Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Synthesis and antimicrobial activity of novel bis-benzimidazolium salts

Amélie Dubreuil, Pierre Mas and Andreea R. Schmitzer*

SUPPORTING INFORMATION

Département de chimie, Université de Montréal, CP 6128, Succursale Centre-Ville,

Montréal, Québec, H3C 3J7, Canada

Table of content

Experimental part

Materials and methods

^{1}H- and ^{13}C-NMR spectra were recorded on 400 and 75 MHz spectrometers, respectively, in the indicated solvent. Chemical shifts are reported in ppm with internal reference to TMS, and *J* values are given in Hertz. The purity of final compounds used in biological assays was determined by ESI/LC-MS on Quantum TSQ Ultra instruments (C18 (2.1 x 100 mm) column, water +0.1% formic acid). Chemicals were purchased from Sigma-Aldrich and used without further purification.

Synthesis and characterization

1-Methylbenzimidazole (2): To a solution of benzimidazole (3.00 g, 25.4 mmol) in 12.7 mL of DMF, KOH (2.85 g, 50.8 mmol) was added. The mixture was stirred for 15 min at 20 °C and iodomethane (3.96 g, 27.9 mmol) was added dropwise under vigorous stirring. After 12 h at 80 °C, the mixture was diluted with 50 mL of water and the organic layer was extracted with ethyl acetate (3 x 25 mL). The organic layers were combined and washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the product was purified by flash chromatography (100 % ethyl acetate) to obtain 1-methylbenzimidazole as a brown oil in 94 % yield. ^{1}H NMR (400 MHz, DMSO) δ 8.18 (s, 1H), 7.66 (dt, *J* = 7.8, 1.0 Hz, 1H), 7.58 – 7.51 (m=dt, *J* = 7.8, 1.0 Hz 1H), 7.27 (td, *J* 7.6, 1.3 Hz, 1H), 7.21 (td, *J* = 7.2, 1.3 Hz, 1H), 3.83 (s, 3H).^{13}C NMR (101 MHz, DMSO) δ 162.78, 145.00, 143.78, 135.04, 122.68, 121.88, 119.73, 110.61, 31.08.LCMS (ESI): calcd. for $[M+H]^+$ $C_8H_8N_2$: 133.0760, found 133.0759.

1-Butylbenzimidazole (3): To a solution of benzimidazole (3.00 g, 25.4 mmol) in 11.5 mL of DMSO, KOH (2.85 g, 50.8 mmol) was added. The mixture was stirred for 15 min at 20 °C and 1-bromobutane (3.83 g, 27.9 mmol) was added dropwise under vigorous stirring. After 12 h at 80 °C, the mixture was diluted with 50 mL of water and the organic layer was extracted with ethyl acetate (3 x 25 mL). The organics layers were combined and washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the product was purified by flash chromatography (100 % ethyl acetate) to obtain 1-butylbenzimidazole as a brown oil in 83 % yield. ^{1}H NMR (400 MHz, DMSO) δ 8.22 (s, 1H), 7.65 (dt, *J* = 7.8, 1.0 Hz, 1H), 7.58 (dt, *J* = 7.8, 1.0 Hz 1H), 7.24 (td, *J* = 7.6, 1.3 Hz, 1H), 7.19 (td, *J* = 7.1, 1.3 Hz, 1H), 4.23 (t, *J* = 7.1 Hz, 2H), 1.82 – 1.70 (p, *J* = 7Hz 2H), 1.31 – 1.17 (h, *J* = 7Hz 2H), 0.87 (t, *J* = 7.4 Hz, 3H).^{13}C NMR (101 MHz, DMSO) δ 144.47, 143.95, 134.29, 122.63, 121.98, 121.80, 119.89, 110.83, 44.25, 31.92, 19.83, 13.88. ; LCMS (ESI): calcd. for $[M+H]^+$ $C_{11}H_{14}N_2$: 175.1230, found 175.1227.

1-Octylbenzimidazole (4): To a solution of benzimidazole (6.00 g, 50.8 mmol) in 23.0 mL of DMSO, KOH (5.70 g, 102 mmol) was added. The mixture was stirred for 15 min at 20 °C and 1-bromooctane (10.8 g, 55.9 mmol) was added dropwise under vigorous stirring. After 12 h at 80 °C, the mixture was diluted with 50 mL of water and the organic layer was extracted with ethyl acetate (3 x 25 mL). The organic layers were combined and washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the product was purified by flash chromatography (100 % ethyl acetate) to obtain 1-octylbenzimidazole as a brown oil in 77 % yield.^{1}H NMR (400 MHz, DMSO) δ 8.21 (s, 1H), 7.65 (dt, *J* = 7.5, 1.3 Hz, 1H), 7.58 (dt, 7.5, 1.3 Hz, 1H), 7.23 (td, *J* = 7.6, 1.3 Hz, 1H), 7.18 (td, *J* = 7.5, 1.3 Hz, 1H), 4.21 (t, *J* = 7.1 Hz, 2H), 1.76 (p, *J* = 7.1 Hz, 2H), 1.28 – 1.14 (m, 11H), 0.81 (t, *J* = 6.8 Hz, 3H).^{13}C NMR (101 MHz, DMSO) δ 144.44, 143.98, 134.27, 122.59, 121.76, 119.89, 110.76, 44.52, 31.63, 29.84, 29.05, 28.94, 26.58, 22.51, 14.34.; LCMS (ESI): calcd. for $[M+H]^+$ $C_{15}H_{22}N_2$: 231.1856, found 231.1855.

((1,3-phenylenebis(ethyne-2,1-diyl))bis(4.1-phenylene))dimethanol (6) : To a solution of 4-iodobenzyl alcohol (2.56 g, 10.09 mmol) in degassed piperidine (15 mL, copper iodide (I) (16.3 mg, 85.6 µmol), bis(triphenylphosphine) palladium (II) chloride (57.8 mg, 82.3 µmol) were added. The solution was cooled down to 0 °C under nitrogen and 1.3-diethynylbenzene (600 mg, 4.76 mmol) was added dropwise over 5 min. The reaction mixture was warmed to room temperature and stirred for 3h. The crude was dissolved in acetone and precipitated in water. The product was isolated by filtration to afford the diol as white powder in 100 % yield.^{1}H NMR (400 MHz, DMSO) δ 7.71 (t, *J* = 1.7 Hz, 1H), 7.60 – 7.51 (m, 6H), 7.47 (t, J=8Hz, 1H), 7.39 (d, *J* = 7.9 Hz, 4H), 5.31 (t, *J* = 5.7 Hz, 2H), 4.54 (d, *J* = 5.7 Hz, 4H).^{13}C NMR (101 MHz, DMSO) δ 144.23, 134.30, 131.82, 131.76, 129.84, 127.11, 123.54, 120.60, 90.80, 88.35, 62.97; LCMS (ESI): calcd. for $[M+Na]^+$ $C_{24}H_{18}O_2$: 361.1199, found 361.1197.

1.3-bis((4-(bromomethyl)phenyl)ethynyl)benzene (7): To a solution of ((1.3-phenylenebis(ethyne-2.1-diyl))bis(4.1-phenylene))dimethanol (500 mg, 1.48 mmol) in anhydrous CH_2Cl_2 (5.00 mL), phosphorus tribromide (694 µL, 7.39 mmol) was added dropwise at 0 °C. The reaction was warmed to room temperature. After 2h, the solution was slowly quenched with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3x 20 mL). The organic layers were combined and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude reaction mixture was purified on silica gel (1:99 ethyl acetate / hexane) to yield the dibromide as a yellow powder with 68 % yield.;^{1}H NMR (500 MHz, DMSO) δ 7.80 – 7.73 (m, 1H), 7.64 – 7.47 (m, 11H), 4.76 (s, 4H).^{13}C NMR (126 MHz, DMSO) δ 138.94, 134.00, 132.36, 132.17, 131.76, 129.71, 122.79, 121.81, 89.78, 88.93, 33.81.It was impossible to determine the exact mass because the compound does not ionize.

1.1'-(((1.3-phenylenebis(ethyne-2.1-diyl))bis(4.1-phenylene))bis(methylene))bis(3-methyl-1H-benzo[d]imidazol-3-ium) dibromide (8):

To a solution of 1.3-bis((4-(bromomethyl)phenyl)ethynyl)benzene (100 mg, 215 µmol) in acetonitrile (1.15 mL), 1-methylbenzimidazole (71.2 mg, 539 µmol) was added and the solution was stirred 48 h at 80 °C. The reaction mixture was diluted in CH_2Cl_2 and precipitated in hexane. The product was dried under vacuum to obtain 95 % yield as white-grey powder. Purity 100 %.^{1}H NMR (400 MHz, DMSO) δ 9.86 (s, 2H), 8.05 (dd, *J* = 7.7, 1.5 Hz, 2H), 7.92 (dd, *J* = 7.7, 1.5 Hz, 2H), 7.74 – 7.53 (m, 15H), 7.50 (t, *J* = 8Hz 1H), 5.83 (s, 4H), 4.11 (s, 6H).^{13}C NMR (101 MHz, DMSO) δ 143.15, 134.87, 133.93, 132.08, 131.97, 131.73, 130.69, 128.63, 126.69, 126.62, 122.70, 122.25, 113.81, 113.62, 89.53, 88.91, 49.41, 33.43.; LCMS (ESI): calcd. for [M^{+2}] $C_{40}H_{32}N_4$: 284.1308, found 284.1297; IR (neat cm^{-1}) 3384, 2925,1608, 1509, 1450, 1274, 1092; Melting point: 290-294 °C

1.1'-(((1.3-phenylenebis(ethyne-2.1-diyl))bis(4.1-phenylene))bis(methylene))bis(3-butyl-1H-benzo[d]imidazol-3-ium) dibromide (9):

To a solution of 1.3-bis((4-(bromomethyl)phenyl)ethynyl)benzene (80 mg, 172 µmol) in acetonitrile (1 mL), 1-butylbenzimidazole (75.1 mg, 431 µmol) was added and the solution was stirred 48 h at 80 °C. The reaction mixture was diluted with CH_2Cl_2 and precipitated in hexane. The product was dried under vacuum to obtain 78 % yield as white-grey powder. Purity 100 %. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.01 (s, 2H), 8.11 (d, *J* = 8 Hz, 2H), 7.92 (d, *J* = 8 Hz, 2H), 7.68-7.55 (m, 16H), 5.82 (s, 4H), 4.51 (t, *J* = 8 Hz, 4H), 1.90 (m, 4H), 1.35 (m, 4H), 0.92 (t, *J* = 8 Hz, 6H); ^{13}C NMR (400 MHz, DMSO-d_6) δ: 142.58. 134.87, 133.97, 132.01, 131.75, 131.37, 130.87 129.53, 128.68, 126.77, 126.71, 122.71, 122.27, 113.97, 113.84, 89.55, 88.94, 49.55, 46.64, 30.46, 19.14, 13.44; LCMS (ESI): calcd. for [M^{+2}] $C_{46}H_{44}N_4$: 326.1777, found 326.1765; IR (neat cm^{-1}) 3400, 2956, 1607, 1557, 1445, 1272, 1112; Melting point: 253-258 °C

1.1'-(((1.3-phenylenebis(ethyne-2.1-diyl))bis(4.1-phenylene))bis(methylene))bis(3-octyl-1H-benzo[d]imidazol-3-ium) dibromide (10) :

To a solution of 1.3-bis((4-(bromomethyl)phenyl)ethynyl)benzene (150 mg, 323 µmol) in acetonitrile (1.65 mL), 1-octylbenzimidazole (186 mg, 808 µmol) was added and the solution was stirred 24 h at 80 °C. The reaction mixture was diluted with CH_2Cl_2 and precipitated in hexane. The product was dried under vacuum to obtain 95 % yield as white-grey powder. Purity 99.21 %.^{1}H NMR (400 MHz, DMSO) δ 10.00 (s, 2H), 8.13 (dd, *J* = 7.7, 1.5 Hz, 2H), 7.95 (dd, *J* = 7.7, 1.5 Hz, 2H), 7.76 – 7.63 (m, 5H), 7.63 – 7.54 (m, 10H), 7.49 (t, *J* = 6.8 Hz, 1H), 5.83 (s, 4H), 4.52 (t, *J* = 7.3 Hz, 4H), 1.93 (q, *J* = 7.1 Hz, 4H), 1.42 – 1.11 (m, 21H), 0.84 (t, *J* = 6.6 Hz, 6H).^{13}C NMR (101 MHz, DMSO) δ 142.55, 134.89, 133.92, 131.98, 131.73, 131.33, 130.86, 129.51, 128.64, 126.77, 126.70, 122.69, 122.26, 113.95, 113.82, 89.51, 88.92, 49.52, 46.86, 31.13, 28.50, 28.39, 25.77, 22.05, 13.93.LCMS (ESI): calcd. for $[M^{+2}]$ $C_{54}H_{60}N_4$: 382.2403, found 382.2420; IR (neat cm^{-1}) 3121, 3009, 2952 1605, 1557, 1452, 1219, 1060; Melting point: 239-243 °C

2.6-bis((trimethylsilyl)ethynyl)pyridine (12): To a solution of 2.6-dibromopyridine (300 mg, 1.27 mmol) in degassed toluene (6.00 mL), copper (I) iodine (24.10 mg, 127 µmol), tetrakis(triphenylphosphine) palladium (0) (146 mg, 127 µmol) and piperidine (751 µL, 7.60 mmol) was added. After 5 minutes stirring, ethynyltrimethylsilane (526 µL, 3.80 mmol) was slowly added and the reaction was stirred overnight under nitrogen. The reaction was quenched with water and the organic layer was extracted 3x with CH_2Cl_2. The organic layer was washed with saturated NH_4Cl and dried over Na_2SO_4. The reaction mixture was concentrated on a rotavap and the product was purified by flash chromatographic. (25:75 hexane/DCM) in 100 % yield as a pink-brown powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.83 (t, *J* = 8 Hz, 1H), 7.54 (d, *J* = 8 Hz, 2H), 0.27 (s, 18H);^{13}C NMR (101 MHz, DMSO) δ 142.69, 138.17, 127.51, 103.87, 95.10, 0.04. LCMS (ESI): calcd. for $[M+H]^+$ $C_{15}H_{21}NSi_2$: 272.1285, found 272.1285.

2.6-diethynylpyridine (13): To a solution of 2.6-bis((trimethylsilyl)ethynyl)pyridine (1.50 g, 5.52 mmol) in anhydrous CH_2Cl_2 (27.6 mL), TBAF (3.61 g, 13.8 mmol) was added. The solution was stirred at room temperature for 3-4 h. The reaction was quenched with water and the organic layer was extracted 3x with CH_2Cl_2. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under pressure and the crude reaction mixture was purified on silica gel (50:50 ethyl acetate / hexane) to afford 2.6-diethynylpyridine as white-brown powder in 80 % yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.81 (t, *J* = 10 Hz, 1H), 7.54 (d, *J* = 10 Hz, 2H), 4.36 (s, 2H);^{13}C NMR (126 MHz, DMSO) δ 142.10, 137.78, 127.40, 82.31, 80.85. LCMS (ESI): calcd. for $[M+H]^+$ C_9H_5N: 128.0495, found 128.0495.

((pyridine-2.6-diylbis(ethyne-2.1-diyl))bis(4.1-phenylene))dimethanol (14) : To a solution of 4-iodobenzyl alcohol (1.24 g, 5.31 mmol) in degassed toluene (9.83 mL), triphenylphosphine (77.4 mg, 295 µmol), copper(I) iodine (37.4 mg, 197 µmol), bis(triphenylphosphine) palladium(II) chloride (138 mg, 197 µmol) and triethylamine (0.726 mL, 11.8 mmol) was added. After 5 minutes stirring, 2.6-diethylnylpyridine was slowly added and the mixture was stirred 24 h under nitrogen at ambient temperature. The reaction was quenched with water and the organic layer was extracted 3x with CH_2Cl_2. The combine organic layer was washed with a 1M aqueous NH_4Cl solution and dried over Na_2SO_4. The reaction mixture was concentrated on a rotavap and the product was obtained by flash chromatographic (80:20 hexane/ethyl acetate and 10:35:55 methanol/hexane/ethyl acetate) in 72 % yield as a yellow-orange powder. ^{1}H NMR (400 MHz, DMSO) δ 7.92 (t, *J* = 7.8 Hz, 1H), 7.68 – 7.58 (m, 6H), 7.43 (d, *J* = 8.2 Hz, 4H), 5.35 (t, *J* = 5.7 Hz, 2H), 4.56 (d, *J* = 5.7 Hz, 4H).^{13}C NMR (101 MHz, DMSO) δ 144.92, 143.28, 138.12, 132.07, 127.18, 127.09, 119.69, 89.62, 88.39, 62.93. LCMS (ESI): calcd. for $[M+H]^+$ $C_{23}H_{17}NO_2$: 340.1332, found 340.1344.

2.6-bis((4-(bromomethyl)phenyl)ethynyl)pyridine (15): To a solution of ((pyridine-2.6-diylbis(ethyne-2.1-diyl))bis(4.1-phenylene))dimethanol (330 mg, 972 µmol) in anhydrous CH_2Cl_2 (5.00 mL), phosphorus tribromide (274 µL, 2.92 mmol) was added drop wise to the solution at 0 °C. The reaction was warmed to room temperature. After 2h, the solution was slowly quenched with saturate aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3x 20 mL). The organic layers were combined and dried over Na_2SO_4. The solvent was removed under pressure and the crude reaction mixture was purified on silica gel (70:30 ethyl acetate / hexane) to obtain dibromide as yellow powder in 44 % yield. ^{1}H NMR (400 MHz, DMSO) δ 7.92 (t, *J* = 7.8 Hz, 1H), 7.70 – 7.59 (m, 6H), 7.54 (d, *J* = 8.3 Hz, 4H), 4.75 (s, 4H). ^{13}C NMR (101 MHz, DMSO) δ 144.53, 142.70, 137.83, 131.81, 131.63, 126.73, 119.20, 89.37, 87.82, 62.46. LCMS (ESI): calcd. for $[M+H]^+$ $C_{23}H_{15}Br_2N$: 465.9625, found 465.9644.

1.1'-(((pyridine-2.6-diylbis(ethyne-2.1-diyl))bis(4.1-phenylene))bis(methylene))bis(3-methyl-1H-benzo[d]imidazol-3-ium) dibromide (16): To a solution of 1.3-bis((4-(bromomethyl)phenyl)ethynyl)pyridine (130 mg, 279 µmol) in acetonitrile (1.40 mL), 1-methylbenzimidazole (77.6 mg, 587 µmol) was added and the solution was stirred 48 h at 80 °C. The reaction mixture was diluted in CH_2Cl_2 and crashed in hexane. The product was dried under vacuum to obtain 85 % yield as pale-brown powder. Purity 99.15 %.^{1}H NMR (400 MHz, DMSO) δ 9.94 (s, 2H), 8.08 (dd, *J* = 8.2, 1,7 Hz 2H), 7.99 – 7.90 (m, 3H), 7.76 – 7.63 (m, 10H), 7.61 (d, *J* = 8.2 Hz, 4H), 5.88 (s, 4H), 4.13 (s, 6H).^{13}C NMR (101 MHz, DMSO) δ 143.17, 142.52, 137.84, 135.59, 132.28, 132.06, 130.68, 128.70, 126.69, 126.61, 121.35, 113.82, 113.62, 88.79, 88.27, 49.33, 33.46; LCMS (ESI): calcd. for $[M^{+2}]$ $C_{39}H_{31}N_5$: 284.6284, found 284.6287; IR (neat cm^{-1}) 3406, 2904, 2211, 1618, 1556, 1509, 1444, 1230, 1091; Melting point: 236-238 °C.

1.1'-(((pyridine-2.6-diylbis(ethyne-2.1-diyl))bis(4.1-phenylene))bis(methylene))bis(3-butyl-1H-benzo[d]imidazol-3-ium) dibromide (17):

To a solution of 1.3-bis((4-(bromomethyl)phenyl)ethynyl)pyridine (210 mg, 451 µmol) in acetonitrile (1 mL), 1-butylbenzimidazole (165 mg, 948 µmol) was added and the solution was stirred 48 h at 80 °C. The reaction mixture was diluted with CH_2Cl_2 and crashed in hexane. The product was dried under vacuum to obtain 60 % yield as pink powder. Purity 95.98 %.1H NMR (500 MHz, DMSO) δ 10.04 (s, 2H), 8.14 (dt, *J* = 8.2, 1,7 Hz 2H), 7.94 (dt, *J* = 8.2, 1,7 Hz 2H), 7.74 – 7.60 (m, 8H), 7.58 (m, 7H), 7.49 (t, J = 7.1 Hz, 1H), 5.84 (s, 4H), 4.53 (t, J = 7.3 Hz, 4H), 1.98 – 1.88 (p, J = 7.4 Hz 4H), 1.43 – 1.32 (h, J = 7.4 Hz 4H), 0.94 (t, J = 7.4 Hz, 6H).13C NMR (126 MHz, DMSO) δ 142.58, 134.86, 133.97, 132.01, 131.75, 131.37, 130.87, 129.52, 128.68, 126.77, 126.71, 122.70, 122.27, 113.97, 113.84, 89.54, 88.94, 49.55, 46.64, 30.46, 19.14, 13.43.; LCMS (ESI): calcd. for $[M^{+2}]$ $C_{45}H_{43}N_5$: 326.6754, found 326.6769; IR (neat cm^{-1}) 3402, 2904, 2213, 1609, 1556, 1510, 1441, 1287, 1113; Melting point: 262-266 °C

1.1'-(((pyridine-2.6-diylbis(ethyne-2.1-diyl))bis(4.1-phenylene))bis(methylene))bis(3-octyl-1H-benzo[d]imidazol-3-ium) dibromide (18):

To a solution of 1.3-bis((4-(bromomethyl)phenyl)ethynyl)pyridine (109 mg, 234 µmol) in acetonitrile (1.20 mL), 1-octylbenzimidazole (124 mg, 539 µmol) was added and the solution was stirred 48 h at 80 °C. The reaction mixture was diluted with CH_2Cl_2 and precipitated in hexane. The product was dried under vacuum to obtain 30 % yield as pink powder. Purity 90.69 %. ^{1}H NMR (400 MHz, DMSO) δ 10.00 (s, 2H), 8.13 (dd, *J* = 7.1, 1.9 Hz, 2H), 7.96 (dd, *J* = 7.1, 1.9 Hz 2H), 7.92 (t, *J* = 7.8 Hz, 1H), 7.74 – 7.63 (m, 9H), 7.59 (d, *J* = 8.2 Hz, 4H), 5.85 (s, 4H), 4.52 (t, *J* = 7.3 Hz, 4H), 1.93 (p, *J* = 7.1 Hz, 4H), 1.35 – 1.23 (m, 18H), 0.84 (t, *J* = 7.1 Hz, 6H).^{13}C NMR (101 MHz, DMSO) δ 143.08, 143.01, 138.32, 136.07, 132.80, 131.82, 131.36, 129.16, 127.53, 127.27, 127.19, 121.87, 114.44, 114.28, 89.28, 88.73, 49.96, 47.35, 31.62, 28.97, 28.87, 26.26, 22.52, 14.42; LCMS (ESI): calcd. for $[M^{+2}]$

$C_{53}H_{59}N_5$: 382.7380, found 382.7391; IR (neat cm^{-1}) 3375, 2921, 2212, 1608, 1554, 1509, 1439, 1165, 1113; Melting point: 239-243 °C

LogP measurement

The compounds (5 mg) suspended in 500 uL of 1-octanol, then 500 uL of PBS pH 7.4. The suspension was stirred for 24h, then centrifuged 5 min at 13 000 rpm to allow phase separation. Each phase was equally diluted and then injected in LC-UV for quantification. The peak areas were determined using OpenLab Software and Excel was used for calculations. The partitioning coefficient was calculated using the following formula:

$$\log P = \log \frac{Aoct * Vinj\ aq}{Aaq * Vinj\ oct}$$

Where *Aoct* = Peak Area for Octanol phase signal

Aaq = Peak Area for Aqueous phase signal

Vinj aq = Injected volume of aqueous phase

Vinj oct = Injected volume of octanol phase

LC Conditions: LC System : Agilent 1200 Series, Column : Waters Symmetry C18 3.5 um, 4.6 x 75 mm. Flow Rate : 0.8 mL/min, mobile phase A: Water + 0.1% Formic Acid, mobile phase B: MeOH, gradient : 10 to 90 % B /10.5 mins.

