Supplementary information

Synthesis and Structural Confirmation of Selaginpulvilin X

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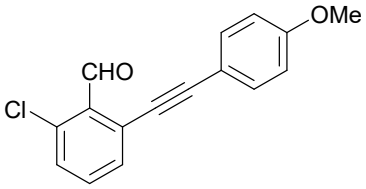
1. General information

All used chemicals were bought from OrgChem, Sigma–Aldrich, Fluorochem, Acros Organics, PENTA Chemicals, Alfa Aesar, BLDpharm, Strem Chemicals, Tokyo Chemical Industry, and Lach:ner. For TLC analysis a UV lamp with a wavelength of 254 nm and TLC plates with F254 bare silica from Silicycle were used. For preparative TLC glass backed TLC plates with 60A F254 silica gel from Silicycle were used. For column chromatography, silica gel 60A (0.040–0.063 mm) was used. All NMR spectra were measured on Bruker Avance III and Bruker Avance NEO 400 MHz spectrometers (400 MHz for ^{1}H and 101 MHz for ^{13}C) and Bruker Avance III 600 MHz spectrometer (600 MHz for ^{1}H and 151 MHz for ^{13}C). MS were obtained on a VG-Analytical ZAB SEQ spectrometer. Melting points were measured on Kofler apparatus KB T300. IR spectra in the KBr mixture were measured on Thermo Scientific Nicolet AVATAR 370 FT-IR spectrometer.

2. Procedures and characterisation data of prepared compounds

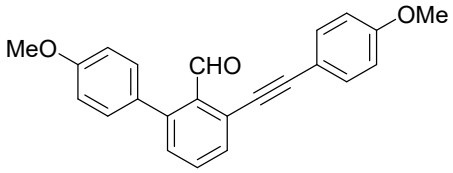
2-chloro-6-((4-methoxyphenyl)ethynyl)benzaldehyde (11)



11

Aldehyde **4** (300 mg, 1.37 mmol, 1 equiv), CuI (13 mg, 0.068 mmol, 0.05 equiv) and $PdCl_2(PPh_3)_2$ (48 mg, 0.068 mmol, 0.05 equiv) were dissolved in Et_3N (10 ml) under an inert atmosphere. The reaction mixture was heated to 80° C. Alkyne **10** (194 mg, 190 µl, 1.47 mmol, 1.1 equiv) was slowly added while stirring and the reaction mixture was left to react for 4 hours. After the reaction completion (TLC), the mixture was quenched with diluted HCl (10 ml, 2M), diluted with water (20 ml) and the mixture was extracted with DCM (3x30 ml). The organic phase was dried with $MgSO_4$ and purified with a chromatography column using 400 mL of EtOAC/hex (1:100) mixture, followed by 500 mL of EtOAC/hex (1:50) mixture as a mobile phase and silica gel as the stationary phase. The title compound was isolated as a pure yellow solid in 59% yield (220 mg). R_f (EtOAc/hex 1:10) = 0.26, ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.65 (s, 1H), 7.53 – 7.48 (m, 3H), 7.43 – 7.34 (m, 2H), 6.88 (d, *J* = 8.8 Hz, 2H), 3.82 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.9, 160.4, 136.1, 133.6, 133.2, 132.6, 132.3, 130.5, 127.7, 114.4, 114.2, 97.5, 84.8, 55.4. HRMS (ESI) for $C_{16}H_{11}ClNaO_2$ [M+Na] calculated 293.0340 and found 293.0340. IR (KBr) 2837, 2224, 2195, 1699, 1508, 1250, 1178, 1026, 791 cm^{-1}. m.p. (EtOAc): 54.1 °C

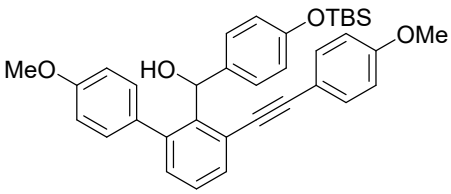
4'-methoxy-3-((4-methoxyphenyl)ethynyl)-[1,1'-biphenyl]-2-carbaldehyde (12)



12

Compound **11** (100 mg, 0.37 mmol, 1 equiv), boronic acid **9** (153 mg, 1.00 mmol, 2.7 equiv), SPhos (15 mg, 0.037 mmol, 0.1 equiv), and $Pd(OAc)_2$ (4 mg, 0.017 mmol, 0.05 equiv) K_2CO_3 (159 mg, 1.15 mmol, 3 equiv) were dissolved in degassed H_2O/toluene 1:5 (8 ml) under an inert atmosphere. The mixture was stirred and heated to 90 °C under reflux for 5 hours. After the reaction completion (TLC) was the mixture diluted with water (20 ml) and extracted with DCM (3x20 ml). The organic phase was then dried with $MgSO_4$ and purified with column chromatography using 1400 ml of EtOAc/hex mixture with a 1-6% gradient of EtOAc as a mobile phase and silica gel as a stationary phase. Compound **12** was prepared as a yellow oily substance in 86% yield (109 mg). R_f (EtOAc/hex 1:10) = 0.16. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.22 (s, 1H), 7.66 – 7.62 (m, 1H), 7.59 (d, *J* = 8.9 Hz, 2H), 7.54 (at, *J* = 7.7 Hz, 1H), 7.34 (dd, *J* = 7.7, 1.3 Hz, 1H), 7.29 (d, *J* = 8.7 Hz, 2H), 7.00 (d, *J* = 8.7 Hz, 2H), 6.92 (d, *J* = 8.9 Hz, 2H), 3.89 (s, 3H), 3.86 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.2, 160.2, 159.7, 145.2, 134.6, 133.6, 132.7, 132.0 131.0, 130.9, 130.6, 124.6, 115.2, 114.2, 114.0, 95.3, 86.6, 55.5, 55.4. HRMS (ESI) for $C_{23}H_{18}NaO_3$ [M+Na] calculated 365.1148 and found 365.1156. IR (KBr) 2835, 2200, 1695, 1512, 1248, 1176, 1030, 829 cm^{-1}

(4-((tert-butyldimethylsilyl)oxy)phenyl)(4'-methoxy-3-((4-methoxyphenyl)ethynyl)-[1,1'-biphenyl]-2-yl)methanol (15)

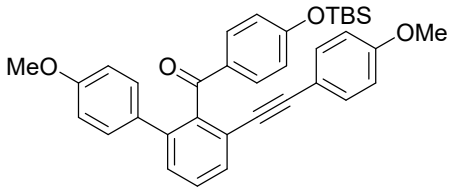


15

In a dry flask filled with an inert atmosphere bromide **14** was dissolved (121 mg, 0.42 mmol, 1.5 equiv) in dry THF (2 ml). The flask was placed inside a dry ice/acetone bath and cooled to -78 °C. Then *t*-BuLi (590 µl, 1.00 mmol, 3.5 equiv) was slowly added and the mixture was stirred for 45 min. Then aldehyde **12** (98 mg, 0.29 mmol, 1 equiv) dissolved in dry THF (2 ml) was added. The reaction mixture was then left to warm up to RT and was left to react for 30 min. After the completion (TLC) was the reaction mixture quenched with a solution of saturated NH_4Cl (10 ml) and extracted with DCM (5x20 ml). The organic phase was dried with $MgSO_4$ and purified with column chromatography using 250 ml of hex/EtOAc (3%) mixture, followed by 600 ml of hex/EtOAc (5%) mixture and 500 ml of hex/EtOAc (7%) mixture as mobile phases and silica gel as stationary phase. Compound **15** was isolated

as an amorphous yellow solid in 80% yield (126 mg). R_f (EtOAc/hex 1:5) = 0.32. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.34 (at, *J* = 7.6 Hz, 1H), 7.29 – 7.25 (m, 1H), 7.17 (m, 4H), 7.09 (d, *J* = 8.8 Hz, 2H), 6.86 (d, *J* = 8.8 Hz, 2H), 6.78 (dd, *J* = 8.8, 2.1 Hz, 4H), 5.99 (d, *J* = 11.8 Hz, 1H), 3.81 (s, 3H), 3.79 (s, 3H), 0.97 (s, 9H), 0.19 – 0.05 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.0, 159.2, 154.6, 142.6, 142.4, 137.3, 133.0, 132.84, 132.82, 130.7, 130.5, 127.9, 127.3, 122.1, 119.7, 114.6, 114.1, 113.8, 96.3, 87.1, 72.6, 55.4, 25.8, 18.4, -4.26, -4.29. HRMS (ESI) for $C_{35}H_{39}O_4Si$ [M+H] calculated 551.2612 and found 551.2613. IR (KBr) 3541, 2954, 2929, 2202, 1510, 1250, 914, 833 cm^{-1}

