공학석사학위논문

Synthetic process for isoimerubrine and its analogs, and their anti-cancer activities

아이소이메루브린과 이의 유사체화합물의 전합성 경로 개발과 이들의 항암효과에 관한 연구

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서울대학교 대학원

화학생물공학부

박 종 범

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지도 교수 김 영 규

이 논문을 공학석사 학위논문으로 제출함

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박종범의 공학석사 학위논문을 인준함

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Synthetic process for isoimerubrine and its analogs, and their anti-cancer activities

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Abstract

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Due to the aging of population many people have been suffered from arthritis, cancer and other diseases. To treat this problem humanity has been developed various medicine, but it has not yet been completely conquered.

Menispermaceae plants have long been used as folk remedies for pain, \mmation, and digestive disorders, etc. Therefore, there were several studies carried out to identify the structure of these plant extracts, and to confirm their biological activity. Tropoloisoquinolines and azafluoranthenes were both isolated from *Menispermaceae*, and their structure was identified. And several studies have been conducted to synthesize them. Our group also synthesized pareitropone via radical anionic coupling.[13]

As an extension of the study, total synthesis of chlorine derivative of isoimerubrine was accomplished by 10 steps overall reaction from 3,4,5-trimethoxy benzyl alcohol. It was confirmed that chlorine derivatives react with methoxide to form isoimerubirne. It was also confirmed that azafluoranthene with the same origin from *Menispermaceae* was synthesized. This suggests that chlorinated derivatives are potentially a platform compound to synthesize of a

variety of alkaloid derivatives.

The synthesized alkaloid derivatives were tested for bioactivity related to anti-cancer activity. As a result, it was found that isomerubrine and its isomers have inhibitory effect on solid cancer and hematological cancer cells. However, the azafluoranthene derivatives produced during the synthesis did not have specific biological activity against cancer cells.

It is believed that synthesizing various derivatives using chlorine derivatives as a platform will greatly contribute to the synthesis of new drugs in the pharmaceutical industry.

Keyword: tropoloisoquinoline, azafluoranthene, radical anionic coupling, biological activity

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List of Abbreviations

C.pareira	Cissampelos pareira
DBU	1,8−Diazabicyclo(5.4.0)undec−7−ene
NBS	N−Bromosuccinimide
TLC	Thin layer chromatography
DDQ	2,3−Dichloro−5,6−dicyano−1,4−benzoquinone
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
NMR	Nuclear magnetic resonance spectroscopy
IC_{50}	Half maximal inhibitory concentration
TsCl	*p*−Toluenesulfonyl chloride
THF	Tetrahydrofuran
TIPSCl	Triisopropylsilyl chloride

1. Introduction

1.1. Isoquinoline alkaloids

Cissampelos pareira is a species of flowering plant in the family *Menispermaceae*. Several alkaloids were isolated from this plant like tropoloisoquinoline, aporphine, proaporphine, and azafluoranthene which have similar structure such as isoquinoline moiety as shown in Figure 1.[1]

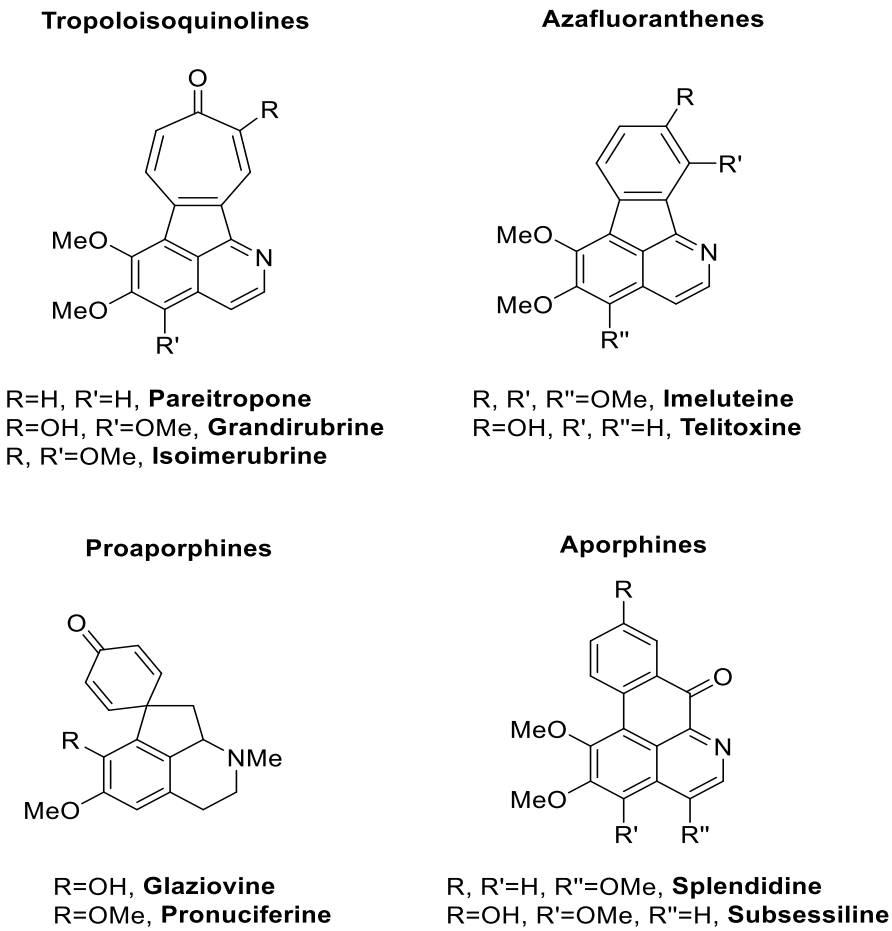


Figure 1. Isoquinoline alkaloids isolated from *Menispermaceae*

C.pareira have been used as a traditional medicine to treatment of inflammation, cancer, pain, etc. Several pharmaceutical researches were also conducted with *C.pareira* extracts using various organic solvents such as ethanol, methanol, and petroleum ether.[2] The extracts were effective against many diseases like anti-anxiety[3], anti-nociceptive[4], anti-inflammatory[5], and anti-cancer[6]. To investigate remedial effect, some of tropoloisoquinoline and azafluoranthene alkaloids were isolated.

Tropoloisoquinoline is a compound containing a heptagonal tropone ring condensed with isoquinoline and following seven natural products have been identified up to date (Figure 2). Imerubrine, grandirubrine, and pareitropone were first isolated from *Abuta imene*, *Abuta grandifolia*, *C.pareira* in 1972, 1980 and 1995 respectively.[7] Recently neotatarin was isolated from rhizome of *A corus calamus L* as ethanol extract in 2017.[8]

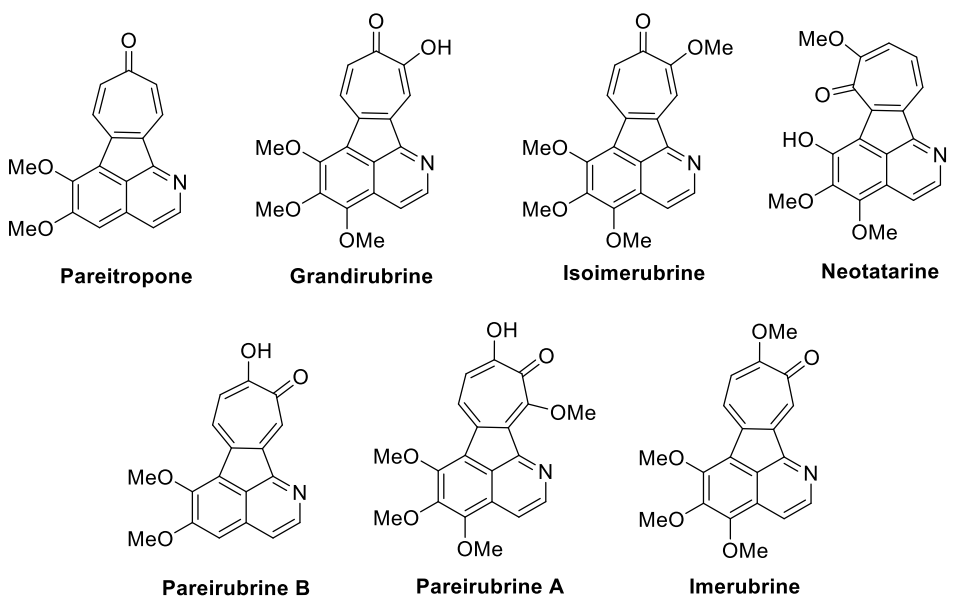


Figure 2. Isolated natural tropoloisoquinoline alkaloids

Azafluoranthene, also isolated from Menispermaceae, is

similar as tropoloisoquinoline but it has benzene ring condensed with isoquinoline not a tropone ring like tropoloisoquinoline (Figure 3). Those alkaloids were tested on several cancer cell line like ACHN (renal carcinoma), HCT-116 (colon adenocarcinoma), A549 (lung carcinoma).[9] Its similar structure, condensed aromatic ring with isoquinoline and methoxy functional group, seemed to have a biological activity. Therefore, I tried to synthesize various tropoloisoquinoilne and azafluoranthene derivatives as well as natural products through one platform compound, a chlorine derivative of isoimerubrine.

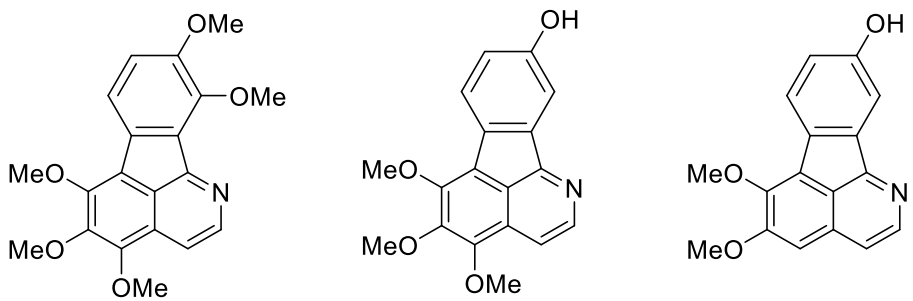
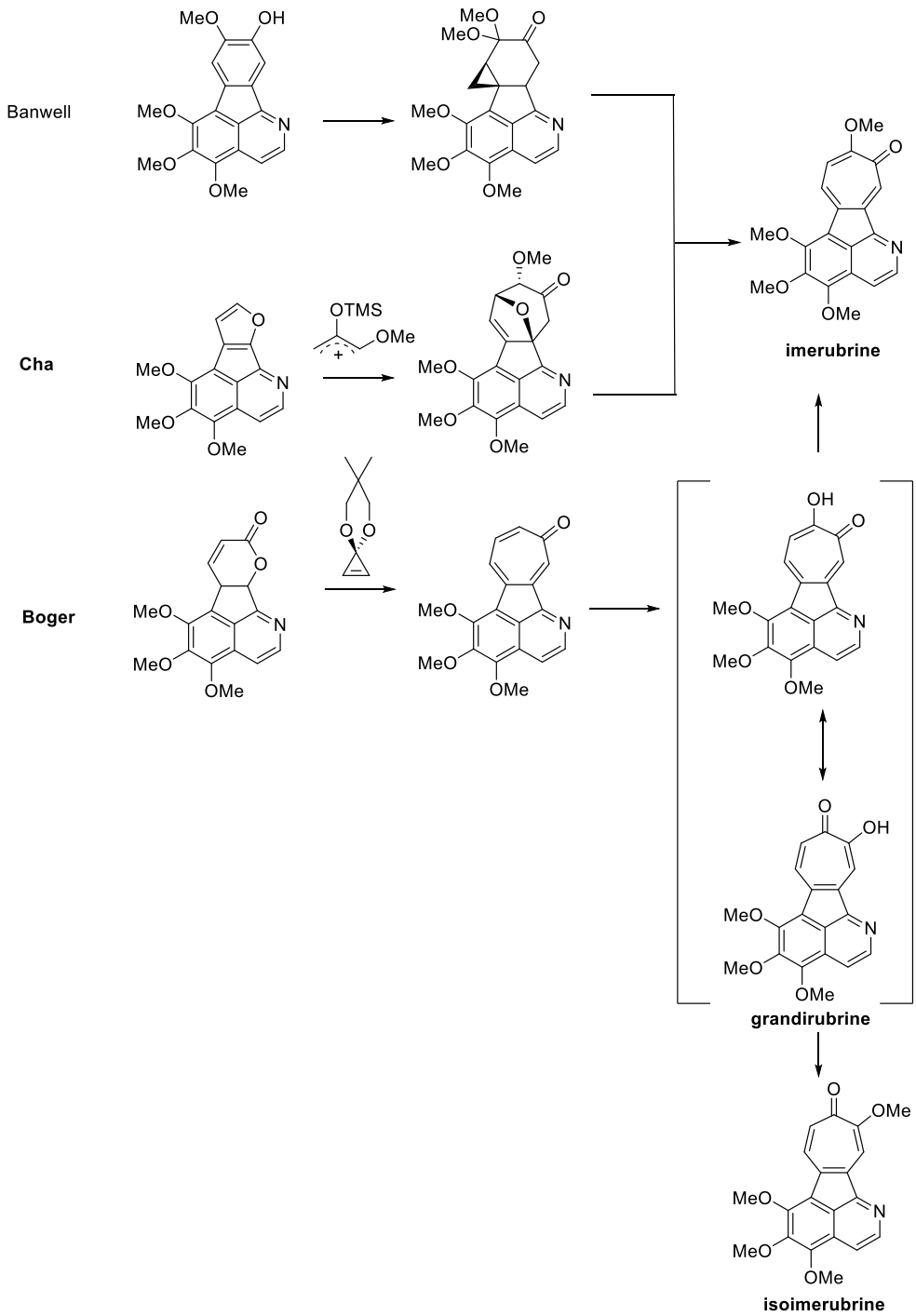


Figure 3. Naturally isolated azafluoranthene alkaloids

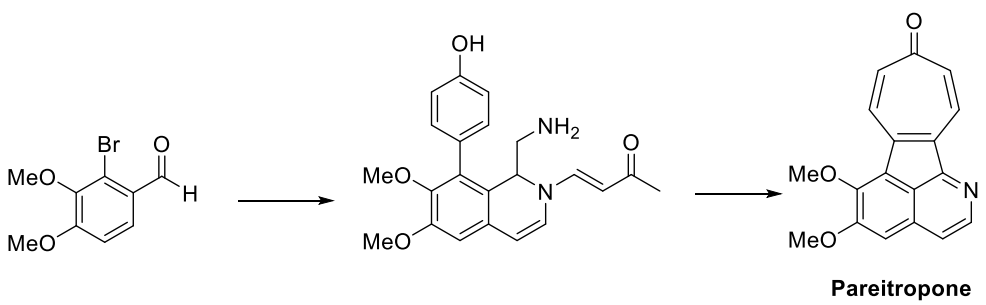
1.2. Previous study

Due to therapeutic action on various disease including cancer and inflammation, many studies have been conducted to synthesize isoquinoline alkaloid (Scheme 1). In 1994, Banwell *et al*. succeeded in regioselective synthesis of imerubrine from dihydroazafluoranthene as an extension of their previous research.[10] Few years later, Boger *et al*. and Cha *et al*. successfully synthesized imerubrine, isoimerubrine and grandirubrine in 1995 and 2001, respectively.[11] The first total synthesis of pareitropone was conducted by K. S. Feldman in 2002, from 2,3,4-trimethoxybenzoic acid. It took total 14 steps reaction and 7% overall yield.[12]



Scheme 1. Previous total synthesis of tropoloisoquinolines[13b]

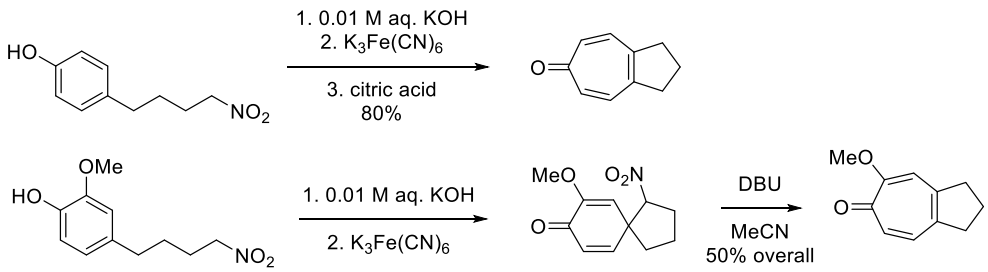
Recently, our group have synthesized pareitropone through radical anionic coupling reaction as a key step (Scheme 2).[13] Using iso vanillin as a basic starting material simple bromination and O-methylation make 2-bromoisovaniline. Then following palladium catalyzed Suzuki coupling make biaryl compound. Functionalization of aldehyde to amide and successive Pomeranz-Frisch annulation make isoquinoline moiety. Precursor of radical anionic coupling was synthesized by Reissert-type nitromethylation. Radical anionic coupling using excess amount of base afford desired pareitropone 30% in 9 steps.



Scheme 2. Synthetic route of pareitropone[13]

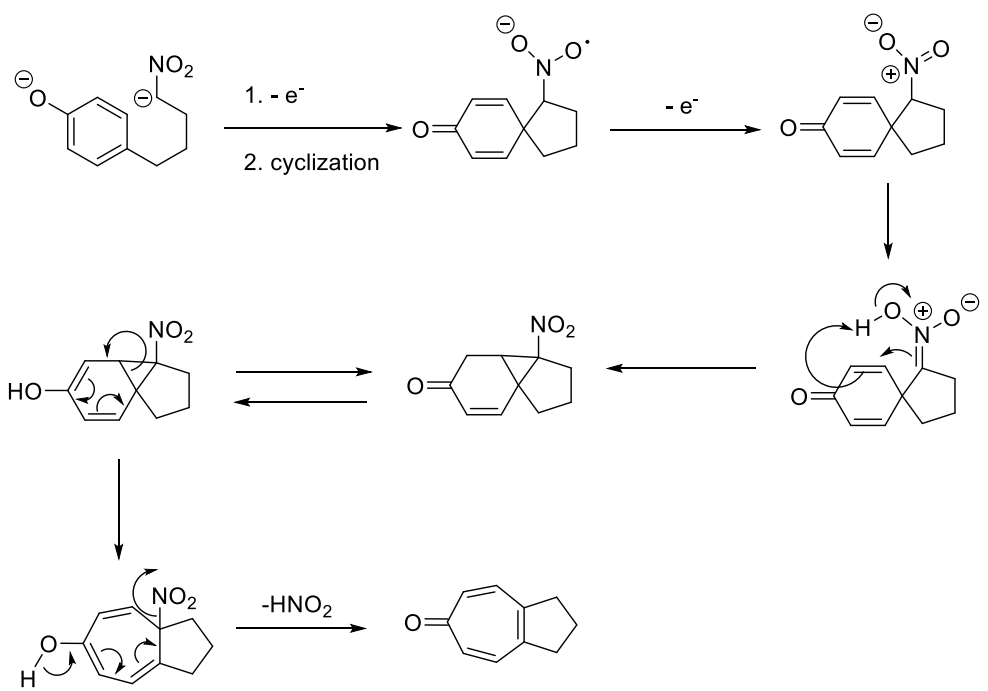
1.3. Kende radical anionic coupling

This reaction was first reported by A. S. Kende and K. Koch by intramolecular radical cyclization of phenolic nitrates in 1986.[14] Reaction of dilute alkaline solution and phenolic nitronate with $K_3Fe(CN)_6$ afford spirocyclic hexadienone. Following rearrangement of hexadienone make tropone and tropolone derivatives (Scheme 3).



Scheme 3. Radical anionic annulation of phenol nitrate

Since formation of dianion is important for overall annulation, the excess amount of base is required even though only 2 equivalents of base is needed. Then cyclization was initiated by $K_3Fe(CN)_6$ as a oxidant. Followed rearrangement with DBU and loss of NO_2 afford desired tropone ring (Scheme 4).

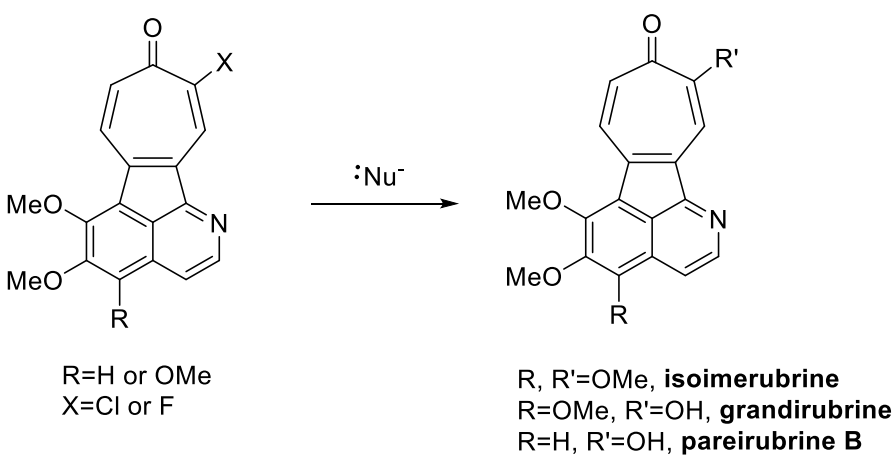


Scheme 4. Proposed mechanism of kende coupling[14]

2. Results and Discussion

2.1. Synthetic strategy for platform chemical

The structural similarities of tropoloisoquinoline alkaloids and total synthesis of pareitropone via radical anionic coupling in our group stimulated to develop platform chemical for various tropoloisoquinoline. If the radical anionic coupling reaction is used, it is possible to make various troporoisoquinoline derivatives by changing the substituent of the tropone ring and isoquinoline. However, the difference in reactivity on each functional group in the reaction step, there was a disadvantage that other optimized conditions were required. It was time consuming process to specify each reaction steps. Therefore, we introduced a halogen substituent in tropone ring which can convert into other functional group after synthesis of tropone moiety.



Scheme 5. Synthetic strategy for various tropoloisoquinoline

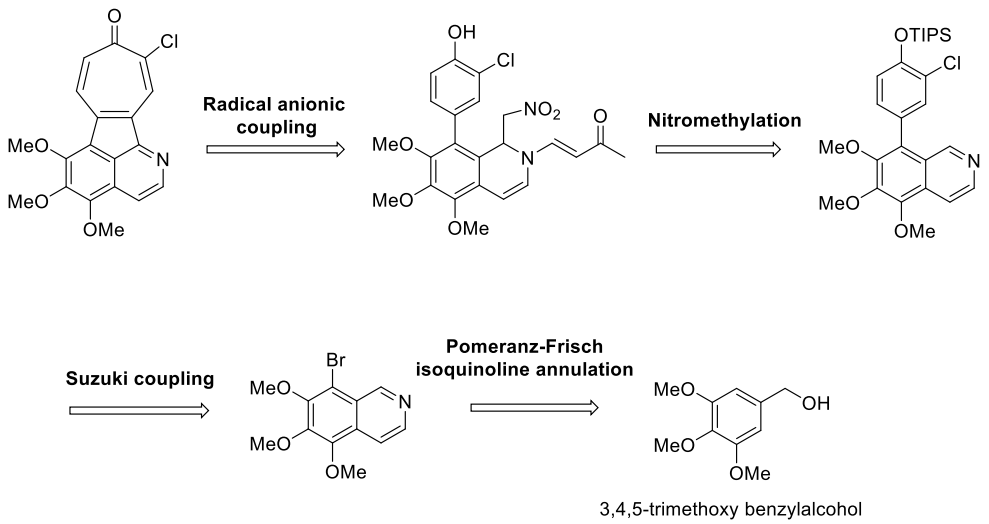
With our newly designed synthetic strategy, halogen derivative of tropoloisoquinoline was synthesized and it can convert into isoimerubrine with magnesiuim methoxide. In addition, during this process we confirmed that azafluoranthene derivatives were afforded. It is very interest that tropoloisoquinoline and azafluoranthene were synthesized at a time.

Despite the remarkable cytotoxicity against P388 leukemia cell, further study on activity of pareitropone has not been reported since the first discovery, only the synthesis of tropoloisoquinoline and the structurally related derivatives were tested by D.L. Boger.[15] They referred that the nitrogen of the isoquinoline and the methoxy substituents may not affect the bioactivity.

We also screened the biological activity of tropoloisoquinoline and azafluoranthene derivatives on A549, HCT119, K562, HL60 cell line.

2.2. Retrosynthetic analysis

Using Kende's radical anionic coupling to synthesize various tropoloisoquinoline, the phenolic nitronate condensed with isoquinoline was required. The phenolic nitronate was prepared by Reissert-type nitromethylation. Biaryl compound was synthesized by palladium catalyzed Suzuki coupling of 8-bromoisoquinoline and sillyl protected 2-chlophenol boronic acid. The isoquinoline was prepared from amination of commercially available 3,4,5-trimethoxy benzylalcohol (Scheme 6).

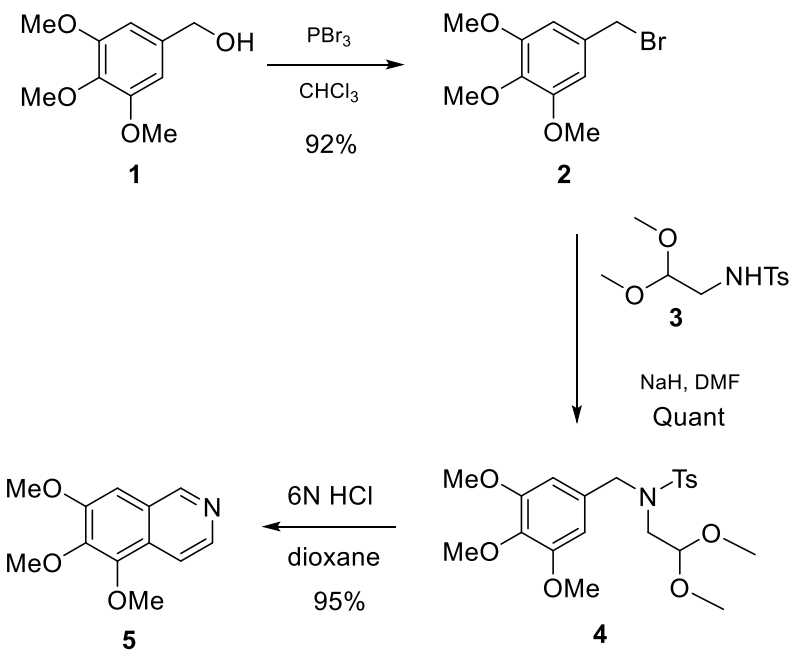


Scheme 6. Retrosynthetic analysis of tropoloisoquinoline derivative

2.3. Synthetic route

Total synthesis of chlorine derivative of isoimerubrine was started from commercially available 3,4,5-trimethoxybenzylalcohol **1**. Firstly, benzyl alcohol was converted into bromine by PBr_3 in 92% yield. The corresponding benzylbromide **2** was coupled with early prepared tosylated amino acetal **3** to afford tosylated benzyl amine **4**. Then benzyl amine **4** was subjected to the Pomerantz-Fritsch annulation. This reaction condition to afford various isoquinoline was screened in our groups.[16] In case of 3,4-dimeethoxy benzyl amine, 2,4-dinitrobenzenesulfonic acid was the best choice. However, when additional methoxy group was exist, unspecified side product was also produced, and it was not purified via column chromatography. Also, the high dilution condition for minimized the intermolecular side reaction consumed a large amount of solvent. It was not efficient, so I changed the condition to use 6 N hydrochloric acid. Hydrochloric

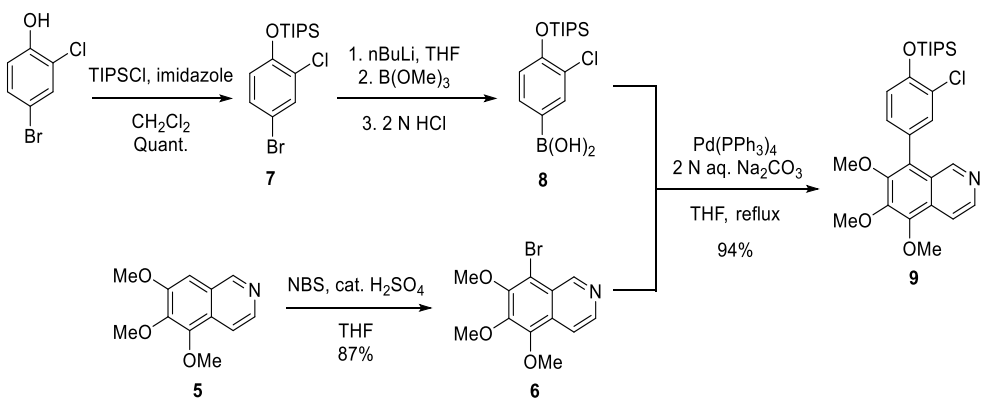
acid was very easy to remove via basic work up using $NaHCO_3$. No significant intermolecular side reactions occurred even if the amount of solvent was greatly reduced and the unspecified side product was not appeared. In addition, isoquinoline **5** could be obtained in increased yields about 95% yield compared to 2,4-dinitrobenzenesulfonic acid 65% (Scheme 7).



