Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supplementary Information

Modular Assembly of Cytotoxic Acetogenin Mimetics by Click Linkage with Nitrogen Functionalities

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List of Contents

1. Experimental details and characterizations of new compounds

General: Optical rotations were measured at room temperature. Infrared spectra (IR) were recorded on a Fourier transform infrared spectrometer (FTIR). ^{1}H NMR spectra were recorded at 300 MHz and are reported in ppm (δ) downfield relative to $CDCl_3$ as internal standard, and ^{13}C NMR spectra were recorded at 75 MHz and assigned in ppm (δ). HRMS spectra were recorded on Agilent 6210 TOF-MS. Elemental analyses were preformed on VARIO EL Elemental Apparatus. All melting points were uncorrected.

Compound 13: To a stirred solution of **12** (1.36 g, 6.12 mmol) in dry THF (29 mL) was added 1M $BH_3 \cdot THF$ complex in THF (12.24 mL, 12.24 mmol) at 0 ^{o}C under N_2. After the reaction was stirred for 3 hrs, H_2O (5 mL) was cautiously added dropwise followed by 15% NaOH (9 mL) and 30% H_2O_2 (9 mL). The mixture was stirred for 2.5 hrs at room temperature and then quenched by aq. $Na_2S_2O_3$ solution (20 mL), and extracted with ethyl ether for three times. The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (PE-EA, 2:1) to give **13** (0.88 g, 60%) as a white wax.

$[\alpha]_D^{25}$ = +36.5 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.29 (10H, brs), 1.39 (3H, d, *J* = 6.9 Hz), 1.52-1.61 (5H, m), 2.24-2.29 (2H, m), 3.62 (2H, t, *J* = 6.6 Hz), 4.98 (1H, dq, *J*=1.8, 6.9 Hz), 6.98 (1H, d, *J* = 1.2 Hz) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 174.0, 149.0, 134.1, 77.4, 62.8, 32.6, 29.3, 29.3, 29.1, 29.0, 27.3, 25.6, 25.0, 19.1 ppm; IR (KBr): 3309, 2923, 2850, 1743, 1471, 1325, 1081, 1031, 951 cm^{-1}; MS (ESI, *m/z*): 263 ($M+Na^+$); HRMS (ESI, *m/z*) calcd. for $C_{14}H_{24}O_3$ ($M+Na^+$): 263.1618, found: 263.1615.

Compound 14: To a solution of **13** (708 mg, 2.95 mmol) in CH_2Cl_2 (50 mL) was added triethylamine (0.534 mL, 3.84 mmol) and methanesulfonyl chloride (0.274 mL, 3.54 mmol) at 0 ^oC. The mixture was stirred at the same temperature for 3.5 hrs, and gradually warmed to room temperature. The mixture was poured into water (20 mL), and extracted with DCM for three times. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography (PE-EA, 4:1) to afford mesylate **14** (844 mg, 90%) as a white wax.

$[\alpha]_D^{25}$ = +23.1 (*c* = 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ: 1.30-1.42 (10H, brs), 1.41 (3H, d, *J* = 6.9 Hz), 1.50-1.57 (2H, m), 1.70-1.79 (2H, m), 2.24-2.29 (2H, m), 3.0 (3H, s), 4.22 (2H, t, *J* = 6.6 Hz), 5.0 (1H, dq, *J* = 1.5, 6.6 Hz), 6.99 (1H, d, *J* = 1.5 Hz) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.8, 149.1, 134.0, 77.4, 70.2, 37.2, 29.1, 29.0, 29.0, 28.8, 27.3, 25.3, 25.0, 19.1 ppm; IR (KBr): 2921, 2852, 1744, 1473, 1340, 1326, 1169, 981, 945 cm^{-1}; MS (ESI, *m/z*): 341 ($M+Na^+$); HRMS (ESI, *m/z*) calcd. for $C_{15}H_{26}O_5S$ ($M+H^+$): 319.1574, found: 319.1571

Compound 15: To a solution of azide **14** (700 mg, 2.2 mmol) in anhydrous EtOH (60 mL) was added NaN_3 (286 mg, 4.4 mmol). After the addition, the reaction mixture was heated to 90 ^oC and stirred for 12 hrs. Solvent was removed under the reduced pressure; the residue was extracted with ether (50 mL x 3). The combined organic layers were washed with H_2O and brine, and dried over Na_2SO_4. After filtration and concentration, the residue was purified by column chromatography (PE-EA, 8:1) to afford **15** (501 mg, 86%) as a colorless oil.

$[\alpha]_D^{25}$ = +23.7 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.30 (10H, brs), 1.41 (3H, d, *J* = 6.9 Hz), 1.50-1.64 (5H, m), 2.23-2.29 (2H, m), 3.25 (2H, t, *J* = 6.6 Hz), 5.0 (1H, dq, *J* = 1.8, 6.9 Hz), 6.99 (1H, d, *J* = 1.5 Hz) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.8, 148.9, 134.1, 77.3, 51.3, 29.2, 29.1, 29.0, 29.0, 28.7, 27.3, 26.6, 25.0, 19.1 ppm; IR (KBr): 2929, 2856, 2096, 1755, 1456, 1319, 1075, 1027, 951 cm^{-1}; MS (ESI, *m/z*): 288 ($M+Na^+$); HRMS (ESI, *m/z*) calcd. for $C_{14}H_{23}N_3O_2$ ($M+Na^+$): 288.1682, found: 288.1680.

Compound 16: A solution of **11** (88 mg, 0.212 mmol) in methanol (2.5 mL) was treated with conc. HCl (0.65 mL) overnight at room temperature. Aqueous $NaHCO_3$ was added to quench the reaction. Solvent was evaporated, and the mixture was extracted with ethyl acetate (30 mL x 3). The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography (PE-EA, 1.5:1) to afford **16** (61 mg, 88%) as a white wax.

$[\alpha]_D^{25}$ = +20.1 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.88 (3H, t, *J* = 6.6 Hz), 1.26-1.45 (18H, m), 2.04 (1H, t, *J* = 2.7 Hz), 2.43-2.46 (2H, m), 2.68 (1H, d, *J* = 2.4 Hz), 2.97 (1H, d, *J* = 4.2 Hz), 3.29-3.35 (1H, m), 3.49-3.56 (2H, m), 3.61-3.73 (5H, m), 3.76-3.79 (1H, m), 3.95-3.99 (1H, m) ppm; ^{13}C NMR (75 MHz, CDCl3) δ: 80.3, 75.9, 74.1, 70.5, 70.3, 70.3, 70.1, 68.5, 32.9, 31. 8, 29.6, 29.5, 29.5, 29.2, 25.5, 23.2, 22.6, 14.0 ppm; IR (neat): 3419, 3312, 2924, 2855, 2120, 1467, 1355, 1249, 1170, 940, 916 cm^{-1}; MS (ESI, *m/z*): 351 ($M+Na^+$); HRMS (ESI, *m/z*) calcd. for $C_{19}H_{36}O_4$ ($M+Na^+$): 351.2506, found: 351.2515.

Compound 17: To a stirred solution of **16** (86 mg, 0.262 mmol) in CH_2Cl_2 (5 mL) at 0 ^oC

was added pyridine (0.064 mL, 0.786 mmol), acetic anhydride (0.062 mL, 0.655 mmol), and DMAP (cat.). The reaction mixture was then allowed to stir at room temperature for 4 hrs. The mixture was poured into cold water, and extracted with CH_2Cl_2 (3 × 30 mL). The organic layer was then washed with water and brine. Removal of solvent under reduced pressure followed by column chromatography (PE-EA 6:1) afforded the corresponding acetate **17** (98 mg, 91%) as a colorless oil.

$[\alpha]_D^{25}$ = +11.1 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.88 (3H, t, *J* = 6.6 Hz), 1.25 (16H, brs), 1.56-1.60 (2H, m), 1.99 (1H, s), 2.07 (3H, s), 2.09 (3H, s), 2.51-2.62 (2H, m), 3.49-3.70 (8H, m), 5.00-5.09 (2H, m) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.6, 170.2, 79.2, 72.6, 72.4, 70.8, 70.7, 70.5, 70.3, 70.3, 31.8, 30.7, 29.5, 29.4, 29.4, 29.2, 25.2, 22.6, 21.1, 20.9, 20.6, 14.0 ppm; IR (neat): 3312, 3274, 2925, 2856, 2123, 1740, 1467, 1373, 1240, 1121, 1042, 958 cm^{-1}; MS (ESI, *m/z*): 435 ($M+Na^+$); HRMS (ESI, *m/z*) calcd. for $C_{23}H_{40}O_6$ ($M+H^+$): 413.2898, found: 413.2891

Compound 18: A solution of $KMnO_4$ (305 mg, 1.929 mmol) in 20 mL of H_2O was cooled in an ice-water bath. In one portion, a solution of alkyne **17** (265 mg, 0.643 mmol), HOAc (3 mL) and TBAB (10 mg, 0.032 mmol) in pentane (15 mL) was added. Without replenishing the ice in the cooling bath, the reaction mixture was stirred for 8 h. After cooling in an ice bath, 2 g of Na_2SO_3 and 3 mL of 6 N HCl were carefully added to the reaction mixture. After the mixture was stirred for 10 min, the reaction mixture was diluted with 20 mL of H_2O and extracted with pentane. The combined organic layers were dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography on silica gel (DCM-CH_3OH,

40:1) to afford **18** (198 mg, 71%) as a yellowish oil.

$[\alpha]_D^{25}$ = +4.4 (*c* = 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ: 0.88 (3H, t, *J* = 6.6 Hz), 1.25 (16H, brs), 1.55-1.59 (2H, m), 2.07 (3H, s), 2.08 (3H, s), 2.72-2.76 (2H, m), 3.49-3.71(8H, m), 5.01-5.05 (1H, m), 5.31-5.37 (1H, m) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ: 174.8, 171.0, 170.3, 72.7, 72.4, 71.3, 70.7, 70.5, 68.9, 35.5, 31.8, 30.7, 29.5, 29.5, 29.4, 29.4, 29.2, 25.2, 22.6, 21.1, 20.9, 14.0 ppm; IR (film): 3460, 2926, 2855, 1743, 1465, 1374, 1240, 1120, 1182, 1045, 958 cm^{-1}; MS (ESI, *m/z*): 455 ($M+Na^+$); HRMS (ESI, *m/z*) calcd. for $C_{22}H_{40}O_8$ ($M+Na^+$): 455.2615, found: 455.2611

Compound 19: To a solution of azide **15** (212 g, 0.8 mmol) in anhydrous tetrahydrofuran (15 ml) was added Ph_3P (419 mg, 1.6 mmol) at room temperature. The mixture was allowed to stir for 3 hrs. Water (50 μl) was then added to the reaction mixture and stirred for additional 17 hrs. The reaction mixture was poured into 2N aq. HCl and extracted with diethyl ether (3×30 ml). The aqueous layer was then adjusted to basic solution (pH 10) with aq. NaOH and extracted with diethyl ether (5×30 ml). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to yield **19** (130 mg, 68%) as a pale yellow liquid. Without further purification, this crude product was used for the next step.