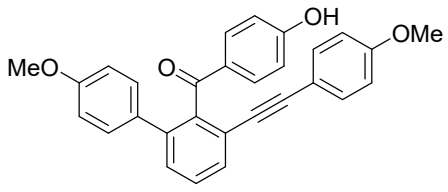
(4-((tert-butyldimethylsilyl)oxy)phenyl)(4'-methoxy-3-((4-methoxyphenyl)ethynyl)-[1,1'-biphenyl]-2-yl)methanone (16)



16

PCC (99 mg, 0.46 mmol, 2 equiv) was dissolved in dry DCM (3 ml). Alcohol **15** (125 mg, 0.23 mmol, 1 equiv) dissolved in dry DCM (5 ml) was added and the reaction mixture was stirred for 18 hours. After the reaction was completed (TLC), the mixture was filtered through a silica plug using 200 ml of EtOAc/hexane (1:5) mixture as the mobile phase. Compound **16** was prepared as a yellow amorphous paste in quantitative yield (124 mg). R_f (EtOAc/hex 1:5) = 0.32. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* = 8.6 Hz, 2H), 7.53 (d, *J* = 7.7 Hz, 1H), 7.45 (at, *J* = 7.7 Hz, 1H), 7.36 (d, *J* = 7.7 Hz, 1H), 7.21 (d, *J* = 8.7 Hz, 2H), 7.05 (d, *J* = 8.8 Hz, 2H), 6.87 – 6.65 (m, 6H), 3.77 (s, 3H), 3.74 (s, 3H), 0.95 (s, 9H), 0.18 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 196.6, 160.4, 159.8, 159.1, 141.7, 140.3, 133.0, 132.3, 132.1, 131.6, 130.4, 130.2, 129.8, 129.0, 121.8, 119.9, 115.1, 113.9, 113.8, 94.4, 86.4, 55.4, 55.3, 25.7, 18.3, -4.3. HRMS (ESI) for $C_{35}H_{37}O_4Si$ [M+H] calculated 549.2456 and found 549.2456. IR (KBr) 2954, 2929, 2204, 1666, 1595, 1510, 1254, 908, 833 cm^{-1}

(4-hydroxyphenyl)(4'-methoxy-3-((4-methoxyphenyl)ethynyl)-[1,1'-biphenyl]-2-yl)methanone (17)

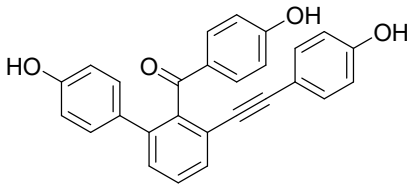


17

Ketone **16** (58 mg, 0.11 mmol, 1 equiv) was dissolved in a plastic flask in dry THF (5 ml). Then TBAF (130 µl, 0.13 mmol, 1.2 equiv) was added and the reaction mixture was stirred for 1 hour. After the reaction was completed (TLC), the reaction was quenched with a saturated solution of $NaHCO_3$ (4 ml) and acidified via a saturated solution of NH_4Cl (8 ml). The mixture was diluted with water (8 ml) and then extracted with DCM (3x20 ml). The organic phase was

dried with $MgSO_4$ and purified with column chromatography using 250 ml of EtOAc/hex (1:3) mixture, followed by 150 ml of EtOAc/hex (1:1) mixture as mobile phase and silica gel as stationary phase. Compound **17** appeared as a yellow amorphous solid and was obtained in an 87% yield (40 mg) with approximately 5% of unidentified impurity. R_f (EtOAc/hex 1:1) = 0.54. ^{1}H NMR (400 MHz, MeOD-d4) δ 7.63 (d, *J* = 8.8 Hz, 2H), 7.56 – 7.49 (m, 2H), 7.40 (dd, *J* = 7.1, 1.9 Hz, 1H), 7.20 (d, *J* = 8.7 Hz, 2H), 7.04 (d, *J* = 8.8Hz, 2H), 6.83 – 6.74 (m, 6H), 3.75 (s, 3H), 3.72 (s, 3H). ^{13}C NMR (101 MHz, MeOD-d4) δ 198.9, 164.4, 161.5, 160.7, 142.8, 141.5, 133.8, 133.7, 133.3, 131.2, 131.1, 130.8, 130.2, 122.9, 116.4, 116.1, 115.9, 115.0, 114.7, 95.2, 87.0, 55.75, 55.64. HRMS (ESI) for $C_{29}H_{22}NaO_4$ [M+Na] calculated 457.1410 and found 457.1409. IR (KBr) 3410, 2841, 2528, 2202, 1647, 1512, 1250, 1030, 835 cm^{-1}

(4'-hydroxy-3-((4-hydroxyphenyl)ethynyl)-[1,1'-biphenyl]-2-yl)(4-hydroxyphenyl)methanone (5)