Scheme 7. Synthetic route for isoquinoline moiety

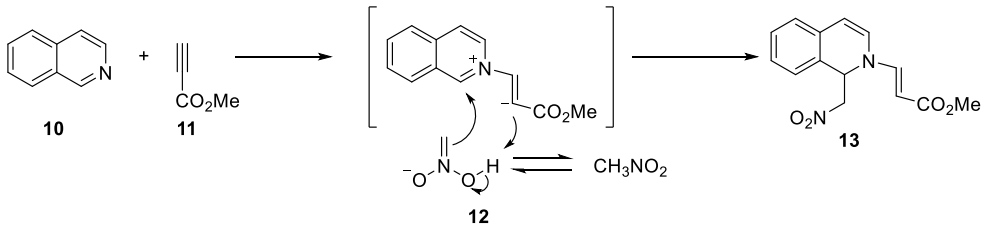
Regioseletive bromination of isoquinoline was conducted with NBS as a bromination reagent. However, the amount of sulfuric acid slightly increased, the reaction vessel changed to cloudy and only small amount of isoquinoline was brominated. It might be the reason that the acidity of sulfuric acid too strong to protonate nitrogen of isoquinoline. To solve this problem, we use 1~2 drops of sulfuric acid. Then we could obtain Suzuki precursor of brominated isoquinoline **6** with increased yield, 87%. To synthesize the chlorine substituted

biaryl compound 9, palladium catalyzed Suzuki coupling reaction was conducted. The silyl protected boronic acid 8 for coupling with isoquinoline 6 was obtained through a three steps reaction from silyl protected 4-bromo-2-chlorophenol. Since the boronic acid was unstable, we immediately used it in the next reaction after the extraction steps without isolation.[17] As a result, the coupled compound 9 was obtained in 94% yield (Scheme 8).



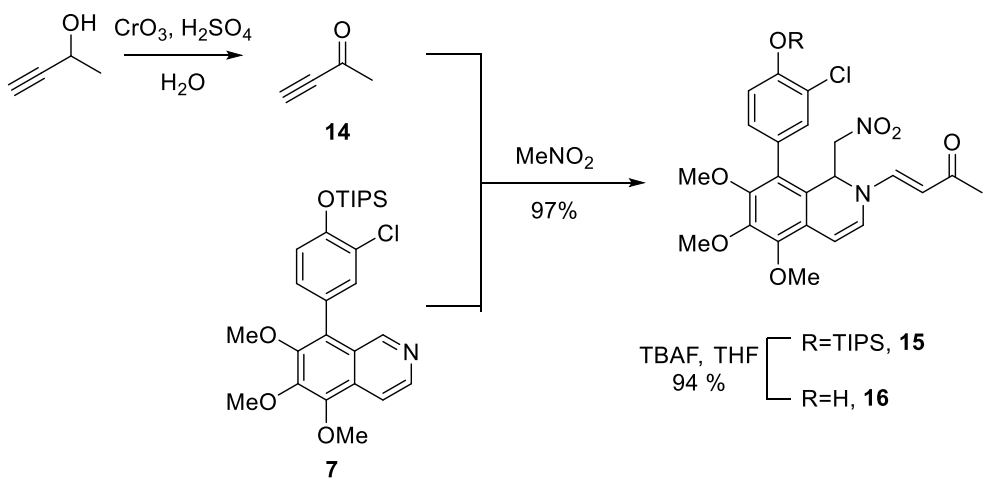
Scheme 8. Synthesis of biaryl compound 9 from isoquinoline 6

Followed nitromethylation was conducted by early reported Ydav's process.[18] They synthesized nitromethyl derivates of 1,2-dihydroisoquinolines via a three-component coupling with activated alkynes and nitromethane without catalyst in mild condition. They tested this reaction on various substituted isoquinoline, quinoline, and propiolate. Reaction of isoquinoline and propiolate yield zwitter ionic intermediate and simultaneously reacts with nitromethane to afford nitromethyl derivatives (Scheme 9).[18]



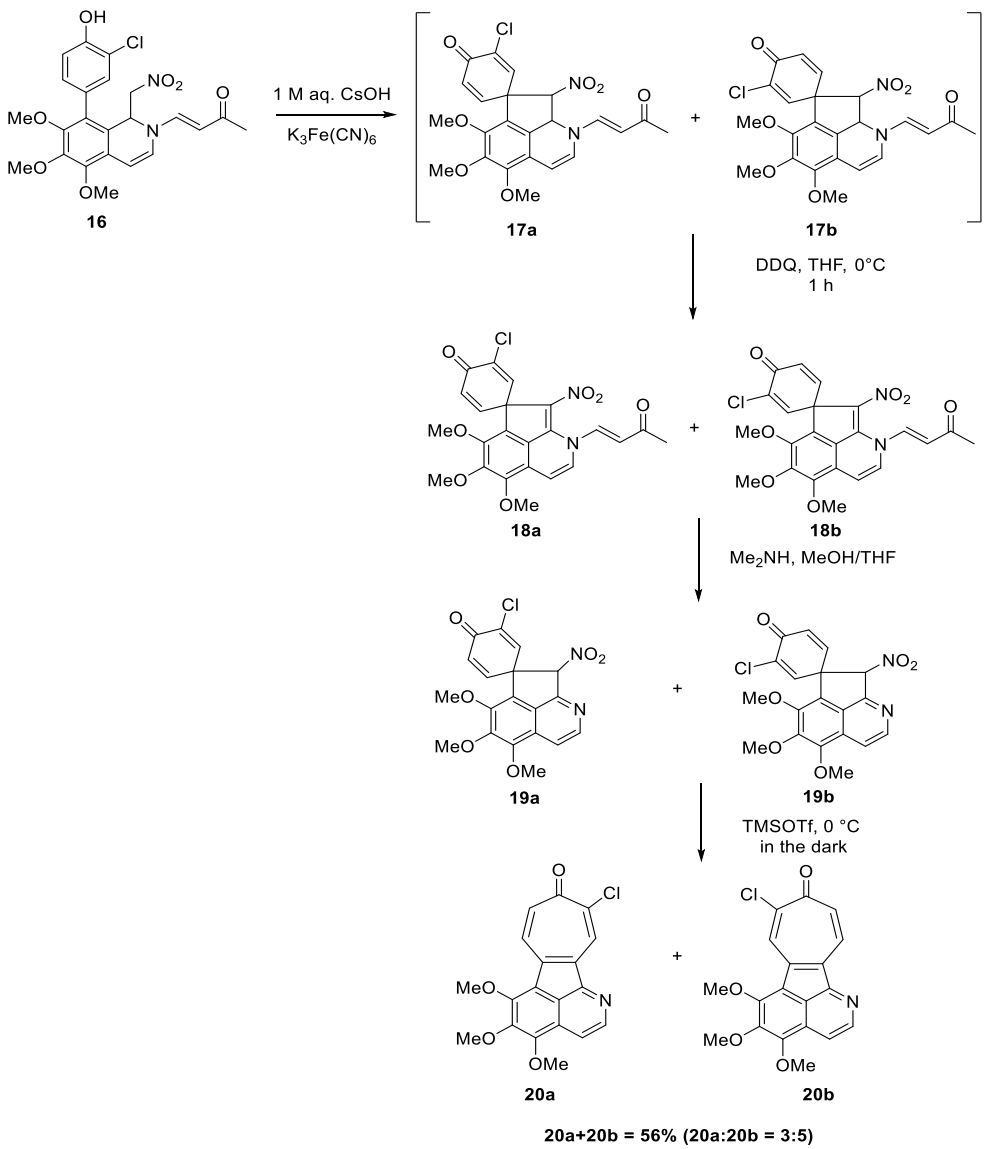
Scheme 9. Proposed mechanism of Reissert type reaction of isoquinoline and propiolate[18]

However, we applied this process in our research the solubility of biaryl compound 9 was poor in $MeNO_2$. In small scale of reaction, it did not affect the reaction yield, but the yield was decreased when the scale was increased. Therefore, we added small amount of CH_2Cl_2 to dissolve reagent fully. As a result, I could afford desired nitromethylated isoquinoline 15 in moderated yield. Then, deprotection of silyl group using TBAF furnished the radical anionic coupling precursor 16 in 94% yield.



Scheme 10. Synthesis of radical anionic precursor 16

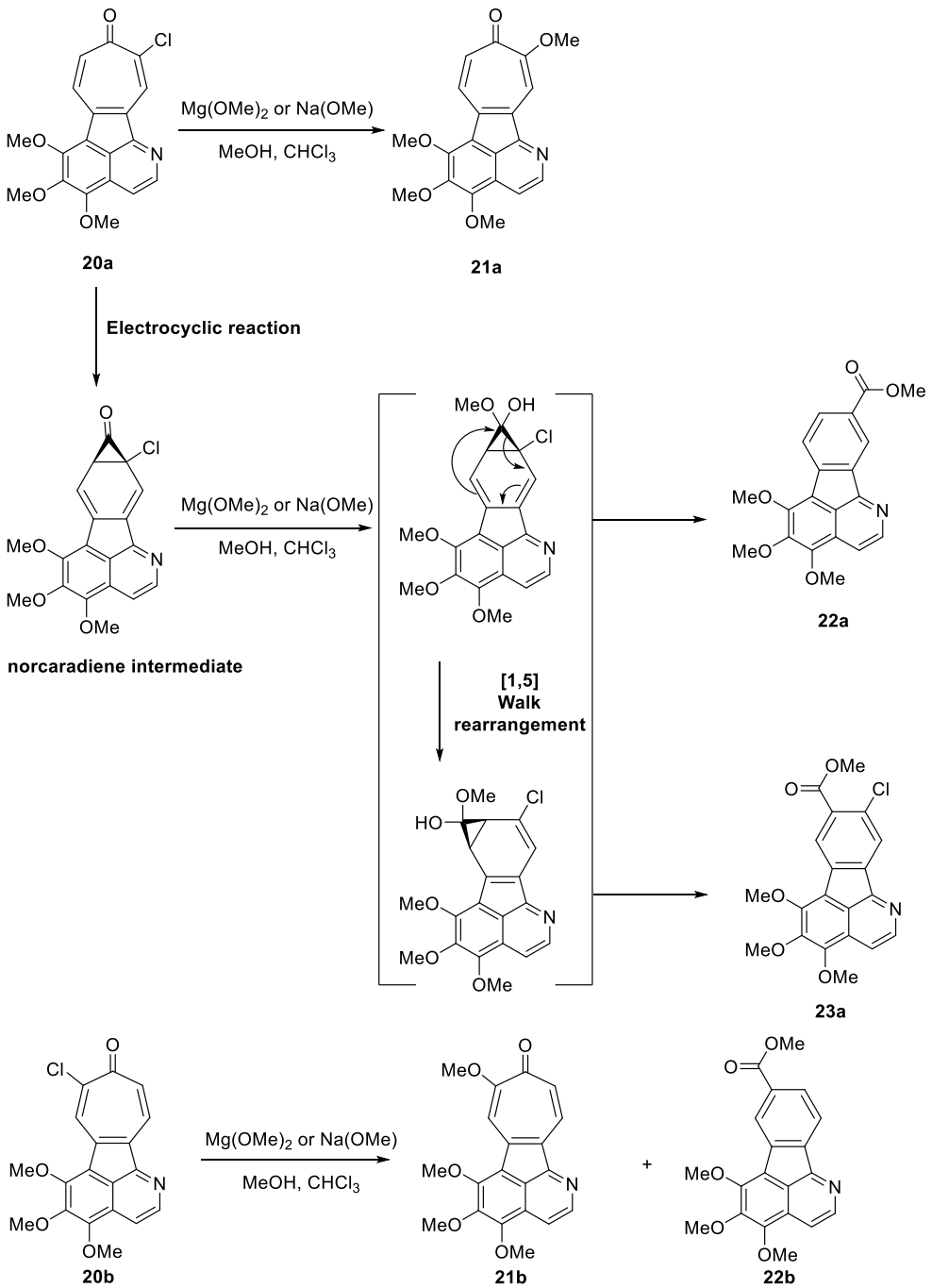
Finally, prepared coupling precursor **16** was dissolved in diluted CsOH aqueous solution to form dianion. The dianion was subjected into $K_3Fe(CN)_6$ solution to furnish spirocycle intermediates **17**. However, unlike unsubstituted tropones, chlorine-substituted derivatives were formed into two isomers (**17a** and **17b**) as they formed into spirocycles. Those intermediates were also unstable in the atmosphere and therefore decomposed to black on the TLC plate in a matter of minutes. Therefore, we added DDQ to furnish the stable spirocyclic nitroolefin intermediate **18a** and **18b**. In order to avoid decomposition of spirocycles, DDQ was added directly to the vessel reacted with $K_3Fe(CN)_6$, but in this case spiroolefin was not produced properly but rather the yield was decreased. It was assumed that the water present in the reaction vessel inhibited the reaction of the intermediate with DDQ. Therefore, we removed the water in the reaction vessel through the extraction then added DDQ. After two hours of stirring in 0 °C, the solution was briefly purified through extraction and dimethylamine was added immediately to remove butynone. The [3,3]-sigmatropic rearrangement of spiroolefin was conducted with TMSOTf. To prevent decomposition of product in hard reaction condition it was conducted in the dark at 0 °C. Then we could afford the desired chlorinated tropone derivatives **20a** and **20b** in 56% yield in successive three steps.



Scheme 11. Synthesis of chlroine derivatives of isoimerubrine[a]

[a]The ratio of **20a** and **20b** was determined by ^{1}H NMR

In order to demonstrate that chlorine derivatives **20** can be converted into other derivatives as platform compounds, the reaction of replacing chlorine groups with methoxy groups was carried out. Reaction of **20** with commercially available NaOMe or $Mg(OMe)_2$ and in situ generation of $Mg(OMe)_2$ by early reported method[19] afford similar result. Substitution of chloride to methoxide was successfully conducted. However, after the reaction unidentified yellow spots were detected by UV (365 nm) on TLC plates and they were isolated via aluminum oxide column chromatography. NMR and single crystal crystallography analysis confirmed that the yellow spot is an isomer with azafluoranthene structure. When **20a** and methoxide were reacted, two kinds of compounds azafluoranthene derivatives were produced **22a, 23a** with isoimerubrine **21a**. **22a** was believed to be produced via norcaradene by an electrocyclic reaction of the tropone ring followed by substitution. **23a** is considered to have been generated after a further [1,5]−walk rearrangement in norcaradiene. However, in the case of the **20b**, the reaction was carried out under the same conditions of **20a**, no product through the walk rearrangement was produced (Scheme 12).



Scheme 12. Reaction pathway for substitution of 20a, 20b to methoxide

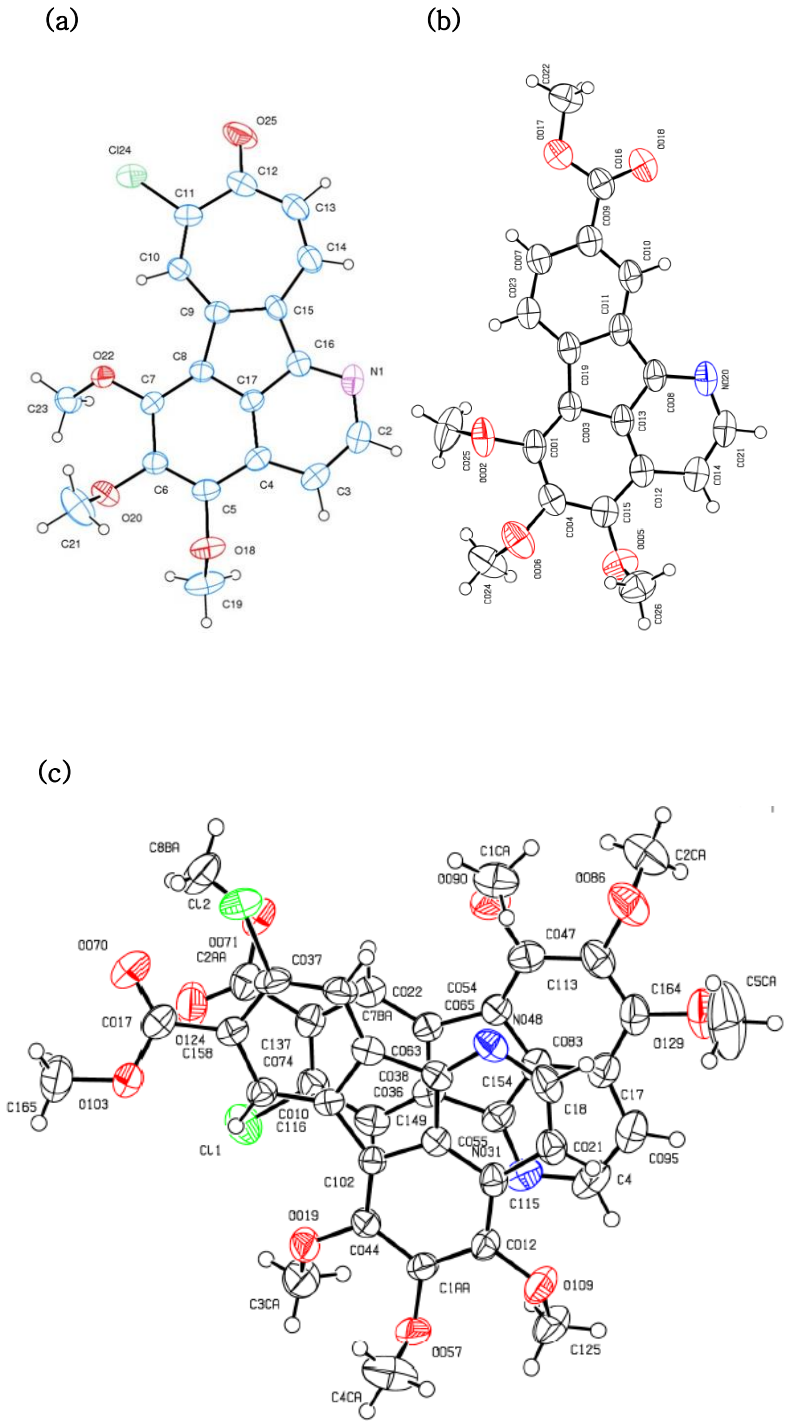


Figure 4. X-ray crystal structure of compound (a) **20a**, (b) **22a**, (c) **23a**[*]

*The x-ray structures of the 23a compounds were confirmed to be overlapped with each other.

2.4. Biological activity

Biological test for anti-cancer effect of synthesized tropoloisoquinoline derivatives were carried out by *J&C Science* in South Korea. To confirm the cytotoxicity of the derivatives, we tested the derivatives in four kinds of cell line (A549, HCT116, K562, HL60).

In the process of dissolving the synthesized compound in DMSO solvent and treating it in the cell line, the compounds were precipitated, and the film formed on the surface due to the solubility problem over 100 μM. Therefore, the test is not performed at the concentration above 100 μM. Tropoloisoquinoline derivatives were red when dissolved and azafluoranthene derivatives were yellow fluorescent (Table 1). As expected, isoimerubirne (**21a**) confirmed the cytotoxicity of cancer cells through cell lines of solid and hematological cancers. It is confirmed that the natural product isoimerubrine has better efficacy in terms of physiological activity than the isomer (**21b**). However, the synthesized azafluoranthene derivatives were found to have no cytotoxicity against cancer cells (Table 2).

Figure 5. DMSO Dilution of Tropoloisoquinoline and Azafluoranthene

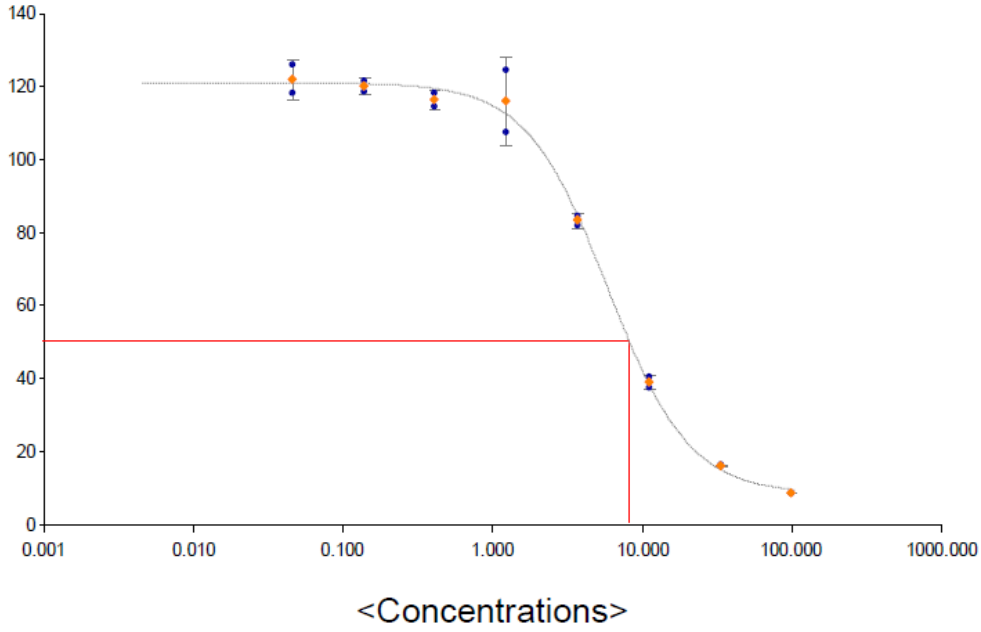
Comp. / Conc. (μM)	21b	22a	22b	23a
100	precipitated	precipitated	precipitated	precipitated
33.3	clear	clear	clear	clear
11.1	clear	clear	clear	clear
3.7	clear	clear	clear	clear
1.23	clear	clear	clear	clear
0.4	clear	clear	clear	clear

Table 1. Dissolution of Compounds with Concentration in DMSO Solvent

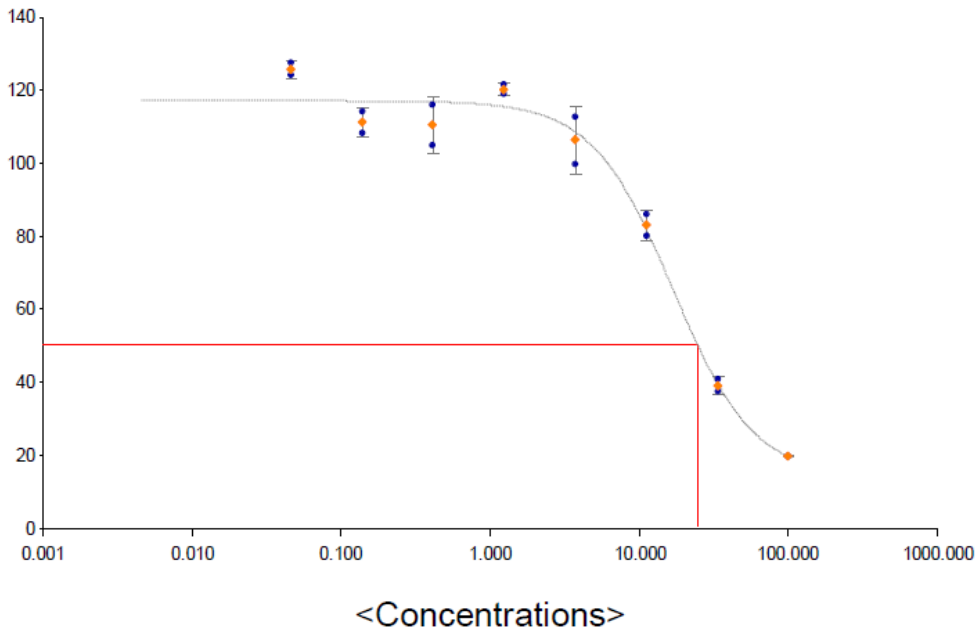
Compound	IC_{50}, μM						
	Solid cancer			Blood cancer		Normal cell	
	H460	A549	HCT 116	K562	HL60	HUVE C	WI-26
21a	8.258	–	8.747	2.832	–	9.689	7.785
21b	24.668	48.69	13.02	3.837	31.57	37.629	24.751
22a	–	>100	>100	>100	>100	>100	>100
22b	–	>100	>100	>100	>100	>100	>100
23a	–	>100	>100	>100	>100	>100	>100
Doxorubicin (Reference)	0.042	–	0.144	0.086	–	0.199	0.033

Table 2. IC_{50} of derivatives on cancer cell line

(a) 21a (isoimerubrine)

