Supporting Information

Tuning In Vitro Cytotoxicity in Diruthenium(I) Bis-Cyclopentadienyl Complexes

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IR and NMR spectra of diruthenium complexes

Figure S1. IR spectrum (650-4000 cm^{-1}) of $[Ru_2Cp_2(CO)(NH_3)(\mu\text{-}CO)\{\mu\text{-}CNMe(Cy)\}]CF_3SO_3$, [**2**]$CF_3SO_3$ (*cis-E*/*Z* + *trans-E*/*Z* isomers).

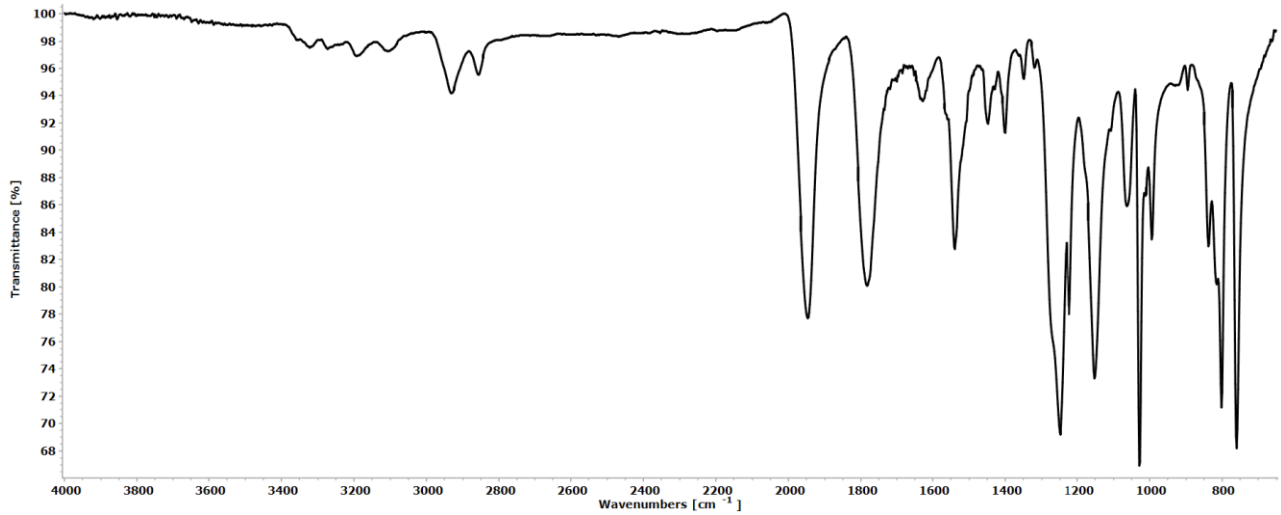


Figure S2. ^1H NMR spectrum (401 MHz, $CDCl_3$) of [**2**]CF_3SO_3 (*cis-E*/*Z* + *trans-E*/*Z* isomers).

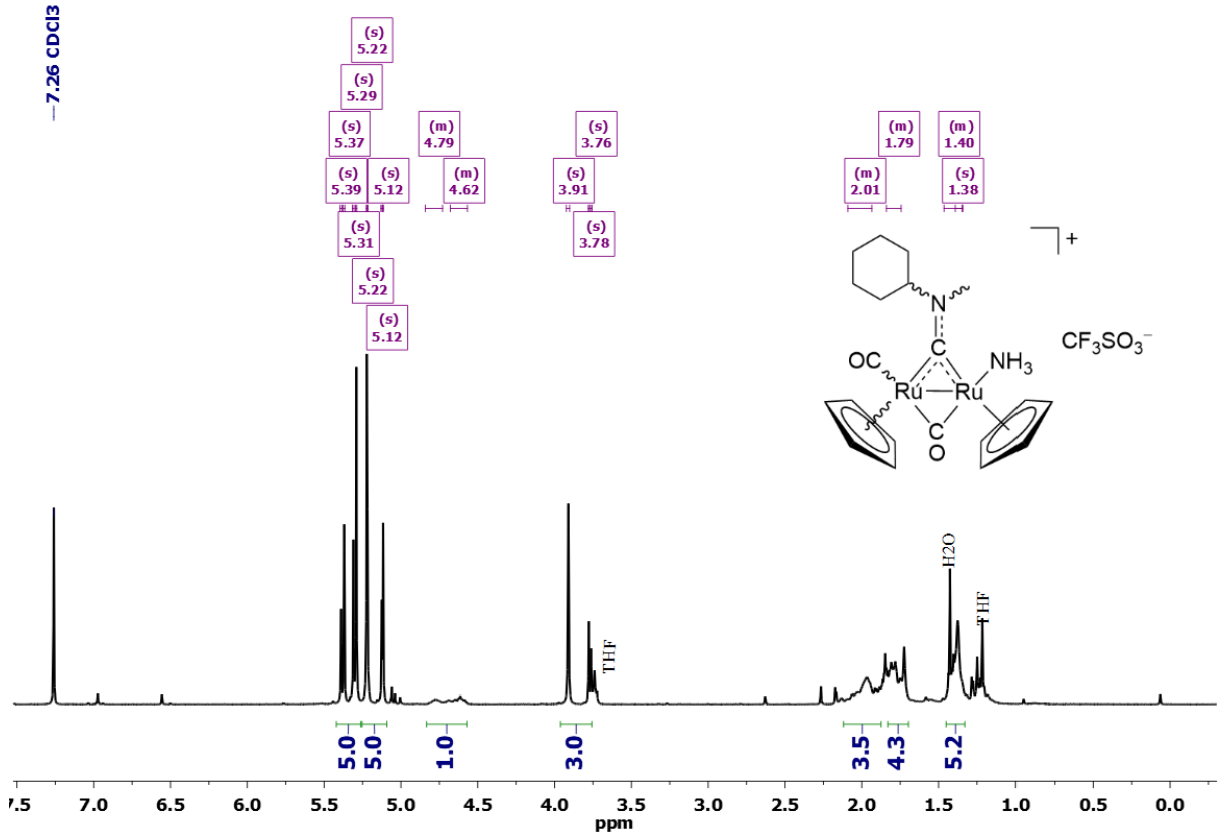


Figure S3. $^{13}C\{^{1}H\}$ NMR spectrum (101 MHz, $CDCl_3$) of [**2**]CF_3SO_3 (*cis-E*/*Z* + *trans-E*/*Z* isomers).

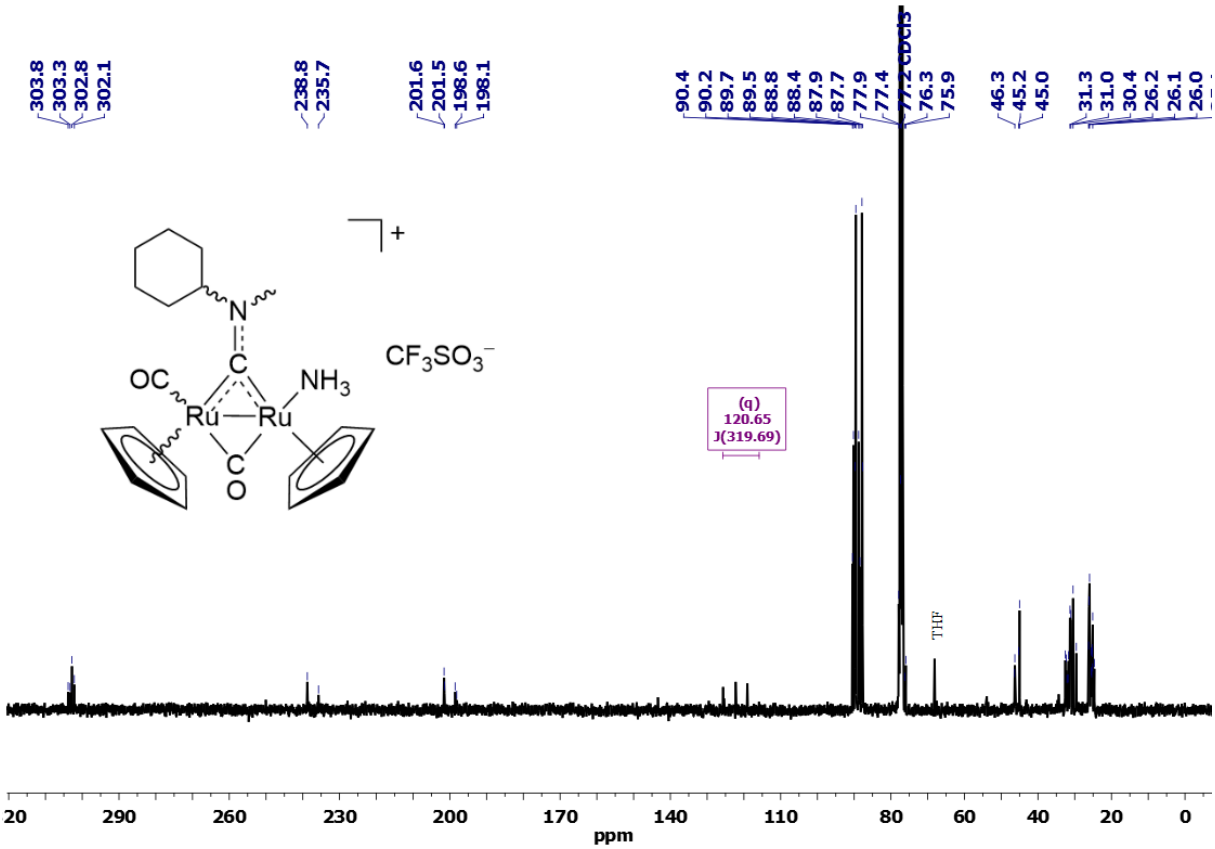


Figure S4. Black line: ^{1}H NMR spectrum (401 MHz, $CDCl_3$) of [**2**]CF_3SO_3. Blue line: ^{1}H NOESY with irradiation at 5.29 ppm (Cp of the *cis-E* isomer). Red line: ^{1}H NOESY with irradiation at 5.22 ppm (Cp^N of the *cis-E* isomer). Green boxes: NOE effects due co-irradiation of Cp and Cp^N resonances of the *cis-Z* isomer. Observed NOEs are indicated by the arrows.

