ABSTRACT

Design, Synthesis, and Biological Evaluation of Novel Benzosuberene Analogues as Potential Cancer Therapeutic Agents

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Microtubules play an important structural role in the endothelial cells lining tumor vasculature. This vasculature is distinct from that of healthy cells and characterized as immature and chaotic, making it an attractive target for anti-cancer therapy. Small molecule anti-cancer agents known as vascular disrupting agents (VDAs) have the ability to starve tumors of the oxygen and nutrients necessary for their survival and proliferation. Benzosuberene analogues, structurally based in part on the natural products combretastatin A-4 (CA4) and colchicine, bind to the colchicine site on beta tubulin, inhibit the formation of microtubules, and thus function as cytotoxic, antiproliferative agents. Four new analogues of **KGP18**, the lead benzosuberene based anti-cancer agent, were designed and prepared by chemical synthesis to extend the structure-activity relationship of the benzosuberene class of compounds. Structural modifications incorporated into these analogues include functional group diversity at the 1-position and 2-position on the 6:7 fused ring system, as well as functional group translocations on the pendant aryl ring. All four new benzosuberene analogues were evaluated for their ability to inhibit tubulin assembly and for their cytotoxicity against three different human cancer cell lines. Encouragingly, one of the new analogues strongly inhibited the polymerization of tubulin (IC_{50} of 1.2 μM), consistent with the activity of the natural product CA4. Although none of the new analogues demonstrated cytotoxicity as pronounced as that of **KGP18**, their synthesis and biological evaluation will facilitate the design of more potent benzosuberene based compounds in the future.

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DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF NOVEL BENZOSUBERENE ANALOGUES AS POTENTIAL CANCER THERAPEUTIC AGENTS

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LIST OF ABBREVIATIONS

δ	Chemical Shift
^{o}C	Degree Celsius
AIA	Angiogenesis Inhibiting Agent
CA1	Combretastatin A-1
CA4	Combretastatin A-4
d	Doublet peak pattern
DCM	Dichloromethane
DIPEA	N,N-Diisopropylethylamine
DMF	Dimethylformamide
DU-145	Prostate carcinoma
DMXAA	5,6-dimethylxanthenone-4-acetic acid
EtOAc	Ethyl acetate
FDA	Food and Drug Administration
GDP	Guanosine diphosphate
GI_{50}	Concentration at which cell growth is inhibited by 50%
GTP	Guanosine triphosphate
IC_{50}	Half maximal inhibitory concentration
M	Molar

m	Multiplet peak pattern
μM	Micromolar
mmol	Millimole
MeOH	Methanol
MHz	Megahertz
NCI-H460	Human large-cell lung carcinoma
n-BuLi	*n*-Butyllithium
NMR	Nuclear magnetic resonance spectroscopy
ppm	Parts per million
q	Quartet peak pattern
s	Singlet peak pattern
SAR	Structure-activity relationship
SK-OV-3	Human ovarian carcinoma
SRB	Sulforhodamine B
t	Triplet peak pattern
TBAF	Tetrabutylammonium fluoride
TBS	*tert*-Butyldimethylsilyl
TBSCl	*tert*-Butyldimethylsilyl chloride
THF	Tetrahydrofuran
TLC	Thin-layer chromatography

TMAH	Tetramethylammonium hydroxide
TMS	Tetramethylsilane
VDA	Vascular Disrupting Agent
VTA	Vascular Targeting Agent

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Finally, I am grateful to Dr. Mary Lynn Trawick and coworkers at Baylor University and Dr. Ernest Hamel at the National Cancer Institute for performing the biological experiments to evaluate the analogues synthesized in this project.

CHAPTER ONE

Introduction

Tumors require an extensive vascular network to provide the supply of oxygen and nutrients necessary for their survival, growth, and proliferation. Tumor vasculature has a distinct structure from that of normal, healthy cells, and it is characterized as immature, discontinuous, and chaotic.[1] The walls of tumor blood vessels are under-developed and highly permeable to macromolecules, resulting in high interstitial blood pressure.[2] This vasculature is also highly branched, often in complex patterns, and lengths between branching points are often unusually long; both of these factors lead to a higher resistance to blood flow and a lower blood flow rate than seen in normal vascular networks.[1] Furthermore, the endothelial cells lining tumor vasculature tend to be irregularly shaped and only loosely connected, resulting in intercellular openings.[2] All of these unusual features make the vasculature of tumors extremely fragile, and as a result, it is an appealing target for anti-cancer therapy.

Vascular Disrupting Agents

Currently, there are two classes of compounds that act as vascular targeting agents (VTAs): angiogenesis inhibiting agents (AIAs) and vascular disrupting agents (VDAs). Angiogenesis, or the formation of new blood vessels, plays a crucial role in the growth and proliferation of tumor cells, and AIAs can inhibit the formation of these new blood vessels.[3] Vascular disrupting agents, on the other hand, can

potently and selectively damage existing tumor vasculature.[3] The exact mechanisms by which these compounds act vary significantly between various classes of VDAs. For example, 5,6-dimethylxanthenone-4-acetic acid (DMXAA, Figure 1) acts only on the actin cytoskeleton of endothelial cells associated with the tumor vasculature.[1] DMXAA stimulates the release of tumor necrosis factor α and other cytokines, ultimately resulting in apoptosis of cancerous cells and a reduction in the size of the tumor.[4]

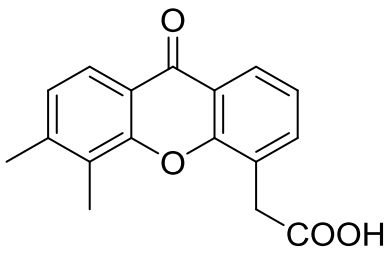


Figure 1: Structure of DMXAA[4]

Several other VDAs, in contrast to DMXAA, exert their anti-cancer effects through the depolymerization of microtubules in the endothelial cells lining the tumor vasculature, leading to reduced blood flow to the tumor, endothelial cell rounding and detachment (Figure 2), and eventual tumor necrosis.[3] The damage VDAs cause to microtubules results in a cell signaling cascade that is thought to be largely responsible for their anti-cancer effects. Although the details of the exact signaling cascade that is responsible are not known, the binding of VDAs to microtubules leads to the activation of the Rho-GTPase, Rho kinase, and mitogen activated protein kinase signaling pathways, and eventually results in the rounding

of the endothelial cells, disruption of cell-cell junctions, and an increase in the permeability of the vasculature to macromolecules.[2-4]

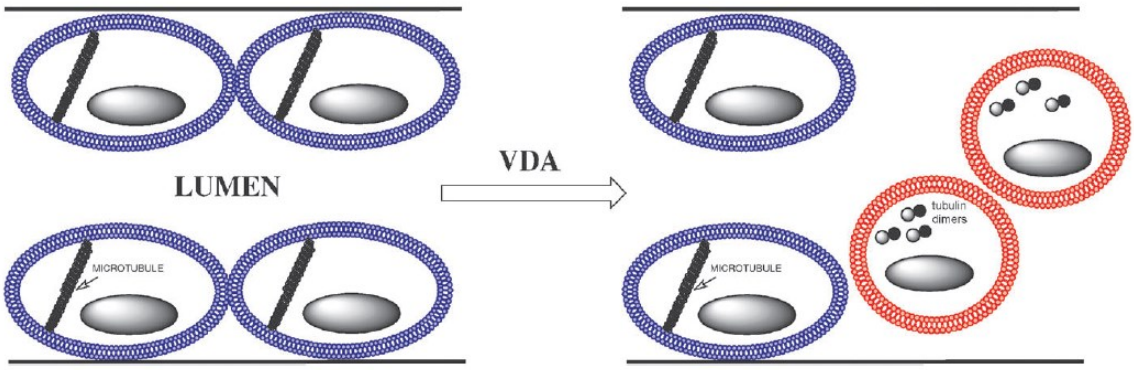


Figure 2: Microtubule depolymerization leading to cell rounding and detachment[3] (Used with permission from Royal Society of Chemistry; license number 3613841191854)

Microtubules as a Target for Anti-Cancer Agents

Microtubules play an important role in the structure of the cytoskeleton of cells, as well as in cell division. They are polymers of the protein tubulin, which has an α,β-heterodimeric structure, and are formed when individual tubulin subunits assemble into protofilaments.[5] These protofilaments then interact laterally and assemble into cylindrical structures, or microtubules.[5] Each tubulin subunit is bound to a guanosine triphosphate (GTP) molecule, one of which can be hydrolyzed to guanosine diphosphate (GDP).[6] When a GTP molecule is hydrolyzed to GDP, there is a conformational change in the protein structure of that individual tubulin subunit, and this structural shift decreases the stability of the whole microtubule protein.[5,6] Because of this process, microtubules are in a constant state of dynamic instability and continuously alternate between growing and shrinking.[7] After

enough GTP has been hydrolyzed from several tubulin subunits, complete depolymerization of a microtubule can result.[7]

One method by which the depolymerization of microtubules can be induced is through the interaction of VDAs with the tubulin-microtubule protein system.[3] In particular, the colchicine binding site on β-tubulin has been shown to be potentially useful in anti-cancer therapy.[8] Colchicine, the first molecule found to bind at this site, is a natural product commonly used to treat gout (Figure 3).[8] Its anti-cancer effects were investigated for use as a potential cancer treatment, but it was ultimately found to be too toxic for such uses.[3,9] No compounds that bind at the colchicine binding site have been approved by the Food and Drug Administration (FDA) for the treatment of cancer, but many have been studied and several are at various phases in clinical trials.[8]

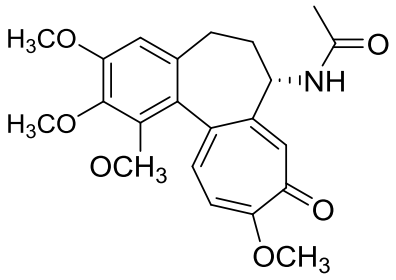


Figure 3: Structure of colchicine[8]

Preclinical studies have demonstrated the vast potential of vascular disrupting agents as cancer therapeutics.[10] Treatment with VDAs results in a nearly instant drop in blood flow to the tumor, and continual treatment, often leads to tumor necrosis.[2] Apart from some slight reductions in blood flow, however, normal tissue is mostly unaffected by VDAs.[1] Although they usually leave behind a viable

rim of cancerous cells, potentially allowing for the regrowth of the tumor, treatments utilizing a combination of VDAs along with traditional cancer chemotherapies, radiotherapy, or angiogenesis inhibiting agents may mitigate this shortcoming.[11] These current conventional treatments are more effective against the well-oxygenated cells at the periphery of tumors due to receiving higher blood flow than cells at the interior of the tumor, giving the conventional treatments greater access to them than VDAs are capable of obtaining.[1] When used in conjunction with some of these other already available treatment options, VDAs have been shown to produce especially promising effects against tumors.[10,11] Studies have also shown that VDAs have a much lower toxicity profile than other chemotherapeutics, and several have undergone or are currently undergoing clinical testing.[2,11] These include a wide variety of potential treatment methods, such as small molecule VDAs and ligand-based VDAs consisting of peptides, antibodies, or growth factors.[1]

