Fall 2015

Total Synthesis of Biologically Active Natural and Unnatural Products

Julia Heimberger

TOTAL SYNTHESIS OF BIOLOGICALLY ACTIVE NATURAL AND UNNATURAL PRODUCTS

by

JULIA HEIMBERGER

(Under the Direction of Abid Shaikh)

ABSTRACT

Herbarin A and B were isolated from the fungal strains of *Cladosporium herbarum* found in marine sponges *Aplysina aerophoba* and *Callyspongia aerizusa*. Total synthesis of Herbarin A and B was achieved by carrying out a multi-step synthesis approach, and the antioxidant properties were evaluated using FRAP assay. Toxicity of these compounds was determined using a zebrafish embryo model. Furthermore, synthesis of C-6 alkyl-azaarene derivatives of nucleosides by Csp^3-H bond functionalization were investigated. Effective incorporation of 2-methylazaarene moiety at the C-6 position of the protected inosine nucleoside provided a new class of compounds with anticipated enhanced biological activity.

INDEX WORDS: Total Synthesis, Antioxidant Properties, Nucleosides, Toxicity, Zebrafish

TOTAL SYNTHESIS OF BIOLOGICALLY ACTIVE NATURAL AND UNNATURAL PRODUCTS

by

JULIA HEIMBERGER

B.S., Georgia Southern University, 2013

A Thesis Submitted to the Graduate Faculty of Georgia Southern University in Partial Fulfilment of the Requirements for the Degree of

MASTER OF SCIENCE

STATESBORO, GEORGIA

TOTAL SYNTHESIS OF BIOLOGICALLY ACTIVE NATURAL AND UNNATURAL PRODUCTS

by

JULIA HEIMBERGER

Major Professor:	Abid Shaikh
Committee:	Karelle Aiken
	Rafael Quirino

Electronic Version Approved:
Fall 2015

DEDICATION

I dedicate this work to my family, for their never ending love and support throughout my life, and for giving me the strength and courage to further my education while reminding me to always stay true to myself.

ACKKNOWLDEGEMENTS

I would like to acknowledge the members of Dr. Shaikh's group; Mark Blocker, Corinne Cade, and David Van Leuven, for their contributions towards the success of this work. I would like to thank my dear friends Megan Dodson, and Kelsey Allmond for their endless help and support. Additionally, I would like to acknowledge Dr. Karelle Aiken, Dr. Brian Koehler, Dr. Shainaz Landge, Dr. Rafael Quirino and last but anything but the least, Dr. Abid Shaikh for their mentorship, and guidance. I would also like to acknowledge Georgia Southern University for the use of their facility, specifically the Department of Chemistry for allowing me to conduct research and the Master of Applied Physical Science Program for financial support and tuition assistance.

TABLE OF CONTENTS

APPENDIX

LIST OF TABLES

LIST OF SCHEMES

LIST OF FIGURES

LIST OF ABBRIVIATIONS

C-6:	Carbon number 6
DBU:	1,8-diazabicyclo [5.4.0] undec-7-ene
DMF:	Dimethyl formamide
DMSO:	Dimethyl sulfoxide
Dpf:	Days post fertilization
E3:	Embryonic medium
FCR:	Folin-Ciocalteu Reagent
$FeCl_3$:	Iron (III) chloride, Ferric chloride
$FeSO_4$:	Iron (II) sulfate, ferrous sulfate
Fe^{2+}-TPTZ:	Ferrous 2,4,6-tripyridyl-s-triazine complex
Fe^{3+}-TPTZ:	Ferric 2,4,6-tripyridyl-s-triazine complex
FRAP:	Ferric Reducing Activity of Plasma
GC-MS:	Gas Chromatograph-Mass Spectrometer
Hpf:	Hours past fertilization
$NaHCO_3$:	Sodium bicarbonate
$NaNH_2$:	Sodium amide
nm:	nanometers
nM:	Nano molar
NMR:	Nuclear Magnetic Resonance
LD50:	50% lethal dose
PG:	Protecting Group
RT:	Room Temperature
RB:	Round Bottom
SeO_2:	Selenium dioxide
S_NAR:	Nucleophillic aromatic substitution
$SOCl_2$:	Thionyl chloride
TBAF:	tetrabutylammonium fluoride
TBDMS-Cl:	*tert*-butyldimethylsilyl chloride

TEAC:	Tetraethylammonium chloride
THF:	Tetrahydrofuran
TLC:	Thin layer chromatography
TPTZ:	2,4,6-tripyridyl-s-triazine
WT:	Wild-type
$Yb(OTf)_3$:	Ytterbium(III) trifluoromethanesulfonate

CHAPTER 1

INTRODUCTION

1.1 NATURAL PRODUCT SYNTHESIS

The first syntheses of Herbarin A and B have been successfully achieved.[1] Herbarin A and B, two new α-pyrone derivatives,were isolated from fungal strains in marine sponge.[2] These compounds were found to exhibit potent toxicity as secondary metabolites of aquatic microorganisms, a rich source of biologically active compounds.[3]

Extensive research continues to test biological activity of structurally unique secondary metabolites from marine sponge as potential natural synthesis products. Studies suggest that there can be a significant increase in the harvest of these potential biologically active metabolites. The marine sponge derivatives, due to the increased degree of biodiversity, demonstrate promise in experimental and clinical models.[3] The sponge-associated fungal metabolites Herbarin A and B, isolated from marine sponges *Aplysina aerophoba* and *Callyspongia aerizusa,* have been found to exhibit inhibitory biological activity against strains of the fungus *Cladosporium herbarum*.[2] Potential applications of Herbarin A and B include uses as antitumor agents, herbicides, and antimicrobials.[3] As a consequence of their remarkable activity and unique chemical structure, a multi-step synthetic route to afford Herbarin A and B in multi-gram scale quantities, a feat not possible from their natural source, was successfully achieved. [3]

Development of a synthetic strategy to permit preparation of multi-gram quantities of the target compounds as well as structural derivatives is useful for exploring structure-activity relationships and compounds' mode of action.[2] The current research demonstrates that Herbarin A and B exhibit antioxidant properties and toxicity in zebrafish embryo models. The amount of compound required for these experiments are relatively large (0.1-1.0 g and more) and are difficult

to obtain from the natural source. Furthermore, the process of isolating pure Herbarins from their natural source is very labor-intensive and time consuming. As such, the chemical syntheses of gram scale quantities of the compounds from commercially available starting material proved highly useful.

1.2 SYNTHESIS OF NUCLEOSIDE DERIVATIVES

Nucleosides, although not toxic alone, have been and continue to be researched as their presence in living systems make excellent scaffolds for chemical modification. Modified nucleoside derivatives can play an important biological role, for example as modulators of adenosine receptors, stimulators of plant cell growth and cell division, inhibitors of DNA polymerases towards normal and cancer cells, and as antiviral compounds.[4-9] Recently, a class of C-6 aryl purine ribonucleosides has been shown to possess cytostatic activity toward T-lymphoblastoid and other cell lines.[11] Owing to the interesting biological properties of modified nucleosides, a number of synthetic protocols have been developed to effectively functionalize the C-6 position. Some of these protocol include palladium-catalyzed C-C bond formation and Suzuki cross coupling reactions.[12-14] These known methods have led to the development of several nucleoside analogues. [19]

The structural core of quinoline is frequently associated with medicinal applications, such as anti-cancer, antimicrobial, HIV-1 integrase inhibitors, HIV protease inhibitors, anti-leishmanial activity, and NK-3 receptor antagonists.[15,16] Owing to their wide applications, the synthesis of quinoline and its derivatives have attracted considerable attention of organic and medicinal chemists for many years.[17,18] In the proposed work, synthetic approaches combine two biologically active cores, nucleosides and quinoline, to produce a new class of compounds with a synergistic

effect that will enhance biological activity. Utilization of a recently developed, novel Csp^3-H functionalization strategy to effectively incorporate alkyl-azaarene moiety at the C-6 position of nucleosides is discussed.[10] Notably, functionalization of nucleosides with azaarenes has not yet been reported in the literature.[4-9]

CHAPTER 2

TOTAL SYNTHESIS OF HERBARIN A AND B, DETERMINATION OF THEIR ANTIOXIDANT PROPERTIES, AND TOXICITY IN ZEBRA FISH EMBRYO MODEL

2.1 INTRODUCTION

Herbarin A and B, were recently isolated by Jadulco and co-workers [2] from two strains of the fungi *Cladosporium herbarum* found in sponges *Aplysina aerophoba* and *Callyspongia aerizusa* respectively. Spectroscopic analysis of ethyl acetate extracts from these fungi revealed two new α-pyrone derived structures, Herbarin A and B. Various spectroscopic techniques such as, NMR and mass-spectrometry were used for structural elucidation. To determine the initial biological activity, a feeding assay was performed on polyphagous pest insect larvae and brine shrimp larvae.[2, 3]

As a consequence of their potent toxicity towards insect and brine shrimp larvae and unique chemical structure, laboratory syntheses of Herbarin A and B (**Figure 1)** was pursued. A multistep synthetic approach provided the target compounds in multi-gram scale quantity, which is not possible from the natural source. Having the larger quantities allowed for further investigation of the potential toxicity and antioxidant properties of these natural products.

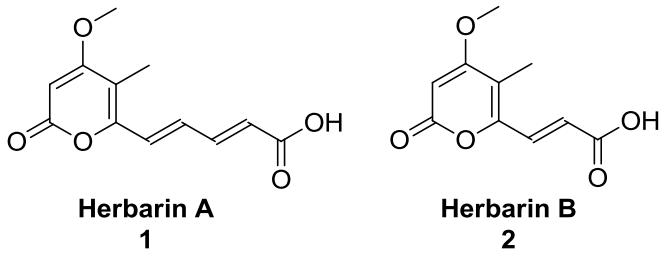
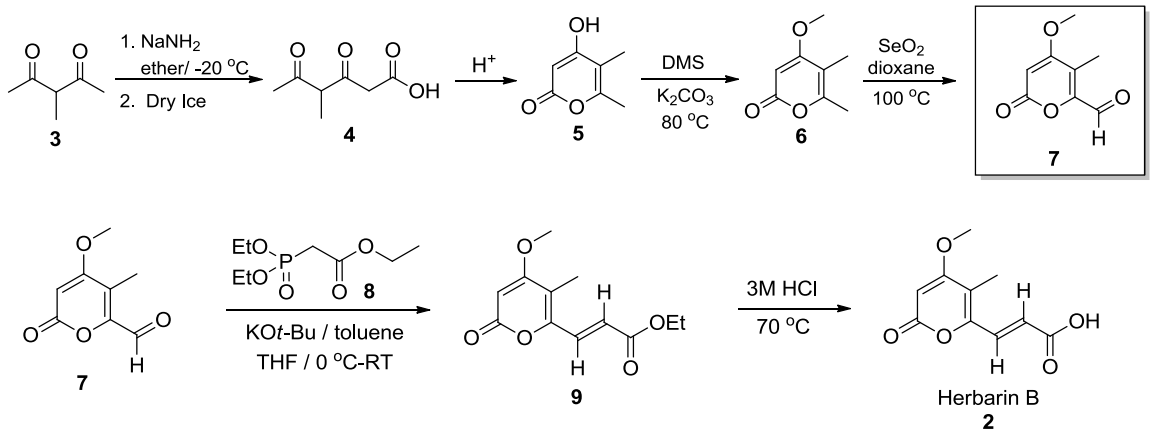


Figure 1. Structures of Herbarin A and Herbarin B

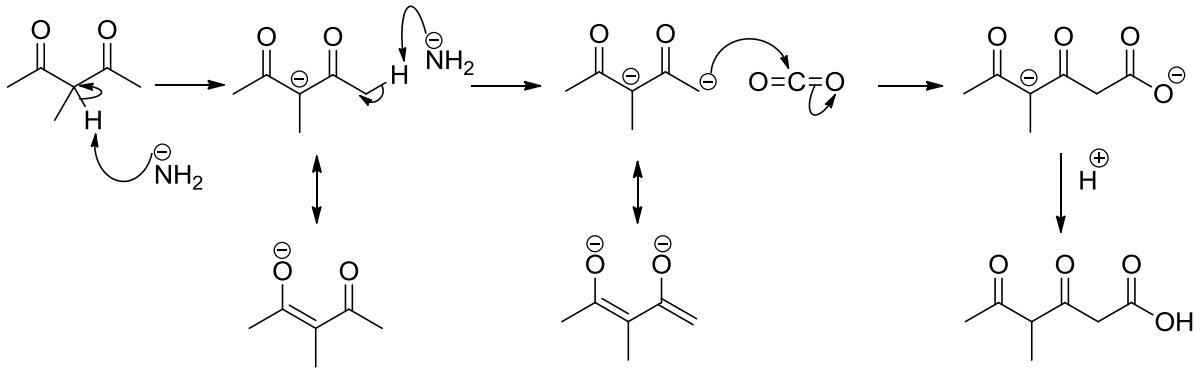
2.2 RESULTS AND DISCUSSION

Herein, synthesis of target compounds using a multi-step reaction sequence has been described, as demonstrated below (**Scheme 1**). Aldehyde (**7**), was identified as the crucial intermediate that can afford both the target compounds.



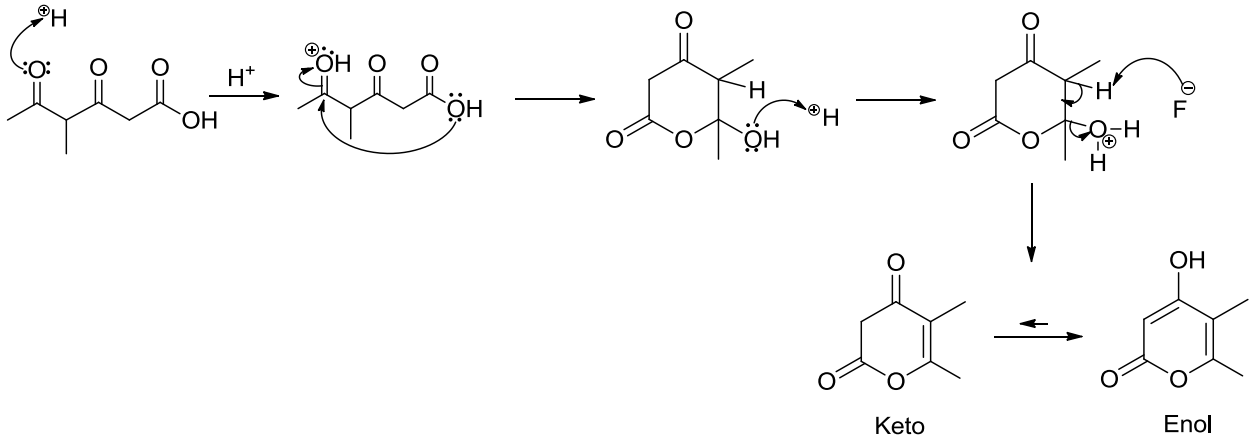
Scheme 1. Synthetic strategy for Herbarin B

As a first step, research efforts were directed towards the synthesis of 4-hydroxypyrone (**5**) by utilizing commercially available starting materials as basic building blocks and using a modified approach described by Wilcox and group.[20] 3-Methyl-2,4-pentanedione (**3**) was treated with $NaNH_2$ and then reacted with dry ice to provide diketo-acid (**4**) in 77% crude yield. A simple acid base reaction (**Scheme 2**) first picks up a proton, providing a dianion mechanism, thus addition of excess base is required. Addition of the nucleophile, followed by treatment with acid provided crude diketo-acid.



Scheme 2. Dianion Carbonyl Mechanism

Diketo-acid (**4**) without any purification was then treated with HF (**Scheme 2**) to afford the 4-hydroxypyrone (**5**) in about 39% yield. The cyclization mechanism is provided below (**Scheme 3**). Upon further treatment with acid, protonation of crude diketo-acid followed by nucleophilic addition of the 2-carbonyl to C-6, closes the ring providing the pyrone core. The closed ring then picks up a proton from acid. The strong nucleophile, Fluorine, picks up a proton resulting in an elimination reaction, providing a double bond between C-5 and C-6. This dehydration reaction provides keto-enol tautomerization products, in which the enol product is favored as it is aromatic, and thus more stable.

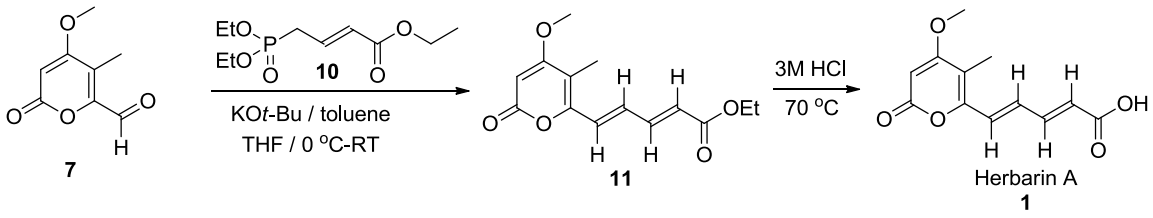


Scheme 3. Cyclization Mechanism

Additionally, etherification provided 4-methoxypyrone (**6**) as a yellow solid in 81% isolated yield, and upon oxidation with SeO_2 selectively converted the methyl group at position C-6 to aldehyde (**7**) in quantitative yield. 4-methoxypyrone (**6**) starting material and product (**7**) were inseparable when subjected to flash chromatography, thus a crude mixture of (**7**) was obtained as a yellow solid in 88% yield. After successful execution of the synthetic scheme, aldehyde (**7**) was synthesized in multi-gram quantities to be utilized in further reactions.