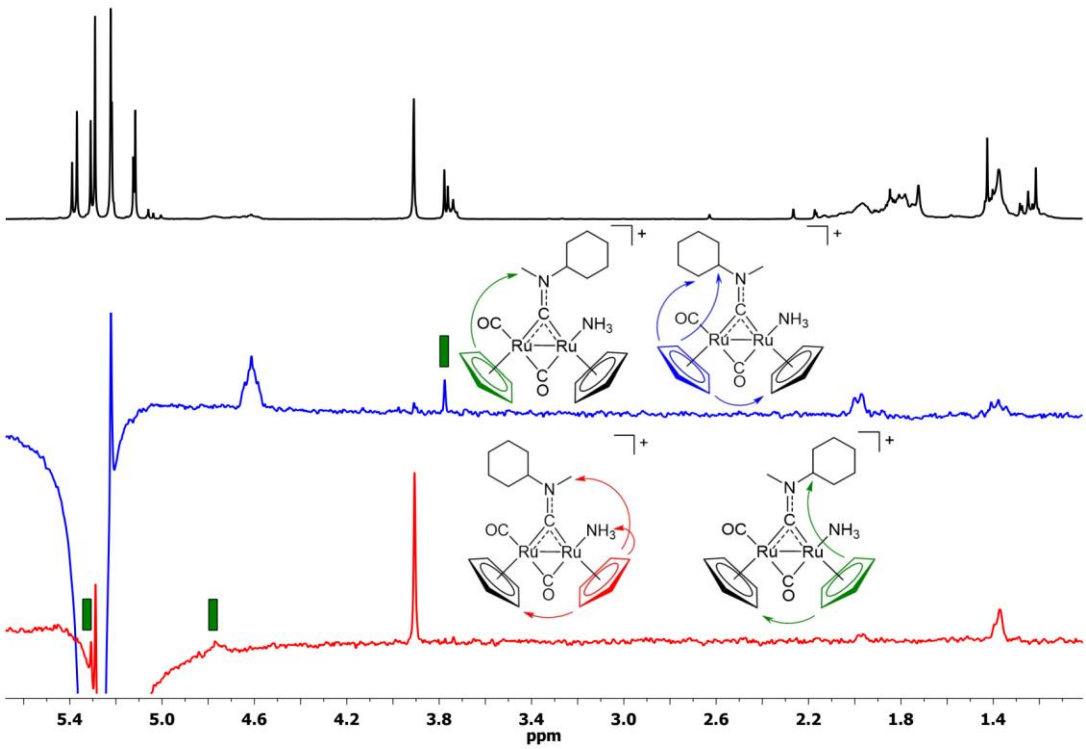


Figure S5. Black line: ^{1}H NMR spectrum (401 MHz, $CDCl_3$) of [**2**]CF_3SO_3. Blue line: ^{1}H NOESY with irradiation at 5.39 ppm (Cp of the *trans-Z* isomer). Red line: ^{1}H NOESY with irradiation at 5.12 ppm (Cp^N of the *trans-E* and *trans-Z* isomers). Green boxes: NOE effects due the *trans-E* isomer. Observed NOEs are indicated by the arrows.

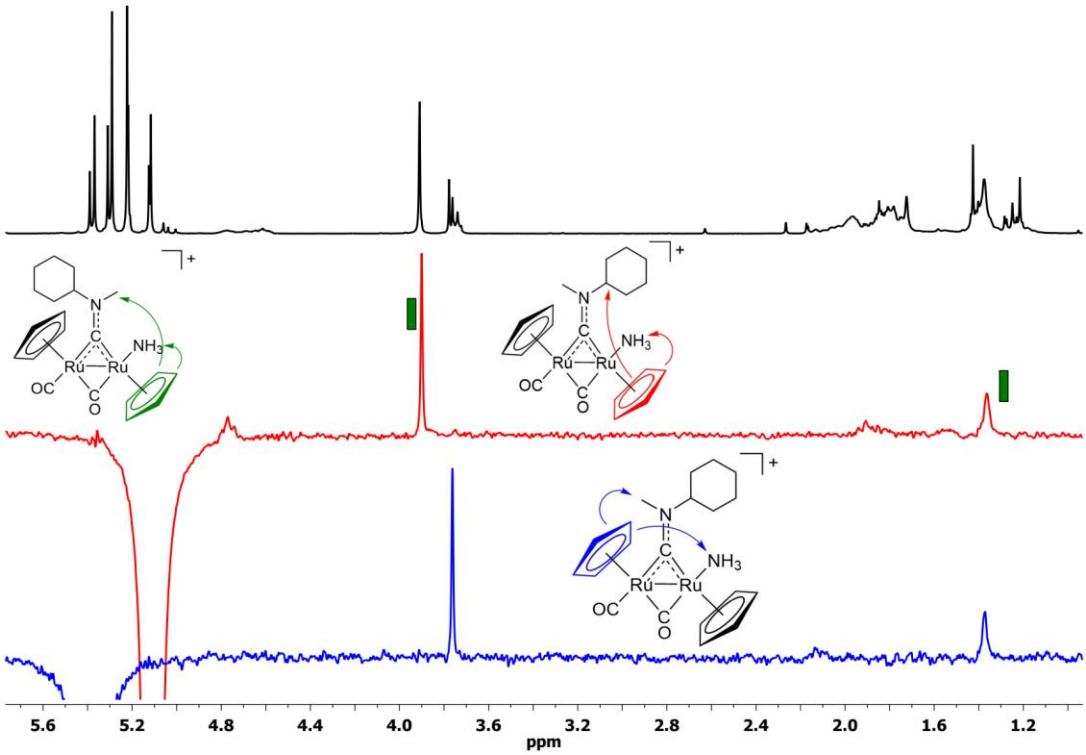


Figure S6. IR spectrum (650-4000 cm^{-1}) of $[Ru_2Cp_2(CO)(Py)(\mu\text{-}CO)\{\mu\text{-}CNMe(Cy)\}]CF_3SO_3$, [**3**]$CF_3SO_3$ (*cis-E*/*Z* + *trans-E*/*Z* isomers).

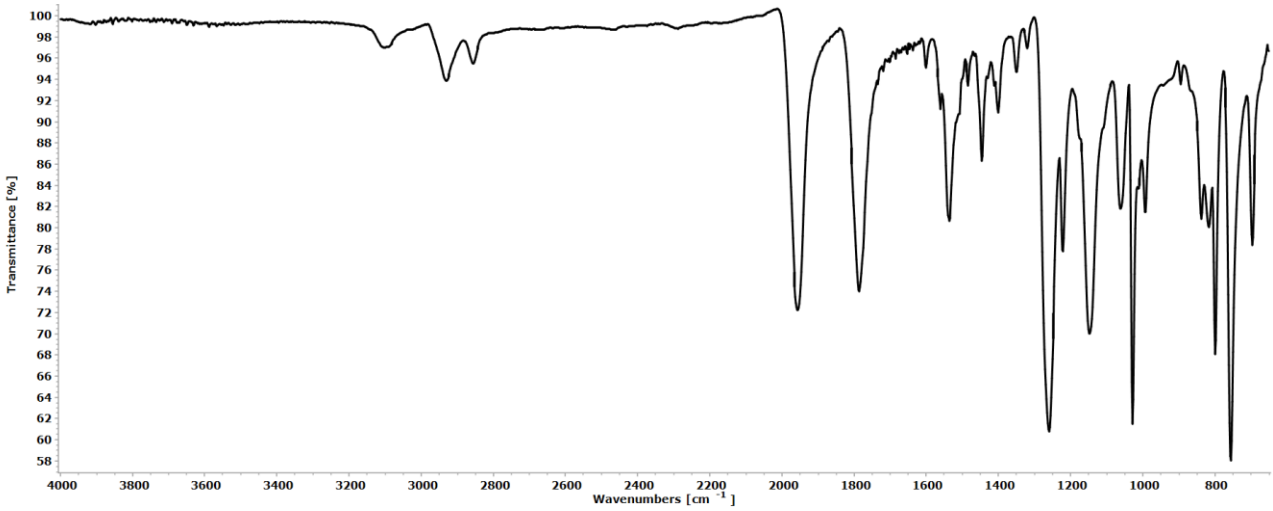


Figure S7. ^{1}H NMR spectrum (401 MHz, $CDCl_3$) of [**3**]CF_3SO_3 (*cis-E*/*Z* + *trans-E*/*Z* isomers). Only signals due to *cis-E*/*Z* isomers are highlighted.

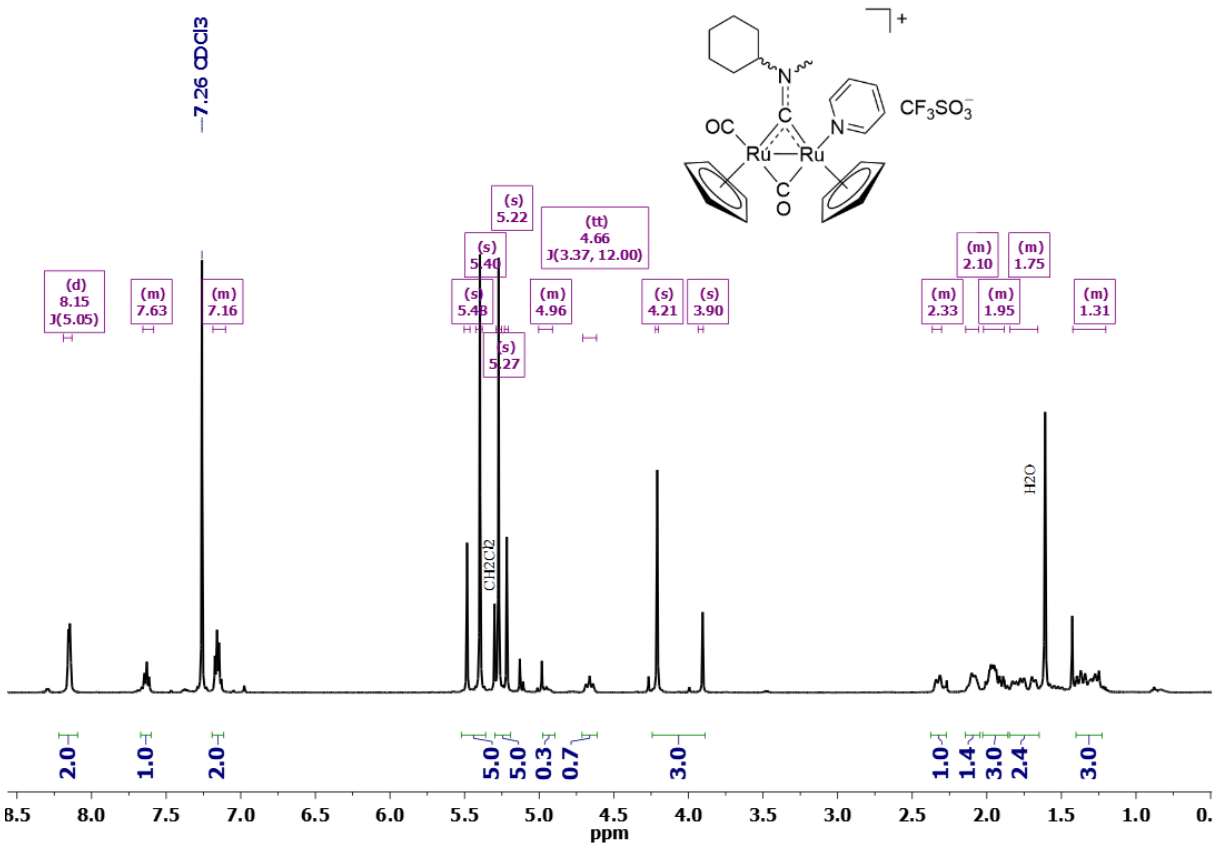


Figure S8. ^{1}H NMR spectrum (401 MHz, $CDCl_3$) of [**3**]CF_3SO_3 (*cis-E*/*Z* + *trans-E*/*Z* isomers). Signals due to *trans-E*/*Z* isomers and total integrals (*cis* + *trans*) are highlighted.

