Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

A-Ring Oxygenation Modulates the Chemistry and Bioactivity of Caged *Garcinia* Xanthones

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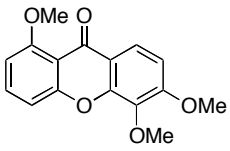
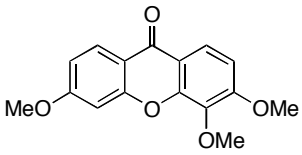
General Chemical Procedures

Unless indicated, all commercially available reagents and anhydrous solvents were purchased at the highest commercial quality and were used as received without further purification. All non-aqueous reactions were carried out under argon atmosphere using dry glassware that had been flame-dried under a stream of argon unless otherwise noted. Anhydrous tetrahydrofuran (THF) and dichloromethane (CH_2Cl_2) were obtained by passing commercially available pre-dried, oxygen-free formulations through activated alumina columns. Flash column chromatography was performed on silica gel (Merck Kieselgel 60, 230-400 mesh) using Hexanes-EtOAc or CH_2Cl_2-MeOH mixtures of increasing polarity. The progress of all the reactions was monitored by thin-layer chromatography (TLC) using glass plates precoated with silica gel-60 F_{254} to a thickness of 0.5 mm (Merck), and compounds were visualized by irradiation with UV light and/or by treatment with a solution of CAM stain or Seebach's stain followed by heating. ^{13}C NMR and ^{1}H NMR spectra were recorded on either 400MHz or 500 MHz Varian instrument or 500 MHz JEOL instrument. $CDCl_3$ was treated with anhydrous K_2CO_3, chemical shifts (δ) are quoted in parts per million (ppm) referenced to the appropriate residual solvent peak reference ($CDCl_3$ or CD_3OD), with the abbreviations s, br s, d, t, q, m, td, dt and qd denoting singlet, broad singlet, doublet, triplet, quartet, multiplet, quartet of doublets, triplet of doublets, doublet of triplets and quartet of doublets respectively. *J* = coupling constants given in Hertz (Hz). High-resolution Mass spectra (HRMS) were recorded on a trisector WG AutoSpecQ spectrometer. Compound **5** was prepared as reported in reference 10.

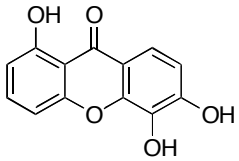
Experimental Procedures: Synthesis



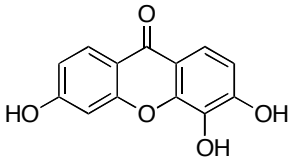
1,5,6-trimethoxy-9*H*-xanthen-9-one (9a). To a suspension of 2,6-dimethoxybenzoic acid **6a** (5 g, 27.1 mmol) in dry CH_2Cl_2 (120 mL), oxalyl chloride (11.6 mL, 136 mmol), and DMF (0.2 mL, 2.7 mmol) were added. The mixture was left stirring for 16 h, concentrated under reduced pressure, and dried under high vacuum for 2 h to afford 2,6-dimethoxybenzoyl chloride **7a**. To a solution of the above synthesized **7a** and 1,2,3-trimethoxy benzene **8** (5.1 g, 30.2 mmol) in dry ether (40 mL), aluminum trichloride (11 g, 82.4 mmol) was added at 0 °C and the solution was left stirring for 12 h at room temperature under inert atmosphere. A mixture of 15% hydrochloric acid and ethyl acetate (200 mL, 1:1) was then added. The ethyl acetate layer was collected, washed with brine, dried over magnesium sulfate, and concentrated under reduced pressure. The residue was suspended in a solution containing methanol (47 mL), water (31 mL), and NaOH (8.3 g, 208.6 mmol) at 0 °C and the solution was acidified with dilute HCl until pH = 2-3. The precipitate was filtered, washed with cold water, and dried to yield **9a** as yellow solid (7.0 g, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 12.0 Hz, 1H), 7.64 (d, J = 8.0 Hz, 1H), 7.11 (d, J = 4.0 Hz, 1H), 7.10 (s, 1H), 6.92 (d, J = 8.0 Hz, 1H), 3.91 (s, 3H), 3.87 (s, 3H), 3.86 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 160.8, 158.0, 157.4, 149.3, 136.1, 135.9, 122.0, 117.8, 111.9, 110.3, 109.9, 107.1, 61.6, 57.0, 56.8; HRMS (ESI) m/e 309.0732 [M+Na$^+$] calcd for $C_{16}H_{14}O_5Na^+$: 309.0733.



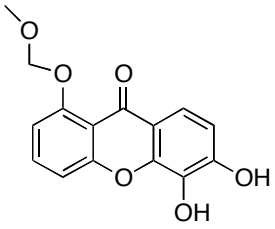
3,4,6-trimethoxy-9*H*-xanthen-9-one (9b). Same procedure as for **9a** was used to yield **9b** as a brown solid (4.5 g, 58% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, J = 8.0 Hz, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.20 (d, J = 4.0 Hz, 1H), 7.10 (s, 1H), 7.01 (dd, J = 8.0 Hz, 4.0 Hz, 1H), 4.03 (s, 3H), 4.00 (s, 3H), 3.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.2, 164.6, 157.4, 157.0, 149.8, 135.8, 127.3, 121.3, 115.5, 113.5, 109.4, 100.5, 60.9, 56.3, 56.1; HRMS (ESI) m/e 287.0912 [M+H$^+$] calcd for $C_{16}H_{15}O_5^+$: 287.0914.



1,5,6-trihydroxy-9*H*-xanthen-9-one (10a). To a solution of 48% hydrobromic acid (34 mL) and acetic acid (65 mL), **9a** (1.0 g, 3.49 mmol) was added and the mixture was stirred for 18 h at 120 ˚C. Upon completion, the solution was cooled to 0 °C and 10% NaOH solution was added until pH = 3-4. The precipitate was filtered, washed with cold water, and dried to yield **10a** as a yellow solid (807 mg, 95% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.73 (t, J = 9.0 Hz, 1H), 7.59 (d, J = 12.0 Hz, 1H), 7.07 (d, J = 9.0 Hz, 1H), 6.99 (d, J = 12.0 Hz, 1H), 6.80 (d, J = 9.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.3, 161.2, 155.8, 152.6, 146.5, 136.8, 132.6, 116.3, 113.2, 109.9, 107.6, 107.1; HRMS (ESI) m/e 243.0298 $[M-H]^-$ calcd for $C_{13}H_7O_5^-$: 243.0299.



3,4,6-trihydroxy-9*H*-xanthen-9-one (10b). Same procedure as for **10a** was used with **9b** (1 g, 3.49 mmol) to yield **10b** as a brown solid (692 mg, 81% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (s br, 1H), 10.31 (s br, 1H), 9.32 (s br, 1H), 7.97 (d, J = 8.0 Hz, 1H), 7.49 (d, J = 8.0 Hz, 1H), 6.85 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.11, 164.0, 158.1, 151.6, 146.9, 133.2, 128.5, 117.0, 115.4, 114.4, 114.3, 113.4, 102.8; HRMS (ESI) m/e 245.0446 $[M+H]^+$ calcd for $C_{13}H_9O_5^+$: 245.0444.

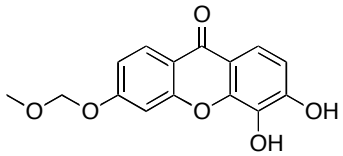


5,6-dihydroxy-1-(methoxymethoxy)-9*H*-xanthen-9-one (11a). To a solution of **10a** (770 mg, 3.15 mmol) in diphenyl ether (28 mL), α,α-dichlorodiphenyl methane (0.97 mL, 5.04 mmol) was added and the mixture was stirred for 4 h at 175 °C. Upon completion, the reaction mixture was

cooled to 60 °C and was poured into petroleum ether (200 mL). The precipitate was filtered, washed with petroleum ether, and dried under vacuum to yield the diphenyl protected diol as a brown solid (1.2 g, 94% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90 (d, J = 8.0 Hz, 1H), 7.64 (m, 3H), 7.57 (t, J = 8.0 Hz, 1H), 7.42 (m, 5H), 7.32 (t, J = 8.0 Hz, 1H), 7.00 (m, 3H), 6.79 (d, J = 8.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 181.5, 162.3, 156.1, 153.4, 141.1, 139.2, 136.8, 134.0, 129.9, 129.8, 128.6, 126.5, 123.4, 121.3, 119.0, 116.9, 111.1, 108.6, 107.1, 106.5; HRMS (ESI) m/e 409.1069 $[M+H]^+$ calcd for $C_{26}H_{17}O_5^+$: 409.1071.

To a solution of the above intermediate (500 mg, 1.22 mmol) in acetone (10 mL), sodium hydride (73 mg, 3.06 mmol) was added and the mixture was stirred for 30 min at 0 °C. To this mixture, MOMCl (0.19 mL, 2.45 mmol) was added dropwise and the reaction left stirring at room temperature for 12 h. Upon completion, the mixture was poured into ice water and the precipitate was filtered, washed with brine, and dried under high vacuum to yield the fully protected xanthone as a yellow powder (546 mg, 99% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, J = 8.5 Hz, 1H), 7.65 (m, 4H), 7.56 (t, J = 8.5 Hz, 1H), 7.41 (m, 6 H), 7.18 (d, J = 8.0 Hz, 1H), 7.05 (d, J = 8.5 Hz, 1H), 6.95 (d, J = 8.5 Hz, 1H), 5.37 (s, 2H), 3.57 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.9, 158.7, 158.0, 152.7, 140.5, 139.9, 135.1, 130.2, 128.9, 127.0, 122.2, 119.9, 111.9, 111.3, 106.5, 96.0, 57.2, 30.2; HRMS (ESI) m/e 475.1151 $[M+Na]^+$ calcd for $C_{28}H_{20}O_6Na^+$: 475.1152.

To a solution of the above intermediate (276 mg, 0.61 mmol) in methanol: THF (6 mL), $NaHCO_3$ (3.05 eq, 256 mg) and 10% Pd/C (27 mg) were added and the mixture was stirred under hydrogen atmosphere for 18 h at room temperature. Upon completion, the mixture was filtered through a plug of celite and was concentrated under reduced pressure. The residue was washed with hexanes and dried under vacuum to yield **11a** as a light brown solid (105 mg, 60% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.56 (t, J = 8.0 Hz, 1H), 7.33 (d, J = 8.5 Hz, 1H), 7.10 (d, J = 8.0 Hz, 1H), 6.93 (d, J = 8.0 Hz, 1H), 6.59 (d, J = 8.5 Hz, 1H), 5.25 (s, 2H), 3.44 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.4, 157.1, 151.1, 145.1, 134.3, 132.2, 128.7, 128.3, 125.9, 116.3, 112.8, 111.0, 110.5, 95.1, 55.9; HRMS (ESI) m/e 311.0524 $[M+Na]^+$ calcd for $C_{15}H_{12}O_6Na^+$: 311.0526.



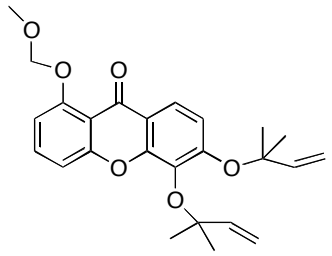
3,4-dihydroxy-6-(methoxymethoxy)-9*H*-xanthen-9-one (11b). Same procedure as for **11a** was used. **10b** (209 mg, 0.86 mmol) yielded diphenyl protected xanthone as a brown solid (290 mg,

83% yield. Diphenyl protected xanthone (217 mg, 0.531 mmol) yielded fully protected xanthone as a yellow powder (230 mg, 95% yield). Fully protected xanthone (350 mg, 0.77 mmol) yielded free diol **11b** as a green powder (121 mg, 54% yield).

The di-phenyl protected intermediate: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.10 (d, J = 9.6 Hz, 1H), 7.86 (d, J = 8.5 Hz, 1H), 7.67 (m, 4H), 7.48 (m, 6H), 7.12 (d, J = 8.5 Hz, 1H), 6.96 (m, 2H); ^{13}C NMR (75 MHz, acetone-d_6) δ 174.0, 163.7, 157.8, 152.3, 145.0, 142.0, 134.5, 139.6, 129.9, 128.8, 128.6, 126.4, 121.4, 119.6, 118.4, 114.8, 113.9, 106.1, 102.5; HRMS (ESI) m/e 409.1072 $[M+H]^+$ calcd for $C_{26}H_{17}O_5^+$: 409.1071.

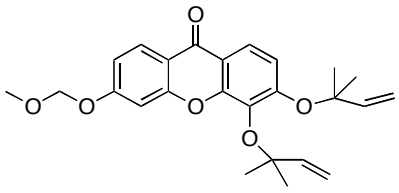
The di-phenyl and MOM protected intermediate: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (d, J = 9.0 Hz, 1H), 7.80 (d, J = 8.5 Hz, 1H), 7.61 (m, 4H), 7.49 (m, 6H), 7.28 (s, 1H), 7.24 (d, J = 8.5 Hz, 1H), 7.11 (d, J = 8.0 Hz, 1H), 5.40 (s, 2H), 3.43 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.8, 162.1, 156.7, 151.6, 140.4, 138.7, 133.1, 129.9, 128.8, 127.9, 125.9, 125.7, 121.2, 119.4, 117.7, 115.4, 114.5, 106.5, 103.1, 94.1, 93.9, 56.1; HRMS (ESI) m/e 453.1329 $[M+H]^+$ calcd for $C_{28}H_{21}O_6^+$: 453.1333.

11b: ^{1}H NMR (400 MHz, acetone-d_6) δ 8.14 (d, J = 8.0 Hz, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.14 (s, 1H), 7.06 (d, J = 8.0 Hz, 1H), 6.96 (d, J = 8.0 Hz, 1H), 5.37 (s, 2H), 3.49 (s, 3H); ^{13}C NMR (100 MHz, acetone-d_6) δ 174.7, 162.4, 157.7, 150.7, 146.5, 132.6, 128.0, 117.2, 116.2, 115.7, 114.2, 112.9, 102.9, 94.5, 55.8; HRMS (ESI) m/e 289.0704 $[M+H]^+$ calcd for $C_{15}H_{13}O_6^+$: 289.0707.

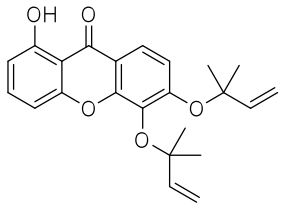


1-(methoxymethoxy)-5,6-bis((2-methylbut-3-en-2-yl)oxy)-9*H*-xanthen-9-one (13a). To a 50 mL round bottom flask was added **11a** (70 mg, 0.24 mmol) followed by dry THF (1.3 mL). The flask was degassed by argon and was placed in an ice water bath. To the homogenous solution was added carbonate **12** (482 mg, 2.43 mmol), via syringe, followed by $Pd(PPh_3)_4$ (28 mg, 0.0243 mmol). The reaction vessel was stirred under argon at 5 ºC for 2 hours. Upon completion, the solvent was removed by rotary evaporation and the crude material was purified through flash chromatography (5-15% EtOAc-hexanes) to yield **13a** as a white solid (64 mg, 63% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, J = 8.0 Hz, 1H), 7.55 (t, J = 8.0 Hz, 1H), 7.14 (d, J = 8.0 Hz, 1H), 7.08 (d, J = 8.0 Hz, 1H), 7.04 (d, J = 8.0 Hz, 1H), 6.31-6.15 (m, 2H), 5.38 (s, 2H), 5.22-5.16

(m, 3H), 5.02 (d, J = 8.0 Hz, 1H), 3.57 (s, 3H), 1.57 (s, 6H), 1.56 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.4, 158.1, 158.0, 156.6, 151.6, 143.9, 143.7, 135.6, 134.5, 121.3, 117.1, 114.3, 113.3, 113.3, 111.7, 110.6, 95.6, 83.7, 82.3, 56.8, 29.9, 27.4, 27.2; HRMS (ESI) m/e 447.1779 $[M+Na]^+$ calcd for $C_{25}H_{28}O_6Na^+$: 447.1778.

