Supporting Information

Covalent Occlusion of the RORγt Ligand Binding Pocket Allows Unambiguous Targeting of an Allosteric Site

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SUPPORTING INFORMATION

S1.0. Experimental section

S1.1 *In silico* measurements

The molecular modeling environment Maestro 11.1 (Schrödinger) was used with no changes to the default parameters throughout. The crystal structure (PDB ID 3L0L) was prepared for the docking simulation using the Protein Preparation Wizard. A receptor grid was generated using the Receptor Grid Generation tool. The examined ligands were drawn in ChemDraw and prepared using the Ligand Preparation tool. Lastly, the prepared ligands were docked into the generated receptor grid using the Covalent Docking tool to obtain the corresponding docking scores.

S1.2. General Chemistry and experiments procedures & characterization

All solvents were supplied by Biosolve and used without further purification. Dry solvent was obtained from a MBRAUN Solvent Purification System (MB-SPS-800). Water was purified by a Millipore purification train. Deuterated solvents were obtained from Cambridge Isotope Laboratories. Solvents were removed *in vacuo* using a Büchi rotary evaporator and a diaphragm pump. All reagents were commercially available and were supplied by Sigma-Aldrich, TCI Chemicals, Fluorochem and Iris Biochem GmbH. Proton (^{1}H) NMR (400 MHz), carbon (^{13}C) NMR (100 MHz) and 2D NMR (400 MHz) were recorded on a Bruker Avance 400 MHz spectrometer. Proton spectra are referenced to tetramethyl silane (TMS). Carbon spectra are referenced to TMS or the solvent peak of the deuterated spectrum. NMR spectra are reported as follows: chemical shift (δ) in parts per million (ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, td = triplet of doublets), coupling constant (J) in Hertz (Hz) (if applicable) and integration (proton spectra only). Peak assignments are based on additional 2D NMR techniques (COSY, HMBC, HSQC). Analytical Liquid

Chromatography coupled with Mass Spectrometry (LC-MS) was performed on a C4 Jupiter SuC4300A 150 x 2.0 mm column using ultrapure water with 0.1% formic acid (FA) and acetonitrile with 0.1% FA, in general with a gradient of 5% to 100% acetonitrile over 10 minutes, connected to a Thermo Fischer LCQ Fleet Ion Trap Mass Spectrometer. The purity of the samples was assessed using a UV detector at 254 nm. Unless otherwise stated all final compounds were >95% pure as judged by HPLC. High resolution mass spectra (HRMS) were recorded using a Waters ACQUITY UPLC I-Class LC system coupled to a Xevo G2 Quadrupole Time of Flight (Q-TOF) mass spectrometer. Manual column chromatography was performed using silica gel with a particle size of 60-200 μm (60 Å). For manual chromatography, solid loading was used. Reaction progress was monitored by thin-layer liquid chromatography (TLC) using Merck TLC silica gel 60 F254 plates. Visualization of the plates was achieved using an ultraviolet lamp (λ_{max} = 254 nm).

General Procedure for Amide Coupling (all compounds, except GW9662, SB1404, 3, 4, 11, 13, 14, 22 and 23)

The amine (2.95 mmol) was dissolved in dry pyridine (2.5 mL) and cooled to 0 °C under argon. 2-Chloro-5-nitrobenzoyl chloride (2.27 mmol, 500 mg) was added to the reaction mixture. The mixture was stirred overnight at room temperature. Subsequently, the mixture was poured into ice and the resulting solid was filtered, washed with water and freeze dried to obtain the product.[1]

GW9662 (compound 1) (2-chloro-5-nitro-*N*-phenylbenzamide)

2-Chloro-5-nitrobenzoyl chloride (1.00 g, 5.56 mmol) was dissolved in a solution of DCM (25 mL) and triethylamine (0.7 mL, 5.0 mmol) at 0 °C under argon. Aniline (0.44 mL, 4.8 mmol) was added dropwise. The reaction mixture was stirred for 5 min at 0 °C followed by 15 min at room temperature. The solution was diluted with EtOAc (60 mL) and washed with 1 M HCl, H_2O, 1 M $NaHCO_3$ and brine (20 mL each). The combined organic phase was dried over $MgSO_4$, filtered

and concentrated *in vacuo*. The crude mixture was purified by recrystallization from EtOAc resulting in the product (1105 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.61 (d, 1H), 8.25 (dd, 1H), 7.84 (s, 1H), 7.75 - 7.57 (m, 3H), 7.41 (t, 2H), 7.22 (t, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 162.32, 146.75, 137.68, 137.01, 136.68, 131.73, 129.41, 126.09, 125.68, 125.41, 120.48; LC-MS (ESI): calc. for $C_{13}H_9ClN_2O_3$ $[M+H]^+$: 277.04, observed 277.17, LC R_t: 6.29 min. HRMS (ESI): calc. for $C_{13}H_9ClN_2O_3$ $[M+H]^+$: 277.0380, observed: 277.0372. Data was consistent with that previously reported.[2]

SB1404 (compound 2) (2-chloro-5-nitro-*N*-methylbenzamide)

2-Chloro-5-nitrobenzoylchloride (500 mg, 2.27 mmol) was dissolved in a solution of DCM (10 mL and triethylamine (0.79 mL, 5.68 mmol) at 0 °C. A solution of methylamine (2.0 M in THF, 2.95 mmol, 1,5 mL) was added dropwise. The reaction mixture was stirred overnight at room temperature under argon. The reaction mixture was diluted with DCM (20 mL) and washed with NH_4Cl (50 mL) and H_2O (50 mL). The combined organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude solid was purified by column chromatography, eluting with 100% DCM to furnish the product (38 mg, 8%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.62 (s, 1H), 8.25 (d, J = 9.5 Hz, 2H), 7.82 (d, J = 8.5 Hz, 1H), 2.80 (d, J = 4.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 164.68, 146.00, 137.84, 137.06, 131.29, 125.35, 123.70, 26.03; LC-MS (ESI): calc. for $C_8H_7ClN_2O_3$ $[M+H]^+$: 215.02, observed 215.00, LC R_t: 3.35 min. HRMS (ESI): calc. for $C_8H_7ClN_2O_3$ $[M+H]^+$: 215.0223, observed: 215.0221. Data was consistent with that previously reported.[3]

Compound 3 (2-chloro-5-nitro-*N*-isopropylbenzamide)

2-Chloro-5-nitrobenzoylchloride (250 mg, 1.24 mmol) was dissolved in THF (8 mL) at 0 °C. Isopropylamine (0.061 mL, 1.49 mmol) and triethylamine (0.1 mL, 1.49 mmol) were added

dropwise. The reaction mixture was stirred overnight at room temperature under argon. Saturated $NaHCO_3$ (50 mL) was added to the reaction mixture and it was extracted twice with DCM (2 x 50 mL). The combined organic phase was washed with aqueous saturated NaCl (50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude solid was purified by recrystallization from 1:1 water/EtOH, resulting in the product (105 mg, 35%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.45 (d, *J* = 2.7 Hz, 1H), 8.17 (dd, *J* = 8.8, 2.7 Hz, 1H), 7.57 (d, *J* = 8.8 Hz, 1H), 5.99 (s, 1H), 4.37-4.25 (m, 1H), 1.30 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 163.40, 146.54, 137.48, 136.92, 131.33, 125.45, 125.04, 42.71, 29.71, 22.56; LC-MS (ESI): calc. for $C_{10}H_{11}ClN_2O_3$ $[M+H]^+$: 243.06, observed 243.08, LC R_t: 4.73 min. HRMS (ESI): calc. for $C_{10}H_{11}ClN_2O_3$ $[M+H]^+$: 243.0536, observed: 243.0540.[3]

Compound 4 (*N*-allyl-2-chloro-5-nitrobenzamide)

Allylamine (1.61 mmol) was dissolved in dry pyridine (1.4 mL) and the reaction mixture was cooled to 0 °C under argon. 2-Chloro-5-nitrobenzoyl chloride (1.24 mmol, 250 mg) was added to the reaction mixture. The mixture was stirred overnight at room temperature. Subsequently, the mixture was poured into ice. The resulting solid was filtered, washed with water and freeze dried to furnish the product (32 mg, 11%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.51 (d, *J* = 2.7 Hz, 1H), 8.21 (dd, *J* = 8.8, 2.7 Hz, 1H), 7.59 (d, *J* = 8.8 Hz, 1H), 6.30 (s, 1H), 6.01-5.88 (m, 1H), 5.37-5.20 (m, 2H), 4.16-4.09 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 164.05, 146.57, 137.51, 136.37, 133.05, 131.47, 125.71, 125.29, 117.52, 42.73; LC-MS (ESI): calc. for $C_{10}H_9ClN_2O_3$ $[M+H]^+$: 241.04, observed 241.00, LC R_t: 4.55 min. HRMS (ESI): calc. for $C_{10}H_9ClN_2O_3$ $[M+H]^+$: 241.0380, observed: 241.0378.[1]

Compound 5 (2-chloro-5-nitro-*N*-cyclohexylbenzamide)

According to the General Procedure for amide coupling, cyclohexylamine (2.95 mmol) was reacted with 2-chloro-5-nitrobenzoyl chloride to furnish the product (69 mg, 11%). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.55 (d, *J* = 7.7 Hz, 1H), 8.25 (dd, *J* = 8.8, 2.8 Hz, 1H), 8.18 (d, *J* = 2.7 Hz, 1H), 7.82 (d, *J* = 8.8 Hz, 1H), 3.80-3.66 (m, 1H), 1.90-1.79 (m, 2H), 1.78-1.65 (m, 2H), 1.62-1.52 (m, 1H), 1.40-1.07 (m, 5H); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 163.79, 146.45, 138.70, 137.53, 131.64, 125.61, 123.93, 48.78, 32.55, 25.63, 24.95; LC-MS (ESI): calc. for $C_{13}H_{15}ClN_2O_3$ $[M+H]^+$: 283.09, observed 283.08, LC R_t: 6.01 min. HRMS (ESI): calc. for $C_{13}H_{15}ClN_2O_3$ $[M+H]^+$: 283.0849, observed: 283.0852.

Compound 6 (*N*-benzyl-2-chloro-5-nitrobenzamide)

According to the General Procedure for amide coupling, benzylamine (2.95 mmol) was reacted with 2-chloro-5-nitrobenzoyl chloride to furnish the product (140 mg, 21%). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 9.22 (t, *J* = 5.8 Hz, 1H), 8.33-8.23 (m, 2H), 7.86-7.80 (m, 1H), 7.44-7.32 (m, 4H), 7.32-7.23 (m, 1H), 4.49 (d, J = 6.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 164.86, 146.49, 139.09, 138.13, 137.56, 131.80, 128.85, 127.84, 127.47, 125.93, 124.16, 43.15; LC-MS (ESI): calc. for $C_{14}H_{11}ClN_2O_3$ $[M+H]^+$: 291.06, observed 291.08, LC R_t: 6.47 min. HRMS (ESI): calc. for $C_{14}H_{11}ClN_2O_3$ $[M+H]^+$: 291.0536, observed: 291.0523.

Compound 7 (2-chloro-5-nitro-*N*-(p-tolyl)benzamide)

According to the General Procedure for amide coupling, p-toluidine (2.95 mmol) was reacted with 2-chloro-5-nitrobenzoyl chloride to furnish the product (580 mg, 88%). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 10.61 (s, 1H), 8.44 (d, *J* = 2.6 Hz, 1H), 8.32 (dd, *J* = 8.8, 2.6 Hz, 1H), 7.89 (d, *J* = 8.8 Hz, 1H), 7.58 (d, *J* = 8.2 Hz, 2H), 7.19 (d, *J* = 8.2 Hz, 2H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 163.01, 146.60, 138.30, 137.57, 136.48, 133.71, 131.79, 129.69,

126.08, 124.31, 120.23; LC-MS (ESI): calc. for $C_{14}H_{11}ClN_2O_3$ $[M+H]^+$: 291.06, observed 291.08, LC R_t: 6.22 min. HRMS (ESI): calc. for $C_{14}H_{11}ClN_2O_3$ $[M+H]^+$: 291.0536, observed: 291.0532.

Compound 8 (2-chloro-5-nitro-*N*-(m-tolyl)benzamide)

According to the General Procedure for amide coupling, m-toluidine (2.95 mmol) was reacted with 2-chloro-5-nitrobenzoyl chloride to furnish the product (620 mg, 94%). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 10.62 (s, 1H), 8.43 (d, *J* = 2.7 Hz, 1H), 8.33 (dd, *J* = 8.8, 2.8 Hz, 1H), 7.90 (d, *J* = 8.8 Hz, 1H), 7.56 (s, 1H), 7.48 (d, *J* = 8.3 Hz, 1H), 7.25 (t, *J* = 7.8 Hz, 1H), 6.98 (d, *J* = 7.5 Hz, 1H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 163.18, 146.61, 138.88, 138.59, 138.27, 137.55, 131.80, 129.17, 126.12, 125.39, 124.28, 120.73, 117.45, 21.66; LC-MS (ESI): calc. for $C_{14}H_{11}ClN_2O_3$ $[M+H]^+$: 291.06, observed 291.08, LC R_t: 6.24 min. HRMS (ESI): calc. for $C_{14}H_{11}ClN_2O_3$ $[M+H]^+$: 291.0536, observed: 291.0529.

Compound 9 (2-chloro-5-nitro-*N*-(o-tolyl)benzamide)

According to the General Procedure for amide coupling, o-toluidine (2.95 mmol) was reacted with 2-chloro-5-nitrobenzoyl chloride to furnish the product (421 mg, 64%). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 10.18 (s, 1H), 8.47 (d, *J* = 2.7 Hz, 1H), 8.33 (dd, *J* = 8.8, 2.8 Hz, 1H), 7.89 (d, J = 8.8 Hz, 1H), 7.50 (d, *J* = 7.9 Hz, 1H), 7.30-7.14 (m, 3H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 163.58, 146.62, 138.43, 137.53, 135.78, 133.31, 131.78, 130.93, 126.68, 126.55, 126.37, 126.03, 124.31, 39.99, 18.43; LC-MS (ESI): calc. for $C_{14}H_{11}ClN_2O_3$ $[M+H]^+$: 291.06, observed 291.08, LC R_t: 5.82 min. HRMS (ESI): calc. for $C_{14}H_{11}ClN_2O_3$ $[M+H]^+$: 291.0536, observed: 291.0537.

Compound 10 (2-chloro-5-nitro-*N*-(4-methoxyphenyl)benzamide)

According to the General Procedure for amide coupling, p-anisidine (2.95 mmol) was reacted with 2-chloro-5-nitrobenzoyl chloride to furnish the product (661 mg, 95%). ^{1}H NMR (400 MHz,

DMSO-d_6): δ (ppm) 10.56 (s, 1H), 8.43 (d, *J* = 2.6 Hz, 1H), 8.34 (dd, *J* = 8.8, 2.7 Hz, 1H), 7.89 (d, *J* = 8.8 Hz, 1H), 7.61 (d, *J* = 8.9 Hz, 2H), 6.96 (d, *J* = 8.9 Hz, 2H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 162.78, 156.35, 146.60, 138.34, 137.59, 132.07, 131.79, 126.05, 124.30, 121.79, 114.45, 55.71; LC-MS (ESI): calc. for $C_{14}H_{11}ClN_2O_4$ $[M+H]^+$: 307.05, observed 307.17, LC R_t: 5.78 min. HRMS (ESI): calc. for $C_{14}H_{11}ClN_2O_4$ $[M+H]^+$: 307.0486, observed: 307.0478.

Compound 11 (2-chloro-5-nitro-*N*-(thiophen-3-yl)benzamide)

Thiophen-3-amine hydrochloride (1.61 mmol) was dissolved in dry pyridine (1.3 mL) and the reaction mixture was cooled to 0°C under argon. 2-Chloro-5-nitrobenzoyl chloride (1.24 mmol, 250 mg) was added to the reaction mixture. The mixture was stirred overnight at room temperature. Subsequently, the mixture was poured into ice. The resulting solid was filtered, washed with water and freeze dried to furnish the product (243 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 11.09 (s, 1H), 8.44 (d, *J* = 2.7 Hz, 1H), 8.35 (dd, *J* = 8.8 Hz, 1H), 7.90 (d, *J* = 8.8 Hz, 1H), 7.70 (dd, *J* = 3.2, 1.3 Hz, 1H), 7.56-7.49 (m, 1H), 7.18 (dd, *J* = 5.2, 1.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 162.25, 146.59, 137.73, 137.67, 136.53, 131.86, 126.26, 125.62, 124.40, 122.00, 110.65; LC-MS (ESI): calc. for $C_{11}H_7ClN_2O_3S$ $[M+H]^+$: 283.00, observed 283.00, LC R_t: 5.91 min. HRMS (ESI): calc. for $C_{11}H_7ClN_2O_3S$ $[M+H]^+$: 282.9944, observed: 282.9944.[1]

Compound 12 (5-chloro-2-nitro-*N*-phenylbenzamide)

First, 5-chloro-2-nitrobenzoyl chloride was synthesized by dissolving 5-chloro-2-nitrobenzoic acid (457.5 mg, 2.27 mmol) in thionylchloride (8.3 mL). The reaction mixture was concentrated *in vacuo*, obtaining 5-chloro-2-nitrobenzoyl chloride (100% yield, 500 mg).

According to the General Procedure for amide coupling, aniline (2.95 mmol) was reacted with 5-chloro-2-nitrobenzoyl chloride to furnish the product (545 mg, 87%). ^{1}H NMR (400 MHz, DMSO-

d_6): δ (ppm) 10.71 (s, 1H), 8.20 (d, *J* = 8.8 Hz, 1H), 7.95 (d, *J* = 2.3 Hz, 1H), 7.86 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.68-7.62 (m, 2H), 7.40-7.33 (m, 2H), 7.17-7.09 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 162.99, 145.47, 139.22, 139.07, 134.73, 131.22, 129.63, 129.33, 126.82, 124.59, 120.17; LC-MS (ESI): calc. for $C_{13}H_9ClN_2O_3$ $[M+H]^+$: 277.04, observed 277.00, LC R_t: 5.89 min. HRMS (ESI): calc. for $C_{13}H_9ClN_2O_3$ $[M+H]^+$: 277.0380, observed: 277.0368.

Compound 13 (2-chloro-5-nitro-*N*-(4-nitrophenyl)benzamide)

2-Chloro-5-nitro-*N*-(4-nitrophenyl)benzamide was synthesized as described in the patent of Amemiya et al.[4] 4-Nitroaniline (263 mg, 1.9 mmol) was dissolved in DMA (5 mL). 2-Chloro-5-nitrobenzoylchloride (500 mg, 2.27 mmol) was added and the reaction mixture was stirred overnight at room temperature. Ethyl acetate (5 mL) and saturated aqueous sodium bicarbonate (30 mL) were added and the mixture was stirred for 1 h. H_2O (10 mL) was added and the reaction mixture was stirred again for 1 h. The resulting crystals were filtered and freeze dried to furnish the product (502 mg, 82%). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 11.31 (s, 1H), 8.56 (d, *J* = 2.7 Hz, 1H), 8.36 (dd, *J* = 8.8, 2.8 Hz, 1H), 8.28 (d, *J* = 9.2 Hz, 2H), 7.99-7.87 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 164.04, 146.63, 145.35, 143.31, 137.62, 137.51, 131.89, 126.52, 125.49, 124.57, 120.18; LC-MS (ESI): calc. for $C_{13}H_8ClN_3O_5$ $[M+H]^+$: 322.02, observed 322.17, LC R_t: 4.70 min. HRMS (ESI): calc. for $C_{13}H_8ClN_3O_5$ $[M+H]^+$: 322.0231, observed: 322.0227.

Compound 14 (2-chloro-5-nitrophenyl)(1*H*-pyrrol-1-yl)methanone)

(2-Chloro-5-nitrophenyl)(1*H*-pyrrol-1-yl)methanone was synthesized as described by D'Silva et al.[5] Triethylamine (0.32 mL, 2.3 mmol) and pyrrole (0.21 mL, 3.1 mmol) were dissolved in DCM (1.23 mL, 19.2 mmol). DMAP (27.7 mg, 0.227 mmol) and 2-chloro-5-nitrobenzoyl chloride (500 mg, 2.27 mmol) were then added to the reaction mixture and it was stirred overnight at room temperature. The reaction mixture was diluted in DCM and washed with $NaHCO_3$ and H_2O, dried

over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting solid was freeze dried to furnish the product (406 mg, 72%). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.65 (d, *J* = 2.7 Hz, 1H), 8.43 (dd, *J* = 8.9, 2.7 Hz, 1H), 7.97 (d, *J* = 8.9 Hz, 1H), 7.24 (s, 2H), 6.42 (t, *J* = 4.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 163.30, 146.92, 137.20, 134.68, 131.98, 127.42, 125.03, 121.08, 114.98; LC-MS (ESI): calc. for $C_{11}H_7ClN_2O_3$ $[M+H]^+$: 251.02, observed 251.00, LC R_t: 4.75 min. HRMS (ESI): calc. for $C_{11}H_7ClN_2O_3$ $[M+H]^+$: 251.0223, observed: 251.0231.

Compound 15 (2-chloro-5-nitro-*N*-(2-ethylphenyl)benzamide)

According to the General Procedure for amide coupling, 2-ethylaniline (2.95 mmol) was reacted with 2-chloro-5-nitrobenzoyl chloride to furnish the product (573 mg, 83%). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 10.18 (s, 1H), 8.46 (d, *J* = 2.8 Hz, 1H), 8.33 (dd, *J* = 8.8, 2.8 Hz, 1H), 7.89 (d, *J* = 8.9 Hz, 1H), 7.51-7.48 (m, 1H), 7.33-7.21 (m, 3H), 2.68 (q, *J* = 7.5 Hz, 2H), 1.17 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 164.04, 146.63, 139.48, 138.49, 137.51, 135.07, 131.80, 129.22, 127.29, 127.16, 126.57, 126.02, 124.25, 24.31, 14.99; LC-MS (ESI): calc. for $C_{15}H_{13}ClN_2O_3$ $[M+H]^+$: 305.74, observed 305.25, LC R_t: 6.27 min. HRMS (ESI): calc. for $C_{15}H_{13}ClN_2O_3$ $[M+H]^+$: 305.0693, observed: 305.0680.

Compound 16 (2-chloro-5-nitro-*N*-(2-(tert-butyl)phenyl)benzamide)

According to the General Procedure for amide coupling, 2-(tert-butyl)aniline (2.95 mmol) was reacted with 2-chloro-5-nitrobenzoyl chloride to furnish the product (593 mg, 71%). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 10.16 (s, 1H), 8.39 (d, *J* = 2.5 Hz, 1H), 8.34 (dd, *J* = 8.7, 2.8 Hz, 1H), 7.90 (d, *J* = 8.8 Hz, 1H), 7.51-7.42 (m, 1H), 7.37-7.25 (m, 3H), 1.39 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 164.86, 147.33, 146.54, 138.44, 137.69, 135.55, 132.16, 131.93, 128.08, 127.30, 127.15, 126.03, 123.86, 35.34, 31.31; LC-MS (ESI): calc. for $C_{17}H_{17}ClN_2O_3$ $[M+H]^+$:

333.79, observed 333.08, LC R_t: 6.63 min. HRMS (ESI): calc. for $C_{17}H_{17}ClN_2O_3$ $[M+H]^+$: 333.1006, observed: 333.0995.

Compound 17 (2-chloro-5-nitro-*N*-(2-fluorophenyl)benzamide)

According to the General Procedure for amide coupling, 2-fluoroaniline (2.95 mmol) was reacted with 2-chloro-5-nitrobenzoyl chloride to furnish the product after recrystallization from ethyl acetate/hexane (50/50) (158 mg, 46%). ^{1}H NMR (400 MHz, Acetone-d_6): δ (ppm) 9.68 (s, 1H), 8.53 (d, *J* = 2.8 Hz, 1H), 8.35 (dd, *J* = 8.8, 2.8 Hz, 1H), 8.31-8.27 (m, 1H), 7.84 (d, *J* = 8.8 Hz, 1H), 7.30-7.20 (m, 3H); ^{13}C NMR (100 MHz, Acetone-d_6): δ (ppm) 164.00, 155.92, 153.47, 147.46, 138.47, 132.19, 126.94, 126.83, 126.77, 126.75, 126.70, 126.67, 126.55, 125.34, 125.07, 124.54, 124.42, 116.29; LC-MS (ESI): calc. for $C_{13}H_8ClFN_2O_3$ $[M+H]^+$: 295.68, observed 295.17, LC R_t: 5.96 min. HRMS (ESI): calc. for $C_{13}H_8ClFN_2O_3$ $[M+H]^+$: 295.0286, observed: 295.0293.

Compound 18 (2-chloro-5-nitro-*N*-(2-methoxyphenyl)benzamide)

According to the General Procedure for amide coupling, 2-methoxyaniline (2.95 mmol) was reacted with 2-chloro-5-nitrobenzoyl chloride to furnish the product after recrystallization from ethanol (212 mg, 30%). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 10.00 (s, 1H), 8.38 (d, *J* = 2.8 Hz, 1H), 8.32 (dd, *J* = 8.8, 2.8 Hz, 1H), 7.98 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.85 (d, *J* = 8.8 Hz, 1H), 7.19 (t, *J* = 7.5 Hz, 1H), 7.10 (d, *J* = 8.3 Hz, 1H), 6.99 (t, *J* = 7.6 Hz, 1H), 3.83 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 163.57, 151.22, 146.46, 138.24, 137.65, 131.66, 126.82, 126.29, 125.94, 124.47, 123.74, 120.73, 112.05, 56.22; LC-MS (ESI): calc. for $C_{14}H_{11}ClN_2O_4$ $[M+H]^+$: 307.71, observed 307.17, LC R_t: 6.19 min. HRMS (ESI): calc. for $C_{14}H_{11}ClN_2O_4$ $[M+H]^+$: 307.0486, observed: 307.0487.

Compound 19 (2-chloro-5-nitro-*N*-(2,6-dimethylphenyl)benzamide)

According to the General Procedure for amide coupling, 2,6-dimethylaniline (2.95 mmol) was reacted with 2-chloro-5-nitrobenzoyl chloride to furnish the product (504 mg, 73%). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 10.14 (s, 1H), 8.38-8.33 (m, 2H), 7.94-7.88 (m, 1H), 7.18-7.10 (m, 3H), 2.30 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 162.93, 146.19, 137.90, 137.01, 135.36, 133.94, 131.55, 127.95, 127.10, 125.69, 123.50, 18.28; LC-MS (ESI): calc. for $C_{15}H_{13}ClN_2O_3$ $[M+H]^+$: 305.74, observed 305.25, LC R_t: 6.09 min. HRMS (ESI): calc. for $C_{15}H_{13}ClN_2O_3$ $[M+H]^+$: 305.0693, observed: 305.0682.

Compound 20 (2-chloro-5-nitro-*N*-(2-(trifluoromethyl)phenyl)benzamide)

According to the General Procedure for amide coupling, 2-(trifluoromethyl)aniline (2.95 mmol) was reacted with 2-chloro-5-nitrobenzoyl chloride to furnish the product after recrystallization from ethanol (124 mg, 32%). ^{1}H NMR (400 MHz, Acetone-d_6): δ (ppm) 9.52 (s, 1H), 8.50 (d, J = 2.7 Hz, 1H), 8.36 (dd, J = 8.8, 2.7 Hz, 1H), 7.98 (d, J = 8.0 Hz, 1H), 7.86 (d, J = 8.9 Hz, 1H), 7.84-7.75 (m, 2H), 7.55 (t, J = 7.5 Hz, 1H); ^{13}C NMR (100 MHz, Acetone-d_6): δ (ppm) 164.75, 147.46, 138.51, 138.32, 135.48, 133.93, 132.37, 130.25, 128.81, 128.00, 127.27, 126.68, 126.10, 125.78, 125.48, 124.87, 123.41; LC-MS (ESI): calc. for $C_{14}H_8ClF_3N_2O_3$ $[M+H]^+$: 345.68, observed 345.17, LC R_t: 6.29 min. HRMS (ESI): calc. for $C_{14}H_8ClF_3N_2O_3$ $[M+H]^+$: 345.0254, observed: 345.0246.

