Supplementary Data

Molecular insight into Mycobacterium tuberculosis resistance to nitrofuranyl amides gained through metagenomics-like analysis of spontaneous mutants

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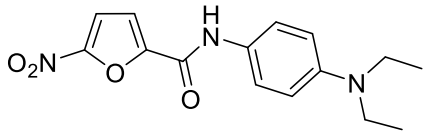
1 Chemistry and NMR – general

For thin layer chromatography (TLC) aluminum sheets pre-coated with silica gel 60 F_{254} (Merck) were used. Flash column chromatography was carried out using silica gel 60 (0.040–0.063 mm, 230-400 mesh ASTM, Merck). Commercially available solvents for reactions, TLC and column chromatography were used after distillation (and were dried if needed) – water, petroleum ether (PE), dichloromethane (DCM) and methyl *tert*-butyl ether (MTBE). Any fine chemical, whose synthesis is not described or mentioned in the references in this study, is commercially available from Sigma-Aldrich, Fluorochem, Fluka, Acros or Alfa Aesar. Melting temperatures were determined in capillary tubes on an Electrothermal MEL-TEMP 1102D-230 VAC apparatus without corrections. The NMR spectra were recorded on a Bruker Avance II+ 600 spectrometer (600.13 MHz for ^{1}H and 150.92 MHz for ^{13}C) and Bruker Neo 600 spectrometer (600.18 MHz for ^{1}H and 150.92 MHz for ^{13}C). In case of $CDCl_3$, TMS was used as internal standard (δ=0.00). For other deuterated solvents ^{1}H spectra were calibrated to the residual solvent peaks (DMSO-d_6 δ=2.50). ^{13}C spectra were calibrated in all cases to the residual solvent peaks ($CDCl_3$ δ=77.00, DMSO-d_6 δ=39.52). ^{1}H and ^{13}C NMR data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), integration, identification, and coupling constants (in Hz). Singlets in ^{13}C NMR spectra were not mentioned with “s” in all cases. Mass spectra (MS) were recorded on a Shimadzu Liquid Chromatograph Mass Spectrometer LCMS-2020. Direct MS-regime was applied; each compound was dissolved in MeOH with concentration 1 mg/ml; injection volume 3-5 µl; mobile phase – 1% formic acid in MeOH; MS detector with electrospray ionization (ESI). MS spectra are reported as fragmentation in m/z with relative intensities (%). The compound names are in agreement with the IUPAC nomenclature.

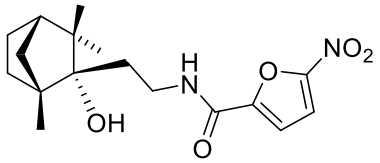
1.1 Synthesis of target compounds

1.1.1 Synthesis of *N*-(4-(diethylamino)phenyl)-5-nitrofuran-2-carboxamide (**8**)



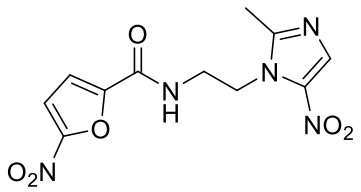
Compound **2** (0.337 g, 2.05 mmol, 1.2 eq.) and Et_3N were dissolved in 20 ml dry DCM. The solution was cooled to 0°C and acyl chloride **1** (0.300 g, 1.71 mmol, 1.0 eq.) was added at once as a solid. The mixture was stirred at r.t. for 18 h (TLC – DCM:MTBE=20:1), then washed with 1% aq. citric acid and water. The organic phase was dried over anhydr. Na_2SO_4 and evaporated. Column chromatography: 1) DCM; 2) DCM:MTBE=20:1 – for product. After column the solid product was washed with hot PE and dried *in vacuo* to give 0.423 g (82%) of pure **8** as red crystals. M.p. 151-152°C. ^{1}H NMR (600.13 MHz, DMSO-d_6, 298 K): δ = 10.36 (br s, 1H, N<u>H</u>), 7.80 (d, 1H, furane C<u>H</u>$_{arom.}$, *J* = 3.9 Hz), 7.56 (d, 1H, furane C<u>H</u>$_{arom.}$, *J* = 3.9 Hz), 7.50 (m, 2H, 2xC<u>H</u>$_{arom.}$), 6.66 (m, 2H, 2xC<u>H</u>$_{arom.}$), 3.32 (q, 4H, 2xC<u>H</u>$_2$, *J* = 7.0 Hz), 1.01 (t, 6H, 2xC<u>H</u>$_3$, *J* = 7.0 Hz). ^{13}C NMR (150.92 MHz, DMSO-d_6, 298 K): δ = 153.84 (1C, <u>C</u>=O), 151.61 (1C, quaternary C$_{arom.}$), 148.61 (1C, quaternary C$_{arom.}$), 144.82 (1C, quaternary C$_{arom.}$), 126.08 (1C, quaternary C$_{arom.}$), 122.47 (2C, <u>C</u>H$_{arom.}$), 115.74 (1C, <u>C</u>H$_{arom.}$), 113.63 (1C, <u>C</u>H$_{arom.}$), 111.50 (2C, <u>C</u>H$_{arom.}$), 43.74 (2C, N-C<u>H</u>$_2$), 12.44 (2C, C<u>H</u>$_3$). MS (ESI+) m/z (rel. int. %): 304 (M+H, 100), 358 (M+Na+MeOH, 98). (ESI-) m/z (rel. int. %): 302 (M-H, 100). Anal. calcd. for $C_{15}H_{17}N_3O_4$ (303.32): C, 59.40; H, 5.65; N, 13.85. Found: C, 59.47; H, 5.60; N, 13.89 %.

1.1.2 Synthesis of *N*-(2-((1*R*,2*R*,4*S*)-2-hydroxy-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl)ethyl)-5-nitrofuran-2-carboxamide (**9**)



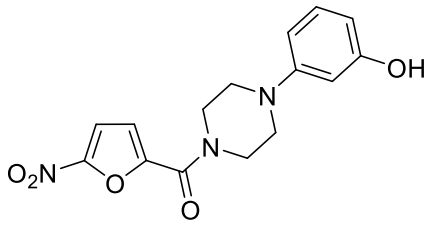
Aminoalcohol **3** (0.340 g, 1.72 mmol, 1.1 eq.) and Et_3N (0.33 ml, 2.35 ml, 1.5 eq.) were dissolved in 20 ml dry DCM and cooled to 5°C. Then acyl chloride **1** was added at once and stirred for 20 h at r.t. Reaction mixture was washed with 1% aq. citric acid and water, dried over anhydr. Na_2SO_4 and evaporated. Column chromatography: 1) DCM; 2) DCM:MTBE = 50:1 – for product. After column the solid product was washed with hot PE and dried *in vacuo* to give pure **9** (0.345 g, 65%) as pale yellow crystals. M.p. 134-135°C. ^{1}H NMR (600.13 MHz, $CDCl_3$, 298 K): δ = 7.36 (d, 1H, furane C<u>H</u>, *J* = 3.8 Hz), 7.30 (apparent br. s, 1H, N<u>H</u>), 7.23 (d, 1H, furane C<u>H</u>, *J* = 3.8 Hz), 3.75 (m, 1H, $CH_2C\underline{H}_2NH$), 3.53 (m, 1H, $CH_2C\underline{H}_2NH$), 2.08 (br s, 1H, O<u>H</u>), 1.98 (m, 1H, $C\underline{H}_2CH_2NH$), 1.75-1.85 (m, 2H, C<u>H</u>$_2$), 1.71 (m, 1H, C<u>H</u>$_2$), 1.66 (m, 1H, C<u>H</u>), 1.60 (m, 1H, C<u>H</u>$_2$), 1.47 (tt, 1H, C<u>H</u>$_2$, *J* = 12.8, 4.7 Hz), 1.11-1.16 (m, 2H, C<u>H</u>$_2$), 1.09 (s, C<u>H</u>$_3$), 1.04 (s, C<u>H</u>$_3$), 1.02 (s, C<u>H</u>$_3$). ^{13}C NMR (150.92 MHz, $CDCl_3$, 298 K): δ = 156.10 (1C, <u>C</u>=O), 151.12 (1C, quaternary furane C), 148.41 (1C, quaternary furane C), 115.61 (1C, furane C<u>H</u>), 112.47 (1C, furane C<u>H</u>), 81.80 (1C, quaternary aliphatic <u>C</u>-OH), 52.72 (1C, quaternary aliphatic C), 49.75 (1C, aliphatic <u>C</u>H), 44.21 (1C, quaternary aliphatic C), 40.90 (1C, <u>C</u>H$_2$), 36.97 (1C, $CH_2\underline{C}H_2NH$), 35.43 (1C, <u>C</u>H$_2$), 30.23 (1C, <u>C</u>H$_2$), 27.88 (1C, <u>C</u>H$_3$), 24.78 (1C, <u>C</u>H$_2$), 22.54 (1C, <u>C</u>H$_3$), 18.00 (1C, <u>C</u>H$_3$). MS (ESI+) m/z (rel. int. %): 376 (M+H+K, 72), 391 (M+Na+MeOH, 56). MS (ESI-) m/z (rel. int. %): 335 (M-H, 100), 381 (M+HCOO, 18). Anal. calcd. for $C_{17}H_{24}N_2O_5$ (336.39): C, 60.70; H, 7.19; N, 8.33. Found: C, 60.66; H, 7.24; N, 8.38%.

1.1.3 Synthesis of *N*-(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl)-5-nitrofuran-2-carboxamide (**10**)



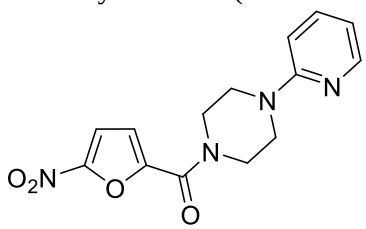
Compound **4** (as dihydrochloride salt, 0.670 g, 2.56 mmol, 1.5 eq.) was suspended in 25 ml dry DCM. Then Et_3N (1.07 ml, 7.70 mmol, 5.4 eq.) and mol. sieve (4Å) were added, cooled to 5°C and stirred for 30 min until clear solution was obtained. Then acyl chloride **1** (0.300 g, 1.71 mmol, 1.0 eq.) was added at once and stirred for 20 h at r.t. Reaction mixture was diluted with 100 ml $CHCl_3$ and filtered through celite pad. The organic phase was washed with water, checked by TLC (DCM:MTBE = 5:1), dried over anhydr. K_2CO_3 and evaporated. The crude product was purified by column chromatography – 1) DCM; 2) DCM:MTBE = 4:1 for product. After column the solid product was washed with hot PE and dried *in vacuo* to give 0.222 g (42%) of pure **10** as pale yellow crystals. M.p. 205-206°C. ^{1}H NMR (600.13 MHz, DMSO-d_6, 298 K): δ = 9.08 (t, 1H, N<u>H</u>, *J* = 6.1 Hz), 8.04 (s, 1H, imidazole C<u>H</u>), 7.74 (d, 1H, furane C<u>H</u>, *J* = 3.9 Hz), 7.35 (d, 1H, furane C<u>H</u>, *J* = 3.9 Hz), 4.43 (t, 2H, NH-CH_2-C<u>H_2</u>, *J* = 5.9 Hz), 3.65 (q, 2H, NH-C<u>H_2</u>-CH_2, *J* = 6.1 Hz), 2.38 (s, 3H, C<u>H_3</u>). ^{13}C NMR (150.92 MHz, DMSO-d_6, 298 K): δ = 156.47 (1C, <u>C</u>=O), 151.54 (1C, quaternary $C_{arom.}$), 151.35 (1C, quaternary $C_{arom.}$), 147.64 (1C, quaternary $C_{arom.}$), 138.62 (1C, quaternary $C_{arom.}$), 133.24 (1C, imidazole <u>C</u>H), 115.91 (1C, furane <u>C</u>H), 113.44 (1C, furane <u>C</u>H), 45.16 (1C, NH-CH_2-<u>C</u>H_2), 38.27 (1C, NH-<u>C</u>H_2-CH_2), 13.81 (1C, <u>C</u>H_3). MS (ESI+) m/z (rel. int. %): 364 (M+Na+MeOH, 100), 310 (M+H, 26). (ESI-) m/z (rel. int. %): 308 (M-H, 100). Anal. calcd. for $C_{11}H_{11}N_5O_6$ (309.24): C, 42.72; H, 3.59; N, 22.65. Found: C, 42.79; H, 3.64; N, 22.60%.

1.1.4 Synthesis of (4-(3-hydroxyphenyl)piperazin-1-yl)(5-nitrofuran-2-yl)methanone (**11**)



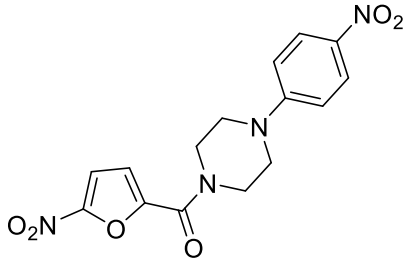
Compound **5** (0.384 g, 2.05 mmol, 1.2 eq.) was suspended in 25 ml dry DCM. Et_3N (0.54 ml, 3.85 mmol, 2.3 eq.) was added and cooled to 5°C. Then acylchloride **1** (0.300 g, 1.71 mmol, 1.0 eq.) was added at once and stirred for 48 h at r.t. Reaction mixture was diluted with DCM and filtered through celite pad. The filtrate was washed with water, dried over anhydr. Na_2SO_4 and evaporated. Crude product was purified by column chromatography – 1) DCM; 2) DCM:MTBE = 10:1 – for product. The solid product was washed with PE and dried in vacuo to give 0.135 g (25%) of pure **11** as pale yellow crystals. M.p. 179-180°C. ^{1}H NMR (600.18 MHz, DMSO-d_6, 353 K): δ = 8.89 (s, 1H, O<u>H</u>), 7.69 (d, 1H, furane C<u>H</u>, *J* = 3.8 Hz), 7.26 (d, 1H, furane C<u>H</u>, *J* = 3.8 Hz), 7.01 (t, 1H, aromatic C<u>H</u>, *J* = 8.1 Hz), 6.41 (m, 1H, aromatic C<u>H</u>), 6.36 (t, 1H, aromatic C<u>H</u>, *J* = 2.3 Hz), 6.27 (m, 1H, aromatic C<u>H</u>), 3.81 (m, 4H, piperazine 2xC<u>H_2</u>), 3.21 (m, 4H, piperazine 2xC<u>H_2</u>). ^{13}C NMR (150.92 MHz, DMSO-d_6, 353 K): δ = 157.85 (1C, <u>C</u>=O), 156.37 (1C, quaternary $C_{arom.}$), 151.50 (1C, quaternary $C_{arom.}$), 150.85 (1C, quaternary $C_{arom.}$), 147.51 (1C, quaternary $C_{arom.}$), 129.15 (1C, aromatic <u>C</u>H), 116.48 (1C, furane <u>C</u>H), 112.23 (1C, furane <u>C</u>H), 106.58 (1C, aromatic <u>C</u>H), 106.51 (1C, aromatic <u>C</u>H), 102.71 (1C, aromatic <u>C</u>H), 48.04 (2C, piperazine 2x<u>C</u>H_2), 43.74 (br s, 2C, piperazine 2x<u>C</u>H_2). MS (ESI+) m/z (rel. int. %): 318 (M+H, 16), 340 (M+Na, 98), 372 (M+Na+MeOH, 100). MS (ESI-) m/z (rel. int. %): 316 (M-H, 100), 362 (M+HCOO, 73), 633 (2M-H, 52). Anal. calcd. for $C_{15}H_{15}N_3O_5$ (317.30): C, 56.78; H, 4.77; N, 13.24. Found: C, 56.83; H, 4.79; N, 13.20%.

