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Newly acylated SN-38 homodimers: carrier-free nano-prodrugs for chemotherapy

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Experimental

General

Instruments: Synthesized compounds were characterized by using nuclear magnetic resonance (NMR), and High-resolution mass spectra (HRMS). ^{1}H NMR and ^{13}C NMR spectra were recorded on 400 and 100 MHz (Bruker AVANCE–400 III) spectrometer, respectively, and calibrated using residual deuterated solvent ($CDCl_3$ at δ 7.26 ppm for ^{1}H, δ 77.2 for ^{13}C NMR) and tetramethylsilane as internal reference. High–resolution mass spectrometry (HRMS) was performed using a Bruker MicrOTOF–Q II–S1 using electrospray ionization (ESI) technique. Average particle size, size distributions, and zeta potential were measured using a Malvern Zetasizer nanoZS. Scanning electron microscope (SEM) images were observed using a S-4800 Hitachi. Cell viability was evaluated using a microplate reader (Bio-Rad iMark).

Materials: SN-38, di-*tert*-butyl decarbonate ($(Boc)_2O$) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl), DL-2-methyl butyric acid, valeric acid, isovaleric acid, pivalic acid, cyclopropyl acetic acid and cyclobutanecarboxylic acid were purchased from Tokyo Chemical Industry Co. Pyridine, 2,2′-dithiodiethanol, 1-methylcyclopropane- carboxylic acid, 2-methylcyclopropanecarboxylic acid and (*S*)-(+)-2-methylbutyric acid were purchased from Sigma-Aldrich. 4-Dimethylaminopyridine (DMAP), tetrahydrofuran (THF), dichloromethane (CH_2Cl_2), ethanol (EtOH), trifluoroacetic acid (TFA) and triphosgene were purchased from FUJIFILM Wako Pure Chemical Corporation. Chloroform ($CHCl_3$) and methanol (MeOH) were purchased from Nacalai Tesque, Inc. Dimethyl sulfoxide-d_6 (DMSO-d6), chloroform-d ($CDCl_3$), and Silica gel 60N (230–400 mesh) for column chromatography were purchased from Kanto Chemical co. Silica gel plates 60F254 for thin layer chromatography (TLC), and Whatman® Nuclepore Track-Etched Membrane (ϕ = 0.05 μm) were purchased from Merck. HCT-116 (Human colon cancer) MCF-7 (Human breast cancer) and A-549 (Human lung cancer) cells were purchased from RIKEN cell bank. Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), and trypsin were purchased from Life Technologies. Cell Counting Kit-8 was purchased from Dojindo Molecular Technologies, Inc. Ketamine Hydrochloride was purchased from Daiichi Sankyo Company, Limited. All reagents and solvents were used without further purification.

Synthesis of Boc-SN-38 (1′)

Pyridine (13 mL, 160 mmol) and $(Boc)_2O$ (2.3 mL, 10 mmol) were added into the suspension of SN-38 **1** (3 g, 7.7 mmol) in dichloromethane under an argon environment at room temperature (RT) and stirred for 3 h. Upon completion of the reaction (monitored by TLC), the reaction mixture was diluted with chloroform and subsequently washed with a saturated solution of sodium bicarbonate ($NaHCO_3$) and 0.25 N hydrochloric acid (HCl) concurrently. The organic layer was concentrated under vacuum then purified by Si-gel column chromatography (0 to 2% MeOH in $CHCl_3$) to give Boc-SN-38 (3.67 g, 97%) as pale Yellowish white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 9.2 Hz, 1H), 7.88 (d, *J* = 2.5 Hz, 1H), 7.65 (s, 1H), 7.64 (dd, *J* = 2.5, 9.2 Hz, 1H), 5.74 (d, *J* = 16.3 Hz, 1H), 5.30 (d, *J* = 16.3 Hz, 1H), 5.25 (s, 2H), 3.91 (s, 1H), 3.15 (q, *J* = 7.7 Hz, 2H), 1.97-1.83 (m, 2H), 1.61 (s, 9H), 1.40 (t, *J* = 7.7 Hz, 3H), 1.03 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9, 157.6, 151.9, 151.5, 150.2, 149.9, 147.4, 146.9, 145.3, 132.1, 127.4, 127.3, 125.2, 118.6, 114.1, 98.0, 84.4, 72.8, 66.3, 49.4, 31.6, 27.7, 23.2, 14.0, 7.8; HRMS (ESI–TOF): m/z $C_{27}H_{29}N_2O_7^+$ ($[M+H]^+$) calcd. for 493.1969, found 493.1974.

Synthesis of Boc-SN-38 dimer (3′)

Boc-SN-38 **1′** (1g, 2.02 mmol), triphosgene (240.0 mg, 0.8 mmol) and DMAP (1.24 g, 10 mmol) were dissolved in dichloromethane (40.0 mL) at 0°C. The reaction mixture was stirred at 0 °C for 10 min followed by 1 mL solution of 2,2′-dithiodiethanol (100 µL/mL, 0.84 mmol/mL) in dichloromethane was added in reaction mixture. The reaction mixture was transferred to room temperature and stirred for 1 h. 1 mL of each solution of triphosgene (120 mg/mL) and 2,2′-dithiodiethanol (100 µL/mL) in dichloromethane were added in reaction mixture sequentially and stirred for additional 2 h. The completion of reaction was monitored with TLC. The reaction mixture was diluted with $CHCl_3$, filtered and washed with saturated aqueous solution of NH_4Cl and brine simultaneously. The organic layer was separated, dried over $MgSO_4$ and concentrated *in vacuo* to afford a residue. A portion of MeOH was added to get precipitate which was filtered and washed with MeOH to get Boc-SN-38 dimer (854 mg, 1.43 mmol, 71%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.17 (d, *J* = 9.2 Hz, 1H), 7.89 (d, *J* = 2.5 Hz, 1H), 7.66 (dd, *J* = 2.5, 9.2 Hz, 1H), 7.21 (s, 1H), 5.80 (d, *J* = 17.1 Hz, 1H), 5.35 (d, *J* = 17.2 Hz, 1H), 5.26 (d, *J* = 5.9 Hz, 2H), 4.01-3.87 (m, 2H), 3.16 (q, *J* = 7.6 Hz, 2H), 2.78 (t, *J* = 6.2 Hz, 2H), 2.26-2.03 (m, 2H), 1.61 (s, 9H), 1.40 (t, *J* = 7.6 Hz, 3H), 0.95 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 167.4, 157.3, 153.3, 152.1, 151.6, 150.1, 147.3, 147.3, 145.7, 145.6, 131.9, 127.7, 127.6, 125.4, 120.0, 114.4, 95.7, 84.5, 78.0, 67.2, 66.2, 49.5, 37.1, 32.0, 27.9, 23.3, 14.0, 7.7; HRMS (ESI-TOF): *m/z* calcd for $C_{60}H_{63}N_4O_{18}$ ($[M+H]^+$) 1191.3573, found 1191.3545.

Synthesis of SN-38C0 dimer 3

Boc-SN-38 dimer **3′** (500.0 mg, 0.42 mmol) was dissolved in 10% solution of TFA in dichloromethane (15 mL). The reaction mixture was stirred for 3.5 h at 25 °C. Completion of reaction was monitored with TLC. The reaction mixture was concentrated under vacuum to get yellow viscous liquid. A portion of MeOH was added to get yellow precipitate. The precipitate was sonicated, filtered, and washed with MeOH followed by $CHCl_3$, which was dried under vacuum to get SN-38C0 dimer (412 mg, 0.416 mmol, 99%) as a pale yellow solid. ^{1}H NMR (DMSO, 400 MHz): δ 10.33 (br s, 1H), 7.96 (d, *J* = 9.8 Hz, 1H), 7.37 (dd, *J* = 2.8, 7.3 Hz, 2H), 6.92 (s, 1H), 5.50 (d, *J* = 3.3 Hz, 2H), 5.53 (s, 2H), 4.16 (q, *J* = 5.3 Hz, 2H), 3.04 (q, *J* = 7.5 Hz, 2H), 2.94 (q, *J* = 5.5 Hz, 2H), 2.17-2.09 (m, 2H), 1.26 (t, *J* = 7.6 Hz, 3H), 0.88 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (DMSO, 100 MHz): δ 167.1, 156.8, 156.4, 152.7, 148.4,

147.2, 144.7, 143.5, 142.6, 131.4, 128.1, 127.8, 122.4, 118.1, 104.7, 93.4, 79.2, 77.8, 66.5, 66.1, 49.4, 36.2, 30.3, 22.2, 13.3, 7.5 HRMS (ESI-TOF): *m/z* calcd for $C_{50}H_{47}N_4O_{14}S_2$ ($[M+H]^+$) 991.2525, found 991.2494.

General method for synthesis of SN-38C5 dimers 9-17

SN-38C0 dimer **3** (200.0 mg, 0.202 mmol), EDC·HCl (84.3 mg, 0.440 mmol) and DMAP (4.89 mg, 0.040 mmol) were dissolved in CH_2Cl_2 (2.5 mL, 0.08 M) at room temperature. The corresponding carboxylic acid (0.501 mmol) was added, and reaction mixture and stirred at room temperature for 3.5 h. The reaction mixture was diluted with CH_2Cl_2 after completion the reaction, filtered and washed with saturated aqueous solution of NH_4Cl and brine simultaneously. The organic layer was separated, dried over $MgSO_4$ then concentrated under vacuum and purified by Si-gel column chromatography (0 to 2% MeOH in $CHCl_3$) to give corresponding SN-38C5 dimer.