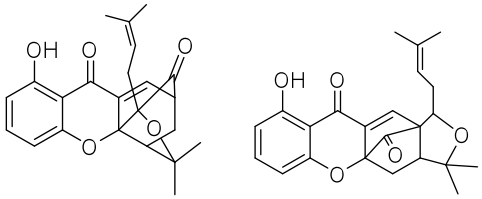


6-(methoxymethoxy)-3,4-bis((2-methylbut-3-en-2-yl)oxy)-9*H*-xanthen-9-one (13b). Same procedure for **13a** was used. **11b** (80 mg, 0.28 mmol) yielded **13b** as a white solid (100 mg, 84% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, J = 8.0 Hz, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.11-7.02 (m, 3H), 6.31-6.15 (m, 2H), 5.28 (s, 2H), 5.22-5.16 (m, 3H), 5.02 (d, J = 8.0 Hz, 1H), 3.52 (s, 3H), 1.58 (s, 6H), 1.56 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.0, 162.2, 157.4, 156.5, 152.5, 143.6, 143.5, 135.8, 128.2, 120.9, 117.2, 116.9, 116.3, 114.0, 113.6, 113.0, 103.3, 94.3, 83.5, 82.1, 56.4, 27.1, 26.9; HRMS (ESI) m/e 447.1776 $[M+Na]^+$ calcd for $C_{25}H_{28}O_6Na^+$: 447.1778.



1-hydroxy-5,6-bis((2-methylbut-3-en-2-yl)oxy)-9*H*-xanthen-9-one (14). To a solution of **13a** (130 mg, 0.31 mmol) in THF (1 mL), $ZnCl_2$ (1M in ether, 0.31 mL, 0.31 mmol) was added and the mixture was left stirring at 40 °C for 2 hours. The solvent was then removed by rotary evaporation and the crude material was purified through flash column chromatography (5% EtOAc-hexane) to yield **14** (110 mg, 93%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (d, J = 8.0 Hz, 1H), 7.54 (t, J = 8.0 Hz, 1H), 7.13 (d, J = 8.0 Hz, 1H), 6.92 (d, J = 8.0 Hz, 1H), 6.76 (d, J = 8.0 Hz, 1H), 6.27-6.14 (m, 2H), 5.23-5.16 (m, 3H), 5.02 (d, J = 8.0 Hz, 1H), 1.57 (s, 6H), 1.56 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.0, 162.0, 157.8, 156.2, 152.6, 143.6, 143.4, 136.4, 135.6,

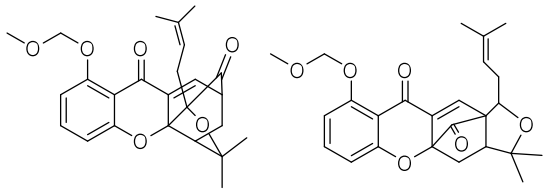
120.5, 116.7, 115.7, 114.4, 113.3, 110.4, 108.6, 107.0, 83.8, 82.5, 27.3, 27.0; HRMS (ESI) m/e 381.1699 $[M+H]^+$ calcd for $C_{23}H_{25}O_5^+$: 381.1697.



8-hydroxy-2,2-dimethyl-3a-(3-methylbut-2-en-1-yl)-1,2-dihydro-1,5-methanofuro[2,3-d]xanthene-4,7(3a*H*,5*H*)-dione (3). A solution of **14** (13 mg, 34 μmol) in DMF (340 μL) was left stirring at 120 °C for 1 hour. The mixture was then cooled to room temperature and the solvent was removed by rotary evaporation. The crude material was then purified by preparative TLC (5% EtOAc-hexanes) to yield the regular caged xanthone **3** (12 mg, 92%) along with the neo isomer (1 mg, 8%).

3: ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.08 (s, 1H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.38 (t, *J* = 8.0 Hz, 1H), 6.51 (m, 2H), 4.38 (m, 1H), 3.51 (m, 1H), 2.62-2.32 (m, 4H), 1.69 (s, 3H), 1.35 (s, 3H), 1.29 (s, 3H), 0.99 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.0, 181.5, 163.0, 159.8, 139.0, 135.5, 135.1, 134.0, 118.9, 109.5, 107.6, 106.3, 90.2, 84.7, 83.8, 49.0, 47.2, 30.5, 29.4, 29.3, 25.7, 25.1, 16.9; HRMS (ESI) m/e 381.1696 $[M+H]^+$ calcd for $C_{23}H_{25}O_5^+$: 381.1697.

neo isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.01 (s, 1H), 7.42 (t, *J* = 8.6 Hz, 1H), 7.30 (d, *J* = 6.9 Hz, 1H), 6.64 (d, *J* = 7.5 Hz, 1H), 6.53 (d, *J* = 7.5 Hz, 1H), 4.99 (m, 1H), 3.78 (m, 1H), 2.54 (d, *J* = 13.2 Hz, 1H), 2.50 (m, 1H), 2.16 (m, 2H), 1.85 (dd, *J* = 13.8 Hz, 10.3 Hz, 1H), 1.72 (s, 3H), 1.60 (s, 3H), 1.38 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.6, 180.0, 163.0, 160.1, 139.4, 136.6, 136.4, 134.6, 117.4, 109.8, 107.9, 106.6, 84.0, 83.9, 79.2, 45.1, 42.2, 33.1, 30.4, 29.7, 26.8, 26.0, 18.3; HRMS (ESI) m/e 381.1698 $[M+H]^+$ calcd for $C_{23}H_2O_5^+$: 381.1697.

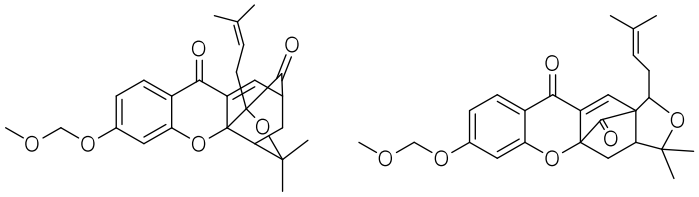


8-(methoxymethoxy)-2,2-dimethyl-3a-(3-methylbut-2-en-1-yl)-1,2-dihydro-1,5-methanofuro[2,3-*d*]xanthene-4,7(3a*H*,5*H*)-dione (15). A solution of **13a** (10 mg, 23.6 μmol) in DMF (240 μL) was left stirring at 120 °C for 1 hour. The mixture was then cooled to room temperature and the solvent was removed by rotary evaporation. The crude material was then

purified by preparative TLC (5% EtOAc-hexanes) to yield the regular caged xanthone **15** (8 mg, 80%) along with the neo isomer **16** (2 mg, 20%).

15: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (t, *J* = 8.0 Hz, 1H), 7.28 (d, *J* = 6.8 Hz, 1H), 6.78 (d, *J* = 8.4 Hz, 1H), 6.71 (d, *J* = 8.4 Hz, 1H), 5.29 (s, 2H), 4.46 (m, 1H), 3.51 (s, 3H), 3.44 (m, 1H), 2.60 (m, 2H), 2.39 (d, *J* = 9.3 Hz,1H), 2.29 (dd, *J* = 13.2 Hz, 4.4 Hz, 1H), 1.68 (s, 3H), 1.37 (s, 3H), 1.27 (s, 3H), 1.25 (m, 1H), 1.07 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.4, 175.8, 161.1, 158.4, 136.5, 136.1, 134.9, 132.6, 118.7, 111.7, 108.7, 94.9, 89.9, 84.5, 83.5, 56.6, 48.6, 46.8, 30.4, 29.1, 25.7, 25.6, 17.1; HRMS (ESI) m/e 447.1777 $[M+Na]^+$ calcd for $C_{25}H_{28}O_6Na^+$: 447.1778.

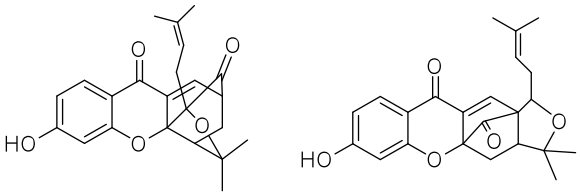
neo isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 (t, *J* = 8.6 Hz, 1H), 7.10 (d, *J* = 6.9 Hz, 1H), 6.86 (d, *J* = 7.5 Hz, 1H), 6.78 (d, *J* = 7.5 Hz, 1H), 5.27 (s, 2H), 5.06 (m, 1H), 3.72 (m, 1H), 3.51 (s, 3H), 2.49 (m, 2H), 2.17 (m, 1H), 2.01 (m, 1H), 1.84 (m, 1H), 1.71 (s, 3H), 1.58 (s, 3H), 1.37 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 200.6, 175.2, 161.9, 158.3, 137.8, 136.6, 136.2, 133.5, 117.5, 112.0, 111.5, 108.8, 95.0, 83.8, 78.9, 56.6, 44.5, 42.5, 32.7, 30.0, 29.7, 26.8, 26.0, 18.3; HRMS (ESI) m/e 447.1780 $[M+Na]^+$ calcd for $C_{25}H_{28}O_6Na^+$: 447.1778.



10-(methoxymethoxy)-2,2-dimethyl-3a-(3-methylbut-2-en-1-yl)-1,2-dihydro-1,5-methanofuro[2,3-*d*]xanthene-4,7(3a*H*,5*H*)-dione (19). A solution of **13b** (15 mg, 39 μmol) in DMF (340 μL) was left stirring at 120 °C for 1 hour. The mixture was then cooled to room temperature and the solvent was removed by rotary evaporation. The crude material was then purified by preparative TLC (5% EtOAc-hexanes) to yield the regular caged xanthone **19** (12 mg, 80%) along with the neo isomer **20** (3 mg, 20%).

19: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 (d, *J* = 10.7 Hz, 1H), 7.40 (d, *J* = 8.2 Hz, 1H), 6.74 (dd, *J* = 8.4 Hz, 2.5 Hz, 1H), 6.65 (d, *J* = 2.5 Hz, 1H), 5.23 (s, 2H), 4.44 (m, 1H), 3.48 (s, 3H), 3.48 (m, 1H), 2.61 (m, 2H), 2.44 (d, *J* = 11.9 Hz,1H), 2.32 (dd, *J* = 16.9 Hz, 5.7 Hz, 1H), 1.71 (s, 3H), 1.33 (s, 3H), 1.30 (s, 3H), 1.00 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.4, 175.5, 163.9, 161.5, 134.9, 134.8, 133.3, 128.9, 118.7, 113.9, 111.6, 103.5, 94.1, 90.8, 84.6, 83.6, 60.5, 56.5, 48.9, 46.8, 30.5, 29.2, 29.1, 25.5, 25.3, 21.2, 16.9, 14.3; HRMS (ESI) m/e 447.1775 $[M+Na]^+$ calcd for $C_{25}H_{28}O_6Na^+$: 447.1778.

neo isomer **20**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (d, *J* = 8.6 Hz, 1H), 7.21 (d, *J* = 6.9 Hz, 1H), 6.81 (d, *J* = 2.3 Hz, 1H), 6.70 (dd, *J* = 7.5 Hz, 2.3 Hz, 1H), 5.25 (d, *J* = 6.9 Hz, 1H), 5.18 (d, *J* = 6.9 Hz, 1H), 5.03 (m, 1H), 3.75 (m, 1H), 3.48 (s, 3H), 2.49 (m, 2H), 2.13 (m, 1H), 2.06 (m, 1H), 1.86 (m, 1H), 1.72 (s, 3H), 1.59 (s, 3H), 1.37 (s, 3H), 1.34 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 200.2, 174.4, 164.3, 162.3, 136.3, 136.0, 134.3, 129.0, 117.5, 112.1, 103.8, 94.2, 84.6, 83.9, 79.1, 56.6, 44.7, 42.1, 33.1, 30.2, 29.7, 26.8. 26.0, 18.3, 14.3; HRMS (ESI) m/e 447.1781 $[M+Na]^+$ calcd for $C_{25}H_{28}O_6Na^+$: 447.1778.



10-hydroxy-2,2-dimethyl-3a-(3-methylbut-2-en-1-yl)-1,2-dihydro-1,5-methanofuro[2,3-*d*]xanthene-4,7(3a*H*,5*H*)-dione (4). To a solution of **19** (1.5 mg, 3.5 μmol) or neo-isomer **20** (1.5 mg, 3.5 μmol) in THF (20 μL), $ZnCl_2$ (10 eq, 1 M in ether) was added and the mixture was left stirring at 40 °C for 24 hours. The solvent was then removed by rotary evaporation and the crude material was purified through preparative TLC (20% EtOAc-hexane) to yield **4** (1 mg, 75%) or neo-isomer **21** (1 mg, 75%).

4: ^{1}H NMR (500 MHz, CD_3OD) δ 7.74 (d, *J* = 8.6 Hz, 1H), 7.33 (d, *J* = 6.9 Hz, 1H), 6.54 (dd, *J* = 8.6 Hz, 2.3 Hz, 1H), 6.39 (d, *J* = 2.3 Hz, 1H), 4.37 (m, 1H), 3.44 (m, 1H), 2.59-2.32 (m, 4H), 1.68 (s, 3H), 1.31 (s, 3H), 1.25 (s, 3H), 1.00 (s, 3H);; ^{13}C NMR (125 MHz, CD_3OD) δ 203.4, 175.8, 166.0, 162.1, 134.9, 134.2, 133.4, 128.7, 118.6, 112.0, 111.3, 102.4, 90.6, 84.4, 83.6, 60.2, 29.3, 28.7, 28.0, 24.7, 24.4, 15.7, 13.1; HRMS (ESI) m/e 381.1696 $[M+H]^+$ calcd for $C_{23}H_{25}O_5^+$: 381.1697.

neo isomer **21**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.72 (d, *J* = 8.6 Hz, 1H), 7.19 (d, *J* = 7.5 Hz, 1H), 6.53 (d, *J* = 10.9 Hz, 1H), 6.48 (s, 1H), 4.94 (m, 1H), 3.83 (m, 1H), 2.47 (d, *J* = 13.2 Hz, 1H), 2.38 (m, 1H), 2.24 (m, 2H), 2.18 (m, 1H), 1.88 (m, 1H), 1.67 (s, 3H), 1.59 (s, 3H), 1.32 (s, 3H), 1.27 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 200.6, 180.1, 166.2, 162.8, 135.9, 135.3, 134.7, 128.7, 117.4, 111.5, 102.6, 101.7, 84.4, 84.0, 79.2, 44.8, 41.6, 32.4, 30.0, 28.5, 25.4, 24.7, 16.7; HRMS (ESI) m/e 381.1699 $[M+H]^+$ calcd for $C_{23}H_2O_5^+$: 381.1697.

Experimental Procedures: Biological Assays

^{3}H-thymidine incorporation assay: CEM cells were plated in a 96-well plate at 20,000 cells/well in RPMI supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 units/ml of penicillin/streptomycin (complete medium). The caged *Garcinia* xanthones were added to the cells at increasing concentrations and 0.1% DMSO was added to control cells. Cells were incubated for 48 h and then pulsed with 3H-thymidine for 7 h. Incorporation of ^{3}H-thymidine was determined in a scintillation counter (Beckman Coulter Inc., Fullerton, CA) after cells were washed and deposited onto glass microfiber filters using a cell harvester M-24 (Brandel, Gaithersburg, MD).

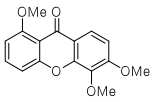
Cells, antibodies and reagents: HeLa cells were grown in DMEM supplemented with 10% FBS. For immunofluorescence and western blot the following antibodies were used: mouse anti-Tom20 (BD Transduction Laboratories, #612278); rabbit anti-cleaved Caspase-9 (Cell Signaling, D315 human spasific); rabbit anti-cleaved Caspase-3 (Cell Signaling, #96645); rabbit anti-beta Tubulin (Abcam, ab6046).

