated. Not every okaramine that does not incorporate a reverse-prenyl group on the indoline ring or azetidine ring (H-M, P) shows severely decreased activity against silkworm larvae. Interestingly, okaramines N, O, and R also show weaker activity. A possible reason for the diminished activity is the absence of a double bond in the 8-member nitrogen heterocycle. Five of the twenty-one members of the okaramine family contain an azetidine ring.

1.2. Biosynthesis

A biogenesis of the okaramine family was proposed by Hayashi et al. (**Scheme 1.1**). In their proposal, tryptophan undergoes dimerization and differentiation by two different reverse-prenyl groups. Oxidative closure of the pyrroloindole ring gives okaramine C. The dihydroindoloazocine ring can be closed to access okaramines N, O, then A, following which azetidine ring closure takes place. This proposed route offers a logical progression for the synthesis of many of the okaramine family.

In contrast to the biogenesis proposed by Hayashi, we had reasoned that the azetidine ring may come by way of a photoinduced [2+2] cycloaddition of the reverse-prenyl unsaturation with the oxindole carbonyl of okaramine R to oxetane **1.1** (**Scheme 1.2**). The cyclization would be

followed by iminium formation to **1.2** to open the oxetane ring and attack from the diketopiperizine nitrogen to form the pyrroloindoline moiety of okaramine E.

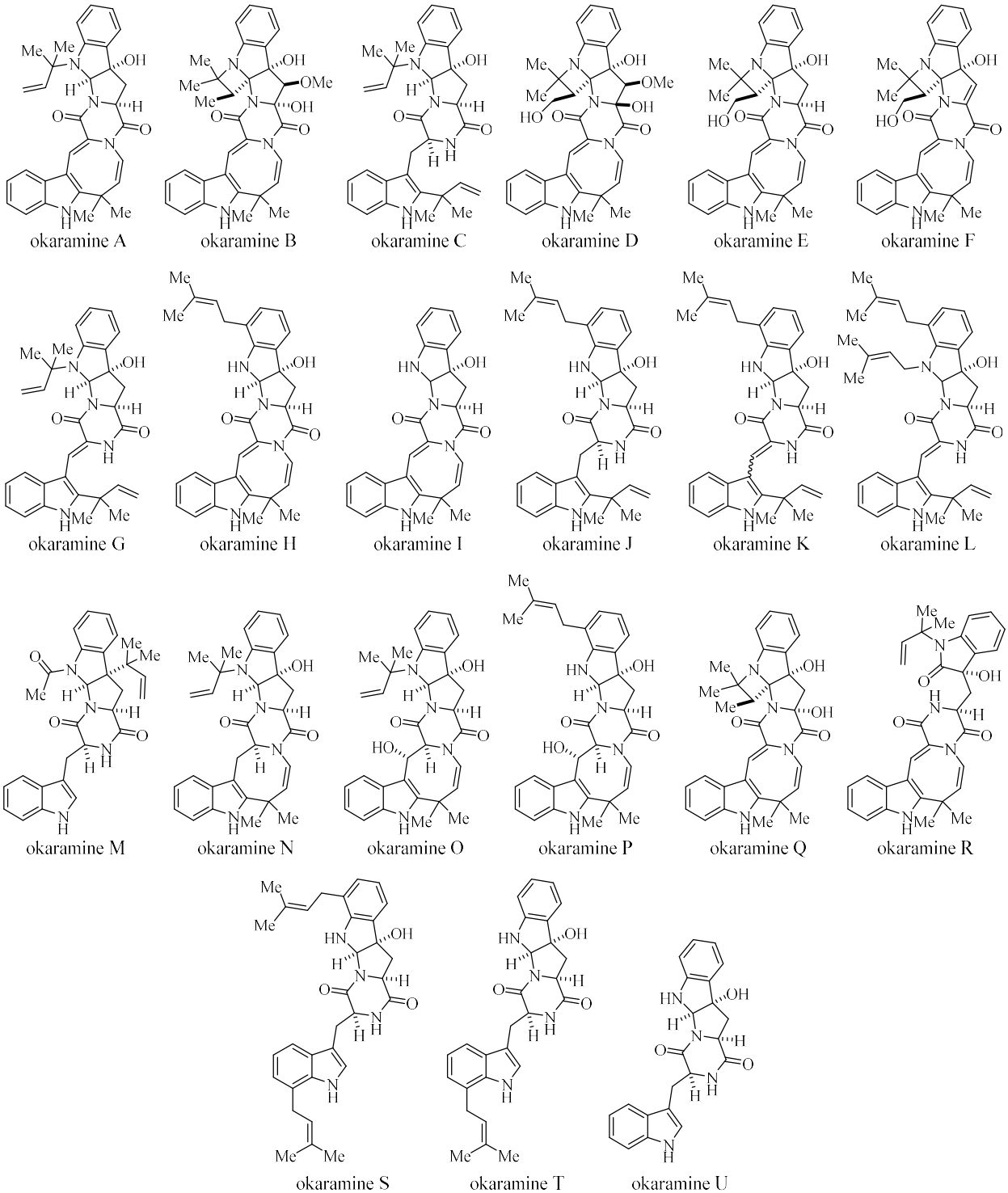
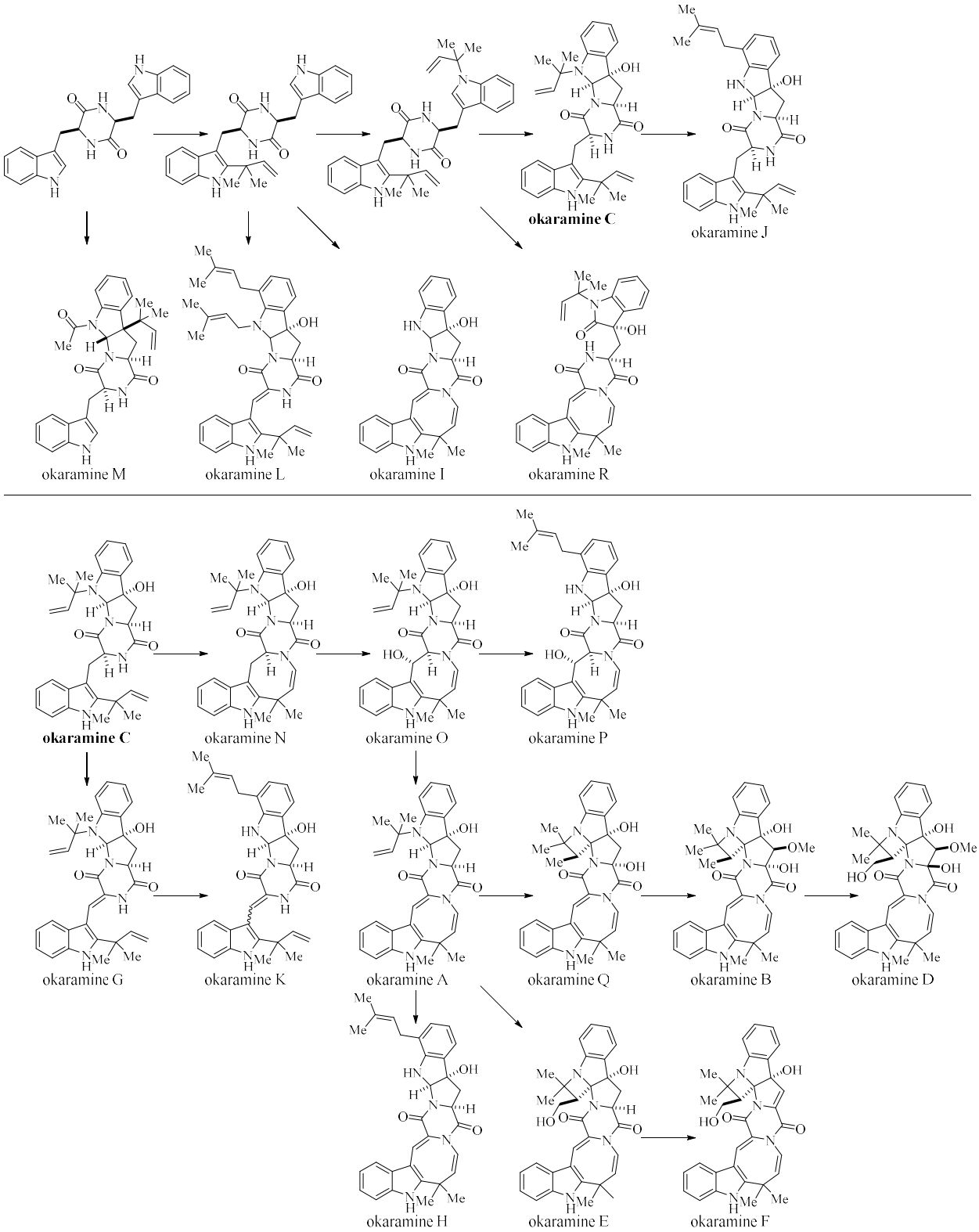
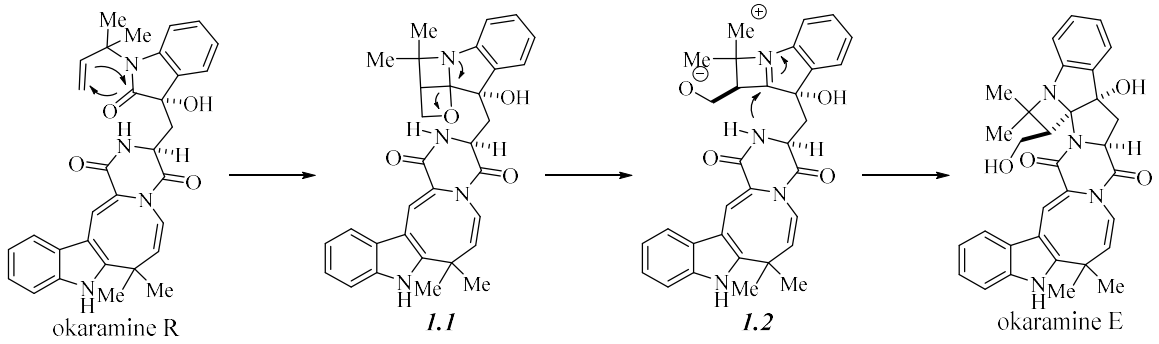


Figure 1.1. The okaramine family.



Scheme 1.1. Proposed biosynthesis of okaramines.

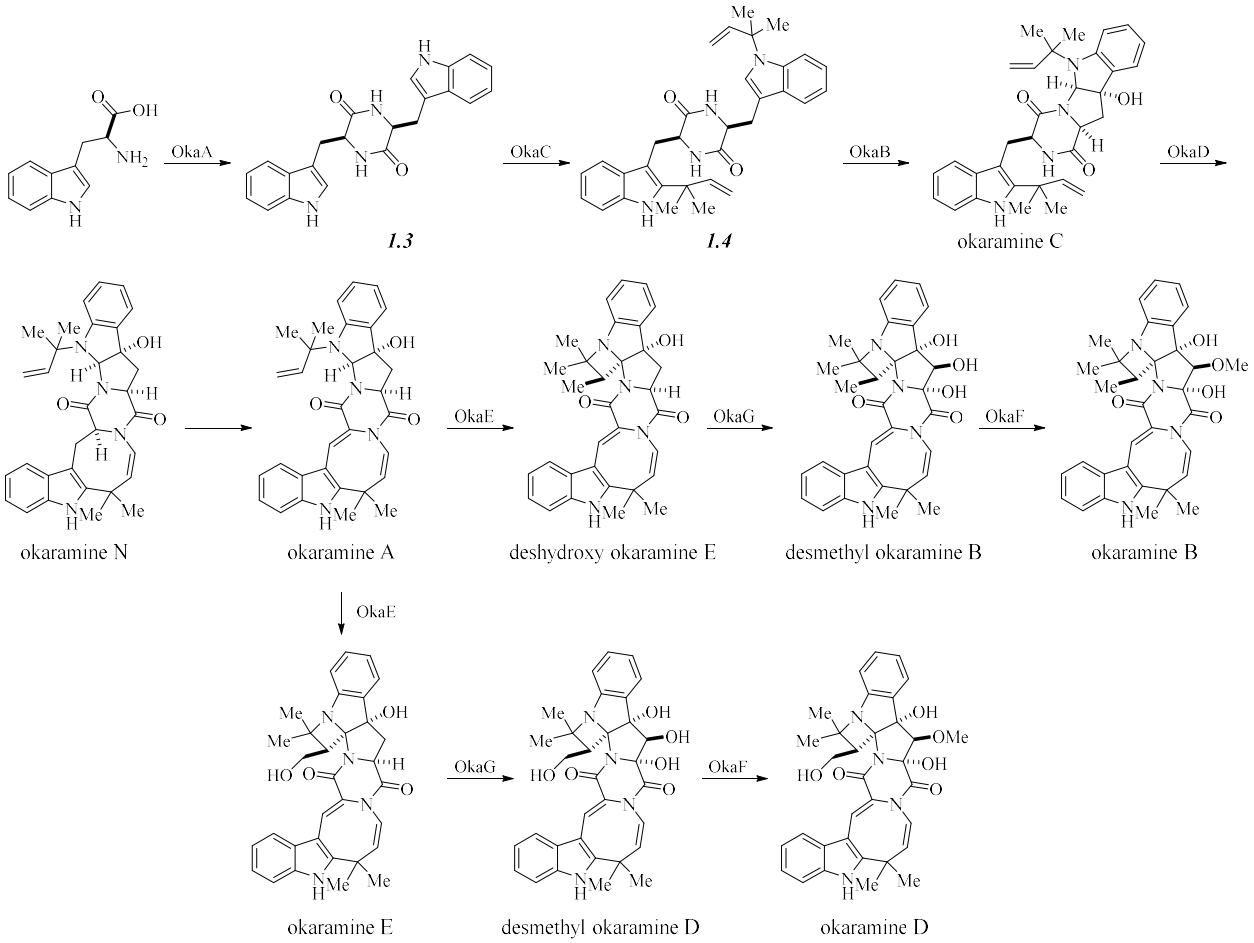


Scheme 1.2. Proposed photochemical mechanism for azetidine ring formation.

Recently, however, an almost complete mapping of the biosynthetic process for the synthesis of the okaramines in *Penicillium simplicissimum* ATCC 90288 (AK-40) and *Aspergillus aculeatus* ATCC 16872 (**Scheme 1.3**) was published.[14] Using clustered regularly interspaced short palindromic repeats (CRISPR)-associated RNA-guided DNA endonucleases (Cas9) to inactivate specific genes to investigate the gene cluster several clues were gathered about the biosynthesis (**Scheme 1.3**). By comparing the gene clusters of the two strains of bacteria under investigation a cluster was identified that had expected non-ribosomal peptide synthases (NRPS) and a flavin-dependent monooxygenase (FMO) for the initial construction of the oxidized and prenylated dipeptide core.

Cutting out enzyme OkaA lead to failure to produce any okaramine products, whereas cutting out OkaC lead to an accumulation of the unmodified dipeptide suggesting OkaA catalyzes formation of the diketopiperazine, and OkaC the consumption of it. By inserting these two genes into yeast it was confirmed; OkaA produces the dipeptide, and OkaC the double reverse-prenylated dipeptide. As further evidence, 6xHis-tagged OkaC was purified and incubated with the dipeptide and dimethylallyl pyrophosphate which produced only the double reverse-prenylated product. OkaB is similar to our own publication on NotB,[15] and other flavin-dependent monooxygenases,

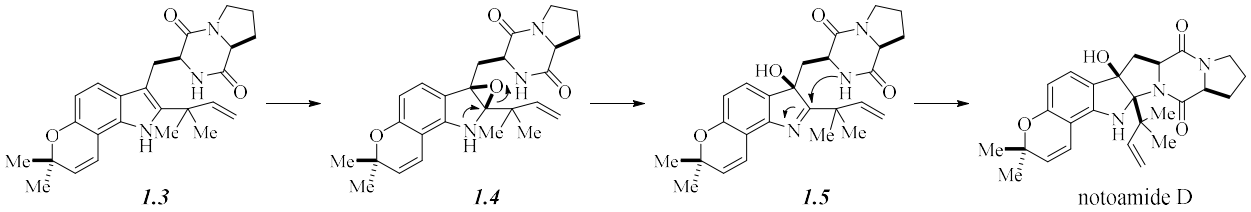
suggesting it would be responsible for the oxidation to the hydroxy-6-5-5 ring structure by way of epoxidation and rearrangement (**Scheme 1.4**). Deletion of the gene encoding OkaB lead to the accumulation of the double reverse-prenylated product as expected.



Scheme 1.3. Biosynthesis of okaramine family

The remaining enzymes in both bacteria, OkaD and OkaE, are both oxygenases, the former a P450 monooxygenase, the latter an α-ketogluterate-dependent dioxygenase. Deletion of OkaD lead to a buildup of okaramine C, the suspected product of the FMO oxidation. Expressing OkaE

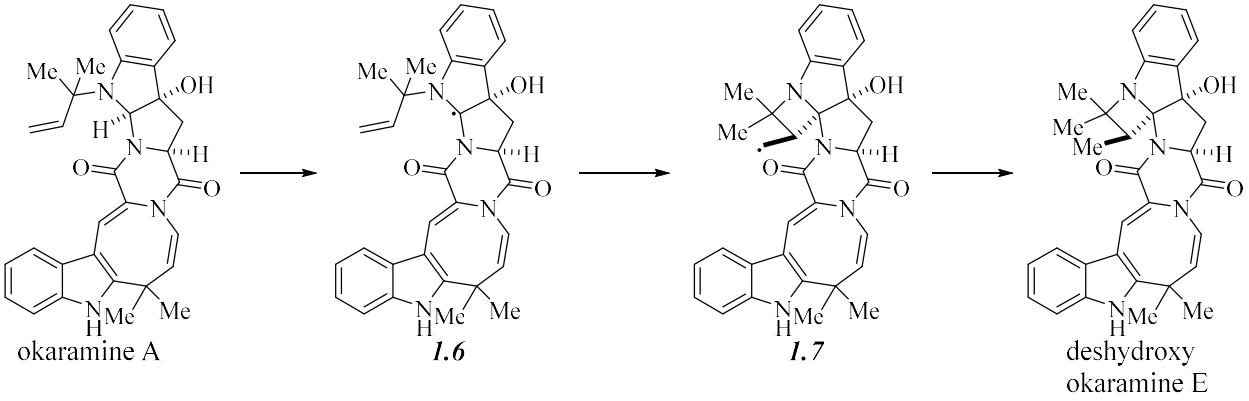
in yeast in the presence of okaramine A gave okaramine E and 12-deshydroxy okaramine E, in part solving the question of how the azetidine ring is constructed in nature.



Scheme 1.4. NotB mechanism to form notoamide D.

Lastly, deleting *okaG* led to a buildup of okaramine E and 12-deshydroxy okaramine E, suggesting the role of OkaG is a formal dihydroxylation. Deletion of *okaF*, gave a buildup of desmethyl okaramine B, which suggests OkaF is a methyltransferase.

Although the immediate precursor to the azetidine ring is now known, the enzyme responsible for the redox neutral transformation is an oxygenase. It remains to solve exactly how the oxygenase is catalyzing a non-oxidative transformation. The hypothesis offered by Lai involves a radical intermediate which cyclizes the azetidine in a 4-exo-trig radical cyclization (**Scheme 1.5**).



Scheme 1.5. Proposed mechanism of cyclization.

Chapter 2. Previous Synthetic Work

2.1. Syntheses by Baran, Roe, Hewitt and Iizuka

To date, only four of the twenty-one okaramines have been synthesized. Baran, Roe, Hewitt and Iizuka reported total syntheses for okaramines N, J, C, and M, respectively.[16-19] In all four cases, the target molecule was disconnected at the amide bonds of the diketopiperazine and prenyl groups (**Figure 2.1**).

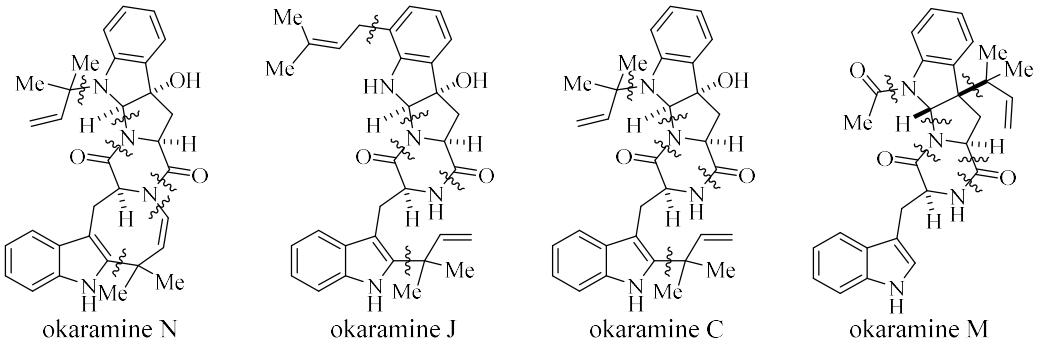
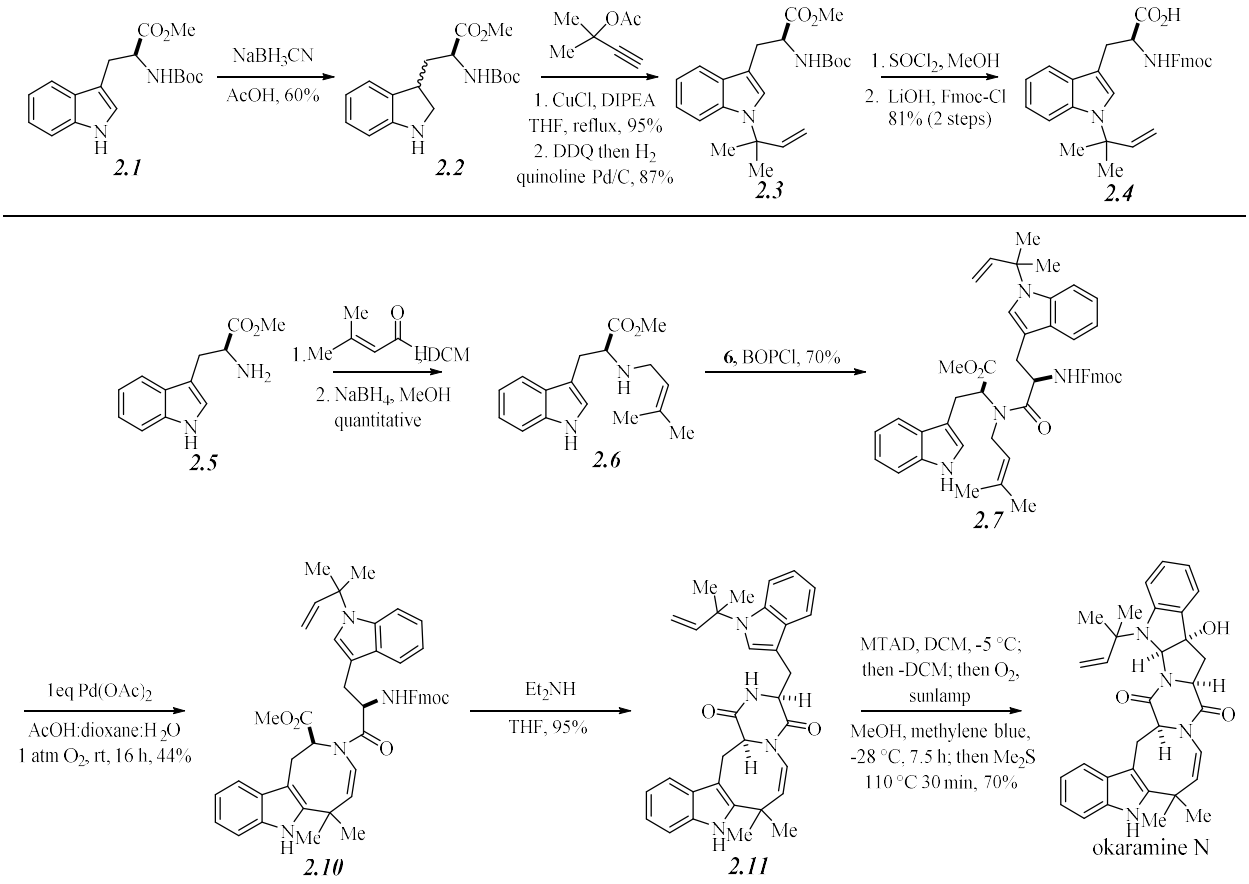


Figure 2.1. Disconnections in the syntheses of okaramine N, J, C and M.

2.1.1. Synthesis of Okaramine N

The synthesis of okaramine N was carried out in nine linear steps by Baran et al. (**Scheme 2.1**). Similar to the biogenesis, two prenyl and two tryptophan molecules were coupled. Starting from the protected L-tryptophan **2.1,** reduction using sodium cyanoborohydride gave indoline **2.2**. Coupling 2-methylbut-3-yn-2-yl acetate with **2.2** was followed by reoxidation to the indole and partial reduction of the alkyne to give **2.3**. Switching nitrogen protecting groups and hydrolysis of the methyl ester produced tryptophan derivative **2.4**. The second prenyl group was installed by reductive amination on the amine nitrogen of L-tryptophan methyl ester **2.5** giving **2.4.** Coupling

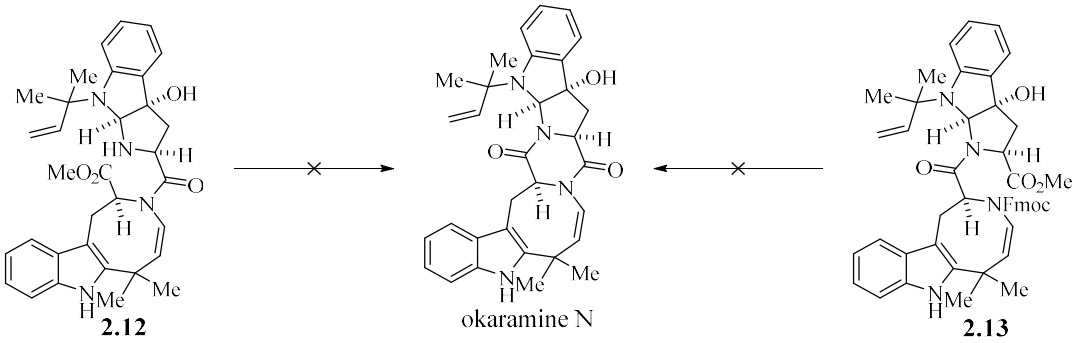
of tryptophan units **2.4** and **2.7** gave dipeptide **2.10**. Sequential closure of the dihydroindoloazocine (**2.10**), diketopiperazine (**2.11**), and pyrrole rings gave okaramine N. This route gave okaramine N in 8% yield from *N*-Boc-tryptophan methyl ester **2.1**.



Scheme 2.1. Synthesis of okaramine N.

This successful route presented several challenges relevant to the synthesis of other okaramine family members. Specifically, it was discovered that the closure of the diketopiperazine ring had certain limitations, namely that it was prone to failure if the pyrroloindole and dihydroindoloazocine rings were already intact (**Scheme 2.2**). Instead, the closure of the pyrrole ring had to be carried out after formation of the diketopiperizine. It was also found that the reverse

prenyl group was prone to Claisen rearrangement in acid or upon heating to 120 °C. Efforts to circumvent this challenge were thwarted, however, as the reverse prenyl group resisted installation late in the synthesis. Due to the structural similarity between the okaramines, much of the synthesis of okaramine N is foundational work for the okaramine family.

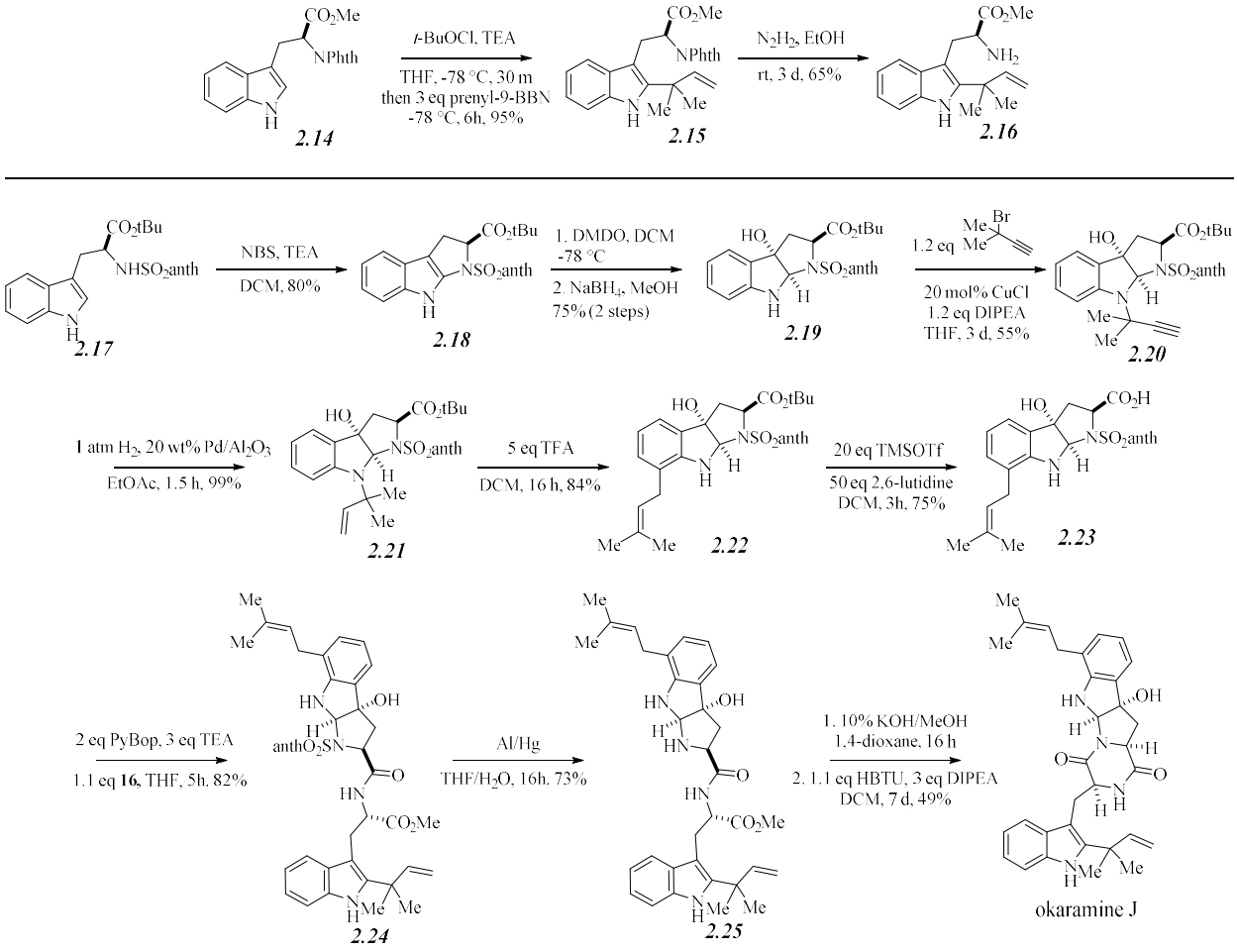


Scheme 2.2. Failed attempts at diketopiperazine ring closure.

2.1.2. Synthesis of Okaramine J

Roe and coworkers originally attempted the synthesis of okaramine C. Similar challenges were faced with regard to diketopiperazine formation, and the same Claisen rearrangement was seen during acidic treatment. Accessing okaramine J, however, required the Claisen rearrangement, which led the authors to switch from the synthesis of okaramine C to the synthesis of okaramine J (**Scheme 2.3**). As with okaramine N, two differentially modified tryptophan units were coupled together to form okaramine J. The first protected L-tryptophan molecule (**2.14**) was coupled with a prenyl group at C-2 of the indole ring to give **2.15**. The phthalimide protecting group was then removed to give amine **2.14.** The other protected L-tryptophan, **2.17**, underwent intramolecular cyclization using NBS to give **2.16.** A net hydration via oxidation using DMDO followed by reduction with $NaBH_4$ yielded alcohol **2.19**. The propargyl predecessor of the reverse

prenyl group was then installed under similar conditions to okaramine N. Partial reduction of the alkyne of **2.20** gave alkene **2.21,** which underwent acid catalyzed Claisen rearrangement and deprotection to give acid **2.23**. Coupling **2.16** and **2.23** furnished dipeptide **2.24,** which was deprotected but resisted ring closure to the diketopiperazine. Only by saponification of ester **2.25** and application of standard peptide coupling conditions was okaramine J able to be synthesized. The successful ten step synthesis gave okaramine J in 6% yield.



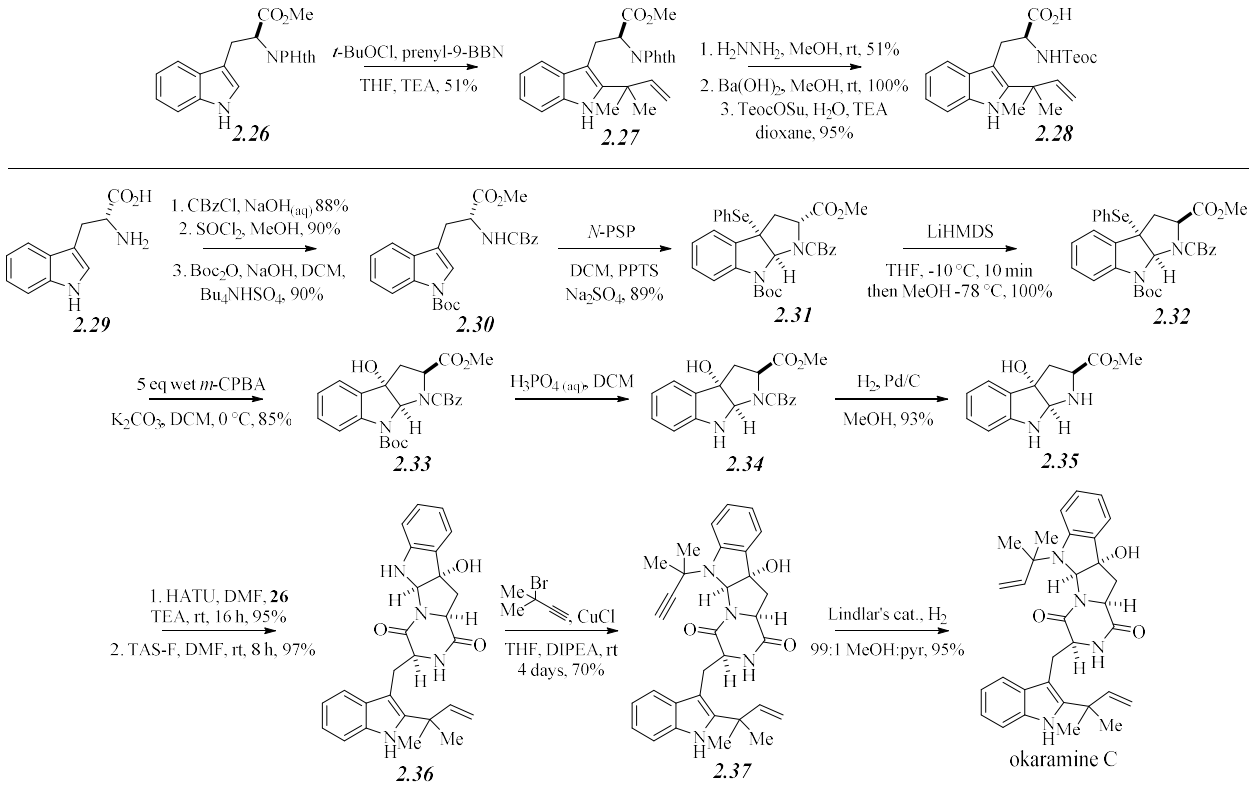
Scheme 2.3. Synthesis of okaramine J.

2.1.3. Synthesis of Okaramine C

Hewitt and coworkers reported the synthesis of okaramine C, which bears strong resemblance to the synthesis of okaramine J (**Scheme 2.4**). The lower half was made by installation of a reverse prenyl group at C-2 of tryptophan **2.26** giving substrate **2.25.** Protecting group manipulation gave coupling fragment **2.26.** D-Tryptophan (**2.29**) was protected and cyclized using *N*-(phenylseleno)phthalimide (*N*-PSP) to furnish **2.30**. The protecting groups on **2.31** were carefully chosen based on earlier work in the same lab to achieve **2.32** in good yield and diastereomeric ratio. Epimerization of the ester moiety followed by oxidative deselenation and Boc deprotection yielded **2.34**, which is identical to **2.19** save for the identity of the protecting groups. CBz-deprotection of **2.34** gave the requisite coupling partner **2.35**. Compounds **2.28** and **2.35** were coupled under standard peptide coupling conditions followed by Teoc deprotection. This underwent diketopiperazine formation at room temperature without any need for peptide coupling reagents, unlike the synthesis of okaramine J, to furnish diketopiperazine **2.34.** Also unlike the synthesis of okaramine N, Hewitt et al. were able to install the reverse prenyl group late in the synthesis of an okaramine using similar conditions to those used in other syntheses. The late stage prenylation allowed Hewitt et al. to finish the synthesis of okaramine C without danger of an unwanted Claisen rearrangement. This route is the longest synthesis of an okaramine; however, it yielded okaramine C in an impressive 31% yield over twelve steps.

2.1.4. Synthesis of Okaramine M

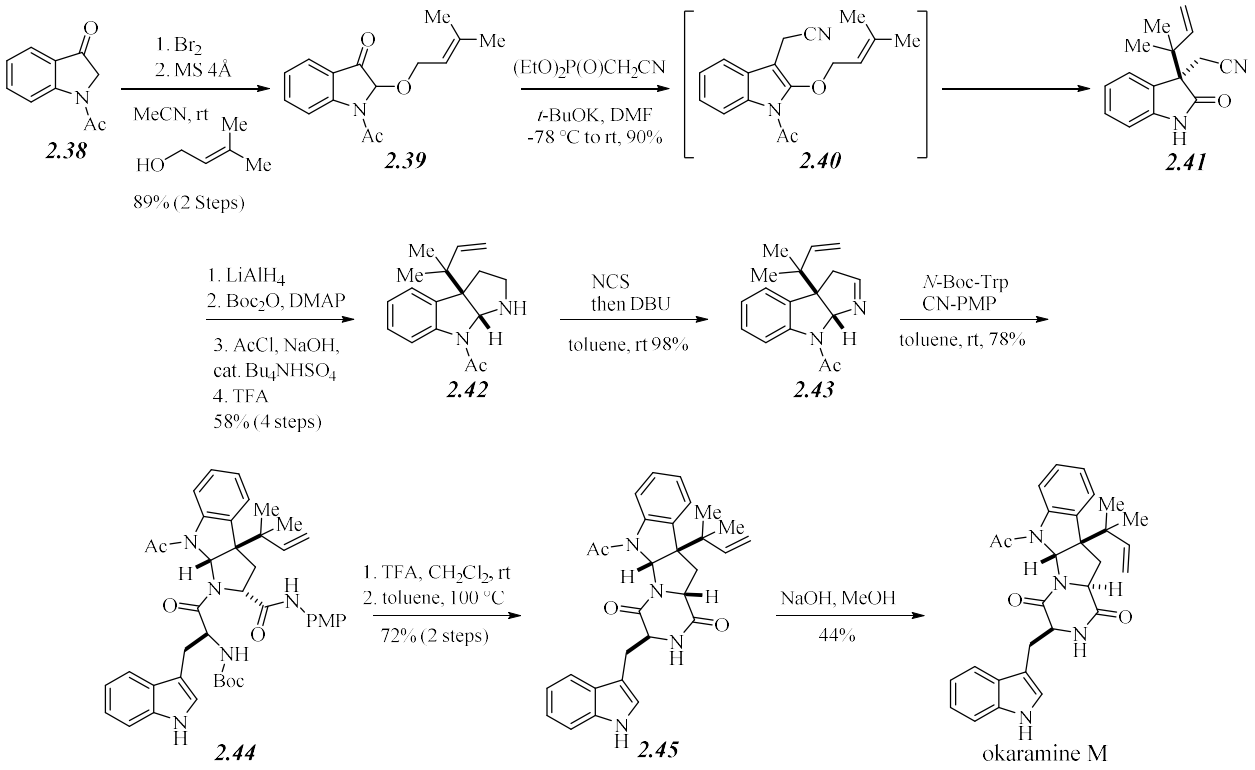
The total synthesis of okaramine M by Iizuka et al. was carried out through a more unique pathway utilizing a reductive cyclization of a 3,3-disubstituted oxindole and a three component Ugi reaction (**Scheme 2.5**). Bromination on C-2 of 3-indolinone **2.38**, followed by substitution



Scheme 2.4. Synthesis of okaramine C.

using prenyl alcohol, gave **2.39**. Horner-Wadsworth-Emmons reaction using cyanomethylphosphonate was followed by spontaneous isomerization to intermediate **2.40**, which underwent a further spontaneous Claisen rearrangement to **2.41**. Reduction using $LiAlH_4$ allowedfor closure of the pyrroloindoline ring. After protecting group manipulation, compound **2.42** was accessed via oxidation to the imine. Using imine **2.43**, *p*-methoxyphenyl isocyanide, and *N*-Boc-tryptophan in an Ugi reaction gave **2.42**. Deprotection of the amine and heating to 100 °C gave diketopiperazine **2.45**. Epimerization of the incorrect diketopiperazine stereocenter gave okaramine M in 44% yield, with only 7% of both diketopiperazine stereocenters being epimerized. Overall, okaramine M was synthesized in twelve steps and 12% yield. In the course of synthesizing okaramine M, Iizuka et al. were able to show that the stereochemistry at C-2 and C-

3 of indoline were wrong in the original characterization. Okaramine M is the only okaramine known to date to have inverted stereochemistry at the C-2 and C-3 positions of indoline.

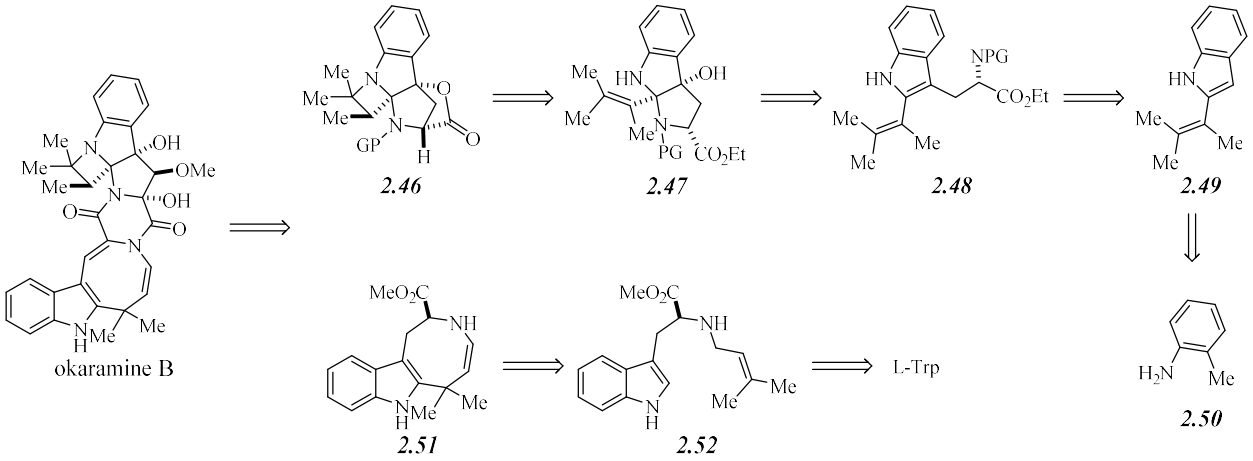


Scheme 2.5. Synthesis of okaramine M.

2.2. Previous Synthesis in the Williams Laboratory

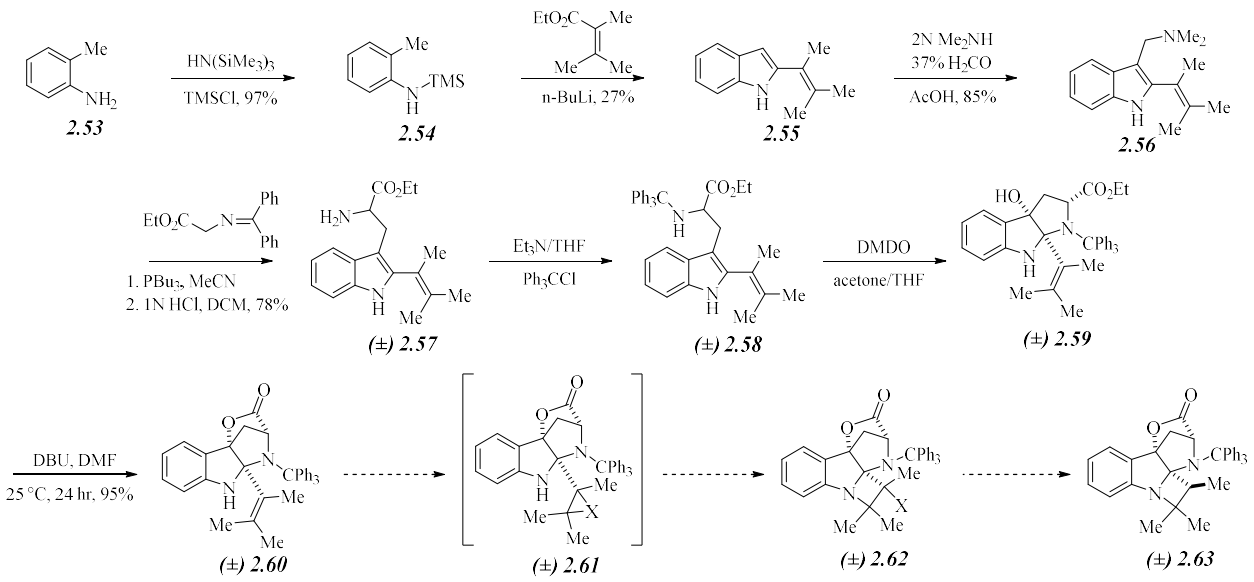
Although okaramine B shares structural similarity with the previously synthesized members of the family, the azetidine and dihydroindoloazocine rings, additional oxidation, and five contiguous stereocenters provide increased synthetic challenges. First attempts at synthesizing okaramine B were performed by Dr. Alan Grubbs. His plan also disconnected the molecule at the diketopiperazine leaving azetidine containing pyrroloindoline fragment **2.46** and dihydroindoloazocine fragment **2.51** similar to the synthesis of okaramine N (**Scheme 2.6**). Compound **2.46** would be made by intramolecular lactonization and hydroamination of **2.47**, which is the product of oxidative cyclization of **2.46.** Compound **2.48** would in turn be constructed

from **2.49**, which is itself constructed from *o*-methylaniline (**2.50**). The lower half would be constructed by palladium mediated ring closure of **2.52** which is made by reductive amination of tryptophan methyl ester onto 3-methyl-2-butenal.



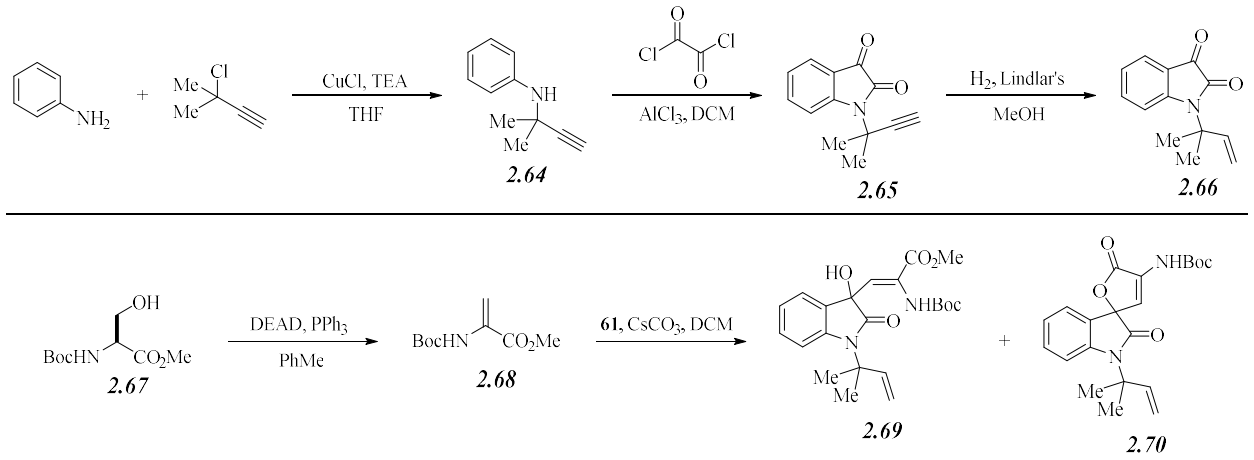
Scheme 2.6. Retrosynthetic analysis of okaramine B.

The plan was carried out racemically to provide a tryptophan derivative with the appropriate olefin installed at the C-2 position of indole (**Scheme 2.7**). Temporary protection of aniline **2.53**, followed by condensation with ethyl 2,3-dimethylbut-3-enoate, gave indole **2.55**. Reaction with *in situ* derived Eschenmoser's salt yielded indole **2.54.** Condensation with ethyl 2-((diphenylmethylene)amino)acetate, followed by hydrolysis of the imine, gave compound **2.55.** After screening protecting groups, it was found that trityl protection allowed for the appropriate stereochemical control upon closure of the pyrrole ring to **2.59,** after which the hydroxyl and acid functionalities were protected as lactone **2.60**. Attempts to close the azetidine ring centered on forming compound **2.61** where X=Br^+, O, or Hg^+, which would undergo rearrangement to **2.62**. Unfortunately, no conditions could be found to effect this transformation.



Scheme 2.7. Synthetic work toward okaramine B.

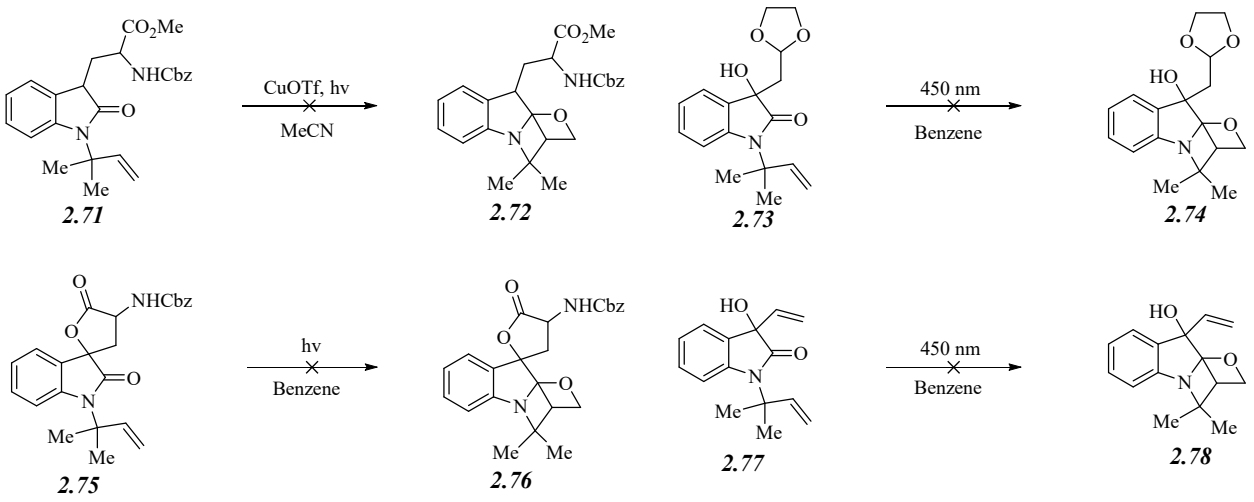
Dr. Timothy McAfoos took over attempts at synthesizing okaramine B and explored multiple routes. Many of the routes featured nucleophilic attacks on an isatin by Grignard reagents, enolates or enamines. One such route featured isatin **2.66** (**Scheme 2.8**).



Scheme 2.8. Progress toward okaramine B.

This isatin fragment was made by first coupling 3-chloro-3-methylbut-1-yne under similar conditions to those above. The anilinic nitrogen of **2.64** displaced a chlorine in oxalyl chloride and then underwent intramolecular Friedel-Crafts acylation to make isatin **2.65**. The alkyne was then reduced to the alkene giving isatin **2.64.** This was then reacted with enamine **2.68,** which is derived from dehydration of protected serine **2.67** under Mitsunobu conditions. Unfortunately, the combination of **2.66** and **2.68** gives only a 2:1 preference for oxindole geometric isomer **2.69** over **2.70** (which underwent spontaneous lactonization) with no enantioenrichment.

Hoping to test our biogenetic hypothesis, Dr. McAfoos attempted to induce photocyclization on the mixture of **2.69** and **2.70** as well as other oxindole fragments as a model for a complete okaramine (**Scheme 2.9**). To date no encouraging results have been obtained.

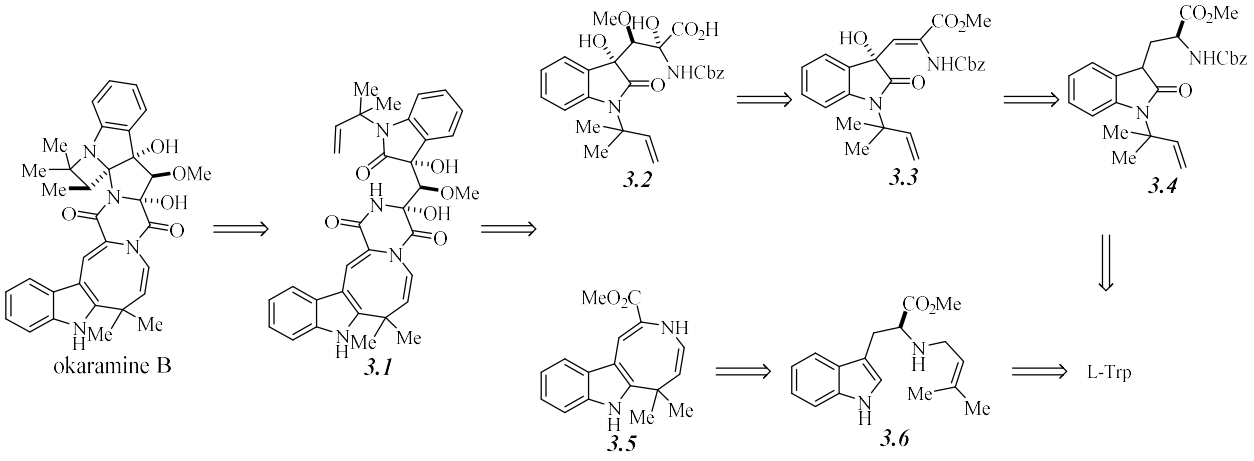


Scheme 2.9. Failed attempts at photocyclization.

Chapter 3. Synthetic Efforts Toward Okaramines B and R

3.1. Recent Synthetic Efforts Toward Okaramine B

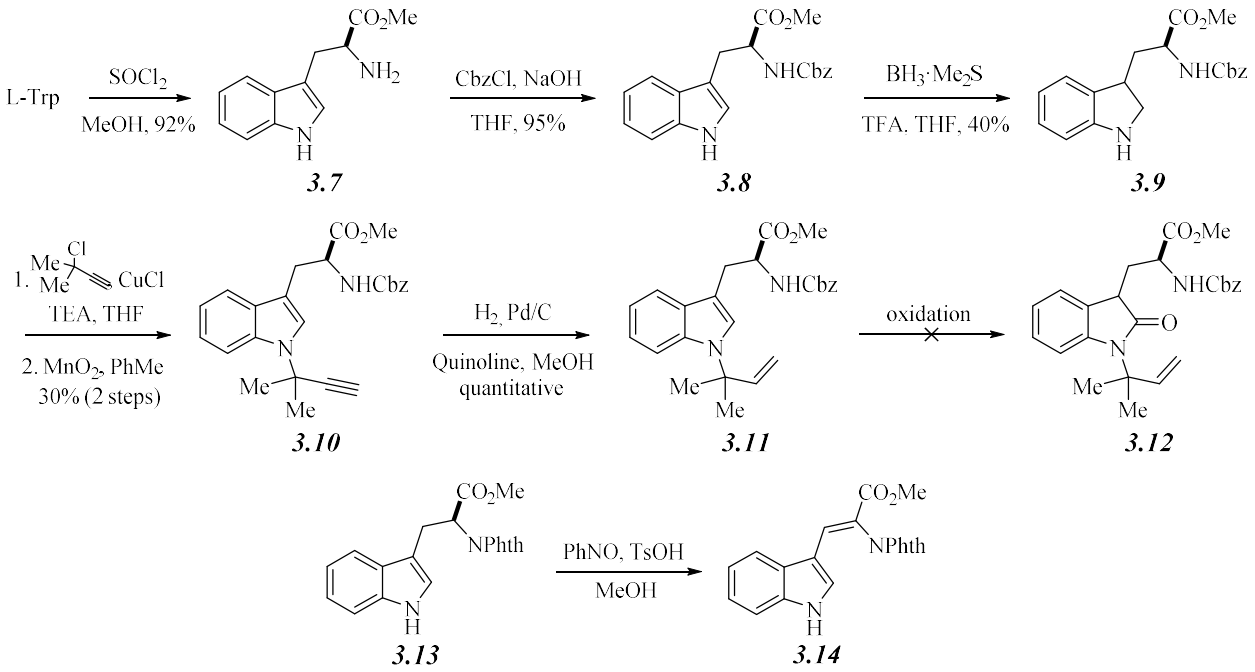
Our recent efforts at synthesizing okaramine B have relied on a new approach (**Scheme 3.1**). Okaramine B would be synthesized by the hypothesized photocyclization of precursor **3.1**, followed by reduction of the resulting primary alcohol. Compound **3.1** would be the product of coupling **3.2** and **3.5**. Fragment **3.2** is the result of oxidation of the α,β unsaturated ester **3.3**, perhaps by epoxidation, followed by ring opening in methanol to yield *trans* stereochemistry of the two oxygen substituents. Compound **3.3** is derived by dehydrogenation of oxindole **3.4**, followed by attack on Davis's oxaziridine by the corresponding enolate. Oxindole **3.4** can be obtained following a similar route to the one utilized for the synthesis of compound **7**, albeit with additional oxidation on the indole. Compound **3.5** would likewise be synthesized parallel to the synthesis of okaramine N.



Scheme 3.1. Synthetic plan toward okaramine B.

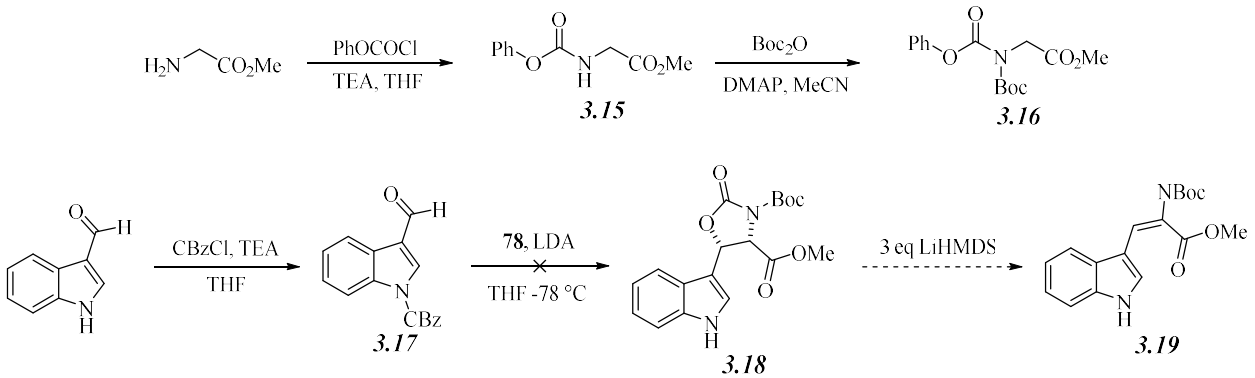
Work on this route began with the esterification of L-tryptophan using thionyl chloride in methanol at reflux (**Scheme 3.2**), which yielded 92% of tryptophan methyl ester **3.5.** Cbz protection of **3.7** gave the expected protected tryptophan **3.8** in 95% yield. Despite several attempts, the reduction of **3.8** to indoline **3.9** using BH_3 in THF/TFA failed to give higher than 40% yield. This product was carried on by installing the propargyl group using 3-chloro-3-methyl-1-butyne, triethylamine and catalytic CuCl. The crude product was oxidized back to the indole using MnO_2 under O_2 atmosphere in just 30% yield over the two steps. Initial attempts to follow a published procedure to reduce the alkyne of **3.10** to the alkene gave a mixture of the alkene and alkane. Roe et al. reported similar problems in the course of synthesizing okaramine J. Rather than resort to careful timing to avoid over reduction we used quinoline and Pd/C in place of Lindlar's catalyst. In 3 hours the alkyne was reduced to the alkene with no evidence by NMR of the alkane. Under these new conditions, reverse prenylated tryptophan **3.11** was obtained quantitatively. Unfortunately, all attempts at oxidizing the indole to oxindole **3.12** have failed. Conditions employed include DMSO/$HCl_{(con)}$, NBS/t-BuOH, Sharpless dihydroxylation, Davis's oxaziridine and Shi epoxidation. Hoping to fare better after dehydrogenation of the tryptophan derivative we attempted to employ work by Baran et al. Unfortunately, conversion of protected tryptophan **3.13** to **3.14** was plagued by persistently low yields leading us to abandon that route altogether.

The first attempt to circumvent the failed nitrosobenzene dehydrogenation involved synthesis of the alkene by formation of an oxaziridine, followed by elimination (**Scheme 3.3**).[18] To this end, protected glycine derivative **3.16** was synthesized from glycine methyl ester. Reaction of ester **3.16** with CBz protected indole-3-carboxaldehyde **3.17** did not provide the desired indole **3.16.** Despite exact precedent, the reaction failed multiple times in our hands.

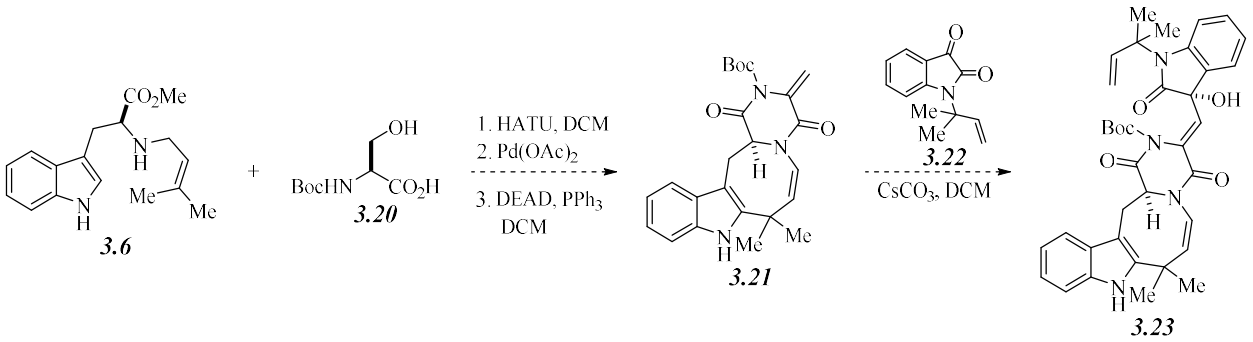


Scheme 3.2. Progress toward the synthesis of okaramine B

We next envisioned using a variation on the enamine strategy employed by Dr. McAfoos (**Scheme 3.4**). We hypothesized that if the enamine of serine were coupled to tryptophan derivative **3.6** we might be able to bias the geometric and enantioselectivity of the product more strongly than previously observed (**Scheme 14**).

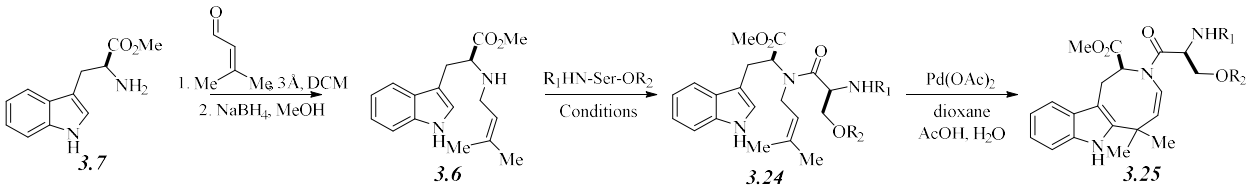


Scheme 3.3. Oxazolidinone route to an oxidized tryptophan derivative.



Scheme 3.4. Enamine route to photochemistry precursor

Work on this route began with reductive amination to install the reverse prenyl group on the amine of tryptophan methyl ester **3.7** (**Scheme 3.5**). Coupling of modified tryptophan **3.6** to *N*-Boc-serine proved problematic and required a coupling reagent screen (**Table 3.1**). $Pd(OAc)_2$ mediated ring closure of the dipeptide has so far yielded only trace amounts of dihydroindoloazocine.

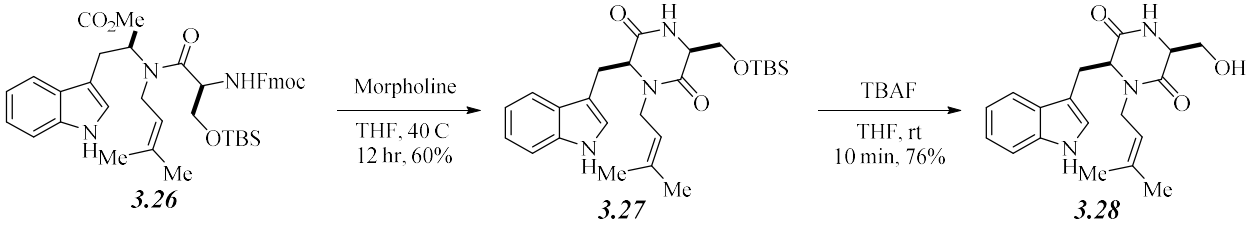


Scheme 3.5. Synthetic progress toward okaramine B.

Desirous to test the proposed 2+2 photocyclization we pressed forward without the dihydroindoloazocine ring system by subjecting **3.26** to deprotection of the Fmoc group with concomitant ring closure to form diketopiperzine **3.27** (**Scheme 3.6**). Deprotection of the silyl protecting group using TBAF afforded **3.28,** which need only be subjected to dehydration to yield a substitute for **3.21**.

Table 3.1. Screening of coupling conditions.

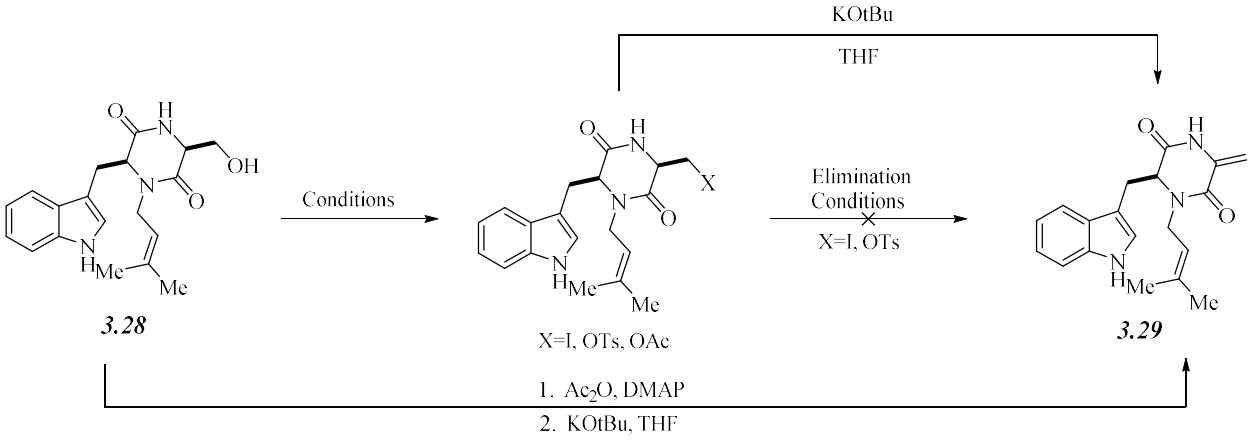
	Conditions	R1	R2	Yield
1	EDCI	Fmoc	H	0%
2	HATU	Fmoc	H	0%
3	BopCl	Fmoc	H	0%
4	HATU	Boc	H	0%
5	HATU	Boc	TBS	0%
6	BopCl	Boc	TBS	20%
7	BopCl	Fmoc	TBS	40%



Scheme 3.6. Synthesis toward an analog of **3.21.**

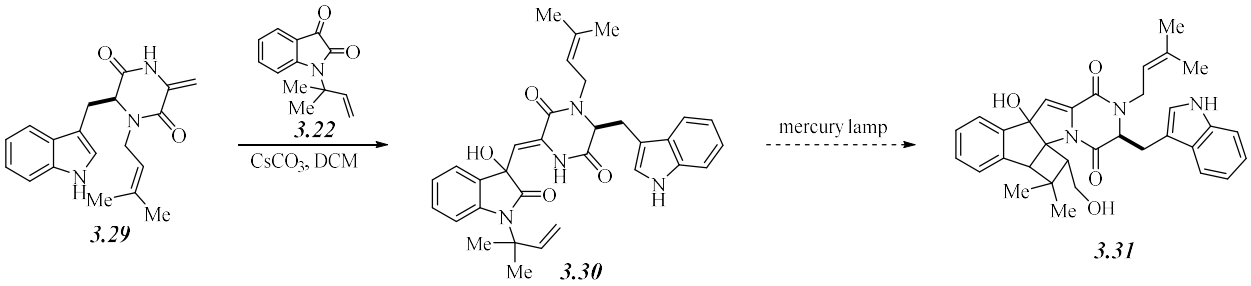
Dehydration of **3.28** proved problematic (**Scheme 3.7**). Conditions that failed to yield **3.29** include the use of Mitsunobu conditions, o-nitrophenylselenocyanate followed by oxidation, and iodination or tosylation followed by elimination. It was found that the acylated product is able to undergo the elimination to **3.29** perhaps by undergoing enolization followed by intramolecular deprotonation. This would be uniquely successful because it does not require nucleophilic attack at the hydroxylated carbon, nor deprotonation at the sterically encumbered adjacent to it.

