Synthesis of unnatural analogues of narciclasine: Chemoenzymatic synthesis of 2-*epi*-1-hydroxymethylnarciclasine

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Abstract

An approach to the synthesis of novel C-1 analogues of narciclasine has been developed. The synthesis relies on chemoenzymatic dihydroxylation of an arene, Stille coupling, and an intramolecular Heck reaction to affect key transformations. The synthesis of the targeted C-1 analogues addresses a gap in literature where few narciclasine analogues have been prepared, with no C-1 homologues existing to date. The synthetic route to 2-*epi*-1-hydroxymethylnarciclasine, as well as a possible route to several other derivatives, is described in detail. Experimental and spectral data are provided for all newly synthesized compounds.

Acknowledgements

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I love you all and hope I have and will continue to make you proud. You've all helped me become a better person.

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List of Abbreviations

µ	Micro
Å	Angstroms
Ac	Acetyl
acac	Acetylacetonate
aq	Aqueous
Ar	Aromatic
atm	Standard atmosphere
B.C.	Before Christ
Bn	Benzyl
BRSM	Based on recovered starting material, yield
Bu	Butyl
Bz	Benzoyl
C	Celsius
Cbz	Carboxybenzyl
cod	Cyclooctadiene
mCPBA	*meta*-Chloroperoxybenzoic acid
d	Doublet
DCM	Dichloromethane
DEAD	Diethyl azodicarboxylate
DIBAL	Diisobutylaluminum hydride
DIPHOS	1,2-*Bis*(diphenylphosphino)ethane
DMAP	4-Dimethylaminopyridine

DMF	*N*,*N*-Dimethylformamide
2,2-DMP	2,2-Dimethoxypropane
DMP	Dess-Martin Periodinane
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPE	1,2-*Bis*(diphenylphosphino)ethane
EI	Electron ionization
eq	Equivalent
ESI	Electrospray ionization
Et	Ethyl
g	Grams
GI_{50}	Concentration for 50% of maximal inhibition of cell proliferation
h	Hour
HMDS	Hexamethyldisilazane
HRMS	High-resolution mass spectroscopy
Hz	Hertz
hν	Light
IC_{50}	Concentration for 50% of maximal inhibition of biological function
IR	Infrared Spectroscopy
L	Litre
LD_{50}	Amount of an ingested substance that kills 50 % of a test population
lit.	Literature
LRMS	Low-resolution mass spectrometry

M	Molar
m	Multiplet
m	Meta
m.p.	Melting point
Me	Methyl
Min	Minute
mol	Mole
Moc	Methoxycarbonyl
MTAD	4-Methyl-1,2,4-triazoline-3,5-dione
NBS	*N*-Bromosuccinimide
NCI	National Cancer Institute
NMO	*N*-Methylmorpholine *N*-oxide
NMR	Nuclear Magnetic Resonance
o	Ortho
p	Para
PEL	Primary effusion lymphoma
q	Quartet
R_f	Retention factor
RNA	Ribonucleic acid
r.t.	Room temperature
s	Singlet
SAR	Structure-activity relationship
t	Triplet

TBAF	Tetra-*n*-butylammonium fluoride
*t*Bu	*Tert*-butyl
TDO	Toluene dioxygenase
TFA	Trifluoracetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMP	2,2,6,6-Tetramethylpiperidine
Ts	Toluenesulfonyl/tosyl
UV	Ultraviolet
v	Wave number

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1. Introduction

This thesis will discuss the synthesis of homologues of narciclasine, a natural product produced in the Amaryllidaceae family of plants. The outlined synthetic route aimed to synthesize the novel unnatural analogue, 2-*epi*-1-hydroxymethylnarciclasine (**2**).

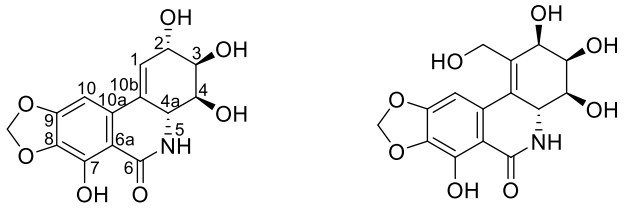


Figure 1. Narciclasine (**1**) and the novel target analogue, 2-*epi*-1-hydroxymethylnarciclasine (**2**).

The intent of the project was to prepare novel synthetic analogues of narciclasine, specifically those bearing modification at the C-1 position. Our approach incorporates themes from existing approaches to narciclasine and its related congeners (**Figure 2**) and has exploited other synthetic techniques to allow access to the desired C-1 derivative family. The project sought to utilize chemoenzymatic synthesis, a technique used frequently within the Hudlicky group, to establish the enantiospecificity of the synthesis. The advantages of using such an "unconventional" technique in organic synthesis will be discussed further, along with further insight into other synthetic methods that have been incorporated in the synthesis.

2. Historical

2.1. Overview of the Amaryllidaceae Alkaloids

The Amaryllidaceae family of plants has served as a particularly rich source of bioactive compounds for millennia. This grouping contains mainly bulbous, flowering plants such as spider lilies and daffodils, among many others. The family is comprised of approximately 75 genera and 1600 distinct species which are native mainly to tropical areas, but also extending into the sub-tropics.[1] Plants from the Amaryllidaceae family have seen use as ornamental flowers throughout many cultures and, perhaps most interestingly, they also produce a vast array of alkaloids that have excited scientists worldwide. Several structural groupings of these alkaloids have been identified, possessing activities ranging from analgesia to antitumor, antifungal, and antibacterial.[2] Of particular importance to the current project are the isocarbostyrils (**1**, **3-6**, **Figure 2**), a group containing phenanthridone-type natural products such as narciclasine (**1**) and pancratistatin (**5**), which have been found to possess potent and selective anti-cancer activity.[3]

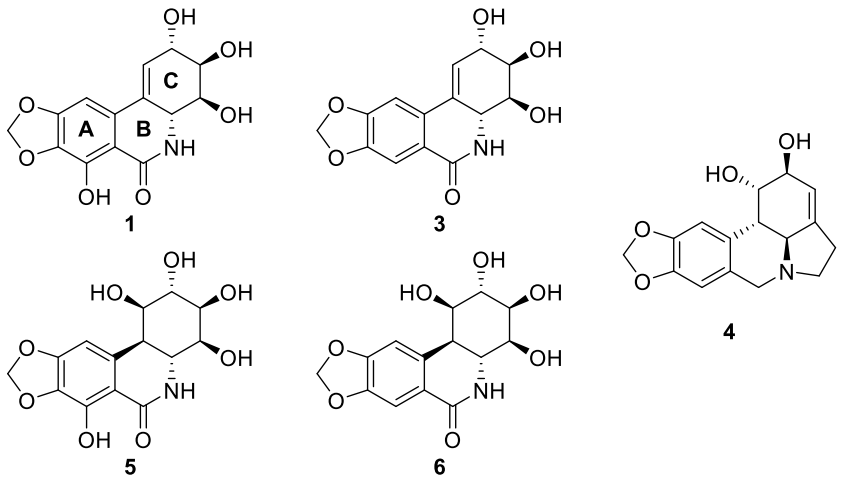


Figure 2. The structures of narciclasine (**1**) and its related Amaryllidaceae congeners: lycoricidine (**3**), lycorine (**4**), pancratistatin (**5**), and 7-deoxypancratistatin (**6**).

The medicinal value of these plants was recognized as far back as 5th century B.C., when Hippocrates reported using their extracts for treatment of tumors.[4,5] He, alongside his successors in ancient Greece and those practicing early medicine throughout the Mediterranean, began popularizing the administration of oils derived from *Narcissus* species to patients with inflammatory tumors or other cancer-like symptoms.[6] With the longstanding use of these plants to treat severe, generally cancer-like illnesses, modern research has shifted to investigating the specific compounds responsible for this observed action. The isocarbostyril-type alkaloids have been identified as the primary source for the observed therapeutic effects of Amaryllidaceae extracts and have thus been a central focus in natural product research in the years since their discovery and isolation.[4] Two members of this subgroup, also being two of the major constituents of Amaryllidaceae extracts, have been studied particularly extensively in large part because of their impressive anti-cancer profiles – namely pancratistatin (**5**) and narciclasine (**1**). Their isolation, synthesis, biological activity, and derivatization will be discussed further in the following sections.

2.2. Isolation, Identification, and Proposed Biosynthesis

The first isolation of an alkaloid from the Amaryllidaceae family came in 1877, when lycorine (**4**) was purified from the extract of wild daffodils, *Narcissus pseudonarcissus*.[7] The alkaloid was recognized as a likely contributor to the observed toxicity of some Amaryllidaceae plants towards animals who ingest them. This finding also helped to explain the horticultural observation that many other flowers are incapable of surviving when placed in water shared by *narcissus* (daffodil) flowers – an earlier clue to the growth inhibitory effect of the exudate of these plants.[4] With the latter uncovering of

the alkaloid's therapeutic potential (possessing both antitumor and antiviral activity),[8] research into this class of natural products intensified.

Narciclasine (**1**), another major Amaryllidaceae constituent, was first isolated in 1967 by Ceriotti.[9] He set out to investigate what chemical constituents of Amaryllidaceae species caused the observed growth inhibition of other plants, as well as an explanation for their historical use in folk/pre-modern medicine. Based on qualitative chemical tests, IR, UV, mass spectrometric, and NMR studies, Ceriotti proposed structure **7**.[10] Alongside this new compound, which he named narciclasine, he also isolated a secondary non-basic constituent, which he named narciprimine, with initially assigned structure **8**.

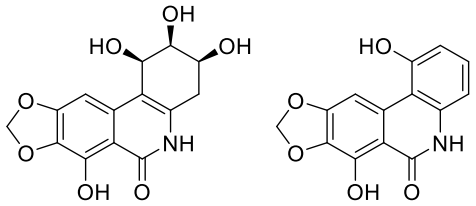


Figure 3. Structural proposals of narciclasine (**7**) and narciprimine (**8**) by Ceriotti.[10]

Less than a year later, Okamoto isolated a cytotoxic compound from the red spider lily, *Lycoris radiata*, naming it lycoricidinol.[11] In the communication, Okamoto proposed structure **9** lacking stereochemical assignment, and also noted its similarity and possible overlap with Ceriotti's narciclasine isolate.[10]

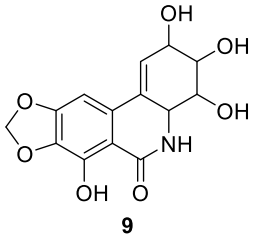


Figure 4. Structure of narciclasine proposed by Okamoto.[11]

The initial structures for both identified natural products would be revised only a few years after their initial isolation. The structure of narciclasine was ultimately confirmed by X-ray analysis of the natural product's tetraacetate derivative **10** (also confirming that narciclasine and lycoricidinol were, in fact, identical),[12] while narciprimine's structure was adjusted (**11**) based on a discrepancy in data after chemical synthesis of the proposed structure.[13]

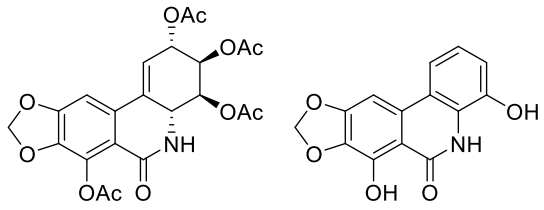
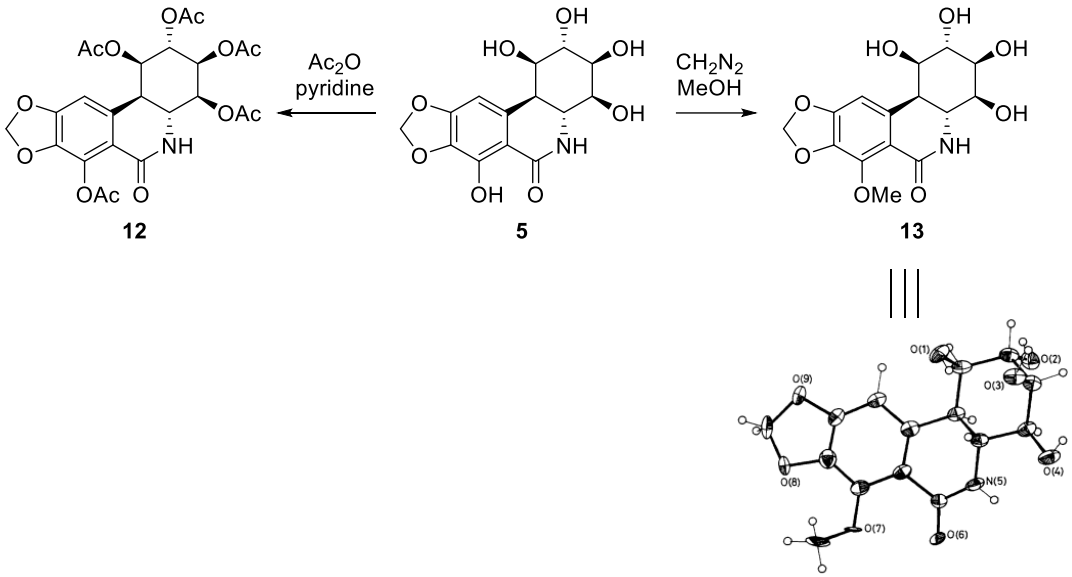


Figure 5. Structural revisions of narciclasine tetraacetate (**10**) and narciprimine (**11**) by Fuganti[12] and Krohn,[13] respectively.

Later, in 1984, a new, closely related anticancer agent was discovered as part of an exploratory endeavor directed by the U.S. National Cancer Institute (NCI). The "exploratory plant evaluation programme" sought to investigate possible bioactive properties of known plant species. Extracts from some Amaryllidaceae plants demonstrated anticancer activity in preliminary *in vitro* assays, where various cancer cell lines were treated with individual plant extracts.[14] These "hits" within the Amaryllidaceae family were studied by George R. Pettit – a prominent figure in natural product discovery who was renowned for his career-long pursuit of naturally-isolated anticancer agents.[15] Pettit and his colleagues were searching for the chemical compound(s) responsible for the significant anticancer activity of the Hawaiian beach spider lily, *Pancratium littorale* (now known as *Hymenocallis littoralis*). Following extraction from the lily's bulbs, selective

solubility studies, gel permeation chromatography, and final crystallization, 6.5 g of the new natural product were isolated.[14,16] The compound was given the name pancratistatin, relating to the parent plant's Latin name but also meaning "all powerful breaker or stopper" and "victory by any and all means", in Latin and Greek etymologies, respectively.[17]

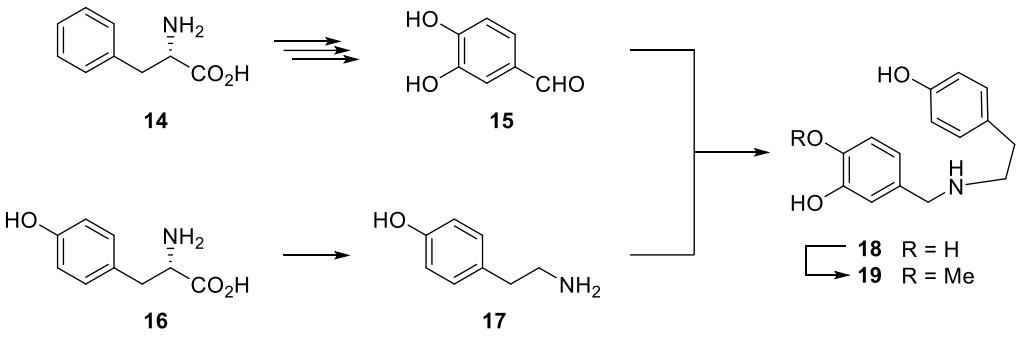
Pancratistatin's structure was elucidated by Pettit and was reported in the first publication, alongside its discovery. The natural product was converted to its corresponding pentaacetate (**12**) and phenolic methyl ether (**13**) derivatives, which were studied by NMR, IR, and elemental analysis. Ultimately, the structure was indisputably determined via X-ray analysis of the C-7 methyl ether (**13**, **Scheme 1**).[14]



Scheme 1. Pancratistatin transformations and crystal structure reported by Pettit.[14]

Biosynthesis of the alkaloids found in the Amaryllidaceae family of plants has been partially elucidated thanks to the efforts of multiple groups in conducting feeding and labeling studies.[18] An interesting trait of the biosynthesis of these molecules lies in their complexity and diversity, with at least twelve distinct ring systems having been observed.

A likely explanation for this is the different couplings undergone by one of the recognized common intermediates of Amaryllidaceae alkaloid biosynthesis, *O*-methylnorbelladine (**19**). *O*-methylnorbelladine (**19**) itself is biosynthesized from L-phenylalanine (**14**) and L-tyrosine (**16**) via protocatechuic aldehyde (**15**) and tyramine (**17**), respectively.[4,19]

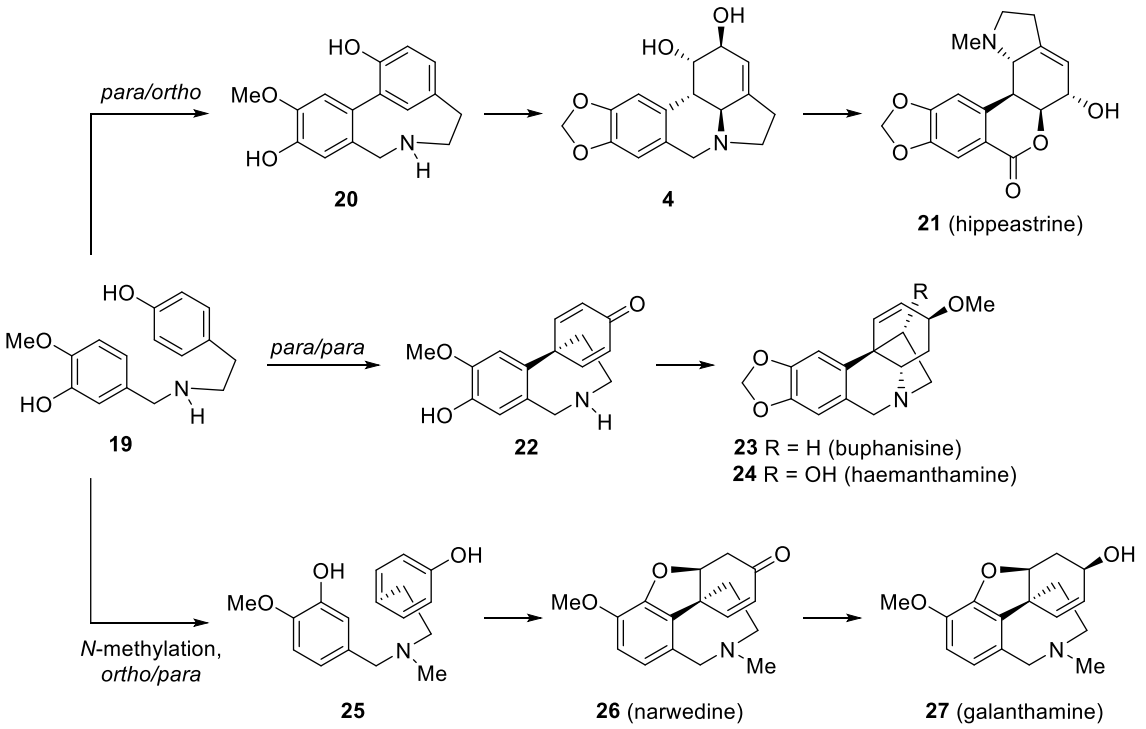


Scheme 2. Biosynthesis of Amaryllidaceae alkaloid precursor, norbelladine (**18**).[4]

This common intermediate is then proposed to undergo a series of different coupling reactions, giving rise to the various ring systems of the alkaloids found in Amaryllidaceae species.[18c-m] The proposed coupling options and some of the alkaloids and ring systems generated by these routes are summarized in **Scheme 3**.

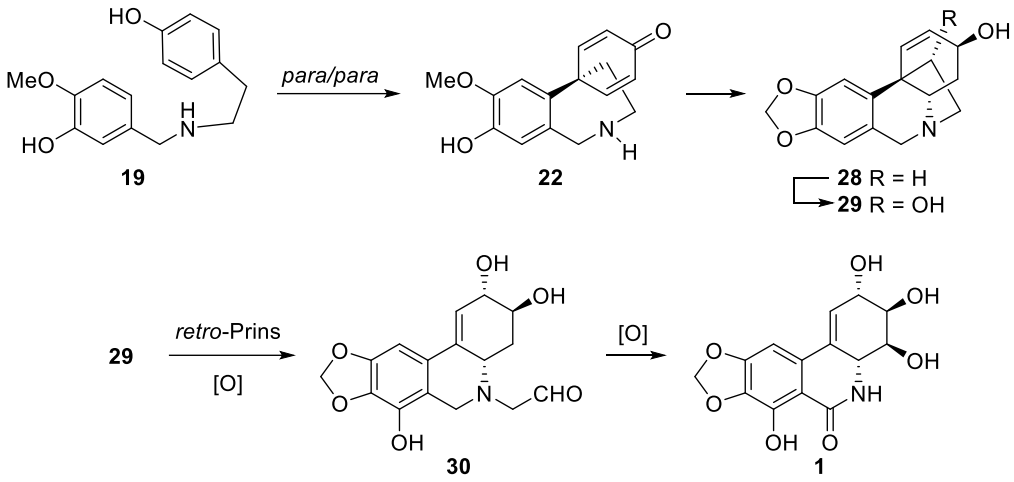
The biosynthesis of narciclasine (**1**), though evidently related to some of the other Amaryllidaceae alkaloids, has still not been fully elucidated. Based on the structure of narciclasine (**1**), it was initially believed that its ring system was likely constructed by the *para-para* or *para-ortho* coupling pathways (**Scheme 3**). The presence of alkaloids from the lycorine (**4**) and haemanthamine (**24**) families in narciclasine-containing species also hinted toward a related biosynthetic pathway.[18n] Tritium labelling and deuterium exchange experiments have been carried out, extending as far back as the 1970s, in order to establish

the biosynthetic precursors to the narciclasine backbone.[18o-r] These studies found that narciclasine is formed through the *para-para* coupling pathway of *O*-methylnorbelladine



Scheme 3. Proposed intramolecular couplings of *O*-methylnorbelladine in alkaloid biosynthesis.[4,18]

(**19**), followed by the elimination of two carbon atoms (the so-called "ethano bridge") and late-stage oxidations.[18o] This was confirmed by feeding experiments using ^{3}H-labelled 11-hydroxyvittatine (**29**), resulting in tritium retention in the haemanthamine and narciclasine produced.[18q] From this confirmed intermediate, it was then proposed that the remaining biosynthesis may proceed through a *retro*-Prins reaction, facilitating the "ethano bridge" cleavage, and subsequent oxidations (**Scheme 4**).[18r] Further support for this proposed pathway comes from the observation of similar "ethano bridge"-cleaved metabolites with C-13 backbones in the same species, such as ismine.[4]



Scheme 4. Proposed biosynthetic origin of narciclasine.[4,18,19]

Though these biosynthesis studies were completed as far back as the 1960s and 1970s, little additional progress has been made in elucidating the full pathway to narciclasine or its related congener, pancratistatin. The existing information does provide a relatively clear picture of how the different ring systems and natural products arise in Amaryllidaceae species, though, so further investigations appear to be a lesser priority.

One ongoing area of Amaryllidaceae alkaloid research is the addressal of low natural abundance of these bioactive compounds and the difficulties it creates for their study and development as lead drug candidates. Despite the presence of both narciclasine (**1**) and pancratistatin (**5**) in several Amaryllidaceae species, they tend to be produced in minute amounts. It is worth noting that alkaloid concentration varies across different plant tissues, with peak content being found in the bulbs and roots. The content can also vary based on season and stage of plant growth, as higher concentrations of some alkaloids have been observed in flowering plants during springtime.[4] **Table 1** summarizes the abundance and distribution of these compounds, alongside their mean GI_{50} values against a panel of cancer cell lines.

Table 1. Distribution of narciclasine and its congeners in Amaryllidaceae plants.[4,20,21,22,23]

	Producing Species	Peak Content (mg/kg)[a]	Mean GI_{50} (μM)
Narciclasine (**1**)	21	200 (*Narcisssus spp.*)	0.016[b]
Pancratistatin (**5**)	11	144 (*H. littoralis*)	0.091[b]
Lycoricidine (**3**)	> 4	222 (*H. littoralis*)	0.15[b]
7-Deoxypancratistatin (**6**)	> 4	3 (*H. kalbreyeri*)	0.32[c]

[a] Content extracted from fresh bulbs.
[b] U.S. National Cancer Institute 60 cancer cell line screen. The screen is composed of 60 human cancer cell lines used for the detection and characterization of anticancer activity in pure compounds and crude extracts.[4,23]
[c] Average of activity against P388 leukemia, lung NCI-H460, colon KM20L2.[21]

The numbers in the above table tell a brief story of the Amaryllidaceae alkaloids: the compounds have impressive biological activities against cancer cells (which will be discussed further in subsequent sections), but their study and possible progression into clinical trials has largely been hindered by their poor accessibility from natural sources. Because of this, many researchers have sought a brief, efficient route to access these compounds by synthetic means. Also worthy of noting are biotechnological efforts by Pettit, who arranged a large-scale cloning operation of *H. littoralis* bulb tissue.[24] This operation showed some promise, converting 1.5 kg of cultivated wild bulbs to approximately 60,000 bulb clones – which were raised in greenhouse conditions before transplantation to fields. The cloned plants were found to successfully produce pancratistatin, narciclasine, and lycoricidine, albeit in significantly lower amounts than the native Hawaiian specimen.[24] Even with these impressive efforts, the pursuit of the natural products via synthesis has continued. These endeavors continue alongside more recent

exploration of unnatural analogue synthesis, in hopes of improving biological activity while attempting to address the supply issue.

2.3. Biological Activity Profile

Alkaloids found in plants of the Amaryllidaceae family have been intensely scrutinized in the past few decades, owing to their impressive biological activities. Although the medicinal value of these plants was recognized as far back as *ca*. 400 B.C., more concentrated focus has been placed on studying the alkaloids they secrete in recent years. Antiviral, antibiotic, antifungal, and antiparasitic activities had been suggested based on traditional uses of the extracts, but the anticancer activity of narciclasine-containing plants has been the most prominent of all.[4]

Indeed, Ceriotti's early studies on the biological activity of narciclasine helped to spark interest in the use of these alkaloids as modern medicines.[9] His initial investigations identified the potent antimitotic activity of narciclasine toward mouse sarcoma cells when injected subcutaneously and, perhaps more importantly, pointed to a good therapeutic index for its use. The study showed that administration of narciclasine in doses of 0.9 mg/kg led to the halting of cancer cell division within 4 h, with no apparent side effects in the mouse subjects. The LD_{50} in mice was determined to be 5 mg/kg via subcutaneous injection, as Ceriotti also noted an increase in observable "disrupted cells" (nuclear envelope rupturing) with increased dosage.[9] Okamoto independently (and near-simultaneously) confirmed these results, reporting on the cytostatic activity of narciclasine and lycoricidine on Ehrlich carcinoma cells.[11] Vazquez's group attributed this activity to narciclasine's potent inhibition of protein biosynthesis. They found, through a [^{14}C]phenylalanine incorporation assay, that narciclasine inhibited peptide synthesis in

yeast and rabbit ribosomes, but not in *E. coli* – pointing to specificity for eukaryotic organisms.[25] Their work also showed that narciclasine blocked ribosomal binding of a 5'-capped donor fragment, but had little effect on binding of 3'-capped or uncapped fragments. This result pointed to narciclasine disrupting binding of the 3' end of donor substrates to the ribosome. This was confirmed by narciclasine's inhibition of anisomycin, an antibiotic known to be a specific inhibitor of substrate binding in eukaryotic ribosomes (and thereby a protein synthesis inhibitor).[25] Vazquez's team also showed that mutant yeast cells bearing altered peptidyl transferase (located within the 60S ribosomal subunit) were resistant to narciclasine, thus confirming the biological target of the alkaloid.[26]

The same group (Jimenez *et al.*) that elucidated this mechanism of action later showed that narciclasine and its closely related congeners also inhibit protein synthesis *in vivo* in cancer stem cells (Krebs II ascites cells). The alkaloids' protein synthesis inhibition was shown to be a largely specific process, as they exhibited no effect on RNA synthesis and little effect on DNA synthesis.[27] The weaker influence of narciclasine on DNA synthesis was proposed to be a secondary effect of protein synthesis inhibition, as translation is needed for DNA synthesis to continue unimpeded. The findings lead to the conclusion that narciclasine exerts its effect on protein synthesis at the stage of peptide bond formation, rather than at an earlier point in transcription or translation.

These early insights into the biological activity of narciclasine caught the attention of the NCI, who began examining the alkaloid's potential as a chemotherapeutic agent. The NCI's preclinical studies consisted of daily treatment of tumor-grafted mice (either mouse syngeneic or human xenograft) with narciclasine. Although an increase in survival rate and

decrease in tumor mass were reported in many of the models, the benefits were marginal relative to the observed toxicity. This led to the termination of the NCI's study.[2b]

Advancement in understanding narciclasine's mechanism of action has been a slow, but gradual, process over the years and has likely contributed to impeding further clinical investigation of the Amaryllidaceae alkaloids. Despite this, one characteristic that has garnered them continued attention is their selective action on cancerous cells. Although the therapeutic index of these compounds was recognized as early as 1967,[9] it was the work of Pandey that confirmed their selective toxicity.[28] Their studies showed that pancratistatin affected apoptosis in Jurkat cells (T-cell leukemia model cell line) in micromolar concentrations and lower, yet had no perceivable cytotoxicity toward "normal" fibroblasts or endothelial cells.[28a] Similar results were later found in *ex vivo* leukemia samples obtained from clinical patients.[28d] Also, they found that pancratistatin did not cause early-stage DNA degradation, unlike commonly prescribed genotoxic cancer therapies, but rather caused DNA fragmentation later, as a result of apoptosis.

This finding was significant as it highlighted a distinct advantage of these natural products over existing cancer treatments such as Taxol © and Vepesid ©, which both show indiscriminate toxicity.[28b] The authors cited an increase in reactive oxygen species (ROSs) as a possible mechanism of action for pancratistatin, as this increase was observed only in neuroblastoma cells, but not in normal human fibroblasts (NHFs). The increase in ROSs after administration of pancratistatin pointed to the mitochondria as a possible target, as increased ROSs may be tied to mitochondrial dysfunction. The publication concluded by vaguely alluding to "many differences in the mitochondria of cancer and normal cells",

such as membrane permeabilization and altered molecular composition of cancer cell mitochondria, as possible bases for the selective action of pancratistatin.[28c]

Kiss, Kornienko, and Evidente later worked to further the understanding of these alkaloids' mechanism of action.[2b] They chose to focus their studies on human brain cancers (glioblastoma and melanoma brain metastases) as these were among the most responsive to treatment with the natural isocarbostyrils (across the NCI 60 human cancer cell line *in vitro* screen).[2b,4] Their findings emphasized the selective, therapeutic effects of narciclasine on xenografted mouse models when administered in non-toxic doses, citing the natural product as a "cytostatic agent that acts as a cancer cell-specific selective modulator of GTPases".[2b] GTPases act as regulators of actin cytoskeleton assembly, which is in turn responsible for cell structural support and cellular motility. Narciclasine was found to modulate one such GTPase, RhoA, responsible for actin cytoskeleton organization and the formation of actin stress fibers – the structures that allow cells to move. Overexpression of Rho proteins has been associated with various cancers, specifically to metastasis. Narciclasine's ability to modulate this pathway seemingly contributes to its anticancer activity.[2b]

Kiss and colleagues continued their investigation by studying narciclasine's interaction with eEF1A – an elongation factor complex involved in actin cytoskeleton organization. eEF1A is also responsible for delivery of tRNAs to the ribosome, and therefore peptide elongation. Kiss' group found that narciclasine directly binds to eEF1A, effectively inhibiting both actin fiber formation and peptide synthesis. While GTPases have been found to play a crucial part in cancer cell proliferation and metastasis, they also have a significant role in actin-based contractions of the heart and other muscles. Therefore, the

observed action of narciclasine as a GTPase modulator at least partially accounts for the alkaloid's selective anticancer activity at low doses and toxic effects at greater doses.[2b]

Table 2: Dose-dependant therapeutic effects of the Amaryllidaceae isocarbostyrils.[2b]

Dose	**Anti-Cancer Activity**	**Side Effects**
Pharmacological Scale (1 µM in vitro *or* 10 mg/kg in vivo)	Proapoptotic Cytotoxic	Severe toxicity
Physiological Scale (50 nM in vitro *or* 1 mg/kg in vivo)	Cytostatic	None observable

Years later, Pandey, Hudlicky, and McNulty would collaborate to investigate the mechanism of action of pancratistatin and its unnatural analogues (**Figure 6**), providing further insight into their selectivity.[3] Their work showed that pancratistatin **5** and its unnatural homologues **31-33** were able to induce apoptosis in multiple cancer cell lines, while exerting no observable effect on peripheral blood mononuclear cells (PBMCs) from healthy volunteers. Pancratistatin selectivity was found to be linked to action of the mitochondria, specifically via the caspase-dependant apoptosis pathway. Apoptogenic factors, such as cytochrome c, can be released from dysfunctional mitochondria in early stages of cancer detection – leading to caspase-induced cleavage of vital cellular proteins. Pancratistatin's ability to trigger this pathway was further supported by a noted decrease in mitochondrial membrane potential (MMP), which translates to increased mitochondrial permeability for the release of apoptogenic factors. Pancratistatin and its analogues were again shown to increase production of ROSs in cancer cells, creating oxidative stress and leading to the release of cytochrome c (and subsequent apoptosis).

Pandey and coworkers also explored the role of mitochondrial electron transport chain (ETC) functional complexes II and III in pancratistatin-induced apoptosis. Inhibitors of functional complex II or III led to suppression of the apoptotic effects of pancratistatin analogues, indicating that their anticancer activity is dependant on these complexes.[3] Despite these findings, no direct molecular interaction was proposed. A final comment on the compounds' mechanism of action was provided, stating, "Inhibiting anti-apoptotic proteins, mimicking pro-apoptotic proteins, or targeting ETC complexes could be potential mechanisms employed by [pancratistatin] analogues".[3]

Perhaps the most important finding of these studies was the effectiveness of pancratistatin analogues in decreasing tumor growth in xenografted colorectal cancer and glioblastoma mouse models. Mice treated with these compounds showed decreased tumor growth and similar overall mass gain to a control subject. This represented an *in vivo* proof of pancratistatin anticancer activity, while also demonstrating that these compounds could be tolerated by the subjects at therapeutic doses.[3] Additionally, the analogues studied were shown to have better activity profiles and greater selectivity toward cancer cells than natural pancratistatin and several standard chemotherapeutic agents.

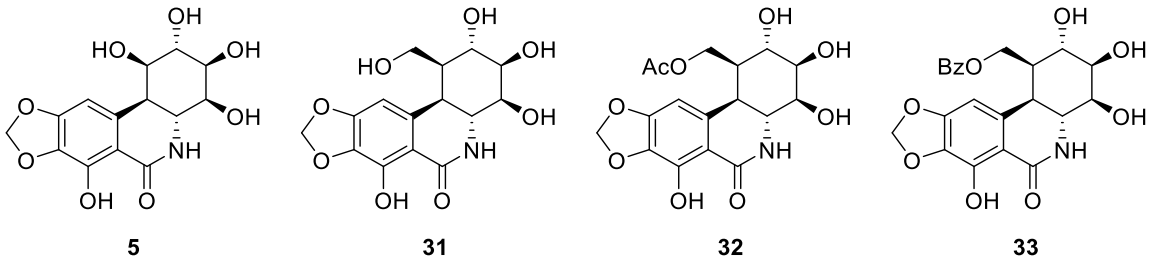


Figure 6. Pancratistatin (**5**) and its unnatural analogues screened in detailed biological evaluation studies.[3]

Further investigation of isocarbostyril activity in animal models was performed recently, analyzing the efficacy of narciclasine in treating primary effusion lymphoma (PEL). PEL is considered an aggressive form of cancer with generally poor prognosis, with median survival times of only 4.8 months.[29] The research confirmed narciclasine's *in vitro* cytotoxicity and selectivity for cancer cells over normal cells, and also examined the *in vivo* potential of the natural product and some of its related congeners. Narciclasine, lycorine, and lycoricidine were all tested for treatment of mice with cell line-derived and patient-derived xenografted tumors. Narciclasine was found to be the best performing of the three natural products, extending specimen survival time by > 50 % on average, while also inhibiting tumor growth (confirmed by bioluminescence imaging). Narciclasine and its related isocarbostyrils showed preferential activity against PEL, representing a crucial finding as traditional chemotherapy is consistently unsuccessful in the treatment of this cancer. Narciclasine's impressive activity against PEL is believed to arise from downregulation of MYC genes – proto-oncogenes that are prevalent in many cancers.[29]

To date, entry of narciclasine-type Amaryllidaceae alkaloids into clinical trials has largely been stalled because of low natural abundance and (still) limited knowledge about the mechanism of action.[4] Synthesis efforts have attempted to address the supply issue but have been unsuccessful to date. These efforts will be discussed in brief in the next section.

2.4. Total Synthesis

While the existing syntheses have brought forth some new chemical strategies, have been academically interesting, and have improved on the efficiency (steps and overall yield), none have been capable of being "efficient enough" to rival nature's production of even low-abundance natural products. Some unnatural analogues have been prepared

through these synthetic endeavors, though, which have demonstrated promising activity profiles, some topping the activity of the natural products themselves.[3,20] Sadly, none of these derivatives have been made in a short enough sequence to allow for the production of large quantities for clinical trials. So, at least for now, the most active analogues can only serve as a template or guide for future investigation and provide information about the pharmacophore and structure-activity relationship (SAR) of these compounds.

2.4.1. Narciclasine and its congeners

Despite the isolation, characterization, and recognition of the biological activity of narciclasine in 1967, synthetic efforts did not come to fruition until three decades later.[30] Since then, several groups have worked on the total synthesis of narciclasine and its congeners, with the most recent being completed by Sarlah in 2019.[31] **Table 3** summarizes the syntheses of narciclasine and lycoricidine, its 7-deoxy relative. In this section, a select few select total synthesis efforts will be discussed; either because of their efficiency, elegance, or relation to the works of this thesis.

Table 3. Summary of published syntheses of narciclasine and lycoricidine.

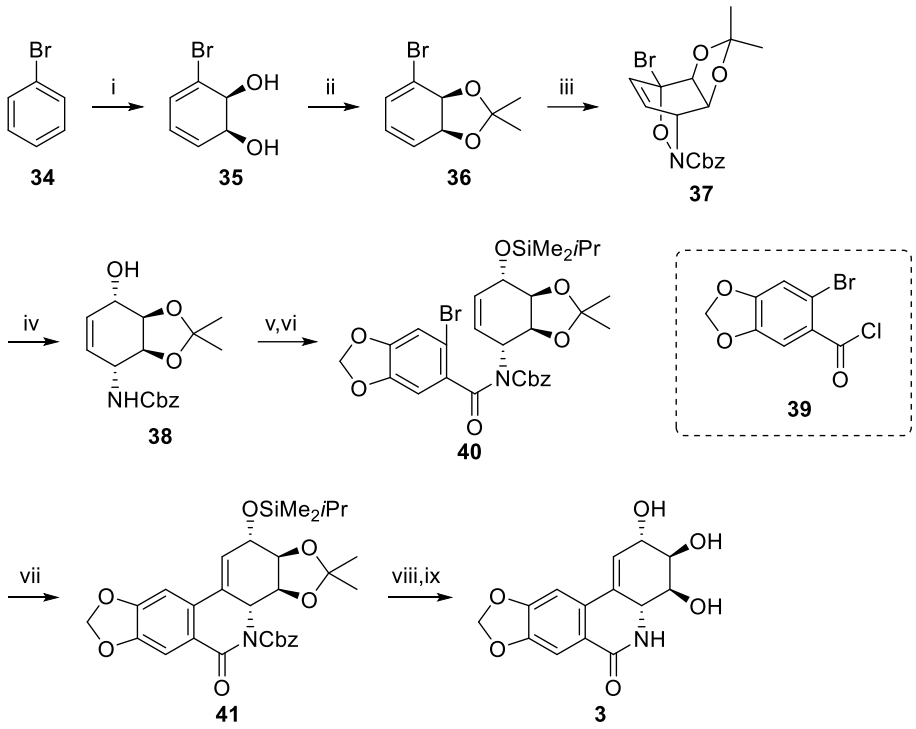
Author	Isocarbostyril	Year	Step count	Overall Yield	Ref.
Rigby	Narciclasine	1997	22	0.2 %	[30,32]
Hudlicky	Narciclasine	1999	12	0.6 %	[33]
Keck	Narciclasine	1999	12	16 %	[34]
Yan	Narciclasine	2002	12	17 %	[35]
Banwell	Narciclasine	2008	11	7 %	[36]
Yamamoto	Narciclasine	2015	14	0.2 %	[37]
Sarlah	Narciclasine	2017	10	3.9 %	[38]

Sarlah	Narciclasine	2019	6	15 %	[39]
Ohta	Lycoricidine	1975	19	1.5 %	[40]
Paulsen	Lycoricidine	1982	13	4 %	[41]
Schubert	Lycoricidine	1987	17	7.2 %	[42]
Ogawa	Lycoricidine	1991	24	0.04 %	[43]
Hudlicky	Lycoricidine	1992	9	12 %	[44]
Martin	Lycoricidine	1993	11	4 %	[45]
Keck	Lycoricidine	1996	14	11 %	[46]
Keck	Lycoricidine	1999	9	27 %	[34]
Yan	Lycoricidine	2002	15	11 %	[47]
Padwa	Lycoricidine	2007	13	10 %	[48]
Banwell	Lycoricidine	2007	11	13 %	[49]
Yadav	Lycoricidine	2010	14	4.2 %	[50]
Sun	Lycoricidine	2017	11	12 %	[51]
Shaw	Lycoricidine	2017	12	7.8 %	[52]
Sarlah	Lycoricidine	2017	9	15 %	[38]
Sarlah	Lycoricidine	2019	6	26 %	[39]
Yan	Lycoricidine	2019	11	10 %	[53]

Ohta completed the first racemic synthesis of lycoricidine in 1975,[40] while the first asymmetric synthesis was accomplished 16 years later by Ogawa, in 24 steps.[43] Shortly after, Hudlicky reported another asymmetric synthesis which exploited a chemoenzymatic transformation to induce enantiospecificity.[44] This technique allowed the step count to be greatly reduced compared to existing syntheses at that time, assembling the natural product in only nine steps. Rigby completed the first total synthesis of narciclasine in 1997, spanning 22 steps.[30] Hudlicky would complete an additional synthesis of narciclasine in 1999, again employing a chemoenzymatic approach and shortening the route to 12 steps.[33] These syntheses by Hudlicky, among others, will be detailed in brief.

Hudlicky – Lycoricidine – 1992

Hudlicky's synthesis of lycoricidine (**Scheme 5**)[44] stood as the shortest route for many years, both improving upon previous syntheses and standing the test of time in terms of efficiency. The approach utilized a biotransformation at the start of the synthesis, converting bromobenzene (**34**) to the corresponding *cis*-dihydrocatechol (**35**). The use of this chiral starting material proved highly advantageous as it set two of the C-ring stereocenters immediately, while also yielding a functionally dense building block for further elaboration. The diol was protected as an acetonide (**36**), and this functional group dictated the stereochemical outcomes of subsequent reactions, again demonstrating the value of this approach. Cleavage of the *N*-*O* bridge, acylation with the A-ring fragment, closure via an intramolecular Heck reaction, and full deprotection yielded the desired alkaloid, lycoricidine (**3**) in only nine steps.



Scheme 5. Hudlicky's synthesis of lycoricidine.[44] Reagents and conditions: i) *pseudomonas putida*; ii) DMP, acetone, TsOH; iii) CbzNHOH, Bu_4NIO_4, DCM; iv) Al(Hg), THF; v) $ClSiMe_2iPr$, imidazole, DCM; vi) *n*-BuLi, **39**, THF; vii) $Pd(OAc)_2$, TlOAc, DIPHOS, anisole; viii) Pd/C, cyclohexene, EtOH; ix) TFA, 0 °C.

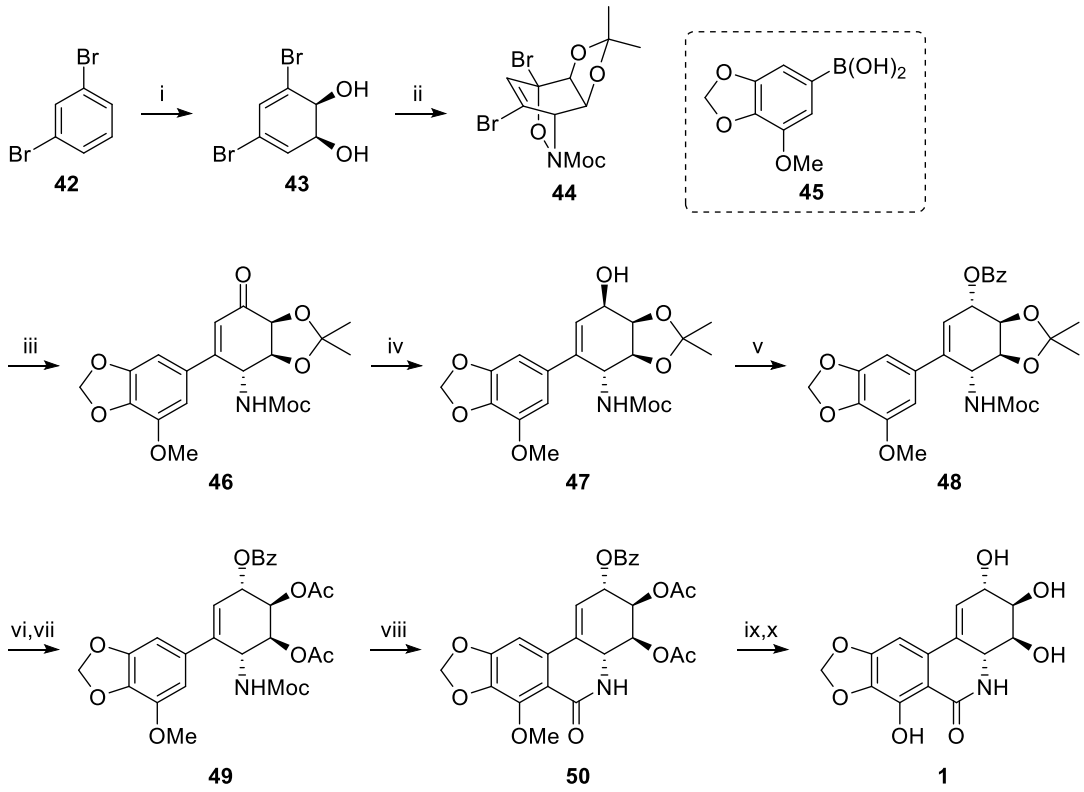
This approach by Hudlicky was by far the most straightforward synthesis of an Amaryllidaceae constituent at the time. The authors mention the pursuit of an efficient, cost-effective, environmentally-friendly, scalable, and broadly amenable synthetic route to the isocarbostyrils – an endeavor which Hudlicky has continued to pursue with his group's many syntheses of these alkaloids.[20]

Hudlicky – Narciclasine – 1999

Hudlicky would continue with the goal of accessing the major Amaryllidaceae constituents via chemoenzymatic synthesis, as he published syntheses of 7-deoxypancratistatin (**6**)[54] and pancratistatin (**5**)[55] throughout the 1990s. In 1999, he reported a synthesis of narciclasine (**1**, **Scheme 6**)[33] which greatly improved upon the efficiency of the lone existing synthesis of the time,[30] shortening the route from 22 steps to 12 steps.

Once again, the strategy relied on the microbial oxidation of an arene to provide an enantiopure building block for use in the synthesis. In a similar fashion to Hudlicky's lycoricidine synthesis (**Scheme 5**), a chemoenzymatically-derived diol (**43**) was protected and subjected to a nitroso Diels-Alder reaction, furnishing bicyclic oxazine **44**. This oxazine possessed all stereocenters and correct relative and absolute stereochemistry of the C-ring of narciclasine. A one-pot Suzuki coupling and *N*-*O* bond reduction was performed, leading to enone **46**, holding the full carbon backbone of the desired natural product. This intermediate was reduced under Luche conditions, and the alcohol was subsequently inverted by a Mitsunobu protocol (**48**). A protecting group exchange was then affected prior to the use of Banwell's modified Bischler-Napieralski conditions, for closure of the B-ring lactam (**50**). Full deprotection was carried out in two steps, leading to narciclasine (**1**) in twelve steps overall.[33]

Hudlicky's work on the Amaryllidaceae alkaloids has been significant, synthesizing multiple natural products and, more recently, numerous unnatural analogues. These syntheses, among those of other analogues, will be discussed in following sections.



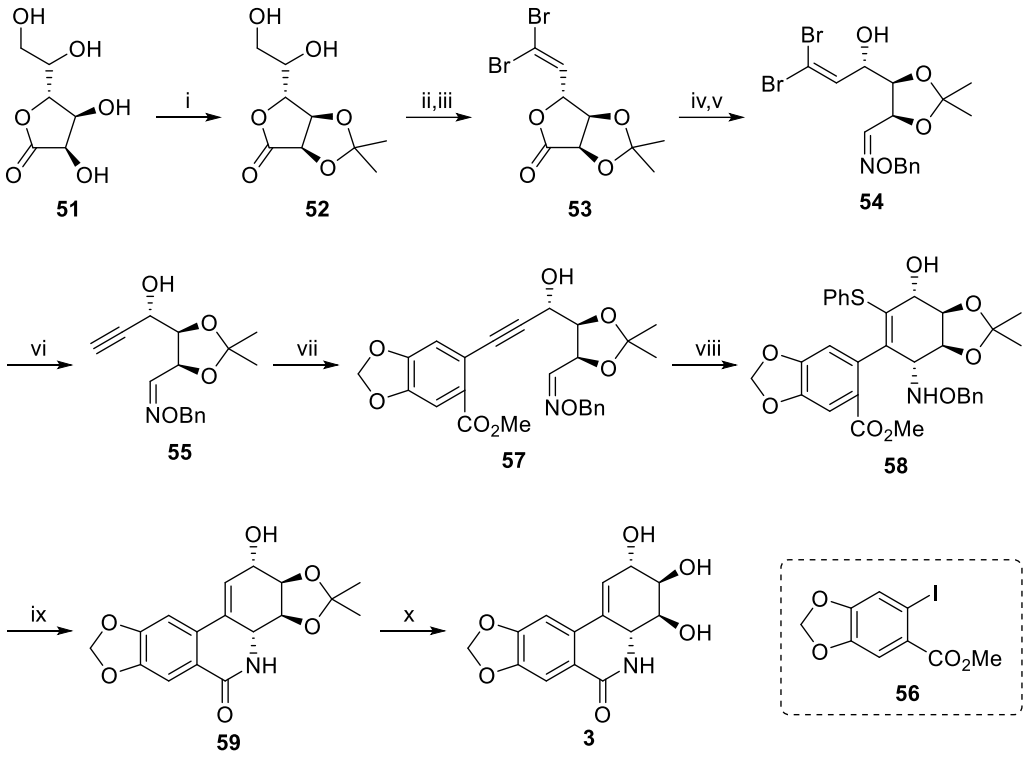
Scheme 6. Hudlicky's synthesis of narciclasine.[33] Reagents and conditions: i) *E. coli* JM109 (pDTG601A); ii) DMP, acetone, TsOH *then* MocNHOH, $NaIO_4$, MeOH, H_2O; iii) **45**, $Pd(PPh_3)_4$, aq. Na_2CO_3, benzene, EtOH, reflux *then* $Mo(CO)_6$, MeCN, reflux; iv) $NaBH_4$, $CeCl_3$, MeOH 0 °C; v) DEAD, Bu_3P, BzOH, THF; vi) DOWEX 50X8-100, MeOH; vii) Ac_2O, pyridine, DMAP, DCM; viii) Tf_2O, DMAP, DCM, 0 °C; ix) Amberlyst A21, MeOH; x) LiCl, DMF, 120 °C.

Keck – narciclasine and lycoricidine – 1999

Another major contributor to Amaryllidaceae alkaloid synthesis is Keck, who has published several syntheses, including those of lycoricidine,[46,34] narciclasine,[34] and 7-deoxypancratistatin.[56] His 2nd generation synthesis of lycoricidine (**Scheme 7**), alongside a closely related route to narciclasine (**Scheme 8**), is noteworthy for its low step count and

interesting approach to constructing the B- and C-rings. The approach was also concise and high yielding, proceeding in nine steps and 44 % overall yield. Stereo- and enantiocontrol in the synthesis were derived from the starting material, D-gulonolactone. The route relied on a Corey-Fuchs reaction followed by Sonogashira coupling to connect the eventual A- and C-ring fragments. The key sequence to establish both the B- and C-rings involved a radical cyclization followed by SmI_2-mediated reductive cyclization. The radical closure was initiated by thiophenol attack of acetylene **55**, generating the more stable benzylic vinyl radical. This reactive intermediate could then react with the *O*-benzyl oxime in a 6-*exo* cyclization, thereby closing the C-ring with all correct stereocenters established (**58**).

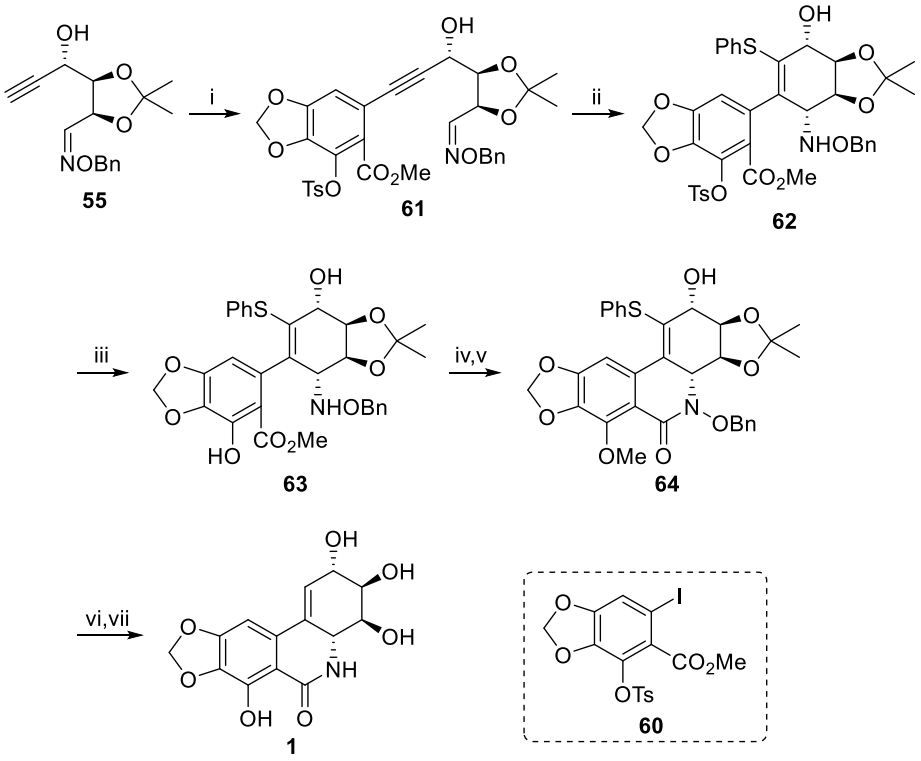
The second key reaction involved SmI_2-mediated reduction, achieving multiple structural conversions in a single reaction. This reaction led to cleavage of the thioether at C-1, *N*-*O* bond cleavage, and cyclization of the resultant amine to the pendant A-ring ester. This sequence provided the full cyclized backbone of lycoricidine, an impressive feat in the rapid building of chemical complexity.



Scheme 7. Keck's 2nd generation synthesis of lycoricidine.[34] Reagents and conditions: i) 2 steps;[57] ii) $NaIO_4$, DCM; iii) CBr_4, PPh_3, Et_3N; iv) L-selectride, Et_2O, -78 °C; v) $H_2NOBn{\cdot}HCl$, pyridine; vi) *n*-BuLi, Et_2O, -90 °C; vii) **56**, $Pd(OAc)_2$, PPh_3, CuI, Et_3N, THF; viii) PhSH, hν, toluene; ix) SmI_2, THF; x) TFA.