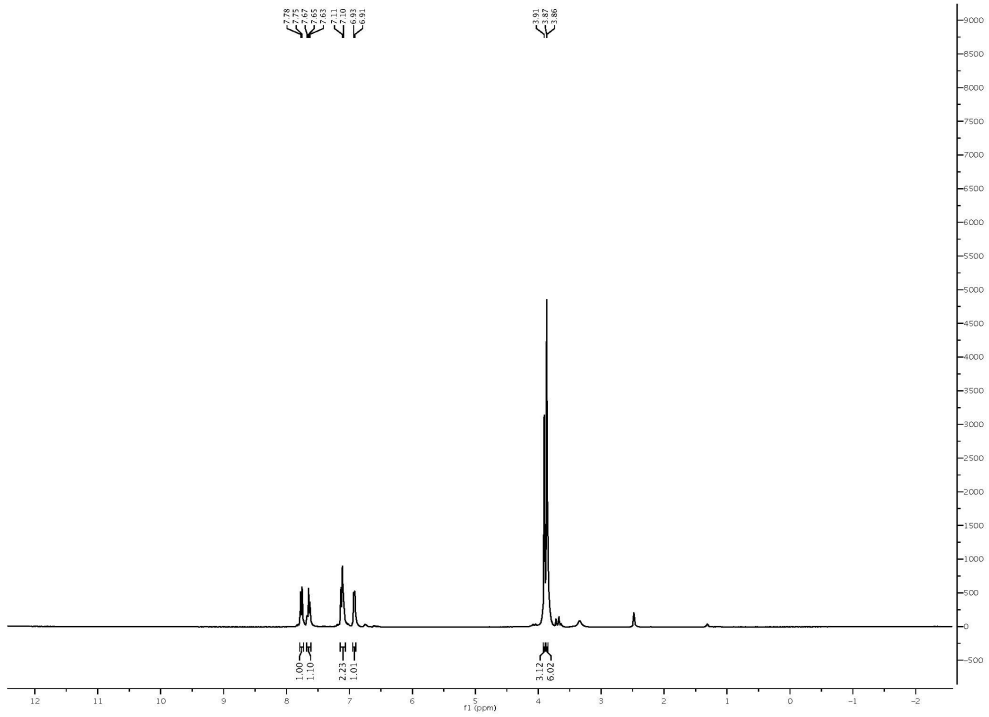
Immunofluorescence analysis: HeLa cells plated on 12mm glass coverslips were fixed with 4% formaldehyde, permeabilized with 0.1% TritonX100 in PBS for 5 min, then incubated in blocking buffer (PBS containing 5% fetal goat serum) for 30 min at room temperature. The cells were then incubated for 1 h at room temperature in primary antibody diluted in blocking buffer. The cells were then washed three times with PBS and incubated with secondary antibody, diluted in blocking buffer, for 1 h at room temperature. Alexa Fluor 594 goat anti mouse (1:500) from Molecular Probes was used. Cells were washed three times with PBS containing Hoechst (1:100,000) (H33342, Molecular Probes) to stain DNA. Coverslips were then mounted onto glass slides and visualized using a Zeiss Observer Z1 156 inverted microscope with 63× objective controlled by 157 AxioVision software (Zeiss, Thornwood, NY).

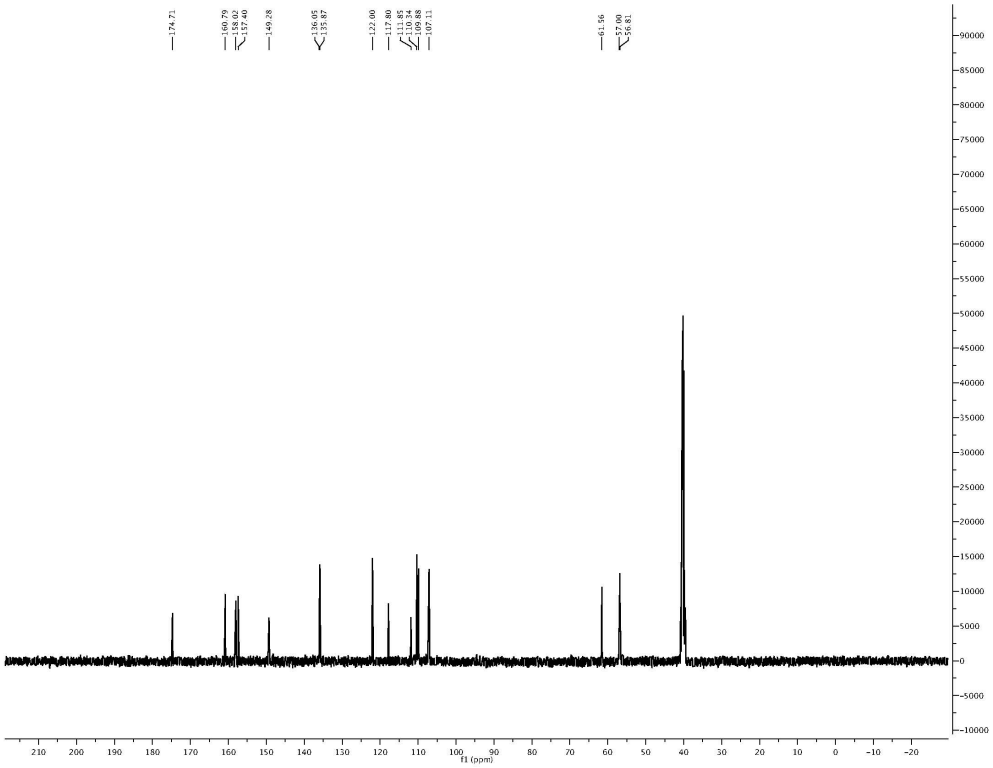
Immunoblot blot analysis: Cells were lysed with PAGE loading buffer (60 mM Tris, pH 6.8, 5% 2-mercaptoethanol, 2% SDS, 0.01% Bromophenol blue, and 10% glycerol). Proteins in the lysate were separated by SDS–PAGE using a 12% running gel. Proteins were transferred on a nitrocellulose membrane (Western Blot) (60 min, 350 mA) that was then kept in blocking buffer (50 mN, Tris, pH 7.5, 150 mM NaCl, 0.05% Tween 20, and 3% BSA) for 1 h. The membrane was incubated for overnight at 4 °C with the primary antibodies diluted in blocking buffer. After washing 3 times with TBS-T buffer, the membranes were incubated for 1 h at room temperature in secondary antibody (anti rabbit IgG HRP-linked, GE Healthcare #NA934V) diluted in

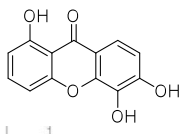
blocking buffer. After 3 more washing steps in TBS-T, the reagent for ECL was added (Perkin-Elmer). Kodak Biomax films were used for exposure.

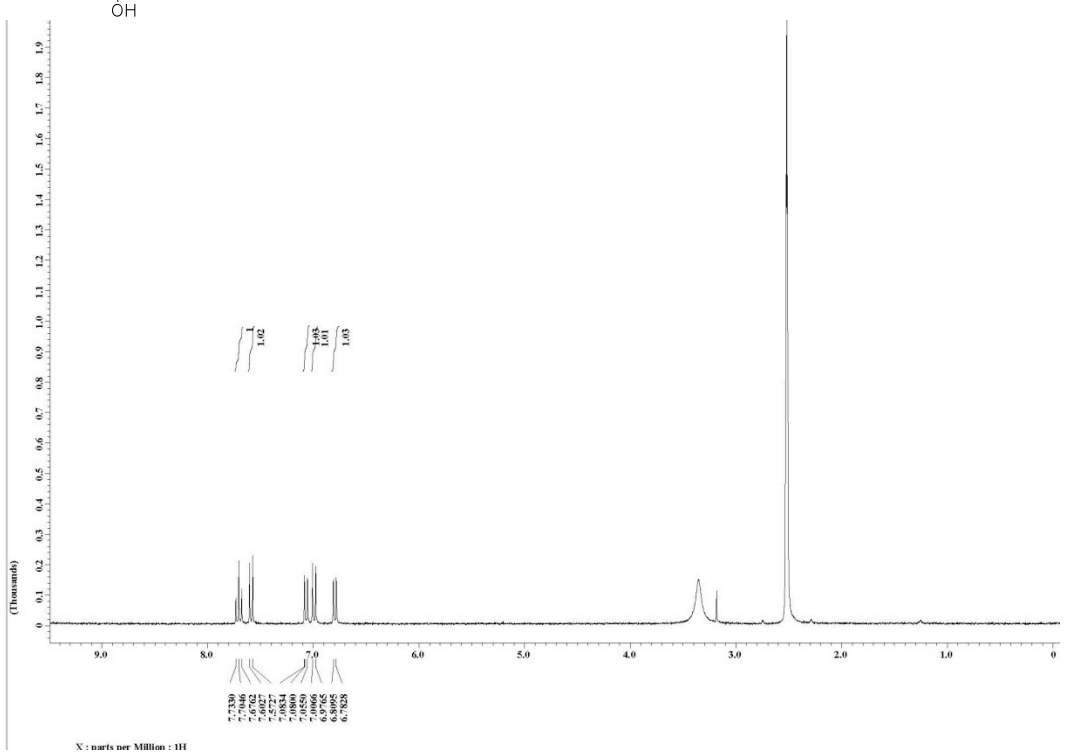
Hsp90 client assay: SKBR3 cells were seeded at 250,000 cells per well in a 6-well plate and were left to grow in DMEM/F12 supplemented with 10% fetal bovine serum for 48 hours. Different concentrations of GA and CLV were prepared in DMSO and used to treat the SKBR3 cells for 24 hours, upon which the cells were trypsinized and lysed in a Nonidet P-40 lysis buffer (1% NP-40, 20 mM HEPES [pH 7.5], 0.12 M NaCl, 1 mM EDTA, 2.5 mM glycerophosphate, 10 mM NaF, 1 mM sodium vanadate, 1 mM phenylmethylsulfonyl fluoride and protease inhibitors). The lysates were cleared at 4 °C for 10 min and the concentration of protein quantified by the Bradford assay. Samples of 20 μg were analyzed in appropriate percentages of SDS–polyacrylamide gels, transferred to PVDF membranes (Immobilon-P, Millipore, Bedford, MA) and blocked for 1 h at room temperature with 5% nonfat dry milk in TBS buffer (20 mM Tris–HCl [pH 7.5], 0.5 M NaCl). Incubation with the primary antibodies was done overnight at 4 °C, while the appropriate secondary antibody (horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit IgG) was done for 2 h at room temperature. The blots were treated with the enhanced chemiluminescence reagent (Pierce) and exposed to x-ray film (Kodak) for detection. Antibodies used were: Akt, pAkt(S473), Her2 (Cell Signaling, Beverly, MA), Raf-1, Hsp90 (SPA-830) and Hsp70 (SPA-822) (Stressgen, Victoria, Canada).

