Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.


# Chemiluminescence Molecular Probe with a Linear-Chain-Reaction Amplification Mechanism

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# Supporting Information

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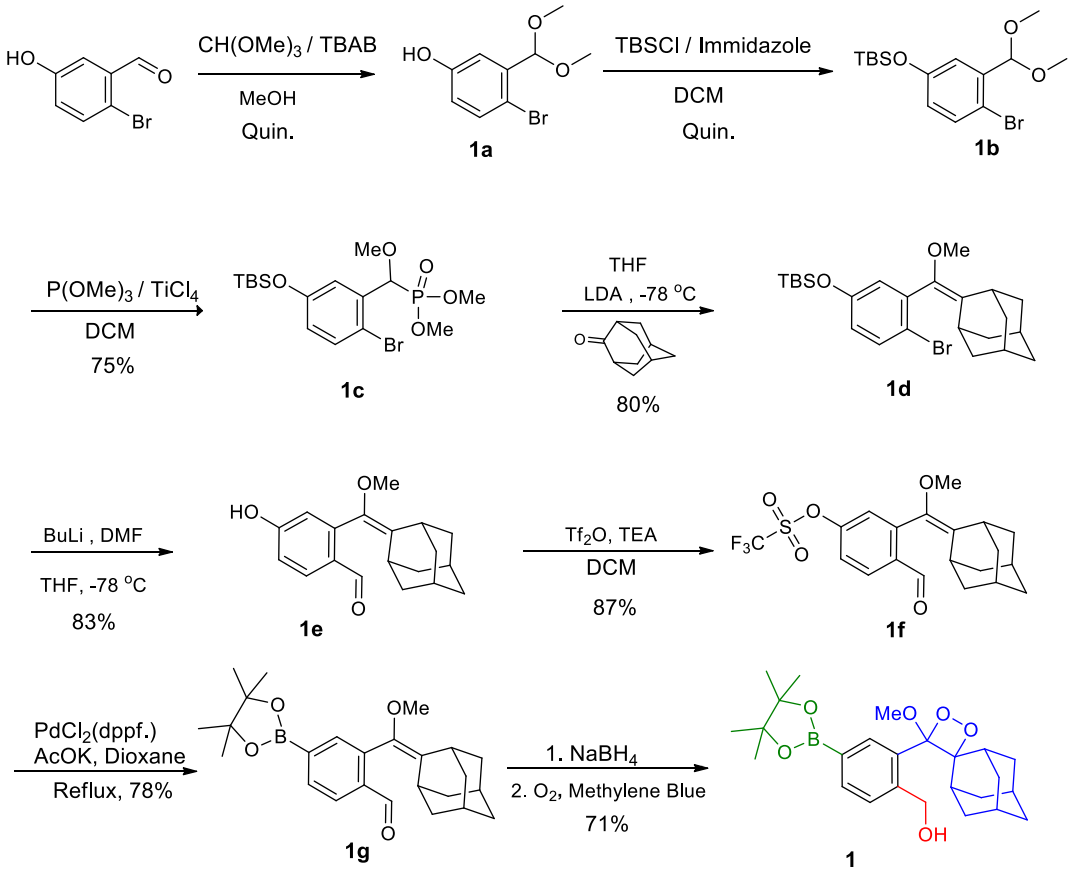
## 1. General methods

Materials and instrumentations: All reactions requiring anhydrous conditions were performed under an argon atmosphere. All reactions were carried out at room temperature unless stated otherwise. Chemicals and solvents were either A.R. grade or purified by standard techniques. Thin layer chromatography (TLC): silica gel plates Merck 60 F254: compounds were visualized by irradiation with UV light. Flash chromatography (FC): silica gel Merck 60 (particle size 0.040-0.063 mm), eluent given in parentheses. Reverse-phase high pressure liquid chromatography (RP-HPLC): C18 5u, 250x4.6 mm, eluent given in parentheses. Preparative RP-HPLC: C18 5u, 250x21 mm, eluent given in parentheses. $^{1}$H-NMR spectra were recorded using Bruker Avance operated at 400 MHz. $^{13}$C-NMR spectra were recorded using Bruker Avance operated at 100 MHz. Chemical shifts were reported in ppm on the δ scale relative to a residual solvent ($CDCl_3$: δ = 7.26 for $^{1}$H-NMR and 77.16 for $^{13}$C-NMR, DMSO-$d_6$: δ = 2.50 for $^{1}$H-NMR and 39.52 for $^{13}$C-NMR). Mass spectra were measured on Waters Xevo TQD. Fluorescence and chemiluminescence were recorded on Molecular Devices Spectramax i3x. All reagents, including salts and solvents, were purchased from Sigma-Aldrich.

Abbreviations: ACN - acetonitrile, DCM - dichloromethane, DMF - N,N'-dimethylformamide, EtOAc - ethyl acetate, Hex - hexanes, MeOH - methanol, TFA - trifluoroacetic acid, THF – tetrahydrofuran, DMAP – 4,4,dimethylaminopyridine, DBTL - Dibutyltin dilaurate.

## 2. Synthesis procedures

### Synthetic route of probe 1:




Synthesis of compound (**1a**)

To a solution of 2-bromo-5-hydroxybenzaldehyde (200 mg, 0.99 mmol, 1 equiv.) and trimethyl orthoformate (120 µL, 1.1 mmol, 1.1 equiv.) in methanol (5 mL) was added tetrabutylammonium tribromide (3.2 mg, 0.01 mmol, 0.01 equiv.). The homogeneous reaction was left at room temperature, and the progress of the reaction was monitored by TLC (Hex:EtOAc 80:20). After completion, the reaction mixture was poured into water and the product extracted with EtOAc (2 x 25 mL). The organic layer was separated, dried over anhydrous sodium sulfate, and concentrated. Further purification was achieved by passing through a short column of silica gel, to give 220 mg of **1a** (yield 89%). $^{1}$H-NMR (400 MHz, $CDCl_3$) δ 7.37 (d, $J$ = 8.6 Hz, 1H), 7.11 (d, $J$ = 3.1 Hz, 1H), 6.70 (dd, $J$ = 8.6, 3.0 Hz, 1H), 5.51 (s, 1H), 3.41 (s, 6H). $^{13}$C-NMR (100 MHz, $CDCl_3$) δ 156.03, 138.35, 134.50, 118.40, 116.01, 113.64, 103.97, 55.05. MS (ESI-) m/z 244.9, 246.9 [M-H$^+$] calc. for $C_9H_{10}BrO_3$ 244.9 246.9.

Synthesis of compound (**1b**)

Mixture of compound **1a** (2.2 g, 8.9 mmol, 1 equiv.) and imidazole (1.81 g, 27.3 mmol, 3 equiv.) was dissolved in DCM (20 mL) and then *tert*-butyl dimethyl silyl chloride (1.62 g, 10.7 mmol, 2 equiv.) was added and the reaction mixture was stirred at room temperature for 1 h. Upon completion, pure water was added to the stirring mixture. The mixture was extracted with DCM (3 x 60 mL). The organic layer was dried over anhydrous sodium sulfate, solvent was evaporated and the residue was purified by silica gel column chromatography, to obtain compound **1b**, 3.2 g (yield 99%). $^{1}$H-NMR (400 MHz, $CDCl_3$) δ 7.39 (d, $J$ = 8.6 Hz, 1H), 7.11 (d, $J$ = 3.0 Hz, 1H), 6.70 (dd, $J$ = 8.6, 3.0 Hz, 1H), 5.49 (s, 1H), 3.37 (s, 6H), 0.98 (s, 9H), 0.20 (s, 6H). $^{13}$C-NMR (100 MHz, $CDCl_3$) δ 156.47, 141.40, 131.82, 124.93, 119.74, 115.57, 103.52, 56.65, 25.66, 18.47.

Synthesis of compound (**1c**)

Trimethyl phosphite (8.6 mL, 84 mmol. 1.4 equiv.) was added to a stirred solution of compound **1b** (18 g, 60 mmol, 1 equiv.) in DCM at room temperature. 15 min later, $TiCl_4$ (8.6 mL, 84 mmol. 1.4 equiv.) was added dropwise to the reaction mixture, which was stirred for additional 30 min. The reaction mixture was diluted with DCM (400 mL) and extracted first with saturated solution of $NaHCO_3$ (200 mL) then with brine (200 mL). The combined organic phases were dried over anhydrous $Na_2SO_4$. After removal of the solvent, the residue was purified by silica gel flash column chromatography to obtained product **1c** as a white solid (18 g, yield 78%). $^{1}$H-NMR (400 MHz, $CDCl_3$) δ 7.36 (dd, $J$ = 8.7, 1.0 Hz, 1H), 7.14–7.09 (m, 1H), 6.68 (ddd, $J$ = 8.7, 2.9, 1.9 Hz, 1H), 5.01 (d, $J$ = 15.7 Hz, 1H), 3.75 (d, $J$ = 10.7 Hz, 3H), 3.60 (d, $J$ = 10.5 Hz, 3H), 3.31 (s, 3H), 0.93 (d, $J$ = 2.9 Hz, 9H), 0.17 (d, $J$ = 4.2 Hz, 6H). $^{13}$C-NMR (100 MHz, $CDCl_3$) δ 156.17, 135.51, 134.09, 123.12, 122.09, 116.28, 80.02, 59.41, 59.25, 54.44, 54.37, 26.35, 18.94. MS (ESI+) m/z 439.0, 441.1 [M-H$^+$] calc. for $C_{16}H_{29}BrO_5PSi$ 439.0, 441.0.

