ABSTRACT

Design, Synthesis, and Biological Evaluation of Indole-Based Vascular

Disrupting Agents for the Potential Treatment of Cancer

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An established strategy in therapeutic oncology entails selectively targeting the tubulin-microtubule system, resulting in inhibition or hyperstimulation of tubulin polymerization effecting cell cycle arrest at anaphase. More recently, it has been found that small molecule inhibitors of tubulin assembly, known as vascular disrupting agents (VDAs), damage tumor vasculature by inducing cell morphology changes in the endothelial cells lining tumor vasculature. Tumor vascular is poorly established compared to normal tissue vasculature, and is disproportionately susceptible to this form of disruption, allowing such compounds to be selective. A particular benchmark VDA isolated from the *Combretum caffrum* tree is the natural product combretastatin A-4 (CA4), which binds at the colchicine site located on the β subunit of the αβ tubulin heterodimer. Previous investigations in the Pinney Laboratory led to the discovery of an indole-based derivative of CA4 known as OXi8006 which demonstrates comparable inhibitory effects of tubulin polymerization and cancer cell growth to CA4. Herein, we report the design, synthesis, and biological evaluation of two new analogs of OXi8006 as potential VDAs for the treatment of cancer. Additionally, several advanced intermediates were prepared for future synthetic approaches to diversely functionalized analogs of OXi8006. Details of the chemical synthesis and collaborative biochemical evaluation are presented.

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DESIGN, SYNTHESIS, AND BIOLOGICAL EVALUATION OF INDOLE-BASED VASCULAR DISRUPTING AGENTS FOR THE POTENTIAL TREATMENT OF CANCER

A Thesis Submitted to the Faculty of

Baylor University

In Partial Fulfillment of the Requirements for the

Honors Program

By

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ABBREVIATIONS

^{1}H NMR	Proton Nuclear Magnetic Resonance
^{13}C NMR	Carbon Nuclear Magnetic Resonance
^{19}F NMR	Fluorine Nuclear Magnetic Resonance
ATA	Angiogenic Targeting Agent
$^{\circ}$C	Degrees Celsius
CA4	Combretastatin A-4
CA1P	Combretastatin A-1 Phosphate
CA4P	Combretastatin A-4 Phosphate
$CDCl_3$	Deuterated Chloroform
$(CD_3)_2SO$	Deuterated Dimethyl Sulfoxide
CF_3	Trifluoromethyl
CH_3	Methyl
CH_2Cl_2	Dichloromethane
DMAP	N,N-Dimethylaminopyridine
cm	Centimeter
Et_3N	Triethylamine
EtOAc	Ethyl acetate
g	Gram
GI_{50}	Half Maximal Growth Inhibition
h	Hour
IC_{50}	Half Maximal Inhibitory Concentration

MHz	Megahertz
min	Minute
mL	Milliliter
mm	Millimeter
mmol	Millimole
Na_2SO_4	Sodium sulfate
nM	Nanomolar
NO_2	Nitro
nm	Nanometer
rt	Room temperature
OCH_3	Methoxy
OH	Hydroxyl
OTBS	*tert*-Butyldimethylsilyloxy
OXi8006	2-(3'-hydroxy-4'-methoxyphenyl)-3-(3",4",5"-trimethoxybenzoyl)-6-methoxyindole
TBAF	Tetrabutylammonium Fluoride
TBSCl	*tert*-Butyldimethylsilyl Chloride
TLC	Thin Layer Chromatography
µL	Microliter
VDA	Vascular Disrupting Agent
VTA	Vascular Targeting Agent

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I am grateful to Dr. Mary Lynn Trawick and coworkers for their assistance with the biochemical evaluation for this project. Your assistance gave me an opportunity to expand my learning experience to further areas of chemistry.

CHAPTER ONE

Introduction

Microtubules are critical to cell shape, transport, and division. They are comprised of globular α- and β-tubulin subunits which polymerize in units of heterodimer pairs. These subunits are largely analogous and both bind GTP, which is exchangeable in the β-tubulin subunit but not in the α-tubulin subunit. Hydrolysis of β-tubulin bound GTP to GDP drives attachment of additional heterodimers and ultimately polymerization into protofilaments. Microtubules, are generally comprised of 13 of these protofilaments. A third subunit, γ-tubulin, has been shown to be involved in the initial organization and nucleation of microtubules but is not part of the actual polymerized microtubule.[1,2] Microtubules are said to be dynamically unstable, as they switch infrequently between a phase in which they readily grow and another in which they rapidly shrink. Microtubules have distinct plus and minus ends based on the asymmetric orientation of the dimers. Elongation and shortening occur much more rapidly at the plus end of the microtubule, which is protected by a GTP cap, wherein the most recently added dimer contains GTP at its β-subunit. GTP is hydrolyzed to GDP only after the next subunit is added, such that the end contains at least one subunit with GTP. It is disruption of this cap which switches a microtubule into its shrinking phase, causing rapid depolymerization of the microtubule. Cells possess an array of microtubule-associated proteins (MAPs) which regulate the growth of microtubules and the stability of the GTP cap.[2,3]

The tubulin-microtubule system has become an attractive target for anticancer agents over the past several decades. Disrupting the normal dynamic instability of

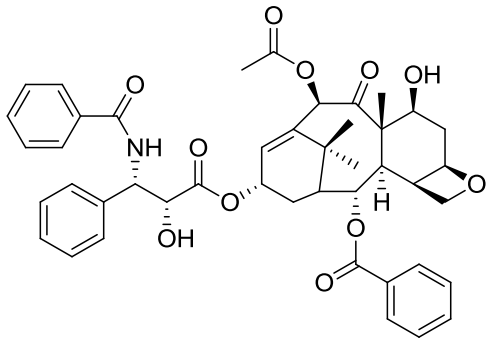
microtubules, either by inhibiting polymerization or by inducing hyperassembly such that normal depolymerization is greatly disrupted can limit normal cell regulation of microtubule length. Microtubules forming the mitotic spindle are particularly susceptible to disruption, since they must rapidly polymerize leading to the point at which chromosomes have aligned at the metaphase plate, and then rapidly depolymerize to separate the chromosomes at anaphase. By disrupting dynamic instability, the cell cycle can be reliably arrested at anaphase. This property has made the microtubule into one of the most promising targets for anticancer agents to date, and the key mechanism of action for many existing anticancer agents which target the microtubule.[4,5]

More recent research has identified other promising mechanisms for the treatment of cancer with microtubule-targeting agents. Tumor vasculature is greatly disorganized relative to normal blood vessels, and as a result, it is significantly more susceptible to disruption than normal tissues, such that drugs targeting the tubulin-microtubule protein system can be selective for tumor vasculature. Normal vasculature is carefully organized to allow an even flow through vascular networks to all cells. By contrast, tumor vasculature, which develops under heightened concentrations of pro-angiogenic factors, creates more haphazard networks which lack normal hierarchy and form vessels of inconsistent shape and diameter.[6] Tumors are reliant on their own vasculature for blood supply and strategies which result in decreased tumor vasculature can starve the underlying tumor.[7-9] One strategy for disrupting tumor vasculature, in line with earlier research into anticancer agents which target tubulin, is to directly induce apoptosis through cell cycle arrest. A second, more recent strategy, is to instead disrupt cytoskeletal tubulin to induce changes in vascular endothelial cell shape.[6] Drugs which target tumor

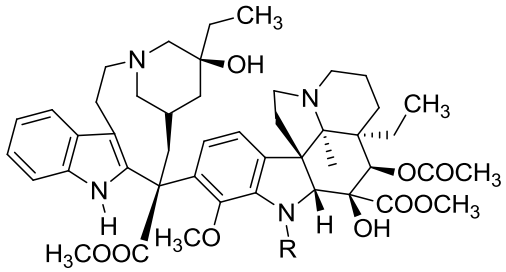
vasculature are collectively known as vascular targeting agents (VTAs), a category which includes angiogenic targeting agents (ATAs), which attempt to inhibit new vasculature, and vascular disrupting agents (VDAs), which attempt to disrupt existing tumor vasculature.[7-9] ATAs tend to stop a tumor from developing sufficient vasculature to support further growth, without destroying the underlying tumor. VDAs, by disrupting existing vasculature, tend to starve the underlying tumor causing necrosis in the core, but leave a viable rim of tumor cells at the outside of the tumor where vasculature is more dense. Both strategies have the potential to be used in combination with existing chemotherapy treatments which address their individual limitations, and preclinical studies of combined treatments involving VDAs have shown greater antitumor activity without increased toxicity.[10,11]

The search for new tubulin binding agents is ongoing as issues related to toxicity, resistance, and drug delivery have limited the effectiveness of all tubulin-binding agents to date in their use as anticancer agents.[12] Three distinct major binding sites on the tubulin αβ-heterodimer have been identified and are used as drug targets for the tubulin microtubule system: the taxol, vinblastine, and colchicine sites.[3] Taxol, vinblastine, and colchicine are shown in Figure 1. The taxol site is found on the β-subunit, but compounds binding the taxol site generally bind strongly only to the polymerized heterodimer and weakly to the unpolymerized subunit.[3,13,14] Its binding site is on the inner surface of the polymerized microtubule and it is able to bind in a 1:1 manner to individual tubulin heterodimers, but only a very small relative concentration is actually needed to achieve high microtubule stability.[3] Compounds binding the taxol site stimulate polymerization and are the furthest developed class, with taxol, also known as paclitaxel, being one of

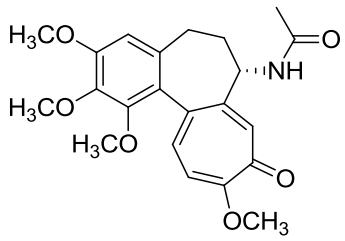
the best-selling anticancer drugs in the world.[4,15] Additionally, several taxol analogs are also in clinical use, while other taxol-site binding agents, including docetaxel, epothilones, and discodermolide are in various phases of clinical development. The vinblastine site is located on the β-subunit, and, additionally, vinblastine molecules are also able to bind the very ends of microtubules.[2,6] Vinblastine site compounds are tubulin inhibitors, preventing polymerization, but not directly causing depolymerization. Several, including vinblastine, vincristine, and vinorelbine are in clinical use, with other compounds in various stages of clinical testing. Vinblastine and vincristine are both natural products which have been have been in clinical use for the previous half-century, but which are neurotoxic yielding significant side effects. Both taxol-site and vinblastine-site tubulin-binding compounds generally act as antimitotic agents, arresting the cell cycle by interfering with the function of the mitotic spindle. Cells are unable to pass from metaphase into anaphase, eventually leading to apoptosis.[3]



Taxol[13]



Vinblastine (R = CH_3)[12]
Vincristine (R = CHO)[12]



Colchicine[11]

Figure 1: Taxol, Vinblastine, and Colchicine

The colchicine binding site is located mostly in the β-subunit but near the αβ interface and compounds binding to it cause tubulin depolymerization.[2,16] Colchicine, which has been known since the 1930s, is clinically as a treatment for gout, but has proven too toxic for use in treatment of cancer.[3] No colchicine-binding compounds are FDA-approved for the treatment of cancer today, but a number of such compounds are in clinical trials. Unlike taxol-site or vinblastine-site binding agents, which act as selective antimitotic agents, useful colchicine-site binding agents are VDAs which act primarily to disrupt tumor vasculature in order to starve the tumor. Compounds related to combretastatin A-4 (CA4) are the furthest advanced in clinical studies among colchicine binding agents. CA4 is a natural product isolated from the *Combretum caffrum* tree in the late 1970s and is a small molecule VDA. Its two aryl rings and the substituents thereof are critically important to its activity against tubulin.[1,17-25] Combretastatin A-4-phosphate (CA4P), a phosphate salt prodrug to CA4, has shown significant success in phase I and phase II clinical trials and is currently in phase III testing.[6,9,10] CA4, CA4P, and CA1P, a close analog currently in phase I clinical trials, are shown in Figure 2.

