Supporting Information

Spiropyrimidinetrione DNA gyrase inhibitors with potent and selective antituberculosis activity

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1. Illustrations of moxifloxacin and QPT-1 crystal structures

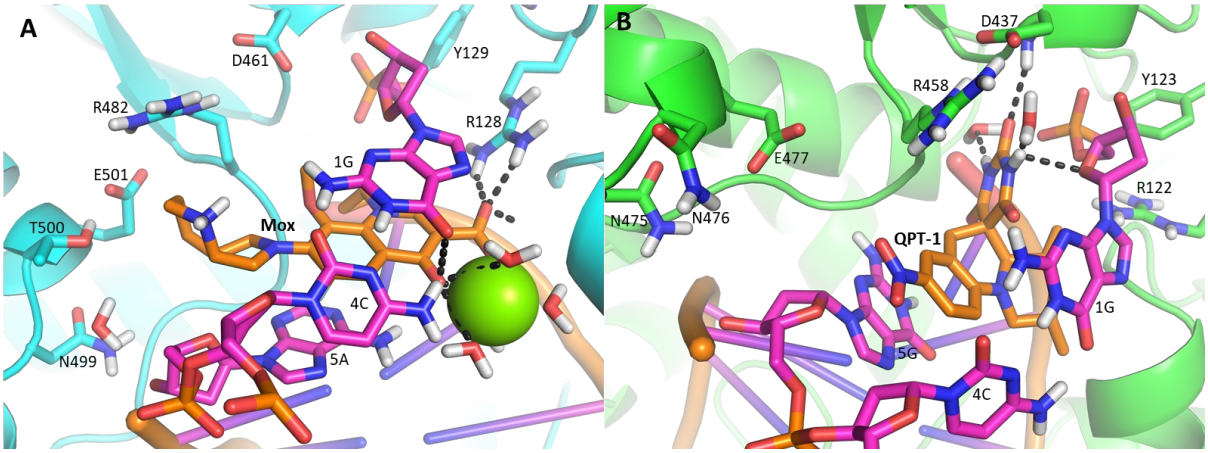


Figure S1 (A) Illustration of the moxifloxacin (orange carbons) and Mg^{2+} binding mode in *Mtb* DNA-gyrase (from PDB 5BS8). (B) Illustration of the QPT-1 (orange carbons) binding mode in *S. aureus* DNA gyrase (from PDB 5CDM).

2. Illustration of QPT-1 docking validation in *Mtb* DNA Gyrase

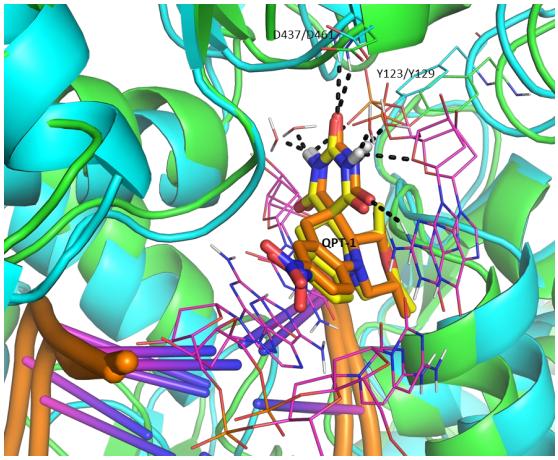
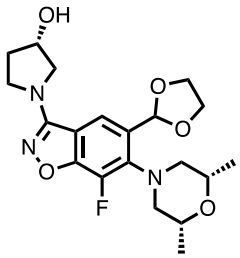


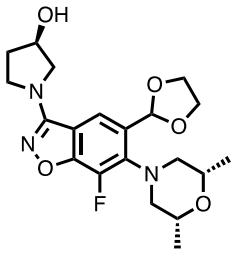
Figure S2: Overlay of QPT-1 (orange) docked into the transposed *Mtb* gyrase (cyan) onto the co-crystal structure of QPT-1 (yellow) bound to *S. aureus* gyrase (PDB ID: 5CDM). The docked QPT-1 ligand structure aligned very closely with the co-crystal QPT-1with an RMSD = 0.636 Å and key binding interactions were maintained.

3. Synthesis and characterization of intermediates

Intermediates from Scheme 2

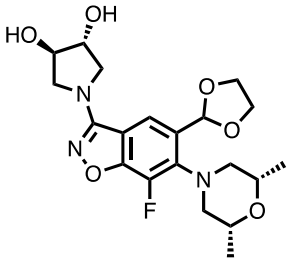


*(S)-1-(6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)pyrrolidin-3-ol (**48a**).* In a sealed tube, a mixture of **47** (497 mg, 1.39 mmol), and 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine (0.48 mL, 5.58 mmol) and (*S*)-pyrrolidine-3-ol (243 mg, 2.81 mmol) in acetonitrile (2 ml) were heated to 90 °C for 16h. A red-brown solution was obtained and cooled to RT. Solvent was removed to afford a brown residue. The material was purified by flash chromatography on silica gel using hexane:EtOAc (20:80 gradient elution) to give the title compound as a red-brown oil (492 mg, 87%). RP-HPLC t_R = 2.564 min (method 1, purity 80%); LC-MS ESI, *m*/*z* 408.0 $[M+H]^+$ (anal. calcd. for $C_{20}H_{26}FN_3O_5$, *m*/*z* 407.4).

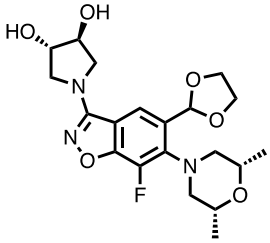


*(R)-1-(6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)pyrrolidin-3-ol (**48b**).* Prepared following the preparation of **48a** using **47** (147 mg, 0.41 mmol), 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine (0.25 mL, 1.64 mmol) and (*R*)-pyrrolidine-3-ol (72 mg, 0.82 mmol). Column chromatography on silica gel using hexane:EtOAc (20:80 gradient). Red-brown oil (116 mg, 69%). RP-HPLC t_R = 3.501 min

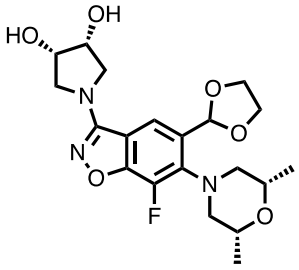
(method 1, purity 99%); LC-MS ESI, *m*/*z* 408.1 $[M+H]^+$ (anal. calcd. for $C_{20}H_{26}FN_3O_5$, *m*/*z* = 407.4).



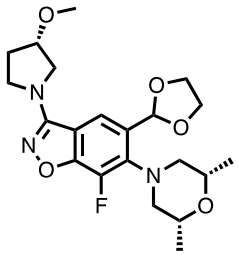
*(3R,4R)-1-(6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)pyrrolidine-3,4-diol (**48c**).* Prepared following the preparation of **48a** using **47** (501 mg, 1.40 mmol), 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine (0.25 mL, 1.69 mmol) and (3*R*,4*R*)-pyrrolidine-3,4-diol (290 mg, 2.81 mmol). Column chromatography on silica gel using DCM:MeOH (95:5 gradient). Off-white solid (481 mg, 81%). RP-HPLC t_R = 2.760 min (method 1, purity 95%); LC-MS ESI, *m*/*z* 424.0 $[M+H]^+$ (anal. calcd. for $C_{20}H_{26}FN_3O_6$, *m*/*z* = 423.4).



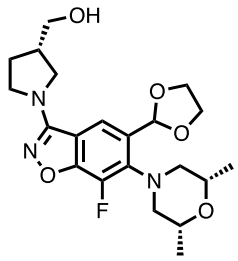
*(3S,4S)-1-(6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)pyrrolidine-3,4-diol (**48d**).* Prepared following the preparation of **48a** using **47** (143 mg, 0.40 mmol), 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine (0.072 mL, 0.48 mmol) and (3*S*,4*S*)-pyrrolidine-3,4-diol (83 mg, 0.80 mmol). Column chromatography using DCM:MeOH (95:5 gradient). Off-white solid (170 mg, 95%). RP-HPLC t_R = 2.922 min (method 1, purity 95%); LC-MS ESI, *m*/*z* 424.0 $[M+H]^+$ (anal. calcd. for $C_{20}H_{26}FN_3O_6$, *m*/*z* = 423.4).



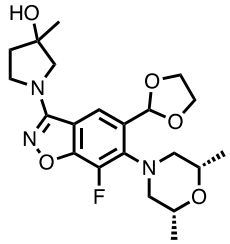
*(3R,4S)-1-(6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)pyrrolidine-3,4-diol (**48e**).* Prepared following the preparation of **48a** using **47** (146 mg, 0.41 mmol), 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine (0.20 mL, 1.31 mmol) and (3*R*,4*S*)-pyrrolidine-3,4-diol hydrochloride (114 mg, 0.82 mmol). Column chromatography on silica gel using DCM:MeOH (95:5 gradient). Off-white solid (134 mg, 76%). RP-HPLC t_R = 3.501 min (method 1, purity 98%); LC-MS ESI, *m*/*z* 424.0 $[M+H]^+$ (anal. calcd. for $C_{20}H_{26}FN_3O_6$, *m*/*z* = 423.4).



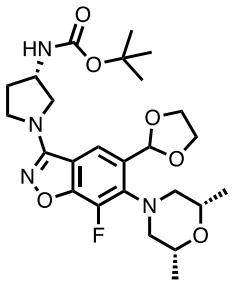
*6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluoro-3-((S)-3-methoxypyrrolidin-1-yl)benzo[d]isoxazole (**48f**).* Prepared following the preparation of **48a** using **47** (147 mg, 0.41 mmol), 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine (0.075 mL, 0.50 mmol) and (*S*)-3-methoxypyrrolidine (84 mg, 0.83 mmol). Column chromatography on silica gel using DCM:MeOH (95:5 gradient). Off-white solid (121 mg, 68%). RP-HPLC t_R = 3.495 min (method 1, purity 98%); LC-MS ESI, *m*/*z* 422.2 $[M+H]^+$ (anal. calcd. for $C_{21}H_{28}FN_3O_5$, *m*/*z* = 421.5).



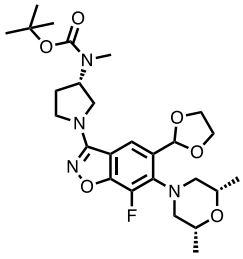
*((S)-1-(6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)pyrrolidin-3-yl)methanol (**48g**).* Prepared following the preparation of **48a** using **47** (300 mg, 0.84 mmol), 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine (0.51 mL, 3.36 mmol) and (*S*)-pyrrolidin-3-ylmethanol (170 mg, 1.68 mmol). Column chromatography on silica gel using hexane:EtOAc (20:80 gradient). Off-white solid (279 mg, 73%). RP-UPLC t_R = 1.014 min (method 2, purity 92%); LC-MS ESI, *m*/*z* 422.1 $[M+H]^+$ (anal. calcd. for $C_{21}H_{28}FN_3O_5$, *m*/*z* = 421.5).



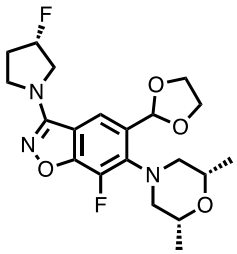
*1-(6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)-3-methylpyrrolidin-3-ol (**48h**).* Prepared following the preparation of **48a** using **47** (304 mg, 0.85 mmol), 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine (0.51 mL, 3.41 mmol) and 3-methylpyrrolidin-3-ol (172 mg, 1.70 mmol). Column chromatography on silica gel using DCM:MeOH (90:10 gradient). Pale-yellow oil (323 mg, 87%). RP-UPLC t_R = 1.058 min (method 2, purity 98%); LC-MS ESI, *m*/*z* 422.2 $[M+H]^+$ (anal. calcd. for $C_{21}H_{28}FN_3O_5$, *m*/*z* = 421.5).



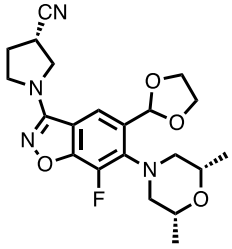
*tert-butyl ((S)-1-(6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)pyrrolidin-3-yl)carbamate (**48i**).* Prepared following the preparation of **48a** using **47** (302 mg, 0.85 mmol), 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine (0.51 mL, 3.39 mmol) and *tert*-butyl (*S*)-pyrrolidin-3-ylcarbamate (315 mg, 1.69 mmol). Column chromatography on silica gel using hexane:EtOAc (20:80 gradient). Off-white solid (192 mg, 43%). RP-UPLC t_R = 1.203 min (method 2, purity 95%); LC-MS ESI, *m*/*z* 507.1 $[M+H]^+$ (anal. calcd. for $C_{25}H_{35}FN_4O_6$, *m*/*z* = 506.6).



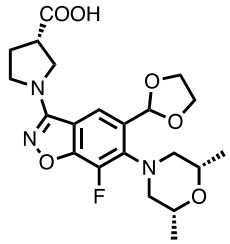
*tert-butyl ((S)-1-(6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)pyrrolidin-3-yl)(methyl)carbamate (**48j**).* Prepared following the preparation of **48a** using **47** (303 mg, 0.85 mmol), 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine (0.51 mL, 3.40 mmol) and *tert*-butyl (*R*)-methyl(pyrrolidin-3-yl)carbamate (340 mg, 1.70 mmol). Column chromatography on silica gel using hexane:EtOAc (20:80 gradient). Off-white solid (365 mg, 79%). RP-UPLC t_R = 1.269 min (method 2, purity 96%); LC-MS ESI, *m*/*z* 521.1 $[M+H]^+$ (anal. calcd. for $C_{26}H_{37}FN_4O_6$, *m*/*z* = 520.6).



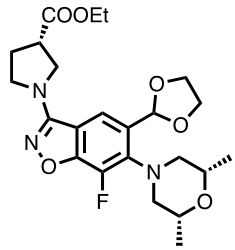
*6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluoro-3-((S)-3-fluoropyrrolidin-1-yl)benzo[d]isoxazole (**4k**).* Prepared following the preparation of **48a** using **47** (308 mg, 0.86 mmol), 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine (0.52 mL, 3.45 mmol) and (*S*)-3-fluoropyrrolidine (154 mg, 1.72 mmol). Column chromatography on silica gel using hexane:EtOAc (20:80 gradient). Pale-yellow oil (247 mg, 68%). RP-UPLC t_R = 1.168 min (method 2, purity 97%); LC-MS ESI, *m*/*z* 410.2 $[M+H]^+$ (anal. calcd. for $C_{20}H_{25}F_2N_3O_4$, *m*/*z* = 409.4).



*(S)-1-(6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)pyrrolidine-3-carbonitrile (**48l**).* Prepared following the preparation of **48a** using **47** (142 mg, 0.40 mmol), 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine (0.13 mL, 0.88 mmol) and (*S*)-pyrrolidine-3-carbonitrile (105 mg, 0.80 mmol). Column chromatography on silica gel using hexane:EtOAc (20:80 gradient). Off-white solid (152 mg, 90%). RP-HPLC t_R = 3.509 min (method 1, purity 99%); LC-MS ESI, *m*/*z* 417.0 $[M+H]^+$ (anal. calcd. for $C_{21}H_{25}FN_4O_4$, *m*/*z* = 416.5).

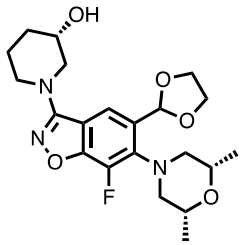


*(S)-1-(6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)pyrrolidine-3-carboxylic acid (**48m**).* Prepared following the preparation of **48a** using **47** (300 mg, 0.84 mmol), 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine (0.51 mL, 3.36 mmol) and (*S*)-pyrrolidine-3-carboxylic acid (194 mg, 1.68 mmol). Column chromatography on silica gel using DCM:0.5M NH_3 in MeOH (80:20 gradient). Off-white solid (280 mg, 77%). RP-UPLC t_R = 1.018 min (method 2, purity 97%); LC-MS ESI, *m*/*z* 436.1 $[M+H]^+$ (anal. calcd. for $C_{21}H_{26}FN_3O_6$, *m*/*z* = 435.5).

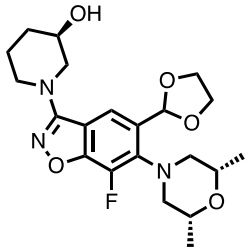


*ethyl (S)-1-((2R,4S,4aS)-11-fluoro-2,4-dimethyl-2',4',6'-trioxo-1,1',2,3',4,4a,4',6'-octahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidin]-8-yl)pyrrolidine-3-carboxylate (**S1**).* Prepared following the preparation of **8** using **48m** (355 mg, 0.79 mmol) and pyrimidine-2,4,6(1H, 3H, 5H)-trione (111 mg, 0.86 mmol). Chiral column chromatography using hexane:EtOH:EtOAc (70:10:20, isocratic, 15mL/min, Diacel IA column). White solid (11 mg, 3%). ^{1}H NMR (300 MHz, MeOD) δ 7.19 (s, 1H), 4.25 – 4.17 (m, 2H), 4.09 – 4.02 (m, 1H), 3.97 – 3.85 (m, 1H), 3.85 – 3.71 (m, 3H), 3.72 – 3.56 (m, 2H), 3.19 – 3.06 (m, 2H), 3.06 – 2.95 (m, 2H), 2.44 – 2.20 (m, 2H), 1.32 – 1.26 (m, 4H), 1.22 (d, *J* = 6.2 Hz, 3H), 1.01 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (151 MHz, MeOD) δ 173.40, 171.78, 168.40, 158.15, 153.18 (d, *J*= 12.8 Hz), 150.37, 134.73 (d, *J*= 239.9 Hz), 134.65, 120.99, 115.54 (d, *J*=

3.3 Hz), 108.29, 72.72, 72.22, 64.93, 60.72, 56.51 (d, *J*= 9.7 Hz), 50.29, 42.86, 42.14, 39.61, 28.32, 17.35, 17.04, 13.07, 10.47. RP-UPLC t_R = 1.027 min (method 2, purity 99%); LC-MS ESI, *m*/*z* 528.2 $[M-H]^-$ (anal. calcd. for $C_{22}H_{28}FN_5O_7$, *m*/*z* = 529.5). $[\alpha]_D^{20}$ = -128.4º (c 0.14, MeOH).

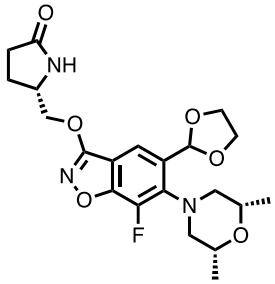


*(S)-1-(6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)piperidin-3-ol (**48n**).* Prepared following the preparation of **48a** using **47** (307 mg, 0.86 mmol), 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine (0.52 mL, 3.44 mmol) and (*S*)-piperidin-3-ol (174 mg, 1.72 mmol). Column chromatography on silica gel using hexane:EtOAc (20:80 gradient). Off-white solid (109 mg, 30%). RP-HPLC t_R = 3.905 min (method 1, purity 98%); LC-MS ESI, *m*/*z* 422.0 $[M+H]^+$ (anal. calcd. for $C_{21}H_{28}FN_3O_5$, *m*/*z* = 421.5).

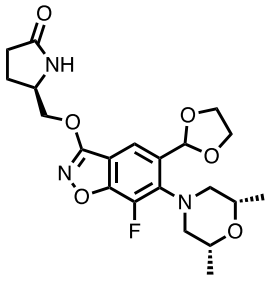


*(R)-1-(6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)piperidin-3-ol (**48o**).* Prepared following the preparation of **48a** using **47** (300 mg, 0.84 mmol), 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-*a*]azepine (0.51 mL, 3.37 mmol) and (*R*)-piperidin-3-ol (170 mg, 1.68 mmol). Column chromatography using hexane:EtOAc (20:80

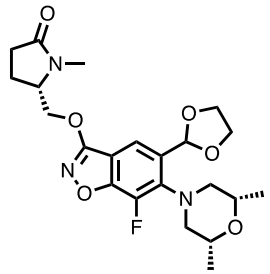
gradient). Off-white solid (251 mg, 71%). RP-HPLC t_R = 3.887 min (method 1, purity 100%); LC-MS ESI, *m*/*z* 422.0 $[M+H]^+$ (anal. calcd. for $C_{21}H_{28}FN_3O_5$, *m*/*z* = 421.5).



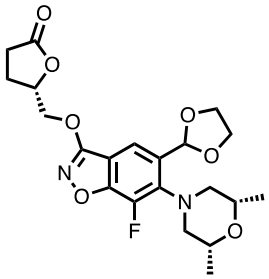
(S)-5-(((6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)oxy)methyl)pyrrolidin-2-one ***(48p)***. In a sealed tube, a mixture of (*S*)-5-(hydroxymethyl)pyrrolidin-2-one (194 mg, 1.68 mmol) and sodium hydride 60% dispersion in mineral oil (135 mg, 3.36 mmol) in *N*,*N*-dimethylformamide (2mL) were stirred at 0°C for 30min. This was followed but the slow addition of **47** (300 mg, 0.841 mmol) in *N*,*N*-dimethylformamide (1mL) at 0°C and warmed to room temperature. The reaction mixture was then stirred at 30°C for 1 h. The reaction was cooled and a dark orange suspension was obtained. The reaction mixture was diluted with 10mL ethyl acetate and washed with 2x10mL of 10% LiCl solution. The organic fractions were combined, dried over Na_2SO_4 and solvent was removed to afford a pale yellow residue. The material was purified by flash chromatography on silica gel using DCM:MeOH (90:10 gradient elution) to give the title compound as a pale yellow oil (289 mg, 73%). RP-UPLC t_R = 1.036 min (method 2, purity 92%); LC-MS ESI, *m*/*z* 436.1 $[M+H]^+$ (anal. calcd. for $C_{21}H_{26}FN_3O_6$, *m*/*z* 435.5).



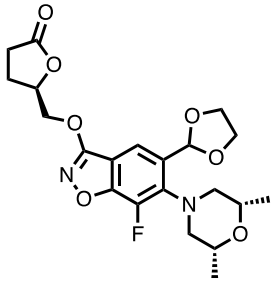
(R)-5-(((6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)oxy)methyl)pyrrolidin-2-one ***(48q)***. Prepared following the preparation of **48p** using **47** (300 mg, 0.84 mmol), sodium hydride 60% dispersion in mineral oil (37 mg, 0.92 mmol), and (*R*)-5-(hydroxymethyl)pyrrolidin-2-one (194 mg, 1.68 mmol). Column chromatography on silica gel using DCM:MeOH (90:10 gradient). Pale-yellow oil (346 mg, 95%). RP-UPLC t_R = 1.037 min (method 2, purity 100%); LC-MS ESI, *m*/*z* 436.1 $[M+H]^+$ (anal. calcd. for $C_{21}H_{26}FN_3O_6$, *m*/*z* = 435.5).



(S)-5-(((6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)oxy)methyl)-1-methylpyrrolidin-2-one ***(48r)***. (*S*)-5-(((6-((2*R*,6*S*)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[*d*]isoxazol-3-yl)oxy)methyl)pyrrolidin-2-one **48p** (300 mg, 0.69 mmol) was dissolved in *N*,*N*-dimethylformamide (5mL) and cooled to 0ºC. Sodium hydride 60% dispersion in mineral oil (41 mg, 1.03 mmol) was added to the reaction mixture and stirred for 1 h. Then, iodomethane (643 *u*L, 1.03 mmol) was added slowly. The mixture was warmed to room temperature and stirred for another 1 h at this temperature. The reaction mixture was diluted with 10mL ethyl acetate and washed with 2x10mL of 10% LiCl solution. The organic fractions were combined, dried over Na_2SO_4 and solvent was removed to afford a dark residue. The material was purified by flash chromatography on silica gel using DCM:MeOH (90:10 gradient elution) to give the title compound as a pale yellow oil (202 mg, 65%). RP-UPLC t_R = 1.080 min (method 2, purity 100%); LC-MS ESI, *m*/*z* 450.1 $[M+H]^+$ (anal. calcd. for $C_{22}H_{28}FN_3O_6$, *m*/*z* = 449.5).

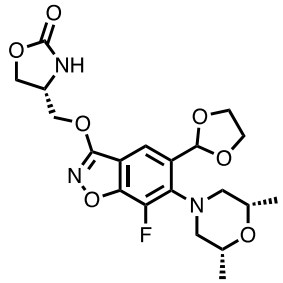


*(S)-5-(((6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)oxy)methyl)dihydrofuran-2(3H)-one (**48s**).* In a sealed tube, a mixture of (*S*)-5-(hydroxymethyl)dihydrofuran-2(3*H*)-one (423.1 mg, 3.64 mmol), **47** (650 mg, 1.82 mmol) and cesium carbonate (2.37 g, 7.29 mmol) in *N*,*N*-dimethylformamide (2mL) were heated to 90 ºC for 32h. The reaction mixture was cooled to room temperature and solvent was removed to afford an orange residue. The material was purified by flash chromatography on silica gel using hexane:EtOAc (20:80 gradient elution) to give the title compound as a pale yellow oil (247 mg, 31%). RP-UPLC t_R = 1.115 min (method 2, purity 100%); LC-MS ESI, *m*/*z* 437.1 $[M+H]^+$ (anal. calcd. for $C_{21}H_{25}FN_2O_7$, *m*/*z* 436.4).

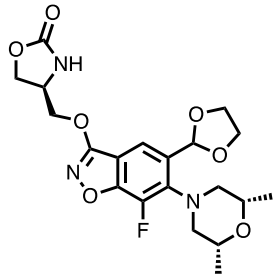


*(R)-5-(((6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)oxy)methyl)dihydrofuran-2(3H)-one (**48t**).* Prepared following the preparation of **48p** using **47** (650 mg, 1.82 mmol), (*R*)-5-(hydroxymethyl)dihydrofuran-2(3*H*)-one (423 mg, 7.29 mmol), and cesium carbonate (2.37 g, 7.29 mmol). Column chromatography on silica gel using hexane:EtOAc (20:80 gradient). Pale-yellow oil (191 mg, 24%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.60 (d, *J* = 0.8 Hz, 1H), 6.13 (s, 1H), 4.98 (tt, *J* =

7.2, 3.4 Hz, 1H), 4.69 – 4.50 (m, 2H), 4.18 – 3.94 (m, 4H), 3.82 – 3.69 (m, 2H), 3.07 (d, *J* = 11.0 Hz, 2H), 2.90 – 2.78 (m, 2H), 2.64 – 2.56 (m, 2H), 2.45 – 2.25 (m, 1H), 2.20 – 2.03 (m, 1H), 1.09 (d, *J*= 6.2 Hz, 6H). RP-UPLC t_R = 1.064 min (method 2, purity, 99%); LC-MS ESI, *m*/*z* 437.0 $[M+H]^+$ (anal. calcd. for $C_{21}H_{25}FN_2O_7$, *m*/*z* = 436.4).

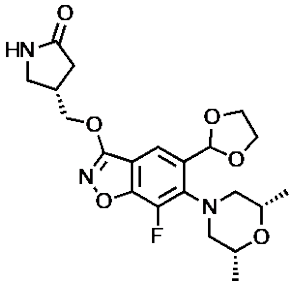


*(R)-4-(((6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)oxy)methyl)oxazolidin-2-one (**48u**).* Prepared following the preparation of **48p** using **47** (300 mg, 0.84 mmol), sodium hydride 60% dispersion in mineral oil (67 mg, 1.68 mmol), and (*R*)-4-(hydroxymethyl)oxazolidin-2-one (197 mg, 1.68 mmol). Column chromatography on silica gel using hexane:EtOAc (40:60 gradient). Pale-yellow oil (276 mg, 72%). RP-UPLC t_R = 1.025 min (method 2, purity 96%); LC-MS ESI, *m*/*z* 438.0 $[M+H]^+$ (anal. calcd. for $C_{20}H_{24}FN_3O_7$, *m*/*z* = 437.4).

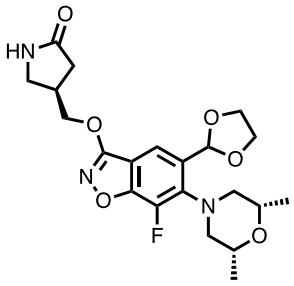


*(S)-4-(((6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)oxy)methyl)oxazolidin-2-one (**48v**).* Prepared following the preparation of **48p** using **47** (300 mg, 0.84 mmol), sodium hydride 60% dispersion in mineral oil (67 mg, 1.68 mmol), and (*S*)-4-(hydroxymethyl)oxazolidin-2-one (197 mg, 1.68 mmol). Column chromatography on

silica gel using hexane:EtOAc (40:60 gradient). Pale-yellow oil (281 mg, 74%). RP-UPLC t_R = 1.024 min (method 2, purity 97%); LC-MS ESI, *m/z* 438.0 $[M+H]^+$ (anal. calcd. for $C_{20}H_{24}FN_3O_7$, *m/z* = 437.4).

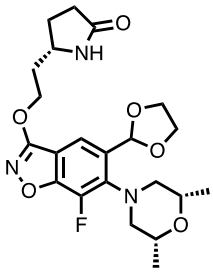


*(S)-4-(((6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)oxy)methyl)pyrrolidin-2-one (**48w**).* Prepared following the preparation of **48p** using **47** (300 mg, 0.84 mmol), sodium hydride 60% dispersion in mineral oil (175 mg, 4.37 mmol), and (*S*)-4-(hydroxymethyl)pyrrolidin-2-one[1] (252 mg, 2.19 mmol). Column chromatography on silica gel using DCM:MeOH (90:10 gradient). Pale-yellow oil (282 mg, 77%). RP-UPLC t_R = 1.026 min (method 2, purity 100%); LC-MS ESI, *m/z* 436.1 $[M+H]^+$ (anal. calcd. for $C_{21}H_{26}FN_3O_6$, *m/z* = 435.5).

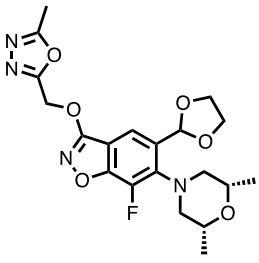


*(R)-4-(((6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)oxy)methyl)pyrrolidin-2-one (**48x**).* Prepared following the preparation of **48p** using **47** (300 mg, 0.84 mmol), sodium hydride 60% dispersion in mineral oil (67 mg, 1.68 mmol), and (*R*)-4-(hydroxymethyl)pyrrolidin-2-one[1] (174 mg, 1.51 mmol). Column chromatography on silica gel using DCM:MeOH (90:10 gradient). Pale-yellow oil

(224 mg, 59%). ^{1}H NMR (300 MHz, DMSO) δ 7.59 (d, *J* = 5.4, 2H), 6.11 (s, 1H), 4.42 (d, *J* = 6.8, 2H), 4.18 – 3.93 (m, 4H), 3.82 – 3.65 (m, 2H), 3.51 – 3.41 (m, 1H), 3.21 – 3.15 (m, 1H), 3.10 – 2.93 (m, 3H), 2.88 – 2.76 (m, 2H), 2.37 (dd, *J* = 16.7, 9.0, 1H), 2.12 (dd, *J* = 16.7, 6.8, 1H), 1.09 (d, *J* = 6.2, 7H). RP-UPLC t_R = 1.020 min (method 2, purity 97%); LC-MS ESI, *m*/*z* 436.1 $[M+H]^+$ (anal. calcd. for $C_{21}H_{26}FN_3O_6$, *m*/*z* = 435.5).



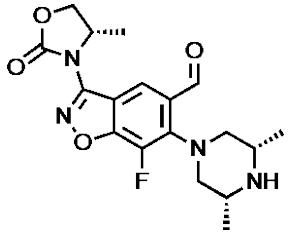
*(S)-5-(2-((6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)oxy)ethyl)pyrrolidin-2-one (**48y**).* Prepared following the preparation of **48p** using **47** (359 mg, 0.80 mmol), sodium hydride 60% dispersion in mineral oil (67 mg, 1.68 mmol), and (*S*)-5-(2-hydroxyethyl)pyrrolidin-2-one (217 mg, 1.68 mmol). Column chromatography on silica gel using DCM:MeOH (90:10 gradient). White solid (360 mg, 75%). RP-UPLC t_R = 1.064 min (method 2, purity 79%); LC-MS ESI, *m*/*z* 450.1 $[M+H]^+$ (anal. calcd. for $C_{22}H_{28}FN_3O_6$, *m*/*z* = 449.5).



*6-((2R,6S)-2,6-dimethylmorpholino)-5-(1,3-dioxolan-2-yl)-7-fluoro-3-((5-methyl-1,3,4-oxadiazol-2-yl)methoxy)benzo[d]isoxazole (**48z**).* Prepared following the preparation of **48p** using **47** (300 mg, 0.841 mmol), sodium hydride 60% dispersion in mineral oil (67 mg, 1.68

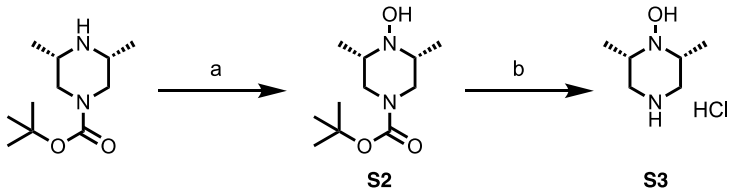
mmol), and (5-methyl-1,3,4-oxadiazol-2-yl)methanol (192 mg, 1.68 mmol). Column chromatography on silica gel using hexane:EtOAc (20:80 gradient elution). White solid (77 mg, 21%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.61 (s, 1H), 6.13 (s, 1H), 5.74 (s, 2H), 4.15 – 3.93 (m, 4H), 3.83 – 3.68 (m, 2H), 3.11 – 3.03 (m, 2H), 2.89 – 2.76 (m, 2H), 2.55 (s, 3H), 1.09 (d, J = 6.2 Hz, 6H). RP-UPLC t_R = 1.468 min (method 2, purity 100%); LC-MS ESI, m/z 435.1 $[M+H]^+$ (anal. calcd. for $C_{20}H_{23}FN_4O_6$, m/z = 434.4).

Intermediates from Scheme 3:

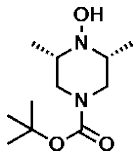


*6-((3S,5R)-3,5-dimethylpiperazin-1-yl)-7-fluoro-3-((S)-4-methyl-2-oxooxazolidin-3-yl)benzo[d]isoxazole-5-carbaldehyde (**50a**).* In a microwave tube, K_2CO_3 (294 mg, 2.13 mmol) and commercially available (2*R*,6*S*)-2,6-dimethylpiperazine (146 mg, 1.23 mmol) were added to a solution of **49** (300 mg, 1.06 mmol) in acetonitrile (3 mL). The resulting reaction mixture was irradiated in a microwave at 80 °C for 30 min. Then, the reaction mixture was cooled and diluted with water (10 mL) and extracted with EtOAc (3x15 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and solvent removed under reduced pressure. The material was purified by flash chromatography on silica gel using CH_2Cl_2:MeOH (0-20%) gradient to afford the title compound (270 mg, 65%) as a light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.27 (s, 1H), 8.61 (d, J = 1.1 Hz, 1H), 4.79 – 4.71 (m, 2H), 4.26 – 4.20 (m, 1H), 3.23 – 3.09 (m, 4H), 3.06 – 2.97 (m, 2H), 1.58 (d, J = 5.9 Hz, 3H), 1.14 (d, J = 6.1 Hz, 6H). RP-UPLC t_R = 3.503 min (method 1, purity 100%); LC-MS ESI, m/z 377.2 $[M+H]^+$ (anal. calcd. for $C_{18}H_{21}FN_4O_4$, m/z = 376.4).

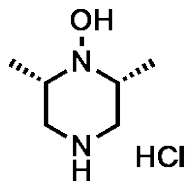
Scheme S1: Synthesis of amines S2 and S3.[a]



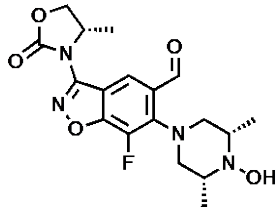
[a]Reagents and conditions: (a) i) SeO_2, H_2O_2, MeOH, 25 °C, 8 h, ii) $NaBH_4$, MeOH, 25 °C, 2 h, 37%; (b) 4M HCl, CH_2Cl_2, 25 °C, 16 h, 76%.



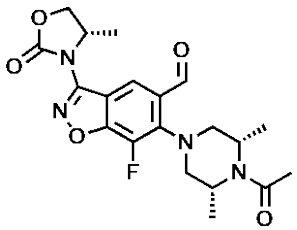
*Tert-butyl (3S,5R)-4-hydroxy-3,5-dimethylpiperazine-1-carboxylate (**S2**).* To a stirring solution of commercially available *tert*-butyl (3*S*,5*R*)-3,5-dimethylpiperazine-1-carboxylate (3.00 g, 14.0 mmol) with MeOH (22 mL) in presence of selenium dioxide (780 mg, 0.700 mmol) at 0 °C under N_2 was added dropwise a 30% hydrogen peroxide (1.79 mL, 17.5 mmol) in 15 min. The mixture was then stirred at 25°C for 8 h. Sodium borohydride (222 mg, 5.88 mmol) was added to the above mixture and stirring continued for 2 h. After removal of the solvent, the residual mixture was taken up in sat. K_2CO_3 solution (10 mL) and extracted with CH_2Cl_2/MeOH (9:1) (4x15 mL). The combined organic layers were dried (Na_2SO_4), concentrated and the residual liquid was purified by flash chromatography on silica gel using CH_2Cl_2:MeOH (0-20%) gradient to afford the title compound (1.20 g, 37%) as an off white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.87 (s, 1H), 3.81 (d, J = 13.5 Hz, 2H), 2.58 – 2.53 (m, 2H), 2.37 – 2.32 (m, 2H), 1.40 (s, 9H), 1.03 (d, J = 6.0 Hz, 6H).



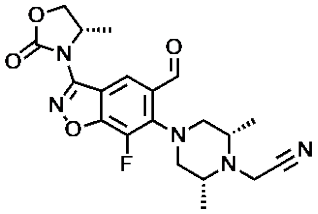
*(2S,6R)-2,6-dimethylpiperazin-1-ol hydrochloride (**S3**)*. To a solution of **S2** (700 mg, 3.04 mmol) in CH_2Cl_2 (10 mL) was added 4M HCl in 1,4-dioxane (7.60 mL, 30.4 mmol). The solution was stirred at 25 °C for 16 h. The solvent was removed under reduced pressure and the residue was triturated with diethyl ether to afford the title compound (300 mg, 76%) as a pale-yellow HCl salt. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.98 (br m, 2H), 3.93 – 3.85 (m, 1H), 3.57 – 3.48 (m, 2H), 3.30 – 3.25 (m, 1H), 3.19 – 2.98 (m, 2H), 1.31 (d, J = 6.3 Hz, 3H), 1.26 (d, J = 6.5 Hz, 3H).



*7-fluoro-6-((3S,5R)-4-hydroxy-3,5-dimethylpiperazin-1-yl)-3-((S)-4-methyl-2-oxooxazolidin-3-yl)benzo[d]isoxazole-5-carbaldehyde (**50b**).* A mixture of **49** (500 mg, 1.77 mmol), **S3** (443 mg, 2.66 mmol) and Et_3N (1.48 mL, 10.6 mmol) in DMSO (5 mL) was heated at 110 °C for 2 h. Then, the reaction mixture was cooled, diluted with EtOAc (20 mL) and washed with water (3x15 mL). The organic phases were combined, dried over Na_2SO_4, filtered and solvent removed under reduced pressure. The material was purified by flash chromatography on silica gel using CH_2Cl_2:MeOH (0-20%) gradient to afford the title compound (300 mg, 41%) as a brown solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.18 (s, 1H), 8.49 (d, J = 1.0 Hz, 1H), 7.92 (s, 1H), 4.80 – 4.63 (m, 2H), 4.30 – 4.20 (m, 1H), 3.31 – 3.27 (m, 2H), 3.19 – 3.10 (m, 2H), 2.85 – 2.71 (m, 2H), 1.45 (d, J = 5.9 Hz, 3H), 1.07 (d, J = 6.0 Hz, 6H). RP-UPLC t_R = 3.698 min (method 1, purity 96%); LC-MS ESI, m/z 393.1 $[M+H]^+$ (anal. calcd. for $C_{18}H_{21}FN_4O_5$, m/z = 392.3).



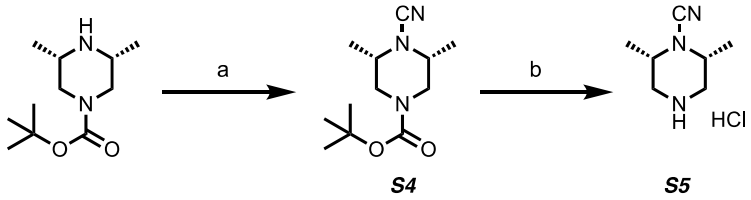
*6-((3S,5R)-4-acetyl-3,5-dimethylpiperazin-1-yl)-7-fluoro-3-((S)-4-methyl-2-oxooxazolidin-3-yl)benzo[d]isoxazole-5-carbaldehyde (**50c**).* Acetic anhydride (115 µL, 1.22 mmol) and pyridine (74 µL, 917 mmol) was added dropwise to a solution of **50a** (230 mg, 0.611 mmol) in DCM (10 mL) and resulting reaction mixture was stirred at 25 °C for 24 h. Reaction mixture was diluted with DCM (15 mL) and washed with 1N HCl to remove excess of pyridine. The organic layer was isolated, dried over Na_2SO_4, filtered and solvent removed under reduced pressure. The material was purified by flash chromatography on silica gel using CH_2Cl_2:MeOH (0-20%) gradient to afford the title compound (220 mg, 80%) as a yellow solid. RP-UPLC t_R = 0.784 min (method 2, purity 93%); LC-MS ESI, *m/z* 419.2 $[M+H]^+$ (anal. calcd. for $C_{20}H_{23}FN_4O_5$, *m/z* = 418.4).



*2-((2S,6R)-4-(7-fluoro-5-formyl-3-((S)-4-methyl-2-oxooxazolidin-3-yl)benzo[d]isoxazol-6-yl)-2,6-dimethylpiperazin-1-yl)acetonitrile (**50d**).* Bromoacetonitrile (125 µL, 1.79 mmol) and K_2CO_3 (330 mg, 2.39 mmol) were added to a solution of **50a** (450 mg, 1.20 mmol) in Acetone (20 ml) and resulting reaction mixture was stirred at 25 °C for 16 h. After completion of reaction, solvent was removed under reduced pressure and residue was taken in EtOAc (50 mL) and washed with water (20 mL). Organic phase was dried over Na_2SO_4, filtered and solvent removed under reduced pressure. The material was purified by flash chromatography

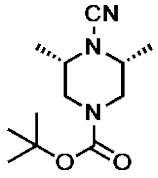
on silica gel using CH_2Cl_2:MeOH (0-5%) gradient to afford the title compound (350 mg, 68%) as a light yellow solid. RP-UPLC t_R = 1.095 min (method 2, purity 97%); LC-MS ESI, *m*/*z* 416.1 $[M+H]^+$ (anal. calcd. for $C_{20}H_{22}FN_5O_4$, *m*/*z* = 415.4).

Scheme S2: Synthesis of amines S4 and S5.[a]



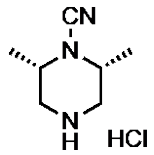
S4 ***S5***

[a]Reagents and conditions: (a) CNBr, K_2CO_3, acetone, 25 °C, 16 h, 79%; (b) HCl solution, CH_2Cl_2, 25 °C, 16 h, 51%.



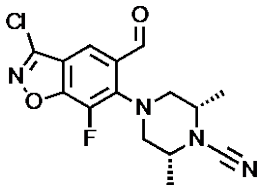
*tert-butyl (3S,5R)-4-cyano-3,5-dimethylpiperazine-1-carboxylate (**S4**).* To a suspension of commercially available *tert*-butyl (3*S*,5*R*)-3,5-dimethylpiperazine-1-carboxylate (5.90 g, 27.5 mmol) and K_2CO_3 (5.71 g, 41.3 mmol) in acetone (50 mL), cyanogen bromide (4.37g, 41.3mmol) was syringed and the reaction mixture stirred at 25 °C for 16 h. The following day a white precipitate was observed. Then, the solvent was evaporated to dryness to afford a white solid. The solid was taken up in dichloromethane (50 mL) and washed with water, followed by brine. The organic layer was isolated, dried over $MgSO_4$, filtered and solvent removed under reduced pressure. The resulting solid was suspended in hexane (50 mL) and stirred at 25 °C for 2 h. The solid was filtered, washed with hexane, dried *in vacuo* to afford the title compound (5.22 g, 79%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.90 (d, *J* = 13.3 Hz, 2H),

3.23 – 3.06 (m, 2H), 2.64 – 2.42 (m, 2H), 1.41 (s, 9H), 1.22 (d, J = 6.5 Hz, 6H). LC-MS ESI, m/z 240.2 $[M+H]^+$ (anal. calcd. for $C_{12}H_{21}N_3O_2$, m/z = 239.3).



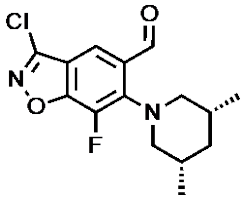
*(2S,6R)-2,6-dimethylpiperazine-1-carbonitrile hydrochloride (**S5**). To* a solution of tert-butyl (3*S*,5*R*)-4-cyano-3,5-dimethylpiperazine-1-carboxylate (**S4**) (5.22g, 21.8 mmol) in dichloromethane (5 mL), Hydrogen chloride solution (9.85 mL, 283 mmol) in dioxane was syringed. The reaction mixture was stirred at 25 °C for 16 h. The following day a white precipitate was observed, filtered, washed with cold DCM and dried *in vacuo* to afford the title compound (1.98 g, 52% yield) as a white HCl salt. *Note: The product is hygroscopic.* ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.24 – 9.81 (m, 2H), 3.74 – 3.49 (m, 2H), 3.33 – 3.13 (m, 2H), 2.75 – 2.54 (m, 2H), 1.23 (d, J = 6.6 Hz, 6H). LC-MS ESI, m/z 140.2 $[M+H]^+$ (anal. calcd. for $C_7H_{13}N_3$, m/z = 139.2).

Intermediates from Scheme 4

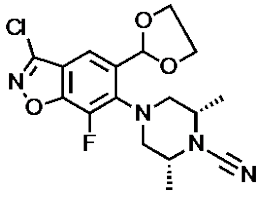


*(2R,6S)-4-(3-chloro-7-fluoro-5-formylbenzo[d]isoxazol-6-yl)-2,6-dimethylpiperazine-1-carbonitrile (**52a**).* To a pressure tube containing a solution of **51** (3.80 g, 17.5 mmol) in acetonitrile (20 mL), K_2CO_3 (9.66 g, 69.9 mmol) and **S5** (4.60 g, 26.2 mmol) were added. The resulting reaction mixture was heated at 110 °C for 5 h. Then, the reaction mixture was cooled and diluted with water (10 mL) and extracted with EtOAc (3x15 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and solvent removed under reduced pressure. The

material was purified by flash chromatography on silica gel using hexane:EtOAc (0-95%) gradient to afford the title compound (4.78 g, 81%) as an off-white solid. RP-UPLC t_R = 2.856 min (method 1, purity 99%); LC-MS ESI, *m/z* 312.8 $[M\text{-}CN]^+$ (anal. calcd. for $C_{14}H_{15}ClFN_3O_2$, *m/z* = 311.7).

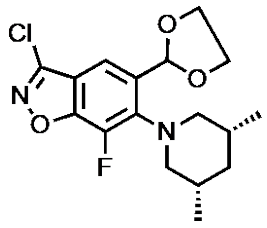


*3-chloro-6-((3R,5S)-3,5-dimethylpiperidin-1-yl)-7-fluorobenzo[d]isoxazole-5-carbaldehyde (**52b**).* A suspension of **51** (1.00 g, 4.60 mmol), K_2CO_3 (1.27 g, 9.19 mmol) and commercially available (3*R*,5*S*)-3,5-dimethylpiperidine (520 mg, 4.60 mmol) in acetonitrile (60mL) was heated to 90 °C for 1 h. The mixture was cooled, solvent removed, diluted with EtOAc (50 mL) and washed with brine (50 mL). The organic layer was isolated, dried over $MgSO_4$, filtered, solvent removed under reduced pressure. The material was purified by flash chromatography on silica gel using hexane:EtOAc (0-10%) gradient to afford the title compound (1.12 g, 77%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.24 (s, 1H), 7.93 (d, *J*= 1.0, 1H), 3.23 (ddd, *J*= 11.8, 4.0, 1.9, 2H), 2.87 (dd, *J*= 11.9, 3.3, 2H), 2.01 – 1.79 (m, 2H), 1.16 – 0.68 (m, 8H). RP-UPLC t_R = 1.404 min (method 2, purity 98%); LC-MS ESI, *m/z* 311.0 $[M+H]^+$ (anal. calcd. for $C_{15}H_{16}ClFN_2O_2$, *m/z* = 310.8).

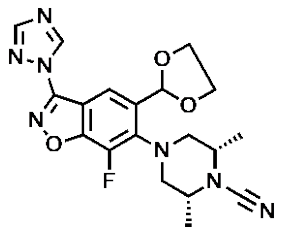


*(2R,6S)-4-(3-chloro-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-6-yl)-2,6-dimethylpiperazine-1-carbonitrile (**53a**).* A mixture of **52a** (451 mg, 1.34 mmol), ethane-1,2-

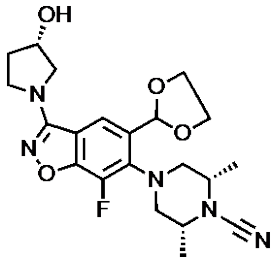
diol (299 µL, 5.36 mmol) and *p*-TSA (13 mg, 0.067 mmol) in toluene (50 mL) was heated to 130 °C for 16 h with azeoptropic removal of water, using a Dean-Stark Trap. The mixture was cooled, solvent removed, diluted with EtOAc (10 mL) and washed with $NaHCO_3$ (10 mL)and water (10 mL). The organic layer was isolated, dried over Na_2SO_4, filtered, solvent removed under reduced pressure. The material was purified by flash chromatography on silica gel using hexane:EtOAc (0-40%) gradient to afford the title compound (328 mg, 57%) as a pale yellow solid. RP-UPLC t_R = 3.751 min (method 1, purity 89%); LC-MS ESI, *m*/*z* 381.1 $[M+H]^+$ (anal. calcd. for $C_{17}H_{18}ClFN_4O_3$, *m*/*z* = 380.8).



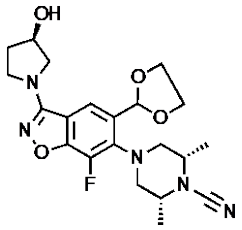
*3-chloro-6-((3R,5S)-3,5-dimethylpiperidin-1-yl)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazole (**53b**).* Prepared as described for **53a** with **52b** (1.12 g, 3.60 mmol), ethane-1,2-diol (1.21 mL, 21.6 mmol) and *p*-TSA (48 mg, 0.250 mmol). Flash column chromatography using hexane:EtOAc (90:10), afforded the title compound (1.16 g, 87%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (d, *J*= 1.0, 1H), 6.17 (s, 1H), 4.30 – 4.15 (m, 2H), 4.15 – 4.01 (m, 2H), 3.21 – 3.12 (m, 2H), 2.74 (td, *J*= 11.0, 3.4, 2H), 1.84 (ddt, *J*= 10.7, 3.5, 1.9, 2H), 0.99 – 0.69 (m, 8H). RP-UPLC t_R = 1.460 min (method 2, purity 96%); LC-MS ESI, *m*/*z* 355.0 $[M]^+$ (anal. calcd. for $C_{17}H_{20}FN_2O_3$, *m*/*z* = 354.8).