The Combretastatins

Compounds related to the class of natural products known as the combretastatins are some of the farthest along in clinical trials. Combretastatin A-1 (CA1, Figure 4) and combretastatin A-4 (CA4, Figure 5), originally isolated from the bark of the South African bush willow tree, *Combretum caffrum,* are two of the most potent vascular disrupting agents that bind tubulin at the colchicine site.[12,13] Assays have shown that these two compounds both inhibit the polymerization of microtubules and inhibit cell growth in cancer cell lines.[14] Notably different from

colchicine and other tubulin-binding compounds, however, CA1 and CA4 have been found to exert anti-vascular effects at much lower doses, making them likely safer and potentially more effective for use in the treatment of cancer.[15,16] Water soluble phosphate prodrugs of the combretastatins have also been developed.[16,22] Several analogues of the various compounds in the combretastatin class have already been designed and synthesized, and many are potent inhibitors of tubulin assembly and exhibit significant anti-cancer activity in various cell lines as well.[17,18]

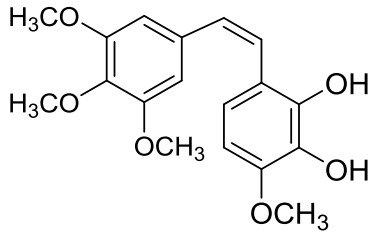


Figure 4: Structure of combretastatin A1[12]

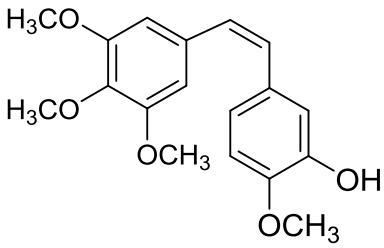


Figure 5: Structure of combretastatin A4[13]

Although the combretastatins have shown much success in clinical trials, an issue related their molecular structures makes them potentially less than ideal candidates for use as therapeutics. While the *Z* isomers of the combretastatins have demonstrated the ability to potently inhibit tubulin polymerization, the alkene bridge connecting the two aromatic rings in the structure allows for the possibility

of isomerization of the molecule to the *E* isomer.[18] The *E* isomers of CA1 and CA4 are significantly less potent, and many analogues of the combretastatins attempt to combat this problem and prevent isomerization.

Benzosuberenes

A long-standing project in the Pinney group at Baylor University led to discovery of the benzosuberene compounds for use as potential VDAs.[19, 23-24] These molecules feature a seven-membered ring fused to one aryl ring and pendant to a second aryl ring, potentially increasing their structural stability and effectiveness for use as cancer therapeutics. Lead benzosuberene **KGP18** (Figure 6), structurally based on colchicine and the combretastatins, was synthesized by Pinney and coworkers as part of a project to design inhibitors of tubulin polymerization. It has been found to be a potent and an effective VDA, with an IC_{50} value of 1.7 μM in assays for inhibition of tubulin assembly.[19] It has also been shown to display significant cytotoxicity in several human cancer cell lines.[19]

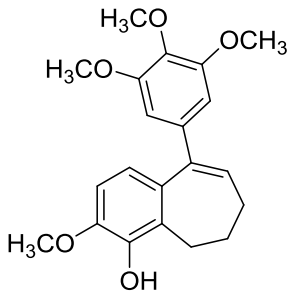


Figure 6: Structure of **KGP18**

In an effort to gain a more thorough understanding of the structure-activity relationship of **KGP18** and other benzosuberene-based compounds, four new

analogues of **KGP18** were designed and synthesized (Figure 7). These compounds have also been evaluated for their cytotoxicity against three human cancer cell lines as well as for their inhibition of tubulin assembly. Ultimately, the synthesis and study of these four new analogues should facilitate the design of more potent benzosuberene-based compounds in the future.

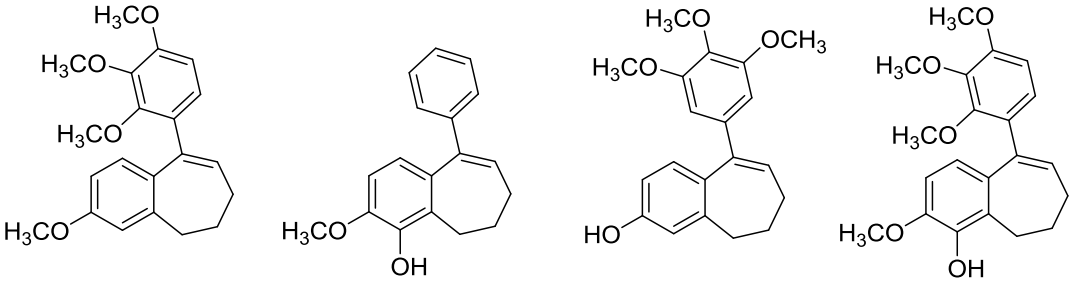


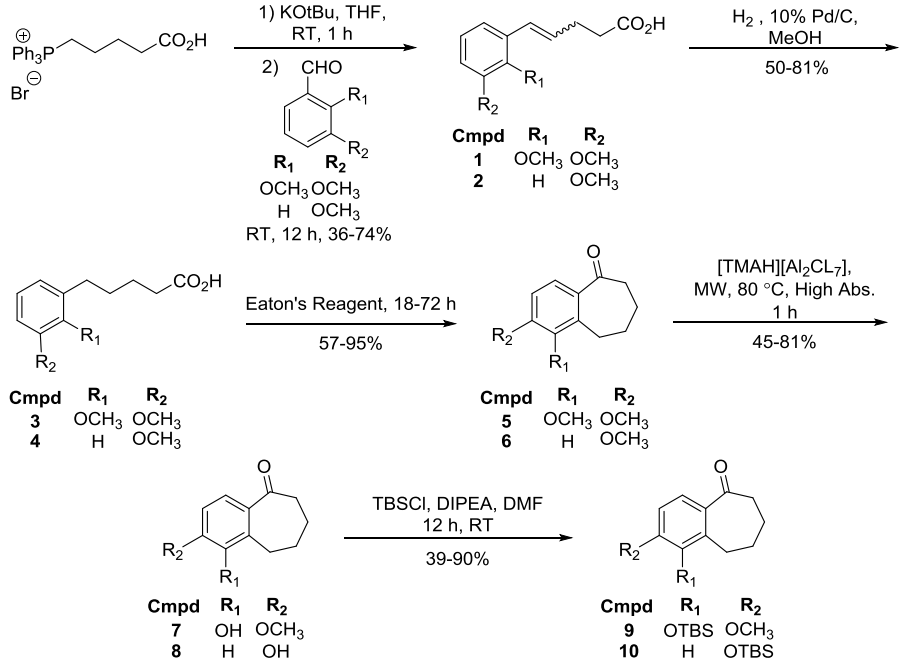
Figure 7: Newly synthesized benzosuberene analogues

CHAPTER TWO

Results and Discussion

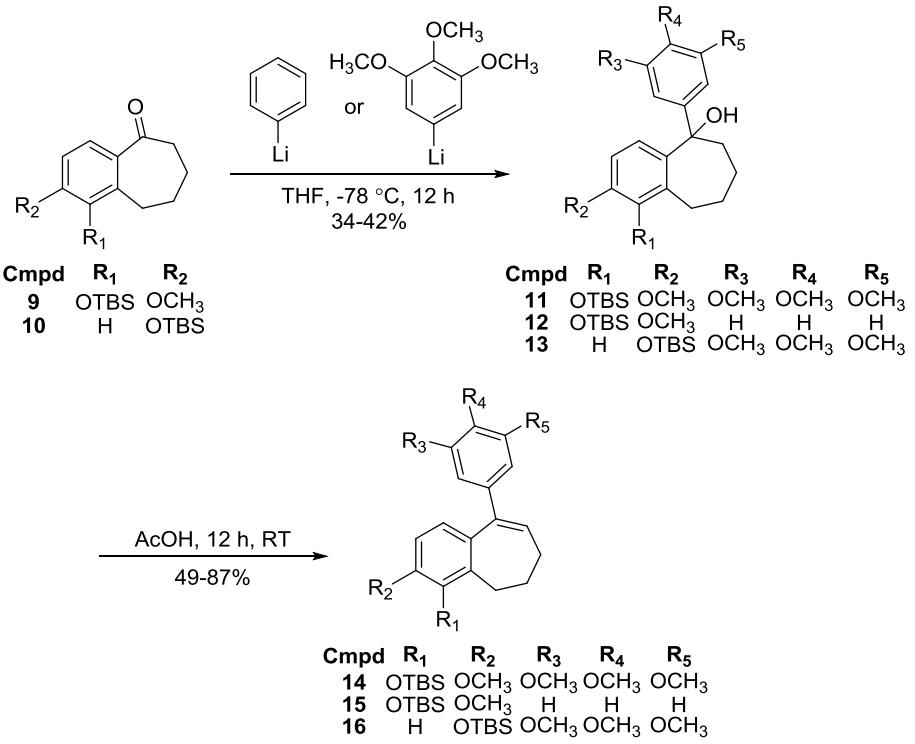
*Synthesis of **KGP18** and New Benzosuberene Analogues*

New benzosuberene analogues **19** and **22-24,** as well as **KGP18** (**21**), were prepared by chemical synthesis (the re-synthesis of **KGP18** by this researcher is not yet complete). Carboxylic acids **1** and **2** were produced by a Wittig reaction and subsequently reduced by palladium-catalyzed hydrogenation to yield compounds **3** and **4** (Scheme 1). Cyclization of reduced carboxylic acids **3** and **4** using Eaton's reagent yielded ketones **5** and **6**. A microwave reaction with [TMAH][Al_2Cl_7] afforded demethylated compounds **7** and **8**, which were protected using TBSCl to form TBS-protected compounds **9** and **10**.