Aldehyde (**7**) was then reacted with phosphonate ester (**8**) using Horner–Wadsworth–Emmons coupling strategy to obtain an exclusively *trans*-alkene (**9**) in about 90% yield.[21] Hydrolysis of the ester group with 3M HCl yielded Herbarin B (**2**) as a white solid in 98% yield. All products were purified using column chromatography and structure elucidation was carried out using various spectroscopic techniques including NMR and mass-spectrometry. Lastly, the spectroscopic data for Herbarin B was compared with literature data to confirm the product formation. Herbarin B was deliberately chosen as the first synthetic target considering its lesser complexity compared to Herbarin A.

After successfully accomplishing the synthesis of Herbarin B, Herbarin A was targeted by using a similar synthetic scheme. The aldehyde intermediate (**7**) was treated with commercially available predominantly *trans*-phosphonate ester (**10**) to form an exclusively *trans*-*trans*-ester (**11**) in 81% yield, which on further hydrolysis with 3M HCl provided Herbarin A in 67% yield (**Scheme 4**).



Scheme 4. Synthetic strategy for Herbarin A

2.3 DETERMINATION OF ANTIOXIDANT PROPERTIES

Ferric Reducing Ability of Plasma (FRAP) Assay: A wide range of assays is known for measuring the reducing capacity of antioxidants. The assays are carried out at acidic (FRAP), neutral (TEAC), or basic (total phenols assay FCR) conditions. The pH values have an important effect on the reducing capacity of antioxidants.[22] Herbarin A and B both have carboxylic acid groups and to maintain the integrity of these molecules, we choose to use the acidic FRAP assay. Also FRAP assay has several advantages such as high reproducibility, simple, rapidly performed and showed the highest efficiency for acids and phenols.[23] Therefore, it would be an appropriate technique for determining antioxidant activity for Herbarin A and B.

The FRAP assay, is presented as a novel method for assessing ‘‘antioxidant power.’’ Ferric to ferrous ion reduction at low pH causes a colored ferrous-tripyridyltriazine complex to form. FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. Absorbance changes are linear over a wide concentration range with antioxidant mixtures, including plasma, and with solutions containing one antioxidant in purified form.[24] The FRAP assay is inexpensive, reagents are simple to prepare, results are highly reproducible, and the procedure is straightforward and speedy. The FRAP assay offers a putative index of antioxidant, or reducing potential of organic compounds within the technological reach of every laboratory.

The FRAP assay was employed as described in the literature.[25] The mechanism of this method is based on the reduction of ferric 2,4,6-tripyridyl-s-triazine complex (Fe^{3+}-TPTZ) to its ferrous form (Fe^{2+}-TPTZ) in the presence of antioxidants (**Figure 2**).

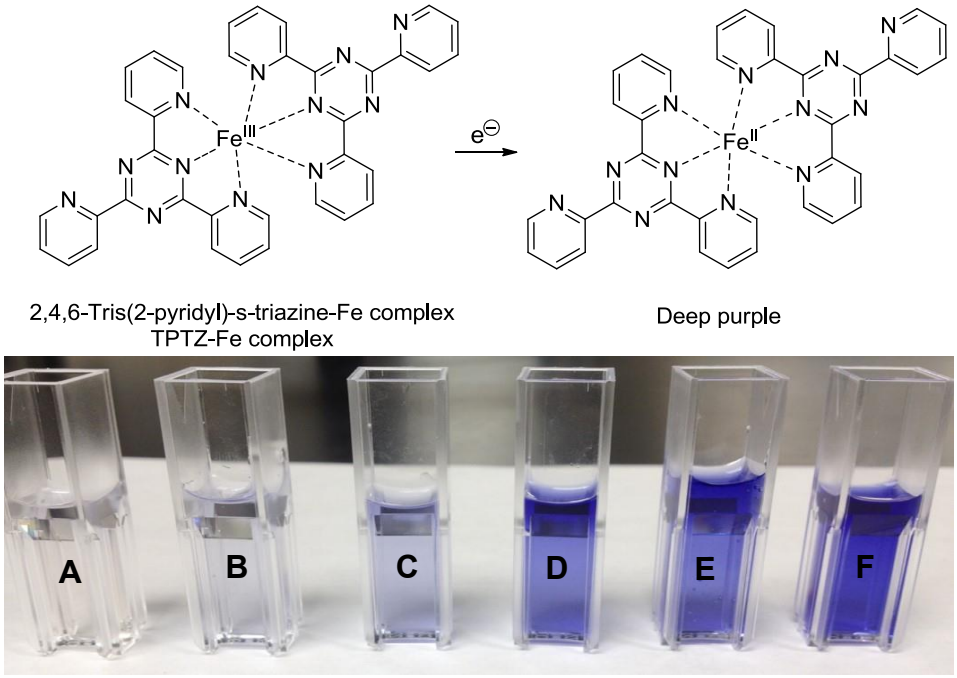


Figure 2. Reduction of Fe^{3+}-TPTZ to Fe^{2+}-TPTZ: Cuvette A contained FRAP reagent and dH_2O as a blank, and cuvettes B-F contained a series of dilutions with Herbarin B at 10, 20, 40, and 80 μg/mL respectively.

FRAP reagent solution was prepared by addition of 25 mL acetate buffer, 2.5 mL of Fe^{3+}-tripyridyl-triazine (Fe^{3+}-TPTZ) (10 mmol/L in 40 mmol/L of HCl, and 2.5 mL $FeCl_3$ (20 mmol/L). Herbarin A was then added to (300 μL) of the FRAP reagent solution (at 10, 20, 40, and 80 μg/mL), followed by 30 μL distilled H_2O and absorbance of the samples were recorded at 593 nm. Herbarin B followed the same procedure and dilution series as Herbarin A, exactly (**Figure 2**).

The electron-donating capacity of the antioxidant was measured by the change in absorbance at 593 nm; the blue-colored Fe^{2+}-tripyridyl-s-triazine (Fe^{2+}-TPTZ) compound was formed from the initial colorless solution (Fe^{3+}-TPTZ). The absorbance of the reaction mixture was measured spectrophotometrically at 593nm after incubation at room temperature for 1 hour. Calibration curves were generated from aqueous solutions of $FeSO_4$ at different concentrations

ranging from 0.1 to 1 mM. A series of dilution standards were prepared from a fresh 1mM solution of $FeSO_4.7H_2O$ in dH_2O to construct a linear regression (absorbance against concentration) and compare FRAP values (units, mM Fe(II) per litre) of the samples to the known Fe(II) concentration of the calibration standard. Both Herbarin A and B showed activity towards antioxidant assay at 0.1 to 1 mM concentrations (**Figure 3**).

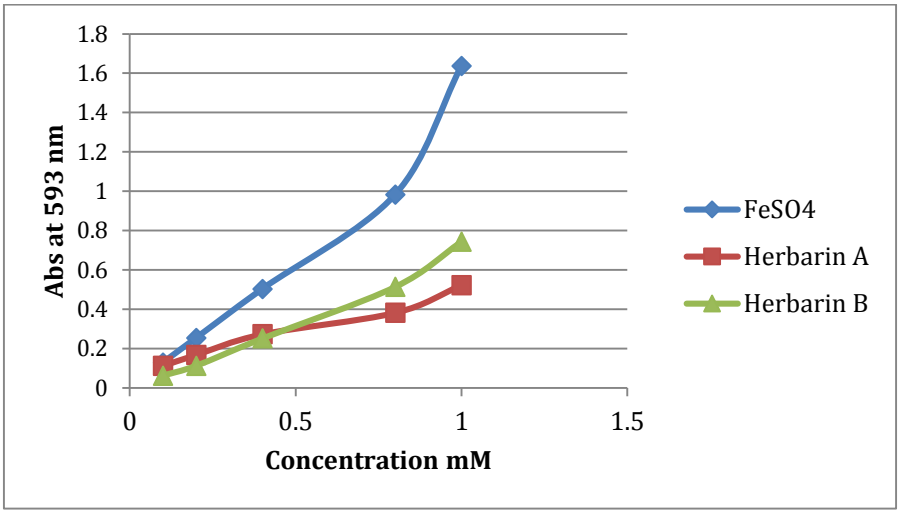


Figure 3. Antioxidant activity of Herbarin A and B

2.4 TOXICITY ASSAY

To assess the biological value of Herbarin A and B, we used a zebrafish embryo screening assay. Herbarin A and B were tested for toxicity and teratogenicity in zebrafish embryos.[26-30] These assays were performed using an established literature procedure.[26-30] Briefly, wild-type zebrafish embryos were collected from spawn tanks and allowed to grow until 6 hpf (hours post fertilization) at standard conditions.[31] At 6 hpf, embryos were observed under a microscope for embryonic development and only normally developing embryos were transferred to wells in 24-well plate and allowed to grow in 1 mL of treatment solution for up to 2 dpf (days post fertilization). Observations

were made at 24 hpf and solutions were replaced as necessary. Herbarin A and B were dissolved in 100% DMSO after purification, further they were re-suspended in E3 (embryonic medium) at various concentrations (**Figure 4 and 5**) for zebrafish assay. Therefore our controls were WT (wild-type) embryos in E3 with same concentrations of DMSO (**Figure 5, A and B**) as in Herbarin A and B solutions. This study had identified that Herbarin B is more biologically active than Herbarin A (**Figure 4 and 5**).

Lethality assay was performed at concentrations from 9 nM to 475 nM and these studies revealed that Herbarin B has an LD50 (50% lethal dose) of 190 nM (**Figure 4**). Herbarin B displayed dynamically increasing lethality from 9 nM to 190 nM concentrations beyond which it is completely lethal (**Figure 4**). Herbarin B was also 100% teratogenic at concentrations of 9 nM to 190 nM (**Figure 5**). Teratogenic phenotypes were specific showing a shortened body axis resembling gastrulation defects during early development, dorsal curvature of the trunk, tail malformations and pericardial edema (**Figure 5, E and F**; black arrows denote dorsal curvature of trunk and tail malformations). While Herbarin B was lethal and teratogenic, Herbarin A appeared to be a docile compound with no observed toxicity and teratogenicity at similar concentrations **(Figure 5, C and D**). The zebrafish assay was performed in triplicate. Statistical analyses using student t-test revealed significant difference (Figure 3; *$p<0.001$) between DMSO control and Herbarin B observations and no significant differences (Figure 3; **$p<0.001$) between DMSO control and Herbarin A treatment. In summary, the zebrafish assays have identified that Herbarin B is more biologically active compared to Herbarin A.

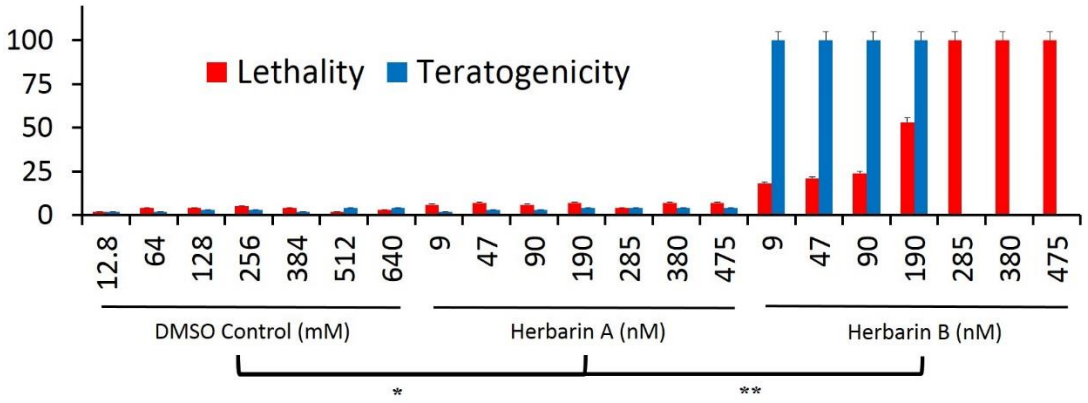


Figure 4: Quantitative summary of lethality and teratogenicity in zebrafish embryos

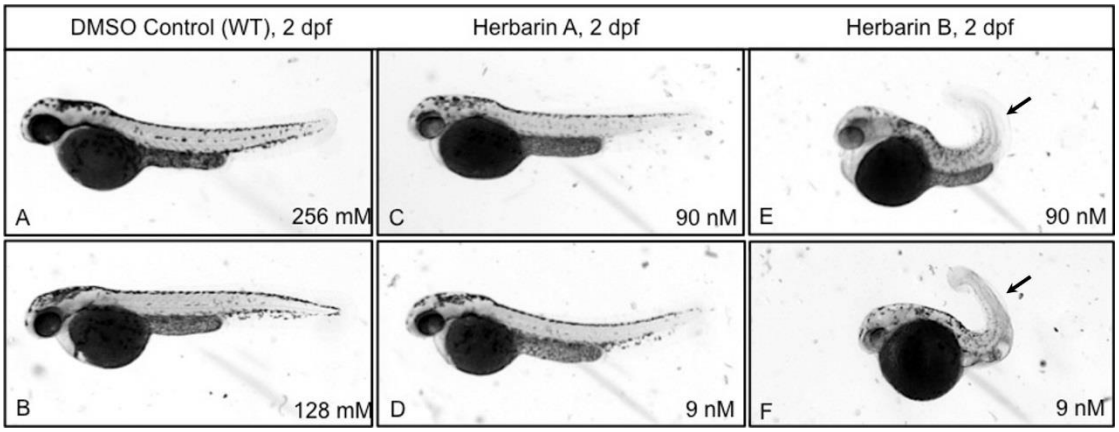


Figure 5: Zebrafish Development in Herbarin A and Herbarin B Treatment.

CHAPTER 3

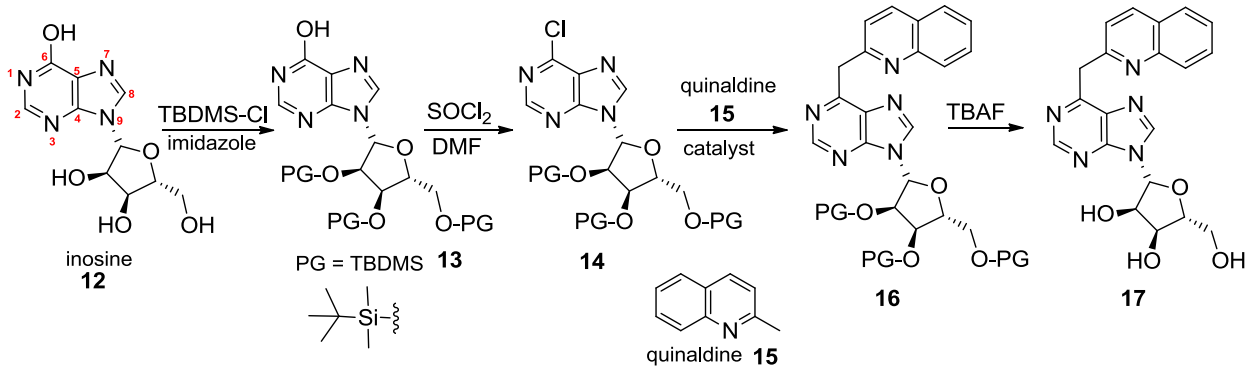
FUNCTIONALIZATION OF NUCLEOSIDES WITH 2-METHYL AZAARENES AT C-6 POSITION

3.1 INTRODUCTION

Recent investigations carried out in our laboratory proved the vital role of a Lewis acid $Yb(OTf)_3$ as a catalyst in Csp^3-H bond functionalization of methyl azaarenes with α-trifluoromethylated carbonyl compounds.[9] We utilized novel Csp^3-H bond functionalization of 2-methyl azaarenes with ethyl trifluoropyruvate and found the addition of 5 mol% of $Yb(OTf)_3$ catalyst (under optimized conditions) provided quantitative product yields. This methodology has been successfully extended for the functionalization of nucleosides with various azaarenes. Various reaction conditions including Lewis/Brønsted acids and bases were utilized to determine optimal reaction conditions for this synthesis.

3.2 RESULTS

Strategy 1: Inosine (**12**) was chosen as the nucleoside and quinaldine (**15**) as its azaarene counterpart. For the first step, inosine was reacted with TBDMS-Cl to fully protect all hydroxyl groups on the ribose (**13**). Treatment with $SOCl_2$ provided conversion of the hydroxyl group at C-6 position to –Cl, creating a better leaving group. The halogenated nucleoside product (**14**) was then subjected to Csp^3-H functionalization with quinaldine (**15**) in presence of various catalyst and reaction conditions for the displacement of chloride from the C-6 position as described below (**Scheme 5**).



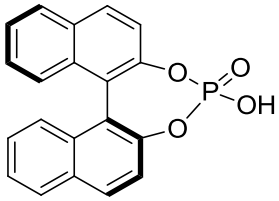
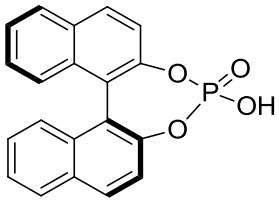
Scheme 5. Proposed synthetic plan for C-6 alkyl-quinoline nucleosides using C-6 chloro-inosine derivative

Conversion of the hydroxyl group at the C-6 position of the protected inosine product (**13**) was investigated. Optimization trials were then conducted by treatment with $SOCl_2$ under various solvent and reaction conditions. Protected inosine (100 mg, 0.1637 mmol) was dissolved in 10 mL dry DCM under argon, followed by the addition of DMF (0.5 mL). Reaction temperature was then reduced to 0°C in an ice bath, followed by the addition of $SOCl_2$ (1.2 equiv, 14.25 µL, 0.19646 mmol). The reaction mixture was then warmed to room temperature and allowed to proceed for 2 h. Solvent was then evaporated and the concentrated product was used as starting material for reaction optimization trials, targeting product (**16**) as depicted above (**Scheme 5**). Trial reactions with DBU under various temperature conditions of 80°C and 110°C for 12 h did not provide the expected product. Trials with NEt_3 (2 Equiv, 45.63 µL, 0.32744 mmol) were utilized instead of DBU. All other reaction conditions were carried out as described above.