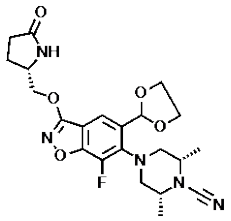
*(2S,6R)-4-(5-(1,3-dioxolan-2-yl)-7-fluoro-3-(1H-1,2,4-triazol-1-yl)benzo[d]isoxazol-6-yl)-2,6-dimethylpiperazine-1-carbonitrile (**54a**).* In a sealed tube, a mixture of **53a** (300 mg, 0.788 mmol), 1H-1,2,4-triazole (54 mg, 0.788 mmol) and sodium hydride (38 mg, 1.58 mmol) in DMF (2 mL) were heated to 90 °C for 2h. A dark brown solution was obtained and cooled to RT. Solvent was removed to afford a brown residue. The residue was adsorbed onto flash silica and purified by flash chromatography using DCM:MeOH (95:5). The last fraction was isolated, solvent removed to afford the title compound (192 mg, 55 %) as an off-white solid. RP-UPLC t_R = 1.051 min (method 2, purity 93%); LC-MS ESI, *m*/*z* 414.2 $[M+H]^+$ (anal. calcd. for $C_{19}H_{20}FN_7O_3$, *m*/*z* = 413.2).



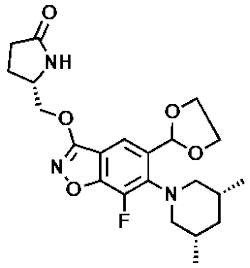
*(2R,6S)-4-(5-(1,3-dioxolan-2-yl)-7-fluoro-3-((S)-3-hydroxypyrrolidin-1-yl)benzo[d]isoxazol-6-yl)-2,6-dimethylpiperazine-1-carbonitrile (**54b**).* In a sealed tube, a mixture of **53a** (301 mg, 0.789 mmol), commercially available (*S*)-pyrrolidin-3-ol (138 mg, 1.58 mmol) and DBU (238 μL, 1.58 mmol) in acetonitrile (2 mL) were heated to 110 ºC for 16 h. A The mixture was cooled, solvent removed, diluted with EtOAc (10 mL) and washed with and water (10 mL). The organic layer was isolated, dried over Na_2SO_4, filtered, solvent removed under reduced pressure. The material was purified by flash chromatography on silica gel using hexane:EtOAc (5:95) gradient to afford the title compound (262 mg, 77%) as an off-white solid. RP-UPLC t_R = 3.06 min (method 2, purity 100%); LC-MS ESI, *m*/*z* 432.2 $[M]^+$ (anal. calcd. for $C_{21}H_{26}FN_5O_4$, *m*/*z* = 431.5).



*(2R,6S)-4-(5-(1,3-dioxolan-2-yl)-7-fluoro-3-((R)-3-hydroxypyrrolidin-1-yl)benzo[d]isoxazol-6-yl)-2,6-dimethylpiperazine-1-carbonitrile (**54c**).* Prepared as described for **54b** with **53a** (302 mg, 0.792 mmol), commercially available (*R*)-pyrrolidin-3-ol (138 mg, 1.58 mmol) and DBU (241 mg, 1.58 mmol). Flash column chromatography using hexane:EtOAc (20:80), afforded the title compound (237 mg, 69%) as an off-white solid. RP-UPLC t_R = 3.06 min (method 1, purity 100%); LC-MS ESI, *m*/*z* 432.2 $[M+H]^+$ (anal. calcd. for $C_{21}H_{26}FN_5O_4$, *m*/*z* = 431.5).

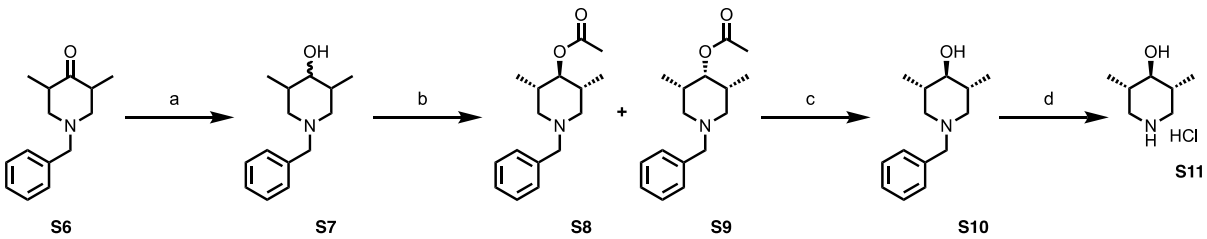


*(2R,6S)-4-(5-(1,3-dioxolan-2-yl)-7-fluoro-3-(((S)-5-oxopyrrolidin-2-yl)methoxy)benzo[d]isoxazol-6-yl)-2,6-dimethylpiperazine-1-carbonitrile (**54d**).* In a sealed tube, a mixture of (*S*)-5-(hydroxymethyl)pyrrolidin-2-one (272 mg, 2.36 mmol) and sodium hydride 60% dispersion in mineral oil (94 mg, 2.36 mmol) in DMF (4 mL) were heated to 30 °C for 30 min. This was followed but the addition of **53a** (300 mg, 0.788 mmol) and the reaction mixture was stirred at 30 °C for 16 h. The mixture was cooled, solvent removed and the material was purified by flash chromatography on silica gel using DCM:MeOH (90:10) gradient to afford the title compound (312 mg, 85%) as a colourless oil. RP-UPLC t_R = 1.016 min (method 2, purity 99%); LC-MS ESI, *m*/*z* 460.1 $[M+H]^+$ (anal. calcd. for $C_{22}H_{26}FN_5O_5$, *m*/*z* = 459.5).

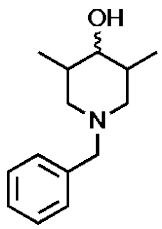


*(S)-5-(((6-((3R,5S)-3,5-dimethylpiperidin-1-yl)-5-(1,3-dioxolan-2-yl)-7-fluorobenzo[d]isoxazol-3-yl)oxy)methyl)pyrrolidin-2-one (**54e**).* Prepared as described for **54b** with **53b** (300 mg, 0.846 mmol), (*S*)-5-(hydroxymethyl)pyrrolidin-2-one (195 mg, 1.69 mmol) and sodium hydride 60% dispersion in mineral oil (68 mg, 1.69 mmol). Flash column chromatography using hexane:EtOAc (20:80), afforded the title compound (358 mg, 91%) as a pale-yellow oil. RP-UPLC t_R = 1.256 min (method 2, purity 93%); LC-MS ESI, *m*/*z* 434.1 $[M+H]^+$ (anal. calcd. for $C_{22}H_{28}FN_3O_5$, *m*/*z* = 433.5).

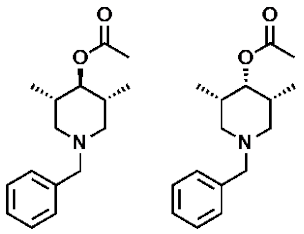
Scheme S3: Synthesis of amine **S11**.[a]



[a]Reagents and conditions: (a) $NaBH_4$, MeOH, 0-20 °C, 0.5h, 99%; (b) Ac_2O, DMAP, Pyr, 110°C, 2h, 19-28%; (c) NaOH, EtOH, 100°C, 24h, 99%; (d)$Pd(OH)_2$/C, conc. HCl, MeOH, 27 °C, 3h, 82%.



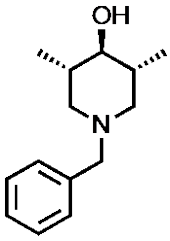
*1-benzyl-3,5-dimethylpiperidin-4-ol (**S7**).* To a mixture of commercially available *rel*-(3*R*,5*S*)-1-benzyl-3,5-dimethylpiperidin-4-one (1.17 g, 5.38 mmol) **(S6)** in methanol (25 mL), sodium borohydride (285 mg, 7.54 mmol) was added at 0 ºC, and stirred at 20 ºC for 30 min. The reaction mixture was quenched with sat. $NaHCO_3$ solution and extracted with EtOAc (3x50 mL), dried over sodium sulfate, filtered and solvent removed to afford the title compound as a mixture of diastereomers. White solid (1.10 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 – 7.23 (m, 10H), 3.59 (s, 1H), 3.54 (s, 2H), 3.49 (s, 2H), 2.89 – 2.78 (m, 2H), 2.71 (t, *J* = 8.9 Hz, 1H), 2.57 – 2.52 (m, 2H), 2.05 – 1.92 (m, 4H), 1.79 – 1.64 (m, 4H), 0.99 – 0.94 (m, 12H). LC-MS ESI, *m*/*z* 220.3 $[M+H]^+$ (anal. calcd. for $C_{14}H_{21}NO$, *m*/*z* = 219.3). *Note: NMR shows presence of both diastereomers.*



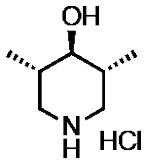
To a pressure tube containing a solution of **S7** and 4-(dimethylamino)pyridine (9.36 mg, 0.0766 mmol) in pyridine (7 mL), acetic anhydride (1.09 mL, 11.5 mmol) was added and the reaction mixture heated to 110 ºC for 2 h. The reaction mixture was cooled and excess pyridine evaporated. The residue was diluted with water and extracted with chloroform (2x60 mL). The organic phases were isolated, combined, dried over sodium sulfate, filtered and solvent removed under reduced pressure to afford a mixture of diastereomers. The isomers were separated by flash chromatography using hexane:EtOAc (0-10%) gradient to afford two diastereomers. *Trans-(3S,4r,5R)-1-benzyl-3,5-dimethylpiperidin-4-yl acetate (**S9**).* Colourless oil (687 mg, 28%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 – 7.23 (m, 5H), 4.33 (t, *J* = 9.7 Hz, 1H), 3.50 (s, 2H), 2.90 – 2.84 (m, 2H), 2.11 (s, 3H), 1.95 – 1.73 (m, 4H), 0.83 (d, *J* = 6.1 Hz, 6H).

RP-UPLC t_R = 0.241 min (method 2, purity 82%); LC-MS ESI, *m/z* 262.3 $[M+H]^+$ (anal. calcd. for $C_{16}H_{23}NO_2$, *m/z* = 261.4).

*Cis-(3S,4s,5R)-1-benzyl-3,5-dimethylpiperidin-4-yl acetate (**S8**).* White solid (514 mg, 19%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.24 (m, 5H), 5.09 (s, 1H), 3.53 (s, 2H), 2.57 (d, *J* = 7.8 Hz, 2H), 2.12 (s, 3H), 2.08 – 1.87 (m, 4H), 0.82 (d, *J* = 6.4 Hz, 6H). RP-UPLC t_R = 0.206 min (method 2, purity 74%); LC-MS ESI, *m/z* 262.3 $[M+H]^+$ (anal. calcd. for $C_{16}H_{23}NO_2$, *m/z* = 261.4).



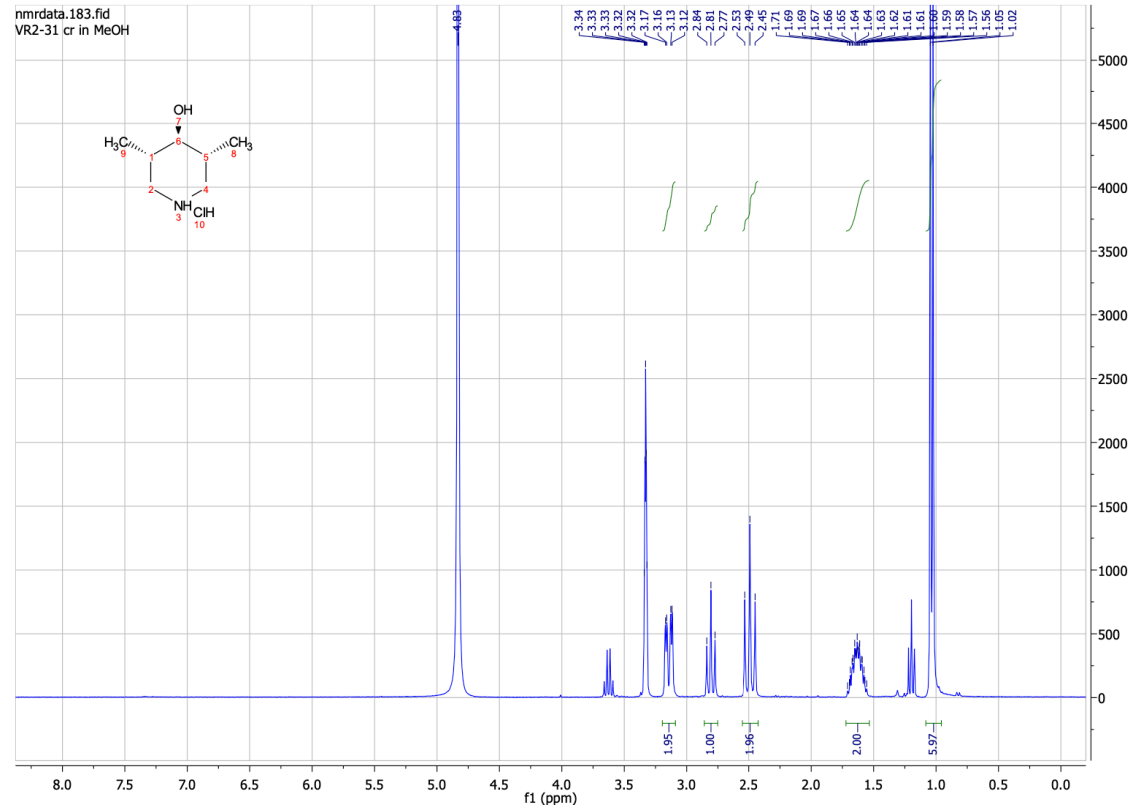
*(3S,4r,5R)-1-benzyl-3,5-dimethylpiperidin-4-ol (**S10**).* To a solution **S9** (680 mg, 2.60 mmol) in ethanol (0.700 mL) was added 5N sodium hydroxide (3.67 mL, 18.4 mmol) in a pressure vial. This reaction mixture was heated to 100 ºC for 24 h. The reaction mixture was cooled, diluted with water and extracted with EtOAc (2x50 mL). The organic phases were isolated, dried over sodium sulfate, filtered and solvent removed to afford the title compound (570 mg, 99%) as a yellow solid. ^{1}H NMR (300 MHz, MeOD) δ 7.37 – 7.24 (m, 5H), 3.50 (s, 2H), 2.89 – 2.80 (m, 2H), 2.59 (t, *J* = 9.3 Hz, 1H), 1.79 – 1.57 (m, 4H), 0.96 (d, *J* = 6.2 Hz, 6H). LC-MS ESI, *m/z* 220.2 $[M+H]^+$ (anal. calcd. for $C_{14}H_{21}NO$, *m/z* = 219.3).



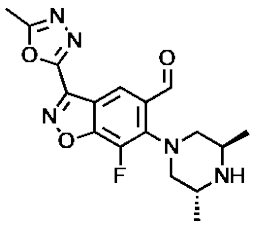
*(3S,4r,5R)-3,5-dimethylpiperidin-4-ol hydrochloride (**S11**).* To a solution of **S10** (565 mg, 2.58 mmol) in methanol (12 mL) was added palladium hydroxide on carbon (72 mg, 0.520 mmol),

followed by 6 drops of conc. HCl and stirred for 3 h at 27 ºC under a hydrogen atmosphere. The reaction mixture was filtered through a pad of Celite, washed with MeOH and solvent evaporated under reduced pressure. EtOH was added and evaporated once again to afford the title compound (350 mg, 82%) as a white solid. ^{1}H NMR (300 MHz, MeOD) δ 3.14 (dd, J = 12.6, 2.9 Hz, 2H), 2.81 (t, J = 9.9 Hz, 1H), 2.49 (t, J = 12.4 Hz, 2H), 1.72 – 1.53 (m, 2H), 1.04 (d, J = 6.5 Hz, 6H). LC-MS ESI, m/z 130.2 $[M+H]^+$ (anal. calcd. for $C_7H_{15}NO$, m/z = 129.2).

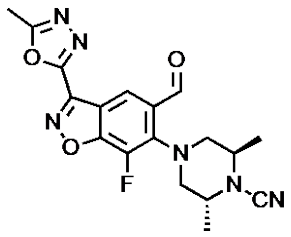
Figure S2: ^{1}H NMR spectrum of S11.



Intermediates from Scheme 5

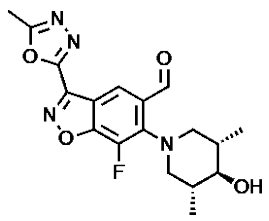


*6-((3R,5R)-3,5-dimethylpiperazin-1-yl)-7-fluoro-3-(5-methyl-1,3,4-oxadiazol-2-yl)benzo[d]isoxazole-5-carbaldehyde (**56**).* In a sealed tube, a mixture of 6,7-difluoro-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1,2-benzoxazole-5-carbaldehyde (500 mg, 1.89 mmol),[2] commercially available (2R,6R)-2,6-dimethylpiperazine dichloride (388 mg, 2.07 mmol) and K_2CO_3 (781 mg, 5.66 mmol) in a mixture of acetonitrile (20 mL):water (1 mL), was heated to 90 °C for 13 h. Then, the reaction mixture was cooled and solvent was removed under reduced pressure. The residue was taken up in EtOAc (50 mL) and washed with water (2x50 mL). The aqueous layers were combined and extracted with fresh EtOAc (50 mL), which was then washed with water and brine. The organic layers were combined, dried over $MgSO_4$, filtered, and solvent removed under reduced pressure. The material was purified by flash chromatography on silica gel using hexane:CH_2Cl_2 (0-10%) gradient to afford the title compound (623 mg, 92%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.32 (s, 1H), 8.32 (s, 1H), 3.02 – 2.92 (m, 2H), 2.72 (s, 3H), 1.12 (d, J = 6.4 Hz, 6H). *(Note: 4 aliphatic protons masked by water peak at 3.3ppm and NH peak not observed).* RP-UPLC t_R = 0.639 min (method 2, purity 100%); LC-MS ESI, *m*/*z* 360.1 $[M+H]^+$ (anal. calcd. for $C_{17}H_{18}FN_5O_3$, *m*/*z* = 359.4).



*(2R,6R)-4-(7-fluoro-5-formyl-3-(5-methyl-1,3,4-oxadiazol-2-yl)benzo[d]isoxazol-6-yl)-2,6-dimethylpiperazine-1-carbonitrile (**57**).* Cyanic bromide (273 mg, 2.58 mmol) and K_2CO_3 (357 mg, 2.58 mmol) were added to a solution of **56** (618 mg, 1.72 mmol) in acetone (10 mL) and resulting reaction mixture was stirred at 27 °C for 9 h. Then the solvent was removed under reduced pressure and residue was taken up in DCM (20 mL) and washed with water (2x10

mL). The aqueous phases were combined and re-extracted with fresh DCM (20 mL). The organic phases were combined, dried over Na_2SO_4, filtered and solvent removed under reduced pressure. The material was purified by flash chromatography on silica gel using hexane:EtOAc (0-90%) gradient to afford the title compound as a white solid (506 mg, 77%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.37 (s, 1H), 8.40 (s, 1H), 3.87 – 3.75 (m, 2H), 3.53 (d, J = 12.5 Hz, 2H), 3.21 – 3.08 (m, 2H), 2.72 (s, 3H), 1.32 (d, J = 6.6 Hz, 6H). RP-UPLC t_R = 1.041 min (method 2, purity 100%); LC-MS ESI, m/z 385.1 $[M+H]^+$ (anal. calcd. for $C_{18}H_{17}FN_6O_3$, m/z = 384.4).



*7-fluoro-6-((3S,4r,5R)-4-hydroxy-3,5-dimethylpiperidin-1-yl)-3-(5-methyl-1,3,4-oxadiazol-2-yl)benzo[d]isoxazole-5-carbaldehyde (**58**).* In a sealed tube, a mixture of 6,7-difluoro-3-(5-methyl-1,3,4-oxadiazol-2-yl)-1,2-benzoxazole-5-carbaldehyde (300 mg, 1.13 mmol),[2] **S13** (225 mg, 1.36 mmol) and K_2CO_3 (391 mg, 2.83 mmol) in a mixture of acetonitrile (12 mL):water (1.2 mL), was heated to 90 °C for 1 h. Then, the reaction mixture was cooled and solvent was removed under reduced pressure. The residue was taken up in EtOAc (50 mL) and washed with water (2x50 mL). The organic layers were combined, dried over $MgSO_4$, filtered, and solvent removed under reduced pressure, to afford the title compound (381 mg, 89%) as a pure yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.33 (s, 1H), 8.58 (d, J = 1.0 Hz, 1H), 3.36 – 3.26 (m, 2H), 3.20 – 3.11 (m, 2H), 2.99 (t, J = 9.7 Hz, 1H), 2.76 (s, 3H), 1.99 – 1.84 (m, 2H), 1.09 (d, J = 6.5 Hz, 6H). ^{19}F NMR (377 MHz, $CDCl_3$) δ -144.37. ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.38, 165.34, 156.28, 155.41 (d, J = 12.4 Hz), , 146.21, 142.59 (d, J = 5.8 Hz), 142.41 (d, J = 256 Hz), 131.74, 120.71, 117.15, 80.19, 59.60 (d, J = 4.7 Hz), 39.33, 15.07, 11.06. RP-

UPLC t_R = 1.050 min (method 2, purity 99%); LC-MS ESI, *m*/*z* 374.4 $[M+H]^+$ (anal. calcd. for $C_{18}H_{19}FN_4O_4$, *m*/*z* = 375.1).

4. Characterization of inactive isomers with unwanted configuration (2*S*,4*R*,4a*R*) and positive optical rotation:

*(2S,4R,4aR)-11-fluoro-8-((S)-3-hydroxypyrrolidin-1-yl)-2,4-dimethyl-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**8E**).* White solid (120 mg, 21%). ^{1}H NMR (300 MHz, MeOD) δ 7.22 (s, 1H), 4.60 – 4.50 (m, 1H), 4.24 – 4.14 (m, 1H), 4.10 - 4.00 (m, 1H), 3.98 – 3.63 (m, 4H), 3.58 – 3.45 (m, 1H), 3.40 – 3.30 (m, 2H), 3.20 – 3.04 (m, 2H), 2.24 – 1.98 (m, 2H), 1.22 (d, *J* = 6.2 Hz, 3H), 1.01 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, MeOD) δ 172.73, 169.43, 159.95, 154.71 (d, *J* = 12.9 Hz), 151.12, 136.33 (d, *J* = 240.0 Hz), 136.12, 122.23, 117.27, 110.08, 74.29, 73.85, 71.73, 66.51, 58.12 (d, *J* = 9.8 Hz), 57.80, 55.50, 47.86, 41.21, 35.00, 18.98, 18.66. RP-HPLC t_R = 2.963 min (method 1, purity 99%); LC-MS APCI, *m*/*z* 472.1 $[M-H]^-$ (anal. calcd. for $C_{22}H_{24}FN_5O_6$, *m*/*z* = 473.5). $[\alpha]_D^{20}$ = +158.3° (c 0.27, MeOH).

*(2S,4R,4aR)-11-fluoro-8-((R)-3-hydroxypyrrolidin-1-yl)-2,4-dimethyl-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**9E**).* White solid (50 mg, 19%). ^{1}H NMR (300 MHz, MeOD) δ 7.21 (s, 1H), 4.63 – 4.50 (m, 1H), 4.23 – 4.15 (m, 1H), 4.09 – 4.02 (m, 1H), 3.98 – 3.84 (m, 1H), 3.85 – 3.77 (m, 1H), 3.77 – 3.61 (m, 4H), 3.57 – 3.44 (m, 1H), 3.20 – 3.06 (m, 2H), 2.24 – 1.99 (m, 2H), 1.22 (d, *J* = 6.2 Hz, 3H), 1.01 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, MeOD) δ 171.18, 167.84, 158.43, 153.16 (d, *J* = 13.2 Hz), 149.57, 134.82 (d, *J* = 239.7 Hz), 134.60, 120.68, 115.66, 108.56, 72.68, 72.25, 70.14, 64.90, 56.53 (d, *J* = 9.5 Hz), 56.31, 53.88, 46.23,

39.61, 33.42, 17.36, 17.03. RP-HPLC t_R = 2.920 min (method 1, purity 99%); LC-MS APCI, *m/z* 472.1 $[M-H]^-$ (anal. calcd. for $C_{22}H_{24}FN_5O_6$, *m/z* = 473.5). $[\alpha]_D^{20}$ = +142.2° (c 0.27, MeOH).

*(2S,4R,4aR)-8-((3R,4R)-3,4-dihydroxypyrrolidin-1-yl)-11-fluoro-2,4-dimethyl-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**10E**).* White solid (99mg, 18%).^{1}H NMR (300 MHz, MeOD) δ 7.10 (s, 1H), 4.15 – 4.04 (m, 3H), 3.97 – 3.92 (m, 1H), 3.84 – 3.63 (m, 4H), 3.57 – 3.48 (m, 1H), 3.47 – 3.41 (m, 2H), 3.07 – 2.94 (m, 2H), 1.11 (d, *J* = 6.2 Hz, 3H), 0.90 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, MeOD) δ 172.73, 169.42, 160.12, 154.77 (d, *J* = 12.0 Hz), 151.13, 136.36 (d, *J* = 239.9 Hz), 136.17, 122.37, 117.19, 110.05, 76.60 (2C), 74.28, 73.84, 66.52, 58.55, 58.11 (d, *J* = 9.8 Hz), 55.55 (2C), 41.22, 18.98, 18.65. RP-HPLC t_R = 2.829 min (method 1, purity 99%); LC-MS APCI, *m/z* 488.1 $[M-H]^-$ (anal. calcd. for $C_{22}H_{24}FN_5O_7$, *m/z* = 489.5). $[\alpha]_D^{20}$ = +160.8° (c 0.27, MeOH).

*(2S,4R,4aR)-8-((3S,4S)-3,4-dihydroxypyrrolidin-1-yl)-11-fluoro-2,4-dimethyl-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**11E**).* Off-white solid (10 mg, 6%). ^{1}H NMR (300 MHz, MeOD) δ 7.24 (s, 1H), 4.23 (dd, *J* = 5.9, 2.9 Hz, 3H), 4.06 (d, *J* = 8.9 Hz, 1H), 4.00 – 3.79 (m, 4H), 3.53 (d, *J* = 10.8 Hz, 2H), 3.17 – 3.06 (m, 2H), 1.22 (d, *J* = 6.2 Hz, 3H), 1.01 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (151 MHz, MeOD) δ 171.17, 167.84, 158.54, 153.19, 149.57, 135.61, 134.66, 134.02, 120.72, 115.64, 108.48, 74.94, 72.70, 72.29, 64.86, 56.54, 54.46, 53.97, 53.87, 39.58, 17.36, 17.04. RP-HPLC t_R = 2.835 min (method 1, purity 99%); LC-MS APCI, *m/z* 488.1 $[M-H]^-$ (anal. calcd. for $C_{22}H_{24}FN_5O_7$, *m/z* = 489.5). $[a]_D^{20}$ = +117.8° (c 0.23, MeOH).

*(2S,4R,4aR)-8-((3R,4S)-3,4-dihydroxypyrrolidin-1-yl)-11-fluoro-2,4-dimethyl-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**12E**).* White solid (52mg, 8%).^{1}H NMR (300 MHz, MeOD) δ 7.18 (s, 1H), 4.38 – 4.25 (m, 2H), 4.24 – 4.09 (m, 1H), 4.09 – 4.00 (m, 1H), 3.98 – 3.85 (m, 1H), 3.83 – 3.73 (m, 2H), 3.60 – 3.47 (m, 2H), 3.41 – 3.34 (m, 1H), 3.19 – 3.04 (m, 2H), 1.87 – 1.63 (m, 1H), 1.22 (d, J = 6.2 Hz, 3H), 1.01 (d, J = 6.3 Hz, 3H). ^{13}C NMR (151 MHz, MeOD) δ 171.14, 167.84, 158.32, 153.13 (d, J = 12.6 Hz), 149.54, 134.73 (d, J = 239.9 Hz), 134.60, 120.76, 115.57, 108.26, 72.67, 72.24, 70.83, 64.88, 56.51 (d, J = 9.7 Hz), 56.54, 53.83, 52.77, 52.49, 39.53, 17.36, 17.04. RP-HPLC t_R = 2.849 min (method 1, purity 99%); LC-MS APCI, m/z 488.1 $[M-H]^-$ (anal. calcd. for $C_{22}H_{24}FN_5O_7$, m/z = 489.5). $[\alpha]_D^{20}$ = +108.5° (c 0.25, MeOH).

*(2S,4R,4aR)-11-fluoro-8-((S)-3-methoxypyrrolidin-1-yl)-2,4-dimethyl-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**13E**).* Off-white solid (36 mg, 12%). ^{1}H NMR (300 MHz, MeOD) δ 7.17 (s, 1H), 4.20 – 4.10 (m, 2H), 4.09 – 4.99 (m, 1H), 4.00 – 3.74 (m, 2H), 3.73 – 3.56 (m, 5H), 3.38 (s, 3H), 3.19 – 3.02 (m, 2H), 2.20 – 2.10 (m, 2H), 1.22 (d, J = 6.2 Hz, 3H), 1.01 (d, J = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, MeOD) δ 171.14, 167.80, 158.60, 153.31 (m), 149.46, 134.91 (d, J = 240.5 Hz), 134.57, 120.76, 115.55, 108.65, 79.79, 72.68, 72.21, 65.08, 56.56 (d, J = 9.7 Hz), 55.45, 54.05, 53.34, 46.36, 39.73, 30.30, 17.34, 16.99. RP-HPLC t_R = 3.046 min (method 1, purity 96%); LC-MS APCI, m/z 486.1 $[M-H]^-$ (anal. calcd. for $C_{23}H_{26}FN_5O_6$, m/z = 487.5). $[\alpha]_D^{20}$ = +160.6° (c 0.25, MeOH).

*(2S,4R,4aR)-11-fluoro-8-((S)-3-(hydroxymethyl)pyrrolidin-1-yl)-2,4-dimethyl-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**14E**).* Off-white solid (76 mg, 23%). ^{1}H NMR (300 MHz, MeOD) δ 7.19 (s, 1H), 4.24 – 4.12 (m, 1H), 4.09 – 4.01 (m, 1H), 3.98 – 3.75 (m, 2H), 3.74 – 3.45 (m, 6H), 3.42 – 3.34 (m, 1H), 3.20 – 3.03 (m, 2H), 2.67 – 2.46 (m, 1H), 2.24 – 2.07 (m, 1H), 1.94 – 1.75 (m, 1H), 1.22 (d, *J* = 6.2 Hz, 3H), 1.01 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (151 MHz, MeOD) δ 171.11, 167.83, 158.32, 153.13 (d, *J* = 13.0 Hz), 149.51, 134.77 (d, *J* = 239.8 Hz), 134.54, 120.61, 115.76, 108.54, 72.66, 72.24, 64.86, 63.27, 56.50 (d, *J* = 9.6 Hz), 53.88, 50.95, 47.17, 41.13, 39.62, 27.52, 17.36, 17.04. RP-HPLC t_R = 3.015 min (method 1, purity 99%); LC-MS APCI, *m*/*z* 486.1 $[M-H]^-$ (anal. calcd. for $C_{23}H_{26}FN_5O_6$, *m*/*z* = 487.5). $[\alpha]_D^{20}$ = +126.9° (c 0.26, MeOH).

*(2S,4R,4aR)-8-((S)-3-aminopyrrolidin-1-yl)-11-fluoro-2,4-dimethyl-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**16E**).* Beige solid (9 mg, 5%). ^{1}H NMR (300 MHz, MeOD) δ 7.20 (s, 1H), 4.25 – 4.15 (m, 1H), 4.10 – 3.99 (m, 1H), 3.96 – 3.84 (m, 1H), 3.84 – 3.70 (m, 3H), 3.70 – 3.57 (m, 1H), 3.31 – 3.27 (m, 1H), 3.18 – 3.06 (m, 3H), 2.35 – 2.21 (m, 1H), 1.92 (s, 2H), 1.22 (d, *J* = 6.2 Hz, 3H), 1.01 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, MeOD) δ 171.68, 168.27, 158.34, 153.21 (d, *J*= 12.5 Hz), 150.21, 134.78 (d, *J*= 240.1 Hz), 134.63, 120.87, 115.61, 108.42, 72.72, 72.23, 64.95, 56.51 (d, *J*= 9.5 Hz), 55.19, 53.80, 50.68, 46.62, 42.15, 39.66, 17.35, 17.03. RP-HPLC t_R = 0.666 min (method 2, purity 99%); LC-MS APCI, *m*/*z* 471.2 $[M-H]^-$ (anal. calcd. for $C_{22}H_{25}FN_6O_5$, *m*/*z* = 472.5). $[\alpha]_D^{20}$ = +116.1° (c 0.21, MeOH).

*(2S,4R,4aR)-11-fluoro-2,4-dimethyl-8-((S)-3-(methylamino)pyrrolidin-1-yl)-1,2,4,4a-tetrahydro-2'H-spiro[isoxazolo[5',4':4,5]benzo[1,2-b][1,4]oxazino[4,3-d][1,4]oxazine-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**17E**).* Off-white solid (37 mg, 11%). ^{1}H NMR (300 MHz, MeOD) δ 7.20 (s, 1H), 4.23 – 4.13 (m, 1H), 4.08 – 3.99 (m, 1H), 3.95 – 3.86 (m, 1H), 3.86 – 3.68 (m, 2H), 3.68 –3.55 (m, 1H), 3.55 – 3.39 (m, 2H), 3.19 – 2.99 (m, 4H), 2.49 (s, 3H), 2.39 – 2.22 (m, 1H), 2.05 – 1.93 (m, 1H), 1.22 (d, *J* = 6.2 Hz, 3H), 1.00 (d, *J* = 6.3 Hz, 3H). ^{13}CNMR (151 MHz, MeOD) δ 171.71, 168.31, 158.28, 153.17 (d, *J*= 12.7 Hz), 150.26, 134.71 (d, *J*= 239.6 Hz), 134.61, 120.89, 115.60, 108.33, 72.71, 72.23, 64.90, 58.91, 56.50 (d, *J*= 9.7 Hz), 53.76, 52.71, 46.68, 39.60, 32.83, 30.07, 17.35, 17.04. RP-HPLC t_R = 2.557 min (method 1, purity 99%); LC-MS APCI, *m/z* 485.1 $[M-H]^-$ (anal. calcd. for $C_{23}H_{27}FN_6O_5$, *m/z* = 486.5). $[\alpha]_D^{20}$ = +97.3° (c 0.26, MeOH).

*(2S,4R,4aR)-11-fluoro-8-((S)-3-fluoropyrrolidin-1-yl)-2,4-dimethyl-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**18E**).* Off-white solid (53 mg, 19%). ^{1}H NMR (300 MHz, MeOD) δ 7.25 (s, 1H), 5.53 – 5.29 (m, 1H), 4.25 – 4.17 (m, 1H), 4.08 – 4.02 (m, 1H), 3.98 – 3.88 (m, 1H), 3.88 – 3.76 (m, 3H), 3.76 – 3.64 (m, 1H), 3.41 – 3.34 (m, 2H), 3.20 – 3.08 (m, 2H), 2.40 – 2.10 (m, 2H), 1.22 (d, *J* = 6.2 Hz, 3H), 1.01 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (151 MHz, MeOD) δ 171.22, 167.89, 158.47, 153.40 (d, *J*= 12.7 Hz), 149.57, 134.89 (d, *J*= 239.7 Hz), 134.67, 121.00, 115.43, 108.49, 93.17, 92.01, 72.68, 72.20, 65.10, 56.57, 54.77 (d, *J*= 23.4 Hz), 46.09, 39.70, 31.76 (d, *J*= 21.8 Hz), 17.32, 16.97. RP-UPLC t_R = 0.965 min (method 2, purity 99%); LC-MS ESI, *m/z* 474.1 $[M-H]^-$ (anal. calcd. for $C_{22}H_{23}F_2N_5O_5$, *m/z* = 475.5). $[\alpha]_D^{20}$ = +9.6° (c 0.27, MeOH).

*(S)-1-((2S,4R,4aR)-11-fluoro-2,4-dimethyl-2',4',6'-trioxo-1,1',2,3',4,4a,4',6'-octahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidin]-8-yl)pyrrolidine-3-carbonitrile (**19E**).* Off-white solid (10 mg, 8%). ^{1}H NMR (300 MHz, MeOD) δ 7.22 (s, 1H), 4.25 – 4.15 (m, 1H), 4.10 – 4.04 (m, 1H), 3.98 – 3.83 (m, 2H), 3.83 – 3.75 (m, 1H), 3.75 – 3.64 (m, 1H), 3.56 – 3.45 (m, 1H), 3.18 – 3.07 (m, 2H), 3.03 – 2.93 (m, 3H), 2.54 – 2.41 (m, 1H), 2.40 – 2.28 (m, 1H), 1.22 (d, *J* = 6.2 Hz, 3H), 1.01 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (151 MHz, MeOD) δ 172.24, 168.80, 158.02, 153.33 (d, *J*= 13.3 Hz), 150.96, 134.80, 134.70 (d, *J*= 240.2 Hz), 121.40, 120.17, 115.30, 107.97, 72.75, 72.22, 64.99, 56.50 (d), 51.20, 42.26, 39.63, 29.39, 27.81, 17.33, 17.03, 10.74. RP-HPLC t_R = 2.750 min (method 1, purity 99%); LC-MS APCI, *m*/*z* 481.1 [M-H]$^-$ (anal. calcd. for $C_{23}H_{23}FN_6O_5$, *m*/*z* = 482.5). $[\alpha]_D^{20}$ = +59.5° (c 0.28, MeOH).

*(2S,4R,4aR)-11-fluoro-8-((S)-3-hydroxypiperidin-1-yl)-2,4-dimethyl-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**21E**).* Off-white solid (82 mg, 65%). ^{1}H NMR (300 MHz, MeOD) δ 7.19 (s, 1H), 4.24 – 4.01 (m, 2H), 3.97 – 3.73 (m, 3H), 3.72 – 3.58 (m, 2H), 3.37 – 3.34 (m, 1H), 3.19 – 2.93 (m, 4H), 2.03 (s, 1H), 1.97 – 1.84 (m, 1H), 1.79 – 1.62 (m, 1H), 1.61 – 1.45 (m, 1H), 1.22 (d, *J* = 6.2 Hz, 3H), 1.01 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 171.90, 168.61, 161.87, 154.58 (d, *J* = 17.6 Hz), 150.30, 135.12, 135.57 (d, *J* = 240.1 Hz), 121.86, 116.27, 116.25, 109.18, 73.45, 73.02, 66.22, 65.68, 57.27 (d, *J* = 9.5 Hz), 55.47, 54.70, 40.44, 32.95, 22.95, 18.15, 17.82. RP-HPLC t_R = 3.029 min (method 1, purity 99%); LC-MS APCI, *m*/*z* 486.1 [M-H]$^-$ (anal. calcd. for $C_{23}H_{26}FN_5O_6$, *m*/*z* = 487.5). $[\alpha]_D^{20}$ = +164.5° (c 0.28, MeOH).

*(2S,4R,4aR)-11-fluoro-8-((R)-3-hydroxypiperidin-1-yl)-2,4-dimethyl-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**22E**).* Off-white solid (91 mg, 31%).H NMR (300 MHz, MeOD) δ 7.20 (s, 1H), 4.19 (dd, *J* = 14.2, 2.2 Hz, 1H), 4.05 (d, *J* = 8.8 Hz, 1H), 3.99 – 3.74 (m, 5H), 3.73 – 3.59 (m, 1H), 3.40 – 3.28 (m, 1H), 3.20 – 2.93 (m, 4H), 2.10 – 1.86 (m, 1H), 1.84 – 1.62 (m, 1H), 1.62 – 1.45 (m, 1H), 1.22 (d, *J* = 6.2 Hz, 3H), 1.01 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, MeOD) δ 171.92, 168.62, 161.85, 154.66 – 154.06 (m), 150.32, 135.59 (d, *J* = 240.5 Hz), 135.14, 121.86, 116.29, 109.20, 73.46, 73.03, 66.16, 65.70, 57.73, 57.29 (d, *J* = 9.5 Hz), 55.46, 54.70, 40.44, 32.95, 22.93, 18.15, 17.83. RP-HPLC t_R = 3.001 min (method 1, purity 99%); LC-MS APCI, *m*/*z* 486.1 $[M-H]^-$ (anal. calcd. for $C_{23}H_{26}FN_5O_6$, *m*/*z* = 487.5). $[a]_D^{20}$ = +122.0º (c 0.26, MeOH).

*(2S,4R,4aR)-11-fluoro-2,4-dimethyl-8-(((S)-5-oxopyrrolidin-2-yl)methoxy)-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**23E**).* Off-white solid (25 mg, 8%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.89 (s, 1H), 7.11 (s, 1H), 4.38 – 4.19 (m, 2H), 4.14 – 3.86 (m, 3H), 3.86 – 3.59 (m, 2H), 3.58 – 3.49 (m, 1H), 3.49 – 3.38 (m, 1H), 2.98 – 2.85 (m, 1H), 2.28 – 2.05 (m, 2H), 1.95 – 1.83 (m, 1H), 1.14 (d, *J* = 6.1 Hz, 3H), 0.89 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 177.33, 171.65, 168.35, 166.30,153.19 (d, J_{CF}= 13. Hz), 150.29, 135.58 (d, J_{CF}= 27.5 Hz), 133.06, 123.27, 114.44, 105.76, 73.27, 72.57, 72.14, 64.96,56.85 (d, J_{CF}= 9.0 Hz), 55.33, 52.58, 49.04, 30.04, 23.16, 18.65, 18.59. RP-UPLC t_R = 0.990 min (method 2, purity 100%); LC-MS ESI, *m*/*z* 500.1 $[M-H]^-$ (anal. calcd. for $C_{23}H_{24}FN_5O_7$, *m*/*z* = 501.5). $[\alpha]_D^{20}$ = +180.5º (c 0.21, MeOH).

*(2S,4R,4aR)-11-fluoro-2,4-dimethyl-8-(((R)-5-oxopyrrolidin-2-yl)methoxy)-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**24E**)*. Beige amorphous solid (72 mg, 18%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.57 (s, 2H), 7.88 (s, 1H), 7.12 (s, 1H), 4.43 – 4.16 (m, 2H), 4.15 – 3.88 (m, 3H), 3.84 – 3.74 (m, 1H), 3.74 – 3.51 (m, 2H), 3.19 – 3.02 (m, 1H), 2.98 – 2.84 (m, 1H), 2.37 – 2.06 (m, 3H), 2.00 – 1.79 (m, 1H), 1.14 (d, J = 6.0 Hz, 3H), 0.89 (d, J = 6.3 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-*d*6) δ 177.35, 171.34, 168.09, 166.30, 153.16 (d, J = 13.0 Hz), 149.92, 135.70, 134.26 (d, J = 240.0 Hz), 123.25, 114.49, 105.77, 73.19, 72.50, 72.15, 64.88, 56.80 (d, J = 8.5 Hz), 53.65, 52.56, 38.94, 30.07, 23.14, 18.66, 18.60.RP-UPLC t_R = 0.915 min (method 2, purity 100%); LC-MS ESI, *m*/*z* 500.1 $[M-H]^-$ (anal. calcd. for $C_{23}H_{24}FN_5O_7$, *m*/*z* = 501.5). $[\alpha]_D^{20}$ = +38.6° (c 0.37, MeOH).

*(2S,4R,4aR)-11-fluoro-2,4-dimethyl-8-(((S)-1-methyl-5-oxopyrrolidin-2-yl)methoxy)-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**25E**)*. Beige solid (56 mg, 24%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.09 (s, 1H), 4.63 – 4.34 (m, 2H), 4.12 – 4.02 (m, 1H), 3.99 – 3.88 (m, 2H), 3.86 – 3.46 (m, 3H), 3.15 – 3.01 (m, 1H), 2.93 – 2.85 (m, 1H), 2.77 (s, 3H), 2.46 – 2.28 (m, 1H), 2.28 – 2.08 (m, 2H), 1.99 – 1.82 (m, 1H), 1.14 (d, J = 6.6 Hz, 4H), 0.88 (d, J = 6.3 Hz, 3H). ^{13}C NMR (151 MHz, DMSO) δ 175.96, 172.17, 168.63, 166.18, 153.09 (d, J_{CF}= 12.8 Hz), 150.82, 135.74, 134.10 (d, J_{CF}= 240.5 Hz), 123.27, 114.09, 105.44, 72.69, 72.36, 70.78, 64.91, 58.74, 56.66 (d, J_{CF}= 9.3 Hz), 53.22, 41.86, 30.14, 28.19, 21.00, 18.38, 18.31. RP-UPLC t_R = 0.927 min (method 2, purity 100%); LC-MS ESI, *m*/*z* 514.2 $[M-H]^-$ (anal. calcd. for $C_{24}H_{26}FN_5O_7$, *m*/*z* = 515.5). $[\alpha]_D^{20}$ = +164.7° (c 0.24, MeOH).

*(2S,4R,4aR)-11-fluoro-2,4-dimethyl-8-(((S)-5-oxotetrahydrofuran-2-yl)methoxy)-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**26E**).* Off-white solid (29 mg, 10%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.79 (s, 1H), 11.47 (s, 1H), 7.15 (s, 1H), 5.03 – 4.88 (m, 1H), 4.62 – 4.53 (m, 1H), 4.51 – 4.40 (m, 1H), 4.08 (d, J = 13.8 Hz, 1H), 3.98 – 3.90 (m, 1H), 3.86 – 3.73 (m, 1H), 3.72 – 3.62 (m, 1H), 3.58 (d, J = 14.3 Hz, 1H), 3.16 – 3.03 (m, 1H), 2.98 – 2.87 (m, 1H), 2.62 – 2.53 (m, 2H), 2.43 – 2.24 (m, 1H), 2.20 – 2.02 (m, 1H), 1.14 (d, J = 6.1 Hz, 3H), 0.89 (d, J = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 177.37, 171.33, 168.08, 166.08, 153.27 (d, J = 13.7 Hz), 149.89, 135.80, 134.10 (d, J = 203.5 Hz), 123.38, 114.36, 105.56, 77.64, 72.52, 72.16, 72.06, 64.88, 56.83 (d, J = 8.5 Hz), 53.63, 39.05, 28.31, 23.44, 18.68, 18.59. RP-UPLC t_R = 0.953 min (method 2, purity 100%); LC-MS ESI, m/z 501.1 $[M-H]^-$ (anal. calcd. for $C_{23}H_{23}FN_4O_8$, m/z = 502.5). $[\alpha]_D^{20}$ = +174.0° (c 0.24, MeOH).

*(2S,4R,4aR)-11-fluoro-2,4-dimethyl-8-(((R)-5-oxotetrahydrofuran-2-yl)methoxy)-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**27E**).* Off-white solid (25 mg, 11%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.14 (s, 1H), 5.02 – 4.88 (m, 1H), 4.61 – 4.50 (m, 1H), 4.50 – 4.40 (m, 1H), 4.13 – 4.03 (m, 1H), 3.99 – 3.89 (m, 1H), 3.84 – 3.72 (m, 1H), 3.72 – 3.63 (m, 1H), 3.63 – 3.53 (m, 1H), 3.19 – 3.03 (m, 1H), 2.98 – 2.84 (m, 1H), 2.63 – 2.53 (m, 2H), 2.43 – 2.24 (m, 1H), 2.17 – 2.04 (m, 1H), 1.14 (d, J = 6.1 Hz, 3H), 0.89 (d, J = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 177.38, 171.36, 168.12, 166.04, 153.28 (d, J = 12.8 Hz), 149.94, 135.80, 134.29 (d, J = 240.1 Hz), 123.42, 114.34, 105.56, 77.60, 72.52, 72.16, 72.01, 64.90, 56.83 (d, J = 9.3 Hz), 53.66, 39.04, 28.32, 23.45, 18.67, 18.59. RP-UPLC t_R = 0.954 min (method 2, purity 100%); LC-MS ESI, m/z 501.1 $[M-H]^-$ (anal. calcd. for $C_{23}H_{23}FN_4O_8$, m/z = 502.5). $[\alpha]_D^{20}$ = +138.5° (c 0.23, MeOH).

*(2S,4R,4aR)-11-fluoro-2,4-dimethyl-8-(((R)-2-oxooxazolidin-4-yl)methoxy)-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**28E**).* Beige solid (29 mg, 9%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.95 (s, 1H), 7.07 (s, 1H), 4.51 – 4.40 (m, 1H), 4.40 – 4.29 (m, 2H), 4.28 – 4.17 (m, 2H), 4.13 – 4.04 (m, 1H), 3.94 (d, J = 8.8 Hz, 1H), 3.86 – 3.73 (m, 1H), 3.71 – 3.56 (m, 2H), 3.17 – 3.03 (m, 1H), 2.95 – 2.85 (m, 1H), 1.14 (d, J = 6.1 Hz, 3H), 0.89 (d, J = 6.2 Hz, 3H). ^{19}F NMR (377 MHz, DMSO) δ -157.50 (s, 1H). RP-UPLC t_R = 0.888 min (method 2, purity 100%); LC-MS ESI, m/z 502.1 $[M-H]^-$ (anal. calcd. for $C_{22}H_{22}FN_5O_8$, m/z = 503.4). $[\alpha]_D^{20}$ = +104.6° (c 0.22, MeOH).

*(2S,4R,4aR)-11-fluoro-2,4-dimethyl-8-(((S)-2-oxooxazolidin-4-yl)methoxy)-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**29E**).* Off-white solid (43 mg, 13%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.95 (s, 1H), 7.07 (s, 1H), 4.50 – 4.40 (m, 1H), 4.39 – 4.29 (m, 2H), 4.29 – 4.19 (m, 2H), 4.12 – 4.03 (m, 1H), 3.98 – 3.91 (m, 1H), 3.84 – 3.74 (m, 1H), 3.71 – 3.57 (m, 2H), 3.16 – 3.04 (m, 1H), 2.95 – 2.85 (m, 1H), 1.14 (d, J = 6.1 Hz, 3H), 0.89 (d, J = 6.3 Hz, 3H). ^{19}F NMR (377 MHz, DMSO) δ -157.57 (s, 1H). RP-UPLC t_R = 0.888 min (method 2, purity 100%); LC-MS ESI, m/z 502.1 $[M-H]^-$ (anal. calcd. for $C_{22}H_{22}FN_5O_8$, m/z = 503.4). $[\alpha]_D^{20}$ = +107.2° (c 0.21, MeOH).

*(2S,4R,4aR)-11-fluoro-2,4-dimethyl-8-(((S)-5-oxopyrrolidin-3-yl)methoxy)-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**30E**).* Off-white solid (67 mg, 21%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.05 (s, 1H), 4.37 – 4.27 (m, 2H), 4.10 – 3.98 (m, 1H), 3.96 – 3.87 (m, 1H), 3.69 – 3.59 (m,

1H), 3.50 – 3.34 (m, 2H), 3.19 – 2.80 (m, 4H), 2.44 – 2.30 (m, 1H), 2.16 – 2.03 (m, 1H), 1.11 (d, J = 6.1 Hz, 3H), 0.86 (d, J = 6.3 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 176.81, 172.13, 168.67, 166.25, 153.08 (d, J = 12.9 Hz), 150.82, 135.70, 134.18 (d, J = 240.1 Hz), 123.31, 114.23, 105.59, 72.62, 72.32, 72.24, 64.95, 56.73 (d, J = 9.3 Hz), 53.30, 44.29, 41.84, 33.55, 33.31, 18.51, 18.46. RP-UPLC t_R = 0.891 min (method 2, purity 100%); LC-MS ESI, m/z 500.1 $[M-H]^-$ (anal. calcd. for $C_{23}H_{24}FN_5O_7$, m/z = 501.5). $[\alpha]_D^{20}$ = +168.8° (c 0.24, MeOH).

*(2S,4R,4aR)-11-fluoro-2,4-dimethyl-8-(((R)-5-oxopyrrolidin-3-yl)methoxy)-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**31E**).* Off-white solid (35 mg, 13%).^{1}H NMR (600 MHz, DMSO-d_6) δ 7.55 (s, 1H), 7.06 (s, 1H), 4.35 – 4.28 (m, 2H), 4.07 – 4.01 (m, 1H), 3.94 – 3.87 (m, 1H), 3.78 – 3.70 (m, 1H), 3.68 – 3.60 (m, 1H), 3.51 – 3.46 (m, 1H), 3.42 – 3.36 (m, 1H), 3.14 – 3.02 (m, 2H), 2.94 – 2.83 (m, 2H), 2.33 – 2.27 (m, 1H), 2.09 – 2.03 (m, 1H), 1.11 (d, J = 6.2 Hz, 3H), 0.85 (d, J = 6.3 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 176.11, 171.93, 168.61, 166.26, 153.13 (d, J = 12.8 Hz), 150.67, 135.72, 134.24 (d, J = 240.0 Hz), 123.41, 114.26, 105.65, 72.58, 72.45, 72.13, 64.94, 56.81 (d, J = 8.9 Hz), 53.42, 44.34, 39.06, 33.70, 33.36, 18.63, 18.62. RP-UPLC t_R = 0.888 min (method 2, purity 98%); LC-MS ESI, m/z 500.1 $[M-H]^-$ (anal. calcd. for $C_{23}H_{24}FN_5O_7$, m/z = 501.5). $[\alpha]_D^{20}$ = +175.9° (c 0.23, MeOH).