(4*S*,4'*S*)-((((Disulfanediylbis(ethane-2,1-diyl))bis(oxy))bis(carbonyl))bis(oxy))bis(4,11-diethyl-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*]quinoline-4,9-diyl) bis(1-methylcyclo-propane-1-carboxylate) (**9**): Yellow solid; Yield 65%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (d, *J* = 9.2 Hz, 1H), 7.78 (d, *J* = 2.4 Hz, 1H), 7.51 (dd, *J* = 2.5, 9.2 Hz, 1H), 7.19 (s, 1H), 5.77 (d, *J* = 17.2 Hz, 1H), 5.33 (d, *J* = 17.1 Hz, 1H), 5.24 (d, *J* = 4.3 Hz, 2H), 3.99-3.87 (m, 2H), 3.13 (q, *J* = 7.6 Hz, 2H), 2.77 (t, *J* = 6.2 Hz, 2H), 2.23-2.03 (m, 2H), 1.51-1.47 (m, 5H), 1.38 (t, *J* = 7.7 Hz, 3H), 0.98-0.90 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 167.5, 157.4, 153.3, 152.0, 150.1, 147.3, 147.3, 145.7, 145.6, 131.8, 127.7, 125.8, 120.0, 114.8, 95.7, 78.0, 77.4, 67.3, 66.2, 49.5, 37.0, 32.0, 23.3, 19.6, 19.1, 18.0, 14.1, 7.7; HRMS (ESI–TOF): m/z $C_{60}H_{59}N_4O_{16}S_2$ ($[M + H]^+$) calcd. 1155.3362, found 1155.3345.

(4*S*,4'*S*)-((((Disulfanediylbis(ethane-2,1-diyl))bis(oxy))bis(carbonyl))bis(oxy))bis(4,11-diethyl-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*]quinoline-4,9-diyl) bis(2-methylcyclo-propane-1-carboxylate) (**10**): Yellow solid; Yield 71%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (d, *J* = 9.2 Hz, 1H), 7.81 (d, *J* = 2.4 Hz, 1H), 7.54 (dd, *J* = 2.5, 9.2 Hz, 1H), 7.19 (s, 1H), 5.77 (d, *J* = 17.1 Hz, 1H), 5.33 (d, *J* = 17.1 Hz, 1H), 5.23 (d, *J* = 4.4 Hz, 2H), 4.00-3.87 (m, 2H), 3.13 (q, *J* = 7.6 Hz, 2H), 2.77 (t, *J* = 6.2 Hz, 2H), 2.22-2.01 (m, 2H), 1.66-1.62 (m, 1H), 1.42-1.31 (m, 5H), 1.23 (d, *J* = 5.6 Hz, 3H), 0.96-0.91(m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 167.5, 157.4, 153.3, 152.0, 150.0, 147.4, 147.3, 145.7, 145.6, 131.8, 127.7, 125.8, 120.0, 114.9, 95.7, 78.0, 77.4, 67.3, 66.2, 49.5, 37.0, 32.0, 23.3, 21.5, 19.0, 18.2, 18.1, 14.1, 7.7; HRMS (ESI–TOF): m/z $C_{60}H_{59}N_4O_{16}S_2$ ($[M + H]^+$) calcd. 1155.3362, found 1155.3344.

(4*S*,4'*S*)-((((Disulfanediylbis(ethane-2,1-diyl))bis(oxy))bis(carbonyl))bis(oxy))bis(4,11-diethyl-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*]quinoline-4,9-diyl) (2*R*,2'*R*)-bis(2-methylbutanoate) (**11**): Yellow solid; Yield 71%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (d, *J* = 9.1 Hz, 1H), 7.79 (d, *J* = 2.4 Hz, 1H), 7.52 (dd, *J* = 2.5, 9.1 Hz, 1H), 7.19 (s, 1H), 5.77 (d, *J* = 17.2 Hz, 1H), 5.33 (d, *J* = 17.1 Hz, 1H), 5.24 (d, *J* = 5.0 Hz, 2H), 3.99-3.85 (m, 2H), 3.14 (q, *J* = 7.7 Hz, 2H), 2.77 (t, *J* = 6.2 Hz, 2H), 2.74-2.69 (m, 1H), 2.23-2.04 (m, 2H), 1.93-1.85 (m, 1H), 1.72-1.65 (m, 1H), 1.38 (t, *J* = 7.7 Hz, 3H), 1.35 (d, *J* = 7.0 Hz, 3H), 1.07 (t, *J* = 7.4 Hz, 3H), 0.94 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.3, 167.5, 157.4, 153.3, 152.1, 150.0, 147.4, 147.3, 145.7, 145.6, 131.9, 127.7, 125.7, 120.0, 114.8, 95.7, 78.0, 77.4, 67.3, 66.2, 49.6, 41.4, 37.1, 32.1, 27.0, 23.3, 16.7, 14.1, 11.9, 7.7; HRMS (ESI–TOF): m/z $C_{60}H_{63}N_4O_{16}S_2$ ([M + H]$^+$) calcd. 1159.3675, found 1159.3647.

(4*S*,4'*S*)-((((Disulfanediylbis(ethane-2,1-diyl))bis(oxy))bis(carbonyl))bis(oxy))bis(4,11-diethyl-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*]quinoline-4,9-diyl) bis(2-methylbutanoate) (**12**): Yellow solid; Yield 58%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, *J* = 9.1 Hz, 1H), 7.79 (d, *J* = 2.4 Hz, 1H), 7.53 (dd, *J* = 2.5, 9.2 Hz, 1H), 7.19 (s, 1H), 5.77 (d, *J* = 17.2 Hz, 1H), 5.33 (d, *J* = 17.1 Hz, 1H), 5.24 (d, *J* = 5.0 Hz, 2H), 3.99-3.86 (m, 2H), 3.14 (q, *J* = 7.7 Hz, 2H), 2.77 (t, *J* = 6.2 Hz, 2H), 2.72 (q, *J* = 6.9 Hz, 1H), 2.23-2.17 (m, 1H), 2.09-2.03 (m, 1H), 1.92-1.85 (m, 1H), 1.72-1.67 (m, 1H), 1.38 (t, *J* = 7.7 Hz, 3H), 1.35 (d, *J* = 7.0 Hz, 3H), 1.07 (t, *J* = 7.4 Hz, 3H), 0.94 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.3, 167.5, 157.4, 153.3, 152.1, 150.0, 147.4, 147.3, 145.7, 145.6, 131.9, 127.7, 125.7, 120.0, 114.8, 95.7, 78.0, 77.4, 67.3, 66.2, 49.6, 41.4, 37.1, 32.0, 26.9, 23.3, 16.7, 14.1, 11.8, 7.7; HRMS (ESI–TOF): m/z $C_{60}H_{63}N_4O_{16}S_2$ ([M + H]$^+$) calcd. 1159.3675, found 1159.3633.

(4*S*,4'*S*)-((((Disulfanediylbis(ethane-2,1-diyl))bis(oxy))bis(carbonyl))bis(oxy))bis(4,11-diethyl-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*]quinoline-4,9-diyl) dipentanoate (**13**): Yellow solid; Yield 58%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, *J* = 9.1 Hz, 1H), 7.80 (d, *J* = 2.4 Hz, 1H), 7.53 (dd, *J* = 2.5, 9.2 Hz, 1H), 7.19 (s, 1H), 5.78 (d, *J* = 17.1 Hz, 1H), 5.33 (d, *J* = 17.1 Hz, 1H), 5.24 (d, *J* = 5.1 Hz, 2H), 4.00-3.86 (m, 2H), 3.14 (q, *J* = 7.7 Hz, 2H), 2.77 (t, *J* = 6.2 Hz, 2H), 2.65 (t, *J* = 7.5 Hz, 2H), 2.22-2.17 (m, 1H), 2.19-2.04 (m, 1H), 1.83-1.75 (m, 2H), 1.51-1.47 (m, 2H), 1.38 (t, *J* = 7.7 Hz, 3H), 0.99 (t, *J* = 7.4 Hz, 3H), 0.95 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 167.5, 157.4, 153.3, 152.1, 149.9, 147.3, 147.3, 145.7, 145.6, 131.9, 127.7, 125.7, 120.0, 114.9, 95.7, 78.0, 77.4, 67.3, 66.2, 49.5, 37.1, 34.3, 32.0, 27.1, 23.3, 22.5, 14.1, 13.9, 7.7; HRMS (ESI–TOF): m/z $C_{60}H_{63}N_4O_{16}S_2$ ([M + H]$^+$) calcd. 1159.3675, found 1159.3642.