Scheme 1: Synthesis of ketones **6**, **9**, and **10**

Ketones **9** and **10** were reacted with one of two aryl lithium reagents (produced through a lithium-halogen exchange reaction) to yield alcohols **12** and **13** (Scheme 2). Ketone **9** will also be used in this reaction to yield alcohol **11** in the synthesis of **KGP18**. Tertiary alcohols **12** and **13** were then dehydrated using acetic acid to afford protected compounds **15** and **16,** and tertiary alcohol **11** will be dehydrated to give compound **14.**



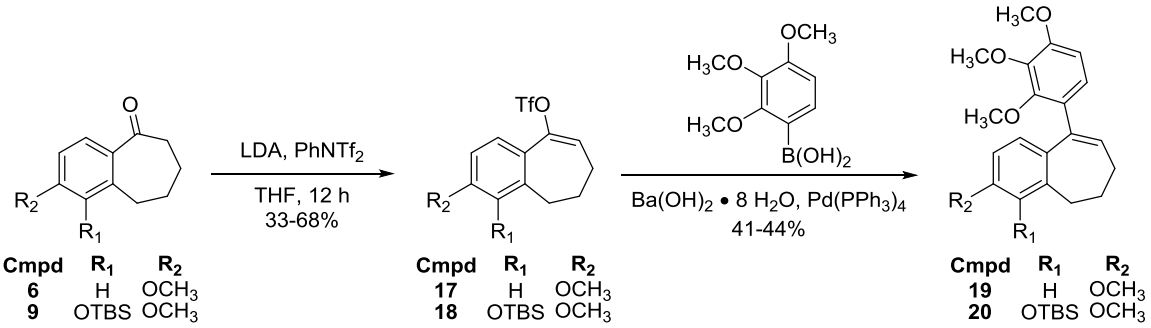
Cmpd	R_1	R_2
9	OTBS	OCH_3
10	H	OTBS

Cmpd	R_1	R_2	R_3	R_4	R_5
11	OTBS	OCH_3	OCH_3	OCH_3	OCH_3
12	OTBS	OCH_3	H	H	H
13	H	OTBS	OCH_3	OCH_3	OCH_3

Cmpd	R_1	R_2	R_3	R_4	R_5
14	OTBS	OCH_3	OCH_3	OCH_3	OCH_3
15	OTBS	OCH_3	H	H	H
16	H	OTBS	OCH_3	OCH_3	OCH_3

Scheme 2: Synthesis of protected compounds **14, 15,** and **16**

Ketones **6** and **9** were enolized and trapped as their corresponding triflate ethers and then reacted in a Suzuki coupling to yield target benzosuberene analogue **19** and protected compound **20** (Scheme 3).



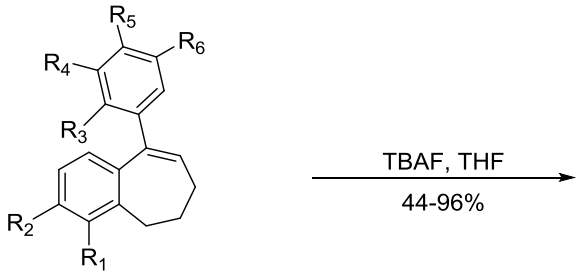
Cmpd	R_1	R_2
6	H	OCH_3
9	OTBS	OCH_3

Cmpd	R_1	R_2
17	H	OCH_3
18	OTBS	OCH_3

Cmpd	R_1	R_2
19	H	OCH_3
20	OTBS	OCH_3

Scheme 3: Synthesis of benzosuberene analogue **19** and protected compound **20**

A final deprotection of compounds **15, 16,** and **20** using TBAF produced desired benzosuberene analogues **22-24** (Scheme 4). The synthesis of **KGP18** (compound **21**) will be completed with the deprotection of phenol **14**.



Cmpd	R_1	R_2	R_3	R_4	R_5	R_6
14	OTBS	OCH_3	H	OCH_3	OCH_3	OCH_3
15	OTBS	OCH_3	H	H	H	H
16	H	OTBS	H	OCH_3	OCH_3	OCH_3
20	OTBS	OCH_3	OCH_3	OCH_3	OCH_3	H

Cmpd	R_1	R_2	R_3	R_4	R_5	R_6
21	OH	OCH_3	H	OCH_3	OCH_3	OCH_3
22	OH	OCH_3	H	H	H	H
23	H	OH	H	OCH_3	OCH_3	OCH_3
24	OH	OCH_3	OCH_3	OCH_3	OCH_3	H

Scheme 4: Deprotection of phenolic intermediates to complete **KGP18** (**21**) and novel benzosuberene analogues **22-24**

Biological Evaluation of Synthesized Analogues

In collaboration with Dr. Mary Lynn Trawick's group at Baylor University, compounds **19, 22, 23,** and **24** were evaluated for their inhibition (GI_{50}) of cancer cell growth in cell lines NCI-H460, (lung) DU-145 (prostate), and SK-OV-3 (ovarian) using a sulforhodamine B (SRB) assay. All four analogues were also evaluated for their inhibition of tubulin assembly (IC_{50}) by Dr. Ernest Hamel at the National Cancer Institute. The corresponding IC_{50} and GI_{50} values, along with literature values for CA4 and **KGP18**, are depicted in Table 1.

Table 1: Biological Evaluation of Synthesized Benzosuberene Analogues

Compound	Inhibition of Tubulin Assembly IC_{50} (μM)	GI_{50} (μM) SRB Assay[a] NCI-H460 (μM)	DU-145 (μM)	SK-OV-3 (μM)
CA4	1.2[20]	0.00223[21]	0.00327[21]	0.00455
KGP18	1.7[19]	0.000028[19]	0.0000032[19]	<0.00003[19]
19	1.2	0.120	0.0562	0.0432
22	>20	0.652	4.40	0.557
23	4.6	0.647	1.02	0.527
24	11	4.24	7.54	3.53

a. Average of n ≥ 3 independent determinations

All four new analogues showed activity against the three human cancer cell lines, although they displayed significantly less cytotoxicity than **KGP18** and CA4. Compound **19** potently inhibited tubulin assembly, with an IC_{50} value comparable to those for **KGP18** and CA4. The complete removal of all three methoxy groups from the pendant aryl ring in compound **22** appears to have greatly reduced its activity. Similarly, simply shifting the methoxy groups to the 2, 3, and 4 positions on the pendant aryl ring significantly reduced the activity of these compounds as well, even when the functional groups on 6:7 fused ring system are the same as those in

KGP18, as in compound **24**. This indicates that the methoxy groups on the pendant aryl ring play an important role in the binding of these compounds to tubulin, and their placement at the 3, 4, and 5 positions on the ring is likely ideal.[25]

CHAPTER THREE

Conclusions

Four new analogues of one of the most potent benzosuberene lead compounds, **KGP18**, were designed and prepared by chemical synthesis. These new analogues featured translocations of the methoxy groups on the pendant aryl ring and functional group modifications at the 1- and 2-positions on the 6:7 fused ring system. The compounds were evaluated for their inhibition of tubulin assembly and for their cytotoxicity against three human cancer cell lines. All four of the analogues were found to be significantly less cytotoxic than **KGP18**, and only one had the ability to inhibit tubulin assembly with an IC_{50} value similar to those of **KGP18** and CA4. Although none of the compounds were more potent than **KGP18** overall, their synthesis and evaluation will ultimately help to extend the structure-activity relationship of the benzosuberenes. The knowledge gained from these studies will be useful in the further design and synthesis of new analogues in search of more potent benzosuberene-based compounds that may be useful as anti-cancer therapeutic agents.

CHAPTER FOUR

Materials and Methods

General Section

Dichloromethane (CH_2Cl_2), methanol (MeOH), dimethylformamide (DMF), and tetrahydrofuran (THF) were used in their anhydrous forms as obtained from chemical suppliers. All reactions were performed under inert atmosphere with nitrogen gas unless stated otherwise. Thin layer chromatography (TLC) plates (pre-coated glass plates with silica gel 60 F_{254}, 0.25 mm thickness) were used to monitor reactions. Microwave radiation was performed using a Biotage Initiator Microwave Synthesizer. Silica gel (200-400 mesh, 60 Å) was used in the purification of intermediates and final products using a Biotage Isolera 1 or 4 flash purification system. Intermediates and final products were characterized by ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data, and TMS was used as internal standard for spectra recorded in $CDCl_3$. All chemical shifts are presented in ppm (δ), and peak patterns are reported as singlet (s), doublet (d), triplet (t), quartet (q), or multiplet (m). Mass spectrometry was carried out under positive ESI (electrospray ionization) using a Thermo Scientific LTQ Orbitrap Discovery Instrument. The purity of target compounds was analyzed at 25°C using an Agilent 1200 HPLC system with a diode-array detector (λ=190-400 nm), a Zorbax XDB-C18 HPLC column (4.6 x 150 mm, 5 μm), and a Zorbax reliance cartridge guard-column; eluents, solvent A: H_2O, solvent B: acetonitrile; gradient, 90% A/10% B to 0%

A/100% B over 0-40 min; flow rate 1.0 mL/min; injection volume 20 µL; monitored at wavelengths of 254, 280, and 300 nm.

Synthesis of Target Compound **19**

(Z)/(E)-5-(3-methoxyphenyl)pent-4-enoic acid **(2)**[23,26]

Potassium *tert*-butoxide (8.2 g, 73 mmol) was added to 3-carboxypropyltriphenyl-phosphonium bromide (15.9 g, 37.1 mmol) dissolved in THF (500 mL). After stirring at room temperature for one hour, 3-methoxybenzalehyde (5.0 g, 37 mmol) dissolved in THF (200 mL) was added to the reaction mixture. The reaction was allowed to stir for 12 hours at room temperature and then was quenched with 2 M HCl. This mixture was extracted with EtOAc (3 x 100 mL), and the organic layer was dried using Na_2SO_4, filtered, and concentrated. Purification by flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (1 CV), 20% A/80% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] yielded the mixture of E/Z-isomers of carboxylic acid **2** (5.63 g, 27.3 mmol, 74%) as a yellow solid.

NMR taken following next step.

5-(3-methoxyphenyl)pentanoic acid **(4)**[23,26,27]

A solution of carboxylic acid **2** (5.63 g, 27.8 mmol) dissolved in dry methanol (200 mL) was prepared and 10% Pd-C (0.44 g) was added. The flask was evacuated under vacuum, and the reaction mixture was stirred for 12 hours at room

temperature under H_2 gas introduced to the flask through balloons. The reaction mixture was filtered through Celite®, extracted with EtOAc (3 x 50 mL) and washed with water, and then dried using Na_2SO_4, filtered, and concentrated. Purification by flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (1 CV), 20% A/80% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] afforded pentanoic acid **4** (4.3 g, 21 mmol, 75%) as a yellow oil.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 11.15 (1H, s), 7.03 (1H, t), 6.62 (2H, t), 6.59 (1H, d), 3.59 (3H, s), 2.45 (2H, d), 2.21 (2H, t), 1.52 (4H, t).

2-Methoxy-benzocycloheptan-5-one **(6)**[19,23,26,27]

Pentanoic acid **4** (4.3 g, 21 mmol) was dissolved in Eaton's reagent (41 mL, P_2O_5 (7.7 wt %) in methanesulfonic acid). The reaction mixture was stirred for 72 hours and then poured over ice and neutralized using $NaHCO_3$. The reaction mixture was extracted with EtOAc (3 x 50 mL) and then dried using Na_2SO_4, filtered, and concentrated. Purification by flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (1 CV), 20% A/80% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] afforded ketone **6** (3.7 g, 19 mmol, 95%).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.59 (1H, d), 6.61 (1H, d), 6.52 (1H, s), 3.63 (3H, s), 2.70 (2H, t), 2.52 (2H, t), 1.66 (2H, m), 1.58 (2H, m).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 204.0, 162.7, 144.3, 131.1, 131.0, 114.7, 111.7, 55.1, 40.5, 32.6, 24.9, 20.5.