*(2S,4R,4aR)-11-fluoro-2,4-dimethyl-8-(2-((S)-5-oxopyrrolidin-2-yl)ethoxy)-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**32E**).* Beige solid (44 mg, 10%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.59 (s, 2H), 7.81 (s, 1H), 7.10 (s, 1H), 4.50 – 4.31 (m, 2H), 4.12 – 4.01 (m, 1H), 3.96 – 3.89 (m, 1H), 3.86 – 3.72 (m, 2H), 3.72 – 3.63 (m, 1H), 3.60 – 3.52 (m, 1H), 3.16 – 3.04 (m, 1H),

2.97 – 2.85 (m, 1H), 2.23 – 2.06 (m, 3H), 2.02 – 1.89 (m, 2H), 1.79 – 1.61 (m, 1H), 1.14 (d, *J* = 6.1 Hz, 3H), 0.89 (d, *J* = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 177.05, 171.39, 168.08, 166.18, 153.11 (d, *J* = 12.5 Hz), 149.94, 135.63, 134.33 (d, *J* = 240.2 Hz), 123.14, 114.34, 105.97, 72.53, 72.16, 68.16, 64.93, 56.93 – 56.72 (m), 53.59, 51.08, 39.07, 35.97, 30.29, 27.31, 18.67, 18.59. RP-UPLC t_R = 0.954 min (method 2, purity 97%); LC-MS ESI, *m*/*z* 514.1 $[M-H]^-$ (anal. calcd. for $C_{24}H_{26}FN_5O_7$, *m*/*z* = 515.5). $[\alpha]_D^{20}$ = +151.7° (c 0.24, MeOH).

*(2S,4R,4aR)-11-fluoro-2,4-dimethyl-8-((5-methyl-1,3,4-oxadiazol-2-yl)methoxy)-1,2,4,4a-tetrahydro-2'H,6H-spiro[isoxazolo[4,5-g][1,4]oxazino[4,3-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**33E**).* Off-white solid (60 mg, 68%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (s, 2H), 7.13 (d, *J* = 1.1 Hz, 1H), 5.66 (s, 2H), 4.12 – 4.03 (m, 1H), 3.98 – 3.91 (m, 1H), 3.84 – 3.73 (m, 1H), 3.73 – 3.62 (m, 1H), 3.59 – 3.52 (m, 1H), 3.15 – 3.05 (m, 1H), 2.97 – 2.88 (m, 1H), 2.54 (s, 3H), 1.14 (d, *J* = 6.2 Hz, 3H), 0.89 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.38, 168.12, 165.61 – 164.97 (m), 161.87, 153.50 (d, *J* = 13.0 Hz), 149.97, 135.96, 134.19 (d, *J* = 240.3 Hz), 114.22 (d, *J* = 3.3 Hz), 105.10, 72.55, 72.16, 64.95, 61.68, 56.83 (d, *J* = 9.4 Hz), 53.50, 18.66, 18.58, 10.93. ^{19}F NMR (377 MHz, DMSO-d_6) δ -157.50. RP-UPLC t_R = 0.983 min (method 2, purity 100%); LC-MS ESI, *m*/*z* 499.1 $[M-H]^-$ (anal. calcd. for $C_{22}H_{21}FN_6O_7$, *m*/*z* = 500.4). $[\alpha]_D^{20}$ = +194° (c 0.27, MeOH).

*(2S,4R,4aR)-11-fluoro-2,4-dimethyl-8-((S)-4-methyl-2-oxooxazolidin-3-yl)-2,3,4,4a-tetrahydro-1H,2'H,6H-spiro[isoxazolo[4,5-g]pyrazino[1,2-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**34E**).* White solid (30 mg, 5.0%). ^{1}H NMR (300 MHz, MeOD) δ 7.63 (s, 1H), 4.69 (dd, *J* = 8.7, 5.8 Hz, 2H), 4.32 – 4.15 (m, 3H), 3.36 – 3.31 (m, 2H), 3.29-3.07 (m, 3H) 1.51 (d, *J* = 5.1 Hz, 3H), 1.25 (d, *J* = 6.3 Hz, 3H), 1.04 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (151

MHz, DMSO-d_6) δ 172.58, 169.41, 154.26, 153.34 (d, J = 12.8 Hz), 152.48, 150.20, 135.79, 133.75 (d, J = 236.5 Hz), 123.42, 118.48, 107.70, 70.55, 66.95, 53.42, 53.50, 52.66, 51.55, 40.56, 39.67, 21.45, 19.40, 17.75. RP-UPLC t_R = 2.93 min (method 1, purity 98%); LC-MS ESI, m/z 487.2 $[M+H]^+$ (anal. calcd. for $C_{22}H_{23}FN_6O_6$, m/z = 486.5). $[\alpha]_D^{20}$ = +131° (c 0.5, MeOH).

*(2S,4R,4aR)-11-fluoro-3-hydroxy-2,4-dimethyl-8-((S)-4-methyl-2-oxooxazolidin-3-yl)-2,3,4,4a-tetrahydro-1H,2'H,6H-spiro[isoxazolo[4,5-g]pyrazino[1,2-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**35E**).* Light pink solid (40 mg, 8.0%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.76 (s, 1H), 11.45 (s, 1H), 8.05 (s, 1H), 7.58 (s, 1H), 4.72 – 4.60 (m, 2H), 4.19 (dd, J = 7.6, 4.3 Hz, 1H), 4.12 – 3.97 (m, 2H), 3.61 (d, J = 14.4 Hz, 1H), 3.11-3.02 (m, 1H), 2.88 (d, J = 14.4 Hz, 1H), 2.78-2.63 (m, 2H), 1.41 (d, J = 5.8 Hz, 3H), 1.10 (d, J = 5.9 Hz, 3H), 0.88 (d, J = 6.3 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.40, 171.54, 168.34, 154.46, 153.87 (d, J = 12.5 Hz), 152.62, 149.97, 135.52 (d, J = 240.3 Hz), 123.21, 118.35, 107.36, 70.71, 64.08, 62.84, 61.52, 54.88, 53.97, 53.31, 39.03, 17.99, 17.19, 16.19. RP-UPLC t_R = 3.606 min (method 1, purity 98%); LC-MS ESI, m/z 503.1 $[M+H]^+$ (anal. calcd. for $C_{22}H_{23}FN_6O_7$, m/z = 502.5). $[\alpha]_D^{20}$ = +136° (c 1.0, MeOH).

*(2S,4R,4aR)-3-acetyl-11-fluoro-2,4-dimethyl-8-((S)-4-methyl-2-oxooxazolidin-3-yl)-2,3,4,4a-tetrahydro-1H,2'H,6H-spiro[isoxazolo[4,5-g]pyrazino[1,2-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**36E**).* White solid (55 mg, 19%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.40 (br s, 2H), 7.63 (s, 1H), 4.87 (s, 1H), 4.74 – 4.63 (m, 2H), 4.28 – 4.18 (m, 1H), 4.11 – 4.00 (m, 1H), 3.93 – 3.76 (m, 2H), 3.64 – 3.54 (m, 1H), 3.45 – 3.40 (m, 2H), 1.89 (s, 3H), 1.42 (d, J = 5.7 Hz, 3H), 1.34 (d, J = 7.2 Hz, 3H), 1.21 (d, J = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.26, 170.31, 169.37, 154.60, 152.68, 150.19, 127.29, 122.67, 120.03, 118.85

(2C), 108.75, 94.16, 70.76, 65.95, 53.23 (2C), 48.57, 43.62, 35.41, 22.92, 21.65, 17.88 (2C). RP-UPLC t_R = 0.777 min (method 2, purity 98%); LC-MS ESI, *m/z* 529.2 $[M+H]^+$ (anal. calcd. for $C_{24}H_{25}FN_6O_7$, *m/z* = 528.4). $[\alpha]_D^{20}$ = +135° (c 0.4, MeOH).

*(2S,4R,4aR)-11-fluoro-8-((S)-3-hydroxypyrrolidin-1-yl)-2,4-dimethyl-2',4',6'-trioxo-1,1',2,3',4,4a,4',6'-octahydro-2'H,3H,6H-spiro[isoxazolo[4,5-g]pyrazino[1,2-a]quinoline-5,5'-pyrimidine]-3-carbonitrile (**40E**).* Off-white solid (135 mg, 27%). ^{1}H NMR (300 MHz, MeOD) δ 7.14 (s, 1H), 4.12 (d, *J* = 9.3 Hz, 1H), 4.09 – 4.01 (m, 1H), 3.76 – 3.35 (m, 7H), 3.28 (dd, *J* = 14.3, 1.7 Hz, 1H), 3.18 – 3.06 (m, 1H), 3.01 (d, *J* = 14.2 Hz, 1H), 2.05 – 1.89 (m, 2H), 1.30 (d, *J* = 6.5 Hz, 3H), 1.09 – 1.05 (m, 3H). ^{13}C NMR (101 MHz, MeOD) δ 172.20, 169.39, 160.00, 154.64, 150.97, 137.95, 135.83, 122.52, 117.30, 114.94, 111.09, 71.70, 65.09, 57.85, 56.50, 55.96, 47.88, 41.15, 35.02, 30.85, 24.41, 16.77, 16.44. RP-UPLC t_R = 2.744 min (method 1, purity 80%); LC-MS ESI, *m/z* 496.1 $[M-H]^-$ (anal. calcd. for $C_{23}H_{24}FN_7O_5$, *m/z* = 497.5). $[\alpha]_D^{20}$ = +139° (c 0.24, MeOH).

*(2S,4R,4aR)-11-fluoro-2,4-dimethyl-2',4',6'-trioxo-8-(((S)-5-oxopyrrolidin-2-yl)methoxy)-1,1',2,3',4,4a,4',6'-octahydro-2'H,3H,6H-spiro[isoxazolo[4,5-g]pyrazino[1,2-a]quinoline-5,5'-pyrimidine]-3-carbonitrile (**42E**).* Off-white solid (33 mg, 9.8%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.87 (s, 1H), 7.13 (d, *J*= 1.2 Hz, 1H), 4.32 – 4.19 (m, 2H), 4.08 (d, *J*= 9.3 Hz, 1H), 4.01 (dt, *J*= 14.3, 2.3 Hz, 1H), 3.95 (dd, *J*= 8.2, 4.4 Hz, 1H), 3.52 – 3.48 (m, 1H), 3.48 – 3.41 (m, 1H), 3.39 (dd, *J*= 9.3, 6.8 Hz, 1H), 3.19 (ddd, *J*= 15.0, 10.7, 1.8 Hz, 1H), 2.93 (dd, *J*= 14.3, 1.5 Hz, 1H), 2.29 – 2.21 (m, 1H), 2.21 – 2.08 (m, 2H), 1.91 – 1.83 (m, 1H), 1.26 (d, *J*= 6.5 Hz, 3H), 1.00 (d, *J*= 6.8 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 177.37, 171.59, 168.78, 166.30, 152.97 (d, *J*= 13.1 Hz), 150.76, 135.59, 134.54 (d, *J*= 240.8 Hz), 123.61, 114.42, 113.67, 106.51, 73.31, 63.46, 55.90 (d, *J*= 8.1 Hz), 54.98, 54.36, 54.21, 52.55, 39.15,

30.04, 23.12, 16.31, 16.22. RP-UPLC t_R = 0.883 min (method 2, purity 100%); LC-MS ESI, *m*/*z* 524.2 $[M-H]^-$ (anal. calcd. for $C_{24}H_{24}FN_7O_6$, *m*/*z* = 525.5). $[\alpha]_D^{20}$ = +190° (c 0.22, MeOH).

*(2R,4S,4aS)-11-fluoro-2,4-dimethyl-8-(((S)-5-oxopyrrolidin-2-yl)methoxy)-2,3,4,4a-tetrahydro-1H,2'H,6H-spiro[isoxazolo[4,5-g]pyrido[1,2-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**43E**).* Beige solid (45 mg, 10%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.73 (s, 1H), 11.39 (s, 1H), 7.89 (s, 1H), 7.51 (s, 1H), 7.07 (s, 1H), 4.28 (qd, *J*= 10.4, 4.7 Hz, 2H), 3.94 (d, *J*= 16.6 Hz, 2H), 3.78 (d, *J*= 9.9 Hz, 1H), 3.56 – 3.42 (m, 2H), 2.95 – 2.82 (m, 2H), 2.21 – 2.10 (m, 2H), 1.92 – 1.66 (m, 4H), 0.90 (d, *J*= 6.4 Hz, 3H), 0.65 (d, *J*= 6.4 Hz, 3H).– **NH Peaks visible.** ^{13}C NMR (151 MHz, DMSO-d_6) δ 178.11, 172.76, 169.21, 167.18, 154.11 (d, *J*= 12.6 Hz), 150.99, 137.68, 135.20 (d, *J*= 239.8 Hz), 124.55, 114.99, 106.15, 74.08, 67.48, 59.46 (d, *J*= 8.5 Hz), 55.45, 53.45, 44.32, 39.94, 33.78, 32.71, 30.99, 24.19, 20.02, 19.74. RP-UPLC t_R = 1.034 min (method 2, purity 96%); LC-MS ESI, *m*/*z* 498.2 $[M-H]^-$ (anal. calcd. for $C_{24}H_{26}FN_6O_6$, *m*/*z* = 499.5). $[\alpha]_D^{20}$ = +137° (c 0.25, MeOH).

*(2R,4R,4aR)-11-fluoro-2,4-dimethyl-8-(5-methyl-1,3,4-oxadiazol-2-yl)-2',4',6'-trioxo-1,1',2,3',4,4a,4',6'-octahydro-2'H,3H,6H-spiro[isoxazolo[4,5-g]pyrazino[1,2-a]quinoline-5,5'-pyrimidine]-3-carbonitrile (**44E**).* White solid (26 mg, 4.1%). RP-UPLC t_R = 0.961 min (method 2, purity 100%); LC-MS ESI, *m*/*z* 493.1 $[M-H]^-$ (anal. calcd. for $C_{22}H_{19}FN_8O_5$, *m*/*z* = 494.4).

*(2S,3R,4R,4aS)-11-fluoro-3-hydroxy-2,4-dimethyl-8-(5-methyl-1,3,4-oxadiazol-2-yl)-2,3,4,4a-tetrahydro-1H,2'H,6H-spiro[isoxazolo[4,5-g]pyrido[1,2-a]quinoline-5,5'-pyrimidine]-2',4',6'(1'H,3'H)-trione (**45E**).* Light yellow solid (49 mg, 18%). ^{1}H NMR (300

MHz, DMSO-d_6) δ 11.55 (brs, 2H), 7.60 (s, 1H), 4.73 (d, J = 7.6 Hz, 1H), 3.98 – 3.93 (m, 2H), 3.74 (d, J = 14.8 Hz, 1H), 3.01 (t, J = 13.0 Hz, 1H), 2.91 (d, J = 14.3 Hz, 1H), 2.75 (q, J = 9.15 Hz, 1H), 2.68 (s, 3H), 1.73 – 1.63 (m, 2H), 1.01 (d, J= 6.4 Hz, 3H), 0.76 (d, J = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.80, 168.44, 165.71, 156.77, 153.53 (d, J = 13.07 Hz), 150.11, 144.76, 137.01, 133.86 (d, J = 242.57 Hz), 126.63, 115.71, 110.48, 78.92, 64.95, 55.86 (d, J = 8.12 Hz), 54.55, 40.90, 38.76, 15.57, 14.78, 11.06. *Note: one of the peak is for two carbons.* ^{19}F NMR (377 MHz, DMSO-d_6) δ -157.68. RP-UPLC t_R = 0.941 min (method 2, purity 97%); LC-MS ESI, m/z 485.2 $[M+H]^+$ (anal. calcd. for $C_{22}H_{21}FN_6O_6$, m/z = 484.4). $[\alpha]_D^{20}$ = -198° (c 0.19, THF).

5. DNA gyrase supercoiling assay.

1 U (unit or the amount of enzyme required to fully supercoil the substrate) of *M. tuberculosis* gyrase was incubated with 0.5 µg of relaxed pBR322 DNA in a 30 µl reaction at 37°C for 30 minutes under the following conditions: 50 mM HEPES-KOH (pH 7.9), 6 mM MgOAc, 4 mM DTT, 1 mM ATP, 100 mM potassium glutamate, 2 mM spermidine and 0.05 mg/ml BSA. Each reaction was stopped by the addition of 30 µl chloroform/iso-amyl alcohol (24:1) and 20 µl Stop Dye (40% sucrose, 100 mM Tris.HCl (pH 7.5), 10 mM EDTA, 0.5 µg/ml bromophenol blue), before being loaded on a 1.0% TAE (Tris-acetate 0.04 mM, EDTA 0.002 mM) gels run at 80V for 3 h. Bands were visualized by ethidium staining for 10 minutes, de-stained for 10 minutes in water and analyzed by gel documentation equipment (Syngene, Cambridge, UK) and quantitated using Syngene Gene Tools software. Raw gel data (fluorescent band volumes) collected from Syngene, GeneTools gel analysis software were calculated as a % of the 100% control (the fully supercoiled DNA band) and converted to % inhibition. The raw gel data was analyzed using SigmaPlot Version 13 (2015). The

global curve fit non-linear regression tool was used to calculate IC_{50} data using the following equation: Exponential Decay, Single, 2 Parameter f = a*exp(-b*x).

6. *In vitro* ADMET assays

6.1 Kinetic solubility. Solubility was performed using a miniaturised shake flask method. 10 mM stock solutions of each compound were used to prepare calibration standards (10-220 µM) in DMSO. The same 10mM stock solutions were accurately dispensed in duplicate into 96-well plates and the DMSO dried down (MiVac GeneVac, 90 min, 37℃). Thereafter, the samples were reconstituted (200 µM) in aqueous solution and shaken (20 hours, 25 °C). The solutions were analysed by means of HPLC-DAD (Agilent 1200 Rapid Resolution HPLC with a diode array detector). Solubility was then determined using the peak areas of the aqueous samples and the best fit calibration curves constructed using the calibration standard.[3]

6.2 Lipophilicity ($LogD_{7.4}$). The lipophilicity assay was performed in triplicate using a shake-flask procedure. 10 mM stock solutions of each test compound were used to spike (100 µM) a 1:1 mixture of phosphate buffer (pH 7.4) and n-octanol. The solutions were shaken vigorously (1500 rpm) on an orbital shaker for 3 hours at room temperature. Thereafter the samples were centrifuged in order to fully separate the two immiscible fluids. The samples were analysed by HPLC-DAD (Agilent 1200 Rapid Resolution HPLC with a diode array detector) and the amount of compound in the buffer and n-octanol were used to determine the partition coefficient, $LogD_{7.4}$.[4]

6.3. Cytotoxicity. Compounds were screened for *in vitro* cytotoxicity against HepG2 cell line using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT)-assay.[5] The

tetrazolium salt MTT was used to measure all growth and chemosensitivity. The tetrazolium ring is cleaved in active mitochondria. Thus, only viable cells are able to reduce the water-soluble yellow coloured MTT to water-insoluble purple coloured formazan. Formazan crystals are dissolved in DMSO. The test samples were tested in triplicate. The test samples were prepared to a 20 mg/mL stock solution in 100% DMSO. Stock solutions were stored at -20 ºC. Further dilutions were prepared in complete medium on the day of the experiment. Samples were tested as a suspension if not completely dissolved. Emetine was used as the reference drug in all experiments. The initial concentration of emetine was 100 µg/mL, which was serially diluted in complete medium with 10-fold dilutions to give 6 concentrations, the lowest being 0.001 µg/mL. The same dilution technique was applied to all test samples. The highest concentration of solvent to which the cells were exposed to have no measurable effect on the cell viability (data not shown). The 50% inhibitory concentration ($IC_{50)}$ values were obtained from full dose-response curves, using a non-linear dose-response curve fitting analysis via GraphPad Prism v.4 software.

7. Sequence alignments

CLUSTAL O(1.2.4) sequence alignment[6] was carried out for GyrA from *Mycobacterium tuberculosis* (MYCTU), *Staphylococcus aureus* (STAAU) and *Escherichia coli* (ECOLI). Residues highlighted in yellow are those referred to in the text. The residues in green represent the QRDR in M. tuberculosis.

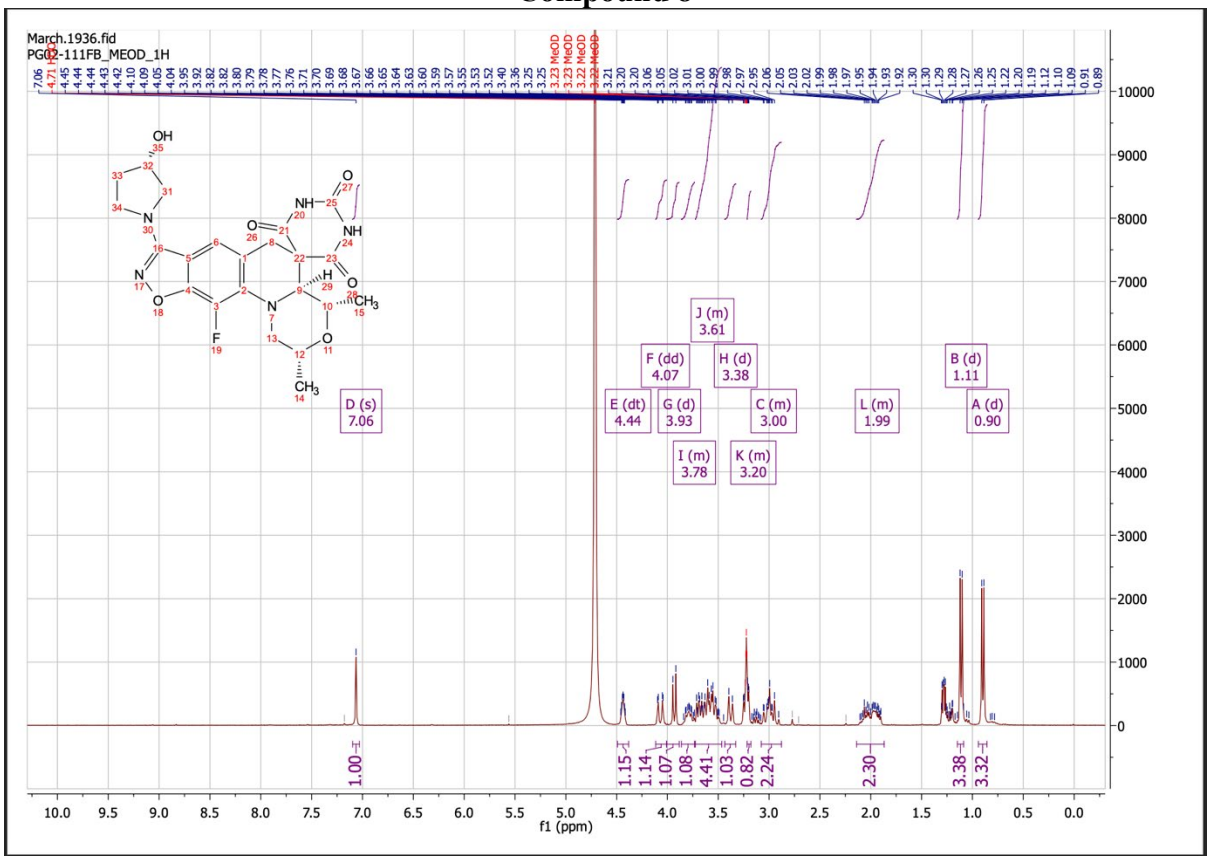
```
SP|P9WG47|GYRA_MYCTU MTDTTLPPDDSLDRIEPVDIEQEMQRSYIDYAMSVIVGRALPEVRDGLKPVHRRVLYAMF 60
SP|P20831|GYRA_STAAU ------MAELPQSRINERNITSEMRESFLDYAMSVIVARALPDVRDGLKPVHRRILYGLN 54
SP|P0AES4|GYRA_ECOLI ------MSDL-AREITPVNIEEELKSSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMN 53
                            :    .*   :* .*:: *::********.****:***********:**.:

SP|P9WG47|GYRA_MYCTU DSGFRPDRSHAKSARSVAETMGNYHPHGDASIYDSLVRMAQPWSLRYPLVDGQGNFGSPG 120
SP|P20831|GYRA_STAAU EQGMTPDKSYKKSARIVGDVMGKYHPHGDSSIYEAMVRMAQDFSYRYPLVDGQGNFGSMD 114
SP|P0AES4|GYRA_ECOLI VLGNDWNKAYKKSARVVGDVIGKYHPHGDSAVYDTIVRMAQPFSLRYMLVDGQGNFGSID 113
                       *   :::: **** *.:.:*:******:::*:::***** :* ** ********** .

SP|P9WG47|GYRA_MYCTU NDPPAAMRYTEARLTPLAMEMLREIDEETVDFIPNYDGRVQEPTVLPSRFPNLLANGSGG 180
SP|P20831|GYRA_STAAU GDGAAAMRYTEARMTKITLELLRDINKDTIDFIDNYDGNEREPSVLPARFPNLLANGASG 174
SP|P0AES4|GYRA_ECOLI GDSAAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTEKIPDVMPTKIPNLLVNGSSG 173
                      .*  ******* *:: :: *:: :::::*:**: ****  : * *:*:::****.**:.*

SP|P9WG47|GYRA_MYCTU IAVGMATNIPPHNLRELADAVFWALENHDADEEETLAAVMGRVKGPDFPTAGLIVGSQGT 240
SP|P20831|GYRA_STAAU IAVGMATNIPPHNLTELINGVLSLSKNP----DISIAELMEDIEGPDFPTAGLILGKSGI 230
SP|P0AES4|GYRA_ECOLI IAVGMATNIPPHNLTEVINGCLAYIDDE----DISIEGLMEHIPGPDFPTAAIINGRRGI 229
                      ************** *: :. :   .:     : ::  :*  : *******.:* *  *

SP|P9WG47|GYRA_MYCTU ADAYKTGRGSIRMRGVVEVEED-SRGRTSLVITELPYQVNHDNFITSIAEQVRDGKLAGI 299
SP|P20831|GYRA_STAAU RRAYETGRGSIQMRSRAVIEE-RGGGRQRIVVTEIPFQVNKARMIEKIAELVRDKKIDGI 289
SP|P0AES4|GYRA_ECOLI EEAYRTGRGKVYIRARAEVEVDAKTGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGI 289
                        **.****.: :*. . :*     **  ::: *:*:***: .:* .*** *:: :: **

SP|P9WG47|GYRA_MYCTU SNIEDQSSDRVGLRIVIEIKRDAVAKVVINNLYKHTQLQTSFGANMLAIVDGVPRTLRLD 359
SP|P20831|GYRA_STAAU TDLRDETSLRTGVRVVIDVRKDANASVILNNLYKQTPLQTSFGVNMIALVNGRPKLINLK 349
SP|P0AES4|GYRA_ECOLI SALRDES-DKDGMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLK 348
                      : :.*::  : *:*:**:::::* ..*::****.:* **.*** **:*: .* *: :.*.

SP|P9WG47|GYRA_MYCTU QLIRYYVDHQLDVIVRRTTYRLRKANERAHILRGLVKALDALDEVIALIRASETVDIARA 419
SP|P20831|GYRA_STAAU EALVHYLEHQKTVVRRRTQYNLRKAKDRAHILEGLRIALDHIDEIISTIRESDTDKVAME 409
SP|P0AES4|GYRA_ECOLI DIIAAFVRHRREVVTRRTIFELRKARDRAHILEALAVALANIDPIIELIRHAPTPAEAKT 408
                      : :  :: *:  *: *** :.****.:*****..*  **  :* :*  ** : *   *

SP|P9WG47|GYRA_MYCTU ----------------------------------GLIELLDIDEIQAQAILDMQLRRLAA 445
SP|P20831|GYRA_STAAU ----------------------------------SLQQRFKLSEKQAQAILDMRLRRLTG 435
SP|P0AES4|GYRA_ECOLI ALVANPWQLGNVAAMLERAGDDAARPEWLEPEFGVRDGLYYLTEQQAQAILDLRLQKLTG 468
                                                       : * *******::*::*:.

SP|P9WG47|GYRA_MYCTU LERQRIIDDLAKIEAEIADLEDILAKPERQRGIVRDELAEIVDRHGDDRRTRIIAA-DGD 504
SP|P20831|GYRA_STAAU LERDKIEAEYNELLNYISELEAILADEEVLLQLVRDELTEIRDRFGDDRRTEIQLGGFED 495
SP|P0AES4|GYRA_ECOLI LEHEKLLDEYKELLDQIAELLRILGSADRLMEVIREELELVREQFGDKRRTEITAN-SAD 527
                      **::::  :  ::   *::*  **.. :    ::*:**  : ::.**.***.*      *

SP|P9WG47|GYRA_MYCTU VSDEDLIAREDVVVTITETGYAKRTKTDLYRSQKRGGKGVQGAGLKQDDIVAHFFVCSTH 564
SP|P20831|GYRA_STAAU LEDEDLIPEEQIVITLSHNNYIKRLPVSTYRAQNRGGRGVQGMNTLEEDFVSQLVTLSTH 555
SP|P0AES4|GYRA_ECOLI INLEDLITQEDVVVTLSHQGYVKYQPLSEYEAQRRGGKGKSAARIKEEDFIDRLLVANTH 587
                      :. **** .*::*:*::. .* *    . *.:*.***:* ..    ::*:: ::.. .**

SP|P9WG47|GYRA_MYCTU DLILFFTTQGRVYRAKAYDLPEASRTARGQHVANLLAFQPEERIAQVIQIRGYTDA-PYL 623
SP|P20831|GYRA_STAAU DHVLFFTNKGRVYKLKGYEVPELSRQSKGIPVVNAIELENDEVISTMIAVKDLESEDNFL 615
SP|P0AES4|GYRA_ECOLI DHILCFSSRGRVYSMKVYQLPEATRGARGRPIVNLLPLEQDERITAILPVTEFEEGVK-V 646
                      * :* *:.:****  * *::** :* ::*  :.* : :: :* *: :: :    .    :

SP|P9WG47|GYRA_MYCTU VLATRNGLVKKSKLTDFDSNRSGGIVAVNLRDNDELVGAVLCSAGDDLLLVSANGQSIRF 683
SP|P20831|GYRA_STAAU VFATKRGVVKRSALSNFSRINRNGKIAISFREDDELIAVRLTSGQEDILIGTSHASLIRF 675
SP|P0AES4|GYRA_ECOLI FMATANGTVKKTVLTEFNRLRTAGKVAIKLVDGDELIGVDLTSGEDEVMLFSAEGKVVRF 706
                      .:** .* **:: *::*.  .  * :*:.: :.***:.. * *. ::::: ::... :**

SP|P9WG47|GYRA_MYCTU SATDEALRPMGRATSGVQGMRFNIDDRLLSLNVVREG--TYLLVATSGGYAKRTAIEEYP 741
SP|P20831|GYRA_STAAU PE--STLRPLGRTATGVKGITLREGDEVVGLDVAHANSVDEVLVVTENGYGKRTPVNDYR 733
SP|P0AES4|GYRA_ECOLI KE--SSVRAMGCNTTGVRGIRLGEGDKVVSLIVPRGD--GAILTATQNGYGKRTAVAEYP 762
                        .::* :*  ::**:*: :  .*.::.* * : .    :*..*..**.*** : :*

SP|P9WG47|GYRA_MYCTU VQGRGGKGVLTVMYDRRRGRLVGALIVDDDSELYAVTSGGGVIRTAARQVRKAGRQTKGV 801
SP|P20831|GYRA_STAAU LSNRGGKGIKTATITERNGNVVCITTVTGEEDLMIVTNAGVIIRLDVADISQNGRAAQGV 793
SP|P0AES4|GYRA_ECOLI TKSRATKGVISIKVTERNGLVVGAVQVDDCDQIMMITDAGTLVRTRVSEISIVGRNTQGV 822
                      ..*. **: :    .*.* :*    * . .::  :*..* ::*  . ::   ** ::**

SP|P9WG47|GYRA_MYCTU RLMNLGEGDTLLAIARNAEESGDDNAVDANGA--------DQTGN--------------- 838
SP|P20831|GYRA_STAAU RLIRLGDDQFVSTVAKVKEDAEDETNEDEQSTSTVSEDGTEQQREAVVNDETPGNAIHTE 853
SP|P0AES4|GYRA_ECOLI ILIRTAEDENVVGLQRVAEPVDEEDLDTIDGS---AAEGDDEIAPEVDVDDEPEEE---- 875
                       *:. .:.: :  : :  *   ::     :.:        ::

SP|P9WG47|GYRA_MYCTU ------------------------------------
SP|P20831|GYRA_STAAU VIDSEENDEDGRIEVRQDFMDRVEEDIQQSLDEDEE 889
SP|P0AES4|GYRA_ECOLI ------------------------------------
```

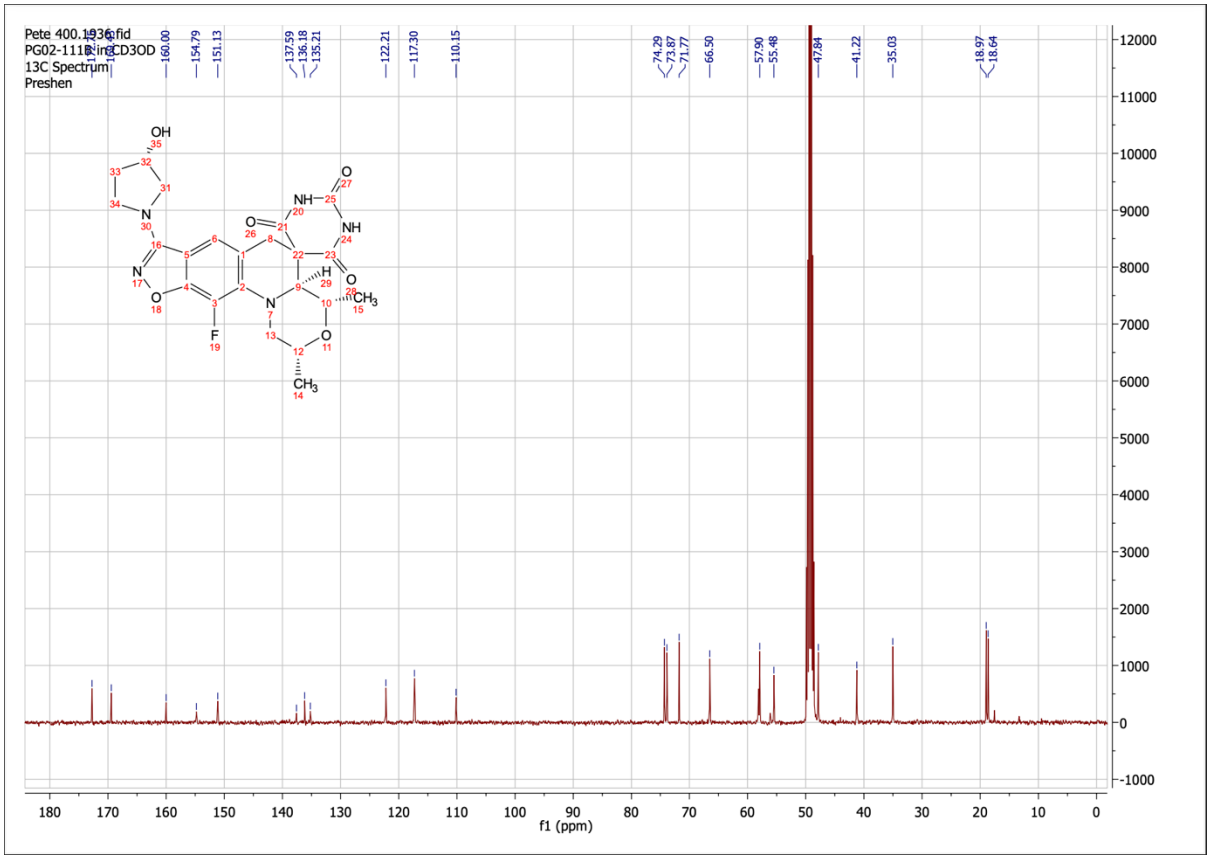
CLUSTAL O(1.2.4) sequence alignment was carried out for GyrB from *Mycobacterium tuberculosis* (MYCTU), *Staphylococcus aureus* (STAAU) and *Escherichia coli* (ECOLI). Residues highlighted in yellow are those referred to in the text. The residues in green represent the QRDR in *M. tuberculosis*.

```
SP|P9WG45|GYRB_MYCTU --MAAQKKKAQDEYGAASITILEGLEAVRKRPGMYIGSTG-ERGLHHLIWEVVDNAVDEA 57
SP|P0A0K8|GYRB_STAAU MVTALSDVNNTDNYGAGQIQVLEGLEAVRKRPGMYIGSTS-ERGLHHLVWEIVDNSIDEA 59
SP|P0AES6|GYRB_ECOLI ---------MSNSYDSSSIKVLKGLDAVRKRPGMYIGDTDDGTGLHHMVFEVVDNAIDEA 51
                          :.*.:..* :*:**:***********.*.   ****:::*:***::***

SP|P9WG45|GYRB_MYCTU MAGYATTVNVVLLEDGGVEVADDGRGIPVATHA-SGIPTVDVVMTQLHAGGKFDSDAYAI 116
SP|P0A0K8|GYRB_STAAU LAGYANQIEVVIEKDNWIKVTDNGRGIPVDIQEKMGRPAVEVILTVLHAGGKFGGGGYKV 119
SP|P0AES6|GYRB_ECOLI LAGHCKEIIVTIHADNSVSVQDDGRGIPTGIHPEEGVSAAEVIMTVLHAGGKFDDNSYKV 111
                     :**:.. : *.:  *. :.* *:*****.  :   *  :.:*::* *******....* :

SP|P9WG45|GYRB_MYCTU SGGLHGVGVSVVNALSTRLEVEIKRDGYEWSQVYEKSEPL-GLKQGAPTKKTGSTVRFWA 175
SP|P0A0K8|GYRB_STAAU SGGLHGVGSSVVNALSQDLEVYVHRNETIYHQAYKKGVPQFDLKEVGTTDKTGTVIRFKA 179
SP|P0AES6|GYRB_ECOLI SGGLHGVGVSVVNALSQKLELVIQREGKIHRQIYEHGVPQAPLAVTGETEKTGTMVRFWP 171
                     ******** *******  **: ::*:     * *::. *   *   . *.***: :**

SP|P9WG45|GYRB_MYCTU DPAVFE-TTEYDFETVARRLQEMAFLNKGLTINLTDERVTQDEVVDEVVSDVAEAPKSAS 234
SP|P0A0K8|GYRB_STAAU DGEIFTETTVYNYETLQQRIRELAFLNKGIQITLRDERDE---------------------- 219
SP|P0AES6|GYRB_ECOLI SLETFTNVTEFEYEILAKRLRELSFLNSGVSIRLRDKRDG---------------------- 211
                     .   *  .* :::* : :*::*::***.*: * * *:*

SP|P9WG45|GYRB_MYCTU ERAAESTAPHKVKSRTFHYPGGLVDFVKHINRTKNAIHSSIVDFSGKGTGHEVEIAMQWN 294
SP|P0A0K8|GYRB_STAAU ---------ENVREDSYHYEGGIKSYVELLNENKEPIHDEPIYIHQSKDDIEVEIAIQYN 270
SP|P0AES6|GYRB_ECOLI ------------KEDHFHYEGGIKAFVEYLNKNKTPIHPNIFYFSTEKDGIGVEVALQWN 259
                                :.  :** **:  :*: :*..*  ** . . :  .  .  **:*:*:*

SP|P9WG45|GYRB_MYCTU AGYSESVHTFANTINTHEGGTHEEGFRSALTSVVNKYAKDRKLLKDKDPNLTGDDIREGL 354
SP|P0A0K8|GYRB_STAAU SGYATNLLTYANNIHTYEGGTHEDGFKRALTRVLNSYGLSSKIMKEEKDRLSGEDTREGM 330
SP|P0AES6|GYRB_ECOLI DGFQENIYCFTNNIPQRDGGTHLAGFRAAMTRTLNAYMDKEGYSKKAKVSATGDDAREGL 319
                      *:  .:  ::*.*   :****  **: *:* .:* *  .    *. .   :*:* ***:

SP|P9WG45|GYRB_MYCTU AAVISVKVSEPQFEGQTKTKLGNTEVKSFVQKVCNEQLTHWFEANPTDAKVVVNKAVSSA 414
SP|P0A0K8|GYRB_STAAU TAIISIKHGDPQFEGQTKTKLGNSEVRQVVDKLFSEHFERFLYENPQVARTVVEKGIMAA 390
SP|P0AES6|GYRB_ECOLI IAVVSVKVPDPKFSSQTKDKLVSSEVKSAVEQQMNELLAEYLLENPTDAKIVVGKIIDAA 379
                      *::*:*  :*:*..*** ** .:**:. *::  .* : .::  **  *: ** * : :*

SP|P9WG45|GYRB_MYCTU QARIAARKARELVRRKSATDIGGLPGKLADCRSTDPRKSELYVVEGDSAGGSAKSGRDSM 474
SP|P0A0K8|GYRB_STAAU RARVAAKKAREVTRRKSALDVASLPGKLADCSSKSPEECEIFLVEGDSAGGSTKSGRDSR 450
SP|P0AES6|GYRB_ECOLI RAREAARRAREMTRRKGALDLAGLPGKLADCQERDPALSELYLVEGDSAGGSAKQGRNRK 439
                     :** **::***:.***.* *:..******** . .*  .*:::**********:*.**:

SP|P9WG45|GYRB_MYCTU FQAILPLRGKIINVEKARIDRVLKNTEVQAIITALGTGIH-DEFDIGKLRYHKIVLMADA 533
SP|P0A0K8|GYRB_STAAU TQAILPLRGKILNVEKARLDRILNNNEIRQMITAFGTGIGGD-FDLAKARYHKIVIMTDA 509
SP|P0AES6|GYRB_ECOLI NQAILPLKGKILNVEKARFDKMLSSQEVATLITALGCGIGRDEYNPDKLRYHSIIIMTDA 499
                      ******:***:******:*::*.. *:  :***:* **  * ::  * ***.*::*:**

SP|P9WG45|GYRB_MYCTU DVDGQHISTLLLTLLFRFMRPLIENGHVFLAQPPLYKLKWQRSDPEFAYSDRERDGLLEA 593
SP|P0A0K8|GYRB_STAAU DVDGAHIRTLLLTFFYRFMRPLIEAGYVYIAQPPLYKLTQGKQK-YYVYNDRELDKLKSE 568
SP|P0AES6|GYRB_ECOLI DVDGSHIRTLLLTFFYRQMPEIVERGHVYIAQPPLYKVKKGKQE-QYIKDDEAMDQYQIS 558
                     **** ** *****:::* *  ::* *:*::*******:.  :..  :  .*.  *

SP|P9WG45|GYRB_MYCTU ------------------------------------------------------------
SP|P0A0K8|GYRB_STAAU ------------------------------------------------------------
SP|P0AES6|GYRB_ECOLI IALDGATLHTNASAPALAGEALEKLVSEYNATQKMINRMERRYPKAMLKELIYQPTLTEA 618


SP|P9WG45|GYRB_MYCTU ------------------------------------------------------------
SP|P0A0K8|GYRB_STAAU ------------------L----------------------------------------- 569
SP|P0AES6|GYRB_ECOLI DLSDEQTVTRWVNALVSELNDKEQHGSQWKFDVHTNAEQNLFEPIVRVRTHGVDTDYPLD 678


SP|P9WG45|GYRB_MYCTU --------------------------------GLK-------------AGKKINKEDGIQR 609
SP|P0A0K8|GYRB_STAAU ------------------------------------------------NPTPKWSIAR 579
SP|P0AES6|GYRB_ECOLI HEFITGGEYRRICTLGEKLRGLLEEDAFIERGERRQPVASFEQALDWLVKESRRGLSIQR 738
                                                                    :    .* *

SP|P9WG45|GYRB_MYCTU YKGLGEMDAKELWETTMDPSVRVLRQVTLDDAAAADELFSILMGEDVDARRSFITRNAKD 669
SP|P0A0K8|GYRB_STAAU YKGLGEMNADQLWETTMNPEHRALLQVKLEDAIEADQTFEMLMGDVVENRRQFIEDNAVY 639
SP|P0AES6|GYRB_ECOLI YKGLGEMNPEQLWETTMDPESRRMLRVTVKDAIAADQLFTTLMGDAVEPRRAFIEENALK 798
                     *******: .:******:*. * : :*.:.**  **: *  ***: *: ** **  **

SP|P9WG45|GYRB_MYCTU VRFLDV 675
SP|P0A0K8|GYRB_STAAU A-NLDF 644
SP|P0AES6|GYRB_ECOLI AANIDI 804
                     .  :*.
```

8. QC data for selected compounds (NMR spectra and UPLC-MS traces)

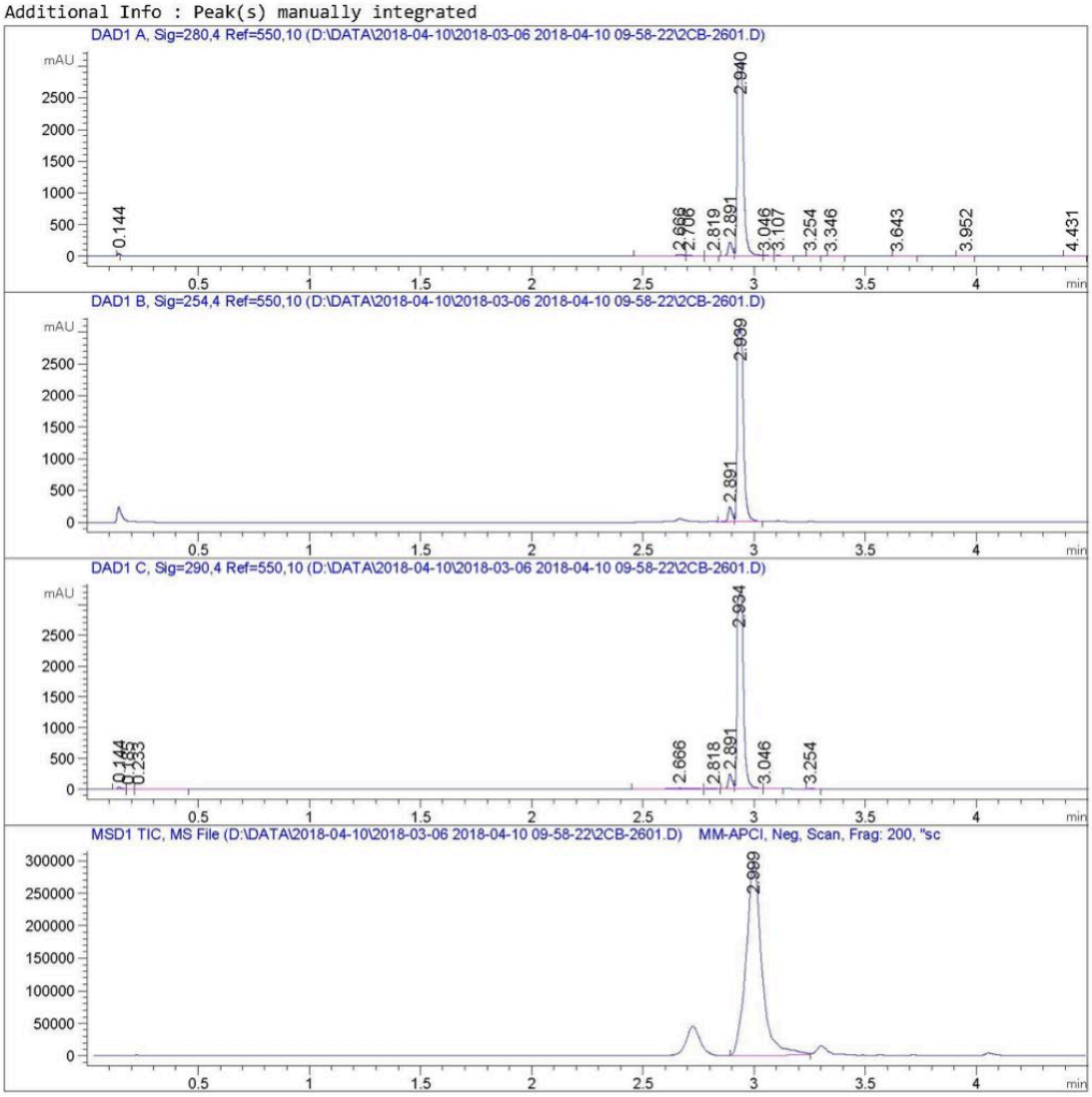
Compound 8



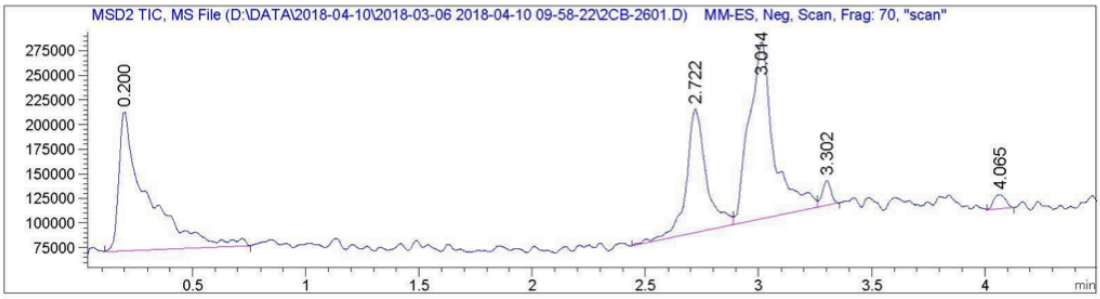

Pete 400.1936.fid
PG02-111B in CD3OD
13C Spectrum
Preshen
160.00
154.79
151.13
137.59
136.18
135.21
122.21
117.30
110.15
74.29
73.87
71.77
66.50
57.90
55.48
47.84
41.22
35.03
18.97
18.64
f1 (ppm)

Data File D:\DATA\2018-04-10\2018-03-06 2018-04-10 09-58-22\2CB-2601.D
Sample Name: PG02-111B-P-NEG

```
==================================================================
Acq. Operator   : SYSTEM                        Seq. Line :  26
Acq. Instrument : Agilent LC-MS                  Location : P2-C-02
Injection Date  : 2018-04-10 12:47:10                 Inj :   1
                                                Inj Volume : 2.000 µl
Method          : D:\DATA\2018-04-10\2018-03-06 2018-04-10 09-58-22\GENERAL METHOD NEG 1.M (
                  Sequence Method)
Last changed    : 2018-04-10 10:14:47 by SYSTEM
Additional Info : Peak(s) manually integrated
```