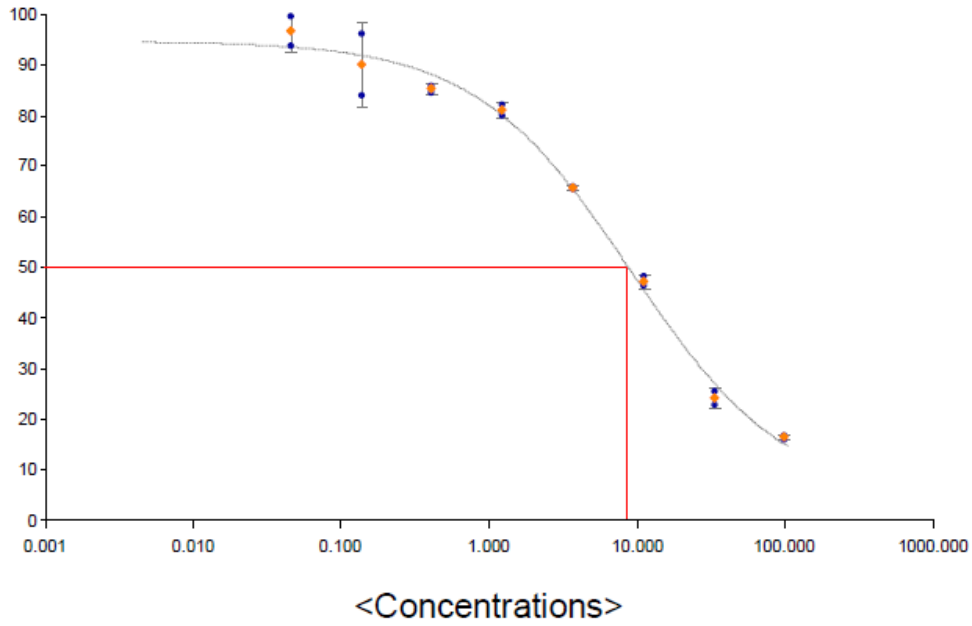


(b) 21b

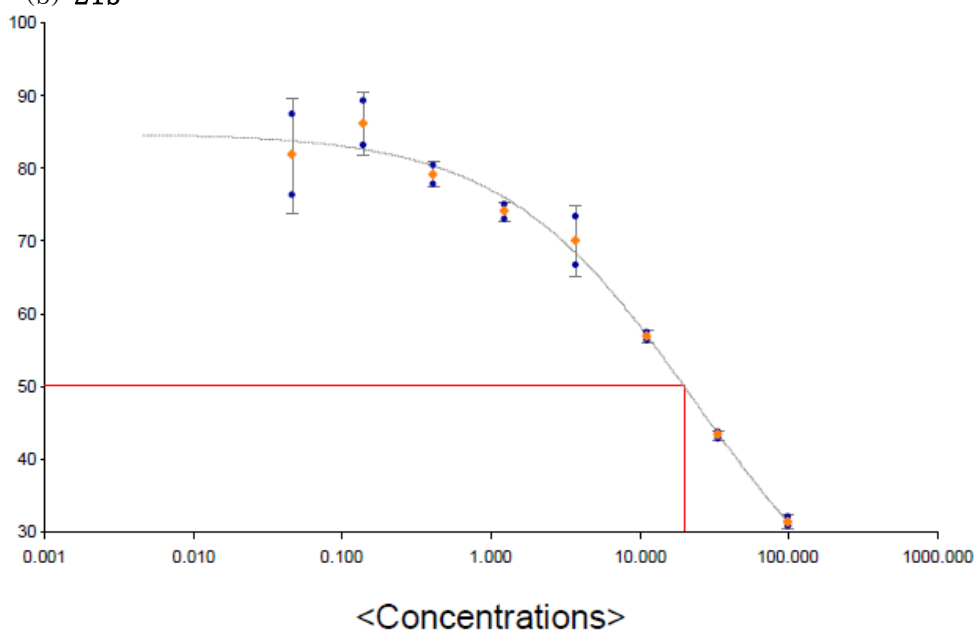


Fiugre 6. IC_{50} Cell viability in H460 cells 3-fold serial dilution of 100 μM, (a) **21a (isoimerubrine)**, (b) **21b**

(a) **21a (isoimerubrine)**

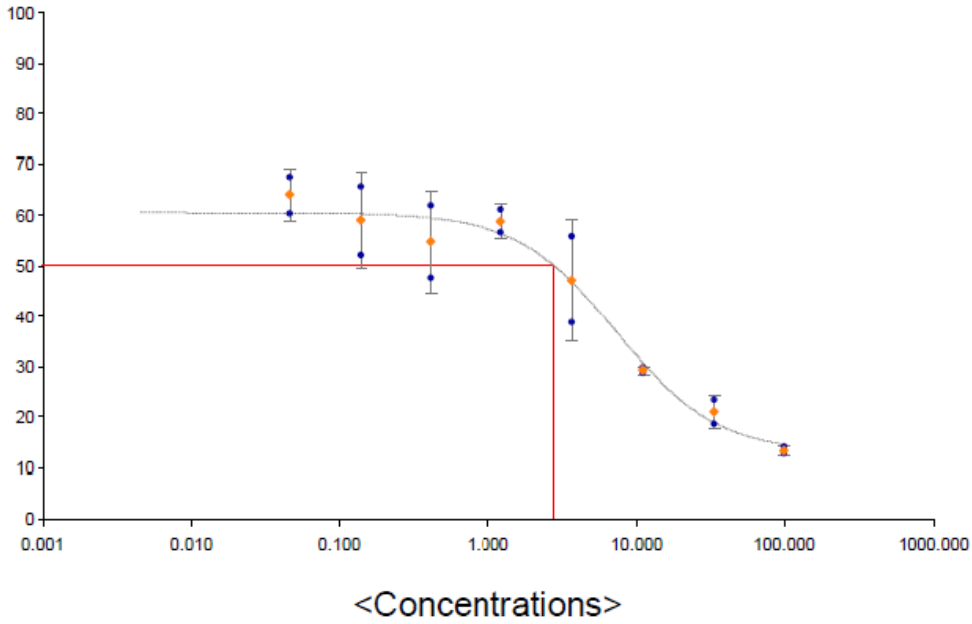


(b) **21b**

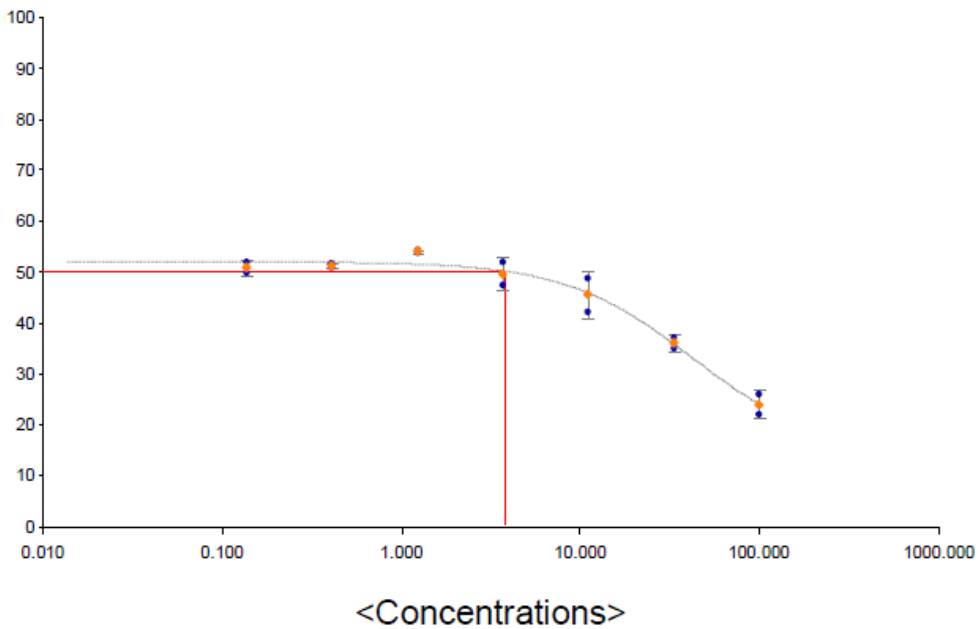


Fiugre 7. IC_{50} Cell viability in HCT-116 cells 3-fold serial dilution of 100 μM, (a) **21a (isoimerubrine)**, (b) **21b**

(a) **21a (isoimerubrine)**



(b) **21b**



Fiugre 8. IC_{50} Cell viability in K562 cells 3-fold serial dilution of 100 μM, (a) **21a (isoimerubrine)**, (b) **21b**

3. Conclusion

A chlorine derivative of isoimerubrine was successfully synthesized from a commercially available benzyl alcohol. By substituting the methoxy group for chlorine derivatives of tropoloisoquinoline, this compound has shown potential as a platform compound to synthesize tropoloisoquinolines with other functional groups. In addition, it was confirmed that azafluoranthene compound was also formed in this process, and it was shown that two alkaloids could be selectively obtained depending on the reaction conditions. This showed that chlorinated derivatives of tropoloisoquinoline can convert into variety of azafluoranthene alkaloid derivatives, which was also extracted from *Menispermaceae*.

The synthesized derivatives were also tested for bioactivity in relation to anti-cancer effects. As a result, it was confirmed that some compounds are effective in solid and hematological cancers.

Although no specific physiological activity was found in the azafluoranthene derivatives generated during the synthetic route process, it is expected that the formation of various azafluoranthene derivatives through the platform compound may contribute to the discovery of compounds having effective biological activity. As a result, chlorinated derivatives of tropoloisoquinoline can provide a variety of alkaloids, which may contribute to the synthesis of new drugs.

4. Experimental Details

4.1. General Information

Materials were obtained from commercial suppliers and were used without further purification. Air or moisture sensitive reactions were conducted under argon atmosphere using oven-dried glassware and standard syringe/septa techniques. All solvents were purified before use. THF and ether were distilled by sodium benzophenone ketyl. Dichloromethane was distilled from CaH_2. MeOH was distilled from potassium carbonate. IR spectra were obtained on a commercially available ATR-FTIR spectrometer. ^{1}H and ^{13}C NMR spectra were measured at 400MHz and 100 MHz, respectively in $CDCl_3$ unless stated otherwise and the data were reported as follows in ppm (δ) from the internal standard (TMS, 0.0 ppm): chemical shift (multiplicity, coupling constant in Hz, integration). High resolution mass spectra were measured by the EI, CI, FAB ionization method. Melting points were determined with an open capillary melting point apparatus and are uncorrected. The reactions were monitored by analytical thin layer chromatography (TLC) using Merck 60 F_{254} glass plates pre-coated with a 0.25 nm thickness of silica gel under UV light (254 nm, 365 nm) followed visualization with a phosphomolybdic acid or ninhydrin staining solution or I_2. Column Chromatography was performed on silica gel 60 (70-230 mesh) or aluminium oxide 90 activated basic (0.063 mm – 0.200 mm), obtained form from *Sigma Aldrich*.

A suitable single crystal was selected on a SuperNova, Dual,

Mo at home/near, AtlasS2 diffractometer. The crystal was kept at 292.15 K during data collection. Using Olex220, the structure was solved with the olex2. Dissolve[21] structure solution program using Charge Flipping and refined with the ShelXL[22] refinement package using Least Squares minimization.

4.2. Biological activity assay

Bioactivity tests related to anti-cancer effects were carried out by the following method by J&C Science in South Korea.

4.2.1. Anti-cancer effect

Cell Culture A549, K562, HL-60, HCT116, WI-26 cells were purchased from ATCC. HUVEC cells were purchased from Korea Research Institute of Bioscience and Biotechnology (KRIBB)

A549, K562, HL-60 cells were maintained in RPMI1640 supplemented with penicillin (100 units/ml) streptomycin (100 μg/ml) and 10% heat-inactivated fetal bovine serum (GIBCO).

HCT116 cells were maintained in McCoy's medium supplemented with penicillin (100 units/ml) streptomycin (100 μg/ml) and 10% heat-inactivated fetal bovine serum (GIBCO).

HUVEC cells were maintained in Human Endothelial Growth Medium supplemented with penicillin (100 units/ml) streptomycin (100 μg/ml) and 10% heat-inactivated fetal bovine serum (GIBCO).

WI-26 cells were maintained in MEM supplemented with penicillin (100 units/ml) streptomycin (100 μg/ml), NEAA and 10% heat-inactivated fetal bovine serum (GIBCO).

The cells were maintained in a humidified 5% CO2 atmosphere at 37 ℃.

Cytotoxicity assay (IC50) Cell cytotoxicity was evaluated using a fluorescence assay. A549 (5 × 103 cells/well), K562 (5 × 103 cells/well), HL-60 (5 × 103 cells/well), WI26 (5 × 103 cells/well), HCT116 (3 × 103 cells/well), HUVEC (3 × 103 cells/well) cells was seeded into 96-well plates and maintained at 37 ℃ for overnight. The plates were incubated for 24 h with various concentrations of the test compounds. The cells were exposed to various concentrations of derivatives. After 24 h of incubation, the Presto Blue cell viability reagent (Invitorgen) was added 10μl to each well and incubated for another 30 min. The fluorescence of the solution was measured at a wavelength of 560 nm using a Model 590 microplate reader (BioTek, USA). The cellular viability was determined from the absorbance value and compared with that of the untreated control group. All experiments were performed in duplicate.

4.3. General synthetic method

5-(bromomethyl)-1,2,3-trimethoxybenzene (2): To 0 ℃ solution of benzyl alcohol **1** (5.0 g, 25.2 mmol) in 90 mL of $CHCl_3$ under argon was added PBr_3 (1.56 mL, 16.6 mmol). The reaction mixture was stirred at 0 ℃ for 15 min, quenched with saturated aqueous $NaHCO_3$ and Na_2SO_3, and extracted with $CHCl_3$ (3 x 30 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo.* Purification by column chromatography (7:2:1 hexane-EtOAc-CH_2Cl_2) afforded benzylbromide **2** as a white solid (6.05 g, 92%) as a white solid: mp 73 ℃; ^{1}H NMR ($CDCl_3$) δ 6.62 (s, 2H), 4.47 (s, 2H), 3.87 (s, 6H), 3.85 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 153.33, 138.30, 133.15, 106.24, 60.85, 56.17, 34.22; HRMS [EI+] calcd for $C_{10}H_{13}BrO_3$ 260.0048, found 260.0046

N-(2,2-dimethoxyethyl)-4-methylbenzenesulfonamide (3):

To 0 ℃ solution of aminoacetaldehyde dimethyl acetal (10 mL, 91.8 mmol) in 100 mL of CH_2Cl_2 was added triethylamine (38 mL, 275.3 mmol), TsCl (21 g, 110 mmol). The reaction mixture was stirred at 0 ℃ for overnight and extracted with CH_2Cl_2 (3 x 30 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (2:1 →4:1 hexane-EtOAc) afforded tosylated amino acetal **3** quantitatively (23.8 g) as a colorless oil to white solid: mp 44 ℃; ^{1}H NMR ($CDCl_3$) δ 7.7 (d, J=8.4, 2H), 7.3 (d, J=8, 2H), 4.67 (br s, 1H), 4.35 (t, J=5.6, 1H), 3.35 (s, 6H), 3.05 (t, J=5.6), 2.45 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 143.6, 136.8, 129.7, 127.1, 102.6, 54.6, 44.6,

21.5; HRMS (FAB) $[M-H]^-$ calcd for $C_{11}H_{16}NO_4S$ 258.0800, found 258.0800

N-(2,2-dimethoxyethyl)-4-methyl-N-(3,4,5-trimethoxybenzyl)benzenesulfonamide (4): To 0 °C solution of benzyl bromide **2** (4 g, 15.3 mmol) in 150 mL of DMF was added NaH (873 mg, 20 mmol). The reaction mixture was stirred 5 min and then tosylated amino acetal **3** (3.37 g, 13 mmol) was added. The reaction mixture was stirred overnight at room temperature. Then quench the reaction using saturated aqueous NH_4Cl (30 mL), then extracted with Et_2O (3 x 20 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (4:1→1:1 hexane-EtOAc) afforded coupled product **4** as a colorless oil. When the oil was dried for a long time, it became white solid: mp 60°C; ^1H NMR ($CDCl_3$) δ 7.7(d, J=7.6, 2H), 7.3(d, J=7.6, 2H), 4.43(s, 2H), 4.4(t, J=5.6, 1H), 3.80(s, 3H), 3.74(s, 6H), 3.29(s, 6H), 3.24(d, J=4.8, 2H), 2.42(s, 3H); ^{13}C NMR ($CDCl_3$) δ 153.23, 143.31, 137.81, 137.41, 131.78, 129.67, 127.21, 105.34, 104.20, 60.82, 55.98, 54.73, 52.75, 48.66, 21.46; HRMS [EI+] calcd for $C_{21}H_{29}NO_7S$ 439.1665, found 439.1665

5,6,7-trimethoxyisoquinoline (5): To a solution of sulfonamide **4** (2 g, 4.6 mmol) in 1,4-dioxane (25 mL) was added 6 N HCl (3.8 mL, 22.8 mmol). Reaction mixture was stirred 1 day then cooled to room temperature, and then quenched with saturated aqueous $NaHCO_3$. After separating the organic layer through extraction with $CHCl_3$(30 mL x 3), the separated organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (1:2

hexane–EtOAc) afforded isoquinoline **5** (948 mg, 95%) as a brown oil. ^{1}H NMR ($CDCl_3$) δ 9.07 (s, 1H), 8.4 (d, J=5.6, 1H), 7.8 (d, J=6, 1H), 7.04 (s, 1H), 4.17 (s, 3H), 4.02 (s, 3H), 4.01 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 153.8, 150.2, 146.6, 144.1, 141.2, 127.5, 125.8, 114.5, 61.4, 61.1, 55.9; HRMS [EI+] calcd for $C_{12}H_{13}NO_3$ 219.0895, found 219.0889

8–bromo–5,6,7–trimethoxyisoquinoline (6): To a solution of isoquinoline **5** (2.2 g, 10 mmol) in distilled THF under argon atmosphere was added NBS (2.67 g, 15 mmol) and one drop of H_2SO_4. The reaction mixture was stirred 1 h at room temperature, and then quenched with saturated aqueous solution of $NaHCO_3$ and Na_2SO_3. After separating the organic layer through extraction with EtOAc (30 mL x 3), the separated organic extracts were dried over Na_2SO_4 and concentrated *in vacuo.* Purification by column chromatography (4:1 hexane–EtOAc) afforded brominated isoquinoline **6** (2.6 g, 87%)as a white solid: mp 66 °C; ^{1}H NMR ($CDCl_3$) δ 9.51 (s, 1H), 8.5 (d, J=6, 1H), 7.84 (d, J=4.8), 4.06 (s, 6H), 4.00 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 151.59, 150.96, 147.60, 146.65, 142.64, 130.28, 123.89, 114.12, 109.83, 61.56, 61.42, 61.25; HRMS [EI+] calcd for $C_{12}H_{12}BrNO_3$ 297.0001, found 297.0005

(4–bromo–2–chlorophenoxy)triisopropylsilane (7): To solution of 4–bromo–2–chlorophenol (20 g, 96.4 mmol) in CH_2Cl_2 (300 mL) was added imidazole (13.1 g 192.8 mmol) and TIPSCl (22.7 mL, 106 mmol). The reaction mixture was stirred overnight at room temperature, then extracted with CH_2Cl_2 (30 mL x 3), and brine. The combined organic extracts were dried over $MgSO_4$ and

concentrated *in vacuo*. Purification by column chromatography (100% hexane) afforded silyl protected phenol **7** (36.06 g, Quant) as a colorless liquid. ^{1}H NMR ($CDCl_3$) δ 7.5 (d, J=2.4, 1H), 7.2 (dd, J_1=8.4, J_2=2.4, 1H), 6.8 (d, J=8.8, 1H), 1.3 (m, 3H), 1.1 (d, J=7.2); ^{13}C NMR ($CDCl_3$) δ 151.40, 132,73, 130.37, 126.46, 121.16, 112.66, 17.86, 12.85; HRMS [EI+] calcd for $C_{15}H_{24}BrClOSi$ 362.0468, found 362.0463

(3-chloro-4-((triisopropylsilyl)oxy)phenyl)boronic acid (8): Dissolve silyl protected phenol **7** (6.56 g, 18 mmol) in distilled THF under argon atmosphere and cooled to −78 ℃. Then ^{n}BuLi was added slowly to the solution. After the stirring the mixture 1 h at −78 ℃, $B(OMe)_3$ was added. The reaction mixture was stirred overnight at −78 ℃ → RT, then cooled to 0 ℃. 2 N HCl was added in the solution and stirring 15 min. The organic layer was separated through extraction with EtOAc (30 mL x 3) and dried over $MgSO_4$. After the evaporation of organic solvent using rotary evaporator, the crude mixture of **8** was just used in next reaction without any purification.

8-(3-chloro-4-((triisopropylsilyl)oxy)phenyl)-5,6,7-trimethoxyisoquinoline (9): To solution of isoquinoline **6** (963 mg, 3.23 mmol) and boronic acid **8** in THF and 2 M aqueous Na_2CO_3 (15 mL) was added at room temperature $Pd(PPh_3)_4$ (149 mg, 0.129 mmol). The reaction mixture was heated at reflux for 5 h, then cooled to room temperature. The crude mixture was extracted with EtOAc (30mL x 3). The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography

(4:1 hexane−EtOAc) afforded biaryl compound **9** (1.52 g, 94%) as a yellow oil: IR 3036, 2944, 2867, 1601, 1501 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.89 (s, 1H), 8.45 (d, J=5.6, 1H), 7.87 (d, J=5.6, 1H), 7.4(s, 1H), 7.15(dd, J_1=8.4, J_2=2, 1H), 7.03 (d, J=8.4), 4.09 (s, 3H), 4.07 (s, 3H), 3.67 (s, 3H), 1.38 (m, 3H), 1.19 (s, 10H), 1.17(s, 8H); ^{13}C NMR ($CDCl_3$) δ 151.72, 151.07, 150.10, 147.10, 146.42, 142.20, 132.53, 129.98, 129.35, 127.27, 126.77, 125.32, 124.84, 119.94, 114.25, 61.58, 61.29, 61.25, 17.95, 12.96; HRMS (EI) [M+] calcd for $C_{27}H_{37}NO_4Si$ 467.2492, found 467.2499

(E)-4-(8-(3-chloro-4-hydroxyphenyl)-5,6,7-trimethoxy-1-(nitromethyl)isoquinolin-2(1H)-yl)but-3-en-2-one (16):

A solution of CrO_3 (747.7 mg, 4.92 mmol) in H_2SO_4/H_2O (1.8 mL/9 mL) was added slowly to a stirring solution of 3-butyn-2-ol (0.32 mL, 4.1 mmol) in H_2SO_4/H_2O (2.5 mL/8 mL) at 0 °C. The mixture was stirred at 2-10 °C for 4 h and then extracted with CH_2Cl_2 (3 mL). The separated organic extracts were dried over $MgSO_4$. The obtained crude mixture of butynone **14** was added to a solution of **9** (1.08 g, 2.1 mmol) in nitromethane (11 mL) at room temperature. The reaction mixture was stirred overnight, and extracted with CH_2Cl_2 (20 mL x 3) and saturated NH_4Cl solution. Combined organic layer was dried over $MgSO_4$, then concentrated *in vacuo.* Purification by column chromatography (2:1 hexane-EtOAc) afforded nitromethylated biaryl compound **15** (1.28 g 97%) as a yellow oil. TBAF·$3H_2O$ (119 mg, 0.456 mmol) was added at room temperature to a solution of **15** (233 mg, 0.37 mmol) in THF (10 mL). The reaction mixture was stirred for 1 h, and then extracted with NH_4Cl (15 mL x 2) and (EtOAc 15 mL x 2). The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (1:1 hexane-EtOAc) and dried in vacuum oven afforded compound **16** (165 mg, 94%) as a yellow bubbled solid: mp 140 °C; 1554, 1610, 2940, 3101 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.22 (d, J=8, 2H), 7.16 (d, J=8.4, 2H), 7.05 (dd, J_1=20.8, J_2=8, 1H), 6.43(s, 2H), 6.17 (br s, 1H), 5.50 (dd, J_1=7.6, J_2=13.6, 1H), 5.41 (br s, 1H), 4.56 (m, 1H), 4.01 (dd, J_1=12.4, J_2=3.2, 1H), 3.98 (s, 6H), 3.61 (d, J=8.8, 3H), 2.05 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 193.71, 196.62, 151.97, 151.90, 151.78, 148.55, 146.67, 146.61, 130.65, 129.82, 128.95, 128.35, 128.24, 126.82, 126.54, 120.83, 120.79,

120.75, 120.42, 117.22, 117.04, 106.11, 102.55, 74.09, 74.04, 61.47, 61.31, 61.27, 60.95, 60.93, 28.63, 28.52; HRMS (FAB) $[M+H]^+$ calcd for $C_{23}H_{24}ClN_2O_7$ 475.1272, found 475.1267

8-chloro-1,2,3-trimethoxy-9H-cyclohepta[a]acenaphthylen-9-one (20a)

10-chloro-1,2,3-trimethoxy-9H-cyclohepta[a]acenaphthylen-9-one (20b): The biaryl phenol **16** (200 mg, 0.42 mmol) was dissolved in 1.0 M aqueous CsOH (3.36 mL) and then diluted with H_2O (6 mL). The prepared dianion was subjected to a solution of $K_3Fe(CN)_6$ (490 mg, 1.5 mmol) in H_2O (10 mL) and $CHCl_3$ (15 mL) over 10 min at 0 ℃ in the dark. After 1 h stirring at 0 ℃, It was quenched by saturated Na_2SO_3 aqueous solution. The reaction mixture was filtered through celite pad, and then the organic layer was separated by extraction with $CHCl_3$ (30 mL x 3). Then DDQ (191 mg, 0.84 mmol) was added to the combined organic layer and stirring 2 h. It was quenched by saturated $NaHCO_3$ aqueous solution and extracted with $CHCl_3$ (30 mL x 3). The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude mixture was subjected to the next reaction without any purification. To a solution of crude mixture **18** in THF (3 mL) was added 2 M Me_2NH in MeOH (3 mL). The mixture was stirred 1 h then it just concentrated by rotary evaporator. Obtained crude mixture **19** was dissolved in distilled THF (7 mL) and cooled to 0 ℃ under argon atmosphere. TMSOTf (0.3 mL, 1.68 mmol) was dropped to the solution and stirred 1 h at 0 ℃. The reaction was quenched by saturated $NaHCO_3$ aqueous solution, extracted with $CHCl_3$ (30 mL x 3). The combined organic extracts were dried over Na_2SO_4 and

concentrated *in vacuo*. Purification by column chromatography (1:2 hexane−EtOAc) afforded a mixture of **20a** and **20b** (83 mg, 56% in 3 steps, **20a** : **20b** =3 : 5) as a reddish solid. The mixture of isomer, **20a** and **20b,** was separated by aluminum oxide column chromatography ($CHCl_3$ 100%). For **20a:** mp 191 ℃; IR 1579, 1601, 2943 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.85 (s, 1H), 8.78 (d, J=6, 1H), 8.30 (d, J=12.4, 1H), 7.82 (d, J=5.6, 1H), 7.40 (d, J=12, 1H), 4.27 (s, 3H), 4.23 (s, 3H), 4.01 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 179.86, 156.98, 156.44, 153.51, 148.68, 145.83, 141.88, 138.27, 136.39, 132.03, 129.90, 125.38, 120.10, 119.47, 115.83, 62.12, 61.53; HRMS (FAB) $[M+H]^+$ calcd for $C_{19}H_{15}ClNO_4$ 356.0690, found 356.0686; For **20b:** mp 193 ℃; IR 1572, 1607, 2950 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.98 (s, 1H), 8.80 (d, J=6, 1H), 8.28 (d, J=12, 1H), 7.84 (d, J=6, 1H), 7.40 (d, J=12, 1H), 4.31 (s, 3H), 4.26 (s, 3H), 4.04 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 179.82, 157.22, 156.35, 153.56, 148.58, 148.41, 146.01, 139.46, 138.89, 135.90, 132.49, 129.48, 125.36, 120.16, 119.27, 115.78, 62.20, 62.16, 61.55; HRMS (FAB) $[M+H]^+$ calcd for $C_{19}H_{15}ClNO_4$ 356.0690, found 356.0688

4,5,6,10−tetramethoxy−9H−azuleno[1,2,3−ij]isoquinolin−9−one (21a), methyl 4,5,6−trimethoxyindeno[1,2,3−ij]isoquinoline−9−carboxylate (22a), methyl 9−chloro−4,5,6−trimethoxyindeno[1,2,3−ij]isoquinoline−8−carboxylate (23a) :