1.1.5 Synthesis of (5-nitrofuran-2-yl)(4-(pyridin-2-yl)piperazin-1-yl)methanone (**12**)



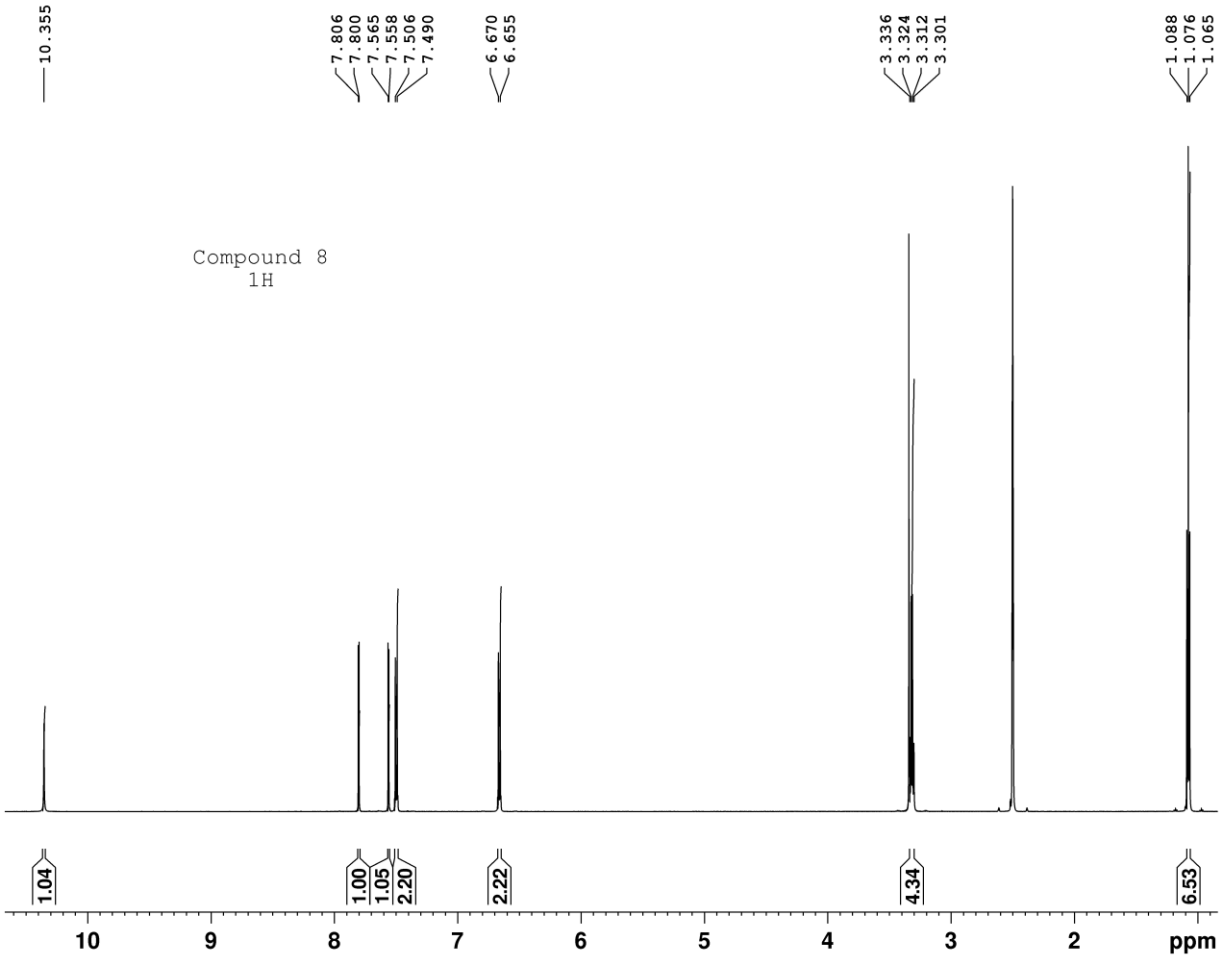
Compound **6** (0.334 g, 2.05 mmol, 1.15 eq.) was dissolved in 25 ml dry DCM. Et_3N (0.54 ml, 3.85 mmol, 2.1 eq.) was added and cooled to 5°C. Then acylchloride **1** (0.317 g, 1.81 mmol, 1.0 eq.) was added at once and stirred for 18 h at r.t. Reaction mixture was washed with water, dried over anhydr. Na_2SO_4 and evaporated. Crude product was purified by column chromatography – DCM:MTBE = 10:1. The solid product was washed with PE and dried *in vacuo* to give 0.411 g (75%) of pure **12** as yellow crystals. M.p. 149-150°C. ^{1}H NMR (600.18 MHz, DMSO-d_6, 353 K): δ = 8.14 (m, 1H, aromatic C<u>H</u>), 7.70 (d, 1H, furane C<u>H</u>, *J* = 3.9 Hz), 7.56 (ddd, 1H, aromatic C<u>H</u>, *J* = 8.9, 7.1, 2.0 Hz), 7.27 (d, 1H, furane C<u>H</u>, *J* = 3.9 Hz), 6.83 (m, 1H, aromatic C<u>H</u>), 6.67 (dd, 1H, aromatic C<u>H</u>, *J* = 7.1, 4.9 Hz), 3.80 (m, 4H, piperazine 2xC<u>H</u>$_2$), 3.64 (m, 4H, piperazine 2xC<u>H</u>$_2$). ^{13}C NMR (150.92 MHz, DMSO-d_6, 353 K): δ = 158.24 (1C, <u>C</u>=O), 156.51 (1C, quaternary $C_{arom.}$), 150.85 (1C, quaternary $C_{arom.}$), 147.51 (1C, quaternary $C_{arom.}$), 147.18 (1C, aromatic <u>C</u>H), 137.13 (1C, aromatic <u>C</u>H), 116.49 (1C, aromatic <u>C</u>H), 112.92 (1C, aromatic <u>C</u>H), 112.22 (1C, aromatic <u>C</u>H), 106.84 (1C, aromatic <u>C</u>H), 44.14 (2C, piperazine 2x<u>C</u>H$_2$), 43.70 (br s, 2C, piperazine 2x<u>C</u>H$_2$). MS (ESI+) m/z (rel. int. %): 303 (M+H, 70), 357 (M+Na+MeOH, 100). MS (ESI-) m/z (rel. int. %): 285 (M-H-O, 100). Anal. calcd. for $C_{14}H_{14}N_4O_4$ (302.29): C, 55.63; H, 4.67; N, 18.53. Found: C, 55.68; H, 4.61; N, 18.57%.

1.1.6 Synthesis of (5-nitrofuran-2-yl)(4-(4-nitrophenyl)piperazin-1-yl)methanone (**13**)



Compound **7** (0.425 g, 2.05 mmol, 1.15 eq.) was dissolved in 25 ml dry DCM. Et_3N (0.36 ml, 2.56 mmol, 1.5 eq.) was added and cooled to 5°C. Then acylchloride **1** (0.300 g, 1.71 mmol, 1.0 eq.) was added at once and stirred for 18 h at r.t. Reaction mixture was washed with water, dried over anhydr. Na_2SO_4 and evaporated. Crude product was purified by column chromatography – DCM:MTBE = 50:1. The solid product was washed with PE and dried *in vacuo* to give 0.540 g (91%) of pure **13** as bright orange crystals. M.p. 200-201°C. ^{1}H NMR (600.18 MHz, DMSO-d_6, 353 K): δ = 8.08 (m, 2H, phenyl aromatic C<u>H</u>), 7.70 (d, 1H, furane C<u>H</u>, *J* = 3.9 Hz), 7.29 (d, 1H, furane C<u>H</u>, *J* = 3.9 Hz), 7.02 (m, 2H, phenyl aromatic C<u>H</u>), 3.88 (m, 4H, piperazine 2xC<u>H</u>$_2$), 3.64 (m, 4H, piperazine 2xC<u>H</u>$_2$). ^{13}C NMR (150.92 MHz, DMSO-d_6, 353 K): δ = 156.53 (1C, <u>C</u>=O), 153.86 (1C, quaternary $C_{arom.}$), 150.88 (1C, quaternary $C_{arom.}$), 147.35 (1C, quaternary $C_{arom.}$), 137.20 (1C, aromatic <u>C</u>H), 125.17 (2C, phenyl aromatic <u>C</u>H), 116.78 (1C, aromatic <u>C</u>H), 112.24 (3C, furane C<u>H</u> and 2 phenyl aromatic <u>C</u>H), 45.56 (2C, piperazine 2x<u>C</u>H$_2$), 43.26 (br s, 2C, piperazine 2x<u>C</u>H$_2$). MS (ESI+) m/z (rel. int. %): 369 (M+Na, 31), 385 (M+K, 28), 401 (M+Na+MeOH, 100). MS (ESI-) m/z (rel. int. %): 391 (M+HCOO, 100). Anal. calcd. for $C_{15}H_{14}N_4O_6$ (346.30): C, 52.03; H, 4.08; N, 16.18. Found: C, 51.97; H, 4.10; N, 16.14%.

2. ^{1}H and ^{13}C NMR spectra of synthesized compounds

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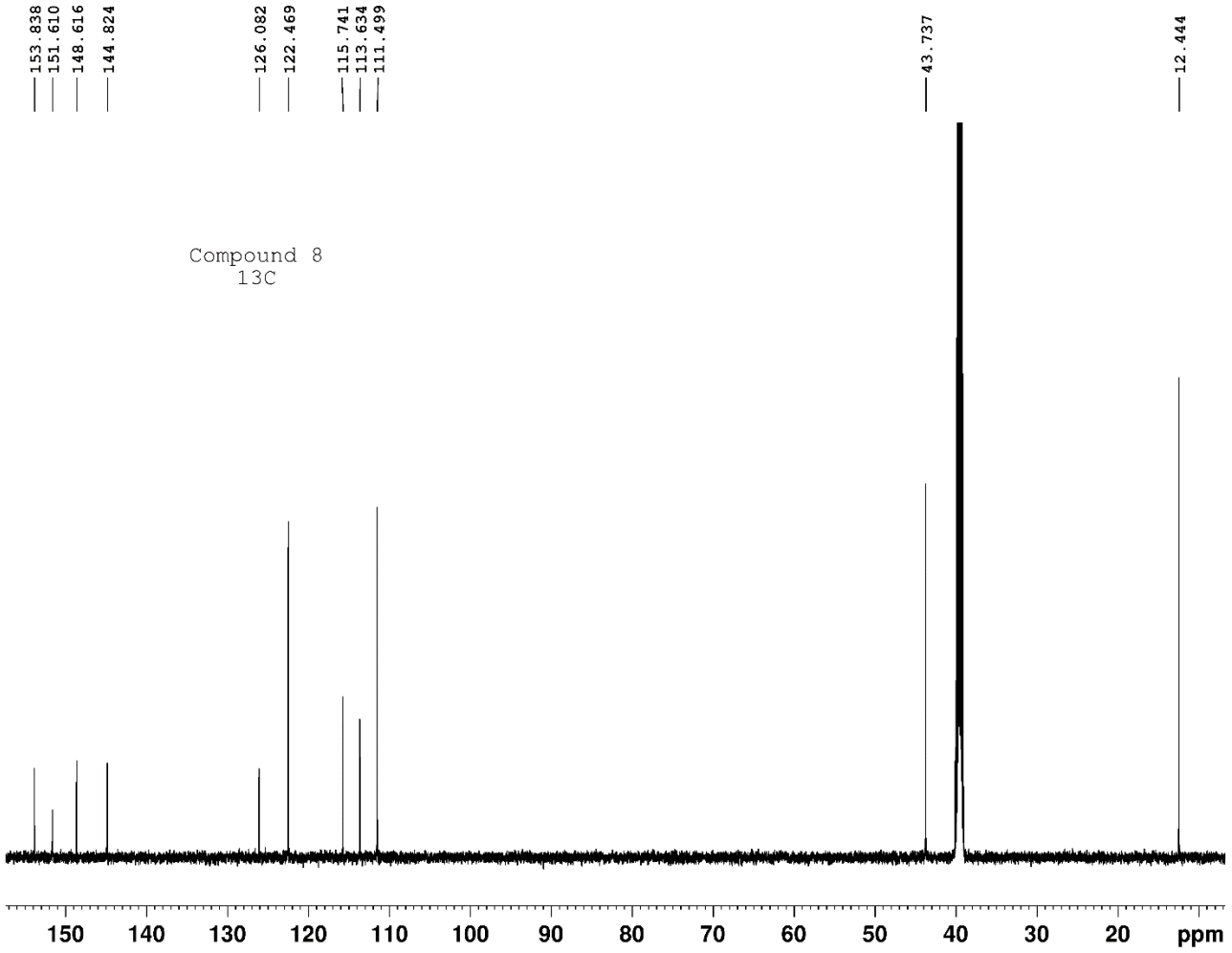