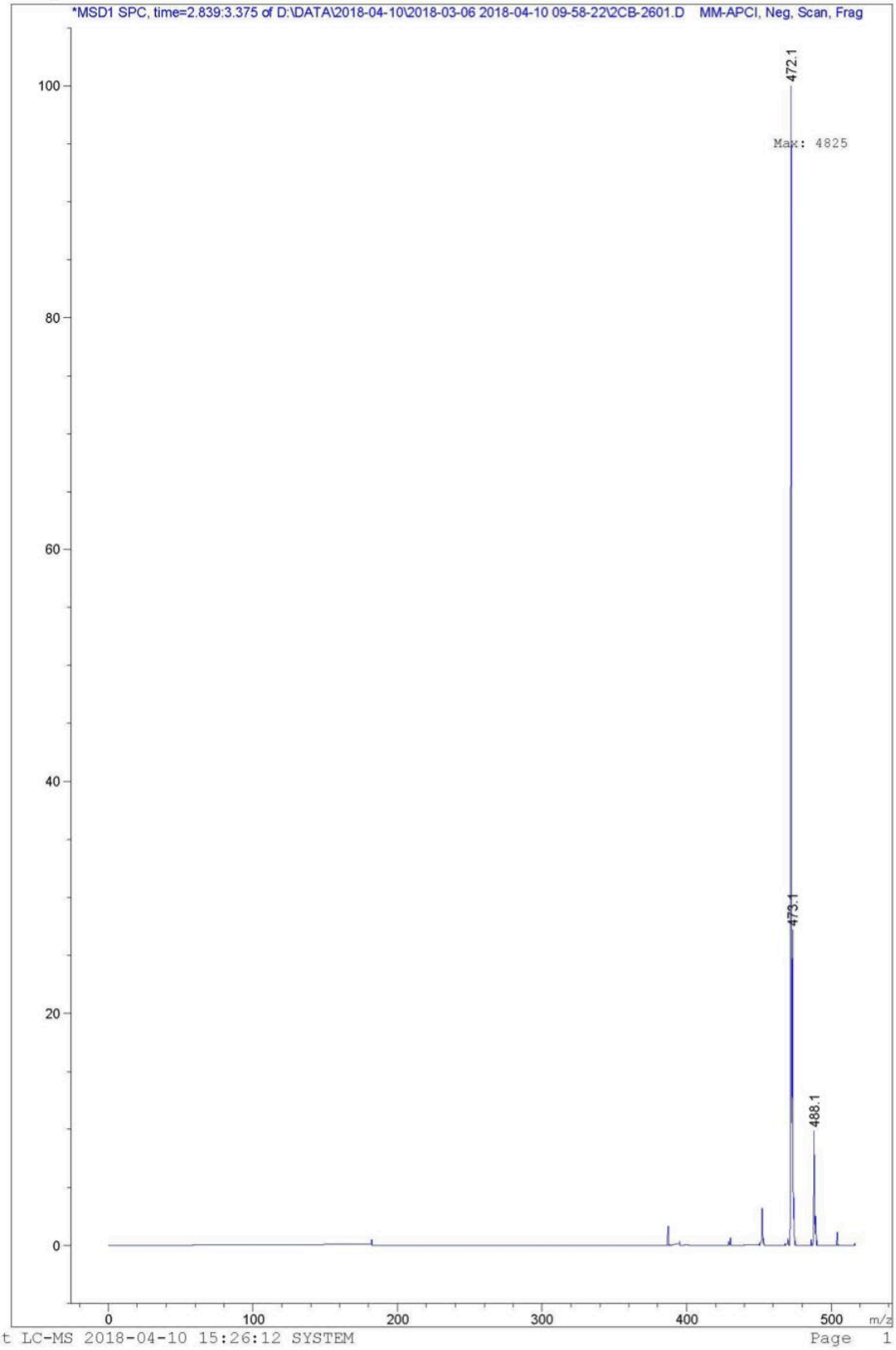


Data File D:\DATA\2018-04-10\2018-03-06 2018-04-10 09-58-22\2CB-2601.D
Sample Name: PG02-111B-P-NEG



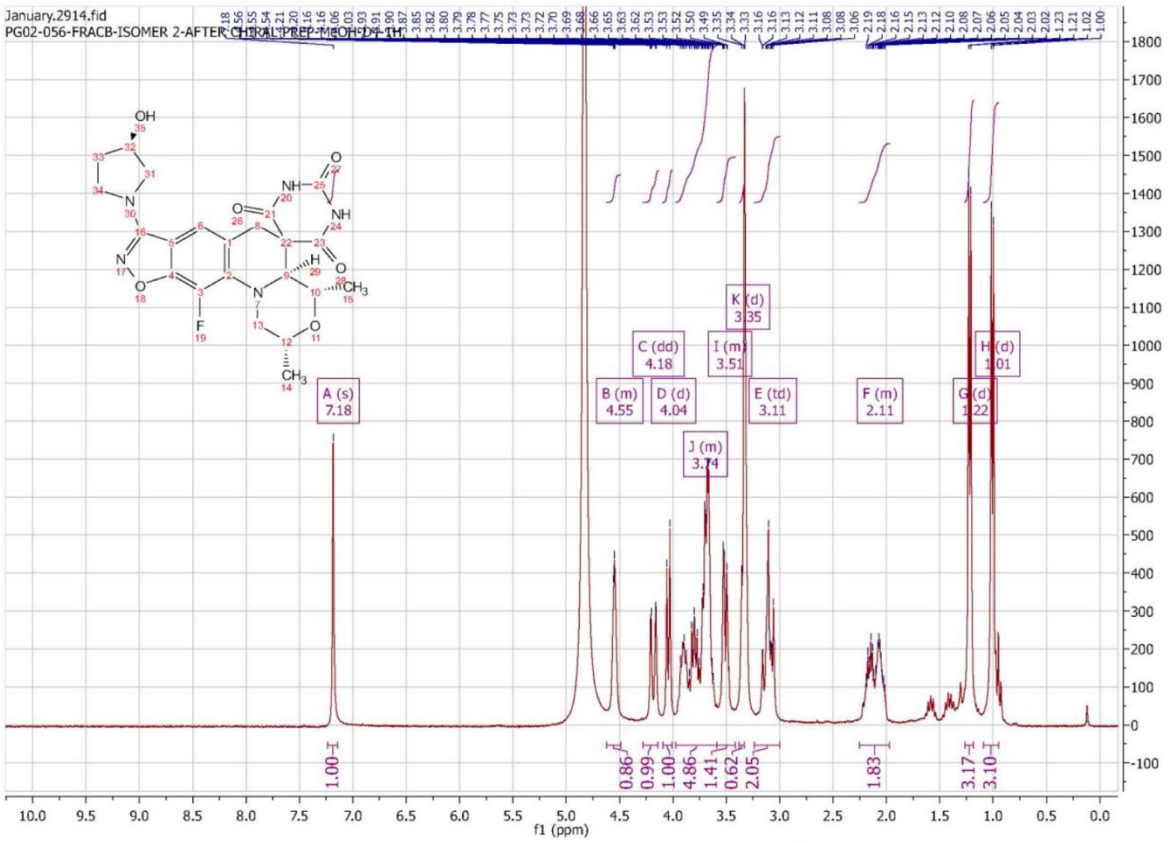

```
=====================================================================
                      Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=280,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.144 BBA  9.36e-3   15.03820   26.78102   0.2370
   2   2.666 BV    0.0414   57.61981   20.09909   0.9082
   3   2.706 VB    0.0320   22.58328   10.31470   0.3559
   4   2.819 BB    0.0249    3.84269    2.19624   0.0606
   5   2.891 BV    0.0190  271.91522  218.72090   4.2857
   6   2.940 VV    0.0276 5941.95898 3066.16211  93.6523
   7   3.046 VB    0.0223    8.99640    5.57408   0.1418
   8   3.107 BB    0.0240    9.67015    6.10827   0.1524
   9   3.254 BB    0.0206    7.44435    5.57200   0.1173
  10   3.346 VB    0.0245    1.26887 7.04945e-1   0.0200
  11   3.643 BB    0.0249 5.41191e-1 2.76090e-1 8.530e-3
  12   3.952 BV    0.0330 6.92319e-1 2.94173e-1   0.0109
  13   4.431 BB    0.0334    3.12759    1.38209   0.0493

Totals :                  6344.69907 3364.18572


Signal 2: DAD1 B, Sig=254,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   2.891 BV    0.0190  291.51050  233.80136   4.6547
   2   2.939 VV    0.0308 5971.25537 3055.35205  95.3453

Totals :                  6262.76587 3289.15341
```

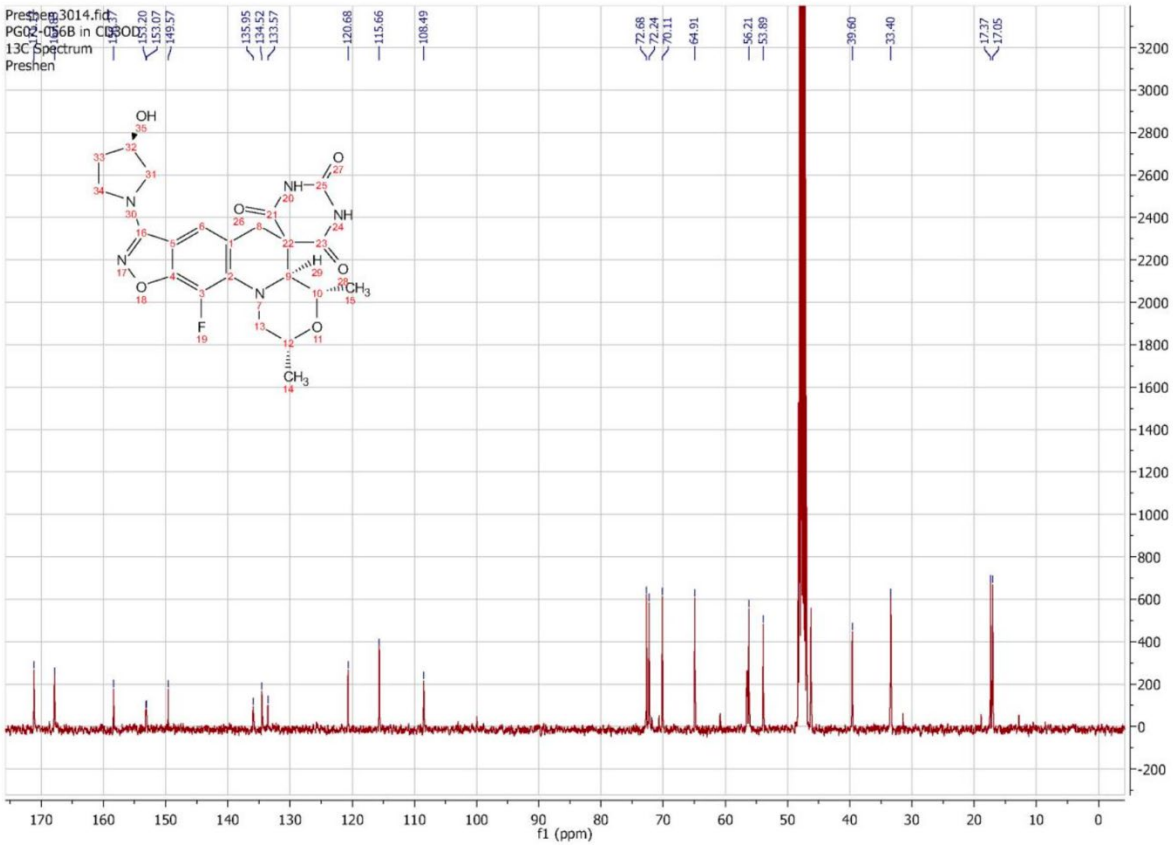
MS Spectrum



ent LC-MS 2018-04-10 15:26:12 SYSTEM Page 1 of 1

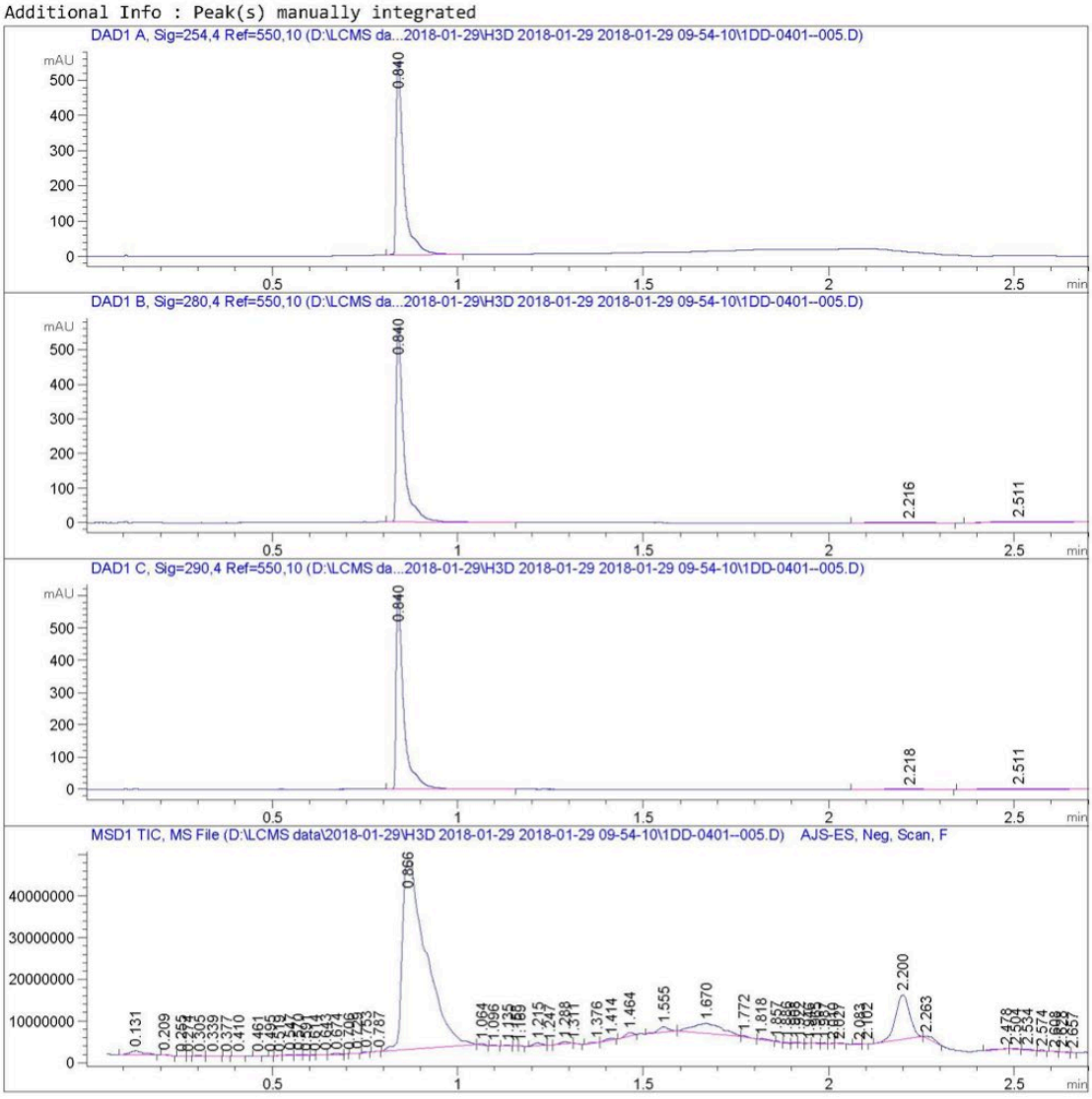
Compound 9


January.2914.fid
PG02-056-FRACB-ISOMER 2-AFTER CHIRAL PREP-MEOH-D4-1H
A (s) 7.18
B (m) 4.55
C (dd) 4.18
D (d) 4.04
E (td) 3.11
F (m) 2.11
G (d) 1.22
H (d) 1.01
I (m) 3.51
J (m) 3.74
K (d) 3.35
1.00
0.86
0.99
1.00
4.86
1.41
0.62
2.05
1.83
3.17
3.10
f1 (ppm)


Preshen3014.fid
PG02-056B in CD3OD
13C Spectrum
Preshen
155.20
153.07
149.57
135.95
134.52
133.57
120.68
115.66
108.49
72.68
72.24
70.11
64.91
56.21
53.89
39.60
33.40
17.37
17.05
f1 (ppm)

Data File D:\LCMS data\2018-01-29\H3D 2018-01-29 2018-01-29 09-54-10\1DD-0401--005.D
Sample Name: PG02-056-FRAC B-ISOMER 2-NEG

```
======================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   5
Acq. Instrument : Calimero                       Location :   P1-D4
Injection Date  : 2018-01-29 10:09:52               Inj :   1
                                               Inj Volume : 1.000 µl
Method          : D:\LCMS data\2018-01-29\H3D 2018-01-29 2018-01-29 09-54-10\NEW GENERAL NEG.
                  M (Sequence Method)
Last changed    : 2018-01-29 09:54:10 by SYSTEM
Method Info     : Standard 2.2min method with 254nm, 280nm and 290nm and positive ESI mode
                  100-800m/z

Additional Info : Peak(s) manually integrated
```



Data File D:\LCMS data\2018-01-29\H3D 2018-01-29 2018-01-29 09-54-10\1DD-0401--005.D
Sample Name: PG02-056-FRAC B-ISOMER 2-NEG

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=254,4 Ref=550,10

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.840	BB	0.0205	775.94153	550.12347	100.0000
Totals :				775.94153	550.12347	

Signal 2: DAD1 B, Sig=280,4 Ref=550,10

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.840	BB	0.0206	799.66559	563.26569	95.3843
2	2.216	BB	0.1030	12.20876	1.42479	1.4563
3	2.511	BBA	0.1278	26.48782	2.46693	3.1595
Totals :				838.36217	567.15740	

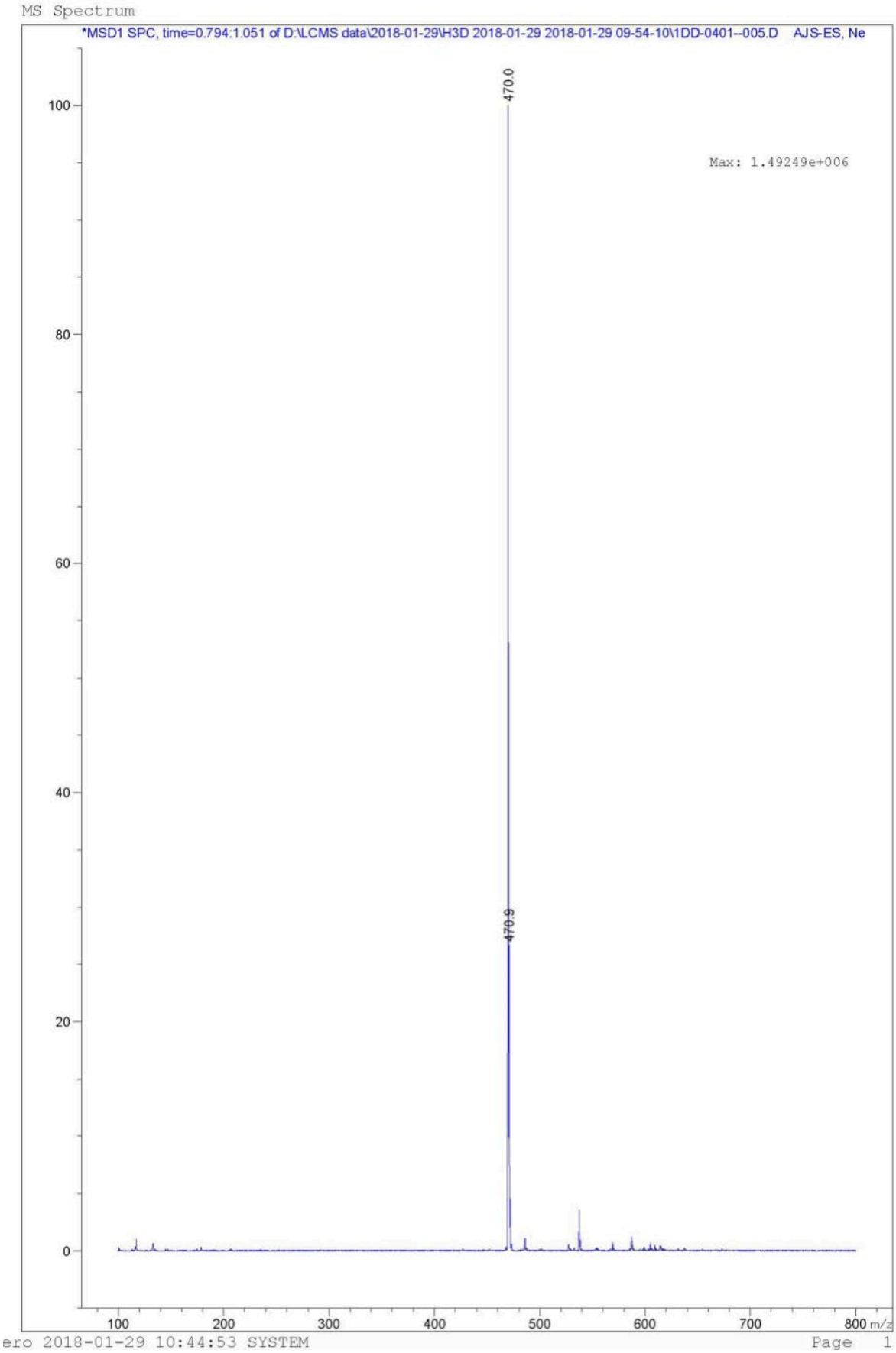
Signal 3: DAD1 C, Sig=290,4 Ref=550,10

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.840	BB	0.0206	856.92084	603.89893	95.9354
2	2.218	BB	0.0927	8.85336	1.13941	0.9912
3	2.511	BBA	0.1315	27.45303	2.52197	3.0735
Totals :				893.22723	607.56031	

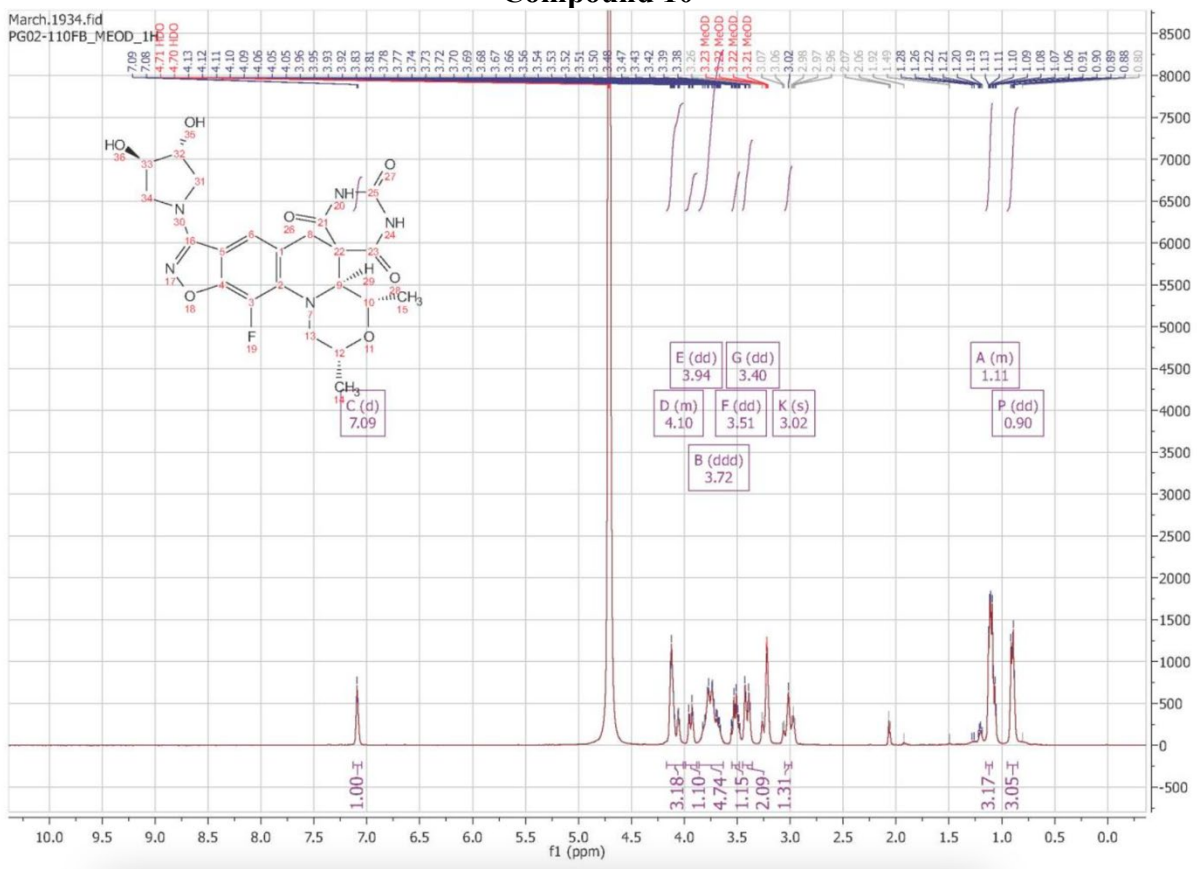
Signal 4: MSD1 TIC, MS File

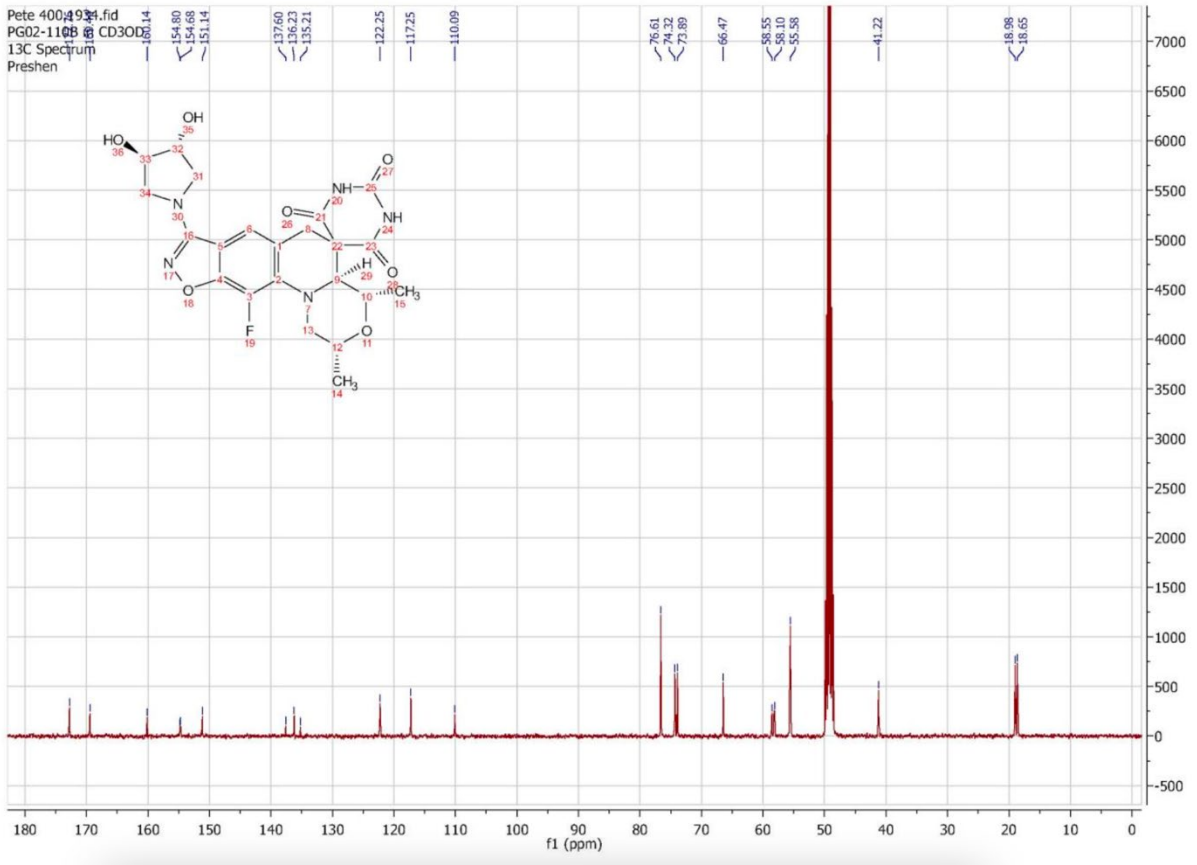
Peak #	RetTime [min]	Type	Width [min]	Area	Height	Area %
1	0.131	BB	0.0338	2.41111e6	9.71374e5	0.9345
2	0.209	BB	0.0224	1.42856e5	1.04632e5	0.0554
3	0.255	BB	0.0116	4.24931e4	6.10307e4	0.0165
4	0.274	BB	7.47e-3	1.22918e4	2.74250e4	4.764e-3
5	0.305	BB	0.0188	2.05701e5	1.94512e5	0.0797

Calimero 2018-01-29 10:44:35 SYSTEM

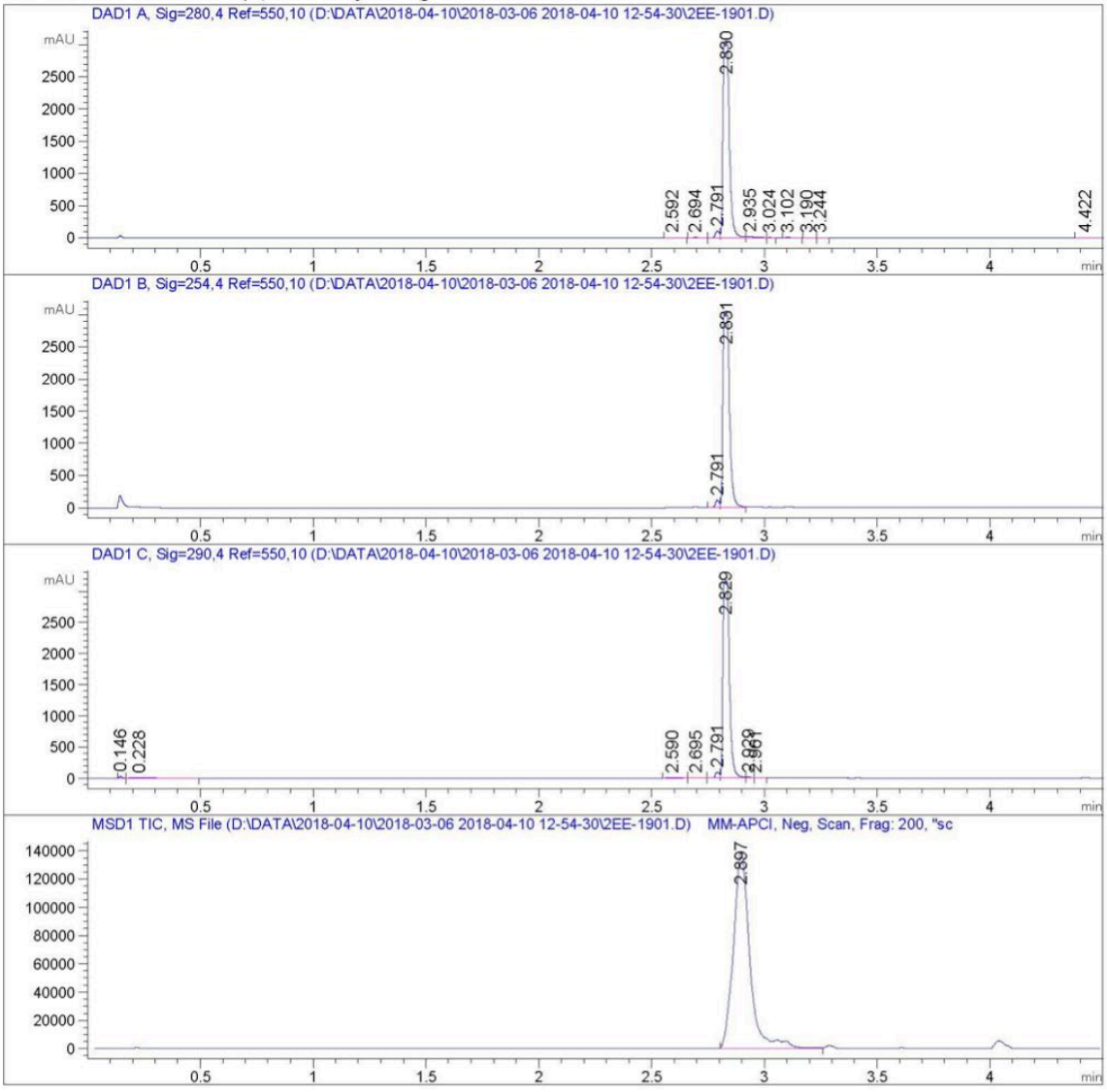

MS Spectrum
*MSD1 SPC, time=0.794:1.051 of D:\LCMS data\2018-01-29\H3D 2018-01-29 2018-01-29 09-54-10\1DD-0401--005.D AJS-ES, Ne
470.0
Max: 1.49249e+006
470.9
100
80
60
40
20
0
100
200
300
400
500
600
700
800 m/z
ero 2018-01-29 10:44:53 SYSTEM
Page 1 of

Compound 10


March.1934.fid
PG02-110FB_MEOD_1H
C (d)
7.09
E (dd)
3.94
G (dd)
3.40
D (m)
4.10
F (dd)
3.51
K (s)
3.02
B (ddd)
3.72
A (m)
1.11
P (dd)
0.90
1.00
3.18
1.10
4.74
1.15
2.09
1.31
3.17
3.05
f1 (ppm)


Pete 400.1934.fid
PG02-110B in CD3OD
13C Spectrum
Preshen
f1 (ppm)

```
==========================================================================
Acq. Operator   : SYSTEM                          Seq. Line :  19
Acq. Instrument : Agilent LC-MS                   Location : P2-E-05
Injection Date  : 2018-04-10 14:56:19                  Inj :   1
                                                 Inj Volume : 2.000 µl
Method          : D:\DATA\2018-04-10\2018-03-06 2018-04-10 12-54-30\GENERAL METHOD NEG 1.M (
                  Sequence Method)
Last changed    : 2018-04-10 12:54:30 by SYSTEM
Additional Info : Peak(s) manually integrated
```



==========

Area Percent Report

==========

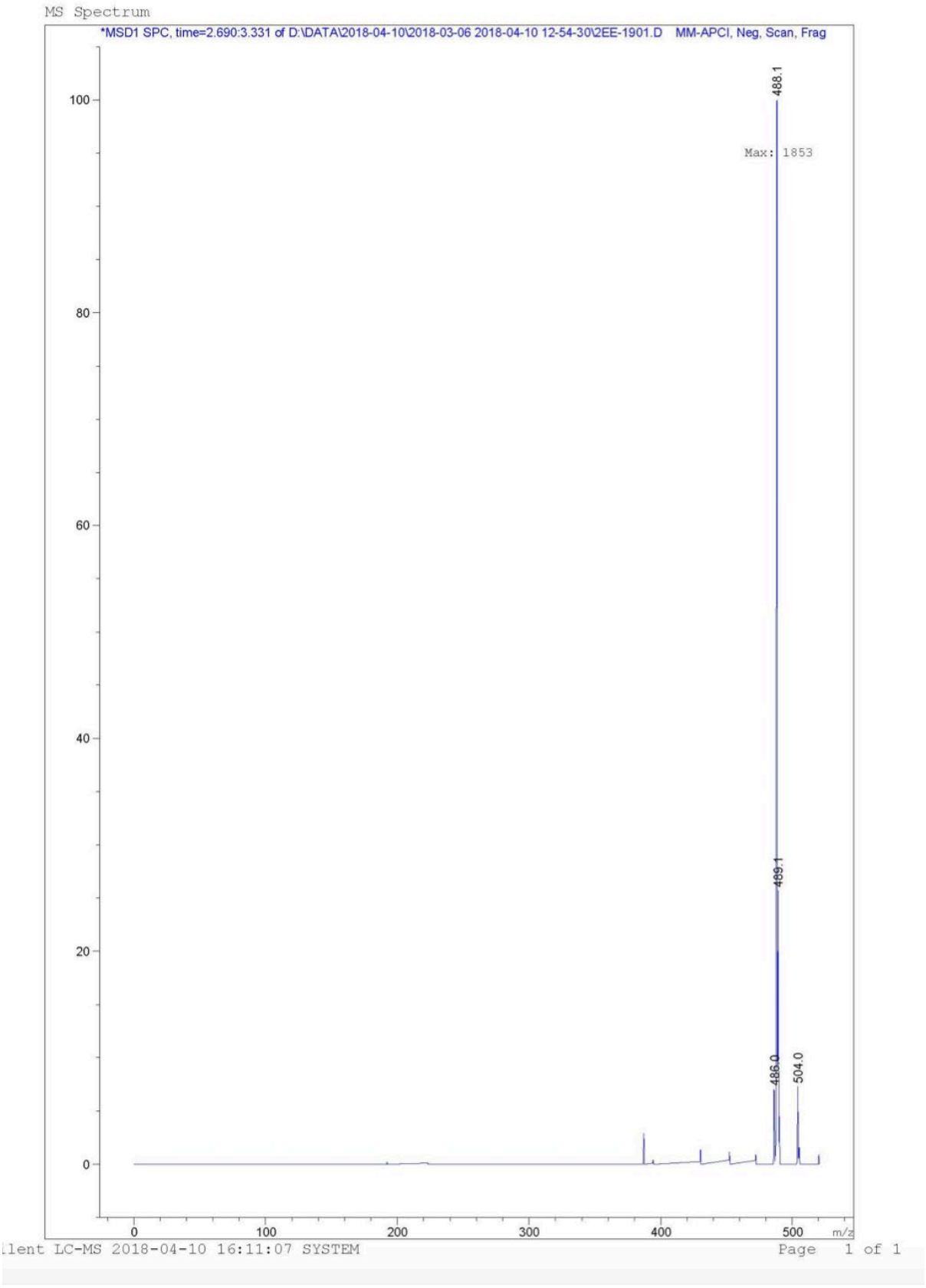
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=280,4 Ref=550,10

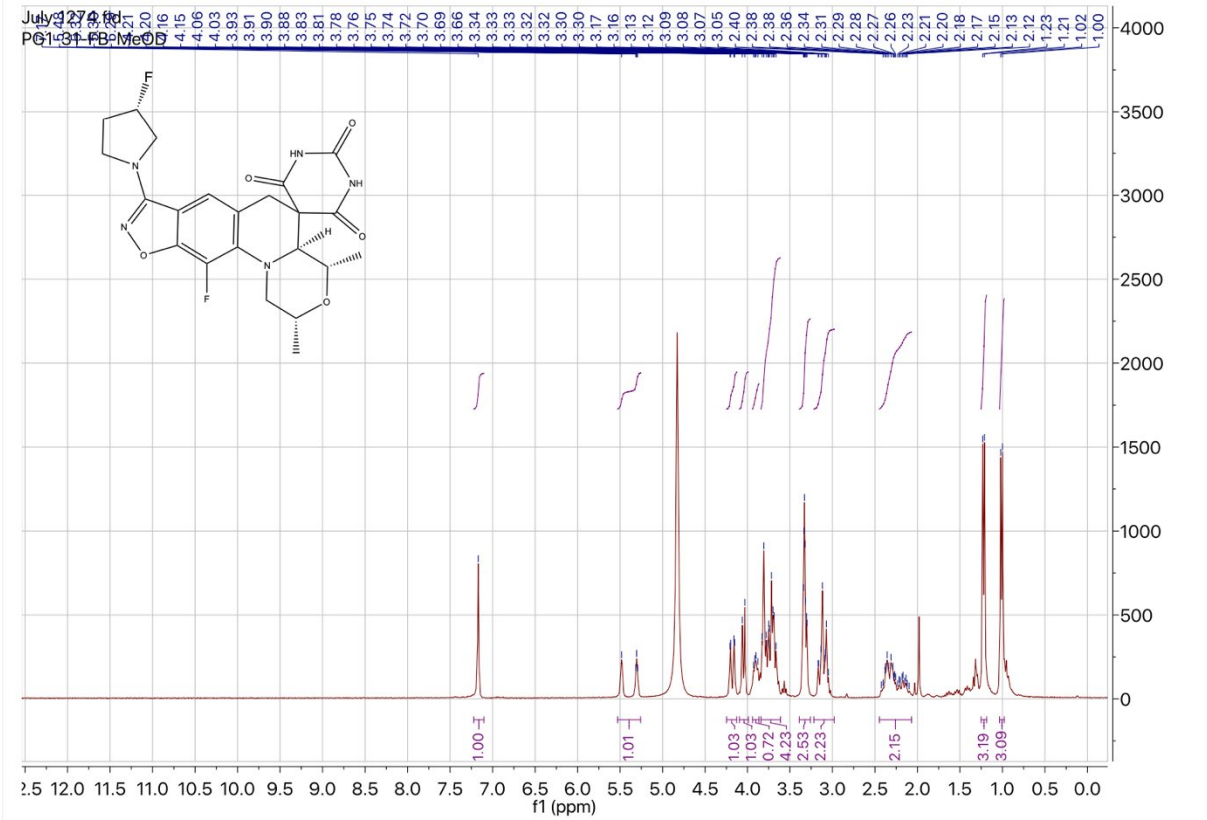
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.592	BB	0.0442	7.71034	2.55734	0.1251
2	2.694	BB	0.0330	11.65389	4.95118	0.1890
3	2.791	BV	0.0185	119.53217	103.22375	1.9390
4	2.830	VV	0.0268	5967.81104	3058.96167	96.8063
5	2.935	VB	0.0378	35.91277	13.62303	0.5826
6	3.024	BB	0.0173	1.76414	1.54401	0.0286
7	3.102	BB	0.0267	12.23887	6.72609	0.1985
8	3.190	BV	0.0355	2.43541	9.15747e-1	0.0395
9	3.244	VB	0.0255	2.25943	1.28436	0.0367
10	4.422	BBA	0.0337	3.37610	1.39767	0.0548
Totals :				6164.69415	3195.18484	

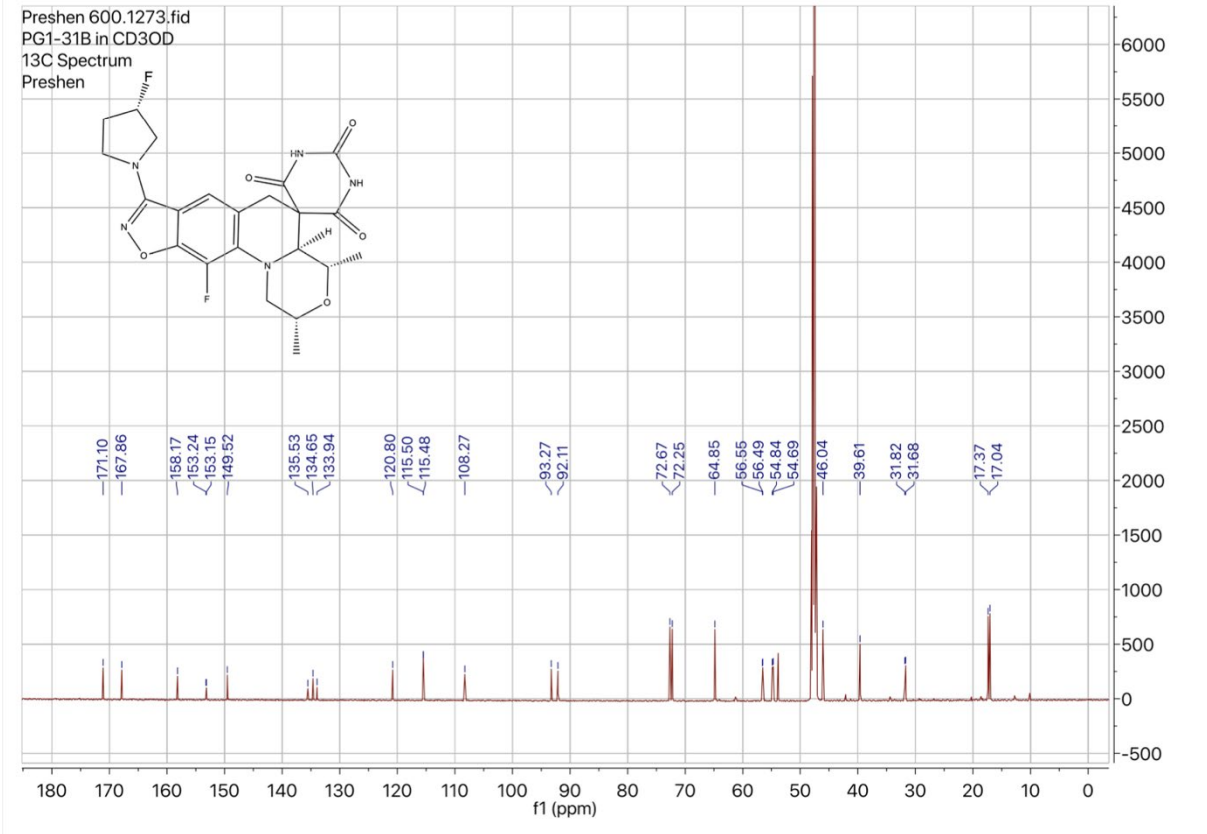
Signal 2: DAD1 B, Sig=254,4 Ref=550,10

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.791	BV	0.0180	129.99344	111.93073	2.0789
2	2.831	VV	0.0279	6122.91748	3054.69824	97.9211
Totals :				6252.91092	3166.62897	


MS Spectrum
*MSD1 SPC, time=2.690:3.331 of D:\DATA\2018-04-10\2018-03-06 2018-04-10 12-54-30\2EE-1901.D MM-APCI, Neg, Scan, Frag
488.1
Max: 1853
489.1
486.0
504.0
100
80
60
40
20
0
0
100
200
300
400
500
m/z
lent LC-MS 2018-04-10 16:11:07 SYSTEM
Page 1 of 1

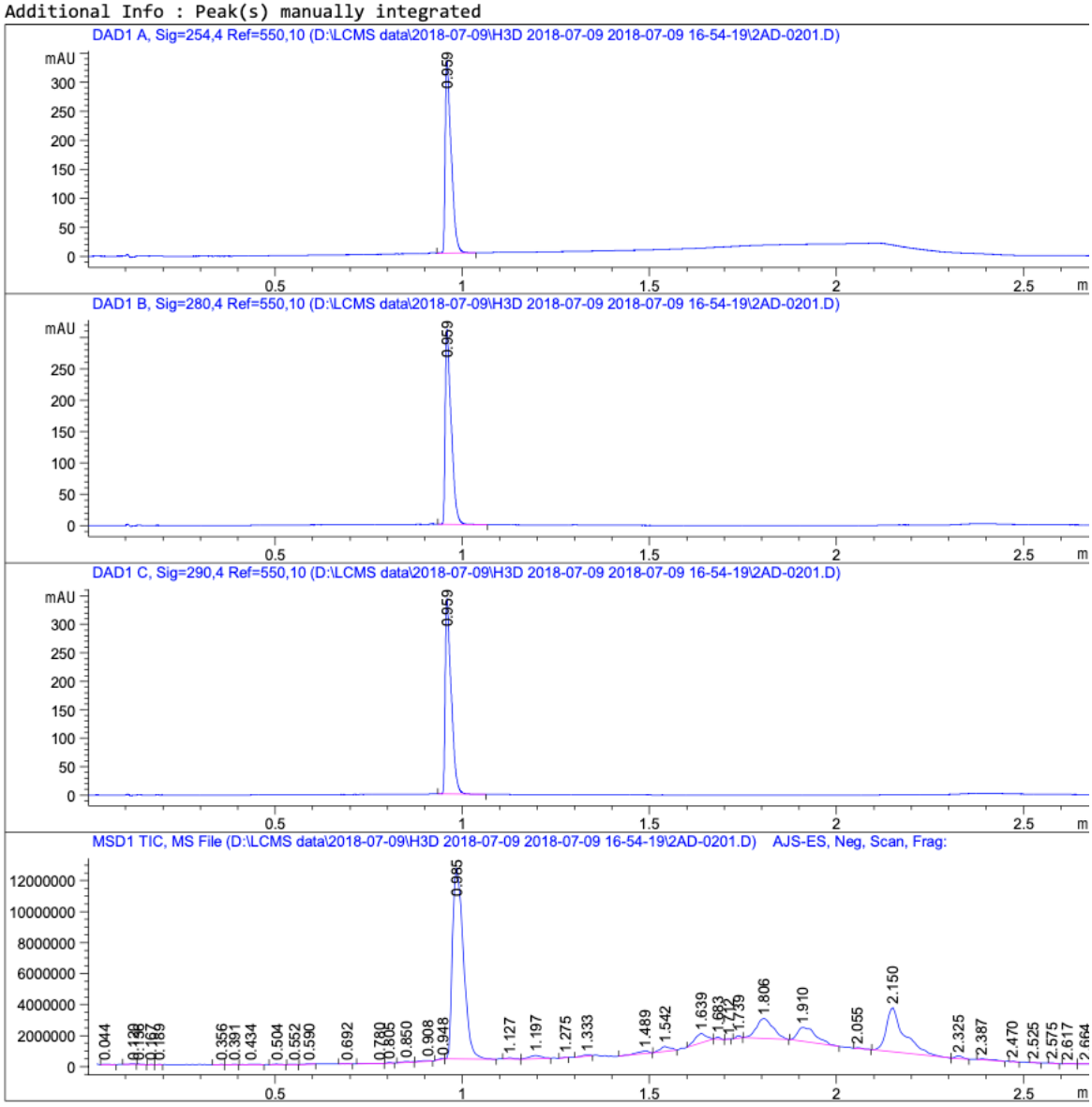
Compound 18

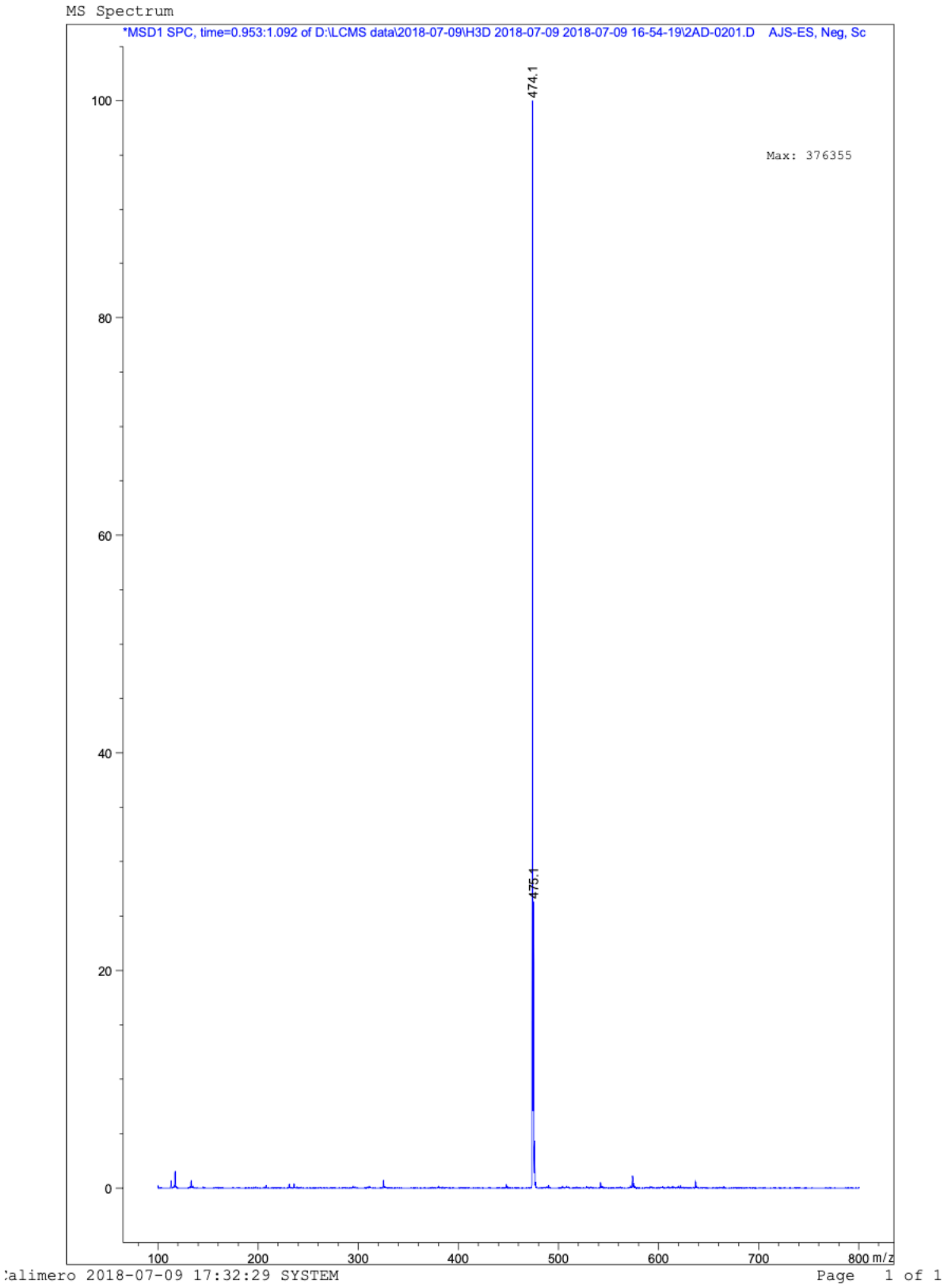

July 1274.fid
PG1-31-1B MeOD
f1 (ppm)