Coupling between **3.22** and **3.29** leads to **3.30** as a complex mixture of diastereomers (**Scheme 3.8**). Although the NMR was not solved the mass was confirmed. **3.30** is a close analogue of **3.23** and we began attempts at photocyclization under Hg lamp.

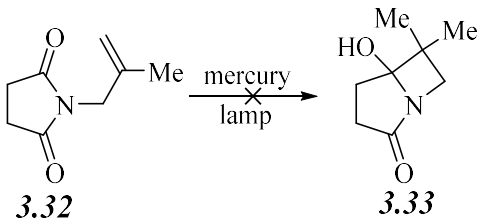


Scheme 3.7. Synthesis of **3.29**.

All attempts at cyclizing **3.30** to **3.31** have met with failure. In an attempt to better understand the conditions and variables of photocyclization we turned to work published previously by Maruyama et al in 1975.[20] In that work succinimide **3.32** was irradiated using a 120 W low-pressure Hg lamp for 5 days giving oxetane **3.33** quantitatively (**Scheme 3.9**). Unfortunately, we were unable to repeat these findings in our photoreactor using different wavelength sets of 14W low-pressure Hg. Only decomposition was seen at shorter wavelengths, typically in much shorter times than 5 days, or no reaction at longer wavelengths (>400 nm).



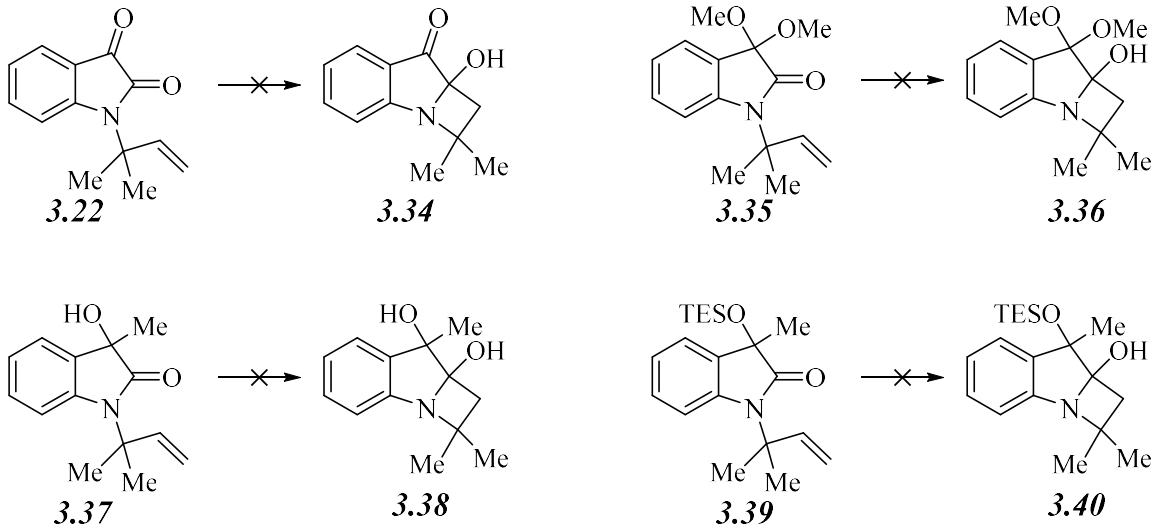
Scheme 3.8. Coupling and photocyclization.



3.32 *3.33*

Scheme 3.9. Failed cyclization of known reaction.

Having tested a variety of variables including oxygenation of the solvent, concentration, vessel material, reaction time, number of 14 W bulbs (maximum number of bulbs was 12), we abandoned focus on succinimide **3.32** and returned to a closer model of compound **3.30**. We began attempts to photocyclize isatin **3.22** to the corresponding oxetane **3.34**, however upon irradiation no reaction was observed (**Scheme 3.10**). In an attempt to alter the electronics of the molecule we protected one carbonyl of **3.22** to make oxindole **3.35**. Photolysis of **3.35** to **3.36** also met with failure and gave only small amounts of the deprotected form of isatin **3.22**. Methylation of isatin **3.22** to oxindole **3.37** proceeded smoothly, however failed to cyclize to **3.38** upon irradiation. TES-protected oxindole **3.39** was prepared from **3.37**, but also resisted cyclization to **3.40**.

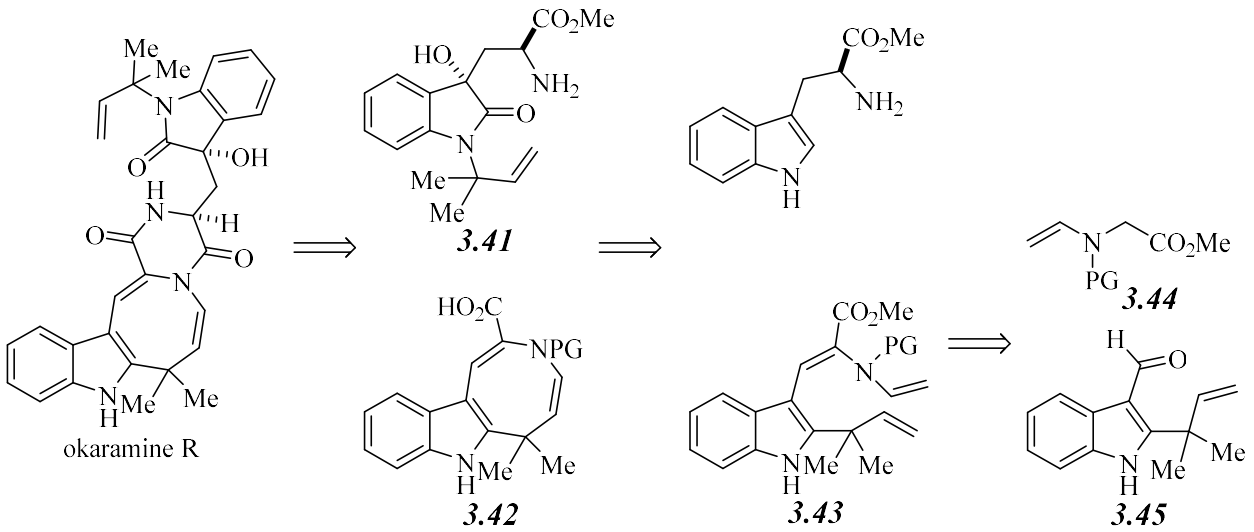


Scheme 3.10. Failed photocylizations.

3.2. Recent Synthetic Efforts Toward Okaramine R

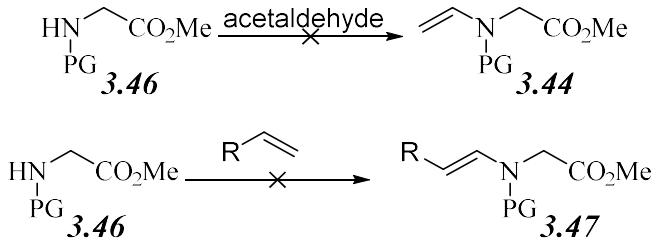
After investigating conditions and substrates for photocyclization it was decided to test the hypothesis directly by synthesizing Okaramine R and attempting photocyclization to Okaramine E (**Scheme 3.11**). We reasoned that Okaramine R could be prepared from peptide coupling and diketopiperizine formation of **3.41** and **3.42**. Compound **3.41** can be made by the oxidation of previously synthesized compound **3.5.** Compound **3.42** could be made using ring closing metathesis on the reverse-prenylated tryptophan enamine derivative **3.43**, which would stem from condensation of the appropriate glycine derivative **3.44** on known reverse-prenylated indole aldehyde **3.45**.

Unfortunately, the synthesis of a protected allylic amine in **3.44** failed under a variety of conditions (**Scheme 3.12**). In addition, attempts to make similar compounds using longer aliphatic chains, which would be irrelevant to the product of the Grubbs reaction, also failed.



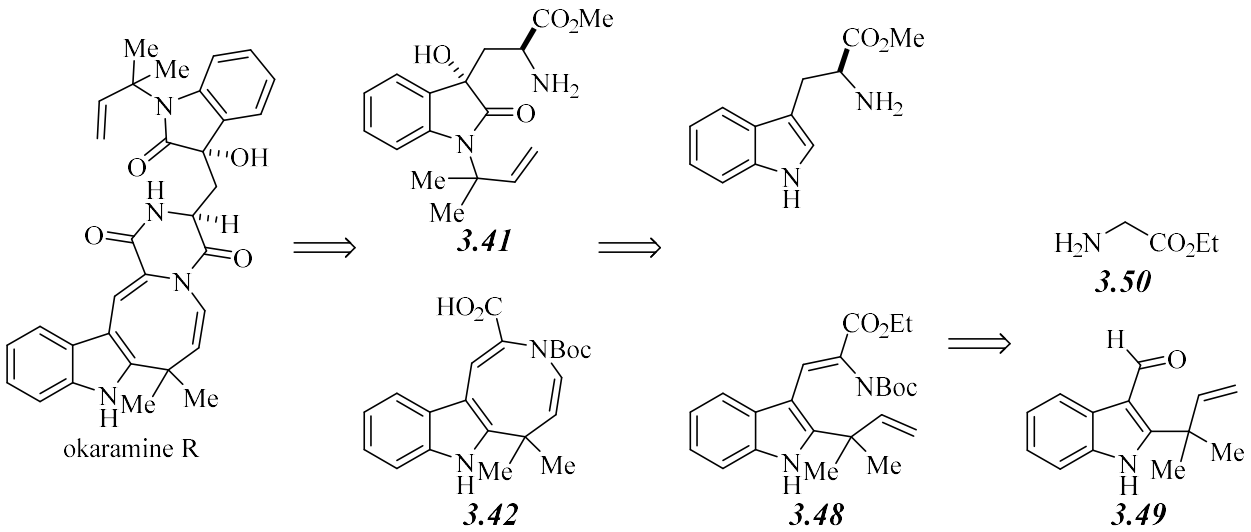
Scheme 3.11. Initial synthetic plan toward okaramine R.

Taking a slightly different direction, the following synthetic plan became the focus (**Scheme 3.13**). In place of a ring closing metathesis to form the 8-membered ring we opted for an oxidative amination to get to **3.42**.

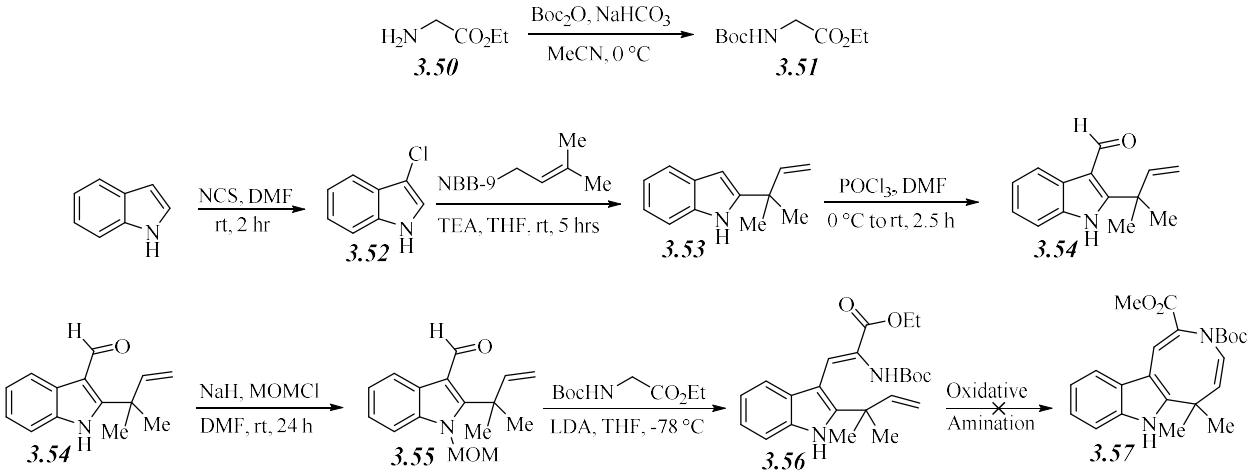


Scheme 3.12. Failed attempts at a Grubbs reaction precursor.

Initial progress toward **3.42** proceeded smoothly (**Scheme 3.14**). Precedented synthesis of **3.54** was followed by MOM protection of the indole nitrogen to **3.55**. Condensation of **3.51**, onto **3.55** was successful, however no attempts to effect an oxidative amination were successful. Palladium catalyzed cyclization did give the 7 membered exomethylene as a single product.

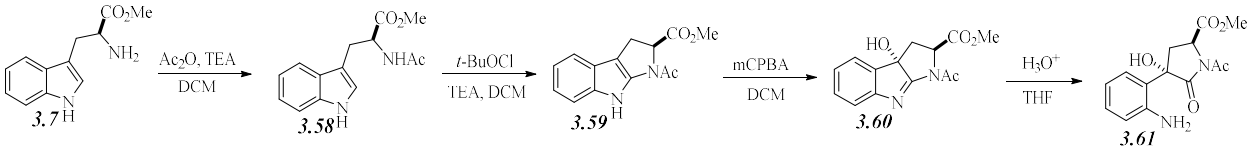


Scheme 3.13. Current synthetic plan toward okaramine R.



Scheme 3.14. Work toward coupling partner **3.42**.

Our efforts toward **3.41** also made good progress initially based on work pioneered by Witkop (**Scheme 3.15**).[21] Protection of **3.7** and cyclization gave tricycle **3.59**. Oxidation with Davis' oxiziridine failed to give useful product, however *m*CPBA gave **3.60** as a single diasteromer. Hoping to gain access to an unprenylated oxindole analog of **3.41** however treatment with aqueous acid gave free **3.61** as the only product.



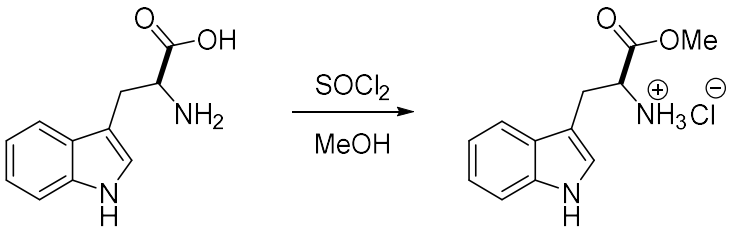
Scheme 3.15. Work toward coupling partner **3.41**.

Chapter 4. Conclusions

The okaramine family of natural products provides several complex synthetic targets. Although four members of the family have been synthesized, none of the azetidine containing okaramines have been synthesized. Of the five azetidine containing okaramines, okaramine B is the most active insecticide and is of synthetic interest. Though the biosynthesis has largely been worked out, the mechanism of the formation of the azetidine ring remains partially unknown. Despite numerous routes being investigated, this target continues to elude us and efforts toward the synthesis of the requisite oxindole are ongoing.

Chapter 5. Experimental

^{1}H and ^{13}C spectra were obtained using 300, 400 and 500 MHz spectrometers. The chemical shifts are given in parts per million (ppm) relative to TMS at δ 0.00 ppm or at residual $CDCl_3$ at δ 5.26 ppm for proton spectra and relative to $CDCl_3$ at δ75.23 ppm for carbon spectra. IRA spectra were recorded on an FT-IR spectrometer as thin films. Mass spectra were obtained using a high/low resolution magnetic sector mass spectrometer. All melting points are uncorrected. Flash column chromatography was performed with silica gel grade 60 (240-400 mesh). Unless otherwise noted, materials were obtained from commercially available sources and used without further purification. Dichloromethane (CH_2Cl_2), tetrahydrofuran (THF), toluene (PhMe), N,N-dimethylformamide (DMF), acetonitrile (CH_2CN), triethylamine (Et_3N), and methanol (MeOH) were all degassed with argon and passed through a solvent purification system containing alumina or molecular sieves.



A slurry of l-tryptophan (25.65g, 125.6 mmol) in methanol was prepared. The flask was cooled in an ice bath and $SOCl_2$ (13.7 mL, 186.6 mmol, 1.5 eq) was added dropwise. The reaction was attached to a water condenser equipped with a drying tube and all to react at 60°C overnight. The solution was condensed to a pink solid which was redissolved in methanol and condensed twice more. The process was then repeated with diethyl ether. The solid was then left on high vacuum overnight and then collected as a pale pink solid (30.63g, 120.3 mmol, 96%). ^{1}H-NMR (300 MHz; DMSO-d_6): δ 11.13 (s, 1H), 6.63 (s, 3H), 5.49 (d, J = 5.7 Hz, 1H), 5.35 (d, J = 6.1 Hz, 1H), 5.24 (d, J = 2.3 Hz, 1H), 5.07 (td, J = 5.5, 0.9 Hz, 1H), 5.01-4.96 (m, 1H), 4.19 (t, J = 4.1 Hz, 1H), 3.63 (s, 3H), 3.29 (t, J = 4.4 Hz, 2H), 2.48 (t, J = 1.7 Hz, 1H).

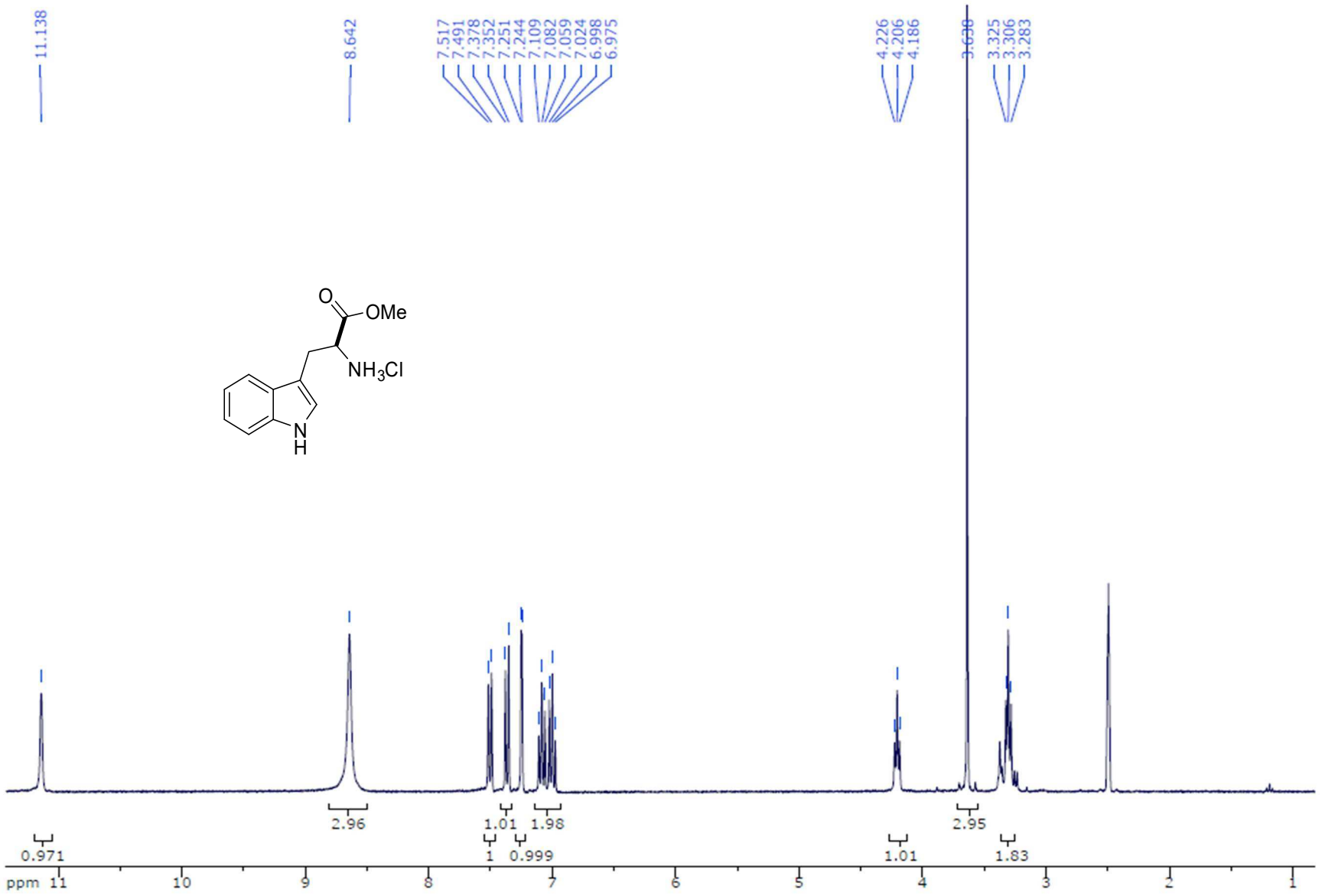


Figure 5.1. ^{1}H NMR of the product.

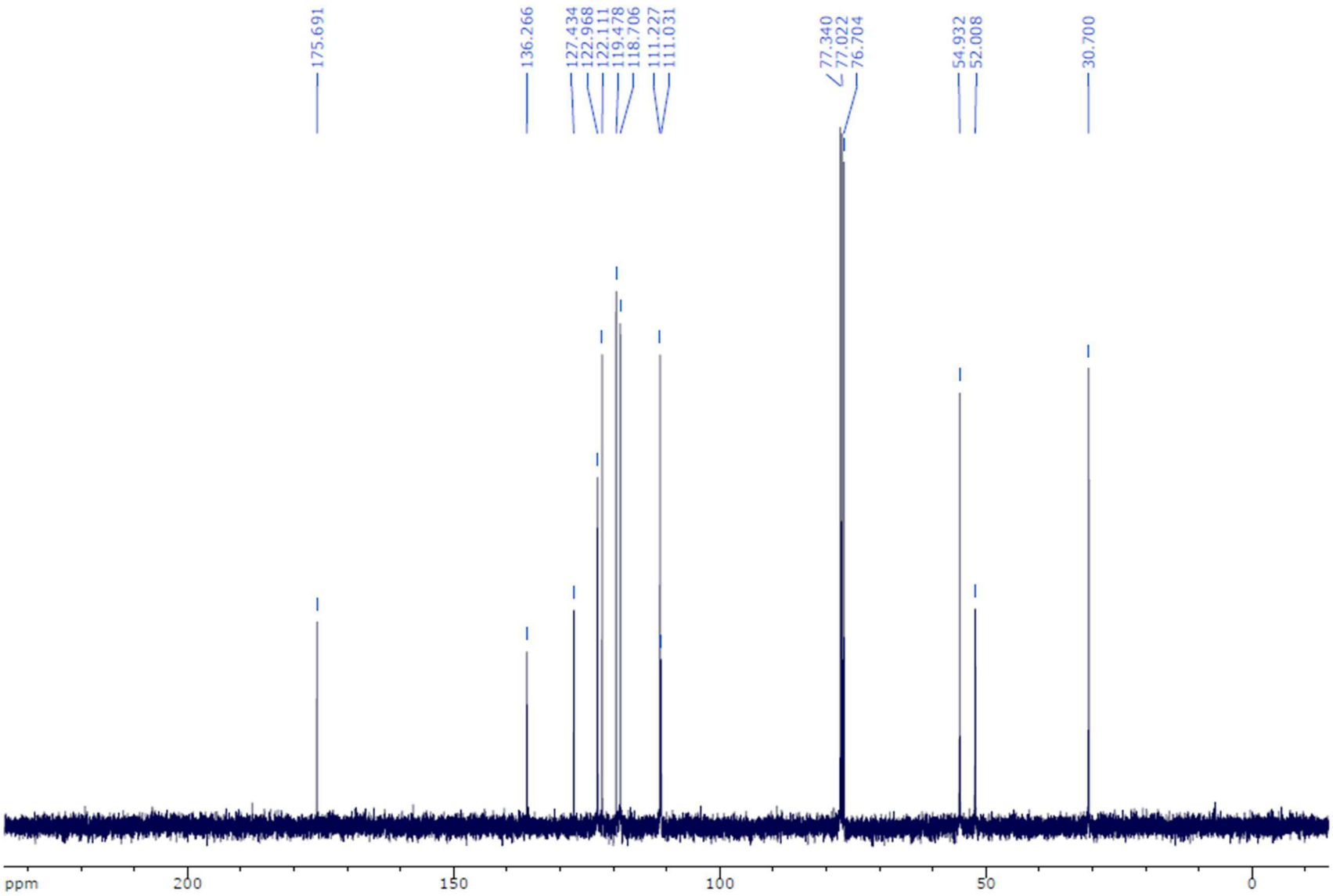
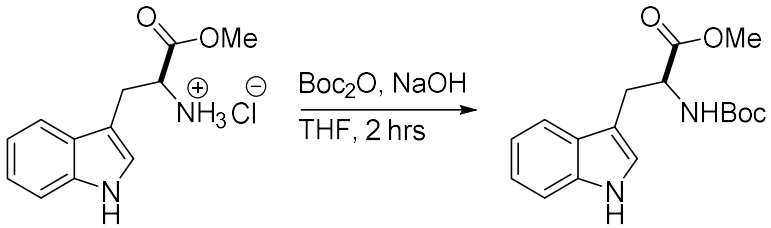


Figure 5.2. ^{13}C NMR of the product.



Suspended methyl ester (2.67 g, 10.5 mmol) in 25 mL THF. Added Boc anhydride (2.41 g, 11.0 mmol, 1.05 eq) and NaOH (10.5 mL 1M solution, 10.5 mmol, 1.0 eq). Stirred at room temperature for 2 hours. Condensed, and acidified with $KHSO_4$. Extracted with EtOAC three times, dried and condensed yielding 2.92 g of clean product (82% yield). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 6.07 (s, 1H), 5.55 (d, J = 5.8 Hz, 1H), 5.35 (d, J = 5.7 Hz, 1H), 5.22-5.17 (m, 1H), 5.14-5.09 (m, 1H), 5.01 (d, J = 2.3 Hz, 1H), 5.07 (d, J = 5.1 Hz, 1H), 4.65 (q, J = 4.6 Hz, 1H), 3.68 (s, 3H), 3.29 (d, J = 5.5 Hz, 2H), 1.43 (s, 8H).

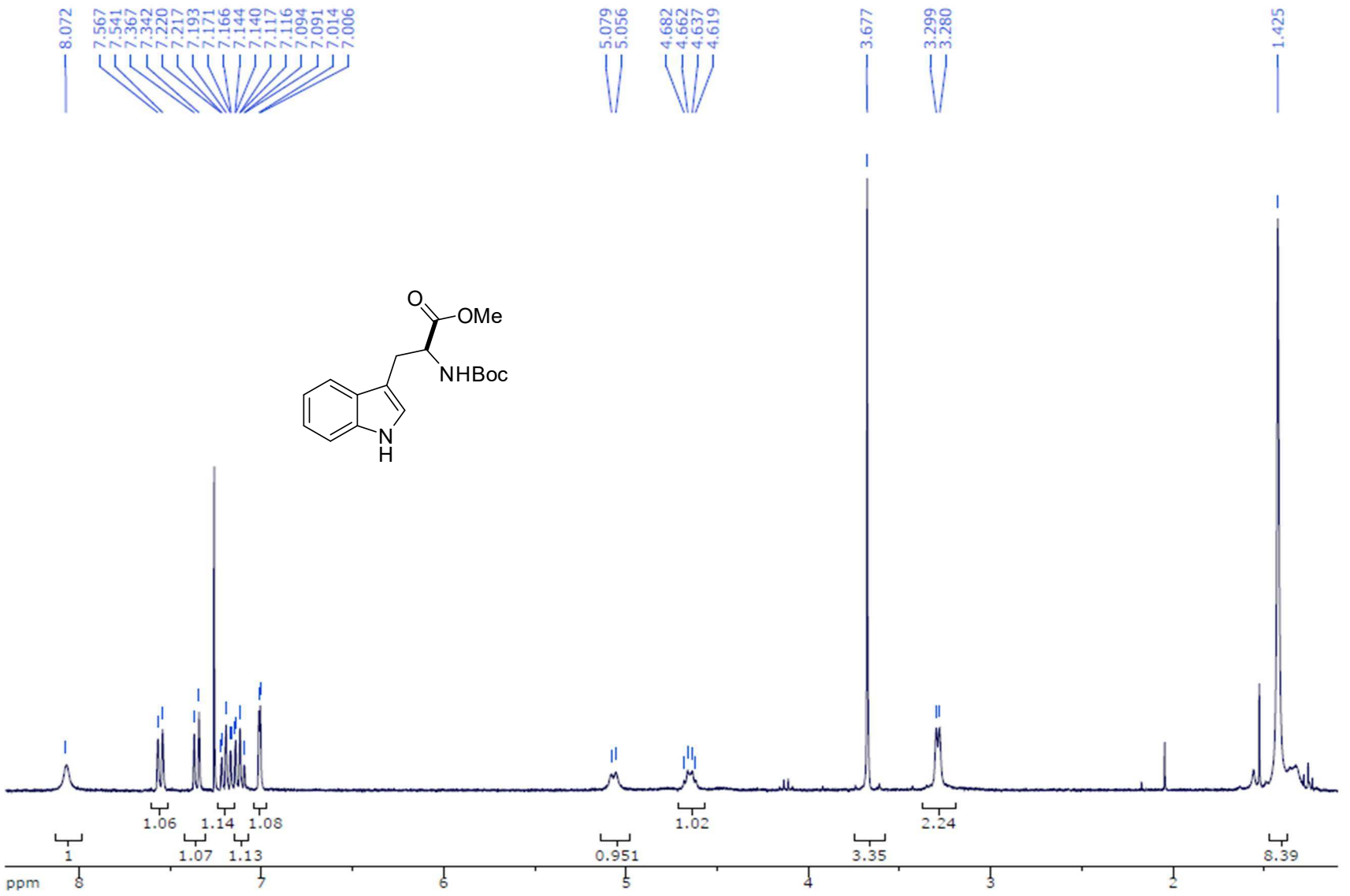


Figure 5.3. ^{1}H NMR of the product.

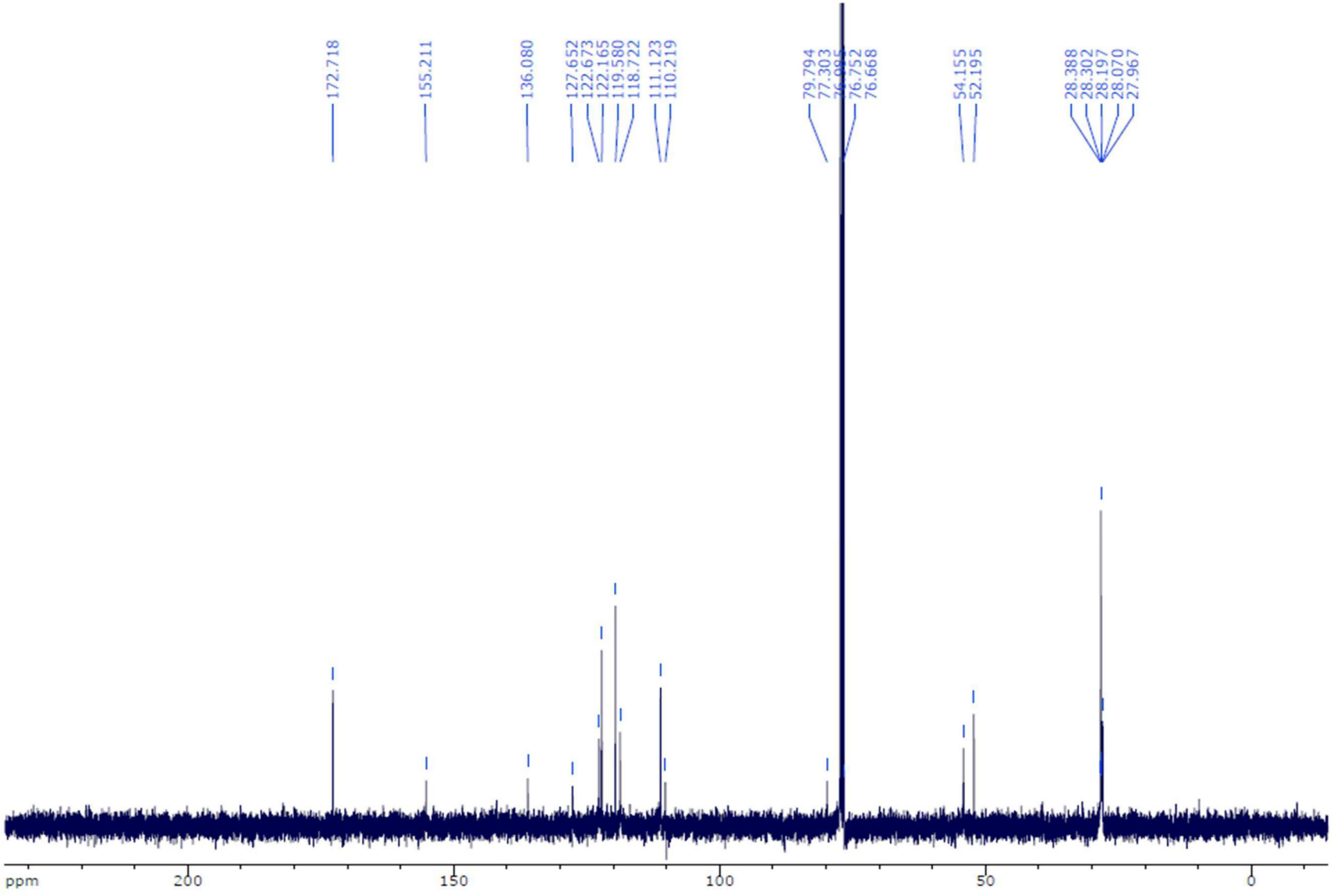
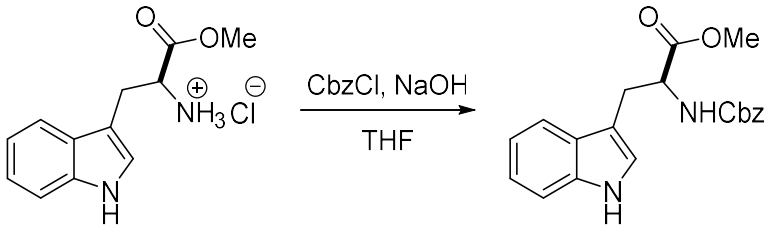


Figure 5.4. ^{13}C NMR of the product.



Trp-OMe-HCl (10g, 39.3 mmol) was suspended in dioxanes. To this was added 1M NaOH (45 mL, 45 mmol, 1.15 eq) and the reaction was cooled in an ice bath. Cbz-Cl (4.4 mL, 44.8 mmol, 1.1 eq) was added followed by another portion of 1M NaOH (45 mL, 45 mmol, 1.15 eq). The solution was stirred for 20 minutes in the ice bath and then allowed to warm to room temperature for 20 minutes. Ethyl acetate was added and the layers were separated. The organic layer was washed with 2x50 mL 1N HCl, then 1x50 mL brine. The solution was dried over $MgSO_4$, filtered through celite and condensed. The orange oil was left on high vacuum and became a foam which collapsed back to an orange oil (13.14 g, 35.3 mmol, 95%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 6.12 (s, 1H), 5.52 (d, J = 5.7 Hz, 1H), 5.33 (s, 5H), 5.21-5.16 (m, 1H), 5.12-5.06 (m, 1H), 4.95 (d, J = 1.7 Hz, 1H), 5.36-5.32 (m, 1H), 5.10 (d, J = 4.4 Hz, 2H), 4.75-4.70 (m, 1H), 3.70 (s, 4H), 3.31 (d, J = 5.4 Hz, 2H).

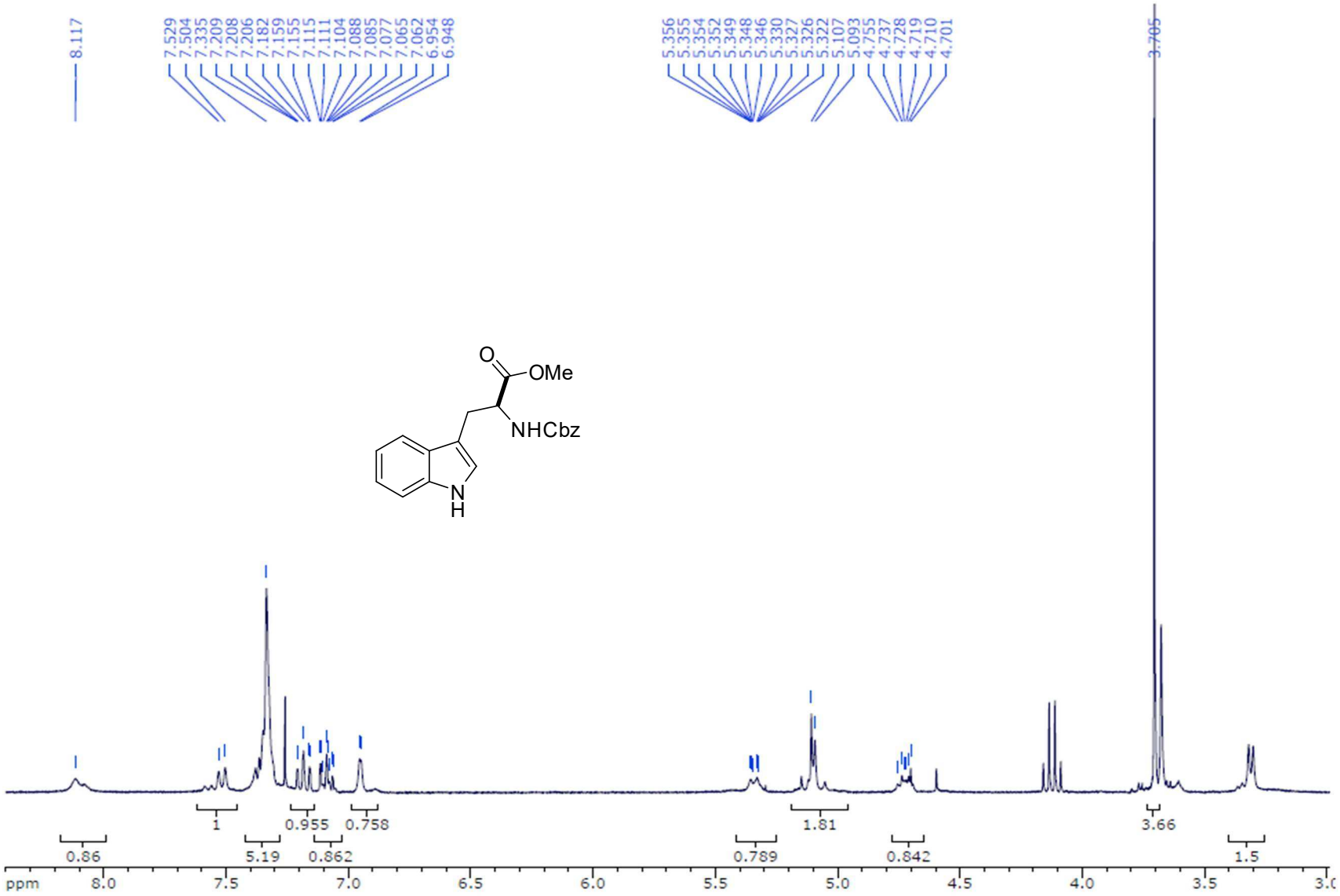
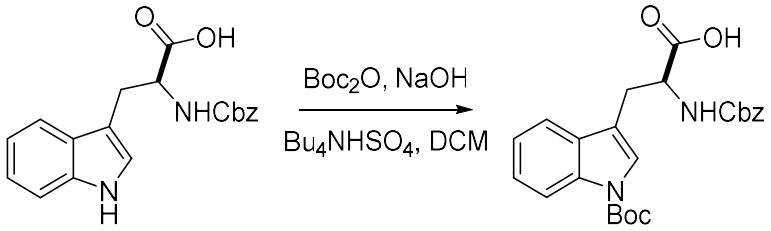
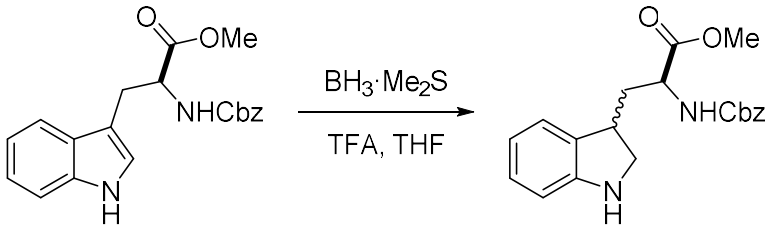


Figure 5.5. ^{1}H NMR of the product.



To a solution of Trp-Cbz-Ome (2.44g, 4.9 mmol) in 50 mL DCM was added Boc_2O (1.56 g, 5.2 mmol, 1.04 eq), Bu_4NHSO_4 (0.350 g, 1.0 mmol, 15 mol%) and 35 mL 1M NaOH. This was left to react at room temperature overnight. The layers were separated, and the aqueous layer was extracted once with DCM. The combined organics were dried over $NaSO_4$ and condensed. Trp-Boc-Cbz-OMe (1.23 g, 2.7 mmol, 39%) was recovered as a white foam.



Dissolved Trp-Cbz-OMe (13.77g, 35.2 mmol) in 20 mL THF at 0°C. 10 mL TFA were added followed by 10M BH_3 in Me_2S (12 mL, 120 mmol, 3.2 eq) and 10 more mL TFA. After 10 minutes the ice bath was removed and the reaction was left to warm to room temperature for 10 more minutes. 50 mL of H_2O were added and solid precipitated. The reaction was condensed and toluene (3x15 mL) was used to azeotrope the TFA off. Ethyl acetate was added and the organic solution was washed three times with 1M NaOH, once with H_2O and once with brine. Dried over Na_2SO_4 and condensed. Flash chromatography (50g silica, 3:1 hexanes/ethyl acetate to 1:1 hexanes/ethyl acetate) yielded Trp-Cbz-OMe-indoline (5.40 g, 15.2 mmol, 41%) as a colorless oil. ^{1}H-NMR (300 MHz; $CDCl_3$): δ 5.26 (s, 4H), 4.98-4.93 (m, 1H), 4.65-4.60 (m, 1H), 4.54 (d, J = 5.7 Hz, 1H), 4.06 (d, J = 6.3 Hz, 1H), 5.05 (s, 1H), 4.42 (t, J = 5.4 Hz, 1H), 3.90 (s, 1H), 3.62 (s, 3H), 3.33-3.15 (m, 2H).

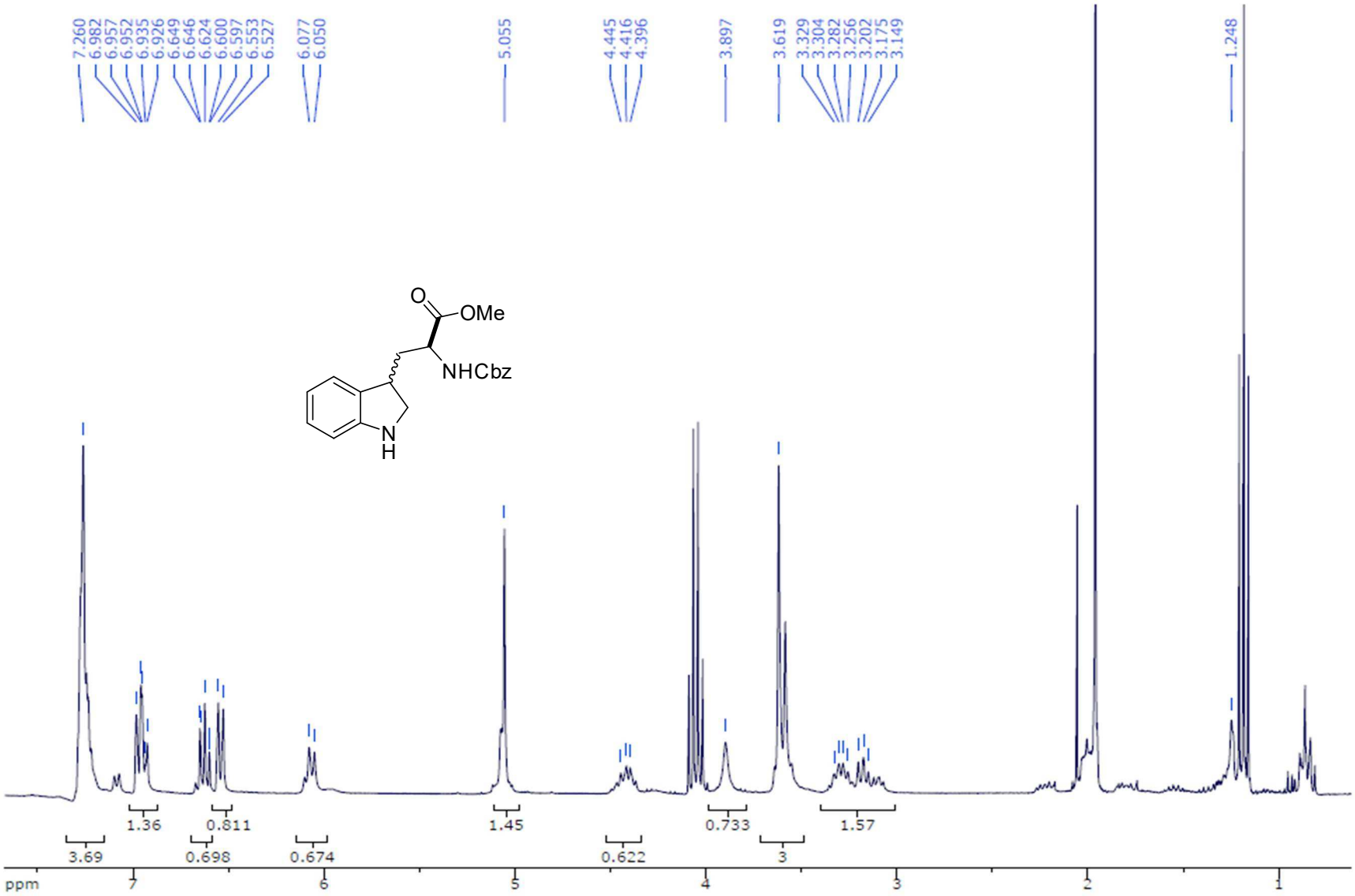


Figure 5.6. ^{1}H NMR of the product.

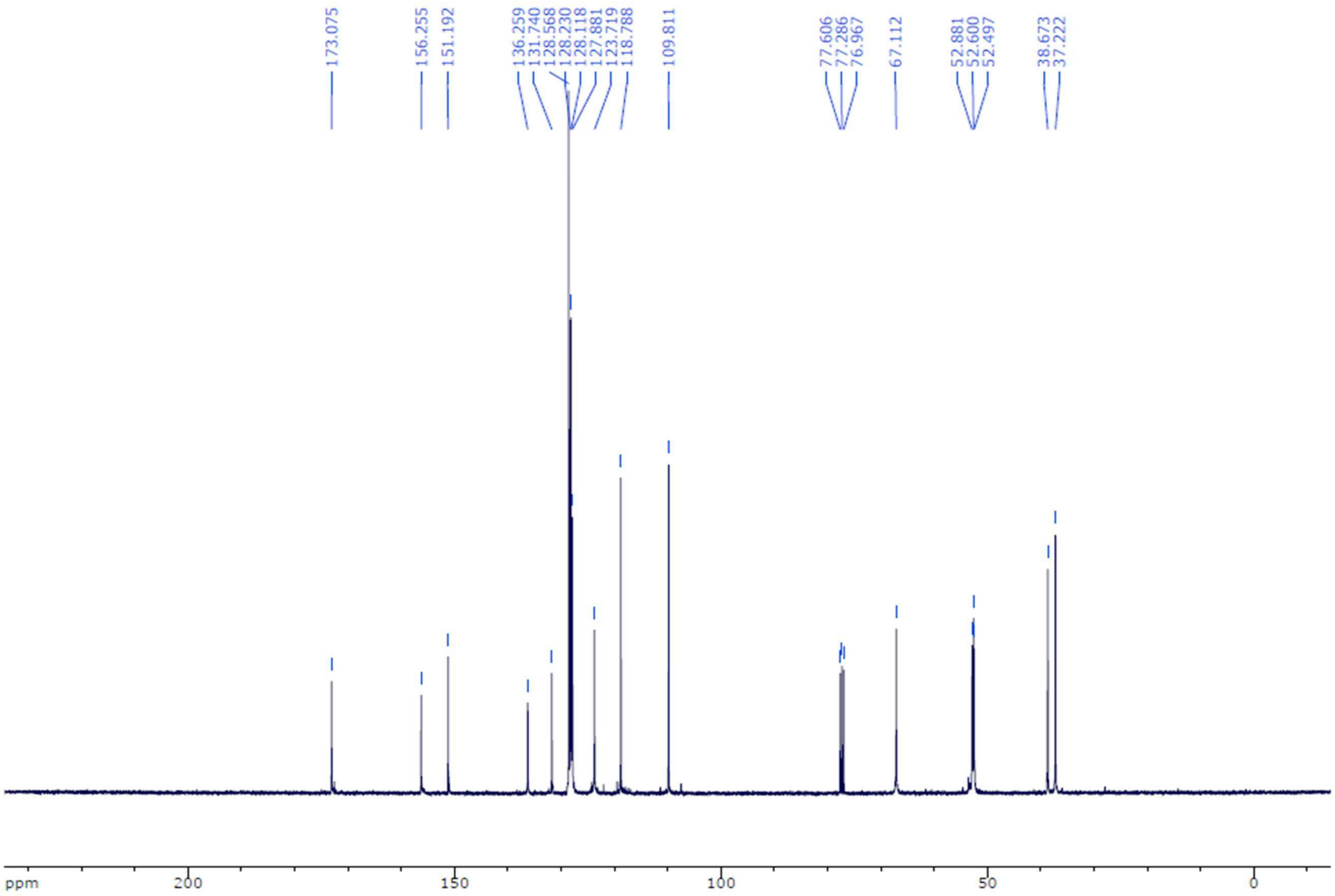
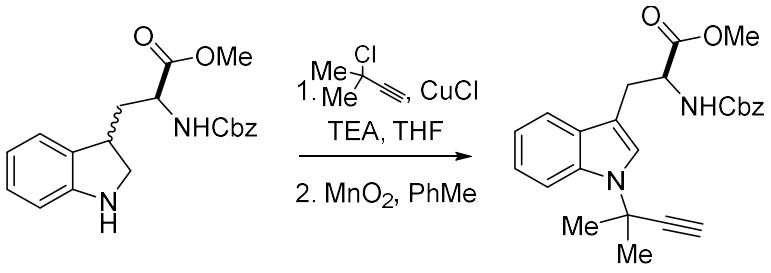


Figure 5.7. ^{13}C NMR of the product.



Trp-Cbz-OMe-Indoline (5.40 g, 15.2 mmol) was dissolved in dry THF. CuCl (0.17 g, 1.7 mmol, 11 mol %) was added under Ar atmosphere and allowed to stir for 5 minutes before adding dry TEA (2.4 mL, 15.2 mmol, 1.1 eq) dropwise. The solution was again allowed to stir for 5 minutes after which 3-chloro-3-methyl-1-butyne (2.1 mL, 16.1 mmol, 1.2 eq) was added and the solution was heated to reflux. TLC of the reaction showed no starting material after one hour and the solvent was evaporated. The residue was taken up in ethyl acetate and filtered through silica. The solution was again condensed and the remaining red oil was dissolved in toluene. To this was added MnO_2 (12 g, 140 mmol, 9 eq) and the suspension was allowed to stir overnight under atmosphere. This was then filtered through celite and the solution was condensed. Column chromatography (200 g, 6:1 hexanes/ethyl acetate to 4:1 hexanes/ethyl acetate) gave Trp-PPren-Cbz-OMe (3.90 g, 9.3 mmol, 61%) as a yellow oil. ^{1}H-NMR (300 MHz; $CDCl_3$): δ 5.83 (d, J = 6.4 Hz, 1H), 5.52 (d, J = 5.9 Hz, 1H), 5.35 (s, 4H), 5.21 (t, J = 5.6 Hz, 1H), 5.11 (t, J = 5.5 Hz, 2H), 5.33 (d, J = 6.2 Hz, 1H), 5.12 (q, J = 11.0 Hz, 2H), 4.73 (q, J = 4.8 Hz, 1H), 3.69 (s, 3H), 3.29 (d, J = 5.5 Hz, 2H), 2.57 (s, 1H), 1.92 (s, 6H).

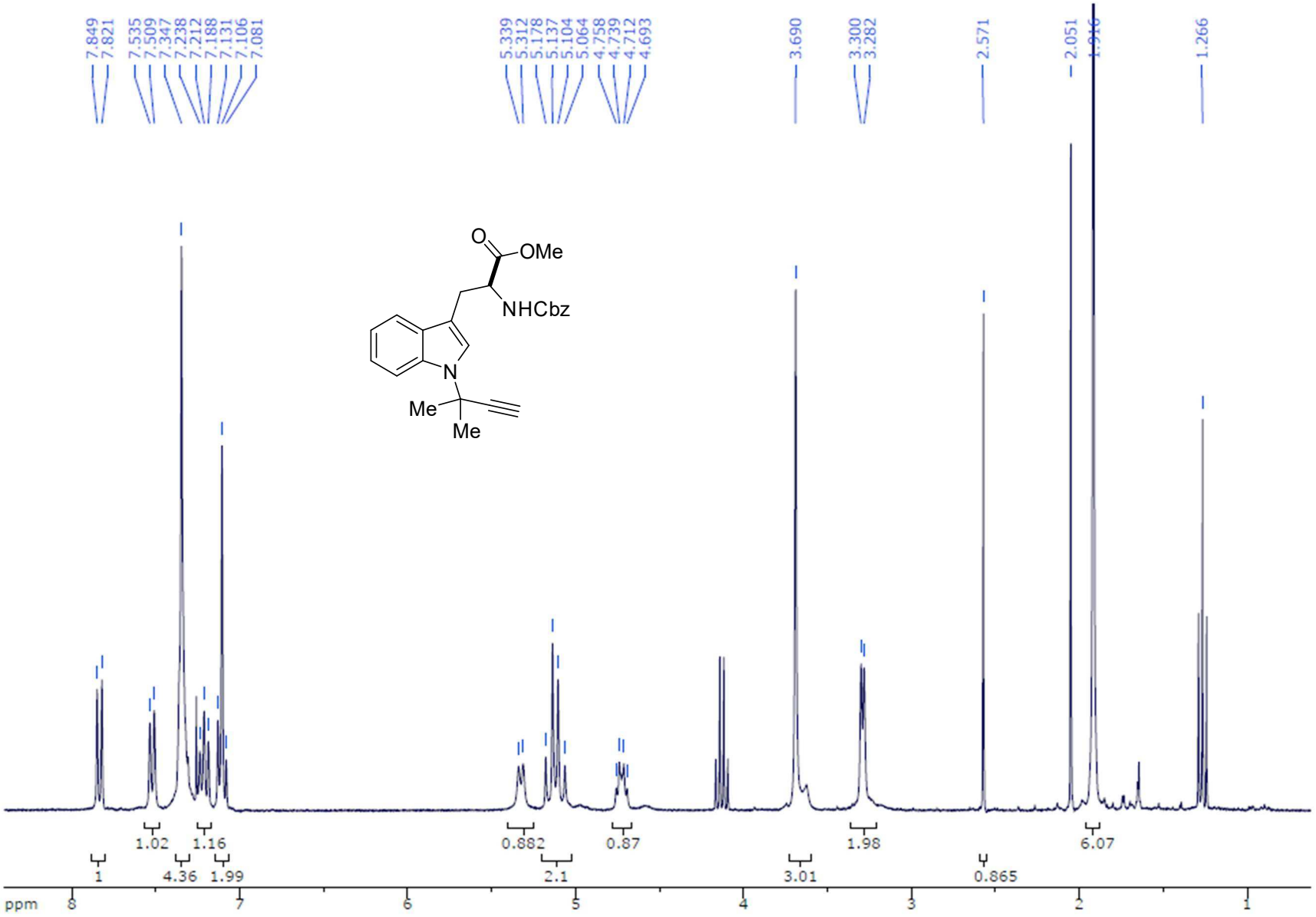
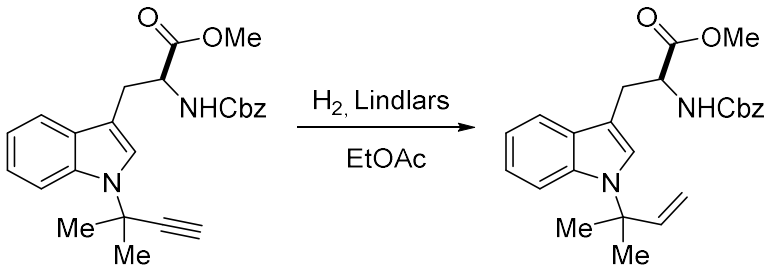


Figure 5.8. ^{1}H NMR of the product.



A solution of Trp-PPren-Cbz-OMe (0.300 g, 0.72 mmol) in 20 mL of methanol and 1 mL distilled quinoline was prepared and the flask was purged with Ar while stirring vigorously. A catalytic amount of Pd/C was added and the Ar was purged with H_2. After 2.5 hours under a H_2 balloon the starting material was consumed according to TLC (longer times will give over-reduction) and the solution was filtered through celite. Ethyl acetate was added and the organic layer was washed twice with 10% H_2SO_4, twice with H_2O and once with brine. The organic layer was dried over $MgSO_4$, filtered through celite and condensed. Trp-Pren-Cbz-OMe (0.300 g, 0.71 mmol, 99%) was recovered as a yellow oil. ^{1}H-NMR (300 MHz; $CDCl_3$): δ 5.59-5.46 (m, 3H), 5.34 (s, 6H), 5.13-5.01 (m, 4H), 4.11 (dd, J = 15.4, 10.7 Hz, 1H), 5.28-5.05 (m, 6H), 4.70 (s, 1H), 3.67 (d, J = 5.5 Hz, 5H), 3.30-3.26 (m, 3H), 1.68 (d, J = 15.0 Hz, 9H).

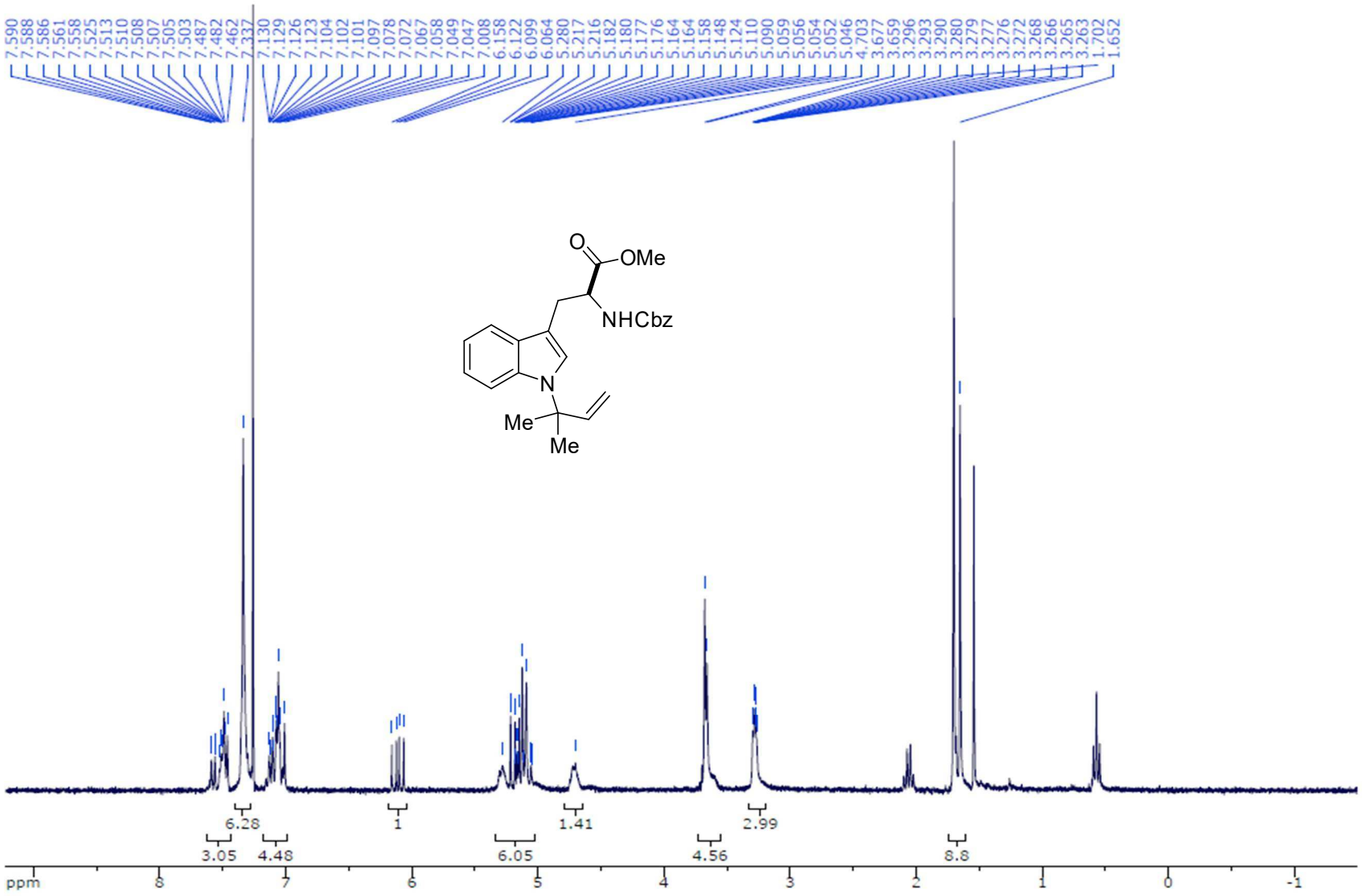


Figure 5.9. ^{1}H NMR of the product.

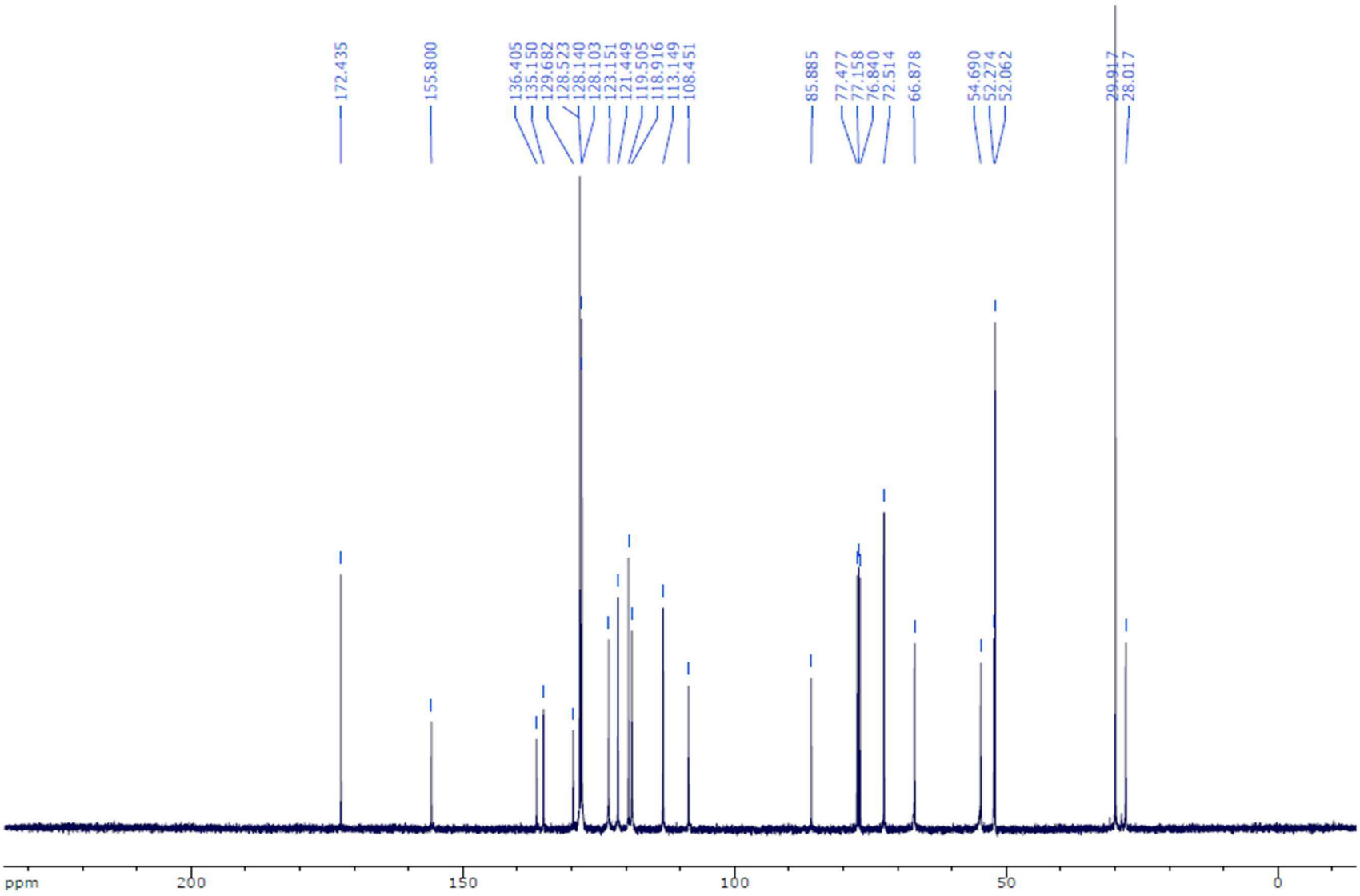
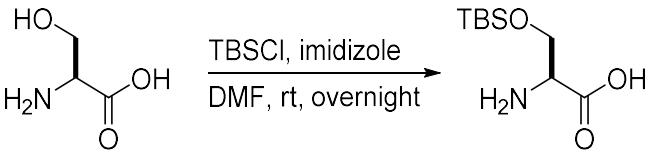
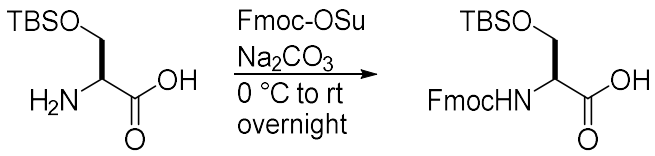


Figure 5.10. ^{13}C NMR of the product.



Add L-serine (38 g, 362 mmol), TBSCl (60 g, 398 mmol, 1.1 eq) and imidizole (54.2g, 796 mmol, 2.2 eq) to dry DMF. Leave overnight. Evaporate DMF under vacuum and partition between hexanesa and water. Stir for 1 hour and separate. Filter the organic layer, condense and leave under vacuum over P_2O_5. Yielded 65 g (82% yield) of product.



Suspend Serine-TBS (29.95 g, 137 mmol), in 150 mL dioxane at 0 °C. Added 60 mL of 10% Na_2CO_3 followed by Fmoc-OSu (45.49 g, 135 mmol) in 180 mL of dioxane dropwise. Left overnight at room temperature. Condense, add H_2O and acidify to pH 3. Add EtOAc, wash once with H_2O and brine. Dry over Na_2SO_3, and condense giving 49 g (82% yield) of product. ^{1}H-NMR (300 MHz; $CDCl_3$): δ 5.77 (d, J = 5.4 Hz, 2H), 5.60 (d, J = 4.1 Hz, 2H), 5.41 (t, J = 5.4 Hz, 2H), 5.31 (t, J = 5.4 Hz, 2H), 5.62 (d, J = 6.0 Hz, 1H), 4.49-4.35 (m, 3H), 4.28-4.23 (m, 1H), 4.17-4.11 (m, 1H), 3.87 (dd, J = 10.1, 4.1 Hz, 1H), 0.90 (s, 10H), 0.08 (d, J = 2.8 Hz, 6H).