Keck would continue his work by applying a similar approach to the synthesis of the more bioactive natural product, narciclasine. They key steps employed were the same in this synthetic route, with the main difference being in the construction of the A-ring fragment with a 7-hydroxy functionality. Penta-substituted arene **60** was constructed in a series of directed metalation steps before submission to Sonogashira coupling with acetylene **55**. The thiophenol initiated radical cyclization again proceeded in high yields and resulted in only a single diastereomer (**62**), setting the stage for the B-ring closure. In the case of the narciclasine synthesis, this conversion had to be conducted in multiple steps,

as problems were encountered in attempting SmI_2 reductive cyclization analogous to that used in the synthesis of lycoricidine. Instead, Lewis acid-catalyzed cyclization was affected, followed by reductive cleavage of the *N-O* bond and global deprotection, affording narciclasine in twelve steps and 26 % overall yield.[34]



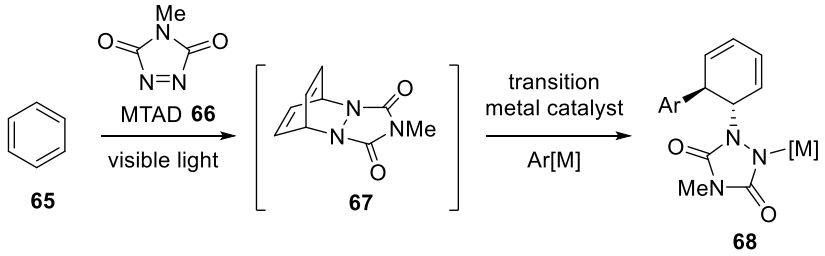
Scheme 8. Keck's synthesis of narciclasine.[34] Reagents and conditions: i) **60**, $Pd(OAc)_2$, PPh_3, CuI, Et_3N, THF; ii) PhSH, hν, toluene; iii) SmI_2, THF, H_2O, 0 °C; iv) MeI, K_2CO_3, DMF; v) Me_3Al, THF, -15-65 °C; vi) SmI_2, MeOH, THF, 0 °C; vii) TFA, 0 °C.

Sarlah – 7-deoxypancratistatin, pancratistatin, lycoricidine, narciclasine – 2019

In 2017, Sarlah reported his group's first syntheses of the Amaryllidaceae constituents lycoricidine and narciclasine.[38] The strategy used an arenophile-mediated dearomative dihydroxylation followed by transpositive Suzuki coupling to functionalize a

simple arene to the eventual functionally dense C-ring of the target alkaloids. The authors drew comparisons of their methodology to the microbial arene dihydroxylation used by Hudlicky[17,33,44,54,55] and others.[20] Although the overall outcome of the two methodologies are similar, it is important to note that the "nature-inspired" enzymatic strategy proceeds in a single step with full regio-, stereo-, and enantiocontrol, while the chemical strategy would be more correctly described as a "cycloaddition, dihydroxylation, cycloreversion" sequence. Nonetheless, Sarlah has completed numerous beautifully executed syntheses of isocarbostyril-type natural products based on this strategy.[38,39]

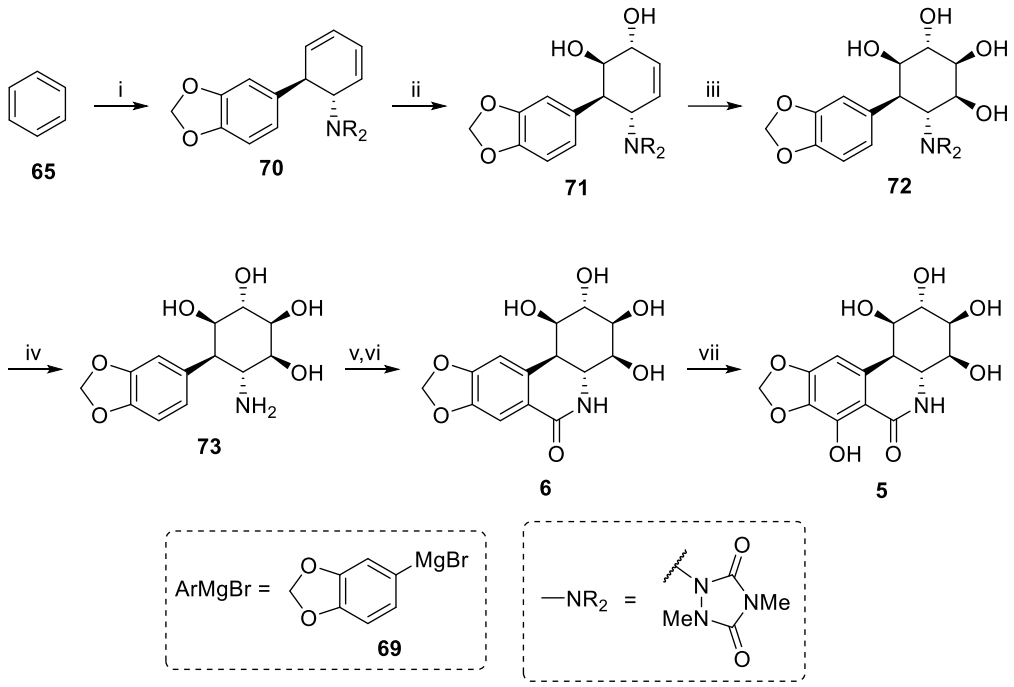
Sarlah greatly improved on his initial approach to the Amaryllidaceae alkaloids in 2019, when he reported syntheses of pancratistatin, 7-deoxypancratistatin, narciclasine, and lycoricidine – all employing a similar dearomative functionalization of benzene (**Scheme 9**). The methodology involved the visible-light induced cycloaddition of *N*-methyl-1,2,4-triazoline-3,5-dione (MTAD, **66**) to benzene, followed by attack of an arene nucleophile (Ar[M]) in the presence of a transition metal catalyst. The overall conversion was referred to as a "dearomative carboamination" and could be made enantioselective by using chiral *P*,*N*-bidentate ligands, opening the door to its use in asymmetric synthesis.



Scheme 9. General strategy for dearomative arene functionalization in Sarlah's 2nd generation Amaryllidaceae syntheses.[39]

Sarlah reported two unique but closely related synthetic routes to pancratistatin and 7-deoxypancratistatin, being executed in nine steps and seven steps, respectively.[39] Despite the high efficiency of the syntheses, the authors noted the aspiration for a streamlined synthesis where both natural products could be accessed by a single route, rather than separate syntheses. They also outlined their objective of using only a single, commercially available reagent for the installation of the A-ring, rather than the requisite synthesis of an arene nucleophile for their first-generation approach to pancratistatin.[39]

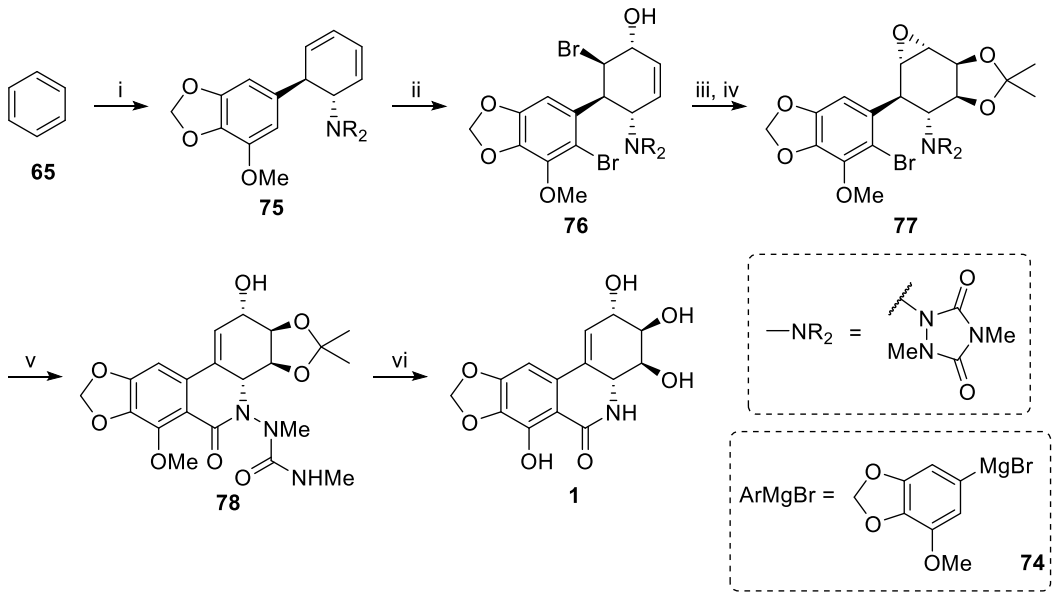
Their second-generation approach (**Scheme 10**) followed similar chemistry to that used for the initial synthesis of 7-deoxypancratistatin, using dearomative carboamination and commercially available Grignard reagent **69**. The synthesis benefited from the avoidance of protecting groups to affect the necessary transformations and construction of the isocarbostyril backbone. The key improvement in the more recent approach was the direct hydroxylation of the C-7 position, making direct conversion of 7-deoxypancratistatin to pancratistatin possible. The technique employed was developed by Uchiyama, involving deprotonative cupration and oxidation.[58] Sarlah's group found the reaction could be applied to the isocarbostyril backbone, whereby the tetraol moiety of 7-deoxypancratistatin was first persilylated using HMDS/I_2, prior to directed cupration of the C-7 position (assisted by the lactam carbonyl) and subsequent oxidation with *t*BuOOH. This transformation allowed the accomplishment of the goal to access both pancratistatins by a single synthetic pathway and completed the synthesis of 7-deoxypancratistatin in six steps and 19 % overall yield and pancratistatin in seven steps and 12 % overall yield.[39]



Scheme 10. Sarlah's improved synthesis of pancratistatins.[39] Reagents and conditions: i) MTAD (**66**), DCM, visible light, -78 °C *then* ArMgBr (**69**), [Ni(cod)$_2$], (*R*,*R*$_p$)-*i*Pr-Phosferrox *then* Me_2SO_4; ii) mCPBA, TsOH, H_2O; iii) OsO_4, NMO; iv) $LiAlH_4$ *then* H_2, Raney-Co; v) Br_2, AcOH; vi) $NaCo(CO)_4$, CO (1 atm), UV light (365 nm); vii) HMDS *then* $(TMP)_2Cu(CN)Li_2$, *t*BuOOH.

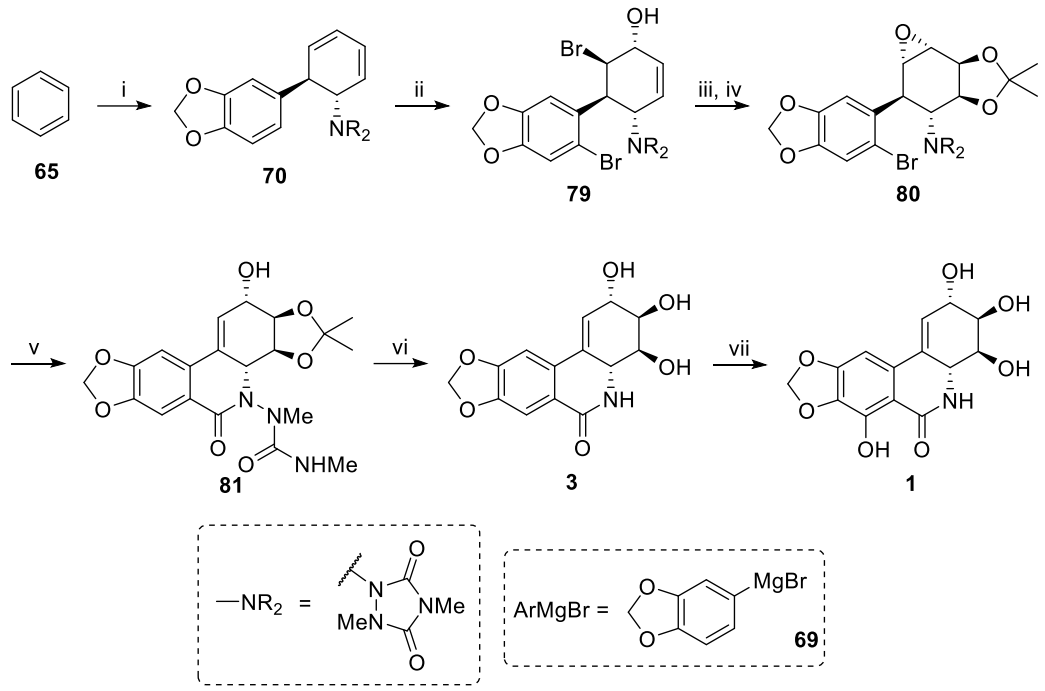
Sarlah continued his progress in preparing Amaryllidaceae alkaloids, moving on to the synthesis of narciclasine-type natural products. His first-generation synthesis of narciclasine (**Scheme 11**) applied much of the same chemistry as seen in the synthesis of pancratistatins. Aside from the dearomative carboamination, the synthesis involved the intramolecular *syn*-elimination/opening of an epoxide with a benzylic anion to form the C-2 allylic alcohol, and simultaneous intramolecular opening of a urazole-type imide with an aryllithium. This served as a new and interesting way of functionalizing the C-ring and

closing the B-ring of the isocarbostyril. The synthesis was completed in six steps and 25 % overall yield, representing a vast improvement over existing syntheses.



Scheme 11. Sarlah's 1st generation approach to narciclasine.[38,39] Reagents and conditions: i) MTAD (**66**), DCM, visible light, -78 °C *then* ArMgBr (**74**), $[Ni(acac)_2]$, (R,R_p)-*i*Pr-Phosferrox *then* Me_2SO_4; ii) NBS, H_2O, iii) OsO_4, NMO *then* K_2CO_3; iv) TsOH, DMP; v) *t*-BuLi; vi) SmI_2 *then* HCl workup.

Once again, a unified approach to access lycoricidine and narciclasine was sought for the second-generation iteration of the synthesis. The application of the direct C-7 hydroxylation used in Sarlah's synthesis of pancratistatins was envisioned, again allowing the use of a simplified aryl Grignard nucleophile in the synthesis. The methodology was successfully applied to the synthesis of lycoricidine and narciclasine in an analogous fashion, accessing the alkaloids in six steps (26 % yield) and seven steps (15 % yield), respectively (**Scheme 12**).[39]



Scheme 12. Sarlah's 2nd generation synthesis of narciclasine, proceeding through lycoricidine.[39] Reagents and conditions: i) MTAD (**66**), DCM, visible light, -78 °C *then* ArMgBr (**69**), [Ni(acac)$_2$], (*R*,*R$_p$*)-*i*Pr-Phosferrox *then* Me_2SO_4; ii) NBS, H_2O, iii) OsO_4, NMO *then* K_2CO_3; iv) TsOH, DMP; v) *t*-BuLi; vi) SmI_2 *then* HCl workup; vii) HMDS *then* $(TMP)_2Cu(CN)Li_2$, *t*BuOOH.

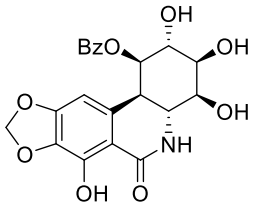
The syntheses discussed showcase some of the excellent progress made in the area of Amaryllidaceae alkaloid synthesis. Several excellent reviews have summarized the syntheses mentioned here, along with many other efforts.[59] Improvements have been made in terms of scalability and conciseness, all the while providing elegant routes to the natural products and developing new synthetic methodologies. Efforts will likely continue as new synthetic tools become available, in hopes of providing a plausible method of supplying scarce natural products.

2.4.2. Synthesis of unnatural analogues

Along with an abundance of efforts to synthesize Amaryllidaceae-derived natural products, there also exist many syntheses of unnatural analogues. These endeavors have allowed for the development of new methods to derivatize the natural products, as well as provided insight into the pharmacophore of the alkaloids. Of the synthesized analogues, summarized nicely in a 2016 review by Hudlicky,[20] only a select few have shown an improvement in biological activity compared to the parent natural products. A select few of these highly active anticancer analogues will be mentioned herein.

2.4.2.1. Pancratistatin analogues

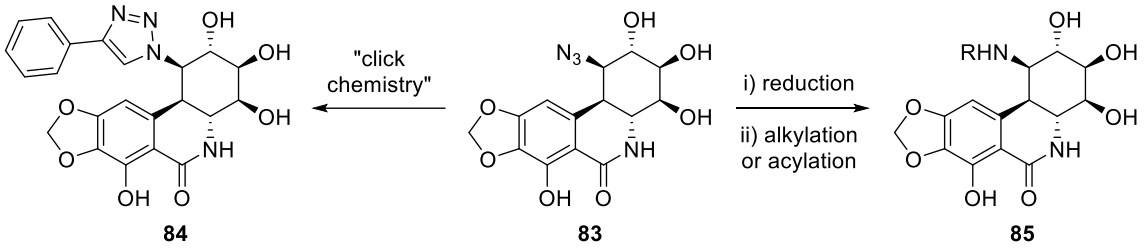
A number of pancratistatin analogues have been synthesized in the past, often serving as stereo- or regiochemical variants of the natural products. There have also been multiple unnatural derivatives prepared bearing functional group deletions, as well as hetero-analogues or homologated compounds. The earliest unnatural analogue to show improvement in anti-cancer activity over that of pancratistatin is the C-1 benzoate (**82**) prepared by Pettit. The compound was made as an intermediate in his reported conversion of narciclasine to the less abundant pancratistatin by chemical means.[60] Intermediates in this conversion were subjected to biological evaluation, and a few were found to have reasonable anticancer activity profiles against the cell lines tested. Benzoate **82** was the best of the new compounds, though, with a recorded ED_{50} value of 1.7 ng/mL against murine leukemia cell lines (compared to 32 ng/mL and 4.4 ng/mL for pancratistatin and narciclasine, respectively).[60] This pointed to the C-1 position as a possible target for future modifications, in hopes of ameliorating biological activity.



82

Figure 7. The C-1 benzoate derivative of pancratistatin prepared by Pettit.[60]

A few years later, a team from a French pharmaceutical company, headed by Marion, would patent a synthetic route to C-1 analogues of pancratistatin.[61] They prepared a small library (35 novel compounds) of nitrogenous pancratistatin analogues in pursuit of bettering the solubility and bioavailability profile of the alkaloid while maintaining or improving its anticancer activity. Their method involved the conversion of a C-1 azide (**83**) to a number of analogues via reduction or "click chemistry", followed by further functionalization (**Scheme 13**).



84 **83** **85**

Scheme 13. Overview of Marion's preparation of nitrogenous pancratistatin derivatives.[61]

The most active compound prepared in this work was the C-1 benzamide analogue (**85**, R = Bz), demonstrating significantly improved activity. This derivative displayed IC_{50} values of 8.7 nM and 4.7 nM against lung and colon cancer cell lines, respectively, representing a five-fold improvement over the activity of narciclasine. Interestingly, many

of the nitrogenous analogues, including benzamide **85** (R = Bz), also showed improved solubility compared to the natural products.[61]

The Hudlicky group would later add to this promising pool of unnatural analogues when his group synthesized a series of C-1 homologues of pancratistatin.[62] The synthesis of these analogues built on earlier results from his group, when previously synthesized C-1 homologues of 7-deoxypancratistatin also showed impressive biological activity values (compared to 7-deoxypancratistatin, which itself is about ten times less potent than pancratistatin).[63] Hudlicky's group reported the synthesis of the C-1 hydroxymethyl, acetoxymethyl, and benzoyloxymethyl derivatives of pancratistatin.[62] The C-1 benzoate homologue (**33**) displayed the best biological activity, with a mean IC_{50} value of 0.03 μmol/L against pancreatic, prostate, lung, and breast cancer cell lines.[61b]

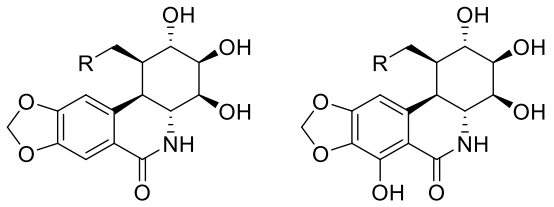


Figure 8. Unnatural C-1 homologues synthesized by Hudlicky.[62,63]

Having demonstrated the possibility of improving biological activity and tailoring the chemical and physical properties of pancratistatin through functionalization at the C-1 position, it seemed as though this site may serve as a reasonable target for further derivatization studies.

2.4.2.2. Narciclasine analogues

Despite being the more naturally abundant of the Amaryllidaceae alkaloids, there have been comparatively few syntheses of narciclasine reported in the literature. The same can be said for derivatization efforts, with only a handful of narciclasine analogues having been prepared to date.

Hudlicky's group reported the synthesis of aza-analogues of narciclasine in 2014 and 2015.[64,65] The syntheses of the 7-aza-nornarciclasine analogues (**88** and **89**)[64] and 10-aza-narciclasine (**90**)[65] were closely related to Hudlicky's previously reported synthesis of lycoricidine,[44] but incorporated the use of pyridine-type aryl fragments. 7-aza analogues **88** and **89** were found to be inactive, potentially due to the lack of methylenedioxy bridge in the A-ring.[64] The 10-aza analogue (**90**), however, displayed anticancer activity comparable (albeit worse) to that of narciclasine, with a mean IC_{50} value of 640 nM across the tested cancer cell lines.[65] This led to the proposal that C-10, another area within the "bay region" of the isocarbostyril backbone, may be another possible area for derivatization.

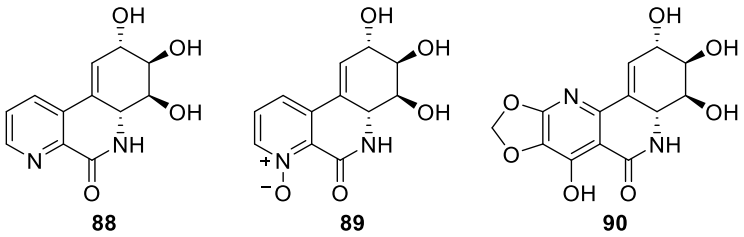


Figure 9. Aza-narciclasine analogues synthesized by Hudlicky.[64,65]

Hudlicky's group has continued their pursuit of unnatural analogues, recently synthesizing a C-10 benzyloxy derivative (**91**)[66] and a C-1 hydroxylated derivative (**92**)[67] of narciclasine. Unfortunately, neither of these analogues showed imprived or maintained

biological activity; with both being a few orders of magnitude less active than their parent natural product, narciclasine.

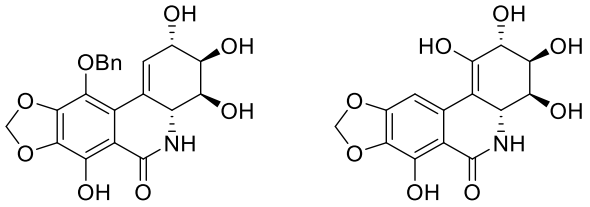


Figure 10. Recent narciclasine analogues synthesized by Hudlicky.[66,67]

2.4.2.3. Summary of SAR studies

With all the past efforts to synthesize unnatural derivatives of the Amaryllidaceae alkaloids, a clearer picture of the compounds' pharmacophore has come to light. The biological evaluation of several analogues has allowed for a cumulative and ongoing SAR study to be performed. Based on the biological activity of these compounds, a set of minimum structural features can be interpreted, and these requirements were summarized well in Hudlicky's 2016 review on Amaryllidaceae alkaloids.[20] **Figure 11** provides a simplified visual representation of the pharmacophore of pancratistatin.

The pharmacophore of narciclasine is less well understood at the current time, as comparatively few derivatization efforts have been made on this natural product. Only one narciclasine analogue has shown even modest activity to this point, being the C-10 aza-derivative (**90**). It is likely that the pharmacophore of narciclasine is similar to that of pancratistatin, however this is largely speculative and can only be applied by analogy until more structural analogues have been prepared and screened for activity.

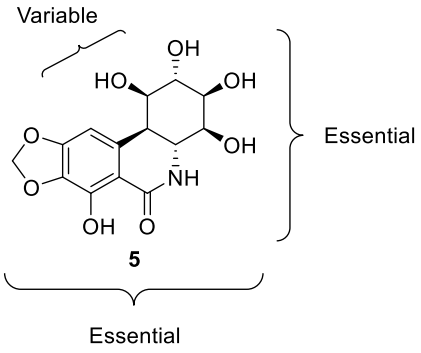


Figure 11. Structure-activity relationship (SAR), as currently understood, for pancratistatin.[20]

Although most of the analogues of Amaryllidaceae alkaloids that have been synthesized have failed to show an improvement in biological activity, the information gained from their assessment is invaluable. The select few derivatives that have shown heightened or maintained anticancer activity will surely inspire the continued pursuit of new and improved analogues in the future. The ultimate achievement of these endeavors would be the attainment of a Amaryllidaceae-derived analogue with suitably potent anticancer activity, along with improved solubility and bioavailability profiles – which could hopefully enter clinical trials and serve as a potential anticancer agent.

2.5 Microbial dihydroxylation of arenes

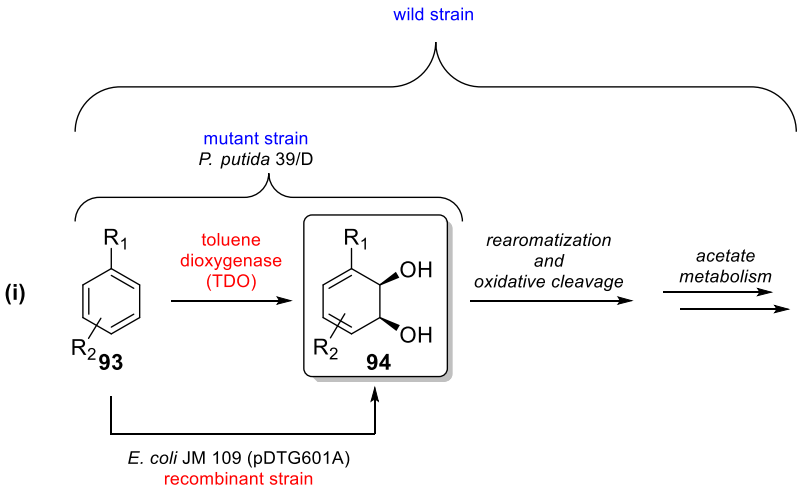
2.5.1 Discovery

Microbial dihydroxylation is a unique chemical transformation carried out in arene metabolism in soil bacteria, *pseudomonas putida*.[68] In the early 1900s, strains of soil bacteria capable of growing on toluene-infused media were isolated, suggesting the presence of a metabolic pathway specific for arenes. It was later pointed out that bacteria

are the likely culprits for the maintenance of hydrocarbon concentrations in soil, preventing detriment to plant viability.[69]

2.5.2 Development

In 1968, Gibson isolated a strain of soil bacteria, *Pseudomonas putida*, capable of growing from an ethylbenzene medium.[70] He would also show that this bacterium was able to metabolize benzene and catechol, suggesting an oxidative metabolic pathway. Gibson would go on to perform feeding experiments with halogenated and otherwise substituted arenes, isolating a stable *cis*-dihydrodiol intermediate.[70b] Only a few years later, he would isolate a mutant strain of *P. putida*, lacking a dehydrogenase enzyme.[71] This served as proof for the occurrence of bacterial dihydroxylation, yielding arene *cis*-dihydrodiols (**94**).



Scheme 14. Chemoenzymatic dihydroxylation reaction carried out by bacteria.[72]

Years later, Gibson would identify the genes responsible for the expression of toluene dioxygenase (TDO), the enzyme responsible for oxidative arene metabolism, in *P. putida*. He would use these findings to prepare a recombinant *E. coli* strain, JM109 (pDTG601A), which overexpressed TDO.[73] This engineered strain provided a more

convenient way to access arene dihydrodiols, as it could be easily grown and used in large scale fermentation reactions. Gibson's findings and development in this area would lead to the eventual use of these arene metabolites in the field of total synthesis.

2.5.3 Use in chemoenzymatic synthesis

The regio-, diastereo-, and enantioselectivity of microbial dihydroxylation has led to its use in the total synthesis of natural products. The arene dihydrodiol (**94**) itself serves as a functionally dense chiral building block, opening the door to enantioselective syntheses and the ability to rapidly construct complex scaffolds. The diene, allylic alcohol, *cis-diol* and R-group functionalities of these small molecules all allow different points for reactivity, and also induce selectivity in a variety of manipulations.[74] Several groups have utilized enzymatically-derived diols as starting materials for the construction of natural products and their analogues in countless sophisticated syntheses. The use of *cis*-dihydrocatechols in natural product synthesis has been reviewed multiple times,[75] with the most recent comprehensive review by Hudlicky in 2022.[72]

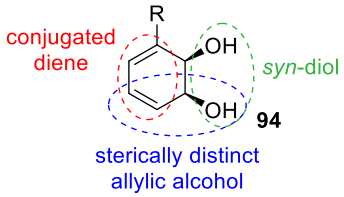


Figure 12. Dense functionality and synthetic utility of dihydrodiols (**94**).[72]

3. Results and Discussion

3.1. Introduction

In recent years, the Hudlicky group has continued their quest to synthesize novel analogues of the Amaryllidaceae alkaloids. Having attained highly bioactive C-1 pancratistatin analogues in the past,[62,63] recent focus has shifted to derivatizing narciclasine. Narciclasine is the more naturally abundant of the Amaryllidaceae alkaloids and has also proven to possess marginally better anticancer activity against a number of cancer cell lines.[4,23] Despite this, there have been relatively few narciclasine analogues synthesized compared to pancratistatin, thereby representing a gap in this field of natural product derivatization and research. Current projects in the group involve the functionalization of natural narciclasine (semi-synthesis) to produce unnatural analogues, as well as chemoenzymatic total synthesis of novel analogues. The current project focuses on the latter, targeting C-1 analogues of narciclasine via total synthesis.

The idea for the project is largely based on the C-1 pancratistatin homologues previously synthesized by Hudlicky and colleagues.[62] These analogues possessed a hydroxymethyl appendage at the C-1 position, either in the form of the free -OH or an acylated derivative (**31**, **32** and **33**). This project was designed with the intent of synthesizing analogous compounds with the narciclasine backbone, thus C-1 (hetero)methylene narciclasine derivatives of type **95** (**Figure 13**). The aminomethyl (**95**, X = NH, R = H) and hydroxymethyl (**95**, X = O, R = H) derivatives were designated as primary targets, with their acylated or alkylated variants coming after.

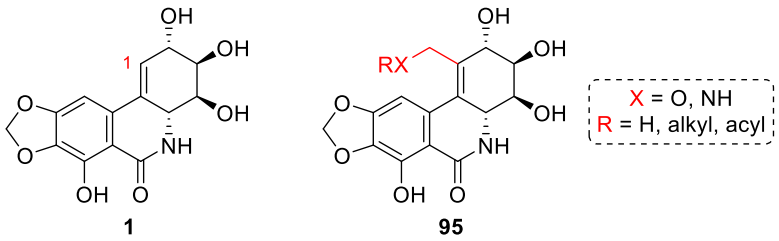


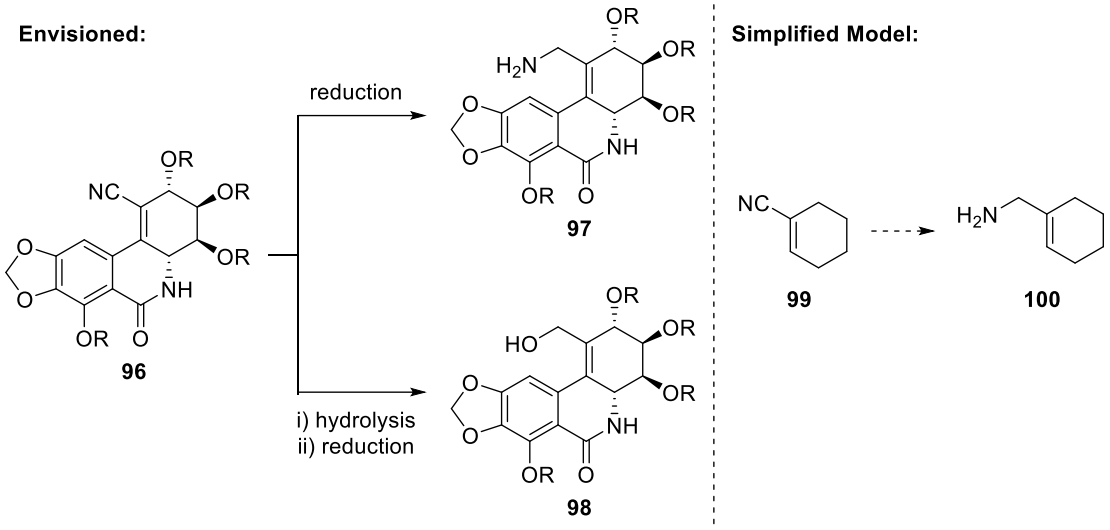
Figure 13. Structures of narciclasine and the target analogue series.

These synthetic efforts were planned to address the issue of poor aqueous solubility, while hoping to also improve overall anticancer activity and bioavailability. The possibility of functionalizing the pendant heteroatom at the C-1 position was foreseen to allow for tailoring/screening these properties, all while furthering the understanding of Amaryllidaceae alkaloid SAR.

3.2 Model studies for the synthesis of C-1 analogues

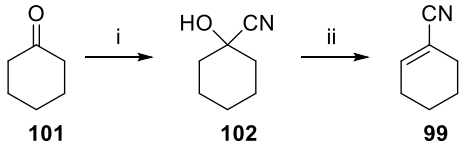
3.2.1. Conversion of vinyl nitriles to allylic amines

While the early stages of the project were being investigated, a C-1 nitrile (**96**) was envisioned as a possible intermediate allowing access to both series of target compounds. A nitrile was seen as a desirable C-1 substituent as it could, theoretically, be converted to a primary amine by full reduction or to an alcohol by hydrolysis and reduction. One of the challenges of this proposed route would be the selective reduction of a nitrile to an amine in the presence of an olefin and other potentially sensitive functional groups. A model study was planned to identify appropriate conditions for the reduction of a vinyl nitrile to an allylic amine (**Scheme 15**).



Scheme 15. Rationale for vinyl nitrile reduction model study.

The model nitrile was prepared in two steps according to a literature protocol[76] (**Scheme 16**), starting from cyclohexanone. The cyanohydrin was prepared by *in situ* generation of HCN and, upon isolation, compound **102** was then dehydrated using thionyl chloride. The pure vinyl nitrile (**99**) was distilled from the mixture, to obtain the desired model substrate in approximately 50 % overall yield.



Scheme 16. Preparation of the model substrate for selective nitrile reduction. Reagents and conditions: i) NaCN, H_2SO_4, H_2O, 60 %; ii) $SOCl_2$, pyridine, 75 %.[76]

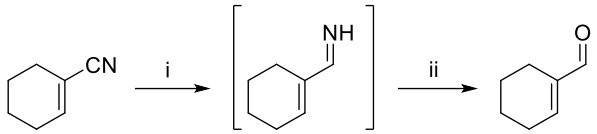
With the model nitrile prepared on large scale, screening for appropriate reduction conditions began. One of the first reductions carried out was with $LiAlH_4$, the only literature procedure[77] for the transformation of the nitrile directly to the allylic amine **100**. The reaction was carried out as a baseline/control experiment, to obtain the allylic amine.

The reaction gave a 25 % yield of amine **100**, which was visible via NMR and IR, and a small amount was purified via Kugelrohr distillation (although heating of the material quickly led to decomposition). These conditions, though, were considered too harsh for use on synthetic intermediates *en route* to narciclasine analogues, and thus milder conditions were screened.

A review of literature on this topic revealed several options for reduction of vinyl nitriles, but many led to full reduction and produced the saturated amine. Another consideration throughout this study was the need for practical conditions for the desired reduction – ones mild enough to be amenable to a narciclasine intermediate. Conditions considered too harsh to be tolerated by narciclasine's functionalities or late-stage protecting groups were eliminated from contention.

The investigation for practical conditions continued with various hydrogenation catalysts. Urushibara Ni, Urushibara Co, Raney Ni, and Raney Co reductions[78] were all attempted under hydrogen atmosphere. To our dismay, none of the heterogeneous reactions furnished the desired amine.

The focus shifted to more common hydride reducing agents, in hopes of finding milder and more selective reduction conditions compared to $LiAlH_4$. It was found that the nitrile could be cleanly reduced to the corresponding enal using DIBAL in toluene at -40 °C and allowing the mixture to slowly warm to r.t. before acidic workup (87 % yield).

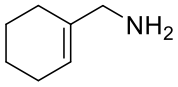
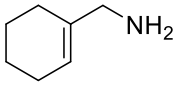
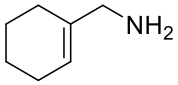
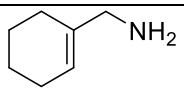
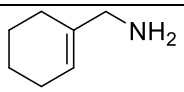


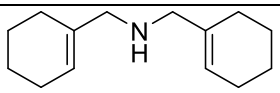
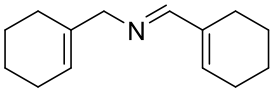
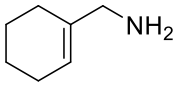
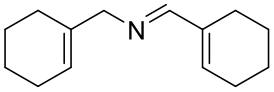
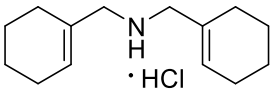
99 103 104

Scheme 17. Reduction of vinyl nitrile (**99**) to enal (**104**). Reagents and conditions: i) DIBAL, toluene -40 °C – r.t.; ii) HCl (aq.) or other aqueous workup, 87 %.

Unfortunately, attempts to further reduce the intermediate imine were rather unsuccessful, and only the aldehyde or small amounts of the desired primary amine could be isolated from the reaction. These results are summarized in **Table 4**.

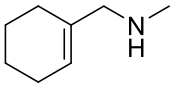
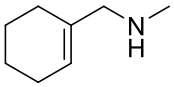
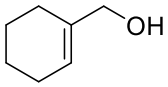
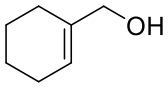
Table 4. Reduction of vinyl nitrile **99** under varying conditions.

Entry	Conditions (for the reduction of nitrile **99**)	Observed/Isolated Products of Interest	Yield
1	i) DIBAL (1 eq.), PhMe, -40 °C ii) $NaBH_4$ (1.5 eq.), MeOH, 0 °C	 **100**	10 %
2	i) DIBAL (1 eq.), PhMe, -40 °C ii) $NaCNBH_3$ (1.5 eq.), MeOH, 0 °C	 **100**	15 %
3	i) DIBAL (1 eq.), PhMe, -40 °C ii) $NaCNBH_3$ (1.5 eq.) (*inverse addition*), MeOH	 **100**	20 %
4	i) DIBAL (1 eq.), PhMe, -40 °C ii) $NaCNBH_3$ (1.5 eq.), MeOH, 0 °C (*under NH_3 atmosphere*)	None, small amount of s.m.	n/a
5	i) DIBAL (1 eq.), PhMe, -40 °C ii) $NaBH(OAc)_3$ (1.5 eq.), MeOH, 0 °C	 **100**	5 %
6[79]	i) $LiBH_4$ (1 eq.), THF/MeOH, reflux	 **100**	5 % 5 %

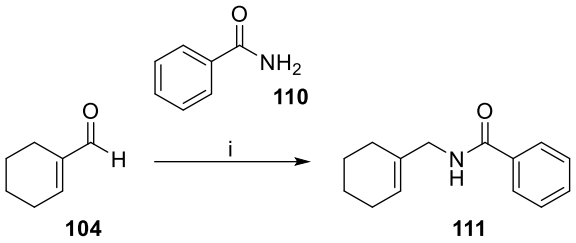
		 **105**  **106**	10 %
7	i) DIBAL (1 eq.), PhMe, -40 °C ii) $LiBH_4$ (1 eq.), THF/MeOH, 0 °C	 **100**  **106**  **107**	5 % 10 % 70 %[80]

Because of the discouraging results from trials to this point, it was postulated that the efficient conversion of nitrile to enal (**104**) could be used to our advantage. With this being the only "clean" conversion performed so far, the use of the enal in reductive amination reactions was investigated. Although redundant in terms of heteroatom exchange, it would theoretically provide a viable means to access nitrogenous models. Reductive amination conditions using primary amine nucleophiles proved successful, however, attempts to perform analogous reactions with ammonia (to form a primary amine) failed. Results of these efforts are summarized in **Table 5**.

Table 5. Attempts at reductive amination of enal **104**.

Entry	Conditions (for the reductive amination of enal **104**)	Observed/Isolated Products of Interest	Yield
1[81]	$MeNH_2 \cdot HCl$, $Ti(OiPr)_4$, Et_3N, $NaCNBH_3$, MeOH	 **108**	25 %
2[82]	$MeNH_2 \cdot HCl$, Et_3N, $NaBH_4$, MeOH	 **108**	60 %
3	NH_3 (generated from NH_4OH/NaOH), $NaBH_4$, MeOH	 **109** + s.m.	60 % 25 %
4	NH_3 (generated from NH_4OH/NaOH), $NaBH(OAc)_3$, MeOH/AcOH	 **109** + s.m.	30 % 25 %
5[83]	NH_4OAc, $NaCNBH_3$, EtOH/NH_4OH	s.m.	n/a

A final option for this conversion was considered, whereby an amide could act as the nucleophile for a reductive amination-type reaction. A review of the literature revealed that such a transformation was already known, presenting as a condensation of amide with a ketone or aldehyde followed by hydrosilylation/reduction of the acyl iminium intermediate.[84] Based on this report, enal **104** was converted to the corresponding *N*-allyl benzamide (**111**), albeit in low yield (**Scheme 18**). With these results in hand, it was time to move on to the synthesis of desired Amaryllidaceae alkaloid analogues.



Scheme 18. Condensation and hydrosilylation reaction of enal **104** and benzamide. Reagents and conditions: i) Et_3SiH, TFA, toluene, 80 °C, 60 %.

3.3. Synthesis of C-1 homologues of narciclasine

The main focus of this project was to synthesize C-1 analogues of narciclasine. The route was based upon previous syntheses of Amaryllidaceae constituents or analogues performed within the Hudlicky group. This synthesis, like many before it in Hudlicky's group, was based on the exploitation of microbial dihydroxylation. This approach allows full enantiospecificity in synthesis, and also provides desirable selectivity in many transformations.

The initial goal was to synthesize novel analogues with general structure **95**, however a number of challenges were encountered along the way. Some of these were overcome, while others seemed insurmountable and shaped the project in unforeseen ways. The eventual outcome was the synthetic route to be detailed herein, primarily targeting 2-*epi*-1-hydroxymethylnarciclasine (**2**).

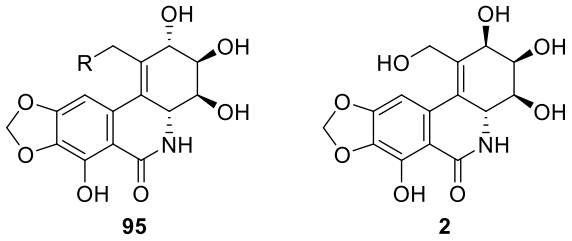
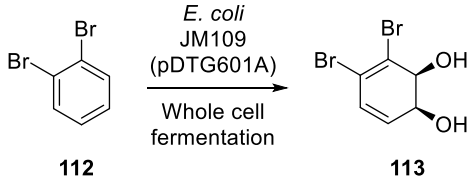


Figure 14. Structures of the planned C-1 analogues (**95**) and the eventual final target, 2-*epi*-1-hydroxymethylnarciclasine (**2**).

3.3.1. Synthesis of 2-*epi*-1-hydroxymethylnarciclasine

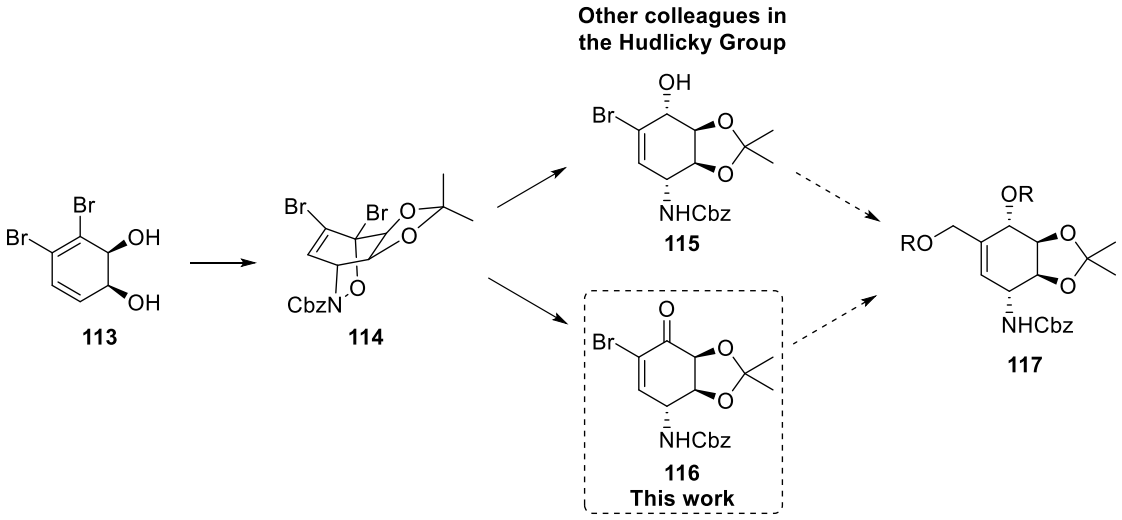
The current synthesis of novel C-1 narciclasine analogues takes inspiration from Hudlicky's 1992 lycoricidine synthesis (**Scheme 5**).[44] In a similar manner to that synthesis, the current approach involved the use of a chemoenzymatically-derived diene diol to induce stereospecificity throughout the synthesis, and employs an acylation-intramolecular Heck strategy to construct the backbone of the natural product. The key difference from the lycoricidine synthesis is, of course, the necessity for the introduction of a C-1 substituent, along with the presence of a C-7 hydroxyl group to furnish a true narciclasine analogue. This was addressed when the synthesis was planned around the use of the 1,2-diboromobenzene-derived diol metabolite (**113**).[85]



Scheme 19. Chemoenzymatic oxidation of 1,2-dibromobenzene.[85]

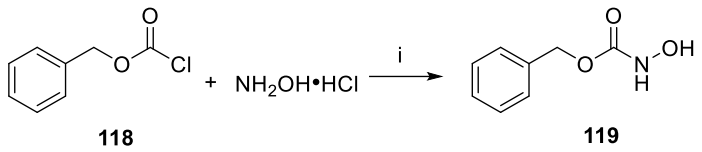
The use of this microbial metabolite was foreseen to provide a pendant vinyl bromide after partial elaboration of the diol (**113**). The chiral C-ring fragment would be

constructed based on the functionality of the diene diol starting material; first undergoing a cycloaddition, followed by cleavage of the heteroatom bridge to form the amino and alcohol functionalities. The reductive cleavage has been performed in a few different ways in Hudlicky's past syntheses, thereby providing two unique but closely related routes (**Scheme 20**). Intermediate **115** is advantageous because it has all desired stereocentres set at an early stage, while **116** provides ample opportunity to functionalize the C1 position via the α-bromo enone. Elaboration of **115** is discussed in a recent thesis by Hudlicky group colleague Lihi Habaz.



Scheme 20. Initial establishment of early steps in the synthetic routes to C-1 narciclasine analogues.

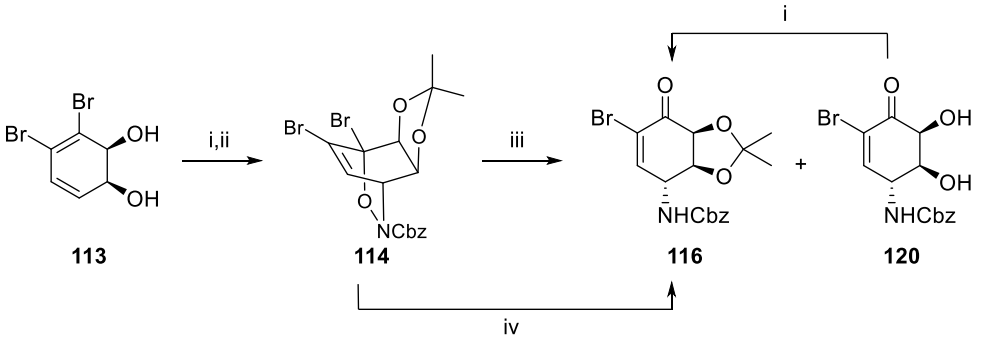
With 1,2-diboromobenzene *cis*-dihydrodiol (**113**) in hand, the synthesis started with the performance of a nitroso Diels-Alder reaction. The dienophile for this reaction, an *N*-acylated hydroxylamine (**119**), was synthesized by a known protocol.[86]



118 119

Scheme 21. Synthesis of the nitroso Diels-Alder dienophile. Reagents and conditions: i) K_2CO_3, Et_2O, H_2O, 75 %, 30 g scale.

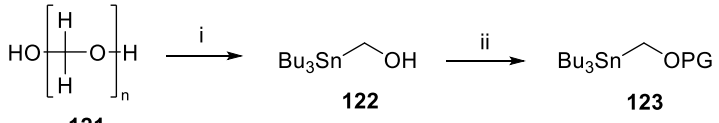
Having prepared CbzNHOH **119**, the oxazine was then synthesized from the 1,2-dibromobenzene diol (**113**) in a one pot, two operation sequence. The reaction was high yielding and scalable, with the possibility of submitting 5 g of diol **113** to the reaction at a time, while maintaining yields. The resulting bicyclic oxazine (**114**) was then reduced with $Mo(CO)_6$ to bromoenone **116**. Upon increasing the reaction scale, yields drop significantly for this reaction. If any more than a trace of water is present, the deprotected diol **120** is produced in greater quantities. This observation was used advantageously to simplify the purification and isolation of bromo-enone **116**, whereby oxazine **114** was intentionally converted to deprotected bromo-enone **120** by use of a MeCN/H_2O solvent system. The crude reaction mixture was then subjected to acetonide reprotection, yielding the desired enone (**116**). This protocol, though more arduous, has provided reproducibility and reasonable yields of this intermediate for further elaboration.



113 114 116 120

Scheme 22. Synthesis of enone **116** from 1,2-dibromobenzene diol **113**. Reagents and conditions: i) 2,2-DMP, *p*TsOH, DCM; ii) $NaIO_4$, CbzNHOH (**119**), MeOH, H_2O, 80 % over two steps; iii) $Mo(CO)_6$, MeCN, reflux, 20-30 % of **116**, 20 % of **120**; iv) $Mo(CO)_6$, MeCN, H_2O, reflux *then* 2,2-DMP, *p*TsOH, DCM, 50 % of **116**.

With enone **116** in hand, focus shifted to the functionalization of the vinyl bromide to install the eventual C-1 substituent. Stille coupling was proposed as a reasonable starting point for initial investigation. The necessary organostannane reagent was prepared from tributyltin hydride and paraformaldehyde in the presence of LDA, furnishing tributylstannyl methanol **122** in near quantitative yields.[87] Stannane **122** was then converted to either a silyl ether[88] or benzoate[89] derivative, providing access to a series of tributyltin reagents suitable for Stille coupling (**123a-c, Scheme 23**).

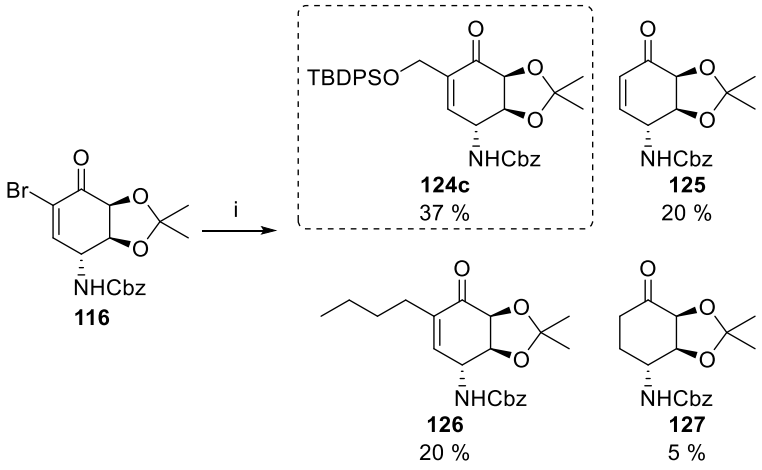


121 122 123

123a	PG-X = benzoyl chloride	base = pyridine, 75 %
123b	PG-X = TBS-Cl	base = imidazole, 77 %
123c	PG-X = TBDPS-Cl	base = imidazole, 71 %

Scheme 23. Synthesis of tributlystannyl methanol derivatives. Reagents and conditions: i) LDA, Bu_3SnH, THF, 0 °C, 95 %; ii) PG-X, base, DCM, 0 °C.

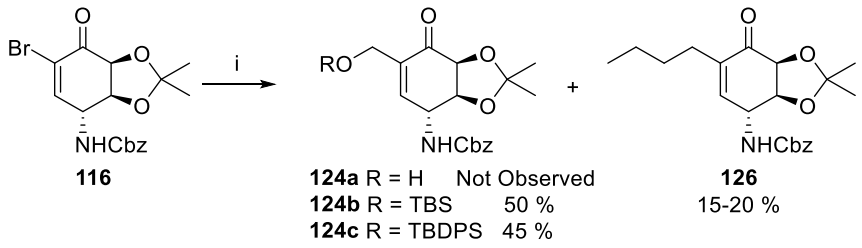
Following the preparation of both the α-bromo enone (**116**) and tin reagents **123a-c**, Stille coupling was attempted, first using the TBDPS-protected reagent **123c**. This reaction gave desired enone **124c** in a disappointing 37 % yield (**Scheme 24**). Side products of the reaction were isolated to help explain the low yield, with the de-halogenated (**125**) and butane addition (**126**) products being the main impurities in the mixture. To make matters worse, desired enone **124** and the butane addition product (**126**) overlap substantially on TLC (regardless of solvent mixture or polarity), and are only barely separable by prep TLC after developing multiple times.



Scheme 24. Initial Stille coupling attempts with bromo enone **116**. Reagents and conditions: i) $PdCl_2(PPh_3)_2$, stannane (**122**, **123b** or **123c**), 1,4-dioxane, reflux.

In an effort to improve the coupling reaction, Pd^0 was used directly in the form of $Pd(PPh_3)_4$. These conditions gave somewhat improved yield and shorter reaction time, but no improvement was observed in selectivity of which R-group was transferred from the tin centre to the bromo enone (**Scheme 25**). Because of these results, and because the presence of butane addition product **126** complicated isolation of the desired product **124c**, other

stannanes were investigated. With the knowledge that the reaction proceeds best with $Bu_3SnCH_2OTBDPS$ using Pd^0, the coupling was attempted analogously using the free tributylstannyl methanol **122** and its TBS derivative **123b**. None of the product bearing the free hydroxyl was observed (**123a**), but the TBS-protected analogue **124b** was isolated in 50 % yield (**Scheme 25**). Perhaps just as important as the product's formation was the fact that its R_f was better resolved from **126** than the previously isolated TBDPS ether **124c**, easing separation of the compounds.



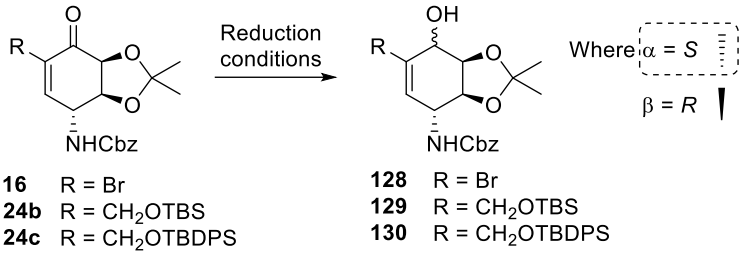
116

124a R = H Not Observed
124b R = TBS 50 %
124c R = TBDPS 45 %

126
15-20 %

Scheme 25. Stille coupling trials and improved outcomes. Reagents and conditions: i) $Pd(PPh_3)_4$, stannane (**122**, **123b** or **123c**), 1,4-dioxane, reflux.

Having obtained the desired C-1 functionalized (pancratistatin numbering) product (**124b**) in modest yields from Stille coupling, it was then necessary to investigate the reduction of the enone functionality. It was foreseen that the undesired β-alcohol may be the favoured product of the reaction due to steric hindrance of the β-face by the acetonide group. This suggests that a hydride can be more easily delivered from the α-face, leading to possible issues in obtaining the desired α-alcohol (**128-130-α**). Therefore, the ability to access one diastereomer selectively was given primary importance in these studies. Various conditions were investigated for the conversion of the enone functionality (**116**, **124b**, **124c**) to the corresponding allylic alcohol (**Scheme 26**), and they are summarized in **Table 6**.



Scheme 26. General scheme of the enone reduction study (see **Table 6**).

Table 6. Results from the reduction of enones to their corresponding allylic alcohols.