UV Detection : Agilent 1100 Series G1315B, wavelength : 280.4 nm.

Methods for the biological assays

Culture conditions and viability tests for bacteria and yeast

MICs were determined in 96-well cell culture plates. The tests were carried out in a growing Lauria Broth (LB) medium at 37°C in triplicate. The optical density of bacterial cells (OD 0.1 to 600 nm) was measured with a Fischer Scientific 40 cell density meter model and UV-vis spectroscopy experiments were performed on a Tecan Infinite M200 microplate reader.

Biofilm inhibition

The *S. aureus* were incubated in a 37° C LB medium for 12 h and re-diluted in the LB medium to be incubated again. After 2 h incubation, the cells were re-diluted at the final concentration (DO 600nm = 0.1-0.15). The biofilms of *S. Aureus* were marked with a Live/Dead stain after 48 h incubation with antibiotics in a growing medium (LB) in an 8-well glass plate. Negative control: DMSO (final concentration 5 % volume). Positive control: 20% Triton X.

Biofilm disruption

The *S. aureus* were incubated in a 37° C LB medium for 12 h and re-diluted in the LB medium to be incubated again. After 2 h incubation, the cells were re-diluted at the final concentration (DO 600nm = 0.1-0.15). After an incubation of 48 h in 8-well chambers, the growth media was removed and the biofilms were washed two times with PBS solution (137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 1.8 mM KH2PO4, in 200 µL of distilled water) to remove planktonic cells. The S. aureus biofilms were incubated with antibiotics for 24 h in PBS solution. Negative control: DMSO (final concentration 5 % volume). Positive control: 20% triton.

Biofilm staining and confocal laser scanning microscopy (LSM) analysis

Biofilms stained with FilmTracer ™ LIVE / DEAD® Biofilm viability Kit (Molecular Probes, Life Technologies Ltd.). A fluorescent marker solution was prepared by adding 1 µL of SYTO® 9 dye and 1 µL of PI dye in 1 ml of filter-sterilized water. One hundred µL of staining solution were added to each well of an 8-well glass plate containing the mature biofilm. After 30 min at room temperature in the dark, the samples were washed with a saline phosphate buffer (PBS) solution (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, in 200 µL of distilled water). The biofilms were then observed with a confocal laser microscope (model Leica TCS SP5; Leica Microsystems CMS GmbH, Mannheim, Germany) using a 20x lens (HC PL FLUOTAR 20.0 x 0.70 DRY). A 483 nm laser was used to excite SYTO® 9, while fluorescent emission was detected from 500 to 540 nm. The PI was

excited with a laser at 535 nm and its fluorescent emission was detected from 600 to 695 nm.

Haemolytic assay

The red blood cells (RBCs) in Alserver's solution were diluted in PBS buffer and centrifuged 10 min. at 2000 rpm. The RBCs were washed two more times with PBS buffer until the surface liquid was crystal clear and were resuspended in PBS at 2% v/v. In a 96 wells plate was added 195 µL of red blood cells solution and 5 µL of compound in DMSO. The plate was incubated with light agitation (25 rpm) for 1 h at 37 °C. The plate was then centrifuged for 10 min. at 2000 rpm and 100 µL of the supernatant was transferred to another plate to analyse the absorbance (λ = 405 nm) with a Tecan Infinite M200 microplate reader.

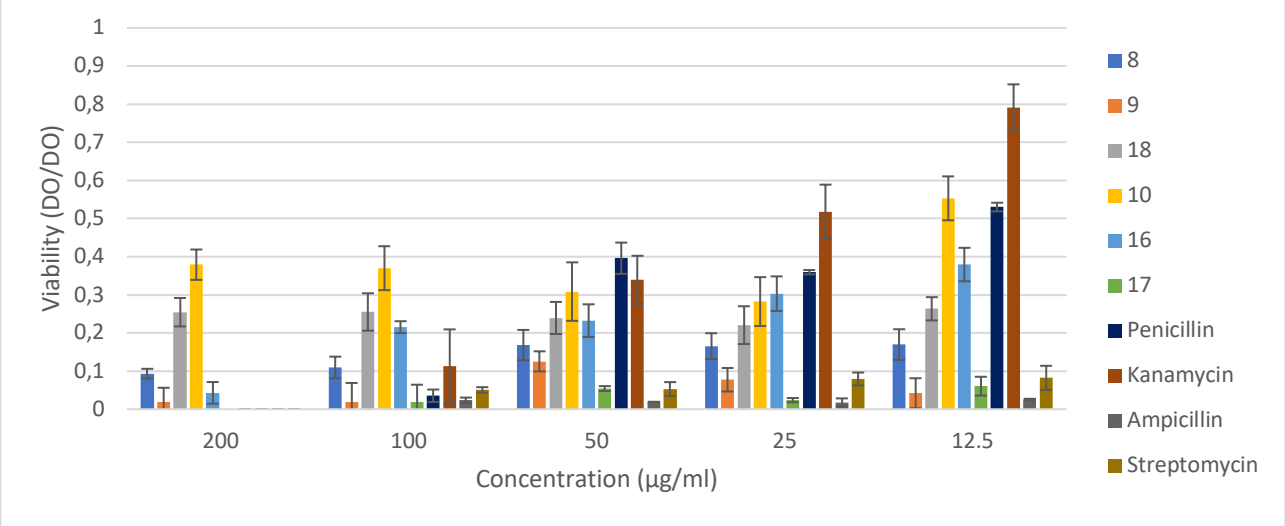


Figure S1: Average percent viability in triplicate for *E. Coli* (SK037).

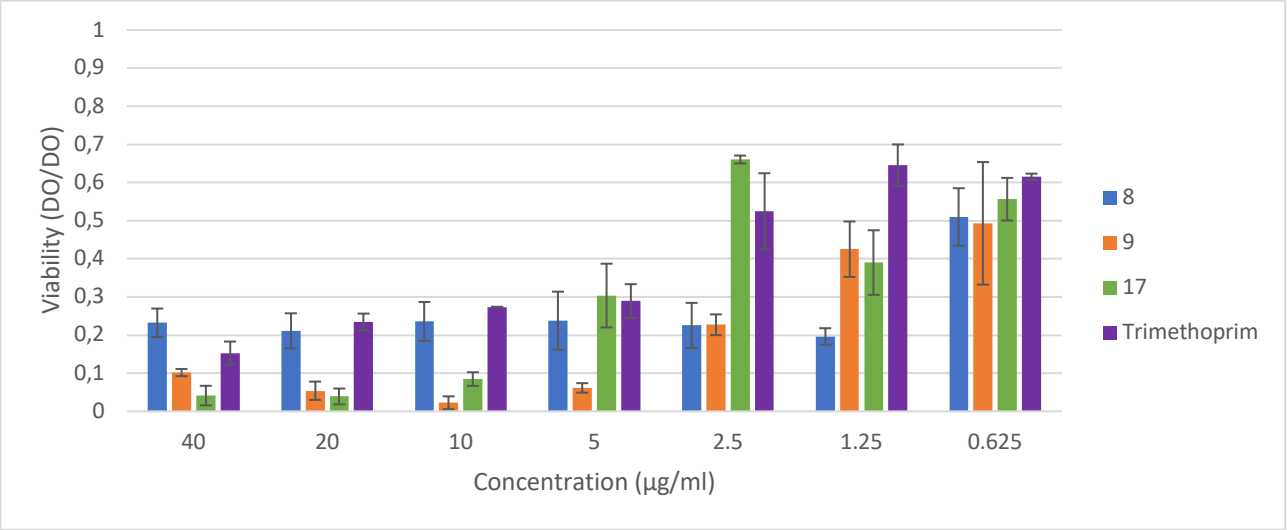


Figure S2: Average percent viability in triplicate for *E. Coli* (SK037).

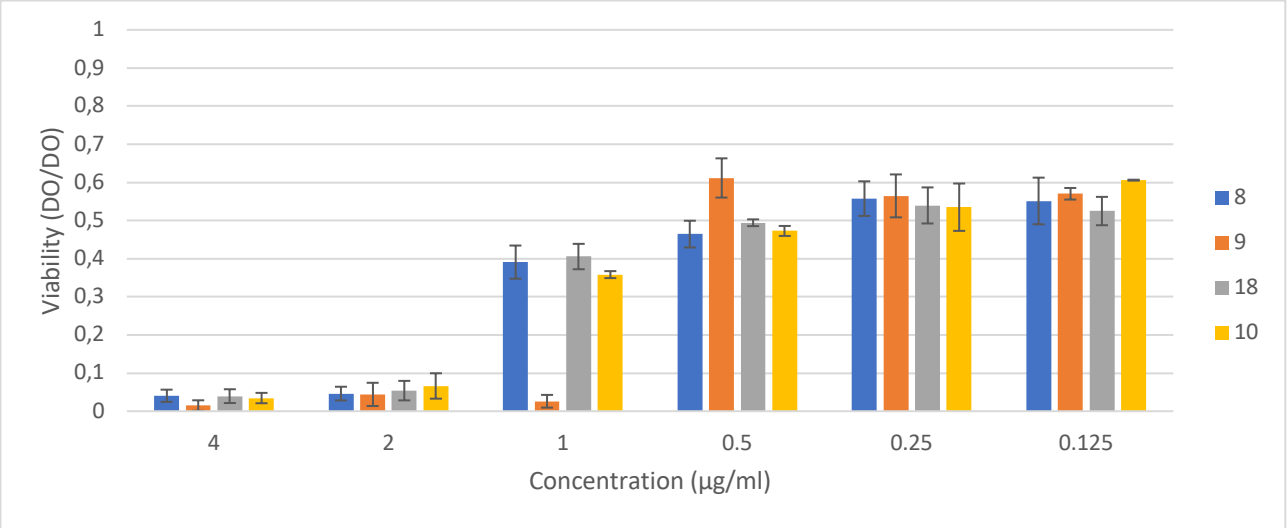


Figure S3: Average percent viability in triplicate for *MRSA = Methicillin-resistant S. aureus* (ATCC 43300).

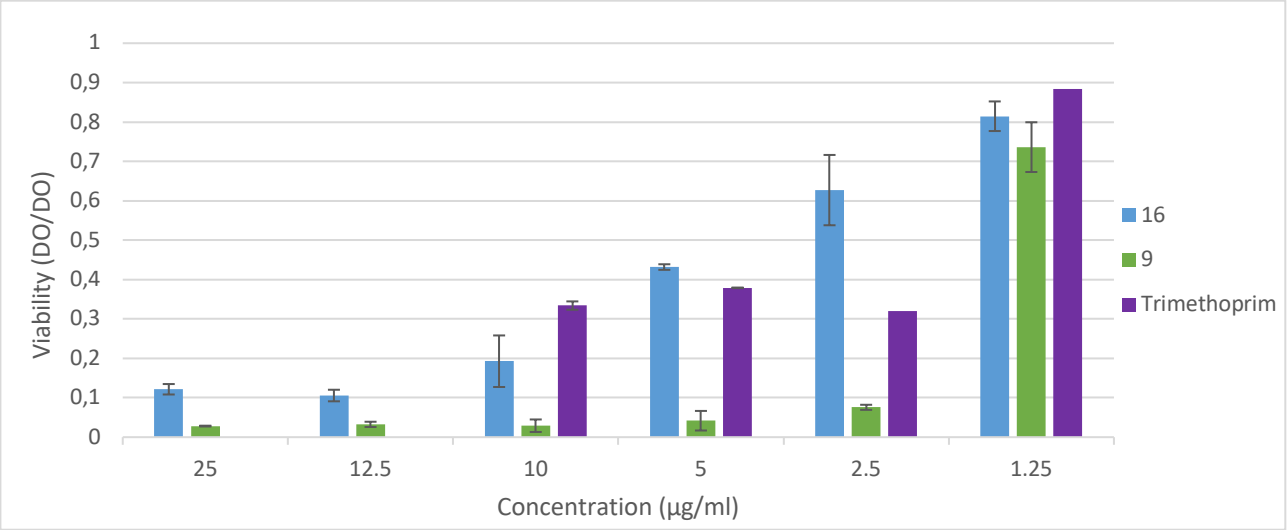


Figure S4: Average percent viability in triplicate for *MRSA = Methicillin-resistant S. aureus* (ATCC 43300).

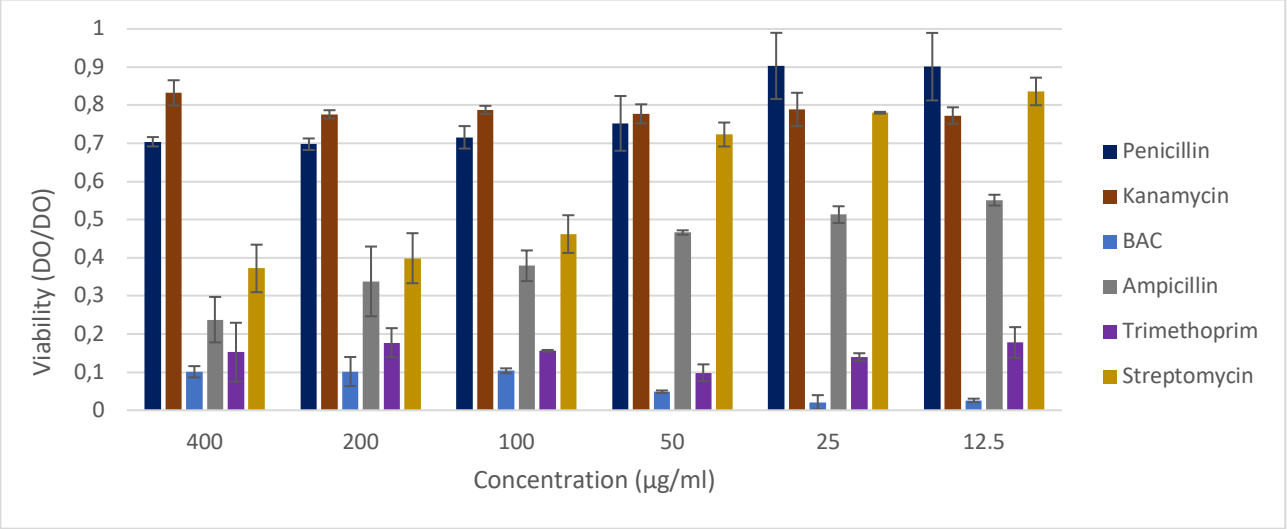


Figure S5: Average percent viability in triplicate for *MRSA = Methicillin-resistant S. aureus* (ATCC 43300).

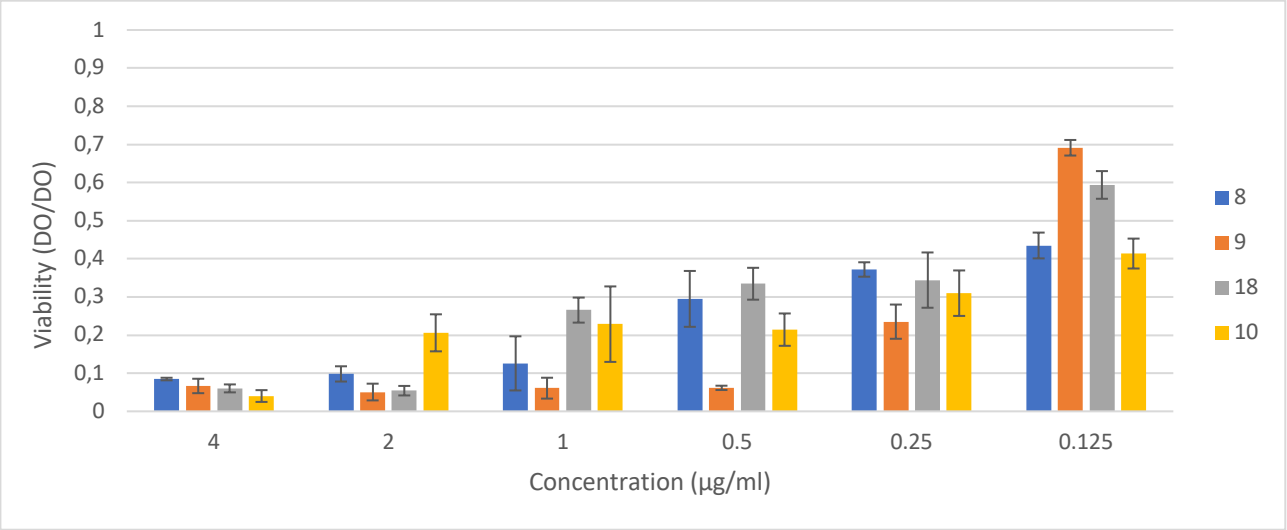


Figure S6: Average percent viability in triplicate for *VRE = vancomycin-resistant E. faecium* (BAA-2316).

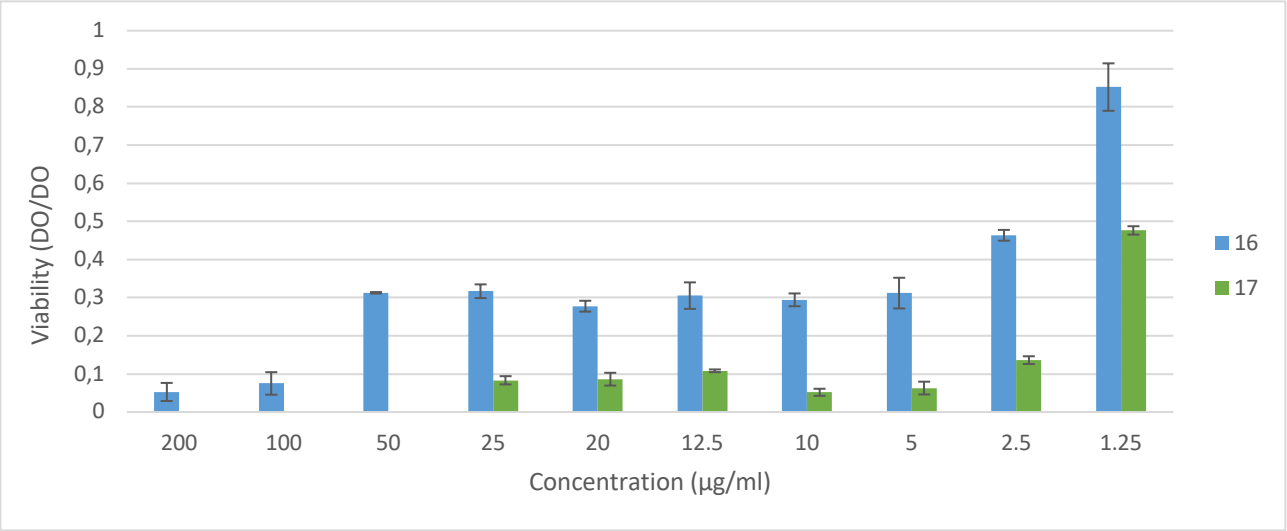


Figure S7: Average percent viability in triplicate for *VRE = vancomycin-resistant E. faecium* (BAA-2316).

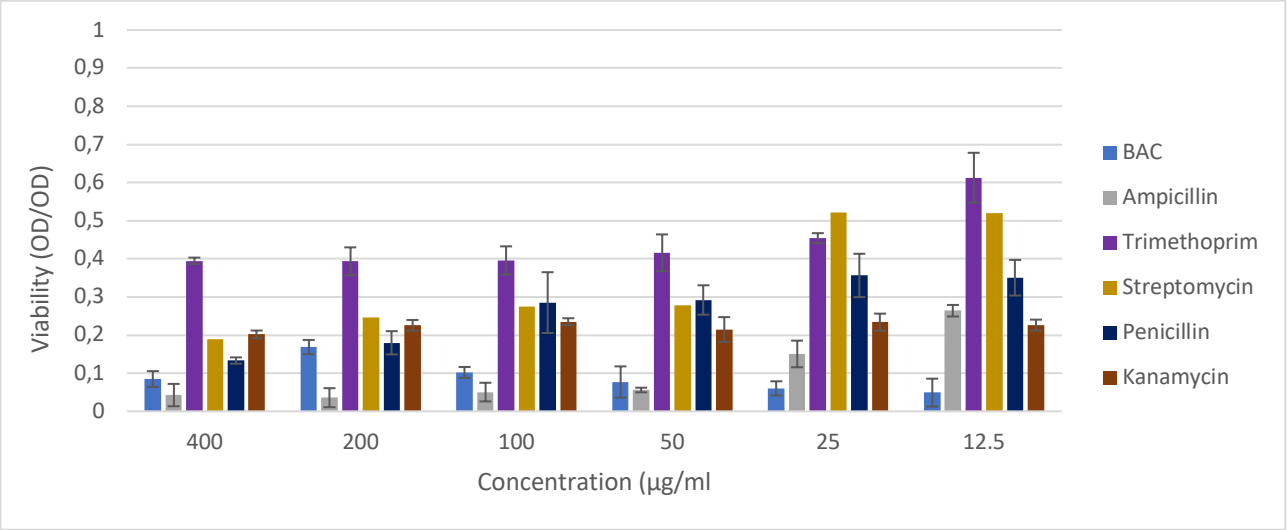


Figure S8: Average percent viability in triplicate for *VRE = vancomycin-resistant E. faecium* (BAA-2316).

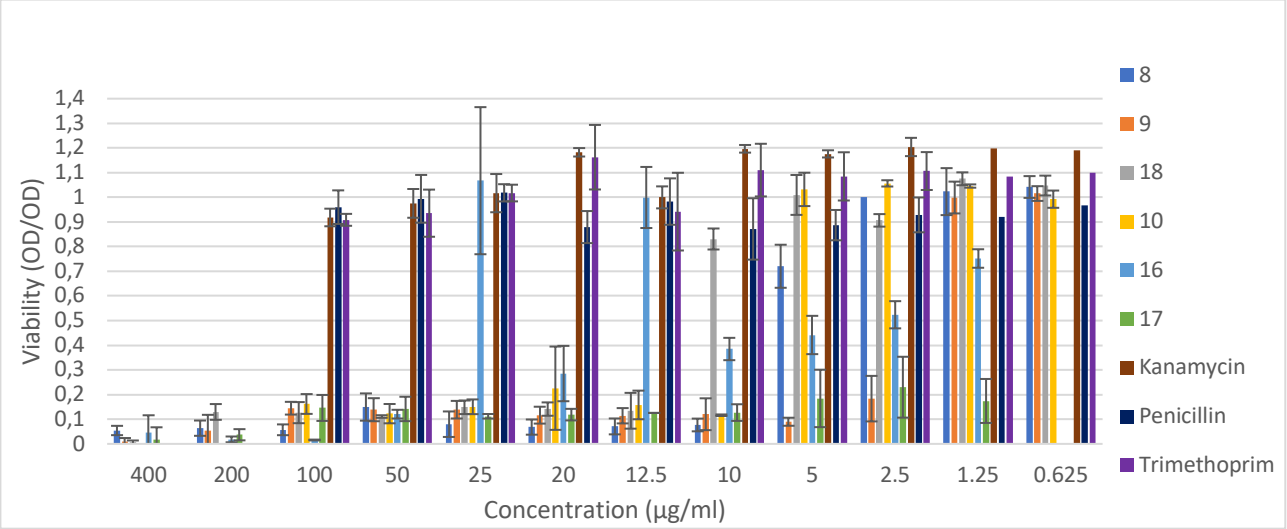


Figure S9: Average percent viability in triplicate for *C. albicans* (SC5314).

