Supplementary Table 1. Summary of gene knockouts identified from genome-scale CRISPR suppressor screens with ML210 and RSL3.

	ML210 screen		RSL3 screen	
gene symbol	median $\log_2$ fold change	median rank percentile	median $\log_2$ fold change	median rank percentile
ACSL4	6.651	0.013	7.291	0.005
ATP2C1	4.610	0.222	1.338	13.323
LPCAT3	4.822	0.164	6.063	0.012
TMEM164	3.122	1.492	4.432	0.178
TMEM165	4.433	0.335	1.348	17.669
TXNRD1	4.476	0.241	-0.863	62.196

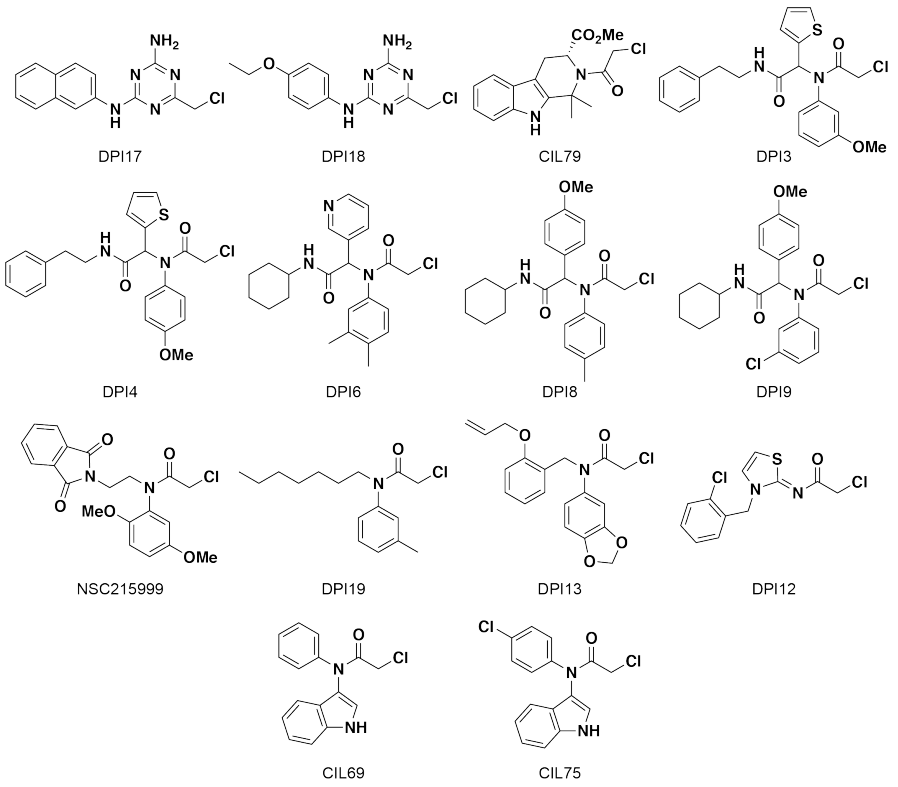
Supplementary Table 2. Summary of guide-level data for hits shown in Supplementary Table 1.

	ML210 screen		RSL3 screen	
gene symbol	median $\log_2$ fold change	median rank percentile	median $\log_2$ fold change	median rank percentile
ACSL4	7.586	0.004	7.839	0.003
ACSL4	6.777	0.011	7.269	0.005
ACSL4	6.525	0.015	7.313	0.004
ACSL4	5.837	0.032	7.168	0.007
ATP2C1	5.287	0.068	0.911	19.319
ATP2C1	5.083	0.094	1.765	7.327
ATP2C1	4.138	0.351	0.894	19.658
ATP2C1	3.940	0.459	2.125	4.755
LPCAT3	7.819	0.003	8.580	0.001
LPCAT3	5.253	0.072	6.568	0.008
LPCAT3	4.391	0.256	5.206	0.027
LPCAT3	1.903	6.491	5.558	0.016
TMEM164	3.528	0.850	4.234	0.242
TMEM164	3.220	1.316	4.163	0.280
TMEM164	3.023	1.668	4.630	0.114
TMEM164	1.248	13.060	4.856	0.063
TMEM165	5.672	0.035	2.469	3.093
TMEM165	5.065	0.095	2.331	3.651
TMEM165	3.801	0.574	0.365	31.687
TMEM165	3.554	0.806	-2.057	78.083
TXNRD1	5.243	0.075	1.231	13.641
TXNRD1	4.731	0.162	0.851	62.001
TXNRD1	4.222	0.320	-0.875	62.390
TXNRD1	2.901	1.948	1.834	75.451

Supplementary Table 3. Physiochemical and pharmacokinetic properties of small-molecule GPX4 inhibitors.

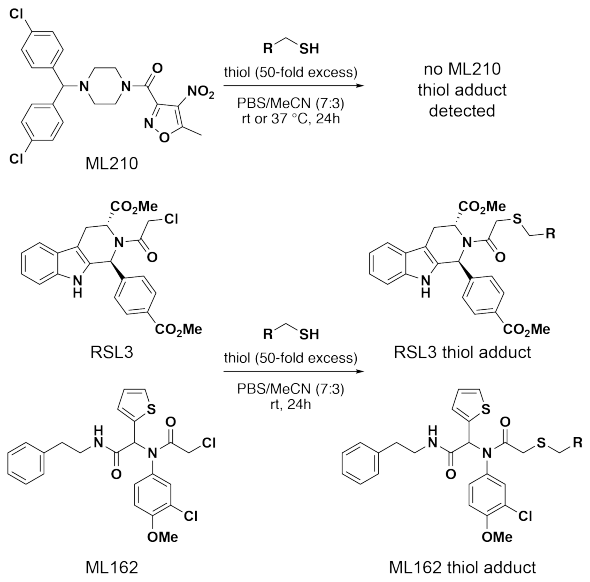
compound	ML210	ML162	RSL3	JKE-1674
MW (g/mol)	475.3	477.4	440.9	451.3
cLogP	4.7	4.8	3.4	4.0
logD (pH 7.5)	4.9	3.6	3.0	3.1
solubility (mg/L)	<0.1	0.2	*	45.6
Caco A-B (nm/s)	*	*	*	113.2
efflux ratio	*	*	*	1.12
plasma stability (%)				
human	21	1	27	>99
rat	76	21	0	>99
mouse	24	27	0	>99
buffer stability (%)				
pH 1	79	>99	87	>99
pH 7	100	n.d.	n.d.	>99
pH 10	0	>99	39	>99
glutathione reactivity?	no	yes	yes	no
cysteine reactivity?	no	yes	yes	no
rat hepatocyte stability (%)	20	<1	<1	76
microsome stability (%)				
human	47	9	7	34
mouse	41	<1	33	91

Values that could not be obtained due to low compound recovery are marked with an asterisk (*). n.d., not determined. cLogP, calculated log(partition-coefficient). LogD, log(distribution-coefficient) determined at pH 7.5.



Supplementary Figure 1. Reported small-molecule GPX4 inhibitors contain reactive alkyl chloride groups, including chloroacetamide and chloromethyltriazine (Yang, W.S. et al. *Cell* **156**, 317-331 (2014); Yang, W.S. et al. *Proc. Natl. Acad. Sci.* **113**, E4966-E4975 (2016); Shimada, K. et al. *Nat. Chem. Biol.* **12**, 497-503 (2016)).

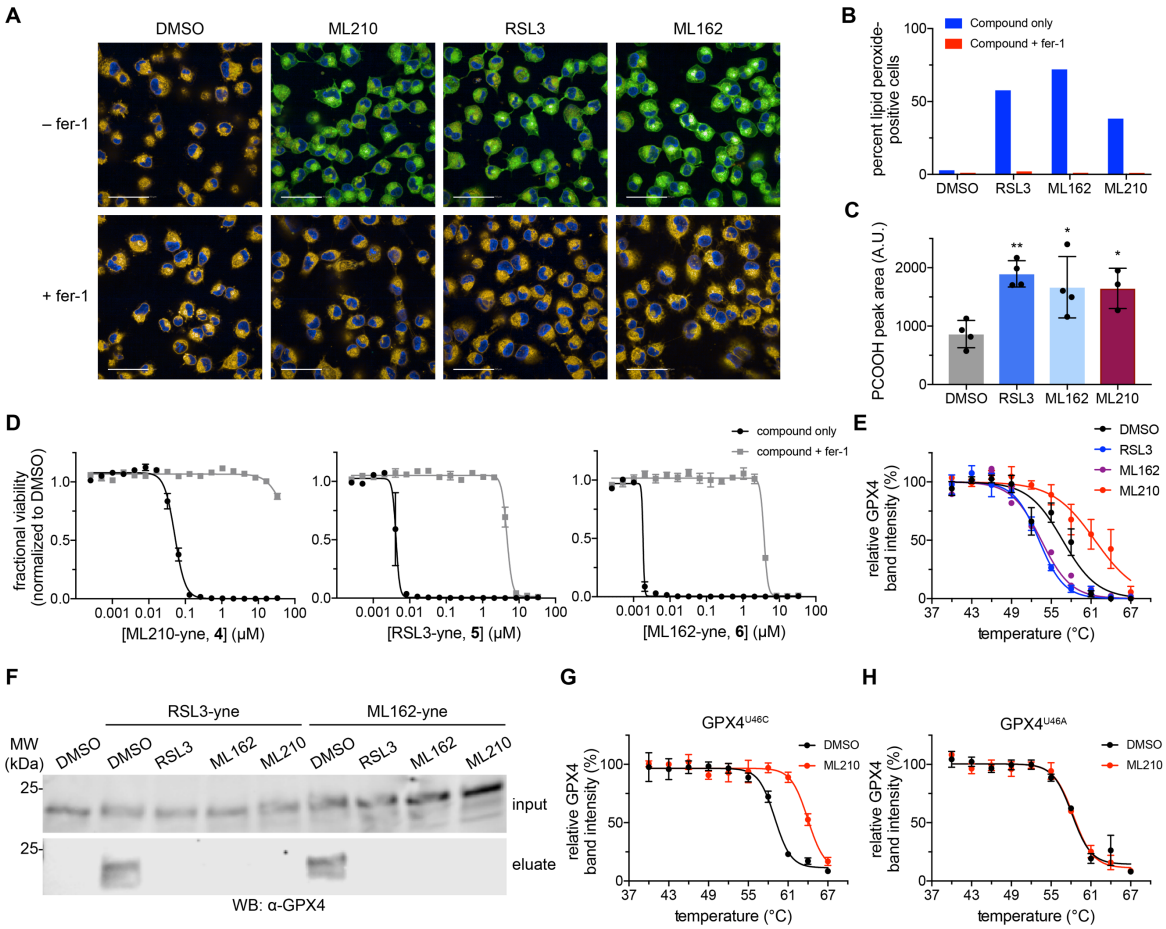
A



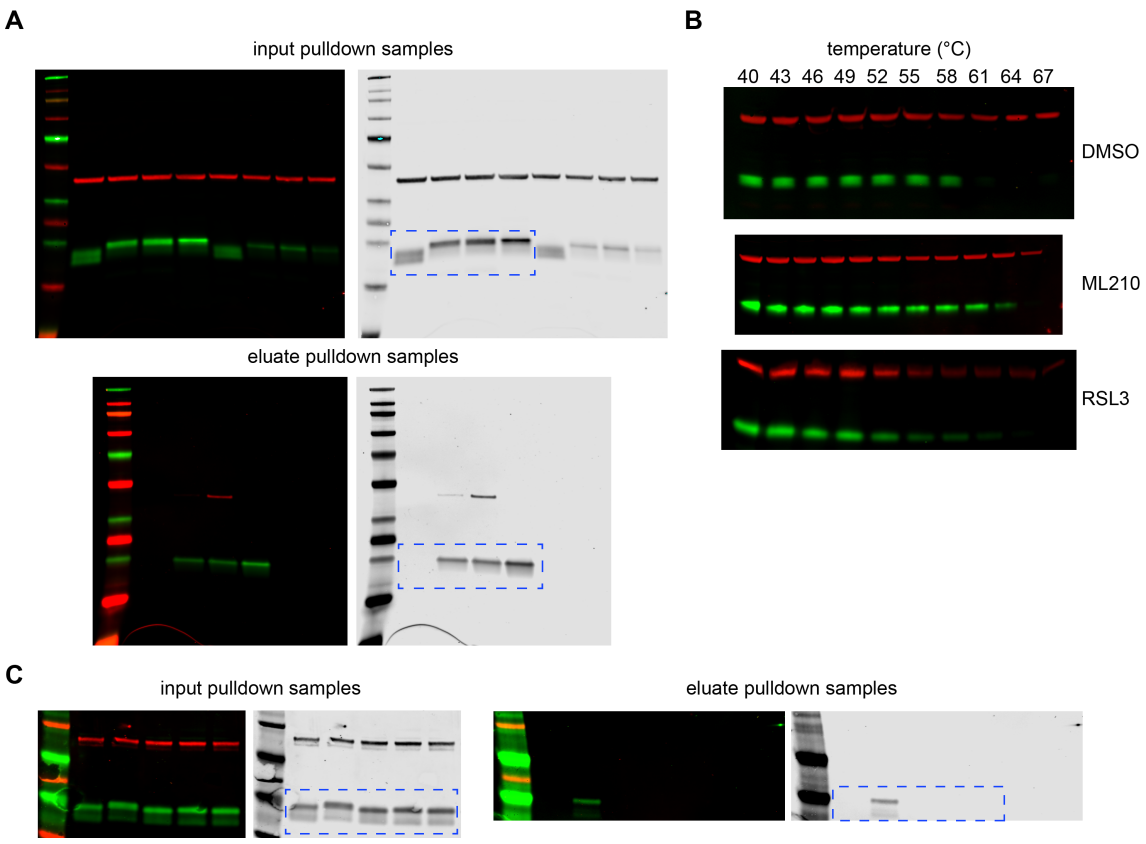
B

compound (MW)	thiol	thiol adduct formation (%)	calculated adduct $[M+H]^+$ (m/z)	observed adduct $[M+H]^+$ (m/z)
ML210 (475.33)	Cys	0	n.a.	n.d.
	NAC	0	n.a.	n.d.
	GSH	0	n.a.	n.d.
RSL3 (440.88)	Cys	100	526.16	526.14
	NAC	51	568.17	568.21
	GSH	100	712.23	712.28
ML162 (477.40)	Cys	100	562.12	562.20
	NAC	100	604.13	604.18
	GSH	100	748.19	748.18

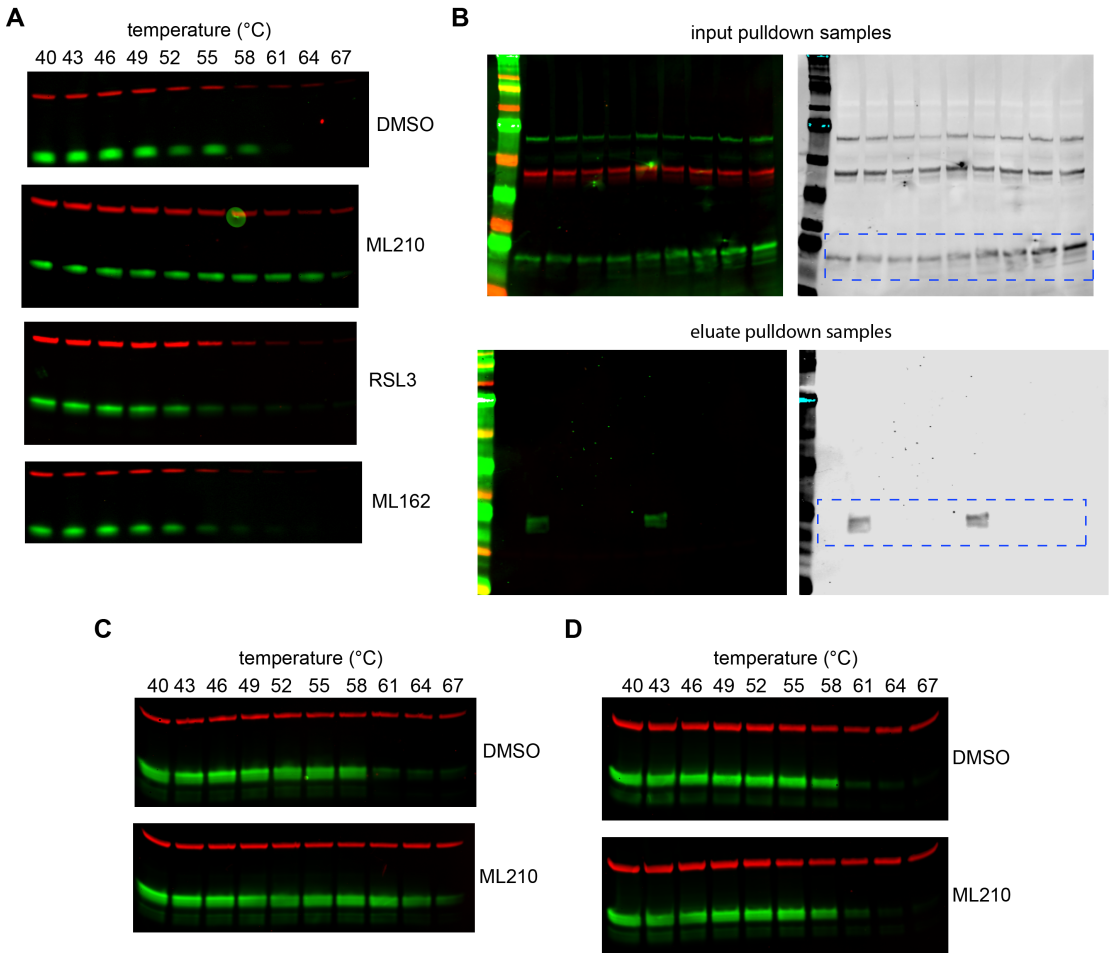
Supplementary Figure 2. ML210 does not react with small-molecule thiols.
(**A**) Schemes depicting reactions of ML210, RSL3, and ML162 (100 μM) with thiols (5 mM) L-cysteine (Cys), L-*N*-acetylcysteine (NAC), and L-glutathione (GSH). ML210 does not react detectibly with these thiols while both RSL3 and ML162 form the indicated thioether adducts at ambient temperature. (**B**) Table of thiol adducts detected by LC-MS.



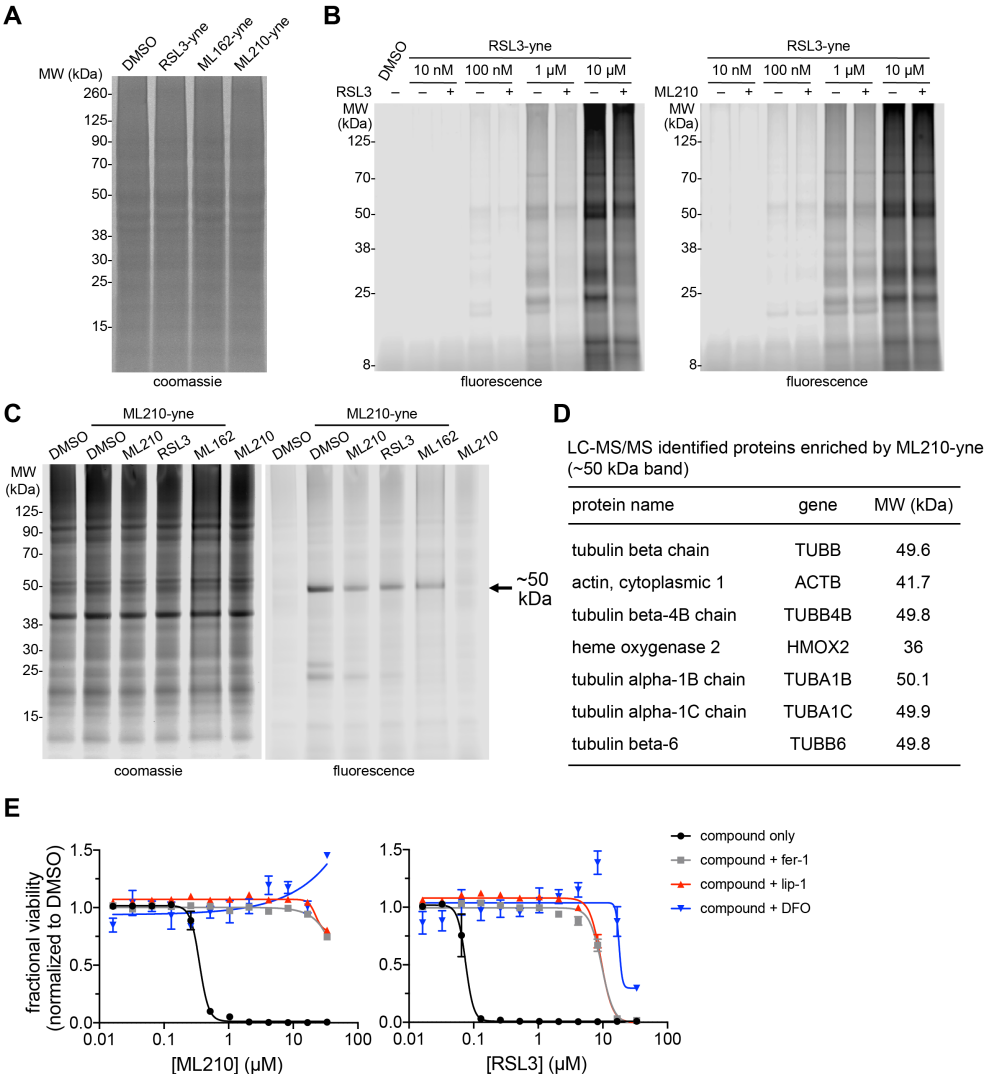
Supplementary Figure 3. Characterization of small-molecule GPX4 inhibitors.
(**A**) Treatment of cells with ML210, RSL3, or ML162 (10 μM, 90 min) leads to accumulation of lipid hydroperoxides in LOX-IMVI cells as assessed by fluorescence imaging with C11-BODIPY 581/591. Co-treatment of cells with ferrostatin-1 (fer-1, 1.5 μM) prevents lipid hydroperoxide accumulation. Scale bars, 50 μm. Top row of images is reproduced from Fig. 1c. (**B**) Treatment of U2OS cells with RSL3, ML162, or ML210 (10 μM, 2 h) leads to accumulation of lipid hydroperoxides, which can be suppressed by fer-1 (1.5 μM), as assessed by flow cytometry. (**C**) Treatment of LOX-IMVI cells with RSL3, ML162, or ML210 (10 μM, 1 h) inhibits the reduction of GPX4-specific substrate phosphatidylcholine hydroperoxide (PCOOH) in LOX-IMVI cell lysates. Data are plotted as the mean ± s.d., $n \geq 3$ technical replicates. *P* values were determined using one-way ANOVA; $^{*}P < 0.05$, $^{**}P < 0.005$ vs. DMSO control. (**D**) Alkyne analogs ML210-yne, RSL3-yne, and ML162-yne exhibit activity in LOX-IMVI cells similar to their respective parent compounds. Data are plotted as mean ± s.e.m., $n = 4$ technical replicates. (**E**) GPX4 CETSA profiles for intact cells (HT-1080) treated with DMSO (black), RSL3 (blue), ML210 (red), or ML162 (purple). Cells were treated with 10 μM compound for 1 h. Data are plotted as mean ± s.e.m., $n = 3$ biologically independent samples except for ML162, which is plotted as individual biological replicates. Representative western blots are shown in Supplementary Fig. 5a. (**F**) Pretreatment of LOX-IMVI cells with RSL3, ML162, or ML210 (10 μM, 30 min) prevents pulldown of GPX4 by RSL3-yne or ML162-yne (10 μM, 30 min). Full-length gel images are shown in Supplementary Fig. 5b. (**G**) GPX4 CETSA of intact cells (LOX-IMVI) expressing 3xFLAG-GPX4^{U46C} protein treated for 1 h with DMSO (black) or ML210 (10 μM; red). Data are plotted as mean ± s.e.m., $n = 3$ biologically independent samples. Representative western blots are shown in Supplementary Fig. 5c. (**H**) GPX4 CETSA of intact cells (LOX-IMVI) expressing 3xFLAG-GPX4^{U46A} protein treated for 1 h with DMSO (black) or ML210 (10 μM; red). Data are plotted as mean ± s.e.m., $n = 3$ biologically independent samples. Representative western blots are shown in Supplementary Fig. 5d.



Supplementary Figure 4. Full images of western blots associated with Figure 1. All gels shown in color depict GPX4 (green) and ACTB (red). Cropped sections of gels are shown with a blue box in grayscale images. (**A**) GPX4 pulldown assay from Fig. 1e. (**B**) Representative intact-cell CETSA blots corresponding to the plot in Fig. 1f. (**C**) GPX4 pulldown assay from Fig. 1g.



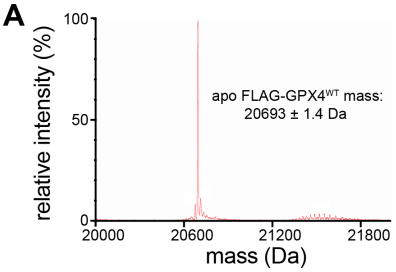
Supplementary Figure 5. Full images of western blots associated with Supplementary Figure 3. All gels shown in color depict GPX4 (green) and ACTB (red). Cropped sections of gels are shown with a blue box in grayscale images. (**A**) Representative intact-cell CETSA blots corresponding to the plot in Supplementary Fig. 3e. (**B**) GPX4 pulldown assay from Supplementary Fig. 3f. (**C**) Representative intact-cell CETSA blots corresponding to the plot in Supplementary Fig. 3g. (**D**) Representative intact-cell CETSA blots corresponding to the plot in Supplementary Fig. 3h.



protein name	gene	MW (kDa)
tubulin beta chain	TUBB	49.6
actin, cytoplasmic 1	ACTB	41.7
tubulin beta-4B chain	TUBB4B	49.8
heme oxygenase 2	HMOX2	36
tubulin alpha-1B chain	TUBA1B	50.1
tubulin alpha-1C chain	TUBA1C	49.9
tubulin beta-6	TUBB6	49.8

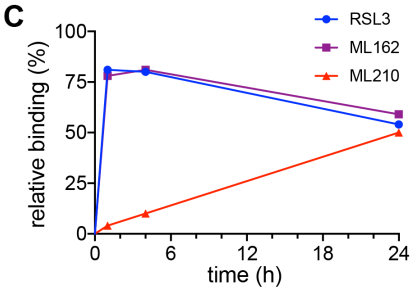
Supplementary Figure 6. Characterization of ML210 selectivity.
(**A**) Coomassie-stained gel corresponding to fluorescence gel in Fig. 1h. (**B**) Cellular proteome reactivity profiles of RSL3-yne in LOX-IMVI cells with pretreatment by RSL3 and ML210 where indicated. Cells were treated with DMSO or inhibitor (10 μM, 1 h) followed by RSL3-yne (0.01-10 μM, 1 h). (**C**) Competitive cellular reactivity profile of ML210-yne in LOX-IMVI cells with pretreatment by ML210, RSL3, and ML162 where indicated. Cells were treated with non-alkyne inhibitors (10 μM, 30 min) followed by treatment with ML210-yne (10 μM, 30 min). Arrow indicates ~50 kDa band, which is not a specific target of ML210-yne. (**D**) Table of proteins identified in ~50 kDa gel band after pulldown with ML210-yne (10 μM, 1 h). The most abundant proteins are cytoskeletal proteins that are typically highly expressed, most of which contain previously characterized reactive cysteine residues (Shrimp, J.H. et al. *ACS Med. Chem. Lett.* **7**, 151-155 (2016); Weerapana, E. et al. *Nature* **468**, 790-797 (2010); Backus, K.M. et al. *Nature* **534**, 570-574 (2016)). These proteins were not detected in a DMSO-treated control sample. (**E**) The LOX-IMVI cell-killing activity of ML210 and RSL3 can be rescued by co-treatment with fer-1 (1.5 μM), lip-1 (1 μM), and DFO (50 μM). Data are plotted as mean ± s.e.m., *n* = 4 technical replicates.

A



FLAG-GPX4 mass (Da)	incubation time (h)	observed mass (Da)	mass difference (Da)
20651	1	20692	41
	4	20693	42
	24	20693	42

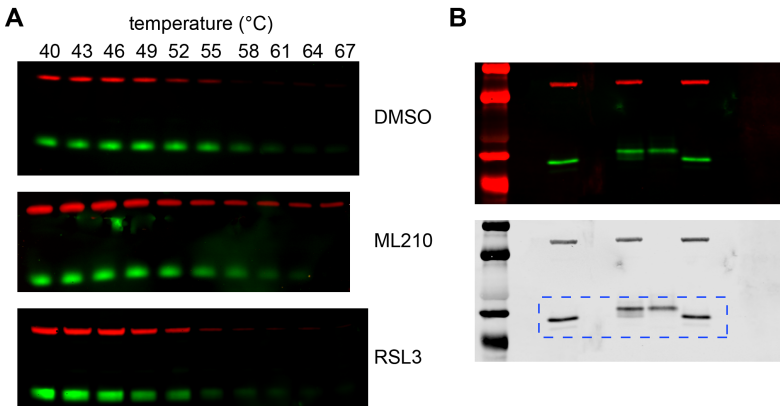
C



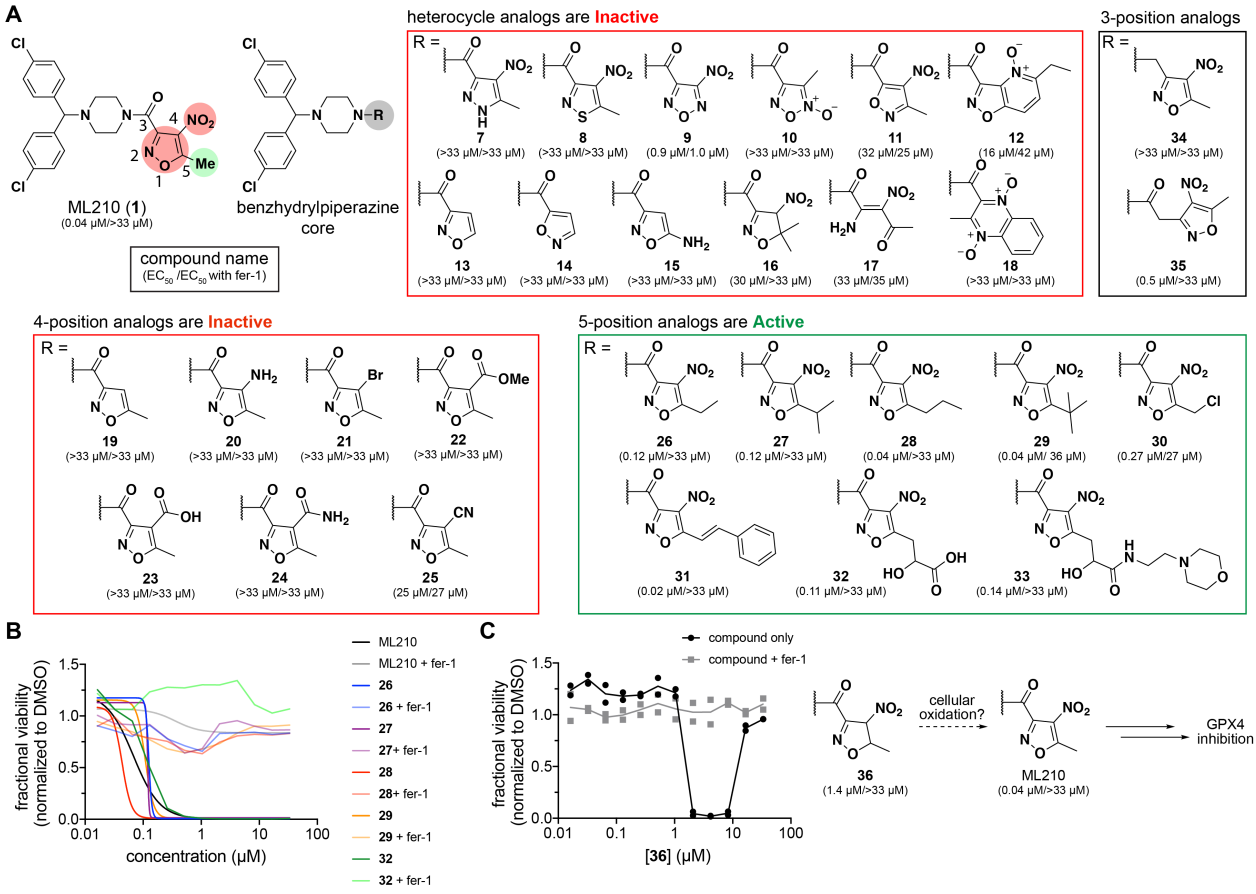
B

incubation time (h)	compound	compound MW (g/mol)	compound concentration (μM)	protein mass (Da)	protein mass + compound (Da)	mass shift (Da)	relative binding intensity (%)
1	DMSO	-----	-----	20692	no binding	-----	-----
	RSL3	440.88	10	20695	21098	403	81
			20	20697	21098	401	83
	ML162	477.40	1	20692	21134	442	48
			10	20694	21135	441	78
			20	20697	21134	437	93
	ML210	475.33	1	20691	no binding	-----	-----
			10	20693	21127	434	4
			20	20693	21127	434	5
4	DMSO	-----	-----	20693	no binding	-----	-----
	RSL3	440.88	10	20695	21098	403	80
			20	20697	21098	401	83
	ML162	477.40	1	20693	21134	441	52
			10	20693	21135	442	81
			20	20696	21135	439	93
	ML210	475.33	1	20692	no binding	-----	-----
			10	20693	21127	434	10
			20	20693	21127	434	12
24	DMSO	-----	-----	20693	no binding	-----	-----
	RSL3	440.88	10	20693	21098	405	54
			20	20694	21098	404	76
	ML162	477.40	1	20692	21134	442	20
			10	20693	21135	442	59
			20	20694	21135	441	84
	ML210	475.33	1	20692	21127	435	5
			10	20693	21127	434	50
			20	20694	21127	433	67

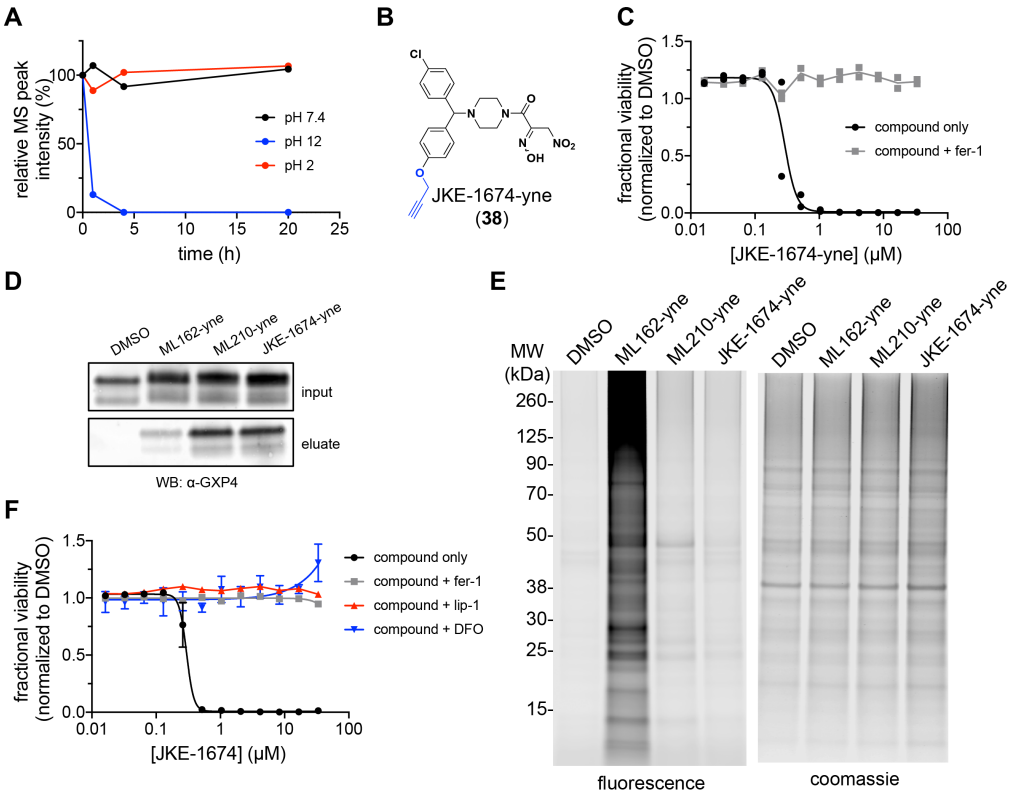
Supplementary Figure 7. Characterization of GPX4 inhibitor binding in cells.
(**A**) Characterization of FLAG-GPX4^{WT} by intact protein mass spectrometry. Upper plot depicts deconvoluted apo FLAG-GPX4^{WT} mass spectrum. The observed protein mass indicates that FLAG-GPX4^{WT} is acetylated in HEK293-6E cells. Apo FLAG-GPX4^{WT} mass is reported as mean ± s.d., n = 48 technical replicates. (**B**) Summary of intact-cell binding experiments in HEK293-6E cells. GPX4 inhibitors (1, 10, or 20 μM) were incubated with cells expressing FLAG-GPX4^{WT} for 1, 4, or 24 hours. Observed RSL3 and ML162 adduct masses are consistent with loss of HCl (36 Da) upon reaction with GPX4. ML210 binding is associated with a loss of 41 Da. (**C**) ML210 (10 μM) interacts with cellular FLAG-GPX4^{WT} more slowly than do chloroacetamides RSL3 and ML162 (10 μM). Data are plotted as single values from the table in Supplementary Fig. 7b.



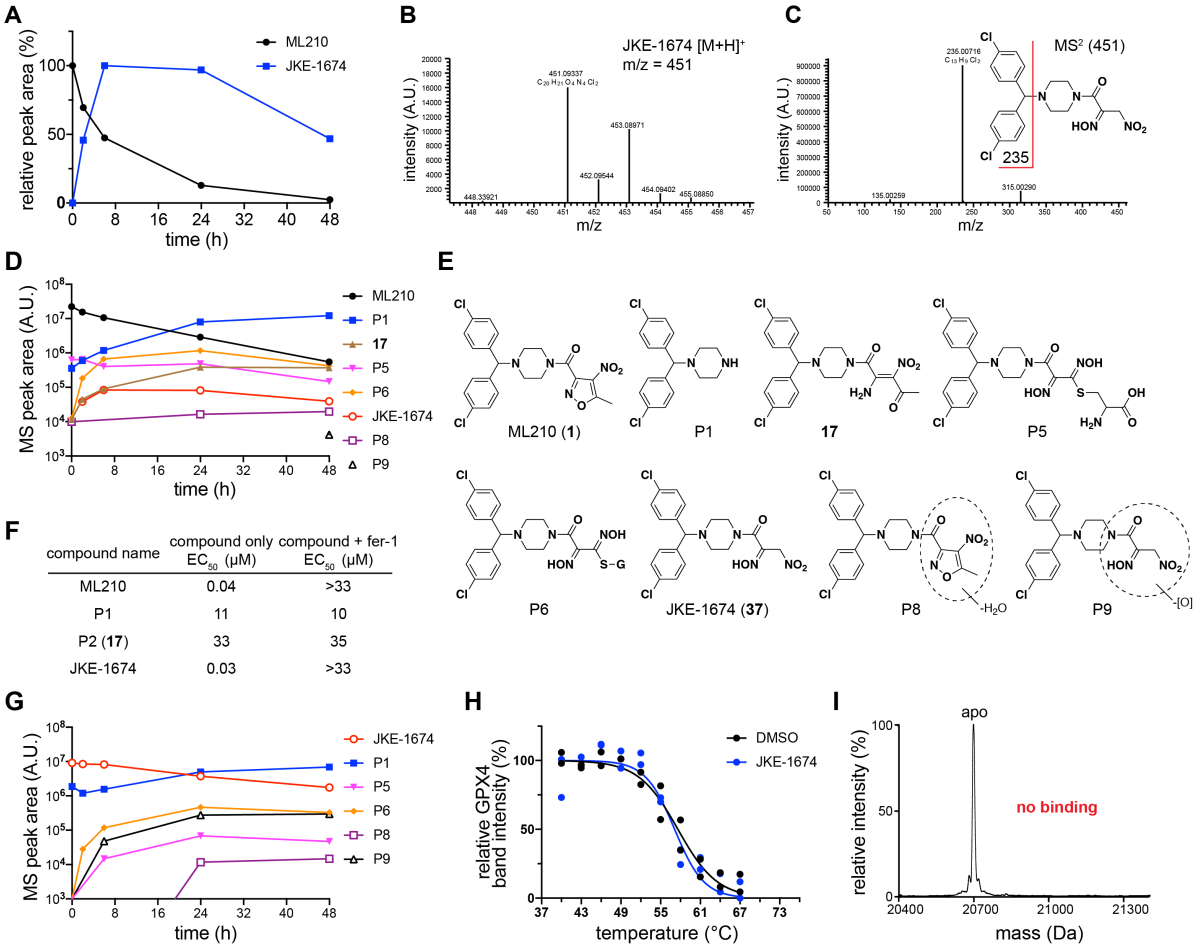
Supplementary Figure 8. Full images of western blots associated with Figure 2. All gels shown in color depict GPX4 (green) and ACTB (red). Cropped sections of gels are shown with a blue box in grayscale images. (**A**) Representative lysate CETSA blots corresponding to the plot in Fig. 2c. (**B**) GPX4 pulldown assay from Fig. 2d.



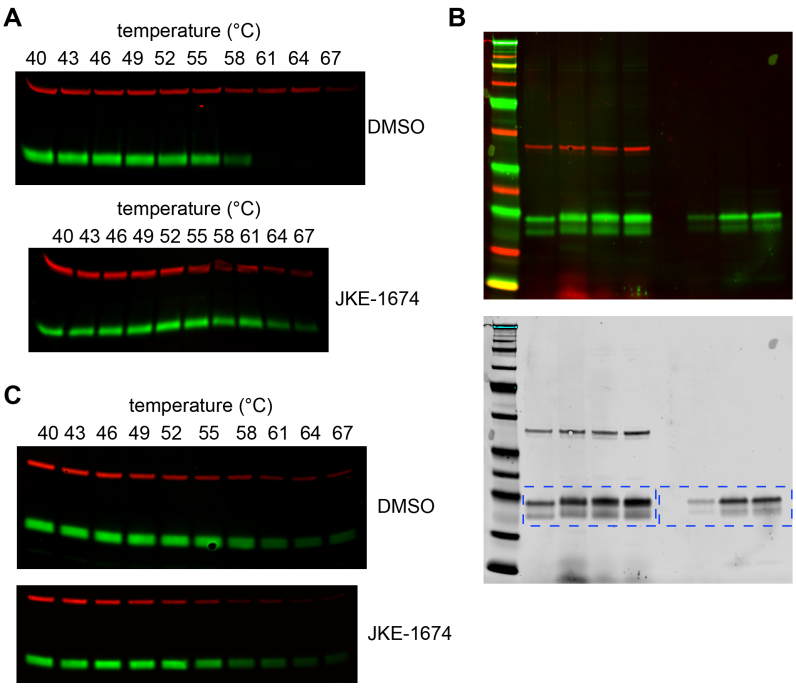
Supplementary Figure 9. Summary of ML210 nitroisoxazole structure–activity relationship (SAR) study. (**A**) ML210 analogs were synthesized and tested in cell viability assays in LOX-IMVI cells. Compounds are classified as inactive GPX4 inhibitors (red boxes) if they have no fer-1 rescuable effect on cell viability. The cellular effects of active analogs (green box) can be rescued by co-treatment fer-1 (1.5 μM). EC_{50} values were determined from 12-point dose-response experiments (n ≥ 2 technical replicates). (**B**) Selected dose-response curves of active 5-position analogs of ML210. (**C**) Dihydroisoxazole analog **36** exhibits cell-killing activity that is rescuable by fer-1 but has an unusual "bell-shaped" dose-response profile. Oxidation of the dihydroisoxazole group to a nitroisoxazole produces ML210, which may explain the cellular effects of **36**. Oxidation of **36** to ML210 occurs upon storage in DMSO at room temperature for extended periods. Data are plotted as two individual technical replicates.



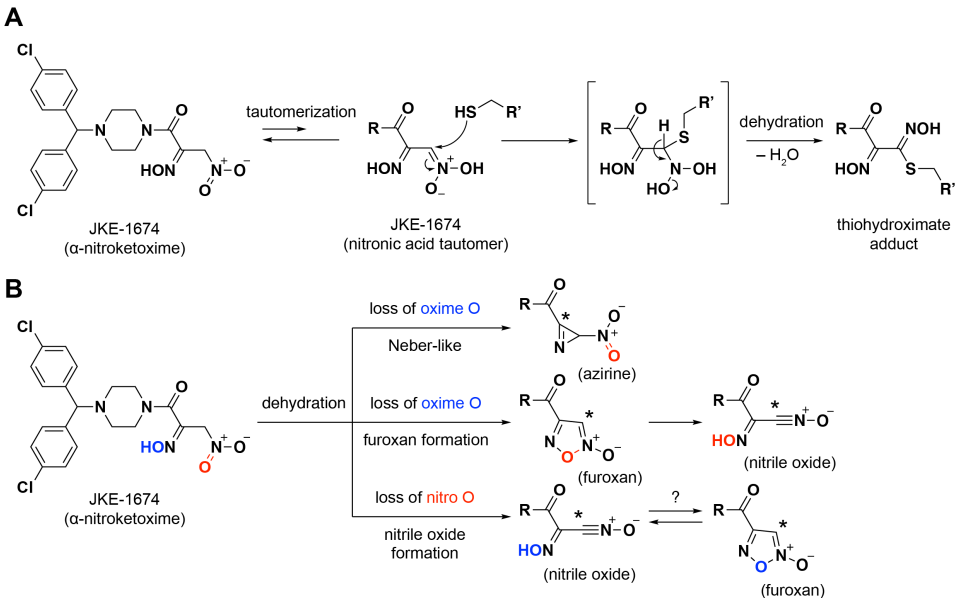
Supplementary Figure 10. Characterization of JKE-1674 and JKE-1674-yne.
(**A**) ML210 rapidly degrades to JKE-1674 (**37**) in pH 12 solution but is stable in acidic and neutral solutions. (**B**) Chemical structure of JKE-1674-yne (**38**). Alkyne group is highlighted in blue. (**C**) JKE-1674-yne exhibits activity in LOX-IMVI cells that is similar to ML210 and JKE-1674. Data are plotted as individual technical replicates. (**D**) JKE-1674-yne (10 μM, 1 h treatment) enables enrichment of GPX4 from LOX-IMVI cells to the same degree as ML210-yne (10 μM, 1 h treatment). (**E**) JKE-1674-yne exhibits proteome-wide reactivity in LOX-IMVI cells similar to ML210-yne. Cells were treated with 10 μM compound for 1 h. (**F**) The cell-killing activity of JKE-1674 is rescued completely by ferroptosis inhibitors fer-1 (1.5 μM), lip-1 (1 μM), and DFO (50 μM). Data are plotted as mean ± s.e.m., *n* = 4 technical replicates.



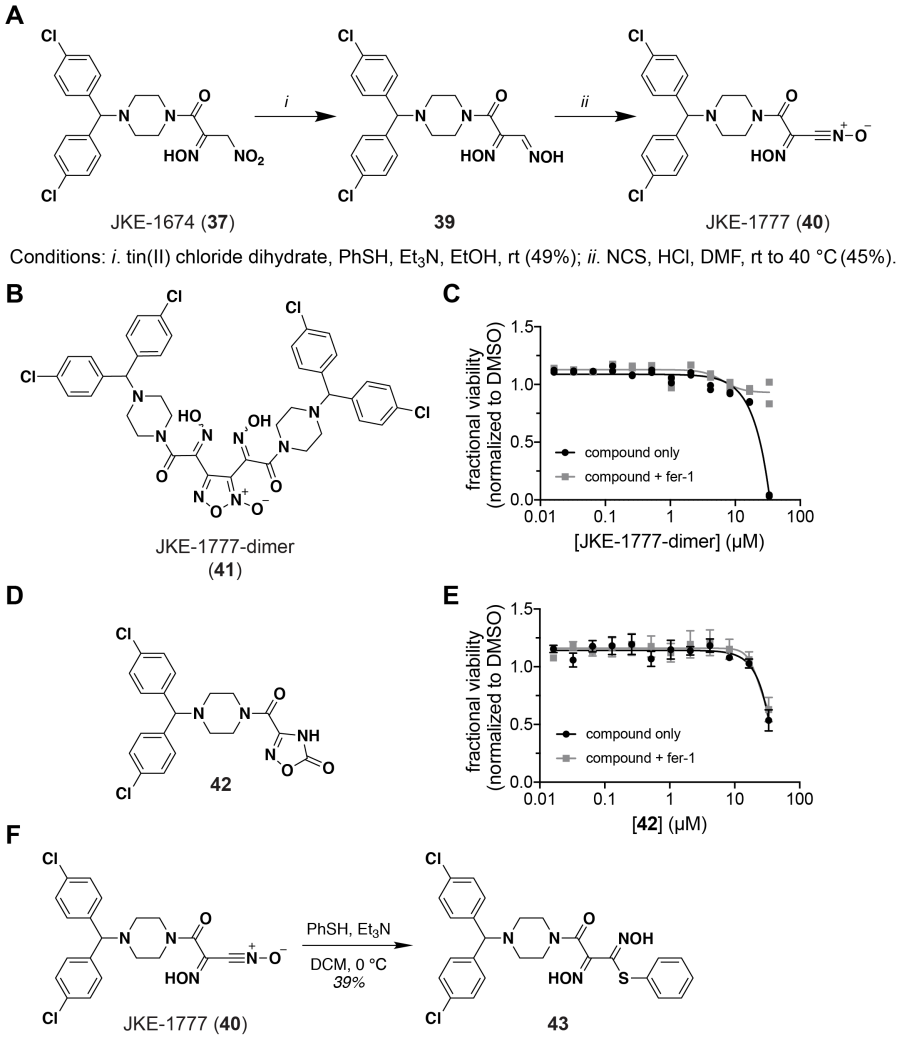
Supplementary Figure 11. JKE-1674 is formed in cells treated with ML210.
(**A**) JKE-1674 forms in a time-dependent manner in LOX-IMVI cells treated with ML210 (10 μM). (**B**) MS spectrum of JKE-1674 observed in cells treated with ML210 (10 μM). This spectrum is consistent with those of a synthetic standard of JKE-1674. (**C**) MS^2 spectrum of JKE-1674 observed in cells treated with ML210 (10 μM). This spectrum is consistent with those of a synthetic standard of JKE-1674. Fragmentation of the benzhydryl group under ESI+ conditions is commonly observed from ML210 analogs in both small-molecule and intact-protein MS experiments. (**D**) Levels of ML210 and seven related metabolites measured over the course of 48 h in LOX-IMVI cells treated with ML210 (10 μM). ML210 and metabolites were detected by LC-MS/MS. (**E**) Chemical structures of ML210-derived metabolites. Glutathione is abbreviated as "G" in structure P6. Identities of metabolites P1, **17**, and P5 were confirmed by comparison of LC-MS/MS spectra to synthetic standards. (**F**) Table of cell viability measurements in LOX-IMVI cells for selected metabolites. Only JKE-1674 exhibits cellular activity comparable to ML210. EC_{50} values were determined from 12-point dose-response experiments performed in duplicate. (**G**) Treatment of cells with JKE-1674 (10 μM) yields similar metabolites as observed upon treatment with ML210, including thiol adducts P5 and P6. (**H**) GPX4 CETSA profiles for LOX-IMVI lysates treated with DMSO (black) or 10 μM JKE-1674 (blue) at ambient temperature for 1 h. Data are plotted as individual replicates from two independent experiments. (**I**) JKE-1674 does not interact with purified wild-type GPX4 protein as determined by intact protein mass spectrometry.



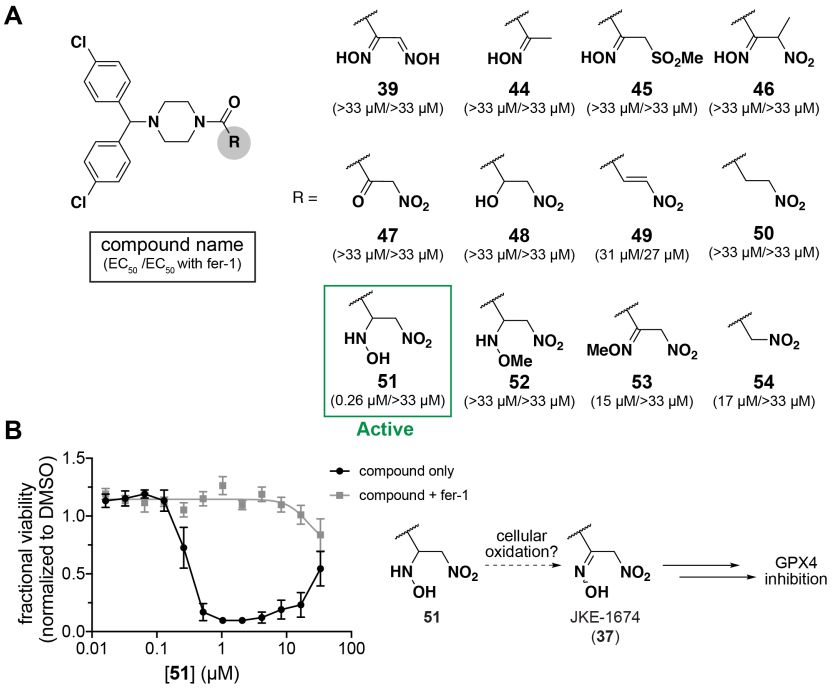
Supplementary Figure 12. Full images of western blots associated with Figure 3, Supplementary Figure 10, and Supplementary Figure 11. All gels shown in color depict GPX4 (green) and ACTB (red). Cropped sections of gels are shown with a blue box in grayscale images. (**A**) Representative intact-cell CETSA blots corresponding to the plot in Fig. 3e. (**B**) GPX4 pulldown assay from Supplementary Fig. 10d. (**C**) Representative lysate CETSA blots corresponding to the plot in Supplementary Fig. 11h.



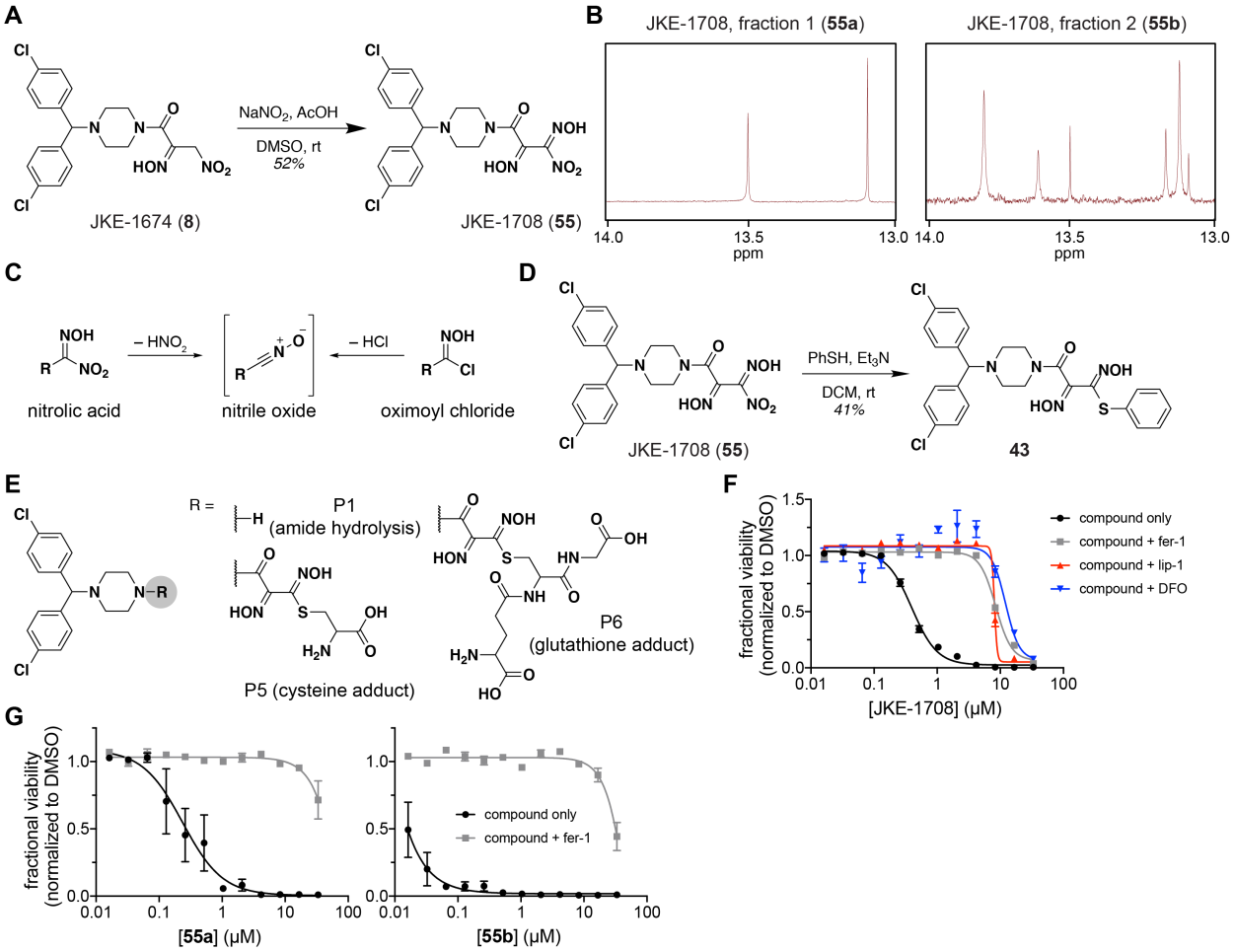
Supplementary Figure 13. Possible JKE-1674 reaction pathways.
(**A**) Possible reaction of JKE-1674 with thiols via a nitronic acid tautomer. The inability of JKE-1674 to react directly with small-molecule thiols or bind purified GPX4 suggests that this mechanism does not explain the observed reactivity of the α-nitroketoxime group in cells. (**B**) Proposed JKE-1674 dehydration pathways. Putative electrophilic sites in the dehydrated products are denoted with an asterisk (*).



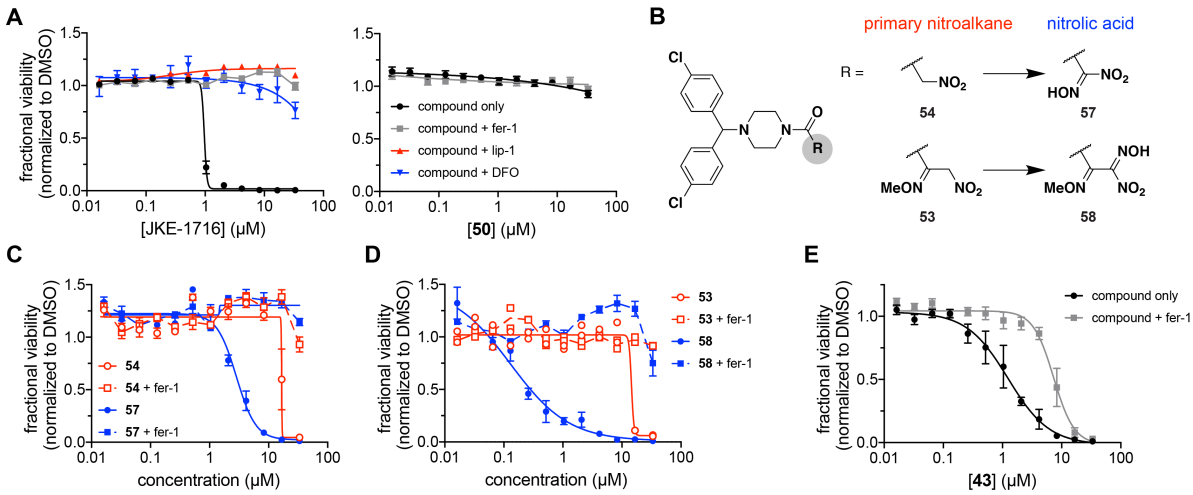
Supplementary Figure 14. Identification of nitrile oxide JKE-1777.
(**A**) Two-step synthesis of JKE-1777 (**40**) from JKE-1674 (**37**). (**B**) Proposed chemical structure of JKE-1777-dimer (**41**). (**C**) Cell viability assessment in LOX-IMVI cells of inactive JKE-1777-dimer (**41**). Data are plotted as two individual technical replicates. (**D**) Chemical structure of **42**, a possible oxadiazolone rearrangement product of JKE-1777. (**E**) Cell viability assessment in LOX-IMVI cells of **42**. Data are plotted as mean ± s.e.m., *n* = 4 technical replicates. (**F**) Nitrile oxide JKE-1777 reacts with thiophenol to produce thiohydroximate adduct **43**.



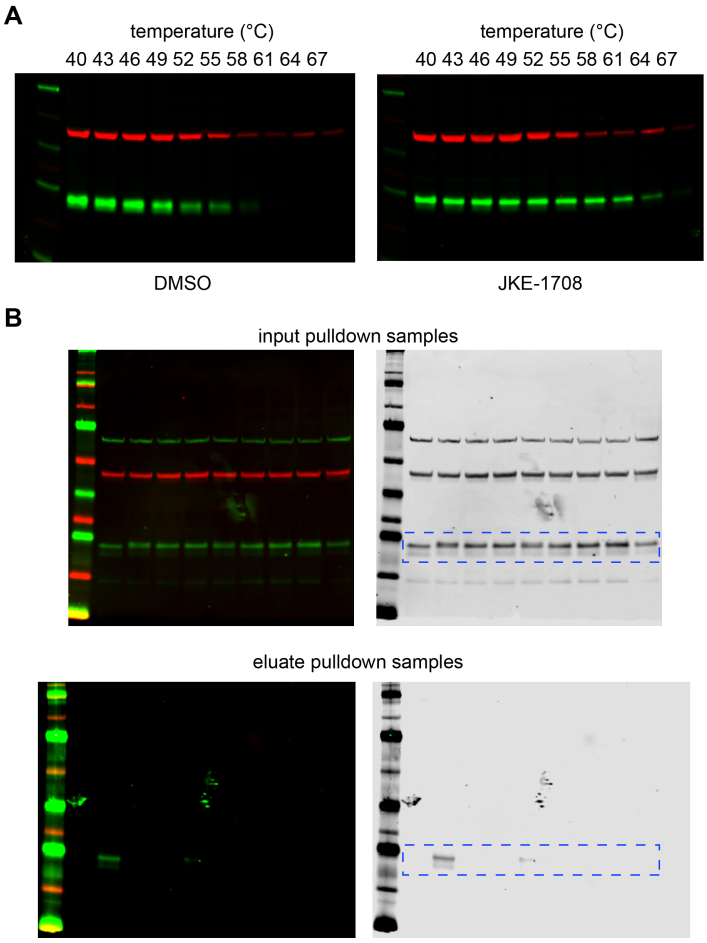
Supplementary Figure 15. Structure–activity relationship (SAR) studies of JKE-1674.
(**A**) Summary of JKE-1674 SAR. EC_{50} values were determined from 12-point dose-response experiments in LOX-IMVI cells (n ≥ 2 technical replicates). (**B**) Hydroxylamine **51** exhibits a "bell-shaped" dose-response in cell viability assays. Oxidation of the hydroxylamine group to an oxime produces JKE-1674, which may account for the cellular effects of **51** in a manner similar to dihydroisoxazole **36** (Supplementary Fig. 9c). Data are plotted as mean ± s.e.m., *n* = 6 technical replicates.



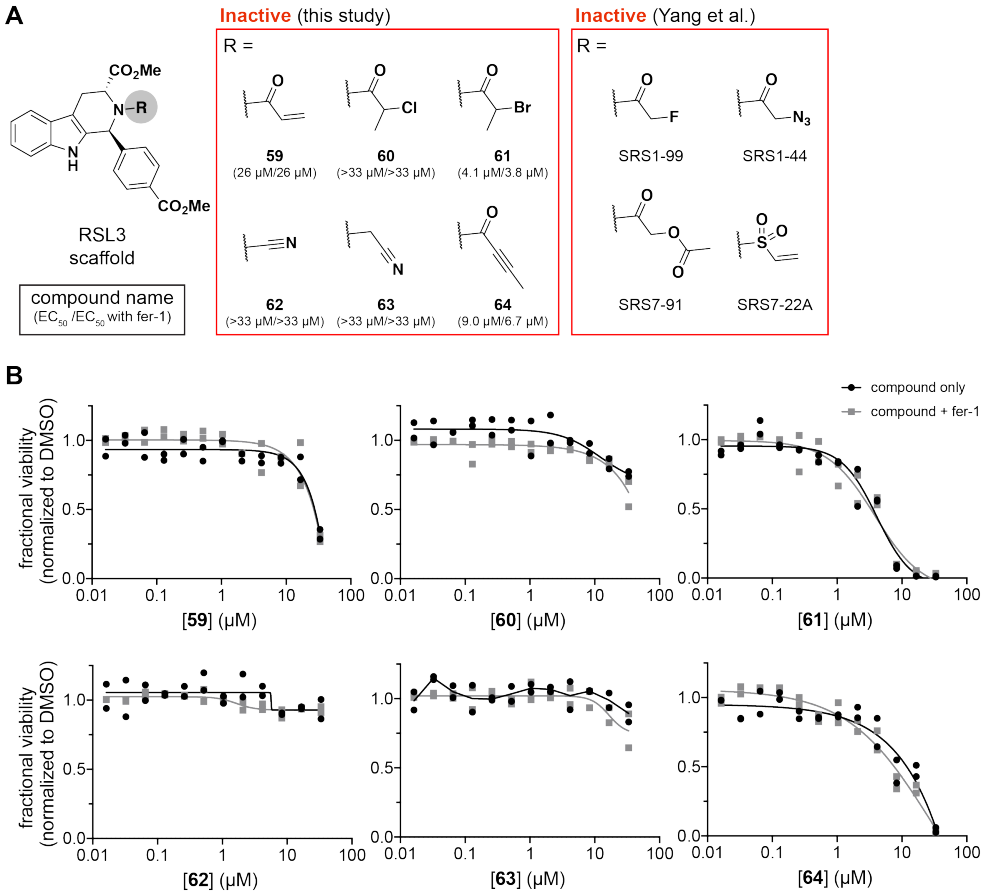
Supplementary Figure 16. Characterization of nitrolic acid JKE-1708 as a direct GPX4 inhibitor.
(**A**) Chemical synthesis of nitrolic acid JKE-1708 (**55**) from JKE-1674. JKE-1708 is obtained as a mixture of isomers and is used without further purification unless otherwise specified. (**B**) ^{1}H NMR spectra of the OH peaks (δ 13-14 ppm) of the two JKE-1708 fractions obtained after purification by flash column chromatography. JKE-1708 fraction 1 (**55a**) contains a single isomer while fraction 2 (**55b**) contains three isomers. The ratio of isomers does not change for either fraction in DMSO-d_6 solution for a period of at least 24 hours at room temperature. (**C**) Nitrolic acids are transformed into nitrile oxides via loss of HNO_2. This is analogous to the formation of nitrile oxides from hydroximoyl chlorides upon loss of HCl. (**D**) Nitrolic acid JKE-1708 reacts with thiophenol to produce the thiohydroximate **43**. This is the same adduct formed between JKE-1777 and thiophenol (Supplementary Fig. 14f). Both reactions produce slightly different mixtures of diastereomers. (**E**) Treatment of LOX-IMVI cells with JKE-1708 (10 μM) leads to the formation of metabolites formed in cells by ML210 and JKE-1674, including thiol adducts P5 and P6. See also Supplementary Fig. 11. (**F**) The cell-killing activity of JKE-1708 in LOX-IMVI cells can be suppressed by ferroptosis inhibitors fer-1 (1.5 μM), lip-1 (1 μM), and DFO (50 μM). Data are plotted as mean ± s.e.m., n = 4 technical replicates. (**G**) **55b** is more potent than **55a** in LOX-IMVI cells. Data are plotted as mean ± s.e.m., n = 4 technical replicates.



Supplementary Figure 17. Characterization of nitrolic acids as GPX4 inhibitors.
(**A**) The LOX-IMVI cell-killing activity of nitrolic acid JKE-1716 (**56**) can be rescued by co-treatment with fer-1 (1.5 μM), lip-1 (1 μM), and DFO (50 μM). Compound **50** is inactive over the tested concentration range. Data are plotted as mean ± s.e.m., *n* = 4 technical replicates. (**B**) Chemical structures of primary nitroalkanes that can be activated by conversion to their corresponding nitrolic acids. (**C**) Conversion of nitroalkane **54** to nitrolic acid **57** results in increased LOX-IMVI cell-killing activity. Data are plotted as mean ± s.e.m., *n* = 3 technical replicates. (**D**) Conversion of nitroalkane **53** to nitrolic acid **58** results in increased LOX-IMVI cell-killing activity. Data are plotted as mean ± s.e.m., *n* = 4 technical replicates for **58** and as two individual technical replicates for **53**. (**E**) Thiohydroximate adduct **43**, formed from thiophenol and either JKE-1777 (Supplementary Fig. 14f) or JKE-1708 (Supplementary Fig. 16d), exhibits LOX-IMVI cell-killing activity that is rescuable by fer-1 (1.5 μM). Data are plotted as mean ± s.e.m., *n* = 4 technical replicates.



Supplementary Figure 18. Full images of western blots associated with Figure 5. All gels shown in color depict GPX4 (green) and ACTB (red). Cropped sections of gels are shown with a blue box in grayscale images. (**A**) Representative intact-cell CETSA blots corresponding to the plot in Fig. 5b. (**B**) GPX4 pulldown assay from Supplementary Fig. 5g.



Supplementary Figure 19. Structure–activity relationship (SAR) studies of RSL3.
(**A**) Replacement of the chloroacetamide warhead of RSL3 with other electrophiles generates inactive analogs (**59–64**) in LOX-IMVI cells. These observations are consistent with the reported inactivity of a non-overlapping set of RSL3 analogs with alternate warheads, including fluoroacetamide, azidoacetamide, and vinylsulfonamide (Yang, W.S. et al. *Cell* **156**, 317-331 (2014)). (**B**) Dose-response curves for RSL3 warhead analogs in LOX-IMVI cells. Data are plotted as two individual technical replicates.

Synthetic Methods

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General Methods

Compounds were synthesized using standard procedures. Air- and moisture-sensitive reactions were performed in oven-dried glassware under a positive pressure of nitrogen. All reagents were obtained from commercial sources and used as received, unless otherwise noted. Anhydrous organic solvents were purchased from Sigma-Aldrich. Flash column chromatography was performed using 20-40 μm silica gel (60 Å mesh) on a Combiflash Rf 150 purification system (Teledyne Isco). Low-resolution mass spectrometry (LRMS) data were obtained with a Waters 2975 LC separations module coupled to a MicroMass ZQ 200 single quadrupole detector operating in ESI+ or ESI– mode. High-resolution mass spectra were acquired with either an Agilent 1290 Infinity separations module coupled to a 6230 time-of-flight (TOF) mass detector operating in ESI+ or ESI– mode or a JEOL AccuTOF 4G LC-plus equipped with an ionSense DART source (MIT Department of Chemistry Instrumentation Facility). Infrared (IR) spectra were recorded on a Nicolet Avatar 370 DTGS FTIR with a Smart Orbit diamond attenuated total reflectance accessory. The thin-film method was used for IR measurements unless otherwise noted. NMR spectra were recorded on Bruker Ultrashield AVANCE 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) or Bruker Ultrashield AVANCE 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometers. NMR solvents were purchased from Cambridge Isotope Laboratories or Sigma-Aldrich. Chemical shifts are reported in parts per million (ppm, δ scale) relative to residual non-deuterated solvent as internal standard: acetone-d_6 (δ_H = 2.05, δ_C = 29.84), chloroform-d ($CDCl_3$; δ_H = 7.26; δ_C = 77.16), dimethylsulfoxide-d_6 (DMSO-d_6; δ_H = 2.50; δ_C = 49.00), methanol-d_4 (MeOD-d_4; δ_H = 3.31; δ_C = 49.00), methylene chloride-d_2 (δ_H = 5.32; δ_C = 54.00), *N*,*N*-dimethylformamide-d_7 (DMF-d_7; δ_H = 8.03, 2.92, 2.75; δ_C = 163.15, 34.89, 29.76). Data for ^{1}H NMR are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constants (*J*) in Hz, and integration.

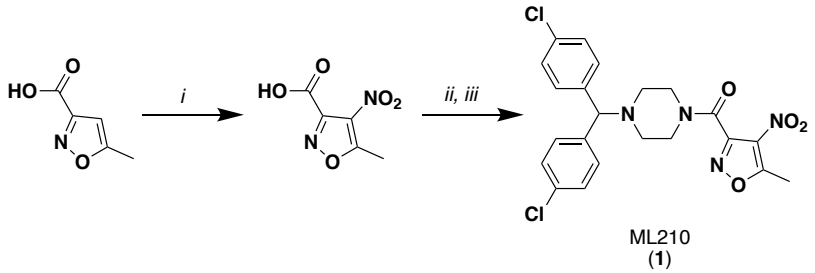
Abbreviations

AcOH	acetic acid
Boc	*tert*-butoxycarbonyl
CDI	1,1'-carbonyldiimidazole
DART	direct analysis in real time
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
DIPEA	*N,N*-diisopropylethylamine
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
EC_{50}	half maximal effective concentration
EDC	*N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide
ESI	electrospray ionization
Et_3N	triethylamine
EtOAc	ethyl acetate
EtOH	ethanol
eq.	equivalents
fer-1	ferrostatin-1
HRMS	high-resolution mass spectra
IR	infrared
LRMS	low-resolution mass spectra
MeCN	acetonitrile
Me_4NNO_3	tetramethylammonium nitrate
MeOH	methanol
MS	molecular sieves
MsCl	methanesulfonyl chloride
NaHMDS	hexamethyldisilazane sodium salt
NaO^tBu	sodium *tert*-butoxide

n-BuLi	*n*-butyllithium
NCS	*N*-chlorosuccinimide
NMR	nuclear magnetic resonance
PhH	benzene
PhMe	toluene
PhSH	thiophenol
PTSA	*p*-toluenesulfonic acid
TBTU	2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate
Tf_2O	trifluoromethanesulfonic anhydride
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TLC	thin-layer chromatography
$TMSCHN_2$	(trimethylsilyl)diazomethane
T3P	2,4,6-tripropyl-1,3,5,2,4,6-trioxatriphosphorinane-2,4,6-trioxide
rt	room temperature
R_t	retention time

Examples

ML210 (1)

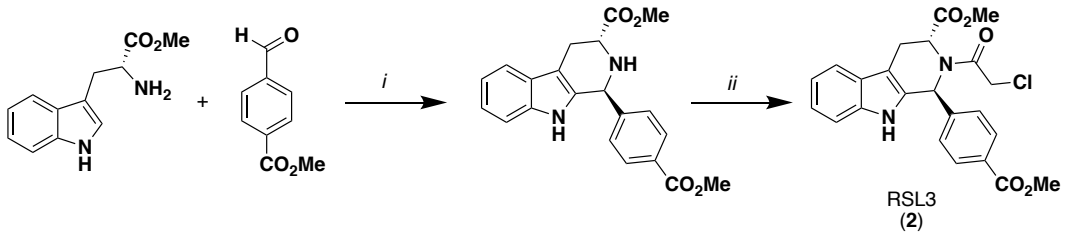


Scheme 1. Synthesis of ML210 (**1**).

Conditions: *i.* KNO_3, H_2SO_4, 50 °C; *ii.* oxalyl chloride, DCM, DMF, 0 °C to rt; *iii.* 1-(bis(4-chlorophenyl)methyl)piperazine, Et_3N, DCM, 0 °C to rt. (82%).

(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)(5-methyl-4-nitroisoxazol-3-yl)methanone (ML210, **1**): Synthesis of ML210 was accomplished according to a procedure adapted from Weïwer et al.[1] 5-Methyl-4-nitroisoxazole-3-carboxylic acid (1.0 g, 5.81 mmol, 1 eq.) was suspended in dry DCM (30 mL) cooled in an ice bath. Oxalyl chloride (1.0 mL, 11.6 mmol, 2 eq.) was slowly added, followed by 1 drop of DMF. The mixture was warmed to rt and stirred overnight. The intermediate acid chloride was isolated after rotary evaporation of the solvent with a rt water bath and was immediately dissolved in 35 mL of dry DCM and cooled in an ice bath. A mixture of 1-(bis(4-chlorophenyl)methyl)piperazine (1.87 g, 5.81 mmol, 1 eq.) and triethylamine (1.1 mL, 14.5 mmol, 1.5 eq.) in dry DCM (15 mL) was slowly added to the flask containing the acid chloride. The mixture was allowed to warm to rt while stirring for 2 h. Purification of the reaction by flash column chromatography (0-30% EtOAc/hexanes) afforded the title compound (**1**, 2.25 g, 82% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (q, *J* = 8.6 Hz, 8H), 4.25 (s, 1H), 3.84 (t, *J* = 5.1 Hz, 2H), 3.36 (t, *J* = 5.0 Hz, 2H), 2.85 (s, 3H), 2.52 (t, *J* = 5.1 Hz, 2H), 2.37 (t, *J* = 5.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.72, 156.50, 153.04, 140.00, 133.24, 129.04, 128.99, 128.79, 74.31, 51.54, 50.98, 46.94, 42.31, 13.47. IR (ATR) vmax (cm^{-1}): 2820, 2060, 2037, 2019, 2004, 1664, 1604, 1525, 1501, 1447, 1413, 1373, 1358, 1290, 1236, 1161, 1143, 1089, 1013, 997, 856, 826, 803, 769, 664, 541, 521, 505. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{22}H_{20}Cl_2N_4O_4$ 475.0940; found 475.0940.

RSL3 (2)

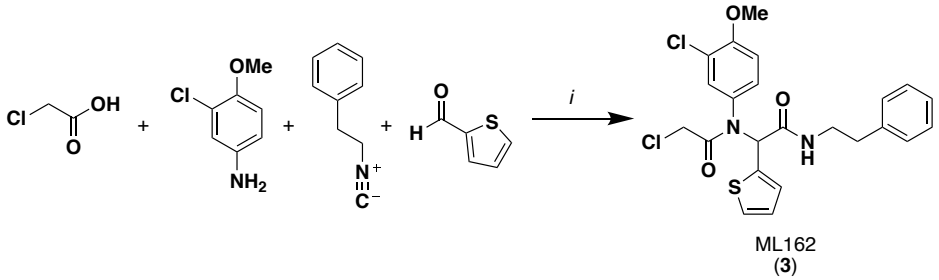


Scheme 2. Synthesis of RSL3 (**2**).

Conditions: *i.* TFA, DCM, reflux; *ii.* chloroacetyl chloride, K_3CO_3.

methyl (1*S*,3*R*)-2-(2-chloroacetyl)-1-(4-(methoxycarbonyl)phenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (RSL3**, 2**): Prepared as described by Yang et al. (ref. [2]). ^{1}H NMR (DMF-d_7, T = 330 K) δ 10.93 (s, 1H), 7.91 (s, 2H), 7.69 (s, 2H), 7.53 (d, *J* = 7.7 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.04 (dt, *J* = 21.4, 7.3 Hz, 2H), 6.29 (s, 1H), 5.48 (s, 1H), 4.74 (d, *J* = 13.8 Hz, 1H), 4.39 (s, 1H), 3.86 (s, 3H), 3.59 (s, 6H). ^{13}C NMR (101 MHz, DMF, T = 330 K) δ 168.41, 166.44, 137.56, 129.67, 126.94, 126.54, 121.81, 119.30, 118.20, 111.62, 57.29, 52.29, 51.78, 43.20. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{23}H_{21}ClN_2O_5$ 441.1217; found 441.1217.

ML162 (3)

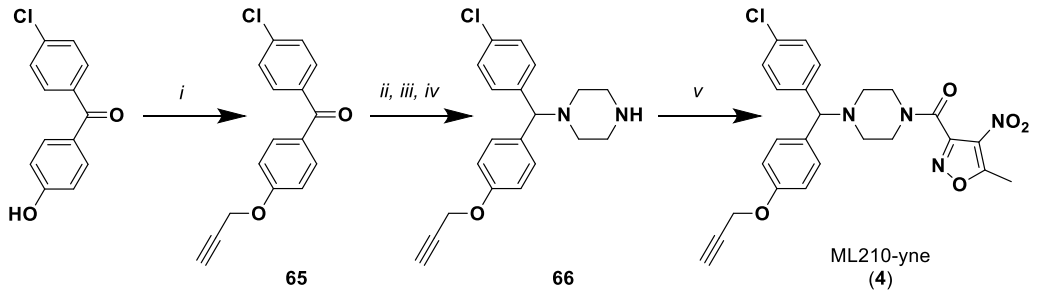


Scheme 3. Synthesis of ML162 (**3**)**.**

Conditions: *i.* MeOH, rt.

2-chloro-*N*-(3-chloro-4-methoxyphenyl)-*N*-(2-oxo-2-(phenethylamino)-1-(thiophen-2-yl)ethyl)acetamide (ML162, **3**): Prepared as described by Weïwer et al. (ref. [1]). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.12 (m, 7H), 6.99 – 6.63 (m, 4H), 6.09 (s, 1H), 6.01 (s, 1H), 3.88 (s, 3H), 3.81 (d, *J* = 2.1 Hz, 2H), 3.64 – 3.46 (m, *J* = 6.7 Hz, 2H), 2.91 – 2.72 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.06, 166.85, 155.66, 138.74, 134.91, 131.57, 131.36, 130.23, 129.48, 128.96, 128.74, 128.41, 126.67, 126.65, 111.86, 60.86, 56.42, 42.44, 41.20, 35.65. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{23}H_{22}Cl_2N_2O_3S$ 477.0806; found 477.0816.

ML210-yne (4)



Scheme 4. Synthesis of ML210-yne (**4**).

Conditions: *i.* propargyl bromide, K_2CO_3, PhMe, DMF, rt (92%); *ii*. $NaBH_4$, MeOH, THF, 0 °C; *iii.* oxalyl chloride, DMF, DCM, 0 °C to rt; *iv.* piperazine, MeCN, reflux (46% over three steps); *v*. 5-methyl-4-nitroisoxazole-3-carboxylic acid, oxalyl chloride, DMF, DCM, 0 °C to rt then **66**, Et_3N, DCM, 0 °C to rt (76%).

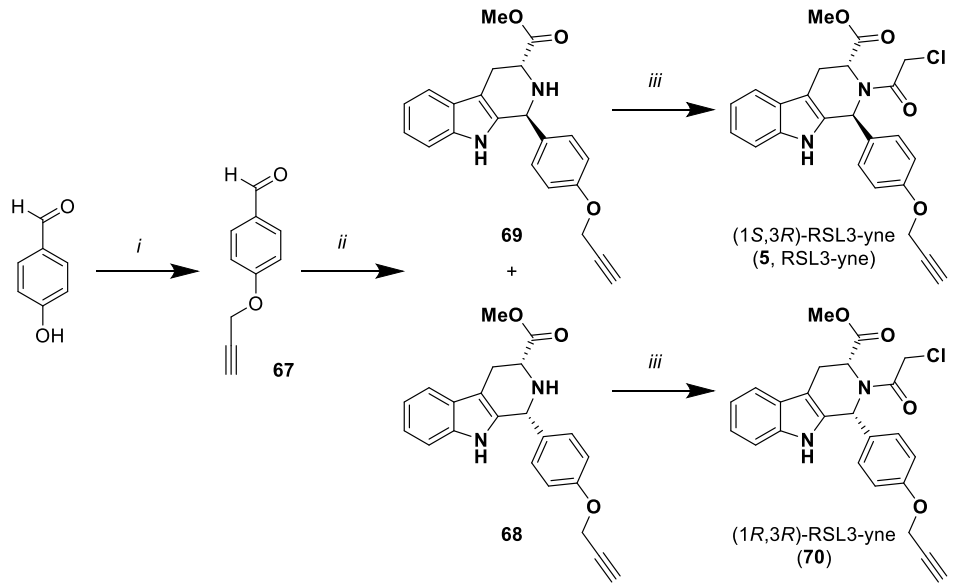
(4-chlorophenyl)(4-(prop-2-yn-1-yloxy)phenyl)methanone (**65**): (*i*) Potassium carbonate (1.07 g, 7.74 mmol, 2 eq.) was added to a solution of 4-chloro-4'-hydroxybenzophenone (900 mg, 3.87 mmol, 1 eq.) in dry DMF (4 mL). Propargyl bromide solution (80 wt% in toluene, 0.83 mL, 7.74 mmol, 1.5 eq.) was added to this and the mixture was stirred overnight at rt. The reaction was partitioned between EtOAc and water and the organic layer was separated. The organic layers were washed several times with water, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting off-white solid (**65**, 945 mg, 90% yield) was carried onto the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 – 7.77 (m, 2H), 7.77 – 7.65 (m, 2H), 7.51 – 7.40 (m, 2H), 7.12 – 7.00 (m, 2H), 4.78 (d, *J* = 2.4 Hz, 2H), 2.57 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 194.34, 161.32, 138.57, 136.55, 132.48, 131.33, 130.77, 128.72, 114.72, 77.88, 76.93, 76.37, 56.07. IR (ATR) vmax (cm-1): 3294, 1651, 1599, 1507, 1485, 1418, 1398, 1375, 1305, 1286, 1265, 1249, 1226, 1171, 1149, 1090, 1016, 927, 851, 837, 763, 677, 652. ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{16}H_{11}ClO_2$ 271.05; found 270.92.

1-((4-chlorophenyl)(4-(prop-2-yn-1-yloxy)phenyl)methyl)piperazine (**66**): (*ii*) A solution of **65** (0.94 g, 3.47 mmol, 1 eq.) in 1:1 THF/MeOH (20 mL) was cooled in an ice bath. $NaBH_4$ (0.13 g, 3.47 mmol, 1 eq.) was added to the reaction in one portion. After stirring for 30 minutes at 0 °C, the reaction was quenched with saturated aqueous ammonium chloride solution (~15 mL). The mixture was extracted with EtOAc, dried over anhydrous Na_2SO_4, filtered, and concentrated to afford crude (4-chlorophenyl)(4-(prop-2-yn-1-yloxy)phenyl)methanol. (*iii*) The resulting crude residue (0.82 g, 3.01 mmol, 1 eq.) was dissolved in dry DCM (10 mL) and cooled in an ice bath. Oxalyl chloride (0.52 mL, 6.01 mmol, 2 eq.) was added, followed by 1 drop of DMF and the reaction was stirred at rt overnight. Evaporation of the volatiles under reduced pressure afforded crude 1-chloro-4-(chloro(4-(prop-2-yn-1-yloxy)phenyl)methyl)benzene, which was immediately carried onto the next step. (*iv*) A solution of 1-chloro-4-(chloro(4-(prop-2-yn-1-yloxy)phenyl)methyl)benzene (0.87 g, 2.99 mmol, 1 eq.) in dry MeCN (30 mL) was refluxed overnight with piperazine (2.57 g, 29.9 mmol, 10 eq.). The reaction was cooled and concentrated under reduced pressure. The crude residue was partitioned between EtOAc and water. The organic layer was separated and washed several times with water. The organic layer was concentrated and the title compound (**66**, 0.56 g, 55% yield over 3 steps) was obtained as a pale-yellow solid after purification by flash column chromatography (0-10% 7 N NH_3 in MeOH/DCM). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 – 7.20 (m, 6H), 6.94 – 6.86 (m, 2H), 4.65 (d, *J* = 2.4 Hz, 2H), 4.17 (s, 1H), 2.89 (t, 4H), 2.52 (t, *J* = 2.4 Hz, 1H), 2.34 (s, 4H), 1.64 (s, 1H). ESI LRMS (m/z): [M+H]+ calculated for $C_{20}H_{21}ClN_2O$ 341.14; found 341.44.

(4-((4-chlorophenyl)(4-(prop-2-yn-1-yloxy)phenyl)methyl)piperazin-1-yl)(5-methyl-4-nitroisoxazol-3-yl)methanone (ML210-yne**, 4**): 5-Methyl-4-nitroisoxazole-3-carboxylic acid (285 mg, 1.66 mmol, 1 eq.) was dissolved in dry DCM (10 mL) and cooled in an ice bath. Oxalyl chloride (480 mg, 0.28 mL, 3.31 mmol, 2 eq.) was added dropwise followed by 1 drop of DMF. The mixture was stirred overnight at rt. The solvent was evaporated to dryness and the crude residue was dissolved in dry DCM (10 mL) and cooled in an ice bath. A solution of **66** (564 mg, 1.66 mmol, 1 eq.) and triethylamine (0.35 mL, 2.48 mmol, 1.5 eq.) in dry DCM (5 mL) was added slowly. The mixture was stirred at rt for several hours, concentrated, and purified by flash column chromatography (30% EtOAc/hexanes). The title compound (**4**, 625 mg, 76% yield) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 (d, *J* = 8.1 Hz, 2H), 7.38 – 7.30 (m, 4H),

6.92 (d, *J* = 8.1 Hz, 2H), 4.73 (d, *J* = 2.5 Hz, 2H), 4.39 (s, 1H), 3.69 (d, *J* = 4.9 Hz, 2H), 3.52 (d, *J* = 2.5 Hz, 1H), 3.35 (d, *J* = 5.0 Hz, 2H), 2.80 (s, 3H), 2.40 (d, *J* = 5.1 Hz, 2H), 2.21 (q, *J* = 5.5, 5.1 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.83, 156.76, 156.55, 153.40, 142.10, 134.78, 131.84, 129.77, 129.18, 129.01, 128.70, 115.40, 79.74, 78.67, 73.17, 55.81, 51.84, 51.13, 46.59, 42.15, 14.02. IR (ATR) vmax (cm-1): 3294, 2923, 2854, 1659, 1605, 1524, 1505, 1447, 1417, 1372, 1357, 1289, 1235, 1218, 1176, 1161, 1144, 1113, 1089, 1028, 1014, 996, 909, 856, 826, 770, 733, 666, 548, 520. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{25}H_{23}Cl_2N_4O_5$ 495.1435; found 495.1424.

RSL3-yne (5)



Scheme 5. Synthesis of (1*S*,3*R*)-RSL3-yne (**5**).

Conditions: *i*. propargyl bromide, K_2CO_3, PhMe, DMF, rt (83%); *D*-tryptophan methyl ester hydrochloride, TFA, 4 Å MS, MeCN, rt to reflux; *iii*. chloroacetyl chloride, DIPEA, DCM, 0 °C to rt (25% combined yield over 2 steps for both diastereomers).

4-(prop-2-yn-1-yloxy)benzaldehyde (**67**) was prepared as described by Pal et al. (ref. [3]). The spectral data were in agreement with reported values. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.91 (s, 1H), 7.92 – 7.80 (m, 2H), 7.15 – 7.03 (m, 2H), 4.78 (d, *J* = 2.4 Hz, 2H), 2.57 (t, *J* = 2.4 Hz, 1H).

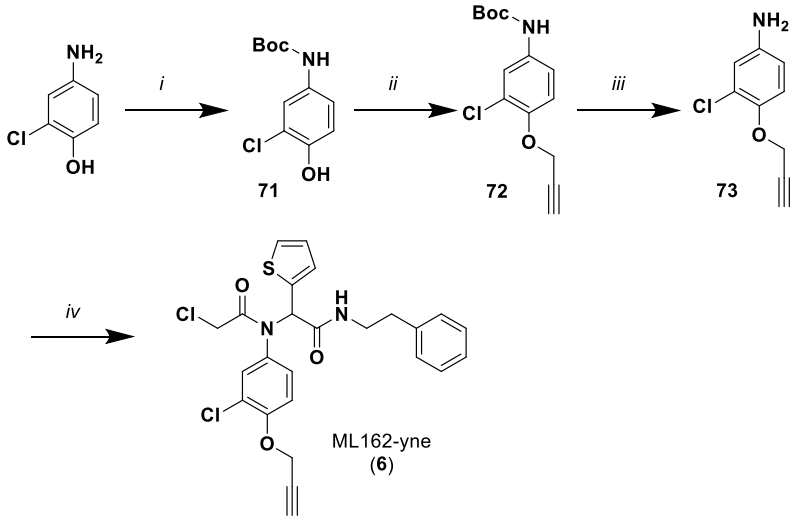
methyl (1*R*,3*R*)-1-(4-(prop-2-yn-1-yloxy)phenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (**68**) and methyl (1*S*,3*R*)-1-(4-(prop-2-yn-1-yloxy)phenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (**69**): *D*-Tryptophan methyl ester hydrochloride (500 mg, 1.96 mmol, 1 eq.) was dissolved in EtOAc (10 mL) and washed with saturated aqueous $NaHCO_3$ (~5 mL × 3). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was dissolved in MeCN (20 mL) with activated 4 Å molecular sieves (500 mg) and 4-(prop-2-yn-1-yloxy)benzaldehyde (314 mg, 1.96 mmol, 1 eq.) was added, followed by 1 drop of TFA. The mixture was refluxed for 1 h before adding another 1 mL of TFA and refluxing was continued overnight. The reaction was cooled, filtered, and diluted with DCM. The organic layer was washed with saturated aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$, filtered, and concentrated. Purification by flash column chromatography (40% EtOAc/hexanes with 1% triethylamine) afforded, in order of elution, (1*R*,3*R*)-intermediate (**68**, 313 mg, 44% yield) and (1*S*,3*R*)-intermediate (**69**, 298 mg, 42% yield). Each product was carried separately on to the subsequent chloroacetylation step. (1*R*,3*R*) isomer (**68**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 – 7.47 (m, 1H), 7.44 (s, 1H), 7.36 – 7.26 (m, 2H), 7.27 – 7.16 (m, 1H), 7.21 – 7.06 (m, 2H), 7.02 – 6.93 (m, 2H), 5.21 (t, *J* = 2.2 Hz, 1H), 4.70 (d, *J* = 2.4 Hz, 2H), 3.97 (dd, *J* = 11.1, 4.3 Hz, 1H), 3.81 (s, 3H), 3.22 (ddd, *J* = 15.2, 4.3, 1.9 Hz, 1H), 3.00 (ddd, *J* = 15.2, 11.1, 2.6 Hz, 1H), 2.53 (t, *J* = 2.4 Hz, 1H), 2.42 (s, 1H). ESI LRMS (m/z): $[M+H]^+$

calculated for $C_{22}H_{20}N_2O_3$ 361.15; found 360.90. (1*S*,3*R*) isomer (**69**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 – 7.49 (m, 2H), 7.28 – 7.06 (m, 5H), 6.99 – 6.88 (m, 2H), 5.37 (s, 1H), 4.68 (d, *J* = 2.4 Hz, 2H), 4.02 – 3.91 (m, 1H), 3.72 (s, 3H), 3.34 – 3.04 (m, 2H), 2.51 (t, *J* = 2.4 Hz, 1H). ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{22}H_{20}N_2O_3$ 361.15; found 361.27.

methyl (1*R*,3*R*)-2-(2-chloroacetyl)-1-(4-(prop-2-yn-1-yloxy)phenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate ((1*R*,3*R*)-RSL3-yne, **70**)**:** Intermediate **68** from the preceding step (200 mg, 0.55 mmol, 1 eq.) was dissolved in dry DCM (5 mL) with DIPEA (0.11 mL, 0.61 mmol, 1.1 eq.) and cooled in an ice bath. Chloroacetyl chloride (0.05 mL, 0.061 mmol, 1.1 eq.) was added dropwise and the mixture was stirred at rt overnight. The organic layer was washed with water, dried over Na_2SO_4, filtered, and concentrated. The title compound (**70**, 135 mg, 55% yield) was isolated after purification by flash column chromatography (EtOAc/hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.64 – 7.56 (m, 1H), 7.31 – 7.25 (m, 1H), 7.24 – 7.13 (m, 4H), 6.94 – 6.74 (m, 3H), 4.91 (s, 1H), 4.62 (d, *J* = 2.4 Hz, 2H), 4.41 – 4.16 (m, 2H), 3.68 (d, *J* = 15.8 Hz, 1H), 3.25 – 3.13 (m, 1H), 3.08 (s, 3H), 2.47 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.19, 167.14, 157.34, 136.56, 132.18, 130.94, 129.79, 126.49, 122.67, 119.93, 118.71, 114.64, 111.19, 107.70, 78.46, 75.75, 55.82, 53.60, 52.42, 51.85, 42.32, 21.72. IR (ATR) vmax (cm-1): 3292, 2950, 1738, 1647, 1625, 1608, 1585, 1506, 1452, 1421, 1372, 1304, 1256, 1237, 1206, 1175, 1115, 1023, 963, 906, 853, 827, 798, 728, 675, 648, 535. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{24}H_{21}ClN_2O_4$ 437.1268; found 437.1267. Stereochemical assignment is further supported by observed cell viability measurements in LOX-IMVI melanoma cells: (EC_{50} = 20 μM; EC_{50} with fer-1 = 20 μM), which is similar to reported activity of (1*R*,3*R*)-RSL3 (ref. [2]).

methyl (1*S*,3*R*)-2-(2-chloroacetyl)-1-(4-(prop-2-yn-1-yloxy)phenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate ((1*S*,3*R*)-RSL3-yne**,** RSL3-yne**, 5**): Intermediate **69** from the preceding step (200 mg, 0.55 mmol, 1 eq.) was dissolved in dry DCM (5 mL) with DIPEA (79 mg, 0.61 mmol, 0.11 mL, 1.1 eq.) and cooled in an ice bath. Chloroacetyl chloride (69 mg, 0.061 mmol, 0.05 mL, 1.1 eq.) was added dropwise. The mixture was stirred at rt overnight. The organic layer was washed with water, dried over sodium sulfate, filtered, and concentrated. The title compound (**5**, 145 mg, 60% yield) was isolated after purification by flash column chromatography (EtOAc/hexanes). ^{1}H NMR (400 MHz, DMF-d_7, T = 325 K) δ 11.10 (s, 1H), 7.70 (d, *J* = 7.8 Hz, 1H), 7.65 (d, *J* = 7.6 Hz, 2H), 7.49 (d, *J* = 7.9 Hz, 1H), 7.22 (dt, *J* = 22.4, 7.3 Hz, 4H), 6.46 (s, 1H), 5.41 (s, 2H), 4.98 (s, 2H), 4.88 (d, *J* = 13.7 Hz, 1H), 4.52 (d, *J* = 13.9 Hz, 1H), 3.76 (s, 3H), 3.47 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) [Note: several peaks appear broad] δ 171.18 (br), 168.93, 157.65 (br), 136.80, 133.70 (br), 132.65 (br), 128.05, 126.36, 122.48, 119.94, 118.50, 115.69 (br), 111.37, 106.90 (br), 78.40, 75.99, 57.41 (br), 55.92, 54.73 (br), 52.73 (br), 42.67 (br), 22.80 (br). ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{24}H_{21}ClN_2O_4$ 437.1268; found 437.1277. Stereochemical assignment is further supported by cell viability measurements in LOX-IMVI melanoma cells: EC_{50} = 0.004 μM (EC_{50} with fer-1 = 5 μM). See Supplementary Figure 3d.

ML162-yne (6)



Scheme 6. Synthesis of ML162-yne (**6**).

Conditions: *i.* Boc_2O, H_2O, rt (84%); *ii.* propargyl bromide, K_2CO_3, PhMe, DMF, rt (85%); *iii*. TFA, DCM, rt (78%); *iv*. chloroacetyl chloride, 2-thiophenecarboxaldehyde, 2-phenethylisocyanide, MeOH, rt (38%).

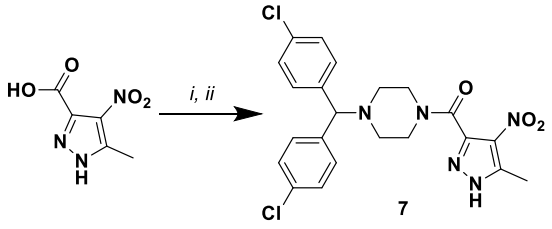
tert-butyl (3-chloro-4-hydroxyphenyl)carbamate (**71**): A suspension of 4-amino-2-chlorophenol (500 mg, 3.48 mmol, 1 eq.) and di-*tert*-butyl dicarbonate (836 mg, 3.83 mmol, 1.1 eq.) in water (4 mL) was stirred at rt for 36 hours. The mixture was extracted with DCM. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. Purification of the residue by flash column chromatography (20% EtOAc/hexanes) afforded the title compound (**71**, 714 mg, 84% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.08 – 6.89 (m, 2H), 6.34 (s, 1H), 5.33 (s, 1H), 1.52 (s, 9H). ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{11}H_{14}ClNO_3$ 244.07; found 244.10.

tert-butyl (3-chloro-4-(prop-2-yn-1-yloxy)phenyl)carbamate (**72**): Potassium carbonate (851 mg, 6.2 mmol, 1.5 eq.) was added to a solution of **71** (1000 mg, 4.1 mmol, 1 eq.) in dry DMF (6 mL). Propargyl bromide solution (80 wt% in toluene, 793 mg, 0.72 mL, 5.3 mmol, 1.3 eq.) was added and the mixture was stirred overnight at rt. The reaction was partitioned between EtOAc and water. The organic layer was separated, washed with water and brine, and concentrated *in vacuo*. Purification by flash column chromatography (20% EtOAc/hexanes) afforded the title compound (**72**, 984 mg, 85% yield) as a pale-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 – 7.47 (m, 1H), 7.21 – 7.12 (m, 1H), 7.05 – 6.98 (m, 1H), 6.39 (s, 1H), 4.73 (d, *J* = 2.4 Hz, 2H), 2.52 (t, *J* = 2.4 Hz, 1H), 1.49 (s, 9H). ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{14}H_{16}ClNO_3$ 282.09; found 282.06.

3-chloro-4-(prop-2-yn-1-yloxy)aniline (**73**): A solution of **72** (984 mg, 3.49 mmol, 1 eq.) in 1:1 DCM/TFA (6 mL) was stirred at rt for 1 h. The solvent was evaporated and the residue was partitioned between EtOAc and saturated aqueous $NaHCO_3$ solution. The organic layer was separated, dried over anhydrous Na_2SO4, filtered, and concentrated *in vacuo* to afford the title compound (**72**, 496 mg, 78% yield) as a pale-yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.96 (d, *J* = 8.7 Hz, 1H), 6.76 (d, *J* = 2.8 Hz, 1H), 6.56 (dd, *J* = 8.7, 2.8 Hz, 1H), 4.68 (d, *J* = 2.4 Hz, 2H), 3.44 (s, 2H), 2.52 (t, *J* = 2.4 Hz, 1H). ESI LRMS (m/z): $[M+H]^+$ calculated for C_9H_8ClNO 182.04; found 184.03.

2-chloro-*N*-(3-chloro-4-(prop-2-yn-1-yloxy)phenyl)-*N*-(2-oxo-2-(phenethylamino)-1-(thiophen-2-yl)ethyl)acetamide (ML162-yne**, 6**): A solution of 2-thiophene-carboxaldehyde (142 mg, 1.27 mmol, 1.2 eq.) and **73** (231 mg, 1.27 mmol, 1.2 eq.) was stirred at rt in MeOH (0.5 mL) for 15 minutes. 2-Chloroacetic acid (100 mg, 1.06 mmol, 1 eq.) and 2-phenethylisocyanide (139 mg, 1.06 mmol, 1 eq.) were then added. The reaction was stirred at rt for 24 h and subsequently concentrated *in vacuo*. Purification by flash column chromatography (30-35% EtOAc/hexanes) afforded the title compound (**6**, 204 mg, 38% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 – 6.57 (m, 11H), 6.08 (d, *J* = 6.5 Hz, 2H), 4.75 (d, *J* = 2.4 Hz, 2H), 3.82 (d, *J* = 1.6 Hz, 2H), 3.65 – 3.45 (m, 2H), 2.90 – 2.71 (m, 2H), 2.59 – 2.54 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.92, 166.62, 153.60, 138.61, 134.77, 132.21, 131.68, 130.13, 129.18, 128.83, 128.71, 128.60, 128.27, 126.57, 126.51, 123.49, 113.77, 77.30, 76.77, 60.81, 56.92, 42.35, 41.08, 35.50. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{25}H_{22}Cl_2N_2O_3S$ 501.0801; found 501.0819.

Compound 7



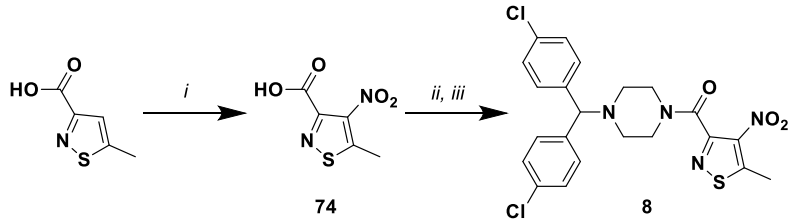
Scheme 7. Synthesis of **7**.

Conditions: *i.* oxalyl chloride, DCM, DMF, rt; *ii.* Et_3N, 1-(bis(4-chlorophenyl)methyl)piperazine, DCM, rt (73% over two steps).

1-[bis(4-chlorophenyl)methyl]-4-(5-methyl-4-nitro-1*H*-pyrazole-3-carbonyl)piperazine (**7**): To a suspension of 5-methyl-4-nitro-1*H*-pyrazole-3-carboxylic acid (54 mg, 315 µmol, 1.0 eq.) in DCM (2 mL)

and 1 drop of DMF was added oxalyl chloride (38 µL, 450 µmol, 1.5 eq.) under a stream of nitrogen. The resultant bubbling mixture was stirred overnight at rt then concentrated from hexanes to obtain a semi-solid oil. The crude acid chloride was re-suspended in DCM (1 mL) and added to a cold solution of 1-[bis(4-chlorophenyl)methyl]piperazine (96.3 mg, 0.3 mmol) and DIPEA (104 µL, 600 µmol, 2.0 eq.) in DCM (2 mL) at 0 °C. The reaction was stirred at rt for 2 h, diluted with DCM, and washed with saturated aqueous sodium bicarbonate solution. The layers were separated and the organic phase was washed with brine, dried over sodium sulfate, filtered, and concentrated. The crude residue was purified by flash chromatography on silica gel (0-100% EtOAc/hexanes) to obtain the title compound (**7**, 104 mg, 73% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.06 (s, 1H), 7.41 – 7.07 (m, 8H), 4.23 (s, 1H), 3.83 (t, *J* = 5.1 Hz, 2H), 3.28 (t, *J* = 5.0 Hz, 2H), 2.49 (t, *J* = 5.1 Hz, 2H), 2.43 (s, 3H), 2.32 (t, *J* = 5.1 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.8, 142.3, 141.5, 140.1, 133.3, 129.7, 129.1, 129.1, 77.5, 77.4, 77.2, 76.8, 74.4, 51.5, 51.1, 47.1, 42.5, 11.4. IR (ATR) vmax (cm^{-1}): 2934, 2856, 1795, 1655, 1587, 1493, 1417, 1357, 1284, 1252, 1167, 1094, 1014, 856, 829, 798, 775, 669. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{22}H_{21}Cl_2N_5O_3$ 474.1100; found 474.1105.

Compound 8



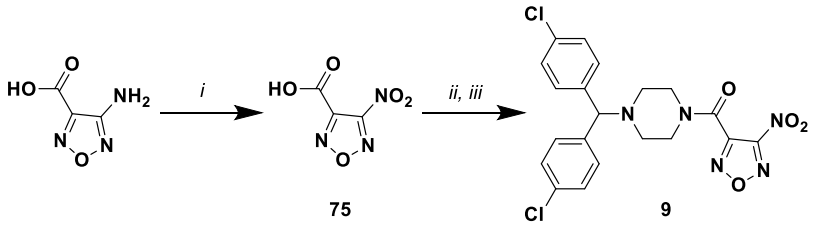
Scheme 8. Synthesis of **8**.

Conditions: *i*. KNO_3, H_2SO_4, 90 °C; *ii*. oxalyl chloride, DCM, DMF, rt; *iii*. Et_3N, 1-(bis(4-chlorophenyl)methyl)piperazine, DCM, rt (24% over three steps).

5-methyl-4-nitroisothiazole-3-carboxylic acid (**74**): 5-Methylisothiazole-3-carboxylic acid (0.145 mg, 1.01 mmol, 1 eq.) was dissolved in concentrated H_2SO_4 (5 mL) with KNO_3 (307 mg, 3.04 mmol, 3 eq.) at rt. The reaction was heated to 90 °C for 48 h and subsequently cooled to rt. The reaction was carefully diluted with water and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$ and evaporated to afford the title compound (**74**, 191 mg, >99% yield) as a yellow solid. The material was carried onto the next step directly without additional purification. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 2.76 (s, 3H). 5-Methylisothiazole-3-carboxylic acid was obtained from Enamine Ltd. (Catalog ID EN300-108424).

(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)(5-methyl-4-nitroisothiazol-3-yl)methanone (**8**): A suspension of **74** (190 mg, 1.01 mmol, 1 eq.) in dry DCM (5 mL) was cooled on an ice bath. Oxalyl chloride (0.26 mL, 2.02 mmol, 2 eq.) was added slowly, followed by 1 drop of DMF. The reaction was warmed to rt overnight while stirring. The solvent was then evaporated under reduced pressure. The residue was dissolved in dry DCM (2 mL) and slowly added to an ice-cold solution of 1-(bis(4-chlorophenyl)methyl)piperazine (324 mg, 1.01 mmol, 1 eq.) and DIPEA (0.23 mL, 1.31 mmol, 1.3 eq.) in DCM (3 mL). The mixture was warmed to rt and stirred overnight. The reaction was dry-loaded onto Celite and purified by flash column chromatography (0-30% EtOAc/hexanes) to afford the title compound (**8**, 117 mg, 24% yield over three steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.22 (m, 8H), 4.25 (s, 1H), 3.82 (t, *J* = 5.1 Hz, 2H), 3.31 (t, *J* = 4.7 Hz, 2H), 2.83 (s, 3H), 2.52 (t, *J* = 5.1 Hz, 2H), 2.36 (t, *J* = 5.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.83, 161.29, 159.61, 141.36, 140.14, 133.18, 129.00, 128.99, 74.42, 51.51, 51.04, 46.69, 42.14, 13.56. IR (ATR) vmax (cm-1): 2915, 2816, 1646, 1593, 1545, 1501, 1487, 1445, 1410, 1382, 1365, 1342, 1290, 1258, 1239, 1182, 1142, 1111, 1088, 1045, 1013, 988, 908, 865, 837, 812, 802, 775, 762, 728, 686, 667, 647, 538, 503. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{22}H_{20}Cl_2N_4O_3S$ 491.0711; found 491.0721.

Compound 9



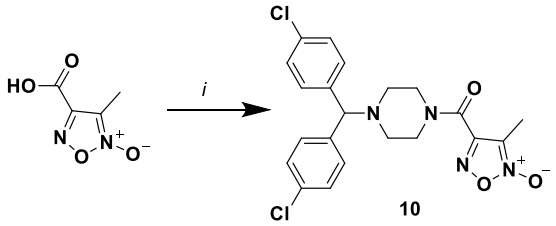
Scheme 9. Synthesis of **9**.

Conditions: *i*. H_2O_2, H_2SO_4, rt; *ii*. oxalyl chloride, DCM, DMF, rt; *iii*. Et_3N, 1-(bis(4-chlorophenyl)methyl)piperazine, DCM, rt (16% over three steps).

4-nitro-1,2,5-oxadiazole-3-carboxylic acid (**75**): An oxidizing solution was prepared by carefully adding 30% H_2O_2 (0.8 mL, 7.75 mmol, 4 eq.) solution to concentrated H_2SO_4 (1.5 mL) at rt. 4-Amino-1,2,5-oxadiazole-3-carboxylic acid (250 mg, 1.94 mmol, 1 eq.) was added in portions. The reaction was stirred overnight at rt and then partitioned between water and EtOAc. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated to afford the title compound (**75**, 194 mg) as a yellow oil. The crude product was carried onto the next step directly.

(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)(4-nitro-1,2,5-oxadiazol-3-yl)methanone (**9**): **75** (190 mg, 1.19 mmol, 1 eq.) was dissolved in dry DCM (2 mL) and cooled on an ice bath. Oxalyl chloride (0.303 mg, 0.2 mL, 2.39 mmol, 2 eq.) was added slowly followed by 1 drop DMF. The mixture was stirred at rt overnight and the solvent was then evaporated under reduced pressure. The residue was dissolved in dry DCM (1mL) and slowly added to an ice-cold solution of 1-(bis(4-chlorophenyl)methyl)piperazine (288 mg, 0.90 mmol, 0.75 eq.) and DIPEA (0.2 mL, 1.16 mmol, 1.3 eq.) in DCM (2 mL). The mixture was allowed to warm to rt overnight. The reaction was concentrated and purified by flash column chromatography (0-20% EtOAc/hexanes) to afford the title compound (**9**, 109 mg, 16% yield over three steps) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.48 – 7.35 (m, 8H), 4.52 (s, 1H), 3.80 – 3.68 (m, 2H), 3.47 – 3.35 (m, 2H), 2.46 – 2.36 (m, 2H), 2.30 – 2.18 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.25, 153.41, 145.56, 141.16, 132.22, 129.92, 129.15, 72.77, 51.90, 51.03, 46.70, 42.56. IR (ATR) vmax (cm-1): 2820, 2362, 1661, 1576, 1542, 1487, 1448, 1411, 1393, 1369, 1343, 1290, 1259, 1258, 1144, 1122, 1107, 1089, 1036, 1014, 996, 865, 822, 813, 802, 756, 541, 504. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{20}H_{17}Cl_2N_5O_4$ 462.0730; found 462.0754.

Compound 10



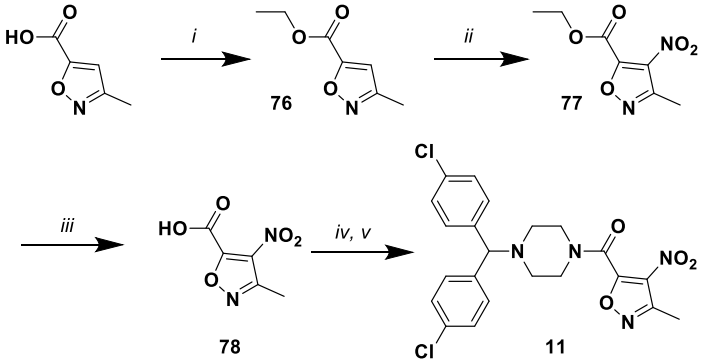
Scheme 10. Synthesis of **10**.

Conditions: *i*. 1-(bis(4-chlorophenyl)methyl)piperazine, DIPEA, T3P, DMF, rt (19%).

4-(4-(bis(4-chlorophenyl)methyl)piperazine-1-carbonyl)-3-methyl-1,2,5-oxadiazole 2-oxide (**10**)**:** To a solution of 4-carboxy-3-methyl-1,2,5-oxadiazole 2-oxide (50 mg, 0.347 mmol, 1.2 eq.) and *N*,*N*-diisopropylethylamine (0.2 mL, 1.16 mml, 4.0 eq.) in DMF (2 mL) was added 1-[bis(4-chlorophenyl)methyl]piperazine (93 mg, 0.289 mmol, 1.0 eq.), followed by T3P (50% in DMF, 0.2 mL, 0.347 mmol, 1.2 eq.). The mixture was stirred overnight at rt and then concentrated. The residue was diluted with water and extracted three times with EtOAc and the combined organic phases were dried over sodium

sulfate, filtered, and concentrated. The crude residue was purified by reverse-phase prep HPLC (65-100% acetonitrile/water with 0.1% formic acid) to obtain the title compound (**10**, 25 mg, 19% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.48 – 7.42 (m, 4H), 7.39 (d, J = 8.4 Hz, 4H), 4.50 (s, 1H), 3.68 (p, J = 2.8 Hz, 4H), 2.39 (t, J = 5.0 Hz, 2H), 2.33 (dd, J = 5.8, 4.0 Hz, 2H), 2.13 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 156.6, 151.7, 139.9, 133.4, 129.2, 129.1, 113.1, 77.4, 77.2, 76.9, 74.5, 52.2, 51.4, 47.3, 42.9, 8.7. ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{21}H_{20}Cl_2N_4O_3$: 447.10; found 447.10.

Compound 11



Scheme 11. Synthesis of **11**.

Conditions: *i*. $SOCl_2$, EtOH, rt (62%); *ii*. Me_4NNO_3, Tf_2O, DCM, rt to 60 °C (88%); *iii*. NaOH, H_2O, THF, rt (91%); *iv*. oxalyl chloride, DMF, DCM, 0 °C to rt; *v*. 1-(bis(4-chlorophenyl)methyl)piperazine, DIPEA, DCM, 0 °C to rt (67% over two steps).

ethyl 3-methylisoxazole-5-carboxylate (**76**): (*i*) Thionyl chloride (0.21 mL, 2.95 mmol, 1.5 eq.) was added to a solution of 3-methylisoxazole-5-carboxylic acid (0.25 g, 1.97 mmol, 1 eq.) in ethanol (5 mL). The mixture was stirred at ambient temperature for 48 h. The solvent was removed under vacuum and the crude residue purified by flash column chromatography to afford the title compound (**76**, 0.19 g, 62% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.78 (s, 1H), 4.42 (q, J = 7.2 Hz, 2H), 2.37 (s, 3H), 1.41 (t, J = 7.1 Hz, 3H). ESI LRMS (m/z): $[M+H]^+$ calculated for $C_7H_9NO_3$ 156.07; found 156.15. These data are in good agreement with literature values (ref. [4]).

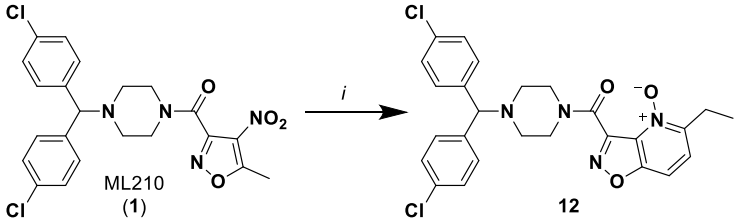
ethyl 3-methyl-4-nitroisoxazole-5-carboxylate (**77**): (*ii*) Using a protocol adapted from Shackelford et al. (ref. [5]), tetramethylammonium nitrate (0.25 g, 1.84 mmol, 1.5 eq.) was suspended in dry DCM (0.3 mL) in a microwave vessel. Triflic anhydride (0.55 g, 0.33 mL, 1.96 mmol, 1.6 eq.) was added followed by DCM (0.2 mL). The suspension was stirred at rt for 1.5 h. Solid ethyl 3-methylisoxazole-5-carboxylate (**76**, 0.19 g, 1.22 mmol, 1 eq.) was added followed by DCM (0.5 mL) and mixed by gentle shaking. The reaction was heated in a microwave reactor at 60 °C for 2 h. After cooling to rt, the contents were poured into saturated aqueous sodium bicarbonate solution (10 mL) and extracted with DCM (3 × 10 mL). The combined organic layers were washed with water (3 × 10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by flash column chromatography (10-15% EtOAc/hexanes) afforded the title compound (**77**, 0.21 g, 88% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.53 (q, J = 7.2 Hz, 2H), 2.58 (s, 3H), 1.14 (t, J = 7.1 Hz, 3H). LRMS (m/z): $[M+H]^+$ calculated for $C_7H_8N_2O_5$ 201.05; found 201.15.

3-methyl-4-nitroisoxazole-5-carboxylic acid (**78**): (*iii*) A solution of ethyl 3-methyl-4-nitroisoxazole-5-carboxylate (**77**, 55 mg, 0.27 mmol, 1 eq.) in THF (0.5 mL) was cooled in an ice bath. A solution of 1 N aqueous NaOH (0.55 mL, 0.55 mmol, 2 eq.) was added and the mixture was stirred for 5 minutes. The reaction was quenched by addition of 4 N HCl (1 mL). After extraction with EtOAc (3 × 2 mL), the combined organic layers were dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The title compound (**78**, 43 mg, 91% yield) was obtained as a white solid without additional purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.62 (s, 3H).

(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)(3-methyl-4-nitroisoxazol-5-yl)methanone (**11**): (*iv*) Oxalyl chloride (40 µL, 0.5 mmol, 2 eq.) was added to a solution of **78** (43 mg, 0.25 mmol, 1 eq.) in dry DCM (3

mL) followed by 1 drop of DMF. The mixture was stirred at rt overnight and the solvent was removed under reduced pressure. (*v*) The crude acid chloride intermediate was dissolved in dry DCM (3 mL) and cooled on ice. A solution of 1-(bis(4-chlorophenyl)methyl)piperazine (80 mg, 0.25 mmol, 1 eq.) and DIPEA (60 µL, 0.32 mmol, 1.3 eq.) in DCM (2 mL) was added slowly to the acid chloride solution. The reaction was warmed to rt and stirred for 4 h. The organic layer was diluted with DCM (10 mL) and washed with water (3 × 10 mL). After concentration of the organic fraction, the crude residue was purified by flash column chromatography (25% EtOAc/hexanes) to afford the title compound (**11**, 80 mg, 67% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.23 (m, 8H), 4.27 (s, 1H), 3.87 – 3.79 (m, 2H), 3.33 – 3.25 (m, 2H), 2.58 (s, 3H), 2.53 (t, *J* = 5.2 Hz, 2H), 2.42 – 2.37 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.59, 154.91, 139.77, 133.38, 130.12, 129.10, 128.94, 74.27, 51.53, 50.87, 46.68, 42.48, 11.05. IR (ATR) vmax (cm-1): 2819, 1664, 1612, 1526, 1488, 1445, 1412, 1379, 1361, 1332, 1291, 1224, 1158, 1143, 1111, 1089, 1055, 1013, 994, 909, 866, 824, 813, 802, 771, 733, 665, 648, 540, 504. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{22}H_{20}Cl_2N_4O_4$ 475.0940; found 475.0941.

Compound 12

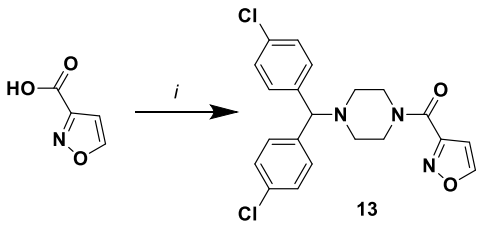


Scheme 12. Synthesis of **12**.

Conditions: *i.* butyraldehyde, piperidine, EtOH, reflux (31%).

3-(4-(bis(4-chlorophenyl)methyl)piperazine-1-carbonyl)-5-ethylisoxazolo[4,5-*b*]pyridine 4-oxide (**12**): ML210 (**1**, 100 mg, 1.06 mmol, 1 eq.) was dissolved in absolute EtOH (1 mL) with butyraldehyde (18 mg, 0.25 mmol, 1.2 eq.) and piperidine (0.1 mL). The reaction was heated to reflux overnight, cooled, and partitioned between EtOAc and water. The organic layer was collected, dried over $MgSO_4$, and concentrated. Purification by flash column chromatography (0-100% EtOAc/hexanes) afforded the title compound (**12**, 34 mg, 31% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.41 (m, 2H), 7.35 – 7.29 (m, 5H), 7.24 (s, 3H), 4.28 (s, 1H), 3.95 – 3.86 (m, 2H), 3.44 – 3.35 (m, 2H), 3.00 (q, *J* = 7.4 Hz, 2H), 2.59 – 2.51 (m, 2H), 2.45 – 2.39 (m, 2H), 1.33 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$)) δ 158.96, 157.37, 150.79, 149.93, 140.31, 133.30, 130.05, 129.13, 125.99, 107.60, 74.38, 51.47, 51.08, 47.13, 42.56, 22.87, 11.00. IR (ATR) vmax (cm-1): 2929, 2818, 1656, 1509, 1479, 1447, 1411, 1360, 1328, 1291, 1247, 1189, 1143, 1088, 1013, 997, 910, 832, 802, 731, 645, 590, 540, 520, 505. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{26}H_{24}Cl_2N_4O_3$ 511.1304; found 511.1318.

Compound 13

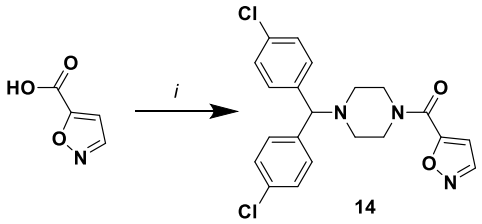


Scheme 13. Synthesis of **13**.

Conditions: *i.* 1-(bis(4-chlorophenyl)methyl)piperazine, TBTU, DIPEA, DCM, rt (73%).

(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)(isoxazol-3-yl)methanone (**13**): A mixture of 1-(bis(4-chlorophenyl)methyl)piperazine (375 mg, 1.17 mmol, 1.1 eq.), isoxazole-5-carboxylic acid (120 mg, 1.06 mmol, 1 eq.), TBTU (511 mg, 1.59 mmol, 1.5 eq.), and DIPEA (0.28 mL, 1.59 mmol, 1.5 eq.) in dry DCM (5 mL) was stirred at rt for 2 h. The reaction was washed with water, concentrated, and purified by flash column chromatography (0-30% EtOAc/hexanes). The title compound (**13**, 323 mg, 73% yield) was obtained as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (d, *J* = 1.7 Hz, 1H), 7.36 – 7.22 (m, 8H), 6.65 (d, *J* = 1.7 Hz, 1H), 4.25 (s, 1H), 3.89 – 3.76 (m, 4H), 2.50 – 2.38 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.12, 158.57, 157.57, 140.00, 133.18, 129.07, 128.98, 105.90, 74.40, 52.14, 51.37, 47.08, 42.70. IR (ATR) vmax (cm-1): 3216, 2914, 2813, 1634, 1550, 1485, 1447, 1396, 1368, 1330, 1290, 1199, 1142, 1110, 1087, 1048, 1031, 1013, 1000, 983, 908, 858, 833, 801, 760, 728, 687, 647, 594, 544, 523, 504. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{21}H_{19}Cl_2N_3O_2$ 416.0932; found 416.0926.

Compound 14



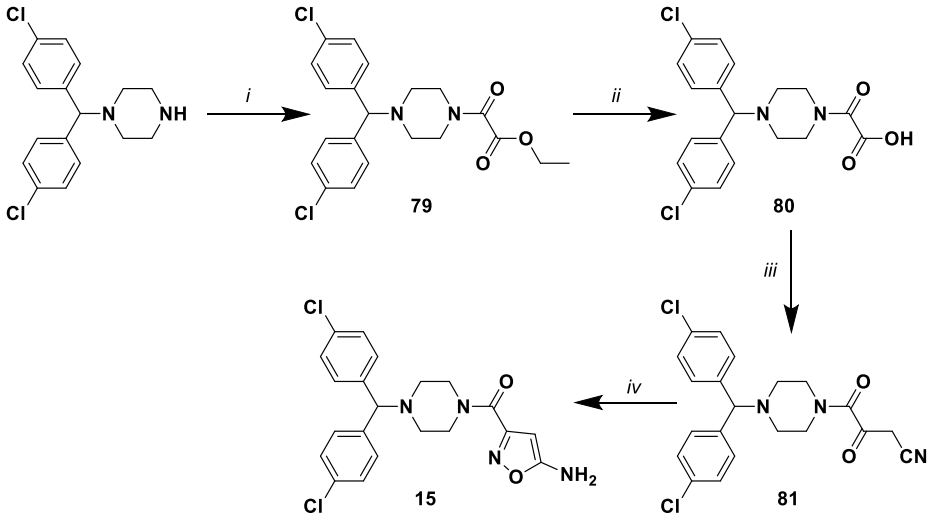
14

Scheme 14. Synthesis of **14**.

Conditions: *i.* 1-(bis(4-chlorophenyl)methyl)piperazine, TBTU, DIPEA, DCM, rt (79%).

(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)(isoxazol-5-yl)methanone (**14**): A mixture of 1-(bis(4-chlorophenyl)methyl)piperazine (500 mg, 1.56 mmol, 1 eq.), isoxazole-5-carboxylic acid (211 mg, 1.87 mmol, 1.2 eq.), TBTU (550 mg, 1.71 mmol, 1.1 eq.), and DIPEA (0.3 mL, 1.71 mmol, 1.1 eq.) in dry DCM (10 mL) was stirred at rt overnight. The reaction was concentrated and purified by flash column chromatography (0-35% EtOAc/hexanes) to afford the title compound (**14**, 512 mg, 79% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, *J* = 1.8 Hz, 1H), 7.35 – 7.21 (m, 8H), 6.73 (d, *J* = 1.8 Hz, 1H), 4.24 (s, 1H), 3.82 – 3.66 (m, 4H), 2.50 – 2.36 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.69, 156.69, 150.10, 139.87, 133.26, 129.04, 129.02, 107.56, 74.36, 52.07, 51.29, 46.66, 42.93. IR (ATR) vmax (cm-1): 2917, 2815, 1641, 1578, 1486, 1426, 1370, 1327, 1290, 1261, 1249, 1202, 1163, 1143, 1108, 1088, 1036, 1013, 1000, 982, 916, 869, 832, 812, 802, 750, 732, 686, 647, 623, 543, 522, 505. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{21}H_{19}Cl_2N_3O_2$ 416.0933; found 416.0926.

Compound 15



Scheme 15. Synthesis of **15**.

Conditions: *i*. ethyl chlorooxoacetate, Et_3N, DCM, 0 °C to rt (92%); *ii*. NaOH, THF (82%); *iii*. *n*-BuLi, MeCN, THF, -78 °C (30%); *iv*. hydroxylamine hydrochloride, DIPEA, EtOH, 70 °C (10 %).

ethyl 2-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-2-oxoacetate (**79**): A solution of 1-(bis(4-chlorophenyl)methyl)piperazine (1.0 g, 3.11 mmol, 1 eq.) and triethylamine (0.51 mL, 3.74 mmol, 1.2 eq.) in dry DCM (20 mL) was cooled in an ice bath. Ethyl chlorooxoacetate (0.38 mL, 3.42 mmol, 1.1 eq.) was added dropwise. The reaction was allowed to warm to rt while stirring overnight. The reaction was directly purified by flash column chromatography (dry-load, 0-30% EtOAc/hexanes) to afford the title compound (**79**, 1.2 g, 92% yield) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.25 (m, 8H), 4.31 (q, *J* = 7.1 Hz, 2H), 4.26 (s, 1H), 3.64 (t, *J* = 5.5 Hz, 2H), 3.45 (t, *J* = 5.0 Hz, 2H), 2.41 (t, *J* = 5.0 Hz, 4H), 1.34 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.66, 160.06, 139.93, 133.21, 129.02, 129.01, 74.29, 62.11, 51.63, 50.94, 46.13, 41.38, 14.00. ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{21}H_{22}Cl_2N_2O_3$ 421.11; found 421.24.

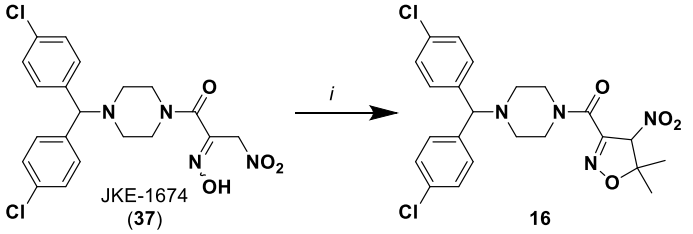
2-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-2-oxoacetic acid (**80**): A 1 N aqueous NaOH solution (2.7 mL, 2.68 mmol, 2 eq.) was added to a mixture of **79** (0.564 g, 1.34 mmol, 1 eq.) in THF (2.7 mL). The mixture was stirred at rt overnight and then partitioned between 1 N aqueous HCl and EtOAc. The organic layer was collected, dried over anhydrous N_2SO_4, filtered, and concentrated to afford the title compound as a white solid (**80**, 430 mg, 82% yield). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.07 – 8.00 (m, 4H), 7.51 – 7.44 (m, 4H), 5.46 (s, 1H), 4.13 – 4.05 (m, 4H), 3.25 – 3.17 (m, 4H). ^{13}C NMR (101 MHz, acetone-d_6) δ 162.81, 160.36, 135.35, 134.36, 130.54, 129.38, 74.48, 51.64, 51.09, 42.88, 38.16. IR (ATR) vmax (cm-1): 2924, 2588, 1731, 1699, 1663, 1595, 1494, 1434, 1282, 1197, 1127, 1093, 1041, 1015, 926, 856, 818, 799, 736, 689, 665, 607, 537, 511, 478. ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{19}H_{18}Cl_2N_2O_3$ 393.08; found 393.24.

4-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-3,4-dioxobutanenitrile (**81**): A solution of *n*-BuLi (2.5 M in hexanes, 0.351 mL, 0.88 mmol, 1.1 eq.) was added to a flask of dry THF (4 mL) at -78 °C under a nitrogen atmosphere. Dry acetonitrile (46 µL, 0.88 mmol, 1.1 eq.) was added dropwise. The mixture was stirred at -78 °C for 1 h. A solution of **80** (336 mg, 0.80 mmol, 1 eq.) in dry THF (1 mL) was added dropwise. The reaction was stirred at -78 °C for an additional 2 h and then quenched with 1 N aqueous HCl and extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by flash column chromatography (0-5% MeOH/DCM) afforded the title compound (**81**, 101 mg, 30% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 – 7.25 (m, 8H), 4.25 (s, 1H), 3.98 (s, 2H), 3.70 – 3.59 (m, 4H), 2.49 – 2.35 (m, 4H). ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{21}H_{19}Cl_2N_3O_2$ 416.09; found 416.18.

(5-aminoisoxazol-3-yl)(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)methanone (**15**): A solution of **81** (75 mg, 0.18 mmol, 1 eq.), DIPEA (50 µL, 0.27 mmol, 1.5 eq.), and hydroxylamine hydrochloride (16 mg, 0.23 mmol, 1.3 eq.) in absolute ethanol (1.5 mL) was heated to 70 °C for 2 h. The reaction was partitioned

between EtOAc and water, and the organic fraction was collected and concentrated. Purification by flash column chromatography (0-5% MeOH/DCM) afforded the title compound (**15**, 8 mg, 10% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 – 7.23 (m, 8H), 5.35 (s, 1H), 4.60 (s, 2H), 4.23 (s, 1H), 3.89 – 3.67 (m, 4H), 2.47 – 2.34 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.76, 160.08, 160.00, 140.18, 133.30, 129.20, 129.09, 81.15, 74.55, 52.33, 51.51, 47.13, 42.61. IR (ATR) vmax (cm-1): 3319, 2921, 2817, 1635, 1488, 1471, 1448, 1410, 1358, 1332, 1290, 1242, 1143, 1089, 1036, 1013, 999, 989, 908, 861, 835, 802, 734, 674, 544, 525, 505. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{21}H_{20}Cl_2N_4O_2$ 431.1036; found 431.1018.

Compound 16

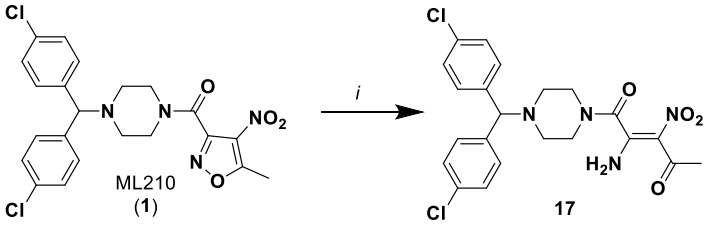


Scheme 16. Synthesis of **16**.

Conditions: *i*. acetone, piperidine, EtOH, 60 °C (31%).

(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)(5,5-dimethyl-4-nitro-4,5-dihydroisoxazol-3-yl)methanone (**16**): JKE-1674 (**37**, 30 mg, 0.07 mmol, 1 eq.) was dissolved in ethanol (0.5 mL) with piperidine (50 µL) and acetone (19 mg, 24 µL, 0.13 mmol, 2 eq.). The reaction was stirred overnight at 60 °C, cooled, and partitioned between EtOAc and saturated aqueous ammonium chloride. The organic layer was collected, dried over $MgSO_4$, and concentrated. Purification by flash column chromatography (0-15% MeOH/DCM) afforded the title compound (**16**, 10 mg, 31% yield) as an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 – 7.25 (m, 8H), 5.78 (s, 1H), 4.23 (s, 1H), 4.12 – 3.93 (m, 2H), 3.87 – 3.76 (m, 1H), 3.65 – 3.55 (m, 1H), 2.58 – 2.30 (m, 4H), 1.53 (s, 3H), 1.48 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.68, 147.70, 140.18, 140.15, 133.37, 129.17, 129.15, 96.78, 88.80, 74.56, 52.29, 51.49, 47.11, 43.04, 26.98, 20.61. IR (ATR) vmax (cm-1): 2923, 2814, 1631, 1559, 1488, 1447, 1410, 1391, 1372, 1331, 1290, 1260, 1236, 1201, 1143, 1105, 1089, 1013, 1000, 982, 951, 910, 865, 813, 802, 778, 732, 688, 647, 631, 619, 576, 536, 505. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{23}H_{24}Cl_2N_4O_4$ 491.1253; found 491.1257.

Compound 17



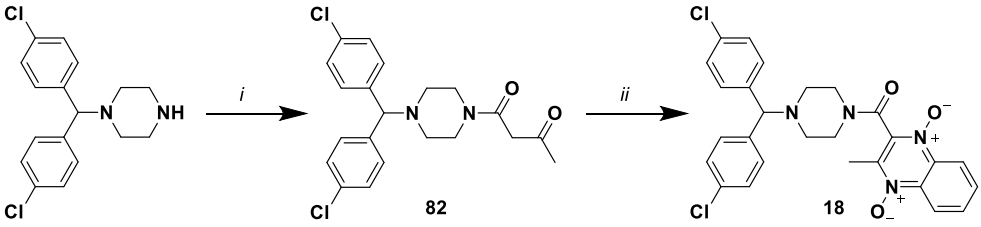
Scheme 17. Synthesis of **17**.

Conditions: *i*. $Mo(CO)_6$, MeCN, H_2O (18%).

(*E*)-2-amino-1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-3-nitropent-2-ene-1,4-dione (**17**): This protocol was adapted from a method described in the literature[6]. A suspension of molybdenum hexacarbonyl (221 mg, 840 µmol) in MeCN (2.8 mL) was heated to 80 °C and stirred for 4 h. The resulting dark brown solution was cooled to rt and to this, was added water (8 µL, 420 µmol, 1.0 eq.), followed by a

solution of (4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)(5-methyl-4-nitroisoxazol-3-yl)methanone (200 mg, 420 µmol) in MeCN (1.3 mL). The reaction was stirred at rt for 3 days and then concentrated. The crude residue was purified by flash column chromatography on silica gel (0-100% EtOAc/hexanes) to obtain the title compound (**17**, 36.9 mg, 18% yield) as a 1:4 mixture of isomers. ^{1}H NMR (400 MHz, acetone-d_6) δ 10.60 (s, 4H), 9.65 (s, 1H), 8.91 (s, 1H), 7.51 (dt, *J* = 8.5, 1.8 Hz, 22H), 7.42 – 7.25 (m, 21H), 4.48 (d, *J* = 3.8 Hz, 5H), 3.73 – 3.28 (m, 14H), 2.62 – 2.31 (m, 36H). ^{13}C NMR (101 MHz, acetone-d_6) δ 195.2, 192.1, 162.9, 162.7, 142.1, 133.5, 130.6, 129.7, 74.7, 51.7, 51.4, 47.2, 42.3. IR (ATR) vmax (cm^{-1}): 2929, 1797, 1755, 1662, 1592, 1491, 1446, 1401, 1359, 1331, 1283, 1157, 1125, 1093, 1014, 927, 861, 799, 758, 678, 625, 527, 504. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{22}H_{22}Cl_2N_4O_4$ 477.1096; found 477.1092.

Compound 18



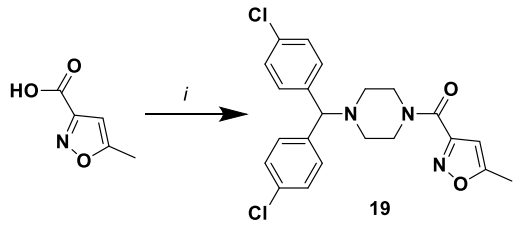
Scheme 18. Synthesis of **18**.

Conditions: *i*. trimethyl-1,3-dioxin-4-one, PhMe, reflux (90%); *ii*. DBU, benzofuroxan, PhMe, rt (67%).

1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)butane-1,3-dione (**82**): 1-(Bis(4-chlorophenyl)methyl)piperazine (400 mg, 1.25 mmol, 1 eq.) was dissolved in toluene (10 mL) with 2,2,6-trimethyl-1,3-dioxin-4-one (177 mg, 1.25 mmol, 1 eq.). The mixture was refluxed for 2 h. After cooling to room temperature, the reaction was concentrated and purified by flash column chromatography (0-100% EtOAc/hexanes) to afford the title compound (**82**, 452 mg, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (q, *J* = 8.2 Hz, 8H), 4.23 (s, 1H), 3.65 (t, *J* = 5.0 Hz, 2H), 3.54 (s, 2H), 3.43 (t, *J* = 4.8 Hz, 2H), 2.38 (t, *J* = 5.0 Hz, 4H), 2.27 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 202.30, 164.89, 140.22, 133.30, 129.17, 129.14, 129.11, 74.53, 51.89, 51.46, 50.17, 46.63, 42.03, 30.36. ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{21}H_{22}Cl_2N_2O_2$ 405.11; found 405.13.

2-(4-(bis(4-chlorophenyl)methyl)piperazine-1-carbonyl)-3-methylquinoxaline 1,4-dioxide (**18**): DBU (169 mg, 166 µL, 1.11 mmol, 1 eq.) was added to a solution of **82** (450 mg, 1.11 mmol, 1 eq.) and benzofuroxan (151 mg, 1.11 mmol, 1 eq.) in toluene (5 mL). The reaction was stirred at room temperature for 24 h and then partitioned between EtOAc and water. The organic layer was separated, concentrated, and purified by flash column chromatography (0-100% EtOAc/hexanes) to afford the title compound (**18**, 387 mg, 67% yield) as an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 – 8.40 (m, 2H), 7.93 – 7.72 (m, 2H), 7.45 – 7.14 (m, 8H), 4.27 (s, 1H), 4.01 – 3.87 (m, 1H), 3.87 – 3.73 (m, 1H), 3.45 – 3.31 (m, 1H), 3.30 – 3.17 (m, 1H), 2.68 – 2.38 (m, 6H), 2.38 – 2.25 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.59, 140.09, 140.07, 139.64, 137.79, 137.14, 136.82, 133.37, 132.45, 131.53, 129.15, 129.08, 129.05, 120.35, 120.24, 74.41, 52.14, 51.25, 46.32, 42.17, 14.66. IR (ATR) vmax (cm-1): 2917, 2814, 2245, 1649, 1518, 1471, 1438, 1410, 1368, 1328, 1278, 1241, 1190, 1168, 1142, 1088, 1070, 1013, 994, 906, 866, 838, 805, 770, 724, 697, 638, 616, 567, 557, 536, 504. ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{27}H_{24}Cl_2N_4O_3$ 523.13; found 523.45.

Compound 19

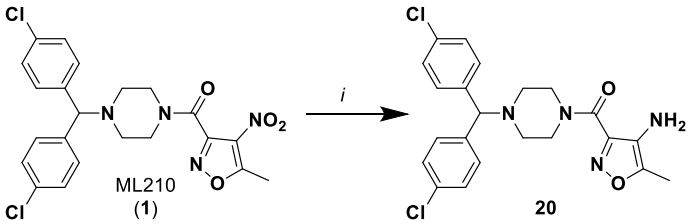


Scheme 19. Synthesis of **19**.

Conditions: *i.* 1-(bis(4-chlorophenyl)methyl)piperazine, TBTU, DIPEA, DCM, rt (74%).

(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)(5-methylisoxazol-3-yl)methanone (**19**): TBTU (165 mg, 0.51 mmol, 1.1 eq.) and DIPEA (98 µL, 0.56 mmol, 1.2 eq.) were added to a solution of 5-methylisoxazole-3-carboxylic acid (150 mg, 0.47 mmol, 1 eq.) in dry DCM (5 mL). Next, 1-(bis(4-chlorophenyl)methyl)piperazine (150 mg, 0.47 mmol, 1 eq.) was added to the mixture in one portion. The reaction was stirred overnight at rt and concentrated. The residue was purified by flash column chromatography (0-30% EtOAc/hexanes) to afford the title compound (**19**, 149 mg, 74% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.22 (m, 8H), 6.25 (d, *J* = 1.0 Hz, 1H), 4.24 (s, 1H), 3.88 – 3.80 (m, 2H), 3.81 – 3.73 (m, 2H), 2.48 – 2.36 (m, 7H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.88, 159.63, 158.61, 140.03, 133.16, 129.07, 128.96, 102.82, 74.41, 52.16, 51.38, 47.02, 42.56, 12.12. IR (ATR) vmax (cm-1): 2915, 2813, 2360, 2341, 1635, 1599, 1486, 1448, 1393, 1364, 1330, 1289, 1262, 1219, 1143, 1105, 1087, 1037, 1013, 1001, 989, 907, 880, 861, 834, 801, 763, 728, 693, 668, 647, 545, 526, 505. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{22}H_{21}Cl_2N_3O_2$ 430.1089; found 430.1102.

Compound 20

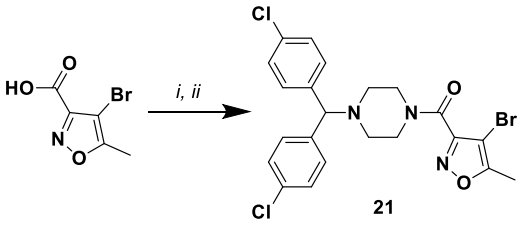


Scheme 20. Synthesis of **20**.

Conditions: *i.* sodium dithionite, THF, H_2O, rt (45%).

(4-amino-5-methylisoxazol-3-yl)(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)methanone (**20**): ML210 (**1**, 50 mg, 0.11 mmol, 1 eq.) and sodium dithionite (55 mg, 0.32 mmol, 3 eq.) were dissolved in 1:1 THF/H_2O (2 mL) and heated to 60 °C for 1 h. The reaction was cooled and partitioned between EtOAc and water. The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (0-5% MeOH/DCM) afforded the title compound (**20**, 21 mg, 45% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.22 (m, 8H), 4.24 (s, 1H), 4.04 (t, *J* = 5.0 Hz, 2H), 3.77 (t, *J* = 5.1 Hz, 2H), 3.57 (s, 2H), 2.44 (dt, *J* = 10.8, 5.1 Hz, 4H), 2.32 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.17, 153.33, 149.94, 140.06, 133.16, 129.07, 129.00, 128.96, 124.76, 74.44, 52.24, 51.48, 47.03, 42.41, 10.12. IR (ATR) vmax (cm-1): 3338, 2920, 2812, 1655, 1577, 1487, 1445, 1410, 1369, 1330, 1289, 1238, 1214, 1174, 1143, 1107, 1088, 1033, 1013, 997, 907, 886, 861, 835, 811, 801, 779, 728, 647, 556, 531, 505. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{22}H_{22}Cl_2N_4O_2$ 445.1198; found 445.1197.

Compound 21

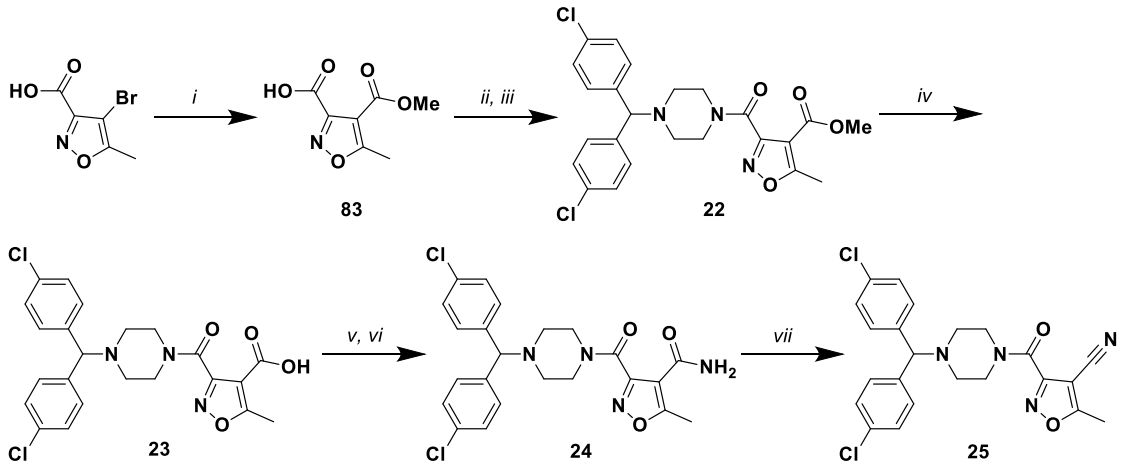


Scheme 21. Synthesis of **21.**

Conditions: *i.* oxalyl chloride, DCM, DMF, rt; *ii*. Et_3N, 1-(bis(4-chlorophenyl)methyl)piperazine, Et_3N, DCM, rt (65% over two steps).

(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)(4-bromo-5-methylisoxazol-3-yl)methanone (**21**): Oxalyl chloride (170 µL, 1.94 mmol, 2.0 eq.) was added to a solution of 4-bromo-5-methylisoxazole-3-carboxylic acid (200 mg, 0.971 mmol) in DCM (4.8 mL), followed by DMF (1 drop). The mixture was stirred at rt overnight and then concentrated to a yellow oil. The crude acid chloride was dissolved in dry DCM (4.8 mL) and to this, was added 1-(bis(4-chlorophenyl)methyl)piperazine (312 mg, 0.971 mmol, 1.0 eq.), followed by triethylamine (1 drop). The yellow mixture was stirred at rt for 2 h, diluted with DCM (10 mL), and washed with saturated aqueous $NaHCO_3$ (10 mL). The aqueous layer was extracted with DCM (3 × 10 mL) and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude oil was purified by flash column chromatography (EtOAc/hexanes) to obtain the title compound (**21**, 324 mg, 65% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.43 (d, J = 8.3 Hz, 4H), 7.37 (d, J = 8.3 Hz, 4H), 4.49 (s, 1H), 3.68 (t, J = 4.8 Hz, 2H), 3.37 (t, J = 4.9 Hz, 2H), 2.44 (s, 3H), 2.37 (t, J = 5.0 Hz, 2H), 2.30 (d, J = 5.0 Hz, 2H).^{13}C NMR (101 MHz, $CDCl_3$) δ 168.2, 158.5, 157.0, 140.1, 133.3, 129.1, 90.5, 77.5, 77.4, 77.2, 76.8, 74.5, 52.2, 51.4, 47.2, 42.3, 11.4. IR (ATR) vmax (cm^{-1}): 2918, 2813, 1648, 1596, 1486, 1442, 1409, 1391, 1362, 1329, 1290, 1261, 1222, 1143, 1087, 1045, 1013, 992, 907, 834, 811, 729, 646, 544, 524, 505. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{22}H_{20}BrCl_2N_3O_2$ 508.0194; found 508.0194.

Compounds 22-25



Scheme 22. Synthesis of **22-25**.

Conditions: *i. n*-BuLi, methyl chloroformate, THF, -78 °C (91 %); *ii.* Oxalyl chloride, DMF, DCM, 0 °C to rt; *iii.* 1-[bis(4-chlorophenyl)methyl]piperazine, DIPEA, DCM, 0 °C to rt (36%, 2 steps); *iv.* NaOH, THF, 50 °C (57%); *v.* oxalyl chloride, DCM, DMF, 0 °C to rt; *vi.* NH_4OH, THF 0 °C to rt (54%, 2 steps); *vii*. TFAA, Et_3N, DCM, rt (86%).

4-(methoxycarbonyl)-5-methyl-1,2-oxazole-3-carboxylic acid (**83**): To a solution of 4-bromo-5-methyl-1,2-oxazole-3-carboxylic acid (1.0 g, 4.85 mmol, 1 eq.) in dry THF (50 mL) at -78 °C was added *n*-BuLi (2.5

M in hexanes, 3.88 mL, 9.70 mmol, 2 eq.) under a stream of nitrogen. The mixture was stirred for 40 min before adding methyl chloroformate (1.37 g, 14.5 mmol, 3 eq.) and the resulting solution was stirred at -78 °C for 3 h. The cooled mixture was quenched with 1 N aqueous HCl then diluted with EtOAc. The aqueous layer was extracted three times with EtOAc and the combined organic layers were washed with water, dried over sodium sulfate, filtered, and concentrated. The crude residue was purified by flash chromatography on silica gel (0-80% EtOAc/hexanes) to obtain the title compound (**83**, 819 mg, 91% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.07 (s, 3H), 2.80 (s, 3H).

methyl 3-{4-[bis(4-chlorophenyl)methyl]piperazine-1-carbonyl}-5-methyl-1,2-oxazole-4-carboxylate (**22**): To a suspension of 4-(methoxycarbonyl)-5-methyl-1,2-oxazole-3-carboxylic acid (**83**, 784 mg, 4.24 mmol, 1.05 eq.) in dry DCM (30 mL) under a stream of nitrogen at 0 °C was added oxalyl chloride (519 µL, 6.06 mmol, 1.5 eq.), followed by DMF (1 drop). The resulting bubbling mixture was stirred at rt for 2 h then concentrated to obtain a yellow solid. The crude acid chloride was dissolved in dry DCM (17 mL) and to this, was added a cold solution of 1-[bis(4-chlorophenyl)methyl]piperazine (1.3 g, 4.04 mmol, 1 eq.) and DIPEA (842 µL, 4.84 mmol, 1.2 eq.) in dry DCM (10 mL) at 0 °C. The reaction was stirred at rt for 1.5 h then concentrated. The crude residue was purified by flash column chromatography on silica gel (30-50% EtOAc/hexanes) to obtain the title compound (**22**, 696 mg, 36% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 – 7.27 (m, 7H), 7.26 – 7.24 (m, 1H), 4.26 (s, 1H), 3.82 (s, 4H), 3.37 – 3.26 (m, 2H), 2.69 (s, 3H), 2.53 – 2.40 (m, 3H), 2.38 – 2.27 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.3, 161.1, 159.2, 157.5, 139.9, 133.24, 129.0, 128.9, 108.0, 102.8, 74.3, 52.1, 51.5, 51.05, 46.9, 42.0, 12.9. IR (ATR) vmax (cm-1): 2819, 2358, 2165, 1727, 1659, 1604, 1488, 1445, 1411, 1308, 1290, 1228, 1117, 1089, 1012, 997, 913, 802, 743. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{24}H_{23}Cl_2N_3O_4$ 488.1144; found: 488.1147

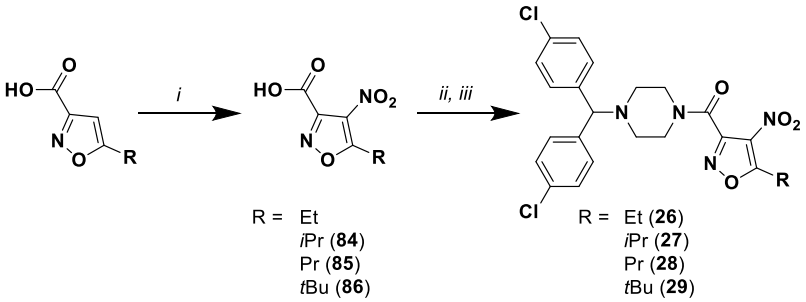
3-{4-[bis(4-chlorophenyl)methyl]piperazine-1-carbonyl}-5-methyl-1,2-oxazole-4-carboxylic acid (**23**): To a solution of **22** (690 mg, 1.41 mmol, 1 eq.) in dry tetrahydrofuran (5 mL) was added 1 N aqueous sodium hydroxide (2.11 mL, 2.11 mmol, 1.5 eq.) dropwise. The mixture was stirred for 1 h at rt and then for 48 h at 50 °C. Then, the mixture was quenched with 1 N aqueous HCl and concentrated. The crude residue was purified by reverse phase flash column chromatography on C18-silica gel (0-100% acetonitrile/water with 0.1% formic acid) to afford the title compound (**23**, 384 mg, 57% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.21 (m, 10H), 4.27 (s, 1H), 4.03 (t, *J* = 4.9 Hz, 2H), 3.87 (t, *J* = 5.0 Hz, 2H), 2.77 (s, 3H), 2.50 (dt, *J* = 13.5, 4.9 Hz, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.4, 161.2, 160.5, 153.8, 139.9, 133.5, 129.2, 129.1, 110.7, 77.4, 77.2, 74.4, 52.1, 51.4, 48.3, 43.9, 13.5. IR (ATR) vmax (cm-1): 2817, 2358, 2343, 1720, 1656, 1596, 1489, 1445, 1339, 1289, 1229, 1136, 1111, 1092, 1013, 997, 973, 911, 858, 837, 802, 769, 734. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{23}H_{21}Cl_2N_3O_4$ 474.0987; found 474.0986.

3-{4-[bis(4-chlorophenyl)methyl]piperazine-1-carbonyl}-5-methyl-1,2-oxazole-4-carboxamide (**24**): To a solution of **23** (350 mg, 737 µmol, 1 eq.) in dry DCM (5 mL) was added at 0 °C oxalyl chloride (96 µL, 1.10 mmol, 1.5 eq.) under a stream of nitrogen, followed by DMF (1 drop). The mixture was stirred overnight at rt then concentrated to obtain a yellow solid. The crude acid chloride was dissolved in dry THF (4 mL) and ammonium hydroxide (28-33% solution, 243 µL, 1.84 mmol, 2.5 eq.) was added dropwise at 0 °C. The resulting mixture was stirred for 2 h at rt then quenched with 1 N aqueous HCl. The aqueous layer was extracted three times with DCM and the combined organic layers were washed with water, dried over sodium sulfate, filtered, and concentrated. The crude residue was purified by flash column chromatography on silica gel (0-80 % EtOAc/hexanes) to obtain the title compound (**24**, 189 mg, 54% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.29 (q, *J* = 8.1 Hz, 9H), 5.58 (s, 1H), 4.25 (s, 1H), 3.82 (t, *J* = 4.9 Hz, 2H), 3.68 (t, *J* = 4.9 Hz, 2H), 2.73 (s, 3H), 2.48 (t, *J* = 5.0 Hz, 2H), 2.42 (t, *J* = 4.9 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.3, 162.6, 160.8, 153.5, 140.0, 133.4, 129.2, 129.1, 111.2, 77.4, 74.5, 52.2, 51.5, 48.1, 43.0, 22.8, 13.2. IR (ATR) vmax (cm^{-1}): 3338, 3183, 2965, 2923, 2807, 2759, 1677, 1637, 1593, 1487, 1446, 1411, 1289, 1270, 1224, 1142, 1108, 1088, 1013, 996, 908, 861, 835, 812, 802, 730. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{23}H_{22}Cl_2N_4O_3$ 473.1147; found 473.1145.

3-{4-[bis(4-chlorophenyl)methyl]piperazine-1-carbonyl}-5-methyl-1,2-oxazole-4-carbonitrile (**25**): To a solution of **24** (138 mg, 291 µmol, 1 eq.) and triethylamine (81 µL, 582 µmol, 2 eq.) in dry DCM (3 mL) was added trifluoroacetic anhydride (76 µL, 582 µmol, 2 eq.). The mixture was stirred at rt for 1 h and concentrated. The resulting oil was suspended in dry DCM (3 mL) and potassium carbonate was added in excess. The mixture was stirred for 1.5 h, then filtered and concentrated. The crude residue was purified by flash column chromatography on silica gel (0-10% methanol/DCM) to obtain the title compound (**25**, 114.7 mg, 86% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.27 (m, 7H), 4.25 (s, 1H), 3.79 (dt, *J* = 10.5, 4.6 Hz, 4H), 2.65 (s, 3H), 2.49 (d, *J* = 5.1 Hz, 2H), 2.43 (t, *J* = 4.9 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.7, 157.4, 156.4, 140.1, 133.4, 129.2, 129.2, 110.0, 77.4, 77.2, 74.5, 52.2, 51.4, 47.1,

43.0, 12.4. IR (ATR) vmax (cm^{-1}): 2359, 2342, 1653, 1489, 997, 913, 743, 682, 669. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{23}H_{20}Cl_2N_4O_2$ 455.1041; found 455.1034.

Compounds 26-29



Scheme 23. Synthesis of **26-29**.

Conditions: *i.* KNO_3, H_2SO_4, 50 C; *ii.* oxalyl chloride, DCM, DMF, 0 °C to rt; *iii.* 1-(bis(4-chlorophenyl)methyl)piperazine, DIPEA or Et_3N, DCM, 0 °C to rt.

(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)(5-ethyl-4-nitroisoxazol-3-yl)methanone (**26**): To a solution of 5-ethyl-4-nitroisoxazole-3-carboxylic acid (Ark Pharm Inc., 86.7 mg, 0.27 mmol) in DCM (1.34 mL) was added oxalyl chloride (46 µL, 0.54 mmol, 2 eq.), followed by DMF (1 drop). The mixture was stirred at rt overnight and concentrated to a yellow semi-solid oil. Separately, a solution of 1-(bis(4-chlorophenyl)methyl)piperazine (86.7 mg, 0.27 mmol, 1 eq.) and triethylamine (75 µL, 0.54 mmol, 2 eq.) in DCM (1.3 mL) was prepared and cooled to 0 °C. The acid chloride was dissolved in DCM (1 mL) and added to the cold mixture containing the piperazine under a stream of nitrogen. The mixture was then warmed to rt and stirred for 2 h before concentrating. Purified by flash column chromatography on silica gel (0-50% EtOAc/hexanes), followed by precipitation from EtOAc/hexanes, to obtain the title compound (**26**, 77 mg, 58% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (d, *J* = 8.3 Hz, 4H), 7.25 (d, *J* = 8.4 Hz, 4H), 4.24 (s, 1H), 3.82 (t, *J* = 5.1 Hz, 2H), 3.35 (t, *J* = 5.0 Hz, 2H), 3.24 (q, *J* = 7.6 Hz, 2H), 2.51 (t, *J* = 5.1 Hz, 2H), 2.36 (t, *J* = 5.0 Hz, 2H), 1.39 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.0, 156.8, 153.3, 140.1, 133.4, 129.2, 129.1, 77.5, 77.4, 77.2, 76.8, 74.5, 51.7, 51.1, 47.1, 42.4, 21.4, 10.6. IR (ATR) vmax (cm^{-1}): 2923, 2818, 1660, 1593, 1523, 1491, 1447, 1417, 1359, 1290, 1146, 1090, 1041, 1013, 997, 957, 909, 825, 813, 802, 755, 736, 687, 666, 541, 520, 505. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{23}H_{22}Cl_2N_4O_4$ 489.1096; found 489.1104.

5-isopropyl-4-nitroisoxazole-3-carboxylic acid (**84**): 5-Isopropylisothiazole-3-carboxylic acid (0.374 mg, 2.41 mmol, 1 eq.) was dissolved in concentrated H_2SO_4 (5 mL) with KNO_3 (487 mg, 4.82 mmol, 2 eq.) at rt. The reaction was heated to 50 °C for 24 h and subsequently cooled to rt. The reaction was diluted with water and carefully extracted with EtOAc. The combined organic layers were dried over $MgSO_4$ and evaporated to afford the title compound (**84**, 362 mg, 87% yield) as a white solid. The material was carried onto the next step directly without additional purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.44 (hept, *J* = 6.8 Hz, 1H), 0.94 (d, *J* = 6.8 Hz, 6H).

(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)(5-isopropyl-4-nitroisoxazol-3-yl)methanone (**27**): A suspension of **84** (100 mg, 0.58 mmol, 1 eq.) in dry DCM (2 mL) was cooled on an ice bath. Oxalyl chloride (0.1 mL, 1.16 mmol, 2 eq.) was added slowly followed by 1 drop of DMF. The reaction was allowed to warm to rt overnight while stirring. The solvent was then evaporated under reduced pressure. The residue was dissolved in dry DCM (1 mL) and slowly added to an ice-cold solution of 1-(bis(4-chlorophenyl)methyl)piperazine (140 mg, 0.44 mmol, 0.75 eq.) and DIPEA (0.1 mL, 0.57 mmol, 1.3 eq.) in DCM (2 mL). The mixture was allowed to warm to rt overnight. The reaction was concentrated and purified by flash column chromatography (0-30% EtOAc/hexanes) to afford the title compound (**27**, 150 mg, 68% yield over two steps) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.26 (m, 8H), 4.28 (s, 1H), 3.95 – 3.82 (m, 3H), 3.43 – 3.34 (m, 2H), 2.60 – 2.50 (m, 2H), 2.45 – 2.36 (m, 2H), 1.44 (s, 3H), 1.42 (s,

3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.86, 156.72, 153.20, 140.00, 133.26, 129.04, 128.97, 127.11, 74.35, 51.54, 50.99, 46.96, 42.30, 28.02, 19.51. IR (ATR) vmax (cm-1): 2980, 2816, 1657, 1588, 1522, 1488, 1467, 1446, 1413, 1390, 1358, 1327, 1290, 1234, 1170, 1140, 1108, 1088, 1069, 1042, 1013, 933, 907, 857, 824, 812, 801, 777, 727, 647, 607, 541, 521, 504. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{24}H_{24}Cl_2N_4O_4$ 503.1253; found 503.1256.

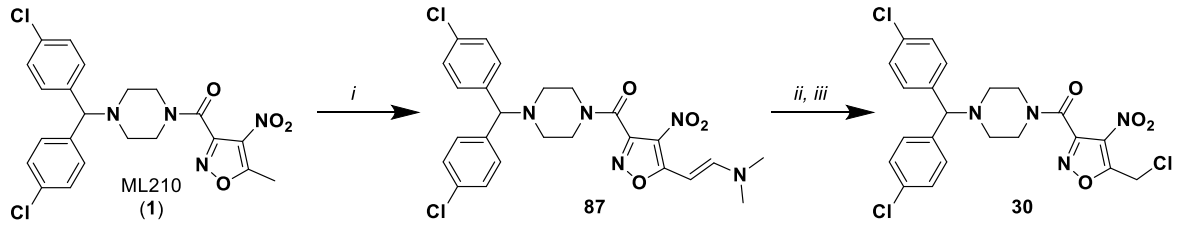
4-nitro-5-propylisoxazole-3-carboxylic acid (**85**): Potassium nitrate (206 mg, 2.04 mmol, 3 eq.) was dissolved in sulfuric acid (1.44 mL). After complete dissolution, a slurry of 5-propylisoxazole-3-carboxylic acid (0.106 g, 683 µmol, 1.0 eq.) in a minimal amount of sulfuric acid was then added and the mixture was slowly heated to 50 °C. The reaction was monitored by ^{1}H NMR in DMSO-d_6. After stirring overnight, the mixture was cooled to rt and quenched with ice. The aqueous layer was extracted three times with EtOAc and the combined organic layers were washed with water, dried over sodium sulfate, filtered, and concentrated to obtain the title compound (**85**, 121 mg, 89% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.65 (t, *J* = 7.3 Hz, 2H), 1.48 (q, *J* = 7.4 Hz, 2H), 0.86 (t, *J* = 7.4 Hz, 3H).

(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)(4-nitro-5-propylisoxazol-3-yl)methanone (**28**): To a solution of 4-nitro-5-propylisoxazole-3-carboxylic acid (**85**, 56.6 mg, 283 µmol) in DCM (1.34 mL) was added oxalyl chloride (75.2 µL, 540 µmol, 2 eq.), followed by DMF (1 drop). The yellow mixture was stirred at rt overnight. After 18 hours, the mixture was concentrated to give the acid chloride as a yellow oil. Separately, a solution of 1-(bis(4-chlorophenyl)methyl)piperazine (86.7 mg, 0.27 mmol, 1 eq.) and triethylamine (75 µL, 540 µmol, 2 eq.) in DCM (1.34 mL) was prepared and cooled to 0 °C. The acid chloride was dissolved in DCM (1 mL) and added to the cold mixture containing the piperazine under a stream of nitrogen. The mixture was then warmed to rt and stirred for 2 h before concentrating. Purified by flash column chromatography on silica gel (0-50% EtOAc/hexanes), followed by precipitation from EtOAc/hexanes, to obtain the title compound (**28**, 53.6 mg, 40% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.22 (m, 8H), 4.25 (s, 1H), 3.87 – 3.80 (m, 2H), 3.39 – 3.32 (m, 2H), 3.23 – 3.14 (m, 2H), 2.52 (t, *J* = 5.1 Hz, 2H), 2.41 – 2.34 (m, 2H), 1.85 (h, *J* = 7.4 Hz, 2H), 1.04 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.2, 156.8, 153.2, 140.1, 133.4, 129.2, 129.1, 128.4, 77.5, 77.4, 77.2, 76.8, 74.5, 51.7, 51.1, 47.1, 42.4, 29.2, 20.2, 13.9. IR (ATR) vmax (cm^{-1}): 2968, 2360, 1665, 1593, 1495, 1447, 1417, 1360, 1287, 1239, 1149, 1091, 1014, 996, 855, 827, 802, 755, 541, 422, 409, 401. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{24}H_{24}Cl_2N_4O_4$ 503.1253; found 503.1256.

5-(tert-butyl)-4-nitroisoxazole-3-carboxylic acid (**86**): Potassium nitrate (189 mg, 1.87 mmol, 3 eq.) was dissolved in sulfuric acid (1.3 mL). After complete dissolution, a slurry of 5-(tert-butyl)isoxazole-3-carboxylic acid (0.106 g, 626 µmol, 1 eq.) in a minimal amount of sulfuric acid was then added and the mixture was slowly heated to 50 °C. The reaction was monitored by ^{1}H NMR in DMSO-d_6. After stirring overnight, the mixture was cooled to rt and quenched with ice. The aqueous layer was extracted three times with EtOAc and the combined organic layers were washed with water, dried over sodium sulfate, filtered, and concentrated to obtain the title compound (**86**, 119 mg, 89% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.16 (s, 9H).

(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)(5-(tert-butyl)-4-nitroisoxazol-3-yl)methanone (**29**): To a solution of 5-(tert-butyl)-4-nitroisoxazole-3-carboxylic acid (**86**, 86.7 mg, 0.27 mmol) in DCM (1.3 mL) was added oxalyl chloride (75 µL, 540 µmol, 2 eq.), followed by DMF (1 drop). The mixture was stirred at rt overnight and concentrated to a yellow semi-solid oil. Separately, a solution of 1-(bis(4-chlorophenyl)methyl)piperazine (86.7 mg, 0.27 mmol, 1 eq.) and triethylamine (75 µL, 540 µmol, 2 eq.) in DCM (1.3 mL) was prepared and cooled to 0 °C. The acid chloride was dissolved in DCM (1 mL) and added to the cold mixture containing the piperazine under a stream of nitrogen. The mixture was then warmed to rt and stirred for 2 h before concentrating. Purified by flash column chromatography on silica gel (0-50% EtOAc/hexanes), followed by precipitation from EtOAc/hexanes, to obtain the title compound (**29**, 77.2 mg, 55% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, *J* = 8.2 Hz, 4H), 7.25 (d, *J* = 8.2 Hz, 4H), 4.24 (s, 1H), 3.80 (s, 2H), 3.39 (t, *J* = 5.0 Hz, 2H), 2.50 (t, *J* = 5.1 Hz, 2H), 2.38 (t, *J* = 5.1 Hz, 2H), 1.49 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.2, 157.0, 154.7, 140.1, 133.4, 129.2, 129.1, 128.6, 77.5, 77.4, 77.2, 76.8, 74.5, 51.7, 51.1, 47.1, 42.4, 35.9, 27.1. IR (ATR) vmax (cm^{-1}): 2976, 2359, 1658, 1568, 1492, 1446, 1411, 1367, 1354, 1289, 1236, 1177, 1142, 1090, 1014, 991, 859, 834, 754, 667, 542, 522, 505. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{22}H_{22}Cl_2N_4O_3$ 461.1147; found: 461.1150.

Compound 30



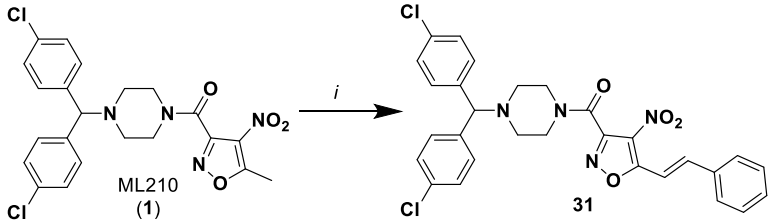
Scheme 24. Synthesis of **30**.

Conditions: *i*. DMF dimethyl acetal, rt (95%); *ii*. NCS, DCM, rt; *iii*. SiO_2, HCl, 45 °C (65%, 2 steps).

(*E*)-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)(5-(2-(dimethylamino)vinyl)-4-nitroisoxazol-3-yl)methanone (**87**): The following protocol was adapted from a reported procedure (ref. [7]). To a solution of ML210 (1 g, 2.1 mmol, 1 eq.) in DMF (2 mL) was added 1,1-dimethoxy-*N*,*N*-dimethylmethanamine (334 µL, 2.52 mmol, 1.2 eq.) at rt. The resulting dark green solution was stirred at rt for 20 min and then concentrated three times from toluene. The crude residue was purified by flash column chromatography on silica gel (0-100% EtOAc/hexanes) to obtain the title compound (**87**, 1.12 g, 95% yield) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* = 13.0 Hz, 1H), 7.31 (d, *J* = 8.6 Hz, 4H), 7.25 (d, *J* = 8.7 Hz, 5H), 5.84 (d, *J* = 13.1 Hz, 1H), 4.23 (s, 1H), 3.81 (t, *J* = 5.1 Hz, 2H), 3.36 (t, *J* = 5.0 Hz, 2H), 3.23 (s, 3H), 3.01 (s, 3H), 2.49 (t, *J* = 5.1 Hz, 2H), 2.35 (t, *J* = 5.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.4, 158.1, 153.5, 152.7, 140.3, 133.3, 129.1, 120.1, 81.4, 77.5, 77.4, 77.2, 76.8, 74.5, 51.6, 51.1, 46.9, 45.9, 42.2, 37.5. IR (ATR) vmax (cm^{-1}): 2926, 2362, 2179, 2156, 2011, 2019, 1979, 1668, 1623, 1578, 1484, 1436, 1396, 1375, 1295, 1238, 1125, 1093, 1014, 821, 751, 541. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{25}H_{25}Cl_2N_5O_4$ 530.1362; found 530.1365.

4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)(5-(chloromethyl)-4-nitroisoxazol-3-yl)methanone (**30**): A mixture of **87** (0.4 g, 754 µmol, 1 eq.) and *N*-chlorosuccinimide (105 mg, 791 µmol, 1.05 eq.) in DCM (3.01 mL) was stirred at rt for 2 h. Then, silica gel (1.83 g, 30.5 mmol, 40.5 eq.) and 1 N aqueous HCl (188 µL, 188 µmol, 0.25 eq.) were consecutively added and the solvent was evaporated under reduced pressure. The mixture was kept at 45 °C for 2 h in a rotary evaporator and subsequently purified by flash column chromatography (0-50% EtOAc/hexanes) to obtain the title compound (**30**, 0.251 g, 65% yield) as a white solid. The compound degrades after prolonged storage at ambient conditions and must be used immediately or stored long term at -20 °C. ^{1}H NMR (400 MHz, MeOD-d_4) δ 7.73 (d, *J* = 8.0 Hz, 4H), 7.47 (d, *J* = 8.0 Hz, 4H), 5.38 (s, 1H), 5.16 (s, 2H), 3.81 (s, 2H), 3.26 – 3.18 (m, 4H), 3.09 (s, 2H). ^{13}C NMR (101 MHz, MeOD-d_4) δ 170.5, 157.9, 153.9, 136.6, 131.2, 130.9, 129.8, 75.6, 53.0, 52.5, 49.5, 49.3, 49.1, 34.1, 30.6. IR (ATR) vmax (cm^{-1}): 2943, 2388, 1795, 1669, 1593, 1533, 1492, 1446, 1417, 1358, 1282, 1246, 1177, 1118, 1094, 1014, 926, 856, 828, 798, 781, 741, 677, 525, 506. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{22}H_{19}Cl_3N_4O_4$ 509.0550; found 509.0517.

Compound 31

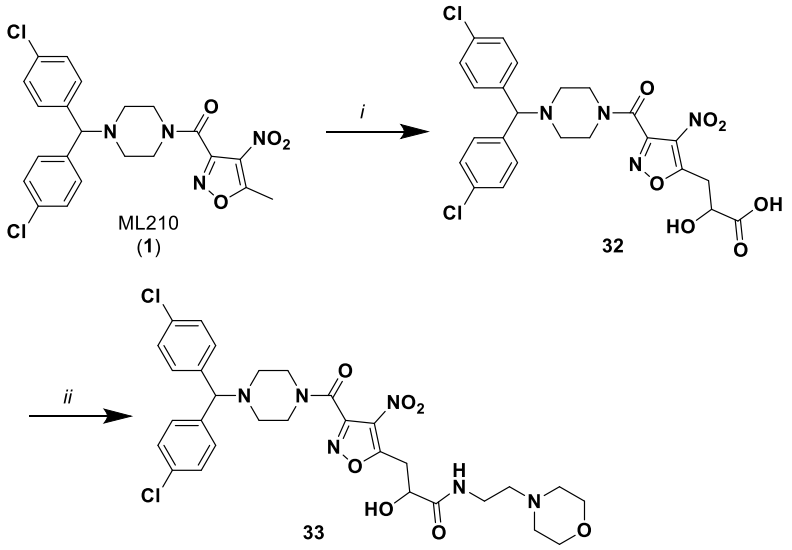


Scheme 25. Synthesis of **31**.

Conditions: *i*. benzaldehyde, EtOH, 60 °C (40%).

(*E*)-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)(4-nitro-5-styrylisoxazol-3-yl)methanone (**31**): The following protocol was adapted from a literature procedure (ref. [8]). To a solution of ML210 (**1**, 0.295 g, 620 µmol, 1 eq.) in ethanol (3 mL) was added benzaldehyde (100 µL, 744 µmol, 1.2 eq.) and the mixture was heated to 60 °C and stirred overnight. The mixture was cooled to rt and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (EtOAc/hexanes) to obtain the title compound (**31**, 141 mg, 40% yield) as an orange solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.01 (d, J = 16.6 Hz, 1H), 7.90 – 7.80 (m, 2H), 7.62 (d, J = 16.6 Hz, 1H), 7.51 (p, J = 3.6 Hz, 3H), 7.47 – 7.41 (m, 4H), 7.40 – 7.34 (m, 4H), 4.50 (s, 1H), 3.71 (s, 2H), 3.41 (s, 2H), 2.41 (s, 2H), 2.23 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.0, 156.7, 153.7, 144.8, 140.1, 134.1, 133.4, 131.8, 131.4, 130.2, 129.4, 129.3, 129.2, 129.1, 128.9, 128.8, 128.6, 126.3, 109.9, 77.5, 77.4, 77.2, 76.8, 74.5, 51.7, 51.1, 47.1, 42.5. IR (ATR) $_{vmax}$ (cm^{-1}): 2928, 2818, 1665, 1626, 1580, 1567, 1498, 1448, 1416, 1356, 1331, 1290, 1238, 1134, 1090, 1013, 997, 973, 859, 826, 802, 752, 692, 542, 520. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{29}H_{24}Cl_2N_4O_4$ 563.1253; found 563.1248.

Compounds 32 and 33



Scheme 26. Synthesis of **32** and **33**.

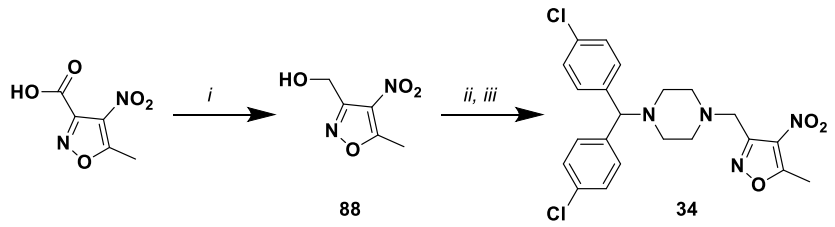
Conditions: *i.* glyoxylic acid monohydrate, DBU, EtOH, rt (67%); *ii.* TBTU, DIPEA, 4-(2-aminoethyl)propanamide, DCM rt (69%).

3-(3-(4-(bis(4-chlorophenyl)methyl)piperazine-1-carbonyl)-4-nitroisoxazol-5-yl)-2-hydroxypropanoic acid (**32**): ML210 (**1**, 250 mg, 0.53 mmol, 1 eq.) was dissolved in absolute ethanol (10 mL) with glyoxylic acid monohydrate (53 mg, 0.58 mmol, 1.1 eq.). DBU (94 µL, 0.63 mmol, 1.2 eq.) was added and the mixture was stirred at rt for 1 h. The reaction was partitioned between EtOAc and 1 N aqueous HCl. The organic layer was separated, concentrated, and purified by flash column chromatography (dry-load, 0-10% MeOH/DCM) to yield the title compound (**32**, 194 mg, 67% yield) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.96 (s, 1H), 7.55 – 7.20 (m, 8H), 5.90 (s, 1H), 4.58 – 4.39 (m, 2H), 3.74 – 3.58 (m, 3H), 3.51 – 3.39 (m, 1H), 3.36 – 3.31 (m, 2H), 2.45 – 2.35 (m, 2H), 2.28 – 2.16 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.0, 171.5, 157.4, 152.6, 138.3, 134.0, 130.1, 129.4, 129.3, 77.4, 76.9, 74.7, 68.1, 51.6, 51.1, 46.4, 41.9, 32.1. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{24}H_{22}Cl_2N_4O_7$ 549.0944; found 549.0943.

3-(3-(4-(bis(4-chlorophenyl)methyl)piperazine-1-carbonyl)-4-nitroisoxazol-5-yl)-2-hydroxy-*N*-(2-morpholinoethyl)propanamide (**33**): TBTU (32 mg, 0.1 mmol, 1.1 eq.) was added to a solution of **32** (50 mg, 0.09 mmol, 1 eq.) in dry DCM (1 mL). DIPEA (19 µL, 0.11 mmol, 1.2 eq.) and 4-(2-aminoethyl)morpholine (13 µL, 0.1 mmol, 1.1 eq.) were added in succession. The mixture was stirred at rt overnight. The reaction was then diluted with DCM, washed with water, dried over anhydrous Na_2SO_4, and concentrated. Flash column chromatography (0-10% MeOH/DCM) afforded the title compound (**33**, 42 mg, 69% yield) as an

off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 – 7.23 (m, 10H), 4.59 – 4.45 (m, 1H), 4.24 (s, 1H), 3.87 – 3.65 (m, 7H), 3.58 – 3.47 (m, 1H), 3.45 – 3.25 (m, 4H), 2.61 – 2.42 (m, 8H), 2.38 – 2.30 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.87, 171.49, 156.79, 153.09, 140.04, 133.45, 129.64, 129.20, 129.10, 74.44, 69.19, 66.72, 56.96, 53.38, 51.63, 51.07, 47.15, 42.55, 35.50, 33.04. ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{30}H_{34}N_6O_7$ 661.19; found 661.28. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{30}H_{34}N_6O_7$ 661.1944; found 661.1940.

Compound 34



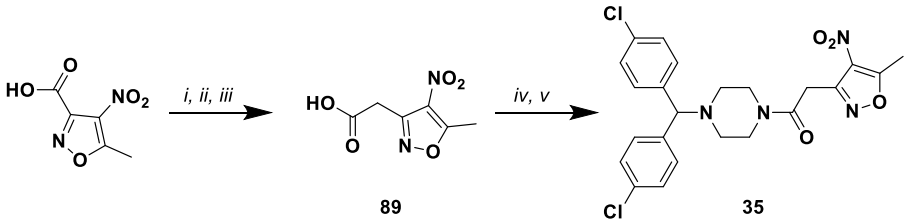
Scheme 27. Synthesis of **34**.

Conditions: *i.* BH3•THF, THF, rt to 40 °C (11%); *ii.* MsCl, DCM, 0 C; *iii.* DIPEA, 1-[bis(4-chlorophenyl)methyl]piperazine, DCM, rt (91%).

(5-methyl-4-nitro-1,2-oxazol-3-yl)methanol (**88**): To a solution of 5-methyl-4-nitro-1,2-oxazole-3-carboxylic acid (172 mg, 1 mmol) in THF (6 mL) was added borane-THF complex (2.50 mL, 2.50 mmol, 1 M) and the reaction progress was monitored by TLC (2% acetic acid/ EtOAc). The solution was stirred at rt for 2 h, then overnight at 40 °C. The mixture was cooled to rt and quenched with methanol. Concentrated three times from methanol and purified by flash chromatography on silica gel (0-50% EtOAc/hexanes) to obtain the title compound (**88**, 18 mg, 11% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.95 (s, 2H), 2.85 (s, 3H).

1-[bis(4-chlorophenyl)methyl]-4-[(5-methyl-4-nitro-1,2-oxazol-3-yl)methyl]piperazine (**34**). To a solution of **88** (20 mg, 126 µmol, 1 eq.) in DCM (1.25 mL) at 0 °C was added DIPEA (54.8 µL, 315 µmol, 2.5 eq.), followed by methanesulfonyl chloride (15 µL, 189 µmol, 1.5 eq.) under a stream of nitrogen. After 90 min, 1-[bis(4-chlorophenyl)methyl]piperazine (61 mg, 189 µmol, 1.5 eq.) was added to the reaction mixture. The mixture was stirred at rt for 24 h then diluted with DCM and washed with saturated aqueous $NaHCO_3$. The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated. The crude residue was purified by flash column chromatography on silica gel (0-100% EtOAc/hexanes) to obtain the title compound (**34**, 53 mg, 91% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 – 7.26 (m, 4H), 7.25 – 7.20 (m, 4H), 4.19 (s, 1H), 3.90 (s, 2H), 2.80 (s, 3H), 2.62 (d, J = 5.1 Hz, 4H), 2.38 (s, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.2, 155.4, 140.7, 133.0, 129.9, 129.2, 129.2, 129.1, 128.9, 77.5, 77.4, 77.2, 76.8, 74.6, 53.3, 51.9, 51.7, 14.0. IR (ATR) vmax (cm^{-1}): 2929, 2352, 1720, 1657, 1594, 1522, 1492, 1415, 1369, 1282, 1215, 1166, 1092, 1014, 927, 906, 853, 831, 800, 755, 690, 666, 537, 510. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{22}H_{22}Cl_2N_4O_3$ 461.1147; found 461.1150.

Compound 35



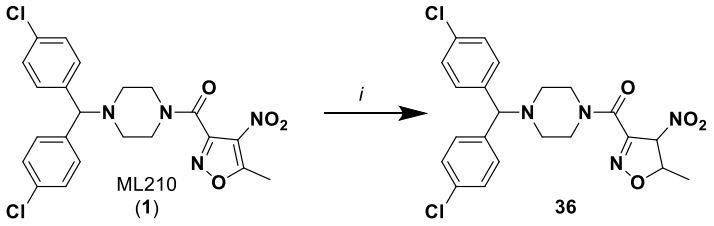
Scheme 28. Synthesis of **35**.

Conditions: *i.* oxalyl chloride, DMF, DCM, 0 °C to rt; *ii.* $TMSCHN_2$, THF, 0 °C to rt; *iii.* AgOAc, Et_3N, THF, 0 °C to rt (22%, 3 steps); *iv.* oxalyl chloride, DMF, DCM, 0 °C to rt; *v.* 1-[bis(4-chlorophenyl)methyl]piperazine**,** DIPEA, DCM, 0 °C to rt (61%, 2 steps).

2-(5-methyl-4-nitro-1,2-oxazol-3-yl)acetic acid (**89**): (*i*) To a solution of 5-methyl-4-nitroisoxazole-3-carboxylic acid (258 mg, 1.49 mmol) in DCM (6 mL) at rt under a stream of nitrogen, was added 1 drop of DMF, followed by oxalyl chloride (152 µL, 1.78 mmol, 1.2 eq.). The mixture was stirred at rt overnight and concentrated to obtain the acid chloride as a yellow semi-solid oil that was used directly. (*ii*) The crude residue was dissolved in THF (7.5 mL), cooled to 0 °C, and treated with a solution of (trimethylsilyl)diazomethane (1.5 mL, 3.0 mmol, 2 M in hexane) under a stream of nitrogen. The reaction was stirred overnight, warming to rt. The reaction was carefully quenched with 10% aqueous citric acid solution and the aqueous phase was extracted three times with ether. The combined organic phases were washed with brine, dried over sodium sulfate, filtered and concentrated. (*iii*) The resulting red oil was dissolved in THF (15 mL) and cooled to 0 °C. A slurry of silver acetate (62 mg, 372 µmol) in triethylamine (5 mL, 35.7 mmol, 24 eq.) was then added and the dark mixture was stirred overnight at rt. The mixture was diluted with 1 N aqueous HCl and extracted three times with EtOAc. The combined organic phases were washed with brine, dried over sodium sulfate, filtered, and concentrated. The crude residue was purified by reverse-phase chromatography on C18-silica gel (5-40% acetonitrile/water with 0.1% formic acid) to obtain the title compound (**89**, 62 mg, 22% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.00 (s, 1H), 3.97 (s, 2H), 2.82 (s, 3H).

1-{4-[bis(4-chlorophenyl)methyl]piperazin-1-yl}-2-(5-methyl-4-nitro-1,2-oxazol-3-yl)ethan-1-one (**35**): (*iv*) To a solution of 2-(5-methyl-4-nitro-1,2-oxazol-3-yl)acetic acid (**89**, 60 mg, 322 µmol) in DCM (1.5 mL) at rt under a stream of nitrogen, was added 1 drop of DMF, followed by oxalyl chloride (33 µL, 386 µmol, 1.2 eq.). The mixture was stirred at rt overnight and concentrated to obtain an oil. (*v*) The crude acid chloride was dissolved in a minimal amount of DCM and added to a cold solution of 1-[bis(4-chlorophenyl)methyl]piperazine (0.1 g, 311 µmol) and DIPEA (65 µL, 373 µmol, 1.2 eq.) in DCM (2 mL) at 0 °C. The reaction was stirred at rt for 2 h and then concentrated under reduced pressure. The crude residue was purified by reverse-phase flash chromatography on C18-silica gel (10-100% acetonitrile/water with 0.1% formic acid) to obtain the title compound (**35**, 95 mg, 61% yield) as a beige solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (d, *J* = 8.3 Hz, 4H), 7.30 – 7.23 (m, 5H), 4.25 (s, 1H), 3.98 (s, 2H), 3.63 (t, *J* = 5.1 Hz, 2H), 3.58 (t, *J* = 4.6 Hz, 2H), 2.81 (s, 3H), 2.47 (t, *J* = 5.1 Hz, 2H), 2.39 (t, *J* = 5.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.0, 165.1, 154.4, 140.3, 133.3, 130.4, 129.1, 77.5, 77.4, 77.2, 76.8, 74.6, 51.9, 51.5, 46.2, 42.3, 30.9, 13.9. IR (ATR) vmax (cm^{-1}): 2924, 1650, 1611, 1515, 1489, 1435, 1370, 1275, 1231, 1167, 1144, 1127, 1110, 1090, 1040, 1014, 1001, 927, 909, 828, 814, 802, 753, 687, 666. ESI HRMS (m/z): $[M+H]^+$ Calculated for $C_{23}H_{22}Cl_2N_4O_4$ 489.1096; found: 489.1103.

Compound 36



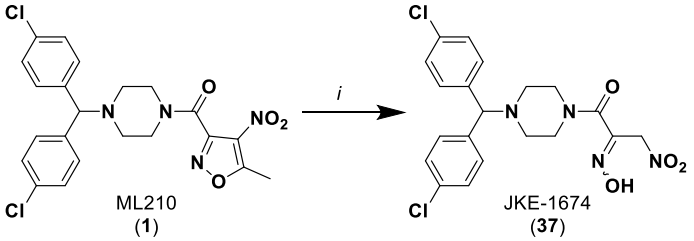
Scheme 29. Synthesis of **36**.

Conditions: *i.* $NaBH_4$, MeOH, THF, 0 °C to rt (81%).

(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)(5-methyl-4-nitro-4,5-dihydroisoxazol-3-yl)methanone (**36**): ML210 (**1**, 99 mg, 0.21 mmol, 1 eq.) was dissolved in 1:1 MeOH/THF (1 mL) and cooled in an ice bath. $NaBH_4$ (8 mg, 0.21 mmol, 1 eq.) was added in one portion and the reaction was stirred for 30 min while warming to rt. The reaction was quenched with saturated aqueous ammonium chloride (2 mL) and

extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, concentrated, and purified by flash column chromatography (EtOAc/hexanes). The title compound (**36**, 80 mg, 81% yield) was obtained as an approximately 2:1 mixture of diastereomers. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 – 7.24 (m, 8H), 6.04 (d, *J* = 9.6 Hz, 1H, minor), 5.85 (d, *J* = 4.2 Hz, 1H, major), 5.22 – 5.06 (m, 1H, major), 5.00 – 4.83 (m, 1H, minor), 4.27 – 4.21 (m, 1H), 4.14 – 3.77 (m, 3H), 3.70 – 3.57 (m, 1H), 2.57 – 2.31 (m, 4H), 1.55 – 1.44 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) Major diastereomer: δ 157.62, 146.84, 140.10, 140.07, 133.34, 129.15, 129.13, 94.89, 82.80, 74.51, 52.21, 51.43, 47.13, 47.04, 43.03, 42.94, 19.35; ^{13}C NMR (101 MHz, $CDCl_3$) Selected minor diastereomer peaks: δ 157.29, 148.72, 92.09, 81.07, 13.13. IR (ATR) vmax (cm-1): 2921, 2815, 1632, 1558, 1488, 1446, 1410, 1371, 1329, 1290, 1235, 1143, 1088, 1013, 999, 946, 906, 865, 826, 812, 801, 782, 726, 648, 618, 569, 534, 504. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{22}H_{22}Cl_2N_4O_4$ 477.1096; found 477.1088.

JKE-1674 (37)

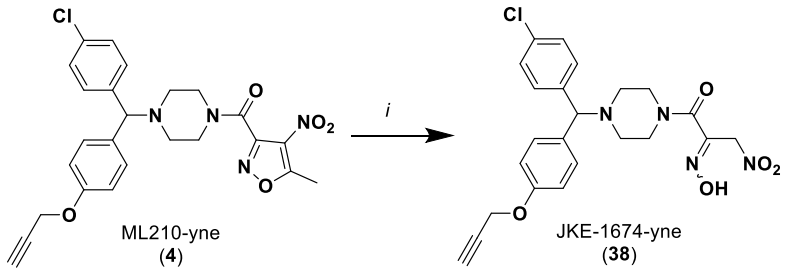


Scheme 30. Synthesis of JKE-1674 (**37**).

Conditions: *i.* NaOH, H_2O, EtOH, 60 °C (91%).

1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-2-(hydroxyimino)-3-nitropropan-1-one (JKE-1674**,** **37**): ML210 (**1**, 2.19 g, 4.61 mmol, 1 eq.) was suspended in a mixture of water (12 mL) and ethanol (17 mL). A 1 N aqueous solution of NaOH was added and the reaction was stirred for 2h at 60 °C. The reaction was cooled to rt and partitioned between EtOAc and 1 N aqueous HCl. The EtOAc layer was collected, washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by flash column chromatography (0-30% EtOAc/hexanes) and the title compound (**37**, 1.90 g, 91% yield) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.55 (s, 3H), 12.29 (s, 1H), 7.48 – 7.41 (m, 17H), 7.41 – 7.34 (m, 17H), 5.55 (s, 9H), 4.47 (d, *J* = 5.2 Hz, 4H), 3.72 (s, 6H), 3.59 (s, 8H), 2.33 (s, 18H). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.35 – 7.30 (m, 4H), 7.29 – 7.24 (m, 6H), 5.52 (s, 1H), 5.32 (s, 1H), 4.23 (s, 1H), 3.84 – 3.76 (m, 1H), 3.76 – 3.63 (m, 2H), 3.57 – 3.44 (m, 1H), 2.50 – 2.37 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.98, 160.48, 143.37, 142.96, 140.93, 140.70, 131.66, 131.63, 129.48, 129.40, 128.66, 128.63, 75.44, 72.47, 68.70, 51.56, 51.17, 50.84, 50.70. IR (ATR) vmax (cm-1): 3023, 1638, 1560, 1490 1434, 1371, 1093, 1014, 801, 752, 535. ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{20}H_{20}Cl_2N_4O_4$ 451.09; found 451.24. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{20}H_{20}Cl_2N_4O_4$ 451.0934; found 451.0933.

Compound 38

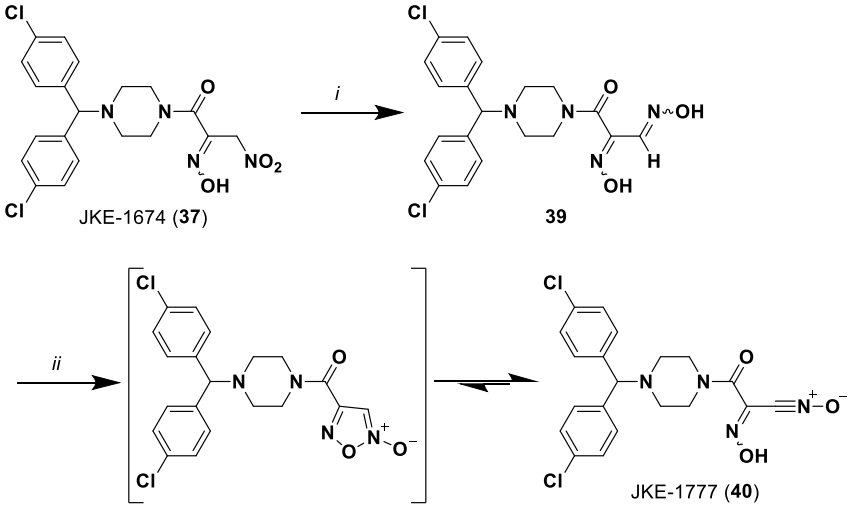


Scheme 31. Synthesis of JKE-1674-yne (**38**).

Conditions: *i.* NaOH, H_2O, THF, 60 °C (29%).

1-(4-((4-chlorophenyl)(4-(prop-2-yn-1-yloxy)phenyl)methyl)piperazin-1-yl)-2-(hydroxyimino)-3-nitropropan-1-one (JKE-1674-yne, **38**): ML210-yne (**4**, 50 mg, 0.1 mmol, 1 eq.) was dissolved in THF (0.25 mL) and a 1 N aqueous solution of NaOH (0.51 mL, 0.51 mmol, 5 eq.) was added. The mixture was heated to 60 °C for 1 h then cooled to rt. The reaction was partitioned between EtOAc and 1 N aqueous HCl. The organic layer was collected, dried over Na_2SO_4, filtered, and concentrated *in* vacuo. Purification by flash column chromatography (30% EtOAc/hexanes) afforded the title compound (**38**, 14 mg, 29% yield) as a white solid as an inseparable mixture of oxime isomers. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.13 – 9.62 (m, 1H), 7.36 – 7.23 (m, 6H), 6.95 – 6.84 (m, 2H), 5.50 (s, 1H), 5.30 (s, 1H), 4.69 – 4.58 (m, 2H), 4.19 (s, 1H), 3.86 – 3.63 (m, 3H), 3.58 – 3.44 (m, 1H), 2.51 (t, *J* = 2.4 Hz, 1H), 2.49 – 2.37 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.41, 160.64, 157.08, 144.46, 143.81, 140.84, 140.65, 134.45, 134.25, 133.09, 129.17, 129.11, 129.05, 128.97, 115.30, 78.61, 75.78, 74.99, 74.86, 74.79, 67.33, 55.97, 52.34, 51.82, 51.36, 47.41, 46.89, 42.74, 41.93. IR (ATR) vmax (cm-1): 3294, 2045, 2921, 2820, 2359, 1616, 1560, 1508, 1488, 144, 1411, 1371, 1290, 1219, 1178, 1144, 1112, 1090, 1027, 1014, 997, 909, 859, 810, 734, 669, 565, 541. ESI LRMS (m/z): [M+H]+ calculated for $C_{23}H_{23}ClN_4O_5$ 471.14; found 471.31. ESI HRMS (m/z): [M+H]+ calculated for $C_{23}H_{23}ClN_4O_5$ 471.1430; found 471.1425.

Compounds 39 and JKE-1777 (40)



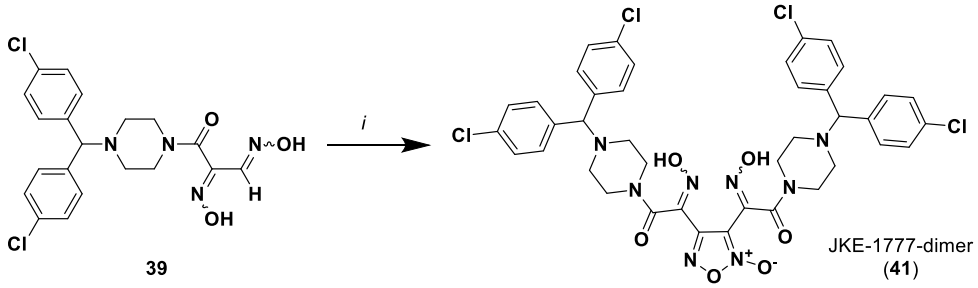
Scheme 32. Synthesis of **39** and JKE-1777 (**40**).

Conditions: *i.* tin(II) chloride dihydrate, PhSH, Et_3N, EtOH (49%); *ii.* NCS, HCl, DMF, rt to 40 °C (45%).

3-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-2-(hydroxyimino)-3-oxopropanal oxime (**39**): JKE-1674 (**37**, 250 mg, 0.55 mmol, 1 eq.) was suspended in absolute ethanol (2 mL) at rt. Tin(II) chloride dihydrate (250 mg, 1.11 mmol, 2 eq.), thiophenol (0.34 mL, 3.32 mmol, 6 eq.), and triethylamine (0.46 mL, 3.32 mmol, 6 eq.) were added in succession. The reaction was stirred at rt for 25 minutes and concentrated. Purification of the residue by flash column chromatography (0-15% MeOH/DCM) afforded the title compound (**39**, 117 mg, 49% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) Major diastereomer: δ 12.16 (s, 1H), 11.91 (s, 1H), 8.19 (s, 1H), 7.45 – 7.33 (m, 8H), 4.42 (s, 1H), 3.64 – 3.47 (m, 2H), 3.36 – 3.16 (m, 2H), 2.34 – 2.19 (m, 4H). Selected signals of minor diastereomer: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.96 (s, 1H), 11.83 (s, 1H), 7.71 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) Major diastereomer: δ 161.68, 148.19, 140.70, 138.05, 131.69, 131.66, 129.43, 128.64, 72.58, 51.35, 50.72, 46.12, 41.01. IR (ATR) vmax (cm-1): 3244, 2829, 1624, 1556, 1489, 1445, 1413, 1368, 1279, 1265, 1215, 1131, 1111, 1091, 1038, 989, 960, 916, 856, 838, 814, 800, 785, 754, 738, 715, 689, 640, 617, 565, 538, 505. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{20}H_{20}Cl_2N_4O_3$ 435.0991; found 435.1002.

2-(hydroxyimino)-3-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-3-oxopropanenitrile oxide (JKE-1777, **40**): NCS (39 mg, 0.3 mmol, 1.1 eq.) was added to a solution of **39** (117 mg, 0.27 mmol, 1 eq.) in dry DMF (1 mL). One drop of concentrated HCl was added and the mixture was stirred at 40 °C for 1 h. The reaction was partitioned between ice-cold 1 N aqueous HCl and EtOAc. The organic layer was separated, washed with ice-cold 1 N aqueous HCl, and dried over anhydrous Na_2SO_4. Purification by flash column chromatography (0-100% EtOAc/hexanes) afforded the title compound (**40**, 52 mg, 45% yield). ^{1}H NMR (300 MHz, methylene chloride-d_2) δ 7.42 – 7.16 (m, 9H), 4.26 (s, 1H), 3.77 – 3.34 (m, 4H), 2.55 – 2.26 (m, 4H). IR (ATR) vmax (cm-1): 2922, 2817, 2282, 1634, 1488, 1445, 1410, 1368, 1290, 1261, 1133, 1090, 1013, 996, 953, 857, 831, 801, 737, 711, 537, 504. ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{20}H_{18}Cl_2N_4O_3$ 433.08; found 433.17. Note: the title compound is unstable when stored as a solid at rt and rapidly degrades in aqueous or DMSO solutions. Storage for several weeks as a solid at -20 °C results in degradation.

Compound 41

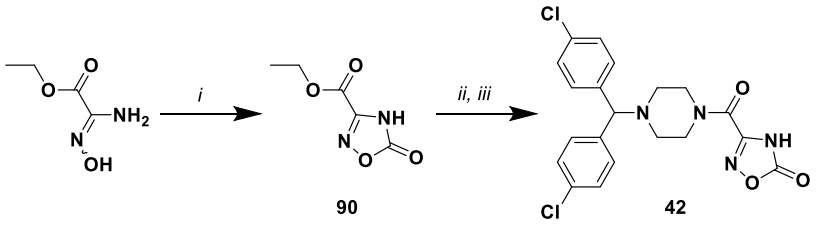


Scheme 33. Synthesis of **41**.

Conditions: *i.* NCS, DMF, rt to 40 °C (18%).

3,4-bis(2-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-1-(hydroxyimino)-2-oxoethyl)-1,2,5-oxadiazole 2-oxide (JKE-1777-dimer, **41**): A mixture of **39** (61 mg, 0.14 mmol, 1 eq.) and NCS (22 mg, 0.17 mmol, 1.2 eq.) was stirred in DMF at 40 °C overnight. The reaction was partitioned between EtOAc/water and the organic layer was separated. After washing the organic layer several times with water, the mixture was concentrated and purified by flash column chromatography (0-100% EtOAc/hexanes) to afford the title compound (**41**, 11 mg, 18% yield) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.05 (s, 0.65H), 12.94 (s, 0.18H), 12.84 (s, 0.65H), 12.44 (s, 0.18H), 7.48 – 7.31 (m, 16H), 4.46 (m, 2H), 3.54 (m, 4H), 3.21 (m, 1H), 2.30 (m, 8H). ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{40}H_{36}Cl_4N_8O_6$ 865.1590; found 865.1581.

Compound 42



Scheme 34. Synthesis of **42**.

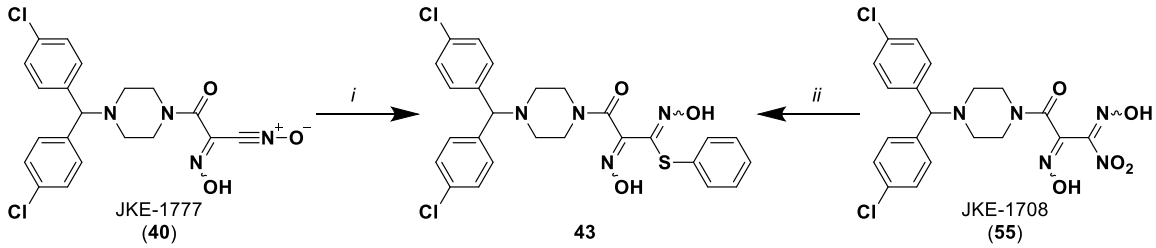
Conditions: *i.* CDI, DBU, 1,4-dioxane, 80 °C (96%); *ii*. NaOH, H_2O, MeOH, rt; *iii*. 1-(bis(4-chlorophenyl)methyl)piperazine, TBTU, DIPEA, DMF, rt (10% yield over 2 steps).

ethyl 5-oxo-4,5-dihydro-1,2,4-oxadiazole-3-carboxylate (**90**): (*i*) Ethyl 2-oximinooxamate (1.00 g, 7.57 mmol, 1 eq.) was dissolved in 1,4-dioxane (7 mL) and CDI (1.35 g, 8.33 mmol, 1.1 eq.) and DBU (0.65 mL,

8.33 mmol, 1.1 eq.) were added in succession. The reaction was heated to 80 °C for 1.5 h, then cooled and quenched with 1 N aqueous HCl. After extraction with EtOAc, the combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo* to afford a yellow oil. The title compound (**90**, 1.15 g, 96% yield) was used directly without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.14 (s, 1H), 4.50 (q, *J* = 7.2 Hz, 2H), 1.44 (t, *J* = 7.1 Hz, 3H). ESI LRMS (m/z): $[M+H]^+$ calculated for $C_5H_6N_2O_4$ 159.04; found 159.16.

3-(4-(bis(4-chlorophenyl)methyl)piperazine-1-carbonyl)-1,2,4-oxadiazol-5(4*H*)-one (**42**): (*ii*) Hydrolysis of **90** (570 mg, 3.61 mmol, 1 eq.) was accomplished stirring with 1 N aqueous NaOH (3.61 mL, 1 eq.) in MeOH overnight at rt. The mixture was then acidified by 1 N aqueous HCl and extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to obtain a crude residue (~400 mg) that was used directly. (*iii*) The residue was dissolved in dry DMF (1 mL) and to this was added 1-(bis(4-chlorophenyl)methyl)piperazine (491 mg, 1.53 mmol, 0.5 eq.), TBTU (540 mg, 1.68 mmol, 1.1 eq.), and DIPEA (0.32 mL, 1.84 mmol, 1.2 eq.). The reaction was stirred at rt for 2 h and partitioned between EtOAc and water. The organic layer was concentrated and the crude residue was purified by flash column chromatography (0-50% EtOAc/hexanes), affording the title compound (**42**, 149 mg, 10% yield) as a solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.95 (s, 1H), 7.34 – 7.26 (m, 7H), 4.26 (s, 1H), 4.16 – 4.09 (m, 2H), 3.81 – 3.73 (m, 2H), 2.51 – 2.43 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.42, 153.04, 152.72, 139.56, 133.40, 129.07, 129.00, 74.20, 51.81, 51.14, 46.55, 43.81. ESI HRMS (m/z): $[M–H]^–$ calculated for $C_{20}H_{18}Cl_2N_4O_3$ 431.0678; found 431.0665.

Compound 43



Scheme 35. Synthesis of **43**.

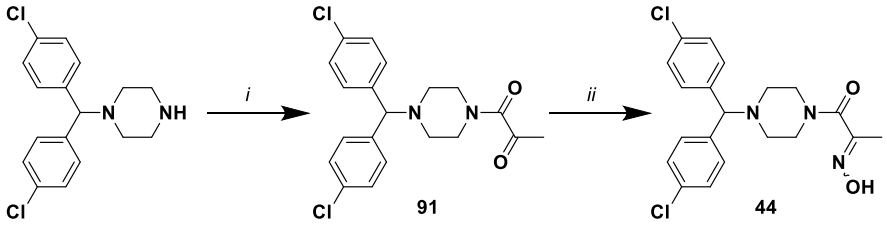
Conditions: *i.* PhSH, Et_3N, DCM, 0 °C (39%); *ii.* PhSH, Et_3N, DCM, rt (41%).

phenyl 3-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-*N*-hydroxy-2-(hydroxyimino)-3-oxopropanimidothioate (**43a**): A suspension of JKE-1777 (**40**, 50 mg, 0.12 mmol, 1 eq.) in dry DCM (2 mL) was cooled in an ice bath. Thiophenol (12 µL, 0.12 mmol, 1 eq.) was added followed by triethylamine (18 µL, 0.13 mmol, 1.1 eq.) and the reaction became homogenous. The reaction was stirred for 15 minutes at 0 °C and subsequently concentrated *in vacuo.* Purification by flash column chromatography (0-50% EtOAc/hexanes) afforded the title compound (**43a**, 44 mg, 70% yield) as a mixture of two oxime isomers ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.60 – 9.72 (m, 2H), 7.60 – 7.51 (m, 2H), 7.29 – 7.19 (m, 11H), 4.10 (s, 1H), 3.49 (s, 2H), 3.15 (s, 2H), 2.27 (s, 2H), 2.11 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.72, 146.55, 144.15, 139.68, 135.12, 133.37, 129.65, 129.26, 129.18, 129.15, 128.42, 75.05, 52.15, 51.58, 46.53, 42.40. IR (ATR) vmax (cm-1): 3210, 2820, 1626, 1488, 1441, 1411, 1371, 1282, 1203, 1126, 1110, 1091, 1056, 1013, 989, 965, 908, 852, 829, 812, 801, 748, 733, 690, 540. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{26}H_{24}Cl_2N_4O_3S$ 543.1024; found 543.1024.

phenyl 3-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-*N*-hydroxy-2-(hydroxyimino)-3-oxopropanimidothioate (**43b**): Thiophenol (8 µL, 0.08 mmol, 1.1 eq.) was added to a solution of JKE-1708 (**55**, 33 mg, 0.07 mmol, 1 eq.) in dry DCM (1 mL) at rt. Triethylamine (13 µL, 0.09 mmol, 1.2 eq.) was added and the mixture was stirred at rt for 6 h. The reaction was concentrated and directly purified by flash column chromatography (0-100% EtOAc/hexanes) to afford the title compound (**43b**, 15 mg, 41% yield) as a white solid as a mixture of four oxime isomers. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.69 (s, 1H), 10.08 (s, 1H), 9.57 (d, *J* = 31.9 Hz, 1H), 9.09 (s, 1H), 7.66 – 7.59 (m, 2H), 7.47 – 7.40 (m, 2H), 7.28 (d, *J* = 12.4 Hz, 24H), 4.17 (d, *J* = 6.4 Hz, 2H), 3.71 (s, 2H), 3.55 (s, 2H), 3.37 – 3.14 (m, 4H), 2.42 (dt, *J* = 10.6, 5.0 Hz, 3H), 2.32 (s,

2H), 2.17 (s, 2H). IR (ATR) vmax (cm-1): 3215, 2822, 1630, 1489, 1441, 1411, 1371, 1290, 1233, 1127, 1091, 1013, 991, 909, 856, 831, 812, 801, 734, 690, 537. ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{26}H_{24}Cl_2N_4O_3S$ 543.10; found 543.19.

Compound 44



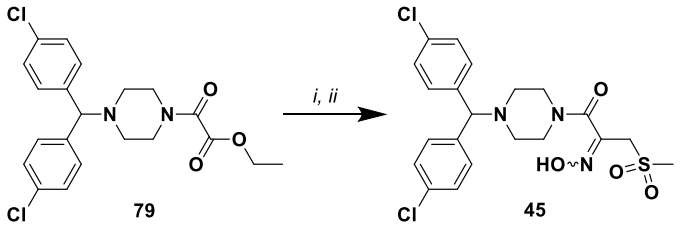
Scheme 36. Synthesis of **44**.

Conditions: *i.* sodium pyruvate, oxalyl chloride, DMF, DCM, rt overnight, then 1-(bis(4-chlorophenyl)methyl)piperazine, DIPEA, DCM, rt (33%); *ii*. hydroxylamine hydrochloride, DIPEA, EtOH, rt (64%).

1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)propane-1,2-dione (**91**): Sodium pyruvate (514 mg, 4.67 mmol, 3 eq.) was suspended in dry DCM (5 mL) and oxalyl chloride (0.53 mL, 6.23 mmol, 4 eq.) was added followed by 1 drop of DMF. The reaction was stirred overnight at rt, filtered, and concentrated *in vacuo*. The residue was suspended in dry DCM (4 mL) and cooled in an ice bath. A solution of 1-(bis(4-chlorophenyl)methyl)piperazine (500 mg, 1.56 mmol, 1 eq.) and DIPEA (0.41 mL, 2.33 mmol, 1.5 eq.) in DCM (1 mL) was added slowly. The reaction was warmed to rt and stirred overnight. After washing the reaction with water, the organic layer was concentrated and dried over Na_2SO_4. Purification by flash column chromatography (0-25% EtOAc/hexanes) afforded the title compound (**91**, 199 mg, 33% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.25 (m, 8H), 4.26 (s, 1H), 3.69 – 3.59 (m, 2H), 3.55 – 3.47 (m, 2H), 2.48 – 2.37 (m, 7H).

1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-2-(hydroxyimino)propan-1-one (**44**): Hydroxylamine hydrochloride (67 mg, 0.96 mmol, 5 eq.) was added to a solution of **91** (75 mg, 0.19 mmol, 1 eq.) in absolute ethanol (1 mL) with DIPEA (0.17 mL, 0.96 mmol, 5 eq.). The mixture was heated to 70 °C for 2 h. After cooling, the reaction was concentrated under reduced pressure and purified by flash column chromatography (0-50% EtOAc/hexanes) to obtain, in order of elution, major (**44a**, 30 mg, 39% yield) and minor (**44b**, 20 mg, 25% yield) isomers of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) Major diastereomer **44a**: δ 7.33 – 7.26 (m, 8H), 7.16 (s, 1H), 4.22 (s, 1H), 3.77 – 3.54 (m, 4H), 2.50 – 2.27 (m, 4H), 2.05 (s, 3H). Minor diastereomer **44b**: δ 7.33 – 7.26 (m, 8H), 6.88 (s, 1H), 4.23 (s, 1H), 3.67 (t, *J* = 5.2 Hz, 2H), 3.36 (t, *J* = 5.0 Hz, 2H), 2.46 – 2.30 (m, 4H), 2.03 (s, 3H). ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{20}H_{21}Cl_2N_3O_2$ 406.1089; found 406.1104 (major) and 406.1099 (minor).

Compound 45

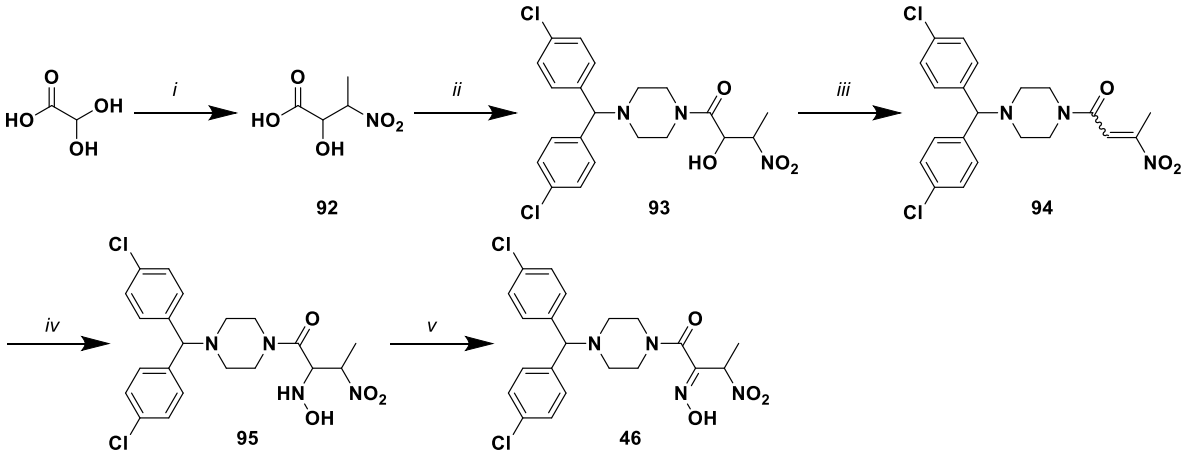


Scheme 37. Synthesis of **45.**

Conditions: *i.* dimethyl sulfone, NaHMDS, THF, -78 °C to 0 °C to rt; *ii*. hydroxylamine hydrochloride, sodium acetate, EtOH (10% over two steps);

1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-2-(hydroxyimino)-3-(methylsulfonyl)propan-1-one (**45**): A solution of methylsulfonylmethane (67 mg, 0.71 mmol, 1.5 eq.) in dry THF (1 mL) was cooled to -78 °C. A solution of NaHDMS (1 M in THF, 0.47 mL, 0.47 mmol, 1 eq.) was added dropwise. The mixture was allowed to warm to 0 °C and stirred for 30 minutes. The deprotonated methylsulfonylmethane solution was added dropwise to a 0 °C solution of **79** (200 mg, 0.47 mmol, 1 eq.) in dry THF (1 mL). The mixture was allowed to warm to ambient temperature while stirring overnight. The reaction was quenched by addition of 1 N aqueous HCl solution and extracted with EtOAc. The organic fraction was dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude residue was passed through a plug of silica and eluted with EtOAc. The filtrate was concentrated to dryness to afford a tan solid (79 mg, 35% crude yield), which was subsequently dissolved in ethanol (0.5 mL). Hydroxylamine hydrochloride (14 mg, 0.20 mmol, 1.2 eq.) was added followed by sodium acetate (17 mg, 0.20 mmol, 1.2 eq.). The reaction was stirred overnight at 50 °C and then cooled to rt. After the reaction was partitioned between EtOAc and water, the organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification of the crude residue by flash column chromatography (0-5% MeOH/DCM) afforded the title compound as a white solid (**45**, 22 mg, 9% yield over two steps). ^{1}H NMR (400 MHz, acetone-d_6) δ 7.54 – 7.47 (m, 5H), 7.36 – 7.31 (m, 4H), 4.50 (s, 2H), 4.41 (s, 1H), 3.75 – 3.64 (m, 4H), 3.04 (s, 3H), 2.40 (q, J = 5.1 Hz, 4H). ^{13}C NMR (101 MHz, acetone-d_6) δ 163.03, 143.57, 141.26, 132.37, 129.48, 128.69, 73.80, 51.84, 51.19, 50.26, 47.03, 42.25, 42.09. IR (ATR) vmax (cm-1): 3188, 3026, 2927, 2819, 1700, 1635, 1487, 1446, 1410, 1370, 1313, 1247, 1197, 1143, 1116, 1089, 1033, 1013, 993, 967, 907, 865, 837, 803, 738, 687, 667, 542, 506. ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{21}H_{23}Cl_2N_3O_4S$ 484.09; found 484.18. ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{21}H_{23}Cl_2N_3O_4S$ 484.0859; found 484.0840.

Compound 46



Scheme 38. Synthesis of **46**.

Conditions: *i.* nitroethane, NaOH, H_2O, rt (55%); *ii.* 1-(bis(4-chlorophenyl)methyl)piperazine, TBTU, DIPEA, DCM, rt (11%, 17-112 and 7%, 17-118); *iii*. MsCl, Et_3N, DCM, -20 °C; *iv*. hydroxylamine hydrochloride, Et_3N, EtOH, rt (85% over two steps); *v*. *p*-benzoquinone, toluene, 50 °C (11%).

2-hydroxy-3-nitrobutanoic acid (**92**): Glyoxylic acid monohydrate (2 g, 21.73 mmol, 1 eq.) was dissolved in water (16 mL). Nitroethane (3.26 g, 3.1 mL, 43.45 mmol, 2 eq.) and solid NaOH (0.91 g, 22.81 mmol, 1.05 eq.) were added and the mixture stirred overnight at rt. The mixture was acidified by addition of 4 N aqueous HCl and extracted with EtOAc several times. The combined organic fractions were dried over anhydrous Na_2SO_4, filtered, and the solvent evaporated. The resulting yellow oil was an approximately equal mixture of diastereomers of the title compound (**92**, 1.78 g, 55% yield). ^{1}H NMR (300 MHz, $CDCl_3$) [Note: two diastereomers are visible) δ 5.09 – 4.87 (m, 3H), 4.44 (d, J = 2.8 Hz, 1H), 3.24 (s, 2H), 1.78 (d, J = 7.0 Hz, 3H), 1.66 (d, J = 6.9 Hz, 3H).

1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-2-hydroxy-3-nitrobutan-1-one (**93**): **92** (1.74 g, 11.64 mmol, 1.1 eq.) was dissolved in dry DCM (100 mL) and TBTU (3.57 g, 11.11 mmol, 1.05 eq.), DIPEA (2.03

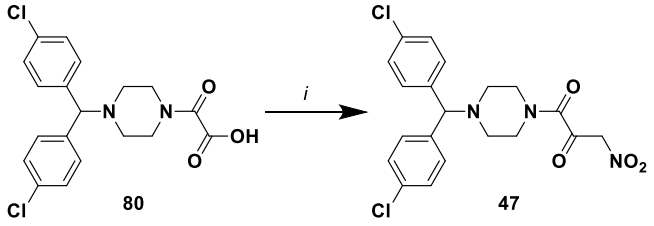
mL, 11.64 mmol, 1.1 eq.), and 1-(bis(4-chlorophenyl)methyl)piperazine (3400 mg, 10.58 mmol, 1 eq.) were added in succession. The reaction was stirred overnight at rt. The reaction was diluted with DCM and washed with water. After concentration under reduced pressure, purification by flash column chromatography (0-25% EtOAc/hexanes) afforded the dehydrated product (**94**, 311 mg, 7% yield) and the title compound (**93**, combined yield 11%, 550 mg) as a mixture of diastereomers. Major diastereomer (354 mg, 7% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 – 7.28 (m, 8H), 5.13 – 4.99 (m, 1H), 4.55 – 4.40 (m, 1H), 4.25 (s, 1H), 3.97 – 3.84 (m, 1H), 3.82 – 3.69 (m, 1H), 3.58 (s, 3H), 2.50 – 2.34 (m, 4H), 1.46 (d, *J* = 6.7 Hz, 3H). Minor diastereomer (196 mg, 4% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (s, 8H), 4.80 – 4.62 (m, 2H), 4.24 (s, 1H), 3.85 – 3.69 (m, 2H), 3.70 – 3.37 (m, 4H), 2.42 (s, 4H), 1.57 (d, *J* = 6.6 Hz, 6H). ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{21}H_{23}Cl_2N_3O_4$ 452.11; found 452.20 (major) and 452.19 (minor).

1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-3-nitrobut-2-en-1-one (**94**): A solution of **93** (550 mg, 1.22 mmol, 1 eq.) in dry DCM (10 mL) was cooled to -20 °C. Triethylamine (0.51 mL, 3.65 mol, 3 eq.) was added followed by the dropwise addition of mesyl chloride (0.28 mL, 3.65 mol, 3 eq.). The reaction was stirred at -20 °C for 1 h. The reaction was then partitioned between water and DCM. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford the title compound (**94**, 530 mg) as a mixture of diastereomers. The crude material was used directly without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 – 7.35 (m, 1H), 7.33 – 7.26 (m, 8H), 4.26 (s, 1H), 3.70 (s, 2H), 3.48 (s, 2H), 2.48 – 2.35 (m, 4H), 2.35 – 2.30 (m, 3H). ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{21}H_{21}Cl_2N_3O_3$ 434.10; found 434.18.

1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-2-(hydroxyamino)-3-nitrobutan-1-one (**95**): Hydroxylamine hydrochloride (170 mg, 2.44 mmol, 2 eq.) was added to a solution of **94** (530 mg, 1.22 mmol, 1 eq.) and triethylamine (0.34 mL, 2.44 mmol, 2 eq.) in 4:1 EtOH/THF (10 mL). The reaction was stirred at rt overnight and partitioned between EtOAc and water. The organic layer was separated and concentrated under reduced pressure. Purification by flash column chromatography (0-100% EtOAc/hexanes) afforded the title compound (**95**, 487 mg, 85% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.19 (m, 8H), 5.87 (s, 1H), 5.66 – 5.06 (m, 1H), 4.79 – 4.57 (m, 1H), 4.55 – 4.41 (m, 1H), 4.25 – 4.13 (m, 2H), 3.88 – 3.48 (m, 4H), 2.51 – 2.28 (m, 4H), 1.62 – 1.45 (m, 3H). ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{21}H_{24}Cl_2N_4O_4$ 467.12; found 467.37.

1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-2-(methoxyimino)-3-nitropropan-1-one (**46**): *p*-Benzoquinone (35 mg, 0.32 mmol, 1 eq.) was added to a solution of **95** (150 mg, 0.32 mmol, 1 eq.) in toluene (1 mL). The mixture was heated to 50 °C for 30 minutes and partitioned between EtOAc and water. The organic layer was concentrated and purified by flash column chromatography (0-100% EtOAc/hexanes) to afford the title compound (**46**, 16 mg, 11% yield) as a mixture of diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$) [Note: spectrum contains two diastereomers.] δ 8.88 (s, 1H), 7.34 – 7.24 (m, 8H), 5.85 – 5.43 (m, 1H), 4.21 (s, 1H), 3.86 – 3.37 (m, 4H), 2.53 – 2.28 (m, 4H), 1.90 (d, *J* = 7.0 Hz, 2H), 1.80 (d, *J* = 7.1 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) [Note: spectrum contains two diastereomers.] δ 161.81, 148.90, 140.13, 140.03, 133.41, 129.17, 129.11, 77.09, 74.80, 74.73, 52.26, 51.77, 51.52, 51.35, 47.31, 46.65, 42.25, 41.68, 16.54. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{21}H_{22}Cl_2N_4O_4$ 465.1096; found 465.1092.

Compound 47



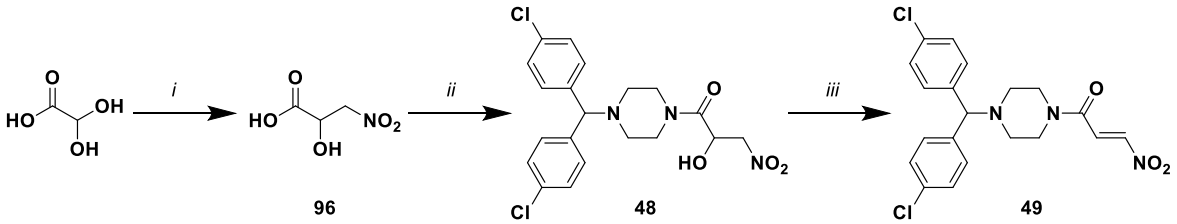
Scheme 39. Synthesis of **47**.

Conditions: *i*. CDI, THF, rt then DBU, nitromethane, THF, 60 °C (84%).

1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-3-nitropropane-1,2-dione (**47**): 2-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-2-oxoacetic acid **80** (1.53 g, 3.89 mmol, 1 eq.) was dissolved in dry THF (9 mL) and CDI (0.69 g, 4.28 mmol, 1.1 eq.) was added. The mixture was stirred for 3h at rt.

Nitromethane (0.63 mL, 11.67 mmol, 3 eq.) was added followed by the slow addition of DBU (2.62 mL, 17.51 mmol, 4.5 eq.). The reaction was stirred at rt for 1 h and then partitioned between EtOAc and 1 N aqueous HCl. The organic fraction was dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by flash column chromatography (0-5% MeOH/DCM) afforded the title compound (**47**, 1.41 g, 84% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.26 (m, 8H), 5.57 (s, 2H), 4.25 (s, 1H), 3.78 – 3.58 (m, 4H), 2.50 – 2.40 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 184.63, 160.85, 139.91, 133.50, 129.22, 129.13, 79.94, 74.48, 51.93, 51.25, 45.96, 42.76. IR (ATR) vmax (cm-1): 2979, 2816, 1643, 1546, 1489, 1411, 1372, 1288, 1236, 1210, 1146, 1091, 1066, 1013, 997, 955, 907, 854, 812, 800, 729, 685, 647, 625, 611, 537, 506. ESI LRMS (m/z): $[M–H]^-$ calculated for $C_{20}H_{19}Cl_2N_3O_4$ 434.07; found 434.28. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{20}H_{19}Cl_2N_3O_4$ 436.0825; found 436.0824.

Compounds 48 and 49



Scheme 40. Synthesis of **48** and **49**.

Conditions: *i.* nitromethane, NaOH, H_2O, rt (42%); *ii.* 1-(bis(4-chlorophenyl)methyl)piperazine, TBTU, DIPEA, DCM, rt (26%); *iii.* MsCl, Et_3N, DCM, -20 °C (70%).

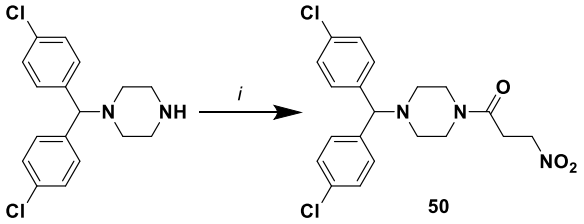
2-hydroxy-3-nitropropanoic acid (**96**): Glyoxylic acid monohydrate (4.00 g, 43.45 mmol, 1 eq.) was dissolved in water (16 mL). Nitromethane (5.30 g, 4.67 mL, 86.91 mmol, 2 eq.) and solid NaOH (1.83 g, 45.63 mmol, 1.05 eq.) were added and the mixture stirred overnight at rt. The mixture was acidified by addition of 4 N aqueous HCl and extracted with EtOAc several times. The combined organic fractions were dried over anhydrous Na_2SO_4, filtered, and evaporated solvent. The residue was precipitated from DCM to afford the title compound (**96**, 2.46 g, 42% yield) as a brown solid, which was used without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.11 (br s, 1H), 6.08 (br s, 1H), 4.86 (dd, J = 13.3, 3.9 Hz, 1H), 4.72 (dd, J = 13.3, 7.0 Hz, 1H), 4.59 (dd, J = 7.0, 3.9 Hz, 1H).

1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-2-hydroxy-3-nitropropan-1-one (**48**): **96** (463 mg, 3.42 mmol, 1.1 eq.) was dissolved in dry DCM (15 mL) and TBTU (1.1 g, 3.42 mmol, 1.1 eq.), DIPEA (0.65 mL, 3.74 mmol, 1.2 eq.), and 1-(bis(4-chlorophenyl)methyl)piperazine (1 g, 3.11 mmol, 1 eq.) were added in succession. The reaction was stirred overnight at rt. The reaction was diluted with DCM and washed with water. After concentration under reduced pressure, purification by flash column chromatography (0-20% EtOAc/hexanes) afforded the title compound (**48**, 350 mg, 26% yield) as a white solid. A small amount (45 mg, 3.4% yield) of the dehydrated product **49** (see below) was also obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.25 (m, 8H), 5.13 – 4.94 (m, 1H), 4.52 – 4.40 (m, 2H), 4.25 (s, 1H), 4.03 (d, J = 7.3 Hz, 1H), 3.81 – 3.68 (m, 1H), 3.65 – 3.43 (m, 3H), 2.51 – 2.29 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.60, 139.82, 139.79, 133.47, 129.19, 129.10, 78.40, 74.40, 66.12, 51.67, 51.24, 45.47, 43.15. IR (ATR) vmax (cm-1): 3377, 2920, 2816, 1640, 1552, 1487, 1409, 1377, 1331, 1290, 1259, 1233, 1207, 1144, 1105, 1087, 1036, 1013, 998, 906, 865, 812, 801, 727, 686, 648, 633, 536, 504. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{20}H_{21}Cl_2N_3O_4$ 438.0987; found 438.0981.

(*E*)-1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-3-nitroprop-2-en-1-one (**49**): A solution of **48** (300 mg, 0.68 mmol, 1 eq.) in dry DCM (1 mL) was cooled to -20 °C. Triethylamine (0.29 mL, 2.05 mol, 3 eq.) was added followed by the dropwise addition of mesyl chloride (0.16 mL, 2.05 mol, 3 eq.). The reaction was stirred for 1 h at -20 °C. The reaction was then partitioned between water and DCM. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by flash column chromatography (0-20% EtOAc/hexanes) afforded the title compound (**49**, 200 mg, 70% yield) as a pale-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.80 (d, J = 13.2 Hz, 1H), 7.69 (d, J = 13.2 Hz, 1H), 7.48 – 7.34 (m, 8H), 4.47 (s, 1H), 3.63 – 3.50 (m, 4H), 2.36 – 2.26 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ

160.15, 147.72, 139.64, 133.38, 129.07, 129.00, 127.46, 74.26, 51.84, 51.09, 46.48, 42.48. ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{20}H_{19}Cl_2N_3O_3$ 420.09; found 420.38. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{20}H_{19}Cl_2N_3O_3$ 420.0876; found 420.0874.

Compound 50

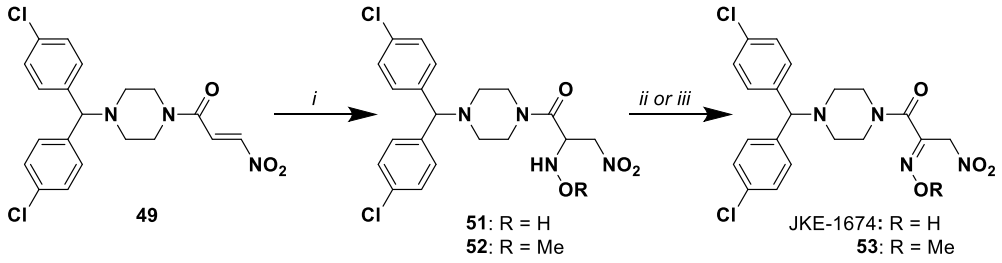


Scheme 41. Synthesis of **50**.

Conditions: *i.* 3-nitropropionic acid, TBTU, DIPEA, DCM, rt (85%).

1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-3-nitropropan-1-one (**50**): 3-Nitropropionic acid (741 mg, 6.23 mmol, 1.25 eq.) was dissolved in dry DCM (40 mL). TBTU (2.08 g, 6.47 mmol, 1.3 eq.), DIPEA (1.1 mL, 6.47 mmol, 1.3 eq.), and 1-(bis(4-chlorophenyl)methyl)piperazine (1.6 g, 4.98 mmol , 1 eq.) were added in succession. The reaction was stirred overnight at rt then washed with water and concentrated under reduced pressure. The residue was purified by flash column chromatography (0-30% EtOAc/hexanes) to afford the title compound (**50**, 1.78 g, 85% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.23 (m, 8H), 4.68 (t, *J* = 6.2 Hz, 2H), 4.22 (s, 1H), 3.64 – 3.58 (m, 2H), 3.51 – 3.46 (m, 2H), 2.94 (t, *J* = 6.2 Hz, 2H), 2.43 – 2.31 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.67, 140.11, 133.30, 129.13, 129.10, 77.36, 74.48, 70.29, 51.77, 51.39, 45.55, 42.10, 29.79. IR (ATR) vmax (cm-1): 2814, 1642, 1552, 1488, 1467, 1446, 1409, 1375, 1333, 1290, 1256, 1232, 1206, 1144, 1111, 1088, 1039, 1013, 1000, 949, 909, 869, 837, 803, 775, 731, 686, 648, 536, 505. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{20}H_{21}Cl_2N_3O_3$ 422.1038; found 422.1045.

Compounds 51-53 and JKE-1674 (37)



Scheme 42. Synthesis of **51-53** and alternate synthesis of JKE-1674 (**37**).

Conditions: *i.* hydroxylamine hydrochloride or methoxyamine hydrochloride, Et_3N, EtOH, rt (74%, 17-103 and 86%, 17-105); *ii. p*-benzoquinone, PhMe, 50 °C (83%, JKE-1674); *iii.* lead(IV) acetate, PhMe (53%, 17-130).

1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-2-(hydroxyamino)-3-nitropropan-1-one (**51**): Hydroxylamine hydrochloride (33 mg, 0.48 mmol, 2 eq.) was added to a solution of **49** (100 mg, 0.24 mmol, 1 eq.) and triethylamine (0.48 mmol, 2 eq.) in absolute ethanol (1 mL). The reaction was stirred at rt overnight and partitioned between EtOAc and water. The organic layer was separated and concentrated under reduced pressure. Purification by flash column chromatography (0-50% EtOAc/hexanes) afforded the title compound (**51**, 80 mg, 74% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.26 (m, 8H), 5.75 (s, 1H), 5.51 (s, 1H), 4.79 – 4.60 (m, 3H), 4.25 (s, 1H), 3.75 – 3.56 (m, 4H), 2.49 – 2.36 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.71, 139.92, 133.27, 129.04, 129.01, 74.39, 73.12, 59.15, 51.91, 51.32,

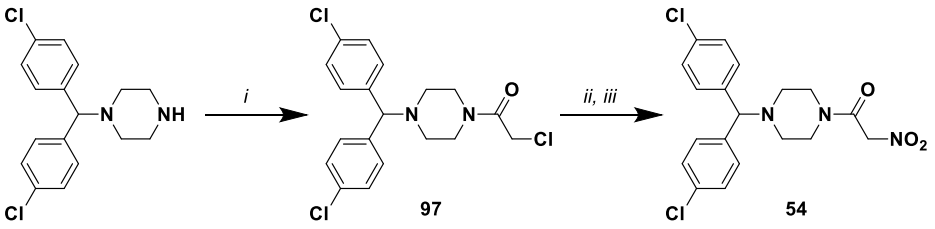
45.96, 42.57. IR (ATR) vmax (cm-1): 3341, 2921, 2818, 1635, 1553, 1488, 1445, 1411, 1377, 1333, 1290, 1242, 1144, 1089, 1034, 1013, 999, 908, 865, 836, 812, 802, 732, 649, 538, 505. ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{20}H_{22}Cl_2N_4O_4$ 453.11; found 453.14. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{20}H_{22}Cl_2N_4O_4$ 453.1091; found 453.1073.

Alternate synthesis of 1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-2-(hydroxyimino)-3-nitropropan-1-one (JKE-1674, **37**): *p*-Benzoquinone (7 mg, 0.07 mmol, 1 eq.) was added to a solution of **51** (30 mg, 0.07 mmol, 1 eq.) in toluene (0.4 mL). The mixture was heated to 50 °C for 30 minutes and partitioned between EtOAc and water. The organic layer was concentrated and purified by flash column chromatography (0-100% EtOAc/hexanes) to afford the title compound (**37**, 25 mg, 83% yield). Spectral (^{1}H and ^{13}C NMR) and LRMS data are identical to material produced via ML210 hydrolysis.

1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-2-(methoxyamino)-3-nitropropan-1-one (**52**): Methoxyamine hydrochloride (255 mg, 0.61 mmol, 1 eq.) was added to a solution of **49** (101 mg, 1.21 mmol, 2 eq.) and triethylamine (0.17 mL, 1.21 mmol, 2 eq.) in absolute ethanol (3 mL). After stirring overnight at rt, the reaction was partitioned between EtOAc and water. Purification by flash column chromatography (0-50% EtOAc/hexanes) afforded the title compound (**52**, 243 mg, 86%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.23 (m, 8H), 6.12 – 5.87 (m, 1H), 4.73 – 4.54 (m, 3H), 4.27 – 4.16 (m, 1H), 3.75 – 3.56 (m, 4H), 3.52 – 3.44 (m, 3H), 2.51 – 2.29 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.47, 140.13, 133.35, 129.14, 129.11, 74.52, 73.43, 62.30, 57.74, 51.99, 51.43, 46.05, 42.63. IR (ATR) vmax (cm-1): 3233, 2933, 2813, 1643, 1552, 1488, 1442, 1411, 1377, 1331, 1290, 1242, 1207, 1144, 1088, 1036, 1013, 998, 910, 865, 836, 812, 802, 732, 648, 538, 505. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{21}H_{24}Cl_2N_4O_4$ 467.1253; found 467.1257.

1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-2-(methoxyimino)-3-nitropropan-1-one (**53**): Lead(IV) acetate (336 mg, 0.76 mmol, 2 eq.) was added to a solution of **52** (177 mg, 0.38 mmol, 1 eq.) in toluene (2 mL). The reaction was stirred at rt for 15 minutes and filtered through a pad of Celite. Flash column chromatography purification (0-35% EtOAc/hexanes) afforded the title compound (**53**, 94 mg, 53% yield) as a mixture of diastereomers. ^{1}H NMR (400 MHz, $CDCl_3$) [Note: spectrum contains a mixture of diastereomers] δ 7.37 – 7.24 (m, 8H), 5.52 – 5.37 (m, 1H), 4.23 (s, 1H), 4.06 – 3.26 (m, 8H), 2.54 – 2.24 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) [Note: spectrum contains a mixture of diastereomers] δ 161.81, 160.23, 143.32, 142.45, 140.36, 140.31, 140.17, 133.29, 129.17, 129.13, 129.09, 74.94, 74.58, 74.53, 74.45, 67.73, 63.65, 63.40, 52.17, 51.70, 51.43, 51.24, 47.51, 46.73, 42.78, 41.75. IR (ATR) vmax (cm-1): 2940, 2819, 1636, 1558, 1488, 1444, 1410, 1371, 1328, 1290, 1226, 1172, 1143, 1109, 1088, 1050, 1030, 1013, 998, 907, 864, 837, 812, 802, 730, 687, 647, 625, 536, 505. ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{21}H_{22}Cl_2N_4O_4$ 465.11; found 464.98. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{21}H_{22}Cl_2N_4O_4$ 465.1091; found 465.1079.

Compound 54



Scheme 43. Synthesis of **54**.

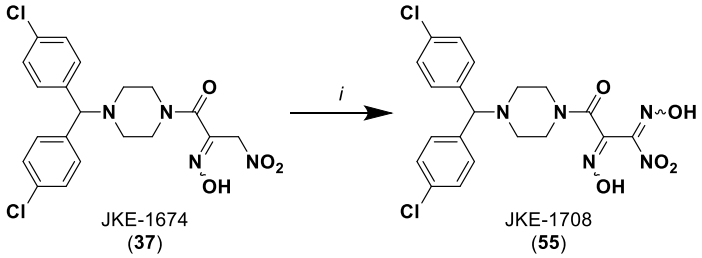
Conditions: *i*. chloroacetyl chloride, Et_3N, DCM, 0 °C to rt (57%); *ii*. KI, acetone, rt; *iii*. $AgNO_2$, THF, Et_2O, rt (44% over two steps).

1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-2-chloroethan-1-one (**97**): A solution of 1-(bis(4-chlorophenyl)methyl)piperazine (0.5 g, 1.56 mmol, 1 eq.) was dissolved in 5 mL dry DCM with triethylamine (0.2 g, 0.28 mL, 2.02 mmol, 1.3 eq.) and cooled in an ice bath. Chloroacetyl chloride (0.21 g, 0.15 mL, 1.86 mmol, 1.2 eq.) was added dropwise. The mixture was stirred overnight at room temperature, concentrated, and purified by flash column chromatography (EtOAc/hexanes) to afford the title compound (**97**, 241 mg,

39% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.15 (m, 8H), 4.21 (s, 1H), 4.01 (s, 2H), 3.55 (m,4H), 2.38 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.06, 140.13, 133.28, 129.11, 129.09, 74.48, 51.77, 51.31, 46.47, 42.31, 40.93. IR (ATR) vmax (cm-1): 1646, 1593, 1488, 1441, 1410, 1369, 1330, 1290, 1247, 1229, 1144, 1109, 1089, 1047, 1013, 999, 960, 907, 864, 838, 801, 727, 686, 661, 646. ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{19}H_{19}Cl_3N_2O$ 397.06; found 397.61.

1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-2-nitroethan-1-one (**54**): Potassium iodide (0.121 g, 0.73 mmol, 1.2 eq.) was added to a solution of **97** (0.241 g, 0.61 mmol, 1 eq.) in acetone (1.2 mL). The reaction was stirred at rt for 6 h and subsequently partitioned between EtOAc and water. The organic layer was collected and washed with water and brine. After drying over anhydrous sodium sulfate, the organics were filtered and concentrated under reduced pressure to afford the iodoacetamide intermediate. The crude product was immediately dissolved in 1:1 Et_2O/THF (8 mL) and silver nitrite (0.140 g, 0.91 mmol, 1.5 eq.) was added. The reaction was stirred in the dark for 24 hours, filtered through a pad of Celite, and concentrated under vacuum. Purification by flash column chromatography (0-30% EtOAc/hexanes) afforded the title compound (**54**, 0.214 g, 86% yield over two steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.23 (m, 7H), 5.25 (s, 2H), 4.24 (s, 1H), 3.66 (t, *J* = 5.1 Hz, 2H), 3.35 (t, *J* = 5.1 Hz, 2H), 2.45 – 2.34 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.05, 139.78, 133.32, 129.07, 128.99, 76.77, 74.25, 51.34, 50.97, 45.93, 42.59. IR (ATR) vmax (cm-1): 2965, 2817, 1660, 1593, 1561, 1488, 1410, 1379, 1322, 1290, 1237, 1208, 1143, 1110, 1088, 1043, 1013, 998, 906, 865, 848, 801, 727, 686, 665, 647, 618, 582, 535, 503. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{19}H_{19}Cl_2N_3O_3$ 408.0882; found 408.0878.

JKE-1708 (55)



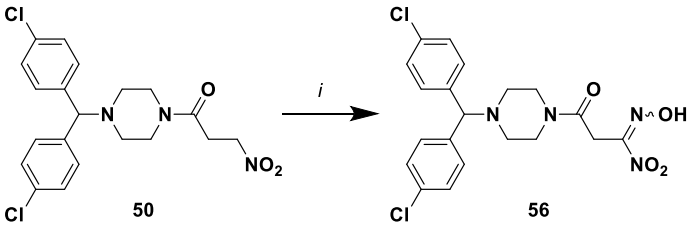
Scheme 44. Synthesis of JKE-1708 (**55**).

Conditions: *i*. $NaNO_2$, AcOH, DMSO, rt (52% yield).

1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-2,3-bis(hydroxyimino)-3-nitropropan-1-one (JKE-1708, **55**): JKE-1674 (**37**, 250 mg, 0.55 mmol, 1 eq.) was dissolved in DMSO (0.5 mL) and acetic acid (0.317 mL, 5.54, 10 eq.) was added followed by sodium nitrite (76 mg, 1.11 mmol, 2 eq.). The mixture was stirred at rt for 20 minutes and was partitioned between EtOAc and water. The organic layer was collected, washed twice with water, and dried over anhydrous Na_2SO_4. After filtration, the solvent was removed *in vacuo* and the residue was purified by flash column chromatography (0-35% EtOAc/hexanes). The title compound was obtained in two fractions (**55a**, Fraction 1: 33 mg, 12% yield, single diastereomer; **55b**, Fraction 2: 106 mg, 40% yield, mixture of diastereomers). The relative amounts of both flash column chromatography fractions varied between preparations. [Note: allowing the reaction to stir at rt for several hours leads to the formation of nitrofurazan **9**. Refluxing JKE-1708 in toluene also affords **9**.] Fraction 1 (**55a**): ^{1}H NMR (400 MHz, acetone-d_6) δ 12.75 – 11.78 (m, 2H), 7.53 – 7.45 (m, 4H), 7.38 – 7.30 (m, 4H), 4.46 (s, 1H), 3.73 – 3.62 (m, 2H), 3.47 – 3.37 (m, 2H), 2.52 – 2.36 (m, 4H). ^{13}C NMR (101 MHz, acetone-d_6) δ 157.39, 148.33, 143.21, 140.92, 132.46, 129.50, 128.72, 73.62, 51.72, 50.96, 45.86, 41.16. IR (ATR) vmax (cm-1): 3153, 3023, 2856, 1686, 1634, 1552, 1487, 1445, 1411, 1365, 1325, 1290, 1259, 1243, 1143, 1127, 1089, 1049, 1013, 996, 950, 893, 844, 813, 802, 737, 686, 621, 537, 505. Fraction 2 (**55b**): ^{1}H NMR (400 MHz, acetone-d_6) δ 12.41 (s, 2H), 7.56 – 7.44 (m, 4H), 7.39 – 7.29 (m, 4H), 4.51 – 4.33 (m, 1H), 3.93 – 3.87 (m, 1H), 3.73 – 3.64 (m, 2H), 3.60 – 3.37 (m, 1H), 2.56 – 2.37 (m, 4H). ^{13}C NMR (101 MHz, acetone-d_6) δ 159.76, 152.77, 141.11, 141.03, 139.70, 132.43, 129.51, 129.49, 128.71, 73.75, 51.83, 51.43, 51.22, 50.88, 46.98, 46.22, 42.42, 41.31. IR (ATR) vmax (cm-1): 3151, 3022, 2823, 1687, 1621, 1547, 1486, 1444, 1410, 1369, 1338,

1290, 1260, 1247, 1089, 1012, 994, 859, 834, 811, 801, 737, 687, 538, 505. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{20}H_{19}Cl_2N_5O_5$ 480.0841; found 480.0842 (**55a**) and 480.0841 (**55b**).

Compound 56

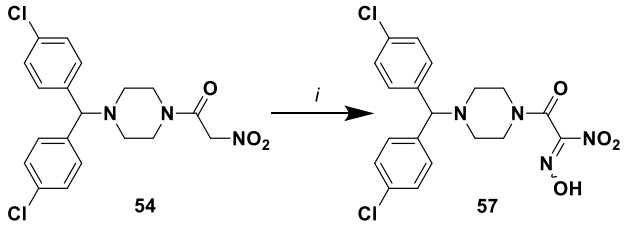


Scheme 45. Synthesis of **56**.

Conditions: *i*. $NaNO_2$, AcOH, DMSO, rt (42%).

1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-3-(hydroxyimino)-3-nitropropan-1-one (**56**): Compound **50** (512 mg, 1.22 mmol, 1 eq.) was dissolved in DMSO (2 mL) with acetic acid (0.65 mL, 11.34 mmol, 10 eq.). Sodium nitrite (235 mg, 3.4 mmol, 3 eq.) was added and the reaction was stirred at rt for 24 h. After partitioning the reaction between EtOAc and water, the organic layer was collected, washed with water, dried over Na_2SO_4, and concentrated under reduced pressure. Purification of the residue by flash column chromatography (0-100% EtOAc/hexanes) afforded the title compound (**56**, 214 mg, 42% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.14 (s, 1H), 7.48 – 7.40 (m, 5H), 7.40 – 7.34 (m, 4H), 4.46 (s, 1H), 4.03 (s, 2H), 3.60 – 3.50 (m, 2H), 3.49 – 3.39 (m, 2H), 2.38 – 2.28 (m, 2H), 2.29 – 2.20 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.85, 158.23, 140.85, 131.66, 129.42, 128.65, 72.49, 51.33, 50.79, 45.13, 41.37, 29.59. IR (ATR) vmax (cm-1): 2859, 2361, 2434, 1619, 1541, 1487, 1446, 1400, 1345, 1291, 1232, 1131, 1110, 1089, 1038, 1013, 999, 951, 861, 839, 800, 746, 686, 669, 583, 536, 504. ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{20}H_{21}Cl_2N_3O_3$ 451.09; found 451.35. ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{20}H_{21}Cl_2N_3O_3$ 451.0934; found 451.0952.

Compound 57



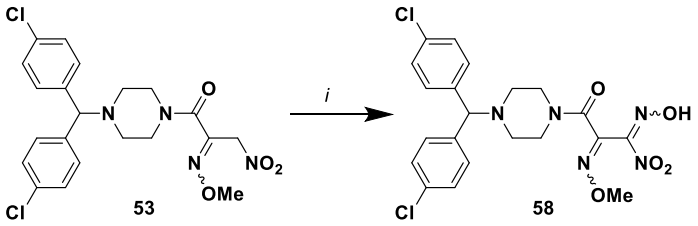
Scheme 46. Synthesis of **57**.

Conditions: *i*. $NaNO_2$, AcOH, DMSO, rt (66%).

1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-2-(hydroxyimino)-2-nitroethan-1-one (**57**): A solution of **54** (214 mg, 0.52 mmol, 1 eq.), sodium nitrite (108 mg, 1.57 mmol, 3 eq.), and acetic acid (471 mg, 0.449 mL, 15 eq.) in dry DMSO (1 mL) was stirred at rt for 1.5 h. The reaction was partitioned between EtOAc and water. The organic layer was collected, washed with water, dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by flash column chromatography (0-30% EtOAc/hexanes) afforded the title compound (**57**, 151 mg, 66% yield) as a mixture of isomers. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.24 (m, 8H), 4.44 – 4.10 (m, 1H), 3.83 – 3.61 (m, 2H), 3.36 (s, 2H), 2.62 – 2.25 (m, 4H). IR (ATR, solid) vmax (cm-1): 2824, 1633, 1547, 1488, 1444, 1411, 1336, 1290, 1262, 1139, 1089, 1043, 1013, 996, 904, 861, 837, 809, 736, 687, 647, 534. A characteristic nitrile oxide peak (2253 cm^{-1}) is observed in the IR spectrum by

thin-film method, likely due to decomposition during measurements. ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{19}H_{18}Cl_2N_4O_4$ 437.08; found 437.20.

Compound 58

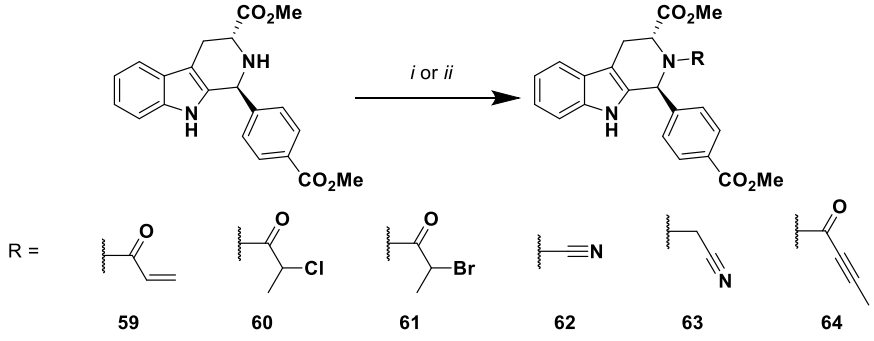


Scheme 47. Synthesis of **58**.

Conditions: *i*. $NaNO_2$, AcOH, DMSO, rt (31%).

1-(4-(bis(4-chlorophenyl)methyl)piperazin-1-yl)-3-(hydroxyimino)-2-(methoxyimino)-3-nitropropan-1-one (**58**): Sodium nitrite (42 mg, 0.61 mmol, 3 eq.) was added to a rt solution of **53** (94 mg, 0.20 mmol, 1 eq.) in DMSO (0.3 mL) and acetic acid (0.81 mL, 2.02 mmol, 10 eq.). The reaction was stirred at rt for 2 h and then partitioned between EtOAc and water. The organic layer was separated and washed with water. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by flash column chromatography (0-30% EtOAc/hexanes) afforded the title compound (**58**, 31 mg, 31% yield) as a complex mixture of isomers. ^{1}H NMR (400 MHz, $CDCl_3$) [Note: complex mixture of isomers] δ 7.43 – 7.26 (m, 9H), 4.43 – 4.22 (m, 1H), 4.14 – 3.54 (m, 7H), 2.68 – 2.33 (m, 4H). IR (ATR) vmax (cm-1): 2945, 1651, 1633, 1556, 1488, 1444, 1411, 1471, 1290, 1140, 1090, 1032, 1013, 997, 801 736, 687, 537, 504. Note: the characteristic nitrile oxide peak (2283 cm^{-1}) is observed in the IR spectrum when the sample is prepared as a thin-film from chloroform solution. This 2283 cm^{-1} peak is not present in the IR spectrum of a solid sample of the title compound. ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{21}H_{22}Cl_2N_4O_4$ 465.11; found 465.73. ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{21}H_{22}Cl_2N_4O_4$ 465.1091; found 465.1079.

Compounds 59-64



Scheme 48. Synthesis of RSL3 warhead analogues.

Conditions: *i*. acid chloride, K_2CO_3, THF, rt; *ii*. alkyl bromide, K_2CO_3, THF, rt.

(1*S*,3*R*)-methyl 2-acryloyl-1-(4-(methoxycarbonyl)phenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (**59**): To a solution of (1*S*,3*R*)-1-(4-(methoxycarbonyl)phenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (25 mg, 0.069 mmol, 1 eq.) in dry THF (1 mL) was added potassium carbonate (47 mg, 0.343 mmol, 5 eq.), followed by acryloyl chloride (28 µL, 0.343 mmol, 5 eq.). The resulting solution was stirred at rt for 90 min and then concentrated. Purification by flash column

chromatography on silica gel (EtOAcl/hexanes with 2% Et_3N) afforded the title compound (**59**, 14 mg, 49% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (s, 3H), 7.70 – 7.34 (m, 3H), 7.24 – 7.03 (m, 3H), 6.49 (d, *J* = 12.6 Hz, 1H), 6.35 – 6.18 (m, 2H), 5.64 (s, 1H), 5.22 (dd, *J* = 5.4, 4.0 Hz, 1H), 3.88 (s, 3H), 3.62 (s, 3H), 3.38 (s, 1H). ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{24}H_{22}N_2O_5$ 419.1607; found 419.1613.

(*1S,*3*R*)-methyl 2-(2-chloropropanoyl)-1-(4-(methoxycarbonyl)phenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (**60**): To a solution of (1*S*,3*R*)-1-(4-(methoxycarbonyl)phenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-b]indole-3-carboxylate (26 mg, 0.071 mmol, 1 eq.) in dry THF (1 mL) was added potassium carbonate (39 mg, 0.285 mmol, 4 eq.), followed by chloroacetyl chloride (14 µL, 0.143 mmol, 2 eq.). The mixture was stirred at rt overnight and the reaction was quenched with methanol (1 mL) and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (basic methanol/DCM) to afford the title compound (**60**, 14 mg, 43% yield) as a 5:1 mixture of diastereomers. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (s, 2H), 7.75 – 7.35 (m, 3H), 7.16 (s, 3H), 6.23 (s, 1H), 5.37 (d, *J* = 44.5 Hz, 1H), 4.66 (q, *J* = 6.6 Hz, 1H), 3.90 (s, 3H), 3.66 (d, *J* = 3.2 Hz, 3H), 3.51 (d, *J* = 5.4 Hz, 1H), 1.63 (s, 3H), 1.59 – 1.55 (m, 1H). ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{24}H_{23}ClN_2O_5$ 455.1374; found 455.1371.

(1*S*,3*R*)-methyl 2-(2-bromopropanoyl)-1-(4-(methoxycarbonyl)phenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (**61**): To a solution of (1*S*,3*R*)-1-(4-(methoxycarbonyl)phenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylate (30 mg, 0.082 mmol, 1 eq.) in dry THF (1 mL) was added potassium carbonate (45.5 mg, 0.329 mmol, 4 eq.), followed by 2-bromopropanoyl chloride (17 µL, 0.165 mmol, 2 eq.). The mixture was stirred at rt overnight and the reaction was quenched with methanol (1 mL) and concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (basic methanol/DCM) to afford the title compound (**61**, 3 mg, 7.3% yield) as a 5:1 mixture of diastereomers. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (s, 2H), 7.66 – 7.35 (m, 3H), 7.25 – 7.07 (m, 3H), 6.22 (s, 1H), 5.37 (s, 1H), 4.64 (q, *J* = 6.6 Hz, 1H), 3.90 (s, 3H), 3.66 (d, *J* = 5.3 Hz, 4H), 3.51 (d, *J* = 5.4 Hz, 1H), 1.78 (s, 3H), 1.56 (s, 2H). ESI LRMS (m/z): $[M+H]^+$ calculated for $C_{24}H_{23}BrN_2O_5$ 499.0869; found 499.0863.

(1*S*,3*R*)-methyl 2-cyano-1-(4-(methoxycarbonyl)phenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (**62**): To a solution of amine (1*S*,3*R*)-1-(4-(methoxycarbonyl)phenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (34 mg, 0.093 mmol, 1 eq.) in dry THF (1 mL) was added potassium carbonate (52 mg, 0.373 mmol, 4 eq.), followed by cyanogen bromide (20 mg, 0.187 mmol, 2 eq.). The resulting solution was stirred at rt overnight and then concentrated. Purification by flash column chromatography on silica gel (basic methanol/DCM) afforded the title compound (**62**, 20 mg, 55% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 – 7.94 (m, 2H), 7.72 (s, 1H), 7.63 – 7.50 (m, 1H), 7.50 – 7.37 (m, 2H), 7.26 – 7.10 (m, 4H), 5.97 (d, *J* = 1.8 Hz, 1H), 4.61 (dd, *J* = 5.3, 3.0 Hz, 1H), 4.00 – 3.87 (m, 3H), 3.76 (s, 3H), 3.49 (dt, *J* = 5.6, 1.8 Hz, 2H). ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{22}H_{19}N_3O_4$ 390.1454; found 390.1454.

(1*S*,3*R*)-methyl 2-(cyanomethyl)-1-(4-(methoxycarbonyl)phenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (**63**): To a solution of (1*S*,3*R*)-1-(4-(methoxycarbonyl)phenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylate (23 mg, 0.063 mmol, 1 eq.) in dry THF (1 mL) was added potassium carbonate (44 mg, 0.316 mmol, 5 eq.), followed by sodium iodide (1 mg, 6.3 µmol, 0.1 eq.). To the resulting yellow mixture was added bromoacetonitrile (22 µL, 0.316 mmol, 5 eq.) and the mixture turned cloudy white. The reaction was stirred at rt for 1 h, then heated to 80 °C and stirred for 6 h. The mixture was cooled to rt and concentrated. Purification by flash column chromatography (basic methanol/DCM) afforded the title compound (**63**, 7 mg, 27% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (d, *J* = 8.3 Hz, 2H), 7.51 (t, *J* = 8.4 Hz, 3H), 7.30 (s, 1H), 7.19 – 7.06 (m, 2H), 5.50 (s, 1H), 4.27 (dd, *J* = 5.6, 2.9 Hz, 1H), 3.92 (s, 3H), 3.88 (d, *J* = 17.3 Hz, 1H), 3.68 (s, 3H), 3.56 (d, *J* = 17.2 Hz, 1H), 3.40 (d, *J* = 7.6 Hz, 2H). ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{23}H_{21}N_3O_4$ 404.1610; found 404.1613.

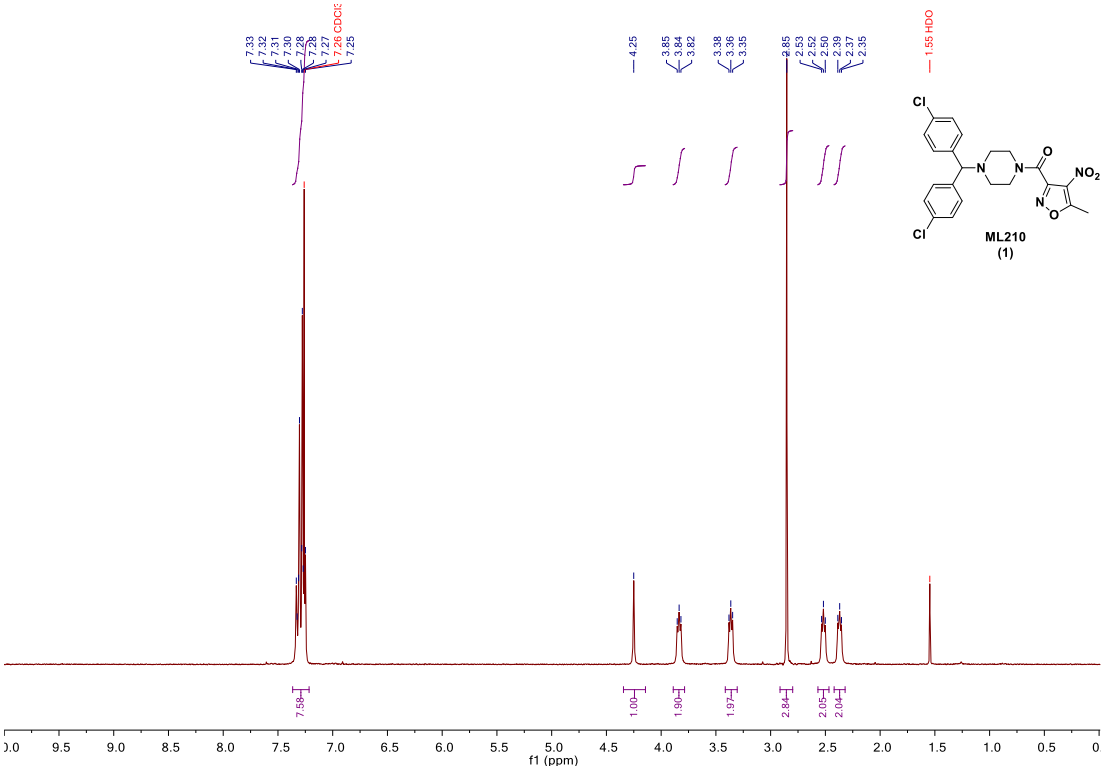
(1*S*,3*R*)-methyl 2-(but-2-ynoyl)-1-(4-(methoxycarbonyl)phenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (**64**): To a solution of butynoic acid (69 mg, 0.823 mmol, 10 eq.) in dry DCM (5 mL) was added oxalyl chloride (2M solution in DCM, 0.41 mL, 0.823 mmol, 10 eq.), followed by 1 drop of DMF. The resulting mixture was stirred for 30 minutes. To this was added (1*S*,3*R*)-1-(4-(methoxycarbonyl)phenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (30 mg, 0.082 mmol, 1 eq.) was then added and the mixture was stirred overnight. Concentration and purification by flash column chromatography on silica gel (basic methanol/DCM) afforded the title compound (**64**, 12 mg, 34% yield). ESI HRMS (m/z): $[M+H]^+$ calculated for $C_{25}H_{22}N_2O_5$ 431.1607; found 431.1611.

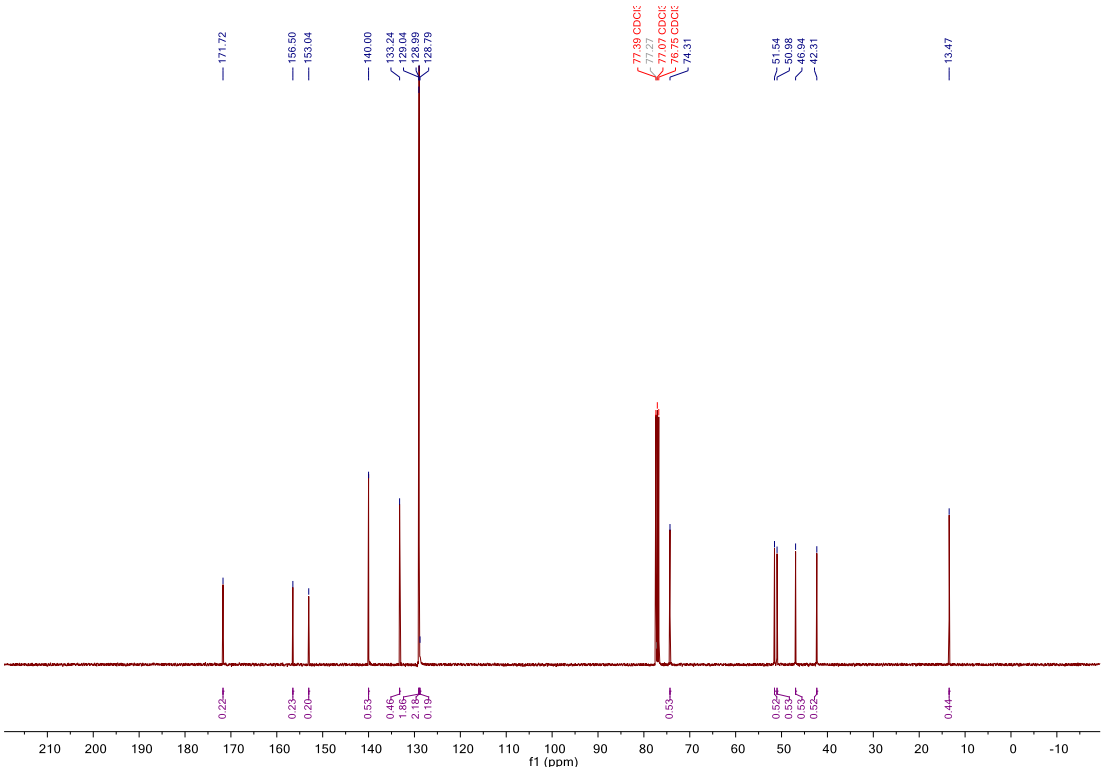
References

1. Weïwer, M. *et al.* Development of small-molecule probes that selectively kill cells induced to express mutant RAS. *Bioorganic Med. Chem. Lett.* **22**, 1822–1826 (2012).
2. Yang, W. S. *et al.* Regulation of ferroptotic cancer cell death by GPX4. *Cell* **156**, 317–331 (2014).
3. Pal, M., Parasuraman, K. & Yeleswarapu, K. R. Palladium-catalyzed cleavage of O/N-propargyl protecting groups in aqueous media under a copper-free condition. *Org. Lett.* **5**, 349–352 (2003).
4. Sekirnik (née Measures), A. R. *et al.* Isoxazole-Derived Amino Acids are Bromodomain-Binding Acetyl-Lysine Mimics: Incorporation into Histone H4 Peptides and Histone H3. *Angew. Chemie - Int. Ed.* **55**, 8353–8357 (2016).
5. Shackelford, S. A. *et al.* Electrophilic tetraalkylammonium nitrate nitration. II. Improved anhydrous aromatic and heteroaromatic mononitration with tetramethylammonium nitrate and triflic anhydride, including selected microwave examples. *J. Org. Chem.* **68**, 267–275 (2003).
6. Scott, M. S., Luckhurst, C. A. & Dixon, D. J. A total synthesis of tarchonanthuslactone exploiting N-pyrrole carbinols as efficient stereocontrolling elements. *Org. Lett.* **7**, 5813–5816 (2005).
7. Dere, R., Monasterolo, C., Moccia, M. & Adamo, M. F. A. Preparation and reactivity of [2-(3-methyl-4-nitro-isoxazol-5-yl)-vinyl]-amines. *Tetrahedron Lett.* **56**, 7168–7171 (2015).
8. Kawai, H., Tachi, K., Tokunaga, E., Shiro, M. & Shibata, N. Trifluoromethylation of aromatic isoxazoles: Regio- and diastereoselective route to 5-trifluoromethyl-2-isoxazolines. *Angew. Chemie - Int. Ed.* **50**, 7803–7806 (2011).

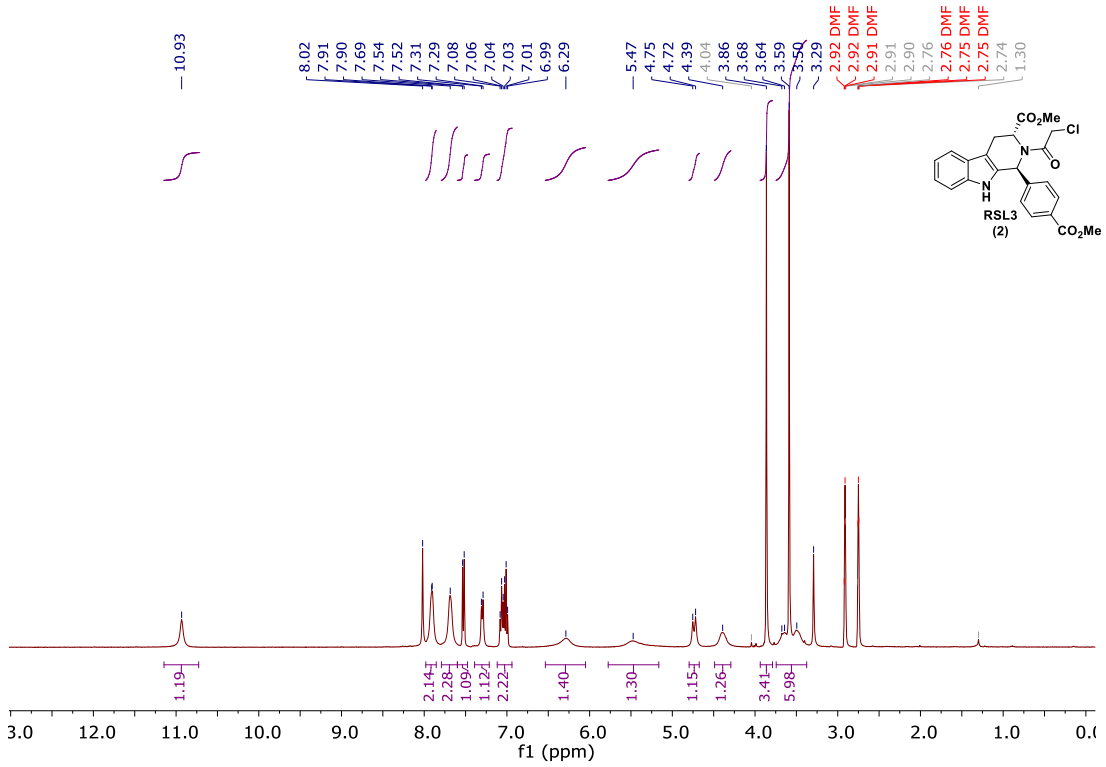
NMR spectra of final compounds

ML210 (1)





RSL3 (2)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7.01
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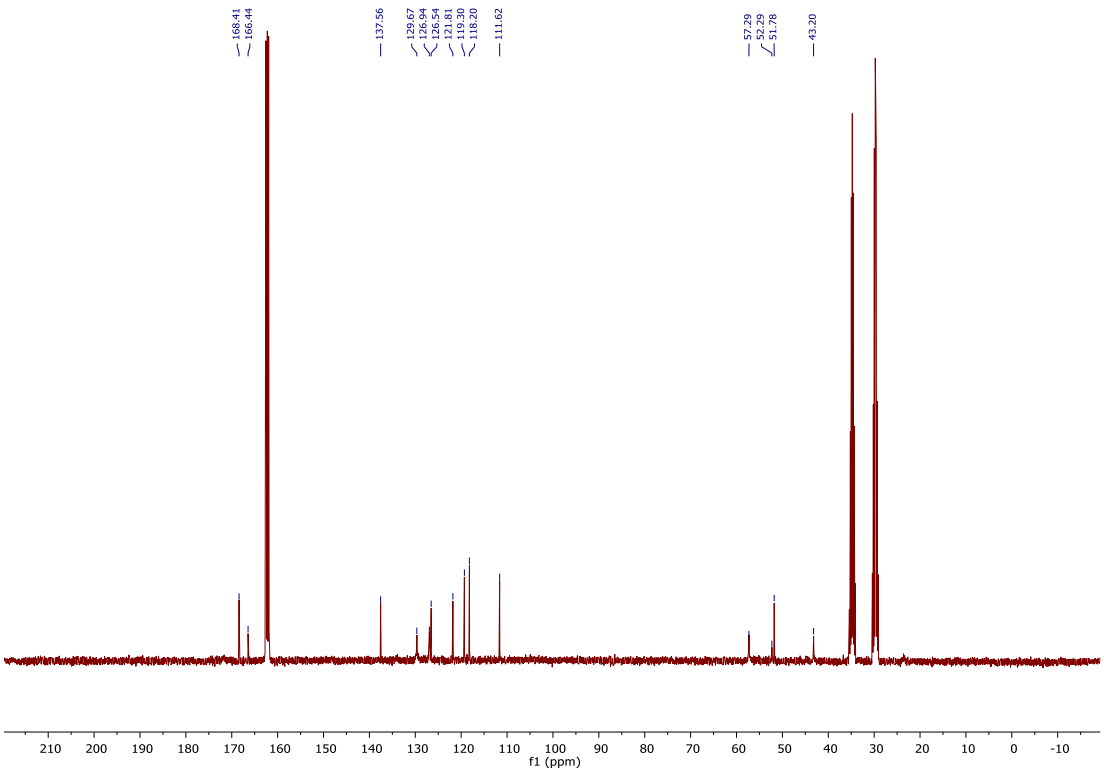
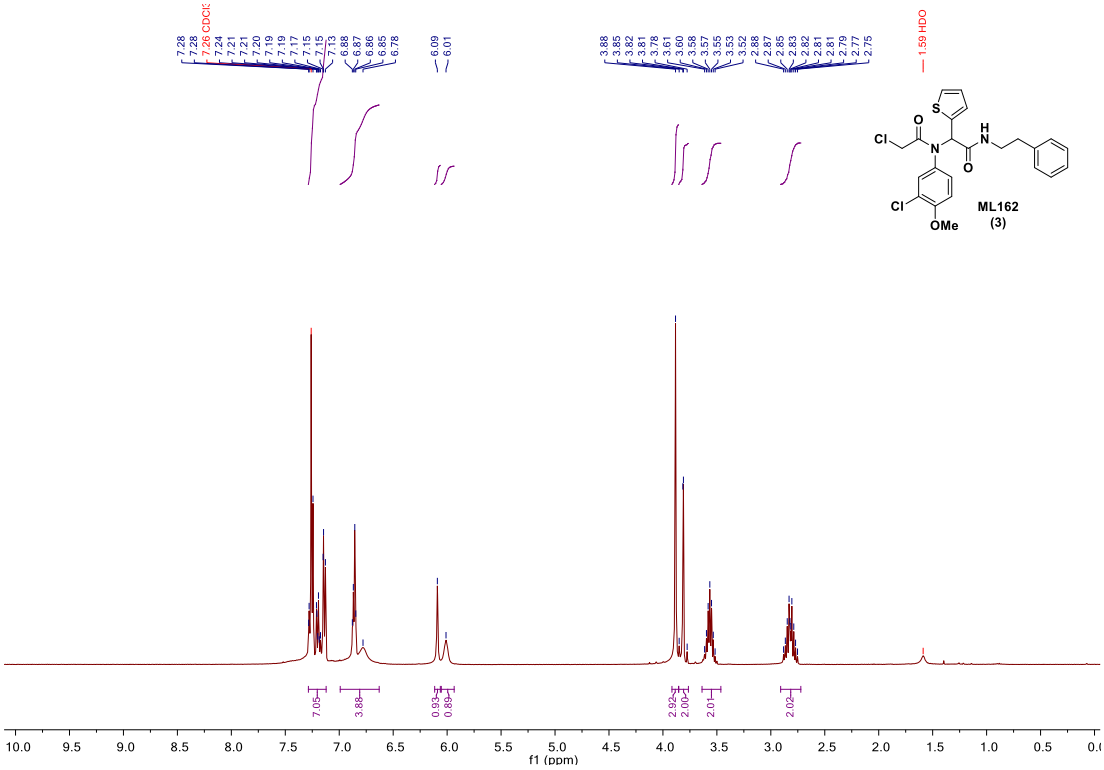
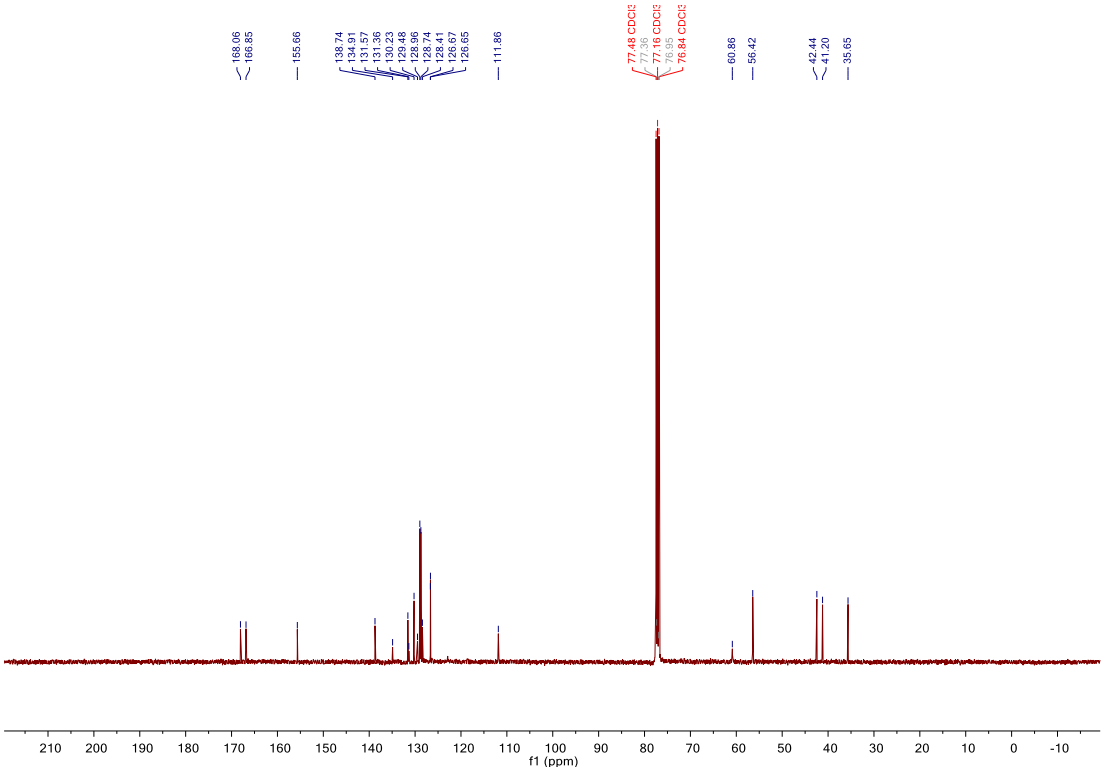
2.76 DMF
2.75 DMF
2.75 DMF
2.74
1.30
1.19
2.14
2.28
1.09
1.12
2.22
1.40
1.30
1.15
1.26
3.41
5.98
f1 (ppm)
RSL3
(2)

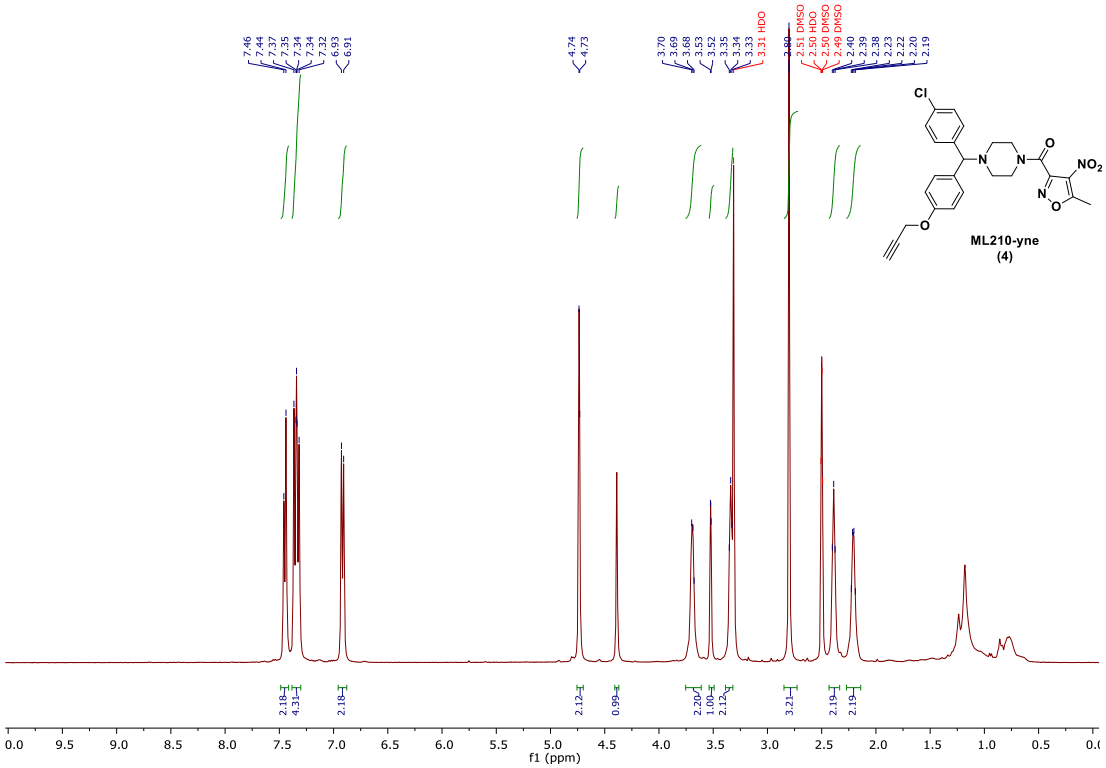

168.41
166.44
137.56
129.67
126.94
126.54
121.81
119.30
118.20
111.62
57.29
52.29
51.78
43.20
f1 (ppm)

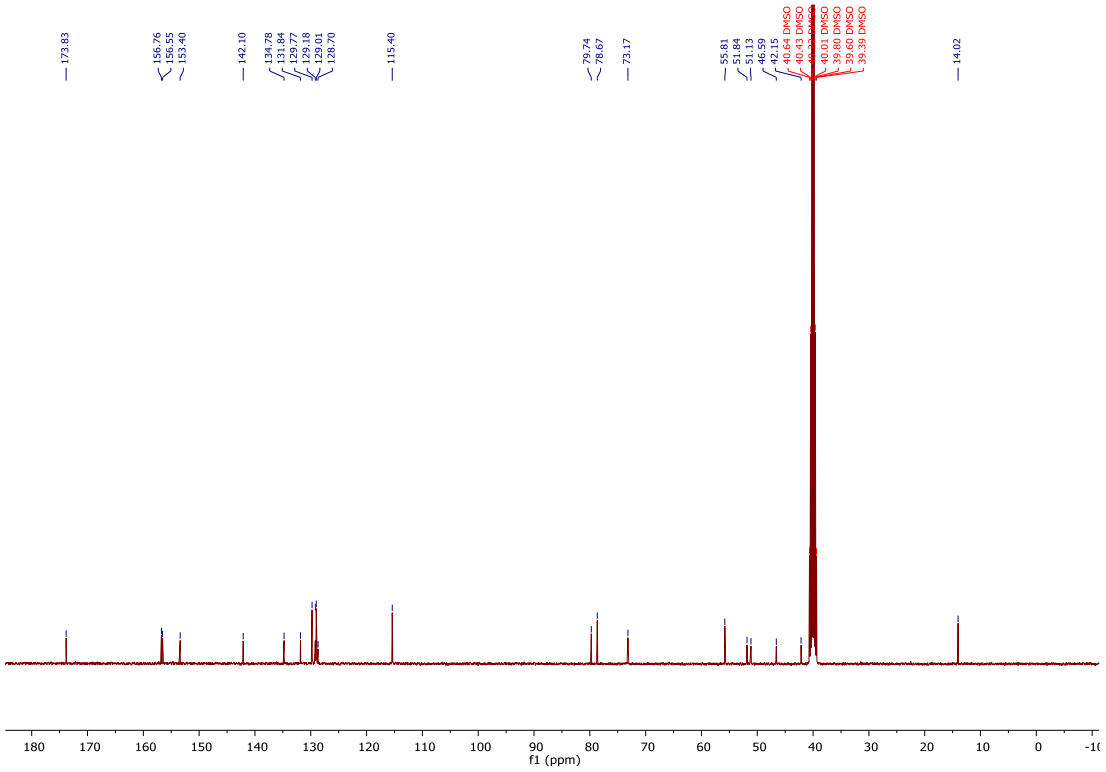
ML162 (3)


7.28
7.28
7.26 CDCl3
7.24
7.21
7.21
7.20
7.19
7.19
7.17
7.17
7.15
7.15
7.13
6.88
6.87
6.86
6.85
6.78
6.09
6.01
3.88
3.85
3.82
3.81
3.78
3.61
3.60
3.58
3.57
3.55
3.53
3.52
2.88
2.87
2.85
2.83
2.82
2.81
2.81
2.79
2.77
2.75
1.59 HDO
ML162
(3)
7.05
3.88
0.93
0.89
2.92
2.00
2.01
2.02
f1 (ppm)

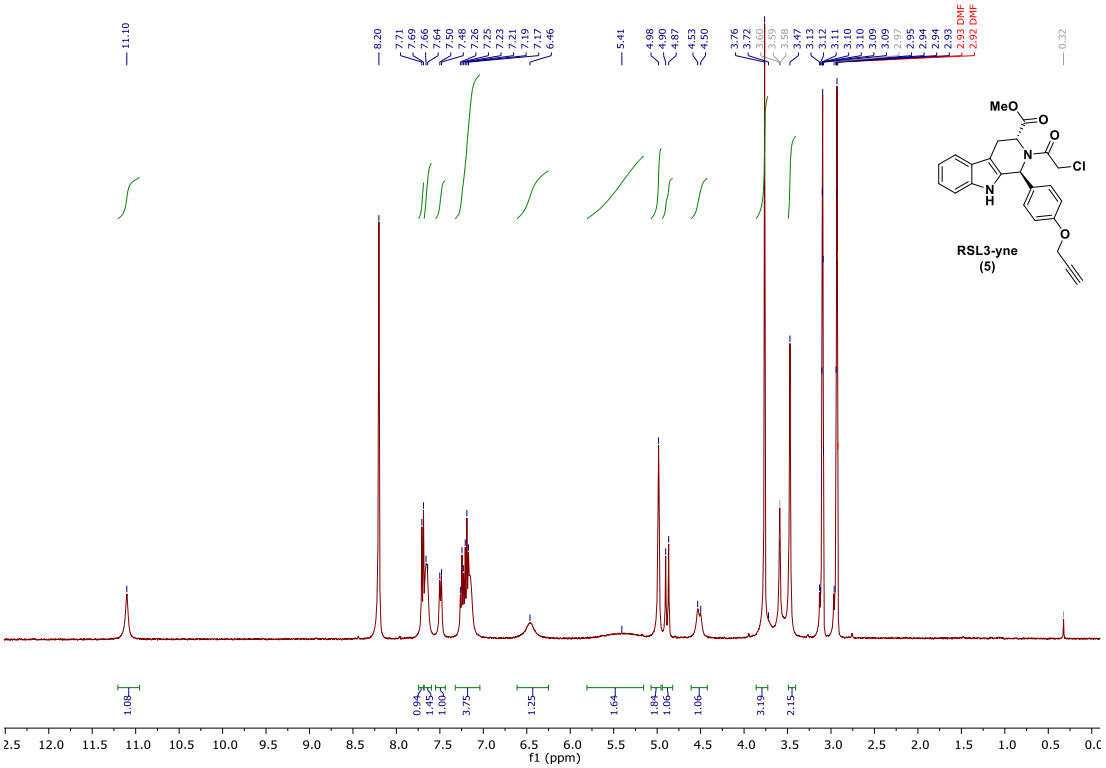

168.06
166.85
155.66
138.74
134.91
131.57
131.36
130.23
129.48
128.96
128.74
128.41
126.67
126.65
111.86
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
60.86
56.42
42.44
41.20
35.65
f1 (ppm)

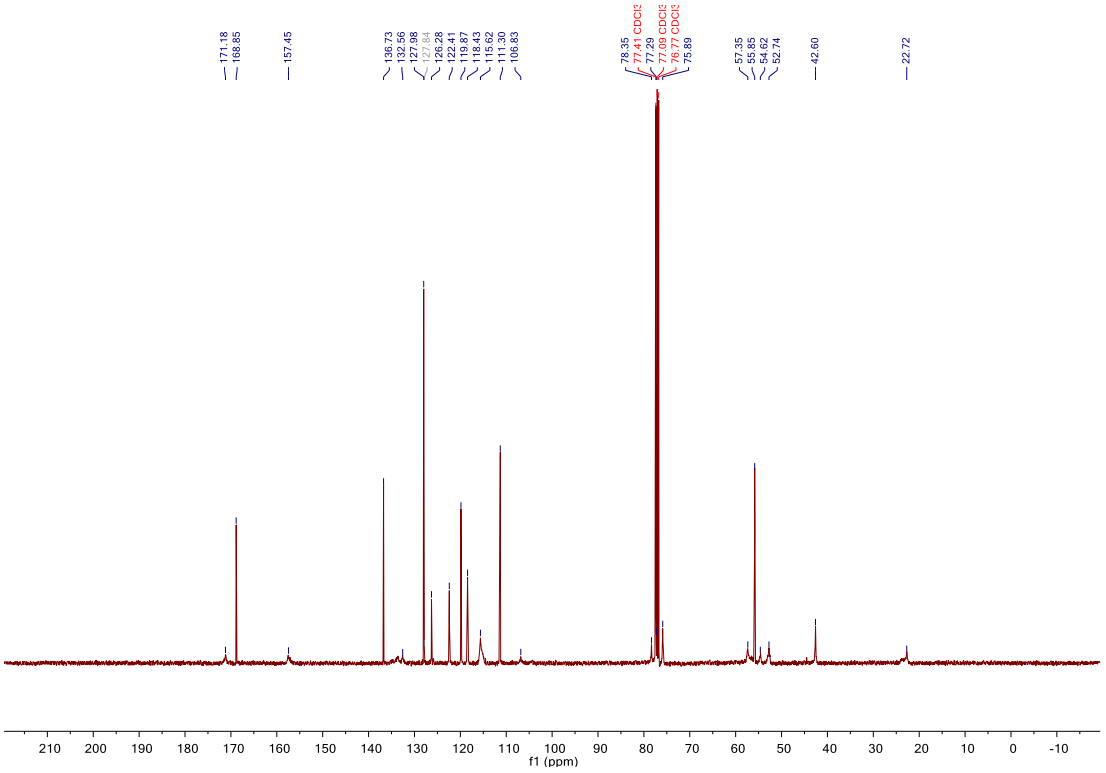
ML210-yne (4)


7.46
7.44
7.37
7.35
7.34
7.34
7.32
6.93
6.91
4.74
4.73
3.70
3.69
3.68
3.52
3.52
3.35
3.34
3.33
3.31 HDO
2.80
2.51 DMSO
2.50 HDO
2.50 DMSO
2.49 DMSO
2.40
2.39
2.38
2.23
2.22
2.20
2.19
Cl
O
N
N
O
NO2
N
O
O
ML210-yne
(4)
2.18
4.31
2.18
2.12
0.99
2.20
1.00
2.12
3.21
2.19
2.19
f1 (ppm)

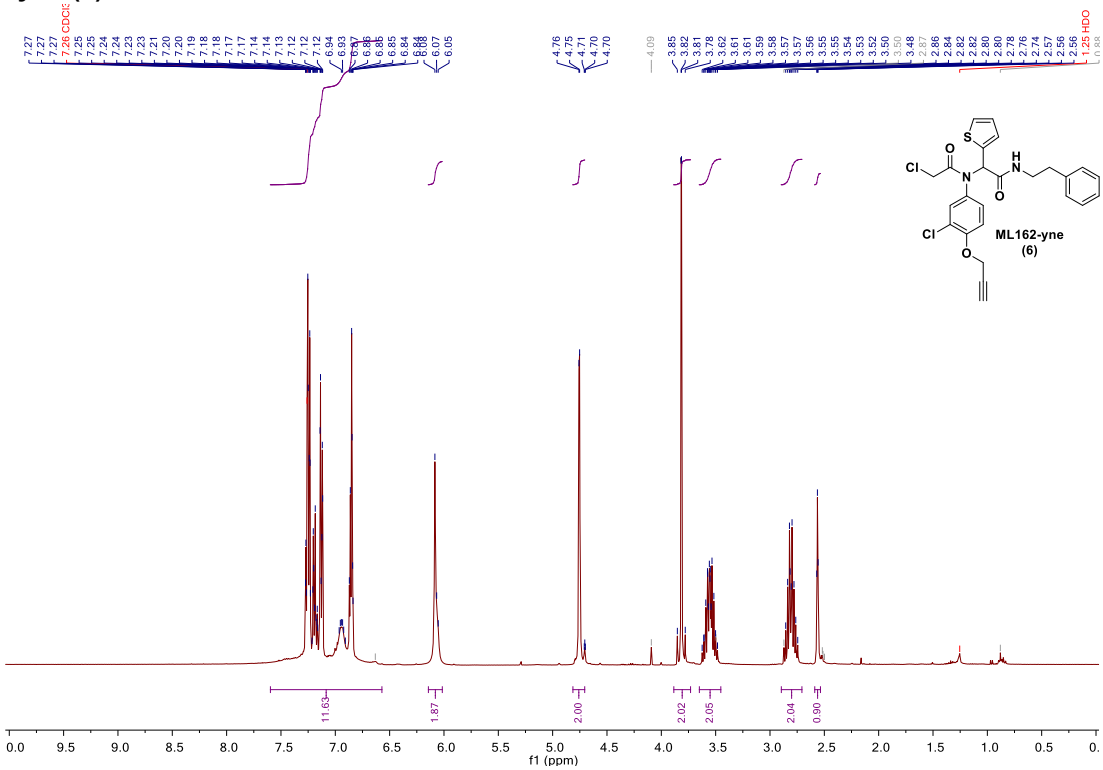

173.83
156.76
156.55
153.40
142.10
134.78
131.84
130.77
129.18
129.01
128.70
115.40
79.74
78.67
73.17
55.81
51.84
51.13
46.59
42.15
40.64 DMSO
40.43 DMSO
40.22 DMSO
40.01 DMSO
39.80 DMSO
39.60 DMSO
39.39 DMSO
14.02
f1 (ppm)

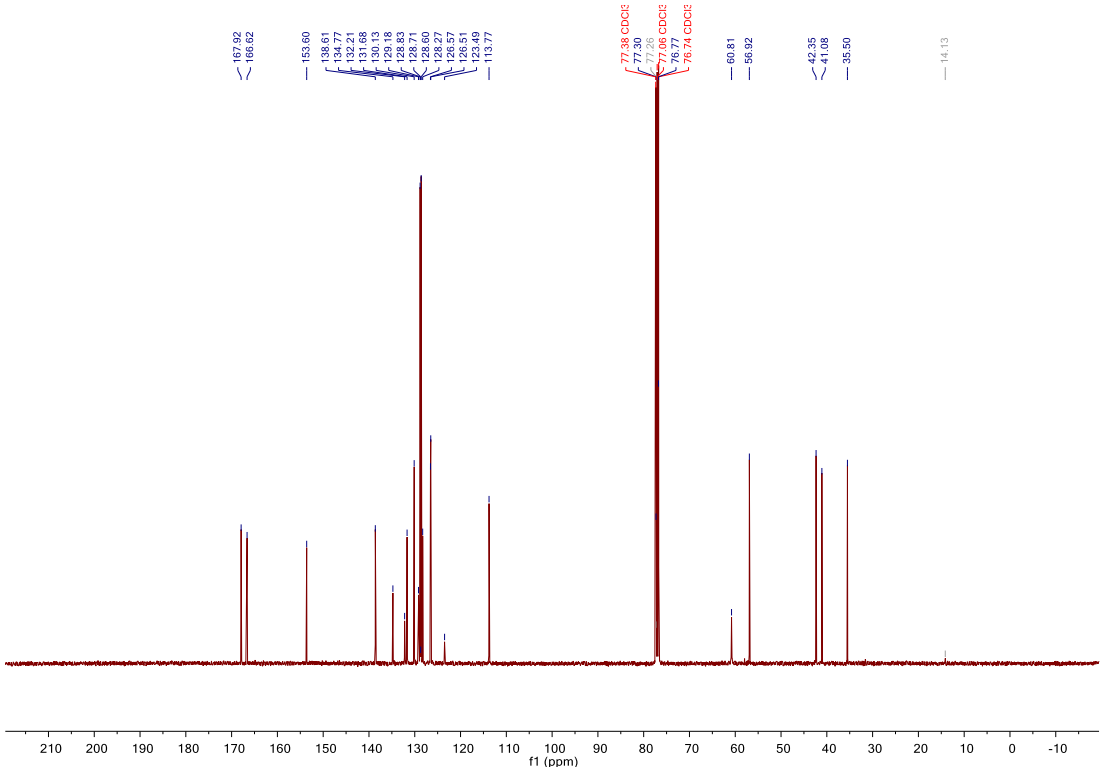
RSL3-yne (5)


11.10
8.20
7.71
7.69
7.66
7.64
7.50
7.48
7.26
7.25
7.23
7.21
7.19
7.17
6.46
5.41
4.98
4.90
4.87
4.53
4.50
3.76
3.72
3.60
3.59
3.58
3.47
3.13
3.12
3.11
3.10
3.10
3.09
3.09
2.97
2.95
2.94
2.94
2.93
2.93 DMF
2.92 DMF
0.32
MeO
O
O
N
Cl
N
H
O
RSL3-yne
(5)
1.08
0.94
1.45
1.00
3.75
1.25
1.64
1.84
1.06
1.06
3.19
2.15
f1 (ppm)

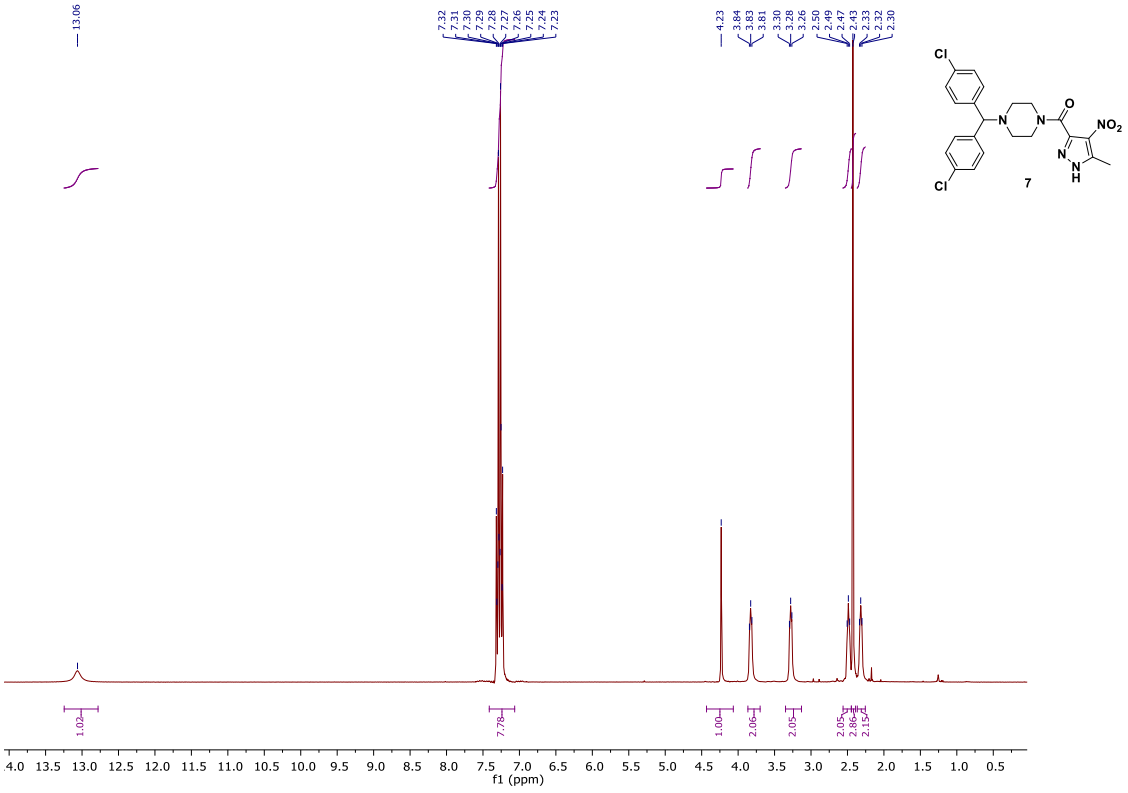

171.18
168.85
157.45
136.73
132.56
127.98
127.84
126.28
122.41
119.87
118.43
115.62
111.30
106.83
78.35
77.41 CDCl3
77.29
77.09 CDCl3
76.77 CDCl3
75.89
57.35
55.85
54.62
52.74
42.60
22.72
f1 (ppm)

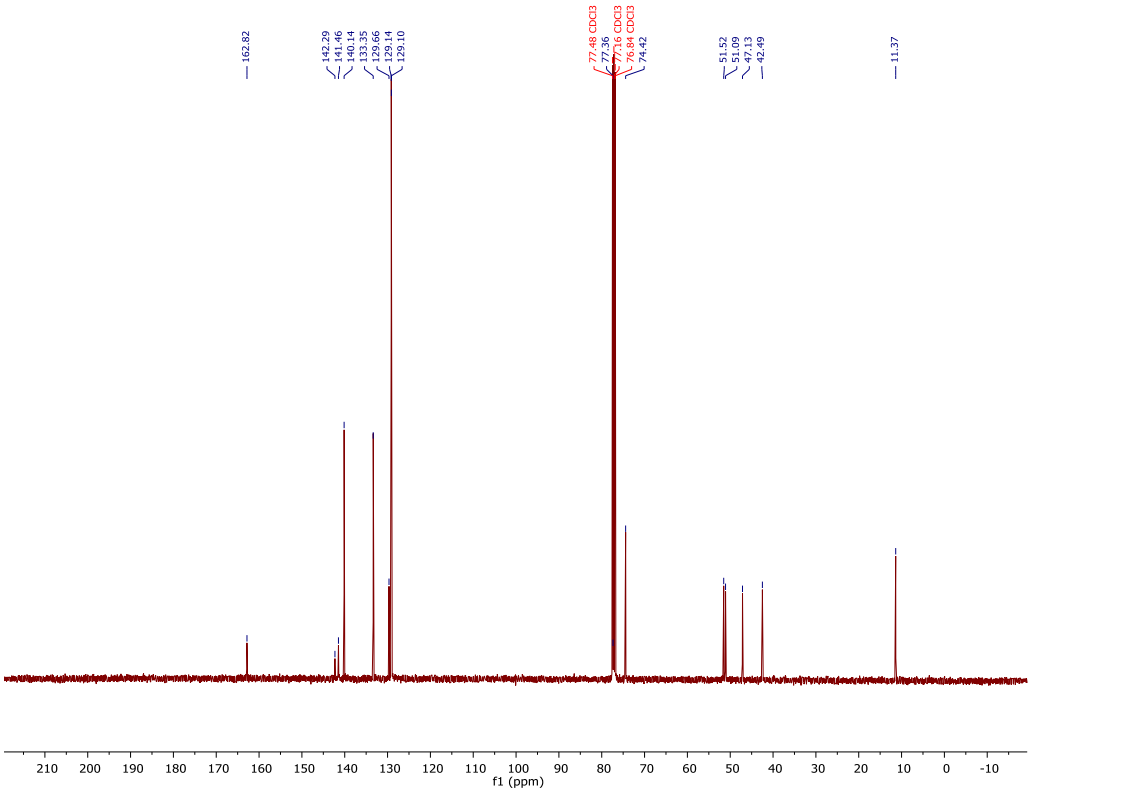
ML162-yne (6)



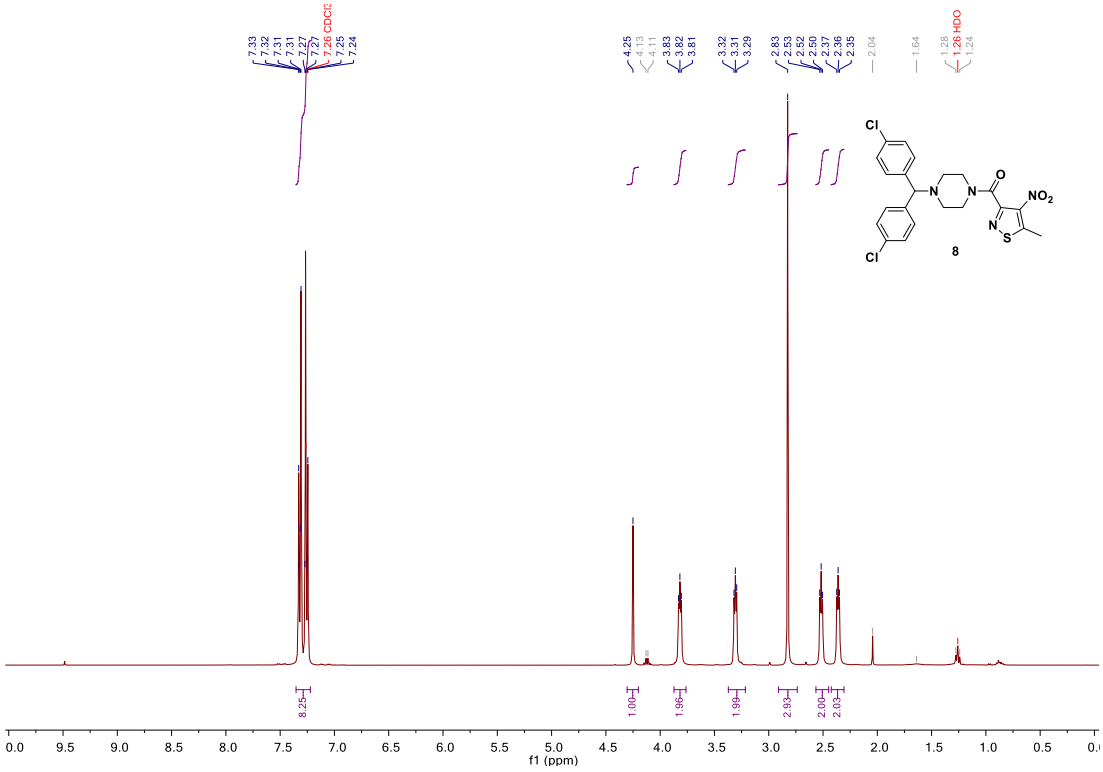


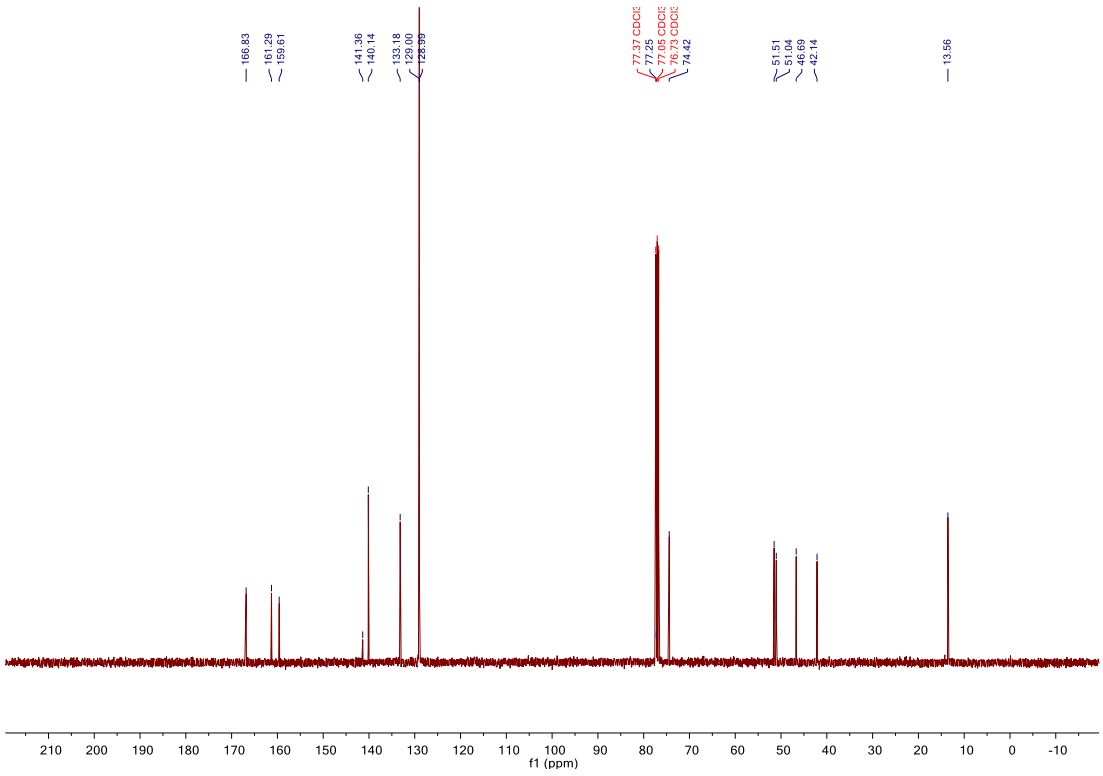
Compound 7


13.06
7.32
7.31
7.30
7.29
7.28
7.27
7.26
7.25
7.24
7.23
4.23
3.84
3.83
3.81
3.30
3.28
3.26
2.50
2.49
2.47
2.43
2.33
2.32
2.30
Cl
Cl
O
N
N
NO2
N
N
H
7
1.02
7.78
1.00
2.06
2.05
2.05
2.86
2.15
f1 (ppm)

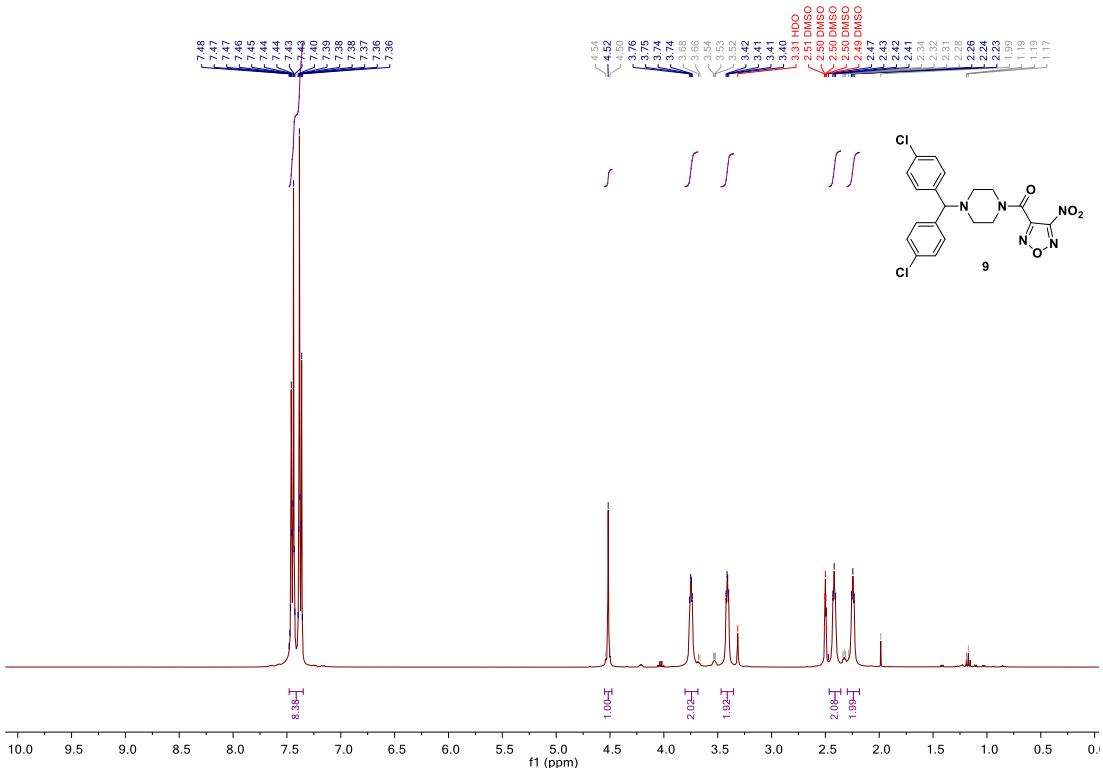

162.82
142.29
141.46
140.14
133.35
129.66
129.14
129.10
77.48 CDCl3
77.36
77.16 CDCl3
76.84 CDCl3
74.42
51.52
51.09
47.13
42.49
11.37
f1 (ppm)

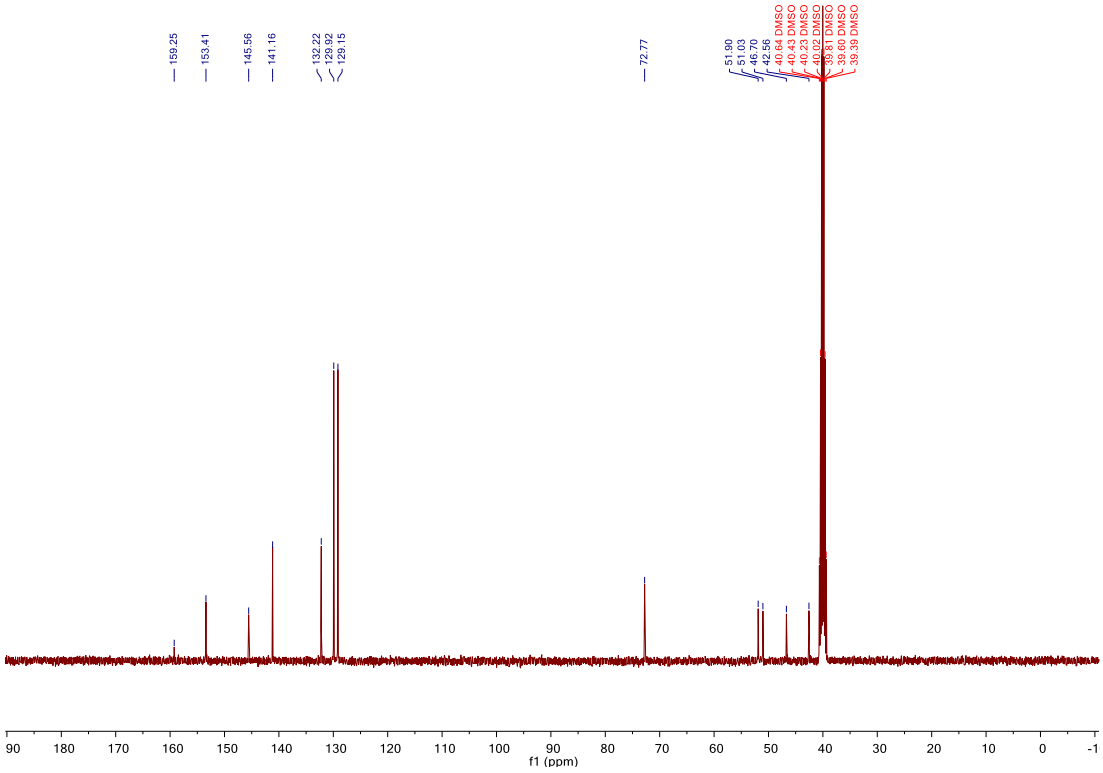
Compound 8


7.33
7.32
7.31
7.31
7.27
7.27
7.26 CDCl3
7.25
7.24
4.25
4.13
4.11
3.83
3.82
3.81
3.32
3.31
3.29
2.83
2.53
2.52
2.50
2.37
2.36
2.35
2.04
1.64
1.28
1.26 HDO
1.24
Cl
Cl
N
N
O
NO2
N
S
8
8.25
1.00
1.96
1.99
2.93
2.00
2.03
f1 (ppm)

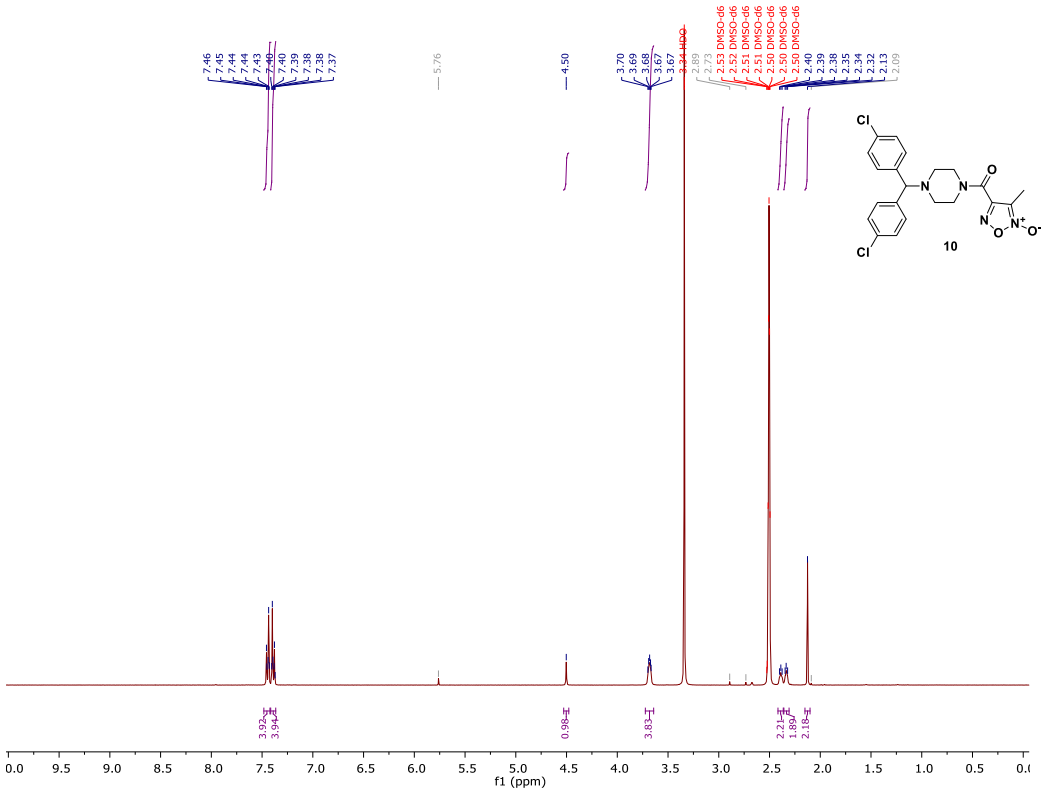

166.83
161.29
159.61
141.36
140.14
133.18
129.00
128.99
77.37 CDCl3
77.25
77.05 CDCl3
76.73 CDCl3
74.42
51.51
51.04
46.69
42.14
13.56
f1 (ppm)

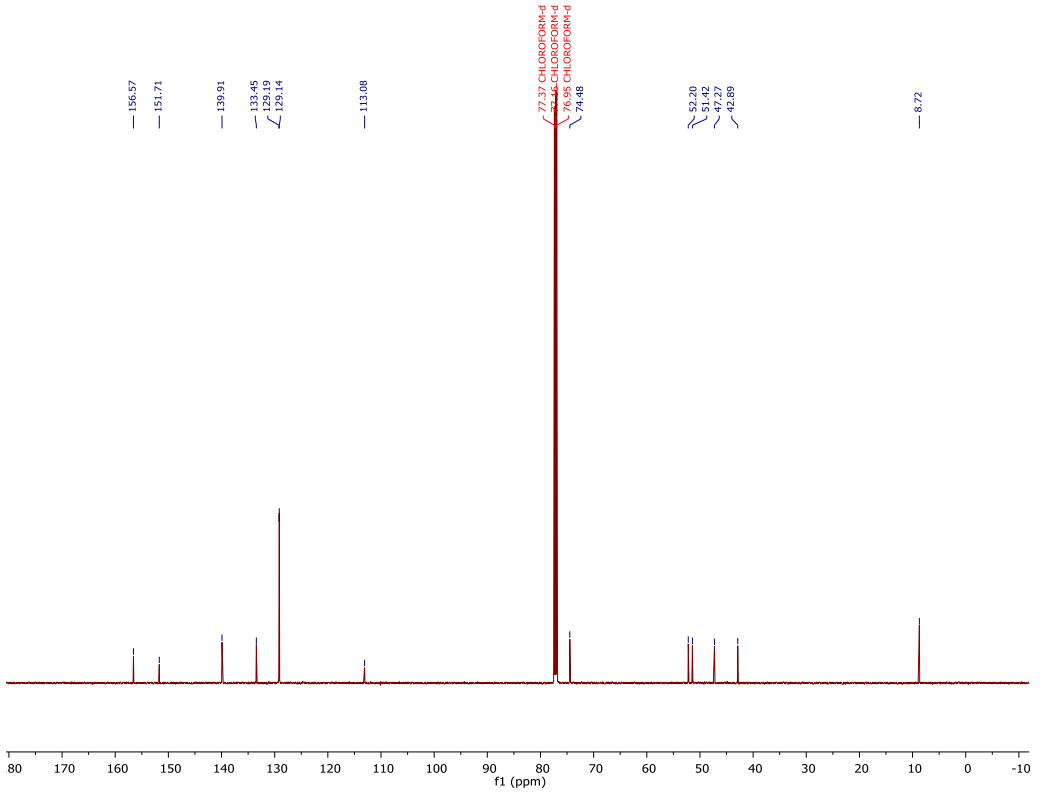
Compound 9


7.48
7.47
7.47
7.47
7.46
7.45
7.44
7.44
7.43
7.43
7.40
7.39
7.38
7.38
7.37
7.36
7.36
4.54
4.52
4.50
3.76
3.75
3.74
3.74
3.68
3.66
3.54
3.53
3.52
3.42
3.41
3.41
3.40
3.31 HDO
2.51 DMSO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.47
2.43
2.42
2.41
2.34
2.32
2.31
2.28
2.26
2.24
2.23
1.99
1.19
1.18
1.17
Cl
Cl
O
NO2
N
N
N
O
N
9
8.38
1.00
2.02
1.92
2.08
1.99
f1 (ppm)

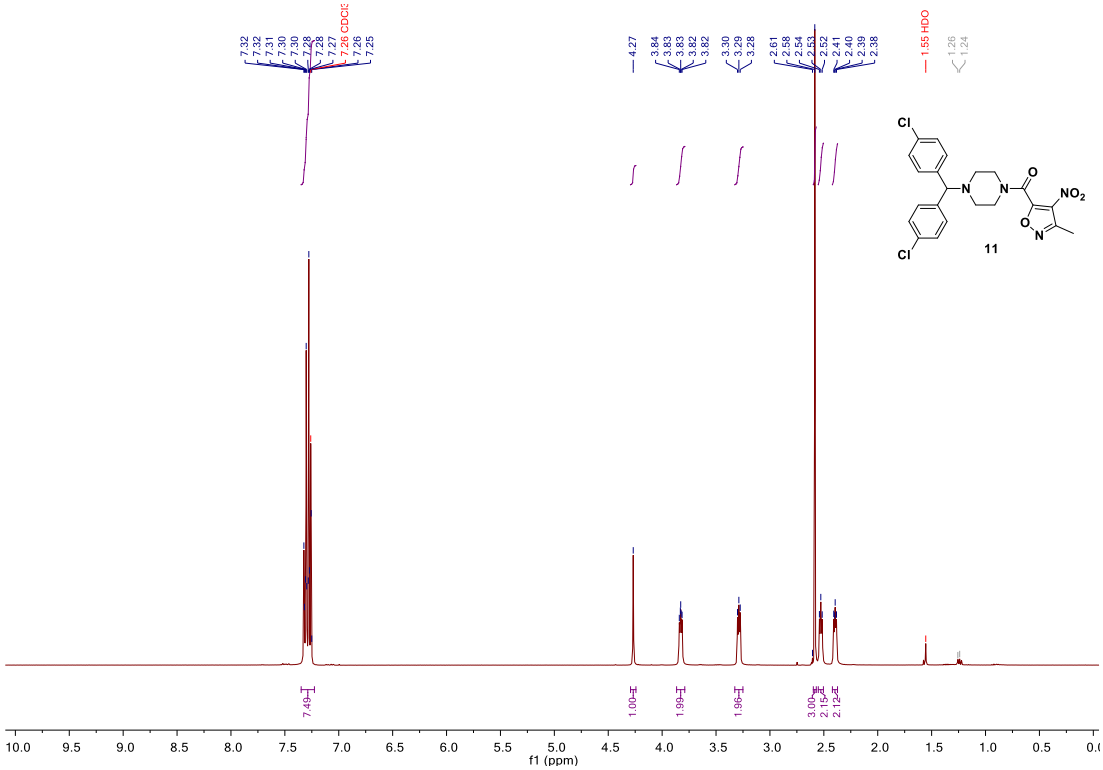

159.25
153.41
145.56
141.16
132.22
129.92
129.15
72.77
51.90
51.03
49.70
42.56
40.64 DMSO
40.43 DMSO
40.23 DMSO
40.02 DMSO
39.81 DMSO
39.60 DMSO
39.39 DMSO
f1 (ppm)

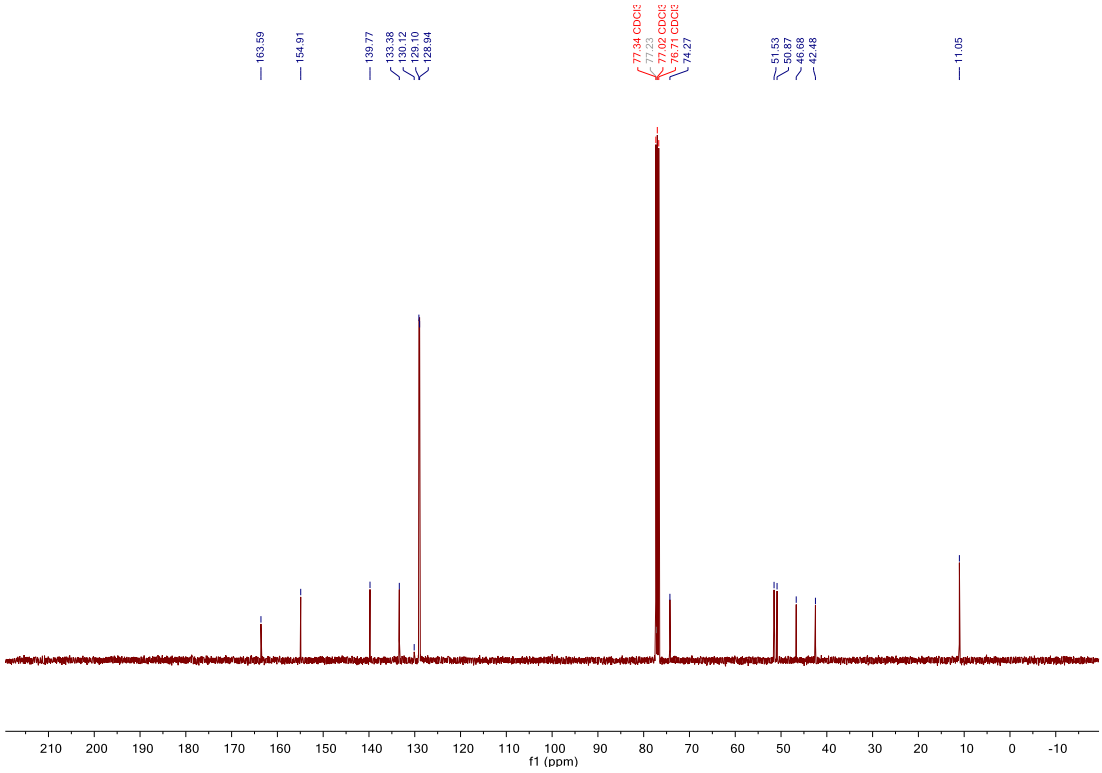
Compound 10


7.46
7.45
7.44
7.44
7.43
7.40
7.40
7.39
7.38
7.38
7.37
5.76
4.50
3.70
3.69
3.68
3.67
3.67
3.21 HDO
2.89
2.73
2.53 DMSO-d6
2.51 DMSO-d6
2.51 DMSO-d6
2.51 DMSO-d6
2.50 DMSO-d6
2.50 DMSO-d6
2.50 DMSO-d6
2.40
2.39
2.38
2.35
2.34
2.32
2.13
2.09
Cl
Cl
O
N
N
N
O
O
10
3.92
3.94
0.98
3.83
2.21
1.89
2.18
f1 (ppm)

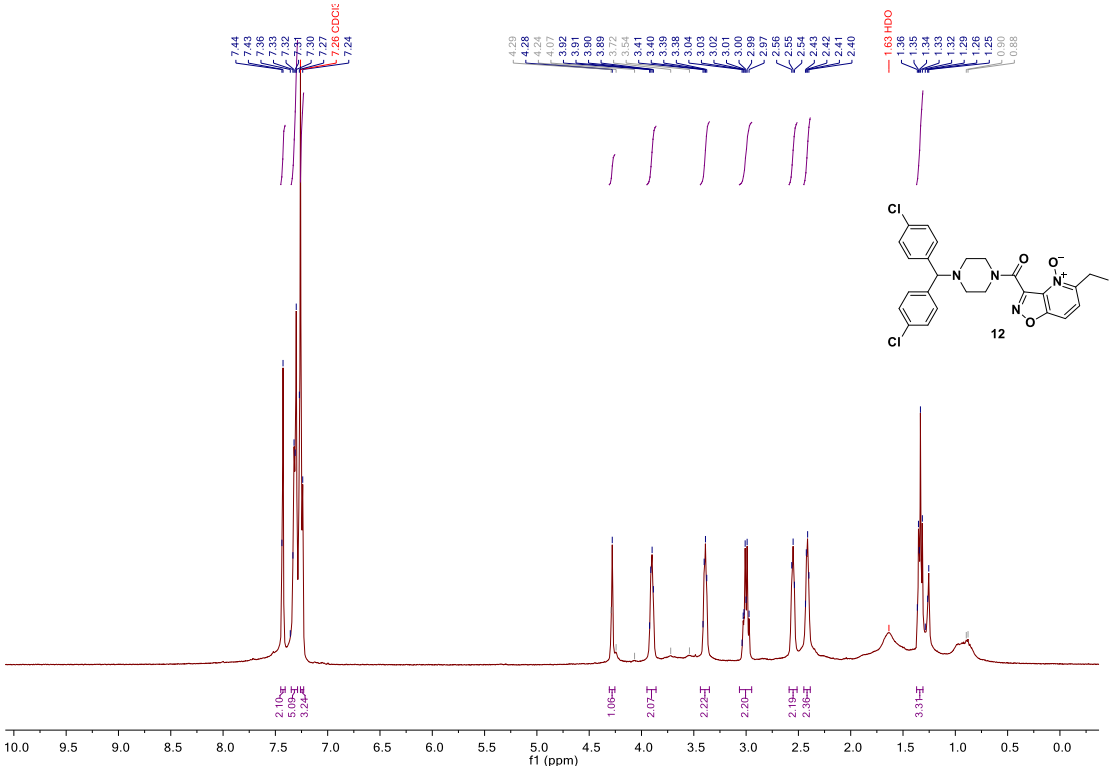

156.57
151.71
139.91
133.45
129.19
129.14
113.08
77.37 CHLOROFORM-d
77.16 CHLOROFORM-d
76.95 CHLOROFORM-d
74.48
52.20
51.42
47.27
42.89
8.72
f1 (ppm)

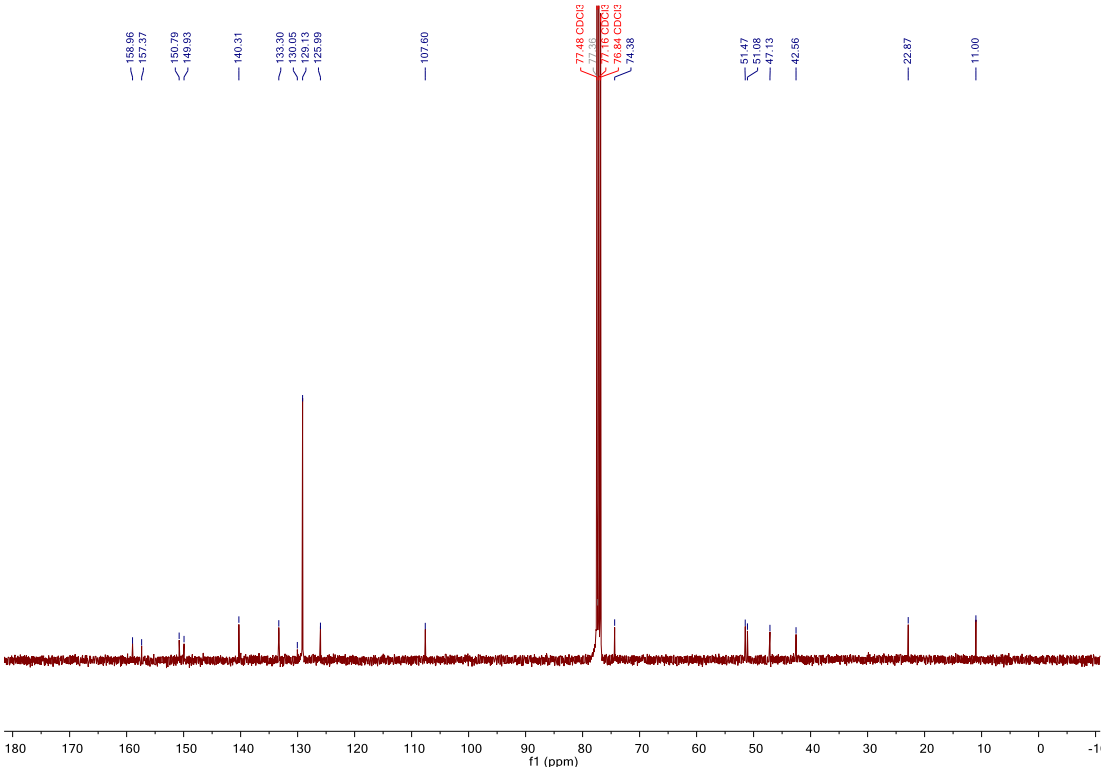
Compound 11


7.32
7.32
7.31
7.30
7.30
7.28
7.28
7.27
7.26 CDCl3
7.26
7.25
4.27
3.84
3.83
3.83
3.82
3.82
3.30
3.29
3.28
2.61
2.58
2.54
2.53
2.52
2.41
2.40
2.39
2.38
1.55 HDO
1.26
1.24
Cl
N
N
O
NO2
O
N
Cl
11
7.49
1.00
1.99
1.96
3.09
2.15
2.12
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

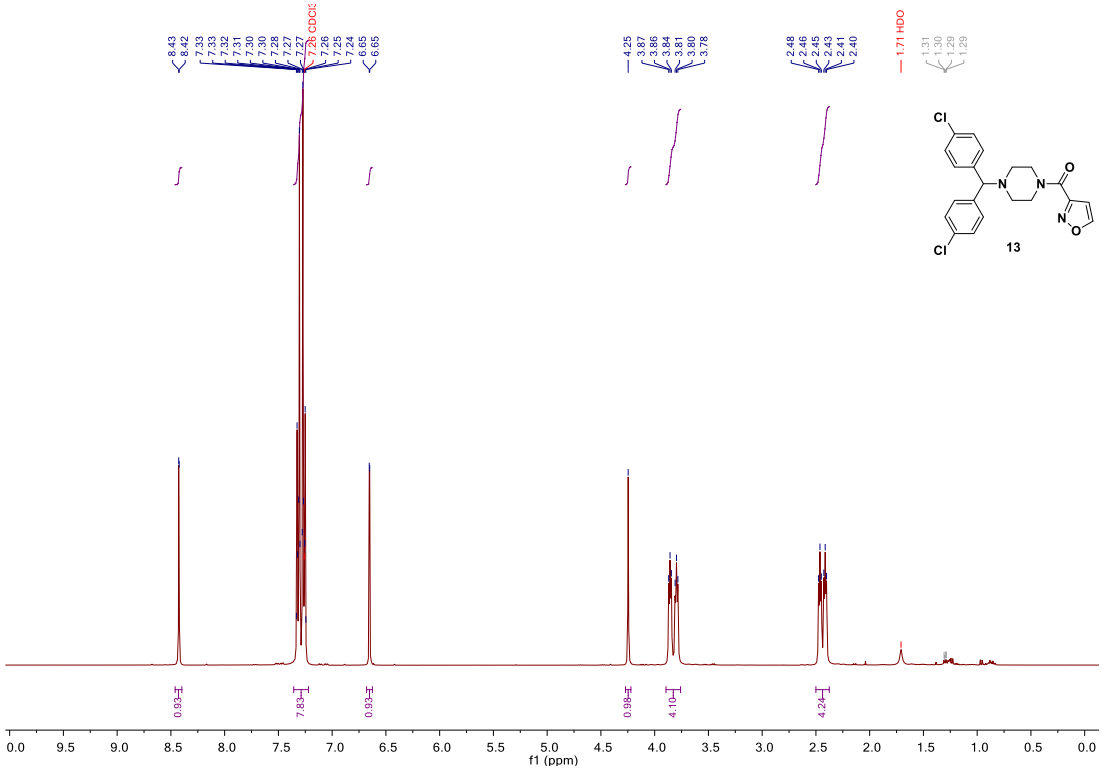

163.59
154.91
139.77
133.38
130.12
129.10
128.94
77.34 CDCl3
77.23
77.02 CDCl3
76.71 CDCl3
74.27
51.53
50.87
46.68
42.48
11.05
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

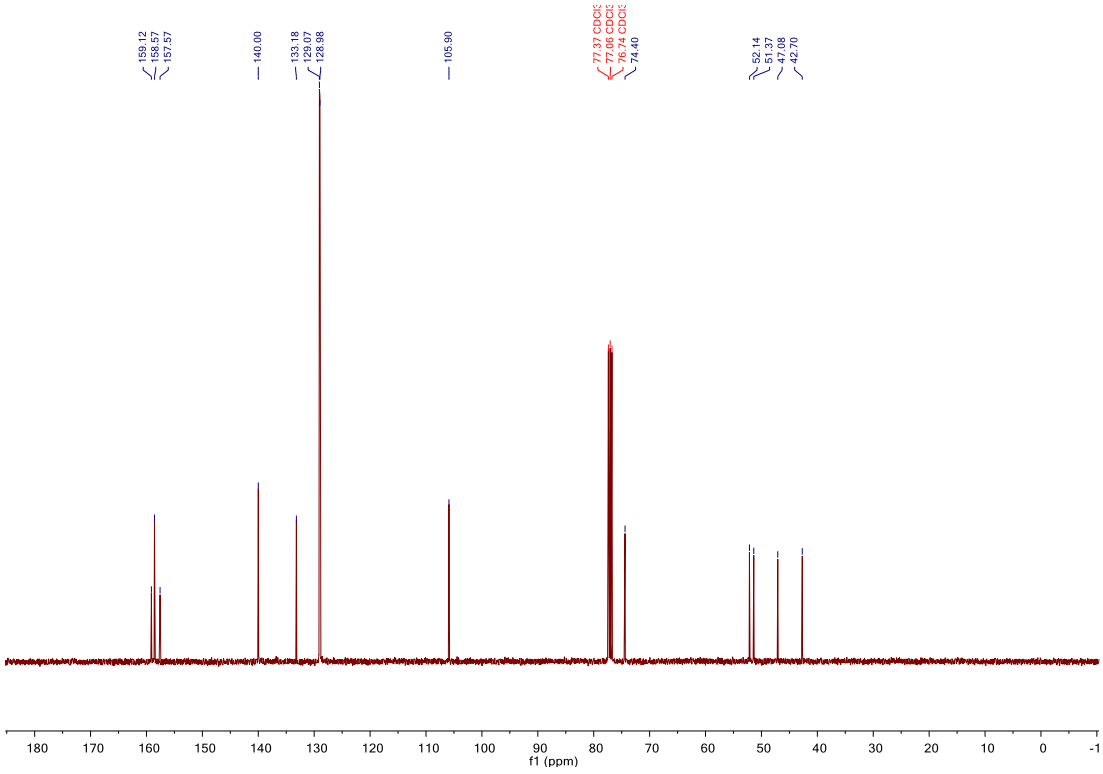
Compound 12


7.44
7.43
7.36
7.33
7.32
7.31
7.30
7.27
7.26 CDCl3
7.24
4.29
4.28
4.24
4.07
3.92
3.91
3.90
3.89
3.72
3.54
3.41
3.40
3.39
3.38
3.04
3.03
3.02
3.01
3.00
2.99
2.97
2.56
2.55
2.54
2.43
2.42
2.41
2.40
1.63 HDO
1.36
1.35
1.34
1.33
1.32
1.28
1.26
1.25
0.90
0.88
Cl
Cl
O
O
N
N
N
N
O
12
2.10
5.09
3.24
1.06
2.07
2.22
2.20
2.19
2.36
3.31
f1 (ppm)

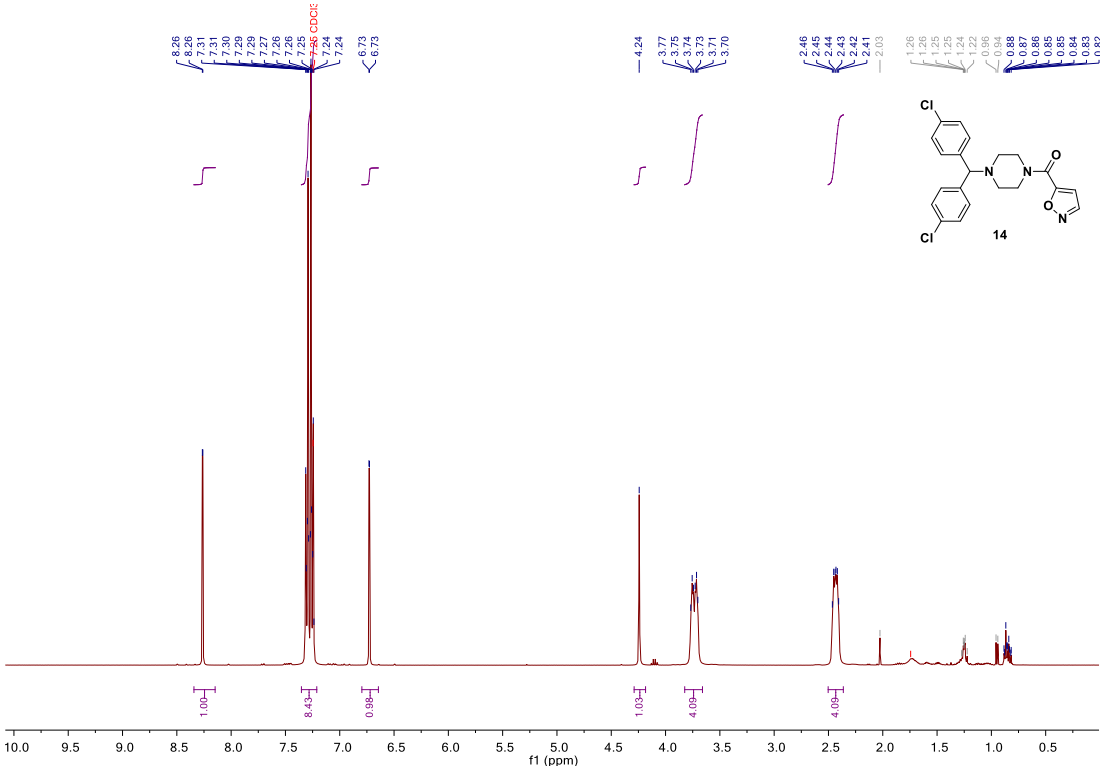

158.96
157.37
150.79
149.93
140.31
133.30
130.95
129.13
125.99
107.60
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
74.38
51.47
51.08
47.13
42.56
22.87
11.00
f1 (ppm)

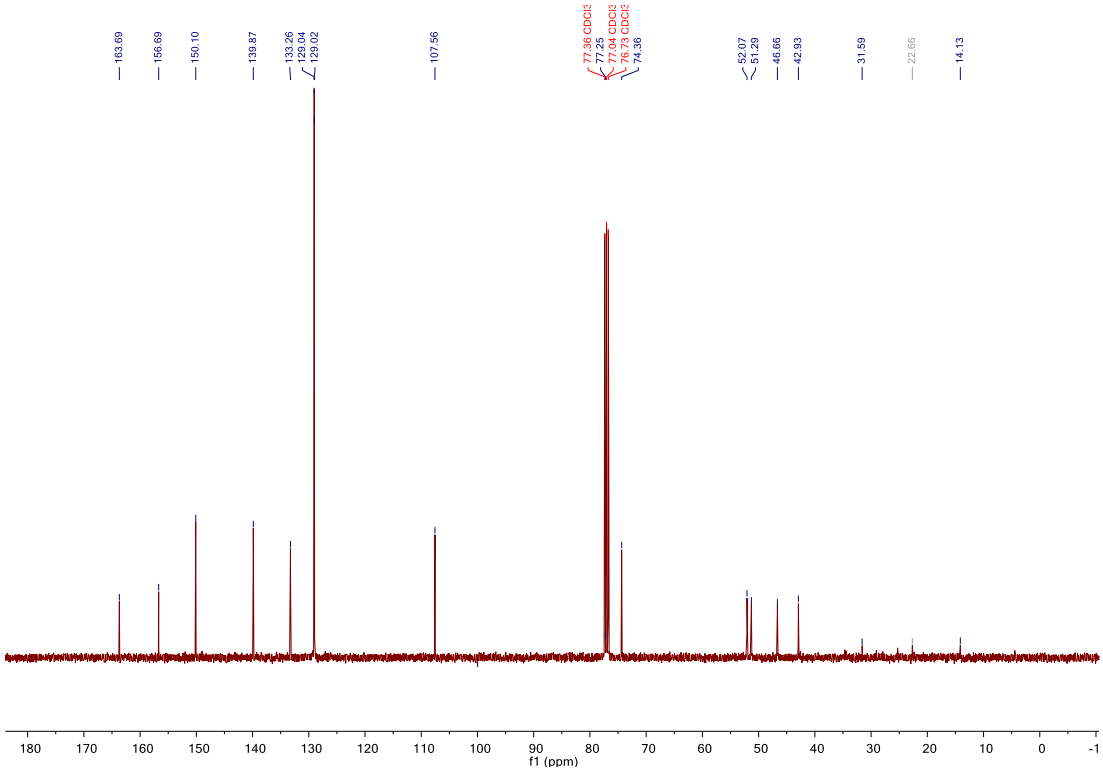
Compound 13


8.43
8.42
7.33
7.33
7.32
7.31
7.31
7.30
7.28
7.27
7.27
7.26 CDCl3
7.26
7.25
7.24
6.65
6.65
4.25
3.87
3.86
3.84
3.81
3.80
3.78
2.48
2.46
2.45
2.43
2.41
2.40
1.71 HDO
1.31
1.30
1.29
1.29
Cl
Cl
N
N
O
N
O
13
0.93
7.83
0.93
0.98
4.10
4.24
f1 (ppm)

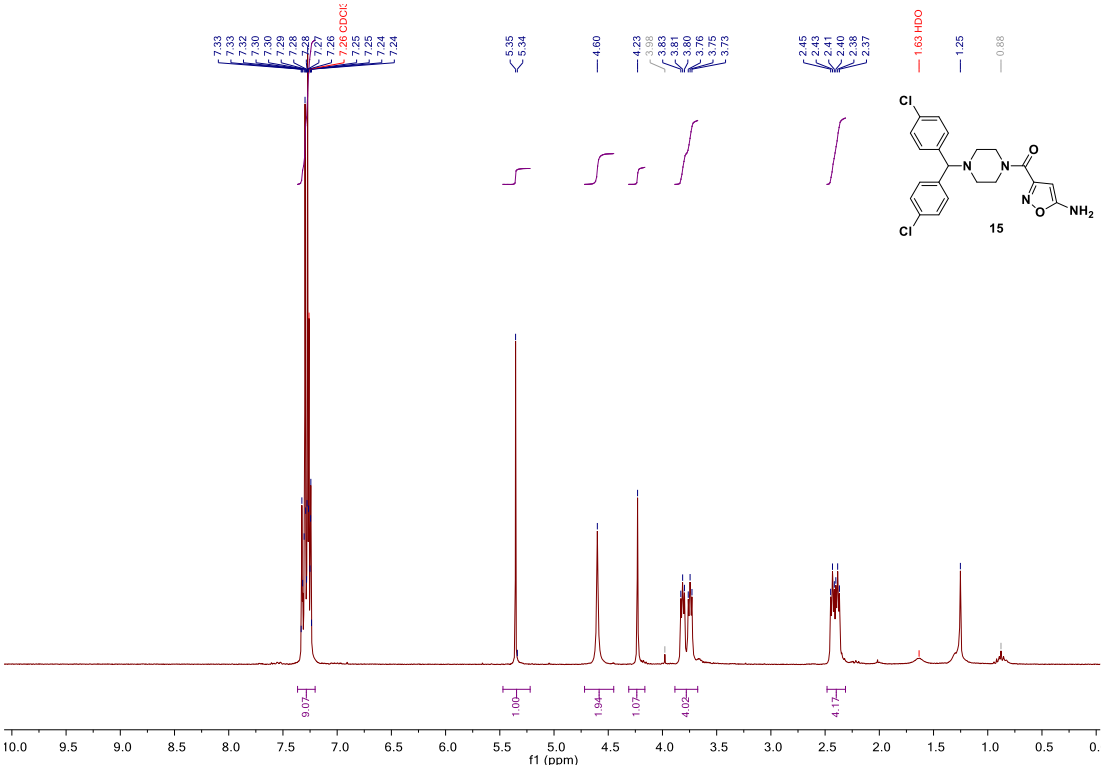

159.12
158.57
157.57
140.00
133.18
129.07
128.98
105.90
77.37 CDCl3
77.06 CDCl3
76.74 CDCl3
74.40
52.14
51.37
47.08
42.70
f1 (ppm)

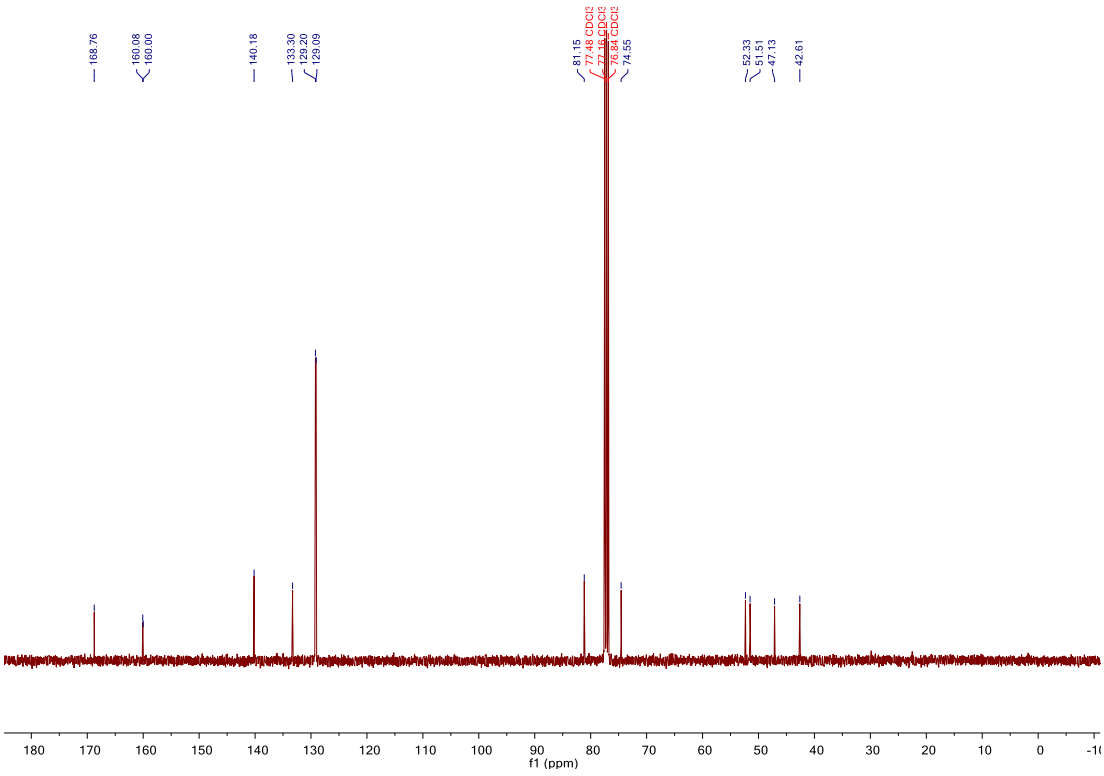
Compound 14


8.26
8.26
7.31
7.30
7.29
7.29
7.27
7.26
7.26
7.25
7.24
7.24
6.73
6.73
4.24
3.77
3.75
3.74
3.73
3.71
3.70
2.46
2.45
2.44
2.43
2.42
2.42
2.41
2.03
1.26
1.25
1.25
1.25
1.24
1.22
0.96
0.94
0.88
0.87
0.86
0.86
0.85
0.85
0.84
0.83
Cl
Cl
N
N
O
O
N
14
1.00
8.43
0.98
1.03
4.09
4.09
f1 (ppm)

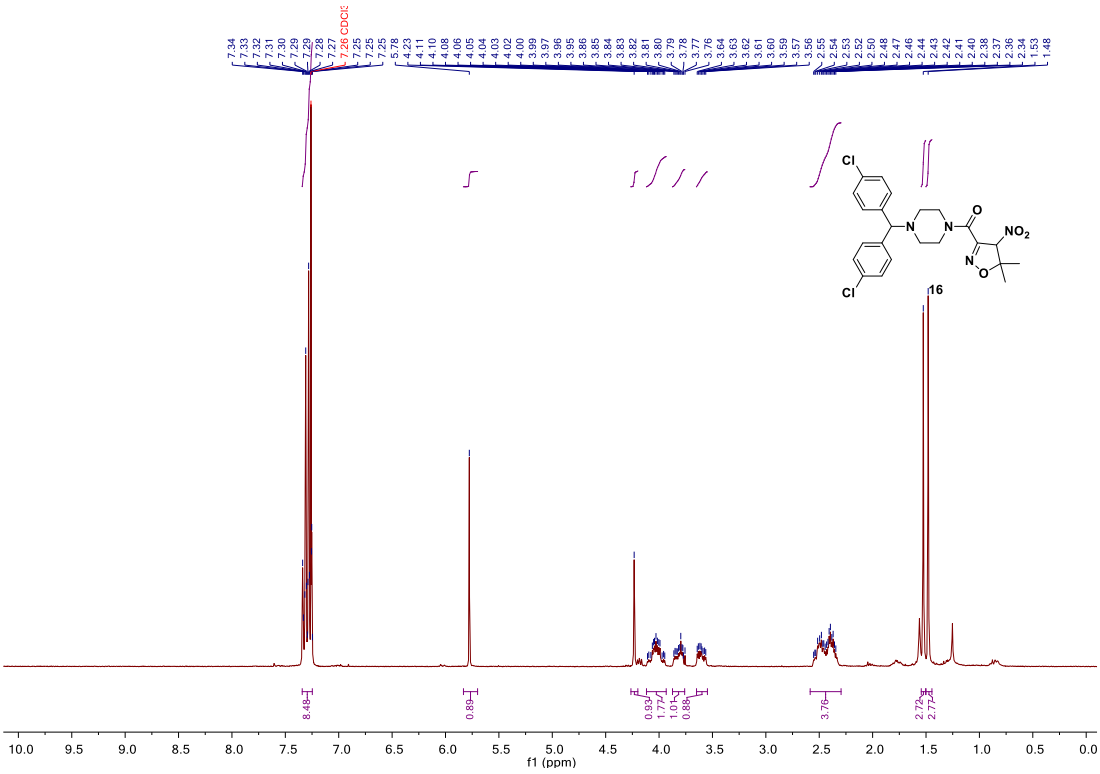

163.69
156.69
150.10
139.87
133.26
129.04
129.02
107.56
77.36 CDCl3
77.25
77.04 CDCl3
76.73 CDCl3
74.36
52.07
51.29
46.66
42.93
31.59
22.66
14.13
f1 (ppm)

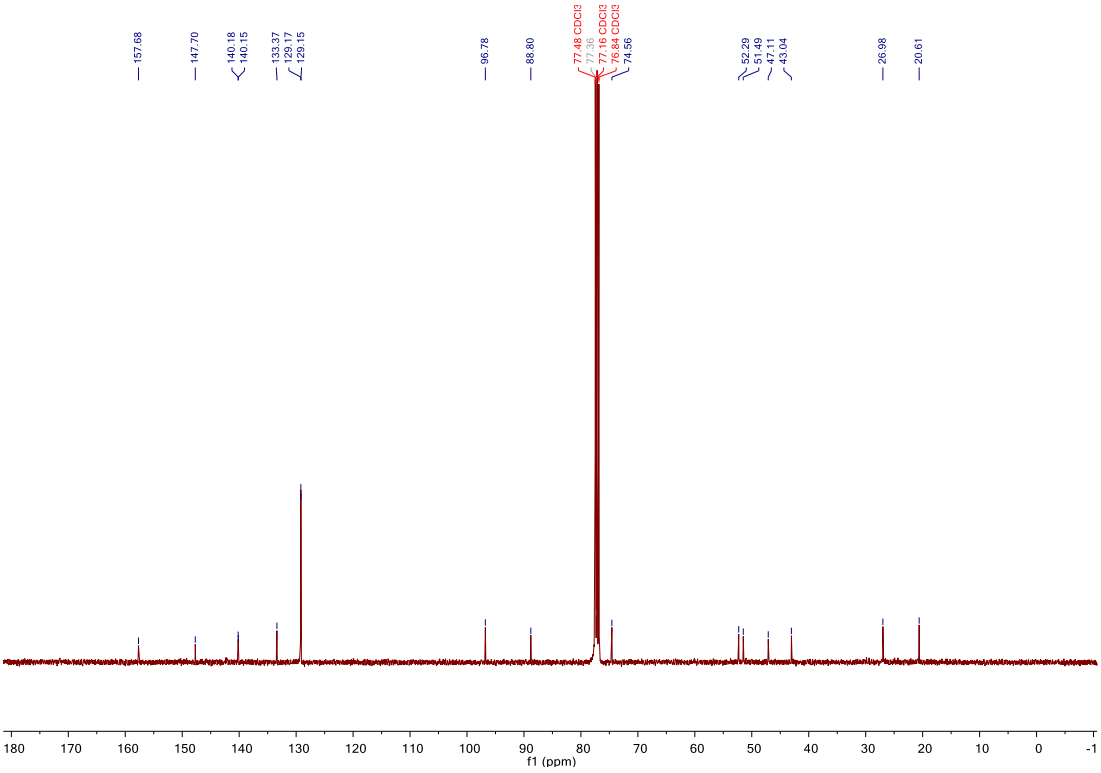
Compound 15


7.33
7.33
7.32
7.30
7.30
7.29
7.28
7.27
7.26 CDCl3
7.26
7.25
7.25
7.24
7.24
5.35
5.34
4.60
4.23
3.98
3.83
3.81
3.80
3.76
3.75
3.73
2.45
2.43
2.41
2.40
2.38
2.37
1.63 H2O
1.25
0.88
9.07
1.00
1.94
1.07
4.02
4.17
f1 (ppm)
Cl
Cl
O
N
N
N
O
NH2
15

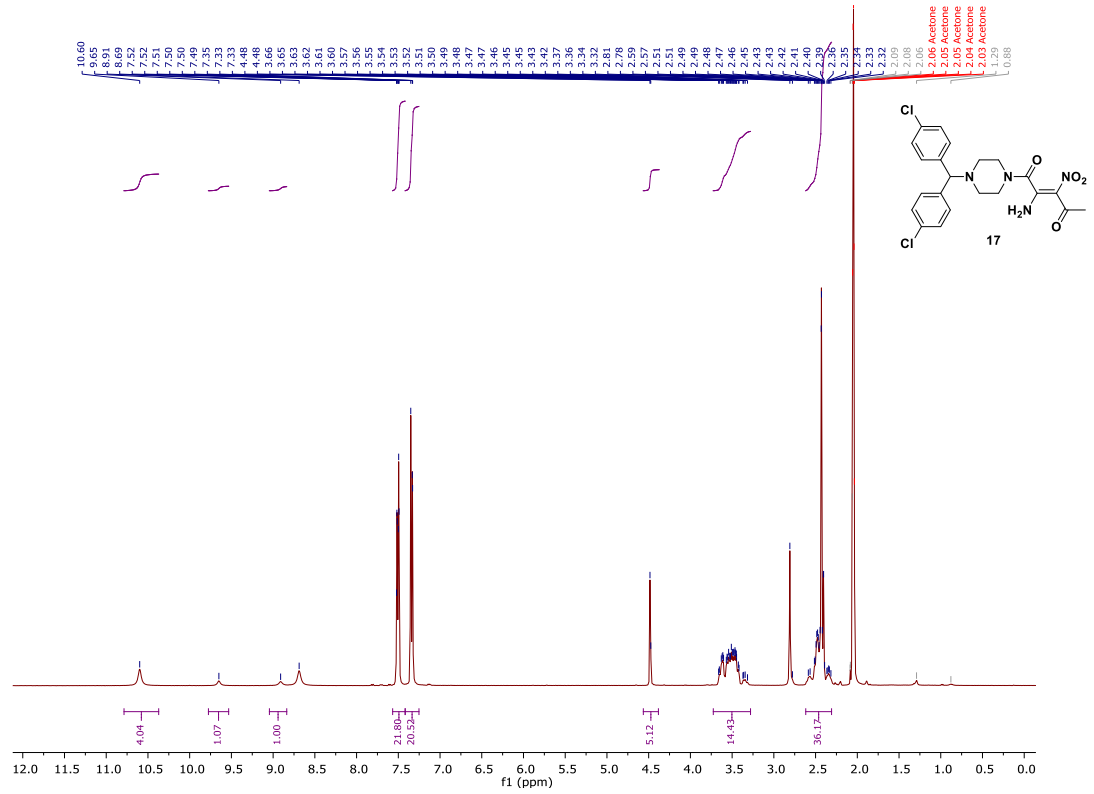

168.76
160.08
160.00
140.18
133.30
129.20
129.09
81.15
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
74.55
52.33
51.51
47.13
42.61
f1 (ppm)

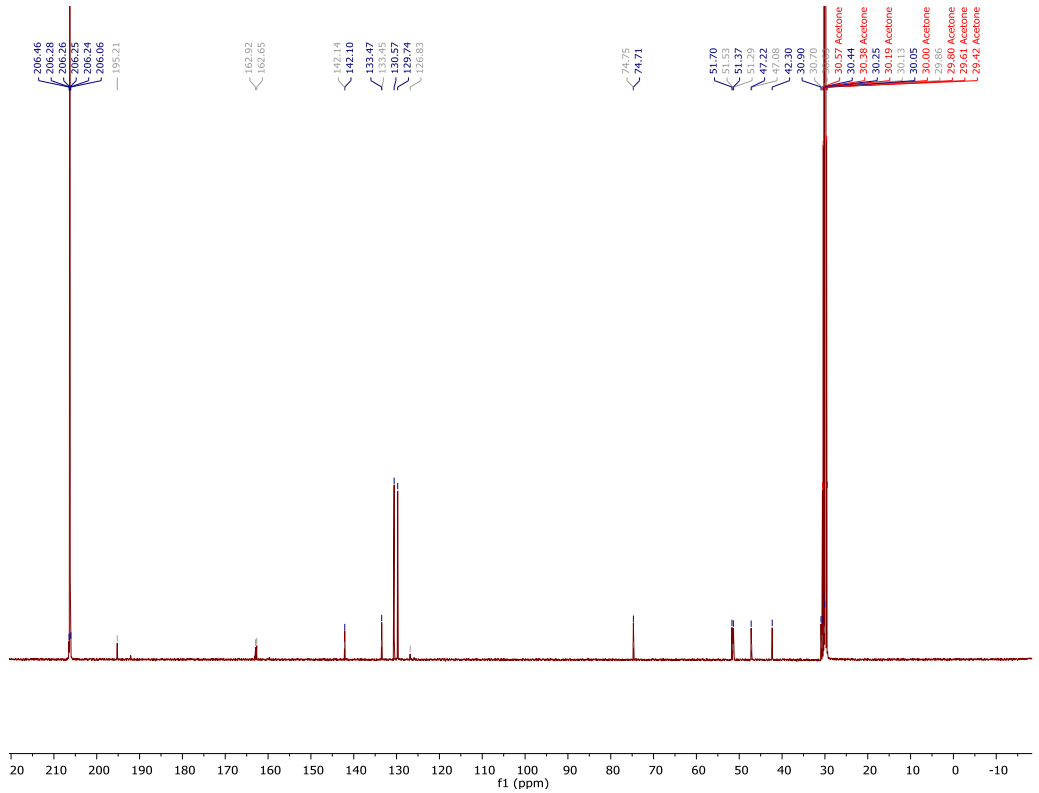
Compound 16


7.34 7.33 7.32 7.31 7.30 7.29 7.29 7.28 7.27 7.26 CDCl3 7.25 7.25 7.25
5.78
4.23 4.11 4.10 4.08 4.06 4.05 4.04 4.03 4.02 4.00 3.99 3.97 3.96 3.95 3.86 3.85 3.84 3.83 3.82 3.81 3.80 3.79 3.78 3.77 3.76 3.64 3.63 3.62 3.61 3.60 3.59 3.57 3.56 3.55
2.55 2.54 2.53 2.52 2.50 2.48 2.47 2.46 2.44 2.43 2.42 2.41 2.40 2.38 2.37 2.36 2.34 1.53 1.48
Cl
N
N
O
NO2
N
O
Cl
16
8.48
0.89
0.93
1.77
1.01
0.88
3.76
2.72
2.73
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

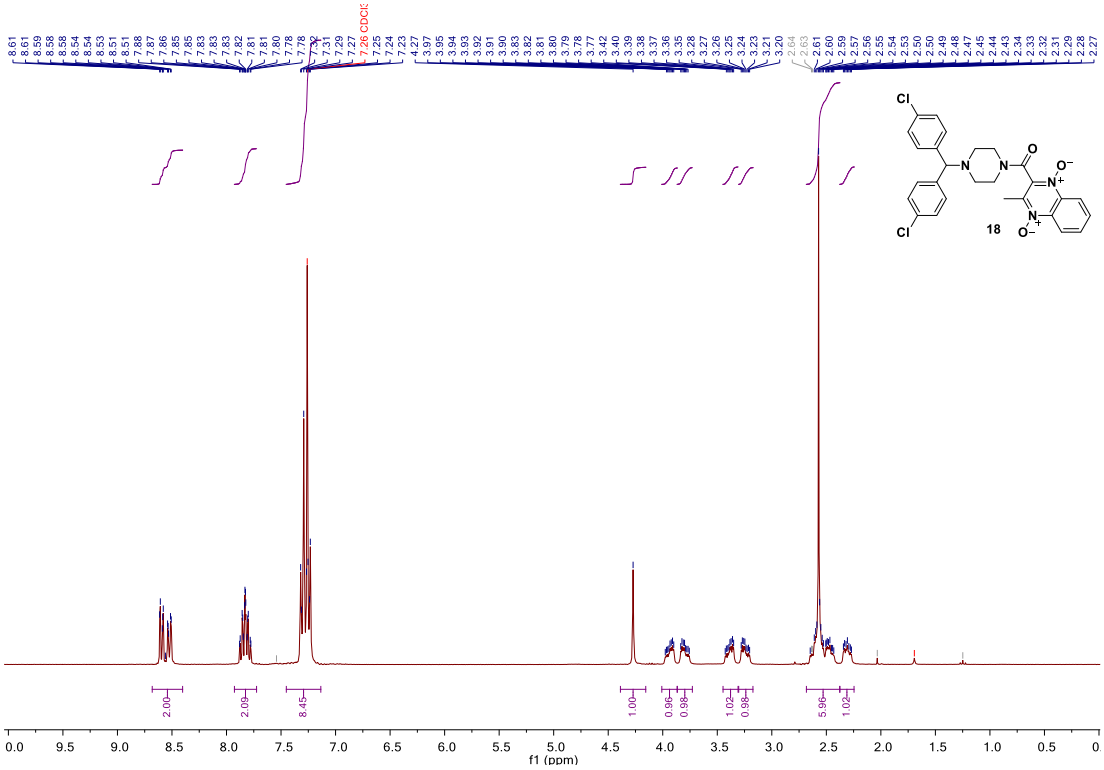

157.68
147.70
140.18
140.15
133.37
129.17
129.15
96.78
88.80
77.48 CDCl3
77.36
77.16 CDCl3
76.84 CDCl3
74.56
52.29
51.49
47.11
43.04
26.98
20.61
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -1
f1 (ppm)

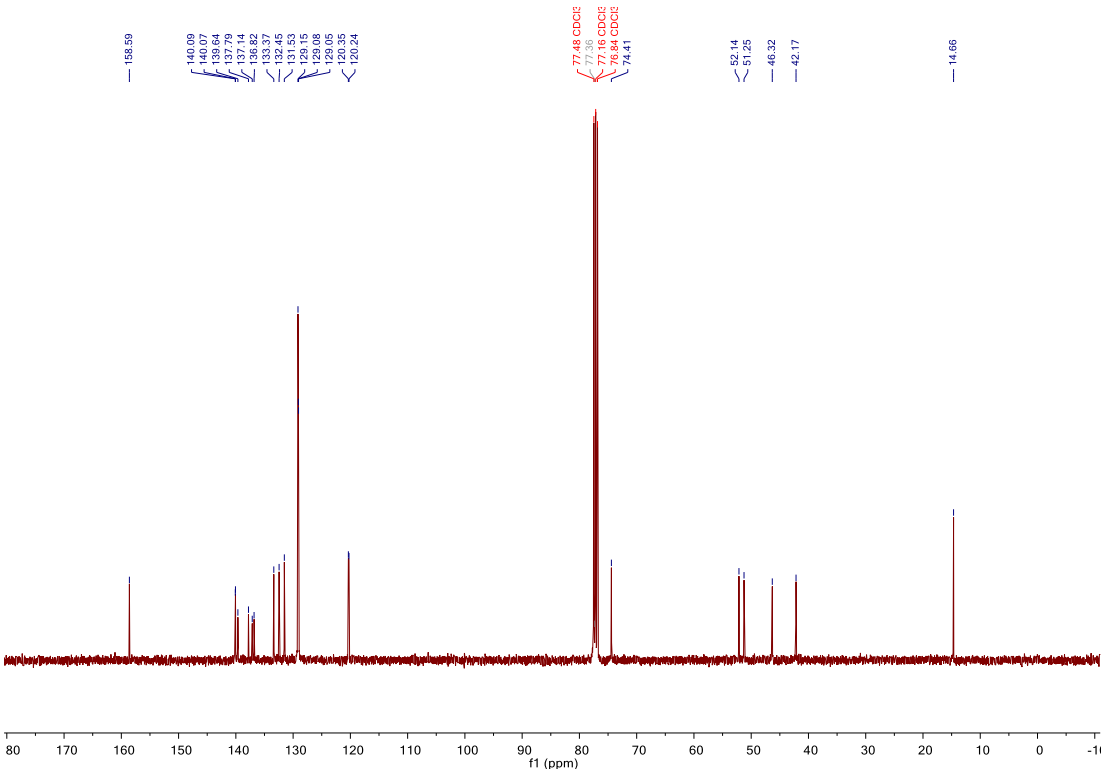
Compound 17


10.60
9.65
8.91
8.69
7.52
7.52
7.51
7.50
7.50
7.49
7.35
7.33
7.33
4.48
4.48
3.66
3.65
3.63
3.62
3.61
3.60
3.57
3.56
3.55
3.54
3.53
3.52
3.51
3.50
3.49
3.48
3.47
3.47
3.46
3.45
3.45
3.43
3.42
3.37
3.36
3.34
3.32
2.81
2.78
2.59
2.57
2.51
2.51
2.49
2.49
2.48
2.47
2.46
2.45
2.43
2.43
2.42
2.41
2.40
2.39
2.36
2.35
2.34
2.33
2.32
2.09
2.08
2.06
2.06 Acetone
2.05 Acetone
2.05 Acetone
2.04 Acetone
2.03 Acetone
1.29
0.88
Cl
Cl
N
N
O
NO2
H2N
O
17
4.04
1.07
1.00
21.80
20.52
5.12
14.43
36.17
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

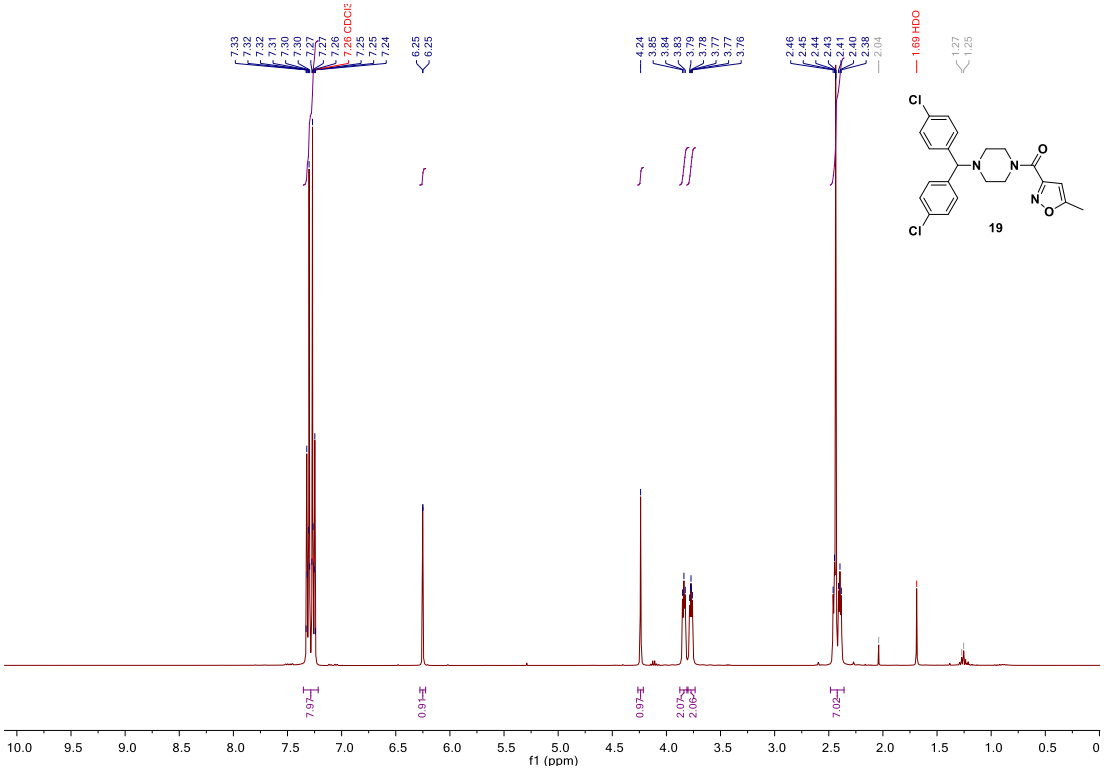

206.46
206.28
206.26
206.25
206.24
206.06
195.21
163.92
162.65
142.14
142.10
133.47
133.45
130.57
129.74
126.83
74.75
74.71
51.70
51.53
51.37
51.20
47.22
47.08
42.30
30.90
30.70
30.57 Acetone
30.44
30.38 Acetone
30.25
30.19 Acetone
30.13
30.05
30.00 Acetone
29.86
29.80 Acetone
29.61 Acetone
29.42 Acetone
20 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

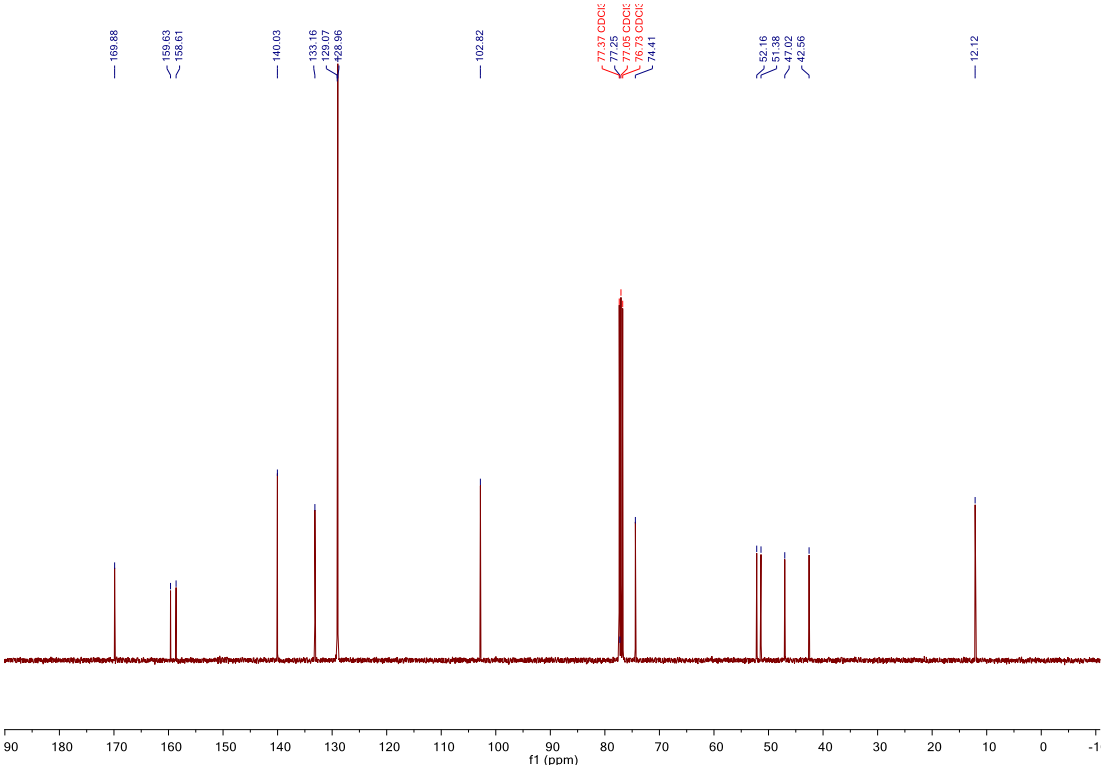
Compound 18


Cl
Cl
O
N
N
O⁻
N⁺
N⁺
O⁻
18
f1 (ppm)

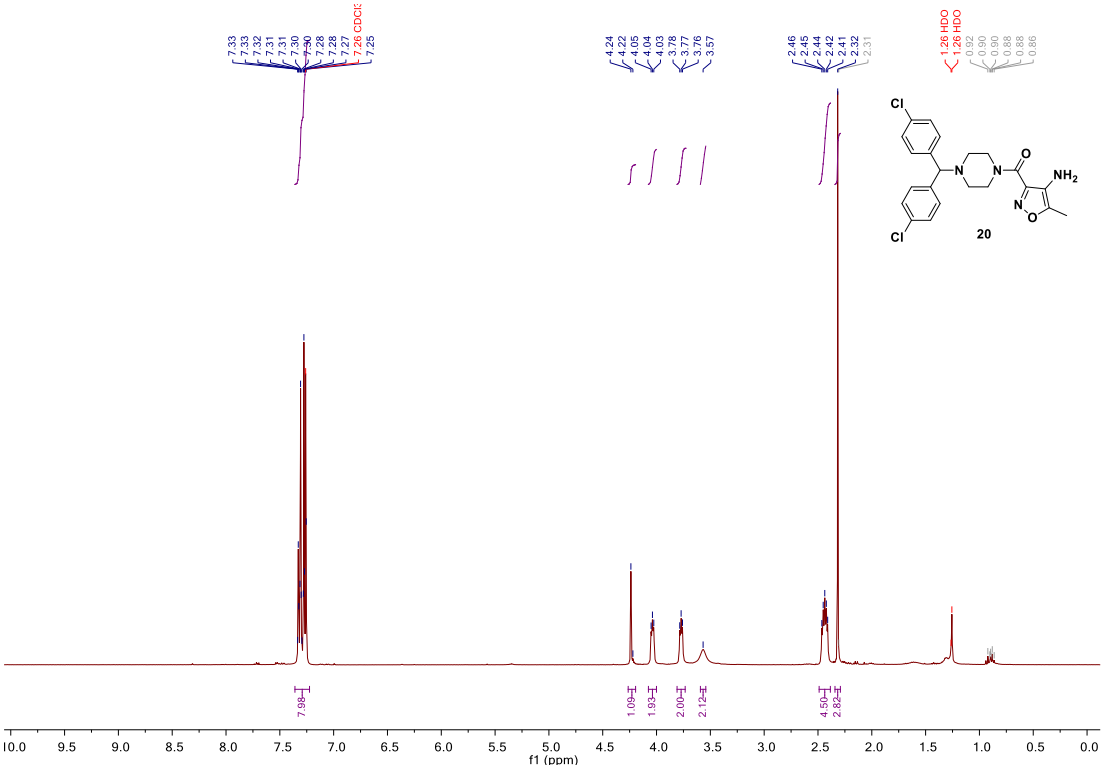

158.59
140.09
140.07
139.64
137.79
137.14
136.82
133.37
132.45
131.53
129.15
129.08
129.05
120.35
120.24
77.48 CDCl3
77.36
77.16 CDCl3
76.84 CDCl3
74.41
52.14
51.25
46.32
42.17
14.66
f1 (ppm)

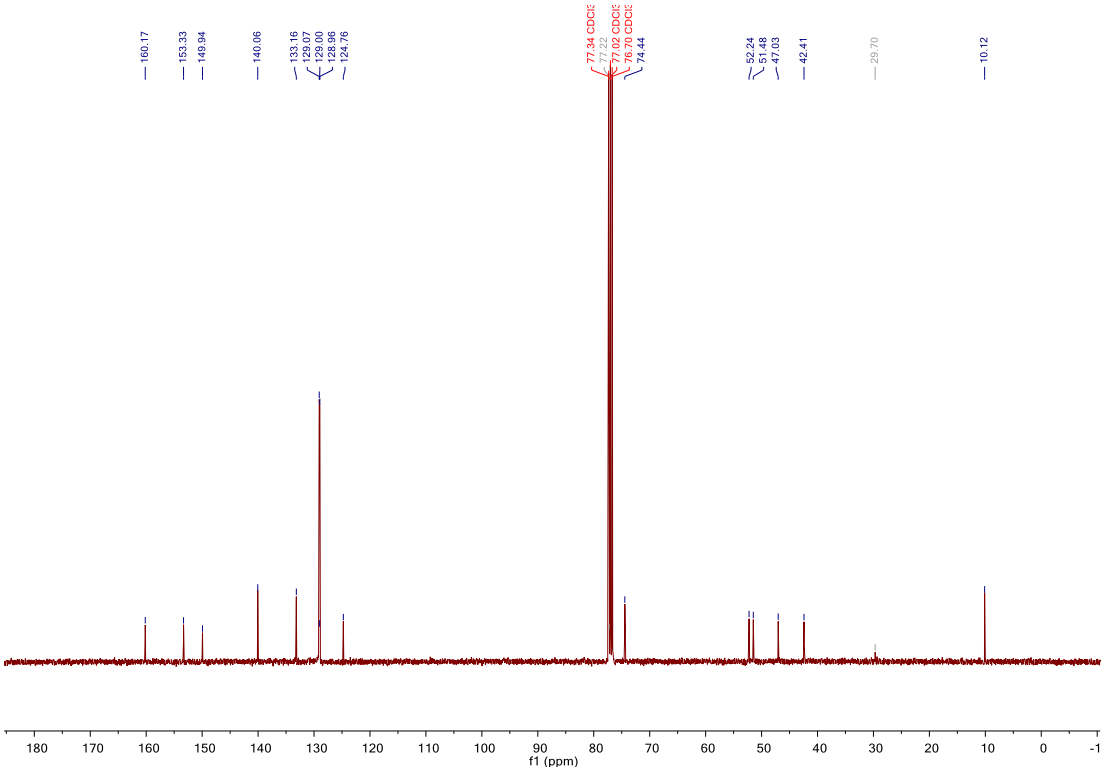
Compound 19


7.33
7.32
7.32
7.31
7.30
7.30
7.27
7.27
7.26
7.26 CDCl3
7.25
7.25
7.24
6.25
6.25
4.24
3.85
3.84
3.83
3.79
3.78
3.77
3.77
3.76
2.46
2.45
2.44
2.43
2.41
2.40
2.38
2.04
1.69 HDO
1.27
1.25
Cl
N
N
O
N
O
Cl
19
7.97
0.91
0.97
2.07
2.06
7.02
f1 (ppm)

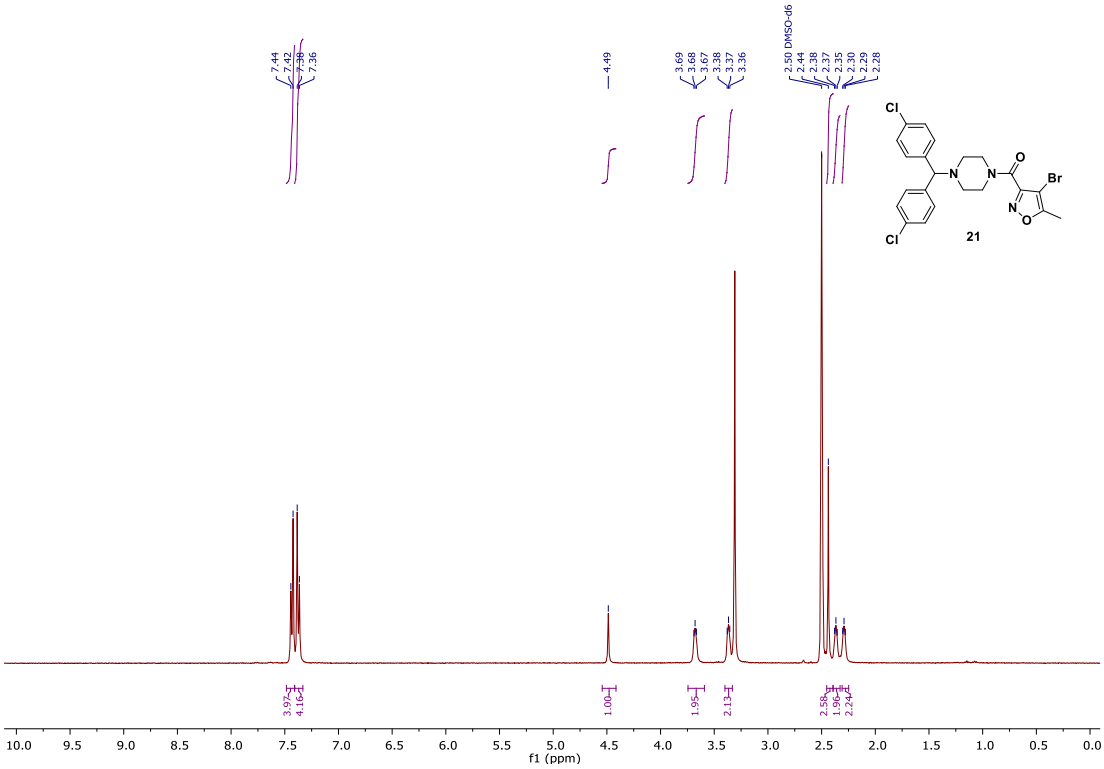

169.88
159.63
158.61
140.03
133.16
129.07
128.96
102.82
77.37 CDCl3
77.25
77.05 CDCl3
76.73 CDCl3
74.41
52.16
51.38
47.02
42.56
12.12
f1 (ppm)

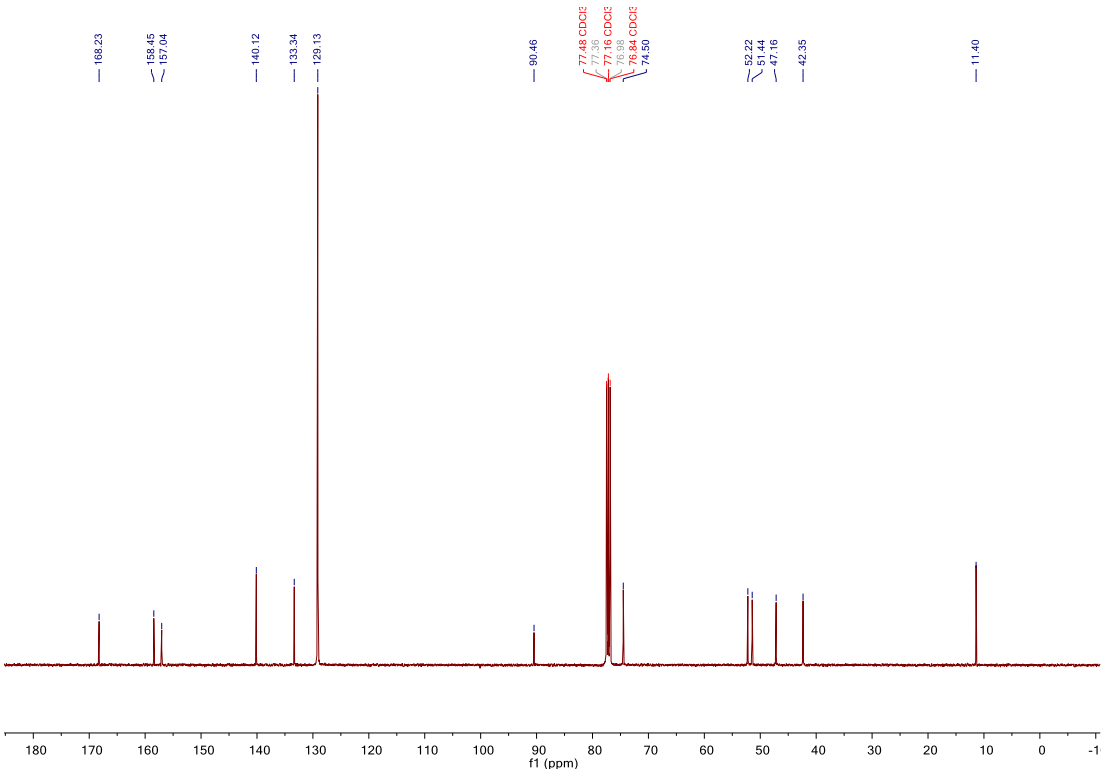
Compound 20


7.33
7.33
7.32
7.31
7.31
7.30
7.30
7.28
7.28
7.27
7.26 CDCl3
7.25
4.24
4.22
4.05
4.04
4.04
4.03
3.78
3.77
3.76
3.57
2.46
2.45
2.44
2.42
2.41
2.32
2.31
1.26 H2O
1.26 H2O
0.92
0.90
0.90
0.88
0.88
0.86
Cl
Cl
N
N
O
NH2
N
O
20
7.98
1.09
1.93
2.00
2.12
4.50
2.82
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

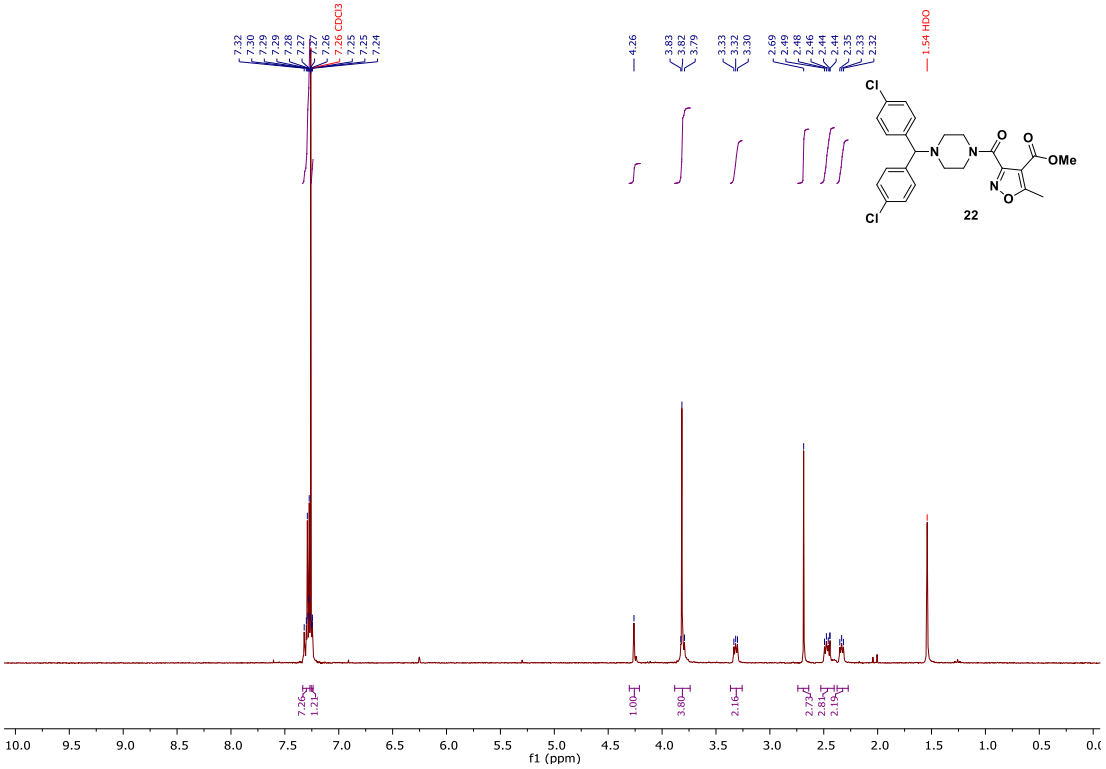

160.17
153.33
149.94
140.06
133.16
129.07
129.00
128.96
124.76
77.34 CDCl3
77.22
77.00 CDCl3
76.79 CDCl3
74.44
52.24
51.48
47.03
42.41
29.70
10.12
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -1
f1 (ppm)

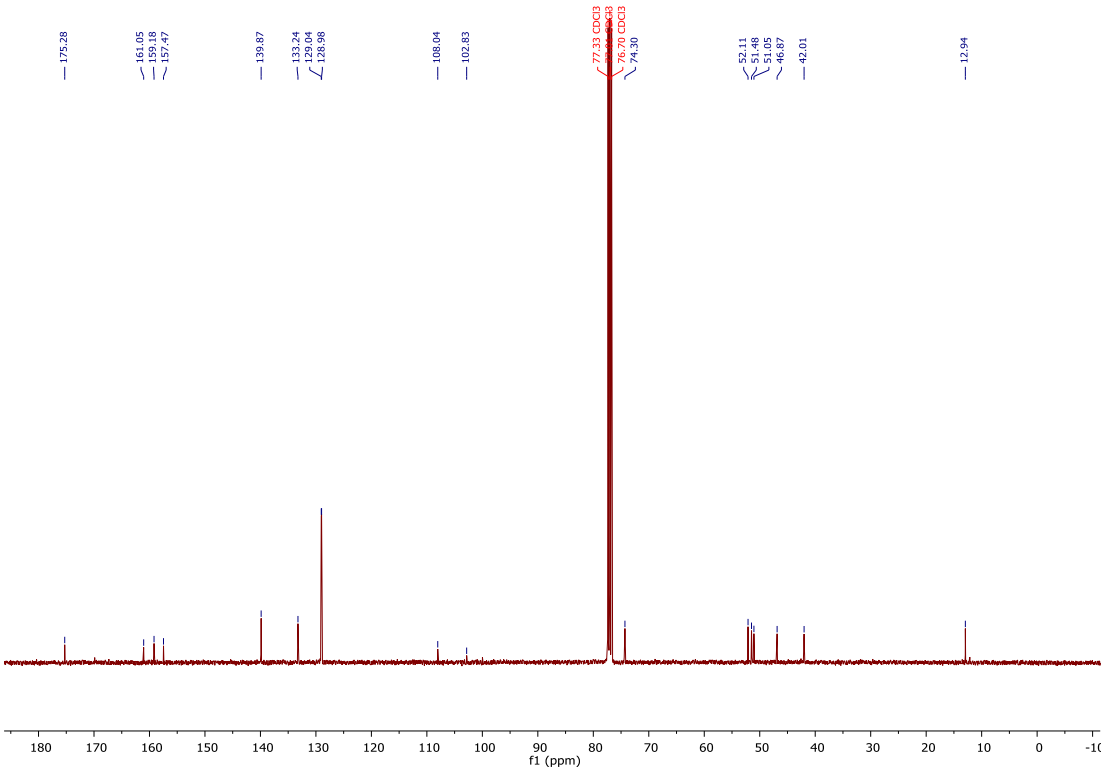
Compound 21


7.44
7.42
7.38
7.36
4.49
3.69
3.68
3.67
3.38
3.37
3.36
2.50 DMSO-d6
2.44
2.38
2.37
2.35
2.30
2.29
2.28
Cl
Cl
N
N
O
Br
N
O
21
3.97
4.16
1.00
1.95
2.13
2.58
1.96
2.24
f1 (ppm)

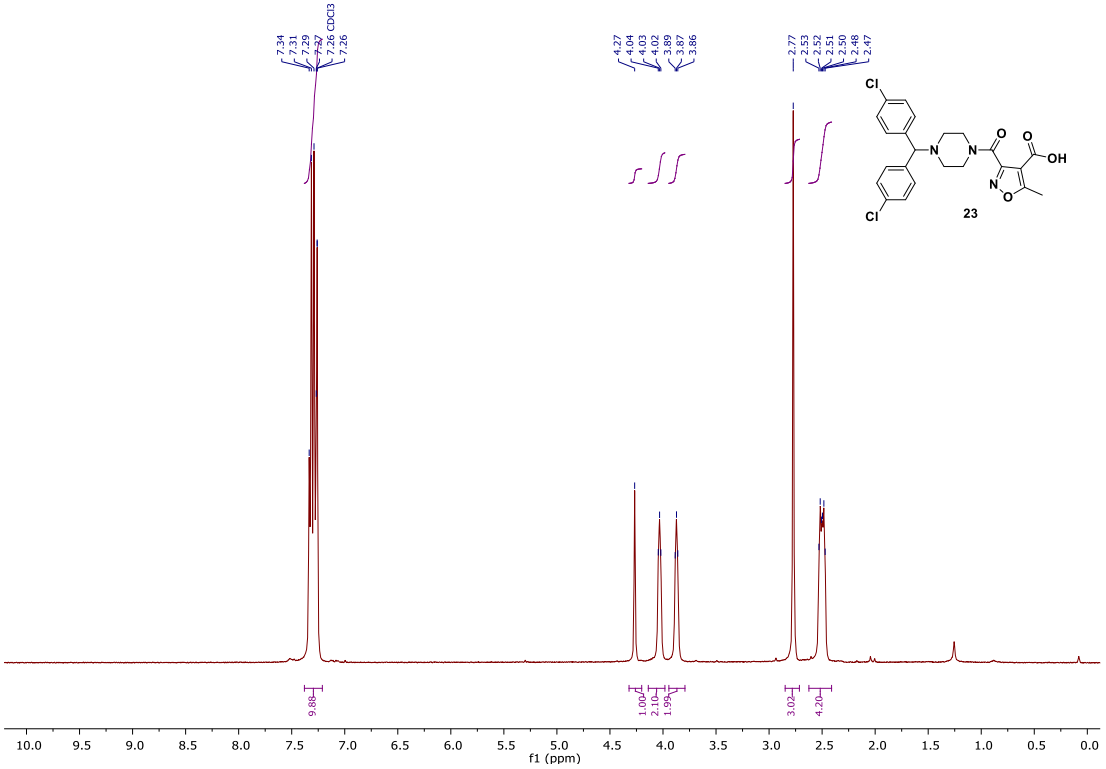

168.23
158.45
157.04
140.12
133.34
129.13
90.46
77.48 CDCl3
77.36
77.16 CDCl3
76.98
76.84 CDCl3
74.50
52.22
51.44
47.16
42.35
11.40
f1 (ppm)

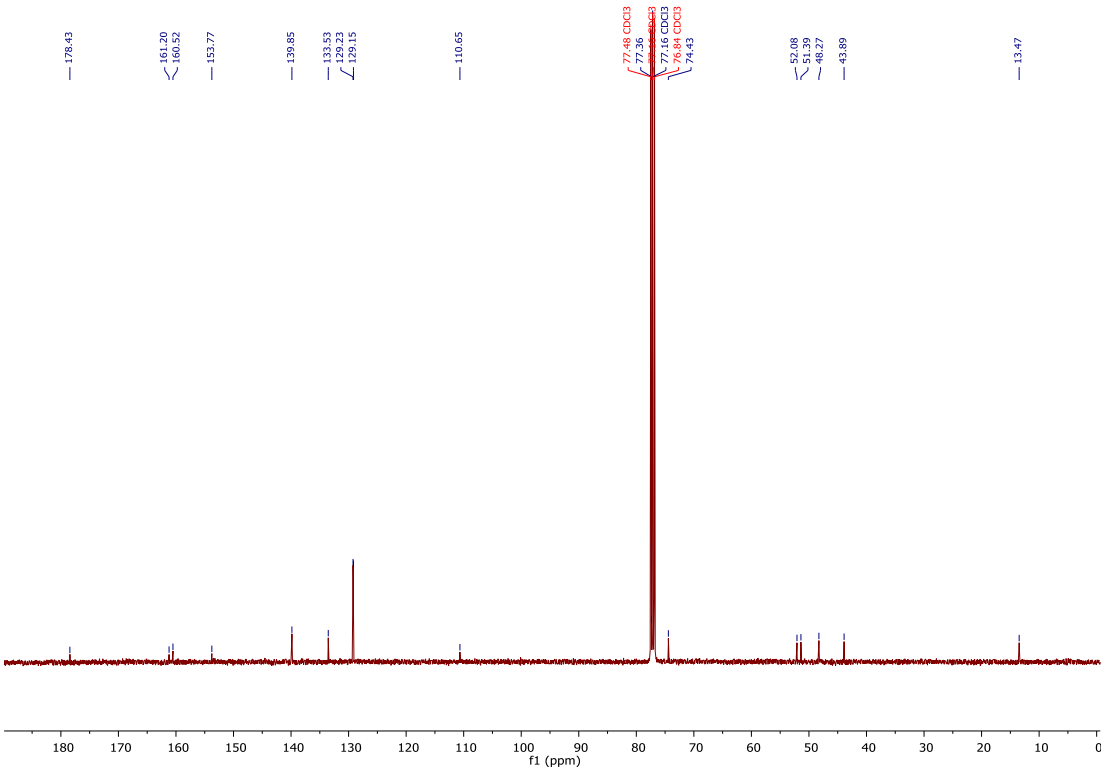
Compound 22


7.32
7.30
7.29
7.29
7.28
7.27
7.27
7.26
7.26 CDCl3
7.25
7.25
7.24
4.26
3.83
3.82
3.79
3.33
3.32
3.30
2.69
2.49
2.48
2.46
2.44
2.44
2.35
2.33
2.32
1.54 HDO
Cl
N
N
O
O
O
OMe
N
O
Cl
22
7.26
1.21
1.00
3.80
2.16
2.73
2.81
2.19
f1 (ppm)

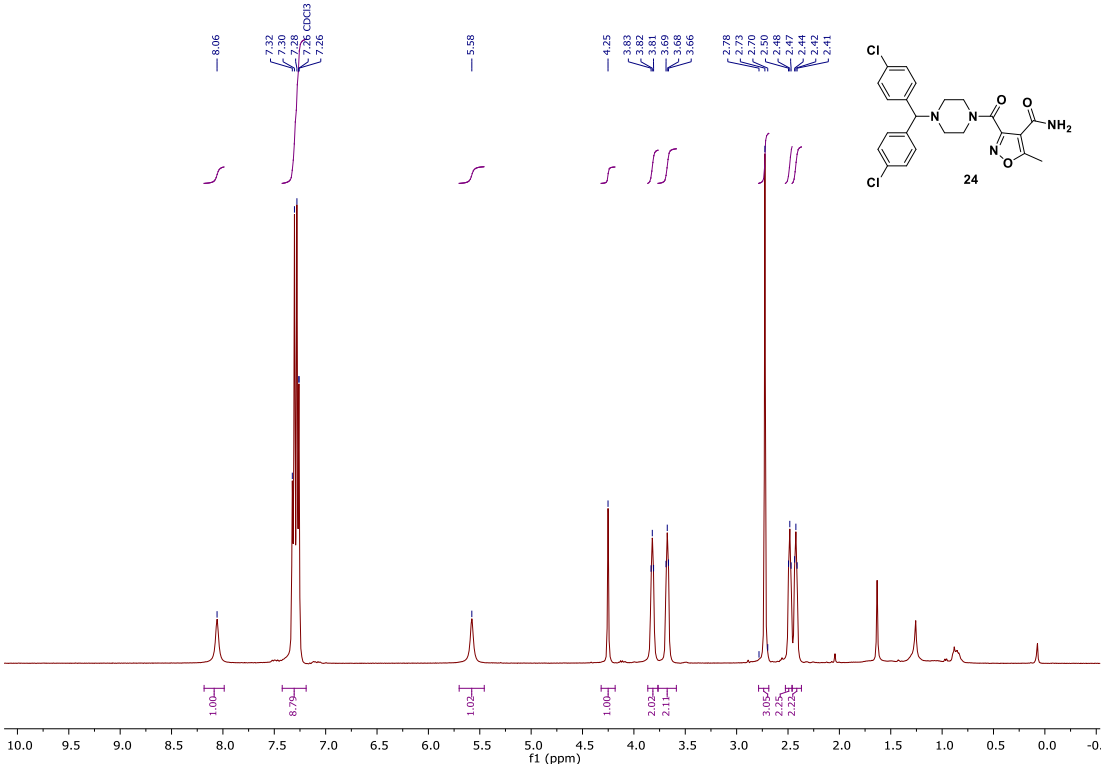

175.28
161.05
159.38
157.47
139.87
133.24
129.04
128.98
108.04
102.83
77.33 CDCl3
76.70 CDCl3
74.30
52.11
51.48
51.05
46.87
42.01
12.94
f1 (ppm)

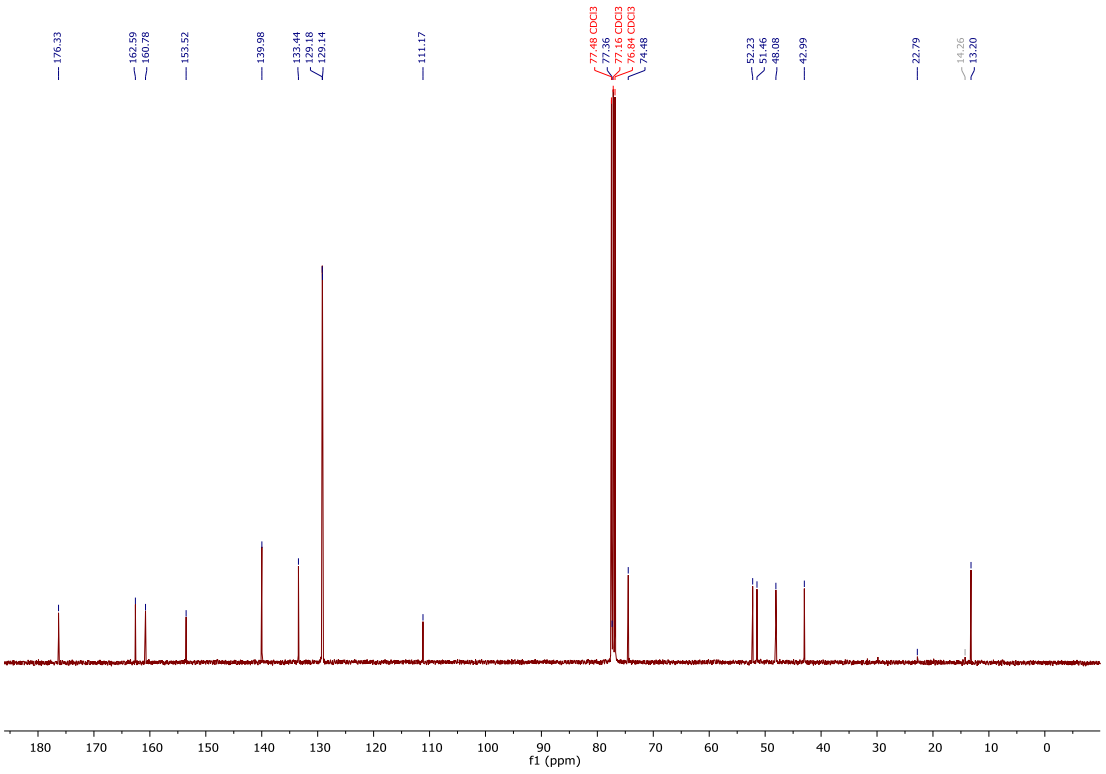
Compound 23


7.34
7.31
7.30
7.27
7.26 CDCl3
7.26
4.27
4.04
4.03
4.02
3.89
3.87
3.86
2.77
2.53
2.52
2.51
2.50
2.48
2.47
Cl
Cl
N
N
O
O
O
OH
N
O
23
9.88
1.00
2.10
1.95
3.02
4.20
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

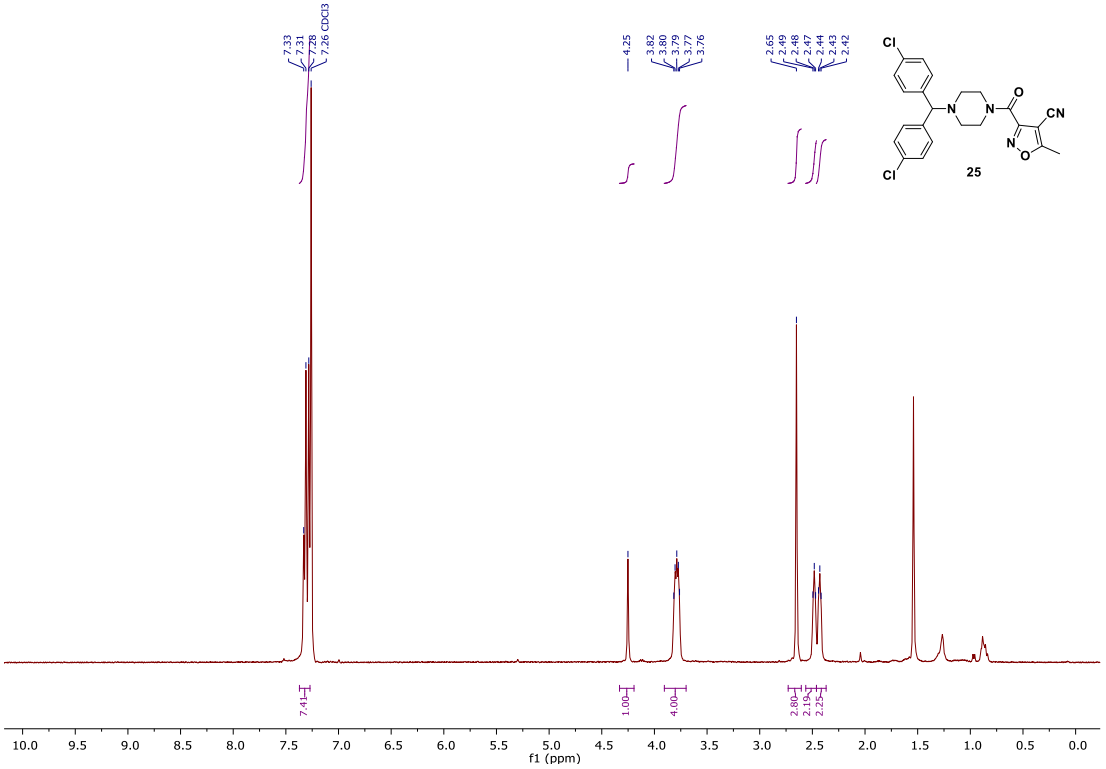

178.43
161.20
160.52
153.77
139.85
133.53
129.23
129.15
110.65
77.48 CDCl3
77.36
77.16 CDCl3
76.84 CDCl3
74.43
52.08
51.39
48.27
43.89
13.47
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

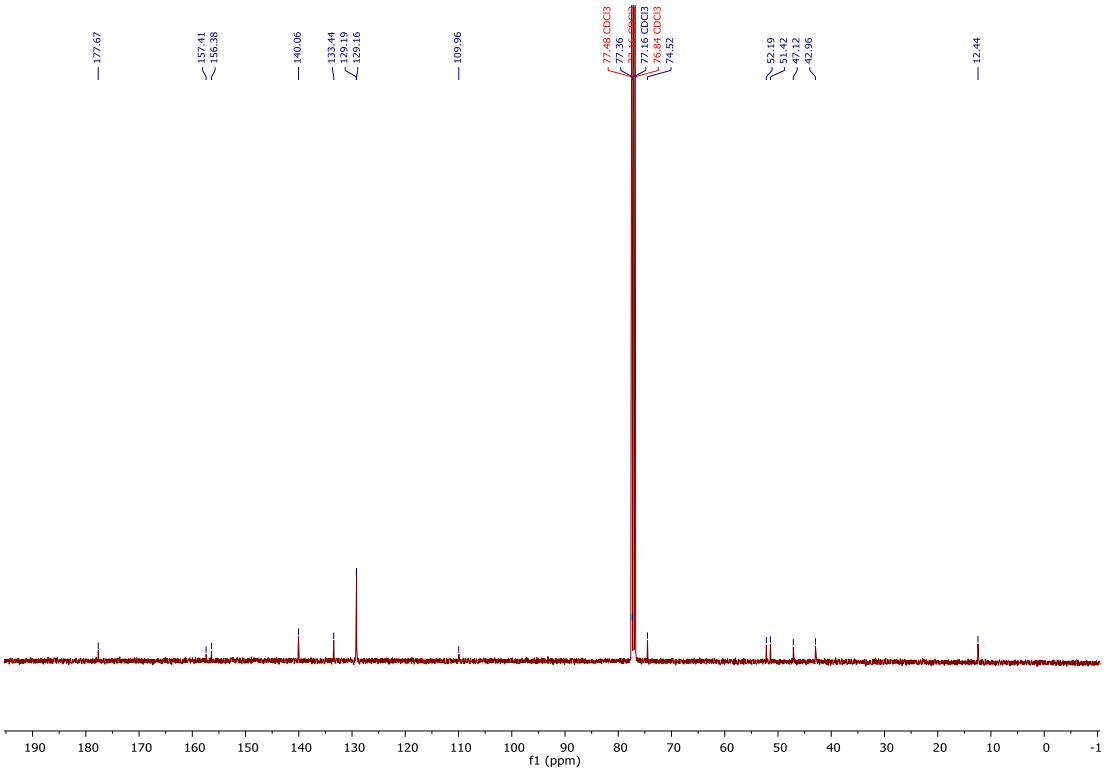
Compound 24


8.06
7.32
7.30
7.28
7.26 CDCl3
7.26
5.58
4.25
3.83
3.82
3.81
3.69
3.68
3.66
2.78
2.73
2.70
2.50
2.48
2.47
2.44
2.42
2.41
Cl
N
N
O
O
NH2
N
O
Cl
24
1.00
8.79
1.02
1.00
2.02
2.11
3.05
2.25
2.23
f1 (ppm)

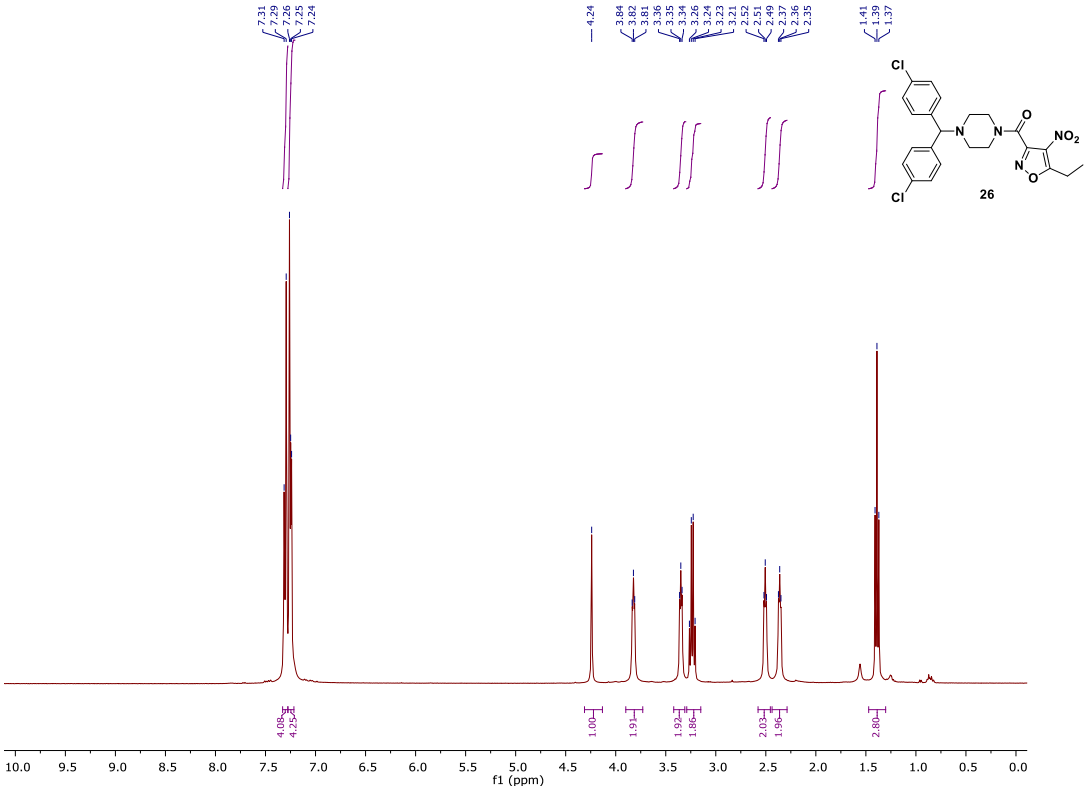

176.33
162.59
160.78
153.52
139.98
133.44
129.18
129.14
111.17
77.48 CDCl3
77.36
77.16 CDCl3
76.84 CDCl3
74.48
52.23
51.46
48.08
42.99
22.79
14.26
13.20
f1 (ppm)

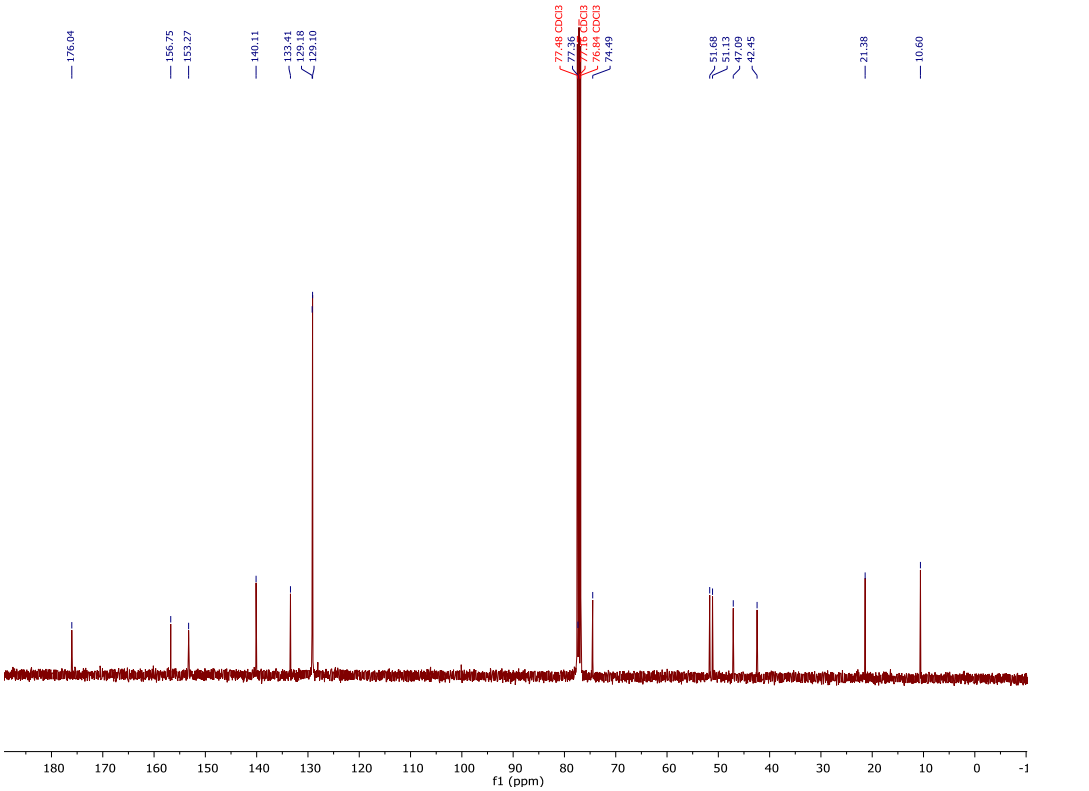
Compound 25


7.33
7.31
7.28
7.26 CDCl3
4.25
3.82
3.80
3.79
3.77
3.76
2.65
2.49
2.48
2.47
2.44
2.43
2.42
Cl
N
N
O
CN
N
O
Cl
25
7.41
1.00
4.00
2.80
2.19
2.25
f1 (ppm)

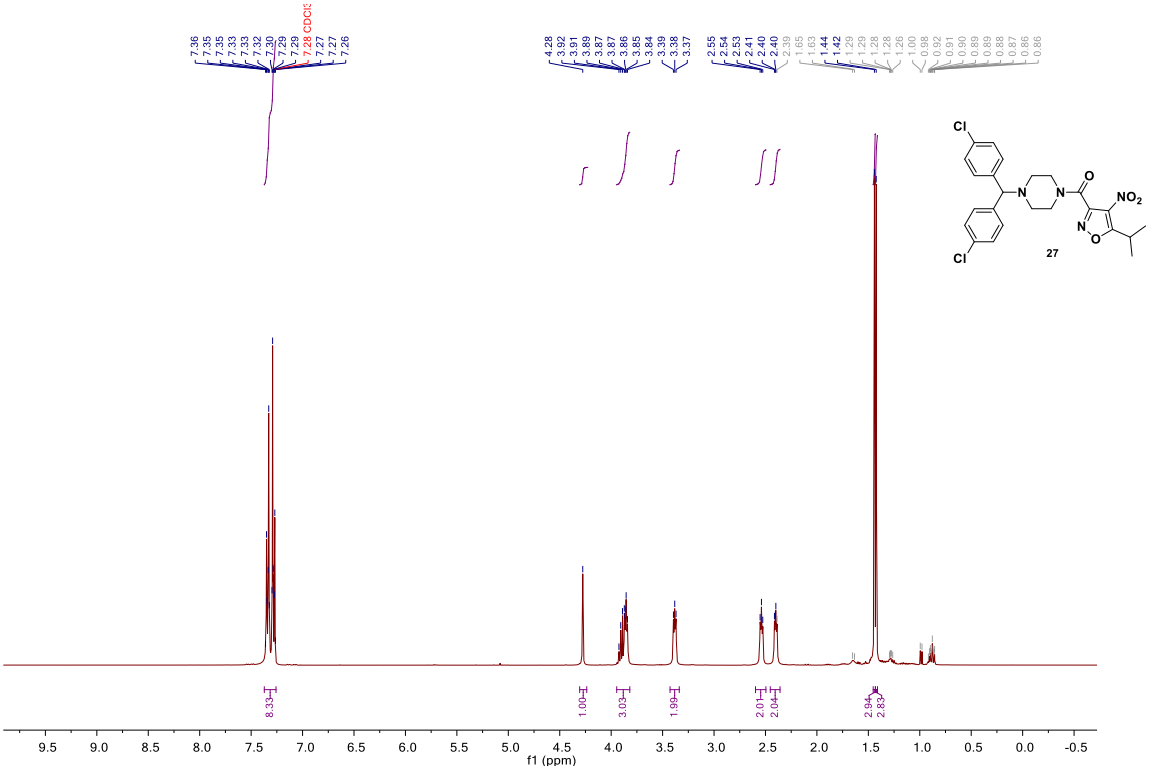

177.67
157.41
156.38
140.06
133.44
129.19
129.16
109.96
77.48 CDCl3
77.36
77.16 CDCl3
76.84 CDCl3
74.52
52.19
51.42
47.72
42.96
12.44
f1 (ppm)

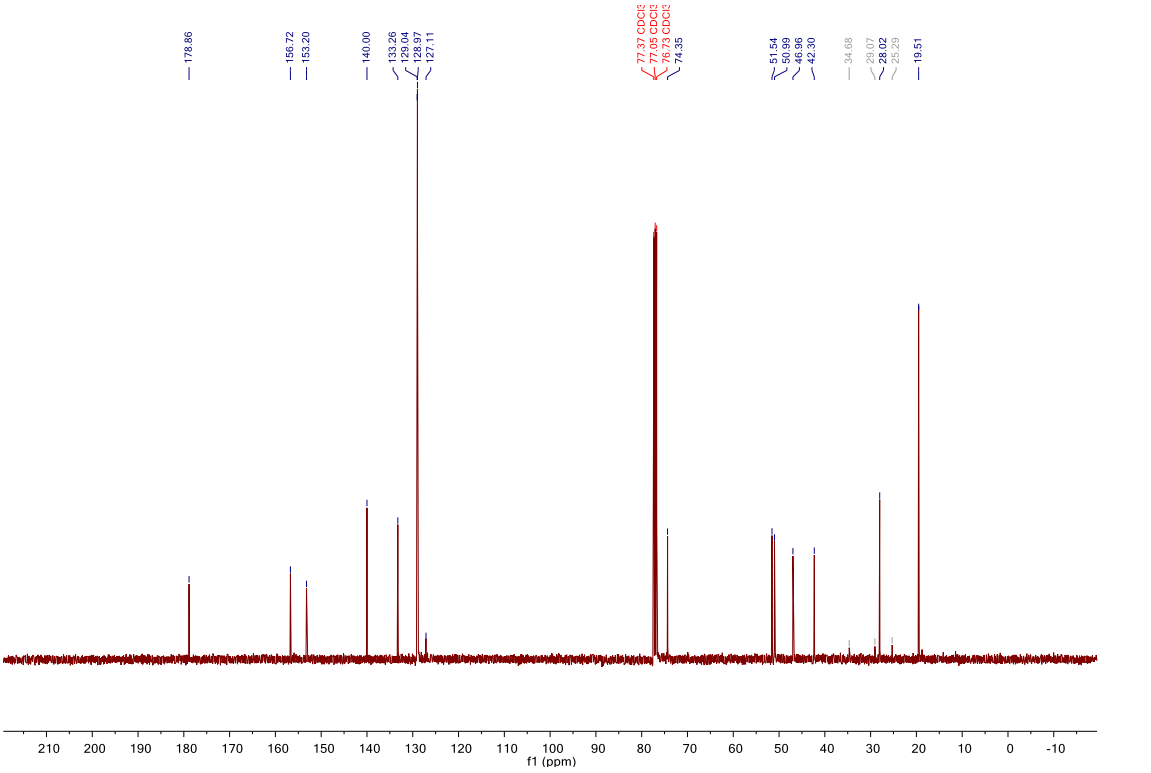
Compound 26


7.31
7.29
7.26
7.25
7.24
4.24
3.84
3.82
3.81
3.36
3.35
3.34
3.26
3.24
3.23
3.21
2.52
2.51
2.49
2.37
2.36
2.35
1.41
1.39
1.37
Cl
Cl
N
N
O
N
O
NO2
26
4.08
4.25
1.00
1.91
1.92
1.86
2.03
1.96
2.80
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

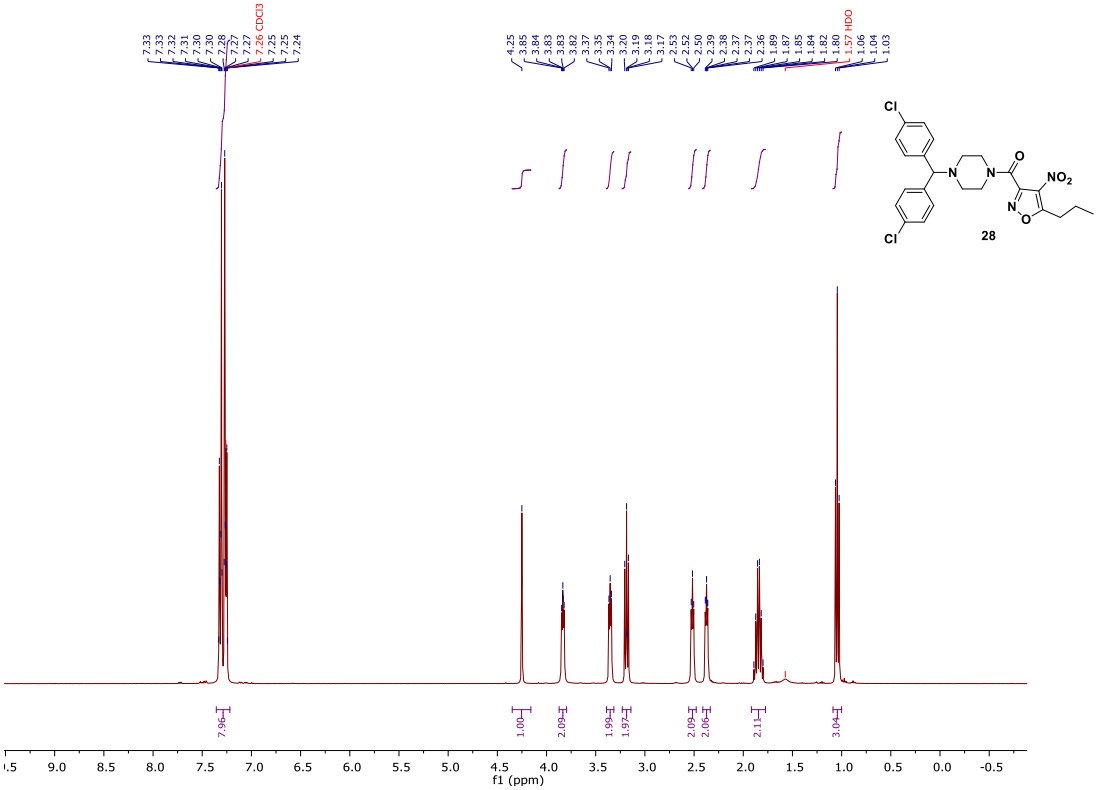

176.04
156.75
153.27
140.11
133.41
129.18
129.10
77.48 CDCl3
77.36
77.16 CDCl3
76.84 CDCl3
74.49
51.68
51.13
47.09
42.45
21.38
10.60
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -1
f1 (ppm)

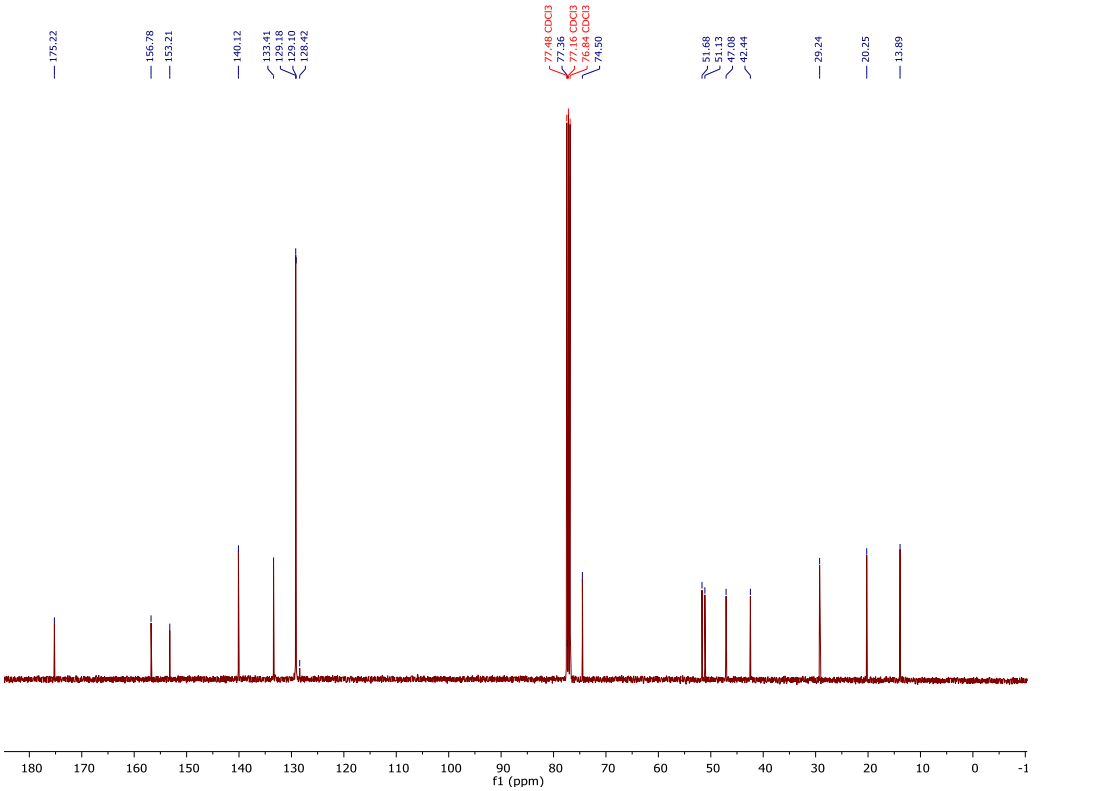
Compound 27


7.36
7.35
7.35
7.33
7.32
7.30
7.29
7.29
7.28 CDCl3
7.27
7.27
7.26
4.28
3.92
3.91
3.89
3.87
3.87
3.86
3.85
3.84
3.39
3.38
3.37
2.55
2.54
2.53
2.41
2.40
2.40
2.39
1.65
1.63
1.44
1.42
1.38
1.29
1.28
1.28
1.26
1.00
0.98
0.92
0.91
0.90
0.89
0.89
0.88
0.87
0.86
0.86
Cl
Cl
N
N
O
N
O
NO2
27
8.33
1.00
3.03
1.99
2.01
2.04
2.94
2.83
f1 (ppm)

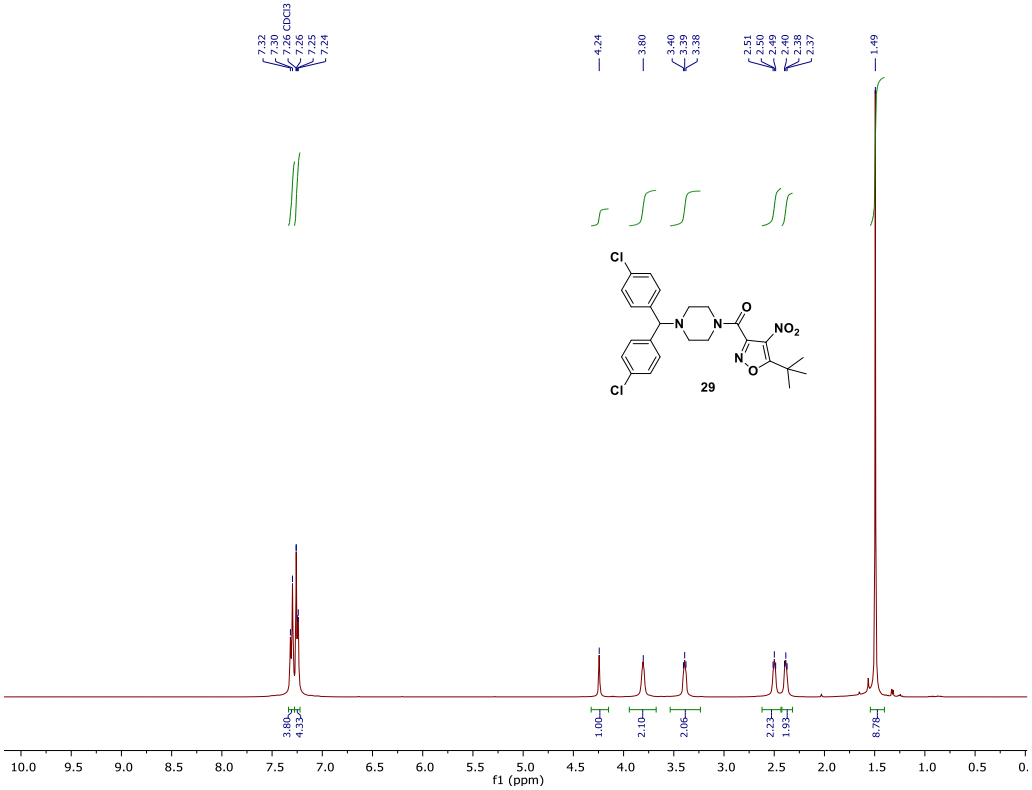

178.86
156.72
153.20
140.00
133.26
129.04
128.97
127.11
77.37 CDCl3
77.05 CDCl3
76.73 CDCl3
74.35
51.54
50.99
46.96
42.30
34.68
29.07
28.02
25.29
19.51
f1 (ppm)

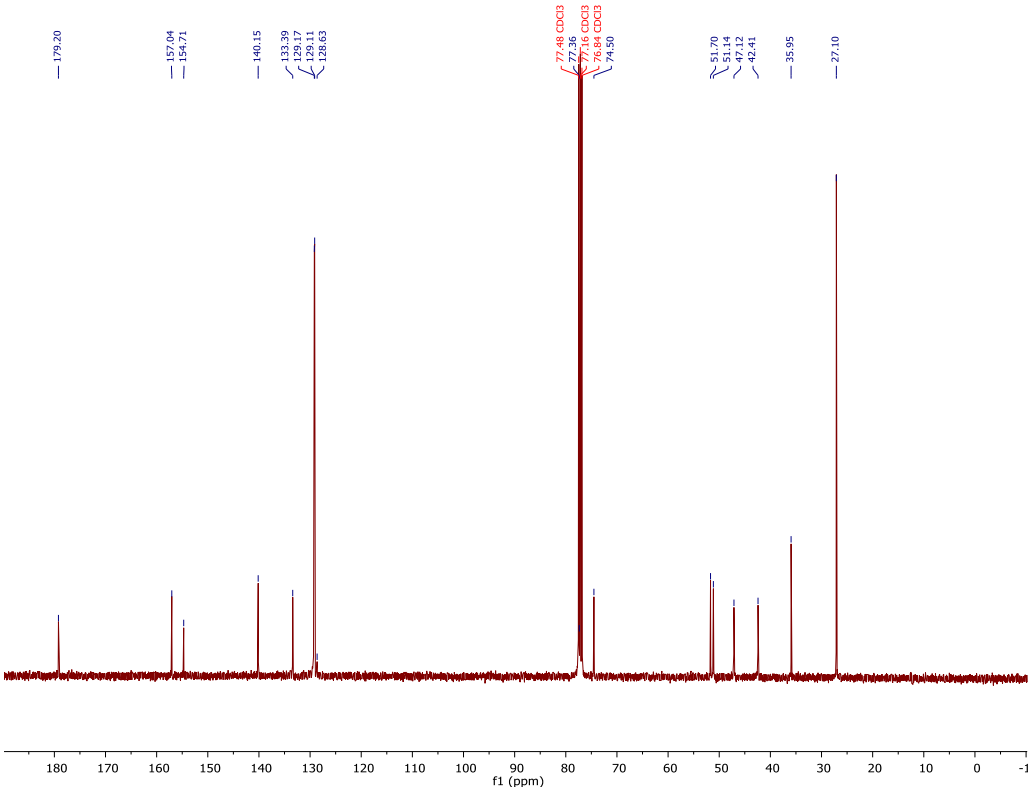
Compound 28


7.33
7.33
7.32
7.31
7.30
7.30
7.28
7.27
7.27
7.26 CDCl3
7.25
7.25
7.24
4.25
3.85
3.84
3.83
3.83
3.82
3.37
3.35
3.34
3.20
3.19
3.18
3.17
2.53
2.52
2.50
2.39
2.38
2.37
2.37
2.36
1.89
1.87
1.85
1.84
1.82
1.80
1.57 HDO
1.06
1.04
1.03
Cl
Cl
N
N
O
O
N
NO2
28
7.96
1.00
2.09
1.99
1.97
2.09
2.06
2.11
3.04
f1 (ppm)

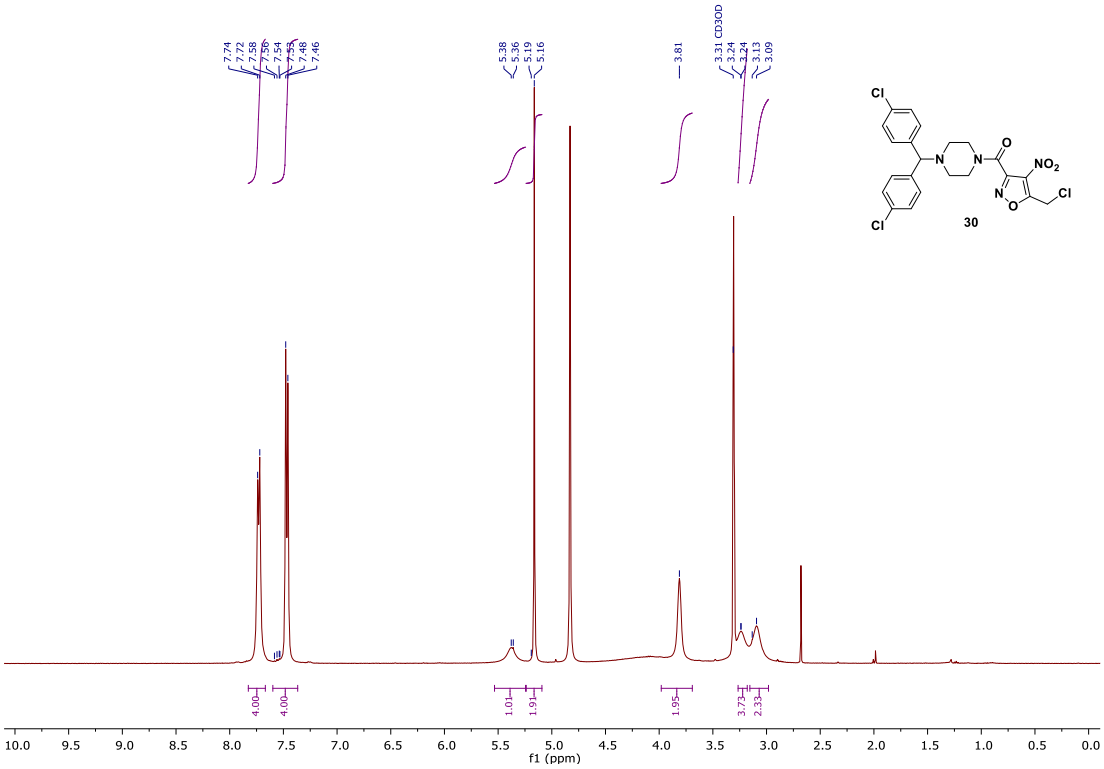

175.22
156.78
153.21
140.12
133.41
129.18
129.10
128.42
77.48 CDCl3
77.36
77.16 CDCl3
76.84 CDCl3
74.50
51.68
51.13
47.08
42.44
29.24
20.25
13.89
f1 (ppm)

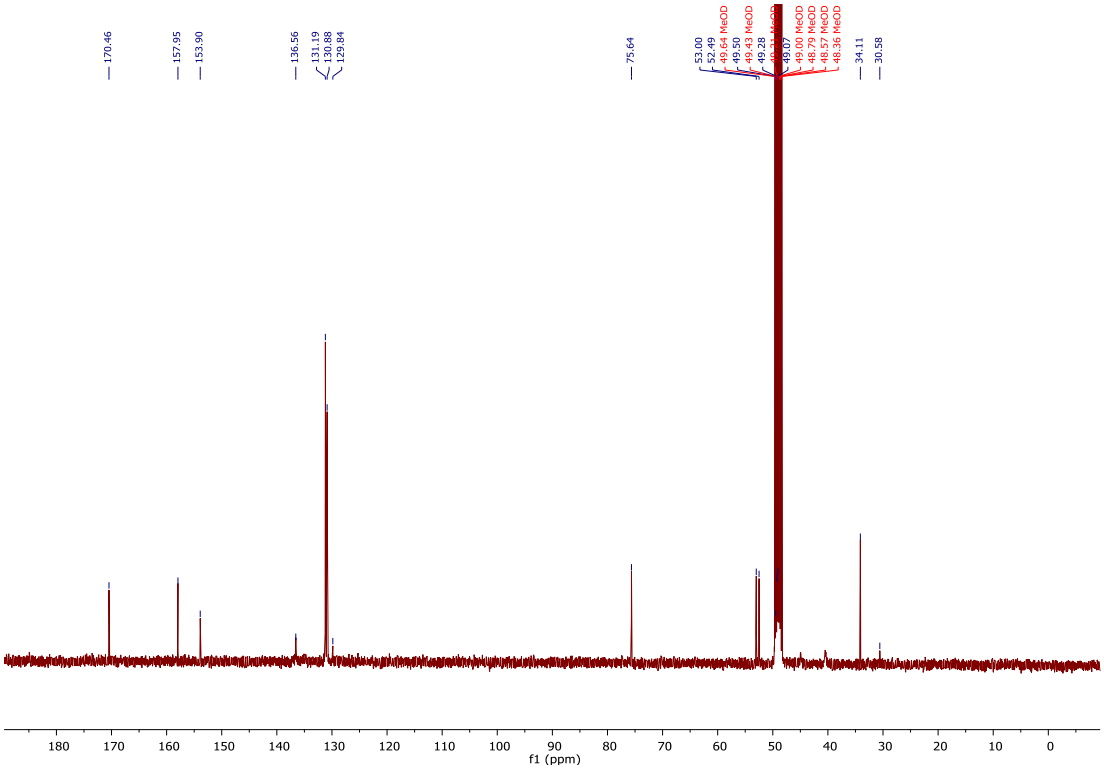
Compound 29


7.32
7.30
7.26 CDCl3
7.25
7.24
4.24
3.80
3.40
3.39
3.38
2.51
2.50
2.49
2.40
2.38
2.37
1.49
Cl
N
N
O
NO2
N
O
Cl
29
3.80
4.33
1.00
2.10
2.06
2.23
1.93
8.78
f1 (ppm)

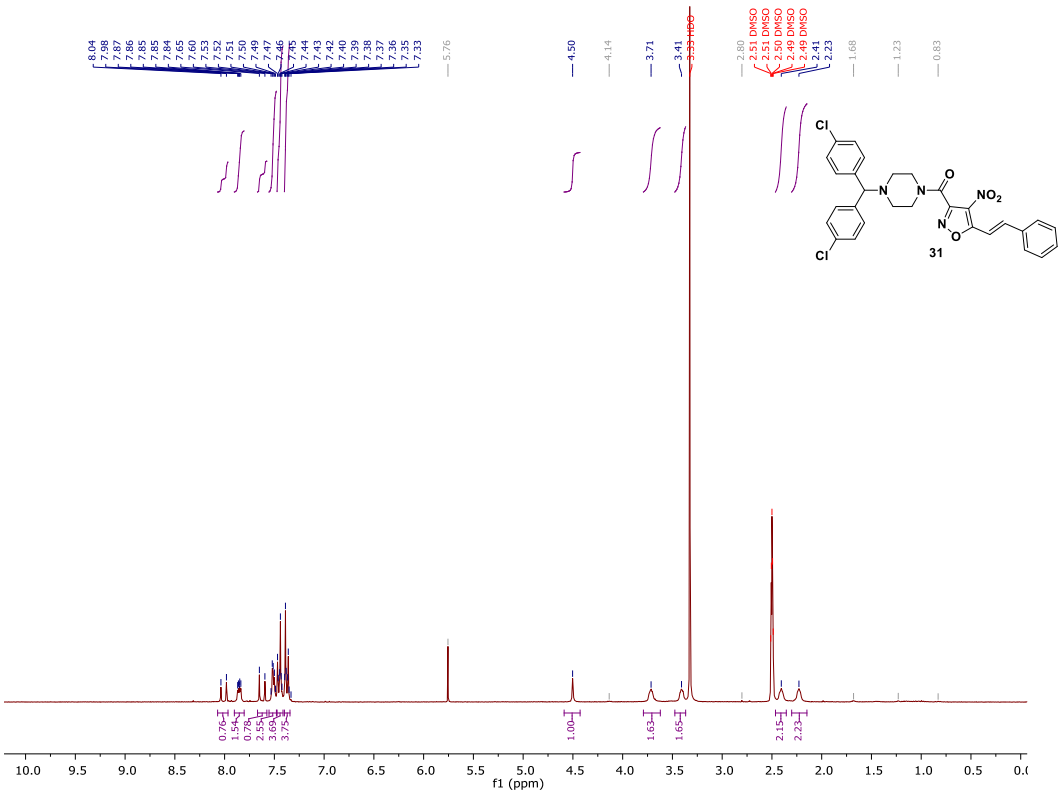

179.20
157.04
154.71
140.15
133.39
129.17
129.11
128.63
77.48 CDCl3
77.36
77.16 CDCl3
76.84 CDCl3
74.50
51.70
51.14
47.12
42.41
35.95
27.10
f1 (ppm)

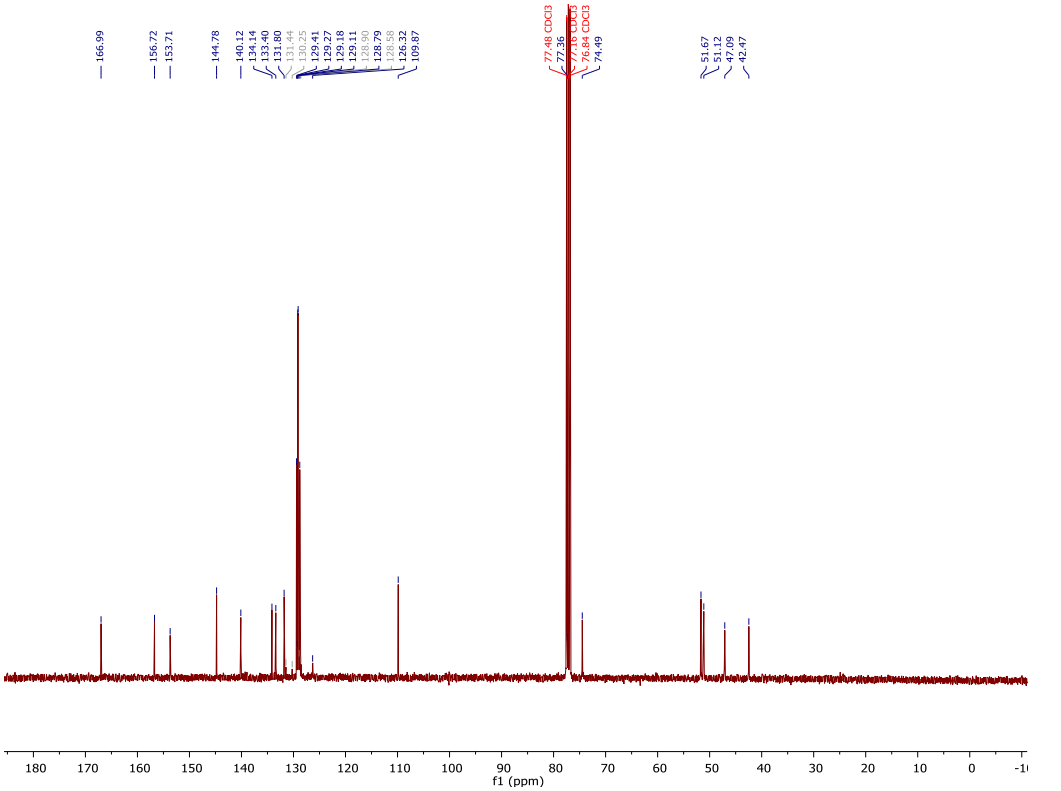
Compound 30


7.74
7.72
7.58
7.56
7.54
7.53
7.48
7.46
5.38
5.36
5.19
5.16
3.81
3.31 CD3OD
3.24
3.24
3.13
3.09
Cl
Cl
N
N
O
NO2
N
O
Cl
30
4.00
4.00
1.01
1.91
1.95
3.73
2.33
f1 (ppm)

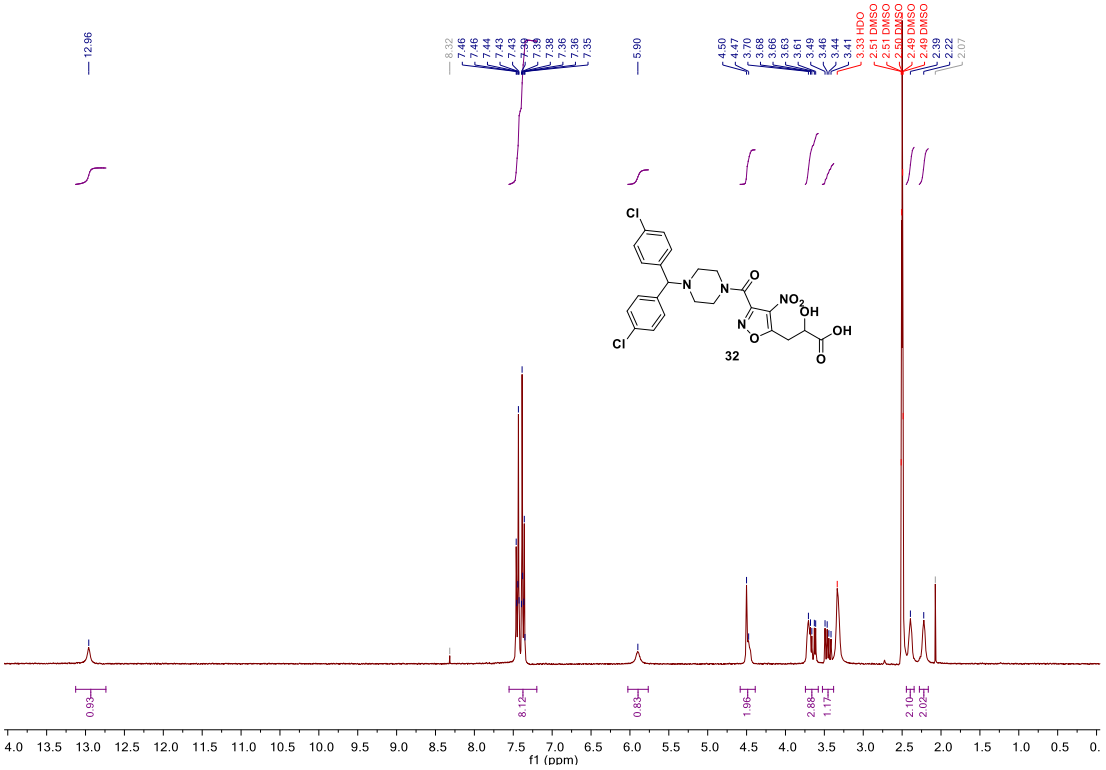

170.46
157.95
153.90
136.56
131.19
130.88
129.84
75.64
53.00
52.49
49.64 MeOD
49.50
49.43 MeOD
49.28
49.07
49.00 MeOD
48.79 MeOD
48.57 MeOD
48.36 MeOD
34.11
30.58
f1 (ppm)

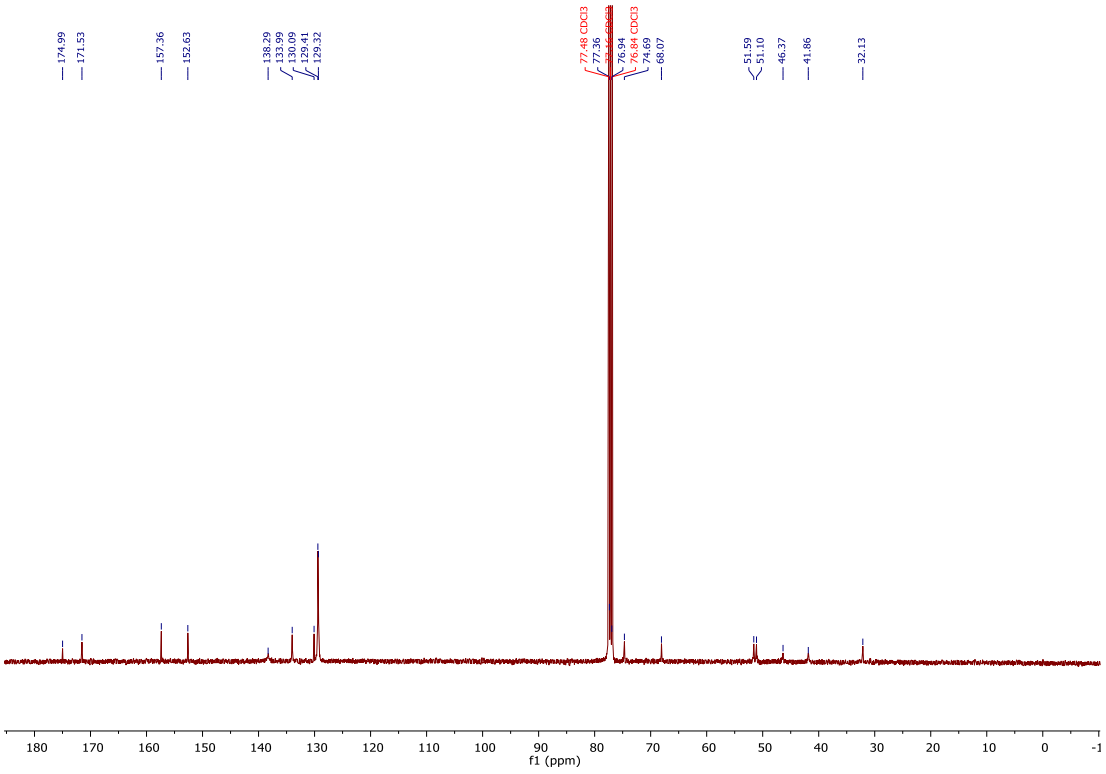
Compound 31


8.04
7.98
7.97
7.86
7.85
7.85
7.84
7.65
7.60
7.53
7.52
7.51
7.50
7.49
7.47
7.46
7.45
7.44
7.43
7.42
7.40
7.39
7.38
7.37
7.36
7.35
7.33
5.76
4.50
4.14
3.71
3.41
3.33 HDO
2.80
2.51 DMSO
2.51 DMSO
2.50 DMSO
2.49 DMSO
2.41
2.23
1.68
1.23
0.83
Cl
Cl
N
N
O
NO2
N
O
31
0.76
1.54
0.78
2.55
3.69
3.75
1.00
1.63
1.65
2.15
2.23
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

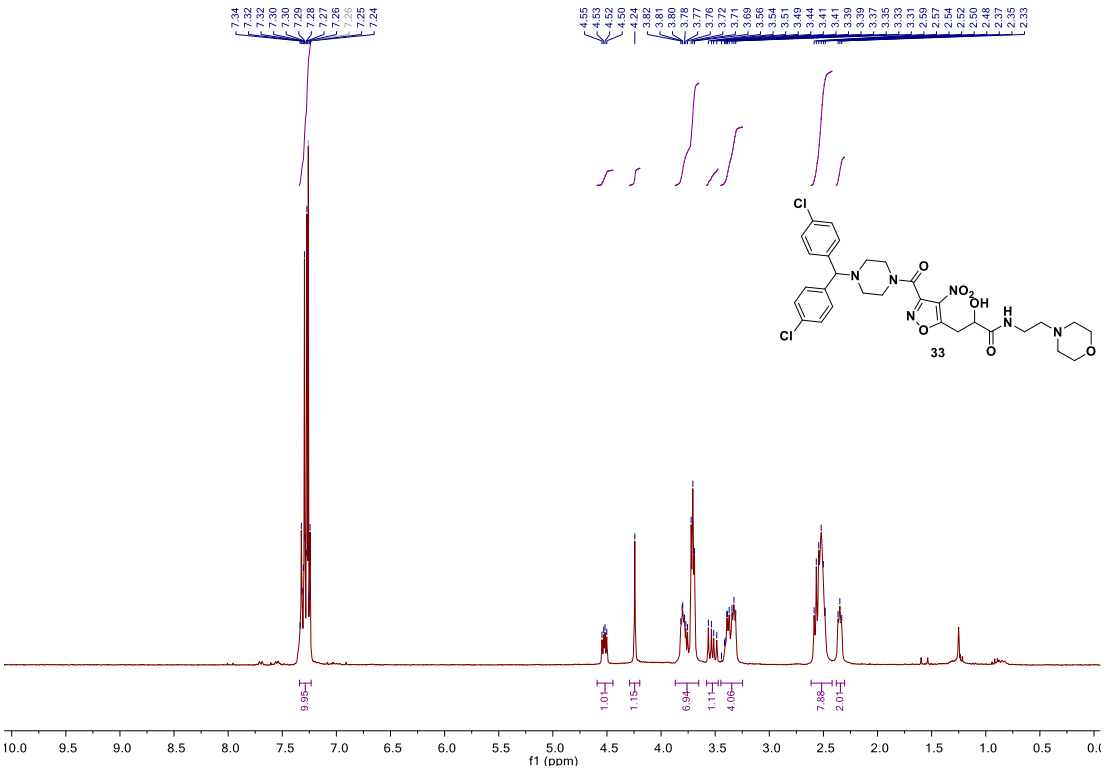

166.99
156.72
153.71
144.78
140.12
134.14
133.40
131.80
131.44
130.25
129.41
129.27
129.18
129.11
128.90
128.79
128.58
126.32
109.87
77.48 CDCl3
77.36
77.16 CDCl3
76.84 CDCl3
74.49
51.67
51.12
47.09
42.47
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

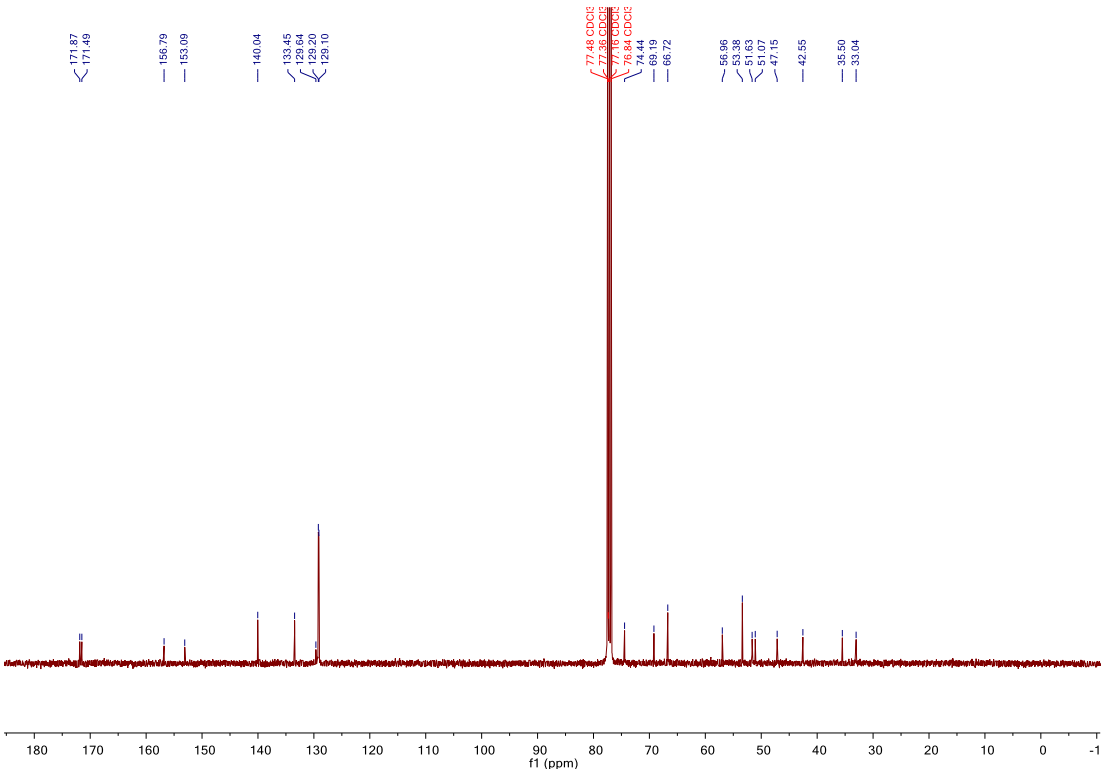
Compound 32


12.96
8.32
7.46
7.45
7.44
7.43
7.39
7.38
7.36
7.36
7.35
5.90
4.50
4.47
3.70
3.68
3.66
3.63
3.61
3.48
3.46
3.44
3.41
3.33 HDO
2.51 DMSO
2.51 DMSO
2.50 DMSO
2.49 DMSO
2.49 DMSO
2.39
2.29
2.07
Cl
Cl
N
N
O
NO2
OH
N
O
OH
O
32
0.99
8.12
0.83
1.96
2.88
1.17
2.10
2.02
f1 (ppm)

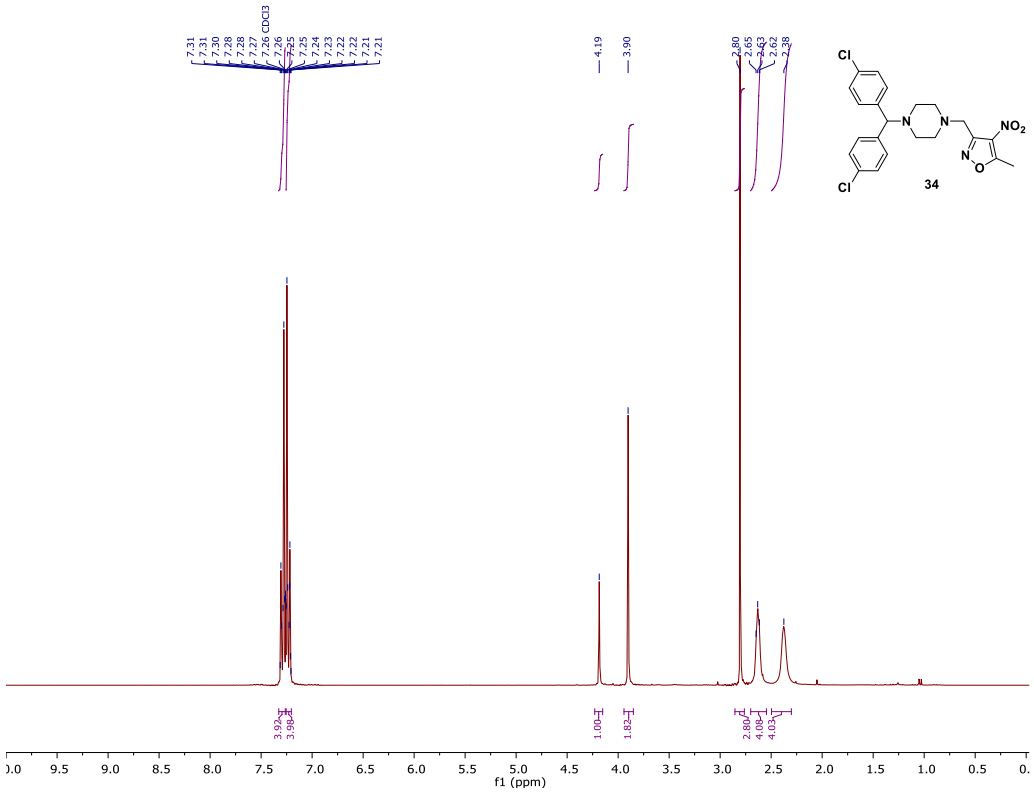

174.99
171.53
157.36
152.63
138.29
133.99
130.99
129.41
129.32
77.48 CDCl3
77.36
76.94
76.84 CDCl3
74.69
68.07
51.59
51.10
46.37
41.86
32.13
f1 (ppm)

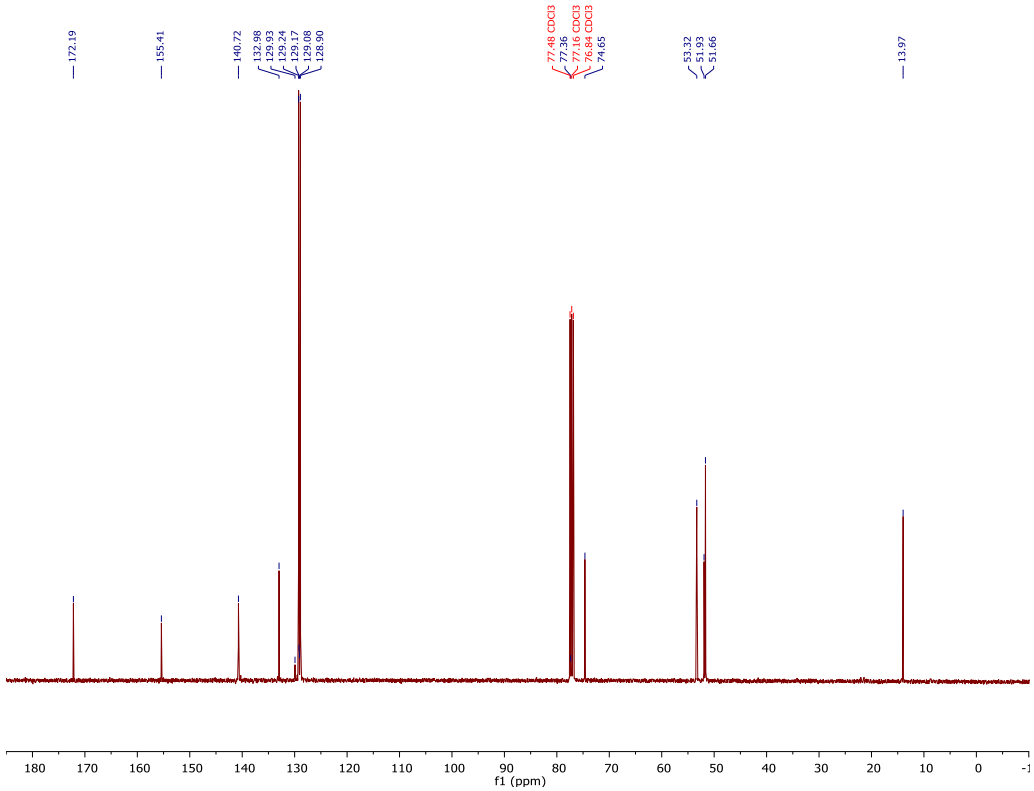
Compound 33


7.34
7.32
7.32
7.30
7.30
7.29
7.28
7.27
7.26
7.25
7.24
4.55
4.53
4.52
4.50
4.24
3.82
3.81
3.80
3.78
3.77
3.76
3.72
3.71
3.69
3.56
3.54
3.51
3.49
3.44
3.41
3.39
3.39
3.37
3.35
3.33
3.31
2.59
2.57
2.54
2.52
2.50
2.48
2.37
2.35
2.33
Cl
Cl
N
N
O
NO2
OH
N
O
H
N
O
N
O
33
9.95
1.01
1.15
6.94
1.11
4.06
7.88
2.01
f1 (ppm)

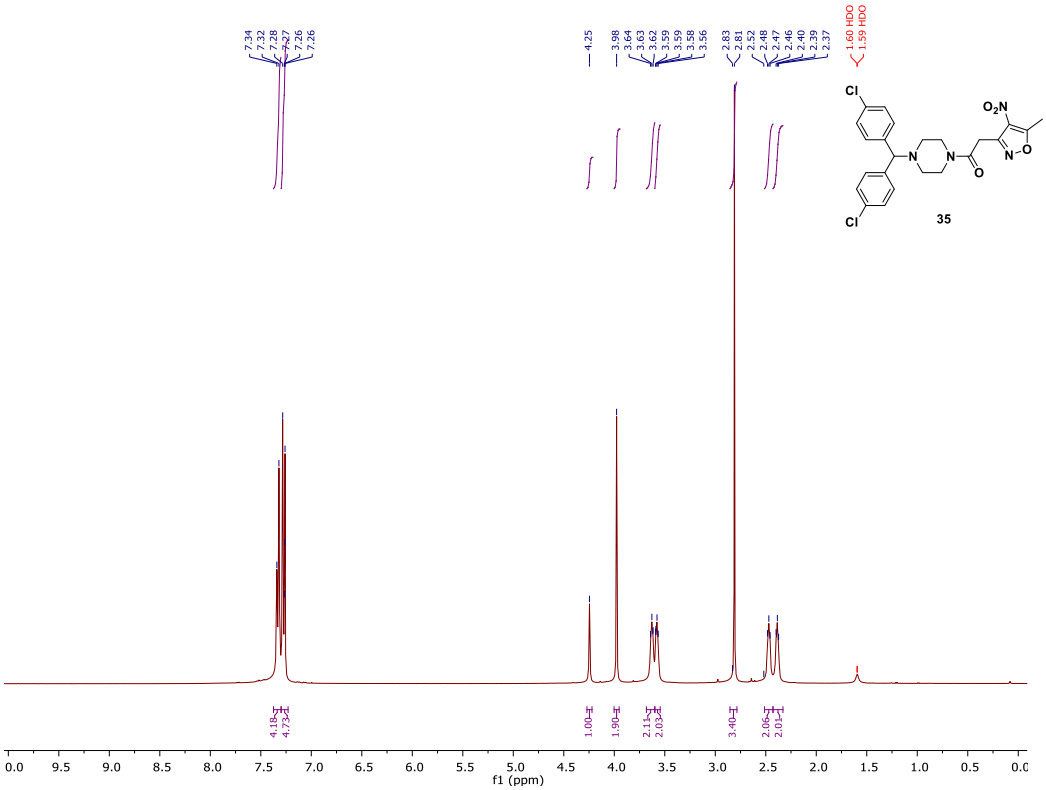

171.87
171.49
156.79
153.09
140.04
133.45
129.64
129.20
129.10
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
74.44
69.19
66.72
56.96
53.38
51.63
51.07
47.15
42.55
35.50
33.04
f1 (ppm)

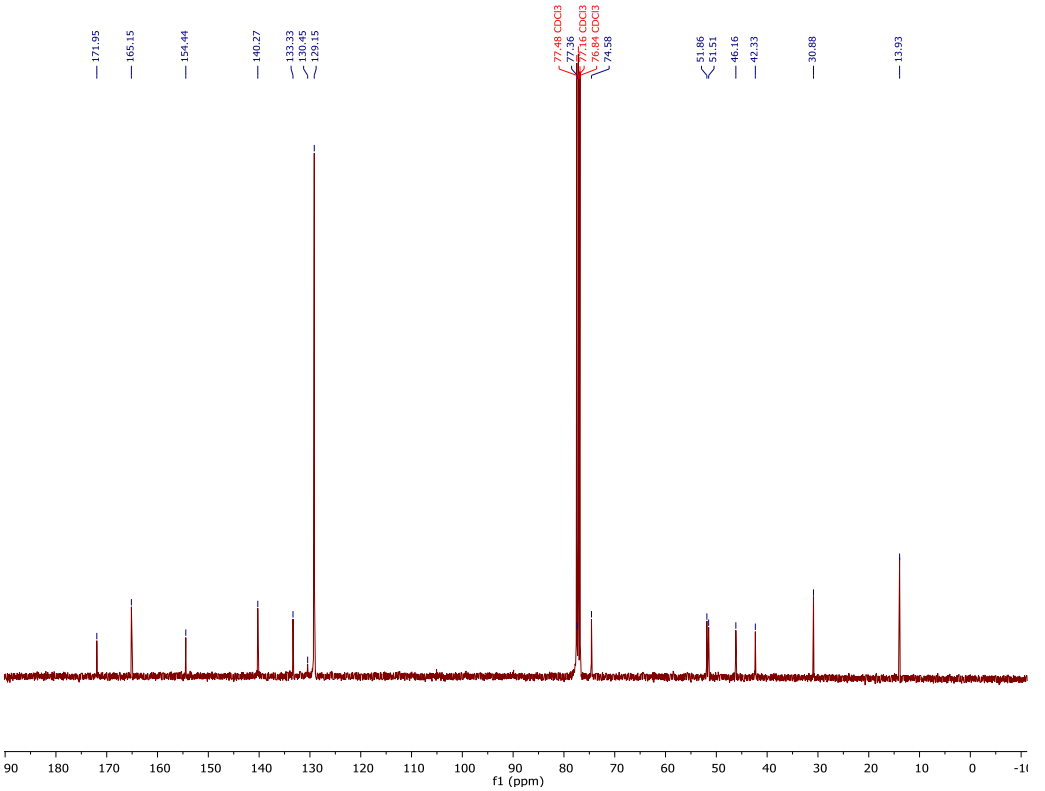
Compound 34


7.31
7.31
7.30
7.28
7.28
7.27
7.26 CDCl3
7.26
7.25
7.25
7.24
7.24
7.23
7.23
7.22
7.22
7.21
7.21
4.19
3.90
2.80
2.65
2.63
2.62
2.38
Cl
Cl
N
N
NO2
N
O
34
3.92
3.98
1.00
1.82
2.80
4.08
4.03
f1 (ppm)

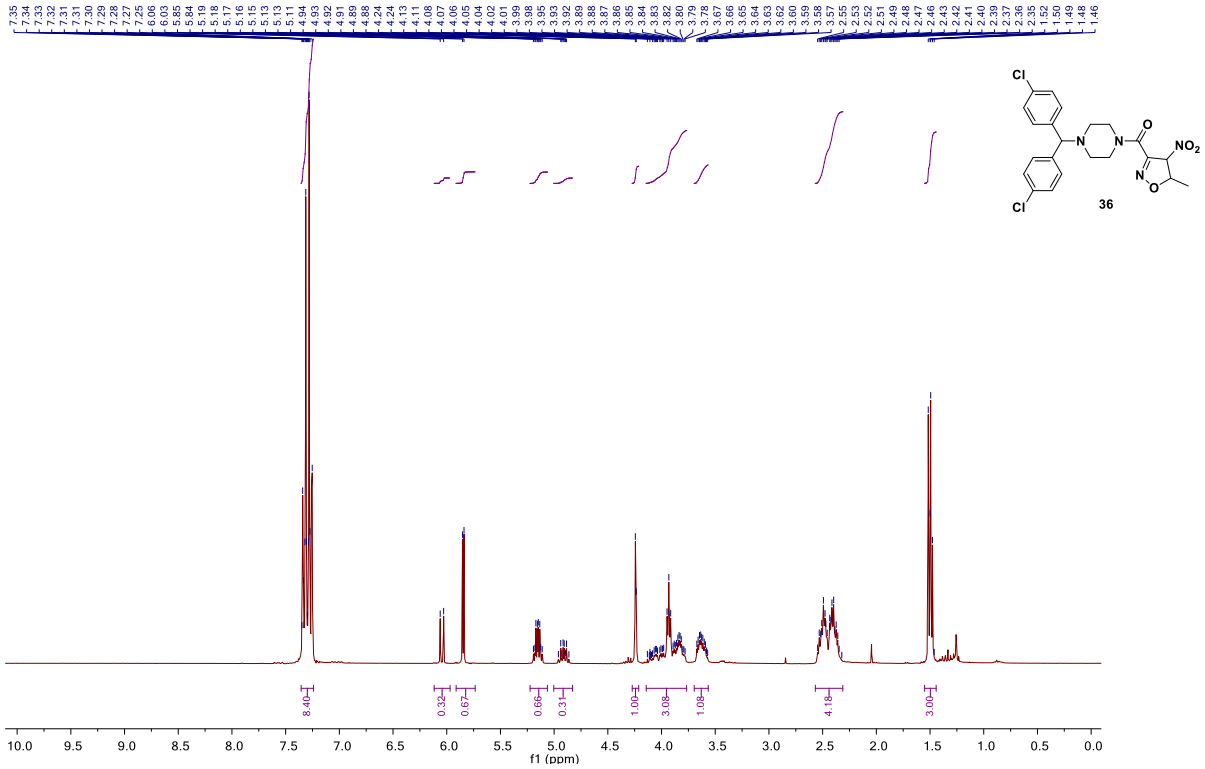

172.19
155.41
140.72
132.98
129.93
129.24
129.17
129.08
128.90
77.48 CDCl3
77.36
77.16 CDCl3
76.84 CDCl3
74.65
53.32
51.93
51.66
13.97
f1 (ppm)

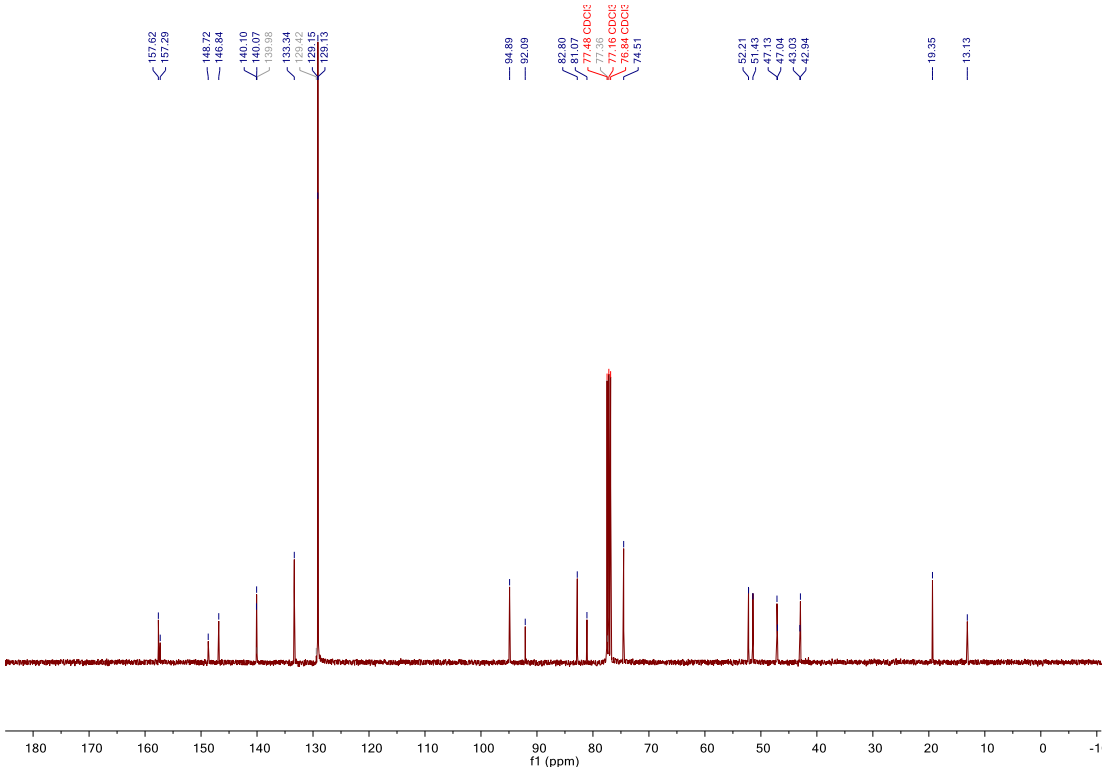
Compound 35


7.34
7.32
7.28
7.27
7.26
7.26
7.26
4.25
3.98
3.64
3.63
3.62
3.59
3.59
3.58
3.56
2.83
2.81
2.52
2.48
2.47
2.46
2.40
2.39
2.37
1.60 HDO
1.59 HDO
Cl
Cl
O_2N
N
N
N
O
O
35
4.18
4.73
1.00
1.90
2.11
2.03
3.40
2.06
2.01
f1 (ppm)

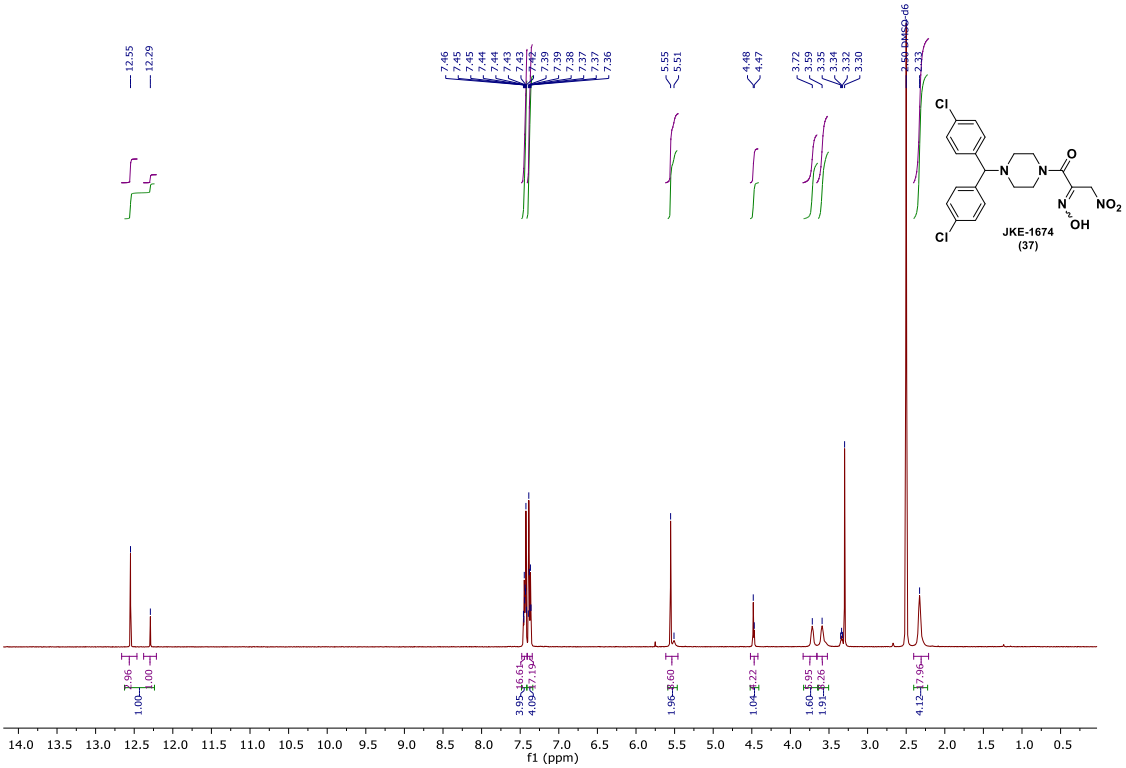

171.95
165.15
154.44
140.27
133.33
130.45
129.15
77.48 CDCl3
77.36
77.16 CDCl3
76.84 CDCl3
74.58
51.86
51.51
46.16
42.33
30.88
13.93
f1 (ppm)

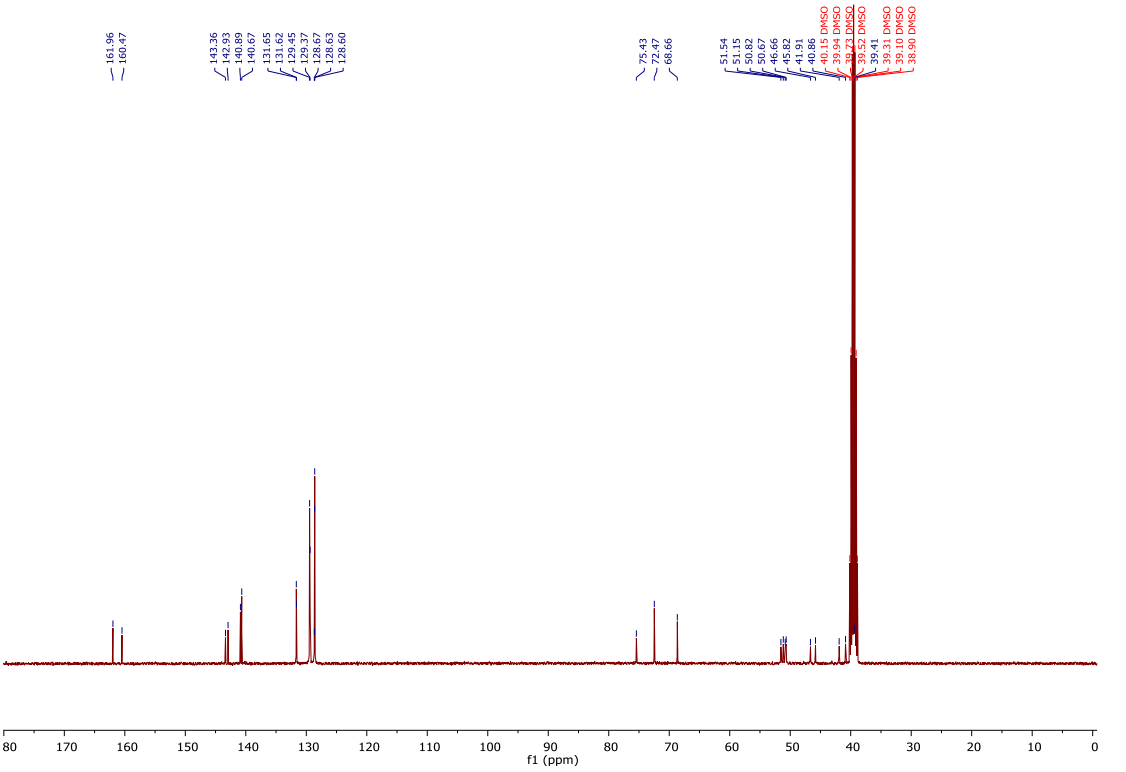
Compound 36


Cl
Cl
N
N
O
NO2
N
O
36
8.40
0.32
0.67
0.66
0.31
1.00
3.08
1.08
4.18
3.00
f1 (ppm)

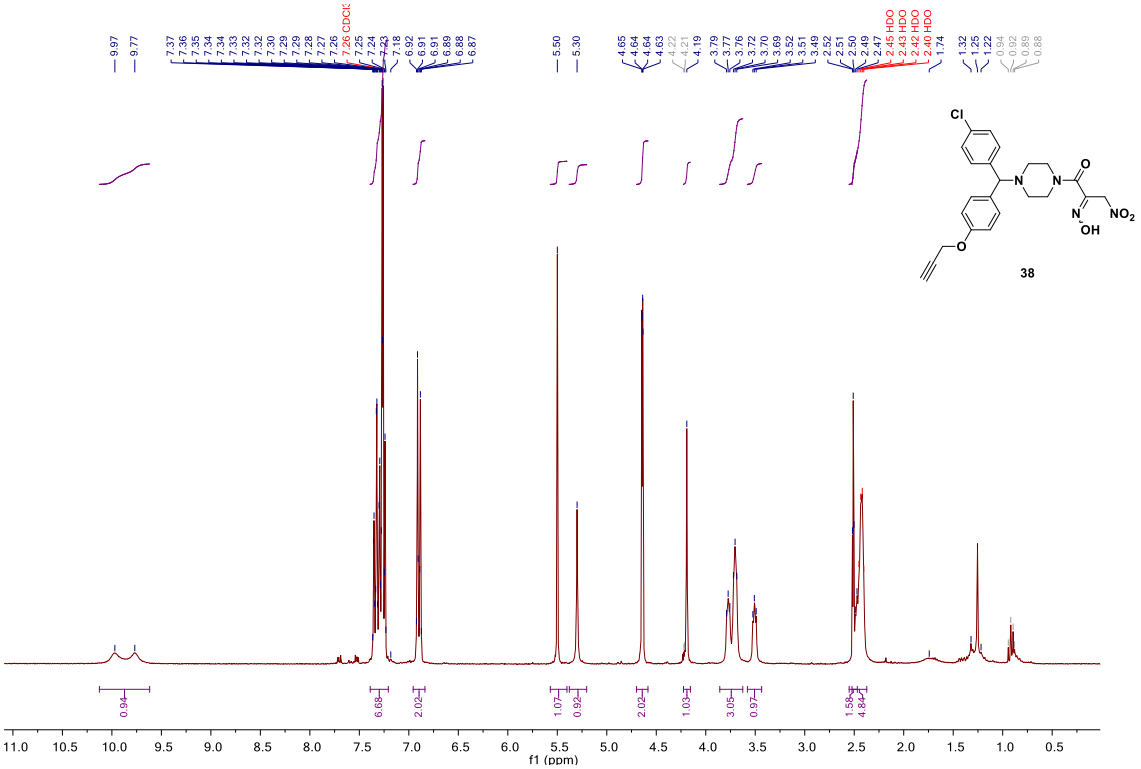

157.62
157.29
148.72
146.84
140.10
140.07
139.98
133.34
129.22
129.15
129.13
94.89
92.09
82.80
81.07
77.48 CDCl3
77.36
77.16 CDCl3
76.84 CDCl3
74.51
52.21
51.43
47.13
47.04
43.03
42.94
19.35
13.13
f1 (ppm)

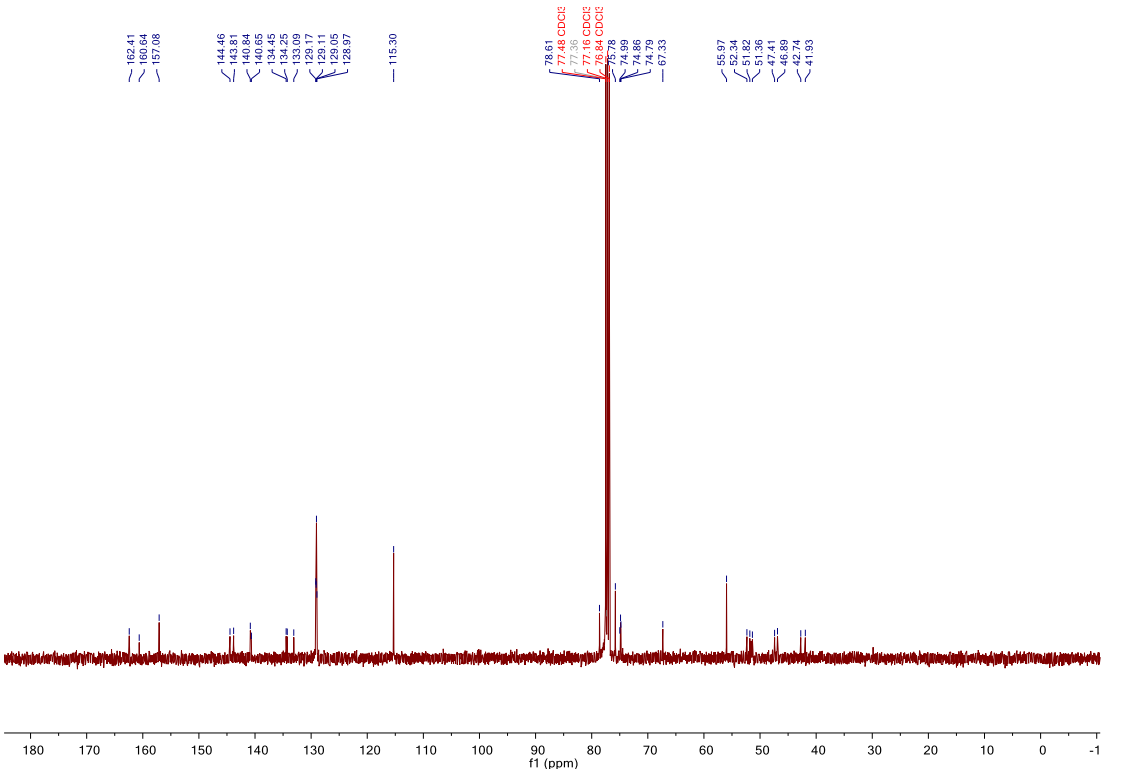
JKE-1674 (37)


12.55
12.29
7.46
7.45
7.45
7.44
7.44
7.43
7.43
7.42
7.39
7.39
7.38
7.37
7.37
7.36
5.55
5.51
4.48
4.47
3.72
3.56
3.35
3.34
3.32
3.30
2.50 DMSO d6
2.33
Cl
Cl
O
N
N
N
N
NO2
OH
JKE-1674
(37)
7.96
1.00
1.00
6.61
7.19
1.60
2.22
1.26
7.96
f1 (ppm)

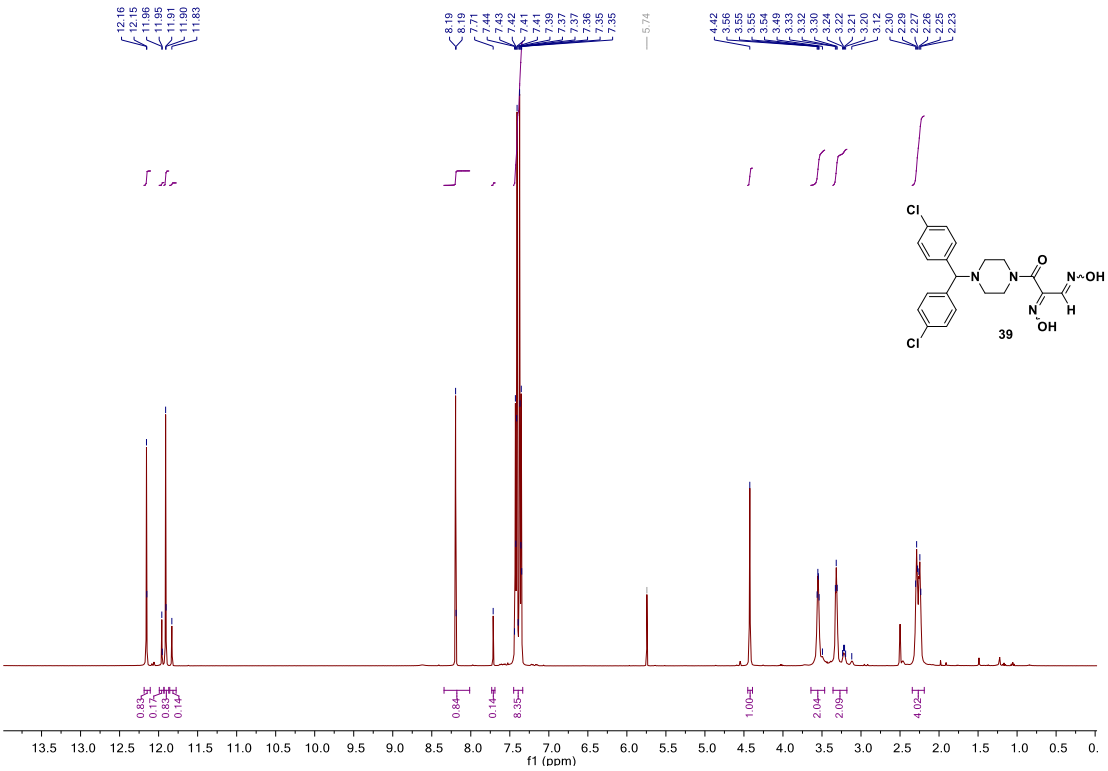

161.96
160.47
143.36
142.93
140.89
140.67
131.65
131.62
129.45
129.27
128.67
128.63
128.60
75.43
73.47
68.66
51.54
51.15
50.82
50.67
46.66
45.82
41.91
40.86
40.15 DMSO
39.73 DMSO
39.52 DMSO
39.41
39.31 DMSO
39.10 DMSO
38.90 DMSO
f1 (ppm)

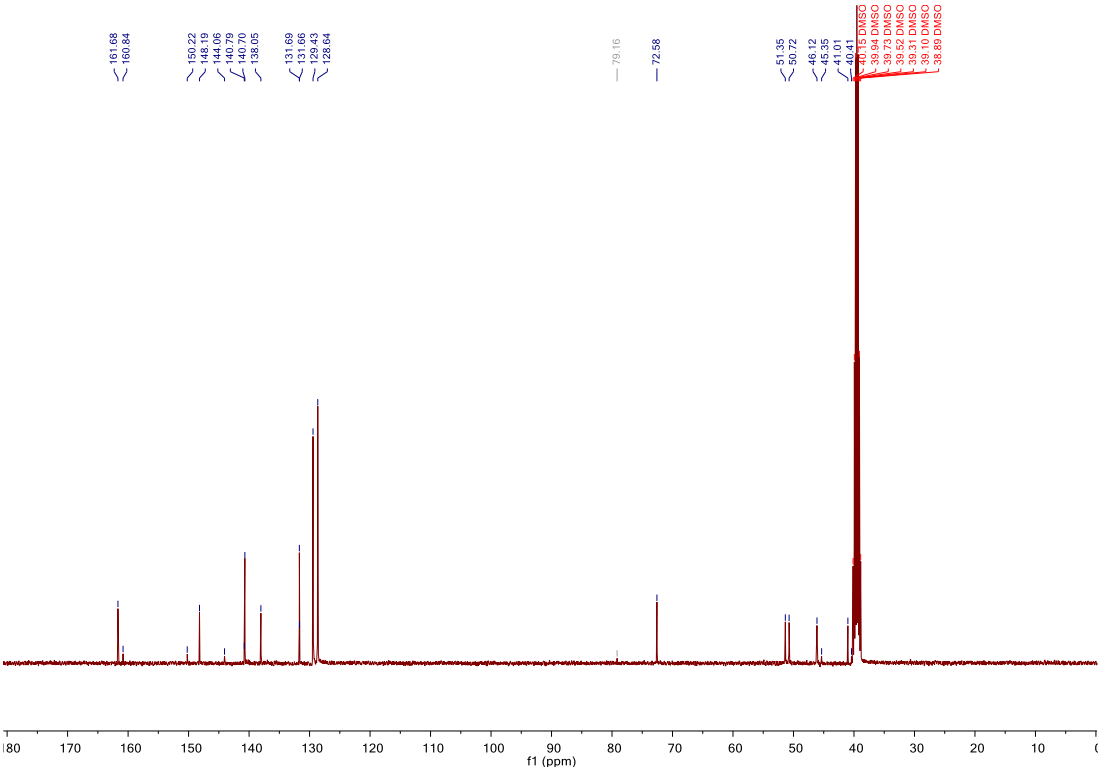
Compound 38


9.97
9.77
7.37
7.36
7.35
7.34
7.34
7.33
7.32
7.32
7.30
7.29
7.29
7.28
7.27
7.26
7.26 CDCl3
7.25
7.24
7.23
7.18
6.92
6.91
6.91
6.89
6.88
6.87
5.50
5.30
4.65
4.64
4.64
4.63
4.22
4.21
4.19
3.79
3.77
3.76
3.72
3.70
3.69
3.52
3.51
3.49
2.52
2.51
2.50
2.49
2.47
2.45 HDO
2.43 HDO
2.42 HDO
2.40 HDO
1.74
1.32
1.25
1.22
0.94
0.92
0.89
0.88
0.94
6.68
2.02
1.07
0.92
2.02
1.03
3.05
0.97
1.58
4.84
f1 (ppm)
Cl
O
N
N
O
N
OH
NO2
O
38

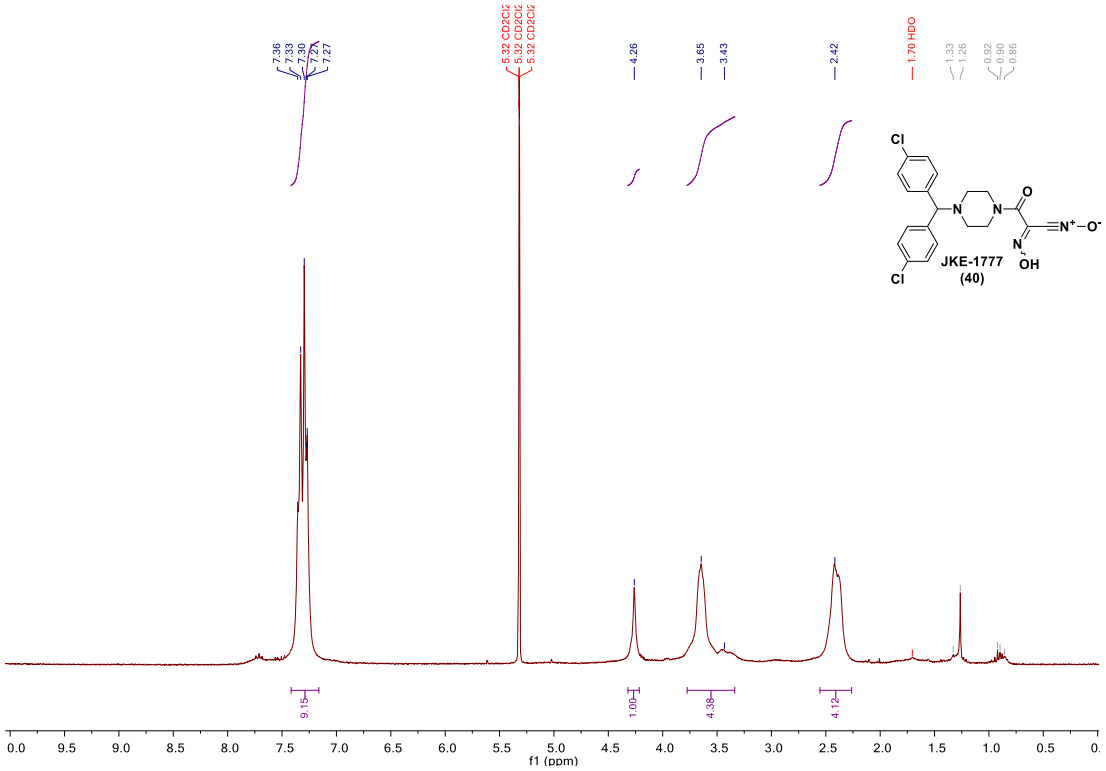

162.41
160.64
157.08
144.46
143.81
140.84
140.65
134.45
134.25
133.09
129.17
129.11
129.05
128.97
115.30
78.61
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
75.78
74.99
74.86
74.79
67.33
55.97
52.34
51.82
51.36
47.41
46.89
42.24
41.93
f1 (ppm)

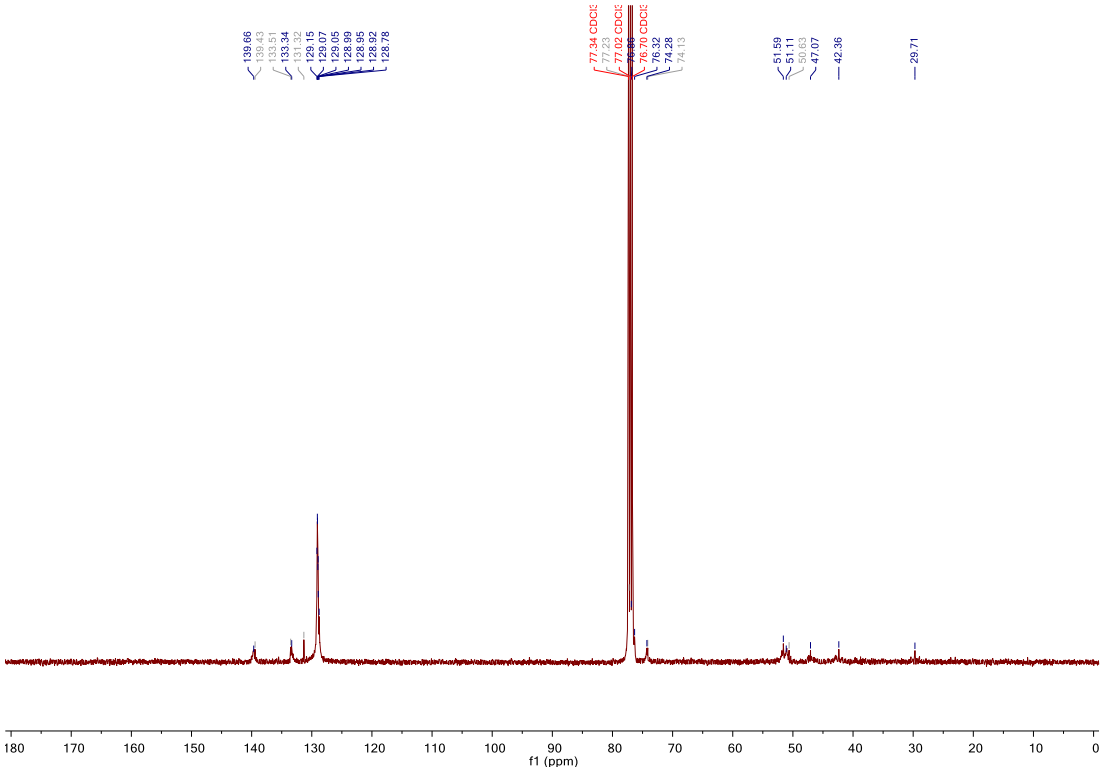
Compound 39


12.16
12.15
11.96
11.95
11.91
11.90
11.83
8.19
8.19
7.71
7.44
7.43
7.42
7.41
7.41
7.39
7.37
7.37
7.36
7.35
7.35
5.74
4.42
3.56
3.55
3.55
3.54
3.49
3.33
3.32
3.30
3.24
3.22
3.21
3.00
3.12
2.30
2.29
2.27
2.26
2.25
2.23
Cl
N
N
O
N
OH
N
H
OH
Cl
39
0.83
0.17
0.83
0.14
0.84
0.14
8.35
1.00
2.04
2.09
4.02
f1 (ppm)

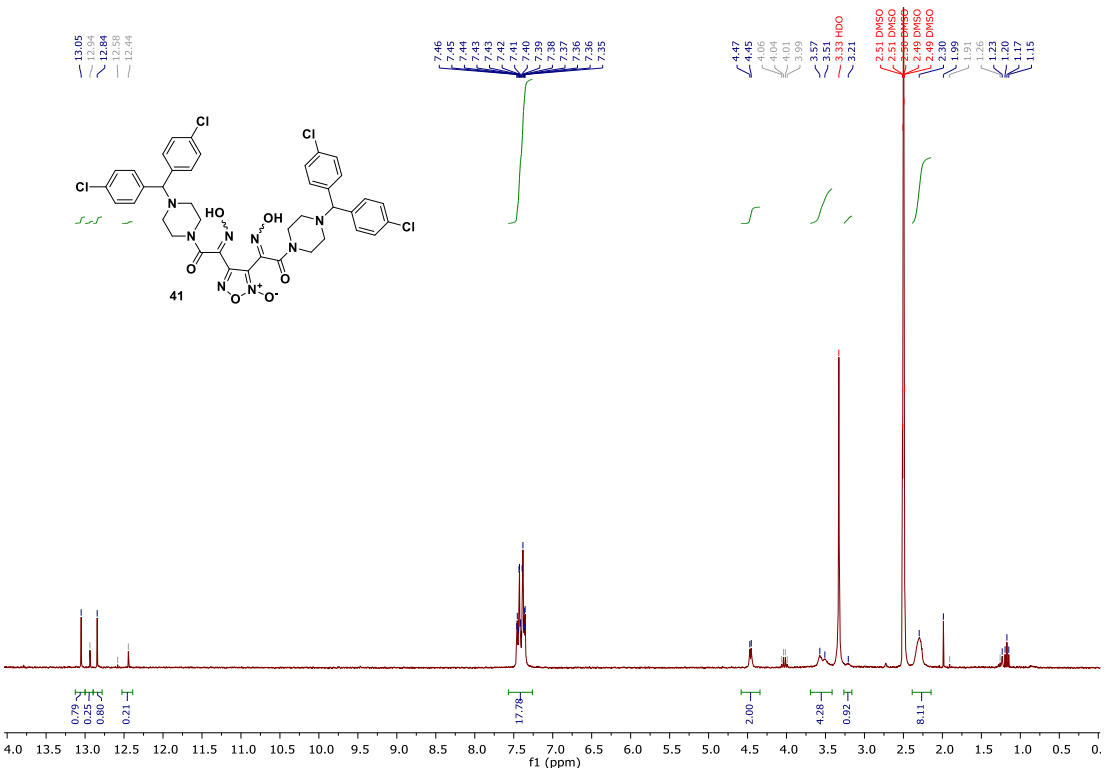

161.68
160.84
150.22
148.19
144.06
140.79
140.70
138.05
131.69
131.66
129.43
128.64
79.16
72.58
51.35
50.72
46.12
45.35
41.01
40.41
40.15 DMSO
39.94 DMSO
39.73 DMSO
39.52 DMSO
39.31 DMSO
39.10 DMSO
38.89 DMSO
f1 (ppm)

JKE-1777 (40)

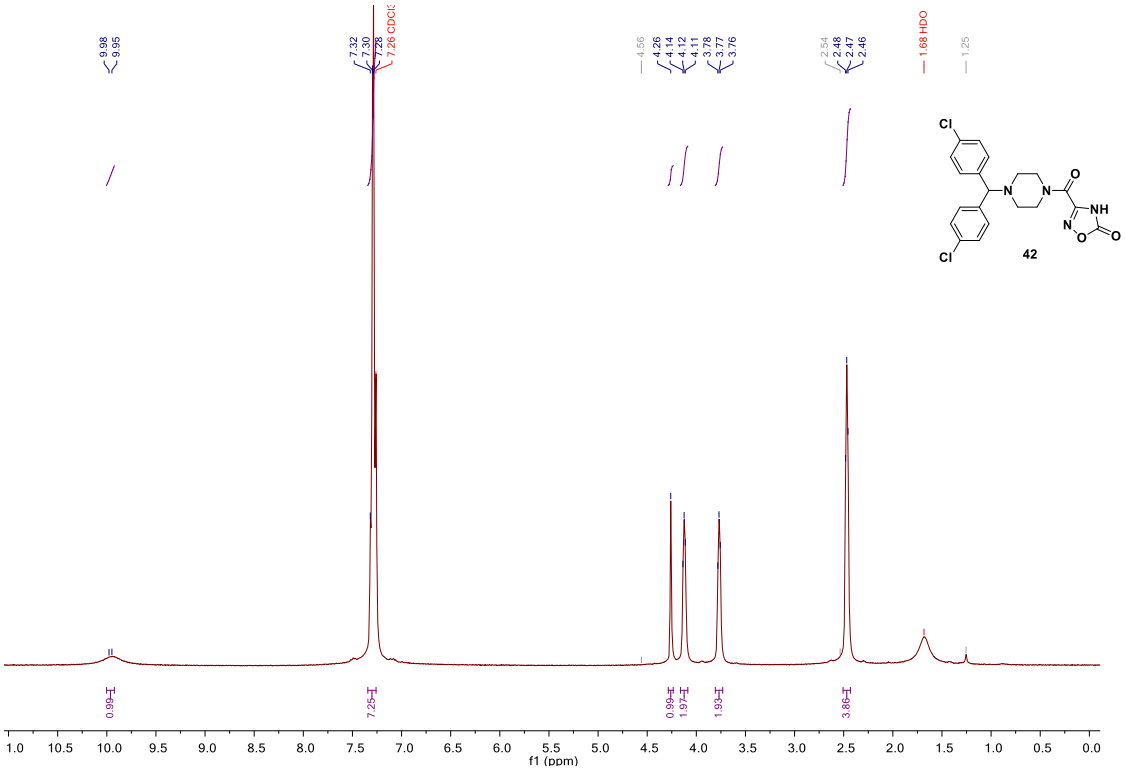

7.36
7.33
7.30
7.27
5.32 CD2Cl2
4.26
3.65
3.43
2.42
1.70 HDO
1.33
1.26
0.92
0.90
0.86
Cl
Cl
JKE-1777
(40)
9.15
1.00
4.38
4.12
f1 (ppm)

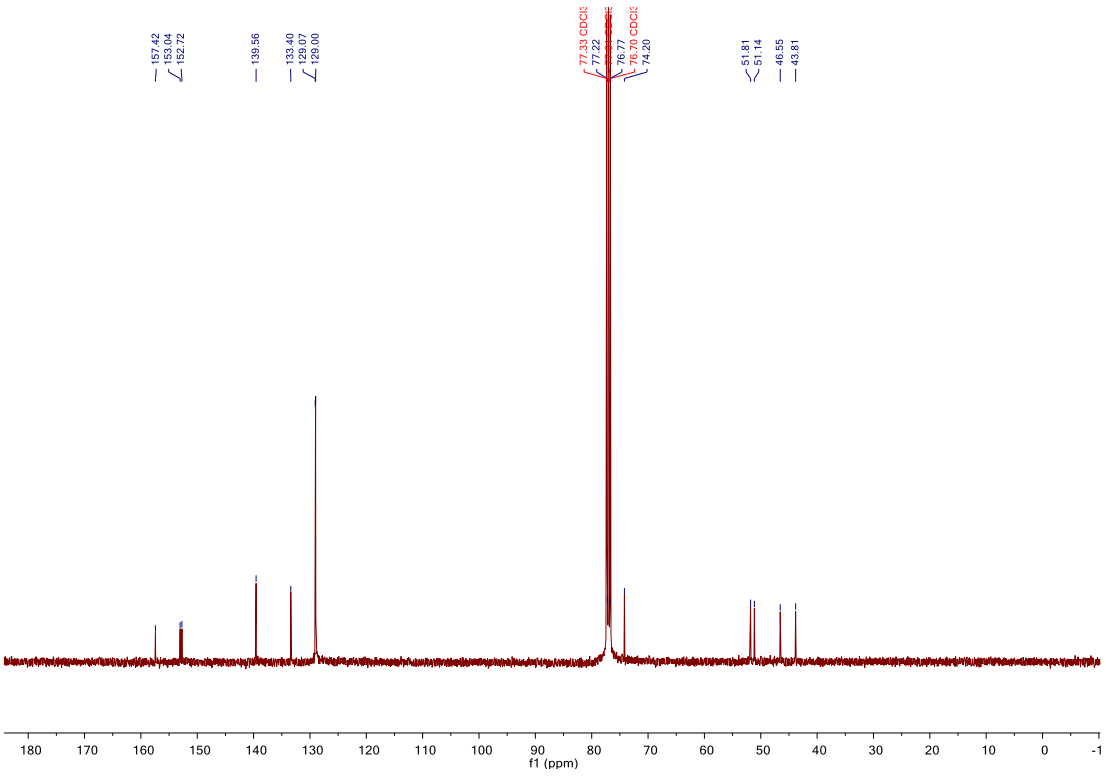

139.66
139.43
133.51
133.34
131.32
129.15
129.07
129.05
128.99
128.95
128.92
128.78
77.34 CDCl3
77.23
77.02 CDCl3
76.70 CDCl3
76.32
74.28
74.13
51.59
51.11
50.63
47.07
42.36
29.71
f1 (ppm)

Compound 41

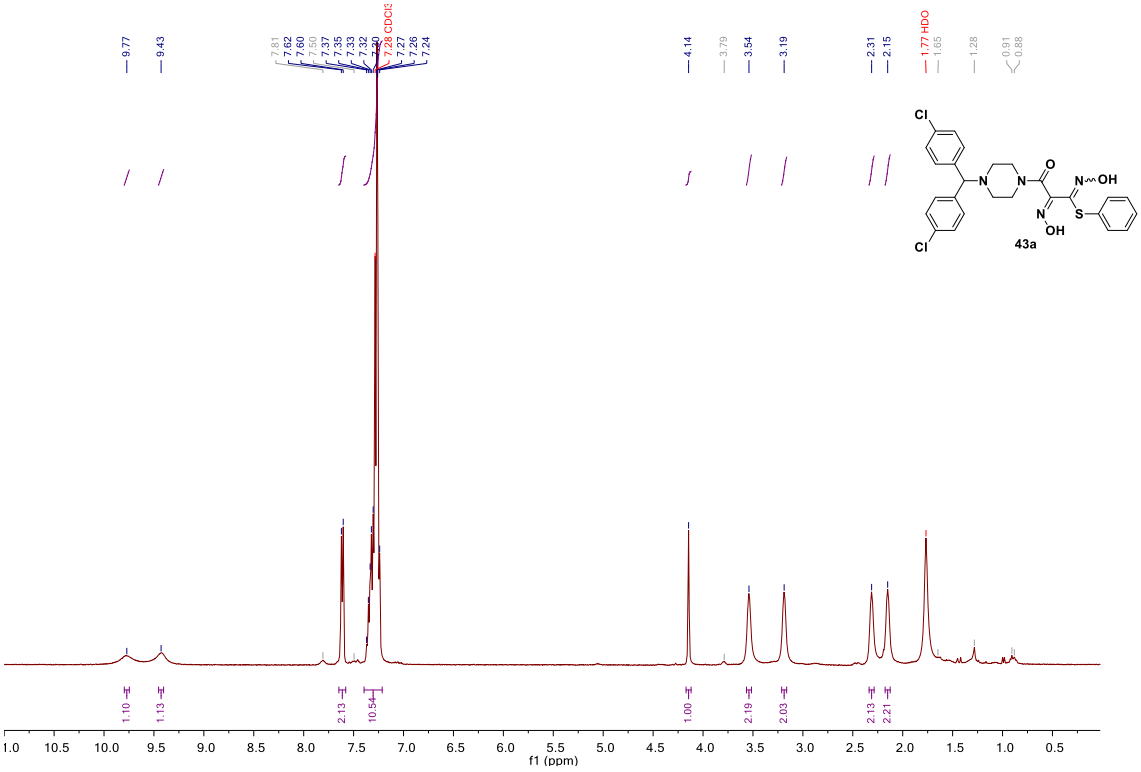


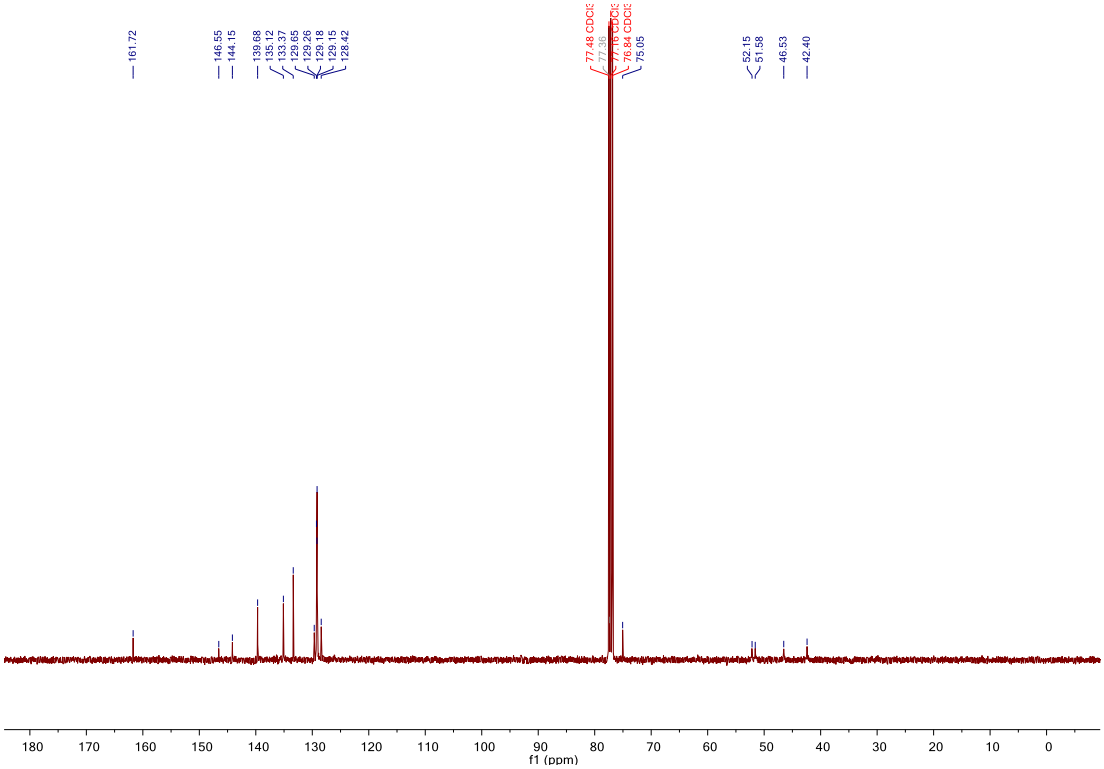
Compound 42


9.98
9.95
7.32
7.30
7.28
7.26 CDCl3
4.56
4.26
4.14
4.12
4.11
3.78
3.77
3.76
2.54
2.48
2.47
2.46
1.68 H2O
1.25
Cl
N
N
O
NH
N
O
O
Cl
42
0.99
7.25
0.99
1.97
1.93
3.86
f1 (ppm)

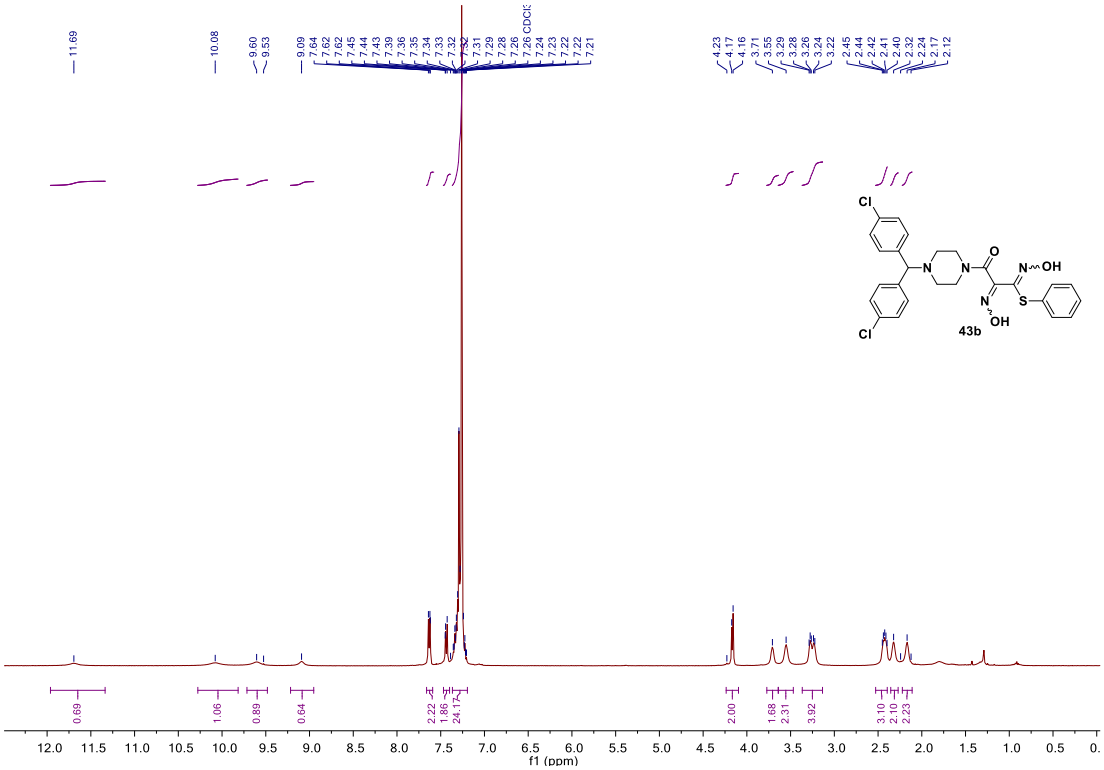

157.42
153.04
152.72
139.56
133.40
129.07
129.00
77.33 CDCl3
77.22
76.77
76.70 CDCl3
74.20
51.81
51.14
46.55
43.81
f1 (ppm)

Compound 43a

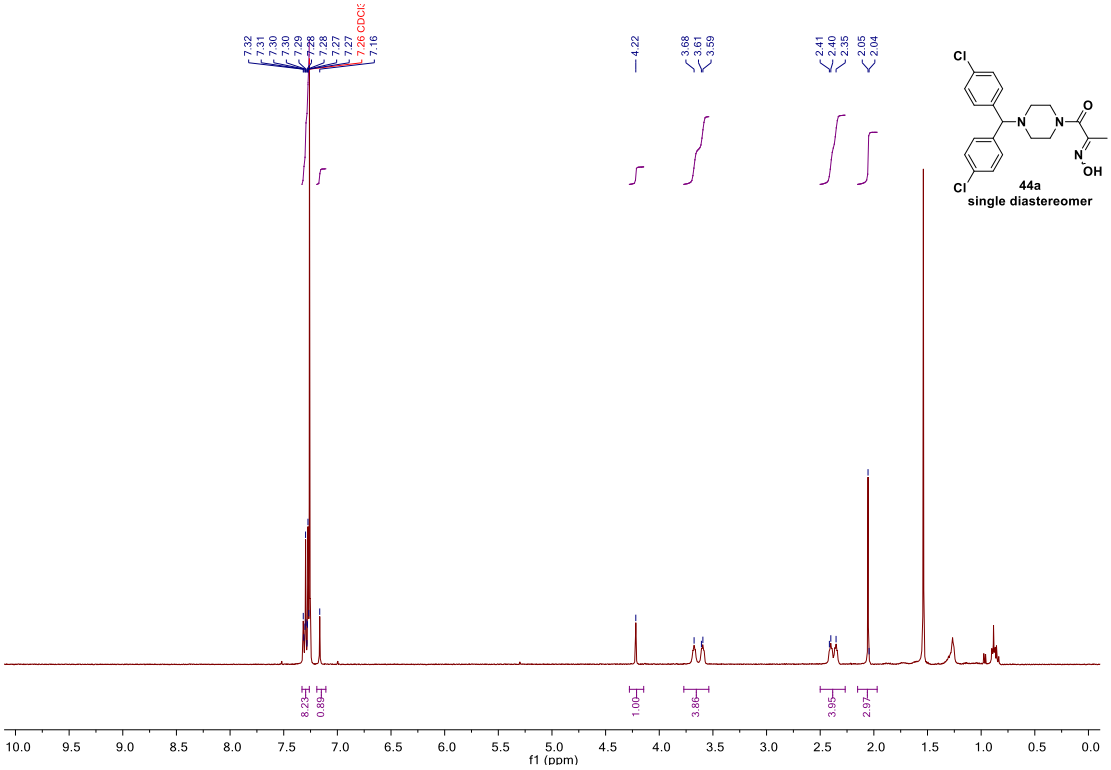


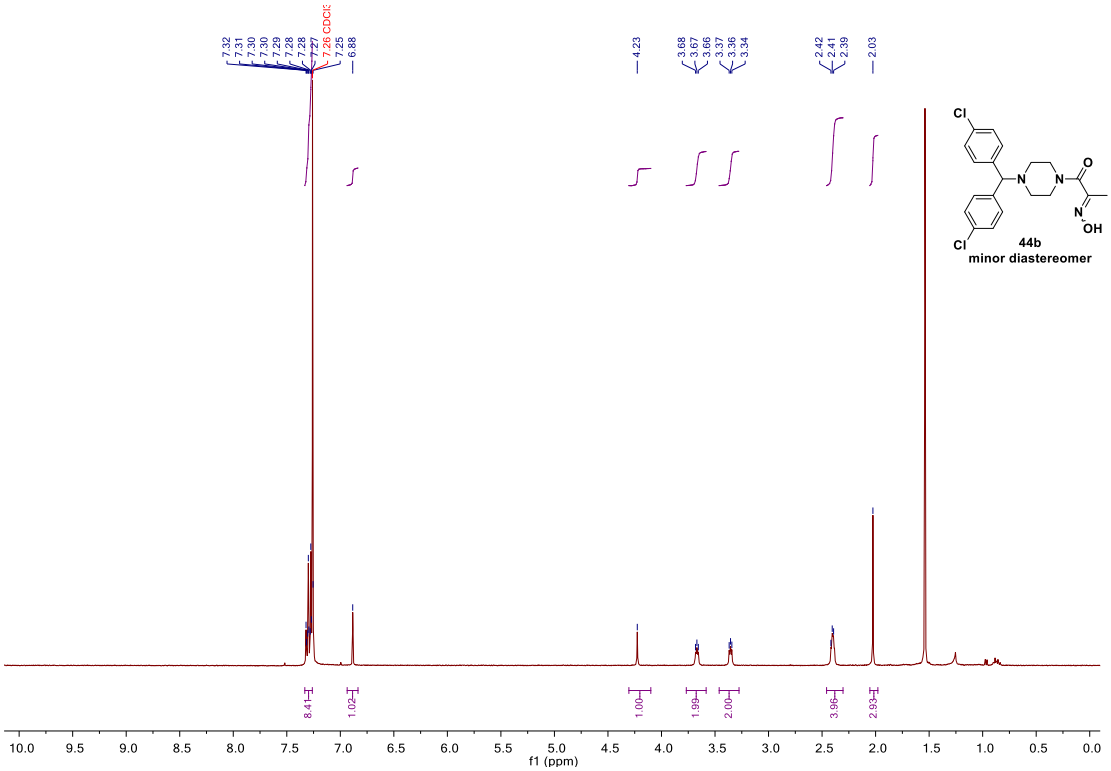


Compound 43b

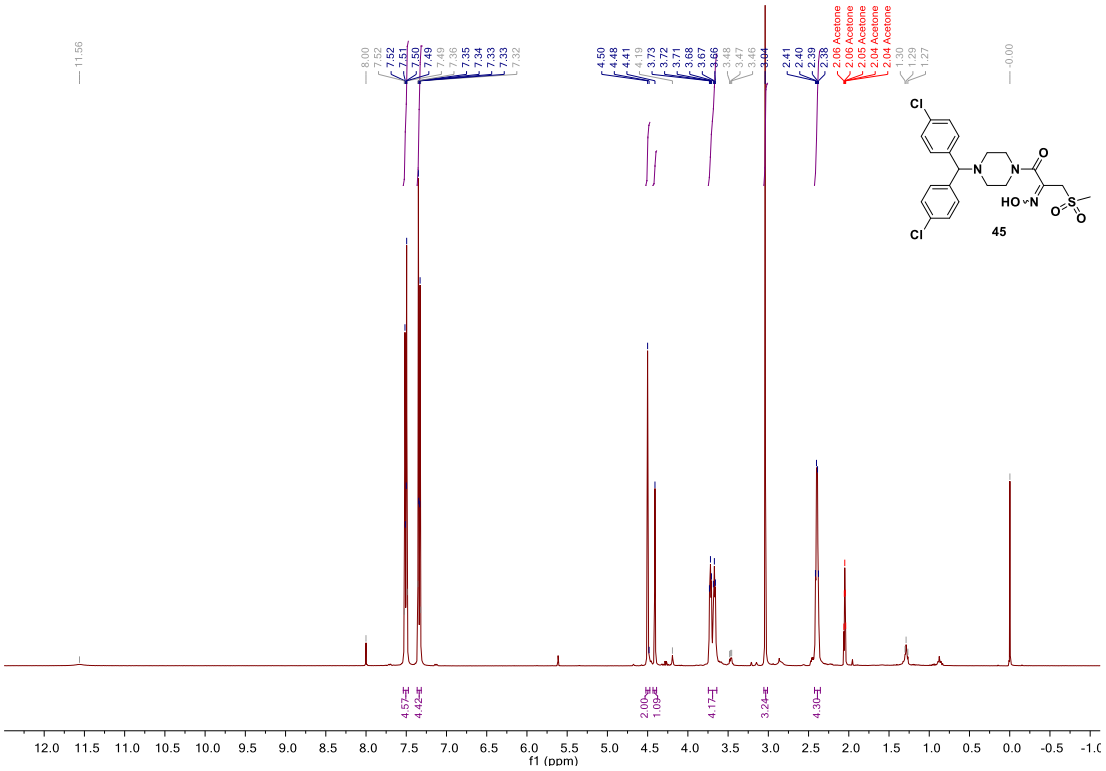


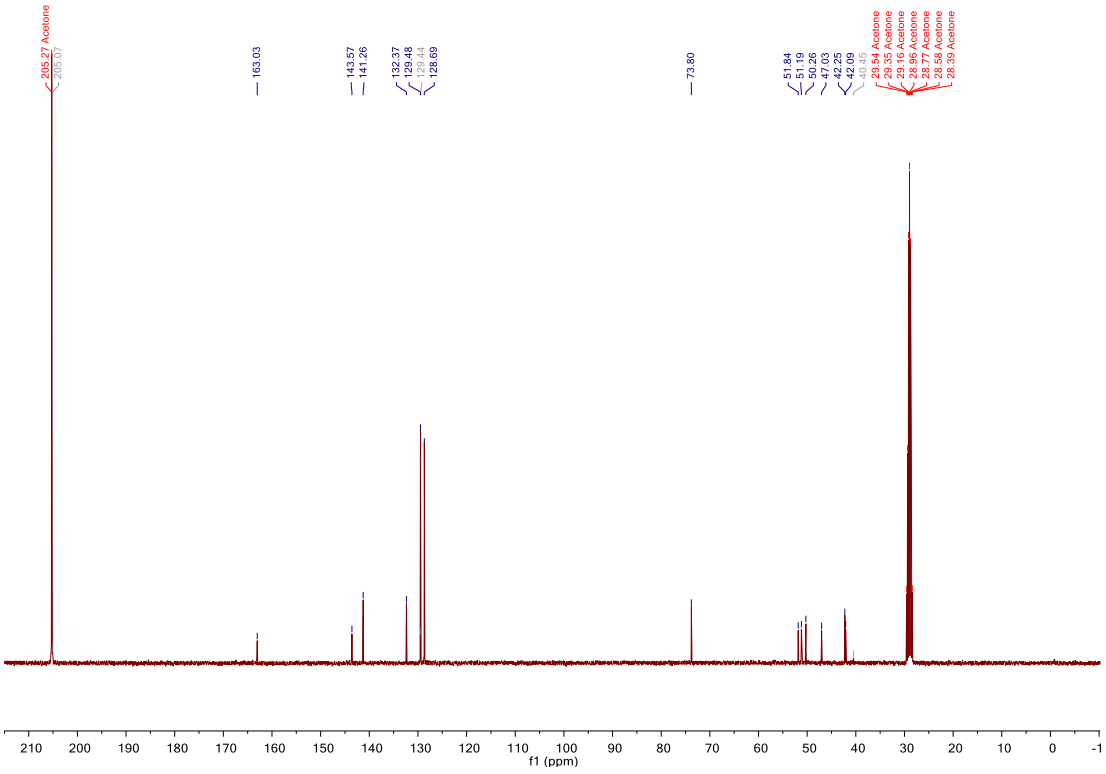
Compound 44


7.32
7.31
7.30
7.30
7.29
7.28
7.28
7.27
7.27
7.26 CDCl3
7.16
4.22
3.68
3.61
3.59
2.41
2.40
2.35
2.05
2.04
Cl
Cl
O
N
N
N
OH
44a
single diastereomer
8.29
0.89
1.00
3.86
3.95
2.97
f1 (ppm)

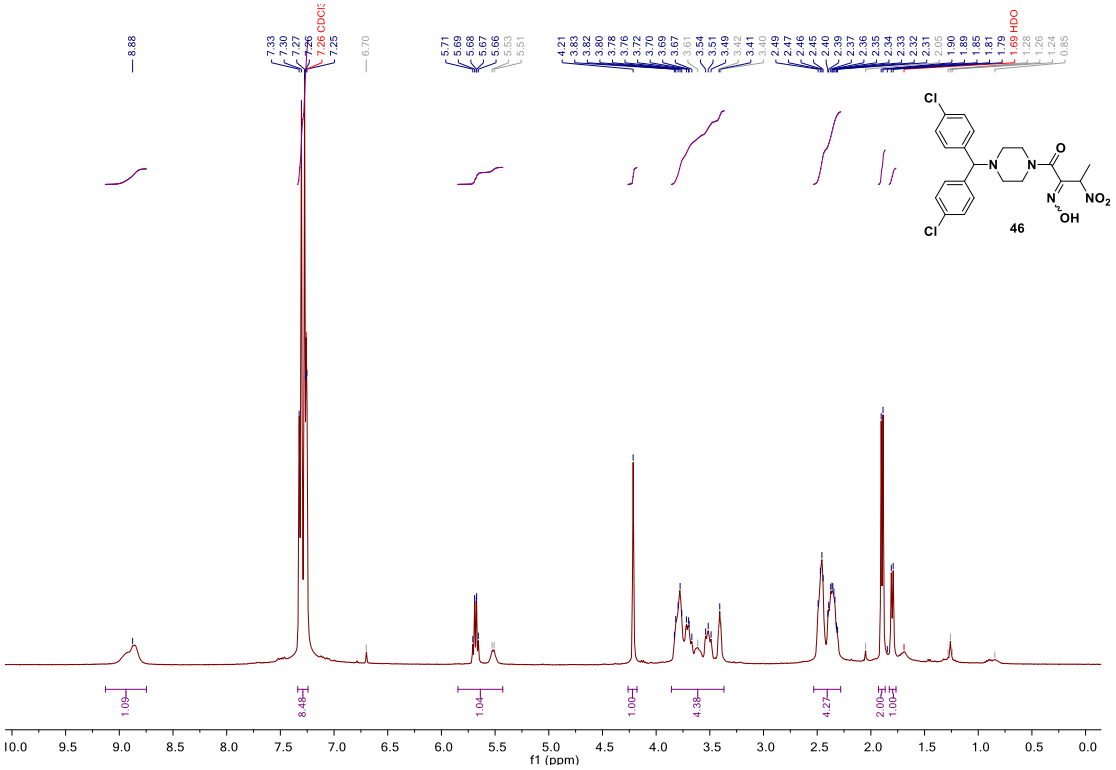

7.32
7.31
7.30
7.30
7.29
7.28
7.28
7.27
7.26 CDCl3
7.25
6.88
4.23
3.68
3.67
3.66
3.37
3.36
3.34
2.42
2.41
2.39
2.03
Cl
Cl
O
N
N
N
OH
44b
minor diastereomer
8.41
1.02
1.00
1.99
2.00
3.96
2.93
f1 (ppm)

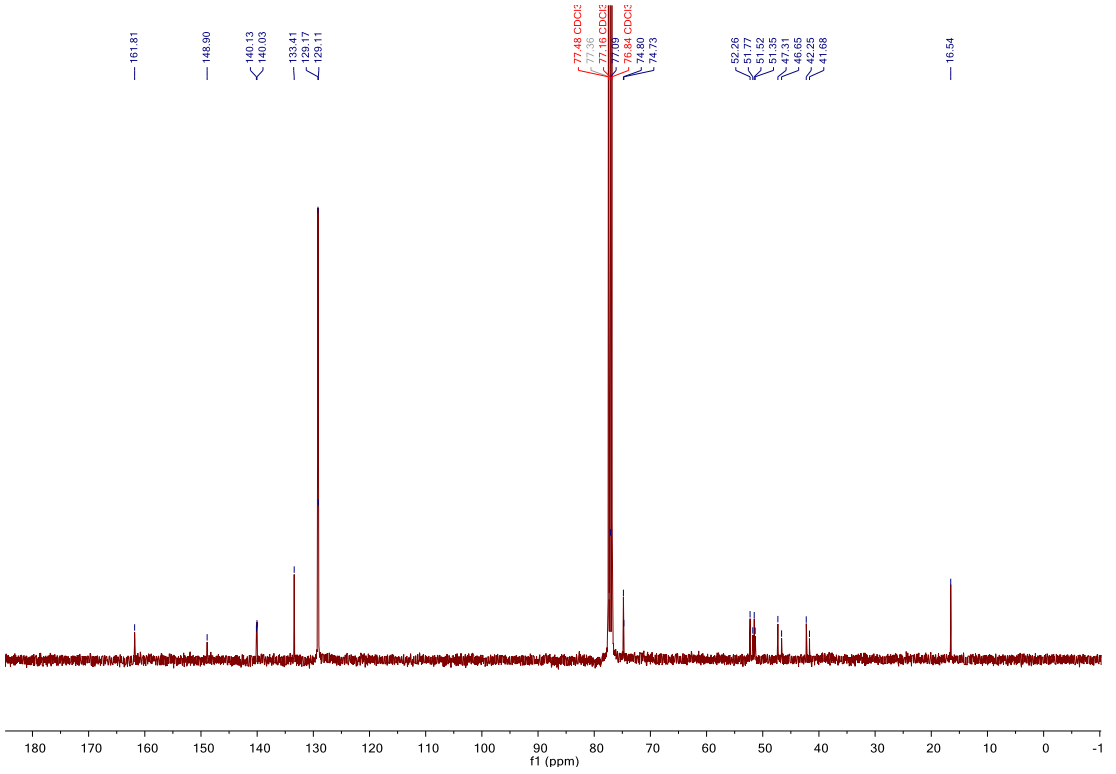
Compound 45


11.56
8.00
7.52
7.51
7.50
7.49
7.36
7.35
7.34
7.33
7.33
7.32
4.50
4.48
4.41
4.19
3.73
3.72
3.71
3.68
3.67
3.66
3.48
3.47
3.46
3.04
2.41
2.40
2.39
2.38
2.06 Acetone
2.06 Acetone
2.05 Acetone
2.04 Acetone
2.04 Acetone
1.30
1.29
1.27
0.00
Cl
Cl
N
N
O
HO
N
S
O
O
45
4.57
4.42
2.00
1.09
4.17
3.24
4.30
f1 (ppm)

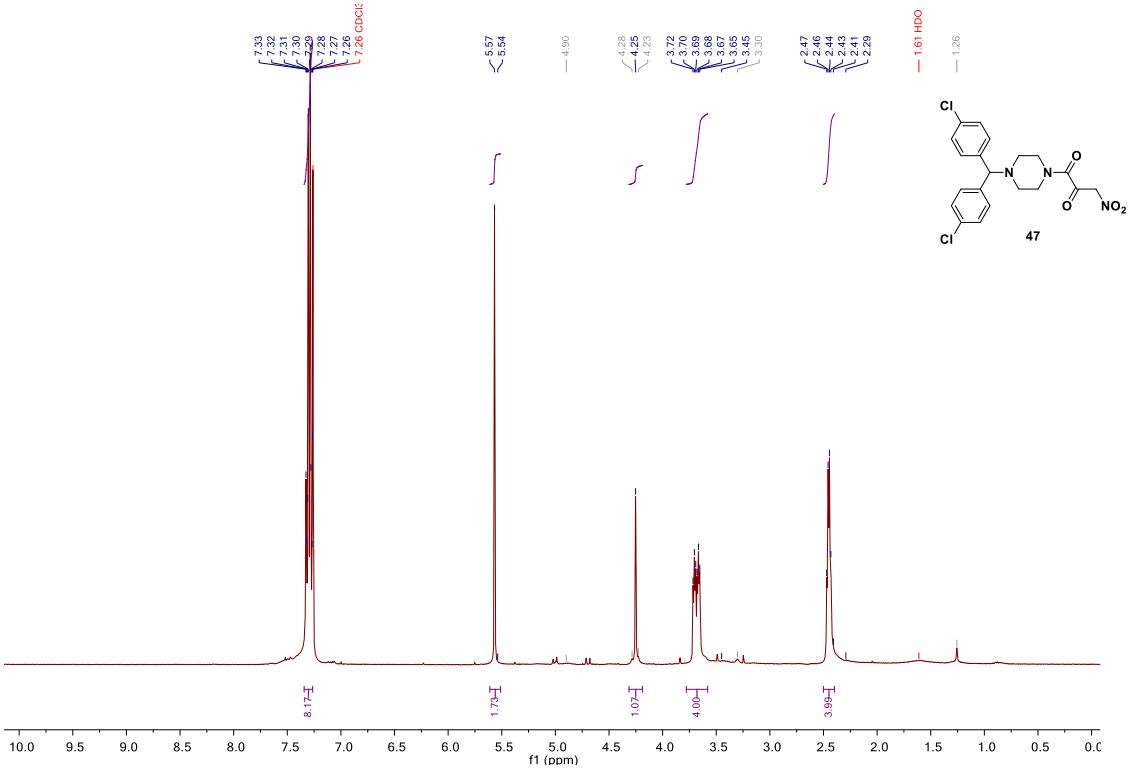

205.27 Acetone
205.07
163.03
143.57
141.26
132.37
129.48
129.44
128.69
73.80
51.84
51.19
50.26
47.03
42.25
42.09
40.45
29.54 Acetone
29.35 Acetone
29.16 Acetone
28.96 Acetone
28.77 Acetone
28.58 Acetone
28.39 Acetone
f1 (ppm)

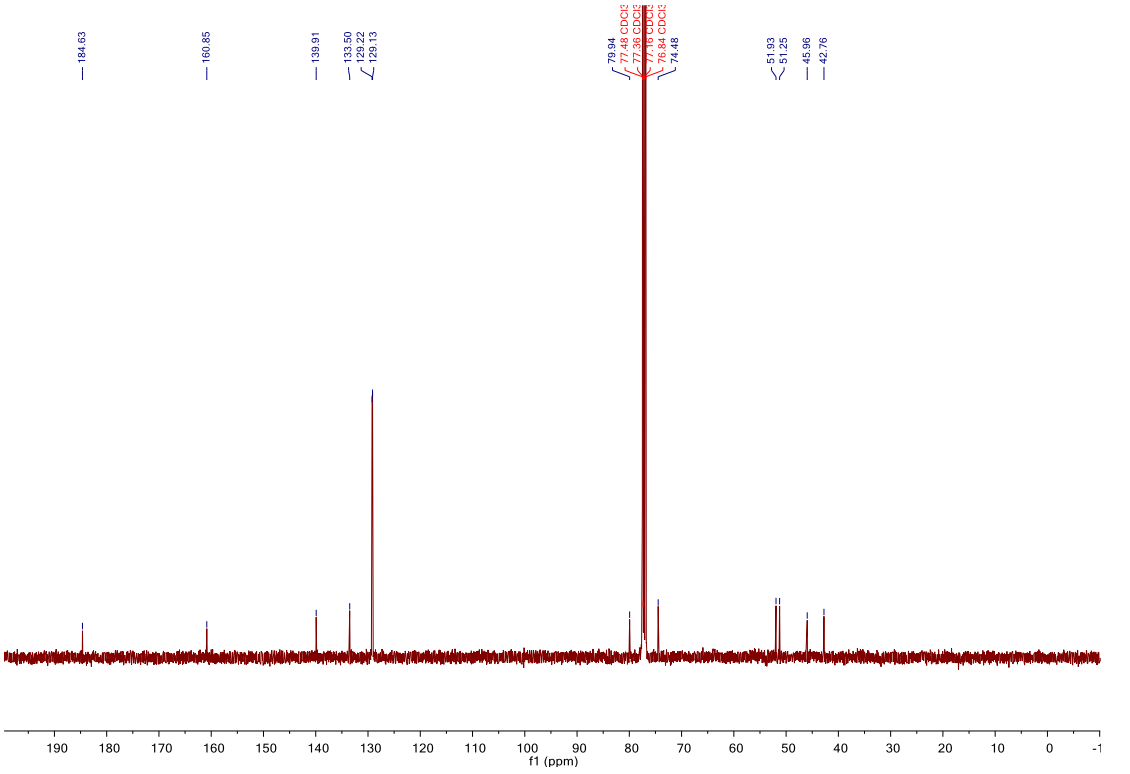
Compound 46


8.88
7.33
7.30
7.27
7.26 CDCl3
7.25
6.70
5.71
5.69
5.68
5.67
5.66
5.53
5.51
4.21
3.83
3.82
3.80
3.78
3.76
3.72
3.70
3.69
3.67
3.61
3.54
3.51
3.49
3.42
3.41
3.40
2.49
2.47
2.46
2.45
2.40
2.39
2.37
2.36
2.35
2.34
2.33
2.32
2.31
2.05
1.90
1.89
1.85
1.81
1.79
1.69 HDO
1.28
1.26
1.24
0.85
Cl
N
N
O
N
OH
NO2
Cl
46
1.09
8.49
1.04
1.00
4.38
4.27
2.00
1.00
f1 (ppm)

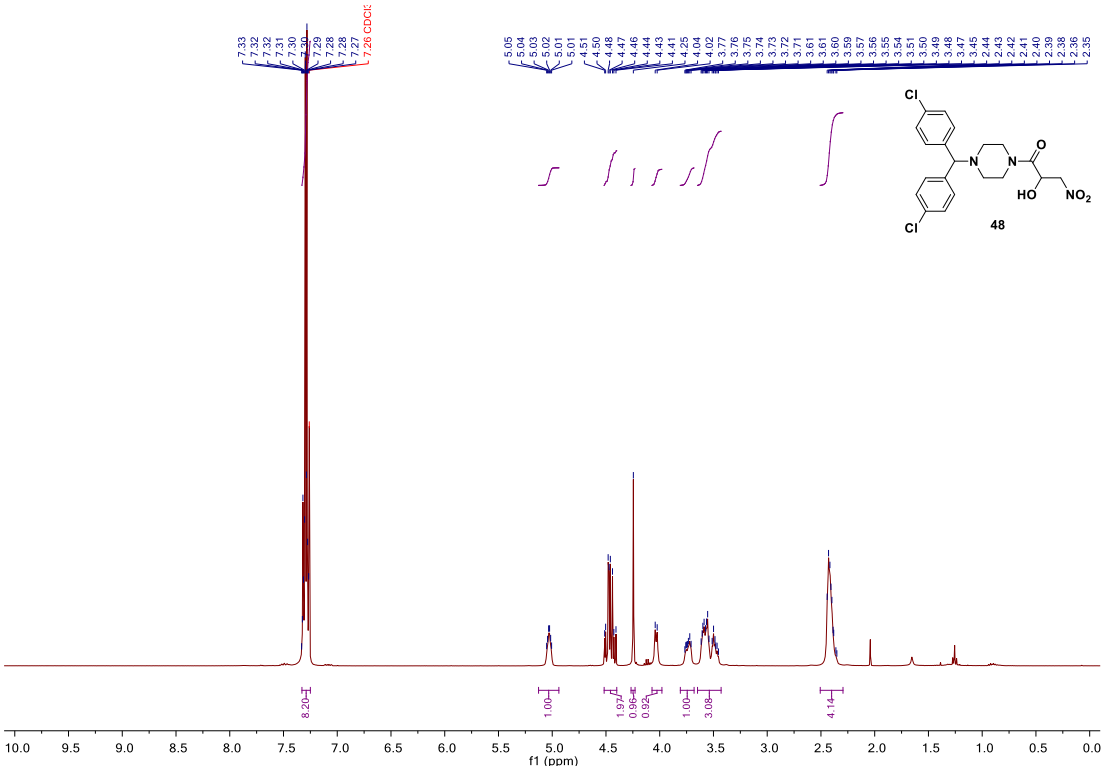

161.81
148.90
140.13
140.03
133.41
129.17
129.11
77.48 CDCl3
77.36
77.15 CDCl3
77.03
76.84 CDCl3
74.80
74.73
52.26
51.77
51.52
51.35
47.31
46.65
42.25
41.68
16.54
f1 (ppm)

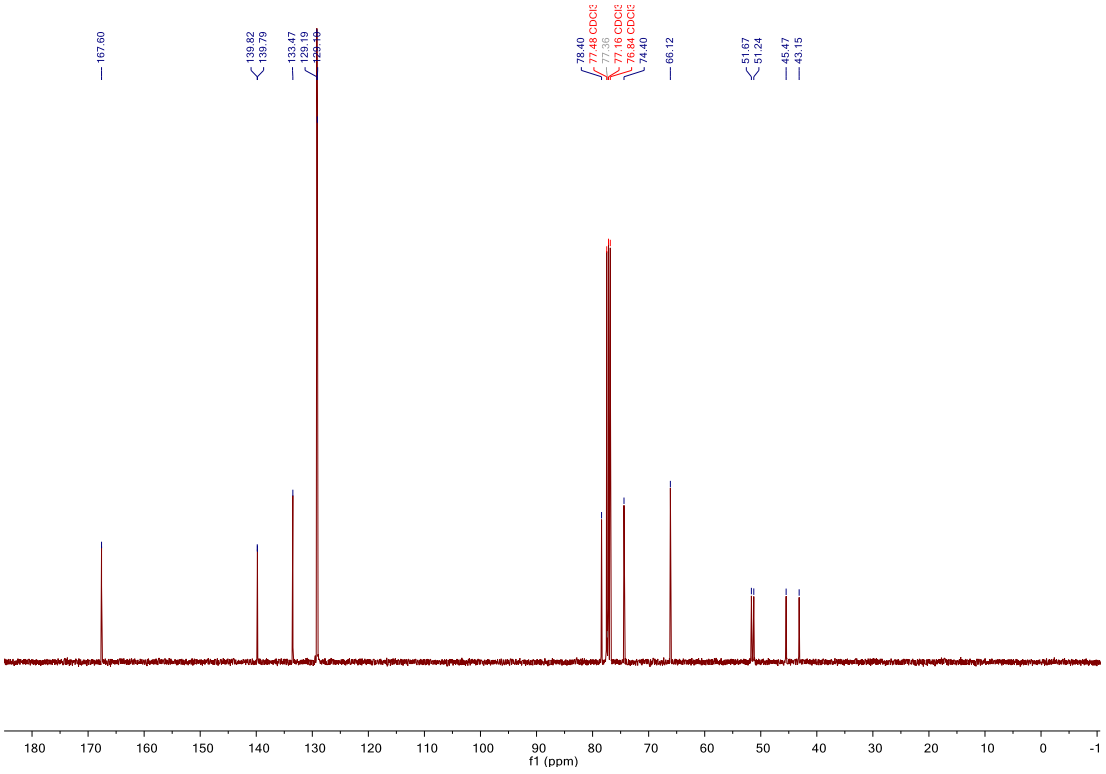
Compound 47


7.33
7.32
7.31
7.30
7.29
7.28
7.27
7.26
7.26 CDCl3
5.57
5.54
4.90
4.28
4.25
4.23
3.72
3.70
3.69
3.68
3.67
3.65
3.45
3.30
2.47
2.46
2.44
2.43
2.41
2.29
1.61 HDO
1.26
8.17
1.73
1.07
4.00
3.99
f1 (ppm)
Cl
N
N
O
O
NO2
Cl
47

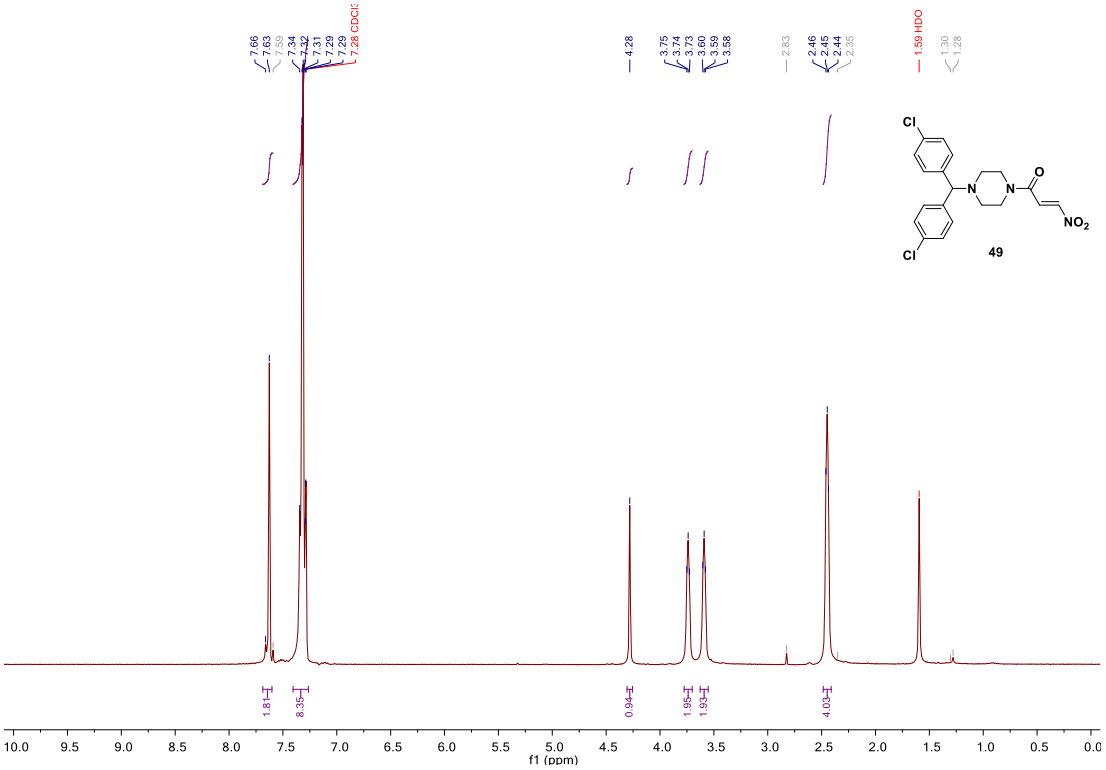

184.63
160.85
139.91
133.50
129.22
129.13
79.94
77.48 CDCl3
77.36 CDCl3
77.16 CDCl3
76.84 CDCl3
74.48
51.93
51.25
45.96
42.76
f1 (ppm)

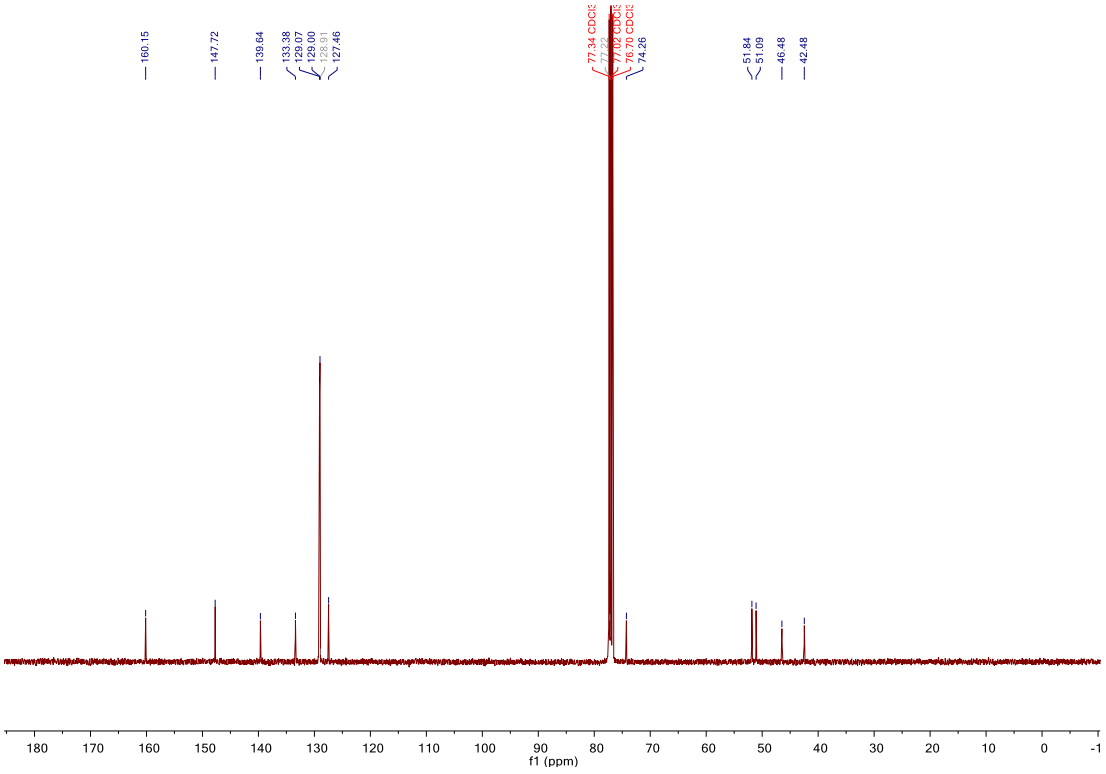
Compound 48


Cl
N
N
O
HO
NO2
Cl
48
f1 (ppm)

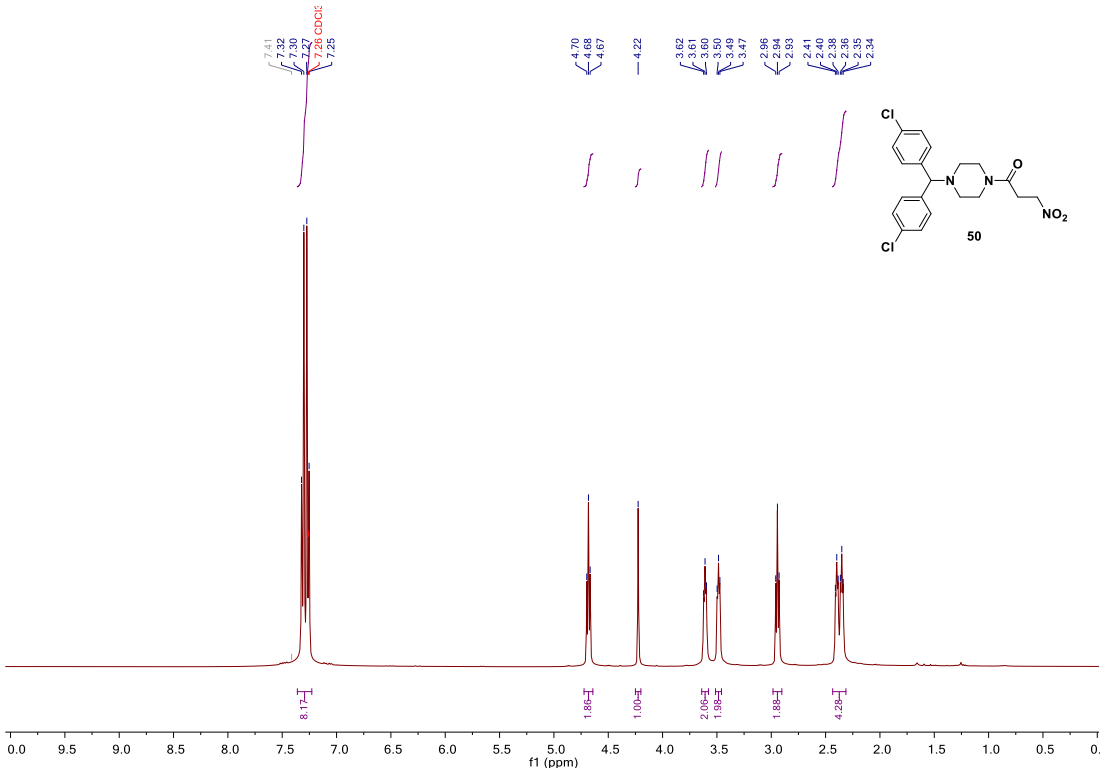

167.60
139.82
139.79
133.47
129.19
78.40
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
74.40
66.12
51.67
51.24
45.47
43.15
f1 (ppm)

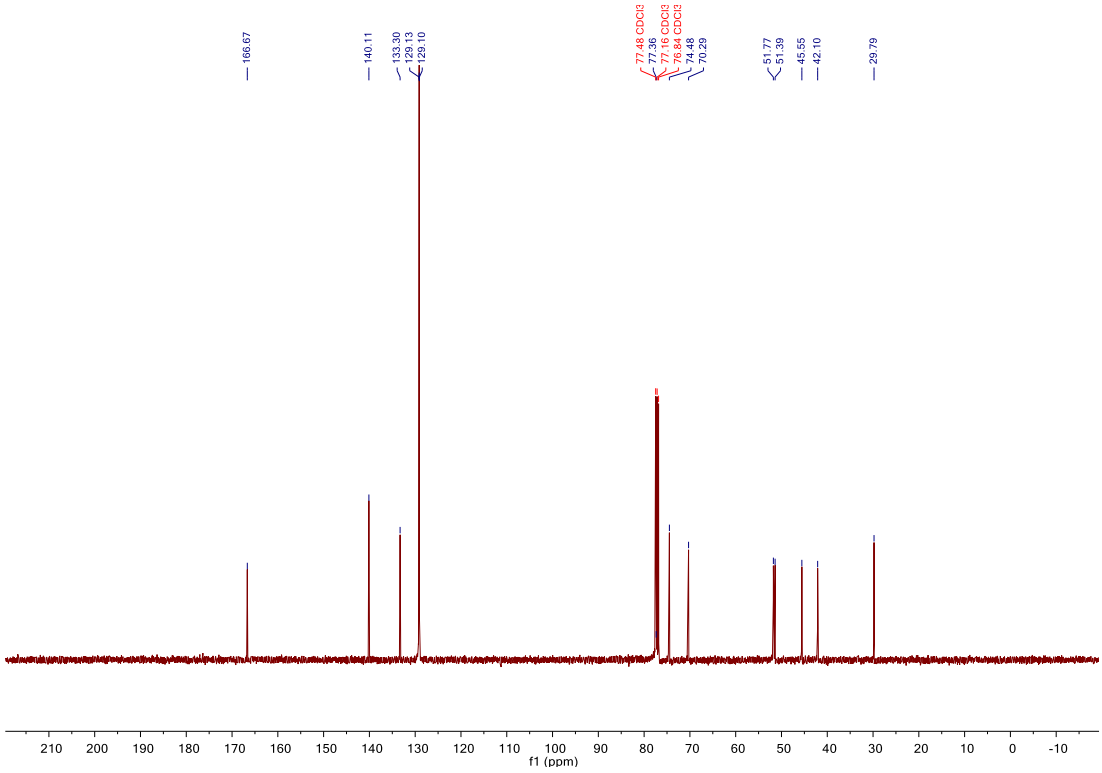
Compound 49


7.66
7.63
7.34
7.32
7.31
7.29
7.29
7.28 CDCl3
4.28
3.75
3.74
3.73
3.60
3.59
3.58
2.83
2.46
2.45
2.44
2.35
1.59 HDO
1.30
1.28
Cl
Cl
N
N
O
NO2
49
1.81
8.35
0.94
1.95
1.93
4.03
f1 (ppm)

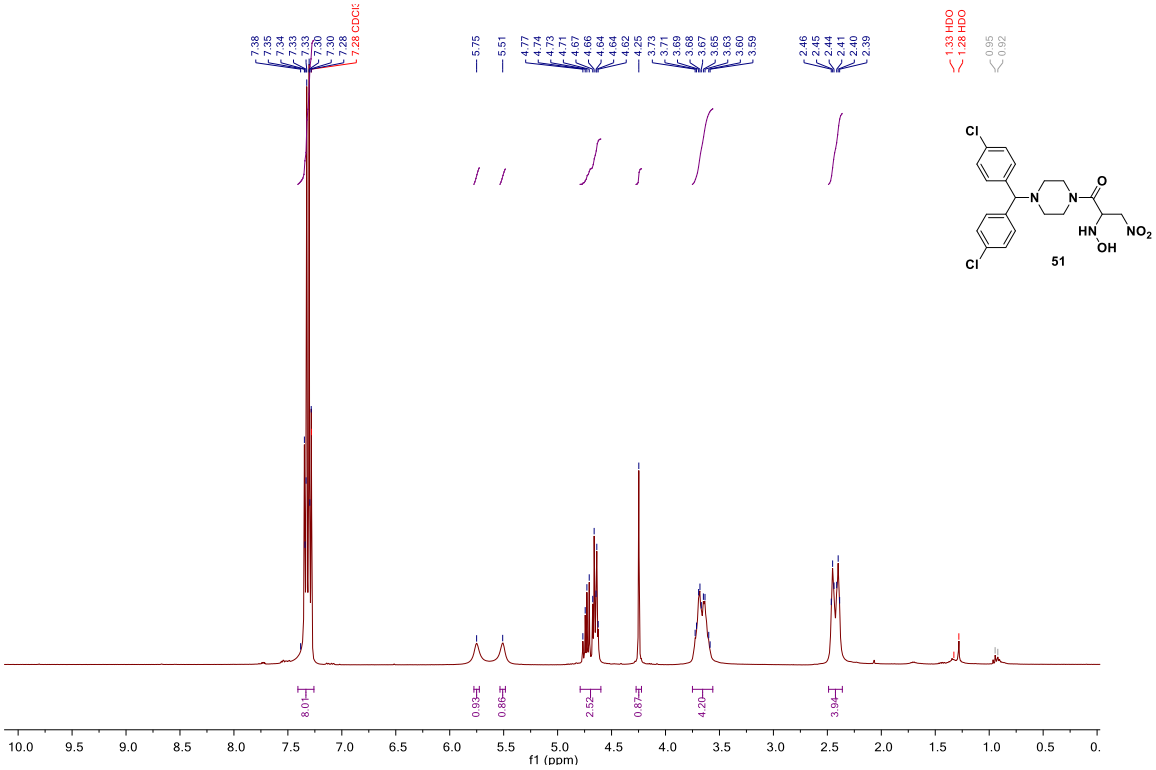

160.15
147.72
139.64
133.38
129.07
129.00
128.91
127.46
77.34 CDCl3
77.02 CDCl3
76.70 CDCl3
74.26
51.84
51.09
46.48
42.48
f1 (ppm)

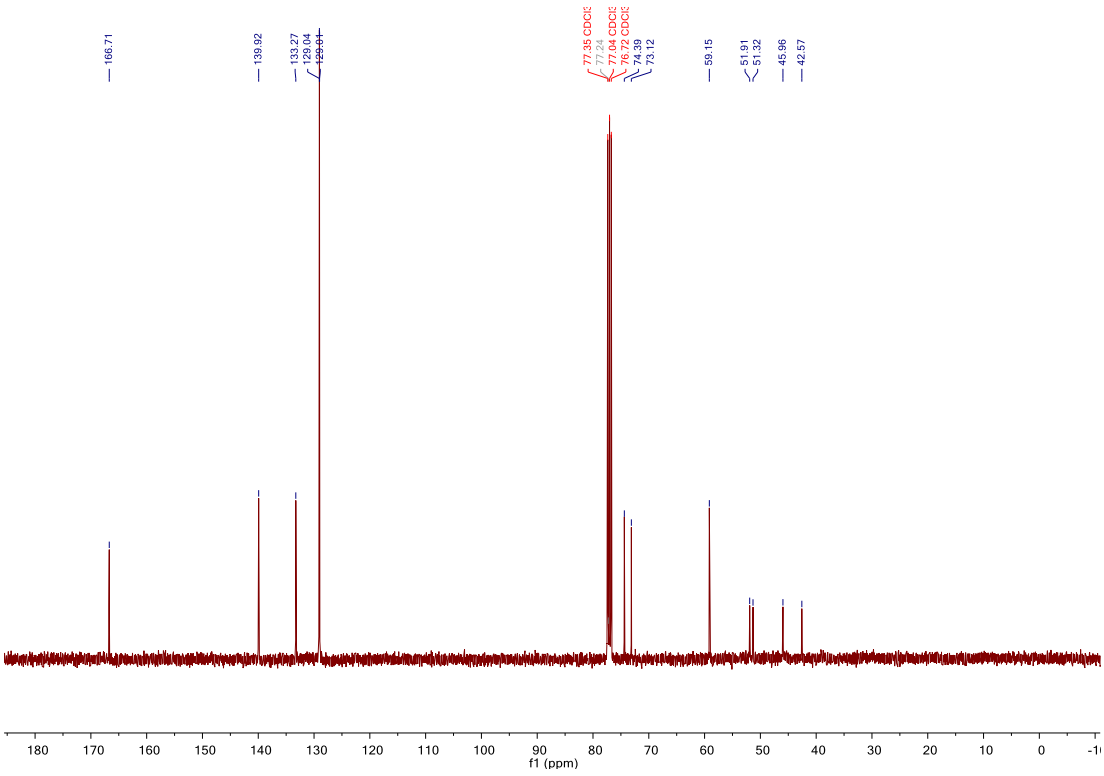
Compound 50


7.41
7.32
7.30
7.27
7.26 CDCl3
7.25
4.70
4.68
4.67
4.22
3.62
3.61
3.60
3.50
3.49
3.47
2.96
2.94
2.93
2.41
2.40
2.38
2.36
2.35
2.34
Cl
N
N
O
NO2
Cl
50
8.17
1.86
1.00
2.06
1.98
1.88
4.28
f1 (ppm)

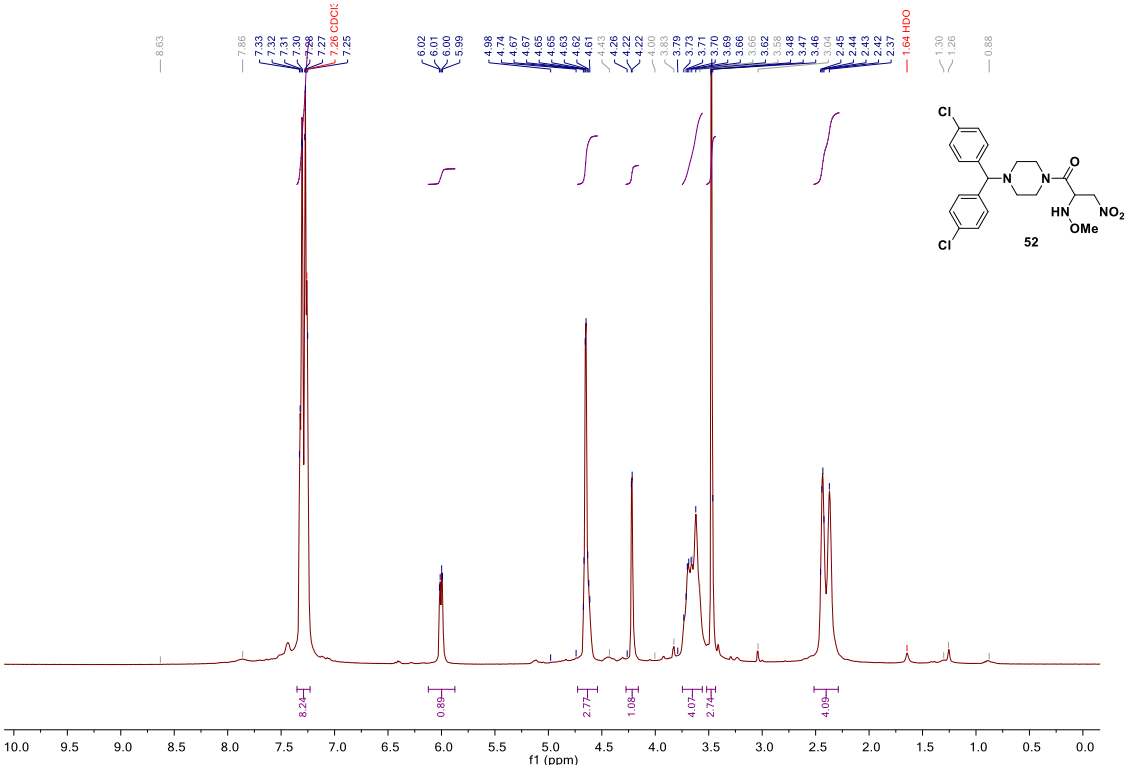

166.67
140.11
133.30
129.13
129.10
77.48 CDCl3
77.36
77.16 CDCl3
76.84 CDCl3
74.48
70.29
51.77
51.39
45.55
42.10
29.79
f1 (ppm)

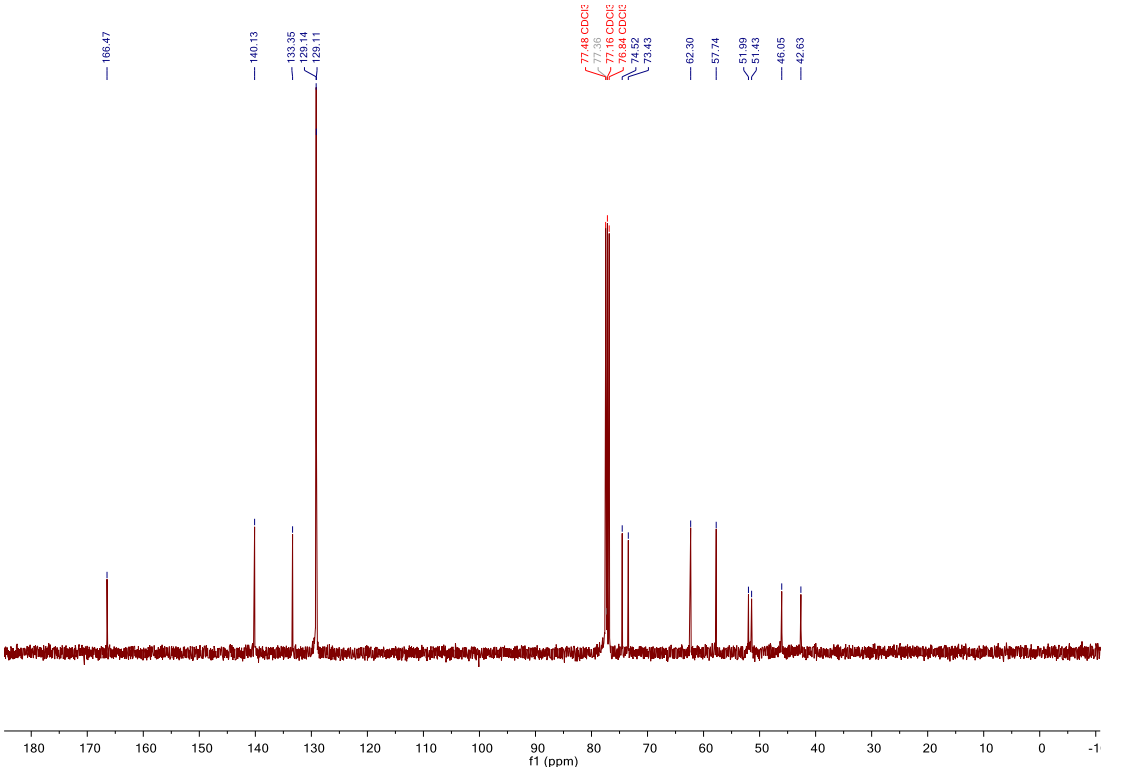
Compound 51


7.38
7.35
7.34
7.33
7.33
7.30
7.30
7.28
7.28 CDCl3
5.75
5.51
4.77
4.74
4.73
4.71
4.67
4.66
4.64
4.64
4.62
4.25
3.73
3.71
3.69
3.68
3.67
3.65
3.63
3.60
3.59
2.46
2.45
2.44
2.41
2.40
2.39
1.33 HDO
1.28 HDO
0.95
0.92
8.01
0.93
0.86
2.52
0.87
4.20
3.94
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.
f1 (ppm)
Cl
Cl
N
N
O
HN
OH
NO2
51

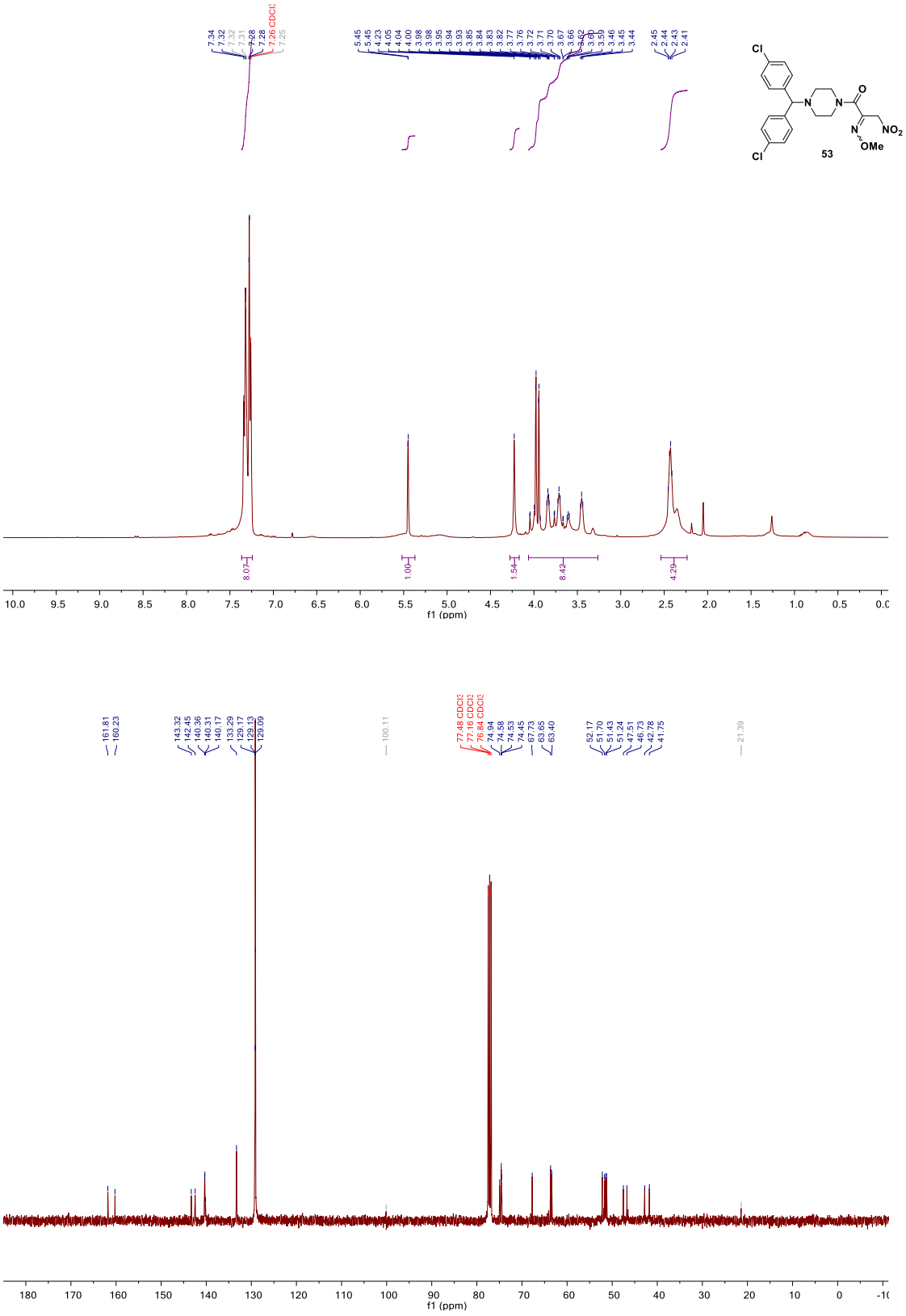

166.71
139.92
133.27
129.04
128.84
77.35 CDCl3
77.04 CDCl3
76.72 CDCl3
74.39
73.12
59.15
51.91
51.32
45.96
42.57
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

Compound 52

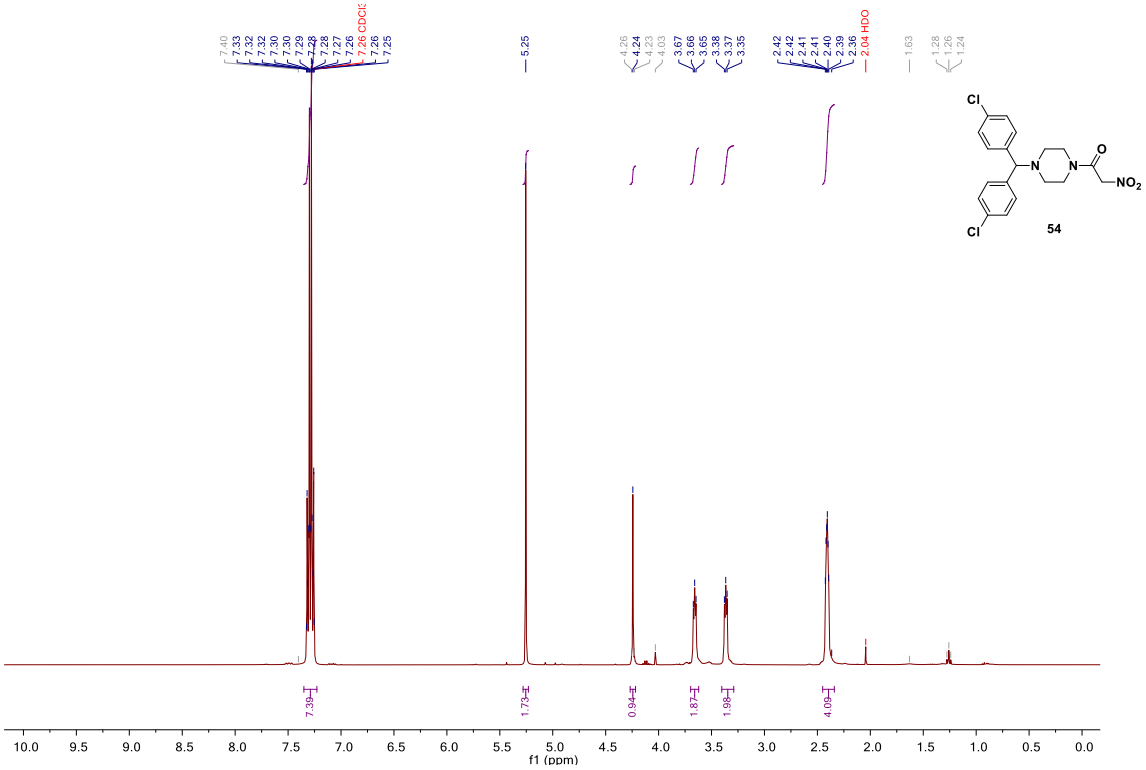

8.63
7.86
7.33
7.32
7.31
7.30
7.28
7.27
7.26 CDCl3
7.25
6.02
6.01
6.00
5.99
4.98
4.74
4.67
4.67
4.65
4.65
4.63
4.62
4.61
4.43
4.26
4.22
4.22
4.00
3.83
3.79
3.73
3.71
3.70
3.68
3.66
3.62
3.58
3.48
3.47
3.46
3.04
2.45
2.44
2.43
2.42
2.37
1.64 HDO
1.30
1.26
0.88
Cl
O
N
N
HN
NO2
OMe
Cl
52
8.24
0.89
2.77
1.08
4.07
2.74
4.09
f1 (ppm)

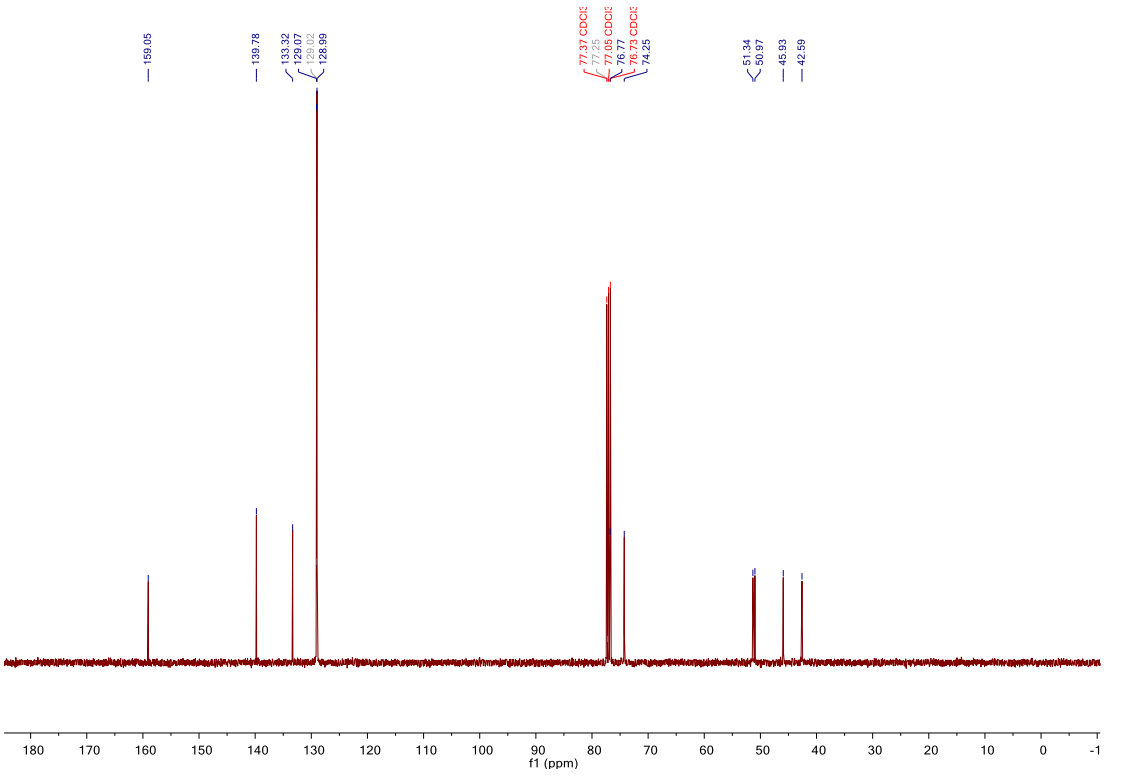

166.47
140.13
133.35
129.14
129.11
77.48 CDCl3
77.36
77.16 CDCl3
76.84 CDCl3
74.52
73.43
62.30
57.74
51.99
51.43
46.05
42.63
f1 (ppm)

Compound 53

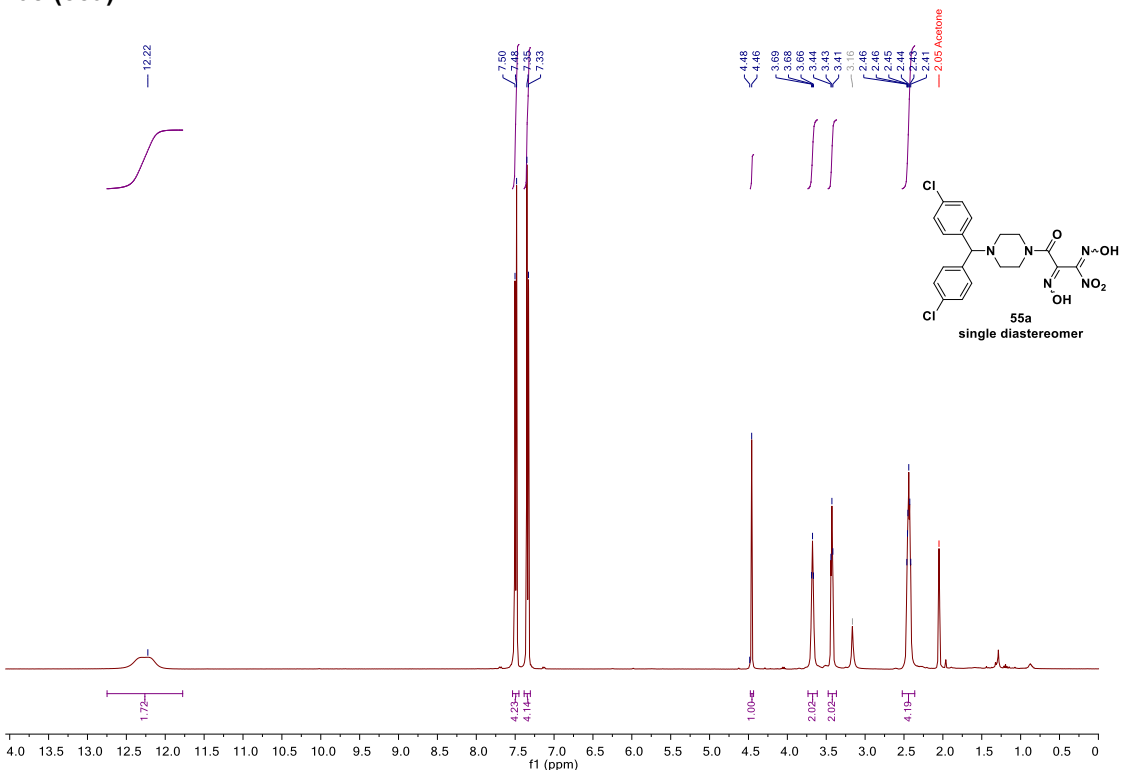

7.34
7.32
7.32
7.31
7.28
7.28
7.26 CDCl3
7.25
5.45
5.45
4.23
4.05
4.04
4.00
3.98
3.96
3.95
3.94
3.93
3.85
3.84
3.83
3.82
3.77
3.76
3.72
3.71
3.70
3.67
3.66
3.62
3.60
3.59
3.46
3.45
3.44
2.45
2.44
2.43
2.41
Cl
Cl
N
N
O
N
NO2
OMe
53
8.07
1.00
1.54
8.42
4.29
f1 (ppm)
161.81
160.23
143.32
142.45
140.36
140.31
140.17
133.29
129.17
129.13
129.09
100.11
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
74.94
74.58
74.53
74.45
67.73
67.65
63.40
52.17
51.70
51.43
51.24
47.51
46.73
42.78
41.75
21.39
f1 (ppm)

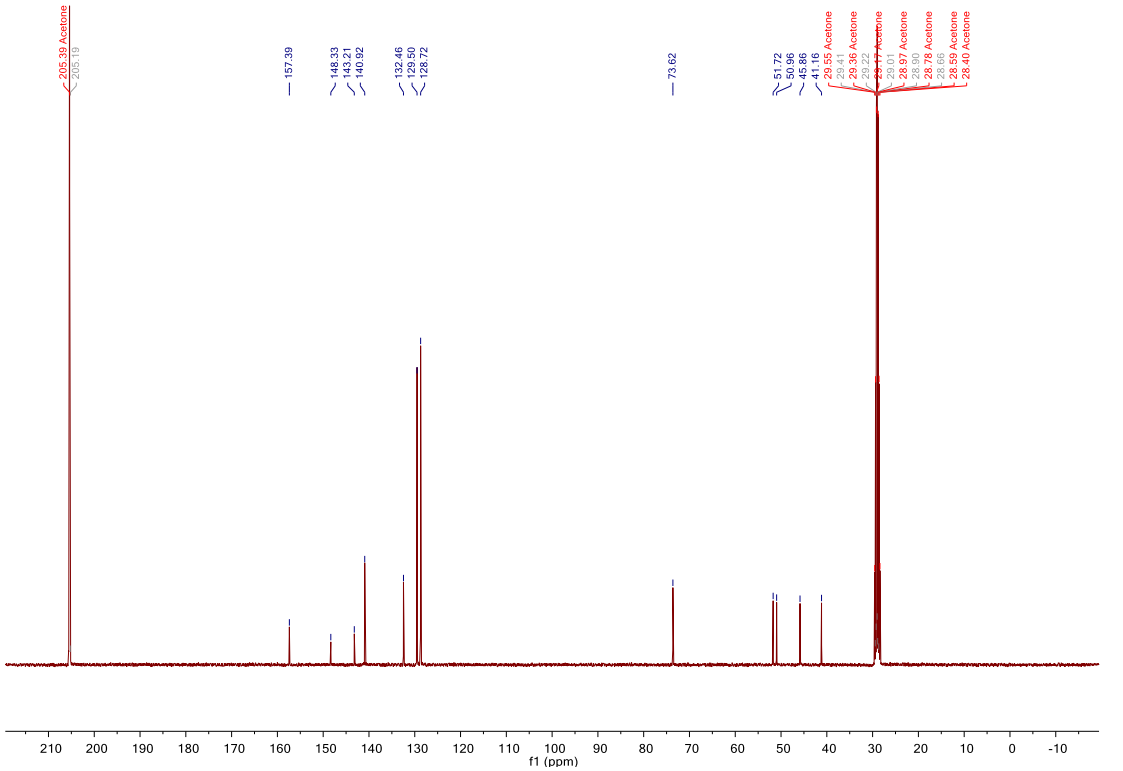
Compound 54


7.40
7.33
7.32
7.32
7.30
7.30
7.29
7.28
7.27
7.26 CDCl3
7.26
7.25
5.25
4.26
4.24
4.23
4.03
3.67
3.66
3.65
3.38
3.37
3.35
2.42
2.42
2.41
2.41
2.40
2.39
2.36
2.04 HDO
1.63
1.28
1.26
1.24
7.39
1.73
0.94
1.87
1.98
4.09
f1 (ppm)
Cl
N
N
O
NO2
Cl
54

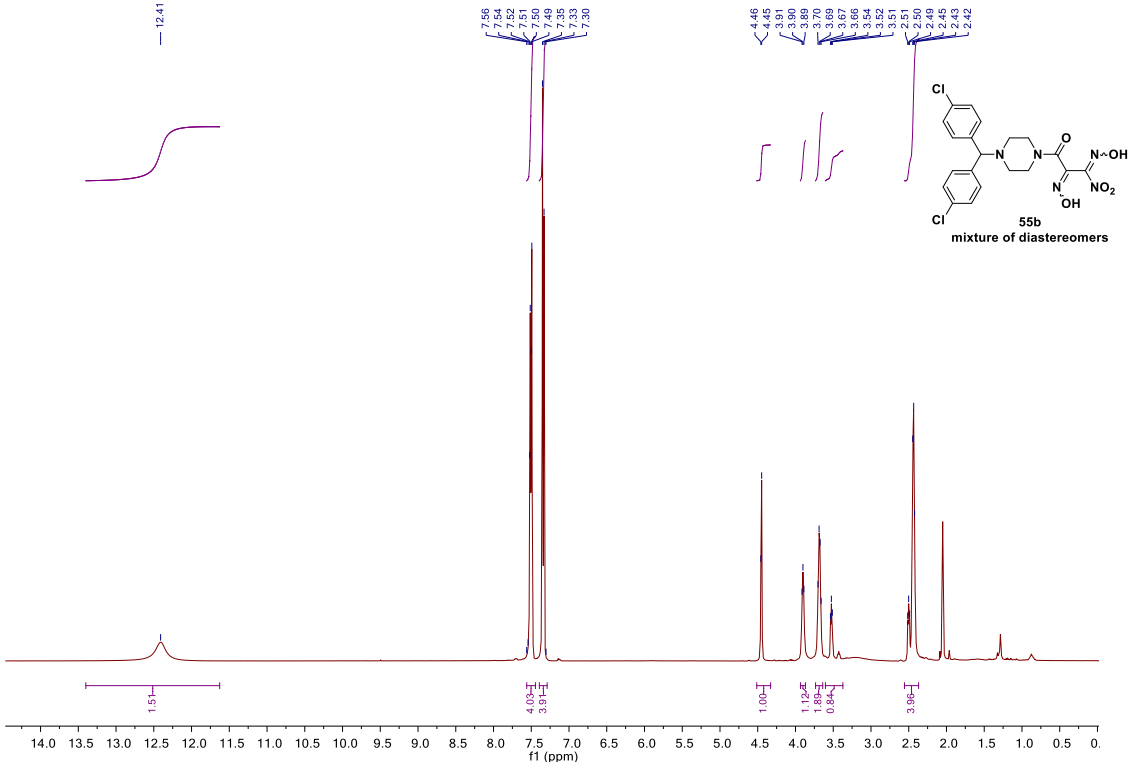

159.05
139.78
133.32
128.07
129.02
128.99
77.37 CDCl3
77.25
77.05 CDCl3
76.77
76.73 CDCl3
74.25
51.34
50.97
45.93
42.59
f1 (ppm)

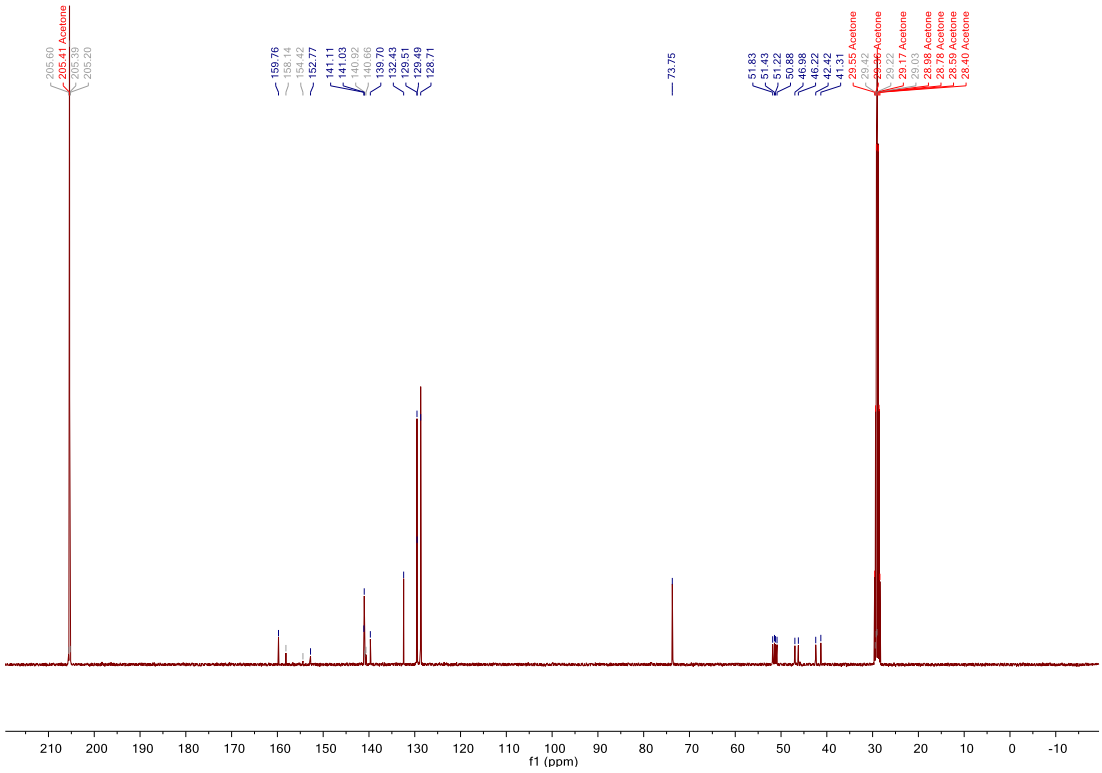
JKE-1708 (55a)


12.22
7.50
7.48
7.35
7.33
4.48
4.46
3.69
3.68
3.66
3.44
3.43
3.41
3.16
2.46
2.46
2.45
2.44
2.43
2.41
2.05 Acetone
Cl
N
N
O
N~OH
N
OH
NO2
Cl
55a
single diastereomer
1.72
4.23
4.14
1.00
2.09
2.02
4.19
f1 (ppm)

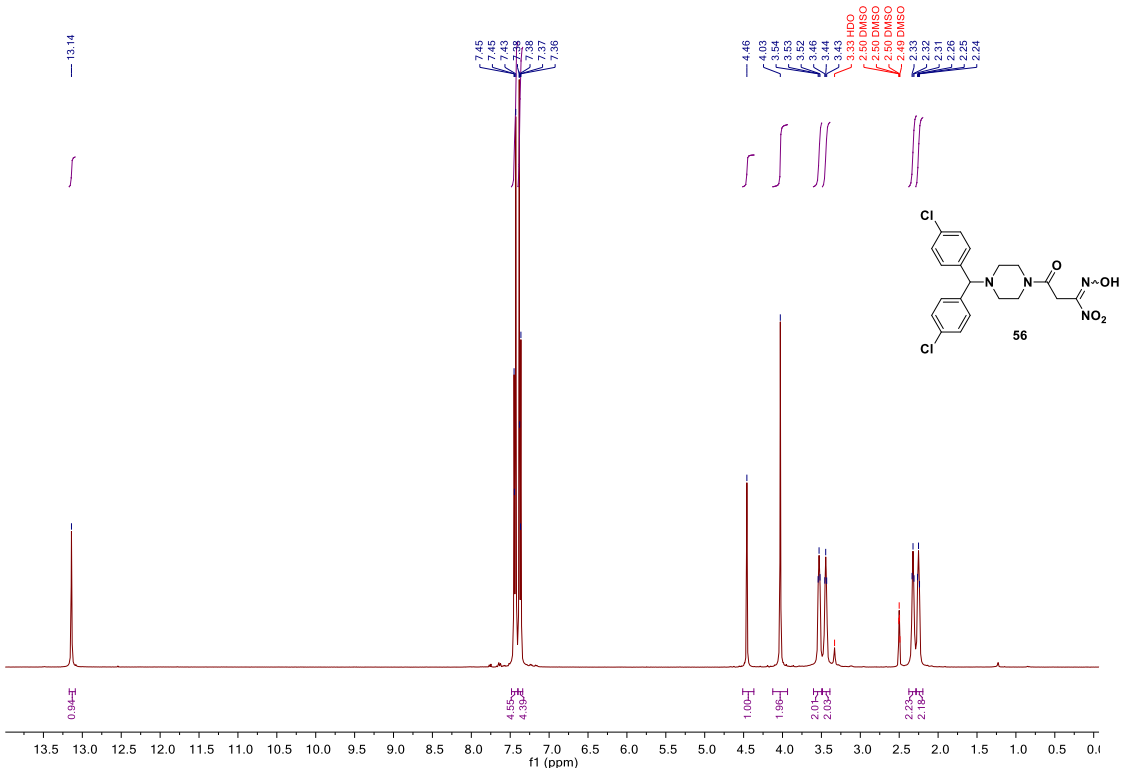

205.39 Acetone
205.19
157.39
148.33
143.21
140.92
132.46
129.50
128.72
73.62
51.72
50.96
45.86
41.16
29.55 Acetone
29.46 Acetone
29.26 Acetone
29.17 Acetone
29.01
28.97 Acetone
28.90
28.78 Acetone
28.66
28.59 Acetone
28.40 Acetone
f1 (ppm)

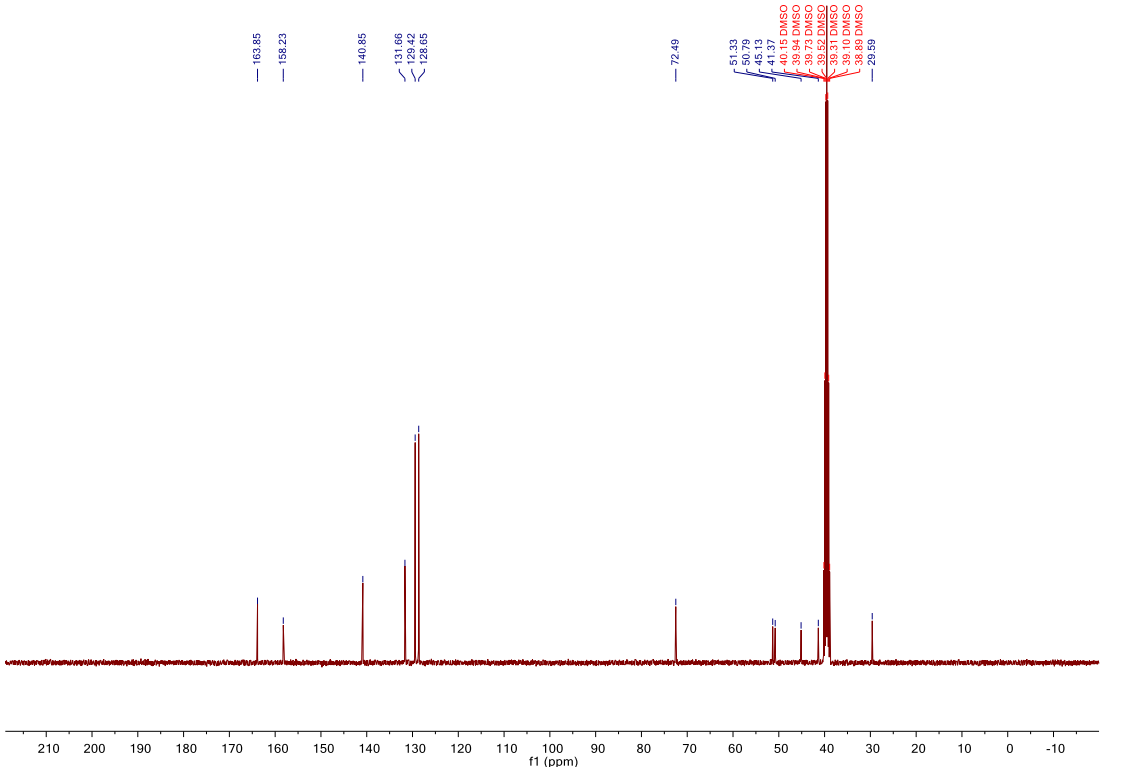
JKE-1708 (55b)


12.41
7.56
7.54
7.52
7.51
7.50
7.49
7.35
7.33
7.30
4.46
4.45
3.91
3.90
3.89
3.70
3.69
3.67
3.66
3.54
3.52
3.51
2.51
2.50
2.49
2.45
2.43
2.42
Cl
Cl
O
N
N
N
N
OH
NO2
OH
55b
mixture of diastereomers
1.51
4.03
3.91
1.00
1.12
1.89
0.84
3.99
14.0 13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.
f1 (ppm)

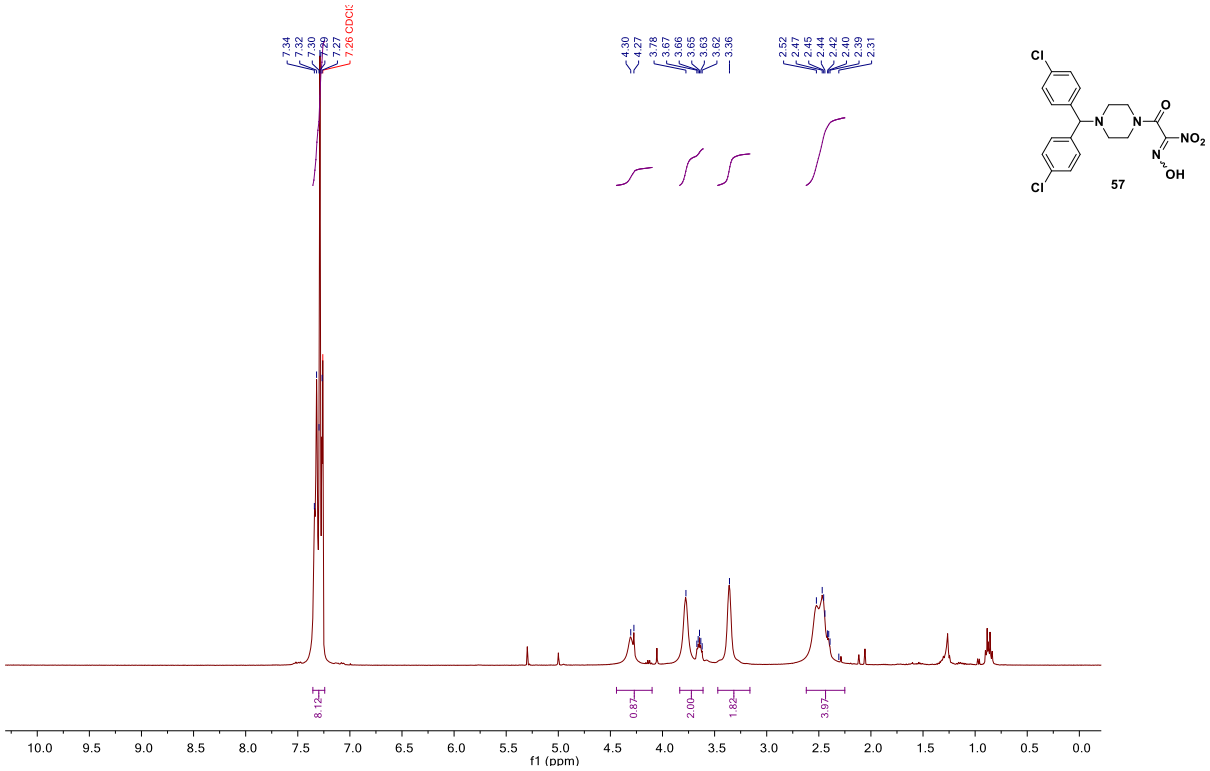

205.60
205.41 Acetone
205.39
205.20
159.76
158.14
154.42
152.77
141.11
141.03
140.92
140.66
139.70
132.43
130.51
129.49
128.71
73.75
51.83
51.43
51.22
50.88
46.98
46.22
42.42
41.31
29.35 Acetone
29.22
29.17 Acetone
29.03
28.98 Acetone
28.78 Acetone
28.59 Acetone
28.40 Acetone
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

Compound 56

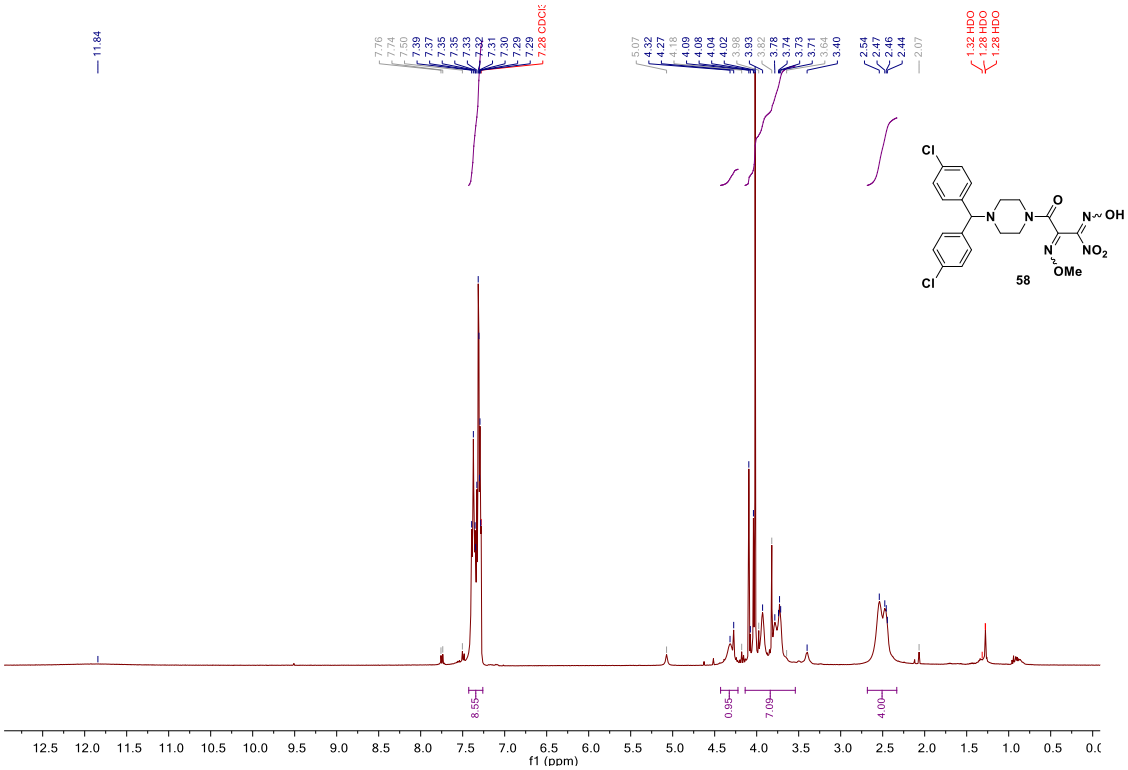

13.14
7.45
7.45
7.43
7.38
7.38
7.37
7.36
4.46
4.03
3.54
3.53
3.52
3.46
3.44
3.43
3.33 HDO
2.50 DMSO
2.50 DMSO
2.50 DMSO
2.49 DMSO
2.33
2.32
2.31
2.26
2.25
2.24
Cl
N
N
O
N~OH
NO2
Cl
56
0.94
4.55
4.39
1.00
1.96
2.01
2.03
2.23
2.18
f1 (ppm)


163.85
158.23
140.85
131.66
129.42
128.65
72.49
51.33
50.79
45.13
41.37
40.13 DMSO
39.93 DMSO
39.73 DMSO
39.52 DMSO
39.31 DMSO
39.10 DMSO
38.89 DMSO
29.59
f1 (ppm)

Compound 57


7.34
7.32
7.30
7.29
7.27
7.26 CDCl3
4.30
4.27
3.78
3.67
3.66
3.65
3.63
3.62
3.36
2.52
2.47
2.45
2.44
2.42
2.40
2.39
2.31
Cl
N
N
O
NO2
N
OH
Cl
57
8.12
0.87
2.00
1.82
3.97
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

Compound 58


11.84
7.76
7.74
7.50
7.39
7.37
7.35
7.35
7.33
7.32
7.31
7.30
7.29
7.29
7.28 CDCl3
5.07
4.32
4.27
4.18
4.09
4.08
4.04
4.02
3.98
3.93
3.82
3.78
3.74
3.73
3.71
3.64
3.40
2.54
2.47
2.46
2.44
2.07
1.32 HDO
1.28 HDO
1.28 HDO
Cl
N
N
O
N OH
N
NO2
OMe
Cl
58
8.55
0.95
7.09
4.00
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)