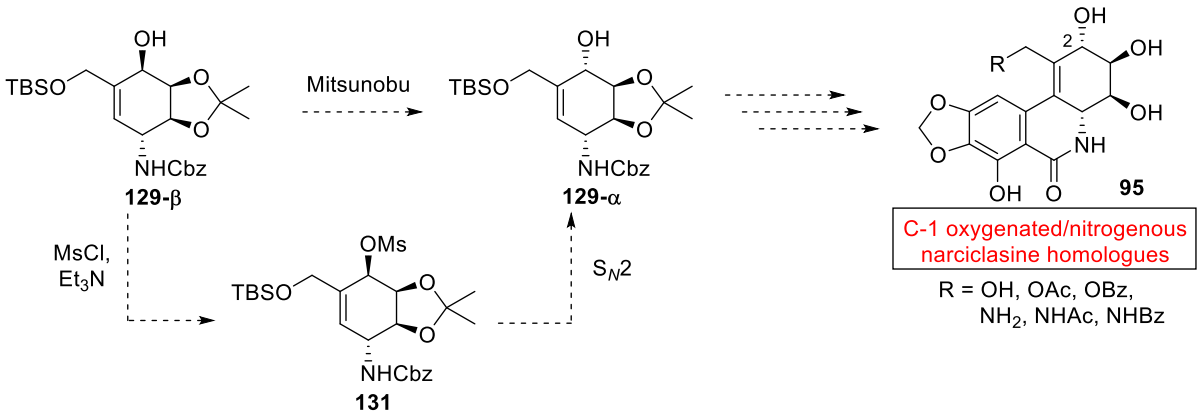
Entry	Starting enone	Reduction conditions	Product*
1	R = Br	$NaBH_4$ (1.5 eq.), $CeCl_3$, MeOH, 0 °C	80:20 β/α
2	R = Br	L-selectride (1.5 eq.), THF, 0 °C	β exclusively
3	R = Br	*S*-CBS/borane (2 eq.), THF, 0 °C	40:60 β/α[a]
4	R = Br	*R*-CBS/borane (2 eq.), THF, 0 °C	60:40 β/α[a]
5	R = $CH_2OTBDPS$	$NaBH_4$ (1.5 eq.), $CeCl_3$, MeOH, 0 °C	80:20 β/α
6	R = $CH_2OTBDPS$	*S*-CBS/borane (2 eq.), THF, 0 °C	traces of β[a]
7	R = $CH_2OTBDPS$	L-selectride (1.5 eq.), THF, 0 °C	β exclusively[a]
8	R = CH_2OTBS	L-selectride (1.5 eq.), THF, 0 °C	β exclusively
9	R = CH_2OTBS	DIBAL (1.5 eq.), THF, -40 °C	β exclusively

[a] Diastereomeric ratio based on ^1H NMR; * R group in product is the same as the starting material for all entries.

The reduction study pointed to the use of either L-selectride or DIBAL for the reduction of enone **124b** (entries 7-9), though these conditions would later prove to be poorly reproducible - with yields frequently dropping below 50 %. These inconsistencies in yield are believed to have been caused by impurities present after Stille coupling, as the

purification of the resultant enones proved difficult. Nonetheless, the reduction screening helped determine conditions to selectively obtain a single diastereomer for further use in the synthesis. The low yields and required inversion were planned to be addressed later.

From **129-β**, the plan was to perform an inversion of the C-2 allylic alcohol to establish the correct stereochemistry needed to match that of narciclasine (**Scheme 27**).



Scheme 27. Proposed approaches to inversion of the C-2 stereocenter.

Attempts were made to invert the C-2 allylic alcohol via a Mitsunobu reaction or through activation (sulfonylation) and S_N2 displacement but, sadly, none of the conditions provided access to **129-α**. With this disappointing result in mind, focus shifted to the synthesis of 2-*epi*-narciclasine homologues, which would still provide useful insight into the SAR of narciclasine.

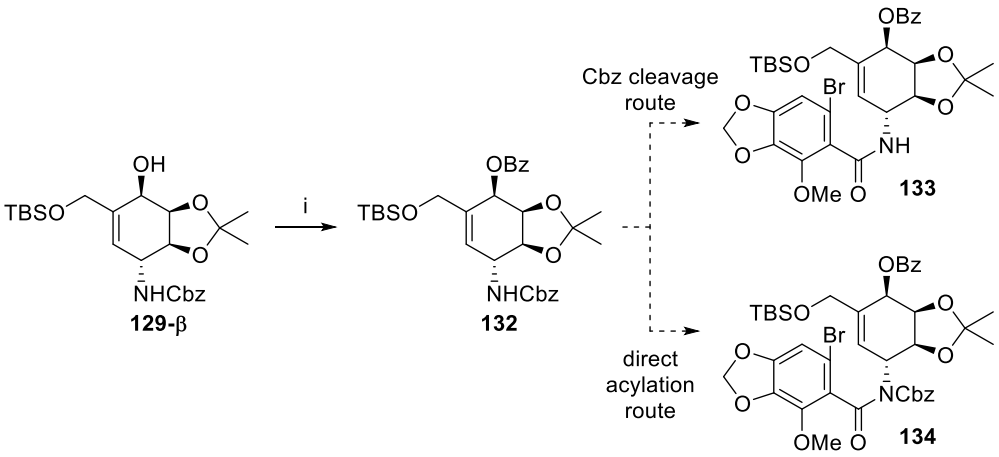
Table 7. Initial attempts at Mitsunobu inversion or sulfonylation of allylic alcohol **129-β**.

Entry	Substrate	Conditions	Result
1*	**129-β**	*p*-NO_2BzOH, THF, r.t.	no conversion
2	**129-β**	TMAD, Bu_3P, *p*-NO_2BzOH, THF, r.t.	no conversion

3	**129-β**	DEAD, Bu_3P, BzOH, THF, r.t.	no conversion
4	**129-β**	DEAD, Bu_3P, BzOH, THF, 35 °C	Low conversion, decomposition only
5	**129-β**	DEAD, Bu_3P, BzOH, THF, 60 °C	Low conversion, decomposition only
6	**129-β**	MsCl, Et_3N, DCM, 0 °C to r.t.	no conversion

*Control reaction to confirm stability of silyl group in the presence of a strong organic acid.

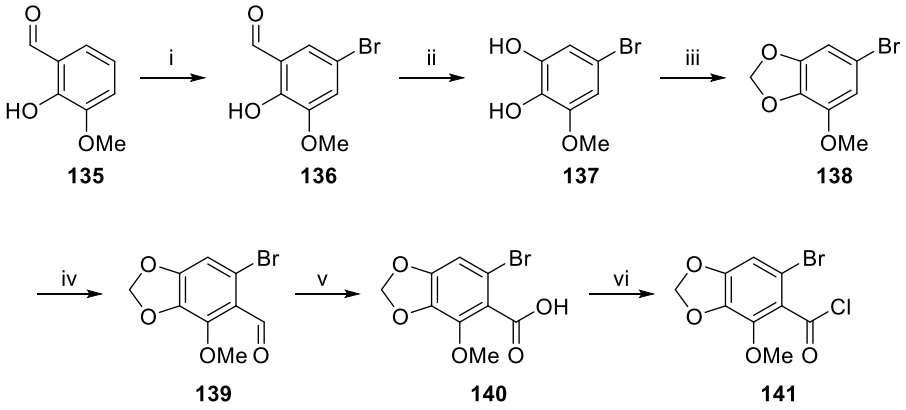
Allylic alcohol **129-β** was then protected as benzoate **132** in preparation for the convergence of A- and C-ring fragments. The initial plan had two possible options from benzoate **132**: cleavage of the Cbz carbamate followed by acylation to an amide or acylation of the carbamate to an imide (**Scheme 28**).



Scheme 28. Planned elaboration of benzoate **132**. Reagents and conditions: i) BzCl, pyridine, DMAP, DCM, 0 °C.

The Cbz cleavage was first attempted according to the conditions reported by Olivo in Hudlicky's 1992 synthesis of lycoricidine (cyclohexene, Pd/C, EtOH).[44] These conditions were successful in cleaving the Cbz group, but unfortunately also reduced the olefin. Other conditions (transfer hydrogenation, dealkylation) led only to unproductive products. Basic hydrolysis of the benzyl carbamate protecting group furnished the desired primary amine, but also deprotected the silyl ether at C-1 and the results were found to be irreproducible. Because of this, the strategy was adjusted and the silyl ether protecting group at C-1 was reconsidered.

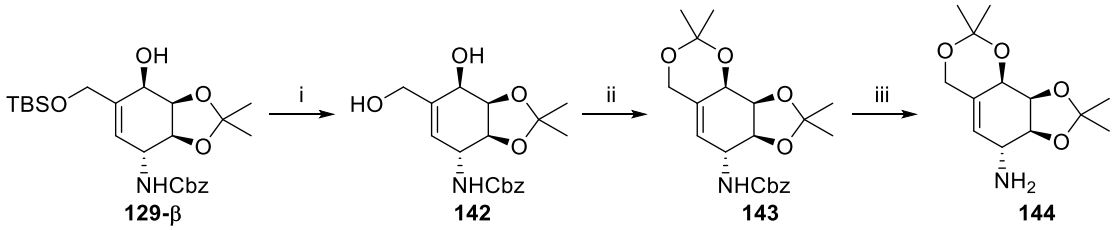
While research on the construction of the narciclasine C-ring was ongoing, it also was necessary to work on the assembly of the aromatic A-ring fragment. The A-ring was constructed by literature protocols,[90,91,92] starting from *o*-vanillin, in a short sequence. The acid chloride (**141**) was proposed to be a suitable coupling partner for an *N*-nucleophile generated from the C-ring fragment.



Scheme 29. Synthesis of the A-ring fragment.[90,91,92] Reagents and conditions: i) Br_2, NaOAc, AcOH, 91 %; ii) H_2O_2, NaOH, H_2O, 0 °C *then* HCl (2 M), 61 %; iii) CH_2I_2, K_2CO_3, DMF, 100 °C, 77 %; iv) Cl_2HCOMe, $SnCl_4$, DCM, -78 °C *then* HCl (3 M), r.t., 55 %; v) $NaClO_2$, NaH_2PO_4, β-amylene, *t*-BuOH, H_2O, 85 %; vi) $(COCl)_2$, DMF, DCM, quant.

Returning to the C-ring synthesis, a change of strategy was required. The elaboration of benzoate **132** proved to not be viable, as the Cbz group was only successfully removed under basic conditions. Instead, it was proposed that the C-ring could be modified to only possess acid-labile (base-stable) protecting groups, aside from the Cbz group. A second acetonide (1,3-dioxane across the C-1 and C-2 allylic alcohols) was considered. To achieve this, allylic alcohol **129-β** was desilylated using TBAF, providing allylic diol **142**. This intermediate was then subjected to conditions for acetonide protection in order to obtain *bis*-acetonide **143**. The *bis*-acetonide was essentially "set up" as a C-ring intermediate bearing only one base- or hydrogenolytic-labile functional group, the carbamate on the nitrogen.

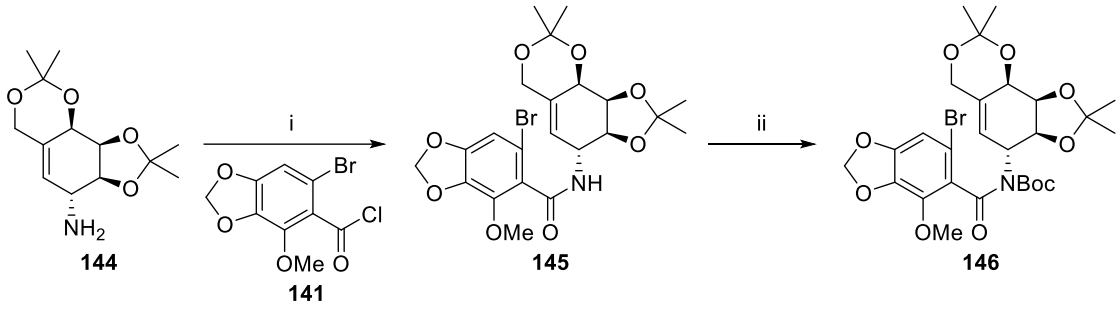
Eventually, the uncovering of suitable conditions for reliable Cbz removal was achieved: a transfer hydrogenation involving $PdCl_2$, Et_3SiH, Et_3N in DCM. These conditions gave the desired selectivity and reproducibility, allowing access to a suitably reactive nucleophile for coupling to the A-ring acid chloride (**141**).



Scheme 30. Conversion of allylic alcohol **129-β** to *bis*-acetonide **144**. Reagents and conditions: i) TBAF, THF, 60 %; ii) 2,2-DMP, *p*TsOH, DCM, DMF, 75 %; iii) 40 % KOH, MeOH, 80 °C, 40 % (irreproducible) *or* Et_3SiH, $PdCl_2$, Et_3N, DCM, 60 %.

With both the A- and C-ring precursors in hand, it was then necessary to connect the fragments and establish the full phenanthridone backbone native to the isocarbostyrils. The strategy to do so was inspired by previous syntheses in the Hudlicky group,[44,64,65,66]

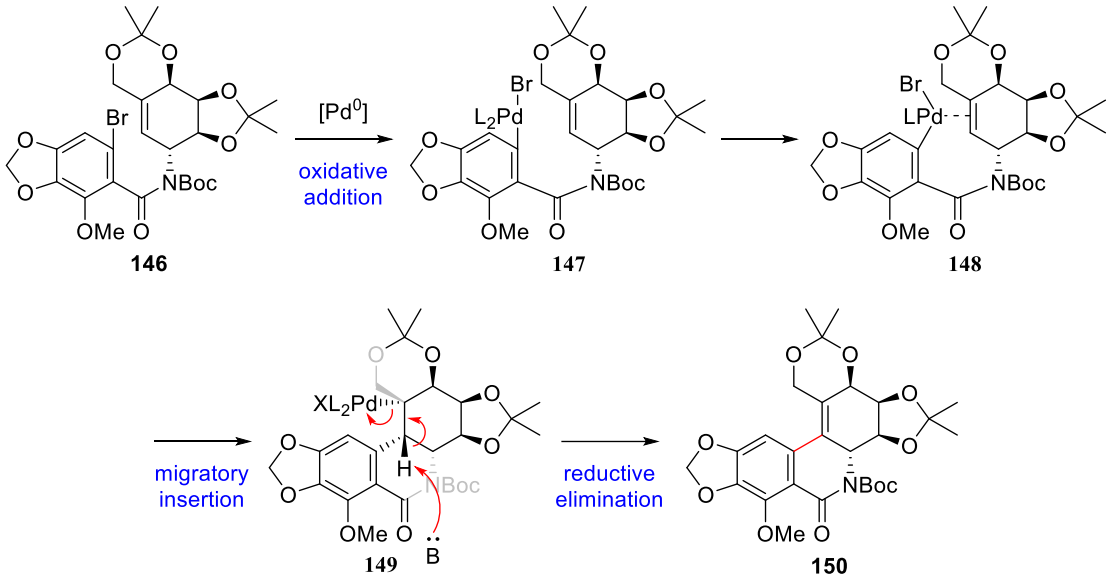
whereby the A- and C-rings are connected via an acylation reaction, followed by an intramolecular Heck-type closure to establish the B-ring lactam. The key amide bond was formed through the reaction of the C-ring precursor, amine **144**, with the A-ring acid chloride (**141**). Amide **145** was subsequently protected as the corresponding Boc imide (**146**), setting the stage for one of the key steps of the synthesis, the intramolecular Heck reaction.



Scheme 31. Convergence of the A- and C-ring fragments. Reagents and conditions: i) **141**, pyridine, DMAP, DCM, 0 °C, 50-60 %; ii) Boc_2O, DMAP, MeCN, r.t., 80 %.

The intramolecular Heck reaction poses a significant challenge in the synthesis, as the reaction must proceed by a stereochemically-disallowed *anti*-elimination in the final step to form the styrene-type double bond (**Scheme 32**).[93] Although a select few examples of this type of reaction are known in the literature, the yields are often modest and the conditions for a successful transformation are highly specific to the substrate.[94] Several mechanisms have been suggested for this elimination step, involving epimerization of the Pd-C bond,[94c] allylpalladium isomerization,[95] homolytic Pd-C cleavage[93] and base-assisted *E2*-type reductive elimination.[94a] The lattermost option seems most likely in the present case and in similar Amaryllidaceae constituent syntheses,[96] whereby a moderate to strong base is used in the intramolecular Heck reaction along with thallium or silver salts (acting

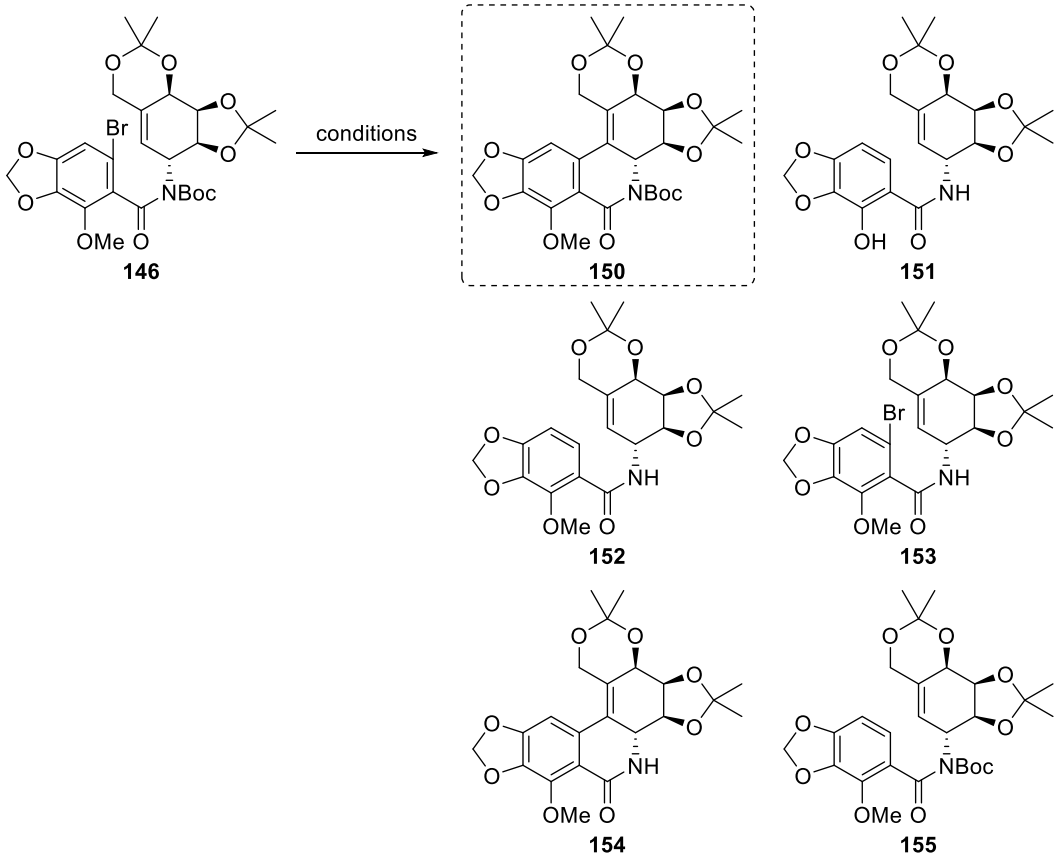
to both promote the cationic pathway of the reaction and to suppress product olefin isomerization).[97]



Scheme 32. Proposed mechanism for envisioned intramolecular Heck coupling.

Various conditions were screened for the Heck reaction, with limited success (**Scheme 33**, **Table 8**). Low conversion of starting material suggested that the oxidative addition step was not proceeding to a great extent, so a few solutions were attempted. First, a replication of Olivo's Heck protocol[44] was performed using stoichiometric Pd catalyst (**Table 8**, **Entry 3a**), but no improvement in reaction progress was observed. The next hypothesis was that the long reaction time and elevated temperature were likely decomposing the catalyst rather quickly, leading to premature cessation of the reaction. Because of these results, the Pd catalyst was added in four portions over the total 40 h reaction time (**Table 8**, **Entry 3b**). These conditions managed to push the reaction to a greater extent, with only approximately 10 % starting material remaining after 40 h, though this only resulted in increased yields of debrominated products **152** and **155** and no

improvement in the isolated yield of desired product **150**. The final attempt at improving the Heck reaction came by using a more electron-rich Pd catalyst, PEPPSI-*i*Pr (**P**yridine-**E**nhanced **P**recatalyst **P**reparation, **S**tabilization and **I**nitiation), in hopes of promoting the oxidative addition step (**Table 8**, **Entry 4**). Again, the use of this catalyst improved the overall conversion, but provided only the dehalogenated products **152** and **155** in increased yields.

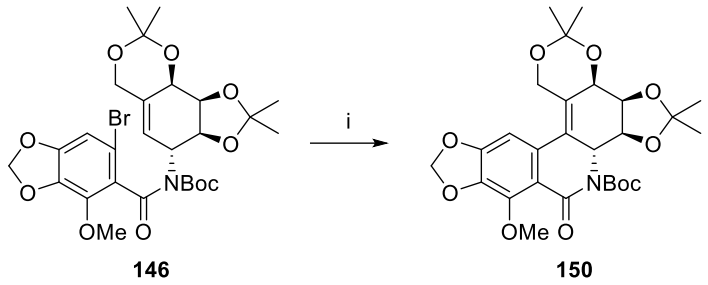


Scheme 33. Intramolecular Heck reaction outcomes, with the desired cyclized product highlighted.

Table 8. Summary of intramolecular Heck results, highlighting the yield of the desired cyclized product, phenanthridone **150**.

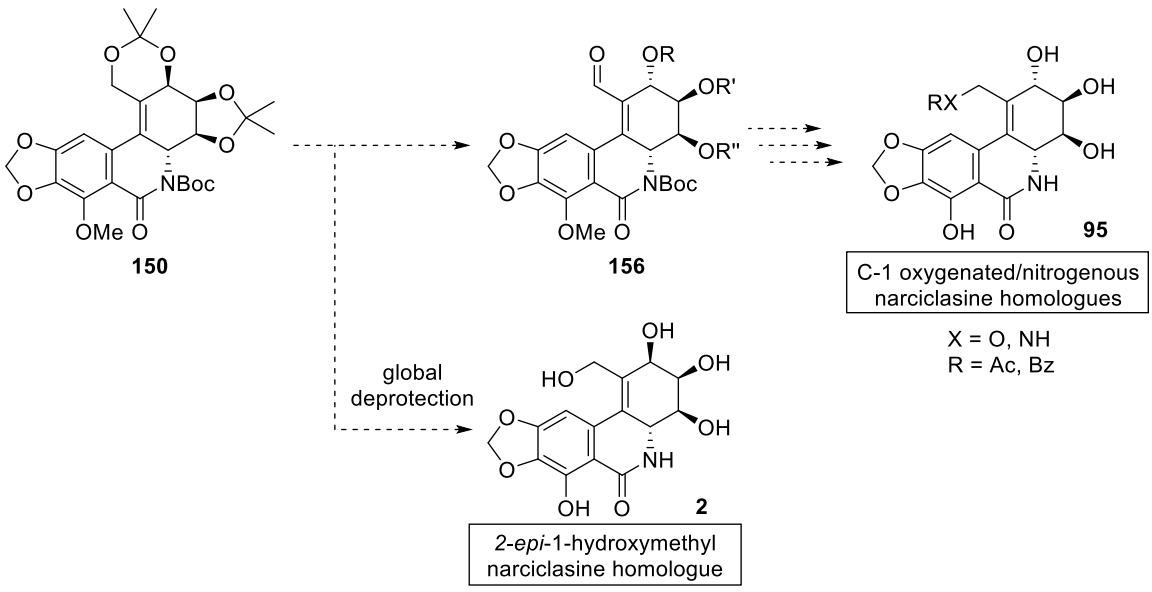
		% Yield						
Entry	Conditions	**146** (s.m.)	**150**	**151**	**152**	**153**	**154**	**155**
1	$Pd(OAc)_2$, DPPE, Ag_3PO_4, toluene, reflux	50 %	Trace	-	10 %	10 %	Trace	10 %
2	$Pd(OAc)_2$, PPh_3, Et_3N, DMF, 120 °C	<5%	-	25 %	20 %	10 %	-	20 %
3	$Pd(OAc)_2$, DPPE, TlOAc, anisole, 130 °C	50 %	5 %	-	10 %	-	Trace	10 %
4	Same as 3. Stoichiometric [Pd]	75 %	7 %	-	<5%	-	Trace	10 %
5	Same as 4. [Pd] added over 40 h	10 %	5 %	-	10 %	-	Trace	20 %
6	PEPPSI-*i*Pr, TlOAc, anisole, 130 °C	25 %	-	-	25 %	-	-	5 %

The conditions used by Hudlicky and Olivo in the synthesis of lycoricidine[44] proved to be the most promising to affect the desired cyclization, though still proceeding in disappointing yield and low conversion. The acquisition of the cyclized product represented a milestone in the synthesis, nonetheless, as the product possessed the fully assembled backbone, all heteroatoms, and stereochemistry of the targeted analogue.

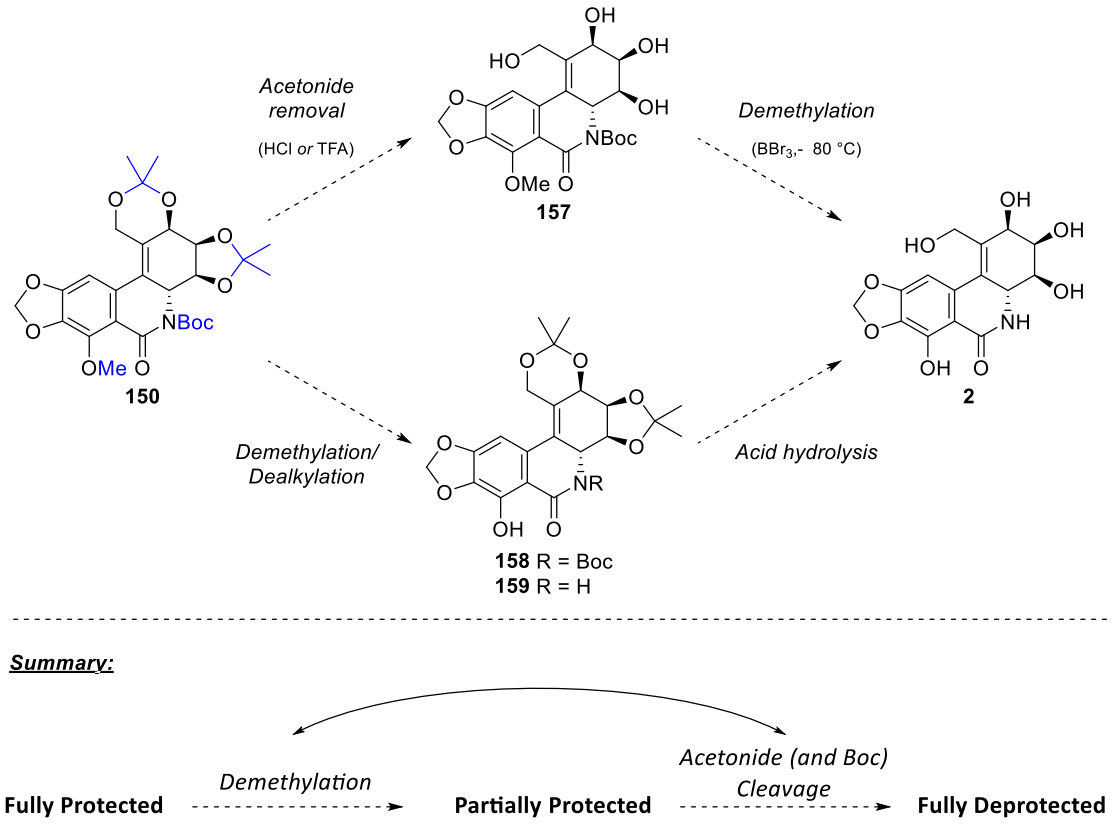


Scheme 34. The successful intramolecular Heck reaction. Reagents and conditions: i) $Pd(OAc)_2$, DPPE, TlOAc, anisole, 130 °C, 5-10 %.

From the phenanthridone intermediate (**150**), all that remained was global deprotection or further functionalization (**Scheme 35**). A tentative plan for the deprotection of this advanced intermediate has been developed (**Scheme 36**), but this research has been delayed by the low supply of intermediate **150** produced thus far.



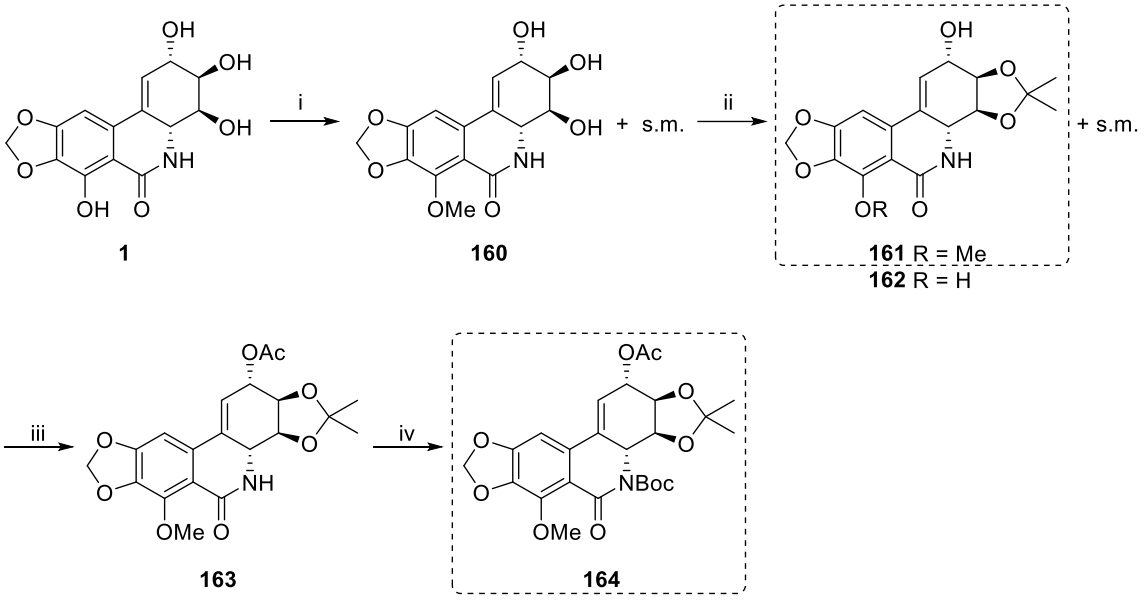
Scheme 35. Planned elaboration of intermediate **150**.



Scheme 36. Planned final deprotection of advanced intermediate **150** to access 2-*epi*-1-hydroxymethylnarciclasine (**2**).

While attempting to amass appreciable amounts of the Heck product (**150**), plausible conditions for the final deprotection steps have been investigated via a model study using narciclasine-derived intermediates. Our group has been very lucky in having the opportunity to work with large amounts of natural narciclasine, isolated as a by-product of processing *narcissus* bulbs to obtain galanthamine. A sample of narciclasine was converted to protected derivative **161** by known methods.[98] The second model substrate, **164**, a fully protected (and perhaps more realistic) model analogous to **150**, was prepared previously by a colleague in the Hudlicky group, in two additional steps (**Scheme 37**). They

generously provided a small sample of this material to be used in the model deprotection study.



Scheme 37. Synthesis of substrates for trial deprotections, **161** and **164**. Reagents and conditions: i) CH_2N_2, EtOH, Et_2O, 50 %; ii) 2,2-DMP, *p*TsOH, DCM, DMF, 60 %; iii) Ac_2O, pyridine, DMAP, 65 %; iv) Boc_2O, DMAP, DCM, 60 %.

The deprotection trials were conducted first on the simpler substrate (**161**, Me narc. acet.), and if successful, were then applied to the fully protected substrate, **164**. A few of the most viable conditions were initially selected, based mainly on previous Amaryllidaceae syntheses with similar deprotection needs. Two main deprotection requirements were identified: demethylation of the phenolic methyl ether at C-7 and an acid-based deprotection for the remaining groups – two acetonides (a 1,3-dioxane ketal and a 1,3-dioxolane ketal) and a Boc carbamate/imide. It should be noted that model substrate **164** bears an acetate at C-2, though this was of little consequence since its removal was foreseen to occur upon treatment with Lewis or aqueous acid.

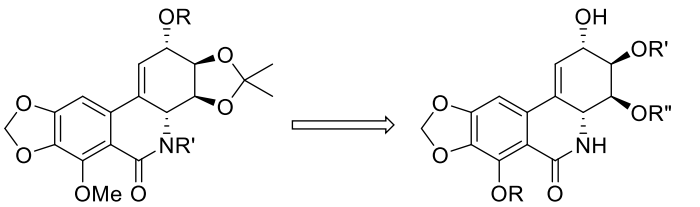
The results of the model deprotections are summarized in **Table 9**. The table is divided into three separate parts: demethylation attempts (**Table 9.1**), acetonide/Boc removal attempts (**Table 9.2**), and miscellaneous trials (**Table 9.3**) – which consist of follow-up entries after successful reactions to remove one or more of the other protecting groups. Entries with an asterisk (*) beside the entry number are considered the most viable reaction conditions from the model study, and are shaded for easier distinction.

Starting with demethylation (**Table 9.1**), the best results were obtained using LiCl or LiI in DMF. The reaction gave clean, though incomplete, conversion to the corresponding demethylated compounds. The lithium halide reactions were also performed on large enough scale to isolate the product, with the LiCl and LiI reactions providing 60 % and 50 % yield of the demethylated product, respectively. TMSI (TMSCl and KI) also successfully demethylated the simpler substrate (**161**) but gave a less clean reaction on the fully protected substrate (**164**) and therefore may not be the most applicable for use on synthetic intermediate **150**. Finally, SmI_2 also gave clean demethylation of 7-methylnarciclasine acetonide (**161**) to narciclasine acetonide (**162**), but with low conversion.

Efforts to remove the acetonide and Boc groups focused mainly on the use of Brønsted acids. Both acetic acid at 70 °C and trifluoroacetic acid at 0 °C were effective in removing the acetonide moiety of **161**; the latter was repeated on heavily protected substrate **164** and resulted in the removal of the acetonide, C-2 acetate, and Boc group. An attempt was made (based on literature precedent) to remove the Boc group using thermal hydrolytic conditions, however, multiple products were observed with varying degrees of deprotection, even after 72 h.

Finally, **Table 9.3** summarizes the results of sequential deprotection steps. Sadly, these entries were largely unsuccessful. One would imagine that treatment of narciclasine acetonide (**162**) with TFA ought to be a safe way to convert the material to fully deprotected narciclasine (**1**). To our disappointment, these trials (*entries 19* and *20*) yielded no narciclasine and resulted in only decomposition to a baseline spot. Milder conditions (dilute HCl, cooling) will be employed should these conditions be applied to the intermediates of the total synthesis. LiCl demethylation of Me narc. (**160,** *entry 18*) also failed, producing no detectable traces of narciclasine (**1**). Thankfully, treatment of **160** with TMSI (*entry 17*) gave clean, but incomplete, demethylation to the desired fully deprotected narciclasine (**1**). With these results in mind, the model study was indeed productive in identifying viable conditions for the forthcoming deprotection of the Heck product (**150**), and will hopefully lead to completion of the synthesis of **2**.

Table 9. Deprotection trials on protected narciclasine models, divided by desired outcome.



161 R = H R' = H
164 R = Ac R' = Boc

160 R = Me R' = R" = H
162 R = H R' = R" = $(C)Me_2$

9.1 Demethylation attempts

			Products			
Entry	**Substrate**	**Conditions**	**160**	**162**	**Other**	**Comments**
1	**161**	NaOBz, H_2O, 100 °C, 7 days	✓	×	-	no demethylation

2	**164**	NaOBz, H_2O, 100 °C, 7 days	✓	×	**161**	no demethylation; thermal cleavage of Boc and OAc
3	**161**	BBr_3, DCM, -78 °C	trace	×	traces of **1**	some s.m. persists through workup
4	**164**	BBr_3, DCM, -78 °C	trace	✓	traces of **161**	multiple spots, no clean conversion to any single product
5*	**161**	TMSCl, KI, MeCN, 60 °C	×	✓	traces of **1**	clean conversion, though incomplete
6	**164**	TMSCl, KI, MeCN, 60 °C	×	✓	traces of **161**	multiple spots, no clean conversion to any single product
7*	**161**	LiCl, DMF, 120 °C	×	✓	traces of s.m.	clean demethylation, 60 % yield
8*	**161**	LiI, DMF, 160 °C	×	✓	traces of s.m.	clean demethylation, 50 % yield

9	**164**	LiCl, DMF, 120 °C	×	×	complex mixture	some demethylation observed, though unclean
10*	**161**	SmI_2, THF, -78 °C	×	✓	s.m.	clean demethylation, but low conversion

9.2 Acetonide and/or Boc removal

			Products			
Entry	**Substrate**	**Conditions**	**160**	**162**	**Other**	**Comments**
11	**164**	H_2O, reflux	×	✓	traces of **161**	thermal cleavage of Boc and OAc
12	**161**	2 M HCl, THF/DCM, 0 °C	×	×	s.m. only	likely needs r.t. or heat, more conc.
13	**161**	Dowex 50WX8-200, MeOH, 60 °C	×	×	s.m. + decomp.	complex mixture
14*	**161**	70 % AcOH, 60 °C	✓	×	some s.m.	clean acetonide deprotection

15*	**161**	TFA, H_2O (5 %), DCM, 0 °C	✓	×	-	clean acetonide deprotection
16*	**164**	TFA, H_2O (5 %), DCM, 0 °C	✓	×	-	cleavage of OAc, acetonide, and Boc; > 50 % yield of **160**

9.3 Miscellaneous trials

			Products			
Entry	**Substrate**	**Conditions**	**160**	**162**	**Other**	**Comments**
17*	**160** *(entry 15)*	TMSCl, KI, MeCN, 60 °C	×	×	**1**	clean conversion, incomplete
18	**160** *(entry 16)*	LiCl, DMF, 120 °C	×	×	-	reaction failed, no narciclasine or s.m.
19	**162** (*entry 7*)	TFA, H_2O (5 %), DCM, 0 °C	×	×	***no narciclas-ine***	reaction failed, decomp. only

20	**162** (*entry 8*)	TFA (neat), -20 °C	×	×	***no narciclas-ine***	reaction failed, decomp. only

-s.m. denotes starting material was visible on TLC and/or recovered from the reaction.

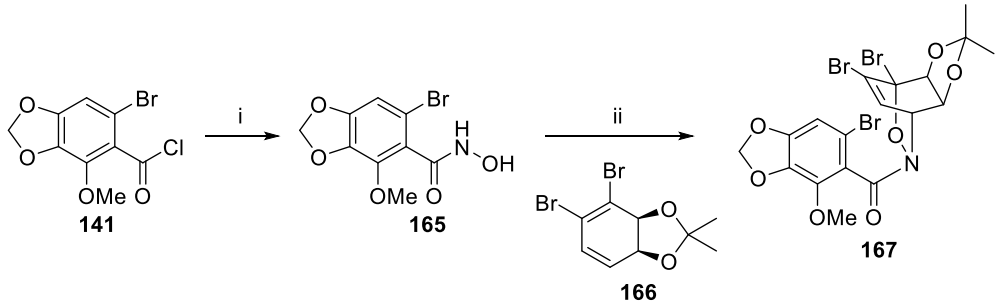
-decomp. denotes decomposition of reaction components, generally observed as three or more products or exclusively undesired/unidentified products.

3.3.2. Alternate routes

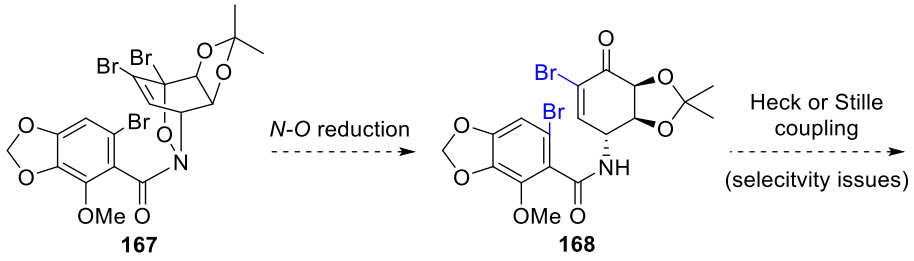
Alongside the efforts detailed thus far, a few alternate routes to the target analogues were conceived. These routes saw limited success, but are noteworthy for their preparation of novel compounds and possible development into further alternative routes.

3.3.2.1. Hydroxamic acid route

One such route that was partially investigated involved the synthesis of hydroxamic acid **165** from the A-ring acid chloride (**141**), followed by oxidation to the acyl-nitroso compound and cycloaddition to the protected 1,2-dibromobenzene diol (**Scheme 38**). Oxazine **167** was isolated and was found to be relatively stable, but this pathway was placed on hold because of the foreseen competition between the vinyl and aryl halides in subsequent coupling reactions (**Scheme 39**).



Scheme 38. Synthesis of oxazine **167** by a nitroso Diels-Alder approach. Reagents and conditions: i) $NH_2OH{\cdot}HCl$, NaOH, THF, H_2O, 80 %; ii) **166**, Bu_4NIO_4, DCM, 0 °C, 70 %.

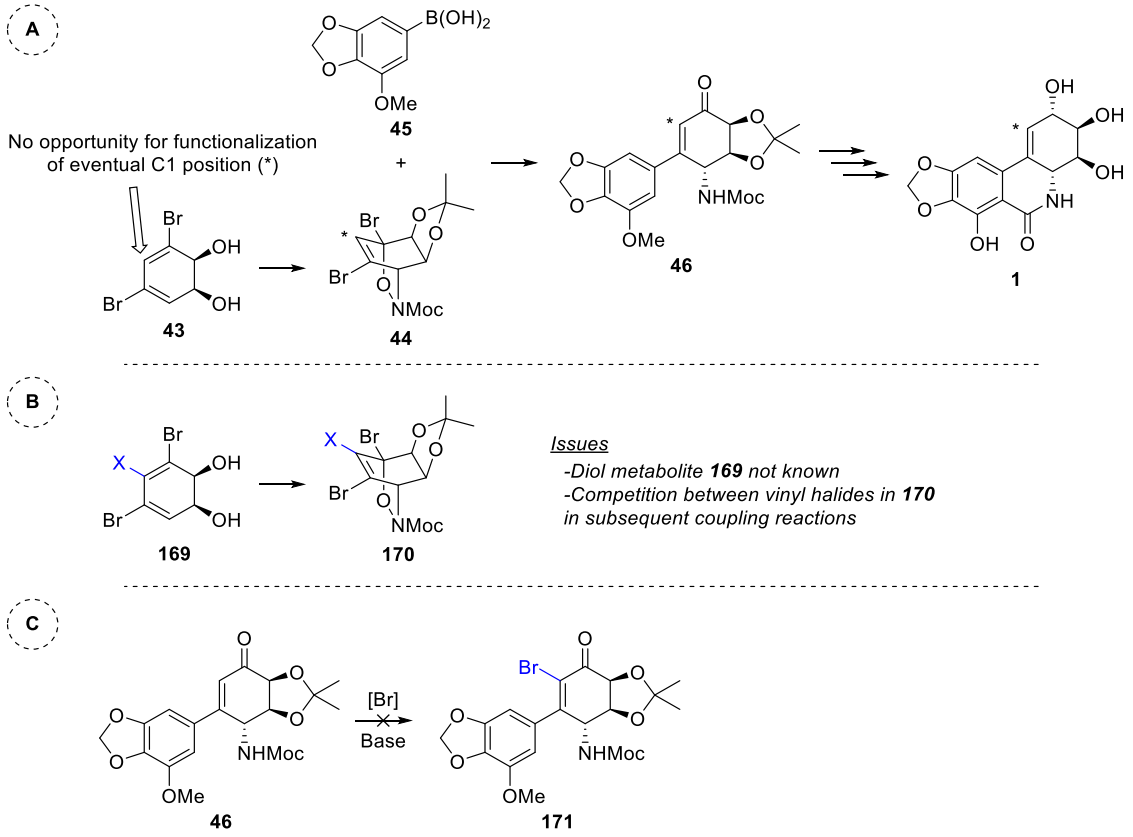


Scheme 39. Foreseeable issues in pursuing elaboration of oxazine **167**.

3.3.2.2. C10a-C10b Route

Over the course of this research, another alternate route was considered, which was based heavily on the Hudlicky group's 1999 synthesis of narciclasine (**Scheme 6**).[33] The idea was to assemble the narciclasine backbone by connecting the A- and C-ring fragments through the C10a-C10b bond first, followed by intramolecular lactam formation. The opposite order of connections was implemented in the route discussed in this thesis, but required an intramolecular Heck reaction to cyclize the B-ring, a significant bottleneck in the synthesis.

An approach like that used by Hudlicky in his previous narciclasine synthesis[33] would avoid the Heck-type B-ring closure. Though this initially appeared to be a promising option, difficulties in functionalizing the C-1 position were foreseen. The synthesis utilizes the 1,3-dibromobenzene-derived diol (**43**), which offers no pendant functionality for elaboration to a C-1 appendage (both vinyl bromides are "used": one being removed during the $Mo(CO)_6$ reduction and the other being essential to the Suzuki coupling to connect the A- and C-rings via the C10a-C10b bond).



Scheme 40. Proposed alternate route to C-1 homologues. A: Overview and reactivity considerations; B: Concept of additional C-1 functionality in starting material and associated issues; C: Concept of installing C-1 functionality at the enone intermediate and associated issues.

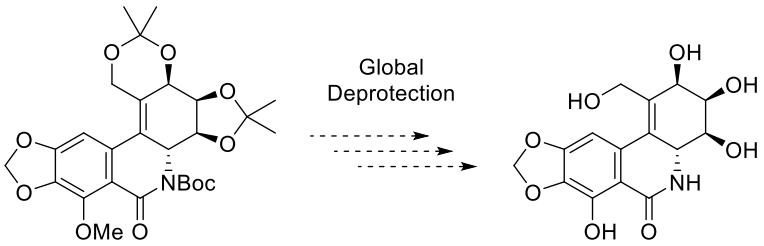
A hypothetical workaround for this issue is shown in **Scheme 40 B**. The use of a more functionalized dihydrodiol (**169**) was proposed, however, such a metabolite is unknown at the time of this writing. Pursuit of such a compound via microbial oxidation of the parent arene was never carried out as further issues were foreseen. Competition between the two vinyl bromides of theoretical oxazine **170** when performing coupling reactions would likely lead to over-substituted products or unfavorable mixtures.

Lastly, an idea was developed whereby the C1 position might be functionalized at the enone stage. This was envisioned to proceed by halogenation-dehydrohalogenation of **46** to obtain the α-bromo enone, followed by cross-coupling to install the desired C1 appendage. Regrettably, attempts to obtain the α-bromo enone were unsuccessful, leading only to the acetonide-cleaved product or halogenation of the electron rich aryl ring.

4. Future Work and Conclusions

4.1. Future Work

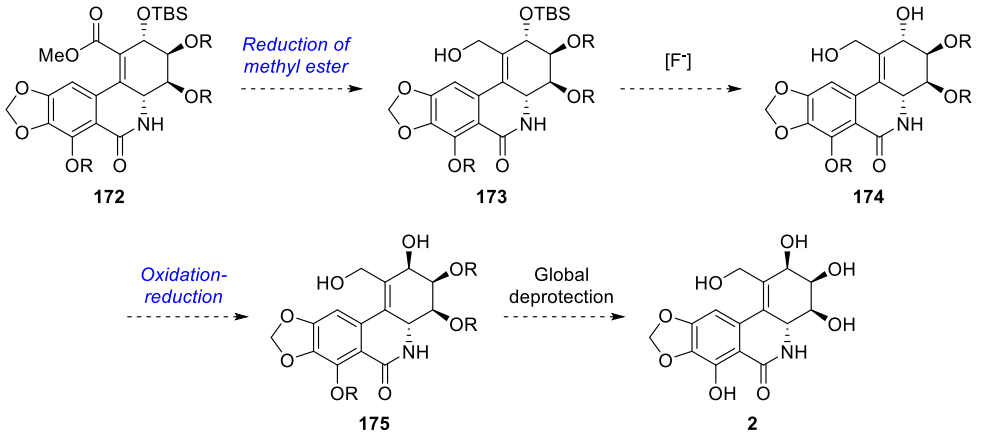
The remaining work associated with the current project revolves around the final deprotection of cyclized intermediate **150**. Should the deprotection steps work well, the synthetic route would give access to a novel C-1 homologue of narciclasine. The biological evaluation of 2-*epi*-1-hydroxymethylnarciclasine, alongside 2-*epi*-narciclasine and any other analogues accessible by this route, will be reported in due course.



Scheme 41. Remaining work for the completion of the project.

4.1.1. Conversion of C-1 ester to 2-*epi*-1-hydroxymethylnarciclasine

A possible alternate route to the synthesis of C-2 *epi*-narciclasine analogues was proposed but has yet to be investigated. The route involves the use of a C-1 methyl ester analogue (**172**) prepared by a colleague (L. Habaz) within the Hudlicky group. The synthesis of the desired analogues from this compound involves two main processes: conversion of the C-1 methyl ester to a hydroxymethyl appendage and an oxidation-reduction sequence to invert the C-2 stereocenter.



Scheme 42. Proposed synthesis of 2-*epi*-1-hydroxymethylnarciclasine from C-1 ester **172**.

This route has yet to be explored, largely because of the low availability of **172** via total synthesis. The synthetic sequence to access this material spans multiple steps and involves a low-yielding (albeit better and more reliable) intramolecular Heck reaction - similar to that discussed in the approach to 2-*epi*-1-hydroxymethylnarciclasine (Section 3.3.1). Even with these limitations in mind, the route may be worth exploring to access the desired C-1 analogues.

4.2. Conclusions

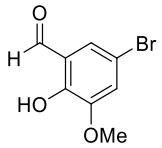
The synthesis reported in this document has no shortage of flaws. Many of the reactions are low-yielding or suffer from irreproducibility, and therefore represent bottlenecks in the route. Despite this, the work reported has established a means of accessing novel C-1 analogues of narciclasine, an as-of-yet untouched territory in Amaryllidaceae alkaloid research. By exploiting established strategies and appending them with updated synthetic tactics, a route to C-1 homologation has been presented; though completion of the synthesis has yet to be achieved. Final deprotection of intermediate **150** remains under investigation and will be reported in due course.

5. Experimental

General Experimental

All reactions run in organic solvents were carried out under Ar or N_2 atmosphere using Schlenk technique to minimize or eliminate exposure to moisture and/or air. All solvents were freshly distilled unless otherwise specified. THF, Et_2O, toluene, anisole, and 1,4-dioxane were distilled from Na/benzophenone ketyl; DCM and DMF were distilled from CaH_2; and MeCN was distilled from K_2CO_3. Thin layer chromatography was performed on EMD Silica Gel 60 Å 250 μm plates with F_{254} indicator, and analyzed under short-wave and/or long-wave ultraviolet light followed by staining with CAM, permanganate, anisaldehyde, or ninhydrin and subsequent charring. Column chromatography was performed using Silicycle Siliaflash P60 (230-400 mesh) silica gel. Melting points were observed from a Hoover Unimelt apparatus and are reported uncorrected. Optical rotations were measured by a Mandel Rudolph Research Analytical Automatic polarimeter at 589 nm with a 50 mm cell length. ^{1}H and ^{13}C NMR spectra were recorded on a 300, 400 or 600 MHz Bruker spectrometer. All chemical shifts are referenced to TMS or residual non-deuterated solvent. NMR data reported as: chemical shift (multiplicity, coupling constant(s) [Hz], integration). ^{13}C NMR spectra were proton decoupled.

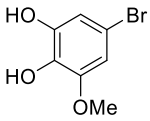
5-Bromo-2-hydroxy-3-methoxybenzaldehyde (136) [90]



o-Vanillin (2-hydroxy-3-methoxybenzaldehyde) **135** (25 g, 0.16 mol), sodium acetate (20.34 g, 0.248 mol) and glacial acetic acid (500 mL) were charged to a round-bottom flask. To the resulting yellow solution was added a solution of bromine (8.46 mL, 0.165 mol) in acetic acid (60 mL) over 45 min. The solution darkened to orange/brown throughout the addition. The mixture was left to stir at room temperature for 16 h. Acetic acid was removed from the reaction by concentrating under reduced pressure. The resulting residue was partitioned to DCM and H_2O (350 mL each). The organic layer was separated, and the aqueous layer was extracted with DCM (5 x 100 mL). The combined organic extracts were washed once with $Na_2S_2O_3$ saturated solution (50 mL) and once with H_2O (100 mL). The solution was then dried with Na_2SO_4, filtered and concentrated under reduced pressure to afford the crude brominated aldehyde **136**, obtained as a dark yellow flaky solid. The odor of acetic acid was detected in this crude product, so the residue was suspended in toluene (100 mL) and concentrated under reduced pressure (repeated twice), yielding **136** (34.55 g, 91% crude) as a light yellow flaky solid. Physical and spectral properties of the product were in accordance with those reported in the literature.[90]

R_f = 0.6 [hexanes/EtOAc (4:1)]; m.p. 119-122 ºC (DCM); lit.[99] m.p. = 119-123 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.01 (s, 1H), 9.86 (s, 1H), 7.32 (d, J = 2.1 Hz, 1H), 7.18 (d, J = 2.1 Hz, 1H), 3.92 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.7, 151.3, 149.7, 126.5, 121.7, 121.3, 111.4, 56.9.

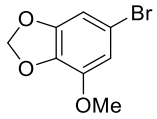
5-Bromo-3-methoxybenzene-1,2-diol (137)[90]



5-Bromo-2-hydroxy-3-methoxybenzaldehyde **136** (18.0 g, 77.9 mmol) was dissolved in a 2% NaOH solution (330 mL) resulting in a bright yellow solution. To this mixture a solution of 35% H_2O_2 (44.3 mL, 513 mmol) in H_2O (350 mL) was added dropwise over 1 h. The reaction mixture became dark crimson throughout the addition. The mixture was allowed to attain room temperature and stirred for 6 h, at which point the consumption of starting material was confirmed by TLC analysis. 3 M HCl (100 mL) was then added, causing a color change to dark orange. The organic layer was extracted with DCM (6 x 150 mL), and the combined layers were washed with Na_2SO_3 saturated solution (150 mL). An iodine-starch paper test yielded a negative result for the presence of peroxides. The resulting organic phase was dried with Na_2SO_4, filtered and concentrated under reduce pressure, yielding a dark oil. The product was purified by column chromatography (hexanes/EtOAc 6:1), affording catechol **137** (10.38 g, 61%) as a light-grey solid. Physical and spectral properties of the product were in accordance with those reported in the literature.[90]

R_f = 0.27 [hexanes/EtOAc (4:1)]; m.p. 75 ºC (EtOAc); lit.[90] m.p. = 76 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.77 (d, J = 2.1 Hz, 1H), 6.61 (d, J = 2.1 Hz, 1H), 5.34 (s, 2H), 3.87 (s, 3H).

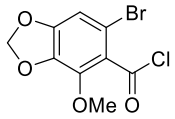
6-Bromo-4-methoxybenzo[*d*][1,3]dioxole (138)[90]



Catechol **137** (17.92 g, 81.81 mmol) and dry potassium carbonate (22.61 g, 163.6 mmol) were charged to a round-bottom flask and dissolved in DMF (90 mL). CH_2I_2 (9.89 mL, 122 mmol) was added, and the mixture was heated to 100 ºC for 2 h. The mixture darkened significantly over the course of the reaction, turning dark green. Consumption of starting material was confirmed by TLC (hexanes/EtOAc 4:1), and the reaction mixture was diluted with H_2O (500 mL). The solution was passed through a plug of Celite® and rinsed thoroughly with DCM. The resulting biphasic mixture was separated, collecting the organic layer. The aqueous layer was then extracted with DCM (3 x 150 mL). The combined organic layers were dried with Na_2SO_4, filtered and concentrated under reduced pressure. The resulting oil was triturated multiple times with toluene to remove residual DMF, giving a dark brown solid. The solid was purified by flash column chromatography (hexanes/EtOAc 9:1) to yield pure **138** as a white crystalline solid (14.6 g, 77%). Physical and spectral properties of the product were in accordance with those reported in the literature.[90]

R_f = 0.76 [hexanes/EtOAc (4:1)]; m.p. 79-80 ºC (EtOAc); lit.[90] m.p. = 80-82 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.68 (m, 2H), 5.97 (s, 2H), 3.88 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.8, 144.5, 135.2, 113.6, 111.3, 106.5, 102.2, 57.1.