Trials utilizing quinaldine (**15**) in presence of various catalyst and reaction conditions to facilitate displacement chloride from the C-6 position as described above (**Scheme 5)** did not provide satisfactory results. Further the role of various Lewis acid and Brønsted acid catalysts was

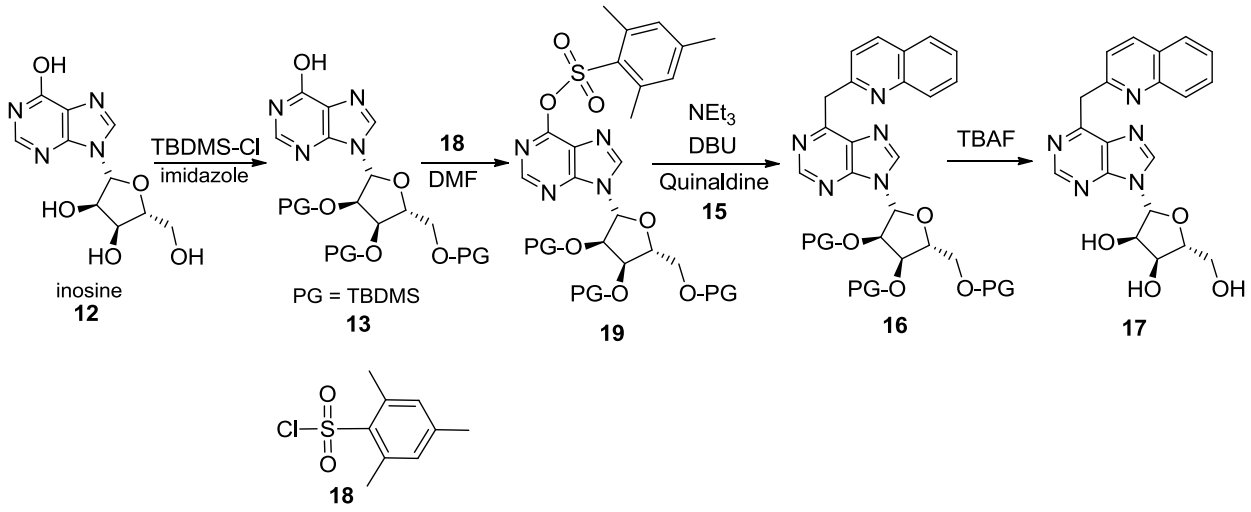
evaluated. Optimization reactions under various solvents, and temperature conditions are described in **Table 1**.

Table 1. Optimization of Various Lewis acid and Brønsted acid catalysts

Entry	Catalyst	Solvent	Temp (°C)	% Yield of (16)
1	$Yb(OTf)_{3.}$	Dioxane	80	0
2	$Yb(OTf)_{3.}$	Dioxane	100	0
3	$Yb(OTf)_{3.}$	Dioxane	110	0
4		Dioxane	80	0
5		Toluene	115	0
6	$RhCl(PPh_3)_3$	Dioxane	80	0
7	$RhCl(PPh_3)_3$	Toluene	115	0
8	$Pd(OAc)_2$	Dioxane	80	0
9	$RuCl_3$	Dioxane	80	0

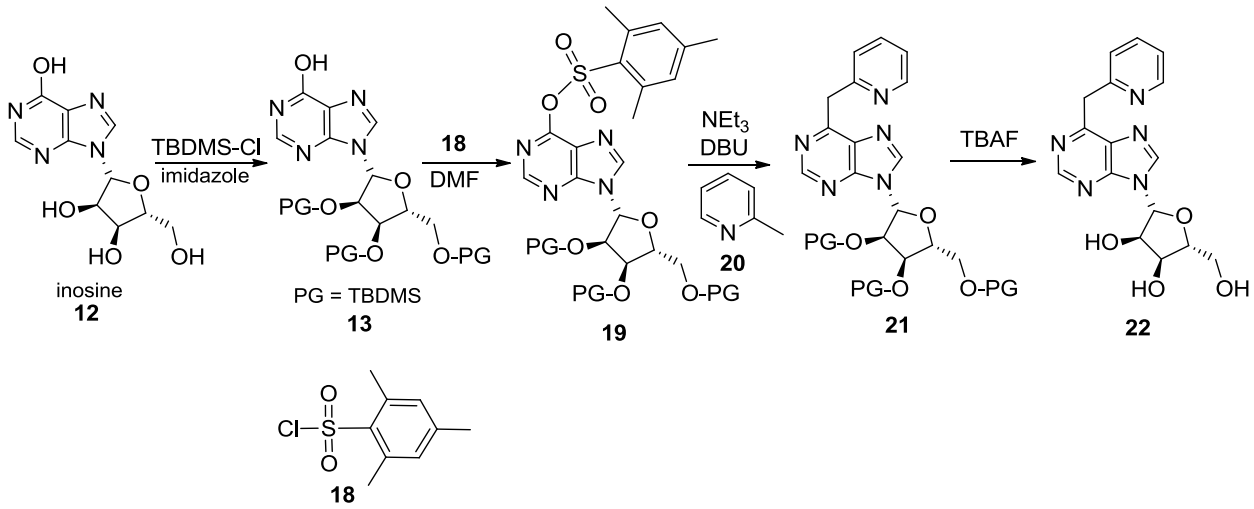
Strategy 2: C-6 hydroxy group was converted to mesitylenesulfonate (**19**) as described in the literature.[18] Further treatment with diazabicycloundecene (DBU) and triethylamine followed by addition of quinaldine (**15**) provided the expected TBDMS protected conjugated product (**16**)

under one pot conditions. Addition of a quinaldine nucleophile under S_NAr displacement mechanism of the mesitylenesulfonate provided C-6 substituted nucleoside. Deprotection with TBAF (Tetrabutylammonium fluoride) provided the 2-methyl azaarene-inosine conjugated product (**17**).



Scheme 6. Proposed synthetic plan for C-6 alkyl-quinoline nucleosides using C-6 mesitylenesulfonate inosine derivative

The above described reaction strategy was then utilized for the Csp^3-H bond functionalization of 2-methyl pyridine (**20**) with C-6 position of inosine (**Scheme 7**). The reaction worked as expected and the resulting conjugated product (**21**) was obtained in good yields. Further deprotection with TBAF provided the 2-methyl pyridine-inosine conjugated product (**22**).



Scheme 7. Proposed synthetic plan for C-6 alkyl-pyridine nucleosides

Conversion of the C-6 hydroxyl group was successfully achieved utilizing the bulky leaving group (**18**) will be subjected to the optimized reaction conditions with various substituted azaarenes. The methodology will also be utilized to functionalize other various nucleosides such as guanosine and 5-methyluridine to get a diverse group of compounds. All the products will be subjected to toxicity assay and also, their anti-viral properties will be determined. External collaboration will be sought in order to determine the anti-viral properties of the products synthesized.

CHAPTER 4

CONCLUSIONS AND FUTURE DIRECTIONS

In conclusion, the synthesis of Herbarin A and Herbarin B has been successfully achieved utilizing commercially available starting materials and following known literature synthetic protocols. All the products were characterized by use of analytical techniques. The antioxidant activity of target compounds was investigated. Furthermore, the toxicity assay using zebrafish embryo was also investigated. Herbarin B was found to exhibit toxicity and be lethal as determined by zebrafish embryo model.

Csp^3-H bond functionalization of 2-methyl azaarenes to protected inosine were found successful utilizing a bulky leaving group at the C-6 position of protected inosine. Future directions include optimization of reaction conditions to afford nucleoside derivatives. Additionally, various 2-methyl azaarenes will be functionalized at the C-6 position as described in the present work. To evaluate the generality of the above method, mesitylenesufonate will be subjected to the optimized reaction conditions with various substituted azaarenes. The methodology will also be utilized to functionalize various other nucleosides such as guanosine and 5-methyluridine to get a diverse group of compounds. All products will be subjected to toxicity assay and anti-viral properties will be determined.

APPENDIX A

ANALTICAL METHODS

MATERIALS: All the starting materials were purchased from Aldrich and used without further purification. Anhydrous solvents were obtained as follows: anhydrous tetrahydrofuran was distilled from sodium metal under argon, anhydrous dichloromethane was dried *via* distillation from CaH_2 immediately prior to use under argon, and anhydrous toluene was stored at 4 Å molecular sieves before use. Other solvents used in synthesis with minimum purity of 99.5% were Aldrich products. $CDCl_3$ and CD_3OD were used as solvents (99.8%) for the NMR studies. Thin Layer Silica Gel Chromatography Plates (60 Å, 250 μm thickness, F-254 indicator). Flash chromatography was performed using 230-400 mesh, 60 Å pore diameter silica gel. Chromatography solvents used were reagent grade.

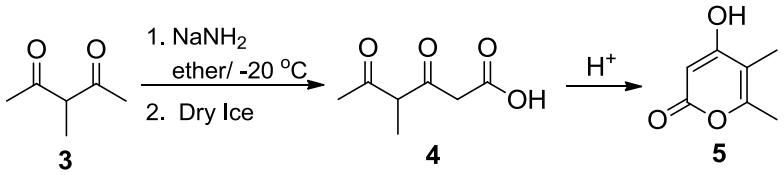
NMR ANALYSIS: The ^{1}H NMR spectra were obtained at 400 MHz using Agilent NMR spectrometer. ^{13}C NMR spectra were recorded at 100 MHz. Chemical shifts are reported in parts per million and are referenced to the deuterated residual solvent peak. NMR data is reported as: δ value (chemical shift, J-value (Hz), integration, where s = singlet, d = doublet, t = triplet, q = quartet, bs = broad singlet).

All the melting points are uncorrected and recorded on MEL-TEMP.

APPENDIX A

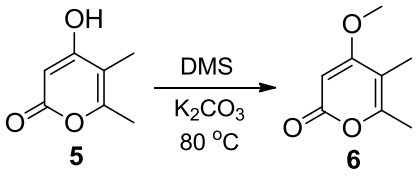
SUPPORTING INFORMATION CHAPTER 2

Synthesis of 4-hydroxy-5, 6-dimethyl-2-pyrone (5)



Compound (**5**) was synthesized using a modified approach as describe by Wilcox (*J. Am. Chem. Soc.* **1988**, *110*, 470-481). 3-Methyl-2,4-pentanedione (4.9 g, 42.9 mmol) was reacted with freshly prepared $NaNH_2$ and dry ice to obtain a diketoacid (**4**). Further treatment of (**4**) with HF provided the hydroxy pyrone (**5**) (2.33 g, 39 %) as red crystals. mp 209.2-210.5 ^{o}C.**^{1}H NMR** (400 MHz, CD_3OD), δ (ppm) 5.39 (s, 1H), 2.23 (s, 3H), 1.91 (s, 3H).**^{13}C NMR** (100.53 MHz, CD_3OD), δ (ppm) 173.4, 168.2, 160.6, 109.4, 89.6, 17.51, 9.56, **MS**-$C_7H_8O_3$ (140.05), m/z (%): Found 140 (M^+)

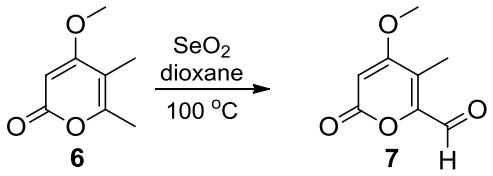
Synthesis of 4-methoxy-5,6-dimethyl-2-pyrone (6)



Hydroxy pyrone (660 mg, 4.7 mmol) and dimethyl sulfate (500 μL, 5.18 mmol) were placed in round bottom flaks along with 10 mL of acetone. Potassium carbonate (4 g, 6 eqv.) was the added and the reaction mixture was refluxed for 15 h under argon. The reaction mixture was filtered through sintered glass funnel; resulting filtrate was concentrated and then subjected to flash chromatography. Product eluted with 1:1 mixture of hexanes and ethyl acetate to get a yellow solid (586 mg, 81%) isolated yield. Yellow solid, mp 101.7-103.0 ^{o}C, **^{1}H NMR** (400 MHz, $CDCl_3$), δ (ppm) 5.38 (s, 1H), 3.76 (s,3H), 2.15

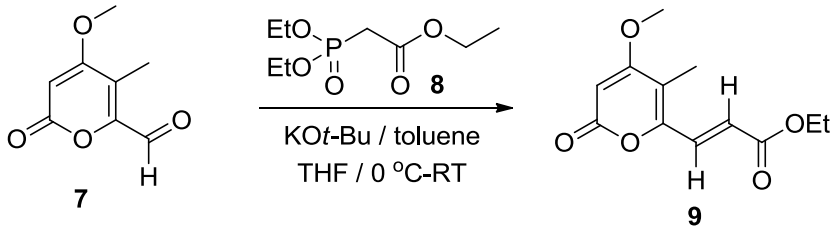
(s, 3H), 1.81 (s, 3H). **^{13}C NMR** (100.53 MHz, $CDCl_3$), δ (ppm) 170.9, 164.6, 157.6, 106.8, 87.8, 56.0, 17.2, 9.4, **MS**-$C_8H_{10}O_3$ (154.06), m/z (%): Found 154 (M^+)

Synthesis of 6-carboxaldehyde-4-methoxy-5-methyl-pyr-2-one (7)



Methoxypyrone (**6**) (535 mg) was placed in a round-bottom flask along with 10 mL 1,4-dioxane. Selenium dioxide (1.54 g, 4 eqv.) was added with vigorous stirring. The reaction mixture was flushed with argon and then refluxed at 100 °C for 12 h. The reaction mixture was filtered through cotton plug. The filtrate was then concentrated under reduced pressure and the crude mixture was subjected to flash chromatography to obtain a yellow solid (513 mg, 88%) yield. The starting material and product were inseparable with chromatography. Yellow solid, mp 94.1-96.8 °C (mixture), **^{1}H NMR** (400 MHz, $CDCl_3$), δ (ppm) 9.78 (s, 1H), 5.73 (s, 1H), 3.87 (s, 3H), 2.26 (s, 3H). **^{13}C NMR** (100.53 MHz, $CDCl_3$), δ (ppm) 169.0, 161.4, 148.2, 118.6, 94.4, 56.8, 8.2, **MS**-$C_8H_8O_4$ (168.04), m/z (%): Found 168 (M^+)

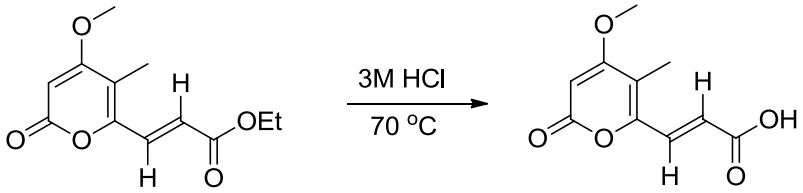
Synthesis of compound ester (9)



Phosphonate ester (**8**) (140 mg, 0.62 mmol) was placed in a round bottom flask along with 10 mL toluene. The mixture was flushed with argon and then cooled to 0 °C, successively potassium *tert*-butoxide (73 mg, 0.65 mmol) was added and the reaction mixture was stirred for 15 min at 0 °C. Aldehyde (**7**) (35

mg, 0.20 mmol) was dissolved in 5 mL tetrahydrofuran and then added drop-wise to above flask for over a period of 5 min. The reaction mixture then stirred at 0 ^{o}C for 1 h. The reaction was quenched with 10 mL water, warmed to room temperature and product was extracted in ethyl acetate. Chromatography purification using 2:8 hexanes/ethyl acetate mixture provided the expected product (**9**) as white solid (42.9 mg, 90%) yield. mp 143.0-144.1 ^{o}C, ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 7.45 (d, *J* = 15.9 Hz, 1H), 6.70 (d, *J* = 15.9 Hz, 1H), 5.59 (s, 1H), 4.22 (q, *J* = 7.20 Hz, 2H), 3.82 (s, 3H), 2.03 (s, 3H), 1.28 (t, *J* = 7.20 Hz, 3H), **^{13}C-NMR** (100.53 MHz, $CDCl_3$), δ (ppm) 169.6, 166.1, 162.4, 151.3, 130.77, 124.2, 113.5, 91.3, 60.9, 56.3, 14.1, 9.3, **MS**-$C_{12}H_{14}O_5$ (238.08), Found 238 (M^+)

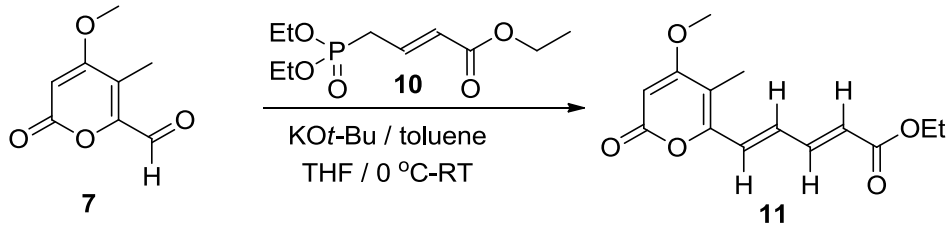
Synthesis of compound (2) (Herbarin B)