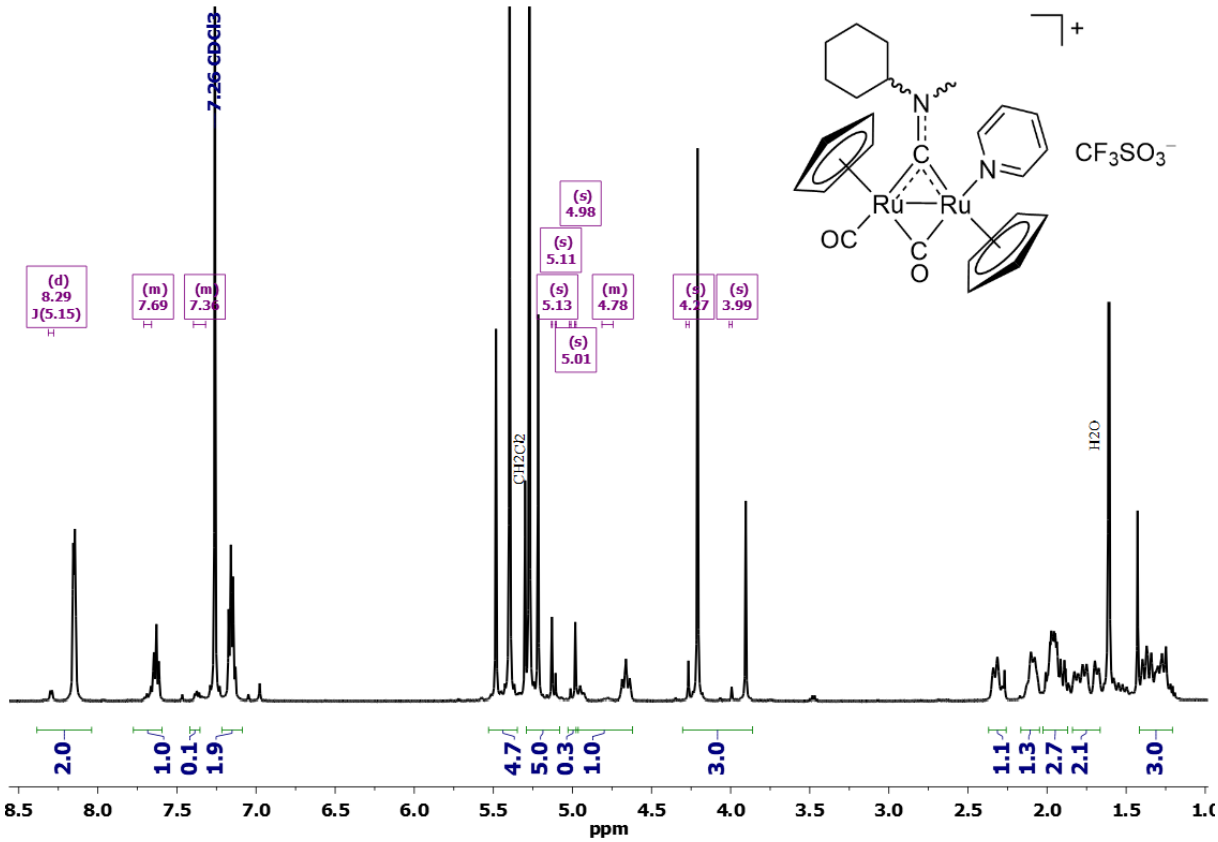


Figure S9. $^{13}C\{^{1}H\}$ NMR spectrum (101 MHz, $CDCl_3$) of [**3**]CF_3SO_3 (*cis-E*/*Z* + *trans-E*/*Z* isomers). Only signals due to *cis-E*/*Z* isomers are highlighted.

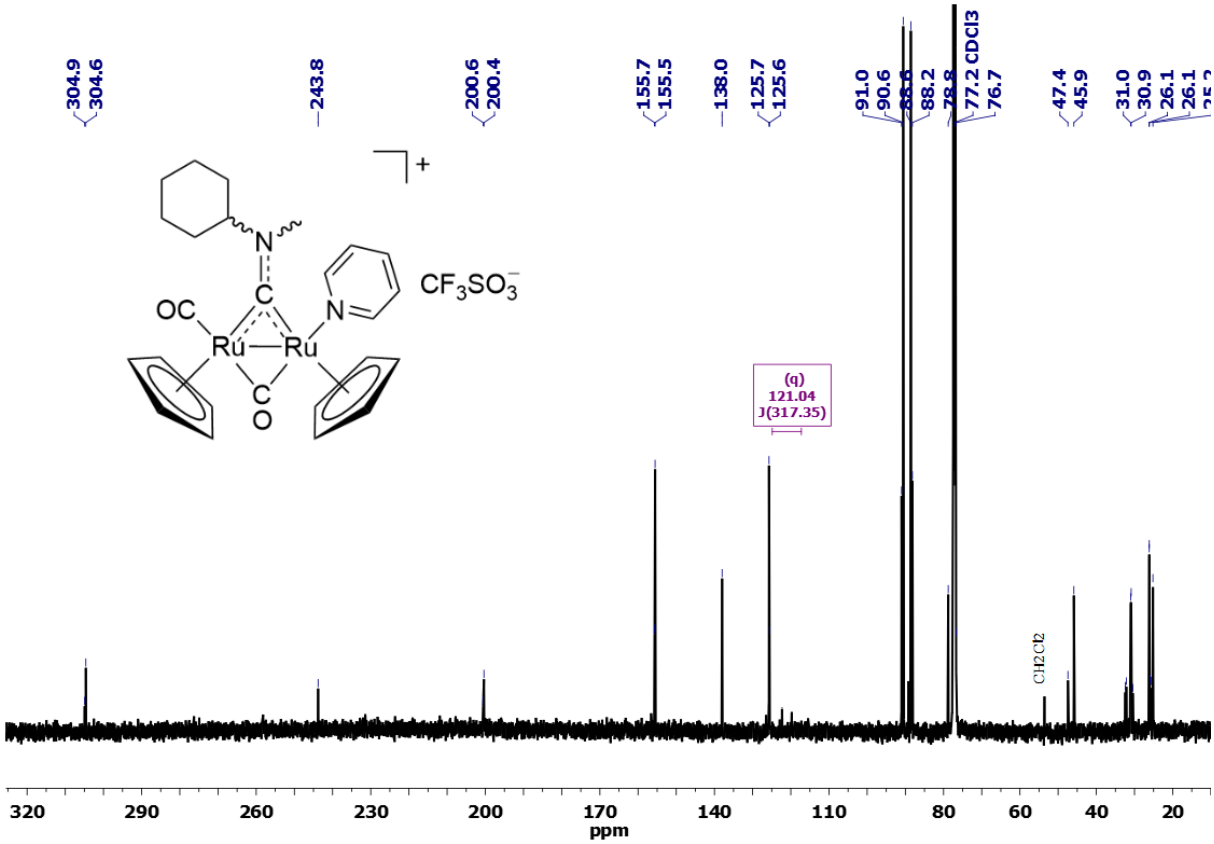


Figure S10. Black line: ^{1}H NMR spectrum (401 MHz, $CDCl_3$) of [**3**]CF_3SO_3. Blue line: ^{1}H NOESY with irradiation at 5.48 ppm (Cp of the *cis-Z* isomer). Observed NOEs are indicated by the arrows.

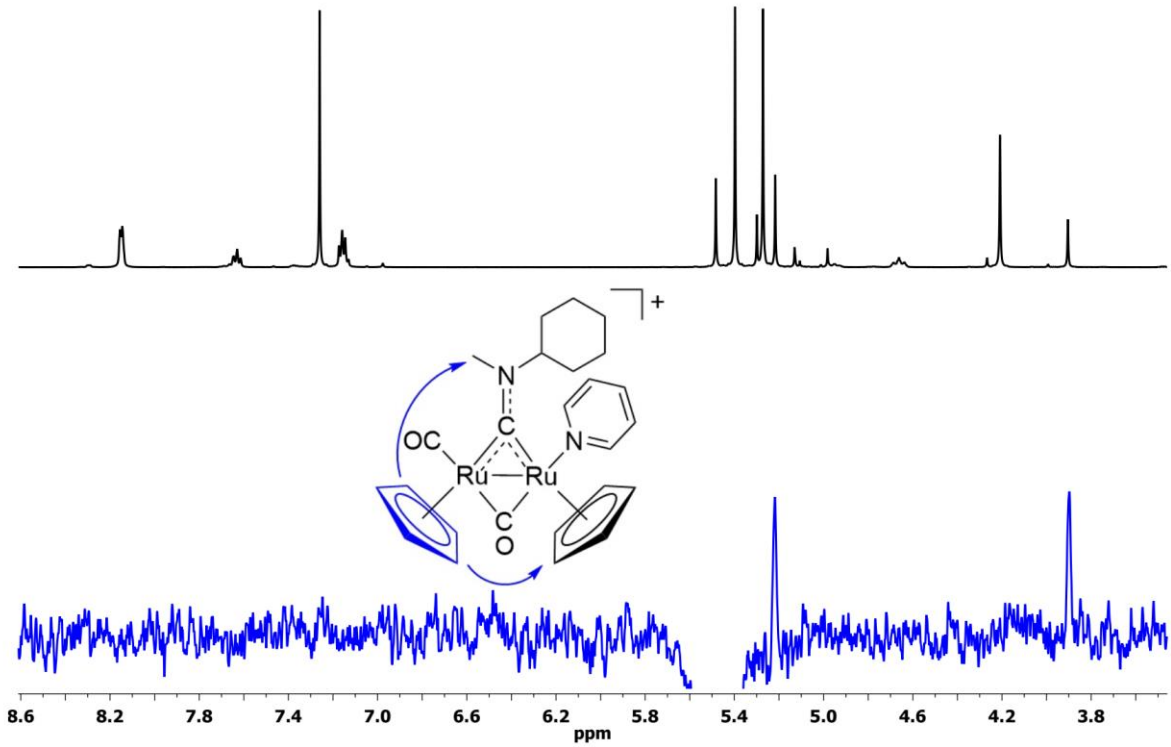


Figure S11. IR spectrum (650-4000 cm^{-1}) of $[Ru_2Cp_2(CO)(PPh_3)(\mu\text{-}CO)\{\mu\text{-}CNMe(Cy)\}]CF_3SO_3$, [**4**]$CF_3SO_3$ (*cis-E* isomer).

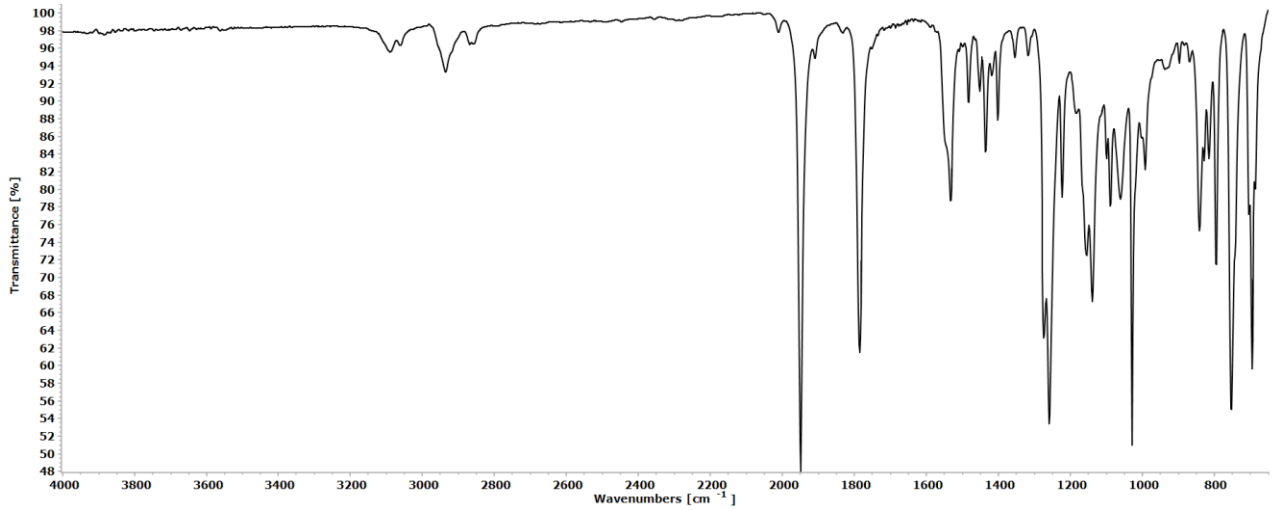


Figure S12. ^1H NMR spectrum (401 MHz, $CDCl_3$) of *cis-E*-[**4**]CF_3SO_3. Minor signals are attributable to the other isomers.

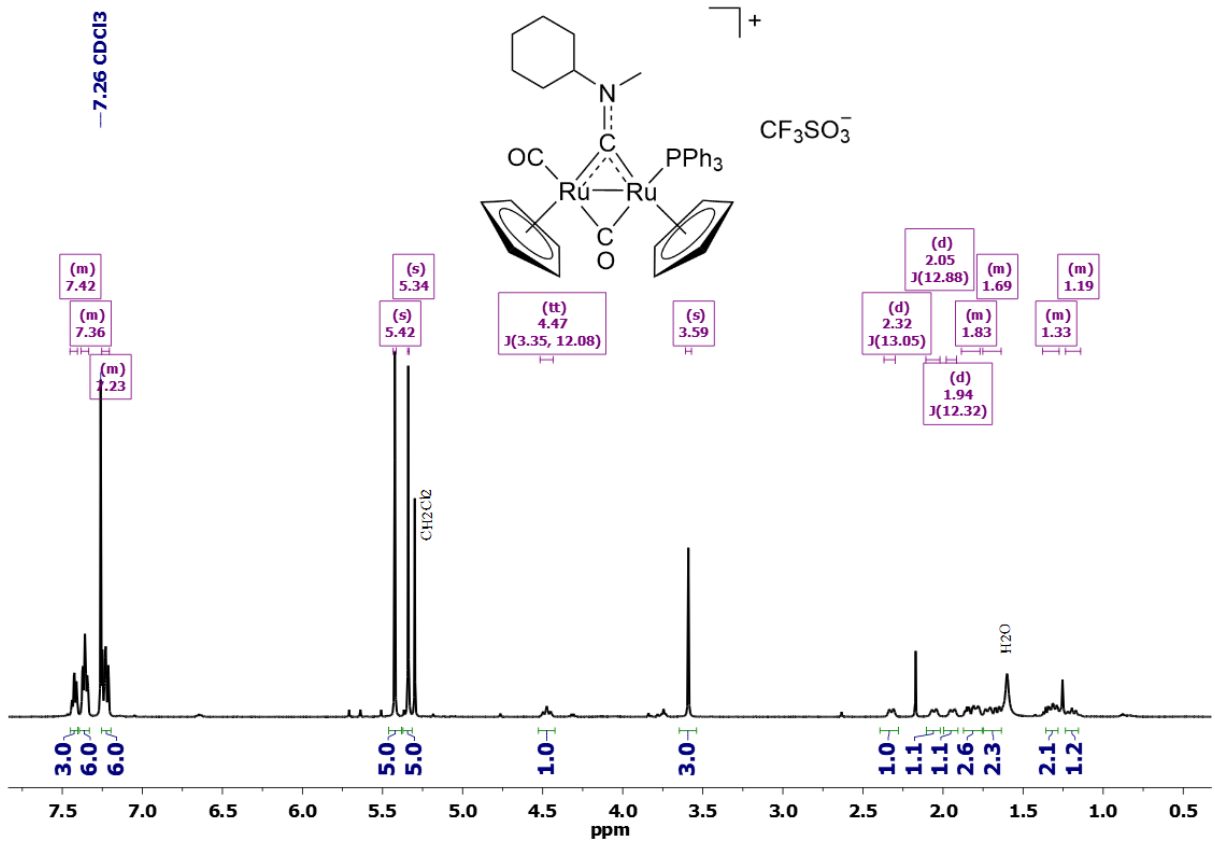


Figure S13. $^{13}C\{^{1}H\}$ NMR spectrum (101 MHz, $CDCl_3$) of *cis-E*-[**4**]CF_3SO_3.

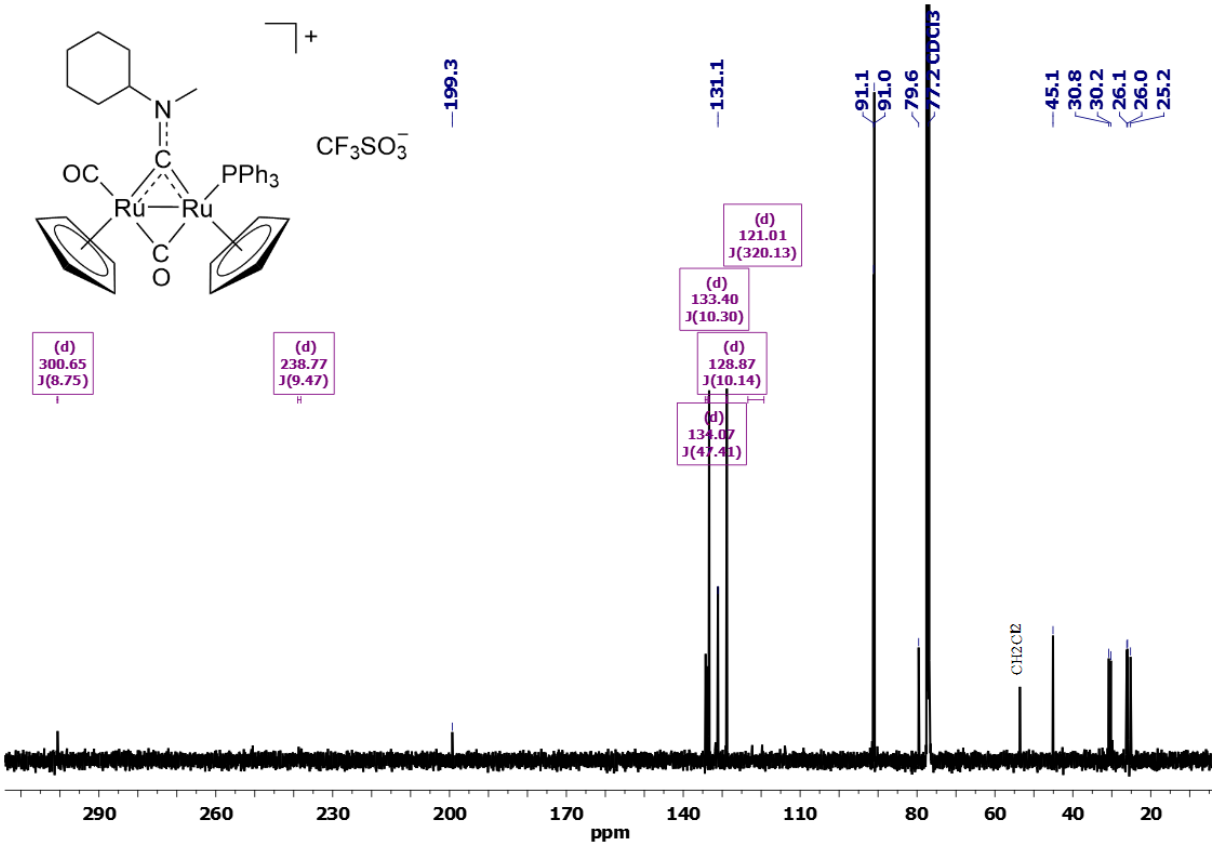


Figure S14. $^{31}P\{^{1}H\}$ NMR spectrum (162 MHz, $CDCl_3$) of *cis-E*-[**4**]CF_3SO_3.

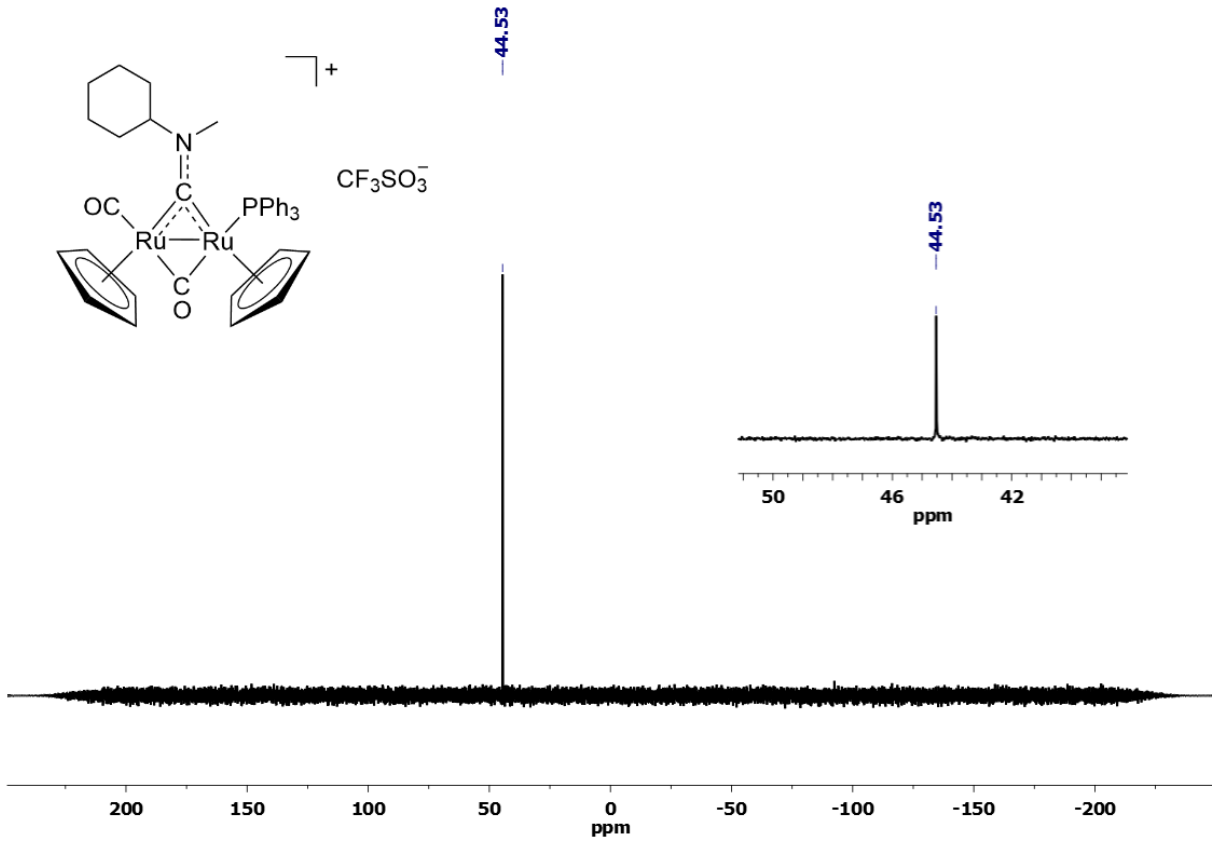


Figure S15. Black line: ^{1}H NMR spectrum (401 MHz, $CDCl_3$) of *cis-E*-[**4**]CF_3SO_3. Blue line: ^{1}H NOESY with irradiation at 5.42 ppm (Cp). Red line: ^{1}H NOESY with irradiation at 5.34 ppm (Cp^P). Observed NOEs are indicated by the arrows.

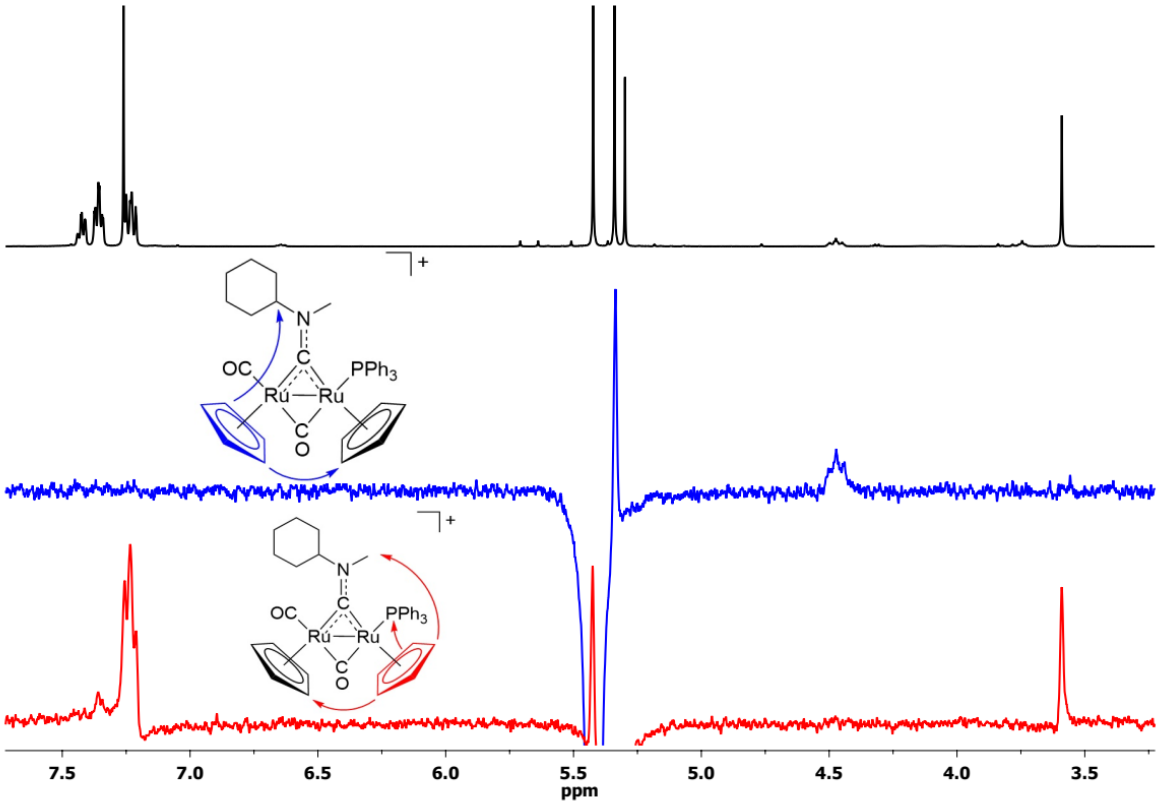


Figure S16. ^{1}H NMR spectrum (401 MHz, $CDCl_3$) of [**4**]CF_3SO_3 (*cis-E*/*Z* + *trans-E*/*Z* isomers).

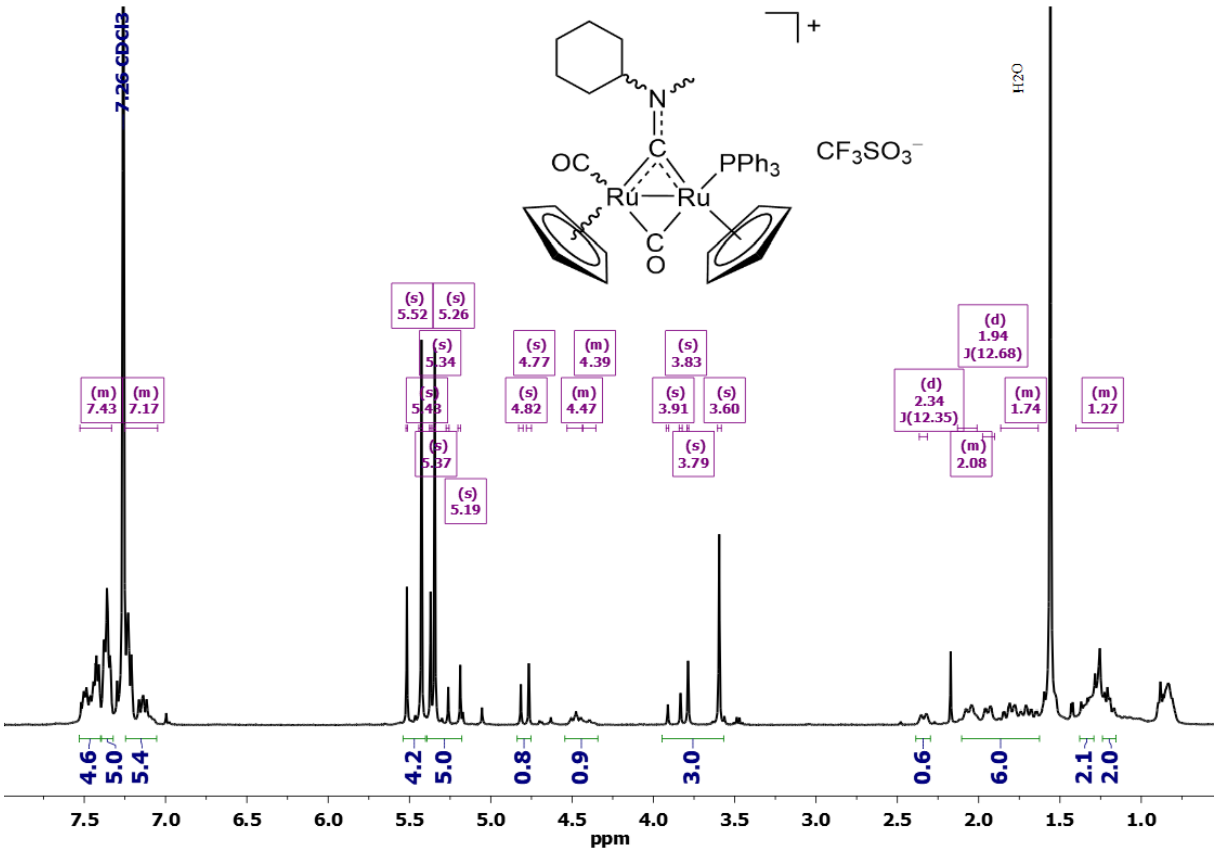


Figure S17. $^{31}P\{^{1}H\}$ NMR spectrum (162 MHz, $CDCl_3$) of [**4**]CF_3SO_3 (*cis-E*/*Z* + *trans-E*/*Z* isomers).

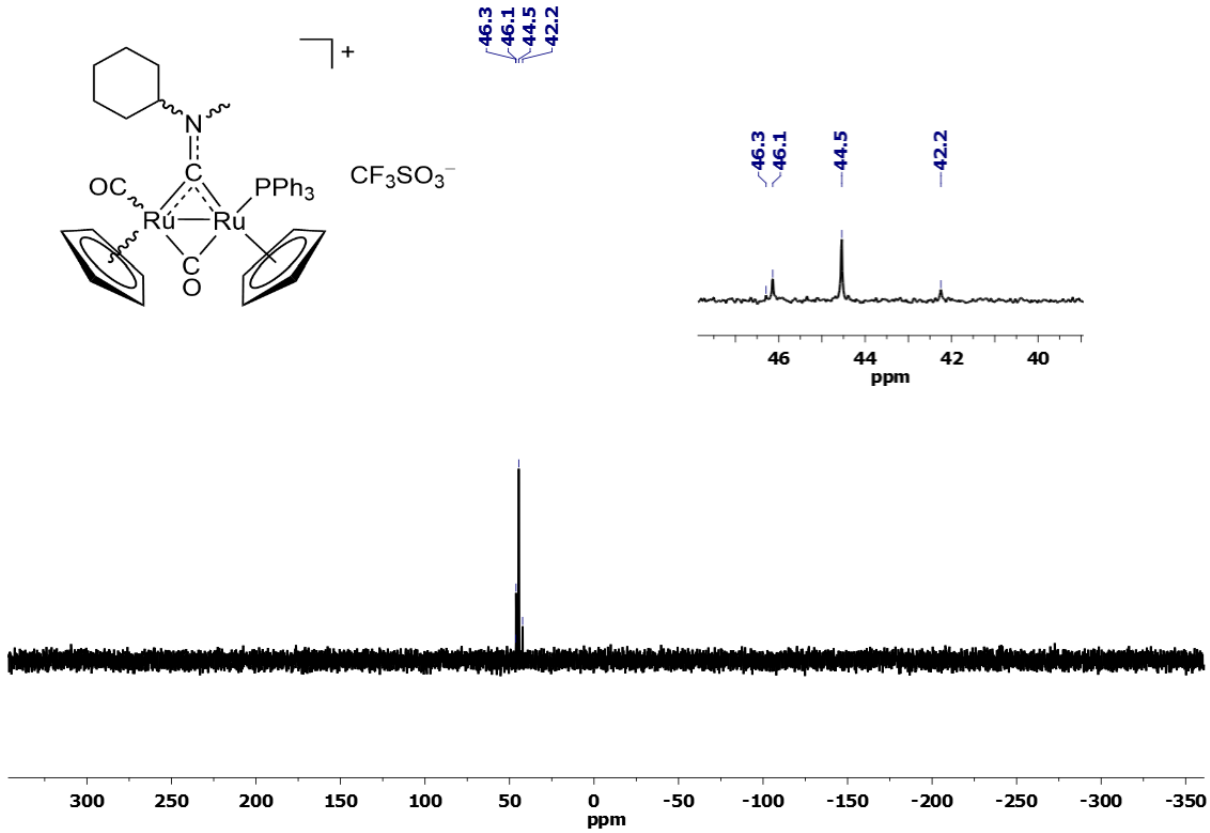


Figure S18. IR spectrum (650-4000 cm^{-1}) of [Ru_2Cp_2(CO){κ*S*-Me_2SO}(μ-CO){μ-CNMe(Cy)}]CF_3SO_3, [**5**]CF_3SO_3 (*cis-E*/*Z* isomers).