Compound 21 (2-chloro-5-nitro-*N*-(naphthalen-1-yl)benzamide)

According to the General Procedure for amide coupling, naphthalen-1-amine (2.95 mmol) was reacted with 2-chloro-5-nitrobenzoyl chloride to furnish the product after recrystallization from ethanol (199 mg, 54%). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 10.76 (s, 1H), 8.62 (d, J = 2.8 Hz, 1H), 8.37 (dd, J = 8.7, 3.0 Hz, 1H), 8.20-8.14 (m, 1H), 8.01-7.96 (m, 1H), 7.93 (d, J = 8.8 Hz, 1H), 7.88 (d, J = 7.9 Hz, 1H), 7.83 (d, J = 7.8 Hz, 1H), 7.63-7.54 (m, 3H); ^{13}C NMR (100 MHz,

DMSO-d_6): δ (ppm) 163.93, 146.24, 138.03, 137.13, 133.76, 132.65, 131.32, 128.22, 128.17, 126.38, 126.23, 126.20, 125.66, 125.58, 124.04, 122.92, 122.80; LC-MS (ESI): calc. for $C_{17}H_{11}ClN_2O_3$ $[M+H]^+$: 327.75, observed 327.25, LC R_t: 6.36 min. HRMS (ESI): calc. for $C_{17}H_{11}ClN_2O_3$ $[M+H]^+$: 327.0536, observed: 327.0524.

Compound 22 (2-chloro-5-nitro-*N*-(2-aminophenyl)benzamide)

N-Boc-1,2-phenylenediamine (0.59 mmol) was dissolved in dry pyridine (13 mL) and the reaction mixture was cooled to 0 °C under argon. 2-Chloro-5-nitrobenzoyl chloride (0.45 mmol, 100 mg) was added to the reaction mixture. The mixture was stirred overnight at room temperature. Subsequently, the mixture was poured into ice. The resulting solid was filtered, washed with water and freeze dried to furnish the product which was recrystallized from methanol (9 mg, 11%). ^{1}H NMR (400 MHz, Acetone-d_6): δ (ppm) 9.23 (s, 1H), 8.56 (d, J = 3.0 Hz, 1H), δ 8.34 (dd, J = 8.8, 2.8 Hz, 1H), 7.84 (d, J = 8.8 Hz, 1H),7.44-7.38 (m, 1H), 7.04 (t, J = 7.6 Hz, 1H), 6.88 (d, J = 8.0 Hz, 1H), 6.70 (t, J = 7.6 Hz, 1H), 4.68 (s, 2H); ^{13}C NMR (100 MHz, Acetone-d_6): δ (ppm) 164.03, 147.51, 143.56, 139.08, 138.50, 132.17, 128.01, 126.83, 126.76, 126.32, 125.03, 123.83, 118.08, 117.55; LC-MS (ESI): calc. for $C_{13}H_{10}ClN_3O_3$ $[M+H]^+$: 292.70, observed 292.17, LC R_t: 4.61 min. HRMS (ESI): calc. for $C_{13}H_{10}ClN_3O_3$ $[M+H]^+$: 292.0489, observed: 292.0483.[1]

Compound 23 (2-chloro-5-nitro-*N*-(2-hydroxyphenyl)benzamide)

2-Aminophenol (6.82 mmol) was dissolved in dry THF (3 ml) and cooled to 0 °C. 2-Chloro-5-nitrobenzoyl chloride (2.27 mmol, 500 mg) was added dropwise to the solution. The reaction mixture was stirred under argon at 0 °C for 3 h. Additionally it was quenched with 5% aqueous hydrochloric acid (2.3 ml), poured in ice and washed with 5% aqueous hydrochloric acid and H_2O. After trituration in a mixture of ethyl acetate/hexane (50/50), the product was obtained (347 mg, 52%). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 9.95 (s, 1H), 9.77 (s, 1H), 8.44 (d, J = 2.2 Hz,

1H), 8.31 (dd, *J* = 8.8, 2.8 Hz, 1H), 7.88 – 7.79 (m, 2H), 7.05 (t, *J* = 7.7 Hz, 1H), 6.93 (d, *J* = 6.6 Hz, 1H), 6.84 (t, *J* = 7.7 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 163.02, 148.97, 145.88, 137.62, 137.13, 131.09, 125.78, 125.38, 125.12, 124.04, 123.63, 118.83, 115.64; LC-MS (ESI): calc. for $C_{13}H_9ClN_2O_4$ $[M+H]^+$: 293.69, observed 293.17, LC R_t: 5.56 min. HRMS (ESI): calc. for $C_{13}H_9ClN_2O_4$ $[M+H]^+$: 293.0329, observed: 293.0326.[1]

Ligands used in binding studies.

The ligands used in binding studies were synthesized as described in literature (**MRL-871**[6] and **FM26**[7], both >95% pure) or they were commercially available (cholesterol derivatives and digoxin).

S1.3. Protein Expression

RORγt-LBD Expression and Purification.

A pET15b expression vector encoding the human RORγt LBD (residues 265-507 (used for mutagenesis and corresponding ligation experiments) or residues 265-518 (used for general ligation experiments and biochemical assays)) with an *N*-terminal His_6-tag was transformed by heat shock into BL21(DE3) *E. coli* cells. Single colonies were used to inoculate pre-cultures of 8 mL LB-media containing 100 μg/mL ampicillin. After over-night incubation at 37 ˚C each pre-culture was transferred to 1L TB media supplemented with ampicillin (100 μg/mL) and incubated at 37 ˚C until an $OD_{600\ nm}$ = 1.0 was reached. Protein expression was then induced with 0.5 mM isopropyl-b-d-thiogalactoside (IPTG) and cultures were incubated for 16 h at 18 ˚C. The cells were collected by centrifugation and suspended in lysis buffer (300 mM NaCl, 20 mM TrisHCl pH 8.0, 20 mM imidazole, 1 mM TCEP, 10% v/v glycerol, cOmplete™, EDTA-free Protease Inhibitor Cocktail tablets (1 tablet/ 50 mL lysate) and benzonase (0.1 μL/ 1 mL)). After lysis using a C3

Emulsiflex-C3 homogeniser (Avestin) the cell lysate was cleared by centrifugation at 4 ˚C and the protein was purified via Ni^{2+} affinity column chromatography. Fractions containing the protein of interest were combined and dialyzed to 150 mM NaCl, 20 mM Tris (pH=8.0), 5 mM DTT and 10% v/v glycerol.

Mutagenesis

Mutagenesis was introduced using the QuickChange Lightning Multi Site-Directed Mutagenesis Kit from Agilent Technologies. The primer with sequence 5'-tgggaaatgtgggaacgtgcggcacatc-acctgacgg-3'was used to mutate the Cys320 residue into an alanine residue. QIAquick Purification Kit from Qiagen was used to purify the mutated DNA.[8]

S1.4. Ligation

Ligation Experiment

Ligation experiments were executed in a buffer with 50 mM TRIS (pH varying from 5.3-5.8), 50 mM NaCl, 5 mM CHAPS and 0.1% Bovine Serum Albumin (BSA). The concentration of RORγt in the buffer was 20 μM and the compound concentration varied from 5 to 10 equivalents (Supporting Table 2). Total volume was 50 μL which was incubated (continuous mixing) at 4°C or RT overnight.

Q-TOF-MS analysis

Purity and exact mass of the ligated RORγt protein was determined using a High-Resolution LC-MS system consisting of a Waters ACQUITY UPLC I-Class system coupled to a Xevo G2 Quadrupole Time of Flight (Q-TOF). The system was comprised of a Binary Solvent Manager and a Sample Manager with Fixed-Loop (SM-FL). A Polaris C18A reverse phase column (2.0 x 100 mm, Agilent) was used (0.3 mL/min) with a 15% to 75% acetonitrile gradient in water

supplemented with 0.1% v/v formic acid before analysis in positive mode in the mass spectrometer. Deconvolution of the m/z spectra was done using the MaxENTI algorithm in the Masslynx v4.1 (SCN862) software. The percentage of ligation has been determined from the ratio between the different mass peaks in the deconvoluted spectra.

S1.5. Biochemical assays

Ligation Experiment for the TR-FRET Coactivator Recruitment Assay – Dose Response

Ligation was conducted using 20 μM His6-RORγt-LBD in buffer containing 50 mM TRIS (pH mentioned in Supporting Table 2), 50 mM NaCl, 5 mM CHAPS and 0.1% Bovine Serum Albumin (BSA). A mix of the probe (10 eq, 200 μM at the maximum concentration) and His6-RORγt-LBD (20 μM) were titrated using a 1.4 x dilution series in Corning white low volume, low binding, 384-well plates at a final volume of 5.71 μL. The final DMSO concentration was 1% v/v throughout. The plate was incubated at 4°C or RT overnight (shown in Supporting Table 2).

TR-FRET Coactivator Recruitment Assay - Dose-Response

Assays were conducted using 100 nM *N*-terminal biotinylated SRC-1 box2 peptide (Biotin-N-PSSHSSLTARHKILHRLLQEGSPSD-$CONH_2$) and 100 nM His_6-RORγt-LBD in buffer containing 10 mM HEPES (pH 7.5), 150 mM NaCl, 5 mM DTT, 0.1% BSA (w/v) and 0.1 mM CHAPS. The highest concentration of probe compound was 1 μM (10 eq). A terbium labelled anti-His antibody (CisBio Bioassays, 61HISTLA) and D2-labelled streptavidin (CisBio Bioassays, 610SADLA) were used at the concentrations recommended by the supplier. Corning white low volume, low binding, 384-well plates at a final volume of 10 μL were used. The final DMSO concentration was 1% v/v throughout. The plate was incubated at room temperature for 60 min and centrifuged before reading (excitation = 340 nm; emission = 665 nm and 620 nm) on a Tecan

infinite F500 plate reader using the parameters recommended by CisBio Bioassays. The data were analyzed with GraphPad Prism 7.0 Software. The dose-response curve was fitted represented by:

$$y = A_1 + \frac{A_1 - A_2}{1 + \frac{x^p}{xo^p}} \quad \text{(eq. 1)}$$

Where y is the FRET ratio ((acceptor/donor)*1000), A_1 is the bottom asymptote, A_2 is the top asymptote, p is the Hill slope, x is the ligand concentration in μM and x_0 is the IC_{50} value in μM. Where dose-response curves did not reach a bottom asymptote, this was fixed at the value of the negative control. The data was normalized with regards to plateau levels (positive and negative control values). The data was recorded in triplicate in two or more independent experiments (one representative dataset shown). Error bars represent the SD of the mean.

Ligation Experiment for the TR-FRET Coactivator Recruitment Assay – Ligand Binding

Ligation was conducted using 20 μM His6-RORγt-LBD in buffer containing 50 mM TRIS (pH mentioned in Supporting Table 2), 50 mM NaCl, 5 mM CHAPS and 0.1% Bovine Serum Albumin (BSA). A mix of the probe (5 eq, 100 μM) and His6-RORγt-LBD (20 μM) was incubated (continuous mixing) at 4°C or RT (shown in Supporting Table 2) overnight, in a total volume of 50 μL. The mixture was rebuffered to TR-FRET assay buffer (10 mM HEPES, 150 mM NaCl) using a PD Spin Trap G-25 column and excess compound was removed.

TR-FRET Coactivator Recruitment Assay - Ligand Binding

Assays were conducted using 100 nM N-terminal biotinylated SRC-1 box2 peptide (Biotin-NPSSHSSLTARHKILHRLLQEGSPSD-CONH2) and 20 nM His6-RORγt-LBD (unligated & ligated) in buffer containing 10 mM HEPES, 150 mM NaCl, 5 mM DTT, 0.1% BSA (w/v) and 0.1 mM CHAPS, pH 7.5. A terbium labelled anti-His antibody (CisBio Bioassays, 61HISTLA) and D2-labelled streptavidin (CisBio Bioassays, 610SADLA) were used at the concentrations recommended by the supplier. Compounds (dissolved in DMSO) were titrated using a 2 x dilution

series in Corning white low volume, low binding, 384-well plates at a final volume of 10 µL. The final DMSO concentration was 2% v/v throughout. The plate was directly measured (excitation = 340 nm; emission = 665 nm and 620 nm) on a Tecan infinite F500 plate reader using the parameters recommended by CisBio Bioassays. The gain was set manually to 141 and 163 for Tb and d2, respectively. Data analysis was performed in an analogous fashion to that described above. The data was recorded in triplicate in two or more independent experiments (one representative dataset shown). Error bars represent the SD of the mean.

Thermal Shift Assay

Thermal shift assays were performed using 40 µL samples containing 5 µM RORγt-LBD, 10 µM compound and 2.5x SYPRO® Orange (Sigma) in buffer containing 150 mM NaCl, 10 mM HEPES (pH 7.5) and 1% DMSO. Hard-Shell® 96-Well PCR Plates (low profile, thin wall, skirted, green/white #hsp9645 were used. The samples were heated from 25 °C to 80 °C at a rate of 0.5 °C per 5 s in a CFX96 Touch Real-Time PCR Detection System (Bio-Rad). After each increment, Sypro Orange intensity was measured using the plate read option in Bio-Rad CFX Manager 3.1. Melting curves and negative derivatives were plotted from the HEX filter using GraphPad Prism 7.0. Melting temperatures were determined by the Bio-Rad CFX Manager 3.1 software in negative mode. The data was recorded in triplicate in three independent experiments (one representative dataset shown). Error bars represent the SD of the mean.

S2.0. Additional information on the design, ligation data and TR-FRET experiments

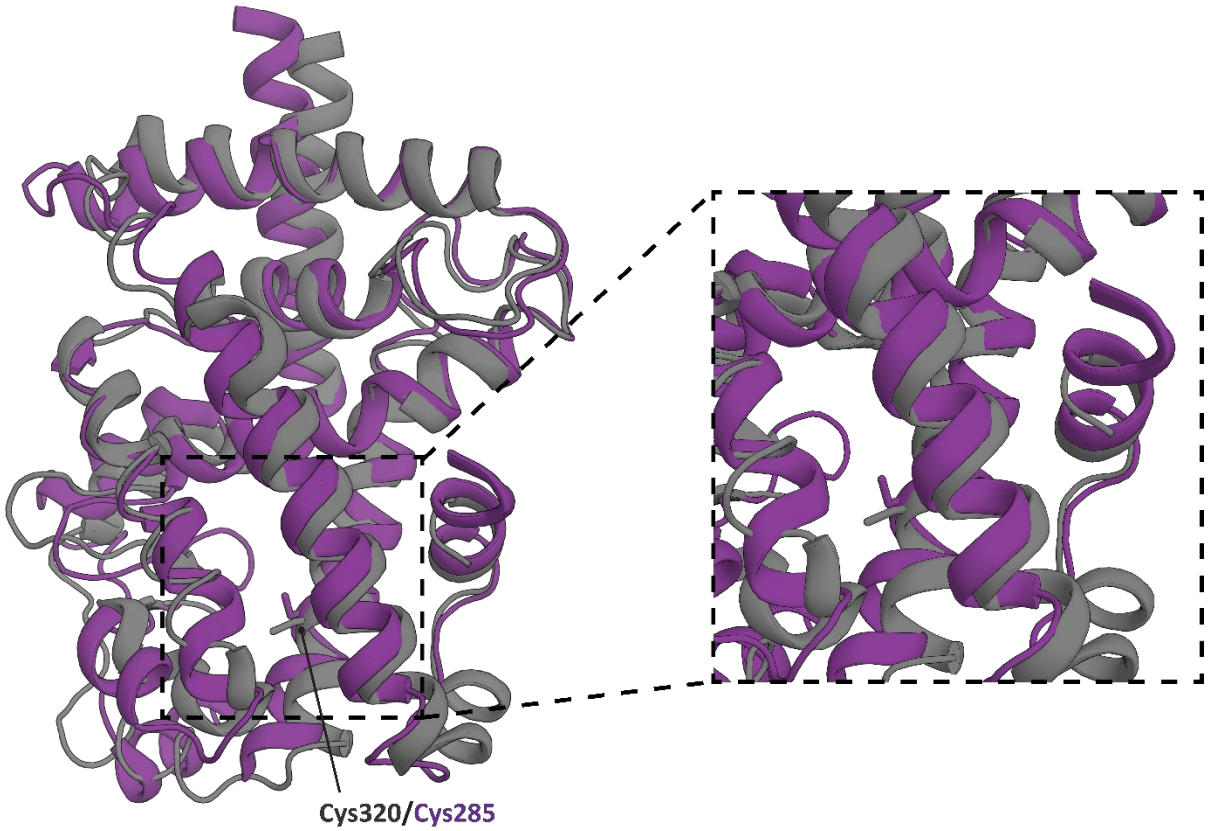


Figure S1. Overlay of the crystal structures of RORγt (grey, PDB: 3L0L) and PPARγ (purple, PDB: 3B0R), demonstrating the similar positioning of the cysteine residue Cys320/Cys285.

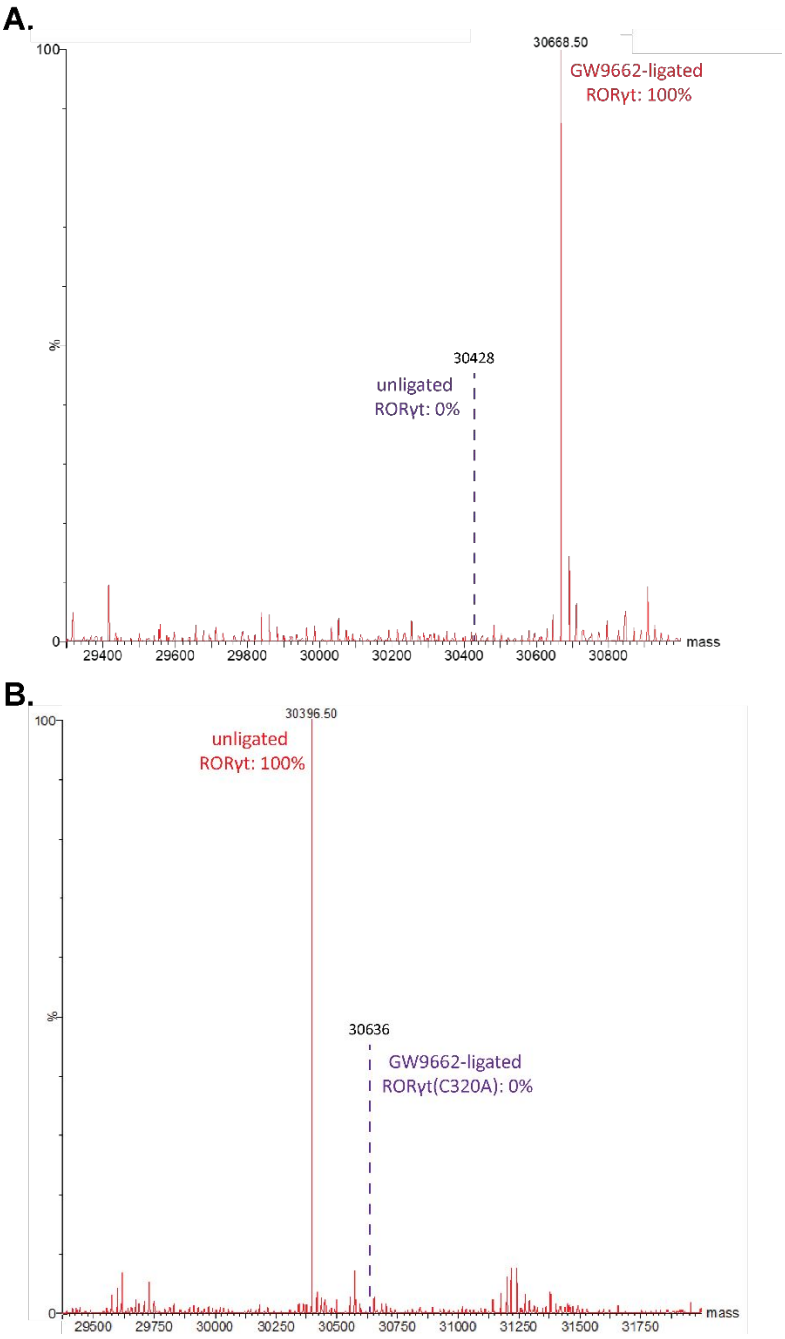
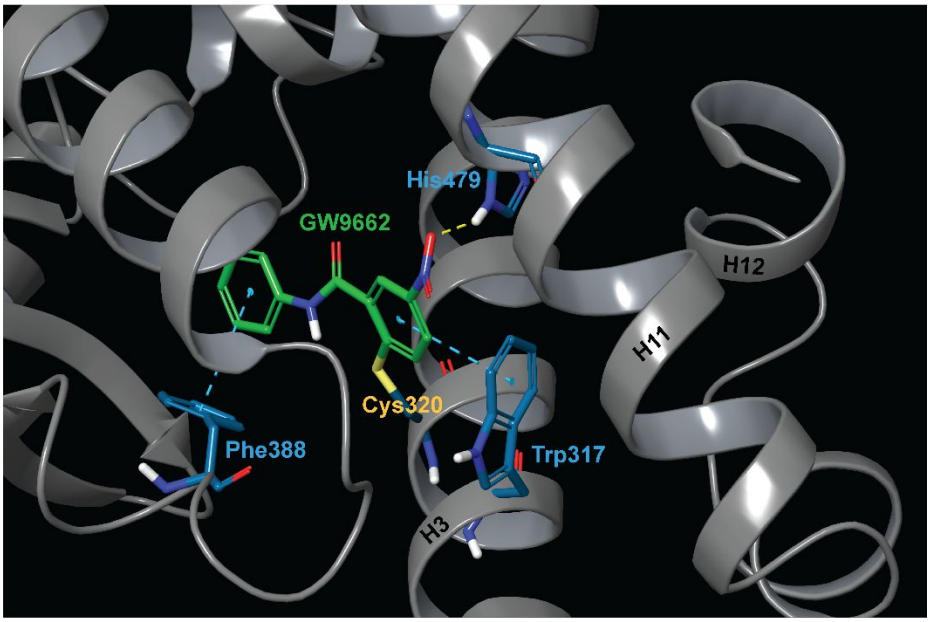


Figure S2. A. Deconvoluted Q-TOF spectrum of RORγt (residues 265-507) ligated to **GW9662**: 100% ligation is observed (mass: 30688.5 Da), while 0% unligated protein is visible (mass: 30428 Da). **B.** Deconvoluted Q-TOF spectrum of RORγt containing a Cys320Ala mutation (265-507), ligated to **GW9662**: 0% ligation is observed (mass: 30636 Da), while 100% unligated protein is visible (mass: 30396.5 Da).

A.



B.

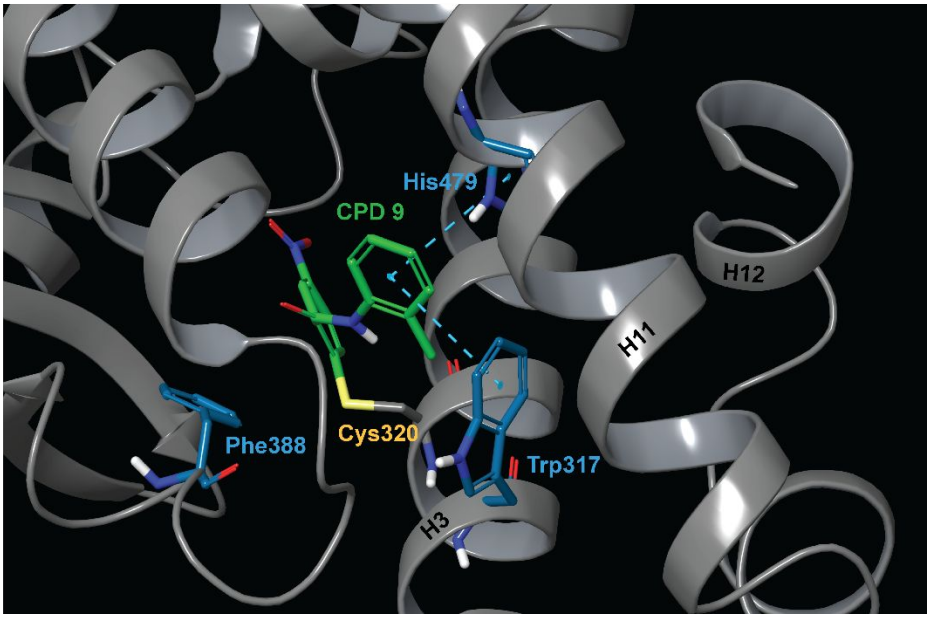


Figure S3. Docking poses of **GW9662** and compound **9** in RORγt (PDB: 3L0L). **A.** Docking pose for **GW9662** (green sticks) (best docking score), making pi-pi interactions (blue dotes lines) with residues Trp317 and Phe388. The warhead is positioned towards H11. **B.** Docking pose for compound **9** (green sticks) (best docking score), making pi-pi interactions (blue dotted lines) with residues Trp317 and His479. The warhead is positioned into the orthosteric pocket.

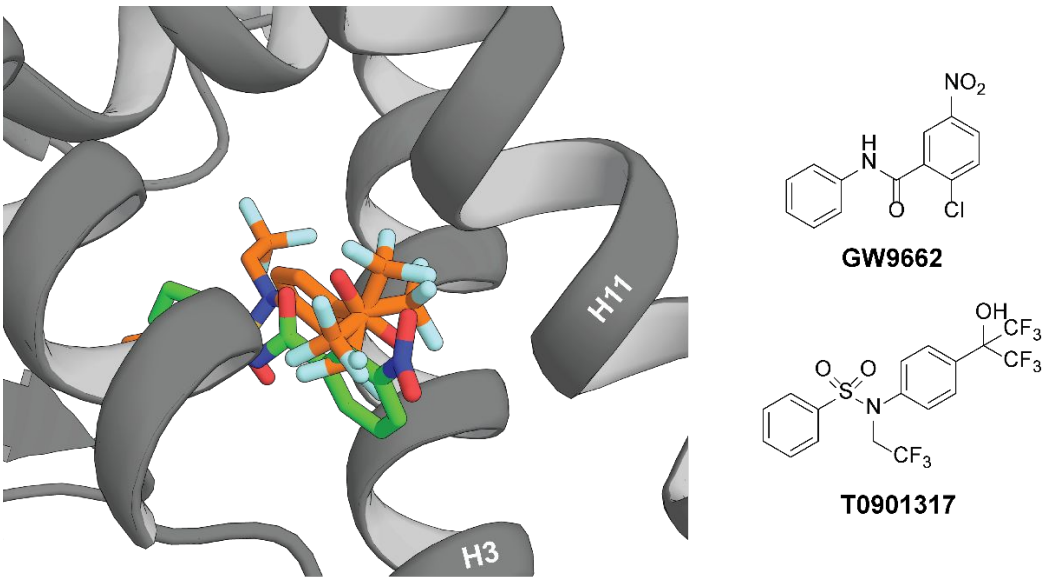


Figure S4. Overlay of the co-crystal structure of RORγt with inverse agonist **T0901317** (orange sticks) (PDB: 4NB6) and docking pose of **GW9662** (orange) (best docking score). The nitro moiety of **GW9662** points towards H11, similar to the CF_3-groups of **T0901317**.