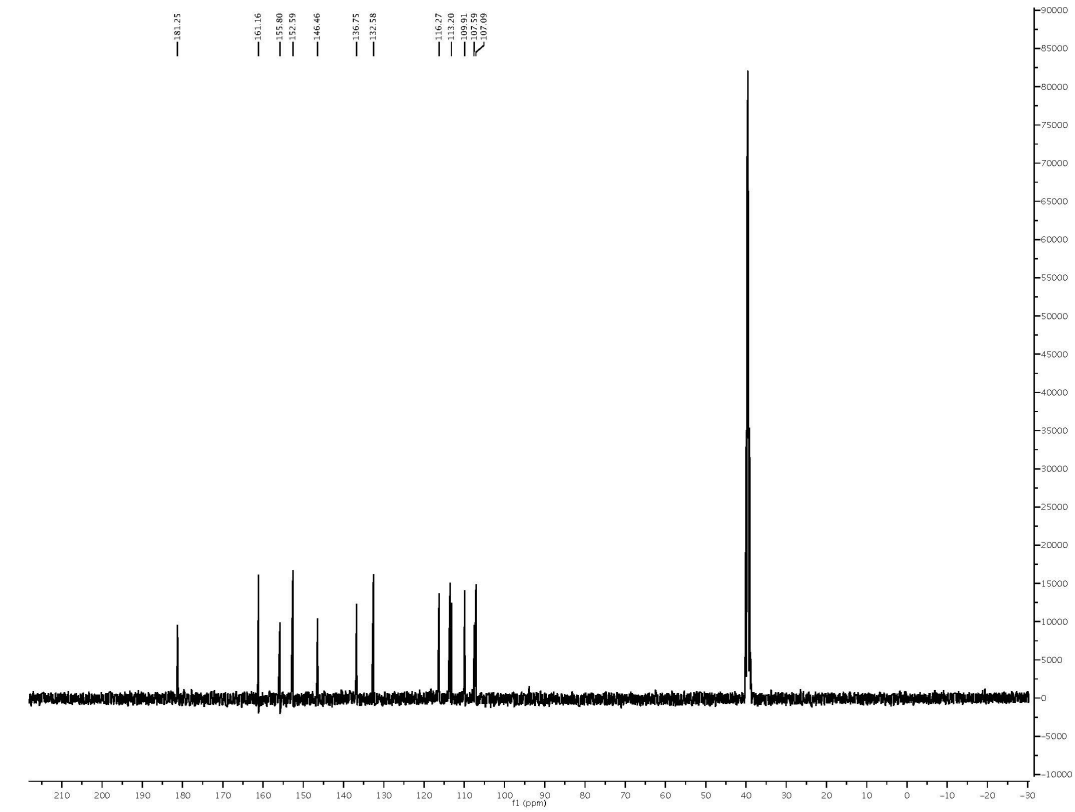


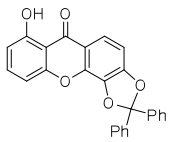


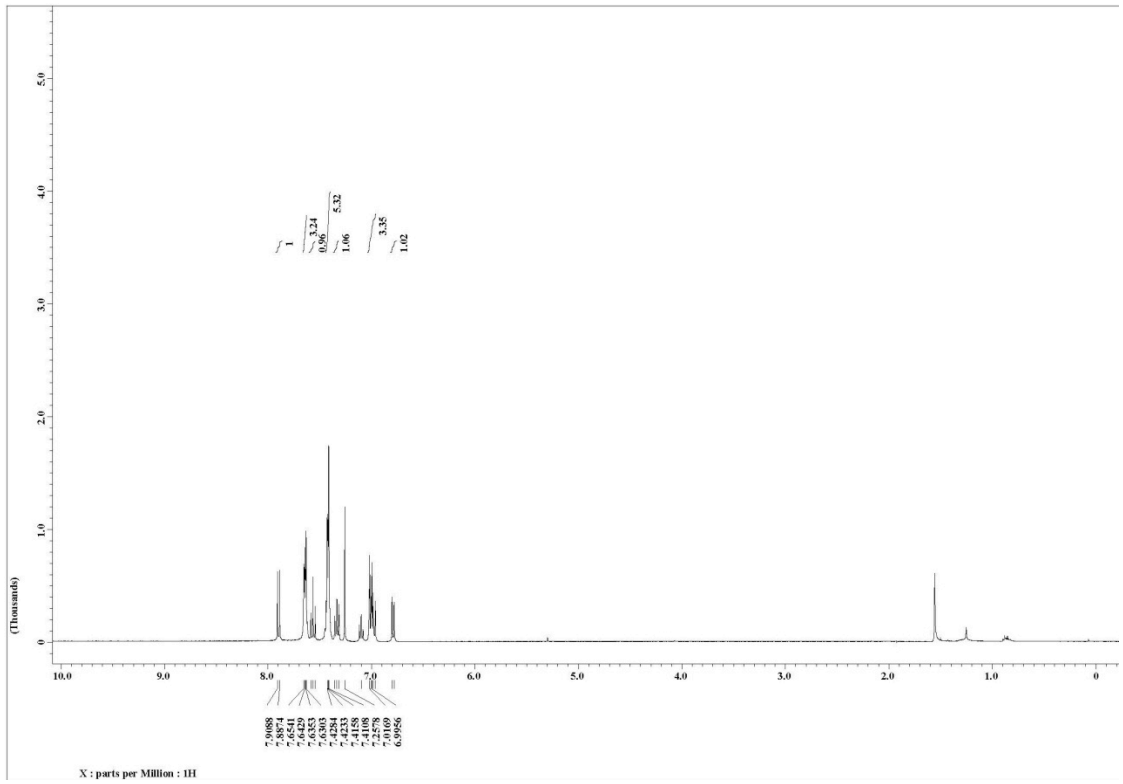


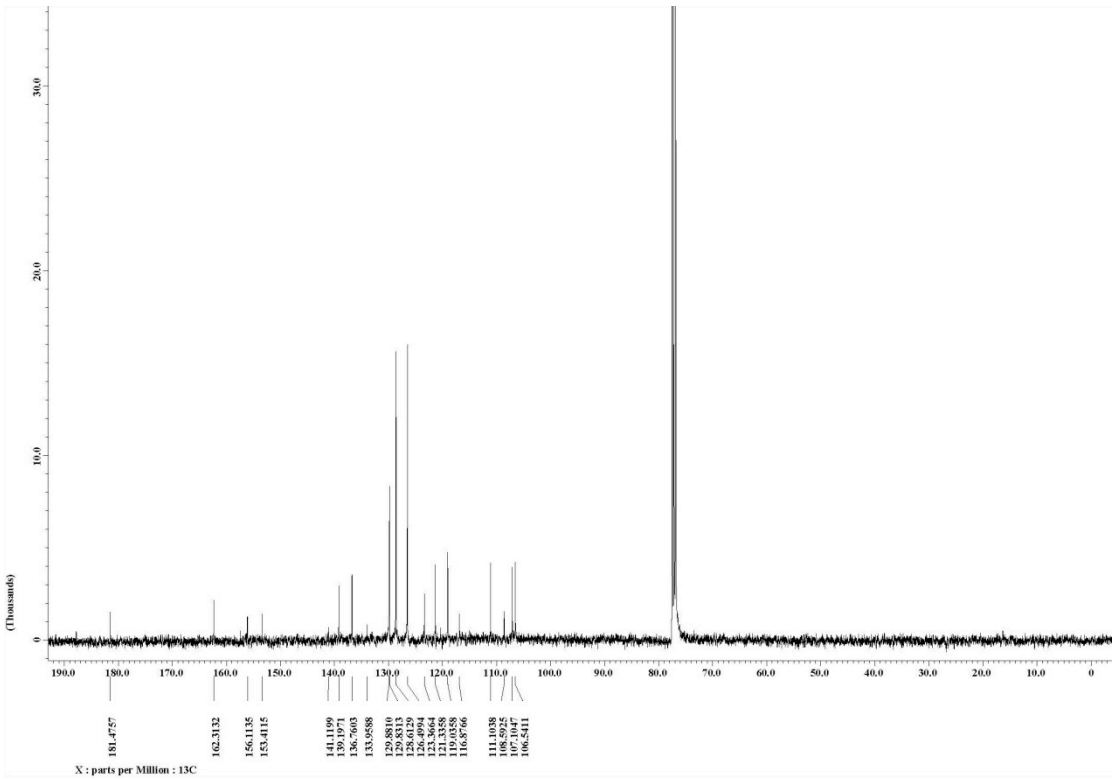


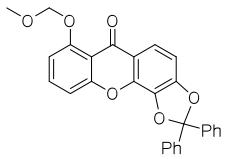


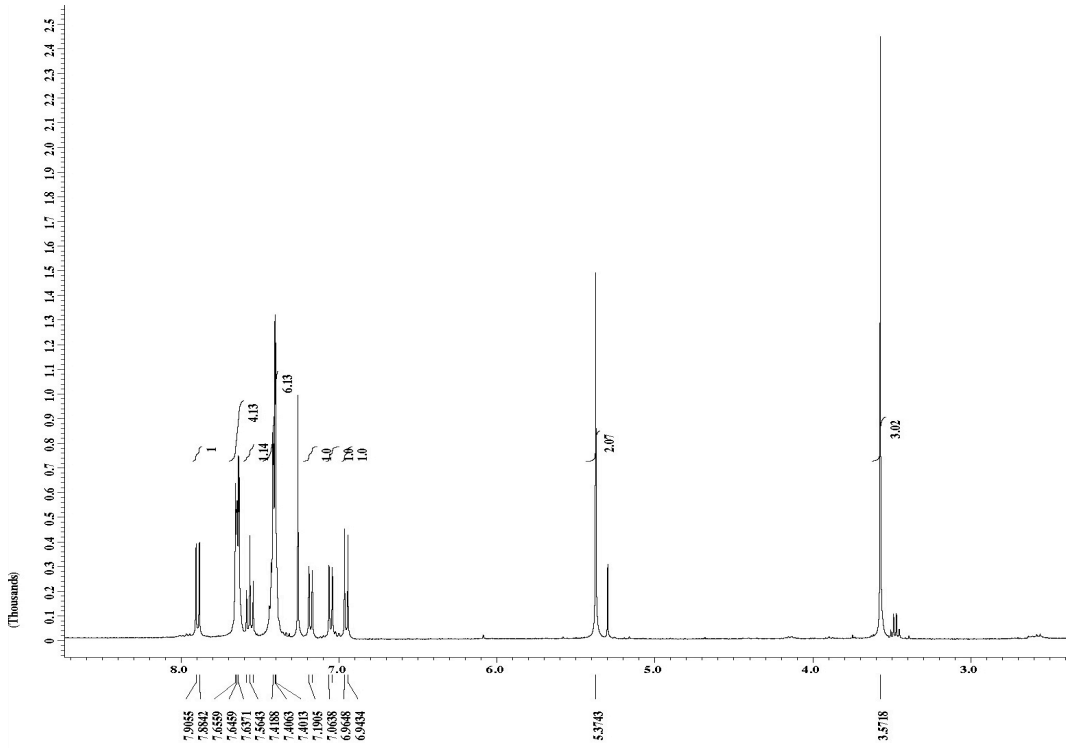


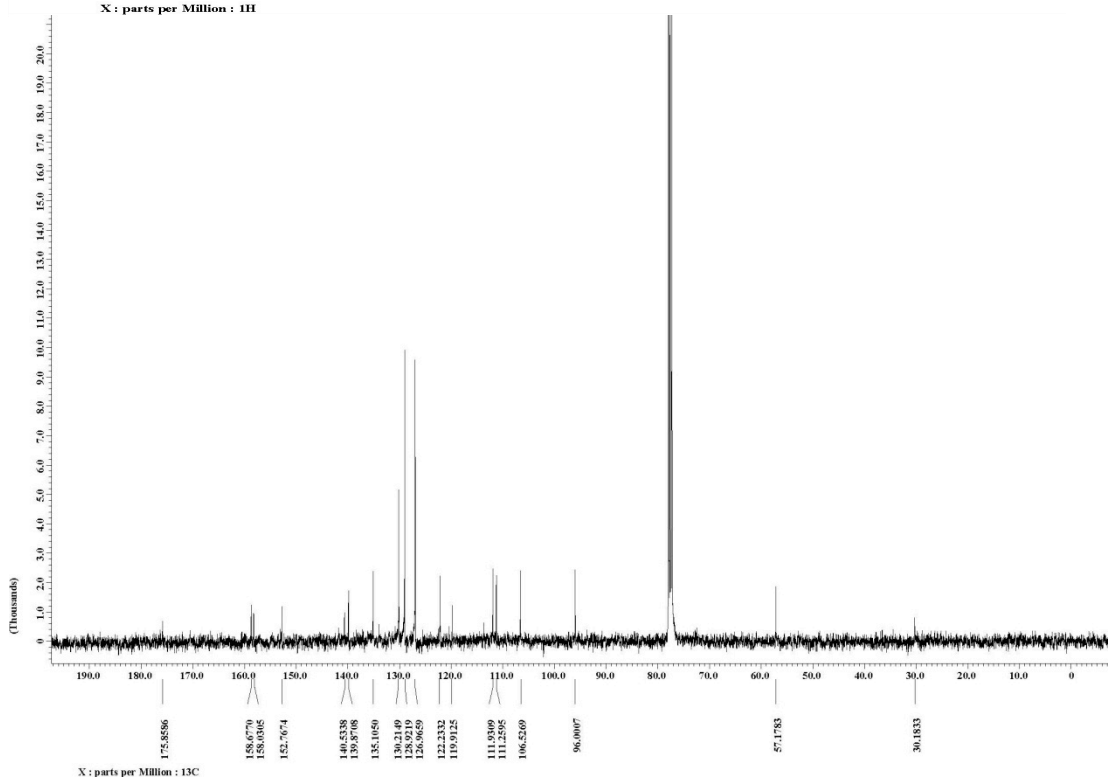


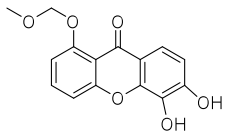


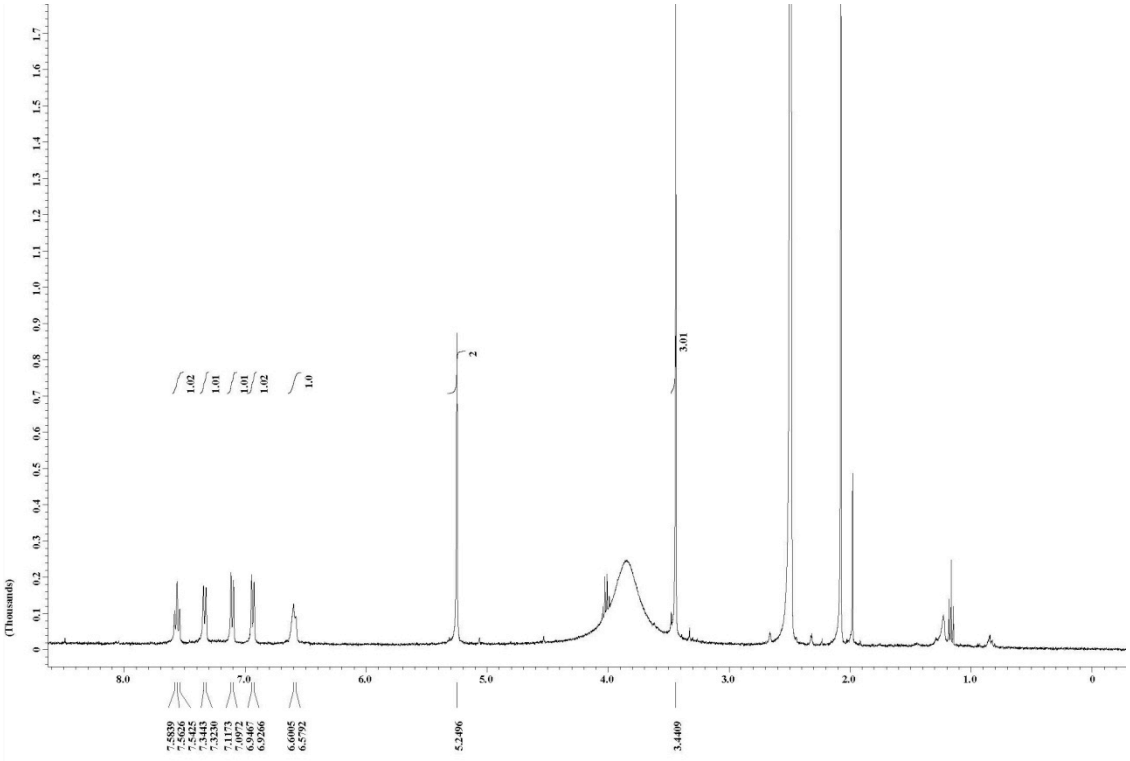


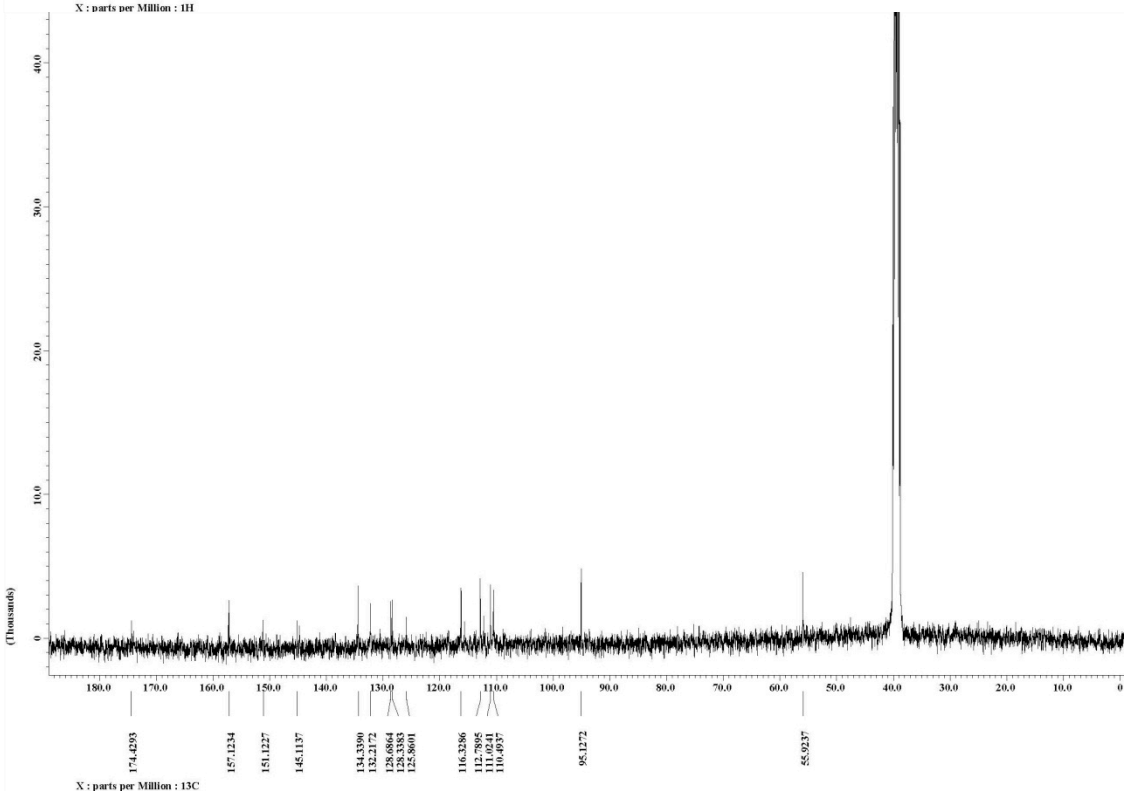