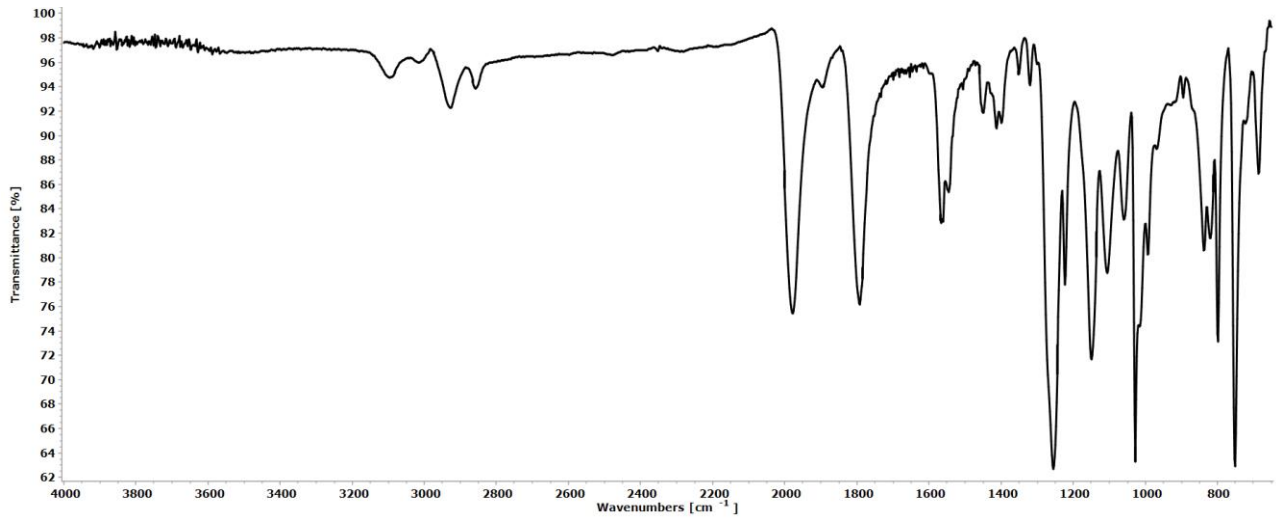


Figure S19. ^{1}H NMR spectrum (401 MHz, acetone-d_6) of [**5**]CF_3SO_3 (*cis-E*/*Z* isomers). Minor signals are presumably attributable to trans isomers.

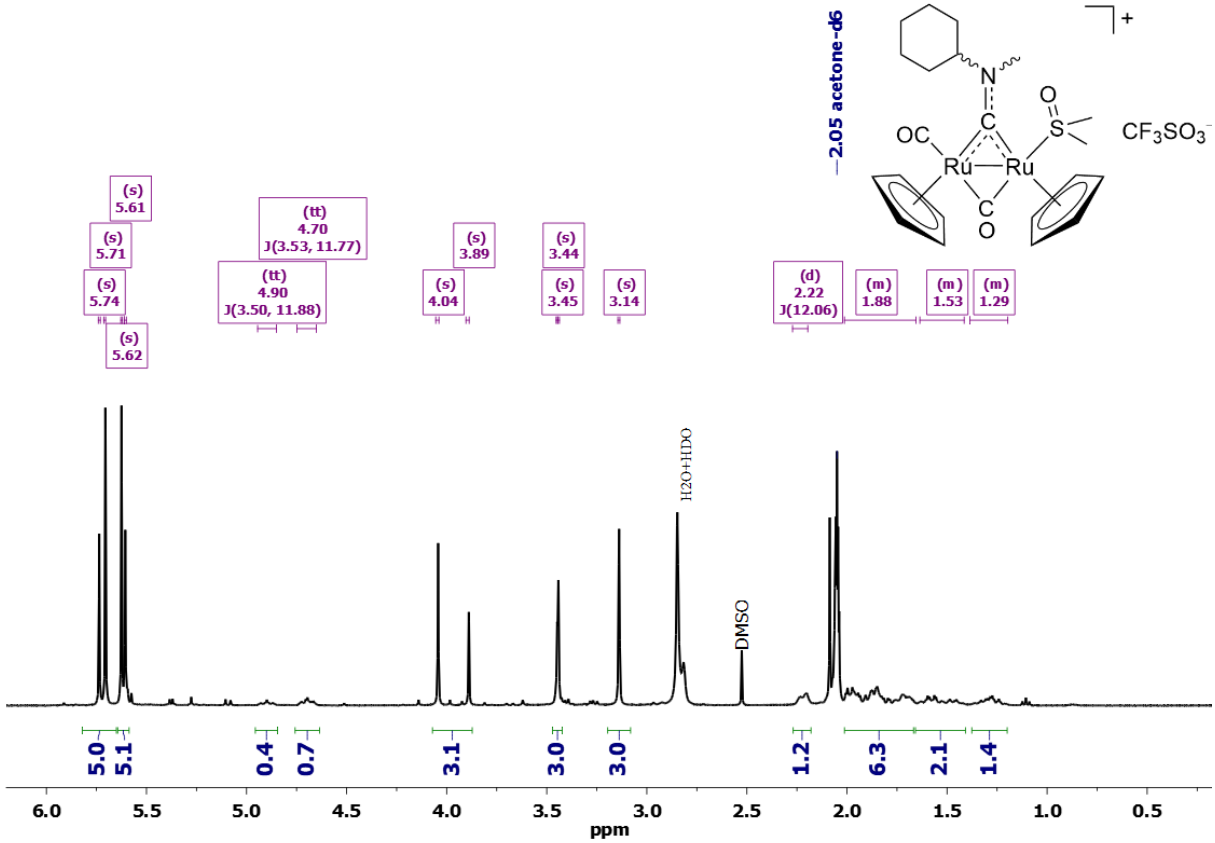


Figure S20. $^{13}C\{^{1}H\}$ NMR spectrum (101 MHz, acetone-d_6) of [**5**]CF_3SO_3 (*cis-E*/*Z* isomers).

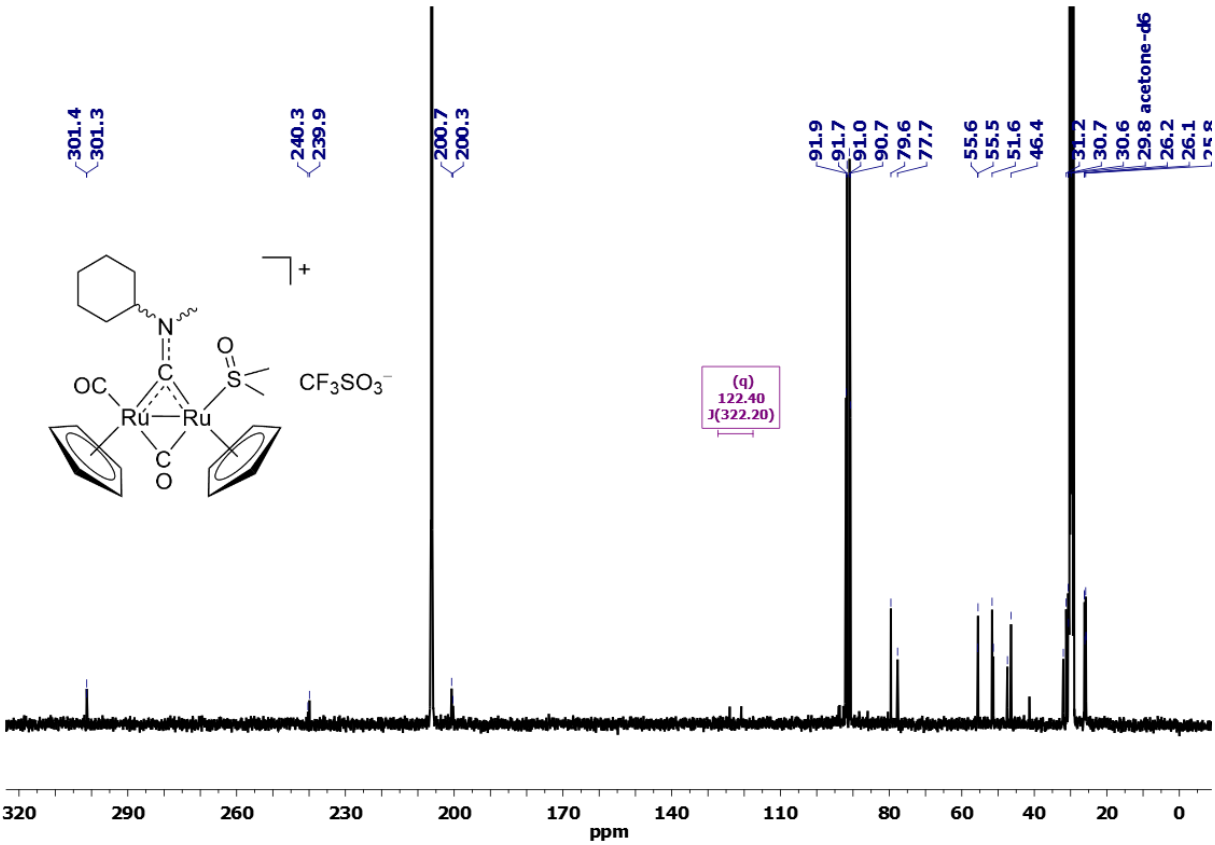


Figure S21. IR spectrum (650-4000 cm^{-1}) of $[Ru_2Cp_2(CO)\{\kappa S\text{-}SC(NH_2)_2\}(\mu\text{-}CO)\{\mu\text{-}CNMe(Cy)\}]CF_3SO_3$, [**6**]$CF_3SO_3$ (*cis-E*/*Z* + *trans-E*/*Z* isomers).