(4*S*,4'*S*)-((((Disulfanediylbis(ethane-2,1-diyl))bis(oxy))bis(carbonyl))bis(oxy))bis(4,11-diethyl-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*]quinoline-4,9-diyl) bis(3-methylbutanoate) (**14**): Yellow solid; Yield 56%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, *J* = 9.2 Hz, 1H), 7.79 (d, *J* = 2.4 Hz, 1H), 7.53 (dd, *J* = 2.5, 9.1 Hz, 1H), 7.19 (s, 1H), 5.78 (d, *J* = 17.2 Hz, 1H), 5.33 (d, *J* = 17.1 Hz, 1H), 5.24 (d, *J* = 5.2 Hz, 2H), 3.99-3.87 (m, 2H), 3.13 (q, *J* = 7.7 Hz, 2H), 2.77 (t, *J* = 6.2 Hz, 2H), 2.53 (d, *J* = 7.1 Hz, 2H), 2.33-2.17 (m, 2H), 2.09-2.04 (m, 1H), 1.38 (t, *J* = 7.7 Hz, 3H), 1.10 (d, *J* = 6.7 Hz, 6H), 0.94 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 167.5, 157.4, 153.3, 152.1, 149.8, 147.4, 147.3, 145.7, 145.6, 131.9, 127.7, 125.8, 120.0, 114.8, 95.8, 78.0, 77.4, 67.3, 66.2, 49.5, 43.5, 37.1, 32.0, 26.0, 23.3, 22.6, 14.1, 7.7; HRMS (ESI–TOF): m/z $C_{60}H_{63}N_4O_{16}S_2$ ([M + H]$^+$) calcd. 1159.3675, found 1159.3651.

(4*S*,4'*S*)-((((Disulfanediylbis(ethane-2,1-diyl))bis(oxy))bis(carbonyl))bis(oxy))bis(4,11-diethyl-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*]quinoline-4,9-diyl) bis(2,2-dimethyl-propanoate) (**15**): Yellow solid; Yield 58%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, *J* = 9.1 Hz, 1H), 7.79 (d, *J* = 2.4 Hz, 1H), 7.51

(dd, *J* = 2.5, 9.1 Hz, 1H), 7.19 (s, 1H), 5.78 (d, *J* = 17.1 Hz, 1H), 5.34 (d, *J* = 17.1 Hz, 1H), 5.26 (d, *J* = 5.0 Hz, 2H), 4.00-3.93 (m, 1H), 3.92-3.88 (m, 1H), 3.16 (q, *J* = 7.8 Hz, 2H), 2.78 (t, *J* = 6.3 Hz, 2H), 2.24-2.18 (m, 1H), 2.10-2.05 (m, 1H), 1.43 (s, 9H), 1.40 (t, *J* = 7.7 Hz, 3H), 0.95 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.1, 167.5, 157.3, 153.3, 152.0, 150.2, 147.3, 147.3, 145.7, 145.6, 131.8, 127.7, 125.7, 120.0, 114.7, 95.7, 78.0, 77.4, 67.2, 66.2, 49.5, 39.4, 37.0, 32.0, 27.3, 23.3, 14.1, 7.7; HRMS (ESI–TOF): m/z $C_{60}H_{63}N_4O_{16}S_2$ ($[M + H]^+$) calcd. 1159.3675, found 1159.3631.

(4*S*,4'*S*)-((((Disulfanediylbis(ethane-2,1-diyl))bis(oxy))bis(carbonyl))bis(oxy))bis(4,11-diethyl-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*]quinoline-4,9-diyl) bis(2-cyclo-propylacetate) (**16**): Yellow solid; Yield 60%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, *J* = 9.2 Hz, 1H), 7.85 (d, *J* = 2.4 Hz, 1H), 7.57 (dd, *J* = 2.5, 9.2 Hz, 1H), 7.19 (s, 1H), 5.79 (d, *J* = 17.1 Hz, 1H), 5.35 (d, *J* = 17.2 Hz, 1H), 5.27 (d, *J* = 5.4 Hz, 2H), 4.02-3.96 (s, 1H), 3.92-2.88 (s, 1H), 3.16 (q, *J* = 7.7 Hz, 2H), 2.79 (t, *J* = 6.2 Hz, 2H), 2.57 (d, *J* = 7.1 Hz, 2H), 2.23-2.19 (m, 1H), 2.11-2.07 (m, 1H), 1.40 (t, *J* = 7.7 Hz, 3H), 1.28-1.19 (m, 1H), 0.96 (t, *J* = 7.5 Hz, 3H), 0.71-0.66 (m, 2H), 0.33-0.31 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 167.5, 157.4, 153.3, 152.1, 149.9, 147.3, 145.7, 145.6, 131.9, 127.7, 127.7, 125.7, 120.0, 114.9, 95.8, 78.0, 77.4, 67.3, 66.2, 49.5, 39.7, 37.1, 32.0, 23.3, 14.1, 7.7, 7.0, 4.7; HRMS (ESI–TOF): m/z $C_{60}H_{59}N_4O_{16}S_2$ ($[M + H]^+$) calcd. 1155.3362, found 1155.3340.

(4*S*,4'*S*)-((((Disulfanediylbis(ethane-2,1-diyl))bis(oxy))bis(carbonyl))bis(oxy))bis(4,11-diethyl-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*]quinoline-4,9-diyl) dicyclobutanecarboxylate (**17**): Yellow solid; Yield 65%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (d, *J* = 9.2 Hz, 1H), 7.81 (d, *J* = 2.4 Hz, 1H), 7.53 (dd, *J* = 2.5, 9.2 Hz, 1H), 7.19 (s, 1H), 5.77 (d, *J* = 17.1 Hz, 1H), 5.33 (d, *J* = 17.1 Hz, 1H), 5.23 (d, *J* = 4.6 Hz, 2H), 3.99-3.95 (m, 1H), 3.92-3.87 (m, 1H), 3.49-3.44 (m, 1H), 3.13 (q, *J* = 7.6 Hz, 2H), 2.77 (t, *J* = 6.2 Hz, 2H), 2.54-2.44 (m, 2H), 2.42-2.33 (m, 2H), 2.22-1.98 (m, 4H), 1.38 (t, *J* = 7.7 Hz, 3H), 0.94 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.0, 167.5, 157.4, 153.3, 152.1, 150.0, 147.4, 147.3, 145.7, 145.6, 131.9, 127.7, 125.7, 120.0, 114.8, 95.7, 78.0, 77.4, 67.3, 66.2, 49.5, 38.3, 37.0, 32.0, 25.5, 25.5, 23.3, 18.6, 14.1, 7.7; HRMS (ESI–TOF): m/z $C_{60}H_{59}N_4O_{16}S_2$ ($[M + H]^+$) calcd. 1155.3362, found 1155.3331.

Fabrication and characterization of nano-prodrugs

The nano-prodrugs of synthesized SN-38C5 dimers **9-17** were fabricated using the reprecipitation method. A solution containing 5 mM of the SN-38C5, which is equivalent to 10 mM of SN-38, was prepared using THF. A volume of 100 μL from this solution was swiftly injected into 10 mL of vigorously stirred deionized water at room temperature, utilizing a micro-syringe. The dispersion of the nanoparticles within the colloidal solution was analyzed using light scattering methods. The morphology and size of the produced nanoparticles were characterized via field emission scanning electron microscopy (FE-SEM). Additionally, the size distribution and zeta potential were measured at a temperature of 25 °C.

***In vitro* cytotoxicity assay**

HCT-116, MCF-7 and A549 cells were cultured in respective culture media under a controlled environment of 5% CO_2 at 37 °C. A volume of 100 μL of the cultured cells was dispensed into 96-well plates at a density of 2×10^4 cells per well. These plates were then incubated under a controlled environment of 5% CO_2 at 37 °C for a duration of 24 h. Subsequently, the supernatant in each well was replaced with 100 μL of a solution (0.04–10 μM based on the SN-38 concentration) containing nano-prodrugs (**9-17**), SN-38, and irinotecan, and the cells were incubated for an additional 48 h. The viability of the cells was assessed using the Cell Counting Kit-8 in conjunction with a microplate reader. The cell viability values were normalized to OD_{450}–OD_{620} for the untreated cells. All assays were conducted in triplicate.