Synthesis of compound (**1d**)

Lithiumdiisopropyl amide (12 mL, 20 mmol, 1.3 equiv.) was added dropwise to the reaction mixture of compound **1c** (7 g, 15.9 mmol, 1 equiv.) dissolved in 20 mL dry THF at -78°C under argon. After stirring of the reaction mixture for 15 min, 2-adamantanone (6.2 g, 20.7 mmol, 1.1 equiv.), dissolved in dry THF (20 mL), was added dropwise to the reaction mixture at -78°C under argon. The reaction mixture was stirred

at room temperature for 2 h. After pouring it into pure water, it was extracted with EtOAc (3x80 mL). The combined organic phases were dried over anhydrous $Na_2SO_4$, the solvent was removed and the residue was purified by silica gel flash column chromatography to give product **1d** (5.5 g, yield 75%). $^1$H-NMR (400 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.6 Hz, 1H), 6.72 (d, *J* = 2.9 Hz, 1H), 6.67 (dd, *J* = 8.6, 2.9 Hz, 1H), 3.30 (s, 3H), 3.25 (m, 1H), 2.35 (m, 1H), 1.93–1.69 (m, 12H), 0.96 (s, 9H), 0.17 (s, 6H). $^{13}$C-NMR (100 MHz, $CDCl_3$) δ 155.32, 142.19, 137.96, 134.07, 131.00, 124.72, 122.19, 117.07, 57.47, 54.13, 39.88, 39.75, 39.39, 39.12, 37.89, 33.54, 30.19, 29.17, 29.01, 26.37, 18.97. MS (ESI+) m/z 463.1, 465.1 [M-H$^+$] calc. for $C_{24}H_{36}BrO_2Si$ 463.1, 465.1.

Synthesis of compound (**1e**)

Compound **1d** (4.5 g, 9.7 mmol, 1 equiv.) was dissolved in THF (20 mL) under $N_2$ atmosphere. The solution was cooled to -78°C and *n*-BuLi (7.8 mL, 2.5 M in Hex) was added. After 15 min of stirring, DMF (2.7 mL) was added. The reaction mixture was heated to room temperature with stirring for 30 min and monitored by TLC. After completion, saturated solution of ammonium chloride (5 mL) was added. The mixture was extracted with EtOAc (3x30 mL). The combined organic layer was washed with brine (25 mL), dried over $Na_2SO_4$ and evaporated under reduced pressure, the product purified by column chromatography to give 3 g of yellow solid (72% yield). $^1$H-NMR (400 MHz, $CDCl_3$) δ 9.99 (s, 1H), 7.80 (dd, *J* = 8.6, 0.8 Hz, 1H), 6.85–6.77 (m, 1H), 6.68 (dd, *J* = 2.3, 0.9 Hz, 1H), 3.21 (m, 4H), 2.17 (s, 1H), 1.91–1.57 (m, 12H). $^{13}$C-NMR (100 MHz, $CDCl_3$) δ 192.52, 162.82, 142.83, 139.36, 134.83, 130.46, 128.35, 118.53, 116.65, 57.94, 39.64, 37.69, 33.31, 30.62, 28.89, 26.39.
MS (ESI-) m/z 297.2 [M-H$^+$] calc. for $C_{19}H_{21}O_3$ 297.2.

Synthesis of compound (**1f**)

Enol ether **1e** (300 mg, 1 mmol, 1equiv.) and triethylamine (192 µL, 1.2 mmol, 1.2 equiv.) were dissolved in 5 mL of DCM and cooled to 0°C. Trifluoromethanesulfonic anhydride (203 µL, 1.1 mmol, 1.1 eqiuv.) was added. Reaction mixture was stirred for 30 min and monitored by TLC. Upon completion, reaction mixture was diluted with DCM (100 mL) and washed with brine (100 mL). The organic phase was dried over $Na_2SO_4$ and concentrated under reduced pressure. Purification by column chromatography (Hex:EtOAc 90:10) afforded compound **1f** as a yellow oil (374 mg, 87% yield). $^1$H-NMR (400 MHz, $CDCl_3$) δ 10.19 (s, 1H), 8.21 (d, *J* = 8.2 Hz, 1H), 7.35

(s, 1H), 7.28 (d, *J* = 2.5 Hz, 1H), 3.28 (d, *J* = 8.1 Hz, 3H), 2.25 (s, 1H), 2.01–1.72 (m, 13H). $^{13}$C-NMR (100 MHz, $CDCl_3$) δ 192.87, 156.43, 156.39, 137.48, 136.59, 131.10, 131.09, 125.68, 122.41, 120.32, 119.44, 118.22, 116.13, 111.40, 60.68, 38.65, 37.92, 33.40, 28.20. MS (ES+): m/z $[M+H]^+$ calc. for $C_{20}H_{22}F_3O_5S$: 514.1; found: 431.2.

Synthesis of compound (**1g**)

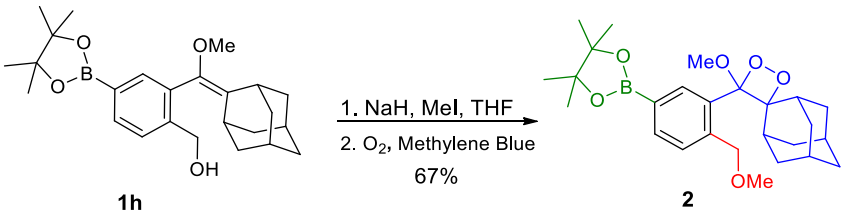
Compound **1f** (215 mg, 0.5 mmol), Bis(pinacolato)diboron (191 mg, 0.75 mmol), potassium acetate (147 mg, 1.5 mmol) and [1,1′-Bis(diphenylphosphino) ferrocene] dichloropalladium(II) (13 mg, 0.015 mmol) were dissolved in 5 mL of dry dioxane and stirred for 1 h at 100℃ under argon atmosphere. Reaction was monitored by RP-HPLC. Upon completion, reaction mixture was diluted with EtOAc (100 mL) and washed with brine. The organic phase was dried over $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (Hex:EtOAc 85:15) which afforded compound **1g** as a pale yellowish solid (160 mg, 78% yield). $^1$H-NMR (400 MHz, $CDCl_3$) δ 10.15 (s, 1H), 7.9 (d, *J* = 8.1 Hz, 1H), 6.90 (d, *J* = 8.2 Hz, 1H), 6.75 (s, 1H), 3.31 (m, 4H), 2.20 (s, 1H), 2.01 – 1.64 (m, 12H), 1.36 (s, 12H). $^{13}$C-NMR (100 MHz, $CDCl_3$) δ 192.87, 138.75, 131.47, 131.46, 131.11, 130.77, 130.55, 123.69, 111.40, 87.49, 87.49, 60.69, 38.66, 38.66, 38.66, 38.66, 37.93, 33.40, 33.40, 28.20, 28.20, 24.70, 24.70, 24.70, 24.70.

Synthesis of compound (**1**)

To a solution of compound **1g** (100 mg, 0.23 mmol, 1 equiv.) in DCM:MeOH mixture (5:3 mL) was added sodium borohydride (15 mg, 0.29 mol, 1.2 equiv.) portion wise at 0°C, and the mixture was stirred at room temperature for 0.5 h and monitored by TLC (Hex:EtOAc 75:25). The reaction mixture was extracted with saturated ammonium chloride and EtOAc (3x20 mL). The organic layer was washed with brine (30 mL) and dried on anhydrous sodium sulfate. The solvent was removed under reduced pressure. The crude residue and few milligrams of methylene blue were dissolved in 10 mL of DCM. Oxygen was bubbled through the solution while irradiating with yellow light. The reaction was monitored by RP-HPLC. The crude product **1** was purified by column chromatography to give 74 mg (yield 71%). $^1$H-NMR (400 MHz, $CDCl_3$) δ 7.86-7.46 (m, 3H), 5.12-4.53 (m, 2H), 3.24 (s, 3H), 3.06 (m, 1H), 2.25 (s, 1H), 2.01–1.72 (m, 12H), 1.34 (s, 12H). $^{13}$C-NMR (100 MHz, $CDCl_3$) δ 155.28, 142.67, 136.37, 134.35,

133.77, 131.22, 123.58, 120.79, 64.66, 58.22, 39.91, 37.76, 33.24, 30.37, 28.96, 26.42, 19.01. MS (ESI+) m/z 443.3 [M+H$^+$] calc. for $C_{25}H_{36}BO_6$ 443.3.

**Synthetic route of probe 2:**




Synthesis of compound (**2**)

To a stirred mixture of compound **1h** (120 mg, 0.29 mmol, 1 equiv.) in anhydrous THF (6 mL) was added NaH (16 mg, 0.44 mmol, 60% in mineral oil, 1.5 equiv.) at 0 °C. After stirring for 30 min, methyl-iodide (58 µL, 0.89 mmol, 3 equiv.) was added. The reaction mixture was stirred at room temperature for 10 h. Upon completion, the reaction mixture was carefully quenched with $H_2O$ (10 mL), then extracted with EtOAc (2 × 30 mL). The organic layer was washed with brine, dried over $Na_2SO_4$, and concentrated under vacuo. The crude residue and few milligrams of methylene blue were dissolved in 10 mL of DCM. Oxygen was bubbled through the solution while irradiating with yellow light. The reaction was monitored by RP-HPLC. The crude product **2** was purified by column chromatography to give 88 mg (yield 67%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, *J* = 9.1 Hz, 1H), 7.62-7.41 (m, 2H), 4.68–4.4 (m, 2H), 3.59 (s, 3H), 3.2 (s, 3H), 3.02 (m, 1H), 2.07–1.59 (m, 13H), 1.37 (s, 12H). $^{13}$C-NMR (100 MHz, $CDCl_3$) δ 137.38, 136.32, 130.05, 130.04, 127.48, 125.91, 120.43, 91.94, 87.49, 87.49, 73.76, 58.33, 52.09, 37.93, 34.88, 34.88, 33.62, 33.62, 33.62, 33.62, 30.31, 30.31, 24.70, 24.70, 24.70, 24.70.

## 3. Chemiluminescence kinetics study

General procedure for chemiluminescent measurement: 1 mM stock solution of the desired compound (**1** and **2**) in DMSO was prepared. Measurements were done at 250 µM concentration in 100 µL buffer 9.5:DMSO (75:25) in the presence of 1EU of AOX. Several concentrations of $H_2O_2$ were added to start the kinetic experiment.