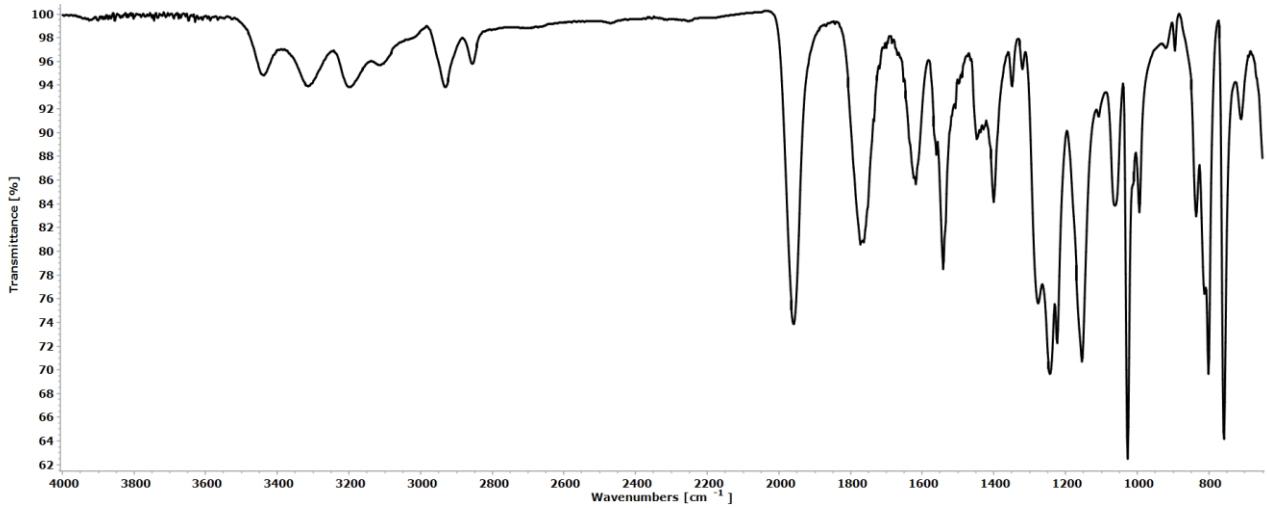


Figure S22. ^1H NMR spectrum (401 MHz, $CDCl_3$) of [**6**]CF_3SO_3 (*cis-E*/*Z* + *trans-E*/*Z* isomers).

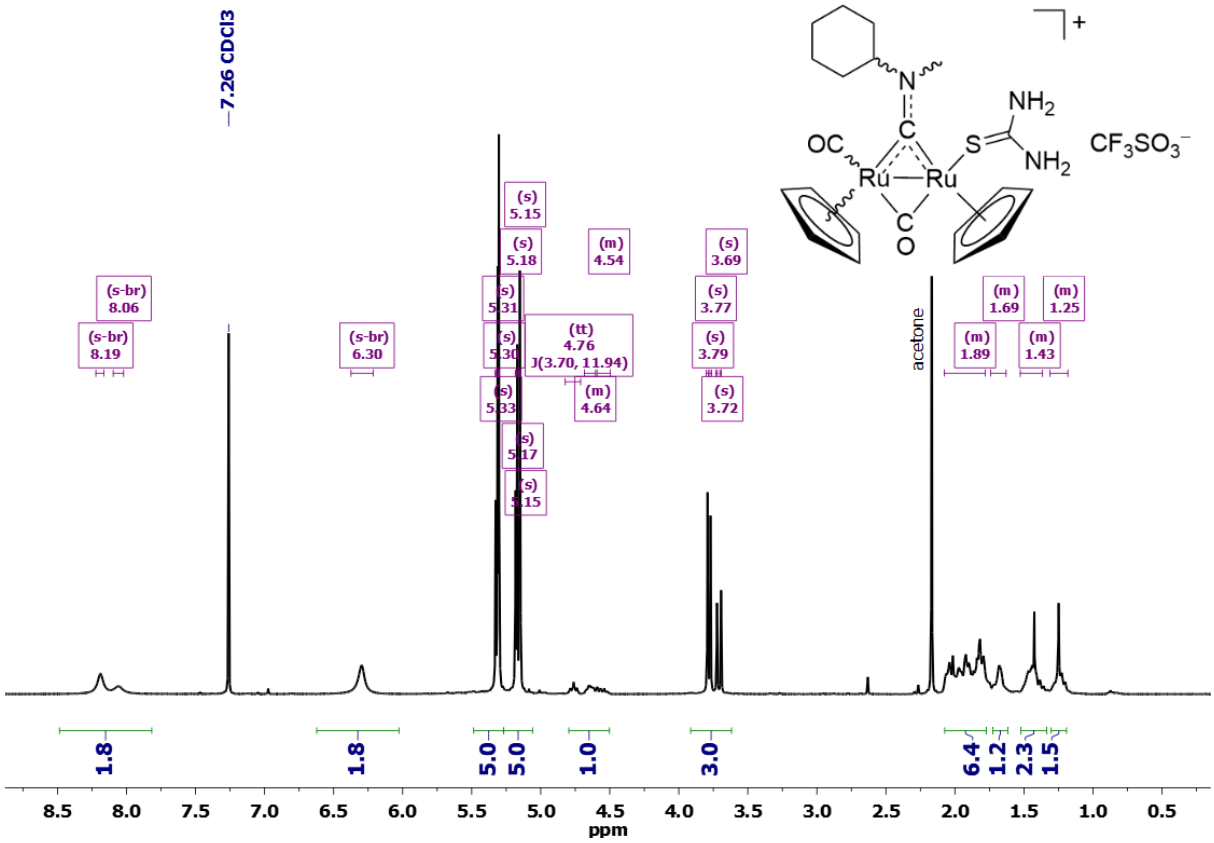


Figure S23. $^{13}C\{^{1}H\}$ NMR spectrum (101 MHz, $CDCl_3$) of [**6**]CF_3SO_3 (*cis-E*/*Z* + *trans-E*/*Z* isomers).

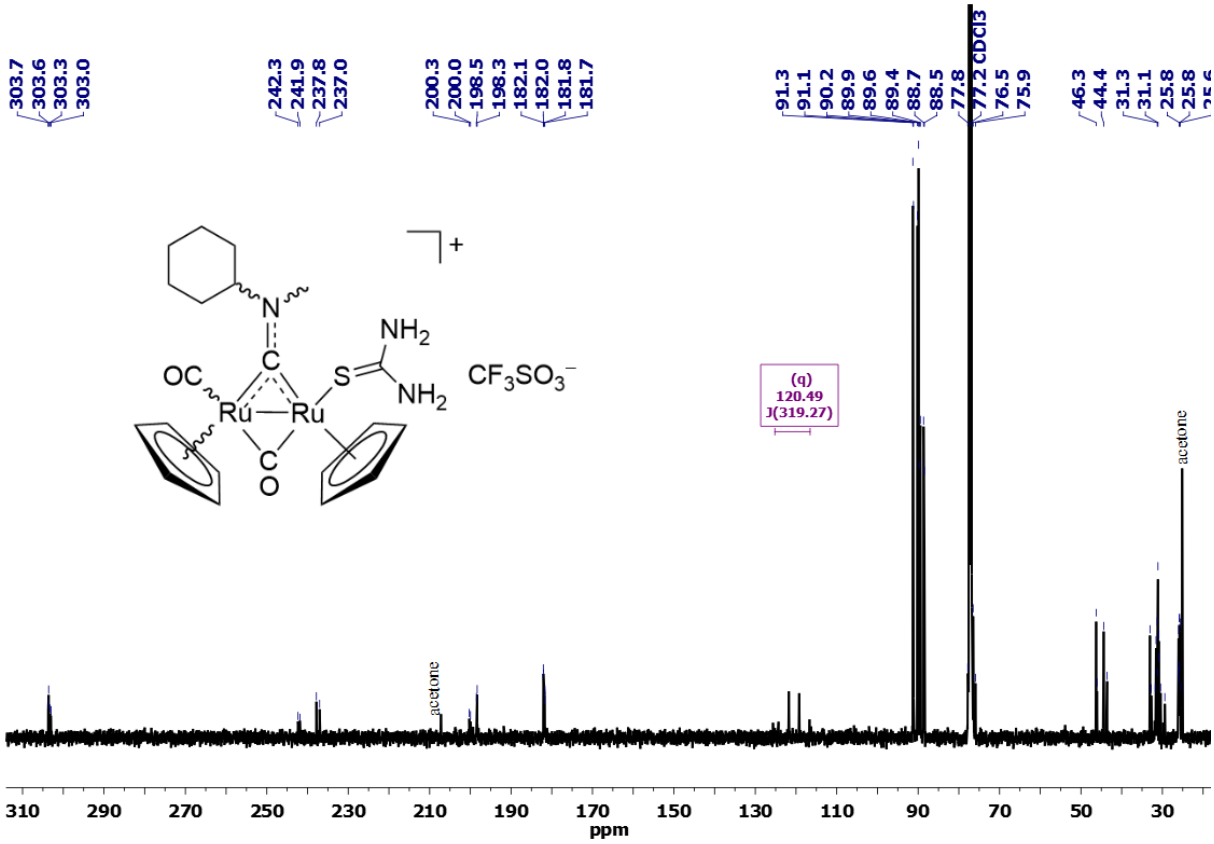


Figure S24. IR spectrum (650-4000 cm^{-1}) of $[Ru_2Cp_2(\mu\text{-}H)(CO)_2\{\mu\text{-}CNMe(Cy)\}]CF_3SO_3$, **7** (*cis*/*trans* isomers).

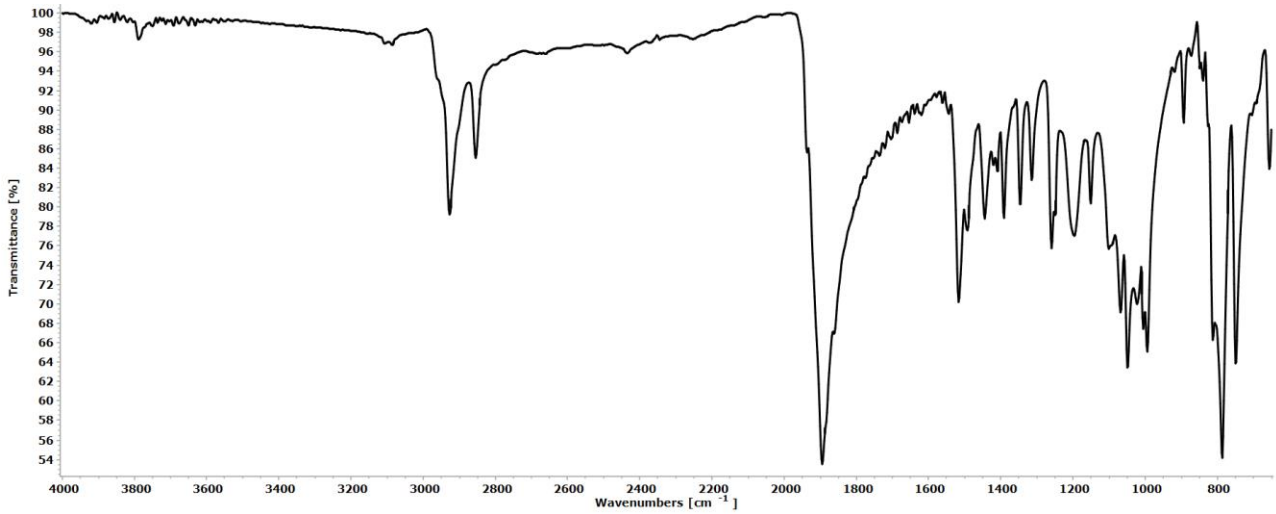


Figure S25. ^{1}H NMR spectrum (401 MHz, acetone-d_6) of **7** (*cis*/*trans* isomers).