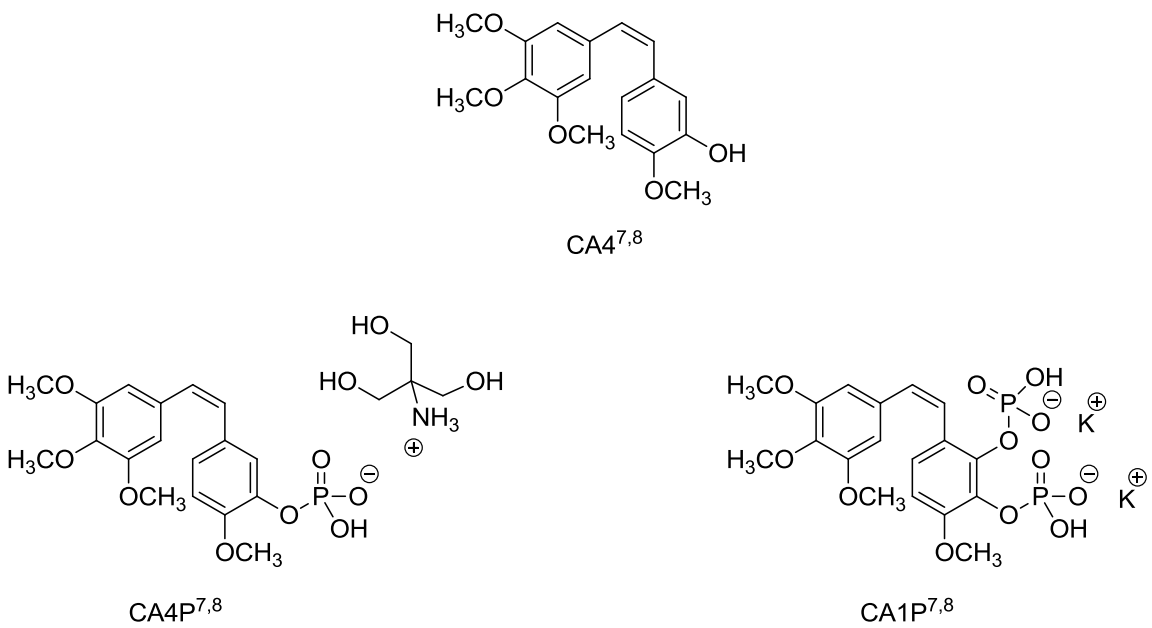


Figure 2: Combretastatin A-4 and Analogs in Clinical Trials

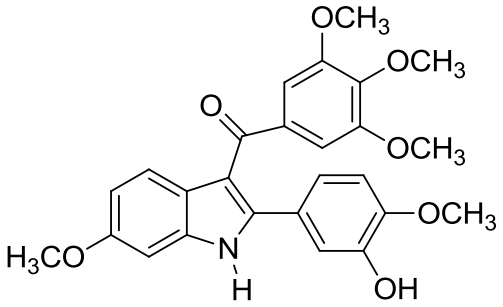
The synthesis and evaluation of indole-based small molecule tubulin-binding compounds, particularly those related to the structures of colchicine and CA4, is a significant area of the search for viable anticancer agents targeting the tubulin-microtubule protein system.[12,20-25] 3-Formyl-2-phenylindoles were synthesized by von Angerer and coworkers by reacting bromoacetophenones with substituted anilines, followed by a formylation step.[20] In testing various combinations of hydroxy and methoxy substituted 3-formyl-2-phenylindoles, it was found that methoxy substituents on the phenyl ring increased tubulin inhibition by an order of magnitude.[12,20] Synthesis and testing of a library of 2-aroylindoles showed a similar trend, with most of the most potent compounds containing one or more methoxy substituents on the phenyl ring.[12,21] Research into diarylindoles has suggested that compounds using an indole as a rigid bridge between aryl rings may be more active than those with a more flexible bridge between these rings.[12,22] 3-Aroylindoles and 1-aroylindoles have been synthesized by

reacting indoles with substituted benzoyl chlorides and aroyl chlorides, respectively, and analysis of these compounds showed that activity varied orders of magnitude based on the locations of methoxy substituents.[12,23] Arylthioindoles, using a sulfur atom to link the two aryl rings, again showed methoxy substituents to be a key determinant of the compounds' activities. Trimethoxy substitutions at the 3, 4, and 5 positions on the aryl ring were critical in inhibition tests against cancer cell lines such that only such arylthioindoles showed meaningful activity.[12,24] 2-Aryl-3-aroylindoles, were synthesized by multiple groups using different synthetic routes. A one-carbon linker between the the indole and the trimethoxyaryl ring was necessary for these compounds to have significant tubulin-binding activity.[12,25]

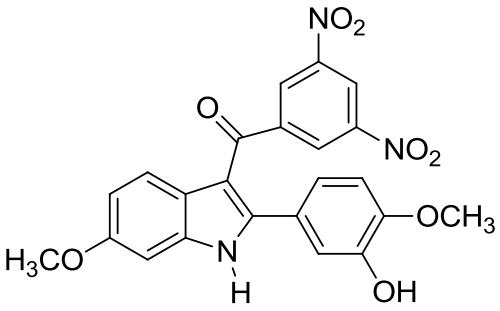
OXi8006, 2-(3'-hydroxy-4'-methoxyphenyl)-3-(3",4",5"-trimethoxybenzoyl)-6-methoxyindole, is one such 2-aryl-3-aroylindole. A carbonyl group serves as the one-carbon linker between the indole and trimethoxyaryl ring.[26-28] It is a VDA, a CA4 analog, and a potent colchicine-site inhibitor of the tubulin-microtubule system.[12] OXi8006 was first synthesized by Pinney and coworkers, and later by Flynn and coworkers in a separate one-pot synthetic route using organometallics.[25-28] The early steps of the Pinney group synthetic route are analogous to those used in von Angerer's of 3-formyl-2-phenylindoles, as the two pathways diverge after linking a phenyl ring to the 2 position of an indole.[20,26] Flynn and coworkers report a tubulin inhibition IC_{50} value of 1.3 ± 0.2 μM for OXi8006 versus 2.1 ± 0.1 μM for CA4, and a human breast carcinoma cell cytotoxicity IC_{50} value of 42 ± 10 nM for OXi8006 versus 11 ± 4 nM for CA4.[25] Given the high activity of OXi8006 and its known synthetic pathway, the compound lends itself as a good starting point in the search for new small molecule colchicine-site inhibitory

agents. Substituting compounds with alternative functional groups at various stages of the synthetic pathway will yield new analogs, which can then be tested for activity against cancer cell lines. In this pathway, alternative anilines can be used to change the final substituents attached to the indole and alternative acyl chlorides can be used in the final steps to change the substituents attached to the linked aryl ring. By working to create analogs to a compound already known to exhibit high activity, there is reasonable hope that a compound can be discovered which exhibits similar or higher levels of activity against one or more cancer cell lines.

In this study, two new OXi8006 analogs were synthesized by substituting commercially available acyl chlorides at a late step in the synthetic pathway designed by Pinney and coworkers for OXi8006. In addition, several advanced indole intermediates were prepared or are currently being purified, which were formed by substituting several different commercially available anilines at an earlier step prior to indole formation. Synthesis of additional analogs based on these advanced intermediates, using both acyl chlorides with trimethoxyl substituents from the pathway to OXi8006 and alternative, commercially available acyl chlorides is currently in progress. Through a collaborative SRB assay with Mary Lynn Trawick and coworkers at Baylor University, the two newly synthesized compounds were tested for relative activity against cancer cell lines to OXi8006.[29,30] The structure of OXi8006 and the tested analog compounds are shown in Figure 3.



OXi8006[25-28]



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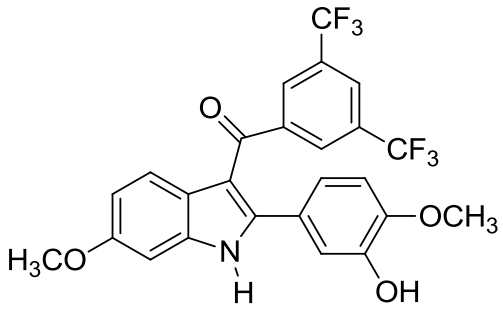

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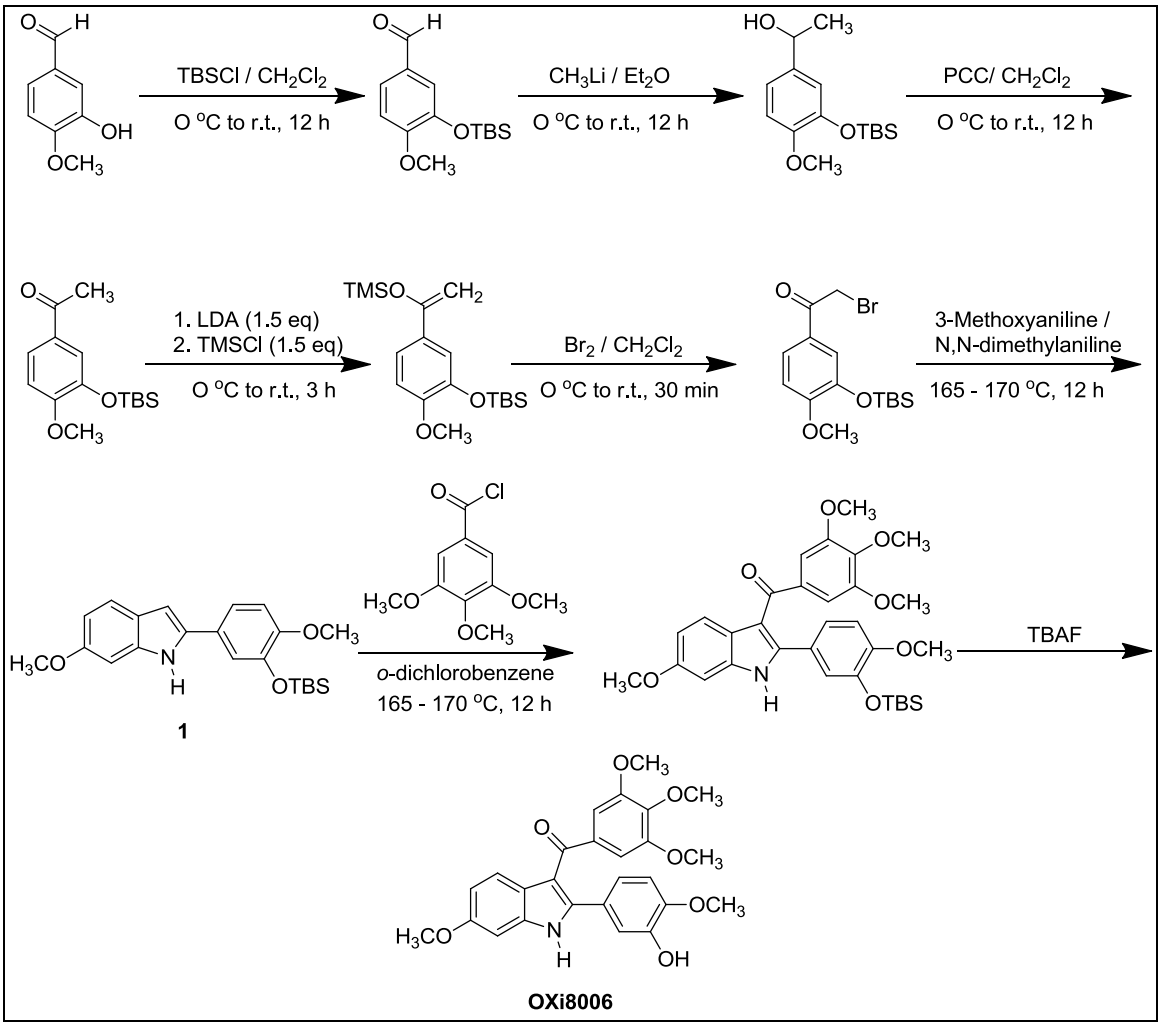
Figure 3: OXi8006 and Synthesized Analogs