9 **2**

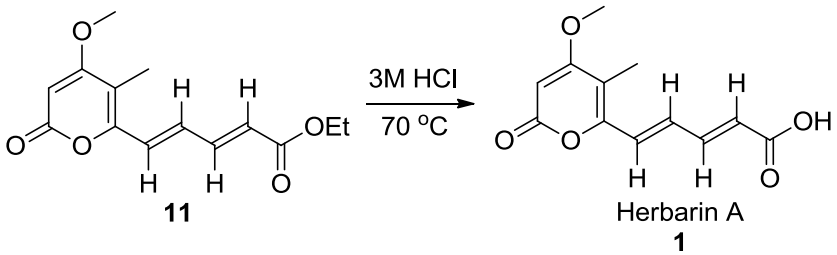
Ester (**9**) (25 mg, 0.105 mmol) was placed in a round-bottom flask along with 10 mL of 3M HCl. The mixture was heated to 70 ^{o}C and stirred for 12 h. The reaction mixture was cooled to 0 ^{o}C. Product was deposited at the bottom as white solid. This solid was washed with water (10 mL x 2), the product was then recrystallized with isopropyl alcohol to obtain a white solid (21.6 mg, 98 %) yield. mp 250.3-252 ^{o}C, **^{1}H-NMR** (400 MHz, CD_3OD) δ (ppm) 7.54 (d, *J* = 15.4 Hz, 1H), 6.61 (d, *J* = 15.4 Hz, 1H), 5.79 (s, 1H), 3.95 (s, 3H), 2.11 (s, 3H), **^{13}C-NMR** (100.53 MHz, CD_3OD), δ (ppm) 172.2, 169.0, 165.2, 152.7, 132.4, 125.4, 115.7, 92.1, 9.4, **MS**-$C_{10}H_{10}O_5$ (210.05), Found 210 (M^+)

Synthesis of compound ester (11)

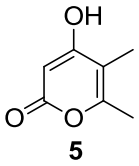


Phosphonate ester (**10**) (308 mg, 1.23 mmol) was placed in a round-bottom flask along with 10 mL toluene. The mixture was flushed with argon and then cooled to 0 ^{o}C, successively potassium *tert*-butoxide (143 mg, 1.27 mmol) was added and the reaction mixture was stirred for 15 min at 0 ^{o}C. Aldehyde (**7**) (69 mg, 0.41 mmol) was dissolved in 5 mL tetrahydrofuran and then added drop-wise to above flask for over a period of 5 min. The reaction mixture then stirred at 0 ^{o}C for 1 h. The reaction was quenched with 10 mL water, warmed to room temperature and product was extracted in ethyl acetate. Chromatography purification using 2:8 hexanes/ethyl acetate mixtures provided the expected product (**11**) as yellow solid (87.8 mg, 81 %) yield. mp 131.5-135.1 ^{o}C, **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm) 7.37 (dd, *J* = 3.67, 14.9 Hz, 1H), 7.19 (dd, *J* = 3.67, 14.92 Hz,1H), 6.68 (d, *J* = 15 Hz, 1H), 6.09 (d, *J* = 15 Hz, 1H), 5.55 (s, 1H), 4.22 (q, *J* = 7.20 Hz, 2H), 3.82 (s, 3H), 1.99 (s, 3H), 1.30 (t, *J* = 7.20 Hz, 3H), **^{13}C-NMR** (100.53 MHz, $CDCl_3$), δ (ppm) 170.1, 166.4, 163.1, 152.7, 142.3, 132.3, 126.2, 125.3, 110.8, 90.1, 60.6, 56.3, 14.2, 9.0, **MS**-$C_{14}H_{16}O_5$ (264.10), Found 264 (M^+)

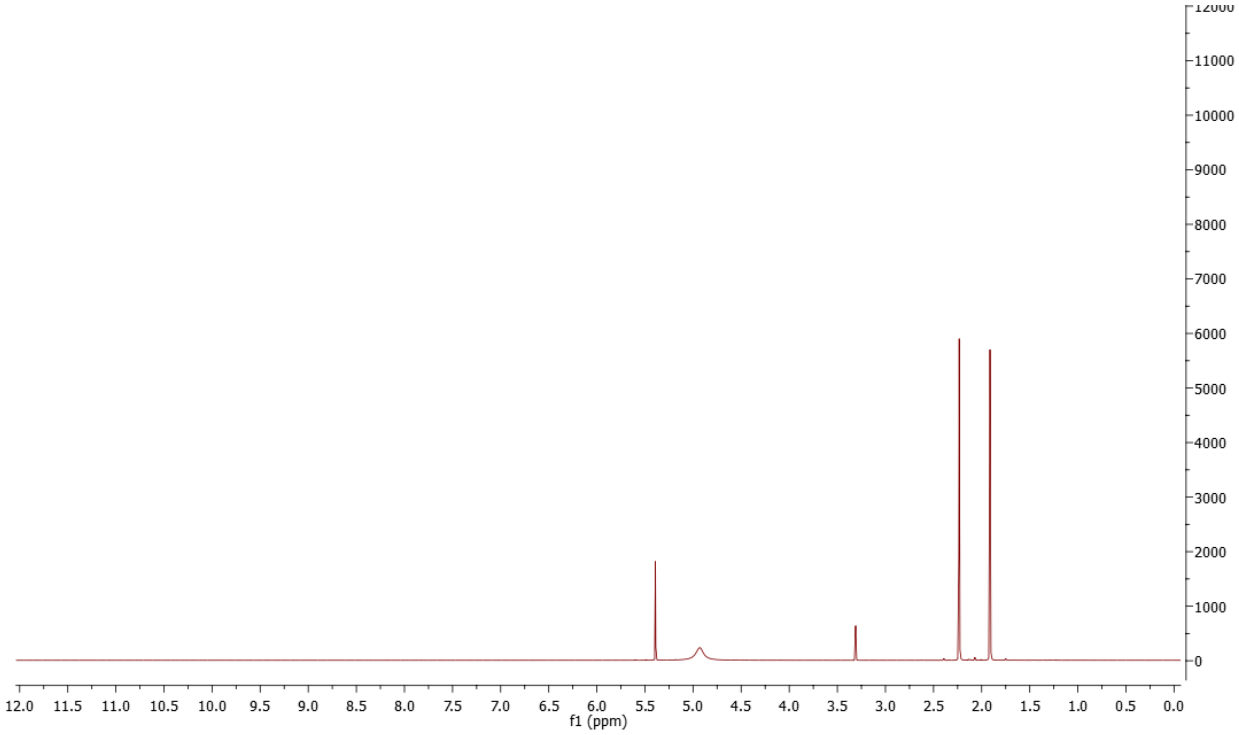
Synthesis of compound (1) (Herbarin A)

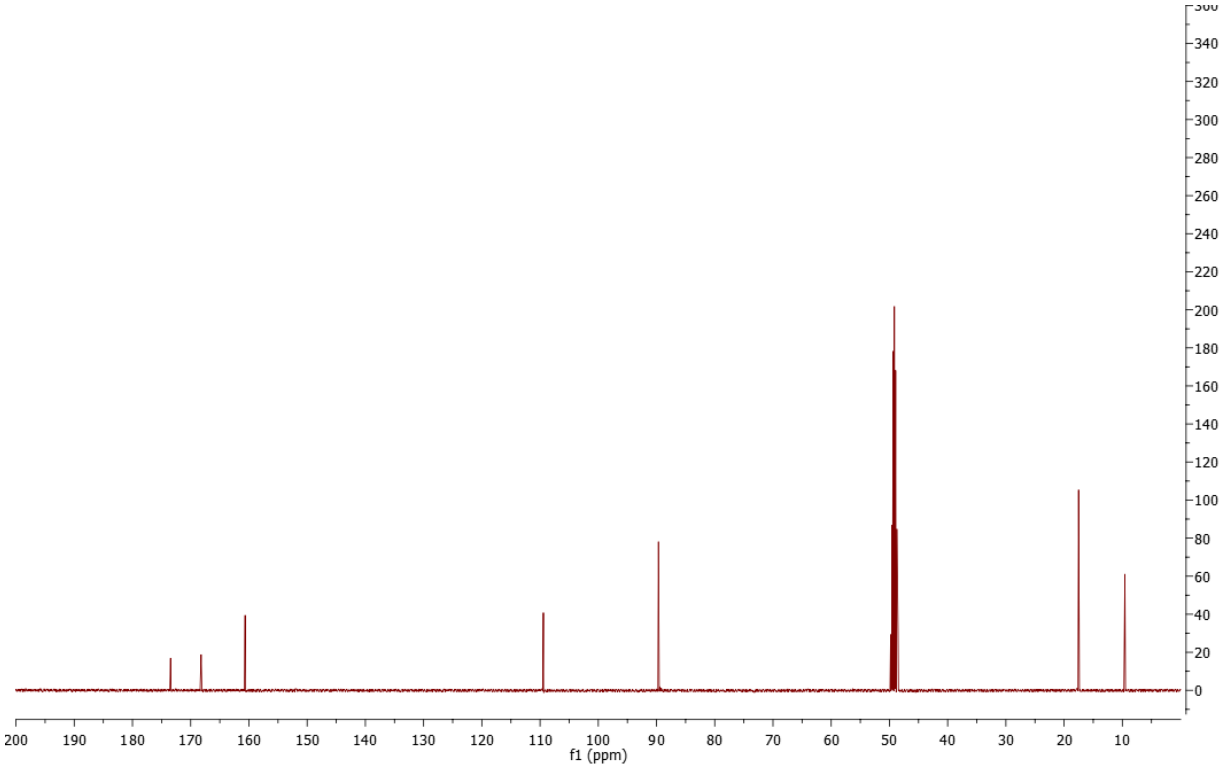


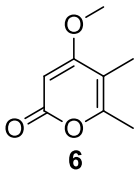
Ester (**11**) (48 mg, 0.182 mmol) was placed in a round-bottom flask along with 10 mL of 3M HCl. The mixture was heated to 70 ^{o}C and stirred for 12 h. The reaction mixture was cooled to 0 ^{o}C. Product was deposited at the bottom as white solid. This solid was washed with water (10 mL x 2), the product was then recrystallized with isopropyl alcohol to obtain a white solid (28.8 mg, 67 %) yield. mp 241.9-243.2 ^{o}C, **^{1}H-NMR** (400 MHz, CD_3OD) δ (ppm) 7.48 (dd, *J* = 3.60, 15 Hz, 1H), 7.19 (dd, *J* = 3.60, 15 Hz,1H), 7.01 (d, *J* = 15 Hz, 1H), 6.20 (d, *J* = 15 Hz, 1H), 5.70 (s, 1H), 3.92 (s, 3H), 2.07 (s, 3H), **^{13}C-NMR** (100.53 MHz, CD_3OD), δ (ppm) 172.2, 168.2, 165.6, 154.3, 144.4, 133.3, 128.3, 126.1, 113.0, 90.8, 57.0, 9.5, **MS**-$C_{12}H_{12}O_5$ (236.08), Found 236 (M^+)


OH
O
O

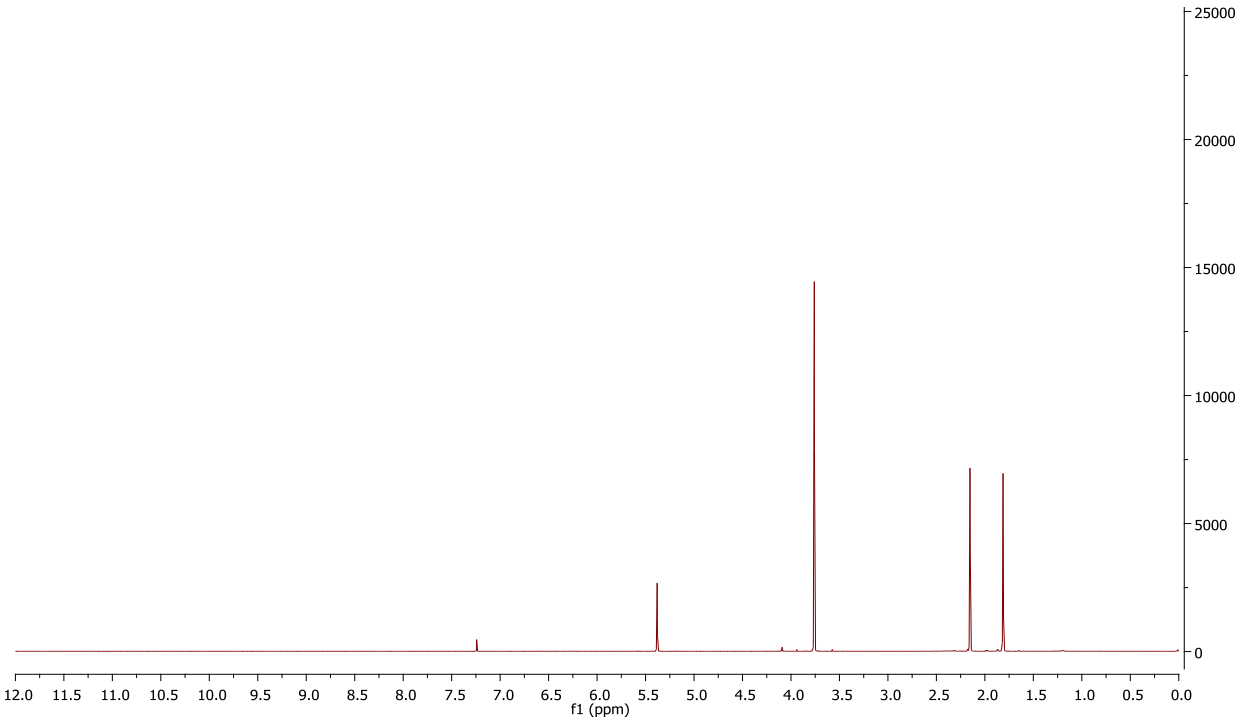
5

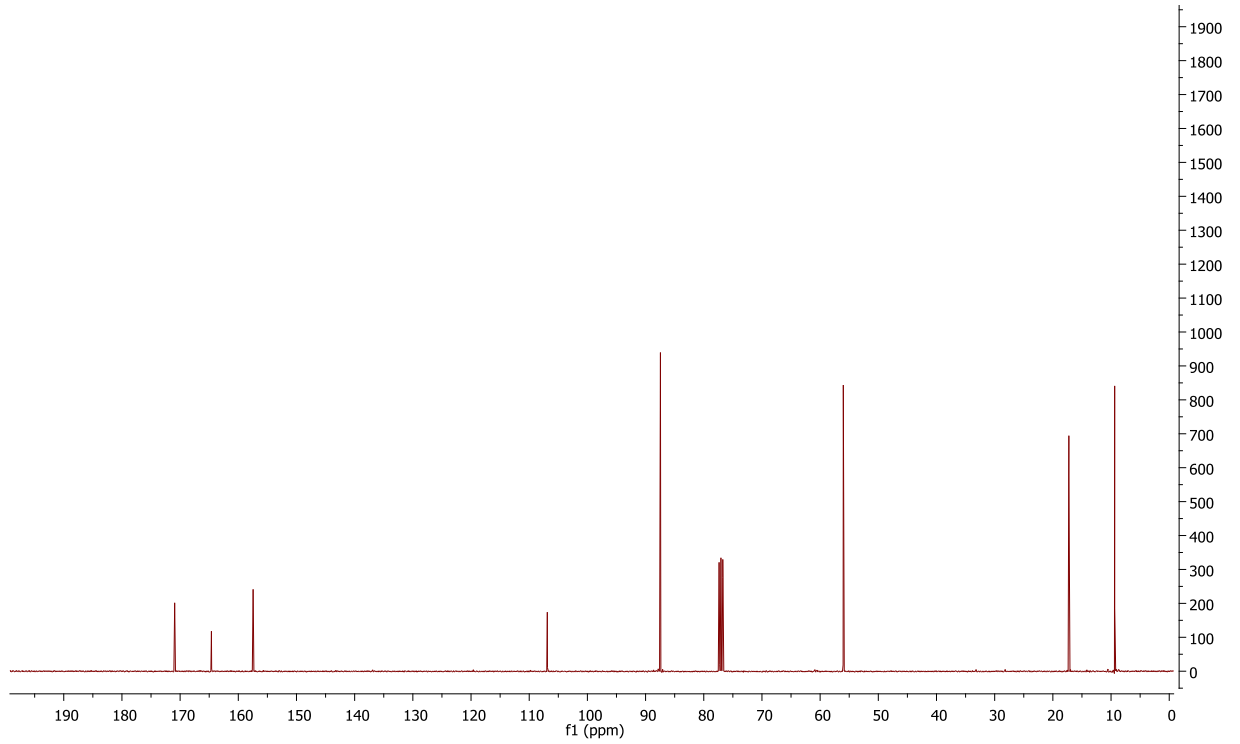

12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

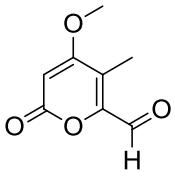

340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)