To solution of tropoloisoquinoline **20a** (30 mg, 0.0845 mmol) in $CHCl_3$ (2 mL) was added 7% $Mg(OMe)_2$ solution in MeOH. The reaction mixture was stirred overnight, quenched with H_2O and diluted with $CHCl_3$ (10 mL). The organic layers were separated with $CHCl_3$ (10 mL x 3). The combined organic extract was dried over

Na_2SO_4 and concentrated *in vacuo*. Purification by aluminium oxide column chromatography (99:1 $CHCl_3$–MeOH) afforded compound **21a** (20 mg, 67%) as a reddish solid, **22a** (4 mg, 13%) and **23a** (4 mg ,12%) as a yellow like fluorescent color of solid. For **21a:** mp 186 ℃; IR 1548, 1582, 1601, 2947 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.74 (d, J=8, 1H), 8.27 (d, J=12, 1H), 7.91 (s, 1H), 7.80 (d, J=8, 1H), 7.40 (d, J=12, 1H), 4.21 (s, 3H), 4.19 (s, 3H), 4.03 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 180.10, 164.29, 158.54, 154.29, 151.23, 149.37, 145.38, 138.99, 136.79, 136.41, 132.49, 125.75, 121.14, 120.91, 115.68, 106.73, 62.12, 61.94, 61.44, 56.79; HRMS [EI+] calcd for $C_{20}H_{17}NO_5$ 351.1107, found 351.1104; For **22a:** mp 115 ℃; ^1H NMR ($CDCl_3$) δ 8.80 (s, 1H), 8.69 (d, J=6, 1H), 8.20 (dd, J_1=8, J_2=1.6, 1H), 8.06 (dd, J_1=7.6, J_2=0.8, 1H), 7.72 (d, J=6, 1H), 4.22 (s, 3H), 4.18 (s, 3H), 4.07 (s, 3H), 3.99 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.94, 158.25, 152.84, 150.64, 149.54, 144.98, 142.43, 138.44, 131.68, 129.07, 126.10, 124.11, 123.39, 123.08, 120.67, 113.90, 62.05, 61.66, 61.48, 52.15, 29.68; HRMS [EI+] calcd for $C_{20}H_{17}NO_5$ 351.1107, found 351.1105; For **23a:** mp 120 ℃; ^1H NMR ($CDCl_3$) δ 8.70 (d, J=6, 1H), 8.36 (s, 1H), 8.19 (s, 1H), 7.76 (d, J=6, 1H), 4.23 (s, 3H), 4.18 (s, 3H), 4.07 (s, 3H), 4.03 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.52, 156.85, 152.44, 150.22, 149.68, 145.15, 141.97, 136.36, 132.72, 130.76, 126.31, 125.70, 124.49, 124.40, 119.82, 114.75, 62.05, 61.74, 61.47, 52.56, 29.68; HRMS [EI+] calcd for $C_{20}H_{16}ClNO_5$ 385.0717, found 385.0716

4,5,6,8-tetramethoxy-9H-azuleno[1,2,3-ij]isoquinolin-9-one (21b), methyl 4,5,6-trimethoxyindeno[1,2,3-ij]isoquinoline-8-carboxylate (22b): Above procedure for **21a** was followed with **20b** (26 mg, 0.732 mmol) to afford compound **21b** (21 mg, 80%) as a reddish solid, **22b** (5 mg, 20%) as a yellow like fluorescent color solid. For **21b:** mp 184 ℃; IR 1548, 1581, 1600, 2942 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.71 (d, J=5.6, 1H), 8.25 (d, J=12, 1H), 8.10 (s, 1H), 7.73 (d, J=5.6, 1H), 7.37 (d, J=12, 1H), 4.29 (s, 3H), 4.24 (s, 3H), 4.16 (s, 3H), 4.05 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 180.08, 165.57, 158.82, 155.16, 152.86, 148.76, 145.54, 142.18, 134.49, 134.10, 129.83, 125.06, 120.99, 120.39, 114.17, 109.60, 62.12, 61.74, 61.50, 56.43; HRMS (FAB) $[M+H]^+$ calcd for $C_{20}H_{18}NO_5$ 352.1185, found 352.1179; For **22b:** mp 140 ℃; ^{1}H NMR ($CDCl_3$) δ 8.70 (d, J=6, 1H), 8.62 (s, 1H), 8.17 (d, J=1.2, 1H), 8.13 (d, J=1.2, 1H), 7.75 (d, J=6, 1H), 4.24 (s, 3H), 4.18 (s, 3H), 4.08 (s, 3H), 4.02 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 167.05, 157.85, 152.37, 149.93, 149.73, 145.00, 142.32, 138.37, 131.35, 129.14, 126.23, 124.69, 124.32, 121.57, 120.77, 114.37, 62.05, 61.74, 61.46, 52.25, 29.68; HRMS [EI+] calcd for $C_{20}H_{17}NO_5$ 351.1107, found 351.1102

REFERENCES

1. (a) M. P. Cava, K. T. Buck, and A. I. DaRocha. *J. Am. Chem. Soc.* 1972, 94, 16, 5931−5931., (b) Jian−Wei Dong, Le Cai, Yun−Shan Fang, Huai Xiao, Zhen−Jie Li, Zhong−Tao Ding, *Fitoterapia.,* 2015, 104, 102–107.

2. Kumari Wimpy, Yadav SK, Mathur Kumkum, Goyal Manoj, *Indian Journal of Pharmacy and Pharmacology*, 2016, 3(4), 152−154.

3. Thakur P, Rana AC., *J Tradit Complement Med*. 2013, 3(3), 188−93.

4. G.Amresh, P.N.Singh, Ch.V.Rao, *Journal of Ethnopharmacology*., 2007, 111, 531–536.

5. G. Amresha, G.D. Reddy, Ch.V. Rao, P.N. Singh, *Journal of Ethnopharmacology*, 2007, 110, 526–531.

6. S. Morris Kupchan, A. C. Patel, Eiichi Fujita, Tumor inhibitors VI. Cissampareine, new cytotoxic alkaloid from Cissampelos pareira. Cytotoxicity of bisbenzylisoquinoline alkaloids. *J. Pharm. Sci.*, 1965, 54, 580−583.

7. (a) M.P. Cava, K.T. Buck, I. Noguchi, M. Srinivasan, M.G. Rao, A.I. DaRocha, The alkaloids of Abuta imene and Abuta rufescens, *Tetrahedron*, 1975, 31(15), 1667−1669. (b) Menachery, Mary D. Cava, Michael P. Grandirubrine, a new tropoloisoquinoline alkaloid. *Heterocycles*, 1980, 14, 943. (c)

Hiroshi Morita, Kouji Matsumoto, Koichi Takeya, Hideji Itokawa, Yoichi Iitaka, *Chem. Pharm. Bull.* 1993, 41(8), 1418-1422.

8. Juan Li, Zhao-Xing Li, Jian-Ping Zhao, Wei Wang, Xiao-Fang Zhao, Bo Xu, Lin Li, Lan Zhang, Jie Ren, Ikhlas A. Khan, Shun-Xiang Li. Novel Tropoloisoquinoline Alkaloid, Neotatarine, from Acorus calamus L. *Chem. Biodiversity*, 2017, 14, e1700201.

9. Swaffar DS, Holley CJ, Fitch RW, Elkin KR, Zhang C, Sturgill JP, Menachery MD. *Planta Med*. 2012, 78(3), 230-2

10. (a) M. F. Mackay, A. K Serelis, *J. Chem. SOC. Perkin Trans. 1*, 1993, 1905. (b) Martin G. Banwell, Neil K. Ireland. *J. Chem. Soc., Chem. Commun.*, 1994, 5, 591-592.

11. (a) Dale L. Boger, Kaqji Takahashi. Total Synthesis of Granditropone, Grandirubrine, Imerubrine, and Isoimerubrine. *J. Am. Chem. Soc*. 1995, 117, 12452-12459. (b) Dale L. Boger, Christine E. Brotherton. *J. Org. Chem.*, 1984, 49(21) 4051-4055. (c) Jae Chol Lee, Jin Kun Cha. *J. Am. Chem. Soc*. 2001, 123, 3243-3246.

12. (a) Ken S. Feldman, Timothy D. Cutarelli, Romina Di Florio. *J. Org. Chem*. 2002, 67, 8528-8537. (b) Ken S. Feldman, Timothy D. Cutarelli. *J. AM. CHEM. SOC*. 2002, 124, 11600-11601.

13. (a) Suk-Koo Hong, Hyeonjeong Kim, Youngran Seo, Sang

Hyup Lee, Jin Kun Cha, Young Gyu Kim. Org Lett. 2010, 12(17), 3954–3956. (b) Nara Shin, Concise total synthesis of tropoloisoquinolines and process development of bio-adipic acid from galactose, Ph.D. Thesis, Seoul National University, South Korea, 2016.

14. (a) Kende, A. S.; Ebetino, F. H.; Ohta, T. *Tetrahedron Lett.* 1985, 26, 3063; (b) Kende, A. S.; Koch, K. *Tetrahedron Lett.* 1986, 27, 6051; (c) LeBoff, A.; Carbonnelle, A. -C.; Alazard, J. -P.; Thal, C.; Kende, A. S. *Tetrahedron Lett.* 1987, 28, 4163; (d) Kende, A. S.; Koch, K.; Smith, C. A. *J. Am. Chem. Soc.* 1988, 110, 2210; (e) Celik, M.; Balci, M. *ARKIVOC* 2007, 8, 150.

15. Vijay Kumar Reddy Kondreddy, Akhilender Naidu Kamatham. *Celecoxib, a COX-2 inhibitor, synergistically potentiates the anti-inflammatory activity of docosahexaenoic acid in macrophage cell line. Immunopharmacol Immunotoxicol*, 2016, 38(2), 153–161.

16. Suk-Koo Hong. Total synthesis of pareitropone and development of bifunctional peptides active on opioid and neurokinin receptors. Ph.D. Thesis, Seoul National University, South Korea, 2010.

17. Eric P. Gillis, and Martin D. Burke. A Simple and Modular Strategy for Small Molecule Synthesis: Iterative Suzuki-Miyaura Coupling of B-Protected Haloboronic Acid Building Blocks. *J. Am. Chem. Soc.*, 2007, 129(21), 6716–6717.

18. Jhillu S. Yadav, Basi V. Subba Reddy, Nagendra Nath Yadav, Manoj K. Gupta. *Synthesis*. 2009, 7, 1131-1136.

19. Mark E. Janik, Susan L. Bane. *Bioorganic & Medicinal Chemistry*. 2002, 10(6), 1895-1903.

20. Dolomanov, O.V., Bourhis, L.J., Gildea, R.J, Howard, J.A.K. & Puschmann, H. *J. Appl. Cryst*. 2009, 42, 339-341.

21. Bourhis, L.J., Dolomanov, O.V., Gildea, R.J., Howard, J.A.K., Puschmann, H. *Acta Cryst*. 2015, A71, 59-75.

22. Sheldrick, G.M. *Acta Cryst*. 2015, C71, 3-8.

APPENDICES

List of ^{1}H NMR Spectra of Selected Compounds

1. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **2**……51

2. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **3**……52

3. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **4**……53

4. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **5**……54

5. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **6**……55

6. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **7**……56

7. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **8**……57

8. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **15**……58

9. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **16**……59

10. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **20a**……60

11. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **20b**……61

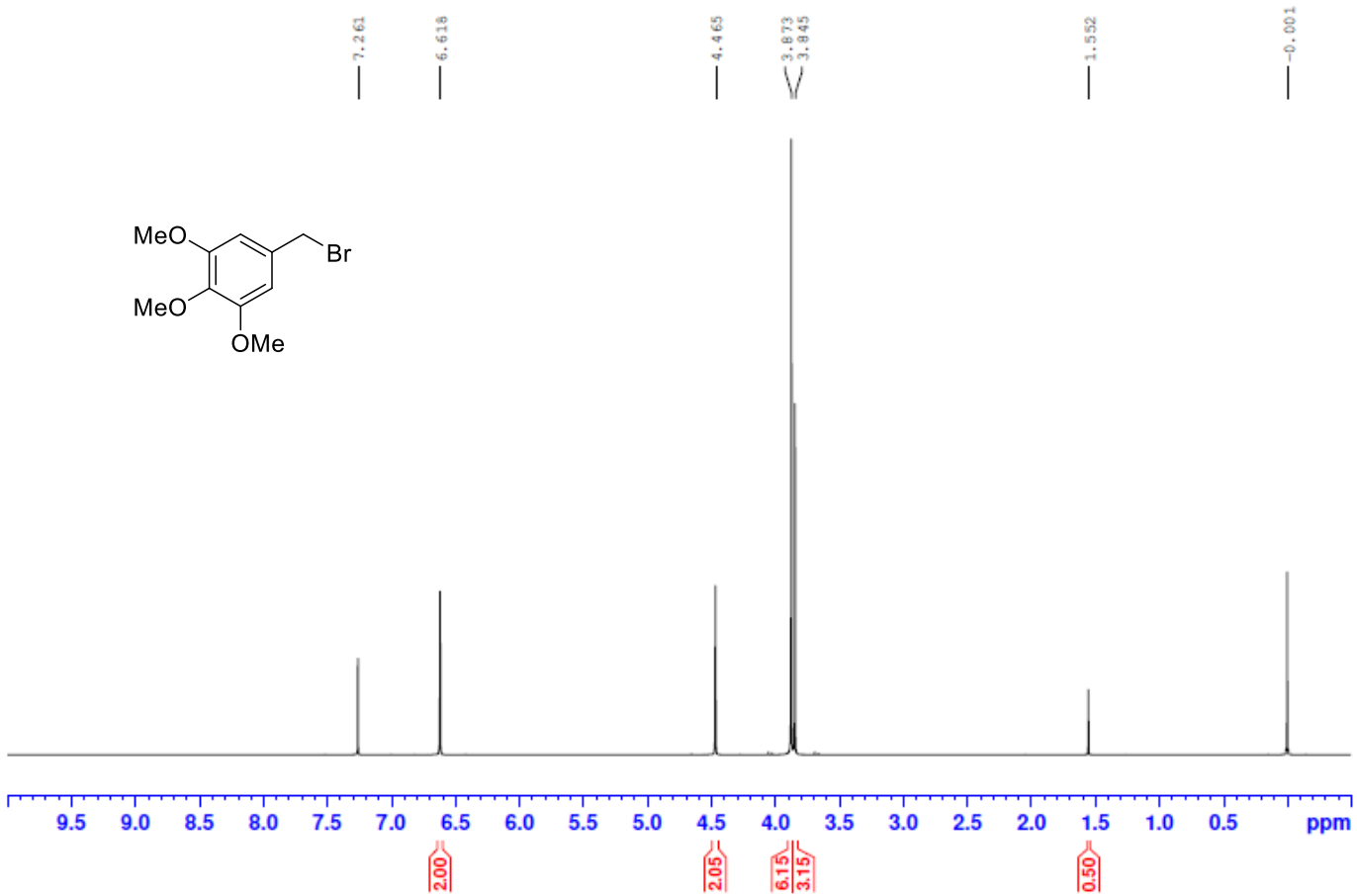
12. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **21a**……62

13. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **21b**……63

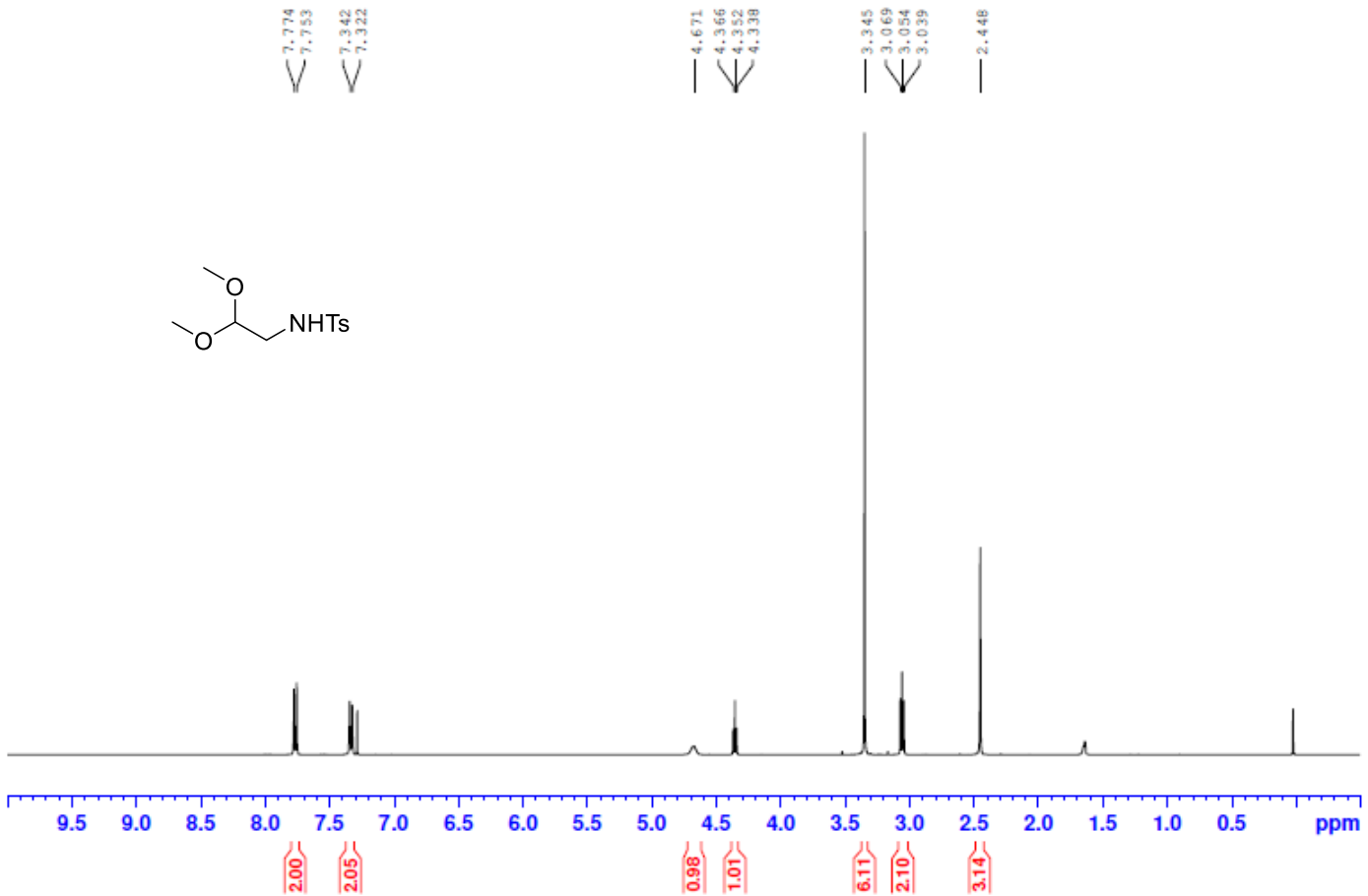
14. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **22a**……64

15. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **22b**……65

16. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **23a**……66



1. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **2**



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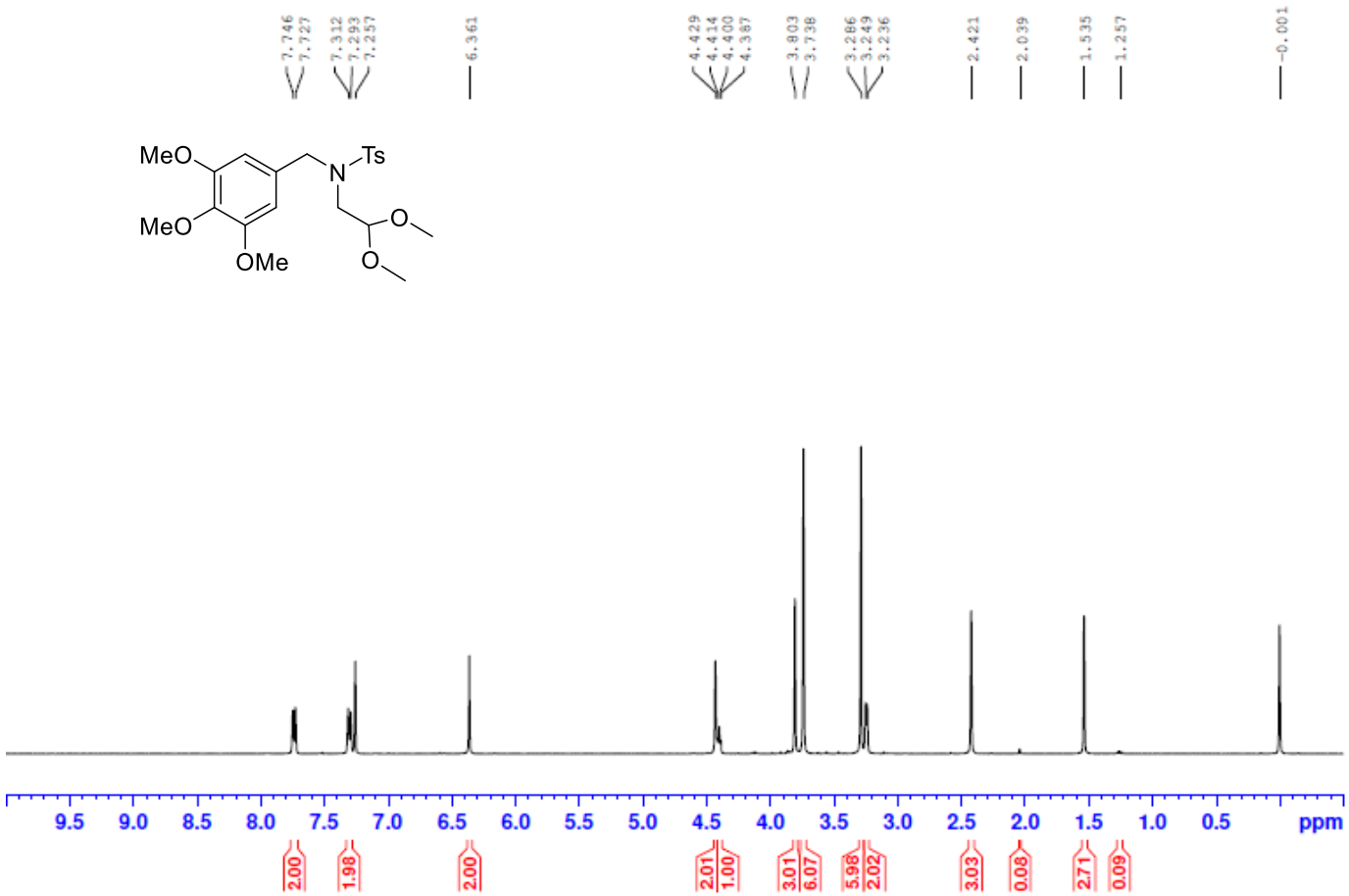
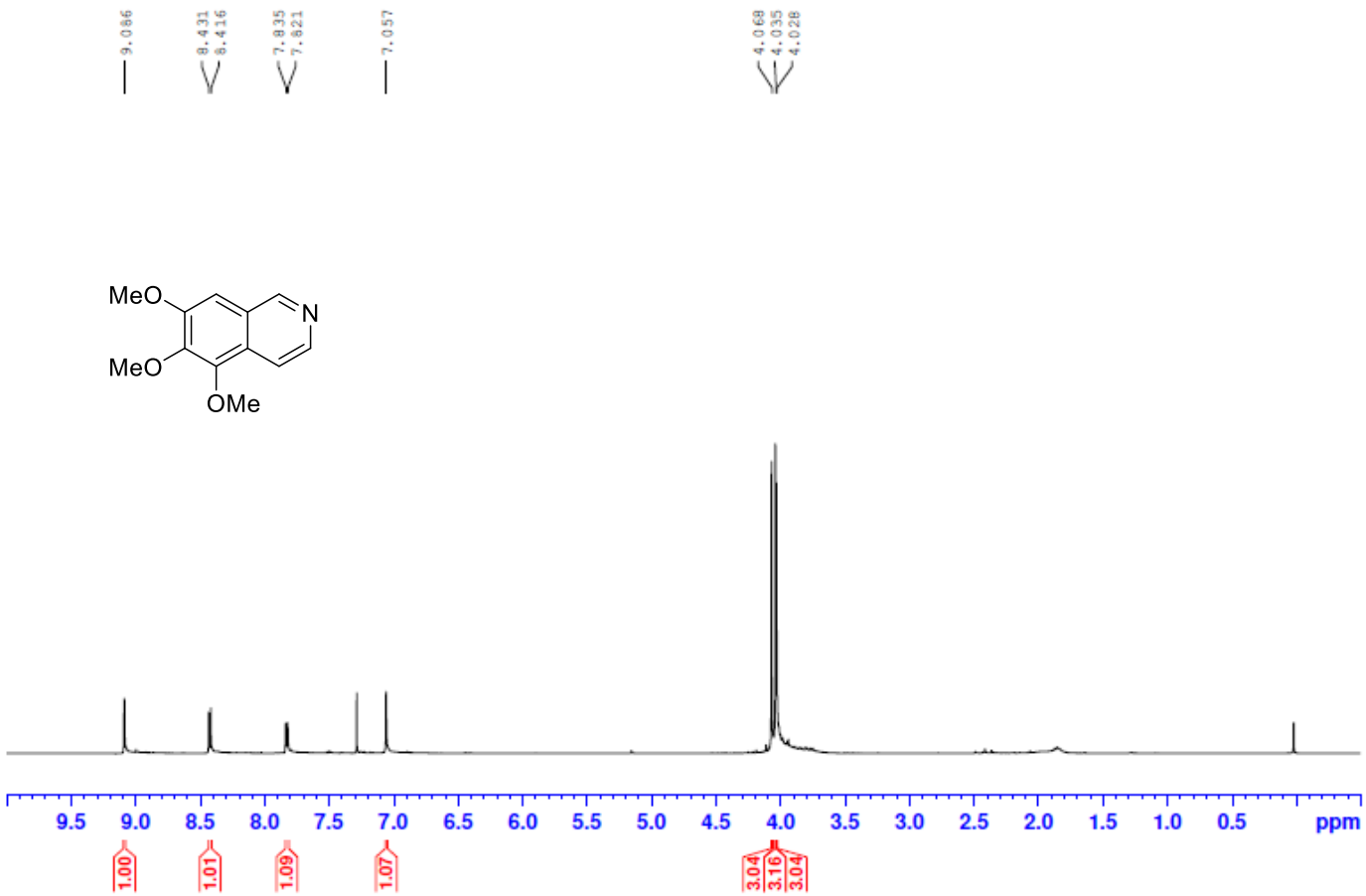
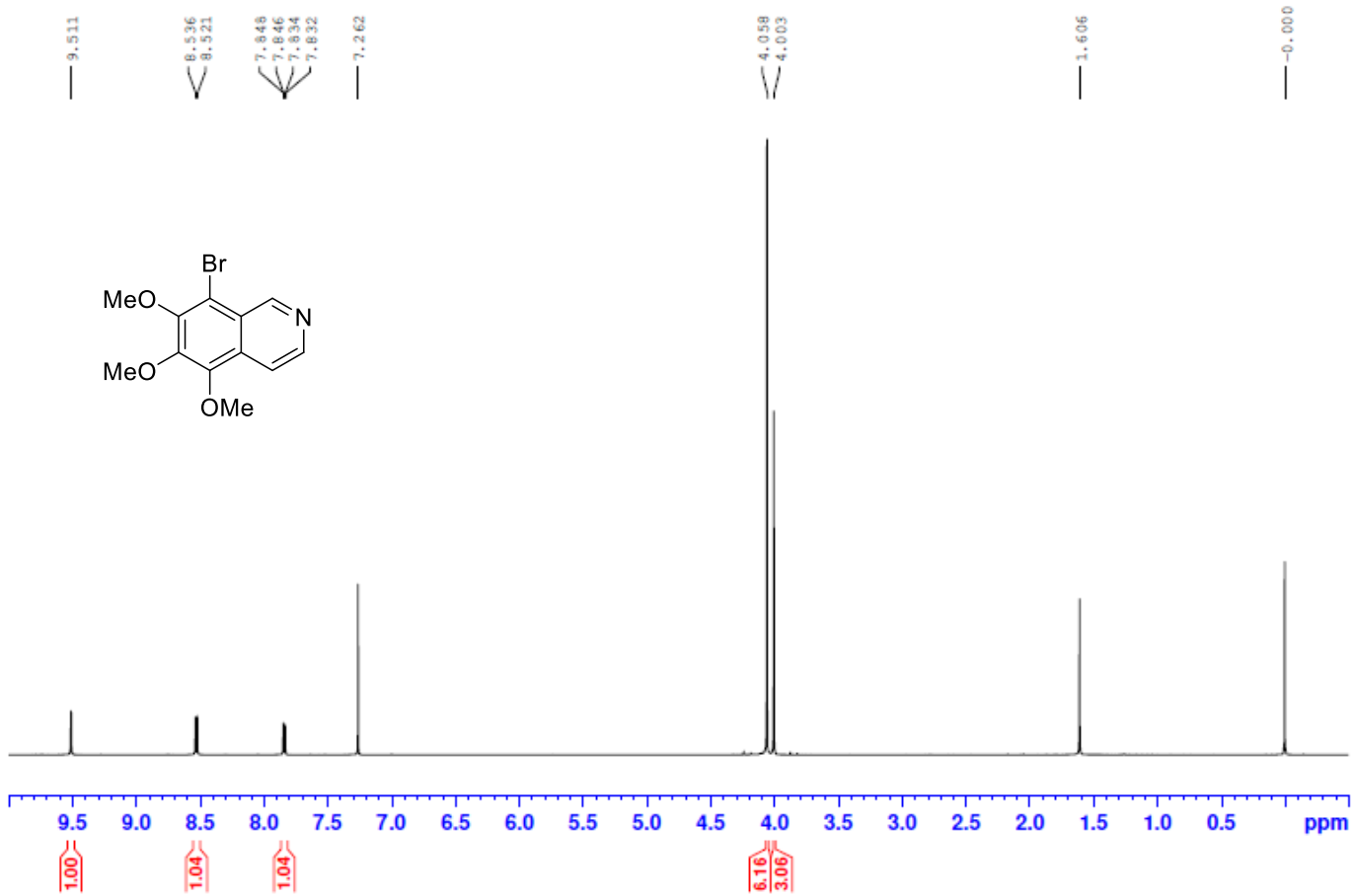