^{1}H, ^{13}C NMR and HRMS spectra of synthesized compounds

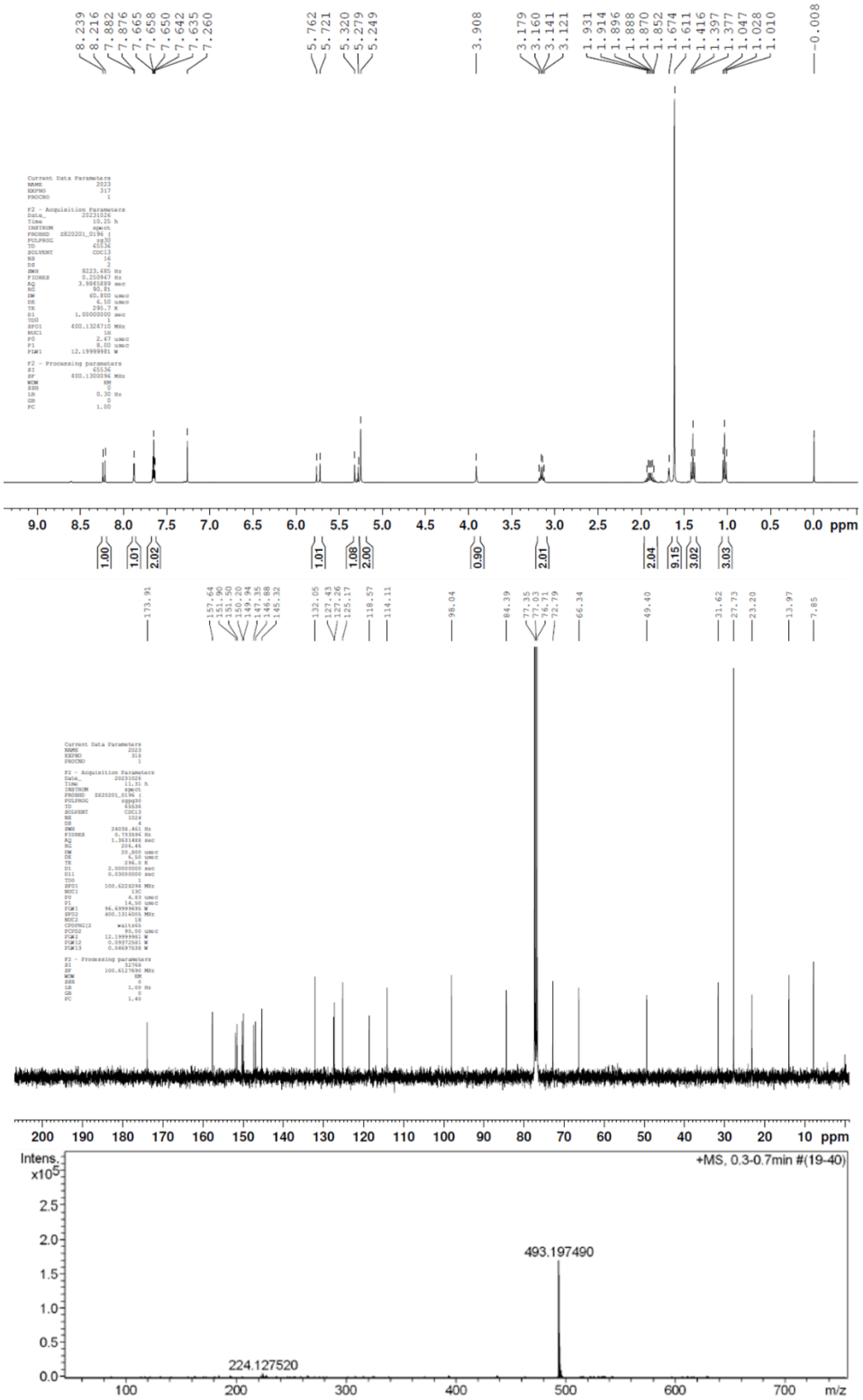

Figure S1. ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) NMR and HRMS spectra of compound Boc-SN38 **1′**.

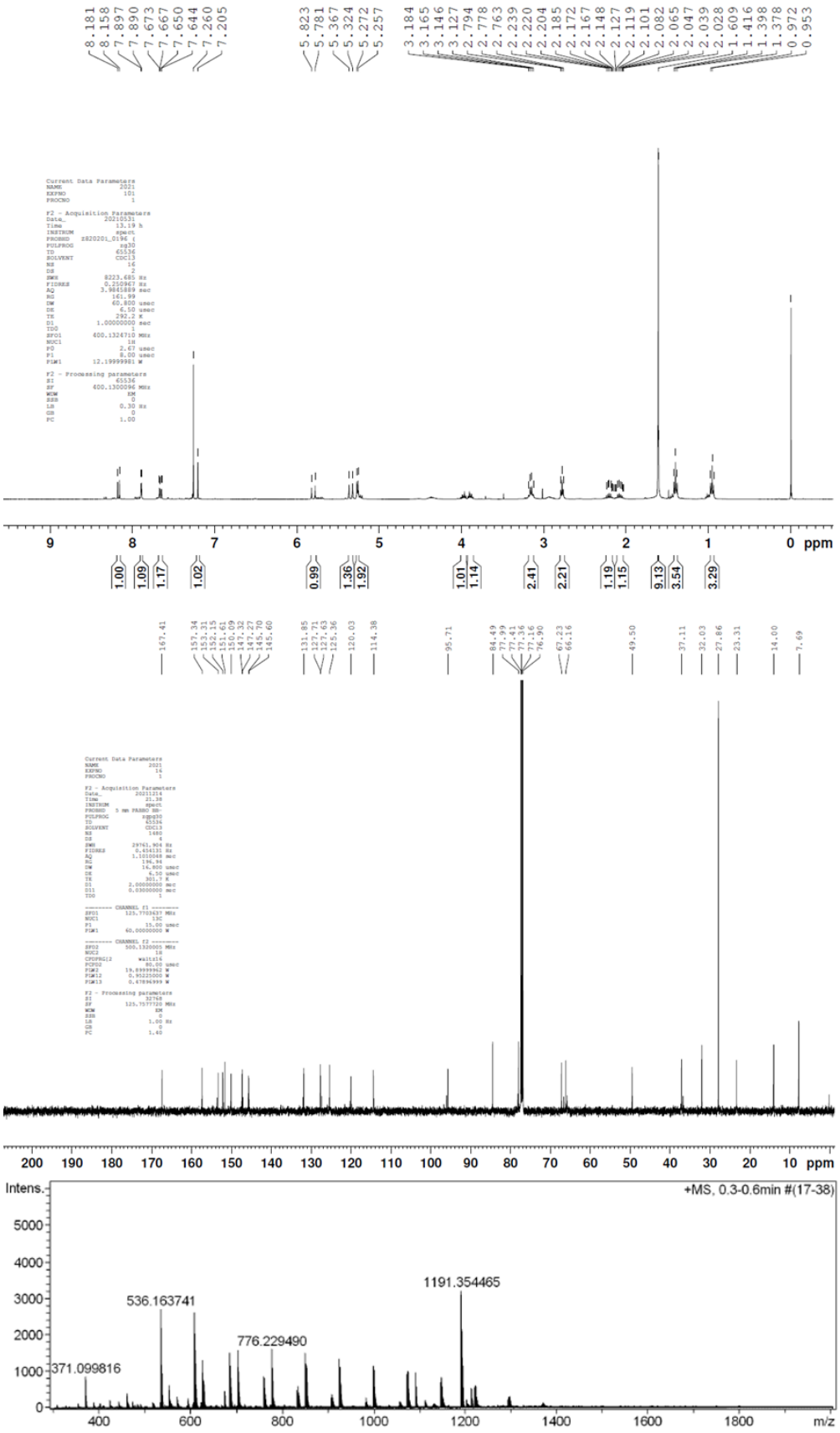

Figure S2. ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) NMR and HRMS spectra of compound Boc-SN38 dimer **3′**.

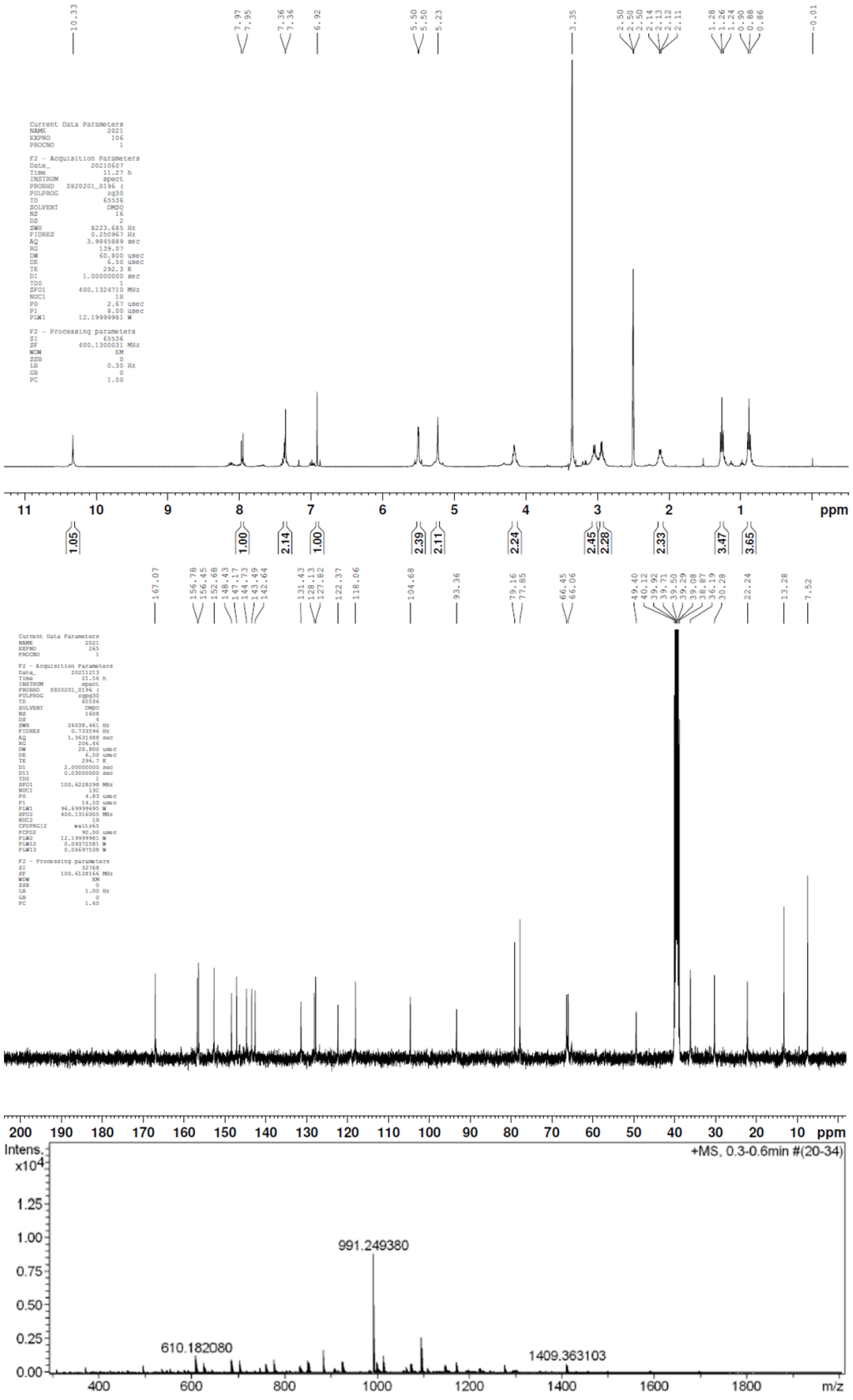

Figure S3. ^{1}H (DMSO, 400 MHz), ^{13}C (DMSO, 100 MHz) NMR and HRMS spectra of compound SN-38C0 dimer **3**.

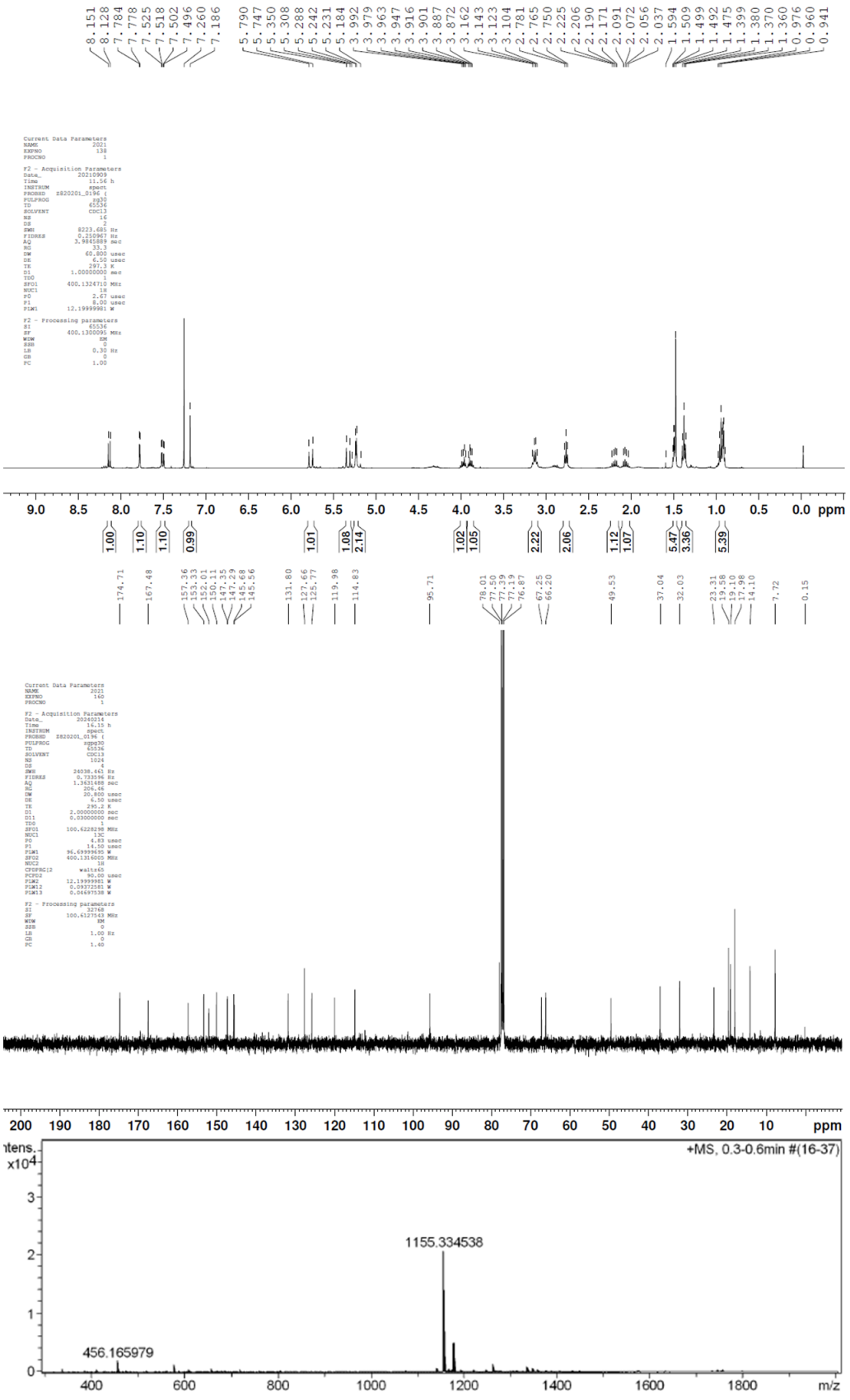

Figure S4. ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) NMR and HRMS spectra of compound **9**.

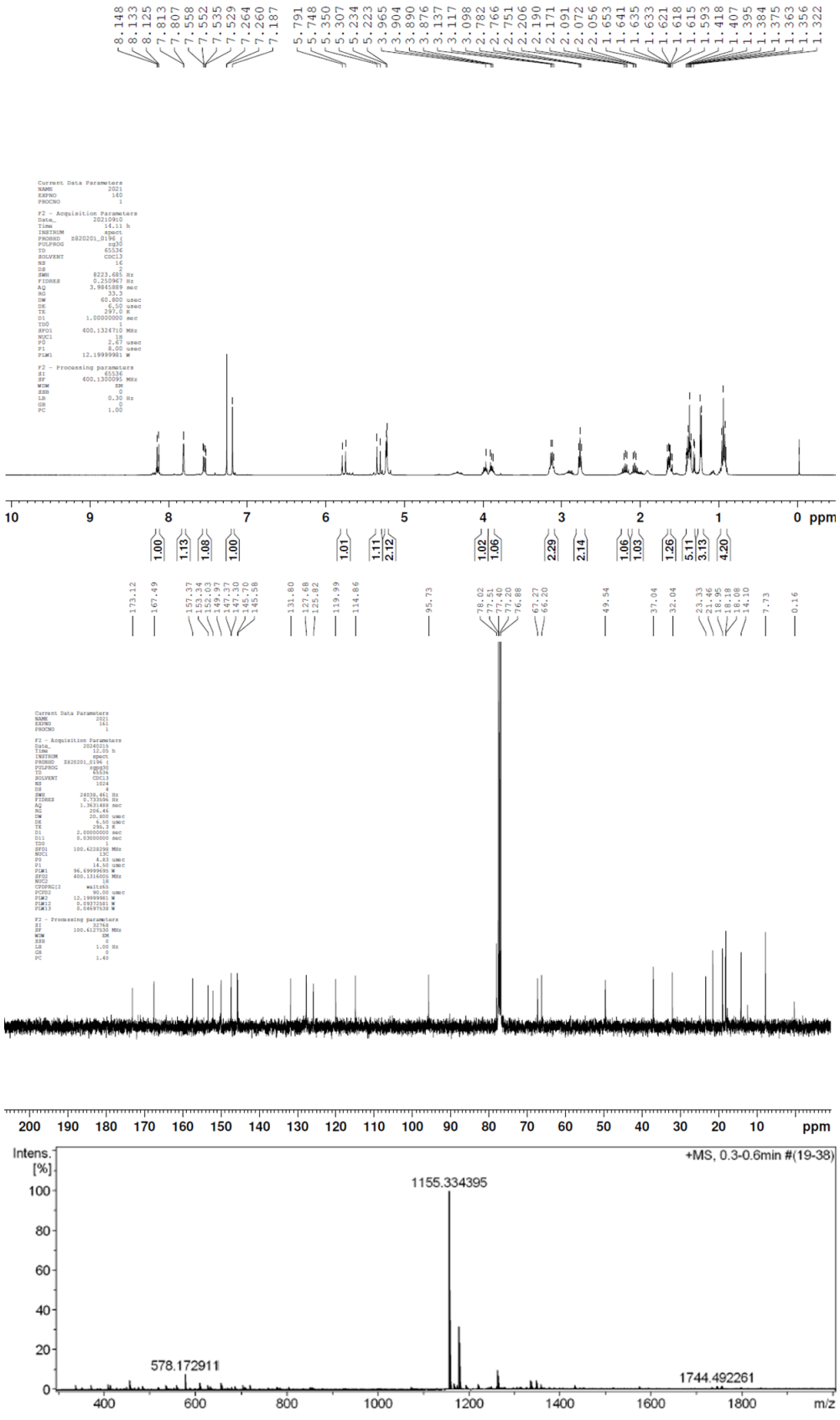

Figure S5. ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) NMR and HRMS spectra of compound **10**.