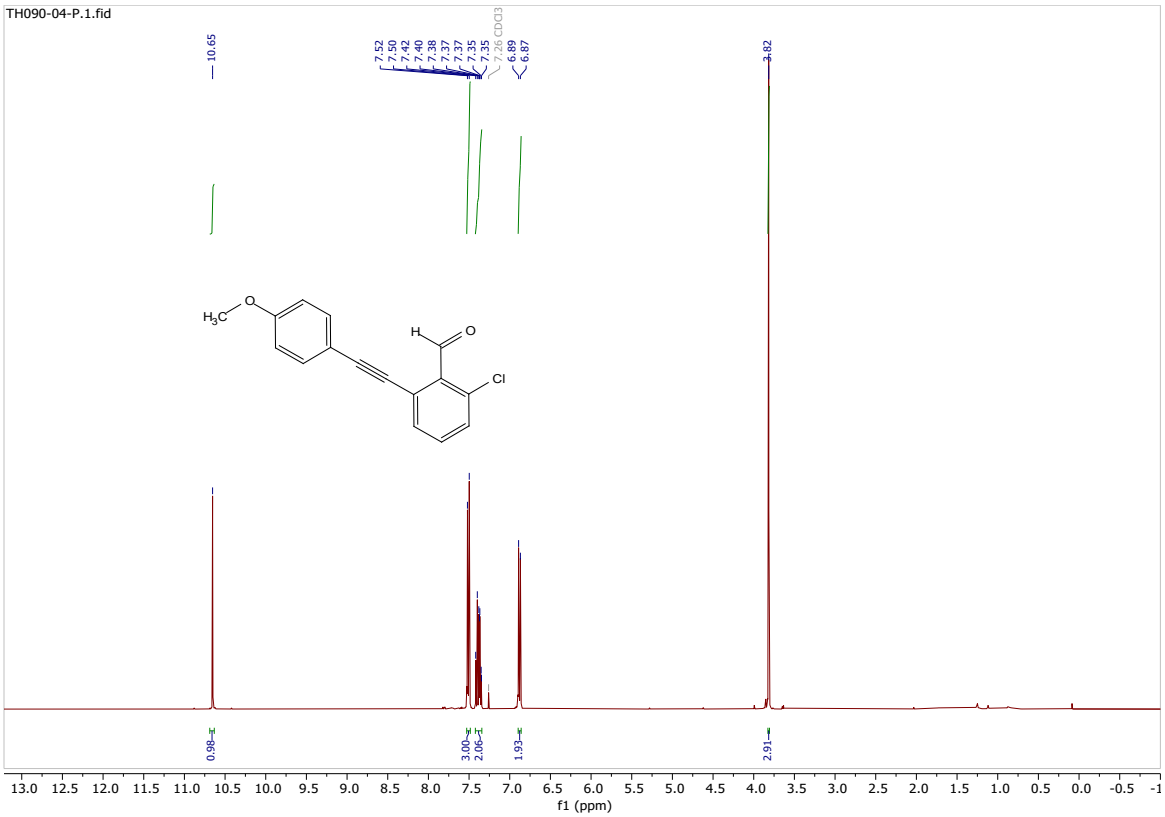


5

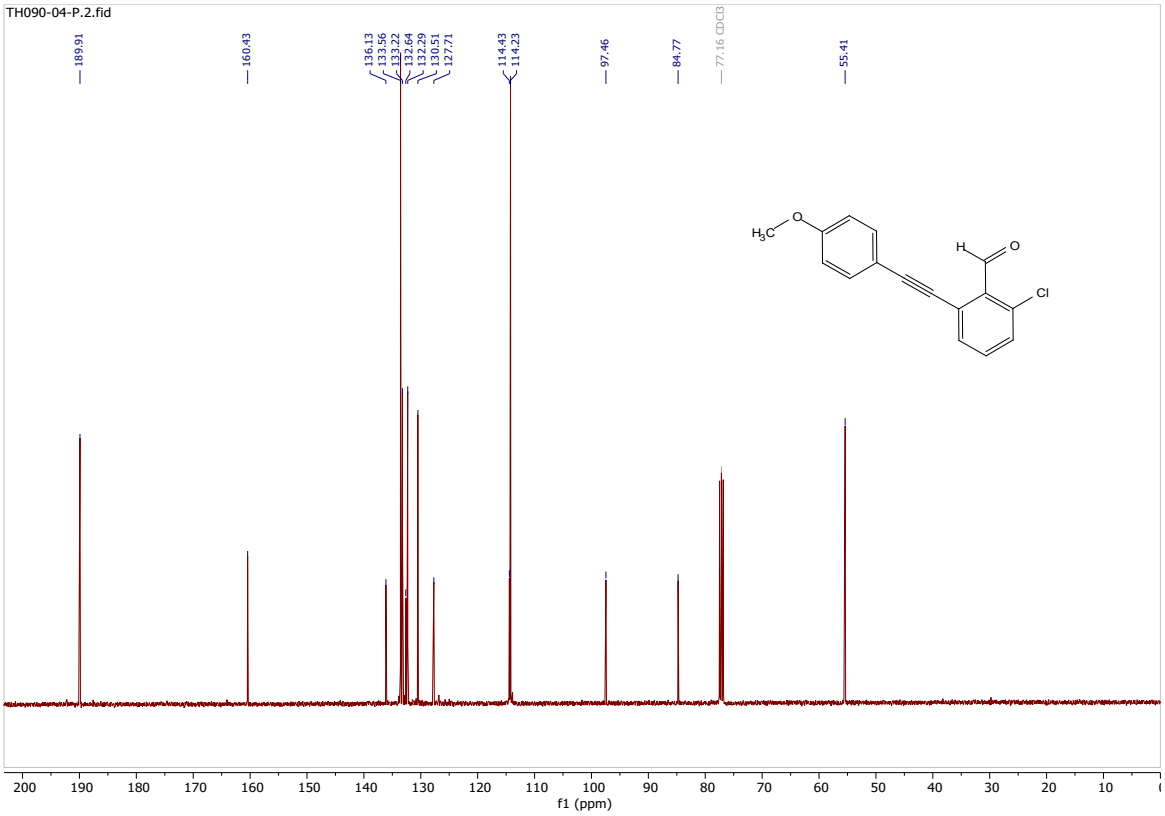
NaH (109 mg, 2.73 mmol, 60 equiv) was dissolved in dry DMF (5 ml) under an inert atmosphere in a dry flask. The reaction mixture was cooled to 0 °C via ice bath. Then EtSH (210 µl, 2.75 mmol, 60 equiv) was dropwise added. The mixture was then left to stir at 0 °C for 1 hour. Then ketone **16** (25 mg, 0.046 mmol, 1 equiv) dissolved in dry DMF was added and the mixture was refluxed at 100 °C for 18 hours. After the reaction was completed (TLC), the reaction mixture was quenched using a saturated solution of NH_4Cl (6 ml), diluted with water (24 ml), and extracted with EtOAc (3x30 ml). Joined organic phases were washed with an aqueous solution of 5% LiCl (3x100 ml). The organic phase was dried with $MgSO_4$ and purified with column chromatography using 125 ml of EtOAc/hex (1:4) mixture, followed by 225 ml of EtOAc/hex (1:2) mixture and 150 ml of EtOAc/hex (1:1.6) mixture as mobile phases and silica gel as stationary phase. Compound **5** in the appearance of a yellow oil was prepared in 43% yield (8 mg). R_f (EtOAc/hex 2:1) = 0.52. ^{1}H NMR (600 MHz, MeOD-d4) δ 7.62 (d, *J* = 8.7 Hz, 2H), 7.54 – 7.49 (m, 2H), 7.39 (dd, *J* = 6.8, 2.2 Hz, 1H), 7.10 (d, *J* = 8.7 Hz, 2H), 6.95 (d, *J* = 8.7 Hz, 2H), 6.77 (d, *J* = 8.7 Hz, 2H), 6.66 (m, 4H). ^{13}C NMR (151 MHz, MeOD-d4) δ 199.1, 164.2, 159.3, 158.2, 142.7, 141.7, 133.9, 133.7, 132.3, 131.2, 130.9, 130.7, 130.6, 130.2, 123.0, 116.31, 116.29, 116.0, 114.6, 95.6, 86.5. The recorded values are in good agreement with the published data.[1]

3. Copies of ^{1}H and ^{13}C NMR spectra of synthesized compounds

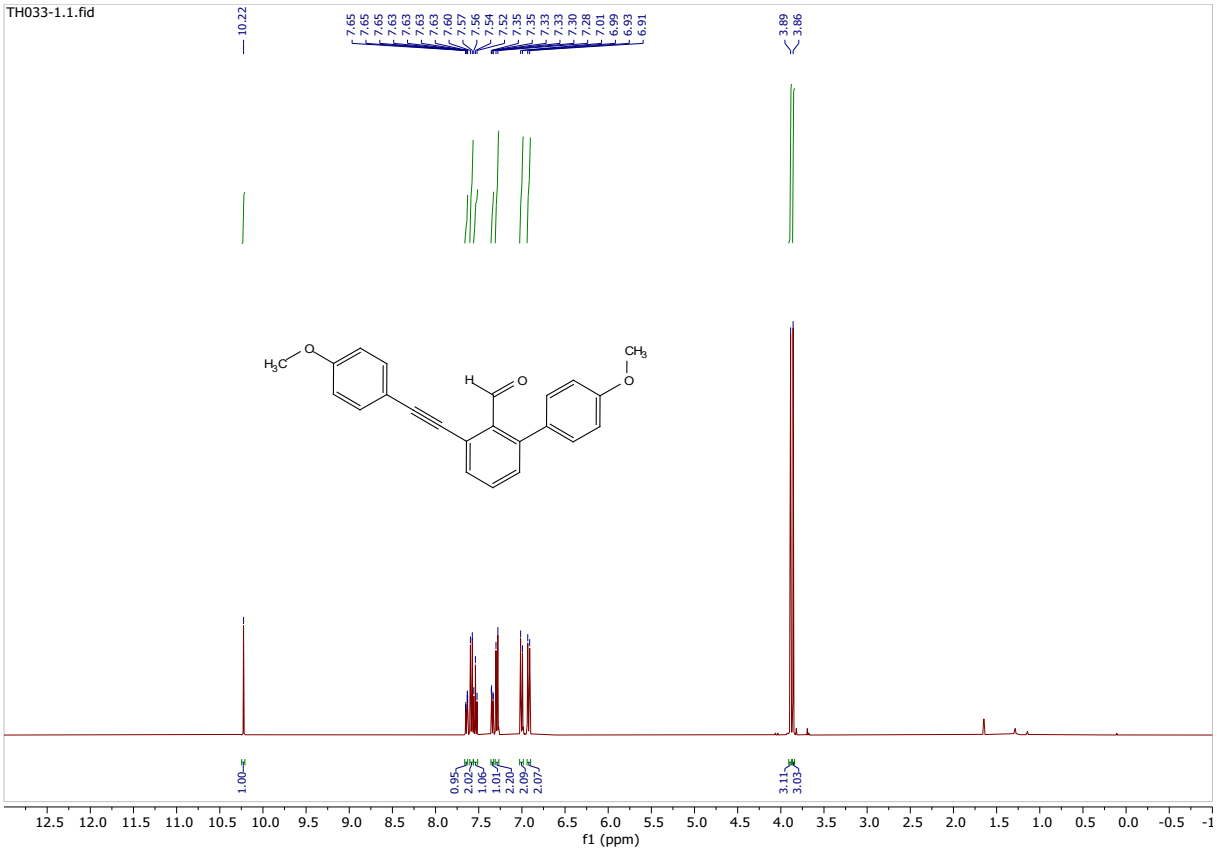
11 ^{1}H NMR (400 MHz, $CDCl_3$)