Compound 25: Under an inert gas, a few drops of neat **23** (2.04 g, 12.75 mmol) was added to predried Mg turnings (0.367 g, 15.3 mmol) in anhydrous diethyl ether (3 mL) to initiate the formation of the organometallic species. The remaining bromide was diluted with diethyl ether (10 mL) and added dropwise to the Mg mixture to maintain gentle reflux. Upon

completion of addition, the Grignard solution was stirred for additional 45 min to give the (cyclopent-3-enylmethyl)magnesium bromide.

To a suspension of CuI (137 mg, 0.722 mmol) in diethyl ether (5 mL) at -40 °C under argon atmosphere was added the freshly prepared Grignard solution (2.6 mL). The mixture was stirred for 10 min, and a solution of compound **10** (100 mg, 0.289 mmol) in diethyl ether (5 mL) was added dropwise to the above solution. The mixture was stirred for 1.5 hrs at the same temperature. The reaction mixture was quenched with saturated aqueous NH_4Cl (6 mL). The separated aqueous layer was re-extracted with diethyl ether and the combined extracts were washed with brine, dried over $NaSO_4$, filtered, and concentrated in vacuo. Purification of the crude product by column chromatography (PE-EA, 6:1) afforded compound **25** (105 mg, 85%) as a colorless oil.

$[\alpha]_D^{25}$ = +2.8 (*c* = 0.99, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.88 (3H, t, *J* = 6.6 Hz), 1.25 (16H, brs), 1.38-1.60 (6H, m), 1.93-1.99 (2H, m), 2.216-2.24 (1H, m), 2.43-2.51 (2H, m), 3.27-3.33 (1H, m), 3.39 (3H, s), 3.50-3.76 (9H, m), 4.66 (1H, d, *J* = 6.9 Hz), 4.78 (1H, d, *J* = 6.9 Hz), 5.65 (2H, s) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ: 129.8, 129.8, 95.9, 76.1, 75.7, 74.0, 70.6, 70.5, 70.3, 55.7, 38.8, 38.8, 37.6, 31.2, 31.9, 31.8, 31.7, 29.6, 29.5, 29.5, 29.2, 25.4, 22.6, 14.0 ppm; IR (film): 3479, 3052, 2924, 2854, 1615, 1466, 1356, 1214, 1142, 1106, 1040, 919 cm^{-1}; MS (ESI, *m/z*): 451 ($M+Na^+$); HRMS (ESI, *m/z*) calcd. for $C_{25}H_{48}O_5$ ($M+Na^+$): 451.3394, found: 451.3399.

Compound 26: MOMCl (0.149 mL, 1.96 mmol) was added to a mixture of **25** (210 mg, 0.49 mmol) and diisopropyl ethylamine (0.405 mL, 2.45 mmol) in dry CH_2Cl_2 (15 mL) at 0 ^oC.

The reaction was stirred at room temperature for 8 hrs, and then quenched with saturated aqueous NH_4Cl. The mixture was extracted with CH_2Cl_2. The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (PE-EA, 8:1) to afford **26** (205 mg, 88%) as a colorless oil.

$[\alpha]_D^{25}$ = -10.7 (*c* = 1.01, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.87 (3H, t, *J* = 6.7 Hz), 1.25-1.56 (22H, m), 1.92-2.00 (2H, m), 2.18-2.23 (1H, m), 2.43-2.51 (2H, m), 3.38 (6H, s), 3.49-3.71 (10H, m), 4.65 (1H, d, *J* = 6.9 Hz), 4.76 (1H, d, *J* = 6.9 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ: 129.7, 129.7, 95.9, 76.2, 76.1, 74.0, 73.9, 70.7, 55.3, 55.3, 38.8, 38.7, 37.6, 32.0, 31.9, 31.8, 30.6, 29.6, 29.5 (2C), 29.2, 25.3, 22.5, 14.0 ppm; IR (film): 3052, 2925, 2854, 1615, 1466, 1356, 1214, 1146, 1105, 1042, 919 cm^{-1}; MS (ESI, *m/z*): 495 ($M+Na^+$); HRMS (ESI, *m/z*) calcd. for $C_{27}H_{52}O_6$ ($M+Na^+$): 495.3656, found: 495.3659

AA005 analogue 1: To a round bottom flask was added alkyne **16** (30 mg, 0.091 mmol), azide **15** (26.5 mg, 0.1 mmol), $CuSO_4{\cdot}5H_2O$ (2.29mg, 0.0091 mmol), sodium ascorbate (3.62mg, 0.0182 mmol), water (2 mL) and THF (2 mL). The mixture was stirred for 12 hrs at room temperature, and then extracted with CH_2Cl_2. The extracts were dried with Na_2SO_4 and concentrated in vacuo. Flash chromatography of the residue on silica gel (DCM-CH_3OH, 30:1) yielded **1** (34 mg, 68%) as a white wax.

$[\alpha]_D^{25}$ = +15.8 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.87 (3H, t, *J* = 6.6 Hz), 1.25-1.42 (33H, m), 1.50-1.56 (2H, m), 1.86-1.91 (2H, m), 2.26 (2H, t, *J* = 7.8 Hz), 2.85-2.93 (2H, m), 3.31 (1H, t, *J* = 9.6 Hz), 3.43-3.71 (7H, m), 3.78-3.80 (1H, m), 4.09-4.12 (1H, m),

4.31 (2H, t, *J* = 7.2 Hz), 4.99 (1H, qd, *J* = 1.5, 6.9 Hz), 7.0 (1H, d, *J* = 1.2 Hz), 7.42 (1H, s) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ: 148.9, 144.3, 134.1, 121.9, 77.4, 75.8, 74.7, 70.5, 70.4, 70.1, 69.5, 50.2, 32.9, 31.8, 30.2, 29.6, 29.5, 29.5, 29.5, 29.3, 29.1, 29.0, 29.0, 28.8, 27.3, 26.4, 25.5, 25.1, 22.6, 19.1, 14.1 ppm; IR (neat): 3465, 2920, 2849, 1734, 1467, 1324, 1116, 1095, 1085 $cm^{-1;}$ MS (ESI, m/z): 594 ($M+H^+$); HRMS (ESI, *m/z*) calcd. for $C_{33}H_{59}N_3O_6$ ($M+H^+$): 594.4477, found: 594.4462.

AA005 analogue 2: To a solution of acid **18** (62 mg, 0.144 mmol) and amine **19** (41 mg, 0.171 mmol) in DMF (2 mL) was added Et_3N (60 μL, 0.43 mmol), EDCI (32.6 mg, 0.171 mmol) and HOBT (27.9 mg, 0.171 mmol) at room temperature. The reaction mixture was stirred for 24 hrs, and quenched with H_2O. The mixture was extracted with ether. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure.

A solution of the crude product in methanol (5 mL) was treated with $NH_2NH_2{\cdot}H_2O$ (1 mL). The mixture was stirred overnight at room temperature. Solvent was evaporated, and the mixture was extracted with DCM (30 mL x 3). The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated. Flash chromatography of the residue on silica gel (DCM-CH_3OH, 25:1) yielded **2** (40 mg, 48% over two steps) as a white wax. $[\alpha]_D^{25}$ = -7.4 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.87 (3H, t, *J* = 6.6 Hz), 1.27 (28H, brs), 1.40 (3H, d, *J* = 6.6 Hz), 1.47-1.53 (2H, m), 2.26 (2H, t, *J* = 7.6 Hz), 2.37-2.39 (2H, m), 3.22 (2H, q, *J* = 6.6 Hz), 3.28-3.34 (1H, m), 3.47-3.70 (7H, m), 3.77-3.80 (1H, m), 4.12-4.16 (1H, m), 4.99 (1H, qd, *J* = 1.5, 6.6 Hz), 6.40 (1H, brs), 6.99 (1H, d, *J* = 1.5Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.8, 171.5, 148.9, 134.2, 77.4, 75.7, 74.3, 70.5, 70.3, 70.2,

67.5, 39.3, 39.2, 33.0, 31.8, 31.8, 29.6, 29.5, 29.5, 29.4, 29.3, 29.1, 29.1, 29.0, 27.3, 26. 8, 25.5, 25.1, 22.6, 19.1, 14.0 ppm; IR (neat): 3412, 3295, 2921, 2849, 1743, 1642, 1469, 1172, 1101, 1084, 1053 cm^{-1}; MS (ESI, m/z): 592 ($M+Na^+$); HRMS (ESI, *m/z*) calcd. for $C_{32}H_{59}NO_7$ ($M+Na^+$) : 592.4184, found: 592.4205.

AA005 analogue 3: To a solution of cycloalkene **26** (89 mg, 0.189 mmol) in THF (3 mL) and water (1.2 mL) at 20 ^oC was added $K_2OsO_8{\cdot}2H_2O$ (7 mg, 0.0189 mmol), sodium periodate (200 mg, 0.943 mmol). After 8 hrs, the reaction was diluted with saturated sodium thiosulfate (10 mL), and then extracted with ethyl acetate. The organic layer was dried with sodium sulfate and concentrated. The crude product was used for the next step without further purification.

To a stirred solution of the crude dialdehyde (95 mg, 0.1885 mmol) and amine **19** (54mg, 0.2262 mmol) in CH_2Cl_2 (2.5 mL) were added $NaBH(OAc)_3$ (35.5 mg, 0.5655mmol) and AcOH (65 μL, 1.131 mmol). After being stirred at room temperature for 3.5 hrs, the reacion mixture was diluted with EtOAc. The mixture was washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (DCM-CH_3OH, 30:1).

A solution of the above product in methanol (3 mL) was treated with conc. HCl (0.6 mL) at room temperature overnight. Aq. $NaHCO_3$ was added to quench the reaction. Solvent was evaporated, and the mixture was extracted with ethyl acetate (30 mL x 3). The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated. Flash chromatography on silica gel (DCM-CH_3OH, 15:1) yielded **3** (31 mg, 26% over three steps)

as a white wax.