Preshen 600.1273.fid
PG1-31B in CD3OD
13C Spectrum
Preshen
f1 (ppm)

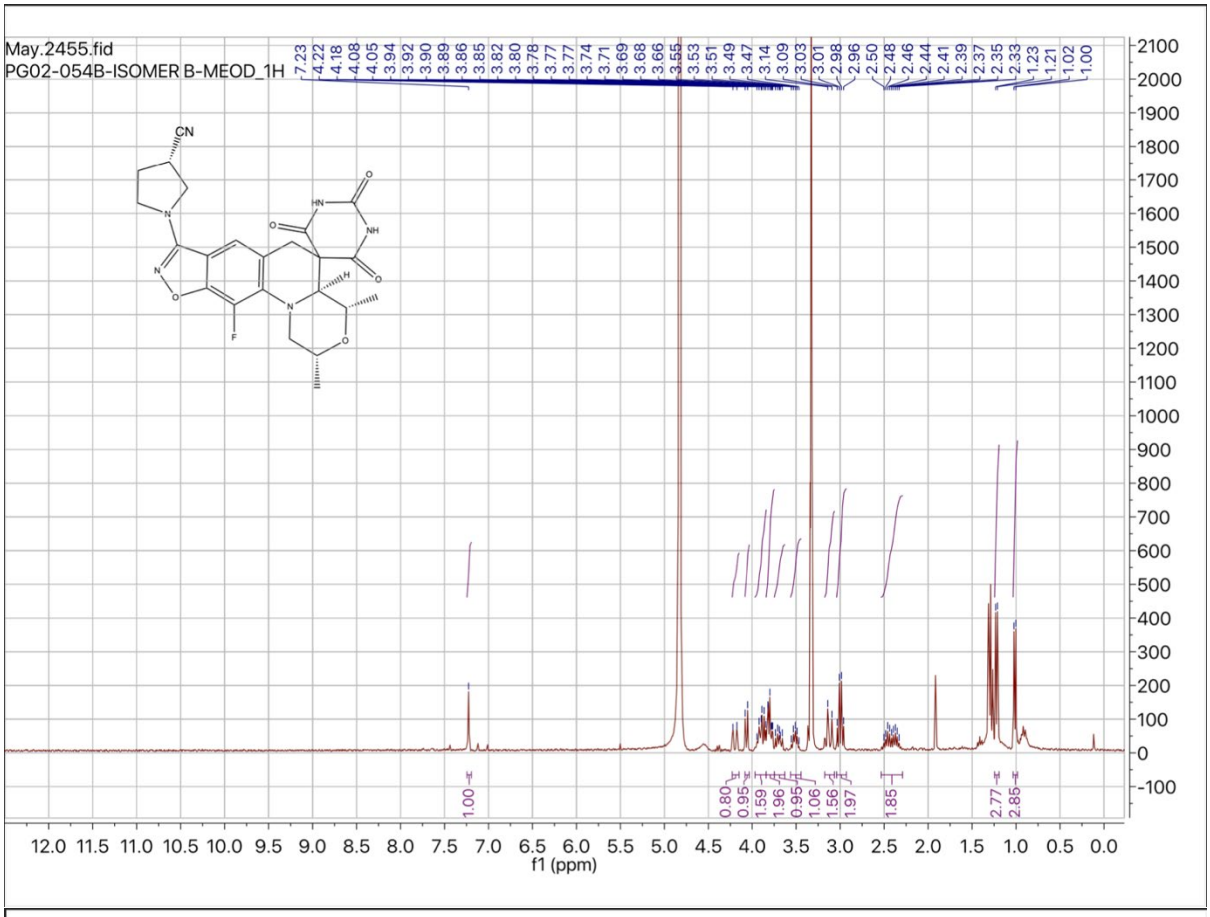
```
=====================================================================
                      Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.959 BB    0.0159  363.88965  330.04791 100.0000

Totals :                   363.88965  330.04791



Signal 2: DAD1 B, Sig=280,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.959 BB    0.0159  341.24991  309.40317 100.0000

Totals :                   341.24991  309.40317



Signal 3: DAD1 C, Sig=290,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.959 BB    0.0159  376.94943  341.80887 100.0000

Totals :                   376.94943  341.80887
```

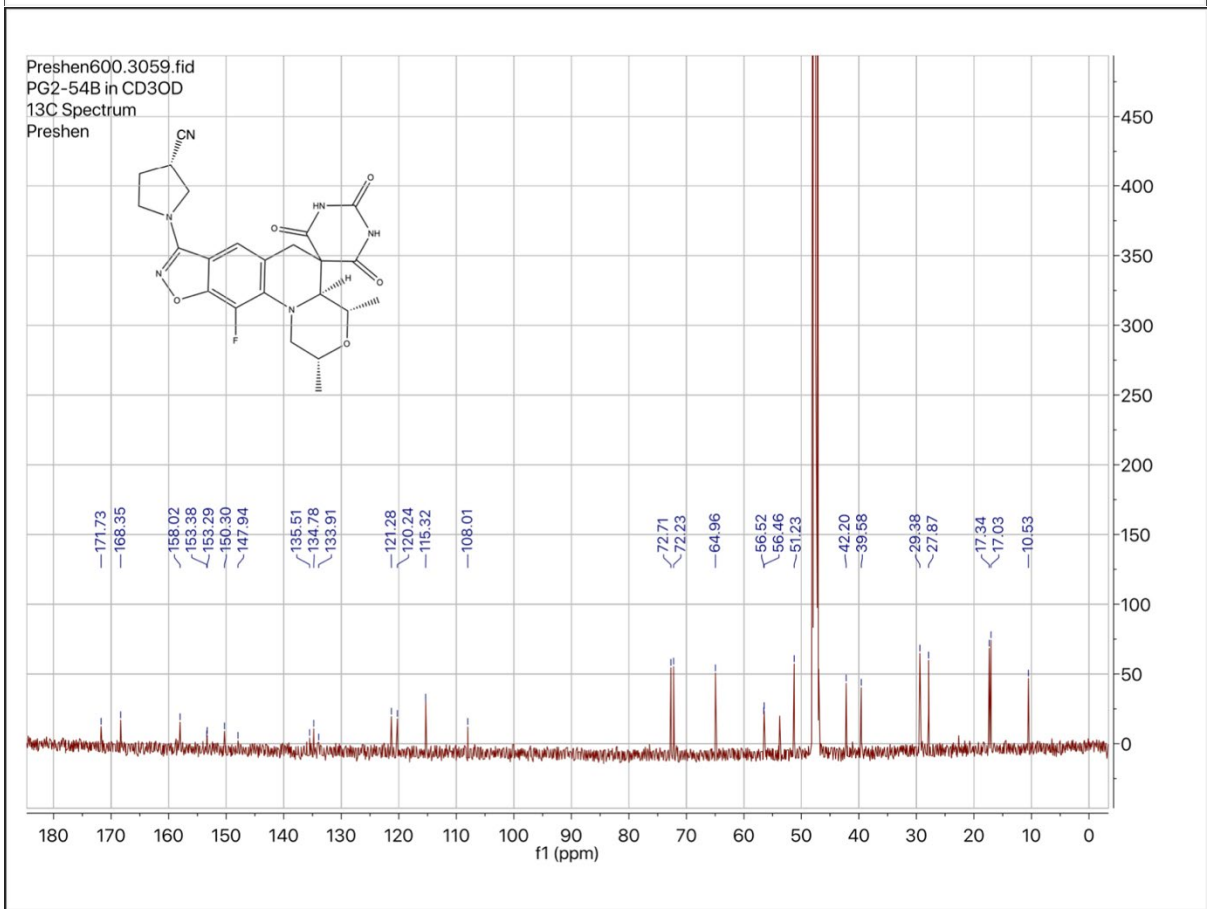
```
==========================================================================
Acq. Operator   : SYSTEM                          Seq. Line :   2
Acq. Instrument : Calimero                        Location :   P2-A4
Injection Date  : 2018-07-09 16:59:17                 Inj :   1
                                                 Inj Volume : 1.000 µl
Method          : D:\LCMS data\2018-07-09\H3D 2018-07-09 2018-07-09 16-54-19\NEW GENERAL NEG.
                  M (Sequence Method)
Last changed    : 2018-07-09 16:54:19 by SYSTEM
Method Info     : Standard 2.2min method with 254nm, 280nm and 290nm and positive ESI mode
                  100-800m/z

Additional Info : Peak(s) manually integrated
```



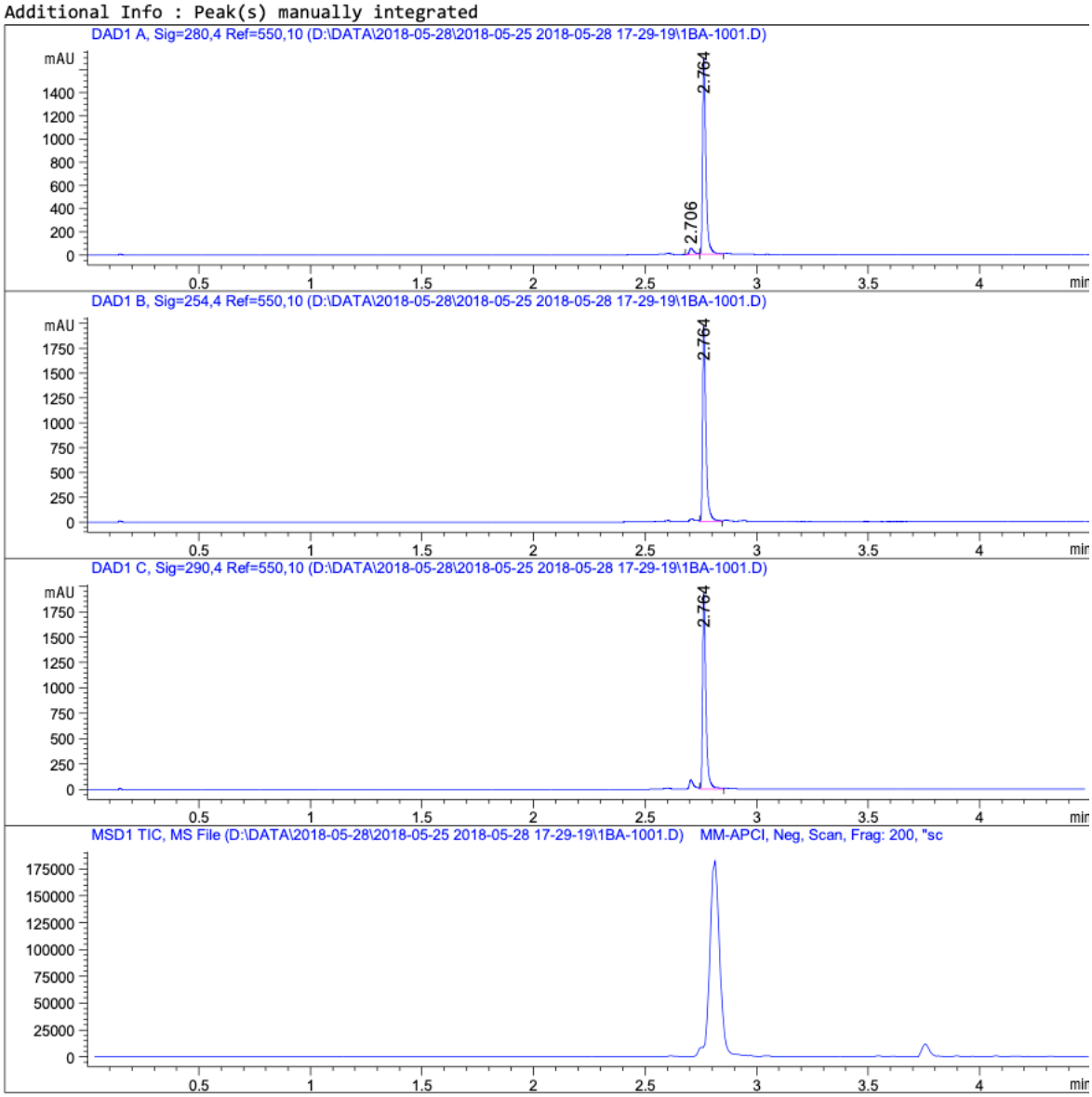

MS Spectrum
*MSD1 SPC, time=0.953:1.092 of D:\LCMS data\2018-07-09\H3D 2018-07-09 2018-07-09 16-54-19\2AD-0201.D AJS-ES, Neg, Sc
474.1
Max: 376355
475.1
100
80
60
40
20
0
100
200
300
400
500
600
700
800 m/z
alimero 2018-07-09 17:32:29 SYSTEM
Page 1 of 1

Compound 19

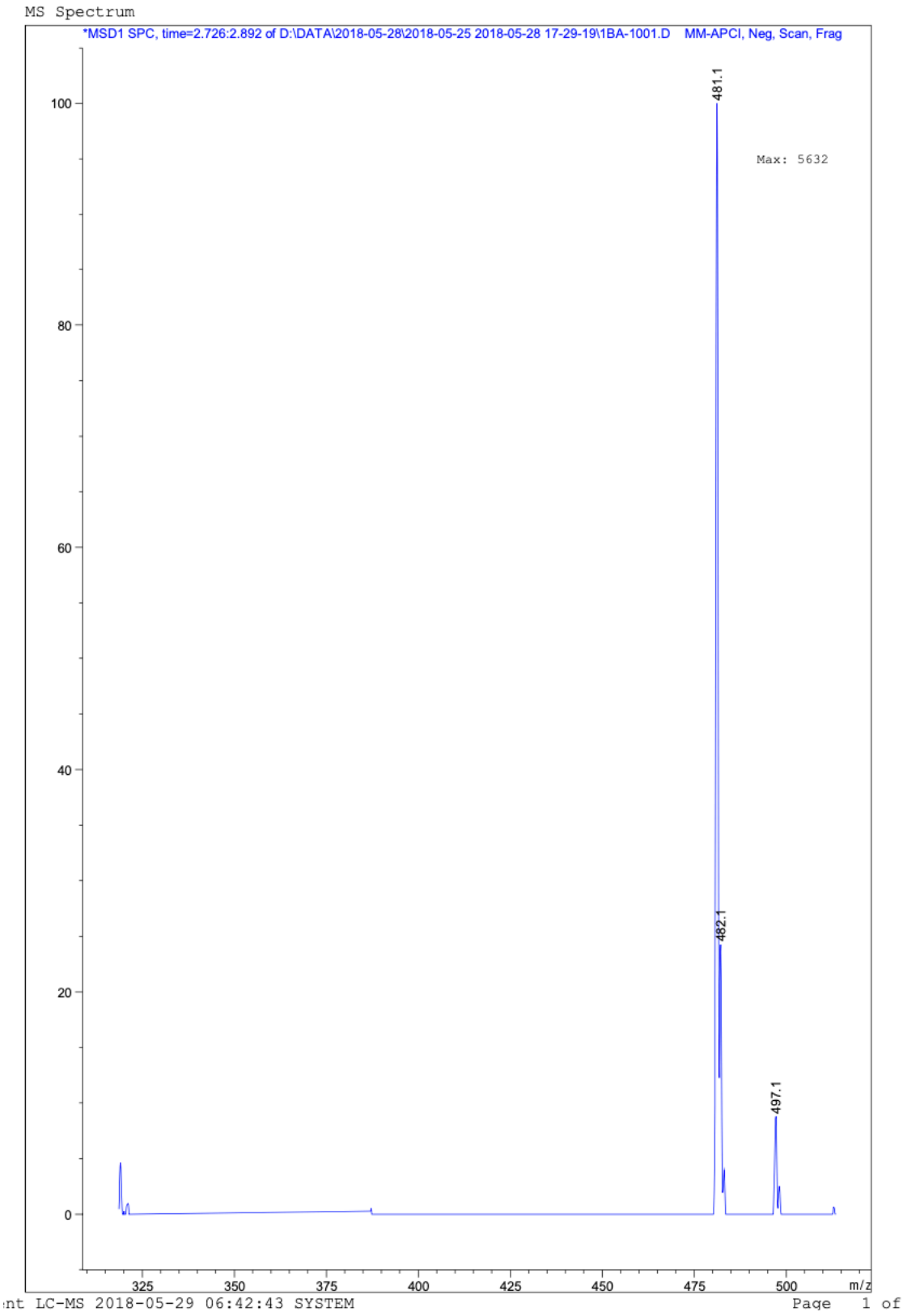

May.2455.fid
PG02-054B-ISOMER B-MEOD_1H
f1 (ppm)


Preshen600.3059.fid
PG2-54B in CD3OD
13C Spectrum
Preshen
f1 (ppm)

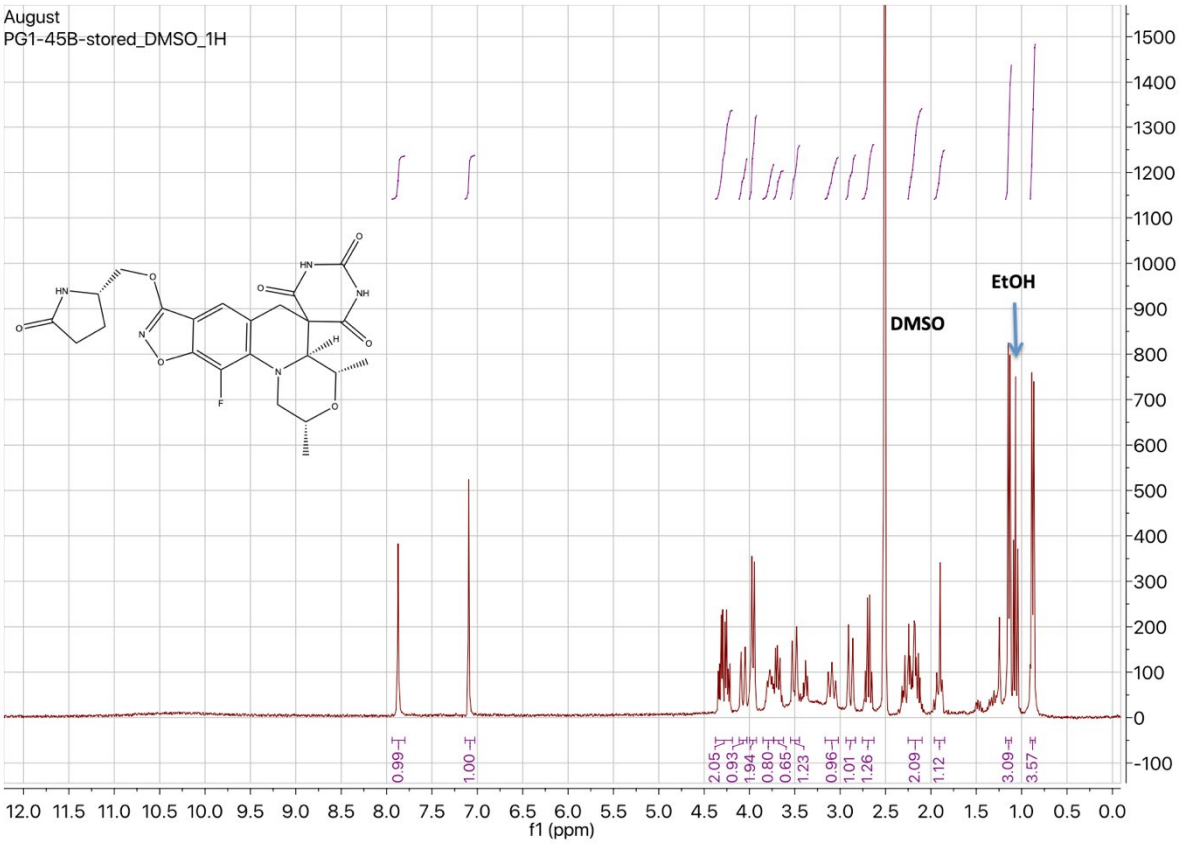
```
================================================================
Acq. Operator   : SYSTEM                        Seq. Line :  10
Acq. Instrument : Agilent LC-MS                  Location : P1-B-01
Injection Date  : 2018-05-28 18:29:53                 Inj :   1
                                                Inj Volume : 2.000 µl
Method          : D:\DATA\2018-05-28\2018-05-25 2018-05-28 17-29-19\GENERAL METHOD NEG 1.M (
                  Sequence Method)
Last changed    : 2018-05-28 17:29:19 by SYSTEM
Additional Info : Peak(s) manually integrated
```

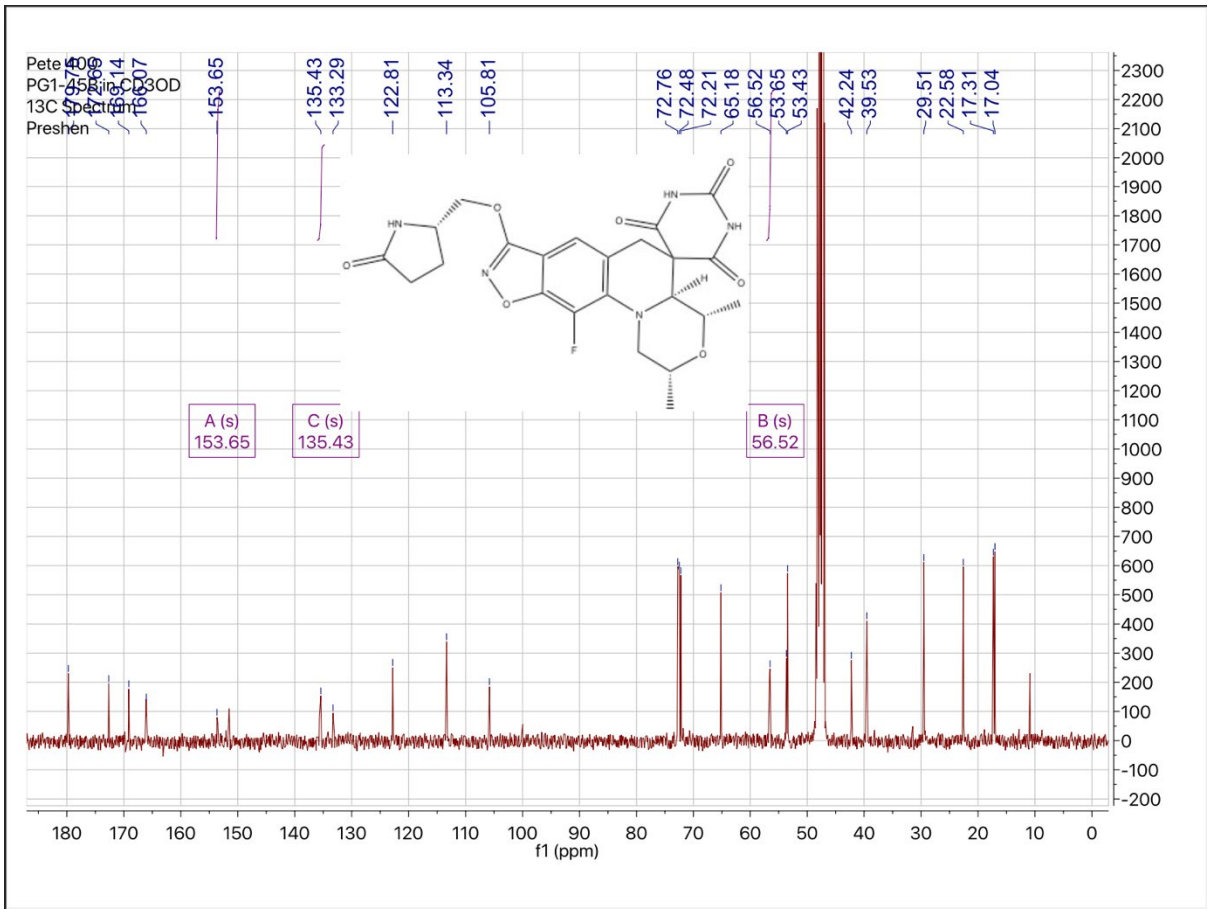


```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=280,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   2.706 BV    0.0214   75.06808   53.25735   4.2451
   2   2.764 VV    0.0153 1693.27087 1671.98340  95.7549

Totals :                   1768.33896 1725.24075



Signal 2: DAD1 B, Sig=254,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   2.764 VV    0.0155 2003.82214 1949.48669 100.0000

Totals :                   2003.82214 1949.48669



Signal 3: DAD1 C, Sig=290,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   2.764 VV    0.0153 1938.09338 1910.02649 100.0000

Totals :                   1938.09338 1910.02649
```

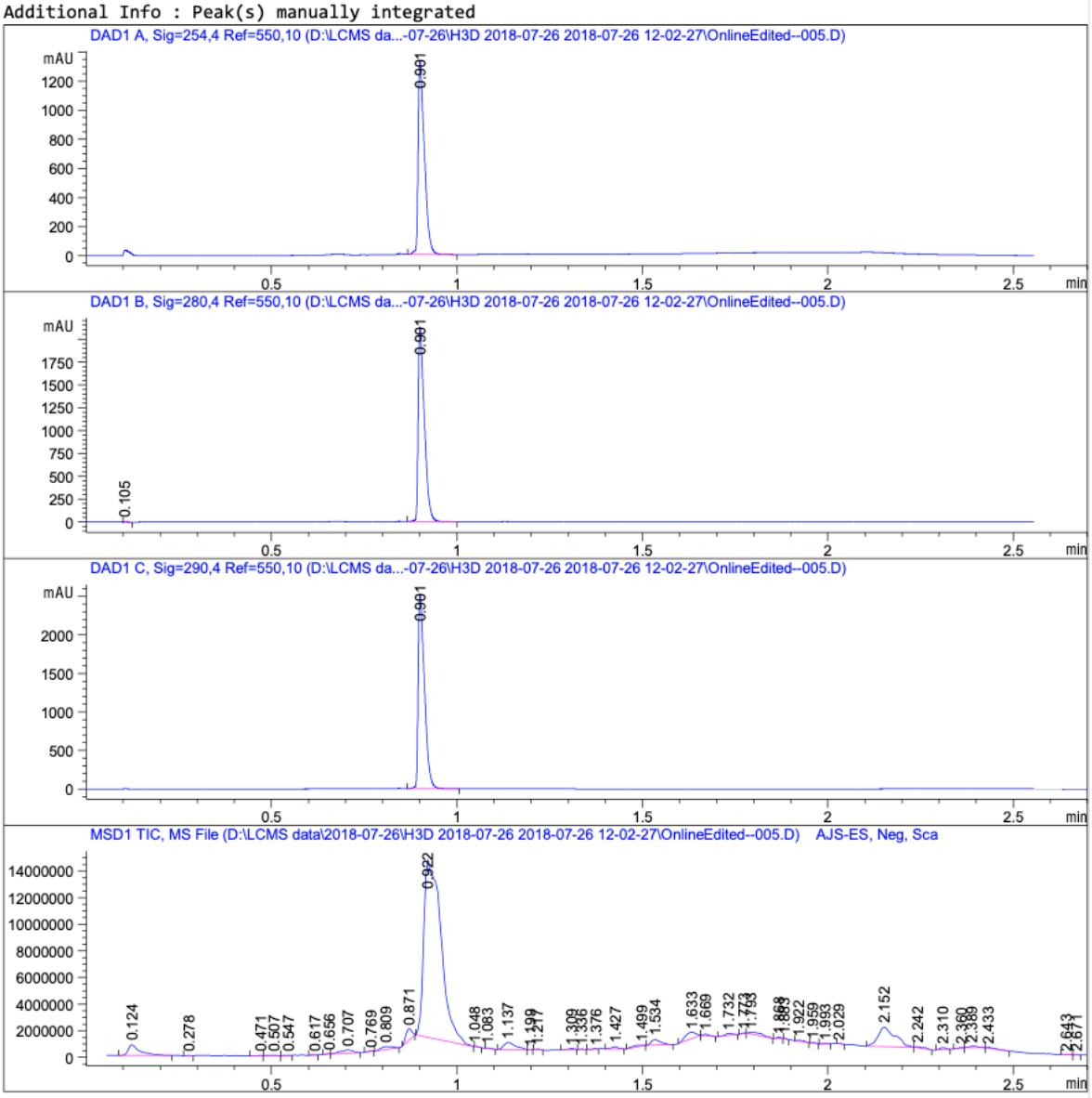

MS Spectrum
*MSD1 SPC, time=2.726:2.892 of D:\DATA\2018-05-28\2018-05-25 2018-05-28 17-29-19\1BA-1001.D MM-APCI, Neg, Scan, Frag
481.1
Max: 5632
482.1
497.1
100
80
60
40
20
0
325
350
375
400
425
450
475
500
m/z
nt LC-MS 2018-05-29 06:42:43 SYSTEM
Page 1 of

Compound 23

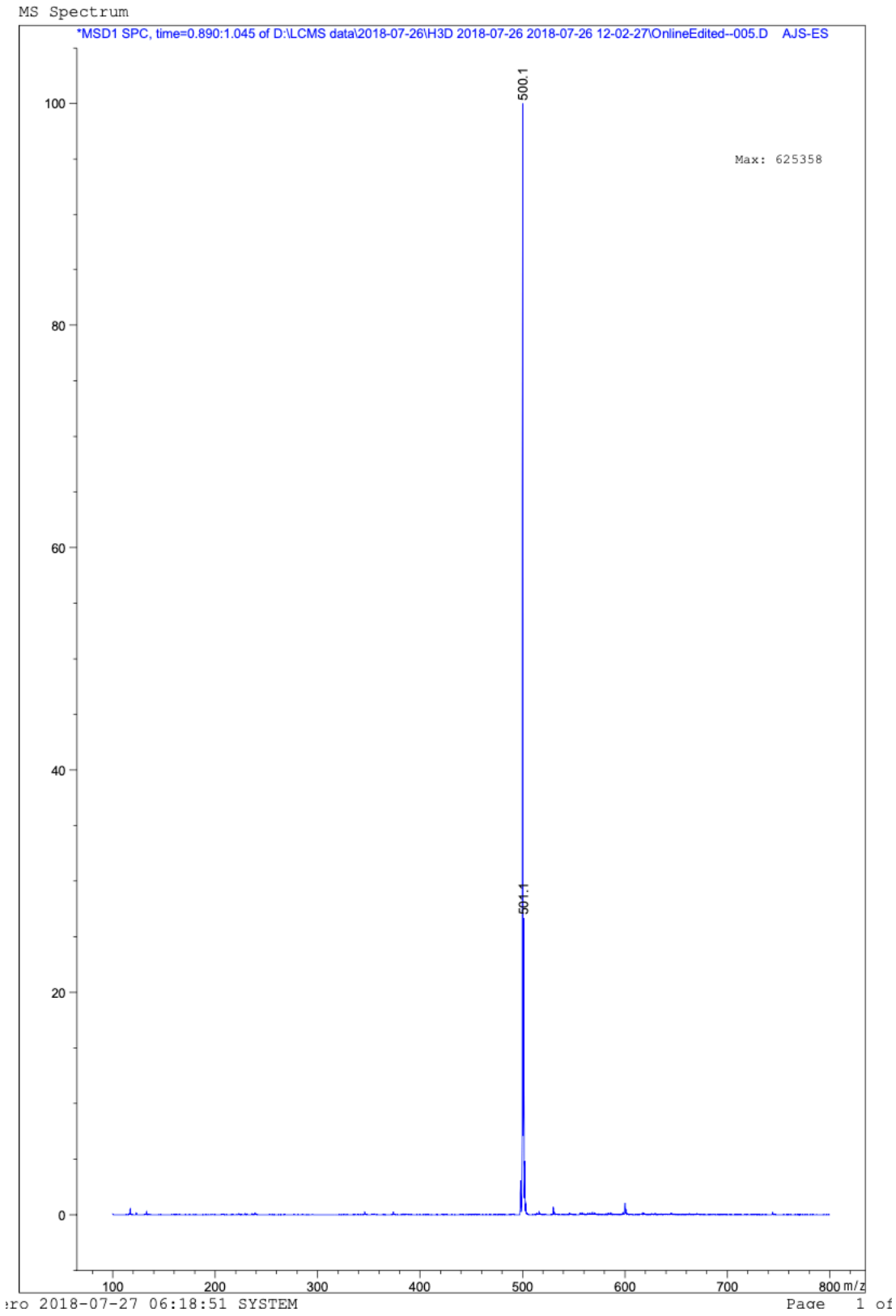

August
PG1-45B-stored_DMSO_1H
DMSO
EtOH
f1 (ppm)


PG1-45B in CD3OD
153.65
135.43
133.29
122.81
113.34
105.81
72.76
72.48
72.21
65.18
56.52
53.65
53.43
42.24
39.53
29.51
22.58
17.31
17.04
A (s)
153.65
C (s)
135.43
B (s)
56.52
f1 (ppm)

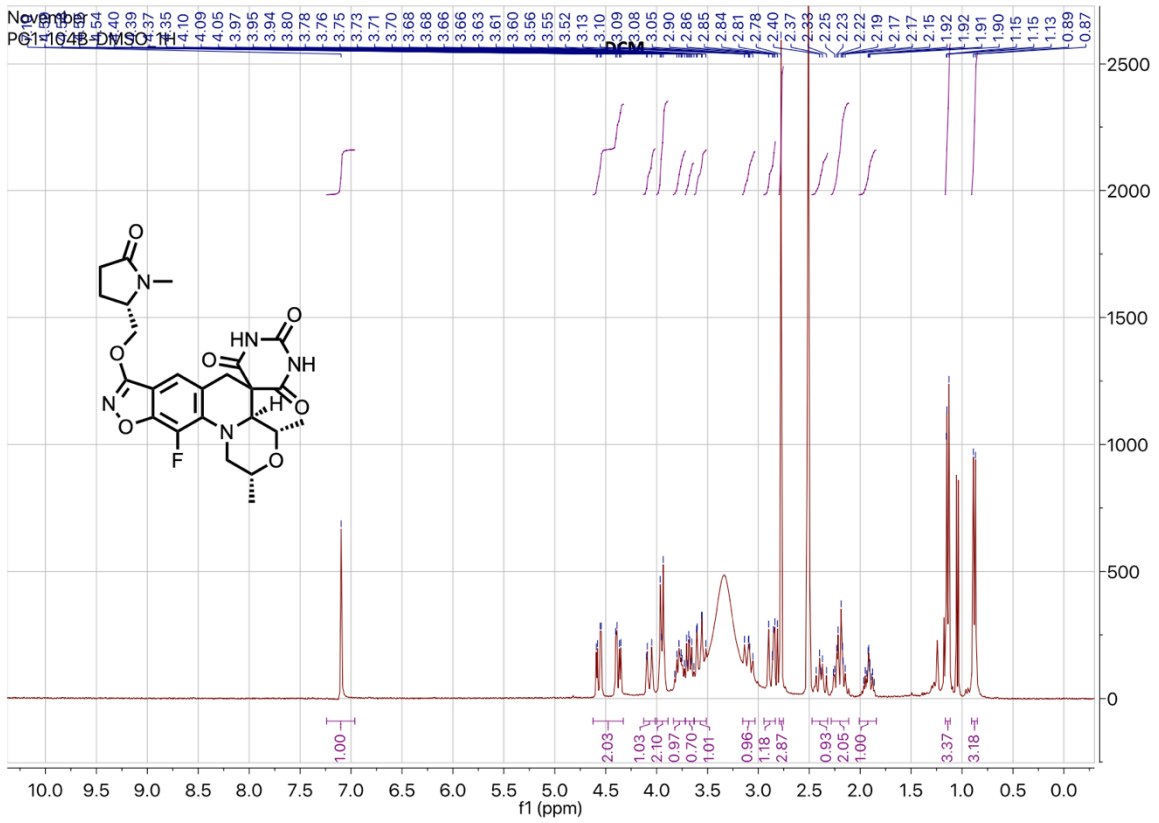
```
================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   5
Acq. Instrument : Calimero                       Location :   P1-D3
Injection Date  : 2018-07-26 12:17:49                Inj :   1
                                                Inj Volume : 1.000 µl
Method          : D:\LCMS data\2018-07-26\H3D 2018-07-26 2018-07-26 12-02-27\NEW GENERAL NEG.
                  M (Sequence Method)
Last changed    : 2018-07-26 12:08:58 by SYSTEM
Method Info     : Standard 2.2min method with 254nm, 280nm and 290nm and positive ESI mode
                  100-800m/z

Additional Info : Peak(s) manually integrated
```

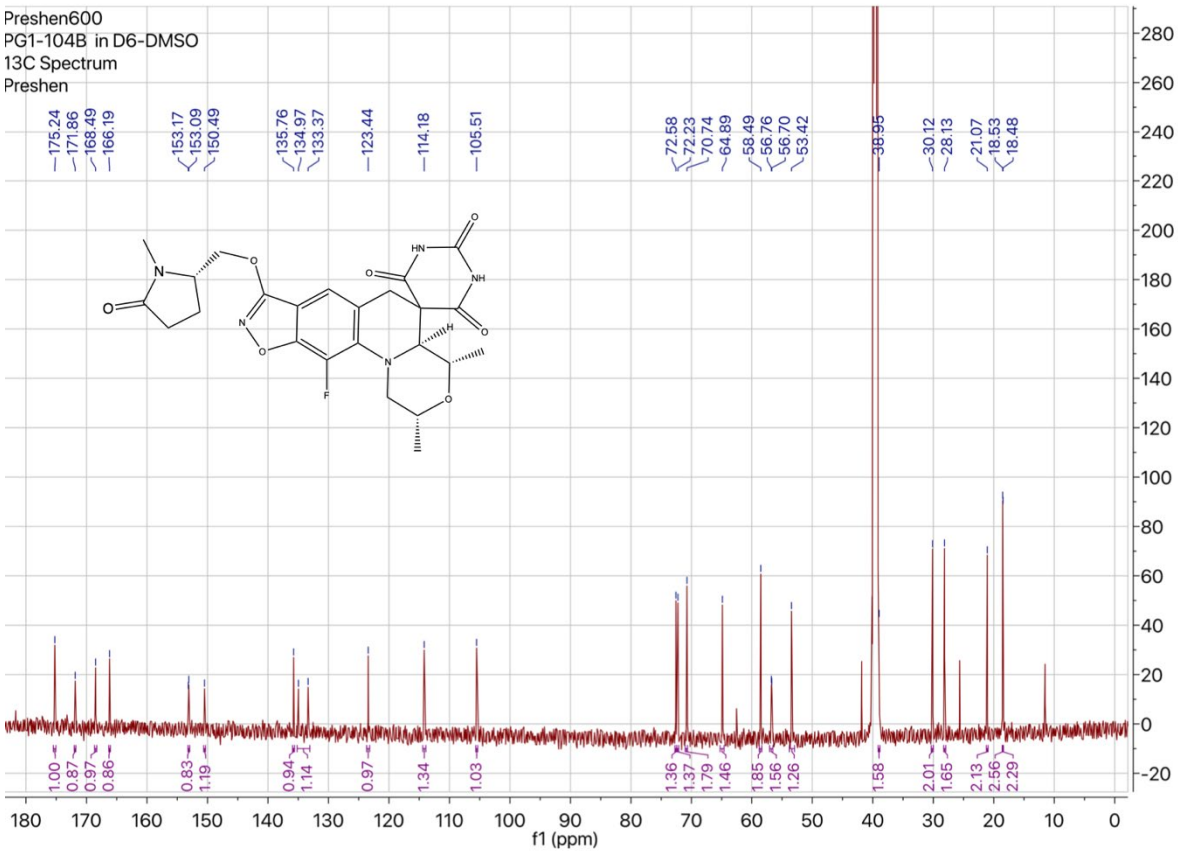


```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.901 BB    0.0168 1564.61304 1322.85315 100.0000

Totals :                   1564.61304 1322.85315



Signal 2: DAD1 B, Sig=280,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.105 BB    0.0114    7.28625   10.08357   0.2964
   2   0.901 BB    0.0166 2450.61865 2107.68970  99.7036

Totals :                   2457.90490 2117.77327



Signal 3: DAD1 C, Sig=290,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.901 BB    0.0173 2972.51392 2512.81714 100.0000

Totals :                   2972.51392 2512.81714
```

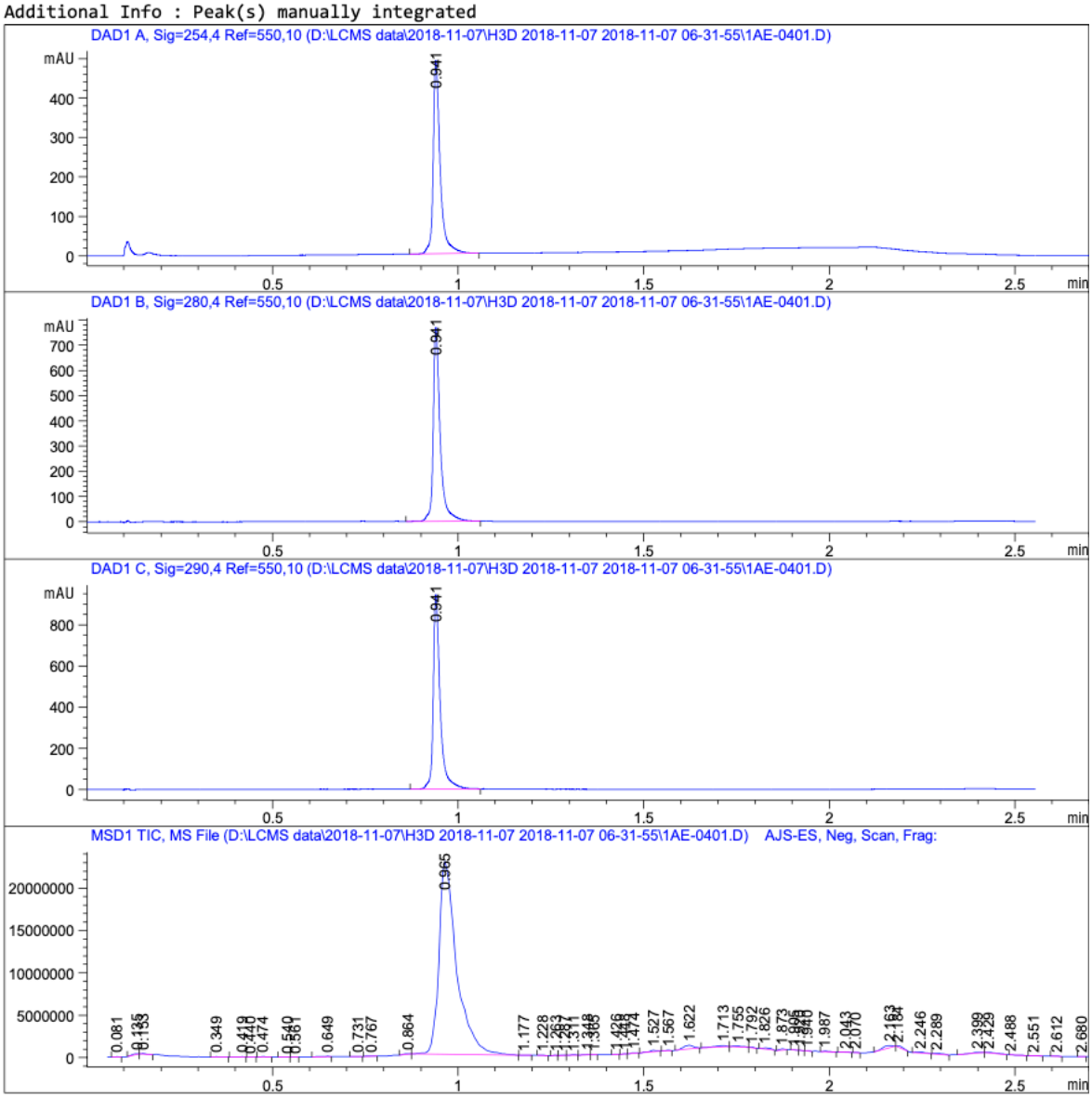

MS Spectrum
*MSD1 SPC, time=0.890:1.045 of D:\LCMS data\2018-07-26\H3D 2018-07-26 2018-07-26 12-02-27\OnlineEdited--005.D AJS-ES
500.1
Max: 625358
501.1
100
80
60
40
20
0
100
200
300
400
500
600
700
800 m/z
ero 2018-07-27 06:18:51 SYSTEM
Page 1 of

Compound 25

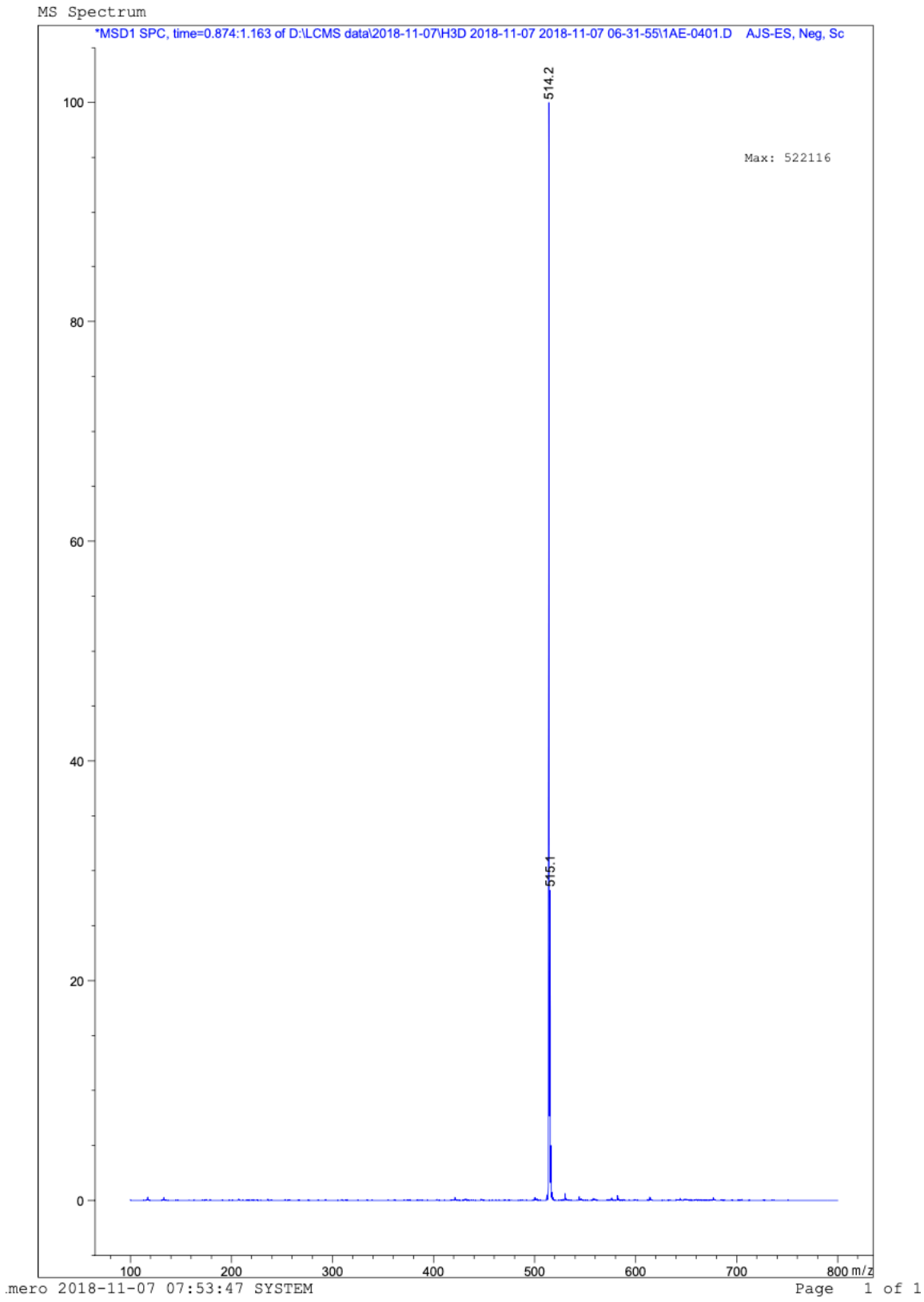

PG1-104B-DMSO-1H
PPM
f1 (ppm)


Preshen600
PG1-104B in D6-DMSO
13C Spectrum
Preshen
f1 (ppm)

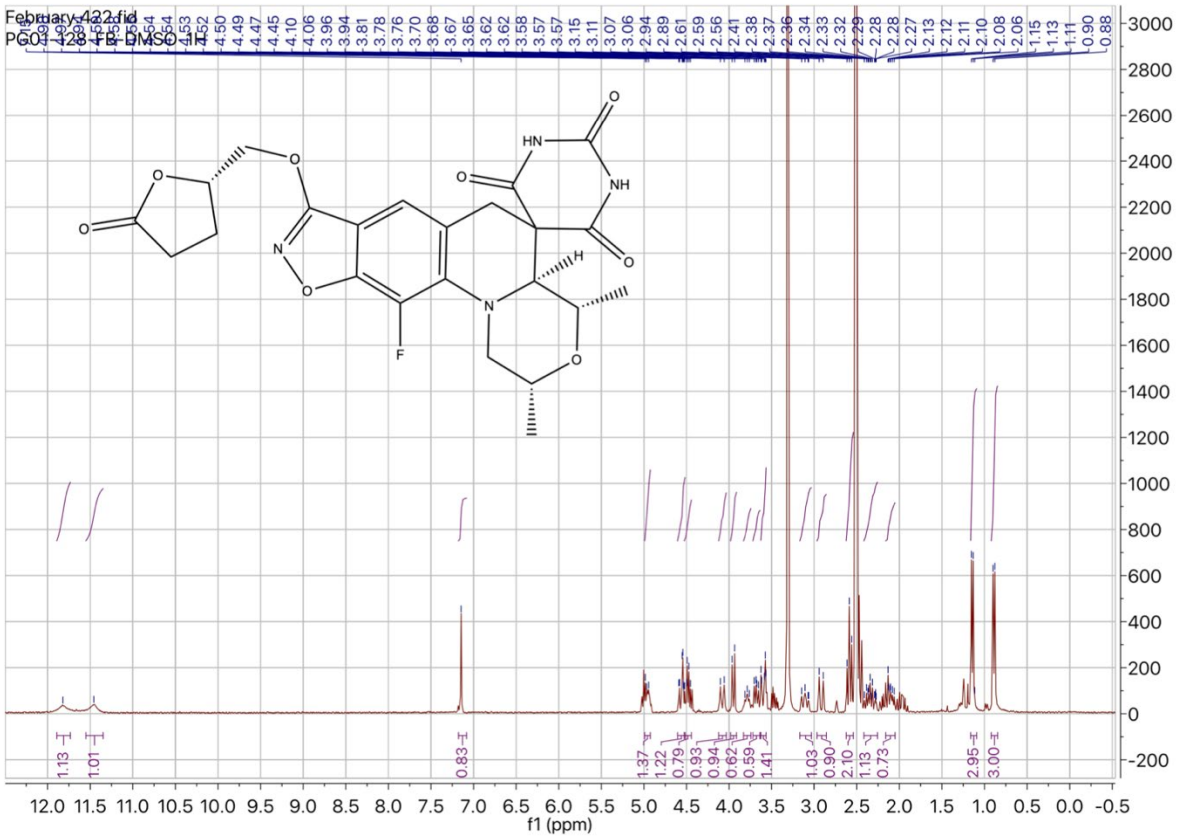
```
==========================================================================
Acq. Operator   : SYSTEM                          Seq. Line :   4
Acq. Instrument : Calimero                        Location :   P1-A5
Injection Date  : 2018-11-07 06:43:38                 Inj :   1
                                                 Inj Volume : 1.000 µl
Method          : D:\LCMS data\2018-11-07\H3D 2018-11-07 2018-11-07 06-31-55\NEW GENERAL NEG.
                  M (Sequence Method)
Last changed    : 2018-11-07 06:31:55 by SYSTEM
Method Info     : Standard 2.2min method with 254nm, 280nm and 290nm and positive ESI mode
                  100-800m/z

Additional Info : Peak(s) manually integrated
```