2-Methoxy-benzocycloheptan-5-trifluoromethanesulfonate **(17)**

A solution of diisopropylamine (1.74 mL, 12.4 mmol) dissolved in THF (50 mL) was prepared and cooled to -78°C. To the solution, *n*-BuLi (5.0 mL, 12 mmol) was added, and the reaction mixture was stirred for 15 minutes. Ketone **6** (2.1 g, 11 mmol) dissolved in THF (10 mL) was added dropwise, and the reaction mixture was then stirred at -78°C for 2 hours. To the reaction mixture, $PhNTf_2$ (4.4 g, 12 mmol) dissolved in THF (20 mL) was then added dropwise and the solution was stirred at -78°C for 12 hours and allowed to warm to room temperature. The reaction mixture was concentrated, washed with water, and extracted with EtOAc (3 x 30 mL). The organic extracts were then dried with Na_2SO_4, filtered, and concentrated. Purification by flash chromatography using a prepacked 10 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (1 CV), 20% A/80% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] afforded triflate **17** (2.3 g, 7.1 mmol, 68%) as a yellow solid.
NMR taken following next step.

2-methoxy-5-(2',3',4'-trimethoxyphenyl)-benzocyclohept-5-ene **(19)**

Boronic acid (1.7 g, 7.8 mmol), $Ba(OH)_2$ (3.4 g, 11 mmol), and $Pd(PPh_3)_4$ (0.24 g, 0.21 mmol) were added to **17** (2.3 g, 7.1 mmol) dissolved in THF (100 mL) and the solution was heated to reflux at 80°C for 2 hours. The reaction mixture was filtered through Celite®, washed with dichloromethane (50 mL), and concentrated. Purification by flash chromatography using a prepacked 10 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (1 CV), 20% A/80% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 25 mL/min; monitored at 254

and 280 nm] yielded benzosuberene analogue **19** (1.1 g, 3.1 mmol, 44%) as a yellow oil.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.03 (1H, d), 6.88 (1H, s), 6.78 (1H, d), 6.58 (2H, s), 6.41 (1H, t), 3.91 (3H, s), 3.83 (3H, s), 3.82 (6H, s), 2.69 (2H, t), 2.21 (2H, m), 2.02 (2H, m).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 158.5, 152.8, 143.6, 142.7, 138.3, 137.4, 132.3, 130.4, 126.7, 113.8, 111.1, 105.2, 60.6, 60.1, 55.8, 54.8, 35.0, 32.7, 25.4, 20.7, 14.1.

HRMS: m/z: obsvd 363.1573 $[M+Na]^+$, calcd for $C_{21}H_{24}O_4Na^+$, 363.1567.

HPLC: 18.23 min.

Synthesis of Target Compound **21**

(Z)/(E)- 5-(2,3-dimethoxyphenyl)pent-4-enoic acid **(1)**[24,26]

Potassium *tert*-butoxide (7.4 g, 66 mmol) was added to 3-carboxypropyltriphenyl-phosphonium bromide (13.4 g, 30.4 mmol) dissolved in THF (500 mL). After stirring at room temperature for one hour, 2,3-dimethoxybenzalehyde (5.0 g, 30 mmol) dissolved in THF (50 mL) was added to the reaction mixture. The reaction was allowed to stir for 12 hours at room temperature and then was quenched with 2 M HCl. This mixture was extracted with EtOAc (3 x 100 mL), and the organic extracts were dried using Na_2SO_4, filtered, and concentrated. Purification by flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (1 CV), 20% A/80% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] yielded

the mixture of E/Z-isomers of carboxylic acid **1** (7.0 g, 30 mmol, 98%) as a yellow oil.

NMR taken following next step.

5-(2,3-dimethoxyphenyl)pentanoic acid **(3)**[24,26,28]

A solution of carboxylic acid **1** (7.0 g, 30 mmol) dissolved in dry methanol (200 mL) was prepared and 10% Pd-C (0.42 g) was added. The flask was evacuated under vacuum, and the reaction mixture was stirred for 12 hours at room temperature under H_2 gas introduced to the flask through balloons. The reaction mixture was filtered through Celite®, extracted with EtOAc (3 x 50 mL) and washed with water, and then dried using Na_2SO_4, filtered, and concentrated. Purification by flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (1 CV), 20% A/80% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] afforded pentanoic acid **3** (4.6 g, 19 mmol, 66%) as a colorless oil.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 11.43 (1H, s), 6.80 (1H, t), 6.60 (2H, d), 3.92 (3H, s), 3.67 (3H, s) 2.50 (2H, s), 2.08 (2H, s), 1.52 (4H, m).

1,2-Dimethoxy-benzocycloheptan-5-one **(5)**[24,26,29]

Pentanoic acid **3** (4.6 g, 19 mmol) was dissolved in Eaton's reagent (38.8 mL, P_2O_5 (7.7 wt %) in methanesulfonic acid). The reaction mixture was stirred for 72 hours and then poured over ice and neutralized using $NaHCO_3$. The reaction mixture was extracted with EtOAc (3 x 50 mL) and then dried using Na_2SO_4, filtered, and concentrated. Purification by flash chromatography using a prepacked 100 g silica

column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (1 CV), 20% A/80% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] afforded ketone **5** (3.5 g, 16 mmol, 83%) as a brown oil.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.35 (1H, d), 6.68 (1H, d), 3.72 (3H, s), 3.62 (3H, s), 2.82 (2H, t), 2.52 (2H, t), 1.64 (2H, m), 1.59 (2H, m).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 203.0, 155.6, 145.5, 135.1, 132.2, 124.8, 109.4, 60.2, 55.2, 40.0, 24.5, 22.7, 20.5.

1-Hydroxy-2-methoxy-benzocycloheptan-5-one **(7)**[24,26,27]

Ketone **5** (1.0 g, 4.5 mmol) was placed in a microwave vial and reacted with [TMAH][Al_2Cl_7][30] (18 mL, 9.1 mmol). The reaction mixture was stirred and microwaved at 80°C on high absorbance for one hour, then poured into water. The product was extracted with EtOAc (3 x 30 mL), dried with Na_2SO_4, filtered, and concentrated.

(Synthesis of benzosuberene **21** [**KGP18**] has not been completed beyond this point.)

Synthesis of Target Compound **22**

See above for synthesis of compounds **1**, **3**, and **5**.

1-Hydroxy-2-methoxy-benzocycloheptan-5-one **(7)**[24,26,27]

Ketone **5** (1.0 g, 4.5 mmol) was placed in a microwave vial and reacted with [TMAH][Al_2Cl_7][30] (18 mL, 9.1 mmol). The reaction mixture was stirred and

microwaved at 80°C on high absorbance for one hour, then poured into water. The product was extracted with EtOAc (3 x 30 mL), dried with Na_2SO_4, filtered, and concentrated. Purification by flash chromatography using a prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (1 CV), 20% A/80% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] yielded phenol **7** (0.76 g, 3.7 mmol, 81%) as a cream-colored solid.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.24 (1H, d), 6.68 (1H, d), 6.26 (1H, s) 3.78 (3H, s), 2.93 (2H, t), 2.61 (2H, t), 1.71 (4H, m).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 205.0, 149.5, 142.5, 133.0, 127.8, 120.7, 107.9, 55.9, 40.6, 24.4, 23.0, 21.2.

1-[(tert-butyldimethylsilyl)oxy]-2-methoxy-benzocycloheptan-5-one **(9)**[24,26]

Phenol **7** (0.76 g, 3.7 mmol) was dissolved in DMF (50 mL) and TBSCl (1.2 g, 7.4 mmol) and DIPEA (1.9 mL, 11 mmol) were added to the solution. The reaction mixture was stirred at room temperature for 12 hours, and then washed with water and extracted with EtOAc (3 x 30 mL), dried using Na_2SO_4, and concentrated. Purification by flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (1 CV), 20% A/80% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] afforded ketone **9** (1.6 g, 4.8 mmol, 90%) as white-colored crystals.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.22 (1H, d), 6.61 (1H, d), 3.66 (3H, s), 2.87 (2H, t), 2.52 (2H, t), 1.63 (4H, m), 0.88 (9H, s), 0.05 (6H, s).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 204.2, 152.9, 141.5, 133.0, 132.7, 122.2, 108.6, 54.6, 40.5, 26.0, 24.6, 23.8, 21.1, 18.8, 4.0.

1-[(tert-butyldimethylsilyl)oxy]-2-methoxy-5-phenyl-benzocycloheptan-5-ol **(12)**

A solution of phenyl bromide (0.69 mL, 6.5 mmol) dissolved in THF (25 mL) was cooled to -78°C. *n*-BuLi (2.7 mL, 6.9 mmol) was added dropwise to the reaction mixture, which was then stirred for one hour at -78°C. Ketone **9** (1.6 g, 4.8 mmol) was dissolved in THF (25 mL) and added dropwise to the reaction mixture, which was then stirred for 12 hours and allowed to warm to room temperature. The reaction mixture was washed with water and extracted with EtOAc (3 x 30 mL). The extracted organic layers were then dried with Na_2SO_4, filtered, and concentrated. Purification by flash chromatography using a prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (1 CV), 20% A/80% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] yielded tertiary alcohol **12** (0.80 g, 2.0 mmol, 42%) as a light yellow oil.

NMR taken following next step.

1-[(tert-butyldimethylsilyl)oxy]-2-methoxy-5-phenyl-benzocyclohept-5-ene **(15)**

Acetic acid (10 mL) was added to tertiary alcohol **12** (0.80 g, 2.0 mmol) and the solution was stirred for 12 hours at room temperature. The reaction mixture was washed with water, extracted with EtOAc (3 x 25 mL), and then dried using Na_2SO_4, filtered, and concentrated. Purification by flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (1

CV), 20% A/80% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] afforded TBS-protected **15** (0.38 g, 1.0 mmol, 49%) as a yellow solid.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.30 (4H, m), 7.26 (1H, t), 6.70 (1H, d), 6.59 (1H, d), 6.37 (1H, t), 3.81 (3H, s), 2.79 (2H, t), 2.13 (2H, m), 1.99 (2H, m), 1.07 (9H, s), 0.26 (6H, s).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 148.6, 143.0, 142.8, 141.6, 134.1, 133.3, 128.0, 126.8, 122.1, 108.4, 54.7, 33.8, 26.2, 25.6, 24.2, 24.0, 21.2, 19.0, 3.8.

1-hydroxy-2-methoxy-5-phenyl-benzocyclohept-5-ene **(22)**

TBAF (1.20mL, 1.20 mmol) was added to protected phenol **15** (0.38 g, 1.0 mmol) dissolved in THF (25 mL). The reaction mixture was stirred for 12 hours at room temperature and then washed with water and extracted with EtOAc (3 x 25 mL). The extracted organic layers were dried with Na_2SO_4, filtered, and concentrated. Purification by flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (1 CV), 20% A/80% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] yielded completed benzosuberene analogue **22** (0.12 g, 0.45 mmol, 44%).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.28 (5H, m), 6.71 (1H, d), 6.55 (1H, d), 6.38 (1H, t), 5.77 (1H, s), 3.91 (3H, s), 2.79 (2H, t), 2.16 (2H, m), 2.00 (2H, m).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 145.0, 142.8, 142.7, 142.4, 134.6, 128.0, 128.0, 127.8, 127.6, 126.9, 120.6, 107.7, 55.9, 33.5, 25.7, 23.5.

HRMS: m/z: obsvd 267.1385 $[M+H]^+$, calcd for $C_{18}H_{19}O_2^+$, 267.1380.

HPLC: 17.89 min.

Synthesis of Target Compound **23**

See above for synthesis of compounds **2**, **4**, and **6.**

2-Hydroxy-benzocycloheptan-5-one **(8)**[26,27]

Ketone **6** (1.0 g, 5.3 mmol) was placed in a microwave vial and reacted with [TMAH][Al_2Cl_7][30] (21 mL, 11 mmol). The reaction mixture was stirred and microwaved at 80°C on high absorbance for one hour, then poured into water. The product was extracted with EtOAc (3 x 30 mL), dried with Na_2SO_4, filtered, and concentrated. Purification by flash chromatography using a prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (1 CV), 20% A/80% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] yielded phenol **8** (0.42 g, 2.4 mmol, 45%) as a white solid.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.72 (1H, d), 6.76 (1H, d), 6.67 (1H, s), 2.87 (2H, t), 2.70 (2H, t), 1.85 (2H, t), 1.79 (2H, d).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 204.9, 159.9, 144.8, 131.6, 131.3, 116.3, 113.7, 40.7, 32.7, 25.0, 20.7.

2-[(tert-butyldimethylsilyl)oxy]-benzocycloheptan-5-one **(10)**[26]

Phenol **8** (0.42 g, 2.4 mmol) was dissolved in DMF (25 mL) and TBSCl (0.72 g, 4.8 mmol) and DIPEA (1.2 mL, 7.1 mmol) were added to the solution. The reaction mixture was stirred at room temperature for 12 hours, and then washed with water

and extracted with EtOAc (3 x 25 mL), dried using Na_2SO_4, and concentrated. Purification by flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (1 CV), 20% A/80% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] afforded ketone **10** (0.53 g, 3.0 mmol, 77%) as a colorless liquid.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.66 (1H, d), 6.68 (1H, d), 6.60 (1H, s), 2.81 (2H, t), 2.64 (2H, t), 1.79 (2H, m), 1.72 (2H, m), 0.94 (9H, s), 0.18 (6H, s).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 204.0, 159.2, 144.0, 132.1, 131.0, 120.9, 117.9, 40.7, 32.6, 25.6, 25.0, 20.7, 18.1, 4.4.

2-[(tert-butyldimethylsilyl)oxy]-5-(3',4',5'-trimethoxyphenyl)-benzocycloheptan-5-ol **(13)**[26]

A solution of 5-bromo-1,2,3-trimethoxybenzene (1.0 g, 4.1 mmol) dissolved in THF (25 mL) was cooled to -78°C. n-BuLi (1.7 mL, 4.3 mmol) was added dropwise to the reaction mixture, which was then stirred for one hour at -78°C. Ketone **10** (0.53 g, 3.0 mmol) was dissolved in THF (5 mL) and added dropwise to the reaction mixture, which was then stirred for 12 hours and allowed to warm to room temperature. The reaction mixture was washed with water and extracted with EtOAc (3 x 25 mL). The extracted organic layers were then dried with Na_2SO_4, filtered, and concentrated. Purification by flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (1 CV), 20% A/80% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] yielded tertiary alcohol **13** (0.47 g, 1.0 mmol, 34%) as a colorless oil.

NMR taken following next step.

2-[(tert-Butyldimethylsilyl)oxy]-5-(3',4',5'-trimethoxyphenyl)-benzocyclohept-5-ene **(16)**[26]

Acetic acid (10 mL) was added to tertiary alcohol **13** (0.47 g, 1.0 mmol) and the solution was stirred for 12 hours at room temperature. The reaction mixture was washed with water, extracted with EtOAc (3 x 25 mL), and then dried using Na_2SO_4, filtered, and concentrated. Purification by flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (1 CV), 20% A/80% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] afforded protected phenol **16** (0.39 g, 0.88 mmol, 87%) as a yellow oil.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.91 (1H, d), 6.77 (1H, d), 6.68 (1H, d), 6.49 (2H, s), 6.34 (1H, t), 3.86 (3H, s), 3.79 (6H, s), 2.60 (2H, t), 2.15 (2H, m), 1.97 (2H, m), 1.00 (9H, s), 0.23 (6H, s).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 154.4, 152.8, 143.7, 142.8, 138.4, 137.3, 133.0, 130.5, 127.0, 120.0, 117.3, 105.2, 60.9, 56.1, 35.0, 32.6, 25.7, 25.5, 18.2, 4.3.

2-hydroxy-5-(3',4',5'-trimethoxyphenyl)-benzocyclohept-5-ene **(23)**[26]

TBAF (1.06 mL, 1.06 mmol) was added to protected phenol **16** (0.39 g, 0.88 mmol) dissolved in THF (25 mL). The reaction mixture was stirred for 12 hours at room temperature and then washed with water and extracted with EtOAc (3 x 25 mL). The extracted organic layers were dried with Na_2SO_4, filtered, and concentrated. Purification by flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (1 CV), 20% A/80% B → 60%

A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] yielded completed benzosuberene analogue **23** (0.13 g, 0.40 mmol, 45%).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.89 (1H, d), 6.78 (1H, s), 6.67 (1H, d), 6.50 (2H, s), 6.33 (1H, t), 6.21 (1H, s), 3.88 (3 H, s), 3.79 (6H, s), 2.59 (2H, t), 2.14 (2H, m), 1.96 (2H, m).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 154.9, 152.8, 144.0, 142.7, 138.7, 137.0, 132.1, 130.7, 127.1, 115.4, 112.9, 105.3, 61.0, 56.1, 35.0, 32.6, 25.5.

HRMS: m/z: obsvd 349.1417 $[M+Na]^+$, calcd for $C_{20}H_{22}O_4Na^+$, 349.1410.

HPLC: 14.30 min.

Synthesis of Target Compound **24**

See above for synthesis of compounds **1, 3, 5, 7,** and **9.**

1-[(tert-butyldimethylsilyl)oxy]-2-methoxy-benzocycloheptan-5-trifluoromethanesulfonate **(18)**

A solution of diisopropylamine (0.18 mL, 1.3 mmol) dissolved in THF (25 mL) was cooled to -78^{o}C. To the solution, *n*-BuLi (0.51 mL, 1.3 mmol) was added, and the reaction mixture was stirred for 15 minutes. Ketone **9** (0.37 g, 1.2 mmol) dissolved in THF (5 mL) was added dropwise, and the reaction mixture was then stirred at -78^{o}C for 2 hours. To the reaction mixture, $PhNTf_2$ (0.45 g, 1.3 mmol) dissolved in THF (5 mL) was then added dropwise and the solution was stirred at -78^{o}C for 12 hours and allowed to warm to room temperature. The reaction mixture was concentrated, washed with water, and extracted with EtOAc (3 x 25 mL). The

organic extracts were then dried with Na_2SO_4, filtered, and concentrated. Purification by flash chromatography using a prepacked 10 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (1 CV), 20% A/80% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] afforded triflate **18** (0.17 g, 0.38 mmol, 33%) as a yellow oil.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.11 (1H, d), 6.79 (1H, d), 6.09 (1H, t), 3.82 (3H, s), 2.88 (2H, t), 2.15 (2H, t), 2.03 (2H, m), 1.04 (9H, s), 0.21 (6H, s).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 150.8, 146.5, 132.9, 130.9, 129.9, 126.0, 120.9, 119.7, 108.8, 54.7, 30.5, 26.0, 25.0, 24.5, 18.9, 4.0.

1-[(tert-butyldimethylsilyl)oxy]-2-methoxy-5-(2',3',4'-trimethoxyphenyl)-benzocyclohept-5-ene **(20)**

Boronic acid (0.09 g, 0.41 mmol), $Ba(OH)_2$ (0.18 g, 0.57 mmol), and $Pd(PPh_3)_4$ (0.013 g, 0.011 mmol) were added to triflate **18** (0.17 g, 0.38 mmol) dissolved in THF (20 mL) and the solution was heated to reflux at 80°C for 2 hours. The reaction mixture was filtered through Celite®, washed with dichloromethane (25 mL), and concentrated. Purification by flash chromatography using a prepacked 10 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (1 CV), 20% A/80% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] yielded protected phenol **20** (0.17 g, 0.15 mmol, 41%).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.92 (1H, d), 6.64 (1H, d), 6.59 (2H, t), 6.45 (1H, d), 6.10 (1H, t), 3.87 (3H, s), 3.83 (3H, s), 3.76 (3H, s), 3.38 (3H, s), 2.89 (2H, t), 2.12 (2H, t), 1.96 (2H, m), 1.05 (9H, s), 0.19 (6H, s).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 153.0, 151.7, 148.5, 142.4, 140.3, 135.8, 132.5, 131.1, 128.2, 124.9, 120.7, 107.9, 106.6, 105.2, 60.6, 60.3, 55.9, 54.6, 33.8, 26.2, 25.5, 24.2, 19.0, 3.9.

1-hydroxy-2-methoxy-5-(2',3',4'-trimethoxyphenyl)-benzocyclohept-5-ene **(24)**

TBAF (0.18 mL, 0.18 mmol) was added to protected phenol **20** (0.068 g, 0.15 mmol) dissolved in THF (20 mL). The reaction mixture was stirred for 12 hours at room temperature and then washed with water and extracted with EtOAc (3 x 25 mL). The extracted organic layers were dried with Na_2SO_4, filtered, and concentrated. Purification by flash chromatography using a prepacked 10 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (1 CV), 20% A/80% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] yielded completed benzosuberene analogue **24** (0.053 g, 0.15 mmol, 96%).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.92 (1H, d), 6.61 (2H, q), 6.38 (1H, d), 6.12 (1H, t), 5.71 (1H, s), 3.86 (6H, d), 3.82 (3H, s), 3.42 (3H, s), 2.87 (2H, t), 2.15 (2H, m), 1.97 (2H, m).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 153.0, 151.8, 144.7, 142.4, 142.4, 140.0, 136.2, 130.9, 128.6, 126.8, 125.0, 119.0, 107.3, 106.6, 60.7, 60.5, 55.9, 55.8, 33.6, 25.6, 23.4.