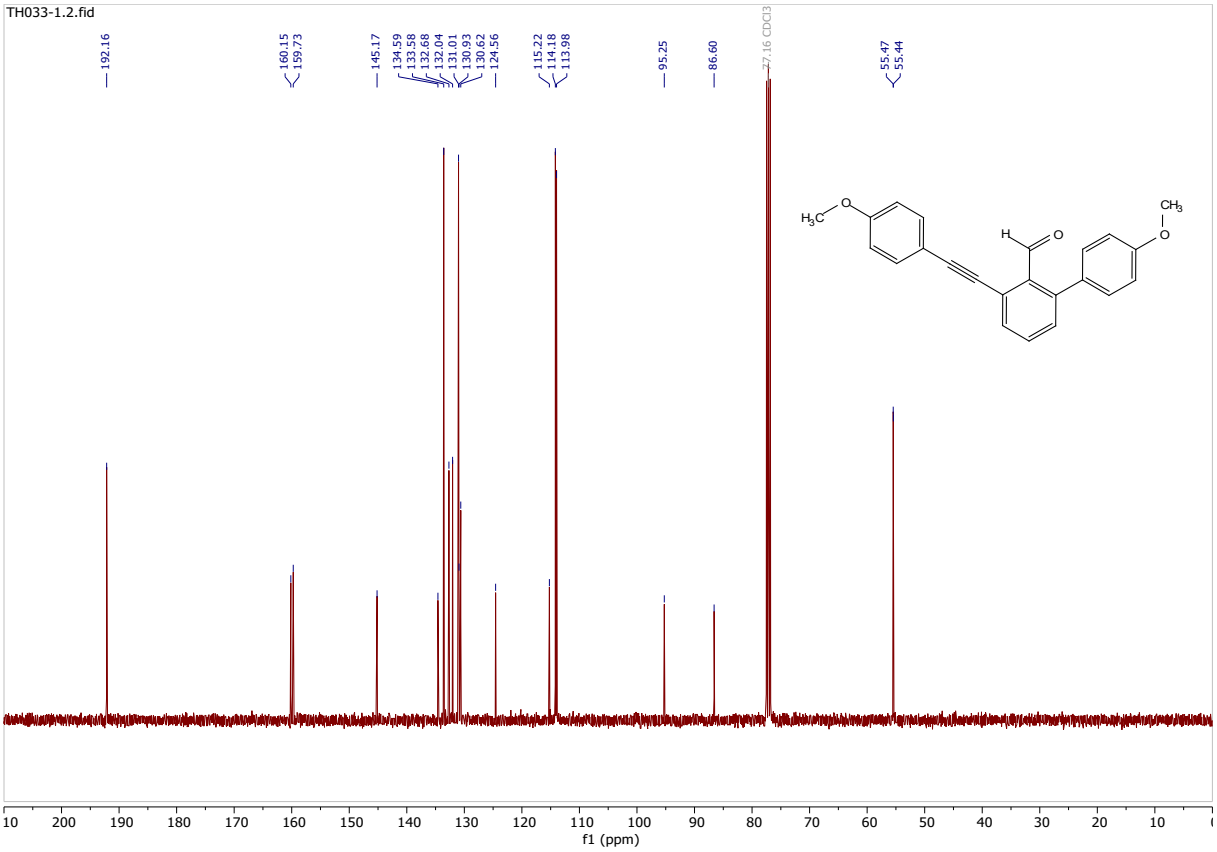
11 ^{13}C NMR (101 MHz, $CDCl_3$)



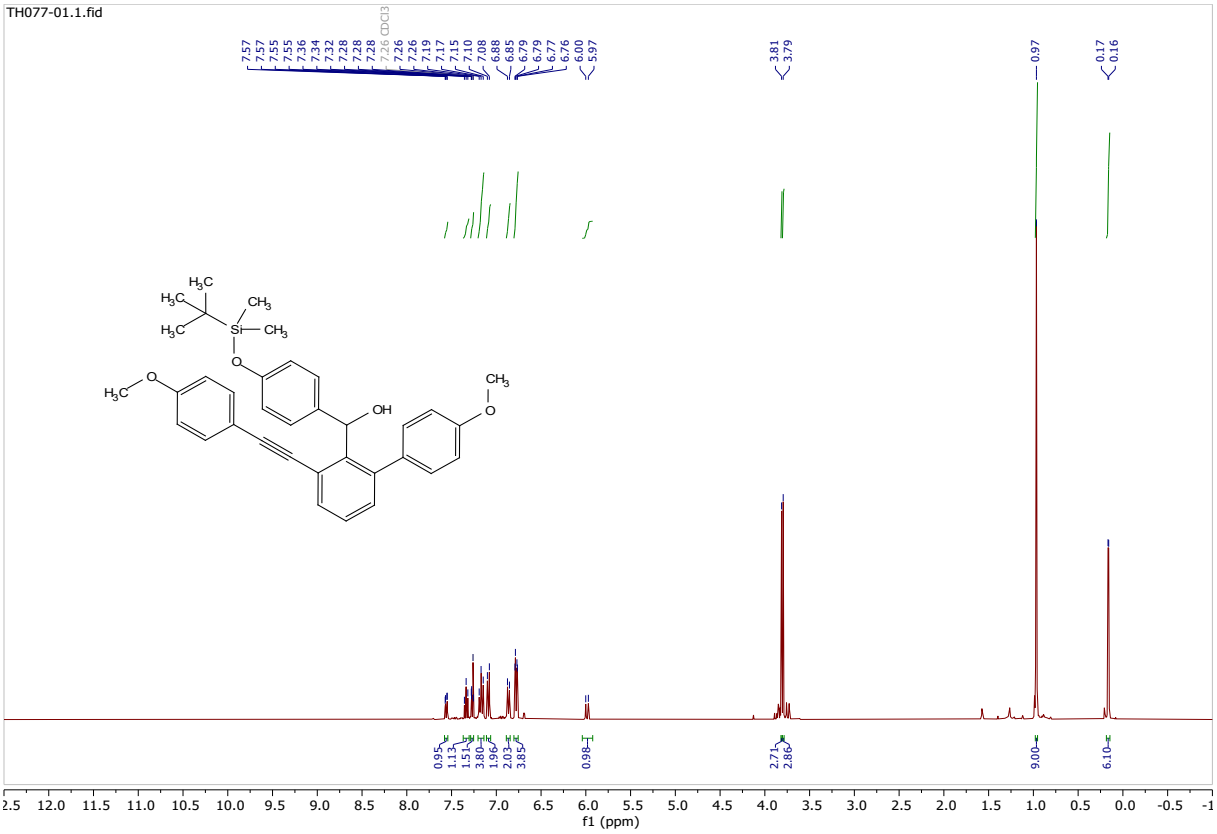
12 ^{1}H NMR (400 MHz, $CDCl_3$)