6-Bromo-4-methoxybenzo[d][1,3]dioxole-5-carbonyl chloride (**141**)

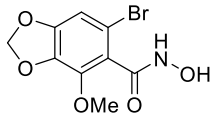


Acid **140** (300 mg, 1.09 mmol) was charged to a round bottom flask which was evacuated and backfilled with argon. The substrate was dissolved in DCM (5 mL). Oxalyl chloride (0.20 mL, 2.2 mmol, 2 eq.) was added dropwise, followed by the careful addition of DMF (3 drops, catalytic). Vigorous evolution of gas was observed upon addition of DMF. The solution was allowed to stir at room temperature for 2 h, after which it was concentrated by rotary evaporation and subsequently dried by repeated concentration from DCM and/or further drying on an oil pump to remove DMF. The crude acid chloride was of sufficient purity for use in further reactions.

The acid chloride is used as crude material to avoid hydrolysis and was characterized only by ^{1}H NMR and IR (to confirm consumption of the starting acid and formation of the desired acid chloride).

R_f = 0.3 (2:1 hexanes:EtOAc); IR (film, cm^{-1}) ν 1806; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.76 (s, 1H), 6.00 (s, 2H), 4.03 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 151.1, 141.5, 136.3, 121.9, 111.3, 107.7, 102.2, 60.6.

6-Bromo-*N*-hydroxy-4-methoxybenzo[d][1,3]dioxole-5-carboxamide (**165**)

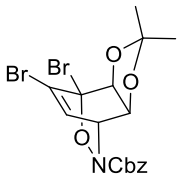


To a solution of $NH_2OH \cdot HCl$ (948 mg, 13.6 mmol, 4 eq.) and NaOH (682 mg, 17.1 mmol, 5 eq.) in 12 mL of a 1:1 mixture of THF/H_2O was added a second solution of crude acid chloride **141** (1.00 g, 3.41 mmol, 1 eq.) in THF (6 mL) in a dropwise manner over 10 min. The reaction was left to stir at room temperature for 3 h, at which time 30 mL of 1 M HCl was added. The mixture was left to stir overnight. EtOAc (20 mL) was added, the layers were separated, and the aqueous layer was washed several times with EtOAc. The combined organic layer was dried with $MgSO_4$, filtered and concentrated. The crude residue was purified by column chromatography (1:2 hexanes:EtOAc) to obtain **165** as a white amorphous solid (820 mg, 83 %).

R_f = 0.4 (1:2 hexanes:EtOAc); IR (film, cm^{-1}) *v* 3338, 3170, 3012, 2950, 2917, 2852, 1619, 1468, 1086, 1027; ^1H NMR (CD_3CN, 300 MHz) δ 8.15 (br s, 1.5H), 6.83 (s, 1H), 6.01 (s, 2H), 3.95 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 161.3, 150.1, 141.2, 136.2, 124.0, 111.6, 107.0, 102.3, 60.1; HRMS (EI) calcd for $C_9H_8BrNO_5$: 288.9586, found 288.9587; Anal. Calcd. for $C_8H_9BrNO_5$: C, 37.27; H, 2.78 Found C, 37.77; H, 2.94.

*^1H NMR spectrum also recorded in $CDCl_3$, 300 MHz: δ 6.77 (s, 1H), 6.00 (s, 2H), 4.02 (s, 3H), 3.12 (s, <1 H, exchangeable), 2.87 (s, <1 H, exchangeable).

(3a*S*,4*S*,7*R*,7a*S*)-Benzyl-4,5-dibromo-2,2-dimethyl-3a,4,7,7a-tetrahydro-4,7-(epoxyimino)benzo[d][1,3]dioxole-8-carboxylate (114)

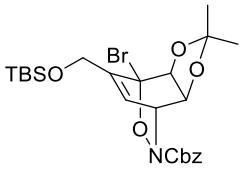


(1*S*,2*S*)-3,4-Dibromocyclohexa-3,5-diene-1,2-diol **113** (5.00 g, 18.7 mmol) was dissolved in DCM (10 mL) and 2,2-DMP (60 mL). To this solution was added *p*-toluenesulfonic acid monohydrate (300 mg, catalytic) and the resulting mixture was stirred for 4 h at room temperature. Water (6 mL) and $NaIO_4$ (3.98 g, 18.7 mmol) were then added and the reaction mixture was cooled to 0 °C. A solution of benzyl hydroxycarbamate **119** (3.43 g, 20.5 mmol) in methanol (15 mL) was then added dropwise to the reaction mixture over 30 min. The reaction was allowed to warm slowly and was stirred for 12 h at room temperature. The reaction was quenched with saturated aqueous $Na_2S_2O_3$ (20 mL) and allowed to stir for 10 min. The resulting mixture was concentrated under reduced pressure to remove methanol and 2,2-DMP. DCM (50 mL) was added to the resulting mixture, the organic layer was separated and the aqueous layer was extracted with DCM (4 x 75 mL). The combined organic layer was dried with $MgSO_4$, filtered and concentrated. The crude residue was purified by column chromatography (gradient 7:1 to 4:1 hexanes:EtOAc as eluent) to obtain **114** as an amber-colored gel (7.93 g, 89 %).

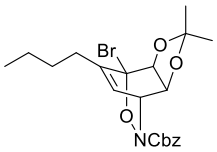
R_f = 0.5 (hexanes:EtOAc 4:1); $[\alpha]_D^{20}$ = -5.6 (*c* 0.33, $CHCl_3$); IR (film, cm^{-1}) *v* 3068, 3033, 2990, 2936, 1757, 1720, 1383, 1266, 1213; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.43 – 7.29 (m, 5H), 6.70 (d, *J* = 6.3 Hz, 1H), 5.32 – 5.15 (m, 2H), 5.08 (dd, *J* = 6.3, 4.1 Hz, 1H), 4.80 (d, *J* = 6.9 Hz, 1H), 4.62 – 4.52 (ddd, *J* = 1.0, 4.1, 6.9, 1H), 1.39 (s, 3H), 1.33 (s, 3H); ^{13}C

NMR ($CDCl_3$, 75 MHz,) δ 157.5, 135.3, 131.4, 128.8, 128.6, 128.1, 122.3, 112.2, 93.9, 82.5, 73.9, 68.9, 55.6, 25.9, 25.7; HRMS (EI) calcd for $C_{17}H_{17}Br_2NO_5$: 472.9473. Found 472.9468; Anal. Calcd for $C_{17}H_{17}Br_2NO_5$: C, 42.97; H, 3.61. Found C, 42.85; H, 3.61.

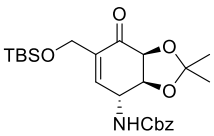
Benzyl (3a*S*,4*R*,7*R*,7a*S*)-4-bromo-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyl-3a,4,7,7a-tetrahydro-4,7-(epoxyimino)benzo[d][1,3]dioxole-8-carboxylate (176)



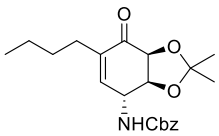
Dibromo-oxazine **114** (487 mg, 1.02 mmol) was charged to a round bottom flask as a solution in DCM and dried thoroughly under high vacuum. The flask was flushed with Ar and the residue was dissolved in 1,4-dioxane (15 mL). *Tert*-butyldimethyl ((tributylstannyl)methoxy)silane **123b** (759 mg, 1.74 mmol, 1.7 eq.) was added, followed by $Pd(PPh_3)_4$ (118 mg, 0.10 mmol, 10 mol %). The solution was then degassed by bubbling with a stream of argon. The mixture was heated to reflux for 18 h. The cooled reaction mixture was passed through a plug of Celite, rinsed thoroughly with EtOAc, and the filtrate was concentrated under reduced pressure. The crude residue was purified by column chromatography (10:1 to 6:1 hexanes:EtOAc as eluent) to obtain **176** as a light yellow oil (17 mg, 11 %) alongside butane addition product **177** (7 %) and the corresponding ring-opened products **124b** (13 %) and **126** (5 %).



177



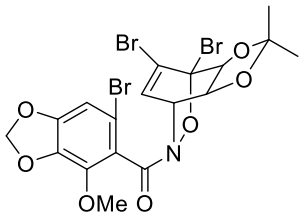
124b



126

R_f = 0.7 (hexanes:EtOAc 4:1); $[\alpha]_D^{24}$ = 12.1 (*c* = 0.85, $CHCl_3$); IR (film, cm^{-1}) *v* 3066, 3033, 2955, 2930, 2856, 1759, 1720, 1382, 1266, 1213, 1084, 838; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.34 – 7.26 (m, 5H), 6.35 (m, 1H), 5.19 (q, *J* = 13.6 Hz, 2H), 5.09 (dd, *J* = 4.0, 5.9 Hz, 1H), 4.64 (d, *J* = 7.0 Hz, 1H), 4.59 (dd, *J* = 3.9, 6.9 Hz, 1H), 4.40 (dd, *J* = 2.4, 16.2 Hz, 1H), 4.19 (dd, *J* = 2.5, 16.2 Hz, 1H), 1.32 (s, 3H), 1.31 (s, 3H), 0.91 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz,) δ 157.8, 141.9, 135.6, 128.7, 128.5, 128.1, 122.6, 111.7, 90.9, 82.4, 74.2, 68.6, 64.1, 53.7, 26.0, 25.8, 25.7, 18.4, -5.2, -5.4; HRMS (EI) calcd for $C_{24}H_{34}BrNO_6Si$: 539.1339; calcd for $C_{20}H_{25}BrNO_6Si^+$ $[M - C_4H_9]^+$ (*t*-butyl): 482.0629. Found 482.0634 $[M - C_4H_9]^+$ (*t*-butyl); LRMS (ESI) Found 557.2 $[M + NH_4]^+$, 562.1 $[M + Na]^+$, 578.1 $[M + K]^+$.

(6-Bromo-4-methoxybenzo[d][1,3]dioxol-5-yl)((3a*S*,4*R*,7*S*,7a*S*)-4,5-dibromo-2,2-dimethyl-3a,4,7,7a-tetrahydro-4,7-(epoxyimino)benzo[d][1,3]dioxol-8-yl)methanone (**167**)



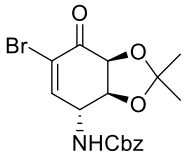
(1*S*,2*S*)-3,4-Dibromocyclohexa-3,5-diene-1,2-diol **113** (139 mg, 0.52 mmol) was dissolved in DCM (0.5 mL) and 2,2-DMP (3 mL). To this solution was added *p*-toluenesulfonic acid

monohydrate (2-3 crystals, catalytic) and the resulting mixture was stirred for 5 h at room temperature. $NaHCO_3$ saturated solution (2 mL) was then added, the layers were separated and the aqueous layer was extracted with DCM (3 x 5 mL). The combined organic layer was dried over $MgSO_4$, filtered and concentrated. The resulting yellow-orange oily residue was immediately used in the nitroso Diels-Alder reaction.

The crude acetonide was dissolved in DCM (5 mL) and Bu_4NIO_4 (225 mg, 0.52 mmol, 1 eq.) was added. The resulting solution was cooled to 0 °C. A solution of hydroxamic acid **165** (300 mg, 1.03 mmol, 2 eq.) in DCM (5 mL) was then added dropwise to the reaction mixture over 30 min. The reaction was allowed to warm slowly and was stirred for 12 h at room temperature. The reaction was quenched with saturated aqueous $Na_2S_2O_3$ (10 mL) and allowed to stir for 10 min. The layers were separated and the aqueous layer was extracted with DCM (2 x 15 mL). The combined organic layer was washed with Na_2CO_3 saturated solution and brine, dried with $MgSO_4$, filtered and concentrated. The crude residue was purified by column chromatography (4:1 to 2:1 hexanes:EtOAc) to obtain **167** as an off-white sticky foam (168 mg, 54 %).

R_f = 0.3 (hexanes:EtOAc 4:1); $[\alpha]^{25}_D$ = -33.5 (*c* 0.79, $CHCl_3$); IR (film, cm^{-1}) *v* 3084, 2990, 2926, 2873, 1666, 1619, 1468, 1263, 1210, 1083, 1023; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.84 (app. t, *J* = 6.4 Hz, 1H), 6.77 – 6.72 (m, 1H), 5.98 (m, 2H), 5.66 (dd, *J* = 4.2, 6.5 Hz, 1H), 4.82 (m, 1H), 4.68 (m, 1H), 4.00 (s, 3H), 1.43 (s, 3H), 1.35 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz,) δ 166.0, 150.9, 140.9, 135.4, 131.2, 122.7, 122.2, 112.7, 107.0, 101.9, 94.5, 82.2, 73.6, 60.3, 52.0, 51.7, 25.8, 25.7; HRMS (EI) calcd for $C_{18}H_{16}Br_3NO_7$: 594.8477. Found 594.8477; LRMS (ESI): 595.9 $[M+H]^+$, 617.8 $[M+Na]^+$, 633.8 $[M+K]^+$. Anal. Calcd for $C_{18}H_{16}Br_3NO_7$: C, 36.15; H, 2.70. Found C, 36.29; H, 2.72.

Benzyl ((3a*S*,4*R*,7a*S*)-6-bromo-2,2-dimethyl-7-oxo-3a,4,7,7a-tetrahydrobenzo[d][1,3]dioxol-4-yl)carbamate (116)

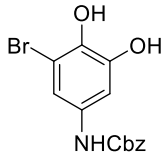


(3a*S*,4*S*,7*R*,7a*S*)-Benzyl-4,5-dibromo-2,2-dimethyl-3a,4,7,7a-tetrahydro-4,7-(epoxyimino)benzo[d][1,3]dioxole-8-carboxylate **114** (1.65 g, 3.46 mmol) was dissolved in MeCN (25 mL). To this solution was added $Mo(CO)_6$ (1.01 g, 3.81 mmol) and the resulting mixture was heated to reflux for 6 h. Upon consumption of starting material (TLC), the mixture was cooled to room temperature before being passed through a plug of Celite. The plug was rinsed thoroughly with MeCN and the filtrate was concentrated under reduced pressure. The crude residue was purified by column chromatography (gradient 4:1 to 2:1 hexanes:EtOAc as eluent) to obtain **116** as a brown foaming oil (703 mg, 52 %).

*[A spot with lower R_f on TLC was observed after workup or chromatography, especially in the presence of any wet solvents. This spot was identified as the acetonide-deprotected enone **120** and thus the column was flushed with pure EtOAc after elution of **116**. The resulting solution was concentrated under reduced pressure to yield a dark brown oily residue. The crude side product was redissolved in DCM and excess 2,2-DMP was added, followed by a catalytic amount of *p*-toluenesulfonic acid. The mixture was stirred until full consumption of the polar product was observed by TLC. The reaction was then quenched by addition of saturated $NaHCO_3$, concentrated to remove MeOH and acetone and purified as described above. This procedure generally lead to the isolation of an additional 10-15% of the desired enone **116**].

R_f = 0.5 (hexanes:EtOAc 2:1); $[\alpha]_D^{20}$ = -55.9 (*c* 0.24, $CHCl_3$); IR (film, cm^{-1}) *v* 3337 (br), 3034, 2988, 2935, 1693 (br), 1518, 1226; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.42 – 7.29 (m, 5H), 7.16 (d, *J* = 4.9 Hz, 1H), 5.30 (d, *J* = 7.8 Hz, 1H), 5.18 – 5.04 (m, 2H), 4.67 (d, *J* = 15.5 Hz, 1H), 4.60 – 4.46 (m, 2H), 1.39 (s, 3H), 1.37 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz,) δ 187.8, 155.6, 145.0, 135.7, 128.8, 128.6, 128.4, 124.5, 110.6, 77.8, 75.3, 67.7, 49.8, 27.5, 25.9; HRMS (EI) calcd for $C_{17}H_{18}BrNO_5$: 395.0368. Found 395.0368; Anal. Calcd for $C_{17}H_{18}BrNO_5$: C, 51.53; H, 4.58. Found C, 51.74; H, 4.85.

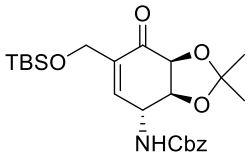
Benzyl (3-bromo-4,5-dihydroxyphenyl)carbamate (178)



A small amount of catechol **178** was obtained when workup of the $Mo(CO)_6$ reduction of **116** was performed by stirring the mixture with SiO_2 overnight prior to filtration through Celite and purification by column chromatography. Isolated as a brown oil.

R_f = 0.4 (hexanes:EtOAc 2:1); IR (film, cm^{-1}) *v* 3267, 3066, 3034, 2958, 2927, 1706, 1608, 1526, 1417, 1251, 1223, 987; ^1H NMR (*d*$_6$ - DMSO, 600 MHz) δ 7.42 – 7.32 (m, 5H), 7.07 (s, 1H), 7.00 (s, 1H), 5.11 (s, 2H); ^{13}C NMR (*d*$_6$ - DMSO, 600 MHz,) δ 153.3, 146.4, 138.5, 136.7, 131.7, 128.4, 128.1, 128.0, 112.3, 109.5, 105.7, 65.6; HRMS (EI) calcd for $C_{14}H_{12}Br_2NO_4$: 336.9950. Found 336.9945.

Benzyl((3a*S*,4*R*,7a*S*)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-2,2-dimethyl-7-oxo-3a, 4,7,7a-tetrahydrobenzo[d][1,3]dioxol-4-yl)carbamate (124b)

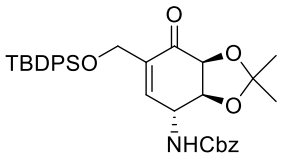


Benzyl ((3a*S*,4*R*,7a*S*)-6-bromo-2,2-dimethyl-7-oxo-3a,4,7,7a-tetrahydrobenzo[d][1,3] dioxol-4-yl)carbamate **116** (1.90 g, 4.80 mmol) was charged to a Schlenk flask as a solution and dried thoroughly under high vacuum. The vessel was flushed with N_2 and the residue was dissolved in 1,4-dioxane (50 mL). *Tert*-butyldimethyl((tributylstannyl)methoxy)silane **123b** (4.18 g, 9.59 mmol) was added, followed by $Pd(PPh_3)_4$ (277 mg, 5 mol %). The resulting solution was degassed by subjection to three freeze-pump-thaw cycles, before backfilling the vessel with N_2. The mixture was heated to reflux for 6 h, until full consumption of starting material was observed (TLC). The mixture was cooled to room temperature before being passed through a plug of Celite. The plug was rinsed thoroughly with EtOAc and the filtrate was concentrated under reduced pressure. The crude residue was purified by column chromatography (gradient 6:1 to 4:1 hexanes:EtOAc as eluent) to obtain **124b** as a light yellow oil (885 mg, 40 %). The procedure was repeated on smaller scale (230 mg of **116**) and a yield of as much as 55 % was obtained.

R_f = 0.7 (hexanes:EtOAc 2:1); $[\alpha]_D^{20}$ = - 47.0 (*c* = 0.35, $CHCl_3$); IR (film, cm^{-1}) *v* 3325 (br), 2986, 2954, 2928, 2856, 1720, 1679, 1521, 1456, 1382, 1227; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.40 - 7.30 (m, 5 H), 6.75 (dd, *J* = 4.2, 1.5 Hz, 1H), 5.12 (m, 2H), 5.08 (s, 1H), 4.61 (br s, 1H), 4.51 (m, 1H), 4.45 (m, 1H), 4.40 (m, 1H), 4.34 (m, 1H), 1.39 (s, 3H), 1.38 (s, 3H), 0.91 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz,) δ 193.9, 155.6,

139.4, 138.5, 136.0, 128.7, 128.5, 128.4, 110.3, 77.8, 74.9, 67.4, 59.7, 48.3, 27.5, 26.0, 25.9, 18.4, - 5.3; HRMS (EI) calcd for $C_{24}H_{35}NO_6Si$: 461.2234. Found 461.2226.

Benzyl-((3a*S*,4*R*,7a*S*)-6-(((*tert*-butyldiphenylsilyl)oxy)methyl)-2,2-dimethyl-7-oxo-3a,4,7,7a-tetrahydrobenzo[d][1,3]dioxol-4-yl)carbamate (124c)

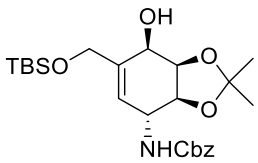


Benzyl-((3a*S*,4*R*,7a*S*)-6-bromo-2,2-dimethyl-7-oxo-3a,4,7,7a-tetrahydrobenzo[d][1,3] dioxol-4-yl)carbamate **116** (210 mg, 0.53 mmol) was charged to a Schlenk flask as a solution and dried thoroughly under high vacuum. The vessel was flushed with N_2 and the residue was dissolved in 1,4-dioxane (5 mL). *Tert*-butyldiphenyl((tributylstannyl) methoxy)silane **123c** (890 mg, 1.6 mmol) was added, followed by $PdCl_2(PPh_3)_2$ (19 mg, 5 mol %). The resulting solution was degassed by subjection to three freeze-pump-thaw cycles, before backfilling the vessel with N_2. The mixture was heated to reflux for 6 h, until full consumption of starting material was observed (TLC). The mixture was cooled to room temperature before being passed through a plug of Celite. The plug was rinsed thoroughly with EtOAc and the filtrate was concentrated under reduced pressure. The crude residue was purified by column chromatography (gradient 4:1 to 2:1 hexanes:EtOAc as eluent) to obtain **124c** as a light yellow oil (114 mg, 37 %).

R_f = 0.6 (hexanes:EtOAc 2:1); $[\alpha]_D^{20}$ = - 45.0 (*c* = 0.24, $CHCl_3$); IR (film, cm^{-1}) *v* 3331 (br), 3070, 2986, 2956, 2856, 1718, 1681, 1520, 1228, 1113; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.65 - 7.60 (m, 4H), 7.41 – 7.35 (m, 11H), 6.86 (dd, *J* = 4.3, 1.5 Hz, 1H), 5.15 (m, 2H),

4.86 (d, *J* = 7.1 Hz, 1H), 4.62 (br s, 1H), 4.49 (m, 2H), 4.45 (s, 1H), 4.37 (m, 1H), 1.38 (s, 3H), 1.35 (s, 3H), 1.08 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz,) δ 193.7, 155.7, 139.1, 138.5, 135.6, 133.2, 133.0, 130.1, 128.8, 128.6, 128.4, 128.0, 110.4, 74.8, 67.5, 60.6, 48.2, 29.8, 27.6, 27.0, 26.0, 19.4; HRMS (EI) calcd for $C_{34}H_{39}NO_6Si$: 585.2547. Found 585.2541; Anal. Calcd for $C_{34}H_{39}NO_6Si$: C, 69.71; H, 6.71. Found C, 69.87; H, 6.90.

Benzyl ((3a*S*,4*R*,7*R*,7a*R*)-6-(((*tert*-butyldimethylsilyl)oxy)methyl)-7-hydroxy-2,2-dimethyl-3a,4,7,7a-tetrahydrobenzo[d][1,3]dioxol-4-yl)carbamate (**129-β**)

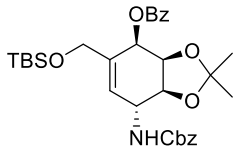


Enone **124b** (1.81 g, 3.92 mmol) was charged to a round bottom flask as a solution and was subsequently dried under vacuum. The residue was dissolved in THF (30 mL) and cooled to 0 °C. L-Selectride (1M solution in THF, 5.1 mL, 5.1 mmol, 1.3 eq.) was added dropwise to the solution over 15 min. The mixture was allowed to gradually attain room temperature and stirred for 12 h. H_2O (5 mL) was added and the mixture was concentrated by rotary evaporation. Residual water was removed by addition of toluene and repeated concentration. The crude residue was purified by column chromatography (gradient 4:1 to 2:1 hexanes:EtOAc as eluent) to obtain **129-β** as a light yellow oil (738 mg, 41 %).

R_f = 0.3 (hexanes:EtOAc 2:1); $[\alpha]_D^{24}$ = - 44.6 (*c* 0.4, $CHCl_3$) IR (film, cm^{-1}) *v* 3450, 3328, 3032, 2953, 2929, 2856, 1701, 1524, 1380, 1253, 1061, 1040, 838, 777; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.37 – 7.33 (m, 5H), 5.81 (s, 1H), 5.11 (s, 2H), 4.78 (br s, 1H), 4.39 (m, 4H), 4.26 (s, 2H), 2.75 (d, *J* = 4.8 Hz, 1H), 1.46 (s, 3H), 1.37 (s, 3H), 0.90 (s, 9H), 0.08 (s, 6H);

^{13}C NMR ($CDCl_3$, 75 MHz,) δ 156.0, 142.0, 136.4, 128.7, 128.3 (2), 123.9, 109.9, 77.4, 75.8, 67.1, 65.8, 64.1, 50.7, 26.6, 26.0, 24.6, 18.5, -5.2, -5.3 ; HRMS (EI) calcd for $C_{24}H_{37}NO_6Si$: 463.2390, found 463.2385; LRMS (ESI) found 464 $[M+H]^+$, 481 $[M+NH_4]^+$, 486 $[M+Na]^+$, 502 $[M+K]^+$; Anal. Calcd for $C_{24}H_{37}NO_6Si$: C, 62.17; H, 8.04. Found C, 62.44; H, 8.06.

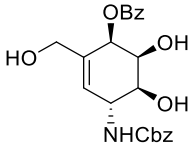
(3a*R*,4*R*,7*R*,7a*S*)-7-(((Benzyloxy)carbonyl)amino)-5-(((*tert*-butyldimethylsilyl)oxy) methyl)-2,2-dimethyl-3a,4,7,7a-tetrahydrobenzo[d][1,3]dioxol-4-yl benzoate (**132**)



Allylic alcohol **129-β** (140 mg, 0.30 mmol) was dissolved in dry DCM (2 mL). Pyridine (70 μL, 0.91 mmol, 3 eq.) was added followed by DMAP (2-3 small crystals, catalytic) and the resulting solution was cooled to 0 °C. Benzoyl chloride (70 μL, 0.60 mmol, 2 eq.) was then added to the cooled solution in a dropwise manner, and the temperature was maintained at 0 °C for 1 h. The mixture was then allowed to gradually attain room temperature and was stirred for 12 h. Following completion of the reaction, $NaHCO_3$ saturated solution (1 mL) was added and the organic layer was separated. The aqueous layer was extracted with DCM (5 x 10 mL) and the combined organic layer was dried with $MgSO_4$, filtered and concentrated. The residue was thoroughly dried on an oil pump to remove residual pyridine. The crude residue was purified by column chromatography (SiO_2, 4:1 hexanes:EtOAc) to obtain **132** as a glossy oil (92 mg, 54 %).

R_f = 0.5 (hexanes:EtOAc 4:1); $[\alpha]^{25}_D$ -77.3 (c 0.6, $CHCl_3$); IR (film, cm^{-1}) *v* 3343, 3065, 3034, 2953, 2930, 2895, 2856, 1719, 1523, 1260, 1068, 836; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.06 (d, *J* = 6.5 Hz, 2H), 7.54 (t, *J* = 6.8 Hz, 1H), 7.44 (m, 2H), 7.40 – 7.29 (m, 5H), 5.94 (s, 1H), 5.77 (s, 1H), 5.14 (m, 3H), 4.68 (s, 1H), 4.41 (m, 2H), 4.26 (m, 2H), 1.34 (s, 6H), 0.89 (s, 9H), 0.07 (s, 3H), 0.06 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz,) δ 166.1, 156.0, 138.6, 136.4, 133.2, 130.2, 129.9, 128.9, 128.7, 128.6, 128.4 (2 C), 110.6, 77.8, 74.6, 67.1 (2 C), 63.9, 52.5, 26.5, 26.0, 24.7, 18.5, -5.2, -5.3; HRMS (EI) calcd for $C_{31}H_{41}NO_7Si$: 567.2652, found 567.2643; LRMS (ESI) found 585 $[M+NH_4]^+$, 590 $[M+Na]^+$, 606 $[M+K]^+$.

(1*R*,4*R*,5*S*,6*S*)-4-(((Benzyloxy)carbonyl)amino)-5,6-dihydroxy-2-(hydroxymethyl)cyclohex-2-en-1-yl benzoate (**179**)*

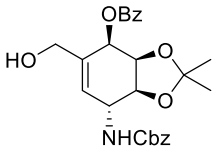


Benzoate **132** (32 mg, 0.06 mmol) was charged to a round bottom flask and the vessel was evacuated under vacuum and backfilled with argon (repeated twice). The material was dissolved in dry MeCN (3 mL). TMSCl (0.5 M solution in MeCN, 0.15 mL, 0.07 mmol) was added followed by KI (9 mg, 0.06 mmol). The vessel was fitted with a cold-water condenser and heated to 60 °C for 2 h. Despite observing incomplete consumption of the starting material, the reaction was halted to prevent the formation of a greater number of products (multiple faint spots forming on TLC). The mixture was allowed to slowly cool to room temperature and was then further cooled to 0 °C. The reaction was quenched with H_2O (1 mL) and was then transferred to a separatory funnel. The organic layer was

extracted with EtOAc (3 x 5 mL) and the combined organic extracts were dried with Na_2SO_4, filtered and concentrated. The crude residue was purified by column chromatography (SiO_2, 100:1 DCM:MeOH to 10:1 DCM:MeOH gradient) to obtain **179** as a cloudy glass-like residue (8 mg, 35 %).

R_f = 0.2 (DCM:MeOH 30:1; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.09-8.07 (m, 5 H), 7.62-7.50 (m, 2 H), 7.48-7.42 (m, 3 H), 5.87 (br s, 1 H), 5.78 (s, 1 H), 5.38 (d, *J* = 4.2 Hz, 1 H), 5.07 (dd, *J* = 4.2, 6.4 Hz, 2 H), 4.60 (br s, 1 H), 4.47 (s, 1 H), 4.13 (s, 2 H), 3.84 (d, *J* = 6.4 Hz, 1 H). LRMS (ESI) calcd for $C_{22}H_{23}NO_7$: 413.1475, found 414 $[M+H]^+$, 431 $[M+NH_3]^+$, 436 $[M+Na]^+$, 452 $[M+K]^+$; HRMS (EI) calcd for $C_{22}H_{23}NO_7$:413.1475, for $C_{22}H_{21}NO_6$: 395.1369 $[M-H_2O]^+$; found 395.1358 $[M-H_2O]^+$.

(3a*R*,4*R*,7*R*,7a*S*)-7-(((Benzyloxy)carbonyl)amino)-5-(hydroxymethyl)-2,2-dimethyl-3a,4,7,7a-tetrahydrobenzo[d][1,3]dioxol-4-yl benzoate (**180**)*

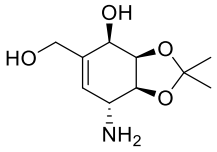


Also isolated from the reaction was alcohol **180** as a result of TBS cleavage (9 mg, 35 %).

*Both compounds **179** and **180** eluted as mixtures with lesser amounts of various impurities and were therefore identified solely based on ^{1}H NMR and HRMS.

R_f = 0.4 (DCM:MeOH 30:1); LRMS (ESI) calcd for $C_{25}H_{27}NO_7$: 453.1788, found 454 $[M+H]^+$, 471 $[M+NH_3]^+$, 476 $[M+Na]^+$; HRMS (EI) calcd for $C_{24}H_{24}NO_7^+$ $[M-CH_3]^+$: 438.1547, found 438.1542 $[M-CH_3]^+$.

(3a*R*,4*R*,7*R*,7a*S*)-7-Amino-5-(hydroxymethyl)-2,2-dimethyl-3a,4,7,7a-tetrahydrobenzo[d][1,3]dioxol-4-ol (**181**)*****

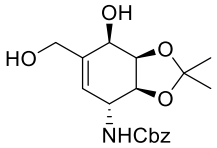


Allylic alcohol **129-β** (27 mg, 0.06 mmol) was dissolved in THF (0.5 mL) and LiOH (1 M aqueous solution, 0.58 mmol) was added. The reaction vessel was fitted with a condenser and heated to 60 °C for 2 d. The mixture was cooled to room temperature and concentrated under reduced pressure, using THF and/or EtOH to azeotropically remove H_2O. The crude residue was purified by column chromatography (10 % deactivated SiO_2, 10:1 to 5:1 DCM:MeOH gradient) to obtain **181** as a light tan semi-solid (8.5 mg ± 1 mg, 68 %).

***Not fully characterized because of poor solubility and rapid decomposition (compound appears to be hygroscopic).**

R_f = 0.2 (DCM: MeOH: NH_4OH 90: 8: 2); $[\alpha]_D^{20}$ = -20.4 (*c* = 0.43, 7:3 MeOH: DMSO); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.81 (d, *J* = 3.4 Hz, 1 H), 4.52 (d, *J* = 4.5 Hz, 1 H), 4.43 (dd, *J* = 4.5, 7.9 Hz, 1 H), 4.23 (s, 2 H), 4.11 (dd, *J* = 5.1, 7.7 Hz, 1 H), 3.79 (s, 1 H), 1.47 (s, 3 H), 1.39 (s, 3 H); ^{1}H NMR (D_6 - DMSO, 300 MHz) δ 8.44 (s, 1 H), 5.56 (s, 1 H), 4.26 (d, *J* = 4.0 Hz, 1 H), 4.18 (dd, *J* = 4.0, 7.7 Hz, 1 H), 4.01 (dd, *J* = 4.6, 7.8 Hz, 2 H), 3.96 (br s, 2 H), 1.85 (s, 1 H), 1.35 (s, 3 H), 1.27 (s, 3 H); HRMS (EI) calcd for $C_{10}H_{17}NO_4$:215.1158, for $C_9H_{14}NO_4^+$: 200.0917 $[M\text{-}CH_3]^+$, found 200.0921 $[M\text{-}CH_3]^+$; LRMS (ESI) found 216 $[M+H]^+$ and 238 $[M+Na]^+$.

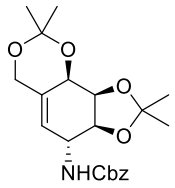
Benzyl-((3a*S*,4*R*,7*R*,7a*R*)-7-hydroxy-6-(hydroxymethyl)-2,2-dimethyl-3a,4,7,7a-tetrahydrobenzo[d][1,3]dioxol-4-yl)carbamate (**142**)



Allylic alcohol **129-β** (131 mg, 0.28 mmol) was charged to a round bottom flask and the vessel was evacuated and backfilled with argon. The compound was dissolved in dry THF (7 mL), followed by the dropwise addition of TBAF (1.0 M in THF, 0.42 mmol, 1.5 eq.). The mixture was left to stir at room temperature for 14 h, darkening from light yellow to brown over the course of the reaction. H_2O (0.7 mL) was added to the mixture and was allowed to stir for 5 min before concentrating under reduced pressure (triturated with THF as needed to remove H_2O). The crude residue was purified by column chromatography (SiO_2, 30:1 to 20:1 DCM:MeOH gradient) to obtain **142** as a white semi-solid residue (75 mg, 76 %).

R_f = 0.7 (DCM:MeOH 10:1); $[\alpha]_D^{25}$ = - 62.7 (*c* = 0.4, $CHCl_3$); IR (film, cm^{-1}) *v* 3524, 3422, 3339, 2992, 2930, 2888, 1688, 1535, 1267, 1035, 731; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.38 – 7.28 (m, 5 H), 5.83 (d, *J* = 3.4 Hz, 1 H), 5.19 (d, *J* = 6.5 Hz, 1 H), 5.08 (s, 2 H), 4.42 (m, 3 H), 4.35 (br s, 1 H), 4.19 (m, 2 H), 3.07 (br s, 2 H), 1.44 (s, 3 H), 1.35 (s, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz,) δ 156.2, 141.9, 136.3, 128.6, 128.32, 128.30, 125.6, 109.9, 75.7, 67.1, 65.9, 64.1, 50.6, 29.8, 26.5, 24.6; HRMS (EI) calcd for $C_{18}H_{23}NO_6$: 349.1525, for $C_{17}H_{20}NO_6$: 334.1285 $[M\text{-}CH_3]^+$, for $C_{18}H_{21}NO_5$: 331.1420 $[M\text{-}H_2O]^+$, found 334.1283 $[M\text{-}CH_3]^+$, 331.1406 $[M\text{-}H_2O]^+$; LRMS (ESI) found 350 $[M+H]^+$, 367 $[M+NH_3]^+$, 372 $[M+Na]^+$, 388 $[M+K]^+$.

Benzyl-((3a*S*,4*R*,9a*R*,9b*R*)-2,2,8,8-tetramethyl-3a,4,9a,9b-tetrahydro-6H-[1,3]dioxolo[4',5':5,6]benzo[1,2-d][1,3]dioxin-4-yl)carbamate (**143**)

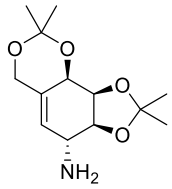


Allylic diol **142** (270 mg, 0.77 mmol) was charged to a round bottom flask, which was then evacuated and backfilled with argon (repeated twice). The compound was dissolved in dry DMF (7 mL), followed by the addition of 2,2-dimethoxypropane (7 mL, 60 mmol, excess) and *p*-toluenesulfonic acid (2-3 small crystals, ~10 mg, catalytic) and left to stir at room temperature for 24 h. $NaHCO_3$ sat. aqueous solution (2 mL) was then added and the mixture was transferred to a separatory funnel. Brine (25 mL) and Et_2O (25 mL) were added and the layers were separated. The aqueous layer was extracted with Et_2O (2 x 50 mL) followed by further extraction with EtOAc (3 x 50 mL). The combined organic extracts were washed once with brine before drying with $MgSO_4$, filtering and concentrating the mixture. The crude residue was purified by column chromatography (SiO_2, 5:1 to 2:1 hexanes:EtOAc) to obtain **143** as a colorless glossy oil (280 mg, 93 %).

R_f = 0.3 (hexanes:EtOAc 2:1); $[\alpha]_D^{25}$ = - 110.3 (*c* = 0.28, $CHCl_3$); IR (film, cm^{-1}) *v* 3326, 3066, 2987, 1699, 1519, 1381, 1219, 1036; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.35 – 7.30 (m, 5 H), 5.60 (d, *J* = 5.8 Hz, 1 H), 5.09 (br s, 2 H), 4.67 (dd, *J* = 4.0, 6.5 Hz, 2 H), 4.51 (br d, *J* = 3.8 Hz, 1 H), 4.46 (app. t, *J* = 2.1 Hz, 1 H), 4.33 (d, *J* = 14.0 Hz, 1 H), 4.20 (app. t, *J* = 6.4 Hz, 1 H), 4.10 (d, *J* = 14.0 Hz, 1 H), 1.47 (s, 3 H), 1.42 (s, 3 H), 1.37 (s, 3 H), 1.33 (s, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz,) δ 156.0, 141.8, 136.2, 128.7, 128.5, 128.4, 115.8, 109.6, 101.1, 76.8, 75.1, 67.2, 65.9, 61.6, 49.2, 26.4, 25.4, 24.6, 23.6; HRMS (EI) calcd for

$C_{21}H_{27}NO_6$: 389.1838, for $C_{20}H_{24}NO_6$: 374.1598 $[M\text{-}CH_3]^+$, found 374.1600 $[M\text{-}CH_3]^+$; LRMS (ESI) found 407 $[M+NH_3]^+$, 412 $[M+Na]^+$, 428 $[M+K]^+$; Anal. calcd. C 64.77, H 6.99, found C 64.55, H 7.00.

(3a*S*,4*R*,9a*R*,9b*R*)-2,2,8,8-Tetramethyl-3a,4,9a,9b-tetrahydro-6H-[1,3]dioxolo[4',5':5,6]benzo[1,2-d][1,3]dioxin-4-amine (**144**)

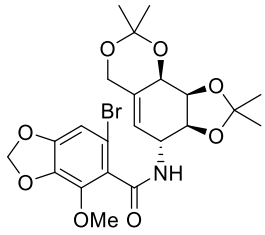


Carbamate **143** (232 mg, 0.60 mmol) was charged to a round bottom flask and dissolved in MeOH (15 mL). KOH (1 M aqueous solution, 6 mmol, 10 eq.) was then added and the vessel was fitted with a reflux condenser. The mixture was heated to 80 °C for 48 h. Efforts to extract the resulting amine proved unsuccessful because of high aqueous solubility. The mixture was concentrated under reduced pressure and then dried thoroughly on an oil pump. The crude residue was purified by column chromatography (10 % deactivated SiO_2, 100:1 to 30:1 DCM:MeOH, followed by 95:4:1 to 90:8:2 DCM:MeOH:NH_4OH) to obtain **144** as a colorless glassy solid (90 mg, 59 %).

R_f = 0.5 (DCM:MeOH:NH_4OH 90:8:2); $[\alpha]_D^{25}$ = -96.6 (*c* = 0.5, $CHCl_3$); IR (film, cm^{-1}) *v* 3369, 3298, 2986, 2924, 2854, 1554, 1456, 1380, 1216, 1056; ^1H NMR ($CDCl_3$, 600 MHz) δ 5.63 (d, *J* = 5.6 Hz, 1 H), 4.73 (dd, *J* = 6.7, 4.6 Hz, 1 H), 4.71 (m, 1 H), 4.35 (dt, *J* = 14.0, 2.1 Hz, 1 H), 4.28 (dt, *J* = 1.3, 6.7 Hz, 1 H), 4.13 (d, *J* = 13.8 Hz, 1 H), 3.59 (d, *J* = 6.0 Hz, 1 H), 1.49 (s, 3 H), 1.45 (s, 3 H), 1.37 (s, 3 H), 1.35 (s, 3 H); ^{13}C NMR ($CDCl_3$,

150 MHz,) δ 139.4, 119.3, 109.2, 100.8, 79.6, 75.6, 66.1, 62.0, 49.0, 26.5, 25.8, 24.6, 23.5; HRMS (EI) calcd for $C_{13}H_{21}NO_4$: 255.1471, for $C_{12}H_{18}NO_4$: 240.1230 $[M\text{-}CH_3]^+$, found 240.1233 $[M\text{-}CH_3]^+$; LRMS (ESI) found 256 $[M+H]^+$, 278 $[M+Na]^+$.

6-Bromo-4-methoxy-*N*-((3aS,4R,9aR,9bR)-2,2,8,8-tetramethyl-3a,4,9a,9b-tetrahydro-6H-[1,3]dioxolo[4',5':5,6]benzo[1,2-d][1,3]dioxin-4-yl)benzo[d][1,3]dioxole-5-carboxamide (**145**)

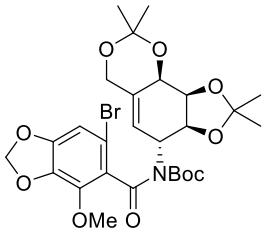


Allylic amine **144** (112 mg, 0.44 mmol) was charged to a round bottom flask which was evacuated and backfilled with argon. The compound was dissolved in DCM (5.5 mL) and the resulting solution was cooled to 0 °C. Pyridine (0.11 mL, 1.3 mmol, 3 eq.) and DMAP (5 mg, 0.04 mmol, 0.1 eq.) were then added, followed by addition of crude acid chloride **141** (194 mg, 0.66 mmol, 1.5 eq.) as a solution in DCM (4.5 mL) dropwise over 10 min. The reaction was allowed to slowly warm to room temperature and continued stirring for 8 h. The mixture was concentrated by rotary evaporation and further drying by an oil pump to remove pyridine. The crude residue was purified by column chromatography (2.5:1 to 1:1 hexanes:EtOAc) to obtain **145** as a slightly cloudy oily solid (169 mg, 75 %).

R_f = 0.4 (1:1 hexanes:EtOAc); $[\alpha]_D^{24}$ = - 97.8 (*c* 1.0, $CHCl_3$); IR (film, cm^{-1}) *v* 3247, 2989, 2936, 2855, 1634, 1536, 1467, 1380, 1260, 1213, 1085, 1041, 752; ^1H NMR ($CDCl_3$, 600 MHz) δ 6.70 (s, 1 H), 5.96 (dd, *J* = 1.3, 4.3 Hz, 2 H), 5.69 (d, *J* = 6.2 Hz, 1 H), 5.47 (d, *J*

= 6.4 Hz, 1 H), 4.75 (dt, *J* = 6.8, 1.3 Hz, 1 H), 4.70 (dd, *J* = 3.9, 6.8 Hz, 1 H), 4.56 (t, *J* = 6.6 Hz, 1 H), 4.51 (p, *J* = 2.0 Hz, 1 H), 4.36 (d, *J* = 13.9 Hz, 1 H), 4.12 (d, *J* = 14.1 Hz, 1 H), 3.99 (s, 3 H), 1.48 (s, 3 H), 1.42 (s, 3 H), 1.39 (s, 3 H), 1.36 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz,) δ 165.6, 150.6, 142.3, 141.3, 136.3, 125.1, 115.8, 111.4, 109.7, 107.4, 102.1, 101.1, 75.8, 75.1, 66.3, 61.7, 60.6, 48.6, 26.5, 25.4, 24.8, 23.7; HRMS (EI) calcd for $C_{22}H_{26}BrNO_8$: 511.0842, for $C_{21}H_{23}BrNO_8$: 496.0602 $[M\text{-}CH_3]^+$, found 496.0603 $[M\text{-}CH_3]^+$; LRMS (ESI) found 512 $[M+H]^+$, 534 $[M+Na]^+$, 550 $[M+K]^+$.

***tert*-Butyl (6-bromo-4-methoxybenzo[d][1,3]dioxole-5-carbonyl)((3a*S*,4*R*,9a*R*,9b*R*)-2,2,8,8-tetramethyl-3a,4,9a,9b-tetrahydro-6H-[1,3]dioxolo[4',5':5,6]benzo[1,2-d][1,3]dioxin-4-yl)carbamate** (**146**)



Amide **145** (170 mg, 0.33 mmol) was charged to a round bottom flask which was evacuated and backfilled with argon. The compound was dissolved in DCM (20 mL), followed by the addition of di-*tert*-butyl dicarbonate (320 mg, 1.47 mmol, 4.4 eq.) and DMAP (178 mg, 1.47 mmol, 4.4 eq.). The resulting solution was stirred at room temperature for 24 h. H_2O (15 mL) was added and the layers were separated. The aqueous layer was extracted with DCM (4 x 20 mL) and the combined organic layer was dried with $MgSO_4$. The crude residue was purified by column chromatography (2:1 to 1:1 hexanes:EtOAc) to obtain **146**

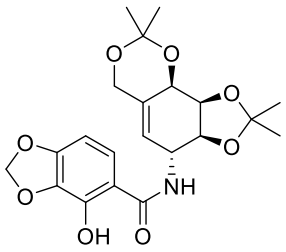
as a slightly cloudy oily solid (128 mg, 64 %) along with 55 mg of the starting amide (93 % BRSM).

R_f = 0.7 (1:1 hexanes:EtOAc); $[\alpha]_D^{24}$ = - 107.7 (*c* = 0.14, $CHCl_3$); IR (film, cm^{-1}) *v* 3093, 2984, 2936, 2851, 1733, 1672, 1621, 1469, 1243, 1156, 1085, 1045, 857, 758; ^1H NMR* ($CDCl_3$, 600 MHz) δ 6.72 (s, 1 H) / 6.66 (s, 1 H), 5.95 (dd, *J* = 1.3, 3.7 Hz, 2 H) / 5.93 (dd, *J* = 1.3, 2.6 Hz, 2 H), 5.34 (m, 1 H) / 5.28 (m, 1 H), 5.12 (m, 1 H), 5.06 (m, 1 H), 4.75 (m, 1 H), 4.65 (dt, *J* = 4.1, 5.5 Hz, 1 H), 4.38 – 4.28 (m, 2 H), 3.99 (s, 3 H) / 3.92 (s, 3 H), 1.51 (s, 3 H), 1.50 (s, 3 H), 1.49 (s, 3 H), 1.40 (s, 3 H), 1.25 (s, 9 H) / 1.23 (s, 9 H); ^{13}C NMR** ($CDCl_3$, 150 MHz,) δ 166.9 /166.6, 152.2 / 152.1, 150.1 / 150.0, 140.9 / 140.1, 136.8 / 136.6, 136.0 / 135.7, 127.2 / 127.1, 114.5 / 114.4, 110.9 / 109.1, 109.7 / 109.6, 107.4 / 106.6, 102.0 / 101.9, 100.5 / 100.4, 84.0 / 83.9, 77.6, 76.5, 66.6, 62.2 / 62.1, 60.2, 58.5 / 58.4, 27.9, 27.6, 26.8 / 26.6, 26.2, 22.3 / 22.1; HRMS (EI) calcd for $C_{27}H_{34}BrNO_{10}$: 611.1366, found 611.1364; LRMS (ESI) found 636 $[M+Na]^+$.

* The ^1H NMR spectrum showed that the compound exists as a mixture of rotamers in solution. The corresponding signals for each rotameric state are reported together as signal (state A) / signal (state B).

** The ^{13}C NMR spectrum showed that the compound exists as a mixture of rotamers in solution. The corresponding signals for each rotameric state are reported together as signal (state A) / signal (state B).

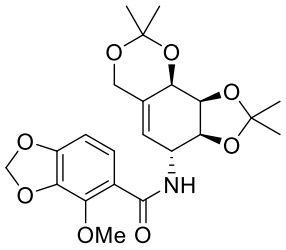
4-Hydroxy-*N*-((3a*S*,4*R*,9a*R*,9b*R*)-2,2,8,8-tetramethyl-3a,4,9a,9b-tetrahydro-6H-[1,3]dioxolo[4',5':5,6]benzo[1,2-d][1,3]dioxin-4-yl)benzo[d][1,3]dioxole-5-carboxamide (151)



Imide **146** (25 mg, 0.041 mmol) was charged to a round bottom flask. The vessel was evacuated and backfilled with argon twice. The substrate was dissolved in DMF (1.5 mL) and $Pd(OAc)_2$ (4 mg, 0.02 mmol, 40 mol %) and Et_3N (20 µL, 0.14 mmol, 3.5 eq.) were added. The resulting solution was degassed by bubbling with a stream of argon for 10 min. PPh_3 (6 mg, 0.02 mmol, 60 mol %) was then added under a stream of argon. The vessel was fitted with an argon-flushed condenser and was heated to 120 °C for 48 h. The mixture was allowed to cool to room temperature, at which point H_2O (10 mL) and Et_2O (20 mL) were added. The layers were separated and the aqueous layer was washed with Et_2O (3 x 20 mL). The combined organic washes were dried over $MgSO_4$, filtered and concentrated. The crude mixture was purified by flash column chromatography (silica, 4:1 hexanes:EtOAc to 1:1 hexanes:EtOAc gradient) to provide multiple products. The main product was phenol **151** (7 mg, 41 %), alongside debrominated amide **152** (4 mg, 23 %), amide **153** (3 mg, 14 %) and traces of cyclized lactam **154** as a mixture with Ph_3PO (< 1 mg, < 5 %). The three latter products were characterized only by ^{1}H-NMR and HRMS (EI) due to the small scale and observed instability of the samples.

151: $[\alpha]_D^{20}$ = -123.7 (*c* 0.25, $CHCl_3$); IR (film, cm^{-1}) *v* 3549, 3373, 2988, 2935, 2857, 1665, 1316, 1064, 731; ^1H NMR ($CDCl_3$, 600 MHz) δ 12.14 (s, 1 H), 6.89 (d, *J* = 8.4 Hz, 1 H), 6.42 (d, *J* = 8.4 Hz, 1 H), 6.06 (s, 2 H), 5.84 (d, *J* = 6.0 Hz, 1 H), 5.65 (d, *J* = 6.0 Hz, 1 H), 4.71 (dd, *J* = 4.1, 6.5 Hz, 1 H), 4.57 (d, *J* = 6.6 Hz, 1 H), 4.56 – 4.53 (m, 2 H), 4.42 (d, *J* = 14.0 Hz, 1 H), 4.19 (d, *J* = 14.1 Hz, 1 H), 1.52 (s, 3 H), 1.48 (s, 3 H), 1.42 (s, 3 H), 1.38 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz,) δ 170.2, 152.6, 146.5, 143.1, 135.3, 120.2, 115.2, 110.5, 110.1, 102.5, 101.4, 100.5, 75.2, 66.0, 61.7, 49.3, 41.6, 26.6, 25.2, 25.0, 23.8; HRMS (EI) calcd for $C_{21}H_{25}NO_8$: 419.1580, for $C_{29}H_{22}NO_8$:404.1340 $[M-CH_3]^+$, found 404.1339 $[M-CH_3]^+$; LRMS (ESI) Found 420 $[M+H]^+$, 442 $[M+Na]^+$, 458 $[M+K]^+$.

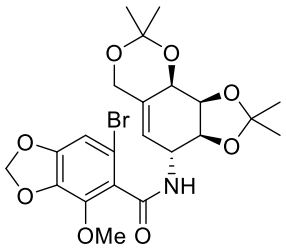
4-Methoxy-*N*-((3a*S*,4*R*,9a*R*,9b*R*)-2,2,8,8-tetramethyl-3a,4,9a,9b-tetrahydro-6H-[1,3]dioxolo[4',5':5,6]benzo[1,2-d][1,3]dioxin-4-yl)benzo[d][1,3]dioxole-5-carboxamide (152)



^1H NMR ($CDCl_3$, 300 MHz) δ 7.77 (d, *J* = 8.3 Hz, 1 H), 7.56 (d, *J* = 6.4 Hz, 1 H), 6.63 (d, *J* = 8.3 Hz, 1 H), 6.01 (s, 2 H), 5.73 (d, *J* = 7.7 Hz, 1 H), 4.70 (dd, *J* = 3.9, 7.0 Hz, 1 H), 4.66 – 4.60 (m, 2 H), 4.58 – 4.53 (m, 1 H), 4.39 (d, *J* = 13.7 Hz, 1 H), 4.17 (d, *J* = 13.9 Hz, 1 H), 4.08 (s, 3 H), 1.51 (s, 3 H), 1.47 (s, 3 H), 1.41 (s, 3 H), 1.35 (s, 3 H); HRMS (EI)

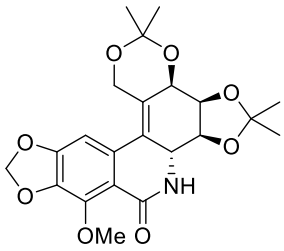
calcd for $C_{22}H_{27}NO_8$: 433.1737, for $C_{21}H_{24}NO_8$: 418.1496 $[M\text{-}CH_3]^+$, found 418.1500 $[M\text{-}CH_3]^+$; LRMS (ESI) Found 434.2 $[M+H]^+$, 456.2 $[M+Na]^+$, 472.1 $[M+K]^+$.

6-Bromo-4-methoxy-*N*-((3a*S*,4*R*,9a*R*,9b*R*)-2,2,8,8-tetramethyl-3a,4,9a,9b-tetrahydro-6H-[1,3]dioxolo[4',5':5,6]benzo[1,2-d][1,3]dioxin-4-yl)benzo[d][1,3]dioxole-5-carboxamide (153)



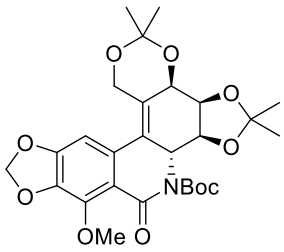
^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.72 (s, 1 H), 5.97 (s, 2 H), 5.69 (d, *J* = 6.0 Hz, 1 H), 5.38 (d, *J* = 6.4 Hz, 1 H), 4.77 (d, *J* = 6.9 Hz, 1 H), 4.70 (dd, *J* = 3.8, 7.1 Hz, 1 H), 4.61 – 4.55 (m, 1 H), 4.54 – 4.49 (m, 1 H), 4.37 (d, *J* = 13.9 Hz, 1 H), 4.13 (d, *J* = 14.0 Hz, 1 H), 4.00 (s, 3 H), 1.49 (s, 3 H), 1.43 (s, 3 H), 1.39 (s, 3 H), 1.37 (s, 3 H); HRMS (EI) calcd for $C_{22}H_{26}BrNO_8$: 511.0842, for $C_{21}H_{23}BrNO_8$: 496.0602 $[M\text{-}CH_3]^+$, found 496.0599 $[M\text{-}CH_3]^+$; LRMS (ESI) Found 512.1 $[M+H]^+$, 534.1 $[M+Na]^+$, 550.1 $[M+K]^+$.

(3a*S*,3b*R*,14a*R*,14b*R*)-6-Methoxy-2,2,13,13-tetramethyl-3b,11,14a,14b-tetrahydro-4H-[1,3]dioxino[5,4-a]bis([1,3]dioxolo)[4,5-c:4',5'-j]phenanthridin-5(3aH)-one (154)



^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.26 (s, 1 H), 6.05 (d, *J* = 1.4 Hz, 1 H), 6.03 (d, *J* = 1.5 Hz, 1 H), 5.72 (br s, 1 H), 4.74 (br s, 1 H), 4.68 (dt, *J* = 15.4, 2.6 Hz, 1 H), 4.54 (t, *J* = 4.2 Hz, 1 H), 4.42 (d, *J* = 15.2 Hz, 1 H), 4.19 – 4.16 (m, 2 H), 4.07 (s, 3 H), 1.55 (br s, 3 H, overlapping with H_2O), 1.451 (s, 3 H), 1.446 (s, 3 H), 1.440 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 164.0, 151.5, 145.1, 138.4, 132.7, 128.7, 122.3, 115.9, 111.7, 102.10, 102.06, 101.96, 77.8, 74.0, 66.1, 61.1, 60.7, 57.4, 28.1, 26.5, 24.4, 23.8; LRMS (ESI) calcd for $C_{22}H_{25}NO_8$ 431.2, found 432.2 $[M+H]^+$, 454.1 $[M+Na]^+$, 470.1 $[M+K]^+$.