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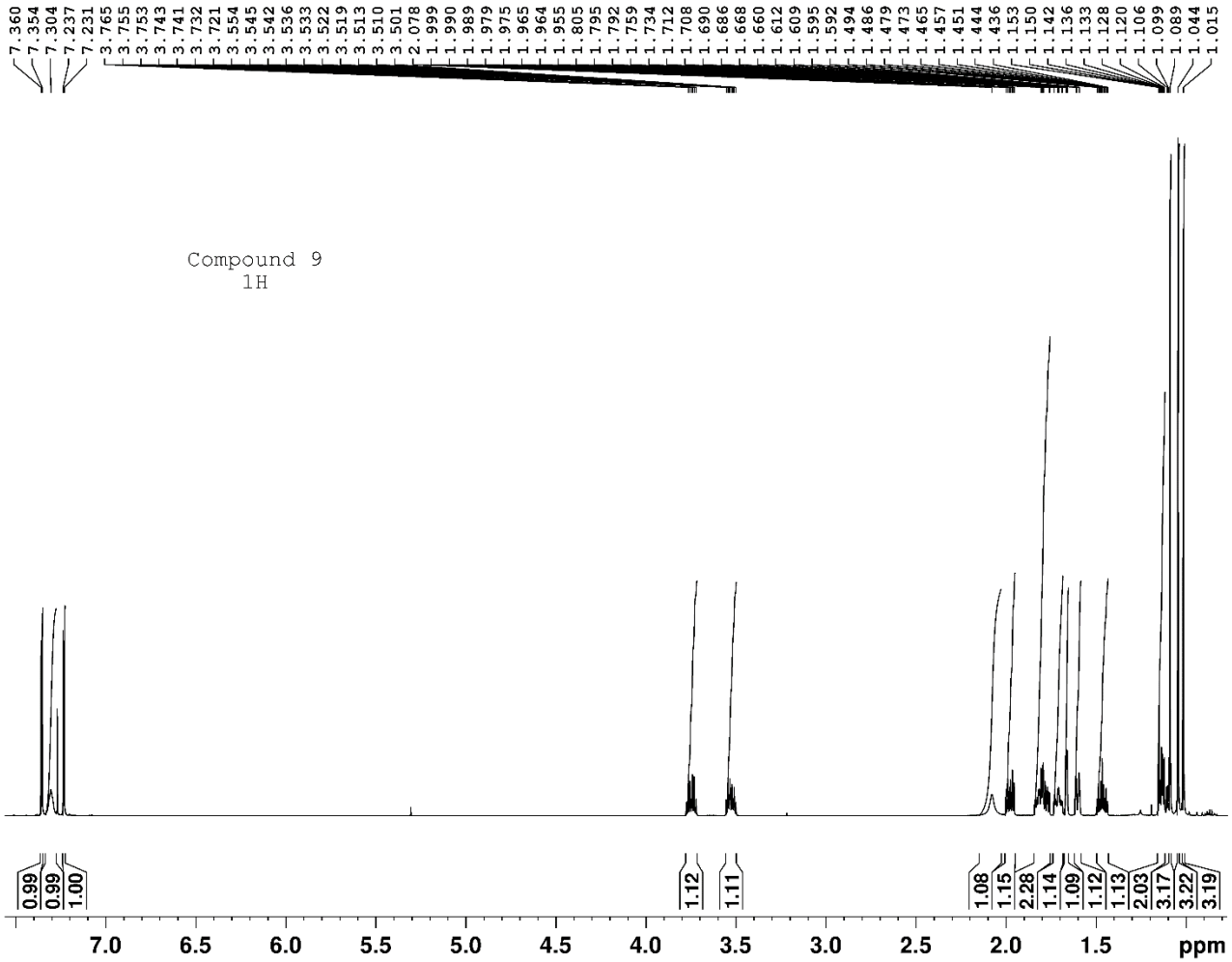


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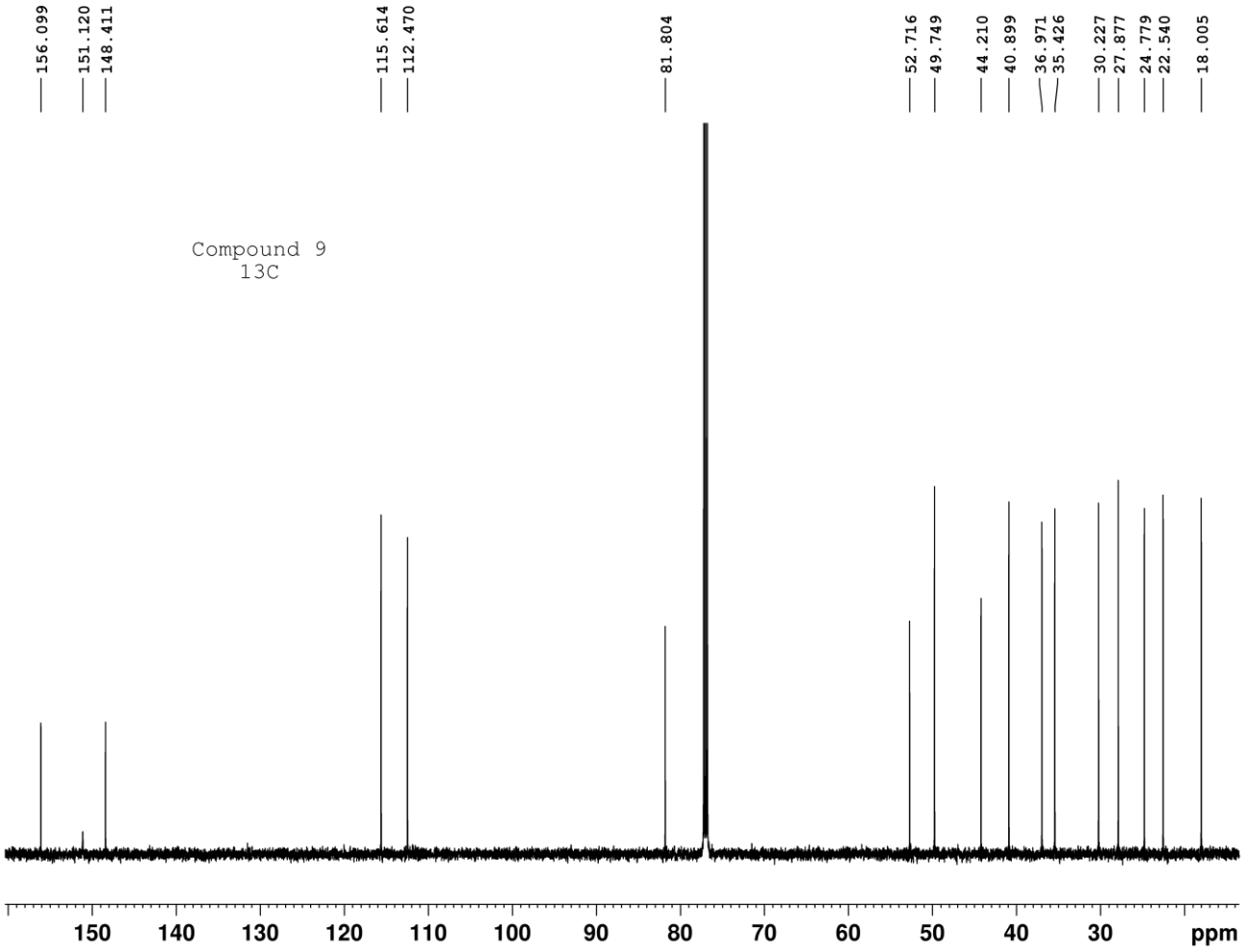
PLW1          20.00000000 W

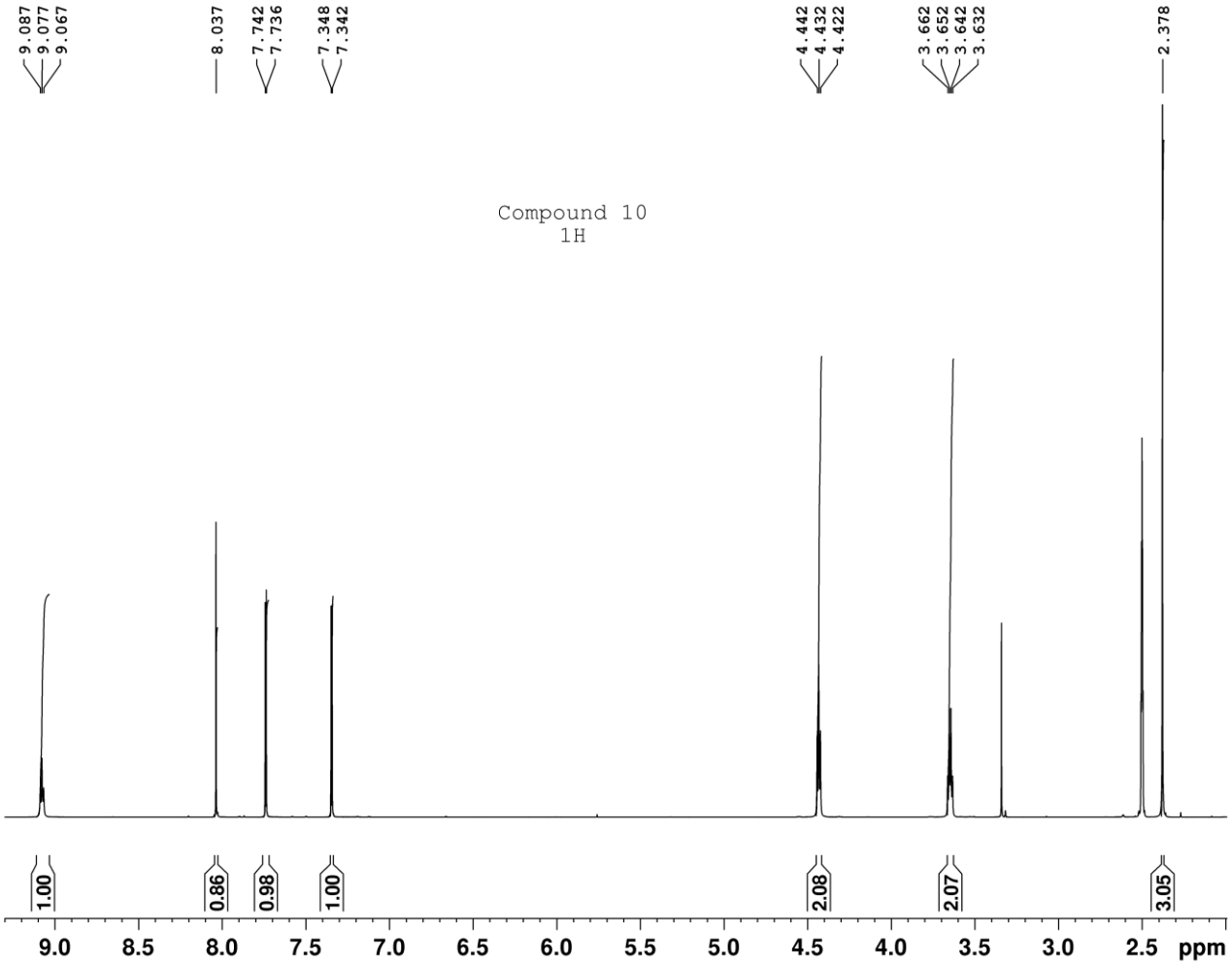
F2 - Processing parameters
SI                  65536
SF           600.0100088 MHz
WDW                    EM
SSB                     0
LB                      0 Hz
GB                      0
PC                   1.00
```




```
Current Data Parameters
NAME                DO-208
EXPNO                   16
PROCNO                   1

F2 - Acquisition Parameters
Date_             20200417
Time                  1.00 h
INSTRUM              spect
PROBHD    Z847801_0047 (
PULPROG             zgdc30
TD                   32768
SOLVENT              CDCl3
NS                     512
DS                       0
SWH              36057.691 Hz
FIDRES            2.200787 Hz
AQ               0.4543829 sec
RG                    2050
DW                  13.867 usec
DE                    6.50 usec
TE                   293.0 K
D1              1.50000000 sec
D11             0.03000000 sec
TD0                      1
SFO1           150.8892338 MHz
NUC1                    13C
P1                    9.80 usec
PLW1           40.00000000 W
SFO2           600.0124004 MHz
NUC2                     1H
CPDPRG[2            waltz16
PCPD2                90.00 usec
PLW2           20.00000000 W
PLW12           0.33800000 W

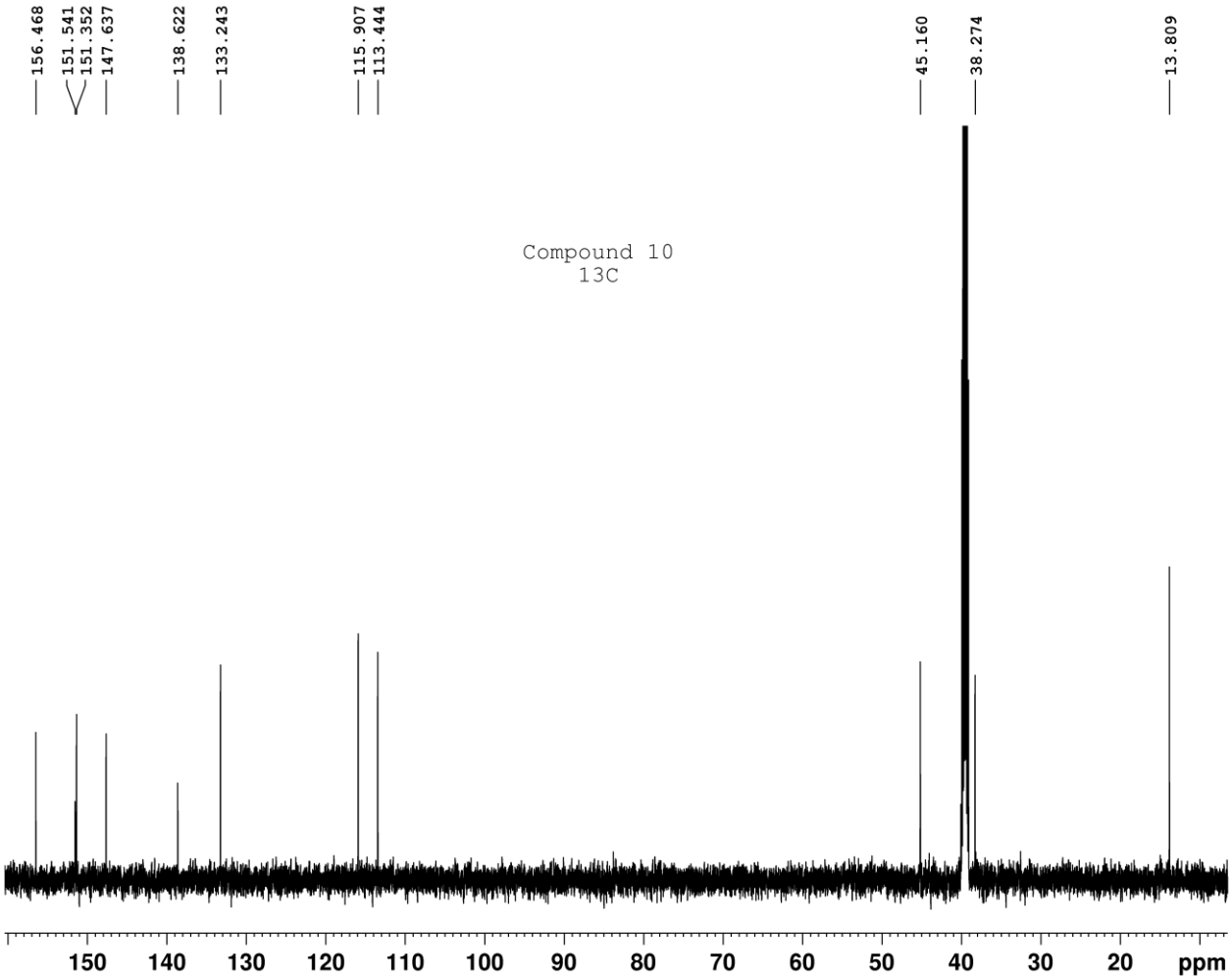
F2 - Processing parameters
SI                    65536
SF             150.8726422 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.40
```




```
Current Data Parameters
NAME              DO-142A
EXPNO                  11
PROCNO                  1