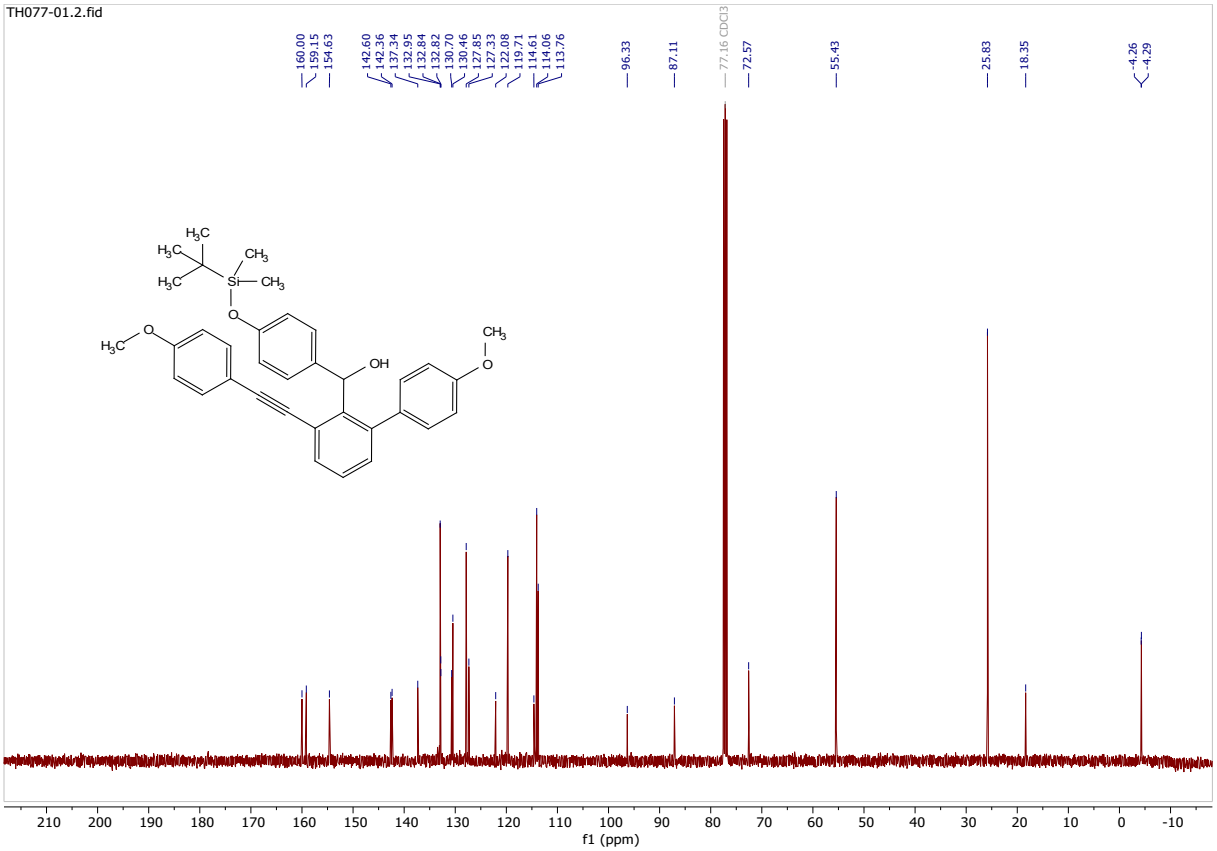
12 ^{13}C NMR (101 MHz, $CDCl_3$)



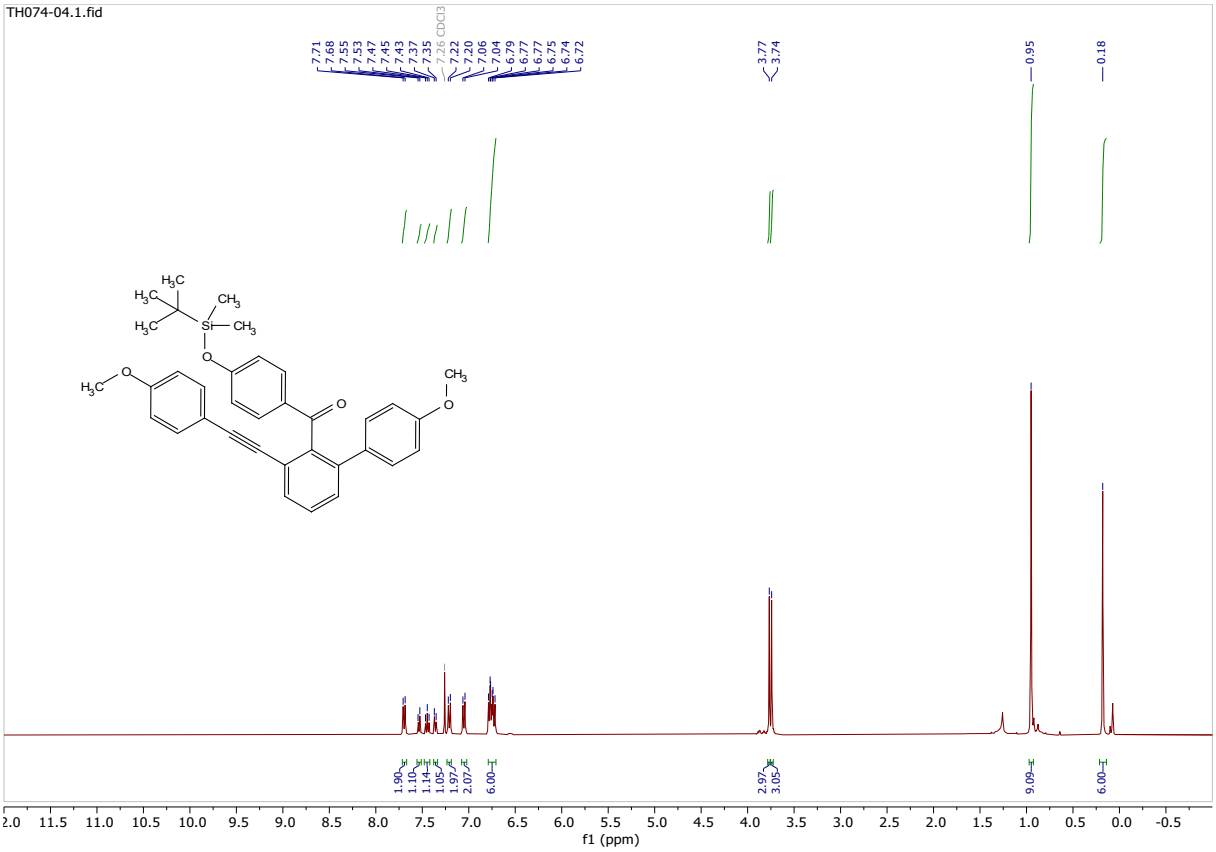
15 ^{1}H NMR (400 MHz, $CDCl_3$)