6

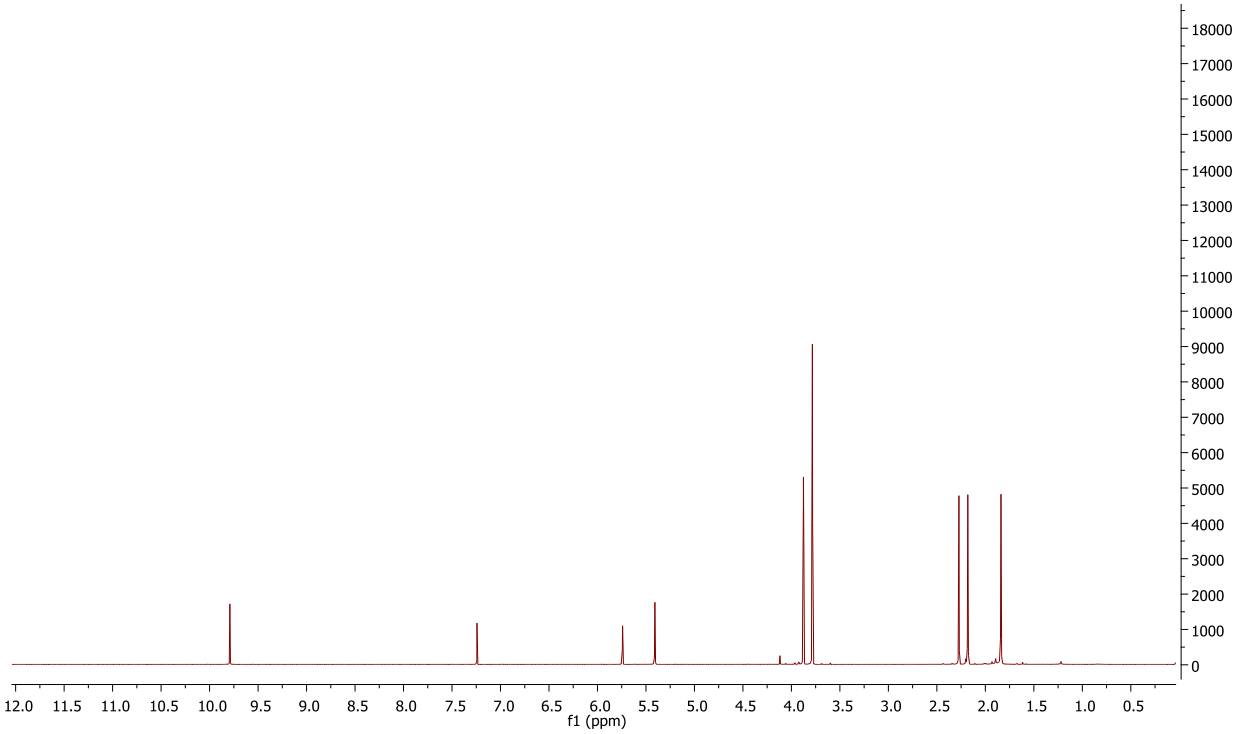

25000
20000
15000
10000
5000
0
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

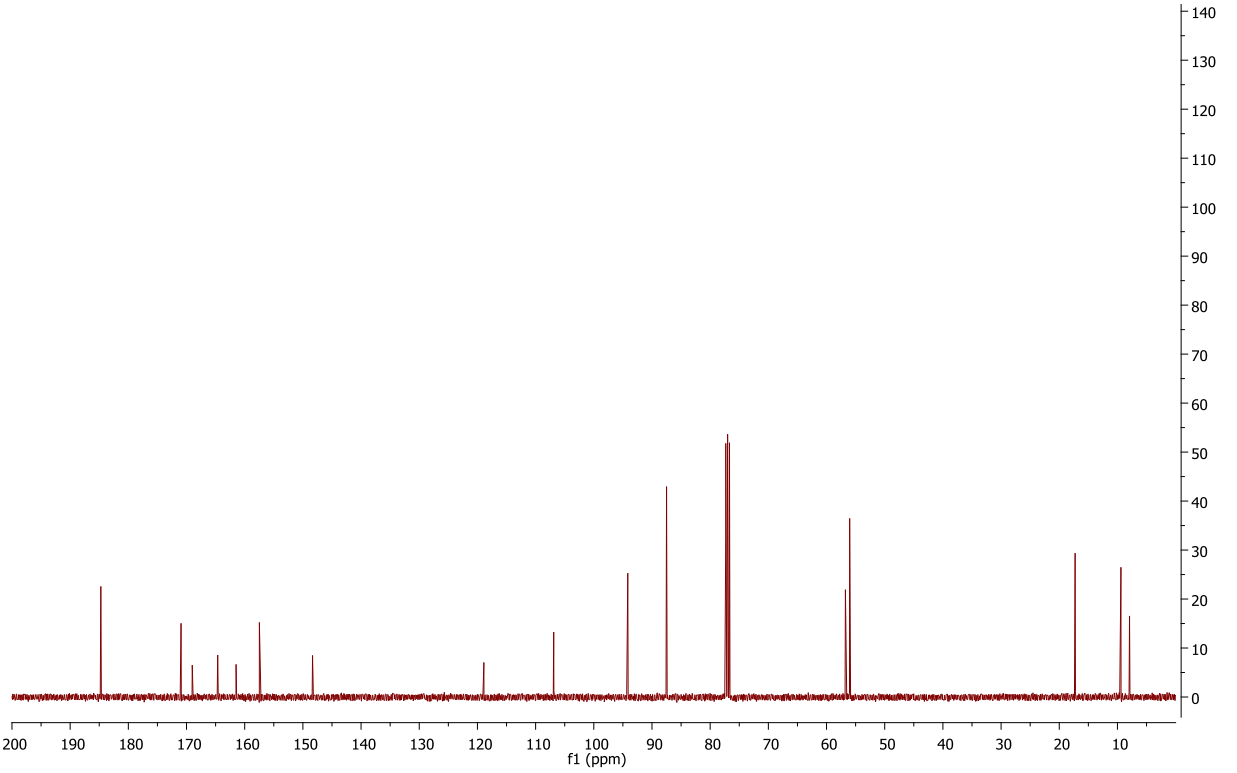

1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

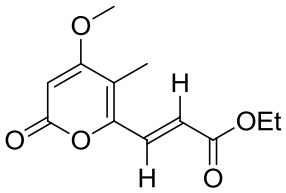


7

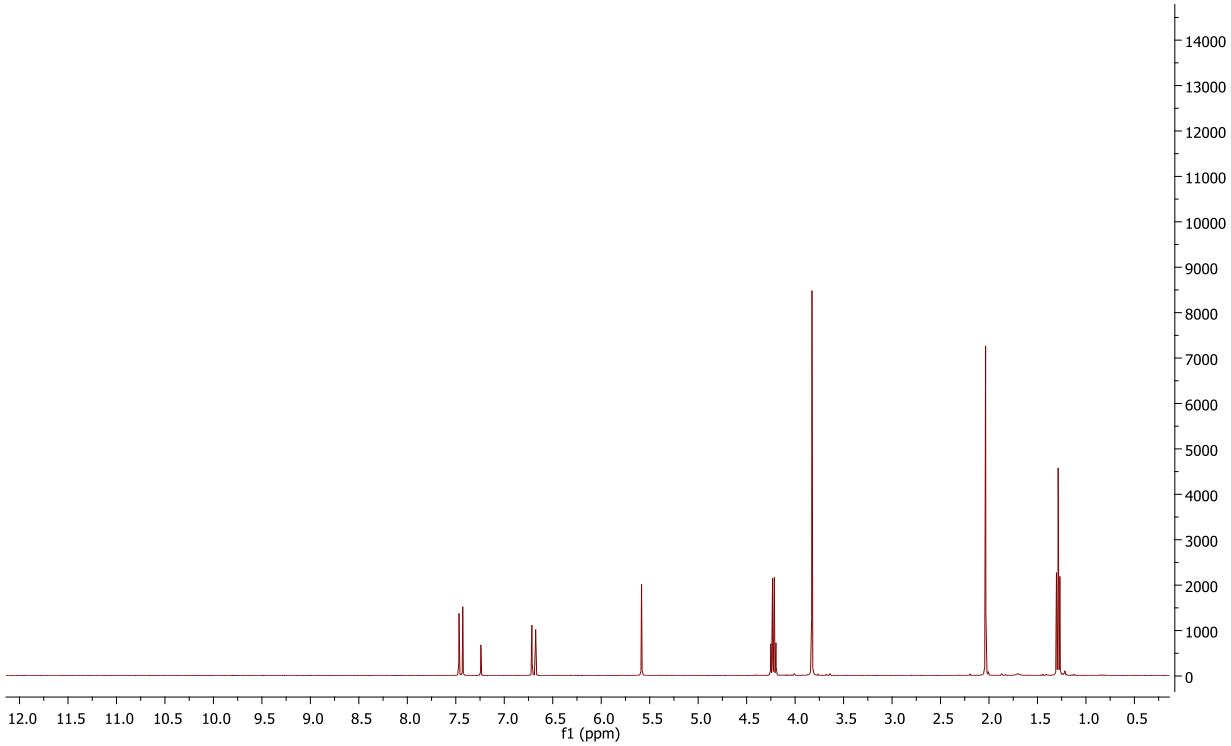
(Mixture 6 and 7)

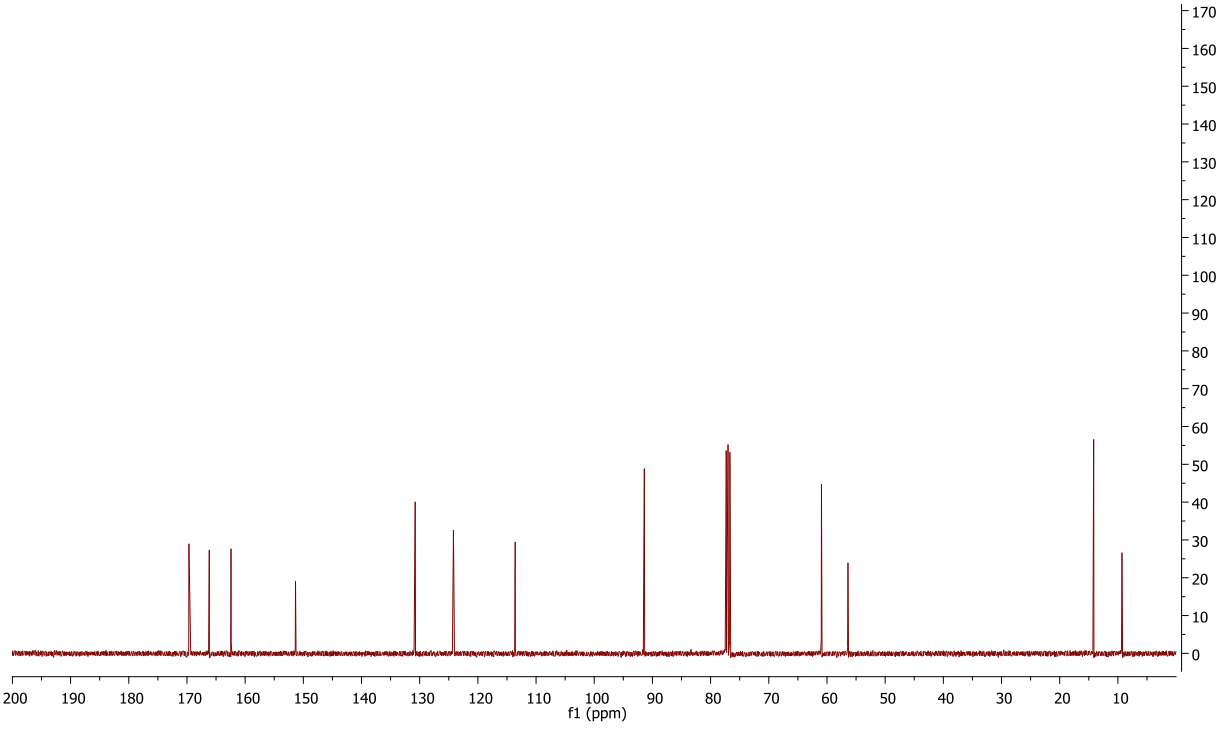


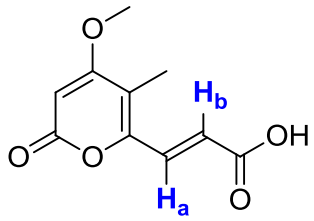



O
H
OEt
O
O
H
O

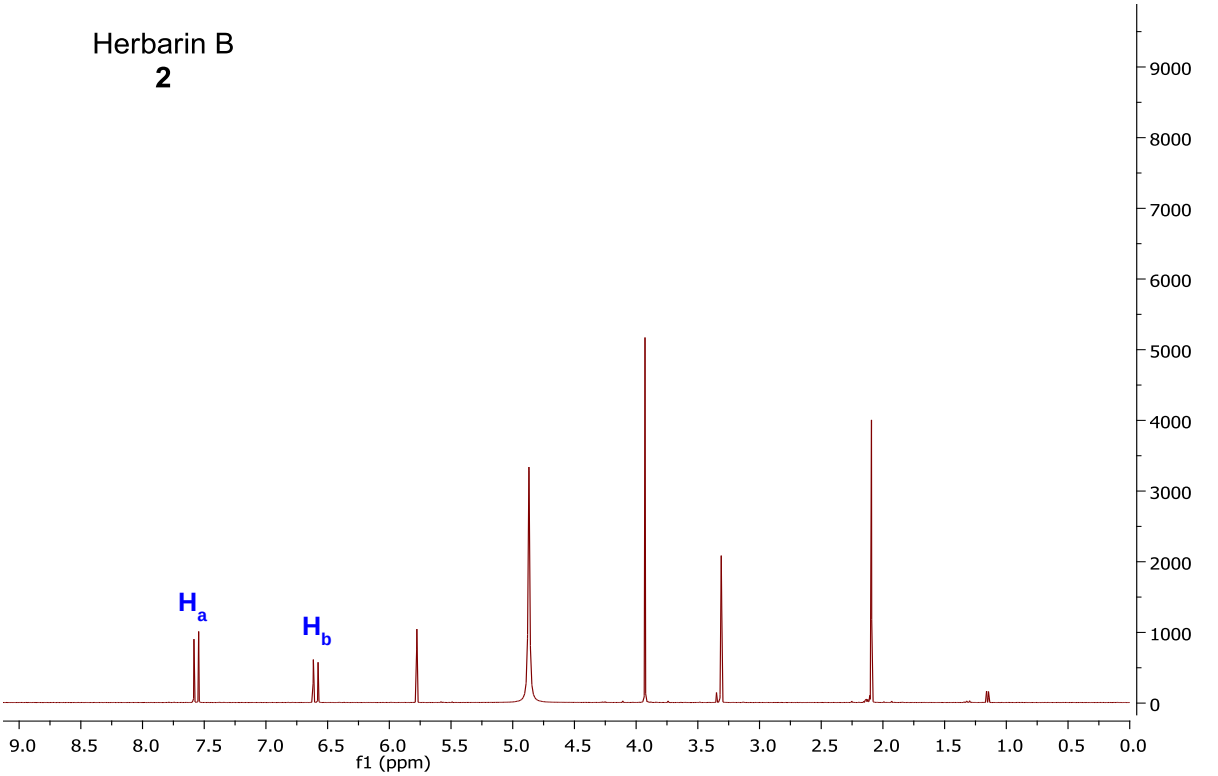
9

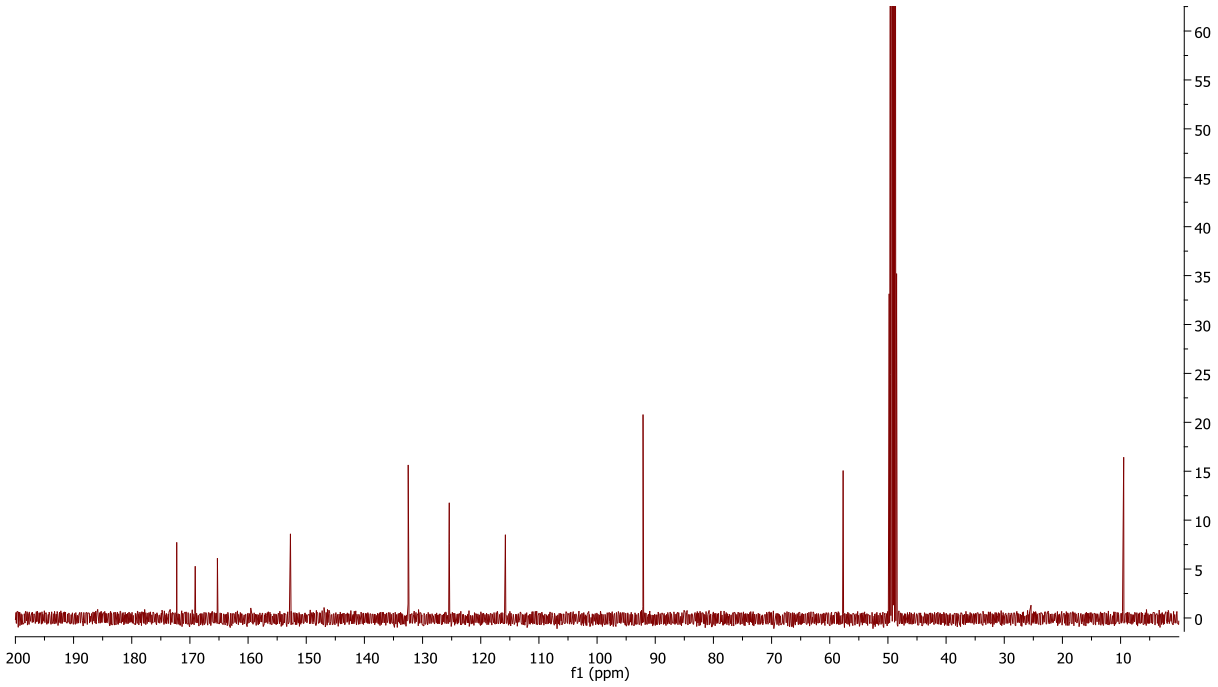

14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

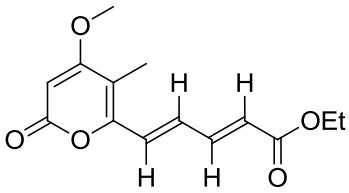

170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)