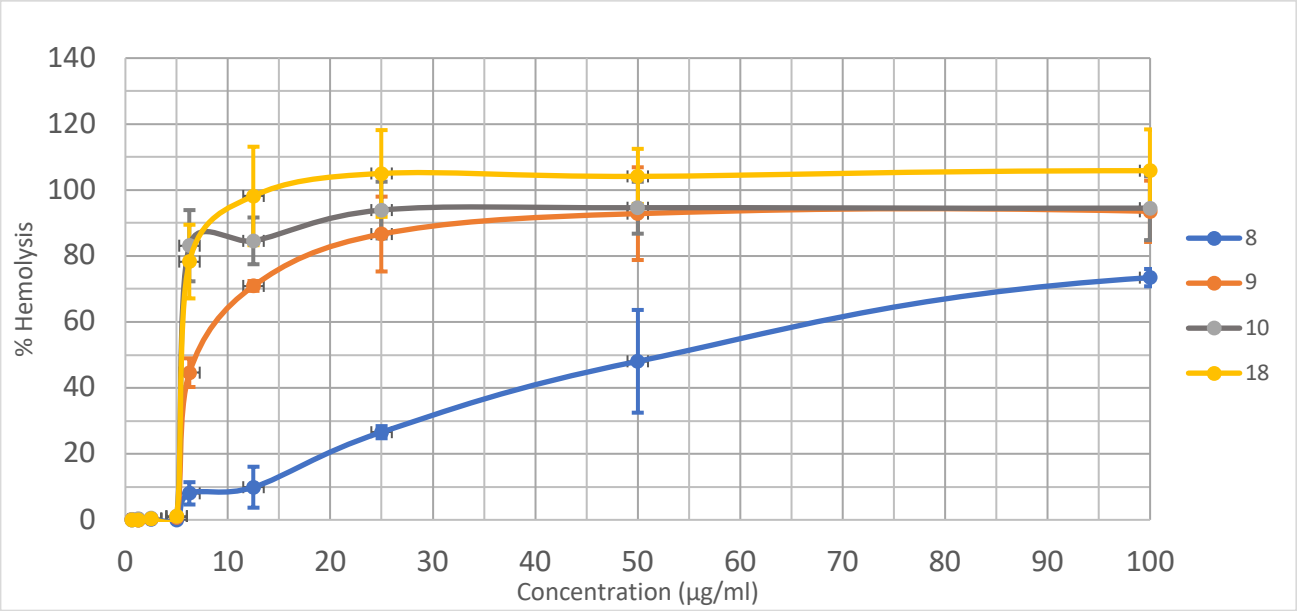
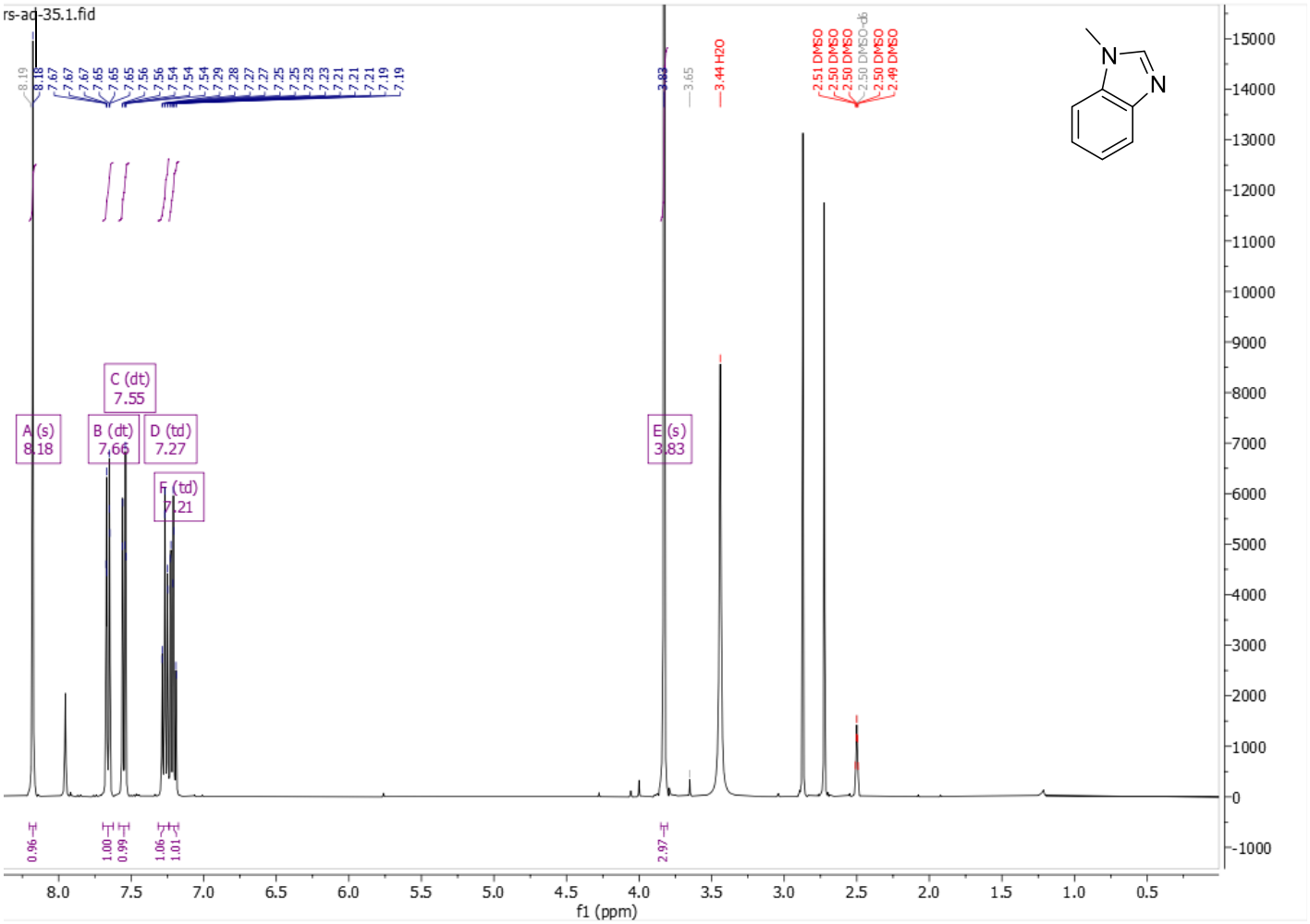
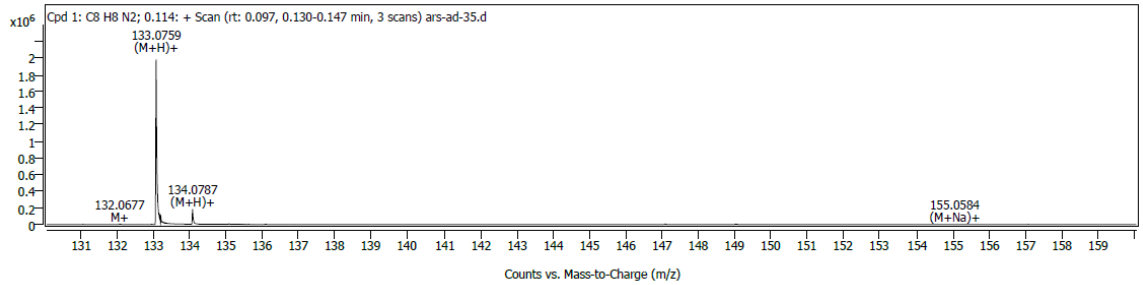


Figure S10: Average percent hemolysis in triplicate for benzimidazolium salts.

NMR spectra and LCMS of the synthesized compounds

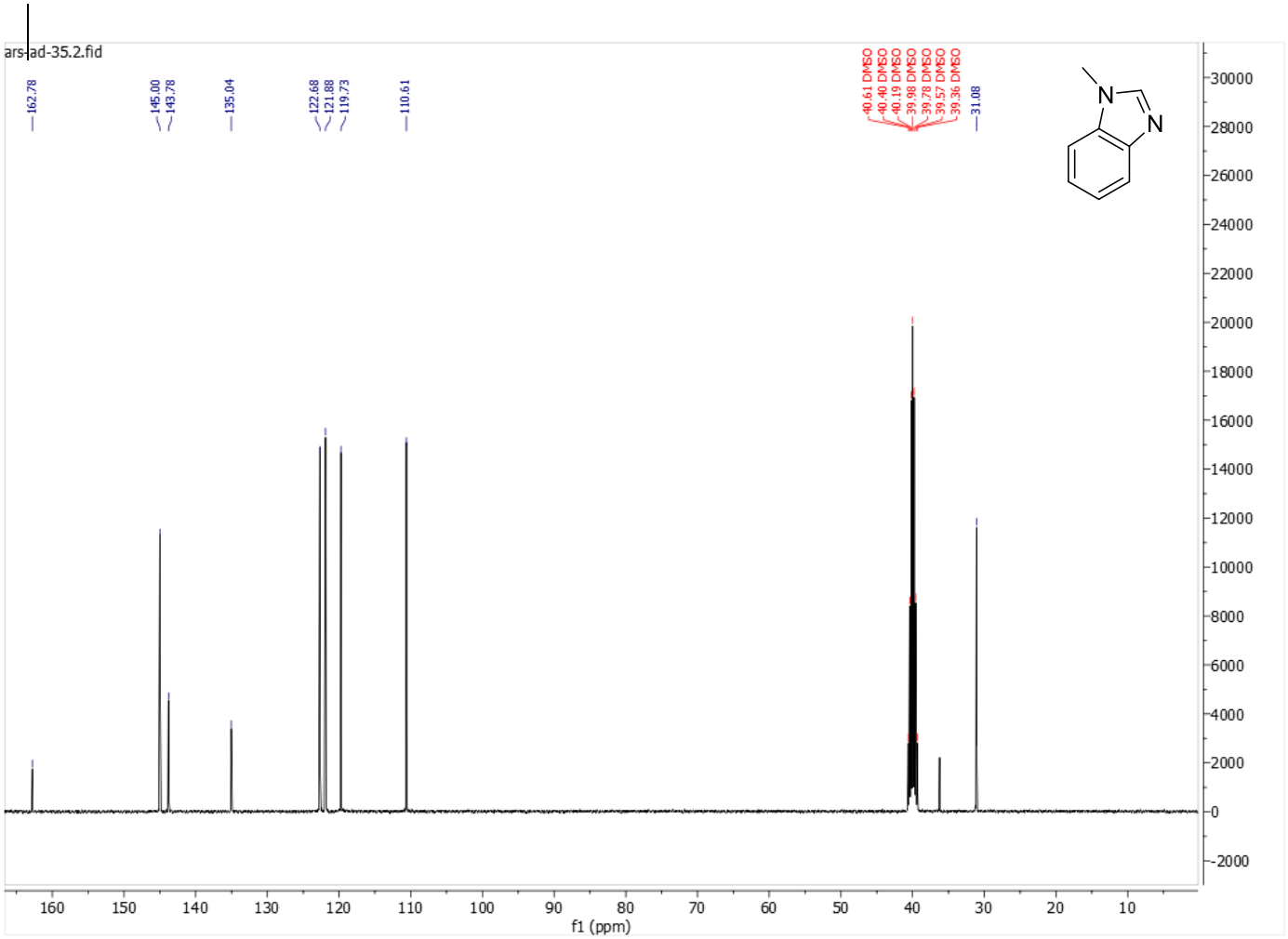
Compound (2):



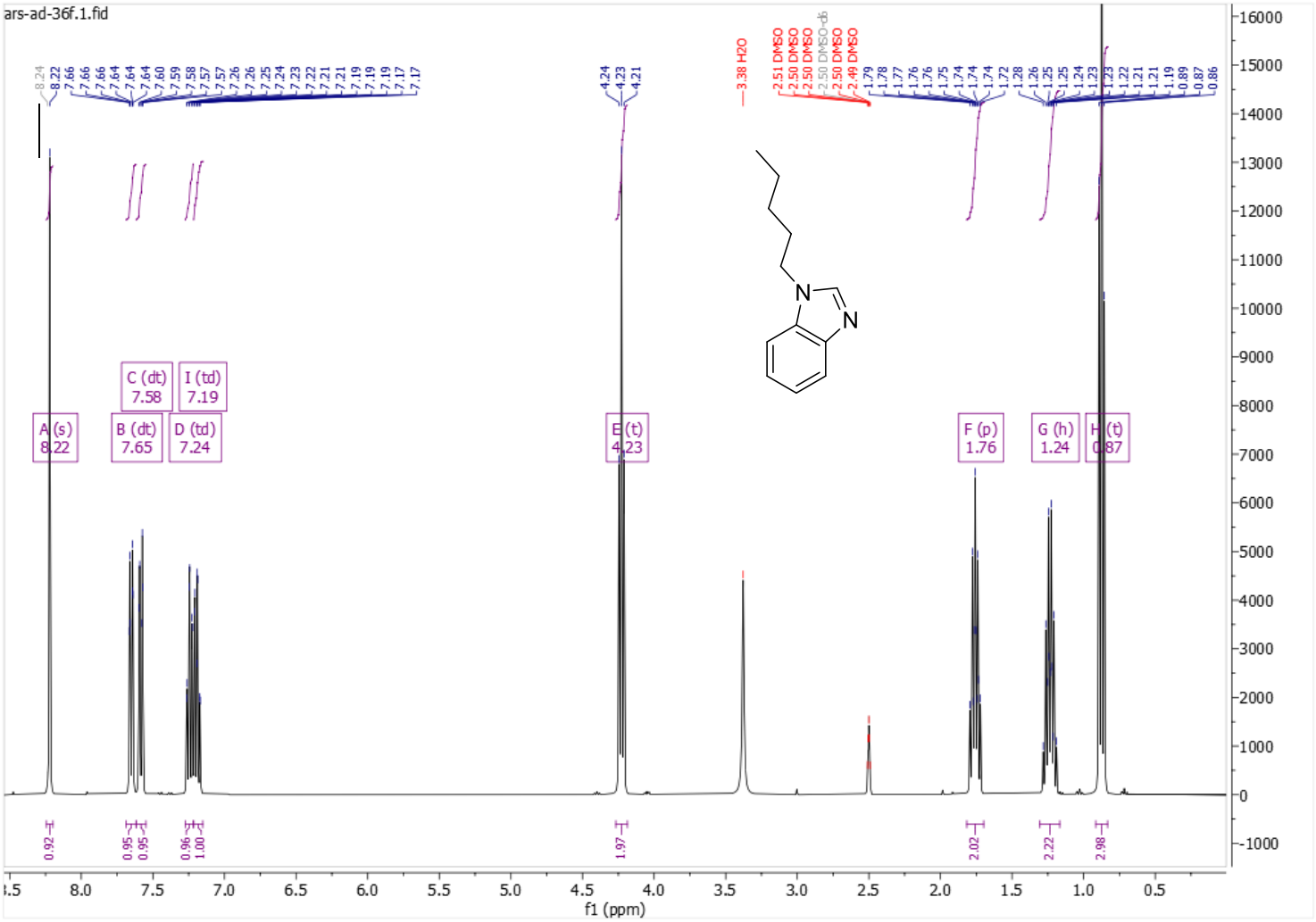


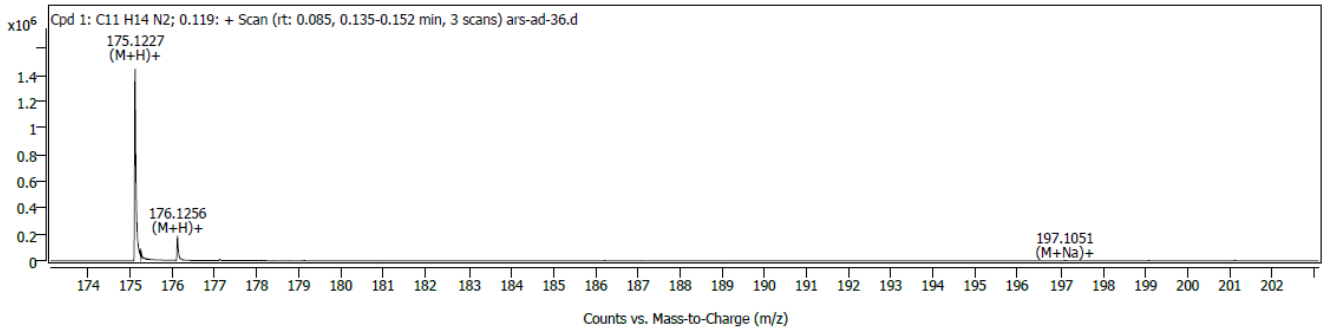
Spectrum Peaks (Max. 1)

Ion Species	Formula	Abund	m/z	m/z (Calc)	Diff (ppm)
(M+H)+	C8H8N2	2048607	133.07588	133.07602	-1.09



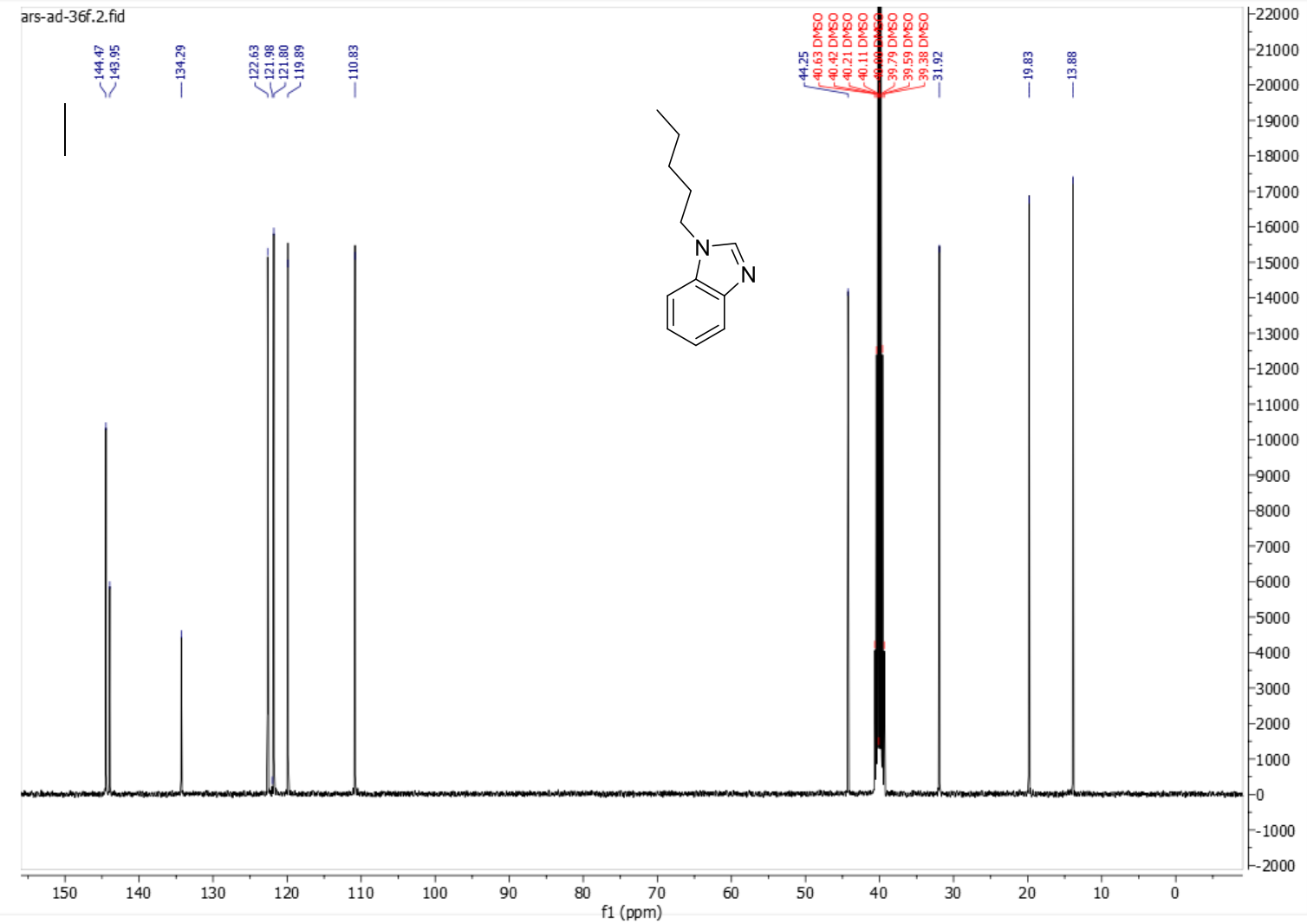
Compound (3):



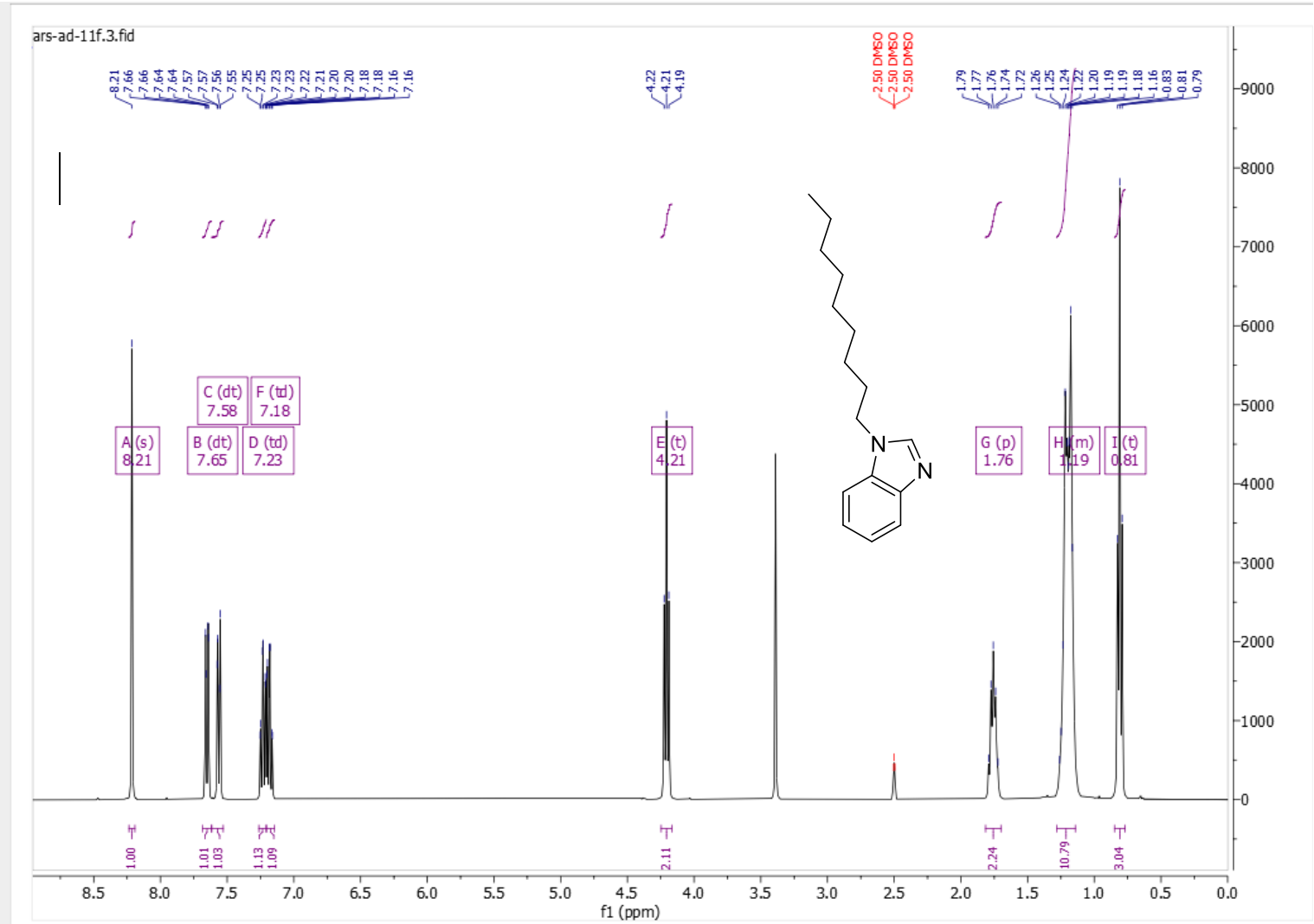


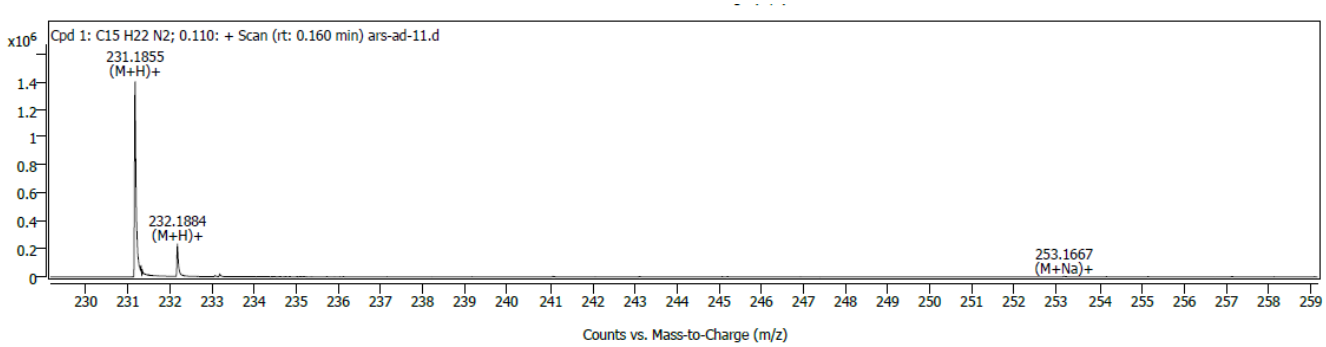
Spectrum Peaks (Max. 1)