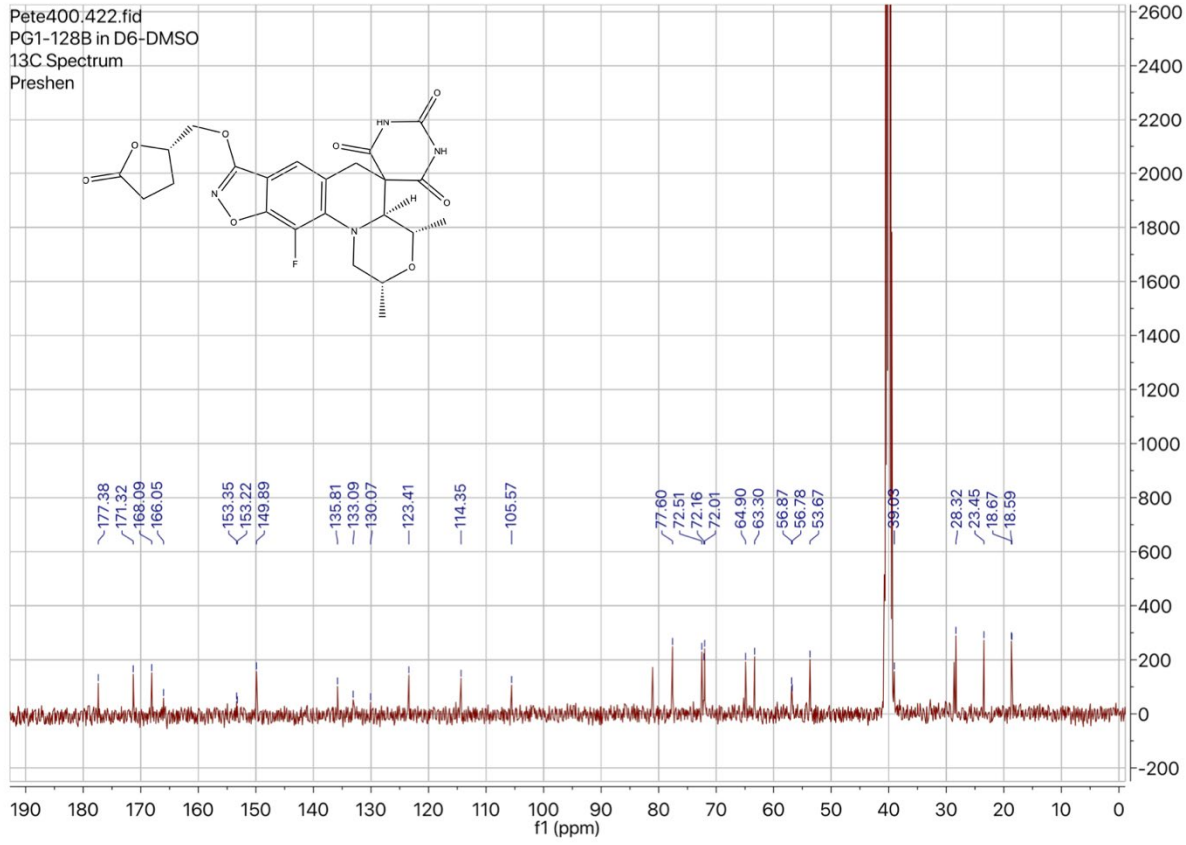


```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.941 BB    0.0193  639.24969  488.06320 100.0000

Totals :                   639.24969  488.06320



Signal 2: DAD1 B, Sig=280,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.941 BB    0.0191  991.06561  765.17273 100.0000

Totals :                   991.06561  765.17273



Signal 3: DAD1 C, Sig=290,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.941 BB    0.0191 1214.28174  940.63983 100.0000

Totals :                  1214.28174  940.63983
```

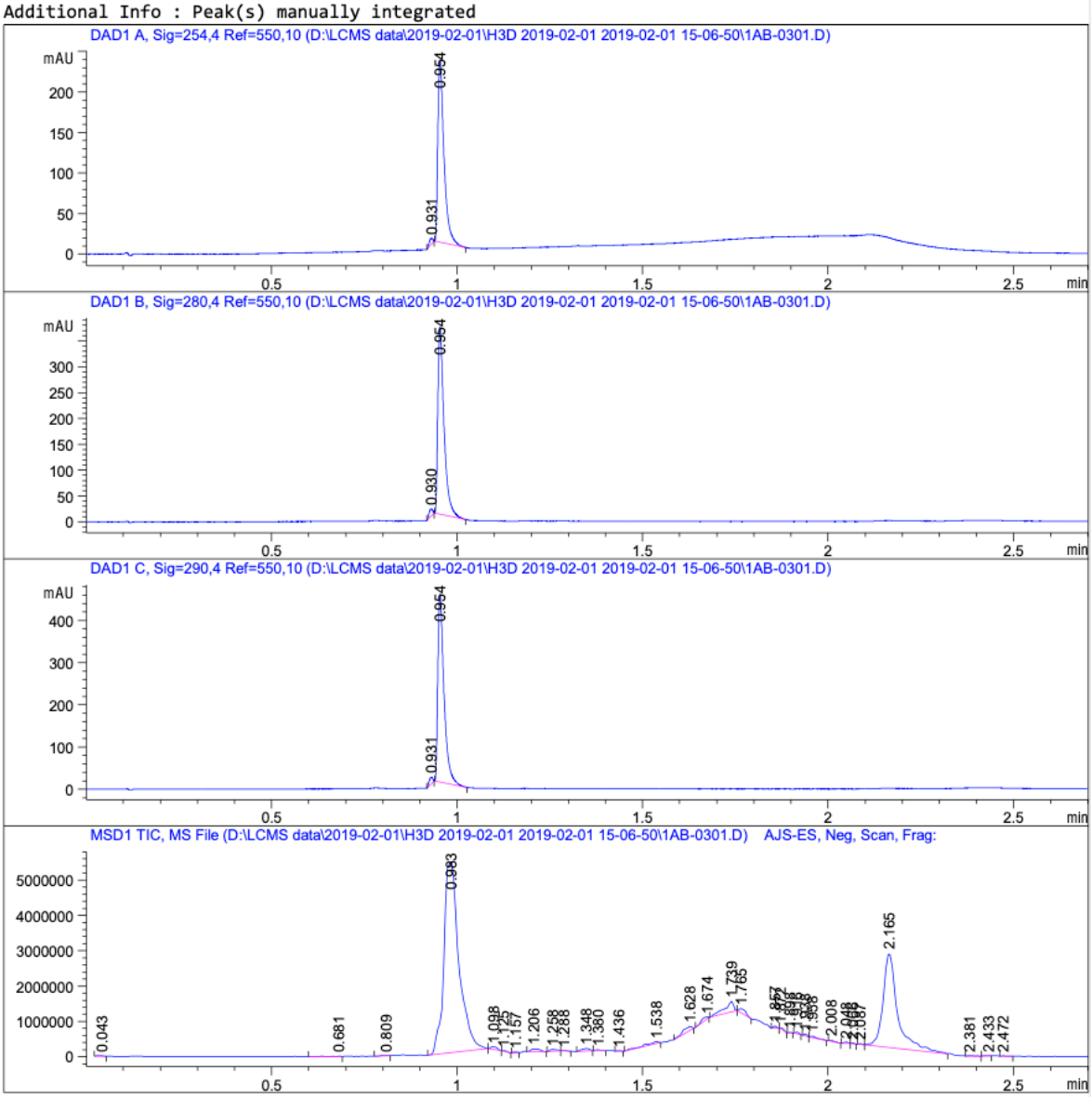

MS Spectrum
*MSD1 SPC, time=0.874:1.163 of D:\LCMS data\2018-11-07\H3D 2018-11-07 2018-11-07 06-31-55\1AE-0401.D AJS-ES, Neg, Sc
514.2
Max: 522116
515.1
100
80
60
40
20
0
100
200
300
400
500
600
700
800 m/z
.mero 2018-11-07 07:53:47 SYSTEM
Page 1 of 1

Compound 26

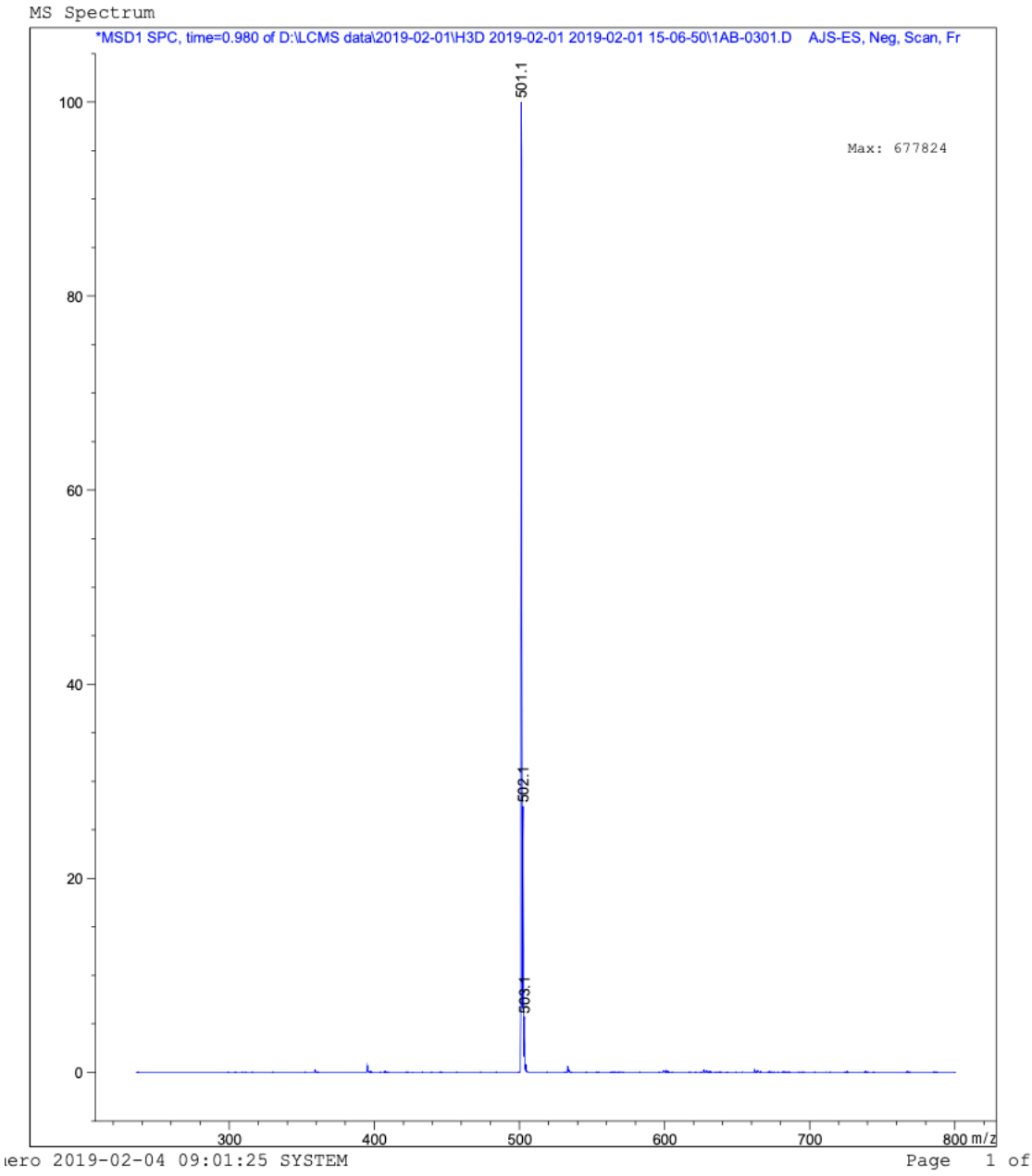

February 422.fid
PG1-128 in DMSO
f1 (ppm)


Pete400.422.fid
PG1-128B in D6-DMSO
13C Spectrum
Preshen
f1 (ppm)

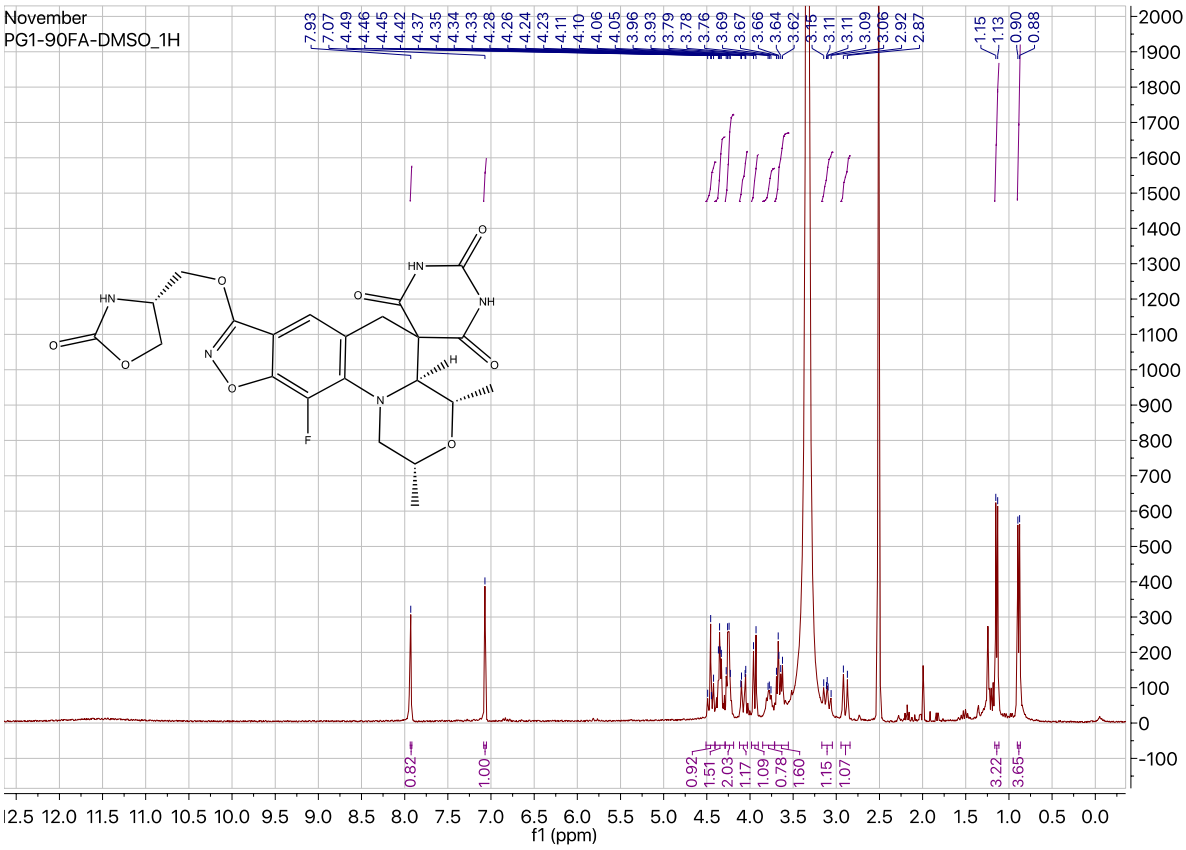
```
==========================================================================
Acq. Operator    : SYSTEM                          Seq. Line :   3
Acq. Instrument  : Calimero                         Location :   P1-A2
Injection Date   : 2019-02-01 15:15:27                  Inj :   1
                                                  Inj Volume : 1.000 µl
Method           : D:\LCMS data\2019-02-01\H3D 2019-02-01 2019-02-01 15-06-50\NEW GENERAL NEG.
                   M (Sequence Method)
Last changed     : 2019-02-01 15:06:50 by SYSTEM
Method Info      : Standard 2.2min method with 254nm, 280nm and 290nm and positive ESI mode
                   100-800m/z

Additional Info : Peak(s) manually integrated
```

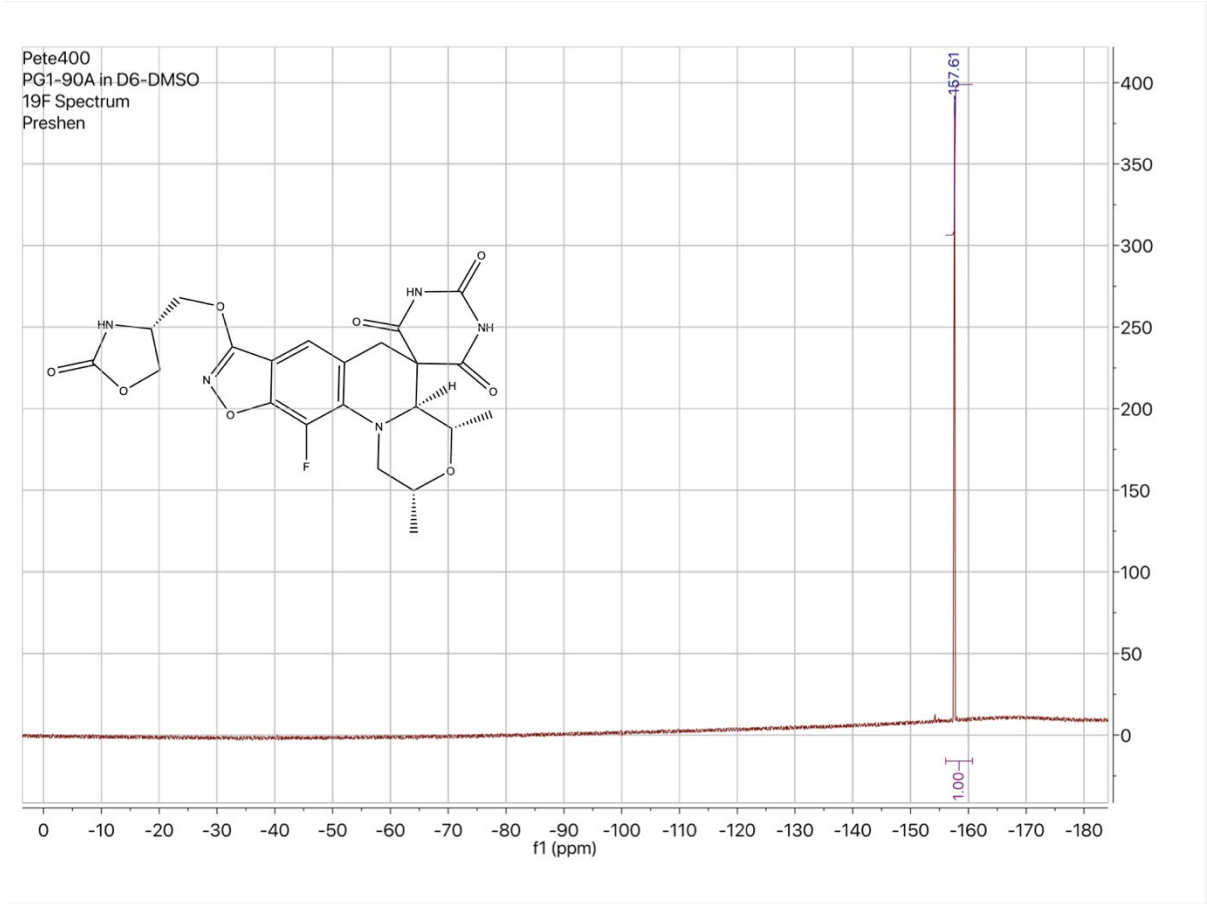


```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.931 BB    0.0120    5.01081    6.89715   1.8720
   2   0.954 BB    0.0177  262.66635  223.68578  98.1280

Totals :                    267.67717  230.58293



Signal 2: DAD1 B, Sig=280,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.930 BB    0.0117    9.42803   13.38401   2.1862
   2   0.954 BB    0.0177  421.82147  359.74792  97.8138

Totals :                    431.24951  373.13193



Signal 3: DAD1 C, Sig=290,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.931 BB    0.0117   10.55772   15.04663   1.9878
   2   0.954 BB    0.0177  520.57440  443.16541  98.0122

Totals :                    531.13212  458.21204
```

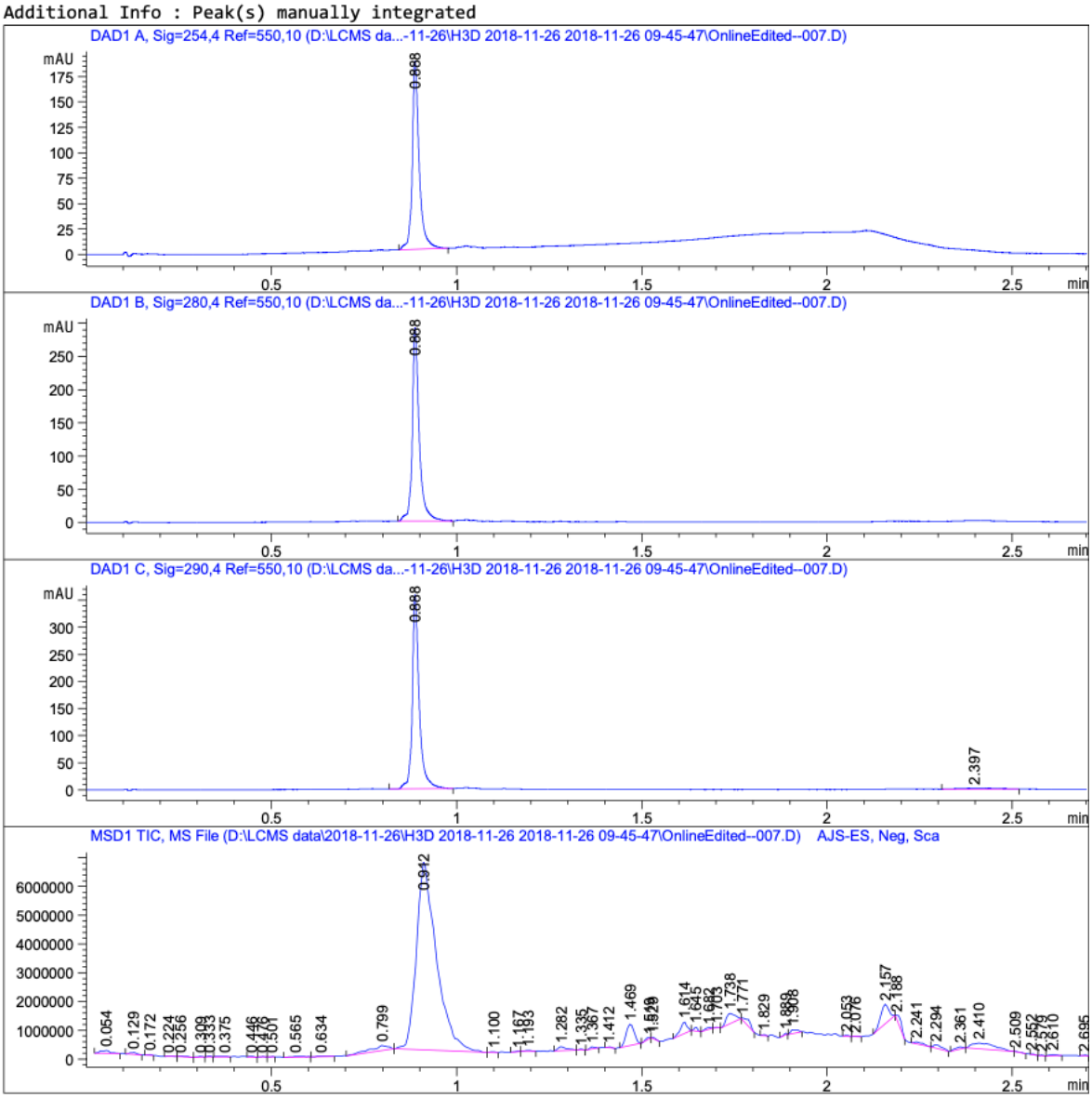

MS Spectrum
*MSD1 SPC, time=0.980 of D:\LCMS data\2019-02-01\H3D 2019-02-01 2019-02-01 15-06-50\1AB-0301.D AJS-ES, Neg, Scan, Fr
Max: 677824
501.1
502.1
503.1
100
80
60
40
20
0
300
400
500
600
700
800 m/z
ıero 2019-02-04 09:01:25 SYSTEM
Page 1 of

Compound 28

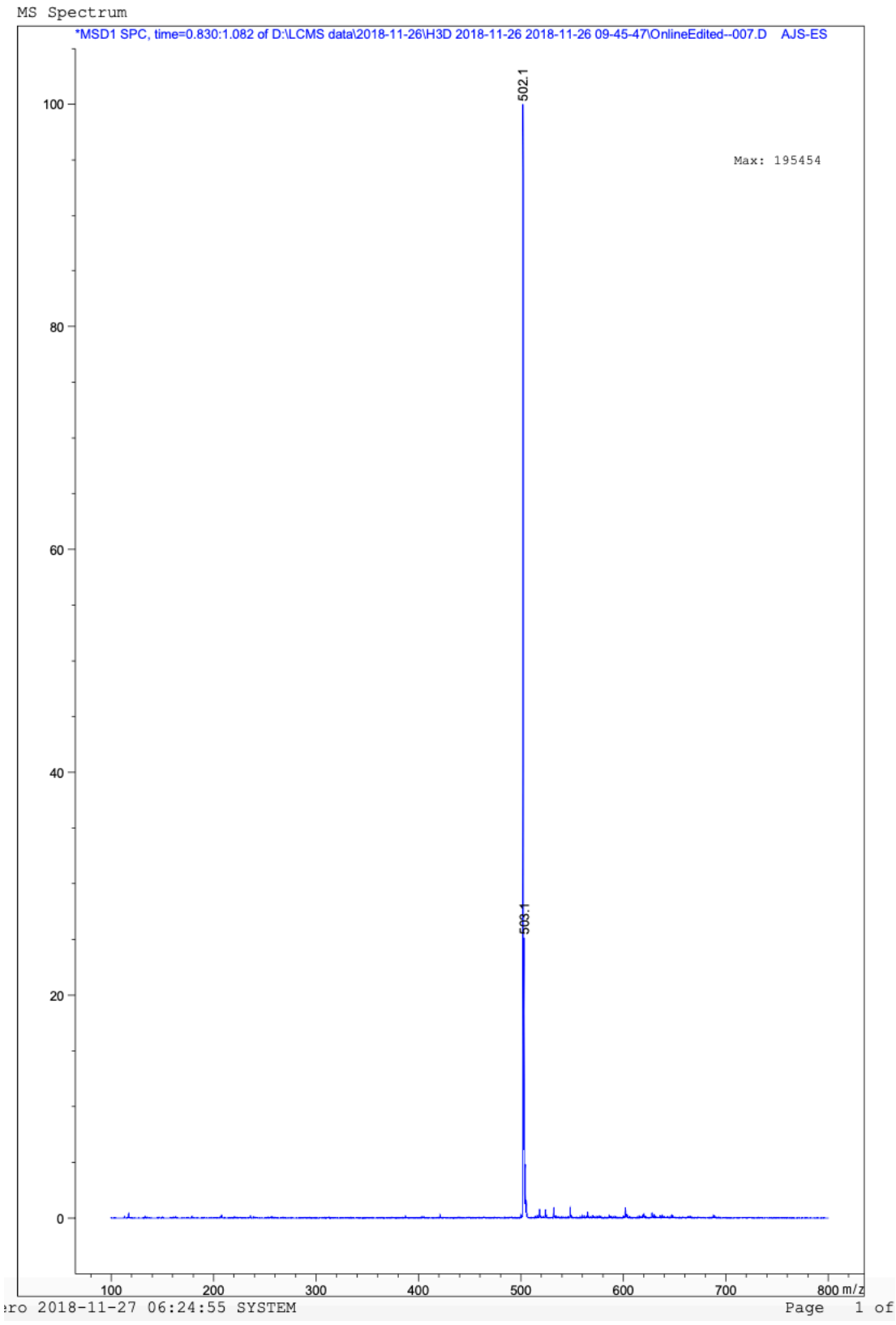

November
PG1-90FA-DMSO_1H
f1 (ppm)


Pete400
PG1-90A in D6-DMSO
19F Spectrum
Preshen
f1 (ppm)

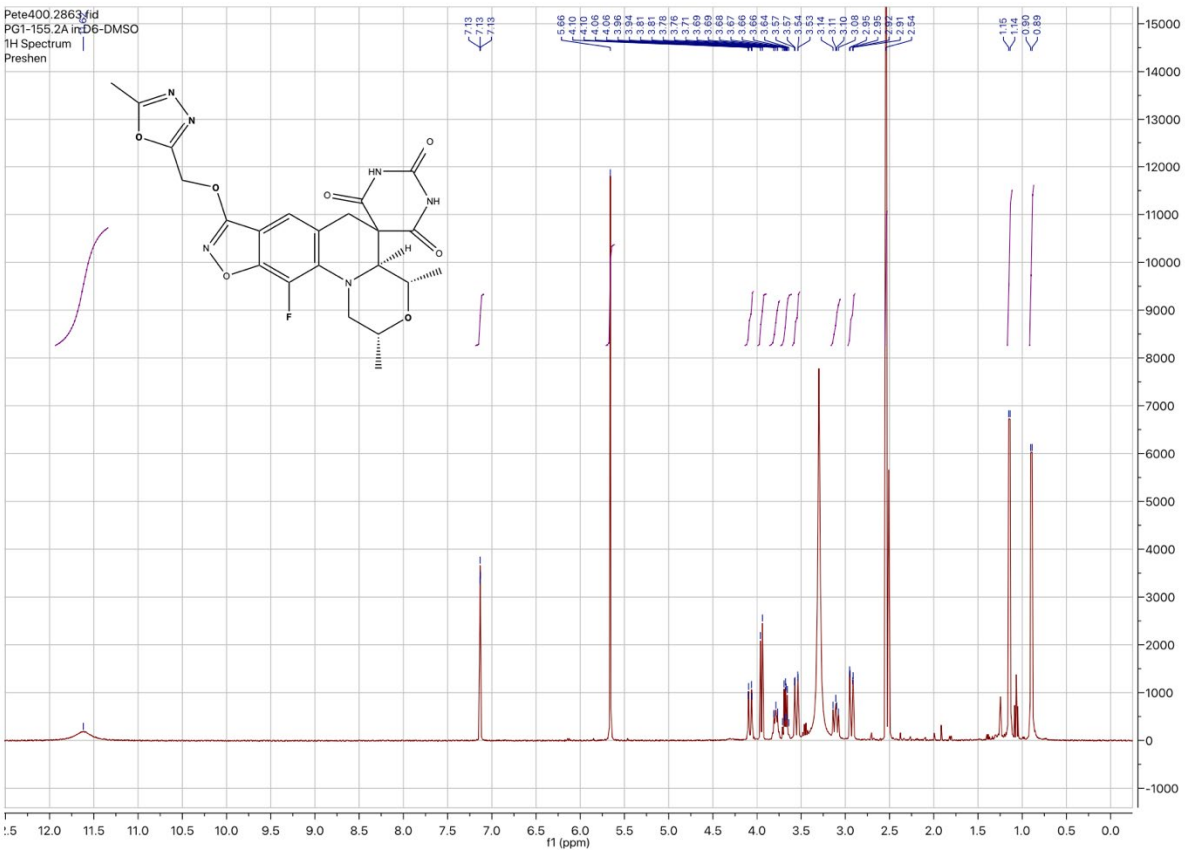
```
==================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   7
Acq. Instrument : Calimero                      Location :   P1-E1
Injection Date  : 2018-11-26 10:08:31               Inj :   1
                                               Inj Volume : 1.000 µl
Method          : D:\LCMS data\2018-11-26\H3D 2018-11-26 2018-11-26 09-45-47\NEW GENERAL NEG.
                  M (Sequence Method)
Last changed    : 2018-11-26 10:07:20 by SYSTEM
Method Info     : Standard 2.2min method with 254nm, 280nm and 290nm and positive ESI mode
                  100-800m/z

Additional Info : Peak(s) manually integrated
```

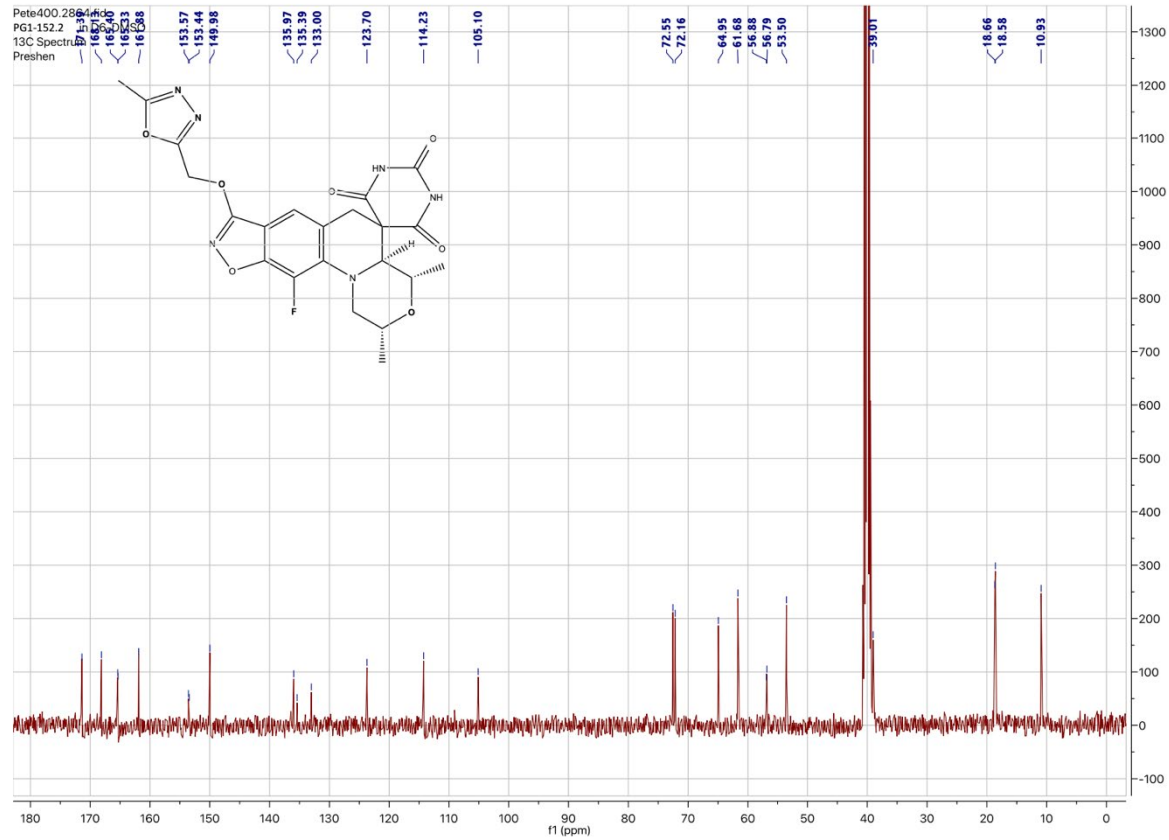


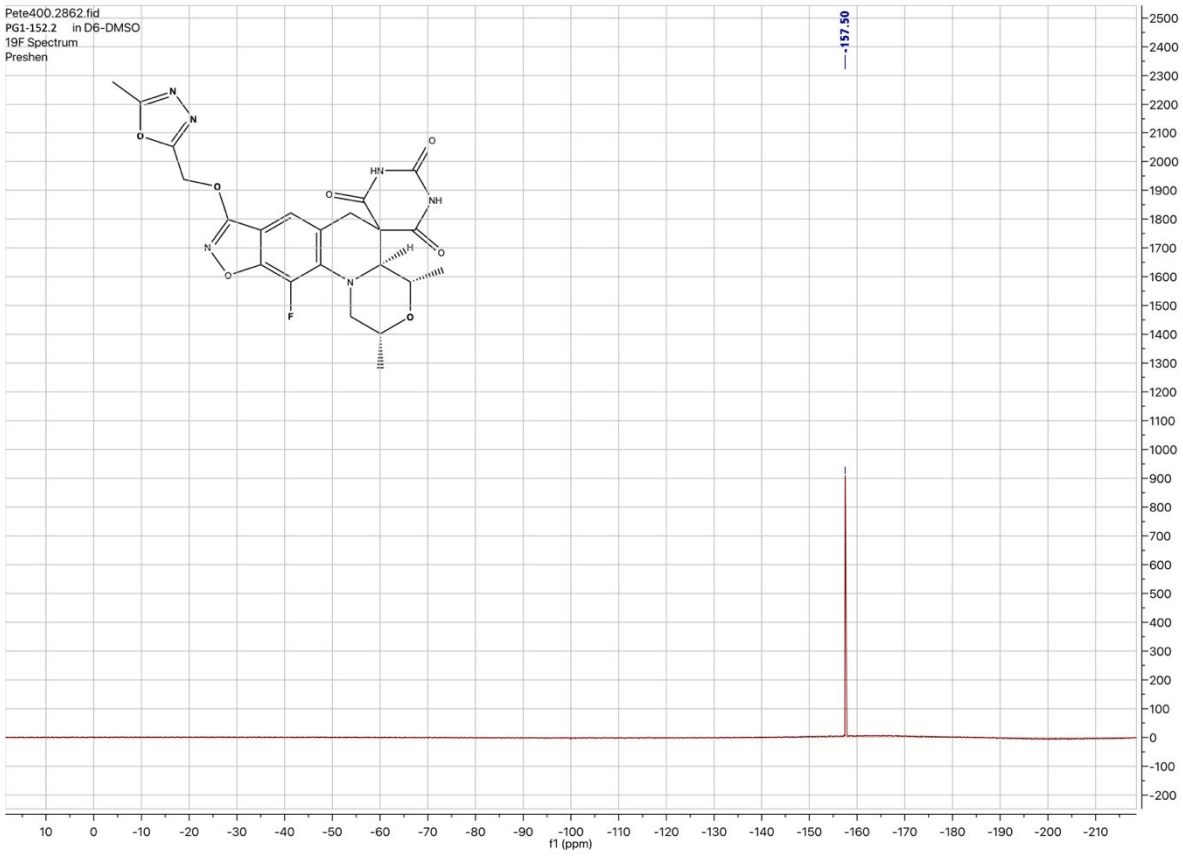
```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.888 BB    0.0187  240.88512  185.01770 100.0000

Totals :                   240.88512  185.01770



Signal 2: DAD1 B, Sig=280,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.888 BB    0.0187  378.45972  290.17822 100.0000

Totals :                   378.45972  290.17822



Signal 3: DAD1 C, Sig=290,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.888 BB    0.0187  466.76984  357.11227  97.2193
   2   2.397 BB    0.0820   13.35058    1.94685   2.7807

Totals :                   480.12041  359.05912
```


MS Spectrum
*MSD1 SPC, time=0.830:1.082 of D:\LCMS data\2018-11-26\H3D 2018-11-26 2018-11-26 09-45-47\OnlineEdited--007.D AJS-ES
502.1
Max: 195454
503.1
100
80
60
40
20
0
100
200
300
400
500
600
700
800 m/z
ro 2018-11-27 06:24:55 SYSTEM
Page 1 of

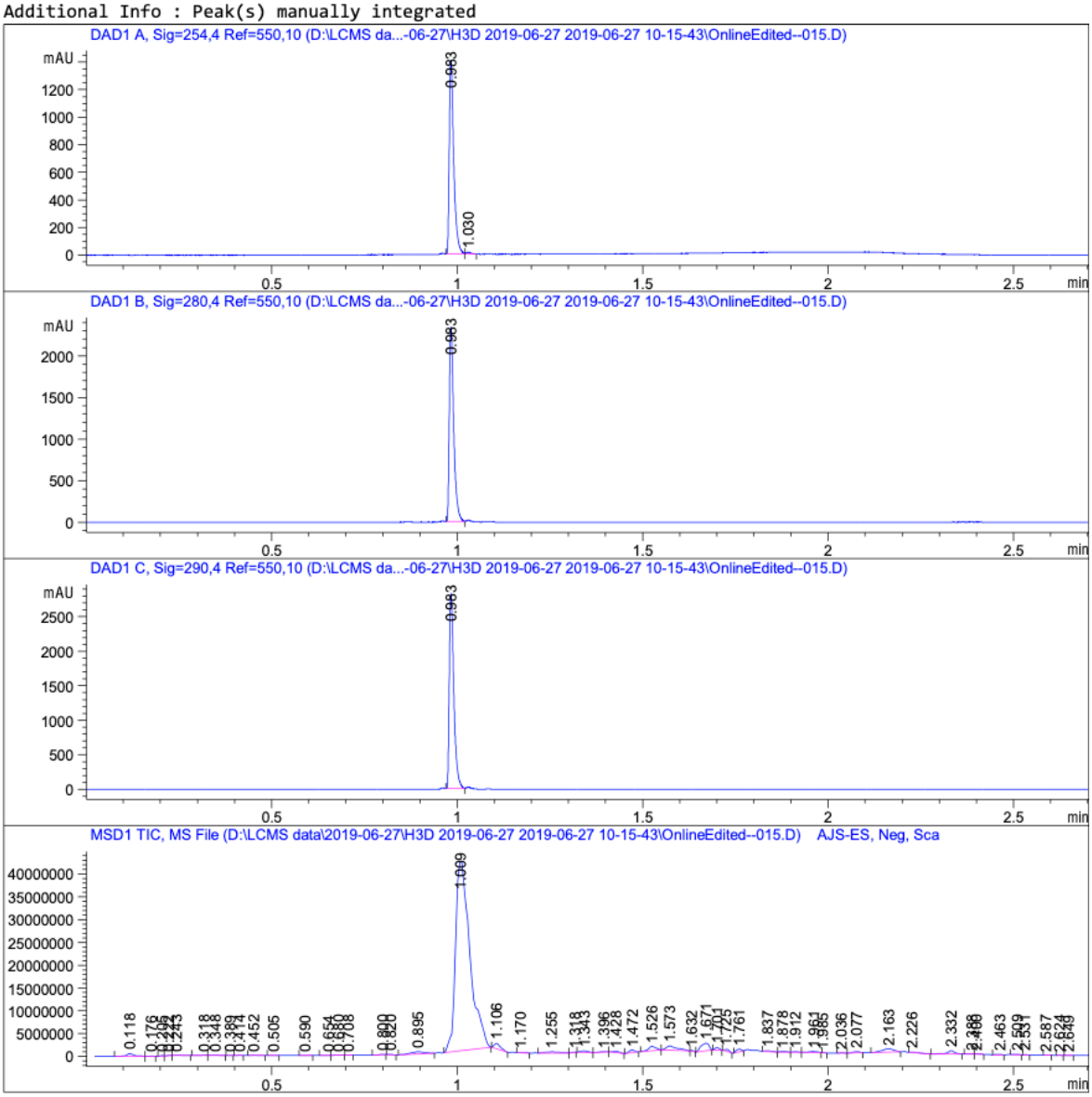
Compound 33


Pete400.2863.fid
PG1-155.2A in D6-DMSO
1H Spectrum
Preshen
f1 (ppm)

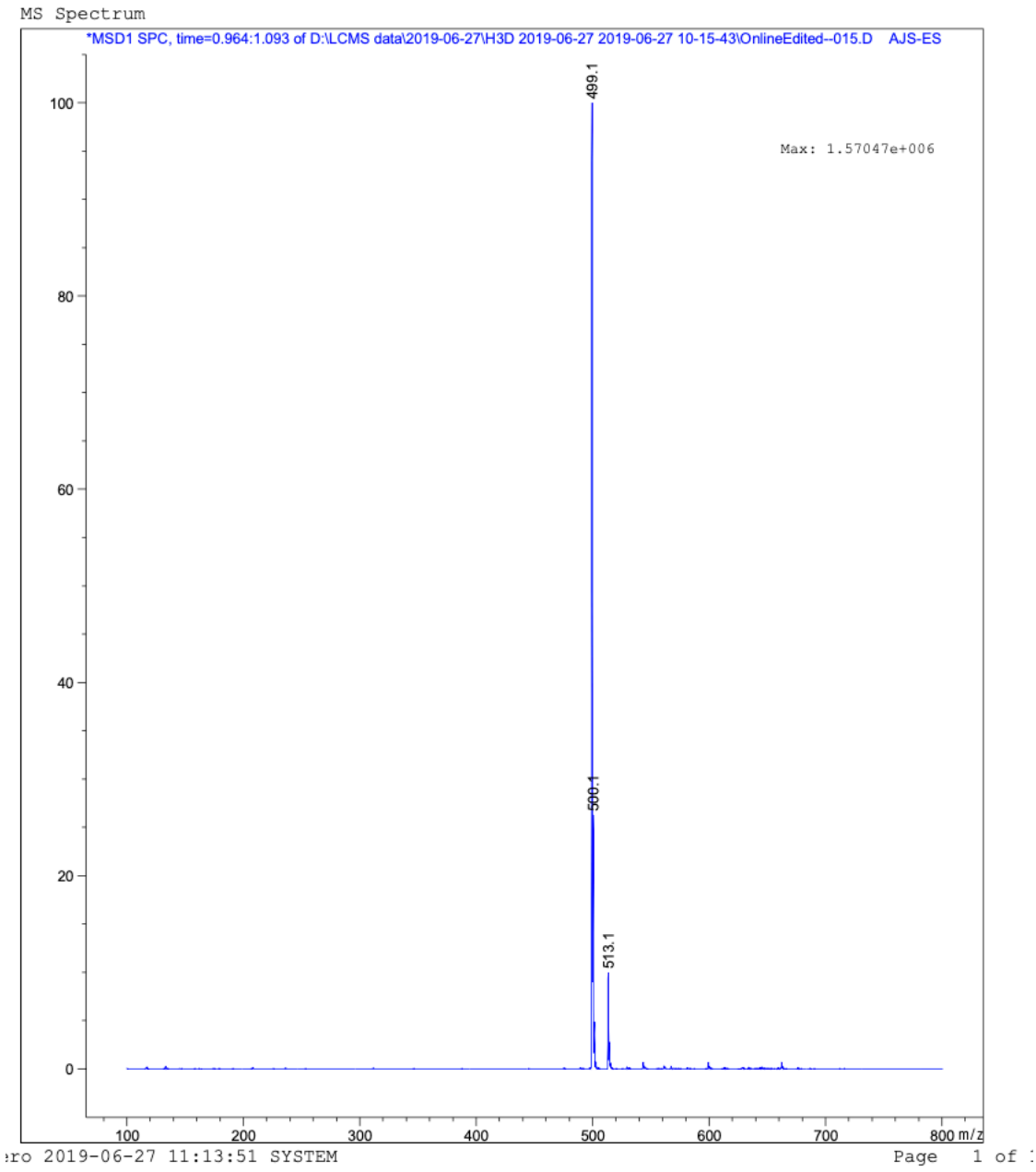

PG1-152.2
13C Spectrum
Preshen
f1 (ppm)


Pete400.2862.fid
PG1-152.2 in D6-DMSO
19F Spectrum
Preshen
-157.50
f1 (ppm)

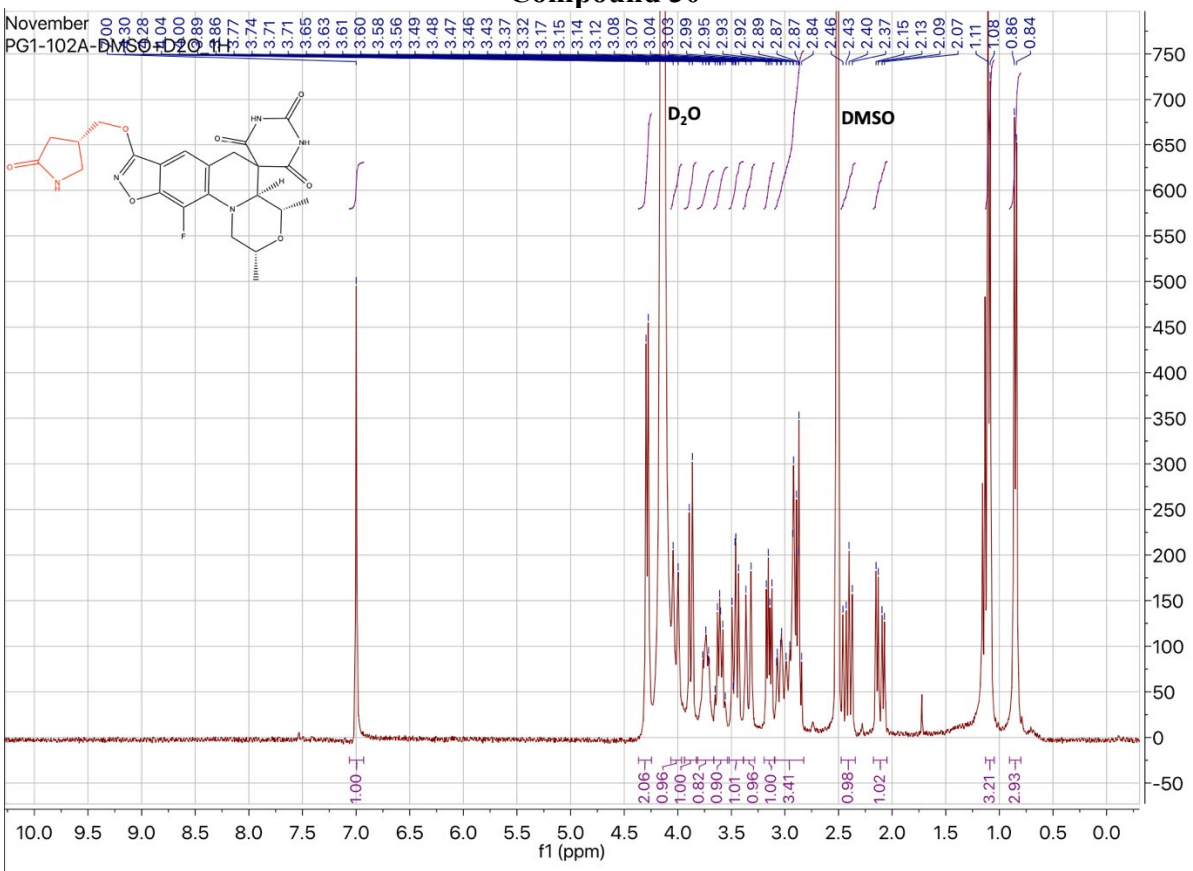
```
================================================================
Acq. Operator   : SYSTEM                        Seq. Line :  15
Acq. Instrument : Calimero                       Location :  P1-D1
Injection Date  : 2019-06-27 11:07:47                Inj :  1
                                                Inj Volume : 1.000 µl
Method          : D:\LCMS data\2019-06-27\H3D 2019-06-27 2019-06-27 10-15-43\NEW GENERAL NEG.
                  M (Sequence Method)
Last changed    : 2019-06-27 10:27:35 by SYSTEM
Method Info     : Standard 2.2min method with 254nm, 280nm and 290nm and positive ESI mode
                  100-800m/z

Additional Info : Peak(s) manually integrated
```