O
O
O
OH
O
Hb
Ha

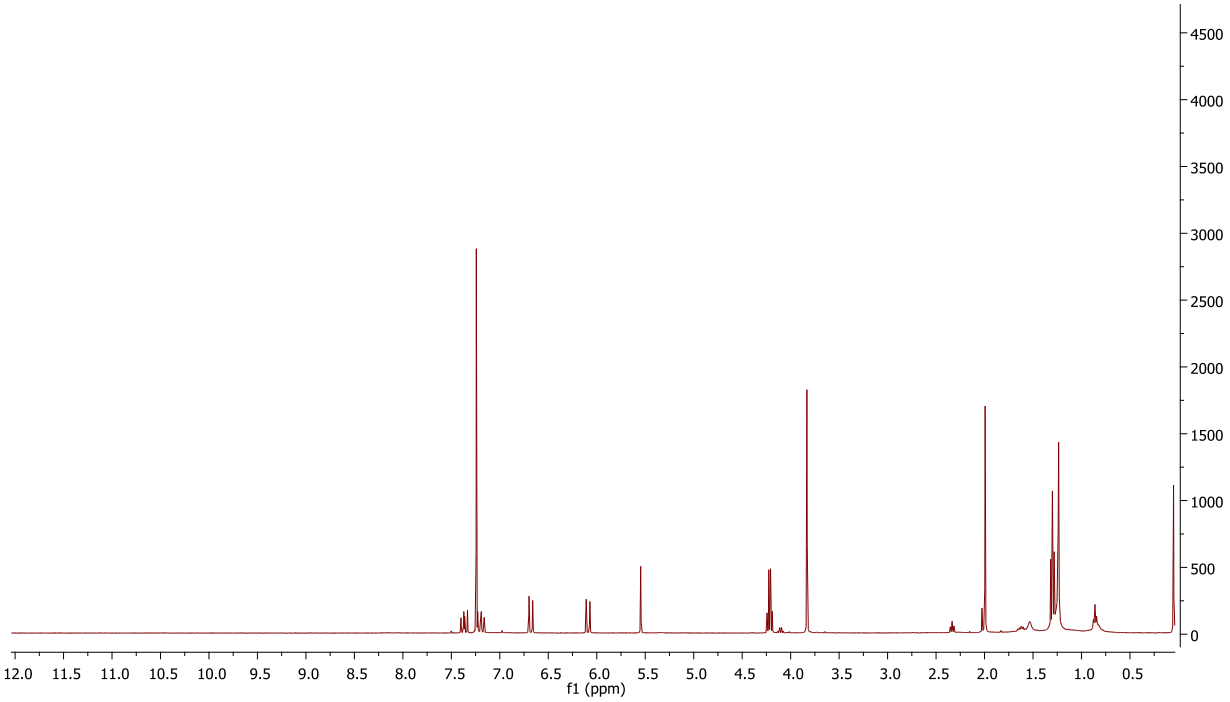
Herbarin B
2

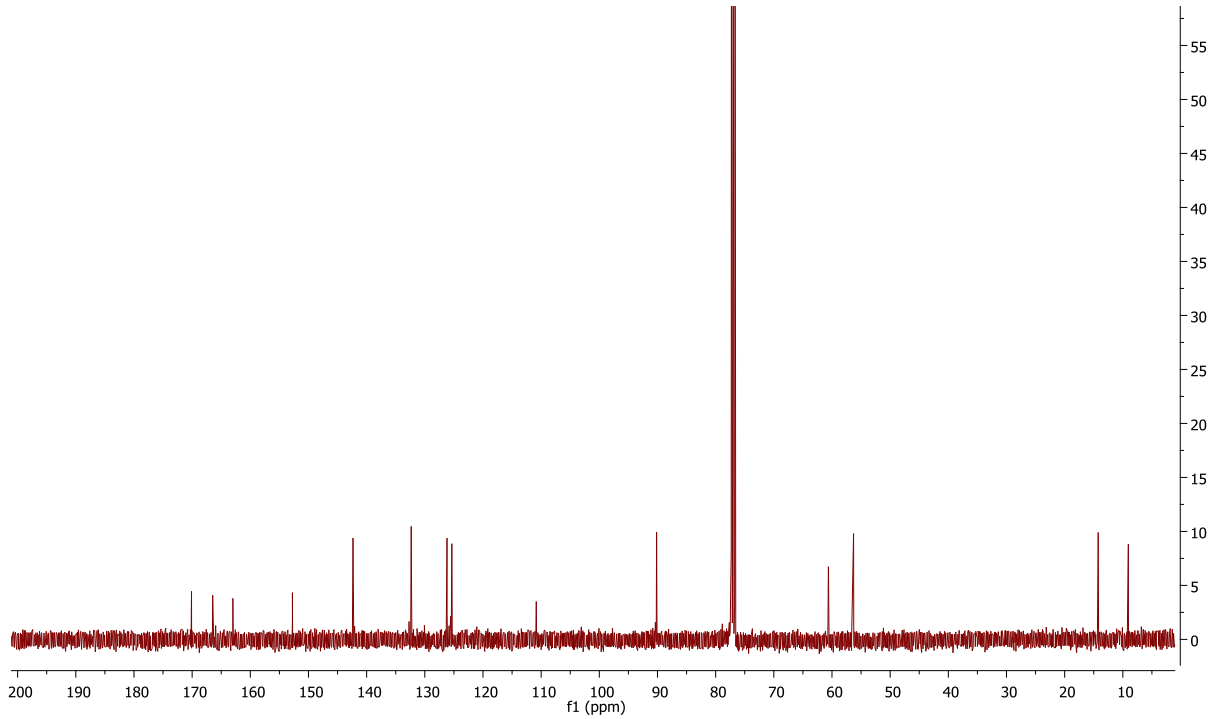

Ha
Hb
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

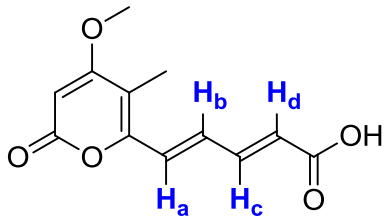

60
55
50
45
40
35
30
25
20
15
10
5
0
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)


O
H
H
OEt
O
O
H
H
O

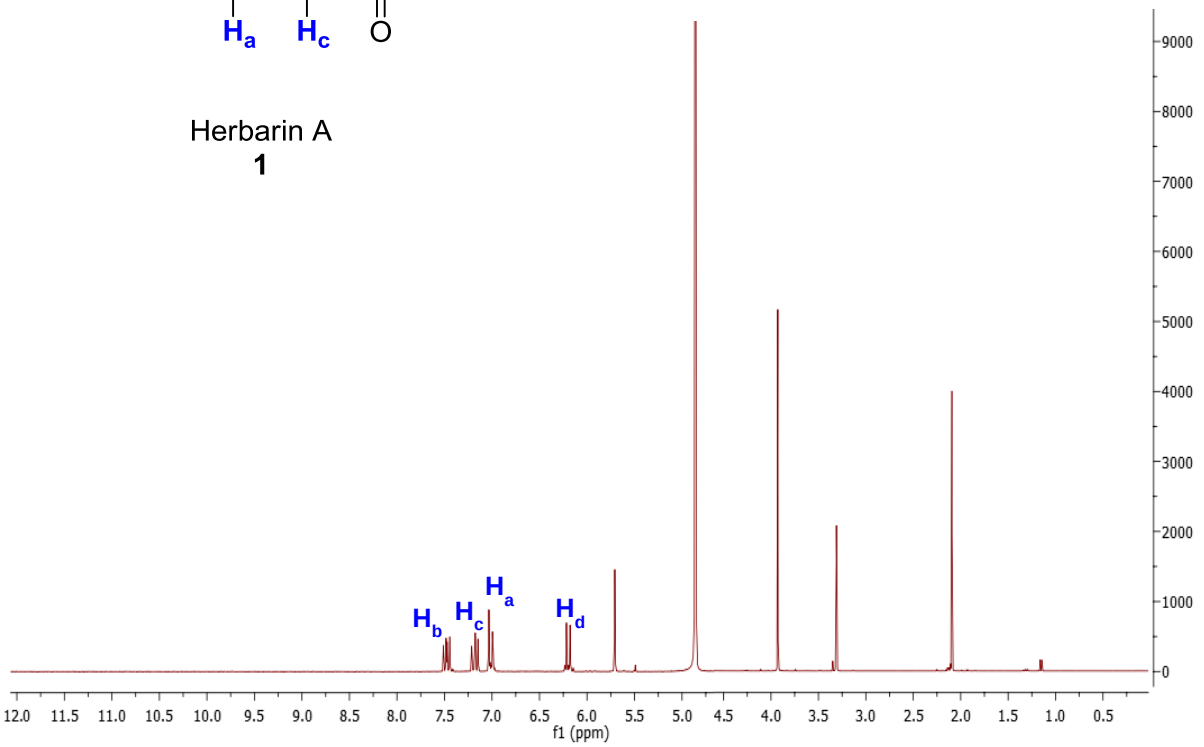
11

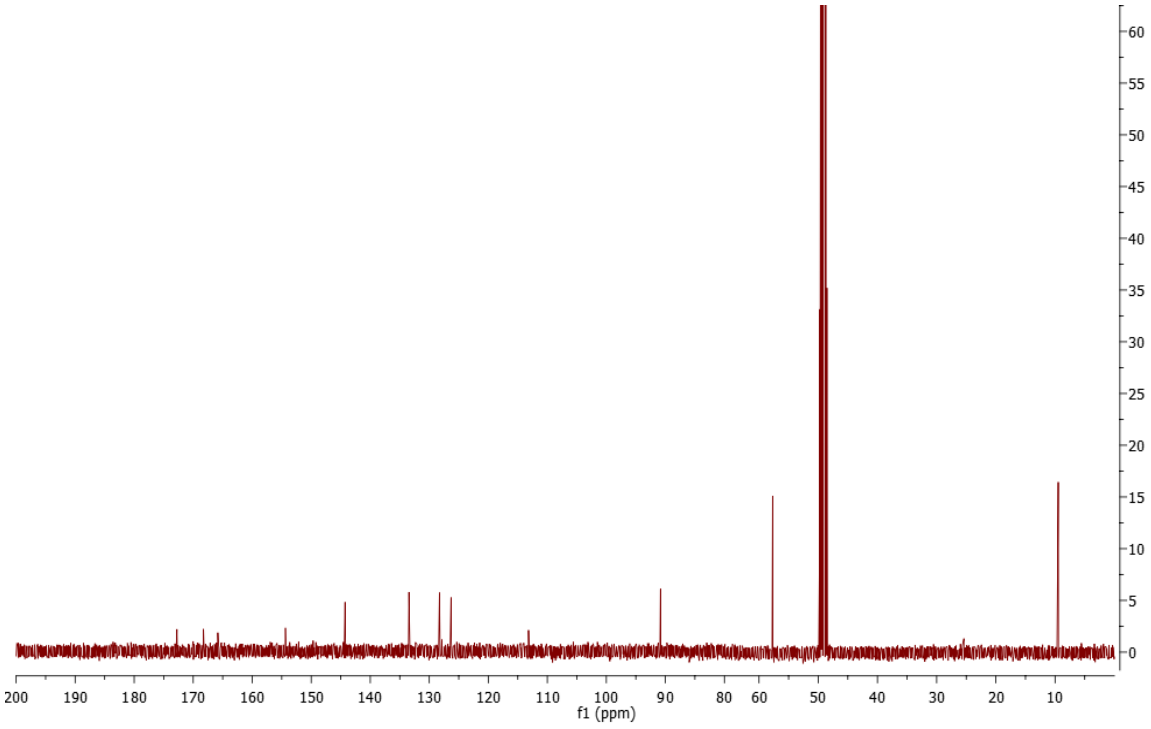

4500
4000
3500
3000
2500
2000
1500
1000
500
0
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)


55
50
45
40
35
30
25
20
15
10
5
0
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)



Herbarin A
1





APPENDIX B

ANALYTICAL METHODS

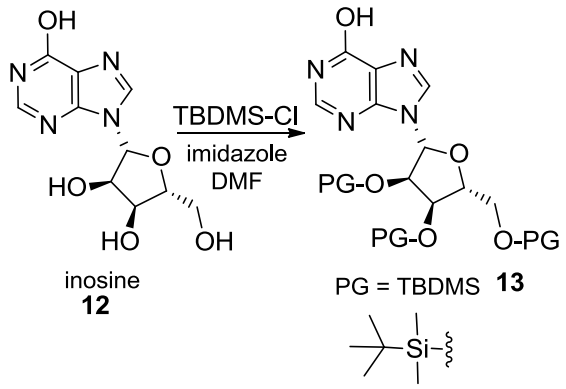
MATERIALS: All the starting materials were purchased from Aldrich and used without further purification. Anhydrous solvents were obtained as follows: Anhydrous tetrahydrofuran and anhydrous dichloromethane were dried *via* distillation from CaH_2 immediately prior to use under argon, and anhydrous DMF was stored at 4 Å molecular sieves before use. Other solvents used in synthesis with minimum purity of 99.5% were Aldrich products. $CDCl_3$ or $(CD_3)_2CO$ were used as a solvents (99.8%) for the NMR studies. Thin Layer Silica Gel Chromatography Plates (60 Å, 250 μm thickness, F-254 indicator). Flash chromatography was performed using 230-400 mesh, 60 Å pore diameter silica gel. Chromatography solvents used were reagent grade.

NMR ANALYSIS: The ^{1}H NMR spectra were obtained at 400 MHz using Agilent NMR spectrometer. ^{13}C NMR spectra were recorded at 100 MHz. Chemical shifts are reported in parts per million and are referenced to the deuterated residual solvent peak. NMR data is reported as: δ value (chemical shift, J-value (Hz), integration, where s = singlet, d = doublet, t = triplet, q = quartet, bs = broad singlet).

APPENDIX B

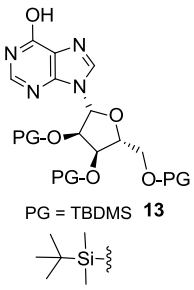
SUPPORTING INFORMATION CHAPTER 3

Synthesis of TBDMS protection of inosine (13)

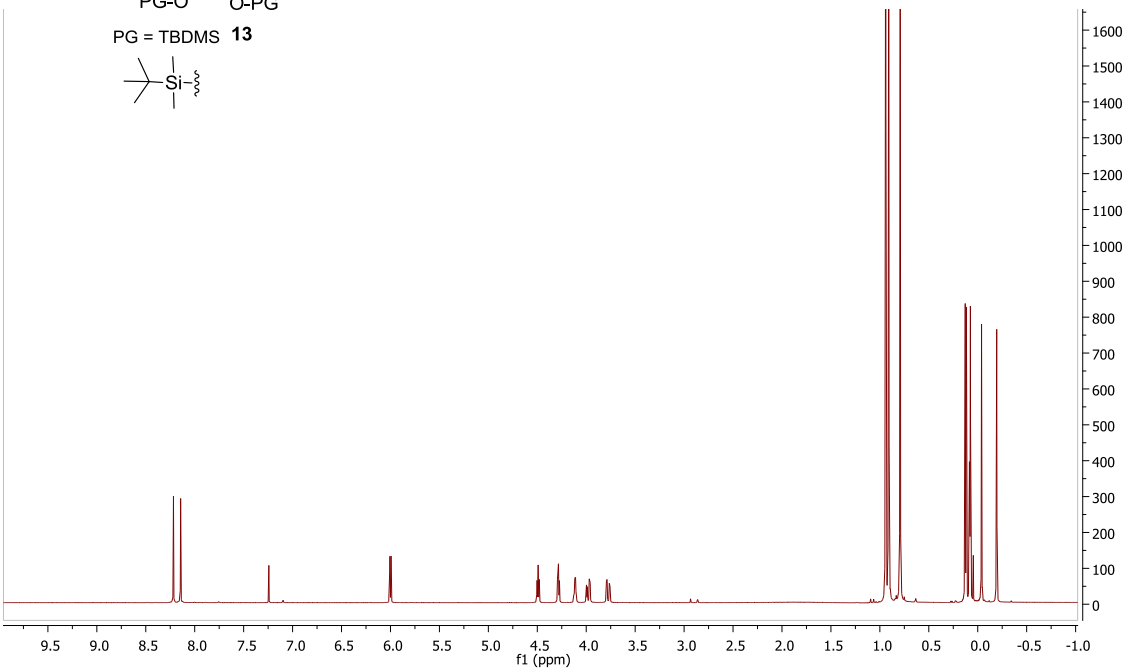


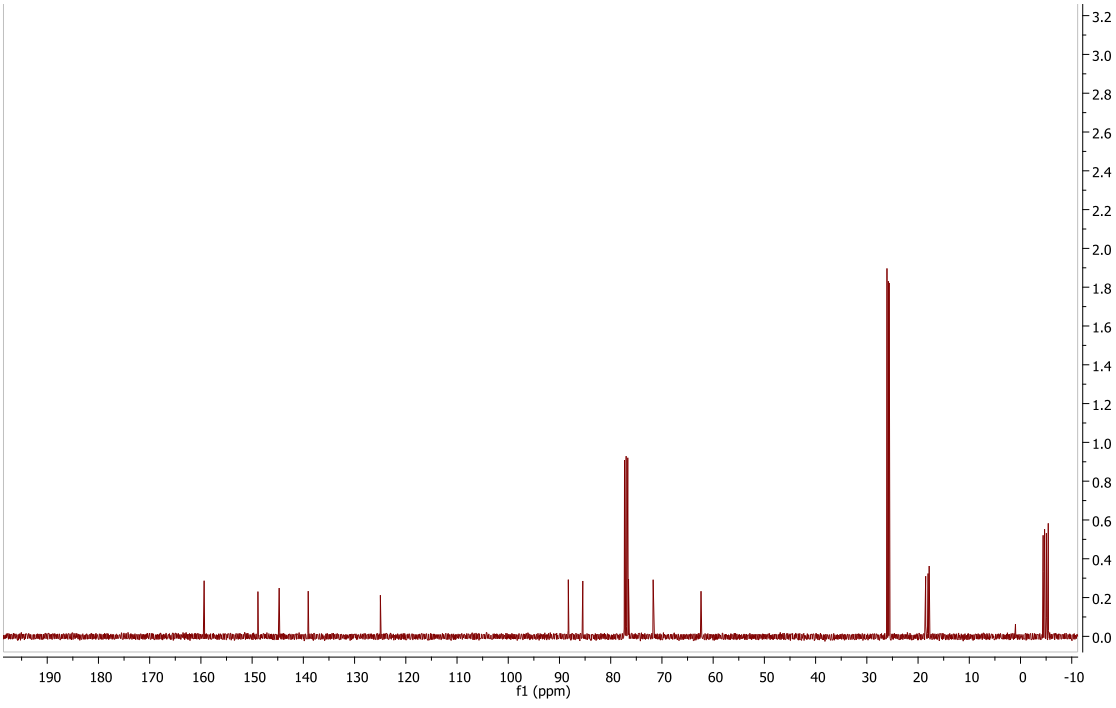
Inosine (**12**) (1g, 0.037 mmol) was placed in a 100 mL RB flask along with 15 mL of DMF. TBDMS-Cl (0.018 mmol) and imidazole (0.026 mmol) were added to the above reaction mixture. The reaction mixture was stirred at room temperature for 12 hours. The crude reaction mixture was then transferred to a separatory funnel, and product was extracted in DCM and washed with water. The organic layer was then passed over sodium sulfate and concentrated to remove residual excess solvent. The white solid was then washed with cold ethanol to remove the traces of TBDMS-Cl, to provide a white solid 634 mg.