F2 - Acquisition Parameters
Date_            20200312
Time                4.08 h
INSTRUM            spect
PROBHD   Z847801_0047 (
PULPROG             zg30
TD                 32768
SOLVENT             DMSO
NS                     32
DS                      0
SWH             9615.385 Hz
FIDRES          0.586877 Hz
AQ            1.7039360 sec
RG                    228
DW                 52.000 usec
DE                 13.95 usec
TE                 293.0 K
D1            1.00000000 sec
TD0                     1
SFO1         600.0145608 MHz
NUC1                  1H
P1                 10.85 usec
PLW1         20.00000000 W

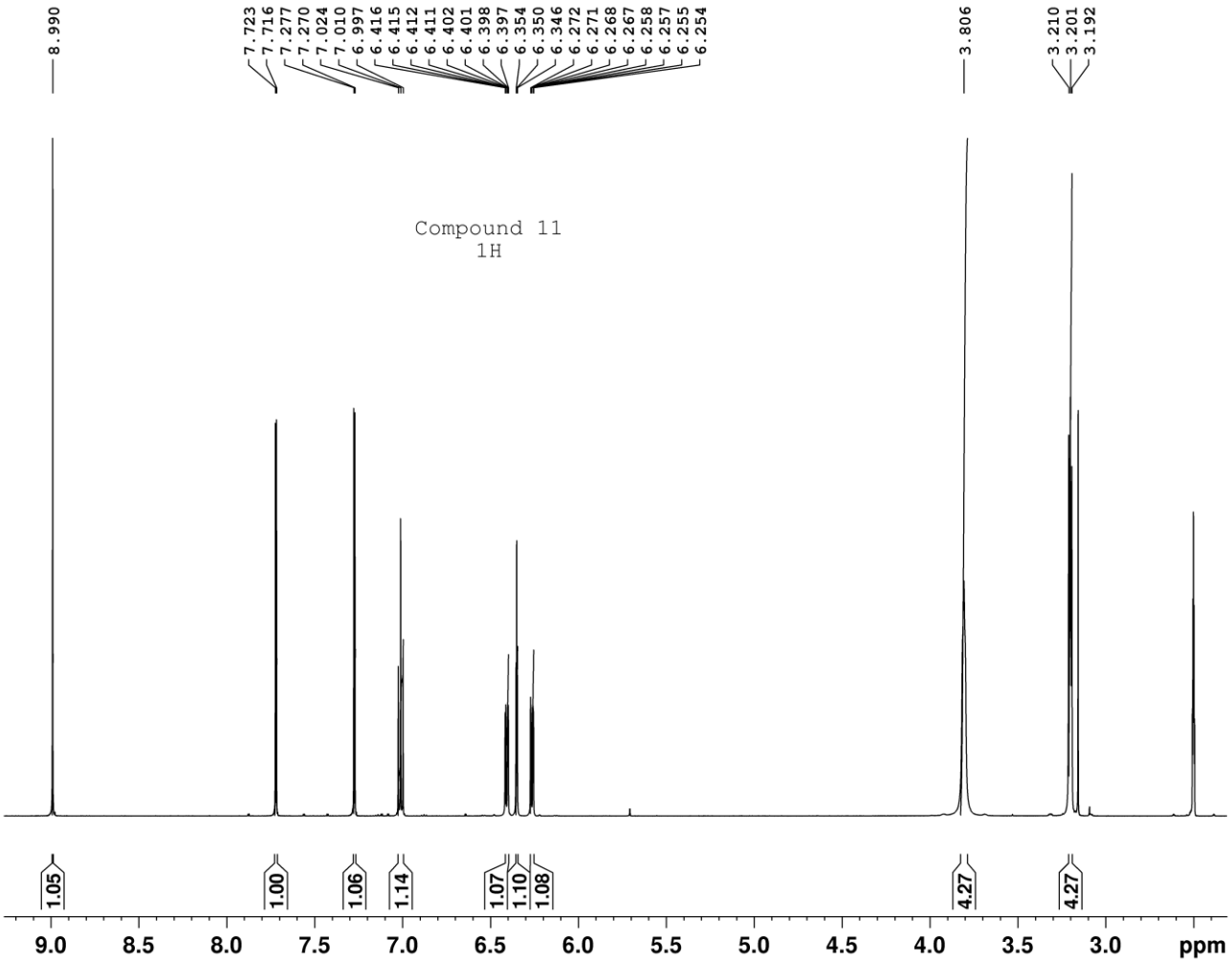
F2 - Processing parameters
SI                  65536
SF           600.0100048 MHz
WDW                    EM
SSB                     0
LB                      0 Hz
GB                      0
PC                   1.00
```




```
Current Data Parameters
NAME              DO-142A
EXPNO                  16
PROCNO                  1

F2 - Acquisition Parameters
Date_             20200312
Time                 4.44 h
INSTRUM             spect
PROBHD    Z847801_0047 (
PULPROG            zgdc30
TD                  32768
SOLVENT              DMSO
NS                    128
DS                      0
SWH            36057.691 Hz
FIDRES          2.200787 Hz
AQ             0.4543829 sec
RG                   2050
DW                 13.867 usec
DE                   6.50 usec
TE                  293.1 K
D1             1.50000000 sec
D11            0.03000000 sec
TD0                     1
SFO1           150.8892338 MHz
NUC1                  13C
P1                   9.80 usec
PLW1          40.00000000 W
SFO2          600.0124004 MHz
NUC2                   1H
CPDPRG[2          waltz16
PCPD2               90.00 usec
PLW2          20.00000000 W
PLW12          0.33800000 W

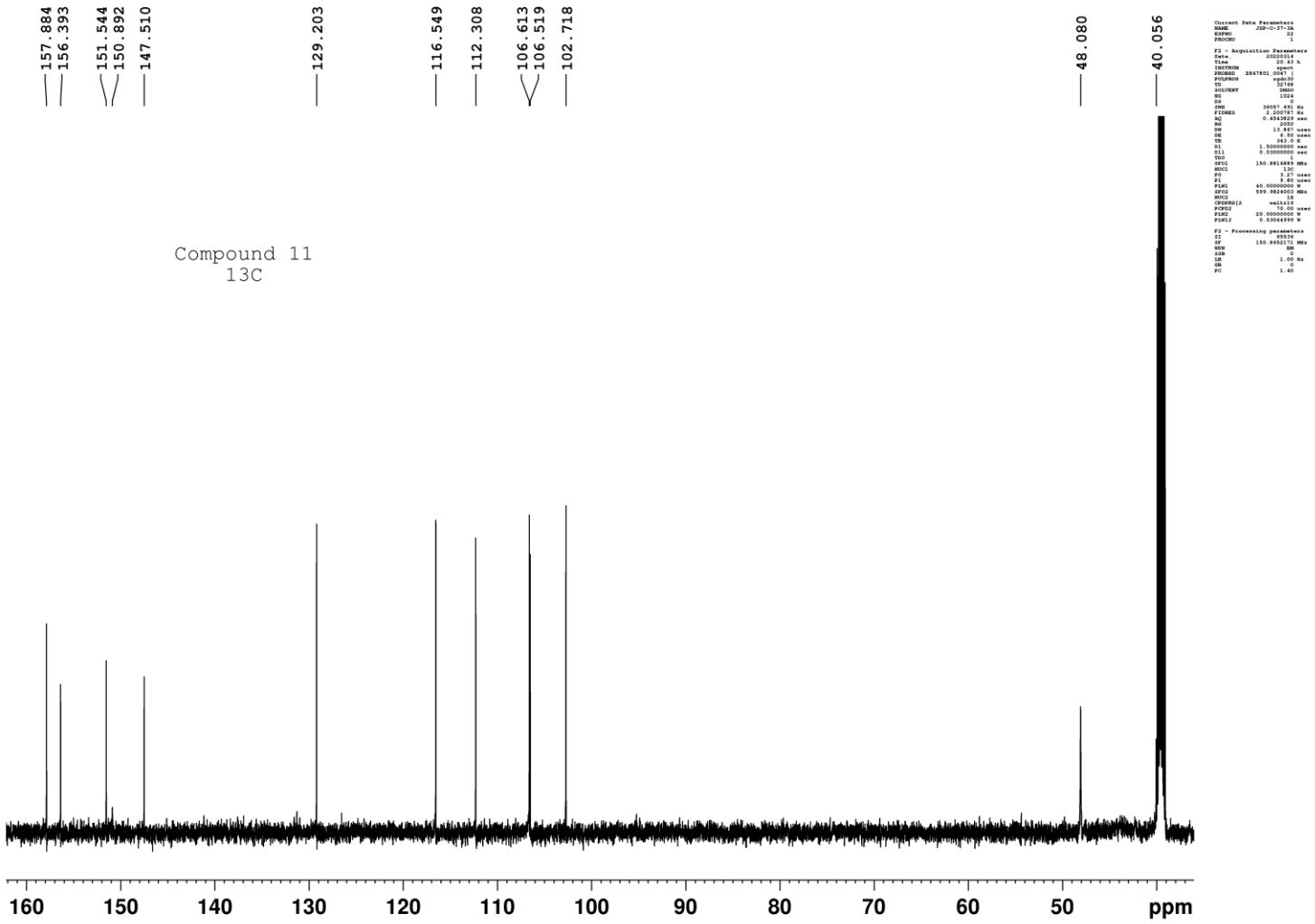
F2 - Processing parameters
SI                  65536
SF            150.8727015 MHz
WDW                    EM
SSB                     0
LB                   1.00 Hz
GB                      0
PC                   1.40
```

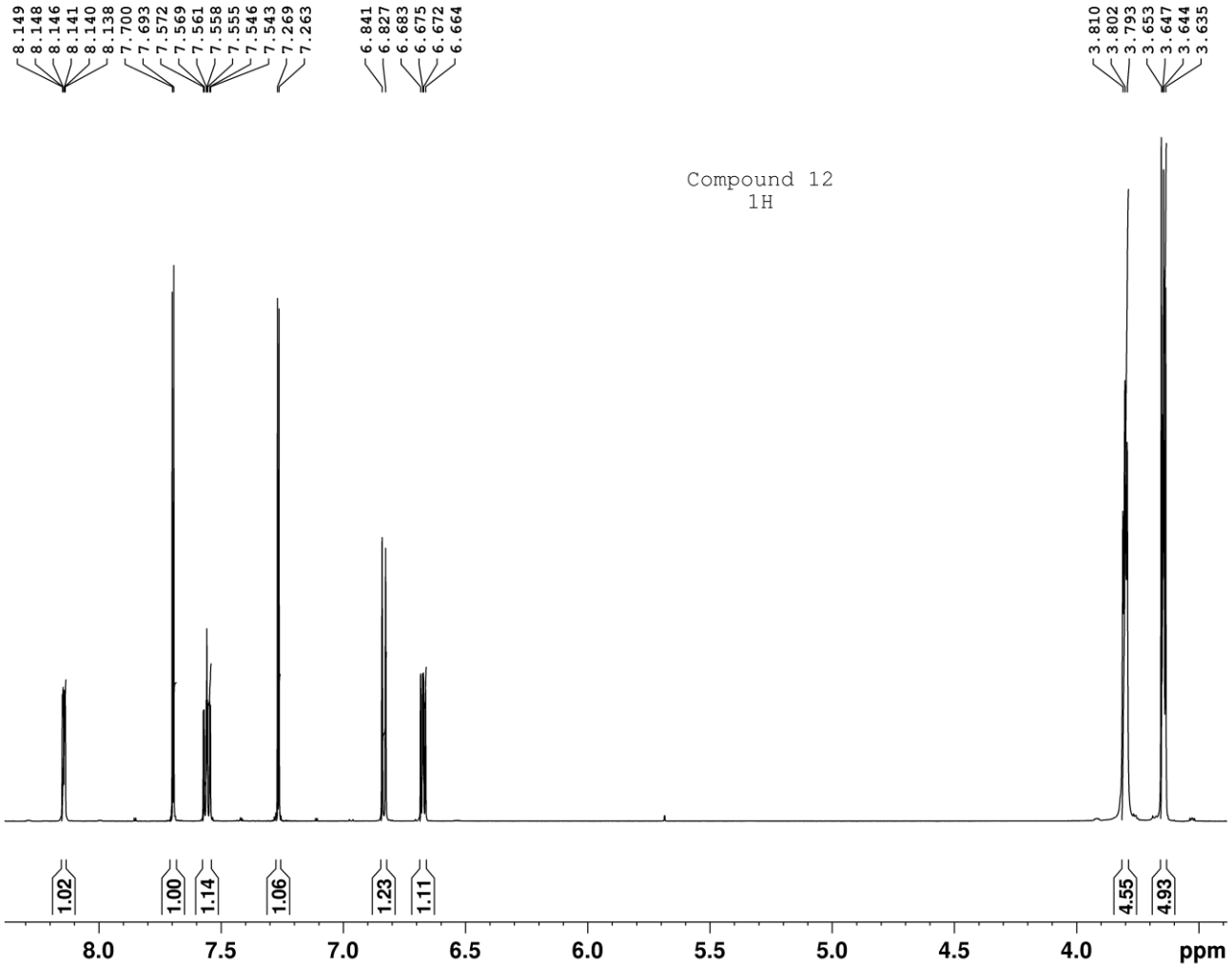



```
Current Data Parameters
NAME              DO-166A
EXPNO                  21
PROCNO                  1

F2 - Acquisition Parameters
Date_            20210903
Time               14.05 h
INSTRUM            spect
PROBHD    Z847801_0047 (
PULPROG            zg30
TD                 32768
SOLVENT             DMSO
NS                     32
DS                      0
SWH              9615.385 Hz
FIDRES           0.586877 Hz
AQ             1.7039360 sec
RG                    228
DW                 52.000 usec
DE                 14.37 usec
TE                 332.9 K
D1             1.00000000 sec
TD0                     1
SFO1           600.0145608 MHz
NUC1                   1H
P0                  3.62 usec
P1                 10.85 usec
PLW1          20.00000000 W