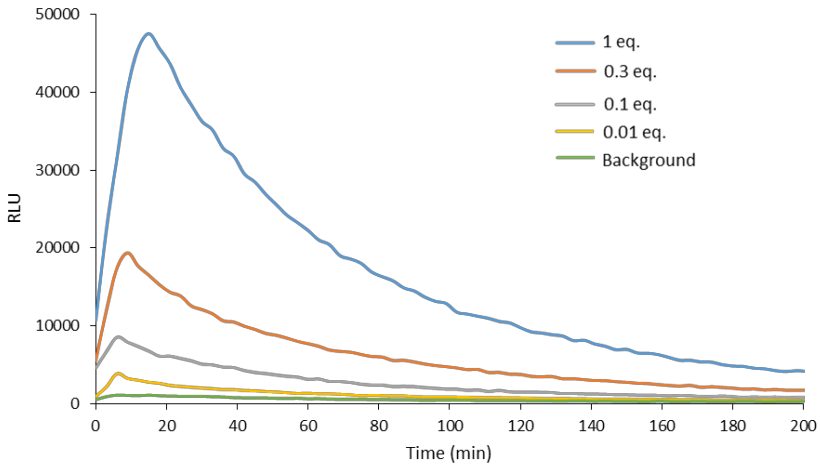



**Figure S1:** Chemiluminescence kinetic of the probe **2** (250 µM) with various concentrations of $H_2O_2$ (0, 2.5, 25, 83 and 250 µM) in the presence of 1 EU AOX in buffer pH 9.5:DMSO (75:25).

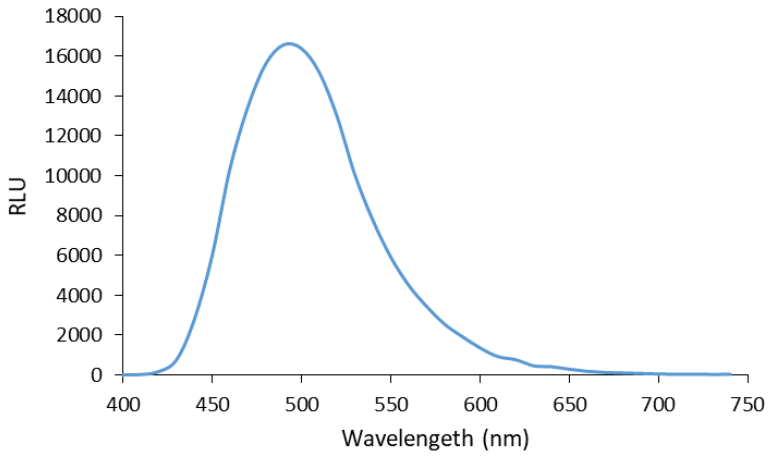



**Figure S2:** Chemiluminescence spectrum of the probe **1** (250 µM) after addition of $H_2O_2$ (250 µM) in buffer pH 9.5:DMSO (75:25).

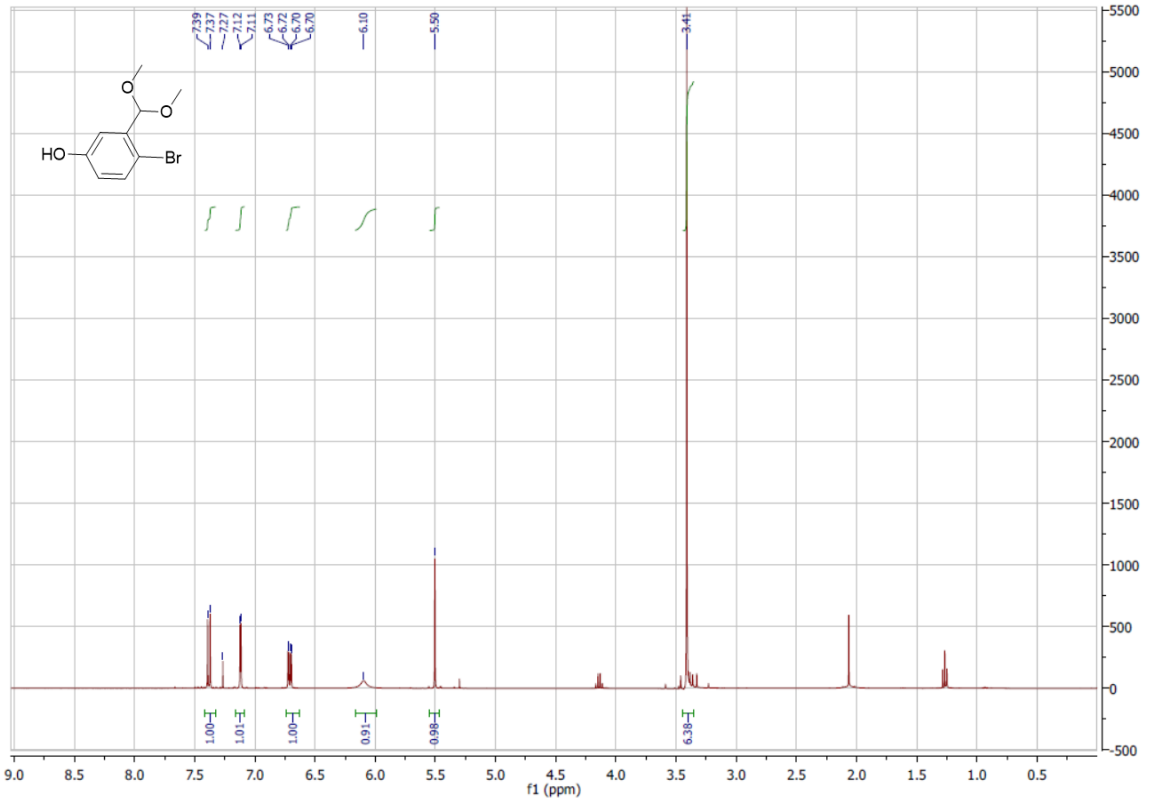
## 4. RP-HPLC kinetics study

The reaction of $H_2O_2$ with the compound **1** was carried out directly in a HPLC vial. The kinetic of the reaction was studied by RP-HPLC spectroscopy.

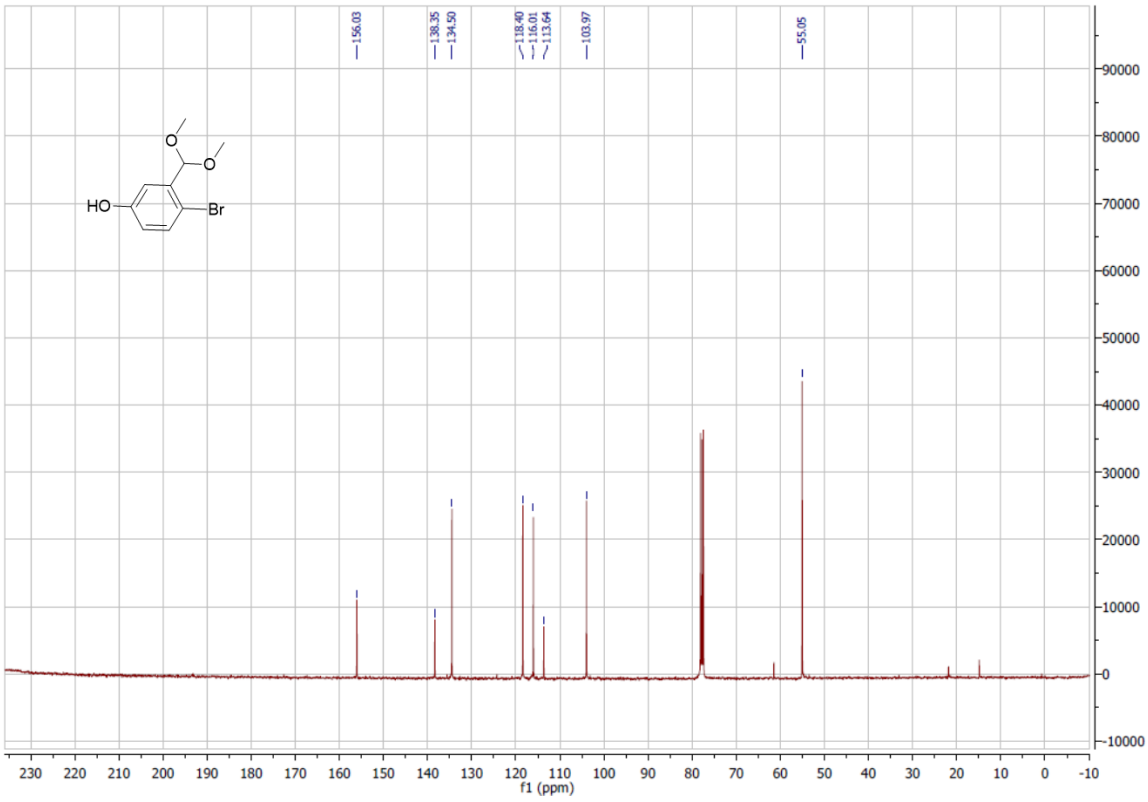
Typical procedure: Measurements were done at 250 µM concentration in 1000 µL buffer 9.5:DMSO (750:250) in the presence of 1EU of AOX and 2.5 µM of $H_2O_2$.