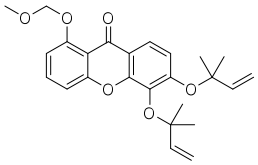


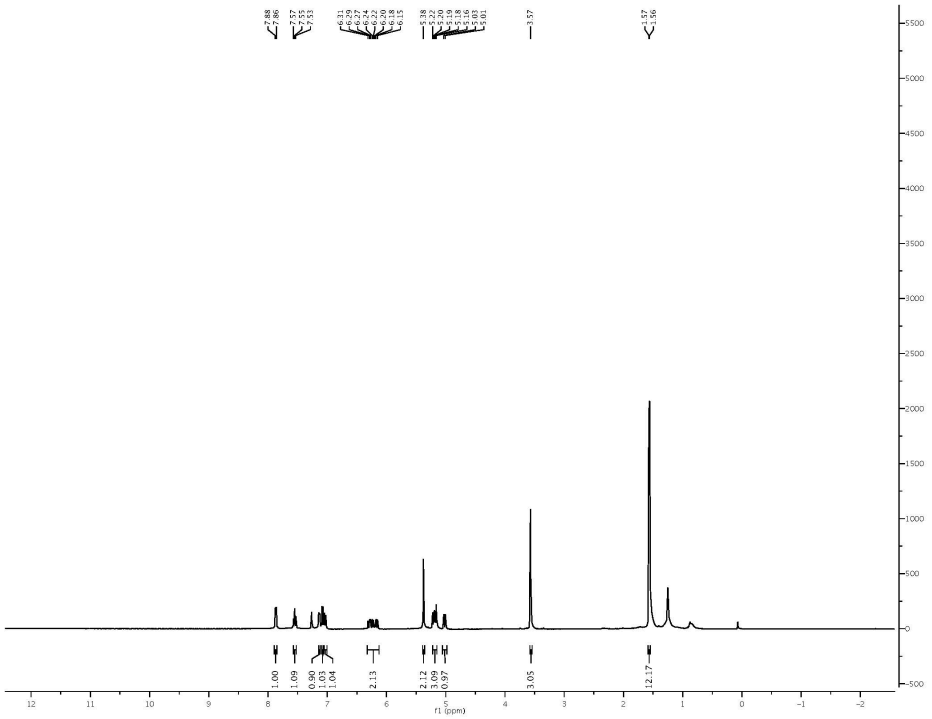


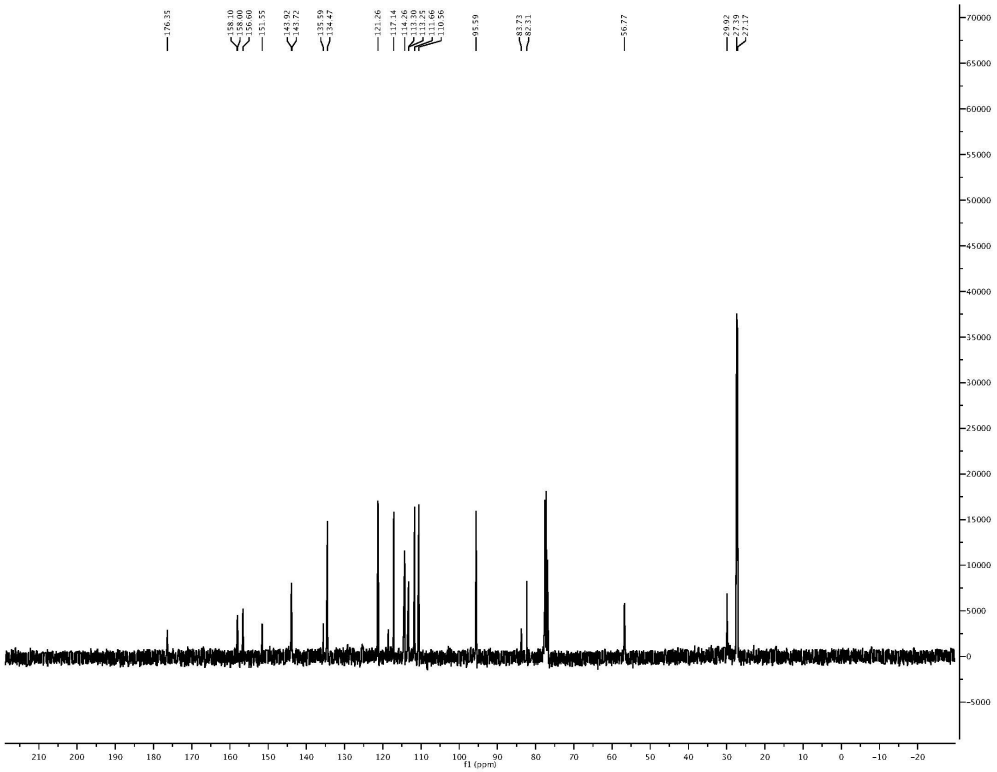


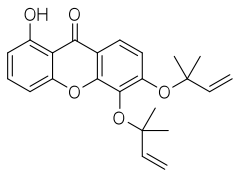


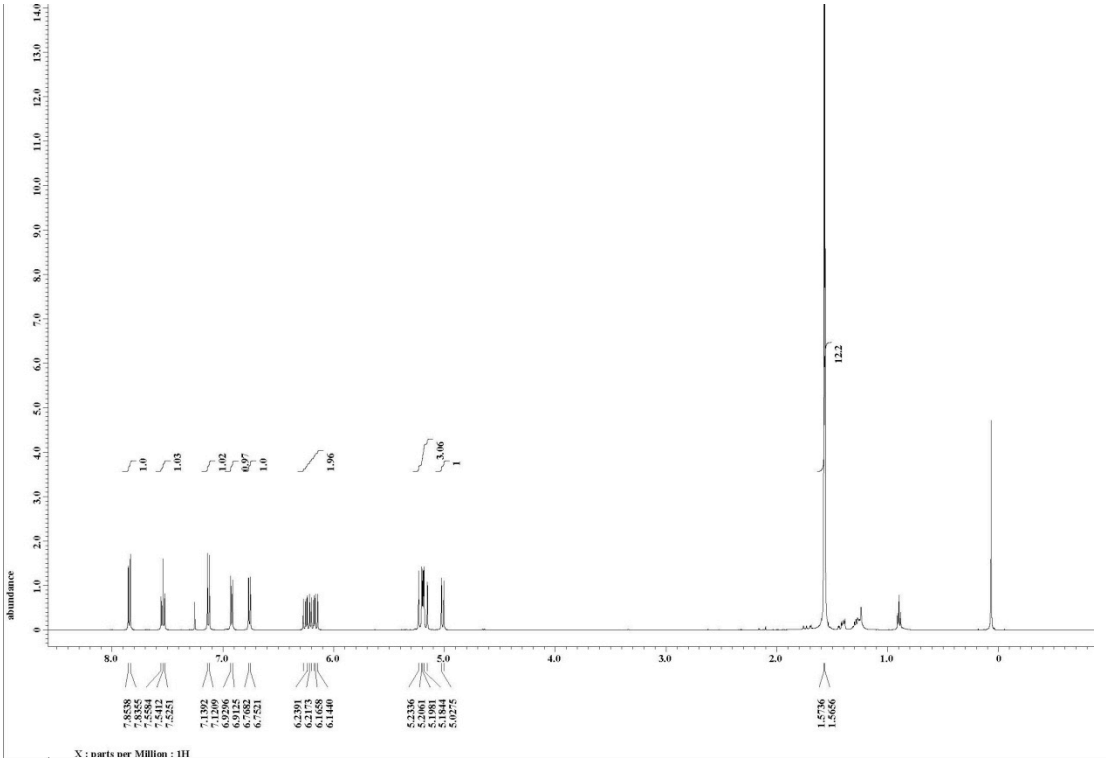


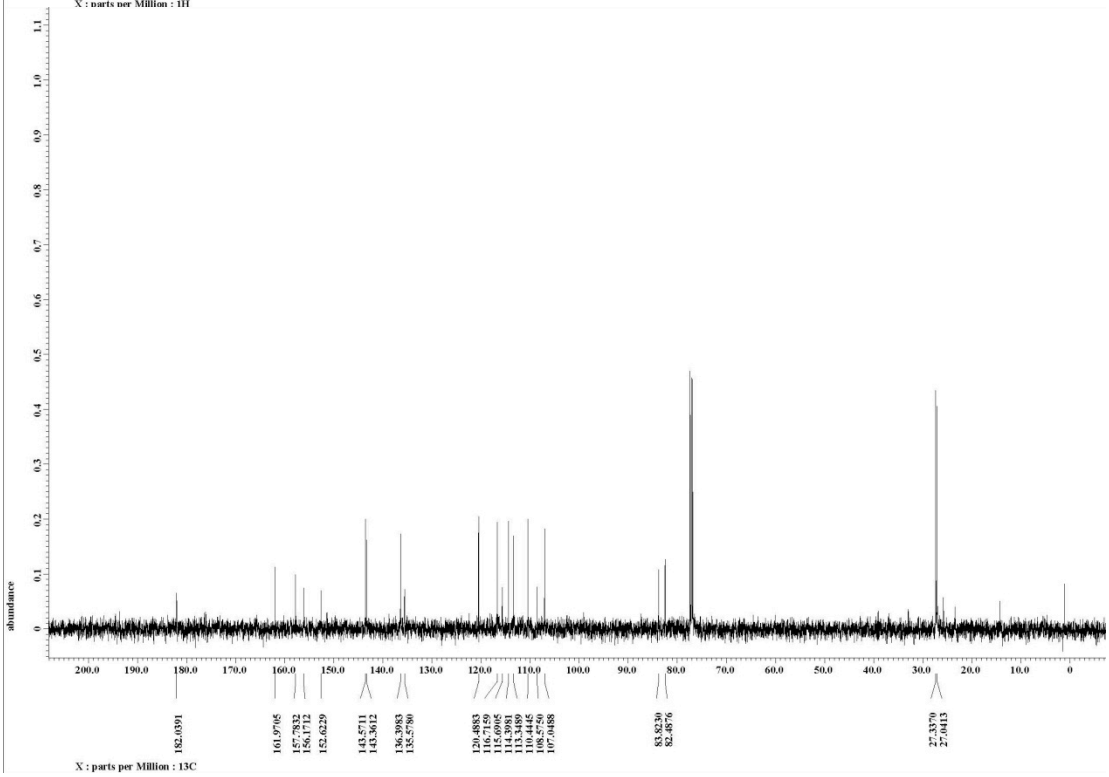


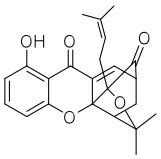


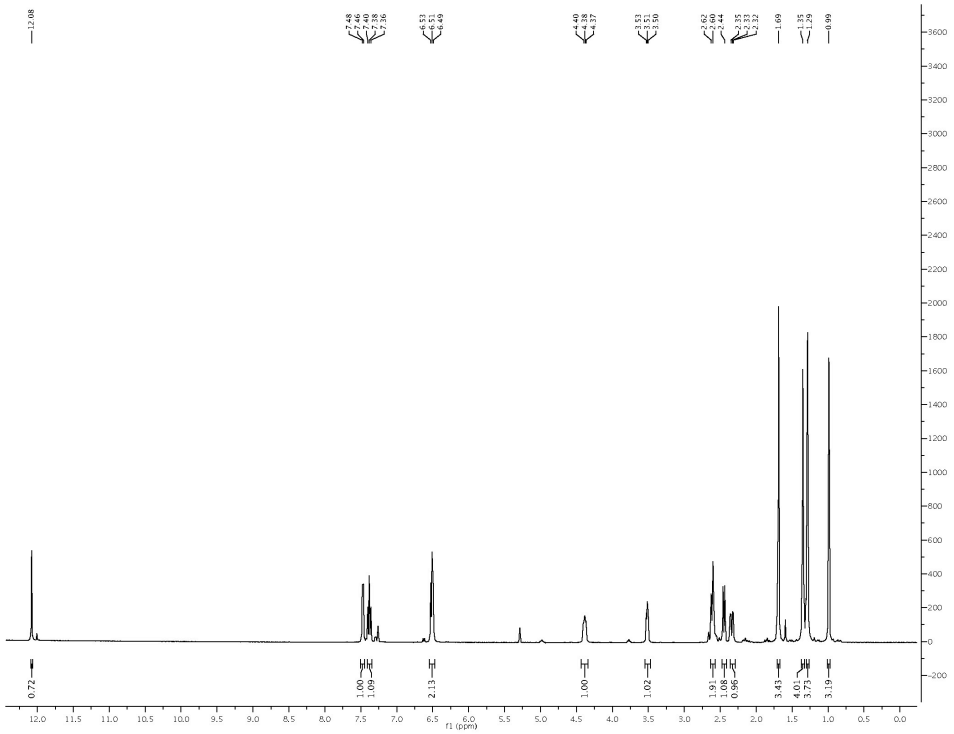


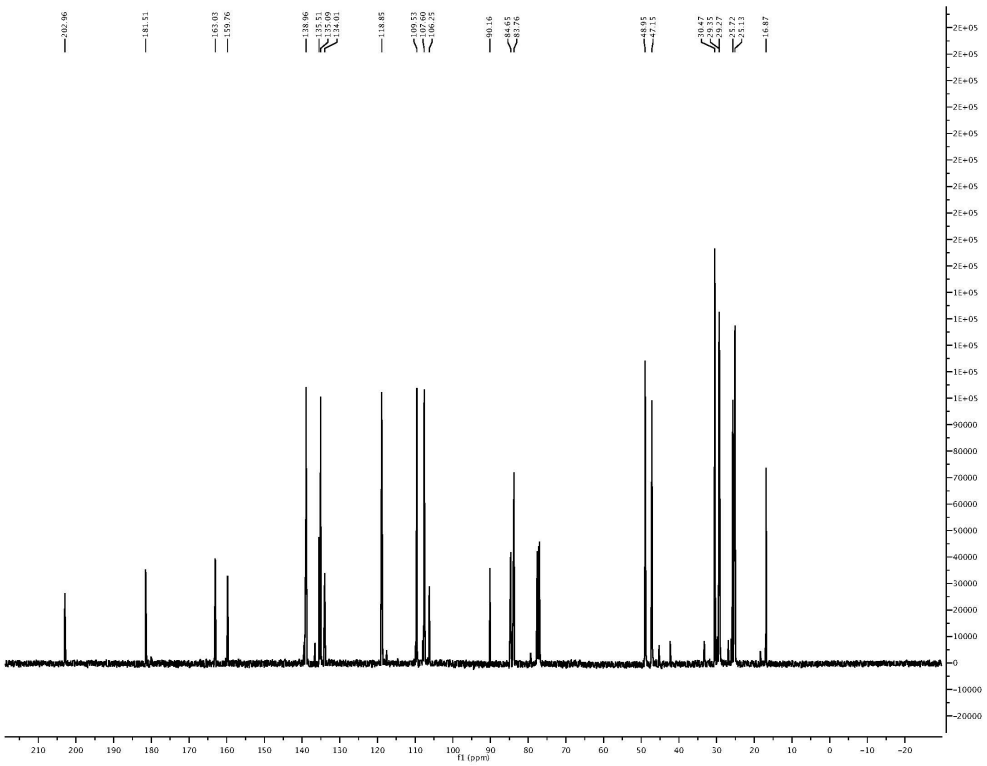


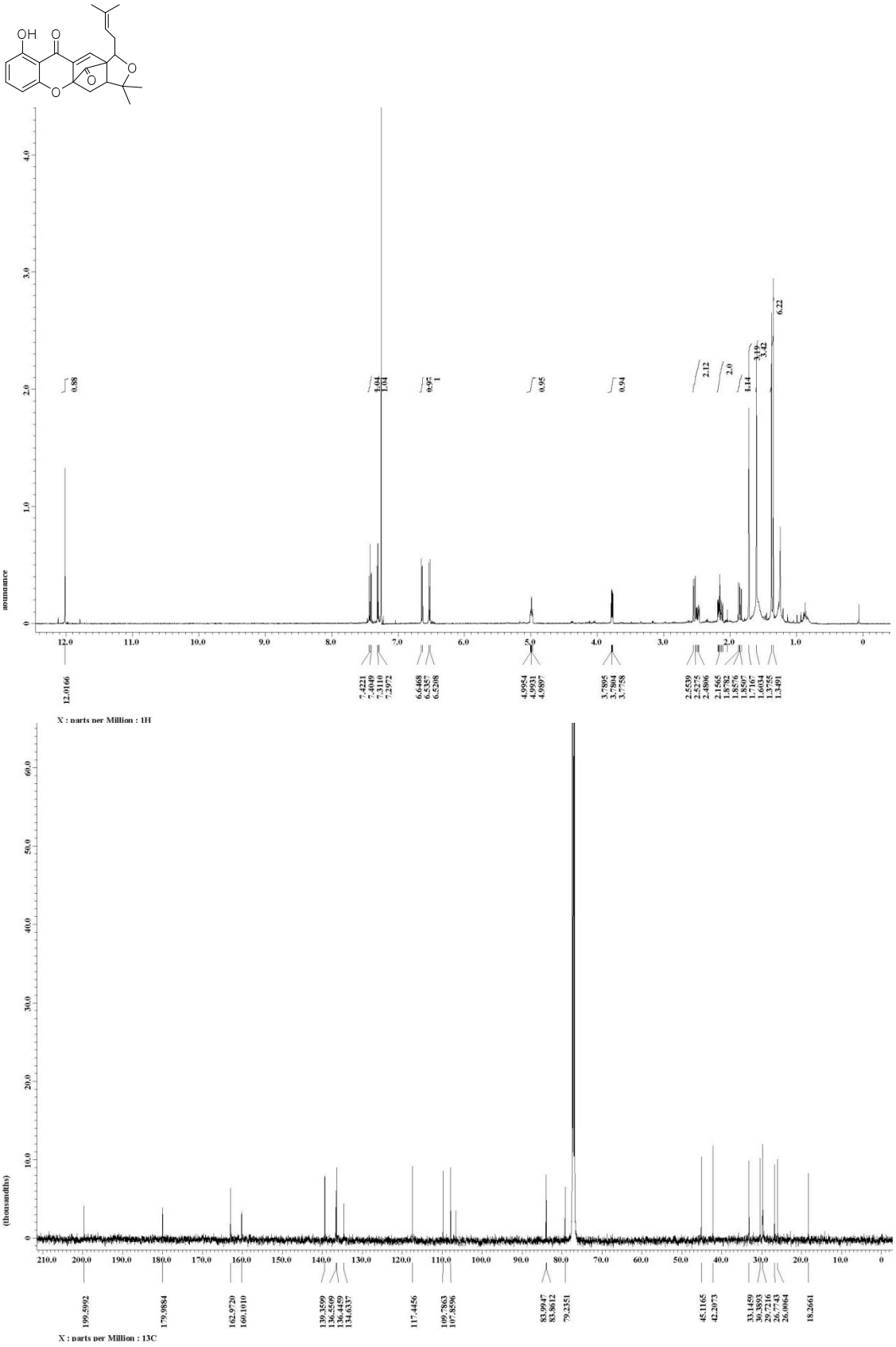


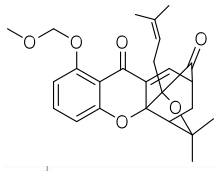