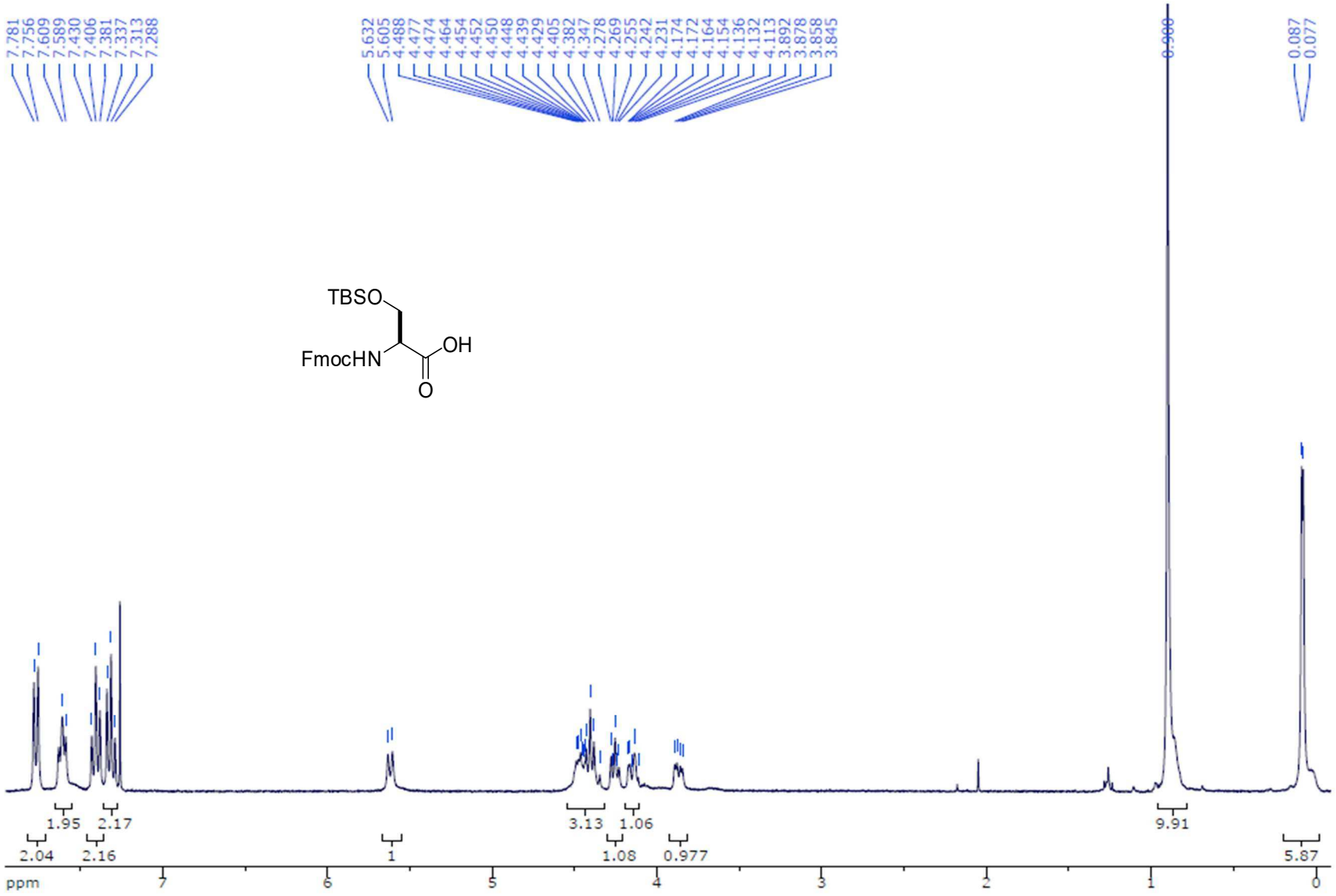


Figure 5.11. ^{1}H NMR of the product.

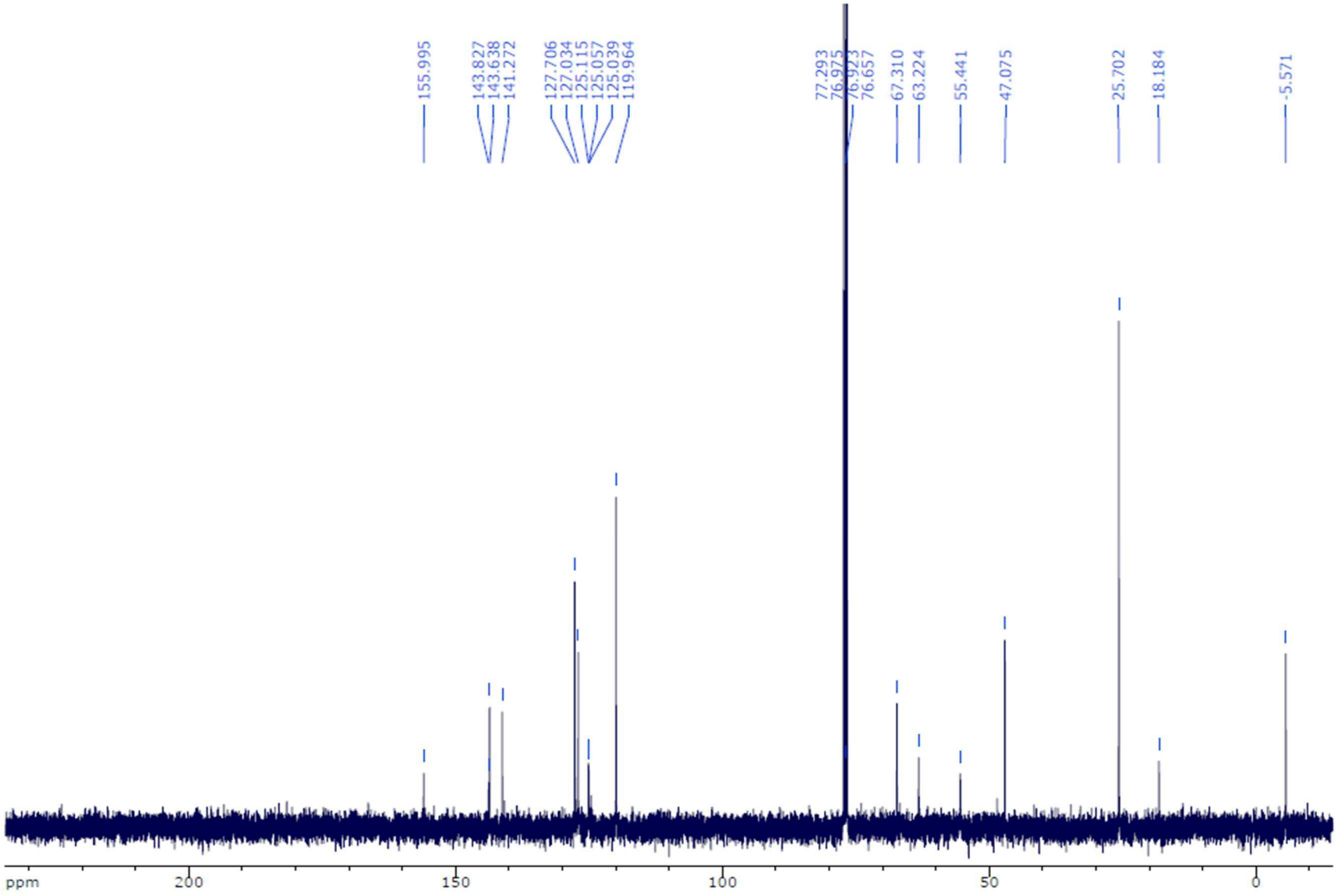
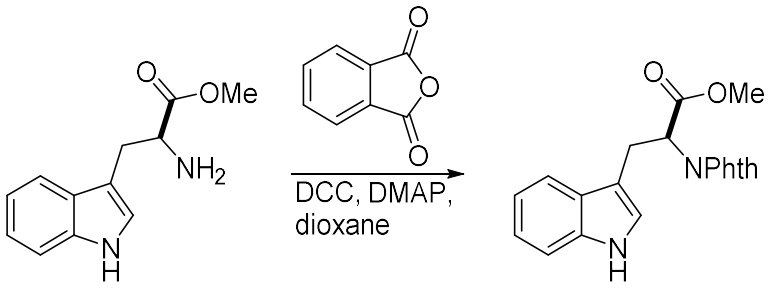


Figure 5.12. ^{13}C NMR of the product.



Dissolved all (4.39 g methyl ester, 5.52 g phthalic anhydride, 10.3 g DCC, catalytic amount of DMAP) in DCM and left for 12 hours. Filter through celite and condense. Column chromatography gave 4.92 g (56% yield) of product. ^{1}H-NMR (300 MHz; $CDCl_3$): δ 5.90 (s, 1H), 5.77-5.74 (m, 2H), 5.68-5.65 (m, 2H), 5.62-5.59 (m, 1H), 5.29-5.25 (m, 4H), 5.16-5.10 (m, 1H), 5.08-5.03 (m, 1H), 5.01 (d, J = 2.4 Hz, 1H), 5.27 (dd, J = 9.1, 4.8 Hz, 1H), 3.80-3.73 (m, 5H).

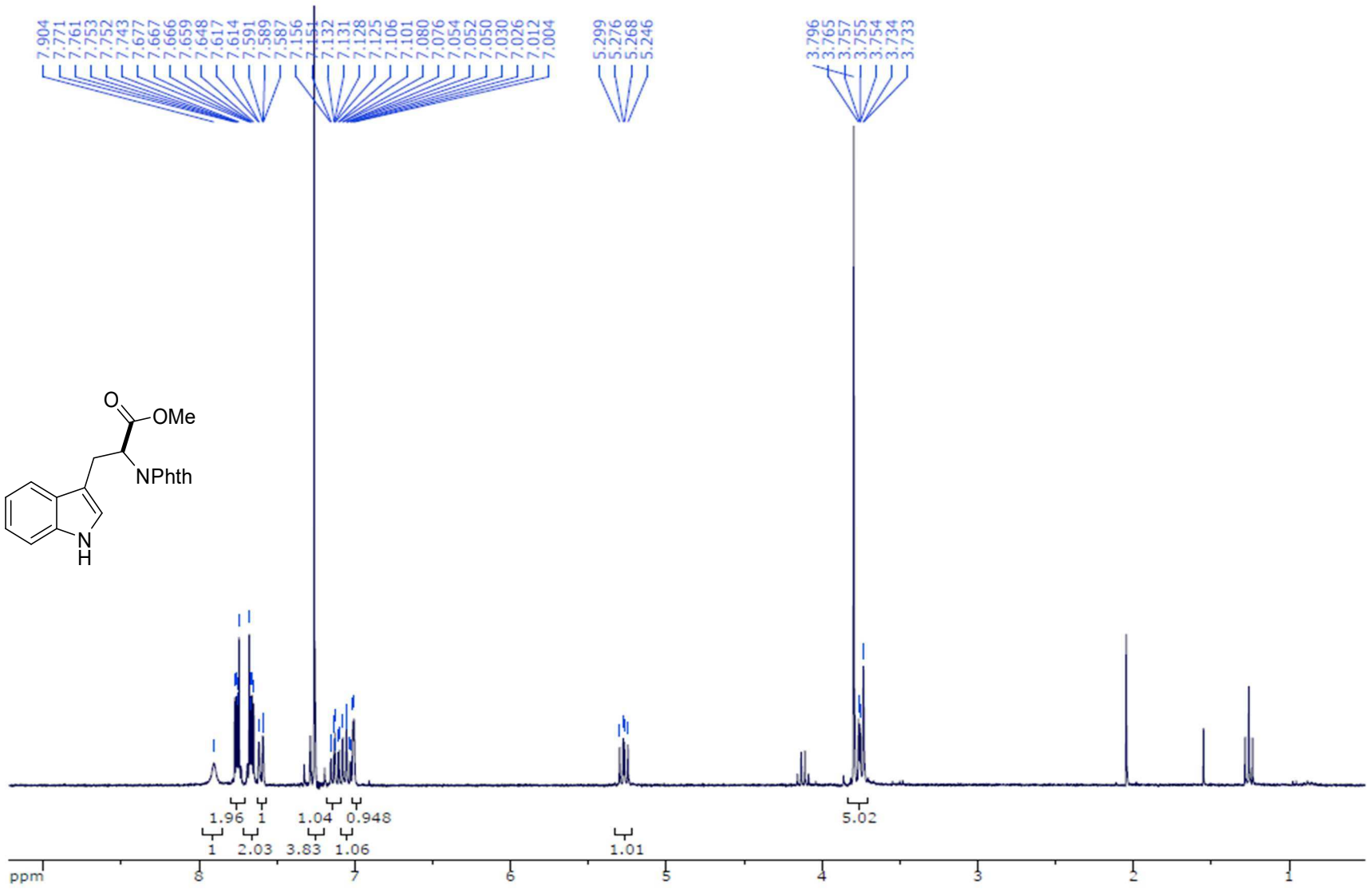
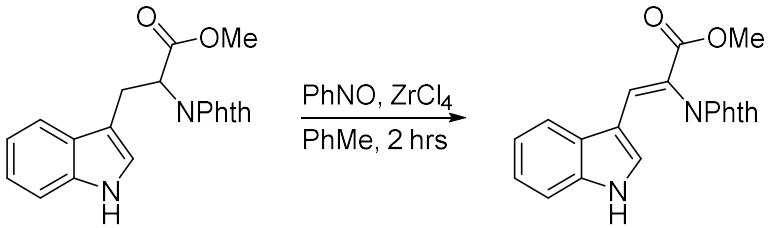


Figure 5.13. ^{1}H NMR of the product.



Dissolved tryptophan in toluene with nitrosobenzene (2.5 eq) at 0 °C. Added $ZrCl_4$ (1.0 eq). After 2 hours filter through Celite. Purification by column chromatography gives only trace amount of product. ^{1}H-NMR (300 MHz; $CDCl_3$): δ 6.55 (s, 1H), 6.46 (s, 1H), 5.96-5.94 (m, 2H), 5.80 (dd, J = 5.6, 3.0 Hz, 3H), 5.33-5.21 (m, 8H), 3.83 (s, 3H).

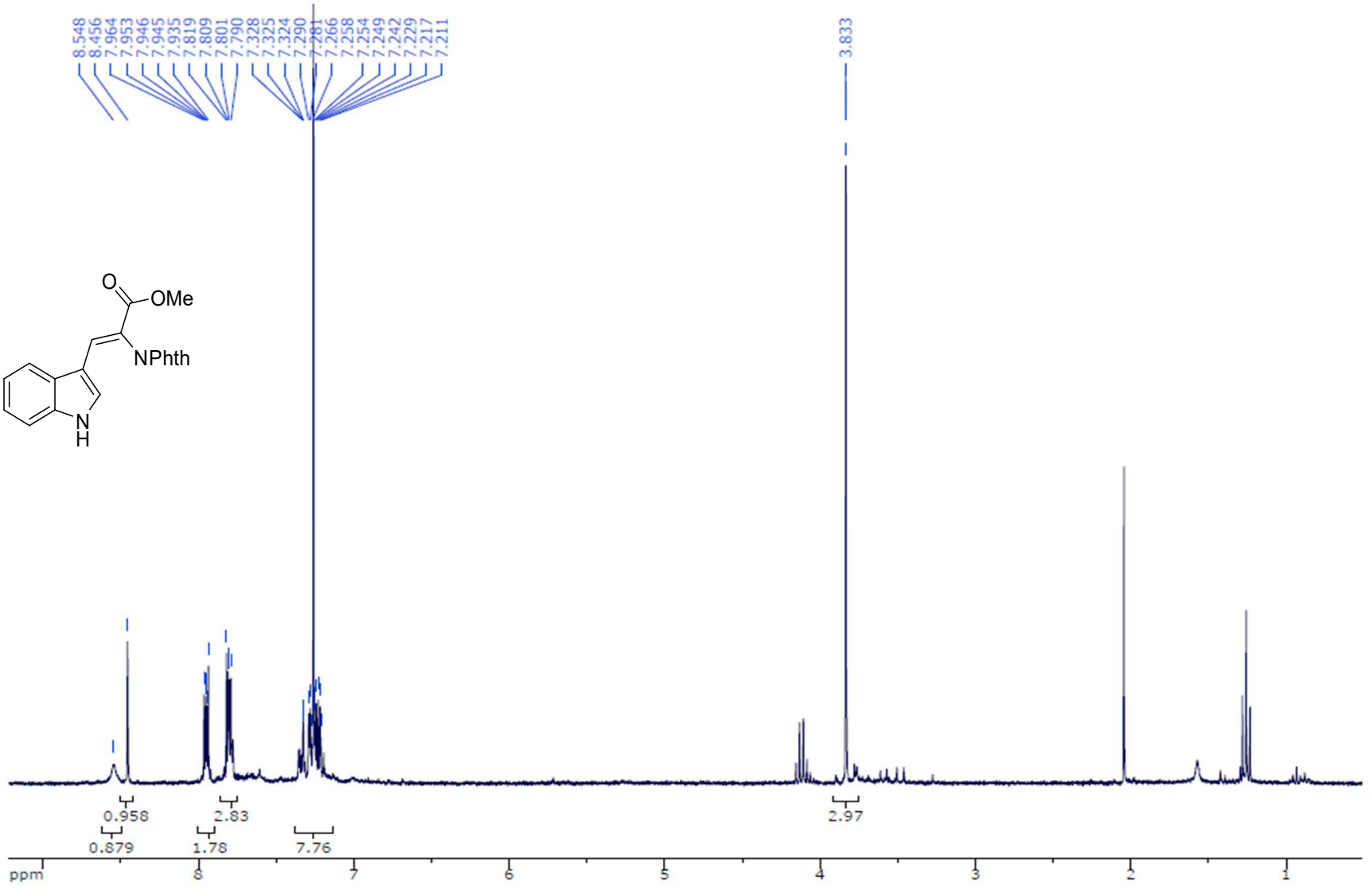
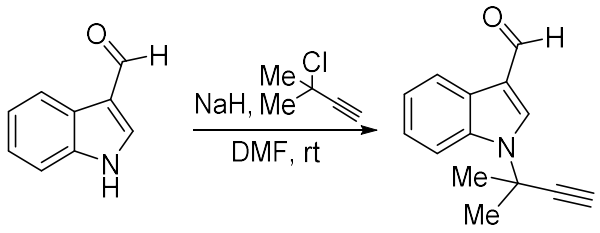
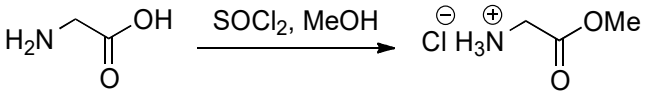


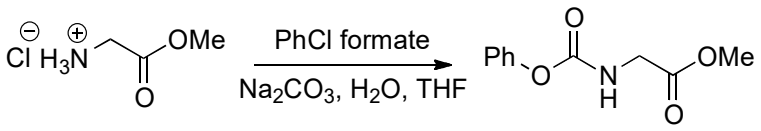
Figure 5.14. [1]H NMR of the product.



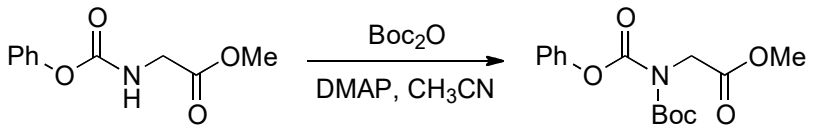
Dissolved indole-3-carboxaldehyde (1.45 g, 1.0 mmol) and 3-chloro-3-methylbutyne (2.03 g, 2.04 mmol) in 20 mL of dry DMF at 0 °C. NaH (1.0 g, 25 mmol) was suspended in a separate 10 mL of DMF and then added to the indole. Let stir at room temperature overnight. The suspension was partitioned between EtOAc and H_2O. The organic layer was washed three times with small portions of H_2O. The organic layer was condensed and purified through column chromatography (10% EtOAc/hex to 20%). Product was pure by NMR.



Dissolved glycine in MeOH at 0 °C. Added $SOCl_2$ dropwise over several minutes. Let stir at room temperature for a few hours. Evaporate the MeOH and carry on to next reaction without further purification.



Dissolve 5.0 g of glycine methyl ester hydrochloride in 1:1 THF:1M $Na_2CO_{3(aq)}$. Add phenyl chloroformate and let stire for 2 hours. Add EtOAc and wash organic layer with H_2O. Extract combined aqueous fractions with EtOAc three times. Wash combined organics with brine once, dry with $MgSO_4$, and condense. Take on to next reaction.



Dissolved product from previous reaction in 150 mL dry CH_3CN at 0 °C. Added Boc_2O and DMAP. Let stir for 3 hours. The solution was condensed and partitioned between EtOAc and sat. NH_4Cl. Aqueous layer was extracted once with EtOAc and the organics were washed once with H_2O and brine. The solution was dried over $MgSO_4$ and condensed. Filter product through silica plug and retain product prior to yellow color. Purification on column (10% EtOAc/hex) yields 3.79 g (31% yield) of pure product. ^{1}H-NMR (400 MHz; $CDCl_3$): δ 5.35 (t, J = 5.7 Hz, 2H), 5.20 (t, J = 5.4 Hz, 1H), 5.13 (d, J = 6.0 Hz, 2H), 4.50 (s, 2H), 3.75 (s, 3H), 1.51 (s, 9H).

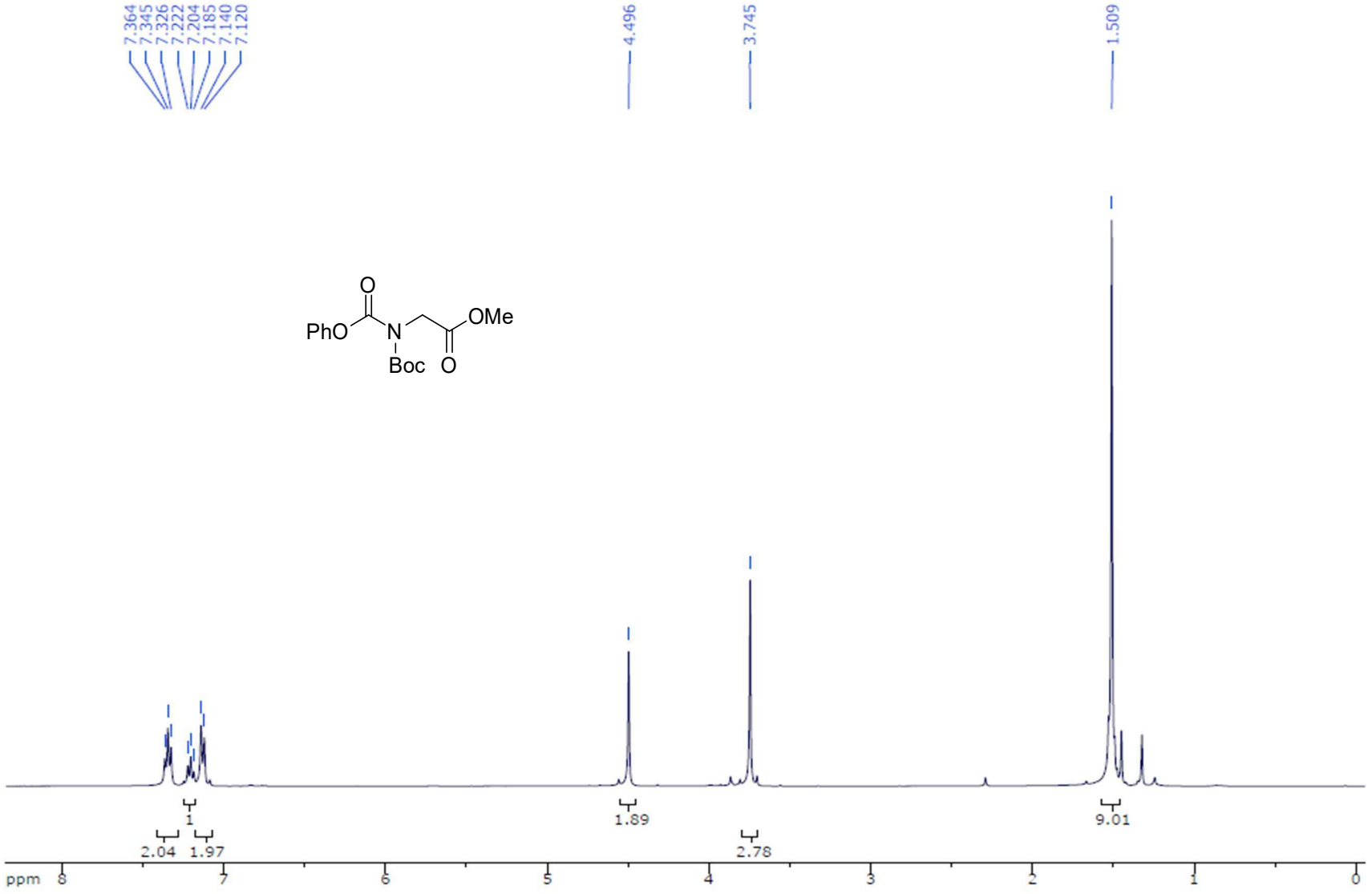
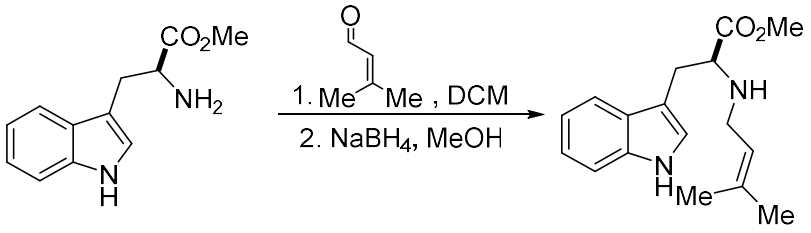


Figure 5.15. ^{1}H NMR of the product.



Dissolved tryptophan methyl ester in dry DCM under argon. Molecular sieves and aldehyde were added to the solution and allowed to react for 3 hrs. The solution was then condensed and dry methanol was added followed by $NaBH_4$. After 1 hr the solution was filtered through silica using DCM. The impure product was then purified using column chromatography. ^{1}H-NMR (300 MHz; $CDCl_3$): δ 6.15 (s, 1H), 5.60 (dd, J = 5.8, 0.7 Hz, 1H), 5.35-5.32 (m, 1H), 5.19 (td, J = 5.5, 1.3 Hz, 1H), 5.11 (td, J = 5.4, 1.1 Hz, 1H), 5.04 (d, J = 2.3 Hz, 1H), 5.19-5.14 (m, 1H), 3.64-3.60 (m, 3H), 3.11-3.07 (m, 5H), 1.67 (s, 4H), 1.56 (s, 3H).

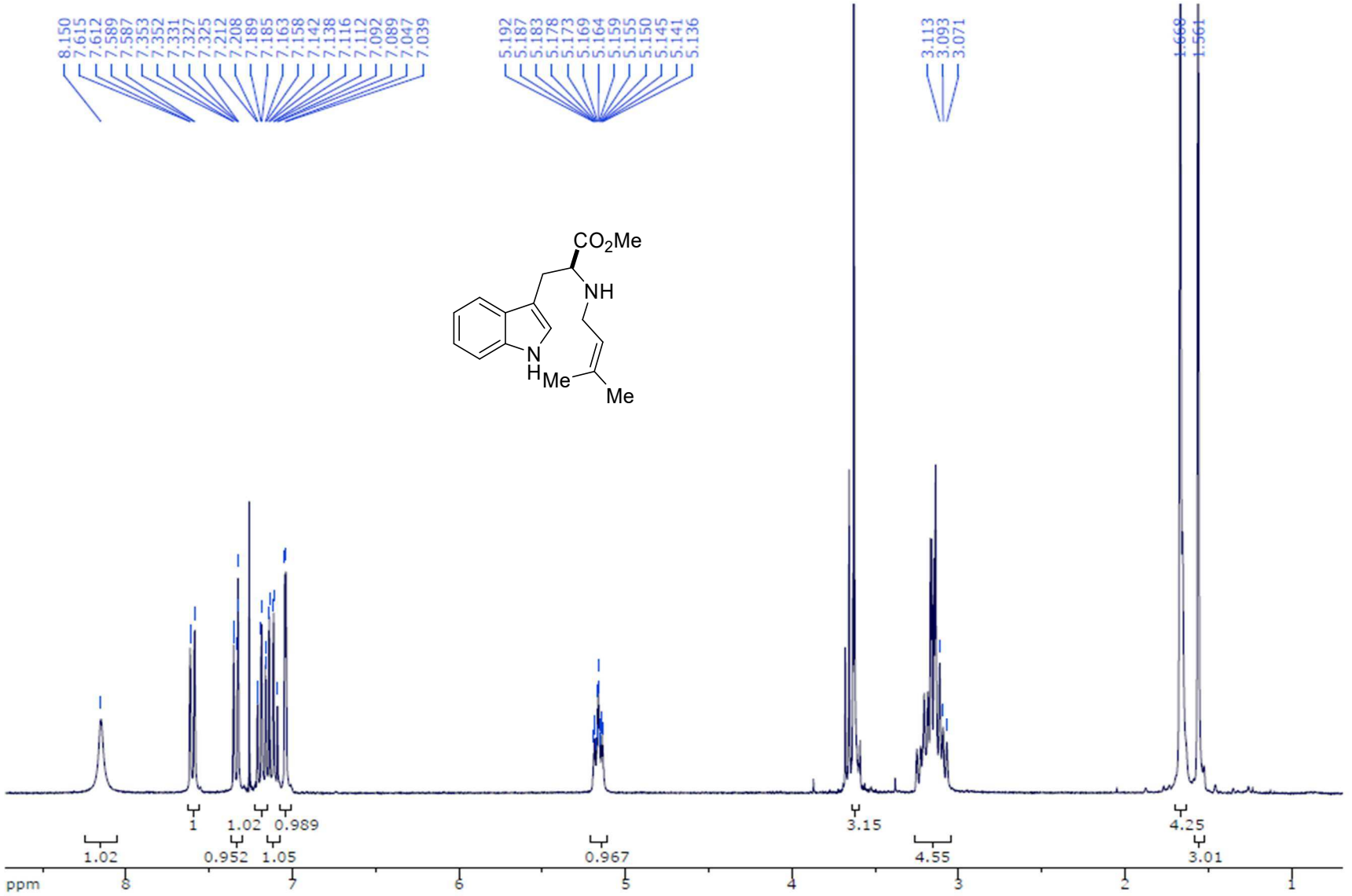
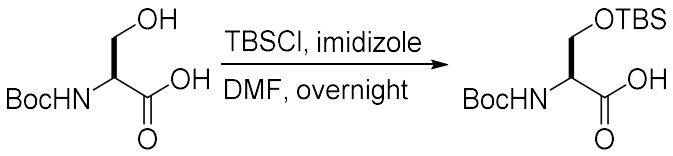


Figure 5.16. ^{1}H NMR of the product.



Dissolved 1.95 g N-Boc-Serine, 1.58 g TBSCl, and 1.40 g imidazole in DMF. Left overnight. Partition between H_2O and EtOAc. Wash with H_2O 5 times, brine, dry and condense. Gave product as a colorless oil. ^{1}H-NMR (300 MHz; $CDCl_3$): δ 10.29 (s, 1H), 5.35 (d, J = 6.4 Hz, 1H), 4.36 (dd, J = 5.7, 2.8 Hz, 1H), 4.09-4.03 (m, 1H), 3.83 (dd, J = 10.1, 3.3 Hz, 1H), 1.43 (s, 10H), 0.85 (s, 12H), 0.02 (d, J = 3.9 Hz, 7H).

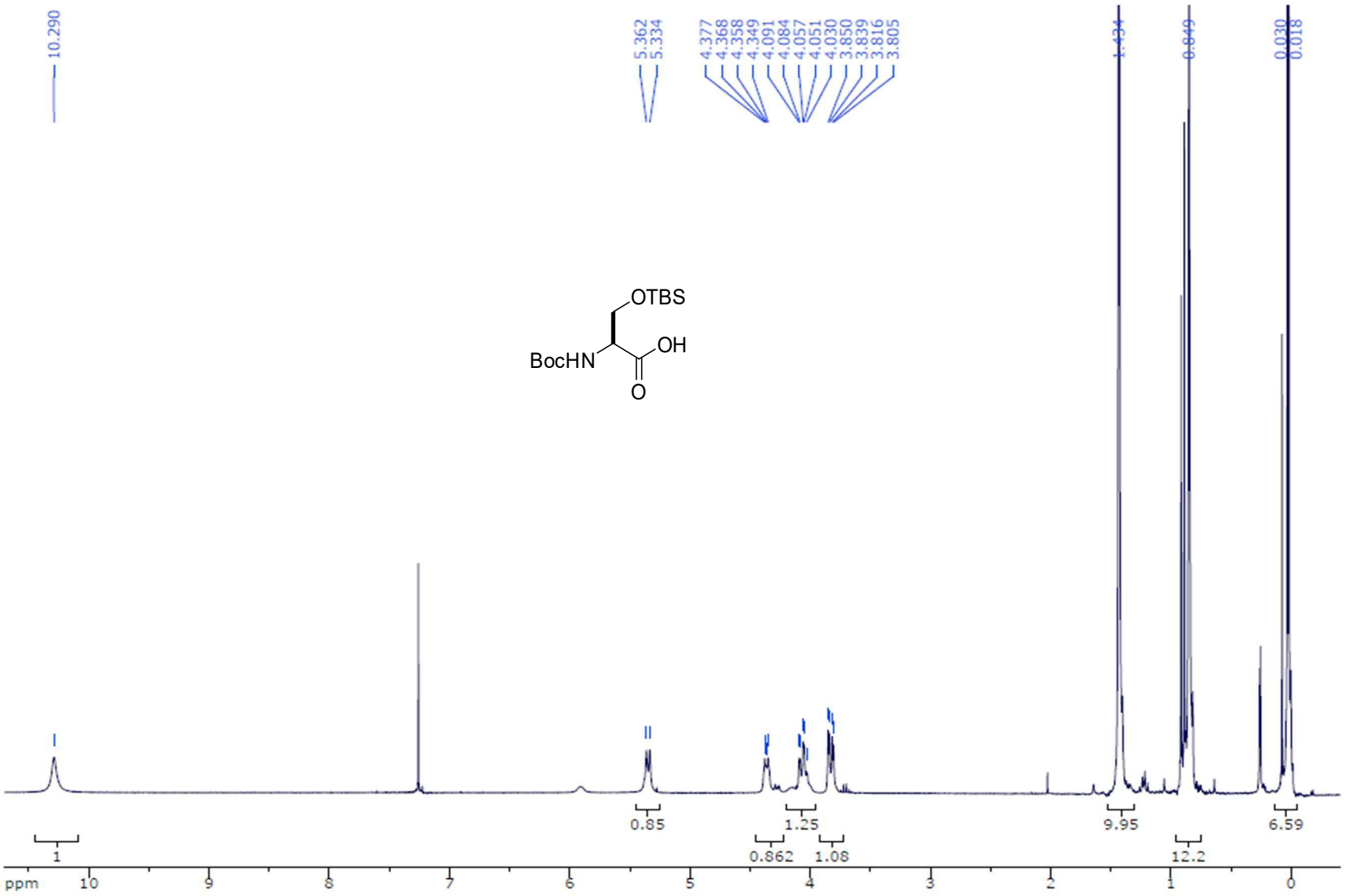
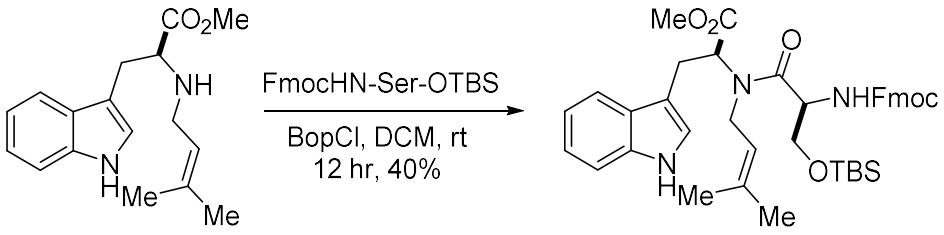


Figure 5.17. ^{1}H NMR of the product.



The tryptophan derivative (116 mg, 0.41 mmol) was dissolved in DCM followed by the serine derivative (188 mg, 0.42 mmol) and BopCl (154 mg, 0.60 mol). The solution was chilled and then DIPEA (0.1 ml, 1.4 mmol) was added dropwise and the solution was left to stir at room temperature overnight. The solution was then partitioned between EtOAc and aqueous $KHSO_4$. The organic layer was washed once with H_2O and brine, dried over Na_2SO_4, filtered and condensed. The impure product was purified using column chromatography. The dipeptide was recovered as a white foam (114 mg, 0.16 mmol, 39%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 6.01 (s, 1H), 5.78 (d, *J* = 5.4 Hz, 3H), 5.61 (t, *J* = 5.9 Hz, 4H), 5.41 (q, *J* = 4.9 Hz, 3H), 5.32 (dd, *J* = 12.6, 5.1 Hz, 4H), 5.20-5.09 (m, 3H), 5.05 (d, *J* = 1.9 Hz, 1H), 5.66 (d, *J* = 6.2 Hz, 1H), 4.94 (d, *J* = 5.6 Hz, 1H), 4.75-4.63 (m, 2H), 4.43-4.36 (m, 3H), 4.22 (t, *J* = 4.9 Hz, 1H), 4.08-4.01 (m, 2H), 3.79 (td, *J* = 10.2, 5.4 Hz, 3H), 3.70-3.65 (m, 4H), 3.59 (td, *J* = 10.5, 4.9 Hz, 3H), 3.27 (dd, *J* = 14.9, 6.6 Hz, 1H), 1.53 (s, 3H), 1.43 (s, 4H), 0.92 (s, 13H), 0.08 (d, *J* = 2.9 Hz, 7H).

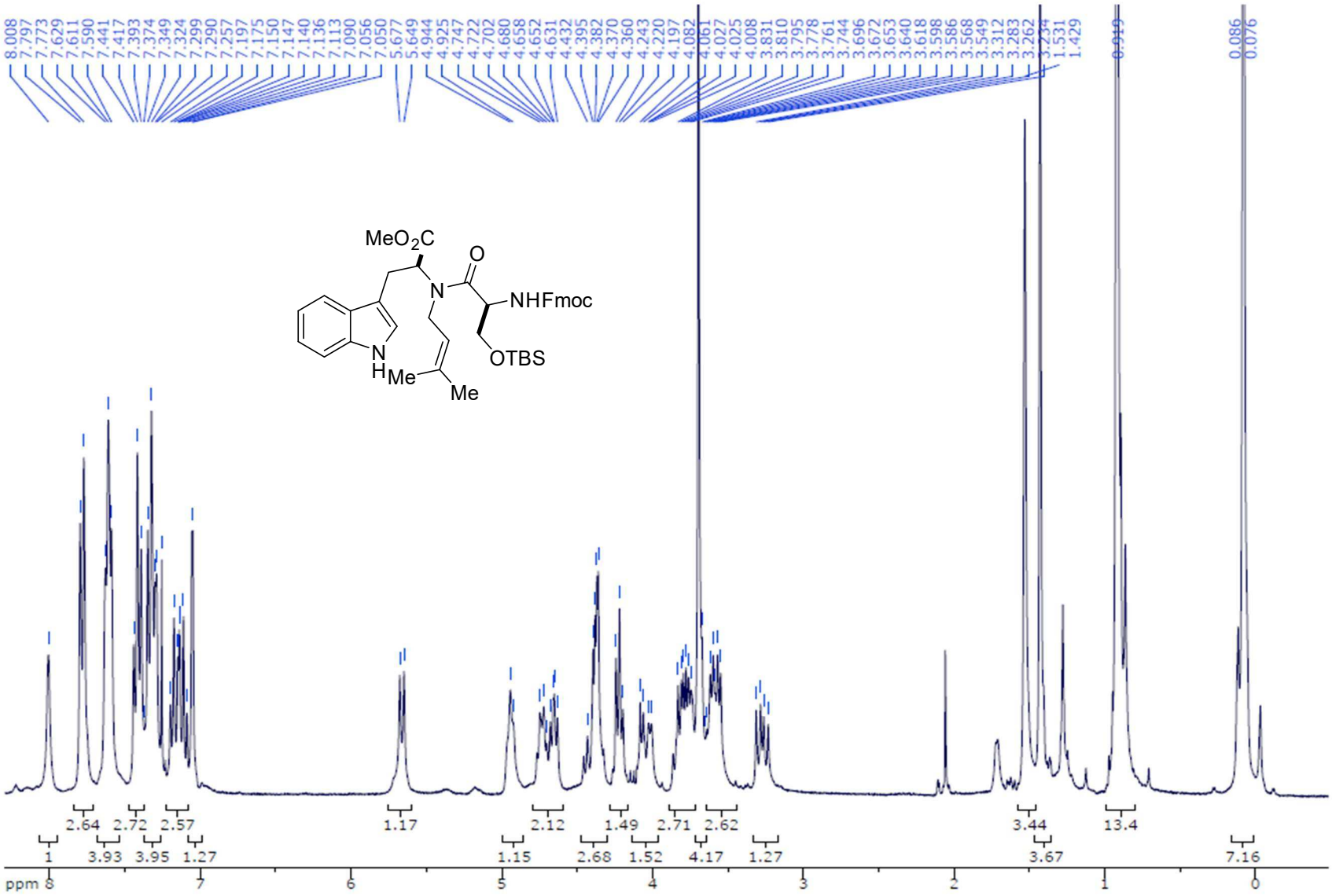


Figure 5.18. ^{1}H NMR of the product.

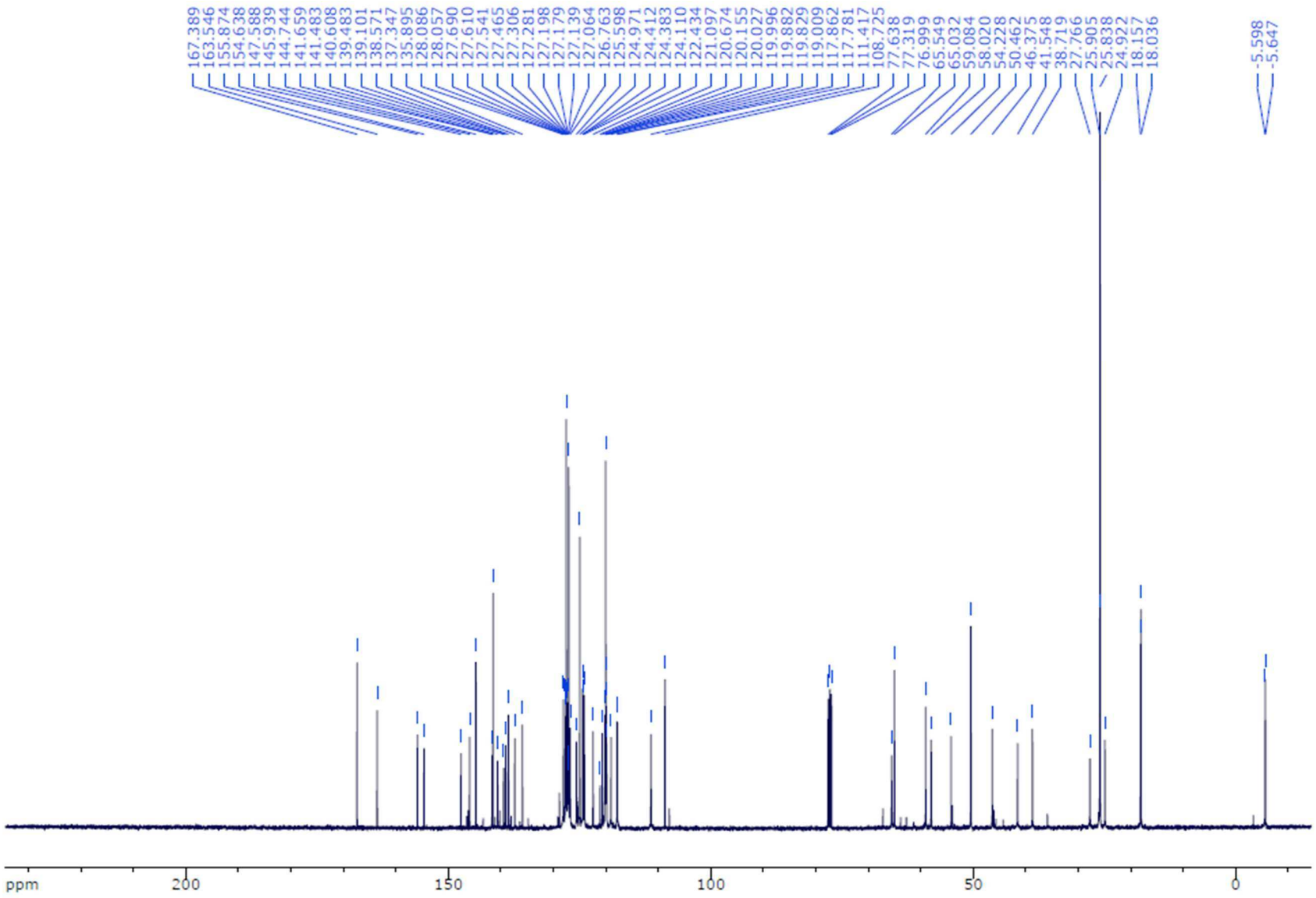
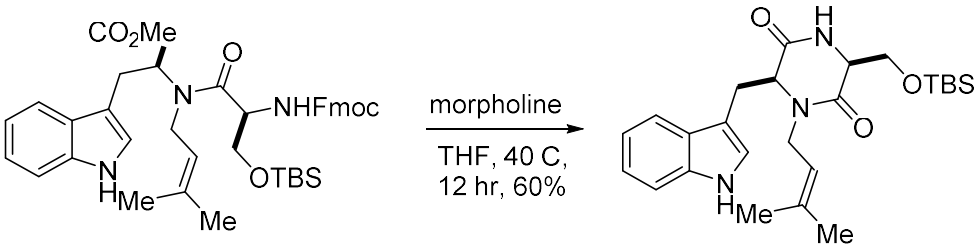


Figure 5.19. ^{13}C NMR of the product.



The dipeptide (213 mg, 0.30 mmol) was dissolved in THF and mopholine (0.04 ml, 0.46 mmol) was added. The solution was left to react overnight at 40 C. The solution was then partitioned between H_2O and EtOAc. The organic layer was washed once with H_2O and brine, dried over Na_2SO_4, filtered and condensed. The crude product was purified using column chromatography (10-50% EtOAc in hex) to yield the diketopiperazine as a white foam (80 mg, 0.18 mmol, 60%).

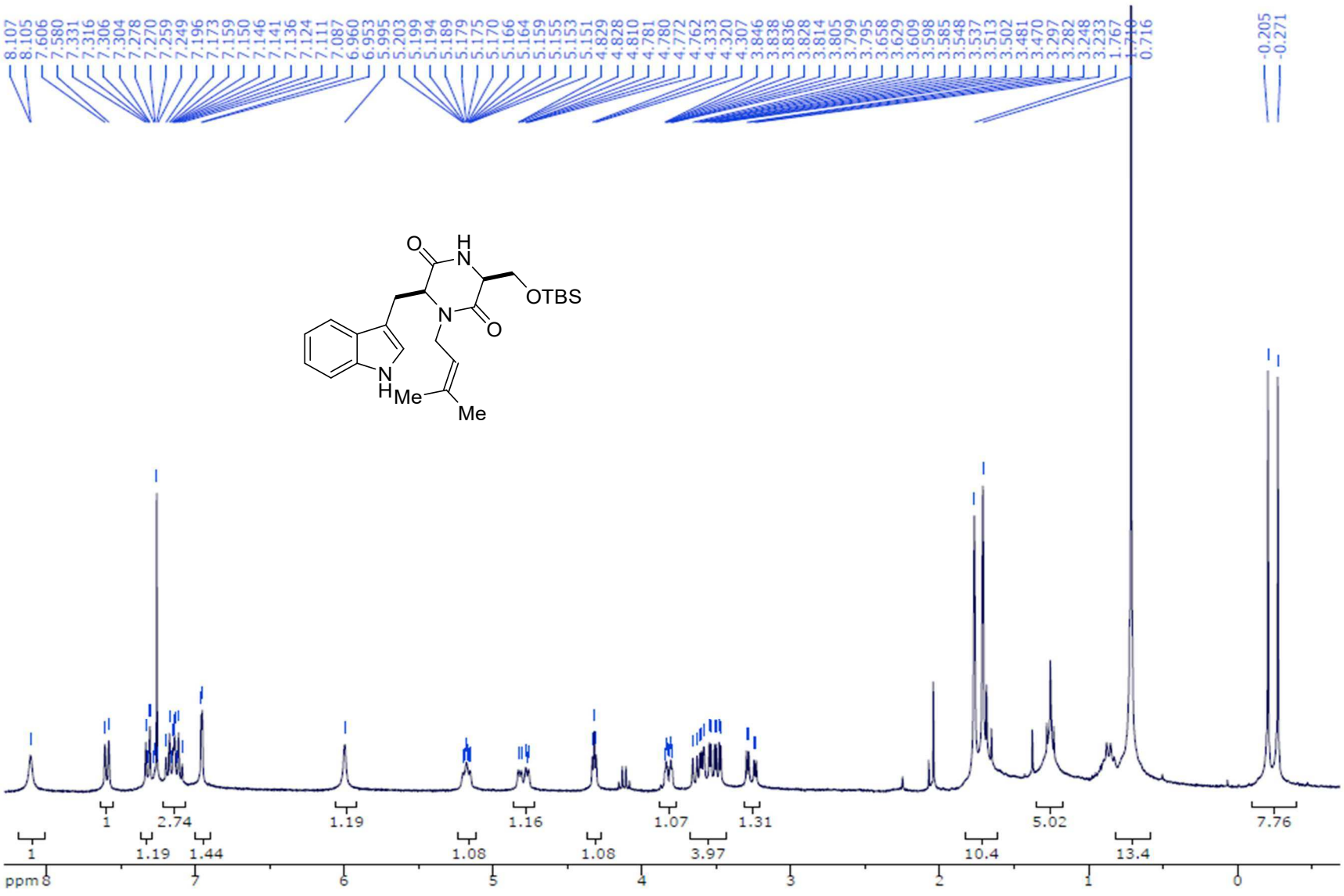
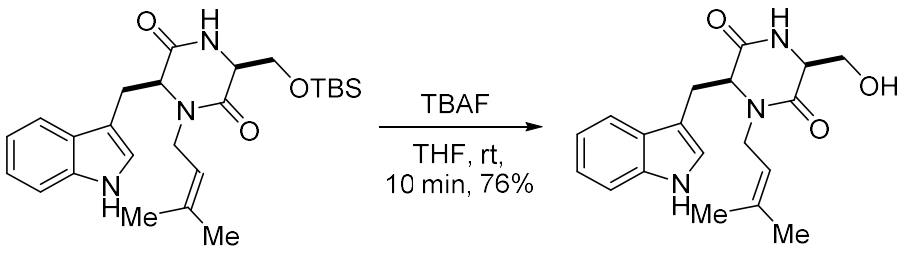


Figure 5.20. ^{1}H NMR of the product.



The protected diketopiperazine (93 mg, 0.20 mmol) was dissolved in a 1M solution of TBAF (2.1 ml, 2.1 mmol) in THF. After the reaction was judged complete by TLC the solution was condensed and purified by column chromatography (EtOAc). Product was recovered as a white powder (52 mg, 0.15 mmol, 76%). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 6.90-6.86 (m, 1H), 5.53 (q, *J* = 6.6 Hz, 1H), 5.32-5.27 (m, 1H), 5.15-5.10 (m, 1H), 5.08-5.02 (m, 1H), 4.96 (dd, *J* = 13.7, 1.5 Hz, 1H), 4.94-4.82 (m, 2H), 5.18-5.11 (m, 1H), 4.77-4.67 (m, 1H), 4.32-4.27 (m, 1H), 3.71 (qd, *J* = 4.1, 4.9 Hz, 1H), 3.61 (dt, *J* = 13.8, 6.7 Hz, 1H), 3.57-3.48 (m, 1H), 3.24 (dd, *J* = 15.0, 4.6 Hz, 1H), 3.03 (s, 1H), 2.97-2.83 (m, 1H), 1.84-1.79 (m, 1H), 1.71 (d, *J* = 14.4 Hz, 3H).

13-C NMR (101 MHz; cdcl3): δ 194.8, 165.7, 164.3, 136.8, 135.7, 125.5, 124.2, 122.3, 119.8, 116.7, 115.4, 111.4, 106.5, 64.4, 59.0, 55.0, 41.4, 25.1, 25.8, 16.1

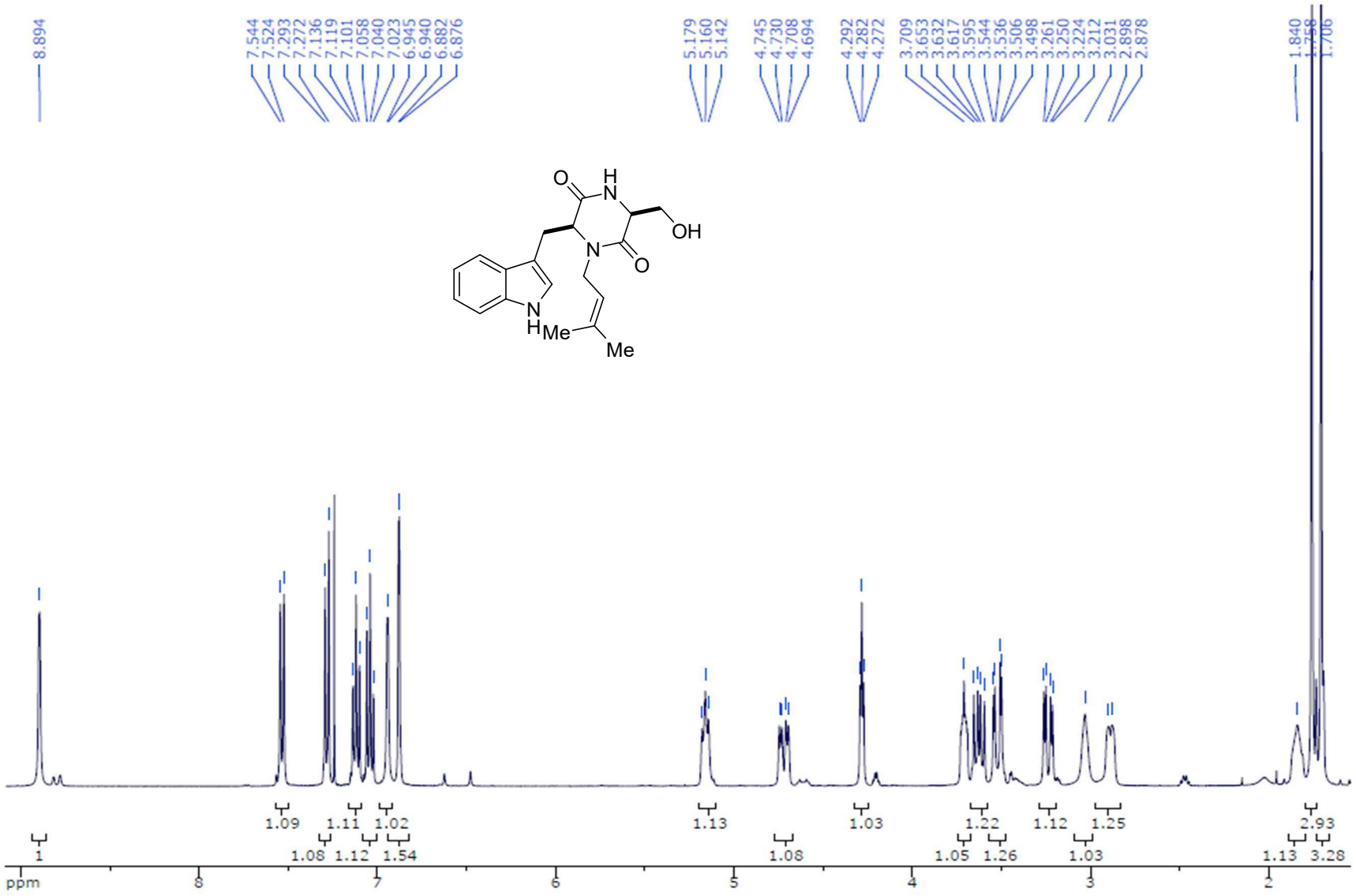


Figure 5.21. ^{1}H NMR of the product.

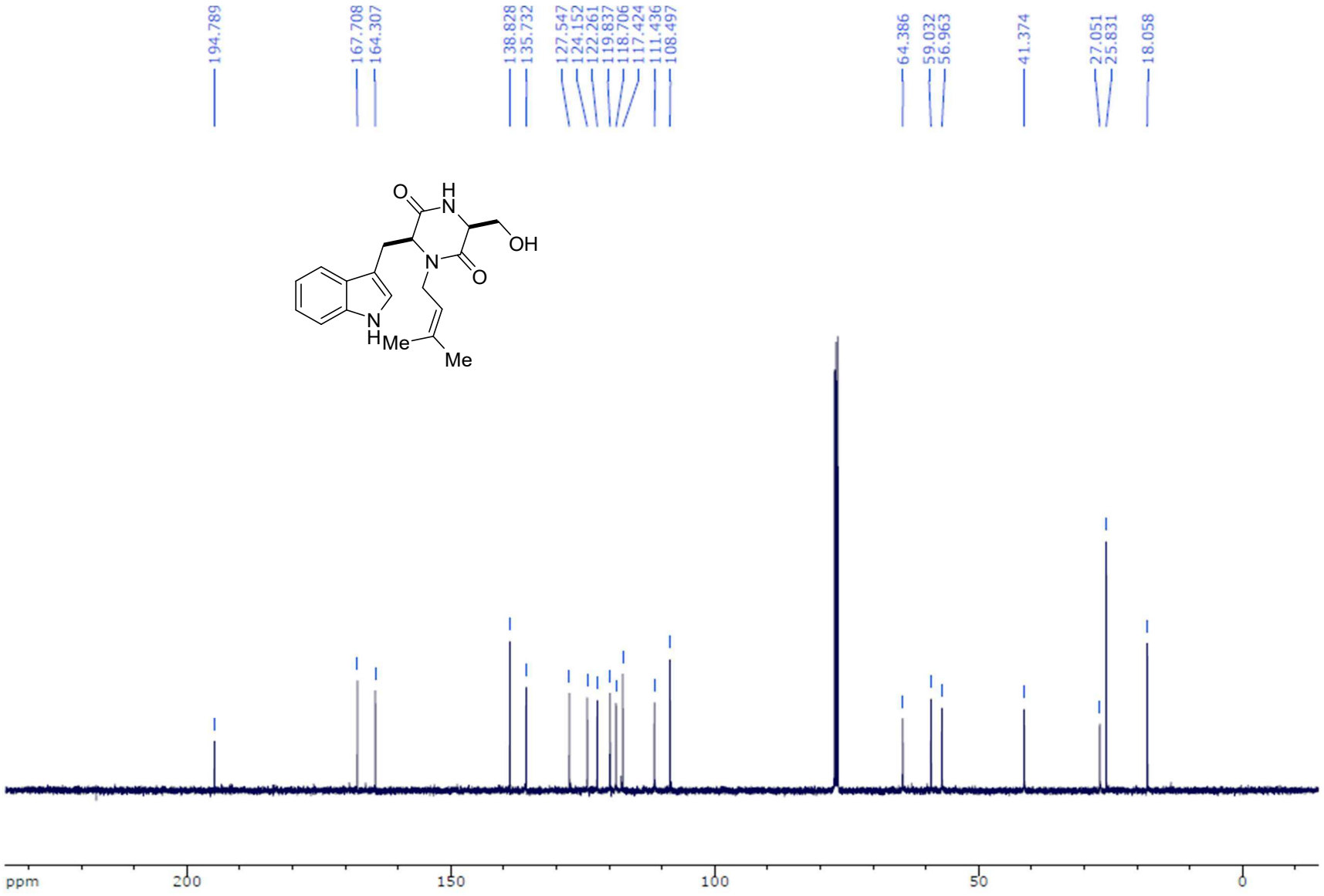
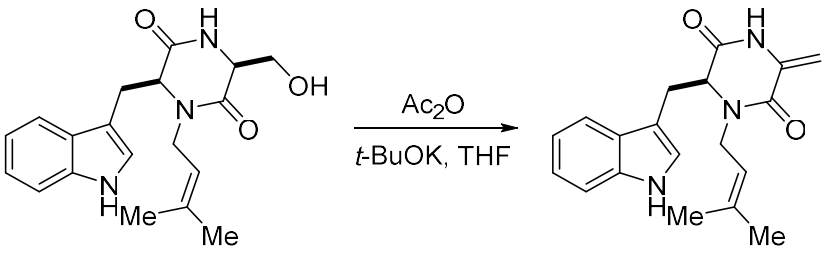


Figure 5.22. ^{13}C NMR of the product.



Dissolve the free alcohol (323 mg, 0.95 mmol) in dry THF with Ac_2O (0.10 mL, 1.1 mmol, 1.1 eq) and DMAP (130 mg, 1.1 mmol, 1.1 eq). After 5 minutes add *t*-BuOK (300 mg, 2.7 mmol, 2.8 eq). After 5 minutes condense, dissolve in DCM and filter through Celite. Purify by column chromatography to obtain product (97 mg, 0.3 mmol, 32% yield). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 6.12 (s, 1H), 5.56 (d, J = 6.0 Hz, 1H), 5.29 (d, J = 6.1 Hz, 1H), 5.15 (t, J = 5.2 Hz, 1H), 5.08 (dd, J = 12.8, 5.0 Hz, 2H), 4.86 (d, J = 2.3 Hz, 1H), 5.21 (t, J = 5.3 Hz, 1H), 5.01 (s, 1H), 4.70 (dd, J = 14.9, 4.3 Hz, 1H), 4.36 (t, J = 3.9 Hz, 1H), 4.01 (s, 1H), 3.64 (dd, J = 14.9, 6.4 Hz, 1H), 3.50 (dd, J = 14.7, 3.3 Hz, 1H), 3.23 (dd, J = 14.8, 4.6 Hz, 1H), 1.77 (s, 3H), 1.72 (s, 2H).

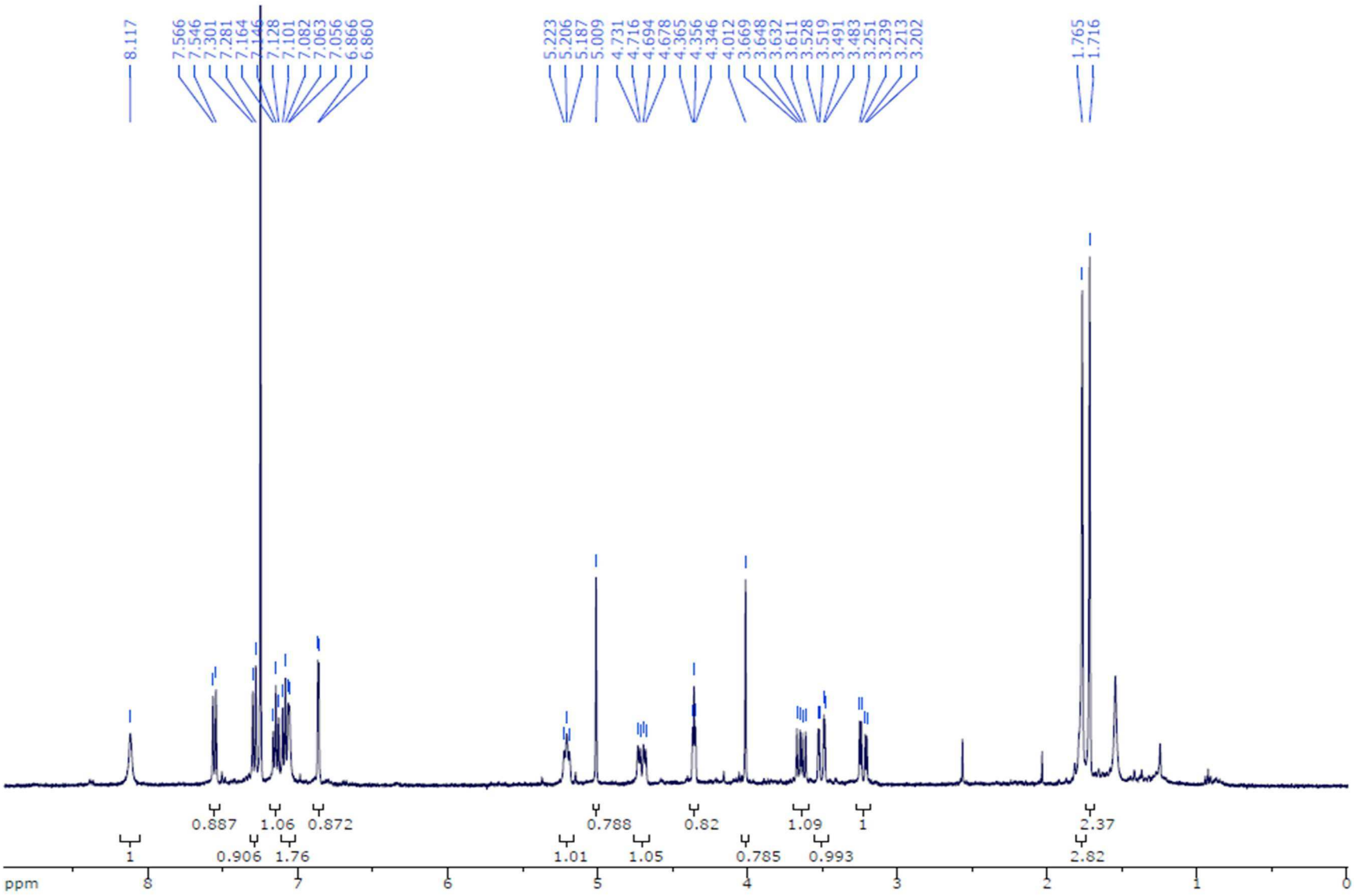


Figure 5.23. ^{1}H NMR of the product.

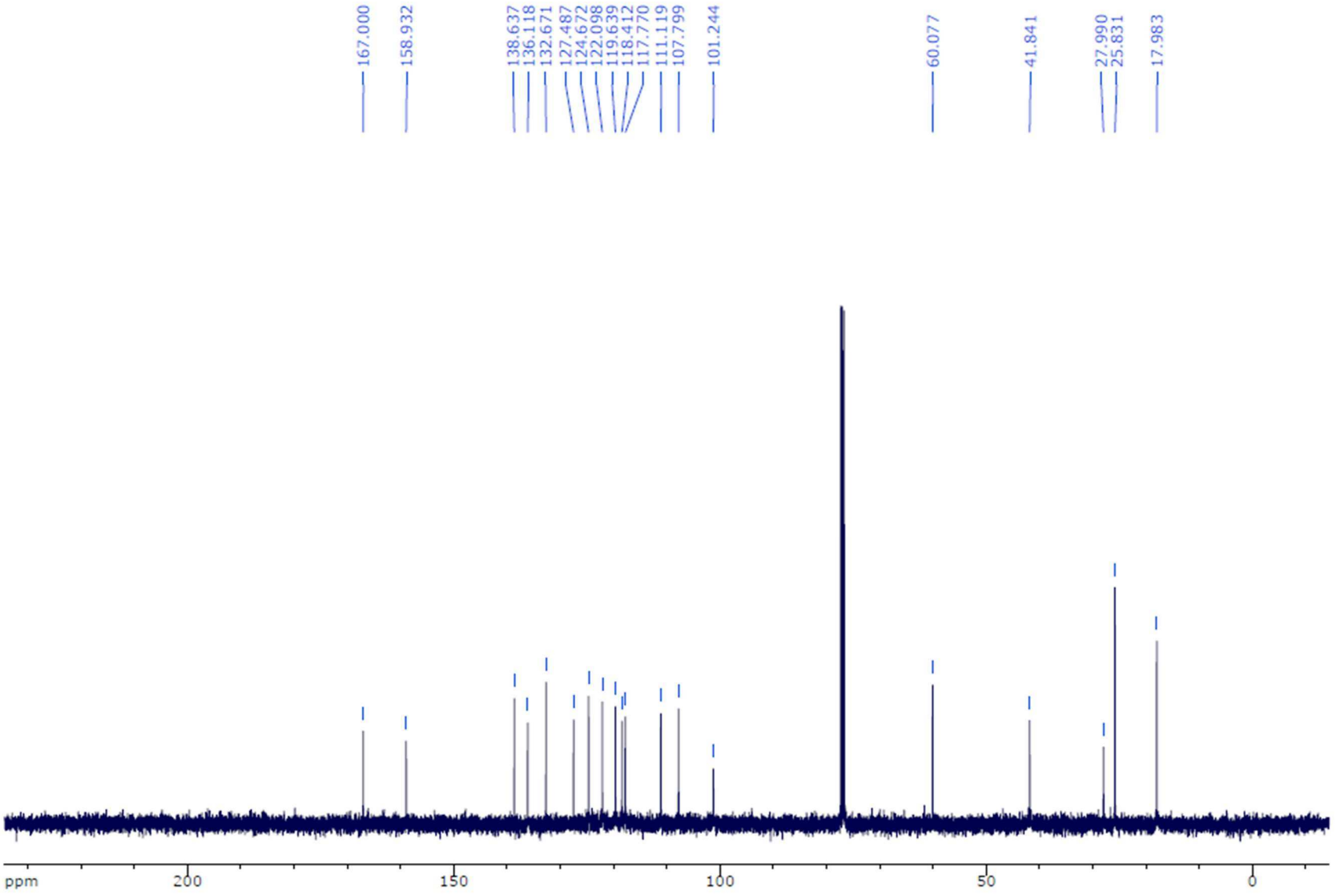
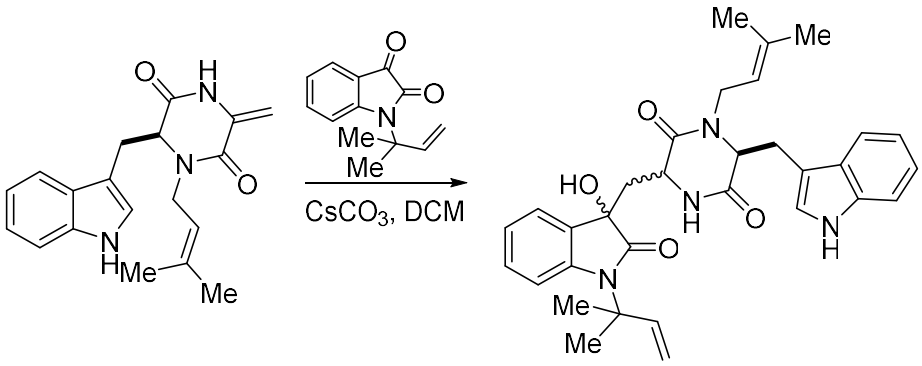


Figure 5.24. ^{1}H NMR of the product.



