Biomimetic Syntheses and Structural Elucidation of the Apoptosis-Inducing Sesquiterpenoid Trimers: (-)-Ainsliatrimers A and B

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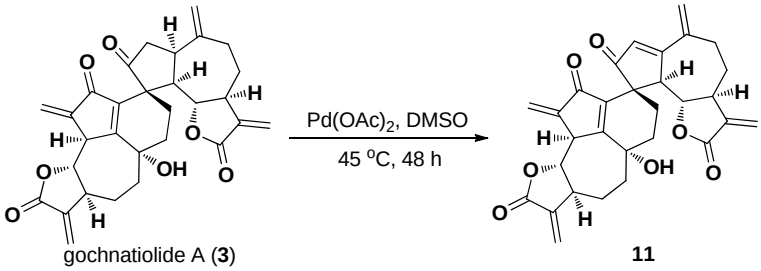
Supporting Information

I) Experimental Section

a) General information:

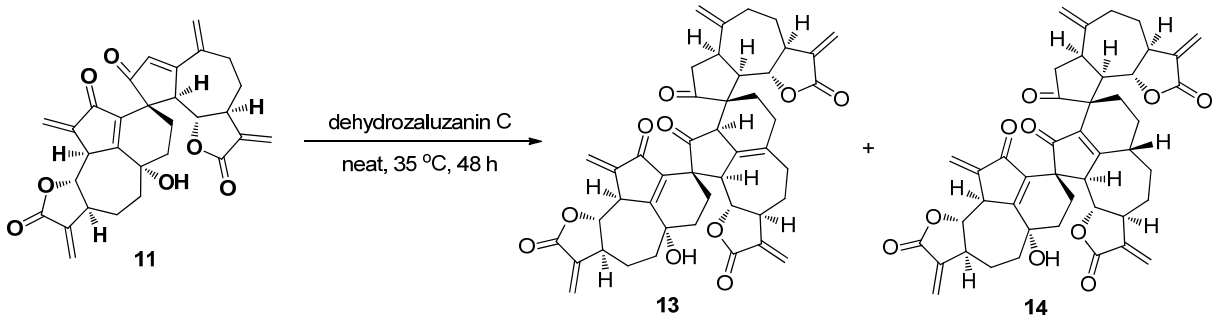
^{1}H NMR spectra were recorded on a Varian 400 MHz spectrometer at ambient temperature with $CDCl_3$ as the solvent unless otherwise stated. ^{13}C NMR spectra were recorded on a Varian 100 MHz spectrometer (with complete proton decoupling) at ambient temperature. Chemical shifts are reported in parts per million relative to chloroform (^{1}H, δ 7.26; ^{13}C, δ 77.00). Data for ^{1}H NMR are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, *br* = broad), coupling constants and integration. Infrared spectra were recorded on a Thermo Fisher FT-IR200 spectrophotometer. High-resolution mass spectra were obtained at Peking University Mass Spectrometry Laboratory using a Bruker APEX Flash chromatography. Optical rotations were recorded on an AUTOPOL III digital polarimeter at 589 nm and are recorded as $[\alpha]_D^{20}$ (concentration in grams/100 mL solvent). The samples were analyzed by HPLC-MS on a Waters Auto Purification LC/MS system (3100 Mass Detector, 2545 Binary Gradient Module, 2767 Sample Manager, and 2998 Photodiode Array (PDA) Detector). The system was equipped with a Waters C18 5μm X-bridge separation column (150*4.6 mm), equilibrated with HPLC grade water (solvent A) and HPLC grade methanol (solvent B) with a flow rate of 1.0 mL/min at room temperature. Preparative HPLC-MS on a Waters Auto Purification LC/MS system (3100 Mass Detector, 2545 Binary Gradient Module, 515 HPLC pump, 2767 Sample Manager, and 2998 Photodiode Array (PDA) Detector). The system was equipped with a Waters C18 5μm X-bridge separation column (150*19 mm). Thin layer chromatography was performed using 0.25 mm silica gel 60-F plates. Flash chromatography was performed using 200-400 mesh silica gel. Yields refer to chromatographically and spectroscopically pure materials, unless otherwise stated. All reagents were used as supplied by Sigma-Aldrich, J&K and Alfa Aesar Chemicals. Methylene chloride, toluene, DMSO and CH_3CN were distilled from calcium hydride; $Pd(OAc)_2$(>99.9%, Sigma-Aldrich) was used without purification. All reactions were carried out in oven-dried glassware under an argon atmosphere unless otherwise noted.

b) Detailed experimental procedures:



Diene 11: To a solution of gochnatiolide A (**3**) (46.0 mg, 0.092 mmol) in DMSO (3.5 mL) was added $Pd(OAc)_2$ (82.3 mg, 0.37 mmol). The resulting mixture was sonicated (about 1 min) until $Pd(OAc)_2$ was completely dissolved, then stirred at 45 ^oC. After 48 h, the reaction mixture was filtered through celite, and washed with EtOAc (50 mL). The filtrate was concentrated in vacuo to 20 mL, then washed with H_2O (20 mL), and brine (20 mL×2). The aqueous layers were further extracted with EtOAc (20 mL×3). The combined organic layers were dried over anhydrous sodium sulfate, and concentrated in vacuo to afford a brown residue, which was purified by flash chromatography (silica gel, EtOAc:PE = 11:9) to afford diene **11** (27.6 mg, 60%) a colorless oil, along with the recovered (-)-gochnatiolide A (**3**) (6.0 mg, 13%).

11: R_f = 0.35 (EtOAc:PE = 3:2); $[\alpha]^{26}_D$ -283.0 (*c* 0.68, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 6.24 (d, 2.4 Hz, 1H), 6.21 (d, 3.6 Hz, 1H), 6.21 (s, 1H); 6.19 (d, 3.2 Hz, 1H), 6.04 (s, 1H), 5.53 (d, 3.2 Hz, 1H), 5.50 (d, 3.2 Hz, 1H), 5.50 (s, 1H), 5.32 (s, 1H), 4.35 (dd, 2.4 Hz, 11.2 Hz, 1H), 4.20 (dd, 9.6 Hz, 11.6 Hz, 1H), 3.87-3.94 (m, 2H), 3.66-3.76 (m, 1H), 3.39 (s, *br*, 1H), 2.91 (m, 1H), 2.58 (m, 1H), 2.36-2.50 (m, 3H), 2.00-2.16 (m, 5H), 1.77 (m, 1H), 1.62 (m, 1H), 1.52 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.3, 194.1, 173.8, 170.1, 169.8, 169.5, 144.1, 142.0, 141.4, 140.2, 138.1, 127.0, 121.6, 121.1, 120.6, 119.6, 83.2, 80.5, 71.4, 54.4, 53.5, 48.8, 48.2, 44.6, 37.3, 35.8, 32.8, 28.4, 26.9, 22.7; IR (neat) ν_{max} 3493, 2934, 2868, 1773, 1698, 1651, 1623, 1453, 1407, 1319, 1268, 1142, 1007, 944, 815, 736 cm^{-1}; HRMS (ESI) [M + H^+] calculated for $C_{30}H_{29}O_7$: 501.1908, found: 501.1910.

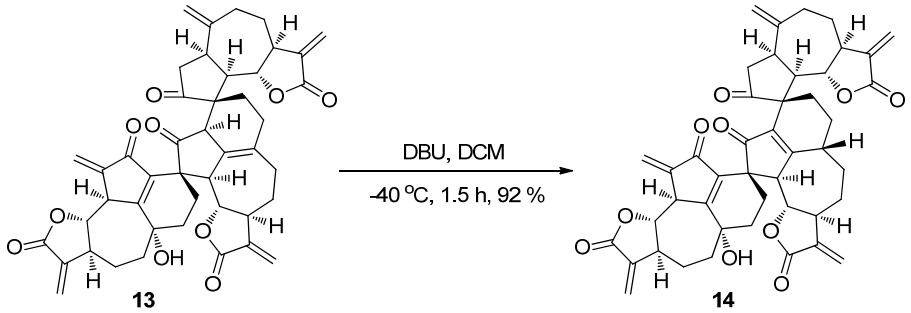


Trimers 13 and 14: A solution of dehydrozaluzanin C (**6**) (105.4 mg, 0.43 mmol) and diene **11** (27.0 mg, 0.054 mmol) in DCM (5.0 mL) was concentrated in vacuo, and the residue was allowed to stand at 35 °C for 48 h. The resulting mixture was purified by flash chromatography (EtOAc:PE = 1:3-3:2) to afford the recovered dehydrozaluzanin C **6** (96.4 mg, 91%) and a mixture of trimers **13** and **14** (23.0 mg). The mixture of trimers **13** and **14** was further purified by preparative HPLC [35% water-65% methanol (2 min)→25% water-75% methanol (13 min), (15 mL/min)] to afford trimer **13** (8.0 mg, 20%) and trimer **14** (8.9 mg, 22%) as white solids.

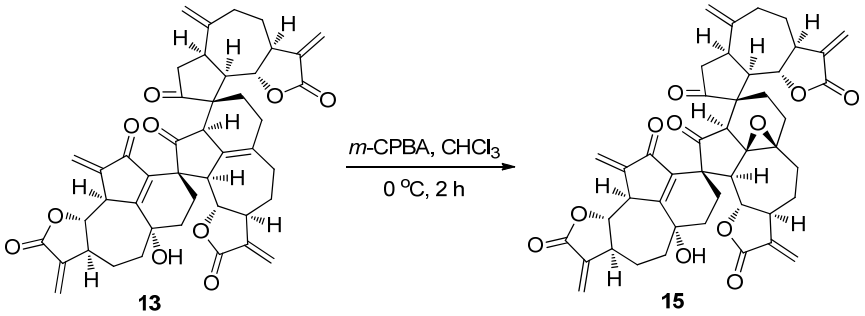
Trimer **13**: mp 150-151 °C; R_f = 0.41 (EtOAc:PE = 4:1); $[\alpha]^{25}_D$ -40.7 (*c* 0.45, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.31 (d, 3.2 Hz, 1H), 6.23 (d, 3.6 Hz, 1H), 6.21 (s, 1H), 6.18 (d, 3.6 Hz, 1H), 5.97 (s, 1H), 5.59 (d, 2.8 Hz, 2H), 5.46 (d, 3.6 Hz, 1H), 5.07 (s, 1H), 4.85 (s, 1H), 4.60 (t, 10.0 Hz, 1H), 4.27 (dd, 8.4 Hz, 10.8 Hz, 1H), 3.92 (d, 10.4 Hz, 1H), 3.90 (s, 1H), 3.86 (m, 1H), 3.70 (d, 10.8 Hz, 1H), 3.64 (t, 10.0 Hz, 1H), 3.42 (t, 10.6 Hz, 1H), 3.13 (m, 1H), 2.96-3.04 (m, 2H), 2.55-2.67 (m, 4H), 1.90-2.44 (m, 14H), 1.73-1.82 (m, 2H), 1.41-1.60(m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 219.5, 217.8, 193.6, 170.2, 169.9, 169.7, 169.6, 150.3, 142.3, 141.8, 140.3, 139.4, 138.5, 130.9, 125.2, 122.0, 121.8, 121.3, 119.6, 113.3, 83.9, 83.4, 79.9, 71.8, 56.7, 53.8, 53.5, 50.4, 49.7, 47.5, 44.5, 44.3, 43.6, 43.4, 39.6, 38.9, 38.6, 35.3, 32.3, 30.6, 29.9, 27.4, 26.3, 26.3, 23.0; IR (neat) ν_{max} 3521, 2934, 2867, 1771, 1695, 1653, 1623, 1405, 1315, 1265, 1138, 1004, 732 cm^{-1}; HRMS (ESI) [M + H$^+$] calculated for $C_{45}H_{45}O_{10}$: 745.3007, found: 745.3007.

Trimer **14**: mp 170 °C (dec.); R_f = 0.41 (EtOAc:PE = 4:1); $[\alpha]^{24}_D$ -174.8 (*c* 0.46, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.28 (d, 3.6 Hz, 1H), 6.21 (s, 1H), 6.18 (d, 3.6 Hz, 2H), 5.99 (s, 1H), 5.56 (d, 2.8 Hz, 1H), 5.53 (d, 3.2 Hz, 1H), 5.47 (d, 3.6 Hz, 1H), 5.06 (s, 1H), 4.72 (s, 1H), 4.43 (dd, 2.4 Hz, 11.6 Hz, 1H), 4.17-4.22 (m, 2H), 3.90 (d, 10.4 Hz, 1H), 3.78 (t, 9.6 Hz, 1H), 3.70 (m, 1H), 3.11-3.16 (m, 3H), 3.01 (m, 1H), 2.90 (m, 1H), 2.55-2.63 (m, 2H), 2.30-2.39 (m, 4H), 2.02-2.24 (m, 5H),

1.80-2.00 (m, 8H), 1.66 (m, 1H), 1.38-1.59 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 221.6, 206.2, 193.9, 173.5, 170.5, 170.2, 169.9, 169.3, 150.6, 141.9, 141.3, 140.3, 138.7, 138.2, 138.2, 121.8, 121.4, 120.2, 119.5, 113.9, 83.6, 83.5, 81.5, 71.2, 54.1, 53.7, 51.0, 50.8, 49.6, 48.2, 44.9, 44.6, 43.4, 40.4, 40.0, 39.6, 37.1, 36.0, 32.1, 31.4, 29.9, 28.7, 27.7, 26.9, 22.7; IR (neat) ν_{max} 3524, 2929, 2864, 1767, 1695, 1643, 1452, 1405, 1306, 1262, 1144, 1007 cm^{-1}; HRMS (ESI) [M + H$^+$] calculated for $C_{45}H_{45}O_{10}$: 745.3007, found: 745.3002.



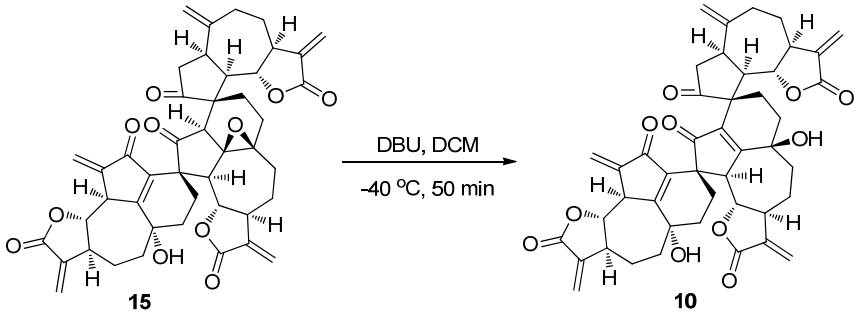
Trimer 14: To a solution of **13** (2.5 mg, 0.0034 mmol) in DCM (0.1 mL) was added a solution of DBU (0.75 μL, 0.005 mmol) in DCM (0.1 mL) at -40 °C. After 1.5 h, the reaction mixture was quenched with sat. NH_4Cl (5 mL). The resulting mixture was extracted with DCM (5 mL), the organic layer was washed with brine (5 mL), and the aqueous layers were extracted with DCM (5 mL×3). The combined organic layers were dried over anhydrous sodium sulfate, concentrated in vacuo and purified by flash chromatography (silica gel, EtOAc:PE = 7:3) to afford trimer **14** (2.3 mg, 92%).



Epoxide 15: To a solution of trimer **13** (9.0 mg, 0.012 mmol) in $CHCl_3$ (0.5 mL) was added *m*-CPBA (70%, 6.5 mg, 0.027 mmol) in four portions over 1 h at 0 °C. The resulting mixture was stirred at 0 °C for an additional 1h. The reaction mixture was diluted with $CHCl_3$ (2.0 mL) and

quenched with sat. $Na_2S_2O_3$ (1.0 mL). The mixture was stirred for 10 min, followed by the addition of sat. $NaHCO_3$ (1.0 mL). The resulting mixture was poured into 10 mL of H_2O, and extracted with DCM (10 mL). The organic layer was washed by brine (10 mL), and the aqueous layers were extracted with DCM (10 mL×3). The combined organic layers were dried over anhydrous sodium sulfate, concentrated in vacuo and purified by preparative TLC (EtOAc:PE = 3:2) to afford the epoxide **15** (7.5 mg, 82%) as a white solid.

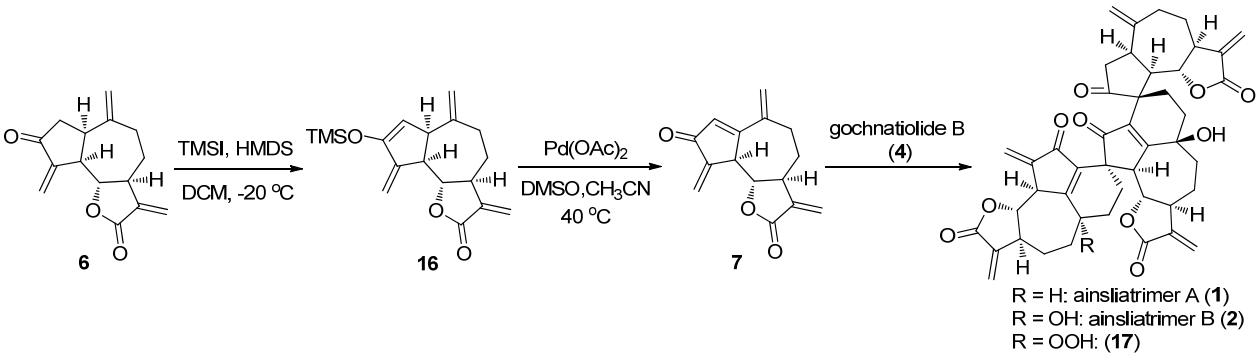
15: mp 189 °C (dec.); R_f = 0.35 (EtOAc:PE = 3:1); $[\alpha]^{25}_D$ +3.7 (*c* 0.33, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.34 (d, 3.6 Hz, 1H), 6.22 (d, 3.6 Hz, 1H), 6.21 (s, 1H), 6.18 (d, 3.6 Hz, 1H), 6.00 (s, 1H), 5.62 (d, 2.8 Hz, 1H), 5.56 (d, 2.8 Hz, 1H), 5.47 (d, 3.2 Hz, 1H), 5.05 (s, 1H), 4.71 (s, 1H), 4.26 (t, 9.4 Hz, 1H), 4.19 (dd, 9.6 Hz, 10.8 Hz, 1H), 3.93 (d, 10.4 Hz, 1H), 3.83 (m, 1H), 3.71 (d, 10 Hz, 1H), 3.66 (s, 1H), 3.57 (d, 10.8 Hz, 1H), 2.99-3.08 (m, 3H), 2.79 (dd, 10.0 Hz, 19.6 Hz, 1H), 2.48-2.64 (m, 4H), 1.94-2.41 (m, 12H), 1.73-1.81 (m, 4H), 1.44-1.62 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 220.2, 216.5, 193.9, 170.8, 170.3, 169.9, 169.3, 149.8, 141.9, 141.7, 140.3, 138.2, 137.6, 122.0, 121.0, 119.6, 113.9, 83.9, 83.7, 81.4, 71.5, 66.4, 62.2, 56.0, 53.3, 52.3, 52.0, 47.8, 47.8, 47.7, 44.2, 43.8, 43.7, 39.5, 39.1, 38.1, 35.6, 32.2, 32.0, 30.6, 25.7, 24.9, 23.0, 22.6; IR (neat) ν_{max} 3512, 2928, 2862, 1762, 1691, 1645, 1311, 1262, 1143, 996 cm^{-1}; HRMS (ESI) [M + H$^+$] calculated for $C_{45}H_{45}O_{11}$: 761.2956, found: 761.2967.



Trimer 10: To a solution of epoxide **15** (6.4 mg, 0.0084 mmol) in DCM (0.3 mL) was added a solution of DBU (1.9 μL, 0.013 mmol) at -40 °C. After 50 min, the reaction was quenched with sat. NH_4Cl (0.5 mL), and the resulting mixture was poured into H_2O (10 mL), and extracted with DCM (10 mL). The organic layer was washed by brine (10 mL), and the aqueous layers were extracted with DCM (10 mL×3). The combined organic layers were dried over anhydrous sodium sulfate,

concentrated in vacuo and purified by preparative TLC (EtOAc:PE = 3:2) to afford trimer **10** (5.9 mg, 92%) as a white solid.

10: mp 205 °C (dec.); R_f = 0.31 (EtOAc:PE = 4:1); $[\alpha]^{24}_D$ -146.5 (*c* 0.26, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 6.29 (d, 3.2 Hz, 1H), 6.20 (s, 1H), 6.19 (d, 3.2 Hz, 1H), 6.17 (d, 3.2 Hz, 1H), 5.99 (s, 1H), 5.57 (d, 3.2 Hz, 1H), 5.53 (d, 2.8 Hz, 1H), 5.47 (d, 3.2 Hz, 1H), 5.08 (s, 1H), 4.72 (s, 1H), 4.54 (dd, 9.6 Hz, 11.6 Hz, 1H), 4.32 (d, 11.6 Hz, 1H), 4.17 (t, 9.2 Hz, 1H), 3.92 (d, 10.0 Hz, 1H), 3.74-3.80 (m, 2H), 3.14-3.24 (m, 2H), 2.99-3.10 (m, 2H), 2.84 (m,1H), 2.58-2.64 (m, 2H), 2.30-2.41 (m, 2H), 1.88-2.28 (m, 15H), 1.73 (m, 1H), 1.41-1.65 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 222.4, 207.3, 193.8, 170.7, 170.5, 169.9, 169.1, 150.4, 141.9, 141.2, 140.3, 138.6, 138.5, 138.4, 121.8, 121.7, 120.0, 119.6, 114.0, 83.6, 83.6, 80.2, 71.4, 68.6, 54.2, 53.6, 50.8, 50.7, 49.7, 48.1, 44.9, 44.3, 43.4, 40.0, 39.5, 37.3, 36.6, 36.4, 35.9, 32.1, 26.9, 25.6, 22.8, 21.1; IR (neat) ν_{max} 3499, 2929, 1763, 1695, 1406, 1311, 1263, 1146, 1003, 756 cm^{-1} ; HRMS (ESI) [M + H^+] calculated for $C_{45}H_{45}O_{11}$: 761.2956, found: 761.2957.



(-)-Ainsliatrimers A (1) and B (2): To a solution of dehydrozaluzanin C **6** (100 mg, 0.41 mmol) in DCM (10.0 ml) was added HMDS (300 μL, 1.41 mmol) at -20 °C. After 3 min, TMSI (190 μL, 1.33 mmol) was added dropwise. After 15 min, to the reaction mixture was added cooled sat. $NaHCO_3$ (10 mL), and the resulting mixture was stirred vigorously at rt for 1 min before H_2O (10 mL) was added. The resulting mixture was extracted with DCM (10 mL), and the organic layer was washed with brine (20 mL). The aqueous layers were extracted with DCM (20 mL×3), and the combined organic layers were dried over anhydrous sodium sulfate, concentrated in vacuo to afford silyl enol ether **16** as a yellow slurry which was used directly without further purification. To the crude silyl enol ether **16** was added $Pd(OAc)_2$ (92.0 mg, 0.41 mmol), CH_3CN (4.0 mL) and DMSO (175 μL, 2.47 mmol). The resulting mixture was sonicated (about 2 min) until $Pd(OAc)_2$ was dissolved

completely, then stirred at 40 °C. The reaction was closely monitored by TLC till it was completed (60 min to 80 min). The reaction mixture was concentrated to about 0.5 mL, and the residue was purified by flash column chromatography (silica gel, EtOAc:PE = 1:2) to afford **7**. The freshly prepared diene **7** was dissolved in toluene (10 mL) and to the resulting solution was added (-)-gochnatiolide B (25.0 mg, 0.05 mmol). The resulting mixture was stirred at 35 °C under air. After 7 days, Me_2S (0.1 mL) and EtOAc (2 mL) was added, and stirred at 35 °C for 12 h. The reaction mixture was concentrated in vacuo, and purified by flash chromatography (silica gel, EtOAc:PE = 9:11-1:1) to afford the recovered gochnatiolide B **4** (4.7 mg, 19%), the crude ainsliatrimer A and the crude ainsliatrimer B. The crude ainsliatrimer A was further purified by preparative TLC (EtOAc:DCM = 23:77) to afford the pure (-)-ainsliatrimer A (**1**) (14.0 mg, 38%). The crude ainsliatrimer B was further purified by preparative TLC (EtOAc:PE = 11:9) to afford the pure (-)-ainsliatrimer B (**2**) (4.2 mg, 11%).

7: a colorless solid;[a] R_f = 0.20 (EtOAc:PE = 3:2); $[\alpha]^{20}_D$ -128.9 (*c* 0.65, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.63 (d, 1.6 Hz, 1H), 6.30 (d, 0.8 Hz, 1H), 6.22 (d, 3.2 Hz, 1H), 5.99 (s, 1H), 5.74 (s, 1H), 5.51 (d, 3.2 Hz, 1H), 5.44 (s, 1H), 3.73-3.81 (m, 2H), 2.93 (m, 1H), 2.76 (m, 1H), 2.37-2.52 (m, 2H), 1.45 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.2, 169.1, 168.3, 142.7, 141.6, 138.5, 130.9, 122.1, 122.0, 119.4, 83.0, 51.3, 49.8, 34.0, 27.2; IR (neat) ν_{max} 3005, 2989, 1769, 1694, 1276, 1261, 1134, 1003, 914 cm^{-1}; HRMS (ESI) [M + H$^+$] calculated for $C_{15}H_{15}O_3$: 243.1016, found: 243.1012.

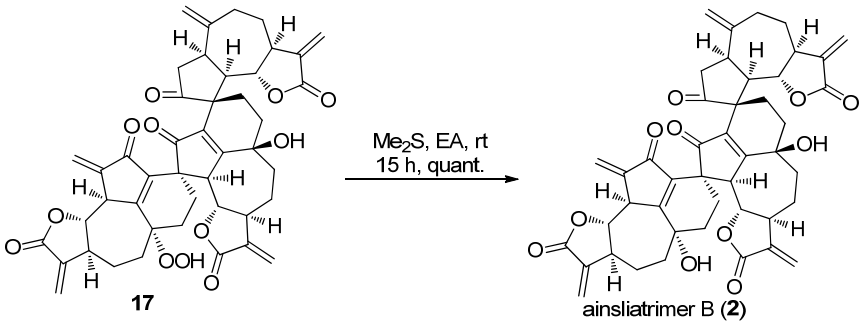
(-)-Ainsliatrimer A (1): mp 176 °C (dec.); R_f = 0.41 (EtOAc:PE = 4:1); $[\alpha]^{20}_D$ -15.7 (*c* 0.21, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.27 (d, 3.6 Hz, 1H), 6.26 (s, 1H), 6.25 (d, 3.6 Hz, 1H), 6.10 (d, 3.2 Hz, 1H), 5.97 (s, 1H), 5.59 (d, 3.2 Hz, 1H), 5.55 (d, 3.2 Hz, 1H), 5.49 (d, 3.2 Hz, 1H), 5.09 (s, 1H), 4.75 (s, 1H), 4.70 (s, *br*, 1H), 4.36 (dd, 9.2 Hz, 10.8 Hz, 1H), 4.22 (t, 9.6 Hz, 1H), 3.79 (t, 9.8 Hz, 1H); 3.43 (d, 10.4 Hz, 1H), 3.09-3.26 (m, 4H), 2.94-3.02 (m, 2H), 2.77 (m, 1H), 2.53-2.69 (m, 4H), 2.29-2.41 (m, 4H), 2.06-2.24 (m, 5H), 1.92-2.00 (m, 3H), 1.77-1.89 (m, 4H), 1.64 (m, 1H), 1.50 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 218.9, 207.6, 193.9, 173.8, 171.9, 169.5, 169.4, 169.4, 149.9, 141.4, 140.2, 139.2, 138.7, 138.5, 138.2, 122.0, 121.6, 120.5, 119.3, 114.4, 84.0, 83.1, 80.7, 68.2, 58.0, 52.3, 52.2, 51.2, 50.5, 50.3, 44.6, 44.0, 43.7, 39.9, 39.5, 36.7, 36.6, 34.5, 31.9, 28.9, 28.6, 27.1, 25.8, 24.9, 21.2; IR (neat) ν_{max} 3468, 2929, 2868, 1772, 1696, 1647, 1404, 1313, 1260, 1142, 1001, 732 cm^{-1}; HRMS (ESI) [M + Na$^+$] calculated for $C_{45}H_{44}NaO_{10}$: 767.2827, found: 767.2818.

[a] Due to the rapid dimerization of **7**, melting point was not determined.

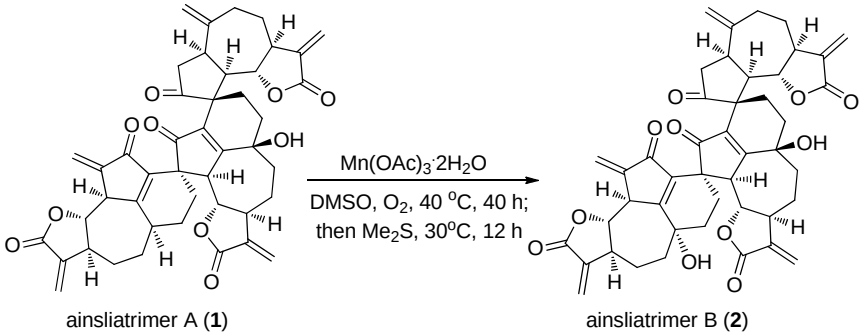
(-)-Ainsliatrimer B (2): mp 180 ^{o}C (dec.); R_f = 0.34 (EtOAc:PE = 4:1); $[\alpha]^{20}_{D}$ -31.9 (*c* 0.54, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.30 (s, 1H), 6.27 (d, 3.6 Hz, 1H), 6.19 (d, 3.6 Hz, 1H), 6.10 (d, 3.6 Hz, 1H), 5.99 (s, 1H), 5.59 (d, 3.2 Hz, 1H), 5.49 (d, 2.8 Hz, 1H), 5.48 (d, 3.6 Hz, 1H), 5.10 (s, 1H), 4.76 (s, 1H), 4.54 (s, *br*, 1H), 4.34 (dd, 9.6 Hz, 11.2 Hz, 1H), 4.22 (t, 9.4 Hz, 1H), 3.86 (d, 10.0 Hz, 1H), 3.77 (m, 1H), 3.61 (t, 9.8 Hz, 1H), 3.16 (d, 11.2 Hz, 1H), 3.14 (m, 1H), 3.10 (m, 1H), 3.00 (m, 1H), 2.93 (t, 9.2 Hz, 1H), 2.55-2.67 (m, 3H), 2.42-2.50 (m, 2H); 2.27-2.39 (m, 2H), 1.88-2.24 (m, 12H), 1.75-1.86(m, 3H), 1.50 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 218.9, 207.2, 194.2, 172.5, 172.2, 169.9, 169.5, 169.3, 149.8, 141.5, 140.1, 139.9, 139.1, 138.5, 138.4, 122.9, 121.7, 119.7, 119.6, 114.5, 84.0, 84.0, 80.5, 72.5, 68.3, 58.2, 52.1, 51.8, 50.5, 50.3, 46.9, 44.5, 43.6, 43.4, 39.8, 39.5, 38.1, 36.5, 36.5, 34.1, 31.9, 31.2, 25.8, 22.8, 21.1; IR (neat) ν_{max} 3475, 2927, 2863, 1766, 1693, 1402, 1307, 1260, 1138, 1107, 1000, 953 cm^{-1}; HRMS (ESI) [M + Na^+] calculated for $C_{45}H_{44}NaO_{11}$: 783.2776, found: 783.2773.

Peroxide 17: To a solution of dehydrozaluzanin C **6** (252 mg, 1.04 mmol) in DCM (25 ml) was added HMDS (0.76 mL, 3.62 mmol) at -20 ^{o}C. After 3 min, TMSI (479 μL, 3.35 mmol) was added dropwise. After 15 min, to the reaction mixture was added cooled sat. $NaHCO_3$ (25 mL), and the resulting mixture was stirred vigorously at rt for 1 min before H_2O (25 mL) was added. The resulting mixture was extracted with DCM (25 mL), and the organic layer was washed with brine (50 mL). The aqueous layers were extracted with DCM (50 mL×3), and the combined organic layers were dried over anhydrous sodium sulfate, concentrated in vacuo to afford silyl enol ether **16** as a yellow slurry which was used directly without further purification. To the crude silyl enol ether **16** was added $Pd(OAc)_2$ (232 mg, 1.04 mmol), CH_3CN (10 mL) and DMSO (0.40 mL, 5.64 mmol). The resulting mixture was sonicated (about 3 min) until $Pd(OAc)_2$ was dissolved completely, then stirred at 40 ^{o}C. The reaction was closely monitored by TLC till it was completed (60 min to 80 min). The reaction mixture was concentrated to about 1.0 mL, and the residue was purified by flash column chromatography (silica gel, EtOAc:PE = 1:2) to afford **7**. The freshly prepared diene **7** and gochnatiolide B **4** (65.0 mg, 0.13 mmol) were dissolved in DCM (3.0 mL), and the mixture was transferred to a 5 mL round bottomed flask and the reaction mixture was stirred at room temperature under air. After 24 h, the solvent was removed in vacuo, and the residue was purified by preparative HPLC to afford peroxide **17** (2.5 mg, 2.5%) as a white solid, along with the recovered (-)-gochnatiolide B **4** (32.0 mg, 49%).

17: mp 165 °C (dec.); R_f = 0.38 (EtOAc:PE = 4:1); $[\alpha]^{22}_D$ -45.7 (*c* 0.11, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, *br*, 1H), 6.30 (s, 1H), 6.28 (d, 3.6 Hz, 1H), 6.20 (d, 3.2 Hz, 1H), 6.11 (d, 3.6 Hz, 1H), 5.98 (s, 1H), 5.60 (d, 3.2 Hz, 1H), 5.50 (d, 3.2 Hz, 1H), 5.48 (d, 2.8 Hz, 1H), 5.09 (s, 1H), 4.76 (s, 1H), 4.48 (s, *br*, 1H), 4.35 (dd, 9.6 Hz, 11.2 Hz, 1H), 4.22 (t, 10.0 Hz, 1H), 3.91 (d, 9.6 Hz, 1H), 3.55-3.58 (m, 2H), 3.14-3.18 (m, 3H), 2.93-3.06 (m, 3H), 2.53-2.69 (m, 3H), 2.45 (m, 1H), 2.30-2.37 (m, 2H), 1.76-2.24 (m, 13H), 1.69 (m, 1H), 1.50 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 219.0, 206.5, 193.9, 171.6, 169.8, 169.5, 169.5, 169.4, 149.9, 142.2, 141.8, 140.2, 139.4, 138.5, 138.5, 122.7, 121.7, 119.8, 119.6, 114.5, 85.0, 84.8, 84.0, 80.7, 68.3, 58.2, 52.2, 51.7, 50.5, 50.3, 47.1, 44.7, 43.7, 43.6, 39.9, 39.5, 36.6, 36.5, 32.4, 31.9, 30.9, 30.4, 25.8, 22.7, 21.0; IR (neat) ν_{max} 3492, 3333, 2926, 2854, 1770, 1696, 1648, 1404, 1312, 1265, 1143, 1004, 959 cm^{-1}; HRMS (ESI) $[M + Na^+]$ calculated for $C_{45}H_{44}NaO_{12}$: 799.2725, found: 799.2723.



Reduction of peroxide 17: To a solution of peroxide **17** (2.5 mg, 0.0032 mmol) in EtOAc (0.2 mL) was added Me_2S (2.3 μL, 0.032 mmol), and the resulting mixture was stirred at rt. After 15 h, the reaction mixture was concentrated in vacuo. The residue was dissolved in DCM (0.2 mL), filtered through a short pad of silica gel and washed with EtOAc/PE (4:1, 20 mL). The filtrate was concentrated in vacuo to afford (-)-ainsliatrimer B (2.5 mg, quant.).



$Mn(OAc)_3 \cdot 2H_2O$ Mediated Allylic Oxidation. To (-)-ainsliatrimer A **1** (3.7 mg, 0.005 mmol) was added a solution of $Mn(OAc)_3 \cdot 2H_2O$ in DMSO (0.1 mL, 2.7 mg/mL, 0.001 mmol), and the reaction mixture was stirred at 40 ^oC for 40 h under an oxygen balloon. The resulting mixture was diluted with EtOAc (5 mL), washed with brine (5 mL×3), and the aqueous layers were extracted with EtOAc (5 mL×3). The combined organic layers were dried over anhydrous sodium sulfate, concentrated in vacuo and purified by prepared TLC (silica gel, EtOAc:PE = 3:2) to afford (-)-ainsliatrimer B **2** (2.1 mg, 56%).

II) Comparison of Natural and Synthetic Products

NMR data (in $CDCl_3$) comparison of natural and synthetic (-)-ainsliatrimer A

^{1}H NMR (Hz)		^{13}C NMR (Hz)	
Natural (400 MHz)	Synthetic (400 MHz)	Natural (100 MHz)	Synthetic (100 MHz)
6.27 (d, 3.0, 1H)	6.27 (d, 3.6, 1H)	218.8	218.9
6.26 (s, 1H)	6.26 (s, 1H)	207.6	207.6
6.25 (d, 3.0, 1H)	6.25 (d, 3.6, 1H)	193.9	193.9
6.10 (d, 3.0, 1H)	6.10 (d, 3.2, 1H)	173.8	173.8
5.97 (s, 1H)	5.97 (s, 1H)	171.9	171.9
5.59 (d, 3.0, 1H)	5.59 (d, 3.2, 1H)	169.4	169.5
5.57 (d, 3.2, 1H)	5.55 (d, 3.2, 1H)	169.4	169.4
5.48 (d, 2.8, 1H)	5.49 (d, 2.8, 1H)	169.4	169.4
5.09 (s, 1H)	5.09 (s, 1H)	150.0	149.9
4.76 (s, 1H)	4.75 (s, 1H)	141.4	141.4
4.36 (t, 10.0, 1H)	4.36 (dd, 9.6, 11.2, 1H)	140.3	140.2
4.22 (t, 10.0, 1H)	4.22 (t, 9.6, 1H)	139.3	139.2
3.78 (t, 10.3, 1H)	3.79 (t, 9.8, 1H)	138.8	138.7
3.43 (d, 10.3, 1H)	3.43 (d, 10.4, 1H)	138.6	138.5
3.21 (m, 1H)	3.21 (m, 1H)	138.2	138.2
3.19 (d, 10.0, 1H)	3.19 (d, 10.0, 1H)	122.0	122.0
3.14 (m, 1H)	3.15 (m, 1H)	121.6	121.6
3.11 (m, 1H)	3.11 (m, 1H)	120.5	120.5
2.99 (m, 1H)	2.98 (m, 1H)	119.3	119.3
2.94 (m, 1H)	2.95 (m, 1H)	114.4	114.4
2.77 (m, 1H)	2.77 (m, 1H)	84.1	84.0
2.65 (m, 1H)	2.64 (m, 1H)	83.2	83.1
2.61 (m, 1H)	2.60 (m, 1H)	80.7	80.7
2.58 (m, 1H)	2.58 (m, 1H)	68.3	68.2
2.55 (m, 1H)	2.55 (m, 1H)	58.1	58.0
2.38 (m, 1H)	2.39 (m, 1H)	52.3	52.3
2.35 (m, 1H)	2.35 (m, 1H)	52.2	52.2
2.34 (m, 1H)	2.34 (m, 1H)	51.2	51.2
2.30 (m, 1H)	2.30 (m, 1H)	50.5	50.5
2.20 (m, 1H)	2.22 (m, 1H)	50.3	50.3
2.17 (m, 1H)	2.17 (m, 1H)	44.6	44.6
2.15 (m, 1H)	2.15 (m, 1H)	44.0	44.0
2.10 (m, 1H)	2.10 (m, 1H)	43.7	43.7
2.06 (m, 1H)	2.08 (m, 1H)	39.9	39.9

2.00 (m, 1H)	1.99 (m, 1H)	39.6	39.5
1.99 (m, 1H)	1.98 (m, 1H)	36.7	36.7
1.94 (m, 1H)	1.94 (m, 1H)	36.6	36.6
1.85 (m, 1H)	1.87 (m, 1H)	34.6	34.5
1. 82 (m, 1H)	1.82 (m, 1H)	31.9	31.9
1. 80 (m, 1H)	1.79 (m, 1H)	28.9	28.9
1. 80 (m, 1H)	1.79 (m, 1H)	28.6	28.6
1.64 (m, 1H)	1.64 (m, 1H)	27.1	27.1
1.50 (m, 1H)	1.50 (m, 1H)	25.8	25.8
		24.9	24.9
		21.2	21.2

$[\alpha]_D$ of Natural and Synthetic Ainsliatrimer A:

	Natural (-)-Ainsliatrimer A	Synthetic (-)-Ainsliatrimer A
$[\alpha]^{20}_D$	-12.0 (*c* 0.55, $CHCl_3$)	-15.7 (*c* 0.21, $CHCl_3$)

NMR data (in $CDCl_3$) comparison of natural and synthetic (-)-ainsliatrimer B

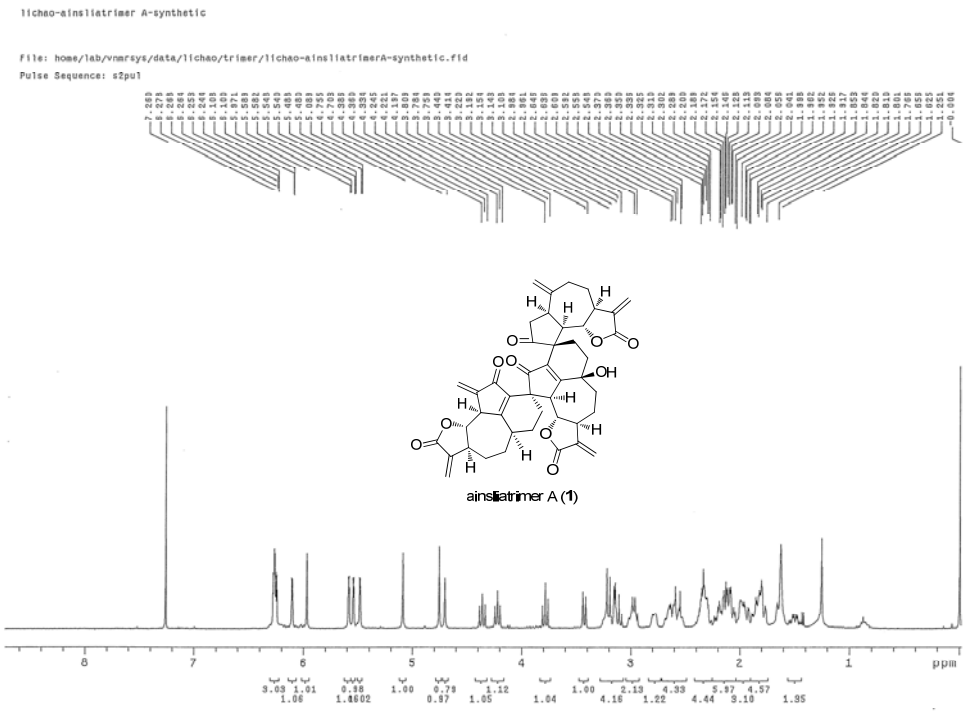
^{1}H NMR (Hz)		^{13}C NMR (Hz)	
Natural (400 MHz)	Synthetic (400 MHz)	Natural (100MHz)	Synthetic (100 MHz)
6.30 (s, 1H)	6.30 (s, 1H)	218.8	218.9
6.28 (d, 3.2, 1H)	6.27 (d, 3.6, 1H)	207.3	207.2
6.19 (d, 3.4, 1H)	6.19 (d, 3.6, 1H)	194.2	194.2
6.11 (d, 3.2, 1H)	6.10 (d, 3.6, 1H)	172.4	172.5
5.99 (s, 1H)	5.99 (s, 1H)	172.3	172.2
5.59 (d, 3.2, 1H)	5.59 (d, 3.2, 1H)	169.8	169.9
5.49 (d, 3.4, 1H)	5.49 (d, 2.8, 1H)	169.4	169.5
5.48 (d, 3.2, 1H)	5.48 (d, 3.6, 1H)	169.3	169.3
5.10 (s, 1H)	5.10 (s, 1H)	149.9	149.8
4.77 (s, 1H)	4.76 (s, 1H)	141.6	141.5
4.35 (t, 9.6, 1H)	4.34 (dd, 9.6, 11.2, 1H)	140.2	140.1
4.22 (t, 9.6, 1H)	4.22 (t, 9.4, 1H)	140.0	139.9
3.87 (d, 10.3, 1H)	3.86 (d, 10.0, 1H)	139.1	139.1
3.78 (m, 1H)	3.77 (m, 1H)	138.6	138.5
3.64 (t, 10.3, 1H)	3.61 (t, 9.8, 1H)	138.5	138.4
3.17 (d, 9.6, 1H)	3.16 (d, 11.2, 1H)	123.0	122.9
3.13 (m, 1H)	3.14 (m, 1H)	121.7	121.7
3.07 (m, 1H)	3.10 (m, 1H)	119.6	119.7
3.00 (m, 1H)	3.00 (m, 1H)	119.5	119.6
2.94 (t, 9.6, 1H)	2.93 (t, 9.2, 1H)	114.5	114.5
2.65 (m, 1H)	2.66 (m, 1H)	84.0	84.0
2.65 (m, 1H)	2.65 (m, 1H)	84.0	84.0
2.63 (m, 1H)	2.63 (m, 1H)	80.6	80.5
2.60 (m, 1H)	2.48 (m, 1H)	72.6	72.5
2.49 (m, 1H)	2.44 (m, 1H)	68.3	68.3
2.34 (m, 1H)	2.34 (m, 1H)	58.3	58.2
2.31 (m, 1H)	2.31 (m, 1H)	52.2	52.1
2.30 (m, 1H)	2.30 (m, 1H)	51.9	51.8
2.23 (m, 1H)	2.22 (m, 1H)	50.5	50.5
2.17 (m, 1H)	2.17 (m, 1H)	50.4	50.3
2.10 (m, 1H)	2.10 (m, 1H)	46.9	46.9
2.09 (m, 1H)	2.09 (m, 1H)	44.5	44.5
2.08 (m, 1H)	2.08 (m, 1H)	43.7	43.6
2.05 (m, 1H)	2.05 (m, 1H)	43.5	43.4
2.05 (m, 1H)	2.05 (m, 1H)	39.9	39.8
1.99 (m, 1H)	1.99 (m, 1H)	39.6	39.5
1.99 (m, 1H)	1.99 (m, 1H)	38.2	38.1

1.93 (m, 1H)	1.93 (m, 1H)	36.6	36.5
1.80 (m, 1H)	1.80 (m, 1H)	36.6	36.5
1.79 (m, 1H)	1.79 (m, 1H)	34.2	34.1
1.78 (m, 1H)	1.78 (m, 1H)	31.9	31.9
1.52 (m, 1H)	1.50 (m, 1H)	31.2	31.2
		25.8	25.8
		22.9	22.8
		21.1	21.1

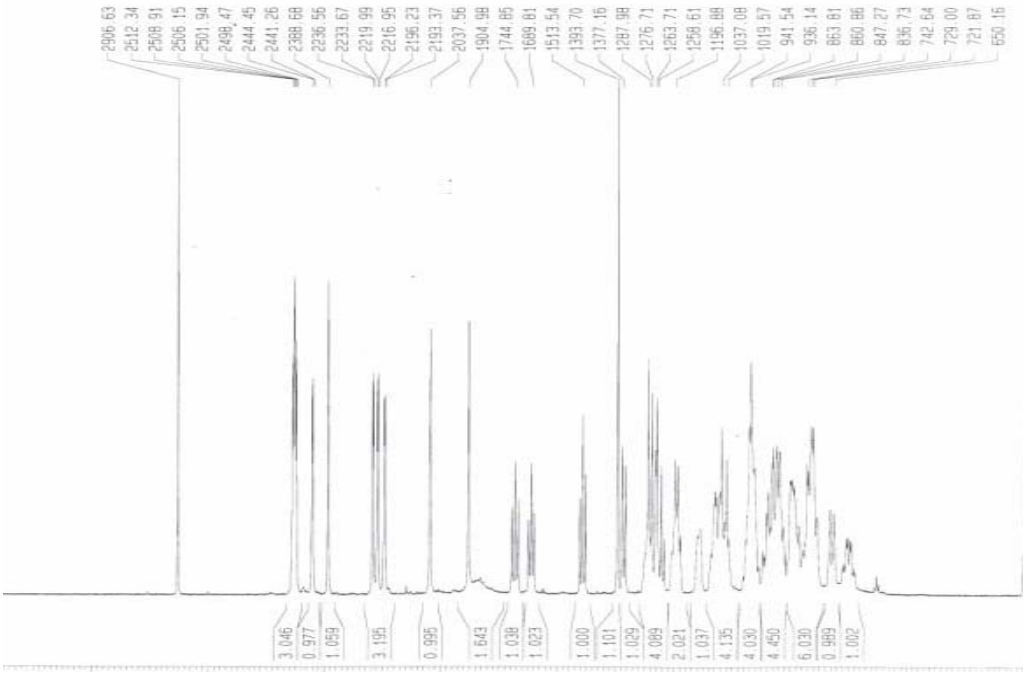
$[\alpha]_D$ of Natural and Synthetic Ainsliatrimer B:

	Natural (-)-ainsliatrimer B	Synthetic (-)-ainsliatrimer B
$[\alpha]^{20}_D$	-25.5 (*c* 0.25, $CHCl_3$)	-31.9 (*c* 0.54, $CHCl_3$)

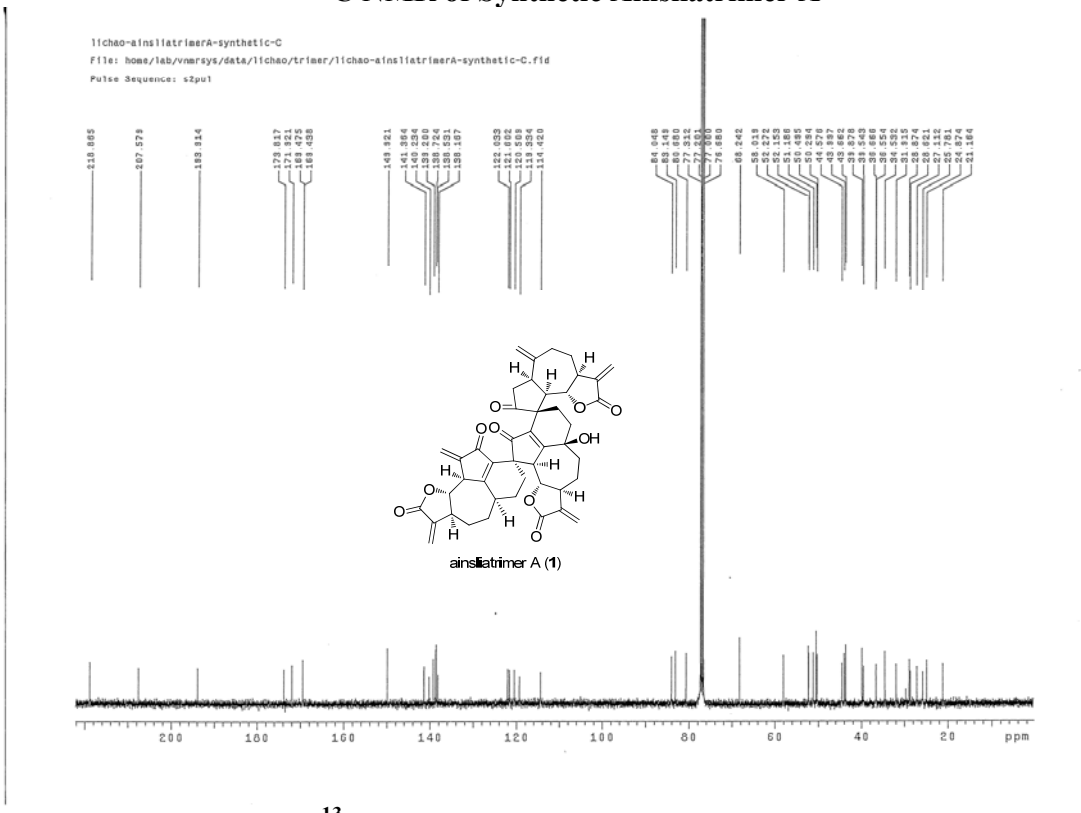
^{1}H NMR of Synthetic Ainsliatrimer A



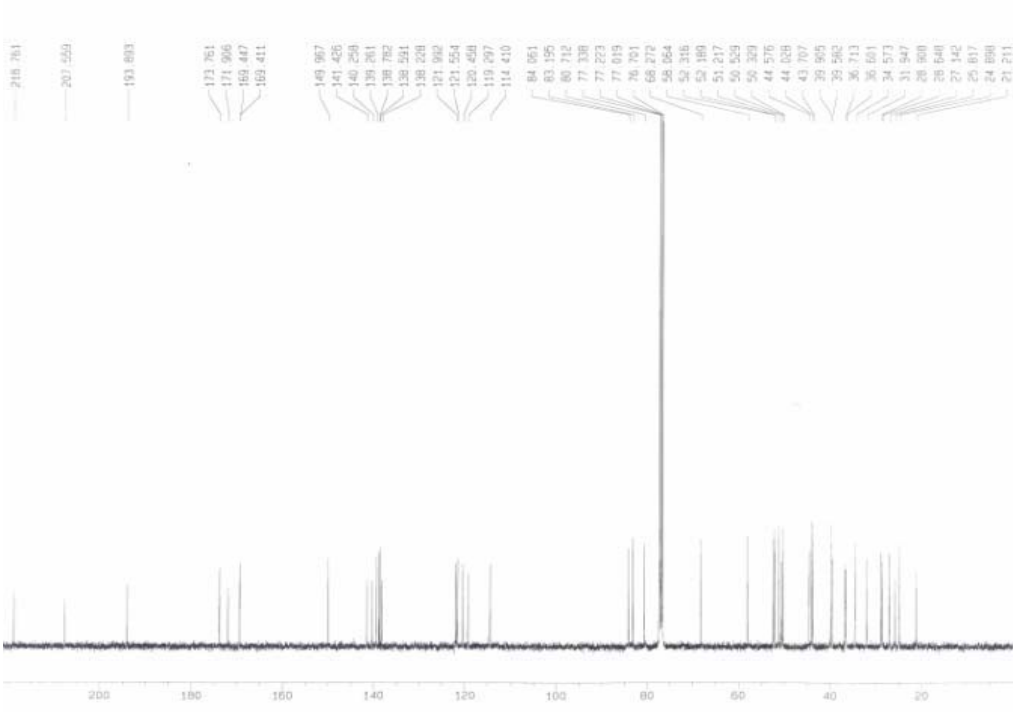
^{1}H NMR of Natural Ainsliatrimer A



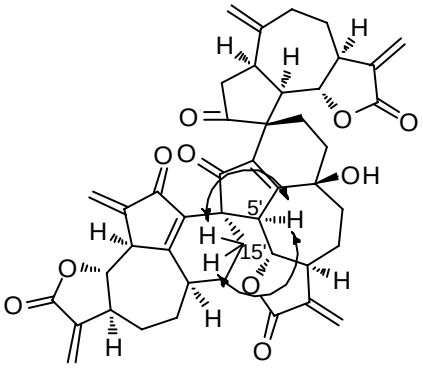
^{13}C NMR of Synthetic Ainsliatrimer A



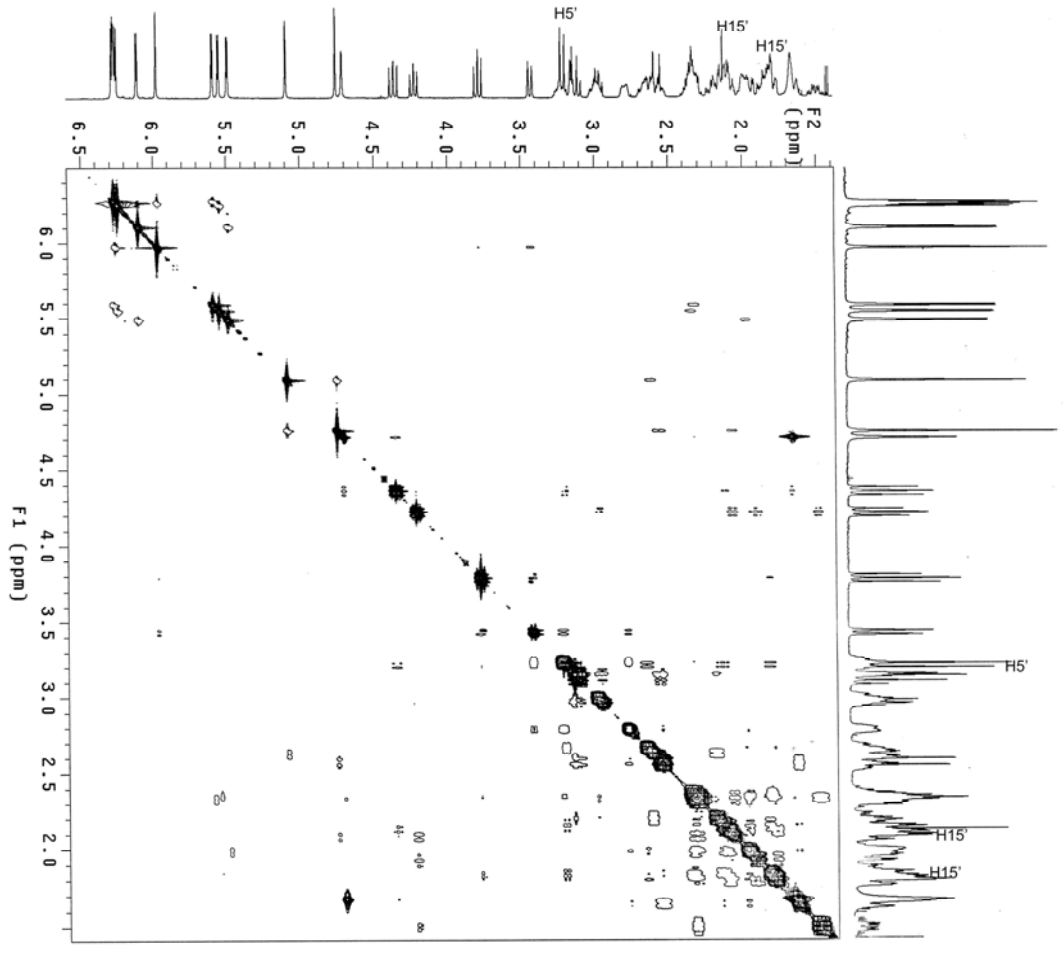
^{13}C NMR of Natural Ainsliatrimer A



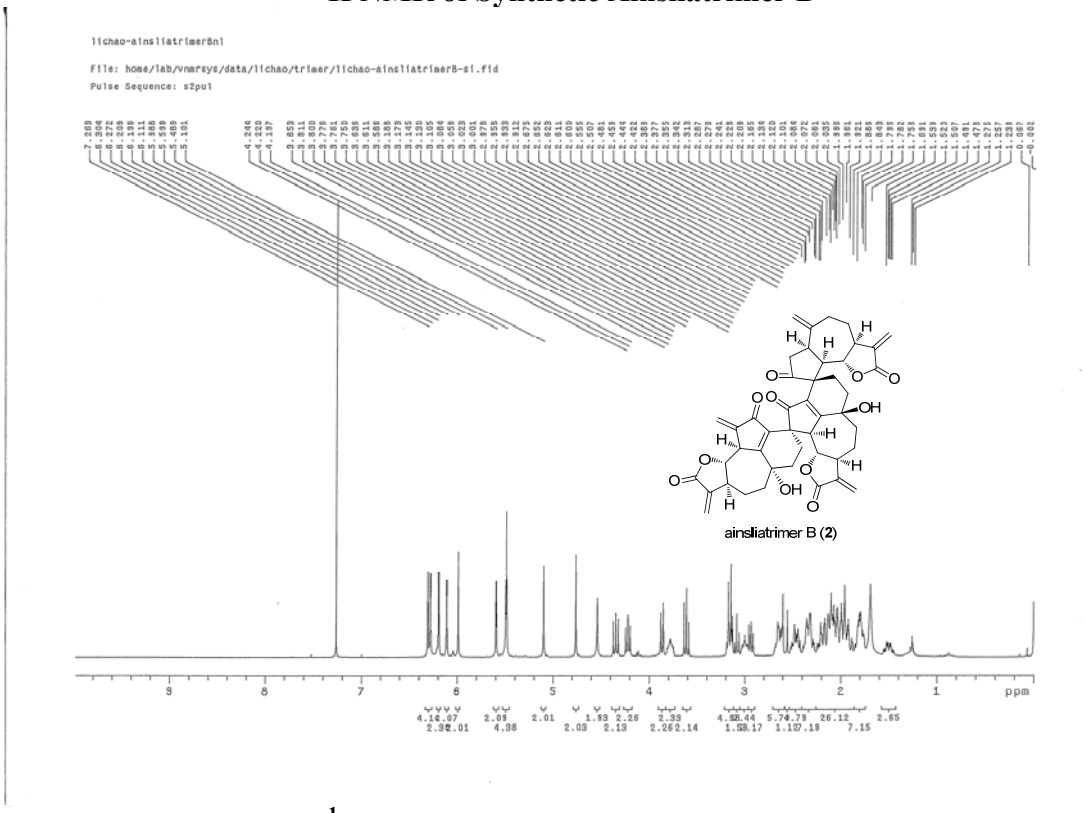
NOESY Spectrum of Synthetic Ainsliatrimer A



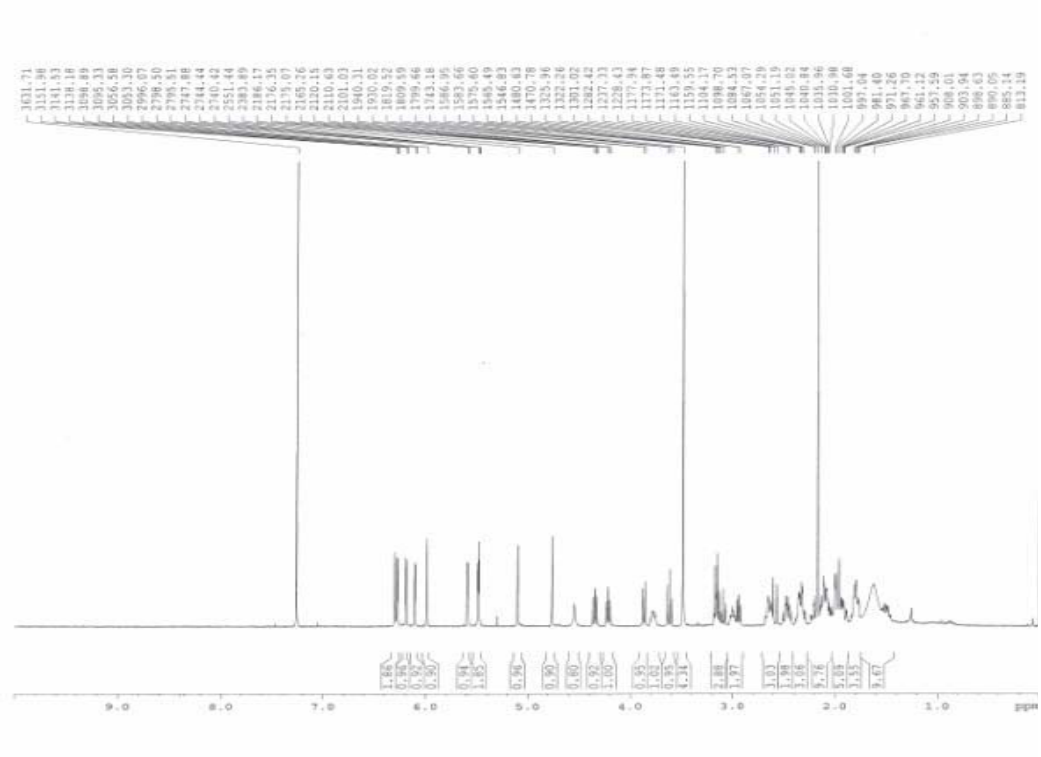
ainsliatrimer A (**1**)



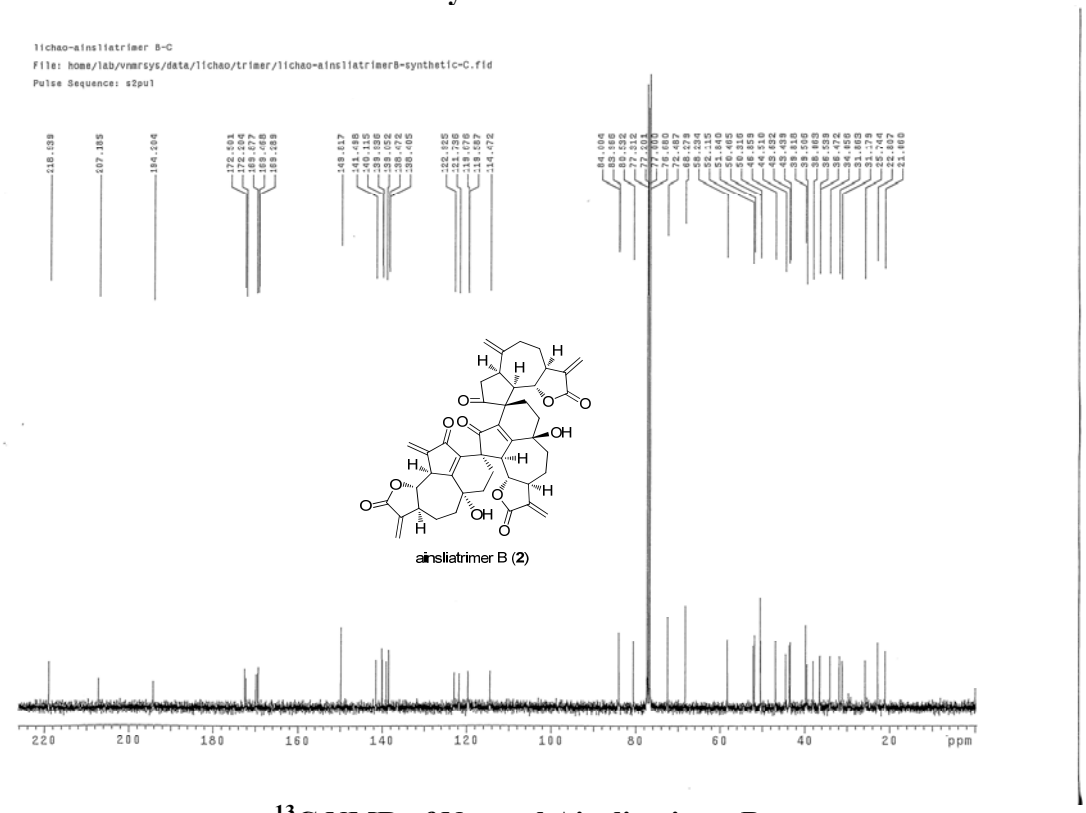
^{1}H NMR of Synthetic Ainsliatrimer B



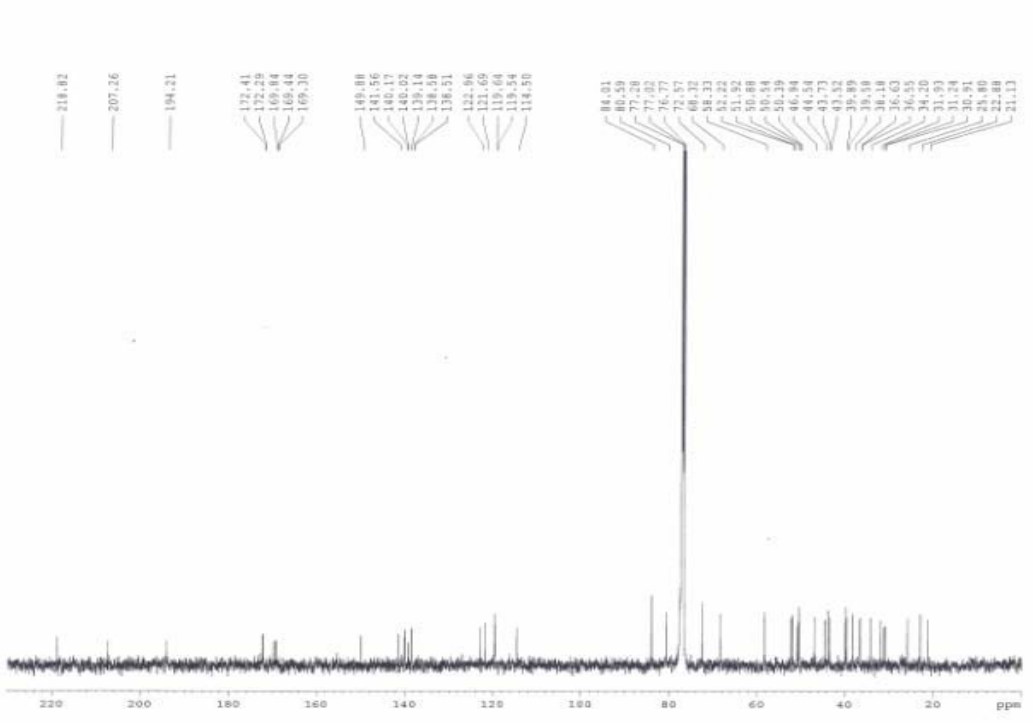
^{1}H NMR of Natural Ainsliatrimer B



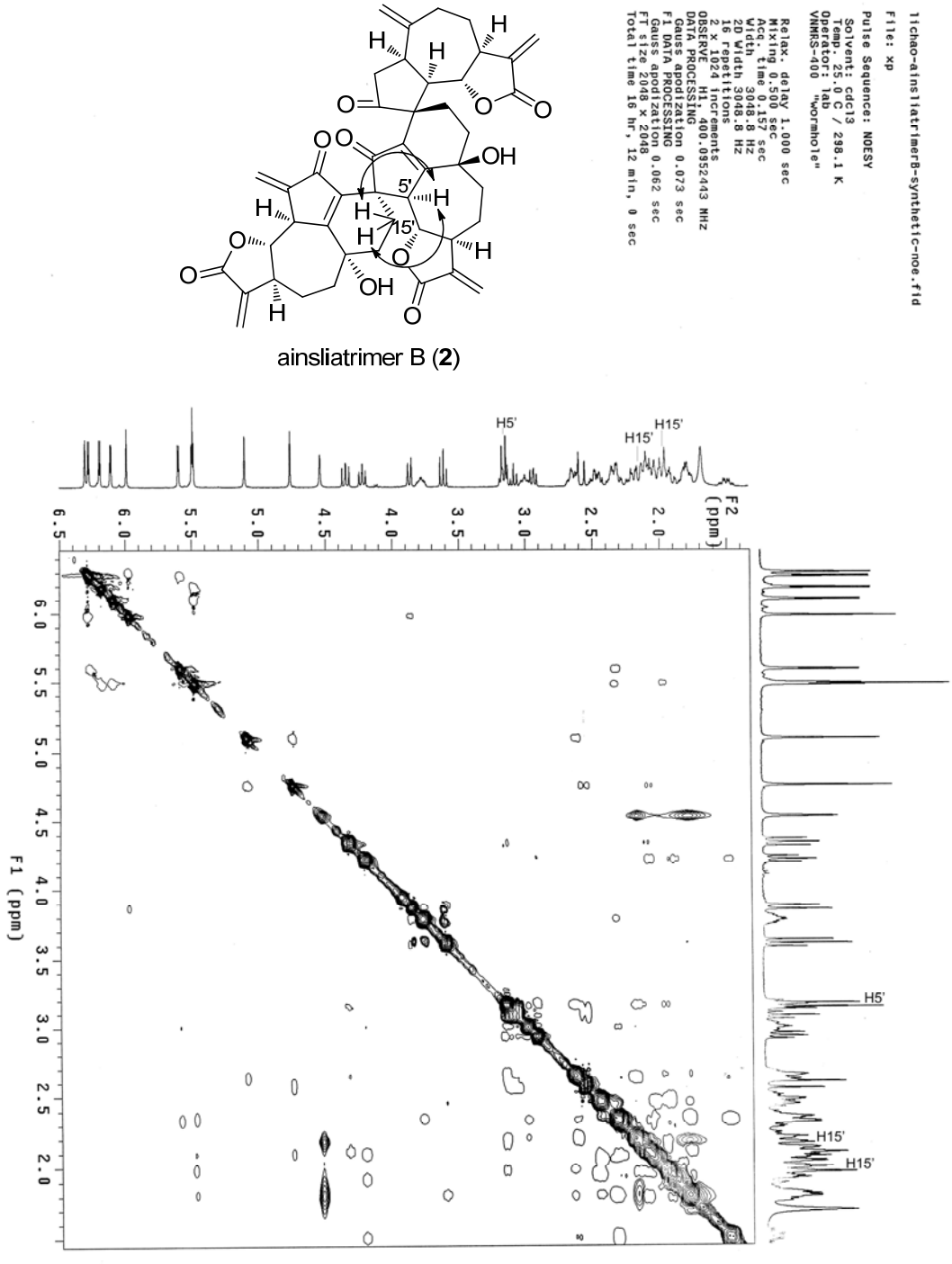
^{13}C NMR of Synthetic Ainsliatrimer B



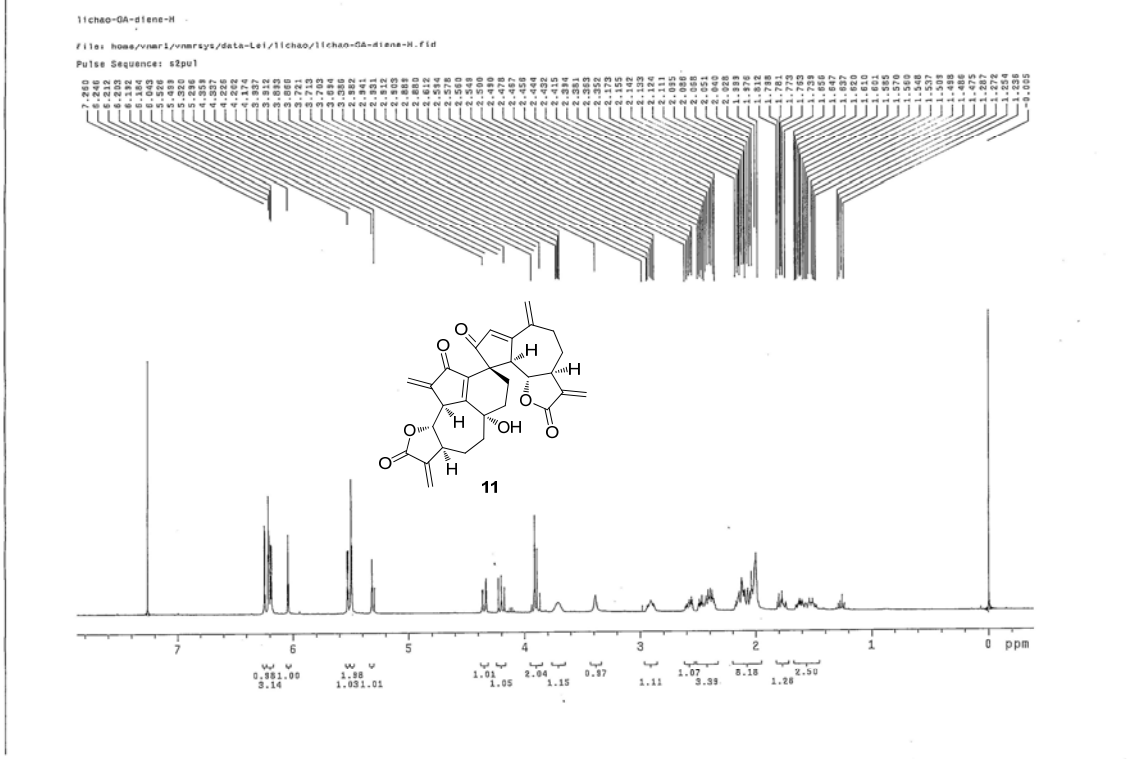
^{13}C NMR of Natural Ainsliatrimer B

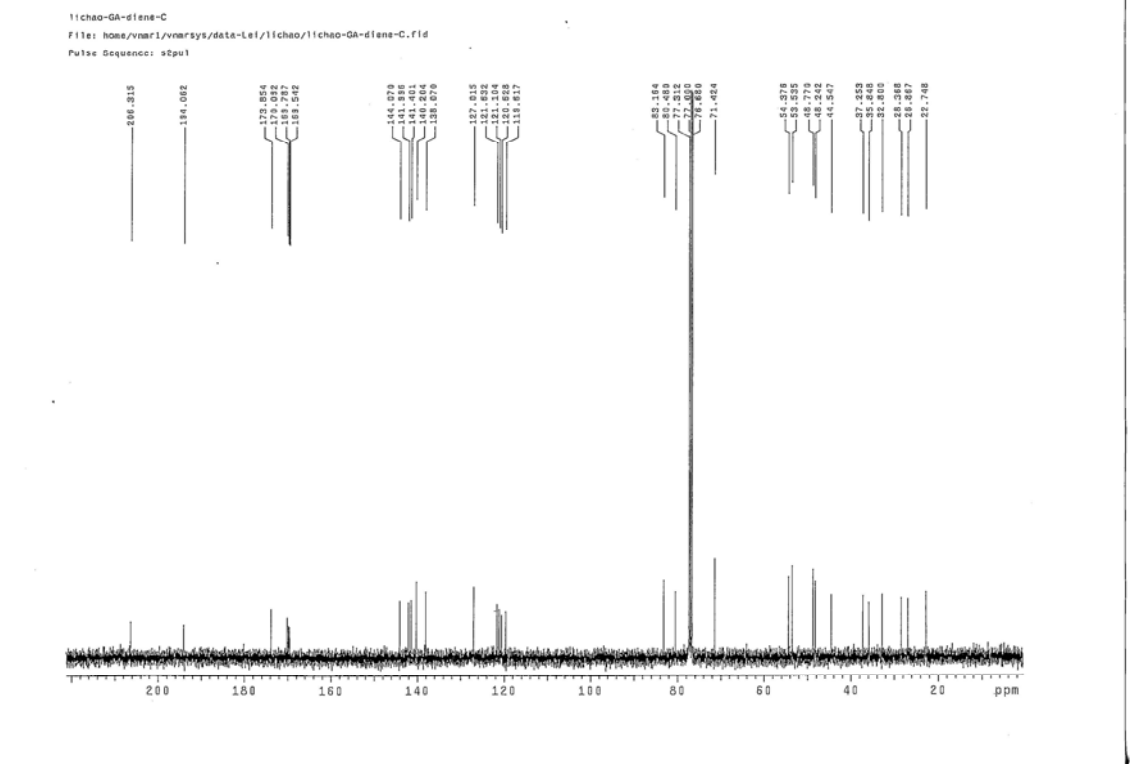


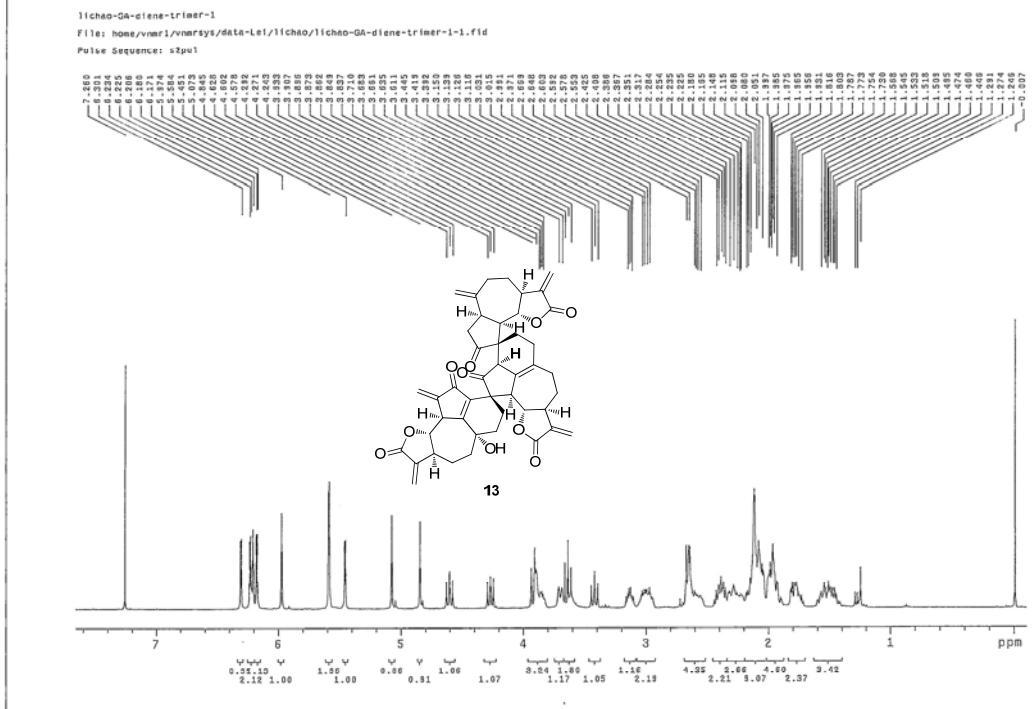
NOESY Spectrum of Synthetic Ainsliatrimer B



(III) ^{1}H and ^{13}C NMR Spectra



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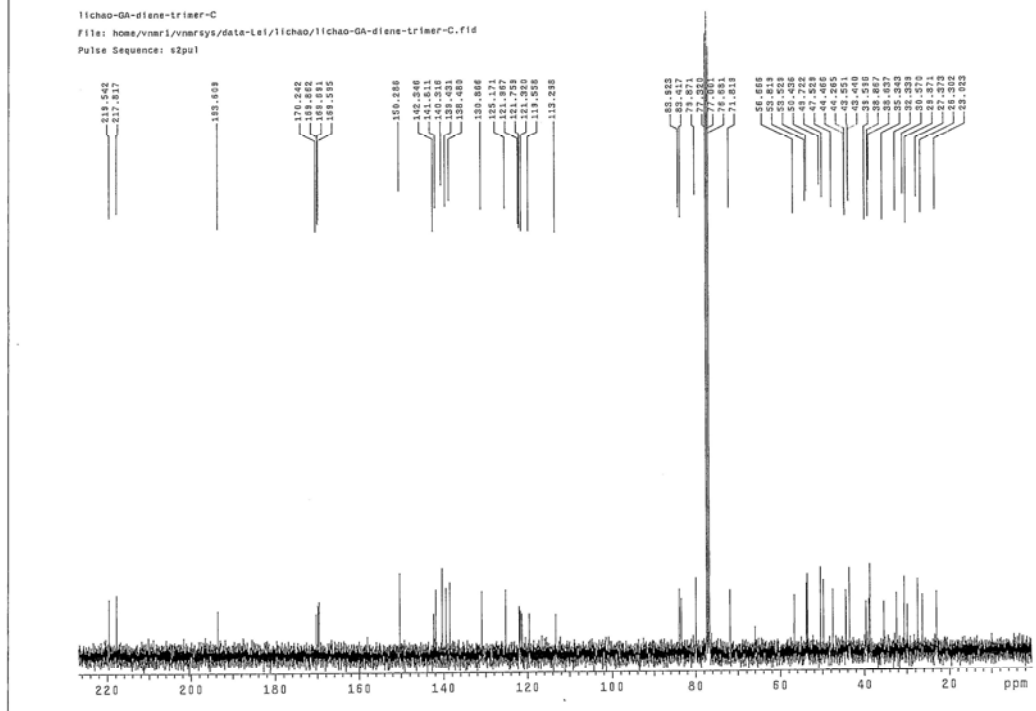
Pulse Sequence: s2pul
13
ppm


lichao-GA-diene-trimer-C
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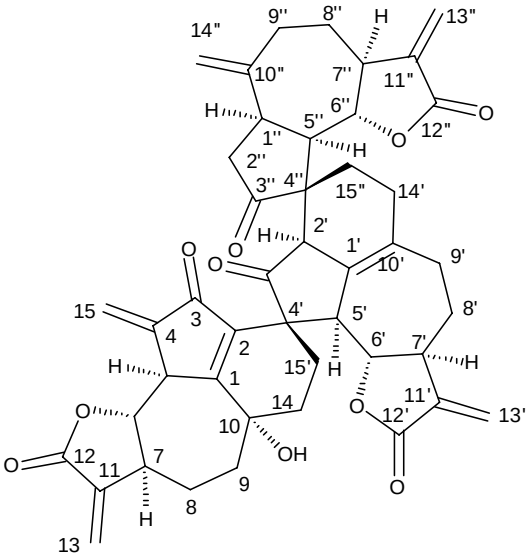
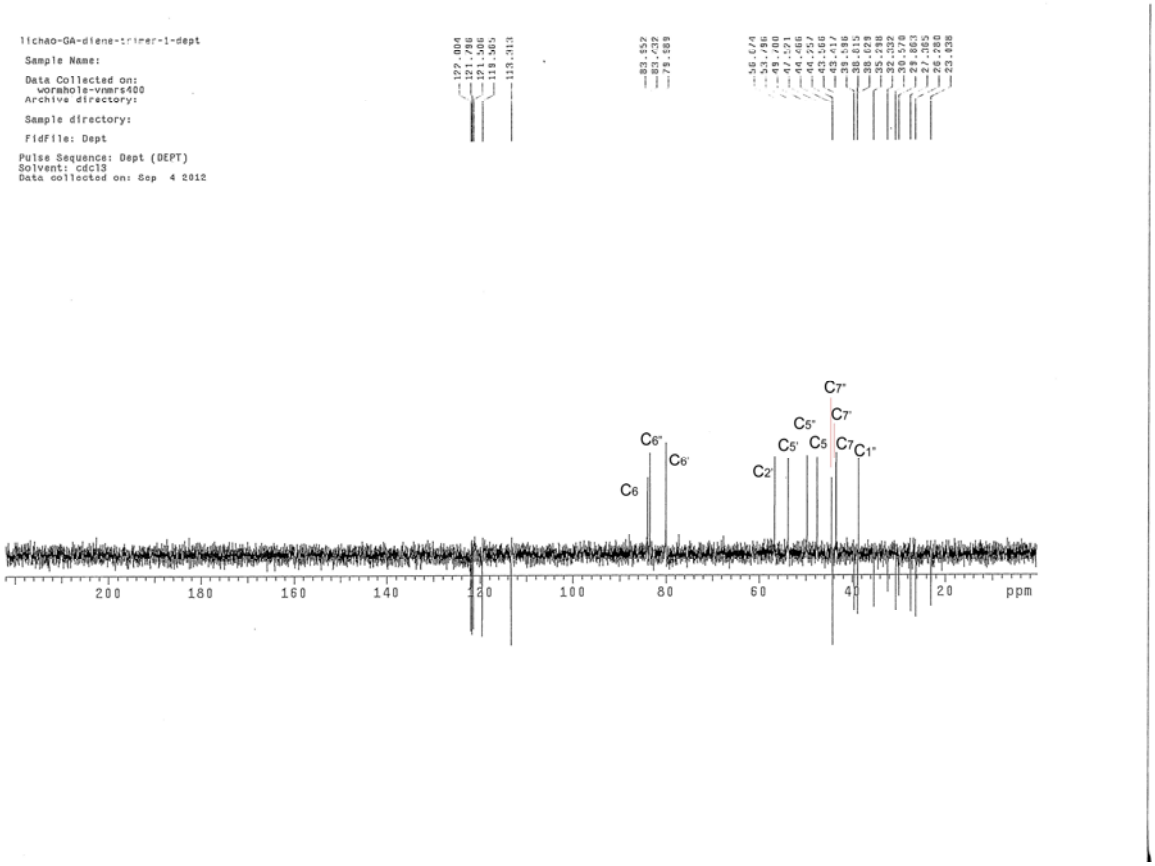
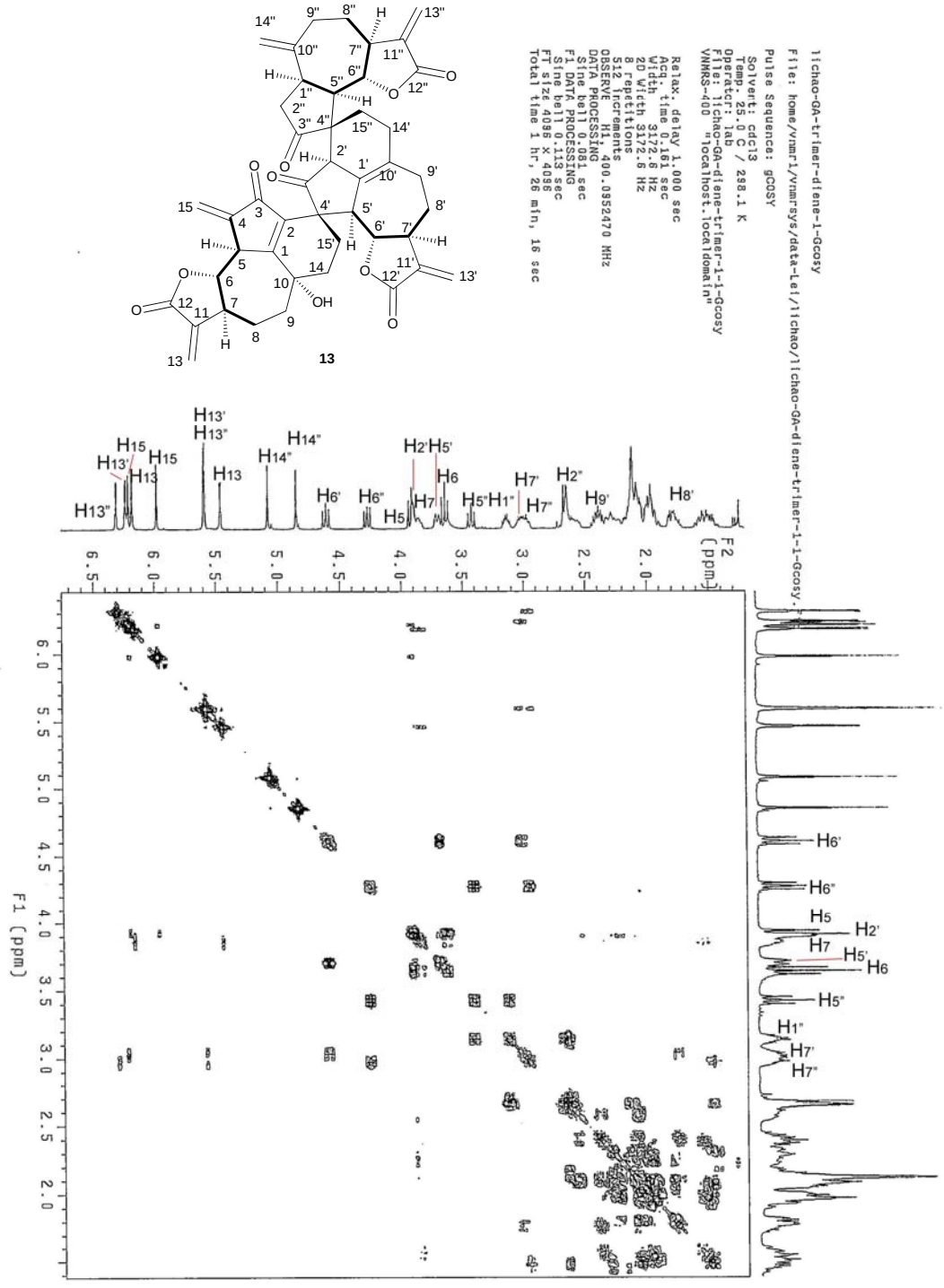
Positional Numbering of Trimer 13



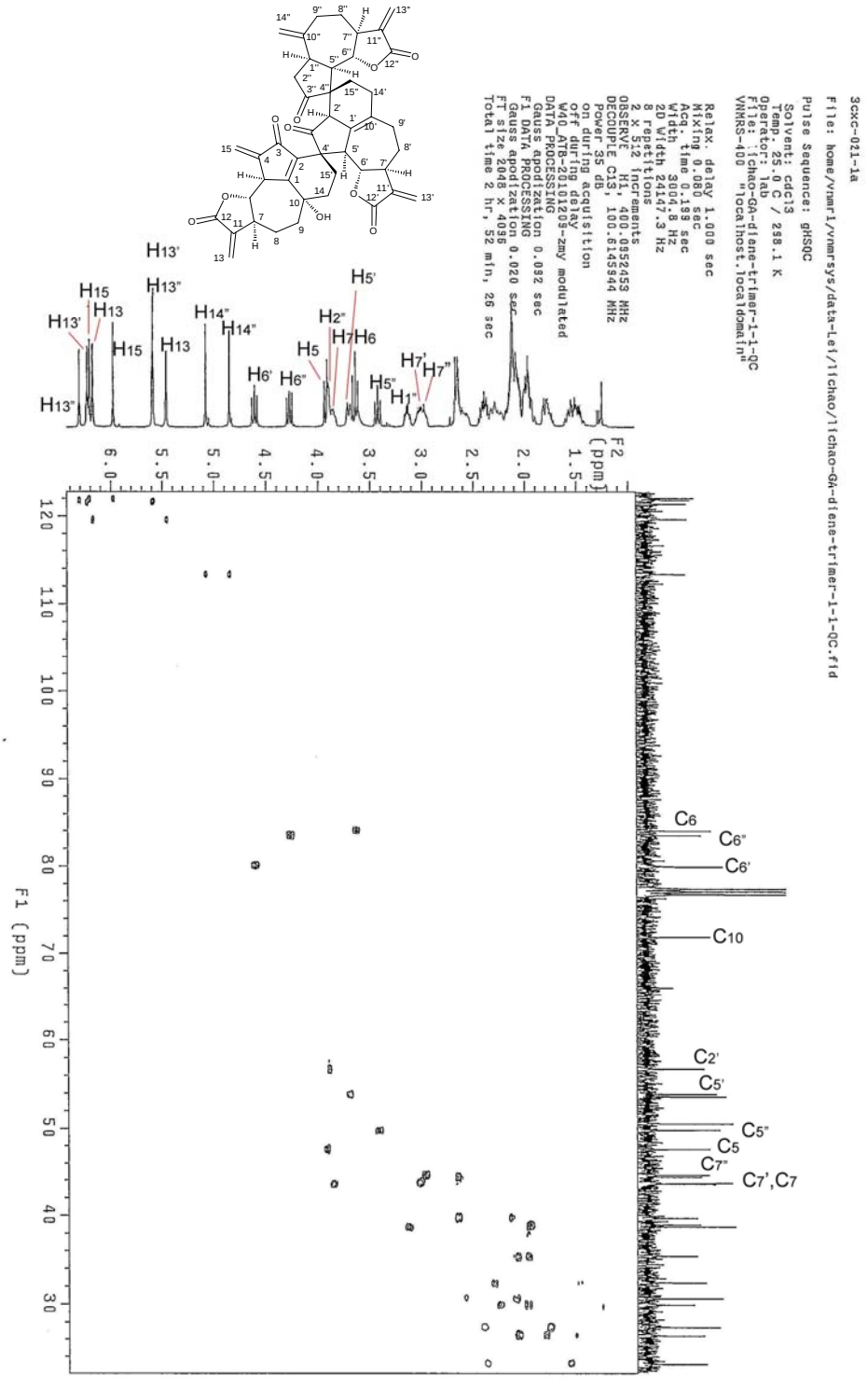
DEPT Spectrum of Trimer 13



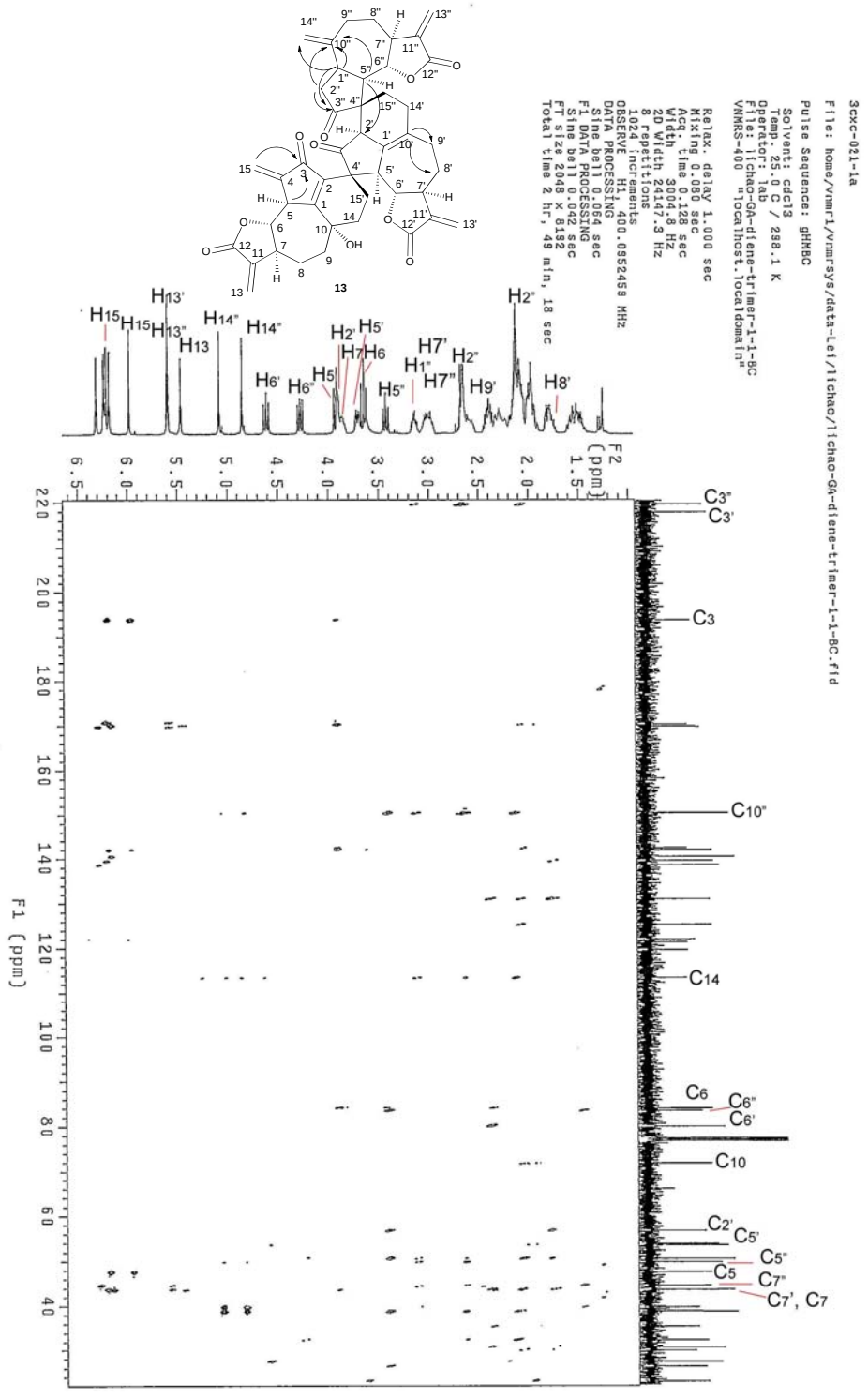
^{1}H-^{1}H COSY Spectrum of Trimer 13 and the Key Correlation (—)



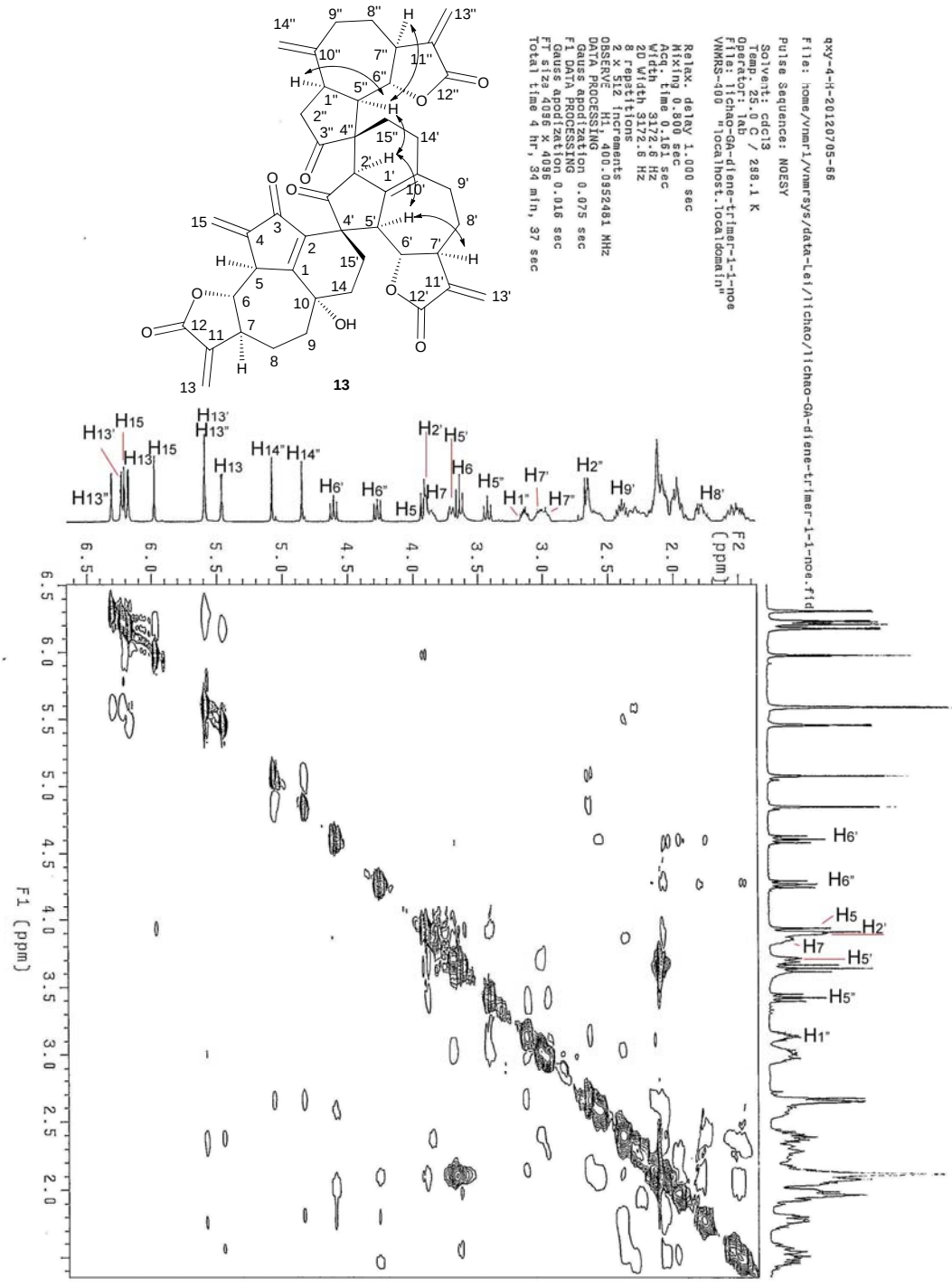
HSQC Spectrum of Trimer 13

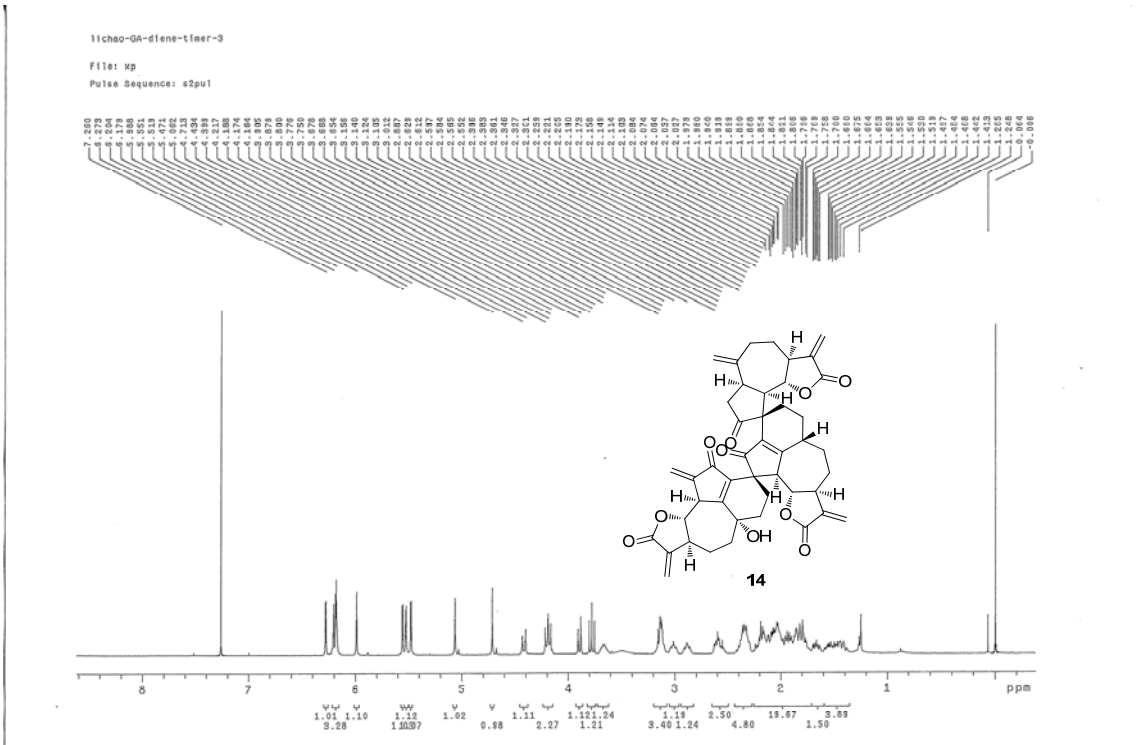


HMBC Spectrum of Trimer 13 and the Key Correlation (H to C) (→)



NOESY of Trimer 13 and the Key Correlation (↔)

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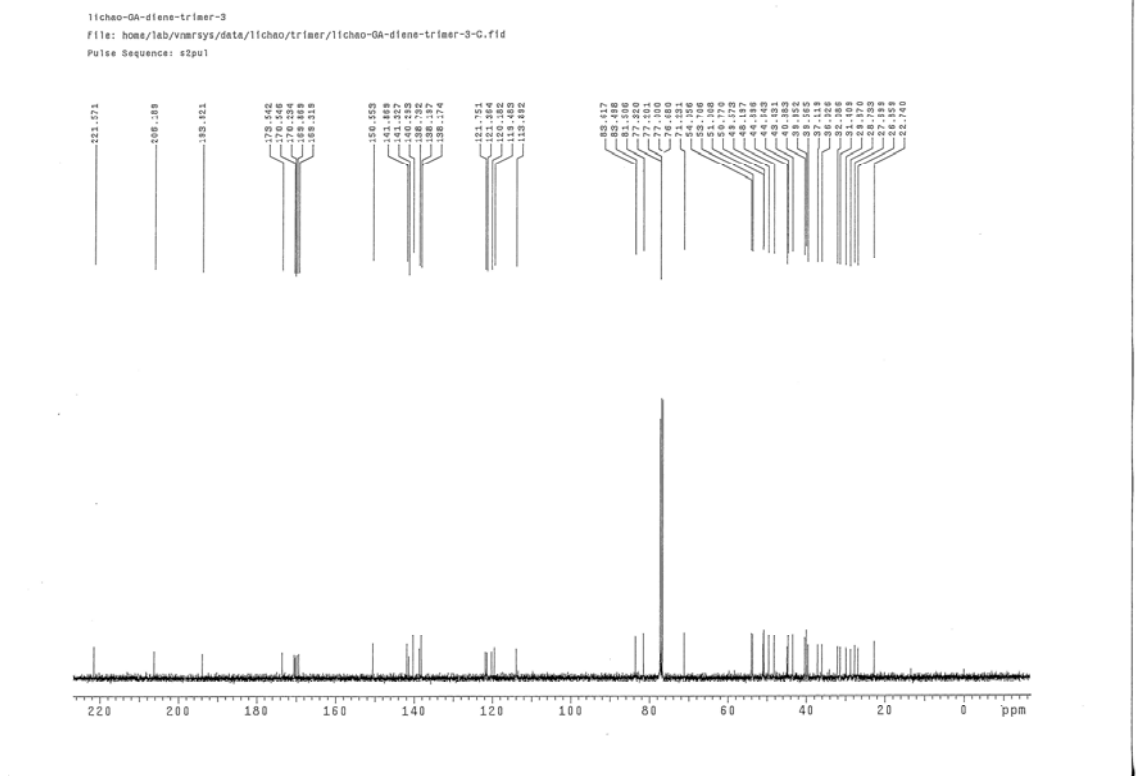
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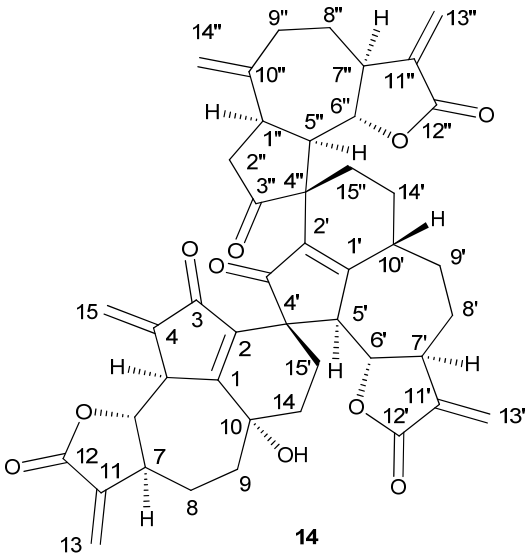
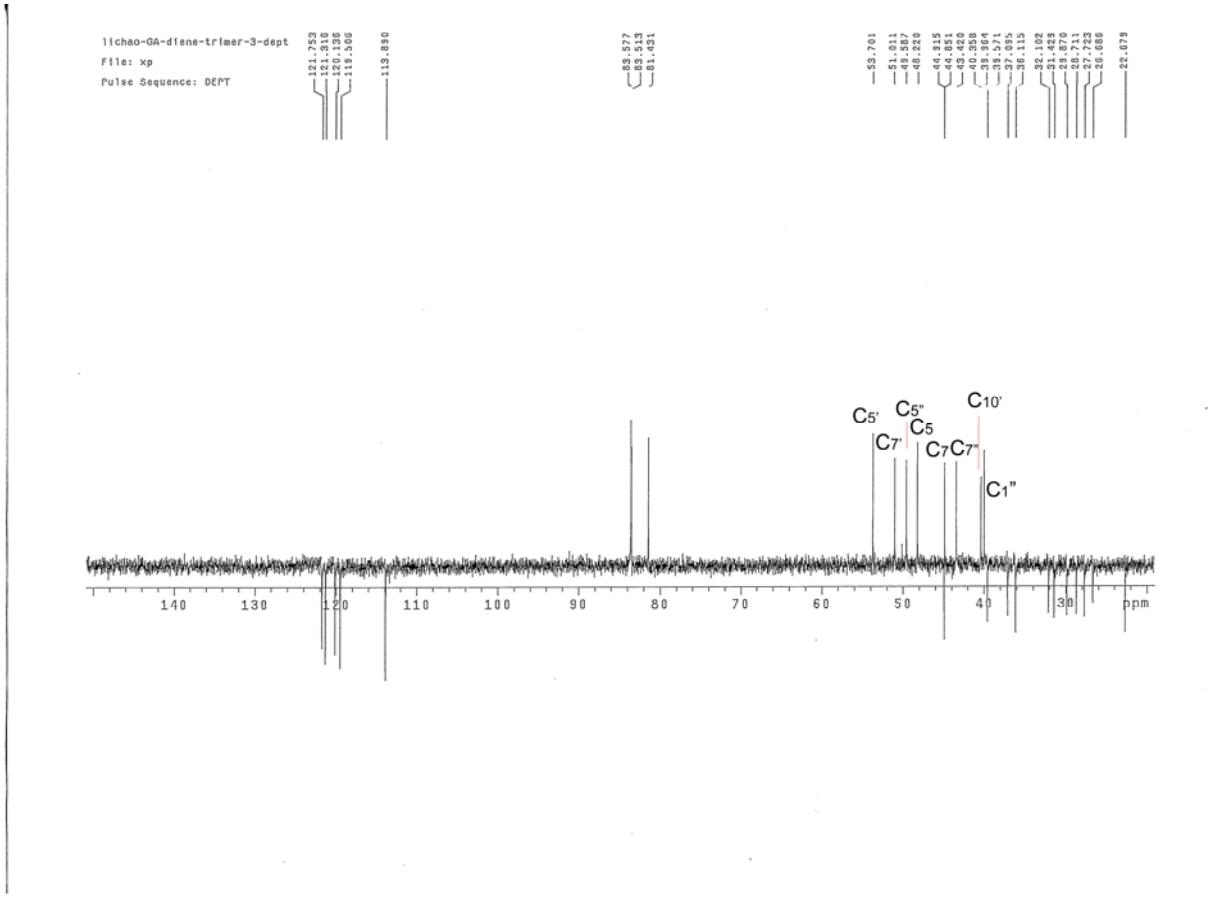
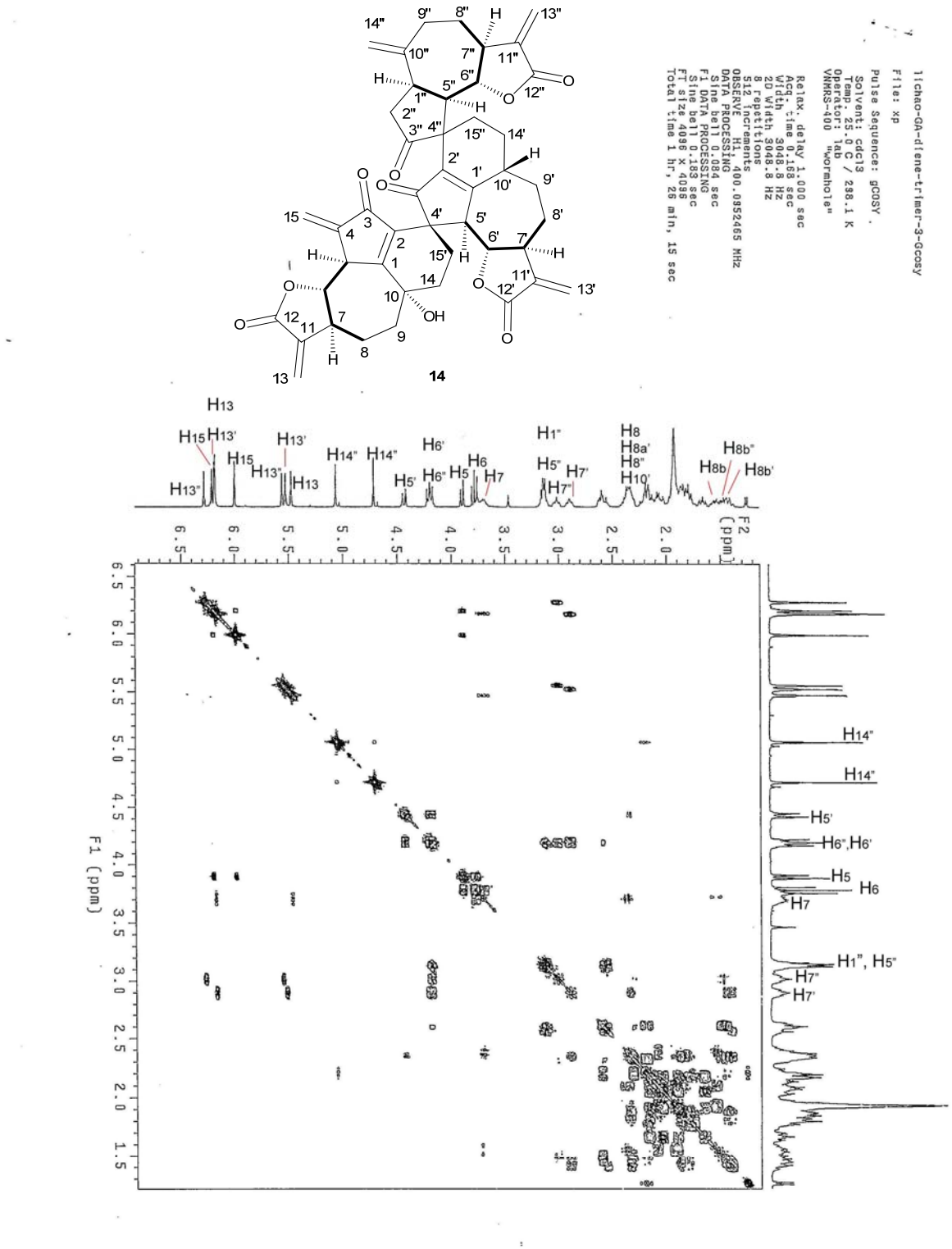
Positional Numbering of Trimer 14



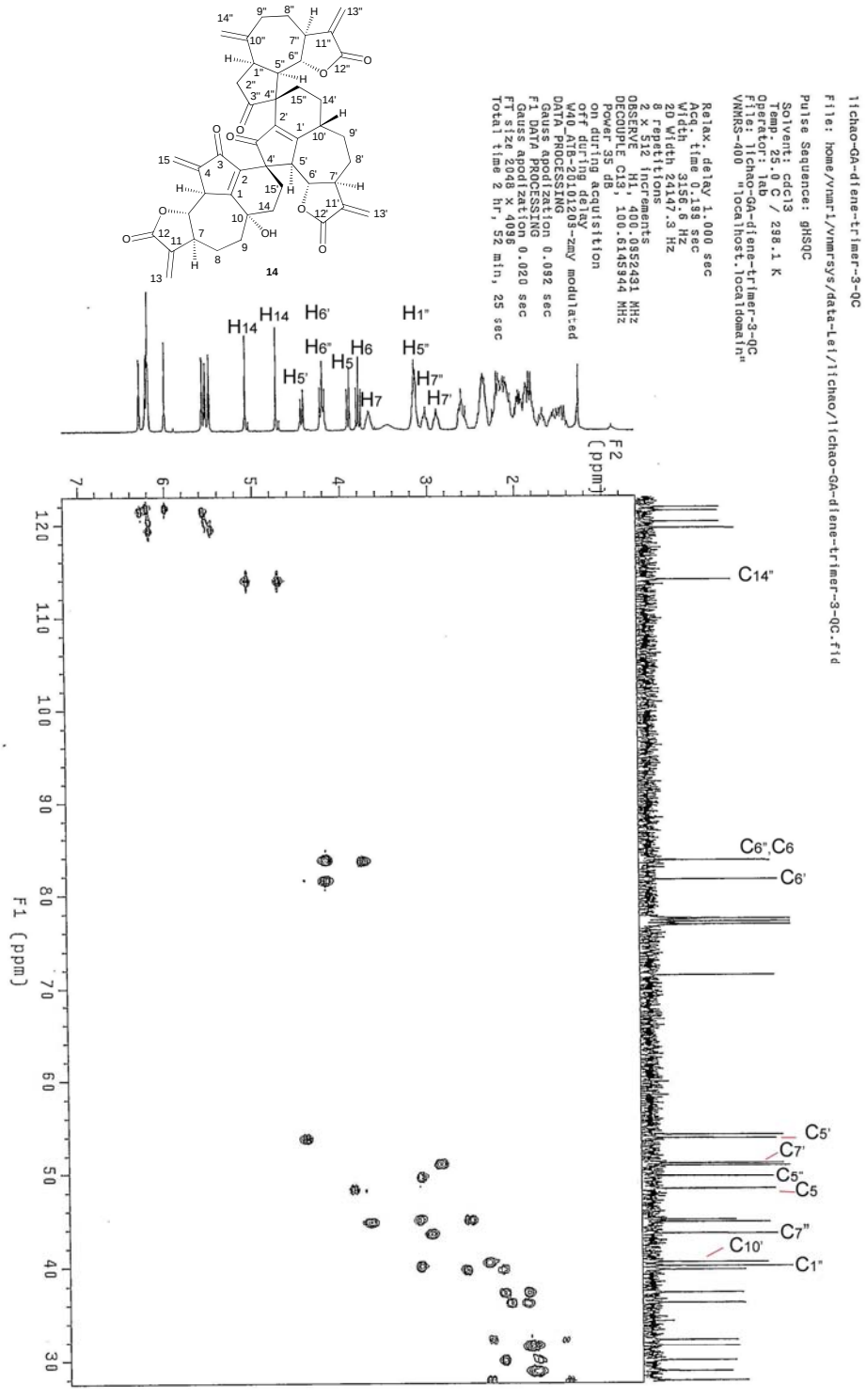
DEPT Spectrum of Trimer 14



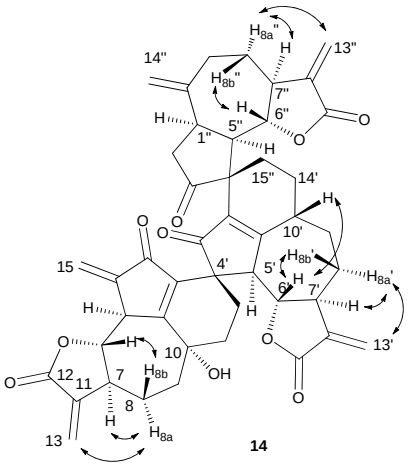
^{1}H-^{1}H COSY Spectrum of Trimer 14 and the Key Correlation (—)

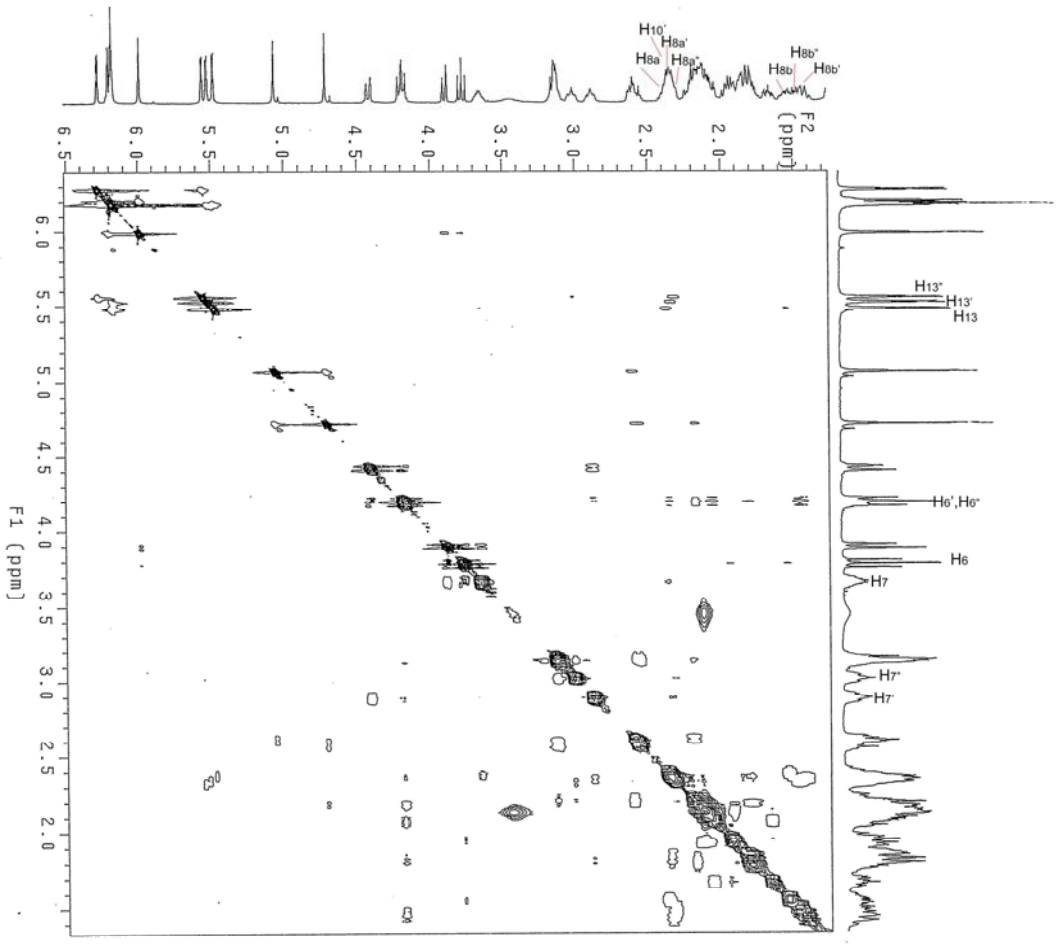


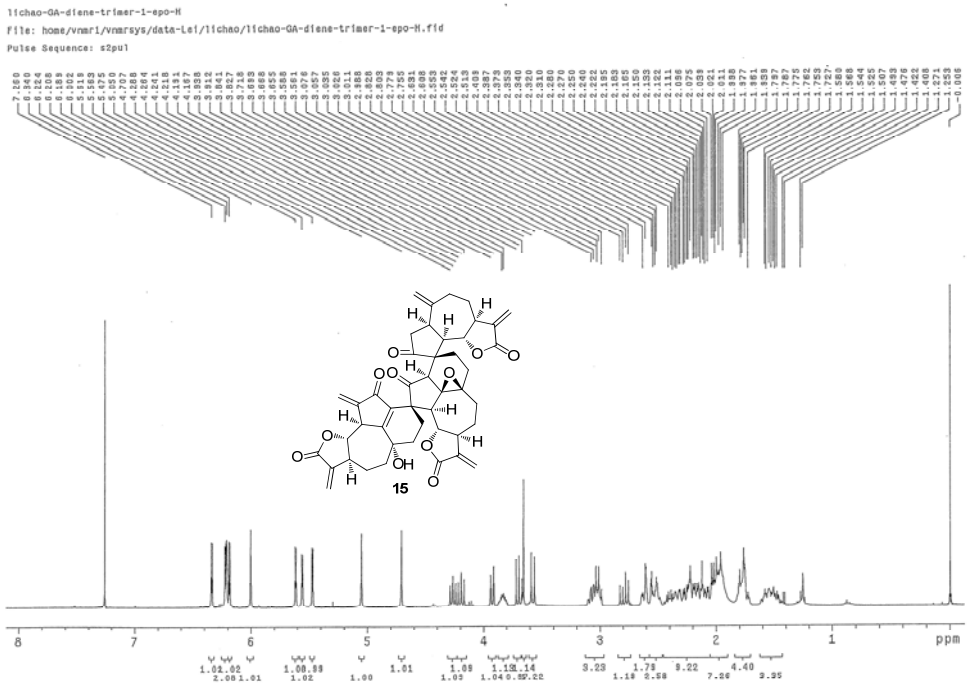
HSQC Spectrum of Trimer 14



NOESY of Trimer 14 and the Key Correlation (↔)



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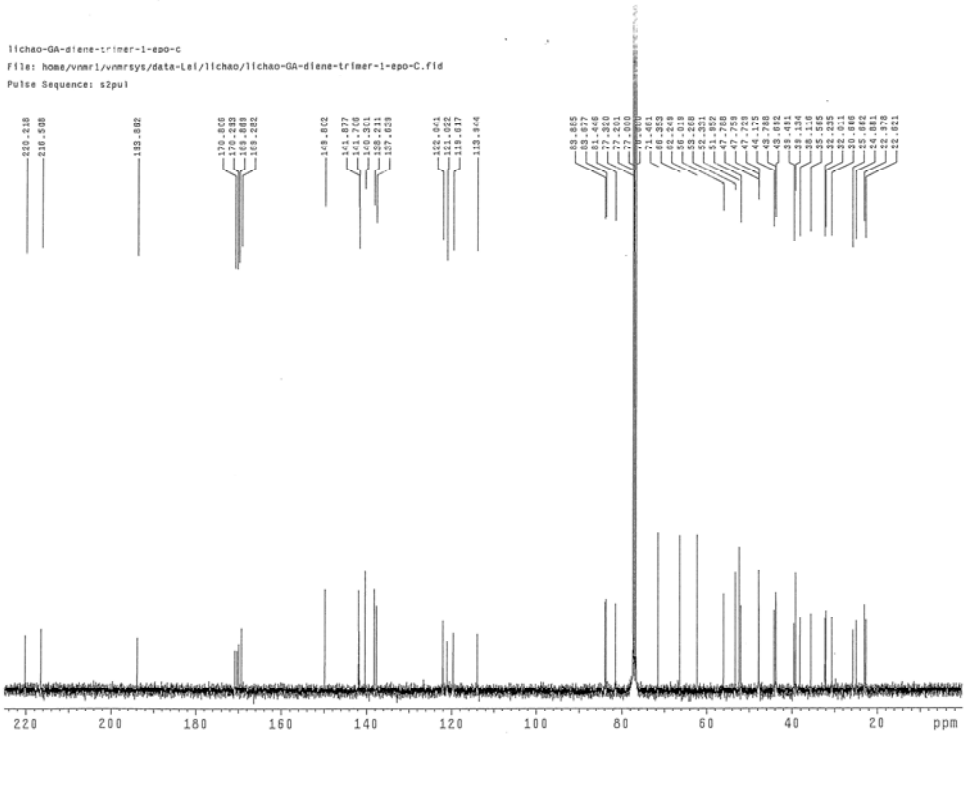
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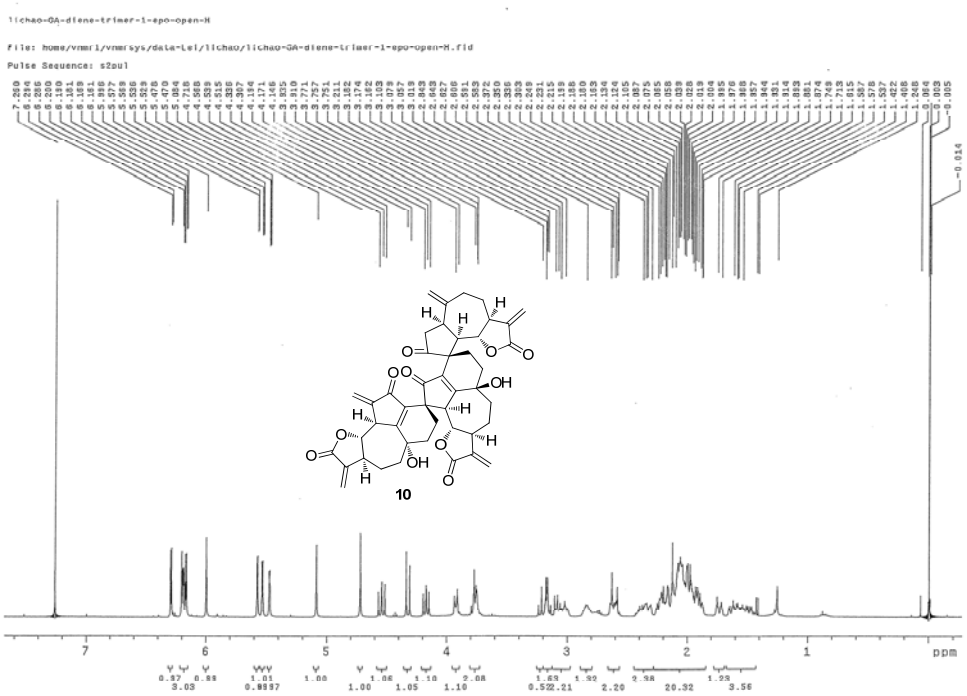
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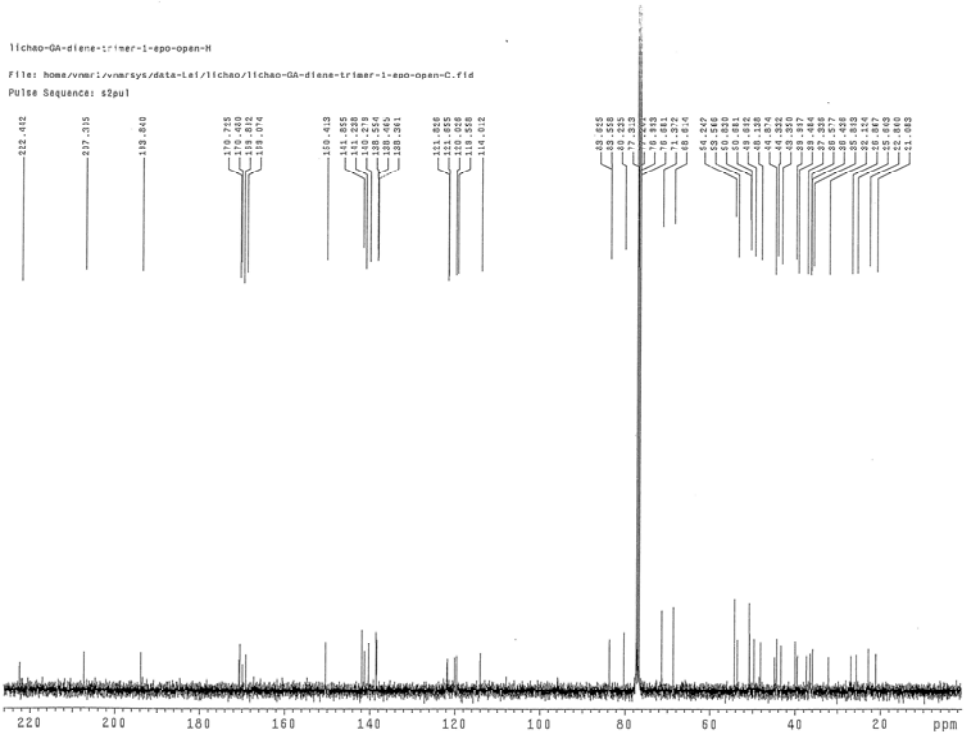
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^{1}H-^{1}H COSY Spectrum of Trimer 10 and the Key Correlation (—)

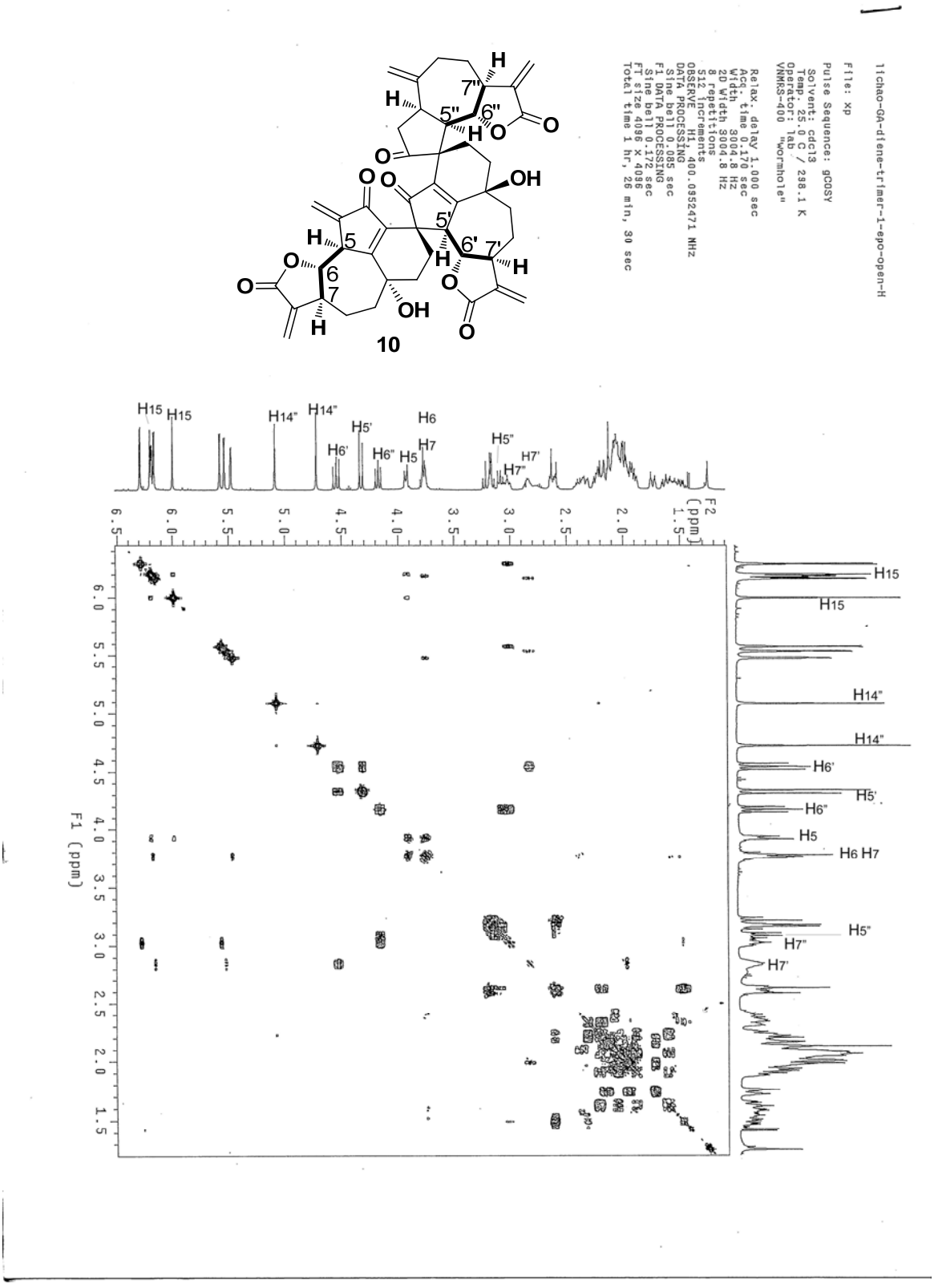
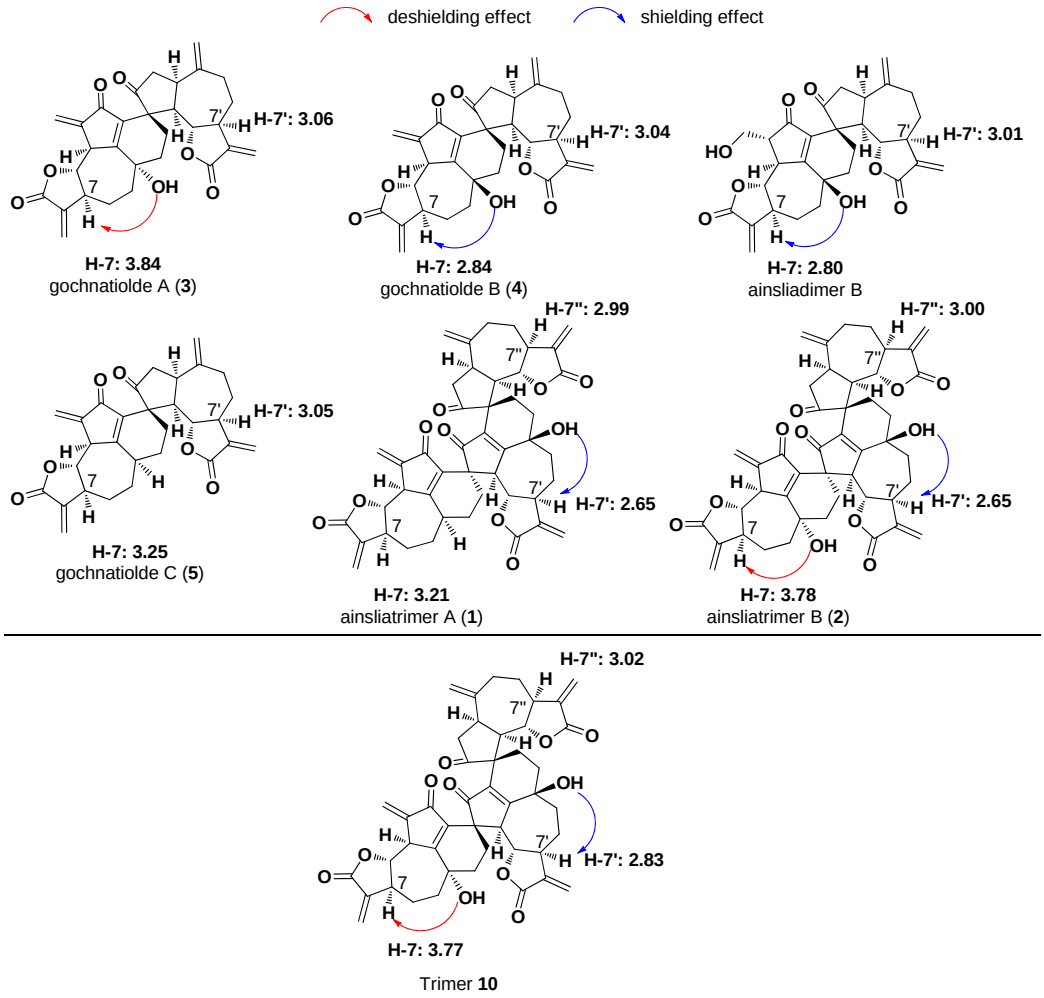
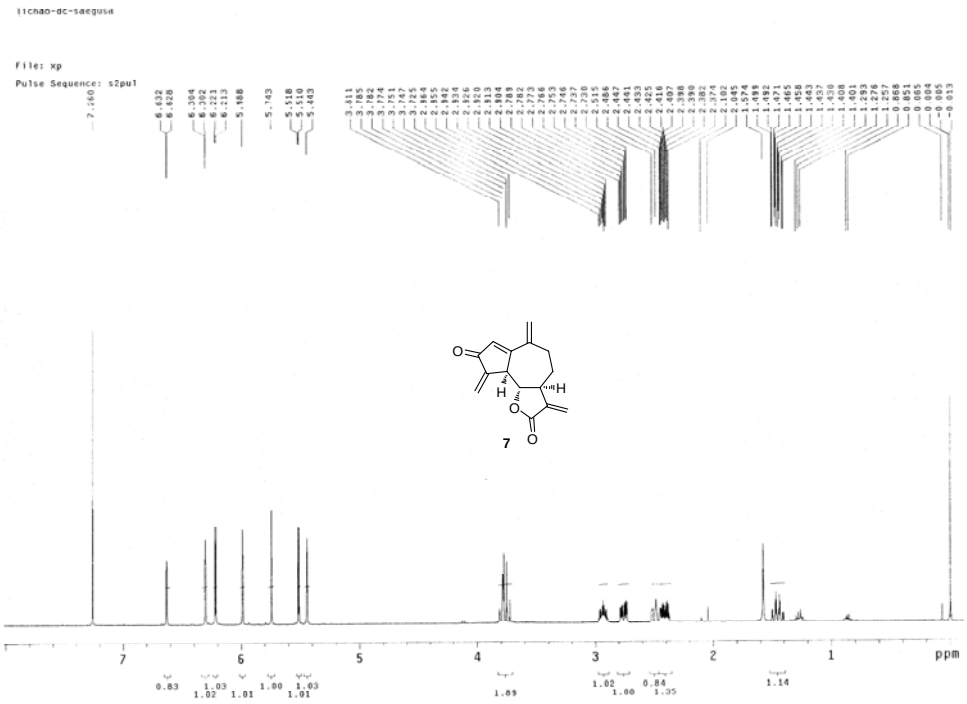


Table S1: Shielding and Deshielding Effects in Guaianolide Dimers and Trimers[b]



[b] For the shielding and deshielding effects in gochnatiolides, see: F. Bohlmann, M. Ahmed, J. Jakupovic, R. M. King, H. Robinson, *Phytochemistry* **1983**, *22*, 191.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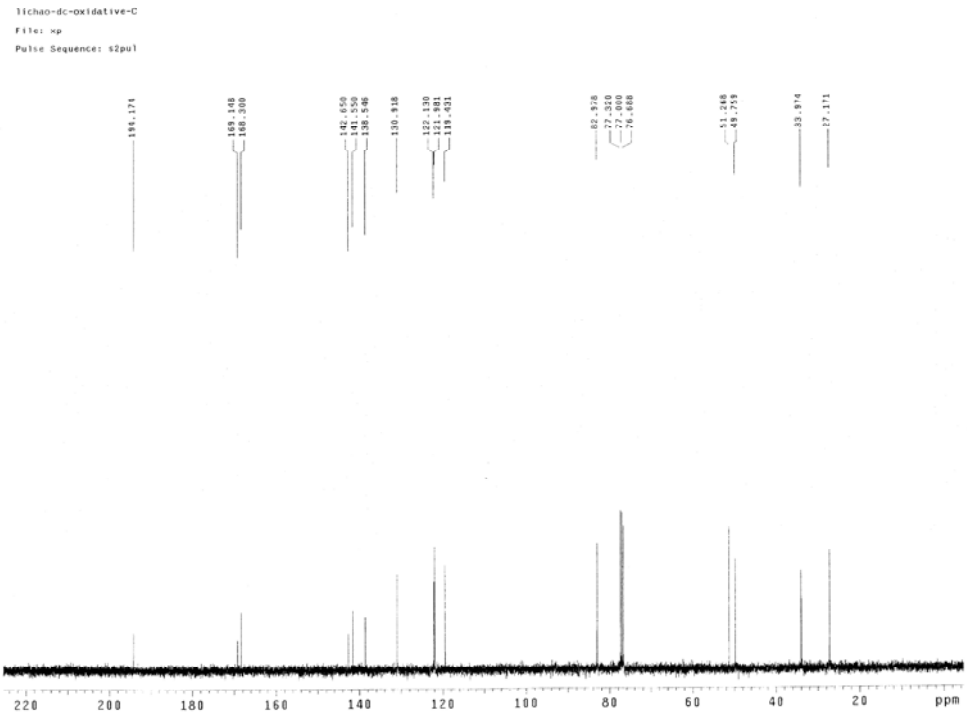
7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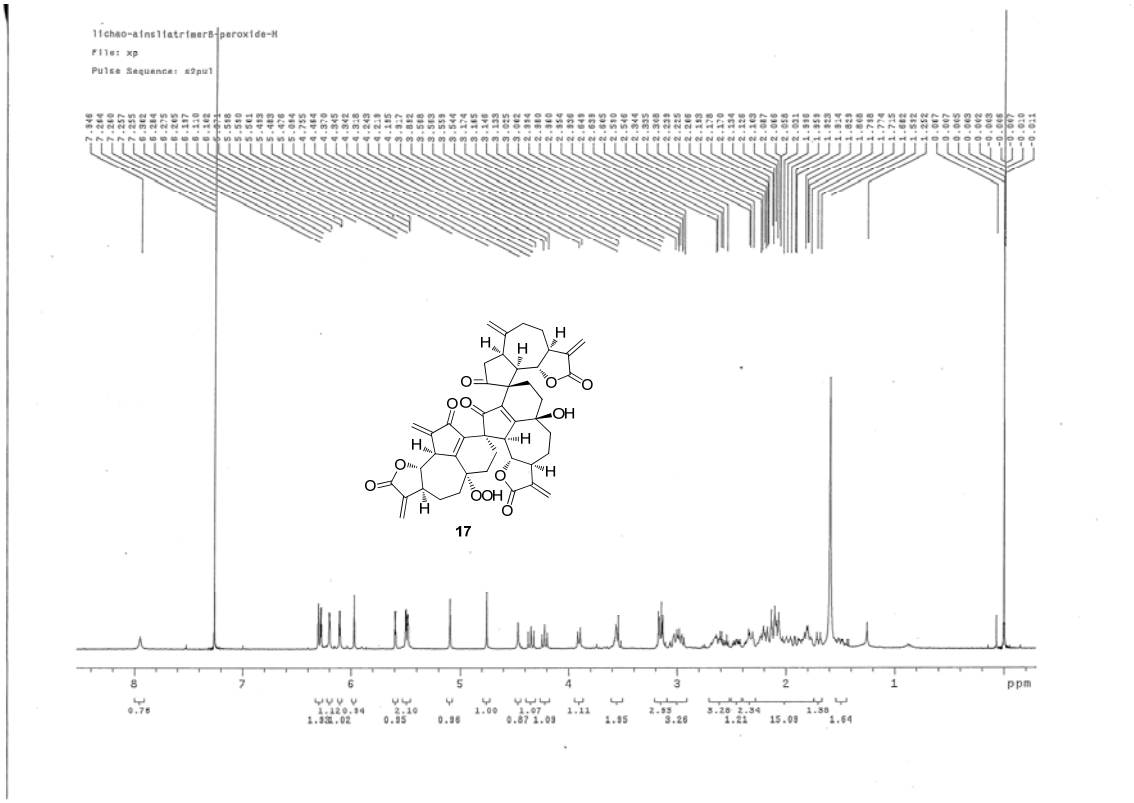
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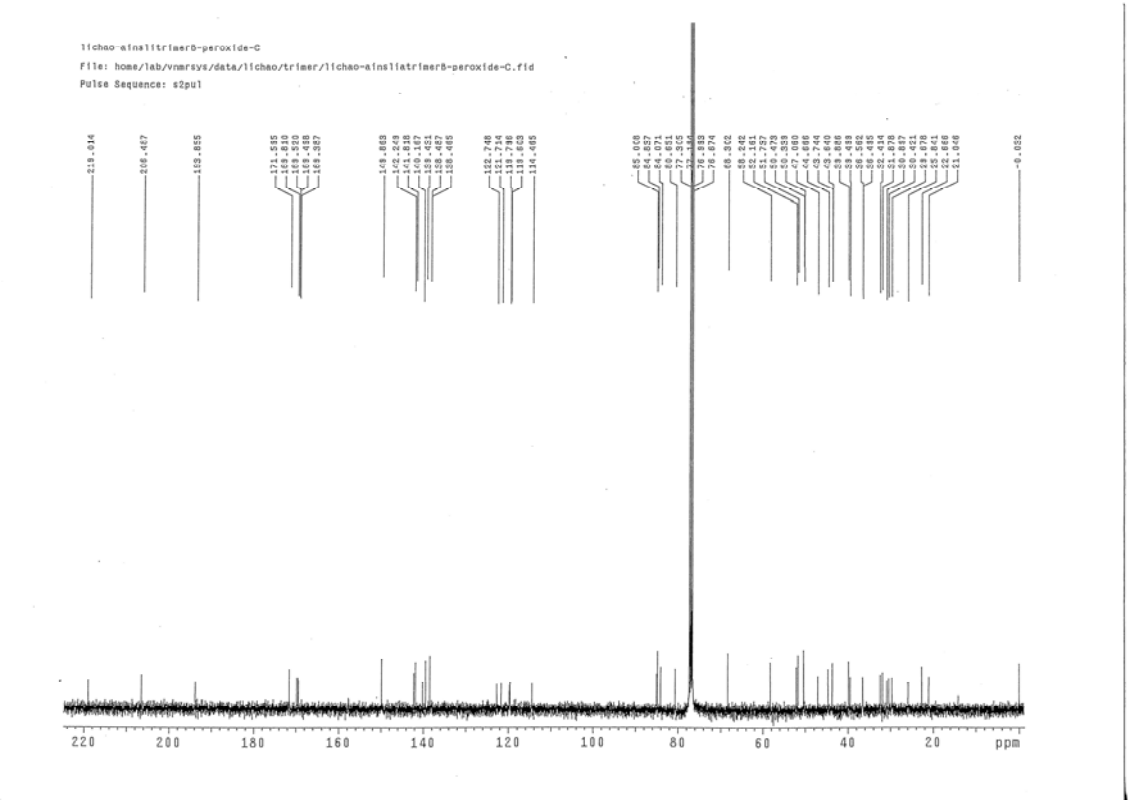
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Ⅳ) Biological Evaluations of (-)-Ainsliatrimers A (1) and B (2), and Trimers 10, 14, 17.

1. Materials and methods

1.1. Reagents and materials

DMEM medium, phosphate buffered saline (PBS), Trypsin and Penicillin/streptomycin were purchased from GIBCO. Fetal bovine serum (FBS) was purchased from Sigma; CCK-8 was purchased from Dojindo; dimethyl sulfoxide (DMSO), LPS and PI cell cycle detection kit were purchased from Sigma; general caspase inhibitor z-VAD-FMK was purchased from Bachem; Necrostatin-1 was purchased from Alexis Biochemicals, 3-AM and BHA were purchased from Sigma; primary antibodies of caspase-3, cleaved PARP and caspase-9 were purchased from Cell Signaling Technology (Beverly, MA, USA); antibody of α-Tubulin was purchased from MBL.

1.2 Cell culture

Hela cells were cultured in DMEM medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 U/mL streptomycin in a humidified 5% CO_2 incubator at 37 °C.

1.3 Cell viability assay

Cell viability assays were measured with CCK-8 method. 100 μL of cell suspensions ($5x10^4$ cells/mL) per well were seeded in 96-well plates, incubated at 37 °C and allowed for attachment for 12 h before treatment. 10 μL of medium containing the indicated compounds were added to each well. The wells containing 110 μL of medium without cells were set as blank controls, and the experiment control cells were treated with 10 μL of medium containing 0.1% DMSO. After certain periods of incubation, 10 μL of CCK-8 was added per well. Plates were incubated at 37°C under 5% CO_2 for 1-4 h. Then the optical density (OD) was read by a multifunction ELISA reader (Beckman) at 450 nm. Four wells per dose were conducted in three independent experiments. IC_{50} values were determined with GraphPad Prism 5.

1.4 Western blot

Hela cells in a 6 well plate (6×10^5) were exposed to 2.5 μM of trimers **1**, **2**, **10**, **14**, **17** for 6 h and harvested by scrapping. Protein concentration of cell lysates was measured by Bradford method. Equal amount of proteins were loaded in sodium dodecyl sulfate-polyacrylamide gels. After electrophoresis, gel was transferred to nitrocellulose membranes. The membranes were washed with PBS containing 0.1% Tween 20 (PBST) thrice 10 min each. Membranes were blocked with 5% nonfat powdered milk for 1 h. Then membranes were incubated with specific primary antibodies for 4 h, washed with PBST thrice 10 min each, and further incubated with anti-rabbit IgG-HRP conjugates secondary antibodies for 2.5 h at RT. Finally, the blots were visualized using enhanced chemiluminescence (ECL) kit (GE Healthcare).

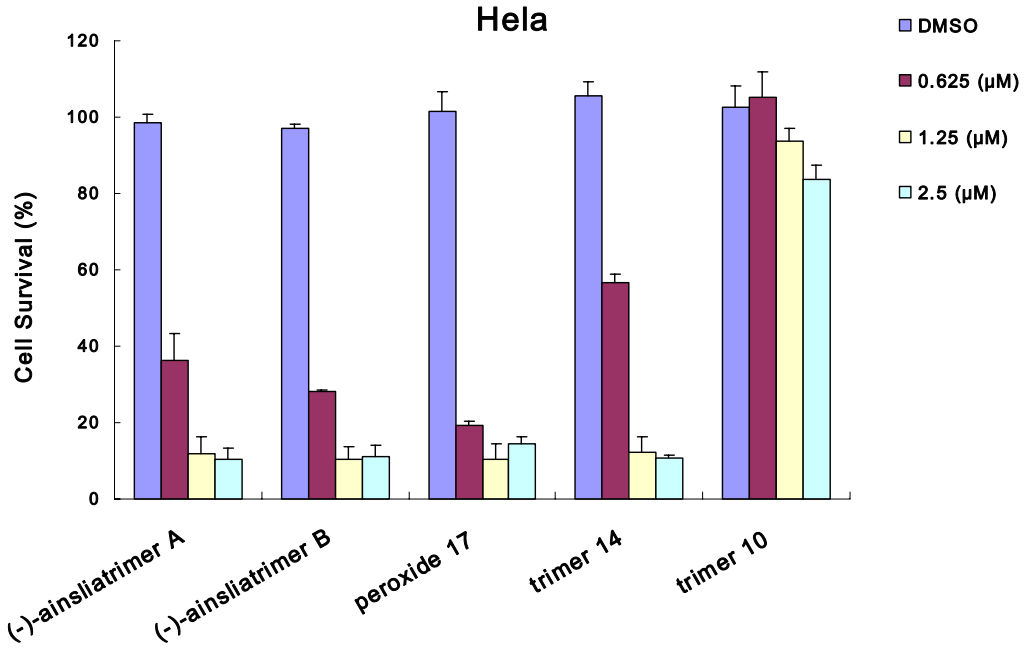
1.5 Cell cycle analysis by PI staining

Cell cycle analysis was evaluated by PI staining. Briefly, about 6×10^5 Hela cells were seeded in a 6 well plate, allowed for attachment for 12 h, and then treated with trimers **1**, **2**, **10**, **14**, **17** for 24 h. After incubation, both floating and attached cells were collected, washed with PBS twice, and fixed in 70% ethanol (v/v) in 4 °C for 24 h. Fixed cells were washed with PBS twice, and then 100 μL RNase A was added and incubated in 37 °C for 30 min to hydrolyze RNA, then cells were stained with PI in dark for another 30 min. Cell cycle was evaluated with FCM and analyzed with WinMDI 2.9. For each analysis, 10,000 events were recorded. The experiments were repeated at least three times independently.

1.6 Statistical analysis

All data were expressed as means ± standard deviation (S.D.). Statistical comparisons among groups were performed by Student's *t*-test. A level of $p < 0.05$ was taken as statistically significant.

2. Supplemental Figures:



Compounds	1	2	17	14	10
IC_{50} (μg/mL)	0.29	0.22	0.17	0.46	16.44

Figure S1. Cytotoxicities of (-)-ainsliatrimer A (**1**), (-)-ainsliatrimer B (**2**), peroxide **17**, trimer **14** and trimer **10** against Hela cells. Hela cells were treated with (-)-ainsliatrimer A (**1**), (-)-ainsliatrimer B (**2**), peroxide **17**, and trimer **14** and trimer **10** at the indicated concentrations for 48 h. Cell viability was determined by CCK-8 assay as detailed in section **1.3**. Results are the representative data of three independent experiments.

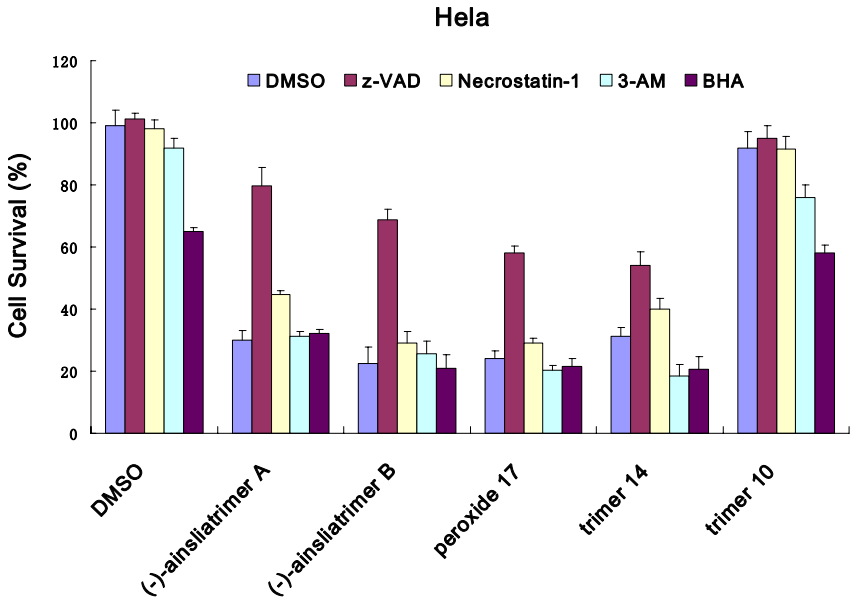


Figure S2. Modulatory profiling of known apoptosis, necrosis or autophagy inhibitor in Hela cells treated with trimers. Hela cells were pretreated with DMSO/20 mM of z-VAD/3 mM of 3-AM/10 mM of Necrostatin-1/15 mM of BHA for 1.5 h, and then the indicated compounds were added at the concentration of 2.5 μM. After 48 h, cell viability was determined by CCK-8 method. Data were represented as mean ± standard deviation of duplicates and all experiments were repeated at least three times with similar results.

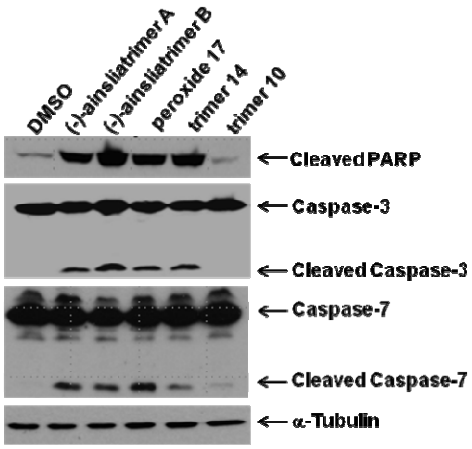
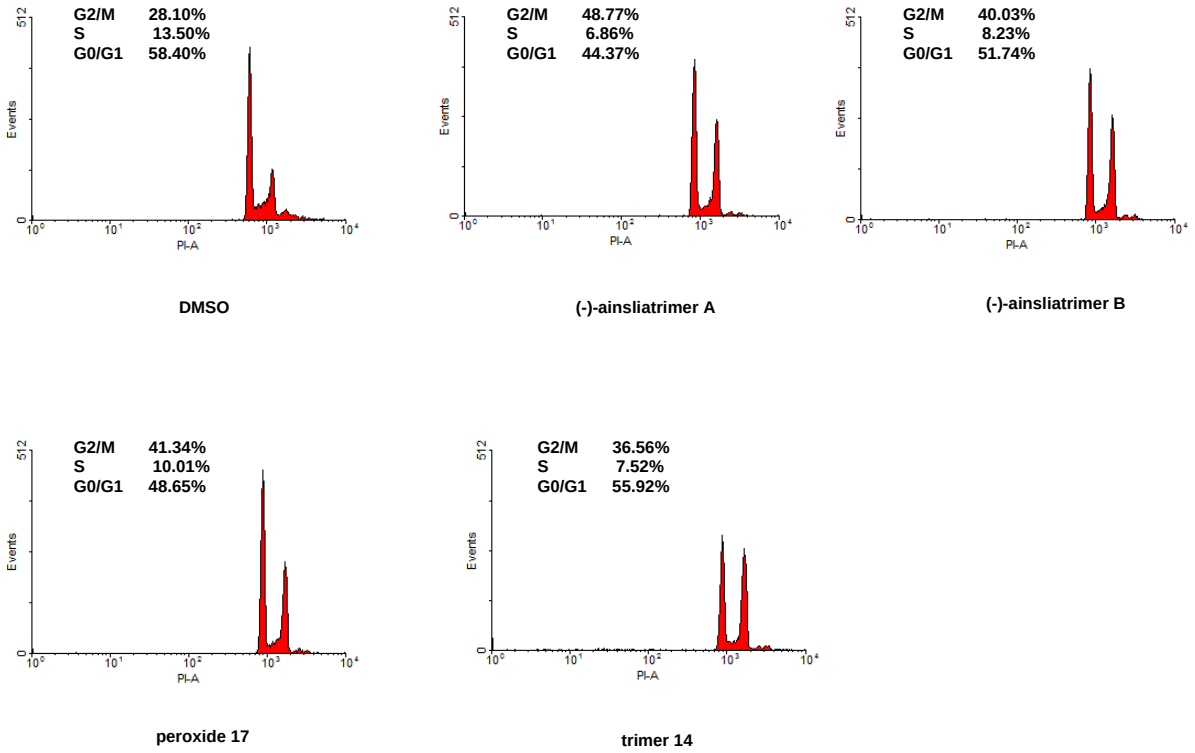


Figure S3. (-)-Ainsliatrimer A (**1**), (-)-ainsliatrimer B (**2**), peroxide **17** and trimer **14** induce apoptosis in Hela cells at the concentration of 2.5 μM. Hela cells were treated with 2.5 μM of the

indicated compounds for 6 h and then the total cell lysates were collected. 60 μg of aliquots were subjected to western blot analysis of caspase-3, caspase-7, cleaved PARP and α-Tubulin levels.



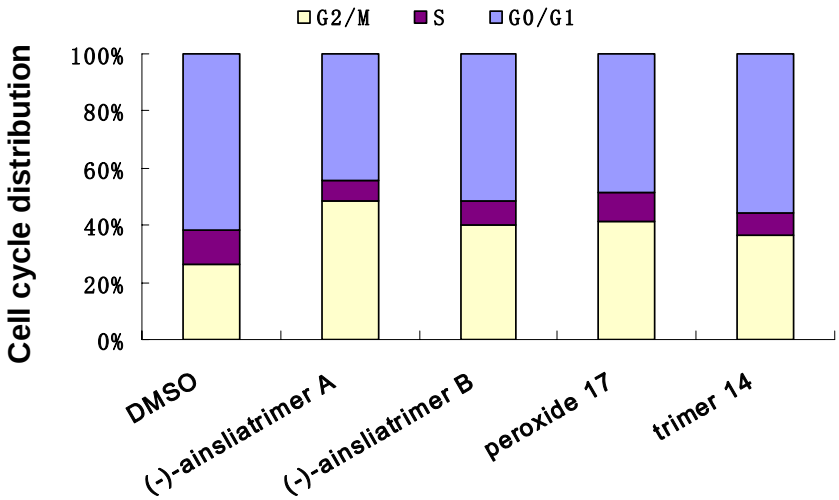


Figure S4. (-)-ainsliatrimers A (**1**) and B (**2**), peroxide **17** and trimer **14** caused cell cycle arrest in G_2/M phase at the concentration of 2.5 μM.