Ion Species	Formula	Abund	m/z	m/z (Calc)	Diff (ppm)
(M+H)+	C11H14N2	1490740	175.12271	175.12297	-1.50


ars-ad-36f.2.fid
144.47
143.95
134.29
122.63
121.98
121.80
119.89
110.83
44.25
40.63 DMSO
40.42 DMSO
40.21 DMSO
40.11 DMSO
39.79 DMSO
39.59 DMSO
39.38 DMSO
31.92
19.83
13.88
N
N
f1 (ppm)

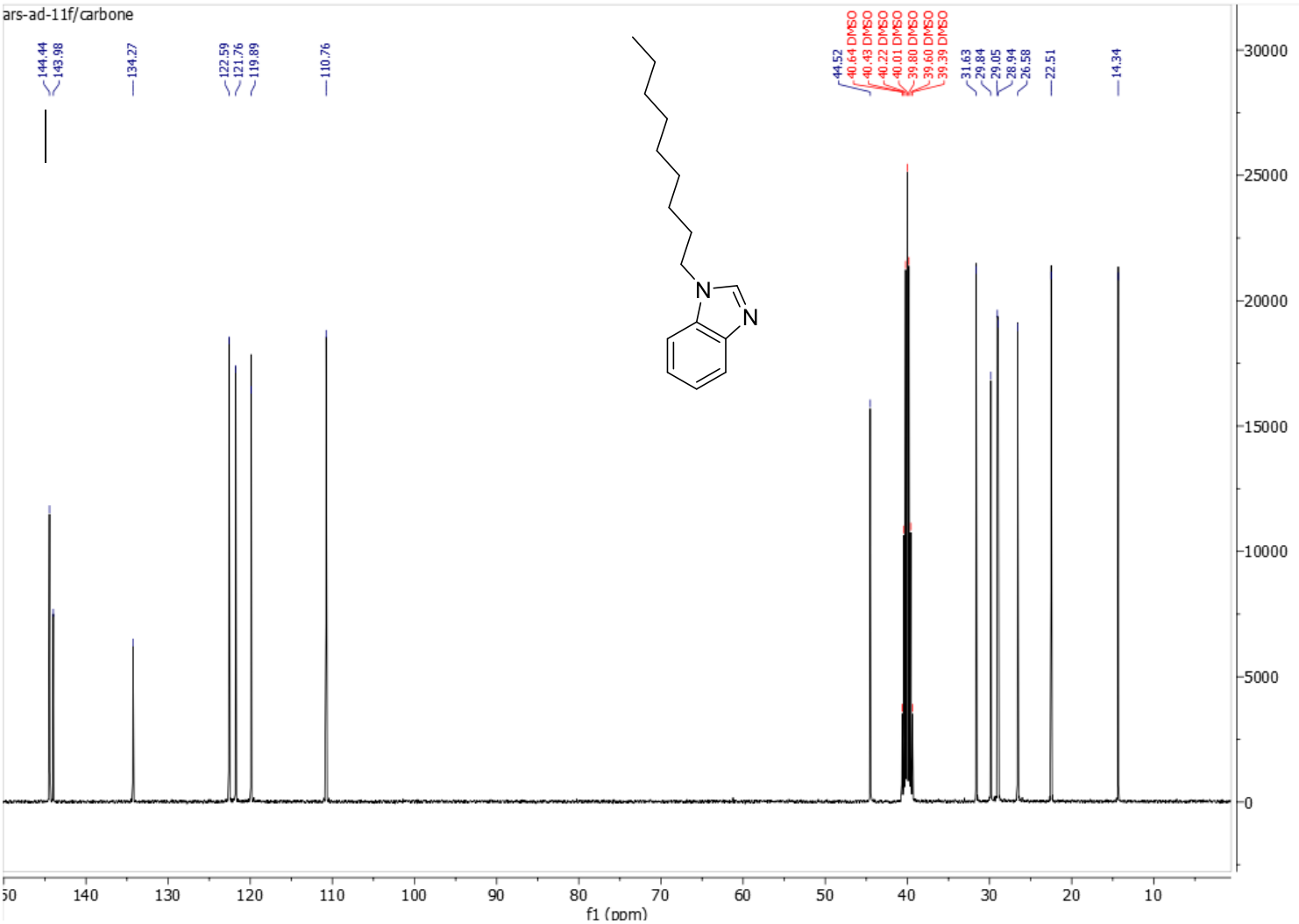
Compound (4):



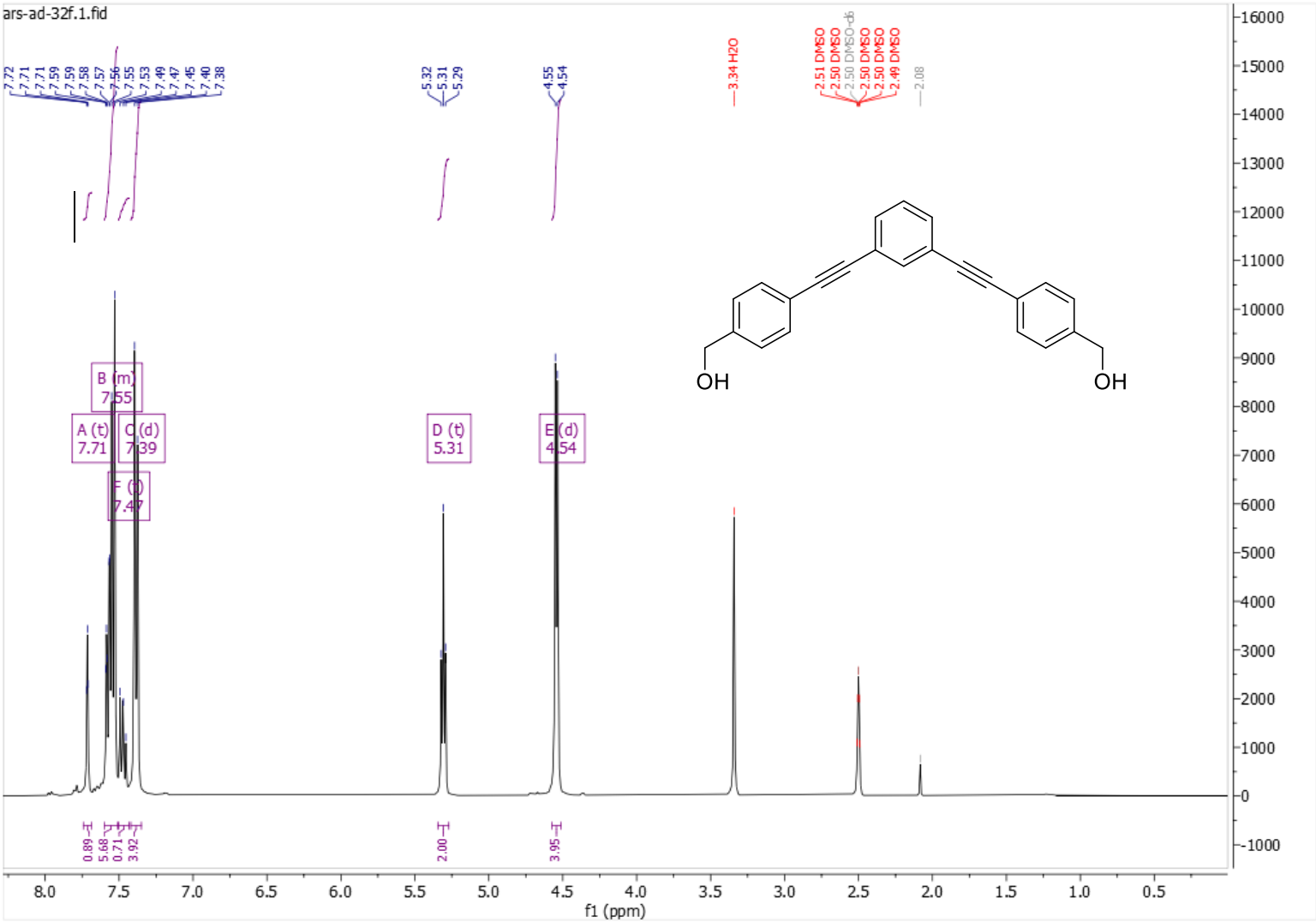


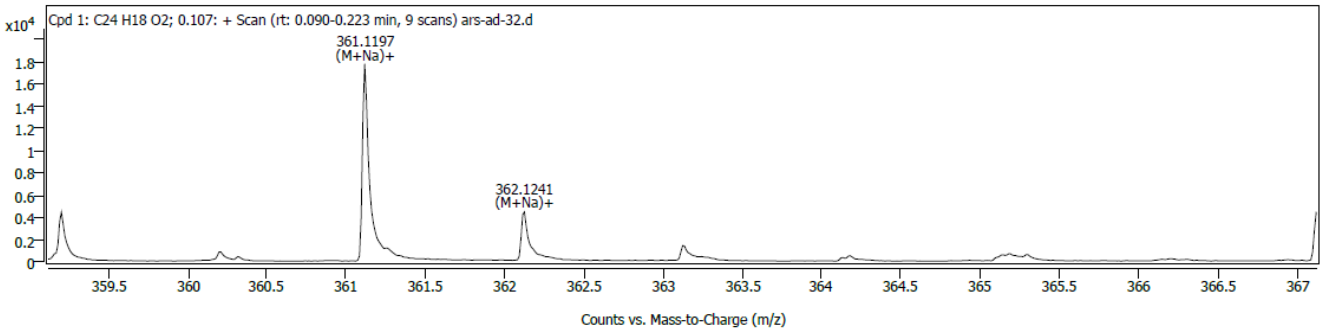
Spectrum Peaks (Max. 1)

Ion Species	Formula	Abund	m/z	m/z (Calc)	Diff (ppm)
(M+H)+	C15H22N2	1418443	231.18548	231.18558	-0.42


ars-ad-11f/carbone
144.44
143.98
134.27
122.59
121.76
119.89
110.76
44.52
40.64 DMSO
40.43 DMSO
40.22 DMSO
40.01 DMSO
39.80 DMSO
39.60 DMSO
39.39 DMSO
31.63
29.84
29.05
28.94
26.58
22.51
14.34
N
N
30000
25000
20000
15000
10000
5000
0
f1 (ppm)

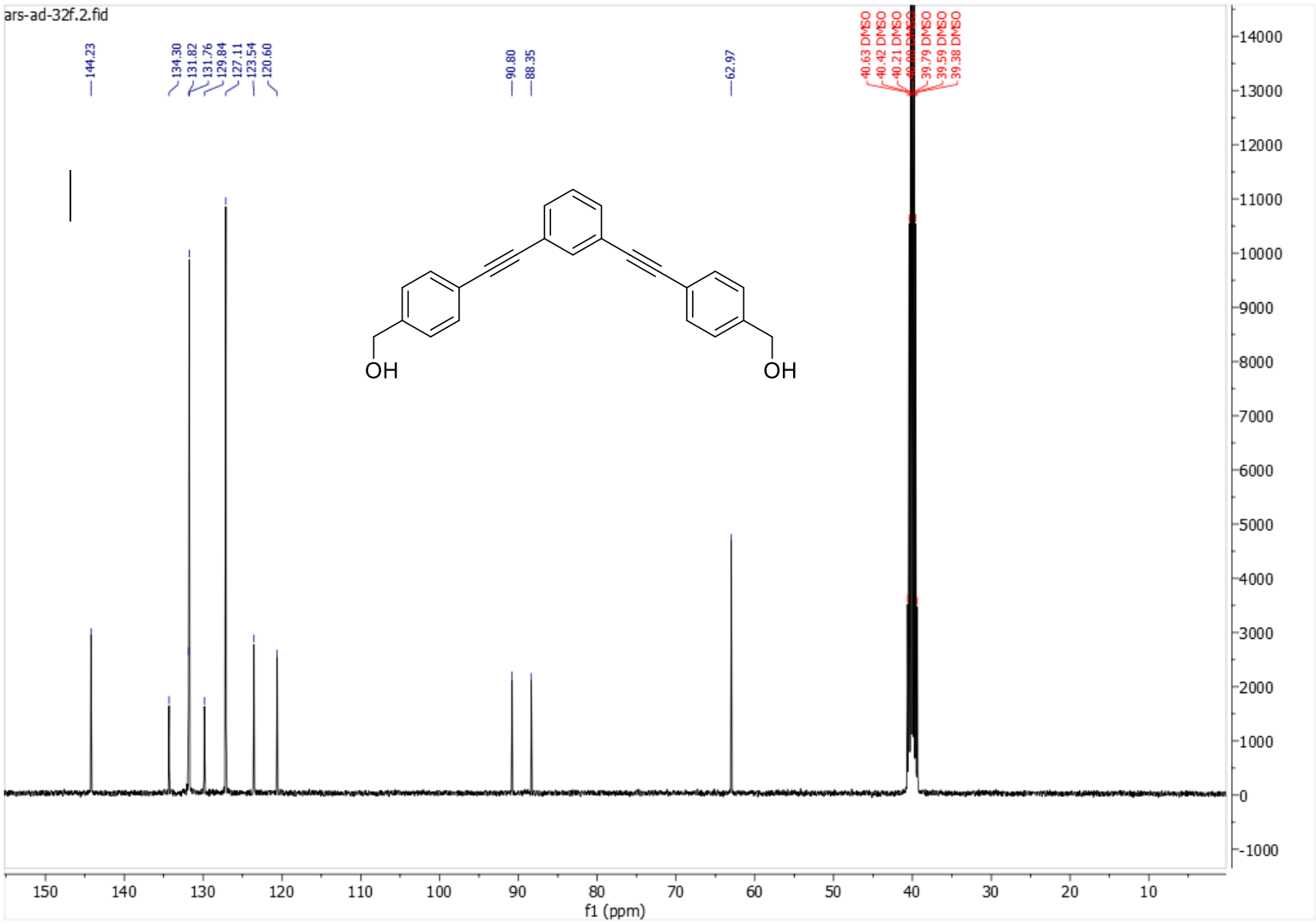
Compound (6):





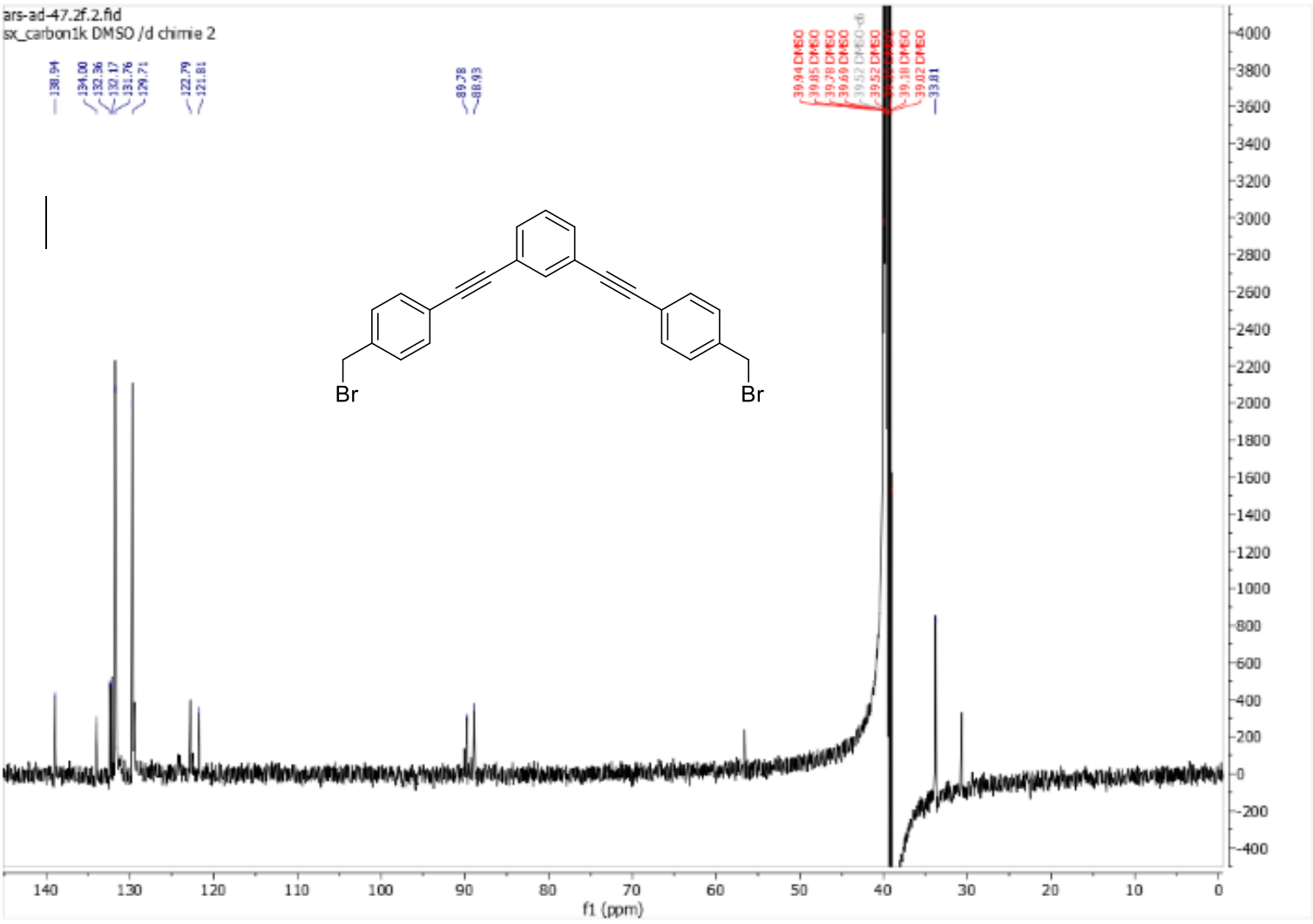
Spectrum Peaks (Max. 1)

Ion Species	Formula	Abund	m/z	m/z (Calc)	Diff (ppm)
(M+Na)+	C24H18O2	17842	361.11975	361.11990	-0.42

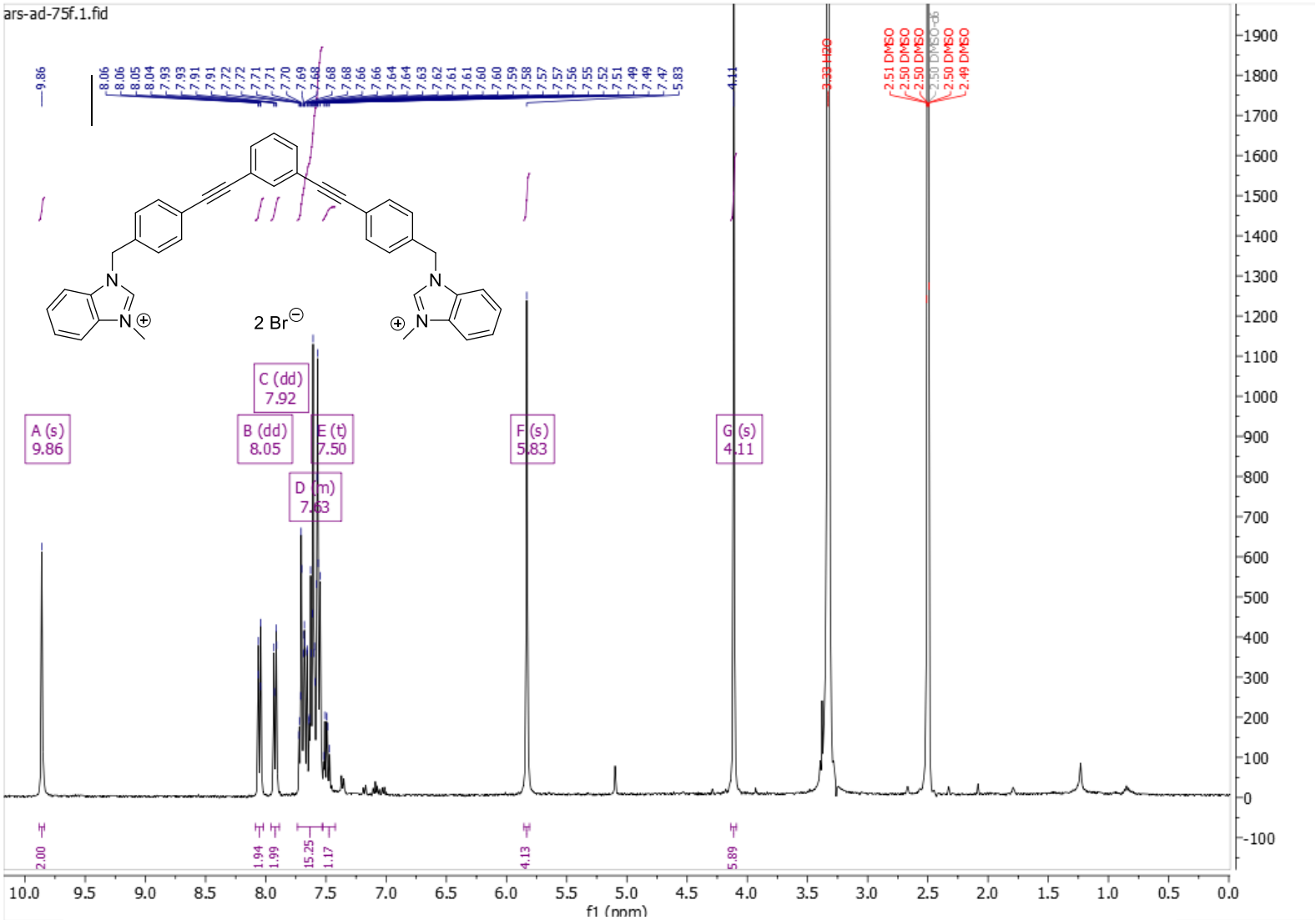

ars-ad-32f.2.fid
144.23
134.30
131.82
131.76
129.84
127.11
123.54
120.60
90.80
88.35
62.97
40.63 DMSO
40.42 DMSO
40.21 DMSO
39.79 DMSO
39.59 DMSO
39.38 DMSO
OH
OH
f1 (ppm)

Compound (7):

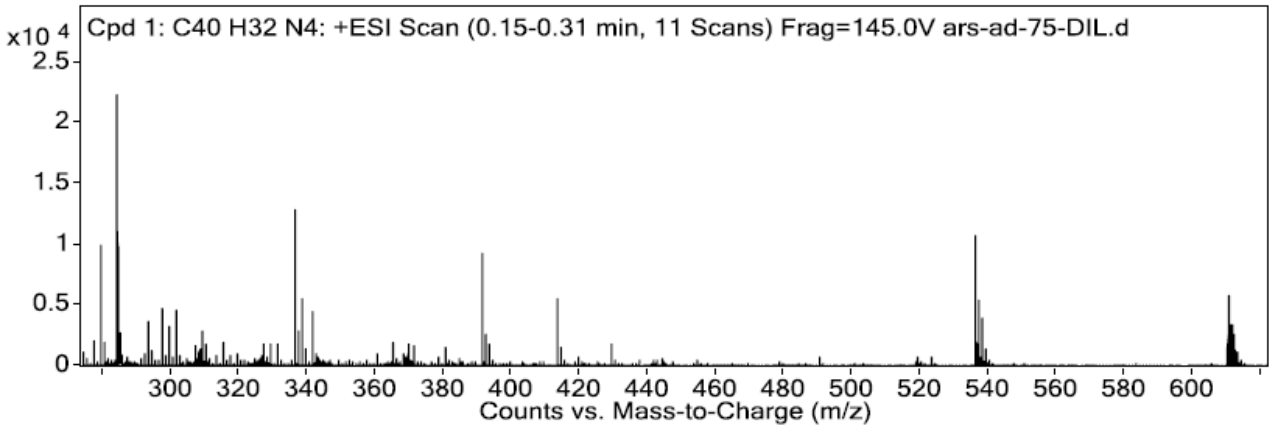

arsead-47-2f-1.fid
bbfo-proton-DMSO-d chimie 2
7.61
7.59
7.58
7.55
7.53
7.52
7.52
7.51
7.49
4.76
Br
Br
C (m)
7.76
B (m)
7.56
A (s)
4.76
1.31
11.00
4.25
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100


ars-ad-47.2f.2.fid
sx_carbon1k DMSO /d chimie 2
138.94
134.00
132.36
132.17
131.76
129.71
122.79
121.81
89.78
88.93
39.94 DMSO
39.85 DMSO
39.78 DMSO
39.69 DMSO
39.52 DMSO-d6
39.52 DMSO
39.38 DMSO
39.02 DMSO
33.81
Br
Br
f1 (ppm)