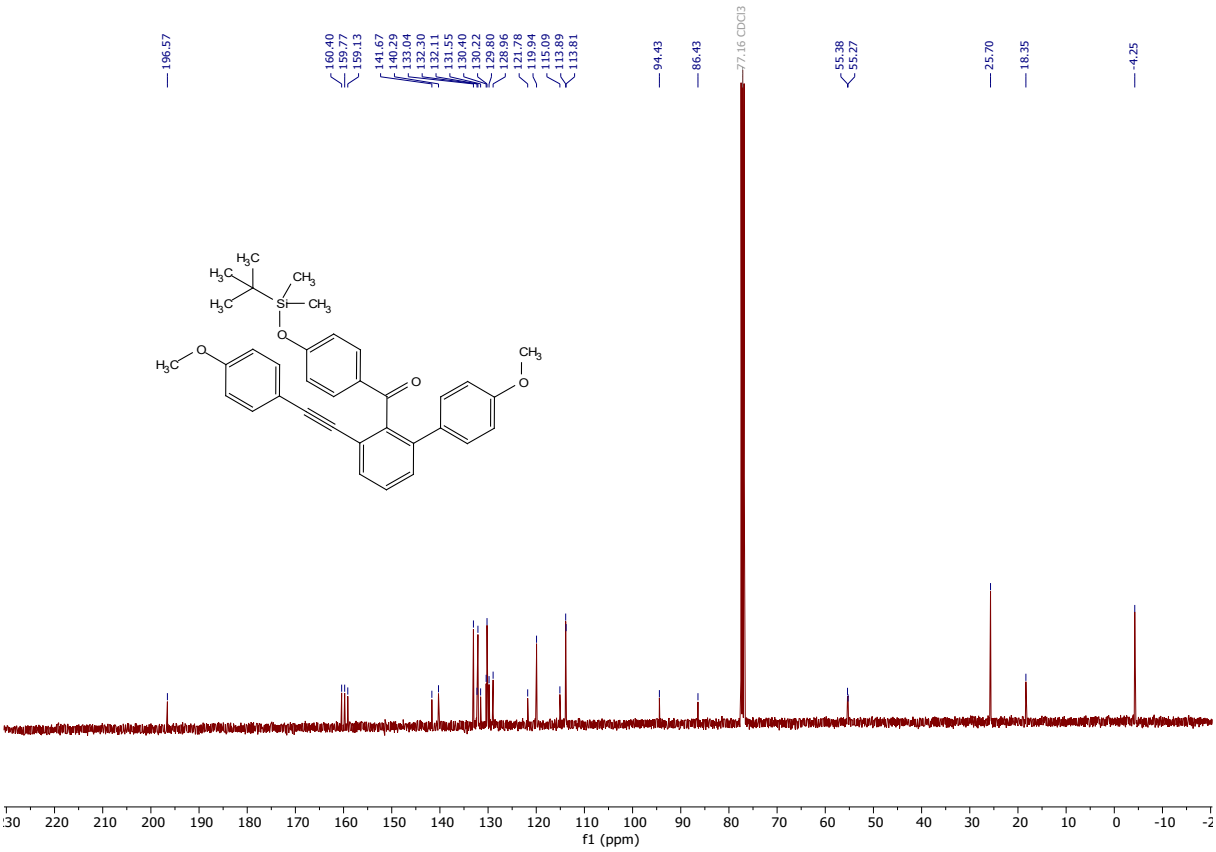
15 ^{13}C NMR (101 MHz, $CDCl_3$)



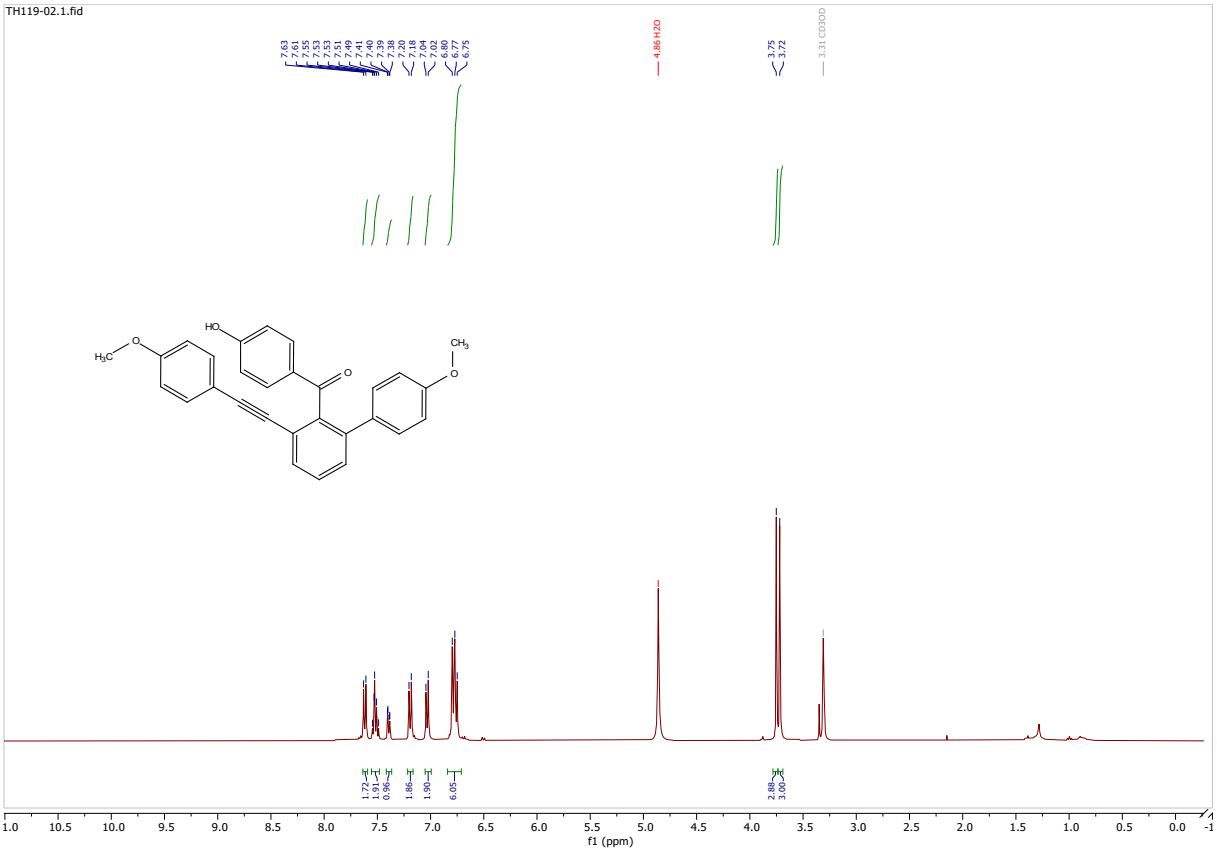
16 ^{1}H NMR (400 MHz, $CDCl_3$)



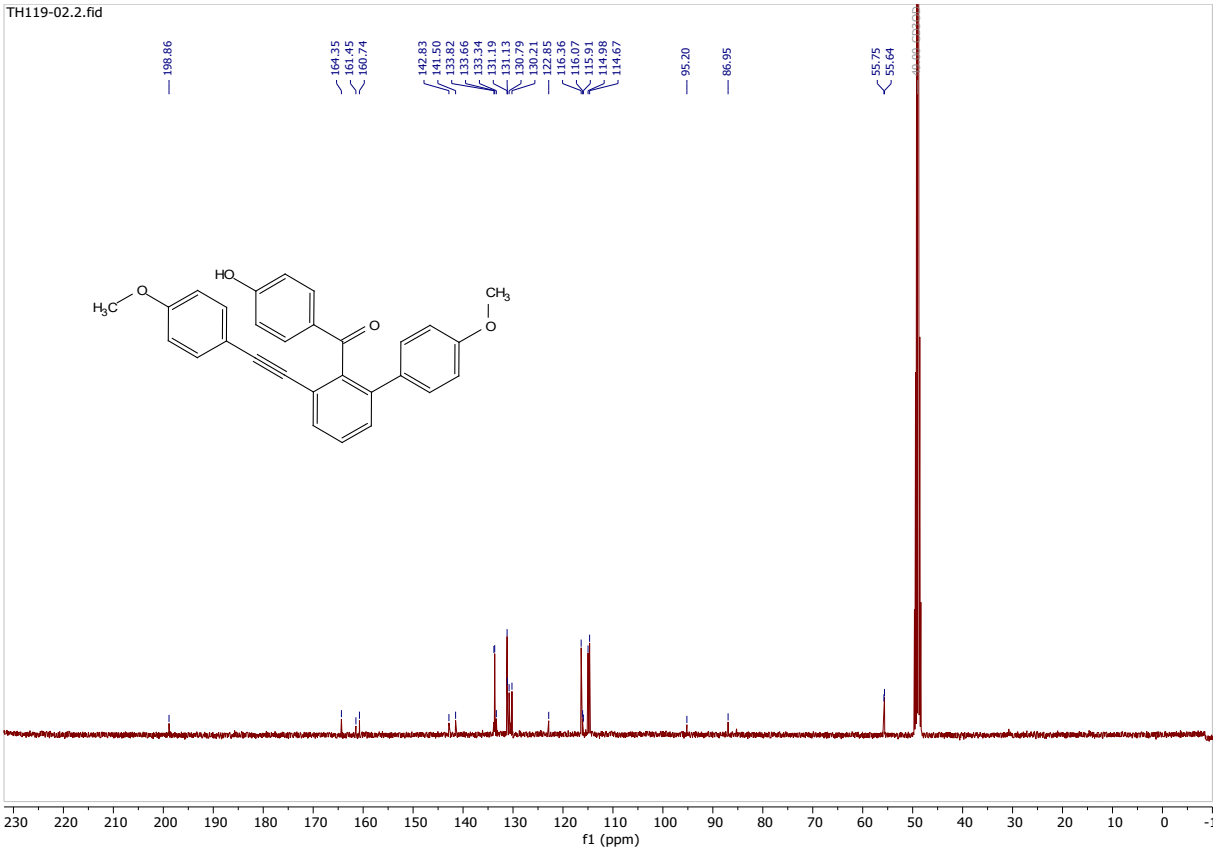
16 ^{13}C NMR (101 MHz, $CDCl_3$)