CHAPTER TWO

Results and Discussion

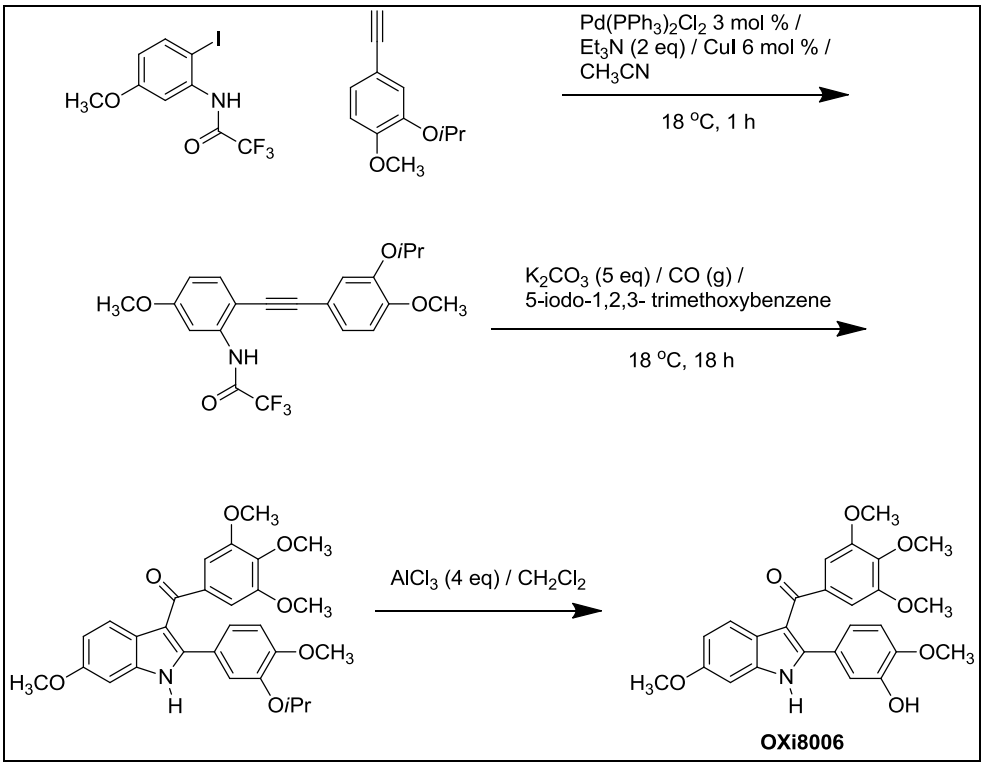
Synthesis of OXi8006 analogs

Two synthetic routes for OXi8006 have been reported. The first, reported by Pinney and coworkers, forms an indole by first synthesizing a protected benzoacetophenones. This protected benzoacetophenone reacts with 3-methoxyaniline in N,N-dimethylaniline to form an indole intermediate. A Friedel-Crafts acylation with 3,4,5-trimethoxybenzoyl chloride yields a protected 2-aryl-3-aroylindole. Deprotection with tetrabutylammoniumfluoride (TBAF) yields the final product. The synthesis by Pinney and coworkers is shown in Scheme 1.[26]



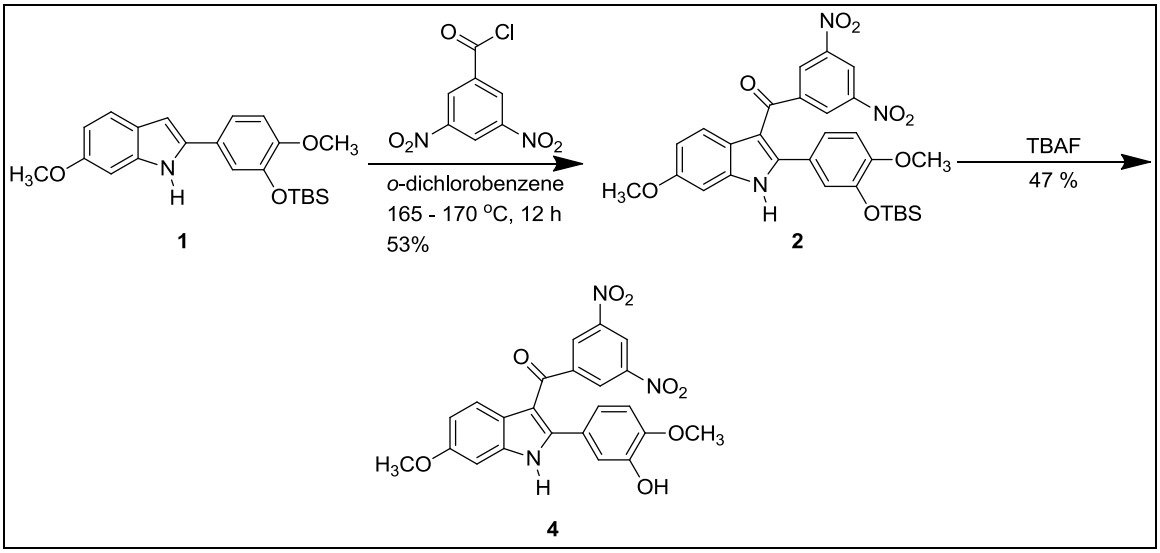
Scheme 1: Pinney Group Synthesis of OXi8006[26]

A second, alternative synthetic route published by Flynn and coworkers uses metallorganics to facilitate a one-pot synthesis of OXi8006.[25] Starting from *o*-iodotrifluoroacetanilide, and an alkyne, Sonogashira conditions yield an alkyne intermediate. Reaction with an aryl iodide forms a 2-aryl-3-aroylindole, and $AlCl_3$ is then used to deprotect it to form OXi8006.



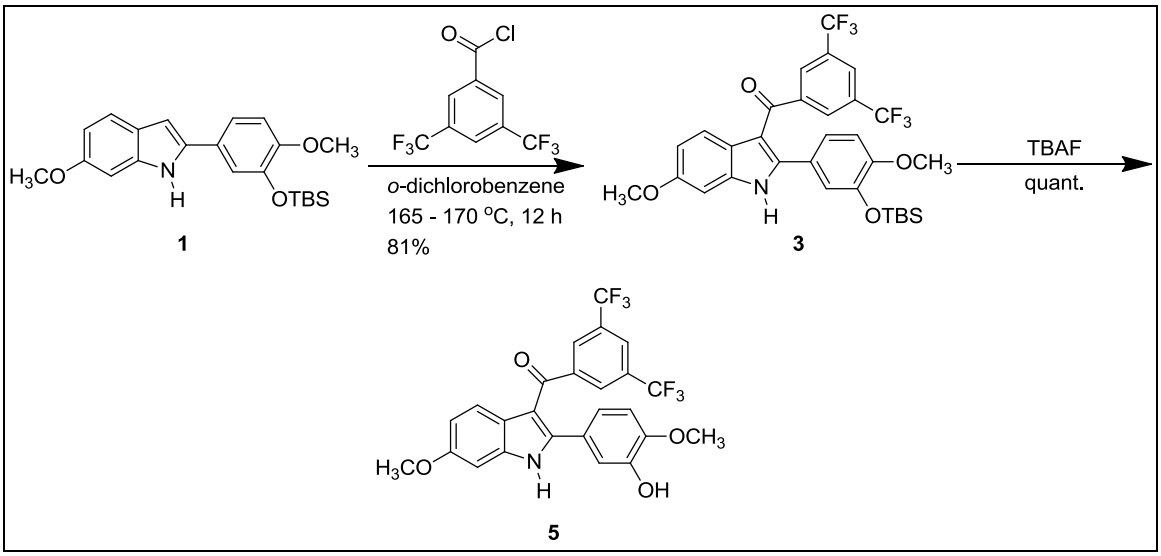
Scheme 2: Flynn and Coworkers Synthesis of OXi8006[25]

The two new analogs to OXi8006 were formed using the synthesis published by Pinney and coworkers, starting from advanced indole **1** prepared by the Pinney group and benzoylchlorides are shown in Scheme 3 and Scheme 4.



Scheme 3: Synthesis of OXi8006 analog **4** from indole **1**

Advanced indole **1** prepared by the Pinney group was used in a Friedel-Crafts acylation using commercially available 3,5-dinitrobenzoylchlorides in order to yield protected benzoyl indole **2**. Deprotection of this compound using TBAF to remove the *tert*-butyldimethylsilyl group yielded final analog **4**.



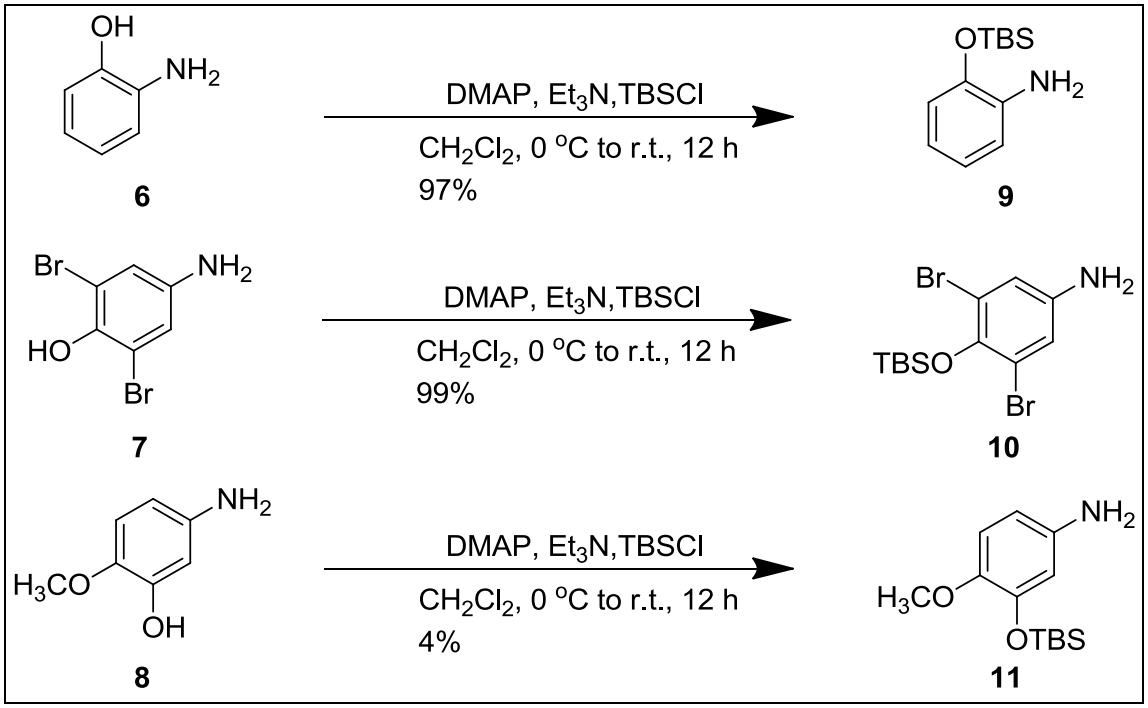
Scheme 4: Synthesis of OXi8006 analog **5** from indole **1**

Advanced indole **1** prepared by the Pinney group was used in a Friedel-Crafts acylation using commercially available 3,5-bistrifluoromethylbenzoylchloride in order to yield protected benzoyl indole **3**. Deprotection of this compound using TBAF to remove the *tert*-butyldimethylsilyl group yielded final analog 5.

Synthesis of Indoles for Planned OXi8006 Analogs

The synthesis by Pinney and coworkers forms an indole intermediate by through a reaction of a protected bromoacetophenone with 3-methoxyaniline. Substituting an

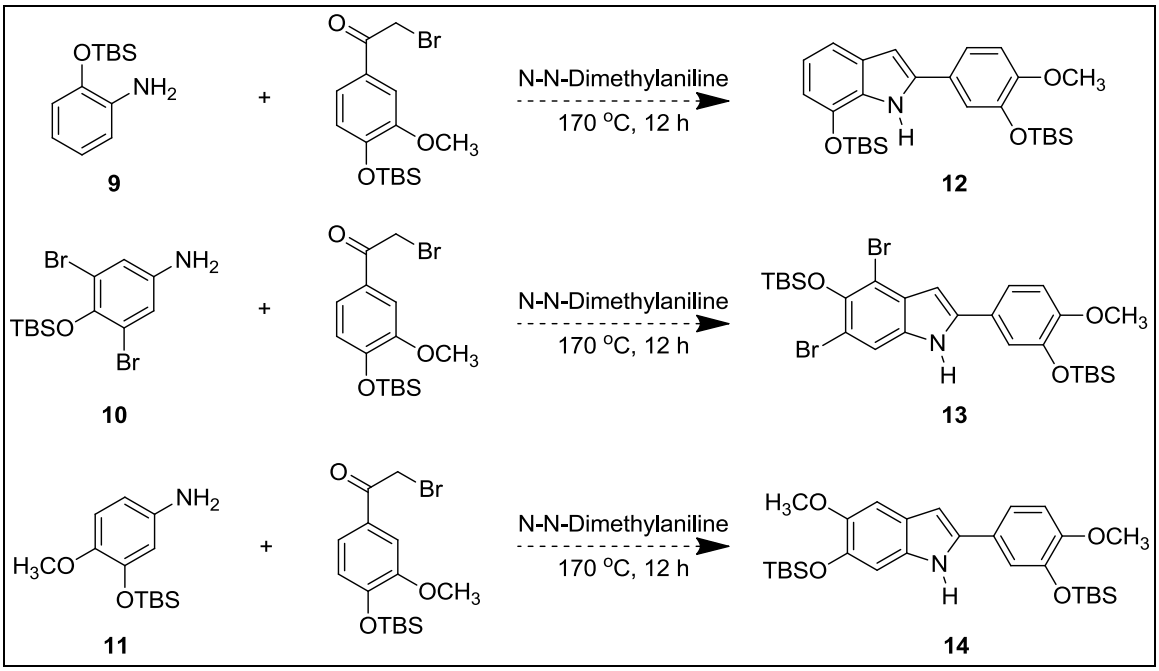
alternative aniline would be expected to lead to a different indole product. Protection of three anilines in preparation for forming indoles is illustrated in Scheme 5.



Scheme 5: Protection of Anilines

Anilines **6-8** were protected in reactions with *tert*-butyldimethylsilyl chloride in a 1:1.1 ratio in order to form protected anilines **9-11**, using DMAP as a catalyst and triethylamine as the necessary base. Compounds **9** and **10** were synthesized in high yield, but compound **11** was synthesized in very low yield, suggesting that aniline **8** may not have been sufficiently soluble in the solvent dicholoromethane.

In-progress and planned synthesis of indoles from the above protected anilines is shown in Scheme 6.



Scheme 6: Planned and In-Progress Synthetic Route of Indoles

Synthesis of indoles **12** and **13** by reaction of protected anilines **9** and **10** by reaction with 2-bromo-1-(4-((tert-butyldimethylsilyl)oxy)-3-methoxyphenyl)ethanone has been attempted, and crude TLC shows apparent presence of the indole products. Purification is in progress, but low yields have made these indoles difficult to isolate. Synthesis of indole **14** is planned pending synthesis of protected aniline **13** in greater yield. Future plans include using indoles **12-14** in a synthetic pathway similar to Scheme 1 in order to yield new OXi8006 analogs.

Biochemical Evaluation of the Synthesized Compounds

Compounds **4** and **5** were evaluated for their ability to inhibit growth of cancer cell lines SK-OV-3, NCI-H460, and DU145 using a standard sulforhodamine B (SRB) assay in a collaboration with Dr. Mary Lynn Trawick's group at Baylor University.[30] The GI_{50} values for this assay and literature values for CA4 are shown in Table 1.

Table 1: SRB Assay Results[8,30]

Compound	GI_{50} (μM)		
	SK-OV-3	NCI-H460	DU-145
4	4.35 ± 0.290	4.08 ± 0.119	5.52 ± 0.106
5	1.45 ± 0.365	2.86 ± 0.104	3.01 ± 0.0829
OXi8006	0.00325 ± 0.00176	0.0338 ± 0.00577	0.0245 ± 0.0102
CA4[8]	0.00013	0.002	0.002

Compounds **4** and **5** showed significant activity against all three lines, but were all approximately two orders of magnitude less active than the values obtained for OXi8006. Compounds **4** and **5** differ from OXi8006 in the substituents of the benzophenone ring, with OXi8006 containing methoxy groups at the 3', 4', and 5' positions, compound **4** containing nitro groups at the 3' and 5' positions, and compound **5** containing trifluorylmethyl groups at the 3' and 5' positions. This suggests that the substituents of this ring system are critical to the activity of these compounds against cancer cell lines.

CHAPTER THREE

Conclusions

Two analogs of indole-based VDA OXi8006 were successfully synthesized from an advanced indole prepared by the Pinney Group at Baylor University. Furthermore, the synthesis of three new indole intermediates is in progress, and future work will involve synthesizing additional analogs of OXi8006 from these intermediates. The two analogs synthesized in this study, compounds **4** and **5**, showed significant activity against the three selected human cancer cell lines, but were less cytotoxic against these cell lines in when compared to OXi8006. Though these two compounds are less cytotoxic, their synthesis and evaluation is useful in the greater context of searching for compounds which are as or more active than OXi8006 and thus may have potential medicinal applications. The results for these compounds will be directly useful in directing further studies with the new indoles currently being synthesized, as the synthetic route after indole formation will determine the benzophenone ring substituents in the resulting analogs.

CHAPTER FOUR

Materials and Methods

General Section

Dichloromethane and tetrahydrofuran (THF) were used in their anhydrous forms, as obtained from the chemical suppliers. Reactions were performed under an inert atmosphere using nitrogen gas, unless specified. Thin-layer chromatography (TLC) plates (precoated glass plates with silica gel 60 F254, 0.25 mm thickness) were used to monitor reactions. Purification of intermediates and products was carried out with a flash purification system using silica gel (200-400 mesh, 60 Å) or RP-C18 prepacked columns. Intermediates and products synthesized were characterized on the basis of their ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), ^{19}F NMR (470 MHz), and ^{31}P NMR (202 MHz) spectroscopic data. TMS was used as an internal standard for spectra recorded in $CDCl_3$, $(CD_3)_2SO$, and $(CD_3)_2CO$. The spectra recorded in D_2O: δ 1H 4.79. All the chemical shifts are expressed in ppm (δ), coupling constants (*J*) are presented in Hz, and peak patterns are reported as broad (br), singlet (s), doublet (d), triplet (t), sextet (sx), double doublet (dd), double triplet (dt), triplet of triplets (tt), doublet of doublets of doublets (ddd), and multiplet (m). Purity of the final compounds was further analyzed at 25 ^{o}C using an Agilent 1200 HPLC system with a diode-array detector (λ = 190-400 nm), a Zorbax XDB-C18 HPLC column (4.6 mm - 150 mm, 5 μm), and a Zorbax reliance cartridge guard-column; eluents, solvent A, 25 mmol TBAB, 0.1% TFA in H_2O, solvent B, 25 mmol TBAB, 0.08% TFA in acetonitrile-H_2O (80:20 (v/v) ratio); gradient, 80%

A/20% B over 0 to 45 min; post-time 15 min; flow rate 1.0 mL/min; injection volume 20 μL; monitored at wavelengths of 210, 254, 230, 280, and 360 nm.

Synthesis of 2-(3'-tert-Butyldimethylsiloxy-4'-methoxyphenyl)-3-(3'',5''dinitrobenzoyl)-6-methoxyindole **2**

To a well-stirred solution of **1** (0.50 g, 1.30 mmol) in *o*-dichlorobenzene (20 mL) was added 3,5-dinitrobenzoylchloride (0.45 g, 1.90 mmol). The reaction mixture was then heated to reflux at 160 ^{o}C for 12 hours. The *o*-dichlorobenzene was distilled off under reduced pressure and the resulting crude dark colored solid was subjected to flash chromatography using a prepacked 50 g silica column [eluents: solvent A, EtOAc, solvent B, hexanes; gradient, 7% A/93% B (4 CV), 7% A/93% B → 60% A/40% B (11 CV), 60% A/40% B (2 CV); flow rate, 40 mL/min; monitored at λ's 254 and 280 nm] resulting in a pale yellow powder (0.40 g, 0.69 mmol, 53%, R_f 0.59 (70:30 hexanes:EtOAc)).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.85 (t, *J* = 2.0 Hz, 1H, Ar<u>H</u>), 8.62 (d, *J* = 2.0 Hz, 2H, Ar<u>H</u>), 8.60 (br s, 1H, N<u>H</u>) 8.15 (d, *J* = 8.5 Hz, 1H, Ar<u>H</u>), 7.00 (dd, *J* = 2.0 Hz, 8.5 Hz, 1H, Ar<u>H</u>), 6.95 (d, 2.0 Hz, 1H, Ar<u>H</u>), 6.87 (dd, *J* = 2.0 Hz, 8.0 Hz, 1H, Ar<u>H</u>), 6.61 (d, *J* = 8.5 Hz, 1H, Ar<u>H</u>), 6.56 (d, *J* = 2.5 Hz, 1H, Ar<u>H</u>), 3.90 (s, 3H, OC<u>H</u>$_3$), 3.70 (s, 3H, OC<u>H</u>$_3$), 0.89 (s, 9H, C(C<u>H</u>$_3$)$_3$), 0.00 (s, 6H, Si(C<u>H</u>$_3$)$_2$).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 187.3, 158.1, 152.1, 147.8, 145.3, 145.1, 143.1, 136.5, 129.1, 123.5, 123.4, 122.7, 122.4, 122.3, 120.1, 112.8, 112.5, 111.8, 95.0, 55.9, 55.5, 25.6, 18.4, -4.8.

Synthesis of 2-(3'-tert-Butyldimethylsiloxy-4'-methoxyphenyl)-3-(3'',5''-(bistrifluoromethyl)benzoyl)-6-methoxyindole **3**

To a well-stirred solution of **1** (0.20 g, 0.52 mmol) in *o*-dichlorobenzene (15 mL) was added 3,5-bis(trifluoromethyl)benzoylchloride (0.14 mL, 0.78 mmol). The reaction mixture was then heated to reflux at 160 ^{o}C for 12 hours. The *o*-dichlorobenzene was distilled off under reduced pressure and the resulting crude dark colored solid was subjected to flash chromatography using a prepacked 50 g silica column [eluents: solvent A, EtOAc, solvent B, hexanes; gradient, 7% A/93% B (4 CV), 7% A/93% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate, 40 mL/min; monitored at λ's 254 and 280 nm] resulting in pale orange crystals (0.26 g, 0.42 mmol, 81%, R_f 0.47 (70:30 hexanes:EtOAc)).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.70 (br s, 1H, NH), 8.07 (d, *J* = 9.0 Hz, 1H, ArH), 8.03 (s, 2H, ArH) 7.74 (s, 1H, ArH), 6.96 (dd, *J* = 2.5 Hz, 9.0 Hz, 1H, ArH), 6.92 (d, *J* = 2.0 Hz, 1H, ArH), 6.85 (dd, *J* = 2.0 Hz, 8.0 Hz, 1H, ArH), 6.65 (d, *J* = 8.5 Hz, 1H, ArH), 6.62 (d, *J* = 2.5 Hz, 1H, ArH), 3.86 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 0.91 (s, 9H, $C(CH_3)_3$), 0.00 (s, 6H, $Si(CH_3)_2$).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 189.5, 157.8, 152.0, 145.3, 144.5, 141.5, 136.6, 131.3 (q, *J* = 33.4 Hz), 129.6 (m), 124.5 (m), 124.1, 123.9, 122.9, 122.7, 122.5, 122.2, 122.0, 112.4, 112.1, 94.9, 55.8, 55.5, 25.6, 18.4, -4.8.

^{19}F NMR ($CDCl_3$, 470 MHz): δ -62.8 (s, 6F, CF_3).

Synthesis of 2-(3'-Hydroxy-4'-methoxyphenyl)-3-(3",5"-dinitrobenzoyl)-6-methoxyindole **4**

To a well-stirred solution of **3** (0.40 g, 0.69 mmol) in THF (10 mL), under nitrogen at 0 ^{o}C was added tetrabutylammoniumfluoride (TBAF) solution (1.03 mL, 1.03 mmol, 1M in THF) dropwise. The reaction mixture was then stirred for 30 minutes

allowing it to warm to room temperature. The reaction mixture was then quenched with water (10 mL) and transferred to a seperatory funnel. The organic phase was extracted using EtOAc (3 x 10 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash column chromatography using a prepacked 50 g silica column [eluents: solvent A, EtOAc, solvent B, hexanes; gradient, 7% A/93% B (4 CV), 7% A/93% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate, 40 mL/min; monitored at λ's 254 and 280 nm] afforded the desired indole free phenol ligand **4** (0.15 g, 0.33 mmol, 47%, R_f 0.12 (70:30 hexanes:EtOAc)) as an yellow powder.

^{1}H NMR ($(CD_3)_2SO$, 500 MHz): δ 12.21 (br s, 1H, NH), 9.06 (br s, 1H, OH), 8.68 (t, *J* = 2.0 Hz, 1H, ArH), 8.41 (d, *J* = 2.0 Hz, 2H, ArH) 8.05 (d, *J* = 8.5 Hz, 1H, ArH), 6.97 (dd, *J* = 2.0 Hz, 1H, ArH), 6.92 (dd, *J* = 2.0 Hz, 8.5 Hz, 1H, ArH), 6.71 (dd, *J* = 2.0 Hz, 8.0 Hz, 1H, ArH), 6.68 (d, *J* = 8.5 Hz, 1H, ArH), 6.54 (d, *J* = 2.0 Hz, 1H, ArH), 3.83 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3).

^{13}C NMR ($(CD_3)_2SO$, 125 MHz): δ 186.7, 156.8, 148.2, 147.1, 146.7, 146.1, 142.8, 136.7, 128.5, 123.3, 121.77, 121.75, 121.7, 119.3, 117.2, 112.0, 111.7, 111.1, 95.0, 55.7, 55.3.

Synthesis of 2-(3'-Hydroxy-4'-methoxyphenyl)-3-(3",5"-(bistrifluoromethyl)benzoyl)-6-methoxyindole **5**

To a well-stirred solution of **3** (0.26 g, 0.42 mmol) in THF (10 mL), under nitrogen at 0 ^oC was added tetrabutylammoniumfluoride (TBAF) solution (0.63 mL, 0.63 mmol, 1M in THF) dropwise. The reaction mixture was then stirred for 30 minutes allowing it to warm to room temperature. The reaction mixture was then quenched with

water (10 mL) and transferred to a seperatory funnel. The organic phase was extracted using EtOAc (3 x 10 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash column chromatography using a prepacked 50 g silica column [eluents: solvent A, EtOAc, solvent B, hexanes; gradient, 7% A/93% B (4 CV), 7% A/93% B → 60% A/40% B (10 CV), 60% A/40% B (5.2 CV); flow rate, 40 mL/min; monitored at λ's 254 and 280 nm] afforded the desired indole free phenol ligand **5** (0.22 g, 0.42 mmol, 100%, R_f 0.21 (70:30 hexanes:EtOAc)) as an orange powder.

^{1}H NMR ($(CD_3)_2SO$, 500 MHz): δ 12.10 (br s, 1H, NH), 9.00 (br s, 1H, OH), 7.99 (m, 2H, ArH), 7.90 (s, 2H, ArH) 6.96 (d, *J* = 2.0 Hz, 1H, ArH), 6.89 (dd, *J* = 2.5 Hz, 9.0 Hz, 1H, ArH), 6.64 (m, 3H, ArH), 3.82 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3).

^{13}C NMR ($(CD_3)_2SO$, 125 MHz): δ 188.4, 156.7, 148.2, 146.3, 146.2, 142.2, 136.7, 129.7 (q, *J* = 32.75 Hz), 128.9 (m), 124.0, 123.7 (m), 123.5, 122.0, 121.9, 121.6, 121.4, 116.9, 111.7, 111.6, 111.0, 94.9, 55.6, 55.3.

^{19}F NMR ($(CD_3)_2SO$, 470 MHz): δ -61.4 (s, 6F, CF_3).

2-(tert-Butyldimethylsilyloxy)-aniline **9**

To a clean dry round bottom flask 2-aminophenol **6** (5.00 g, 32.89 mmol) was dissolved in dry CH_2Cl_2 (100 mL) under nitrogen. The solution was then cooled to 0 ^oC and Et_3N (5.05 mL, 36.18 mmol) was added followed by the addition of N,N-dimethylaminopyridine (DMAP) (0.40 g, 3.29 mmol). The reaction mixture was then stirred for 10 minutes upon which *tert*-butyldimethylsilyl chloride (TBSCl) (5.45 g, 36.18 mmol) was added gradually. The solution was then allowed to warm to room temperature and was stirred for 12 hrs. Upon completion, the reaction was quenched with water (150

mL) and transferred to a separatory funnel where the organic layer was extracted with CH_2Cl_2. The extracted layers were then combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The TBS aniline product **9** (7.61 g, 34.08 mmol, 97% R_f 0.55, (90:10 hexanes:EtOAc)) was isolated as a brown oil and was taken to the next step without purification.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.88 (m, 1H, Ar<u>H</u>), 6.85 (dd, *J* = 1.5 Hz, 8.0 Hz, 1H, Ar<u>H</u>), 6.78 (dd, *J* = 1.5 Hz, 8.0 Hz, 1H, Ar<u>H</u>), 6.72 (m, 1H, ArH), 3.76 (br, s, 2H, N<u>H</u>$_2$), 1.14 (s, 9H, C(C<u>H</u>$_3$)$_3$), 0.35 (s, 6H, Si(C<u>H</u>$_3$)$_2$).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 142.9, 138.2, 121.9, 118.5, 118.3, 115.7, 25.9, 18.3, -4.2.

3,5-(Dibromo)-4-(tert-butyldimethylsilyloxy)-aniline **10**

To a clean dry round bottom flask 2,6-dibromo-4-aminophenol **7** (4.00 g, 14.99 mmol) was dissolved in dry CH_2Cl_2 (100 mL) under nitrogen. The solution was then cooled to 0 ^oC and Et_3N (2.30 mL, 16.48 mmol) was added followed by the addition of N,N-dimethylaminopyridine (DMAP) (0.18 g, 1.49 mmol). The reaction mixture was then stirred for 10 minutes upon which *tert*-butyldimethylsilyl chloride (TBSCl) (2.48 g, 16.48 mmol) was added gradually. The solution was then allowed to warm to room temperature and was stirred for 12 hrs. Upon completion, the reaction was quenched with water (150 mL) and transferred to a separatory funnel where the organic layer was extracted with CH_2Cl_2. The extracted layers were then combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by flash column chromatography using a prepacked 50 g silica column [eluents: solvent A, EtOAc, solvent B, hexanes; gradient, 7% A/93% B (4 CV), 7% A/93% B → 60% A/40% B (10 CV), 60% A/40% B (2.0 CV); flow rate, 40 mL/min; monitored at λ's 254 and 280 nm]

afforded the TBS aniline product **10** (5.70 g, 14.95 mmol, 99%, R_f 0.57, (70:30 hexanes:EtOAc)) as a dark brown oil.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.83 (s, 2H, Ar<u>H</u>), 3.46 (br, s, 2H, N<u>H</u>$_2$), 1.04 (s, 9H, C(C<u>H</u>$_3$)$_3$), 0.32 (s, 6H, Si(C<u>H</u>$_3$)$_2$).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 142.8, 141.8, 119.4, 116.1, 26.4, 19.1, -2.1.

3-(tert-Butyldimethylsilyloxy)-4-methoxyaniline **11**

To a clean dry round bottom flask 3-amino-2-methoxyphenol **8** (4.00 g, 28.75 mmol) was dissolved in dry CH_2Cl_2 (100 mL) under nitrogen. The solution was then cooled to 0 ^oC and Et_3N (4.41 mL, 31.62 mmol) was added followed by the addition of N,N-dimethylaminopyridine (DMAP) (0.35 g, 2.87 mmol). The reaction mixture was then stirred for 10 minutes upon which *tert*-butyldimethylsilyl chloride (TBSCl) (4.77 g, 31.62 mmol) was added gradually. The solution was then allowed to warm to room temperature and was stirred for 12 hrs. Upon completion, the reaction was quenched with water (150 mL) and transferred to a separatory funnel where the organic layer was extracted with CH_2Cl_2. The extracted layers were then combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by flash column chromatography using a prepacked 100 g silica column [eluents: solvent A, EtOAc, solvent B, hexanes; gradient, 7% A/93% B (4 CV), 7% A/93% B → 60% A/40% B (10 CV), 60% A/40% B (2.0 CV); flow rate, 40 mL/min; monitored at λ's 254 and 280 nm] afforded the TBS aniline product **11** (0.32 g, 1.27 mmol, 4%, R_f 0.50, (70:30 hexanes:EtOAc)) as a dark purple oil.

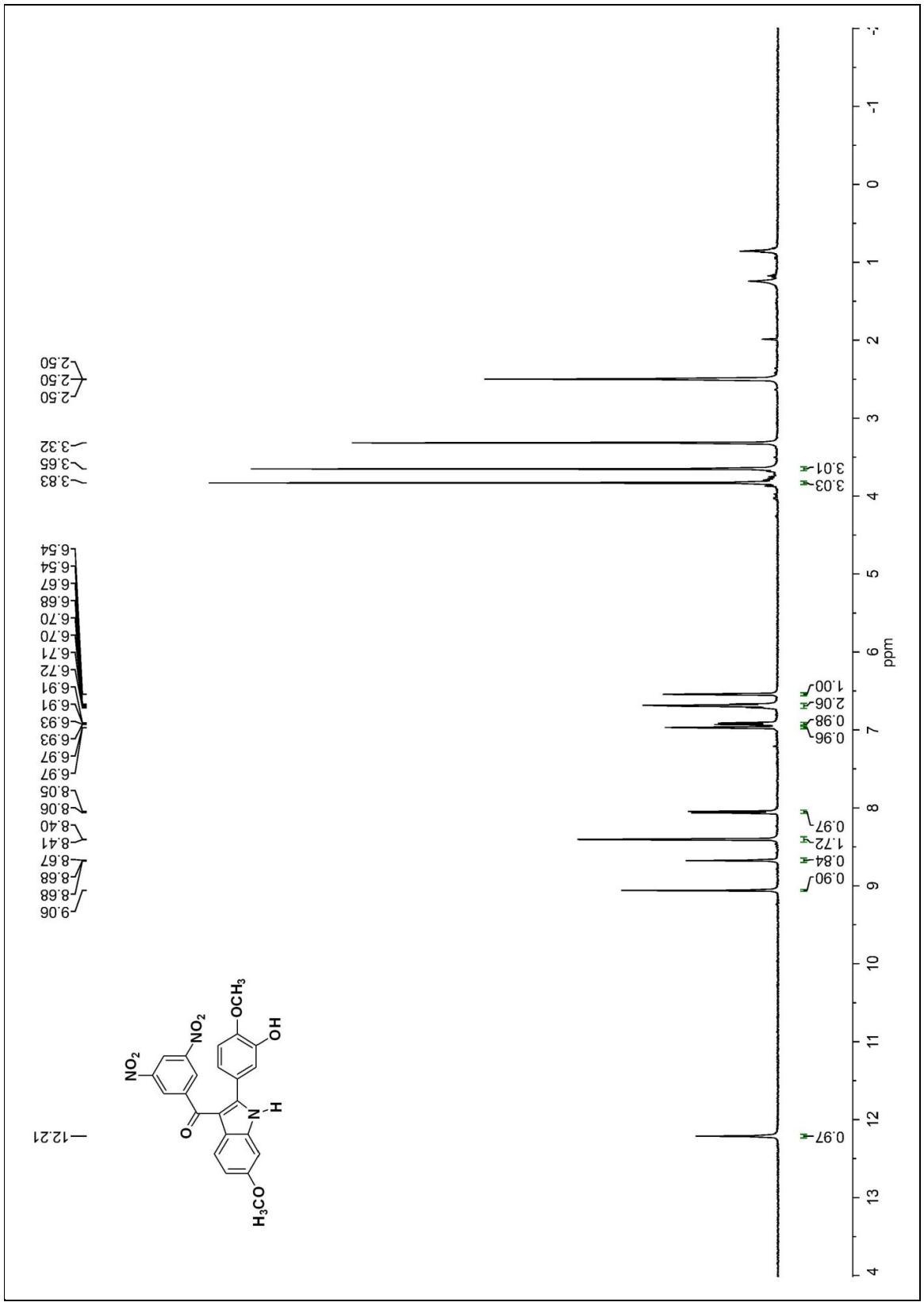
^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.68 (d, *J* = 8.0 Hz, 1H, Ar<u>H</u>), 6.28 (d, *J* = 2.5 Hz, 1H, Ar<u>H</u>), 6.25 (dd, *J* = 3.0 Hz, 8.5 Hz, 1H, Ar<u>H</u>), 3.72 (s, 3H, OC<u>H_3</u>), 3.37 (br, s, 2H, N<u>H_2</u>), 0.99 (s, 9H, C(C<u>H_3</u>)$_3$), 0.15 (s, 6H, Si(C<u>H_3</u>)$_2$).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 146.2, 144.4, 140.8, 114.5, 109.6, 108.3, 56.7, 25.9, 18.6, -4.5.

APPENDIX

APPENDIX

Selected NMR Spectra of Target Molecules **4**, **5**, **9**, **10**, and **11**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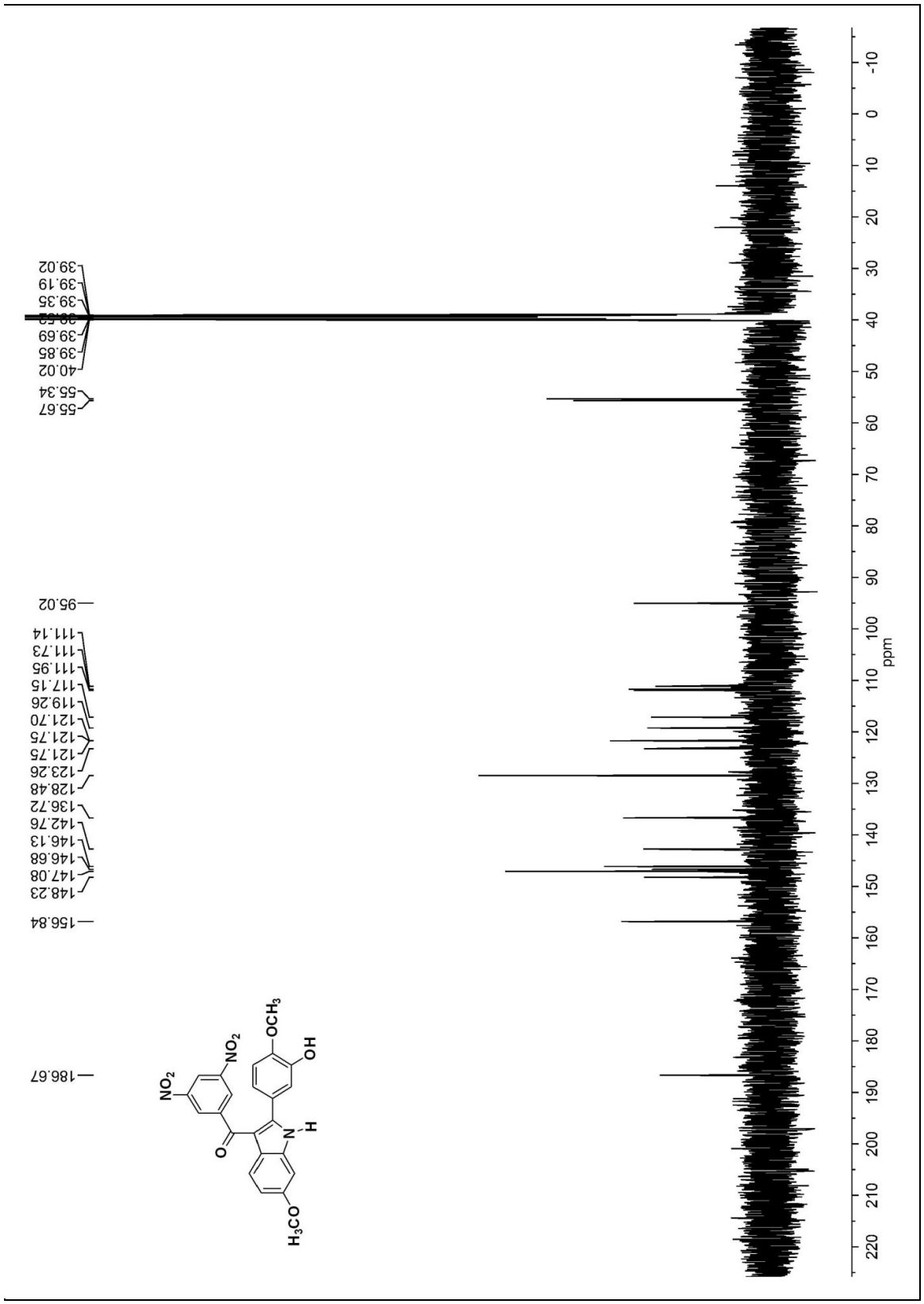
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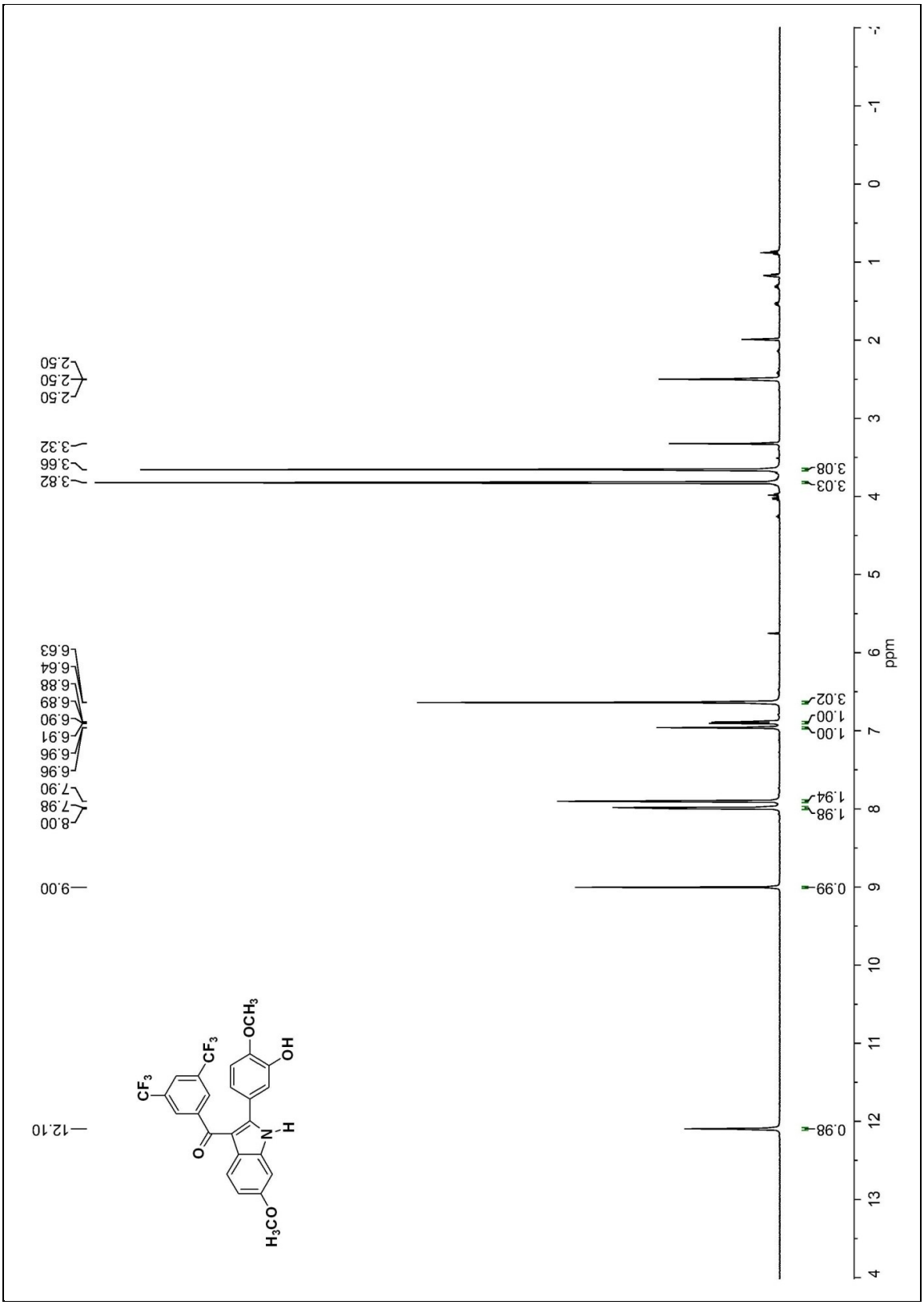
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CF3