Dissolve the diketopiperazine (50 mg, 0.15 mmol) in DCM with the isatin fragment (50 mg, 0.23 mmol, 1.5 eq), and $CsCO_3$ (150 mg, 0.45 mmol, 3 eq). Reflux overnight. Filter through Celite and purify by column chromatography to obtain 34 mg of product (41% yield). The NMR is complicated; however the mass matches the product.

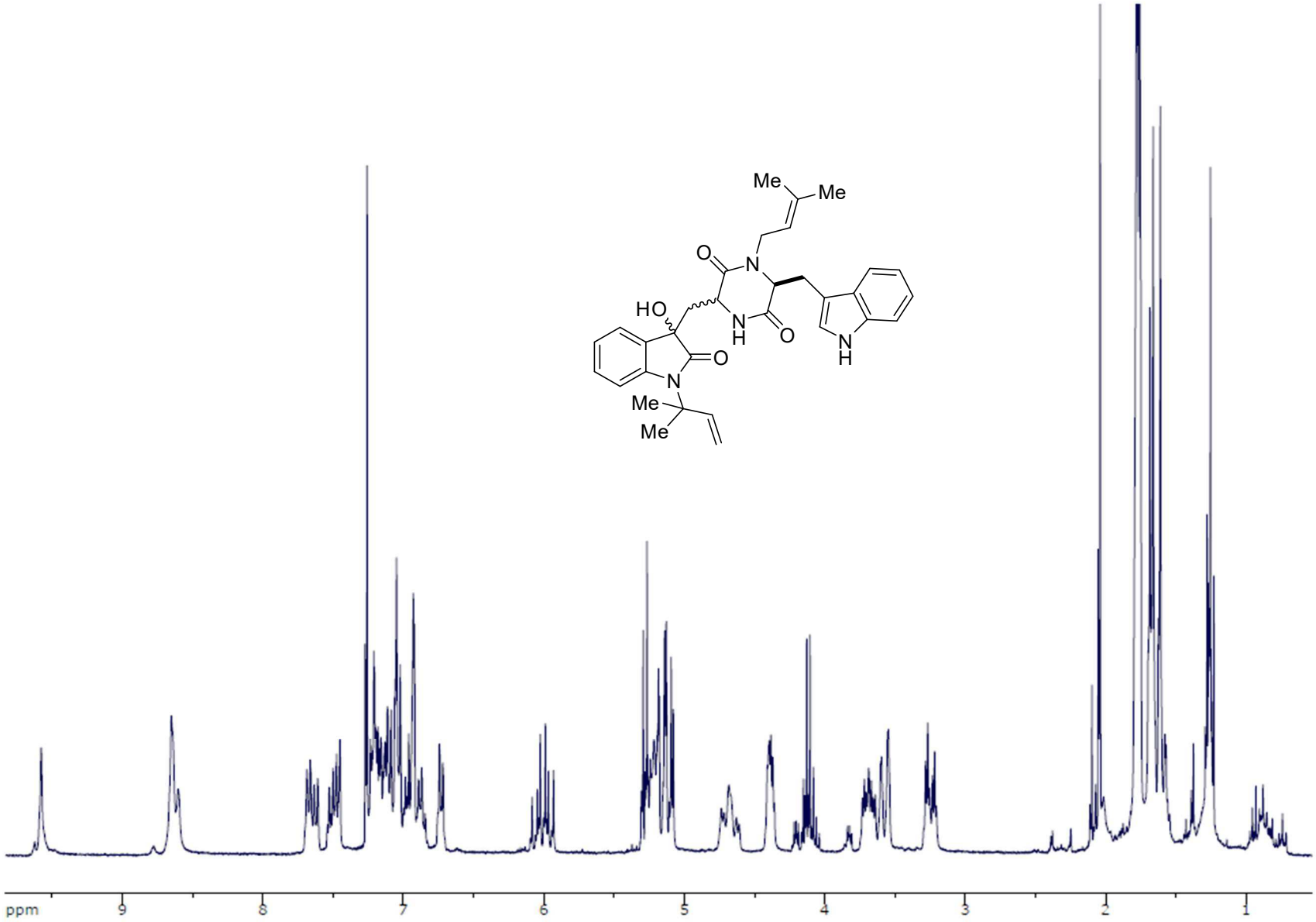
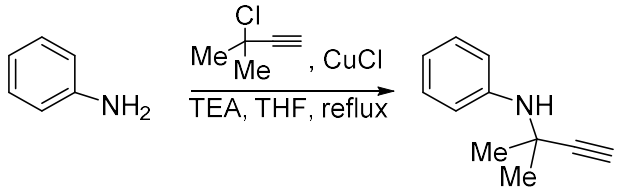


Figure 5.25. ^{1}H NMR of the product.



Added distilled aniline (1.03 g, 11.1 mmol), alkyne (1.25 mL, 10.8 mmol), and CuCl (0.1 g, 1.0 mmol) to THF. Added TEA slowly (1.6 mL, 11.5 mmol). Reaction turned red. Left for 3 hours. Partitioned between H_2O and EtOAc. Extracted once with EtOAc, then washed with brine, dried and condensed. Column chromatography gave 0.658 g of clean product (38% yield). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 5.19 (t, J = 5.8 Hz, 2H), 4.94 (d, J = 6.4 Hz, 2H), 4.79 (t, J = 5.3 Hz, 1H), 3.64 (s, 1H), 2.36 (s, 1H), 1.61 (s, 7H).

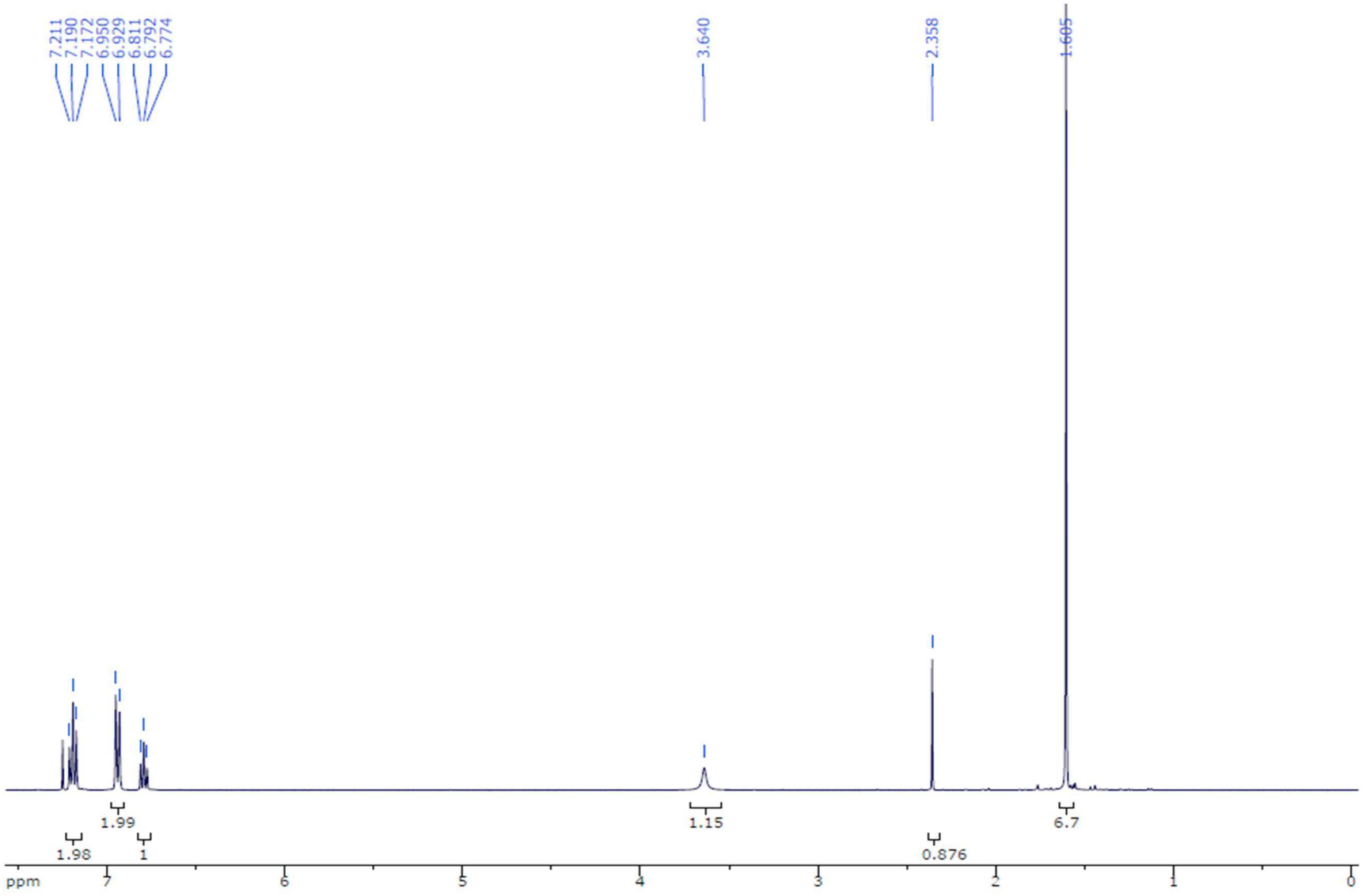
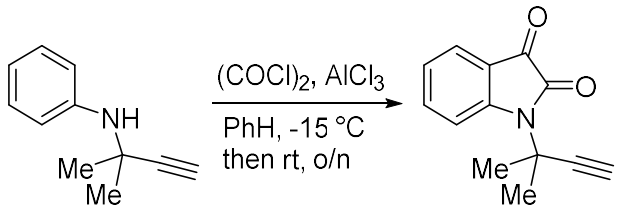


Figure 5.26. ^{1}H NMR of the product.



Dissolve aniline (3.05 g, 19.2 mmol)in benzene at 0 °C. Added $(COCl)_2$ (4.5 g, 35.5 mmol. 1.8 eq) slowly. Remove ice bath and stir 3 hrs. Changed solvent to DCM, cooled to -15 °C and added $AlCl_3$ (3 g, 22.5 mmol, 1.2 eq). After one hour remove the ice bath and leave overnight at room temperature. Purification by column chromatography gave 2.1 g (52% yield) clean product. ^{1}H-NMR (400 MHz; $CDCl_3$): δ 5.77 (d, J = 6.2 Hz, 1H), 5.63 (d, J = 5.4 Hz, 1H), 5.57 (t, J = 5.9 Hz, 1H), 5.12 (t, J = 5.5 Hz, 1H), 2.61 (s, 1H), 2.02 (s, 6H).

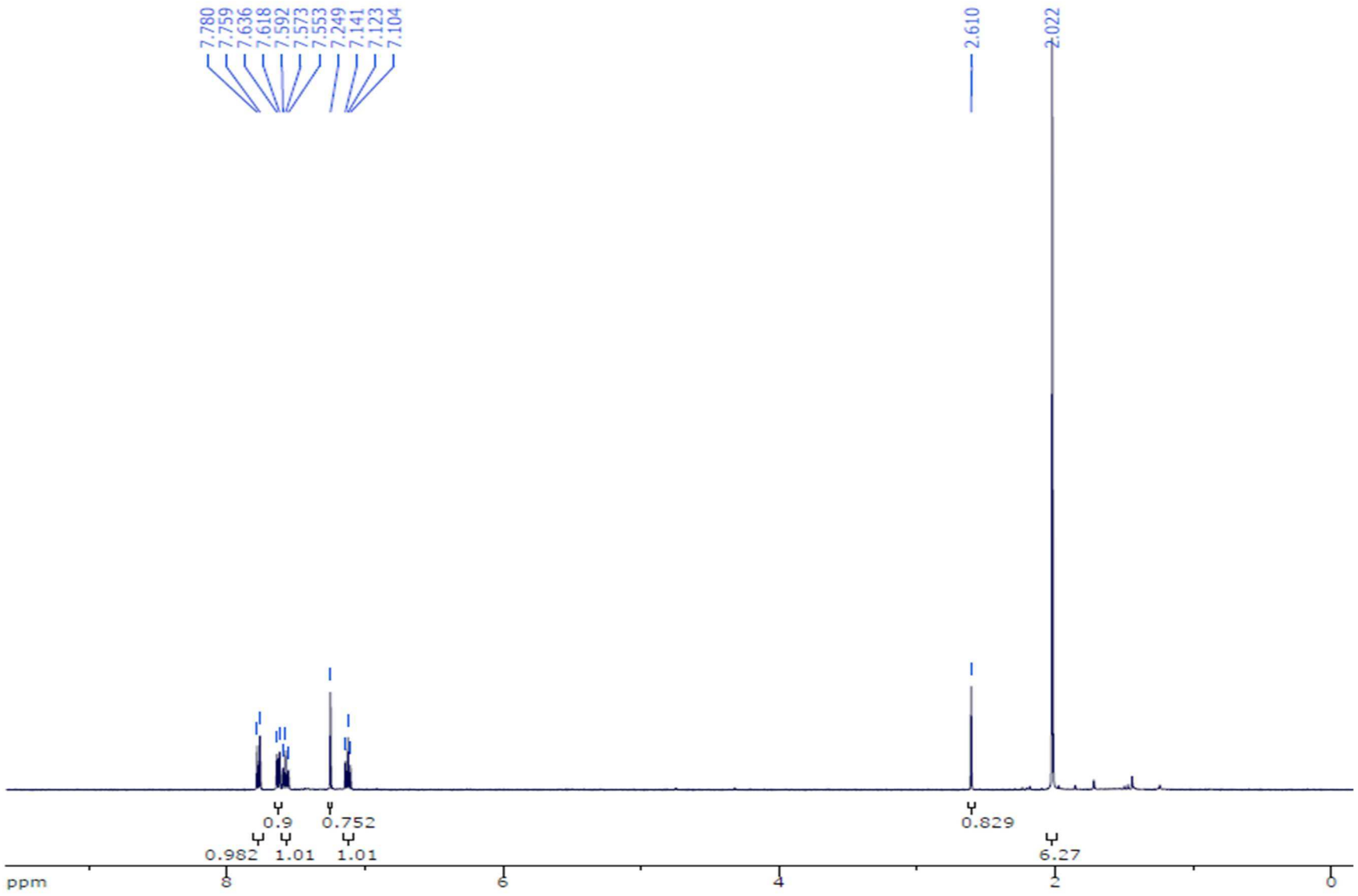
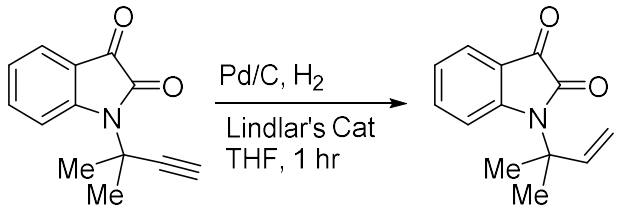
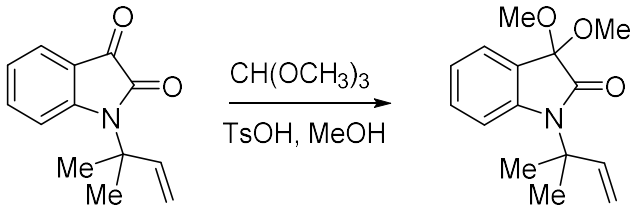


Figure 5.27. ^{1}H NMR of the product.



Dissolved alkyne in THF and purged solvent and flask with hydrgen gas multiple times. Added 33 mass% $CaCO_3$/Pb poisoned catalyst and left to react for 1 hour. Column chromatography gave exclusively the alkene without overreduction to the alkane. Use of other solvents (EtOAc, MeOH) and catalysts ($BaSO_4$ poisoned catalyst) gave overreduction or competitive reduction.



Reverse prenylated isatin was dissolved in dry MeOH with a catalytic amount of TsOH and an equivalent of trimethylorthoformate. The solution was brought to reflux for three days. The organic solution was washed with H_2O and brine, dried over $MgSO_4$ and condensed. The product was purified using column chromatography. ^{1}H-NMR (400 MHz; $CDCl_3$): δ 5.36 (d, J = 5.5 Hz, 1H), 5.19 (dd, J = 14.6, 6.0 Hz, 2H), 5.02 (t, J = 5.3 Hz, 1H), 4.11 (dd, J = 15.6, 10.7 Hz, 1H), 5.17 (dd, J = 14.2, 4.9 Hz, 2H), 3.52 (s, 6H), 1.99 (s, 1H), 1.78 (s, 6H).

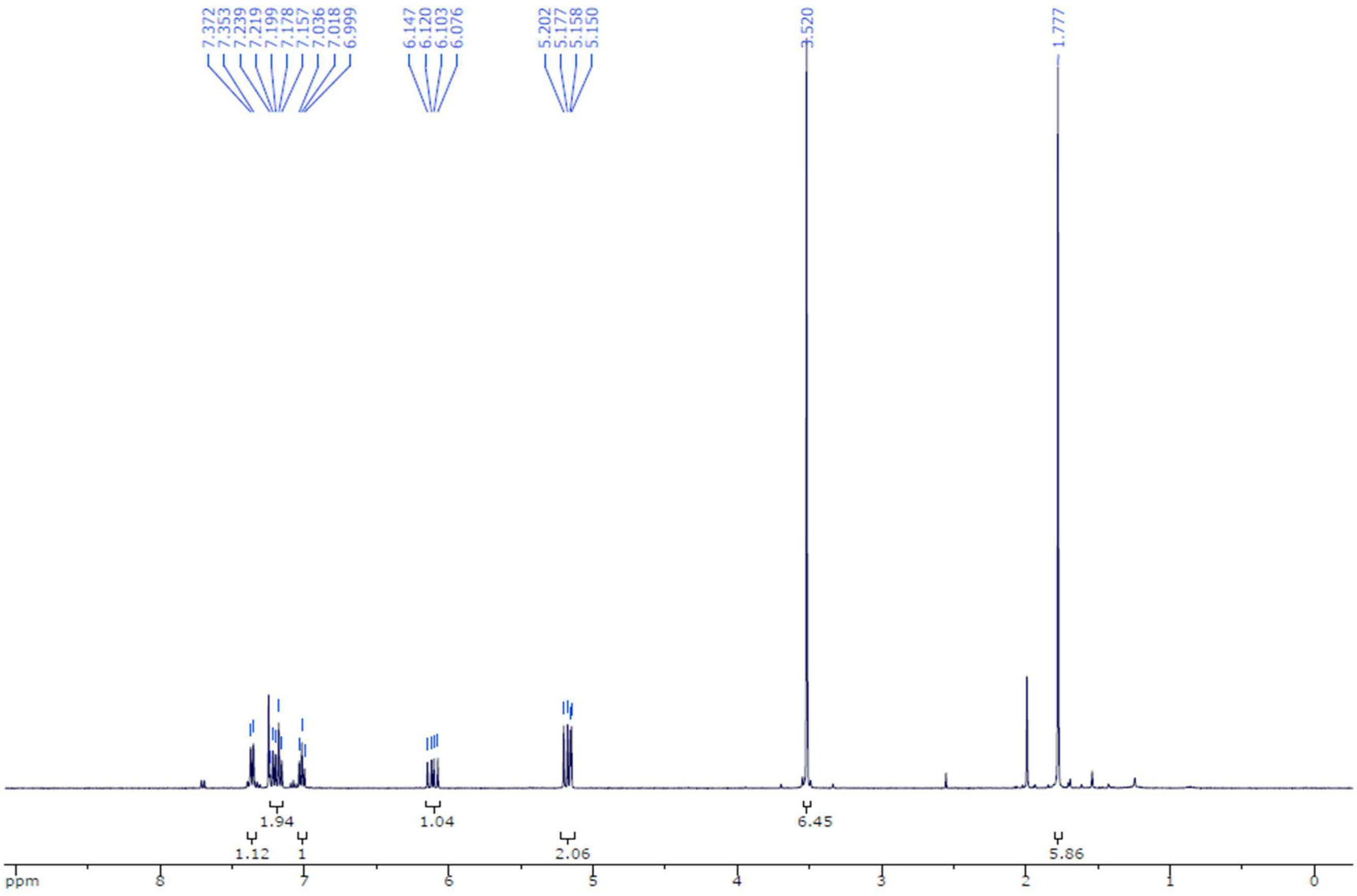
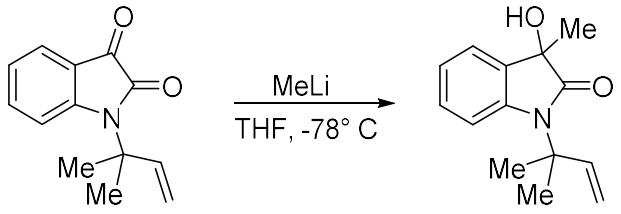
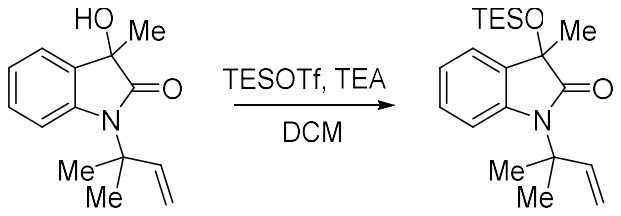


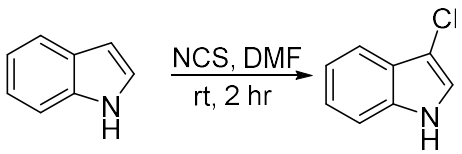
Figure 5.28. ^{1}H NMR of the product.



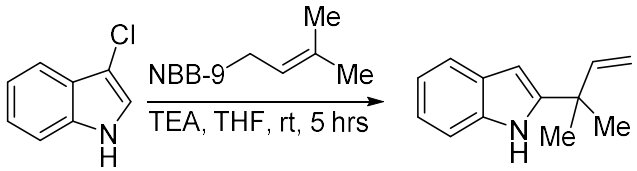
Reverse prenylated isatin (523 mg, 2.4 mmol, 1 eq) was dissolved in dry THF at -78°C. MeLi in a 1.6 M solution of THF (1.5 mL, 2.4 mmol, 1 eq) was added dropwise. After stirring for an hour at the same temperature the solution was partitioned between 1M HCl and EtOAc. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered through Celite and condensed. Purification by column chromatography (0% to 20% EtOAc in hexanes) gave 290 mg of pure product (54% yield).



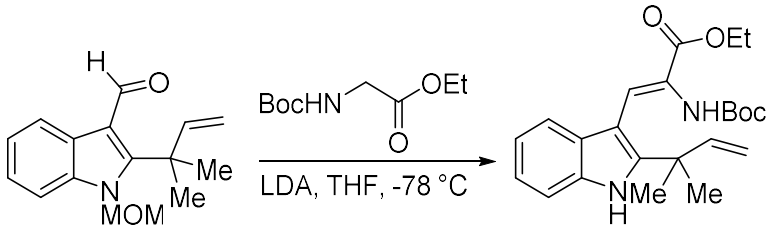
The oxindole (78 mg, 0.34 mmol, 1 eq) was dissolved in dry DCM at rt followed by TESOTf (0.1 mL, 0.53 mmol, 1.6 eq) and TEA (0.06 mL, 0.43 mmol, 1.3 eq). The solution was stirred overnight at room temperature followed by evaporation and column chromatography to obtain the protected oxindole.



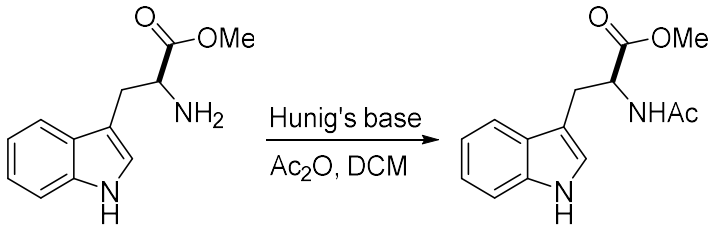
Dissolve Indole (3.13 g, 24.7 mmol) in DMF and add NCS (3.74 g, 26.0 mmol, 1.05 eq) in a flask that is kept in the dark at room temperature. Leave 2 hours. Add brine and extract 3X with EtOAc, wash with water 3X, brine once, dry and condense. Column chromatography gives 3.77 g (93% yield).



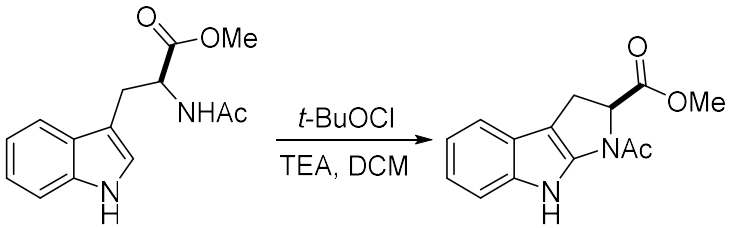
Indole-Cl (3.77 g, 24.9 mmol) dissolved in THF, add TEA (11.3 mL) dropwise. Leave 30 min. Add to solution of prenyl-9BBN (3 eq) and leave 5 hrs. Column Chromatography gave 2.54 g (55% yield) of product.



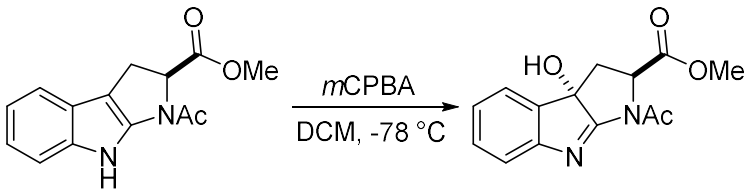
Add *N*-Boc-Gly-OEt (346 mg, 1.7 mmol) in THF dropwise to a solution of LDA (1.7 mL, 2M, 1.7 mmol). Dissolved indole fragment (220 mg, 0.85 mmol) in THF at -78 °C and added dropwise to the previous solution. After 15 minutes allowed to warm to room temperature. Partitioned between NH_4Cl/EtOAc. Column chromatography on the contents of the organics gave 360 mg (95% yield) of product.



Dissolve 4.17 g tryptophan methyl ester in DCM and add hunig's base (10 mL, 2.38 eq). Added Ac_2O (25 mL, 1.08 eq). The addition causes boiling. Stir for 1 hr at room temperature. Partition between DCM and HCl. Dry the organics and reduce to 5.77 g of product (92% yield).



Dissolved protected tryptophan (1.00 g, 3.8 mmol) in 60 mL DCM followed by TEA (4.1 mL, 4 eq) at -10 °C. Dropwise addition of *t*-BuOCl dissolved in 5 mL of CCl_4 over 30 minutes. Left to react overnight. Condense and partition between EtOAc and HCl. Separate, wash with H_2O, brine, condense and dry. Column chromatography gave 0.332 g (34% yield) of product.



Dissolved 800 mg of witkop tricycle in DCM at -78 °C. Added *m*CPBA (1.16 eq), let warm to room temperature. Extraction and column chromatography on the residue gave 25% yield of the alcohol product.

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Part 2. Studies Toward the Synthesis and Biosynthesis of the Hapalindole Family

Chapter 1. Introduction

1.1. Isolation and Activity

Upon discovery that *Hapalosiphon fontinalis* produces an extracellular antialgal compound Moore *et al.* isolated the first two members of the Hapalindole family; Hapalindole A and B.[22] Since the 1984 publication on the elucidation of the Hapalindoles the number of similar natural compounds has expanded to 28 (**Figure 1.1**).[23] Closely related families have also been uncovered including the fischerindoles, ambiguines and hapalindolinones leading to more than 70 discrete molecules.[24]

The hapalindole and related families have a rich background of research focusing on the activity, biosynthesis and synthesis of this extensive family of secondary metabolites. The first hapalindoles were isolated based on antialgal activity, however the activity of the hapalindoles includes insecticidal[25], antimycotic[22, 26-27], and antibacterial[28-29] activity. The related welwitindolinones have also shown anticancer activity[30].

Hapalindoles J (1989, 2012),[31-32] M (1989),[31] U (1990, 2012),[32-33] H (1990, 2014),[33-34] G (1994, 2011),[35-36] O (1994, 2012),[37-38] Q (2001, 2003, 2008, 2014, 2016),[34, 39-42] A (2011),[36] and K (2011)[36] have succumb to total syntheses, along with a number of hapalindole-related structures. Reports on the syntheses of the main group of the hapalindole family follows.

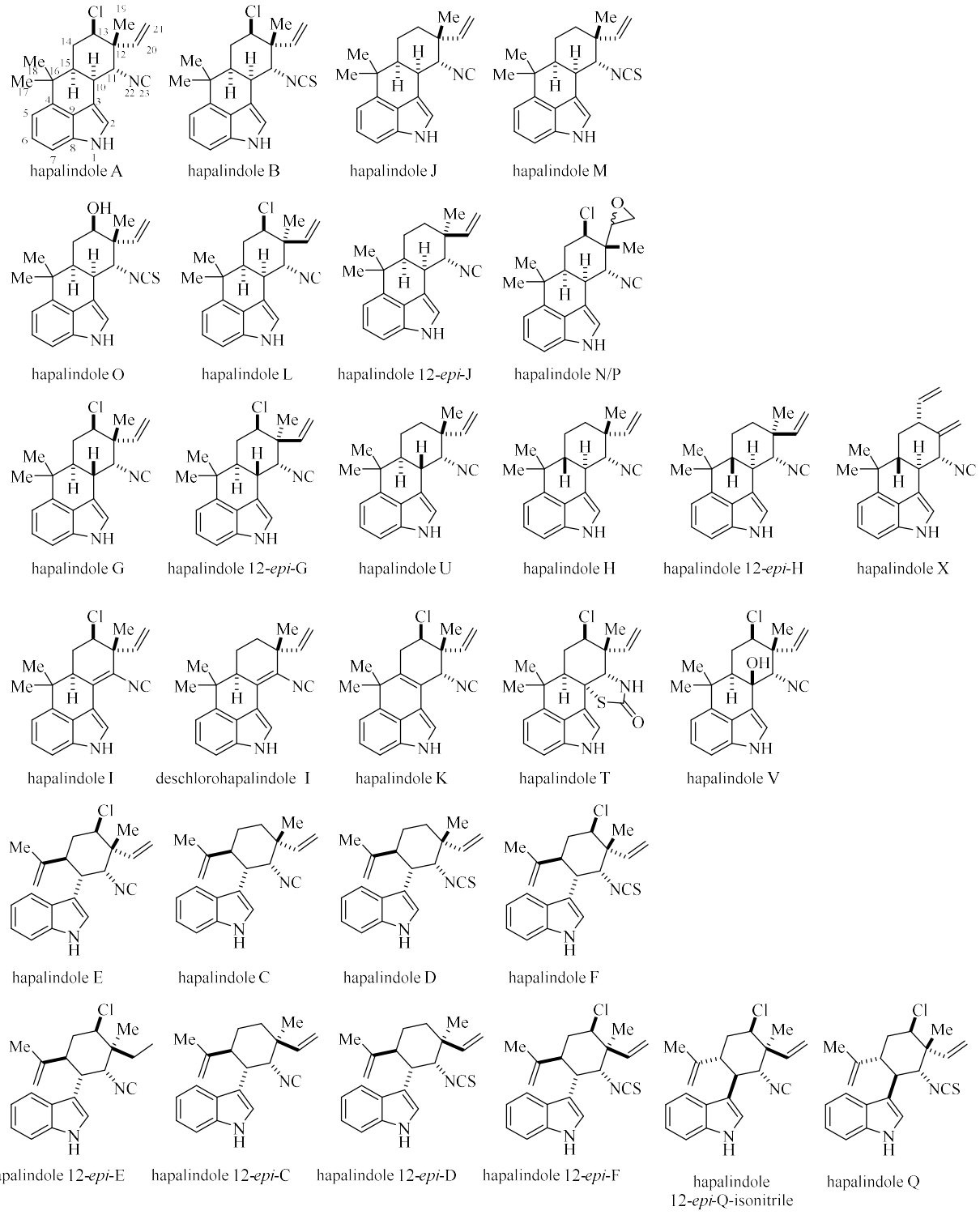
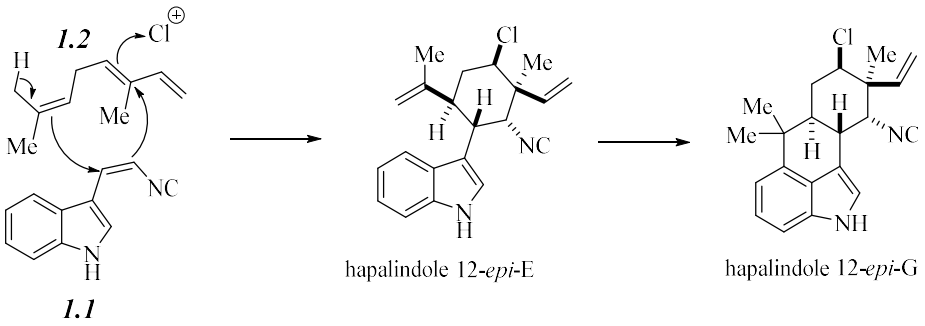


Figure 1.1. The hapalindole family of natural products.

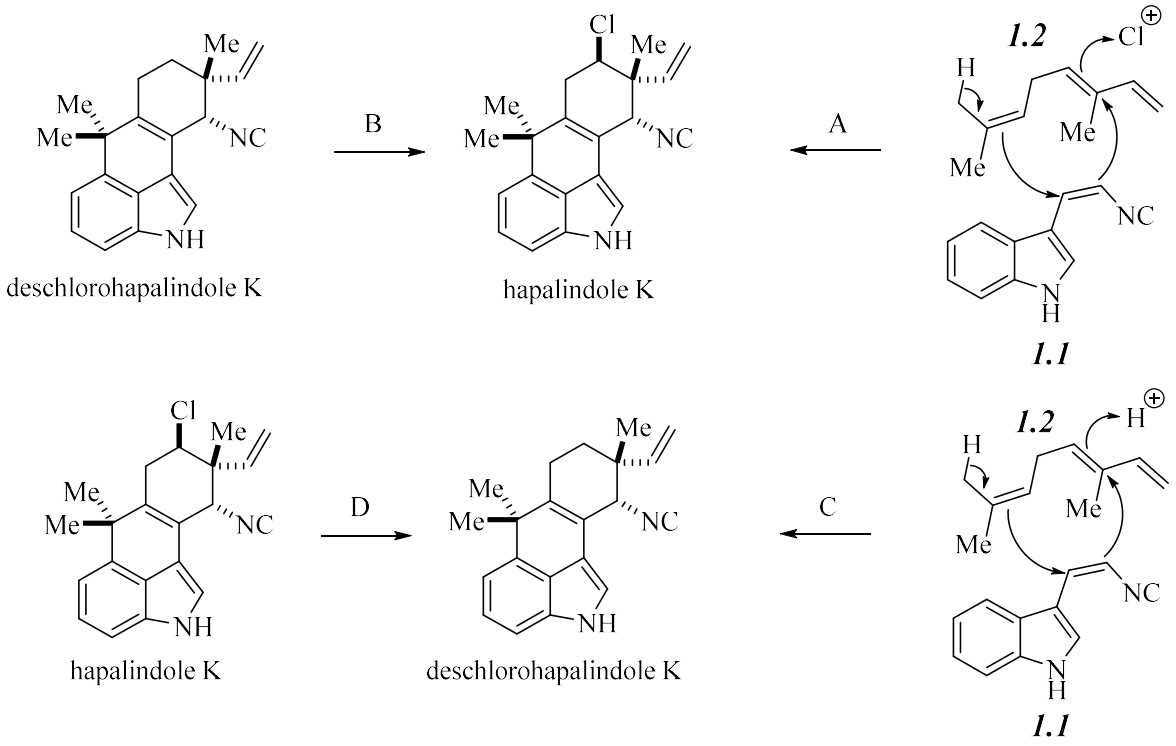
1.2. Biosynthesis

Moore et al. went on to purpose a biosynthetic origin for the tricyclic hapalindole core (**Scheme 1.1**).[30] Tryptophan and geranyl derivatives are proposed to be coupled with an electrophilic chlorine to provide 12-*epi*-Hapalindole E. From 12-*epi*-Hapalindole E the tetracyclic core can be created by aromatic substitution with the disubstituted alkene to make 12-*epi*-Hapalindole G.



Scheme 1.1. Original biogenesis proposal by Moore et al.[30]

The biosynthesis proposed above (applied to hapalindole K in route A of **Scheme 1.2**) requires a dehalogenase to access the deschlorohapalindoles (route D). Alternatively, there could be a second pathway involving hydronium (route C), followed by a late-stage halogenase (route B). We proposed that the chlorine is installed with a halogenase and intended to begin the study of the biosynthesis by testing this hypothesis through the synthesis of deschlorohapalindole K. Upon synthesis of the target it would be subjected to the enzyme believed to be responsible for halogenation.



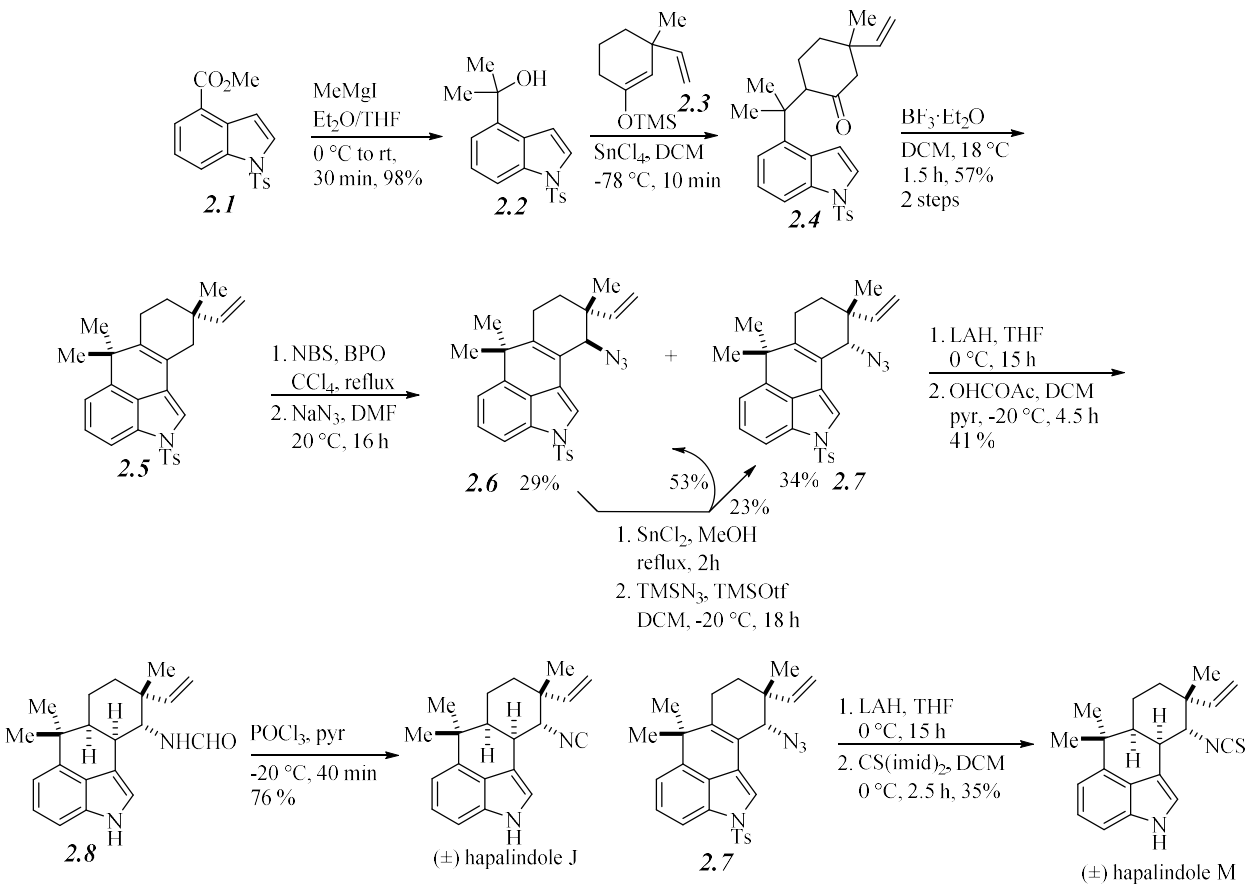
Scheme 1.2. Extension of the original biogenesis proposal.

Chapter 2. Previous Synthetic Work

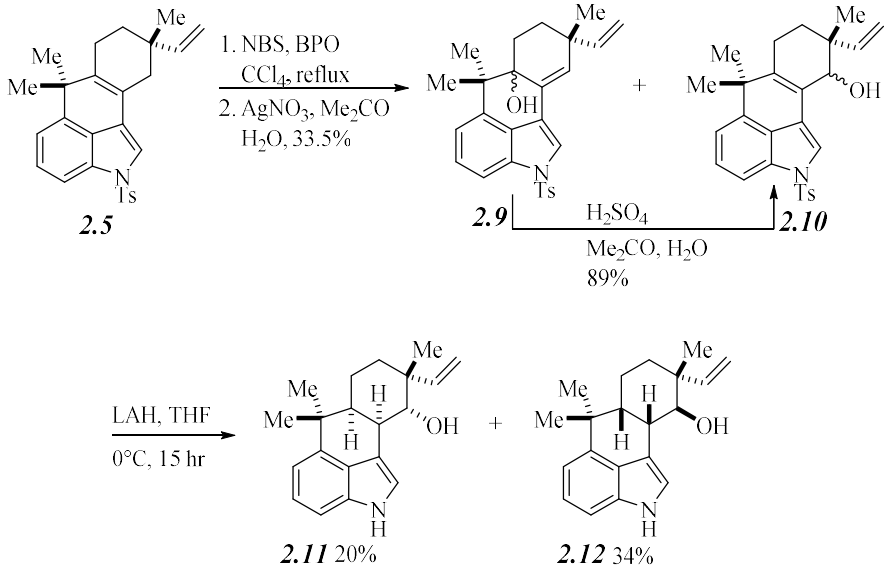
2.1. Syntheses of Hapalindoles (±) J, (±) M, (±) H, (±) U, and O.

Muratake was the first to publish on the synthesis of members of the hapalindole family.[31] In the first of a three part publication the total syntheses of racemic hapalindoles J and M were shown (**Scheme 2.1**). The synthesis begins with C7-functionalized protected indole **2.1**. Double Grignard addition afforded the necessary alcohol to effect a coupling to make the C15–C16 bond of the hapalindoles. The coupling comes between alcohol **2.2** and racemic silyl enol ether **2.3** to make tricycle **2.4**. Lewis-acid catalyzed condensation of the carbonyl in **2.4** leads to tetracycle **2.5** by forming the C3–C10 bond. To establish the correct C-11 oxidation level allylic bromination was used, followed by sodium azide substitution to yield a mixture of C11-epimers **2.6** and **2.5.** A small amount of recycling is possible using $SnCl_2$ in methanol followed by trimethylsilylazide. With **2.7** in hand, LAH reduction of the azide, tosyl protecting group, and C10–C15 unsaturation followed by amidation leads to **2.6.** This unusual LAH olefin reduction was explored separately in part 2 of the series showing some various stereoselectivities for the installed hydrogens at C-10 and C-15.[43] Dehydration using $POCl_3$ lead to racemic hapalindole J. Using the minor product from the C-11 oxidation in the LAH reduction followed by double substitution of $CS(imidazole)_2$ lead to racemic hapalindole M.

In part 3 of the series Muratake revealed the synthesis of racemic Hapalindole U (**Scheme 2.2** and **2.3**). Following the bromination of **2.5** and treatment with $AgNO_3$, followed by aqueous acid isomerization of **2.9**, gave alcohol **2.10**. LAH reduction of this alcohol gave C10–C15 saturated tetracycles **2.11** and **2.12** in 20% and 34% yield respectively (compare to reduction of **2.7**).

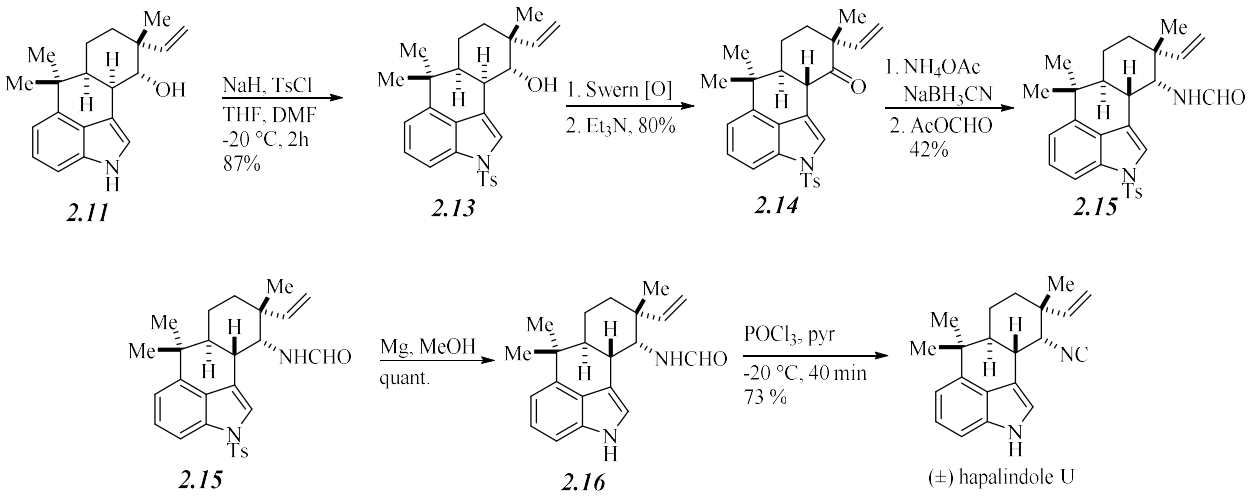


Scheme 2.1. Muratake's first synthesis of racemic hapalindoles J and M.



Scheme 2.2. Muratake's work toward the first synthesis of racemic hapalindole U.

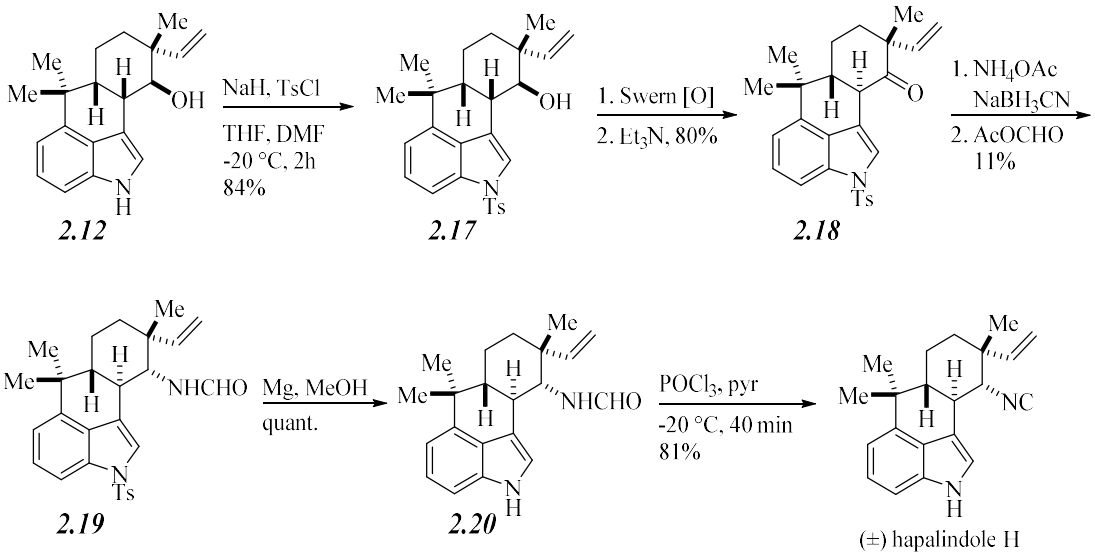
Protection of the indole **2.11** followed by Swern oxidation and C10-epimerization gives *trans*-decalin carbonyl **2.14**. Reductive amination followed by the same amidation used above gave amide **2.15**. Deprotection and dehydration led to racemic hapalindole U.



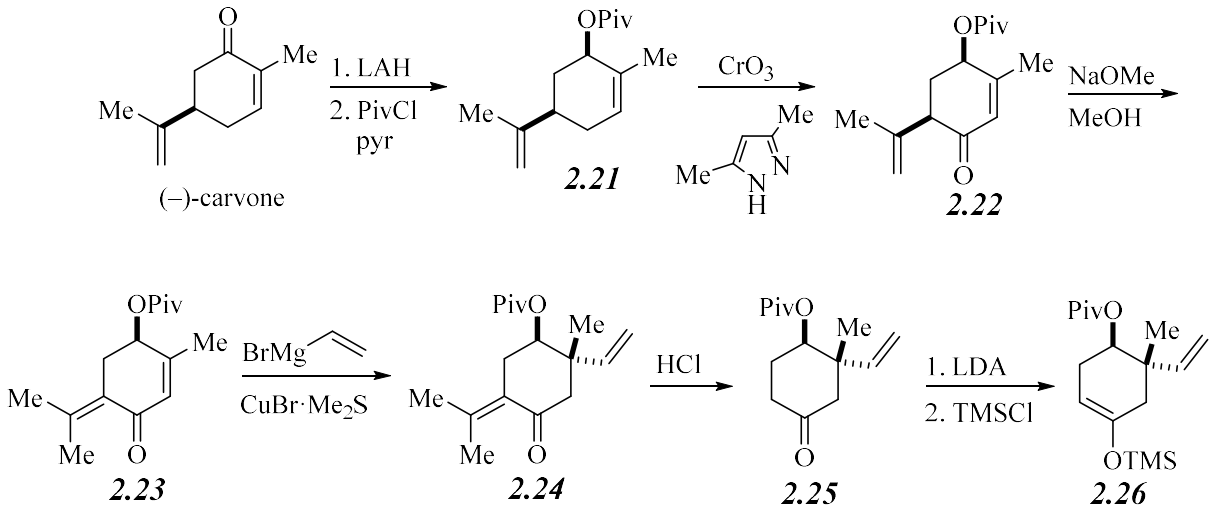
Scheme 2.3. Muratake's first synthesis of racemic hapalindole H.

Hapalindole H was synthesized similarly by using tetracycle alcohol **2.12** from above (**Scheme 2.4**). Protection, oxidation and epimerization gave *trans*-decalin **2.16.** Deprotection and dehydration afforded racemic hapalindole H.

Sakagami continued their efforts in the synthesis of hapalindole O (**Scheme 2.5**).[37] Reduction and Piv-protection of enantiopure (–)-carvone followed by chromium trioxide allylic oxidation gave enone **2.22** in 35% yield. Isomerization using sodium methoxide gave the double enone in 95% yield which underwent conjugate addition as shown before in 77% yield. The unnecessary remaining olefin was cleaved off using aqueous acid in 84% yield, which was enolized in preparation for coupling without purification.



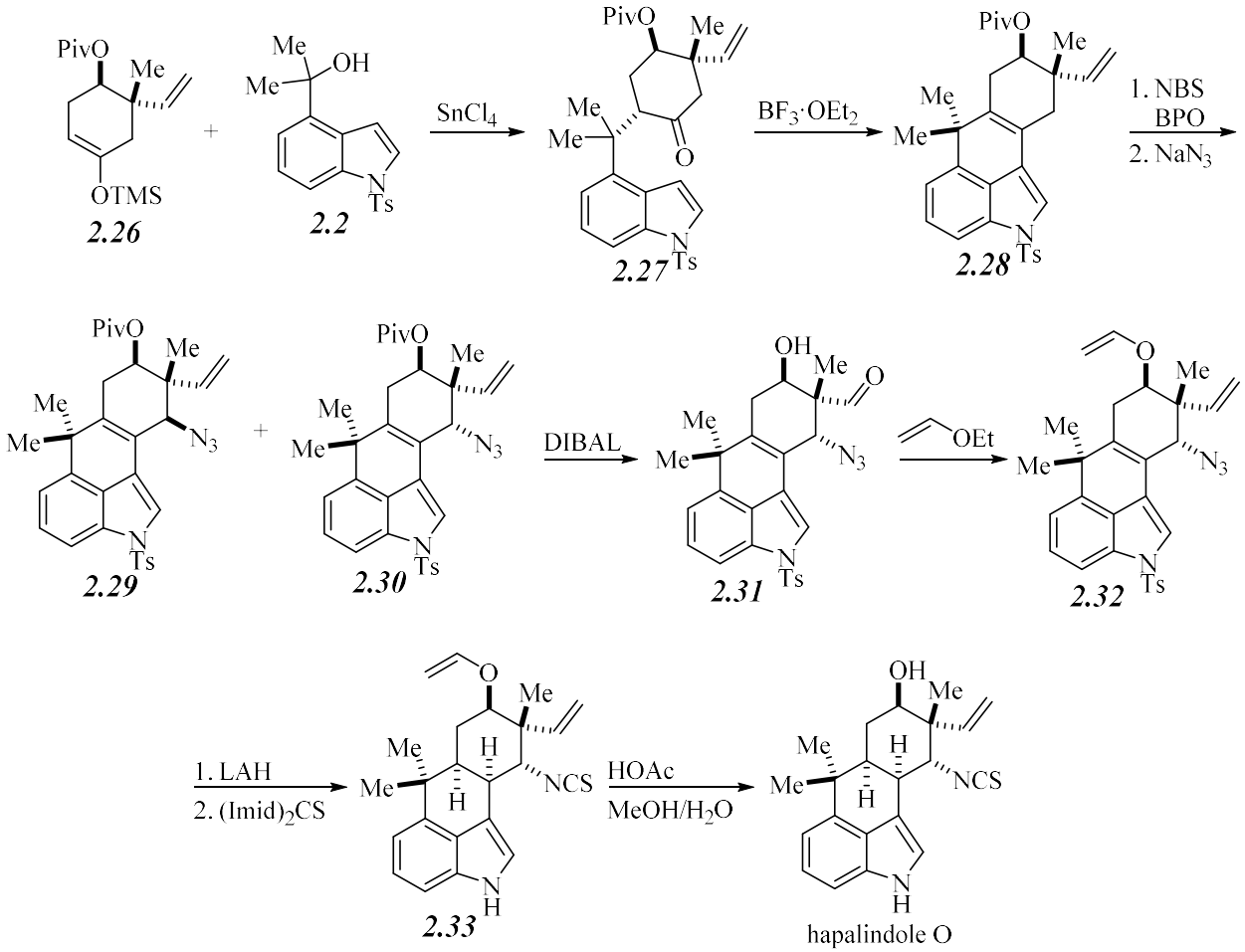
Scheme 2.4. Muratake's first synthesis of racemic hapalindole H.



Scheme 2.5. Synthesis of coupling partner **2.24.**

Coupling of the silyl enol ether to indole **2.2** under the standard tin-mediated coupling gave C13-oxidized tricycle **2.27** in 50% yield (**Scheme 2.6**). Friedel-Crafts type cyclization gave enantiopure tetracycle **2.28** in 66% yield. With the tetracyclic structure complete the previously successful allylic bromination and substitution gave azide diastereomers **2.29** and **2.30** as a 1:1

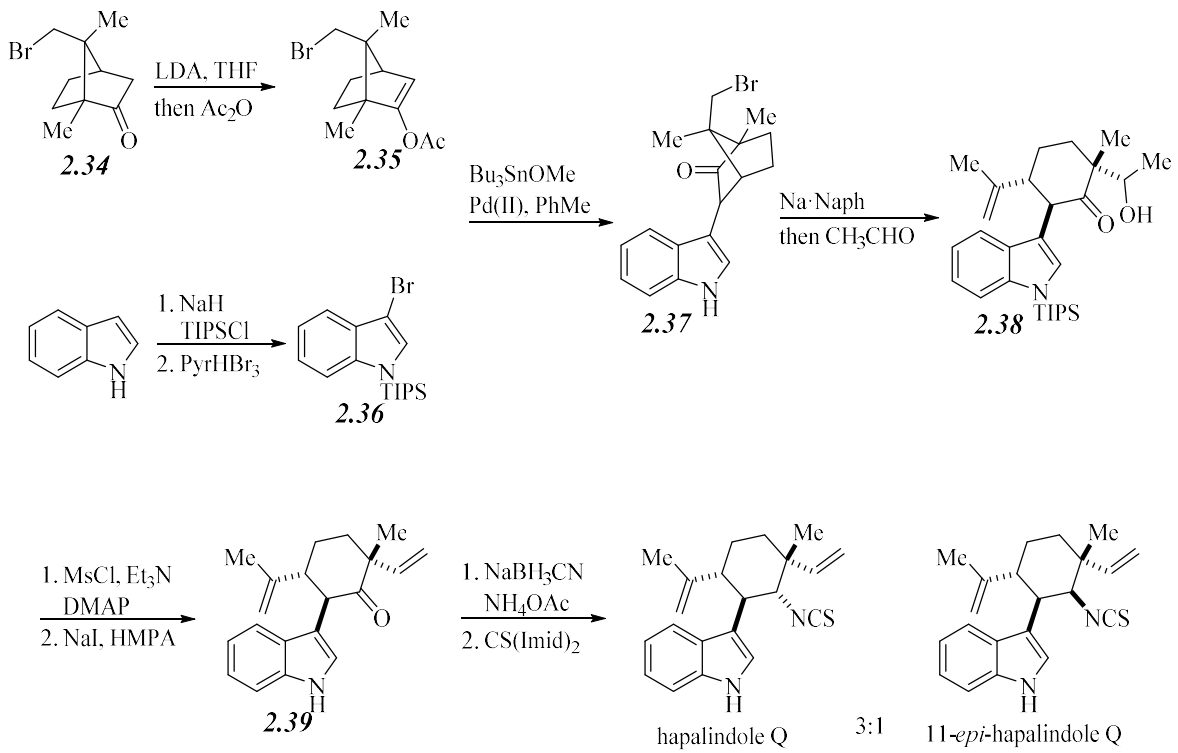
mixture in 78% yield. Deprotection using DIBAL in almost quantitative yield followed by reprotection with SEMCl again in high yield. Reduction of the C10–C15 olefin, azide, and tosyl protecting group followed by thioacyl substitution gave isothiocyanate **2.33** in 48% yield. Deprotection gave another enantiopure synthesis of a member of the hapalindole family; hapalindole O.



Scheme 2.6. Sakagami's completed synthesis of enantiopure hapalindole O.

2.2. Synthesis of Hapalindole Q

The first publication of an enantioselective synthesis was accomplished by Vaillancourt in their unique approach to the hapalindole family in the synthesis of (+)-hapalindole Q (**Scheme 2.7**).[44] Using brominated camphor allowed for stereoselectivity from the chiral pool as follows. (+)-9-bromocamphor was acylated using LDA and acetic anhydride to give the masked enolate **2.35**. Simultaneously indole was protected using TIPSCl, and brominated at C3 using pyridinium tribromide to give **2.34.** The acyl-enolate became the tin-enolate in situ, which underwent coupling using palladium catalysis to make endo camphor-indole intermediate **2.37** in 51% yield, with 10% of the undesired exo product. Fragmentation of the camphor bridge using sodium naphthalenide followed by acetaldehyde to give tricycle **2.38** in 81% yield. Mesylation followed iodination gave elimination to **2.39**, which underwent familiar reductive amination and acyl substitution to give (+)-hapalindole Q in an overall 8% yield allowing for the confirmation of stereochemical assignments.



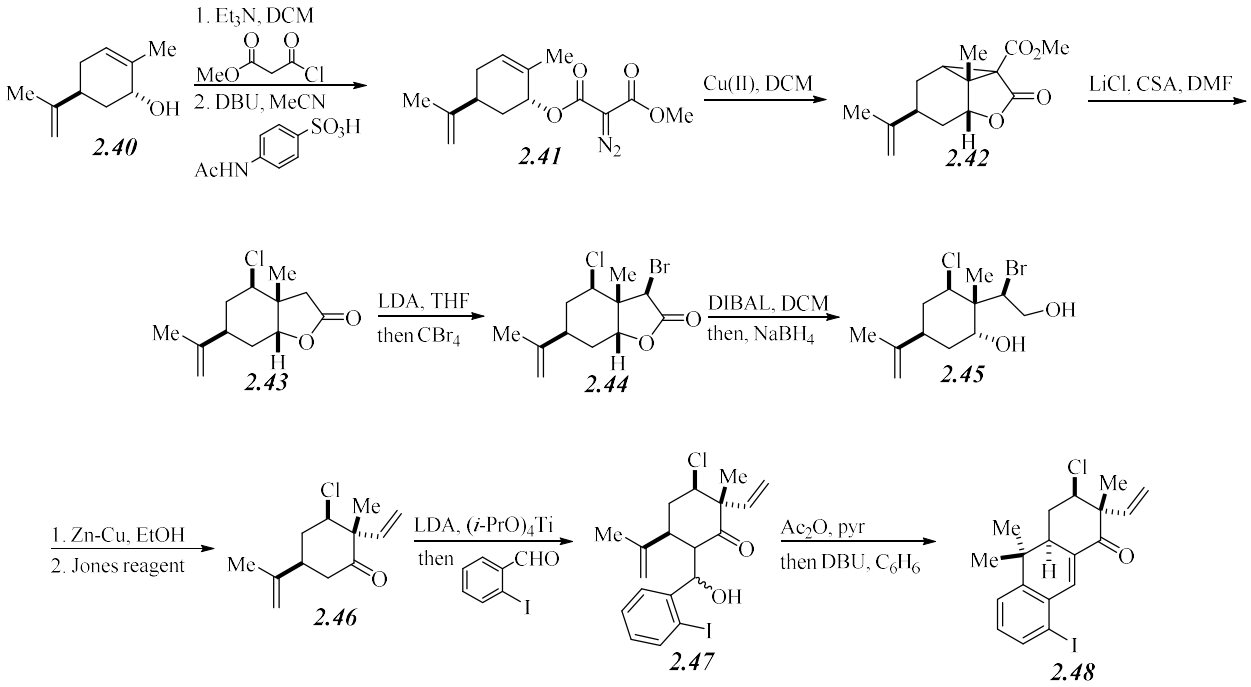
Scheme 2.7. Vaillancourt's synthesis of hapalindole Q.

2.3. Synthesis of Hapalindole G

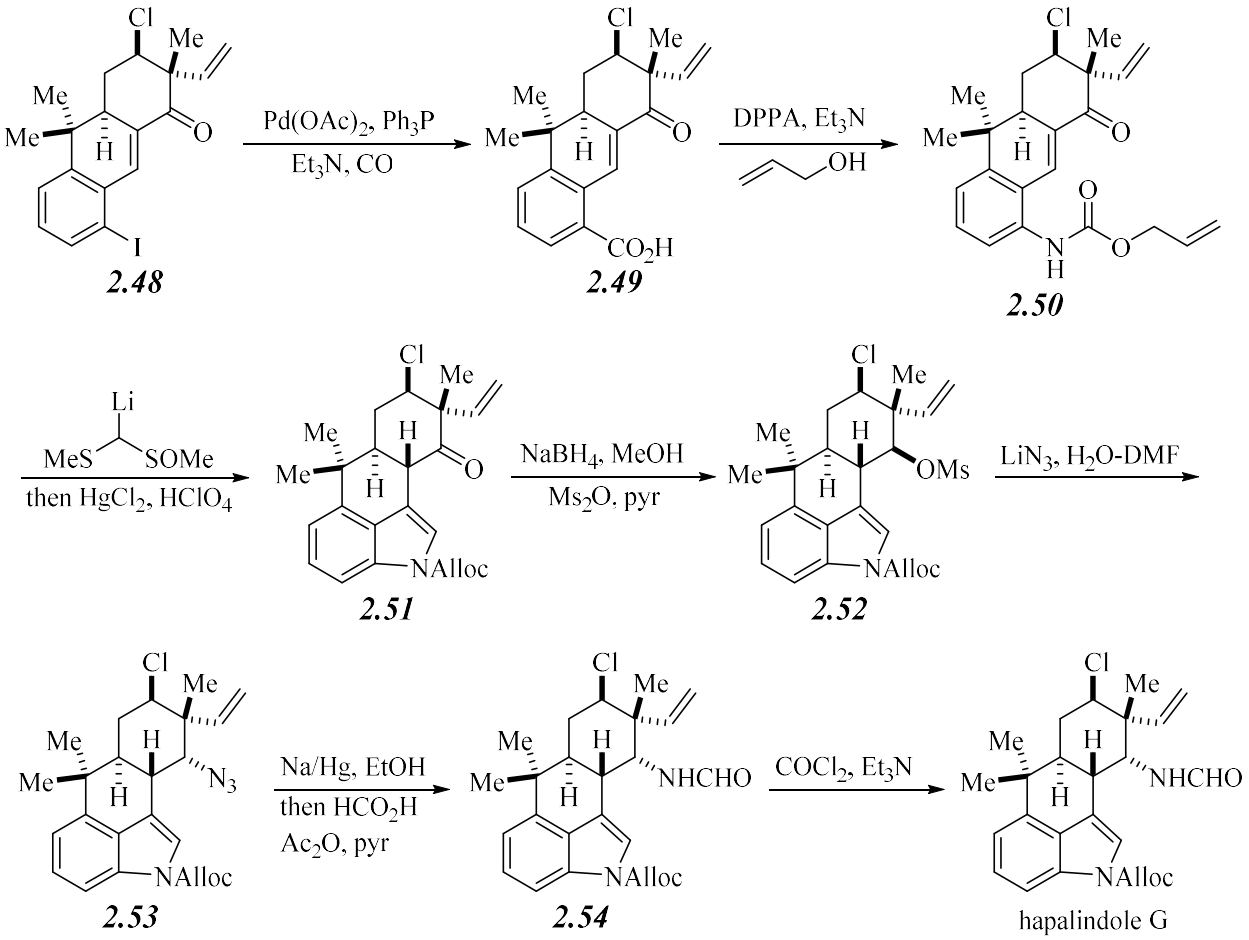
Shortly after this victory Fukuyama published on the synthesis of (-)-hapalindole G (Scheme **2.8** and **2.9**).[35] (-)-*trans*-carveol **2.40** was synthesized from (-)-carvone, followed by acyl substitution on methyl (chloroformyl)acetate and diazo transfer to compound **2.41**. Cu(II) assisted cyclopropanation on the diazo gave **2.42** which underwent a Krapcho-like decarbomethyoxylation to give the C13 chlorination. Enolate attack to CBr_4, reduction of the lactone and elimination of the resulting halohydrin gave a completed cyclohexanone **2.46** for the hapalindole skeleton. Attack into *o*-iodo benzaldehyde and dehydration gave aryl iodide **2.46.**

Aryl iodide **2.48** underwent Pd-catalyzed coupling to carbon monoxide to make carboxylic acid **2.49** in 80%. Schmidt-like rearrangement with allyl alcohol gave **2.50** which underwent conjugate addition and oxidation to complete the tetracyclic skeleton in **2.51**. Reduction,

mesylation, deprotection and substitution to the azide gave **2.53**, similar to structures made previously. Reduction of the azide in a sodium amalgam followed by familiar amidation and dehydration gave enantiopure (-)-hapalindole G.



Scheme 2.8. Fukuyama's work toward the synthesis of hapalindole G.

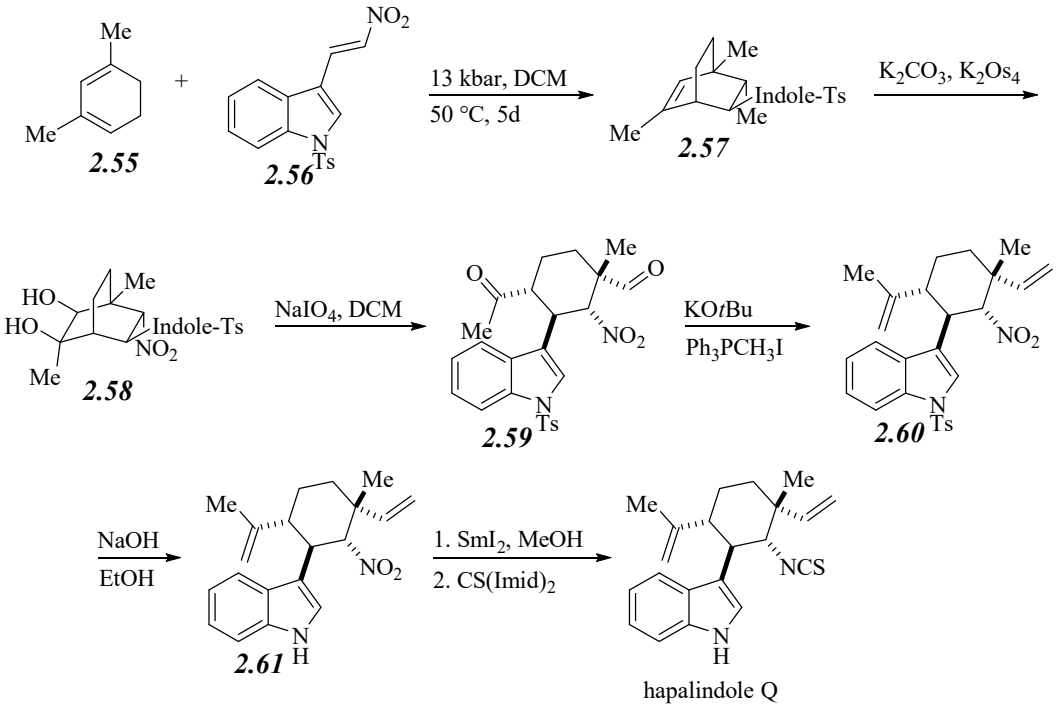


Scheme 2.9. Fukuyama's synthesis of hapalindole G.