## 5. $^{1}$H-NMR and $^{13}$C-NMR spectra

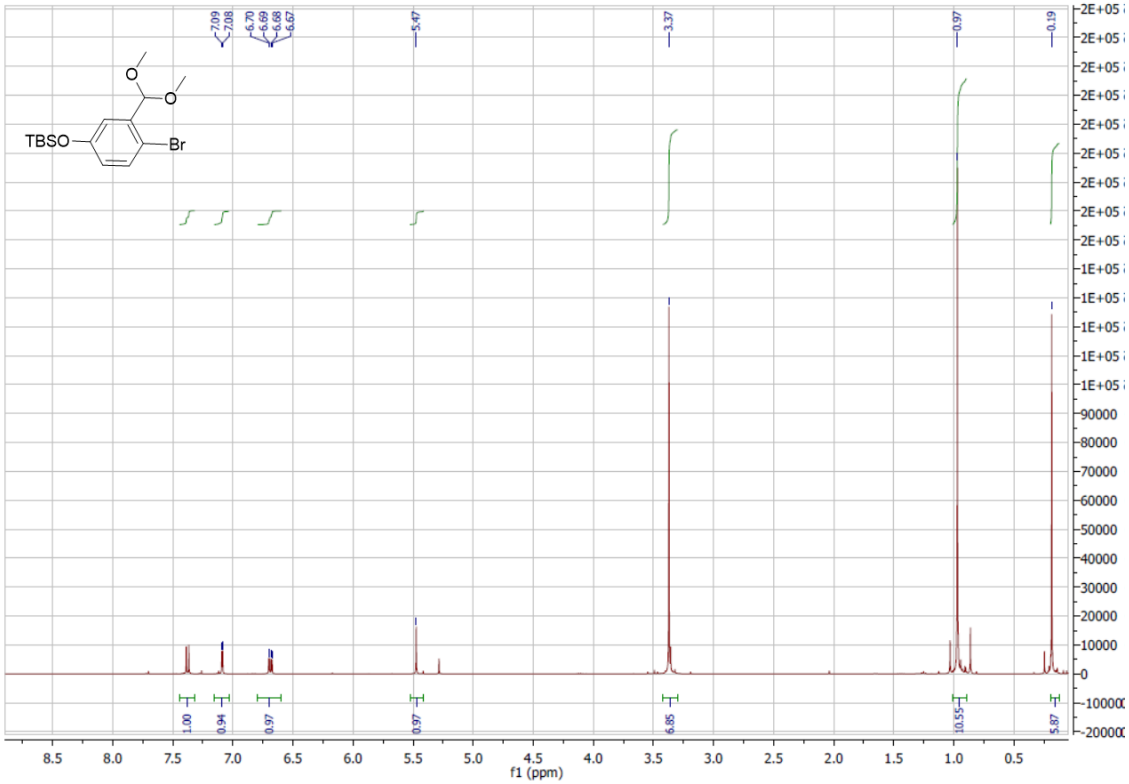
$^{1}$H-NMR Spectrum (400 MHz, $CDCl_3$) of **1a**:



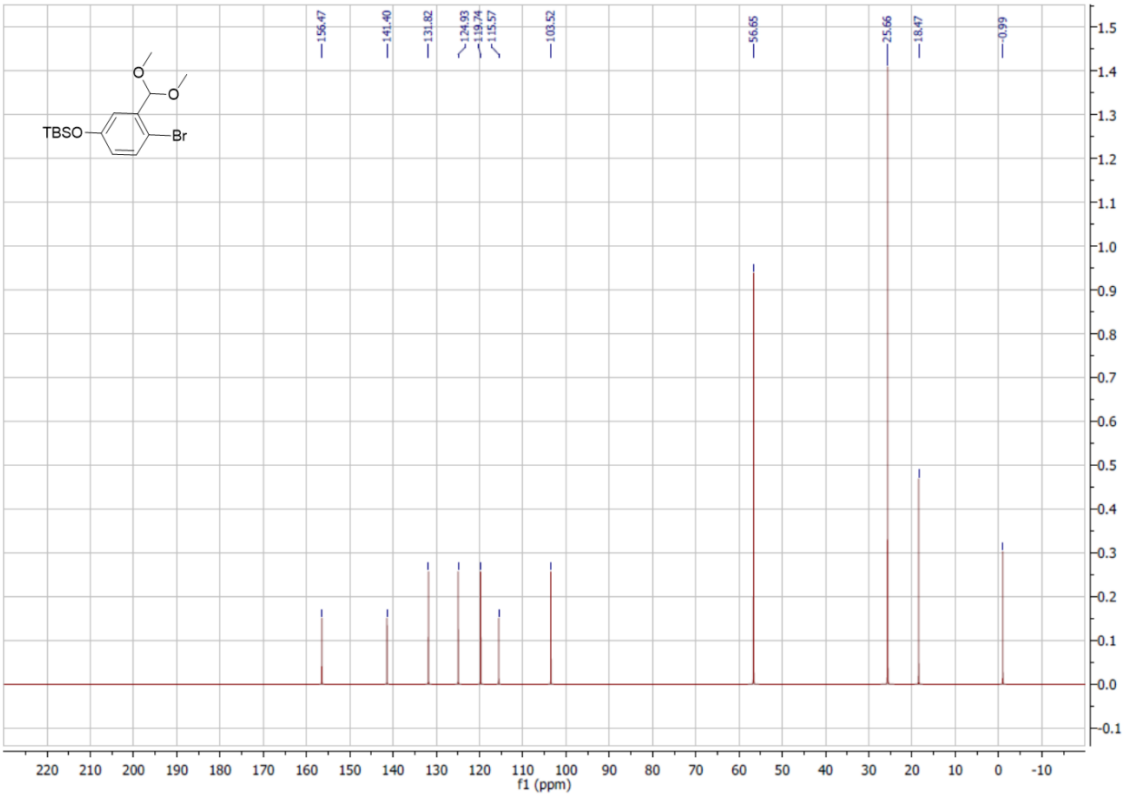

$^{13}$C-NMR Spectrum (100 MHz, $CDCl_3$) **1a**:



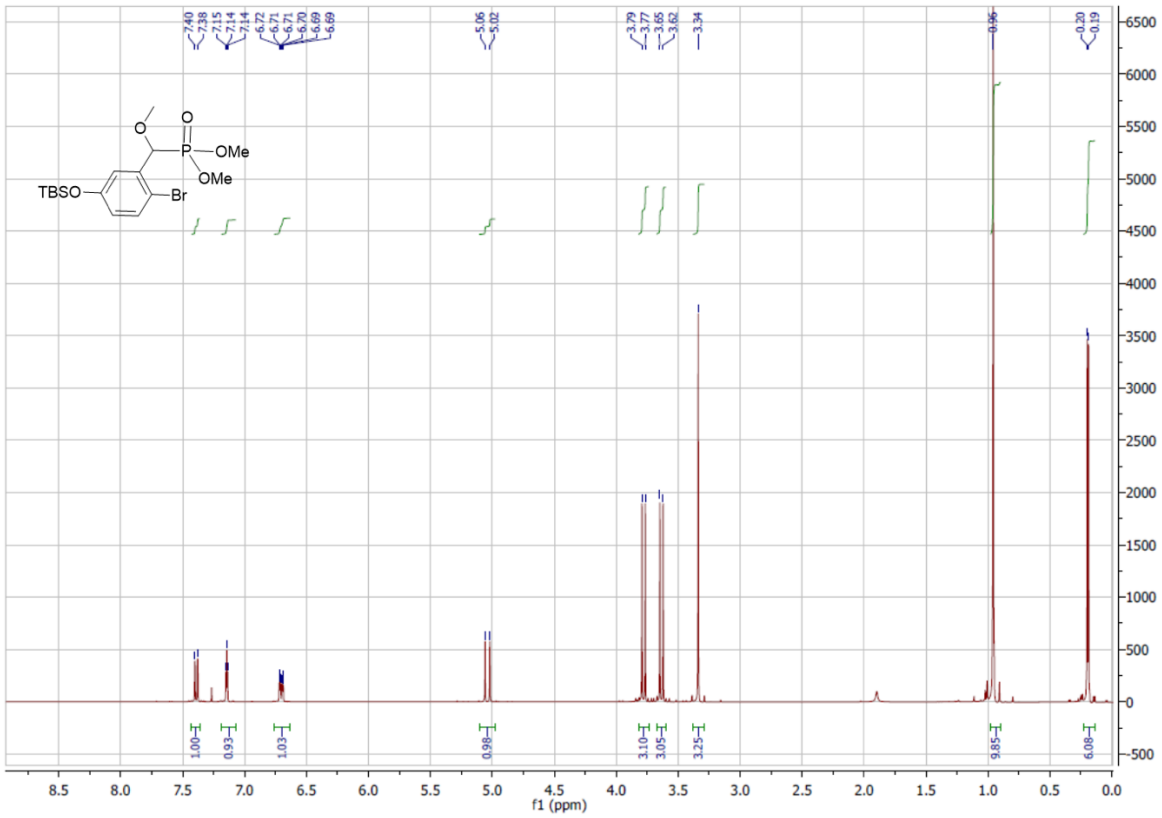
$^{1}$H-NMR Spectrum (400 MHz, $CDCl_3$) of **1b**:



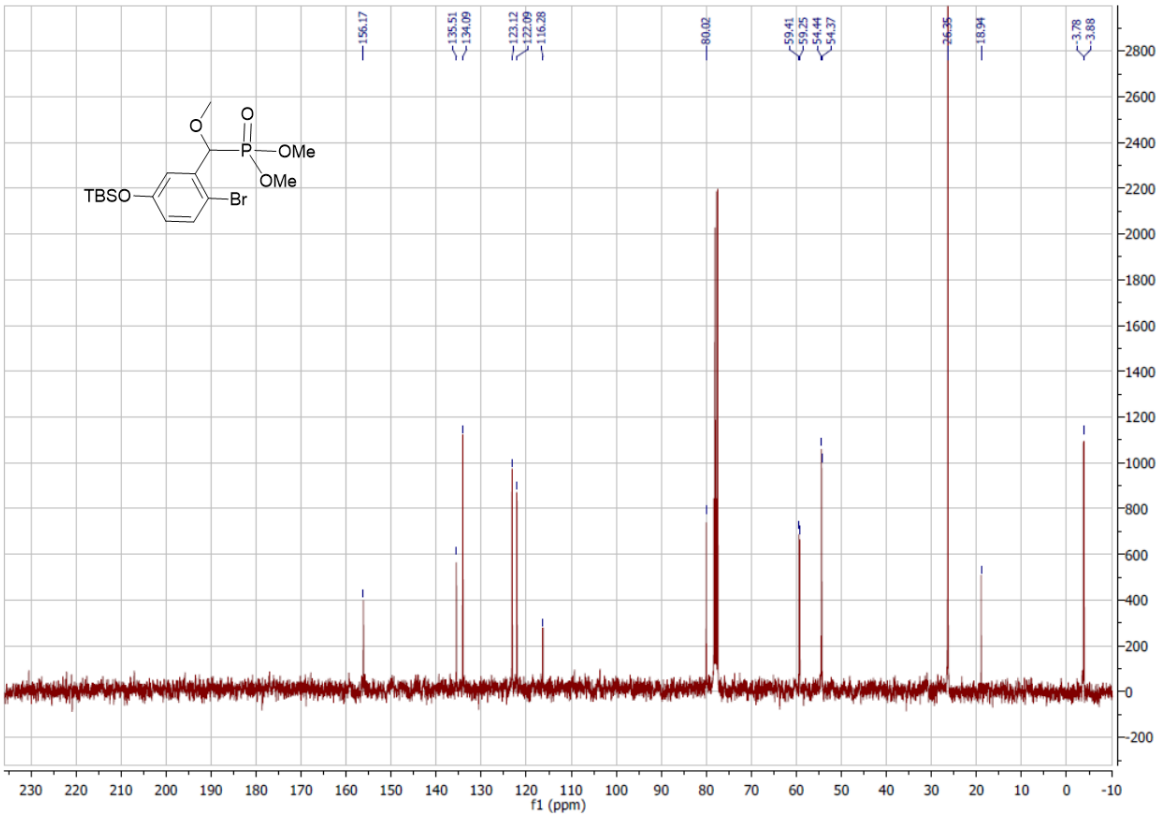

$^{13}$C-NMR Spectrum (100 MHz, $CDCl_3$) of **1b**:



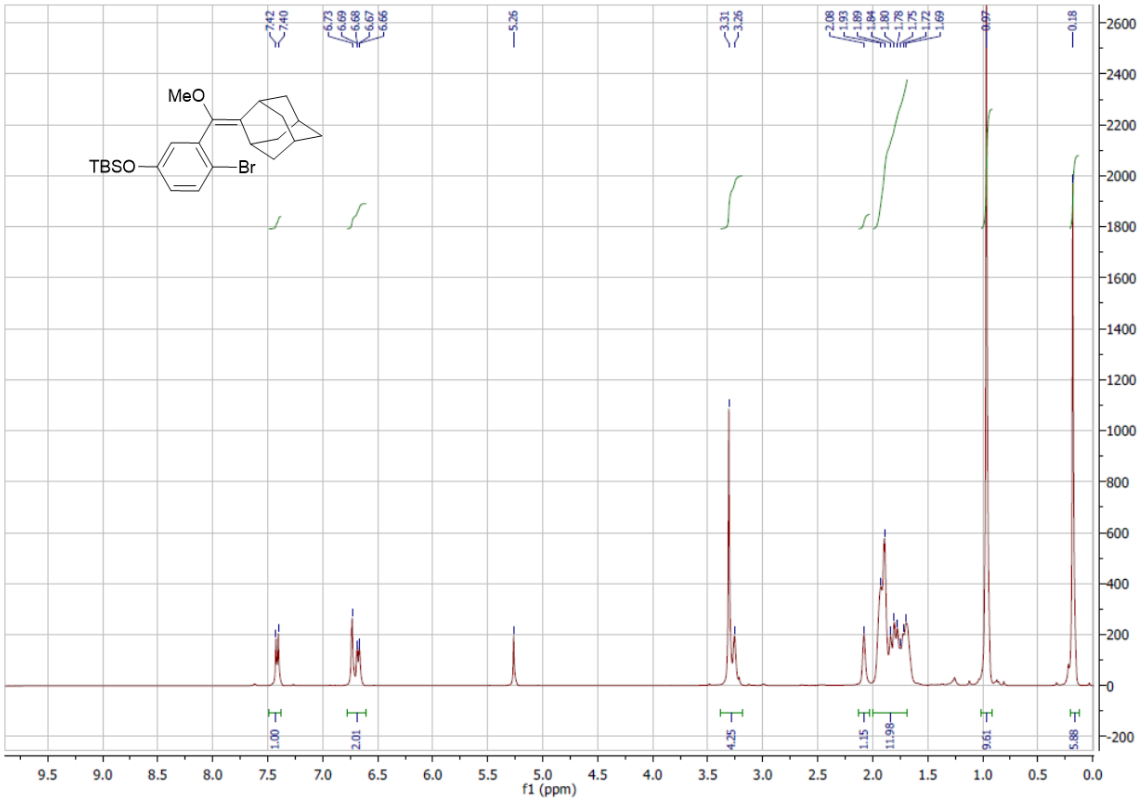
$^{1}$H-NMR Spectrum (400 MHz, $CDCl_3$) of **1c**:



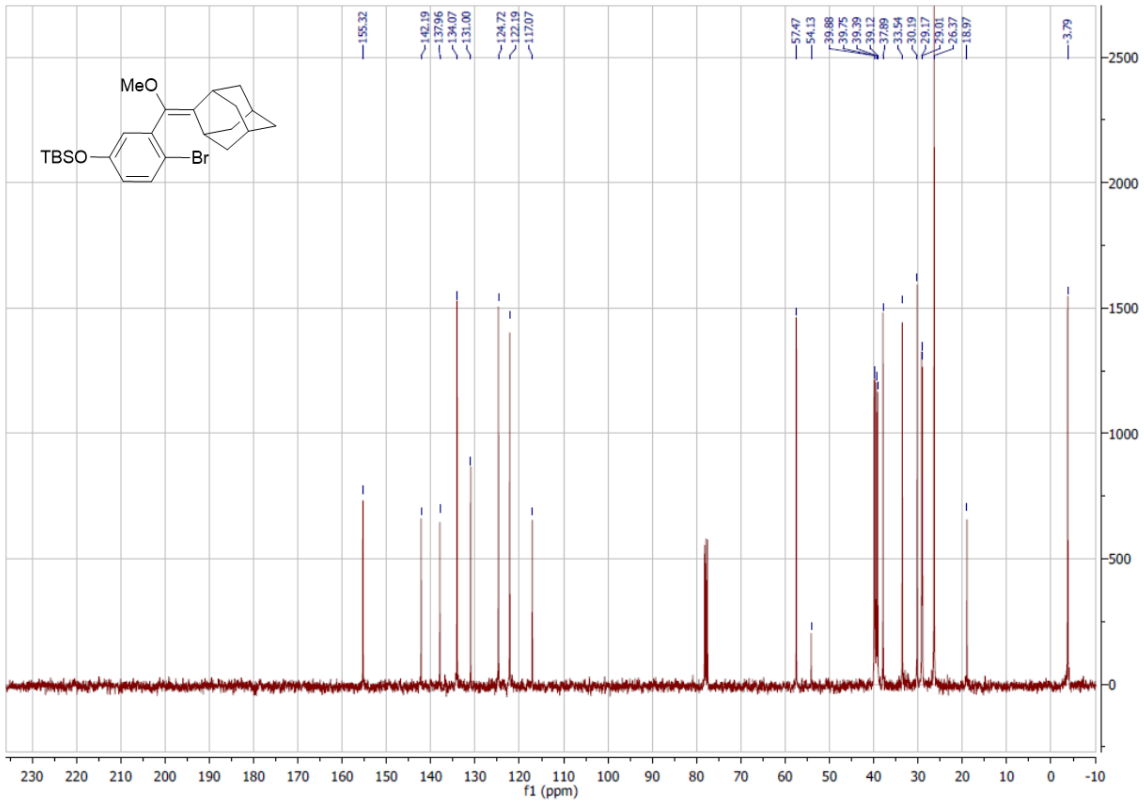

$^{13}$C-NMR Spectrum (100 MHz, $CDCl_3$) of **1c**:



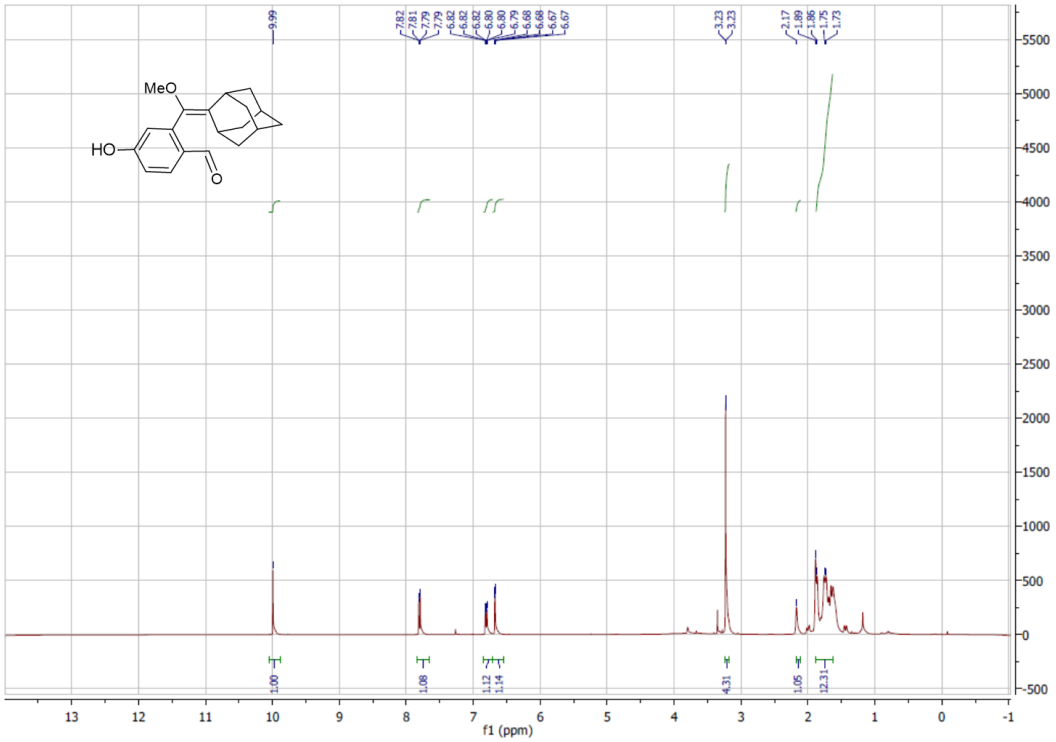
$^{1}$H-NMR Spectrum (400 MHz, $CDCl_3$) of **1d**:



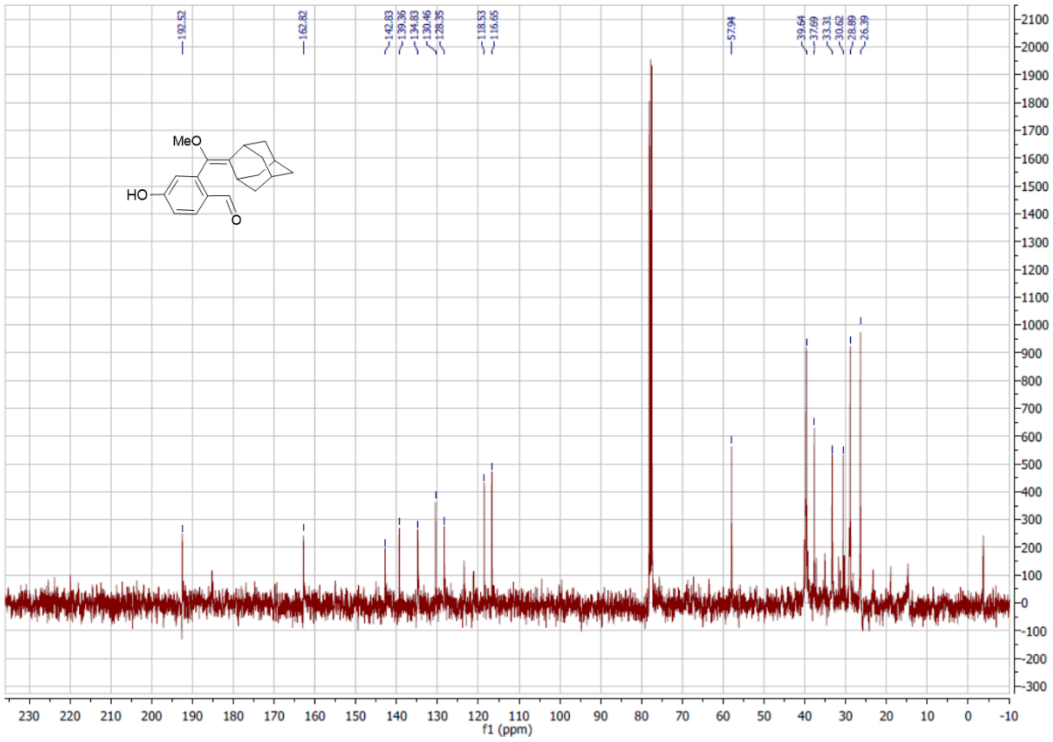

$^{13}$C-NMR Spectrum (100 MHz, $CDCl_3$) of **1d**:



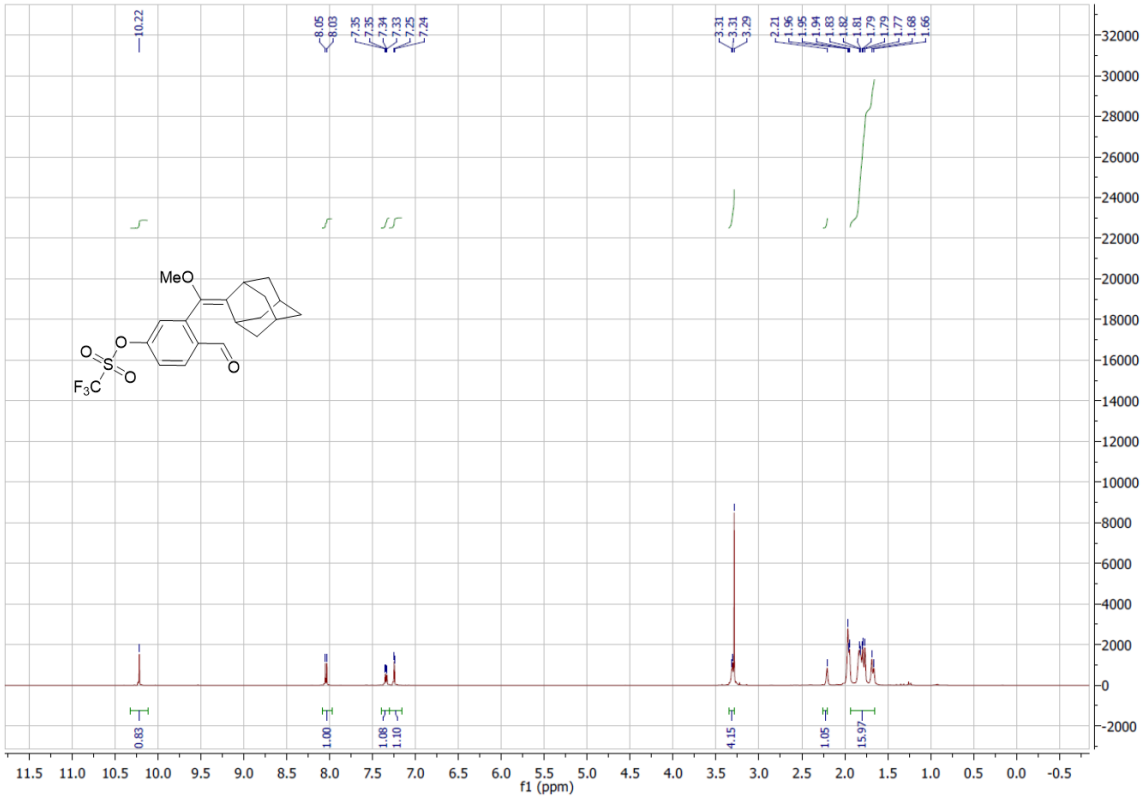
$^{1}$H-NMR Spectrum (400 MHz, $CDCl_3$) of **1e**:



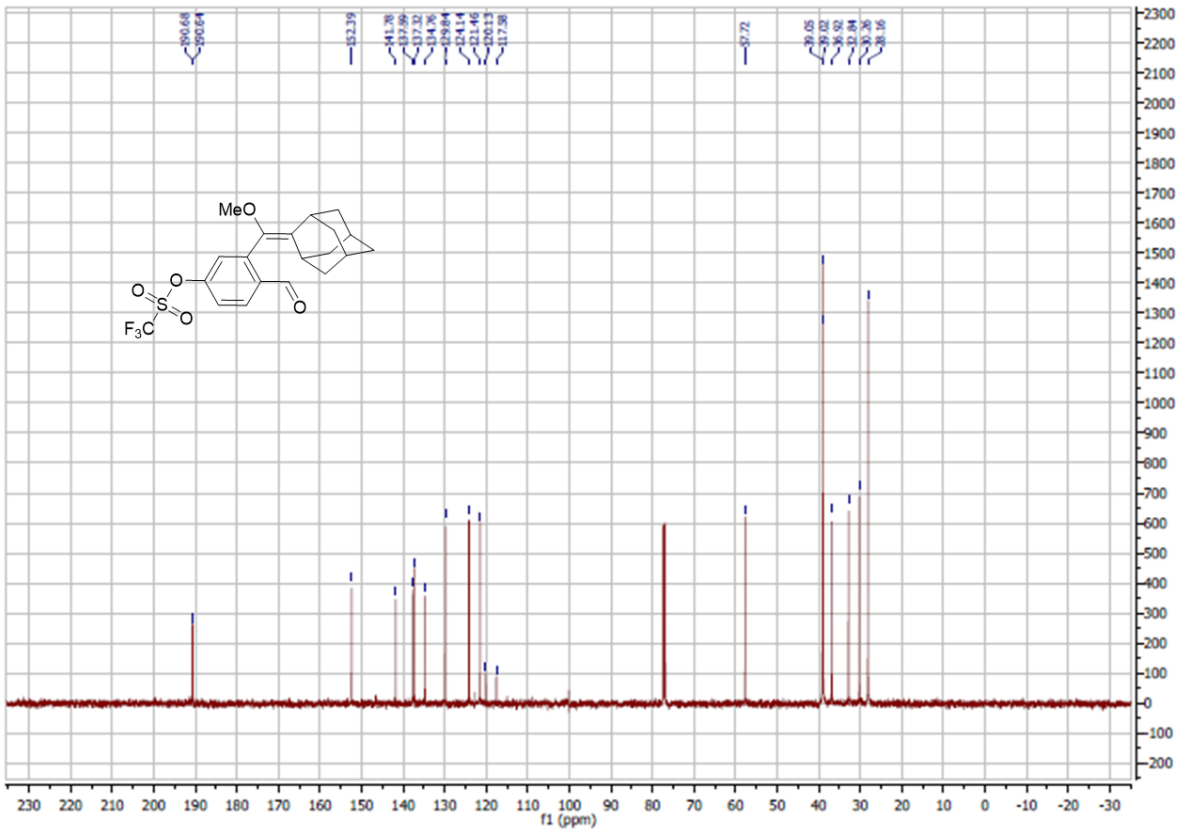

$^{13}$C-NMR Spectrum (100 MHz, $CDCl_3$) of **1e**:



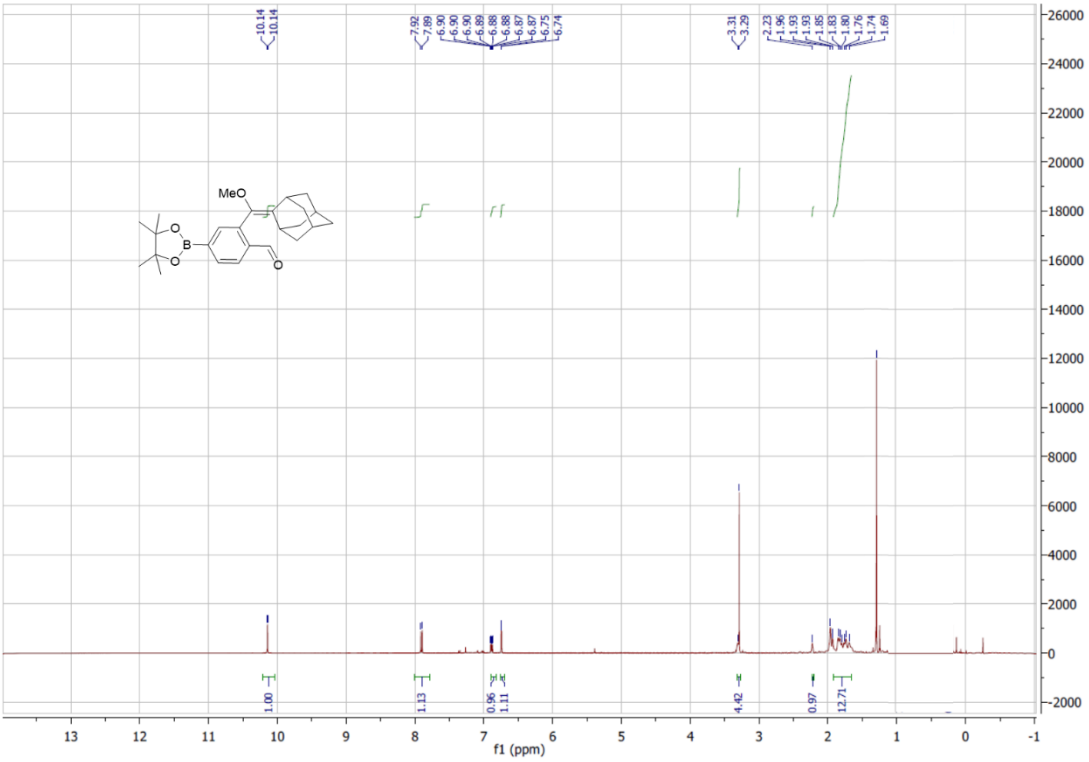
$^{1}$H-NMR Spectrum (400 MHz, $CDCl_3$) of **1f**:



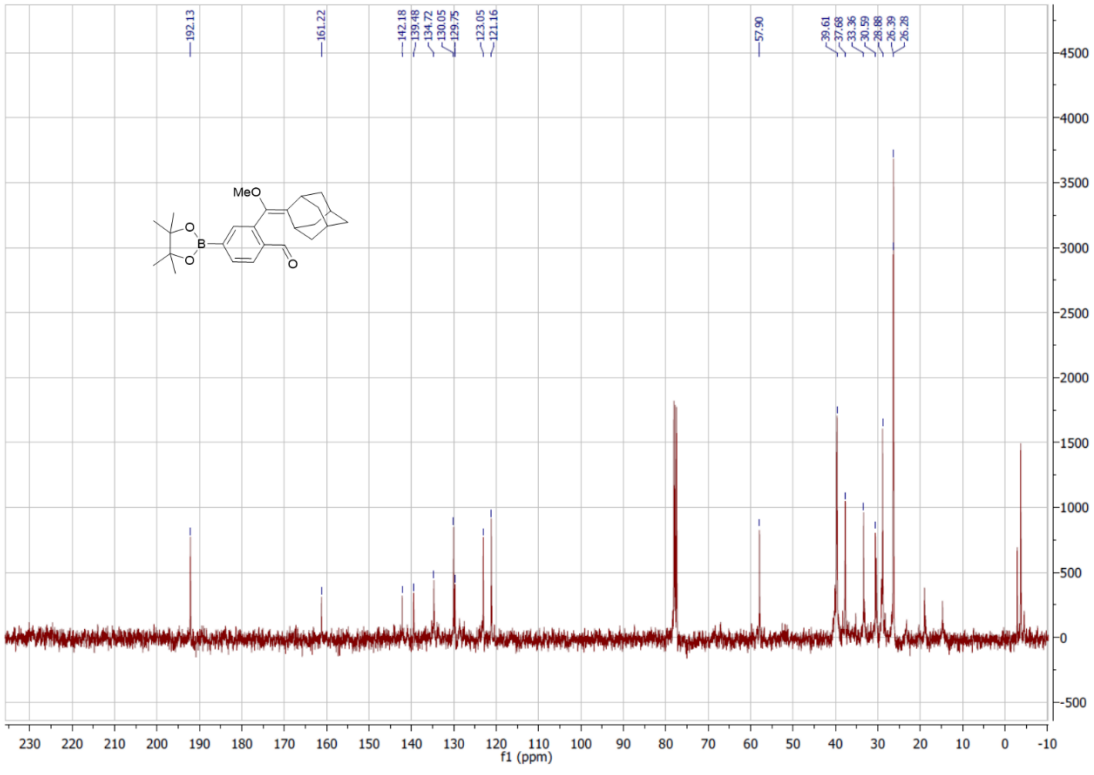

$^{13}$C-NMR Spectrum (100 MHz, $CDCl_3$) of **1f**:



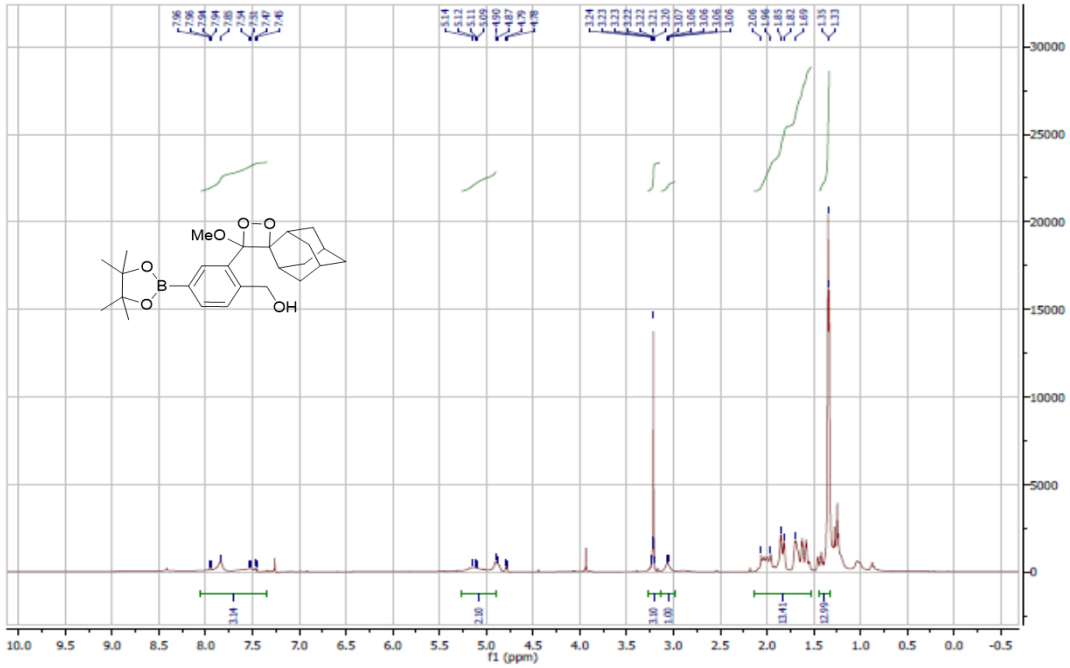
$^{1}$H-NMR Spectrum (400 MHz, $CDCl_3$) of **1g**:



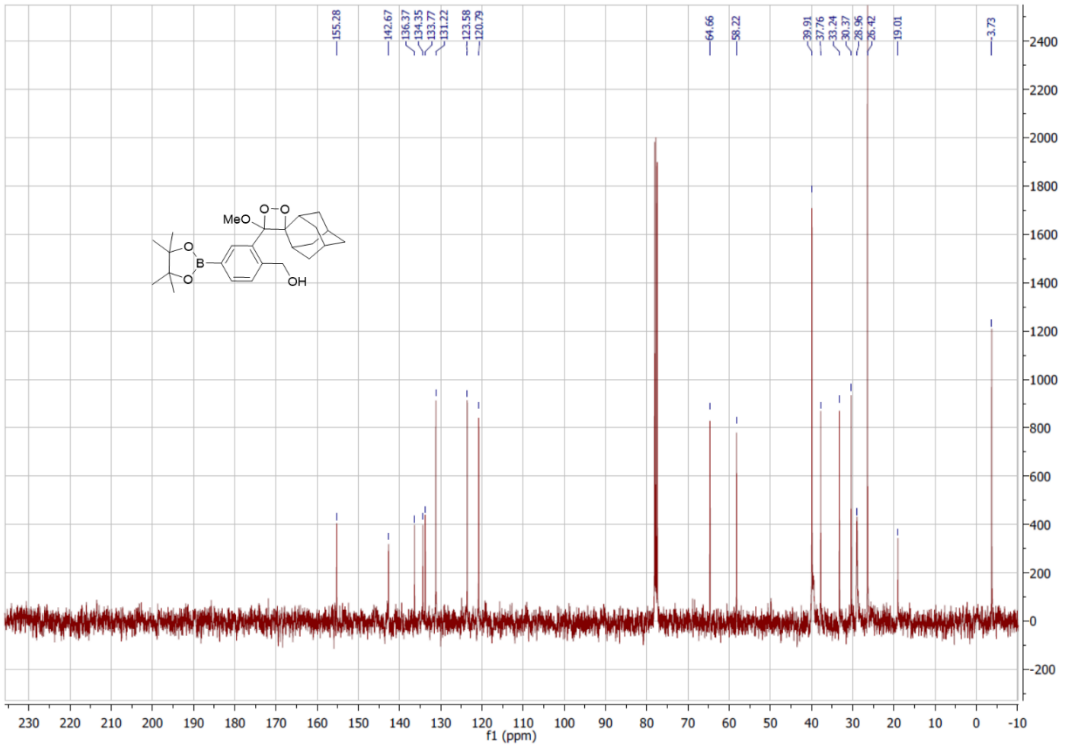

$^{13}$C-NMR Spectrum (100 MHz, $CDCl_3$) of **1g**:



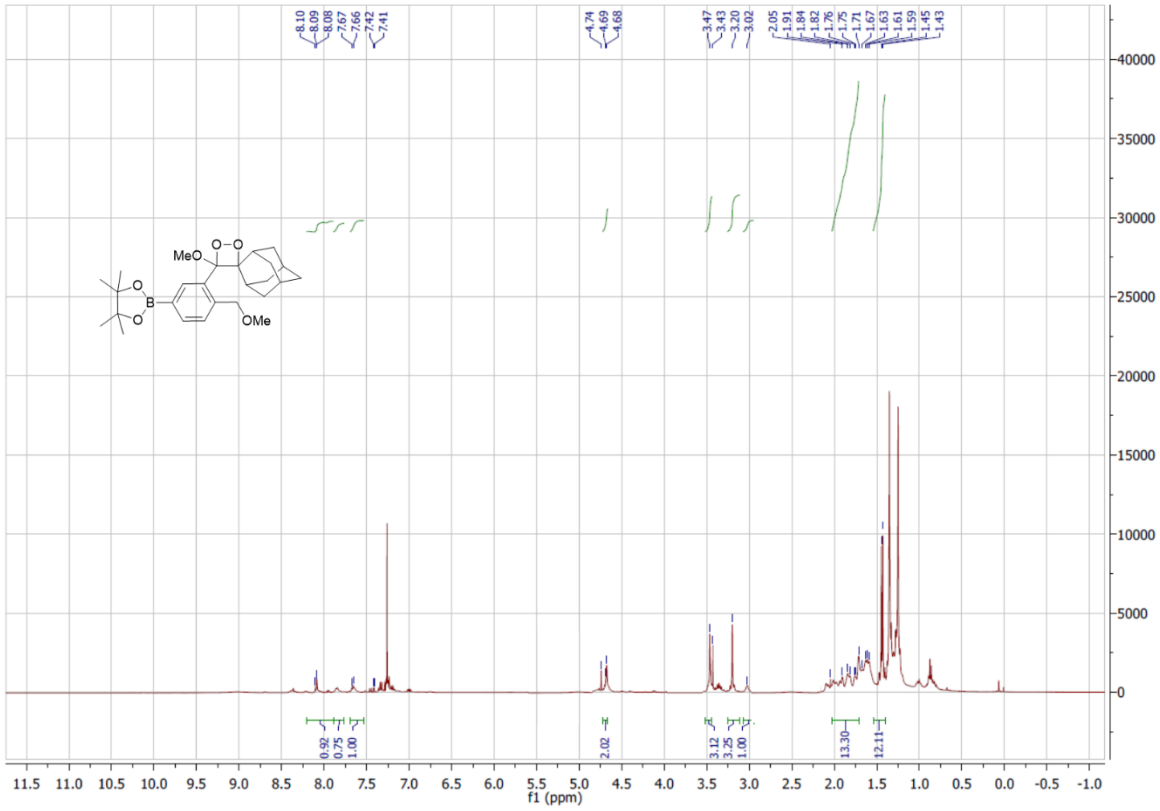
$^{1}$H-NMR Spectrum (400 MHz, $CDCl_3$) of **1**:



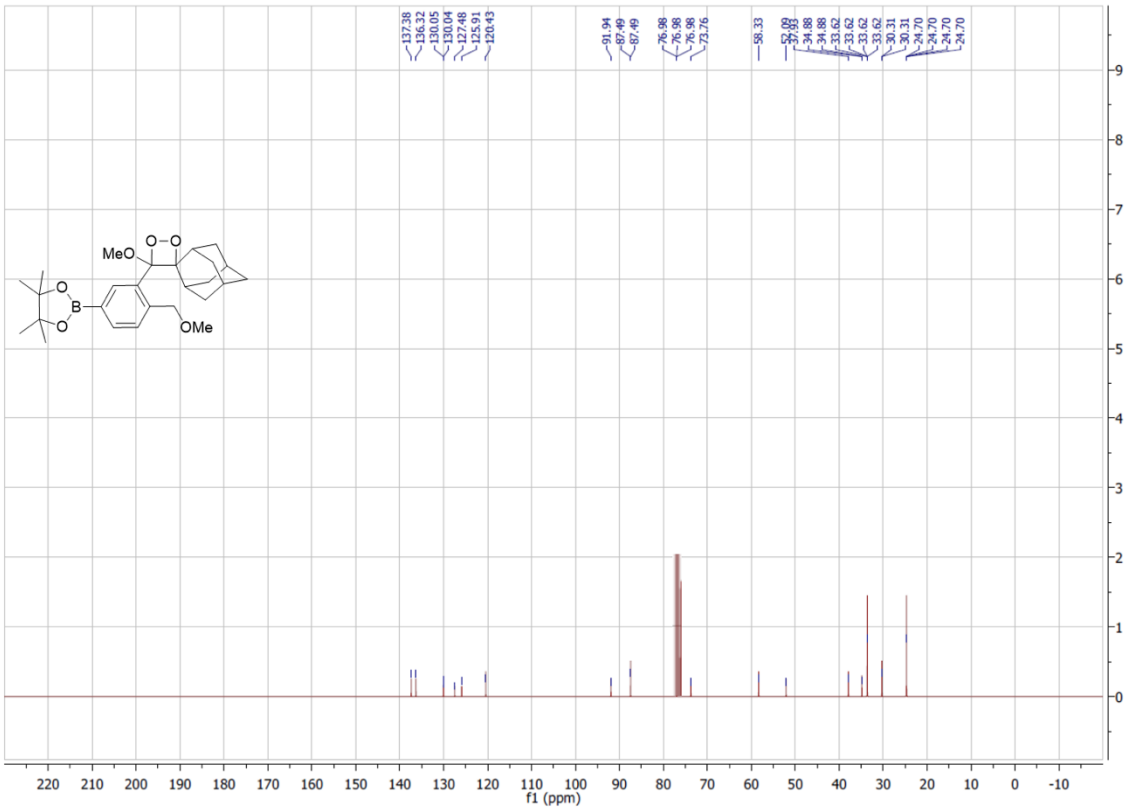

$^{13}$C-NMR Spectrum (100 MHz, $CDCl_3$) of **1**:



$^{1}$H-NMR Spectrum (400 MHz, $CDCl_3$) of **2**:

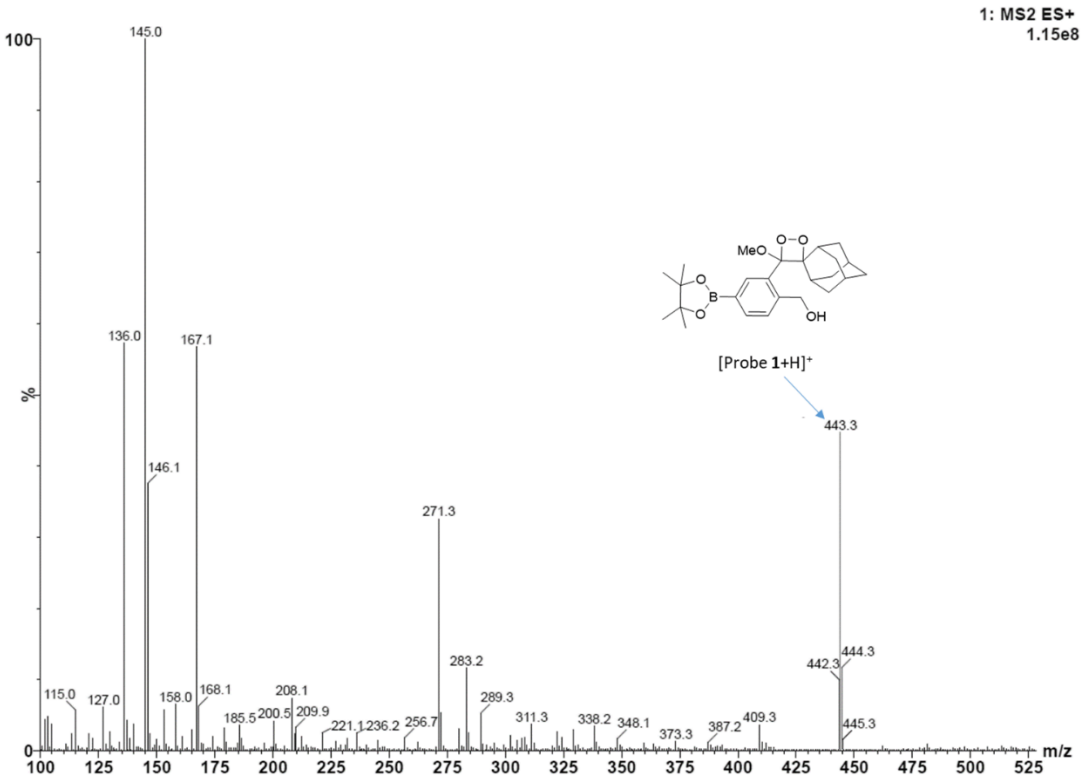



$^{13}$C-NMR Spectrum (100 MHz, $CDCl_3$) of **2**:



## 6. MS spectra

Mass spectrum of probe **1**:




Mass spectrum of probe **2**: