Neutral Rhenium(I) Tricarbonyl Complexes with Sulfur-Donor Ligands: Anti-Proliferative Activity and Cellular Localization

Aviva Levina, Kartika Wardhani, Liam J. Stephens, Melissa V. Werrett, Chiara Caporale, Elena Dallerba, Victoria L. Blair, Massimiliano Massi, Peter A. Lay* and Philip C. Andrews*

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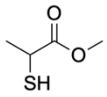
Experimental

Synthesis and characterisation of mercaptocarboxylate methyl esters and the precursor and aqua rhenium tricarbonyl complexes

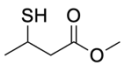
General Considerations

Thiolactic acid was purchased from sigma and used as received, 3-mercaptobutanoic acid was synthesised according to previously published methods.[S1] ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra were recorded on a Bruker Avance III 400 spectrometer with a 9.4 Tesla magnet (400.13 MHz for ^{1}H) at room temperature. The spectra were recorded with a 5 mm broadband autotunable probe with Z-gradients and a BACS 60 tube autosampler. ^{1}H and ^{13}C spectra were internally referenced using the deuterated solvent signal. Infrared spectra were recorded on an Agilent Technologies Cary 630 FT-IR spectrometer in the range of 4000-500 cm^{-1}. IR frequencies are reported in wavenumbers with the relative intensities indicated as strong (s), medium (m) or weak (w). Elemental microanalyses were performed at Monash University on a Perkin Elmer 2400 Series II CHNS/O Elemental Analyser. Mass spectrometry (ESI) was performed on a Micro mass Platform QMS spectrometer with an electrospray source.

To prepare **methyl 2-mercaptopropanoate (MMP*H*)** and **methyl 3-mercaptobutanoate (MMB*H*)**, the corresponding mercaptocarboxylic acids (8 -10 mmol) were dissolved in excess methanol (10 mL) with 2-3 drops concentrated sulfuric acid before being heated at reflux for 4 hr. DCM and water were added to the reaction and the ester was extracted into DCM (3 × 15 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed to isolate the pungent smelling compound as an oil.



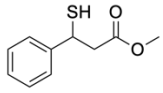
Methyl 2-mercaptopropanoate (MMP*H*) 1.12 g, 94%. ^{1}H NMR (400.1 MHz, $CDCl_3$): δ = 3.65 (3H, s, OC*H*$_3$), 3.43 (1H, m, C*H*), 2.10 (1H, d, *J* = 8.34 Hz, S*H*), 1.43 (3H, d, *J* = 7.04 Hz, CH_3). ν_{max} FT-IR (cm^{-1}): 1168 (s), 1452 (s), 1736 (s) ESI-MS (methanol) for $C_3H_6O_2S$, 106.01. (ESI-): m/z = 104.9 [M-H]$^{-}$.



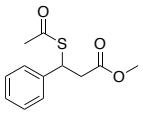
Methyl 3-mercaptobutanoate (MMB*H*) 1.03 g, 77%. ^{1}H NMR (400.1 MHz, $CDCl_3$): δ = 3.70 (3H, s, OC*H*$_3$), 3.37 (1H, app. septet, *J* = 6.7 Hz, C*H*), 2.60 (2H, m, C*H*$_2$), 1.83 (1H, d, *J* = 6.72, S*H*), 1.37 (3H, d, *J* = 6.84, C*H*$_3$). ν_{max} FT-IR (cm^{-1}): 1704 (s), 2926 (w). ESI-MS (methanol) for $C_4H_8O_2S$, 120.02. (ESI-): m/z = 118.9 [M-H]$^{-}$.

Methyl 3-mercapto-3-phenylpropanoate (MMPP*H*) was prepared from commercially available methyl cinnamate. Firstly, methyl cinnamate and thioacetic acid (in excess) were

refluxed overnight before being cooled to room temperature, with the organics extracted with diethyl ether and washed with water. After concentrating the organics under *vacuo*, the subsequent crude mixture that was left over was purified by silica gel chromatography (5% ethyl acetate: 95% petroleum benzine) to yield methyl **3-(acetylthio)-3-phenylpropanoate** as a white solid (62%). To methyl 3-(acetylthio)-3-phenylpropanoate (1.0 equiv.) was then dissolved in methanol before KOH (1.2 equiv.) in methanol was added dropwise. The reaction was stirred for 40 min before being concentrated in *vacuo*. The organics were extracted with ethyl acetate and disregarded, then the subsequent aqueous layer was acidified with HCl, re-extracted with diethyl ether, dried with $MgSO_4$ and concentrated under *vacuo* to yield **methyl 3-mercapto-3-phenylpropanoate (LH3)** as a clear oil (32%).

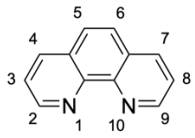


Methyl 3-mercapto-3-phenylpropanoate (MMPP*H*) 58 mg, 32 %. ^{1}H NMR (400.1 MHz, $CDCl_3$): δ = 7.30-7.24 (4H, m, CH_{AR}), 7.23 (1H, app t, CH_{AR}), 4.42 (1H, q, *J* =7.1 Hz, C*H*), 3.61 (3H, s, OCH_3), 2.93 (2H, d, *J* = 7.5 HZ, CH_2), 2.17 (2H, d, *J* = 6.0 HZ, S*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.3, 142.9, 128.9, 127.7, 126.8, 52.0, 44.5, 39.6. ν_{max} FT-IR (cm^{-1}): 697 (s), 1732 (s), 2952 (w). ESI-MS (methanol) for $C_{10}H_{12}O_2S$, 196.1. (ESI-): m/z = 195.1 $[M-H]^-$.

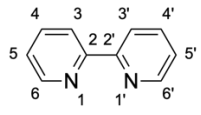


3-(Acetylthio)-3-phenylpropanoate. ^{1}H NMR (400.1 MHz, $CDCl_3$): δ = 7.30-7.19 (5H, m, CH_{AR}), 5.00 (1H, dd, *J* = 8.4, 7.1 Hz, C*H*), 3.56 (3H, s), 3.02-2.90 (2H, m, CH_2), 2.25 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 194.1, 170.7, 140.2, 128.8, 127.8, 127.6, 51.9, 43.8, 40.7, 30.5. ν_{max} FT-IR (cm^{-1}): 699 (s), 1687 (s), 1737 (s), 2953 (w). ESI-MS (methanol) for $C_{12}H_{14}O$, 238.1. (ESI-): m/z = 261.1 $[M+Na]^+$.

NMR referencing scheme



1,10-phenanthroline



2,2'-bipyridine

For IR spectra interpretation. A′(1) = totally symmetric in-phase **ν(CO)**, A′(2) = totally symmetric out-of-phase **ν(CO)**, A′′ = asymmetric vibration of equatorial CO ligands, cm^{-1}.[S2]

The preparation of *fac*-[Re(NN)$(CO)_3$(X)] (NN = 1,10-phenanthroline or 2,2'- bipyridine) was carried out following procedures adapted from previously published work.[S3] Rhenium pentacarbonyl chloride or bromide (1 eq) and the corresponding diimine (**bipy** or **phen**) (1.1

eq) were added to toluene and the mixture was heated at reflux for 4 hr. A yellow solid was filtered off and washed with cold toluene to yield the final complex.

***fac*-[Re(CO)$_3$(phen)Cl]:** 1.77 g, 95 %; ν_{max} FT-IR (cm^{-1}) 3086 w, 2511 w, 2014 s (CO A′(1)), (1925 sh) 1886 s (CO A′(2)/A′′), 1736 m, 1687 m, 1603 m, 1518 m, 1425 m, 1410 w, 1259 m, 1233 w, 1172 m, 1037 m, 850 s, 782 m, 723 s.

***fac*-[Re(bipy)(CO)$_3$Cl]:** 1.56 g, 94 %; ν_{max} FT-IR (cm^{-1}) 2013 m (CO A′(1)), 1874 s (CO A′(2)/A′′), 1602 m, 1492 w, 1471 m, 1444 m, 1314 m, 1246 m, 1220 w, 1156 w, 1123 w, 1106 w, 1071 m, 1000 w, 968 w, 809 w, 766 s, 733 m, 663 w.

***fac*-[Re(CO)$_3$(phen) (CF$_3$SO$_3$)]**: 0.95 g, 72 %; ν_{max} FT-IR (cm^{-1}): 2029 s (CO A′(1)), (1920 sh) 1889 s (CO A′(2)/A′′), 1632 w, 1521 m, 1428 m, 1323 s, 1231 s, 1201 s, 1172 s, 1144 s, 1009 s, 846 s, 777 m, 722 s.

***fac*-[Re(bipy)(CO)$_3$(CF$_3$SO$_3$)]:** 1.17 g, 82 %; ν_{max} FT-IR (cm^{-1}) 3124 w, 3094 w, 2030 s (CO A′(1)), 1894 s (CO A′(2)/A′′), 1604 m, 1498 w, 1474 m, 1447 m, 1337 s, 1318 m, 1286 w, 1235 s, 1197 s, 1160 s, 1111 w, 1076 w, 1038 w, 1011 s, 898 w, 807 w, 767 s, 733 s.

The rhenium aqua derivatives, *fac*-[Re(NN)(CO)$_3$(OH$_2$)]$^+$[CF$_3$SO$_3^-$] (**4a** and **4b**) were synthesised according to previously published methods.[S4] Silver triflate (in acetone) was added to a solution of *fac*-[Re(NN)(CO)$_3$Cl] in acetone, protected from light. The reaction was heated to reflux for 18 hr. Upon cooling, the reaction was filtered through Celite to remove silver chloride. The solvent was removed and the yellow residue was resuspended in water, briefly sonicated and stirred for 2-3 hr. The suspension in water was centrifuged and the supernatant was filtered through Celite to remove any insoluble product. The yellow solution was then lyophilised to isolate a yellow-orange powder. NMR spectral data matched those previously reported.[S5]

4a *fac*-[Re(bipy)(CO)$_3$(OH$_2$)][CF$_3$SO$_3$]: 760 mg, 90.0 %.
ν_{max} FT-IR (cm^{-1}): 3208 w, 3124 w, 2036 s (CO A′ (1)), 1917 s (1902 sh) (CO A′(2)/A′′), 1605 m, 1476 m, 1448 m, 1920 m, 1228 s, 1176 s, 1023 s, 969 w, 772 s, 734 m. ^{1}H NMR (400.1 MHz, D$_2$O): δ = 9.13 (2H, d, J = 4.84 Hz, bipy H$_{6,6'}$), 8.51 (2H, d, J = 8.22 Hz, bipy $H_{3,3'}$), 8.29 (2H, td, J= 7.96, 1.48 Hz, bipy H$_{4,4'}$), 7.72 (2H, m, bipy $H_{5,5'}$). Elemental analysis for C$_{14}$H$_{10}$F$_3$N$_2$O$_7$ReS, calculated: C 28.33, H 1.70, N 4.72; found: C 27.99, H 1.54, N 4.82.

4b *fac*-[Re(CO)$_3$(phen)(OH$_2$)][CF$_3$SO$_3$]: 966 mg, 84 %.
ν_{max} FT-IR (cm^{-1}): 3074 w, 2031 s (CO A′ (1)), 1892 s (CO A′(2)/A′′), 1632 w, 1607 w, 1587 w, 1522 m, 1431 m, 1281 s, 1234 s, 1170 s, 1029 s, 918 w, 847 s, 777 w, 724 s. ^{1}H NMR (400.1 MHz, D$_2$O): δ = 9.50 (2H, dd, J= 5.12, 1.35 Hz, phen $H_{2,9}$), 8.86 (2H, dd, J= 8.31, 1.34 Hz, phen $H_{4,7}$), 8.20 (2H, s, phen $H_{5,6}$), 8.04 (2H, dd, J= 8.30, 5.15 Hz, phen $H_{3,8}$). Elemental analysis for C$_{16}$H$_{10}$F$_3$N$_2$O$_7$ReS, calculated: C 31.12, H 1.63, N 4.54; found: C 31.25, H 1.23, N 4.56.

Supporting Information References

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S5. M. S. Capper, A. Enriquez Garcia, N. Macia, B. Lai, J.-B. Lin, M. Nomura, A. Alihosseinzadeh, S. Ponnurangam, B. Heyne, C. S. Shemanko and F. Jalilehvand, *J. Biol. Inorg. Chem.*, 2020, **25**, 759-776.

Crystallographic Details

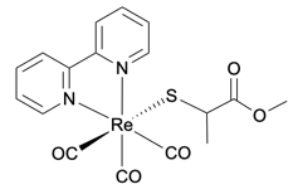
Representative orange to red crystal specimens were mounted on a nylon cryoloop and cooled to 123 K. Diffraction data were collected on a Rigaku Synergy S diffractometer, fitted with a HyPix6000 hybrid photon counting detector, using CuKα radiation, λ = 1.54184 Å (**1a**, **2b**, **3b**) or MoKα radiation, λ = 0.71073 Å (2a). Data were processed, including an empirical multi-scan (**2a**, **2b**, **3b**) or analytical/face-indexed (**1a**) absorption correction using the proprietary software CrysAlisPro (Rigaku OD, Yarnton, UK, 2020). The structure was solved and refined using the SHELX software suite (G. M. Sheldrick, *Acta Cryst., Sect. C*, 2015, 71, 8-3). Hydrogen atoms attached to carbon were placed in calculated positions using a riding model. All data for compounds **1a, 2a, 2b** and **3b** has been deposited with the Cambridge Crystallographic Database with CCDC numbers 2252065-2252068, respectively.