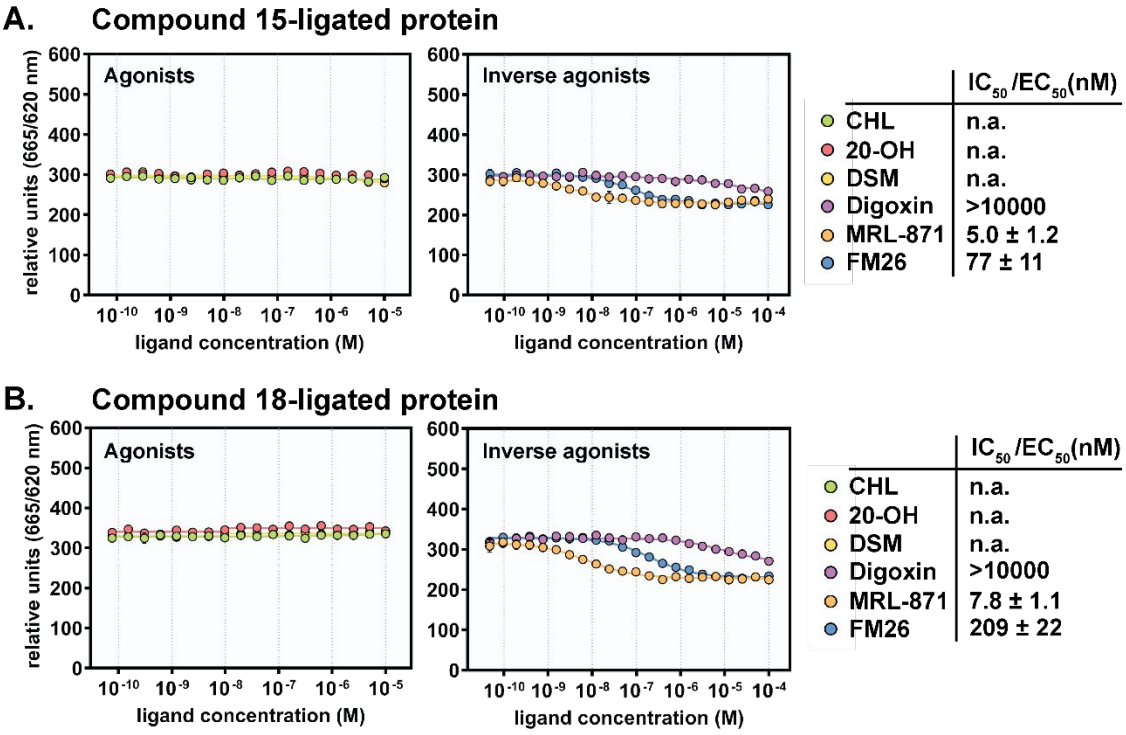


Figure S5. TR-FRET coactivator recruitment ligand binding assay with ligated RORγt by titration of various orthosteric and allosteric ligands (Cholesterol (CHL), 20α-hydroxycholesterol (20-OH), desmosterol (DSM), digoxin, **MRL-871** and **FM26**). Ligand binding assay for **A.** Compound **15**-ligated protein. **B.** Compound **18**-ligated protein. Abbreviations: n.a., not active. Data recorded in triplicate from two independent experiments (one representative dataset shown). Error bars represent the SD of the mean.

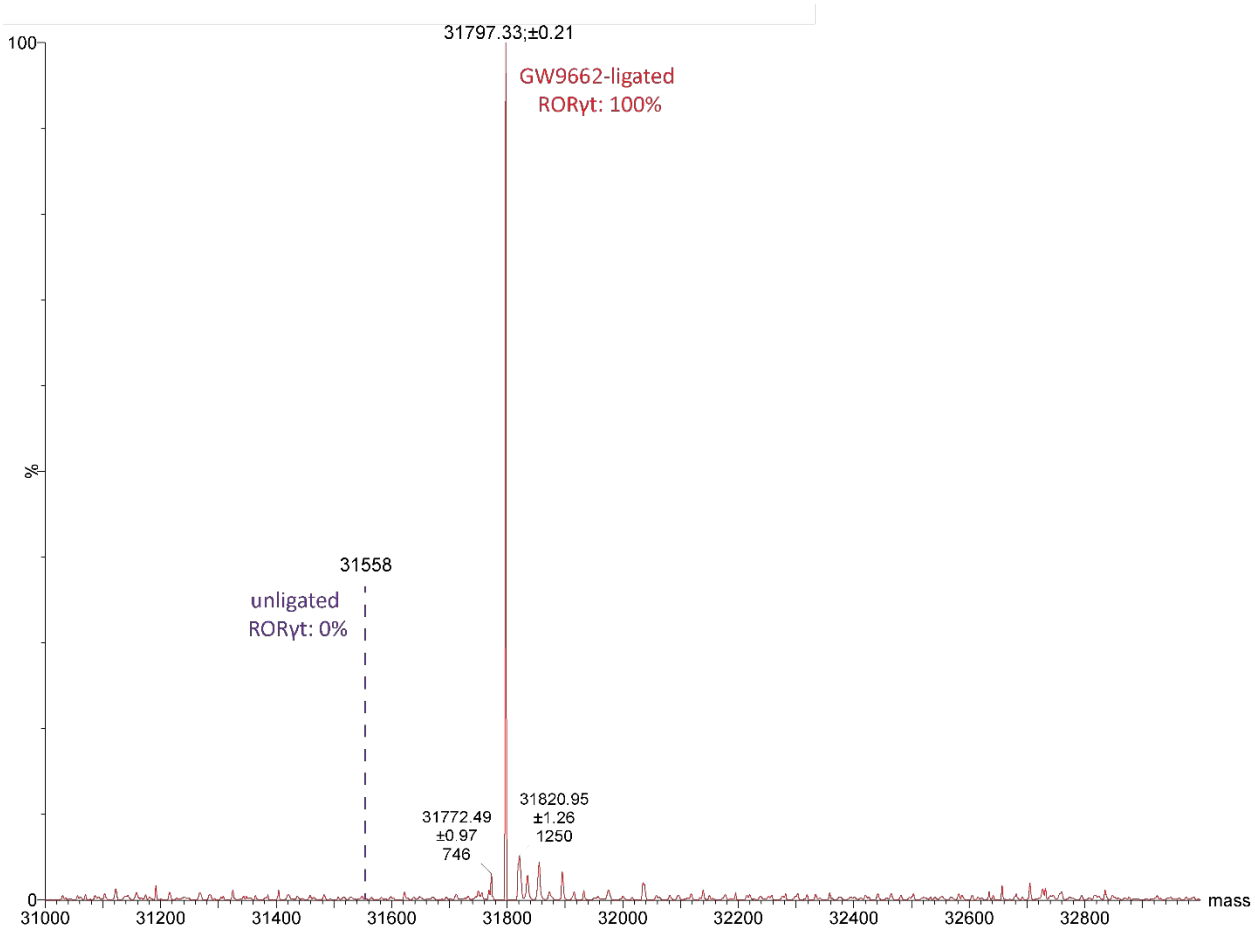


Figure S6. Deconvoluted Q-TOF spectrum of RORγt ligated to **GW9662**: 100% ligation is observed, while 0% unligated protein is visible (mass: 31558.11 Da). Mass expected: 31797.68 Da, mass observed: 31796.33 Da.

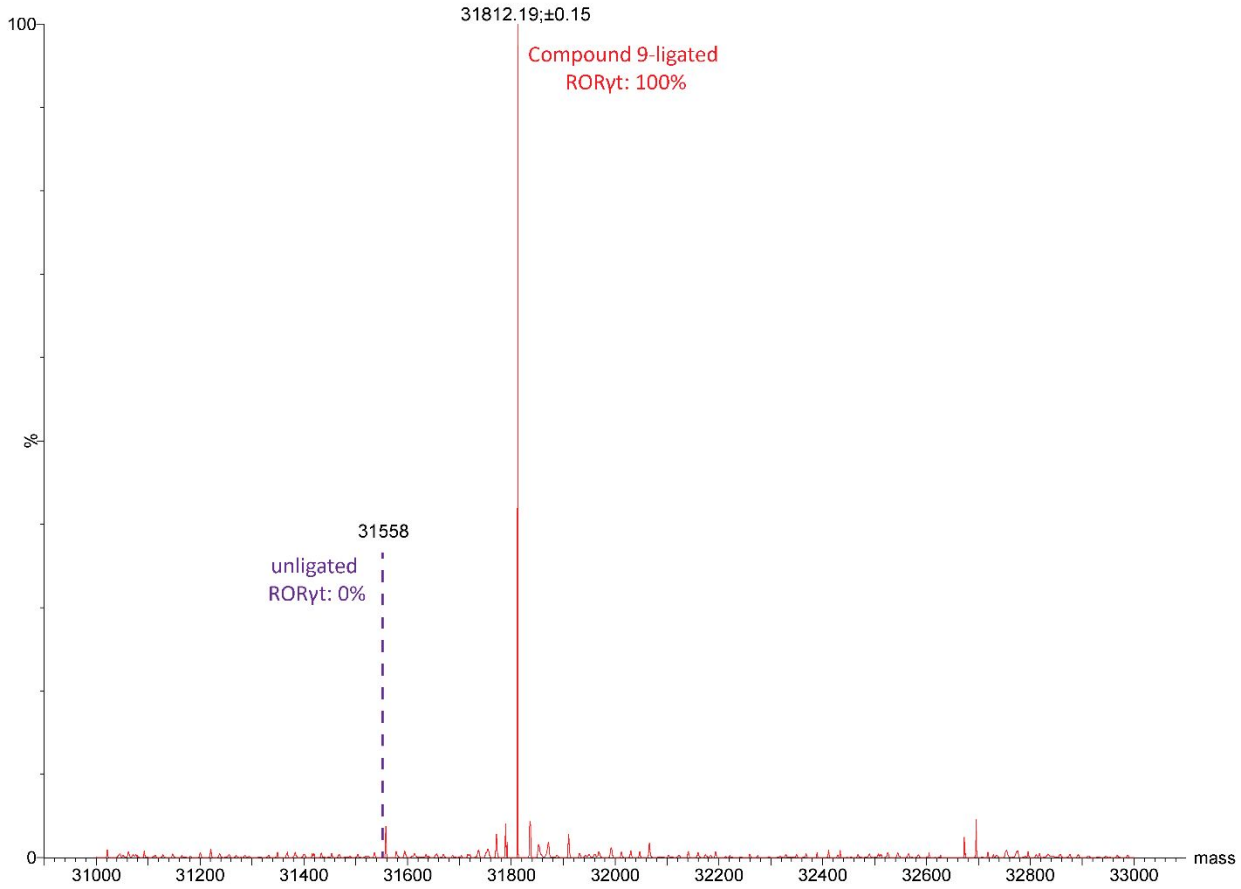


Figure S7. Deconvoluted Q-TOF spectrum of RORγt ligated to compound **9**: 100% ligation is observed, while 0% unligated protein is visible (mass: 31558.11 Da). Mass expected: 31811.70 Da, mass observed: 31812.19 Da.

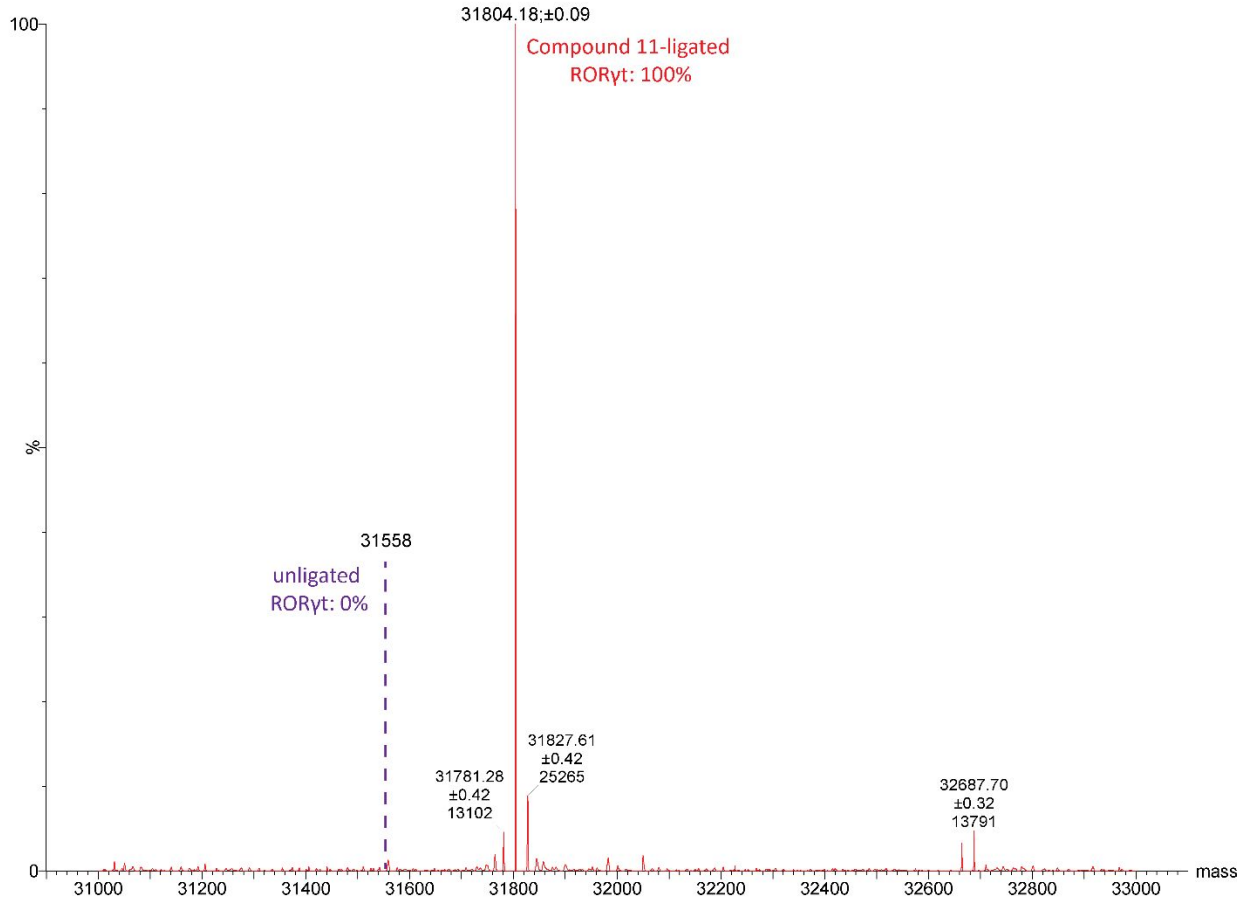


Figure S8. Deconvoluted Q-TOF spectrum of RORγt ligated to compound **11**: 100% ligation is observed, while 0% unligated protein is visible (mass: 31558.11 Da). Mass expected: 31804.35 Da, mass observed: 31804.18 Da.

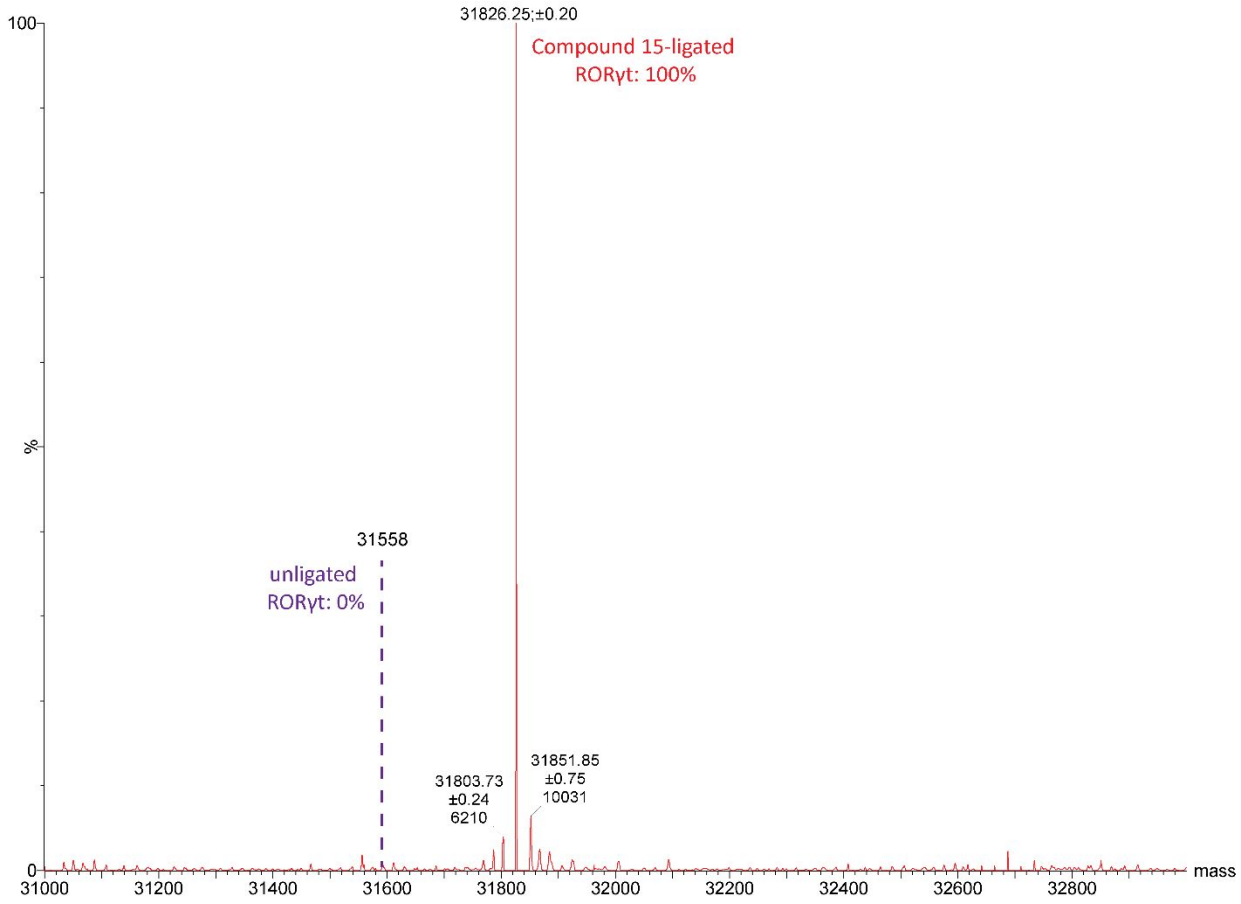


Figure S9. Deconvoluted Q-TOF spectrum of RORγt ligated to compound **15**: 100% ligation is observed, while 0% unligated protein is visible (mass: 31558.11 Da). Mass expected: 31826.38 Da, mass observed: 31826.25 Da.

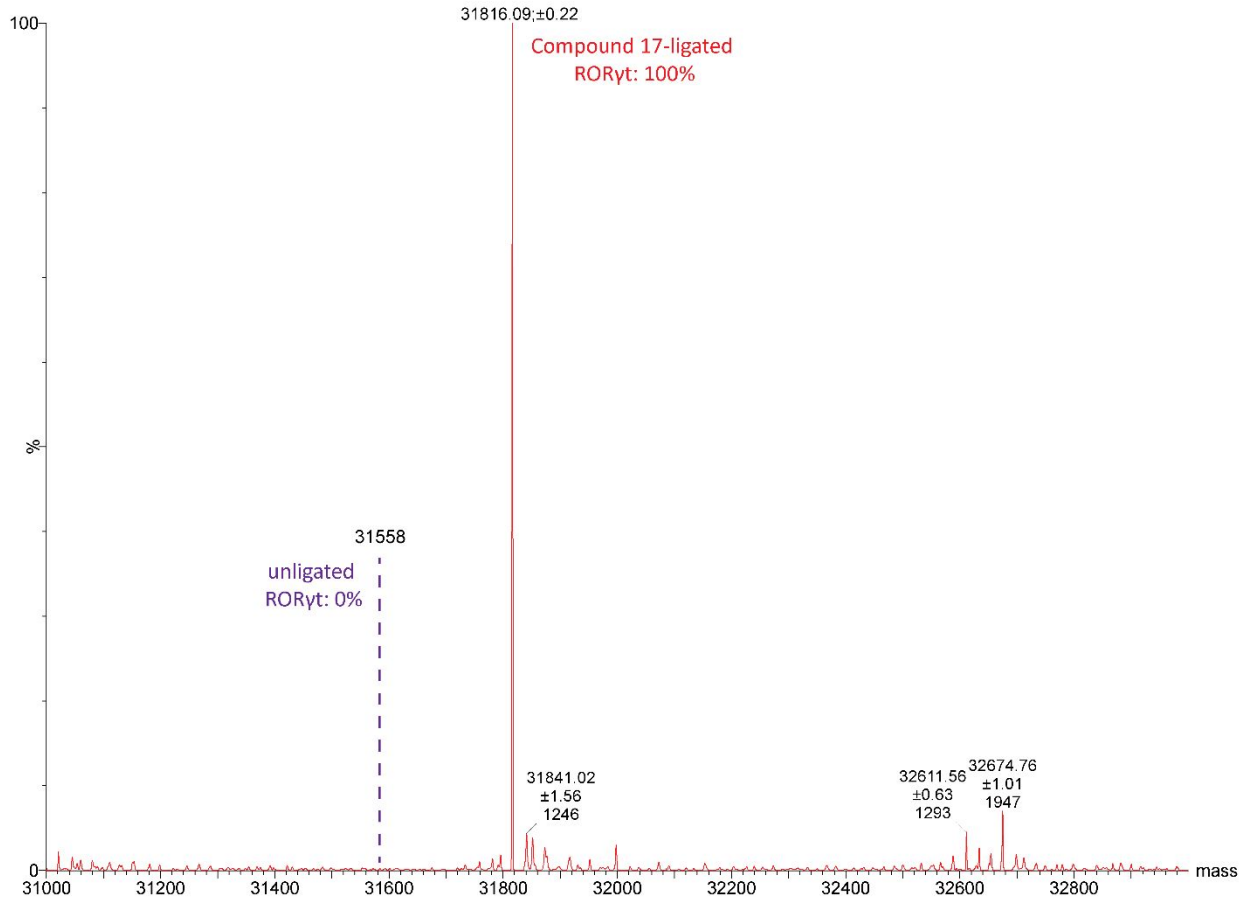


Figure S10. Deconvoluted Q-TOF spectrum of RORγt ligated to compound **17**: 100% ligation is observed, while 0% unligated protein is visible (mass: 31558.11 Da). Mass expected: 31816.32 Da, mass observed: 31816.09 Da.

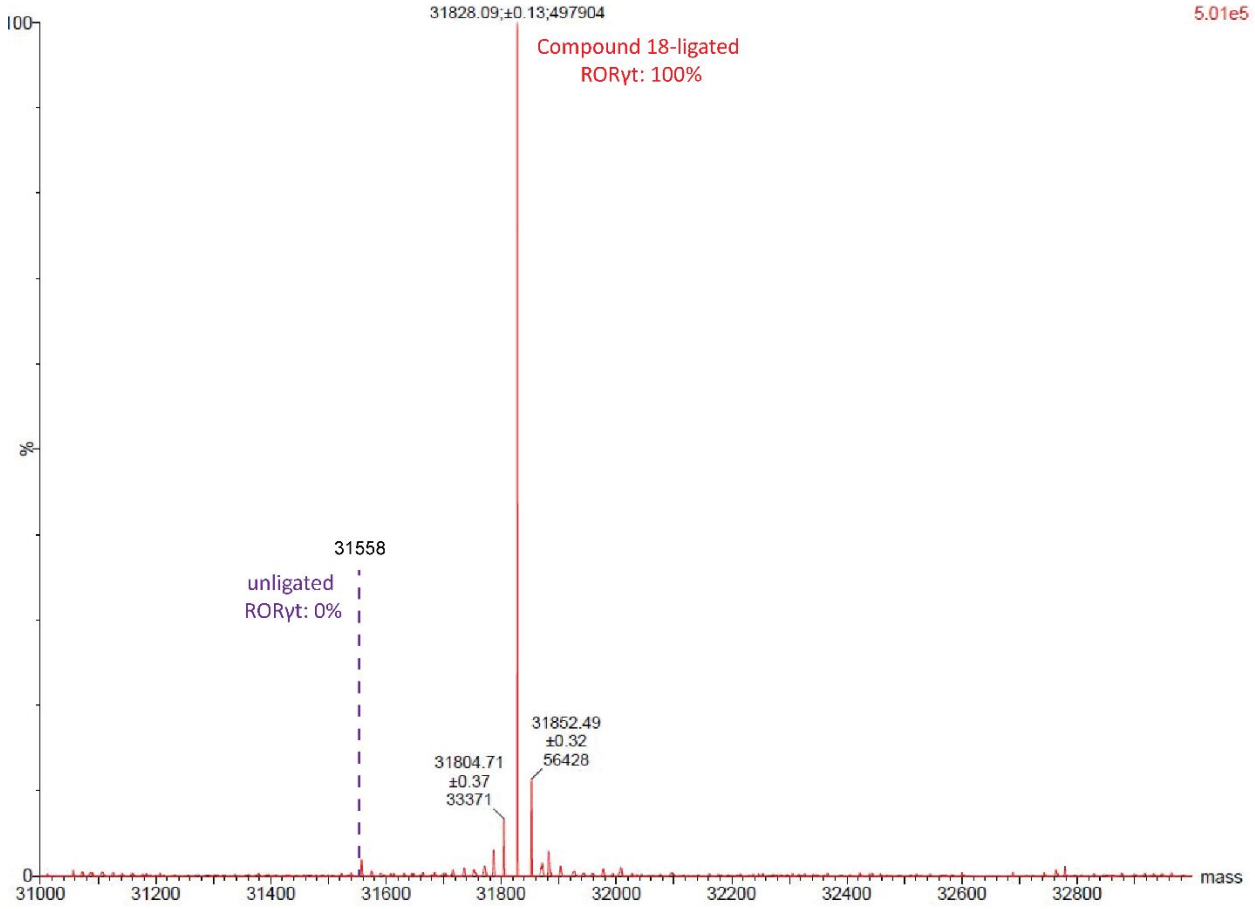


Figure S11. Deconvoluted Q-TOF spectrum of RORγt ligated to compound **18**: 100% ligation is observed, while 0% unligated protein is visible (mass: 31558.11 Da). Mass expected: 31828.35 Da, mass observed: 31828.09 Da.

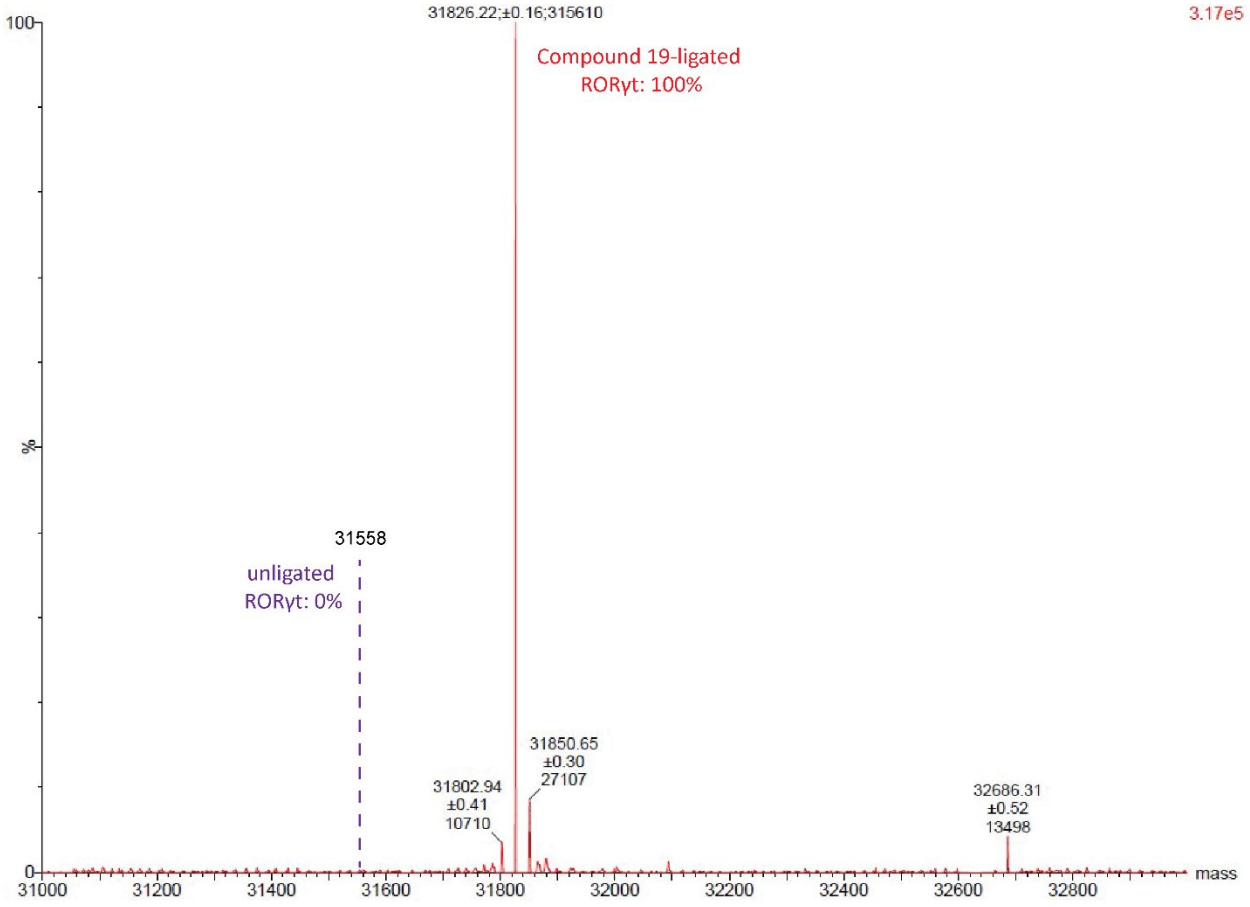


Figure S12. Deconvoluted Q-TOF spectrum of RORγt ligated to compound **19**: 100% ligation is observed, while 0% unligated protein is visible (mass: 31558.11 Da). Mass expected: 31826.38 Da, mass observed: 31826.22 Da.