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 8.22 (s, 1H), 8.14 (s, 1H), 6.01 (d, *J* = 4.9 Hz, 1H), 4.49 (t, *J* = 4.94 Hz, 1H), 4.28 (t, *J* = 4.82 Hz, 1H), 4.11 (dd, *J* = 3.71, 3.03 Hz, 1H), 3.98 (dd, *J* = 11.2, 4.16 Hz, 1H), 3.77 (dd, *J* = 11.2, 2.5 Hz, 1H), 0.94 (s, 9H), 0.91 (s, 9H), 0.74 (s, 9H), 0.13 (s, 1H), 0.12 (s, 1H), 0.08 (s, 1H), 0.07 (s, 1H), -0.04 (s, 1H), -0.19 (s, 1H). **^{13}C NMR** (100.53 MHz, $CDCl_3$), δ (ppm) 159.3, 148.8, 144.7, 139.0, 124.9, 88.2, 85.4, 76.5, 71.7, 62.3, 26.0, 25.8, 25.7, 18.5, 18.0, 17.8, -4.4, -4.7, -4.7, -5.0, -5.4, -5.4

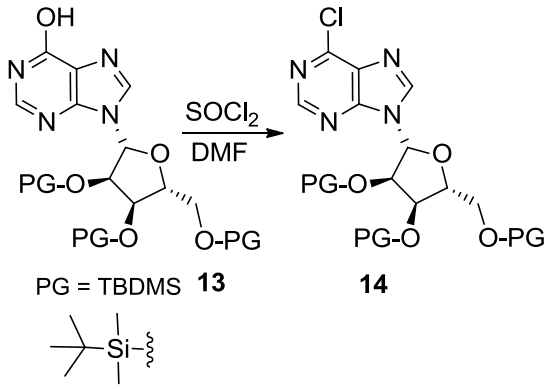


PG = TBDMS **13**



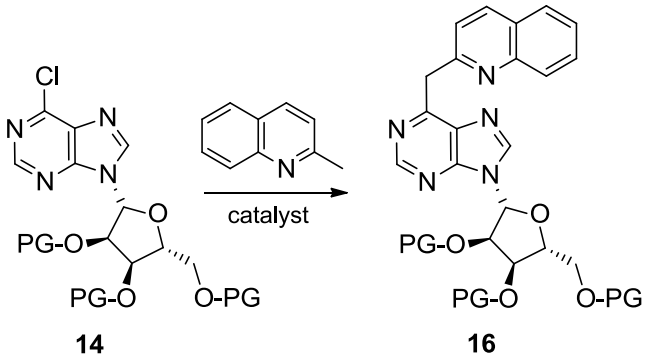


Synthesis of C-6 chloro-inosine (14)



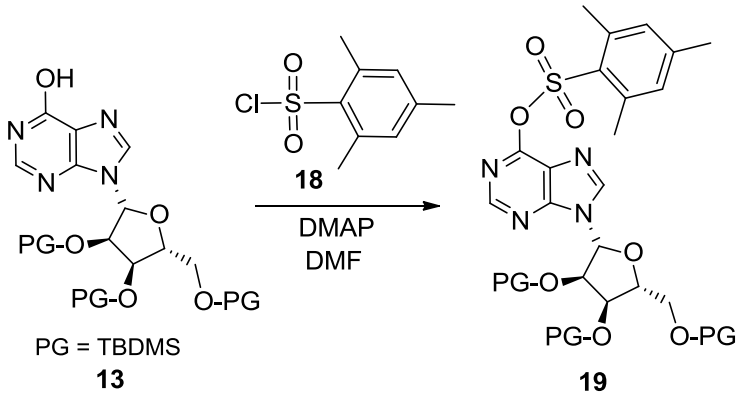
Protected inosine (**13**) (100 mg, 0.16 mmol) was in a 50 mL RB flask along with 10 mL DCM and the reaction mixture was purged with argon. Later 0.5 mL DMF and triethylamine (46 uL, 0.32 mmol) were added successively. The reaction mixture was then cooled to 0˚C and thionyl chloride (15 uL, 0.19 mmol) was added drop-wise. The reaction was allowed to warm to RT and stirred for 3 h. After complete conversion, as indicated on TLC, excess solvent was evaporated under reduced pressure and resulting product was used for next step without further purification.

Synthesis of 2-methyl azaarene C-6 conjugated product (16)



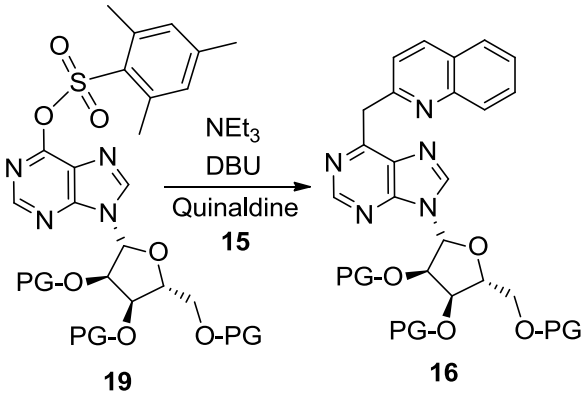
C-6 chloro inosine (**14**) (50 mg) was placed on a 25 mL RB flask and 5 mL of Dioxane was added under argon. DBU (200 uL) was added and the reaction mixture was heated to 80 °C for 12 h. No product formation was observed on TLC so the same reaction mixture was then further heated to 110 °C for 12 h. No product formation was observed under basic conditions. Similar reaction parameters were then tried using a Lewis acid catalyst $Yb(OTf)_3$ at 110 °C, however, the reaction did not provide the expected product.

Reaction C-6 hydroxyl inosine with 2-mesitylenesulfonyl chloride (19)



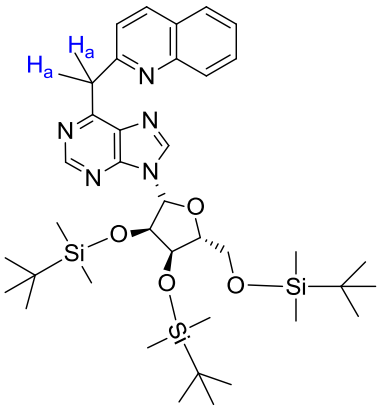
Protected inosine (**13**) (100 mg, 0.16 mmol) was dissolved in 5 mL of DCM, followed by addition of DMAP (2.0 mg, 0.016 mmol) and triethylamine (45.7 uL, 0.32 mmol) into a RB flask under argon. 2-mesitylene sulfonyl chloride (**18**) (71.7 mg, 0.32 mmol) was then added to the reaction mixture and allowed to proceed at RT for 3 hours. After the complete conversion, the reaction mixture was diluted with DCM and poured into a separatory funnel along with 50 mL water. The product was extracted in DCM and washed with water followed by dilute $NaHCO_3$ solution. The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure to obtain a viscous liquid. The product was used further without any purification.

Reaction of C-6 mesitylenesulfonate with quinaldine (16)



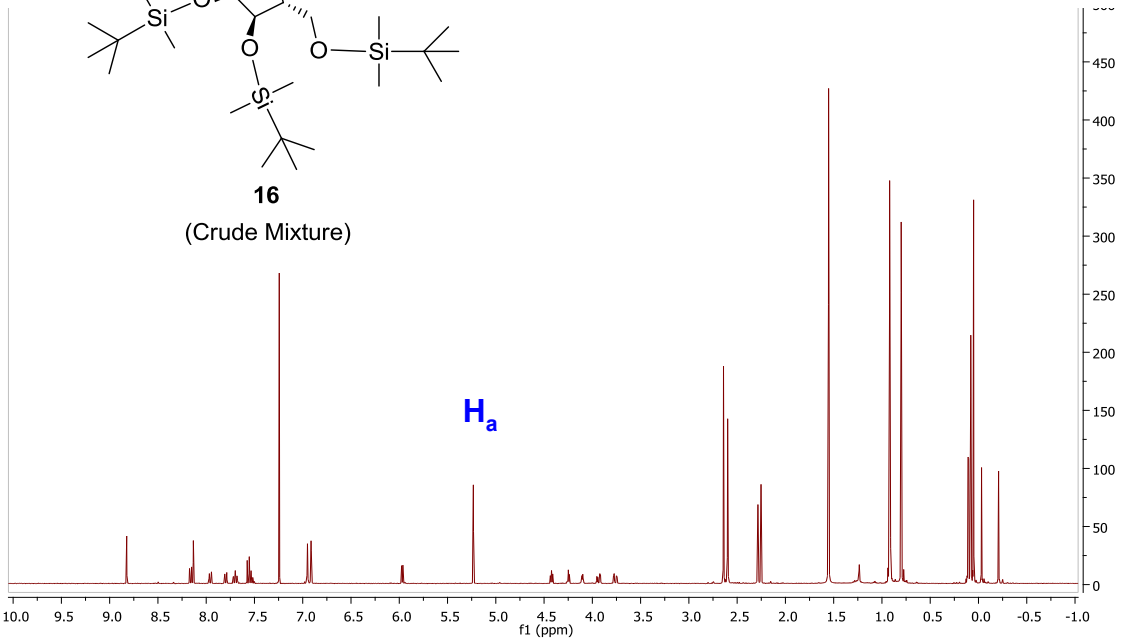
Product (**19**) was dissolved in dichloromethane (30 mL) and was added to quinaldine (31.3 mg) along with triethylamine (365 uL) at 0°C, and the resulting mixture was stirred for 10 min. DBU (30 uL) was added, and stirred for 10 h at RT. The reaction mixture was poured into dichloromethane, washed with water and brine, and concentrated. The crude product was purified using flash chromatography (1:1 ethyl acetate/hexane) to give expected conjugated product (**16**).

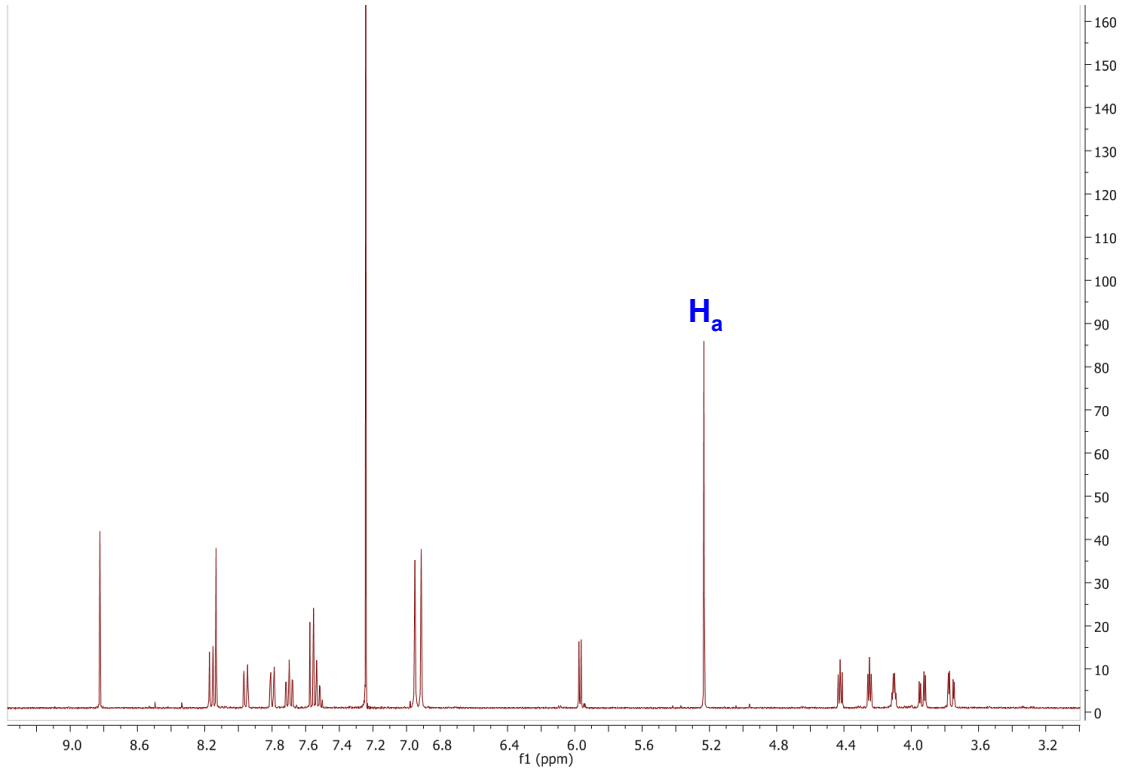
^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 8.82 (s, 1H), 8.16 (t, *J* = 8.04 Hz, 1H), 7.96 (d, *J* = 8.04 Hz, 1H), 7.80 (d, *J* = 7.55 Hz, 1H), 7.70 (dt, 7.92 Hz, 1H), 7.56 (d, *J* = 9.16 Hz, 1H), 7.53 (t, *J* = 7.18 Hz, 1H), 6.93 (d, *J* = 15.3 Hz, 1H), 6.01 (d, *J* = 5.19 Hz, 1H), 5.23 (s, 2H), 4.42 (t, *J* = 4.12 Hz, 1H), 4.24 (t, *J* = 4.19 Hz, 1H), 4.10 (dd, *J* = 3.45, 3.11 Hz, 1H), 3.93 (dd, *J* = 11.8, 4.0 Hz, 1H), 3.76 (dd, *J* = 11.8, 2.11 Hz, 1H), 0.92 (s, 9H), 0.91 (s, 9H), 0.80 (s, 9H), 0.11 (s, 1H), 0.10 (s, 1H), 0.08 (s, 1H), 0.05 (s, 1H), -0.03 (s, 1H), -0.20 (s, 1H). **^{13}C NMR** (100.53 MHz, $CDCl_3$), δ (ppm) 145.1, 143.1, 141.2, 140.0, 138.1, 137.1, 132.2, 131.7, 129.7, 129.0, 128.1, 127.6, 126.9, 119.2, 88.1, 85.6, 71.5, 26.0, 25.7, 25.6, 22.6, 22.5, 0.9, -4.4, -4.6, -4.7, -4.7, -4.7, -5.0, -5.4, -5.4

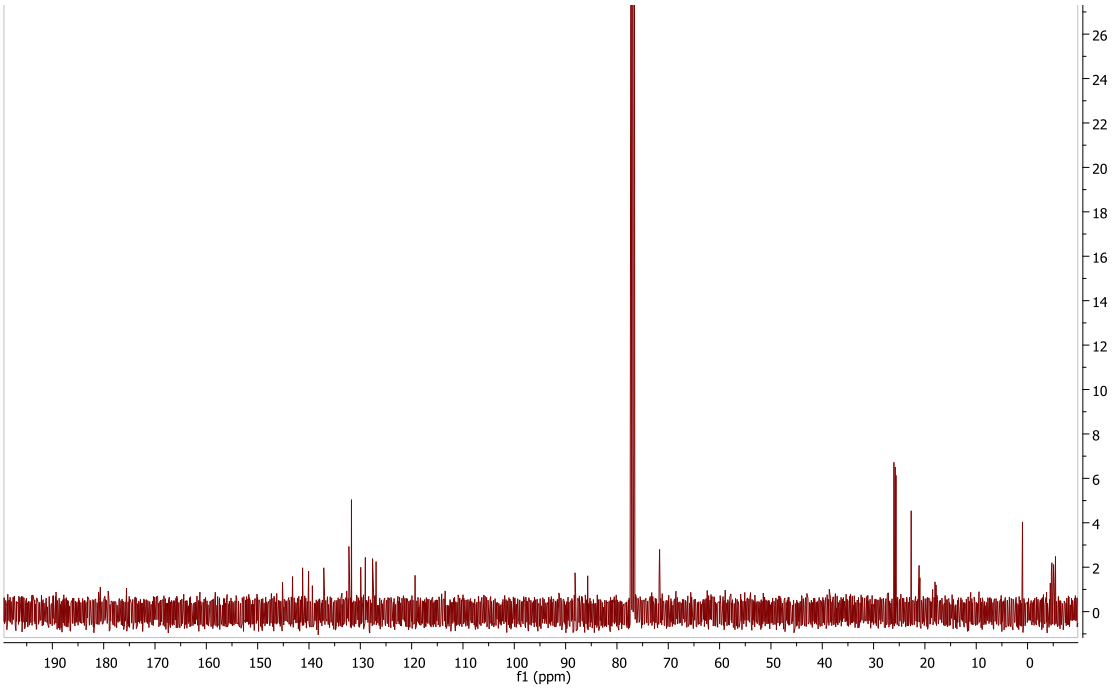


16

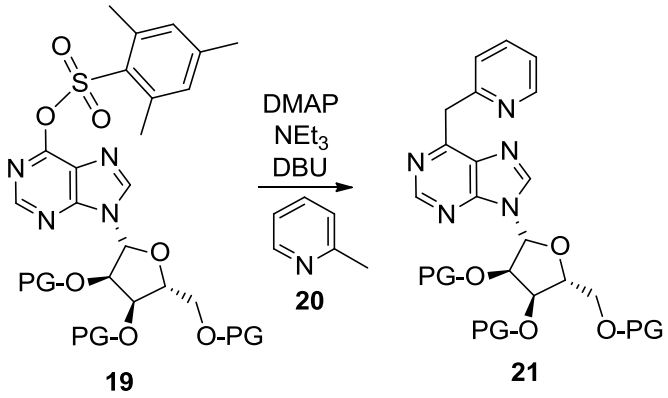
(Crude Mixture)