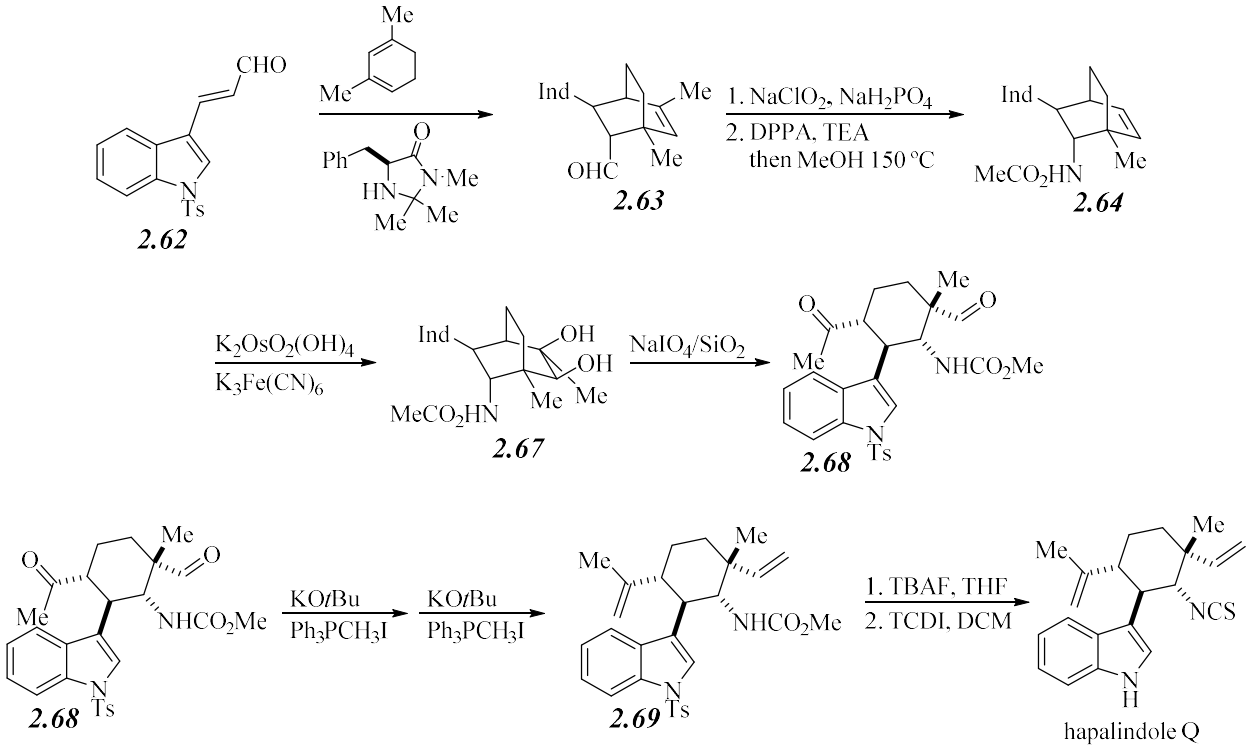
2.4. Synthesis of Hapalindole Q

In 2001 Kinsman published on a new approach to the hapalindoles; a Diels-Alder cyclization to form the tricycle (**Scheme 2.10**).[45] Tosyl-protected 3-(2-nitroethenyl)indole reacted with diene **2.55** to give racemic tricycle **2.57** in 77% yield and 80% diastereomeric excess. Sharpless dihydroxylation gave diol **2.58** which cleaved in better than 95% yield to give intermediate **2.59**. Now nearly complete, the carbonyls were methylenated, and the indole deprotected to give **2.61**. Reduction of the nitro to the amine in SmI_2 and thioacyl substitution gave racemic hapalindole Q in another unique approach to the hapalindole skeleton.

By modifying the Diels-Alder to be enantioselective they achieved the synthesis of (+)-hapalindole Q (**Scheme 2.11**).[46] Using *trans*-aldehyde **2.62** in an organocatalyst-mediated Diels-Alder, bridged bicycle **2.67** was made enantioselectively. Dihydroxylation and $NaIO_4$ oxidation gave dicarbonyl **2.68**, which was methylenated to **2.69**. Vaillancourt's method to convert to an isothiocyanate gave (+)-hapalindole Q in overall 1.7% yield.



Scheme 2.10. Kinsman's synthesis of racemic hapalindole Q

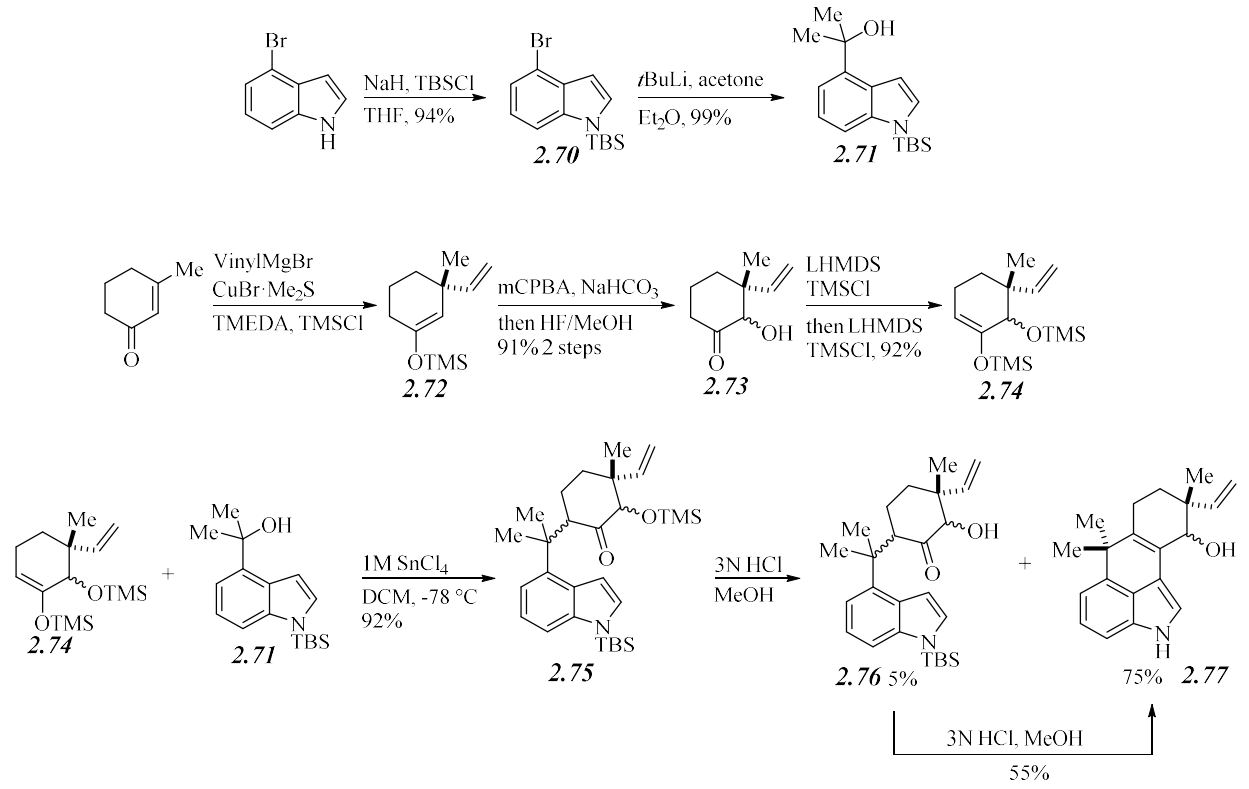


Scheme 2.11. Kinsman's enantioselective synthesis of hapalindole Q.

2.5. Syntheses of Hapalindoles (±) J, (±) U, and (±) O.

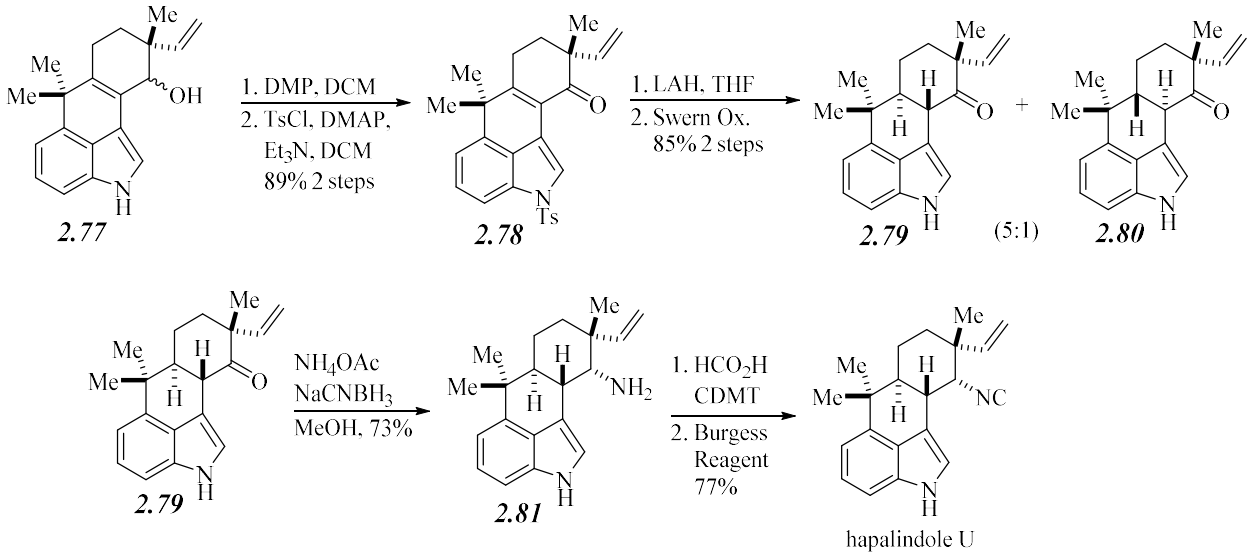
In 2011 Rafferty published on the total syntheses of hapalindole J and U from our laboratory (**Schemes 2.12**–**2.14**).[32] The carbon disconnections were identical to Muratake, however the approach to C11 oxidation and C10–C15 reduction were different. Starting with conjugate addition of vinyl magnesium bromide to 3-methyl-2-cyclohexanone, followed by enolate trapping with trimethylsilyl chloride gave silyl enol ether **2.72**. Rubottom oxidation gave alpha-hydroxy ketone **2.73** in 91% yield over two steps. Treatment with LHMDS and trapping the enolate gave silyl enol ether **2.74** in 92% yield. Coupling partner **2.71** was synthesized starting from 4-bromoindole. TBS protection gave **2.70** in 94% yield. This was followed by lithium-halogen exchange, and treatment with acetone, to give TBS-protected C7-functionalized indole **2.71**. Using similar tin-mediated coupling conditions by Muratake, **2.74** and **2.71** were coupled to

form an unseparated mixture of irrelevant diastereomeric tricycle **2.75**. Treatment with HCl in methanol gave condensation of the carbonyl to another irrelevant diastereomeric mixture; tetracyclic alcohol **2.77**, circumventing the allylic bromination and substitution used previously.



Scheme 2.12. Rafferty's work toward hapalindoles J and U.

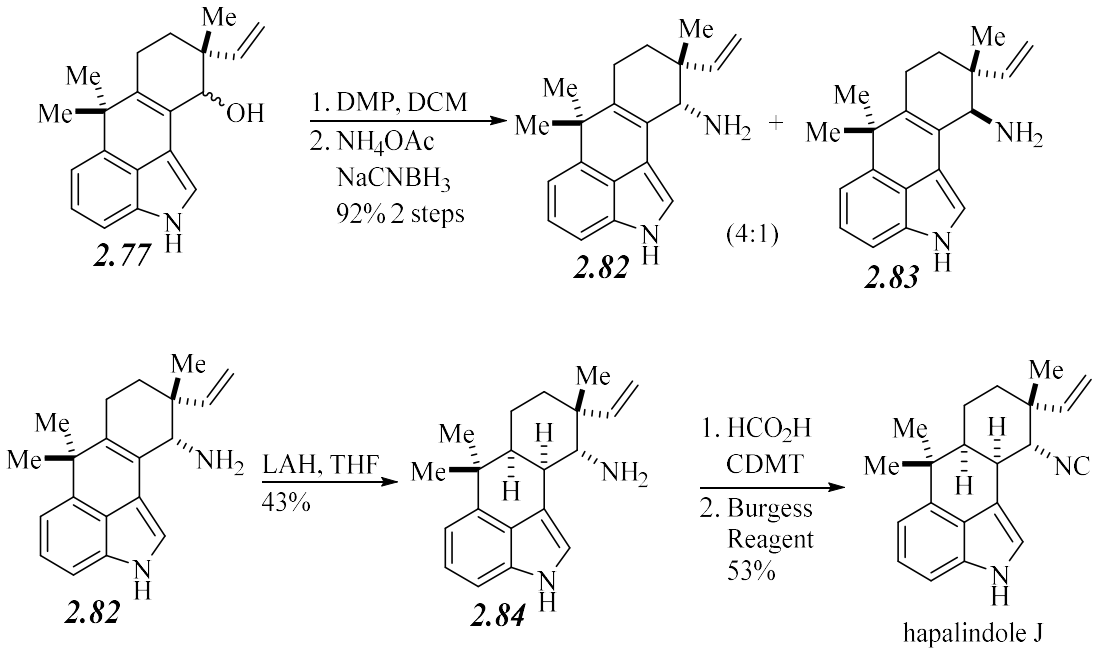
Dess-Martin periodinane oxidation of the diastereomer alcohol followed by indole protection gave tosyl **2.78** in 89% yield over 2 steps (**Scheme 2.13**). LAH reduction of the carbonyl (compare to reductions above) gave the *trans*-decalin connection without the need for C11-epimerization. Swern oxidation gave a 5:1 mixture of the diastereomeric *trans*-decalin rings in 85% yield over two steps. Reductive amination (73% yield) followed by amidation and dehydration using Burgess reagent gave racemic hapalindole U in 25% overall yield.



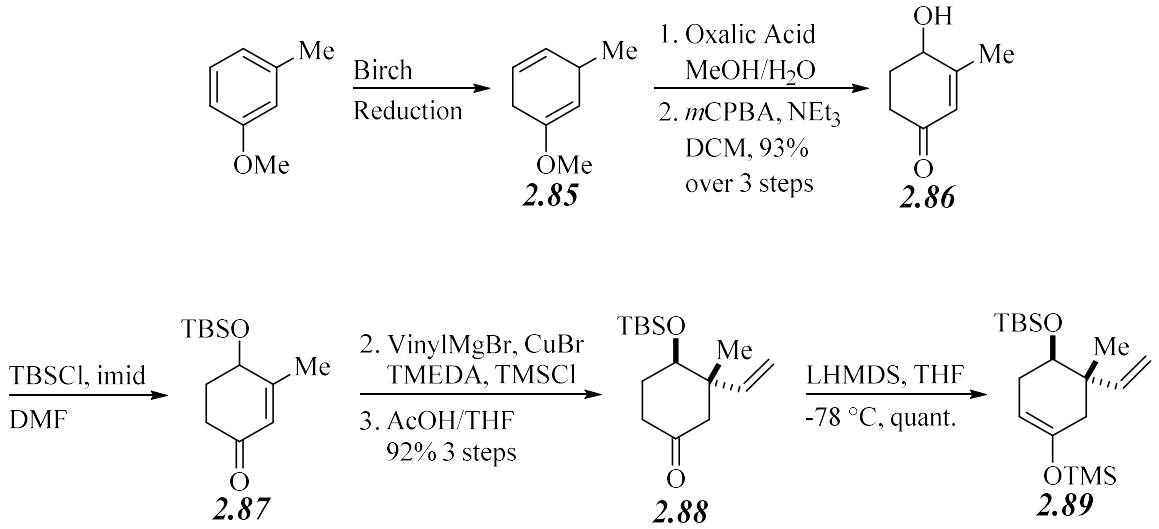
Scheme 2.13. Rafferty's synthesis of racemic hapalindole U.

Using the diastereomeric mixture of tetracycle alcohols (**2.77**) a 4:1 mixture of amines were made by Dess-Martin periodinane oxidation and reductive amination in 92% yield over 2 steps (**Scheme 2.14**). The desired amine **2.82** was treated with LAH, reducing the olefin as shown by Muratake, in moderate 43% yield. Amidation and dehydration gave racemic hapalindole J in an overall yield of 11%.

Rafferty also pursued the synthesis of racemic tetracycles with an oxidized C13 (**Schemes 2.15**–**2.16**). Following chemistry by Rubottom, 3-methylanisole underwent birch reduction and then oxidation to oxidized enone **2.86** in 93% yield over three steps. TBS protection and conjugate addition gave **2.88** in 92% yield over 3 more steps. Silyl enol ether synthesis gave the coupling partner for a C13 oxidized hapalindole; **2.89** quantitatively



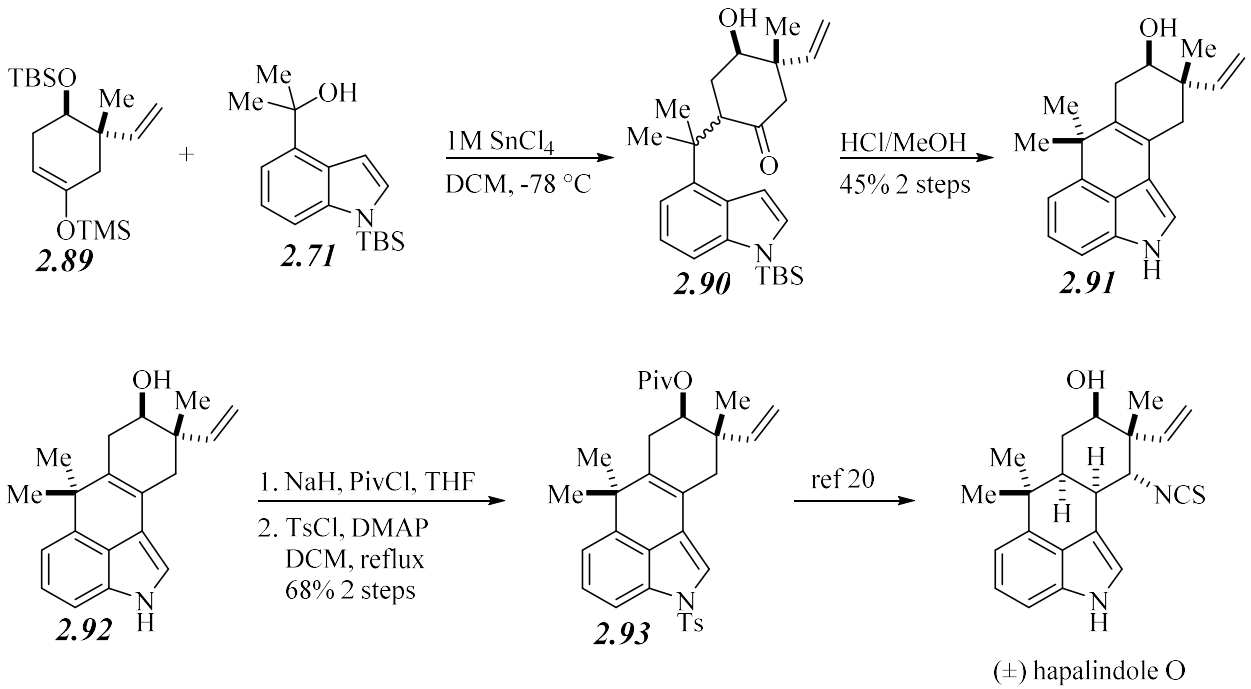
Scheme 2.14. Rafferty's synthesis of racemic hapalindole J.



Scheme 2.15. Rafferty's synthesis of racemic coupling partner **2.86.**

Coupling **2.89** with **2.71** using the $SnCl_4$ protocol gave tricycle **2.90**, which was cyclized as previously done to tetracycle **2.91** in 45 % yield over two steps. Double protection gave

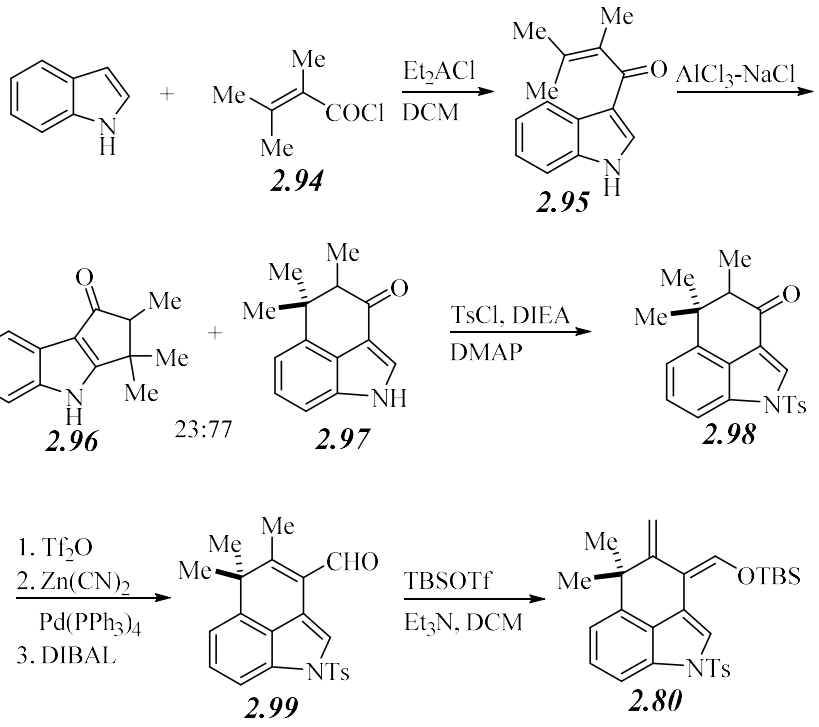
intermediate **2.93** which intercepts the total synthesis of hapalindole O by Muratake, which completed the formal synthesis.



Scheme 2.16. Rafferty's synthesis of racemic hapalindole O.

2.6. Synthesis of Hapalindoles K, A, and G.

Chandra pursued the synthesis of the C13 chlorinated tetracycles hapalindoles K, A and G (**Schemes 2.17–.20**).[47] Starting nucleophilic acyl substitution from C3 of indole and Friedel-Crafts type cyclization tricycle **2.97** was obtained in 70% yield. As a byproduct of the Friedel-Crafts reaction was the Fischerindole skeleton **2.96**, which was obtained in 23% yield. Pd catalyzed coupling of nitrile to a triflate intermediate gave a product that was reduced and hydrolyzed to unsaturated aldehyde **2.99**. Trapping the diene-silyl enol ether gave **2.100** in preparation for a Diels-Alder cyclization.

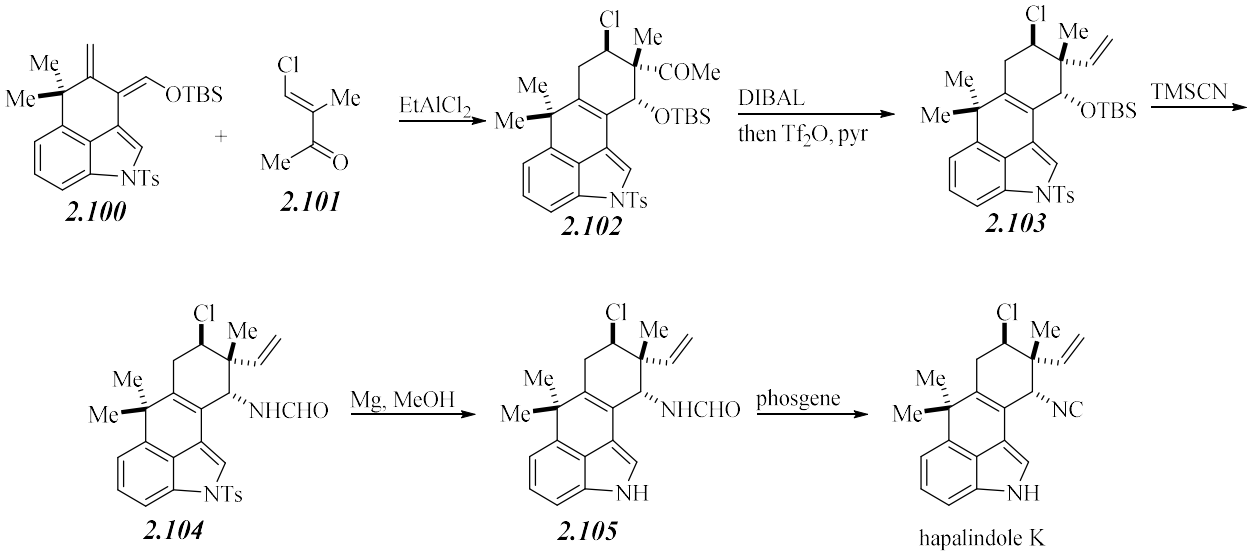


Scheme 2.17. Chandra's work toward a synthesis of hapalindole K.

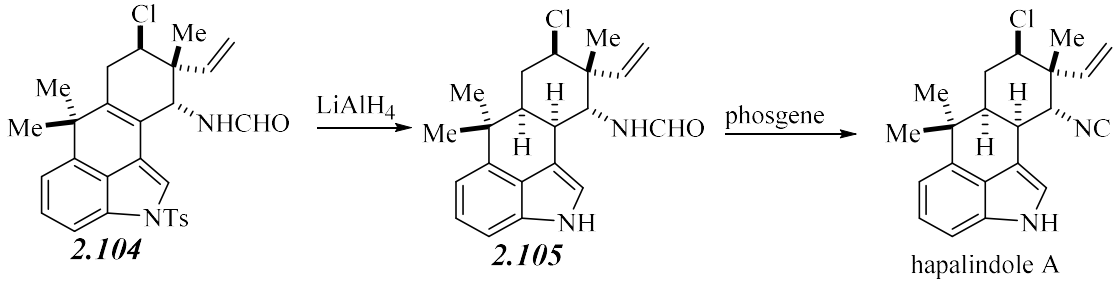
2.100 underwent Diels-Alder cyclization with chlorinated alkene **2.101** gave the tetracyclic core (**Scheme 2.18**). Reduction of the ketone followed by dehydration and deprotection gave the oxidative equivalent of the hapalindole family. Previous methods to convert the alcohol to an amine were unsuccessful and lead only to degradation. A Ritter reaction, however, gave 23:1 selectivity at C11 to give **2.104**. Deprotection and dehydration gave enantiopure hapalindole K.

Reduction of the C10–C15 olefin in **2.104** using chemistry similar to Muratake, followed by dehydration gave hapalindole A (Scheme **2.19**).

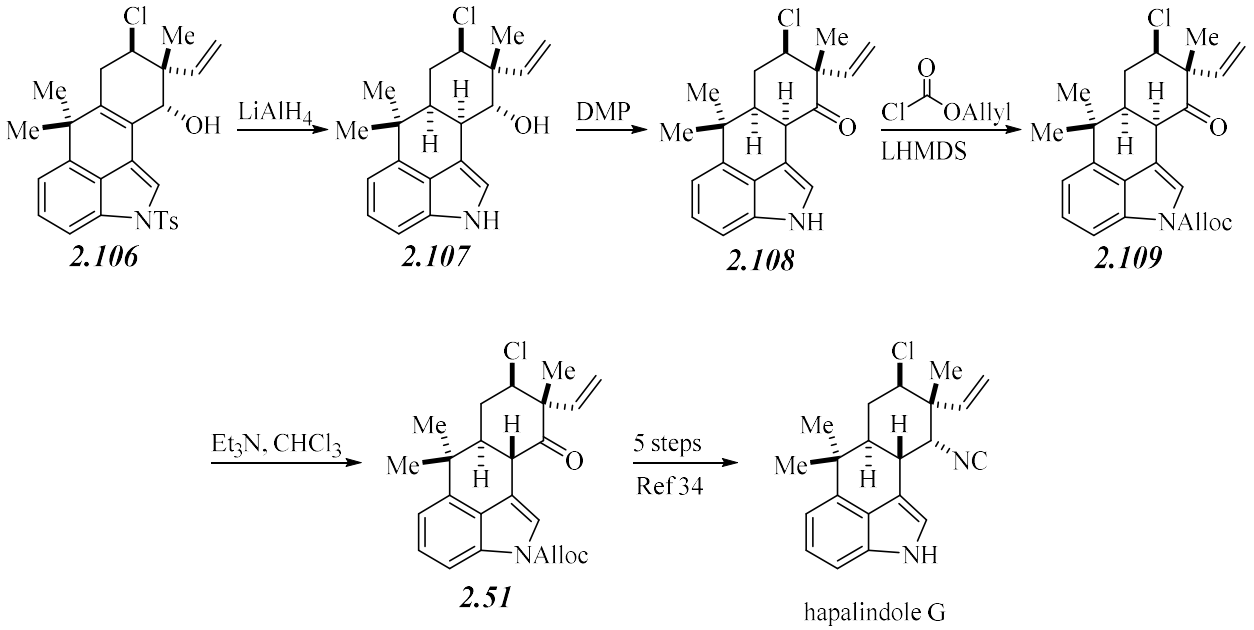
Reduction of the olefin in alcohol **2.106** followed by oxidation of the alcohol gave carbonyl **2.108** (**Scheme 2.20**). Alloc protection and epimerization of C10 intercepted **2.51** from Fukuyama[35] completing a formal synthesis of hapalindole G.



Scheme 2.18. Chandra's synthesis of hapalindole K.



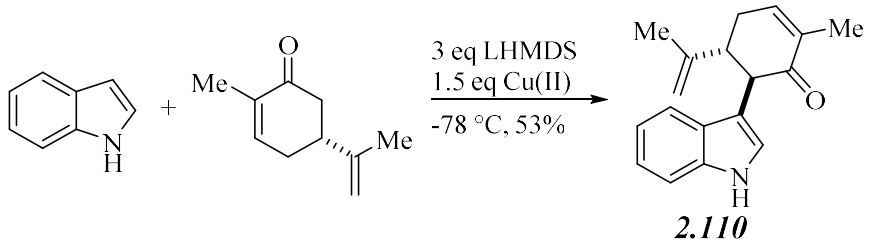
Scheme 2.19. Chandra's synthesis of hapalindole A.



Scheme 2.20. Chandra's synthesis of hapalindole G.

2.7. Synthesis of Hapalindoles Q, U, and Ambiguine H

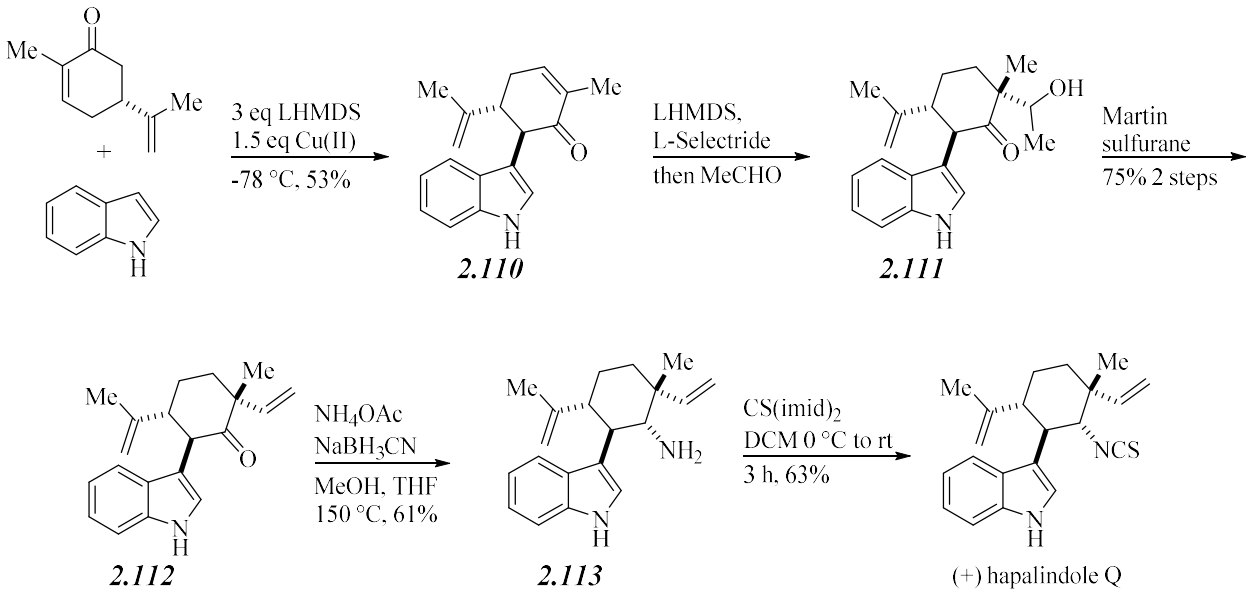
Richter began his work in the synthesis of hapalindoles by developing a novel coupling between indole and carvone to make tricycle **2.110** (**Scheme 2.21**).[48]



Scheme 2.21. Novel copper mediated coupling of carvone and indole.

Using this new coupling they pursued the total synthesis of a number of members of the hapalindole/welwitindolinone/ambiguine super family beginning with an enantioselective

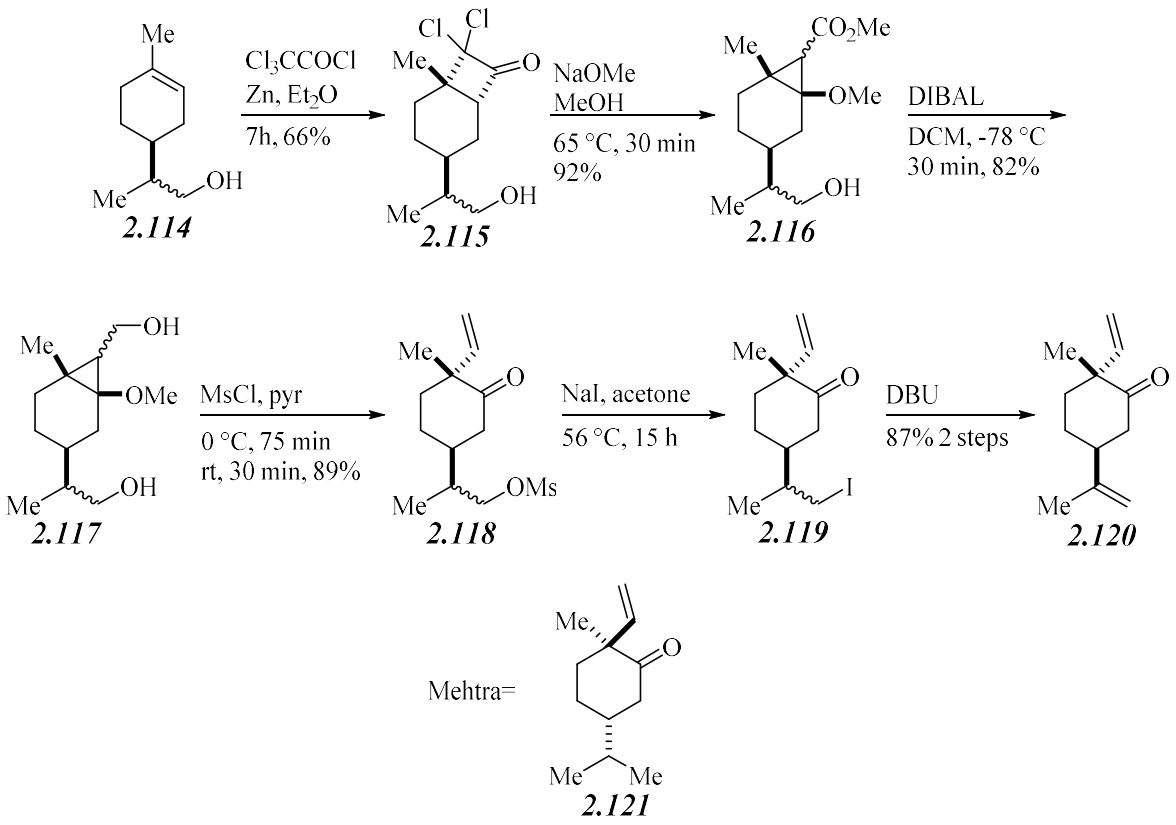
synthesis of (+)-hapalindole Q (**Scheme 2.22**).[49] After coupling indole and carvone, enone **2.110** underwent reduction and treatment with acetaldehyde to make tricycle **2.111**. Martin sulfurane dehydration to **2.112** was accomplished in 75% yield over two steps. Reductive amination as shown previously gave amine **2.113** in a 6:1 dr. Double thioacyl substitution gave (+)-hapalindole Q in 38% yield over two steps, 22% overall.



Scheme 2.22. Richter's synthesis of enantiopure hapalindole Q.

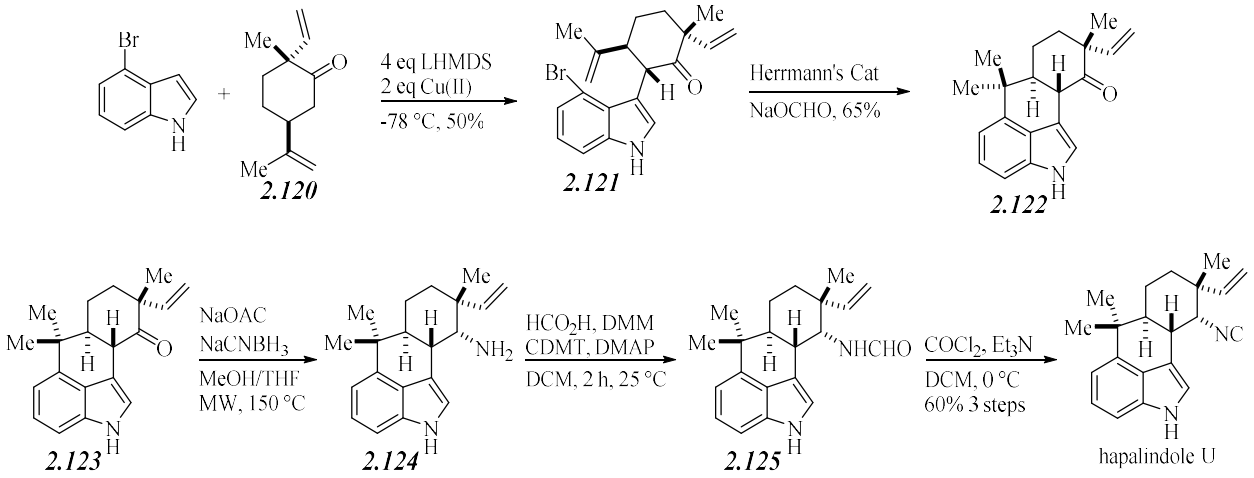
Using a modification on the work on terpenes by Mehtra[50], Richter synthesized an enantiopure coupling partner for indole appropriate for hapalindole U (**Scheme 2.23**). Starting from alcohol **2.114** (commercially available at the time), cyclobutanone **2.115** was synthesized in 66% yield by careful manual control of the temperature by constant addition of ice to the sonication bath. Treatment of the cyclobutanone with sodium methoxide in methanol gave cyclopropane **2.116** in 92% yield. DIBAL reduction of the ester gives diol **2.117** in 82% yield, which was mesylated leading to elimination/ring opening to cyclohexanone **2.118** in 89% yield. Refluxing

sodium iodide in acetone gives alkyl iodide **2.119**, which was treated with DBU without purification to give coupling partner **2.120** in 87% over two steps.



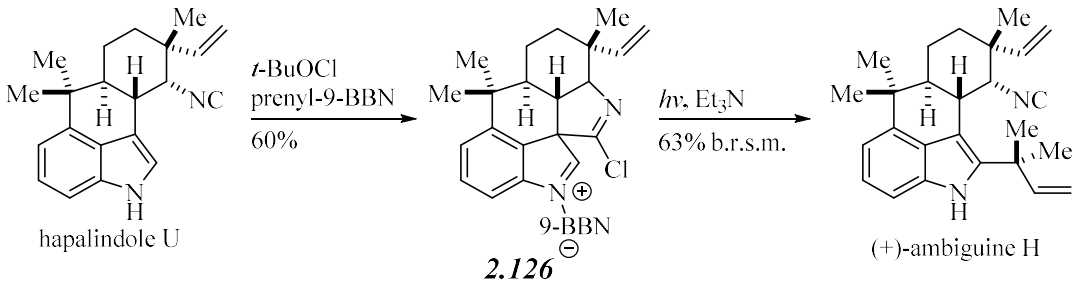
Scheme 2.23. Richter's modification of work by Mehtra toward coupling partner **2.119**.

Coupling the freshly made **2.120** to 4-bromoindole under similar conditions as before yielded C4-bromo substituted tricycle **2.121** in moderate 50% yield (**Scheme 2.24**).[51] After screening several catalysts Richter settled on Herrmann's catalyst to cyclize to the enantiopure tetracycle in 65% yield. Reductive amination under microwave conditions followed by coupling to formamide gave amide **2.125** as a single diastereomer. Dehydration using phosgene gave enantiopure (-)-hapalindole U in 60% yield over three transformations.



Scheme 2.24. Richter's synthesis of enantiopure hapalindole U.

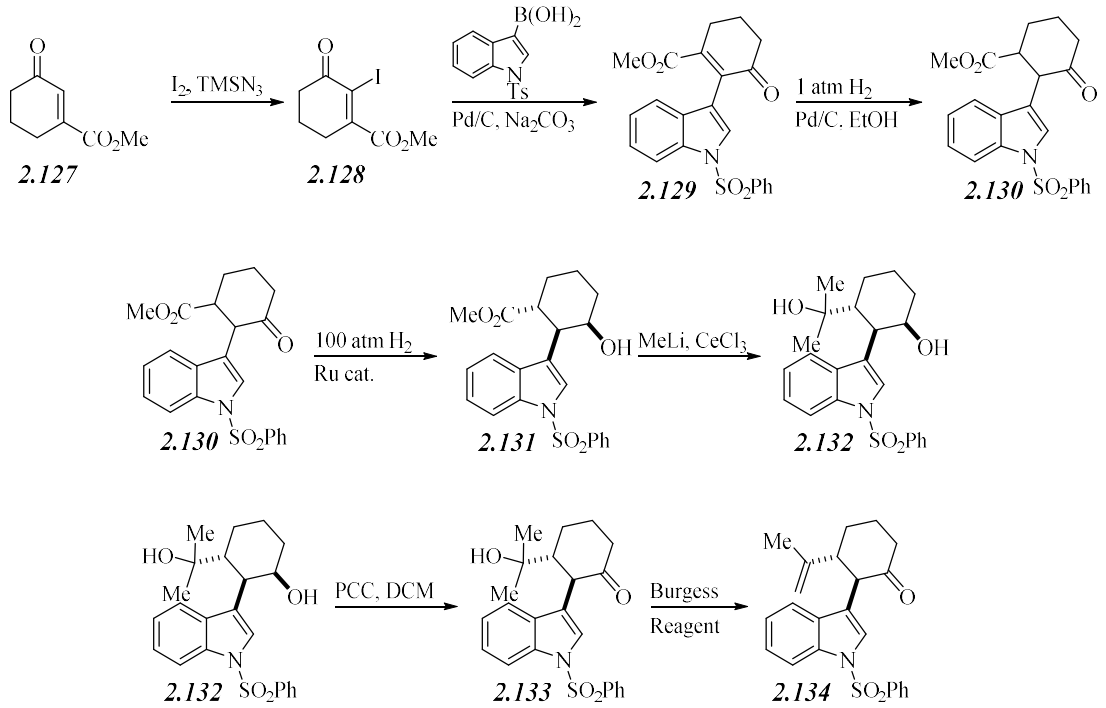
Continuing their work, hapalindole U was treated with *t*-BuOCl and prenyl-9-BBN giving an unusual chlorinated imine **2.124.** Photolytic cleavage gave (+)-ambiguine H in 63% yield based on recovered starting material.



Scheme 2.25. Richter's synthesis of racemic ambiguine H.

In 2016 Liu et al. published on the synthesis of four hapalindole structures including (+)-hapalindole Q and the unnatural enantiomer of hapalindole D (**Scheme 2.26–2.27**).[42] In a somewhat lengthy linear sequence the cyclohexyl ring was partially constructed, coupled by

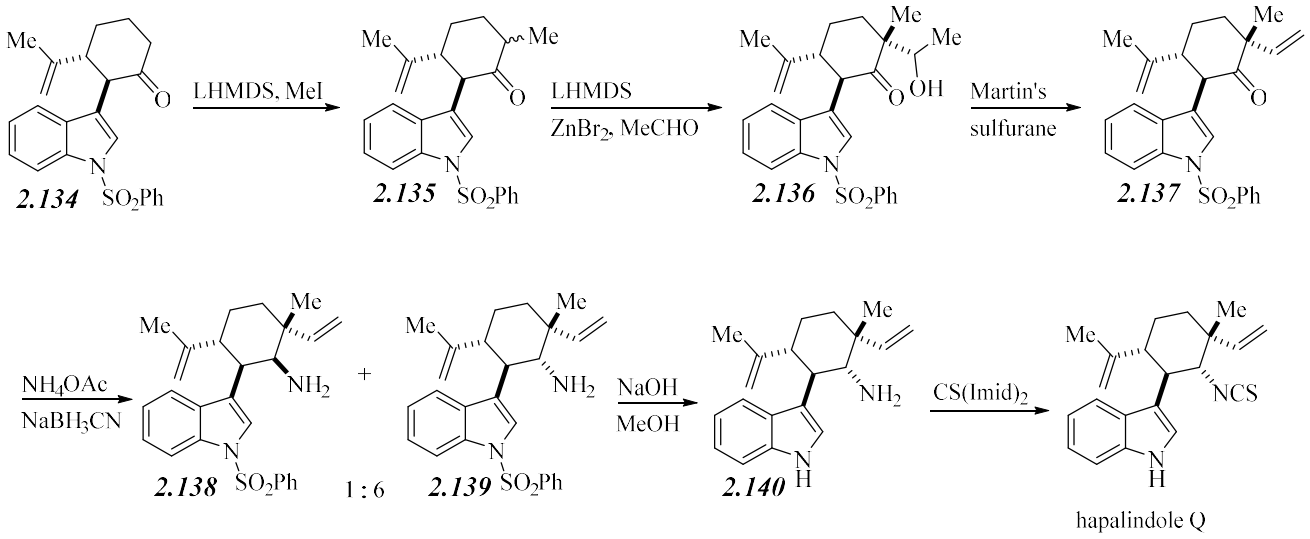
Suzuki coupling, and completed to a protected version of the common tricyclic cyclohexanone intermediate **2.134**.



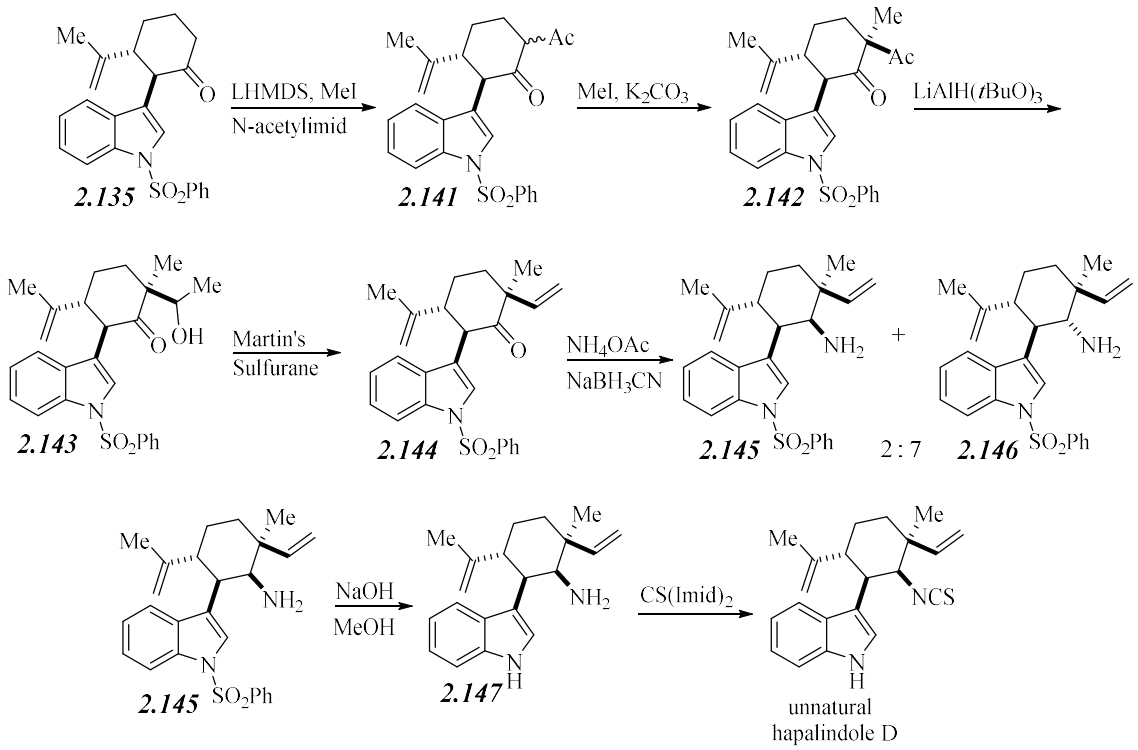
Scheme 2.26. Liu's work toward a synthesis of enantiopure hapalindole Q.

α-Alkalation gave **2.135** as a diastereomeric mixture, followed by attack into acetaldehyde giving **2.136** (**Scheme 2.27**). Dehydration gave **2.137** followed by reductive amination giving the desired diastereomer in a 6:1 mixture. Deprotection and thioacyl substitution gave (+)-hapalindole Q in 13 steps.

Installing the methyl and vinyl groups in reverse order gave tricycle **2.144**, which under reductive amination conditions gave a 7:2 mixture disfavoring the desired product. Following the same sequence as before gave the unnatural enantiomer of hapalindole D in 14 steps.



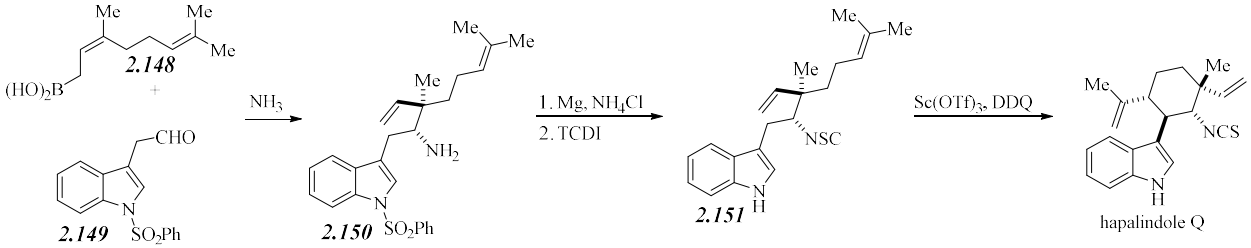
Scheme 2.27. Liu's synthesis of enantiopure hapalindole Q.



Scheme 2.28. Liu's synthesis of unnatural hapalindole D as a minor diasteromer.

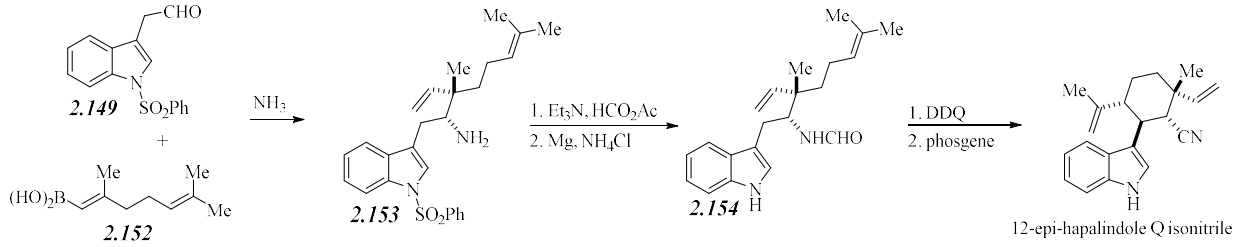
2.8. Synthesis of Hapalindoles Q, H, and 12-epi-hapalindole Q isonitrile

In an intriguing novel approach to the hapalindole family, Lu et al pursued a route that much more closely resembles the biosynthesis that we have elucidated (**Scheme 2.29**). Combining a geranyl boronic acid to aldehyde **2.149** with ammonia under a mannich-type reaction. Deprotection and isothiocyanate formation gives **2.151**. Oxidative cyclization gave hapalindole Q in a short, novel, intriguing approach to the hapalindole family.



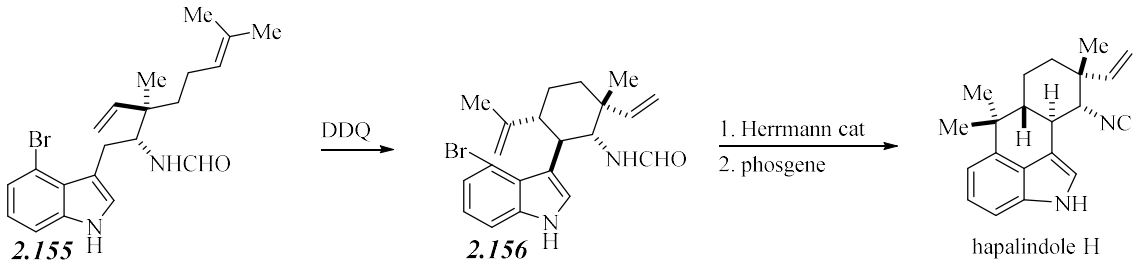
Scheme 2.29. Lu's synthesis of racemic hapalindole Q.

Continuing along a similar vein, the *trans*-geranyl boronic acid gave amine **2.153**, which underwent amidation and deprotection in preparation for oxidative cyclizaion. Treatment with DDQ and phosgene gave 12-*epi*-hapalindole Q isonitrile.



Scheme 2.30. Lu's synthesis of racemic 12-*epi*-hapalindole Q isonitrile.

Preparing the C4-brominated geranyl indole allowed similar cyclization to tricycle **2.154.** Using chemistry established by Richter allowed successful completion of the synthesis of racemic hapalindole H.



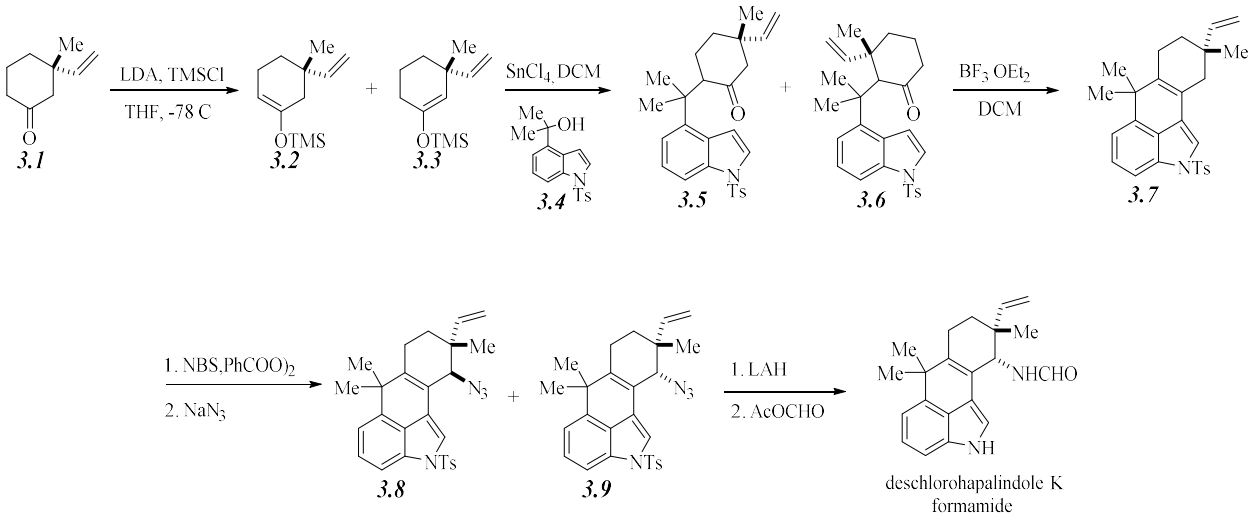
Scheme 2.31. Lu's synthesis of racemic hapalindole H.

With the state of the field as described there were no answers about the biosynthesis of this family of products. Significant progress had been made on the synthesis of these molecules, however some stereochemical combinations had yet to be accessed. Our goals in continuing this field at this point were to explore the biogenesis of the hapalindoles as well as contribute to the synthetic access to these complex molecules.

Chapter 3. Results and Discussion

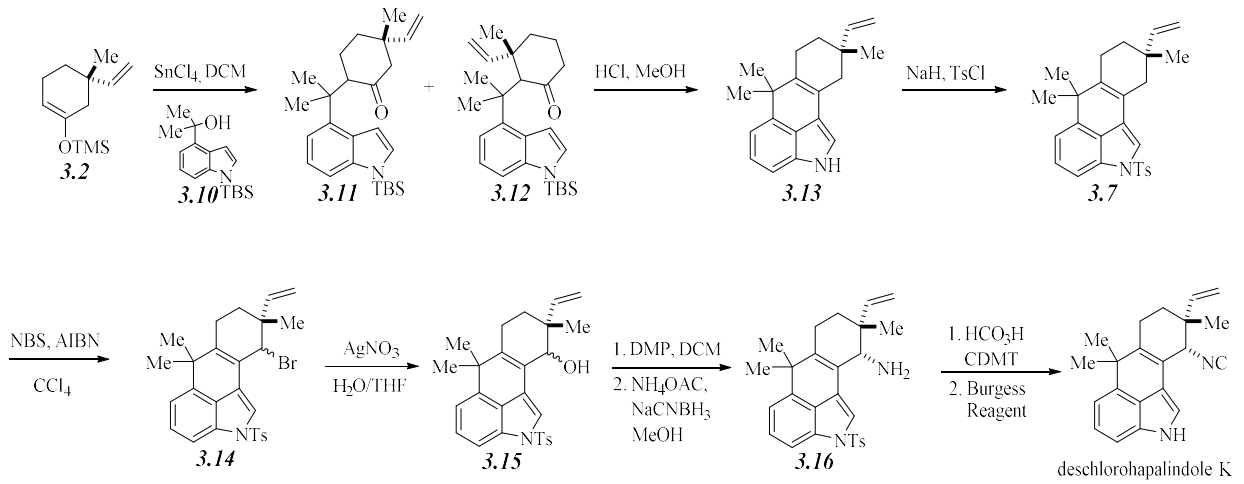
3.1. Synthetic efforts toward deschlorohapalindole K

Our first synthetic target to investigate the biosynthesis was deschlorohapalindole K. The synthesis of deschlorohapalindole K was previously accomplished in our lab by Dr. Ryan Rafferty. This was done similar chemistry to that pioneered by Muratake in the 1990 racemic total syntheses of Hapalindoles J and M.[31] In this publication deschlorohapalindole K formamide was made as an unwanted byproduct in the synthesis of hapalindole J (**Scheme 3.1**). Coupling and cyclization of the appropriate cyclohexanone derived silyl enol ether **3.3** with tertiary alcohol indole fragment **3.4** provided the core of hapalindole K. NBS allylic bromination provided a handle on C-11 to install an azide. LAH reduction lead to deprotection of the tosyl group, reduction of the azide to amine, and a mixture of products resulting from reduction or preservation of the tetrasubstituted alkene. Amidation gave the precursor to hapalindole K.



Scheme 3.1. Muratake synthesis of deschlorohapalindole K formamide

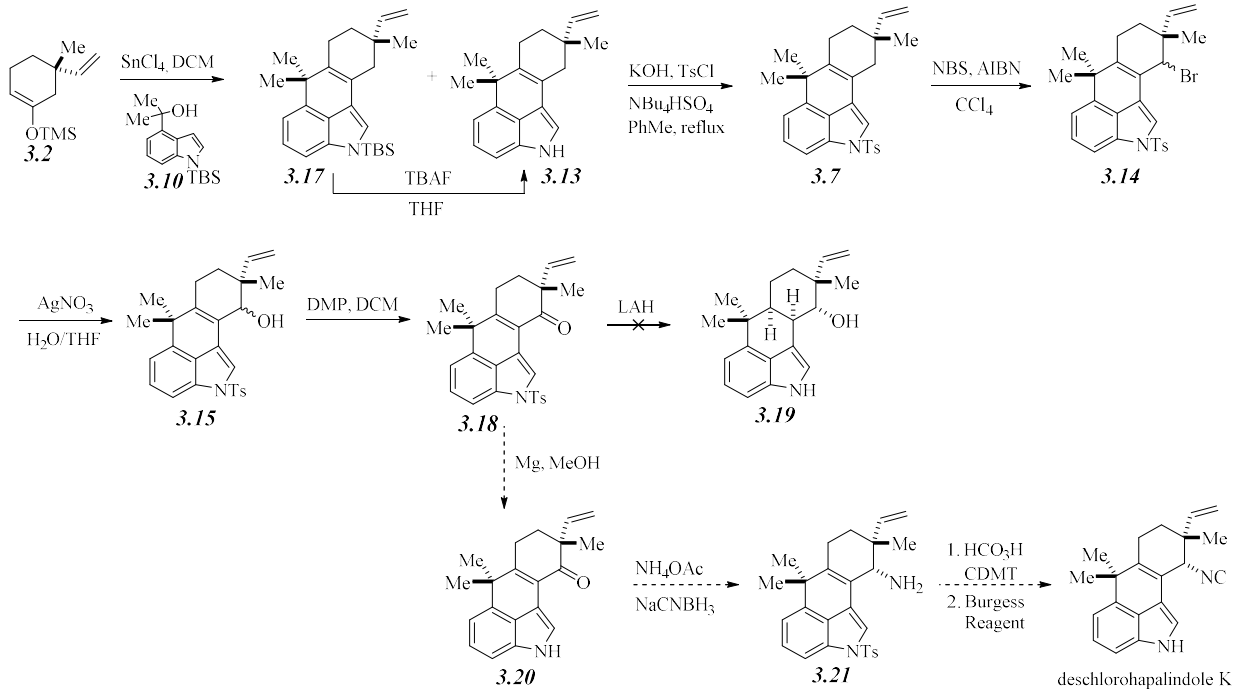
The chemistry that Dr. Ryan Rafferty performed in our lab varied from Mutsaka's work in the end to provide a better yield of the desired deschlorohapalindole K (**Scheme 3.2**). Installation of a hydroxyl group on C-11 allowed for oxidation and stereocontrolled reductive amination to establish the desired stereochemistry. By using $NaCNBH_3$ he was able to avoid the reduction of the tetrasubstituted alkene. Conversion to the isonitrile using formic acid and dehydration successfully yielded the desired product. Authentic samples of deschlorohapalindole K were submitted for testing but appear to have been misplaced. To replace the sample, we began again on this synthesis.



Scheme 3.2. Unpublished efforts by Dr. Rafferty on the synthesis of deschlorohapalindole K.

Recently in our hands, however, some unexpected results have been obtained (**Scheme 3.3**). Among them the coupling using $SnCl_4$ yields a mixture of the protected and unprotected tetracycles (**3.17** and **3.13** respectively). No tricycle was recovered. Tosylation using Dr. Rafferty's conditions failed to protect the indole, though conditions were found to effect this transformation to **3.5.** Out of an interest in the substrate scope of enzymes used in the production

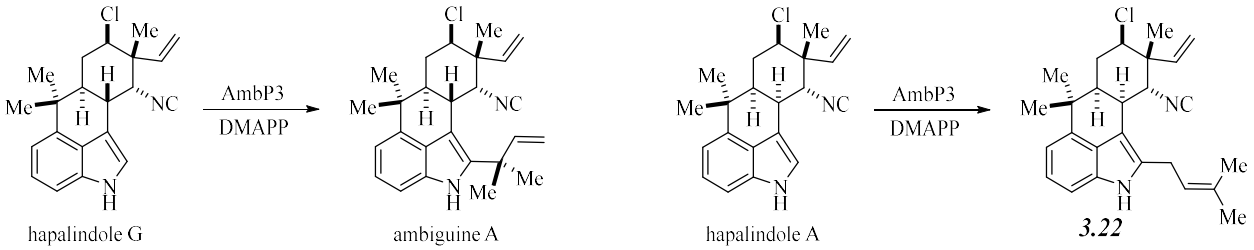
of the hapalindoles we attempted a detour to the C-11 alcohol. LAH reduction of the carbonyl and tetrasubstituted alkene should also cleave the tosyl protecting group. Unfortunately, a variety of conditions using LAH all failed to reduce anything other than the carbonyl. Triethylsilane, super hydride, and dissolving metal reduction conditions, among others, also failed.



Scheme 3.3. Recent efforts on the synthesis of deschlorohapalindole K and related hapalindole structure **3.19**.

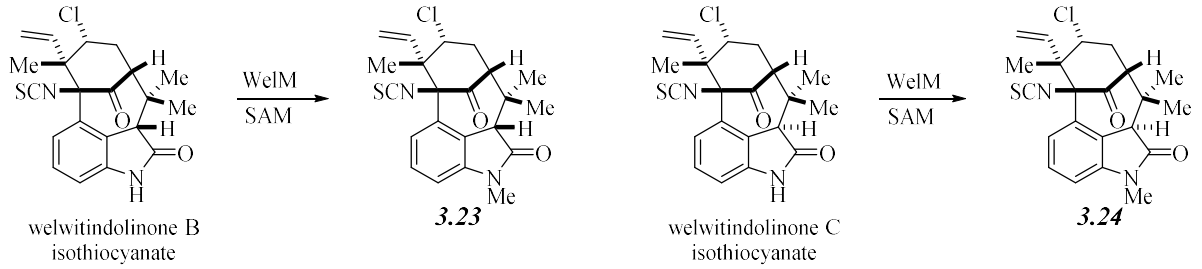
After significant attempts to reduce the C10-C15 olefin all met with failure we turned our attention to the deprotection of the indole ring. Again, multiple attempts failed to yield product; this time leading to decomposition of the starting material. At this point in our efforts Hillwig et al. revealed their first foray into the subject (**Scheme 3.4**).[52] They used genome mining on *Fischerella ambigua* UTEX 1903 to identify genes of interest. Genes for the synthesis of indole isonitrile were identified, along with prenyl transferases and oxygenases. The prenyl transferases

were overexpressed and tested *in vitro* for activity. AmbP3 was active for prenylation of hapalindole molecules to the ambiguine scaffold using dimethylallyl pyrophosphate (DMAPP).



Scheme 3.4. *In vitro* activity of overexpressed gene AmbP3 on hapalindoles A and G

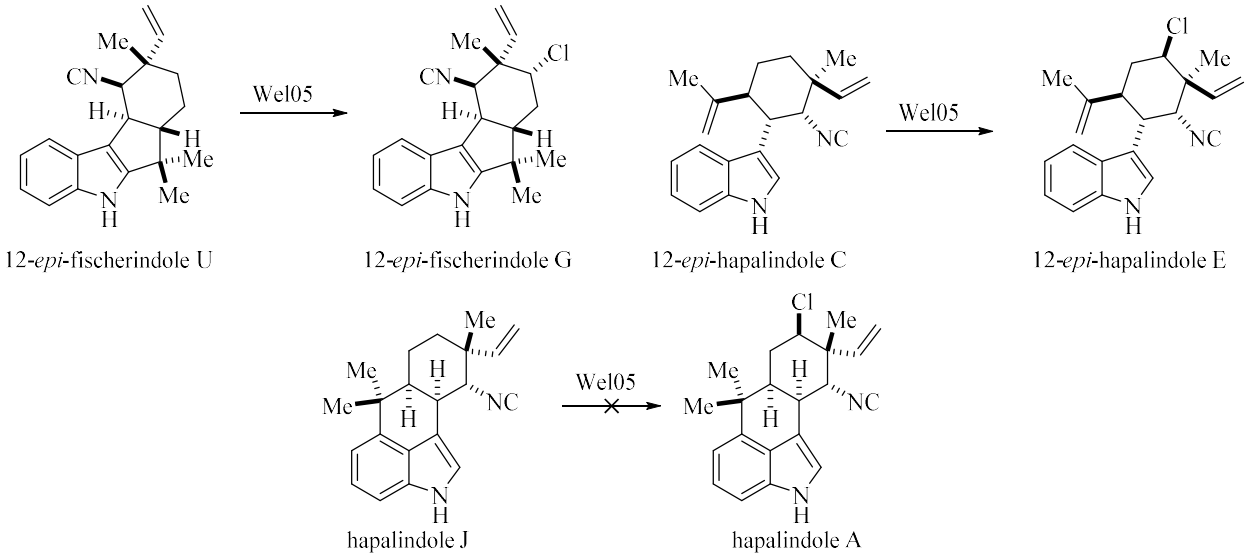
Another attempt made by the same group to study the biogenesis of closely related welwitindolinones (in *Hapalosiphon welwitschii* UTEX B1830) failed to give more definitive insight into the source of the main scaffold of this family, or the oxygenation events. A methyl transferase that acts on the indole nitrogen was identified, however (**Scheme 3.5**).[53]



Scheme 3.5. Identified S-adenosylmethionine (SAM) methyl transferases.