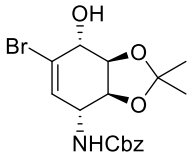
***tert*-Butyl (3a*S*,3b*R*,14a*R*,14b*R*)-6-methoxy-2,2,13,13-tetramethyl-5-oxo-3a,3b,5,11,14a,14b-hexahydro-4H-[1,3]dioxino[5,4-a]bis([1,3]dioxolo)[4,5-c:4',5'-j]phenanthridine-4-carboxylate (150)**



Imide **146** (35 mg, 0.06 mmol) was charged to a round bottom flask. The vessel was evacuated and backfilled with argon twice. The substrate was dissolved in anisole (2.5 mL) and $Pd(OAc)_2$ (13 mg, 0.06 mmol, 1 eq.), 1,2-bis(diphenylphosphino)ethane (46 mg, 0.11 mmol, 2 eq.) and TlOAc (30 mg, 0.11 mmol, 2 eq.) were added sequentially, turning the solution green before developing to yellow. The resulting solution was degassed by bubbling with a stream of argon for 10 min. The vessel was fitted with an argon-flushed condenser and was heated to 130 °C for 18 h. The cooled reaction mixture was passed through a celite plug and rinsed with EtOAc and DCM. The filtrate was concentrated and dried thoroughly to remove residual anisole and was then purified by flash column chromatography (silica, 4:1 hexanes:EtOAc to 1:1 hexanes:EtOAc gradient) to obtain pure **150** as a colourless oil (2.1 mg, 7 %, 28 % [BRSM]).

R_f = 0.6 (1:1 hexanes:EtOAc); $[\alpha]_D^{22}$ = 42.2 (*c* 0.14, $CHCl_3$); IR (film, cm^{-1}) *v* 2983, 2924, 2853, 1761, 1704, 1672, 1606, 1480, 1371, 1266, 1242, 1150, 1108, 1049; ^1H NMR ($CDCl_3$, 600 MHz) δ 6.23 (s, 1 H), 6.04 (s, 1 H), 6.03 (s, 3 H), 4.77 (br s, 1 H), 4.64 – 4.59 (m, 3 H), 4.35 (d, *J* = 14.9 Hz, 1 H), 4.32 (s, 1 H), 4.06 (s, 3 H), 1.57 (s, 9 H), 1.54 (s, 3 H), 1.44 (s, 3 H), 1.42 (s, 3 H), 1.38 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz,) δ 162.5, 152.9, 152.0, 145.7, 138.3, 135.6, 133.9, 121.5, 115.5, 110.6, 102.1, 101.7, 101.4, 84.5, 75.2, 74.3, 66.2, 61.9, 61.1, 60.1, 28.0, 27.7, 26.0, 24.2, 24.1; HRMS (EI) calcd for $C_{27}H_{33}NO_{10}$: 531.2104, for $C_{27}H_{34}NO_{10}$: 532.2177 $[M+H]^+$, found 532.2171 $[M+H]^+$; LRMS (ESI) Found 532.2 $[M+H]^+$, 554.2 $[M+Na]^+$, 570.2 $[M+K]^+$.

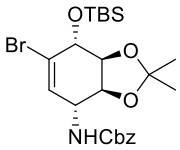
Benzyl((3a*S*,4*R*,7*R*,7a*R*)-6-bromo-7-hydroxy-2,2-dimethyl-3a,4,7,7a-tetrahydro benzo[d][1,3]dioxol-4-yl)carbamate (182)



Oxazine **114** (1.13 g, 2.37 mmol) was charged to a round bottom flask and dissolved in THF (25 mL) and water (2.5 mL). The resulting solution was cooled to 0 °C prior to the addition of aluminum amalgam. The aluminum amalgam was prepared as follows: aluminum turnings were dipped sequentially into 1 M KOH (20 s), distilled water (5 s), 0.5 % $HgCl_2$ aqueous solution (60 s), distilled water (5 s) and then finally added to the reaction mixture. The first addition of aluminum (535 mg, 19.8 mmol, 8.4 eq.) was followed by stirring for 15 h. An additional portion of aluminum (303 mg, 11.2 mmol, 4.7 eq.) was added at 0 °C, followed by stirring at room temperature for 24 h. The resulting mixture was diluted with EtOAc (50 mL) and was passed through a plug of celite. The plug was further rinsed with DCM and the combined filtrate was concentrated. Purification by flash column chromatography (10 % deactivated silica, 4:1 to 2:1 hexane:EtOAc) afforded pure allylic alcohol **182** as a colourless oil (301 mg, 32 %).

R_f = 0.47 (hexanes:EtOAc 2:1); IR (film, cm^{-1}) ν 3348, 3065, 3033, 2987, 2934, 1695, 1510, 1375, 1295, 1214, 1164, 1037, 872, 736, 697; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.38 – 7.30 (m, 5 H), 6.33 (d, *J* = 6.1 Hz, 1 H), 5.52 (d, *J* = 7.1 Hz, 1 H), 5.13 – 5.05 (m, 2 H), 4.49 (d, *J* = 5.6 Hz, 1 H), 4.42 – 4.31 (m, 3 H), 3.46 (br s, 1 H), 1.40 (s, 3 H), 1.31 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 155.8, 136.1, 130.3, 128.7, 128.42, 128.37, 126.9, 109.0, 79.1, 75.9, 73.1, 67.3, 50.9, 26.7, 24.7.

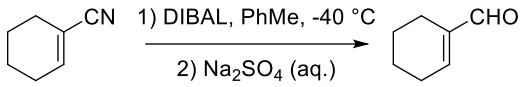
Benzyl ((3a*S*,4*R*,7*R*,7a*S*)-6-bromo-7-((*tert*-butyldimethylsilyl)oxy)-2,2-dimethyl-3a,4,7,7a-tetrahydrobenzo[d][1,3]dioxol-4-yl)carbamate (183)



Allylic alcohol **182** (1.53 g, 3.83 mmol) was charged to a round bottom flask and dissolved in DCM (25 mL). The resulting solution was cooled to 0 °C and imidazole (1.56 g, 22.9 mmol, 6 eq.) and TBSCl (1.73 g, 11.5 mmol, 3 eq.) were added. The resulting solution was stirred at 0 °C for 30 min. before removal of the ice bath and allowing the mixture to stir at room temperature for 18 h. The mixture was then heated to reflux for 2 h if incomplete consumption was observed. The reaction mixture was cooled to room temperature and water (75 mL) was added. The organic layer was separated and the aqueous layer was further extracted with DCM (4 x 50 mL). The combined organic layer was washed once with brine, dired over $MgSO_4$, filtered and concentrated. Purification by flash column chromatography (silica, 15:1 to 9:1 hexane:EtOAc) afforded pure silyl ether **183** as a light yellow oil (1.85 g, 94 %).

R_f = 0.5 (hexanes:EtOAc 9:1); IR (film, cm^{-1}) *v* 3392, 3066, 3034, 2953, 2930, 2898, 2857, 1726, 1504, 1381, 1253, 1213, 1063, 838, 779; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.35 – 7.29 (m, 5 H), 6.47 (d, *J* = 5.1 Hz, 1 H), 5.65 (d, *J* = 4.3 Hz, 1 H), 5.09 (s, 2 H), 4.55 (t, *J* = 5.3 Hz, 1 H), 4.42 (q, *J* = 3.1 Hz, 2 H), 4.33 (s, 1 H), 1.37 (s, 3 H), 1.30 (s, 3 H), 0.87 (s, 9 H), 0.20 (s, 3 H), 0.19 (s, 3 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 155.6, 136.5, 131.4, 128.5, 128.1, 128.0, 127.2, 108.8, 80.0, 76.3, 74.6, 66.9, 49.4, 26.4, 25.7, 24.5, 17.9, -4.3, -5.0.

Nitrile reduction model study

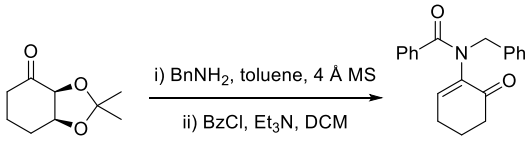


99 **104**

Vinyl nitrile **99** (500 mg, 4.67 mmol) was charged to a flame-dried Schlenk tube under N_2 atmosphere. The nitrile was dissolved in toluene and cooled to -40 °C. DIBAL (1 M solution in toluene, 9.3 mmol) was added dropwise over 15 minutes. The mixture was maintained at -40 °C for 2.5 h then was allowed to gradually warm to r.t. Reaction completion was confirmed by TLC (4:1 hexanes:EtOAc). The mixture was again cooled to -40 °C and was quenched with a saturated aqueous solution of Na_2SO_4 (1 mL). The biphasic mixture was allowed to stir for 1 h, warming to room temperature. $MgSO_4$ was added, and the mixture was passed through a plug of Celite and concentrated under reduced pressure to afford the crude enal. The residue was purified by flash column chromatography (19:1 hexanes:EtOAc) to obtain the pure enal **104** as a colorless oil (418 mg, 81 %), matching published data.

Rigby model study

***N*-benzyl-*N*-(6-oxocyclohex-1-en-1-yl) benzamide** (**185**)



184 **185**

Ketone **184** (174 mg, 1.02 mmol) was charged to a round bottom flask and dissolved in toluene (10 mL). Benzylamine (0.11 mL, 1.0 mmol, 1 eq.) was added and the mixture was heated to reflux for 12 h, throughout which time water was removed by a Dean-Stark

apparatus or by initial addition of 4 Å molecular sieves. The mixture was cooled to room temperature and the solvent was removed by rotary evaporation. The crude imine was dissolved in DCM and cooled to 0 °C. Triethylamine (0.16 mL, 1.1 mmol, 1.1 eq.) and benzoyl chloride (0.12 mL, 1.0 mmol, 1 eq.) were then added sequentially in a dropwise manner. The mixture was allowed to stir at room temperature for 8 h. $NaHCO_3$ saturated solution (10 mL) was added and the phases were separated. The aqueous layer was extracted with DCM (3 x 20 mL) and the combined organic layer was washed with brine followed by drying with $MgSO_4$. The crude residue was purified by column chromatography (4:1 to 3:1 hexanes:EtOAc) to obtain **185** as a colorless oil (175 mg, 56 %) which readily decomposed to a brown oil upon standing.

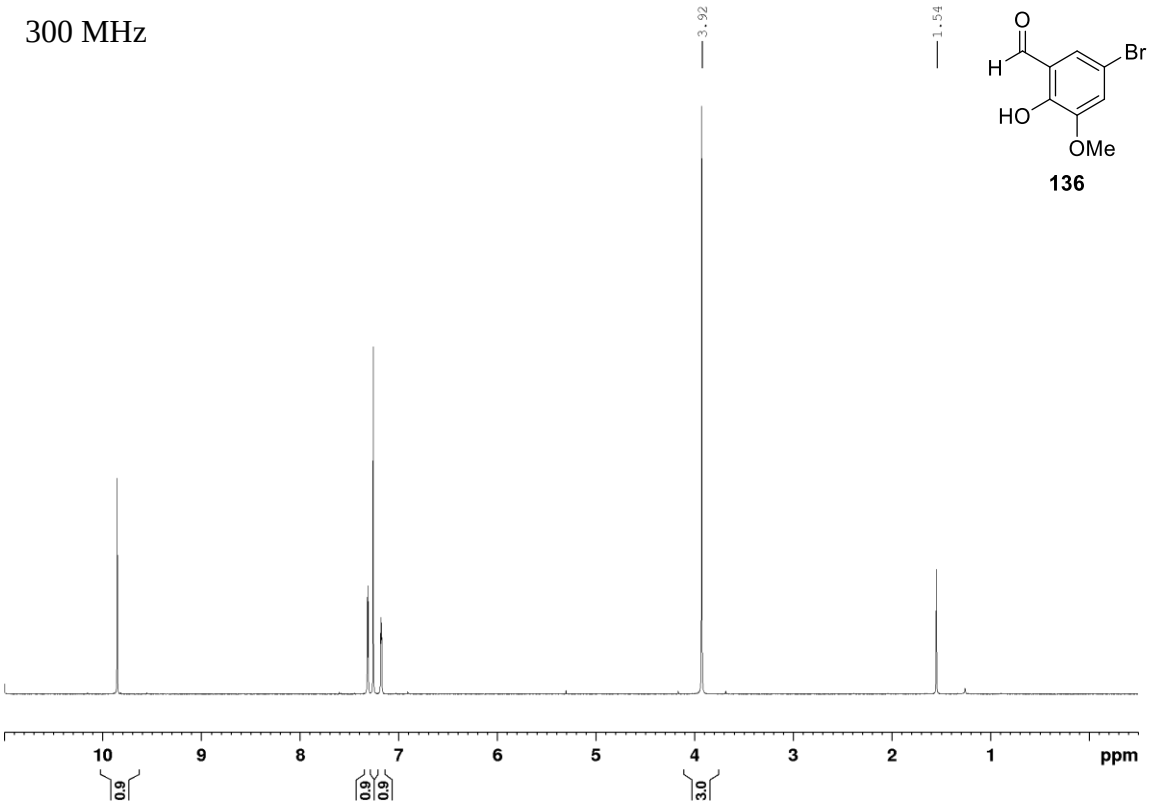
R_f = 0.4 (2:1 hexanes:EtOAc); IR (film, cm^{-1}) *v* 3060, 3029, 2930, 2867, 1686, 1649, 1389, 700; ^1H NMR* ($CDCl_3$, 600 MHz) δ 7.39 – 7.27 (m, 10 H), 6.28 (s, 1 H), 5.50 (br s, 1 H), 4.22 (br s, 1 H), 2.28 (s, 2 H), 2.11 (s, 2 H), 1.74 (s, 2 H); ^{13}C NMR** ($CDCl_3$, 150 MHz,) δ 195.0, 171.4, 149.4, 139.7, 137.5, 136.6, 129.8, 129.0, 128.5, 128.0, 127.6, 127.5, 51.0, 38.4, 25.9, 22.2; HRMS (EI) calcd for $C_{20}H_{19}NO_2$: 305.1416, found 305.1413; LRMS (ESI) found 306 $[M+H]^+$, 328 $[M+Na]^+$, 344 $[M+K]^+$.

* The ^1H NMR spectrum showed broad peaks with no visible coupling constants, suggesting the product was being analyzed as a rotameric mixture. The spectrum was reacquired in d_6-DMSO but little difference was seen.

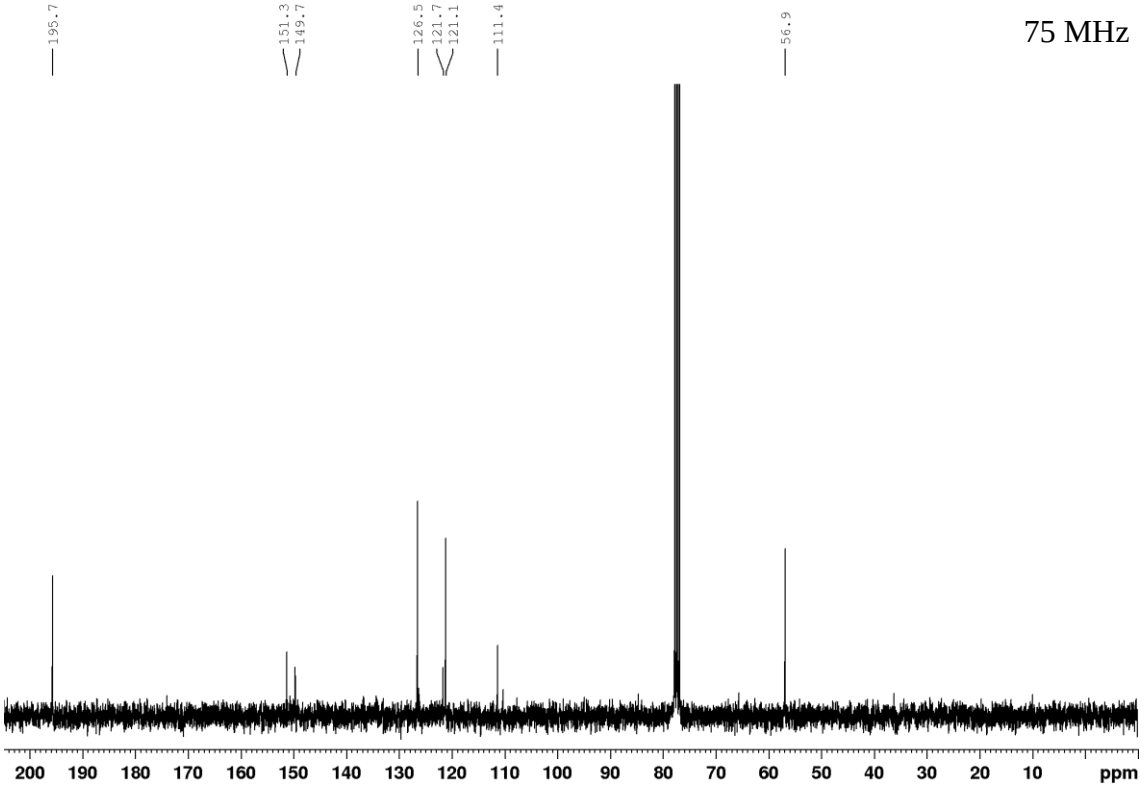
** The ^{13}C NMR spectrum was acquired using a longer relaxation time in order to observe the benzylic carbon signal. Longer experiment times (more scans) were necessary to observe and resolve all aromatic signals.

6. Selected Spectra

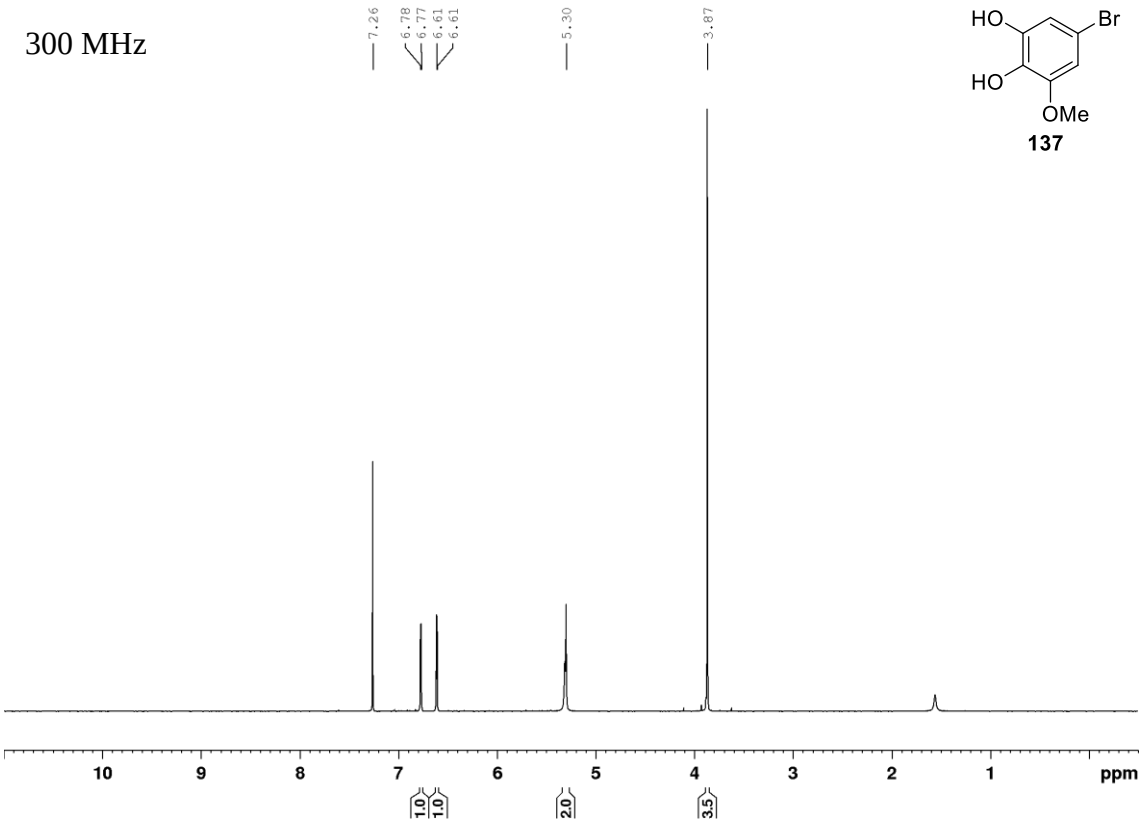
300 MHz



75 MHz

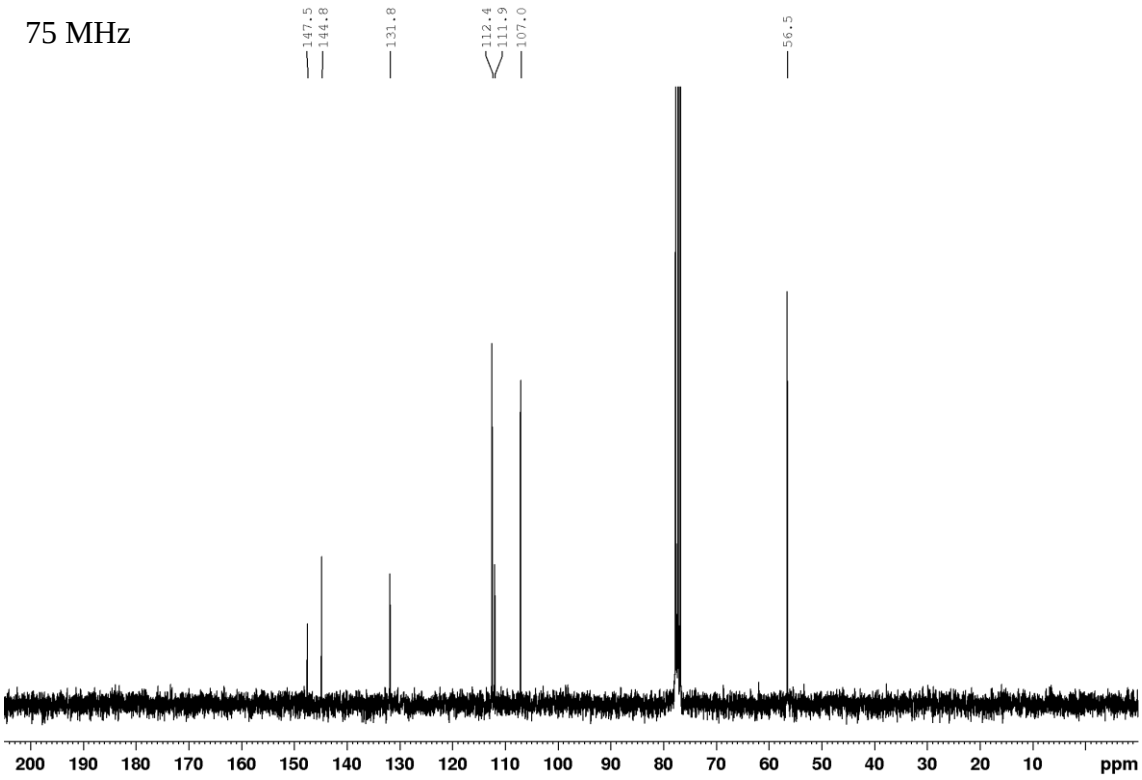


300 MHz

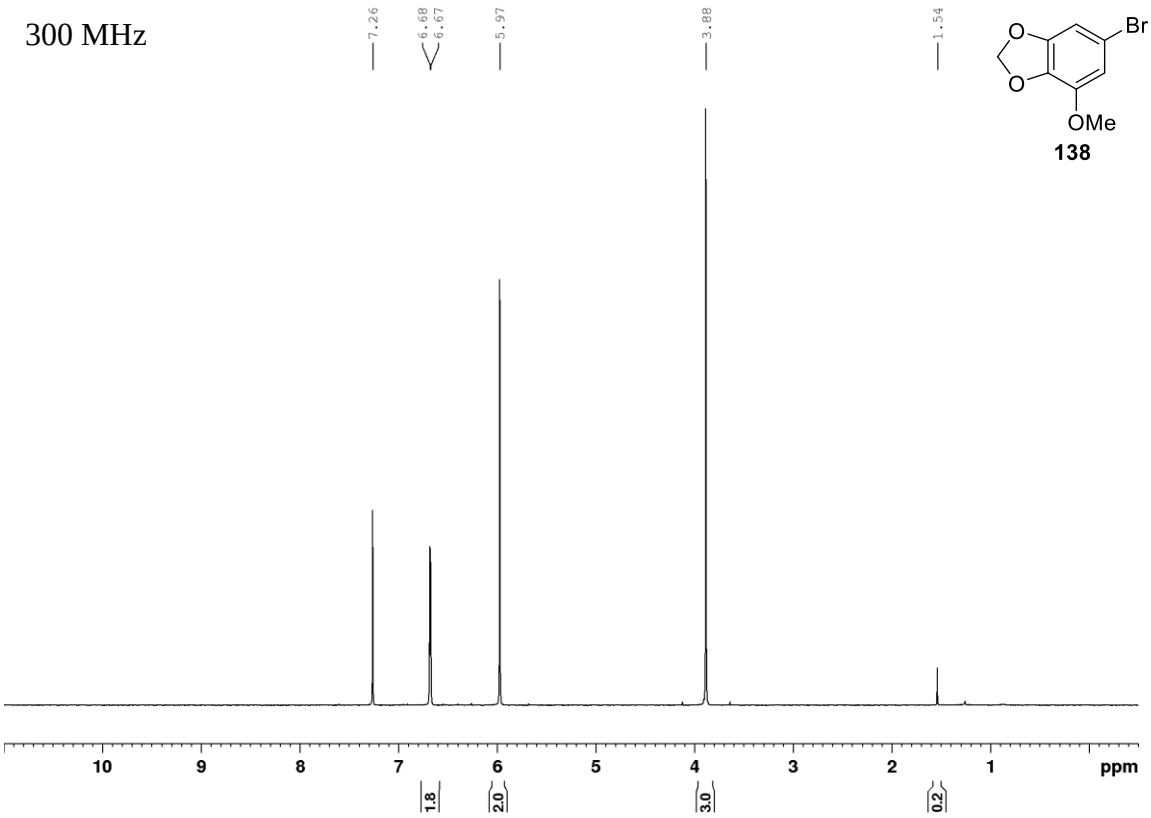


137

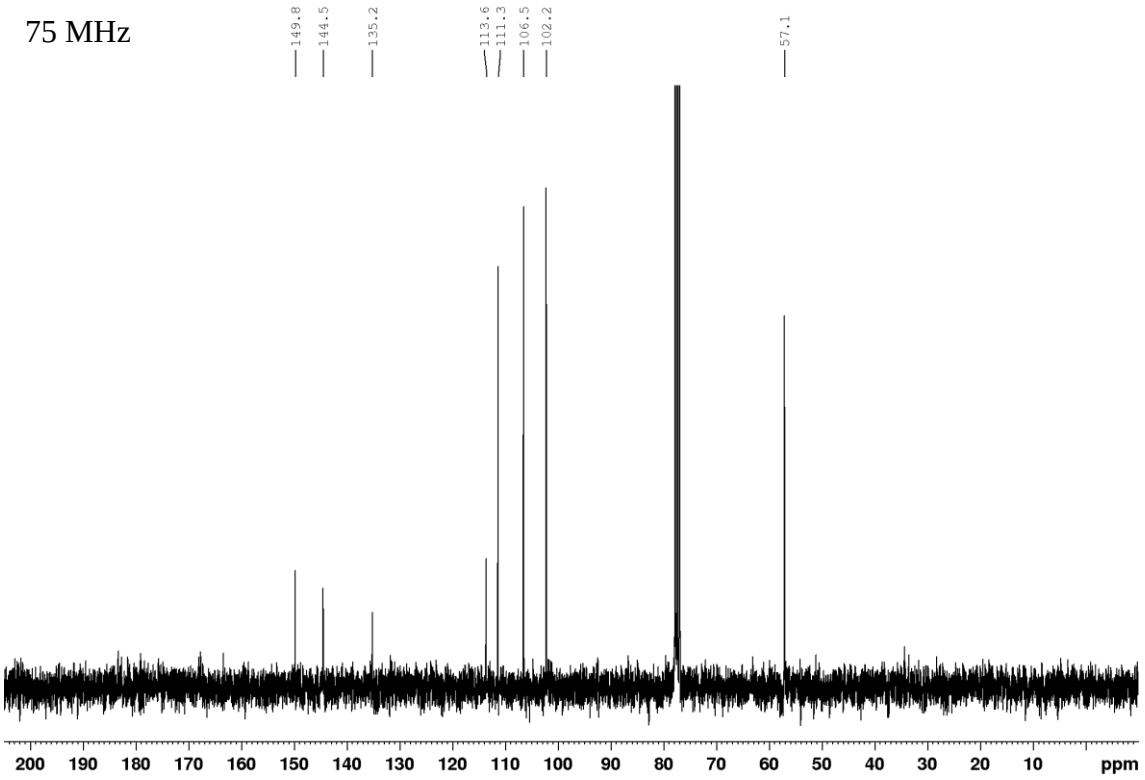
75 MHz



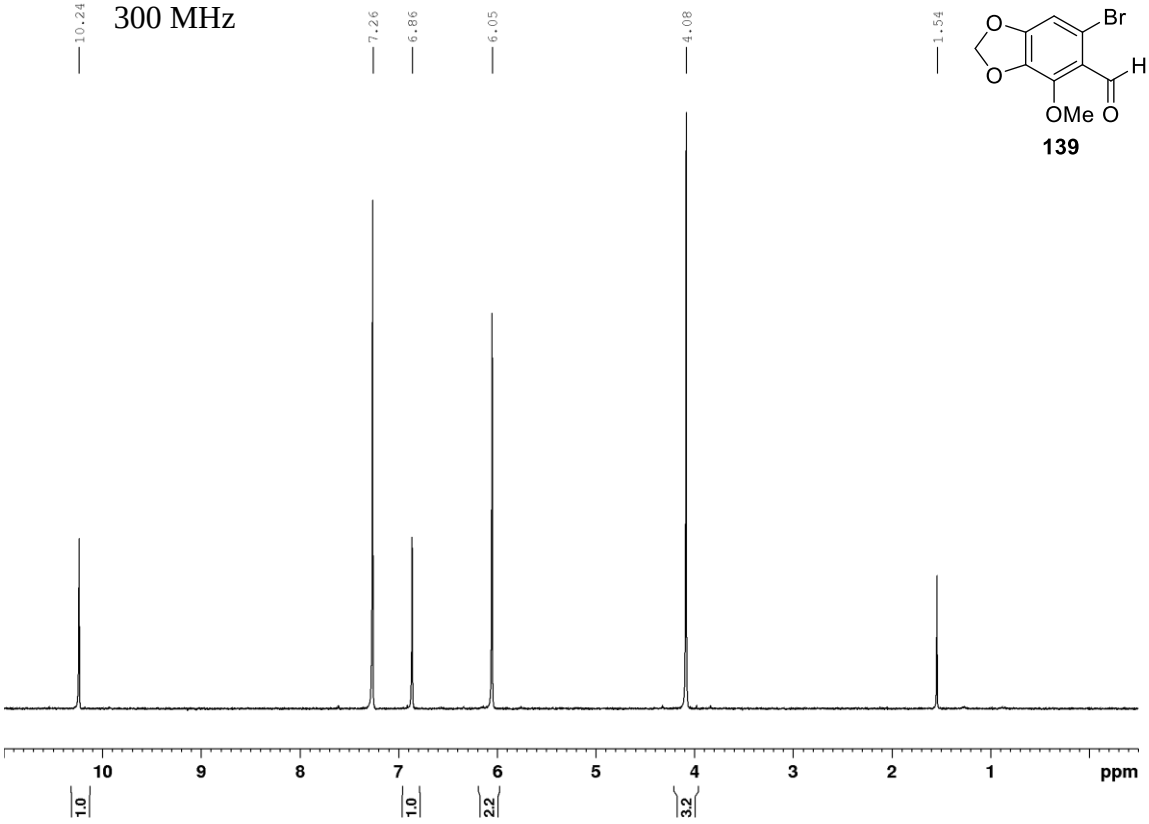
300 MHz



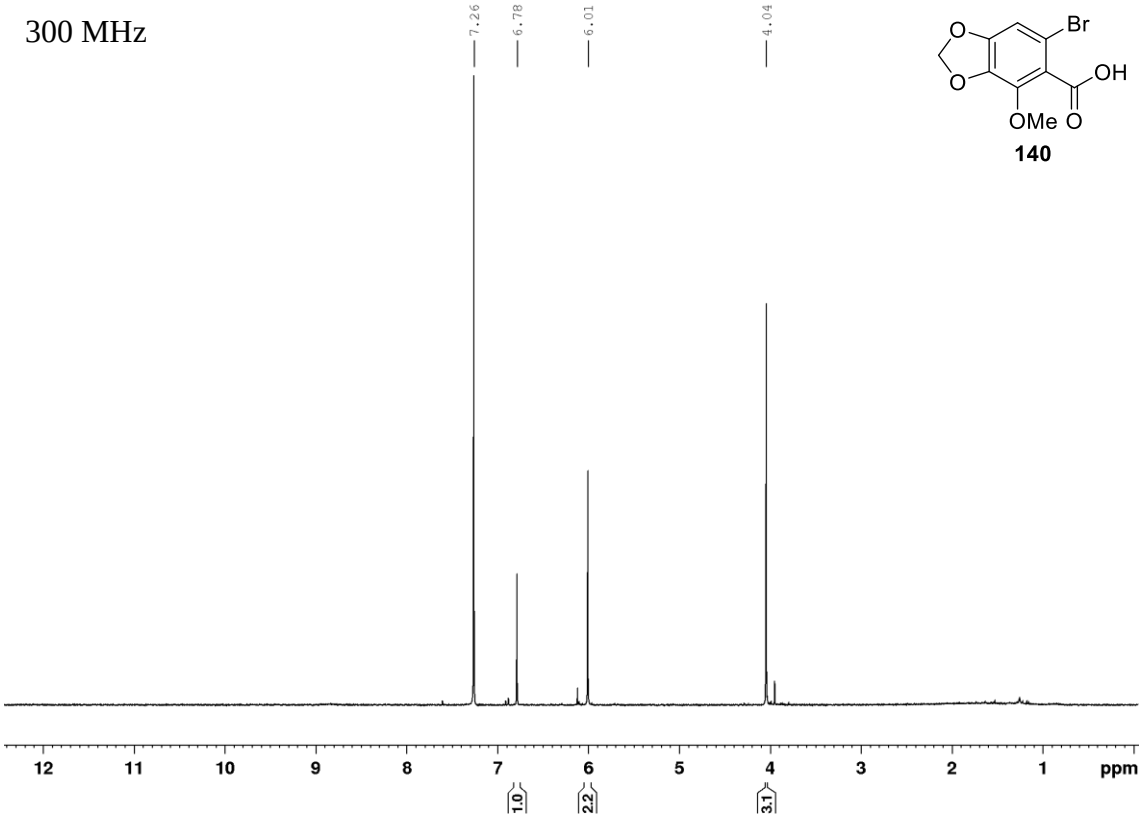
75 MHz



300 MHz

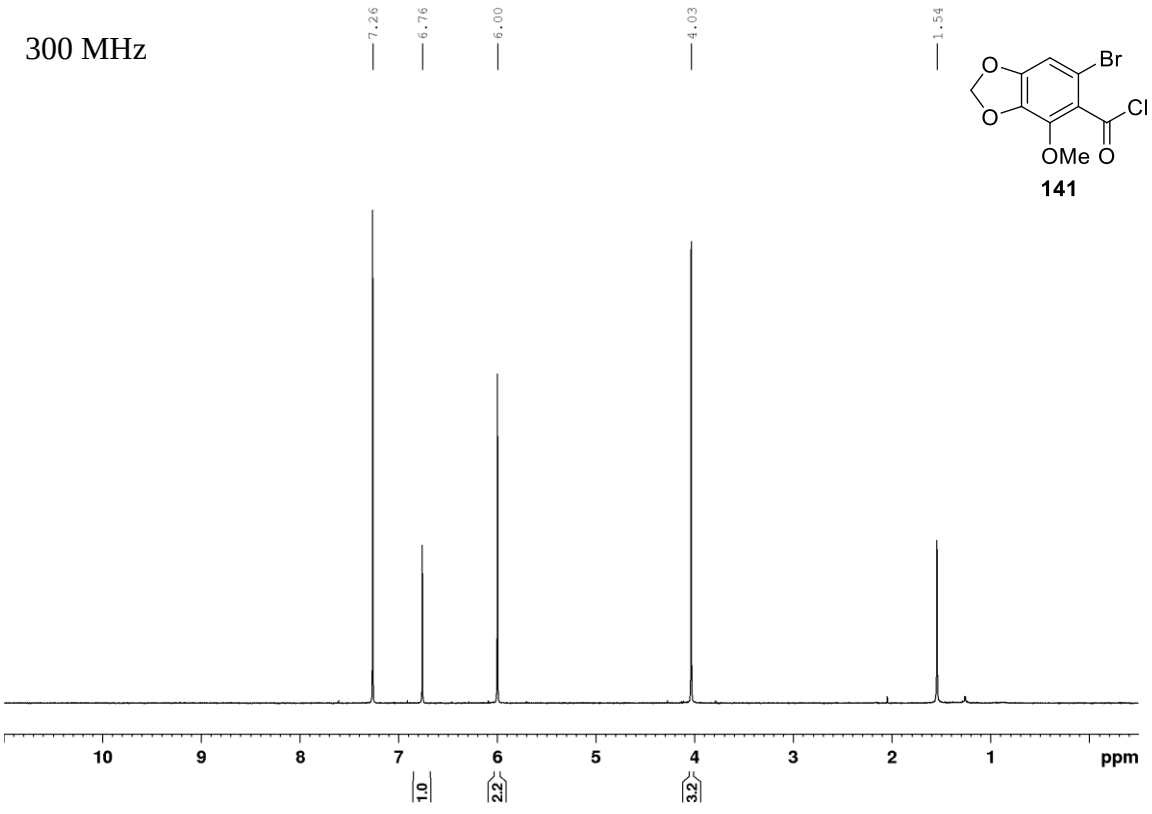

10.24
7.26
6.86
6.05
4.08
1.54
Br
O
O
H
OMe O
139
10
9
8
7
6
5
4
3
2
1
ppm
1.0
1.0
2.2
3.2

300 MHz



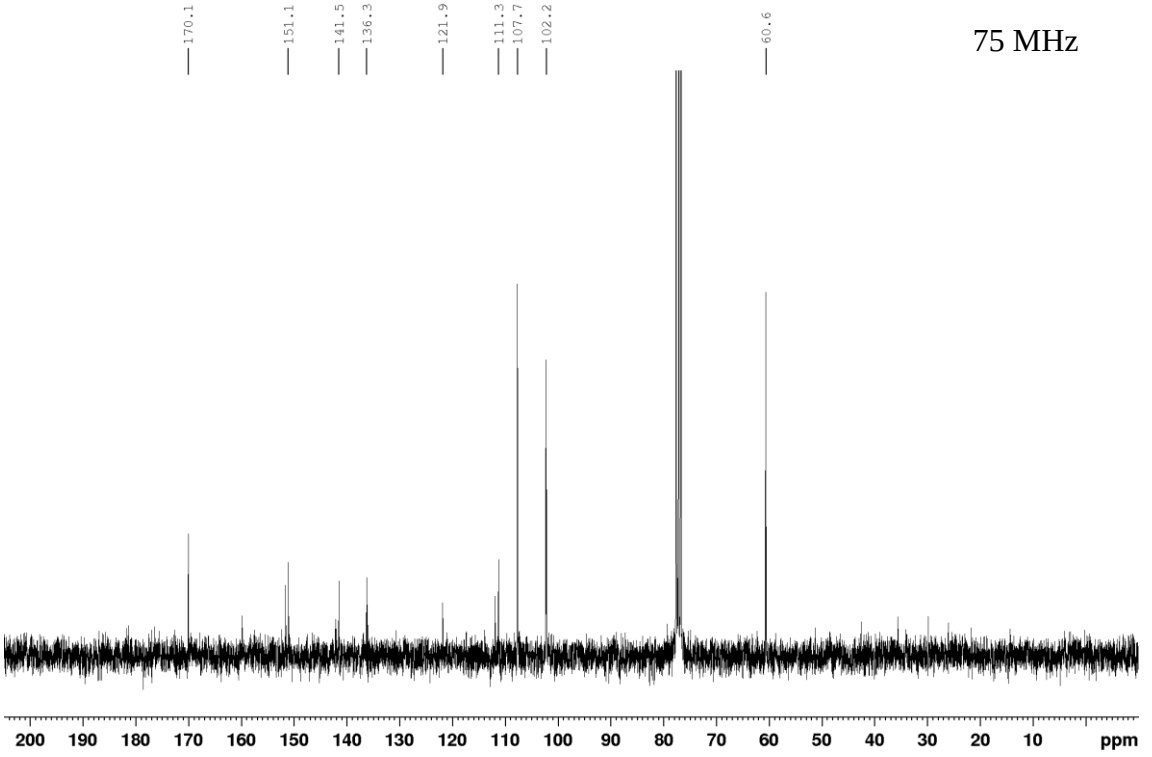
140

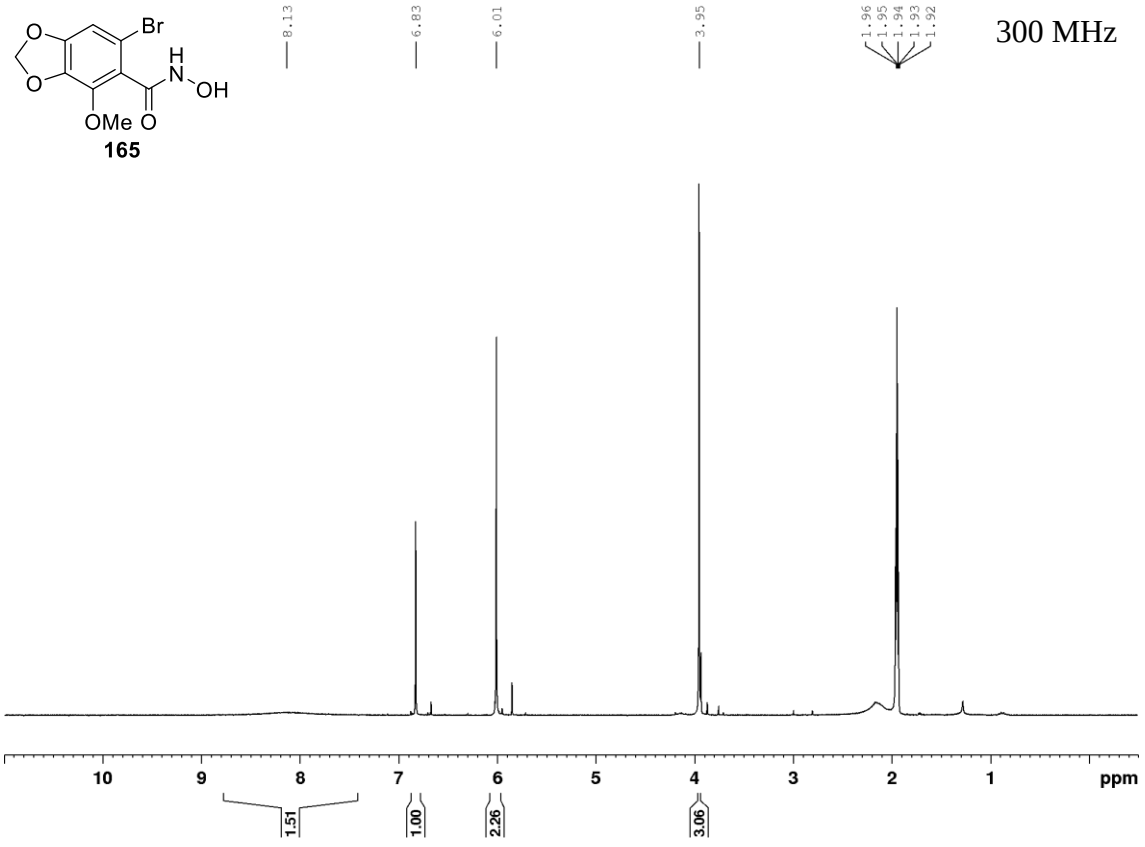
300 MHz

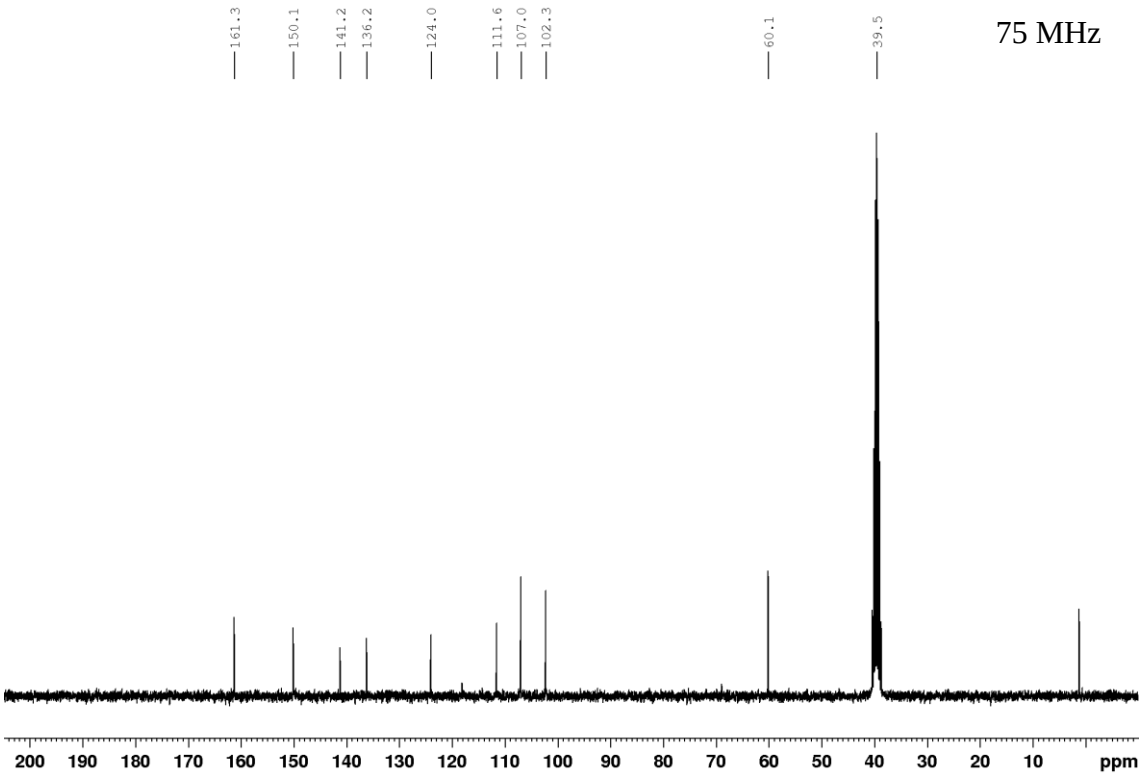


141

75 MHz




Br
O
O
H
N
OH
OMe O
165
8.13
6.83
6.01
3.95
1.96
1.95
1.94
1.93
1.92
300 MHz
10
9
8
7
6
5
4
3
2
1
ppm
1.51
1.00
2.26
3.06


161.3
150.1
141.2
136.2
124.0
111.6
107.0
102.3
60.1
39.5
75 MHz
200
190
180
170
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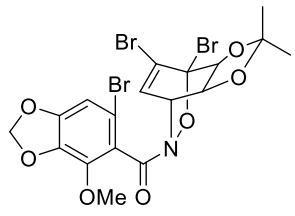
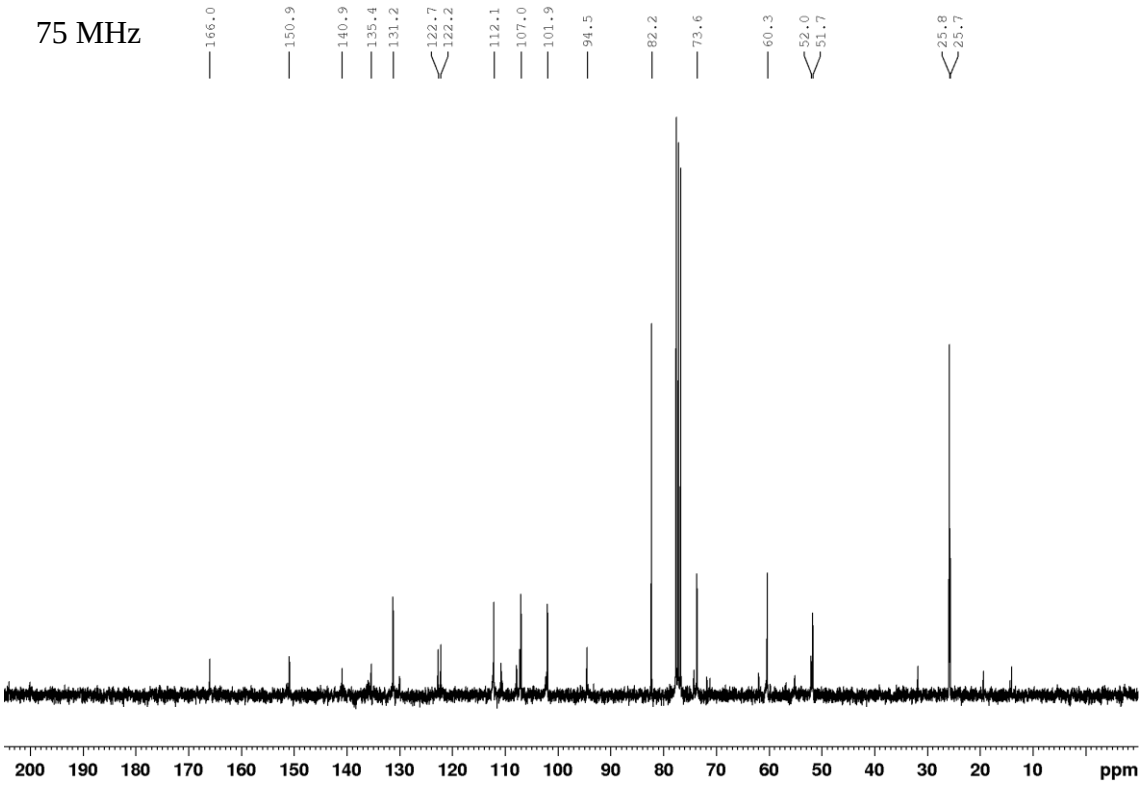
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

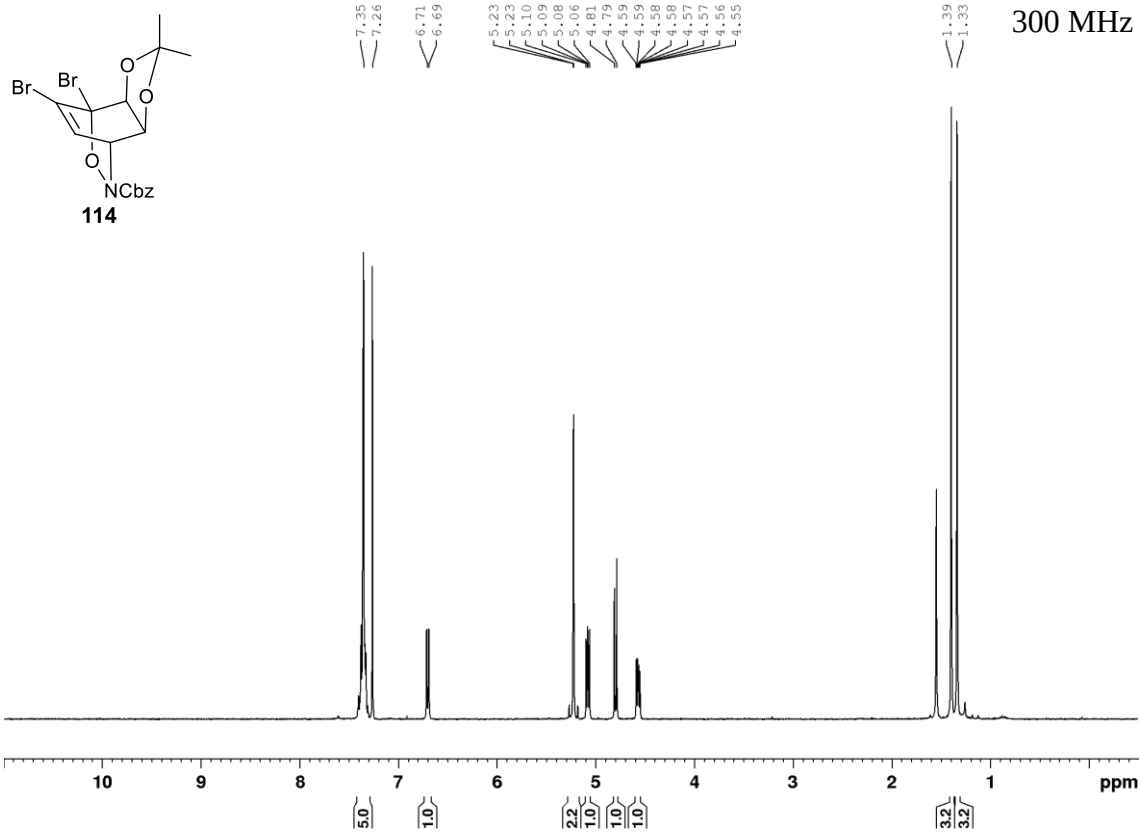


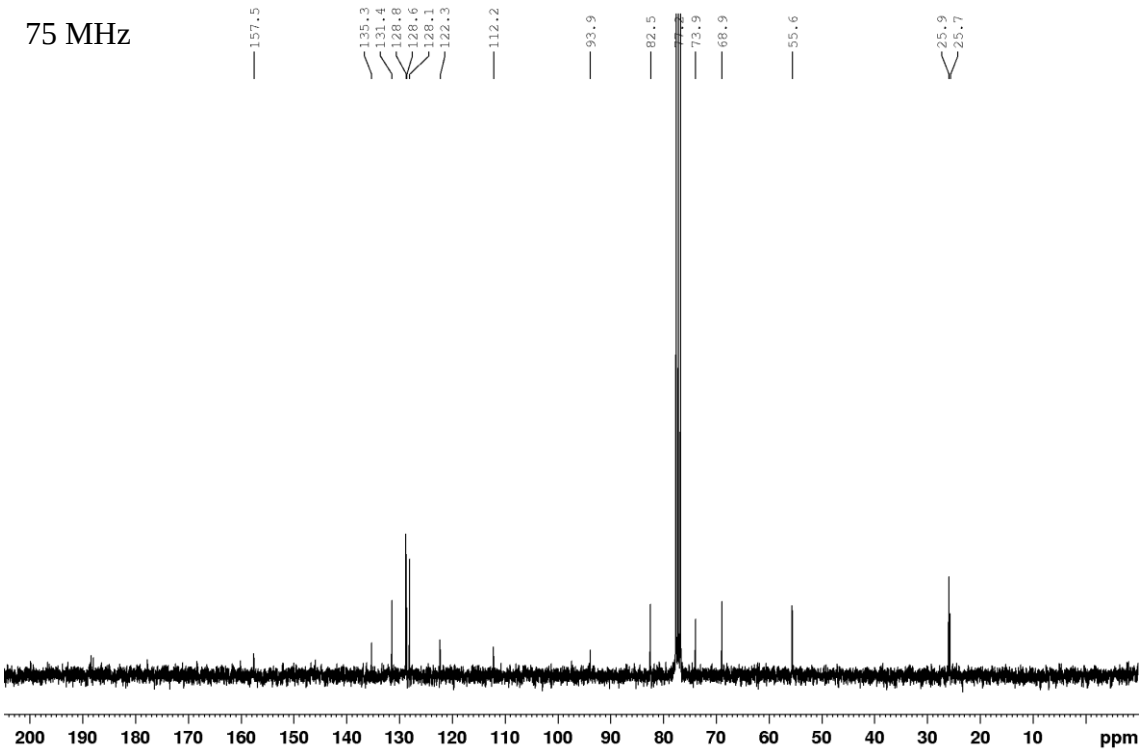
167

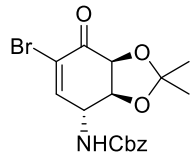
300 MHz

75 MHz




300 MHz
Br
Br
O
O
O
NCbz
114
7.35
7.26
6.71
6.69
5.23
5.23
5.10
5.09
5.08
5.06
4.81
4.79
4.59
4.59
4.58
4.58
4.57
4.57
4.56
4.55
1.39
1.33
10
9
8
7
6
5
4
3
2
1
ppm
5.0
1.0
2.2
1.0
1.0
1.0
3.2
3.2