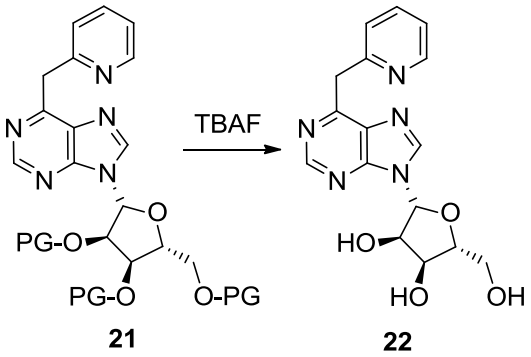


Reaction of C-6 mesitylenesulfonate with 2-methyl pyridine (21)



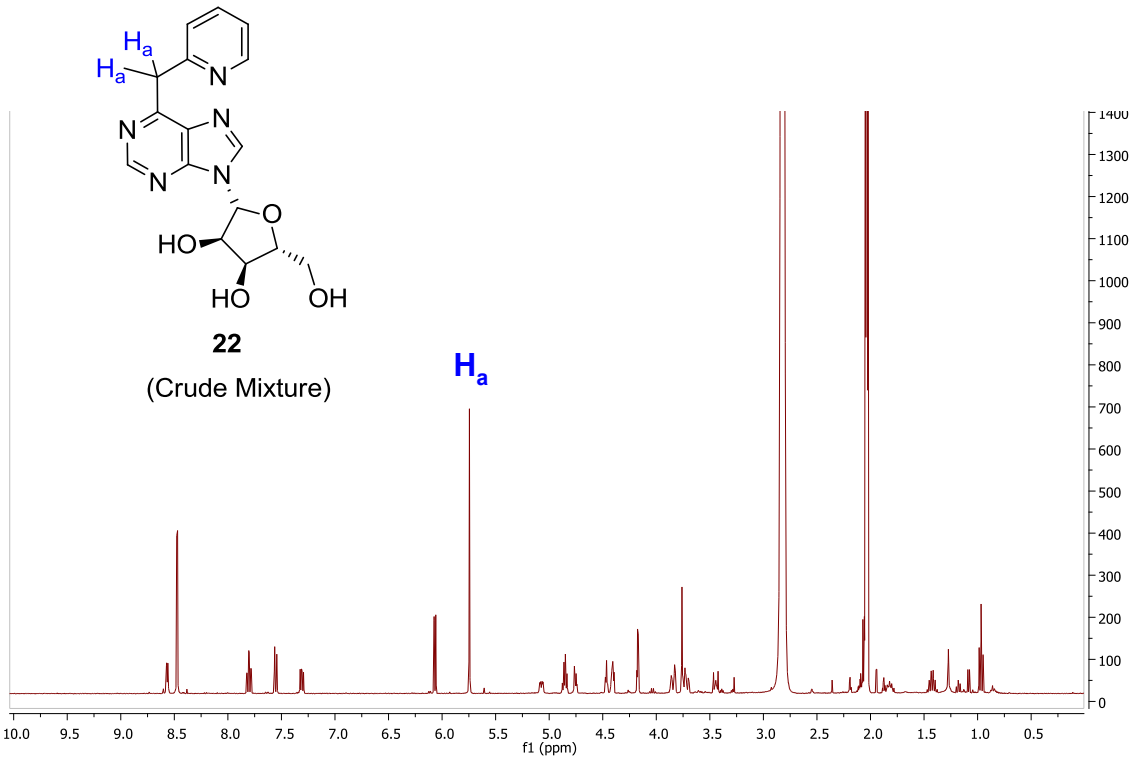
Product (**19**) was dissolved in dichloromethane (30 mL) and was added to picoline (21.47 mg) along with triethylamine (365 uL) at 0°C, and the resulting mixture was stirred for 10 min. DBU (30 uL) was added, and stirred for 10 h at RT. The reaction mixture was poured into dichloromethane, washed with water and brine, and concentrated. The crude product was purified using flash chromatography (1:1 ethyl acetate/hexane) to give expected conjugated product (**21**).

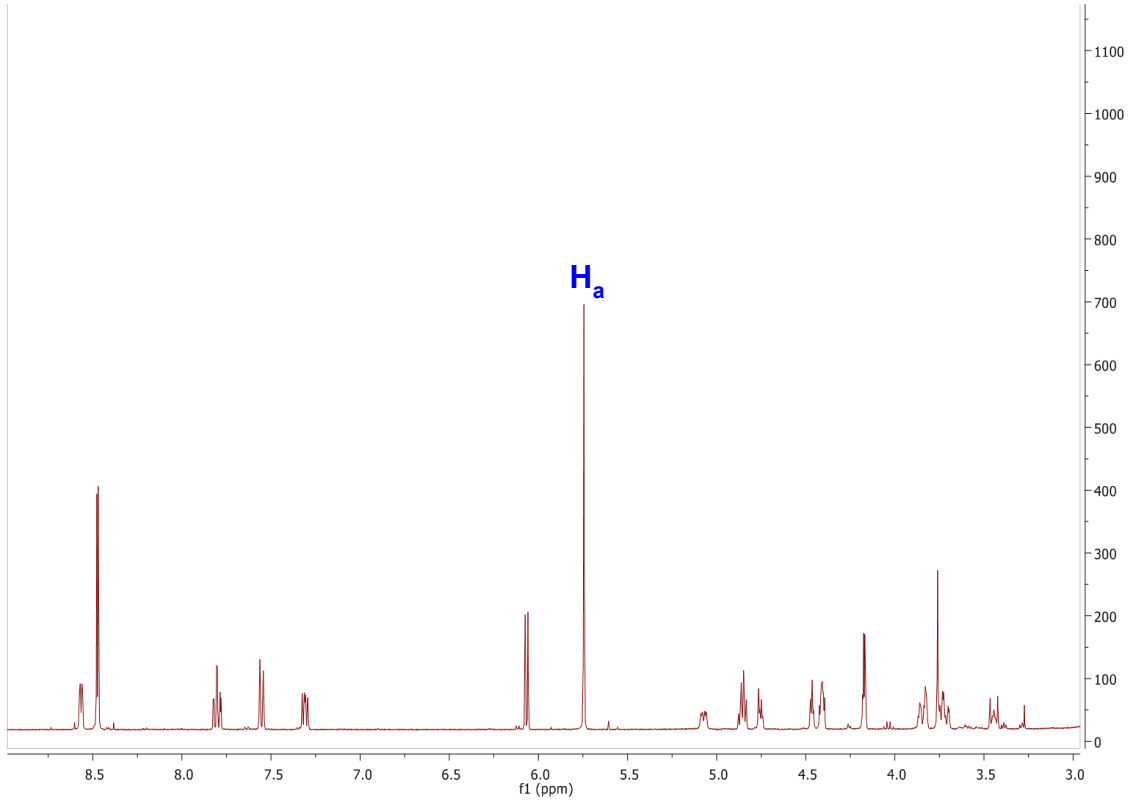
Deprotection of coupled product (21) by TBAF (22)

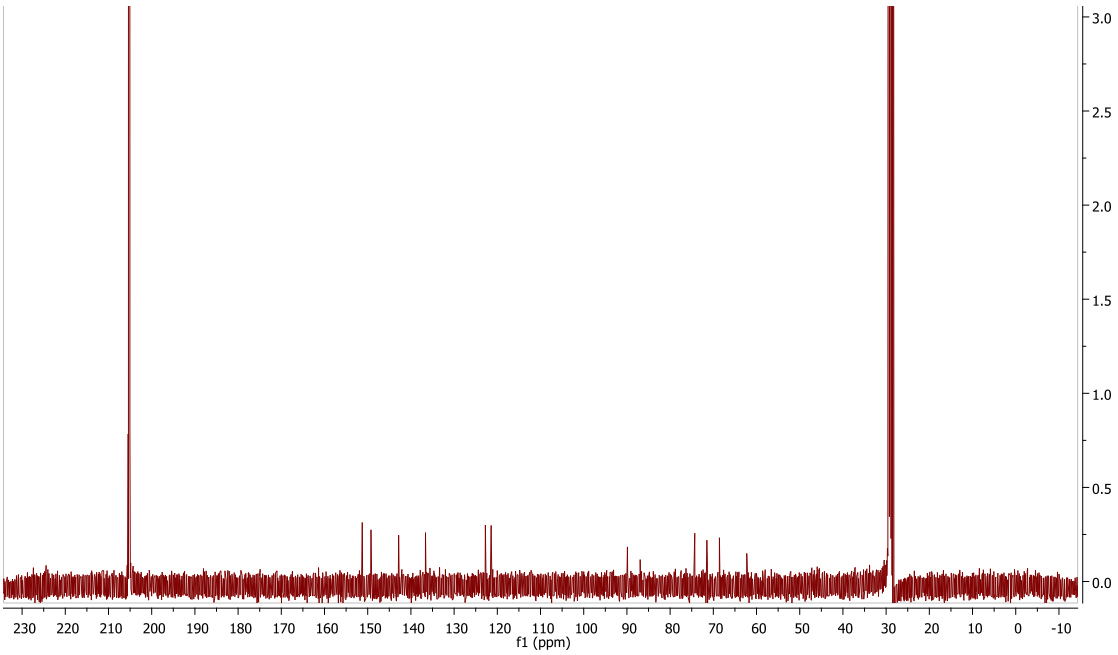


To a solution of product (**21**) (20 mg, 0.011 mmol) in THF (5 mL) was added 1 M TBAF in THF (0.5 mL, 0.05 mmol). The mixture was stirred at room temperature until completion of the reaction as shown by TLC (DCM/MeOH). The solvent was removed under reduced pressure, and the product was isolated by column chromatography on silica gel (DCM/MeOH, 9:1) followed by recrystallization with MeOH/ H_2O (1:1) to afford the title nucleoside (**22**) as a white solid.

^{1}H NMR (400 MHz, $(CD_3)_2CO$), δ (ppm) 8.57 (d, *J* = 4.71 Hz, 1H), 8.47 (d, *J* = 3.33 Hz, 1H), 7.80 (dt, *J* = 7.60, 1.81 Hz, 1H), 7.55 (d, *J* = 7.54 Hz, 1H), 7.31 (dd, *J* = 7.21, 4.73 Hz, 1H), 6.07 (d, *J* = 6.47 Hz, 1H), 5.74 (s, 2H), 5.07 (dd, *J* = 8.48, 3.58 Hz, 1H), 4.85 (dd, *J* = 10.71, 5.83 Hz, 1H), 6.23 (dd, *J* = 6.36, 3.15 Hz, 1H), 4.46 (dd, *J* = 4.21, 2.14 Hz, 1H), 4.41 (dd, *J* = 6.76, 4.10 Hz, 1H). **^{13}C NMR** (100.53 MHz, $(CD_3)_2CO$), δ (ppm) 151.2, 149.6, 142.8, 136.6, 122.7, 121.4, 89.8, 189.79, 86.93, 86.91, 74.2, 71.9, 71.3, 68.5, 62.2, 61.2


Ha
Ha
N
N
N
N
N
O
HO
HO
OH
22
(Crude Mixture)
Ha
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
10.0
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
f1 (ppm)


Ha
1100
1000
900
800
700
600
500
400
300
200
100
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
f1 (ppm)


3.0
2.5
2.0
1.5
1.0
0.5
0.0
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

REFERENCES

1. Heimberger, J.; Cade, H. C.; Padgett, J.; Sittaramane, V.; Shaikh, A. *Bioorg. Med. Chem. Lett*. **2015**, *25*, 1192-1195.
2. Jadulco, R.; Brauers, G.; Edrada, R. A.; Ebel, R.; Sudarsono, V. W.; Proksch, P. *J. Nat. Prod.* **2002**, *65*, 730-733.
3. Lou, J.; Fu, L.; Peng, Y.; Zhou, L. *Molecules* **2013**, *18,* 5891-5935.
4. Daly, J. W. *J. Med. Chem*. **1982**. *25*, 197-207.
5. Jacobson, K. A.; van Galen, P. J. M.; Williams, M. *J. Med. Chem*. **1992**, *35*, 407-422.
6. Miller, C. O. *Annu. Rev. Plant Physiol*. **1961**, *12*, 395-408.
7. Henderson, T. R., Frihart, C. R., Leonard, N. J., Schmitz, R. Y., Skoog, F. *Phytochemistry* **1975**, *14*, 1687-1690.
8. Schnebli, H. P.; Hill, D. L.; Bennett, L. L., Jr. *J. Biol.Chem*. **1967**, *242*, 1997-2004.
9. Wright, G. E.; Dudycz, L. W. *J. Med. Chem*. **1984**, *27*, 175-181.
10. Graves, V. B.; Shaikh, A. *Tetrahedron Lett*. **2013**, *54*, 695-698.
11. Hocek, M.; Holy, A.; Votruba, I.; Dvorakova, H. *J. Med. Chem*. **2000**, *43*, 1817-1825.
12. Hirota, K.; Kitade, Y.; Kanbe, Y.; Maki, Y. *J. Org. Chem*. **1992**, *57*, 5268-5270.
13. Havelkova, M.; Hocek, M.; Cyesnek, M.; Dvorak, D. *Synlett* **1999**, 1145-1147.
14. Lakshman, M.K.; Hilmer, J. H.; Martin, J. Q.; Keeler, J. C.; Dinh, Y. Q. V.; Ngassa, F. N.; Russon, L. M. *J. Am. Chem. Soc*. **2001**, *123*, 7779- 7787.
15. Elderfield, R. C.; Le Von , E. F. *J. Org. Chem*. **1960**, *25*, 1576-1583.
16. Musiol, R.; Jampilek, J.; Buchta, V.; Silva, L.; Niedbala, H.; Podeszwa, B.; Palka, A.; Majerz-Maniecka, K.; Oleksyn, B.; Polanski, J. *Bioorg. Med. Chem*. **2006**, *14*, 3592-3598.
17. Chen, J. J.; Drach, J. C.; Townsend, L. B. *J. Org. Chem*. **2003**, *68*, 4170-4178.

18. Ali, I. A. I. *Arkivoc*, **2008**, 77-89.

19. Lakshman, M. K.; Ngassa, F. N.; Keeler, J. C.; Dinh, Y. Q. V.; Hilmer, J. H.; Russon, L. M. *Org. Lett*. **2000**, *2*, 927-930.

20. Suh, H.; Wilcox, C. S. *J. Am. Chem. Soc*. **1988**, *110*, 470-481.

21. Teichert, A.; Jantos, K.; Harms, K.; Studer, A. *Org. Lett*. **2004**, *6*, 3477-3480.

22. Huang, D.; Ou, B.; Prior, R. L. *J. Agric. Food. Chem.* **2005**, *53*, 1841-1856.

23. Thaipong, K.; Boonprakob, U.; Crosby, K.; Cisneros-Zevallos, L.; Byrne, D. H. *J. Food Comp. Anal.* **2006**, *19*, 669-675.

24. Benzie, I. F. F.; Strain, J. *J. Anal. Biochem*. **1996**, *239*, 70-76.

25. Kpegba, K.; Agbonon, A.; Petrovic, A. G.; Amouzou, E.; Gbeassor, M.; Proni, G.; Nesnas, N. *J. Nat. Prod*. **2011**, *74*, 455-459.

26. He, N.; Li, X.; Feng, D.; Wu, M.; Chen, R.; Chen, T.; Chen, D.; Feng, X. *Chem. Res. Toxicol*. **2013**, *26*, 89-95.

27. Fako, V. E.; Furgeson, D. Y. *Adv. Drug Delivery Rev*. **2009**, *61*, 478-486.

28. Hill, A. J.; Teraoka, H.; Heideman, W.; Peterson, R. E. *Toxicol. Sci*. **2005**, *86*, 6-19.

29. Zhang, J. P.; Meng, J.; Li, Y. P.; Hu, C. Q. *Arch. Pharm*. **2010**, *343*, 553-560.

30. Zhang, J.; Qian, J.; Tong, J.; Zhang, D.; Hu, C. *Chem. Res. Toxicol*. **2013**, *26*, 1168-1181.

31. Westerfield, M. *The Zebrafish Book. A Guide for the Laboratory Use of Zebrafish (Danio rerio)*,4th ed.; University of Oregon Press: Eugene, **2000**.