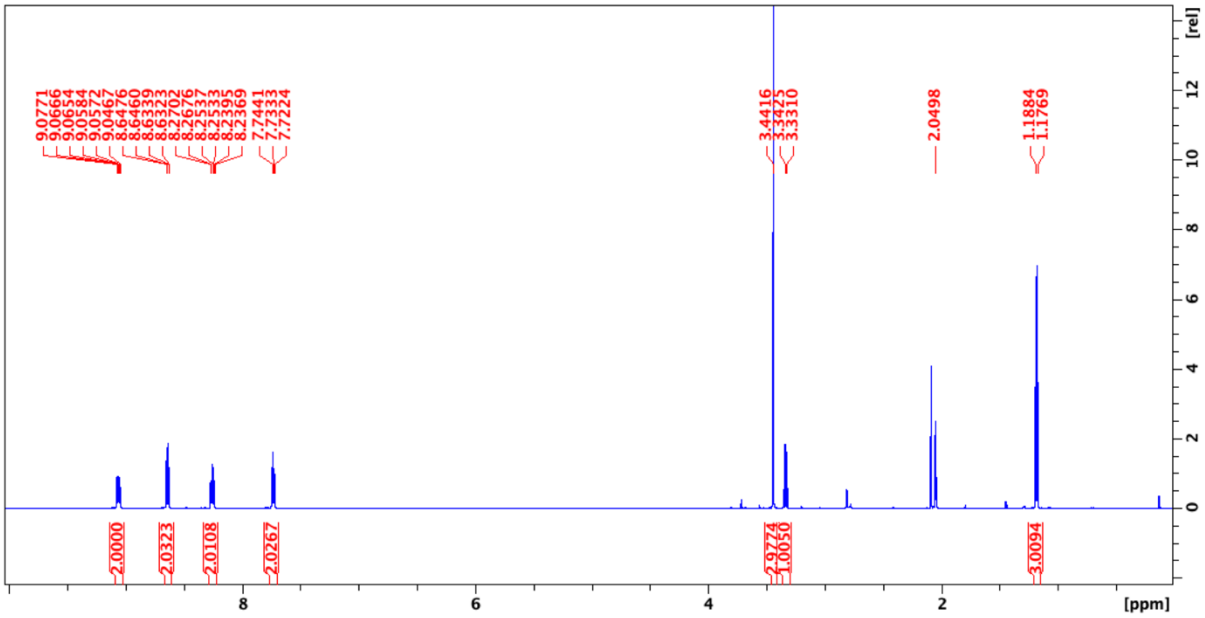
Table S1. Crystal and refinement data

	1a	**2a**	**2b**	**3b**
Formula	$C_{17}H_{15}N_2O_5ReS$	$C_{18}H_{17}N_2O_5ReS$	$C_{20}H_{17}N_2O_5ReS$	$C_{25}H_{19}N_2O_5ReS$
M	545.57	559.59	583.61	645.68
Crystal System	triclinic	monoclinic	monoclinic	triclinic
Space group	*P*-1	$P2_1/c$	$P2_1/c$	*P*-1
a, Å	11.3382(1)	6.5470(4)	6.3860(1)	8.0910(2)
b	12.5869(2)	18.4883(9)	20.3549(2)	10.3371(2)
c	15.1417(2)	15.5201(8)	15.3475(2)	14.8397(3)
α, °	107.918(1)	90	90	103.819(2)
β	95.850(1)	97.050(2)	100.417(1)	91.764(2)
γ	113.688(1)	90	90	106.969(2)
V, Å^3	1818.63(4)	1864.39(18)	1962.08(4)	1146.12(5)
Z	4	4	4	2
d_{calcd}, g.cm^{-3}	1.993	1.994	1.976	1.871
μ, mm^{-1}	14.423	6.662	13.424	11.569
N_{total}	36389	18204	39801	14838
N (R_{int})	7552 (0.059)	4639 (0.085)	4137 (0.071)	4506 (0.058)
N_{obs} ($I > 2\sigma I$)	6977	2825	3887	4267
$R1$ ($F2 > 2sF2$)	0.0501	0.0409	0.0492	0.0387
w$R2$ ($F2$)	0.1276	0.0827	0.1298	0.0989
parameters	473	246	264	308
restraints	0	0	0	0
Δρ(max,min), eÅ^{-3}	3.18, -3.21	1.29, -1.37	2.20, -1.53	1.65, -1.88

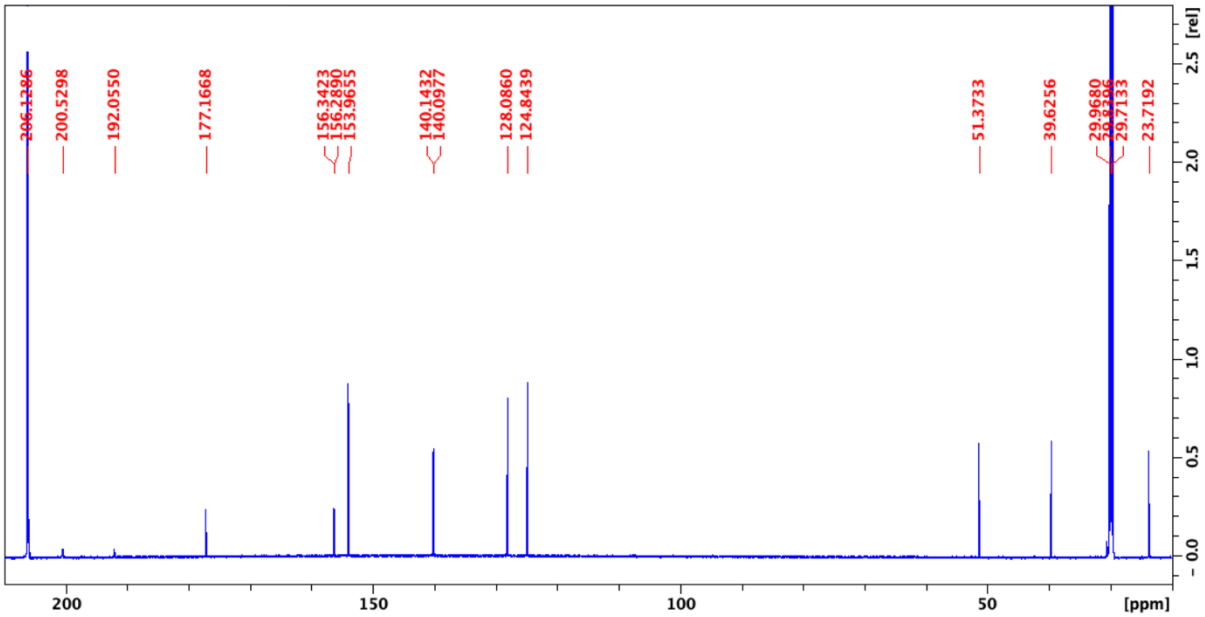
^{1}H and ^{13}C NMR Spectra

Figure S1: ***fac*-[Re(bipy)(CO)$_3$(MMP)], 1a**



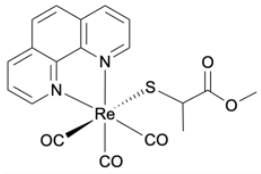


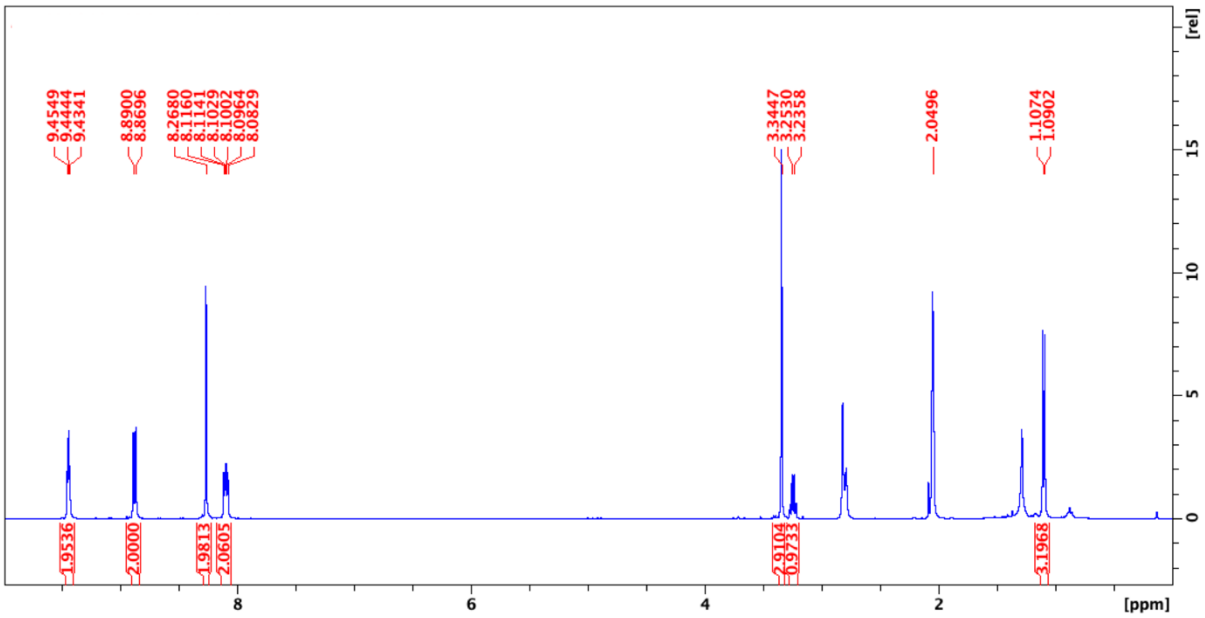
^{1}H NMR spectrum of **1a** in acetone-d$_6$.



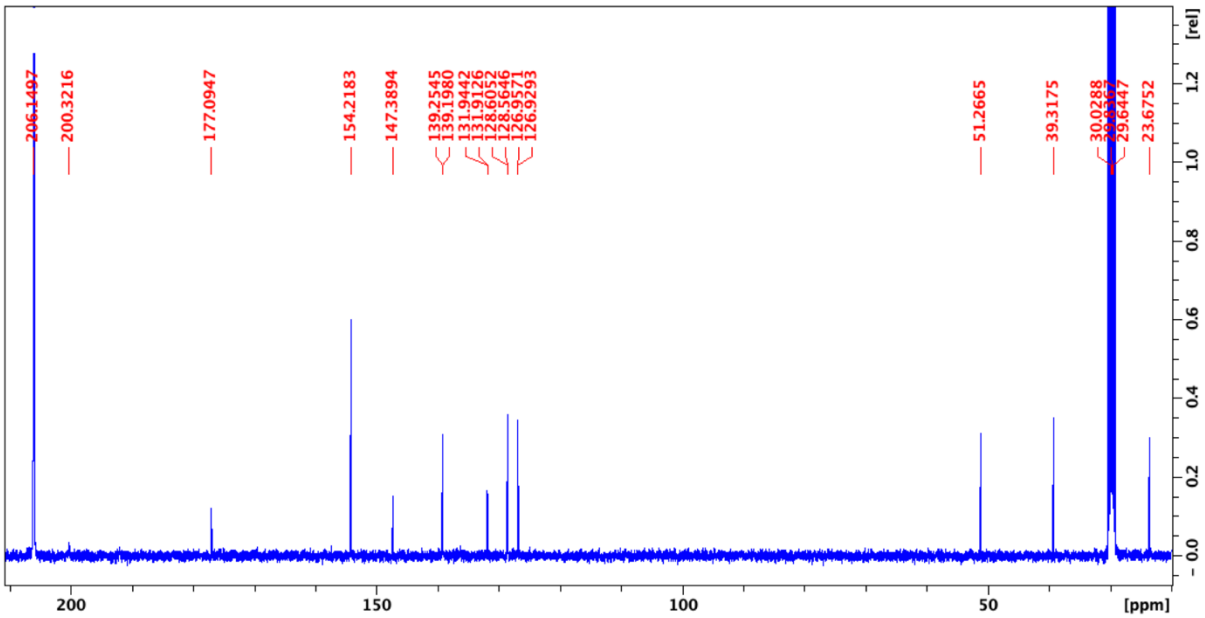
^{13}C NMR spectrum of **1a** in acetone-*d*$_6$.

Figure S2: ***fac*-[Re(CO)$_3$(phen)(MMP)], 1b**

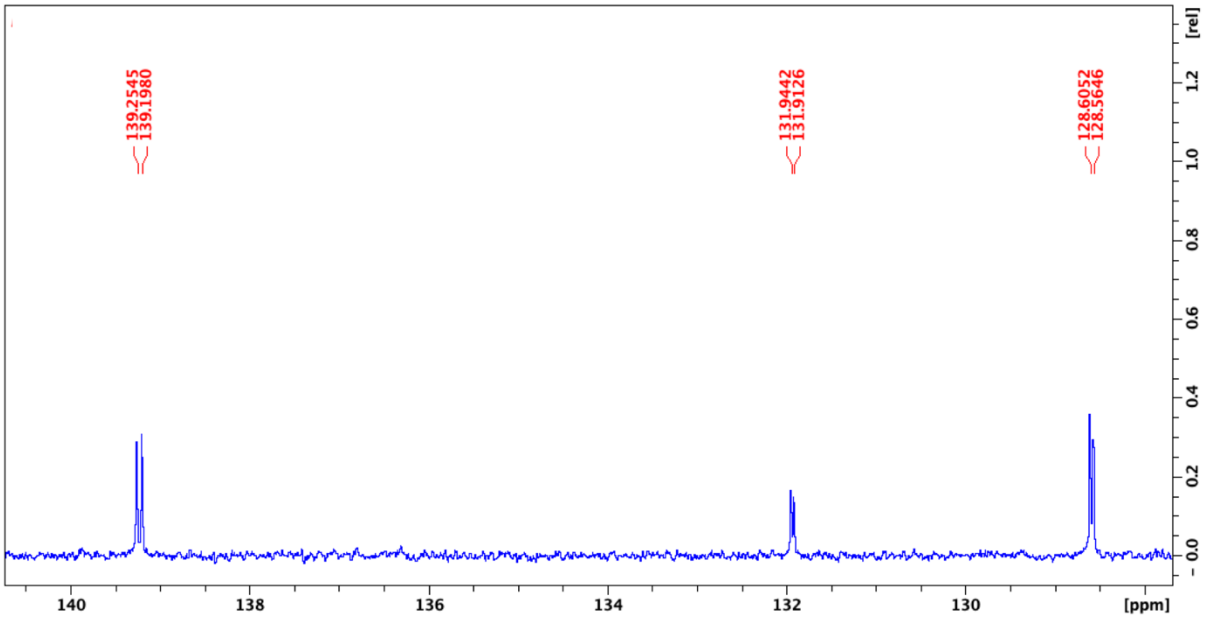




^{1}H NMR spectrum of **1b** in acetone-d$_6$.

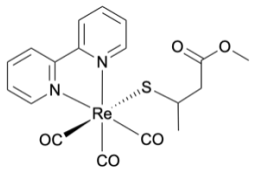


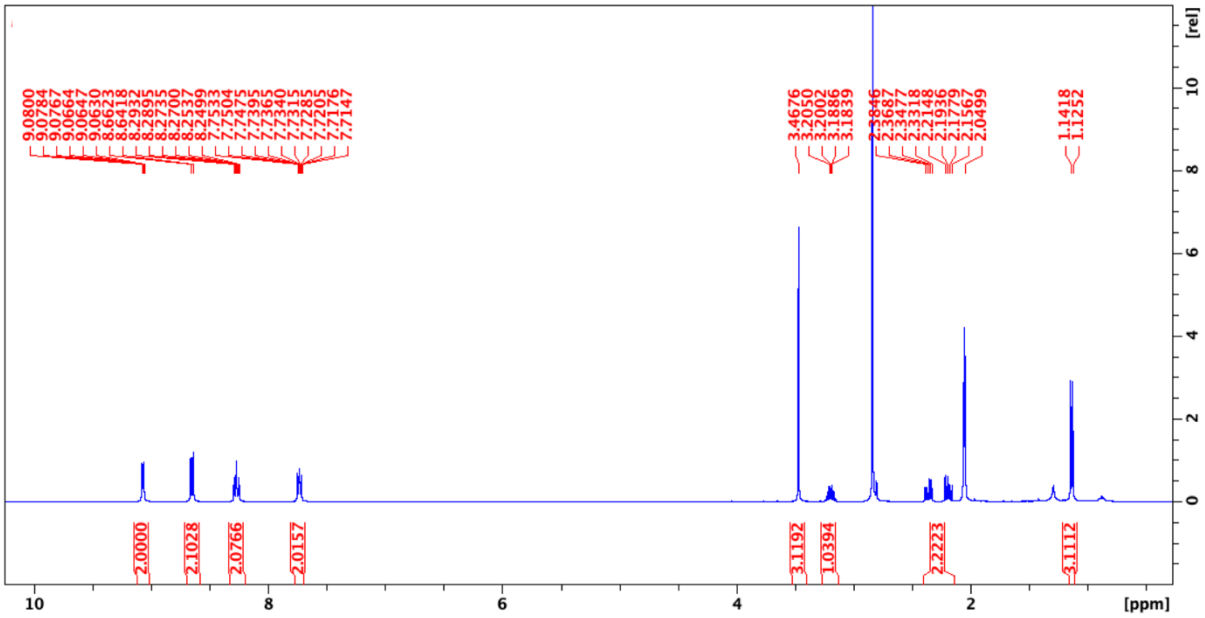
^{13}C NMR spectrum of **1b** in acetone-*d*$_6$.



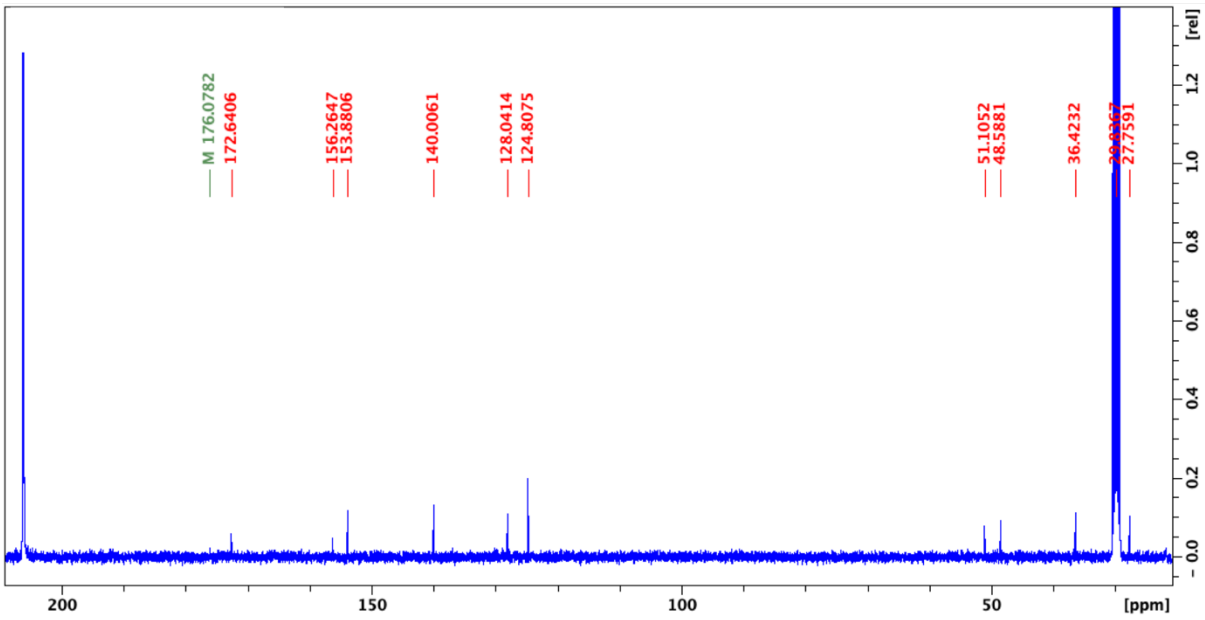
^{13}C NMR spectrum of **1b** in acetone-d_6. Expansion to highlight some of the signal splitting that is observed. Reported in the experimental as either two distinct signals or indicated by using 'split.'

Figure S3: *fac*-[Re(bipy)(CO)$_3$(MMB)], 2a



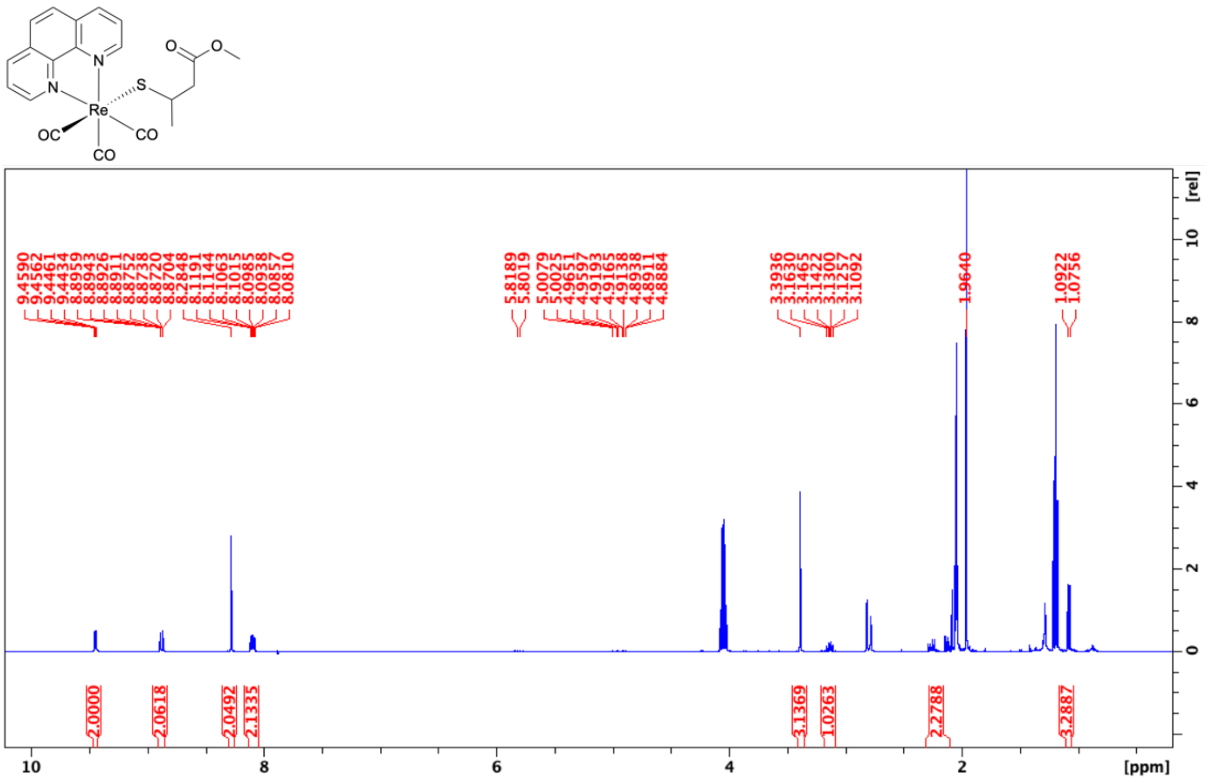


^{1}H NMR spectrum of **2a** in acetone-d_6.

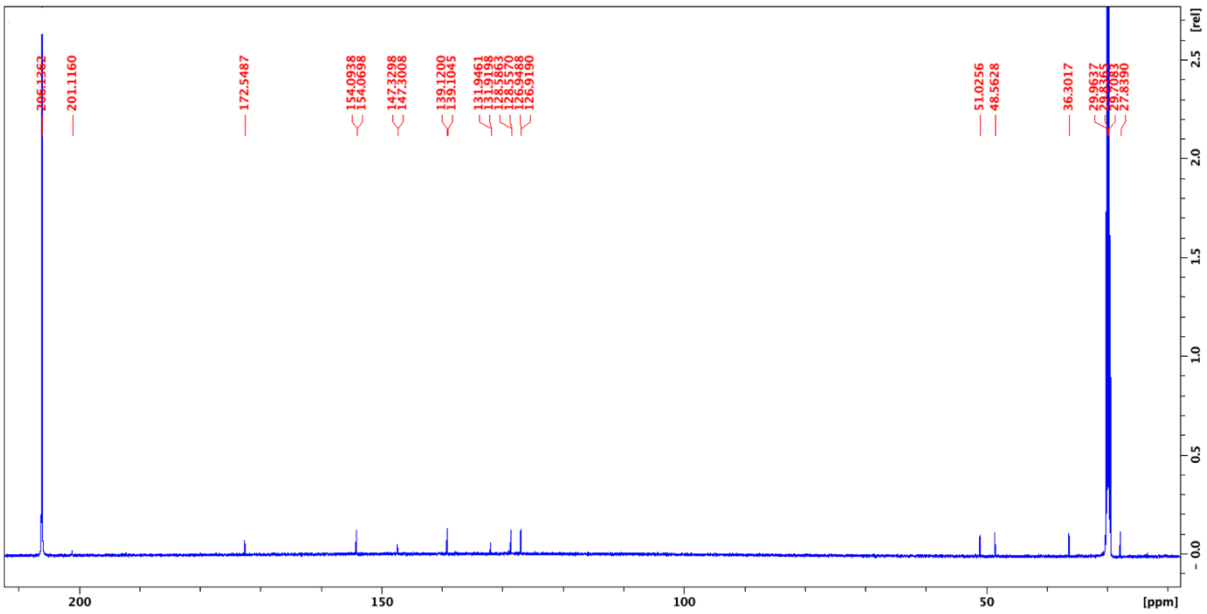


^{13}C NMR spectrum of **2a** in acetone-d_6.

Figure S4: *fac*-[Re(CO)$_3$(phen)(MMB)], 2b

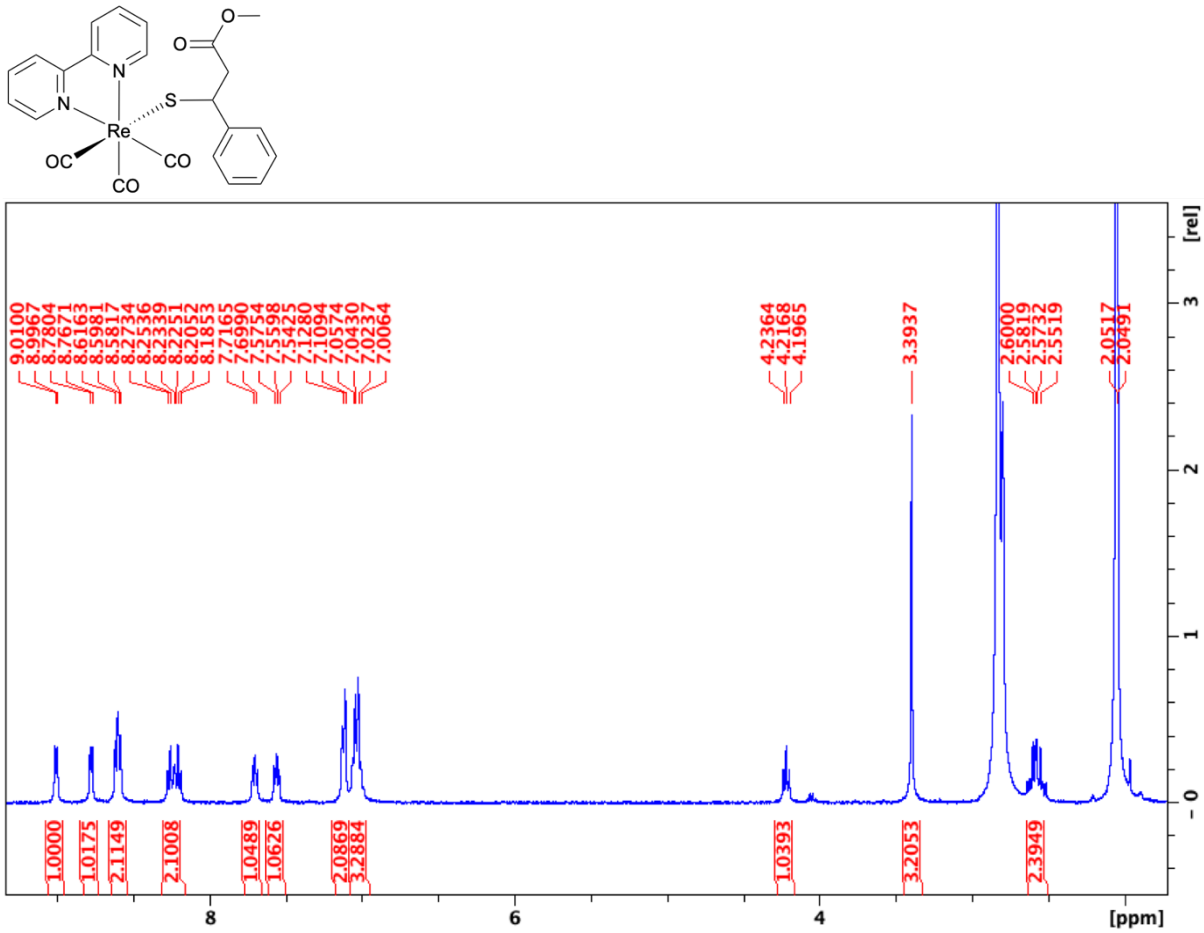


^{1}H NMR spectrum of **2b** in acetone-d_6. Residual ethyl acetate present.

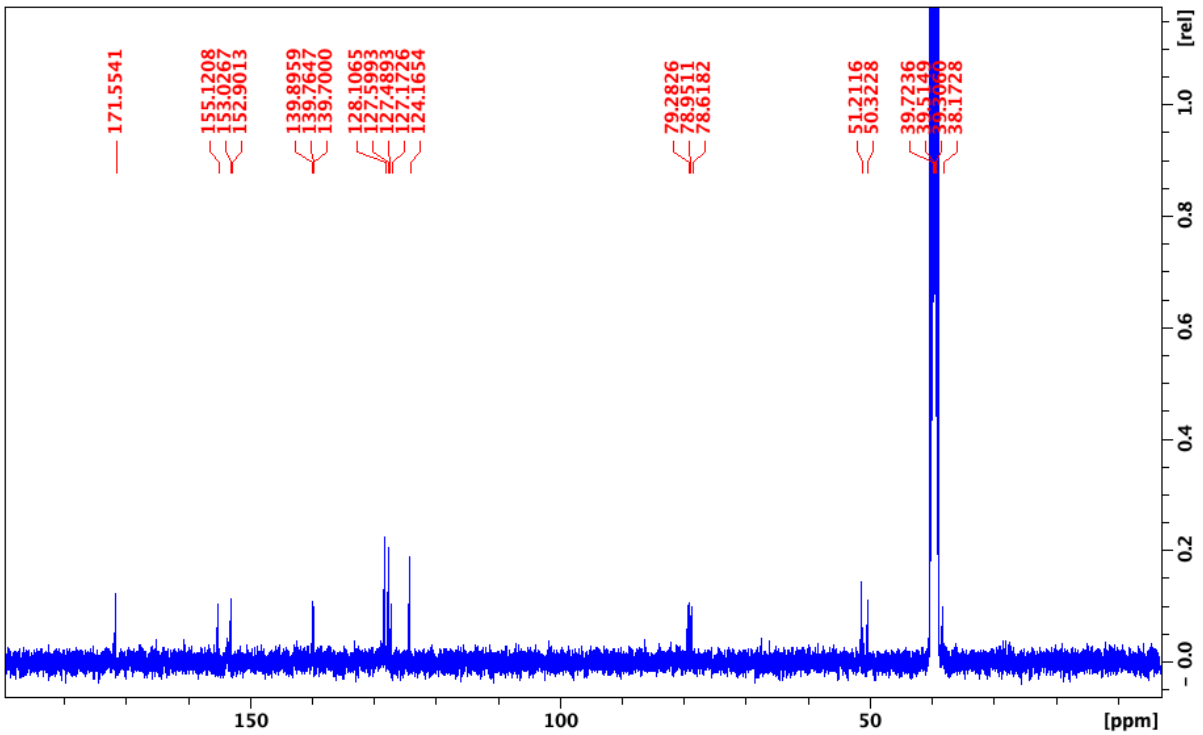


^{13}C NMR spectrum of **2b** in acetone-d_6.