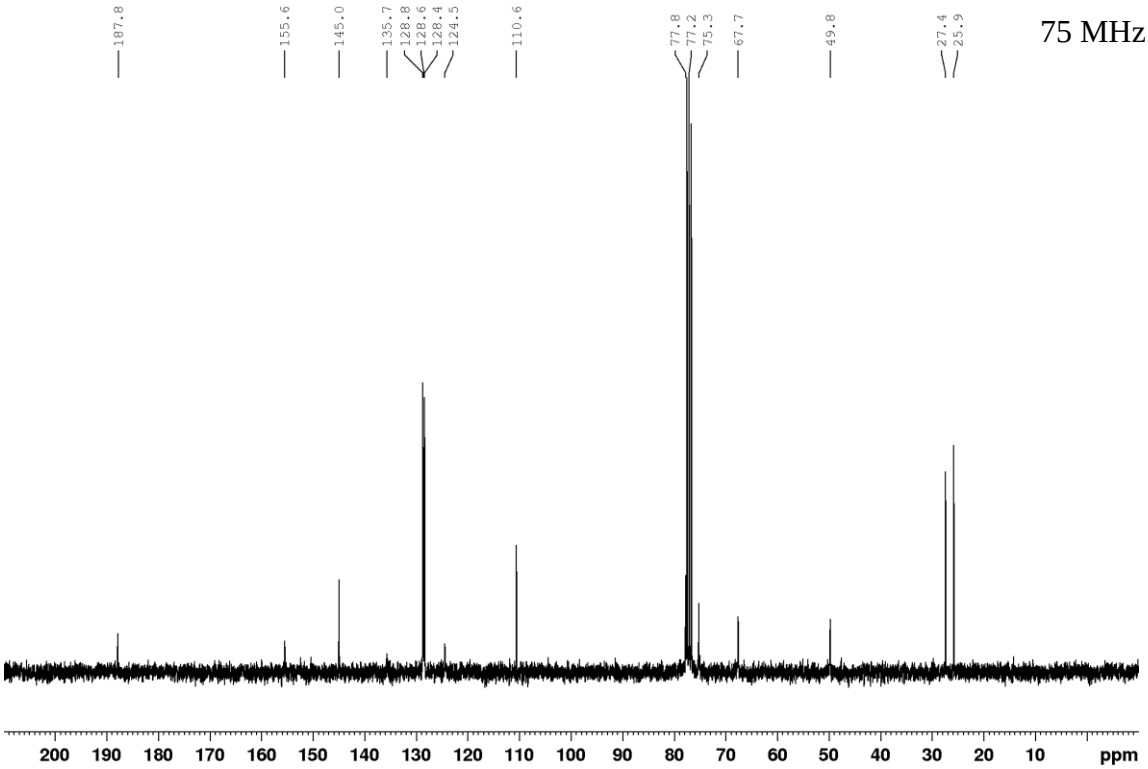

75 MHz
157.5
135.3
131.4
128.8
128.6
128.1
122.3
112.2
93.9
82.5
77.2
73.9
68.9
55.6
25.9
25.7
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

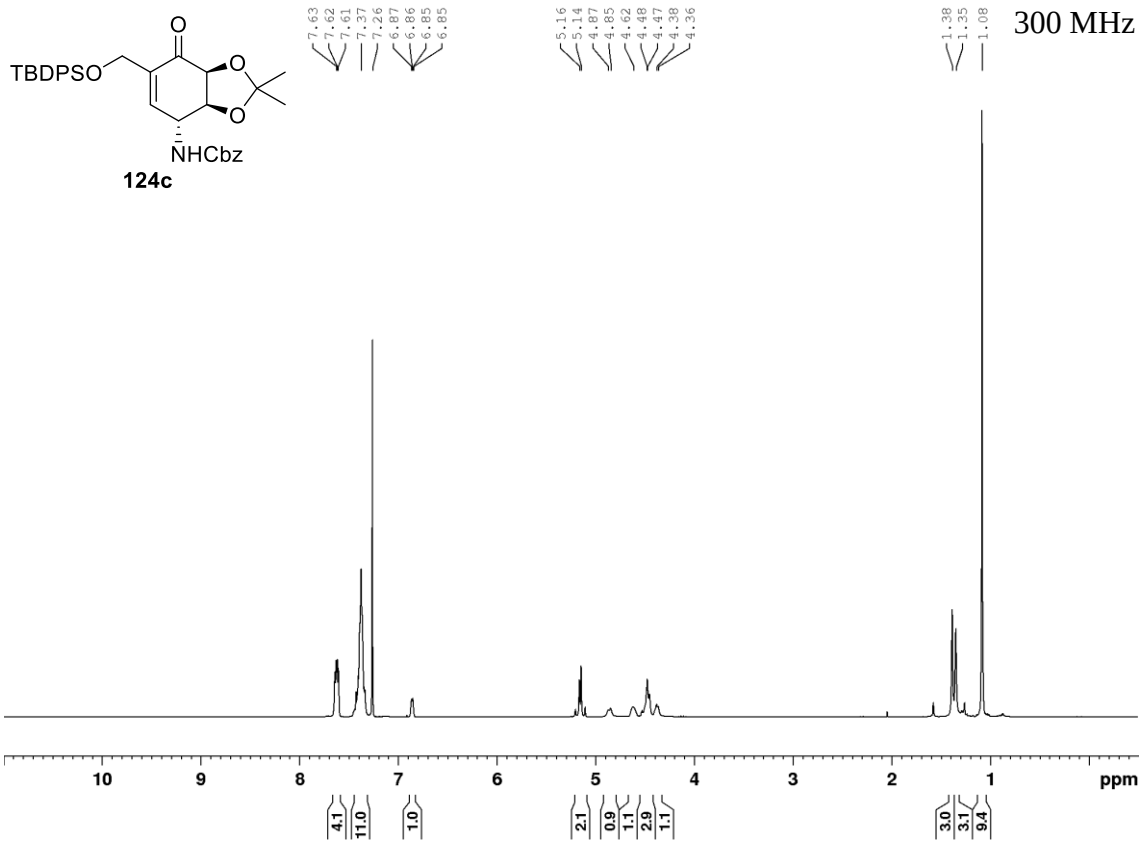


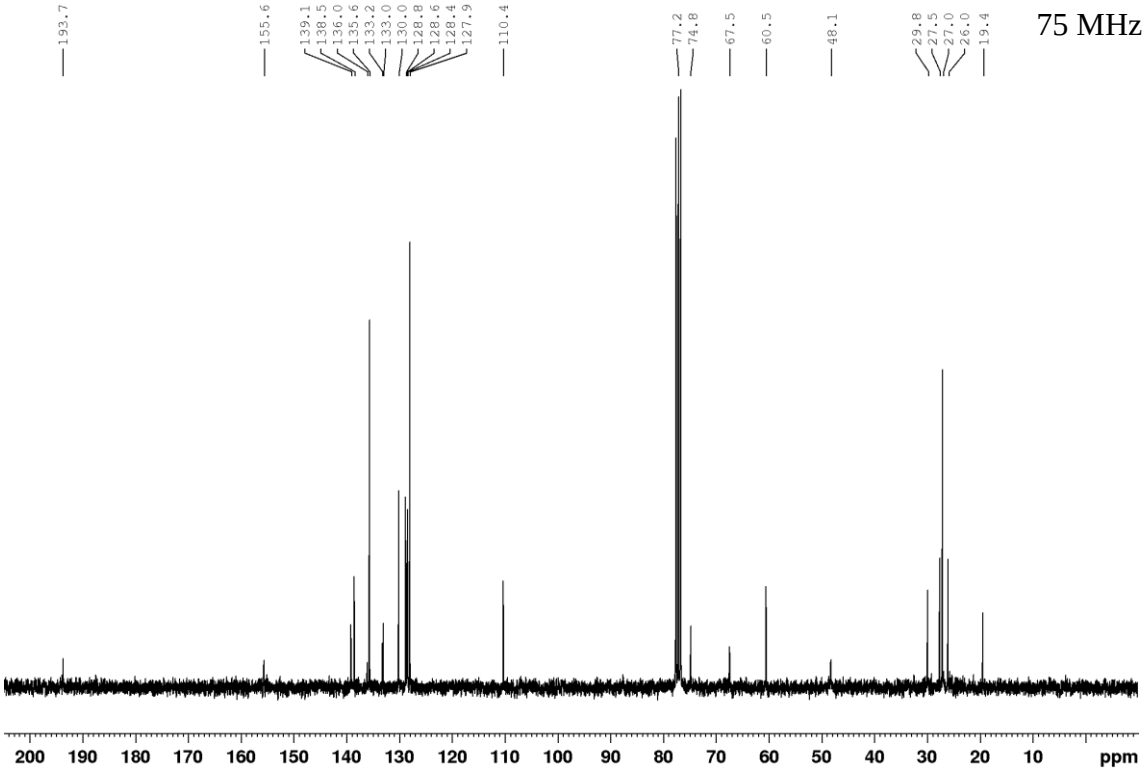
116

300 MHz

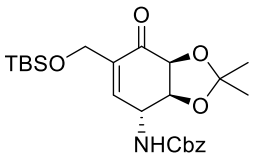
75 MHz




TBDPSO
O
O
O
NHCbz
124c
7.63
7.62
7.61
7.37
7.26
6.87
6.86
6.85
6.85
5.16
5.14
4.87
4.85
4.62
4.48
4.47
4.38
4.36
1.38
1.35
1.08
300 MHz
10
9
8
7
6
5
4
3
2
1
ppm
4.1
11.0
1.0
2.1
0.9
1.1
2.9
1.1
3.0
3.1
9.4

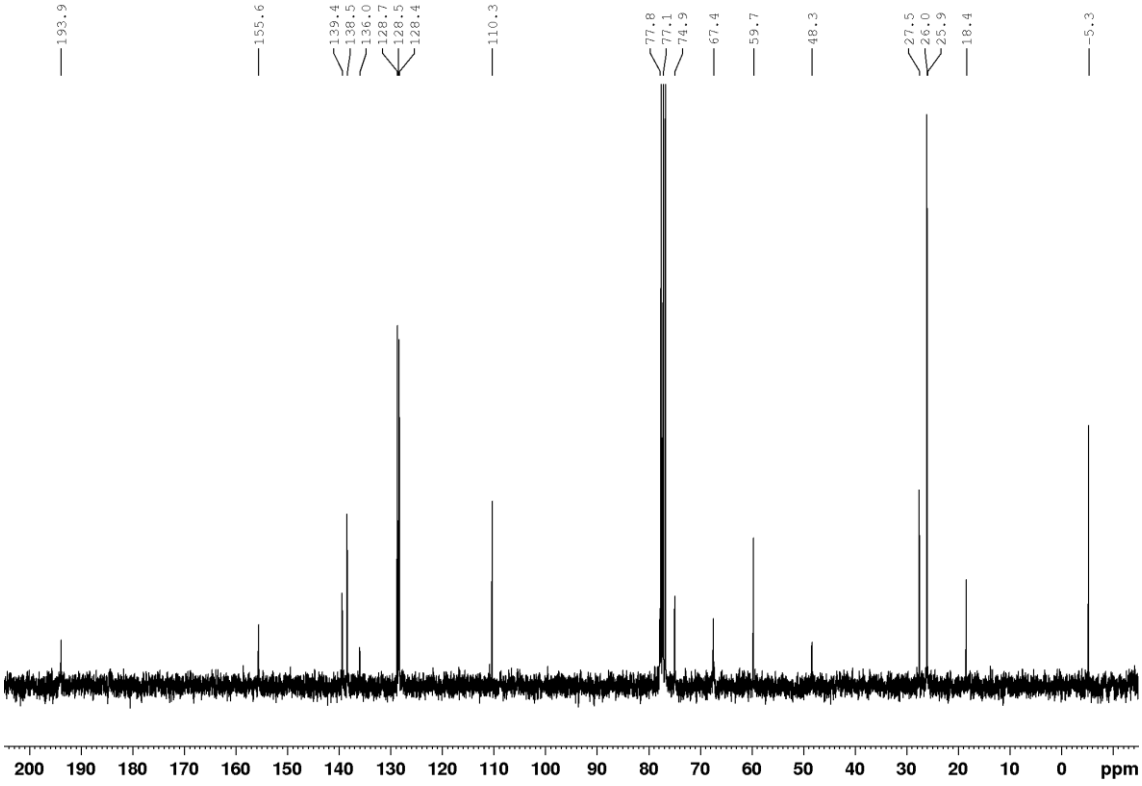

193.7
155.6
139.1
138.5
136.0
135.6
133.2
133.0
130.0
128.8
128.6
128.4
127.9
110.4
77.2
74.8
67.5
60.5
48.1
29.8
27.5
27.0
26.0
19.4
75 MHz
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

300 MHz

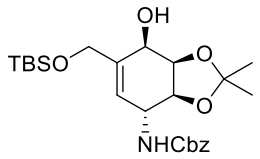


124b

75 MHz

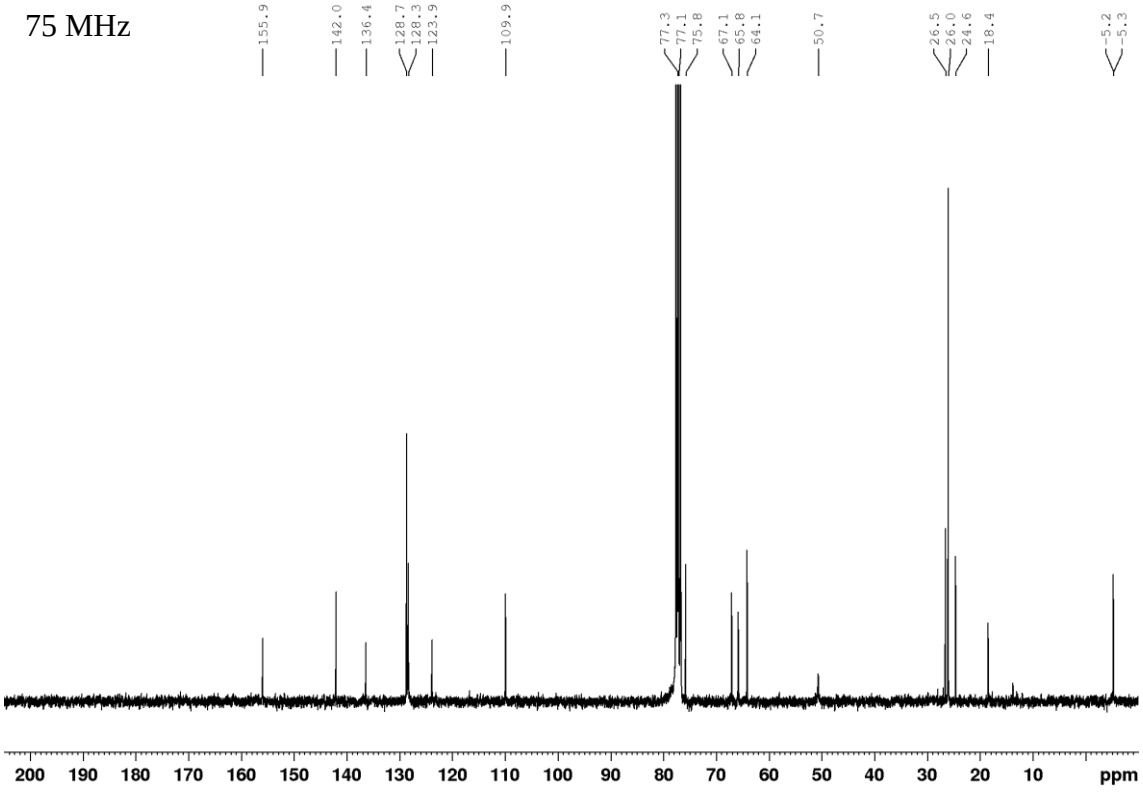


300 MHz

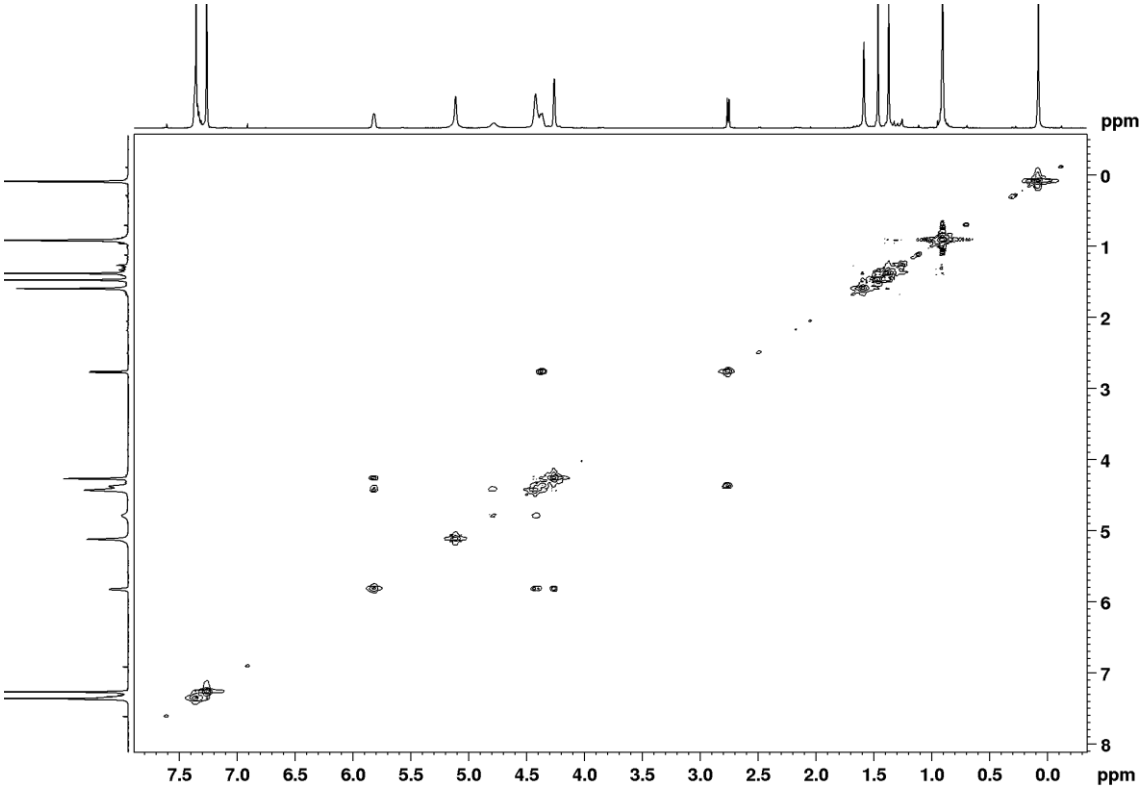


129-β

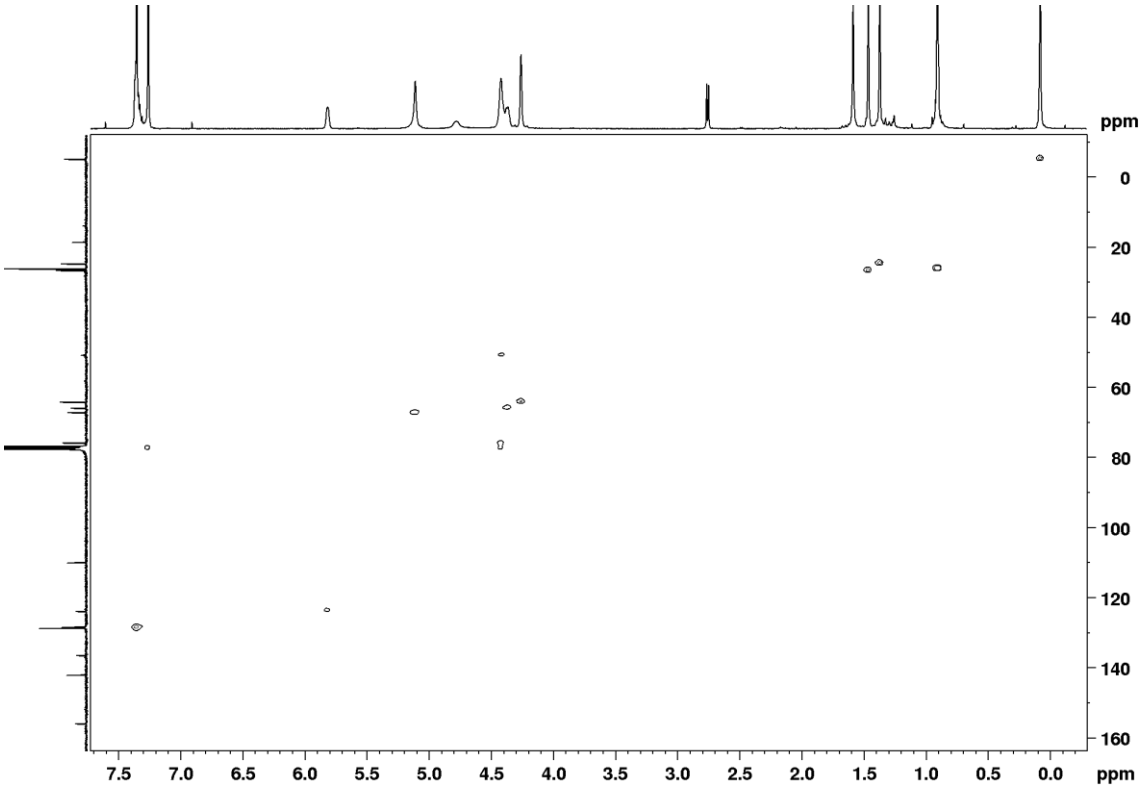
75 MHz



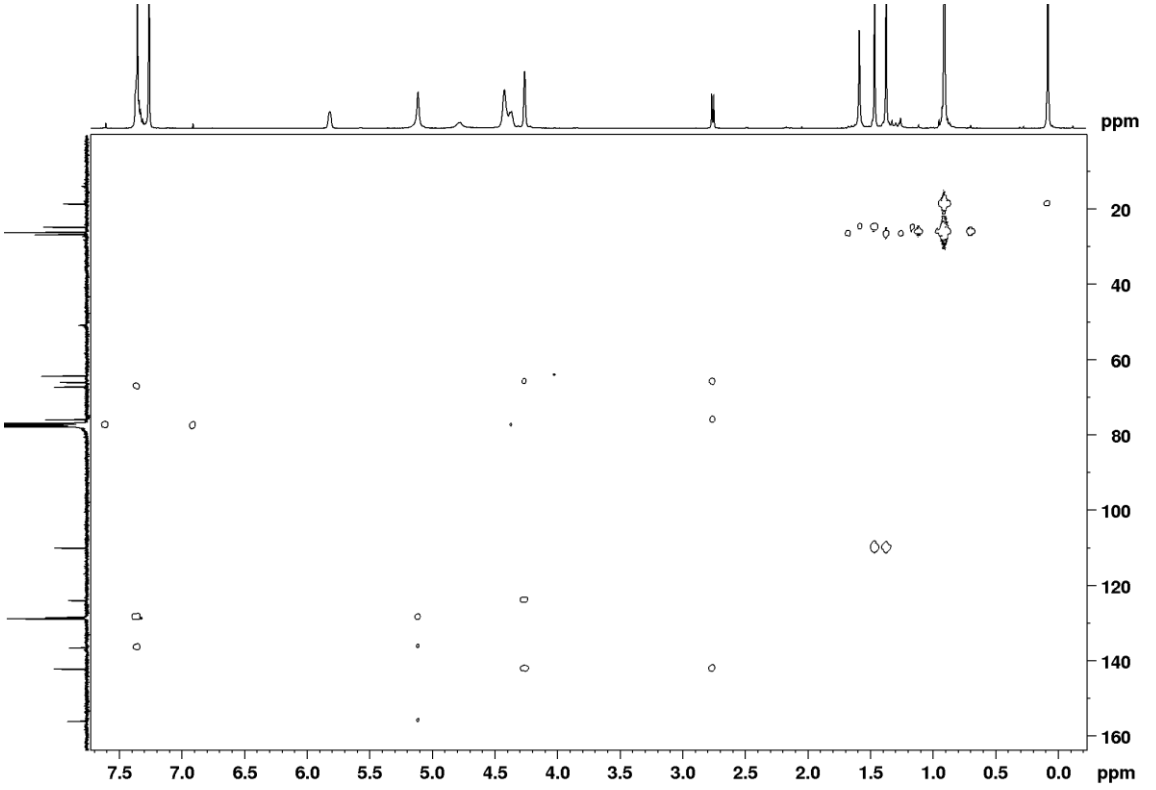
129-β: ^{1}H COSY



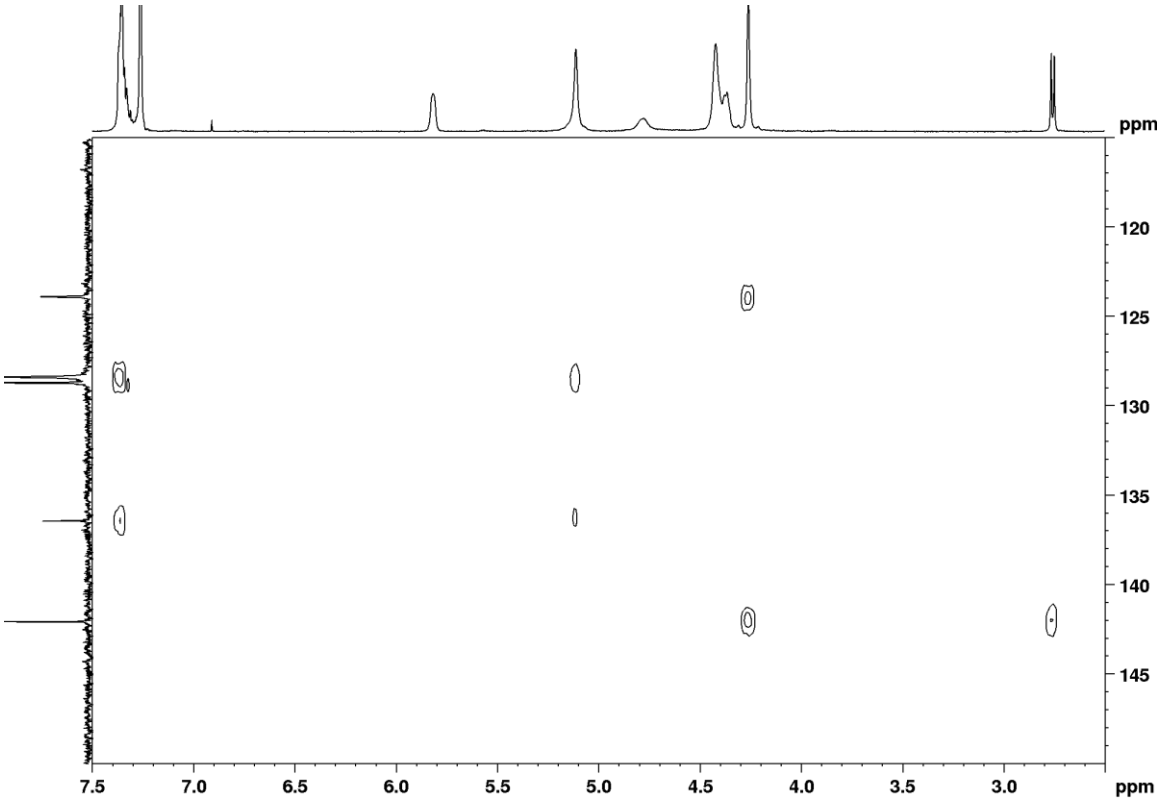
^{1}H-^{13}C HSQC

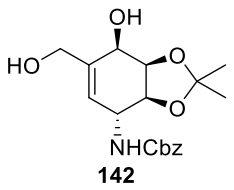


129-β: ^{1}H-^{13}C HMBC



^{1}H-^{13}C HMBC (expansion)

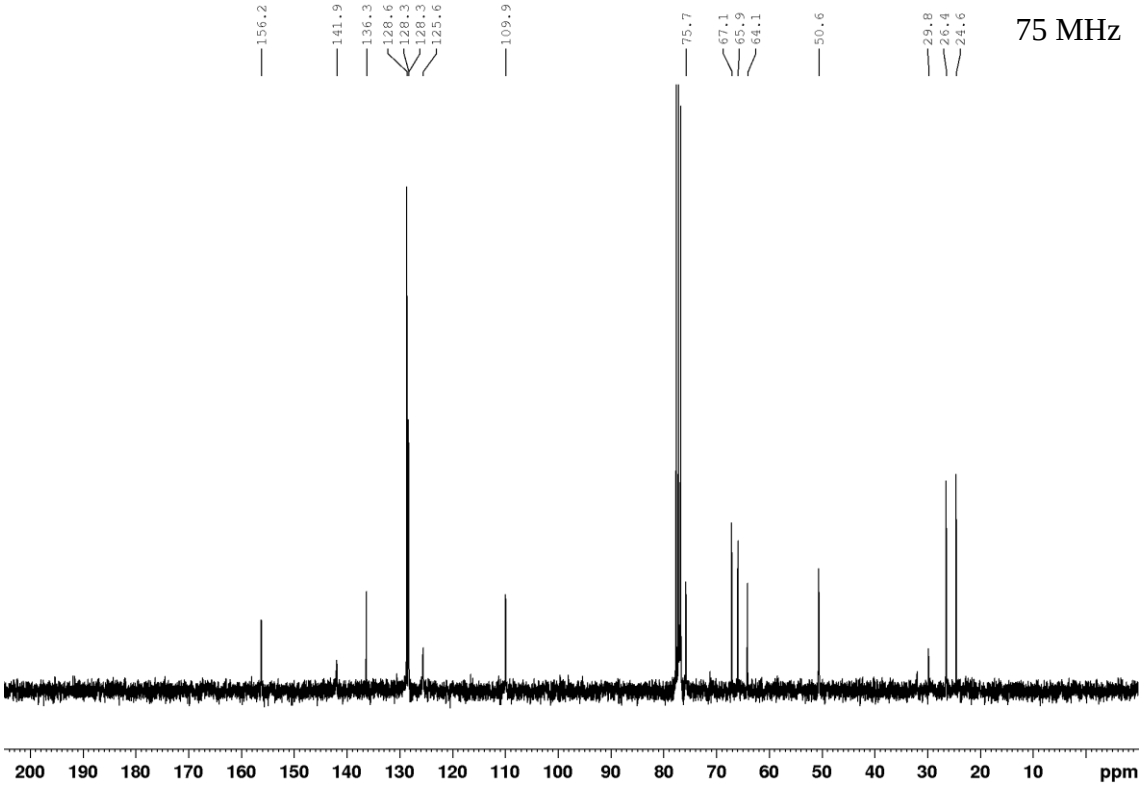


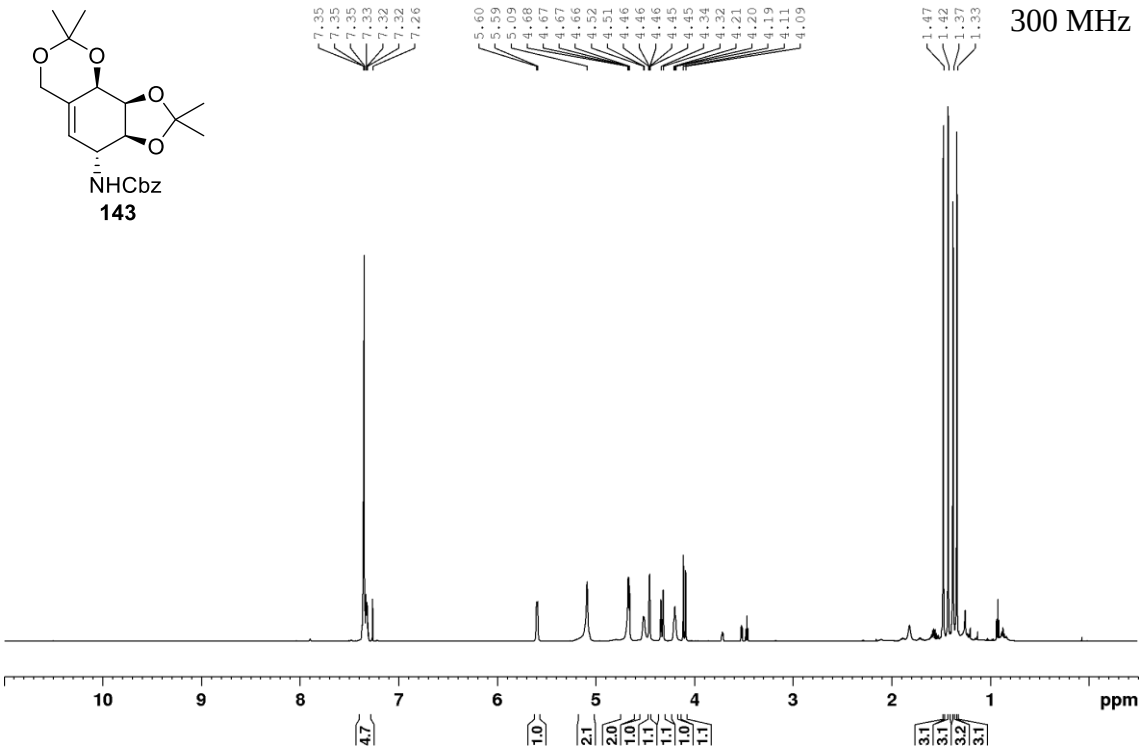


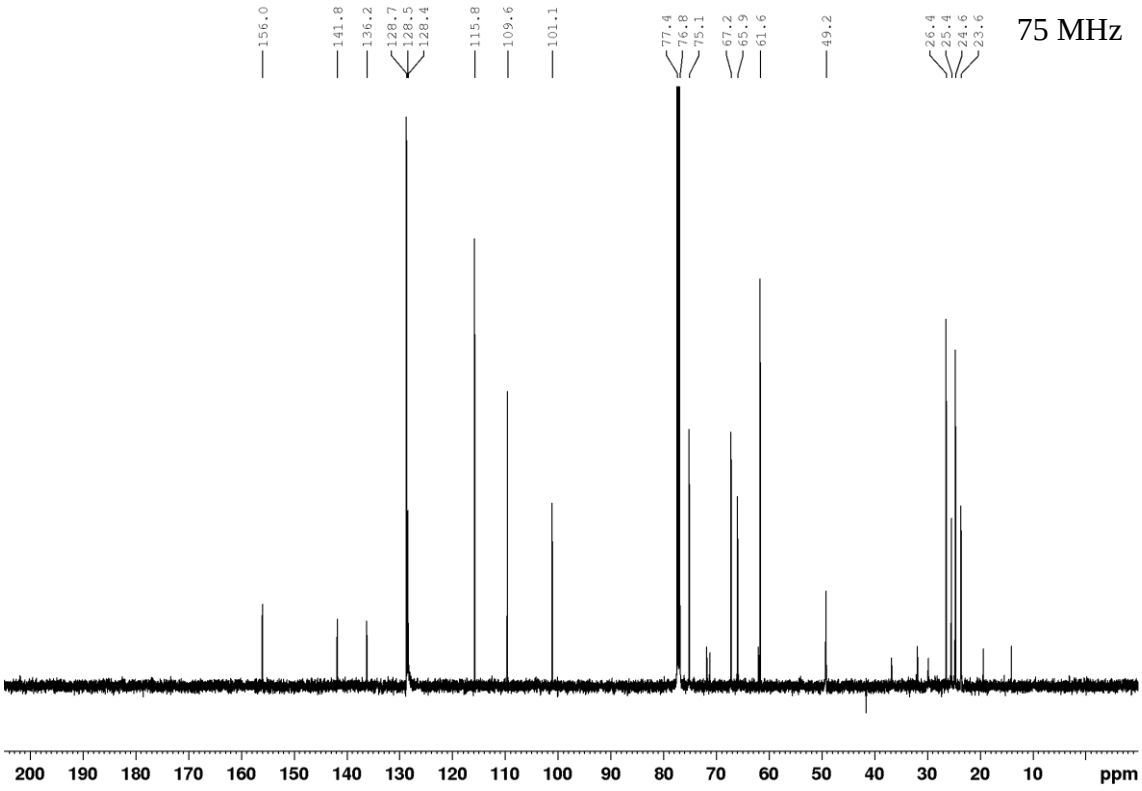
142

300 MHz

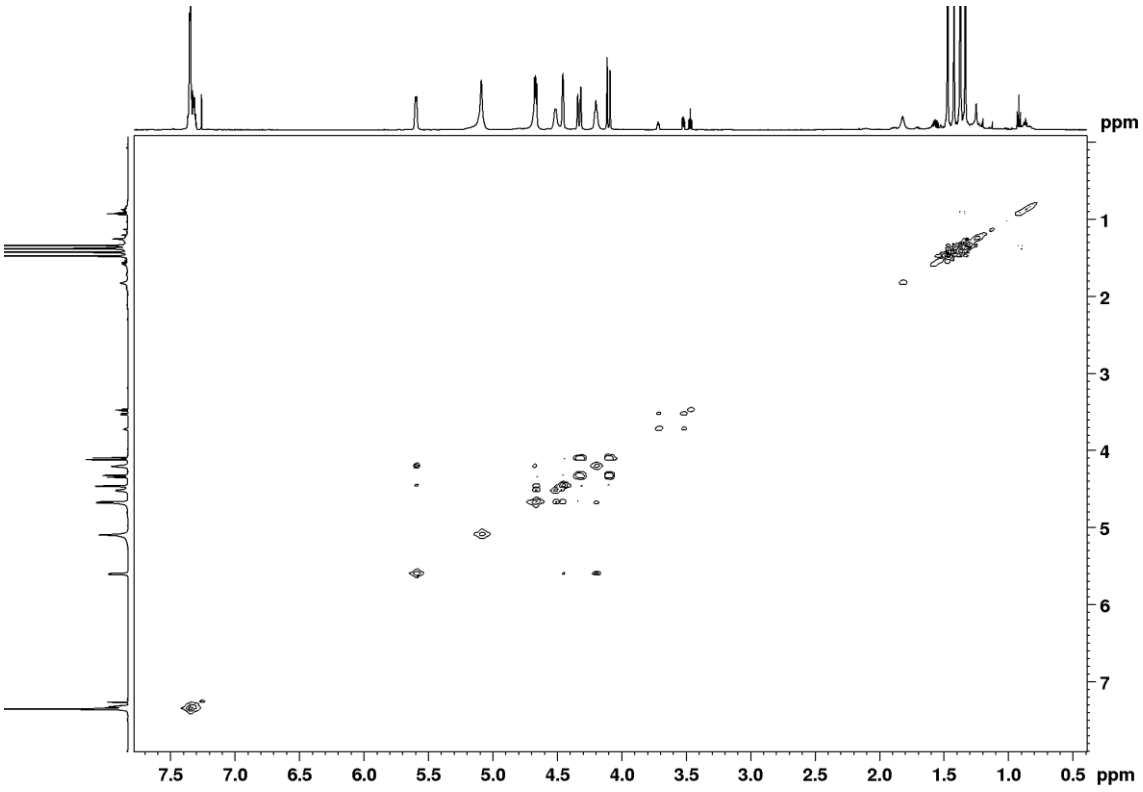
75 MHz



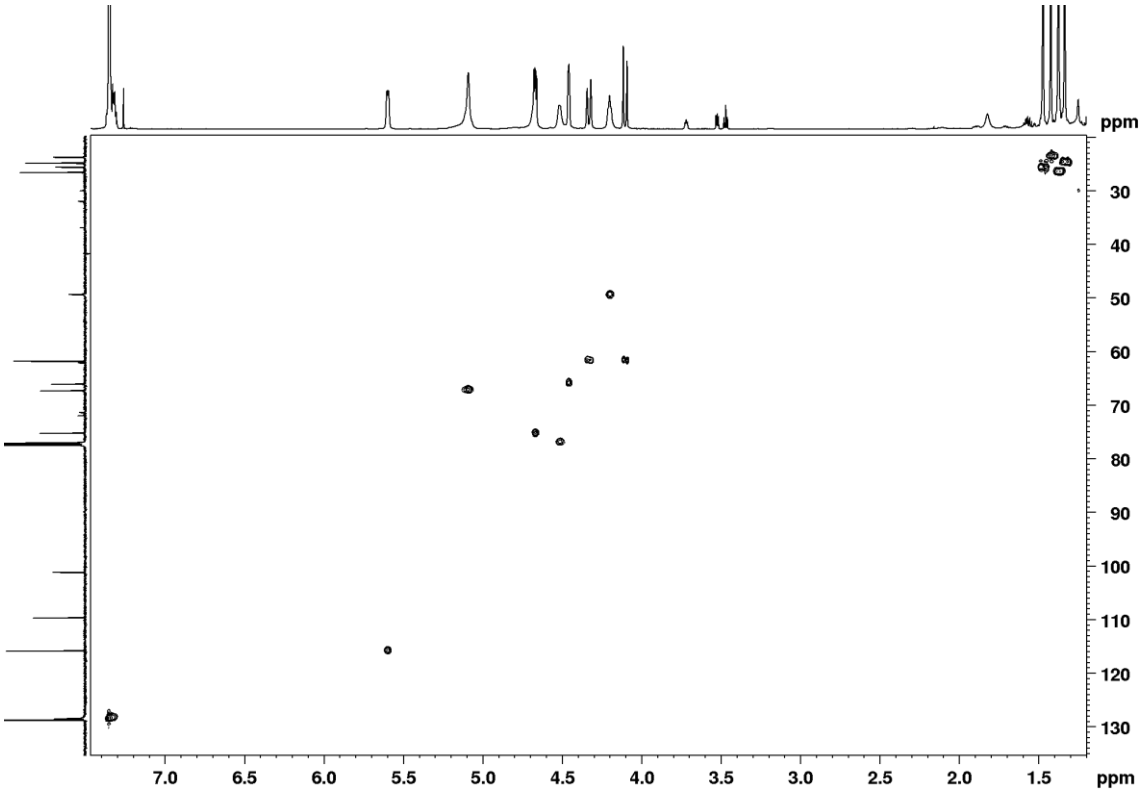

NHCbz
143
300 MHz
7.35
7.35
7.35
7.33
7.32
7.32
7.26
5.60
5.59
5.09
4.68
4.67
4.67
4.66
4.52
4.51
4.46
4.46
4.46
4.45
4.45
4.34
4.32
4.21
4.20
4.19
4.11
4.09
1.47
1.42
1.37
1.33
10
9
8
7
6
5
4
3
2
1
ppm
4.7
1.0
2.1
2.0
1.0
1.1
1.1
1.0
1.1
3.1
3.1
3.2
3.1


75 MHz
156.0
141.8
136.2
128.7
128.5
128.4
115.8
109.6
101.1
77.4
76.8
75.1
67.2
65.9
61.6
49.2
26.4
25.4
24.6
23.6
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

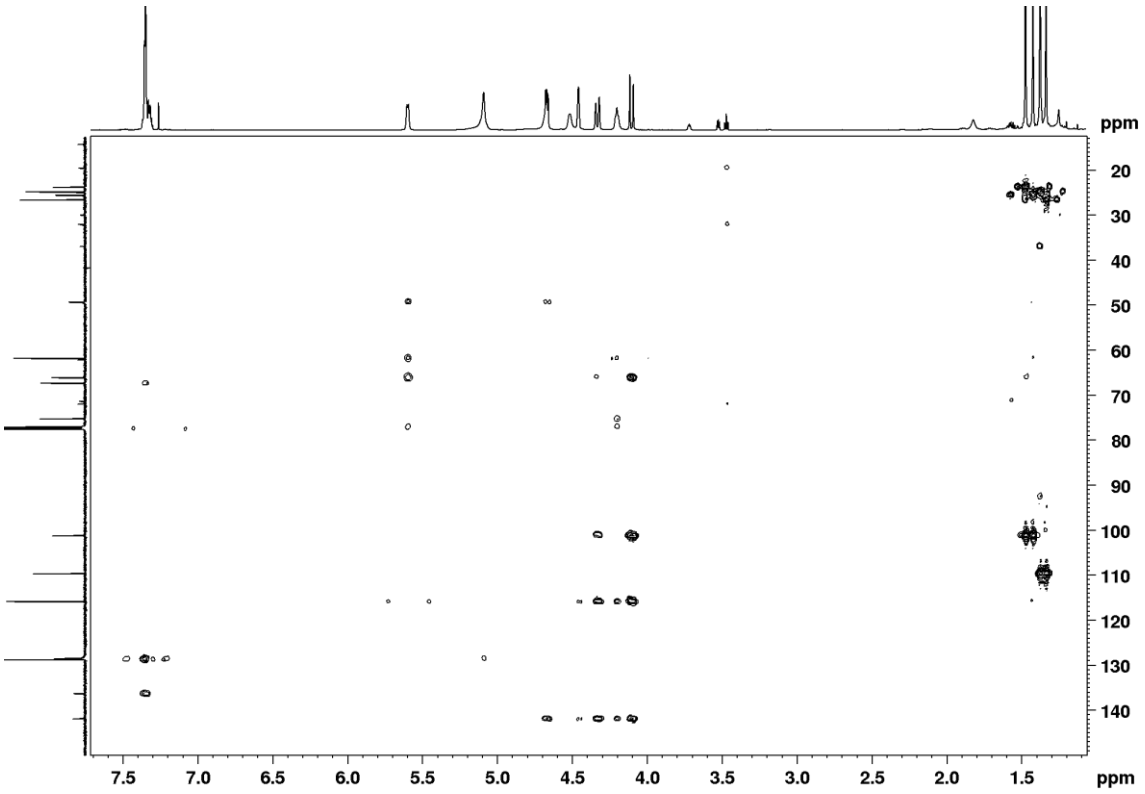
143: ^{1}H COSY



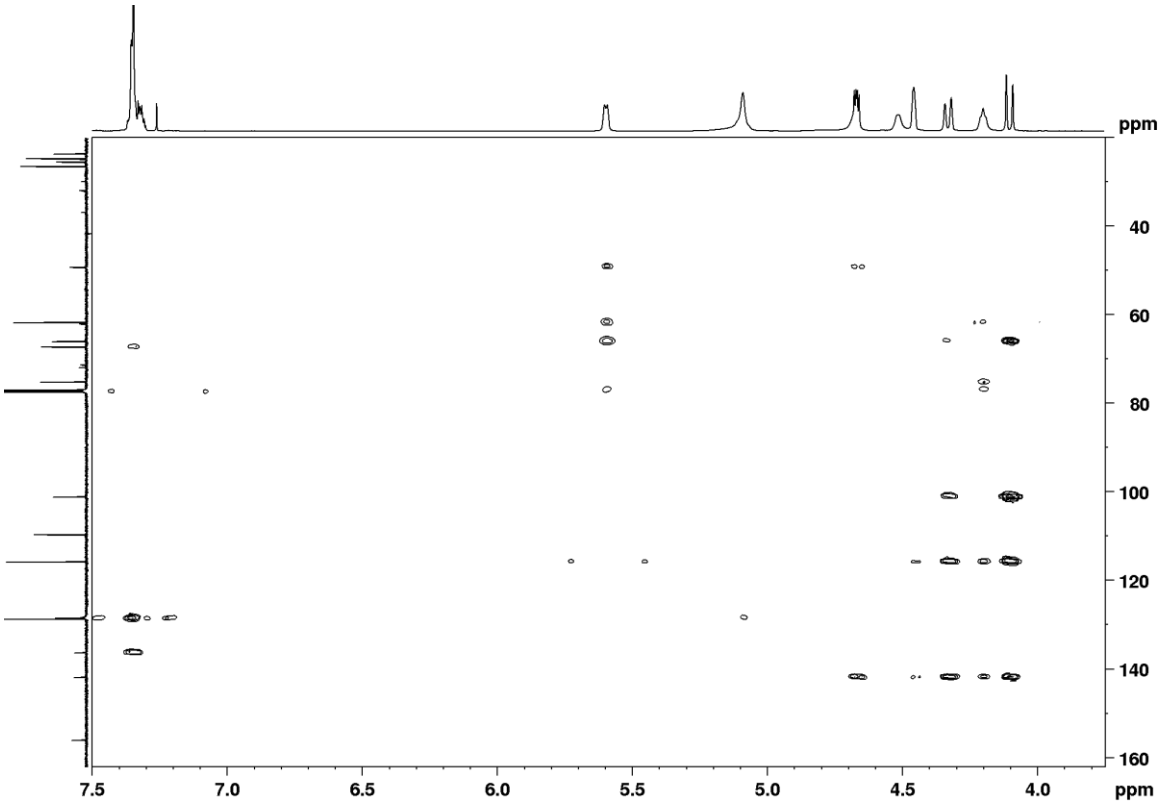
^{1}H-^{13}C HSQC



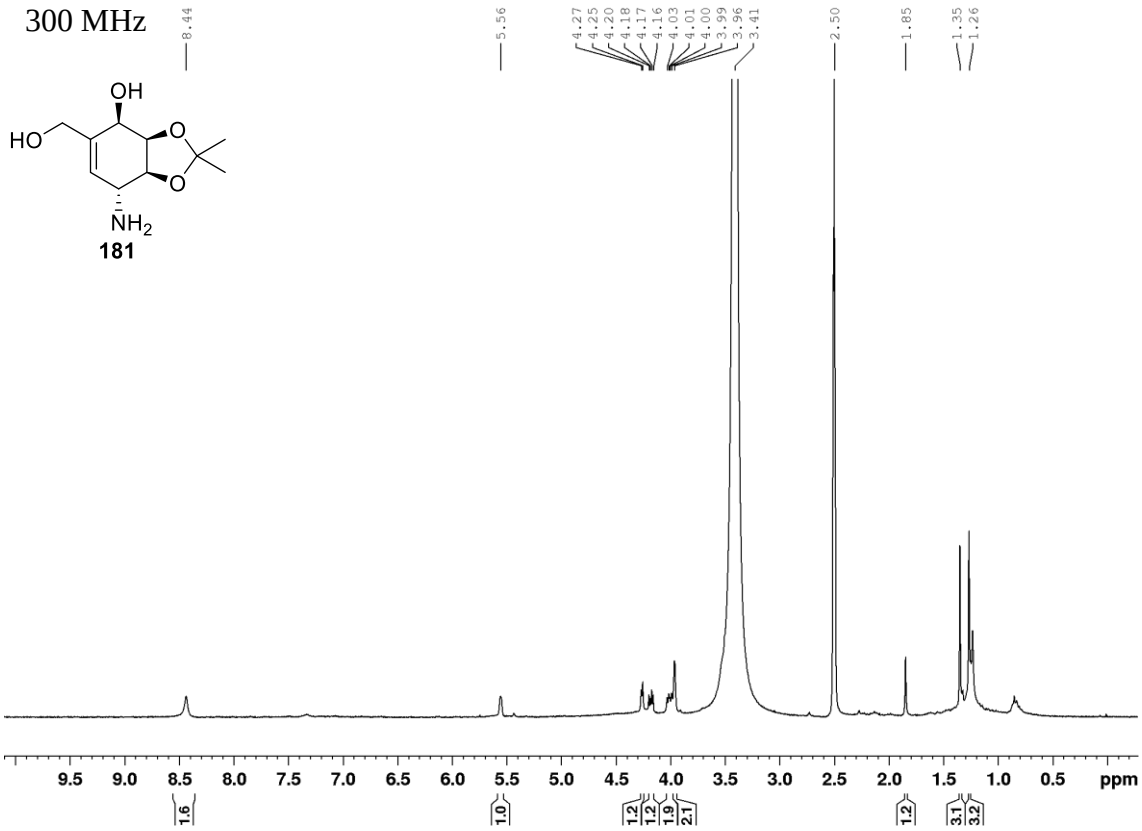
143: ^{1}H-^{13}C HMBC

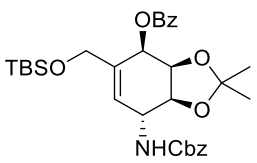


^{1}H-^{13}C HMBC (expansion)



300 MHz

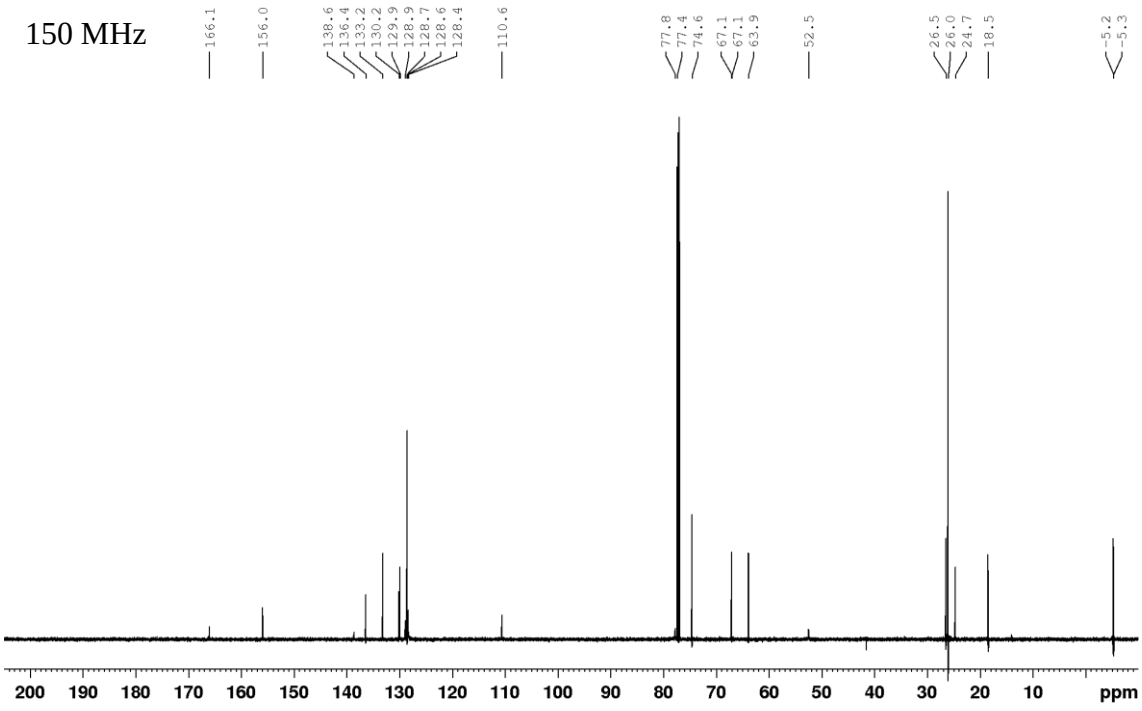

8.44
5.56
4.27
4.25
4.20
4.18
4.17
4.16
4.03
4.01
4.00
3.99
3.96
3.41
2.50
1.85
1.35
1.26
OH
HO
O
O
NH2
181
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.6
1.0
1.2
1.2
1.9
2.1
1.2
3.1
3.2



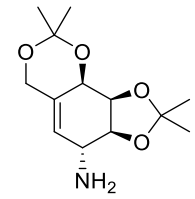
132

600 MHz

150 MHz

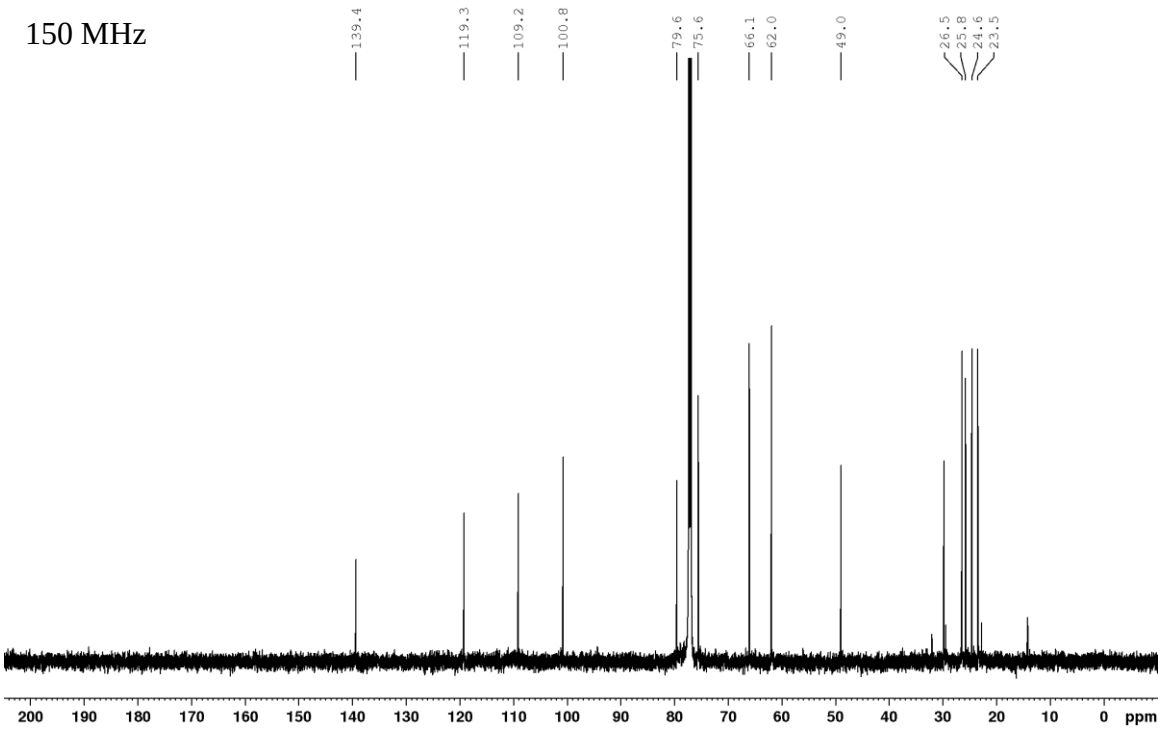


600 MHz

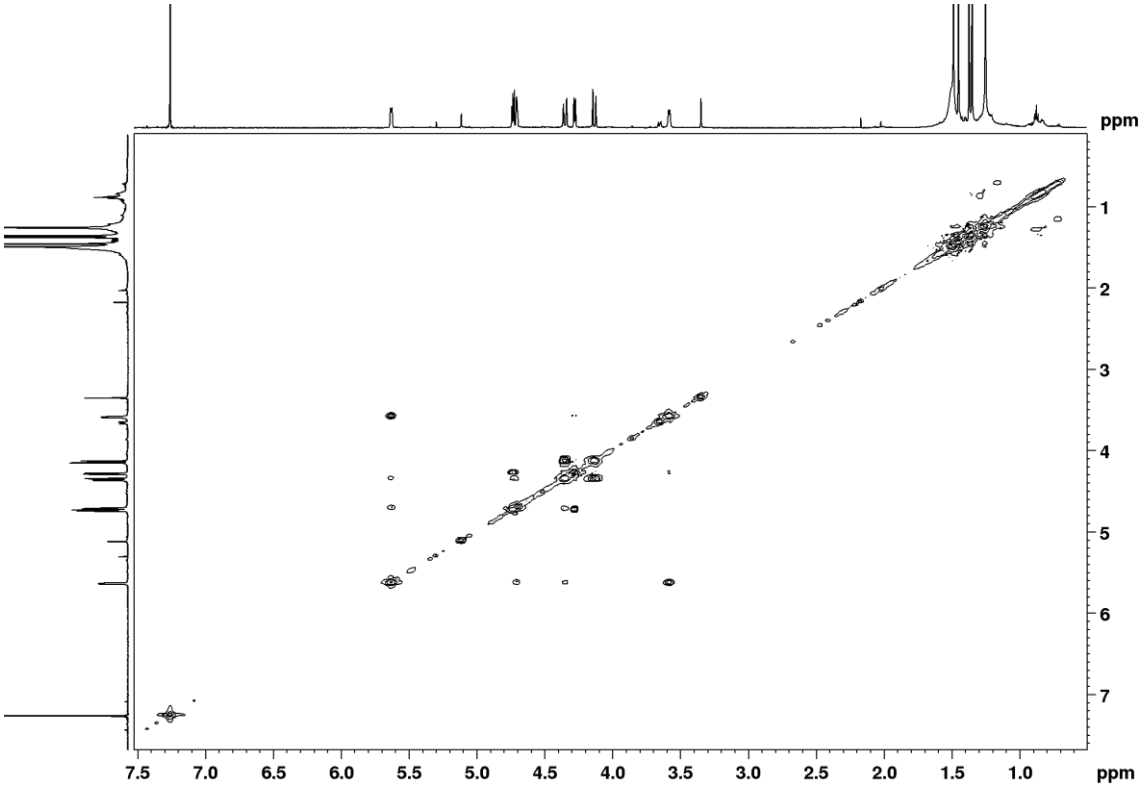


144

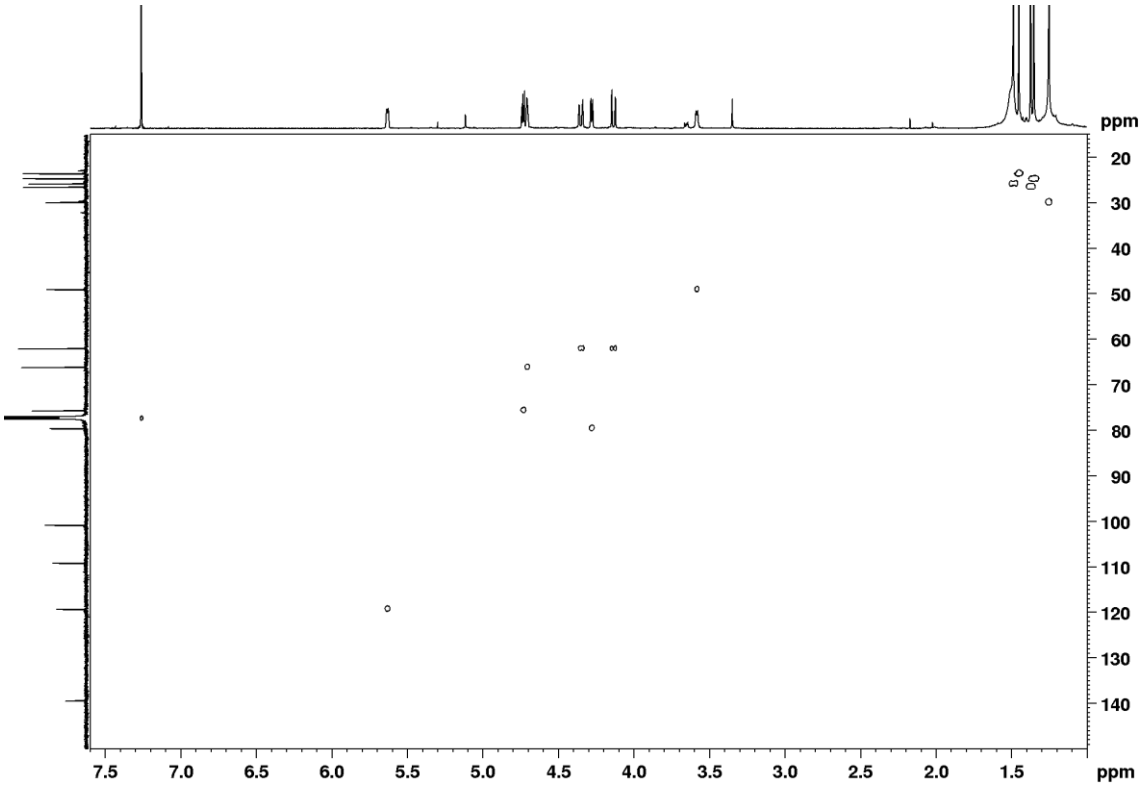
150 MHz



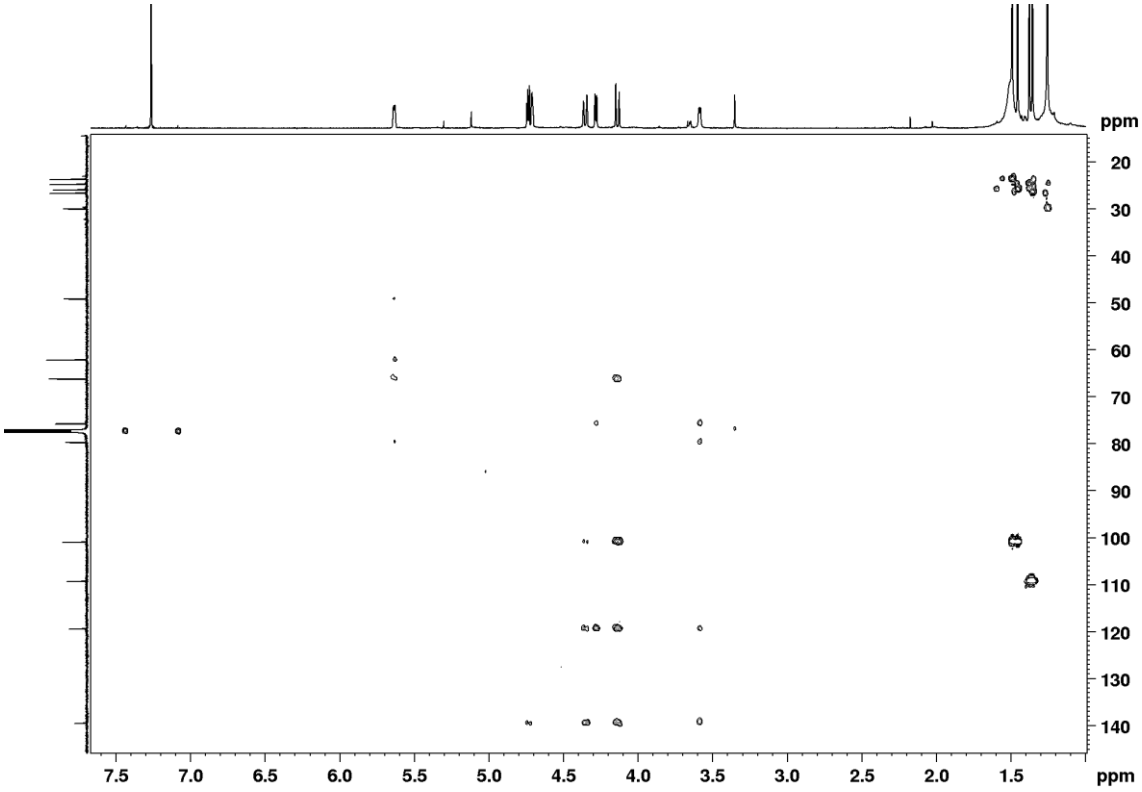
144: ^{1}H COSY



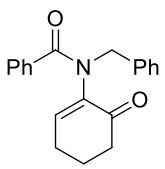
^{1}H-^{13}C HSQC



144: ^{1}H-^{13}C HMBC

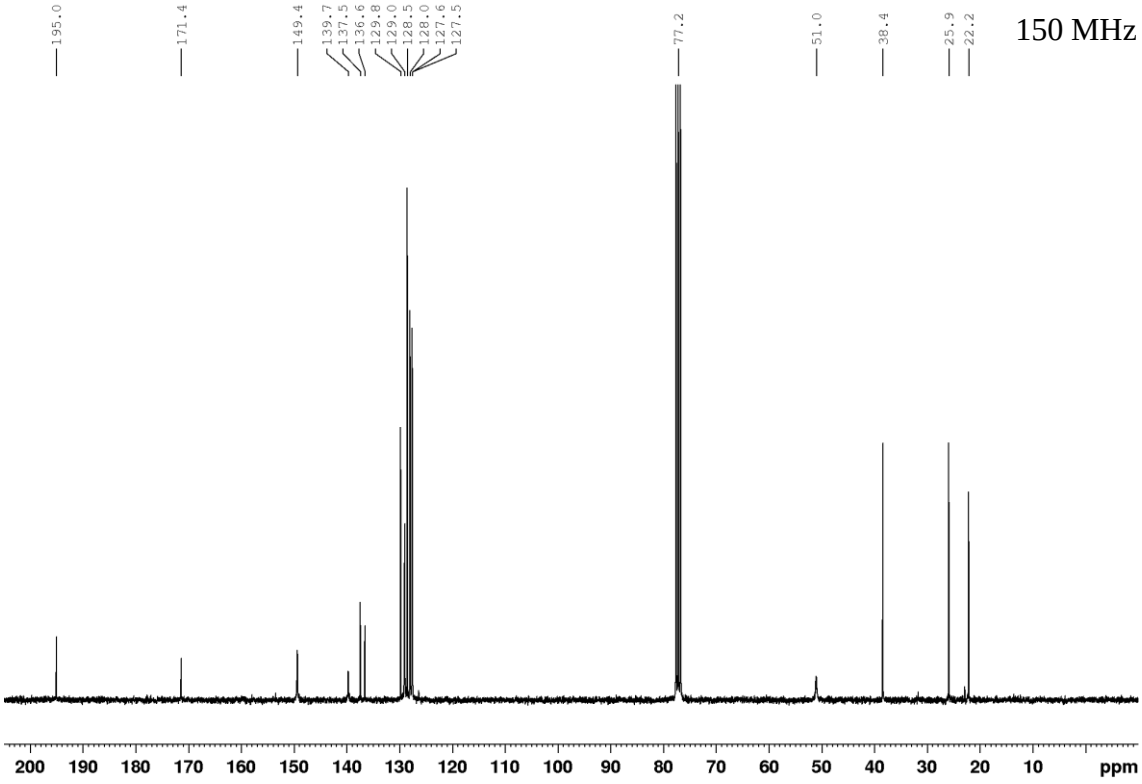


600 MHz

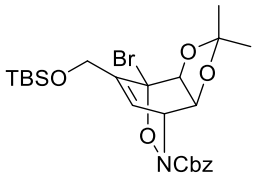


185

150 MHz

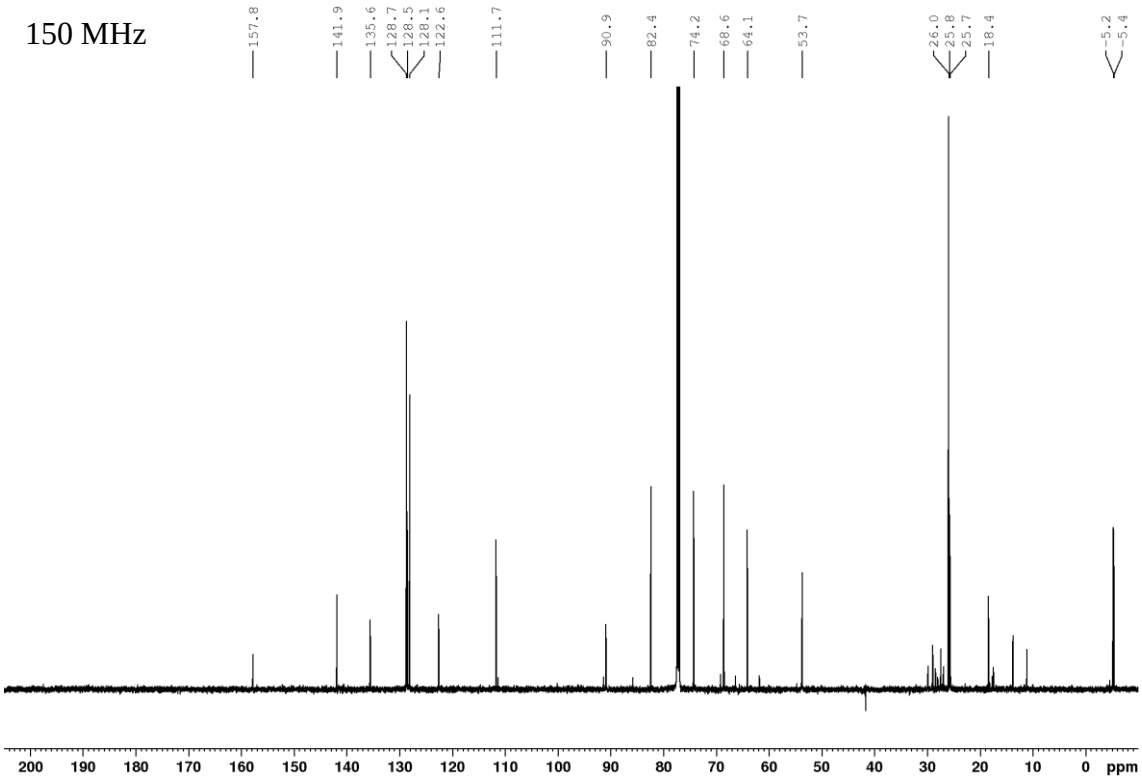


600 MHz

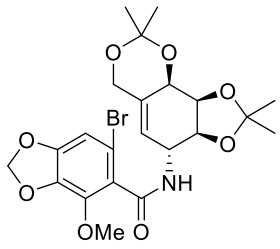


176

150 MHz

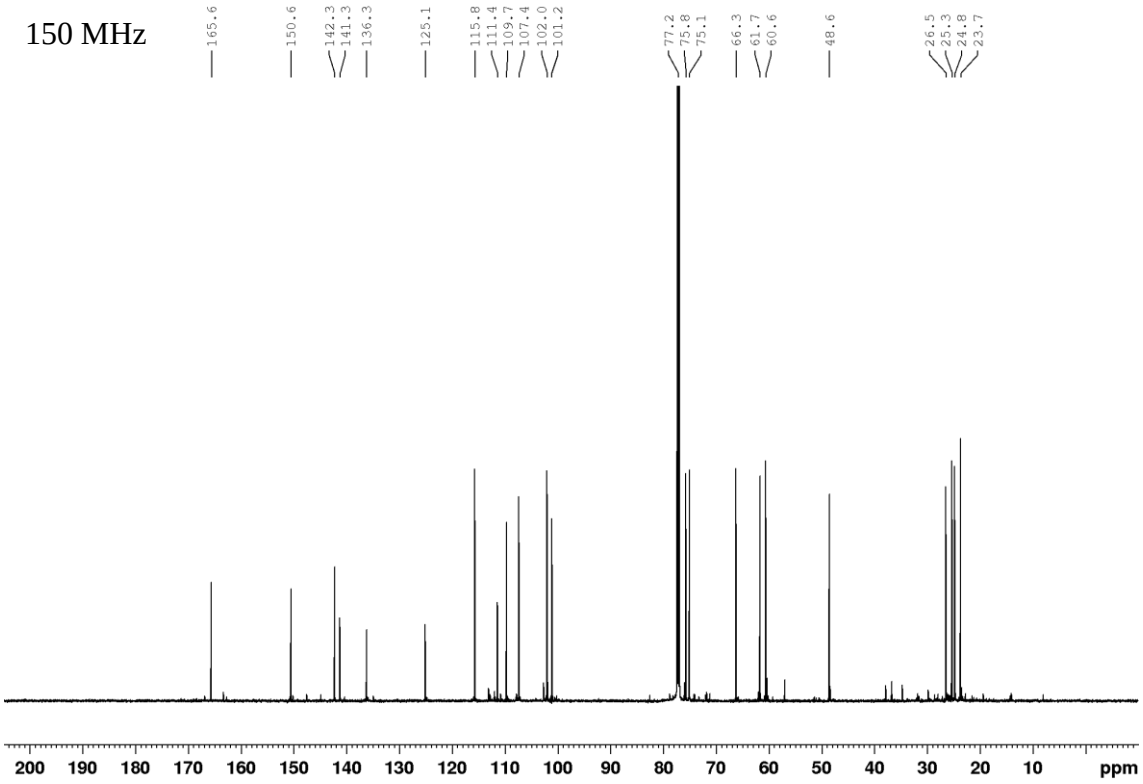


600 MHz

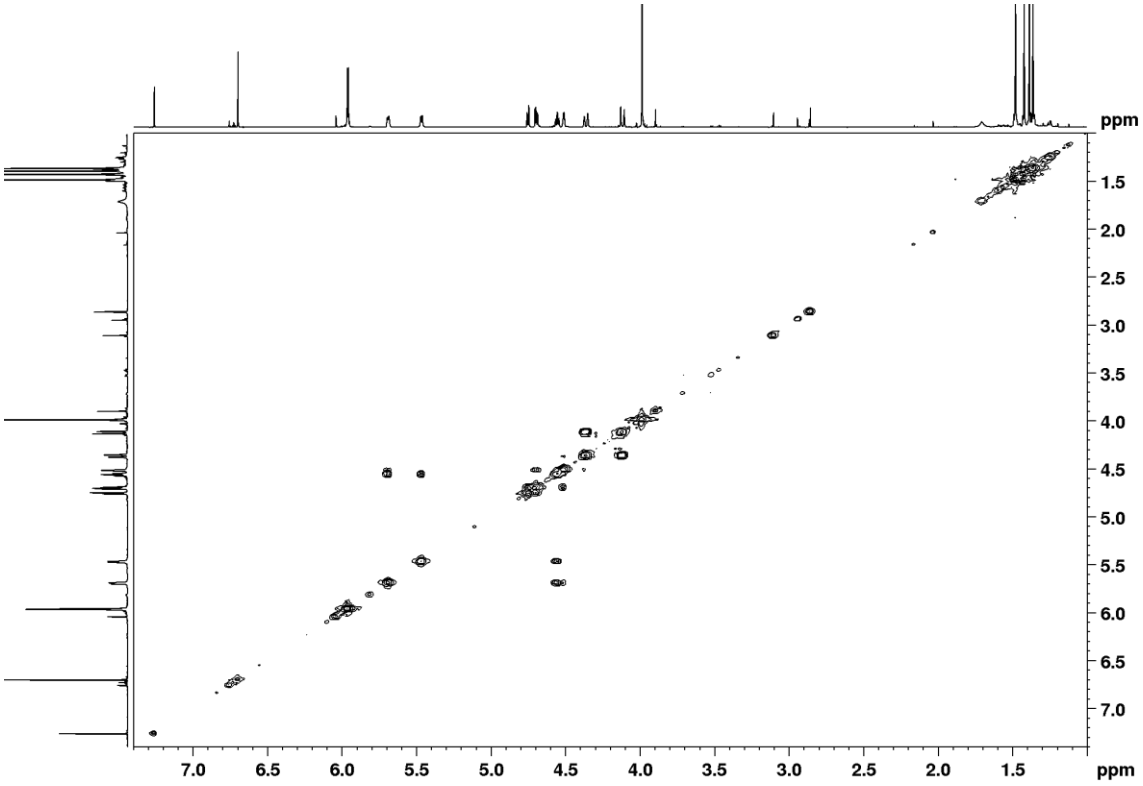


145

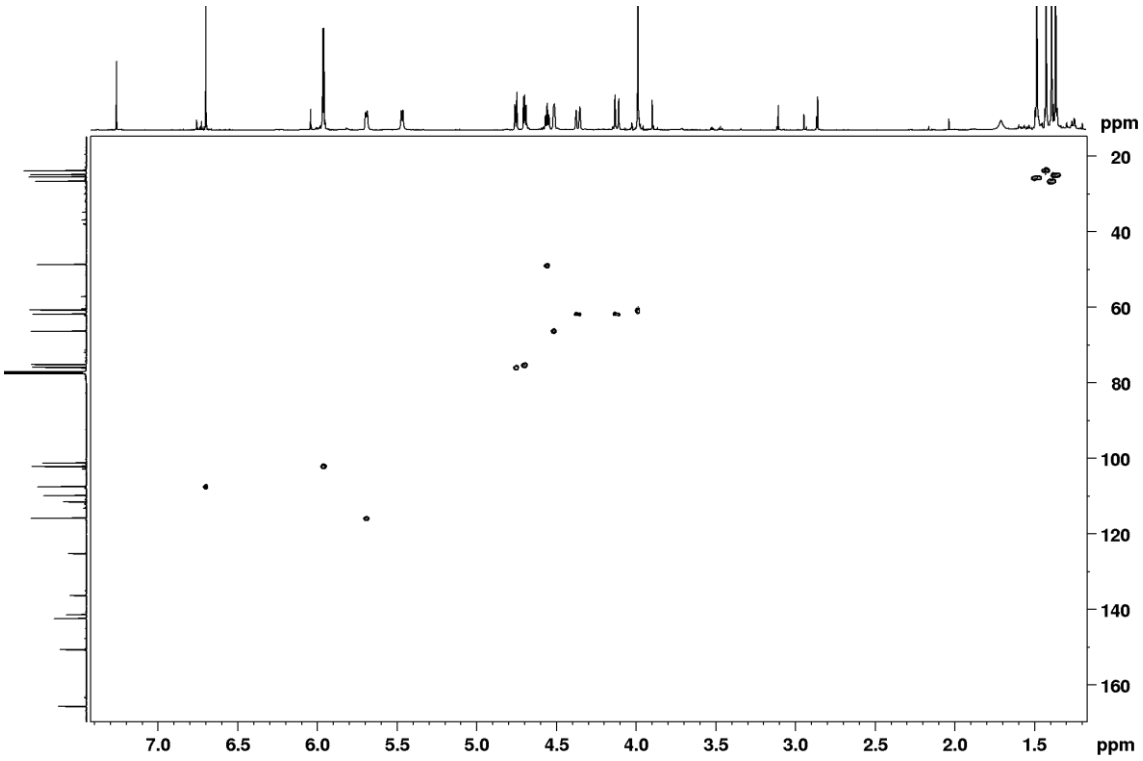
150 MHz



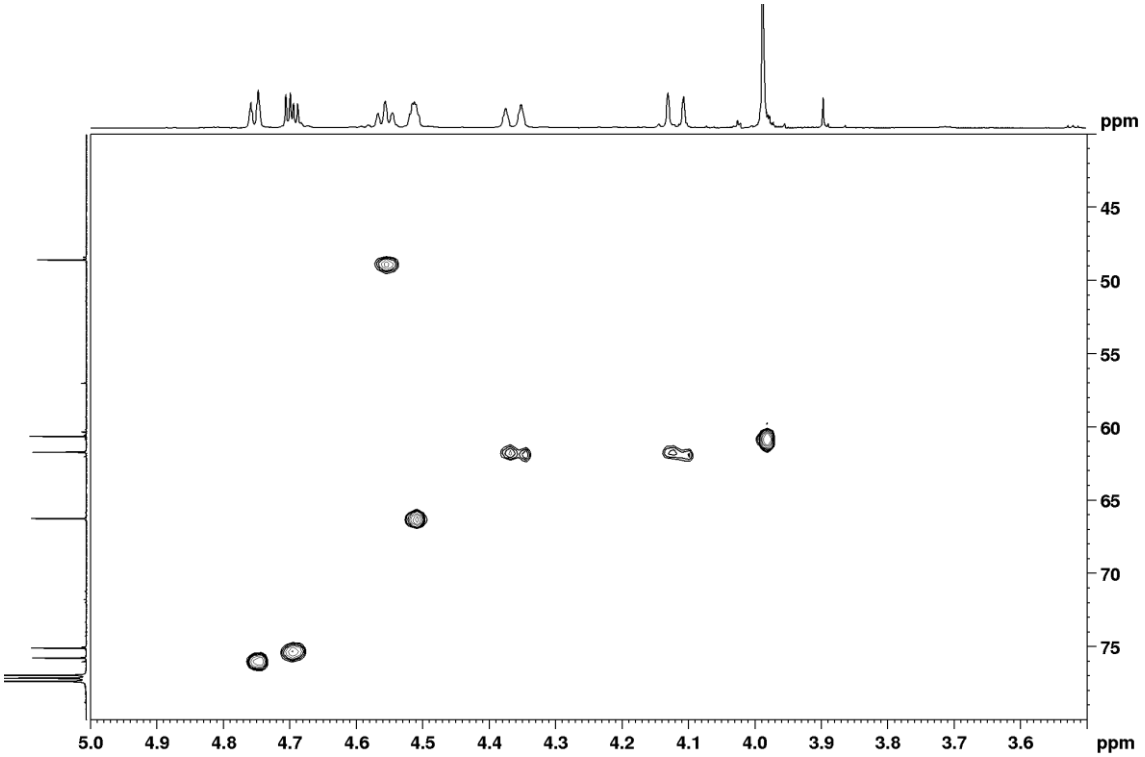
145: ^{1}H COSY



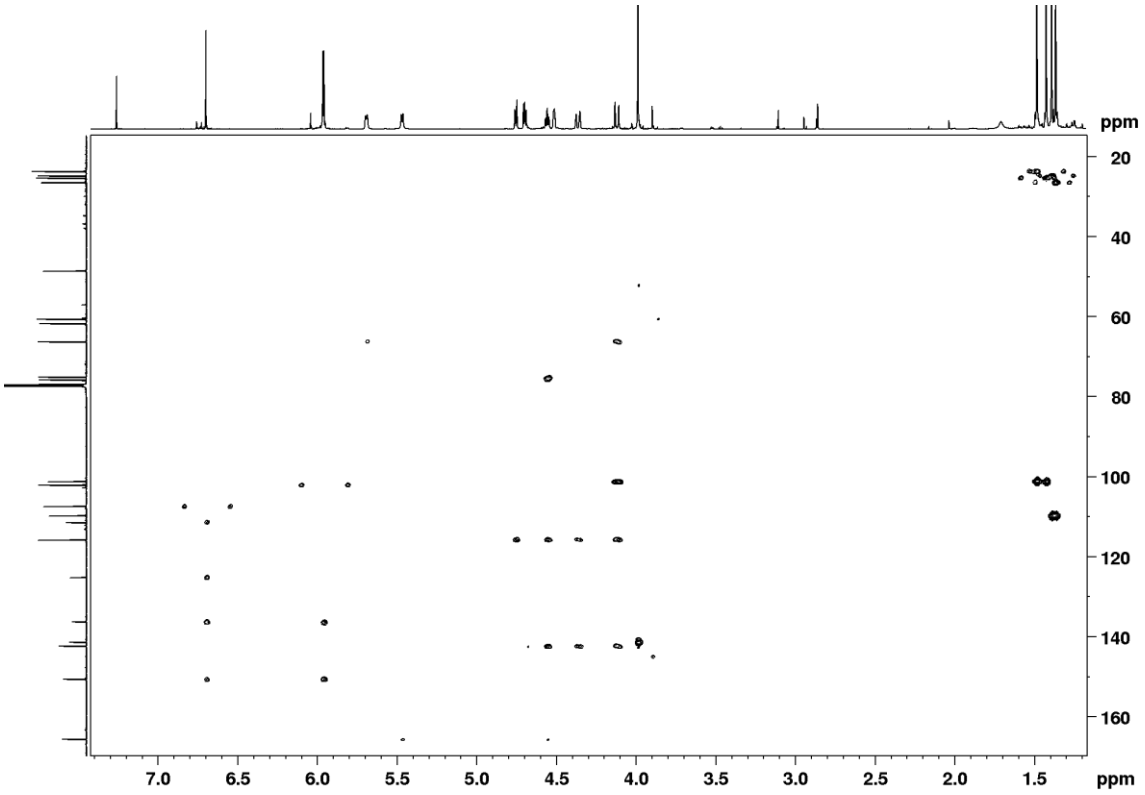
^{1}H-^{13}C HSQC



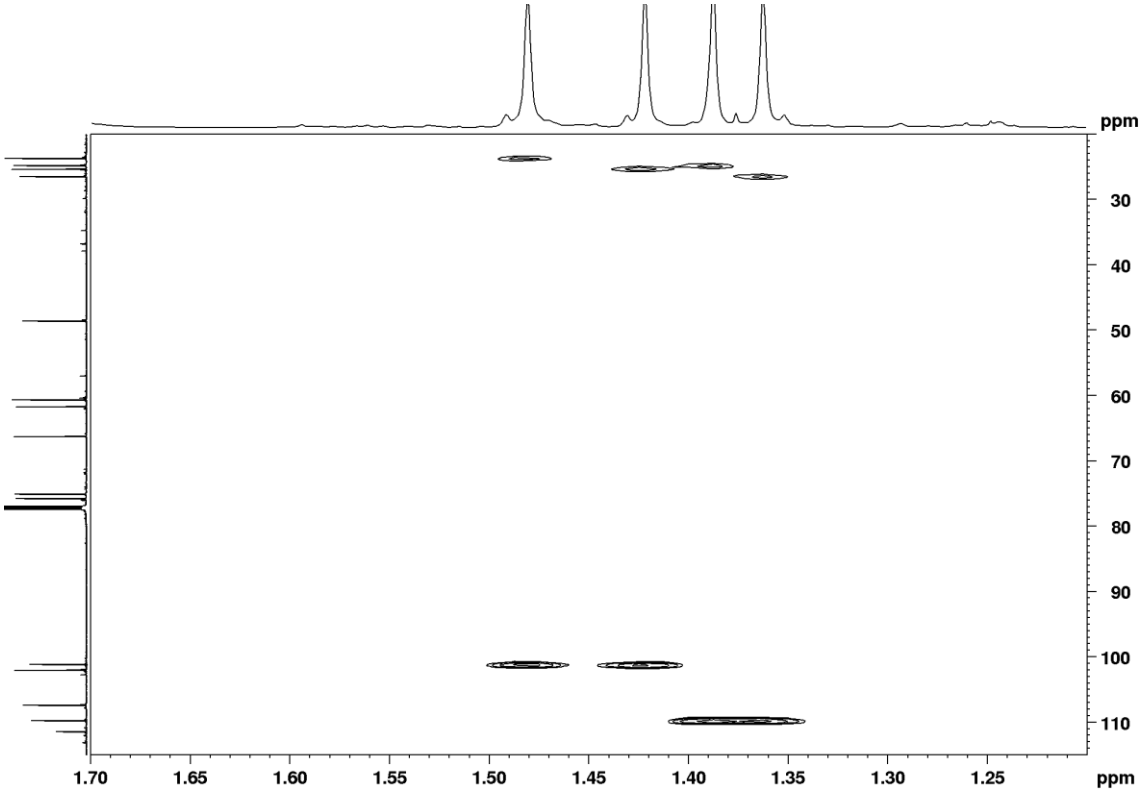
145: ^{1}H-^{13}C HSQC (expansion)



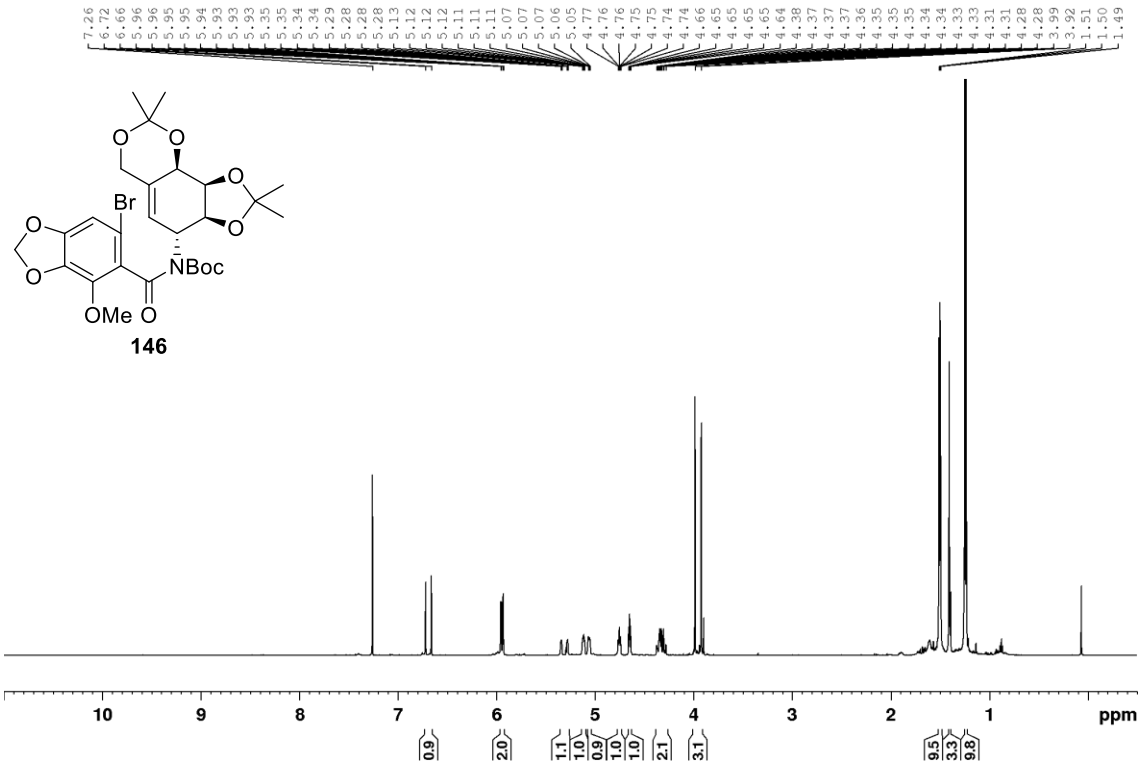
^{1}H-^{13}C HMBC



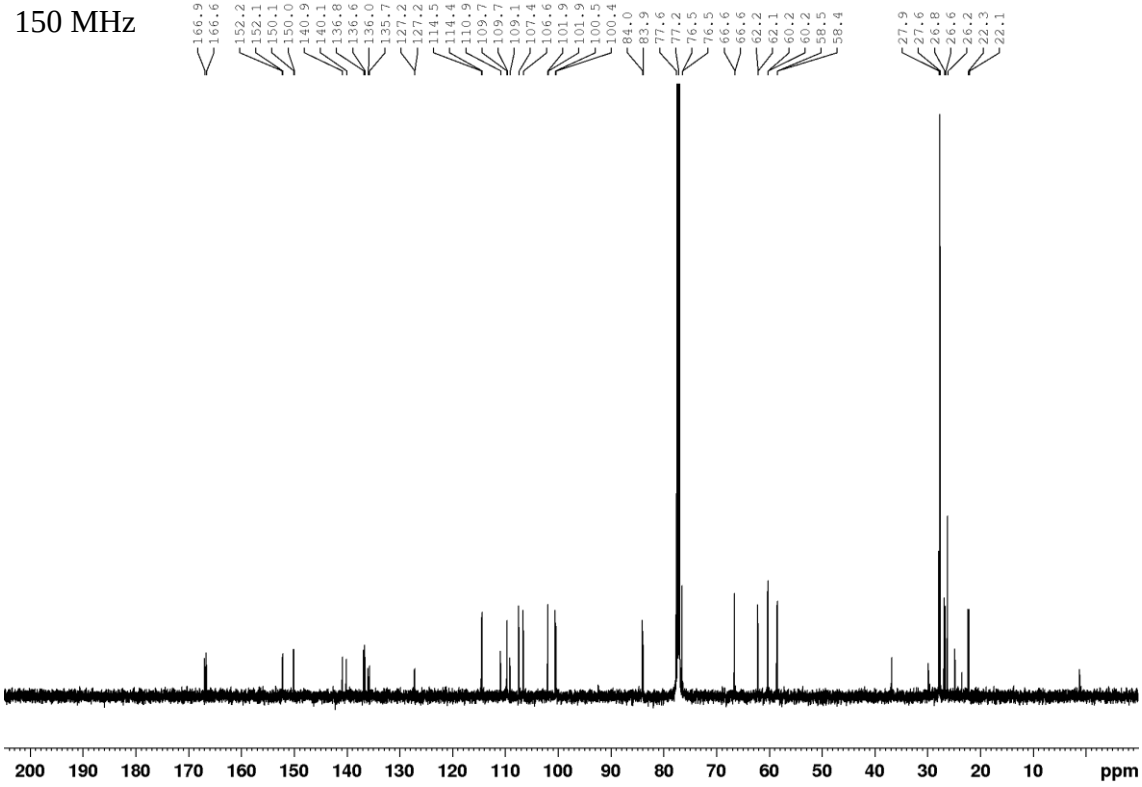
145: ^{1}H-^{13}C HMBC (expansion)



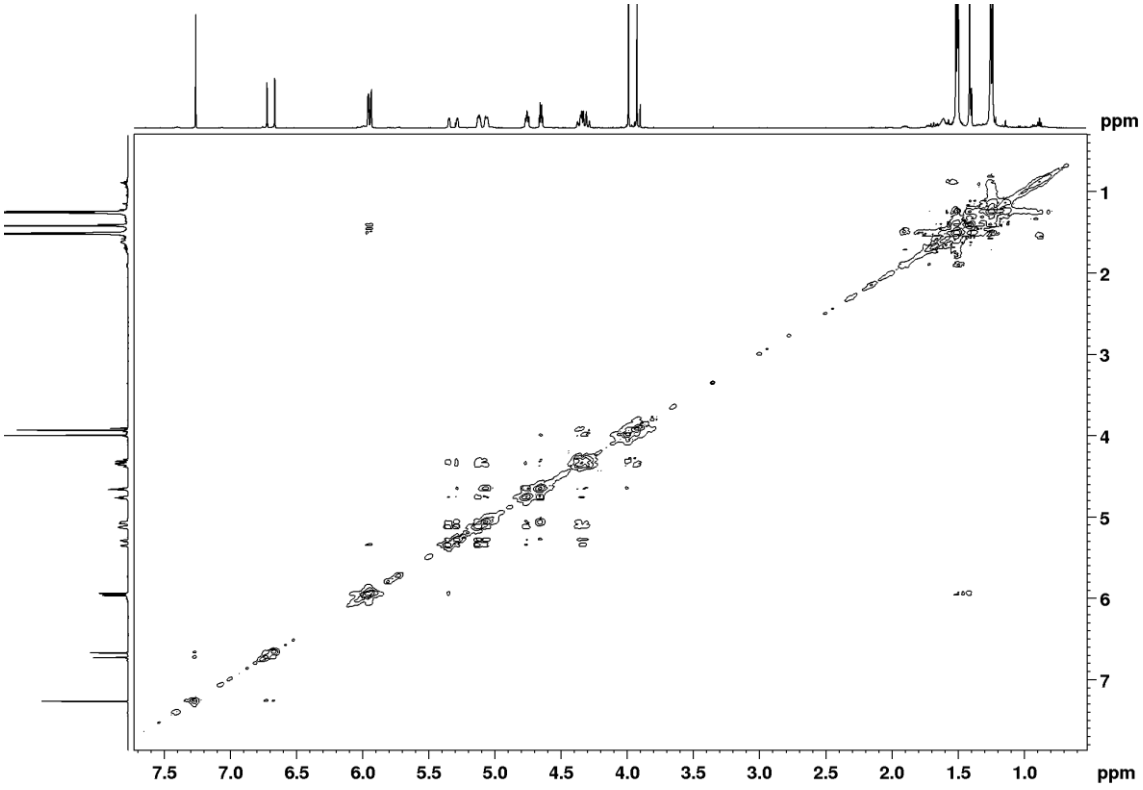
600 MHz



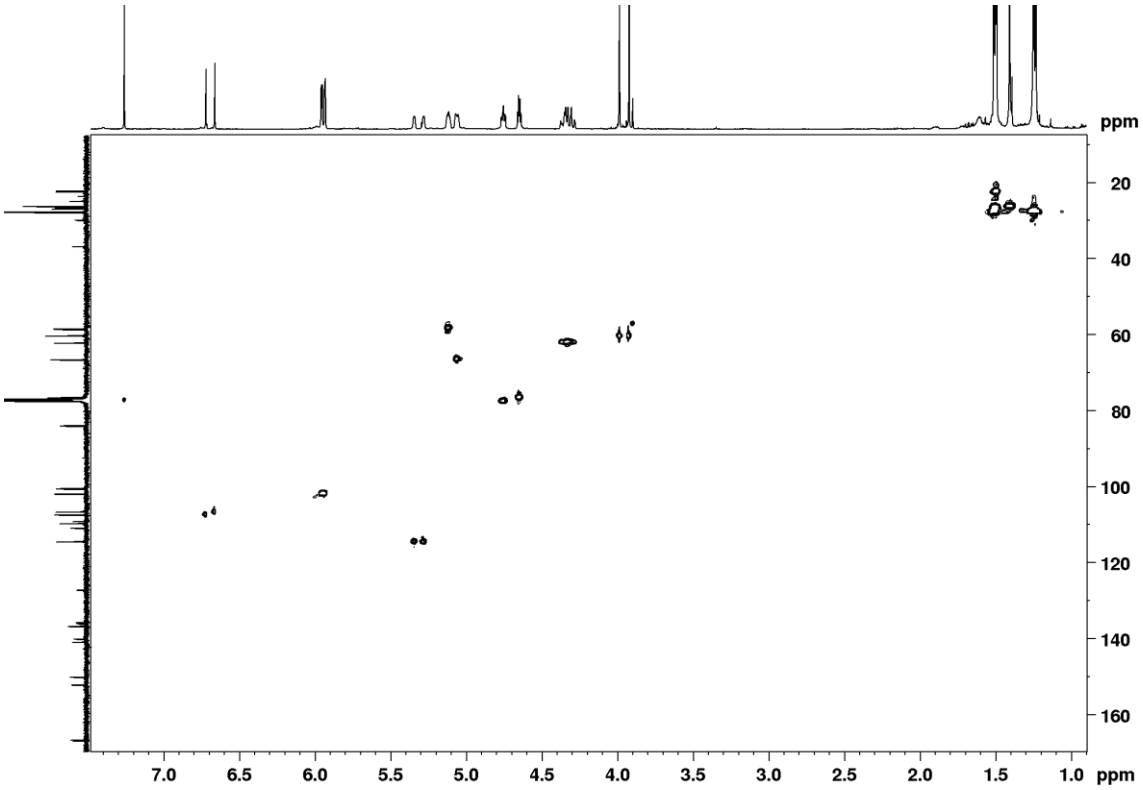
150 MHz



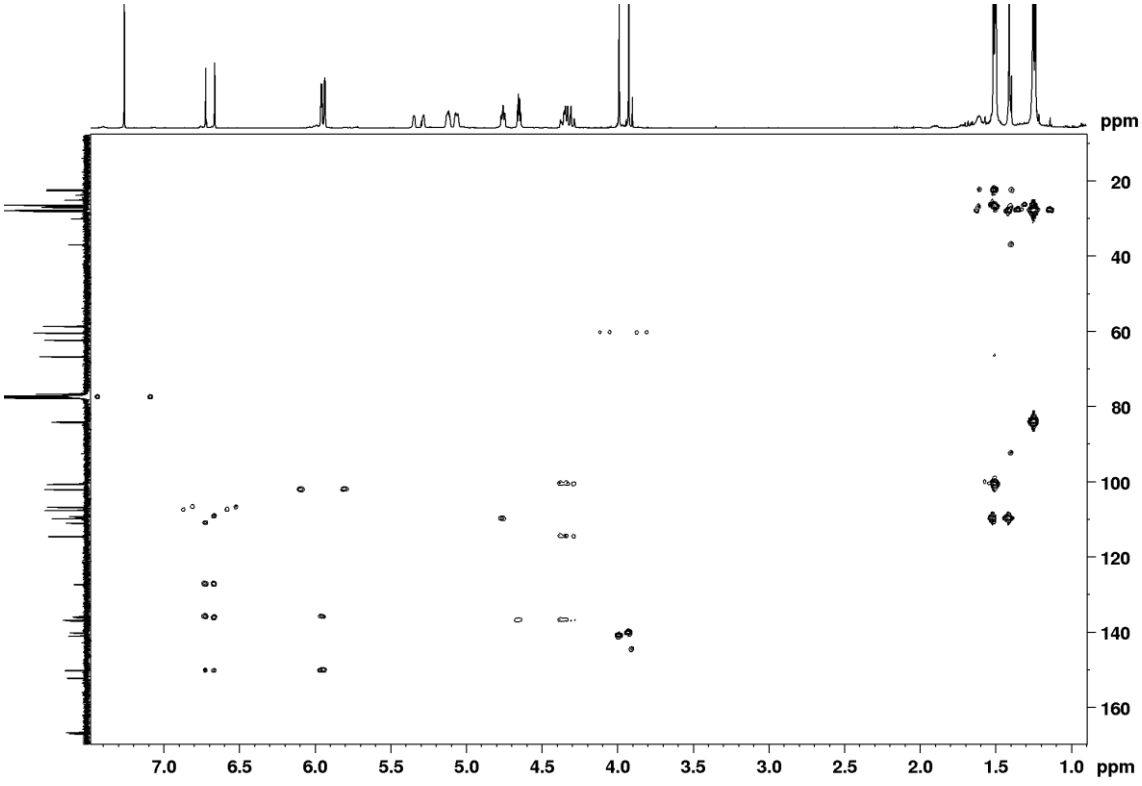
146: ^{1}H COSY



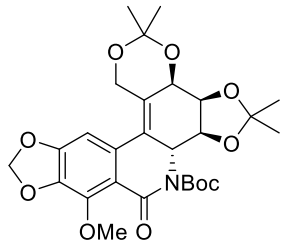
^{1}H-^{13}C HSQC



146: ^{1}H-^{13}C HMBC

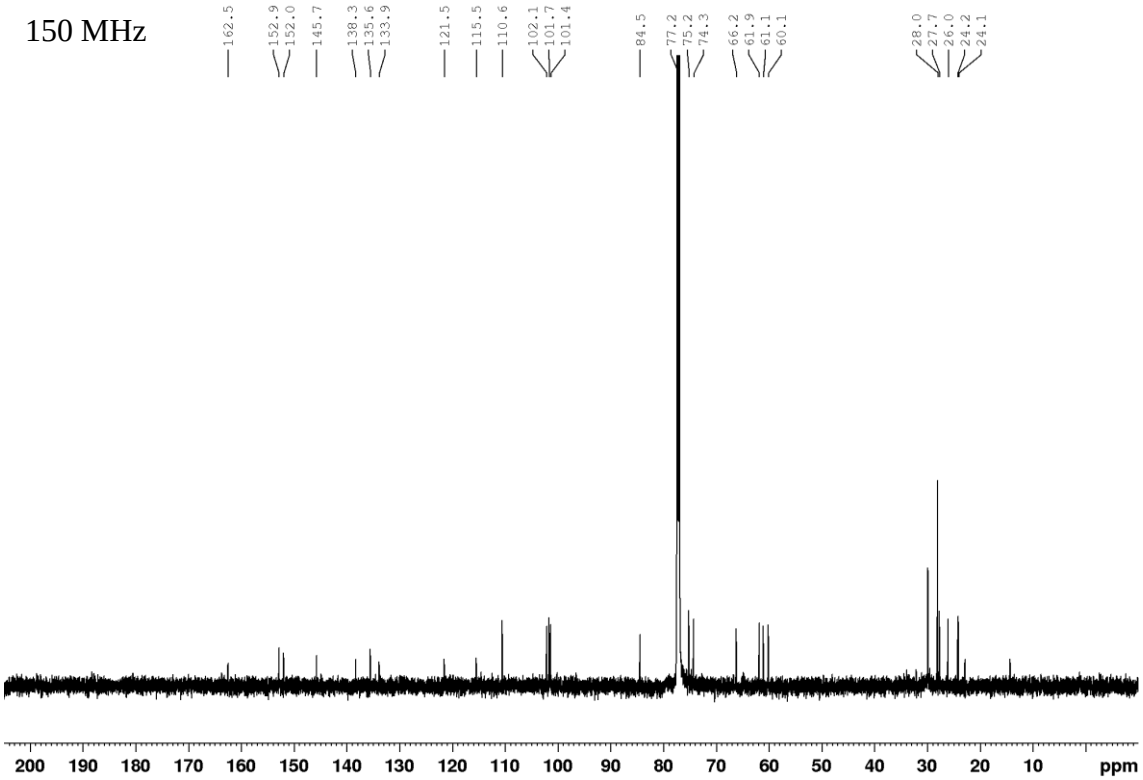


600 MHz

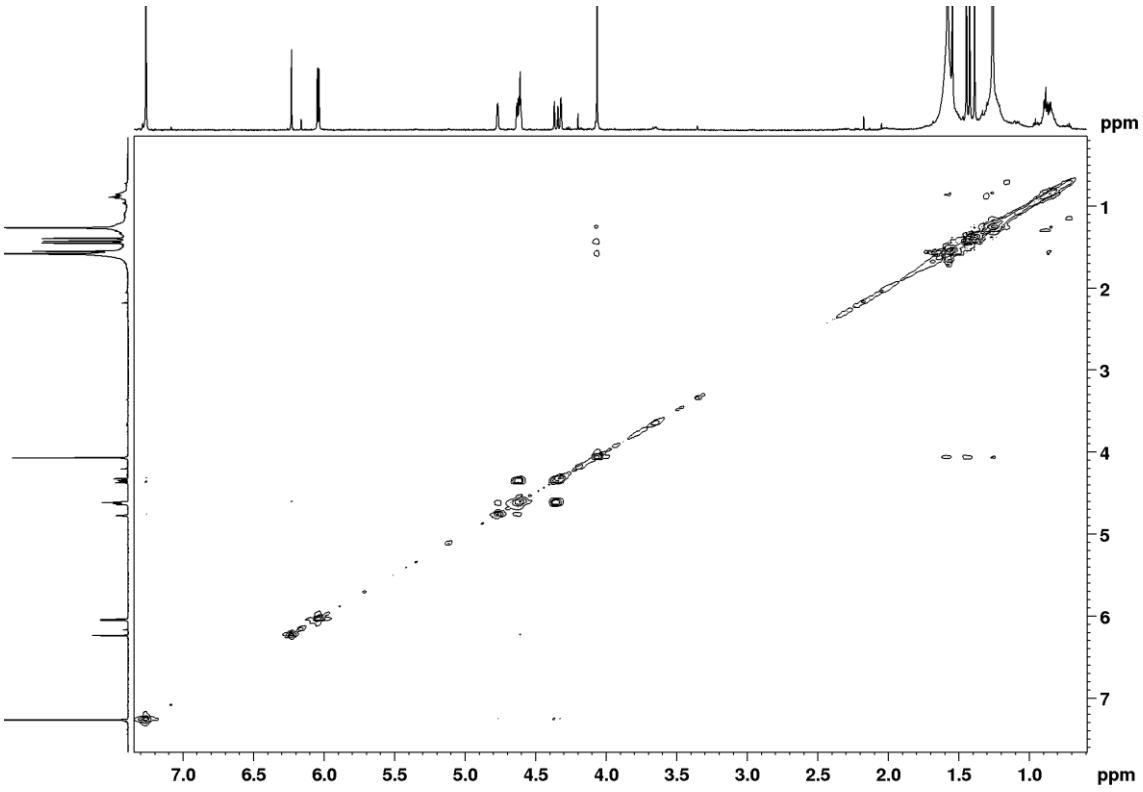


150

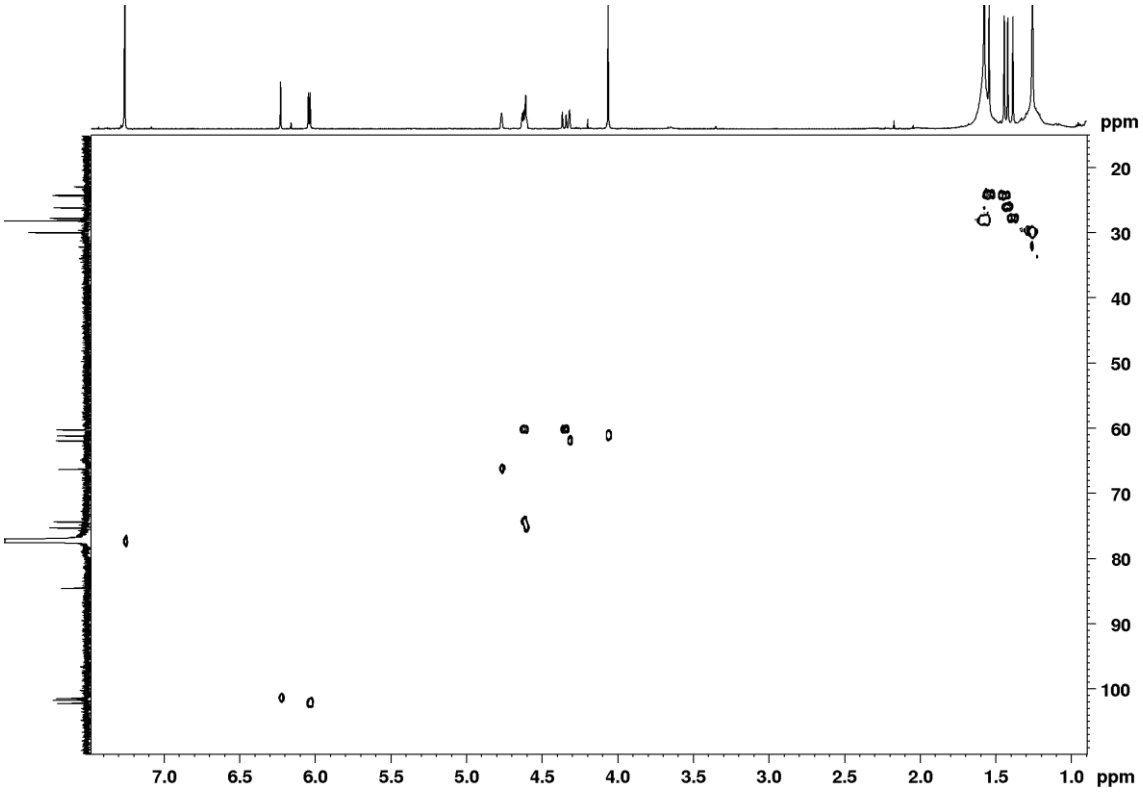
150 MHz



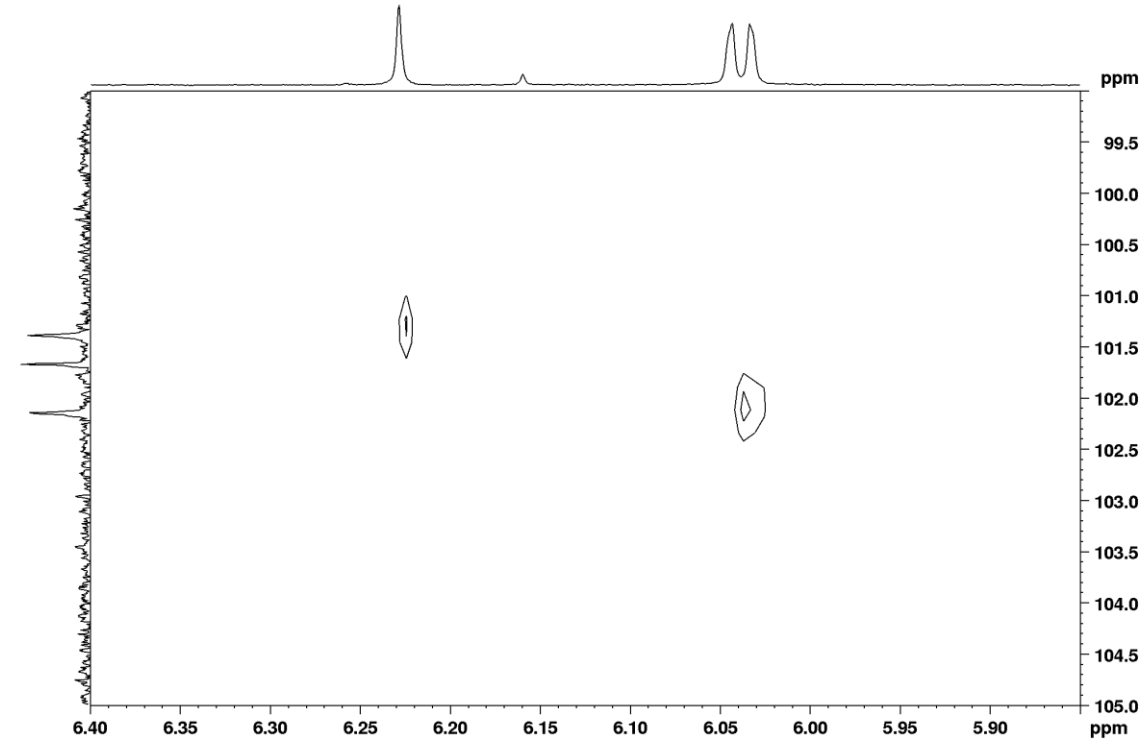
150: ^{1}H COSY

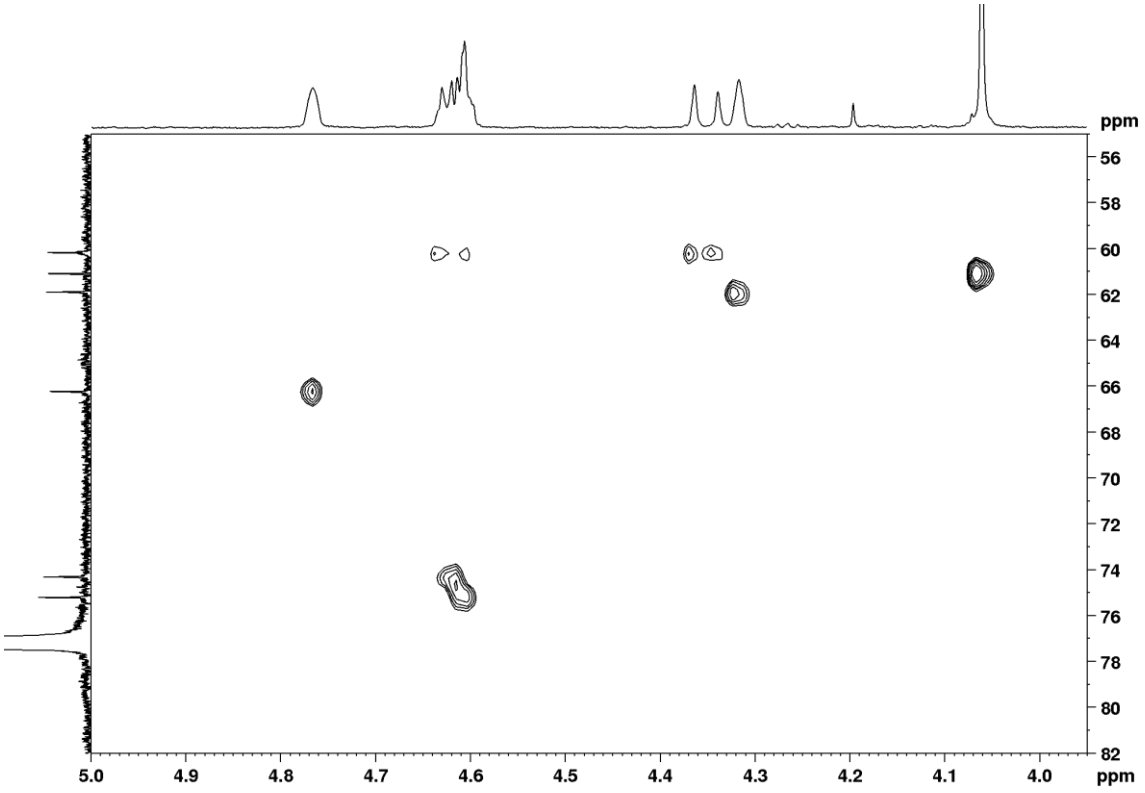


^{1}H-^{13}C HSQC

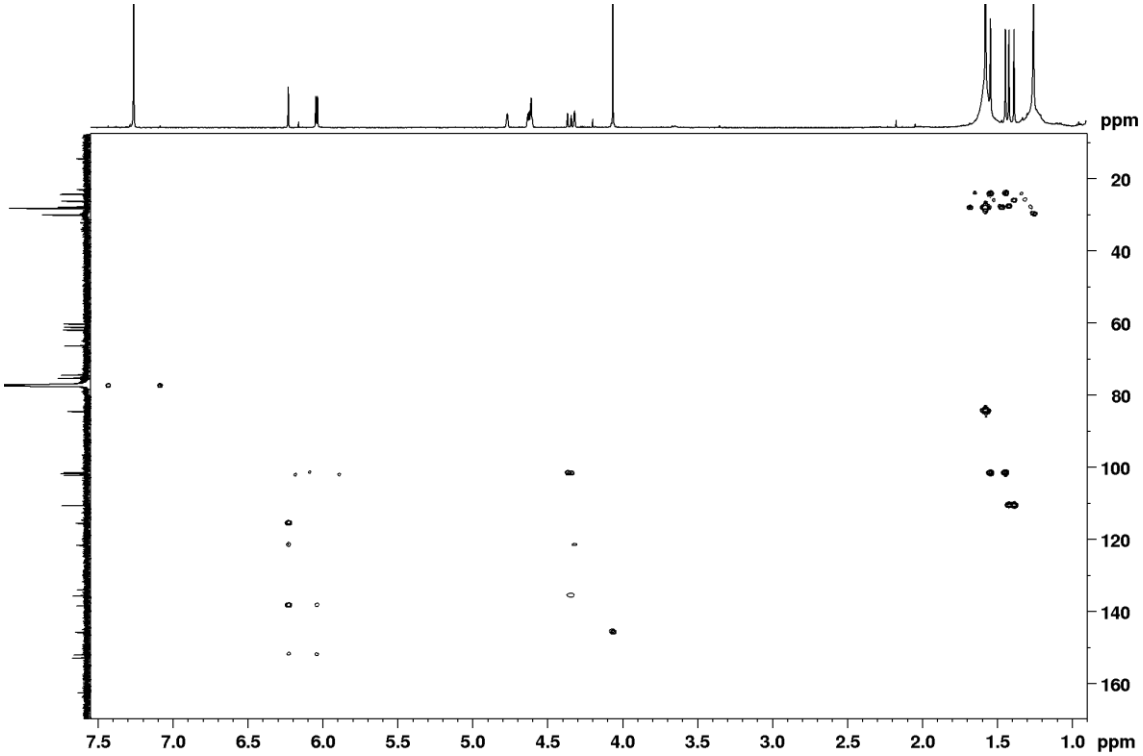


150: ^{1}H-^{13}C HSQC (expansions)