F2 - Processing parameters
SI                  65536
SF             600.0100048 MHz
WDW                    EM
SSB                     0
LB                      0 Hz
GB                      0
PC                   1.00
```

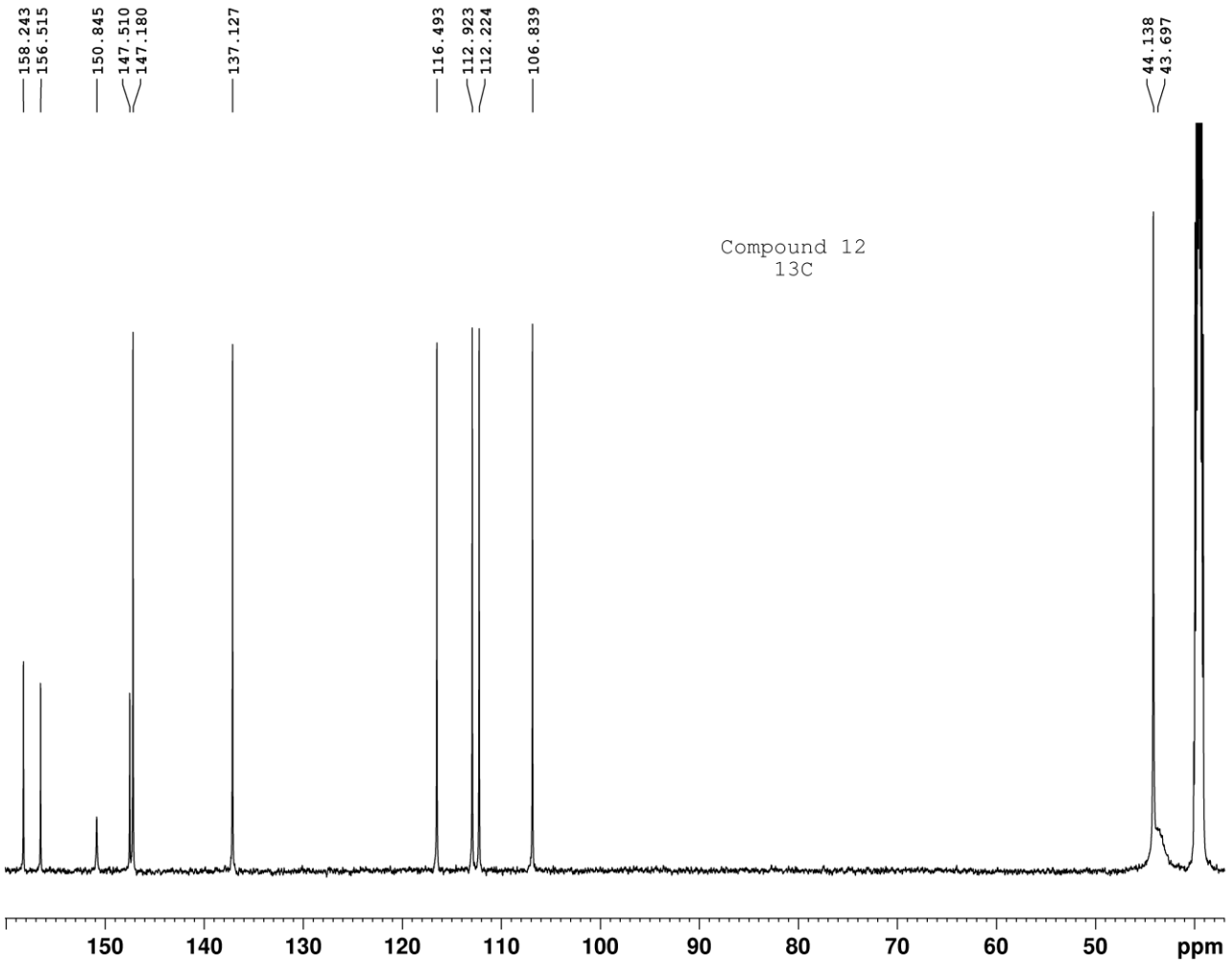

157.884
156.393
151.544
150.892
147.510
129.203
116.549
112.308
106.613
106.519
102.718
48.080
40.056
Compound 11
13C
160
150
140
130
120
110
100
90
80
70
60
50
ppm




```
Current Data Parameters
NAME                 DO-190A
EXPNO                      11
PROCNO                      1

F2 - Acquisition Parameters
Date_              20220328
Time                  17.09 h
INSTRUM              Avance
PROBHD    Z168773_0033 (
PULPROG                 zg30
TD                      32768
SOLVENT                  DMSO
NS                         16
DS                          0
SWH                10000.000 Hz
FIDRES              0.610352 Hz
AQ                  1.6384000 sec
RG                        101
DW                     50.000 usec
DE                     22.95 usec
TE                     353.0 K
D1                 1.00000000 sec
TD0                         1
SFO1            600.1845620 MHz
NUC1                       1H
P0                       4.00 usec
P1                      12.00 usec
PLW1            21.70899963 W

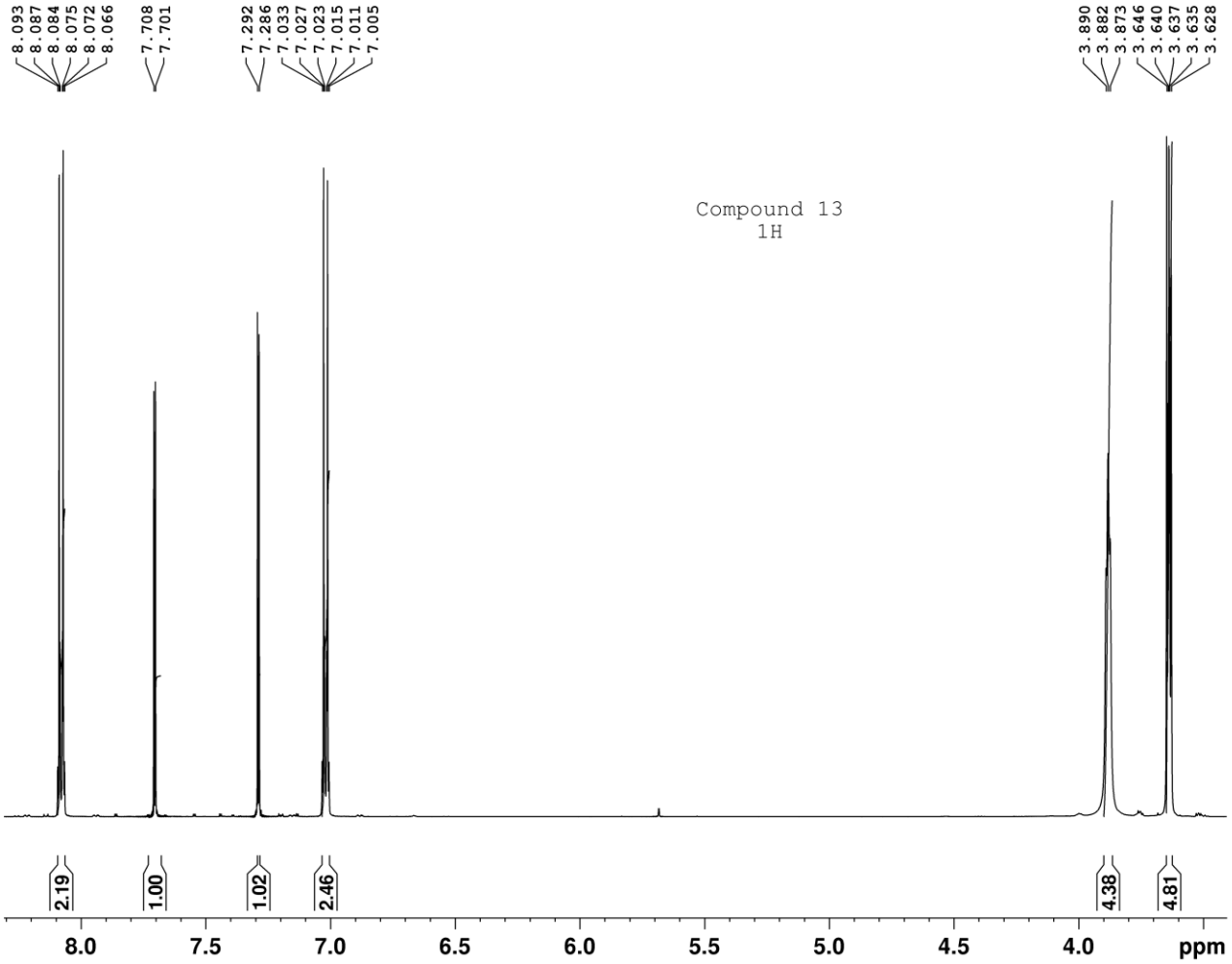
F2 - Processing parameters
SI                      65536
SF              600.1800047 MHz
WDW                        EM
SSB                         0
LB                          0 Hz
GB                          0
PC                       1.00
```




```
Current Data Parameters
NAME              DO-190A
EXPNO                  12
PROCNO                  1

F2 - Acquisition Parameters
Date_           20220328
Time              17.44 h
INSTRUM          Avance
PROBHD   5168773_0033 (
PULPROG          zgdc30
TD                32768
SOLVENT           DMSO
NS                 1024
DS                    0
SWH            35714.285 Hz
FIDRES         2.179827 Hz
AQ             0.45875200 sec
RG                  101
DW               14.000 usec
DE                18.00 usec
TE                353.0 K
D1             1.50000000 sec
D11            0.03000000 sec
TD0                    1
SFO1          150.9319844 MHz
NUC1                 13C
P0                3.33 usec
P1               10.00 usec
PLW1          67.54000092 W
SFO2          600.1624007 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2            80.00 usec
PLW2          21.72701073 W
PLW12          0.49773711 W

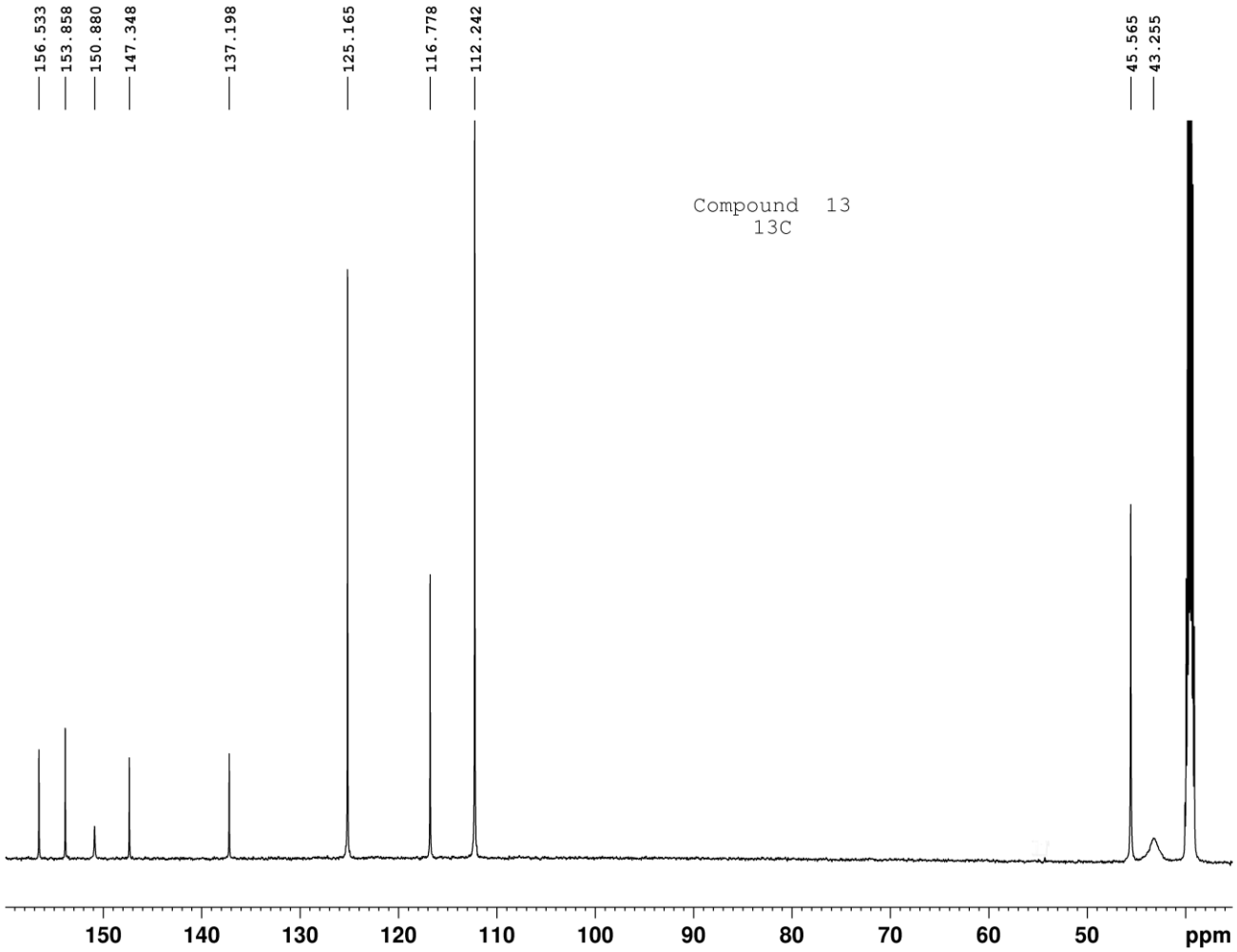
F2 - Processing parameters
SI                 65536
SF            150.9155146 MHz
WDW                   EM
SSB                    0
LB                  5.00 Hz
GB                     0
PC                  0.10
```




```
Current Data Parameters
NAME                DO-209A
EXPNO                    11
PROCNO                    1

F2 - Acquisition Parameters
Date_               20220328
Time                   19.26 h
INSTRUM               Avance
PROBHD    Z168773_0033 (
PULPROG                 zg30
TD                     32768
SOLVENT                 DMSO
NS                        16
DS                         0
SWH                10000.000 Hz
FIDRES              0.610352 Hz
AQ                 1.6384000 sec
RG                       101
DW                    50.000 usec
DE                     22.95 usec
TE                     353.0 K
D1                 1.00000000 sec
TD0                        1
SFO1             600.1845620 MHz
NUC1                      1H
P0                      4.00 usec
P1                     12.00 usec
PLW1             21.70899963 W

F2 - Processing parameters
SI                     65536
SF               600.1800047 MHz
WDW                       EM
SSB                        0
LB                         0 Hz
GB                         0
PC                      1.00
```




```
Current Data Parameters
NAME              DO-209A
EXPNO                  12
PROCNO                  1

F2 - Acquisition Parameters
Date_            20220328
Time               20.01 h
INSTRUM           Avance
PROBHD   I160773_0033 (
PULPROG           zgdc30
TD                 32768
SOLVENT             DMSO
NS                  1024
DS                     0
SWH            35714.285 Hz
FIDRES          2.179827 Hz
AQ             0.4587520 sec
RG                   101
DW                14.000 usec
DE                 18.00 usec
TE                 353.0 K
D1             1.50000000 sec
D11            0.03000000 sec
TD0                    1
SFO1          150.9319844 MHz
NUC1                  13C
P0                  3.33 usec
P1                 10.00 usec
PLW1          67.54000092 W
SFO2          600.1824007 MHz
NUC2                   1H
CPDPRG[2          waltz16
PCPD2              80.00 usec
PLW2          21.72701073 W
PLW12          0.49773711 W