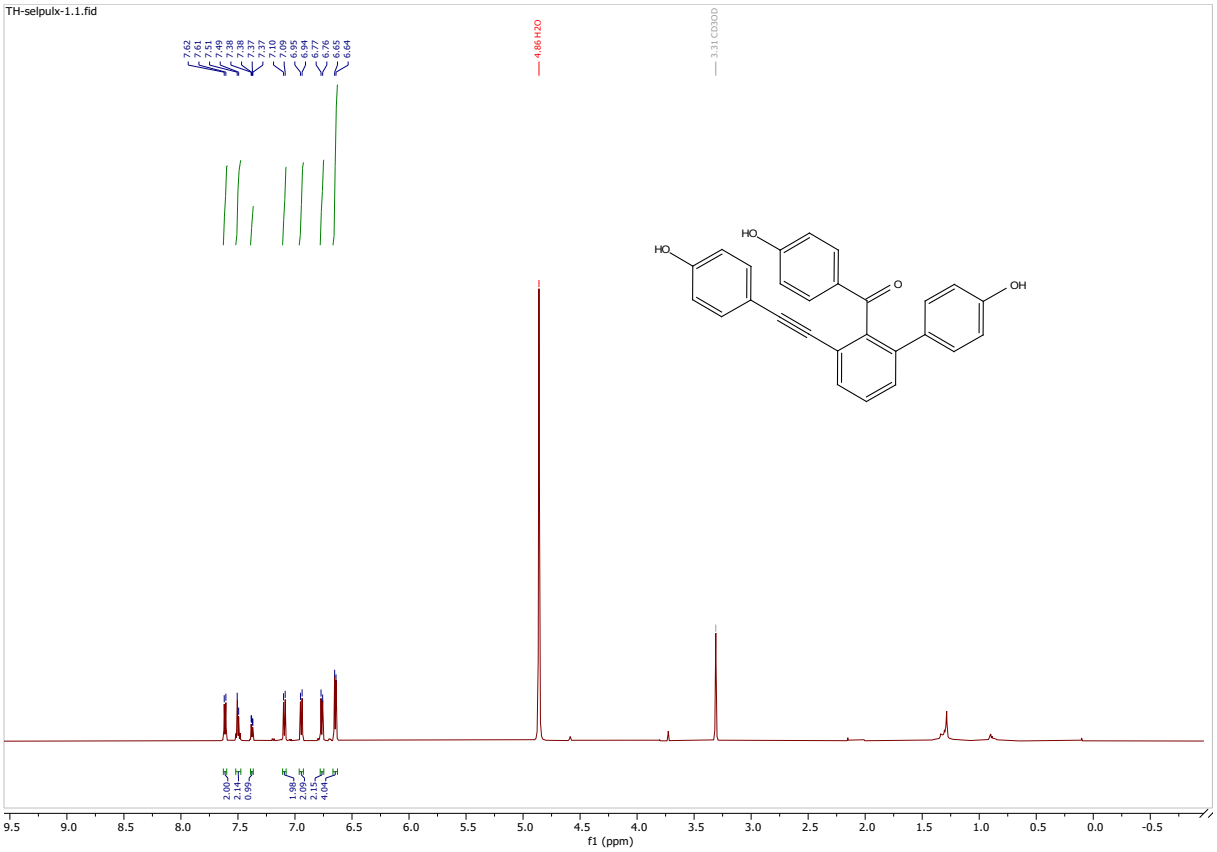
17 ^{1}H NMR (400 MHz, MeOD-d4)



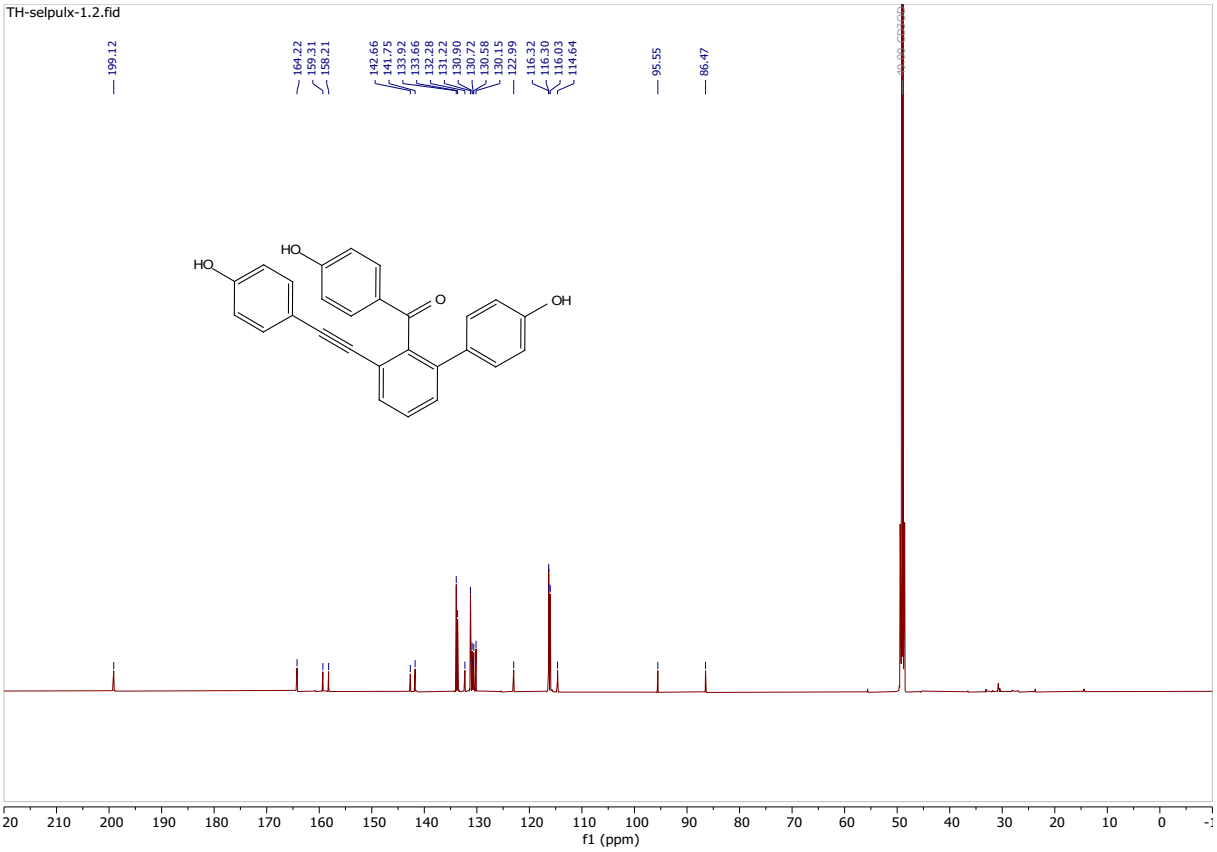
17 ^{13}C NMR (400 MHz, MeOD-d4)