$[\alpha]_D^{25}$ = +5.8 (*c* = 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ: 0.87 (3H, t, *J* = 6.6 Hz), 1.25-1.42 (40H, m), 1.49-1.53 (2H, m), 1.67-1.70 (2H, m), 1.91 (2H, t, *J* =10.8 Hz), 2.23-2.34 (4H, m), 2.95 (2H, d, *J* = 11.1), 3.31 (2H, t, *J*= 9.3 Hz), 3.51-3.80 (6H, m) , 4.99 (1H, qd, *J* = 1.8, 6.9 Hz), 6.98 (1H, d, *J*=1.5 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.8, 148.8, 134.2, 77.3, 75.8, 75.7, 70.4, 70.4, 70.3, 70.2, 59.1, 53.9, 35.7, 32.9, 32.2, 32.1, 32.0, 31.8, 30.1, 29.6, 29.5, 29.5, 29.4, 29.3, 29.3, 29.1, 29.1, 27.6, 27.3, 26.8, 25.5, 25.1, 22.6, 19.1, 14.0 ppm; IR (neat): 3512, 2922, 2850, 1743, 1468, 1378, 1324, 1117, 1102, 1088, 1028, 965 cm^{-1}; MS (ESI, *m/z*): 624 ($M+H^+$); HRMS (ESI, *m/z*) calcd. for $C_{37}H_{69}NO_6$ ($M+Na^+$): 624.5198, found: 624.5195.

2. Details of biological experiments

Reagents: AA005 and AA005 analogues **1-3** (from School of Chemistry and Chemical Engineering, Nanjing University) were dissolved in 75% ethanol as a 6.6 μM stock solution and kept at -80 ^{o}C.

Cell culture and treatment:

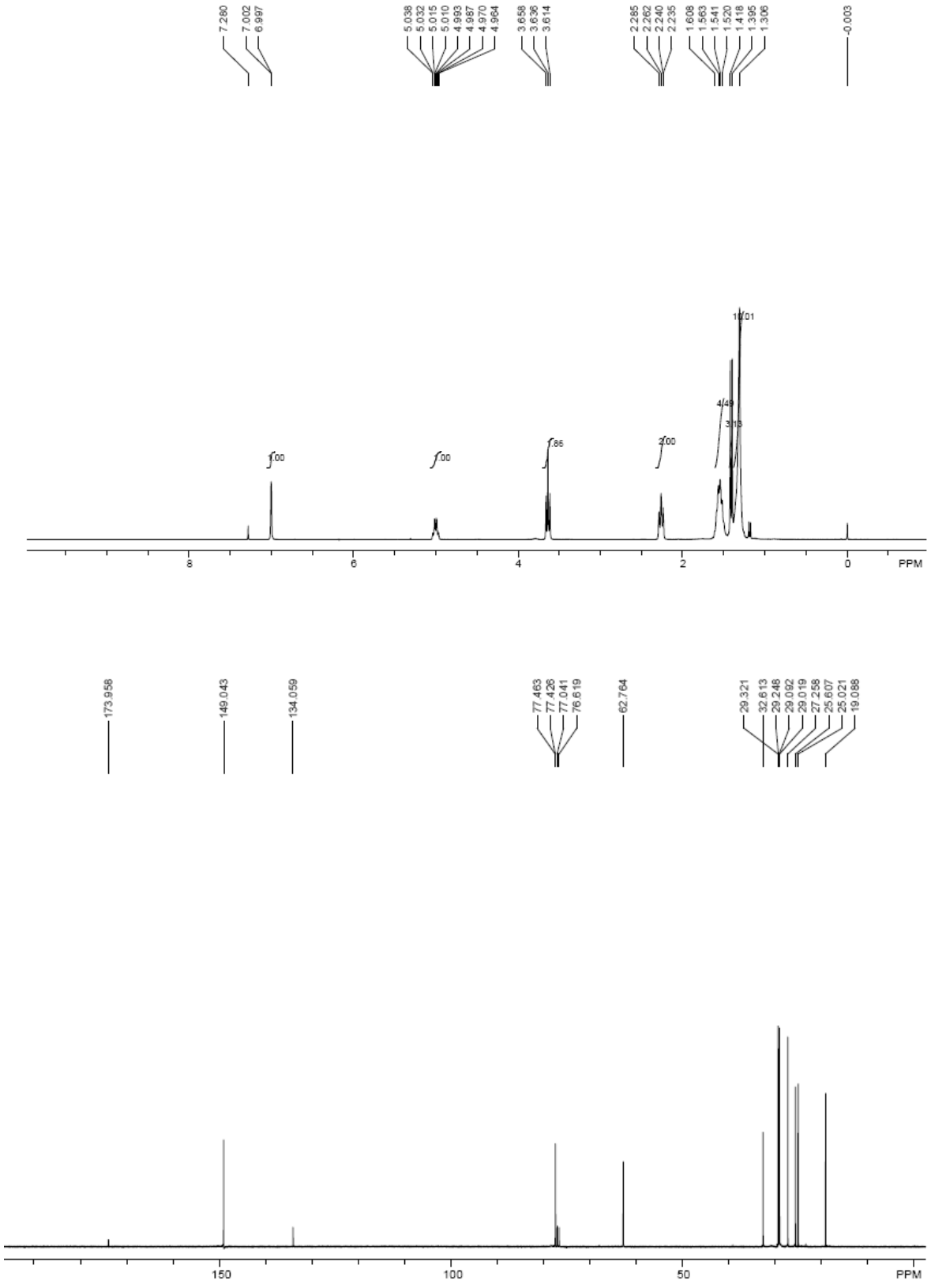
Six leukemic cell lines were used for the current studies. NB4, a APL cell line was kindly provided by Dr. M. Lanotte's in France; U937, an acute myelomonocytic leukemic cell line, and a chronic myeloid leukemic cell line K562 were obtained from Cell Bank of Shanghai Institutes of Biological Sciences (Shanghai, China); Kasumi-1, a t(8;21)-positive M2b-type AML cell line was provided by Dr. Kamada in Japan; as well as HL-60,a human promyelocytic cell line and Jurkat, an acute T cell leukemic cell line were provided by Shanghai Institute of Hematology (Shanghai, China).

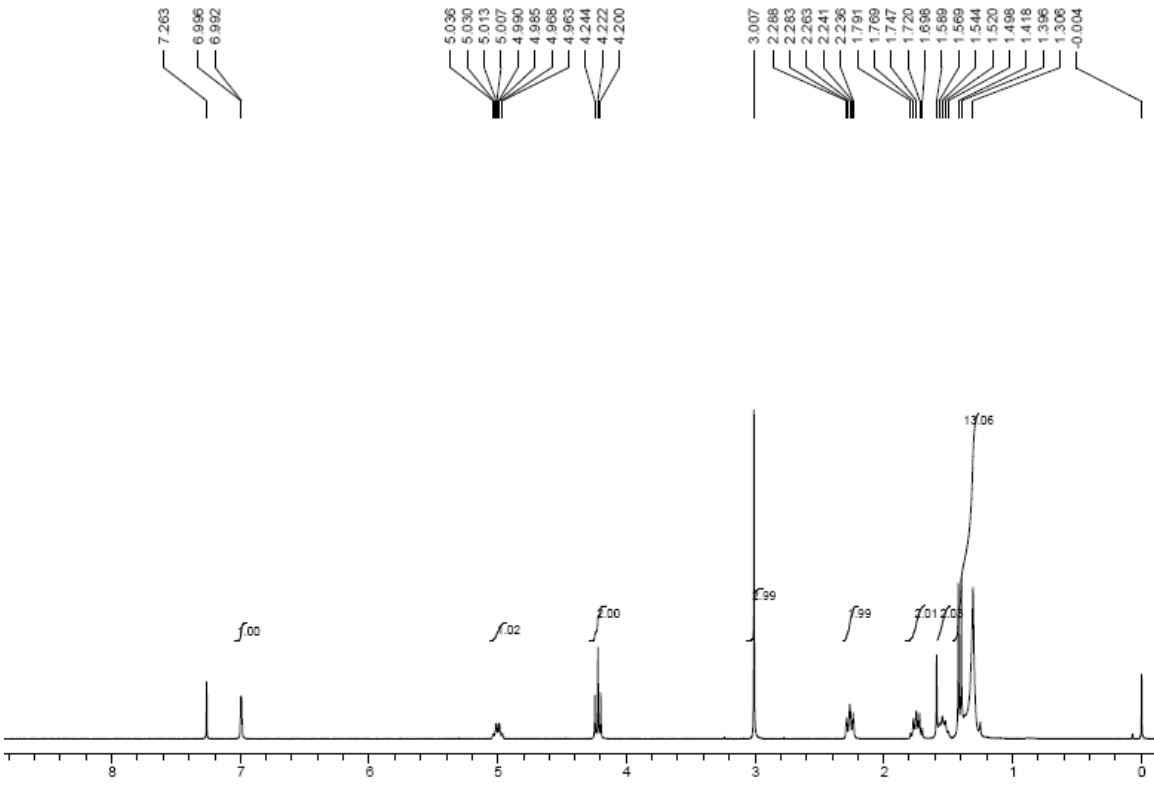
Six adherent cell lines including five breast cancer cell lines MCF-7, MDA-MB-435S, BT-549, BT-474, SK-Br-3 provided by Chinese Academy of Sciences (Shanghai, China), and a colorectal adenocarcinomic cell line SW620 were used.

All cell lines were cultured in RPMI-1640 medium (Hyclone, Logan, UT) supplemented with 10% heat-inactivated fetal calf serum (FCS, HyClone, Logan, UT).

For experiments, cells were seeded into ninety-six-well plates at the initial density of 1×10^4 cells per well for leukemic cell lines and 2×10^3 cells per well for adherent cell lines, followed by incubation with the indicated concentrations of the above compounds and maintained in a 5% CO_2–95% air humidified atmosphere at 37°C. For quantitative analysis of cell growth inhibition ratio, 10 μL of Cell Counting Kit-8 (CCK-8, Dojindo, Kumamoto, Japan) solution was added to each well at 37 ^{o}C for 4h, absorbance at 450 nm was monitored with a microplate reader (Spectra Max Plus 384; Molecular Devices). The values obtained were normalized to those of control cells incubated with vehicle only.