F2 - Processing parameters
SI                 65536
SF            150.9155143 MHz
WDW                   EM
SSB                    0
LB                  5.00 Hz
GB                     0
PC                  1.40
```

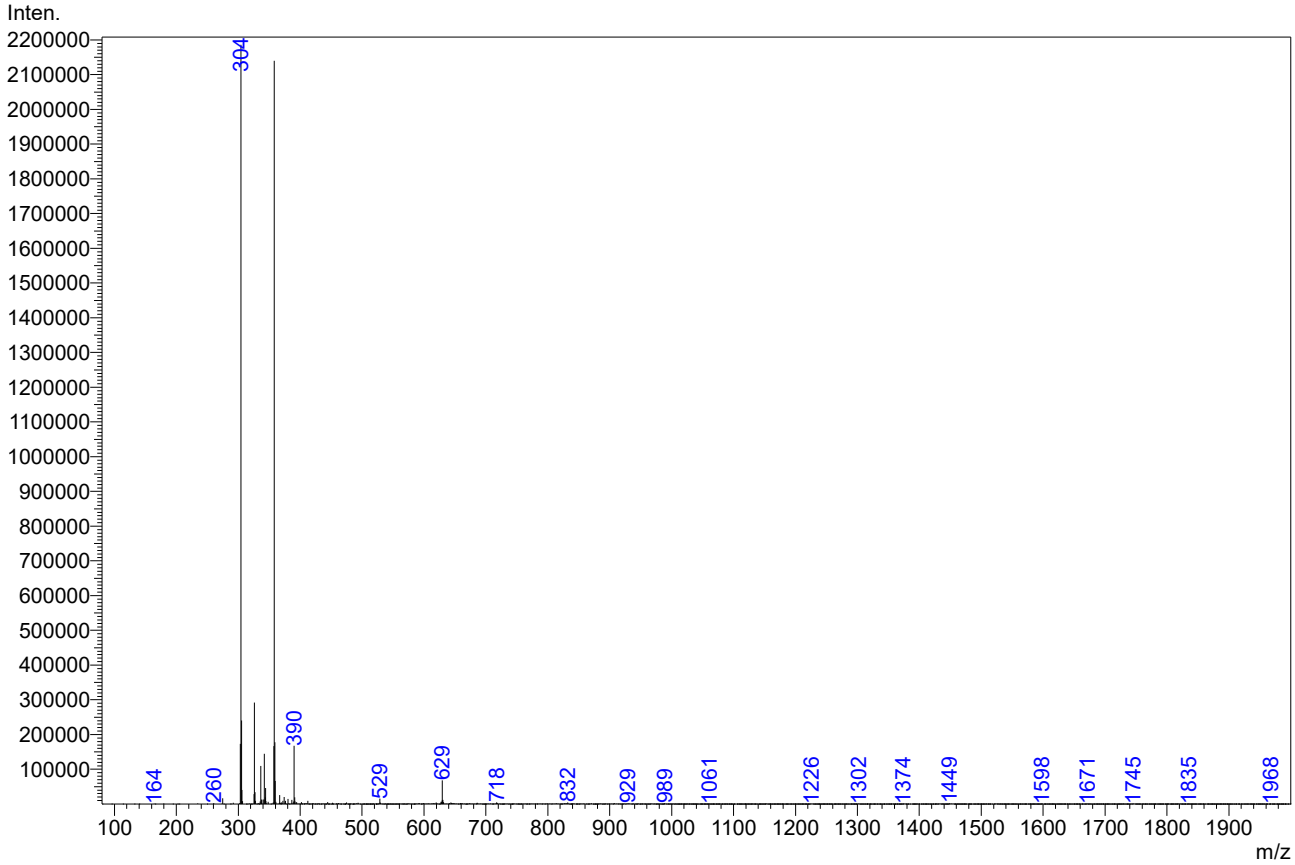
3. MS spectra of synthesized compounds

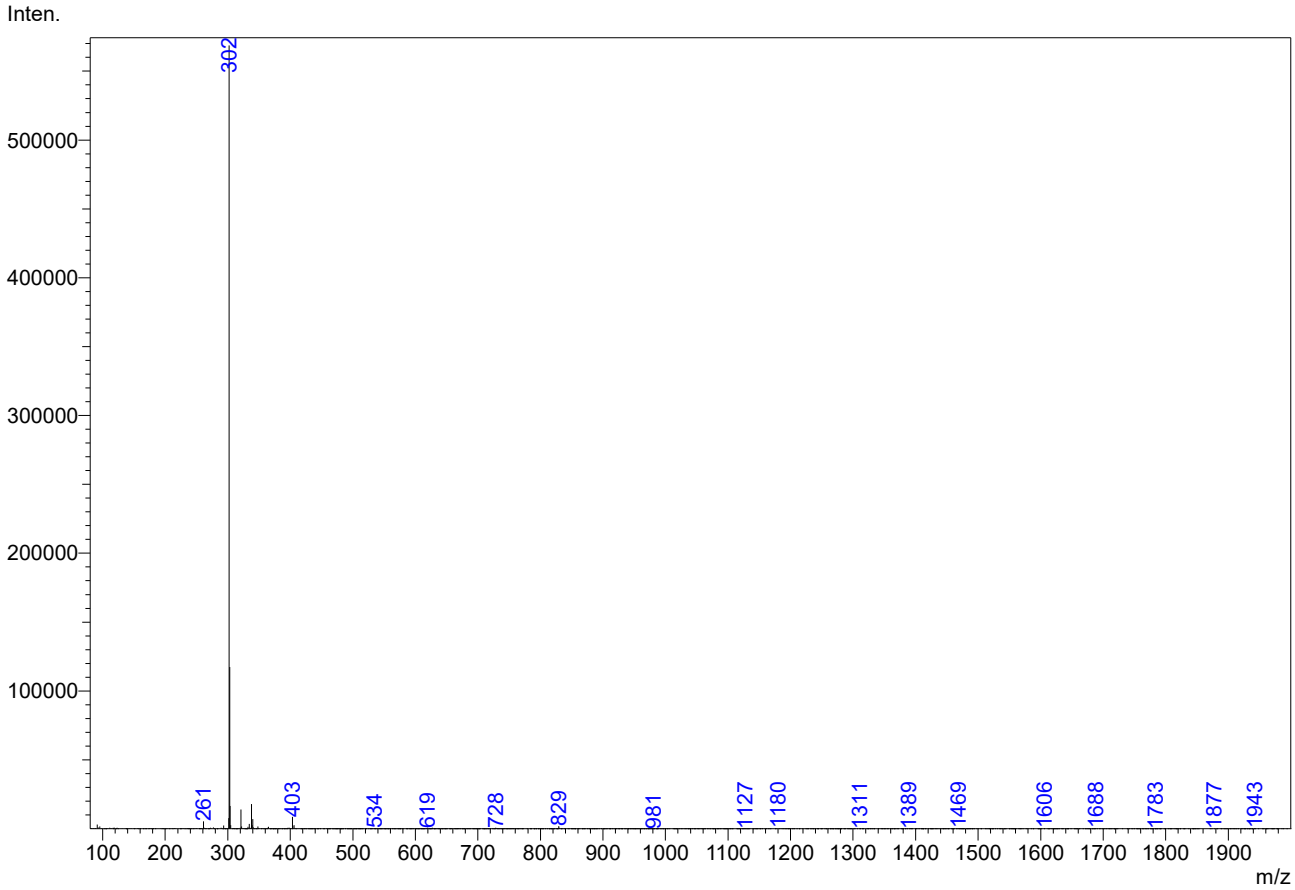
==== Shimadzu LabSolutions Browser Report ====

MS Spectrum(DO-229.lcd)

Compound 8

Ret. Time: 1-1(E+) 1-2(E-) [0.193->0.633]-[0.073->0.960]



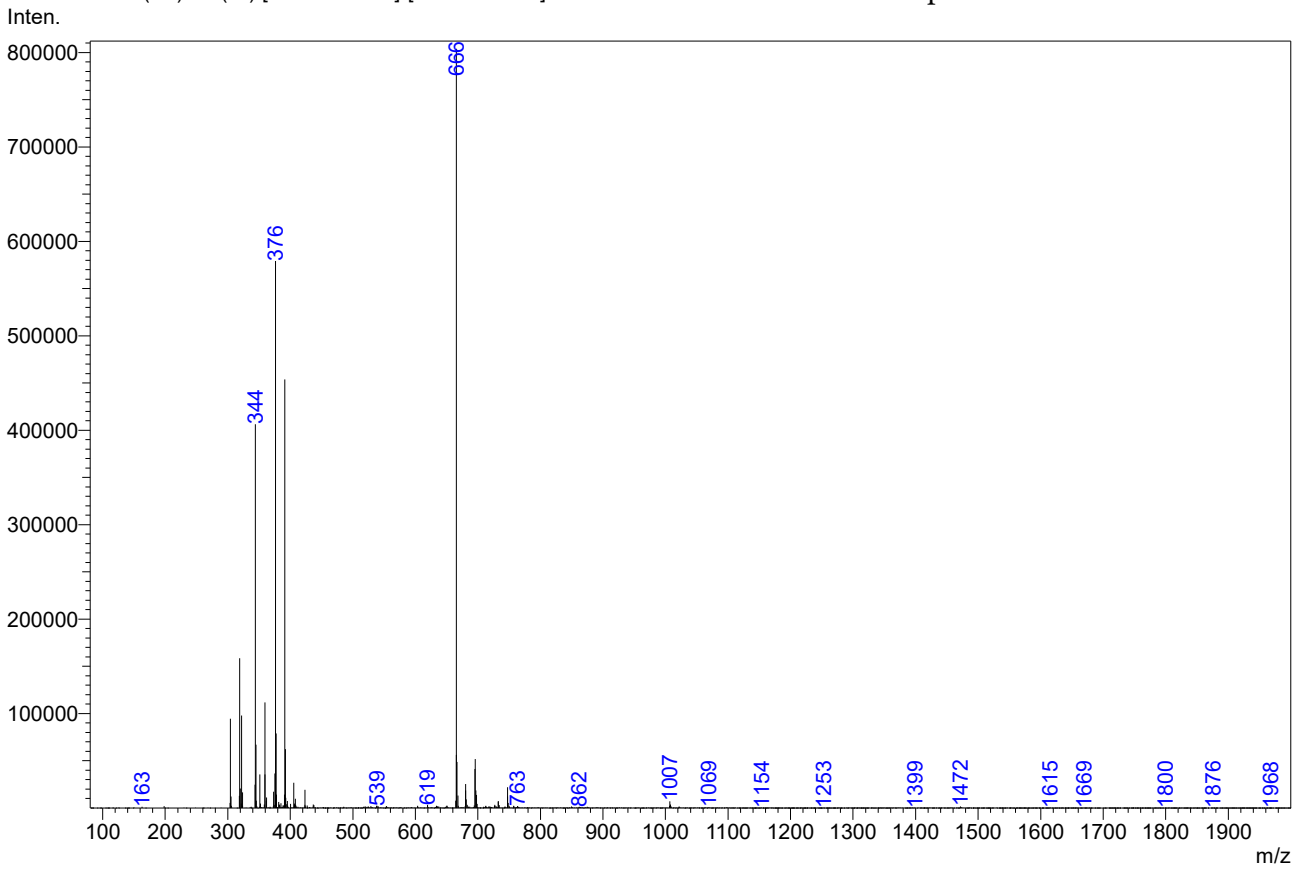