Compound (8):

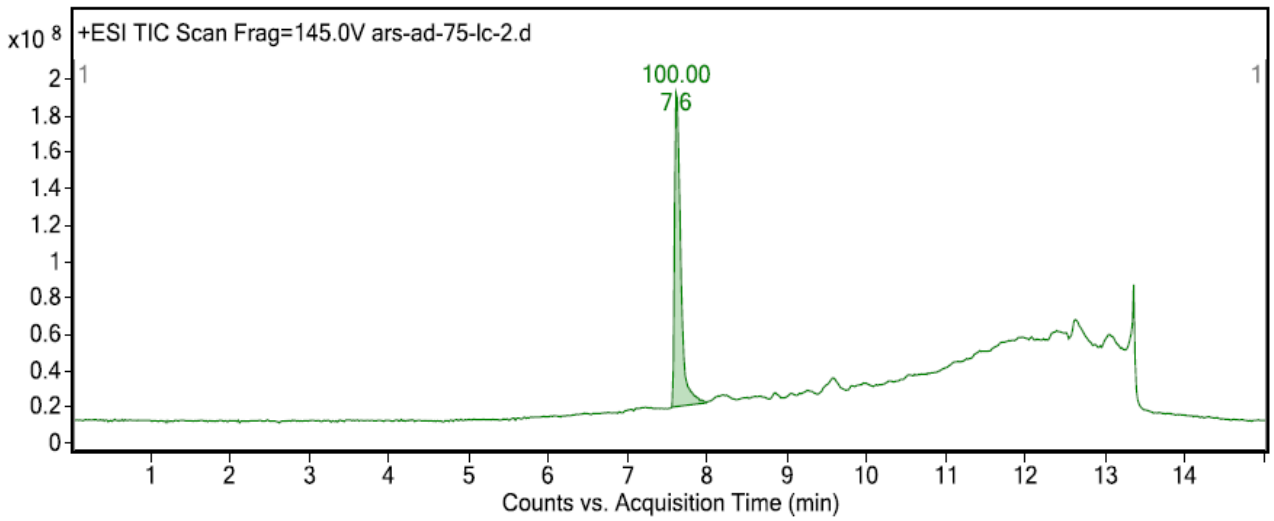


MS Zoomed Spectrum



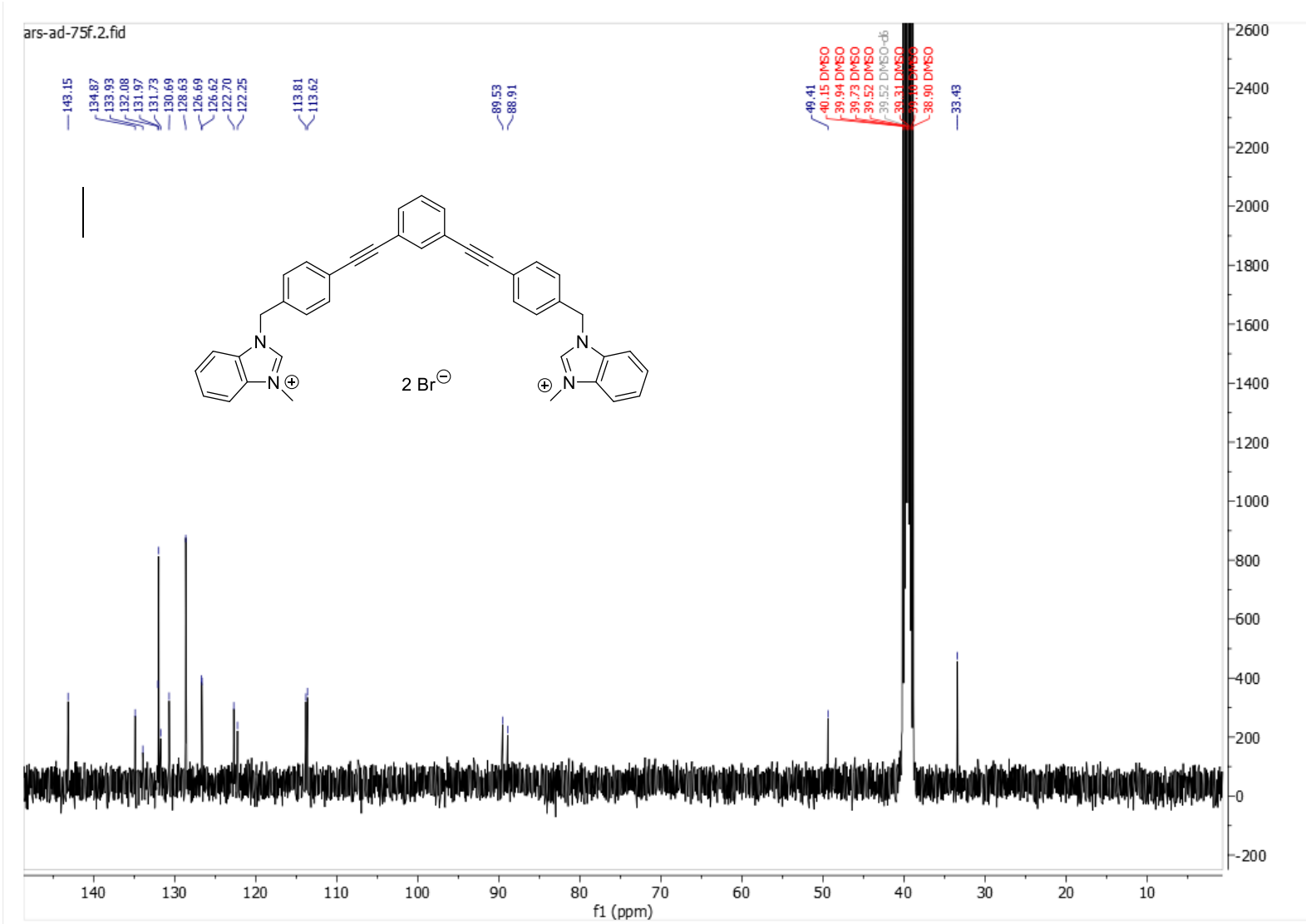
MS Spectrum Peak List

Ion	Formula	Abund	*Expe. m/z*	*Calc. m/z*	Diff(ppm)
M+2	C40H32N4	22378.26	284.12967	284.1308	3.96

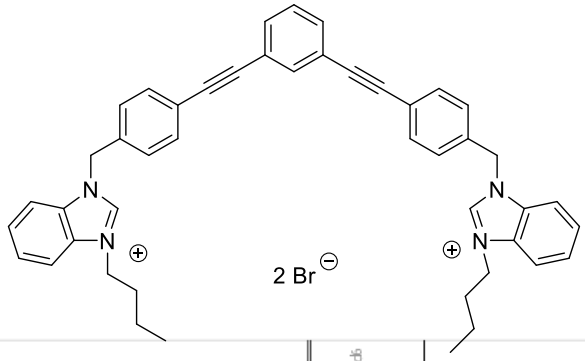


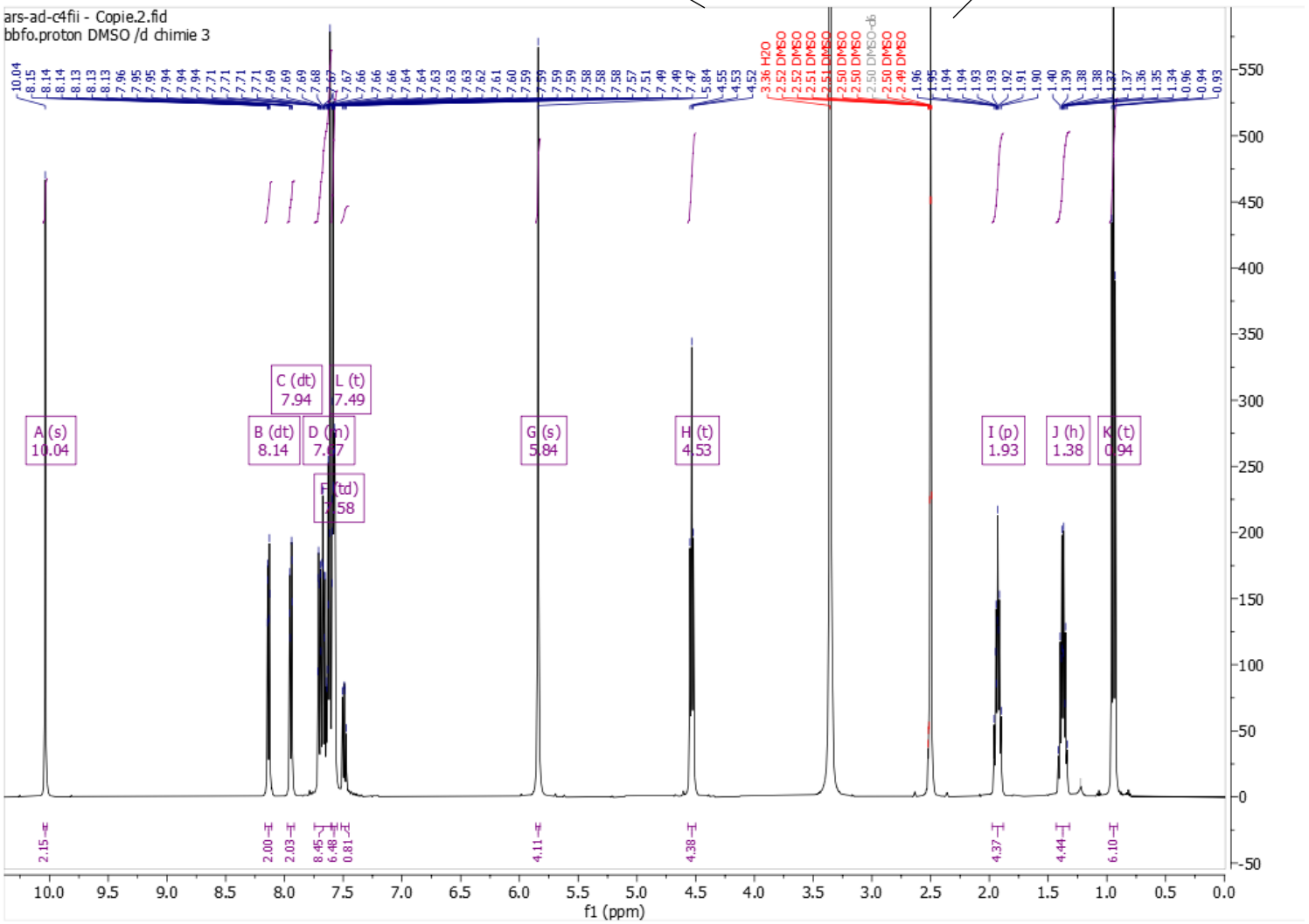
Integration Peak List

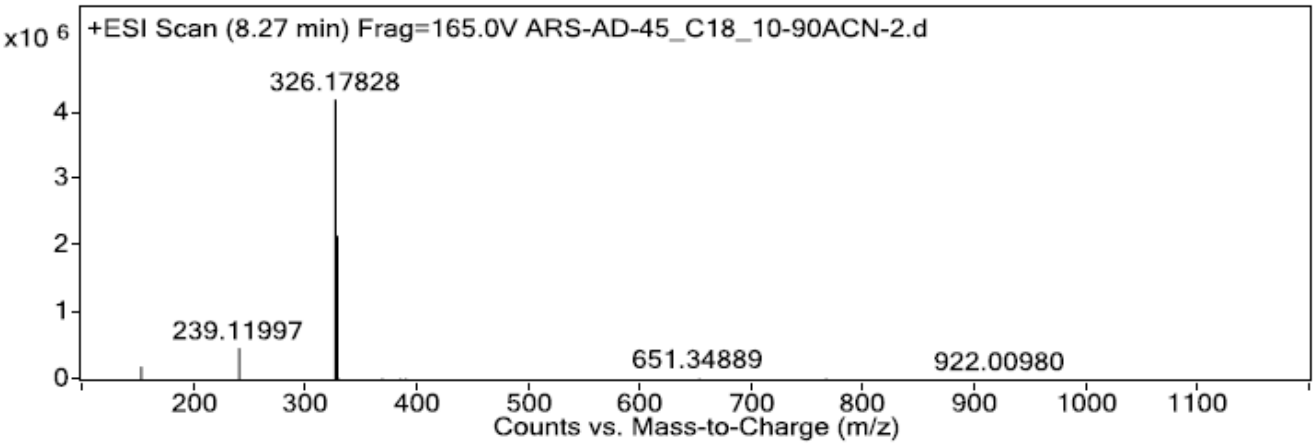
Peak	RT	Height	Area	Area (%)
1	7.6	172285027.3	945141727.6	100



Compound (9):

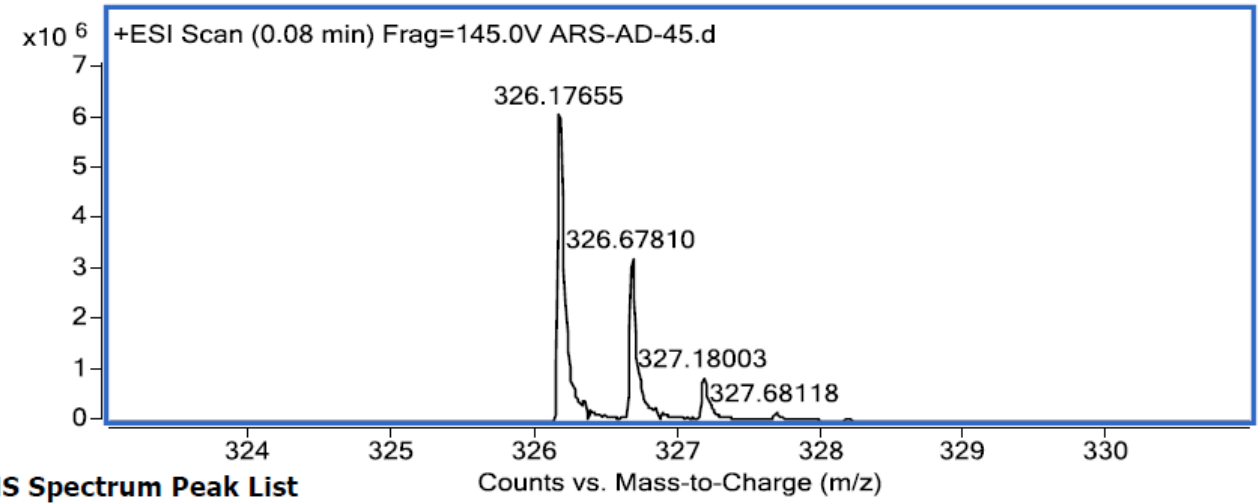

2 Br


ars-ad-c4fii - Copie.2.fid
bbfo.proton DMSO /d chimie 3
A (s) 10.04
B (dt) 8.14
C (dt) 7.94
D (m) 7.67
E (td) 7.58
G (s) 5.84
H (t) 4.53
I (p) 1.93
J (h) 1.38
K (t) 0.94
L (t) 7.49
f1 (ppm)


x10 6
+ESI Scan (8.27 min) Frag=165.0V ARS-AD-45_C18_10-90ACN-2.d
326.17828
239.11997
651.34889
922.00980
Counts vs. Mass-to-Charge (m/z)

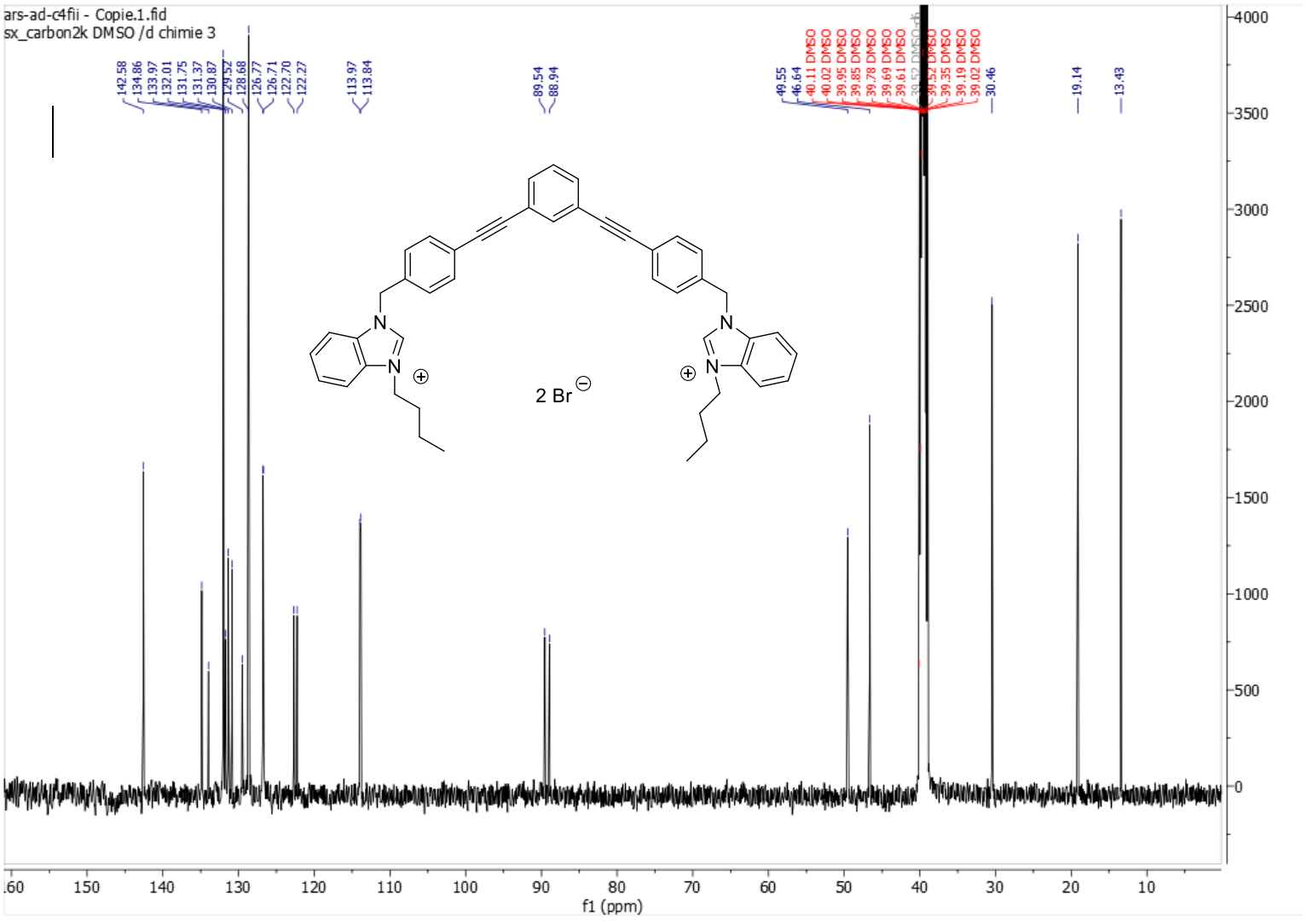
Integration Peak List

Peak	RT	Height	Area	Area (%)
1	8.25	69009985.7	411236010.2	100

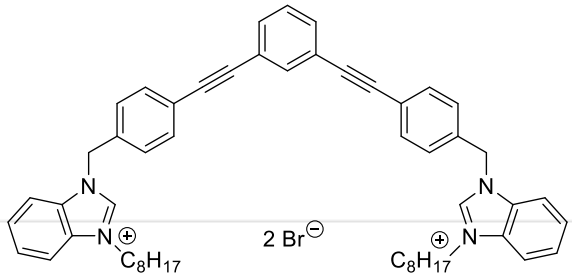


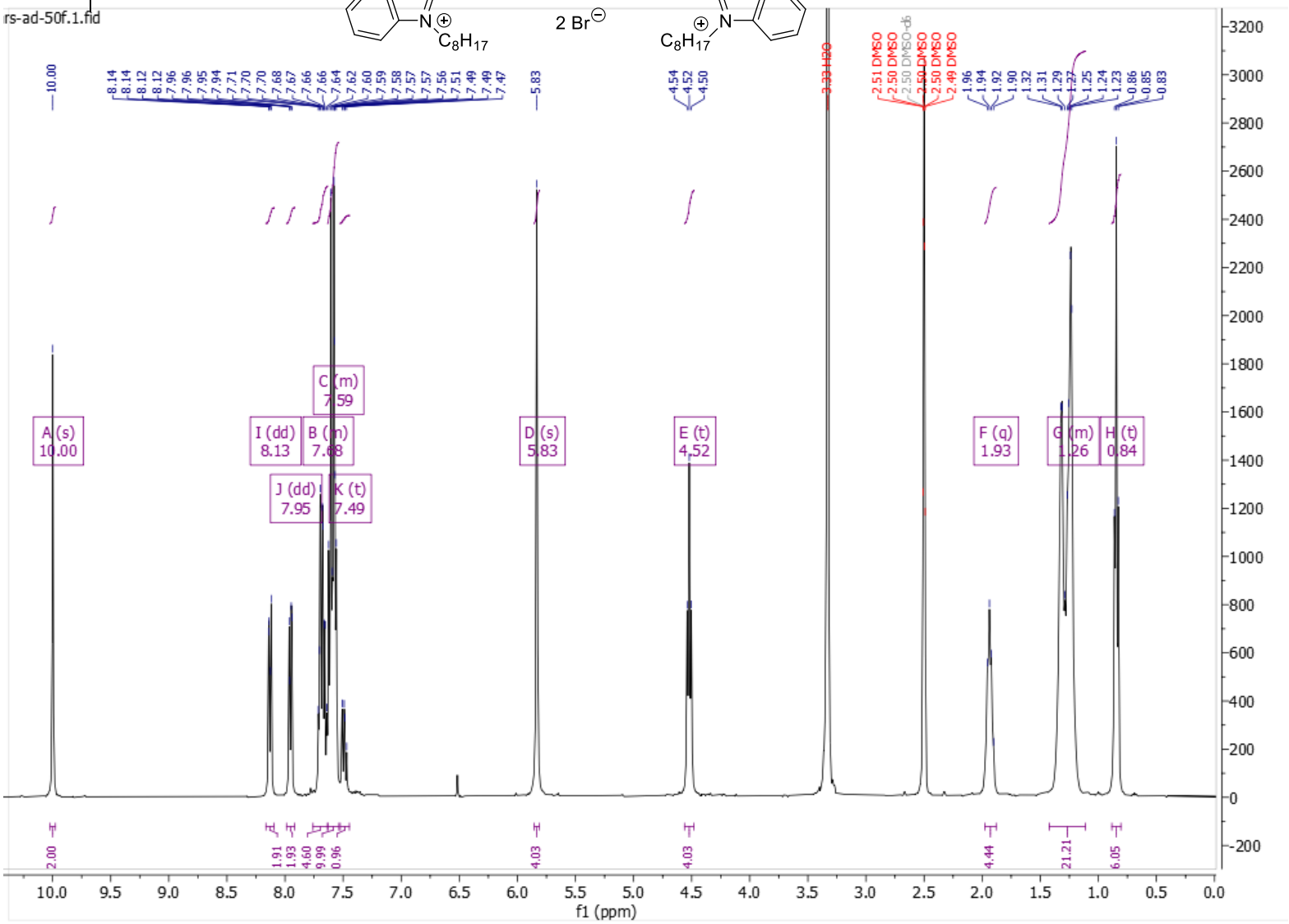
MS Spectrum Peak List

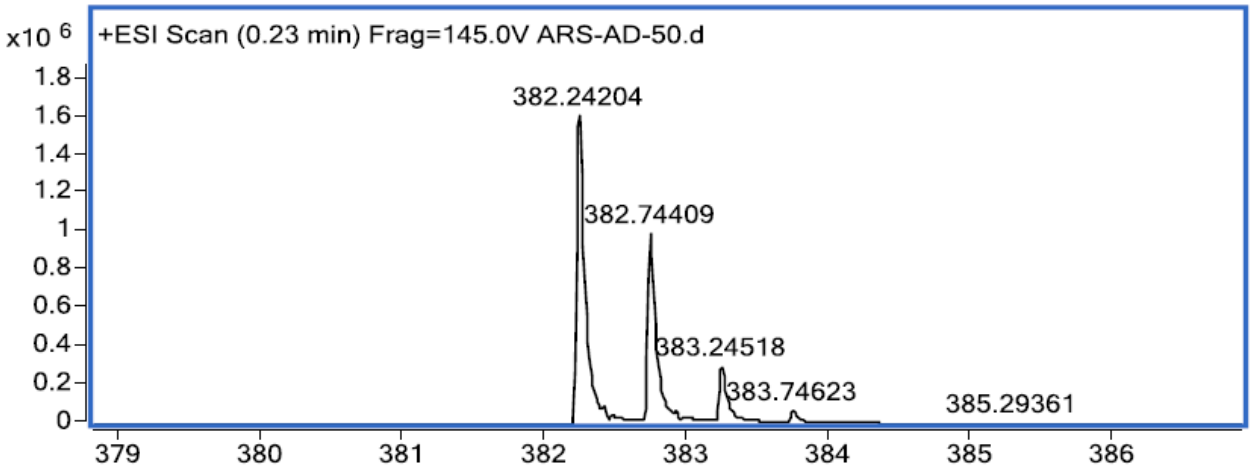
Ion	Formula	Abund	*Expe. m/z*	*Calc. m/z*	Diff(ppm)
(M+2)	C46 H44 N4	n/a	326.17655	326.17775	3.68