HRMS: m/z: obsvd 379.1516 $[M+Na]^+$, calcd for $C_{21}H_{25}O_5Na^+$, 379.1516.

HPLC: 16.18 min.

SRB Assay[31-32]

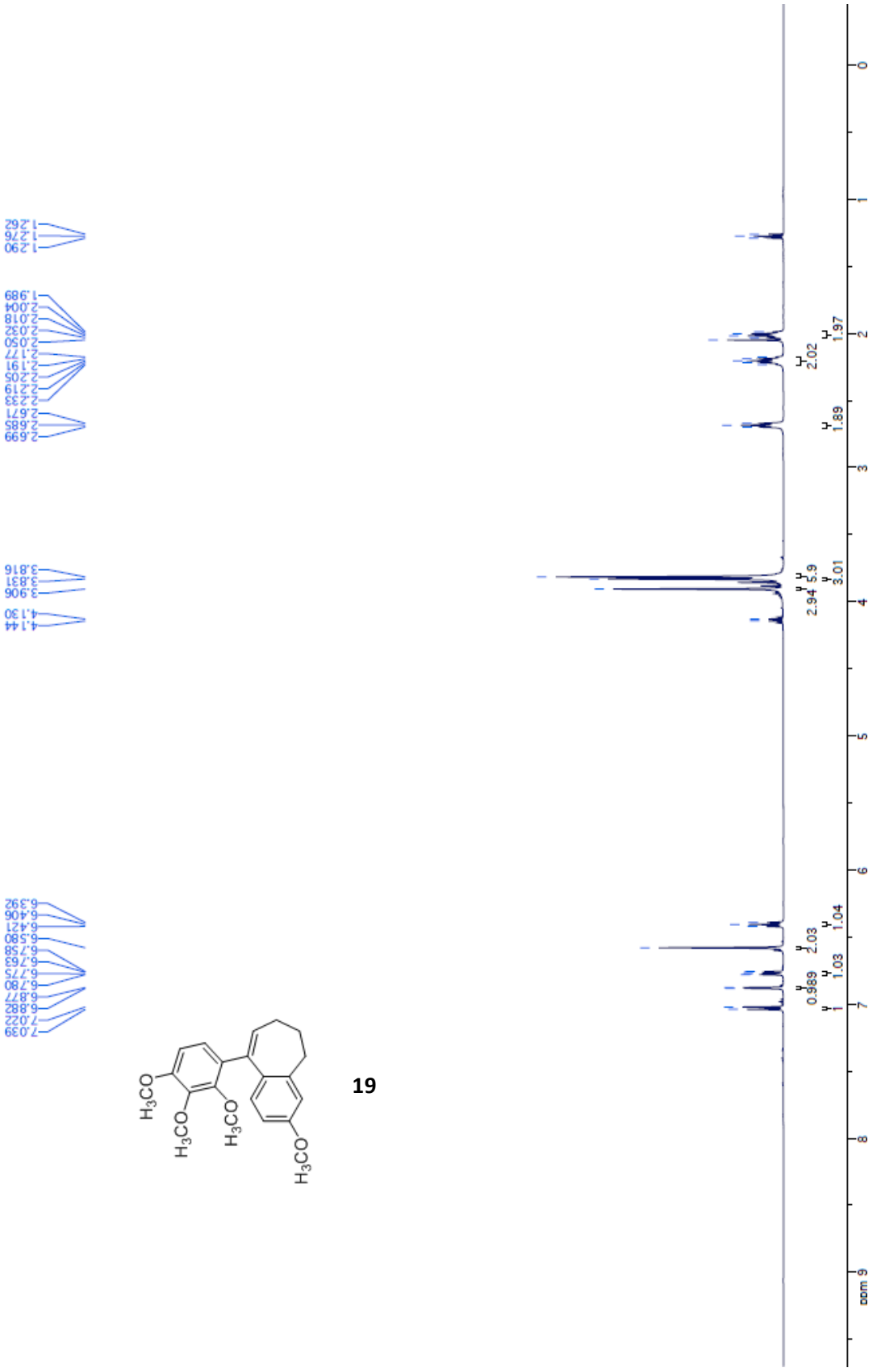
The inhibition of human cancer cell growth was evaluated using the sulforhodamine B assay.[31] Cancer cell lines were plated at 9000 cells/well into 96-well plates using DMEM supplemented with 5% fetal bovine serum/ 1% gentamicin sulfate and incubated for 24 hours, and then serial dilutions of the compounds were added. After 48 hours, the cells were fixed with trichloroacetic acid, washed, dried, stained with sulforhodamine B dye (Acid red 52), solubilized, and read at 540 nm and normalized to 630 nm with an automated Biotek plate reader. A growth inhibition of 50% (GI_{50} or the drug concentration causing 50% reduction in the net protein increase) was calculated from the absorbance data.

Inhibition of Tubulin Polymerization[33]

Experiments to assess the inhibition of tubulin assembly were performed with 0.25 mL reaction mixtures. The mixtures contained purified bovine brain tubulin, tubulin monosodium glutamate, dimethyl sulfoxide, GTP, and varying concentrations of compound. All components except GTP were preincubated for 15 min at 30 °C in a 0.24 mL volume. The mixtures were chilled on ice, and 10 μL of 10 mM GTP was added to each sample. The reaction mixtures were then transferred to cuvettes held at 0 °C in spectrophotometers. The temperature was raised to 30 °C over 30 s, and polymerization was followed turbidimetrically at 350 nM for 30 minutes. IC_{50} values were determined versus the control after 20 min at 30 °C. For complete assay details, see reference 24.

APPENDIX

NMR SPECTRA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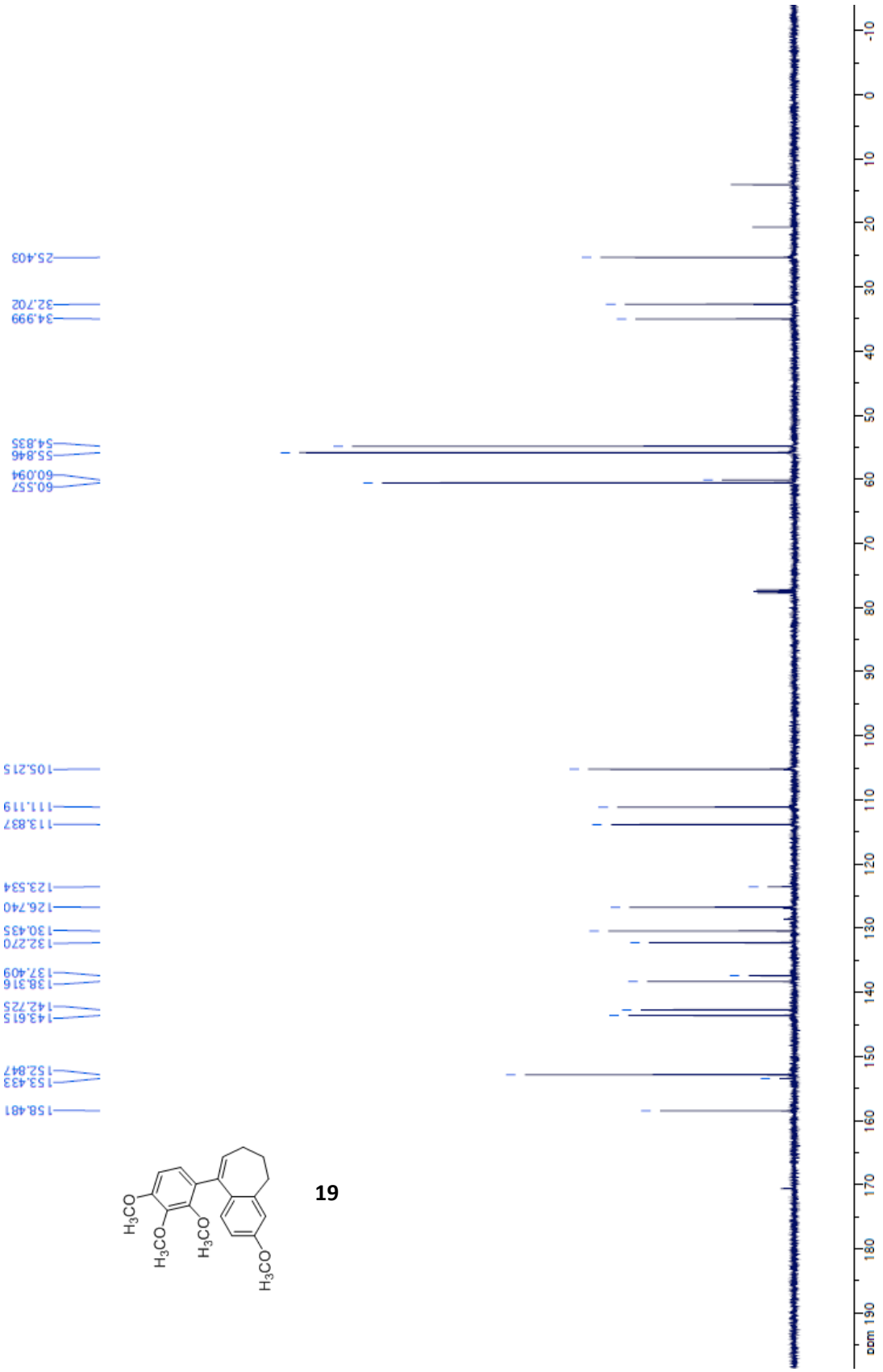
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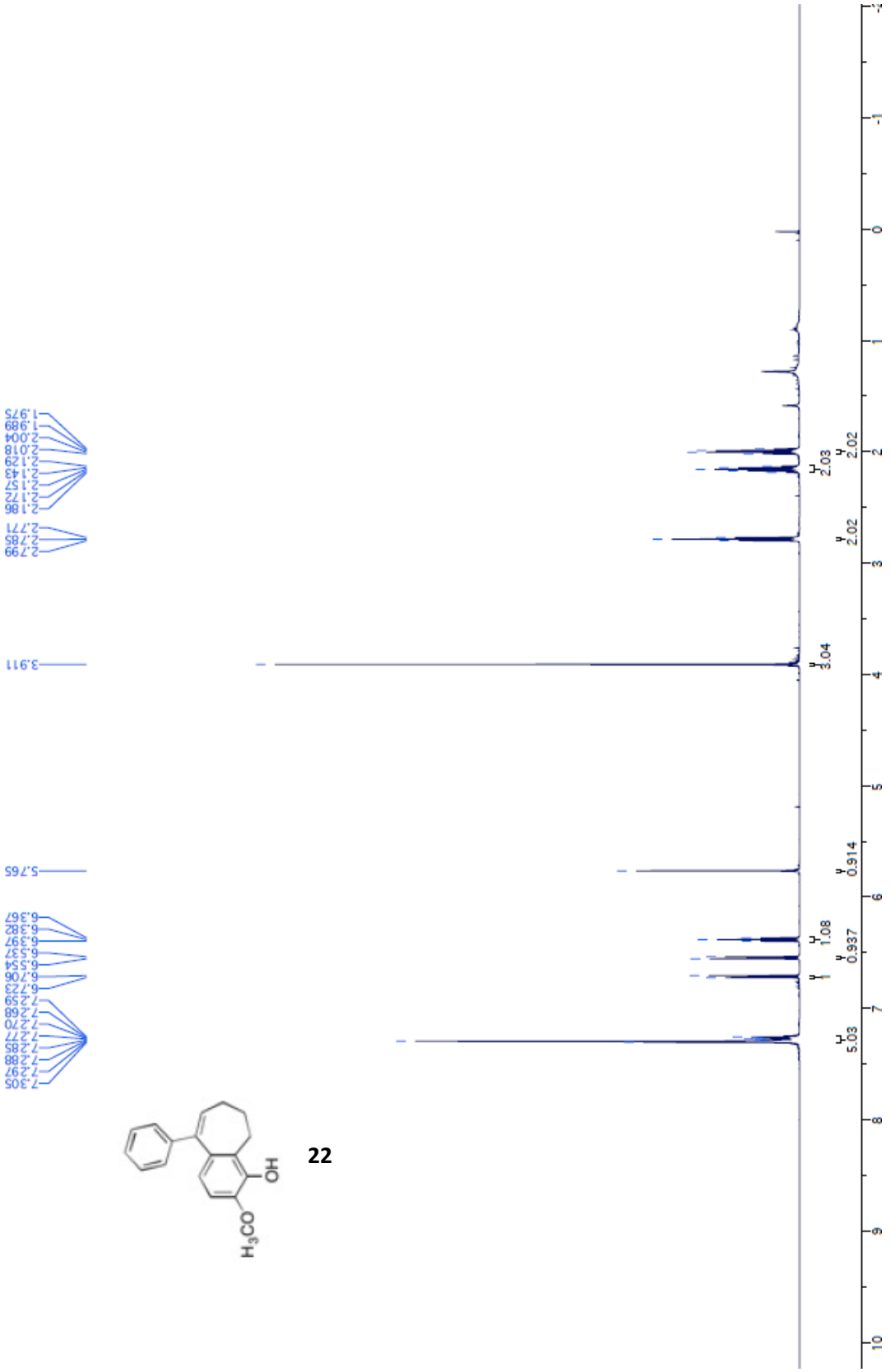
H3CO
H3CO