5 ^{1}H NMR (600 MHz, MeOD-d4)



5 ^{13}C NMR (151 MHz, MeOD-d4)



4. X-Ray details

X-Ray section

The x-ray single crystal experiment of **17** was performed on Bruker D8 VENTURE Kappa Duo PHOTONIII by IμS micro-focus sealed tube CuKα (λ= 1.54178 Å) at low temperature. The position of atoms were determined by direct methods (XT)[2] and refined by full matrix least squares based on F^2 (SHELXL2019).[3] The hydrogen atoms on carbon were calculated into idealized positions (riding model) and assigned temperature factors either $H_{iso}(H)$ = 1.2 U_{eq}(pivot atom) or $H_{iso}(H)$ = 1.5 U_{eq} (pivot atom) for methyl moiety. The hydrogen atom in C-O-H moiety was found on difference Fourier map and was refined under assumption of rigid-body model. The significant intermolecular interaction is hydrogen bond O4-H4…O1$^{(i)}$ 2.7874(11) Å with angle on H4 161° (symmetry code (i) 1/2-x; 1/2+y;3/2-z) along twofold screw axis.

Crystal data for **17,** $C_{29}H_{22}O_4$, M_r = 434.46; Monoclinic, $P2_1/n$ (No 14), a = 9.4717 (5) Å, b = 12.7788 (7) Å, c = 18.5575 (10) Å, β = 100.343 (2)°, V = 2209.6 (2) Å^3, Z = 4, D_x = 1.306 Mg m^{-3}, temperature of sample 120(2) K, colorless prism of dimensions 0.22 × 0.13 × 0.08 mm, multi-scan absorption correction (μ = 0.70 mm^{-1}) T_{min} = 0.80, T_{max} = 0.94; a total of 31736 measured reflections (θ_{max}= 68.3˚), from which 4039 were unique (R_{int} = 0.024) and 3844 observed according to the $I > 2\sigma(I)$ criterion. The refinement converged (Δ/σ_{max}= 0.001) to R = 0.032 for observed reflections and w$R(F^2)$ = 0.081, GOF = 1.04 for 300 parameters and all 4039 diffractions. The final difference map displayed no peaks of chemical significance ($\Delta\rho_{max}$ = 0.22, $\Delta\rho_{min}$ -0.19 e.Å^{-3}).

X-ray crystallographic data have been deposited with the Cambridge Crystallographic Data Centre under deposition number CCDC 2376335 for **17** and can be obtained free of charge from the Centre via its website (https://www.ccdc.cam.ac.uk/structures/).

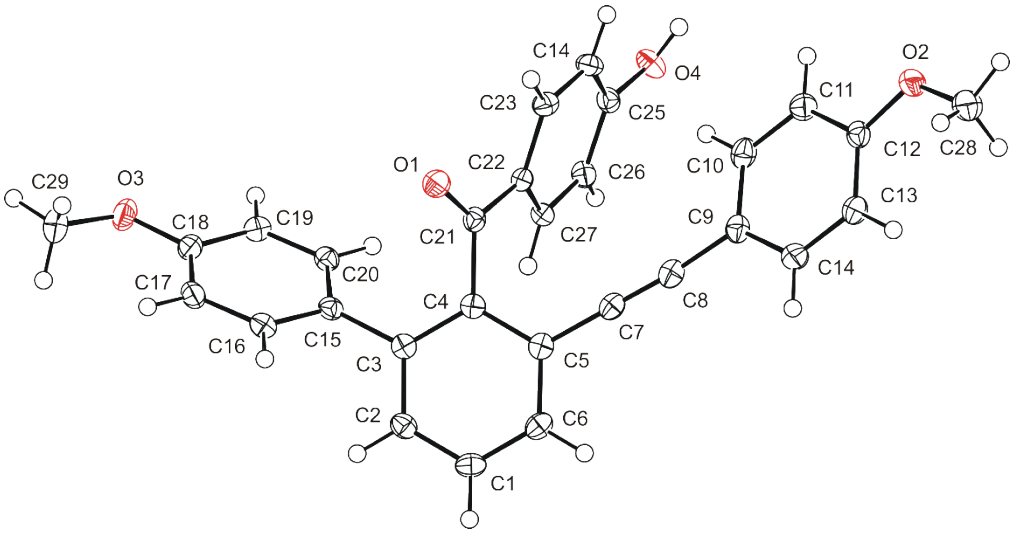


Fig.1. View on molecule of **17.** The displacement ellipsoids at a 50% probability level.

5. Comparisons of analytical data of isolated Selaginpulvilin X (5) and synthesized structure

In the case of ^{1}H spectra of the prepared compound **5**, we detected all the reported signals of the isolated compound **5** (Table 1).[1] We observed doublets with chemical shifts of 7.62, 7.10, 6.95, and 6.77 ppm. One doublet of doublet with a chemical shift of 7.39 ppm was present, and two multiplet areas with values of chemical shifts between 7.54 and 7.49 ppm and 6.66 ppm were observed.

^{1}H spectra of compound 5 in MeOD-d_4 (600 MHz)	
Isolated [ppm] (intensity, multiplicity, *J*)	**Prepared [ppm] (intensity, multiplicity, *J*)**
7.61 (2H, d, *J* = 8.8 Hz)	7.62 (2H, d, *J* = 8.7 Hz)
7.52 – 7.50 (1H, m) 7.51 – 7.48 (1H, m)	7.54 – 7.49 (2H, m)
7.38 (1H, dd, *J* = 6.8, 2.1 Hz)	7.39 (1H, dd, *J* = 6.8, 2.2 Hz)
7.09 (2H, d, *J* = 8.6 Hz)	7.10 (2H, d, *J* = 8.7 Hz)
6.94 (2H, d, *J* = 8.5 Hz)	6.95 (2H, d, *J* = 8.7 Hz)
6.76 (2H, d, *J* = 8.8 Hz)	6.77 (2H, d, *J* = 8.7 Hz)
6.65 (2H, d, *J* = 8.5 Hz) 6.65 (2H, d, *J* = 8.6 Hz)	6.66 (4H, m)

Table 1. Values of ^{1}H chemical shifts for isolated and prepared selaginpulvilin X (**5**)

In the case of ^{13}C spectra of the prepared compound **5**, we were able to detect all the reported signals of isolated compound **5** (Table 2).[1] Our measurement provided the spectra with a cleaner resolution compared to the original report, and therefore, we were able to differentiate a few carbons that were not differentiated in the previous work. For instance, we detected two signals, at 116.29 and 116.31 ppm, while in the original work only one signal at 116.3 ppm is reported for both carbons. Similarly, we could detect signal at 130.7, which was originally not reported by the authors, as it merged with other signal. In total, we detected 21 signals in the ^{13}C spectra which equals to the number of nonequivalent carbons present in selaginpulvilin X (**5**).

^{13}C spectra of compound 5 in MeOD-d_4 (151 MHz)	
Isolated [ppm]	**Prepared [ppm]**
199.1	199.1
164.3	164.2
159.3	159.3
158.2	158.2
142.6	142.7
141.7	141.7
133.9	133.9
133.7	133.7
132.3	132.3
131.2	131.2
130.9	130.9
Present but not reported	130.7
130.6	130.6
130.2	130.2
123.0	123.0
116.3	116.31
116.3	116.29
116.0	116.0
114.6	114.6
95.5	95.6
86.5	86.5

Table 2. Values of ^{13}C chemical shifts for isolated and prepared selaginpulvilin X (**5**)

6. References

1. Q.-F. Zhu, T.-T. Luo, Q. Chen, B.-B. Gao, A.-F. Zeng, J.-L. Ao, G.-B. Xu, S.-G. Liao and X. He, *Chemistry & Biodiversity*, 2023, **20**, e202300109.
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