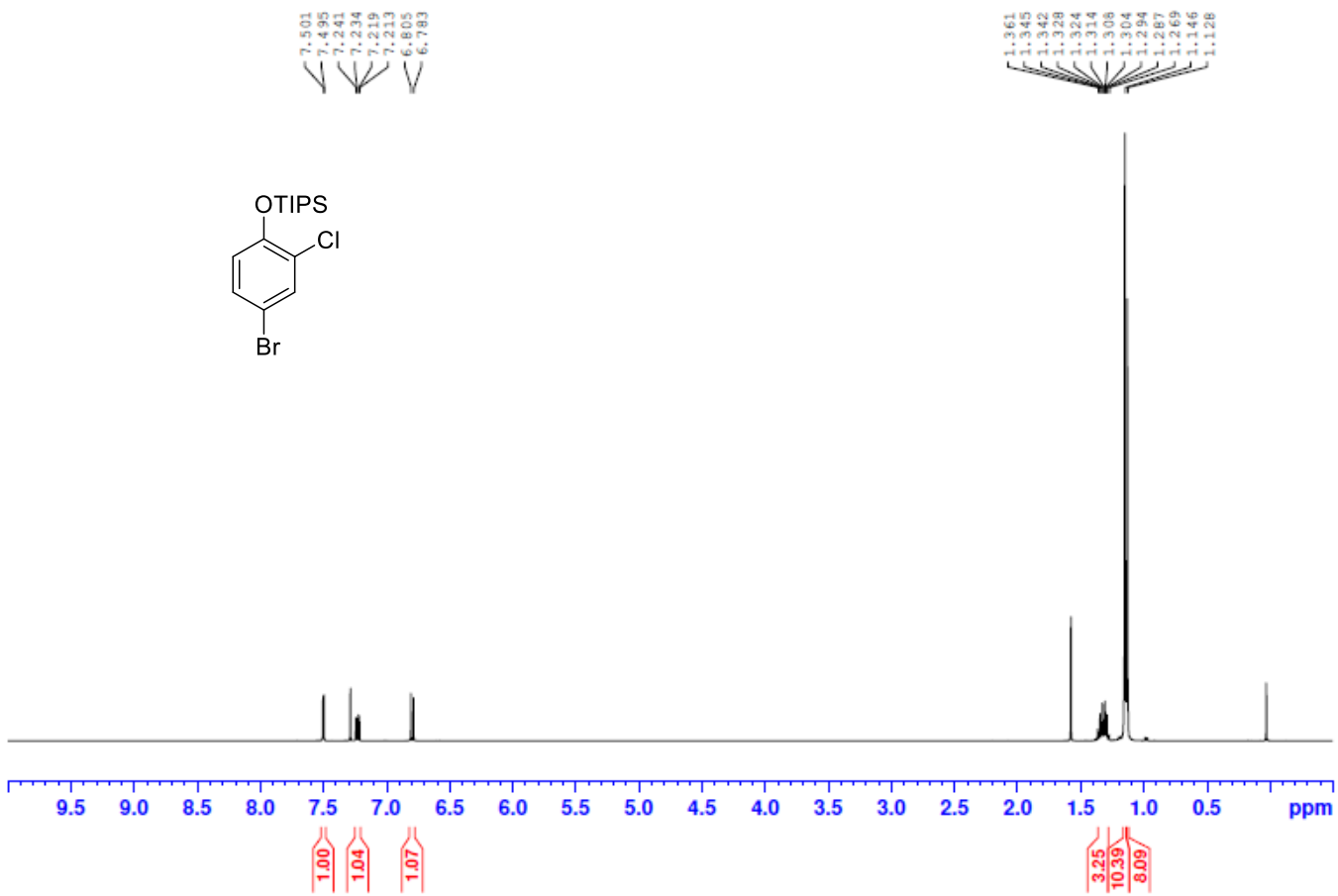
3. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **4**



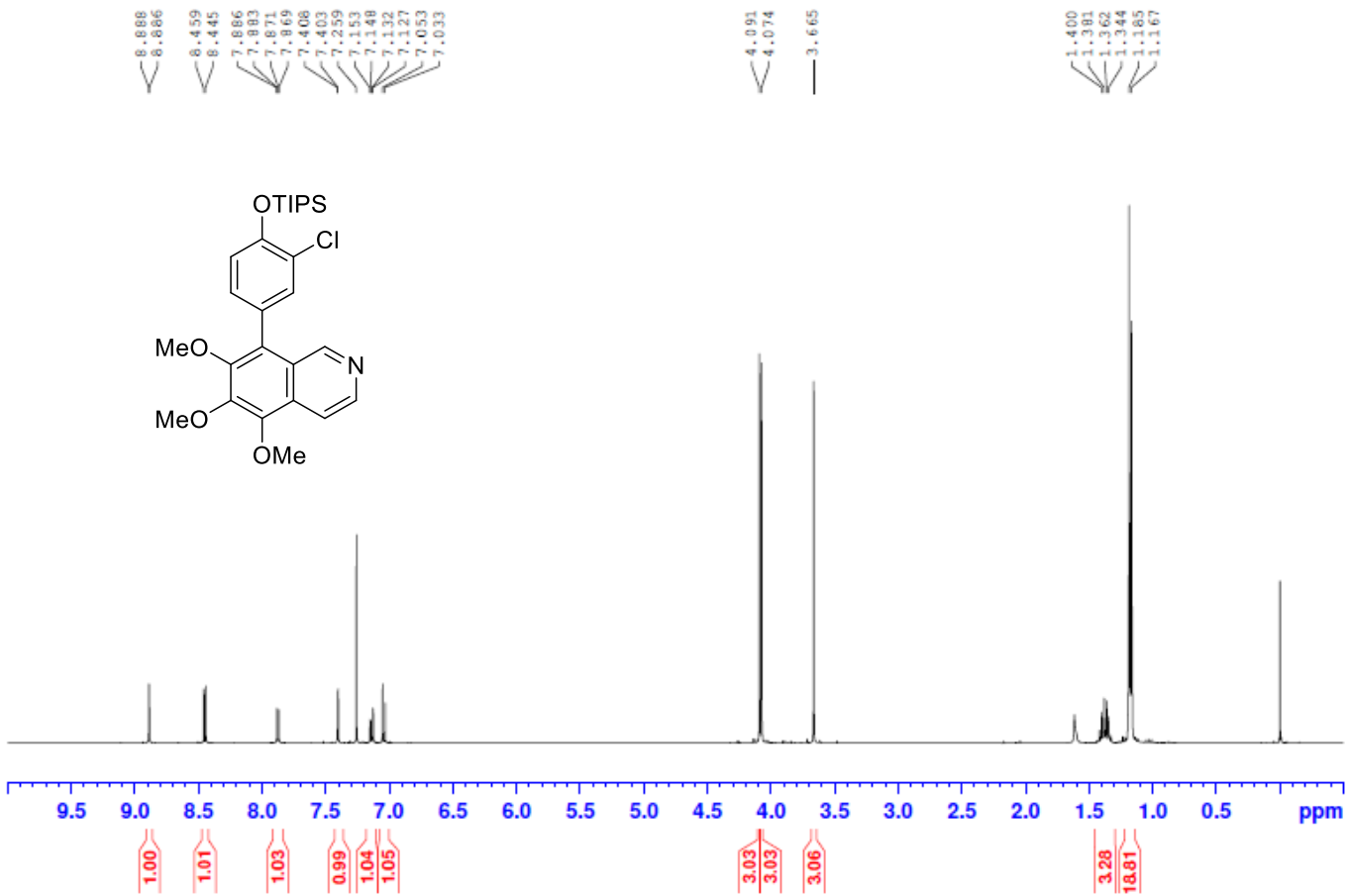
4. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound 5



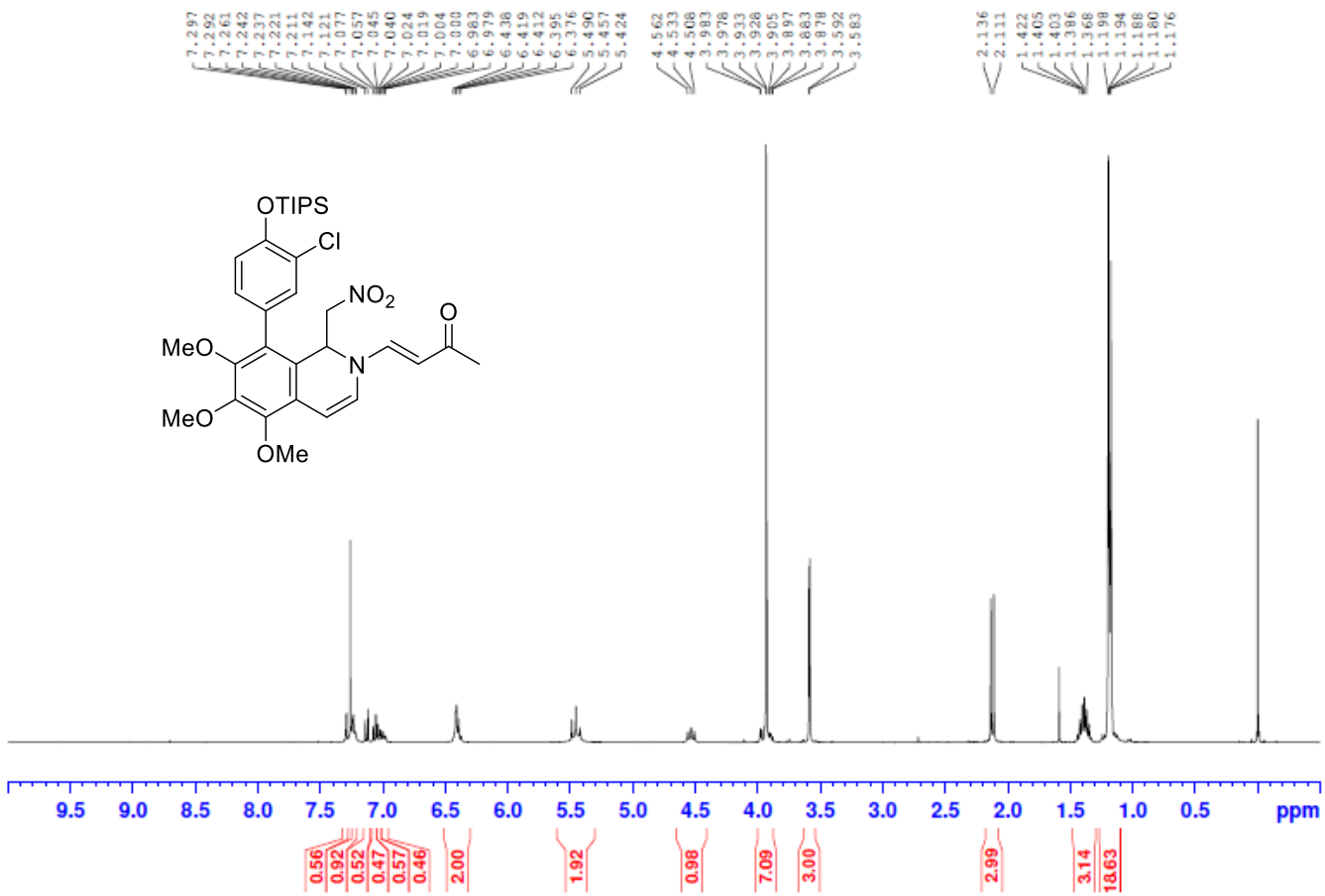
5. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound 6



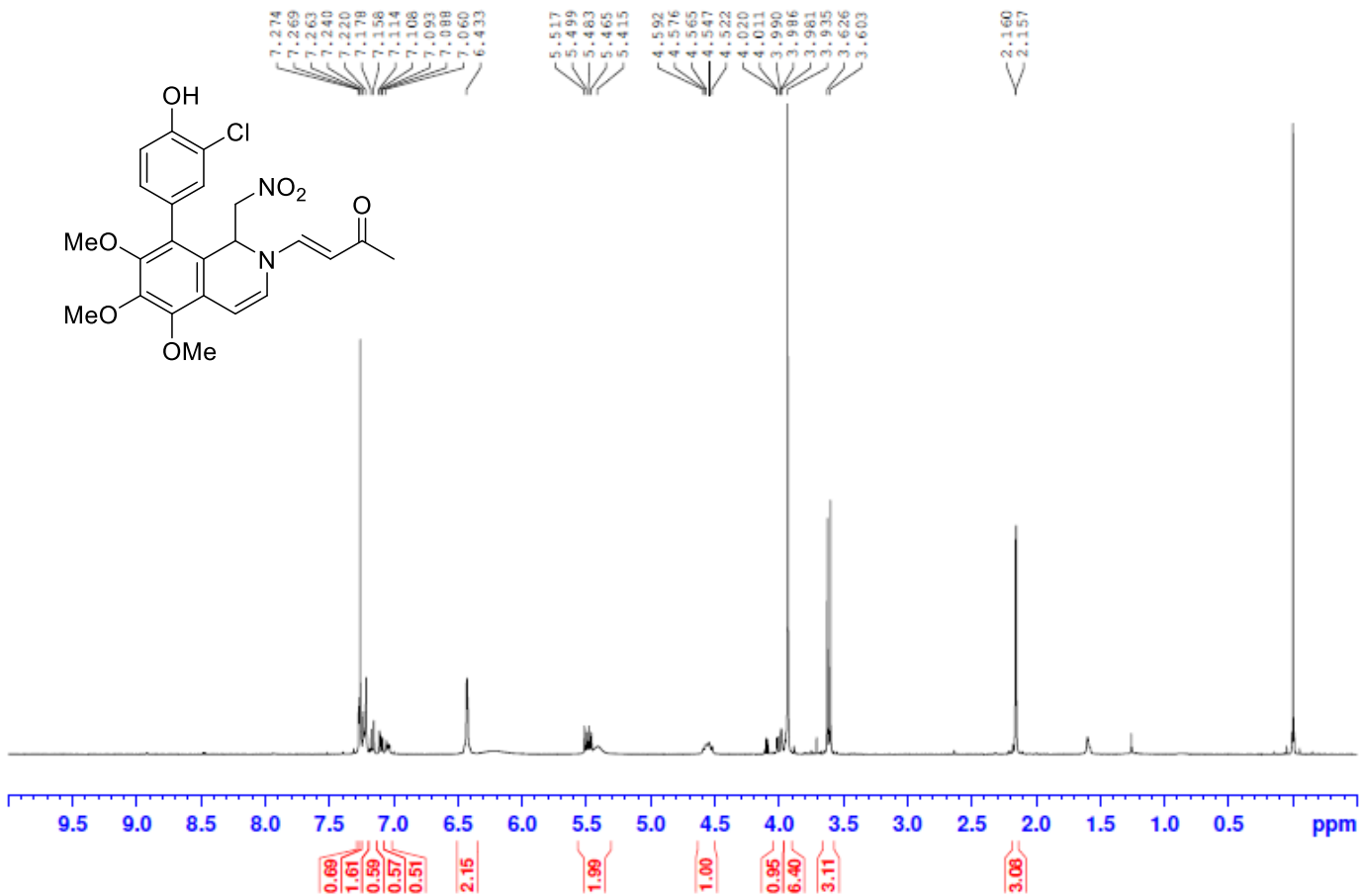
6. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **7**



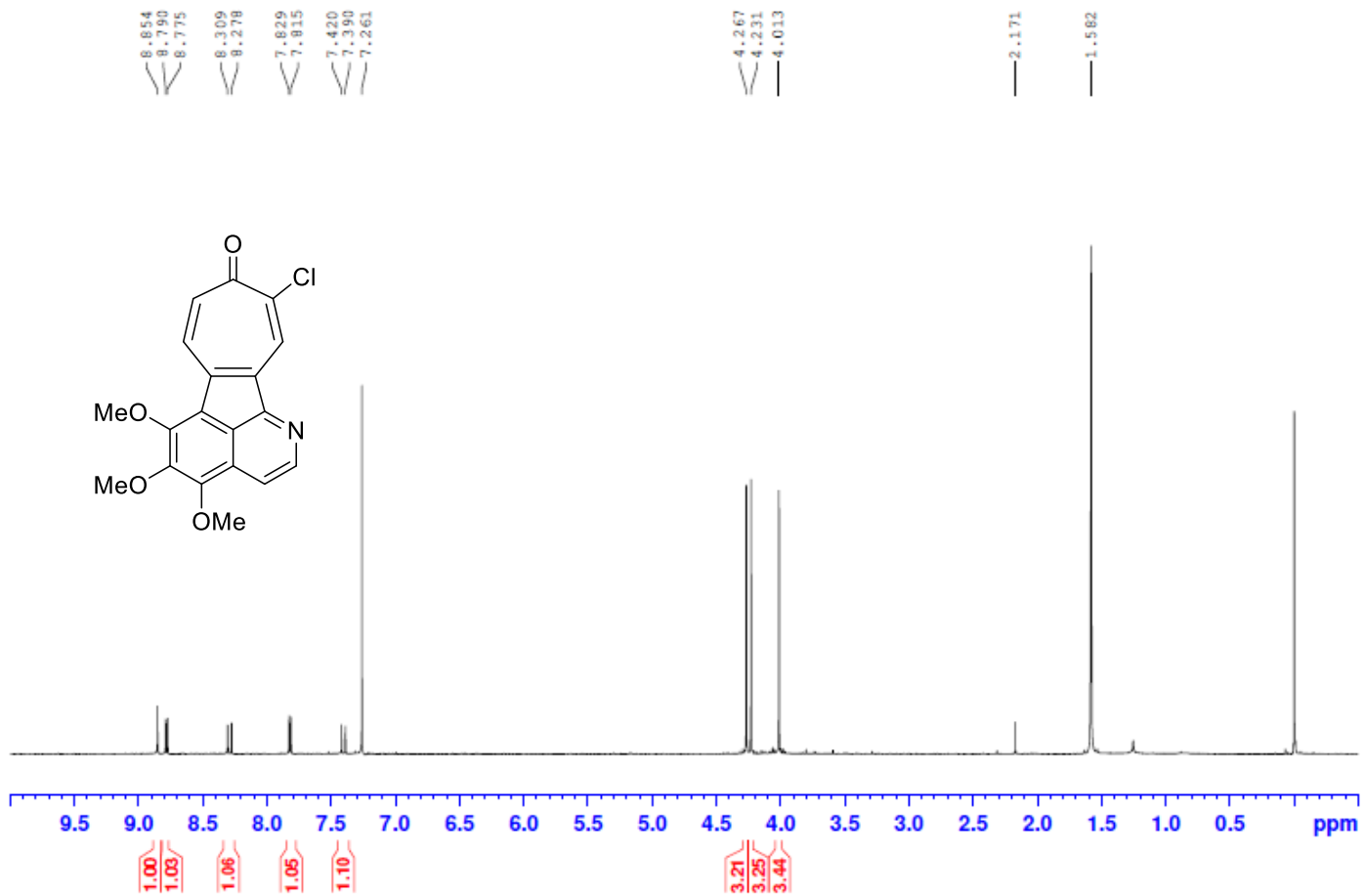
7. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **9**



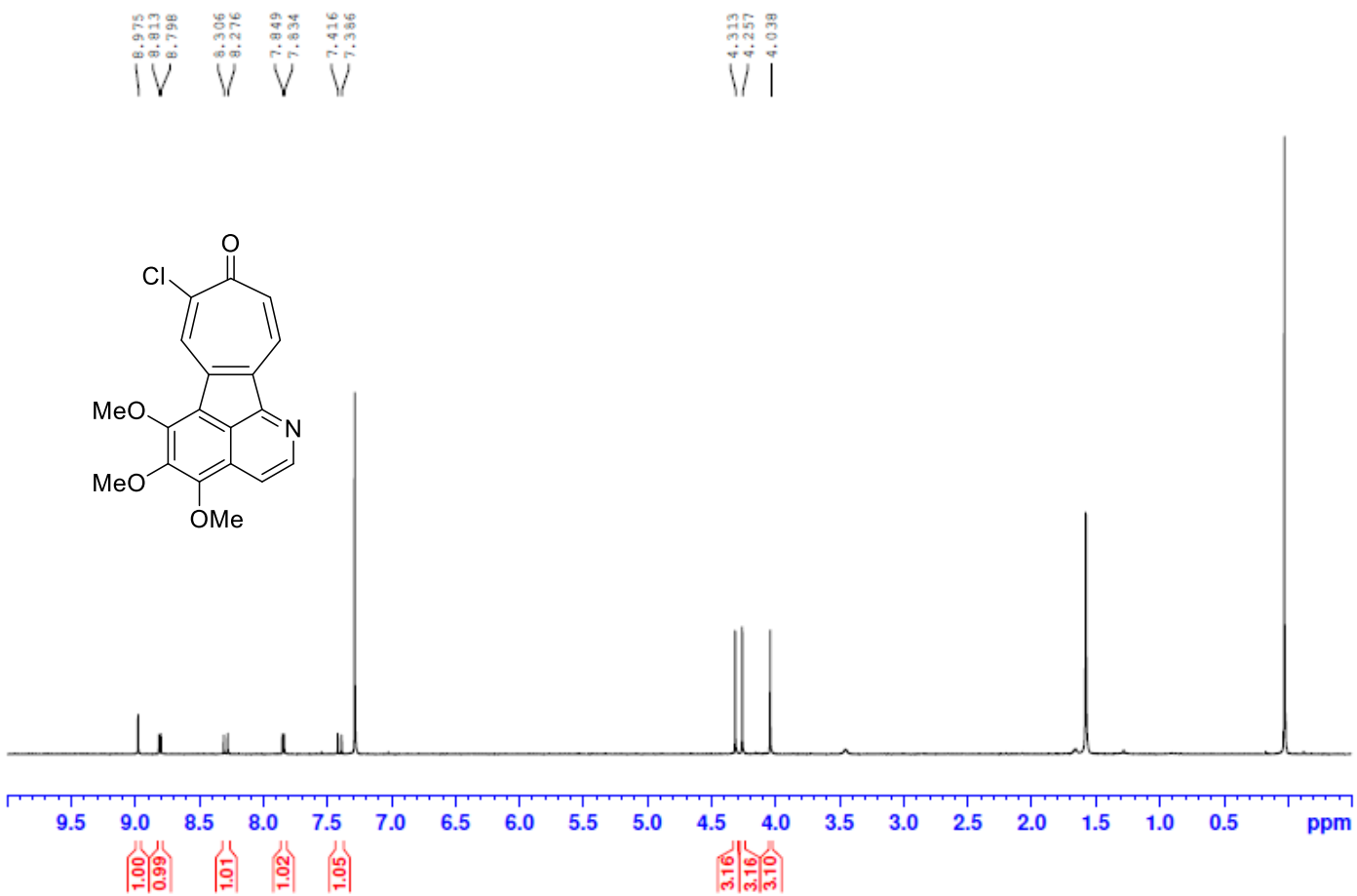
8. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **15**



9. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **16**



10. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **20a**



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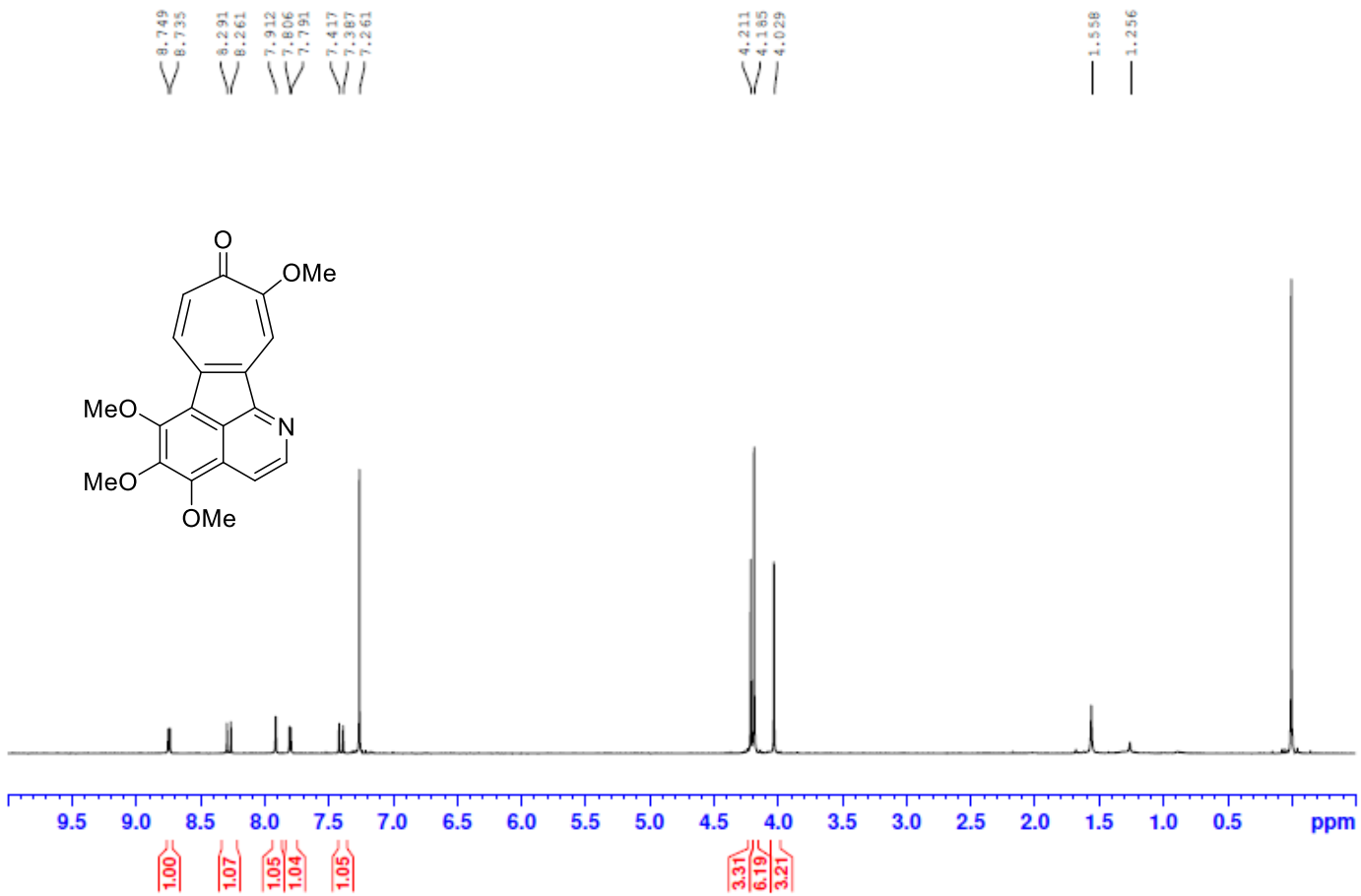
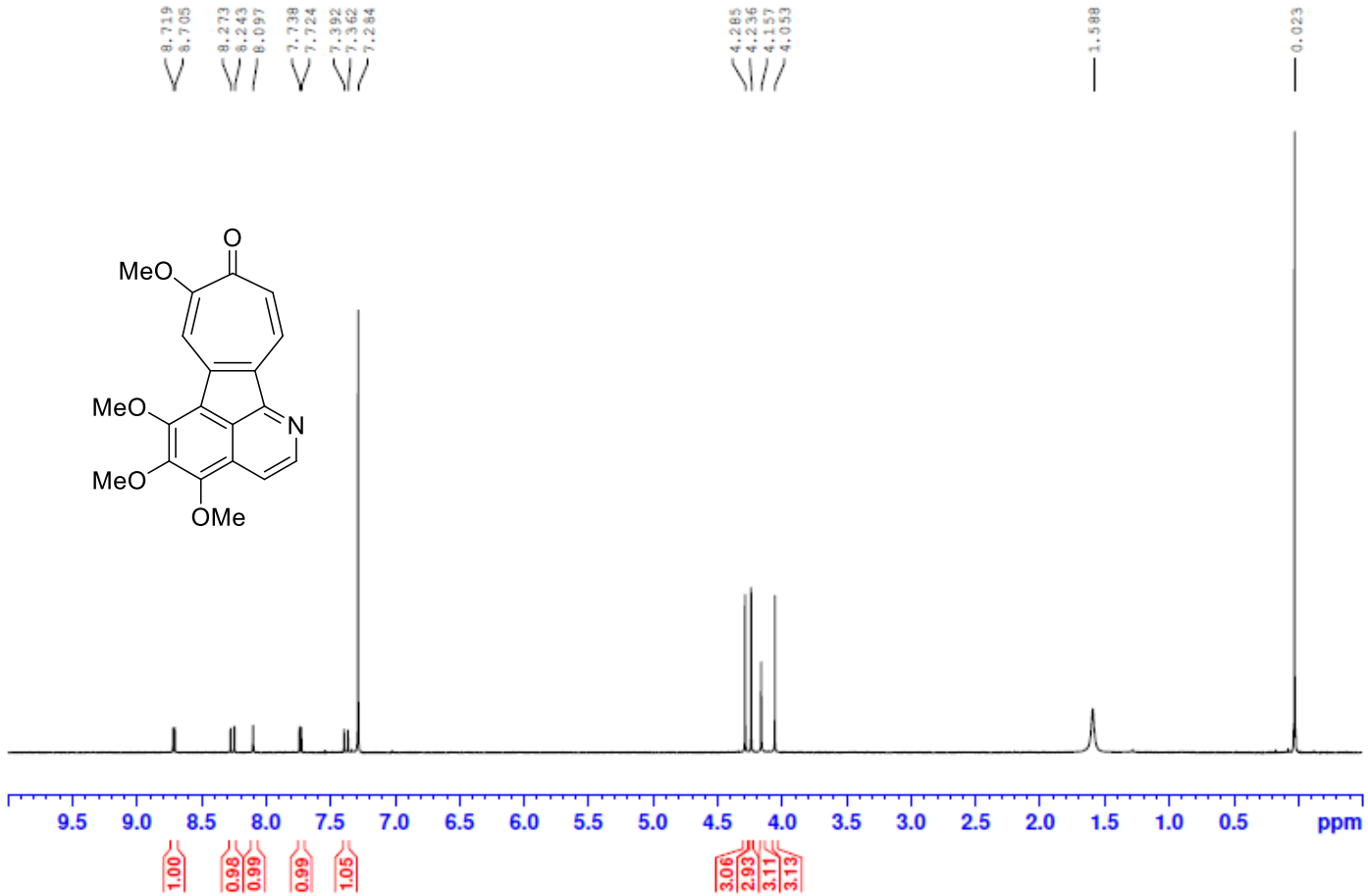
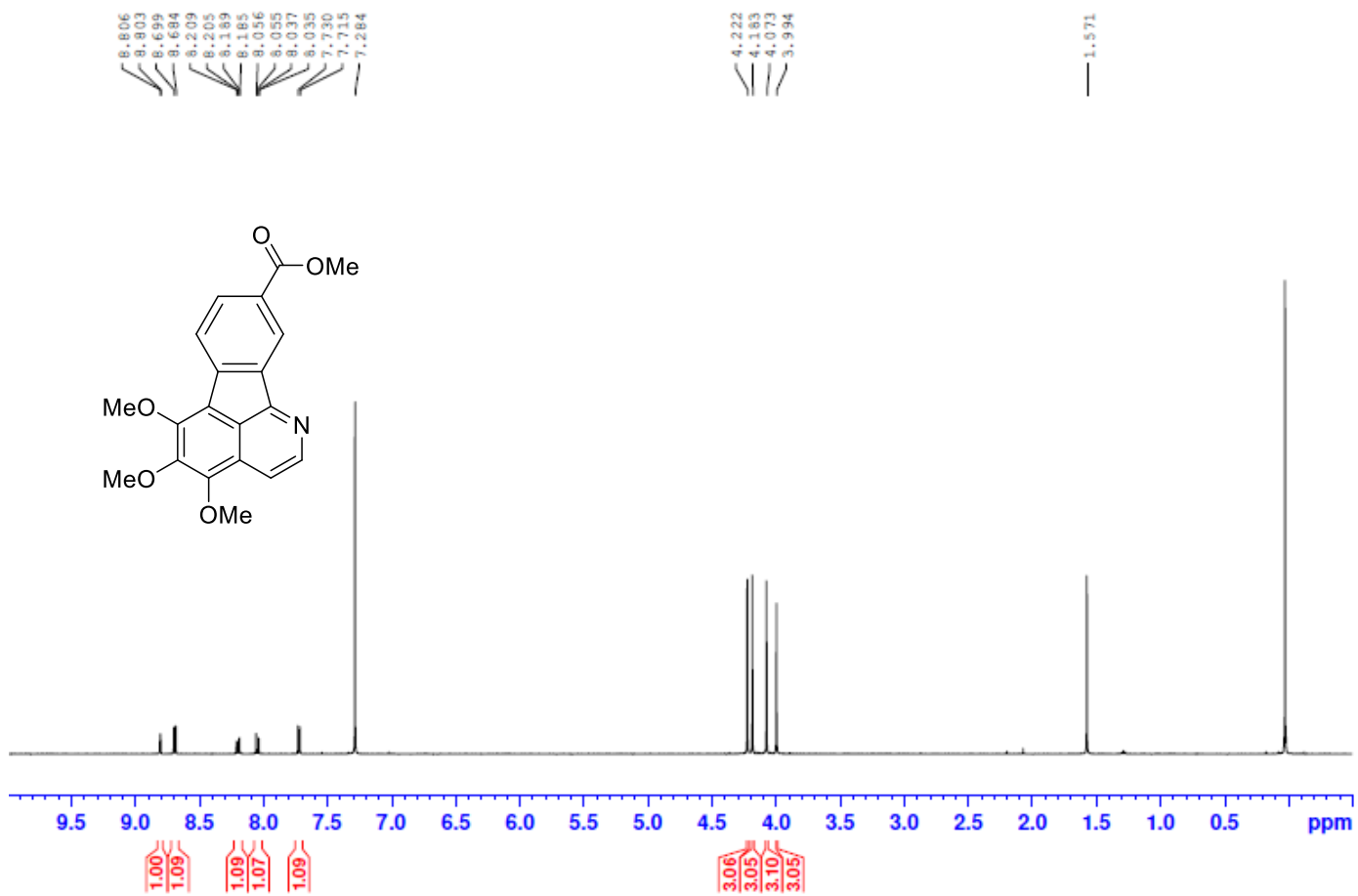


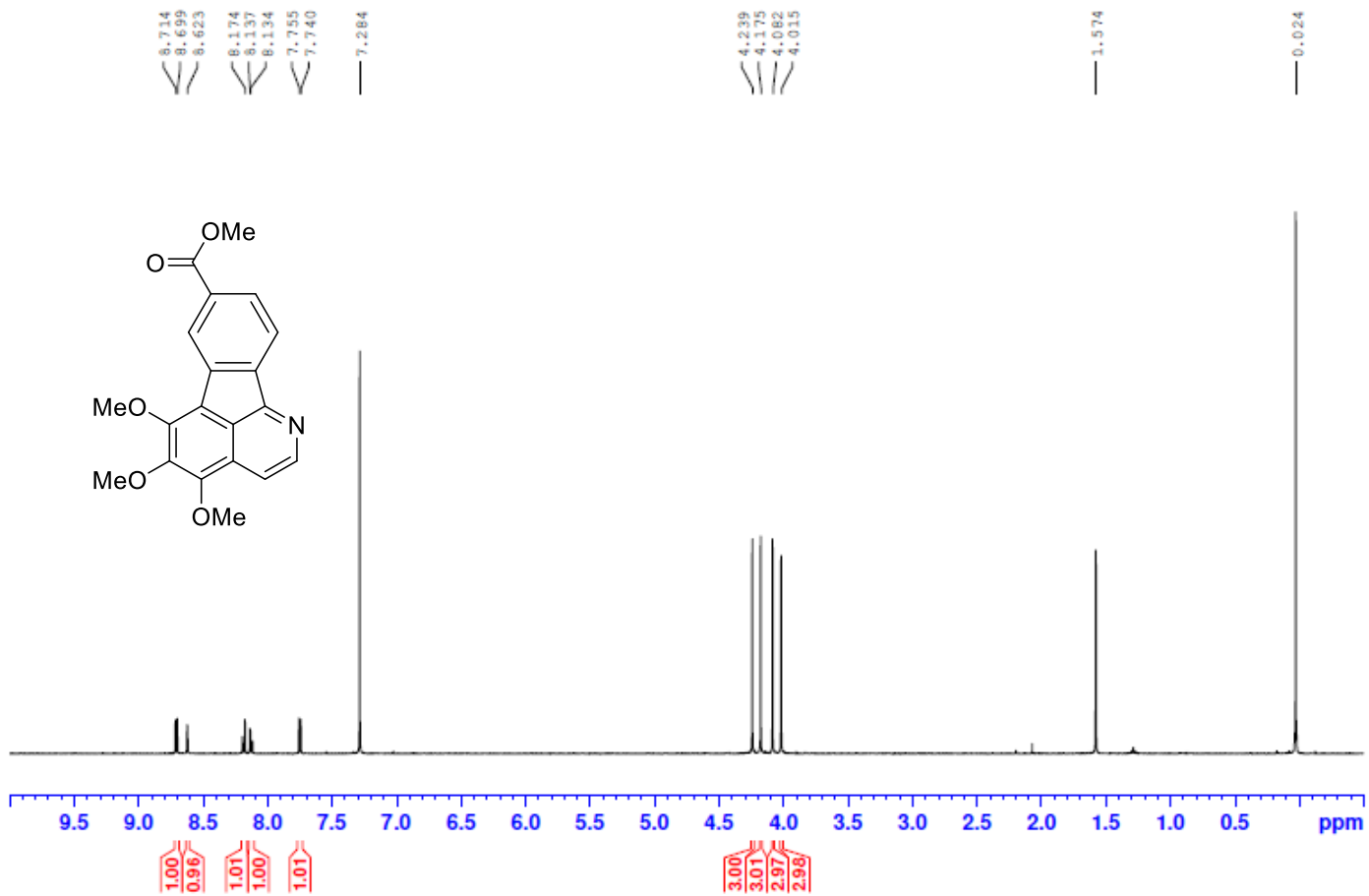
12. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **21a**



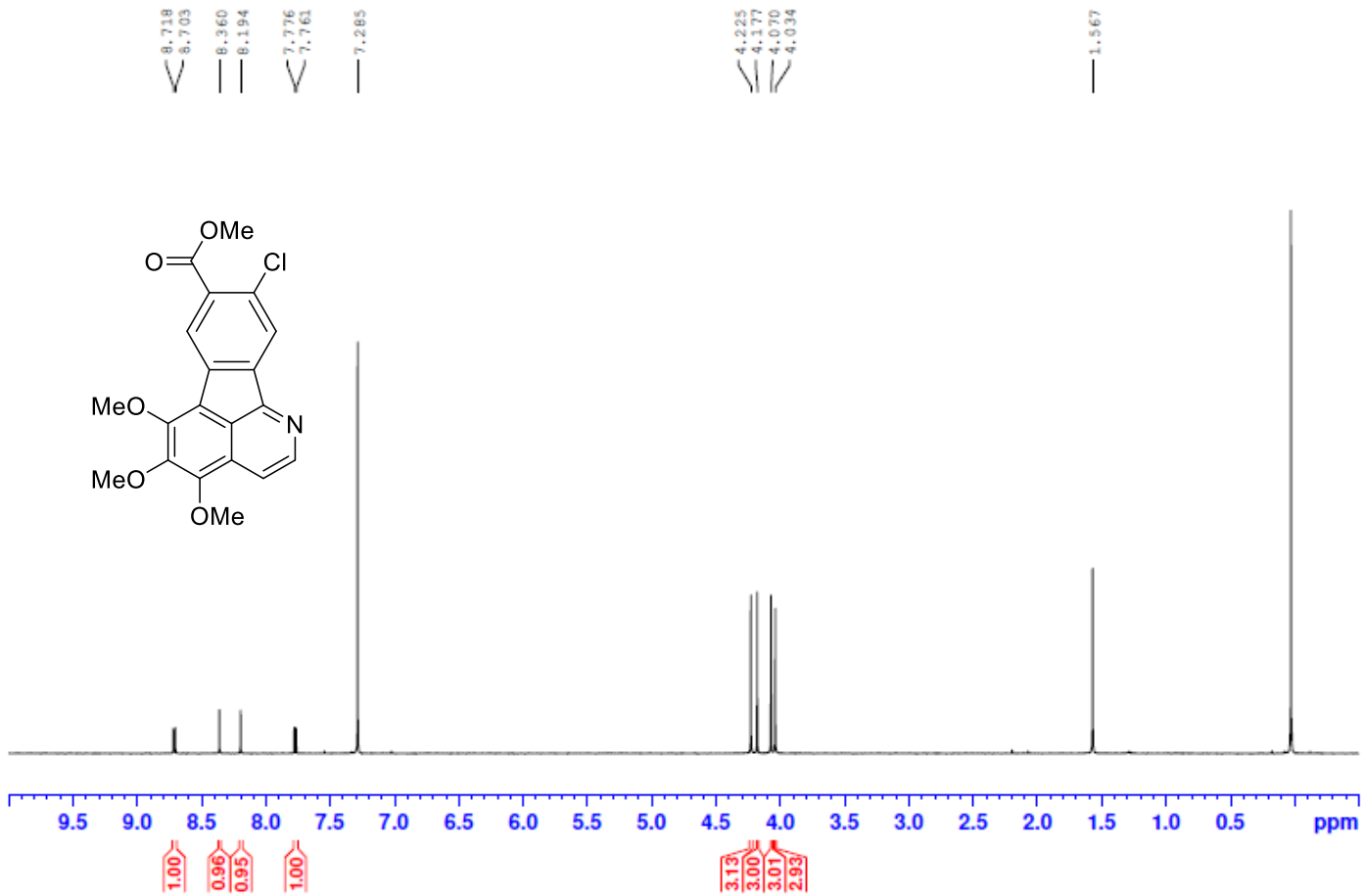
13. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **21b**



14. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **22a**



15. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **22b**



16. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **23a**

List of ^{13}C NMR Spectra of Selected Compounds

1. 100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **2**……68

2. 100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **3**……69

3. 100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **4**……70

4. 100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **5**……71

5. 100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **6**……72

6. 100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **7**……73

7. 100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **9**……74

8. 100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **15**……75

9. 100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **16**……76

10. 100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **20a**……77

11. 100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **20b**……78

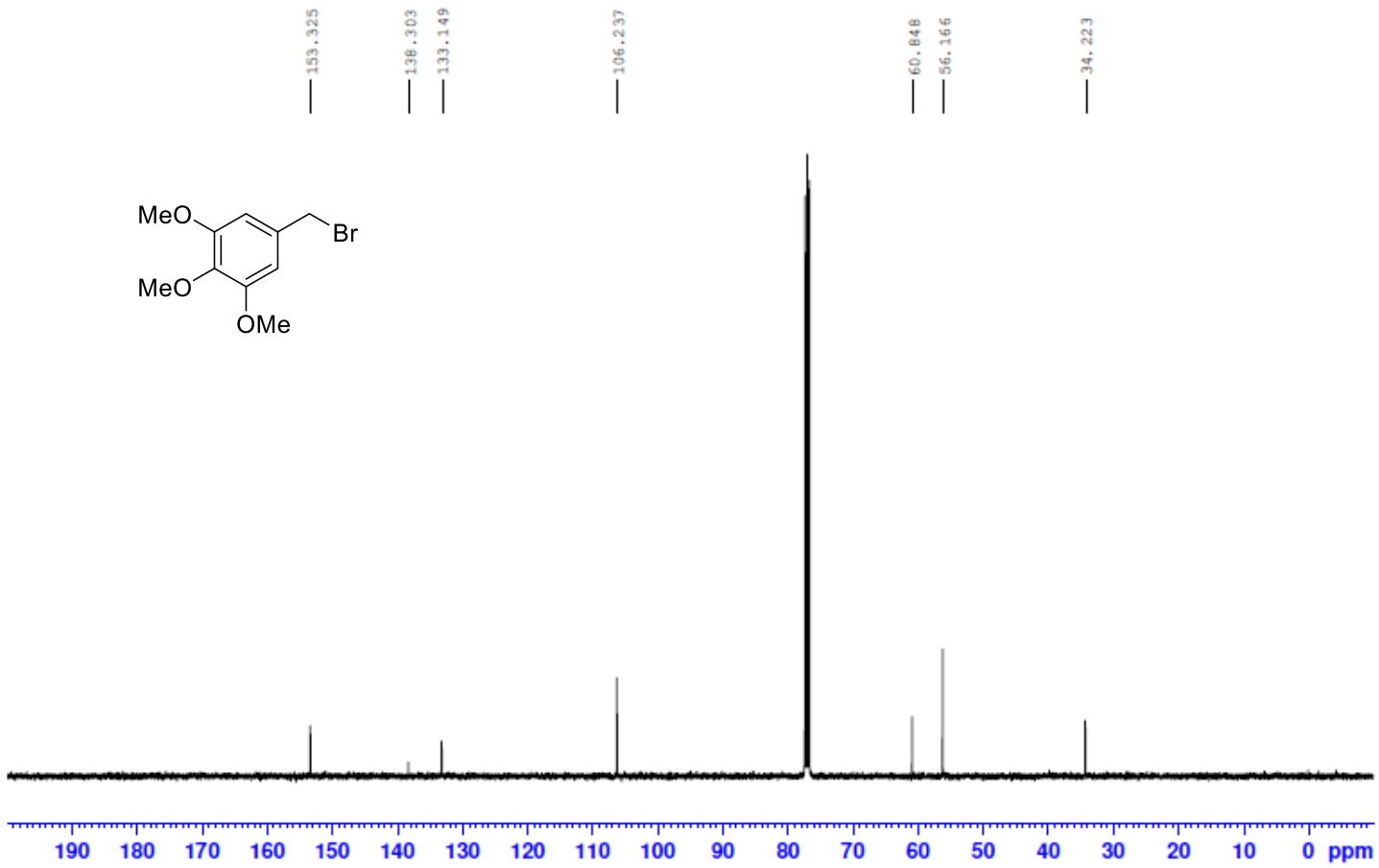
12. 100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **21a**……79

13. 100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **21b**……80

14. 100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **22a**……81

15. 100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **22b**……82

16. 100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **23a**……83



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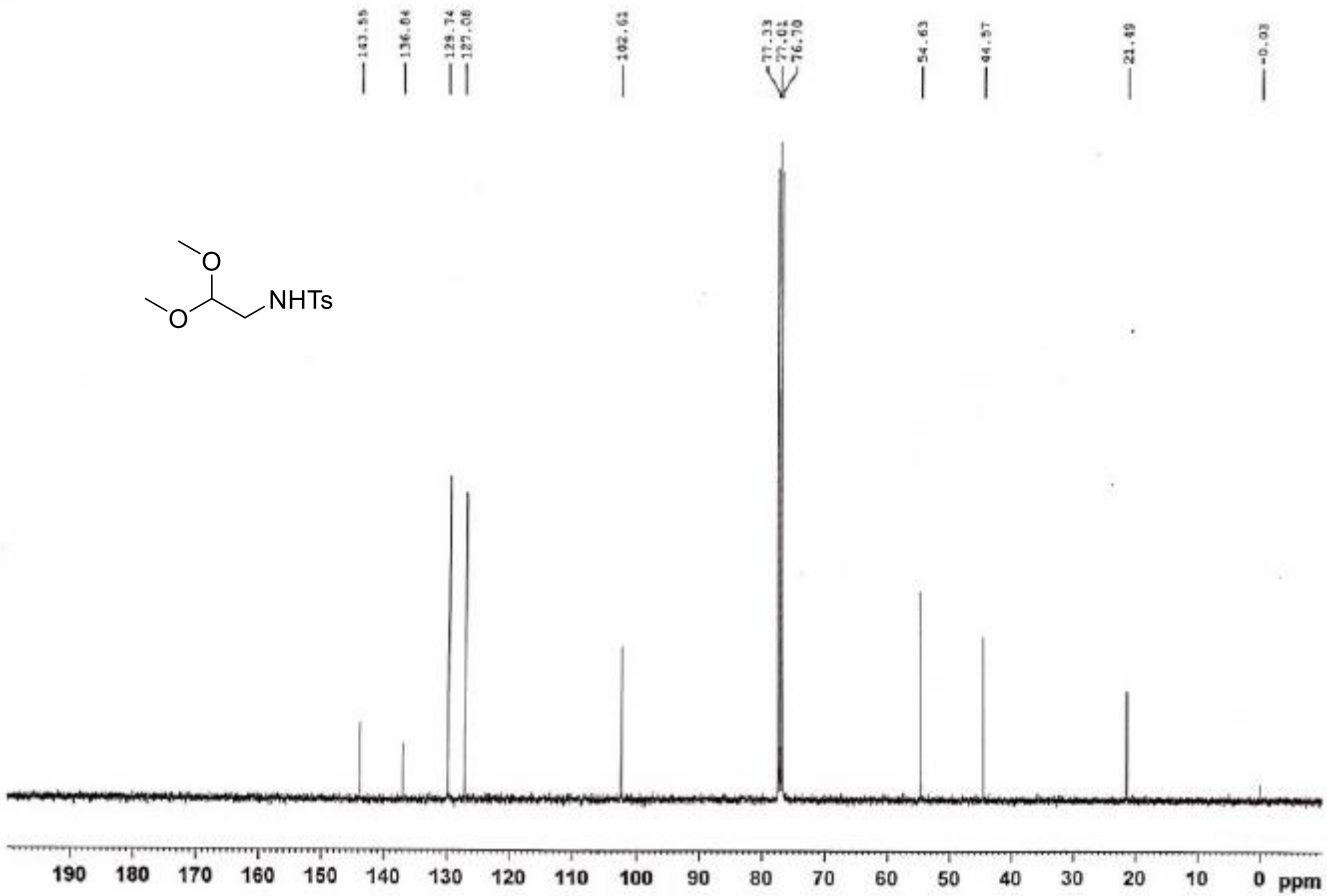
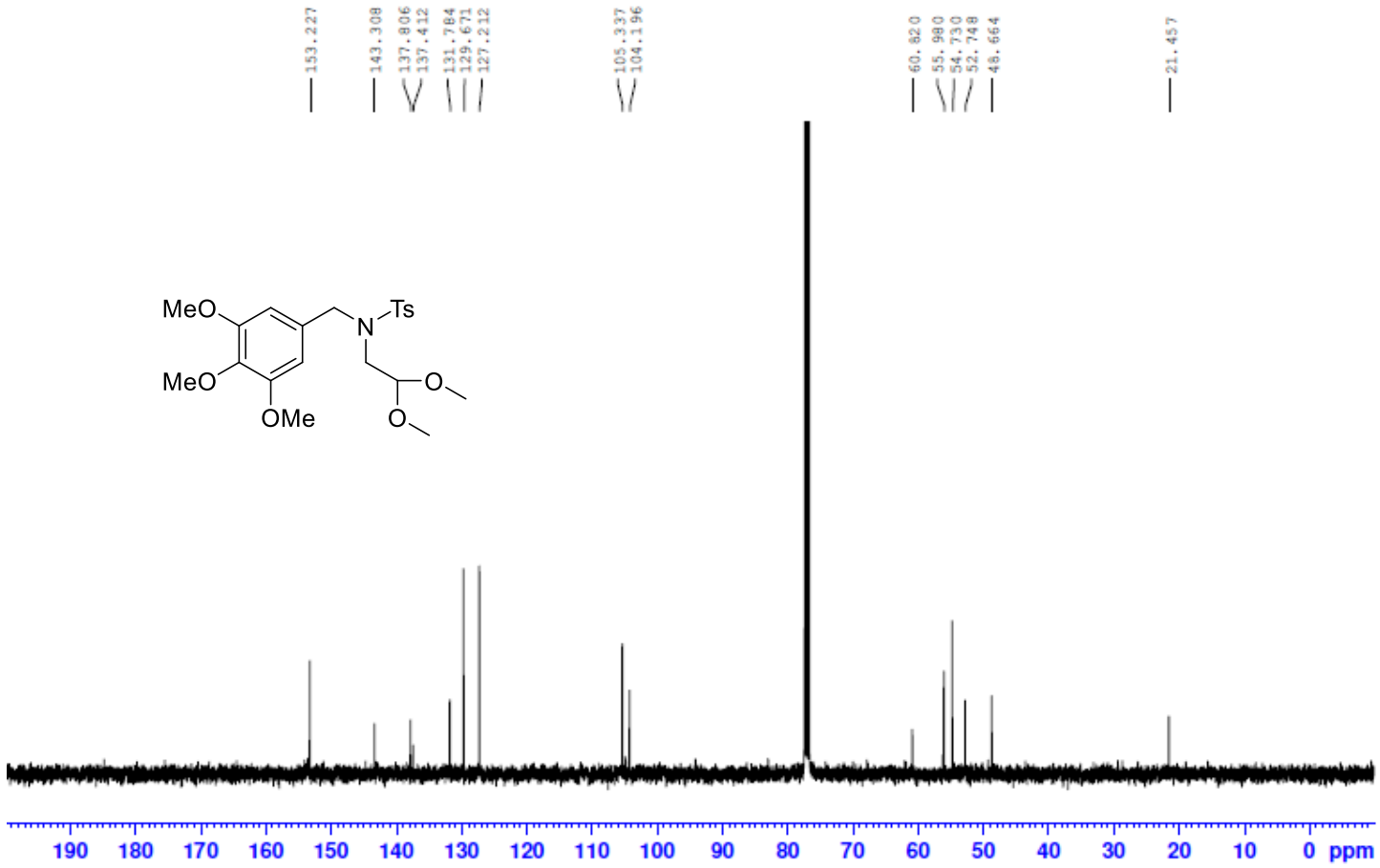
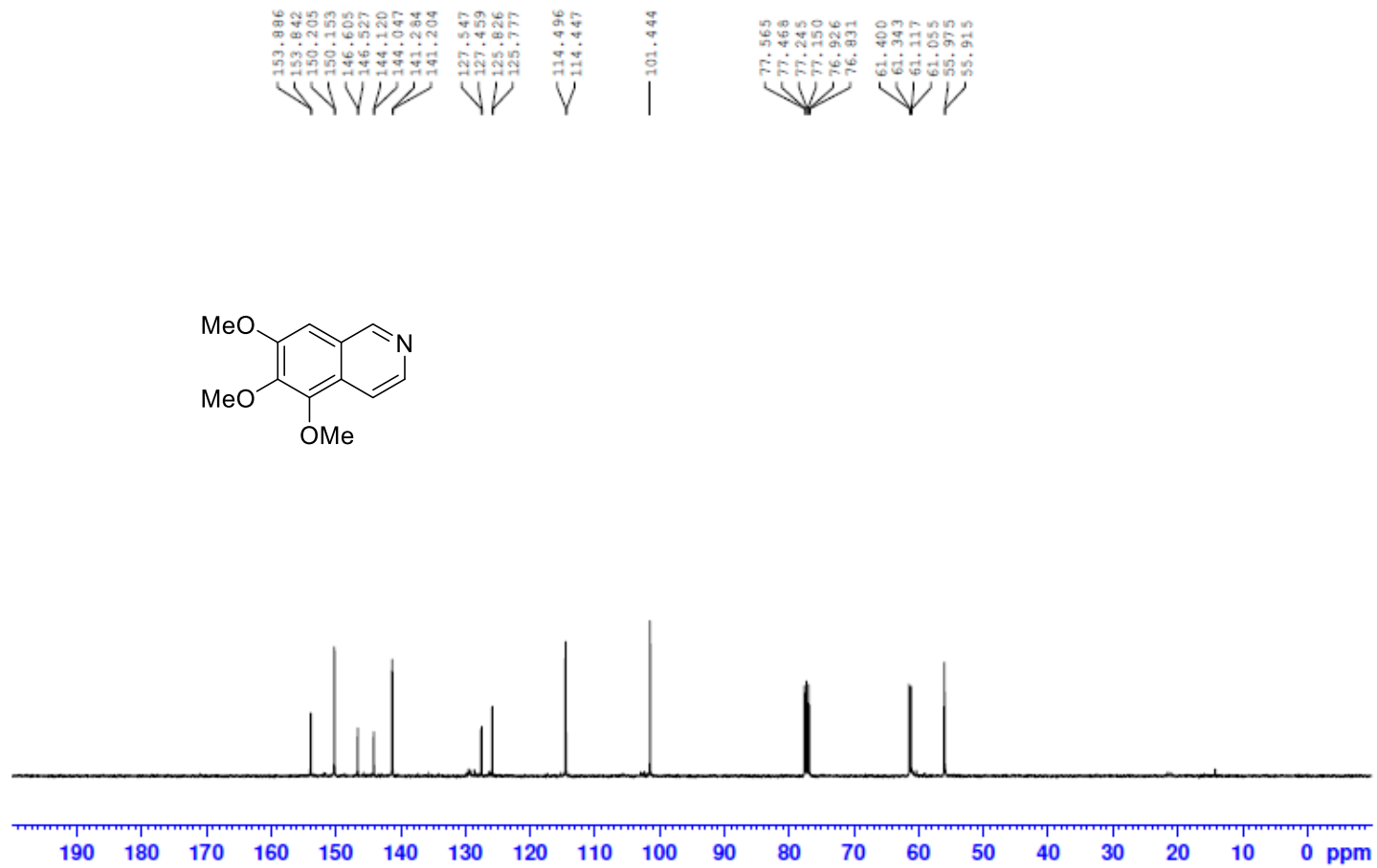


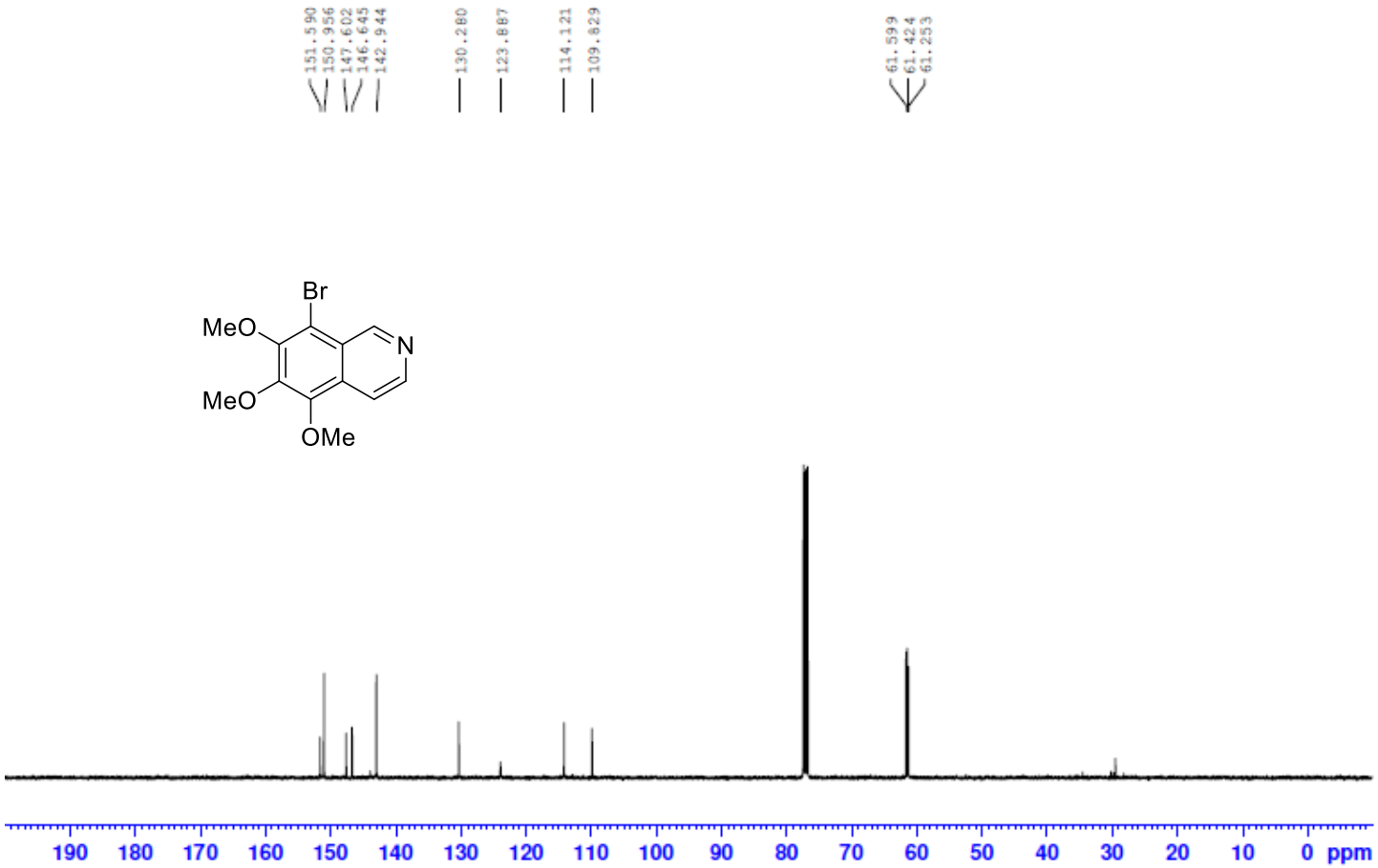
100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **3**



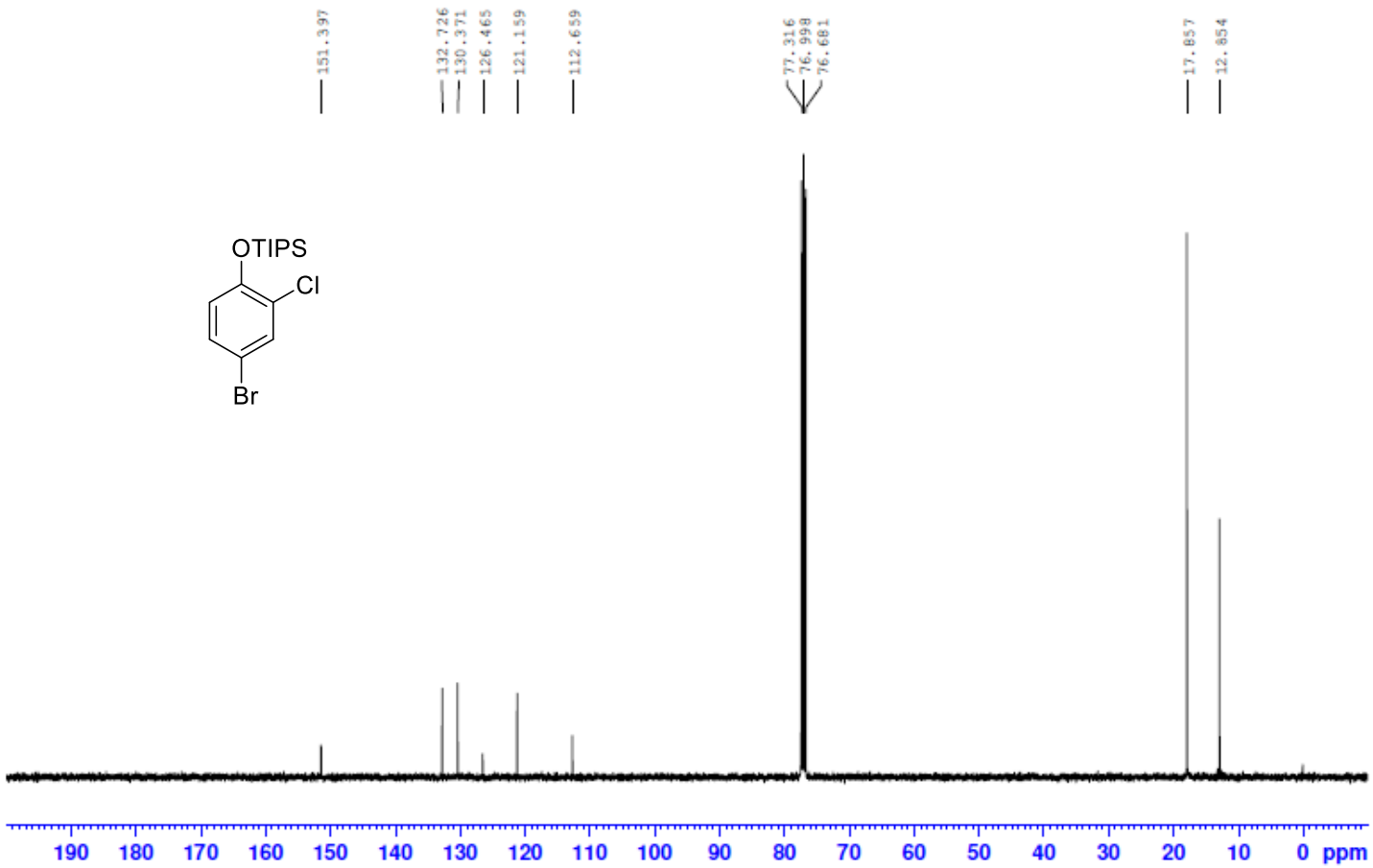
100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **4**



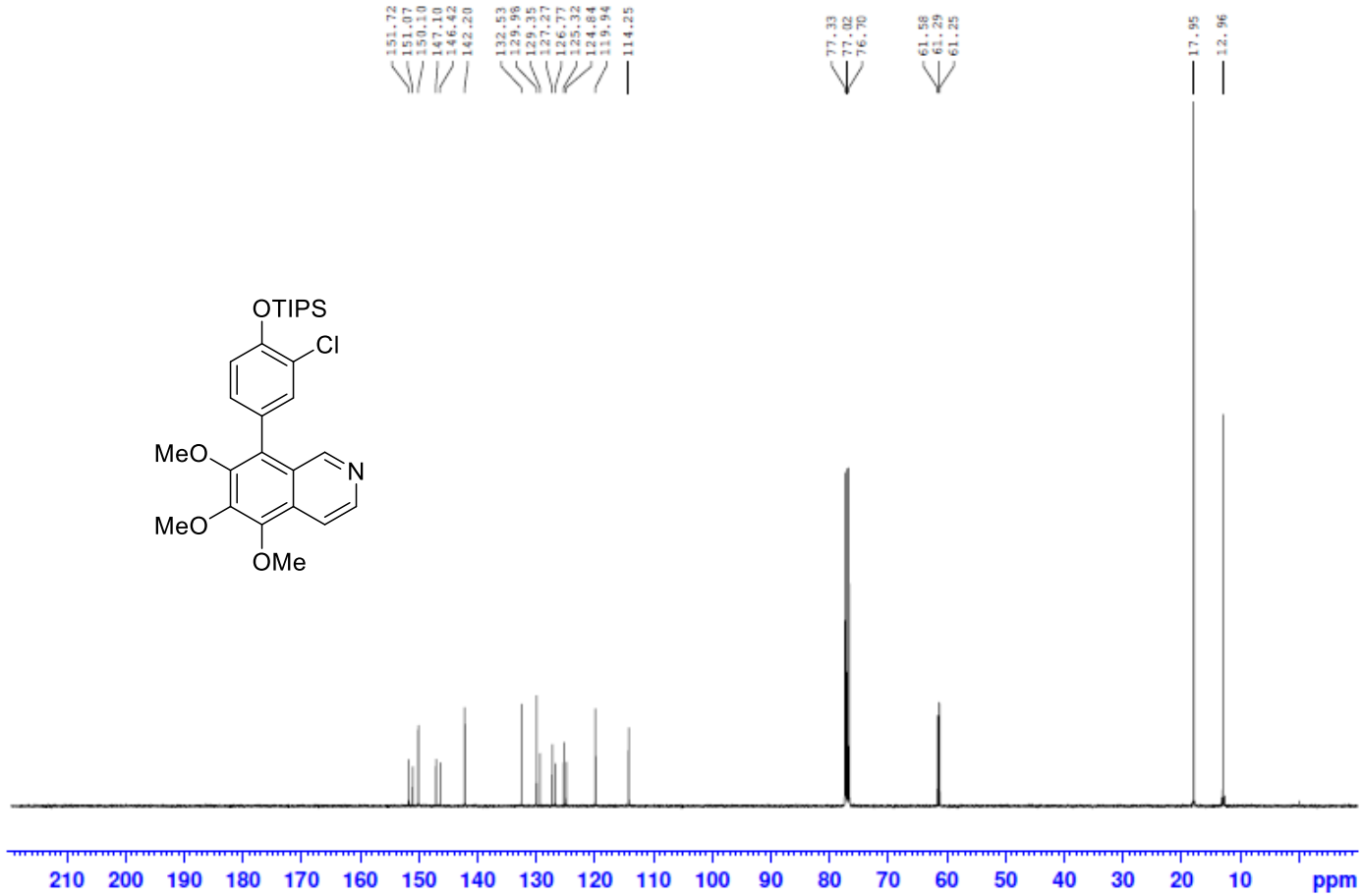
100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **5**



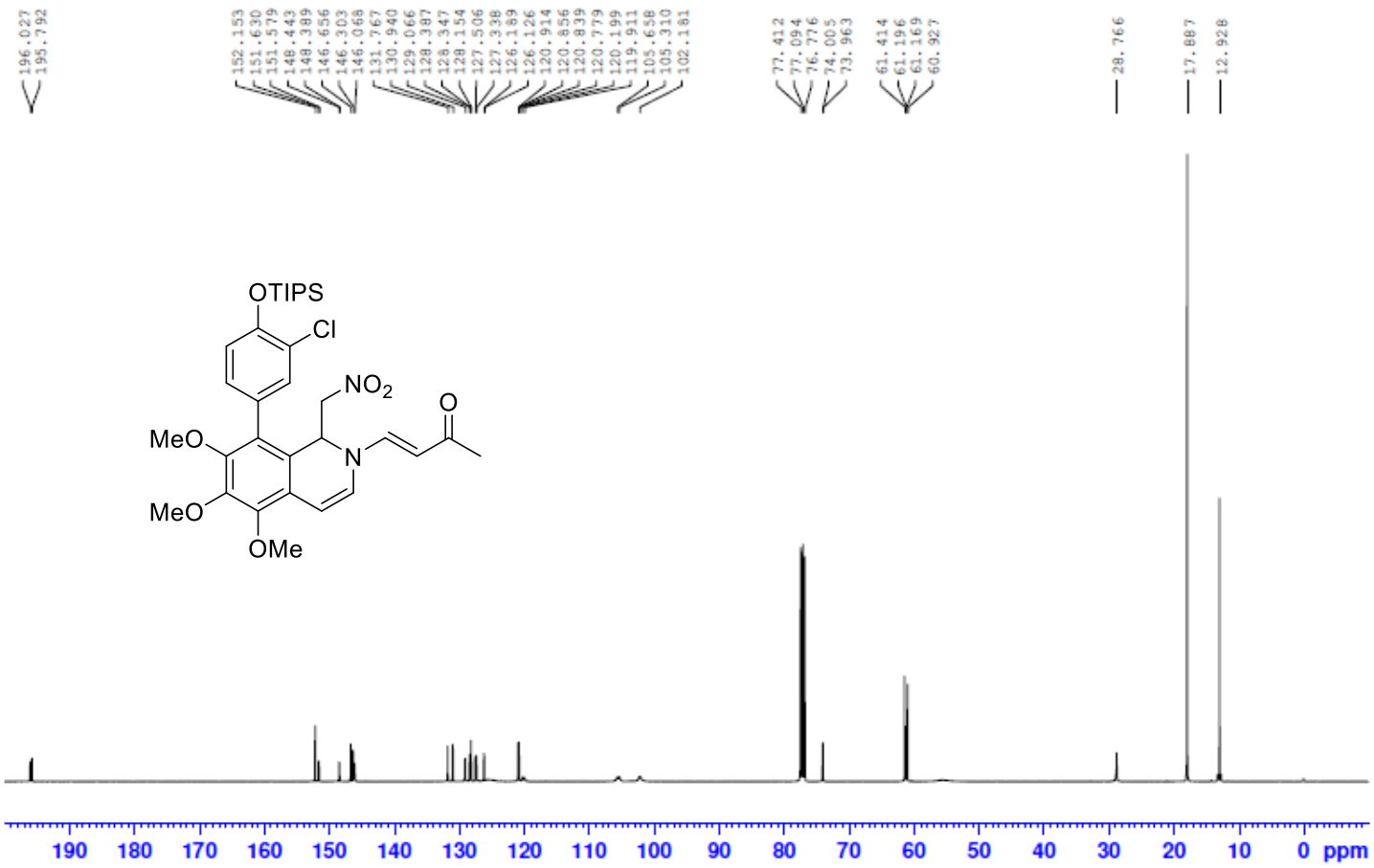
100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound 6



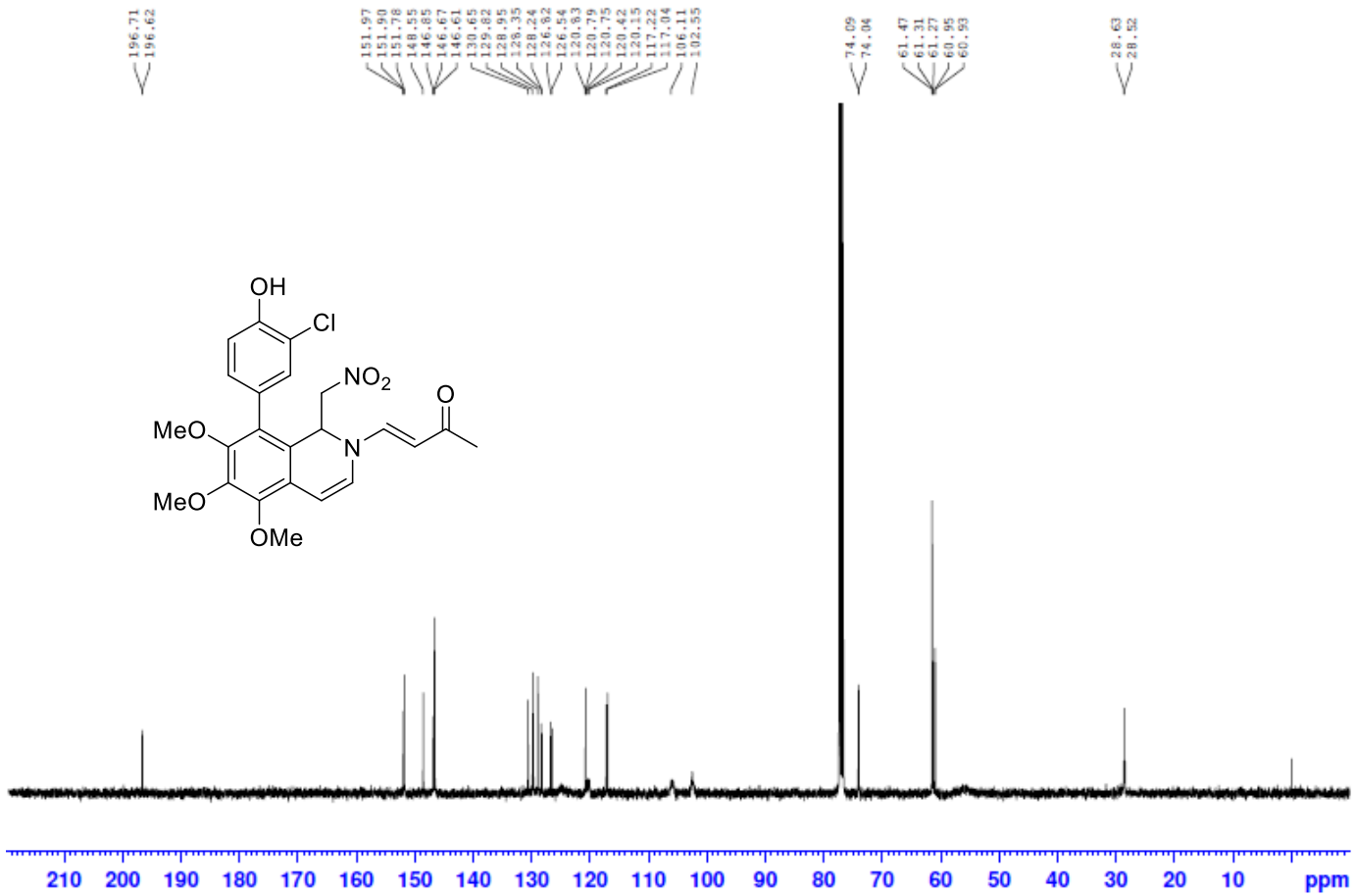
100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **7**



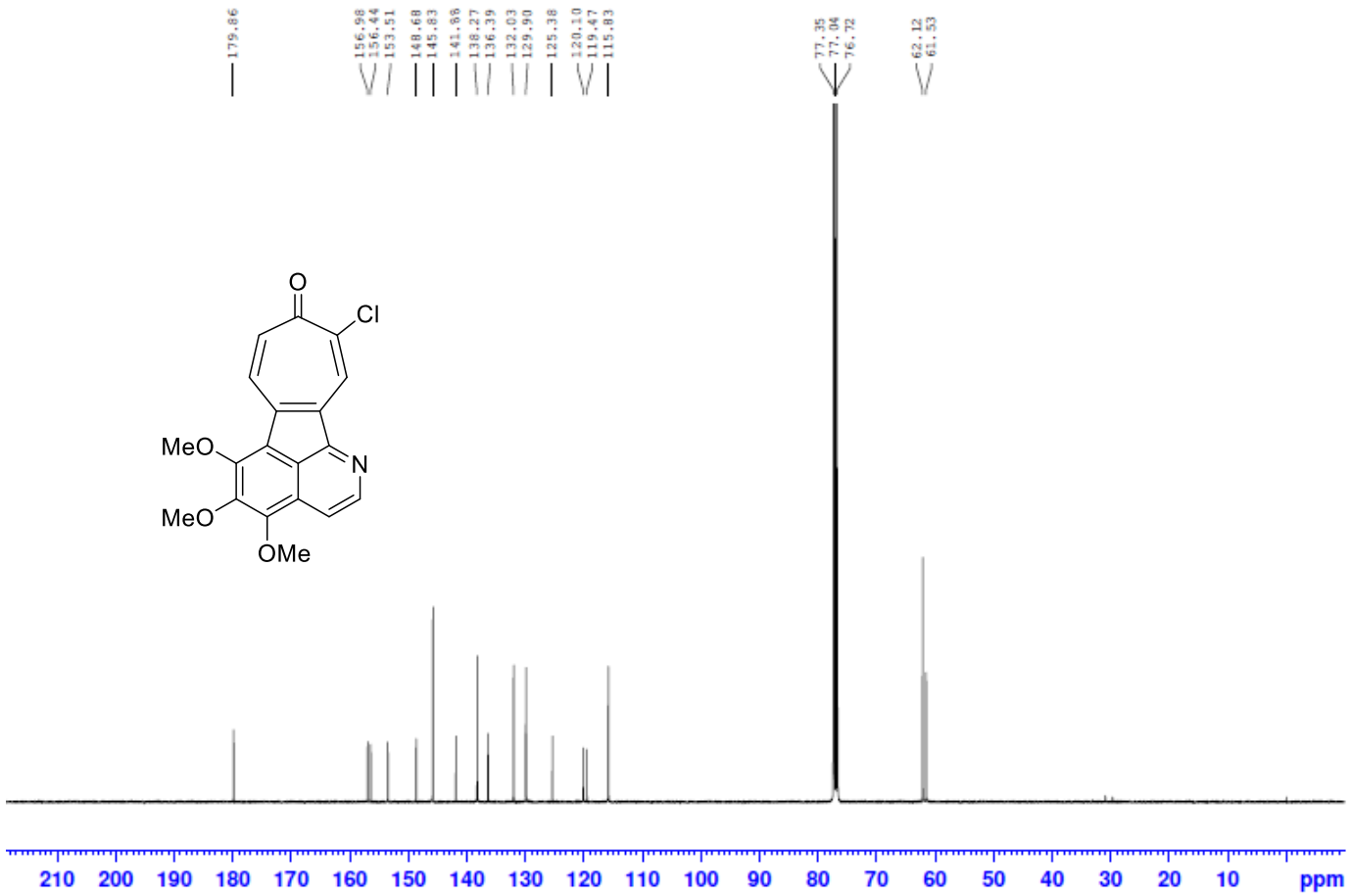
100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **9**