3. NMR Spectra of new compounds

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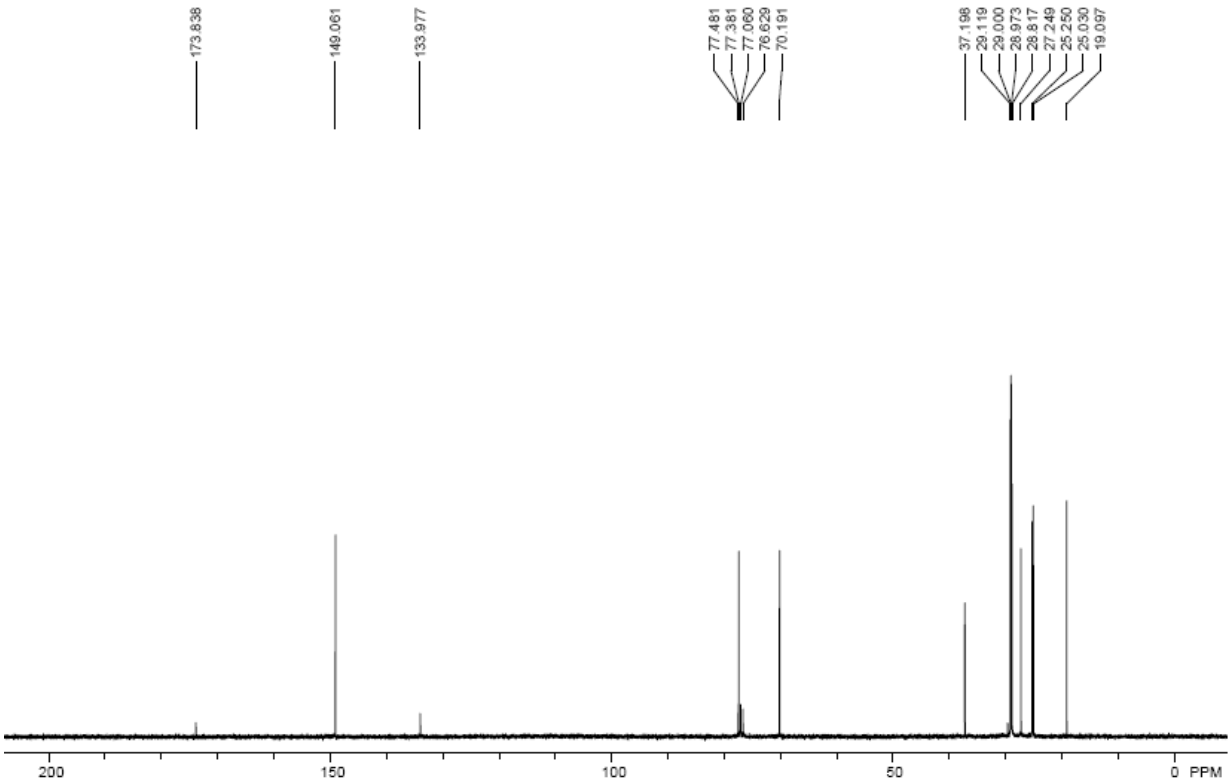
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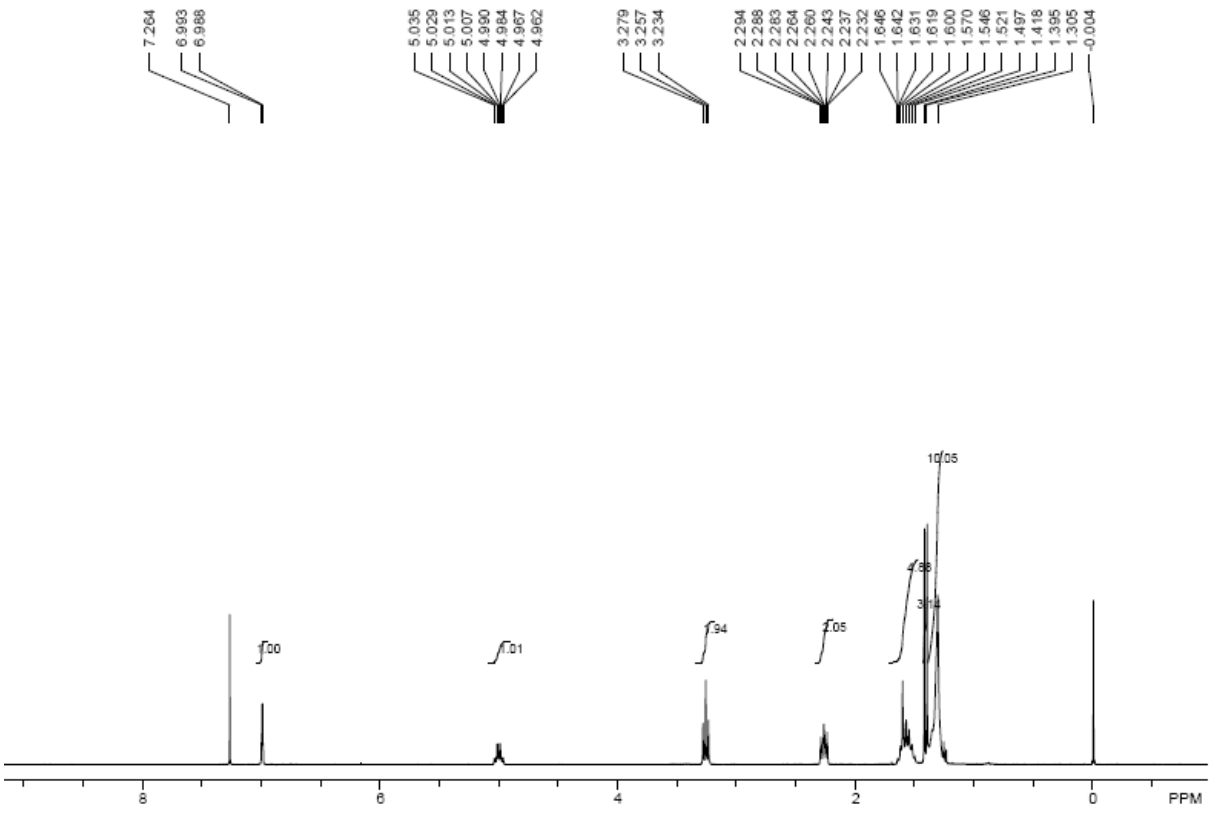