Figure S5: *fac*-[Re(bipy)(CO)$_3$(MMPP)], 3a

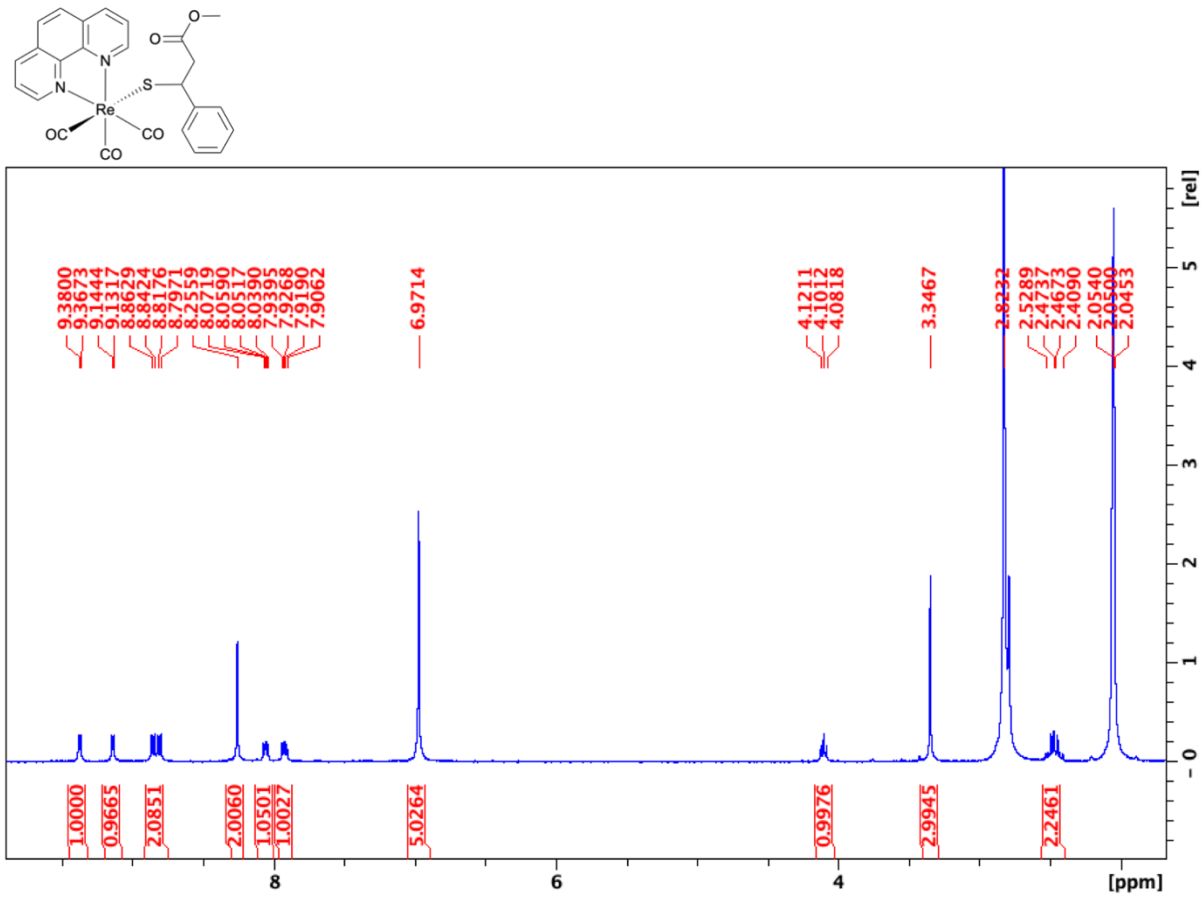


^{1}H NMR spectrum of **3a** in acetone-d_6.



^{13}C NMR spectrum of **3a** in DMSO-d_6

Figure S6: *fac*-[Re(CO)$_3$(phen)(MMPP)], 3b

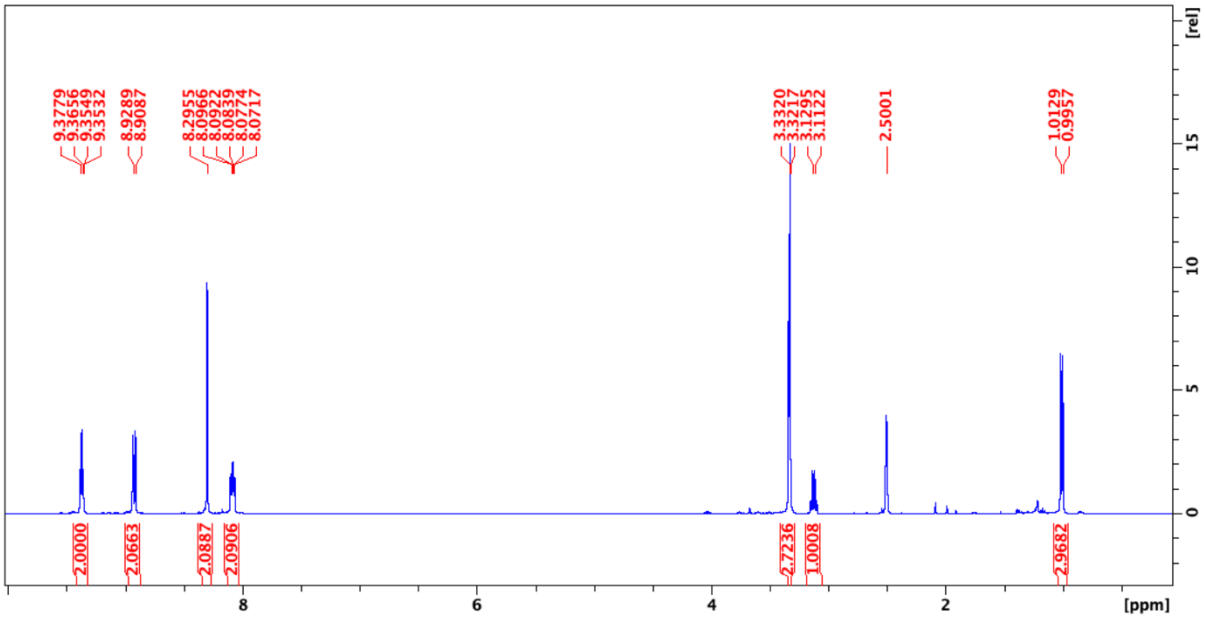


^{1}H NMR spectrum of **3b** acetone-d_6

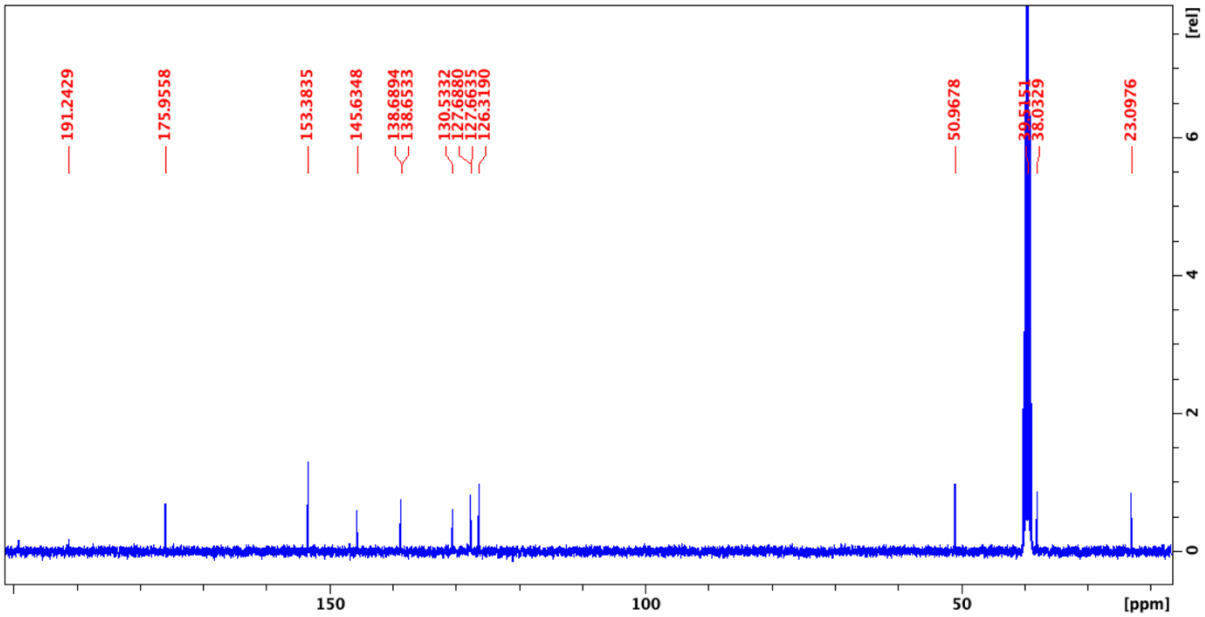
Good quality ^{13}C NMR spectrum of **3b** not obtained due to poor solubility.

Figure S7: Variable Temperature NMR Study of *fac*-[Re(CO)$_3$(phen)(MMP)], 1b

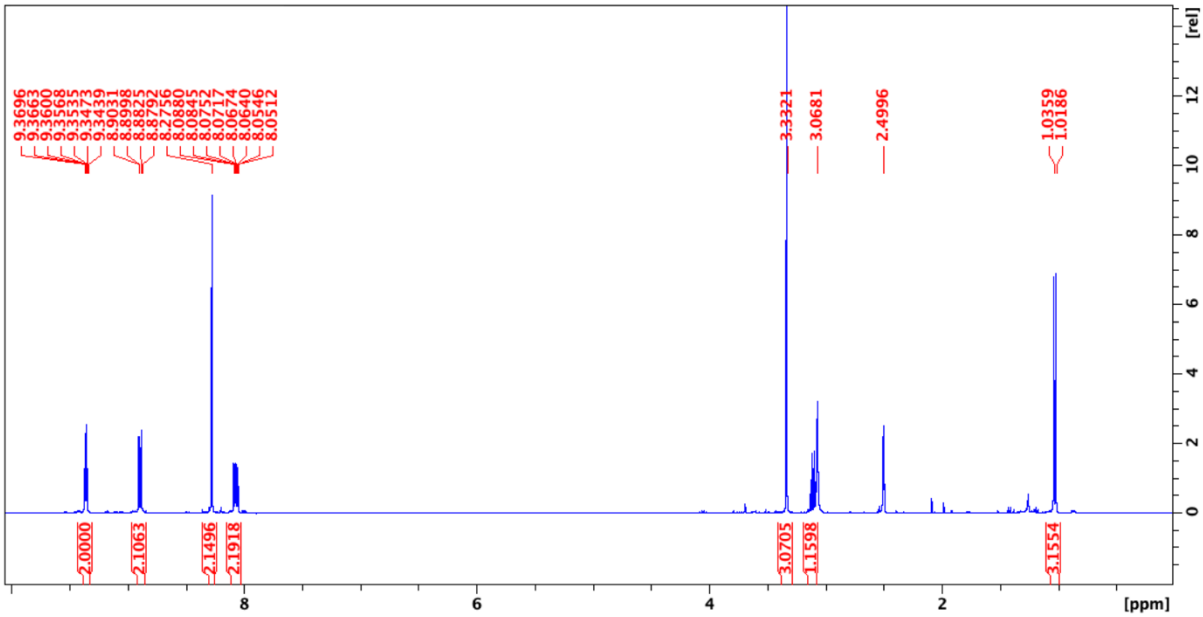
Increase temperature to understand / investigate the split signals seen in the ^{13}C NMR spectrum. All the phen signals are 'split', not seen in the ^{1}H spectrum and no splitting on the S ligand.



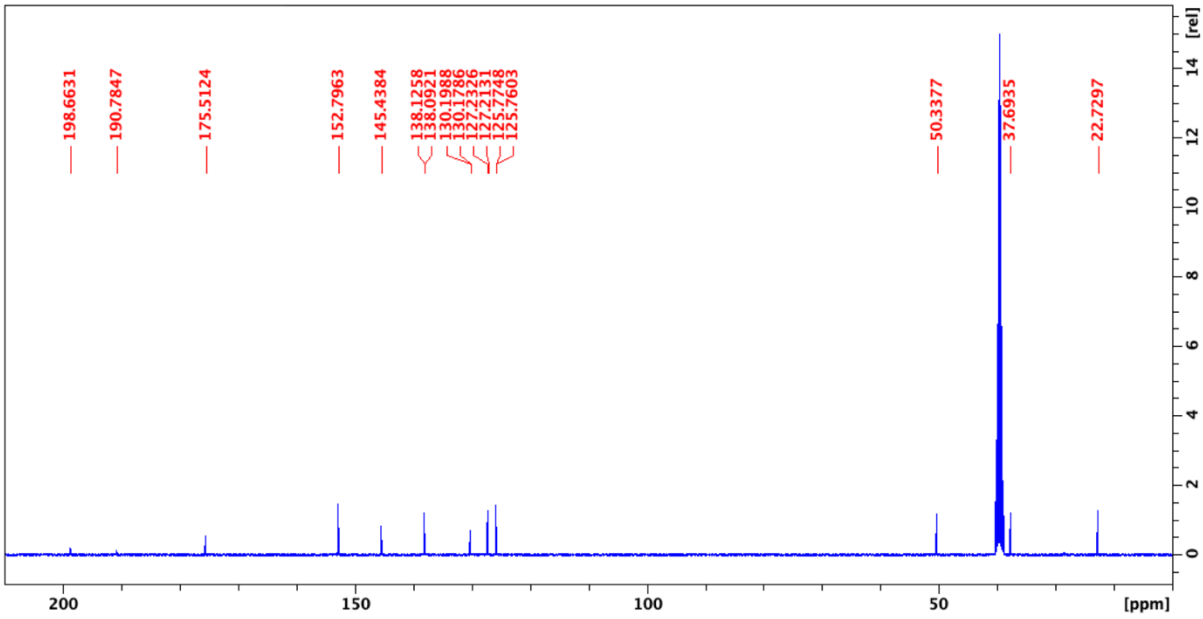
^{1}H NMR spectrum of **1b** in DMSO-d_6 at room temperature.