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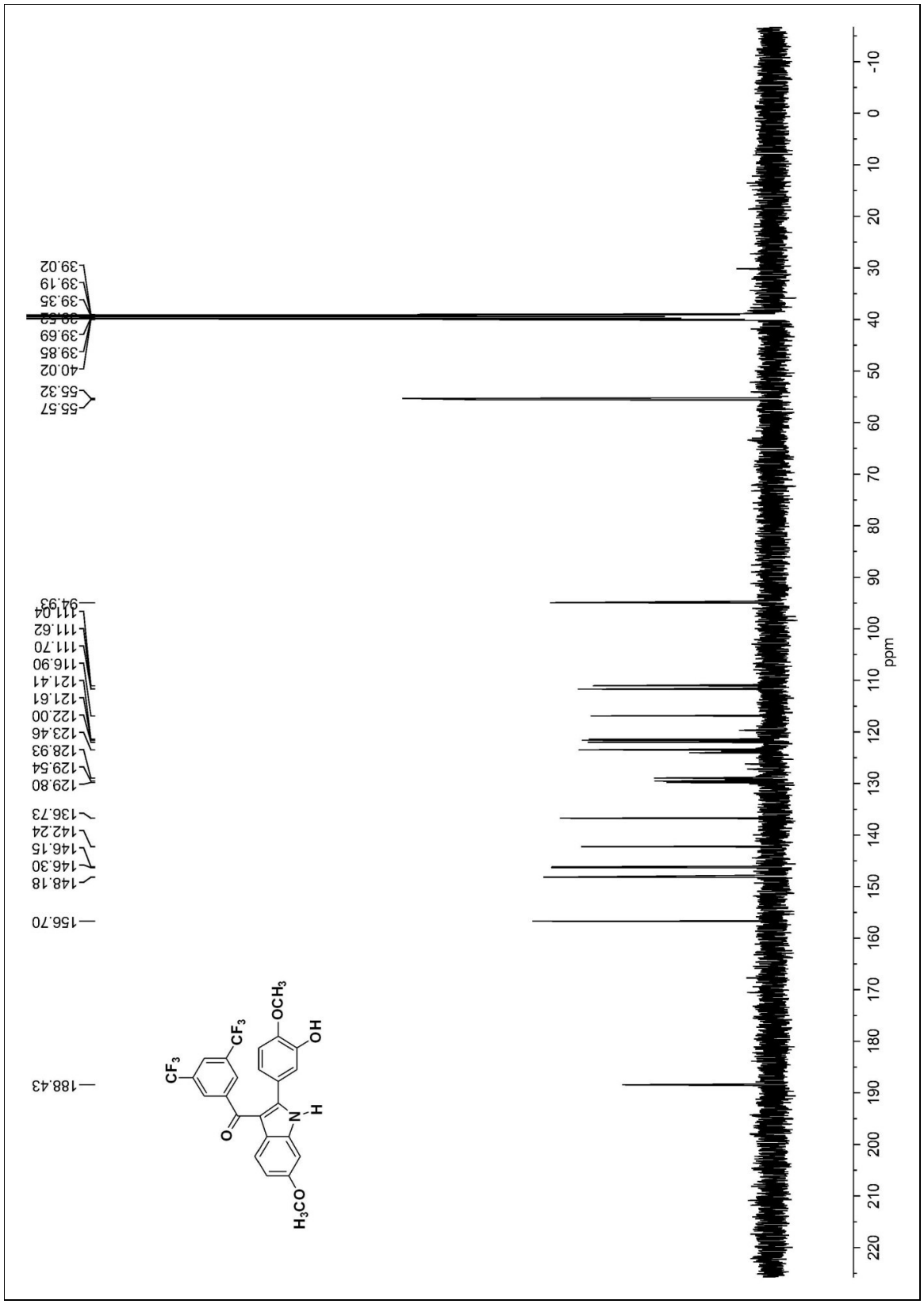
N
H
H3CO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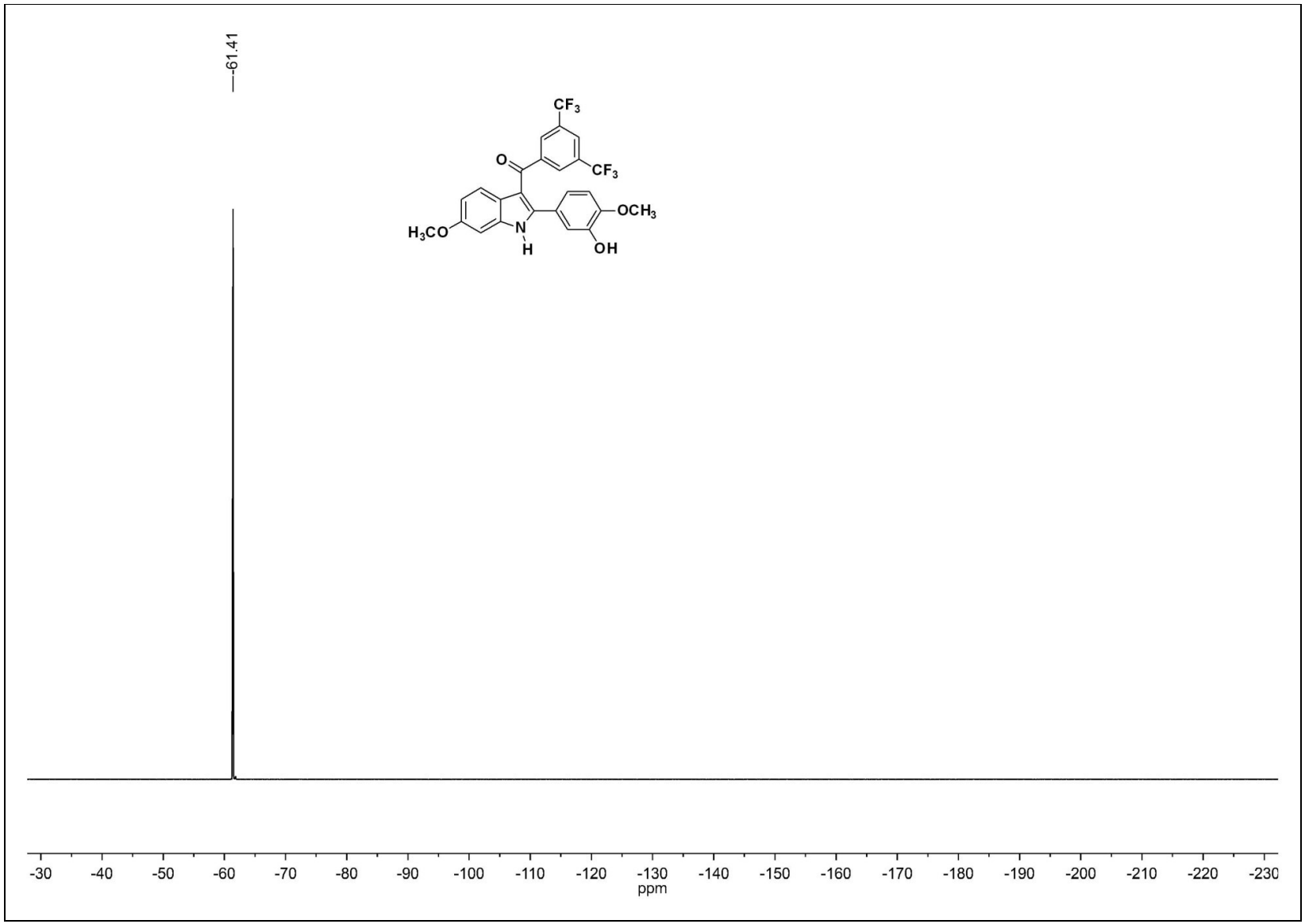
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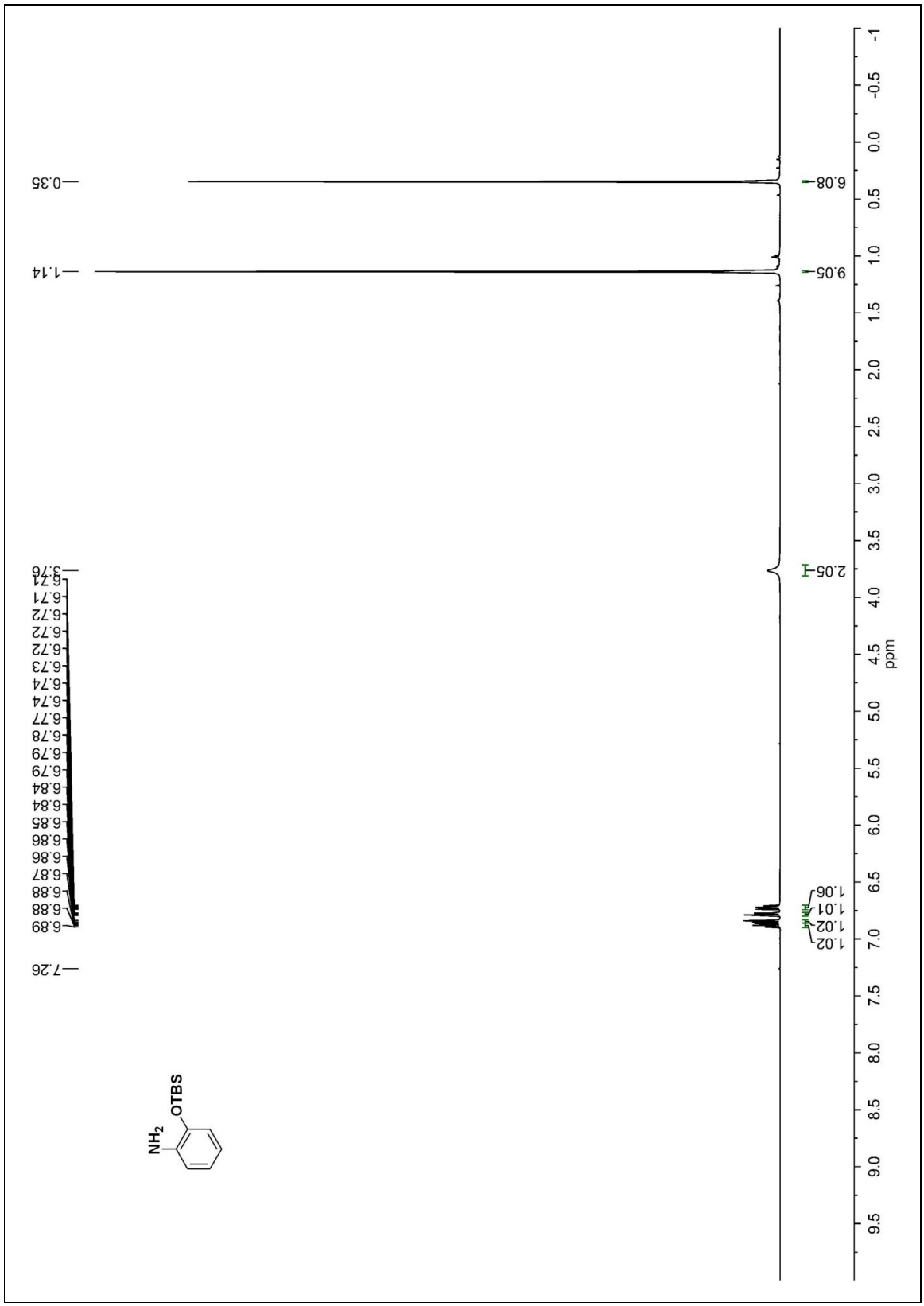
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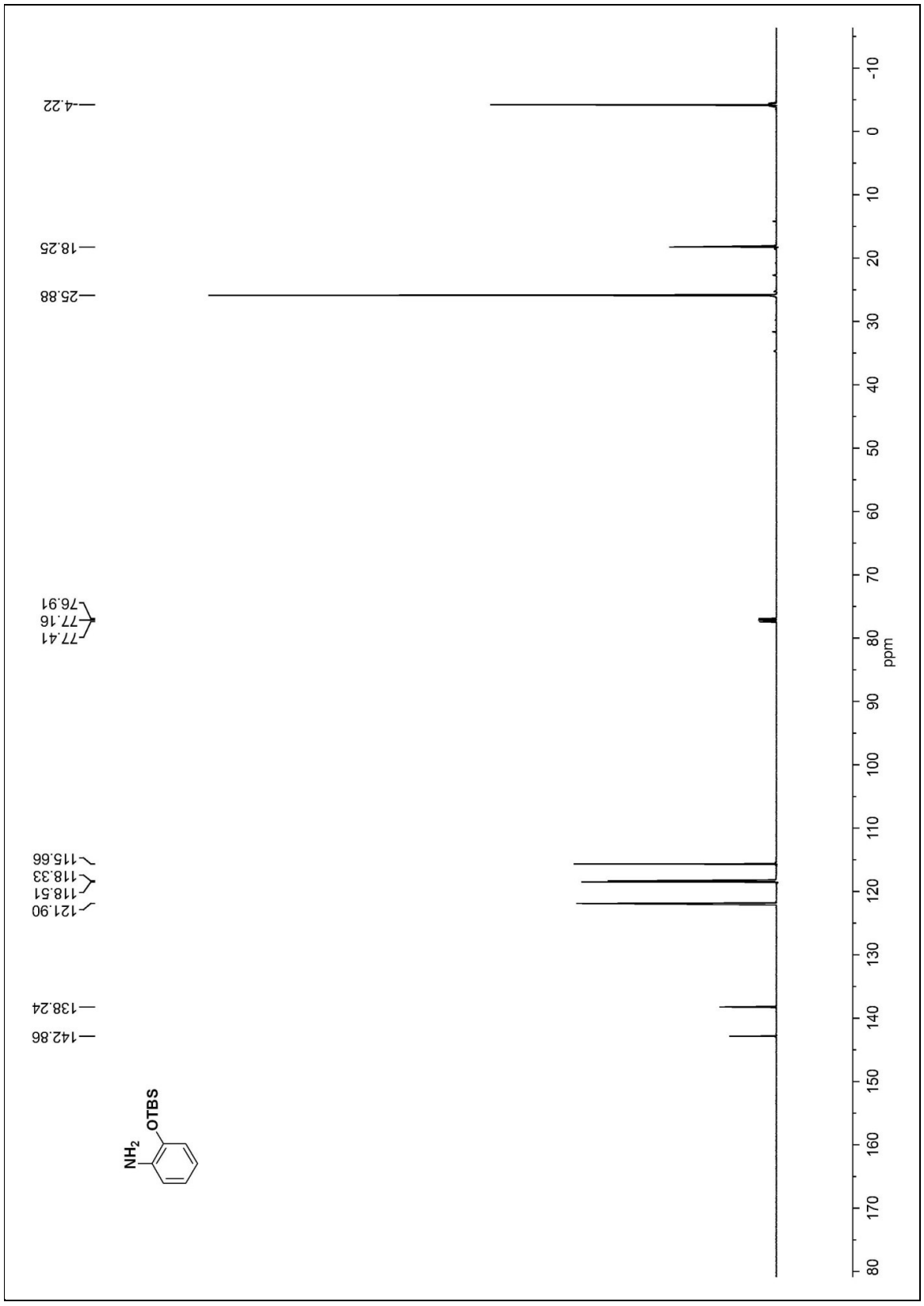