Compound (10):

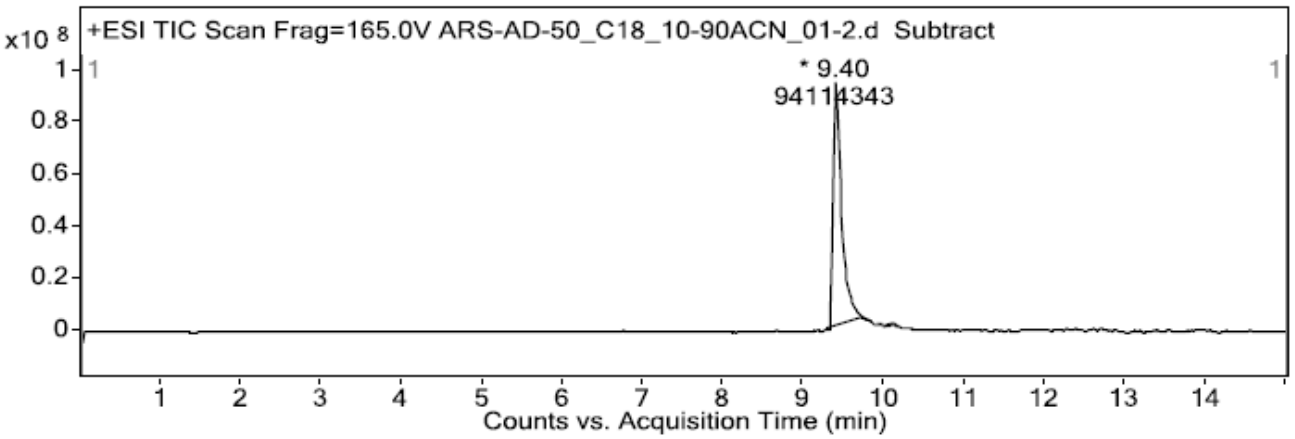






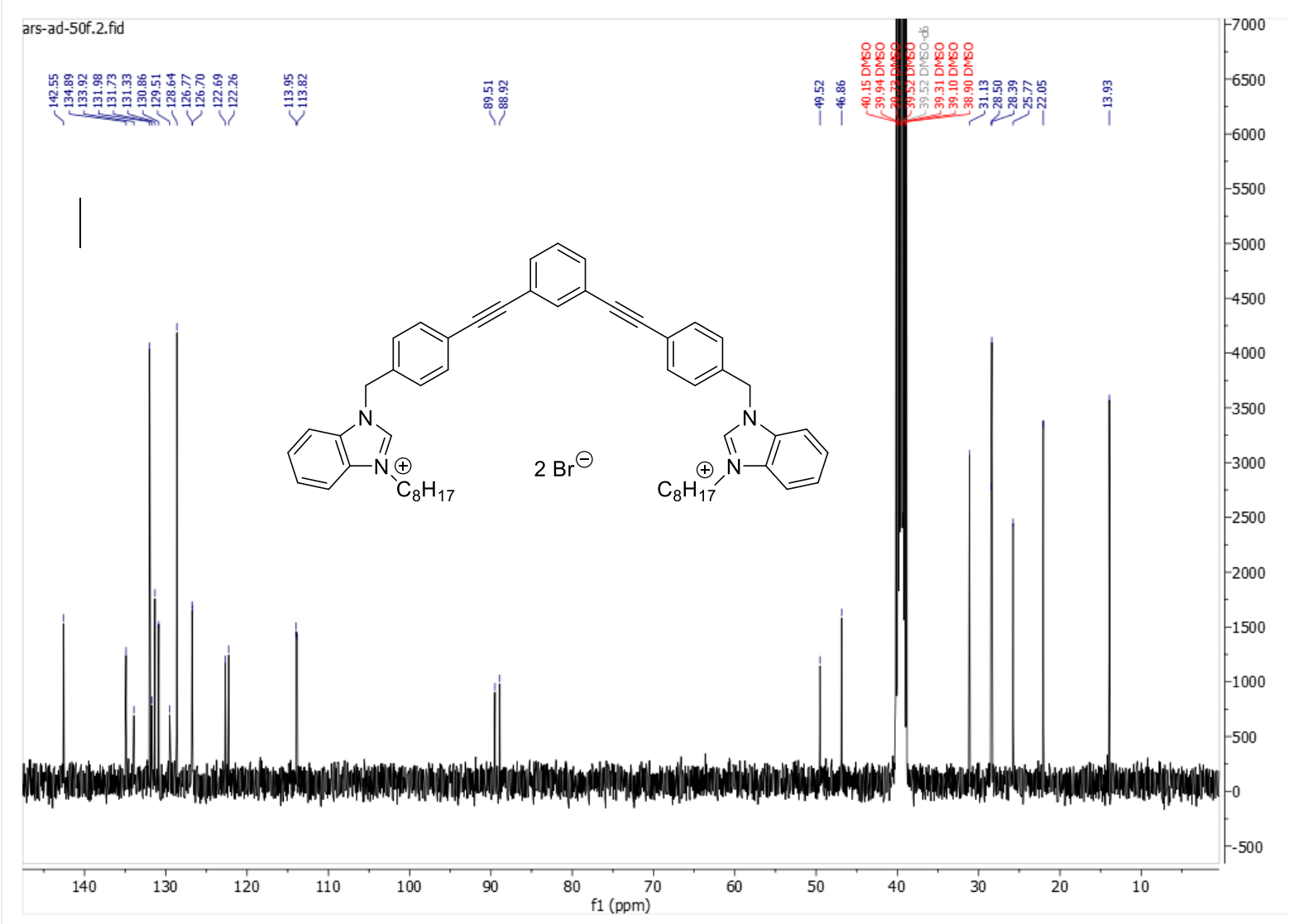
MS Spectrum Peak List

Ion	Formula	Abund	*Expe. m/z*	*Calc. m/z*	Diff(ppm)
(M+2)	C54 H60 N4	n/a	382.24204	382.24035	-4.42

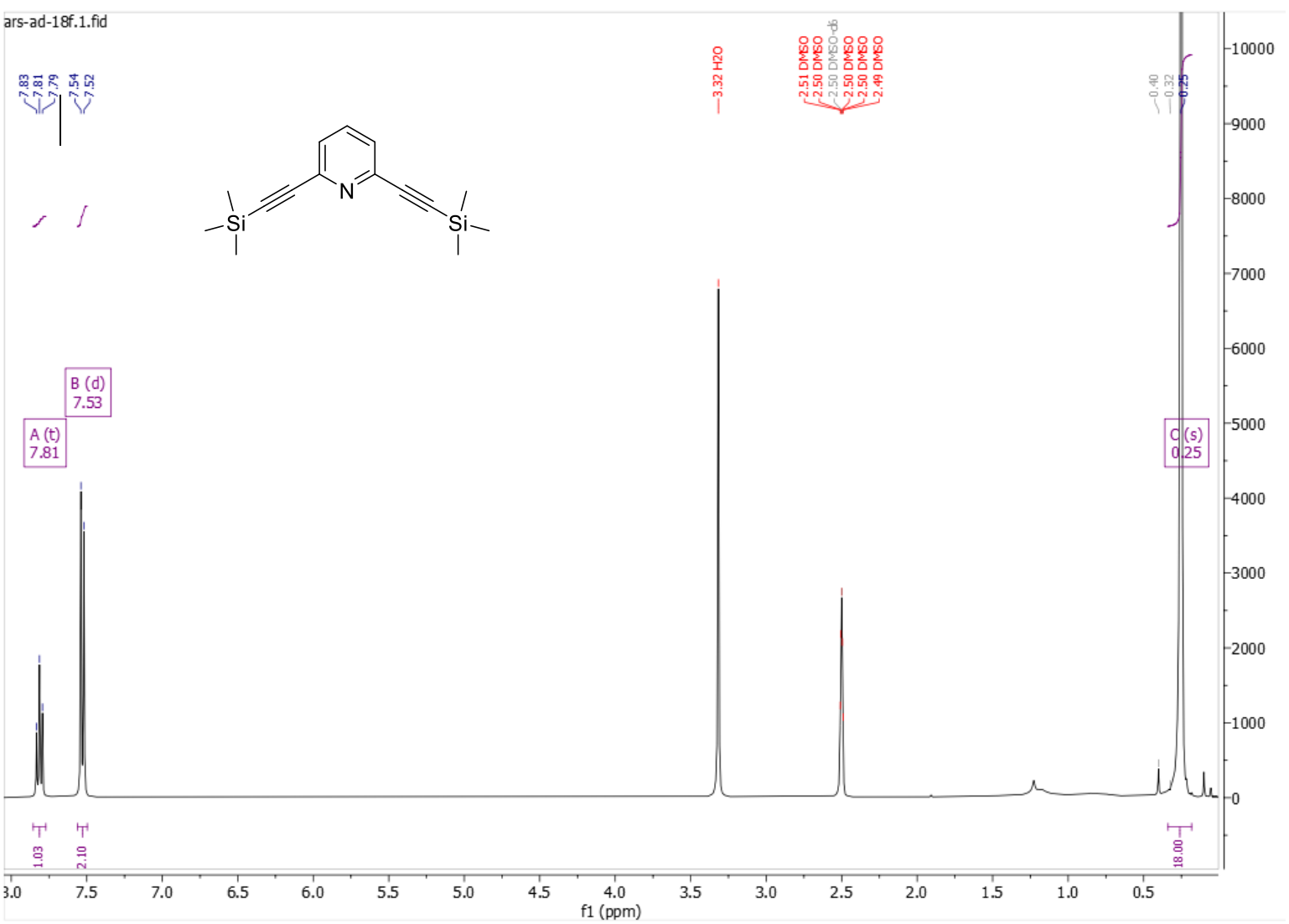


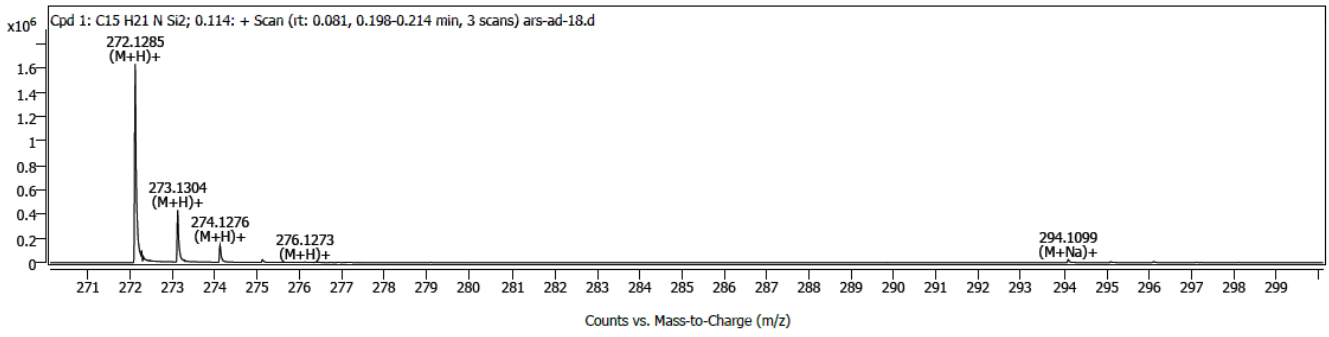
Integration Peak List

Peak	RT	Height	Area	Area (%)
1	9.4	94114343	704309639	99.21
2	9.75	0	297309	0.04
3	9.98	512294	812586	0.11
4	10.08	1140836	4472491	0.63



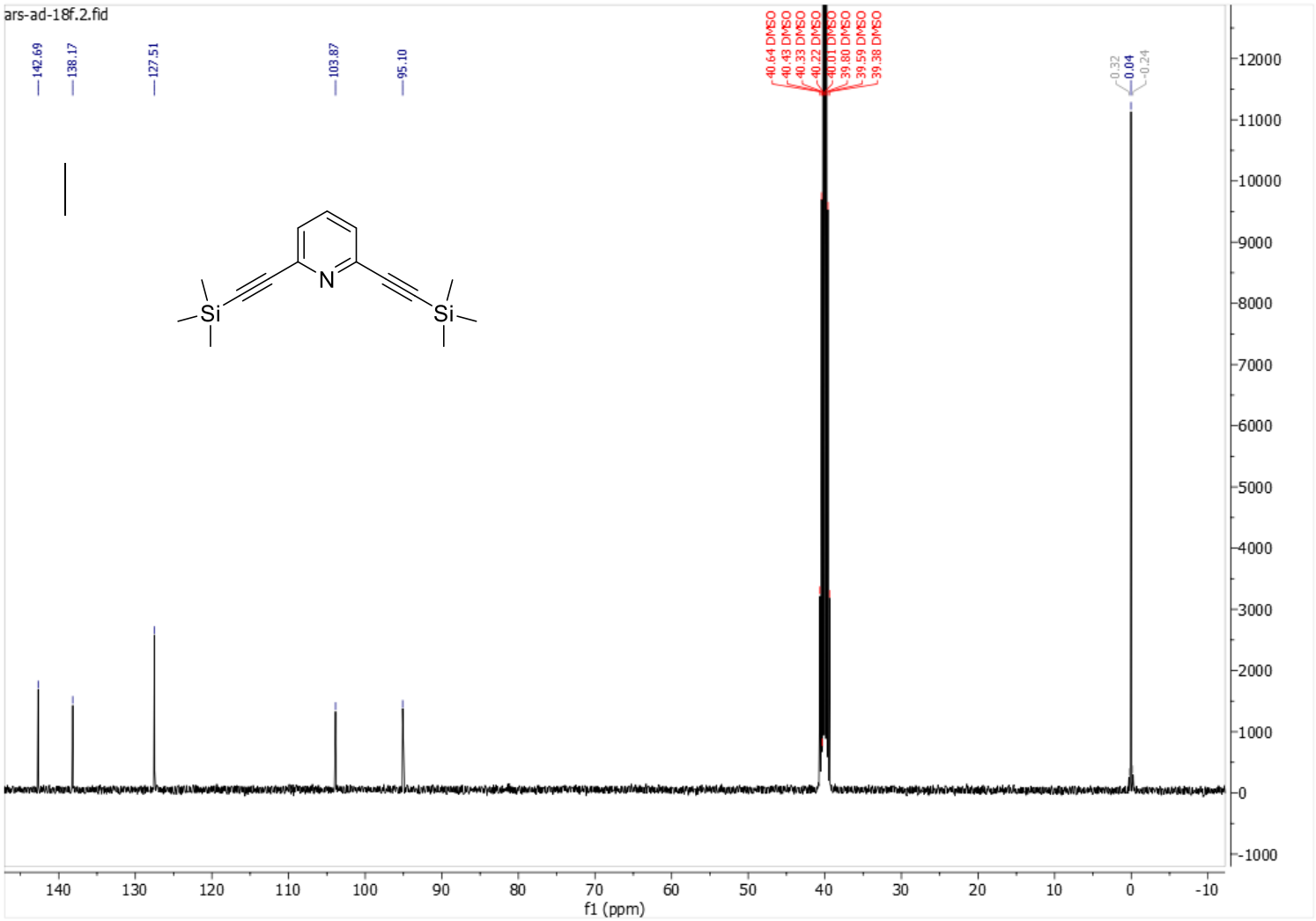
Compound (12):



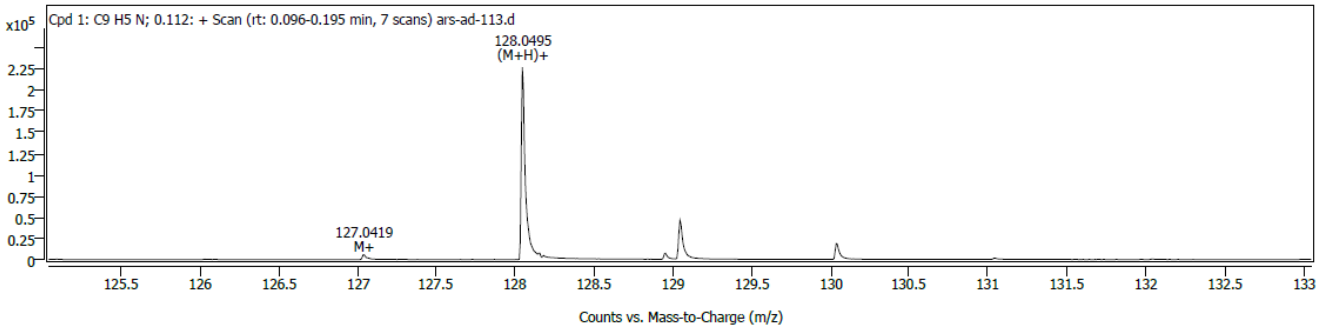


Spectrum Peaks (Max. 1)

Ion Species	Formula	Abund	m/z	m/z (Calc)	Diff (ppm)
(M+H)+	C15H21NSi2	1638420	272.12849	272.12853	-0.15

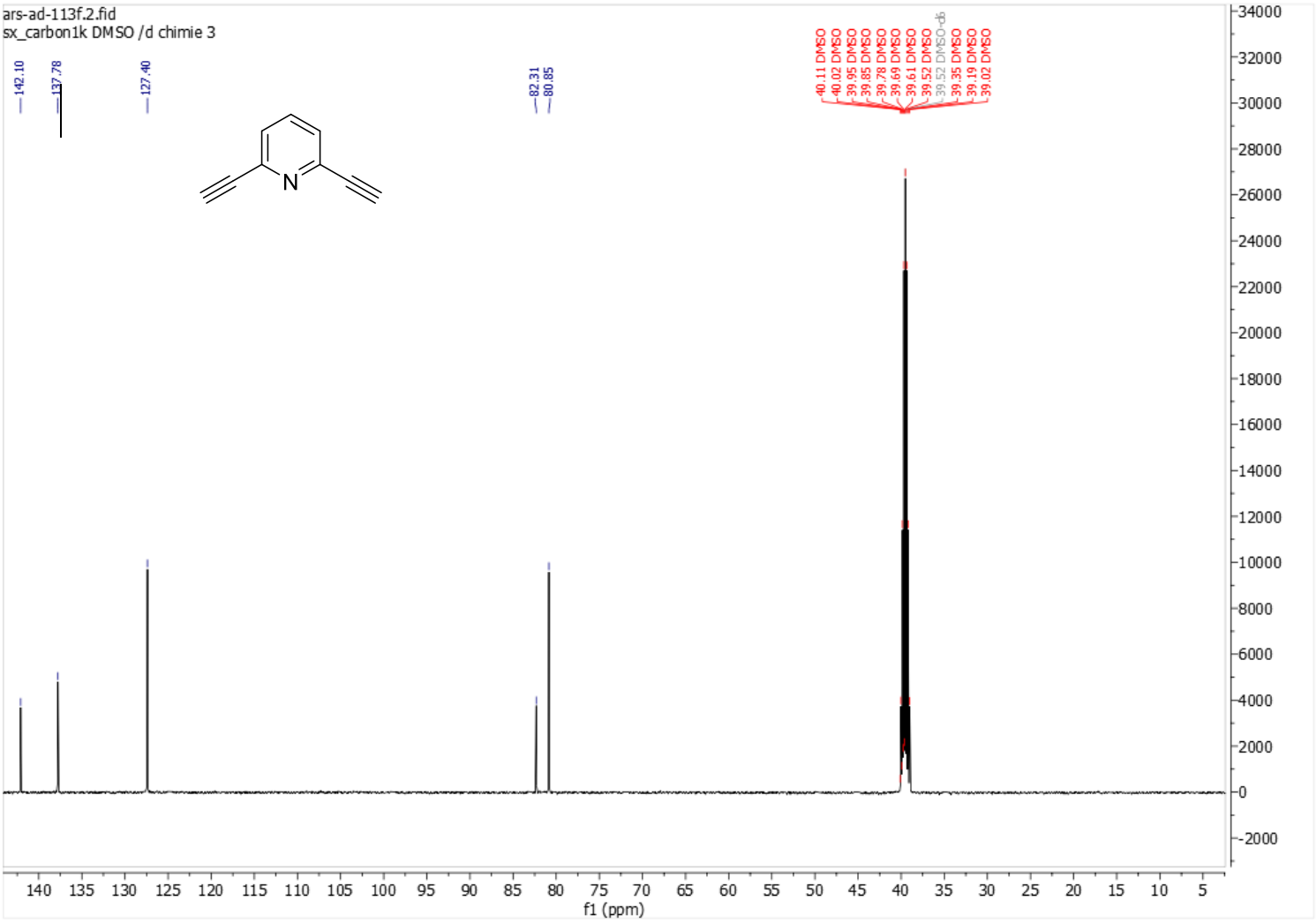

ars-ad-18f.2.fid
142.69
138.17
127.51
103.87
95.10
40.64 DMSO
40.43 DMSO
40.33 DMSO
40.22 DMSO
40.01 DMSO
39.80 DMSO
39.59 DMSO
39.38 DMSO
0.32
0.04
-0.24
N
Si
Si
f1 (ppm)

Compound (13):