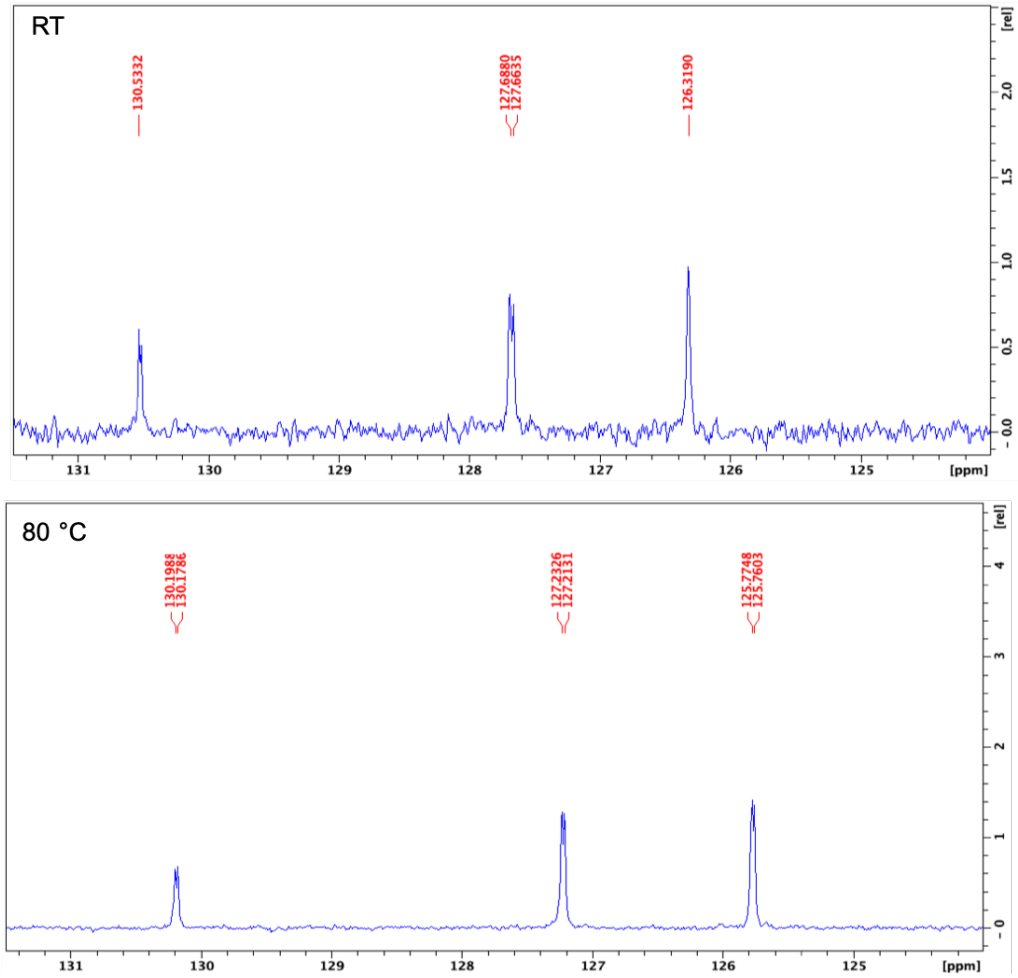
^{13}C NMR spectrum of **1b** in DMSO-d_6 at room temperature.



^{1}H NMR spectrum of **1b** in DMSO-d_6 at 80 °C.



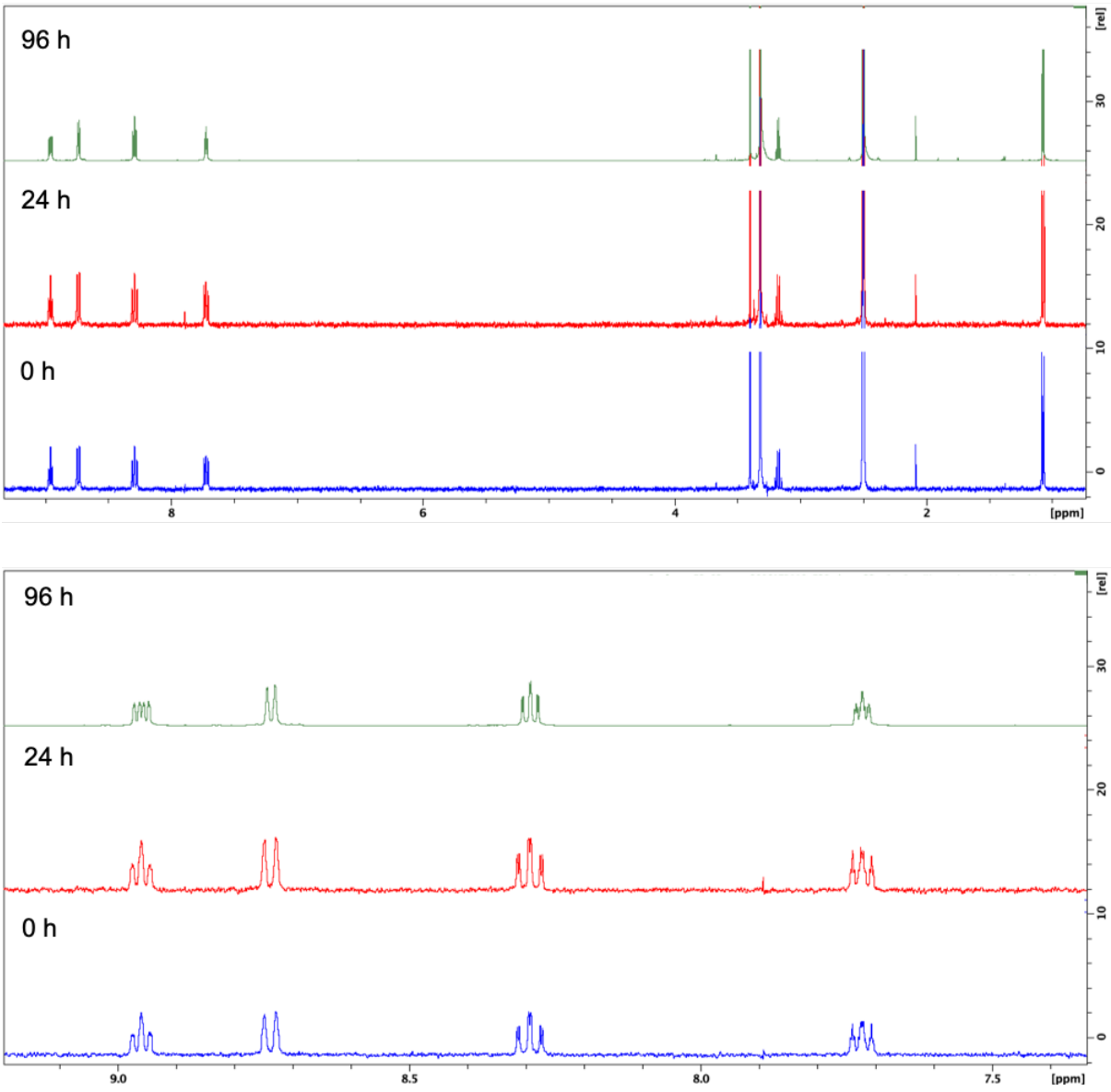
^{13}C NMR spectrum of **1b** in DMSO-d_6 at 80 °C.



^{13}C NMR section of **1b** in DMSO-d_6 at room temperature (top) and at 80 °C (bottom).

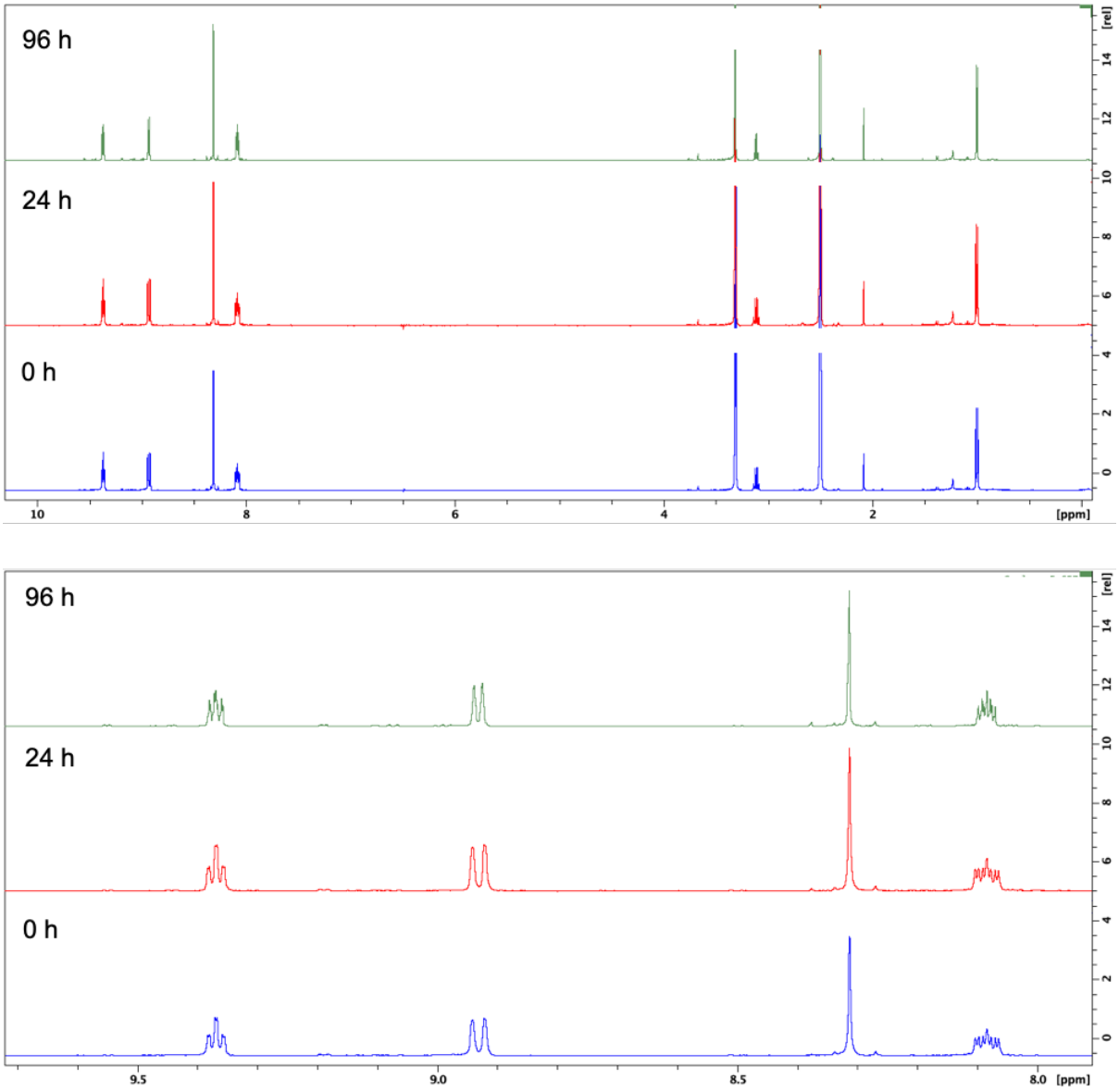
Stability studies of the Re complexes in DMSO-d_6

Figure S8: ***fac*-[Re(bipy)(CO)$_3$(MMP)], 1a**



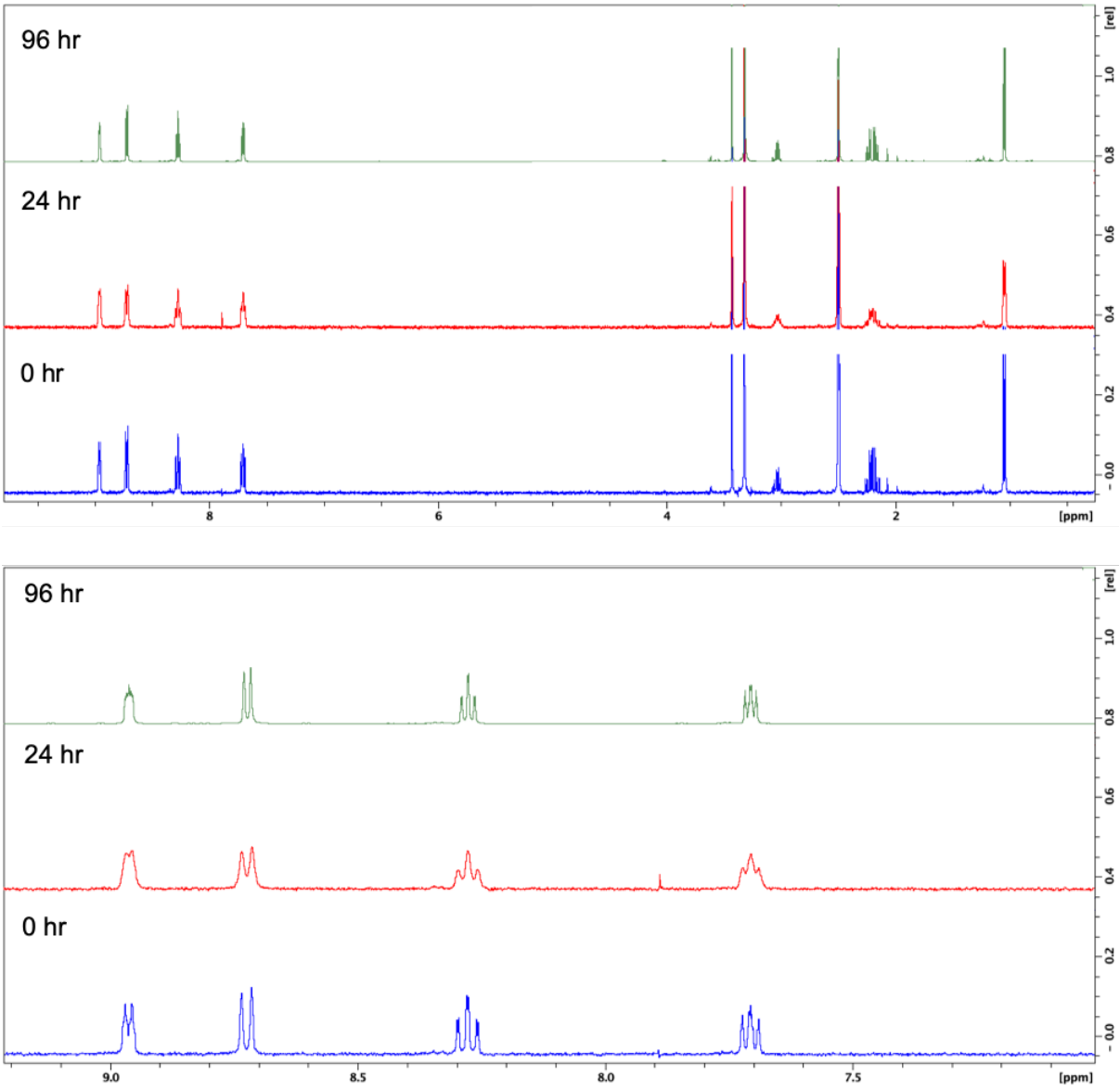
^{1}H NMR spectrum of **1a** in DMSO-d_6 at room temperature, over time. Full spectra (top) and aromatic region (bottom).

Figure S9: ***fac*-[Re(CO)$_3$(phen)(MMP)], 1b**



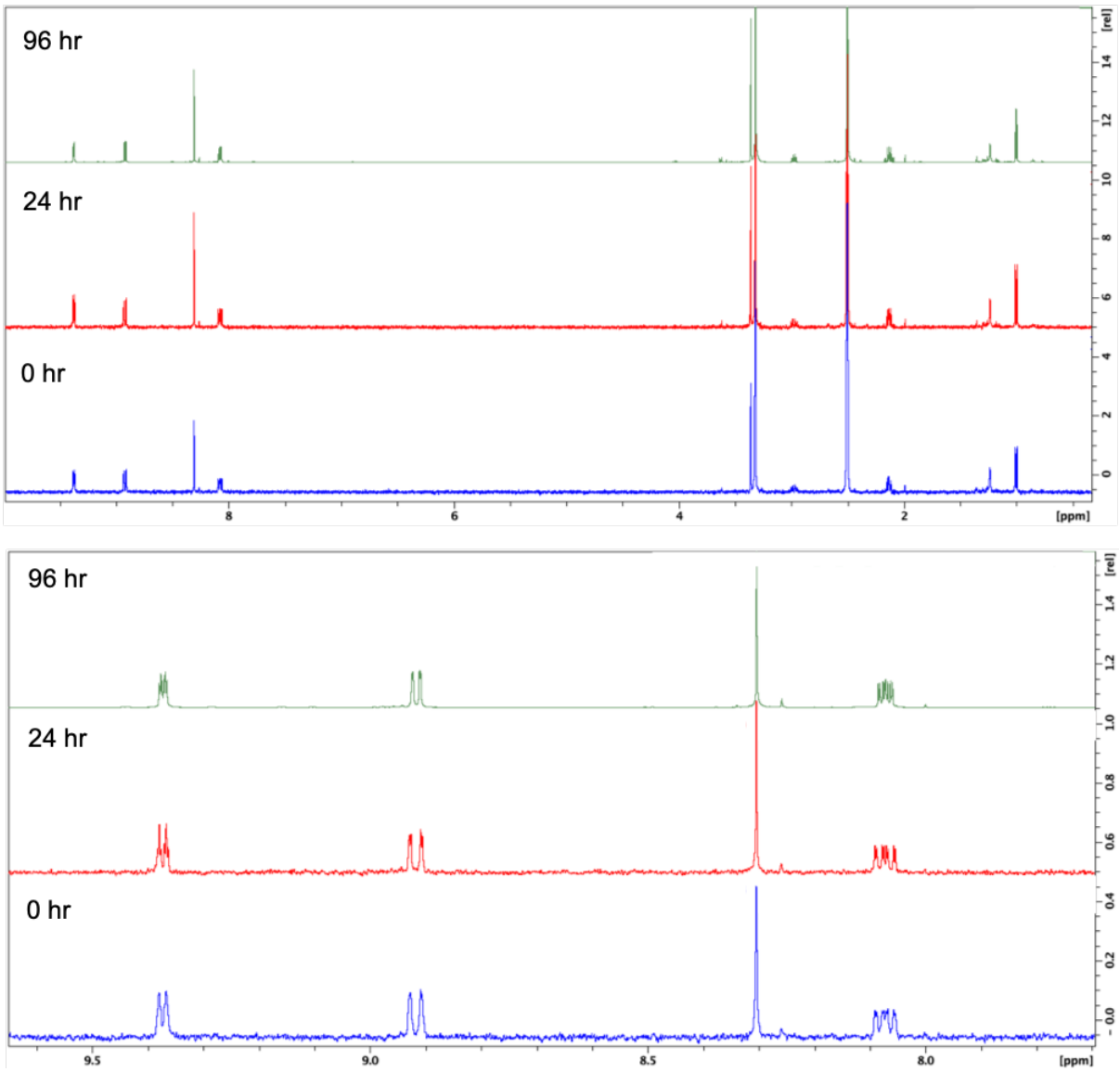
^{1}H NMR spectrum of **1b** in DMSO-d_6 at room temperature, over time. Full spectra (top) and aromatic region (bottom).

Figure S10: *fac*-[Re(CO)$_3$(phen)(MMB)], 2a



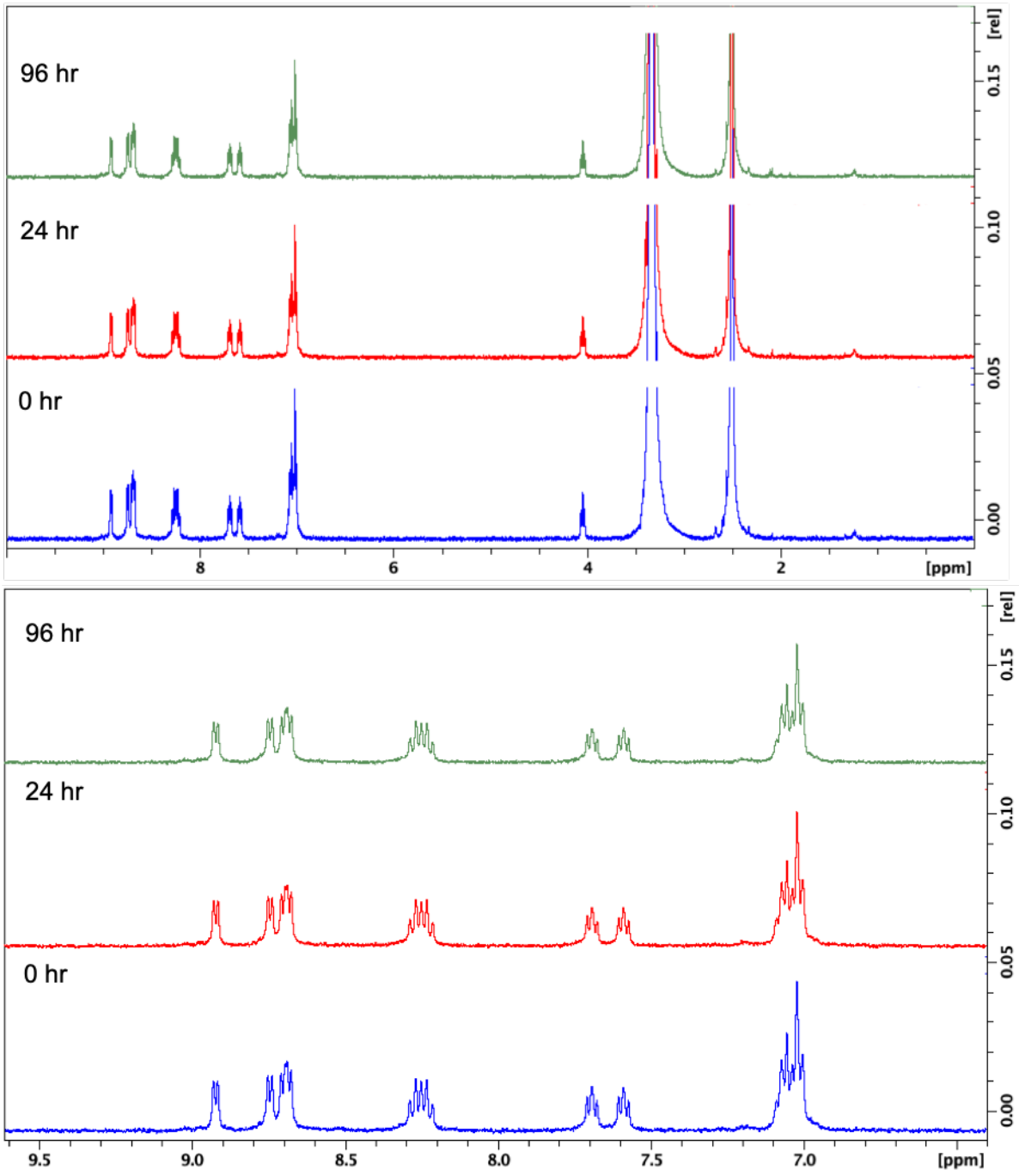
^{1}H NMR spectrum of **2a** in DMSO-d_6 at room temperature, over time. Full spectra (top) and aromatic region (bottom).

Figure S11: *fac*-[Re(CO)$_3$(phen)(MMB)], 2b



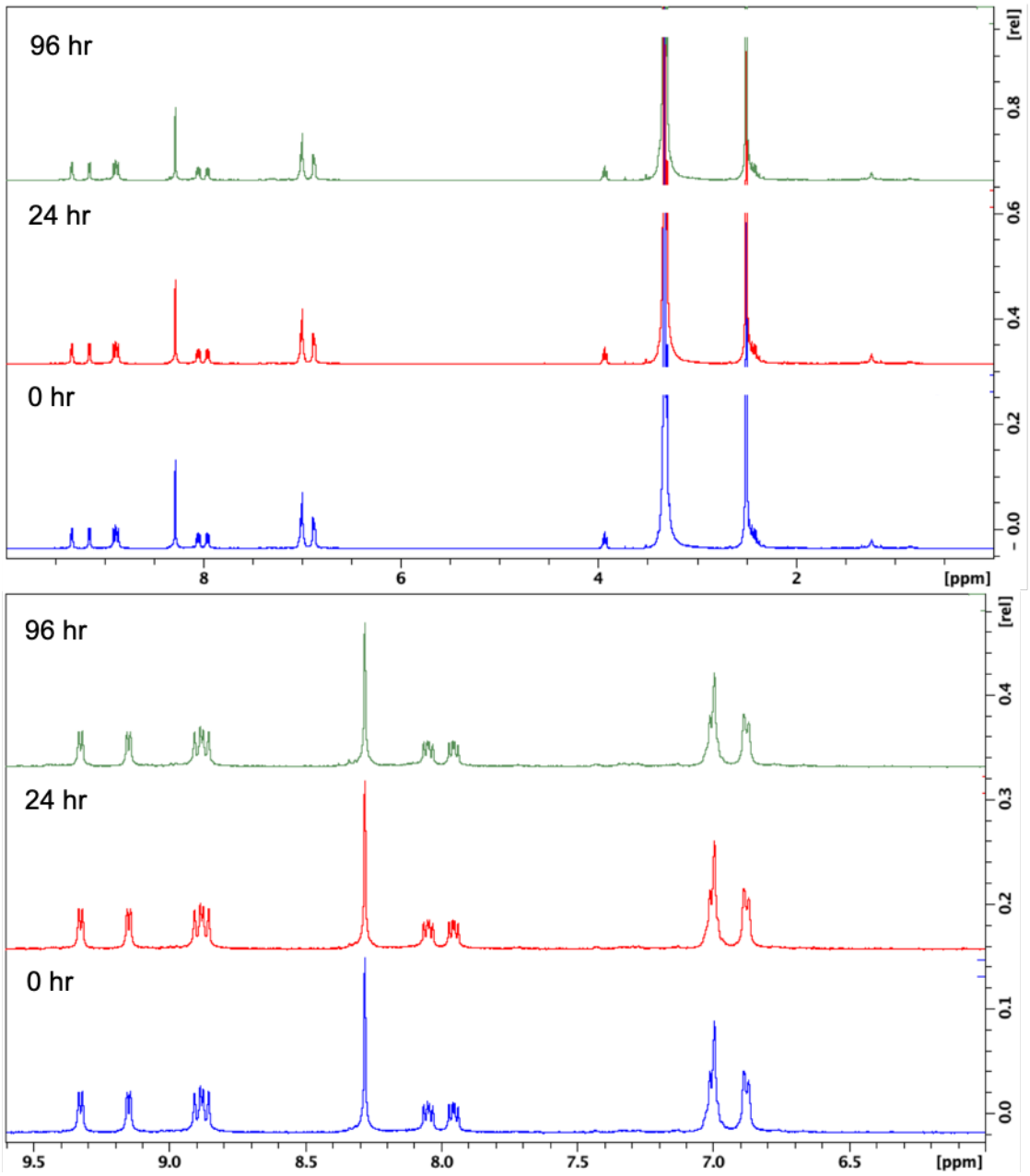
^{1}H NMR spectrum of **2b** in DMSO-d_6 at room temperature, over time. Full spectra (top) and aromatic region (bottom).

Figure S12: *fac*-[Re(bipy)(CO)$_3$(MMPP)], 3a



^{1}H NMR spectrum of **3a** in DMSO-d_6 at room temperature, over time. Full spectra (top) and aromatic region (bottom). *poor solubility*

Figure S13: *fac*-[Re(CO)$_3$(phen)(MMPP)], 3b



^{1}H NMR spectrum of **3b** in DMSO-d_6 at room temperature, over time. Full spectra (top) and aromatic region (bottom).

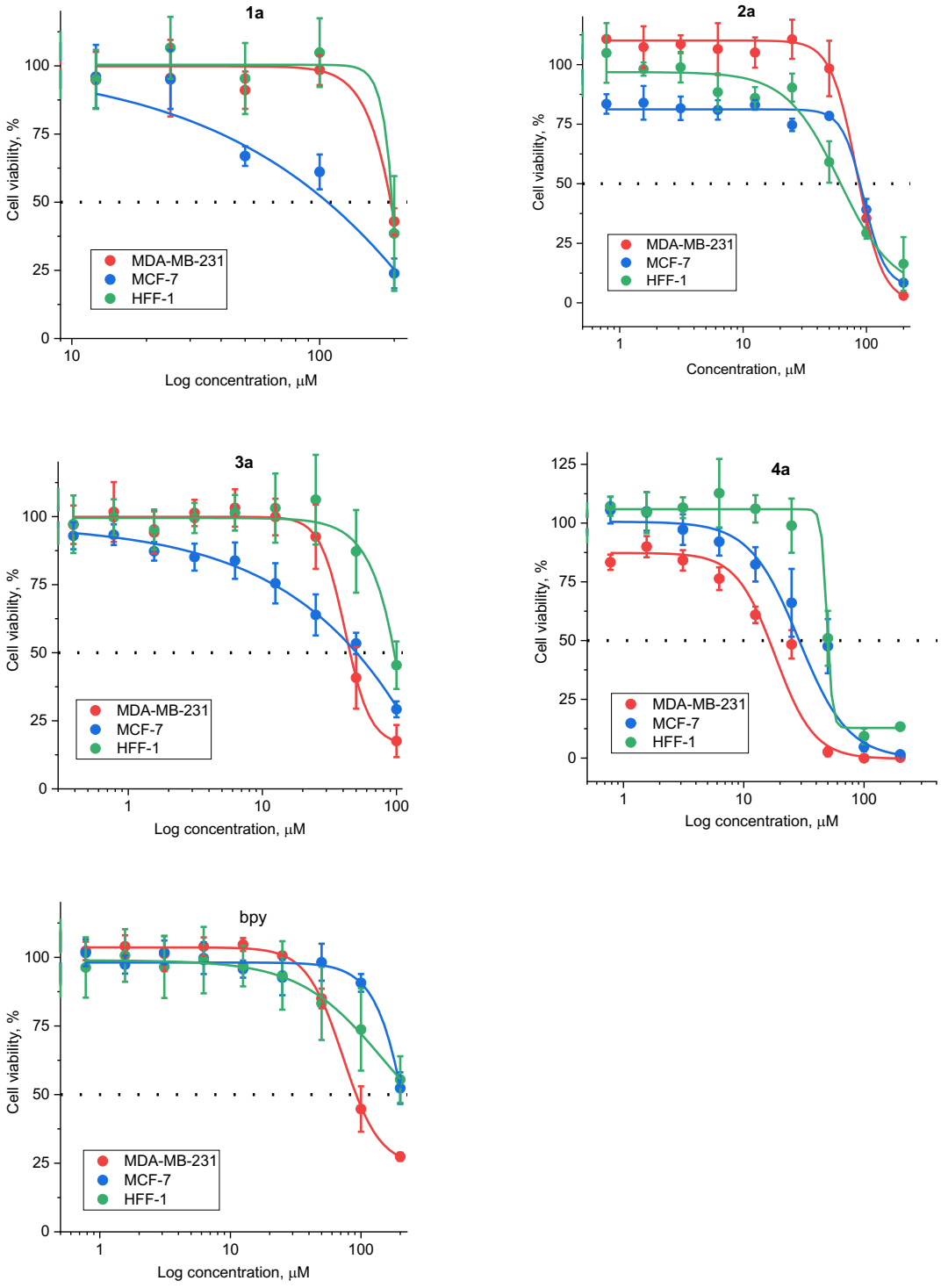


Figure S14. Typical concentration dependencies for cell viabilities in 72 h treatments (MTT assays). Points and error bars represent mean values and standard deviations of six replicate wells, and lines are sigmoidal fits of the experimental data (used for the determination of IC_{50} values, Table S1). Designations of the compounds correspond to Figure 1, main text. The figure is continued at the next page.