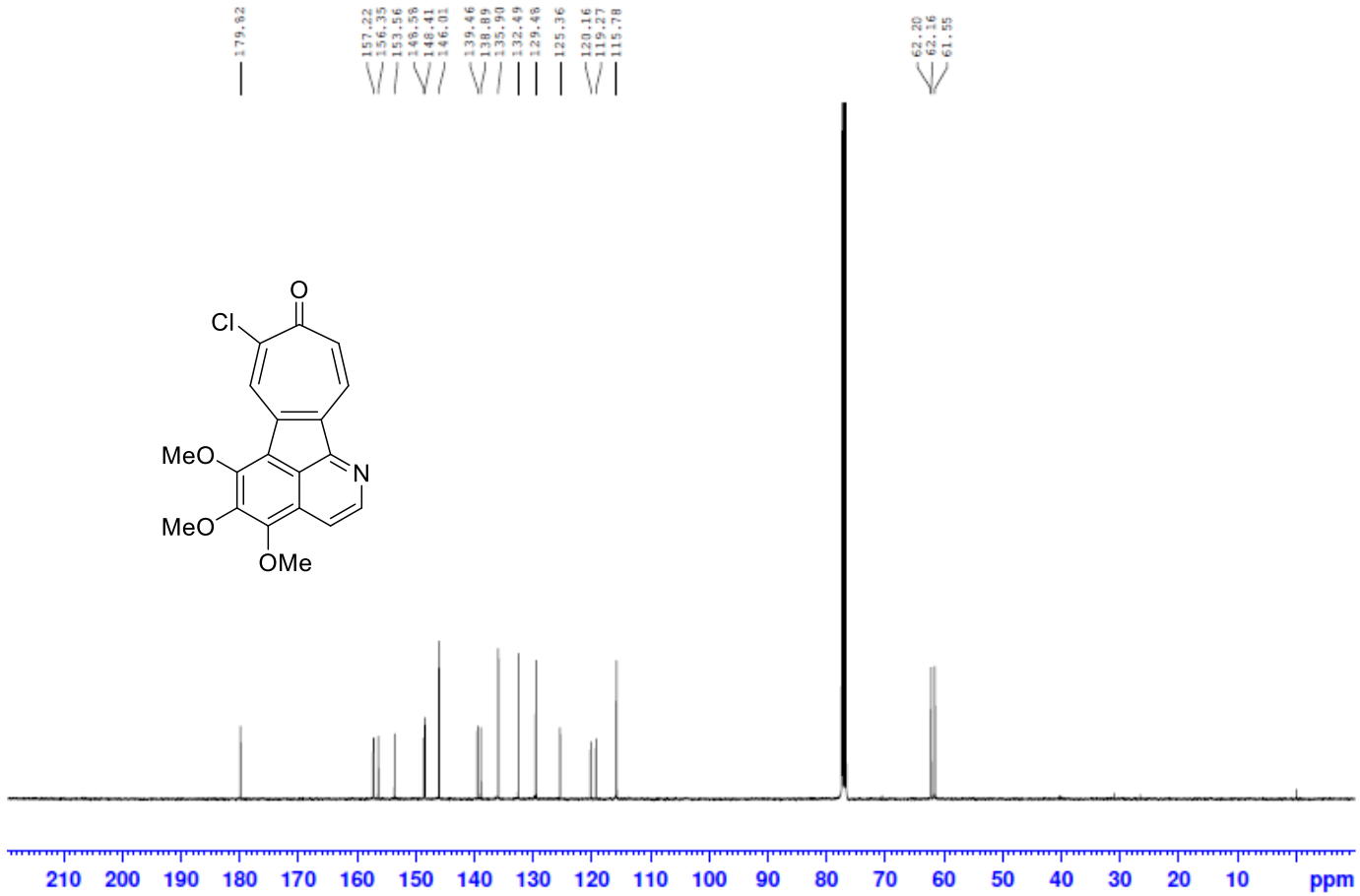
100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **15**



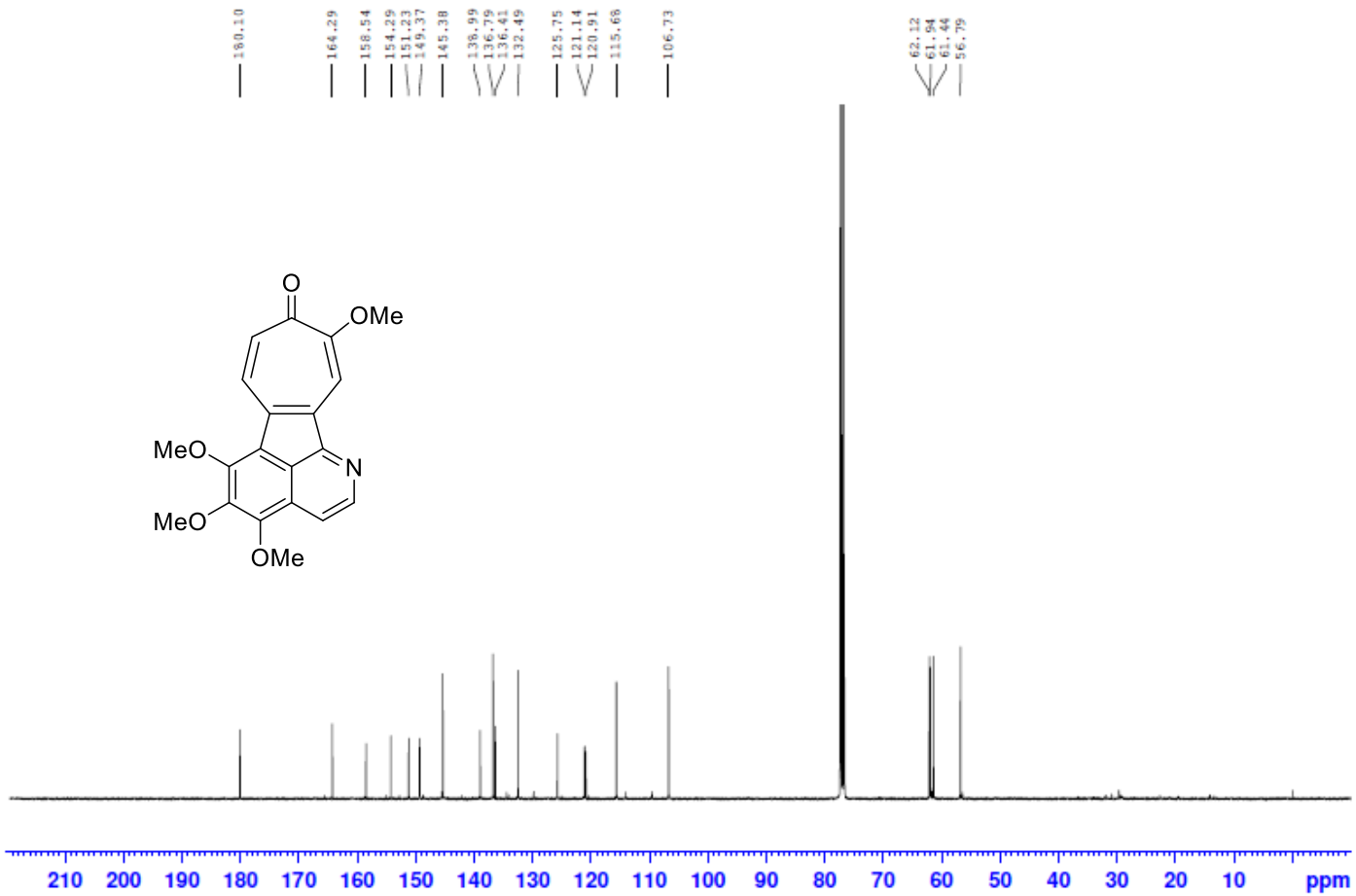
100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **16**



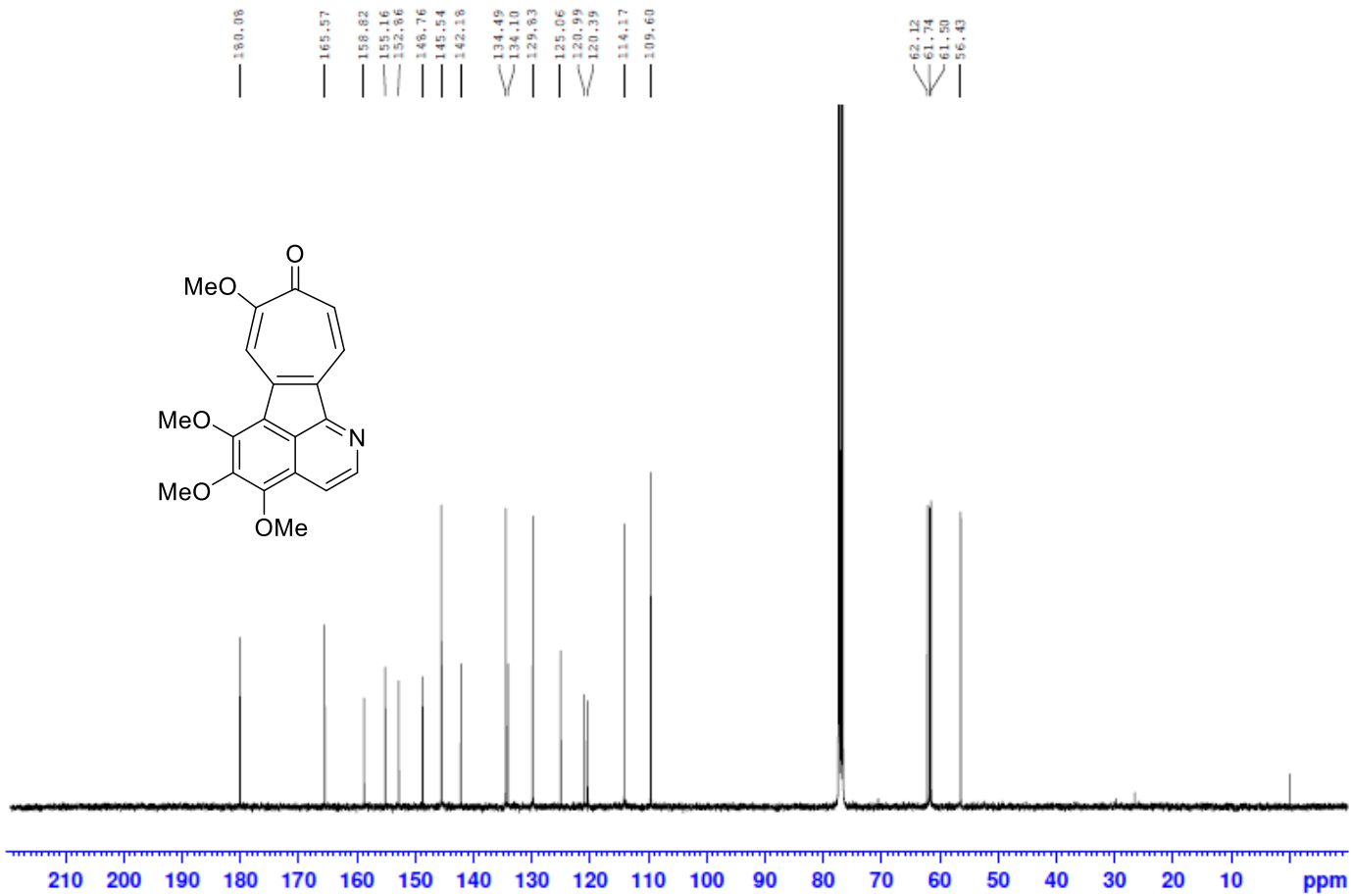
100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **20a**



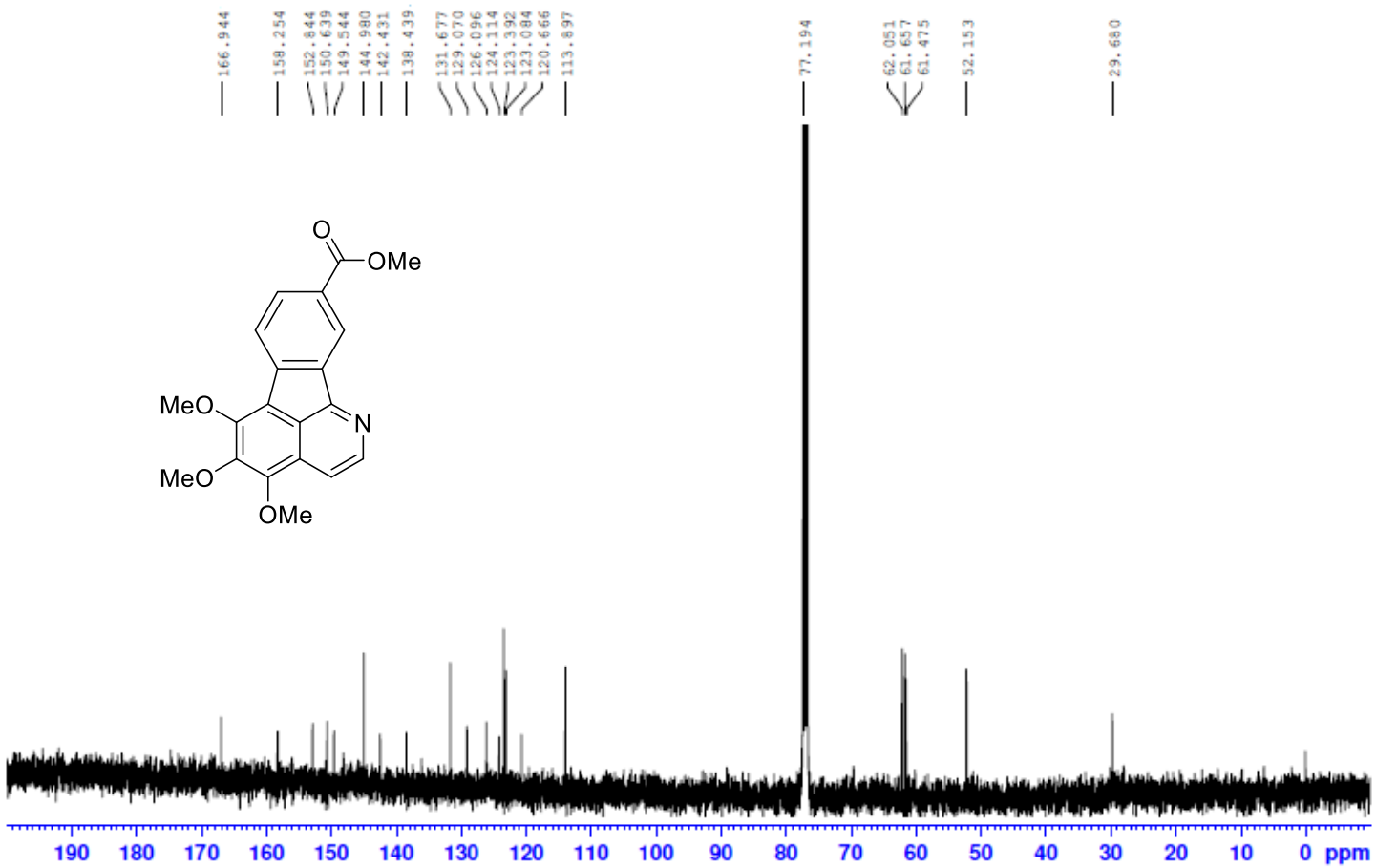
100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **20b**



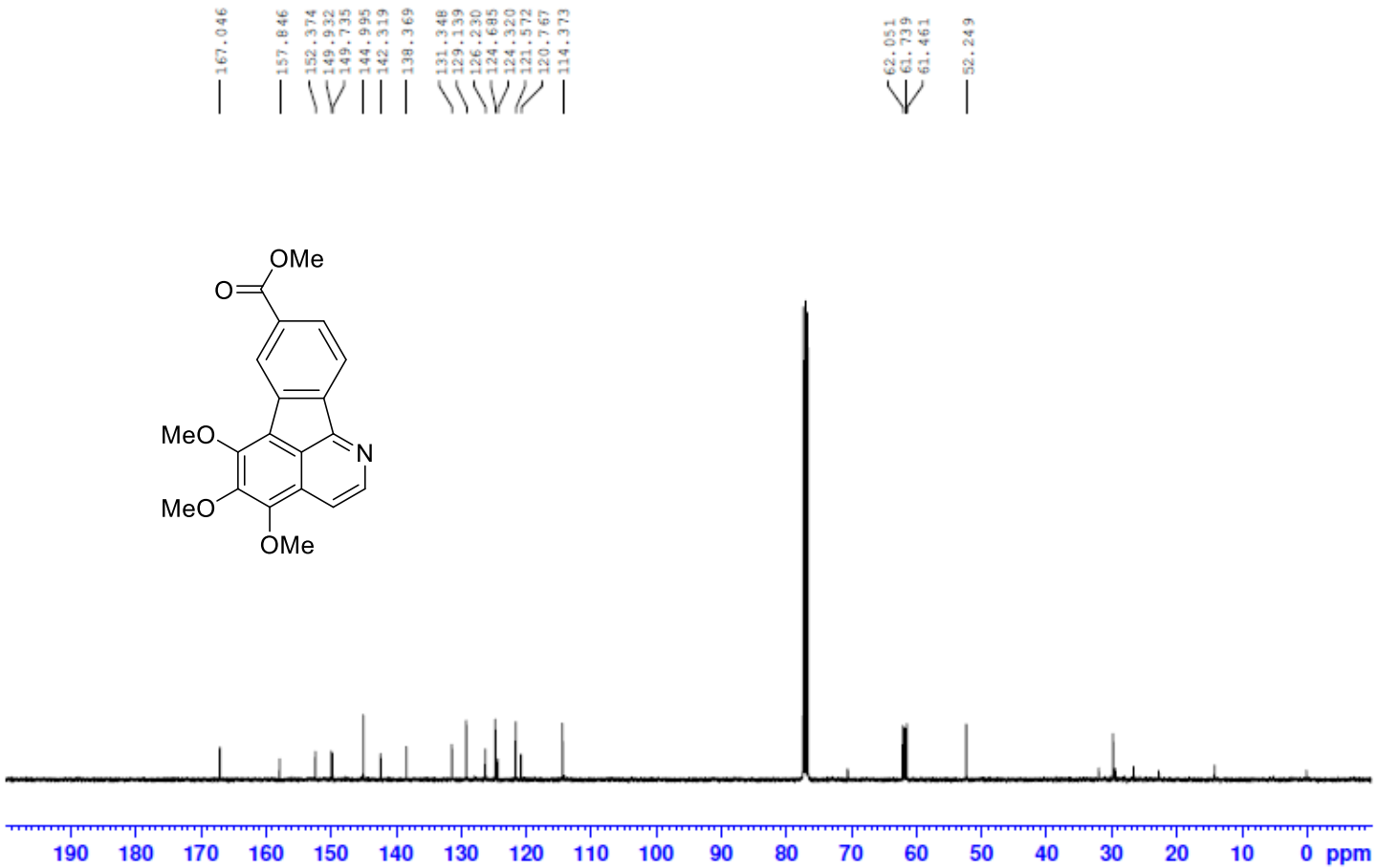
100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **21a**



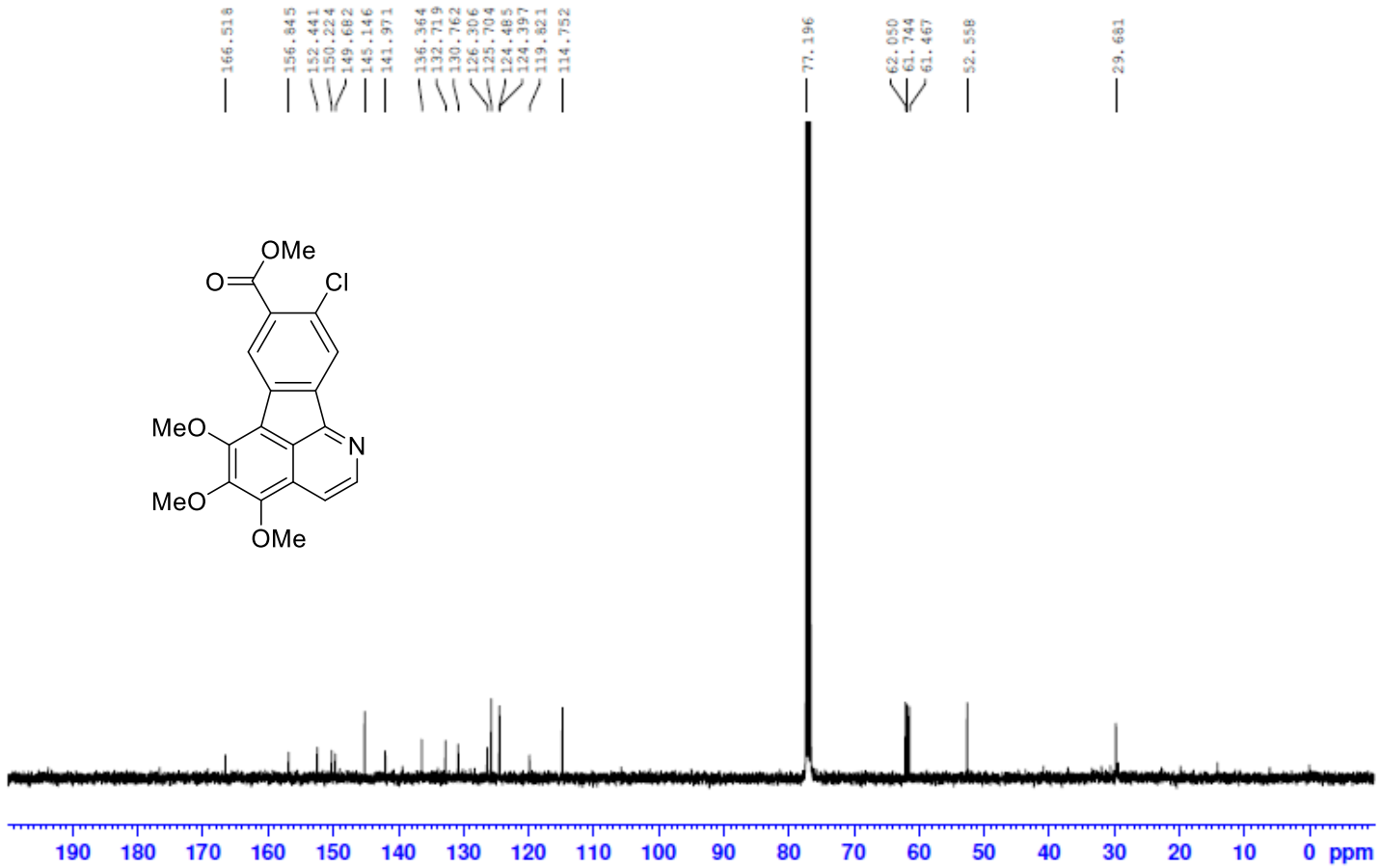
100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **21b**



100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **22a**



100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **22b**



100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **23a**

국 문 초 록

인구의 고령화가 지속되면서 많은 사람들이 암과 관절염으로부터 고통받고 있다. 인류는 전통적으로 이를 치료하기 위해 다양한 약들을 개발하였지만 아직까지 완전히 정복하지는 못했다. 방기과에 속하는 식물들은 오래전부터 민간요법으로 통증, 염증, 소화질환 사용되어왔다. 그래서 이들 방기과 식물의 추출물을 분석하여 이들의 구조를 규명하고 생리활성 능력을 확인하는 연구들이 진행되었다. 트로폴로아이소퀴놀린과 아자플루오란텐은 모두 방기과 식물에서 확인된 화합물로서 이들의 구조가 규명되면서 이들을 합성하는 연구들이 몇몇 진행되었다. 우리 그룹 또한 아이소바닐린으로부터 라디칼 음이온 중합을 주요 반응으로 하여 퍼레이트로폰을 합성하였다. 이 연구의 연장선으로 본 연구에서는 아이소이메루브린의 염소 유도체를 간단한 화합물을 시작으로 10단계의 화학반응을 거쳐 합성하였다. 염소가 도입된 유도체를 플랫폼 화합물로 해서 다양한 트로폴로아이소퀴놀린 유도체들을 합성할 수 있는 가능성이 있다는 것을 보이기 위해 메톡사이드와 반응을 진행하였다. 그 결과 염소가 메톡시기로 치환된 아이소이메루브린이 합성되는 것을 확인할 수 있었고 그 과정에서 같은 기원을 가지는 아자플루오란텐 유도체 역시 생성되는 것을 확인하였다. 이를 통해 염소가 도입된 트로폴로아이소퀴놀린 유도체가 플랫폼 화합물로서 가능성이 있다는 것을 보여주었다.

또한, 합성한 트로폴로아이소퀴놀린과 아자플루오란텐 유도체들의 항암 효과에 대한 테스트를 진행했다. 테스트 결과 합성한 아이소이메루브린과 그 이성질체에서 혈액암과 고형암에 대한 세포독성을 가지고 있는 것을 확인하였고 아이소이메루브린이 이성질체에 비해 좀더 효능이 좋다는 것을 확인할 수 있었다. 다만, 합성 과정에서 생성된 아자플루오란텐 유도체들에서는 암세포에 대한 특별한 생리활성 능력을 가지고 있지 않은 것으로 확인되었다. 생리 활성 능력을 측정하는 실험 단계에서 대부분의 화합물들이 DMSO용매에 용해되지 않고 석출되어 표면에 막이 형

성되는 것이 관찰되었는데 이러한 용해도 문제 때문에 암세포에 대한 세포독성이 다소 낮게 나오는 것으로 보인다. 염소가 도입된 트로폴로아이소퀴놀린을 메톡시기뿐만 아니라 다른 작용기로 치환을 하여 여러 종류의 알칼로이드 유도체들을 합성하게 된다면 이와 같은 용해도 문제를 해결할 수 있을 것으로 생각되고 이를 통해 의약 산업에 있어 다양한 신약 합성으로 큰 기여를 할 수 있다고 판단된다.

주요어: 트로폴로아이소퀴놀린, 아자플루오란텐, 라디칼 음이온 중합, 혈액암, 고형암, 세포독성
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