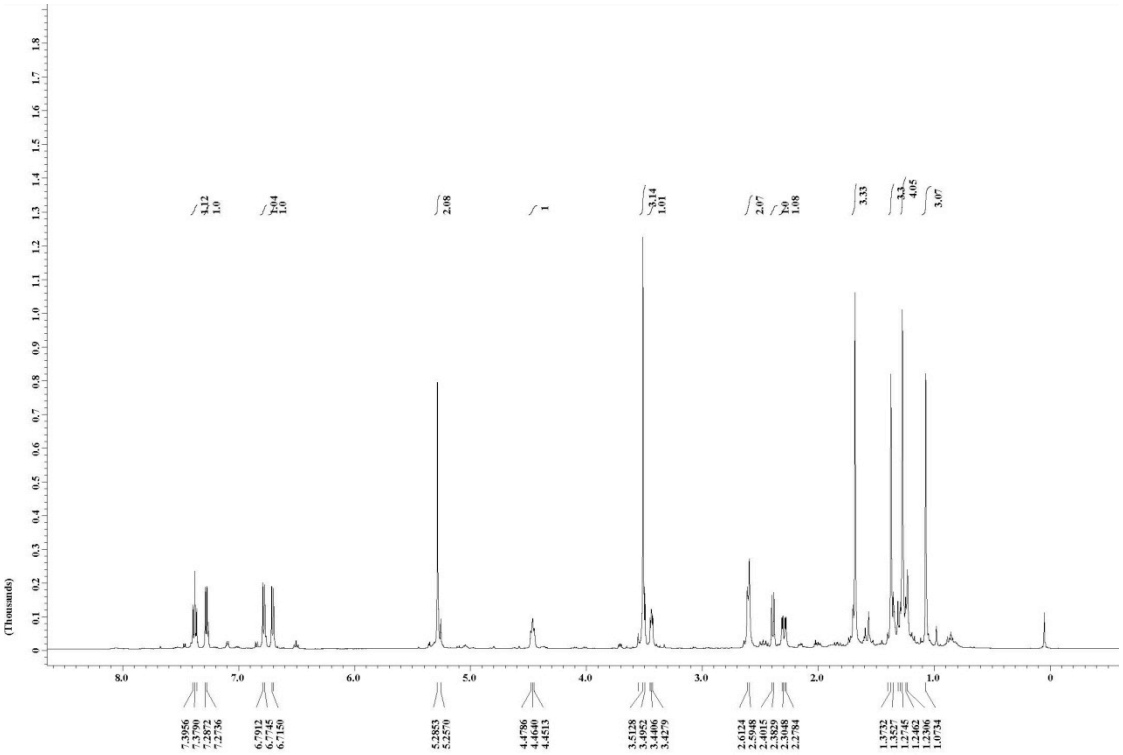








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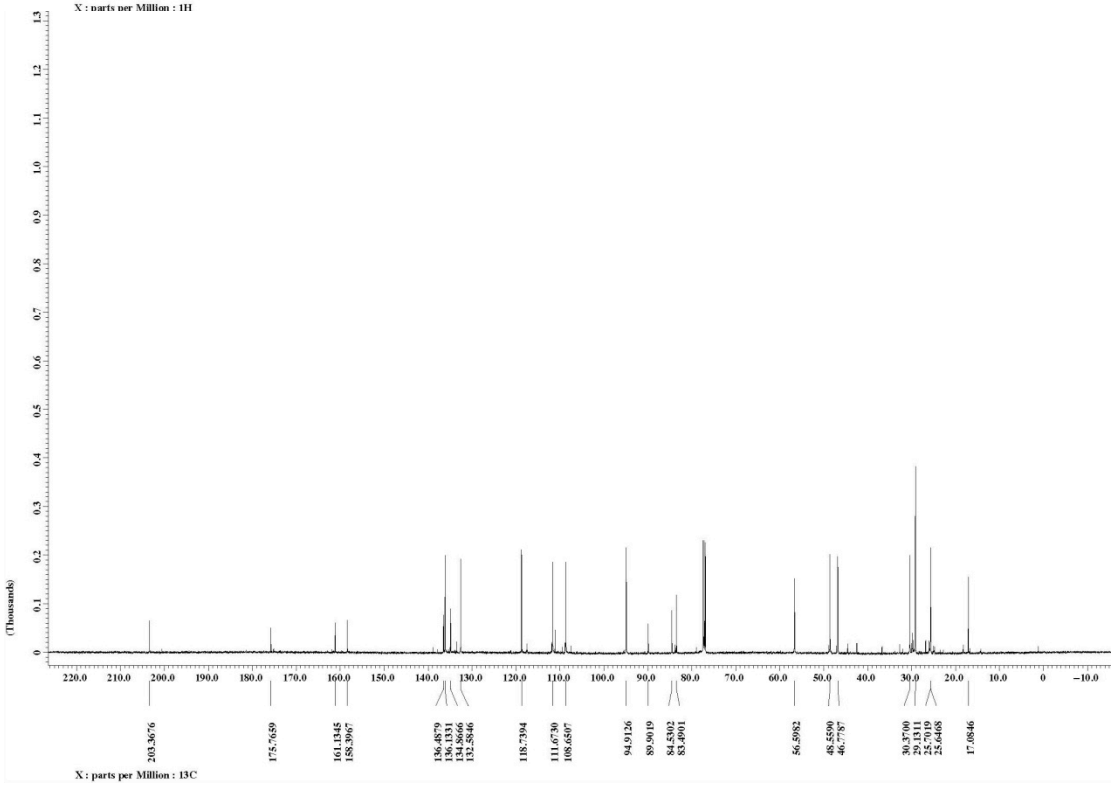
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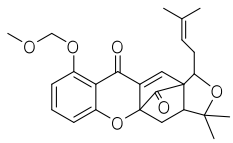
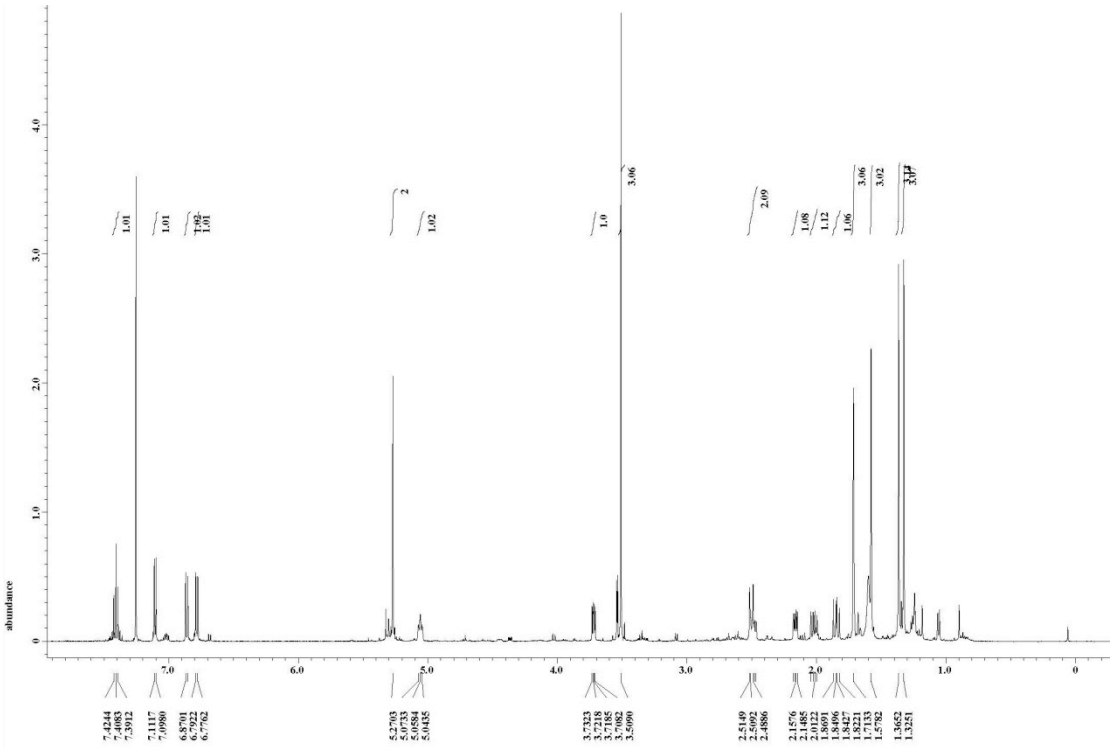
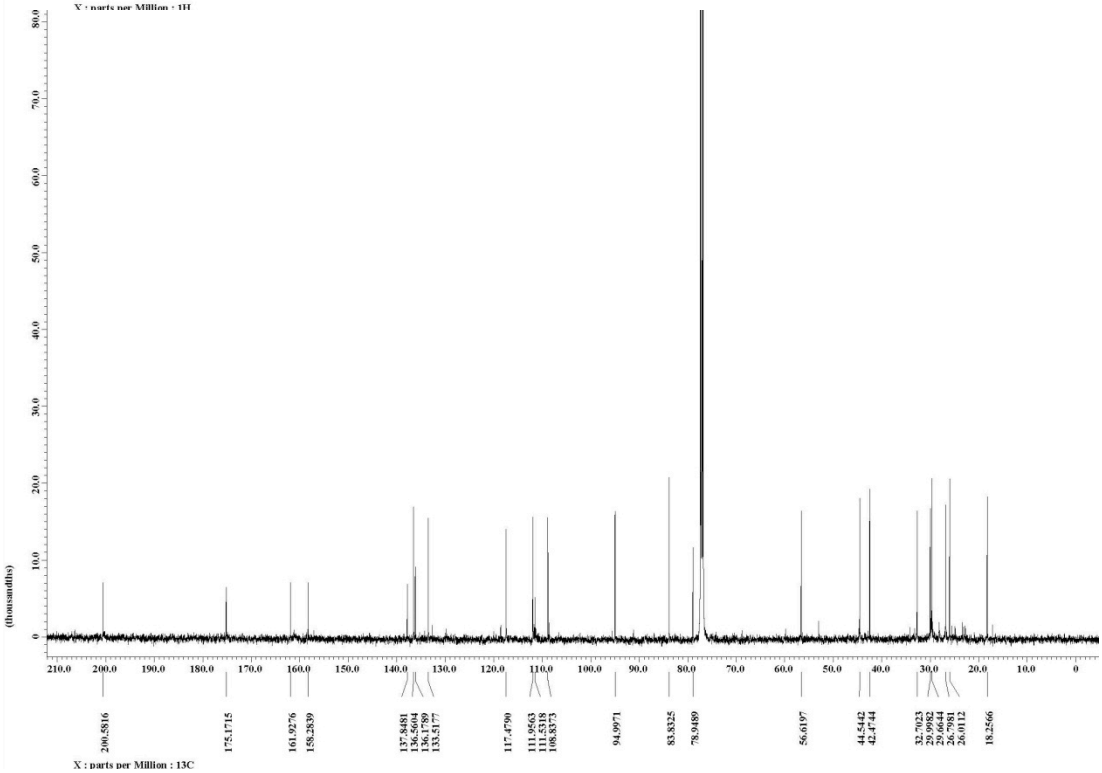
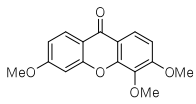
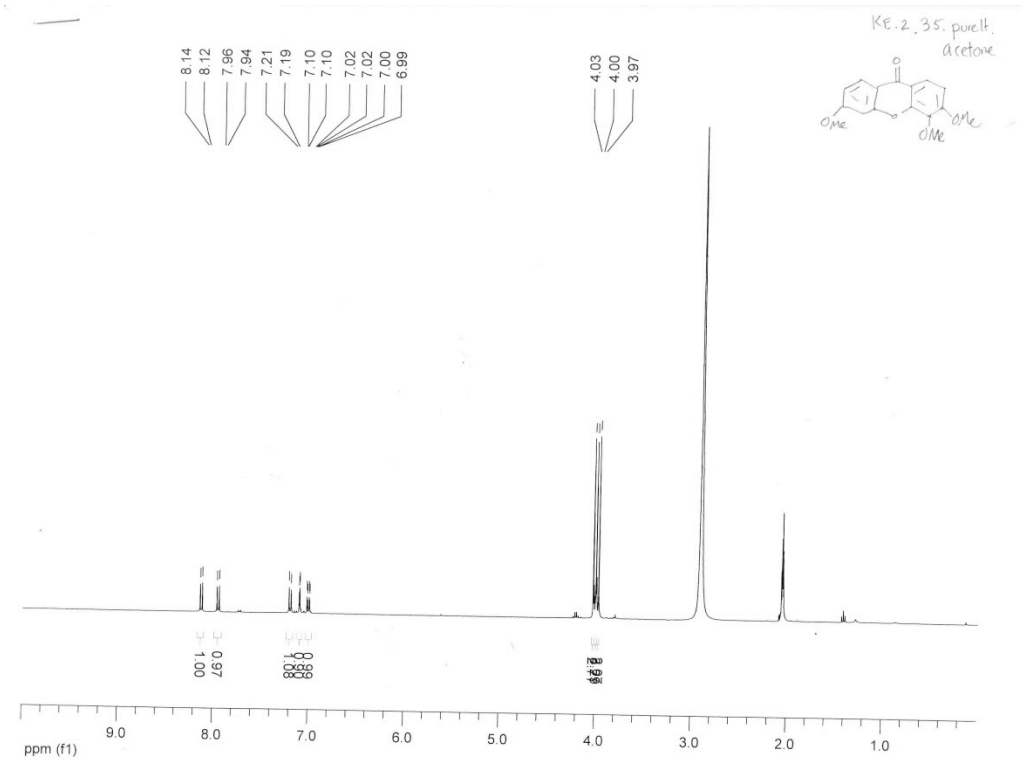
X : parts per Million : 13C