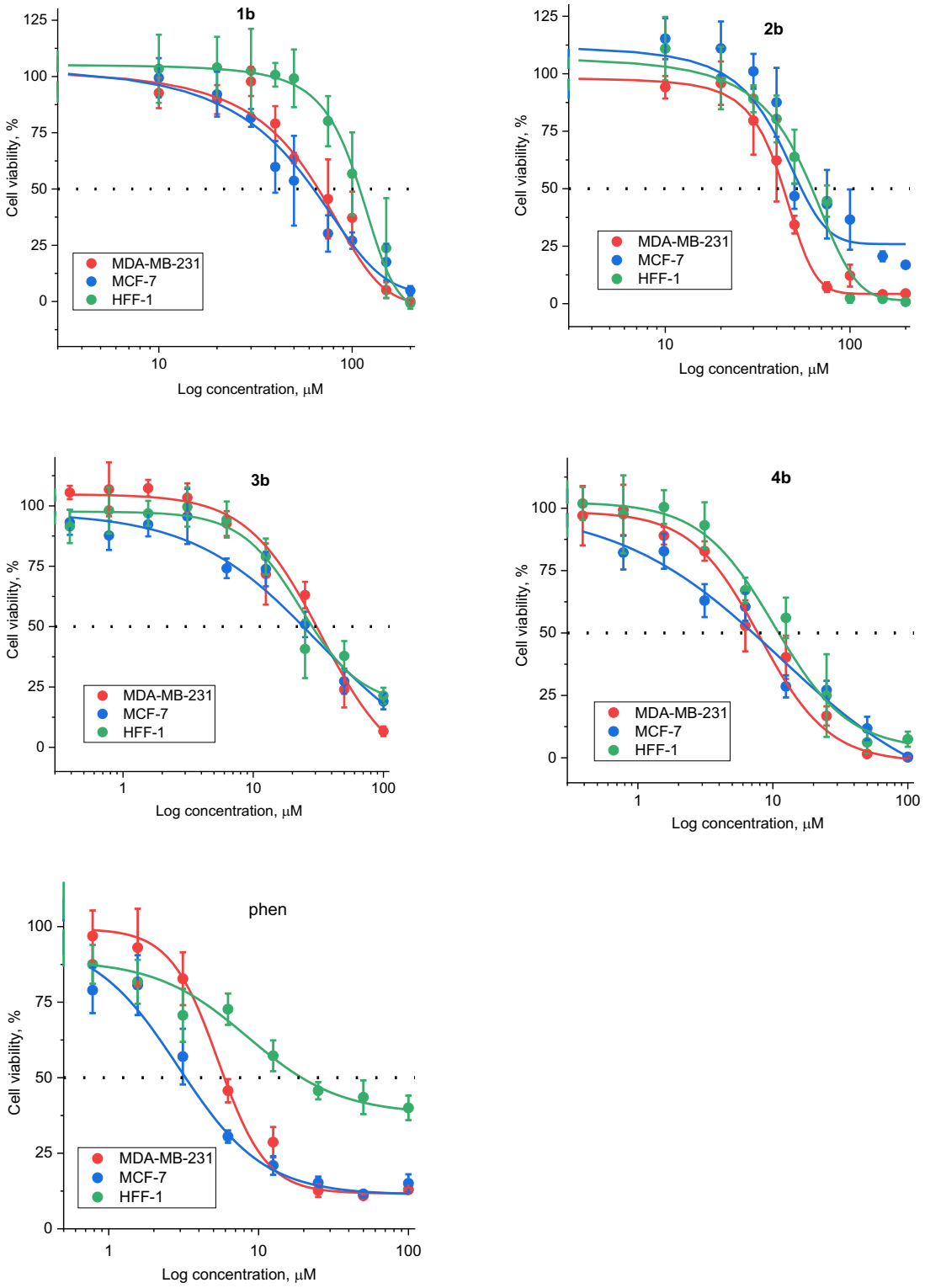


Figure S14 (end).

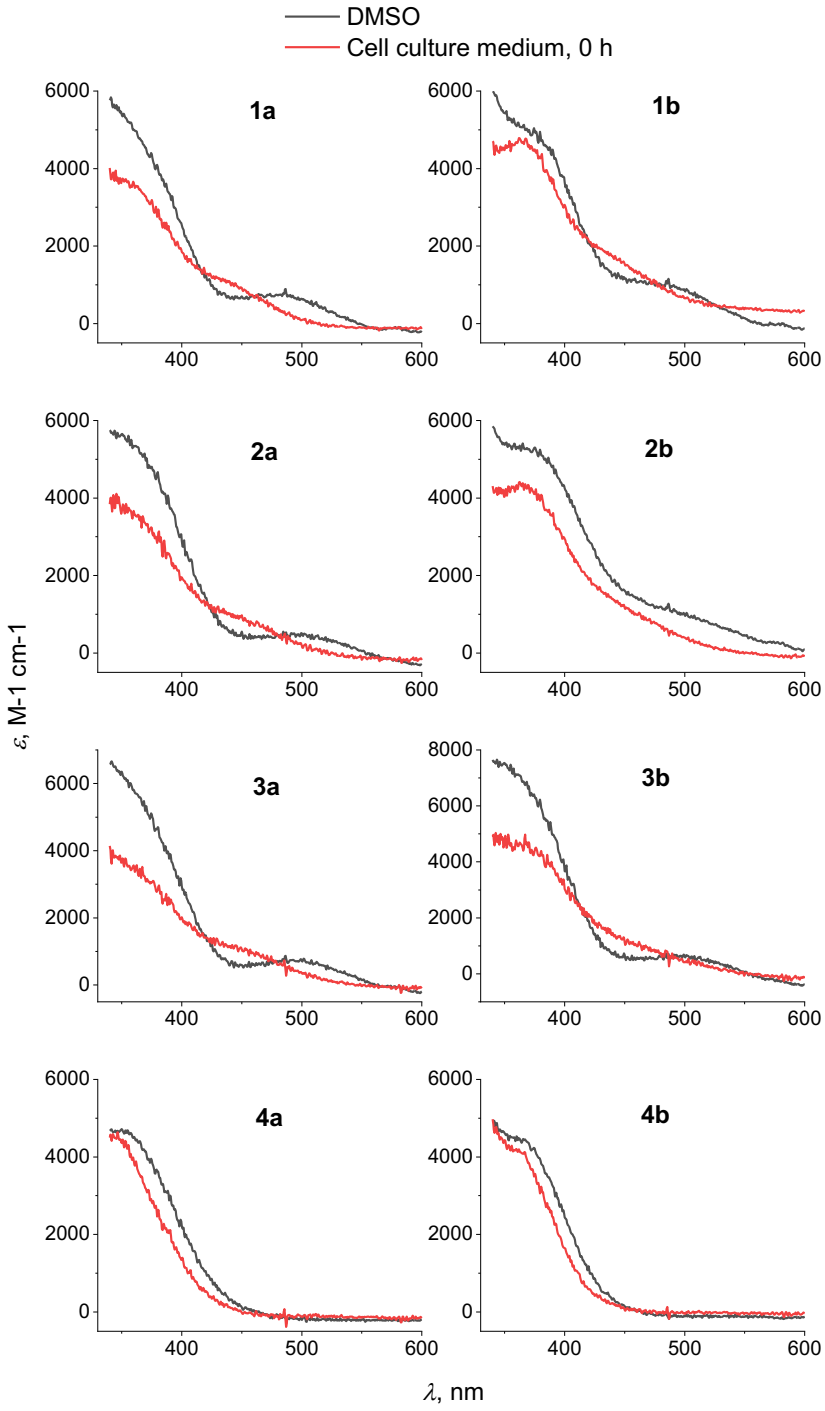


Figure S15. Comparison of UV-vis spectra of Re tricarbonyl complexes (Figure 1 in the main text) in DMSO or in cell culture medium (DMEM without phenol red, supplemented with 1.0% vol. GlutaMax, 1.0% vol. antibiotic-antimycotic, 2.0% vol. FCS and 10 mM HEPES, pH 7.4). Spectra were recorded immediately after the addition of stock solutions of Re complexes in DMSO (100-fold concentration), final [Re] = 15-20 μM (exact concentrations were determined by ICP-MS).

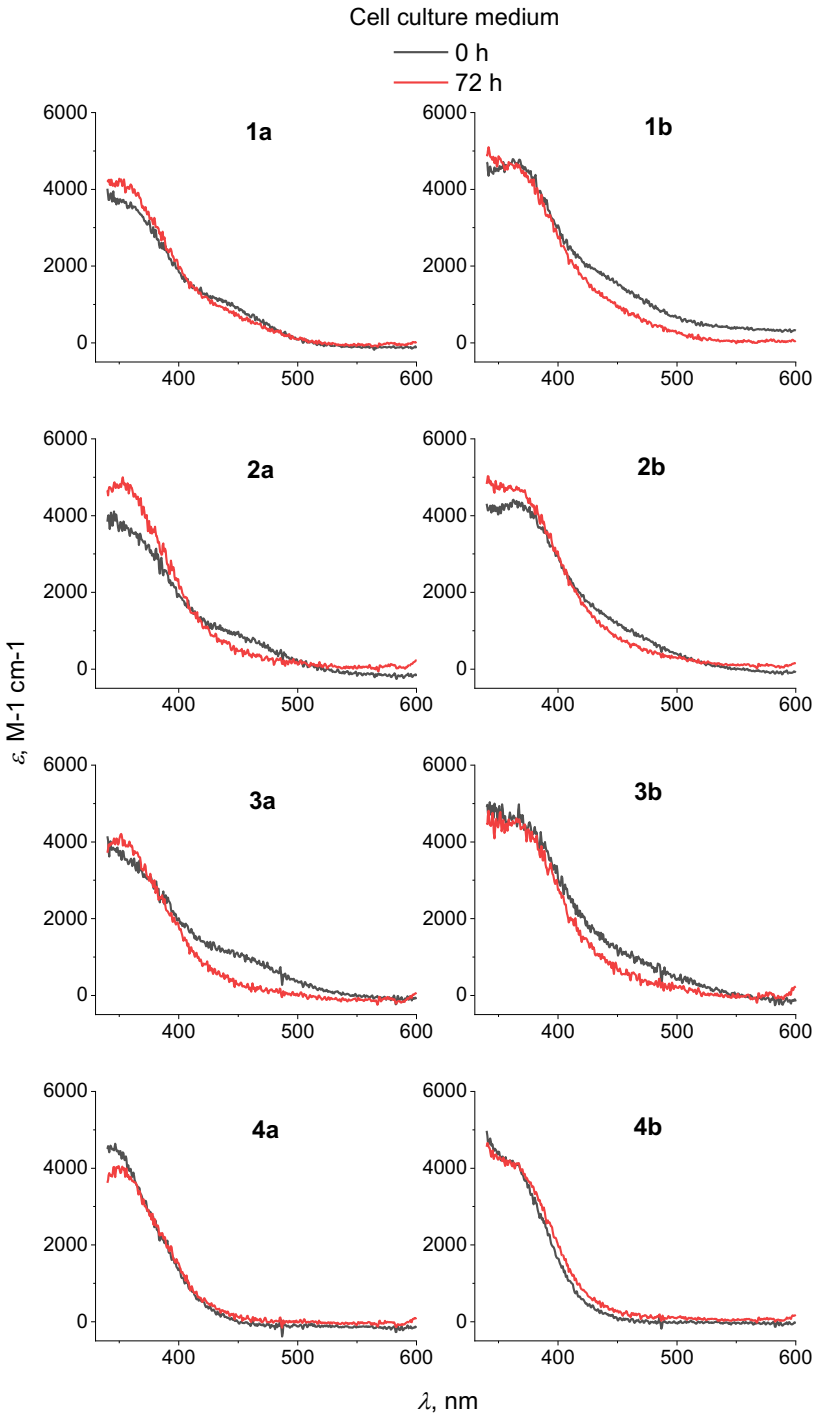


Figure S16. Comparison of electronic absorption spectra of Re tricarbonyl complexes (Figure 1 in the main text) before and after the incubation in cell culture medium for 72 h at 310 K. Other conditions correspond to Figure S15.