19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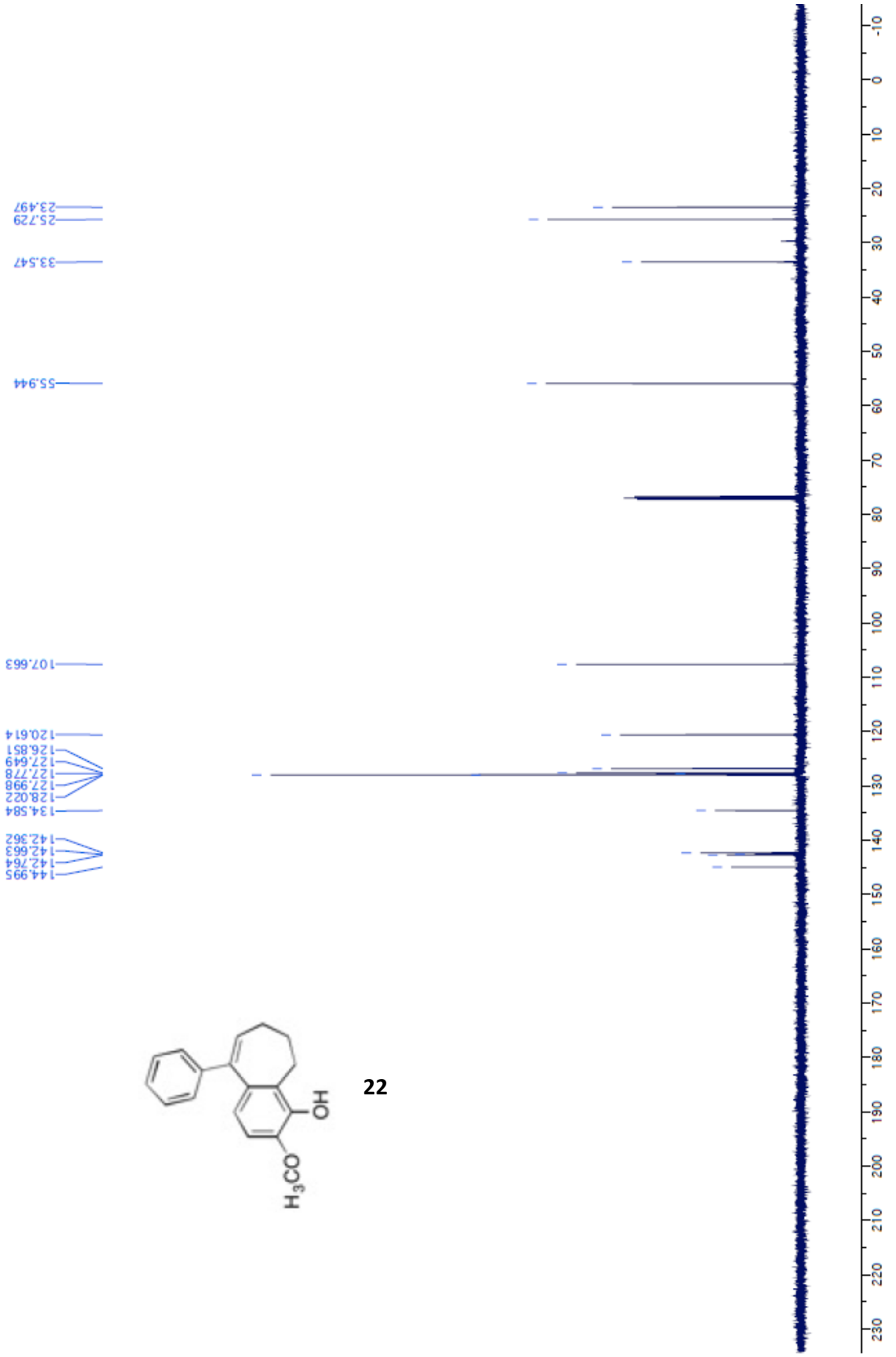
22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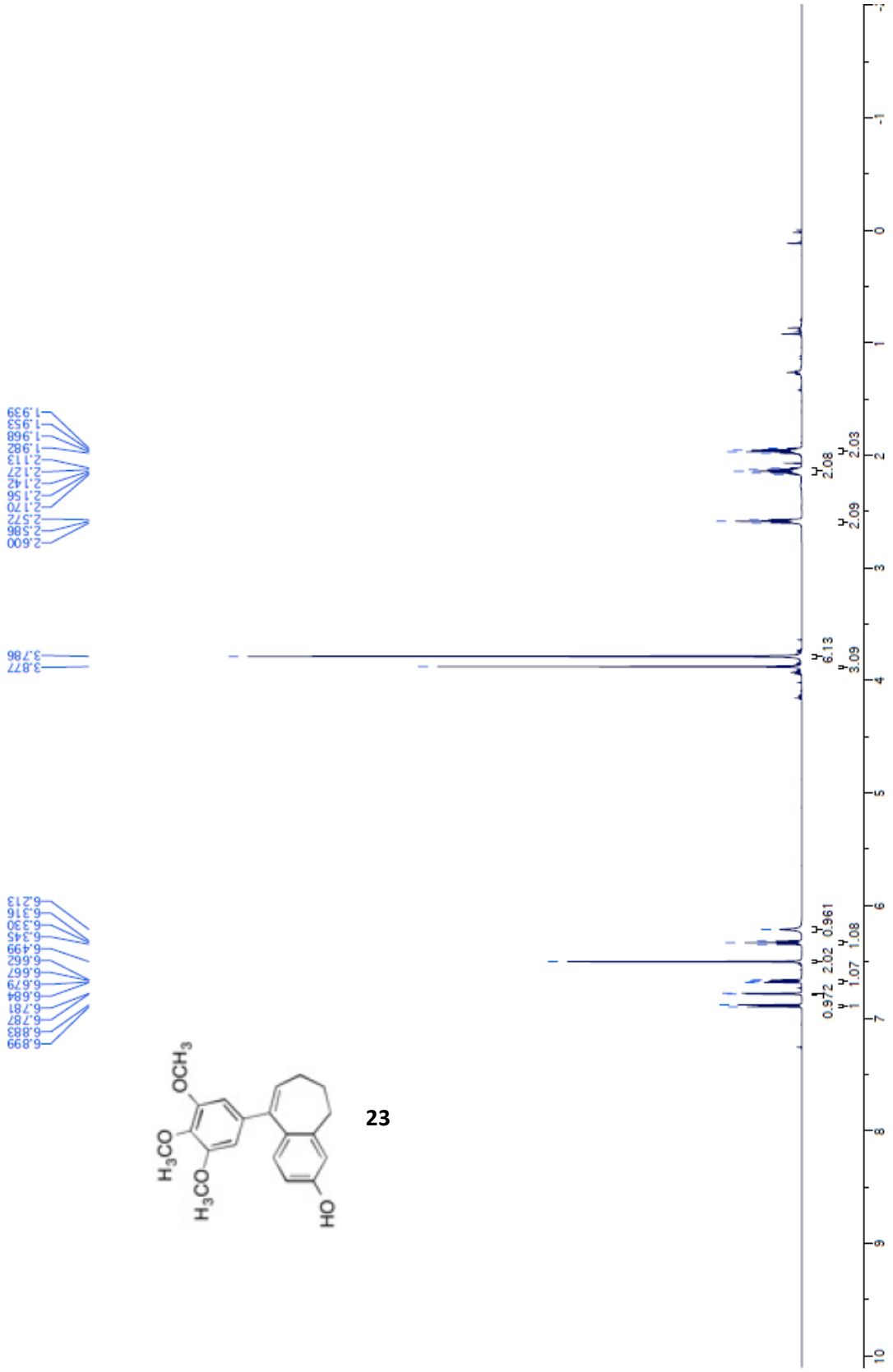
22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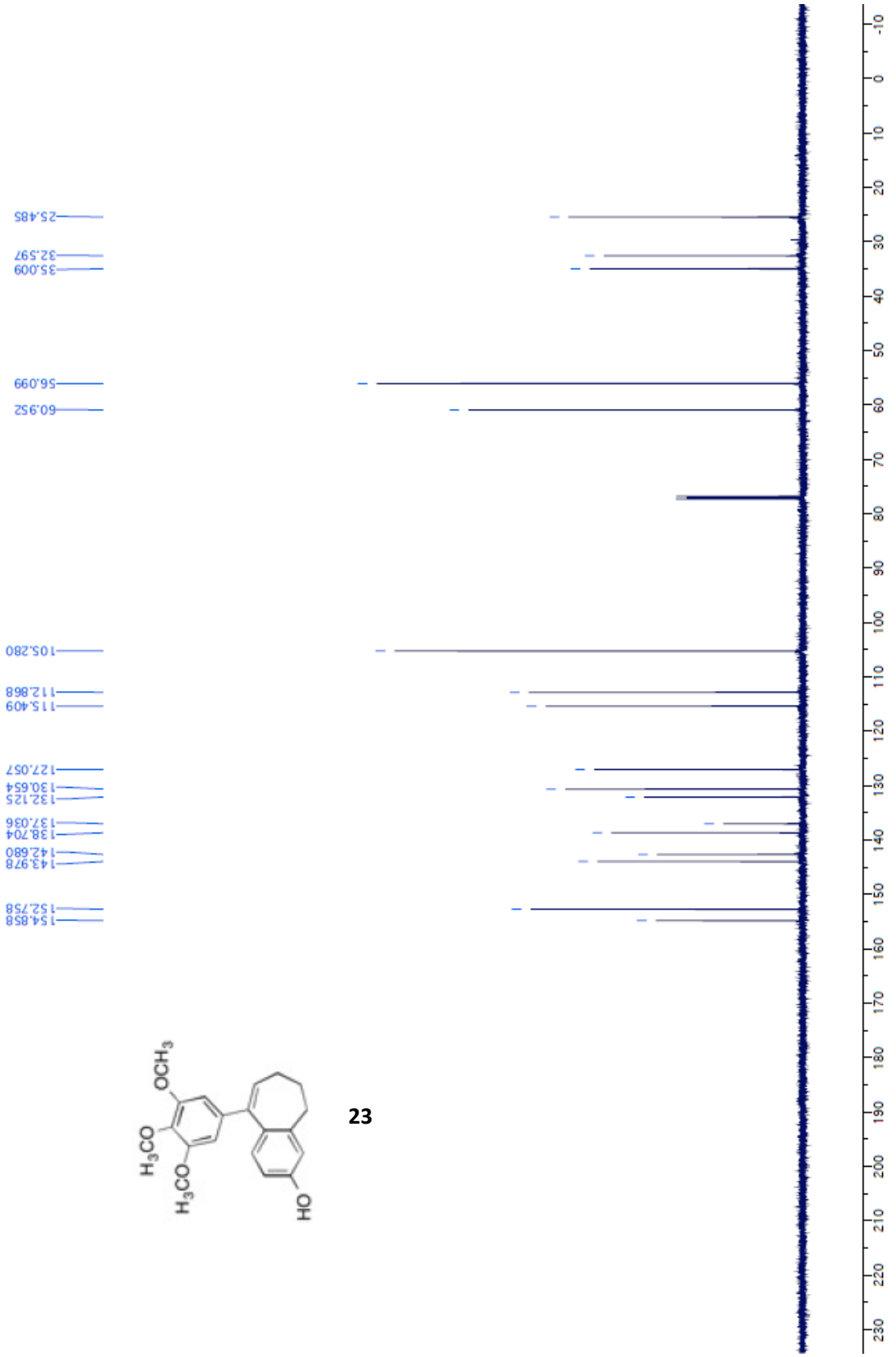
HO