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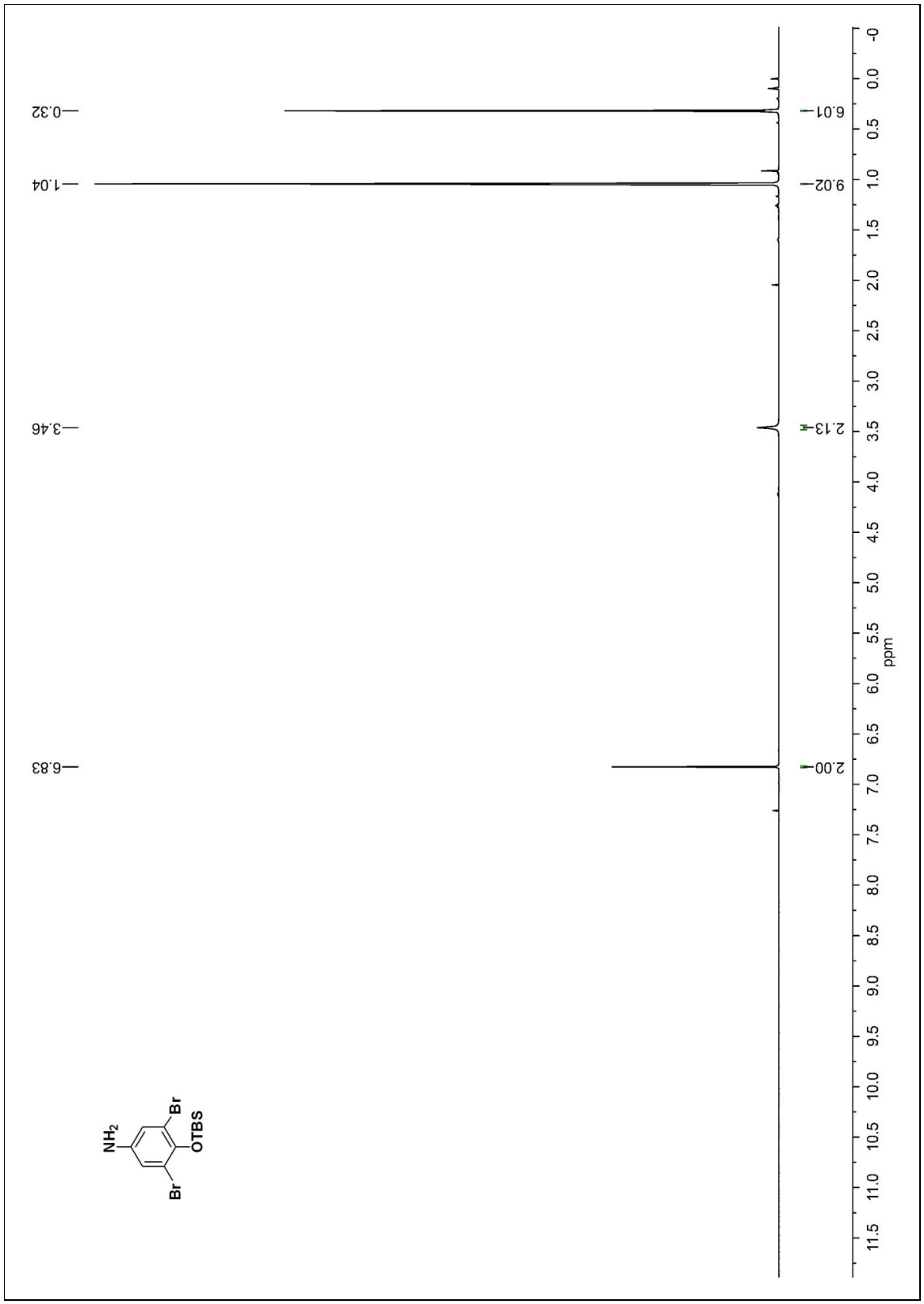
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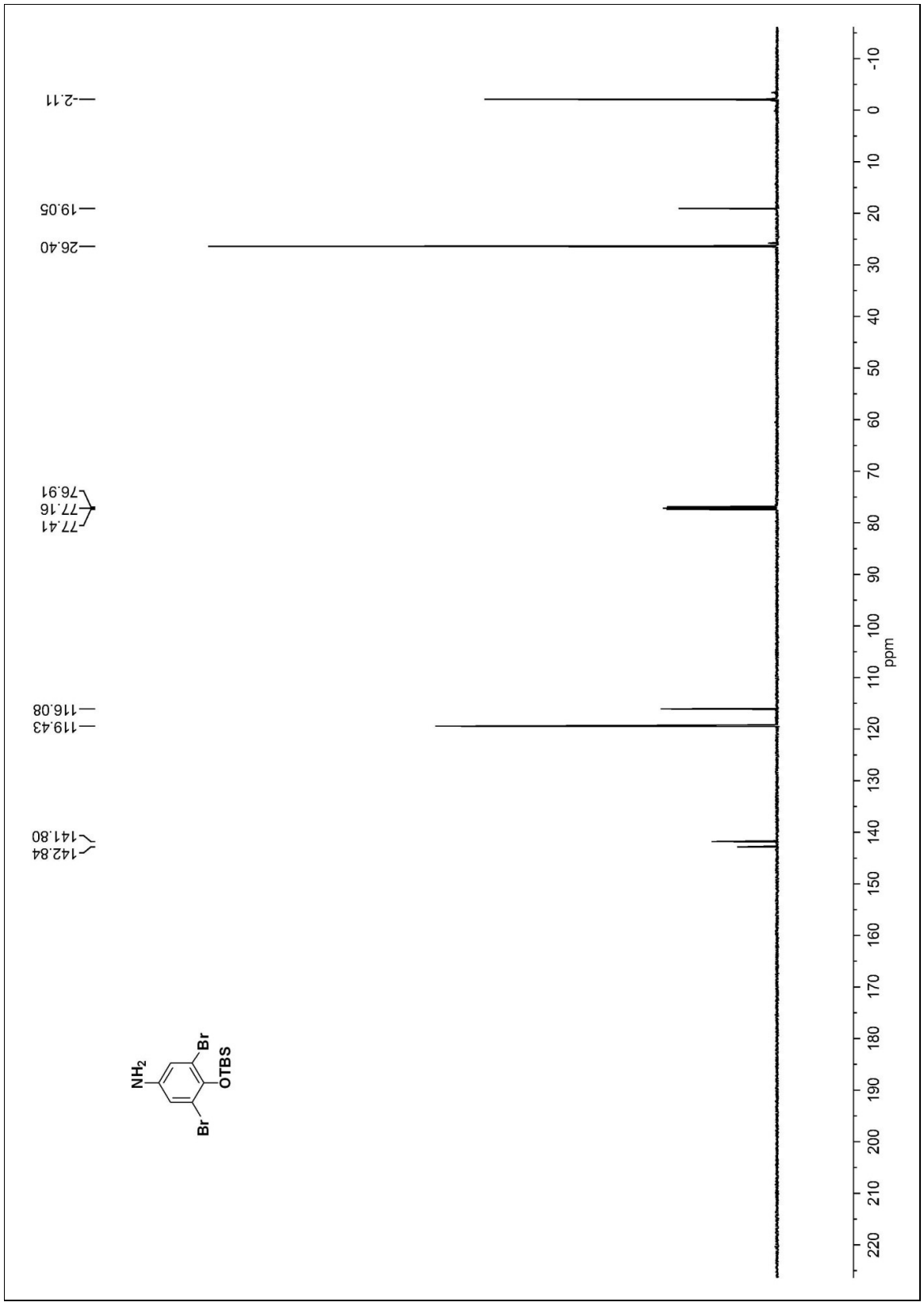
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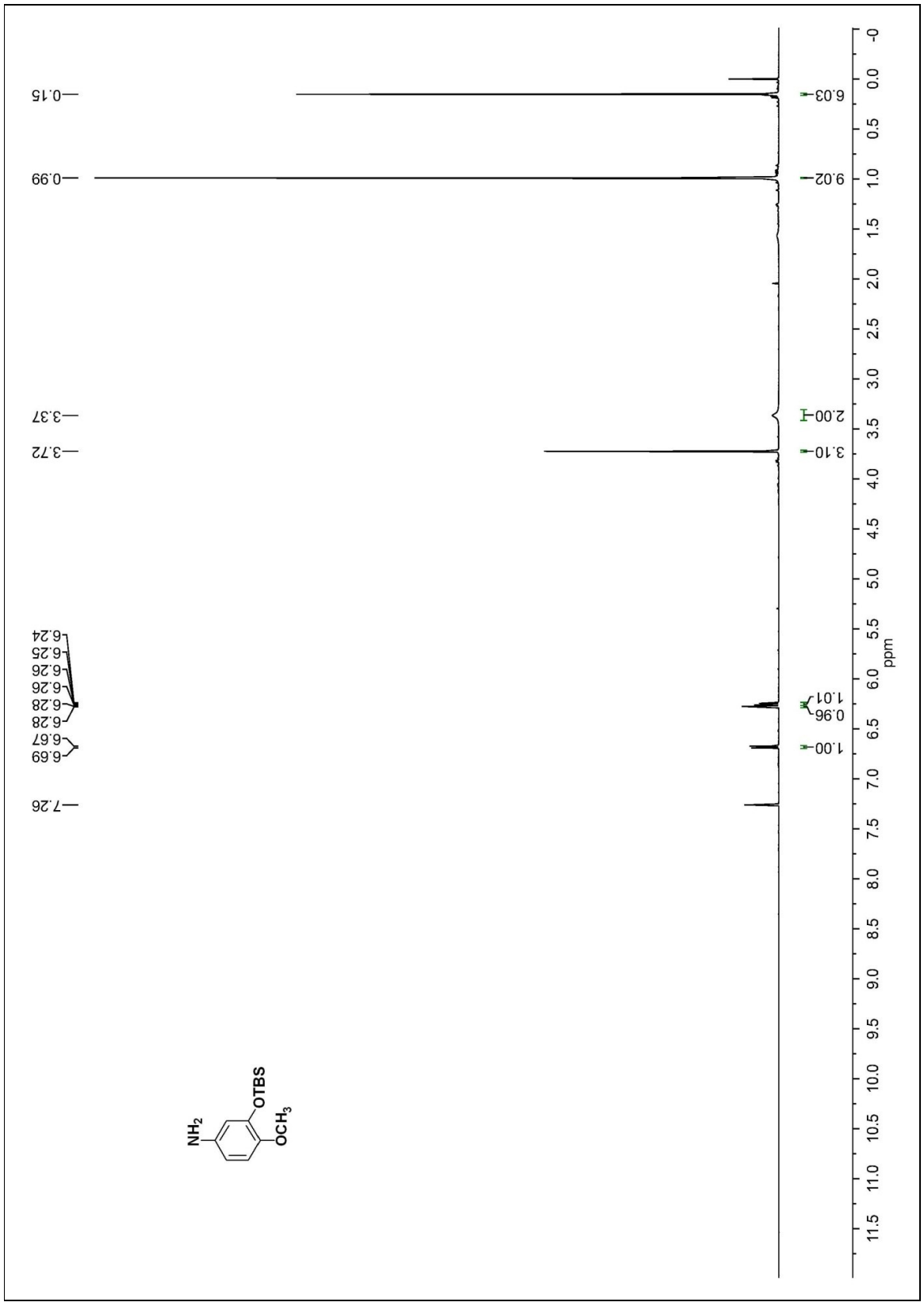
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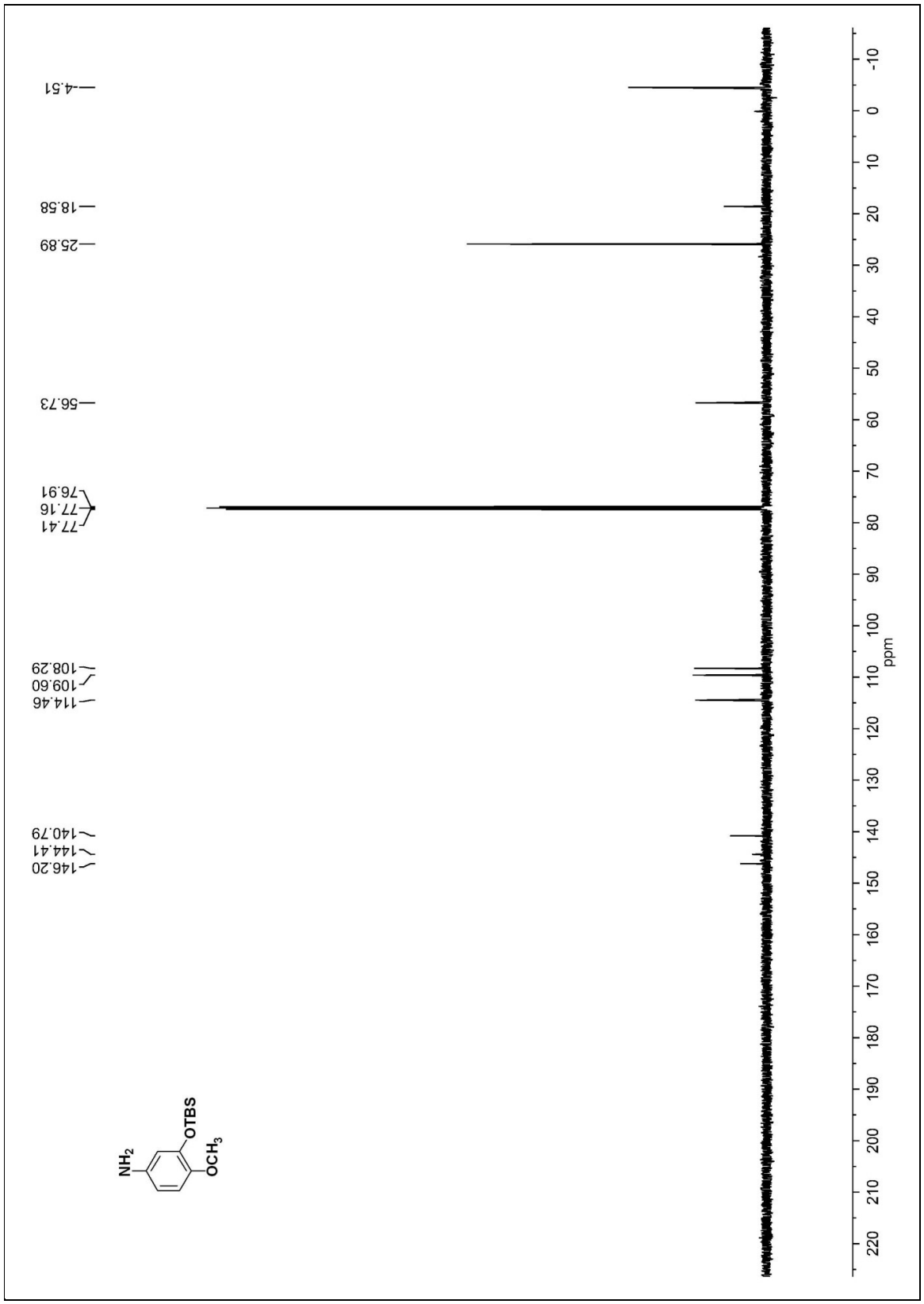
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