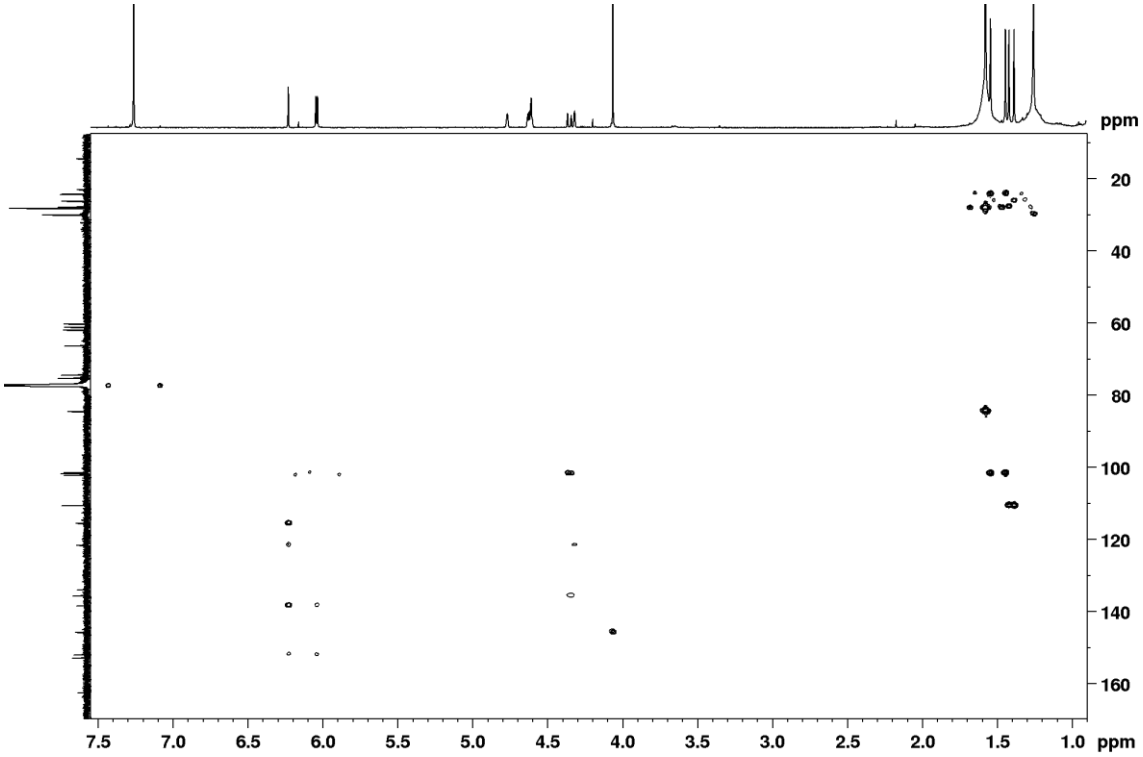




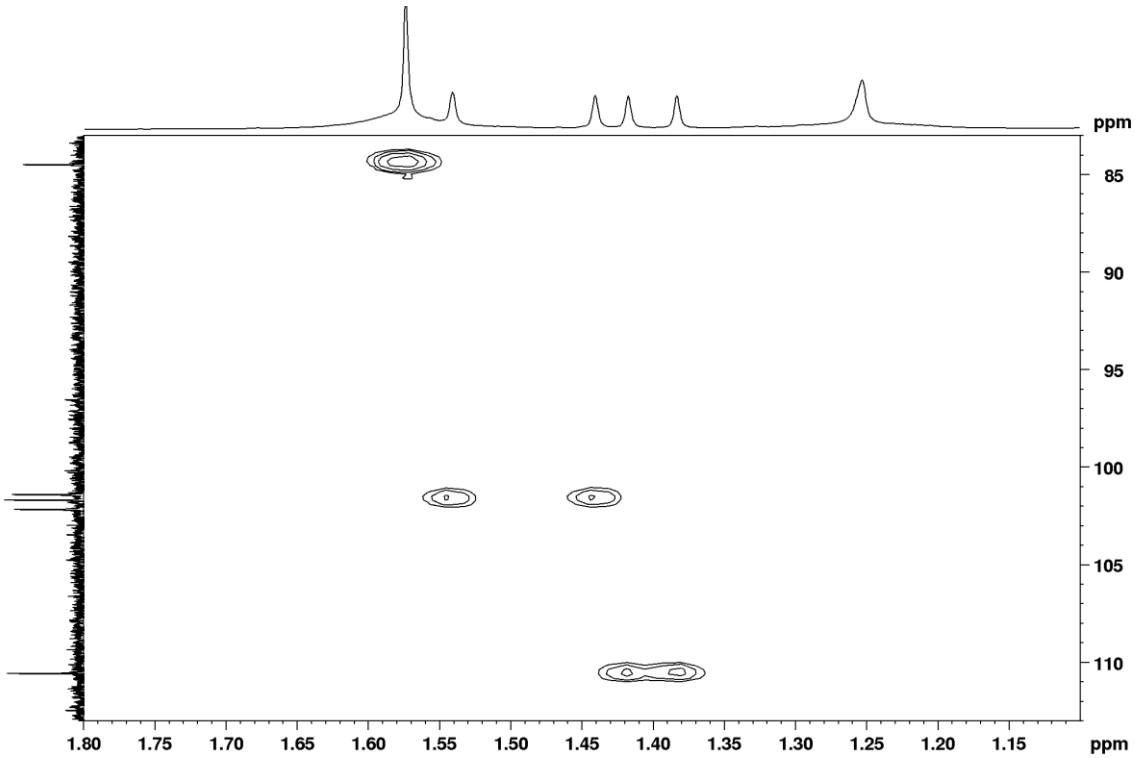
150: ^{1}H-^{13}C HMBC



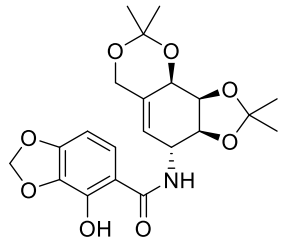
^{1}H-^{13}C HMBC (expansion)



150: ^{1}H-^{13}C HMBC (expansions)

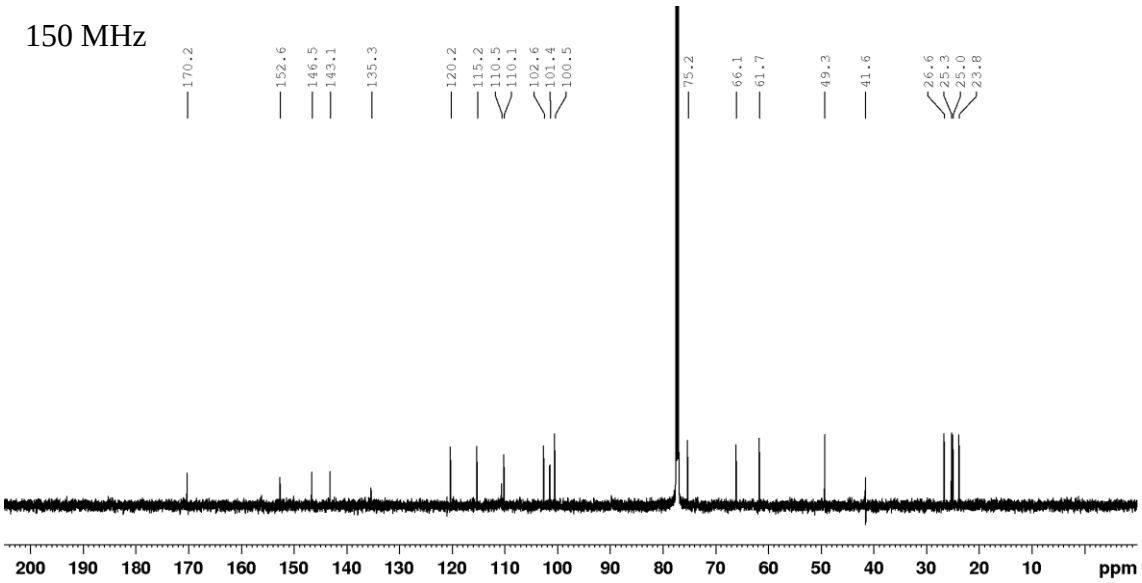


600 MHz



151

150 MHz



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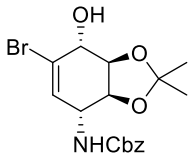
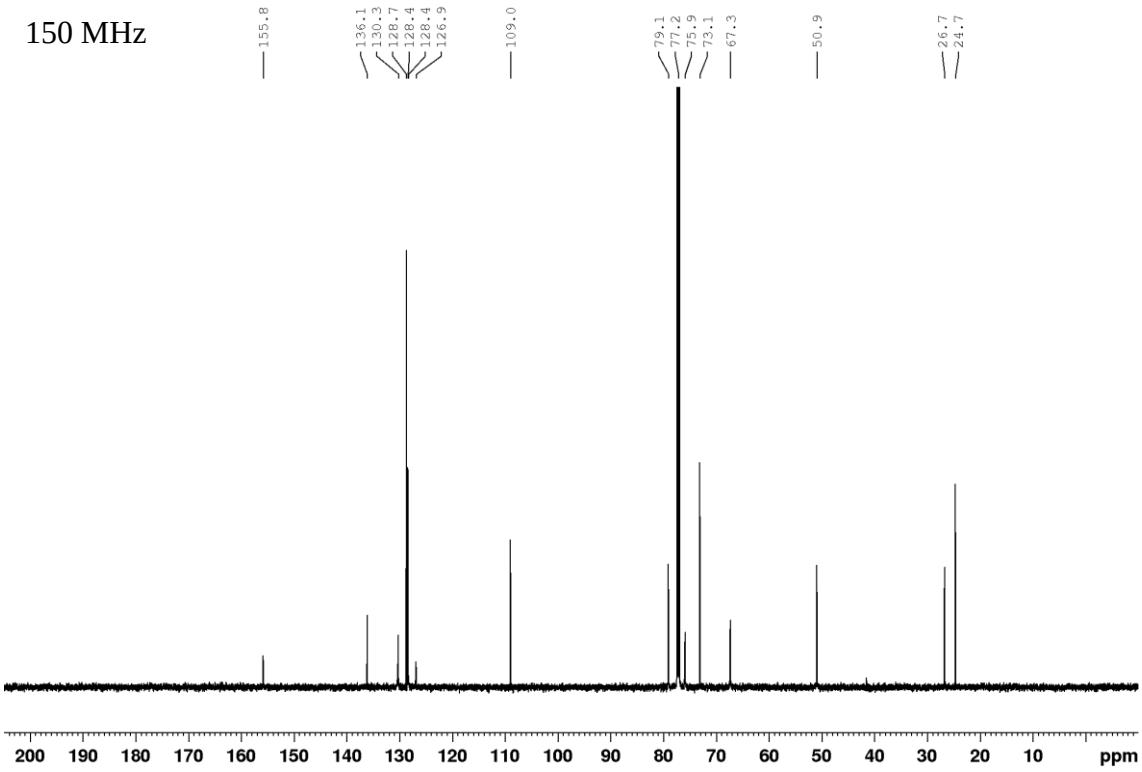
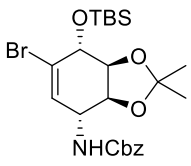


182

150 MHz

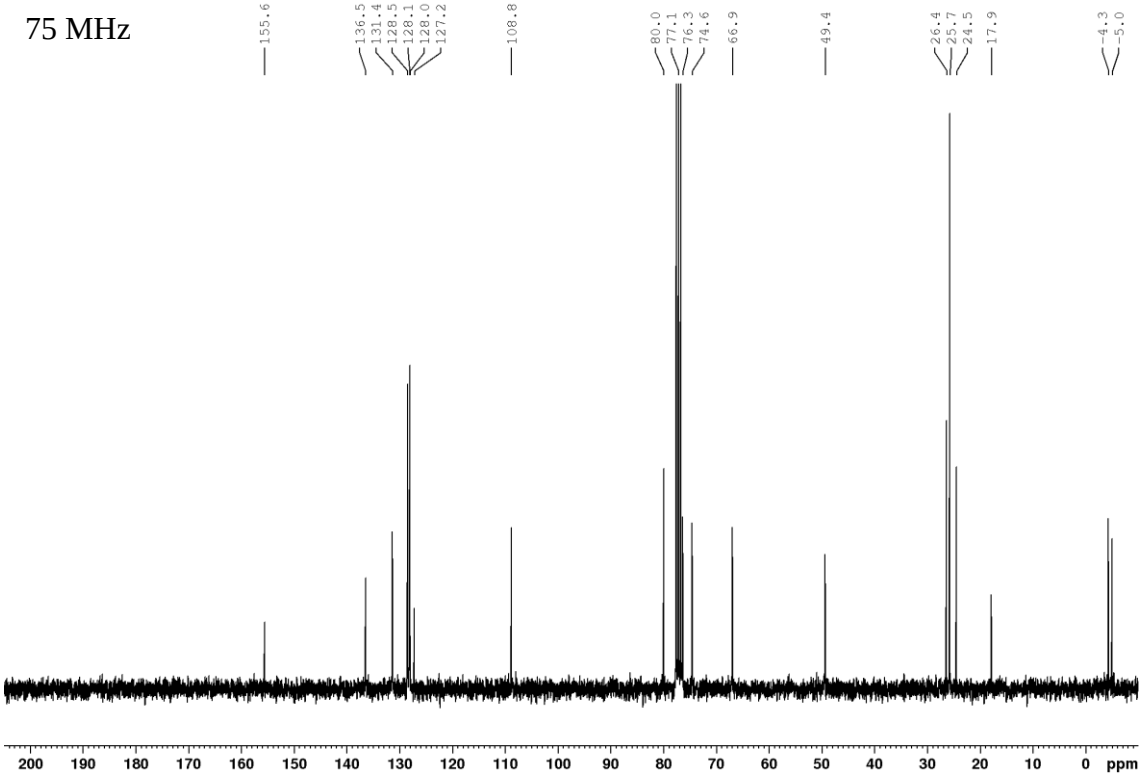


300 MHz



180

75 MHz



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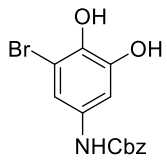
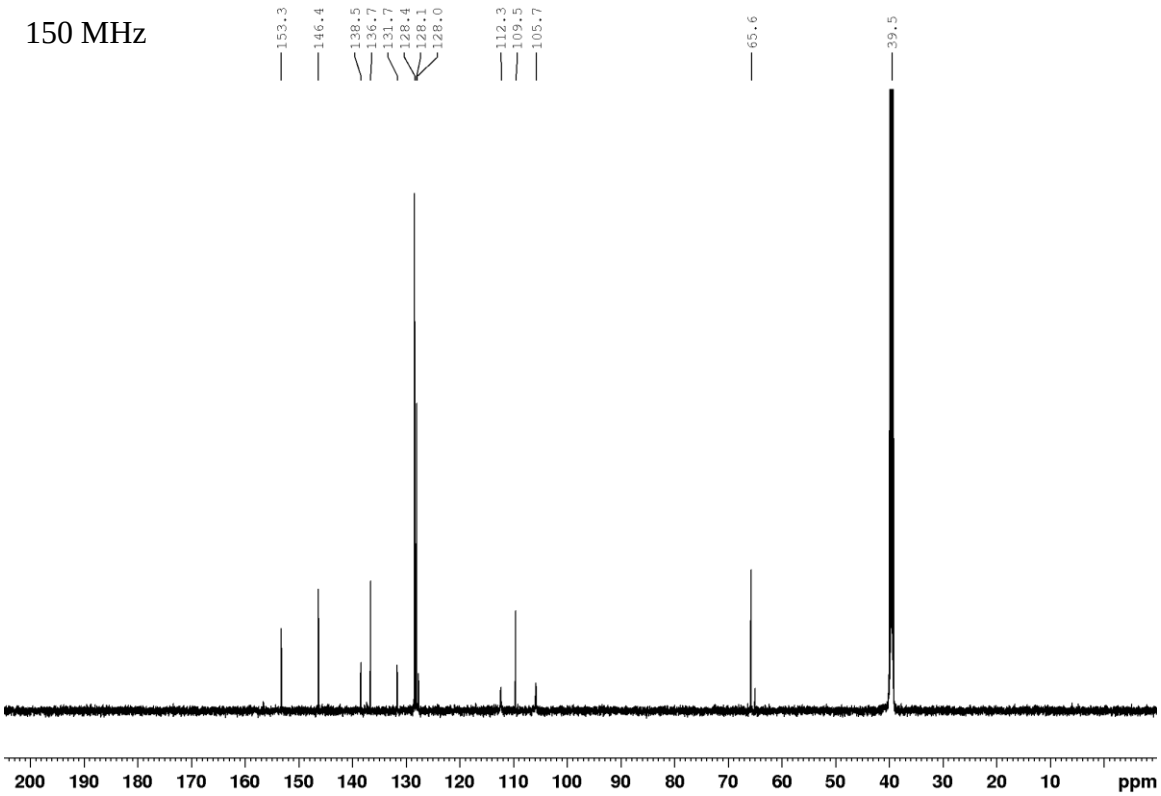
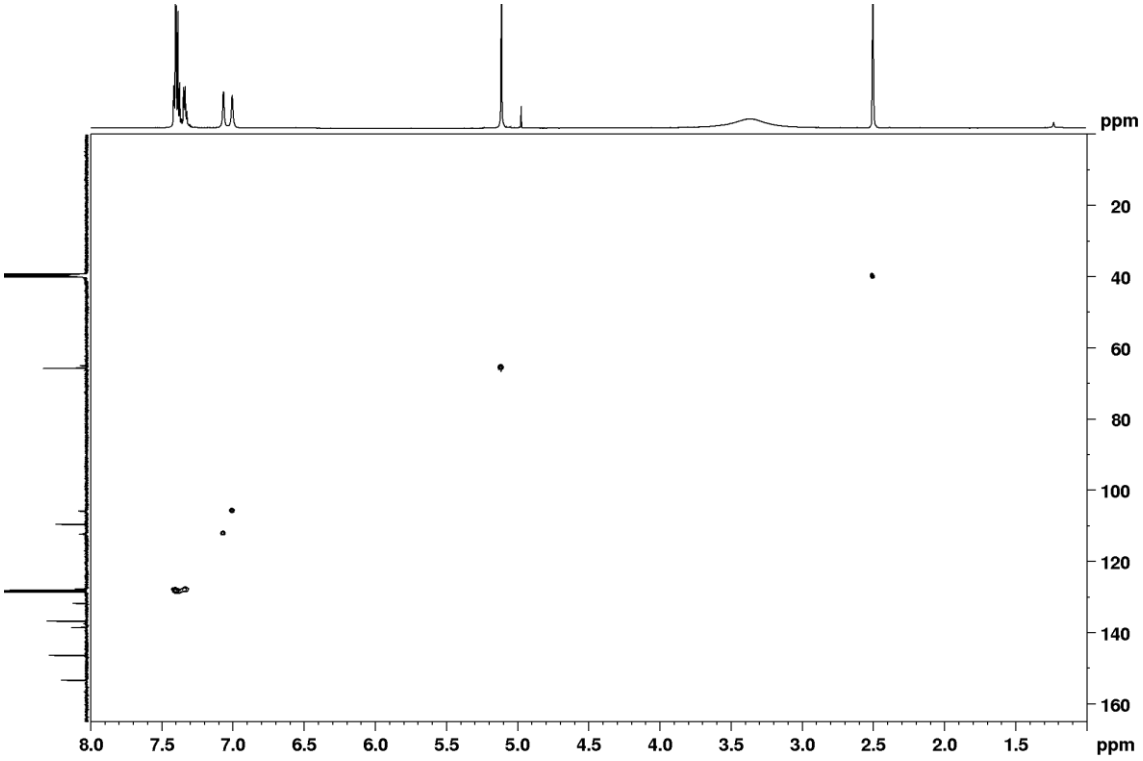


178

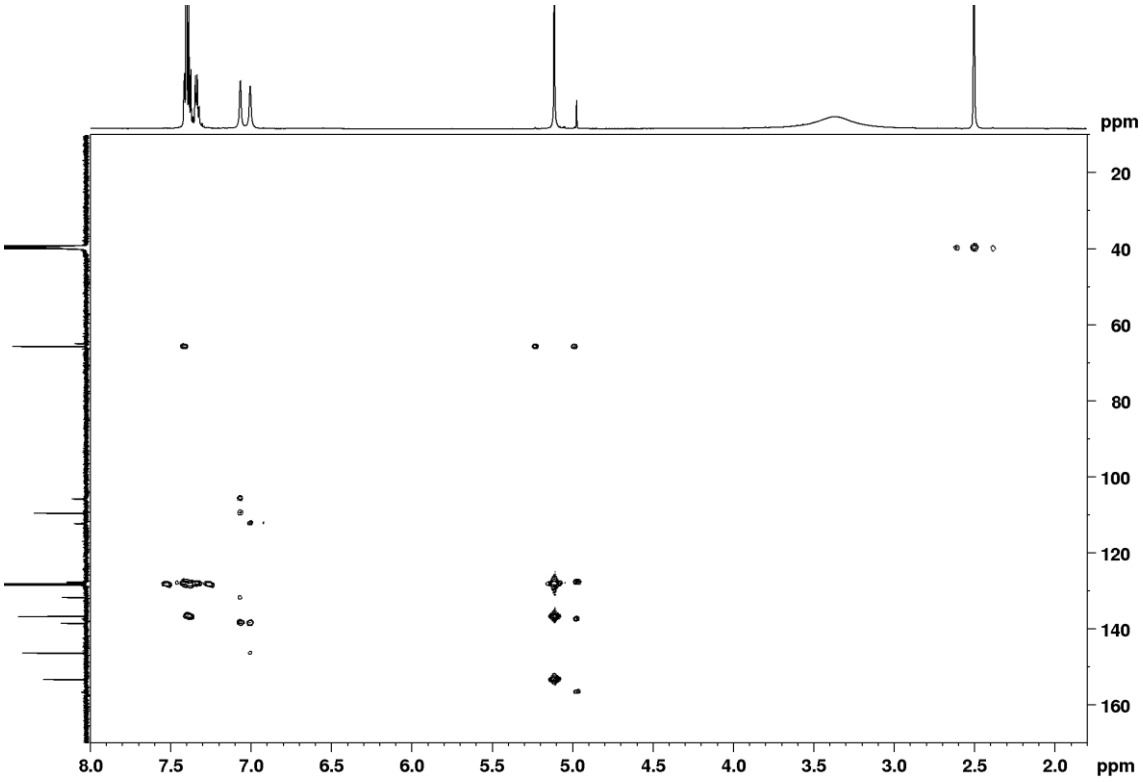
150 MHz



171: ^{1}H-^{13}C HSQC



^{1}H-^{13}C HMBC



7. Vita

Korey Thomas Bedard was born in Welland, Ontario, Canada on September 7, 1994 to parents Wade Thomas Bedard and Lori-Anne Bedard. He graduated from Welland Centennial Secondary School in 2011, before beginning studies at Brock University in St. Catharines, ON. There he completed an undergraduate degree, attaining a Hon. BSc. in Biochemistry in 2017. He then continued his education by transitioning to graduate school in May 2017. He has been a member of the Hudlicky research group since September 2016, having done research towards a BSc., MSc., and PhD. degree. He plans to graduate with a PhD. in Chemistry (Organic) in October 2022, before moving to Vancouver, B.C. to begin a MITACS post-doctoral fellowship at the University of British Columbia in collaboration with NanoVation Therapeutics, under the supervision of Dr. Glenn Sammis and Dr. Marco Ciufolini.

Korey is an animal lover and enjoys the outdoors. He is an avid hockey fan, and enjoys playing the sport frequently. He also enjoys skiing, playing softball and golf, and spending time with friends and family.

8. References

[1] Karakoyun, C.; Masi, M.; Cimmino, A.; Önür, M. A.; Somer, N. U.; Kornienko, A.; Evidente, A. *Nat. Prod. Commun.* **2019**, August, 1.

[2] For articles discussing the biological activity of the Amaryllidaceae alkaloids see: a) Nair, J.J.; Bastida, J.; Codina, C.; Viladomat, F.; van Staden, J. *Nat. Prod. Commun.* **2013**, *8*, 1335; b) Van Goietsenoven, G.; Mathieu, V.; Lefranc, F.; Kornienko, A.; Evidente, A.; Kiss, R. *Med. Res. Rev.* **2013**, *33*, 439; c) Van Goietsenoven, G.; Andolfi, A.; Lallemand, B.; Cimmino, A.; Lamoral-Theys, D.; Gras, T.; Abou-Donia, A.; Dubois, J.; Lefranc, F.; Mathieu, V.; Kornienko, A.; Kiss, R.; Evidente, A. *J. Nat. Prod.* **2010**, *73*, 1223; d) Evidente, A.; Kireev, A. S.; Jenkins, A. R.; Romero, A. E.; Steelant, W. F. A.; Van Slambrouck, S.; Kornienko, A. *Planta Med.* **2009**, *75*, 501; e) Stafford, G. I.; Pedersen, M. E.; van Staden, J.; Jager, A. K.; *J. Ethnopharmacol.* **2008**, *119*, 513; f) Ingrassia, L.; Lefranc, F.; Mathieu, V.; Darro, F.; Kiss, R. *Translational Oncol*. **2008**, *1*, 1.

[3] Ma, D.; Pignanelli, C.; Tarade, D.; Gilbert, T.; Noel, M.; Mansour, F.; Adams, S.; Dowhayko, A.; Stokes, K..; Vshyvenko, S.; Collins, J.; Hudlicky, T.; McNulty, J.; Pandey, S. *Sci. Rep.* **2017**, *7*, 42957.

[4] Kornienko, A.; Evidente, A. *Chem. Rev.* **2008**, *108*, 1982.

[5] Gardeil, J. B., translator. Traduction des Oeuvres Me´dicales d'Hippocrate, Fages, Meilhec et Cie: Toulouse, **1801**; 4 volumes.

[6] a) Duke, J. A.; Duke, P. K. *Medicinal Plants of the Bible*; Trad-Medic Books: New York, **1983**; p 98; b) Hartwell, J. L. *Plants Used Against Cancer*; Quarterman Publication: Lawrence, MA, **1982**; c) Kosteletzky, B. F. *Allgemeine Medizinisch-Pharmazeutische Flora*; Borrosch and Andre´: Prague, **1831-1836**; 6 volumes.

[7] Gerrard, A. W. *Pharm. J.* **1877**, *8*, 214; b) Cook, J. W.; Loudon, J. D. *The Alkaloids*, Volume 2; Holmes, H. L.; Academic Press, New York, **1952**, 331.

[8] a) Arrigoni, O.; Arrigoni Liso, R.; Calabrese, G. *Nature* **1975**, *256*, 513; b) Evidente, A.; Motta, A. *2001 Studies in Natural Products Chemistry*; Elsevier: Amsterdam, **2002**, 581.

[9] Ceriotti, G. *Nature*, **1967**, *213*, 595.

[10] Piozzi, F.; Fuganti, C.; Mondelli, R.; Ceriotti, G. *Tetrahedron*, **1968**, *24*, 1119.

[11] Okamoto, T.; Torii, Y.; Isogai, Y. *Chem. Pharm. Bull*. **1968**, *16*, 1860.

[12] Immirzi, A.; Fuganti, C. *J. Chem. Soc., Chem. Commun*. *1972*, 240.

[13] Mondon, A.; Krohn, K. *Chem. Ber*. **1970**, *103*, 2729.

[14] Pettit, G. R.; Gaddamidi, V.; Cragg, G. M.; Herald, D. L.; Sagawa, Y. I. *J. Chem. Soc., Chem. Commun*. **1984**, 1693.

[15] Byars, R. *Waging War on Cancer.* Victoria, CA: Friesen Press; **2015**.

[16] Note: Although this may seem like an appreciable amount of such a potent anticancer agent, it is worth noting that the isolation process started from 45 kg of plant bulbs.[14] This scarcity speaks to the low natural abundance of these bioactive alkaloids, a feature which has sparked interest in their synthesis and further investigation.

[17] Hudlicky, T.; Tian, X.; Königsberger, K.; Maurya, R.; Rouden, J.; Fan, B. *J. Am. Chem. Soc.* **1996**, *118*, 10752.

[18] For reviews and articles on the elucidation of Amaryllidaceae alkaloid biosynthesis see: a) Bernfeld, P. *Biogenesis of Natural Compounds*; Pergamon Press: Oxford, **1963**; 985; b) Battersby, A. R.; Binks, R.; Breuer, S. W.; Fales, H. M.; Wildman, W. C.; Highet, R. J. *J. Chem. Soc.* **1964**, 1595; c) Battersby, A. R.; Binks, R.; Breuer, S. W.; Fales, H. M.; Wildman, W. C. *Proc. Chem. Soc.* **1961**, 243; d) Kirby, G. W.; Tiwari, H. P. *J. Chem. Soc. (C)* **1966**, 676; e) Barton, D. H. R.; Kirby, G. W.; Taylor, J. B.; Thomas, G. M. *Proc. Chem. Soc.* **1962**, 179; f) Barton, D. H. R.; Kirby, G. W.; Taylor, J. B.; Thomas, G. M. *J. Chem. Soc.* **1963**, 4545; g) Battersby, A. R.; Fales, H. M.; Wildman, W. C. *J. Am. Chem. Soc.* **1961**, *83*, 4098; h) Jeffs, P. W. *Proc. Chem. Soc.* **1962**, 80; i) Fuganti, C. *Tetrahedron Lett.* **1973**, *14*, 1785; j) Murphy, C. F.; Wildman, W. C. *Tetrahedron Lett.* **1964**, *5*, 3863; k) Barton, D. H. R. *Proc. Chem. Soc.* **1963**, 293; l) Barton, D. H. R. *Pure Appl. Chem.* **1964**, *9*, 35; m) Barton, D. H. R.; Kirby, G. W. *Proc. Chem. Soc.* **1960**, 392; n) Fuganti, C. *The Alkaloids*; Academic Press: New York, 1975; p 83; o) Fuganti, C.; Staunton, J.; Battersby, A. R. *Chem. Commun.* **1971**, 1154; p) Fuganti, C.; Mazza, M. *Chem. Commun.* **1971**, 1388; q) Battersby, A. R.; Hebert, R. B.; McDonald, E.; Ramage, R.; Clements, J. H. *Chem. Commun.* **1966**, 603; r) Fuganti, C. *Gazz. Chim. Ital.* **1973**, *103*, 1255.

[19] Desgagné-Penix, I. *Phytochem Rev.* **2021**, *20*, 409.

[20] Ghavre, M.; Froese, J.; Pour, M.; Hudlicky, T. *Angew. Chem. Int. Ed.* **2016**, *55*, 5642.

[21] Hudlicky, T.; Moser, M.; Banfield, S. C.; Rinner, U.; Chapuis, J.-C.; Pettit, G. R. *Canadian Journal of Chemistry*. **2006**, *84*, 1313.

[22] Piozzi, F.; Marino, M. L.; Fuganti, C.; Di Martino, A. *Phytochemistry* **1969**, *8*, 1745.

[23] Pettit, G. R.; Pettit III, G. R.; Backhaus, R. A.; Boyd, M. R.; Meerow, A. W. *J. Nat. Prod.* **1993**, *56*, 1682.

[24] Bakhaus, R. A.; Pettit, G. R., III; Huang, D. S.; Pettit, G. R.; Groszek, G.; Odgers, J. C.; Ho, J.; Meerow, A. *Acta Hortic.* **1992**, 364.

[25] Carrasco, L.; Fresno, M.; Vazquez, D. *FEBS Lett.* **1975**, *52*, 236.

[26] a) Jimenez, A.; Sanchez, L.; Vazquez, D. *FEBS Lett.* **1975**, *55*, 53; b) Jimenez, A.; Sanchez, L.; Vazquez, D. *FEBS Lett.* **1975**, *60*, 66.

[27] Jimenez, A.; Santos, A.; Alonso, G.; Vazquez, D. *Biochim. Biophys. Acta* **1976**, *425*, 342.

[28] a) Kekre, N.; Griffin, C.; McNulty, J.; Pandey, S. *Cancer Chemother. Pharmacol.* **2005**, *56*, 29; b) Pandey, S.; Kekre, N.; Naderi, J.; McNulty, J. *Artif. Cells, Blood Substitutes, Immobilization Biotechnol.* **2005**, *33*, 279; c) McLachlan, A.; Kekre, N.; McNulty, J.; Pandey, S. *Apoptosis* **2005**, *10*, 619; d) Griffin, C.; Hamm, C.; McNulty, J.; Pandey, S. *Cancer Cell Int.* **2010**, *10*, 6.

[29] Gopalakrishnan, R.; Matta, H.; Chaudhary, P. M. *Sci. Rep.* **2020**, *10*, 5712.

[30] Rigby, J.H.; Mateo, M. E. *J. Am. Chem. Soc.* **1997**, *119*, 12655.

[31] Bingham, T. W.; Hernandez, L. W.; Olson, D. G.; Svec, R. L.; Hergenrother, P. J.; Sarlah, D. *J. Am. Chem. Soc.* **2019**, *141*, 657.

[32] Rigby J. H.; Maharoof, U. S. M.; Mateo, M. E. *J. Am. Chem. Soc*. **2000**, *122*¸ 6624.

[33] a) Gonzalez, D.; Martinot, T.; Hudlicky, T. *Tetrahedron Lett*. **1999**, *40*, 3077. b) Hudlicky, T.; Rinner, U.; Gonzalez, D.; Akgun, H.; Schilling, S.; Siengalewicz, P.; Martinot, T. A.; Pettit, G. R. *J. Org. Chem*. **2002**, *67*, 8726.

[34] Keck, G. E.; Wager, T. T.; Rodriguez, J. F. D. *J. Am. Chem. Soc*. **1999**, *121*, 5176.

[35] Elango, S.; Yan, T. H. *J. Org. Chem.* **2002**, *67*, 6954.

[36] Matveenko, M.; Banwell, M. G.; Willis, A. C. *Tetrahedron*, **2008**, *64*, 4817.

[37] Maji, B.; Yamamoto, H. *J. Am. Chem. Soc*. **2015**, *137*, 15957.

[38] Southgate, E. H.; Holycross, D. R.; Sarlah, D. *Angew. Chem. Int. Ed*. **2017**, *56*, 15049.

[39] Bingham, T. W.; Hernandez, L. W.; Olson, D. G.; Svec, R. L.; Hergenrother, P. J.; Sarlah, D. *J. Am. Chem. Soc*. **2019**, *141*, 657.

[40] Ohta, S.; Kimoto, S. *Tetrahedron Lett*. **1975**, *16*, 2279.

[41] a) Paulsen, H.; Stubbe, M. *Tetrahedron Lett*. **1982**, *23*, 3171; b) Paulsen, H.; Stubbe, M.; *Liebigs Ann. Chem*. **1983**, 535.

[42] Ugarkar, B. G.; Dare, J.; Schubert, E. M. *Synthesis* **1987**, 715.

[43] a) Chida, N.; Ohtsuka, M.; Ogawa, S. *Tetrahedron Lett*. **1991**, *32*, 4525; b) Chida, N.; Ohtsuka, M.; Ogawa, S. *J. Org. Chem*. **1993**, *58*, 4441.

[44] a) Hudlicky, T.; Olivo, H. F. *J. Am. Chem. Soc*. **1992**, *114*, 9694; b) Hudlicky, T.; Olivo, H.; McKibben, B. *J. Am. Chem. Soc*. **1994**, *116*, 5108.

[45] Martin, S. F.; Tso, H.-H. *Heterocycles* **1993**, *35*, 85.

[46] Keck, G. E.; Wager, T. T. *J. Org. Chem.* **1996**, *61*, 8366.

[47] Elango, S.; Yan, T.-H. *Tetrahedron* **2002**, *58*, 7335.

[48] Padwa, A.; Zhang, H. *J. Org. Chem.* **2007**, *72*, 2570.

[49] Matveenko, M.; Kokas, O. J.; Banwell, M. G.; Willis, A. C. *Org. Lett*. **2007**, *9*, 3683.

[50] Yadav, J. S.; Satheesh, G.; Murthy, C. V. S. R. *Org. Lett.* **2010**, *12*, 2544.

[51] Cai, S.-L.; Yuan, B.-H.; Jiang, Y.-X.; Lin, G.-Q.; Sun, X.-W. *Chem. Commun.*, **2017**, *53*, 3520.

[52] Saidharredy, P.; Shaw, A. K. *Tetrahedron*, **2017**, *73*, 6773.

[53] Lo, H.-J.; Chang, Y.-K.; Anathan, B.; Lih, Y.-H.; Liu, K.-S.; Yan, T.-H. *J. Org. Chem.* **2019**, *84*, 10065.

[54] a) Tian, X.; Maurya, R.; Konigsberger, K.; Hudlicky, T. *Synlett* **1995**, 1125; b) Hudlicky, T.; Tian, X. R.; Konigsberger, K.; Maurya, R.; Rouden, J.; Fan, B. *J. Am. Chem. Soc*. **1996**, *118*, 10752.

[55] Tian, X.; Hudlicky, T.; Konigsberger, K. *J. Am. Chem. Soc*. **1995**, *117*, 3643.

[56] Keck, G. E.; Wager, T. T.; McHardy, S. F. *J. Org. Chem*. **1998**, *63*, 9164.

[57] Fleet, G. W. J.; Ramsden, N. G.; Witty, D. R. *Tetrahedron* **1989**, *45*, 319.

[58] Tezuka, N.; Shimojo, K.; Hirano, K.; Komagawa, S.; Yoshida, K.; Wang, C.; Miyamoto, K.; Saito, T.; Takita, R.; Uchiyama, M. *J. Am. Chem. Soc*. **2016**, *138*, 9166.

[59] For recent reviews on Amaryllidaceae alkaloid synthesis see: a) Fürst, R. *Planta Med*. **2016**, *82*, 1389; b) He, M. M.; Qu, C. R.; Gao, O. D.; Hu, X. M.; Hong, X. C. *RSC Adv*. **2015**, *5*, 16562; see also references [2f, 4, 20].

[60] Pettit, G. R.; Melody, N.; Herald, D. L. *J. Org. Chem.* **2001**, *66*, 2583.

[61] Marion, F.; Annereau, J.-P.; Fahy, J. (Patent) WO 2010012714 (A1), **2010.**

[62] a) Vshyvenko, S.; Scattolon, J.; Hudlicky, T.; Romero, A.E.; Kornienko, A. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4750; b) Vshyvenko, S.; Scattolon, J.; Hudlicky, T.; Romero, A.E.; Kornienko, A.; Ma, D.; Tuffley, I.; Pandey, S. *Can. J. Chem.* **2012**, 932.

[63] Collins, J.; Rinner, U.; Moser, M.; Hudlicky, T.; Ghiviriga, I.; Romero, A.E.; Kornienko, A.; Ma, D.; Griffin, C.; Pandey, S. *J. Org. Chem.* **2010**, *75*, 3069.

[64] Vshyvenko, S.; Reisenauer, M. R.; Rogelj, S.; Hudlicky, T. *Bioorganic Med. Chem. Lett.* **2014**, *24*, 4236.

[65] Vshyvenko, S.; W'Giorgis, Z.; Weber, A.; Neverova, N.; Hedberg, B.; Hudlicky, T. *Adv. Synth. Catal.* **2015**, *357*, 83.

[66]Ticli, V.; Zhao, Z.; Du, L.; Kornienko, A.; Hudlicky, T. *Tetrahedron*, **2021**, *101*, 132505.

[67] Goulart-Stollmaier, J.; Thomson, J.; Arias, M.-A. E.; Hudlicky, T. *Manuscript in preparation*. **2022**.

[68] a) Stormer, K. *Zentralbl. Bakteriol. Parasitenk. Infek.* **1908**, *20*, 282; b) Gray, P. H. H.; Thornton, H. G. *Zentralbl. Bakteriol. [II]* **1928**, *73*, 74.

[69] Simonart, P.; Batistic, L. *Nature* **1966**, *212*, 1461.

[70] a) Gibson, D. T.; Koch, J. R.; Kallio, R. E. *Biochemistry* **1968**, *7*, 2653; b) Gibson, D. T.; Koch, J. R.; Schuld, C. L.; Kallio, R. E. *Biochemistry* **1968**, *7*, 3795.

[71] Gibson, D. T.; Cardini, G. E.; Maseles, F. C.; Kallio, R. E. *Biochemistry* **1970**, *9*, 1631.

[72] Bedard, K.; Hudlicky, T. Enzymatic dihydroxylation of aromatic compounds: Nature's unique reaction and its impact on the synthesis of natural products. In *Strategies and Tactics in Organic Synthesis*, Volume 15; Harmata, M.; Elsevier, 2022, 53.

[73] Zylstra, G. J.; Gibson, D. T. *J. Biol. Chem.* **1989**, *264*, 14940.

[74] a) Taher, E. S.; Banwell, M. G.; Buckler, J. N.; Yan, Q. *Chem. Rec.* **2018**, *18*, 239; b) Hudlicky, T.; Reed, J. W. *Synlett* **2009**, 685; c) Hudlicky, T.; Gonzalez, D.; Gibson, D. T. *Aldrichimica Acta* **1999**, *32*, 35; d) Hudlicky, T. *ACS Omega*, **2018**, *3*, 17326.

[75] For reviews on enzymatically derived diols and their synthetic application see: (a) Lan, P.; Ye, S.; Banwell, M. G. *Chem. Asian J.* **2019**, *14*, 4001; (b) Taher, E. S.; Banwell, M. G.; Buckler, J. N.; Yan, Q. *Chem. Rec.* **2018**, *18*, 239; (c) Lewis, S. E. *Arene Chemistry*, **2015**, 915; (d) Reed, J. W.; Hudlicky, T. *Acc. Chem. Res.* **2015**, *48*, 674; (e) Lewis, S. E. *Chem. Commun.* **2014**, *50*, 2821; (f) Hassan, H. A. *Curr. Trends Biotechnol. Pharm.* **2012**, *6*, 389; (g) Banwell, M. G.; Lehmann, A. L.; Menon, R. S.; Willis, A. C. *Pure Appl. Chem.* **2011**, *83*, 411; (h) Mortison, J. D.; Sherman, D. H. *J. Org. Chem.* **2010**, *75*, 7041; (i) Hudlicky, T. *Pure Appl. Chem.* **2010**, *82*, 1785; (j) Hudlicky, T.; Reed, J. W. *Chem. Soc. Rev.* **2009**, *38*, 3117; (k) Boyd, D. R.; Bugg, T. D. H. *Org. Biomol. Chem.* **2006**, *4*, 181; (l) Johnson, R. A. Microbial Arene Oxidations. In *Organic Reactions*, Volume 63; John Wiley and Sons, Inc.: New York, **2004**, *63*, 117; (m) Banwell, M. G.; Edwards, A. J.; Harfoot, G. J.; Joliffe, K. A.; McLeod, M. D.; McRae, K. J.; Stewart, S. G.; Vogtle, M. *Pure Appl. Chem.* **2003**, *75*, 223; (n) Boyd, D. R.; Sharma, N. D.; Allen, C. C. R. *Curr. Opin. Biotechnol.* **2001**, *12*, 564; (o) Boyd, D. R.; Sheldrake, G. N. *Nat. Prod. Rep.* **1998**, 309; (p) Hudlicky, T.; Reed, J. W. An evolutionary perspective of microbial oxidations of aromatic compounds in enantioselective synthesis: history, current status, and perspectives. In *Advances in Asymmetric Synthesis*, Volume 1; Hassner, A., Ed.; JAI Press: Greenwich (CT), **1995**, 271; (q) Carless, H. A. J. *Tetrahedron: Asymmetry* **1992**, *3*, 795; (r) Brown, S. M.; Hudlicky, T. The use of arene *cis*-diols in synthesis. In *Organic Synthesis: Theory and Applications*, Volume 2; Hudlicky, T., Ed.; JAI Press: Greenwich (CT), **1993**, 113; (s) Widdowson, D. A.; Ribbons, D. W.; Thomas, S. D. *Janssen Chim. Acta* **1990**, *8*, 3.

[76] McElvain, S.M.; Starn Jr., R.E. *J. Am. Chem. Soc.*, **1955**, *77*, 4571.

[77] Volger, B.; Meller, M.; Bayer, R.; Kraus, W. *J. Org. Chem.* **1989**, *54*, 4165.

[78] Kukula, P.; Koprivovoa, K. *J. Catalysis*, **2005**, *234*, 161.

[79] Soai, K.; Ookawa, A. *J. Org. Chem.* **1986**, *51*, 4000.

[80] The hydrochloride salt of secondary amine **107** was characterized by NMR and Mass Spectrometry, and its aqueous solubility led to the initial conclusion of it likely being a salt. It was initially hoped that the HCl salt of amine **100** had been formed, but the ^{1}H NMR spectrum and MS pattern suggested only the presence of the dimerized product. Partial characterization data: ^{1}H NMR ($CDCl_3$) δ 9.43 (br s, 2H, signal disappeared upon D_2O quench), 5.88 (s, 2H), 3.40 (t, 4H), 2.07-2.17 (m, 8H), 1.58-1.68 (m, 8H); ^{13}C NMR ($CDCl_3$) δ 131.8, 128.7, 51.3, 27.3, 25.4, 22.4, 21.8.

[81] Endoma-Arias, M.; Hudlicky, T. *Chem. Eur. J*. **2016**, *22*, 14540.

[82] Donets, P.A.; Van der Eycken, E.V. *Synthesis*. **2011**, 2147.

[83] Stocker, B.; Timmer, M. *J. Org. Chem.* **2010**, *75*, 5470.

[84] Knipe, P.; Paton, R.; Smith, M.; *Chem. Eur. J.* **2014**, *20*, 3005.

[85] Finn, K. J.; Collins, J.; Hudlicky, T. *Tetrahedron* **2006**, *62*, 7471.

[86] Katritzky, A. R.; Avan, I.; Tala, S. R. *J. Org. Chem.* **2009**, *74*, 8690.

[87] *Org. Syn.* **1993**, *71*, 133-136; **1998**, *9*, 704.

[88] Majeed, A.; Antonsen, O.; Benneche, T.; Undeheim, K. *Tetrahedron.* **1989**, *45*, 993.

[89] Malova-Krizkova, P.; Hammerschmidt, F. *Eur. J. Org. Chem.* **2013**, *23*, 5143.

[90] Schmidt, B.; Riemer, M. *J. Org. Chem.* **2014**, *79*, 4104.

[91] Furneaux, R. H.; Gainsford, G. J.; Mason, J. M. *J. Org. Chem.* **2004**, *69*, 7665.

[92] Cho, Y.-S.; Cho, C.-G. *Tetrahedron* **2008**, *64*, 2172.

[93] Amos, P. C.; Whiting, D. A. *J. Chem. Soc., Chem. Commun.* **1987**, 510.

[94] Examples of syntheses involving a Heck-type closure proceeding by *anti*-elimination: (a) Lautens, M.; Fang, Y.- Q. *Org. Lett.* **2003**, *5*, 3679 ; (b) Enders, D.; Lenzen, A.; Backes, M.; Janeck, C.; Catlin, K.; Lannou, M. – I.; Runsink, J.; Raabe, G. *J. Org. Chem.* **2005**, *70*, 10538; (c) Maeda, K.; Farrington, E. J.; Galardon, E.; John, B. D.; Brown, J. M. *Adv. Synth. Catal*. **2002**, *344*, 104. (d) Shea, K. M.; Lee, K. L.; Danheiser, R.

L. *Org. Lett*. **2000**, *2*, 2353. (e) Ikeda, M.; El Bialy, S. A. A.; Yakura, T. *Heterocycles*, **1999**, *51*, 1957 and references therein.

[95] Shimizu, I.; Tsuji, J. *J. Am. Chem. Soc.*, **1982**, *104*, 5844.

[96] Examples of narciclasine-type Amaryllidaceae alkaloid syntheses involving an intramolecular Heck reaction for B-ring closure: a) McIntosh, M. C.; Weinreb, S. M., *J. Org. Chem*., **1993**, *58*, 4823; b) Friestad, G. K.; Branchaud, B. P. *Tetrahedron Lett.* **1995**, *36*, 7047; see also references.[43,44,45,64,65,66]

[97] Abelman, M. M.; Oh, T.; Overman, L. E. *J. Org. Chem.* **1987**, *52*, 4130; Ashimori, A.; Overman, L. E. *J. Org. Chem.* **1992**, *57*, 4571.

[98] Borra, S.; Lapinskaite, R.; Kempthorne, C.; Liscombe, D.; McNulty, J.; Hudlicky, T. *J. Nat. Prod.* **2018**, *81*, 1451.

[99] Barker, D.; Pilkington, L. I.; Jung, E.-K. *Synthesis*, **2017**, *49*, 1190.