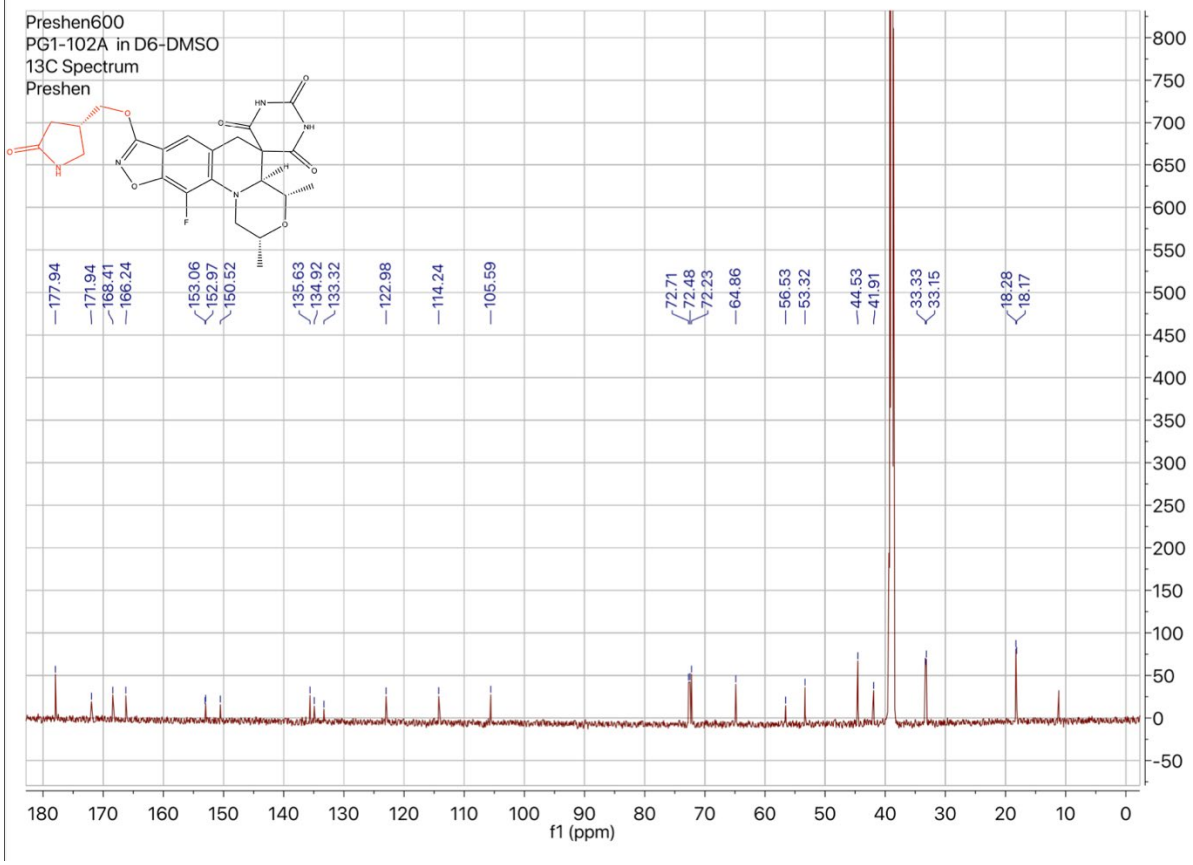


```
=====================================================================
                      Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.983 BB    0.0127 1166.26697 1403.49121  99.2968
   2   1.030 BB    0.0113    8.25875   11.57130   0.7032

Totals :                  1174.52572 1415.06251



Signal 2: DAD1 B, Sig=280,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.983 BB    0.0127 1932.95618 2336.63159 100.0000

Totals :                  1932.95618 2336.63159



Signal 3: DAD1 C, Sig=290,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.983 BB    0.0129 2403.87866 2819.24976 100.0000

Totals :                  2403.87866 2819.24976
```

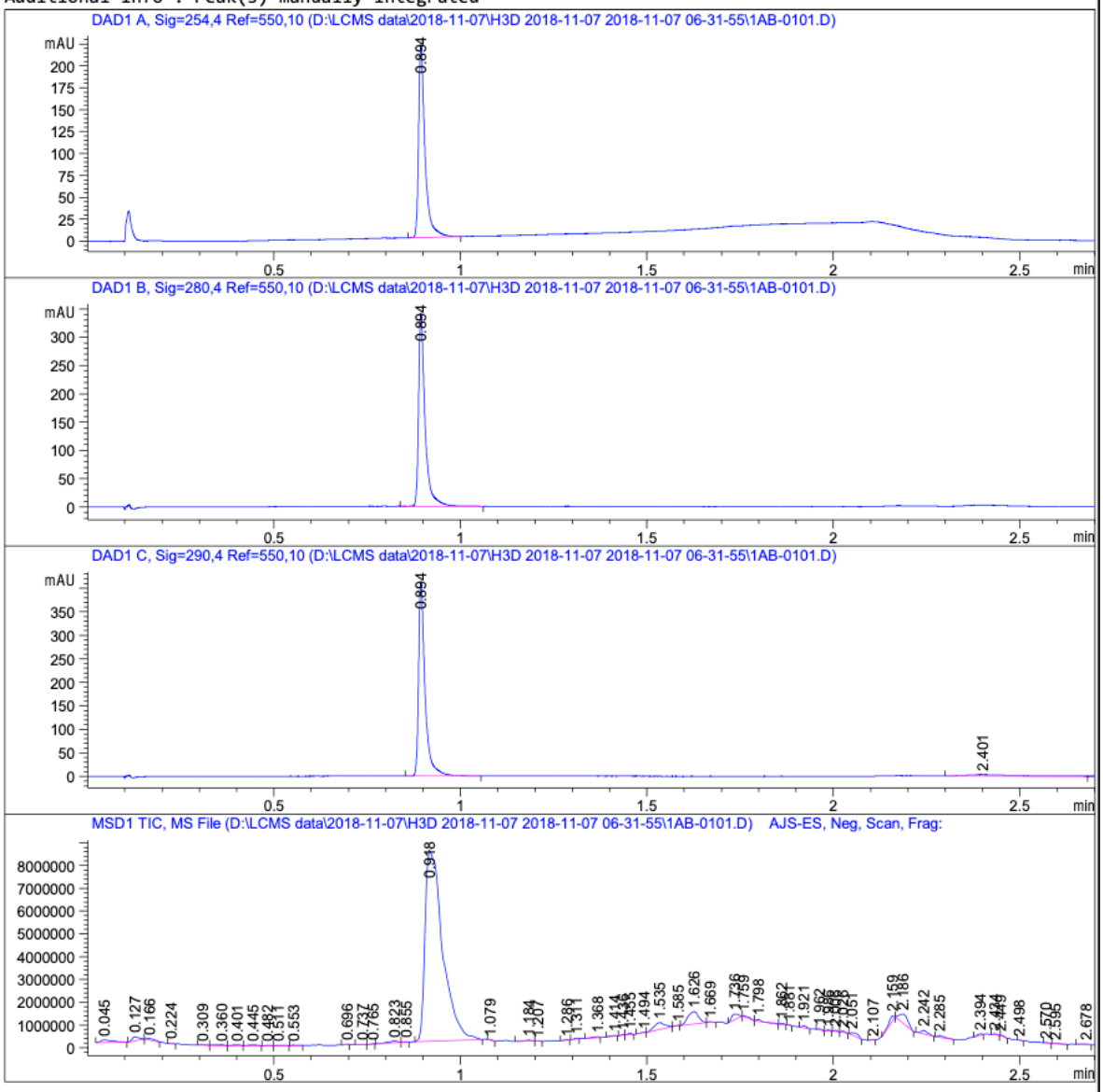

MS Spectrum
*MSD1 SPC, time=0.964:1.093 of D:\LCMS data\2019-06-27\H3D 2019-06-27 2019-06-27 10-15-43\OnlineEdited--015.D AJS-ES
Max: 1.57047e+006
499.1
500.1
513.1
100
80
60
40
20
0
100
200
300
400
500
600
700
800 m/z
ero 2019-06-27 11:13:51 SYSTEM
Page 1 of 1

Compound 30

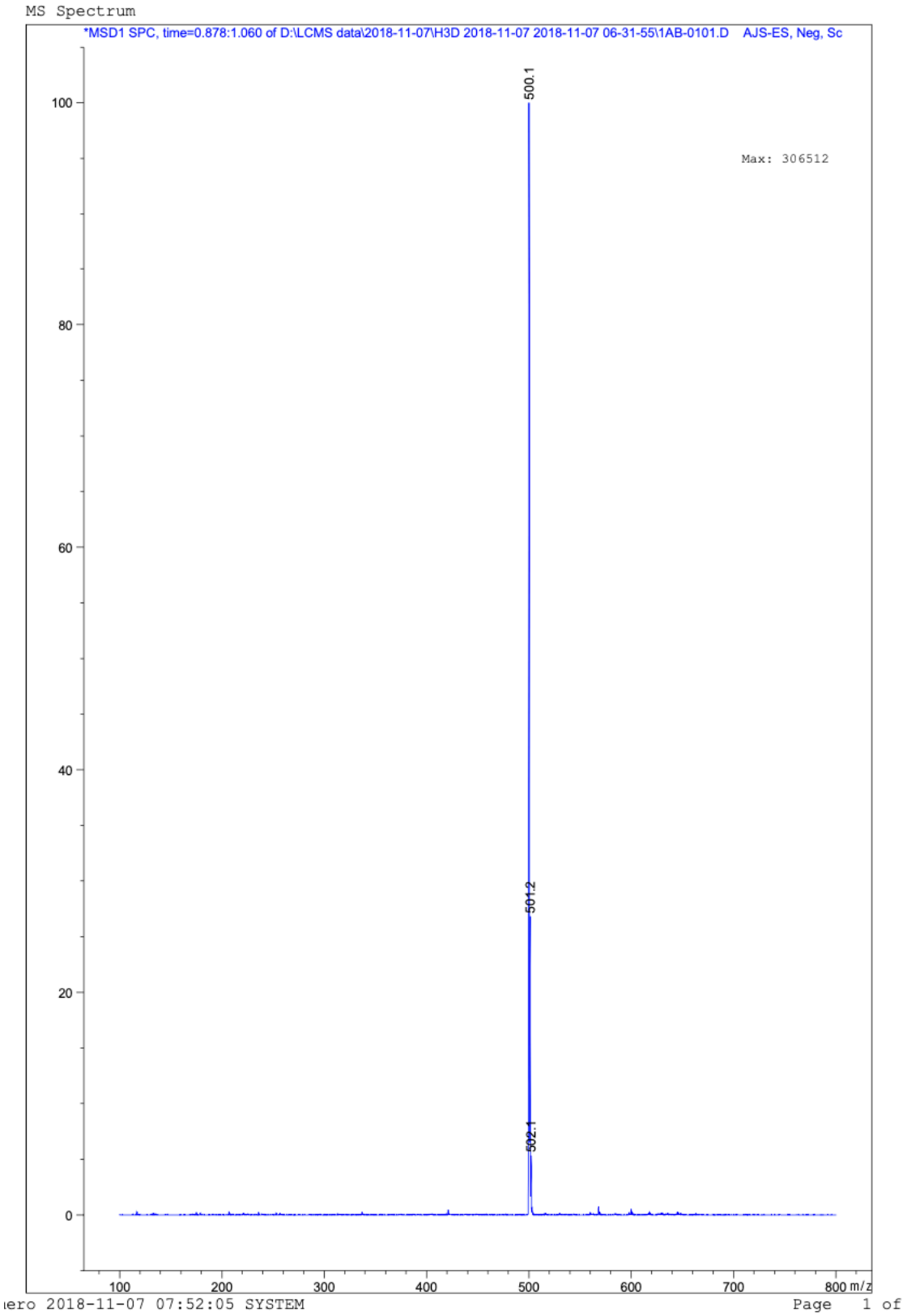

November
PG1-102A-DMSO+D2O 1H
D2O
DMSO
f1 (ppm)


Preshen600
PG1-102A in D6-DMSO
13C Spectrum
Preshen
177.94
171.94
168.41
166.24
153.06
152.97
150.52
135.63
134.92
133.32
122.98
114.24
105.59
72.71
72.48
72.23
64.86
56.53
53.32
44.53
41.91
33.33
33.15
18.28
18.17
f1 (ppm)

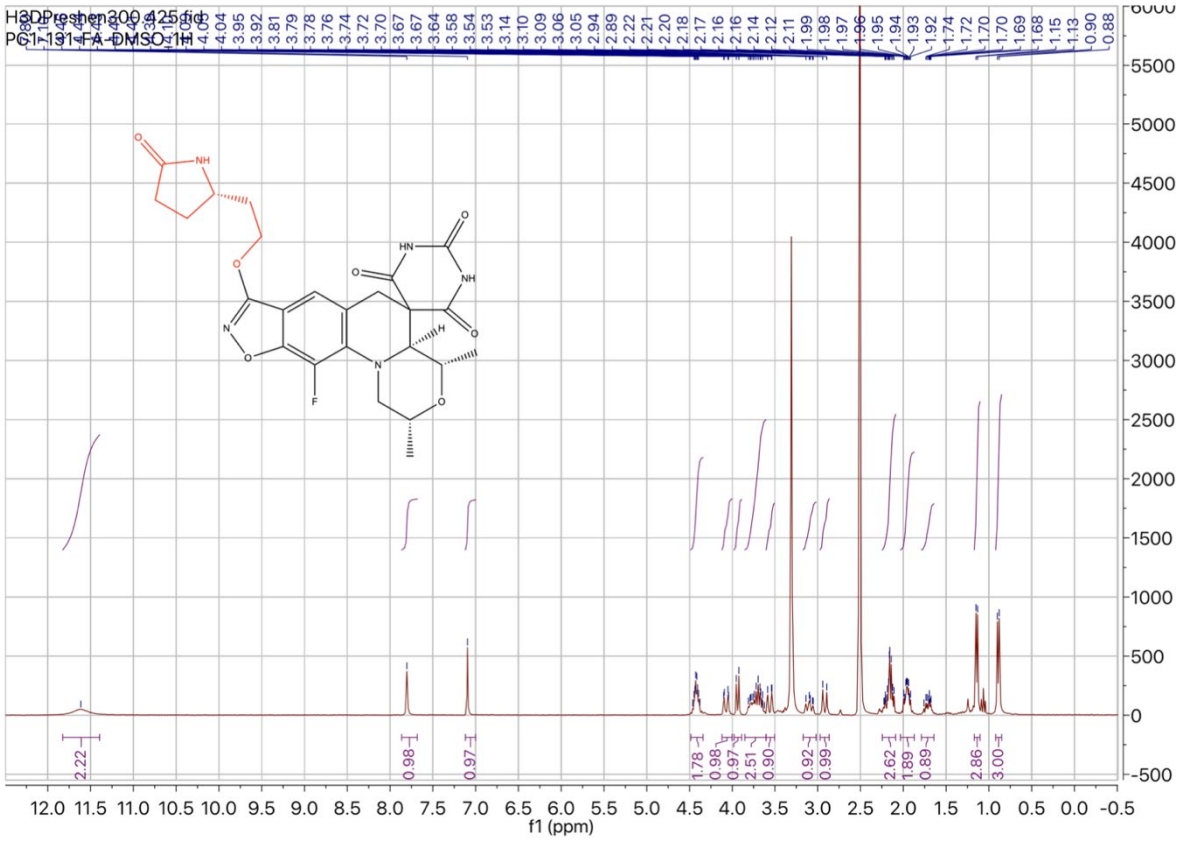
```
======================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   1
Acq. Instrument : Calimero                      Location :   P1-A2
Injection Date  : 2018-11-07 06:33:04               Inj :   1
                                               Inj Volume : 1.000 µl
Method          : D:\LCMS data\2018-11-07\H3D 2018-11-07 2018-11-07 06-31-55\NEW GENERAL NEG.
                  M (Sequence Method)
Last changed    : 2018-11-07 06:31:55 by SYSTEM
Method Info     : Standard 2.2min method with 254nm, 280nm and 290nm and positive ESI mode
                  100-800m/z

Additional Info : Peak(s) manually integrated
```



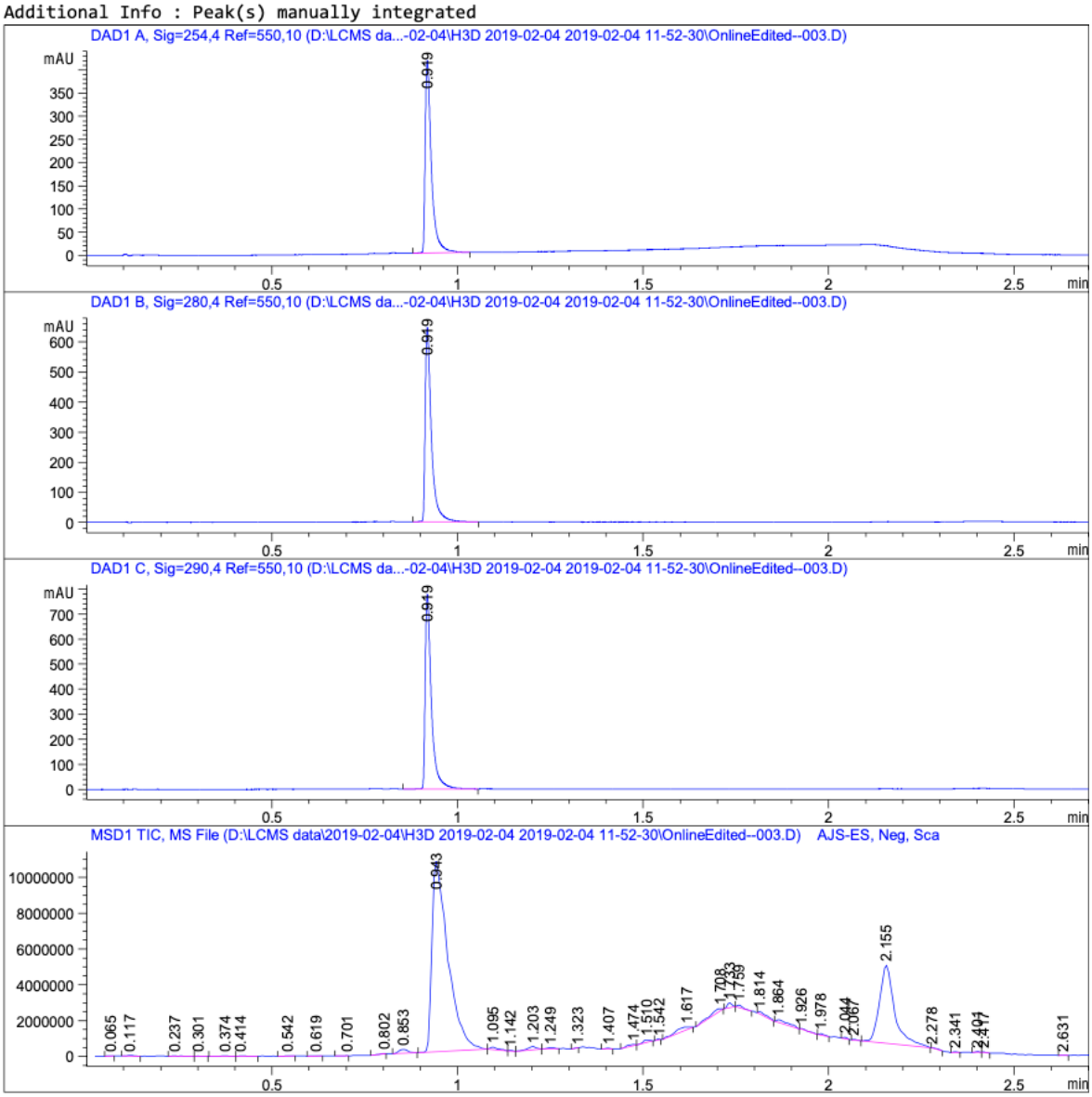
```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.894 BB    0.0181  264.79385  218.42566 100.0000

Totals :                   264.79385  218.42566



Signal 2: DAD1 B, Sig=280,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.894 BB    0.0182  413.66232  339.58585 100.0000

Totals :                   413.66232  339.58585



Signal 3: DAD1 C, Sig=290,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.894 BB    0.0182  500.08047  411.54272  95.3150
   2   2.401 BB    0.1075   24.58017    2.73217   4.6850

Totals :                   524.66064  414.27490
```


MS Spectrum
*MSD1 SPC, time=0.878:1.060 of D:\LCMS data\2018-11-07\H3D 2018-11-07 2018-11-07 06-31-55\1AB-0101.D AJS-ES, Neg, Sc
Max: 306512
500.1
501.2
502.1
100
80
60
40
20
0
100
200
300
400
500
600
700
800 m/z
ıero 2018-11-07 07:52:05 SYSTEM
Page 1 of

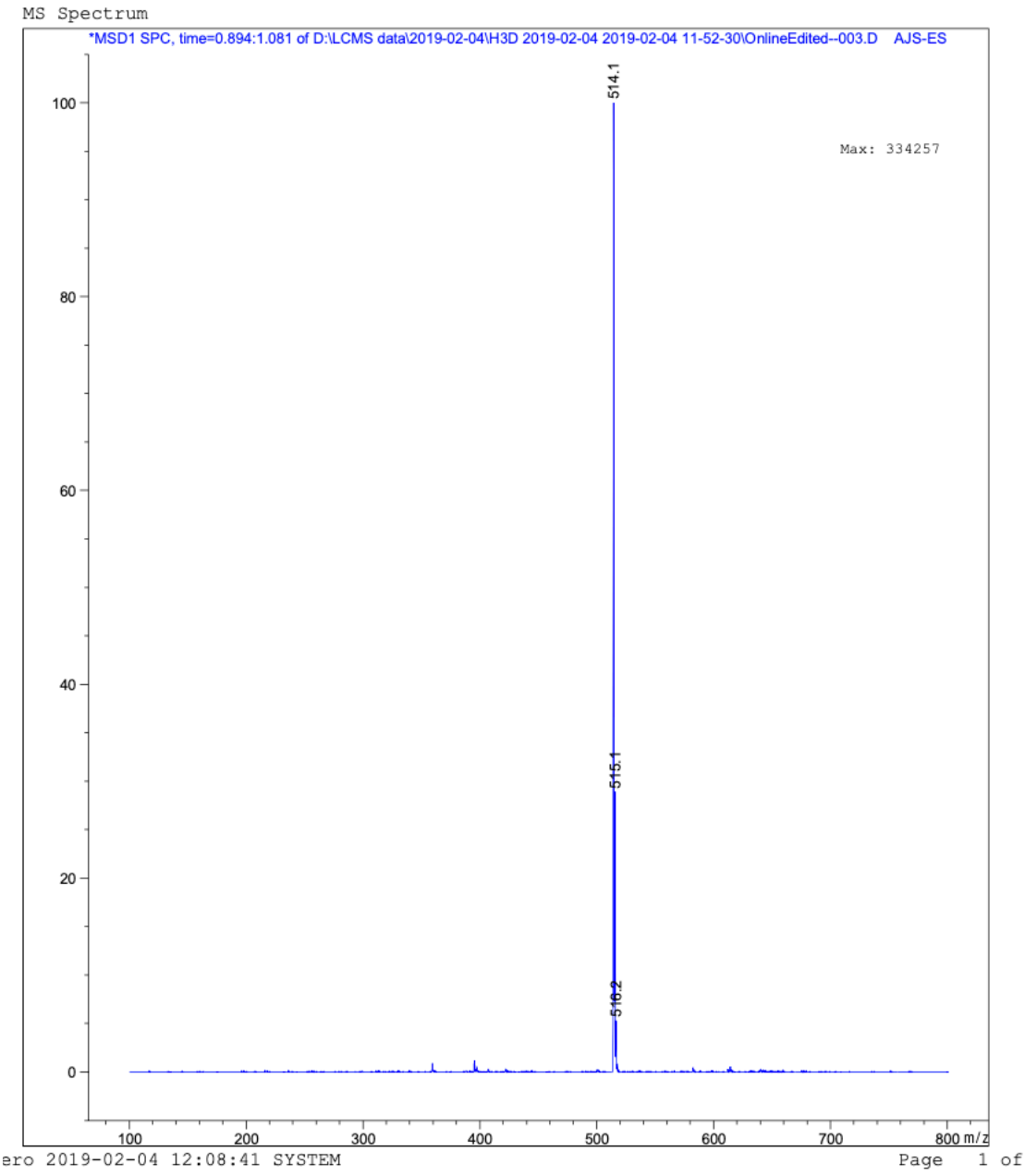
Compound 32


H3DPresher209425.fid
PG1-131-FA-DMSO-1H
f1 (ppm)

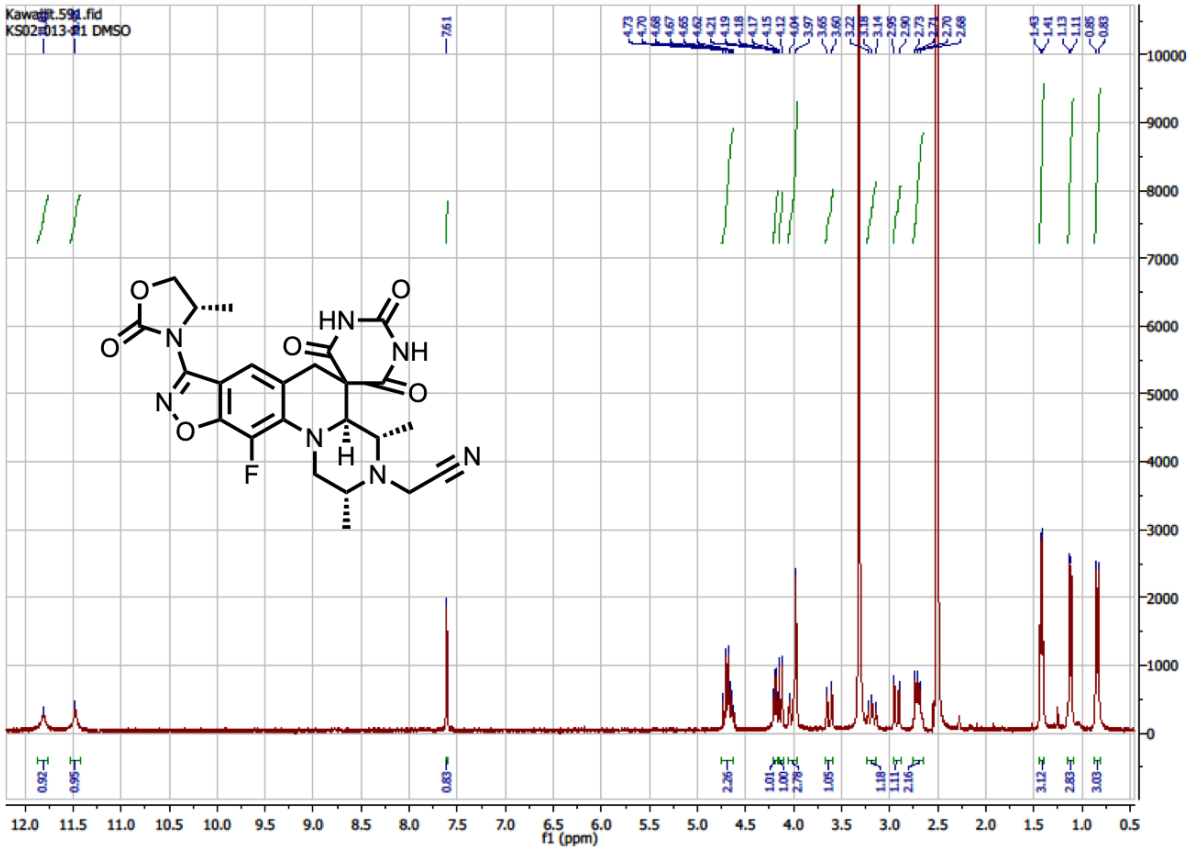
```
======================================================================
Acq. Operator   : SYSTEM                          Seq. Line :   3
Acq. Instrument : Calimero                        Location :   P1-E1
Injection Date  : 2019-02-04 12:00:52                 Inj :   1
                                                 Inj Volume : 1.000 µl
Method          : D:\LCMS data\2019-02-04\H3D 2019-02-04 2019-02-04 11-52-30\NEW GENERAL NEG.
                  M (Sequence Method)
Last changed    : 2019-02-04 11:53:07 by SYSTEM
Method Info     : Standard 2.2min method with 254nm, 280nm and 290nm and positive ESI mode
                  100-800m/z

Additional Info : Peak(s) manually integrated
```



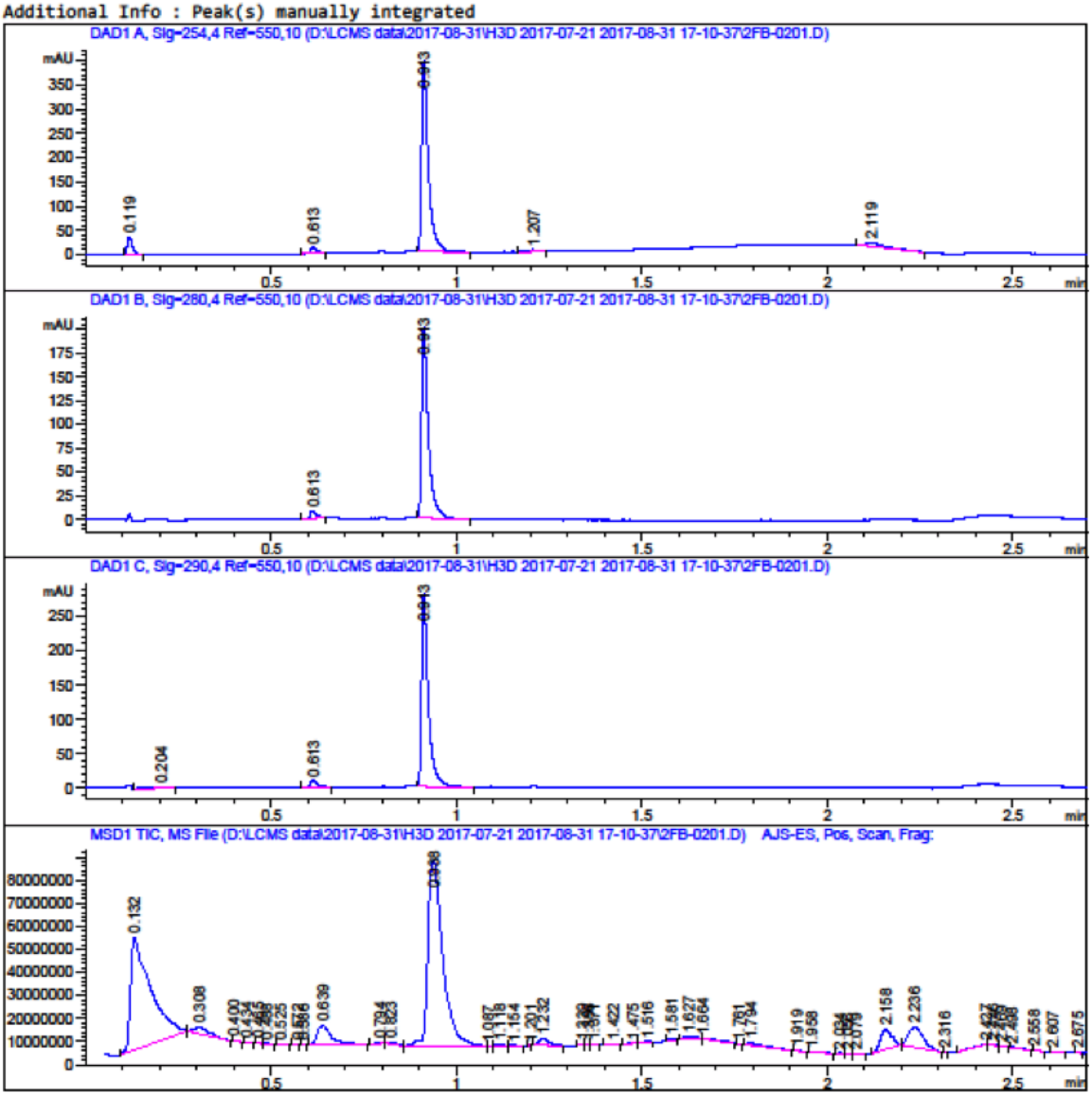
```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.919 BB    0.0178  488.17245  412.54709 100.0000

Totals :                   488.17245  412.54709



Signal 2: DAD1 B, Sig=280,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.919 BB    0.0177  757.15967  642.51489 100.0000

Totals :                   757.15967  642.51489



Signal 3: DAD1 C, Sig=290,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.919 BB    0.0177  909.96307  772.32440 100.0000

Totals :                   909.96307  772.32440
```

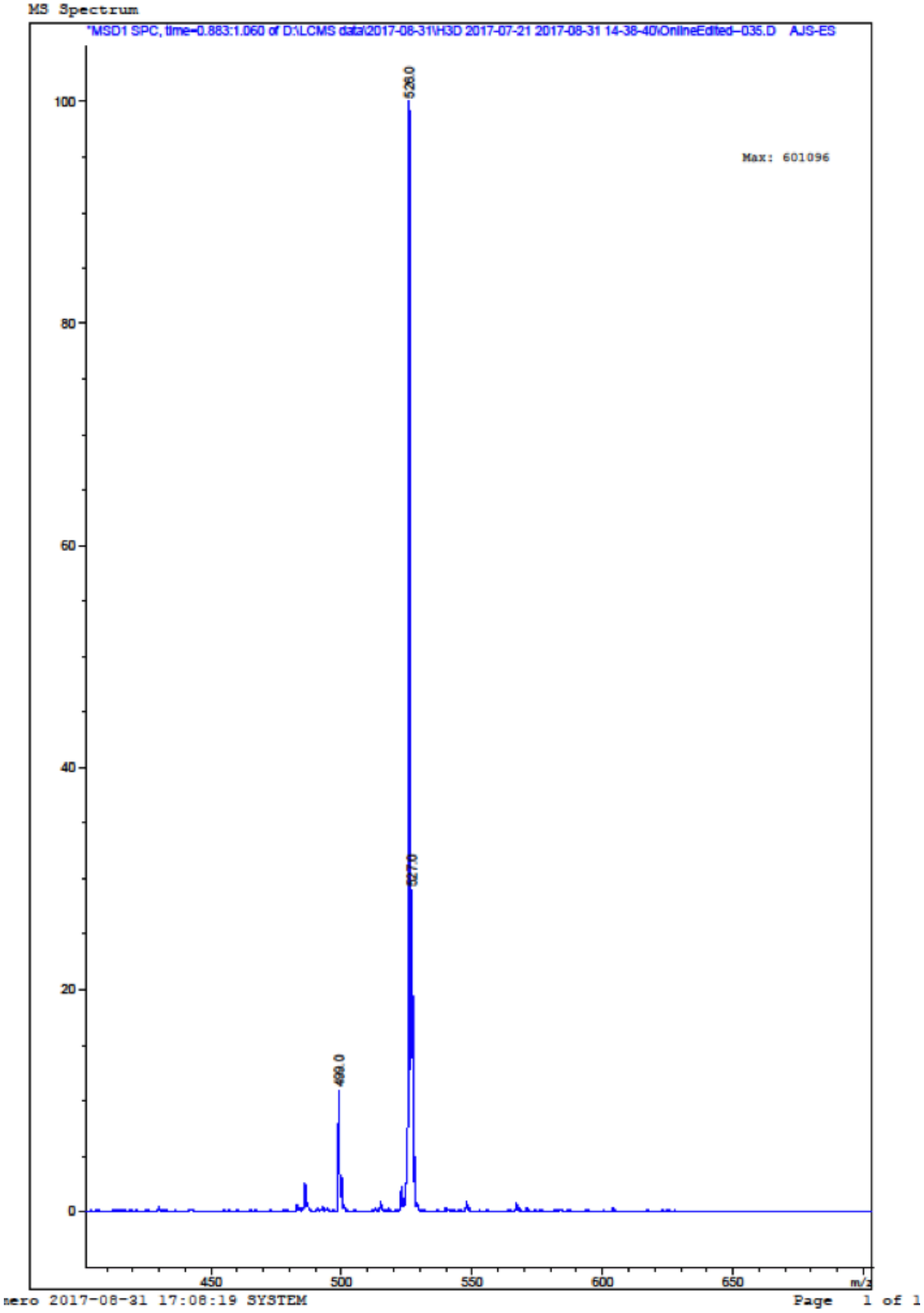

MS Spectrum
*MSD1 SPC, time=0.894:1.081 of D:\LCMS data\2019-02-04\H3D 2019-02-04 2019-02-04 11-52-30\OnlineEdited--003.D AJS-ES
Max: 334257
514.1
515.1
516.2
100
80
60
40
20
0
100
200
300
400
500
600
700
800 m/z
ero 2019-02-04 12:08:41 SYSTEM
Page 1 of

Compound 37

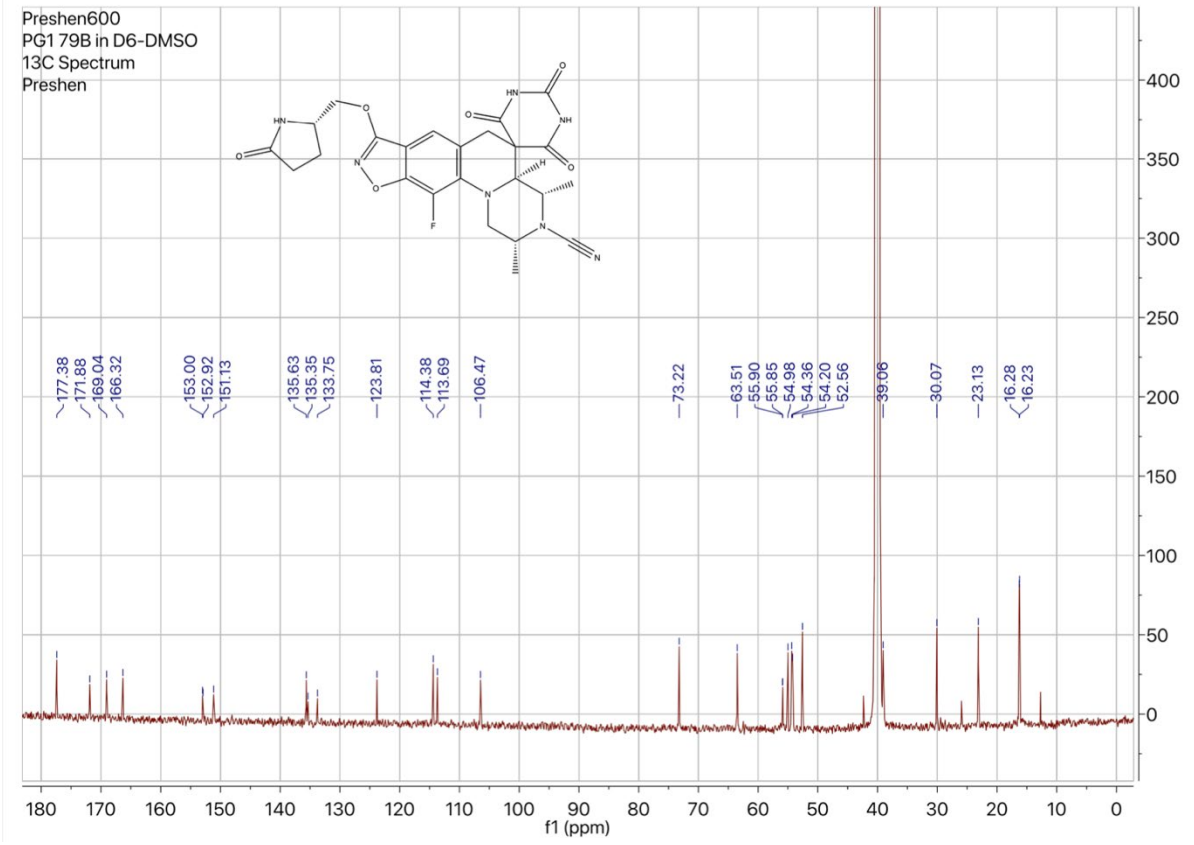


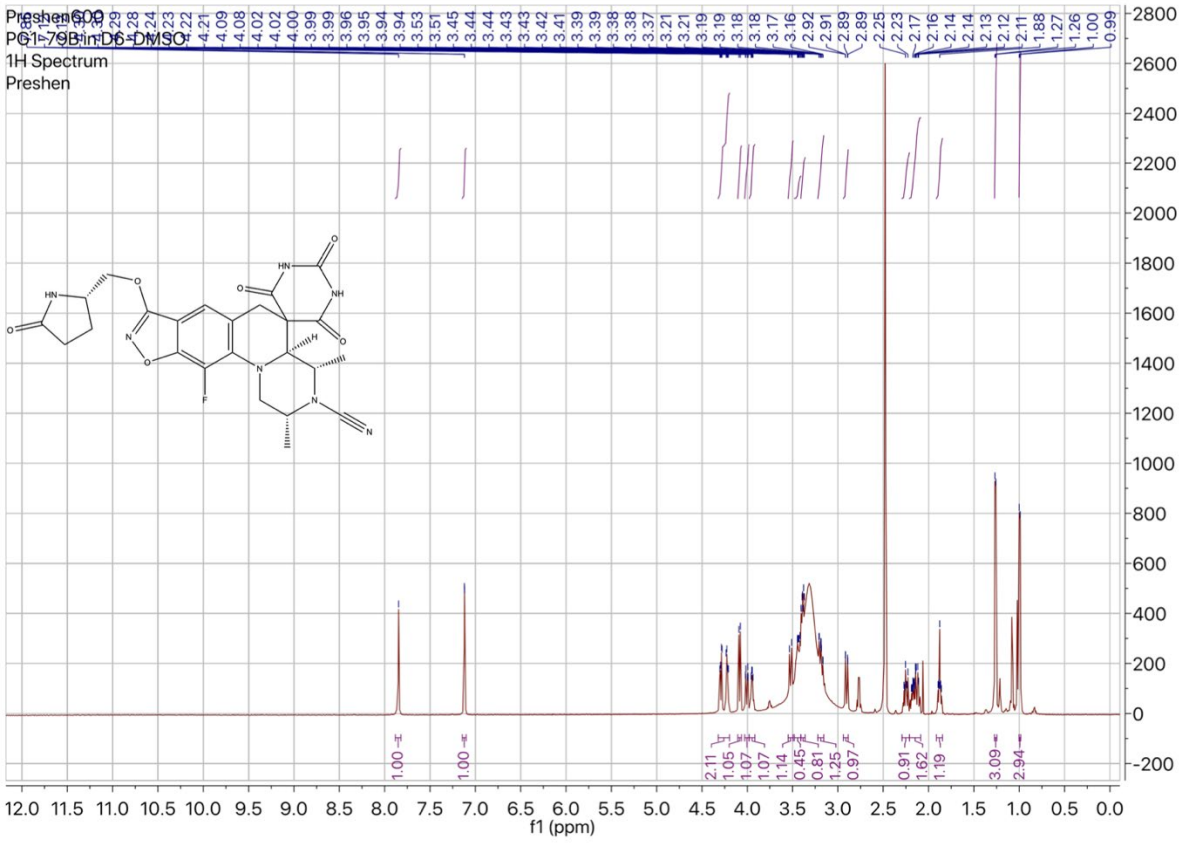
```
================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   2
Acq. Instrument : Calimero                       Location :   P2-F2
Injection Date  : 2017-08-31 17:15:29                Inj :   1
                                               Inj Volume : 1.000 µl
Method          : D:\LCMS data\2017-08-31\H3D 2017-07-21 2017-08-31 17-10-37\NEW GENERAL POS.
                  M (Sequence Method)
Last changed    : 2017-08-31 17:10:37 by SYSTEM
Method Info     : Standard 2.2min method with 254nm, 280nm and 290nm and positive ESI mode
                  100-800m/z

Additional Info : Peak(s) manually integrated
```



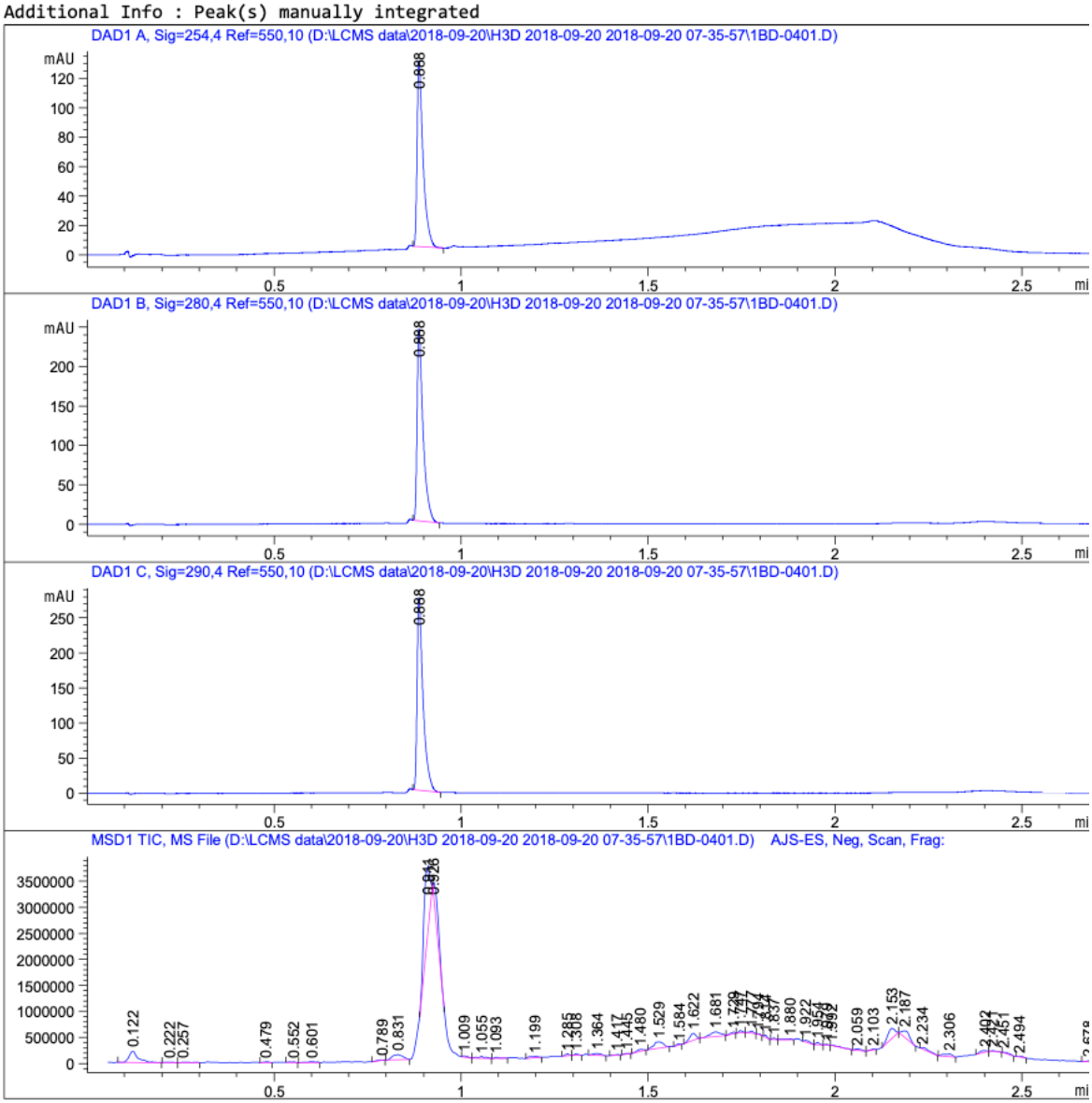

MS Spectrum
*MSD1 SPC, time=0.883:1.060 of D:\LCMS data\2017-08-31\H3D 2017-07-21 2017-08-31 14-38-40\OnlineEdited-035.D AJS-ES
Max: 601096
526.0
527.0
499.0
100
80
60
40
20
0
450
500
550
600
650
m/z
nero 2017-08-31 17:08:19 SYSTEM
Page 1 of 1

Compound 42

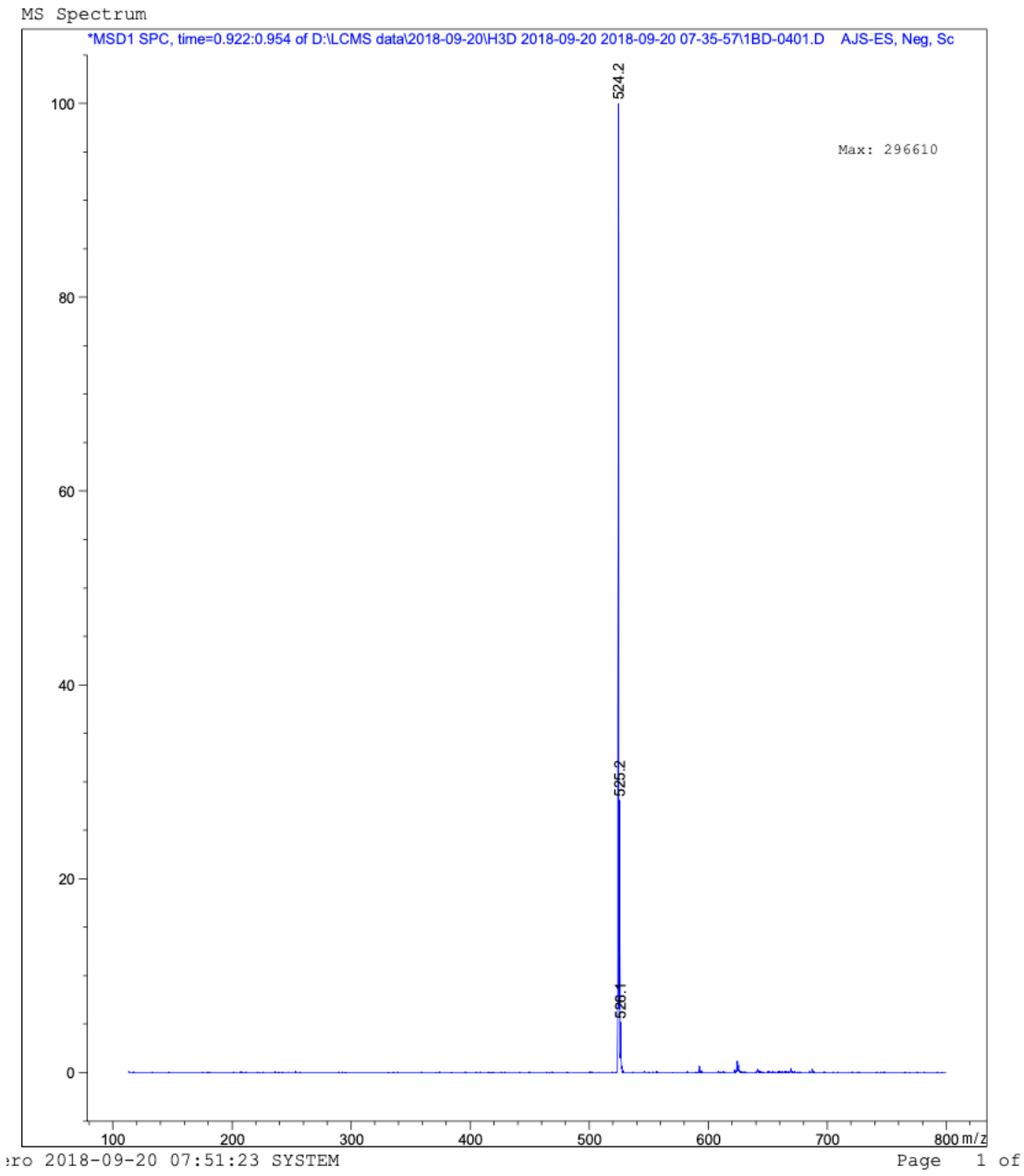

Preshen600
PG1 79B in D6-DMSO
13C Spectrum
Preshen
177.38
171.88
169.04
166.32
153.00
152.92
151.13
135.63
135.35
133.75
123.81
114.38
113.69
106.47
73.22
63.51
55.90
55.85
54.98
54.36
54.20
52.56
39.06
30.07
23.13
16.28
16.23
f1 (ppm)


Preshen600
PG1-79B in D6-DMSO
1H Spectrum
Preshen
1.00
1.00
2.11
1.05
1.07
1.07
1.14
0.45
0.81
1.25
0.97
0.91
1.62
1.19
3.09
2.94
f1 (ppm)