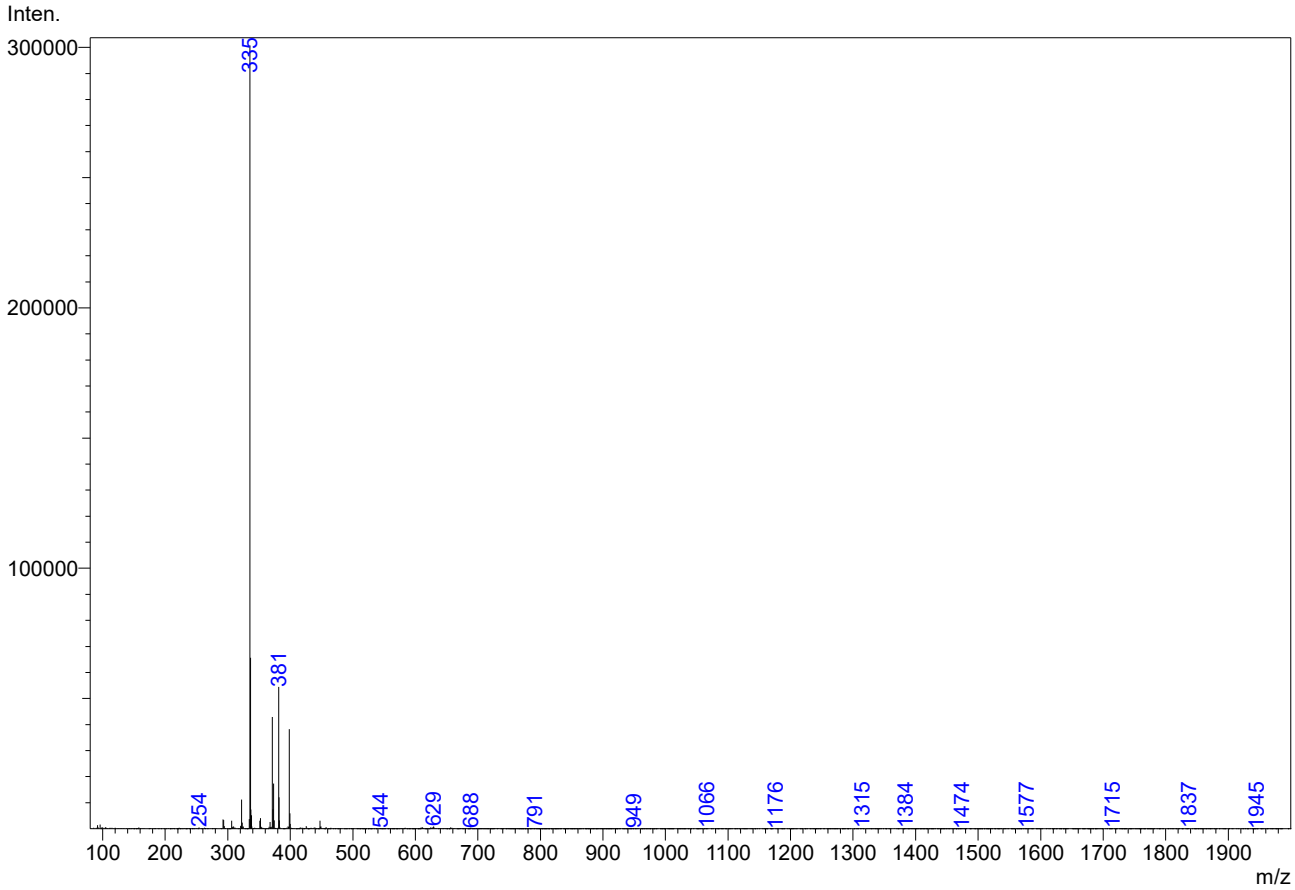
==== Shimadzu LabSolutions Browser Report ====

MS Spectrum(DO-208.lcd)

Ret. Time: 1-1(E+) 1-2(E-) [0.193->0.720]-[0.120->1.013]

Compound 9

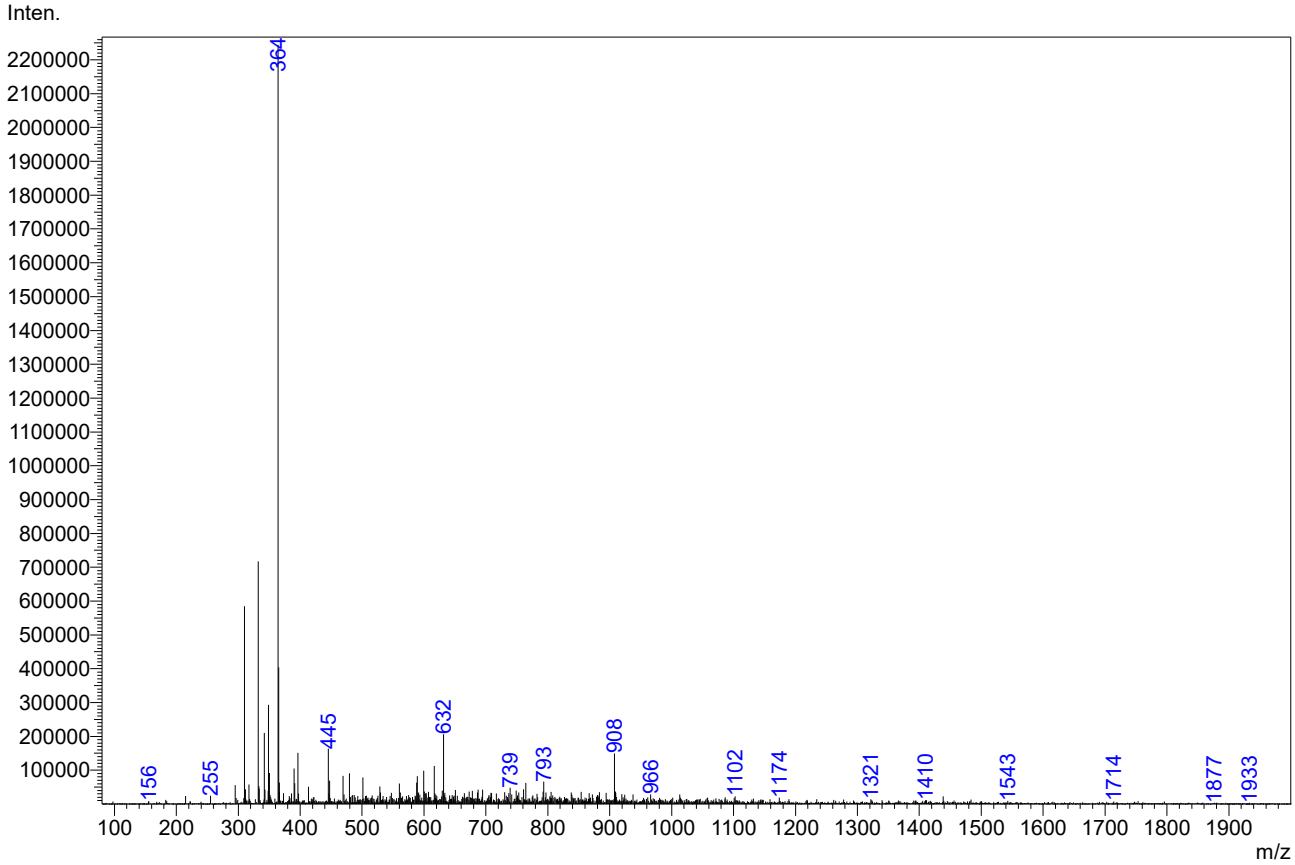


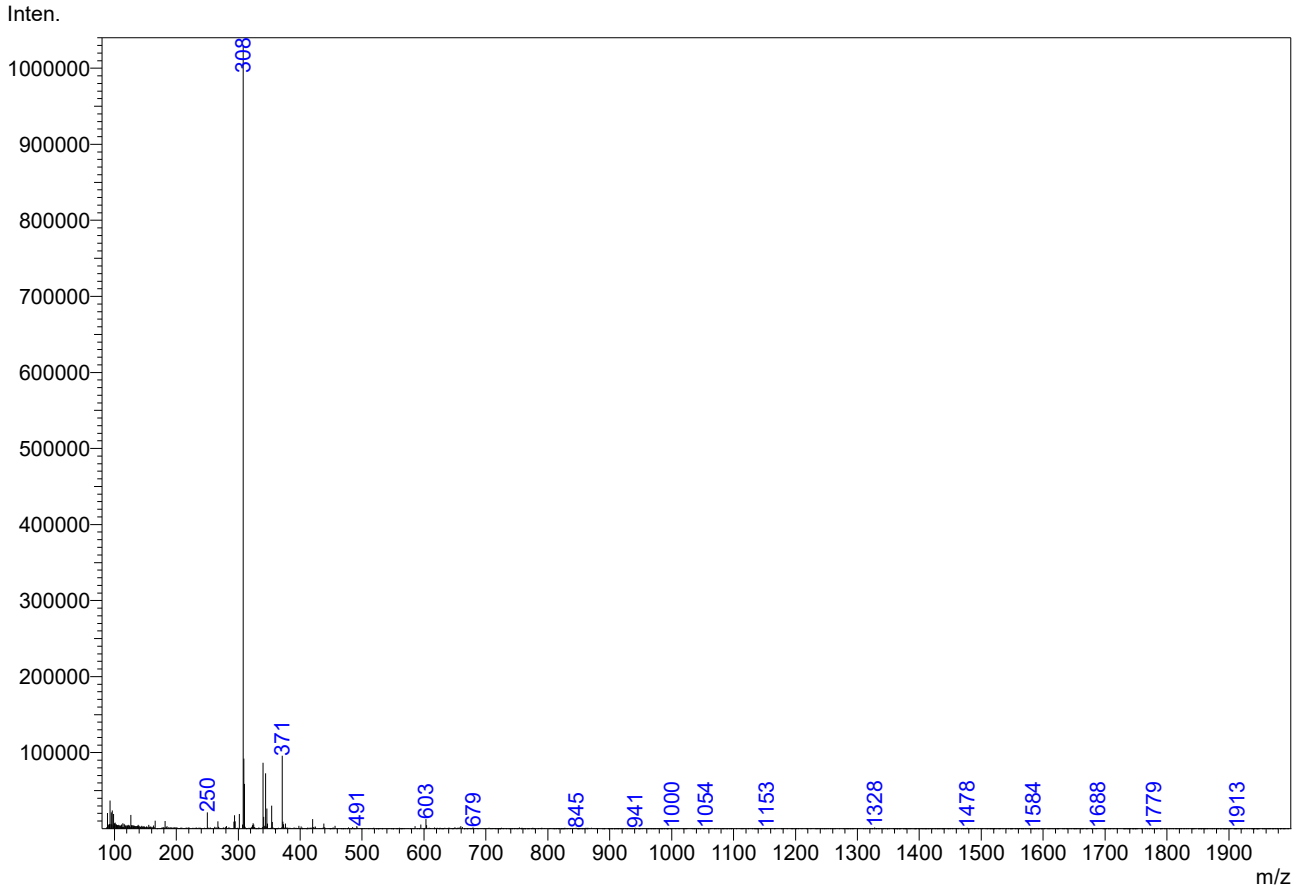


==== Shimadzu LabSolutions Browser Report ====

MS Spectrum(DO-142.lcd) Compound 10

Ret. Time: 1-1(E+) 1-2(E-) [0.347]



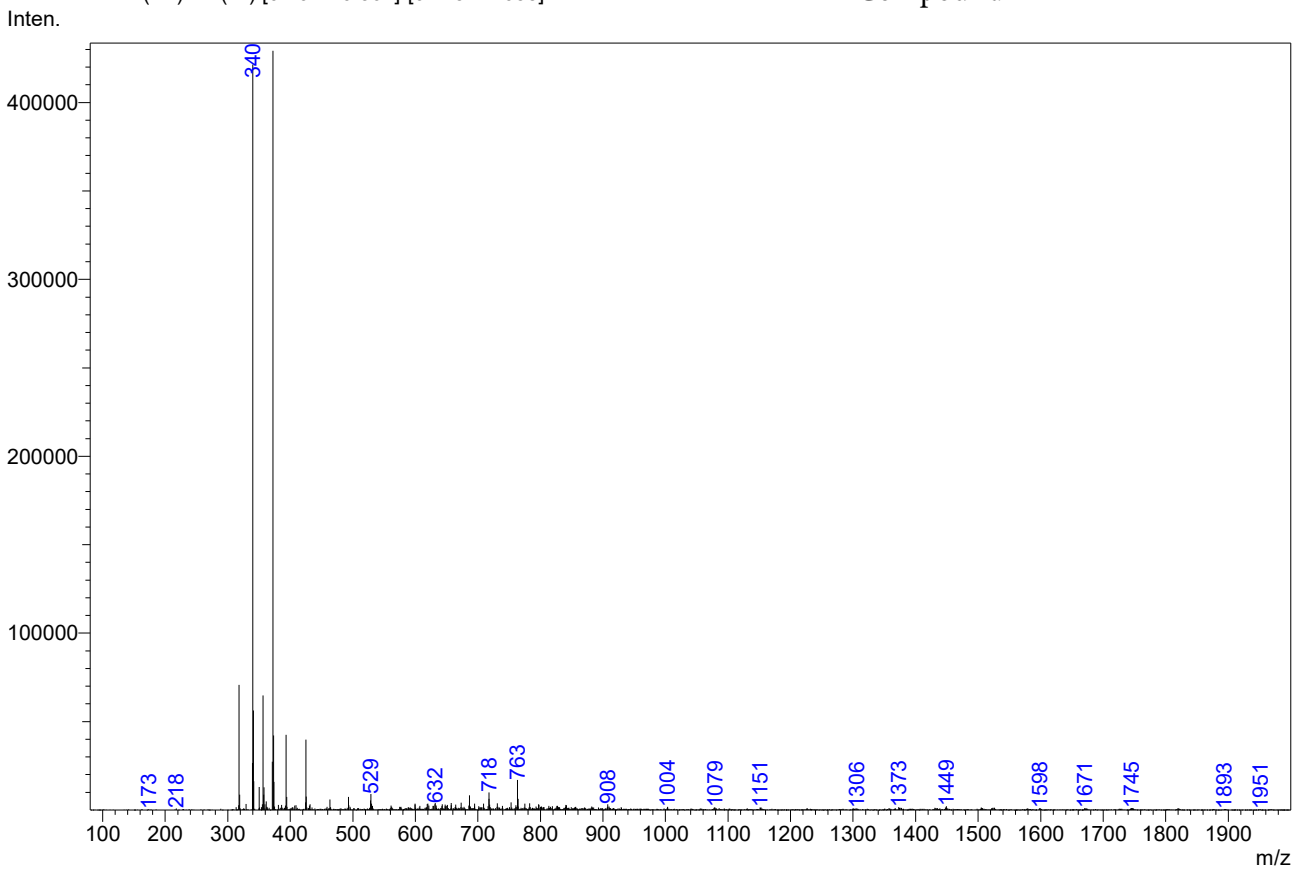


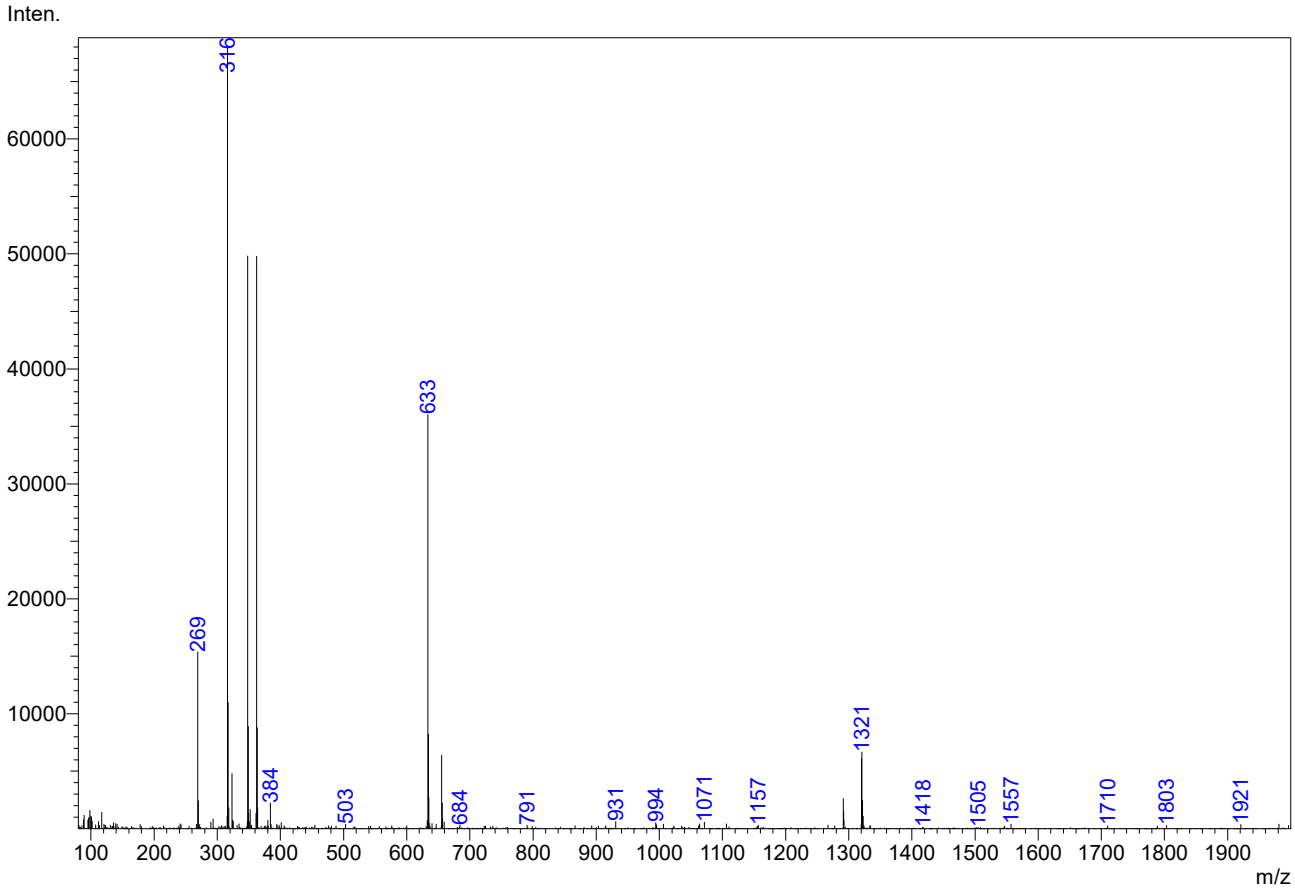
==== Shimadzu LabSolutions Browser Report ====

MS Spectrum(DO-166.lcd)