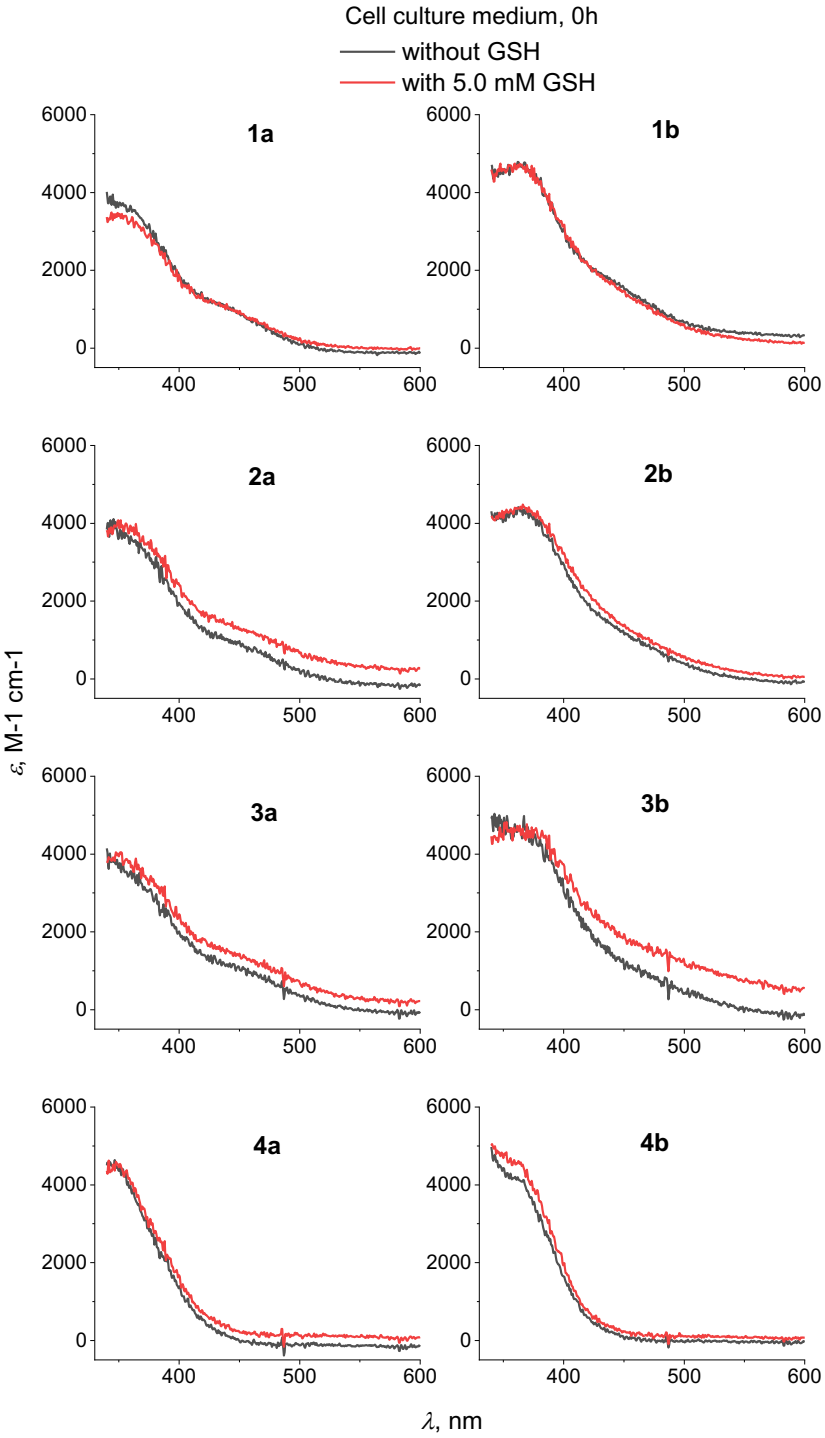


Figure S17. Effect of the addition of GSH to cell culture medium (5.0 mM + 5.0 mM NaOH to compensate for the change in pH) on the electronic absorption spectra of Re tricarbonyl complexes (Figure 1 in the main text). Other conditions correspond to Figure S15.

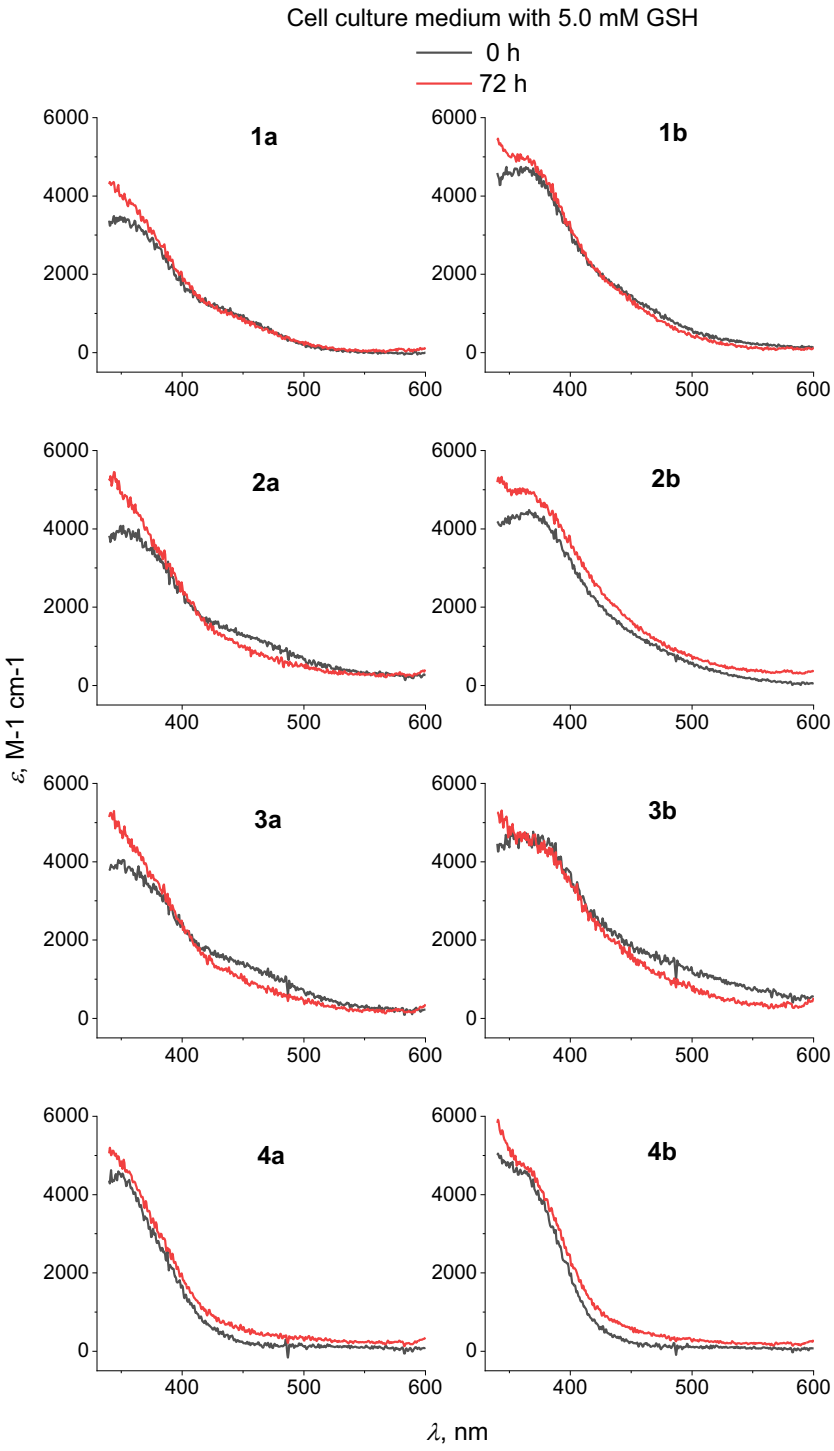


Figure S18. Comparison of electronic absorption spectra of Re tricarbonyl complexes (Figure 1 in the main text) before and after the incubation in cell culture medium containing 5.0 mM GSH for 72 h at 310 K. Other conditions correspond to Figure S15.

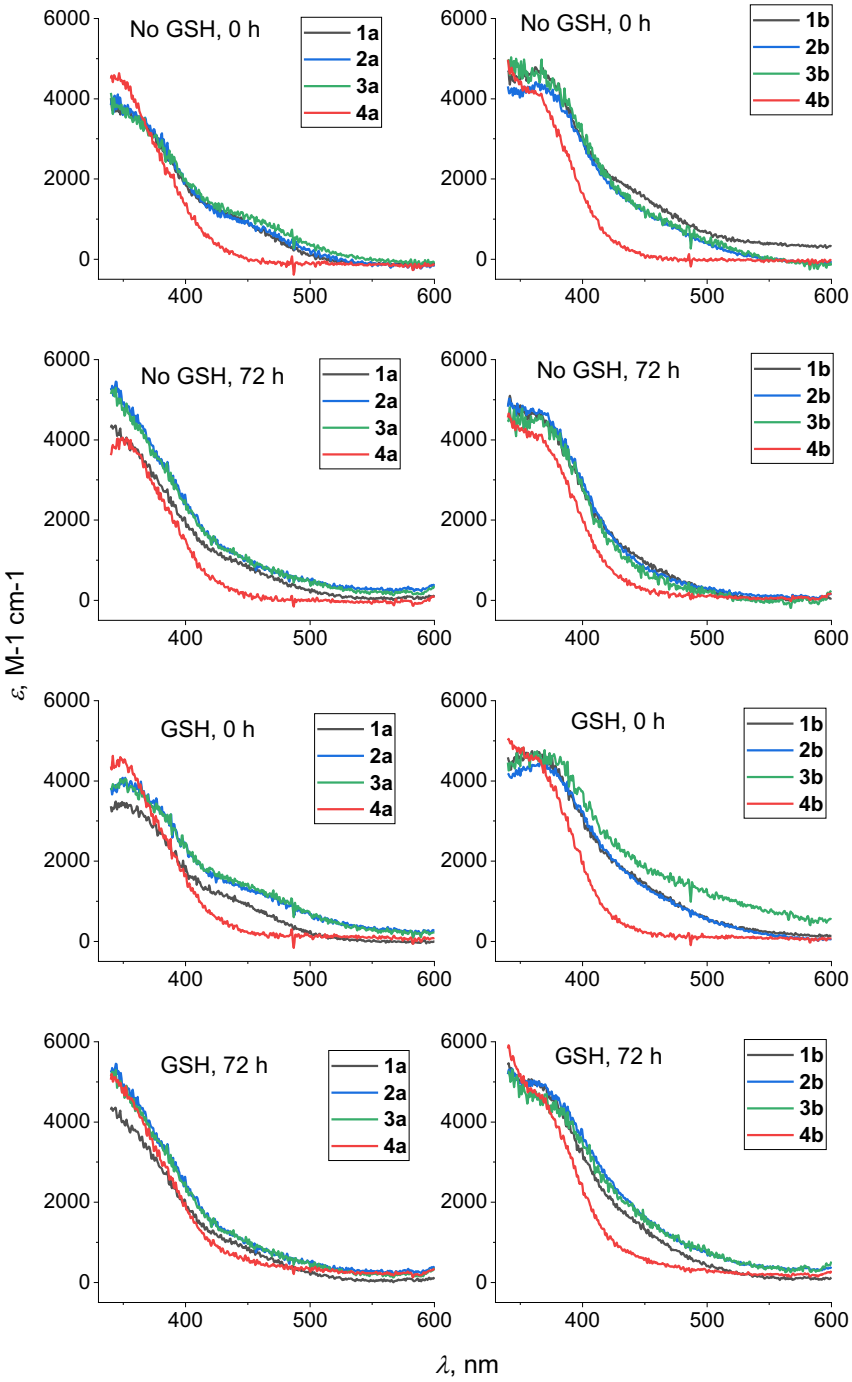


Figure S19. Comparison of electronic absorption spectra of **1a-4a** and **1b-4b** (Figure 1 in the main text) in cell culture medium with or without added GSH (5.0 mM); before or after the incubation at 310 K for 72 h. Other conditions correspond to Figure S15.

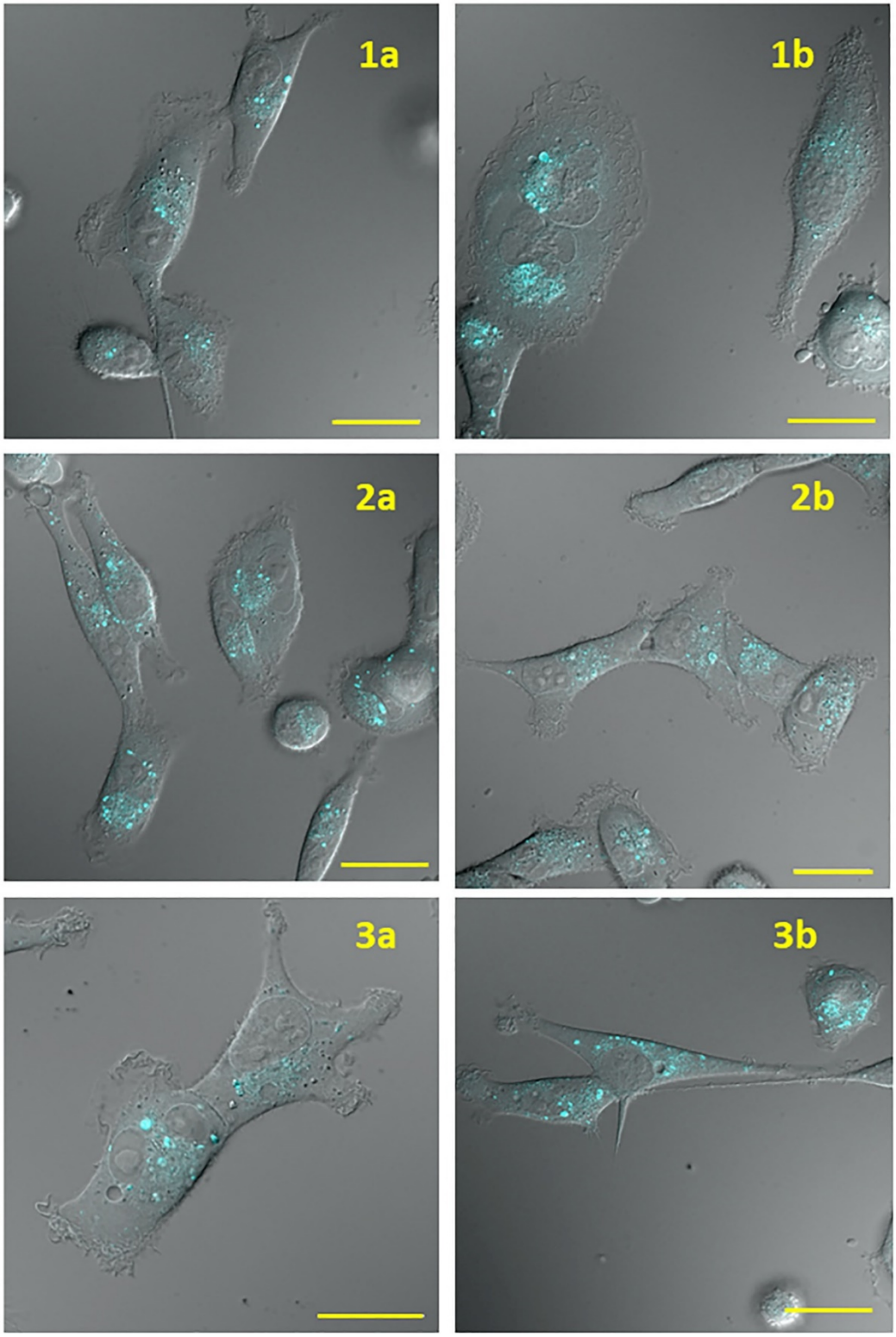


Figure S20. Typical confocal microscopy images (overlays of DIC and Re phosphorescence channels) of MDA-MB-231 cells treated with 25 μM of $[Re(CO)_3(NN)(SR)]$ complexes for 4 h. Designations of the complexes correspond to Figure 1, main text; scale bar, 20 μm.