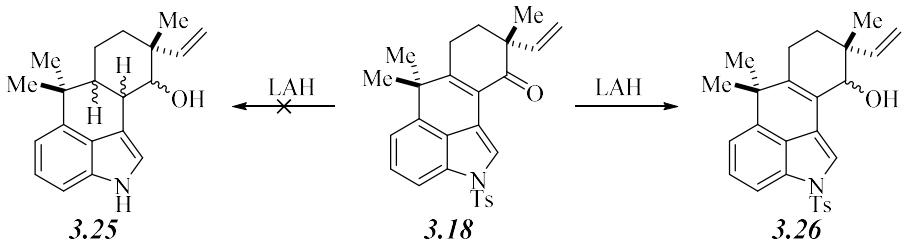
Shortly thereafter the oxygenases in *Hapalosiphon welwitschii* UTEX B1830 were tested for *in vitro* activity on 12-*epi*-fischerindole U, 12-*epi*-hapalindole C, and hapalindole J (**Scheme 3.6**).[54] The first two were natural substrates for the bacterium and produced the corresponding

alkaloids. Hapalindole J, however, is no produced by *Hapalosiphon welwitschii* UTEX B1830 and failed to give any hapalindole A. With the discovery of a halogenase enzyme that works on a hapalindole molecule we abandoned our efforts in this area.



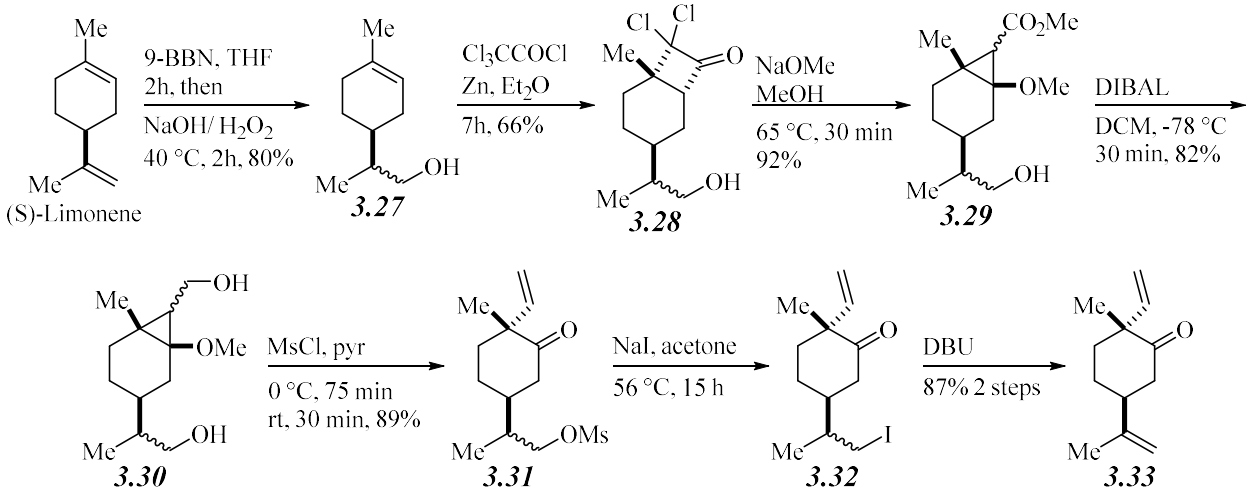
Scheme 3.6. Enzymatic halogenase activity on hapalindole-type alkaloids.

At this same time our collaborators in the Sherman group expressed enzymes for the synthesis of the core of the hapalindoles. As a positive control for the results of their enzymatic reactions we have undertook the synthesis of hapalindole U, which they believe they created through natural expression. The above chemistry is applicable to the synthesis of hapalindole however, unlike published examples, in our hands metal-hydride reducing agents failed to reduce the tosylate or C10-C15 olefin (**Scheme 3.7**). After several trials of fresh LAH from various sources and careful purification of the starting material we decided to use the chemistry pioneered by Richter to get enantiopure material (**Scheme 3.8**).



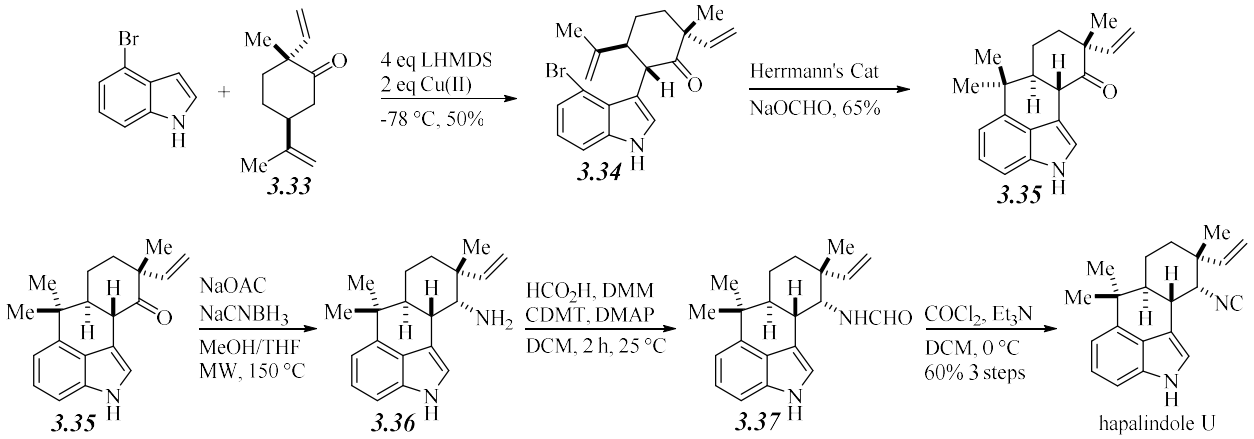
Scheme 3.7. Attempts toward hapalindole U.

Alcohol **3.27** was no longer commercially available, so we applied a previously published oxidation of (R)-limonene to (S)-limonene (**Scheme 3.8**).[55] We then applied the chemistry Richter modified to make enantiopure cyclohexanone **3.33** as described previously.



Scheme 3.8. Synthesis of enantiopure cyclohexanone **3.33** from published works.

Using the copper catalyzed coupling of the cyclohexanone to 4-bromoindole gave the expected tricycle **3.34**, which was cyclized, reduced and elaborated to hapalindole U as previously published.[51]



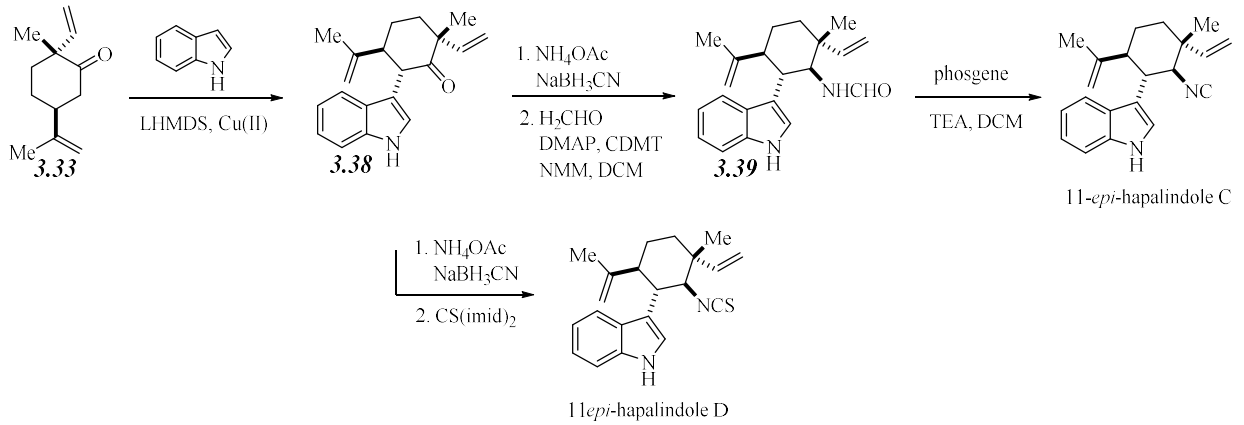
Scheme 3.9. Synthesis of hapalindole U as previously published.[51] Scheme recreated here for convenience.

3.2. Syntheses of Hapalindole D and C

As part of our investigations in the biosynthetic origins of the hapalindole family we wanted to test some potential tricyclic substrates that might lead to tetracyclic hapalindoles. To this end we pursued the synthesis of hapalindoles C and D as possible precursors to hapalindole U and hapalindole U isothiocyanate respectively, as well as synthetic targets that have eluded synthesis. Coupling of cyclohexanone **3.33** to indole proceeded in 50% yield to give tricycle **3.34.** Reductive amination gave the amine with only one detectable diastereomer. Amidation and dehydration unfortunately gave 11-*epi*-hapalindole C. Similarly, thioacylsubstitution gave 11-*epi*-hapalindole D.

Though not a natural substrate for the bacterium we are focusing on (*Fischerella ambigua* UTEX 1903), we submitted the structures for testing but to no effect. Whether the enzymes is selective based on the stereochemistry of the C-11 center, or tricycles are simply not involved in the biosynthesis of tetracyclic hapalindoles, remained a question. At this time, we reported in collaboration with the Sherman lab on recent discoveries in the biosynthesis of the hapalindole

core.[56] A genome mining comparison of *Fischerella ambigua* UTEX 1903 (*fam*) to the previously published gene cluster of *hapalosiphon welwitschii* UTEX B1830[53] (*amb*) revealed a nearly identical array of genes (**Figure 3.1**). The expected genes involved in the synthesis of tryptophan, indole isonitrile **3.40**, and isoprenoids DMAPP/GPP were present, along with prenyltransferases and oxygenases for elaboration, and a series of genes of initially unknown function (*fam* C1-C4).



Scheme 3.10. Synthesis of 11-*epi*-hapalindoles C and D.

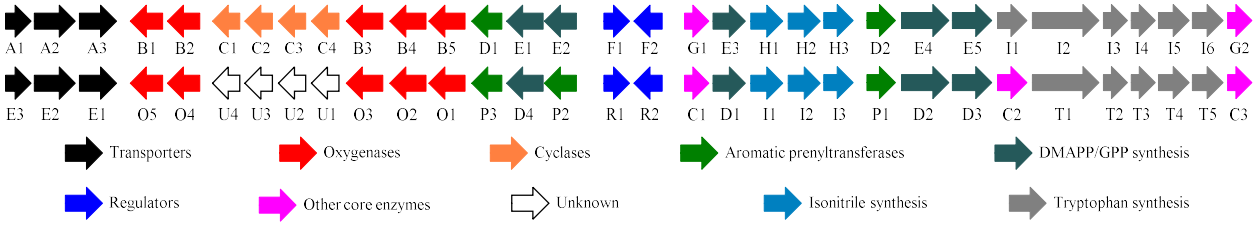
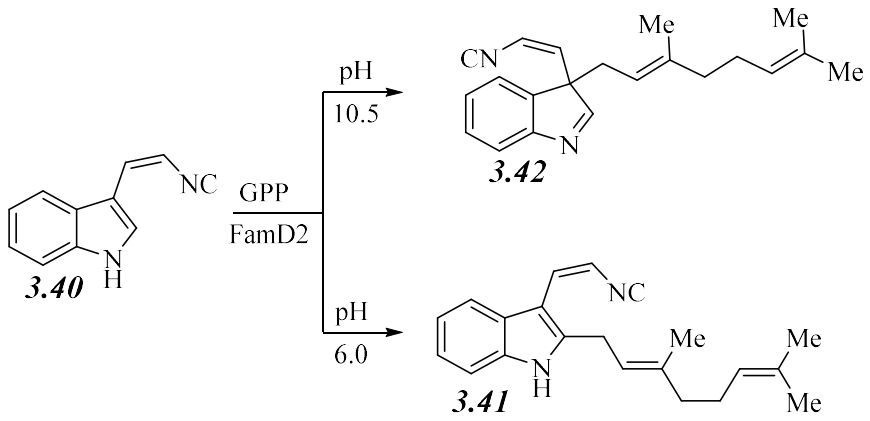


Figure 3.1. Comparison of the gene clusters in *Fischerella ambigua* UTEX 1903 (*fam*, top), and *hapalosiphon welwitschii* UTEX B1830[53] (*amb*, bottom).

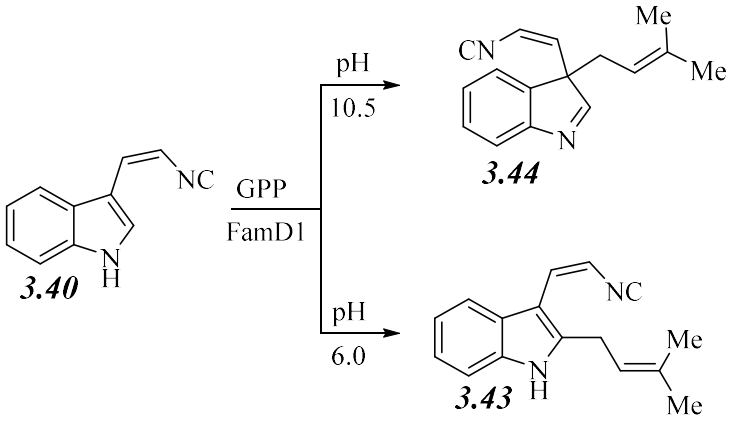
Isolation and overexpression of aromatic prenyltransferases FamD1 and FamD2 was successful allowing for *in vitro* investigations. Indole isonitrile **3.40** was accepted as a substrate by FamD2 with GPP to make C-2 and C-3 geranylated products **3.41** and **3.42** depending on pH (pH 4.0 lead to C-2 prenylation, pH 10.5 gave C-3 geranylation) (**Scheme 3.11**). DMAPP was

also accepted by the enzyme. This flexibility in substrate and pH dependent products is in contrast to the findings of Hillwig with *hapalosiphon welwitschii* UTEX B1830.[53]



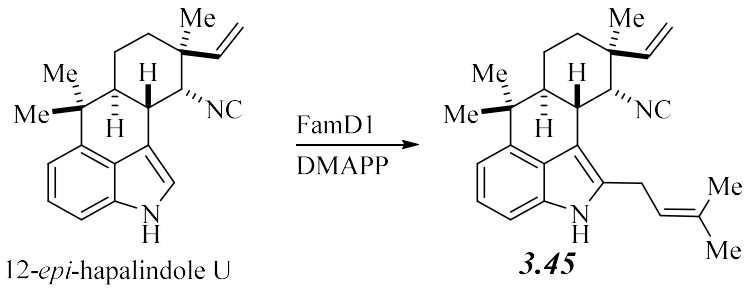
Scheme 3.11. Geranylation using FamD2.[56]

FamD1 showed similar activity with GPP in making C-2 geranylated indole isonitrile **3.40**, however less efficiently (**Scheme 3.12**). Furthermore, in a 1:1:1 mixture of indole isonitrile **3.40**, DMAPP and GPP the preferred substrate was DMAPP.



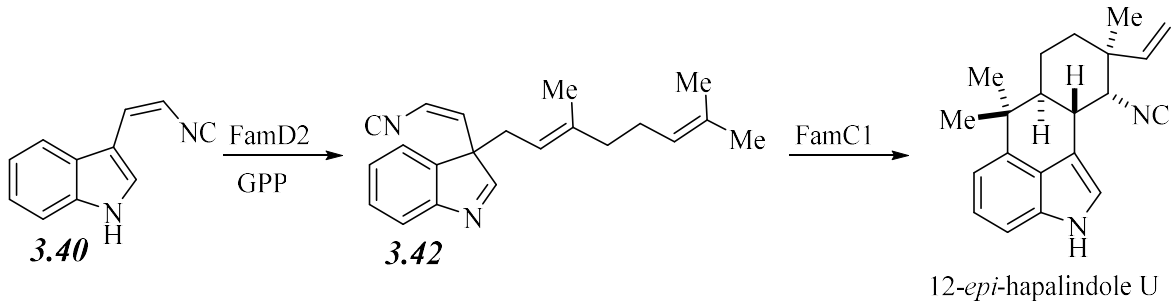
Scheme 3.12. Prenylation using FamD1.[56]

Only FamD1 was able to catalyze the prenylation of hapalindole U, similar to AmbP3 previously published (**Scheme 3.13**).[53]



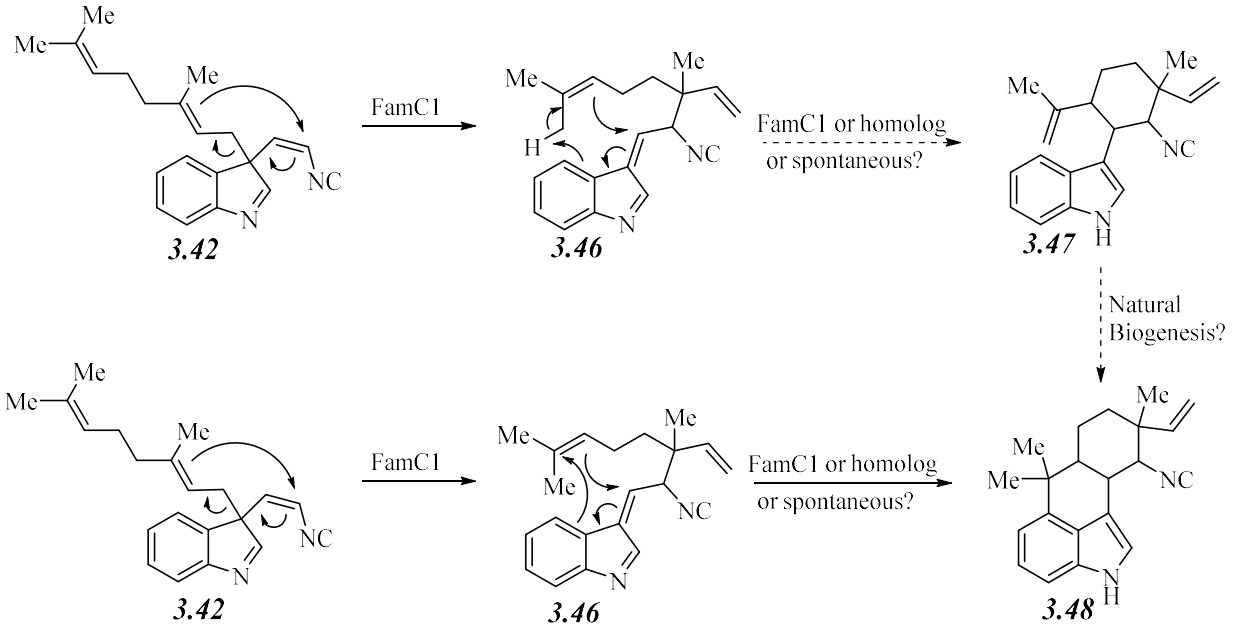
Scheme 3.13. Prenylation of 12-*epi*-hapalindole U.

These discoveries leave the questions of how the scaffold is cyclized. With the rest of the gene cluster identified it was likely FamC1-C4 contained the necessary cyclases to form the hapalindole scaffold from geranylated indole isonitrile **3.40**. Initial attempts to express FamC1 failed, leading to a cell-free lysate as the preferred method of investigation. In a cell free lysate geranylated indole isonitrile **3.40** cyclizes to 12-*epi*-hapalindole U. Purification of the active cyclase and analysis lead to the identification of FamC1 (**Scheme 3.14**).



Scheme 3.14. Biogenesis of 12-*epi*-hapalindole U.

This understanding leaves several questions unanswered (**Scheme 3.15**). Does FamC1 catalyzed a Cope rearrangement followed by a Diels-Alder cyclization/rearomatization to make the tetracyclic scaffold? Or is the second step spontaneous? After the Cope rearrangement does an ene reaction/rearomatization occur to the tricyclic scaffold followed by spontaneous or catalyzed electrophilic aromatic substitution to the tetracyclic scaffold? Or are the tricycles shunt metabolites instead of precursors to the tetracycles?



Scheme 3.15. Remaining questions on the biogenesis of the hapalindole scaffolds.

To answer these questions, we focused our efforts on the synthesis of natural hapalindoles C and D. We thus set out to change the selectivity of the reductive amination to access these tricyclic hapalindoles. An analysis of the previously published efforts to reduce the carbonyl of tricycle and tetracycle hapalindole precursors are compiled in **Figure 3.2**.

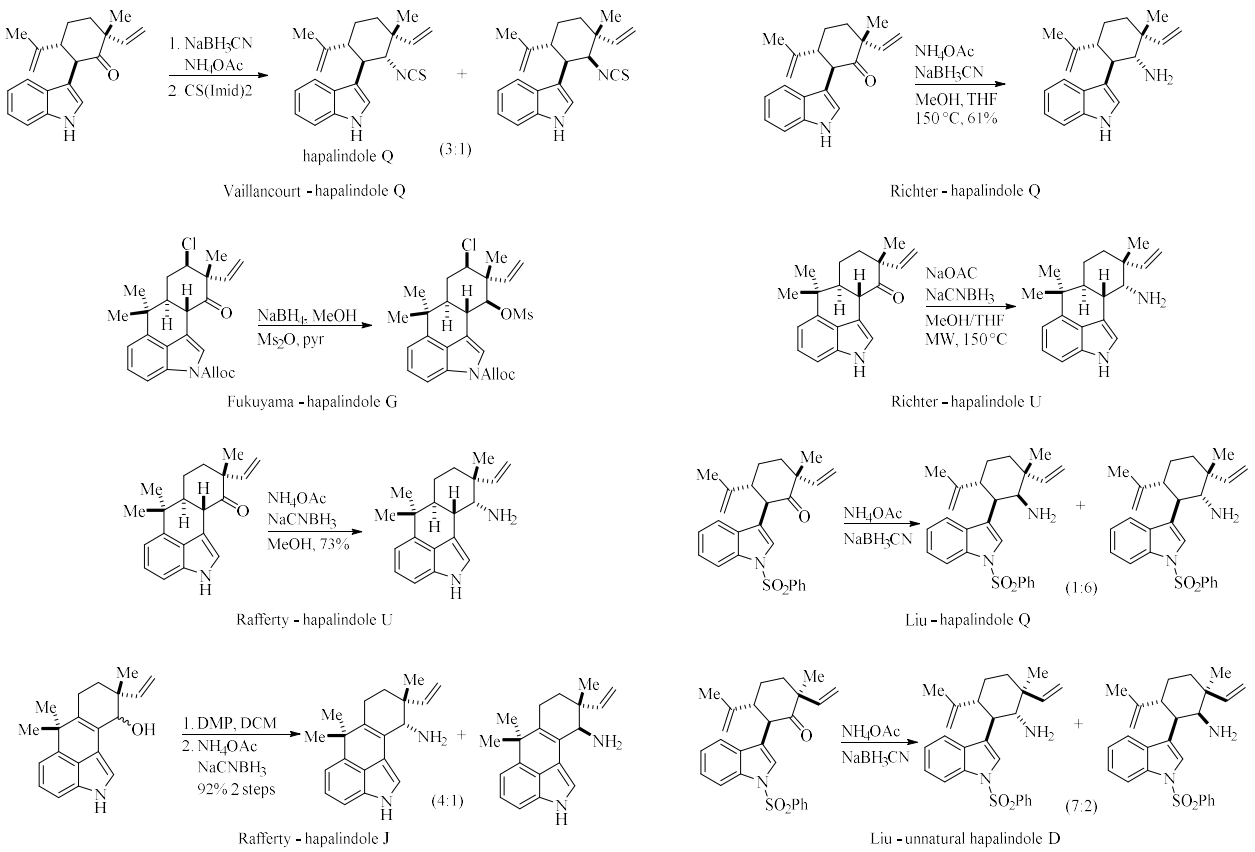
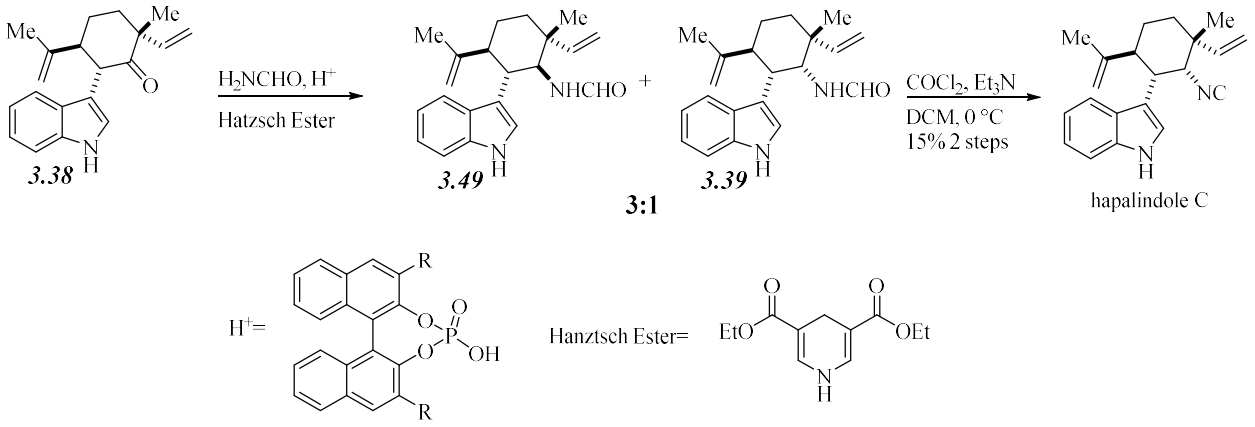


Figure 3.2. Previous attempts to reduce tricyclic and tetracyclic hapalindole scaffolds with a C-11 carbonyl.

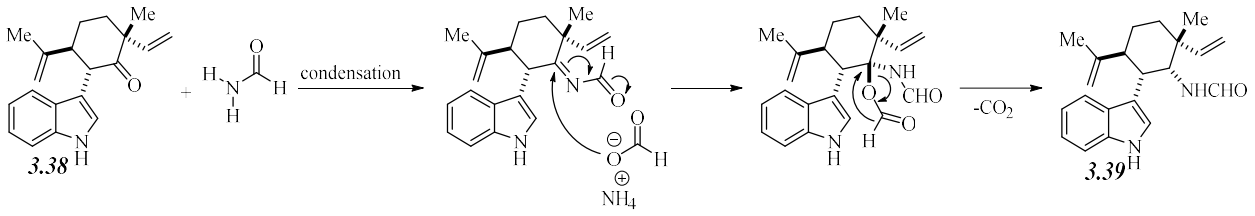
A structural analysis of these examples shows how the stereochemical outcome of reducing a C-11 carbonyl can be understood. In every tricyclic structure the reduction favors a *trans* relationship to the indole ring. In *trans*-decalin tetracyclic structures, however, the reduction approaches from the opposite face, with the interesting exception when C13 has a chlorine. Our reduction results fit the above data, requiring a new approach.

Inspired by examples of enantioselective transfer hydrogenation using the Hantzsch ester,[57] or benzothiazolines,[58-59] we attempted first to take tricycle **3.38** directly to formamide **3.39** (**Scheme 3.16**). The product was dehydrated to give a 1:3 diastereoselectivity, giving 15% hapalindole C.



Scheme 3.16. Synthesis of hapalindole C through a Leuckart reaction.

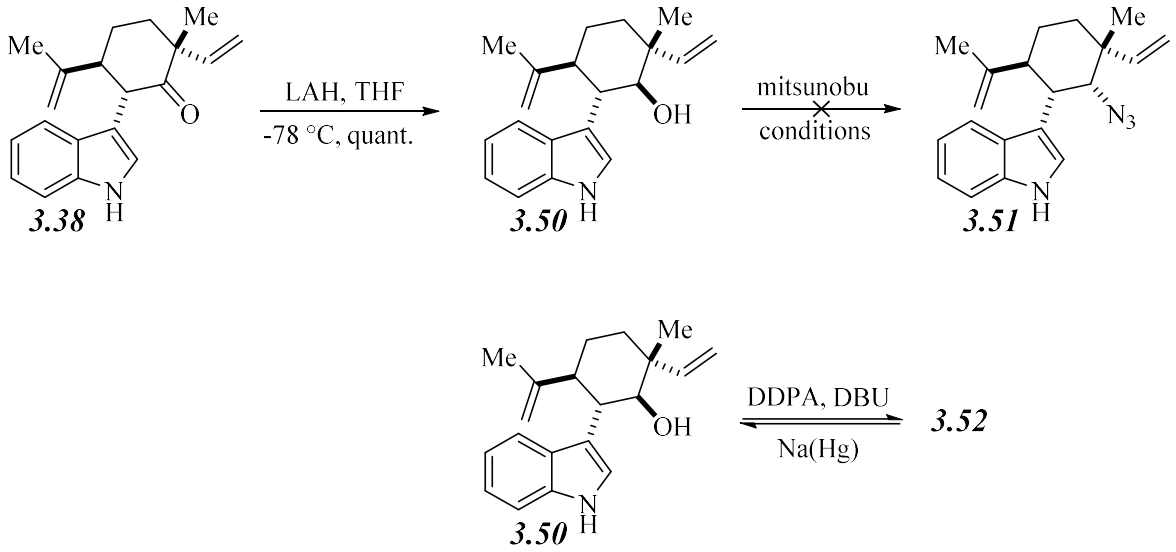
To improve the selectivity, we began to explore the reaction more thoroughly only to discover that the selectivity was the same with or with the catalyst, and the reduction occurred with and without the Hantzsch ester. Disappointingly the reaction can be attributed completely to a Leuckart reaction (**Scheme 3.17**). After screening solvents, temperatures, reaction times, reducing agents, nitrogen source, and trying to sequester the water before formation of formic acid we could not find any way to force the selectivity of the reduction away from the molecules natural tendency. We turned next to using a formal S_N2 to turn an alcohol precursor to the appropriate amine.



Scheme 3.17. Leuckart mechanism for the formation of formamide **6.39**.

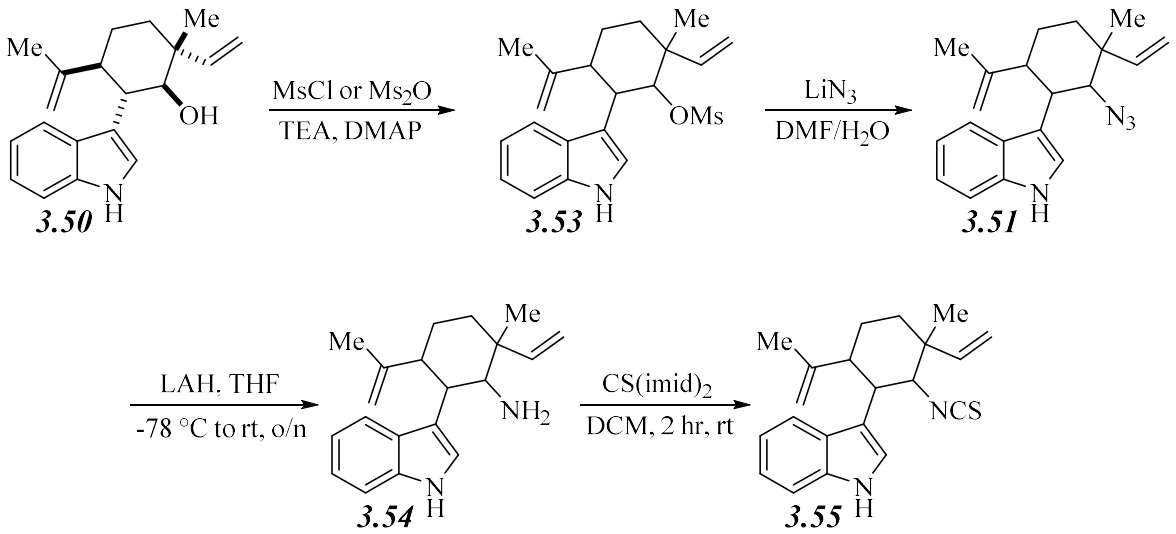
Reduction of carbonyl **3.38** was slow compared to the reduction of **3.51** from Fukuyama's work, so we turned to LAH as a reducing agent (**Scheme 3.18**). Treatment at -78 °C gave full conversion to alcohol **3.42** in quantitative yield in under 5 minutes. We employed a variety of

mitsunobu reaction conditions involving DEAD, DIAD, ADDP, CMMP, PPh_3, PMe_3, HN_3, DPPA, and DBU, with a few results highlighted here. Treating alcohol **3.40** with DEAD/PPh_3/DPPA gave the same product as treatment with DBU/DPPA. Reduction of the product using Na(Hg) amalgam failed to give the desired amine, instead returning the alcohol. To avoid this product we employed a fresh solution of HN_3 ("extremely explosive" if neat, our solution was titrated at a safe 0.2%), however the starting material was never consumed.



Scheme 3.18. Failed attempts to invert the C11 stereocenter using mitsunobu chemistry.

Finally, we resolved to take advantage of the chemistry first performed by Fukuyama in their synthesis of hapalindole G.[35] Attempts at conversion of alcohol **3.50** to mesylate **3.53** made an uncharacterizable product, possibly a mixture of mesylate rotomers, which gave a clean NMR after treatment with LiN_3. The product, which had an appropriate mass, was carried forward toward the synthesis of hapalindole D. Although the mass was again correct, the product is an isomer of hapalindole D.



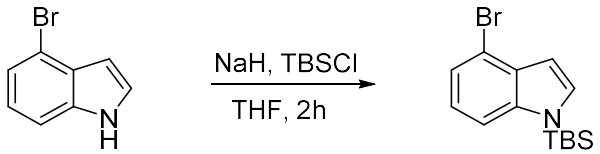
Scheme 3.19. Application of Fukuyama chemistry that resulted in an isomer of hapalindole D.

Chapter 4. Conclusions

We have successfully synthesized hapalindoles D and C using chemistry similar to previous syntheses. The current technology, however, gave very unfavorable diastereoselectivity. After several failed routes to improve the selectivity we have shortened the route and improved the selectivity using a Leuckart reaction. This method could provide a shortcut in other syntheses of isonitrile hapalindole products, especially where the natural facial selectivity is strongly favored.

Chapter 5. Experimental

^{1}H and ^{13}C spectra were obtained using 300, 400 and 500 MHz spectrometers. The chemical shifts are given in parts per million (ppm) relative to TMS at δ 0.00 ppm or at residual $CDCl_3$ at δ 5.26 ppm for proton spectra and relative to $CDCl_3$ at δ75.23 ppm for carbon spectra. IRA spectra were recorded on an FT-IR spectrometer as thin films. Mass spectra were obtained using a high/low resolution magnetic sector mass spectrometer. All melting points are uncorrected. Flash column chromatography was performed with silica gel grade 60 (240-400 mesh). Unless otherwise noted, materials were obtained from commercially available sources and used without further purification. Dichloromethane (CH_2Cl_2), tetrahydrofuran (THF), toluene (PhMe), N,N-dimethylformamide (DMF), acetonitrile (CH_2CN), triethylamine (Et_3N), and methanol (MeOH) were all degassed with argon and passed through a solvent purification system containing alumina or molecular sieves.



0.55 g NaH (60% in mineral oil, 13.8 mmol) was dissolved in dry THF. 4-Bromoindole (1.5 mL, 12 mmol) was added and gas evolved. After 30 minutes TBSCl (2.33 g, 15.5 mmol) was added. The solution was left to stir for 2 hours. The suspension was extracted with hexanes 3 times, washed once with H_2O, and brine. Column chromatography gave product quantitatively (3.53 g). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 5.45 (d, J = 6.3 Hz, 1H), 5.25 (dd, J = 11.0, 3.2 Hz, 2H), 5.01 (t, J = 6.0 Hz, 1H), 4.68 (dd, J = 3.2, 0.9 Hz, 1H), 0.92 (s, 10H), 0.61 (s, 7H).

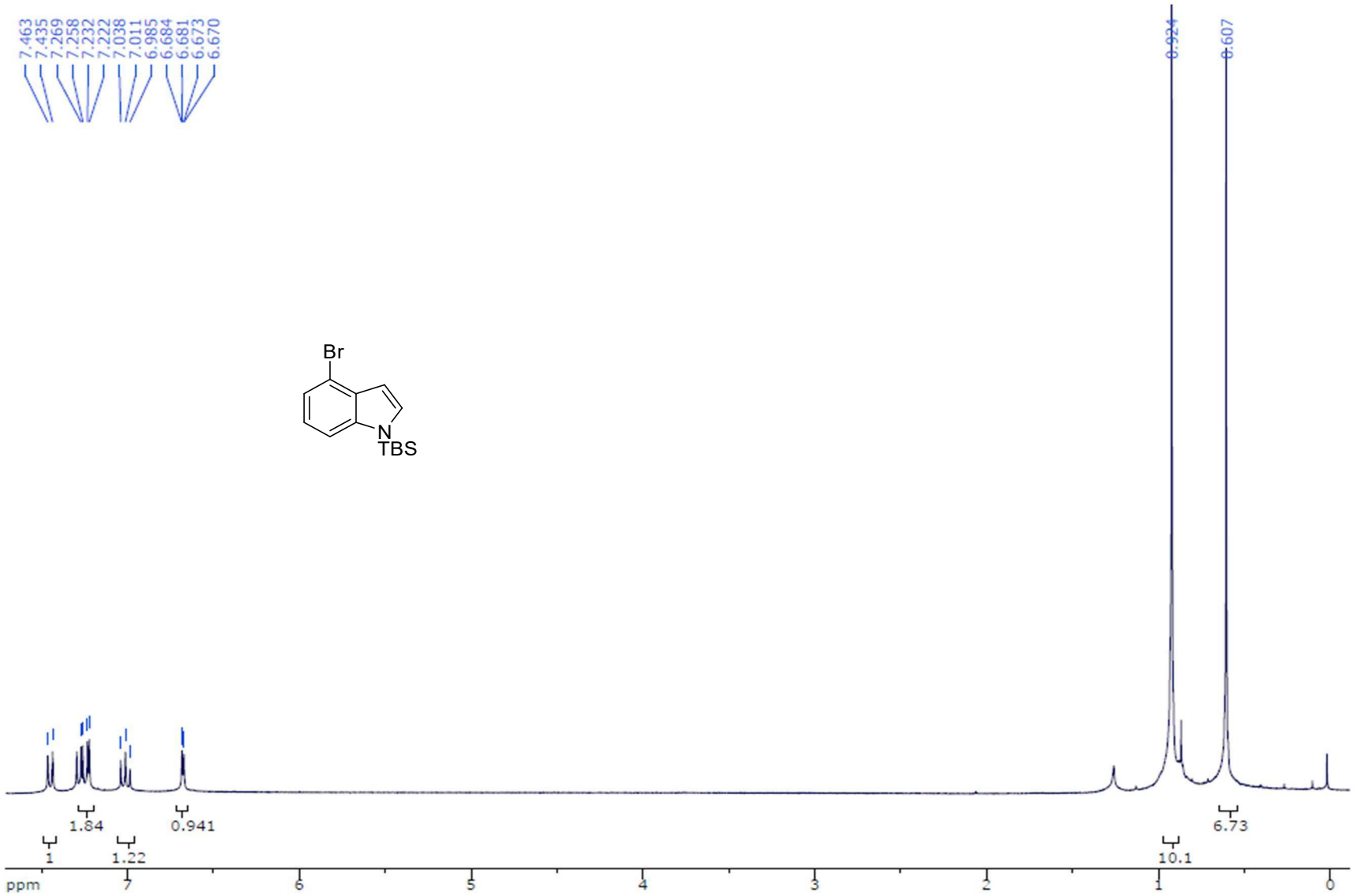
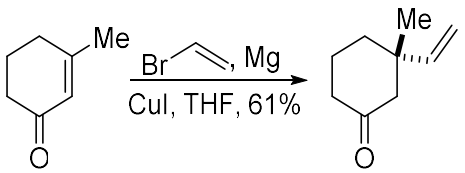


Figure 5.1. ^{1}H NMR of the product.



To Mg (5.4 g) suspended in dry THF was added a trace amount of I_2 and 10 mL of vinylbromide in THF (1 M). Heated until sustained reflux and then added the rest of the vinylbromide dropwise (total 80 mL, 80 mmol). After reflux ended heat back to reflux for 2 hours. Cool to room temperature and add to a solution of CuI (2.29 g, 12 mmol) in THF at -78 °C. After 30 min add a solution of the hexanone (6.5 mL 75 mmol) in 50 mL THF. Leave 1 hour, remove from the bath and leave an hour. Pour into saturated ammonium chloride, wash organic with water and brine, dry over sodium sulfate. Purification by column chromatography with 5% EtOAc in hexanes (61% yield). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 5.69 (dd, J = 15.5, 10.8 Hz, 1H), 5.00-4.93 (m, 2H), 2.43 (d, J = 14.0 Hz, 1H), 2.28-2.14 (m, 4H), 1.85-1.80 (m, 3H), 1.70-1.60 (m, 3H), 1.04 (s, 3H). 13-C NMR (400 MHz; cdcl3): δ 211.3, 145.7, 112.7, 51.6, 41.6, 40.8, 34.5, 25.2, 22.11, 22.09, 22.04

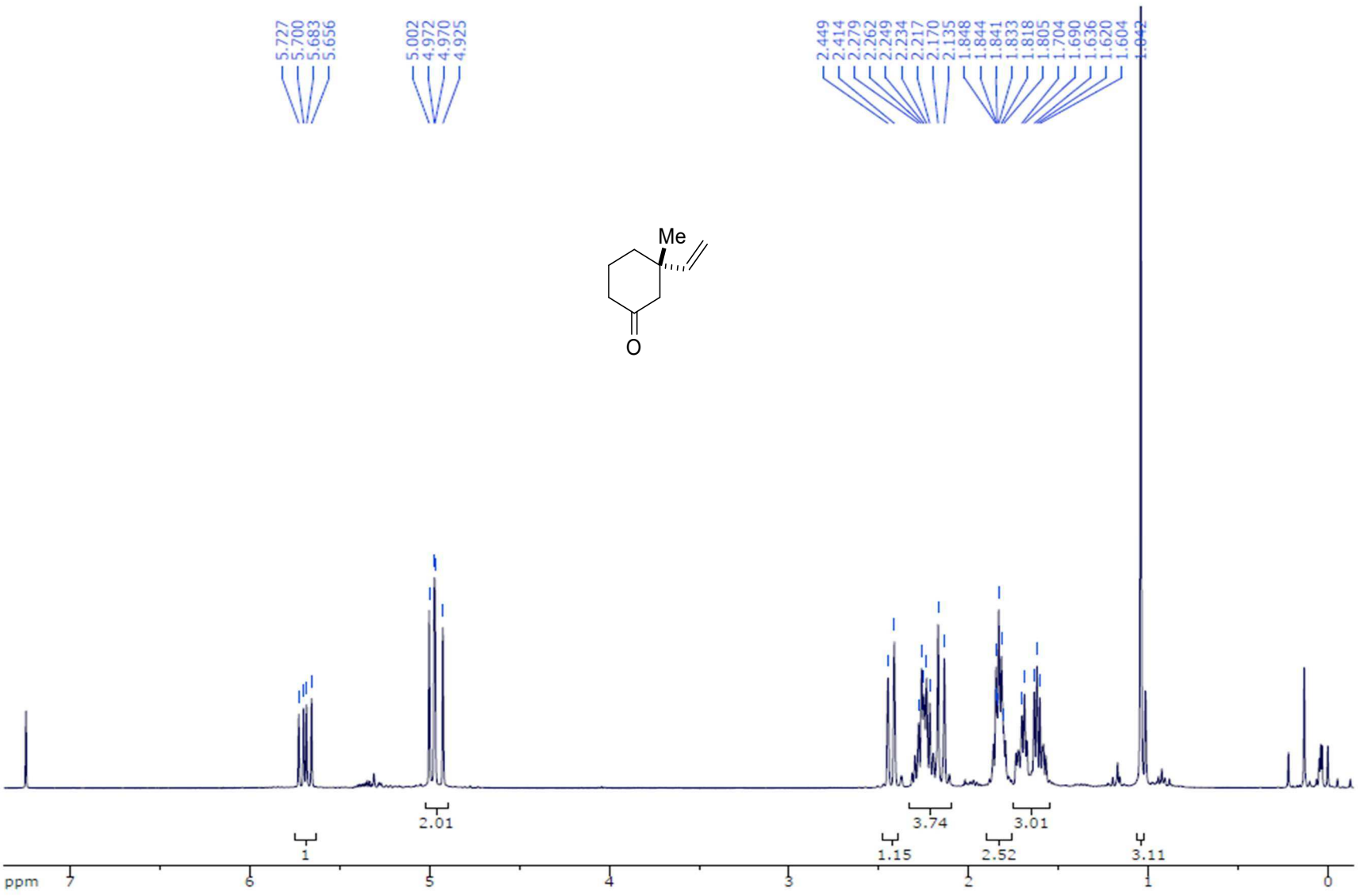


Figure 5.2. ^{1}H NMR of the product.

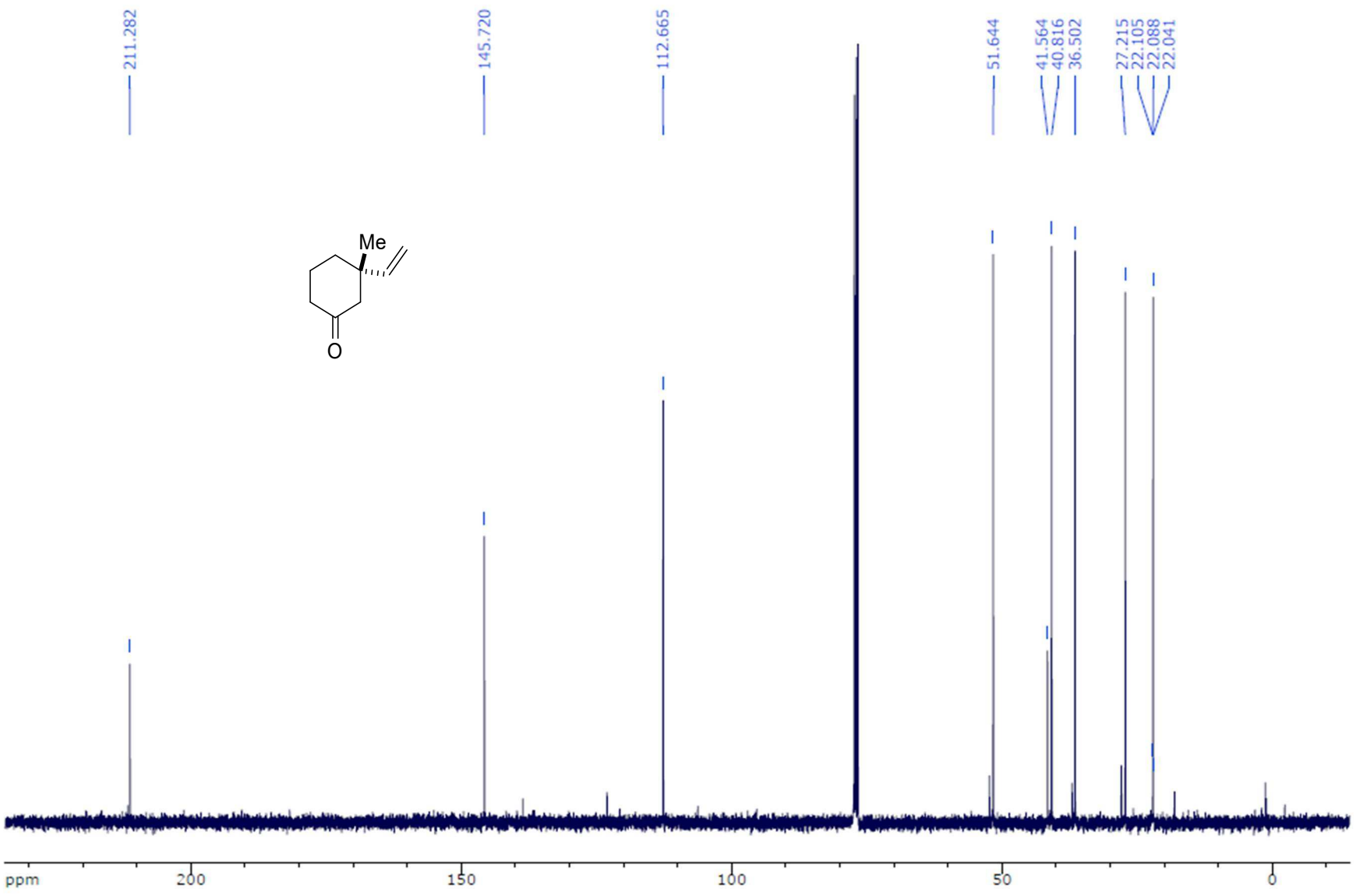
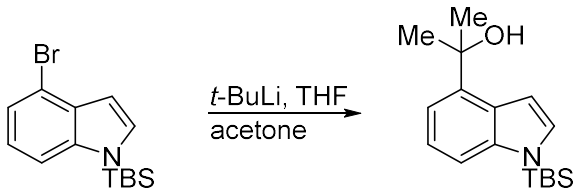


Figure 5.3. ^{13}C NMR of the product.



The protected indole (1.16 g, 3.9 mmol) was dissolved in dry ether (100 mL) at -78 °C. *t*-BuLi was added dropwise and left to react for 15 minutes. Acetone (15 mL) was added and left 45 minutes to react. Reaction was quenched with saturated ammonium chloride, and extracted twice with hexanes. Combined organics were washed with water, brine, and dried over sodium sulfate. Purification by column chromatography (1:3 hexanes:EtOAc) gave quantitative product as a white solid. ^{1}H-NMR (400 MHz; $CDCl_3$): δ 5.44 (d, *J* = 6.0 Hz, 1H), 5.19 (d, *J* = 3.1 Hz, 1H), 5.12 (dt, *J* = 15.2, 5.5 Hz, 2H), 4.96 (d, *J* = 3.2 Hz, 1H), 1.98 (s, 1H), 1.75 (s, 6H), 0.94 (s, 9H), 0.60 (s, 6H). 13-C NMR (101 MHz; cdcl3): δ 141.8, 140.6, 130.4, 125.9, 120.9, 115.2, 113.2, 105.6, 73.5, 30.7, 19.5, -3.9

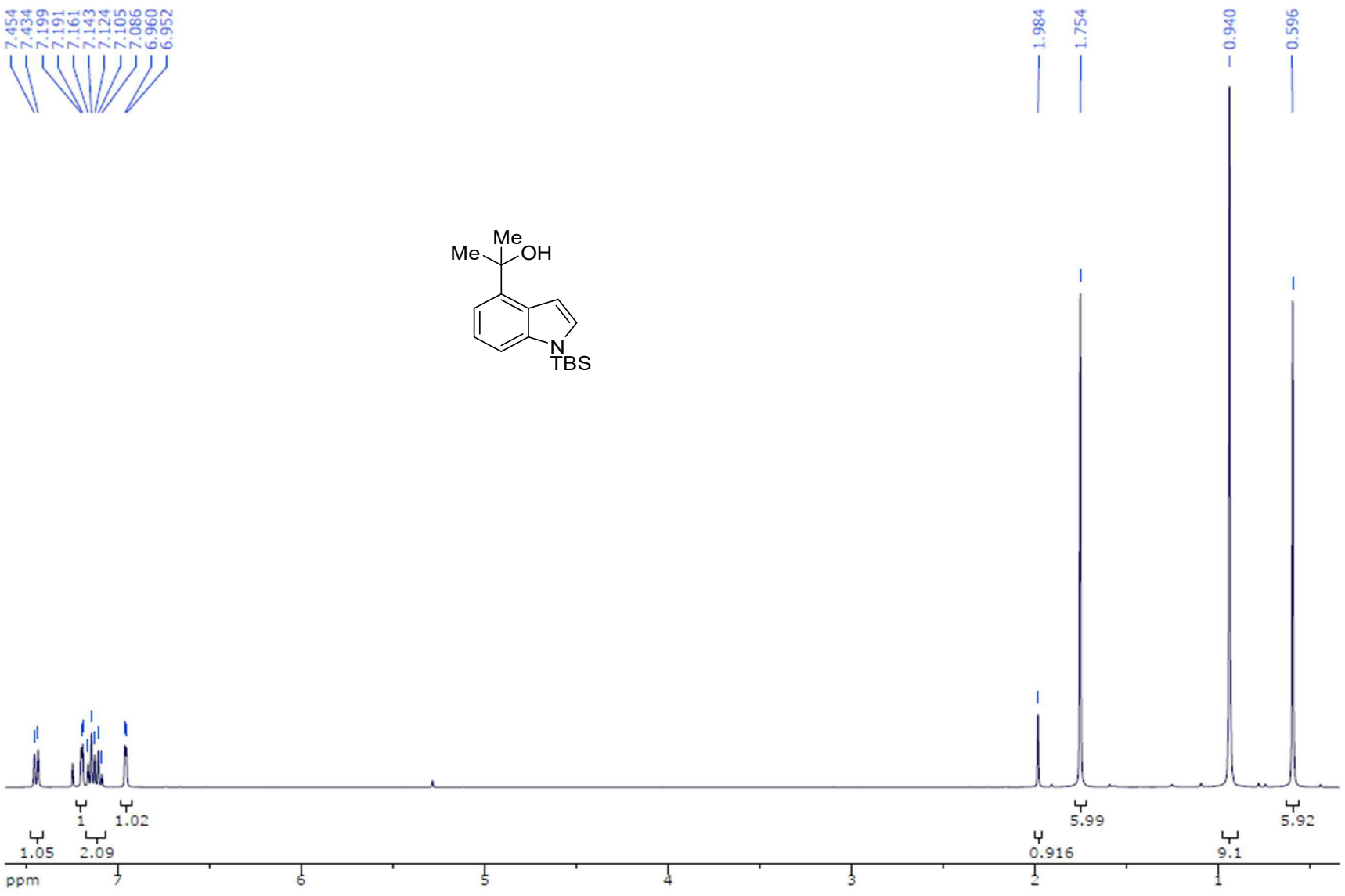


Figure 5.3. ^{1}H NMR of the product.

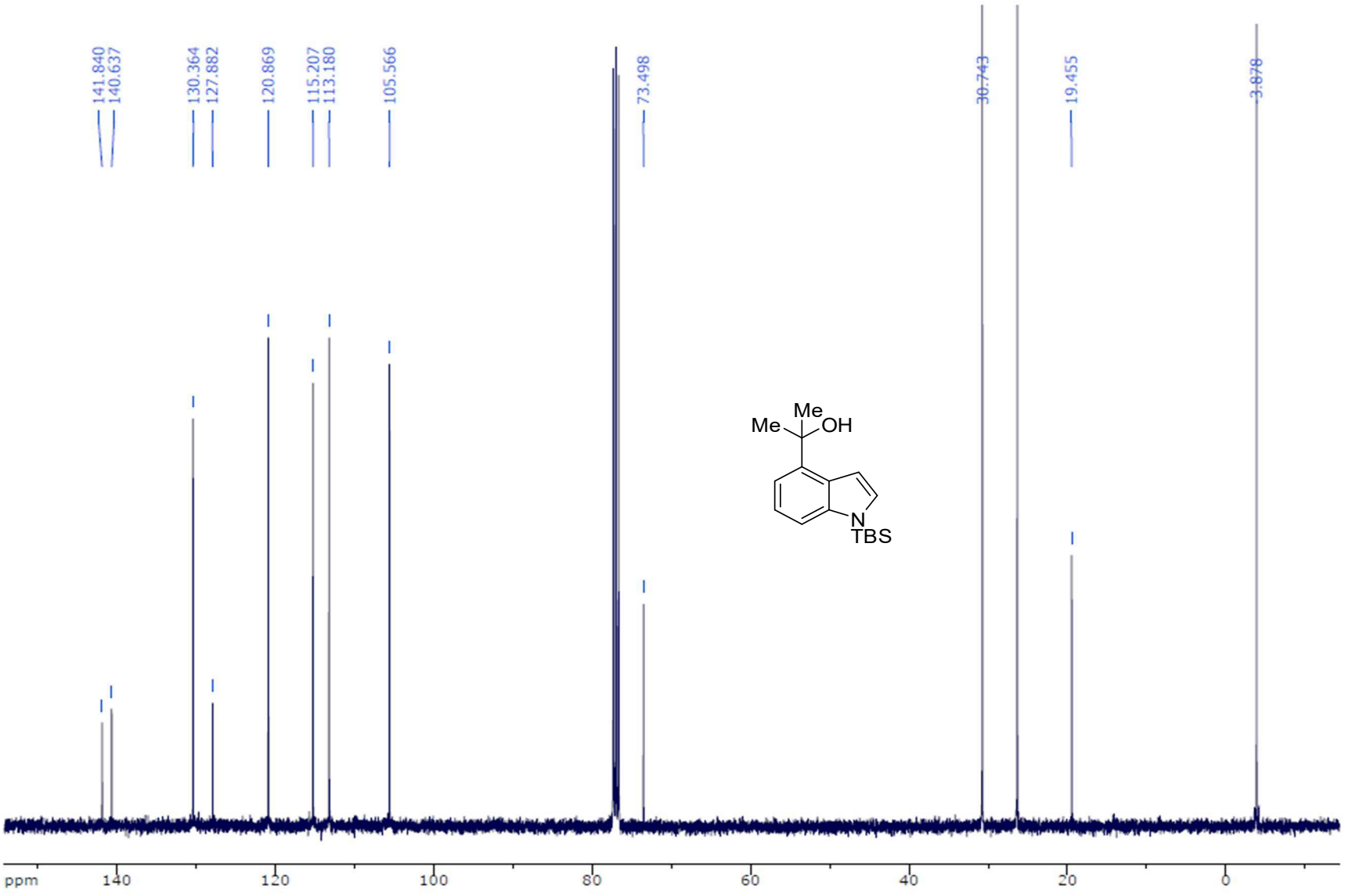
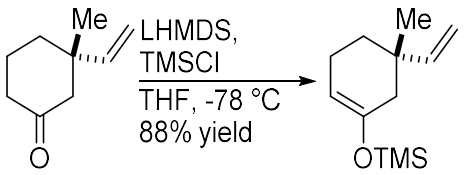


Figure 5.4. ^{13}C NMR of the product.



The ketone (350 mg, 2.5 mmol) was dissolved in dry THF at -78 °C. LHMDS (2.8 mL, 1M in THF, 2.8 mmol) was added dropwise and left at that temperature for 45 minutes. TMSCl (0.35 mL, 2.8 mmol) was added and left another 45 minutes. Add brine and hexanes. Extract with hexanes, combine organics, dry over sodium sulfate and condense. 88% yield, no further purification. Mixture of isomers, not characterized.

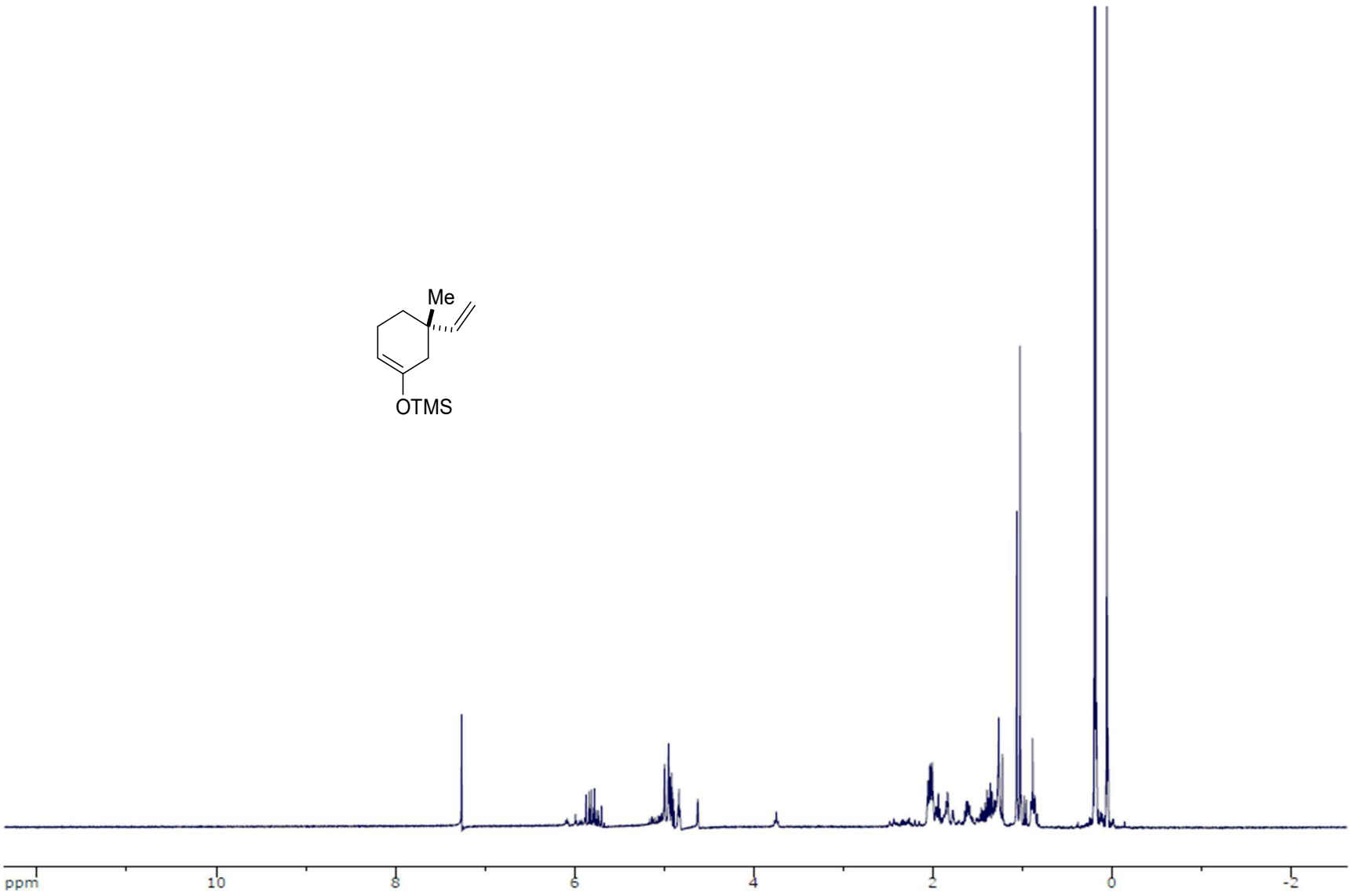


Figure 5.5. ^{1}H NMR of the product.

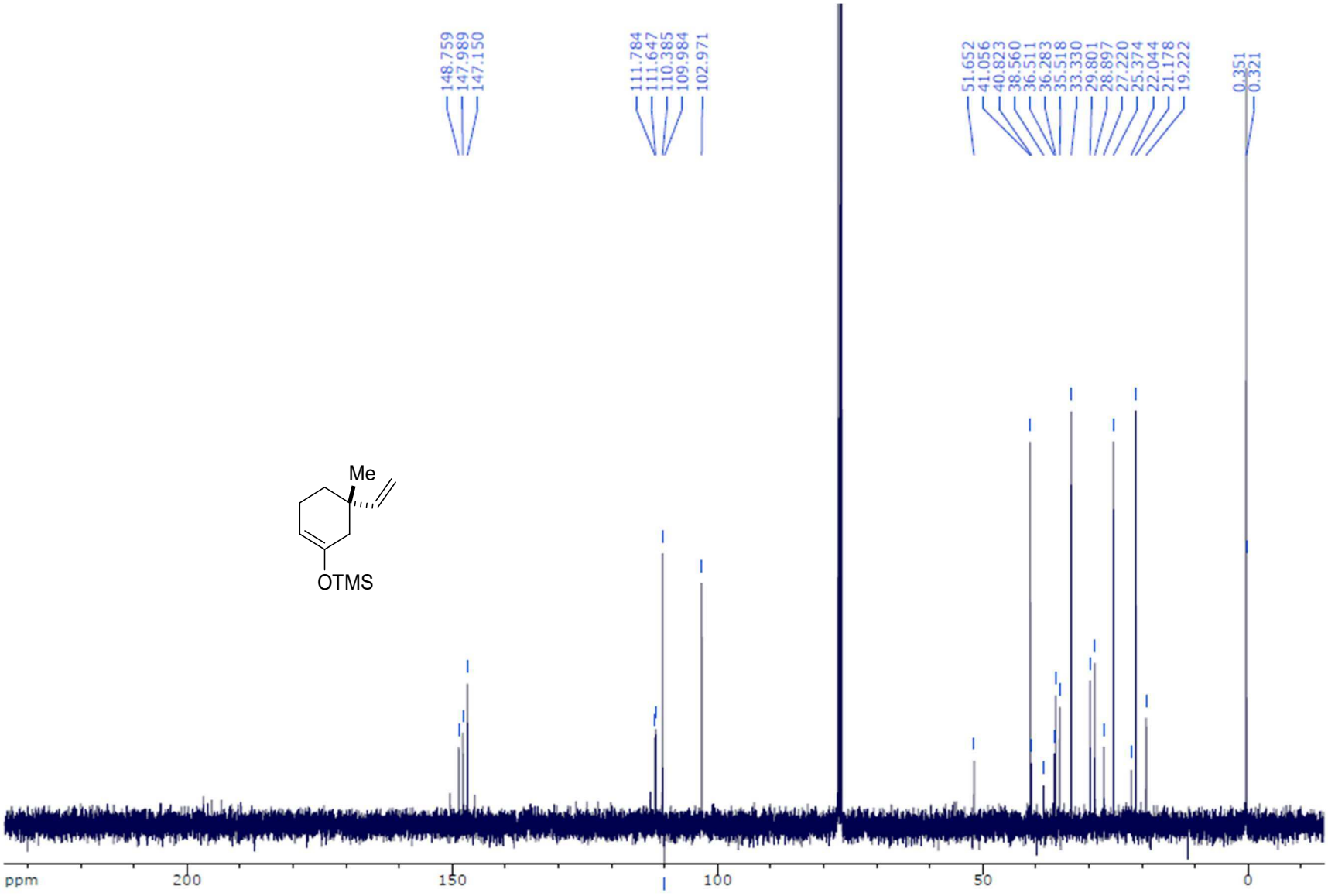
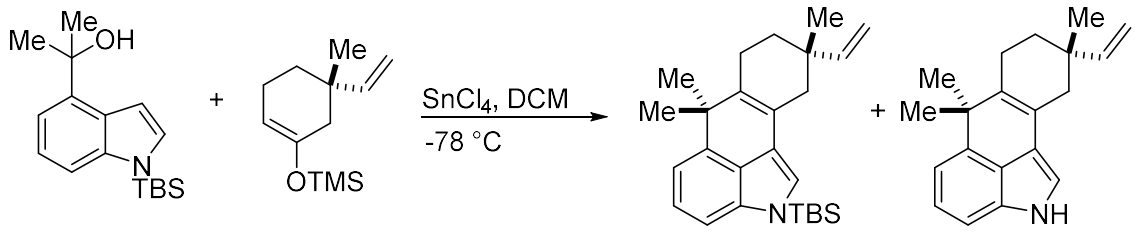


Figure 5.6. ^{13}C NMR of the product.



Dissolve coupling partners (64 mg indole, 100 mg enolate) in DCM at -78 °C and add $SnCl_4$ (1.3 eq). After 15 min quench with $NaHCO_3$ and extract with DCM. Wash organics with water and brine, dry with sodium sulfate and condense. Flash chromatography yields 40% TBS protected product, and 6% of the unprotected product.

TBS product: ^{1}H-NMR (300 MHz; $CDCl_3$): δ 5.18-5.16 (m, 2H), 5.00 (dd, *J* = 5.4, 2.5 Hz, 1H), 4.80 (s, 1H), 5.91 (dd, *J* = 15.5, 10.7 Hz, 1H), 5.03-4.93 (m, 2H), 2.52-2.26 (m, 4H), 1.64 (dt, *J* = 11.3, 5.9 Hz, 2H), 1.45 (d, *J* = 6.2 Hz, 6H), 1.11 (s, 3H), 0.96 (s, 10H), 0.57 (s, 6H).

NH product: ^{1}H-NMR (300 MHz; $CDCl_3$): δ 5.71 (s, 1H), 5.21 (t, *J* = 5.7 Hz, 2H), 5.09 (d, *J* = 6.0 Hz, 1H), 5.00 (d, *J* = 5.3 Hz, 1H), 4.84 (d, *J* = 1.9 Hz, 1H), 5.89 (dd, *J* = 15.5, 10.7 Hz, 1H), 5.01-4.92 (m, 3H), 2.51-2.24 (m, 5H), 1.62 (ddd, *J* = 16.8, 12.7, 5.0 Hz, 3H), 1.45 (d, *J* = 6.5 Hz, 7H), 1.10 (s, 4H).