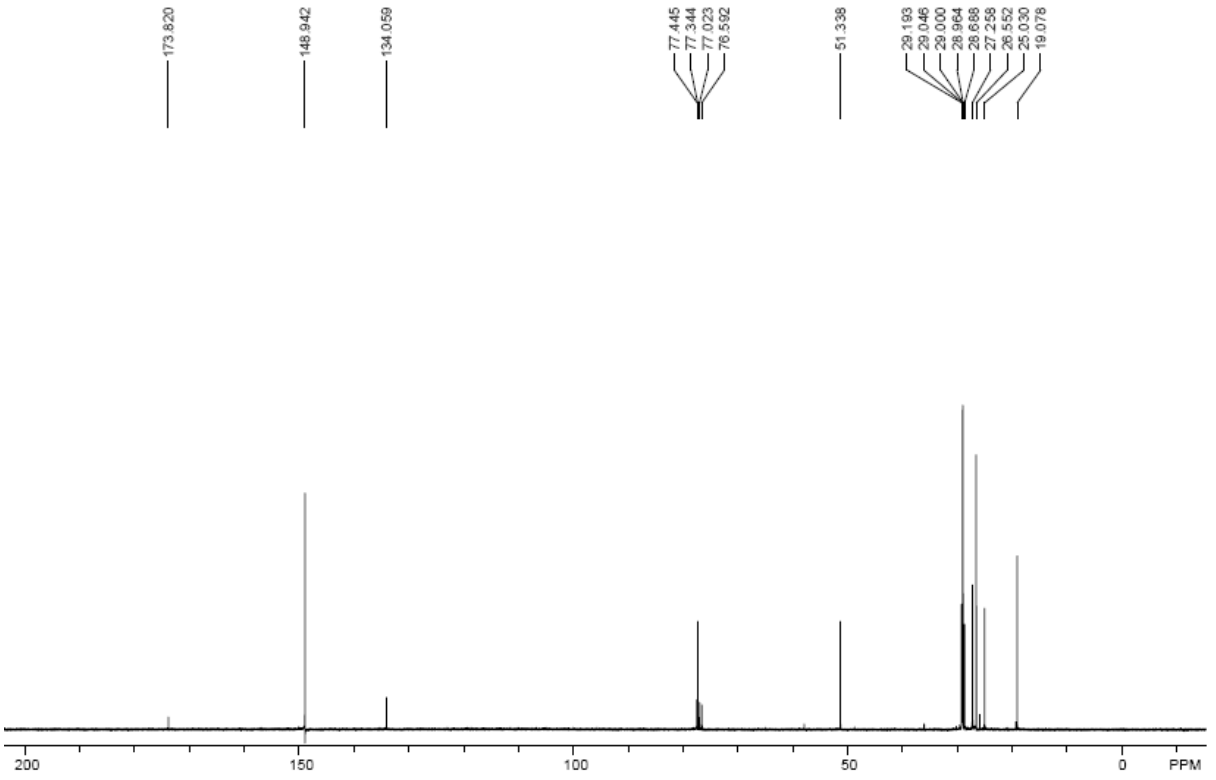

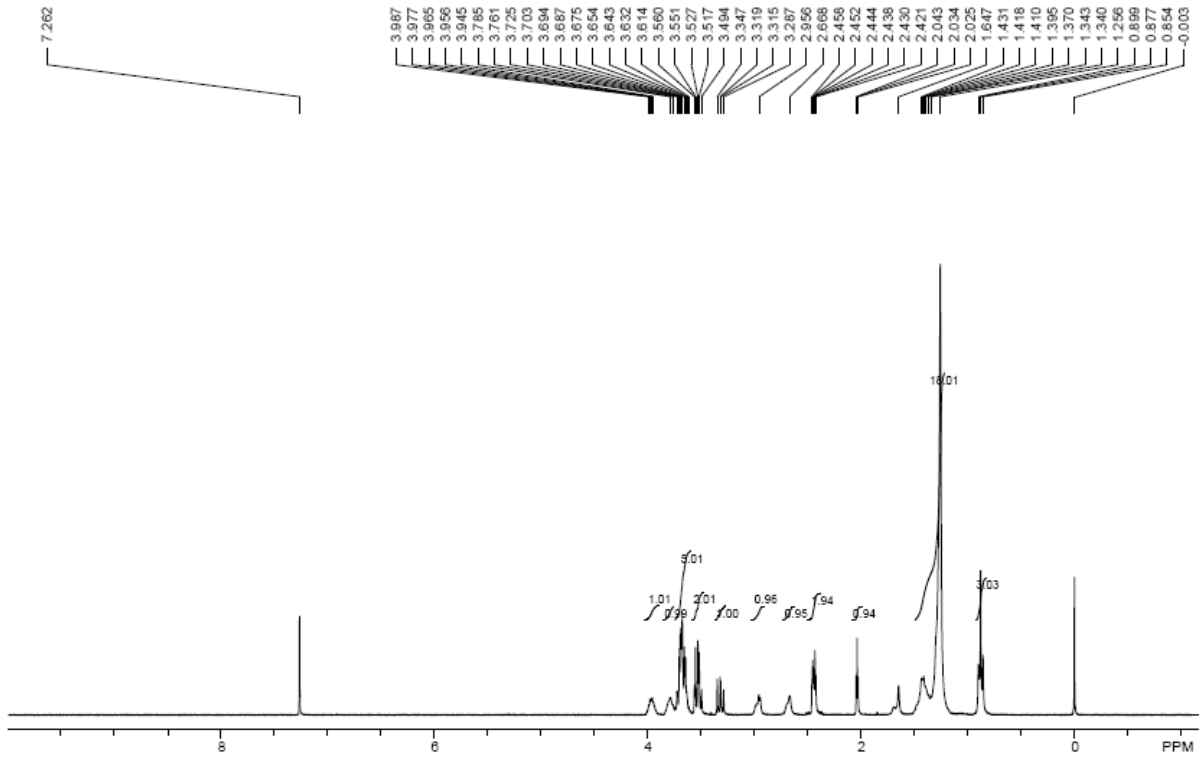
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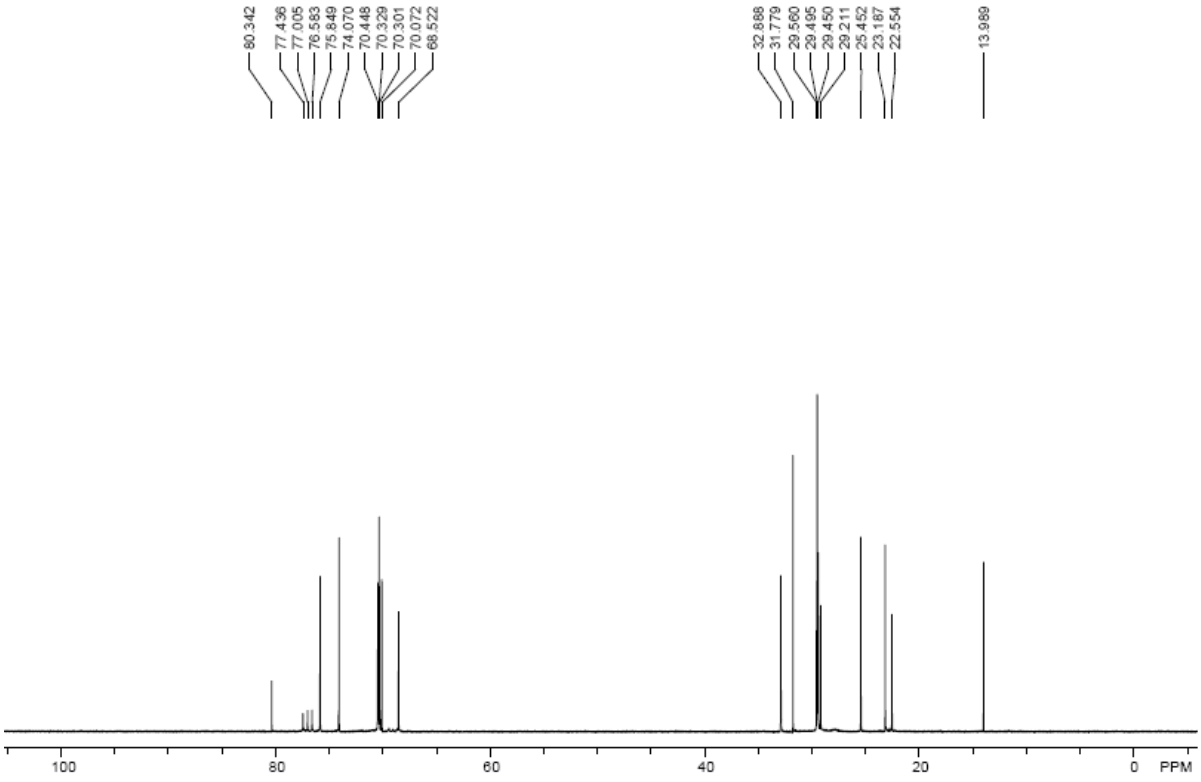
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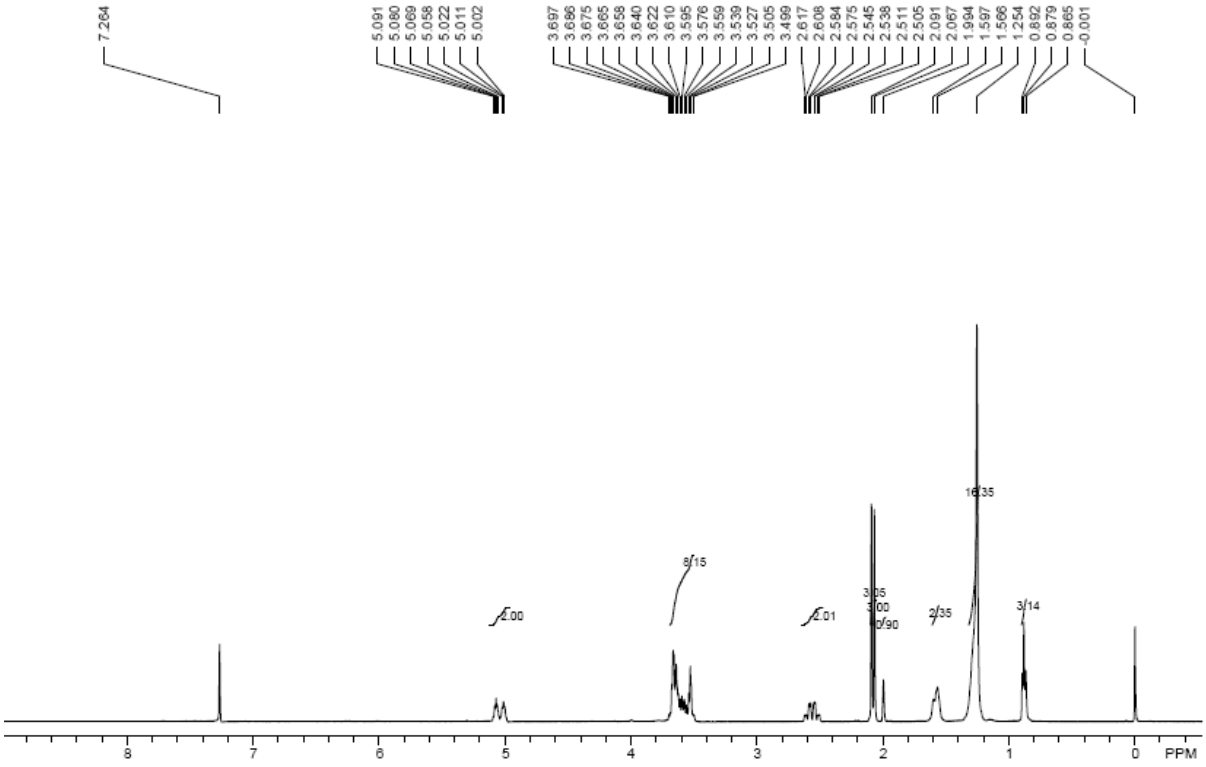
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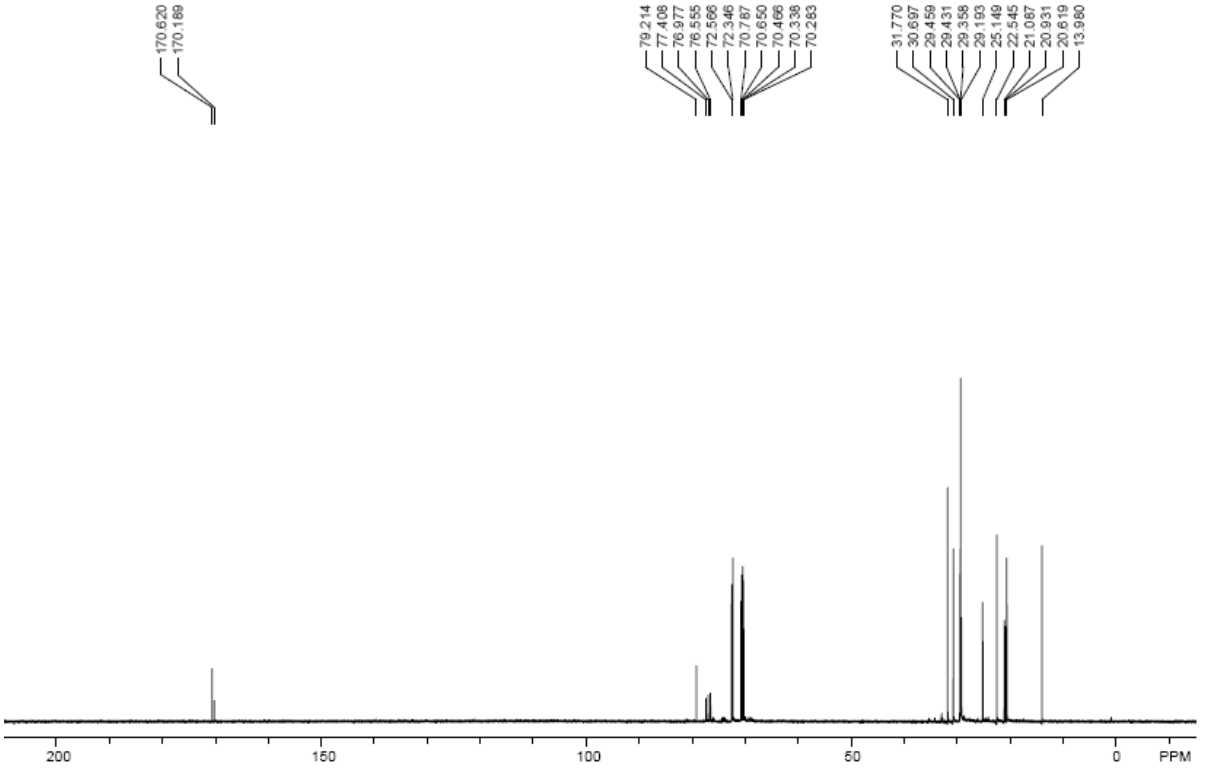
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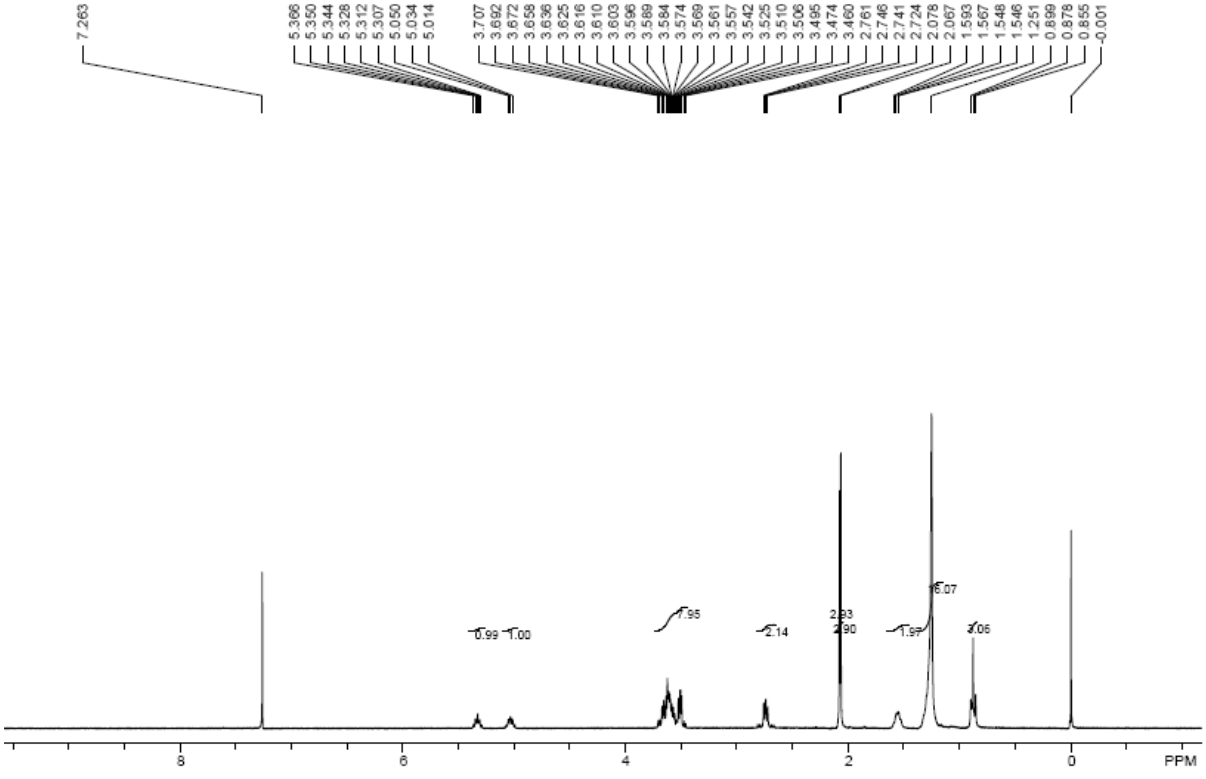
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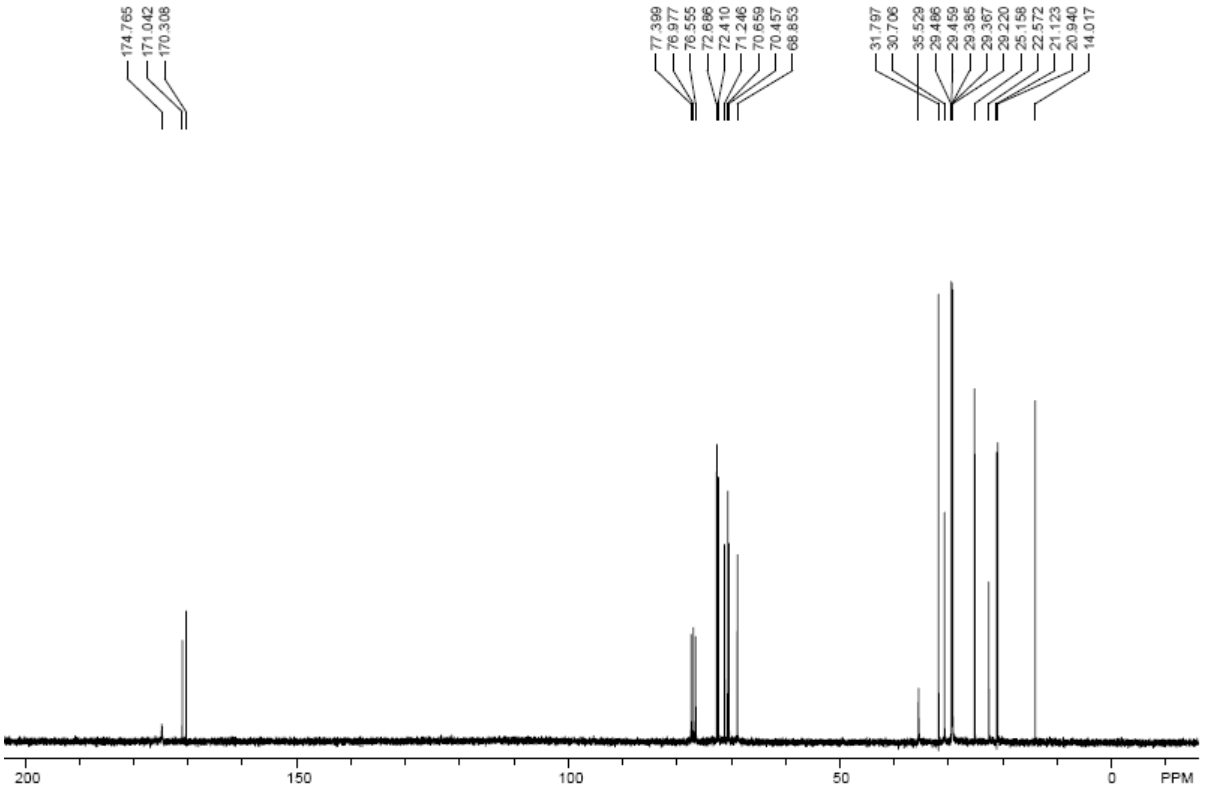
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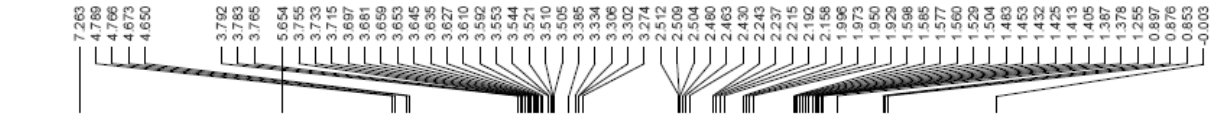
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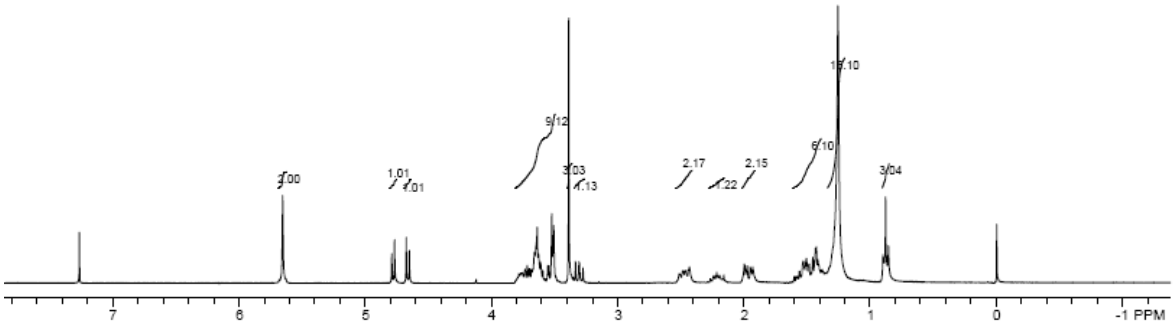
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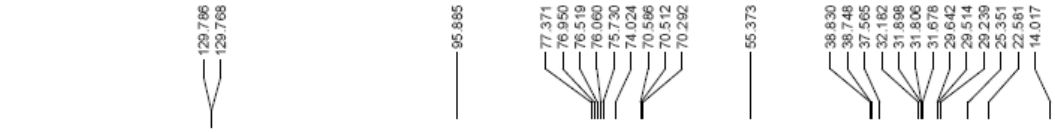
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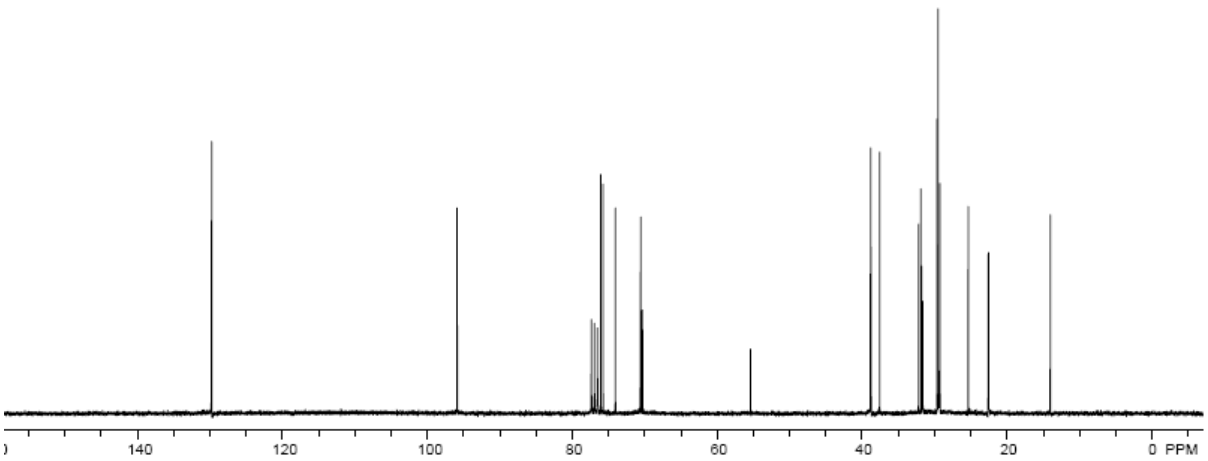