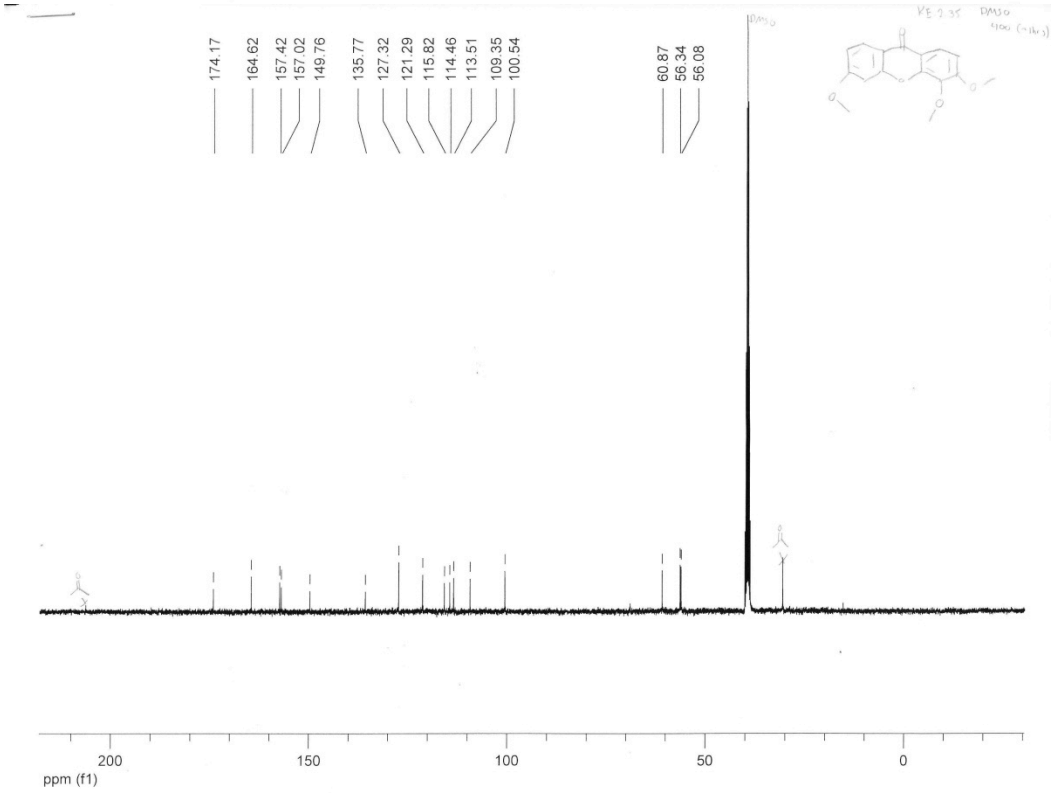


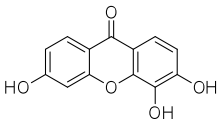


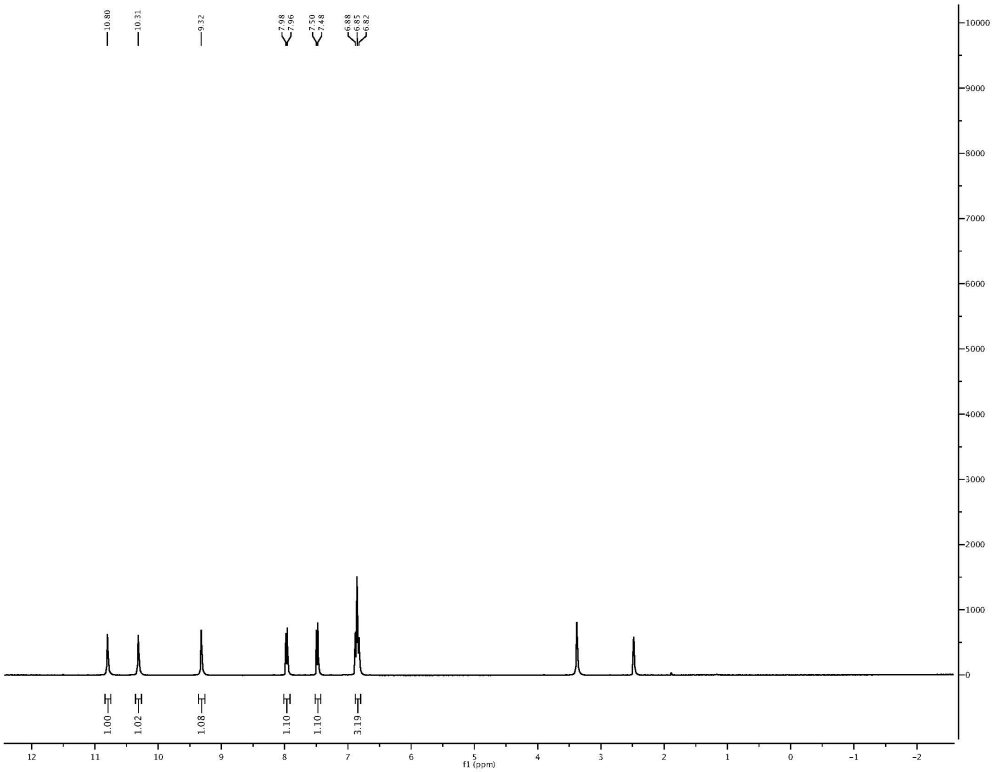


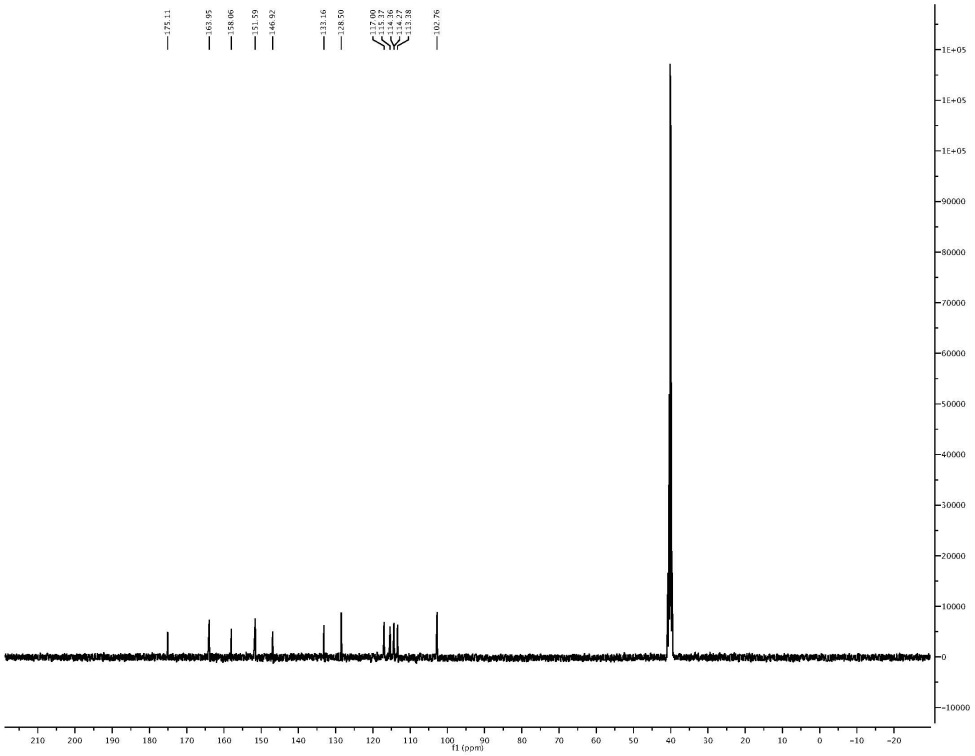

O
MeO
O
OMe
OMe

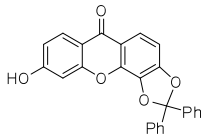

KE.2.35. pure ll. acetone
8.14
8.12
7.96
7.94
7.21
7.19
7.10
7.10
7.02
7.02
7.00
6.99
4.03
4.00
3.97
1.00
0.97
ppm (f1)

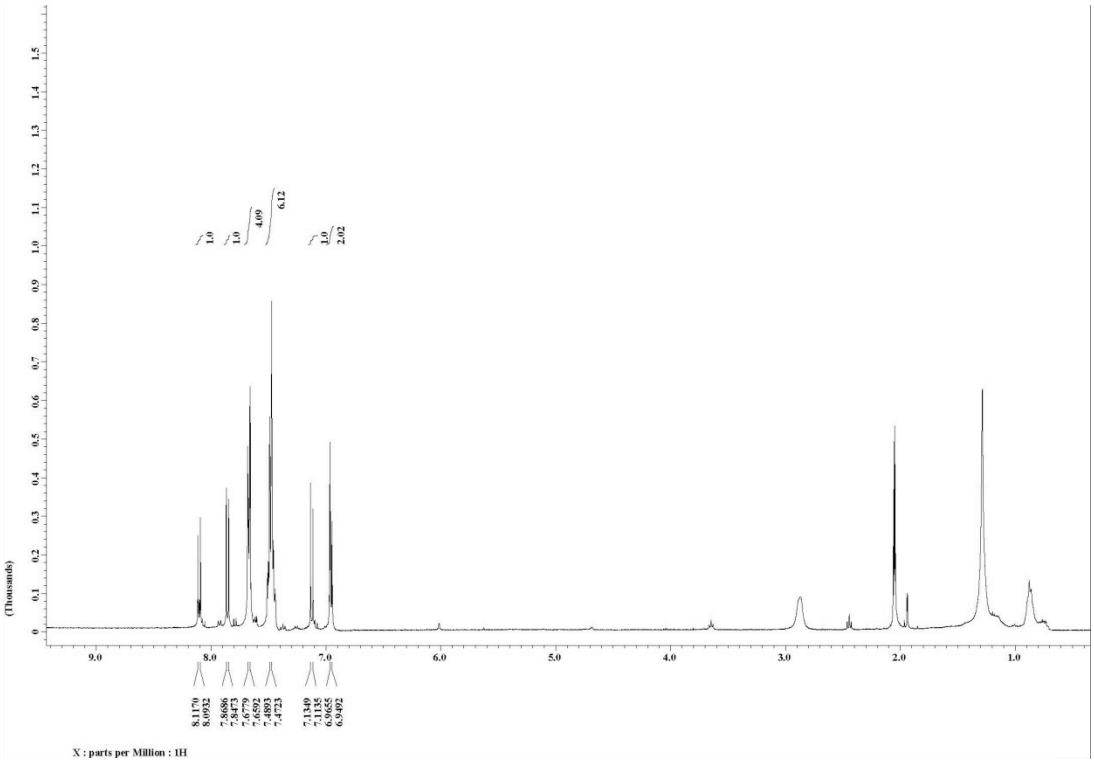

174.17
164.62
157.42
157.02
149.76
135.77
127.32
121.29
115.82
114.46
113.51
109.35
100.54
60.87
56.34
56.08
ppm (f1)