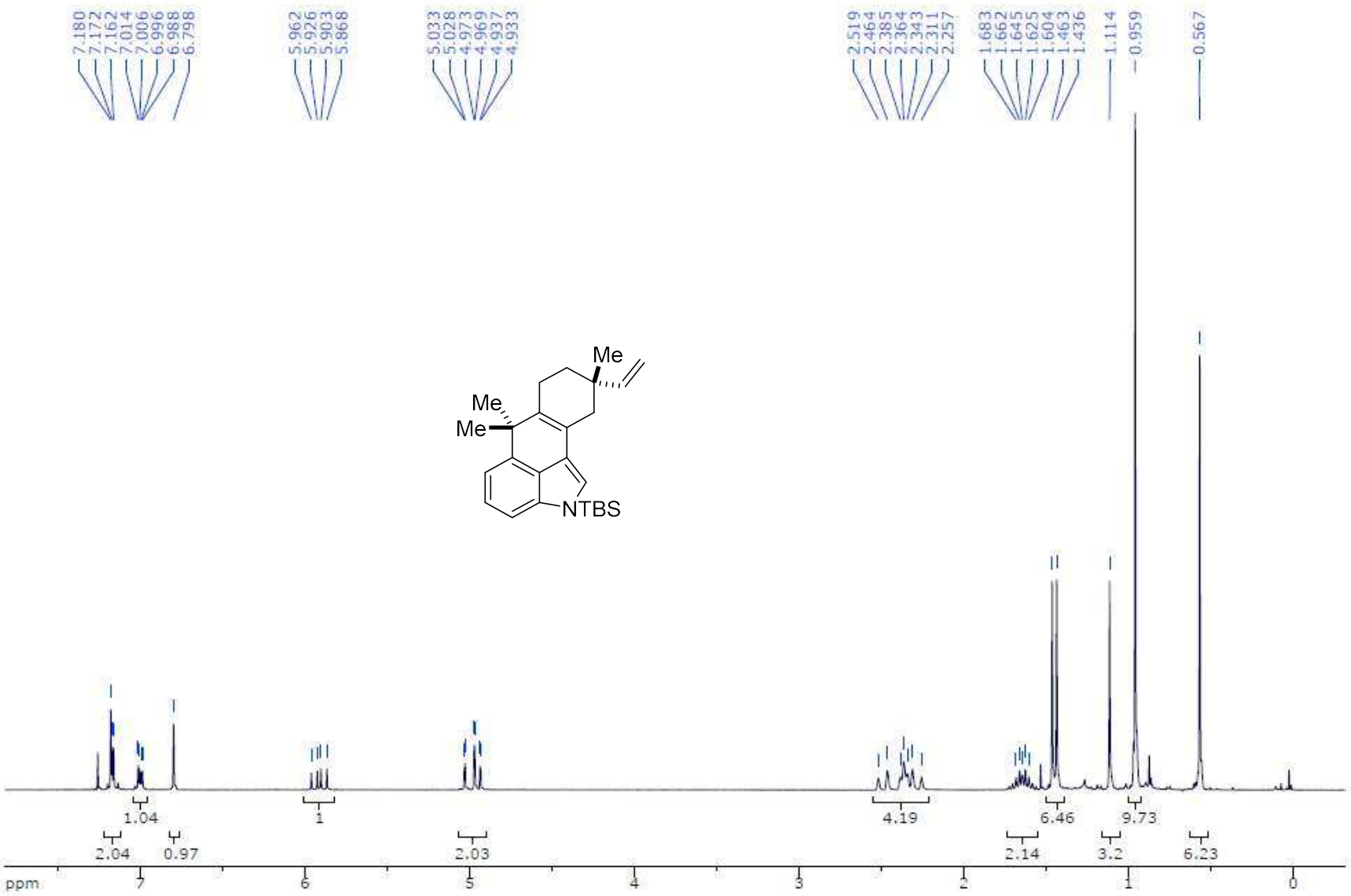


Figure 5.7. ^{1}H NMR of the product.

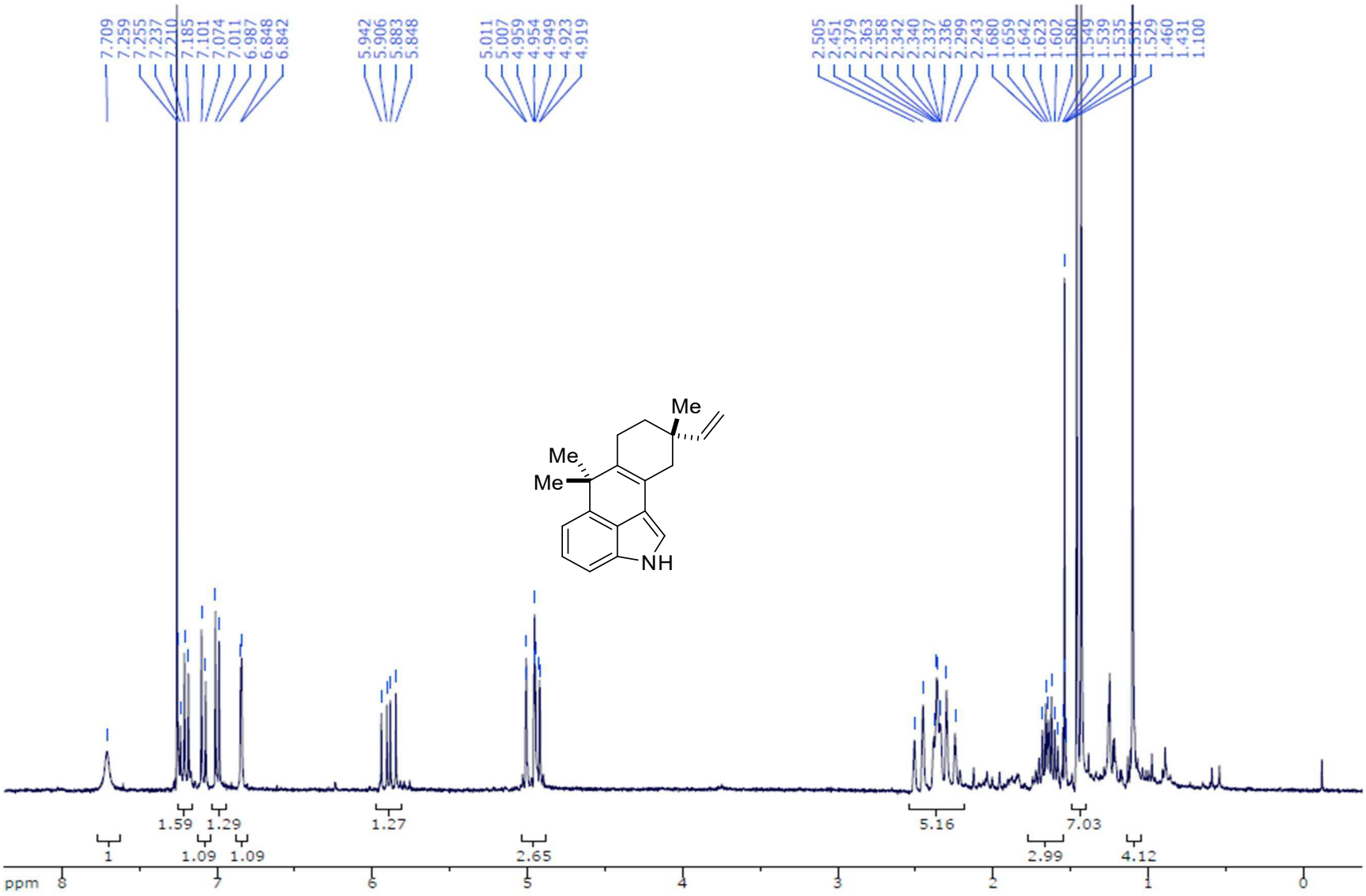
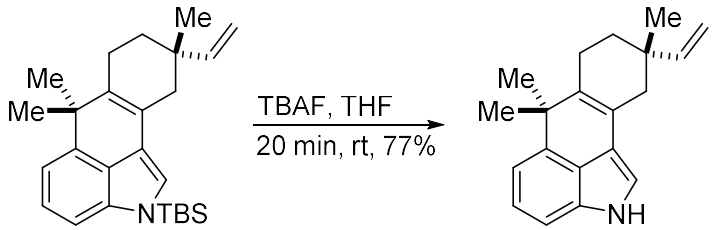
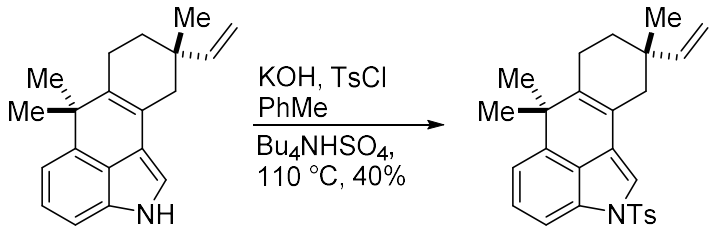


Figure 5.8. ^{1}H NMR of the product.



Protected tetracycle (148 mg, 0.38 mmol) was dissolved in THF at room temperature. TBAF (0.4 mL 1M, 0.4 mmol) was added and left for 20 minutes. The solution was partitioned between H_2O and EtOAc, was with brine and dried over sodium sulfate. Purification through column chromatography gave 77% pure product. : ^{1}H-NMR (300 MHz; $CDCl_3$): δ 5.18-5.16 (m, 2H), 5.00 (dd, *J* = 5.4, 2.5 Hz, 1H), 4.80 (s, 1H), 5.91 (dd, *J* = 15.5, 10.7 Hz, 1H), 5.03-4.93 (m, 2H), 2.52-2.26 (m, 4H), 1.64 (dt, *J* = 11.3, 5.9 Hz, 2H), 1.45 (d, *J* = 6.2 Hz, 6H), 1.11 (s, 3H), 0.96 (s, 10H), 0.57 (s, 6H).



Dissolved starting material (185 mg, 0.67 mmol) in toluene with TsCl (140 mg, 0.73 mmol), Bu_4NHSO_4 (14 mg, 0.04 mmol) and 3 mL of 50% KOH. Heated to 110 °C overnight. Partition between ammonium chloride and ethyl acetate. Purification on column chromatography gave 40% pure tosylated tetracycle. ^{1}H-NMR (400 MHz; $CDCl_3$): δ 5.79 (d, J = 5.7 Hz, 1H), 5.66 (d, J = 6.1 Hz, 1H), 5.30 (t, J = 5.8 Hz, 1H), 5.20 (d, J = 6.0 Hz, 1H), 5.12 (d, J = 5.3 Hz, 1H), 5.84 (dd, J = 15.7, 10.5 Hz, 1H), 4.96-4.91 (m, 1H), 2.45-2.20 (m, 4H), 1.68-1.53 (m, 1H), 1.37 (d, J = 10.9 Hz, 3H), 1.08 (s, 2H). 13-C NMR (101 MHz; cdcl3): δ 144.7, 144.4, 140.8, 140.2, 135.7, 133.4, 129.8, 124.96, 124.77, 124.1, 121.0, 119.1, 116.6, 115.2, 111.0, 110.6, 40.6, 34.7, 34.50, 34.38, 30.02, 29.84, 24.1, 22.6, 21.5

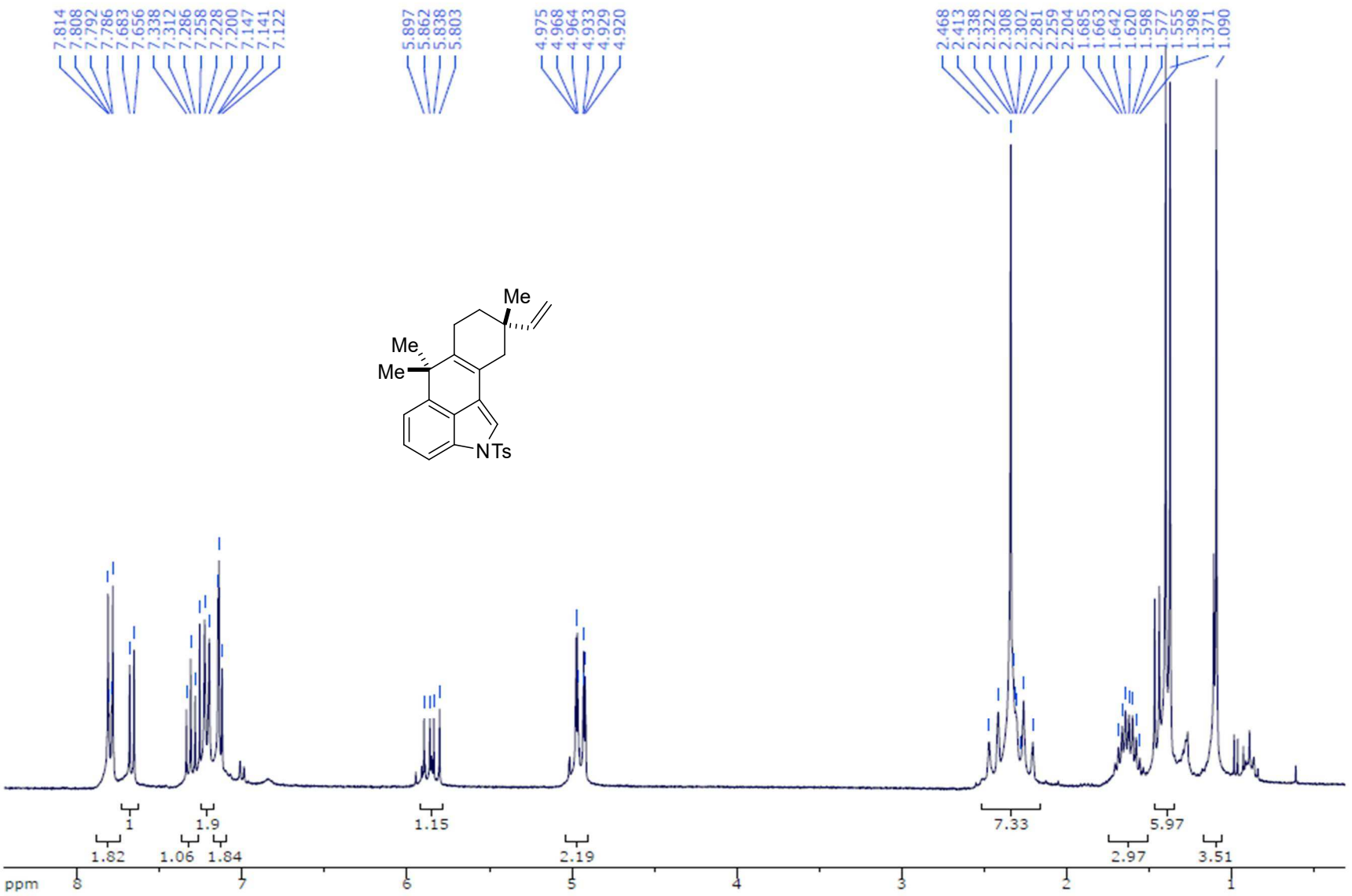


Figure 5.9. ^{1}H NMR of the product.

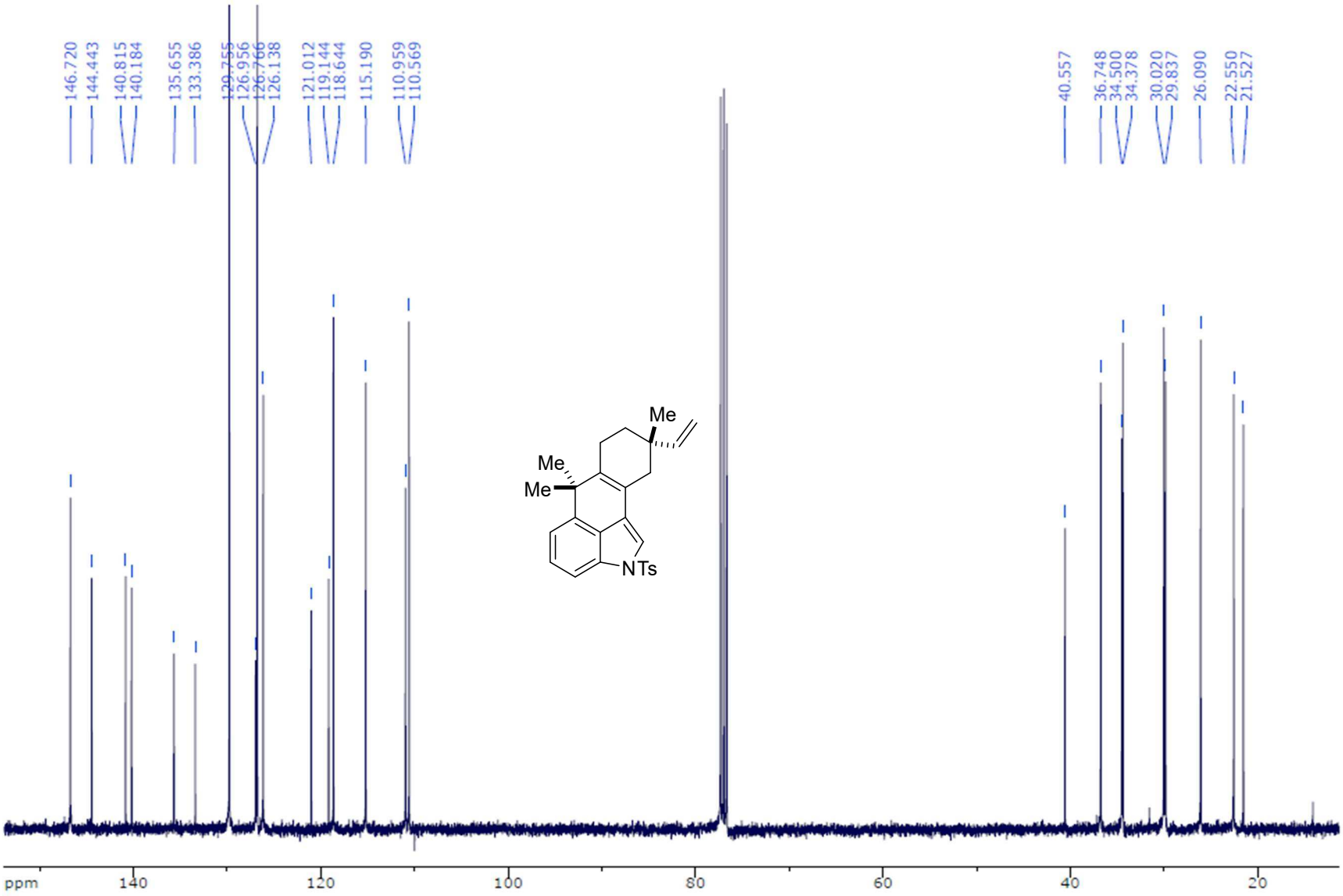
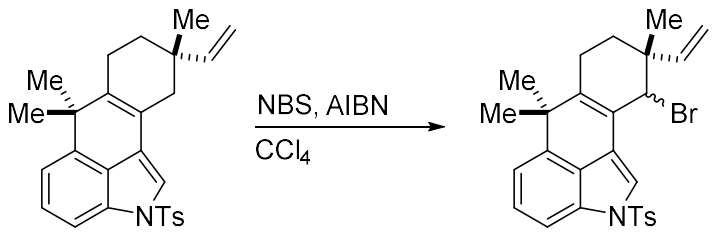


Figure 5.10. ^{13}C NMR of the product.



Tosylated tetracycle (270 mg) was dissolved in carbon tetrachloride followed by NBS (54 mg, 1.1 eq) and AIBN (10 mg, 0.22 eq). Reflux 1 hour. Purification by column chromatography gave 110 mg of a mixture of diastereomers. Not charachterized.

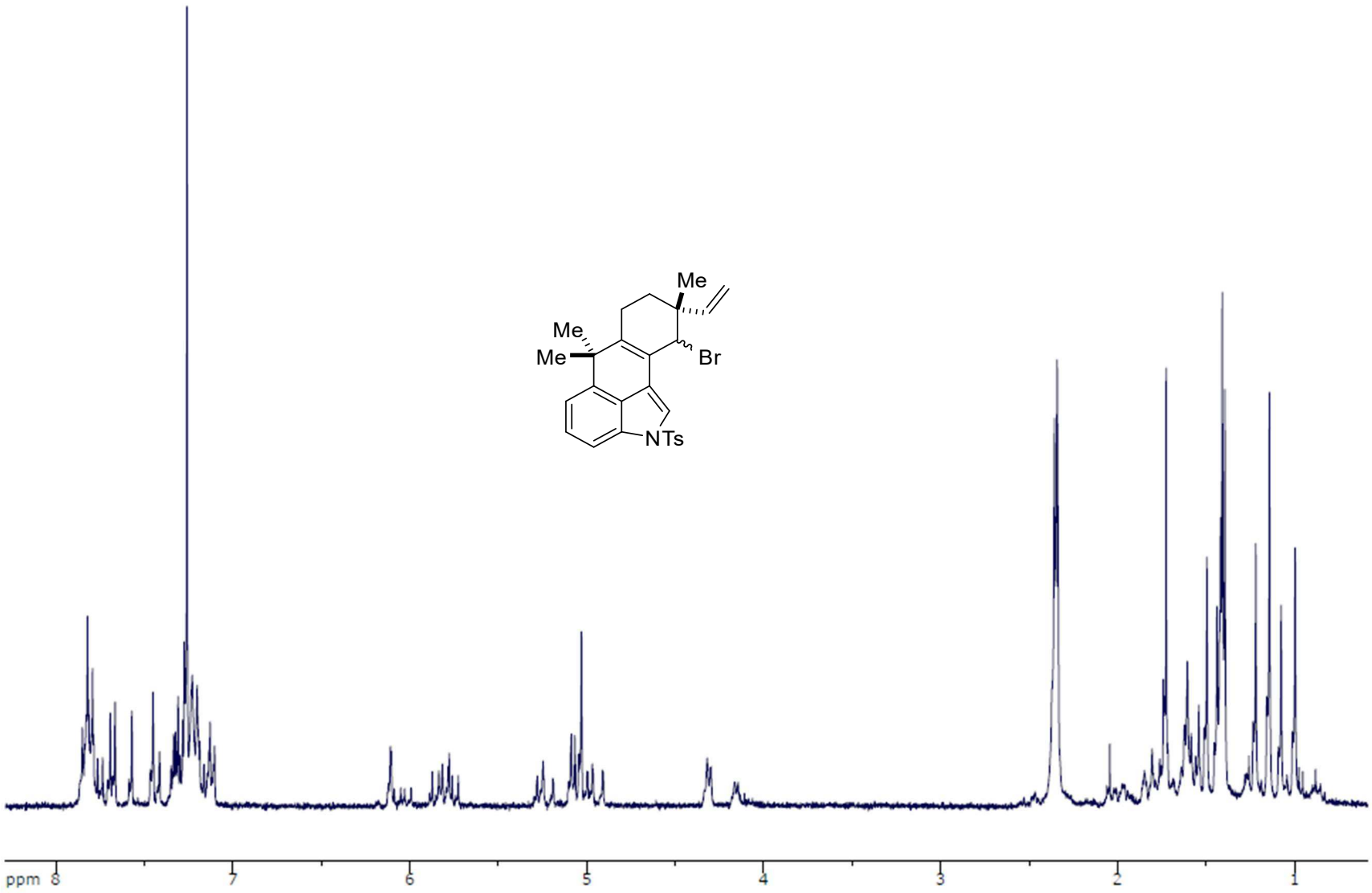
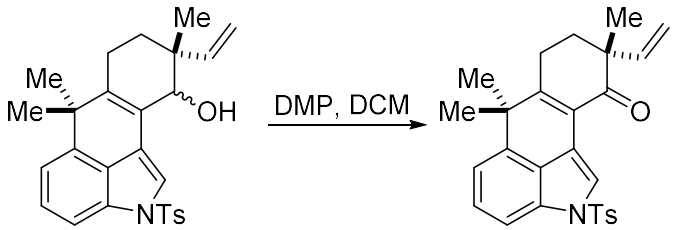


Figure 5.11. ^{1}H NMR of the product.



Dissolve alcohol (37 mg) and DMP (1 eq) in DCM at room temperature for 2 hours. Quench with NaS_2O_3 and was with saturated sodium bicarbonate and then once with NaS_2O_3, water and brine. Dried over sodium sulfate and purify by column chromatography. ^{1}H-NMR (300 MHz; $CDCl_3$): δ 6.13 (s, 1H), 5.83 (d, J = 6.4 Hz, 2H), 5.74 (d, J = 6.2 Hz, 1H), 5.32 (t, J = 5.8 Hz, 1H), 5.22-5.19 (m, 2H), 5.12 (d, J = 5.5 Hz, 2H), 5.94 (dd, J = 15.6, 10.7 Hz, 1H), 5.04 (dd, J = 34.9, 14.2 Hz, 2H), 2.64 (dd, J = 5.0, 5.0 Hz, 2H), 2.33 (s, 3H), 2.11-1.93 (m, 3H), 1.49 (s, 6H), 1.29 (s, 8H).

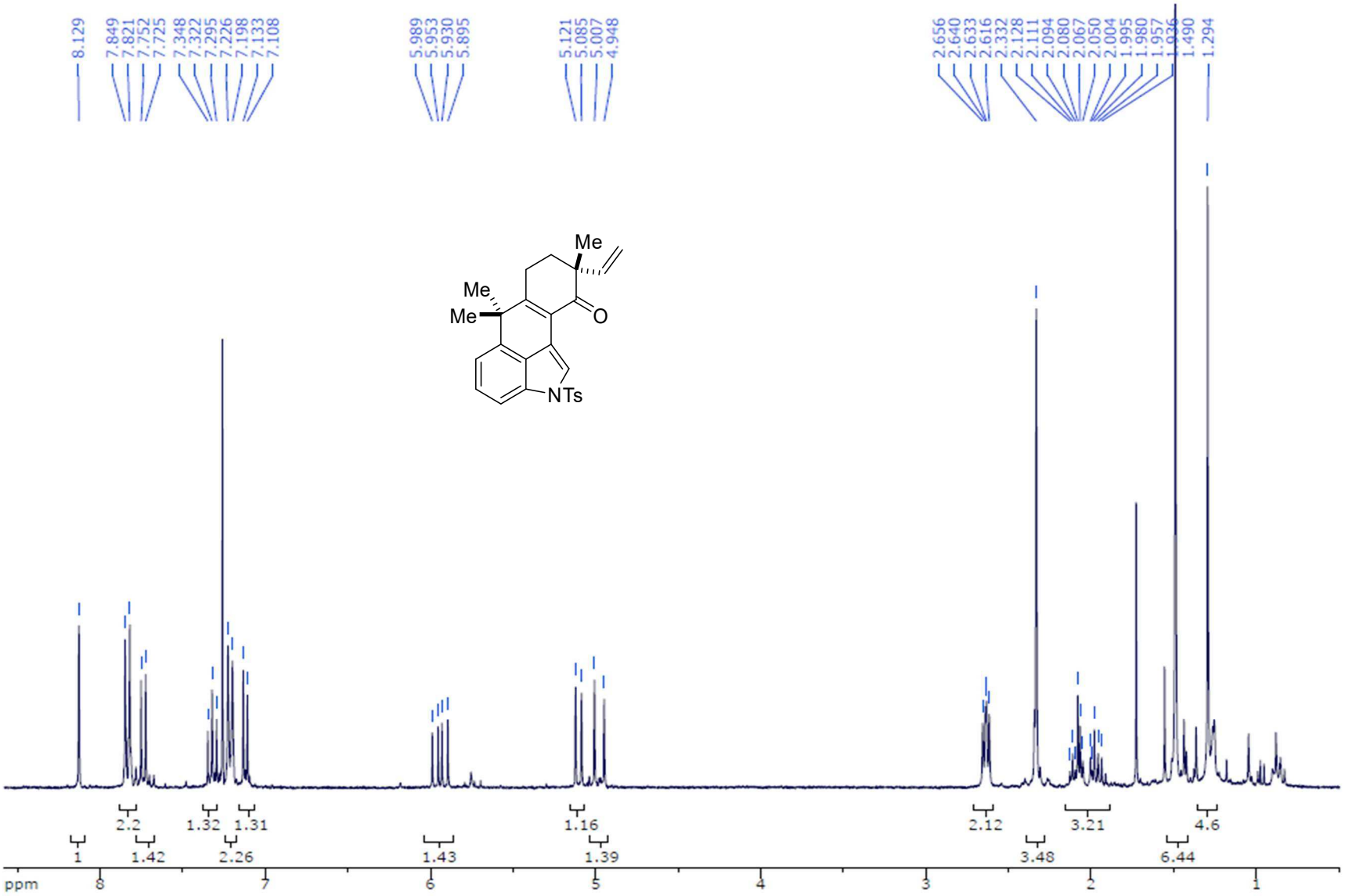
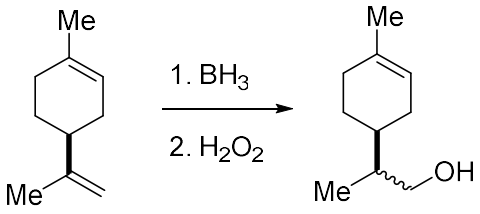
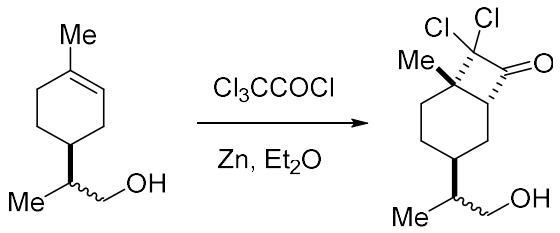


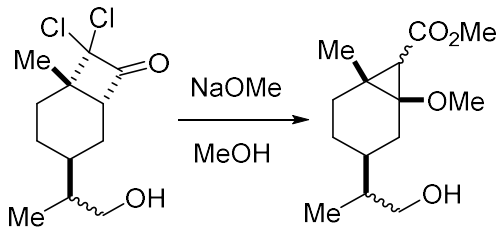
Figure 5.12. ^{1}H NMR of the product.



Cooled a 0.5 M solution of 9-BBN in THF (360 mL, 180 mmol) in an ice bath in a 1L 3-neck flask. Added 23 mL (S)-limonene dissolved in 50 mL THF over the course of an hour. Pulled the ice bath and stirred for 2 hours at room temperature. Added 45 mL 3M NaOH and 45 mL 30% wt H_2O_2 in separate addition funnels while keeping the internal temperature under 10° C by using a dry ice-acetone bath. After the addition the solution was stirred at 40° C for 2 hours. Poured into 100 mL water and 100 mL Et_2O. Separated layers and extracted the aqueous layer with Et_2O (3x100 mL). Combined organics were dried over Na_2SO_4 and condensed. Purification by distillation followed by column chromatography yielded pure product with spectral data matching the literature.



The starting olefin was dissolved in ether in a round bottom flask. Zn dust was added to this solution and the suspension was put in an ultrasound bath regulated to 25° C. While sonicating the trichloroacetyl chloride was added dropwise over an hour to the suspension. Sonication continued for another 6 hours. The dark brown suspension was filtered through celite and condensed. The dark red oil was purified by column chromatography yielding a yellow oil containing a mixture of diasteromers.



The cyclobutanone yellow oil was dissolved in anhydrous methanol to which sodium methoxide (4 eq) was added. The suspension was lowered into a heated oil bath and left to reflux for 2 hours. This solution was partitioned between EtOAc and 1M HCl. The aqueous layer was extracted twice with EtOAc and the combined organics were washed with 1 M HCl, then brine, then dried over Na_2SO_3. The solution was filtered and condensed to yield a yellow oil mixture of diastereomers.

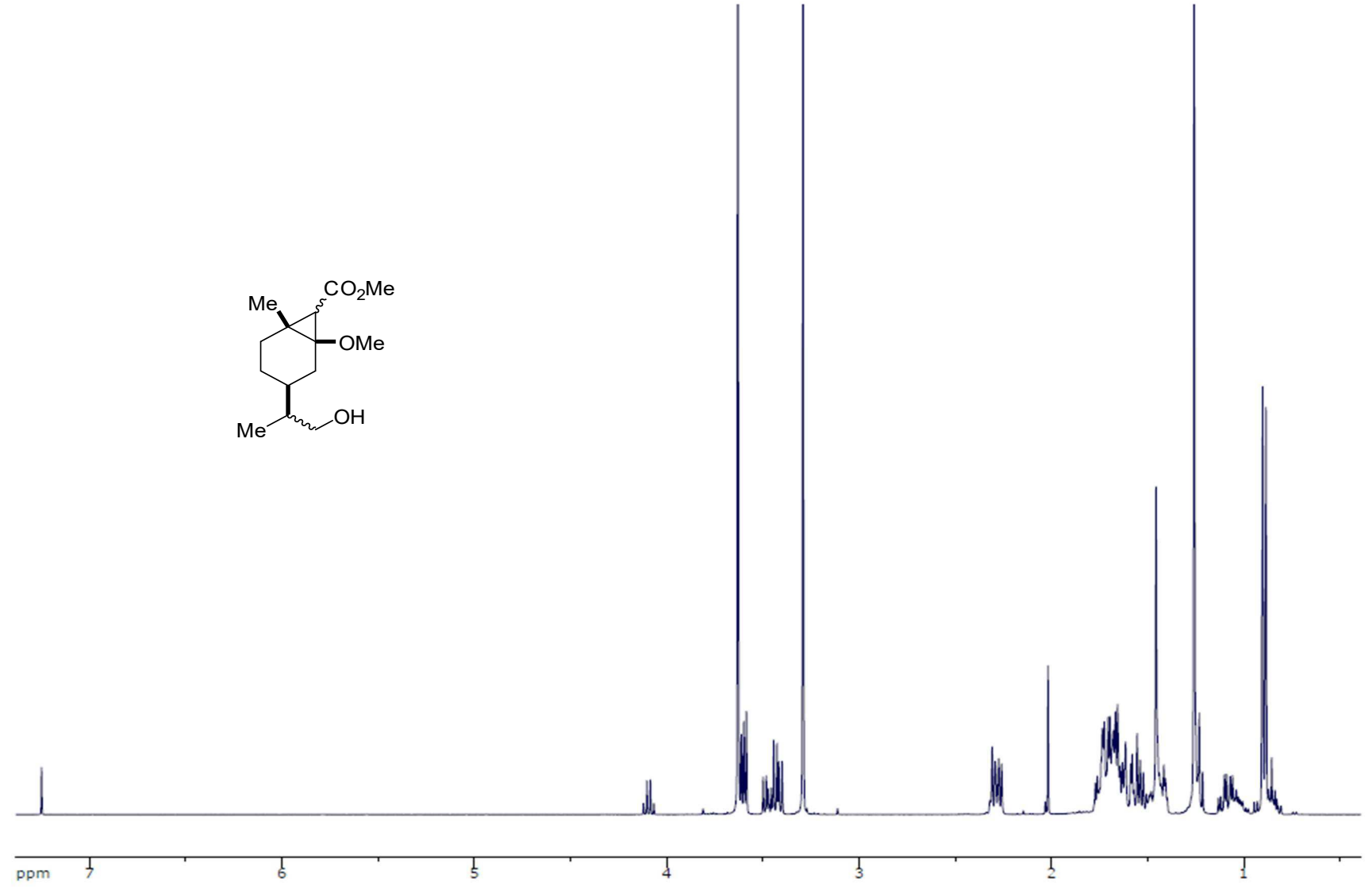


Figure 5.13. ^{1}H NMR of the product.

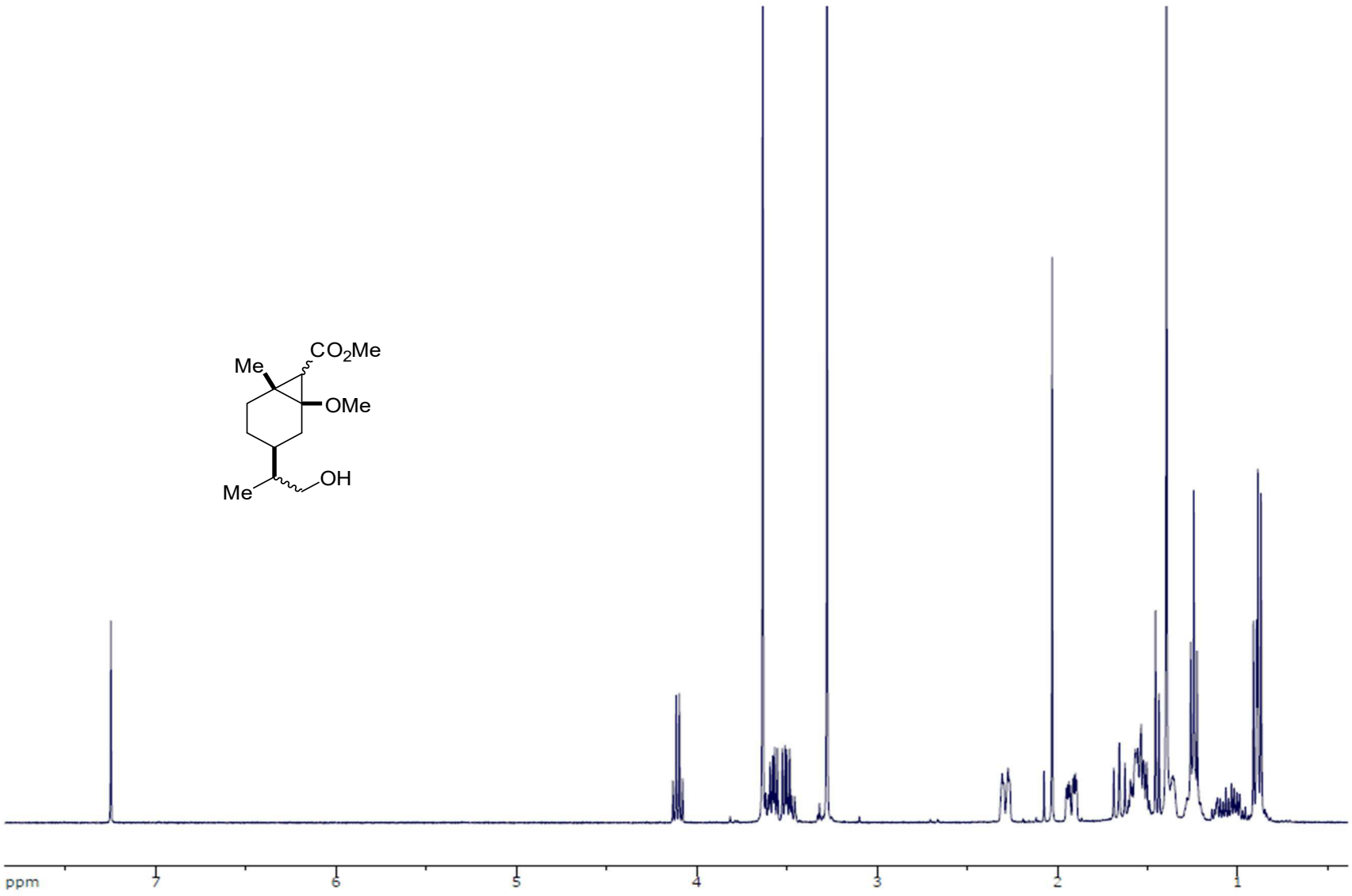
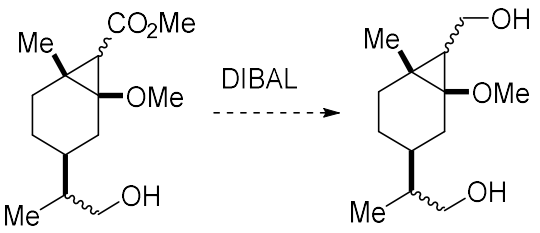


Figure 5.14. ^{1}H NMR of the product.



To a solution of the ester in DCM was added a 1 M toluene solution of DIBAL (5 eq) dropwise at -78 °C. The reaction was stirred for 30 minutes cold and quenched by the dropwise addition of MeOH. The solution was warmed to warm temperature, diluted with EtOAc and Rochelle's salt solution and stirred overnight. The layers were separated and extracted with EtOAc 3 times, and dried with Na_2SO_4. The residue was purified by column chromatography giving a mix of diastereomers.

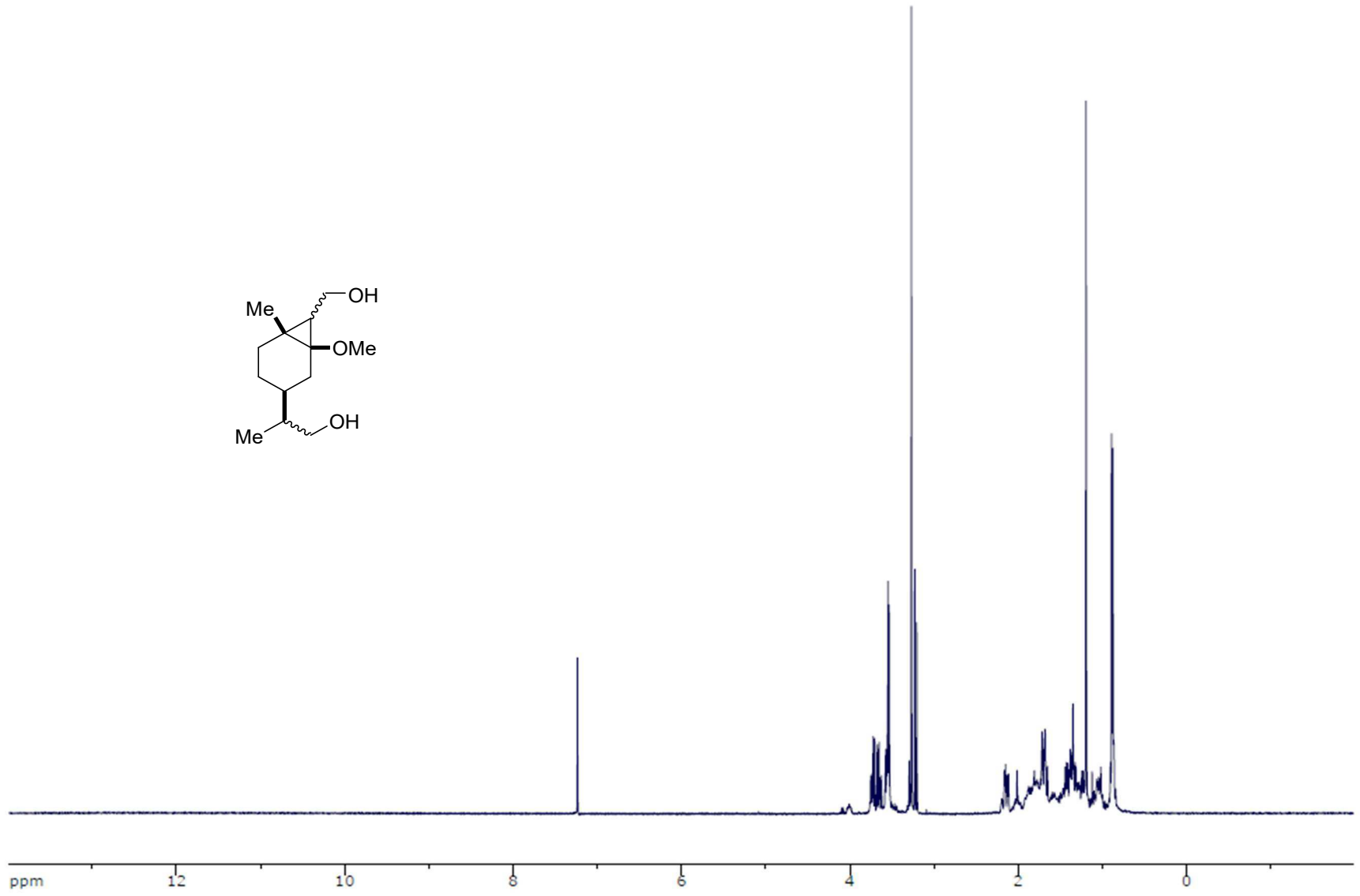


Figure 5.15. ^{1}H NMR of the product.

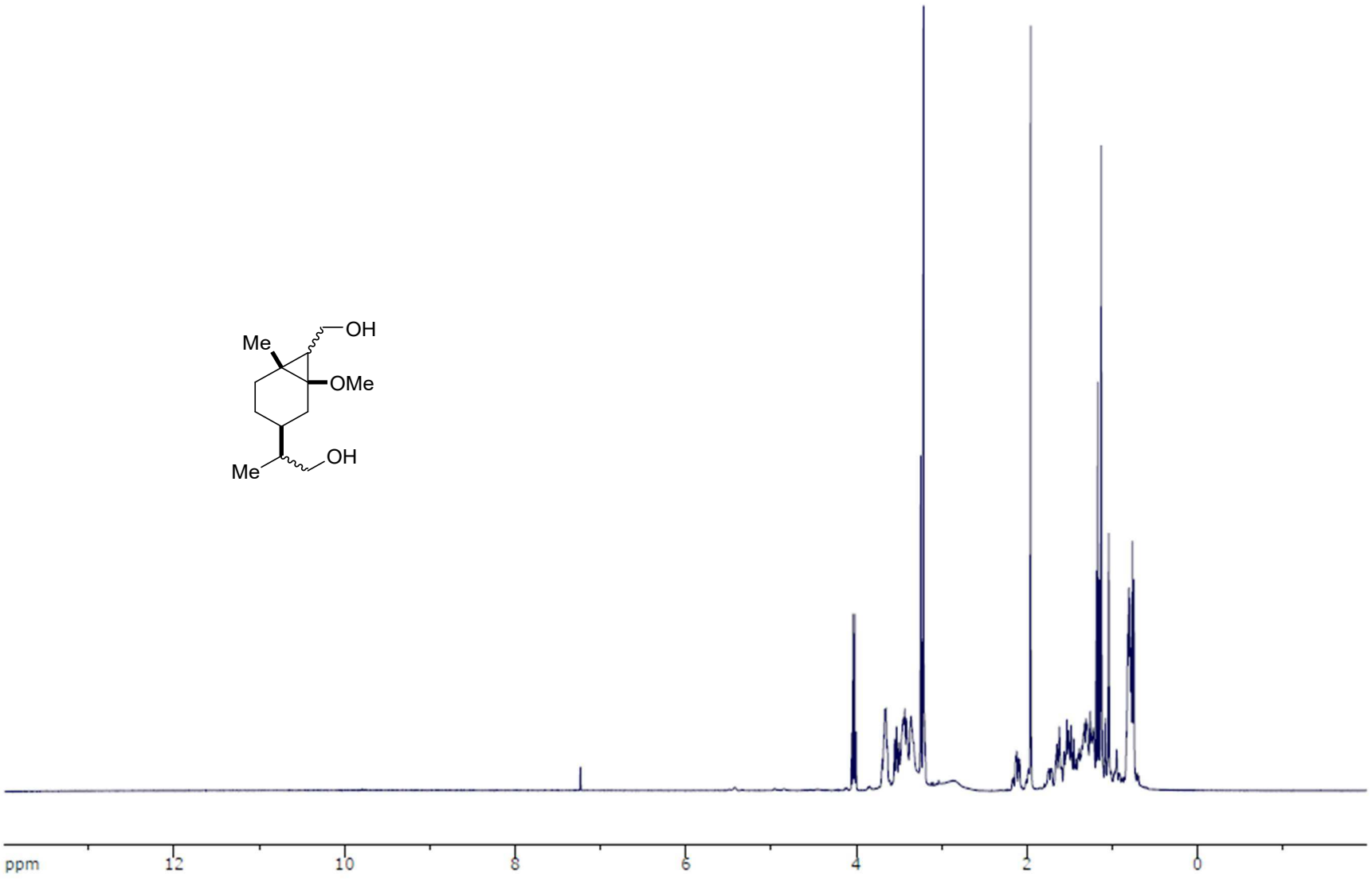
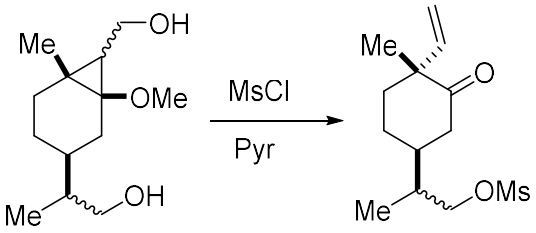


Figure 5.16. ^{1}H NMR of the product.



The diol (1.7g) was dissolved in anhydrous pyridine (200 mL) and cooled in an ice bath. MsCl was added dropwise and the solution was left to react at that temperature over 90 minutes. The reaction was then allowed to warm to room temperature and stirred for another 30 minutes, then poured into 1 M HCl and Et_2O, extracted with Et_2O 4 times and washed with 1M HCl and brine. The solution was condensed, then dissolved in 4:1 AcOH:H_2O and stirred for 2.5 hours at room temperature. The reaction mixture was diluted with Et_2O and H_2O and extracted with Et_2O 4 times. The organic layers were washed with saturated $NaHCO_3$ 4 times, brine twice, and the dried over sodium sulfate. The residue was purified by column chromatography to give a mixture of diastereomers.

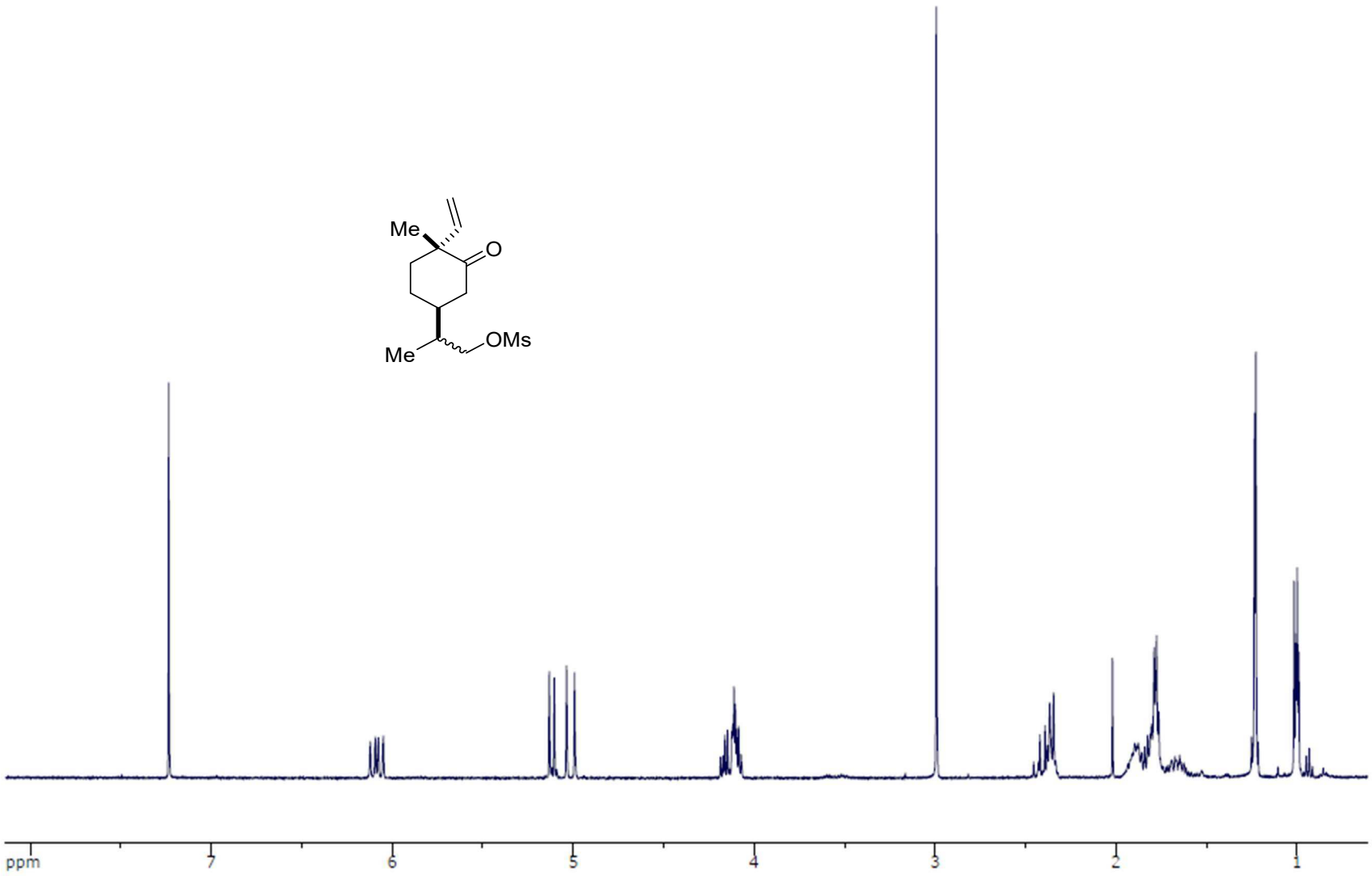
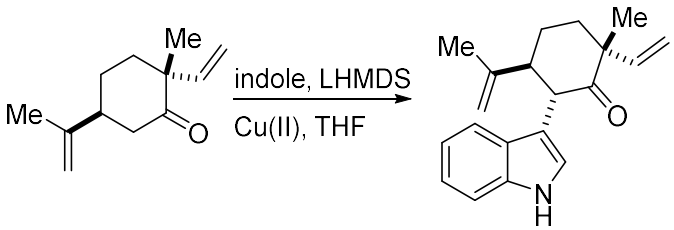


Figure 5.17. ^{1}H NMR of the product.



The cyclohexanone was dissolved in THF with 1.9 eq indole. LHMDS (3.4 eq, 1 M THF) was added at -78 °C dropwise. The reaction was left for 30 minutes at that temperature. Copper(II)-2-ethylhexanoate (0.2 M solution in THF, 1.5 eq) was added in one portion. After 5 minutes the cooling bath was pulled, and the solution was allowed to warm to room temperature. The solution was partitioned between 1M HCl and EtOAc. This was extracted 3 times with EtOAc, washed with HCl, NaOH, and brine, then dried. The residue was purified by column chromatography giving product in 50% yield. ^{1}H-NMR (400 MHz; $CDCl_3$): δ 6.01 (s, 1H), 5.33 (d, *J* = 5.9 Hz, 1H), 5.29 (d, *J* = 6.1 Hz, 1H), 5.12 (t, *J* = 5.1 Hz, 1H), 5.03 (t, *J* = 5.0 Hz, 1H), 4.90 (d, *J* = 2.4 Hz, 1H), 4.27 (dd, *J* = 15.7, 11.0 Hz, 1H), 5.11-5.03 (m, 2H), 4.61 (s, 1H), 4.55 (t, *J* = 1.5 Hz, 1H), 4.21 (d, *J* = 12.3 Hz, 1H), 2.92 (td, *J* = 11.9, 4.1 Hz, 1H), 2.18 (qd, *J* = 12.7, 4.1 Hz, 1H), 2.04 (td, *J* = 13.0, 3.9 Hz, 1H), 1.97 (t, *J* = 3.8 Hz, 1H), 1.90 (ddt, *J* = 16.2, 11.0, 3.6 Hz, 1H), 1.58 (s, 3H), 1.51 (s, 3H). 13-C NMR (101 MHz; cdcl3): δ 211.5, 144.6, 143.0, 134.0, 125.3, 123.1, 121.6, 119.17, 116.99, 112.14, 112.01, 111.7, 111.1, 52.4, 50.6, 45.9, 34.8, 25.6, 23.0, 16.4. $(M+H)^+$ $C_{20}H_{24}NO$ 294.1852 m/z

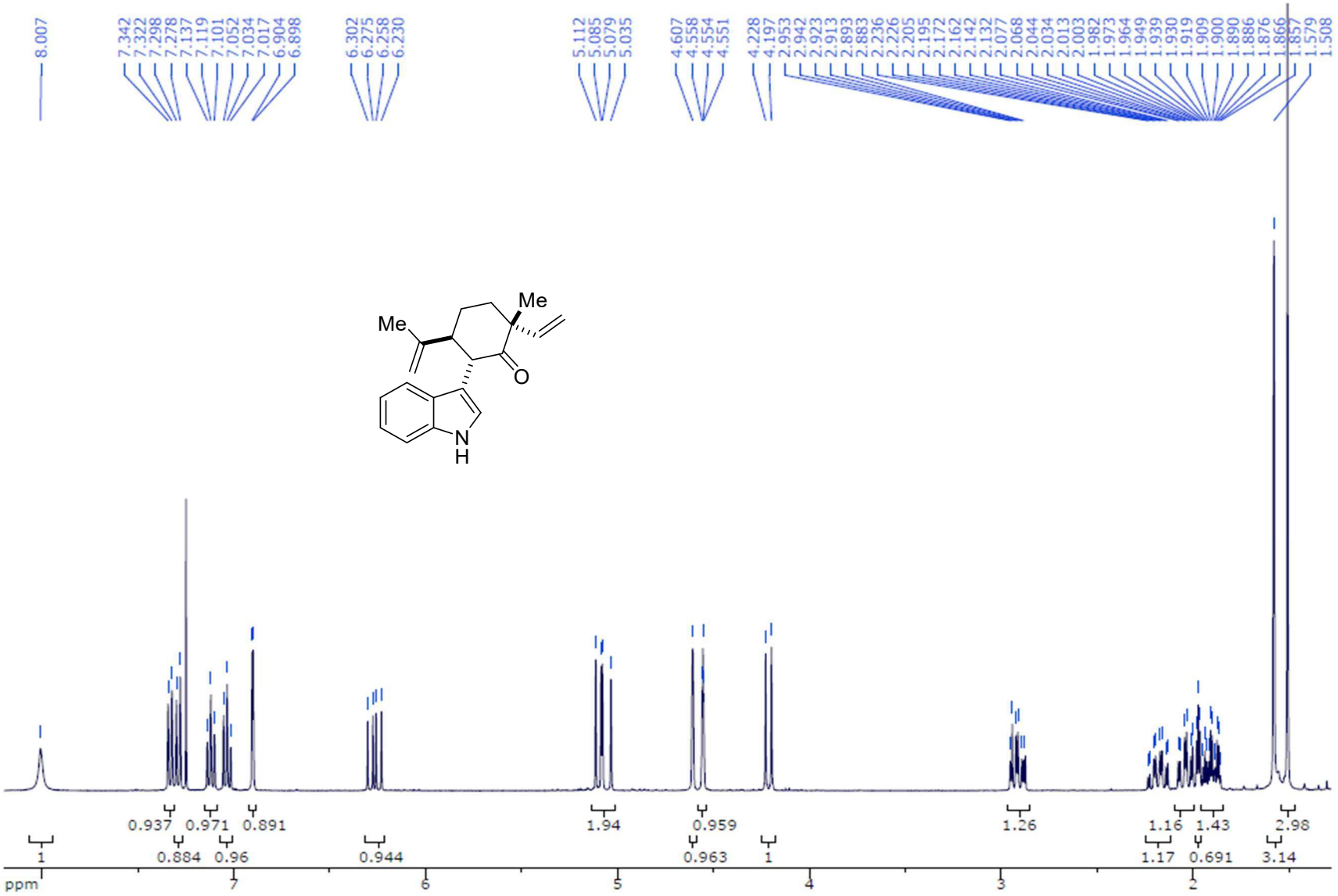


Figure 5.18. ^{1}H NMR of the product.

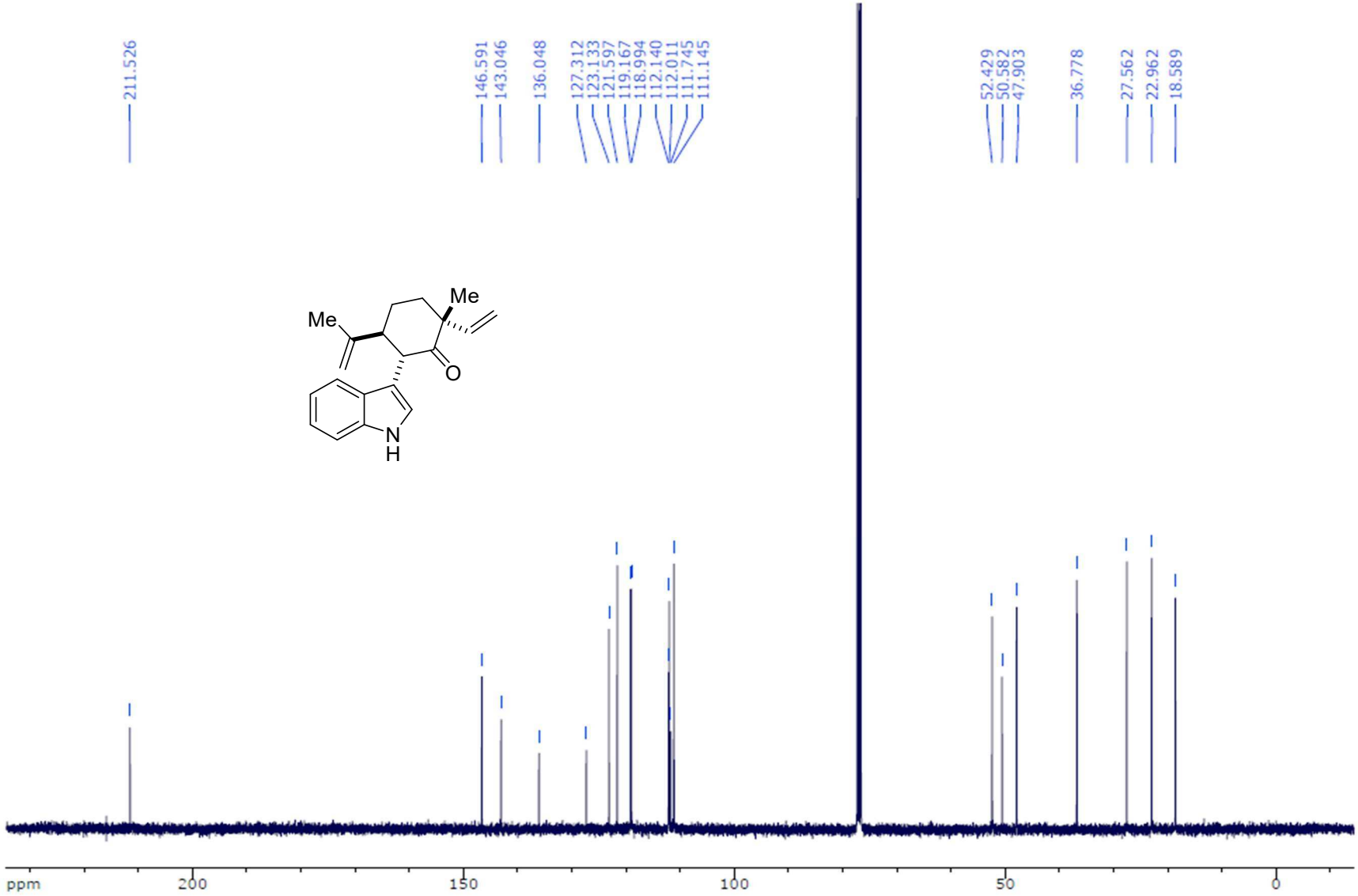
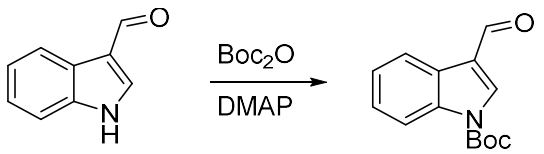
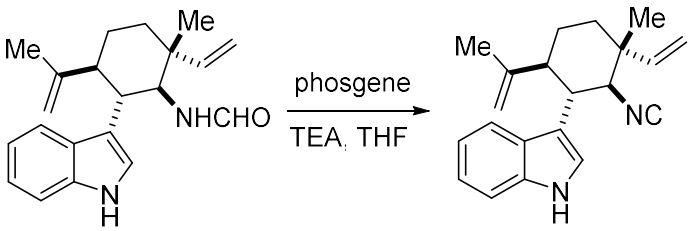


Figure 5.19. ^{13}C NMR of the product.



The aldehyde (3.0 g) was dissolved in acetonitrile (70 mL), followed by addition of DMAP (0.50 g) and boc anhydride (4.6 g). A small amount of gas evolved, following which the solution was left to stir for one hour. After completion of the reaction the acetonitrile was evaporated and the solid was dissolved in ethyl acetate. This was washed with H_2O twice, followed by brine and dried over Na_2CO_3. Concentration of the solution quantitatively yielded orange, mostly pure product which was used without further purification.



The formamide was dissolved in DCM and TEA (20 eq) in a flame dried flask under argon. Phosgene (solution in toluene) was added dropwise at 0 °C until the starting material was consumed by TLC. The reaction was quenched while still cold with $NaHCO_3$ and warmed to room temperature. The solution was extracted with DCM 5 times, washed with brine and dried over sodium sulfate. The solvent was reduced and purified by chromatography to give the pure product. ^{1}H-NMR (400 MHz; $CDCl_3$): δ 6.00 (s, 1H), 5.65 (d, J = 5.9 Hz, 1H), 5.34 (d, J = 6.1 Hz, 1H), 5.17 (td, J = 5.6, 1.0 Hz, 1H), 5.10 (t, J = 5.1 Hz, 1H), 5.02 (d, J = 2.4 Hz, 1H), 5.87 (dd, J = 15.5, 10.8 Hz, 1H), 5.16-5.09 (m, 2H), 4.49 (d, J = 23.0 Hz, 2H), 3.95-3.84 (m, 1H), 3.18 (t, J = 11.1 Hz, 1H), 2.79-2.67 (m, 1H), 1.79-1.64 (m, 4H), 1.53 (s, 3H), 1.30 (s, 3H).

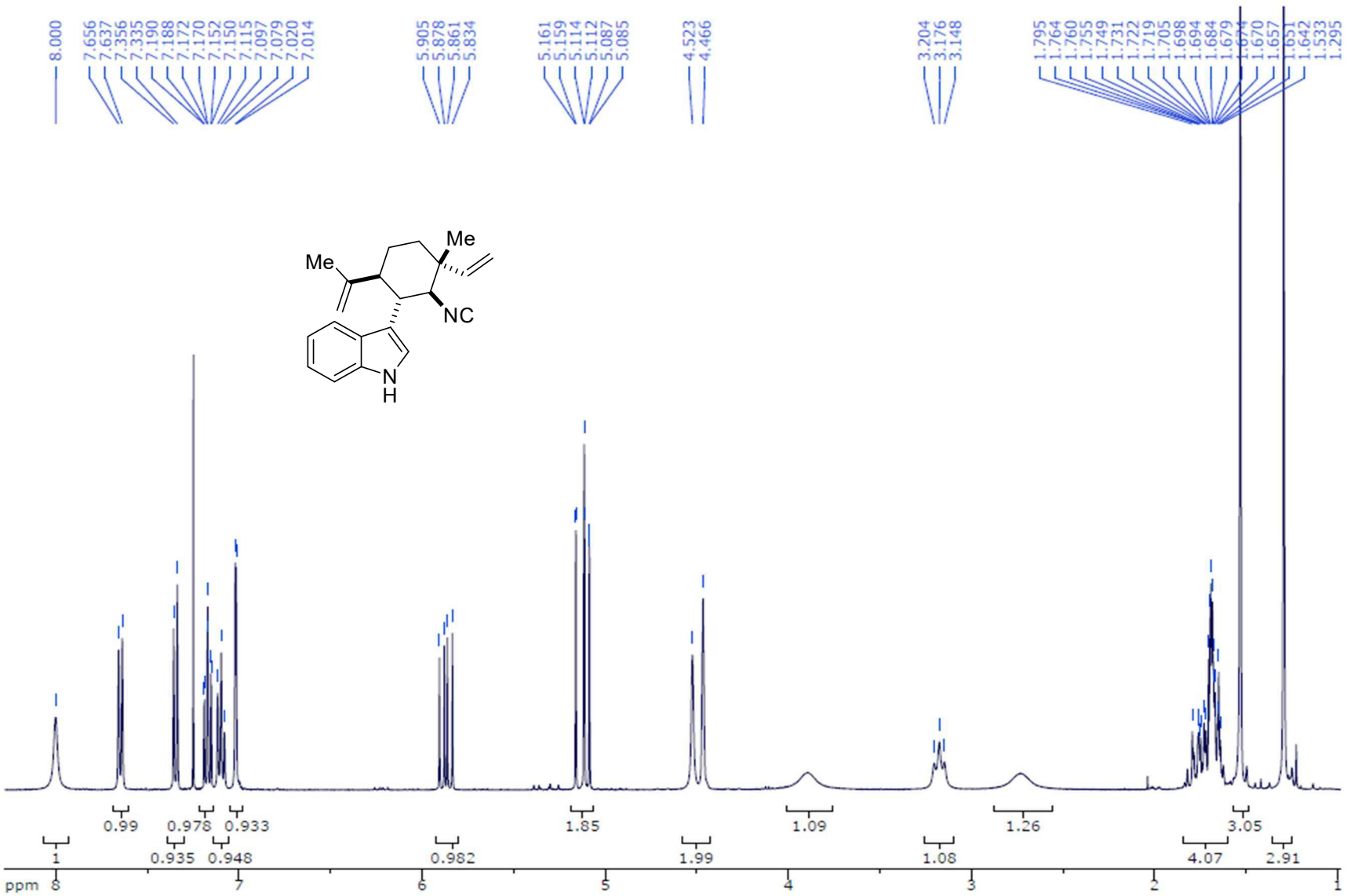


Figure 5.20. ^{1}H NMR of the product.