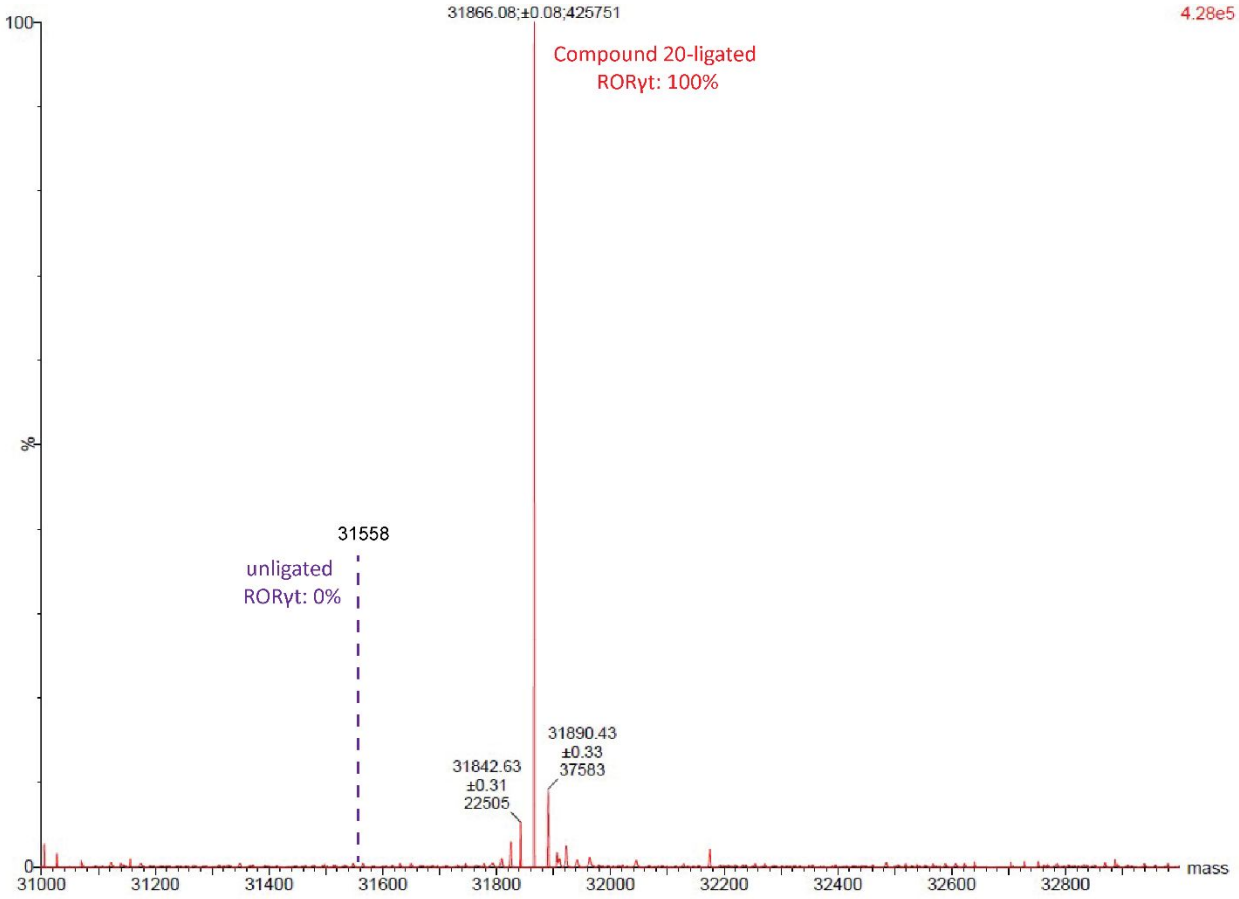


Figure S13. Deconvoluted Q-TOF spectrum of RORγt ligated to compound **20**: 100% ligation is observed, while 0% unligated protein is visible (mass: 31558.11 Da). Mass expected: 31866.32 Da, mass observed: 31866.08 Da.

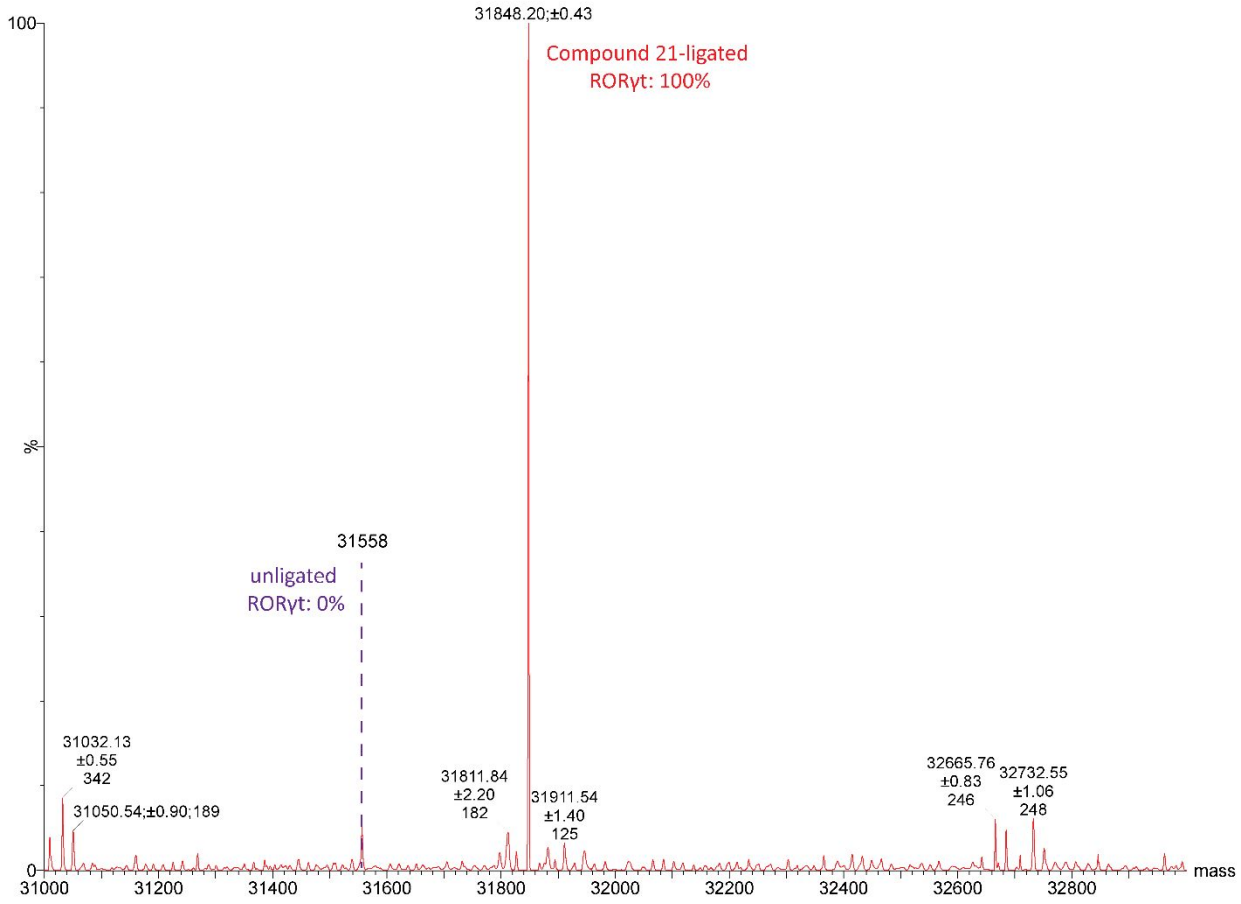


Figure S14. Deconvoluted Q-TOF spectrum of RORγt ligated to compound **21**: 100% ligation is observed, while 0% unligated protein is visible (mass: 31558.11 Da). Mass expected: 31848.39 Da, mass observed: 31848.20 Da.

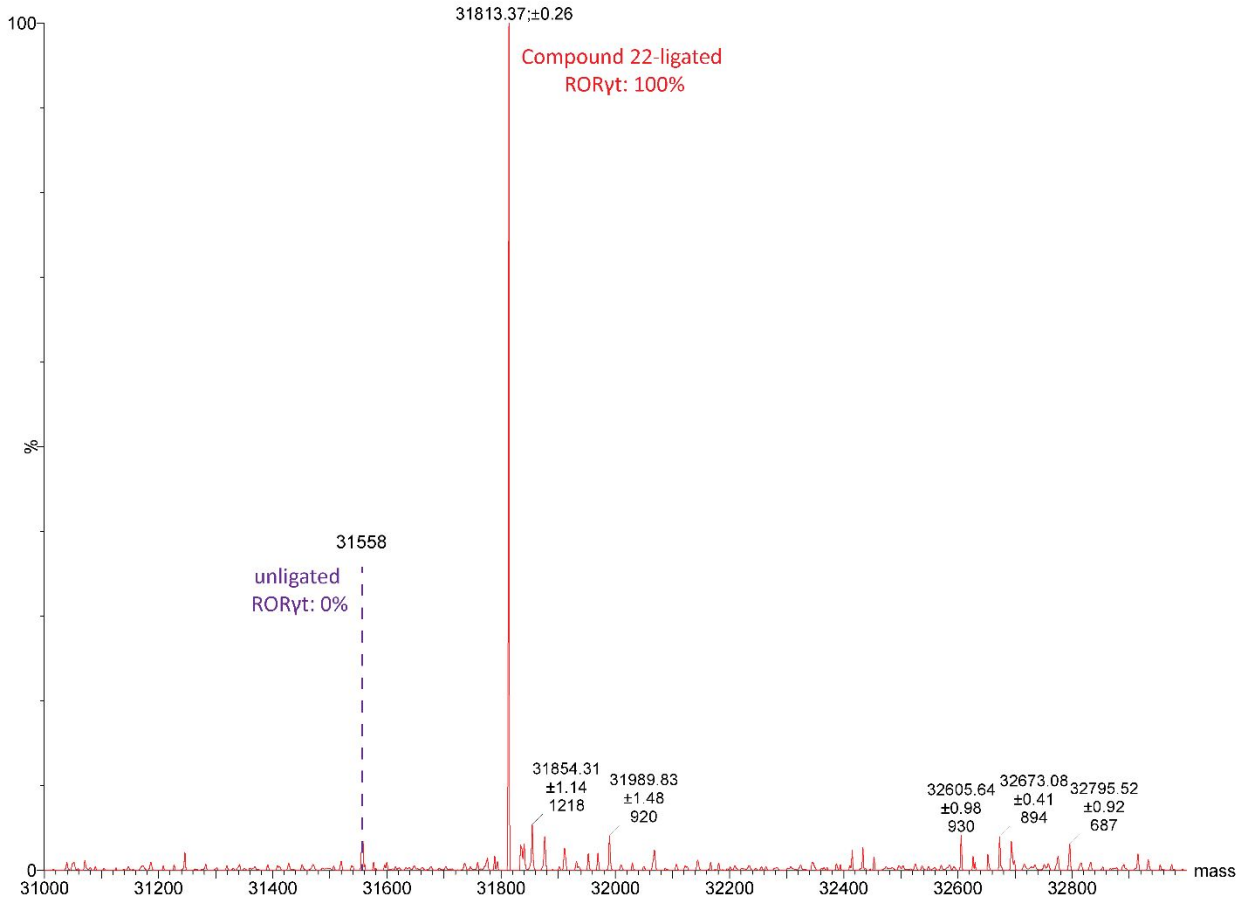


Figure S15. Deconvoluted Q-TOF spectrum of RORγt ligated to compound **22**: 100% ligation is observed, while 0% unligated protein is visible (mass: 31558.11 Da). Mass expected: 31813.34 Da, mass observed: 31813.37 Da.

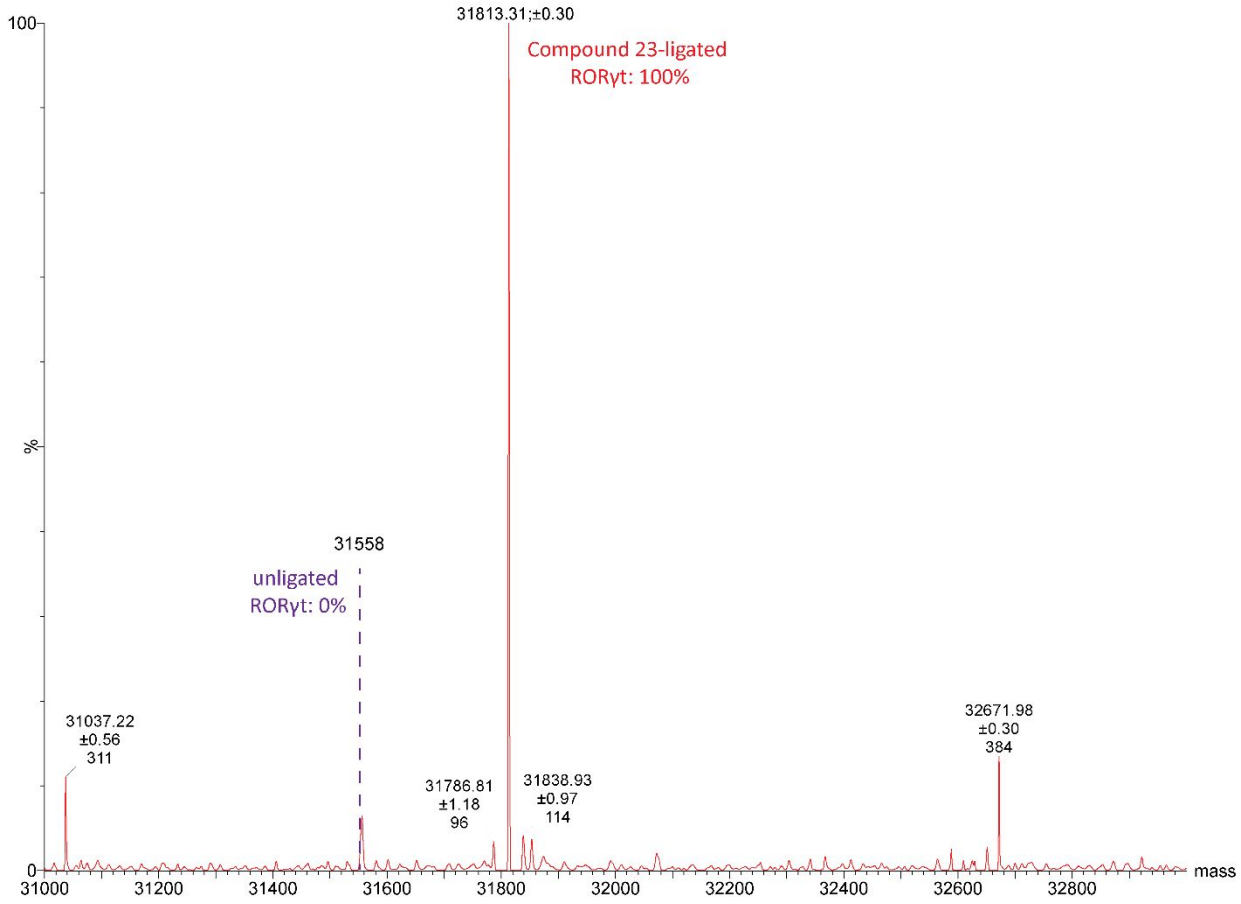
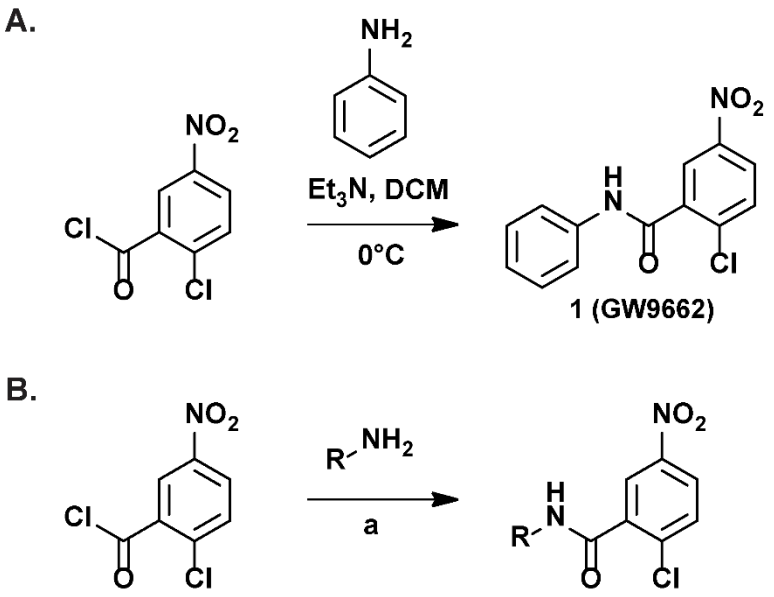


Figure S16. Deconvoluted Q-TOF spectrum of RORγt ligated to compound **23**: 100% ligation is observed, while 0% unligated protein is visible (mass: 31558.11 Da). Mass expected: 31814.33 Da, mass observed: 31813.31 Da.



Scheme S1. A. Synthesis route to obtain compound **GW9662** via an amide coupling reaction. **B.** General synthesis route for all compounds.[a]

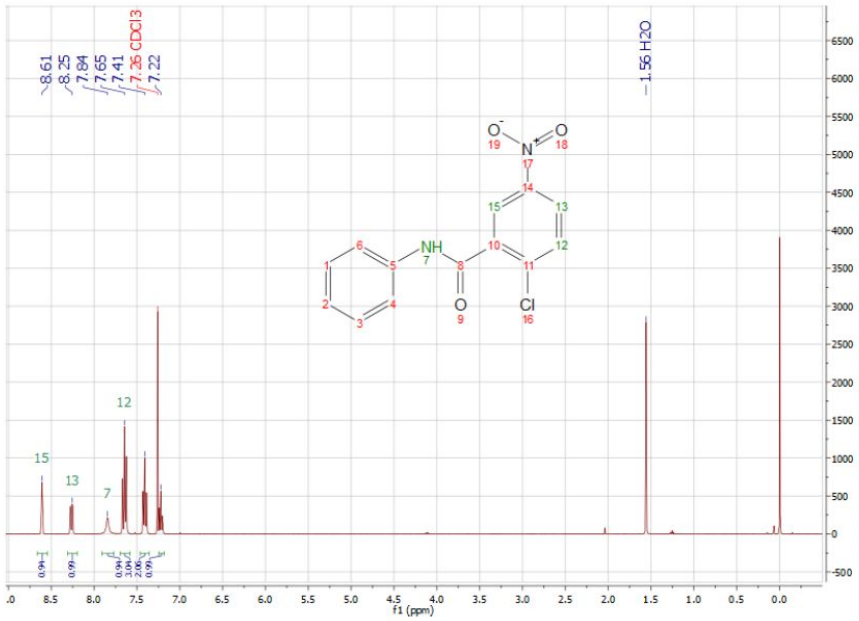
[a] Reagents and conditions: (a) amine, triethylamine, DCM, 0°C, 18h, or: pyridine, 0°C, 18h, or: DMA, RT, 18h, or: Et_3N, DMAP, DCM, RT, 18h, or: THF, RT, 3h. The reaction conditions for each compound are specified in the experimental section.

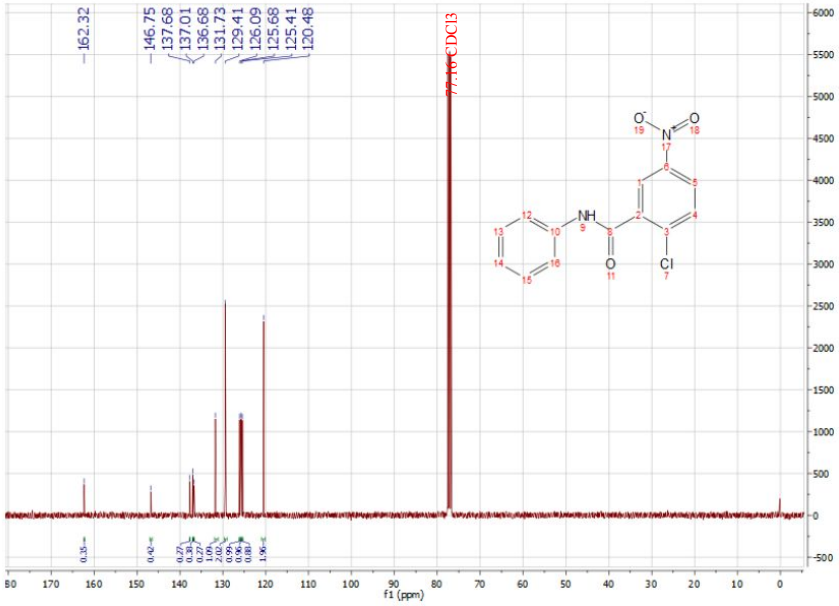
Table S1. Optimal ligation conditions for all compounds.

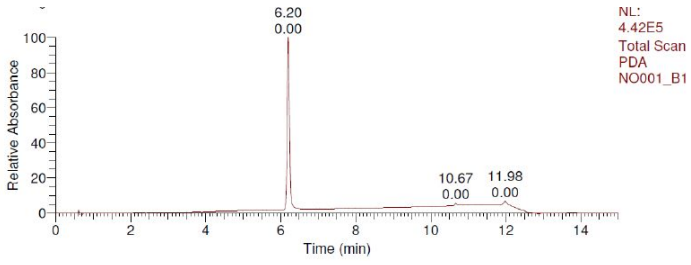
Compound	Ligation conditions
1 (GW9662), 11, 15, 16 & 17	pH=5.8, 5 eq, 4°C
2 (SB1404), 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 18, 19, 20 & 23	pH=5.8, 5 eq, RT
21	pH=5.8, 10 eq, 4°C
22	pH=5.3, 10 eq, RT

S3.0. NMR spectra (^{1}H & ^{13}C) and LC-UV/TIC traces for assayed compounds

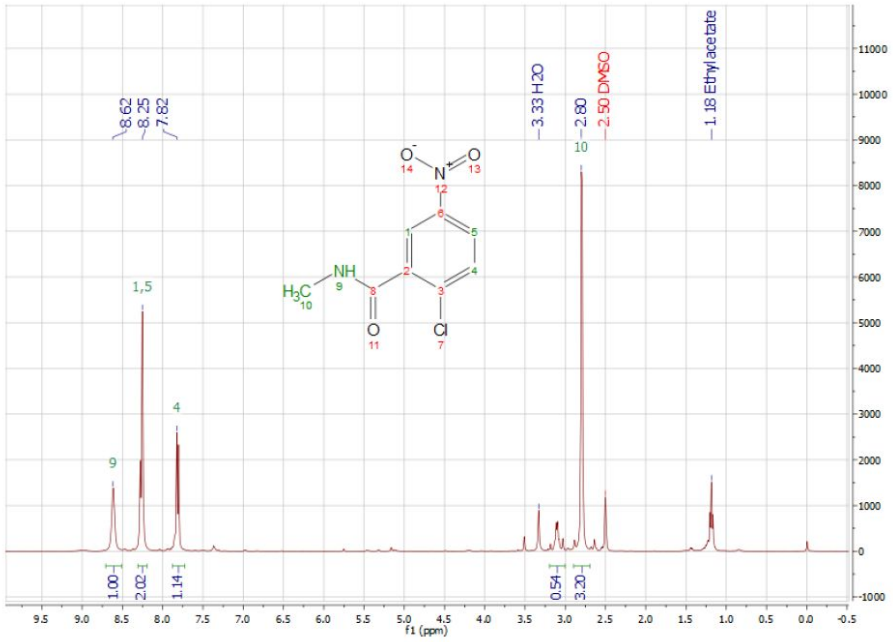
Compound 1 (GW9662)

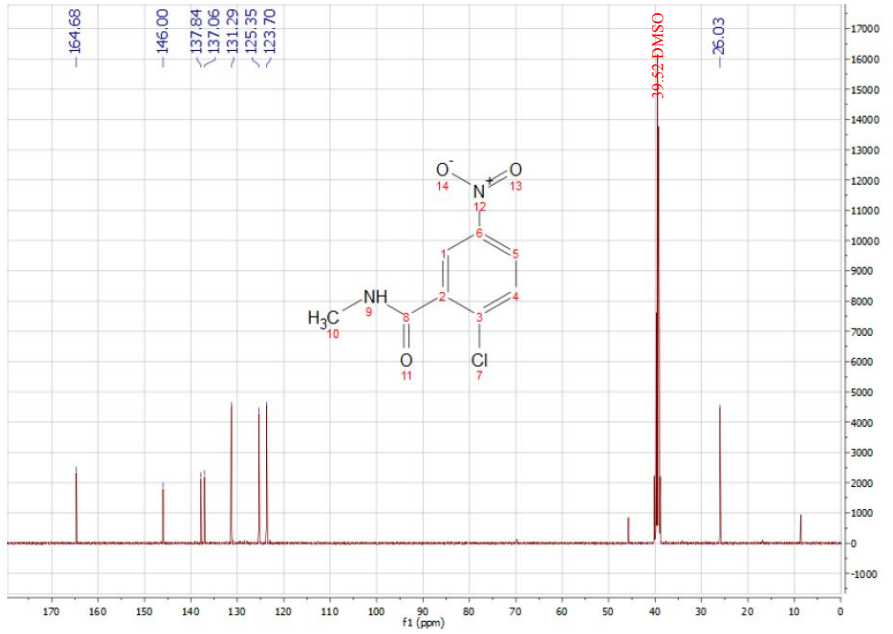


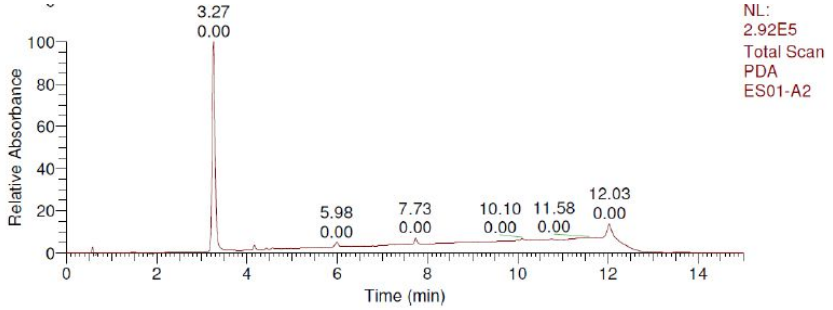




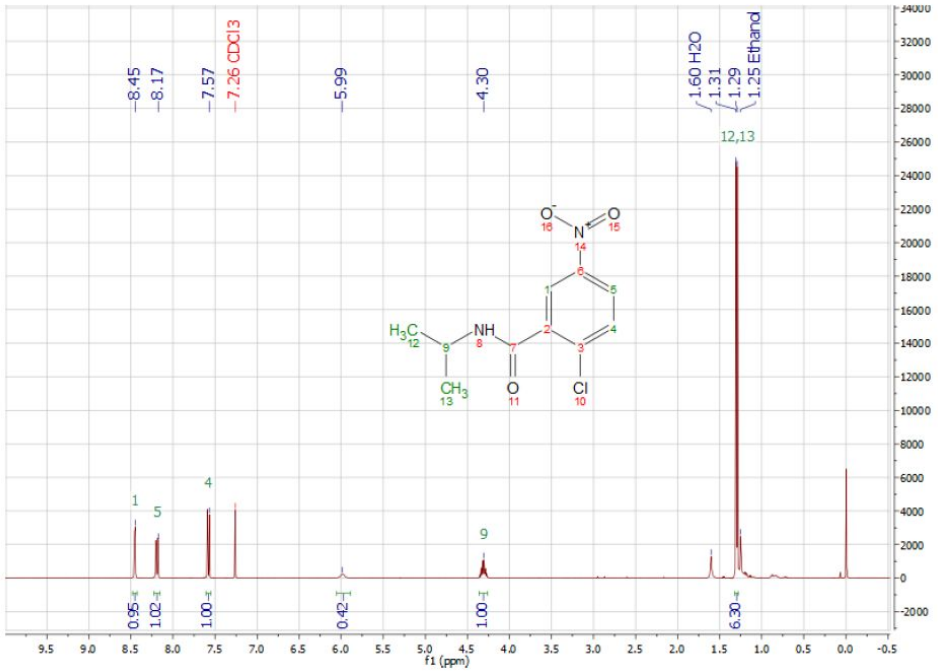
Compound 2 (SB1404)