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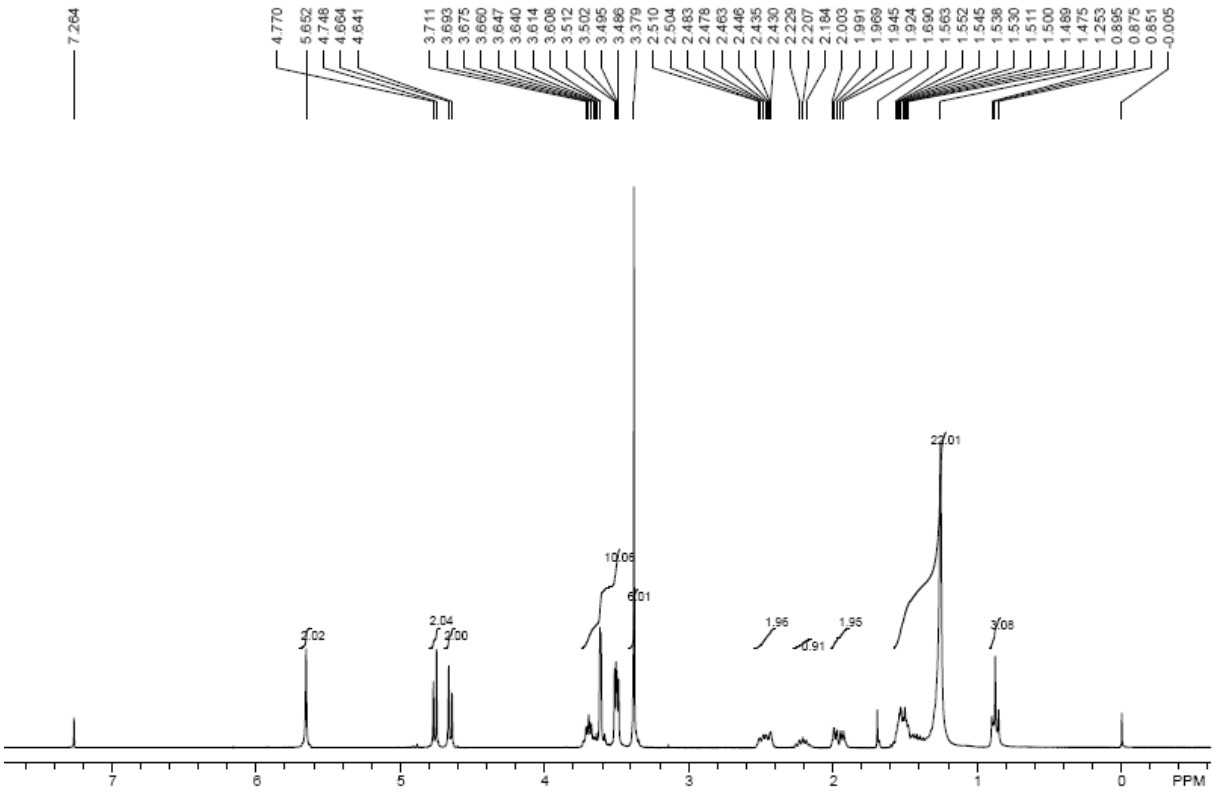
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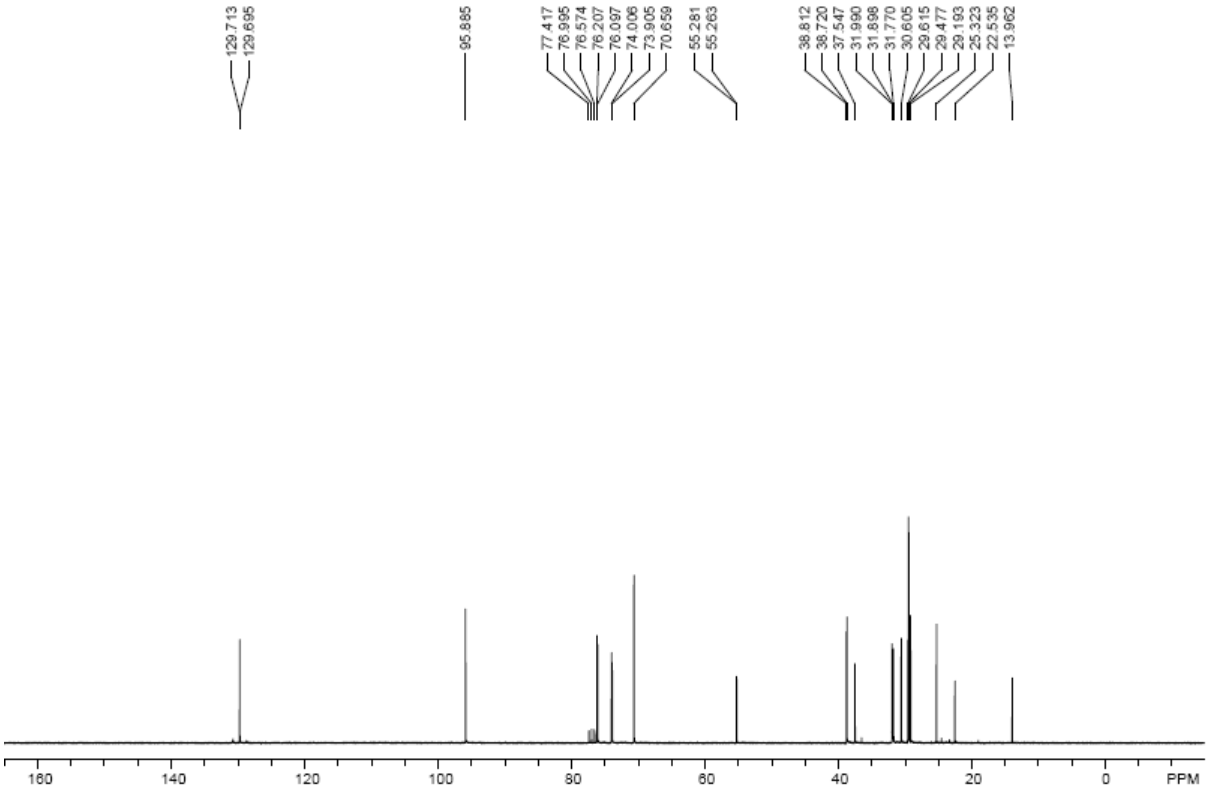
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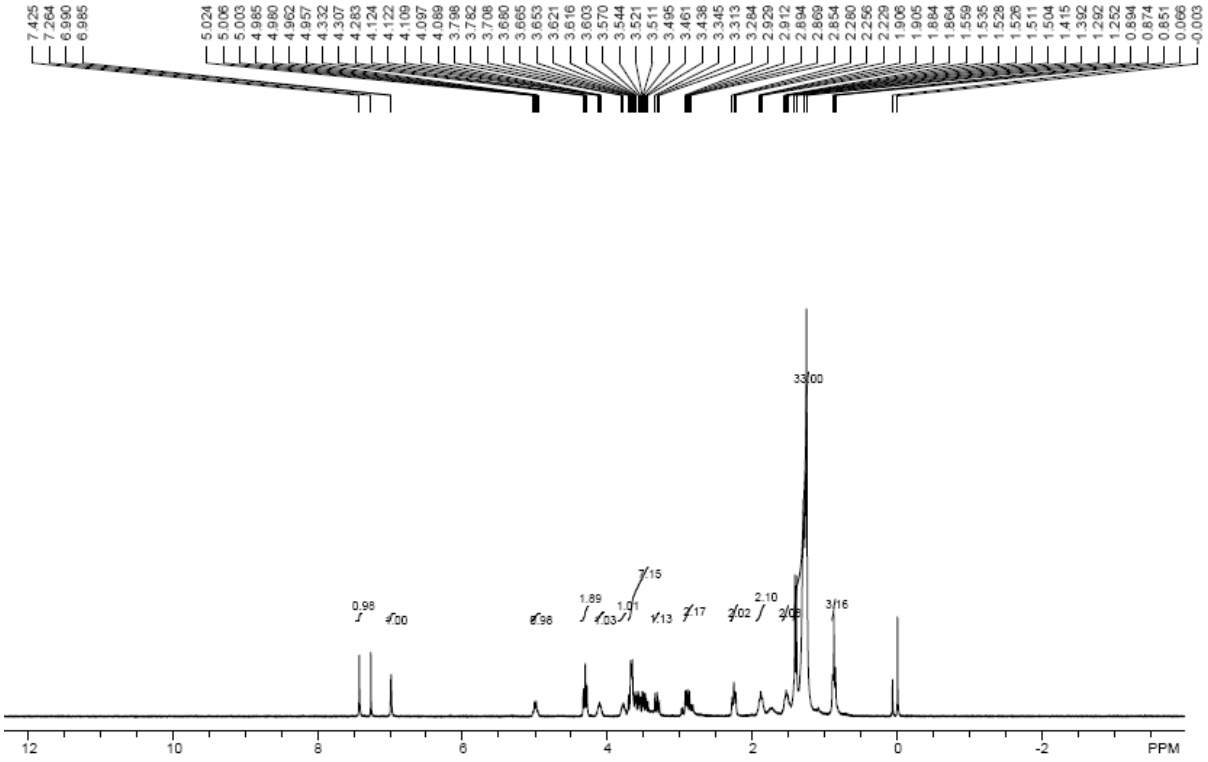
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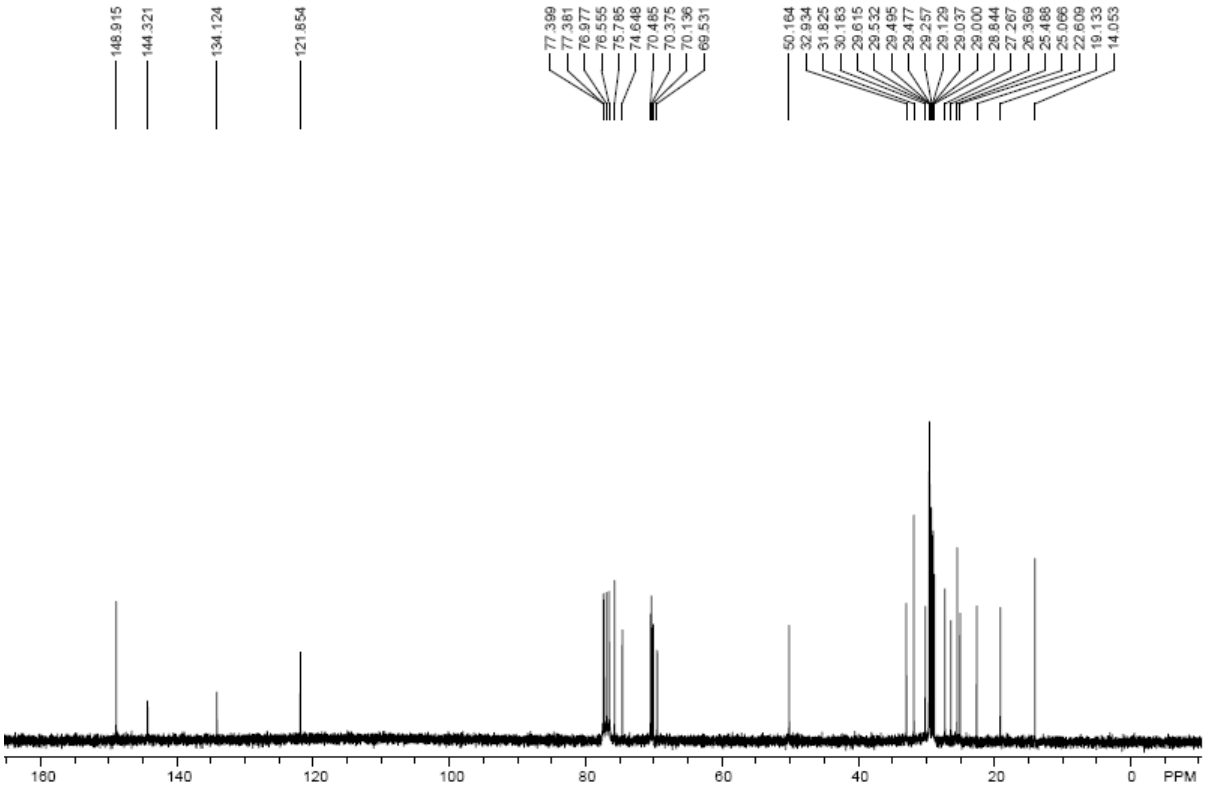
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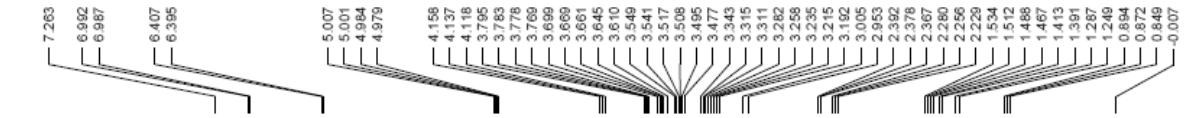