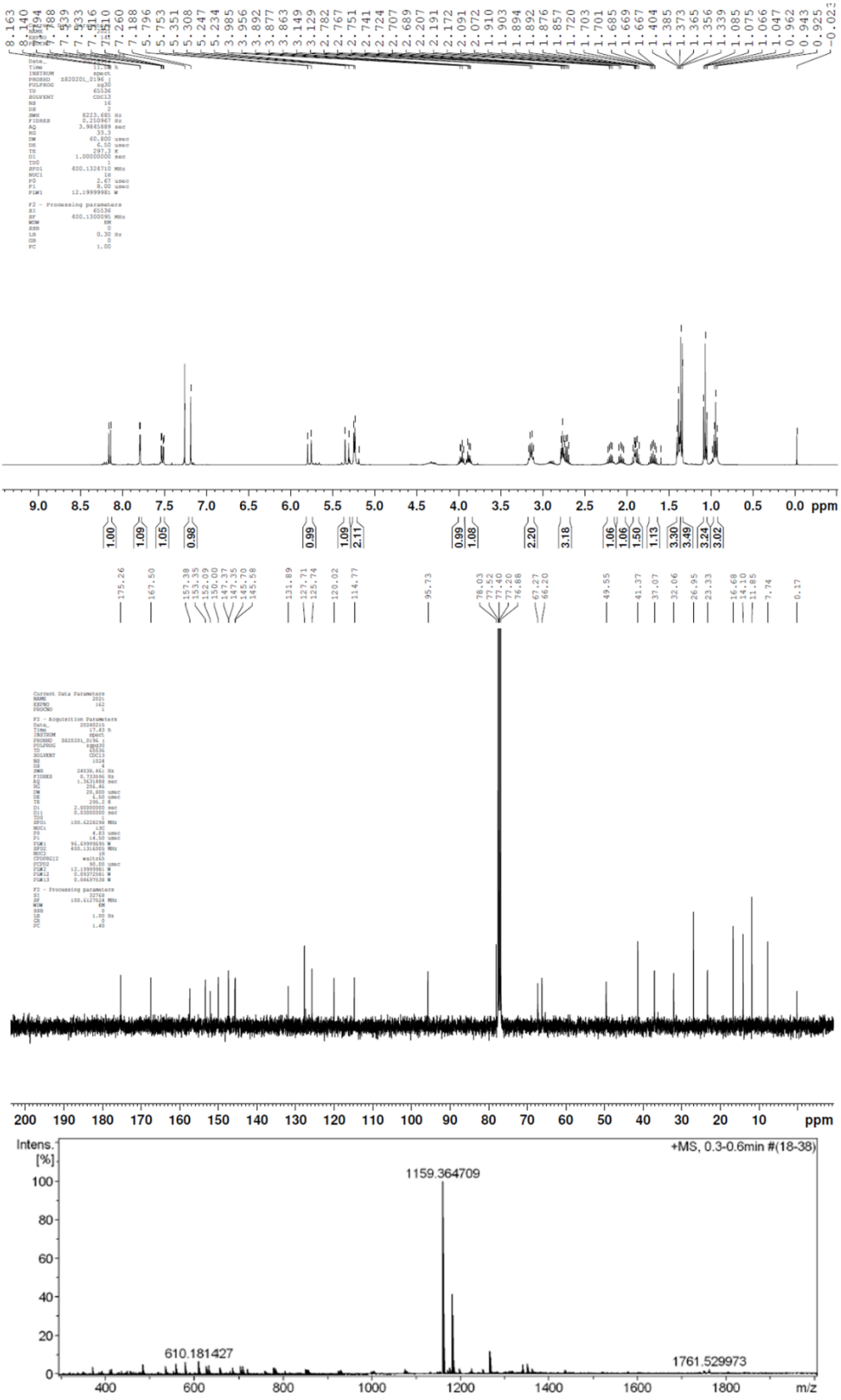

Figure S6. ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) NMR and HRMS spectra of compound **11**.

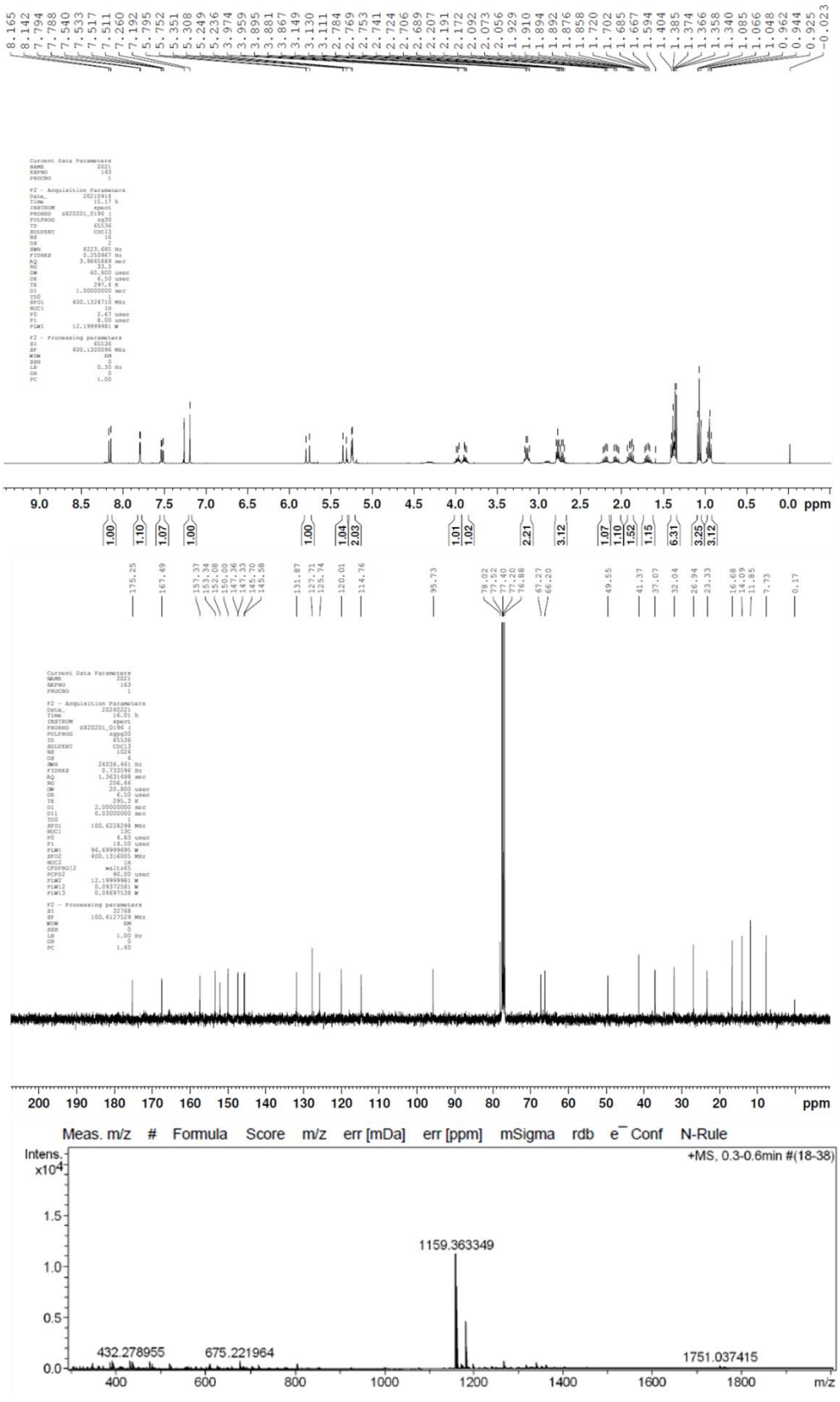

Figure S7. ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) NMR and HRMS spectra of compound **12**.