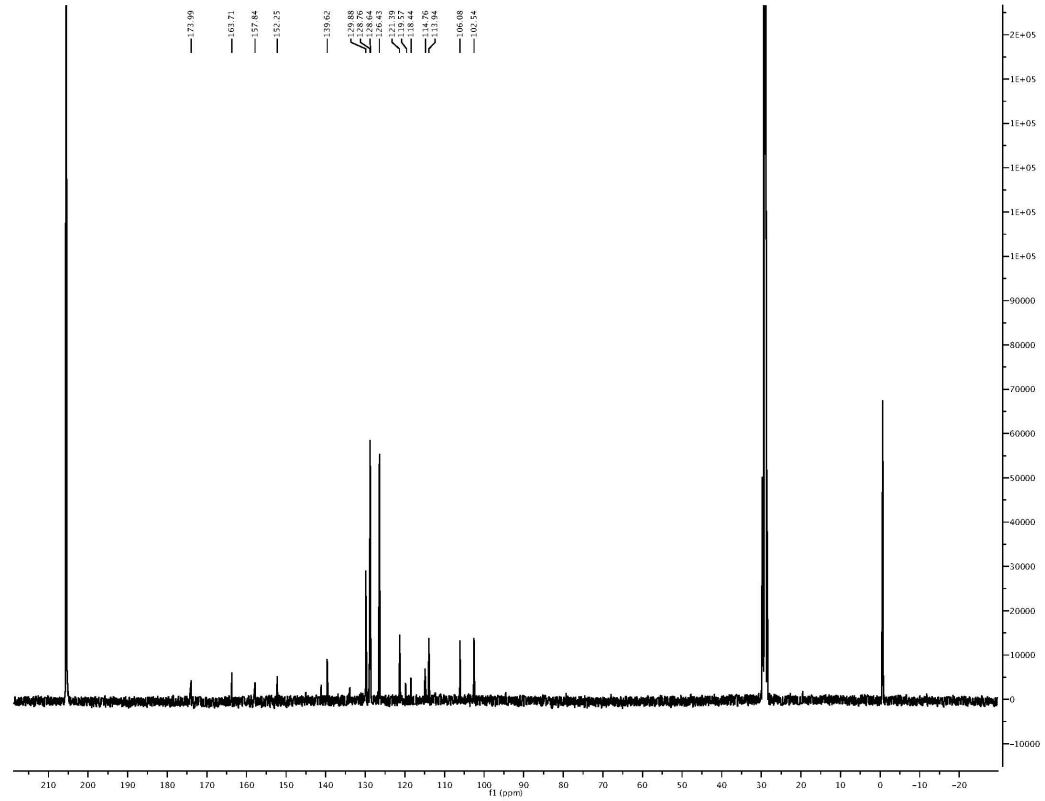


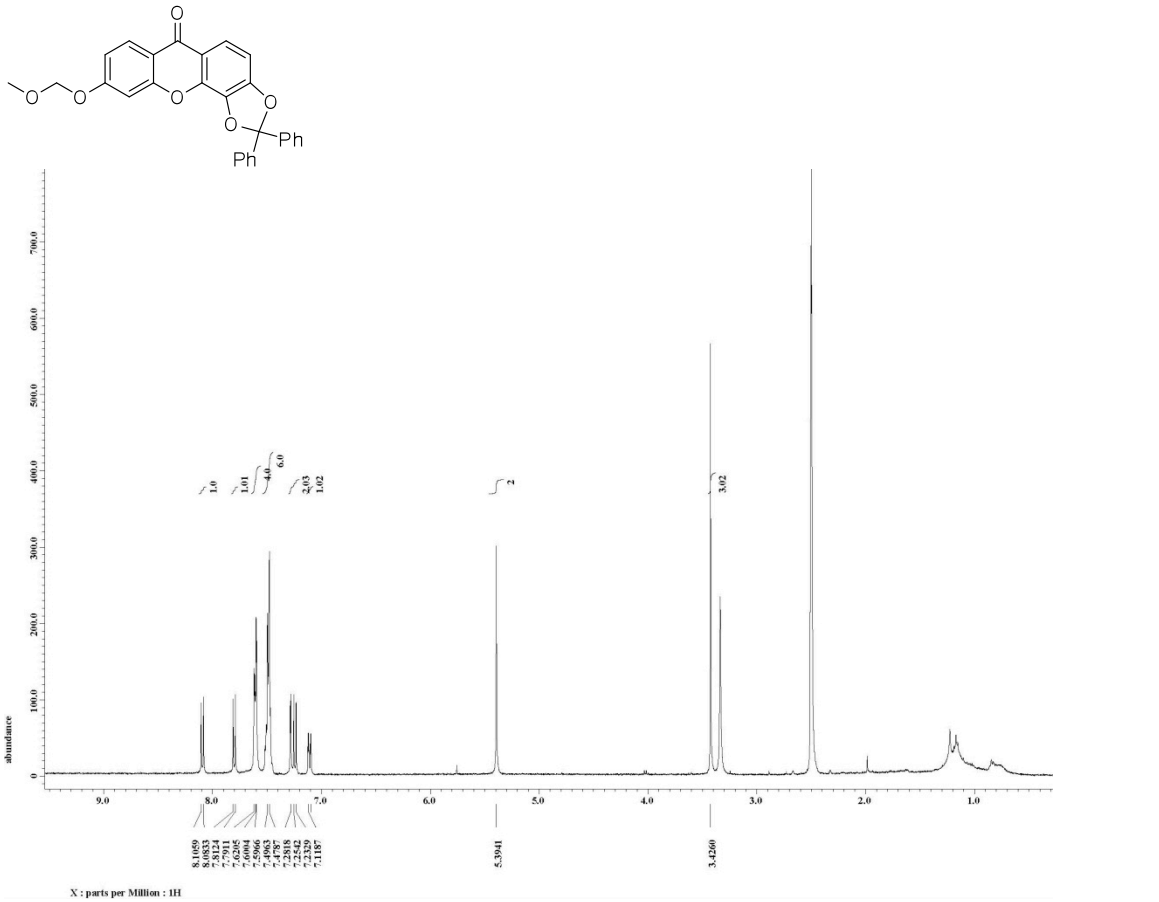


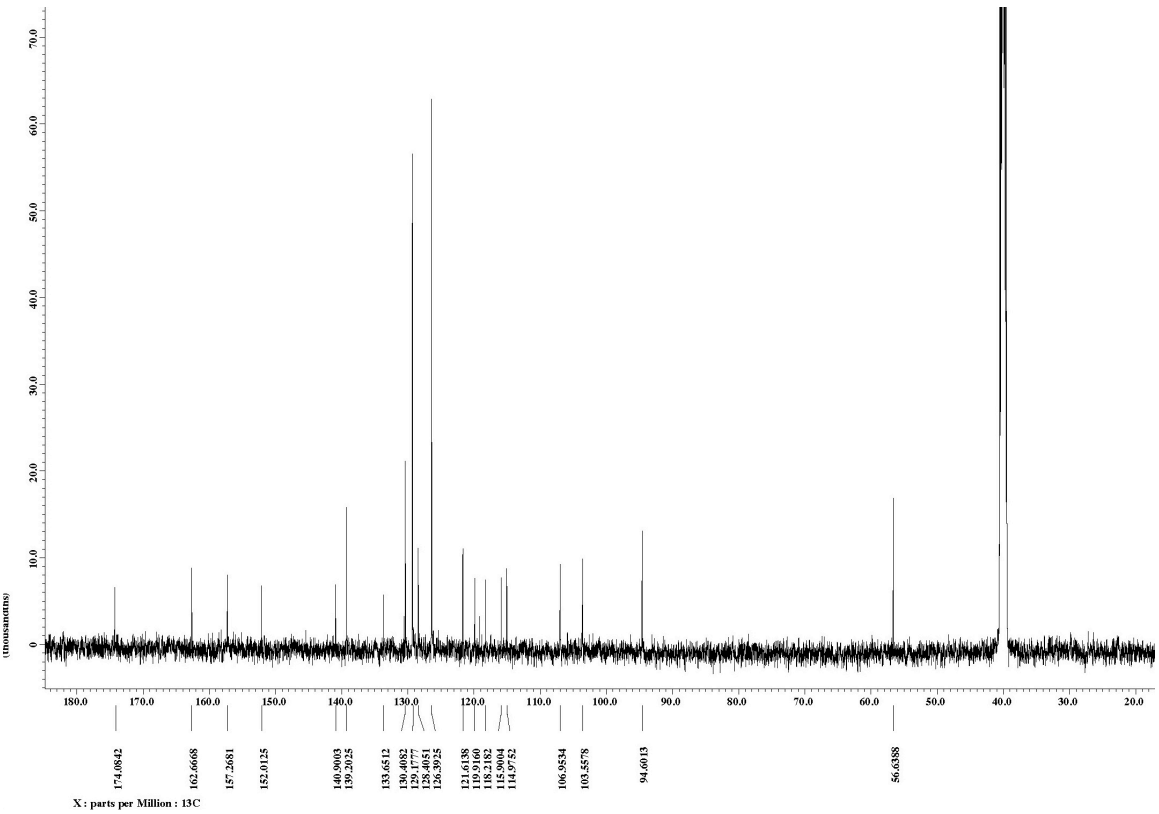


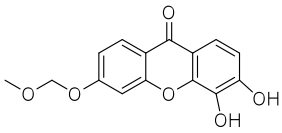

HO
O
O
O
O
Ph
Ph

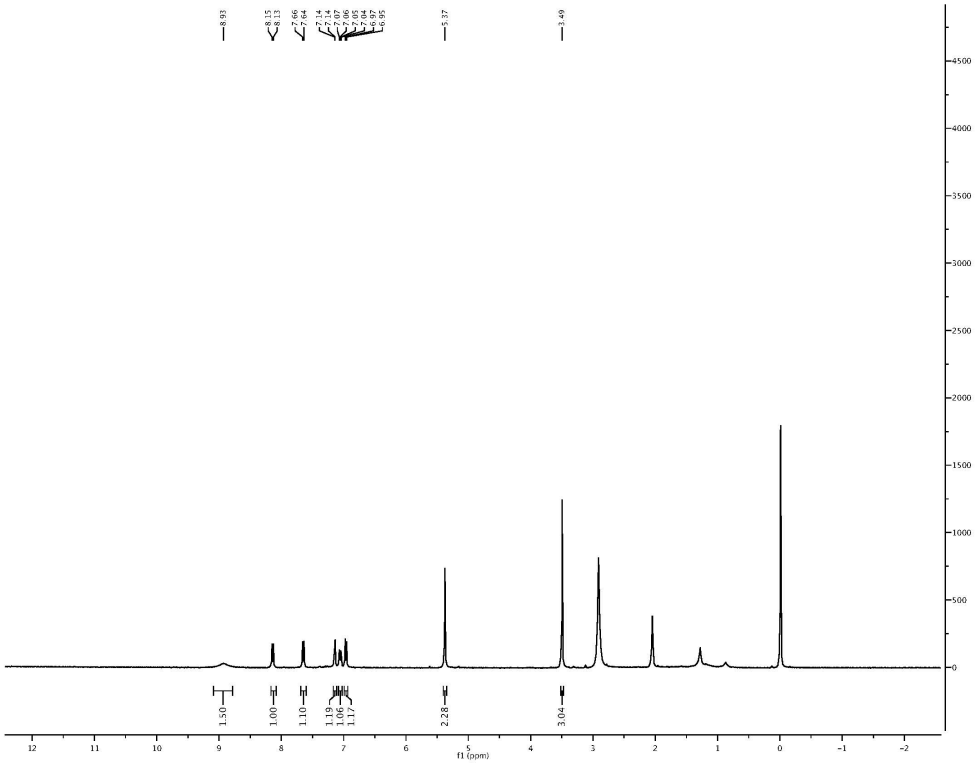

(Thousands)
1.0
1.0
4.09
6.12
1.0
2.02
8.1170
8.0932
7.8686
7.8473
7.6779
7.6592
7.4903
7.4723
7.1349
7.1135
6.9655
6.9492
X : parts per Million : 1H

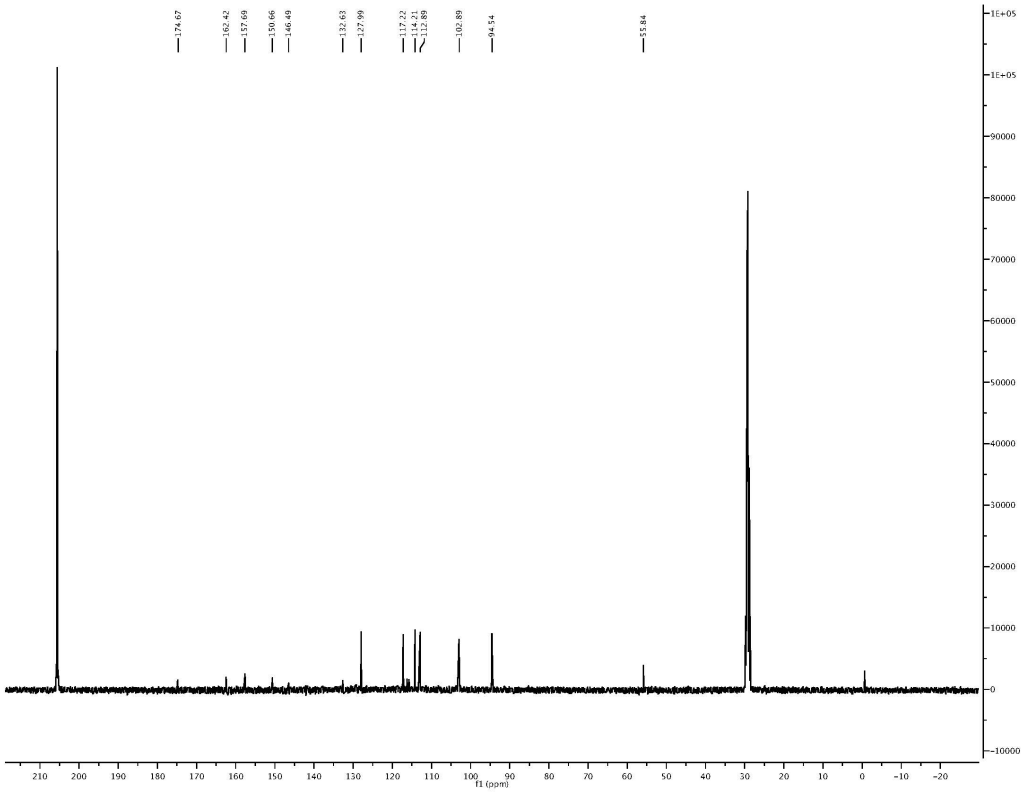

173.99
163.71
157.84
152.25
139.62
129.88
128.76
128.64
126.43
121.39
119.57
118.44
114.76
113.94
106.08
102.54
f1 (ppm)

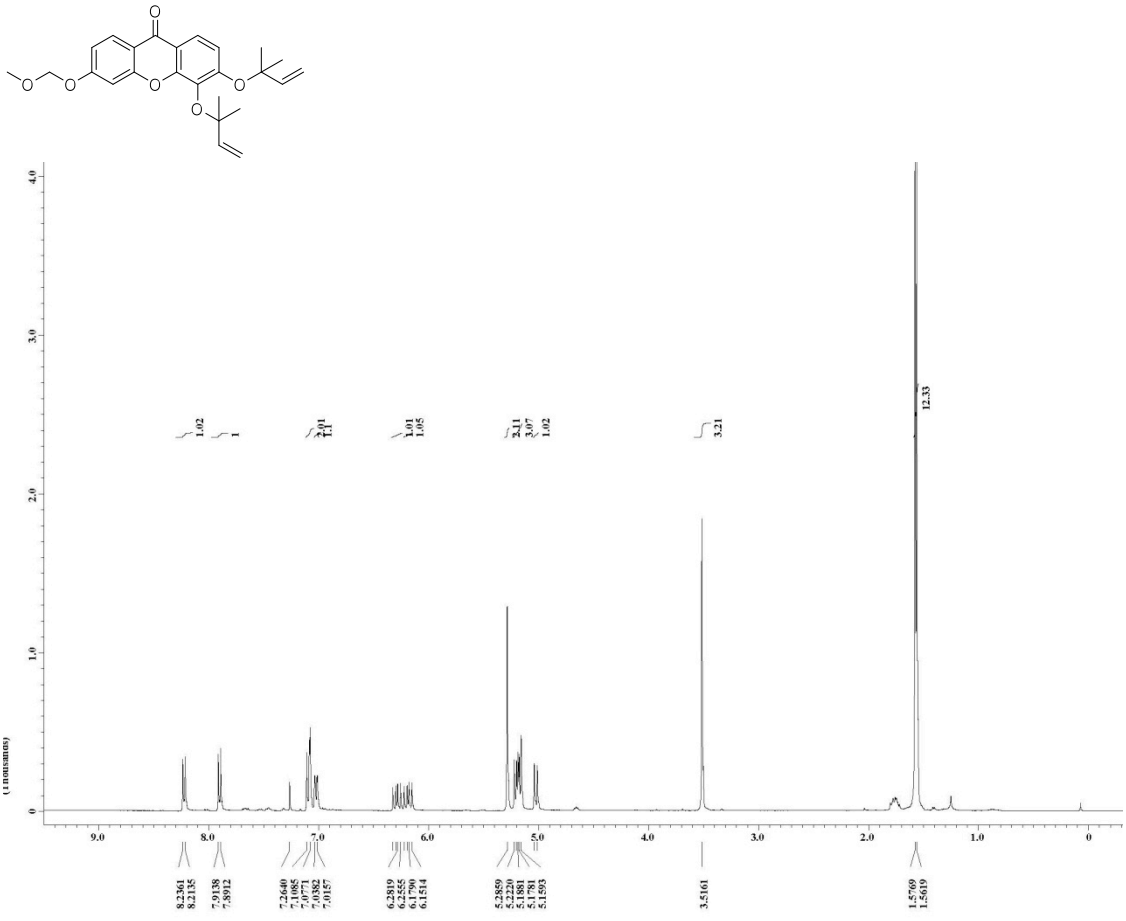


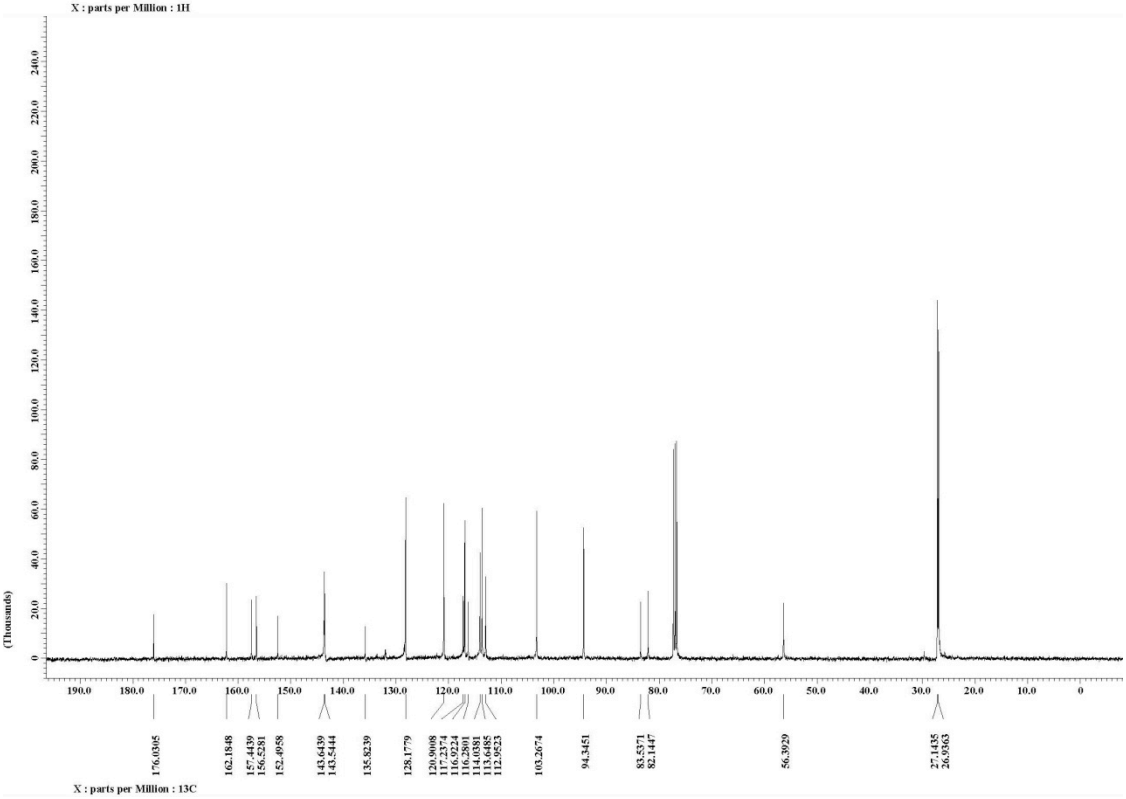


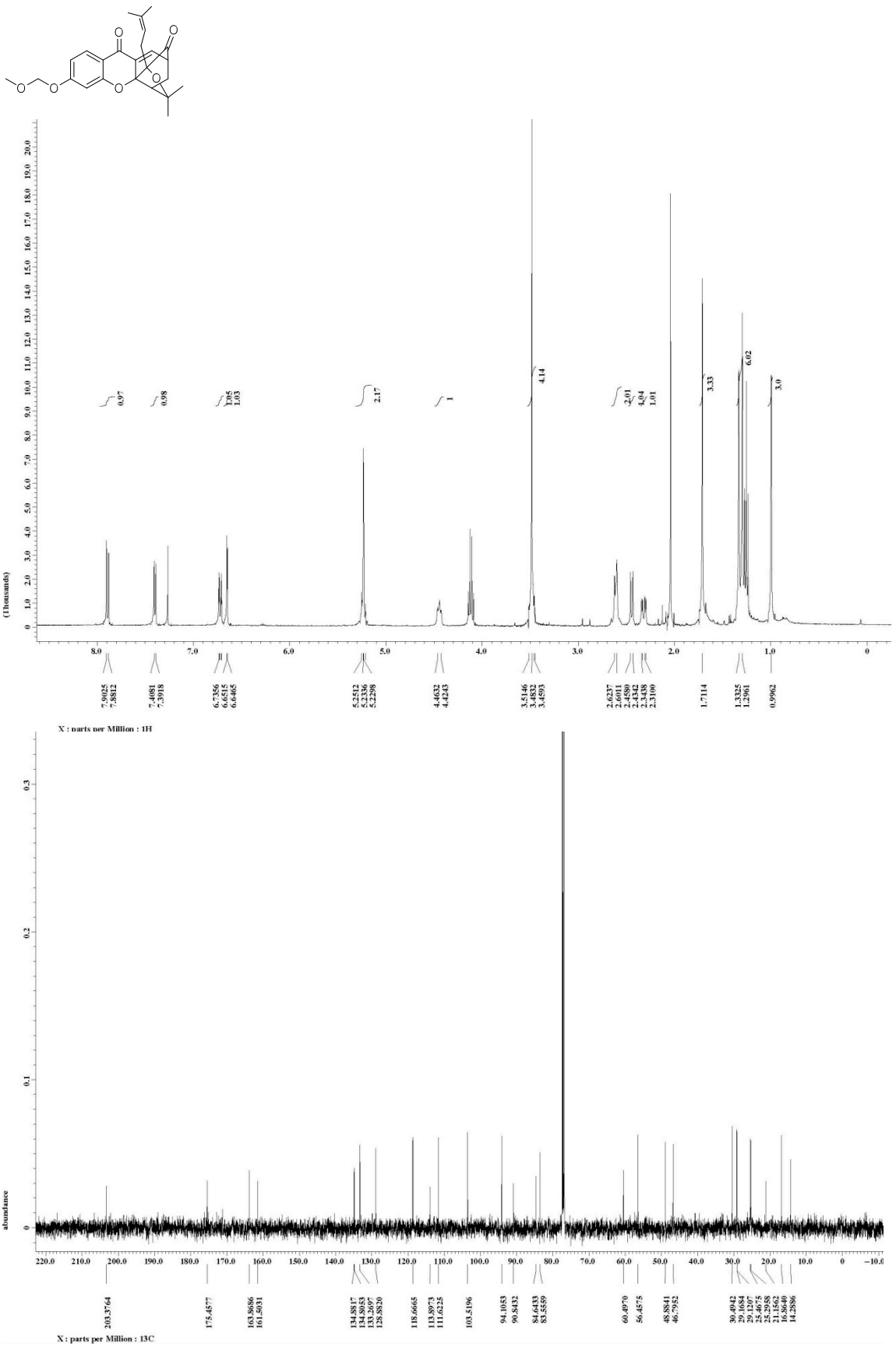











(Thousands)
0.97
0.98
1.05
1.03
2.17
1
4.14
2.01
3.04
1.01
3.33
6.02
3.0
7.9025
7.8812
7.4081
7.3918
6.7356
6.6515
6.6465
5.2512
5.2336
5.2298
4.4632
4.4243
3.5146
3.4832
3.4593
2.6237
2.6011
2.4580
2.4342
2.3458
2.3100
1.7114
1.3325
1.2961
0.9962
X : parts per Million : 1H
abundance
203.3764
175.4577
163.8686
161.5031
134.8817
134.3093
133.2057
128.3820
118.6665
113.8973
111.6225
103.5196
94.1053
90.8432
84.6433
83.5559
60.4970
56.4575
48.8841
46.7952
30.4942
29.1684
29.1207
25.4675
25.2958
21.1563
16.8646
14.2886
X : parts per Million : 13C