Electronic Supplementary Information for

Systematically Altering the Lipophilicity of Rhenium(I) Tricarbonyl Anticancer Agents to Tune the Rate at Which They Induce Cell Death

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Experimental

Physical Measurements.

NMR samples were prepared in DMSO-d_6 and analyzed on either a 500 MHz Bruker AV 3HD spectrometer equipped with a broadband Prodigy cryoprobe or an INOVA NMR spectrometer (300 or 400 MHz). ^{1}H NMR spectra were analyzed using MestReNova and referenced to the residual DMSO peak at 2.50 ppm. Samples were prepared for FTIR spectroscopy as KBr pellets and analyzed on a Thermo Nicolet Avatar 370 DTGS FTIR spectrometer. UV–vis spectra were acquired either using an Agilent Cary 8454 UV–visible spectrophotometer or a Shimadzu UV-1900 spectrophotometer equipped with a Quantum Northwest TC 1 temperature controller. Elemental analysis (CHN) was performed by Atlantic Microlab Inc. (Norcross, GA, USA). High-resolution electrospray mass spectrometry measurements (HR-ESI-MS) were performed on an Exactive Orbitrap mass spectrometer in positive ion mode (ThermoFisher Scientific, Waltham, MA). Absorbance signatures from cell viability and protein concentration assays were measured using a Biotek Synergy HT plate reader. ICP-OES was carried out using an Agilent 5110 ICP-OES. A 10-point standard with respect to rhenium was used and fits were found to be with R^2 of 0.9999. Concentrations were then back-calculated to the stock sample concentration.

Materials and Reagents.

All reagents were purchased from commercial vendors. All reactions were carried out under ambient atmospheric conditions without any efforts to exclude oxygen or water. Solvents used were of ACS grade or higher. **Re-Chains** were synthesized following a modified version of previously reported procedures.[1–11]

Synthesis of Re-C2.

$Re(CO)_5Cl$ (50 mg, 0.14 mmol), picolinaldehyde (13.1 µL, 0.14 mmol), and ethylamine (8.9 µL, 0.14 mmol) were refluxed in 3 mL MeOH for 4 h. The yellow solution was cooled to room temperature and concentrated under reduced pressure to ~0.5 mL, and 20 mL of 1:4 ether:hexane was added to precipitate the rhenium complex. The mixture was vacuum-filtered and washed with hexane to obtain the pure material as an orange powder. Yield: 33 mg (54%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.24 (s, 1H), 9.00 (d, 1H), 8.32 (td, 1H), 8.24 (d, 1H), 7.78 (td, 1H), 4.11 (m, 2H), 1.46 (t, 3H). FTIR (KBr, ν(CO) cm^{-1}): 1880, 1918, 2027. ESI-MS (positive ion mode, MeCN): *m/z* 405.02352, calcd 405.02434 for $[M–Cl]^+$; 433.01852, calcd 433.01926 for $[M–Cl+CO]^+$; 446.04992, calcd 446.05089 for $[M–Cl+MeCN]^+$. Anal. Calcd for **Re-C2** ($C_{11}H_{10}ClN_2O_3Re$): C, 30.61; H, 2.48; N, 6.26. Found: C, 30.58; H, 2.16; N, 6.08%.

Synthesis of Re-C3.

$Re(CO)_5Cl$ (50 mg, 0.14 mmol), picolinaldehyde (13.1 µL, 0.14 mmol), and 1-propylamine (11.4 µL, 0.14 mmol) were refluxed in 3 mL MeOH for 4 h. The yellow solution was cooled to room temperature and concentrated under reduced pressure to ~0.5 mL, and 20 mL of 1:4 ether:hexane was added to precipitate the rhenium complex. The mixture was vacuum-filtered and washed with hexane to obtain the pure material as an orange-yellow powder. Yield: 45 mg (71%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.23 (s, 1H), 9.00 (d, 1H), 8.32 (td, 1H), 8.25 (d, 1H), 7.78 (d, 1H), 4.01 (t, 2H), 1.93 (m, 2H), 0.94 (t, 3H). FTIR (KBr, ν(CO) cm^{-1}): 1887, 1930, 2024. ESI-MS (positive ion mode, MeCN): *m/z* 419.03887, calcd 419.03999 $[M–Cl]^+$; 460.06529, calcd 460.06654 for

[M–Cl+MeCN]$^+$; 478.11220, calcd 477.09309 for [M–Cl+MeCN+NH_3]$^+$. Anal. Calcd for **Re-C3** ($C_{12}H_{12}ClN_2O_3Re$): C, 31.75; H, 2.66; N, 6.17. Found: C, 31.88; H, 2.56; N, 6.26%.

Synthesis of Re-C4.
$Re(CO)_5Cl$ (50 mg, 0.14 mmol), picolinaldehyde (13.1 µL, 0.14 mmol), and 1-butylamine (13.7 µL, 0.14 mmol) were refluxed in 3 mL MeOH for 4 h. The yellow solution was cooled to room temperature and concentrated under reduced pressure to ~0.5 mL, and 20 mL of 1:4 ether:hexane was added to precipitate the rhenium complex. The mixture was vacuum-filtered and washed with hexane to obtain the pure material as an orange-yellow powder. Yield: 31 mg (48%). ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 9.24 (s, 1H), 8.99 (d, 1H), 8.32 (td, 1H), 8.25 (d, 1H), 7.78 (td, 1H), 4.04 (m, 2H), 1.90 (m, 2H), 1.35 (dt, 2H), 0.94 (t, 3H). FTIR (KBr, ν(CO) cm^{-1}): 1889, 1930, 2022. ESI-MS (positive ion mode, MeCN): *m/z* 433.05477, calcd 433.05564 for [M–Cl]$^+$; 461.04965, calcd 461.05056 for [M–Cl+CO]$^+$; 474.08118, calcd 474.08219 for [M–Cl+MeCN]$^+$. Anal. Calcd for **Re-C4** ($C_{13}H_{14}ClN_2O_3Re$): C, 33.37; H, 3.02; N, 5.99. Found: C, 33.55; H, 2.88; N, 5.99%.

Synthesis of Re-C5.
$Re(CO)_5Cl$ (50 mg, 0.14 mmol), picolinaldehyde (13.1 µL, 0.14 mmol), and 1-pentylamine (16 µL, 0.14 mmol) were refluxed in 3 mL MeOH for 4 h. The yellow solution was cooled to room temperature and concentrated under reduced pressure to ~0.5 mL, and 20 mL of 1:4 ether:hexane was added to precipitate the rhenium complex. The mixture was vacuum-filtered and washed with hexane to obtain the pure material as a yellow-orange powder. Yield: 26 mg (40%). ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 9.23 (s, 1H), 9.01 (d, 1H), 8.32 (td, 1H), 8.25 (d, 1H), 7.78 (td, 1H), 4.03 (t, 2H), 1.94 (m, 2H), 1.35 (m, 4H), 0.89 (t, 3H). FTIR (KBr, ν(CO) cm^{-1}): 1905, 1923, 2027. ESI-MS (positive ion mode, MeCN): *m/z* 447.07041, calcd 447.07129 for [M–Cl]$^+$; 465.08107, calcd 465.08186 for [M–Cl+H_2O]$^+$; 488.09690, calcd 488.09784 for [M–Cl+MeCN]$^+$. Anal. Calcd for **Re-C5**·0.1Et_2O ($C_{14.4}H_{17}ClN_2O_{3.1}Re$): C, 35.34; H, 3.50; N, 5.72. Found: C, 35.61; H, 3.33; N, 6.09%.

Synthesis of Re-C6.
$Re(CO)_5Cl$ (50 mg, 0.14 mmol), picolinaldehyde (13.1 µL, 0.14 mmol), and 1-hexylamine (18.2 µL, 0.14 mmol) were refluxed in 3 mL MeOH for 4 h. The yellow solution was cooled to room temperature and concentrated under reduced pressure to ~0.5 mL, and 20 mL of 1:4 ether:hexane was added to precipitate the rhenium complex. The mixture was vacuum-filtered and washed with hexane to obtain the pure material as a yellow-orange powder. Yield: 21 mg (30%). ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 9.23 (s, 1H), 9.01 (d, 1H), 8.32 (td, 1H), 8.25 (d, 1H), 7.78 (td, 1H), 4.03 (m, 2H), 1.92 (m, 2H), 1.32 (m, 6H), 0.87 (t, 3H). FTIR (KBr, ν(CO) cm^{-1}): 1895, 1918, 2019. ESI-MS (positive ion mode, MeCN): *m/z* 461.08593, calcd 461.08694 for [M–Cl]$^+$; 479.09655, calcd 479.09751 for [M–Cl+H_2O]$^+$; 502.11243, calcd 502.11349 for [M–Cl+MeCN]$^+$. Anal. Calcd for **Re-C6** ($C_{15}H_{18}ClN_2O_3Re$): C, 36.33; H, 3.66; N, 5.65. Found: C, 36.36; H, 3.55; N, 5.66%.

Synthesis of Re-C12.
$Re(CO)_5Cl$ (50 mg, 0.14 mmol), picolinaldehyde (13.1 µL, 0.14 mmol), and 1-dodecylamine (26 mg, 0.14 mmol) were refluxed in 3 mL MeOH for 4 h. The yellow solution was cooled to room temperature and concentrated under reduced pressure to ~0.5 mL, and 20 mL of 1:4 ether:hexane was added to precipitate the rhenium complex. The mixture was vacuum-filtered and washed with hexane to obtain the pure material as a yellow-orange powder. Yield: 58 mg (70%). ^{1}H NMR (500

MHz, DMSO-d_6): δ 9.23 (s, 1H), 9.00 (d, 1H), 8.32 (td, 1H), 8.24 (d, 1H), 7.78 (td, 1H), 4.02 (m, 2H), 1.88 (m, 2H), 1.33–1.23 (m, 18H), 0.85 (t, 3H). FTIR (KBr, ν(CO) cm^{-1}): 1888, 1901, 2021. ESI-MS (positive ion mode, MeCN): *m/z* 545.17980, calcd 545.18085 for $[M–Cl]^+$; 586.20631, calcd 586.20739 for $[M–Cl+H_2O]^+$; 603.13772, calcd 603.23394 for $[M–Cl+MeCN]^+$.Anal. Calcd for **Re-C12** ($C_{21}H_{30}ClN_2O_3Re$): C, 43.48; H, 5.21; N, 4.83. Found: C, 43.63; H, 5.10; N, 4.80%.

Determination of Log *P* Values. Water was stirred with 1-octanol for 24 h and then centrifuged (3000 rpm, 5 min) to separate the two phases, yielding water saturated with octanol and also octanol saturated with water. The rhenium complexes were dissolved in the octanol solution (4 mL). After analysis via UV–vis spectroscopy, the mixtures (3 mL) were then stirred with octanol-saturated water (147 mL) for 30 min. The layers were then separated via centrifugation (3000 rpm, 5 min) followed by transferring with a fine-gauge needle, and the octanol layer was analyzed again via UV–vis spectroscopy. The partition coefficient was then determined using the following equation:

$$log\ P = log[(\frac{V_{water}}{V_{octanol}})(\frac{A_f}{A_i - A_f})]$$

General Cell Culture. Cells were cultured as monolayers in a humidified incubator at 37 °C with 5% CO_2. The human cervical cancer, HeLa, cells were obtained from the American Type Culture Collection (Manassas, VA, USA). HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS. Cells were routinely passed at 80–90% confluency using 0.05% trypsin, 0.53 mM EDTA, 1× solution. Cell lines were tested monthly for contamination using the InvivoGen mycoplasma detection kit, PlasmoTest™.

Cytotoxicity. Dose-escalation studies were performed for all the rhenium complexes. Stock solutions were prepared as 20 mM solutions in DMSO. Solutions for dose-dependent studies were prepared by first serially diluting the stock solution 1:4 in DMSO, and then each was further diluted 1:100 into growth media. Cells were seeded in a 96-well plate at a density of 8000 cells/well and allowed to adhere for 24 h. Growth media was removed, and the cells were dosed with 200 µL of compounds in a dose-dependent manner for 48 h, then analyzed using an MTT assay described below.

MTT assays were performed following compound treatment by first replacing growth media with 200 µL MTT (1 mg/mL). Cells were incubated with the dye for 4 h, and growth media was removed to yield purple formazan crystals. The crystals were dissolved in 200 µL of DMSO containing 12% glycine buffer (0.1 M glycine, 0.1 M NaCl, pH 10.5). The absorbance was measured at 570 nm for each well using a Biotek Synergy HT plate reader. Each plate contained six replicates per concentration, and at least three independent experiments were conducted with the same procedures. Data points were fit to the equation:

$$y = E_{inf} + \frac{E_0 - E_{inf}}{1 + (\frac{D}{IC_{50}})^{HS}}$$

where y is cell viability, E_{inf} is the viability at infinite drug concentration, E_0 is the viability at zero drug concentration, D is the drug concentration, IC_{50} is the 50% growth inhibitory concentration, and HS is the Hill slope.[12] E_0 was constrained to lie between 0.9 and 1. The compounds' IC_{50} values were determined by fitting the data and solving for these variables using the curve-fitting program, MagicPlot Pro.

Cellular Uptake. HeLa cells were plated in 100 mm × 20 mm tissue culture dishes and allowed to grow to 80% confluency. Growth media was then replaced with 6 mL of 50 µM compounds in media or with 0.3% v/v DMSO in media as a negative control. Samples were incubated at either 37 °C or 4 °C for 3 h. No visible signs of precipitation were observed during this time. After incubation, the cells were washed with 3 mL of Dulbecco's phosphate-buffered saline solution (DPBS) and harvested using trypsin. Cell lysates were then obtained using modified versions of previously described methods shown below.[13] The bicinchoninic acid (BCA) assay was used to determine protein content for lysate samples.[14] The procedure for measuring protein content was previously described by the manufacturer. In a 96-well plate, 25 µL of cell lysate was combined with 200 µL of working reagent, incubated for 30 min, and then the absorbance was measured at 562 nm. Protein concentrations were then extrapolated from a calibration curve using bovine serum albumin (BSA) standard dilutions. Samples were measured in triplicates.

Whole cell lysates were obtained by centrifuging harvested cells, washing with 1 mL of DPBS, lysing with 1 mL of 4% sodium dodecyl sulfate (SDS) lysis buffer (4% w/v SDS, 150 mM NaCl, 50 mM triethanolamine), and vortexing on the highest setting for 10 s. The supernatant was transferred to a clean tube. 750 µL of the lysate was treated with 11 µL 70% HNO_3 followed by sonication for 10 min. After digestion, rhenium content in each sample was determined using ICP-OES. Results are reported as the mass ratio of metal to protein (ng/µg) in each sample, in which protein content was determined using the BCA assay described above.

Table S1 Percent rhenium taken up via passive transport (4 °C) normalized by the total uptake (37 °C). The error represents the uncertainties from the standard deviation from three replicates.

	Fold Decrease from 37 to 4 °C	Percent Re Content (4 °C/37 °C)
Re-C2	4 ± 2	20 ± 10 %
Re-C3	7 ± 3	14 ± 7 %
Re-C4	5 ± 2	19 ± 6 %
Re-C5	3 ± 2	30 ± 10 %
Re-C6	3 ± 1	40 ± 20 %
Re-C12	2.3 ± 0.3	43 ± 5 %

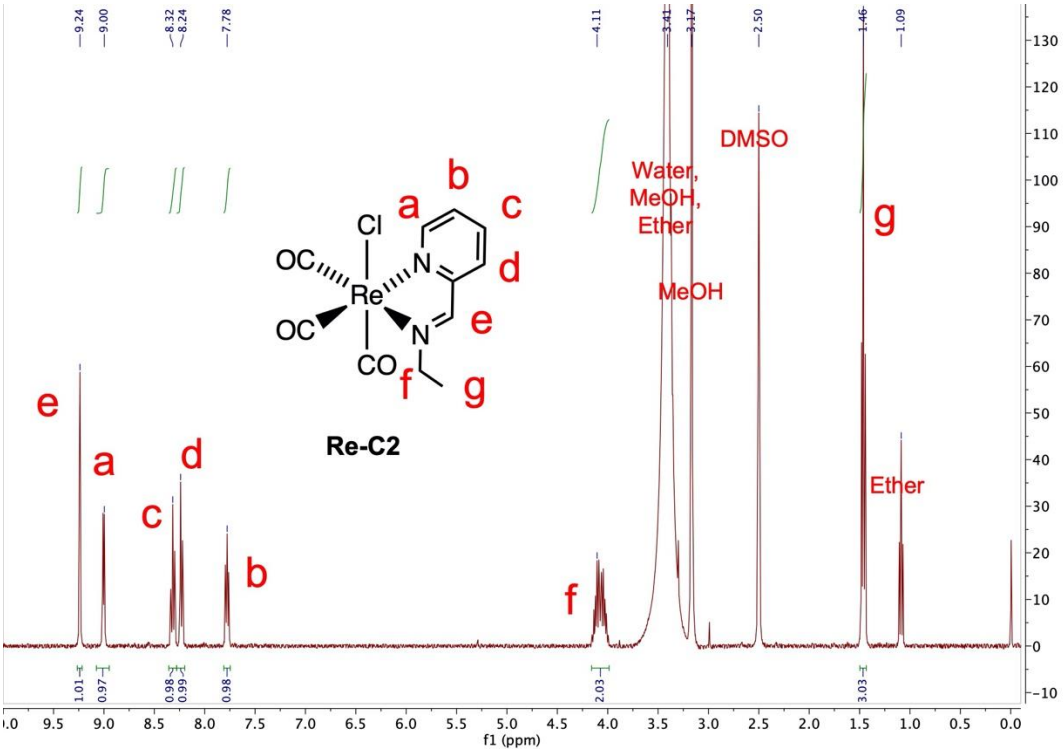

Fig. S1 ^{1}H NMR spectrum of **Re-C2** in DMSO-d_6 (400 MHz, 298 K).

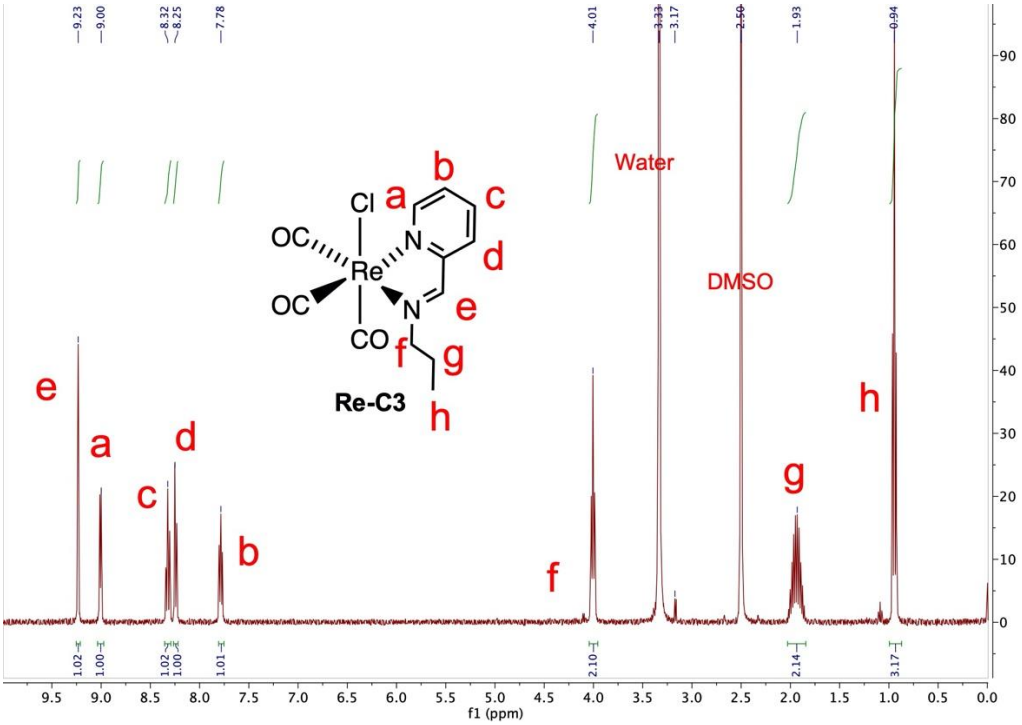

Fig. S2 ^{1}H NMR spectrum of **Re-C3** in DMSO-d_6 (400 MHz, 298 K).

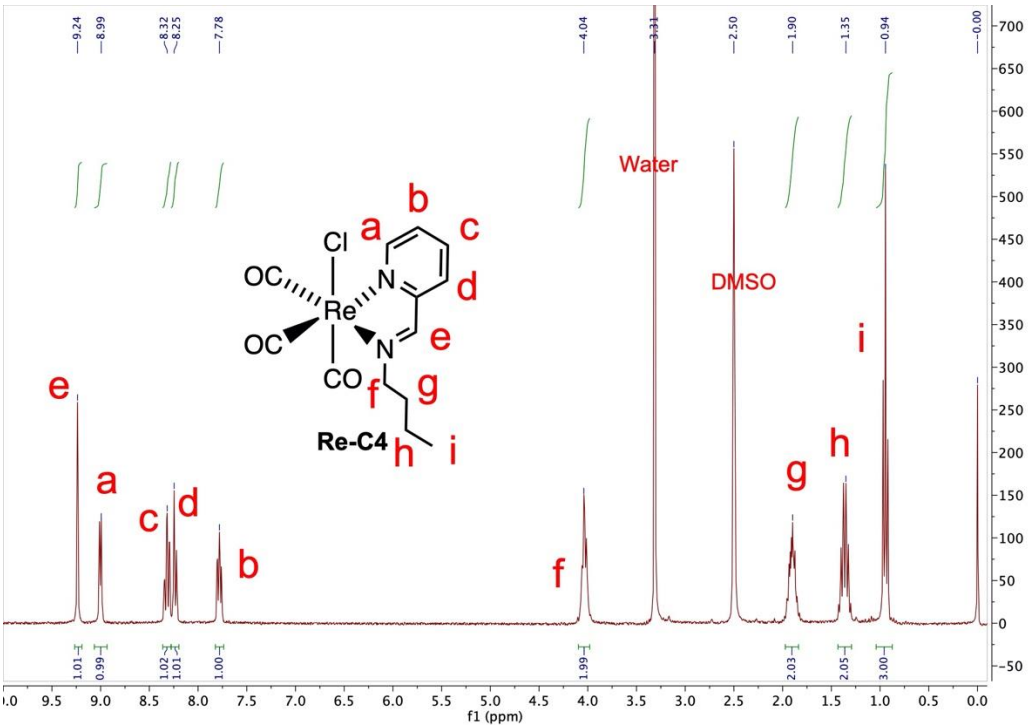

Fig. S3 ^{1}H NMR spectrum of **Re-C4** in DMSO-d_6 (300 MHz, 298 K).

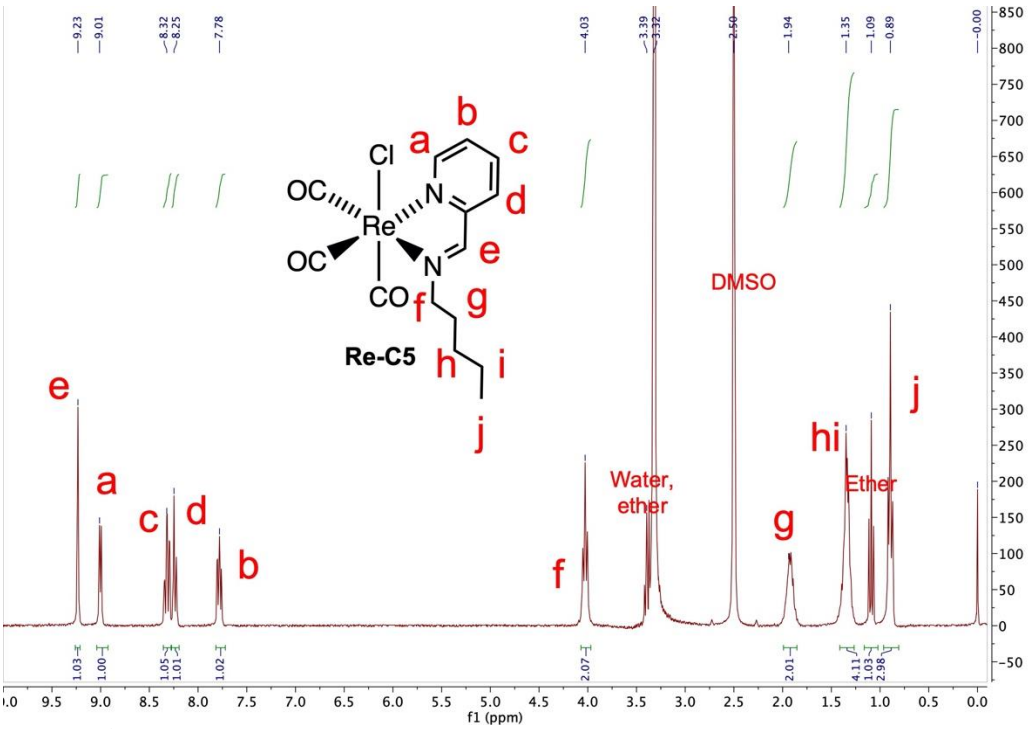

Fig. S4 ^{1}H NMR spectrum of **Re-C5** in DMSO-d_6 (300 MHz, 298 K).

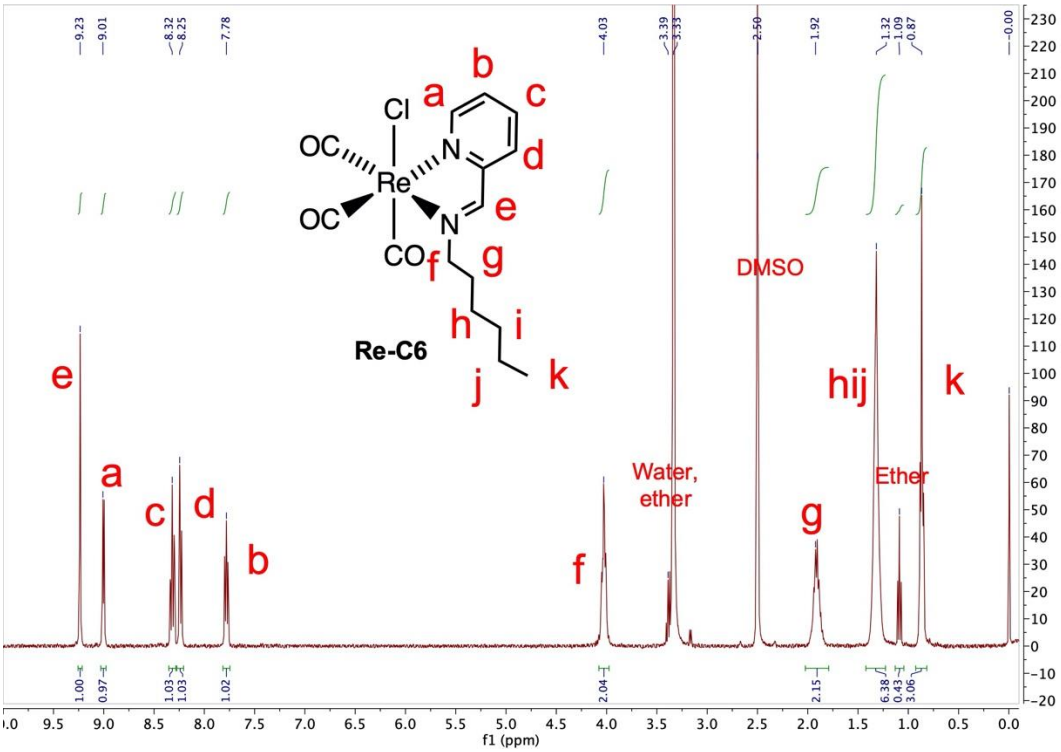

Fig. S5 ^{1}H NMR spectrum of **Re-C6** in DMSO-d_6 (400 MHz, 298 K).

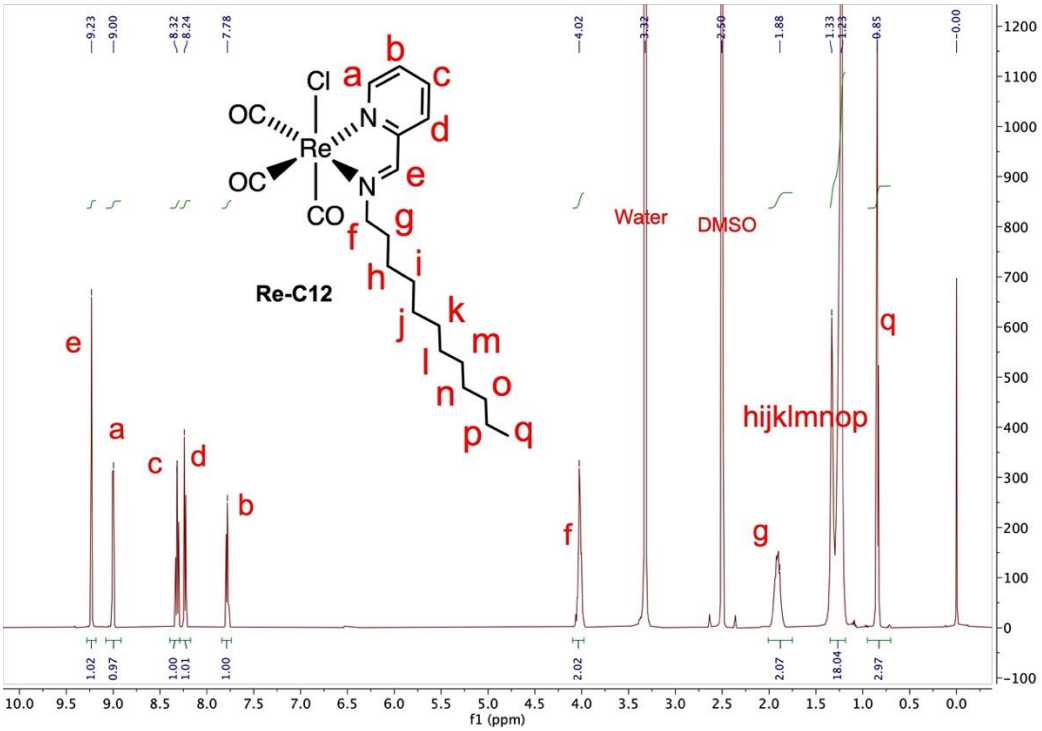

Fig. S6 ^{1}H NMR spectrum of **Re-C12** in DMSO-d_6 (500 MHz, 298 K).

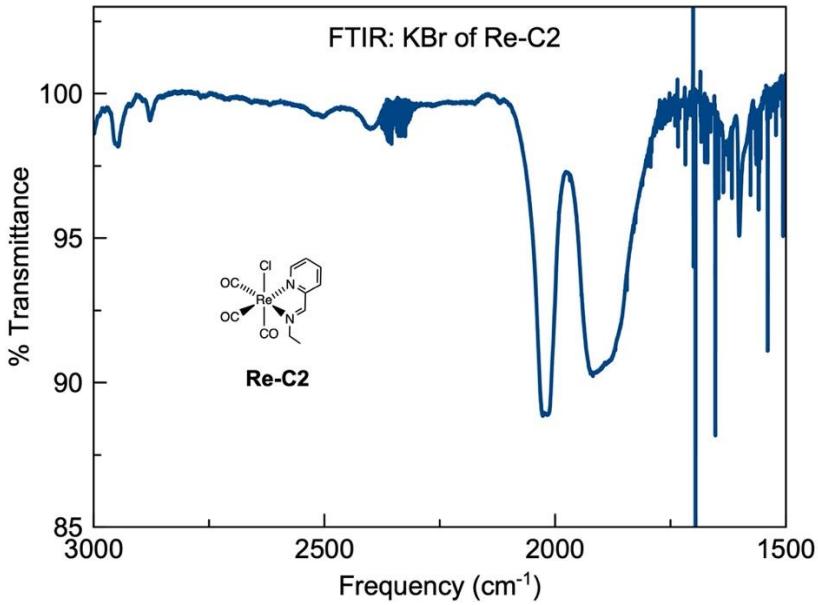

Fig. S7 FTIR spectrum of **Re-C2** (KBr pellet).

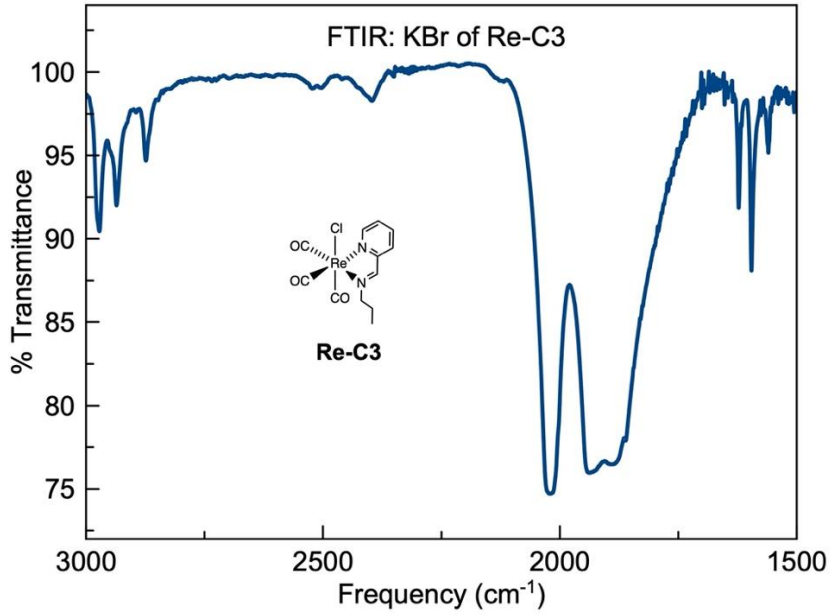

Fig. S8 FTIR spectrum of **Re-C3** (KBr pellet).

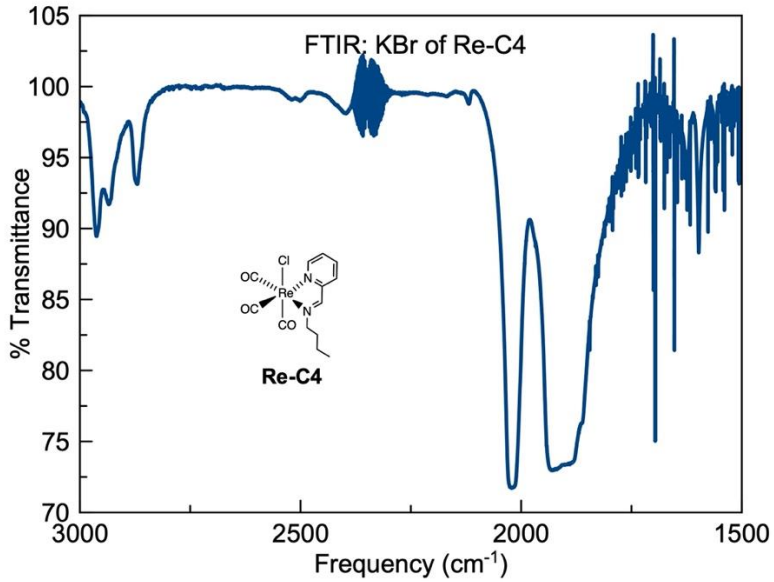

Fig. S9 FTIR spectrum of **Re-C4** (KBr pellet).

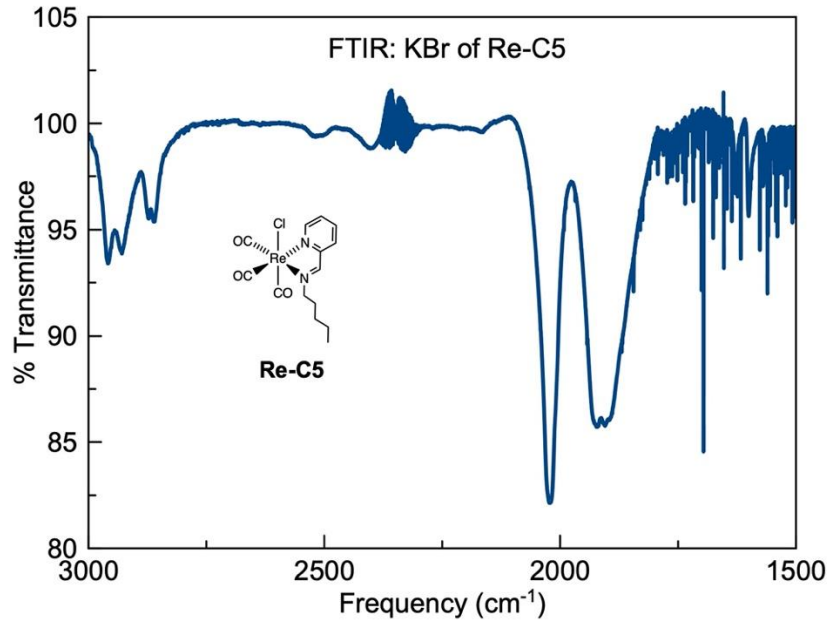

Fig. S10 FTIR spectrum of **Re-C5** (KBr pellet).

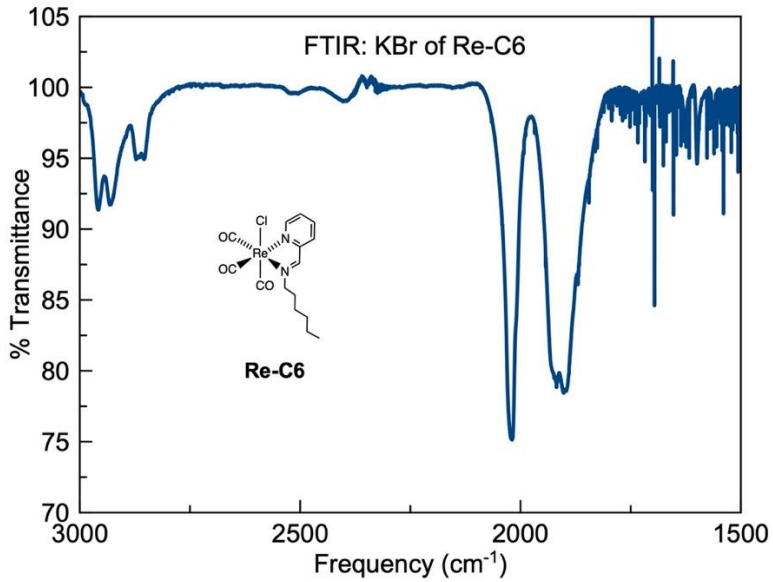

Fig. S11 FTIR spectrum of **Re-C6** (KBr pellet).

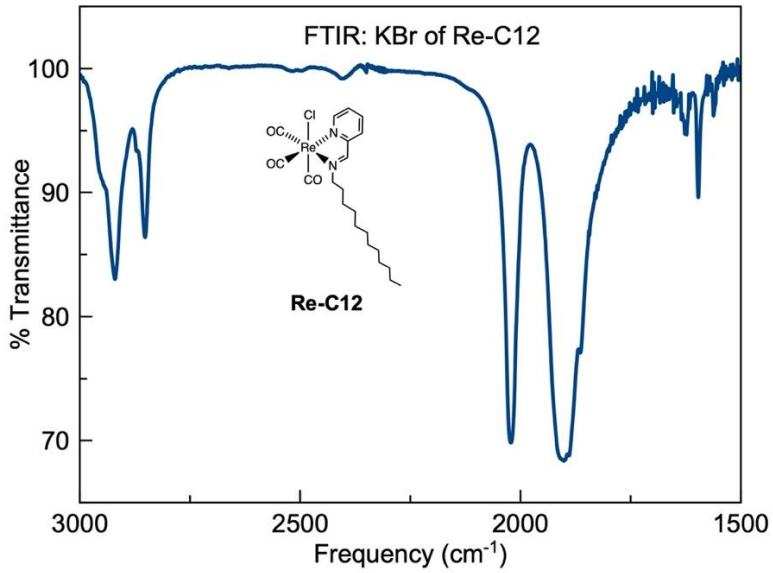

Fig. S12 FTIR spectrum of **Re-C12** (KBr pellet).

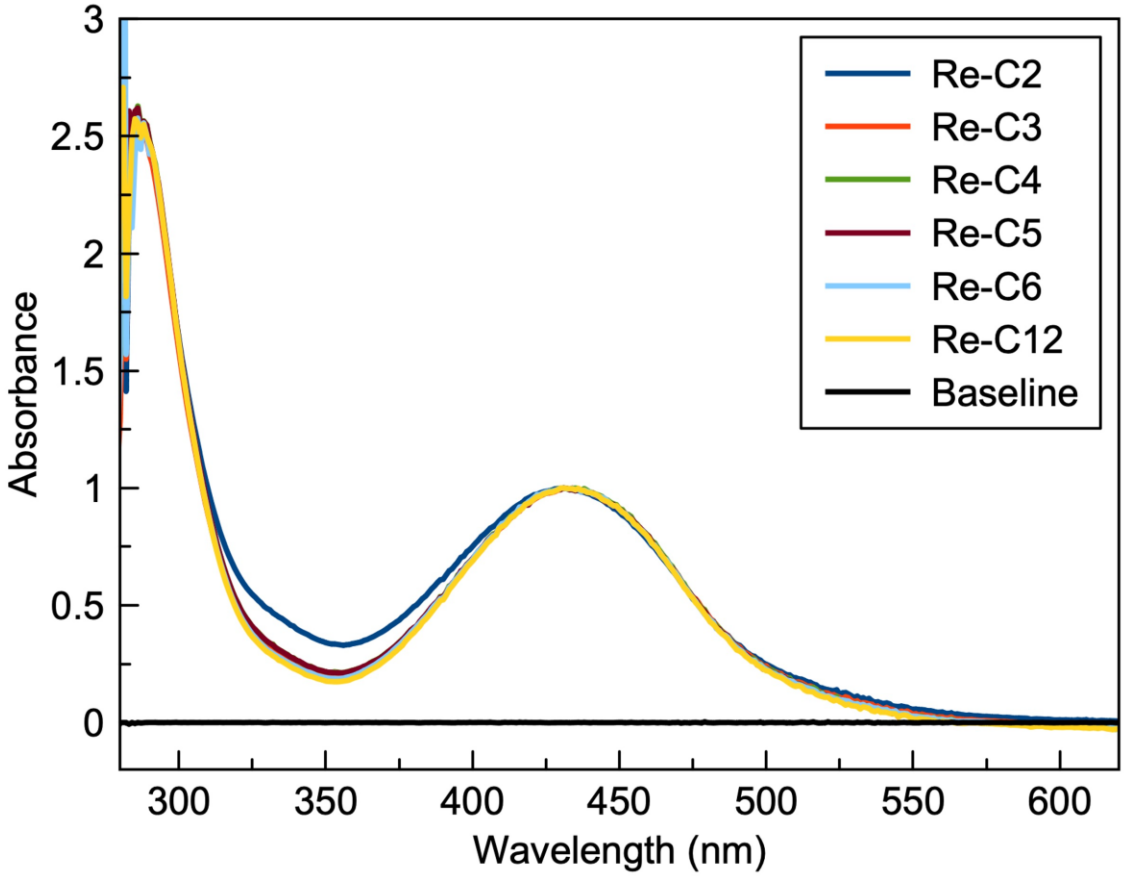

Fig. S13. UV–vis spectra of 20 μM **Re-C2** (navy blue), **Re-C3** (red), **Re-C4** (green), **Re-C5** (maroon), **Re-C6** (light blue), and **Re-C12** (yellow) in toluene.

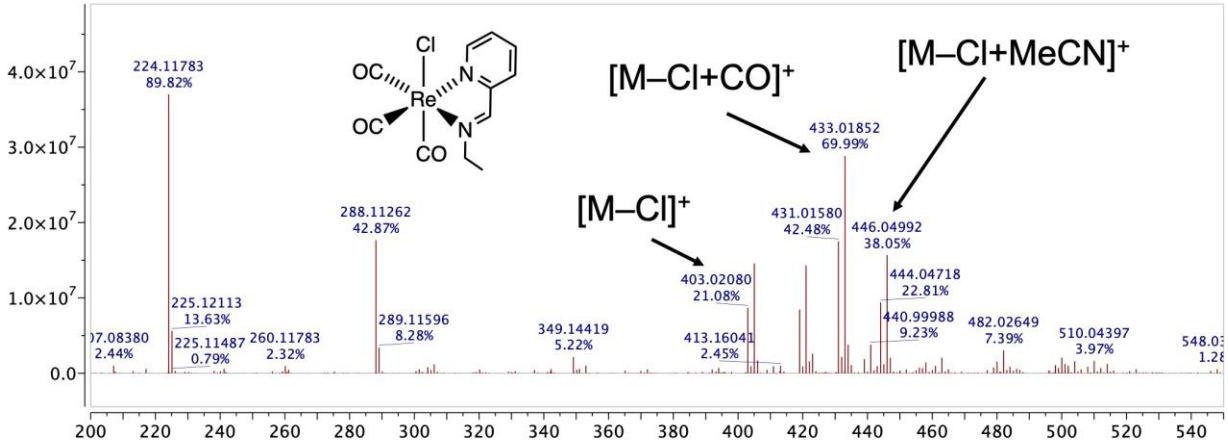

Fig. S14. ESI-MS of **Re-C2** (positive ion mode, MeCN).

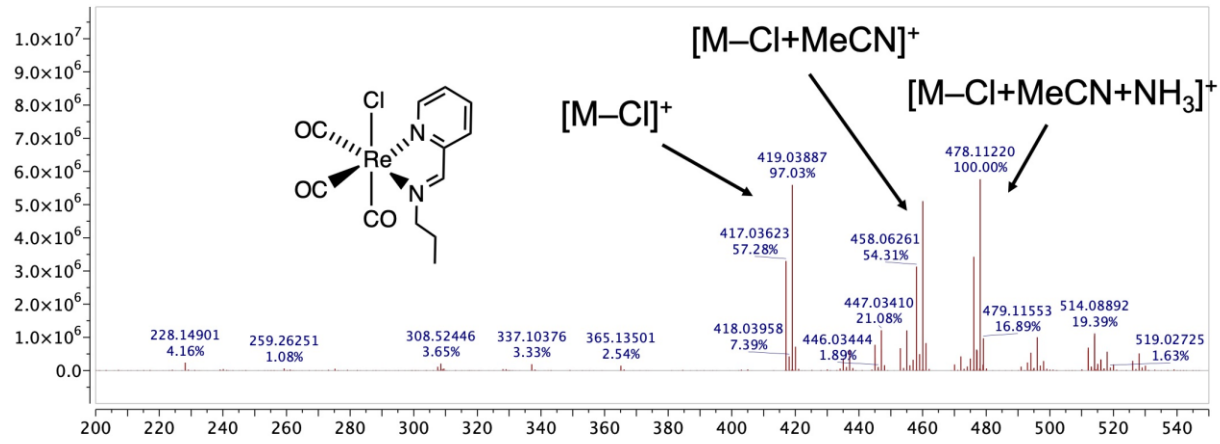

Fig. S15. ESI-MS of **Re-C3** (positive ion mode, MeCN).

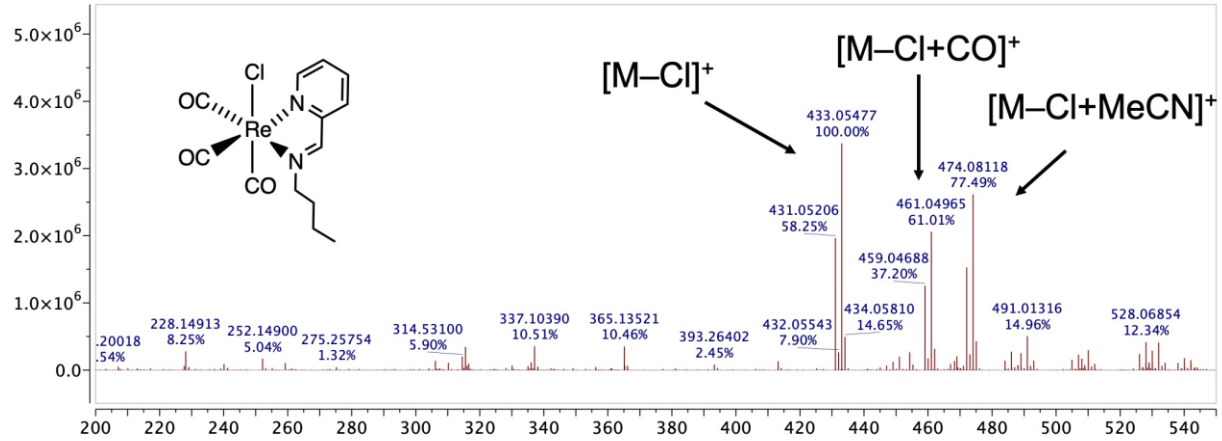

Fig. S16. ESI-MS of **Re-C4** (positive ion mode, MeCN).

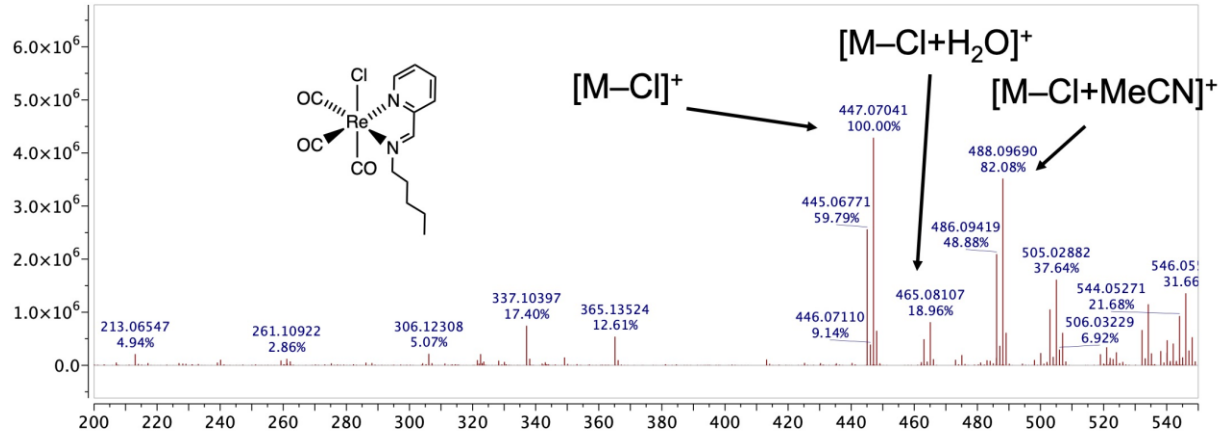

Fig. S17. ESI-MS of **Re-C5** (positive ion mode, MeCN).

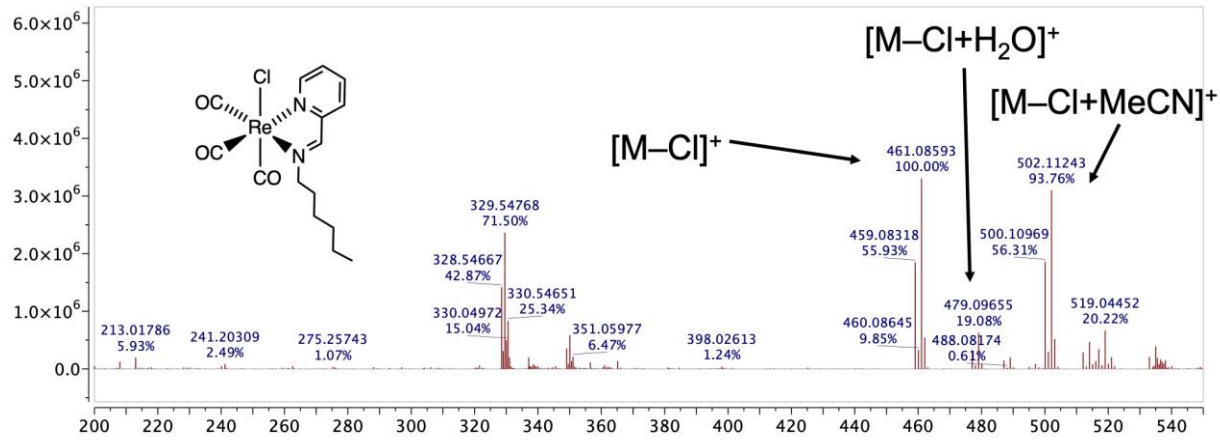

Fig. S18. ESI-MS of **Re-C6** (positive ion mode, MeCN).

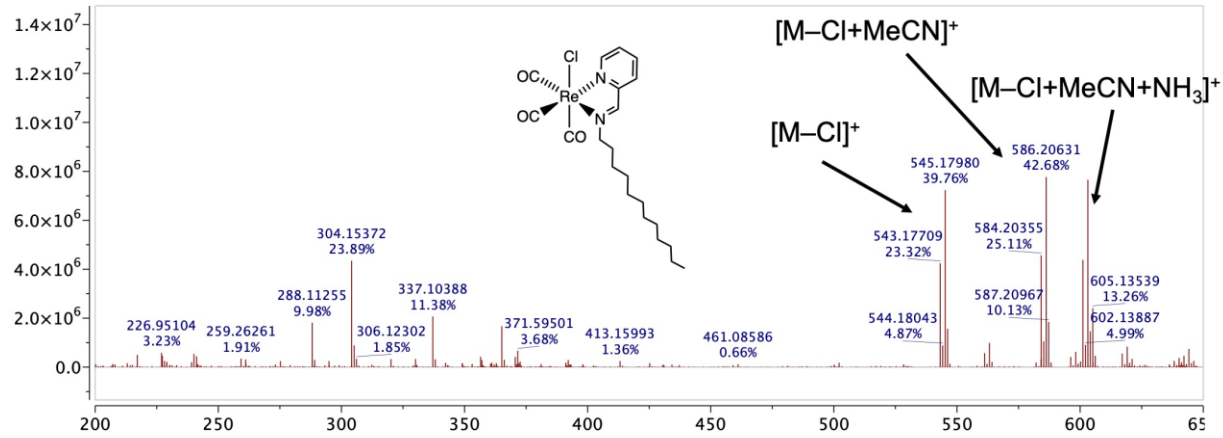

Fig. S19. ESI-MS of **Re-C12** (positive ion mode, MeCN).

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