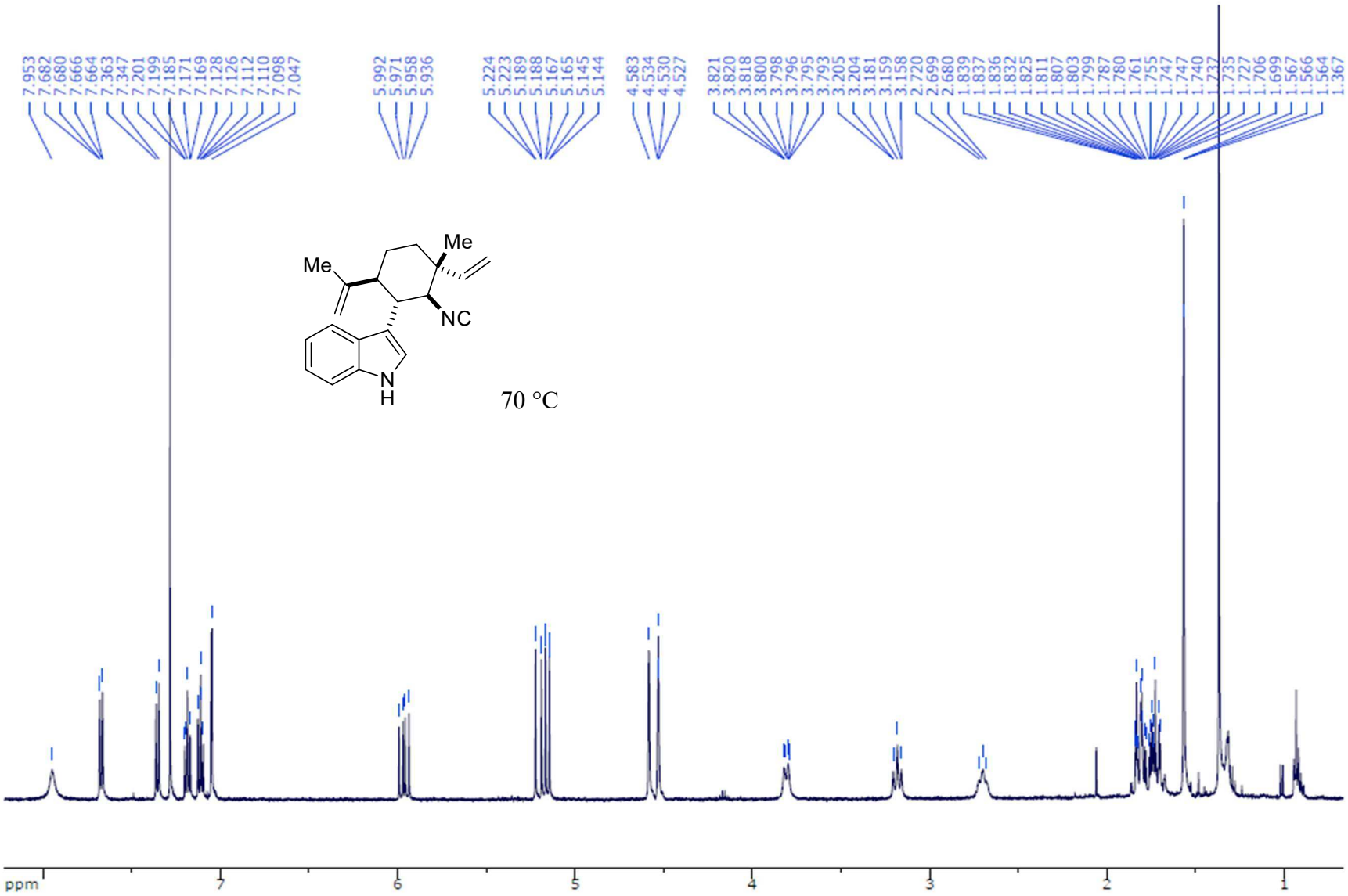
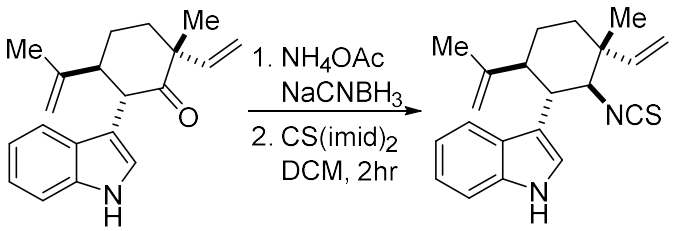
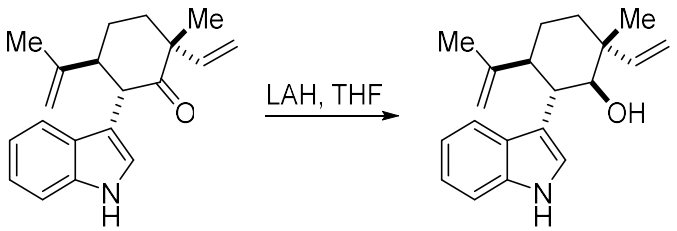


Figure 5.21. ^{1}H NMR of the product.



The carbonyl was dissolved in 1:10 mixture of THF:MeOH with 10 eq $NaCNBH_3$ and 40 eq NH_4OAc and given to microwave irradiation (130 °C for 5 min). This was partitioned between EtOAc and 1N NaOH and the aqueous layer was extracted with EtOAc 3 times. Organics were washed with 1N NaOH and brine, and dried over sodium sulfate. The residue was dissolved in DCM and treated with 1 eq of $CS(imid)_2$ for 2 hrs. The solution was reduced and purified by column chromatography giving 11-epi-hapalindole D.



The carbonyl (42 mg) was dissolved in 3 ml THF at room temperature, followed by addition of 1.0 M LAH in THF (1.5 eq). After 12 h Rochelle's salt was added and left to stir for 2 h. The organics were dried, condensed and purified through column chromatography. 37 mg of pure colorless oil alcohol were recovered. ^{1}H-NMR (400 MHz; $CDCl_3$): δ 6.03 (s, 1H), 5.73 (d, *J* = 5.9 Hz, 1H), 5.34 (d, *J* = 6.1 Hz, 1H), 5.17 (t, *J* = 5.5 Hz, 1H), 5.09 (t, *J* = 5.5 Hz, 1H), 5.02 (d, *J* = 2.3 Hz, 1H), 5.99 (dd, *J* = 15.6, 10.8 Hz, 1H), 5.11 (d, *J* = 15.6 Hz, 1H), 5.04 (d, *J* = 10.8 Hz, 1H), 4.53 (s, 1H), 4.45 (s, 1H), 3.73 (d, *J* = 9.3 Hz, 1H), 3.04 (t, *J* = 11.1 Hz, 1H), 2.70 (t, *J* = 11.2 Hz, 1H), 1.80-1.61 (m, 4H), 1.54 (d, *J* = 10.5 Hz, 4H), 1.23 (s, 3H). 13-C NMR (101 MHz; cdcl3): δ 146.2, 145.6, 134.5, 122.0, 119.7, 119.3, 111.5, 111.30, 111.18, 50.05, 50.02, 41.5, 34.3, 25.6, 19.1, 15.3. $(M+H)^+$ $C_{20}H_{26}NO$, 294.2009 m/z

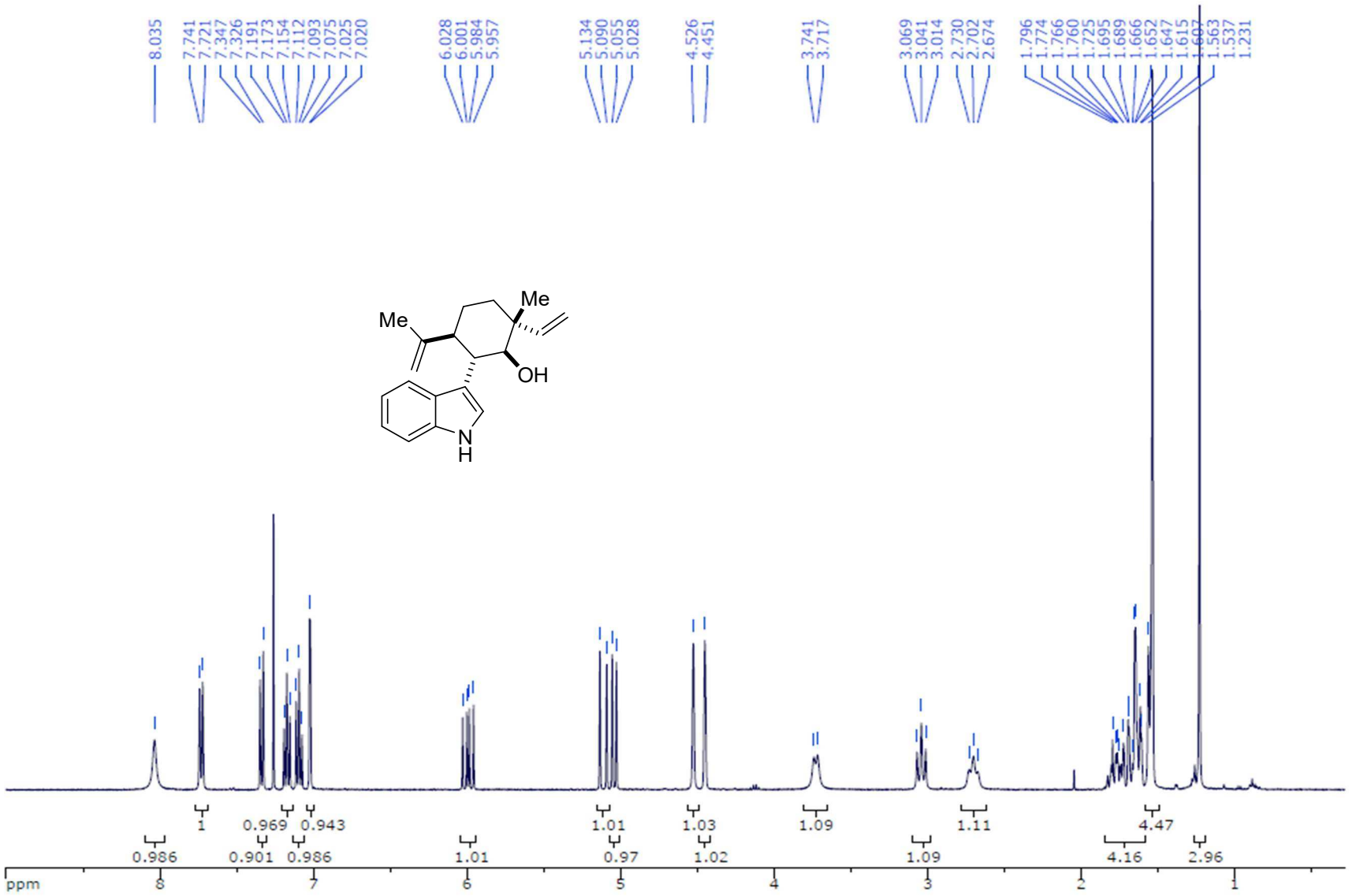


Figure 5.22. ^{1}H NMR of the product.

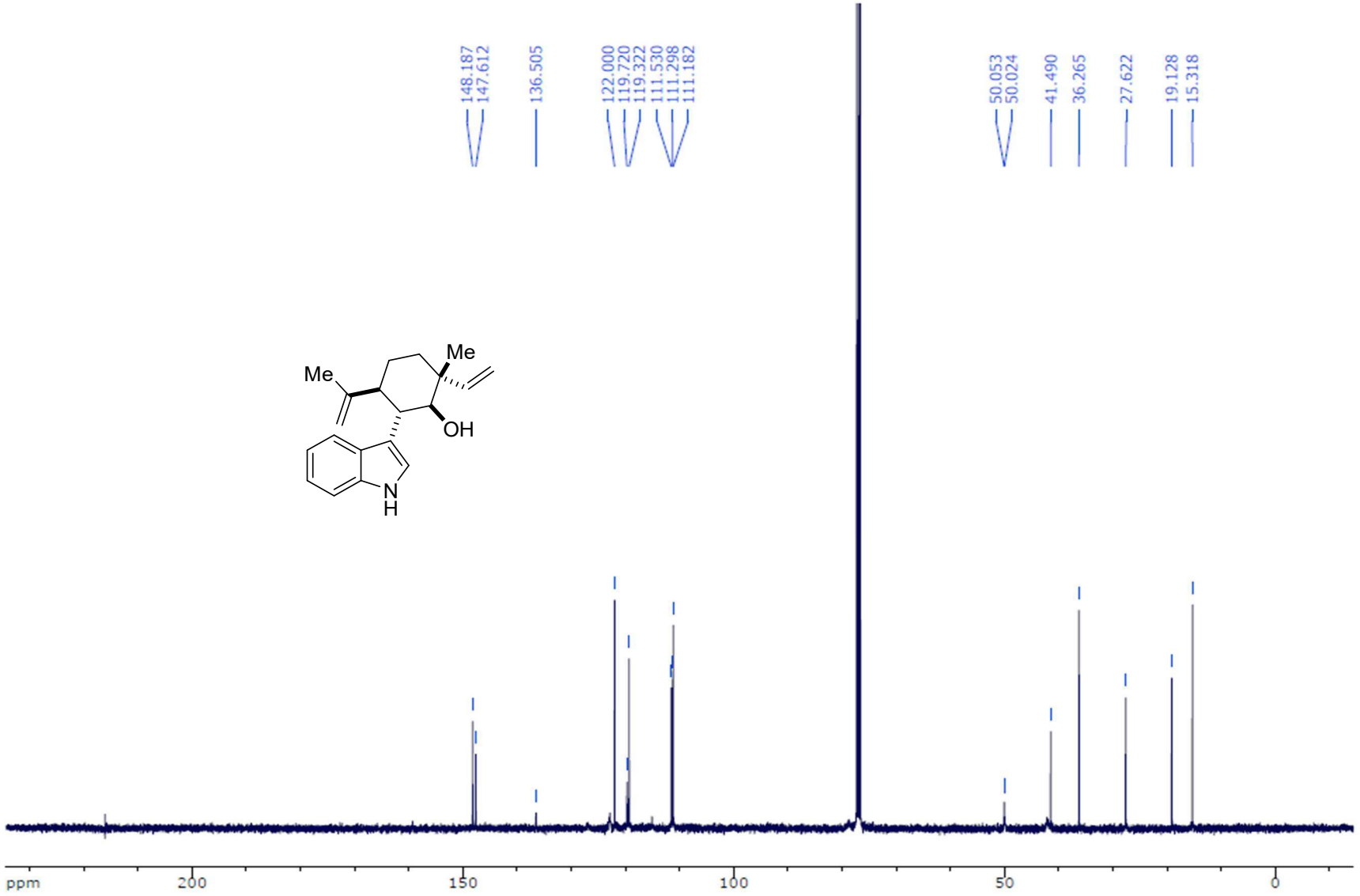
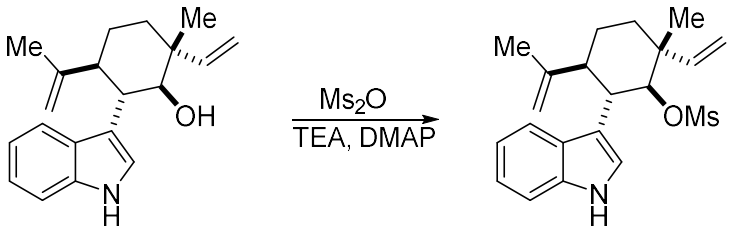


Figure 5.23. ^{13}C NMR of the product.



The alcohol was dissolved in DCM, 1.1 eq TEA and 0.1 eq DMAP. Freshly recrystallized Ms_2O (1 eq) was added and left at room temperature for 2 hrs. The solution was condensed and purified by preparatory TLC. ^{1}H-NMR (400 MHz; $CDCl_3$): δ 6.13 (s, 1H), 5.58 (s, 1H), 5.29 (d, *J* = 5.7 Hz, 1H), 5.17-5.04 (m, 2H), 5.99 (dd, *J* = 14.6, 11.2 Hz, 1H), 5.17-5.12 (m, 2H), 4.55 (d, *J* = 54.7 Hz, 2H), 3.44 (s, 1H), 2.50 (s, 1H), 1.79-1.64 (m, 4H), 1.46 (d, *J* = 0.3 Hz, 4H), 1.37-1.25 (m, 6H). 13-C NMR (101 MHz; cdcl3): δ 145.8, 126.8, 122.2, 119.8, 113.4, 111.9, 111.19, 111.18, 94.5, 75.31, 75.19, 75.16, 75.11, 75.07, 74.99, 74.89, 74.84, 74.82, 74.80, 74.76, 74.67, 74.56, 52.42, 52.39, 42.4, 35.52, 35.47, 35.45, 34.3, 29.7, 25.6, 22.7, 19.2, 15.7, 14.1

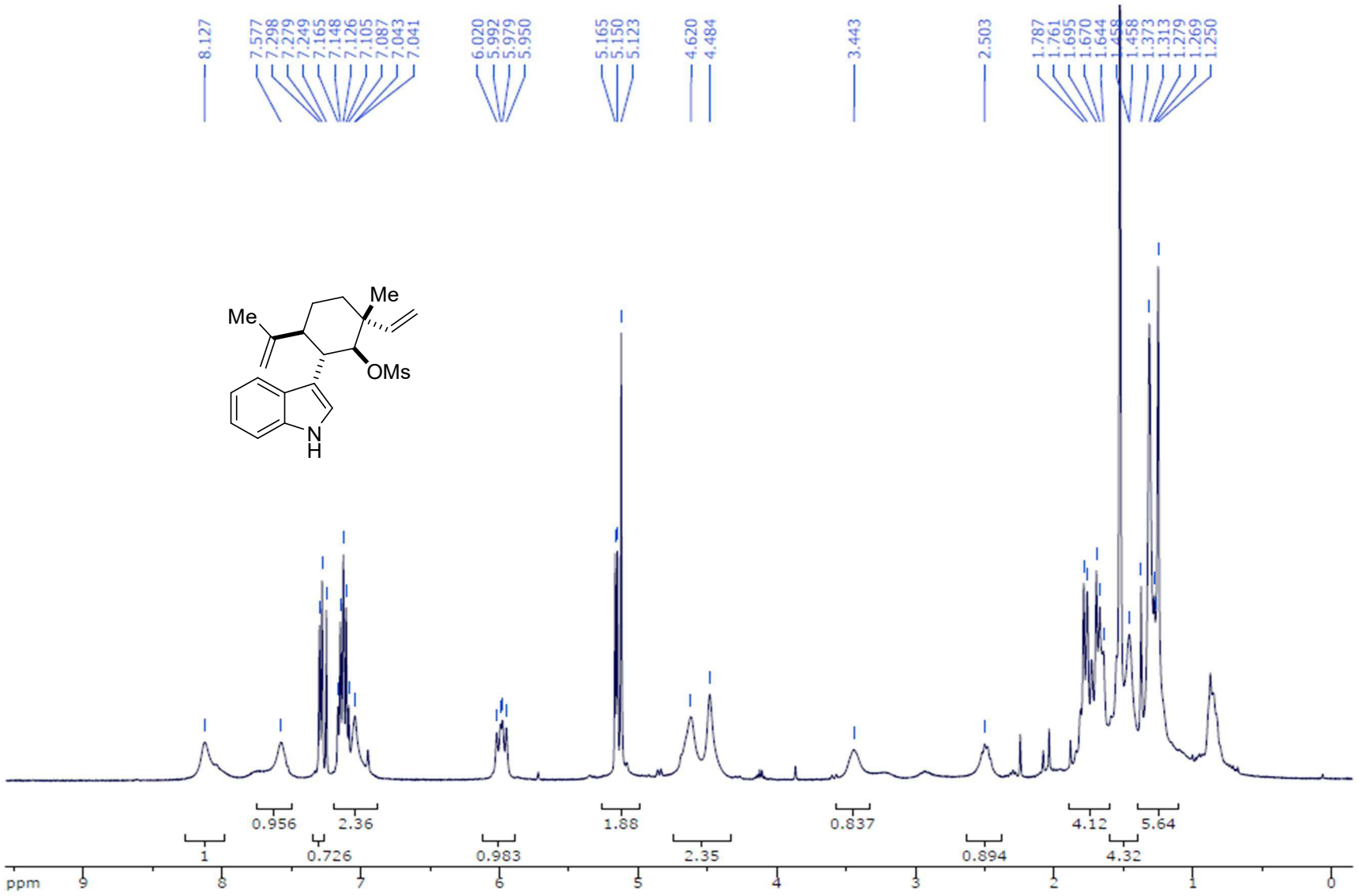


Figure 5.24. ^{1}H NMR of the product.

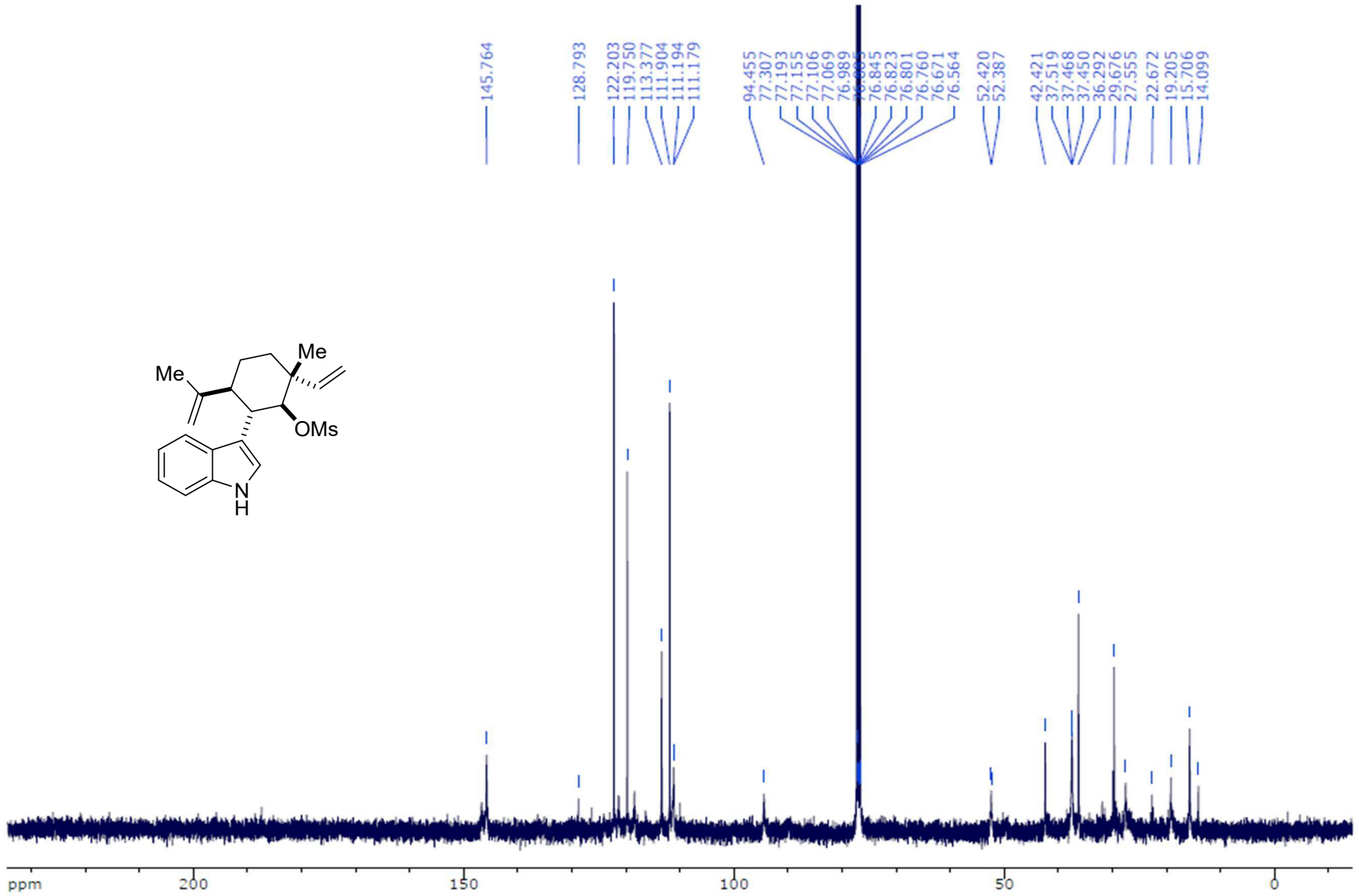
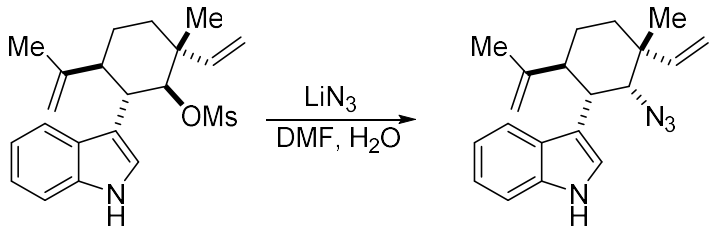
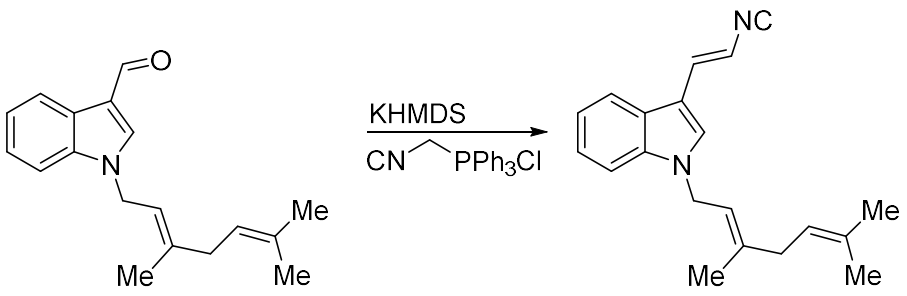


Figure 5.25. ^{13}C NMR of the product.



The mesylate material was dissolved in DMF and LiN_3 dissolved in H_2O was added. The solution was heated to 100 °C and left overnight. The product was condensed and purified by preparatory TLC. $(M+Cl)^-$ $C_{20}H_{24}ClN_4$ 355.1695 m/z



Cis: ^{1}H-NMR (400 MHz; $CDCl_3$): δ 6.04 (s, 1H), 5.65 (d, *J* = 5.9 Hz, 1H), 5.34 (s, 1H), 5.28-5.23 (m, 4H), 5.20 (t, *J* = 5.4 Hz, 1H), 4.75 (d, *J* = 6.2 Hz, 1H), 5.67 (d, *J* = 6.8 Hz, 1H), 5.38 (t, *J* = 4.7 Hz, 1H), 5.04 (s, 1H), 4.77 (d, *J* = 4.8 Hz, 2H), 2.09 (d, *J* = 6.0 Hz, 4H), 1.84 (s, 3H), 1.63 (s, 3H), 1.56 (s, 3H), 1.53 (s, 1H).

Trans: ^{1}H-NMR (400 MHz; $CDCl_3$): δ 5.65 (d, *J* = 5.9 Hz, 1H), 5.35 (d, *J* = 6.1 Hz, 1H), 5.29-5.26 (m, 1H), 5.25-5.20 (m, 2H), 5.08 (d, *J* = 14.3 Hz, 1H), 4.29 (d, *J* = 14.3 Hz, 1H), 5.35 (t, *J* = 4.9 Hz, 1H), 5.05 (s, 1H), 4.68 (d, *J* = 4.9 Hz, 2H), 2.10 (s, 4H), 1.80 (s, 3H), 1.66 (s, 3H), 1.58 (s, 3H).

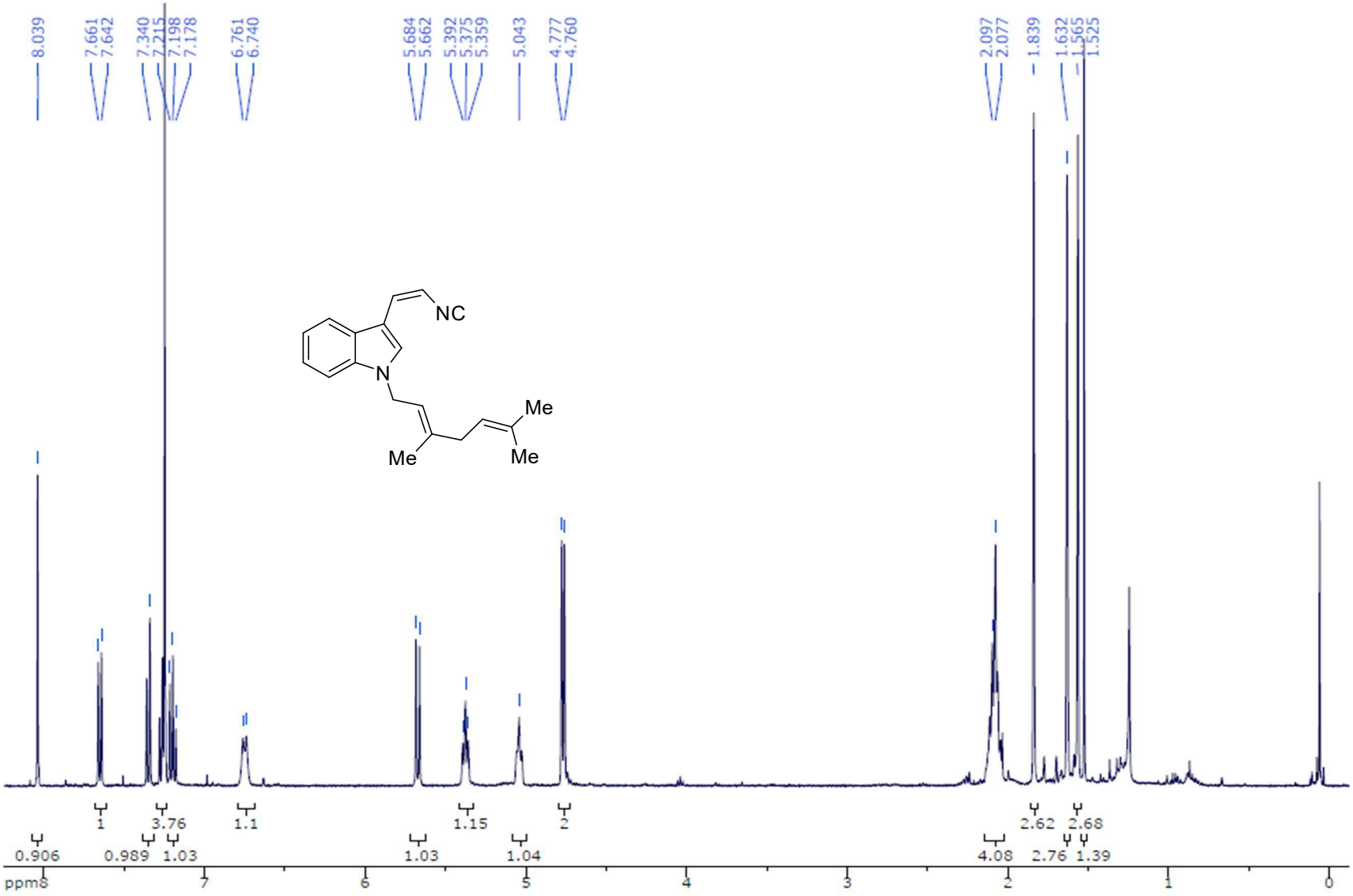


Figure 5.26. ^{1}H NMR of the product.

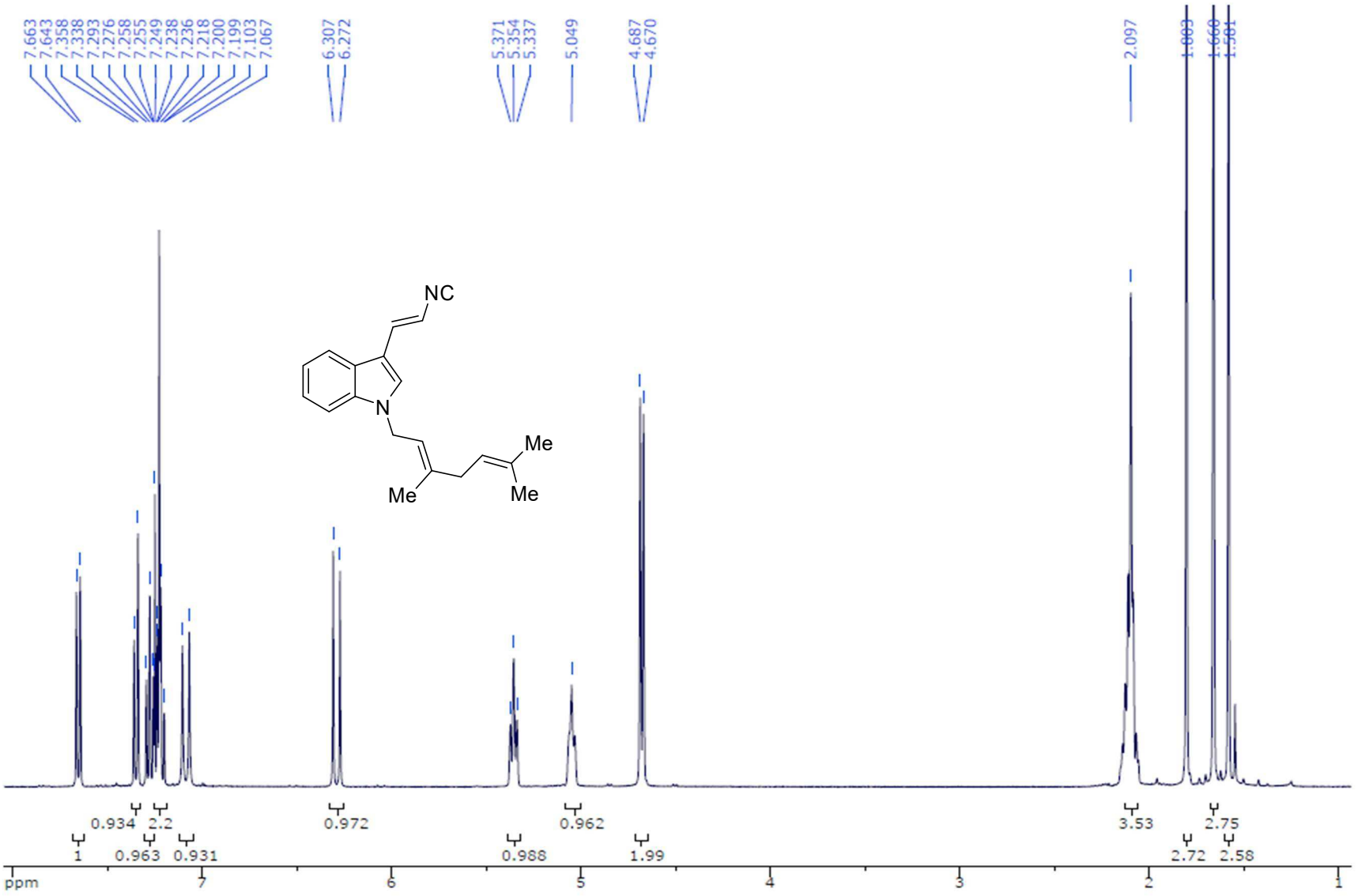
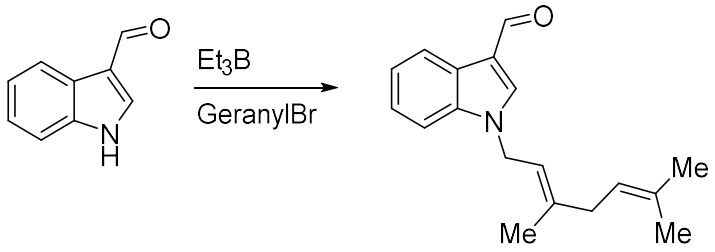


Figure 5.27. ^{1}H NMR of the product.



^{1}H-NMR (400 MHz; $CDCl_3$): δ 9.98 (s, 1H), 6.31-6.29 (m, 1H), 5.72 (s, 1H), 5.38-5.30 (m, 3H), 5.42 (t, *J* = 5.0 Hz, 1H), 5.06 (s, 1H), 4.74 (d, *J* = 5.0 Hz, 2H), 2.13 (s, 4H), 1.82 (s, 3H), 1.66 (s, 3H), 1.59 (s, 3H).

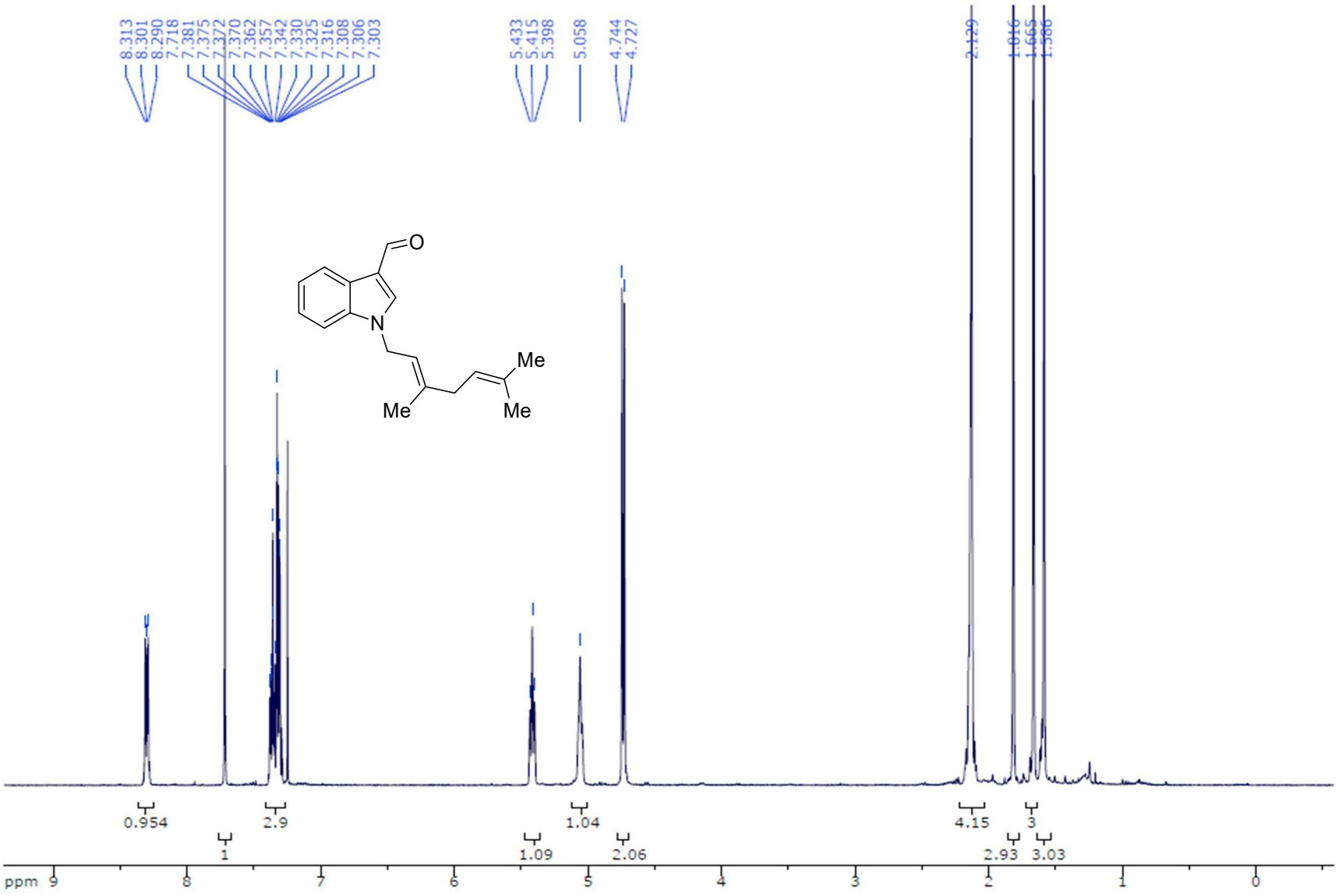
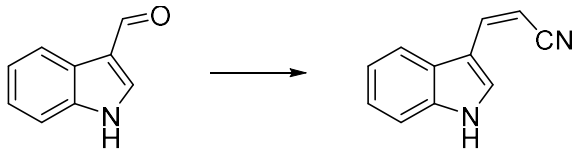


Figure 5.28. ^{1}H NMR of the product.



^{1}H-NMR (400 MHz; $CDCl_3$): δ 6.51 (s, 1H), 6.14 (d, *J* = 2.8 Hz, 1H), 5.67 (d, *J* = 5.8 Hz, 1H), 5.44-5.42 (m, 1H), 5.27 (t, *J* = 5.6 Hz, 1H), 5.22 (t, *J* = 5.4 Hz, 1H), 4.78 (t, *J* = 4.2 Hz, 1H), 5.74 (d, *J* = 6.9 Hz, 1H).

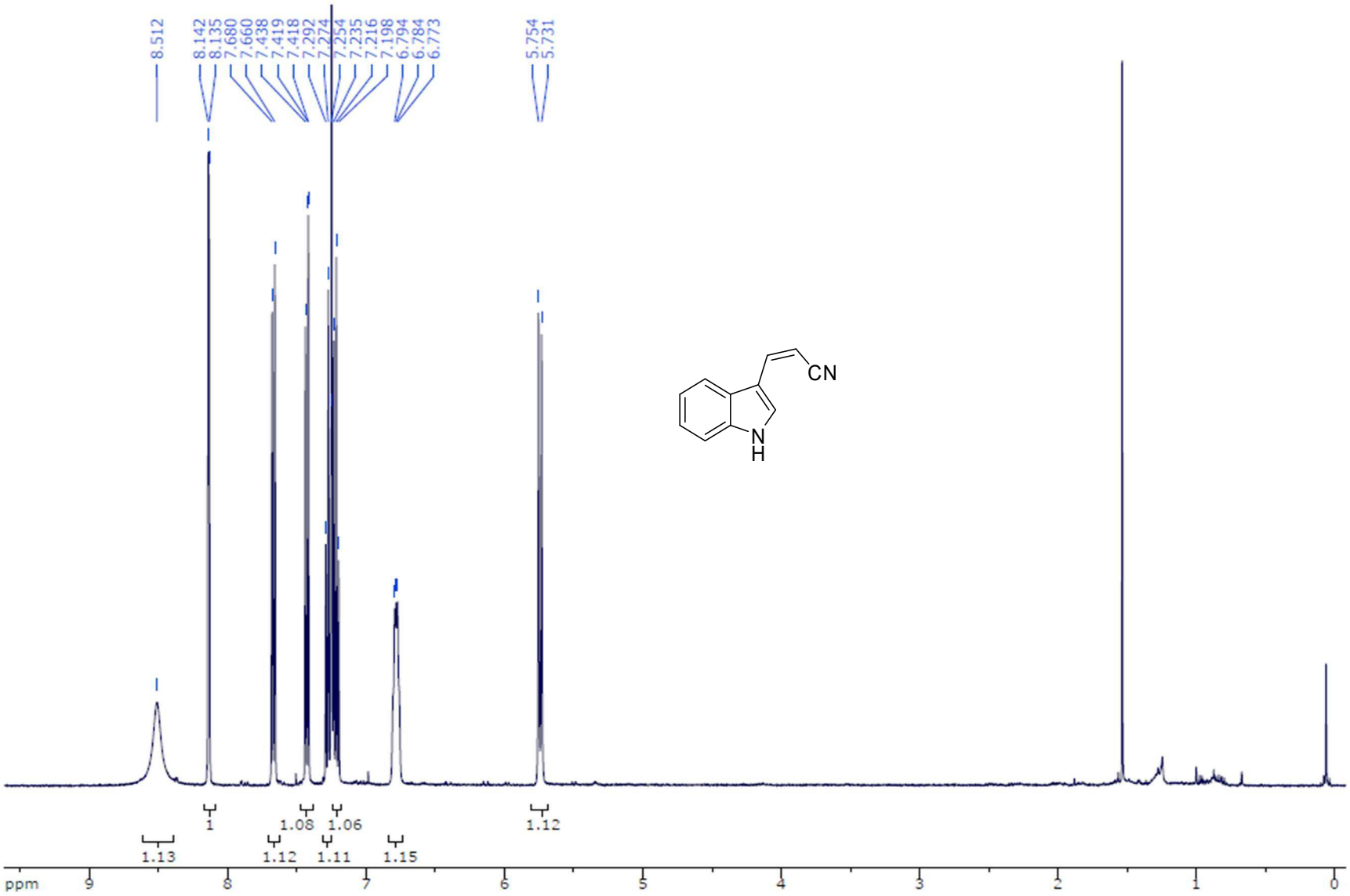


Figure 5.29. ^{1}H NMR of the product.

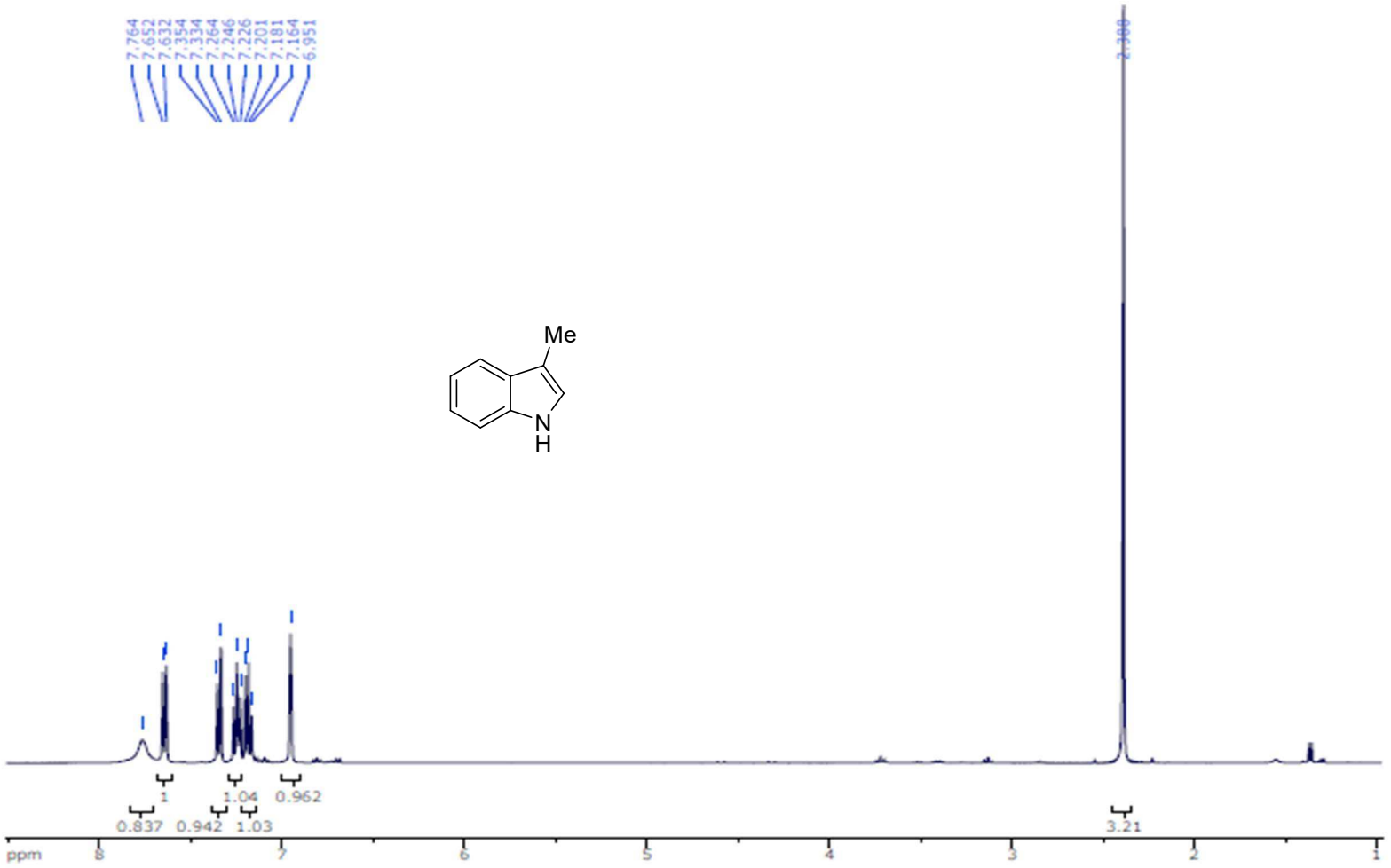


Figure 5.30. ^{1}H NMR of the product.

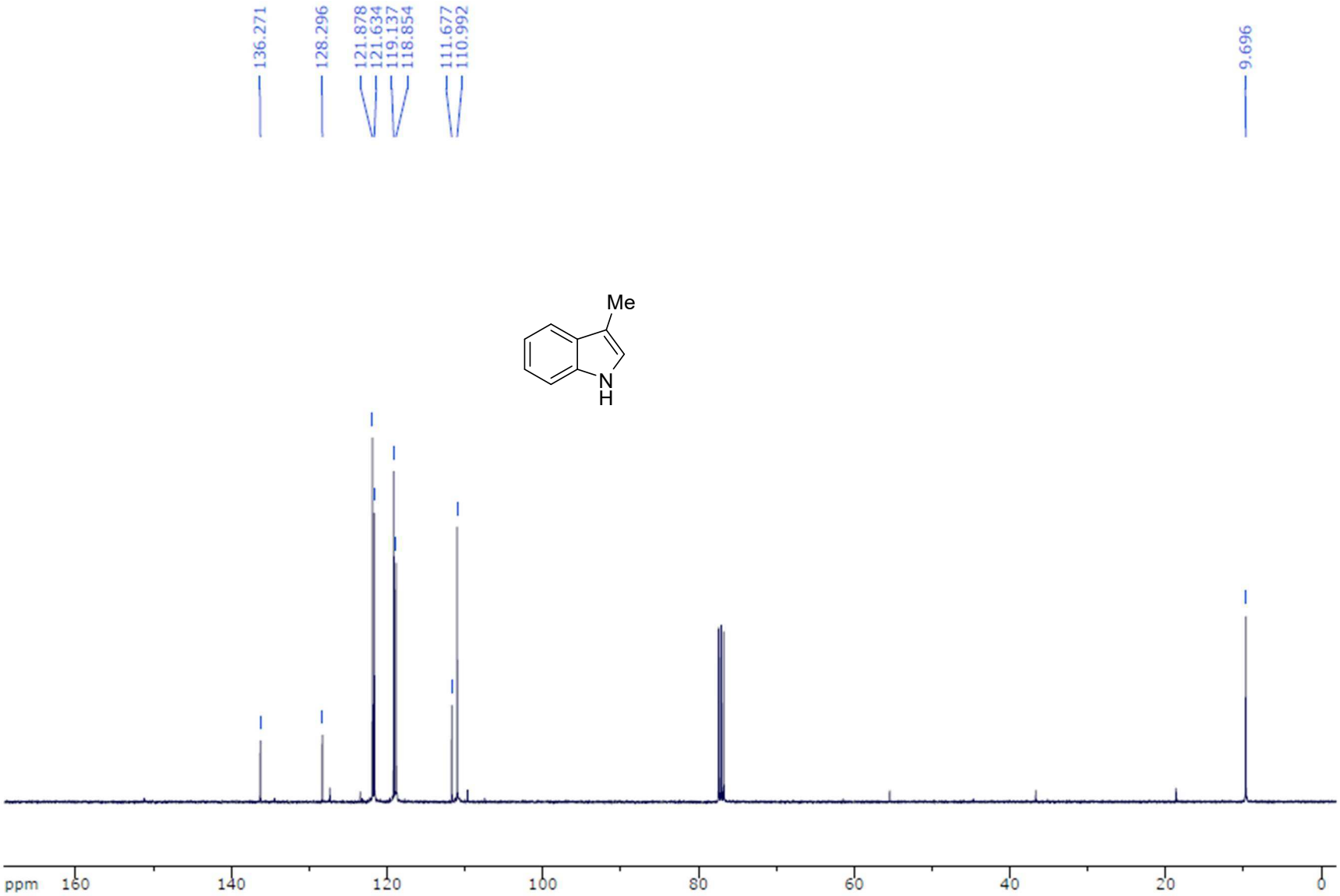


Figure 5.31. ^{13}C NMR of the product.

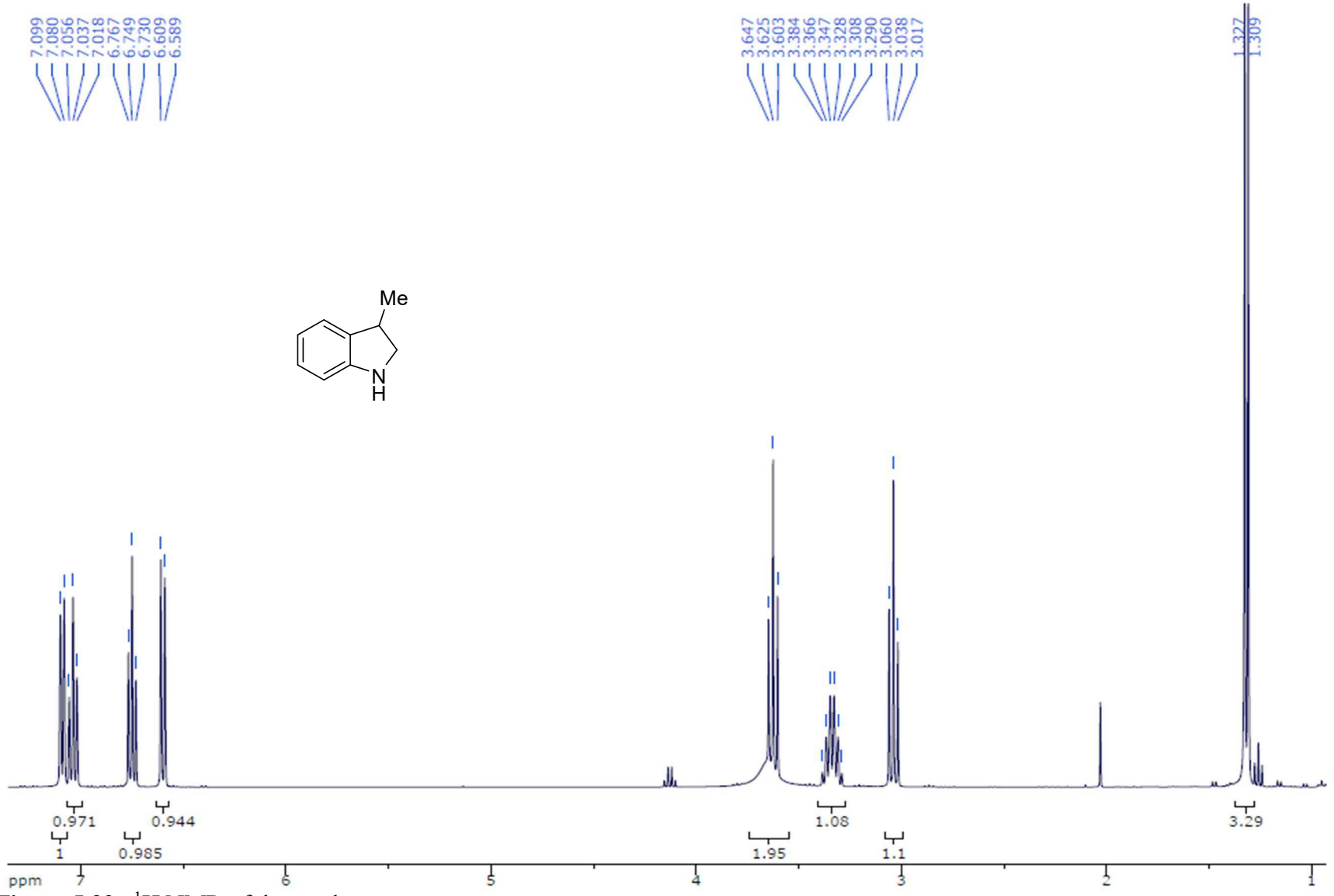


Figure 5.32. ^{1}H NMR of the product.

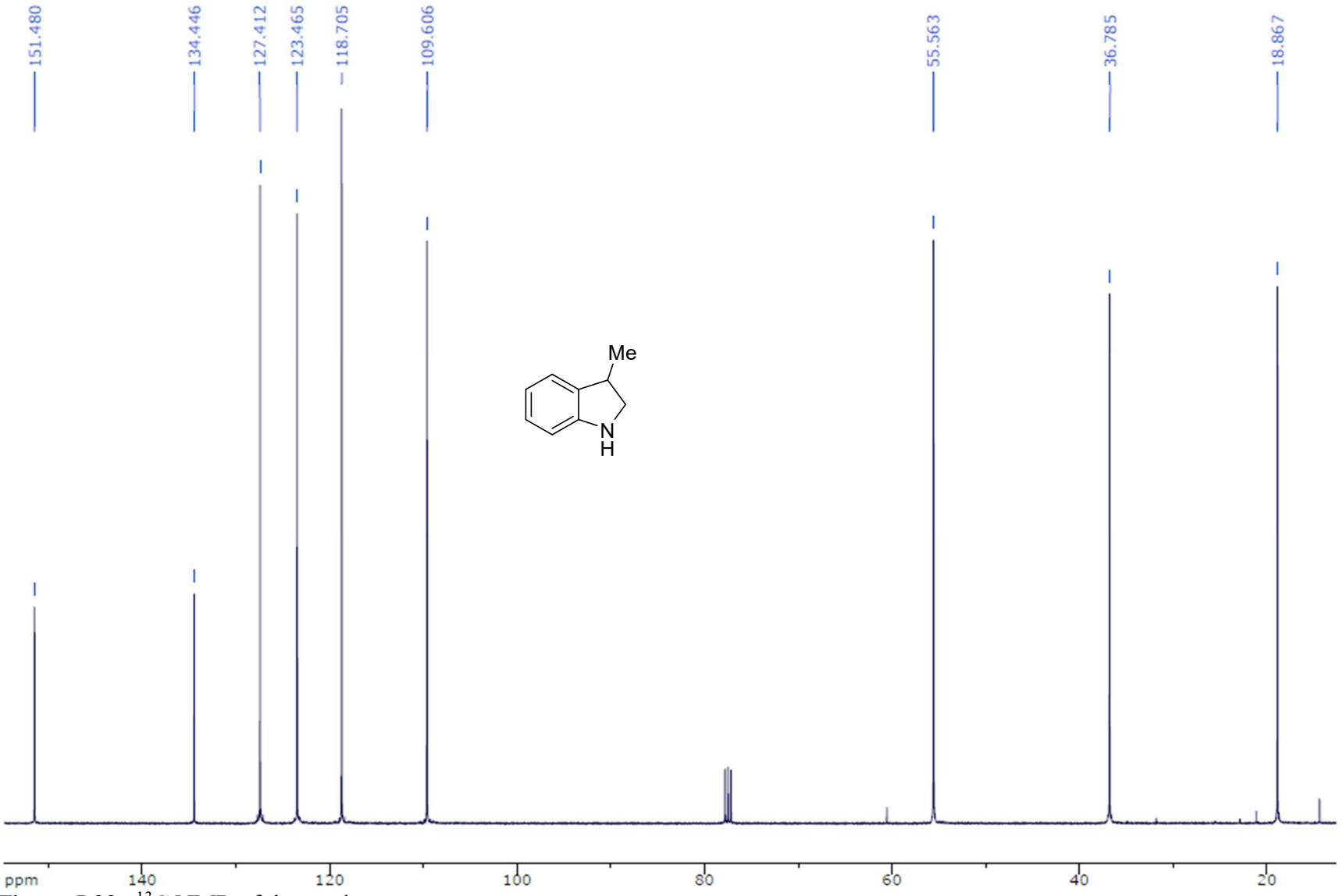


Figure 5.33. ^{13}C NMR of the product.

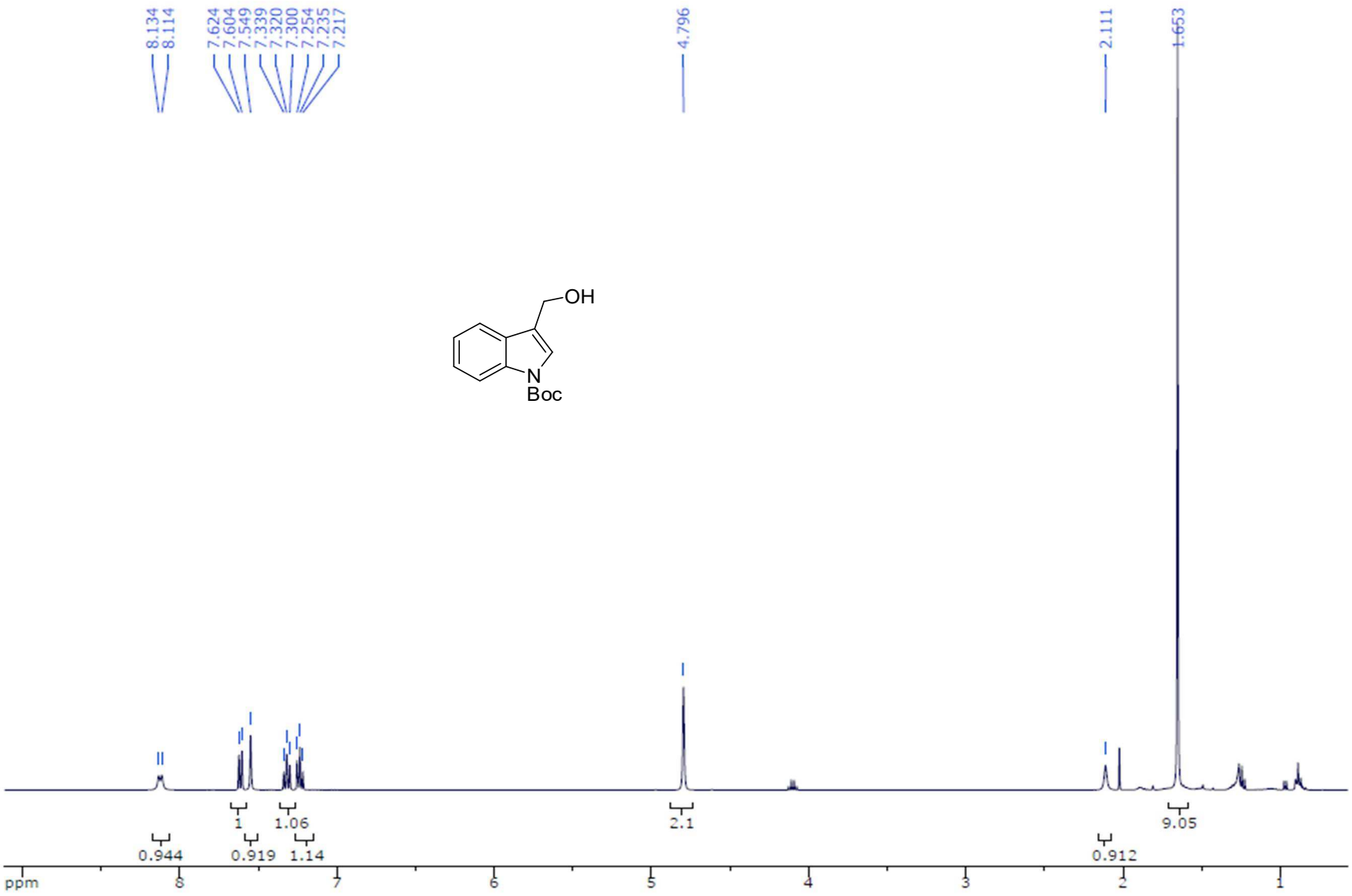


Figure 5.34. ^{1}H NMR of the product.

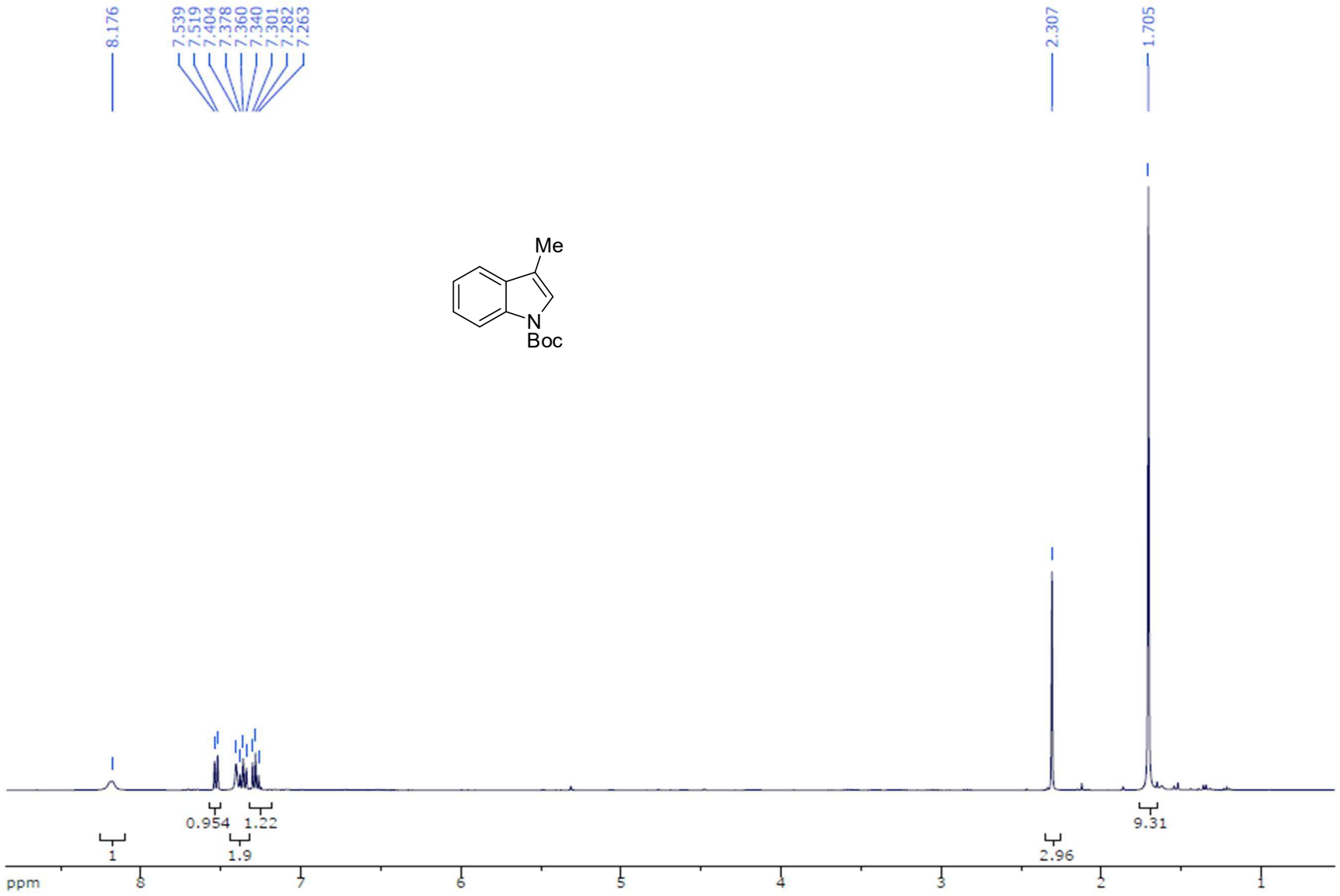


Figure 5.35. ^{1}H NMR of the product.

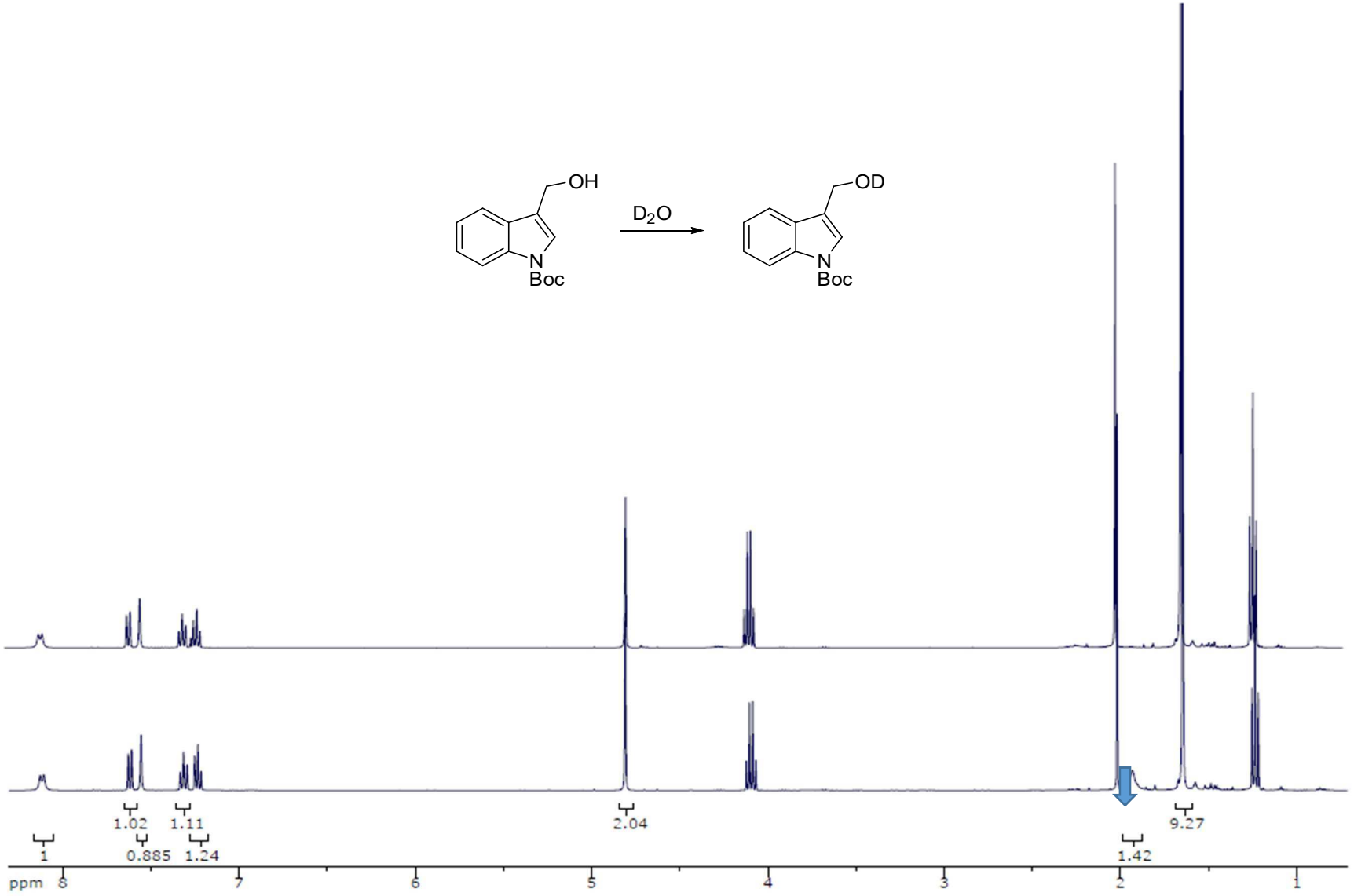


Figure 5.36. ^{1}H NMR of the product.

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