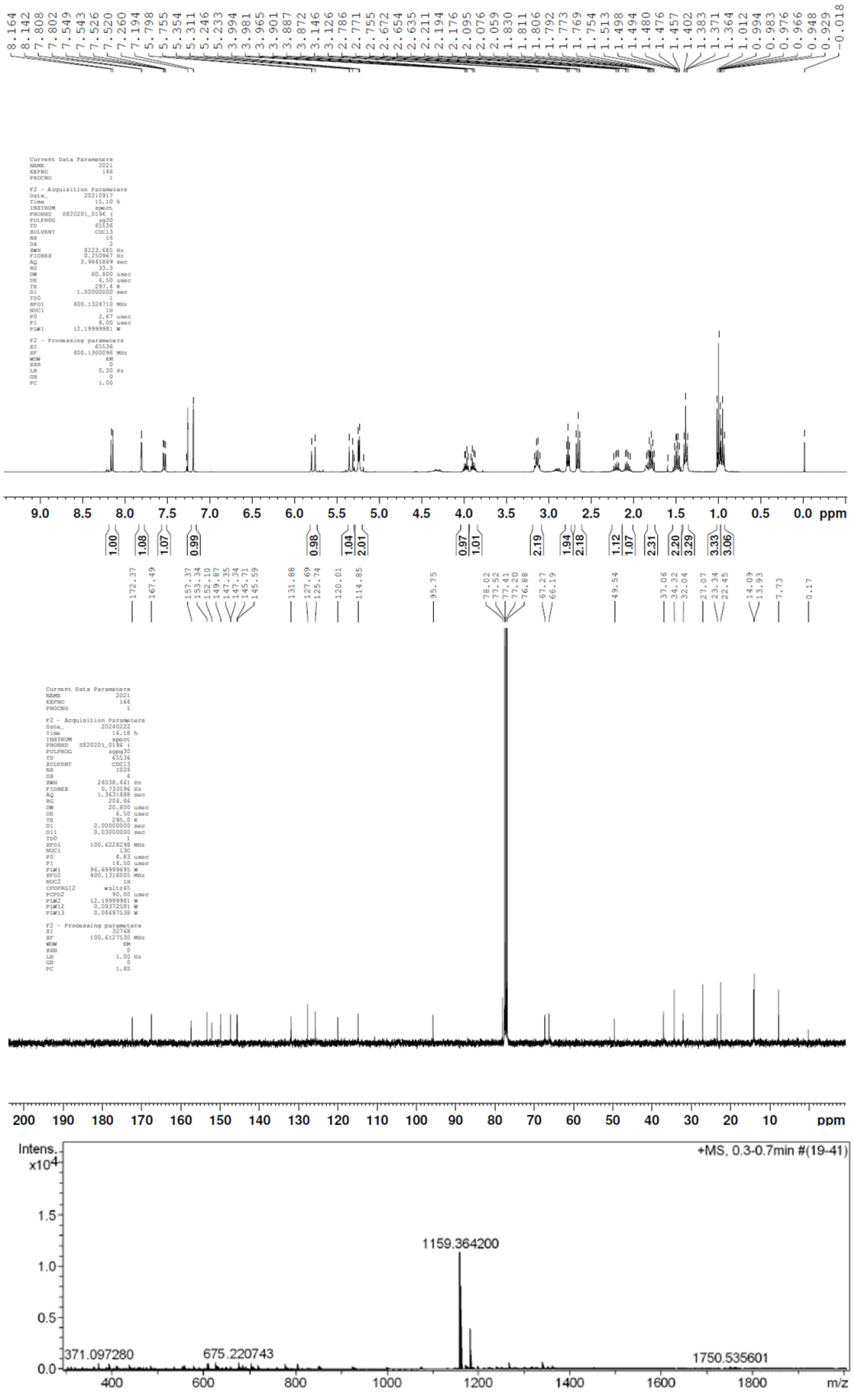

Figure S8. ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) NMR and HRMS spectra of compound **13**.

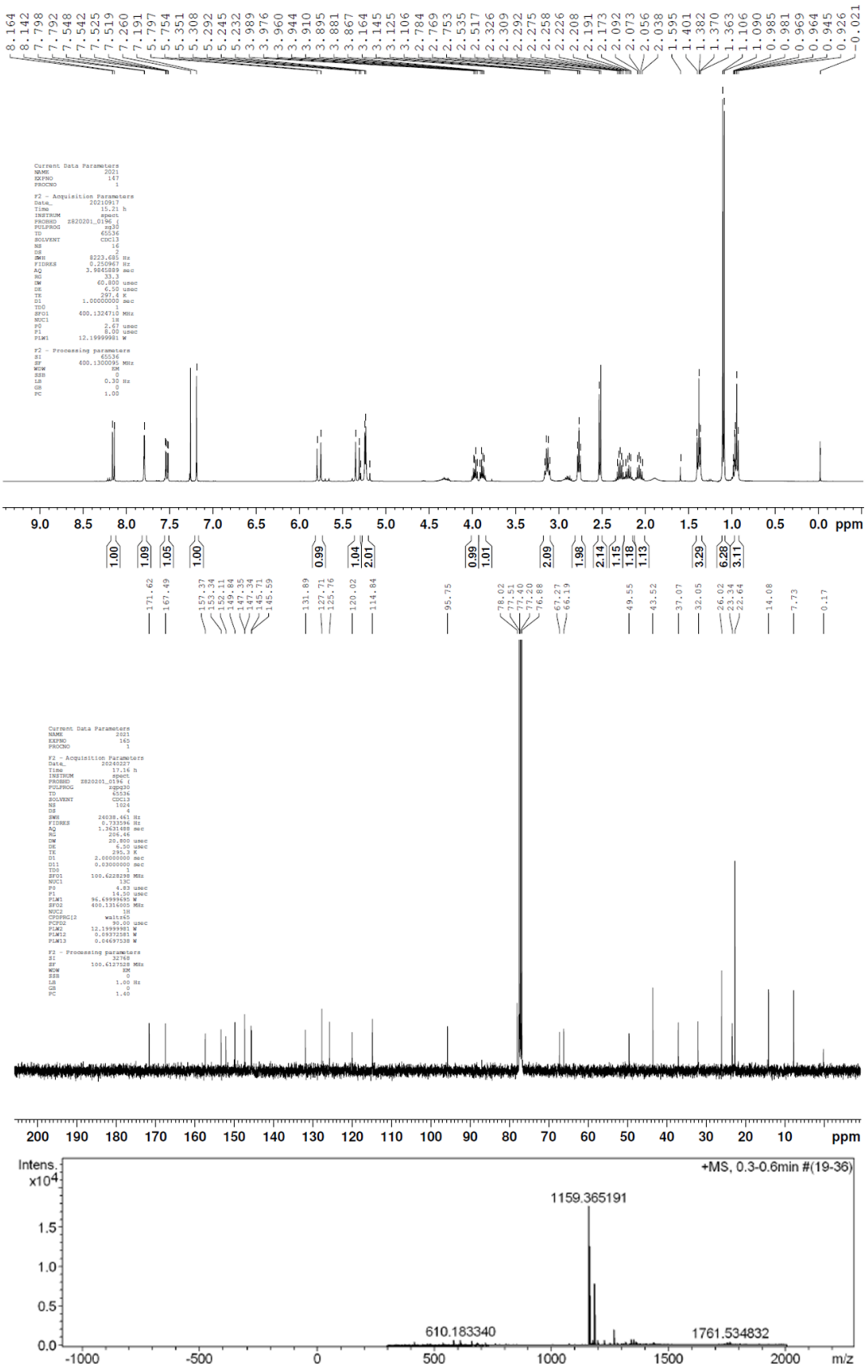

Figure S9. ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) NMR and HRMS spectra of compound **14**.

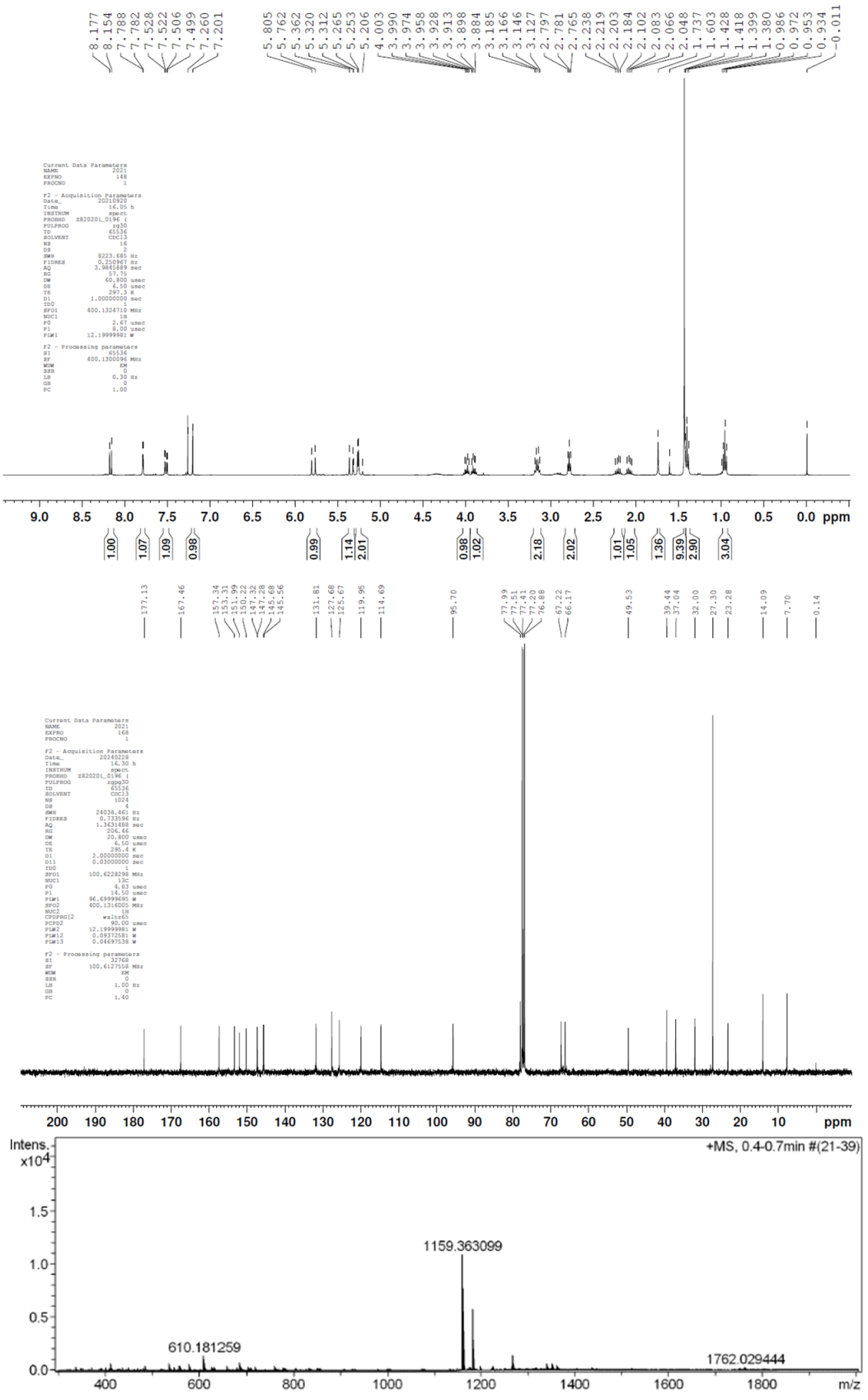

Figure S10. ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) NMR and HRMS spectra of compound **15**.