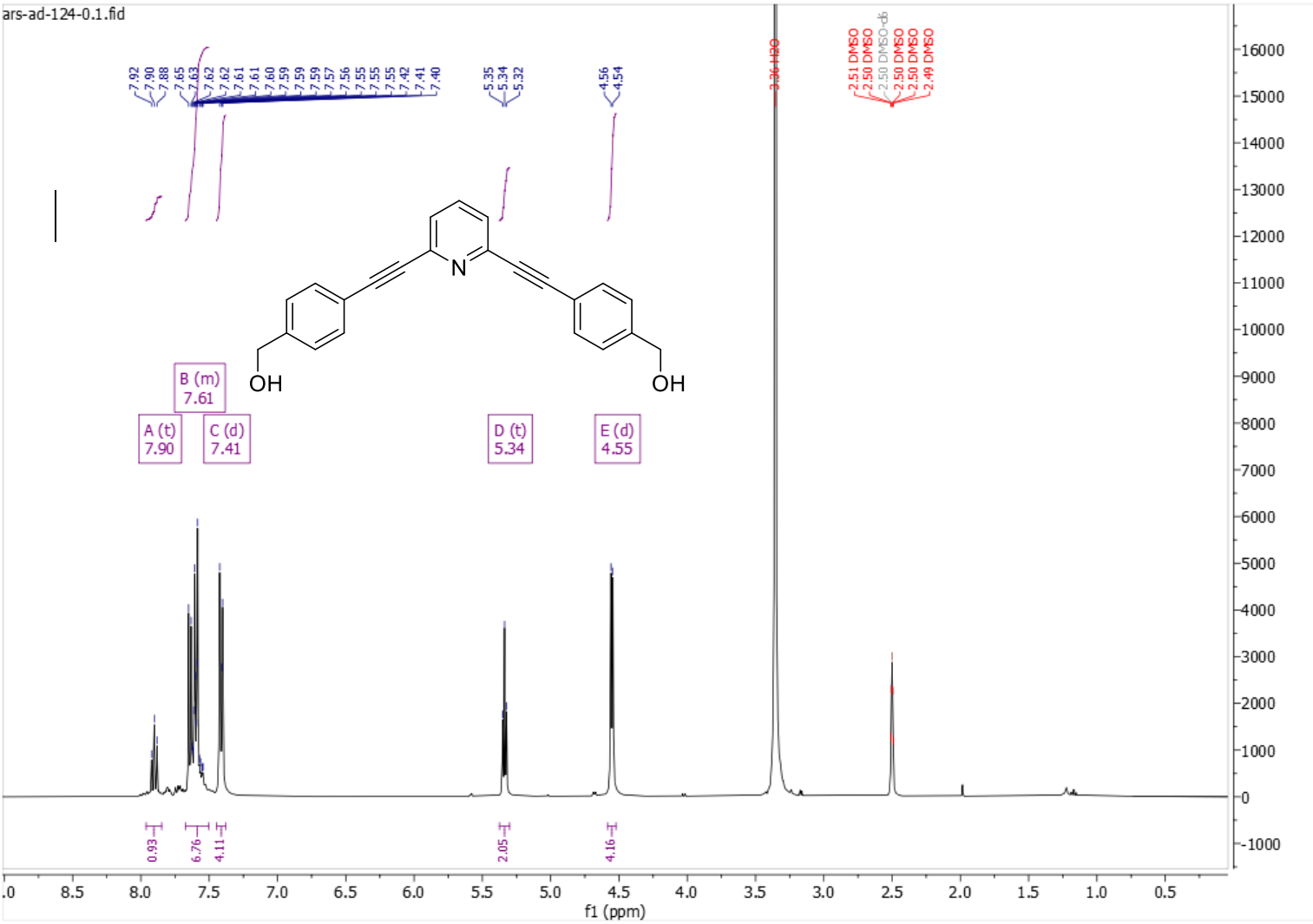


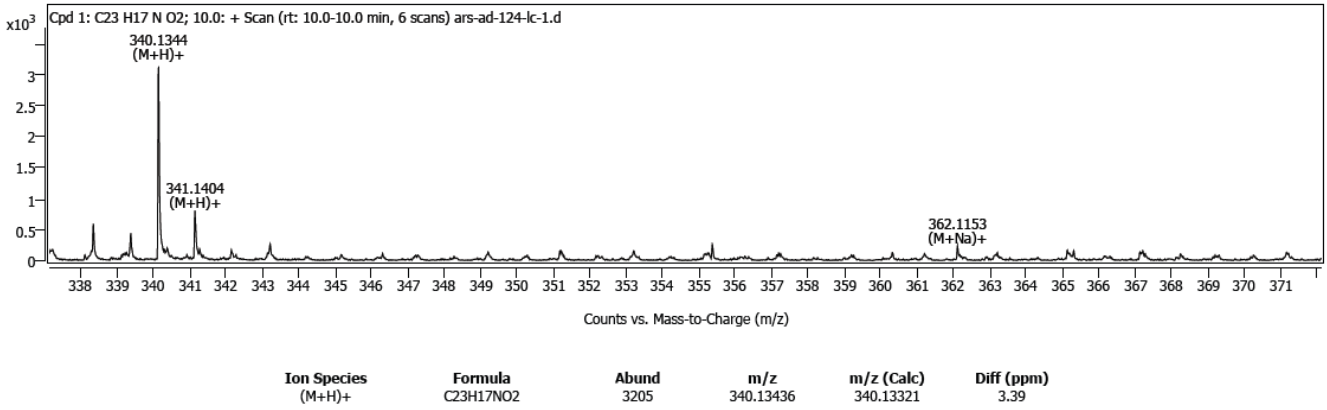
Spectrum Peaks (Max. 1)

Ion Species	Formula	Abund	m/z	m/z (Calc)	Diff (ppm)
(M+H)+	C9H5N	232501	128.04946	128.04948	-0.13

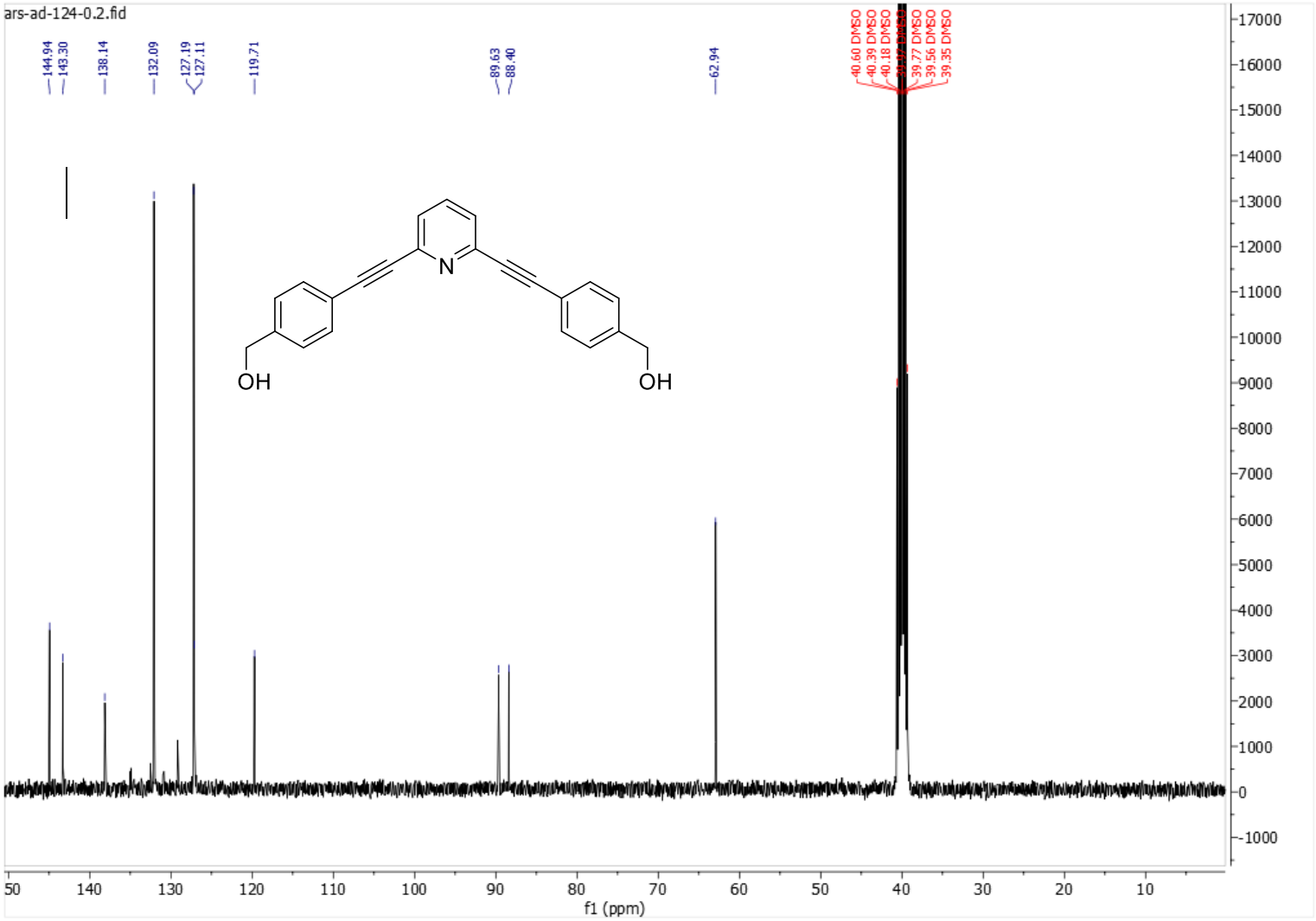

ars-ad-113f.2.fid
sx_carbon1k DMSO /d chimie 3
142.10
137.78
127.40
82.31
80.85
40.11 DMSO
40.02 DMSO
39.95 DMSO
39.85 DMSO
39.78 DMSO
39.69 DMSO
39.61 DMSO
39.52 DMSO
39.52 DMSO-d6
39.35 DMSO
39.19 DMSO
39.02 DMSO
N
f1 (ppm)

Compound (14):

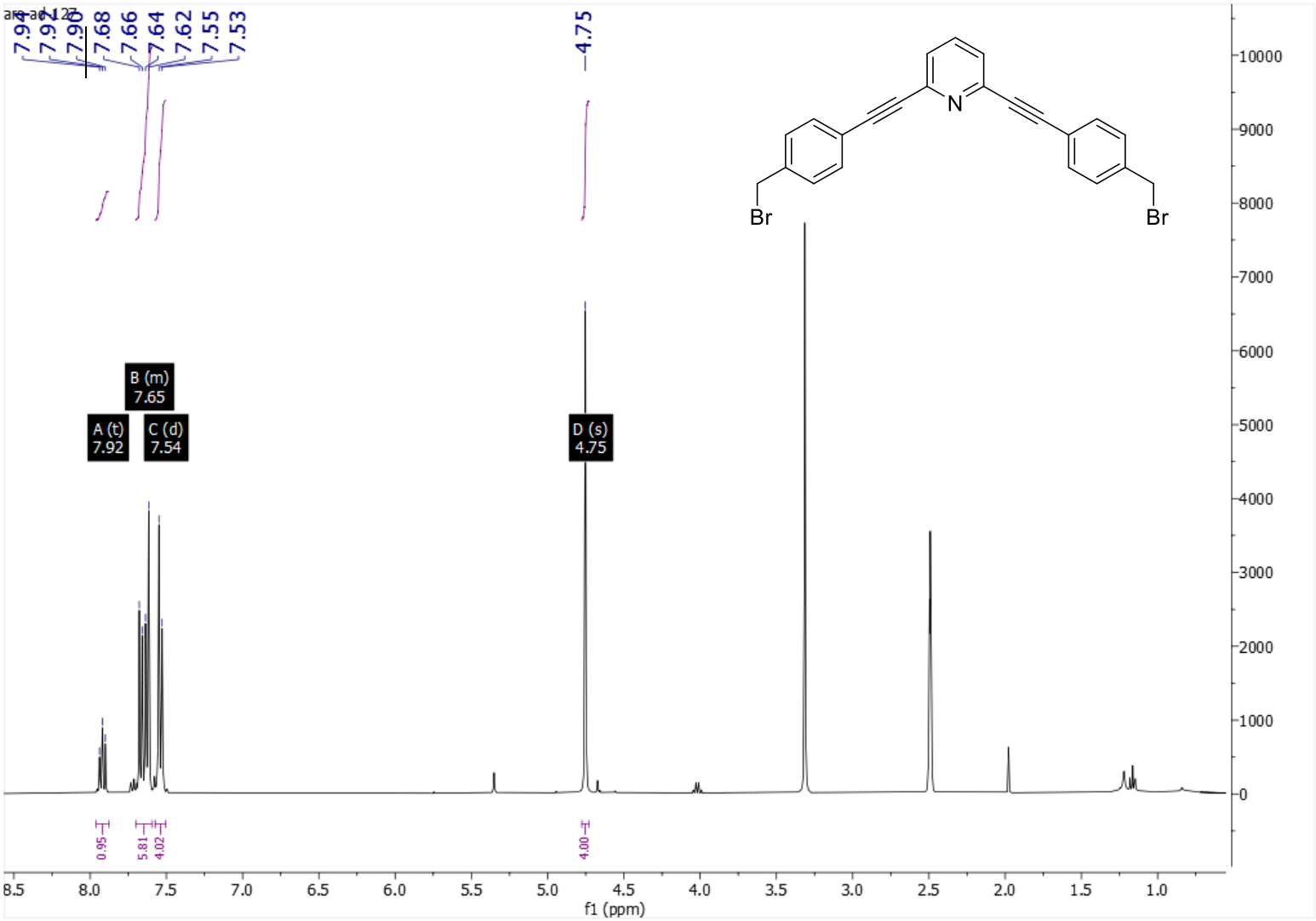


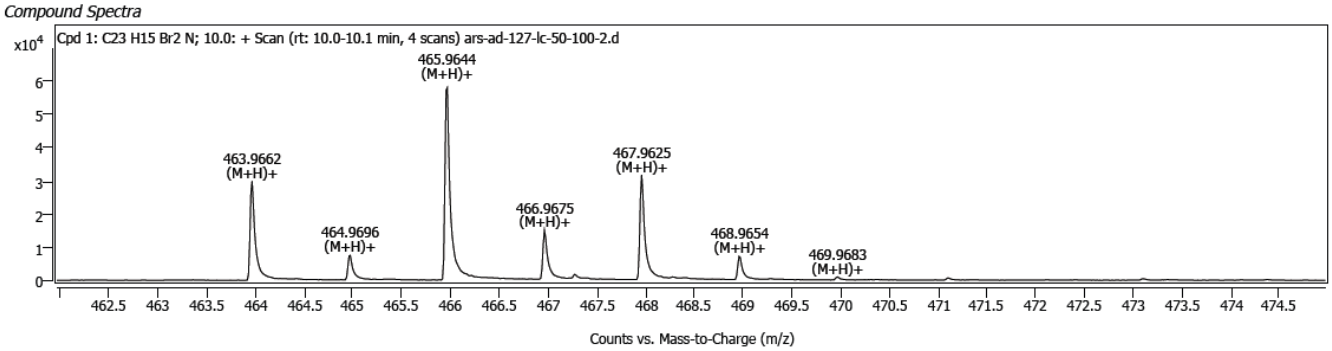


Ion Species	Formula	Abund	m/z	m/z (Calc)	Diff (ppm)
(M+H)+	C23H17NO2	3205	340.13436	340.13321	3.39


ars-ad-124-0.2.fid
144.94
143.30
138.14
132.09
127.19
127.11
119.71
89.63
88.40
62.94
40.60 DMSO
40.39 DMSO
40.18 DMSO
39.77 DMSO
39.56 DMSO
39.35 DMSO
N
OH
OH
f1 (ppm)

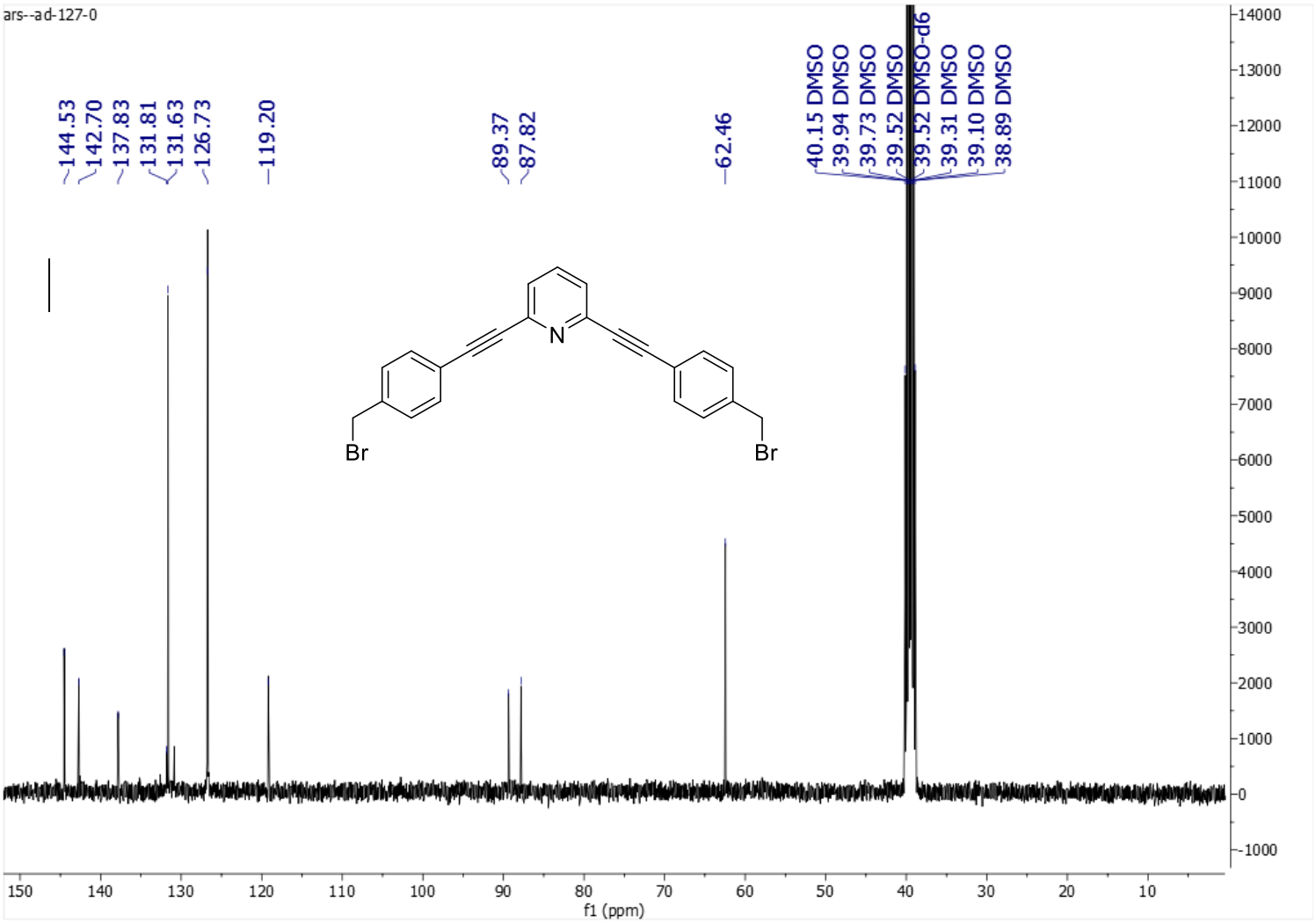
Compound (15):



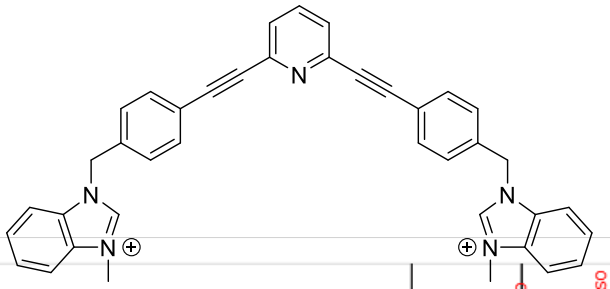


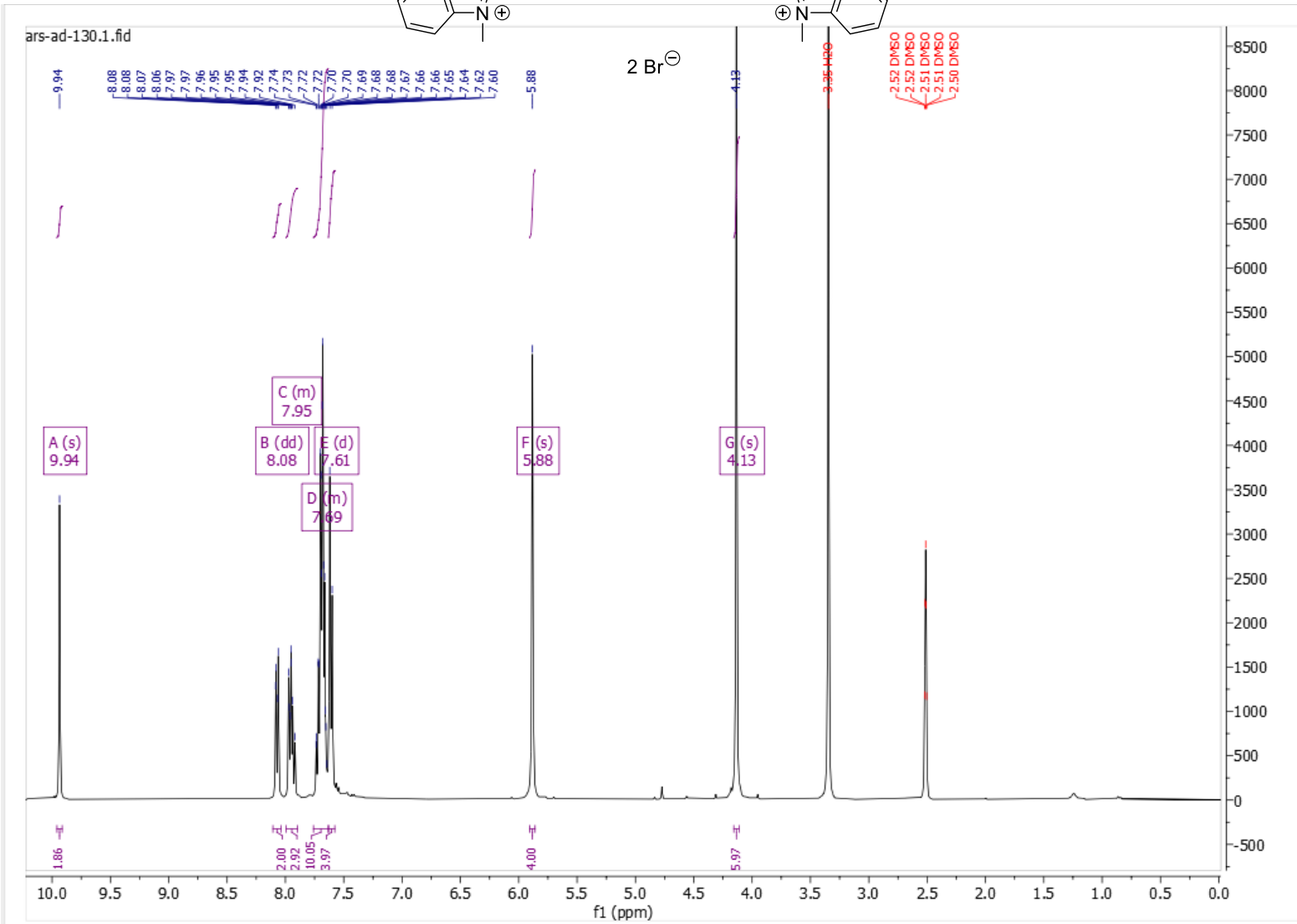
Spectrum Peaks (Max. 1)

Ion Species	Formula	m/z (Calc)	m/z	Abund	Diff (ppm)
(M+H)+	C23H15Br2N	465.96249	465.96437	59754	4.03

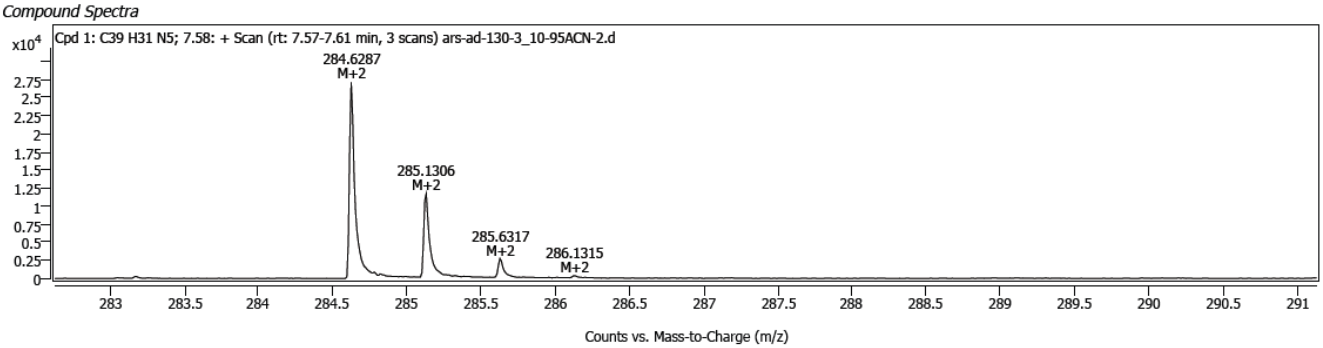

ars--ad-127-0
144.53
142.70
137.83
131.81
131.63
126.73
119.20
89.37
87.82
62.46
40.15 DMSO
39.94 DMSO
39.73 DMSO
39.52 DMSO
39.52 DMSO-d6
39.31 DMSO
39.10 DMSO
38.89 DMSO
N
Br
Br
f1 (ppm)

Compound (16):