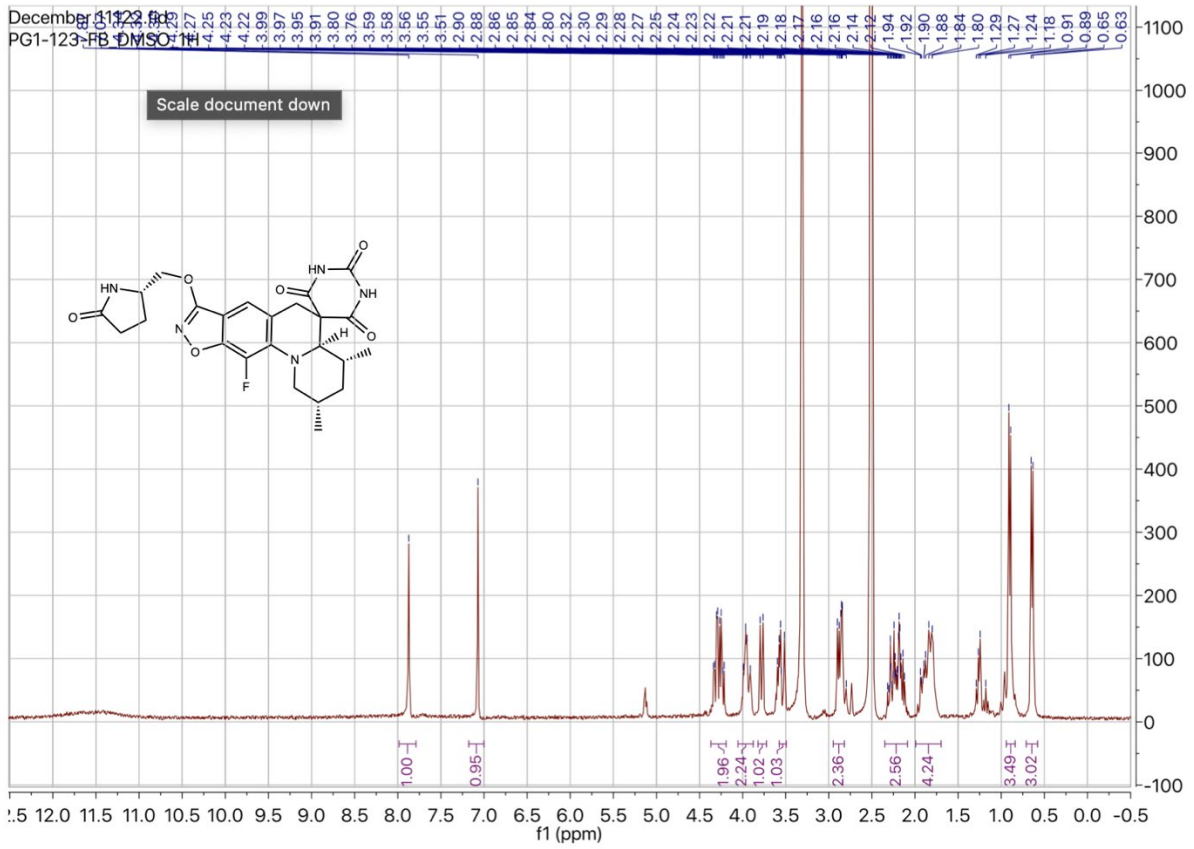
```
==================================================================
Acq. Operator   : SYSTEM                          Seq. Line :   4
Acq. Instrument : Calimero                        Location :   P1-B4
Injection Date  : 2018-09-20 07:47:42                 Inj :   1
                                                Inj Volume : 1.000 µl
Method          : D:\LCMS data\2018-09-20\H3D 2018-09-20 2018-09-20 07-35-57\NEW GENERAL NEG.
                  M (Sequence Method)
Last changed    : 2018-09-20 07:35:57 by SYSTEM
Method Info     : Standard 2.2min method with 254nm, 280nm and 290nm and positive ESI mode
                  100-800m/z

Additional Info : Peak(s) manually integrated
```

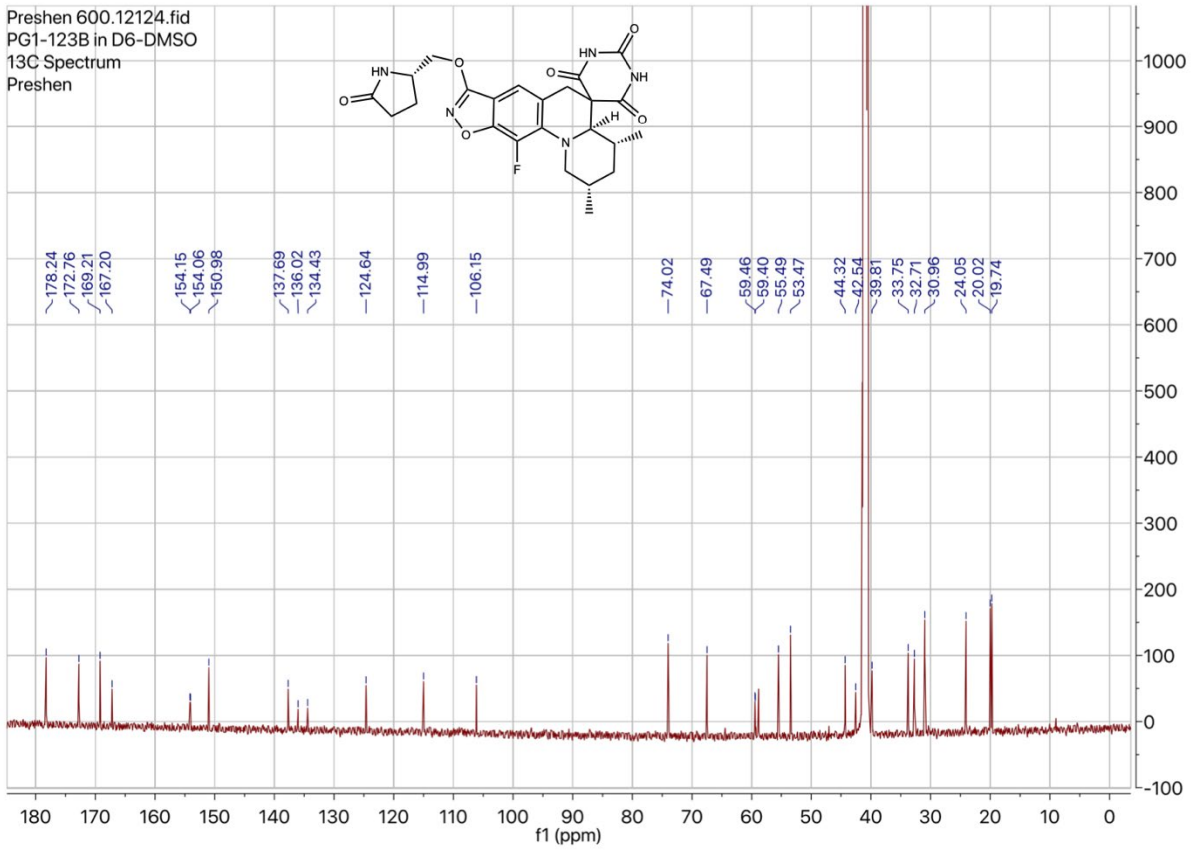


```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.888 BB    0.0164  139.09093  126.17318 100.0000

Totals :                   139.09093  126.17318



Signal 2: DAD1 B, Sig=280,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.888 BB    0.0163  266.93304  242.72917 100.0000

Totals :                   266.93304  242.72917



Signal 3: DAD1 C, Sig=290,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.888 BB    0.0163  301.55054  273.87531 100.0000

Totals :                   301.55054  273.87531
```

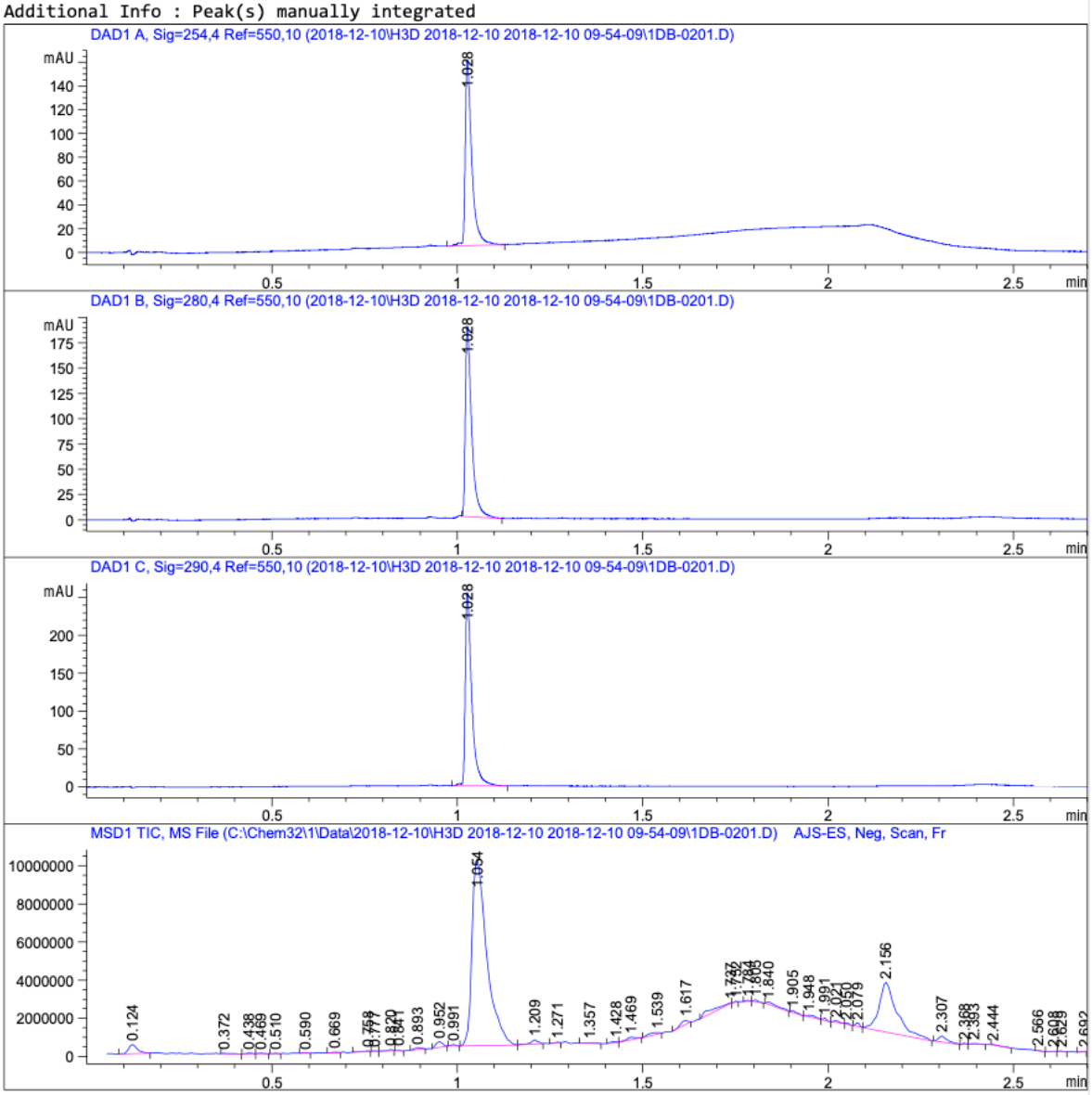

MS Spectrum
*MSD1 SPC, time=0.922:0.954 of D:\LCMS data\2018-09-20\H3D 2018-09-20 2018-09-20 07-35-57\1BD-0401.D AJS-ES, Neg, Sc
Max: 296610
524.2
525.2
526.1
100
80
60
40
20
0
100
200
300
400
500
600
700
800 m/z
ero 2018-09-20 07:51:23 SYSTEM
Page 1 of

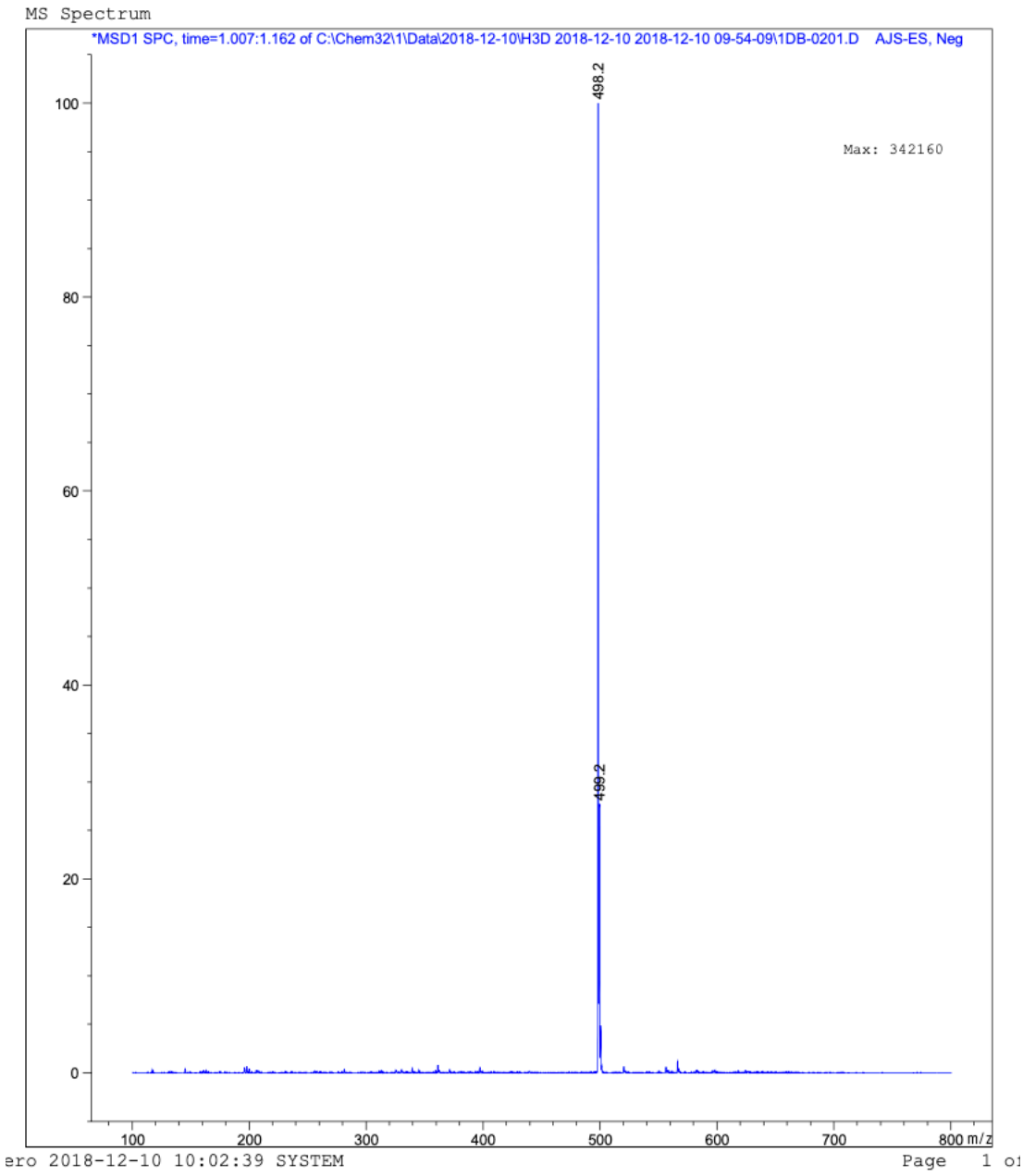
Compound 43


December 1122.fid
PG1-123-FB DMSO 1H
Scale document down
f1 (ppm)

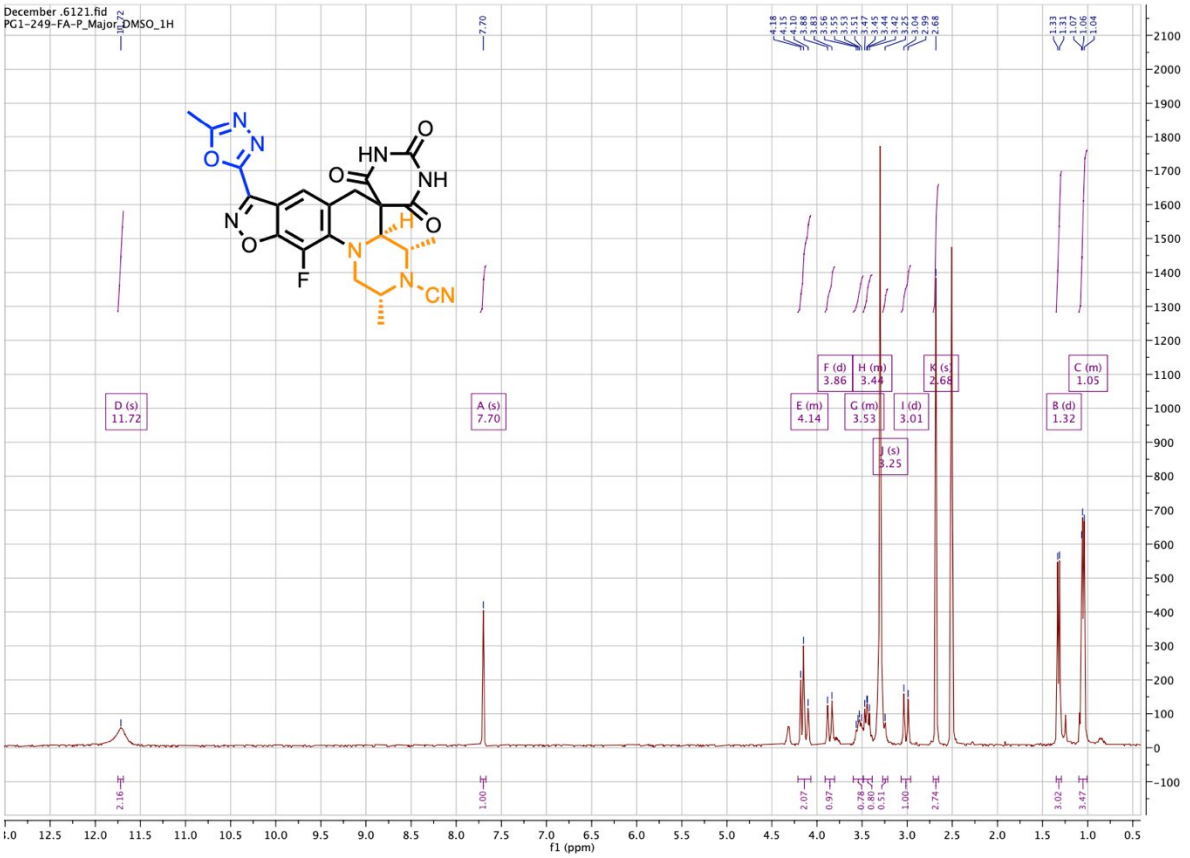

Preshen 600.12124.fid
PG1-123B in D6-DMSO
13C Spectrum
Preshen
178.24
172.76
169.21
167.20
154.15
154.06
150.98
137.69
136.02
134.43
124.64
114.99
106.15
74.02
67.49
59.46
59.40
55.49
53.47
44.32
42.54
39.81
33.75
32.71
30.96
24.05
20.02
19.74
f1 (ppm)

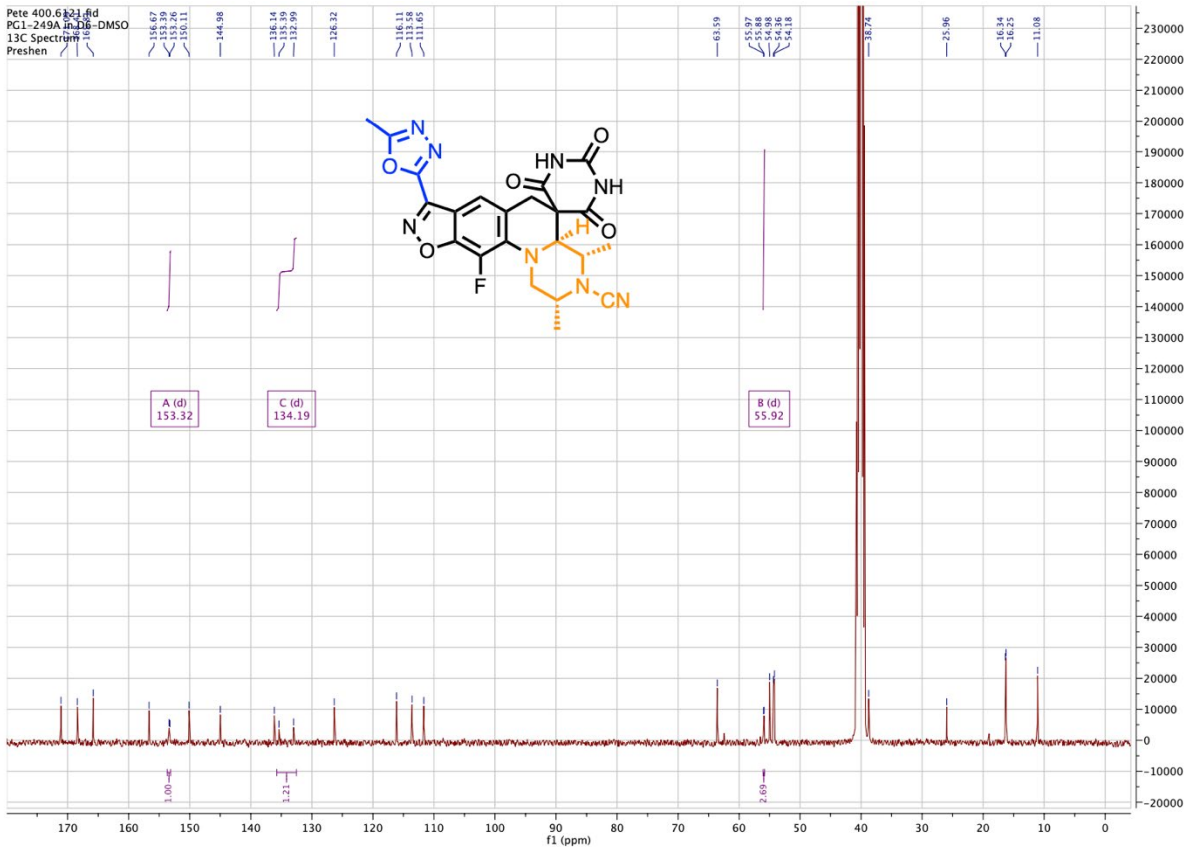
```
================================================================
Acq. Operator    : SYSTEM                          Seq. Line :   2
Acq. Instrument  : Calimero                         Location :   P1-D2
Injection Date   : 2018-12-10 09:58:57                   Inj :   1
                                                  Inj Volume : 1.000 µl
Method           : C:\Chem32\1\Data\2018-12-10\H3D 2018-12-10 2018-12-10 09-54-09\NEW GENERAL
                   NEG.M (Sequence Method)
Last changed     : 2018-12-10 09:54:09 by SYSTEM
Method Info      : Standard 2.2min method with 254nm, 280nm and 290nm and positive ESI mode
                   100-800m/z

Additional Info : Peak(s) manually integrated
```

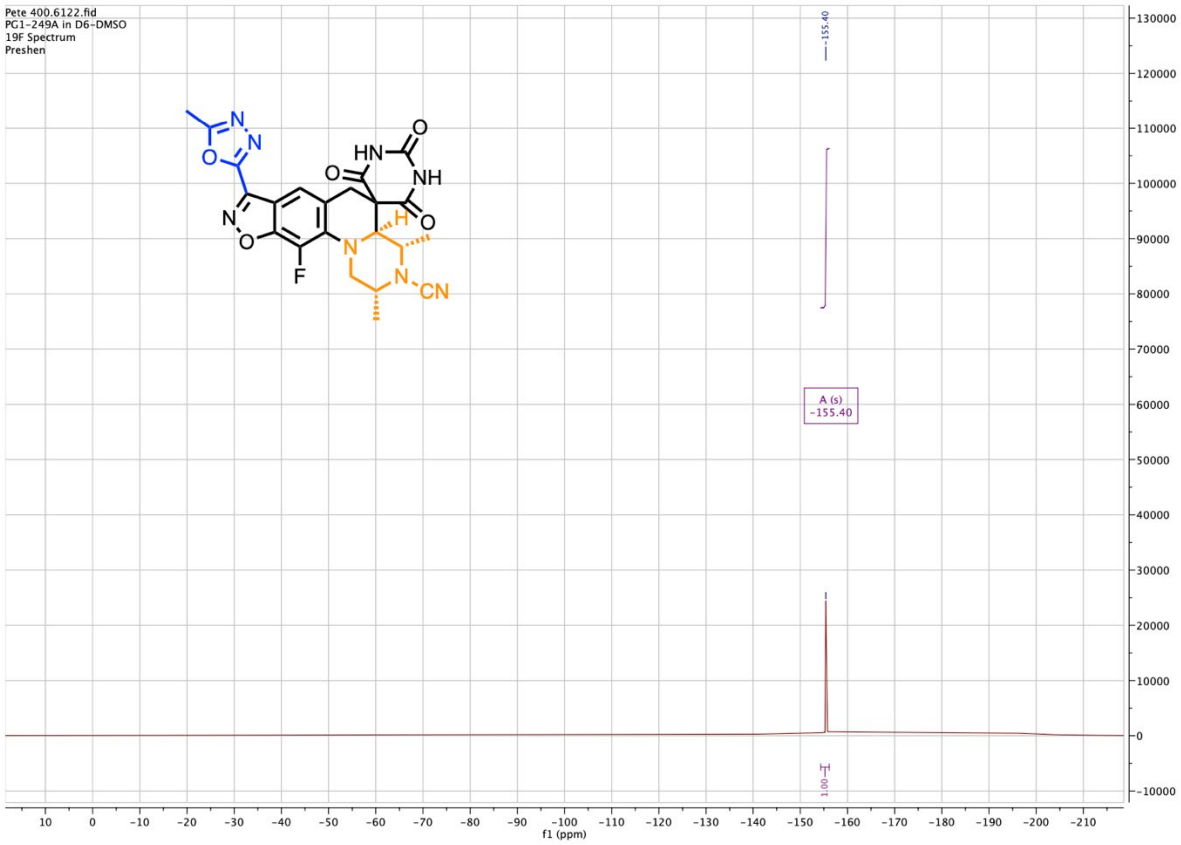



MS Spectrum
*MSD1 SPC, time=1.007:1.162 of C:\Chem32\1\Data\2018-12-10\H3D 2018-12-10 2018-12-10 09-54-09\1DB-0201.D AJS-ES, Neg
Max: 342160
498.2
499.2
100
80
60
40
20
0
100
200
300
400
500
600
700
800 m/z
ero 2018-12-10 10:02:39 SYSTEM
Page 1 o

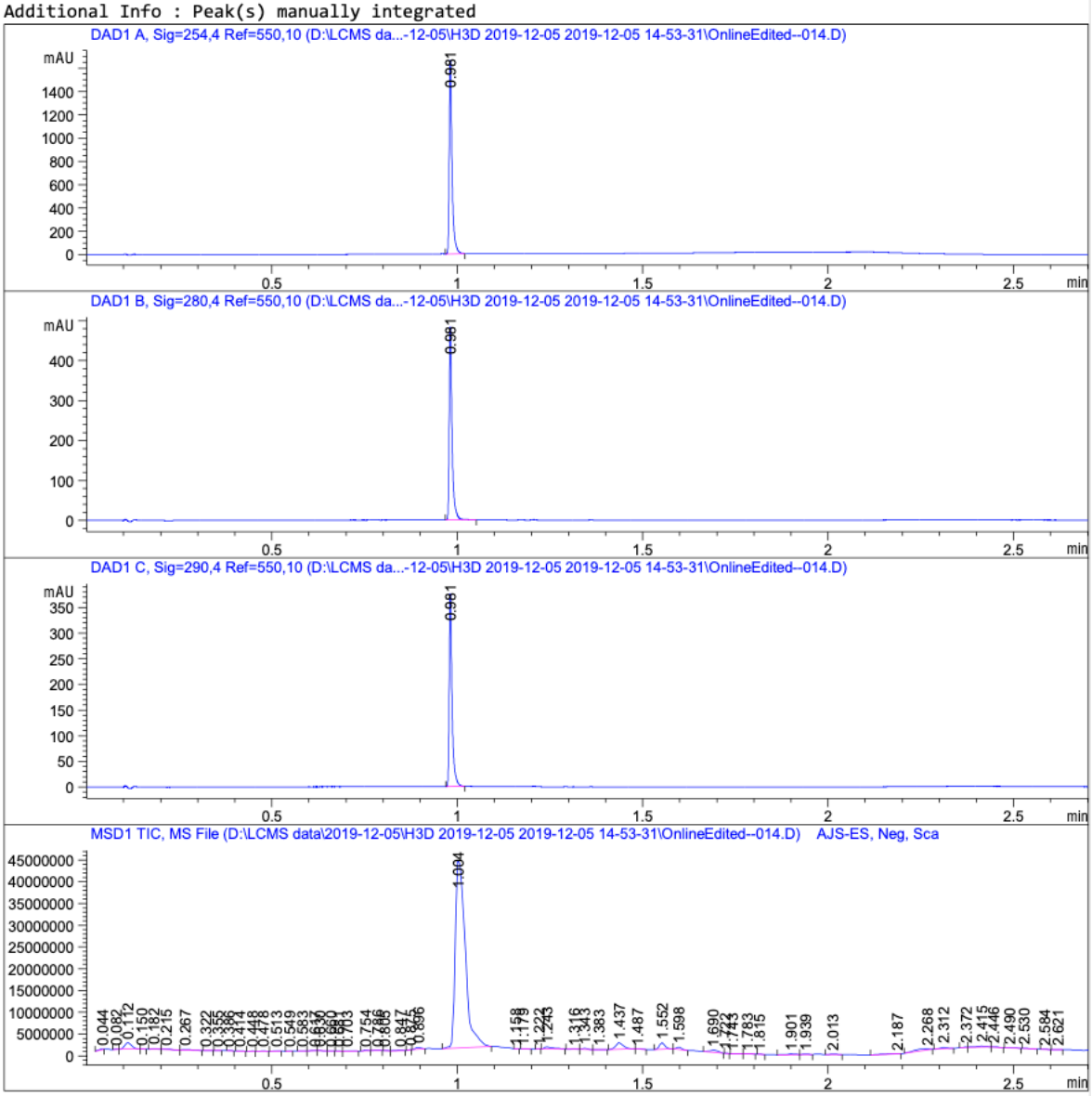
Compound 44


December .6121.fid
PG1-249-FA-P_Major_DMSO_1H
D (s) 11.72
A (s) 7.70
F (d) 3.86
H (m) 3.44
K (s) 2.68
E (m) 4.14
G (m) 3.53
I (d) 3.01
J (s) 3.25
C (m) 1.05
B (d) 1.32
f1 (ppm)

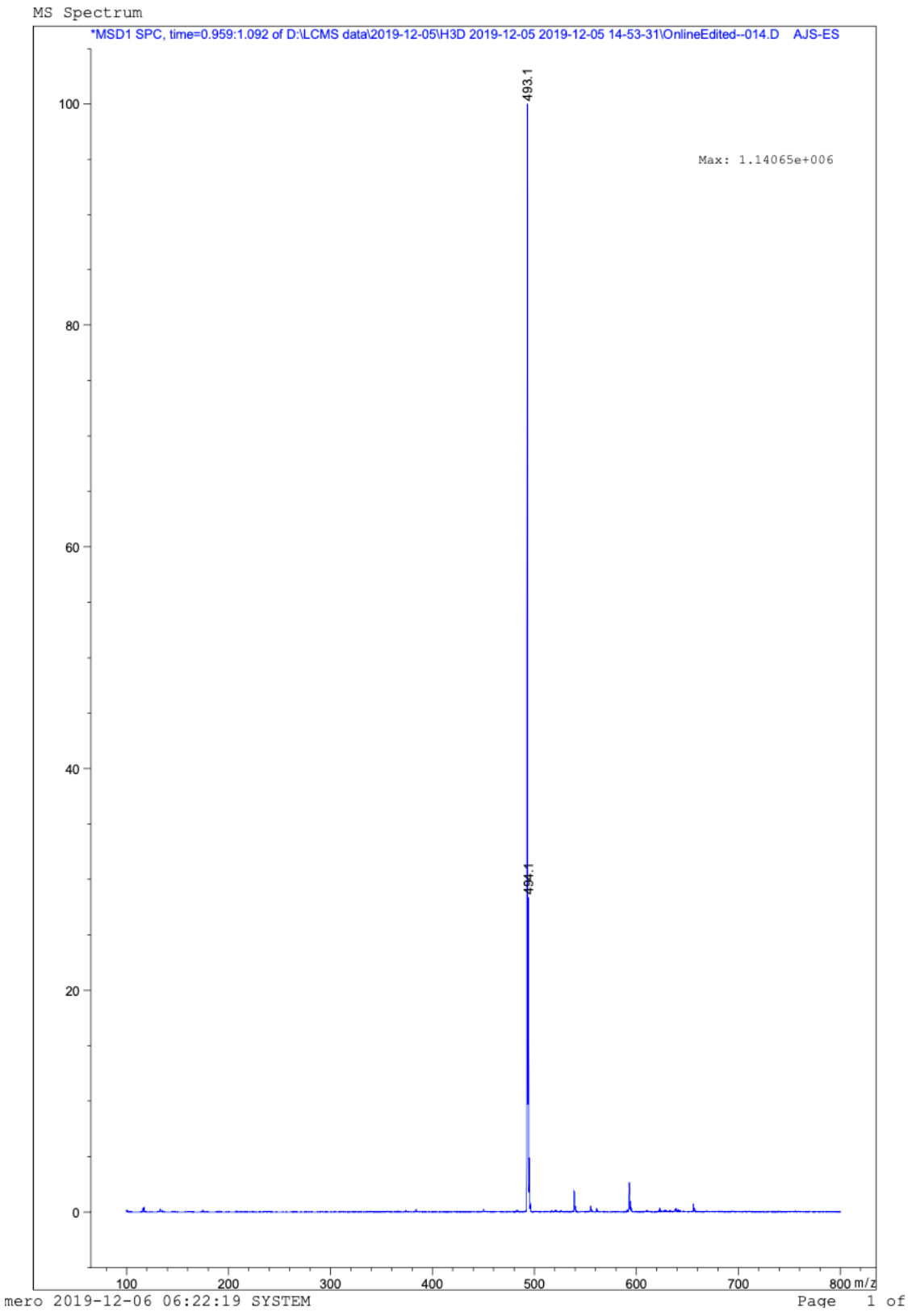

Pete 400.6121.fid
PG1-249A in D6-DMSO
13C Spectrum
Preshen
A (d) 153.32
C (d) 134.19
B (d) 55.92
f1 (ppm)


Pete 400.6122.fid
PG1-249A in D6-DMSO
19F Spectrum
Preshen
-155.40
A (s)
-155.40
1.00
f1 (ppm)

```
====================================================================
Acq. Operator   : SYSTEM                          Seq. Line :  14
Acq. Instrument : Calimero                        Location :   P1-C4
Injection Date  : 2019-12-05 15:43:13                  Inj :   1
                                                 Inj Volume : 1.000 µl
Method          : D:\LCMS data\2019-12-05\H3D 2019-12-05 2019-12-05 14-53-31\NEW GENERAL NEG.
                  M (Sequence Method)
Last changed    : 2019-12-05 15:31:37 by SYSTEM
Method Info     : Standard 2.2min method with 254nm, 280nm and 290nm and positive ESI mode
                  100-800m/z

Additional Info : Peak(s) manually integrated
```



```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=254,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.981 BB   8.27e-3  887.41132 1649.93152 100.0000

Totals :                   887.41132 1649.93152



Signal 2: DAD1 B, Sig=280,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.981 BB   8.35e-3  261.20499  479.99350 100.0000

Totals :                   261.20499  479.99350



Signal 3: DAD1 C, Sig=290,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.981 BB   8.30e-3  201.75490  373.43118 100.0000

Totals :                   201.75490  373.43118
```


MS Spectrum
*MSD1 SPC, time=0.959:1.092 of D:\LCMS data\2019-12-05\H3D 2019-12-05 2019-12-05 14-53-31\OnlineEdited--014.D AJS-ES
493.1
494.1
Max: 1.14065e+006
100
80
60
40
20
0
100
200
300
400
500
600
700
800 m/z
mero 2019-12-06 06:22:19 SYSTEM
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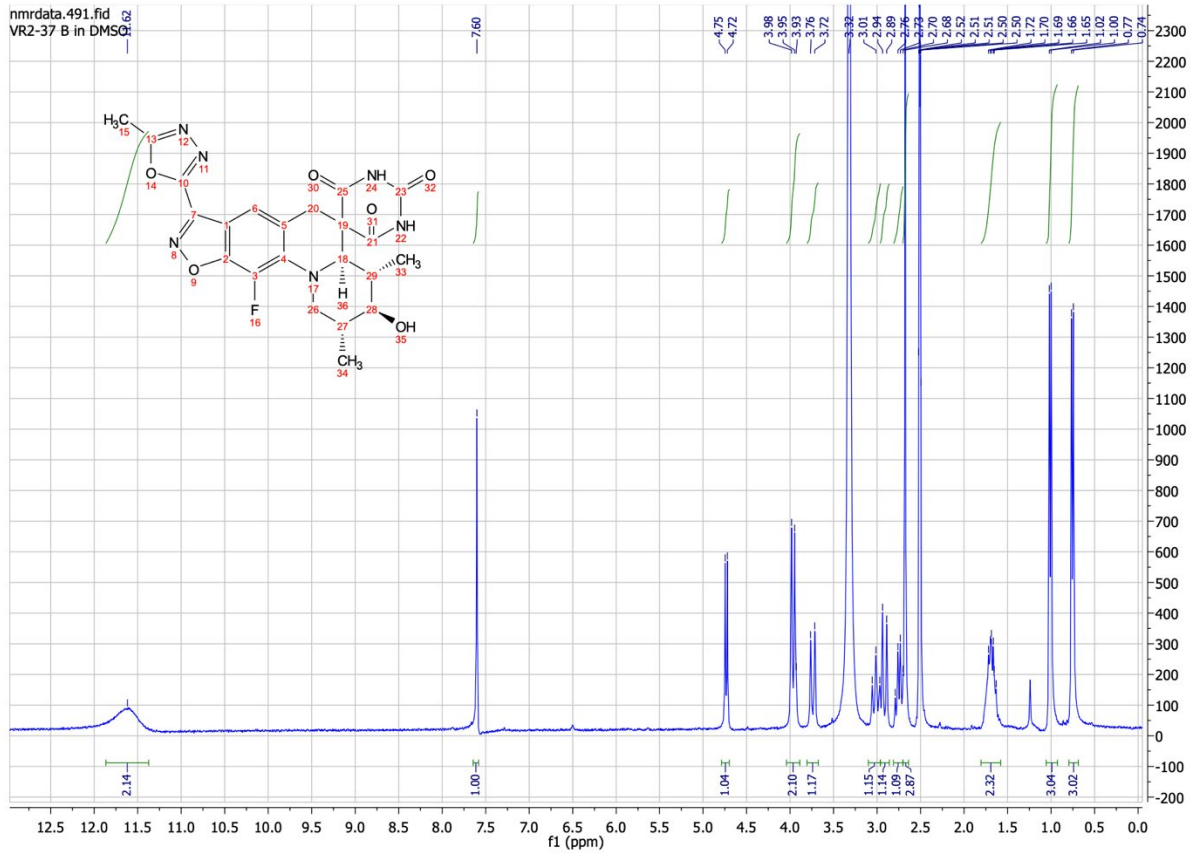
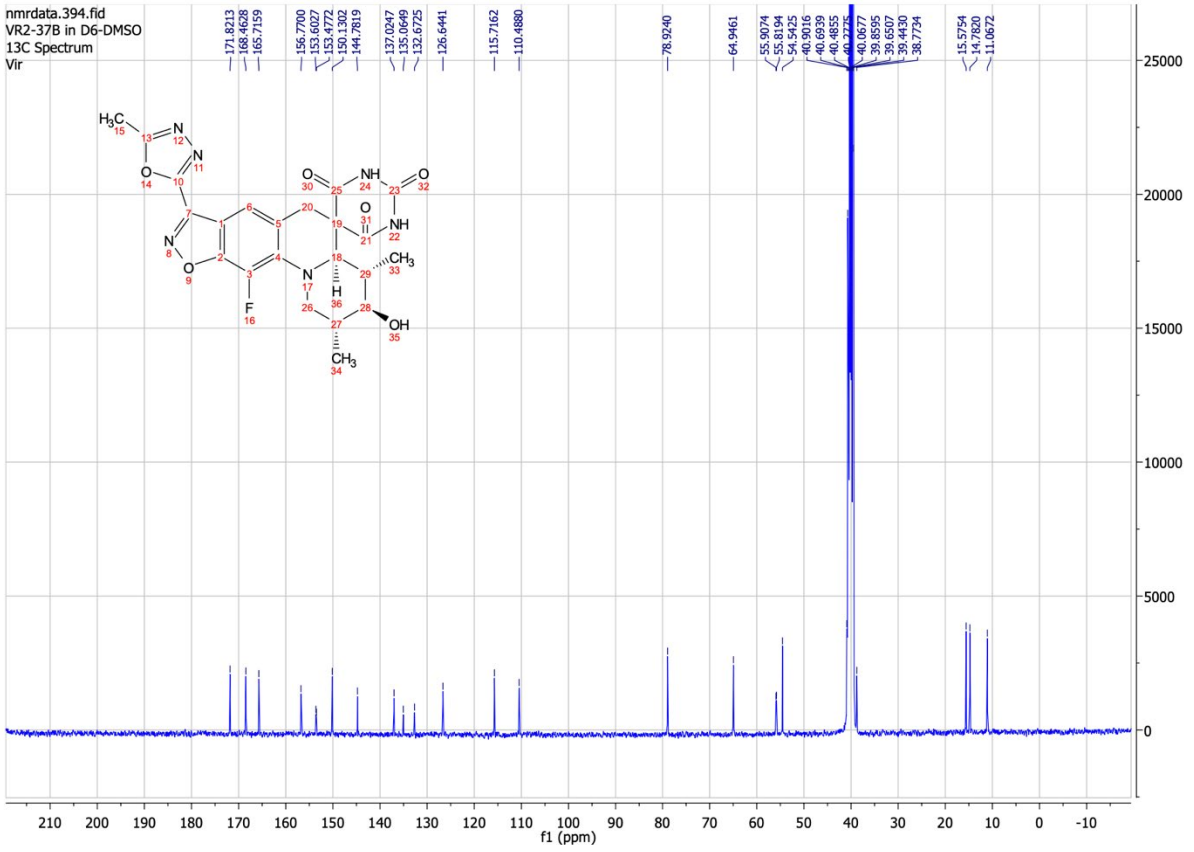
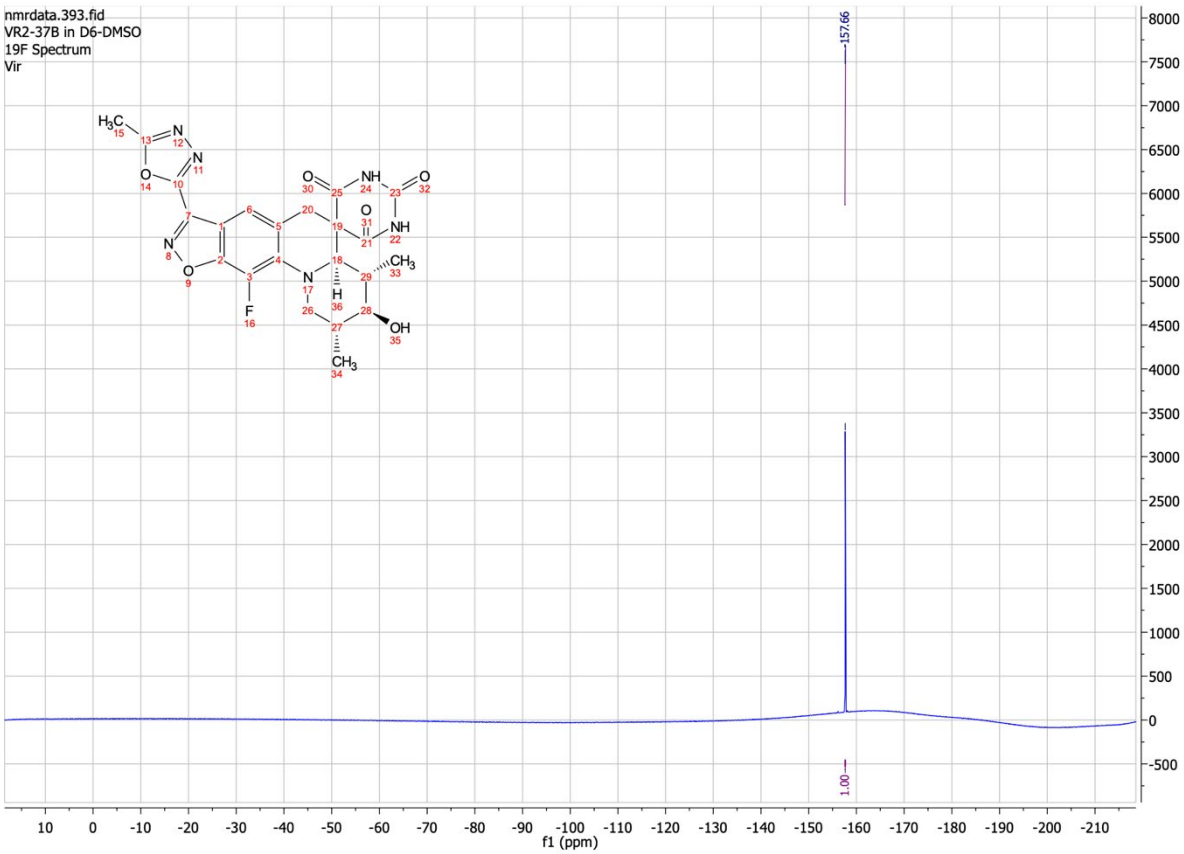
Compound 45

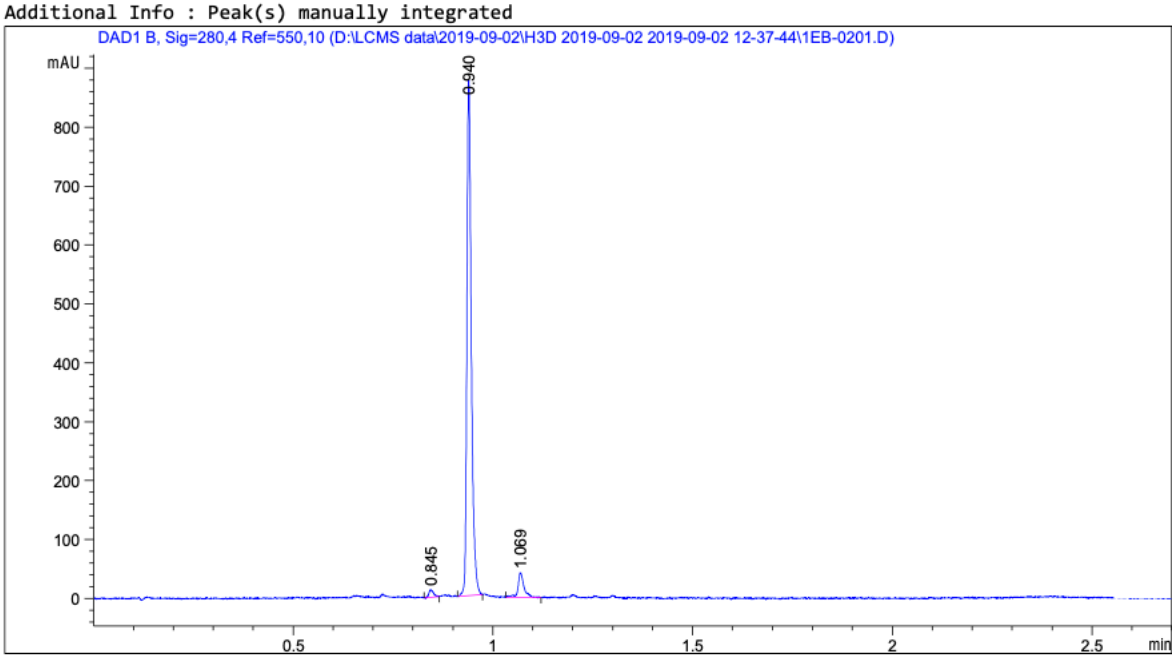

nmrdata.491.fid
VR2-37 B in DMSO
f1 (ppm)


nmrdata.394.fid
VR2-37B in D6-DMSO
13C Spectrum
Vir
f1 (ppm)


nmrdata.393.fid
VR2-37B in D6-DMSO
19F Spectrum
Vir
157.66
1.00
f1 (ppm)

```
======================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   2
Acq. Instrument : Calimero                      Location :   P1-E2
Injection Date  : 2019-09-02 12:42:26               Inj :   1
                                               Inj Volume : 1.000 µl
Method          : D:\LCMS data\2019-09-02\H3D 2019-09-02 2019-09-02 12-37-44\NEW GENERAL POS.
                  M (Sequence Method)
Last changed    : 2019-09-02 12:37:44 by SYSTEM
Method Info     : Standard 2.2min method with 254nm, 280nm and 290nm and positive ESI mode
                  100-800m/z

Additional Info : Peak(s) manually integrated
```



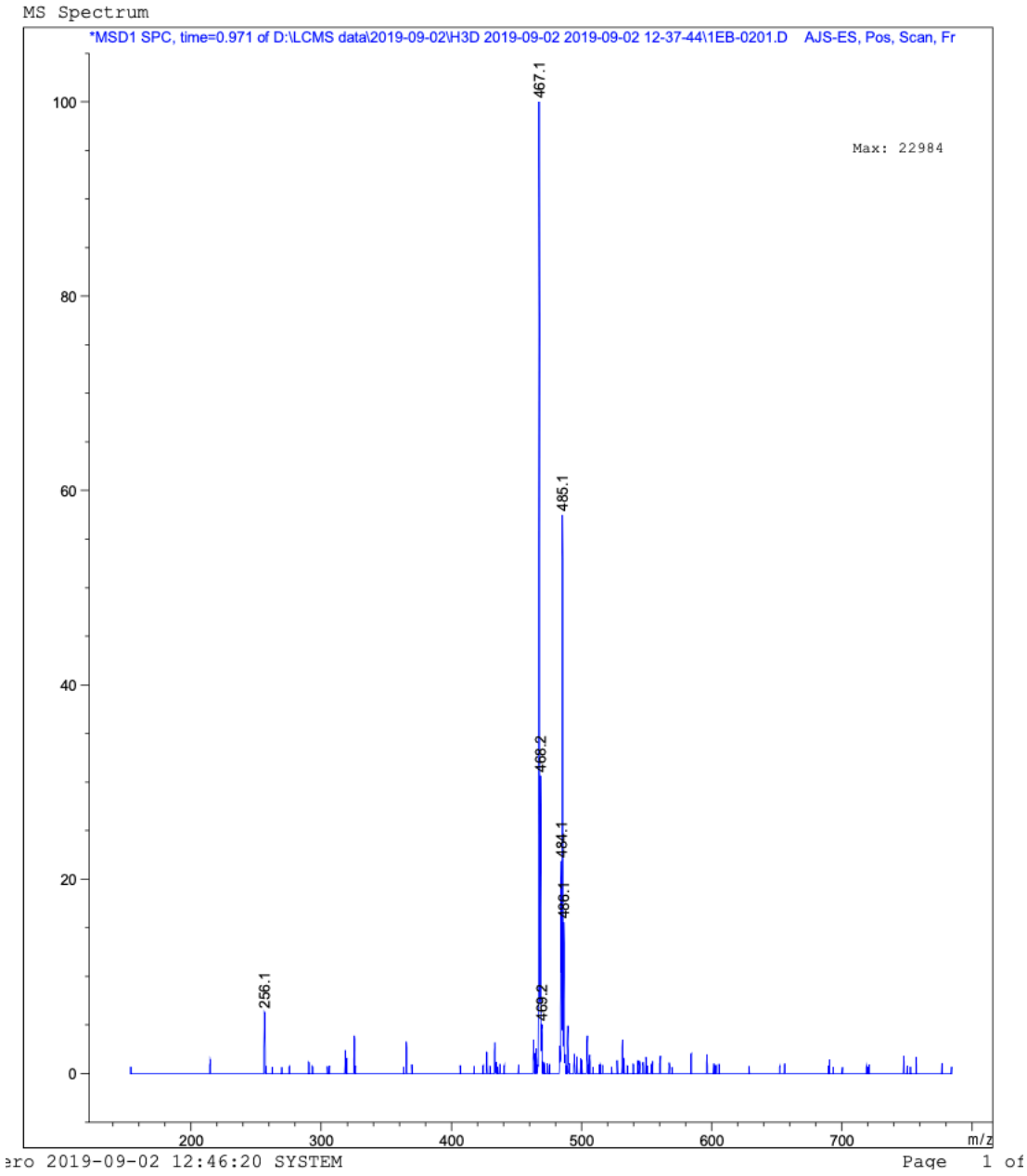

```
======================================================================
                        Area Percent Report
======================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 B, Sig=280,4 Ref=550,10

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   0.845 BB    0.0131   10.11594   12.92509   1.3672
   2   0.940 BB    0.0121  684.22076  872.96301  92.4741
   3   1.069 BB    0.0157   45.56845   41.96892   6.1587

Totals :                   739.90516  927.85703
```


MS Spectrum
*MSD1 SPC, time=0.971 of D:\LCMS data\2019-09-02\H3D 2019-09-02 2019-09-02 12-37-44\1EB-0201.D AJS-ES, Pos, Scan, Fr
Max: 22984
467.1
485.1
468.2
484.1
486.1
256.1
469.2
100
80
60
40
20
0
200
300
400
500
600
700
m/z
ero 2019-09-02 12:46:20 SYSTEM
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9. References

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