Ret. Time: 1-1(E+) 1-2(E-) [0.207->0.567]-[0.113->1.053]

Compound 11



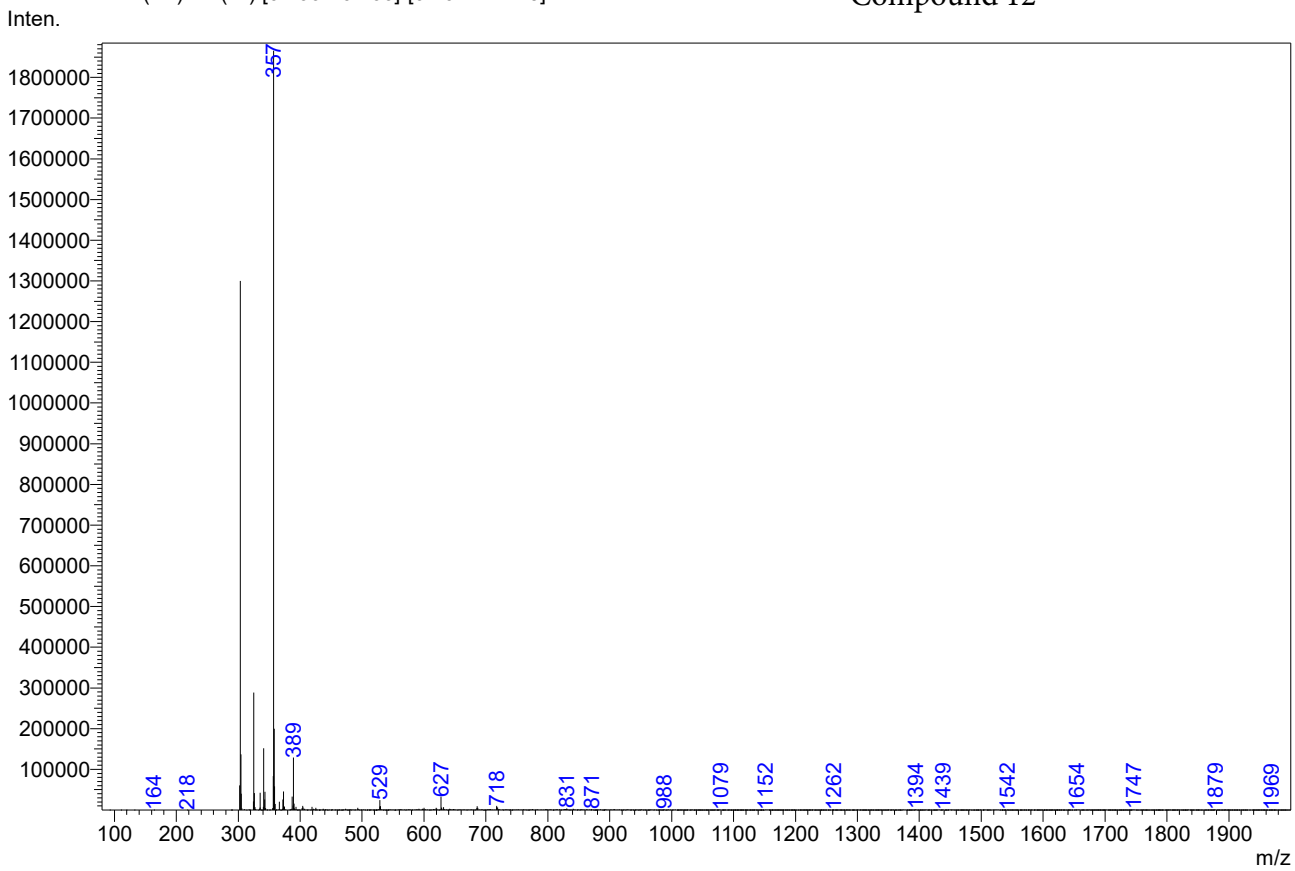


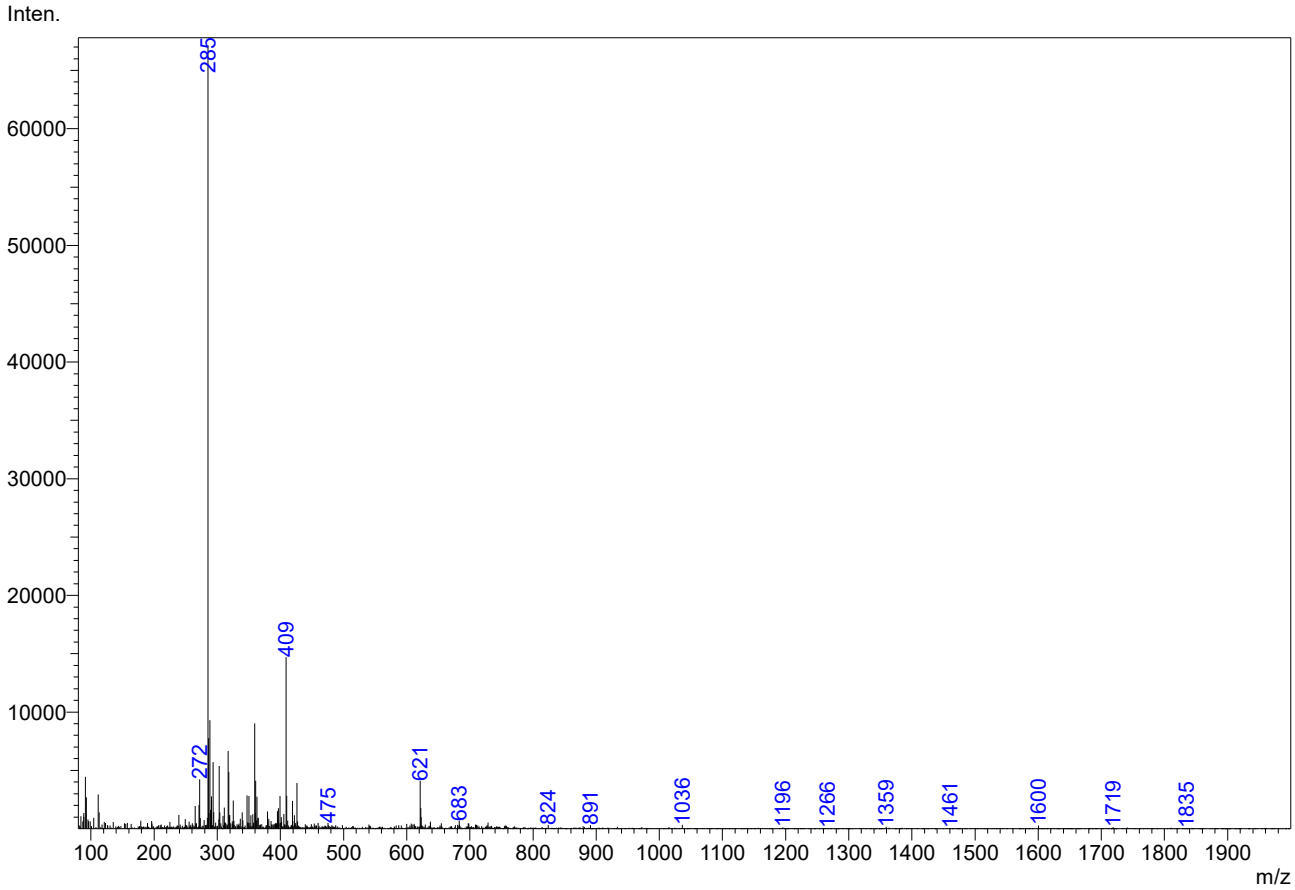
==== Shimadzu LabSolutions Browser Report ====

MS Spectrum(DO-190.lcd)

Ret. Time: 1-1(E+) 1-2(E-) [0.200->0.700]-[0.107->1.273]

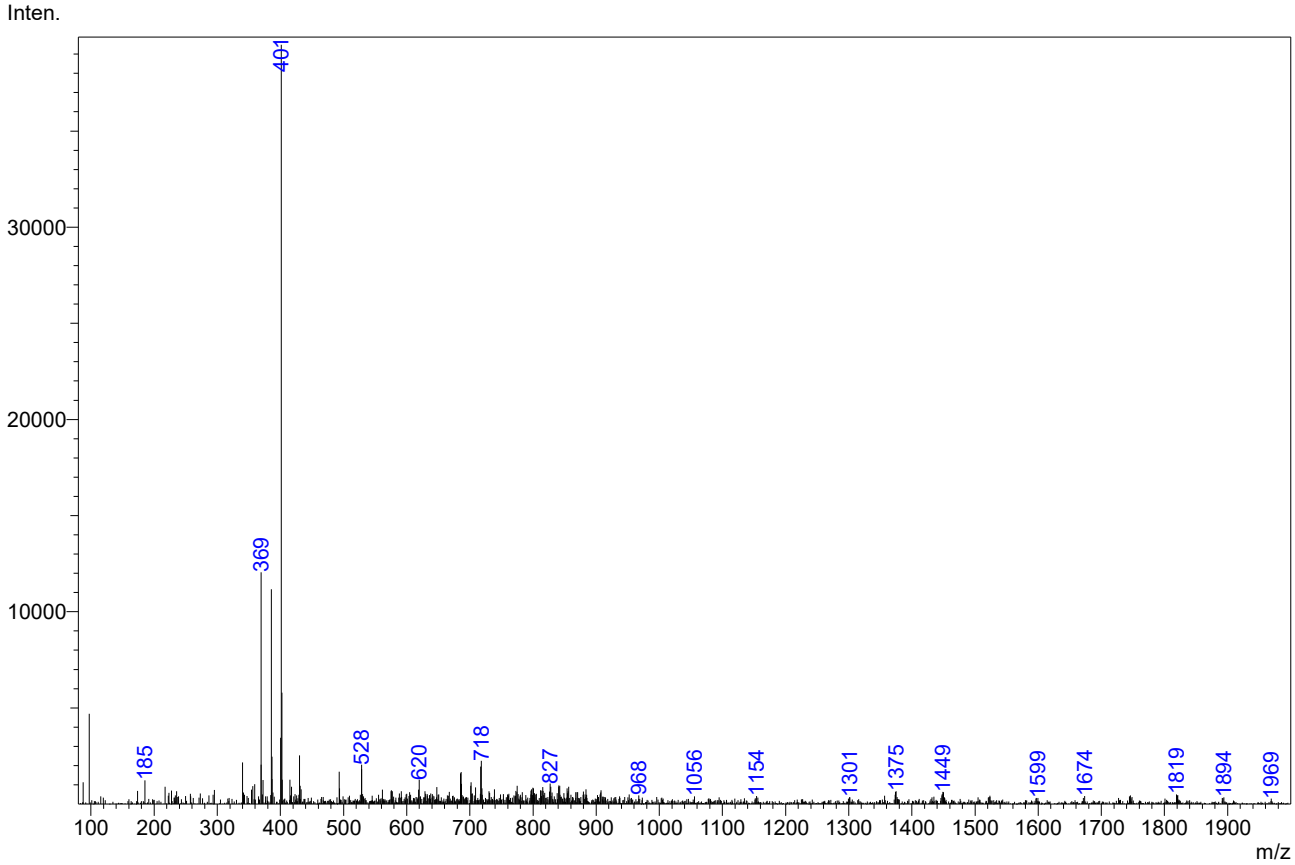
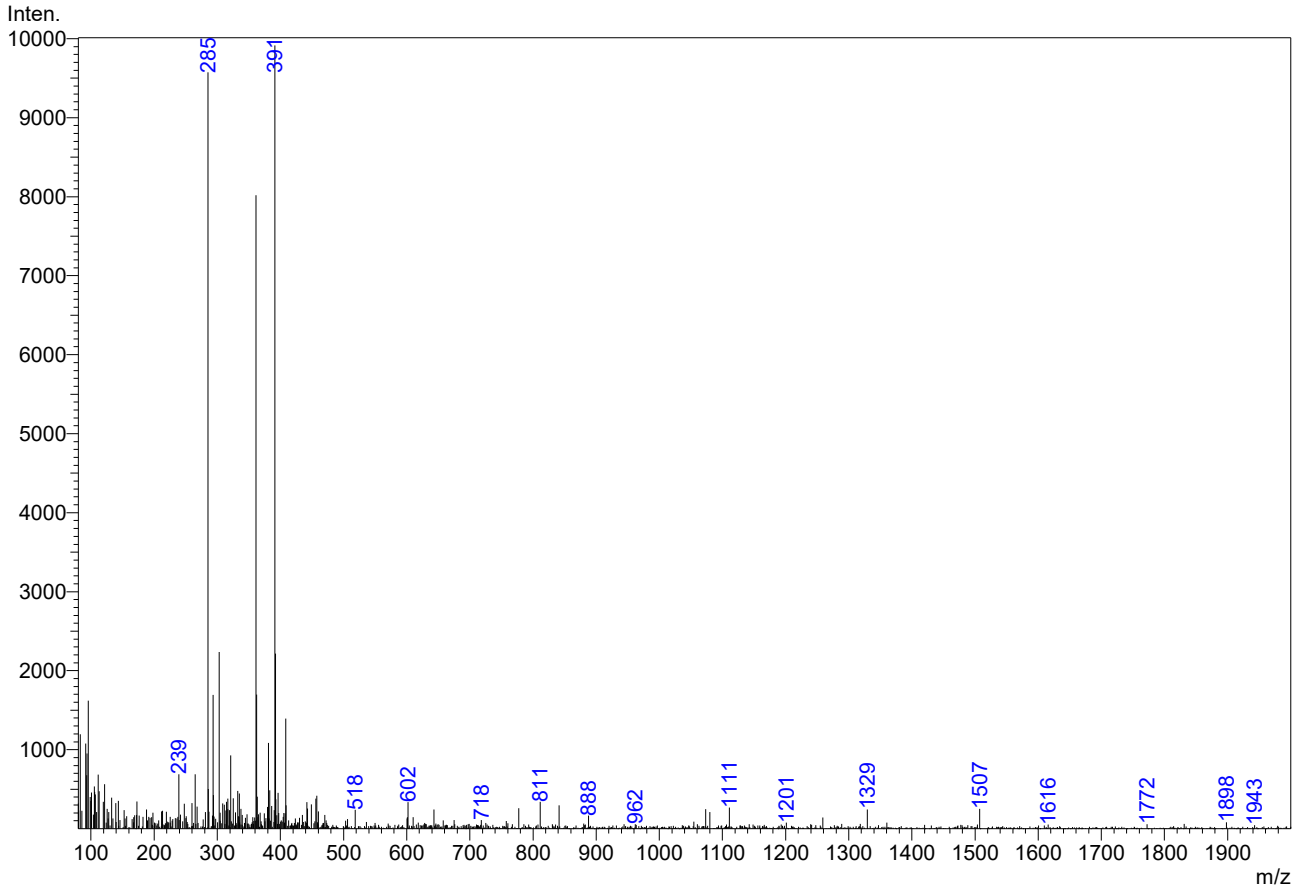
Compound 12





==== Shimadzu LabSolutions Browser Report ====

Ret. Time: 1-1(E+) 1-2(E-) [0.200->0.707]-[0.133->1.153]

MS Spectrum(DO-209.lcd)

Compound 13