23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HO

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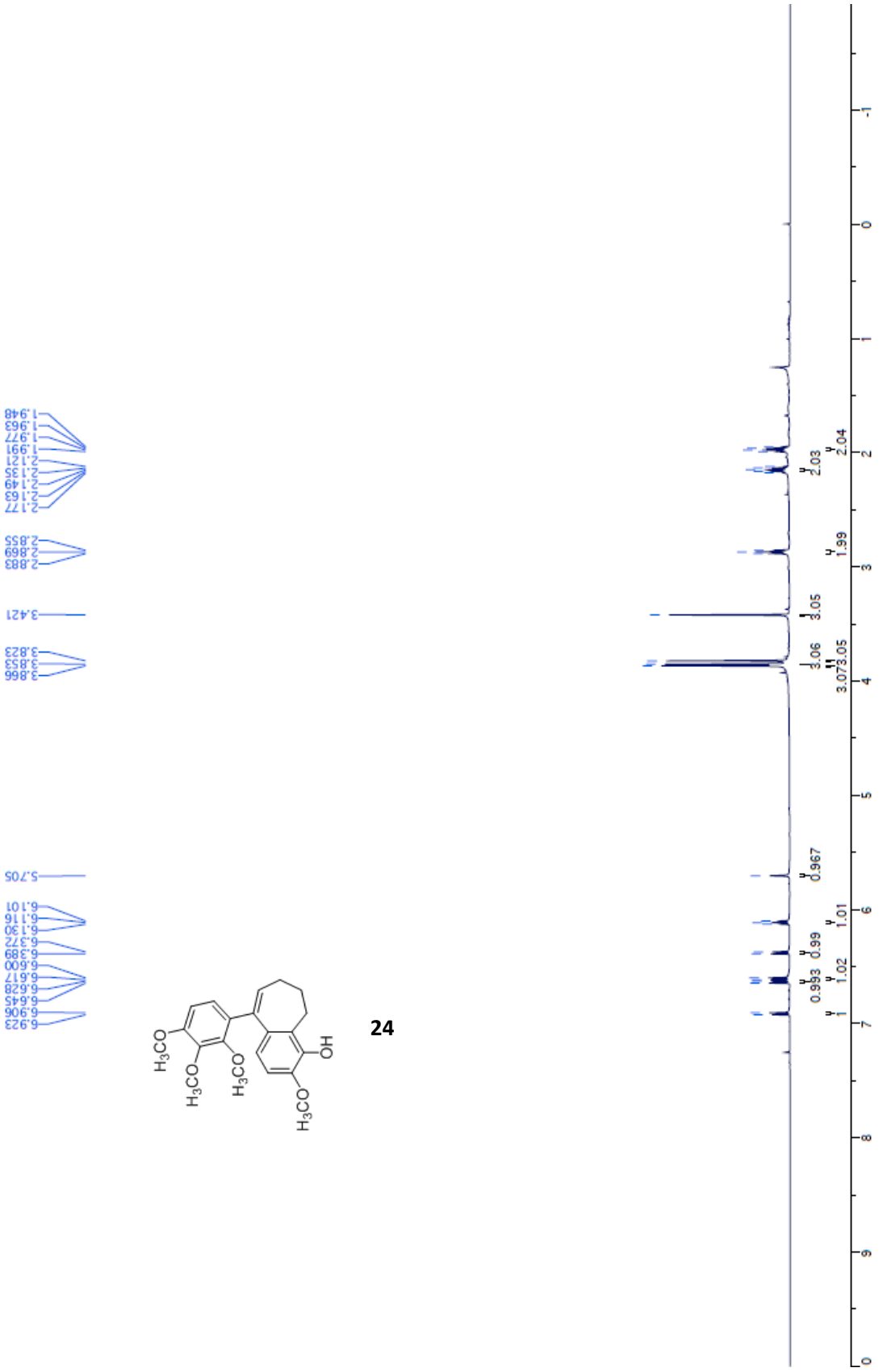
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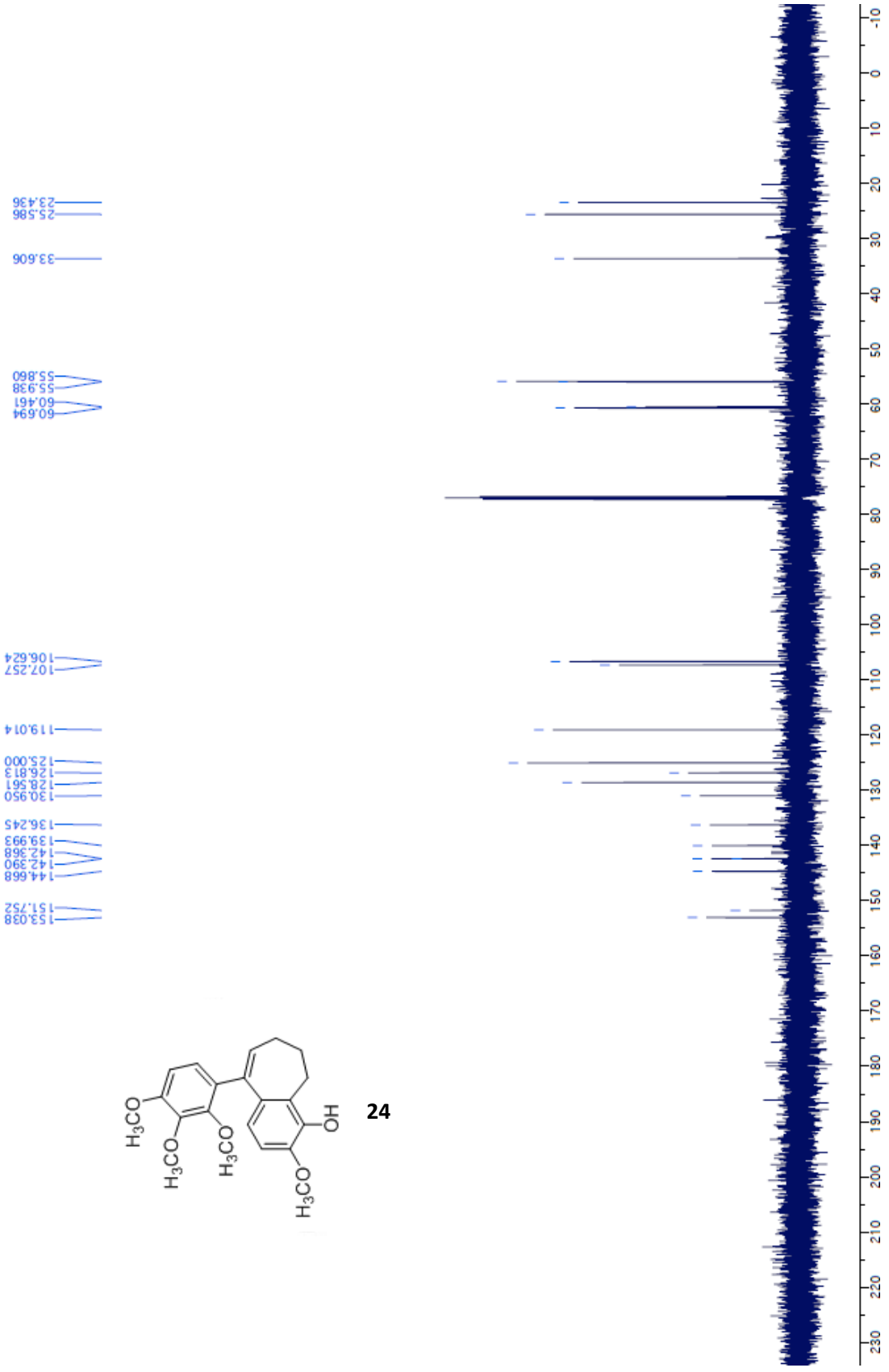
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OH

24

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