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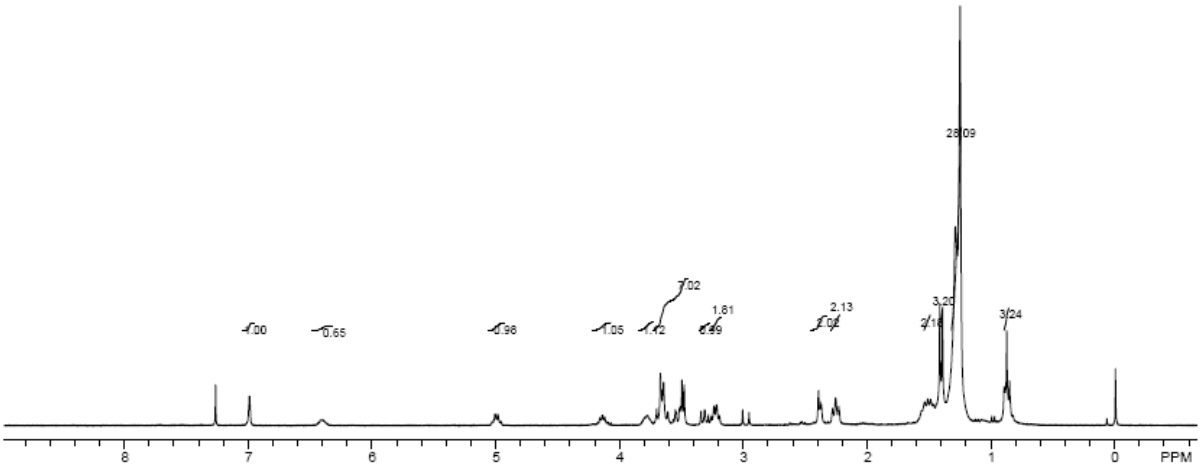
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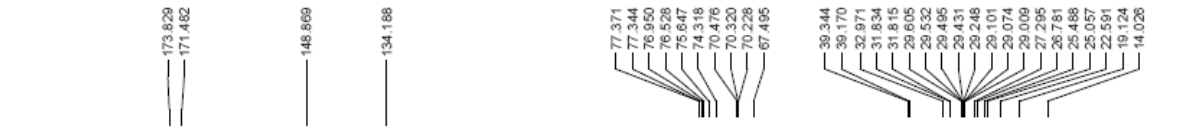
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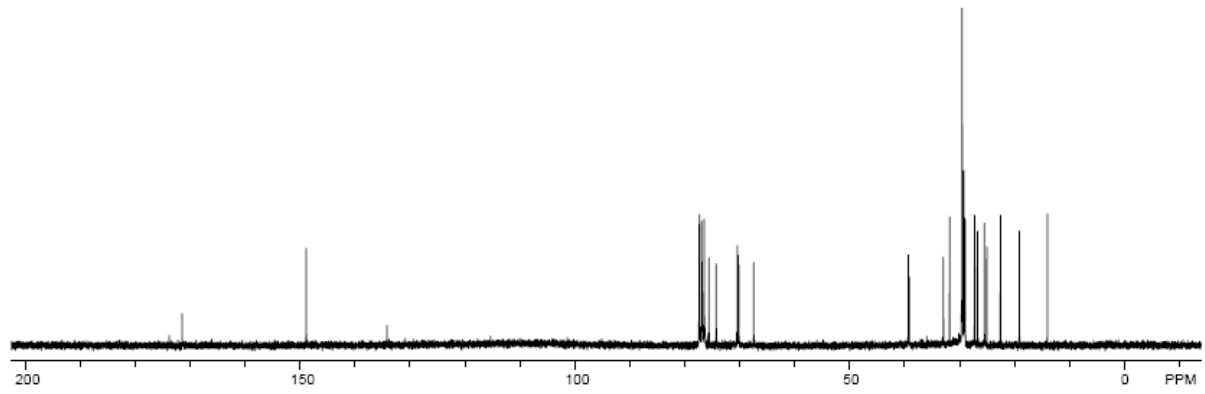
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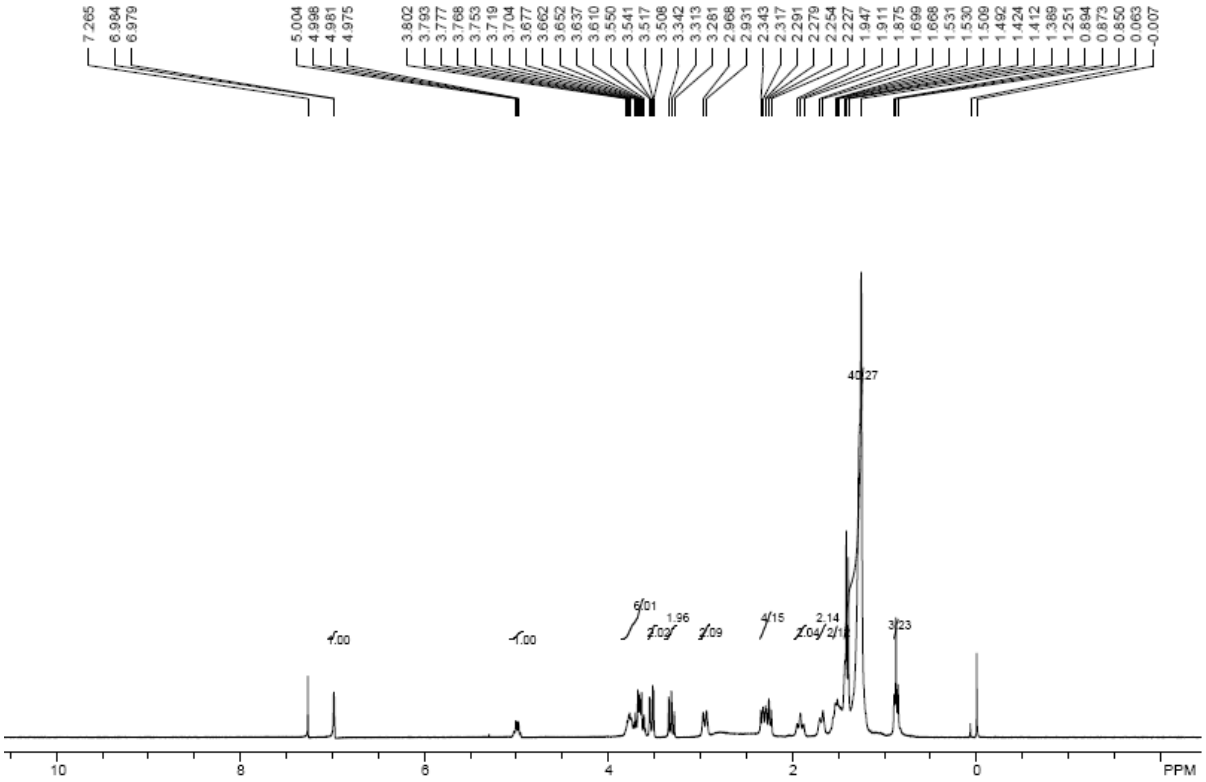
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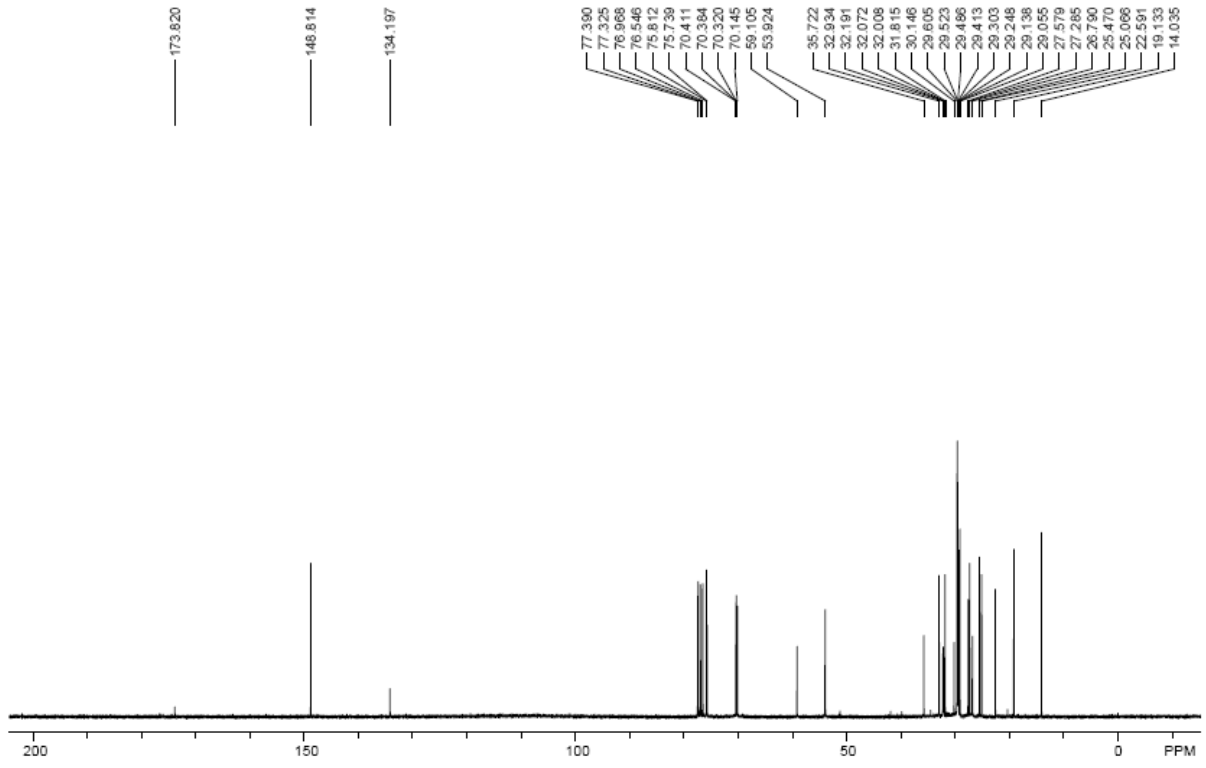
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