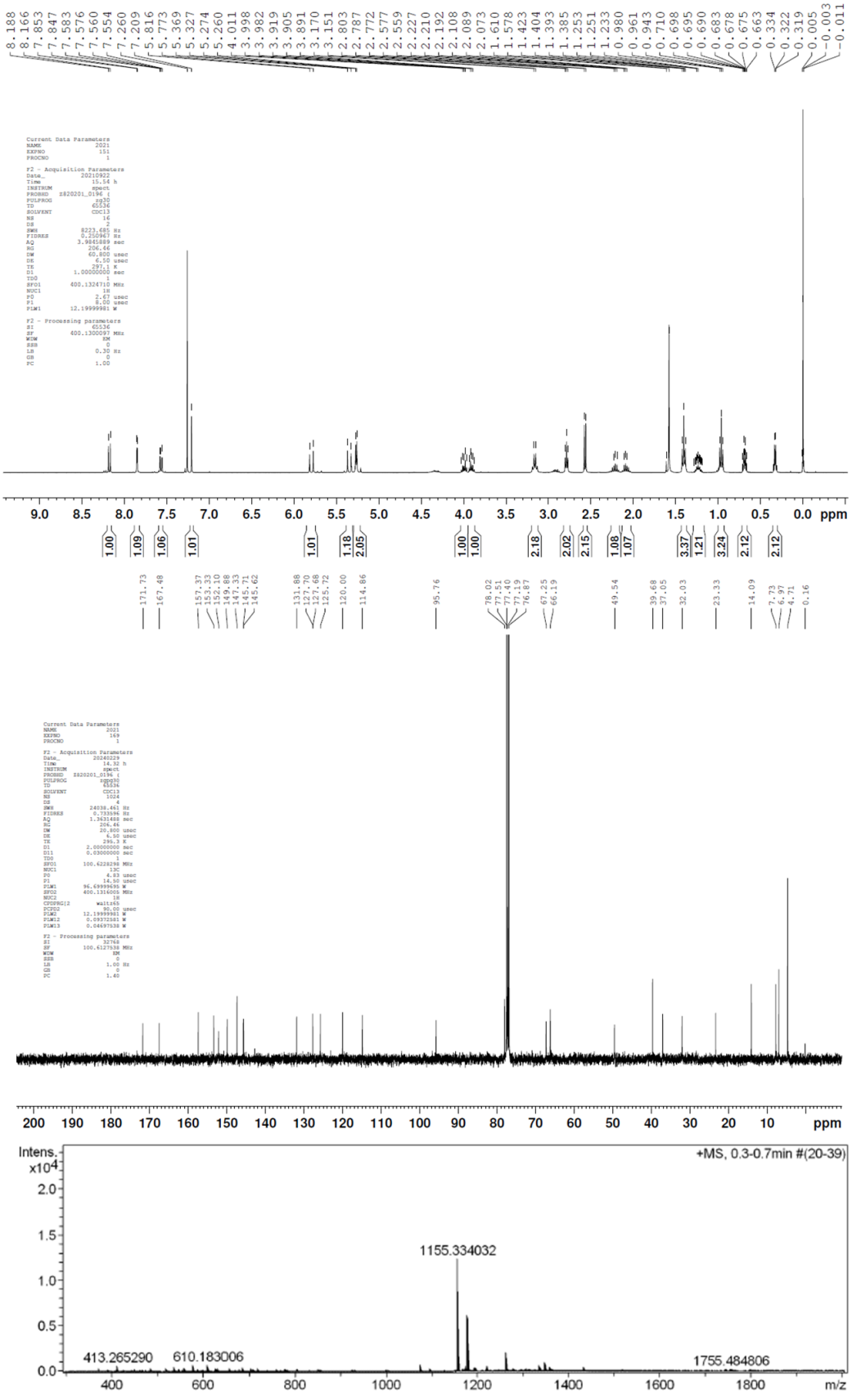

Figure S11. ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) NMR and HRMS spectra of compound **16**.

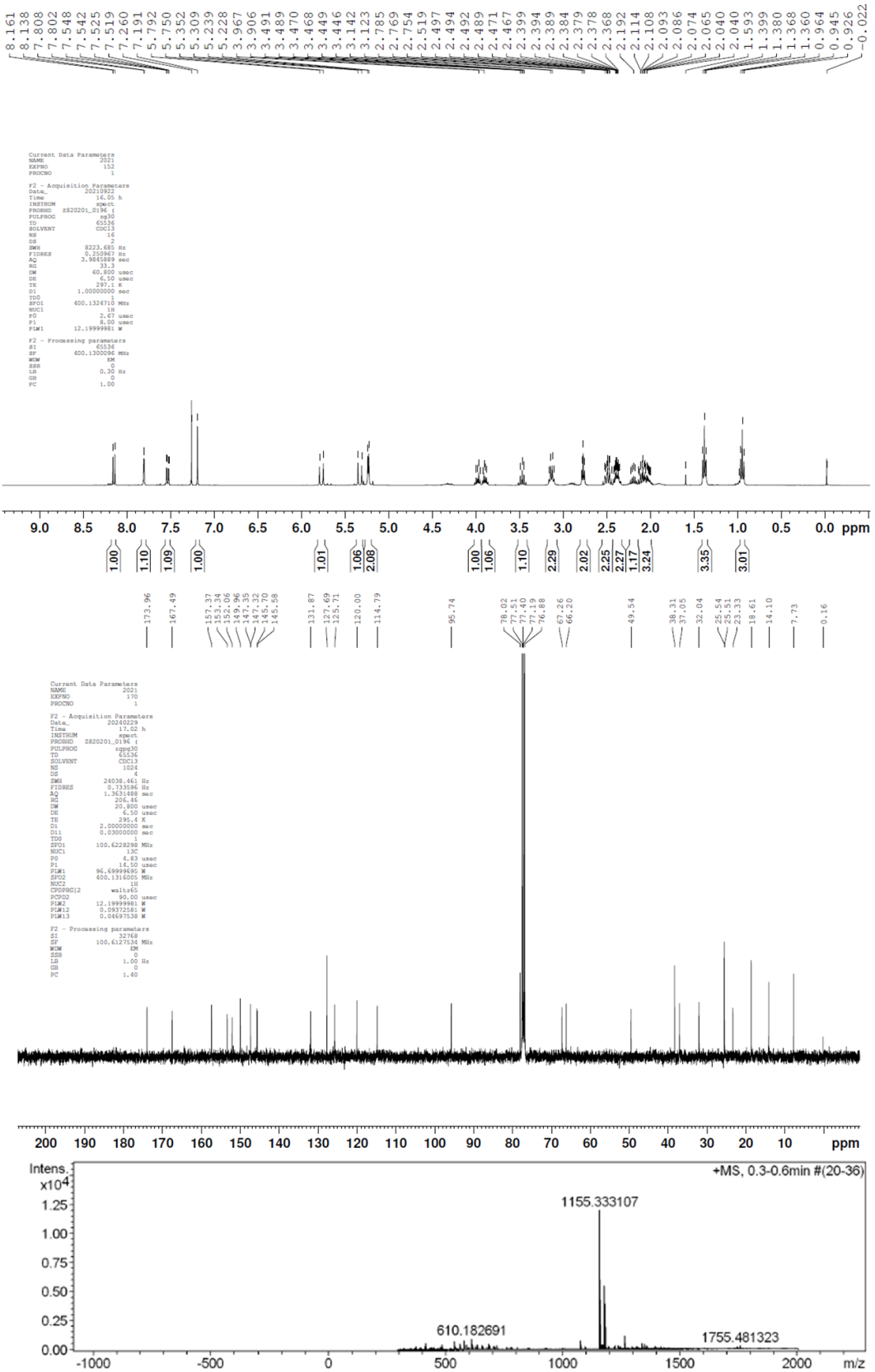

Figure S12. ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) NMR and HRMS spectra of compound **17**.