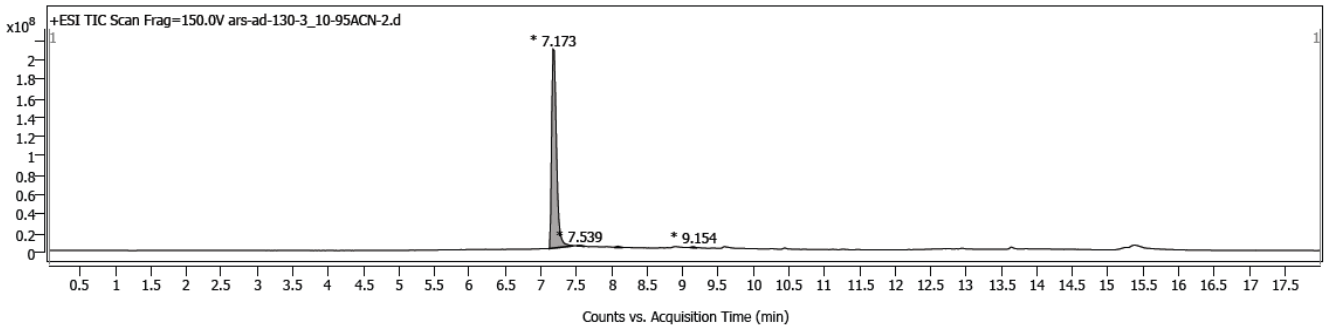


Compound Spectra



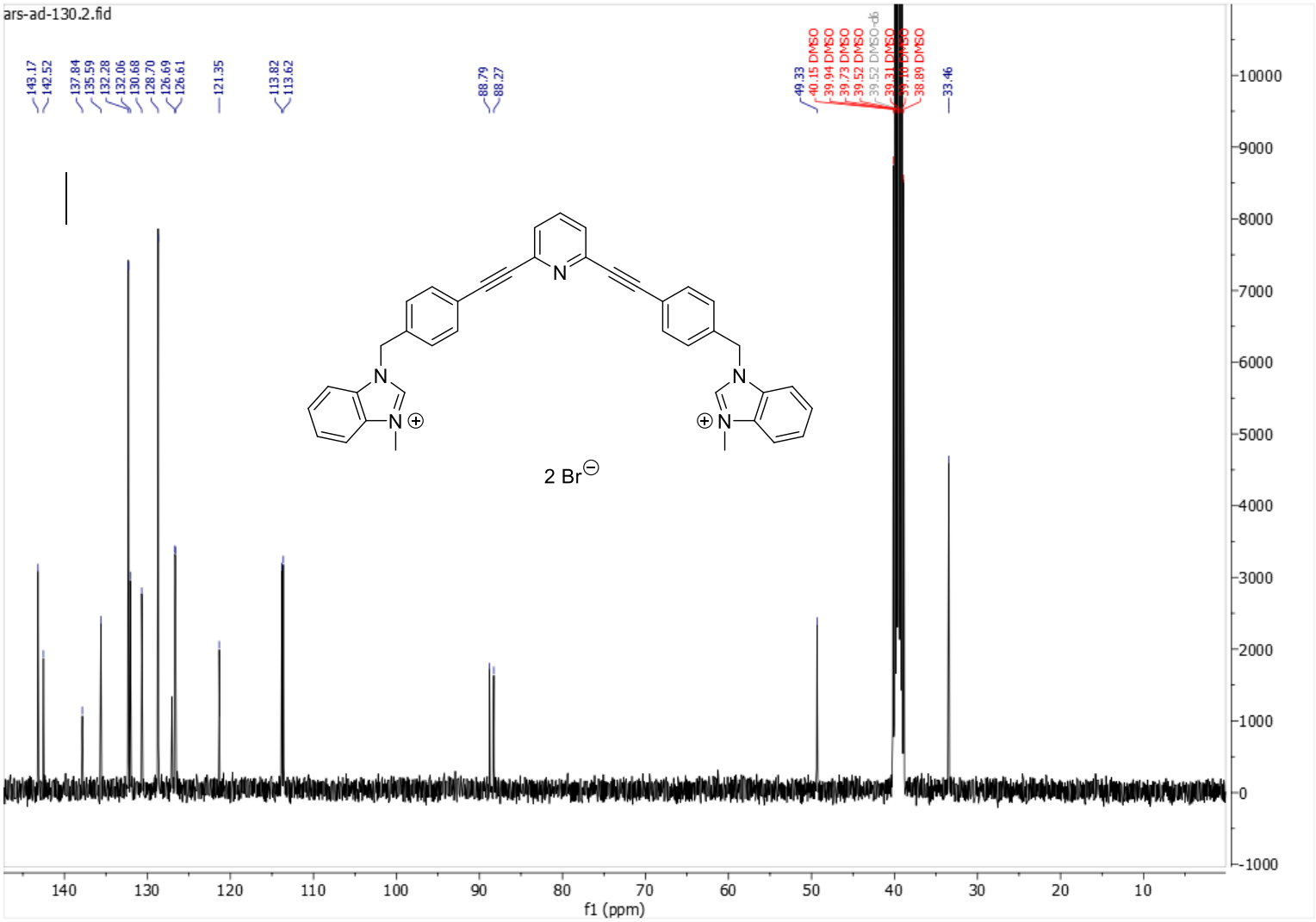
Spectrum Peaks (Max. 1)

Ion Species	Formula	m/z (Calc)	m/z	Abund	Diff (ppm)
M+2	C39H31N5	284.62842	284.62869	27190	0.93

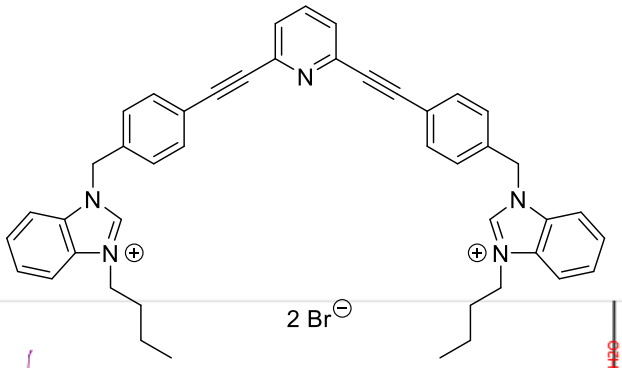


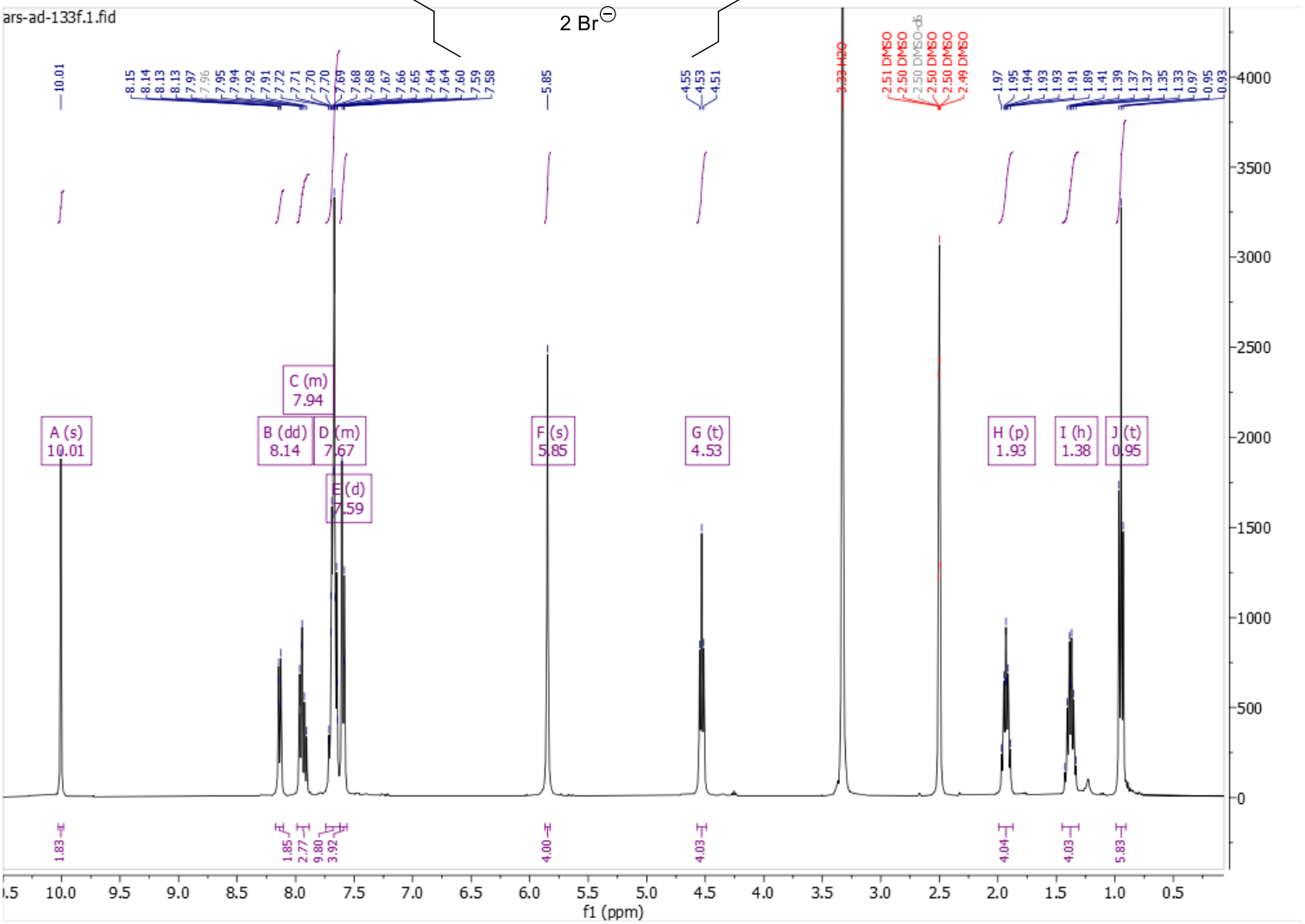
Chromatogram Peaks

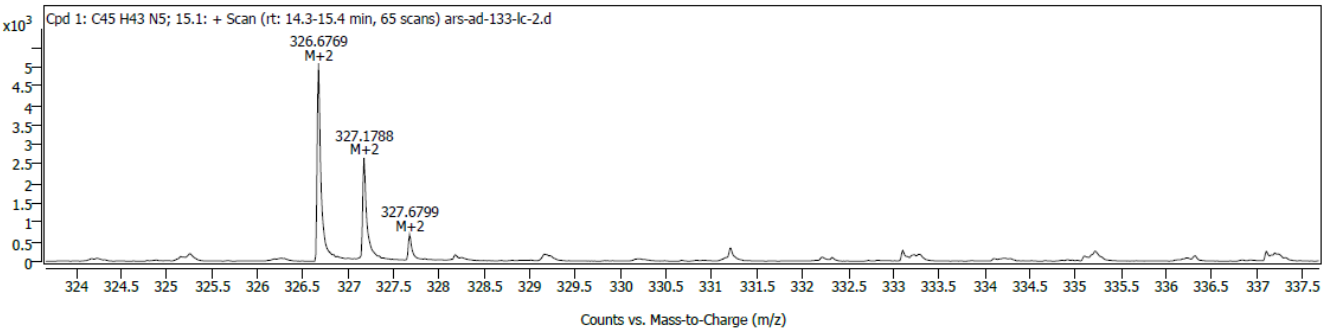
RT	Area	Area Sum %
7.2	981753704	99.15
7.5	2076425	0.21
8.1	3715956	0.38
9.2	2602887	0.26



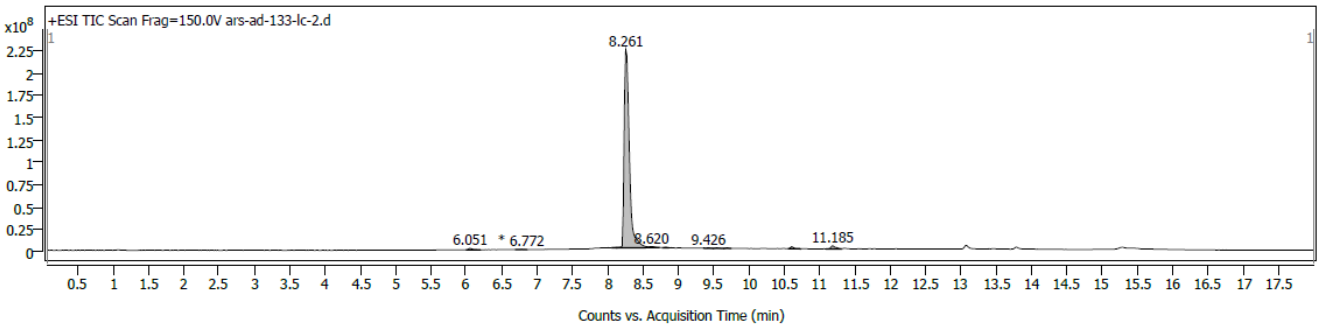
Compound (17):





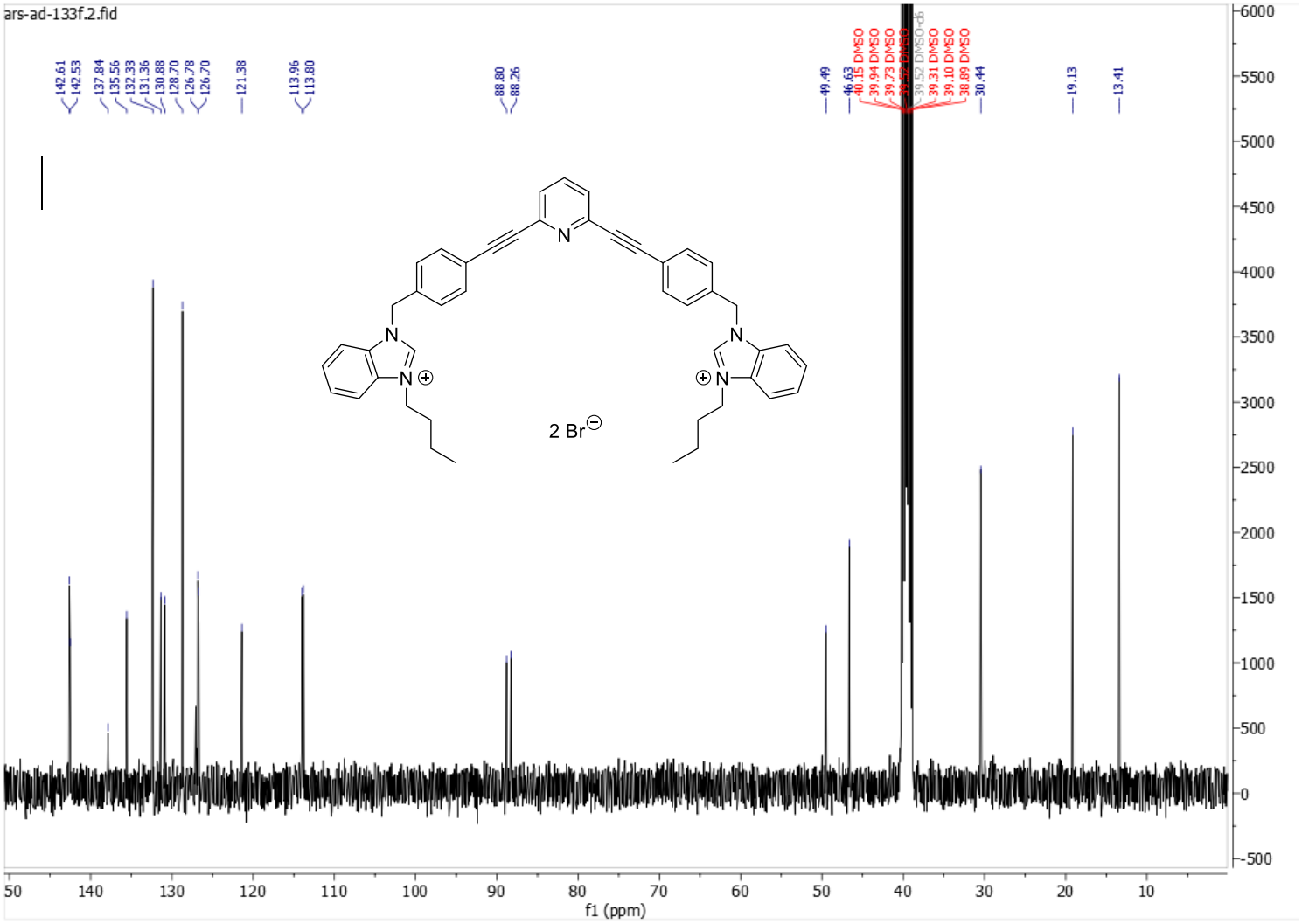


Ion Species	Formula	Abund	m/z	m/z (Calc)	Diff (ppm)
M+2	C45H43N5	5086	326.67689	326.67537	4.64

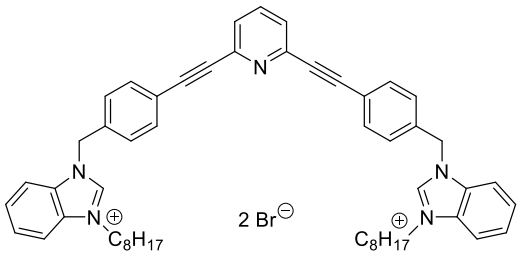


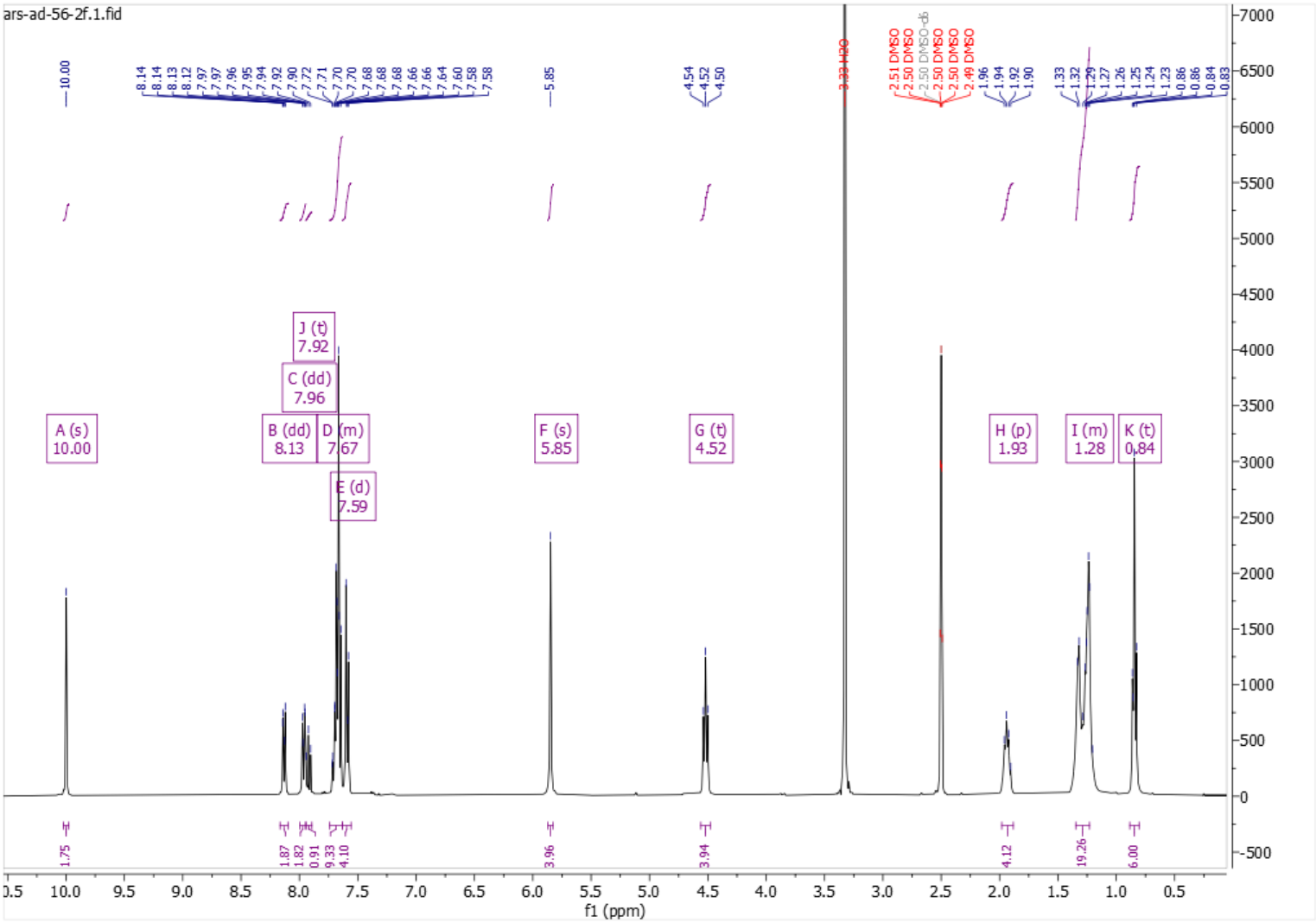
Chromatogram Peaks

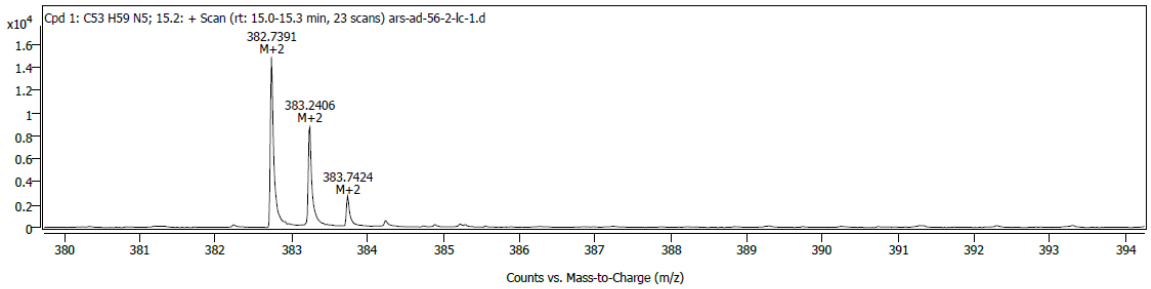
RT	Area	Area Sum %
6.1	6157248	0.52
6.8	1341745	0.11
8.1	1363074	0.12
8.3	1134343213	95.98
8.6	7039232	0.60
8.8	1882220	0.16
9.4	2110547	0.18
9.5	2460479	0.21
9.7	2103395	0.18
10.6	6985387	0.59
11.2	16029074	1.36



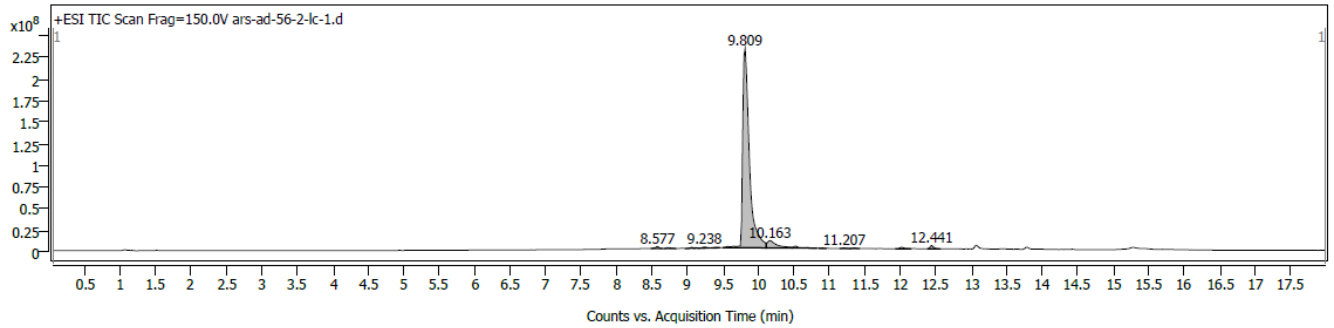
Compound (18):







Ion Species	Formula	Abund	m/z	m/z (Calc)	Diff (ppm)
M+2	C53H59N5	15002	382.73914	382.73797	3.04



Chromatogram Peaks

RT	Area	Area Sum %
8.6	9504689	0.57
8.7	5151674	0.31
9.1	3478022	0.21
9.1	1676855	0.10
9.2	5299264	0.32
9.4	1888570	0.11
9.6	4955105	0.30
9.7	7053915	0.42
9.8	1505815045	90.69
10.2	78469469	4.73
10.5	7943634	0.48
10.7	1957247	0.12
10.9	1782475	0.11
11.2	2692173	0.16
11.4	2649512	0.16
12.0	6957277	0.42
12.4	13085384	0.79