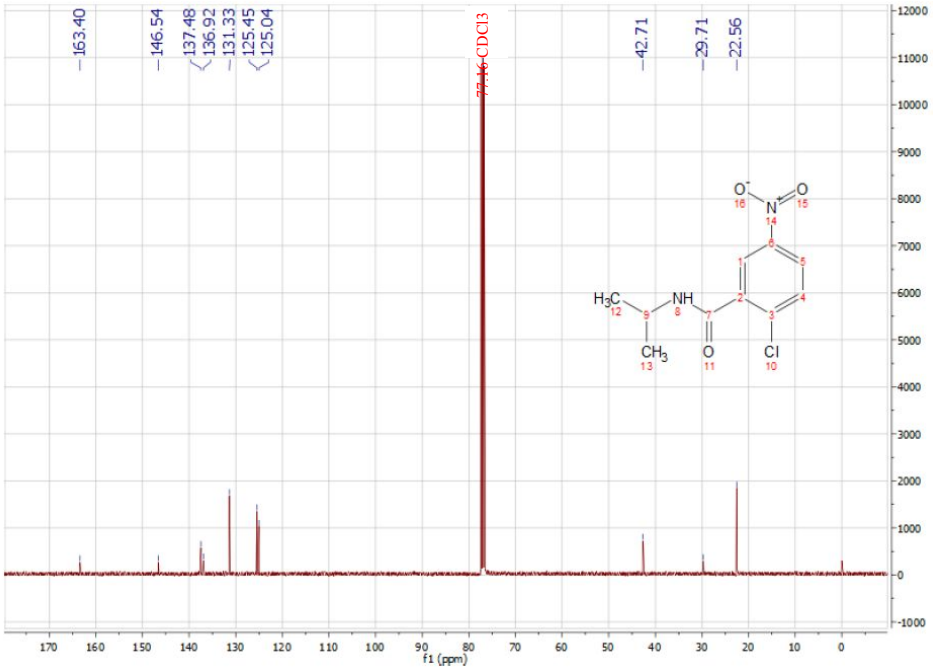


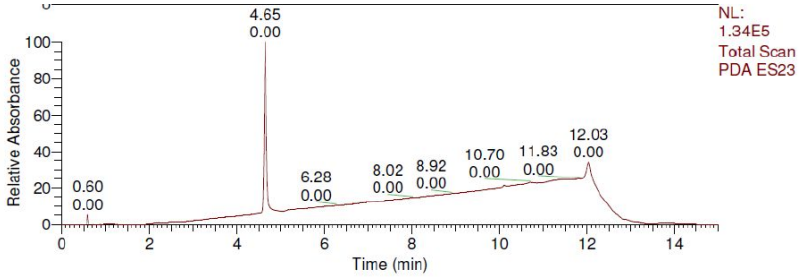




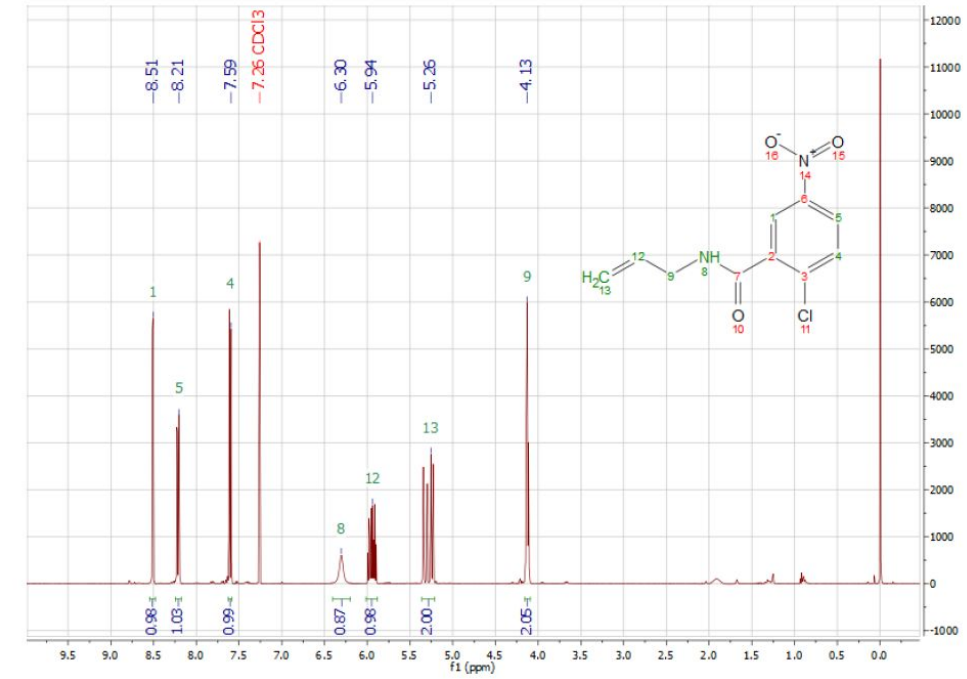
Compound 3

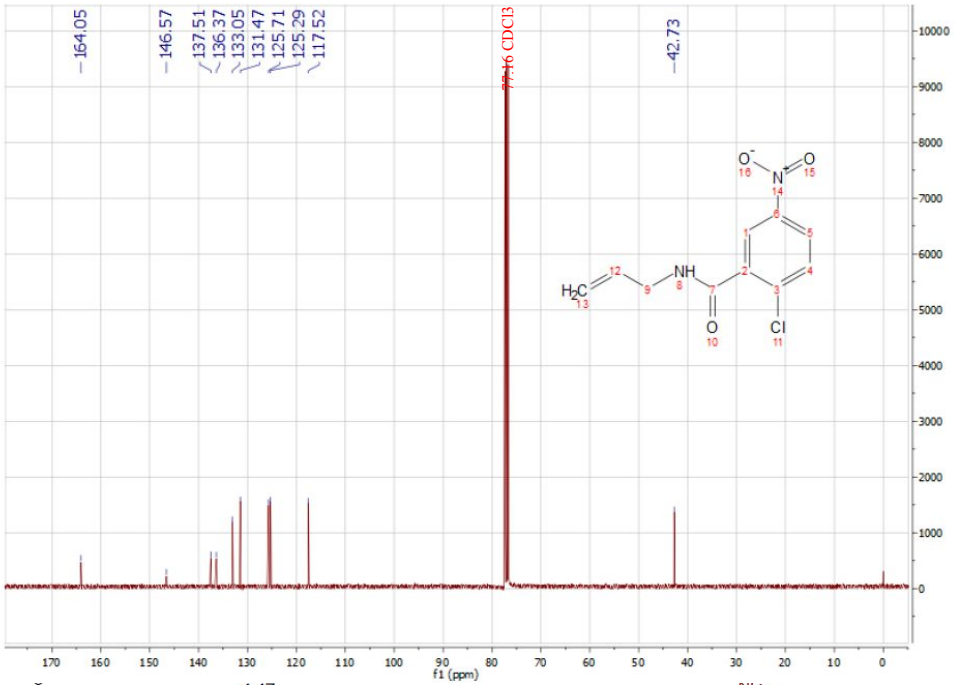

8.45
8.17
7.57
7.26 CDCl3
5.99
4.30
1.60 H2O
1.31
1.29
1.25 Ethanol
12,13
1
5
4
9
0.95
1.02
1.00
0.42
1.00
6.30
f1 (ppm)

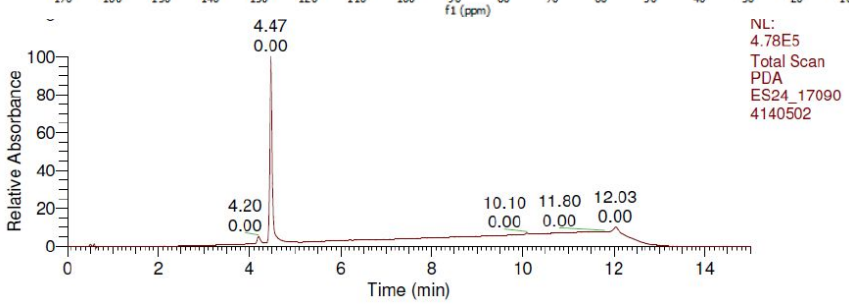

163.40
146.54
137.48
136.92
131.33
125.45
125.04
77.16 CDCl3
42.71
29.71
22.56
f1 (ppm)


NL:
1.34E5
Total Scan
PDA ES23
Relative Absorbance
Time (min)
4.65
0.00
0.60
0.00
6.28
0.00
8.02
0.00
8.92
0.00
10.70
0.00
11.83
0.00
12.03
0.00

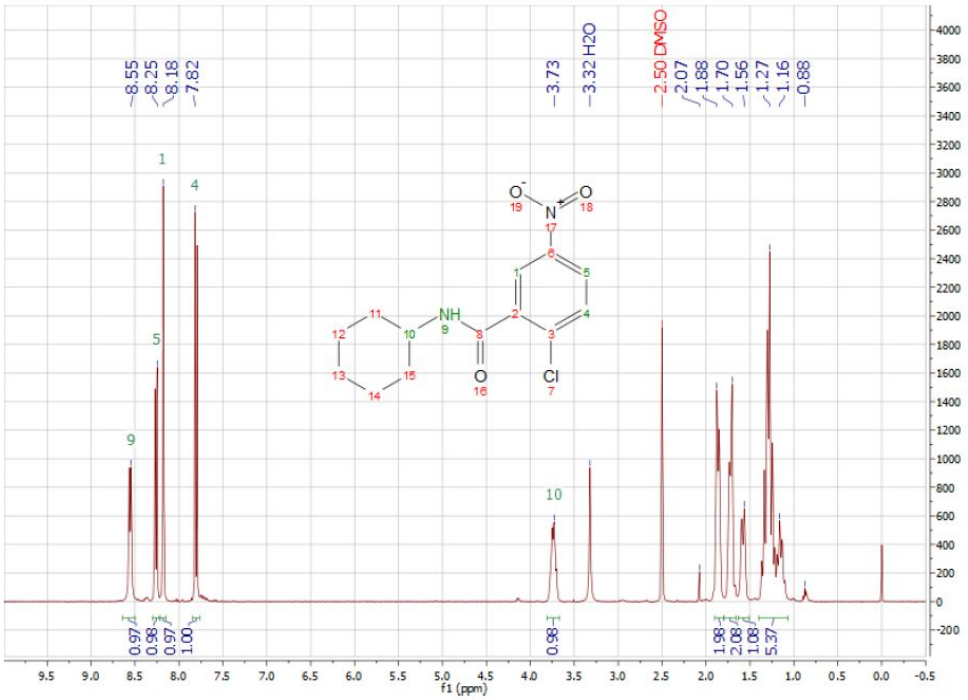
Compound 4

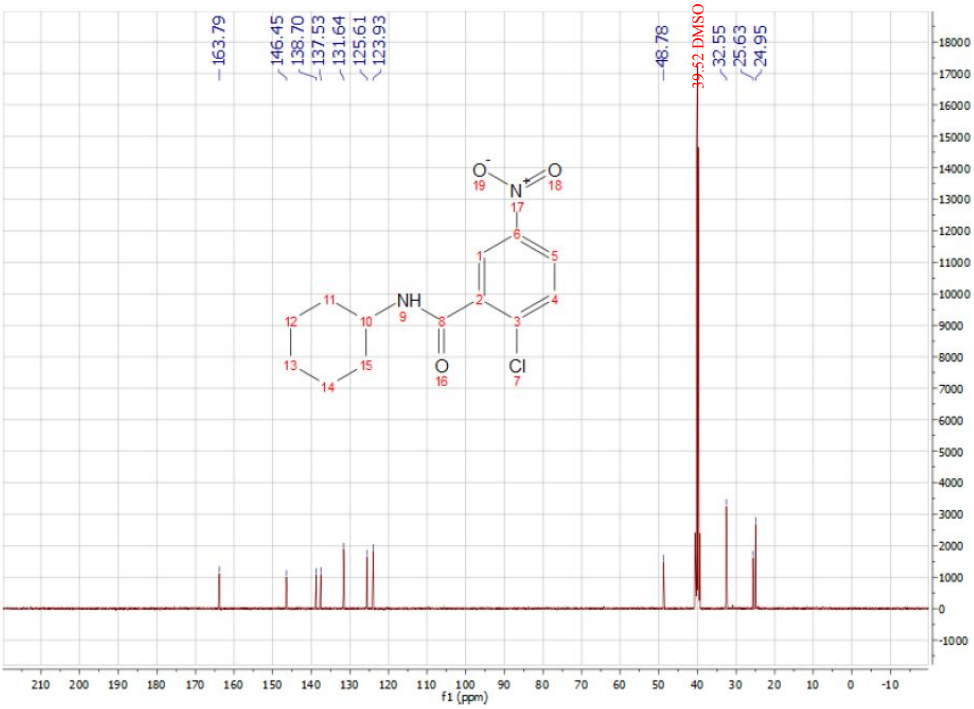

8.51
8.21
7.99
7.26 CDCl3
6.30
5.94
5.26
4.13
f1 (ppm)

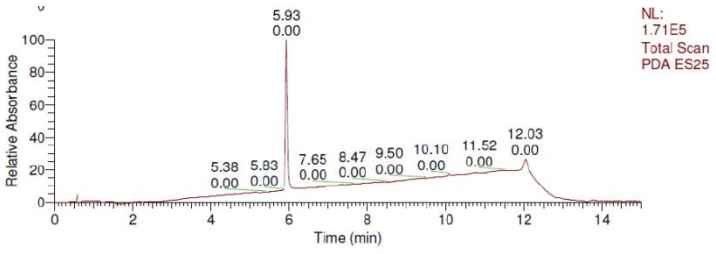

164.05
146.57
137.51
136.37
133.05
131.47
125.71
125.29
117.52
77.16 CDCl3
42.73
f1 (ppm)


4.47
0.00
4.20
0.00
10.10
0.00
11.80
0.00
12.03
0.00
NL:
4.78E5
Total Scan
PDA
ES24_17090
4140502
Relative Absorbance
Time (min)

Compound 5


8.55
8.25
8.18
7.82
3.73
3.32 H2O
2.50 DMSO
2.07
1.88
1.70
1.56
1.27
1.16
0.88
f1 (ppm)


163.79
146.45
138.70
137.53
131.64
125.61
123.93
48.78
39.52 DMSO
32.55
25.63
24.95
f1 (ppm)


5.93
0.00
NL:
1.71E5
Total Scan
PDA ES25
Relative Absorbance
Time (min)
5.38 0.00
5.93 0.00
7.65 0.00
8.47 0.00
9.50 0.00
10.10 0.00
11.52 0.00
12.03 0.00

Compound 6

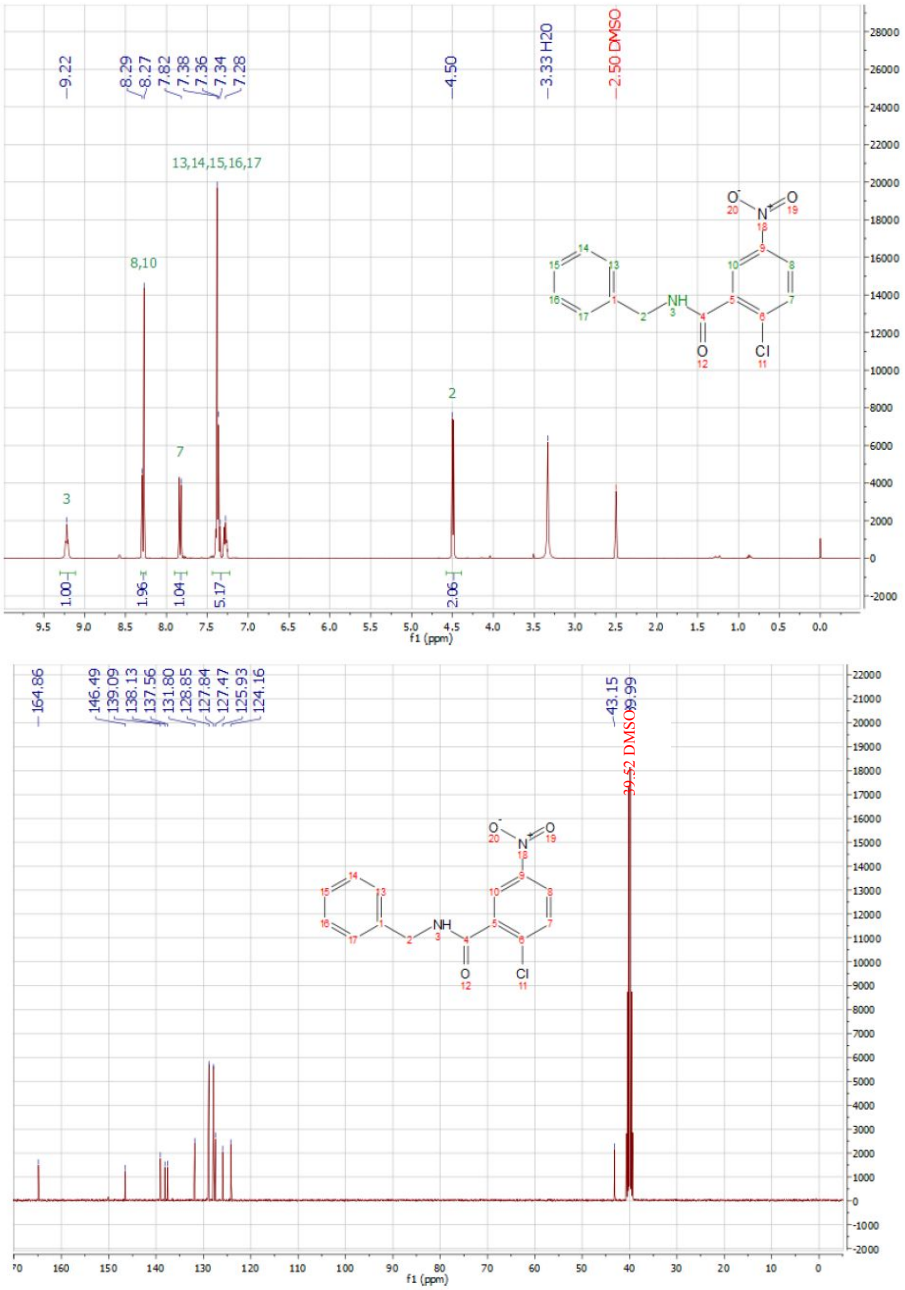
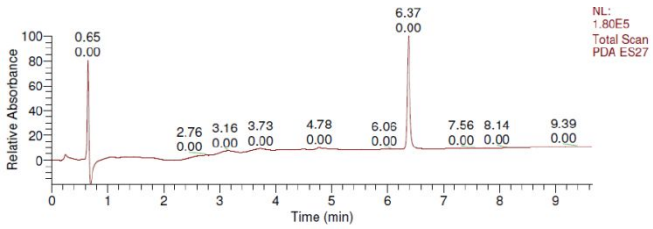
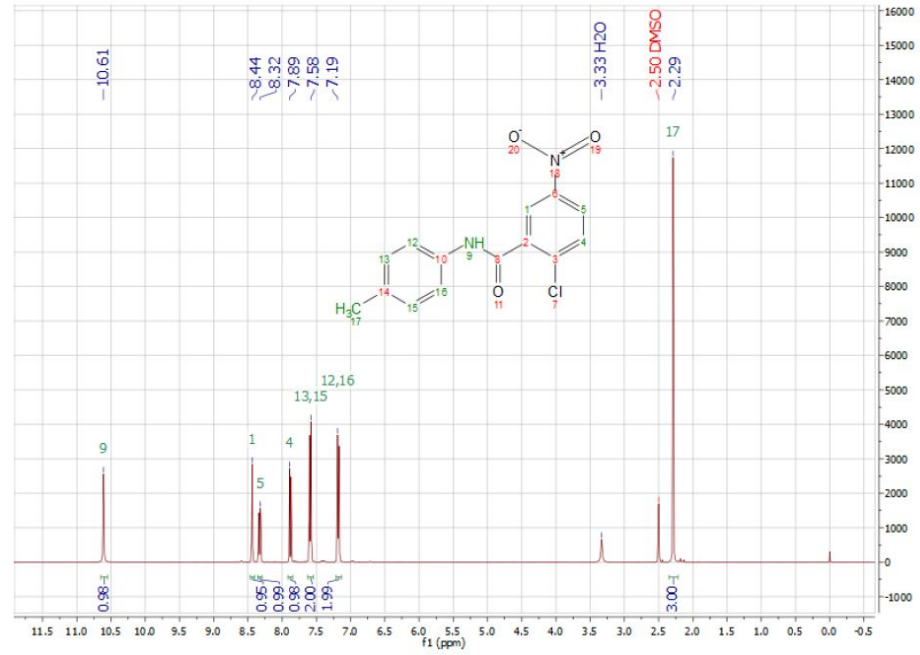
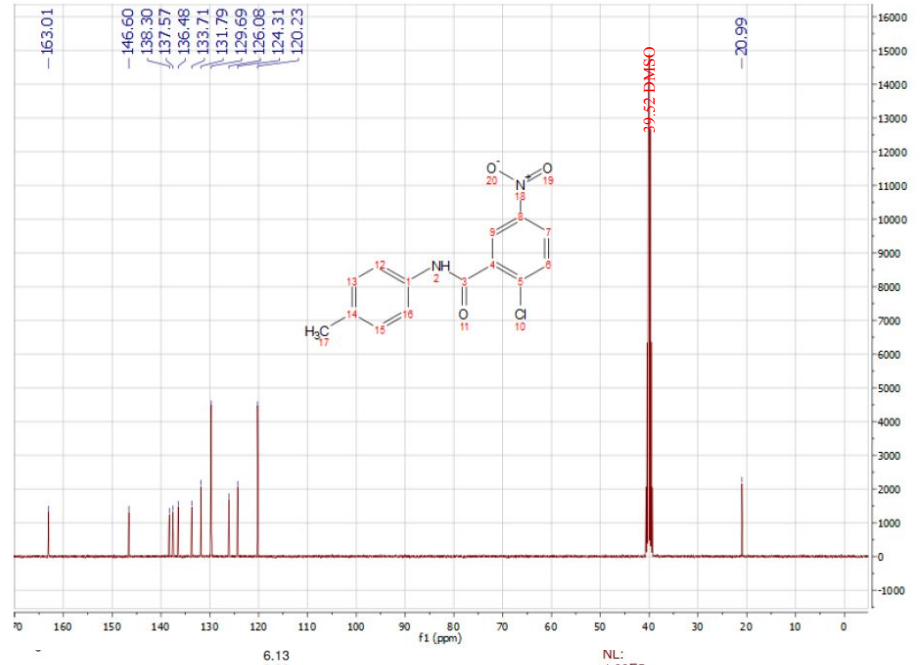


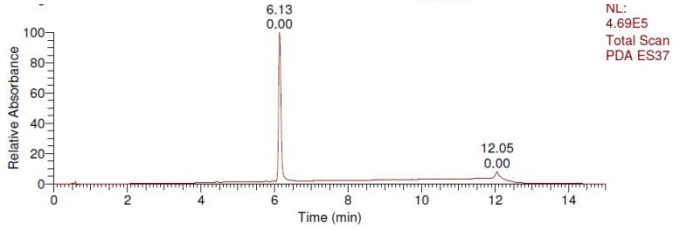
Figure A.20: *^{13}C NMR of ES27.*



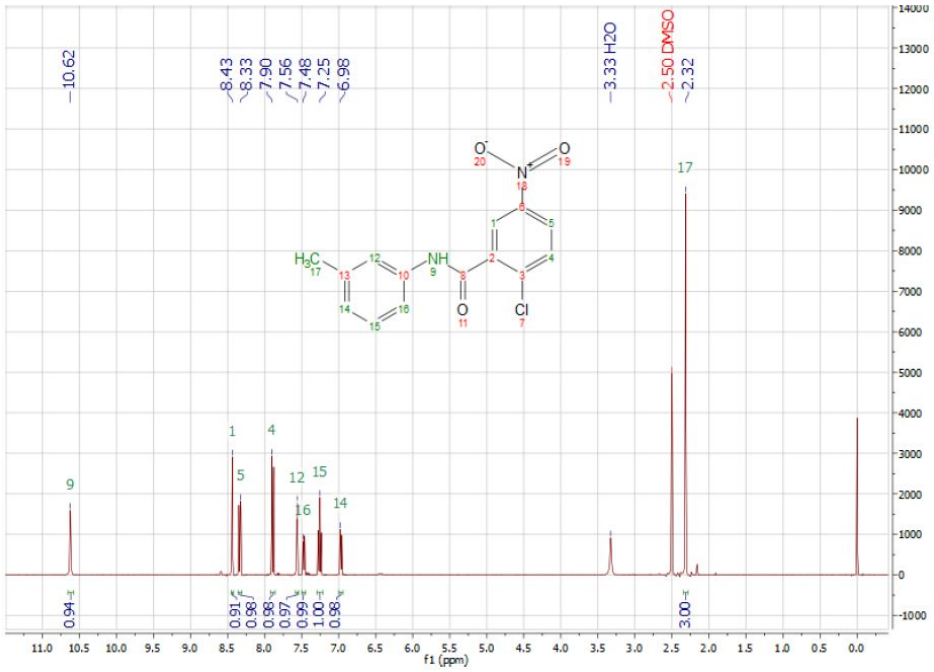
Compound 7

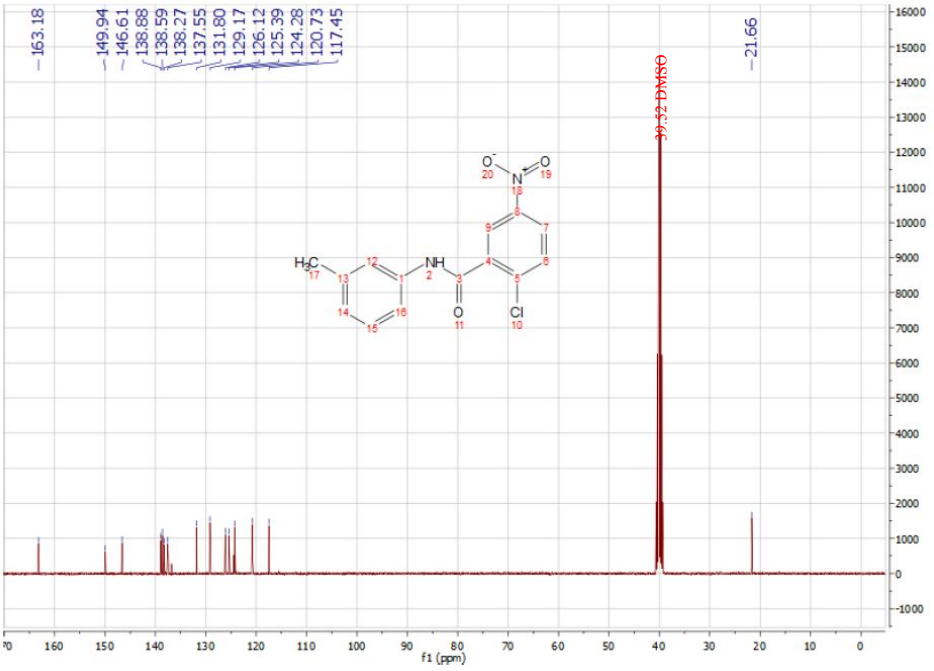

10.61
8.44
8.32
7.89
7.58
7.19
3.33 H2O
2.50 DMSO
2.29
17
12,16
13,15
9
1
4
5
f1 (ppm)

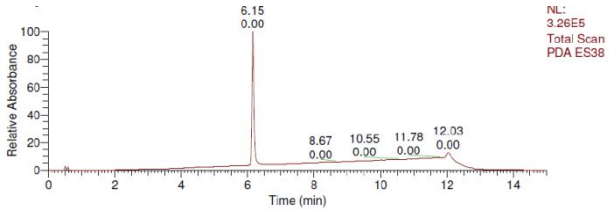

163.01
146.60
138.30
137.57
136.48
133.71
131.79
129.69
126.08
124.31
120.23
39.52 DMSO
20.99
f1 (ppm)


6.13
0.00
12.05
0.00
NL:
4.69E5
Total Scan
PDA ES37
Relative Absorbance
Time (min)

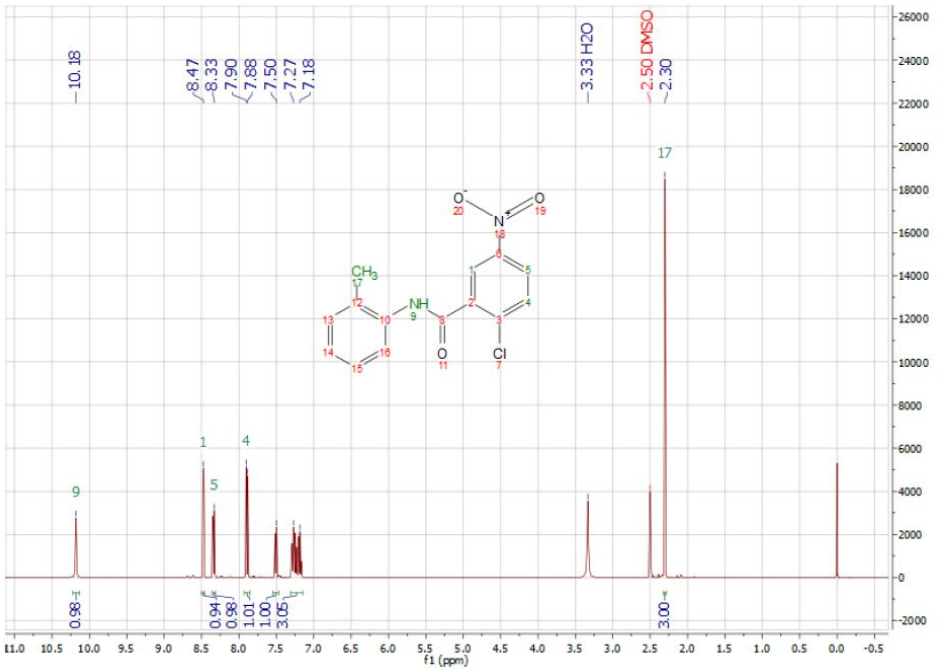
Compound 8

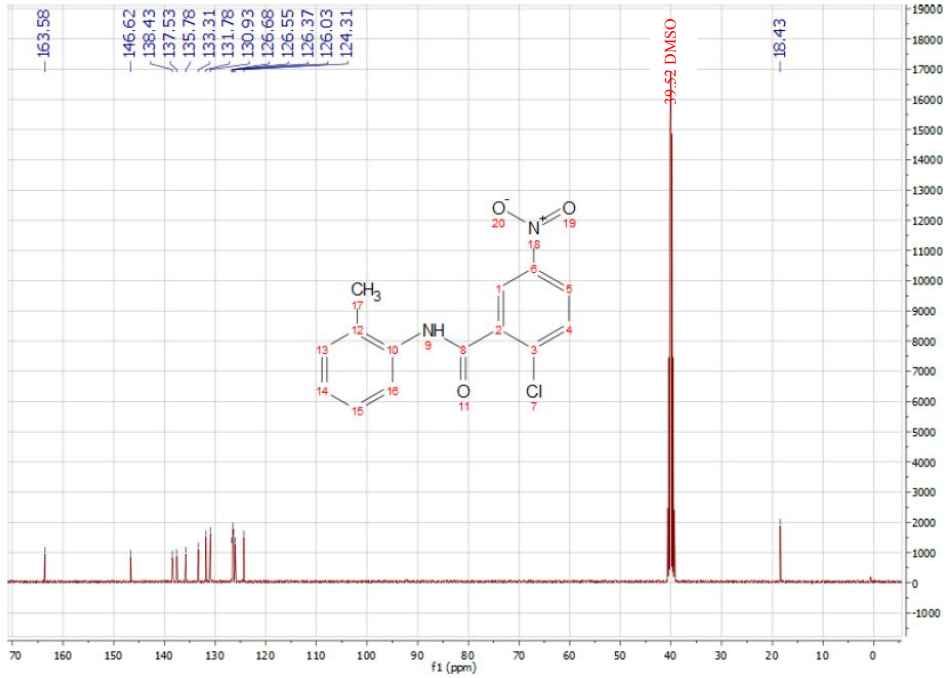

10.62
8.43
8.33
7.90
7.56
7.48
7.25
6.98
3.33 H2O
2.50 DMSO
2.32
17
1
4
5
12
15
16
14
9
0.94
0.91
0.98
0.98
0.97
0.99
1.00
0.98
3.00
f1 (ppm)

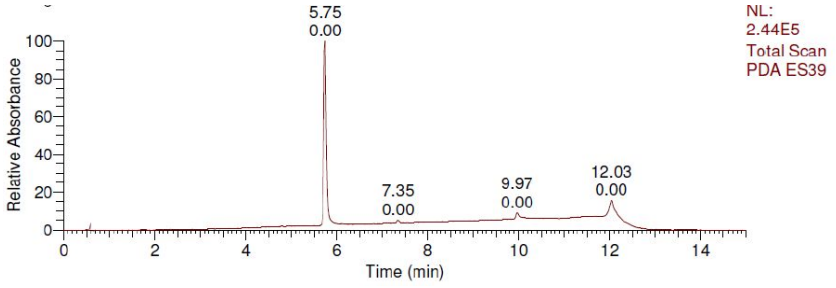

163.18
149.94
146.61
138.88
138.59
138.27
137.55
131.80
129.17
126.12
125.39
124.28
120.73
117.45
39.52 DMSO
21.66
f1 (ppm)


6.15
0.00
8.67
0.00
10.55
0.00
11.78
0.00
12.03
0.00
NL:
3.26E5
Total Scan
PDA ES38
Relative Absorbance
Time (min)

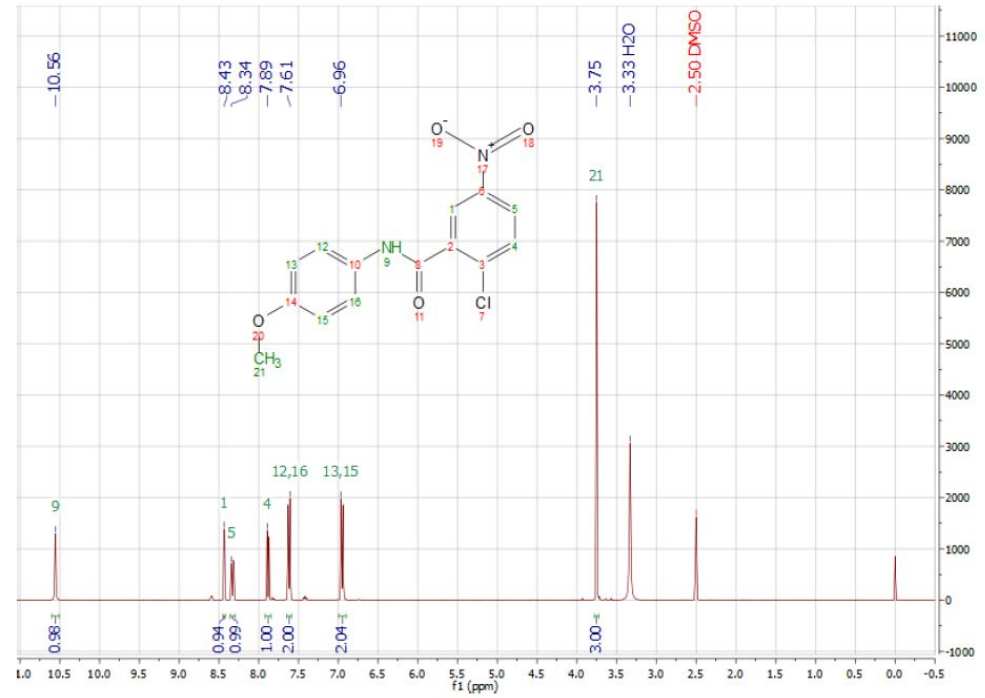
Compound 9

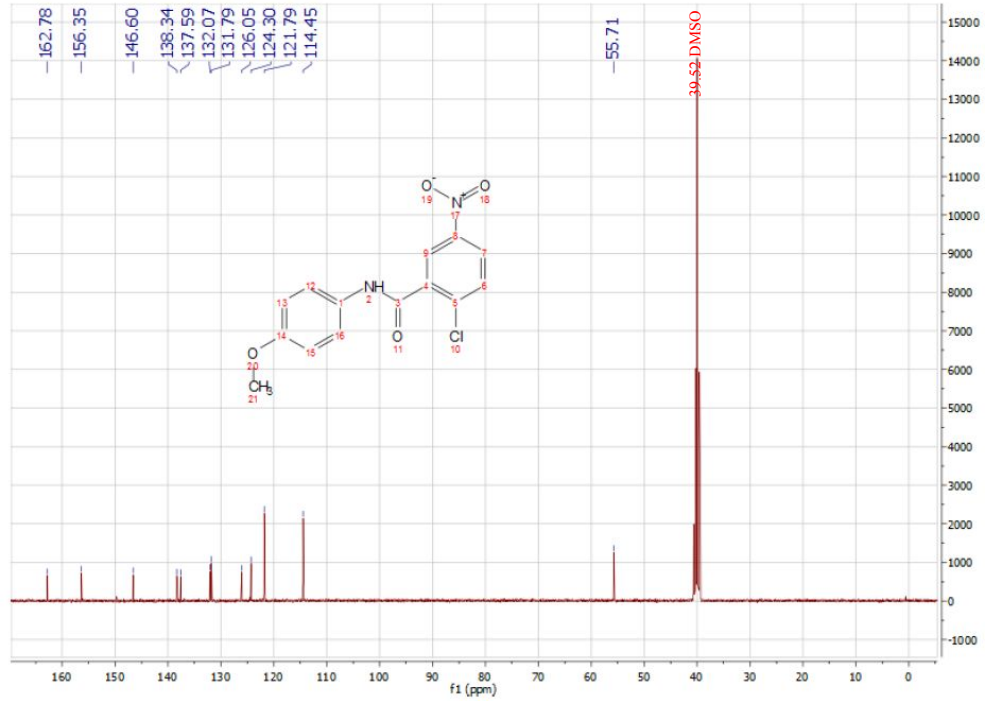

10.18
8.47
8.33
7.90
7.88
7.50
7.27
7.18
3.33 H2O
2.50 DMSO
2.30
17
9
1
5
4
0.98
0.94
0.98
1.01
1.00
3.05
3.00
f1 (ppm)

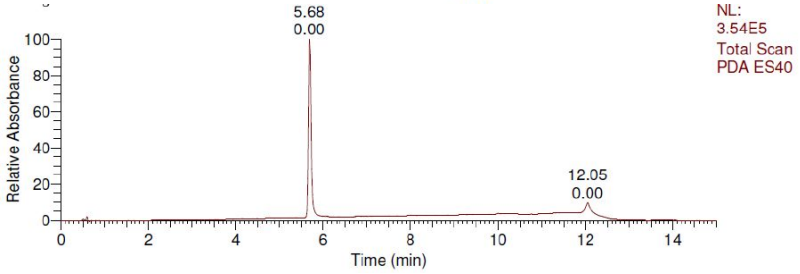

163.58
146.62
138.43
137.53
135.78
133.31
131.78
130.93
126.68
126.55
126.37
126.03
124.31
39.52 DMSO
18.43
f1 (ppm)


5.75
0.00
7.35
0.00
9.97
0.00
12.03
0.00
NL:
2.44E5
Total Scan
PDA ES39
Relative Absorbance
Time (min)

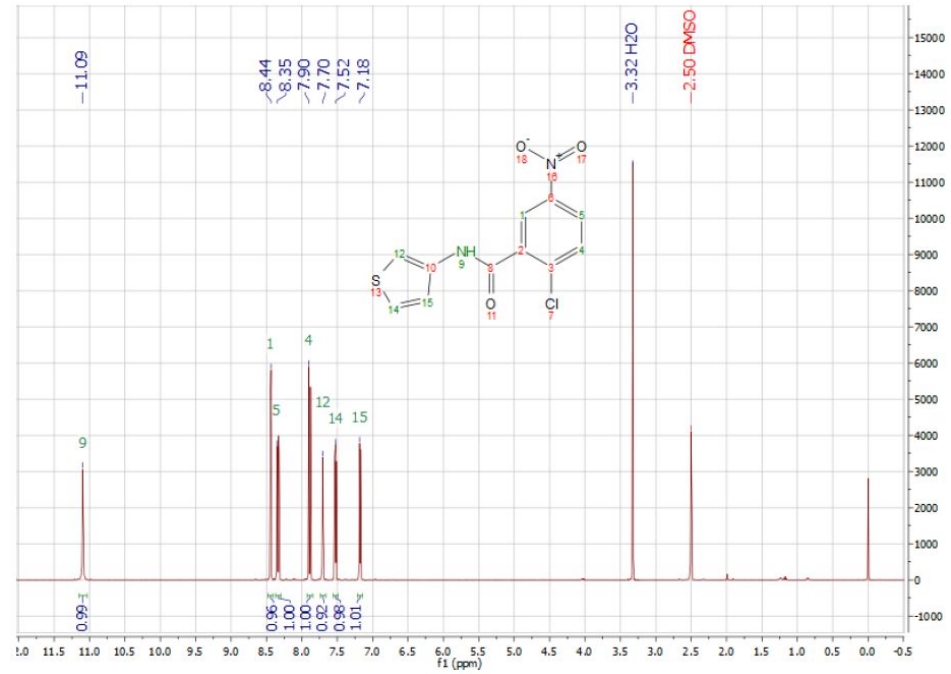
Compound 10

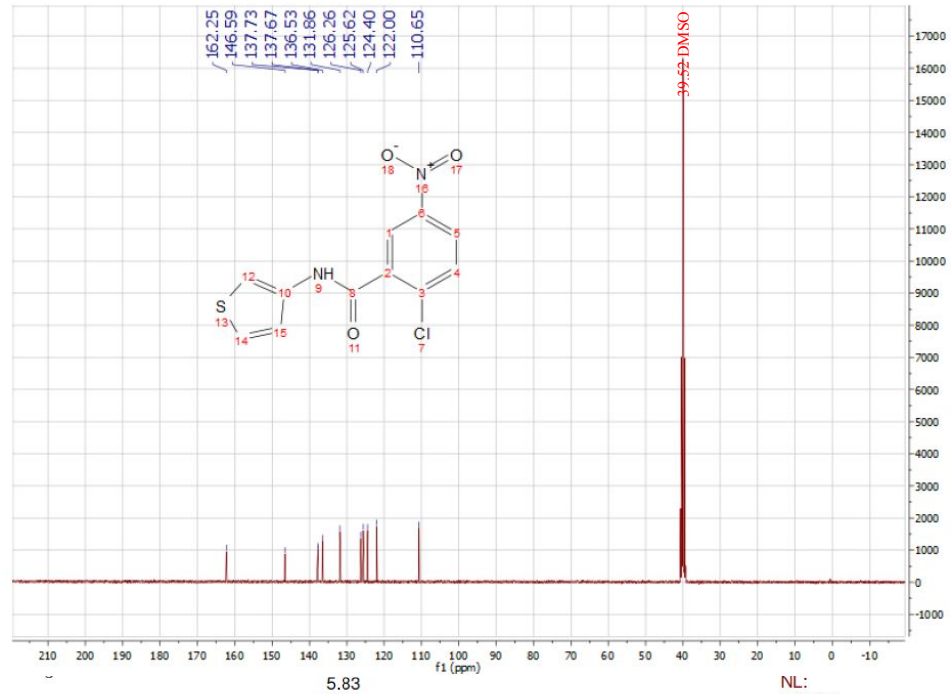

10.56
8.43
8.34
7.89
7.61
6.96
3.75
3.33 H2O
2.50 DMSO
21
12,16
13,15
9
1
4
5
0.98
0.94
0.99
1.00
2.00
2.04
3.00
f1 (ppm)

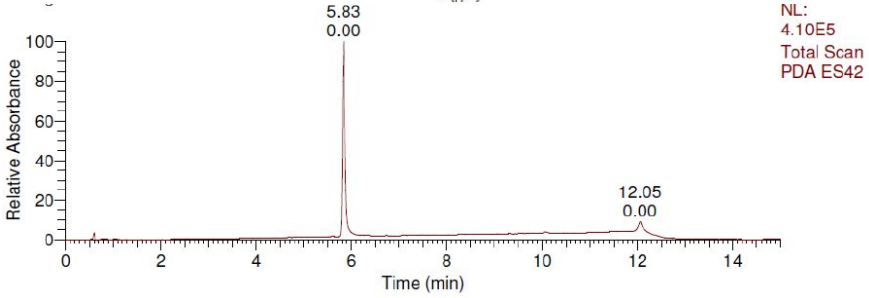

162.78
156.35
146.60
138.34
137.59
132.07
131.79
126.05
124.30
121.79
114.45
55.71
39.52 DMSO
f1 (ppm)


5.68
0.00
12.05
0.00
NL:
3.54E5
Total Scan
PDA ES40
Relative Absorbance
Time (min)

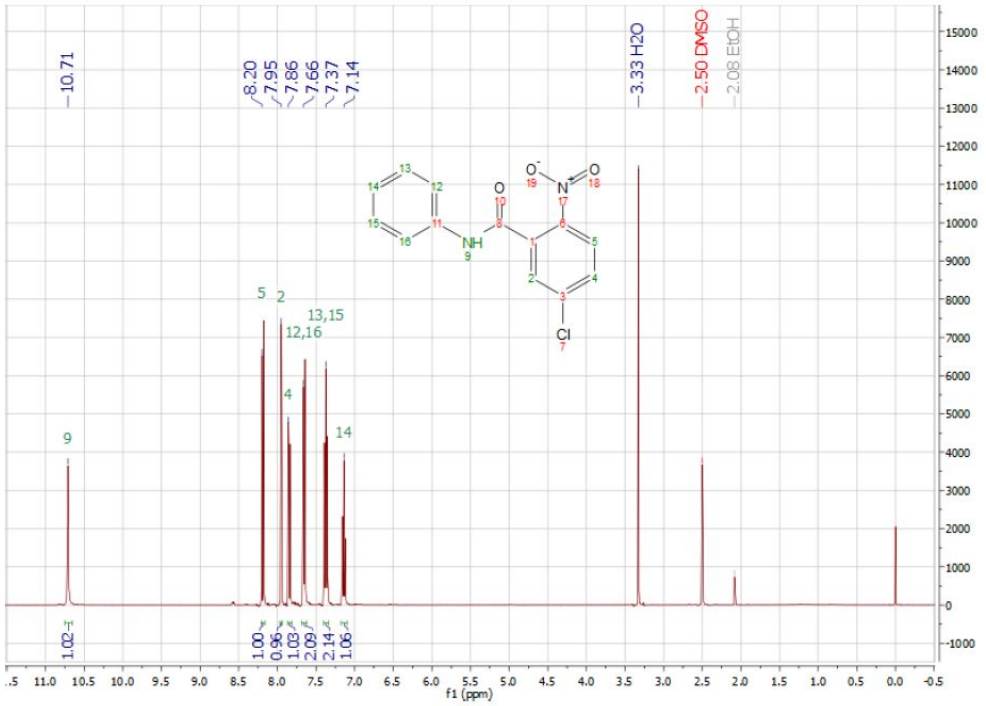
Compound 11

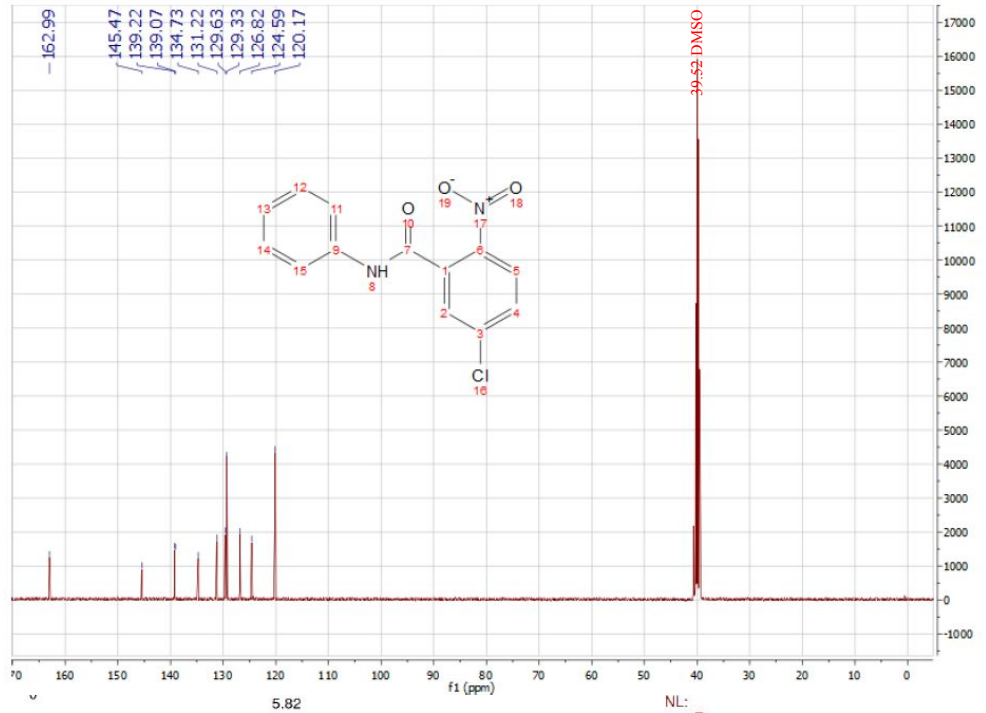

11.09
8.44
8.35
7.90
7.70
7.52
7.18
3.32 H2O
2.50 DMSO
f1 (ppm)

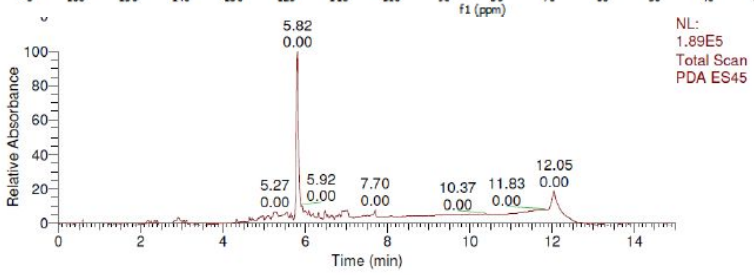

162.25
146.59
137.73
137.67
136.53
131.86
126.26
125.62
124.40
122.00
110.65
39.52 DMSO
f1 (ppm)


5.83
0.00
12.05
0.00
NL:
4.10E5
Total Scan
PDA ES42
Relative Absorbance
Time (min)

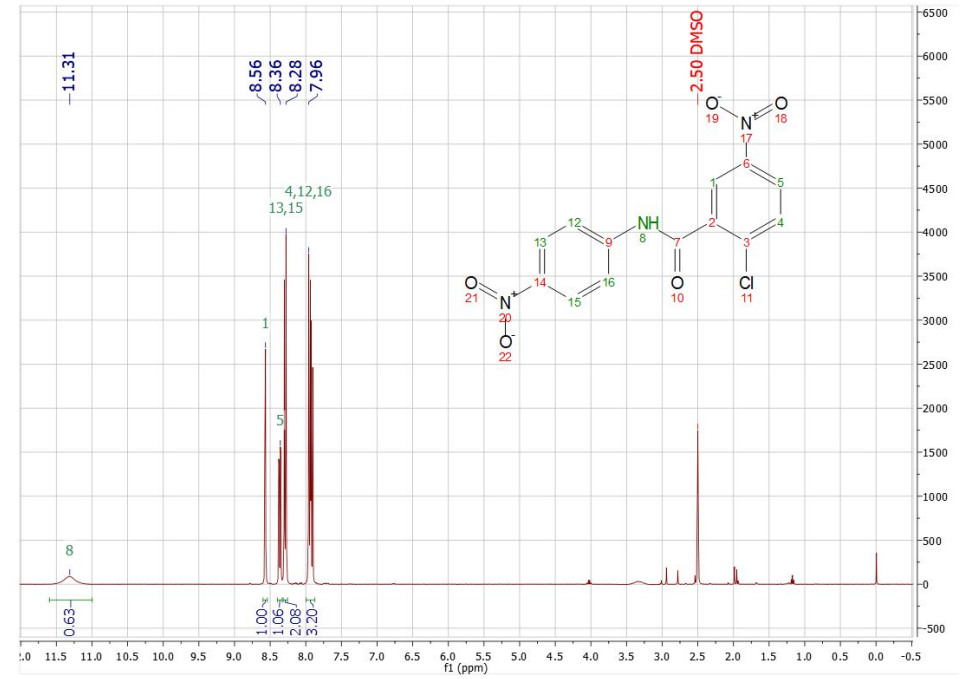
Compound 12

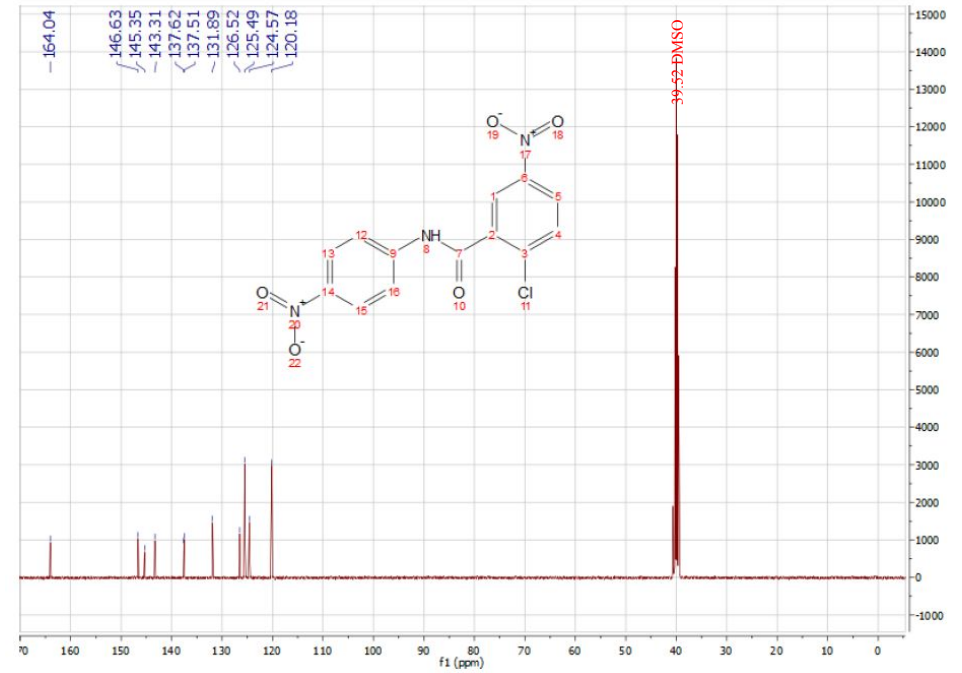

10.71
8.20
7.95
7.86
7.66
7.37
7.14
3.33 H2O
2.50 DMSO
2.08 EtOH
5
2
13,15
12,16
4
14
9
1.02
1.00
0.96
1.03
2.09
2.14
1.06
f1 (ppm)

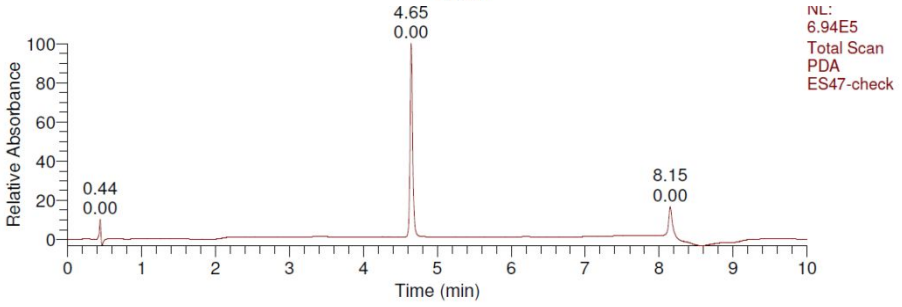

162.99
145.47
139.22
139.07
134.73
131.22
129.63
129.33
126.82
124.59
120.17
39.52 DMSO
f1 (ppm)


5.82
0.00
NL:
1.89E5
Total Scan
PDA ES45
Relative Absorbance
5.27
0.00
5.92
0.00
7.70
0.00
10.37
0.00
11.83
0.00
12.05
0.00
Time (min)

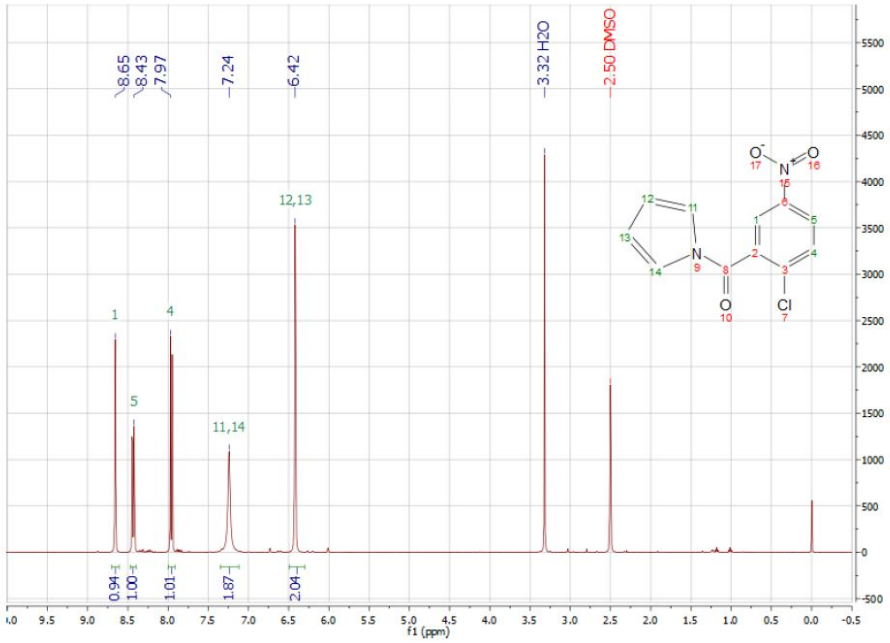
Compound 13

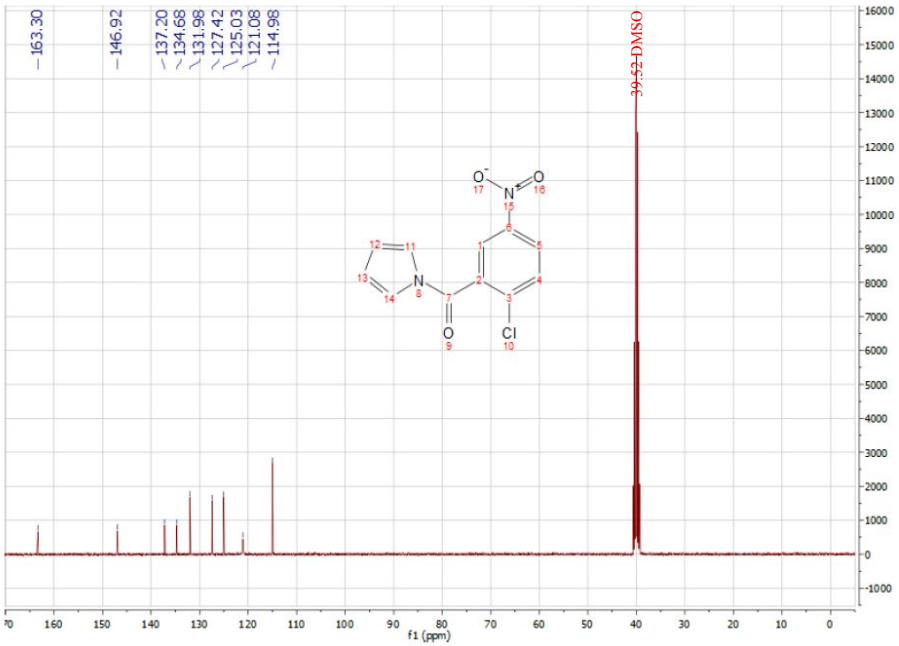

11.31
8.56
8.36
8.28
7.96
2.50 DMSO
4,12,16
13,15
1
5
8
NH
Cl
f1 (ppm)

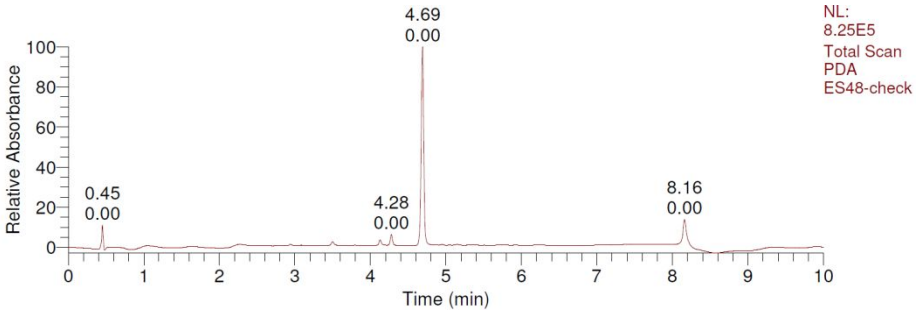

164.04
146.63
145.35
143.31
137.62
137.51
131.89
126.52
125.49
124.57
120.18
39.52 DMSO
NH
Cl
f1 (ppm)


4.65
0.00
NL:
6.94E5
Total Scan
PDA
ES47-check
0.44
0.00
8.15
0.00
Relative Absorbance
Time (min)

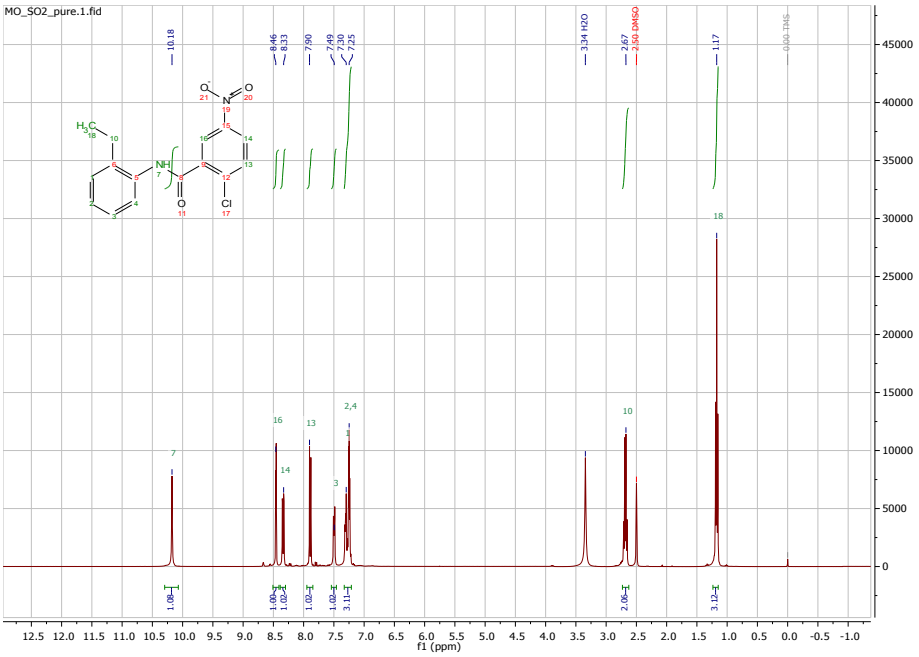
Compound 14

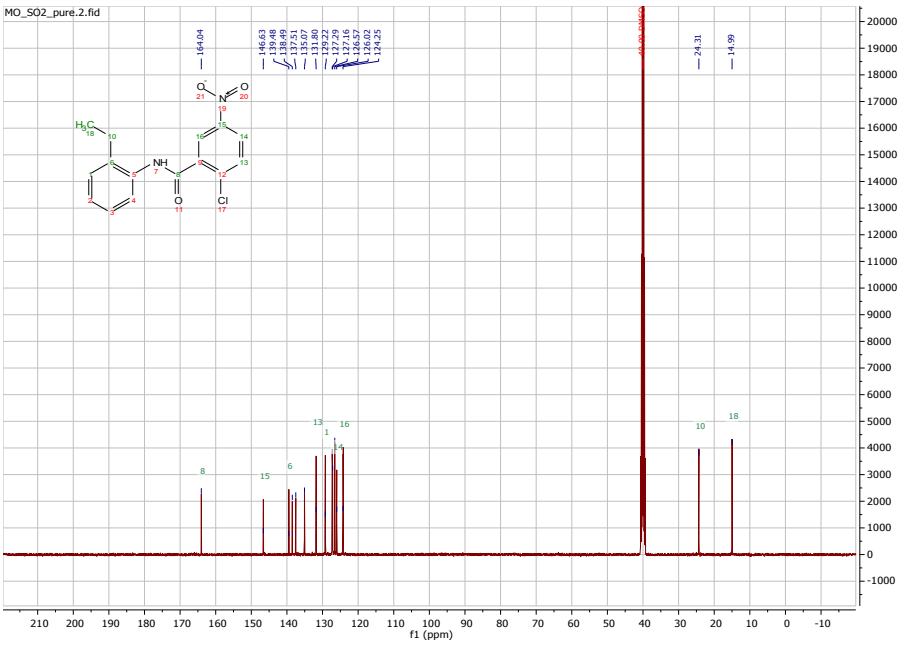

8.65
8.43
7.97
7.24
6.42
3.32 H2O
2.50 DMSO
12,13
1
4
5
11,14
0.94
1.00
1.01
1.87
2.04
f1 (ppm)

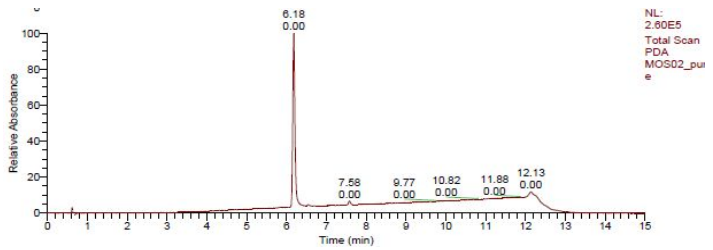

163.30
146.92
137.20
134.68
131.98
127.42
125.03
121.08
114.98
39.52 DMSO
f1 (ppm)


4.69
0.00
NL:
8.25E5
Total Scan
PDA
ES48-check
Relative Absorbance
0.45
0.00
4.28
0.00
8.16
0.00
Time (min)

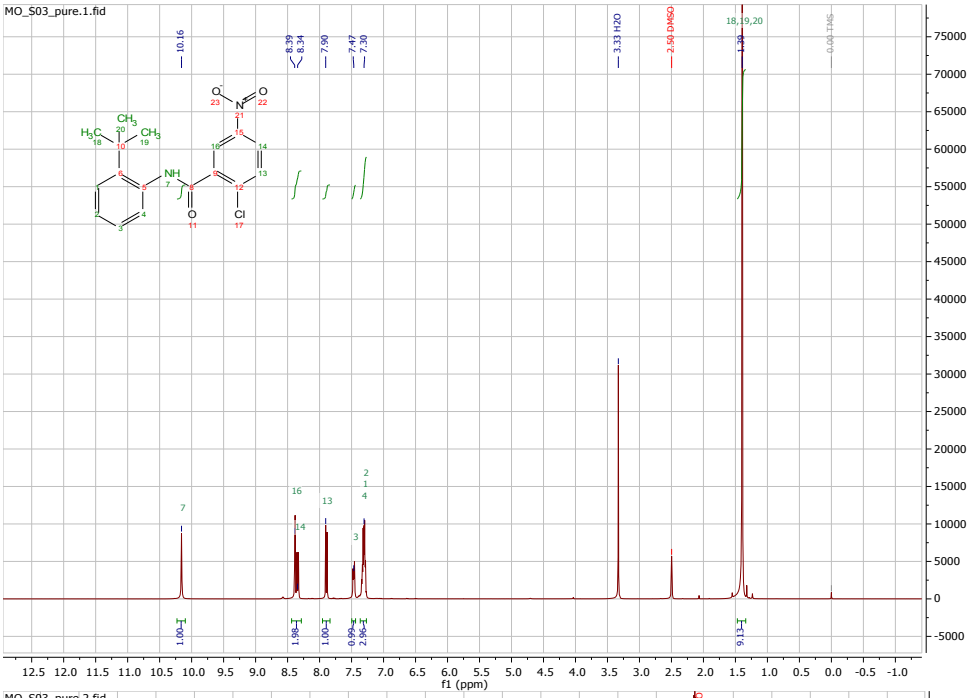
Compound 15

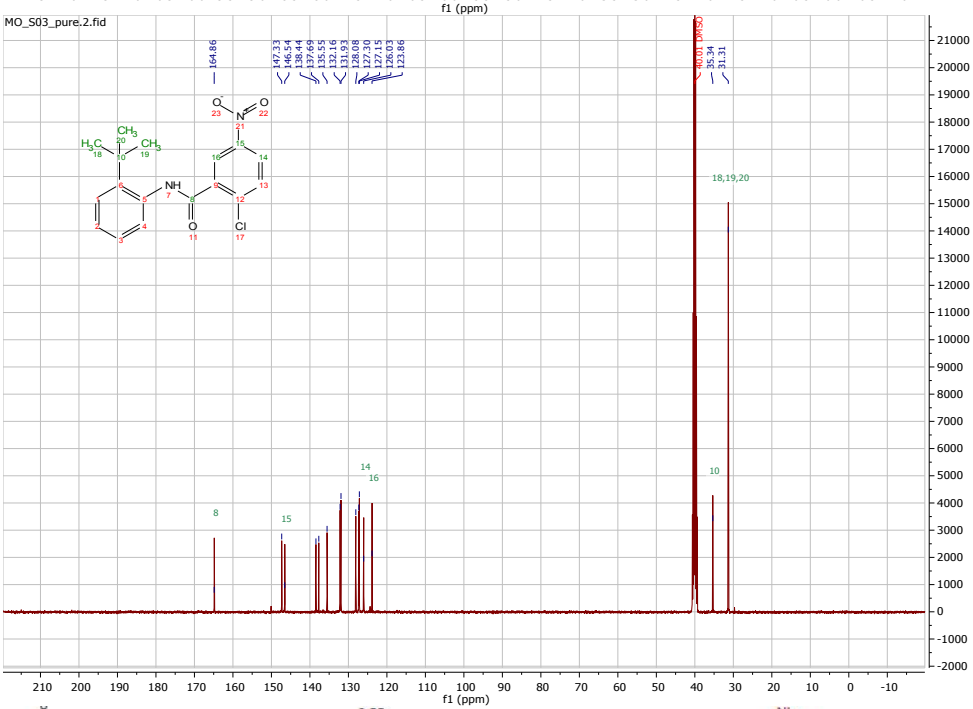

MO_SO2_pure.1.fid
10.18
8.46
8.33
7.90
7.49
7.30
7.25
3.34 H2O
2.67
2.50 DMSO
1.17
0.00 TMS
f1 (ppm)

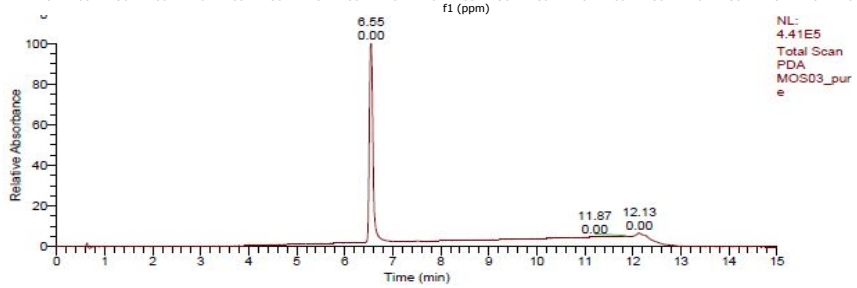

MO_SO2_pure.2.fid
164.04
146.63
139.48
138.49
137.51
135.07
131.88
129.22
127.29
127.16
126.57
126.02
124.25
40.00 DMSO
24.31
14.99
f1 (ppm)


6.18
0.00
7.58
0.00
9.77
0.00
10.82
0.00
11.88
0.00
12.13
0.00
NL:
2.60E5
Total Scan
PDA
MOS02_pur
e
Relative Absorbance
Time (min)

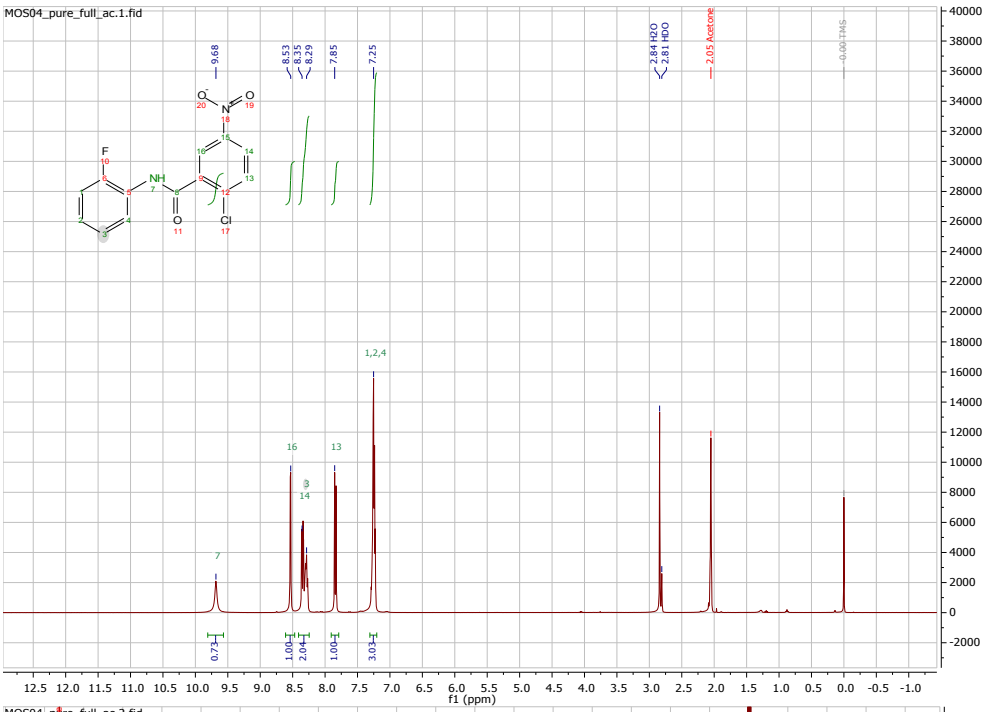
Compound 16

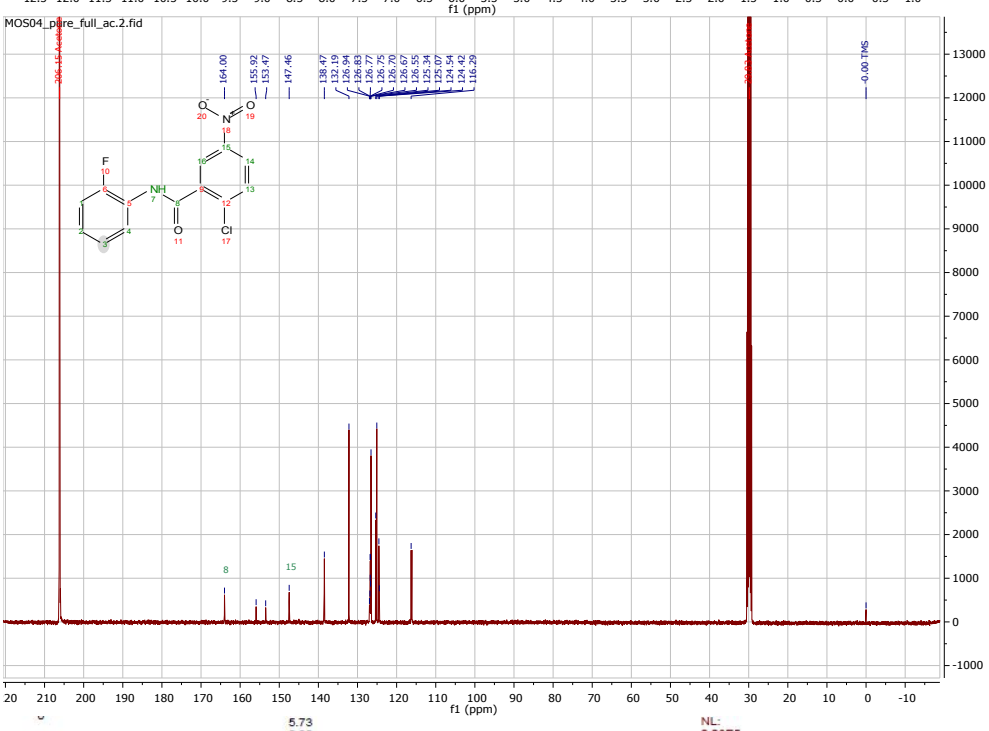

MO_S03_pure.1.fid
f1 (ppm)

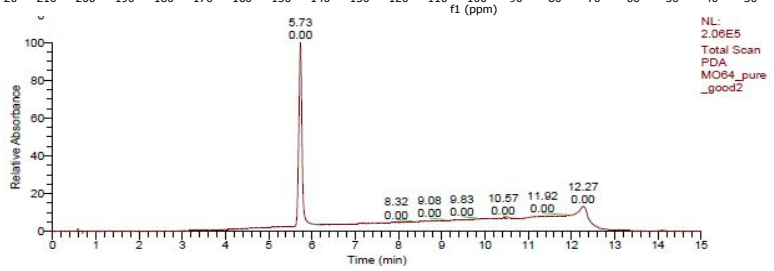

MO_S03_pure.2.fid
f1 (ppm)


6.55
0.00
11.87
0.00
12.13
0.00
NL:
4.41E5
Total Scan
PDA
MOS03_pur
e
Relative Absorbance
Time (min)

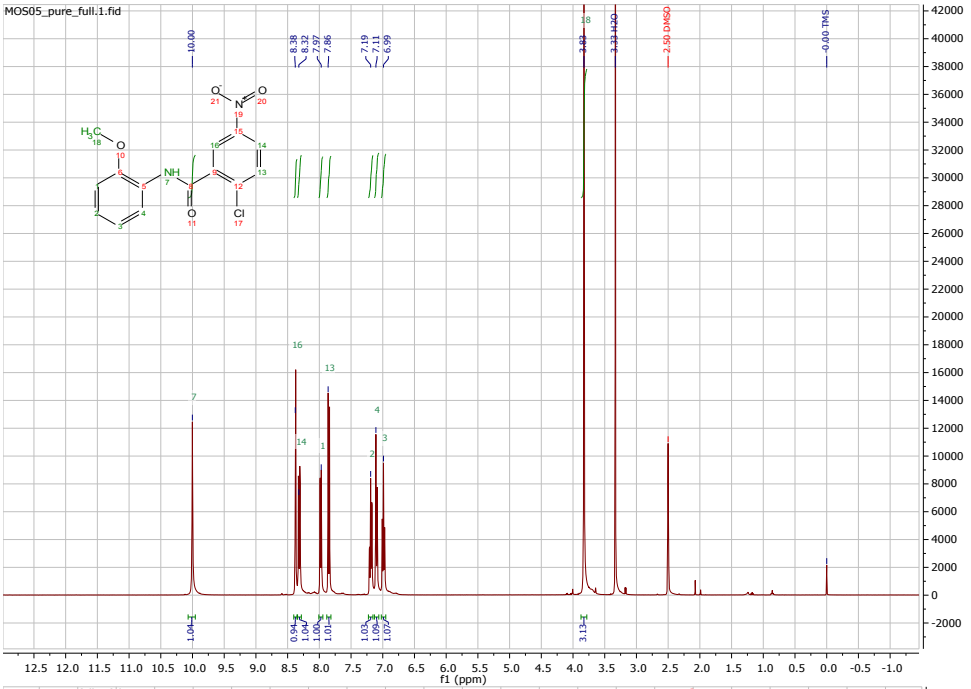
Compound 17

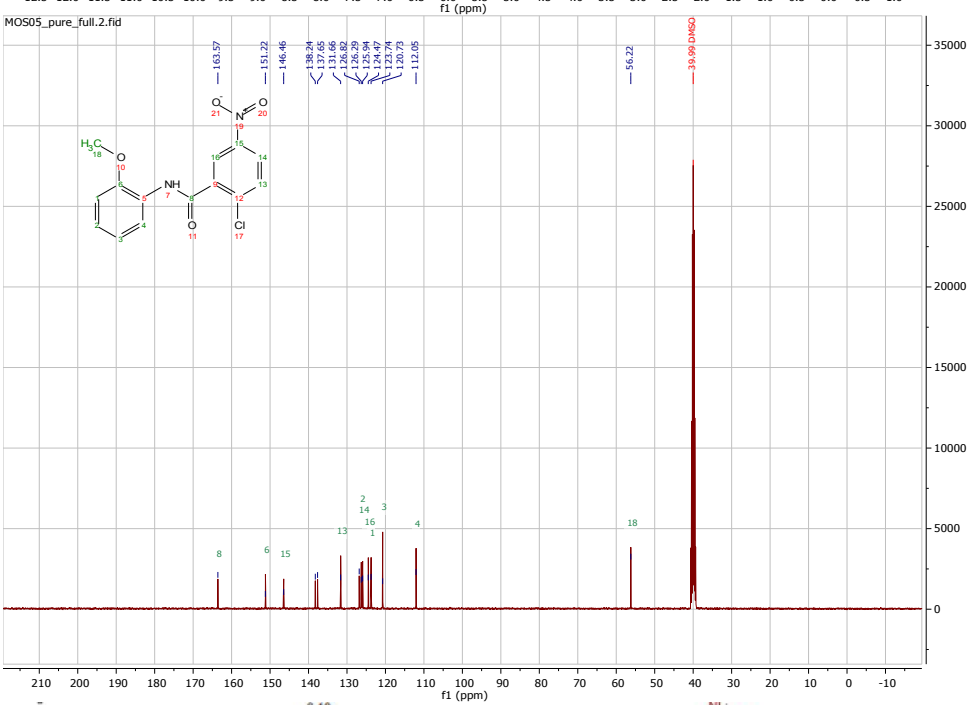

MOS04_pure_full_ac.1.fid
9.68
8.53
8.35
8.29
7.85
7.25
2.84 H2O
2.81 H2O
2.05 Acetone
-0.00 TMS
1,2,4
16
13
14
7
0.73
1.00
2.04
1.00
3.03
f1 (ppm)

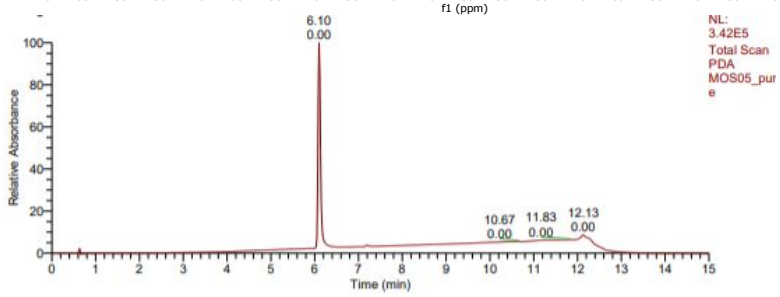

MOS04_pure_full_ac.2.fid
206.15 Acetone
164.00
155.92
153.47
147.46
138.47
132.19
126.94
126.83
126.77
126.75
126.70
126.67
126.55
125.34
125.07
124.54
124.42
116.29
29.92
-0.00 TMS
8
15
f1 (ppm)


5.73
0.00
NL:
2.06E5
Total Scan
PDA
MO64_pure
_good2
Relative Absorbance
8.32
0.00
9.08
0.00
9.83
0.00
10.57
0.00
11.92
0.00
12.27
0.00
Time (min)

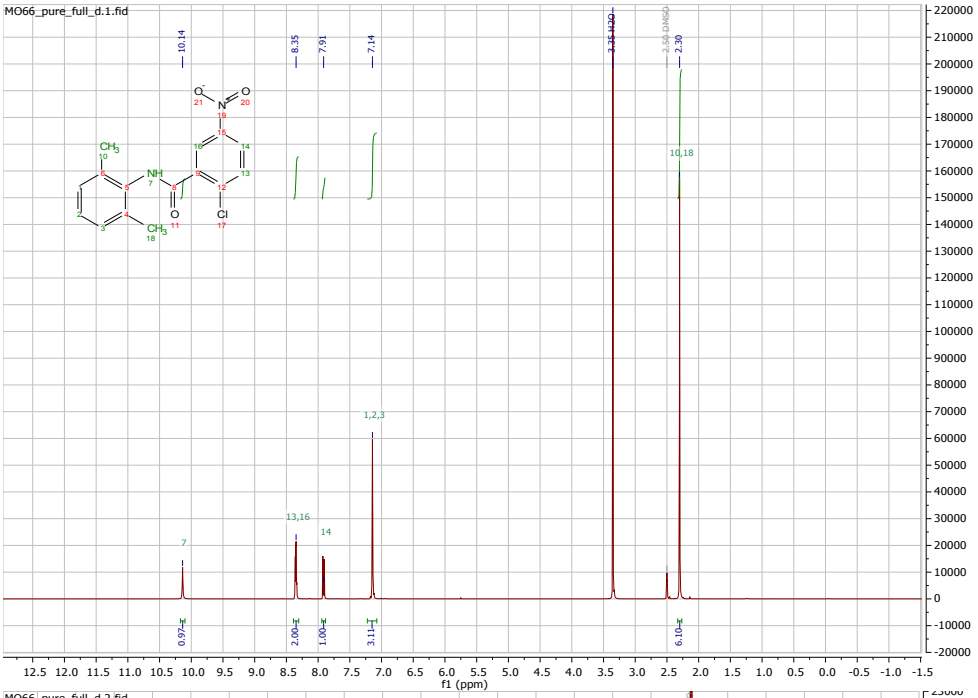
Compound 18

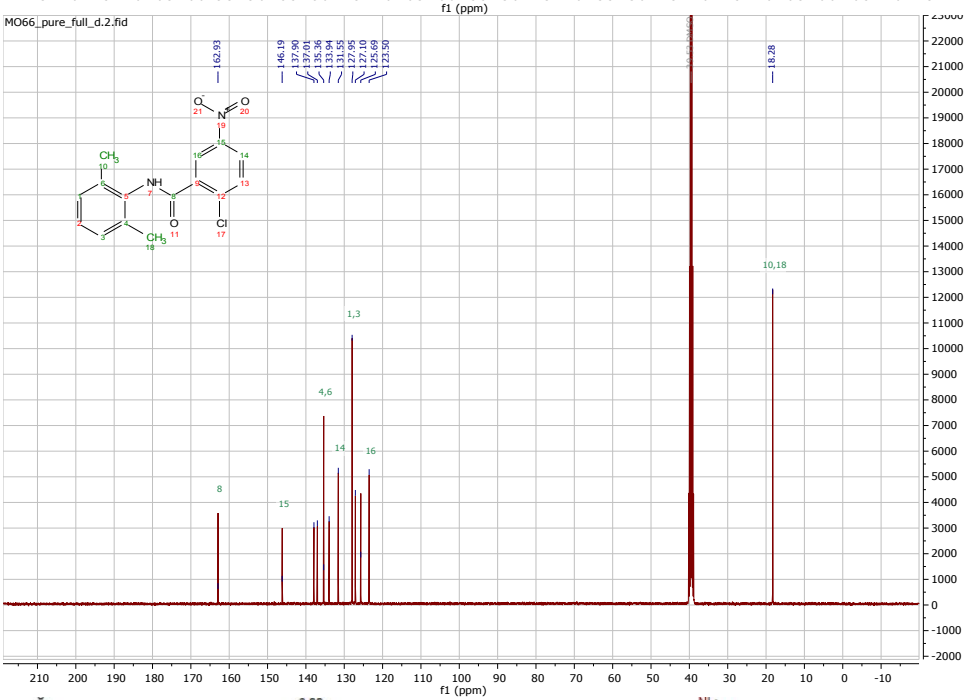

MOS05_pure_full.1.fid
f1 (ppm)

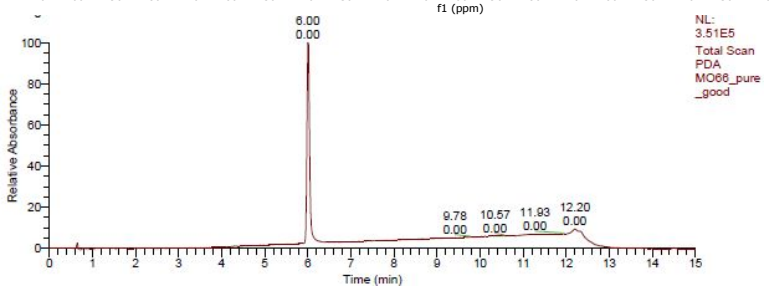

MOS05_pure_full.2.fid
f1 (ppm)


6.10
0.00
10.67
0.00
11.83
0.00
12.13
0.00
NL:
3.42E5
Total Scan
PDA
MOS05_pur
e
Relative Absorbance
Time (min)

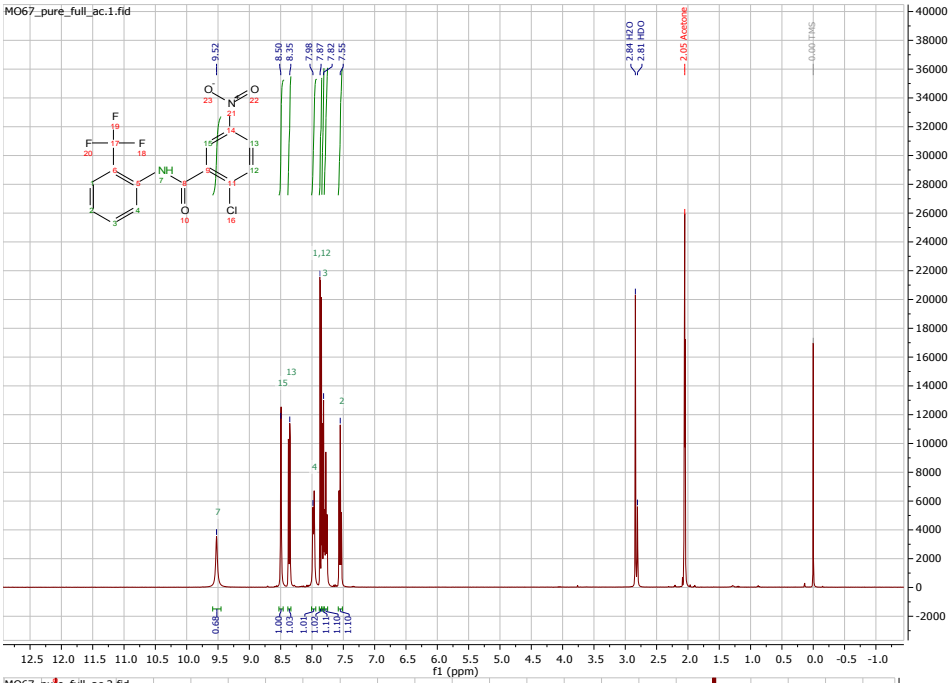
Compound 19

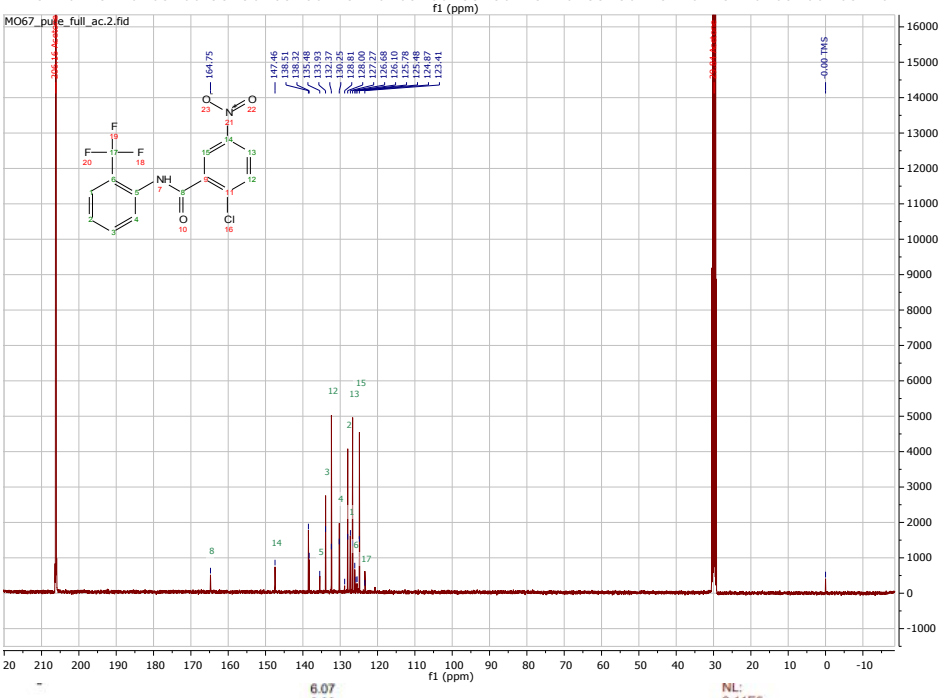

MO66_pure_full_d.1.fid
f1 (ppm)

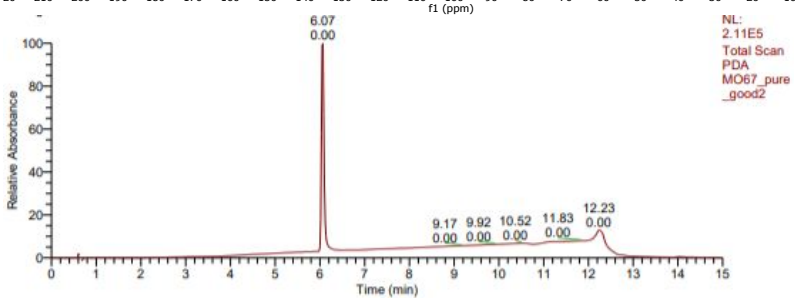

MO66_pure_full_d.2.fid
f1 (ppm)


NL:
3.51E5
Total Scan
PDA
MO66_pure
_good
Relative Absorbance
Time (min)

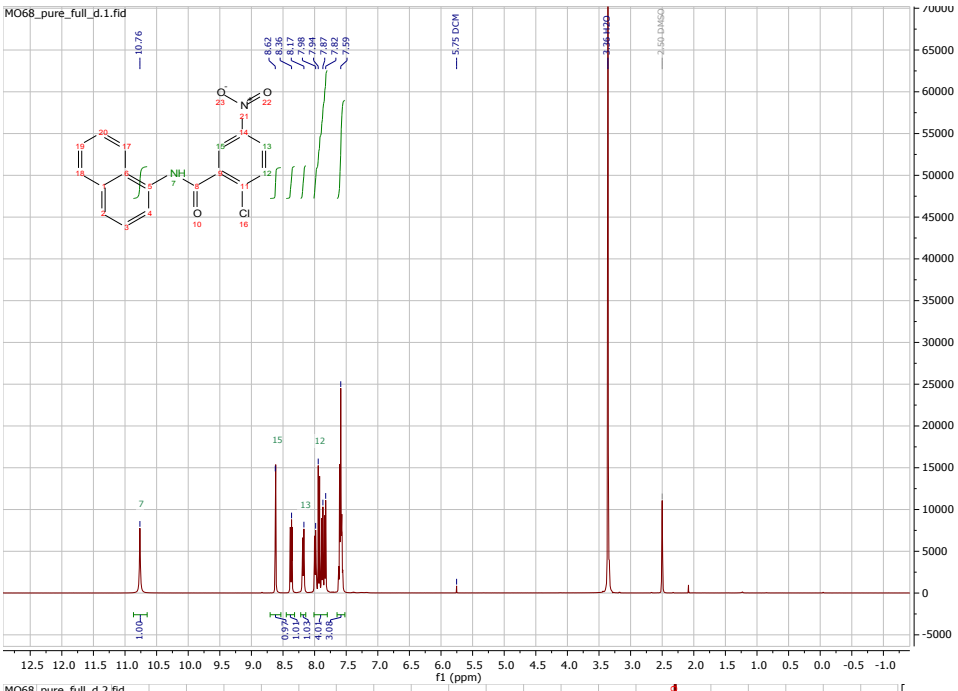
Compound 20

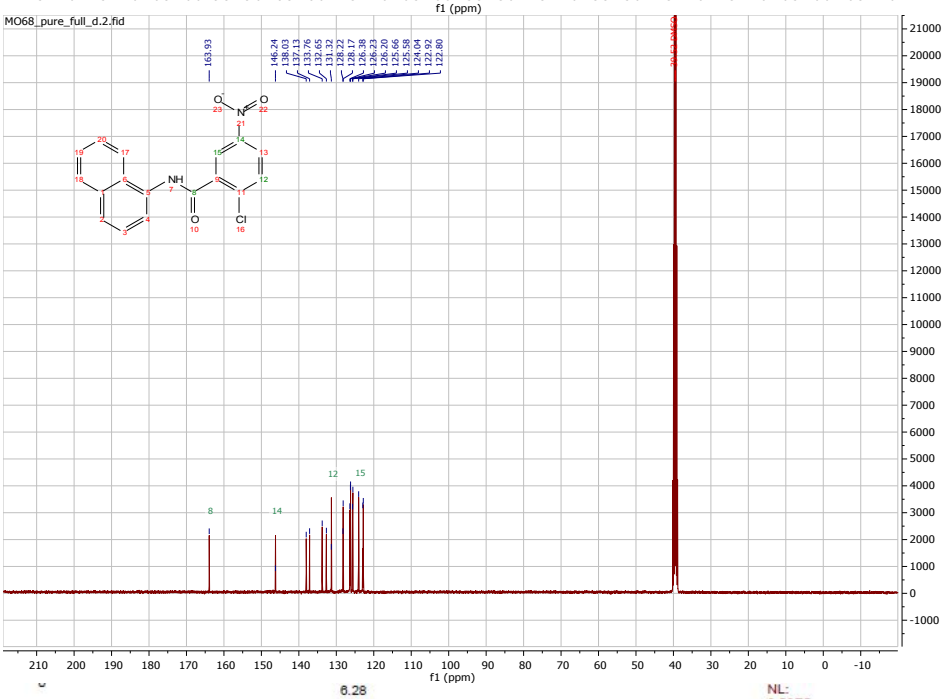

MO67_pure_full_ac.1.fid
9.52
8.50
8.35
7.98
7.87
7.82
7.55
2.84 H2O
2.81 HDO
2.05 Acetone
0.00 TMS
f1 (ppm)

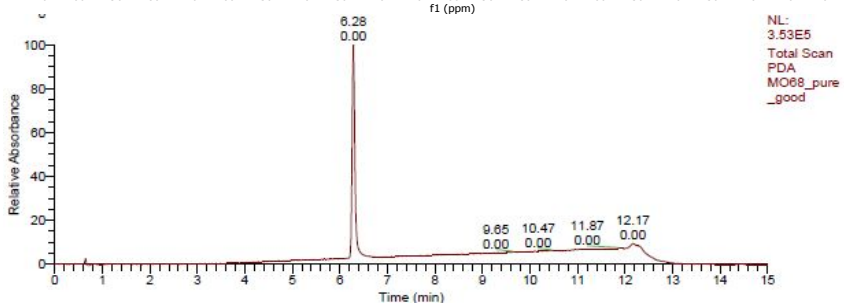

MO67_pure_full_ac.2.fid
164.75
147.46
139.51
138.32
135.48
133.93
133.27
130.25
128.81
128.00
127.27
126.68
126.10
125.78
125.48
124.87
123.41
206.14 Acetone
30.74 Acetone
0.00 TMS
f1 (ppm)


6.07
0.00
NL:
2.11E5
Total Scan
PDA
MO67_pure
_good2
Relative Absorbance
9.17
0.00
9.92
0.00
10.52
0.00
11.83
0.00
12.23
0.00
Time (min)

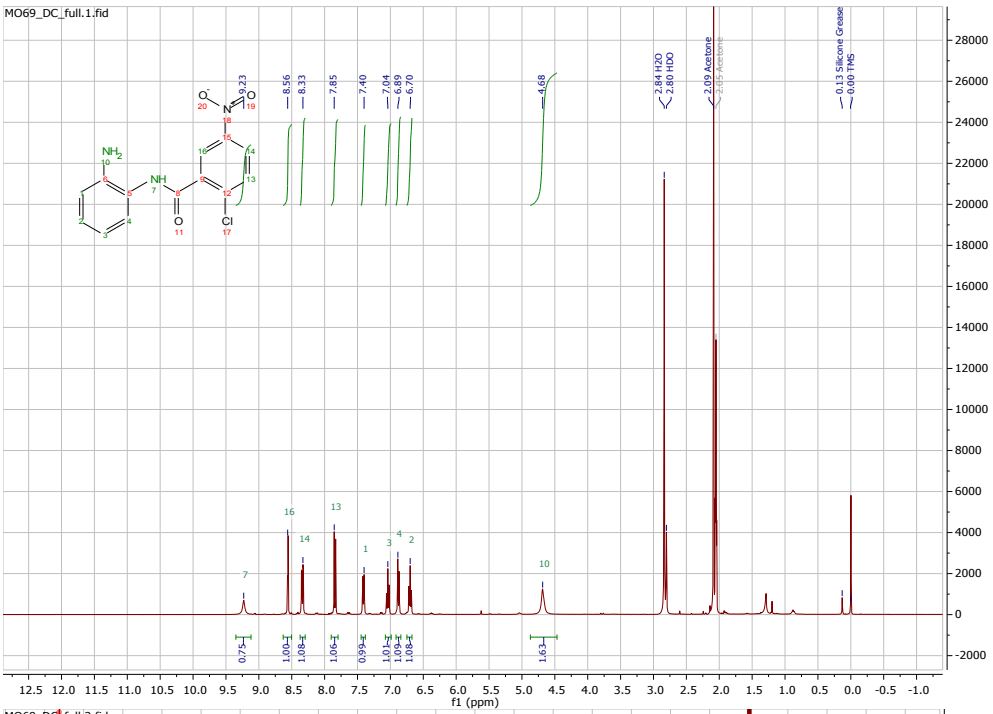
Compound 21

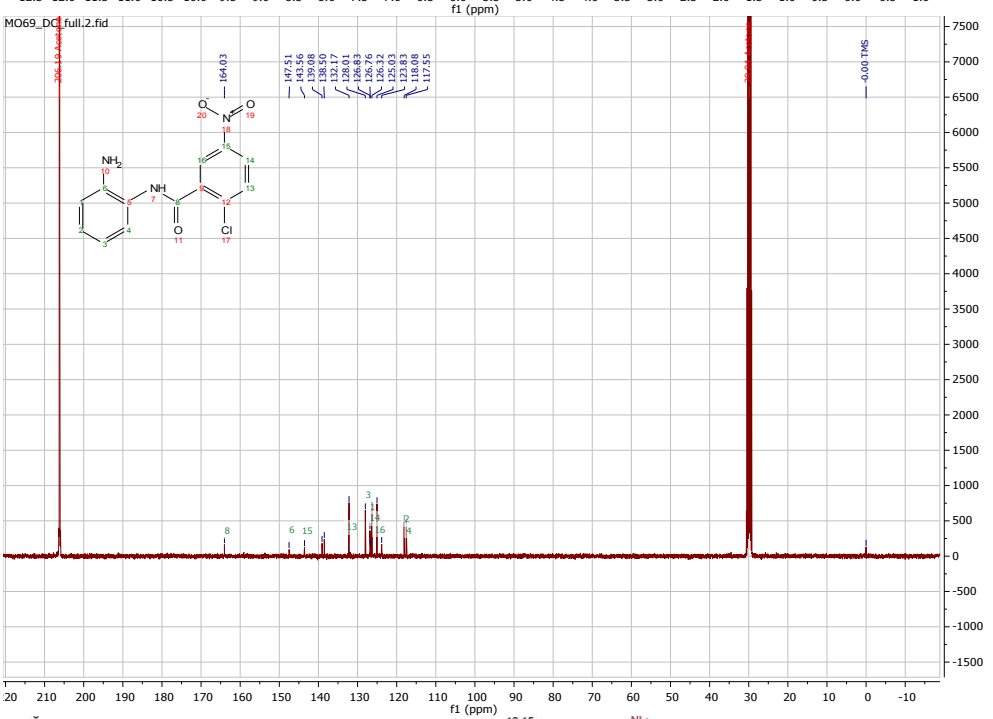

MO68_pure_full_d.1.fid
f1 (ppm)

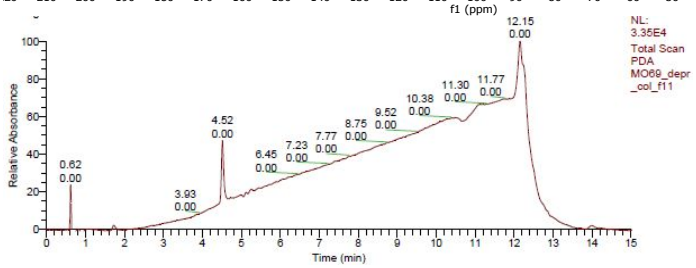

MO68_pure_full_d.2.fid
f1 (ppm)


6.28
0.00
NL:
3.53E5
Total Scan
PDA
MO68_pure
_good
9.65
0.00
10.47
0.00
11.87
0.00
12.17
0.00
Relative Absorbance
Time (min)

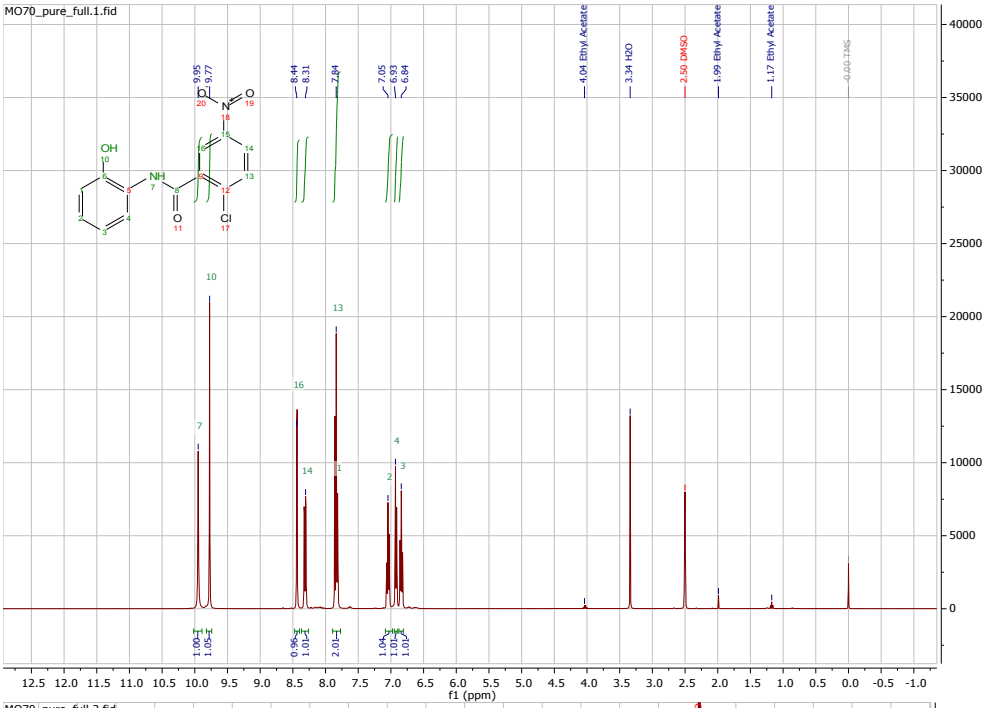
Compound 22

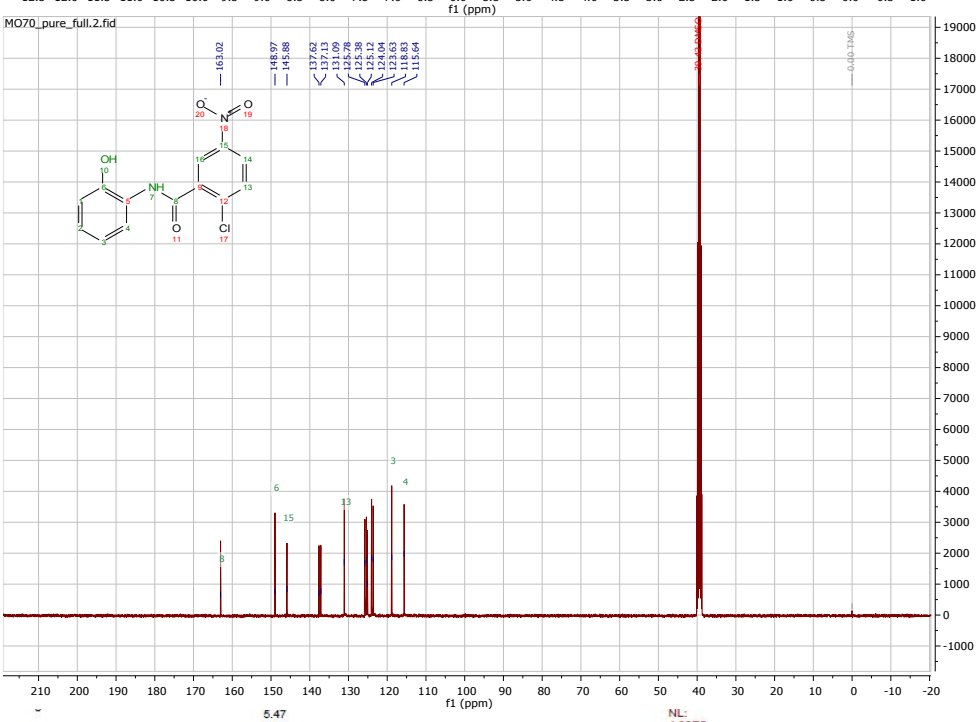

MO69_DC_full.1.fid
8.56
8.33
7.85
7.40
7.04
6.89
6.70
4.68
2.84 H2O
2.80 HDO
2.09 Acetone
0.13 Silicone Grease
0.00 TMS
f1 (ppm)

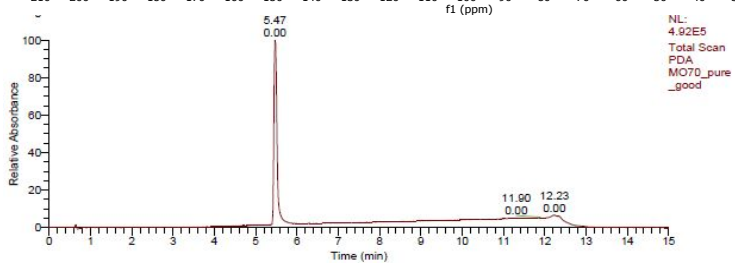

MO69_DC_full.2.fid
206.19 Acetone
164.03
147.51
140.55
139.08
138.50
132.17
128.01
126.83
126.76
126.32
125.03
123.83
118.08
117.55
30.94 Acetone
-0.00 TMS
f1 (ppm)


NL:
3.35E4
Total Scan
PDA
MO69_depr
_col_f11
Relative Absorbance
Time (min)
0.62
0.00
3.93
0.00
4.52
0.00
6.45
0.00
7.23
0.00
7.77
0.00
8.75
0.00
9.52
0.00
10.38
0.00
11.30
0.00
11.77
0.00
12.15
0.00

Compound 23


MO70_pure_full.1.fid
9.95
9.77
8.44
8.31
7.84
7.05
6.93
6.84
4.04 Ethyl Acetate
3.34 H2O
2.50 DMSO
1.99 Ethyl Acetate
1.17 Ethyl Acetate
0.00 TMS
OH
NH
O
Cl
f1 (ppm)


MO70_pure_full.2.fid
163.02
148.97
145.88
137.62
137.13
131.09
125.78
125.38
124.17
124.04
123.63
118.83
115.64
39.43 DMSO
0.00 TMS
OH
NH
O
Cl
f1 (ppm)


5.47
0.00
NL:
4.92E5
Total Scan
PDA
MO70_pure
_good
11.90
0.00
12.23
0.00
Relative Absorbance
Time (min)

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