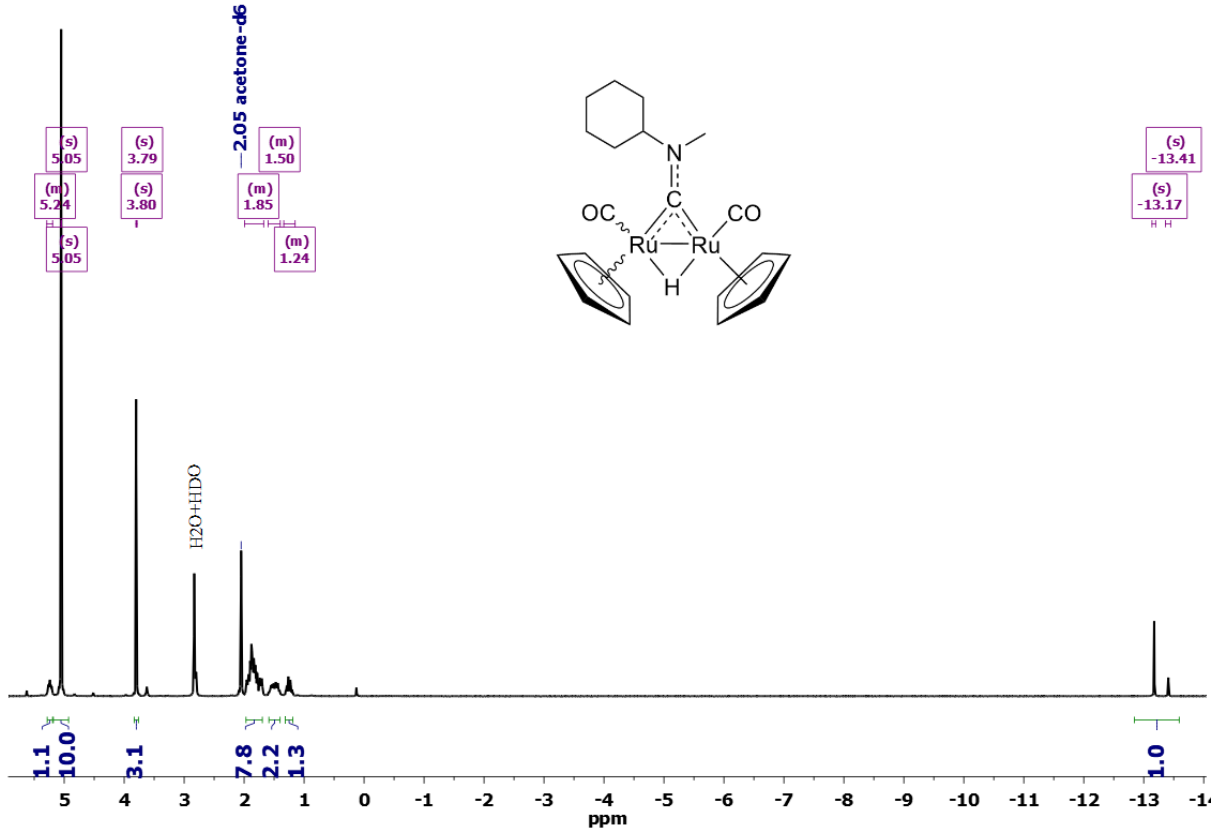


Figure S26. $^{13}C\{^{1}H\}$ NMR spectrum (101 MHz, acetone-d_6) of **7** (*cis*/*trans* isomers).

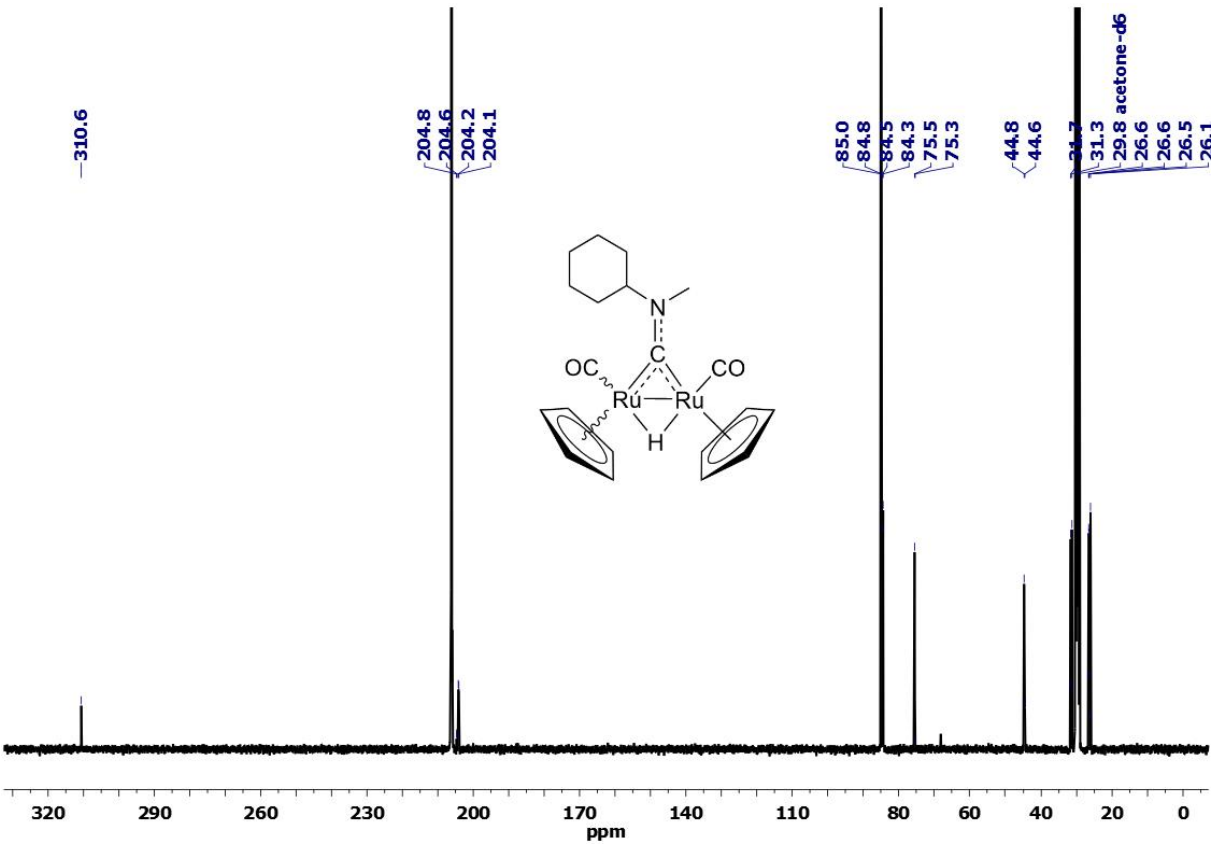


Figure S27. ^{1}H NMR spectrum (401 MHz, acetone-d_6) of [Ru_2Cp_2(CCPh)(CO)(μ-CO){μ-CNMe(Cy)}], **9** (*cis-E/Z* isomers). Signals marked with asterisk (*) are due to by-products, possibly including *trans* isomers of **9**.

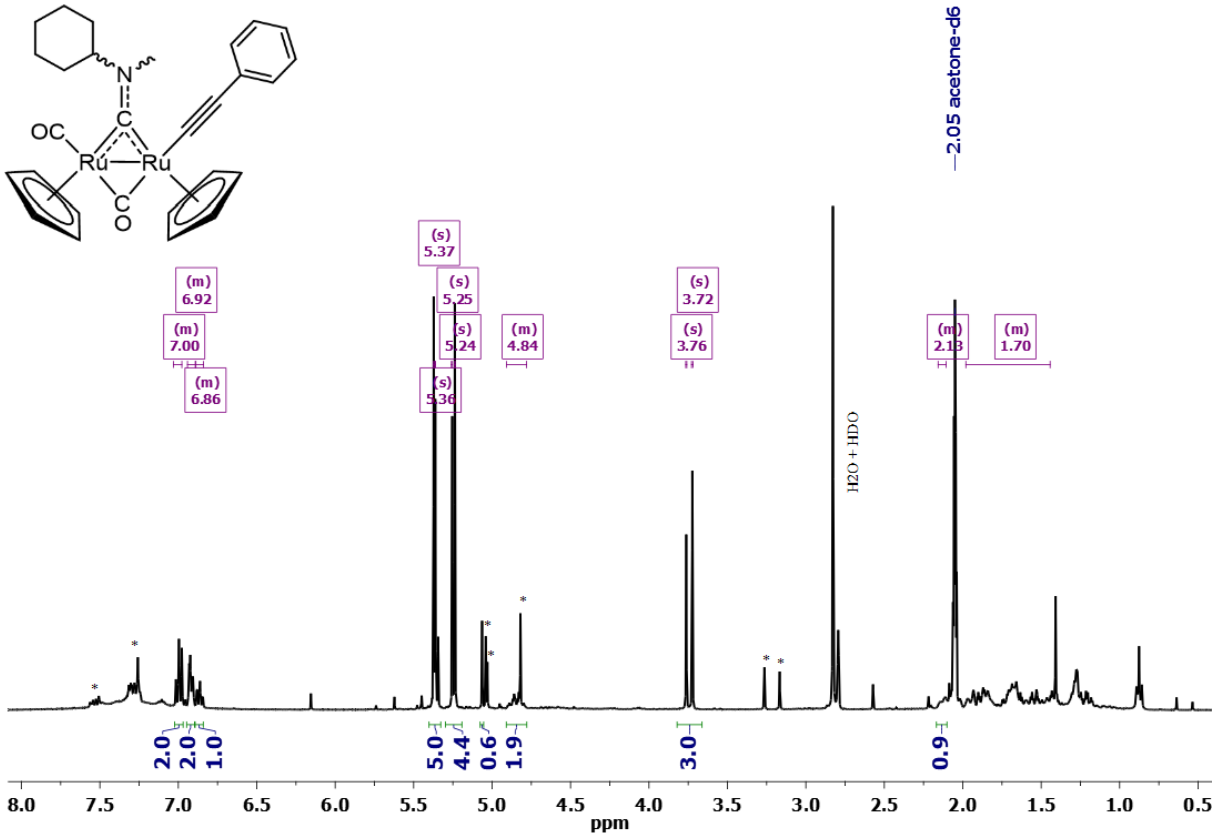


Figure S28. ^{13}C{^{1}H} NMR spectrum (101 MHz, acetone-d_6) of **9** (*cis-E/Z* isomers). Signals marked with asterisk (*) are due to by-products, possibly including *trans* isomers of **9**.

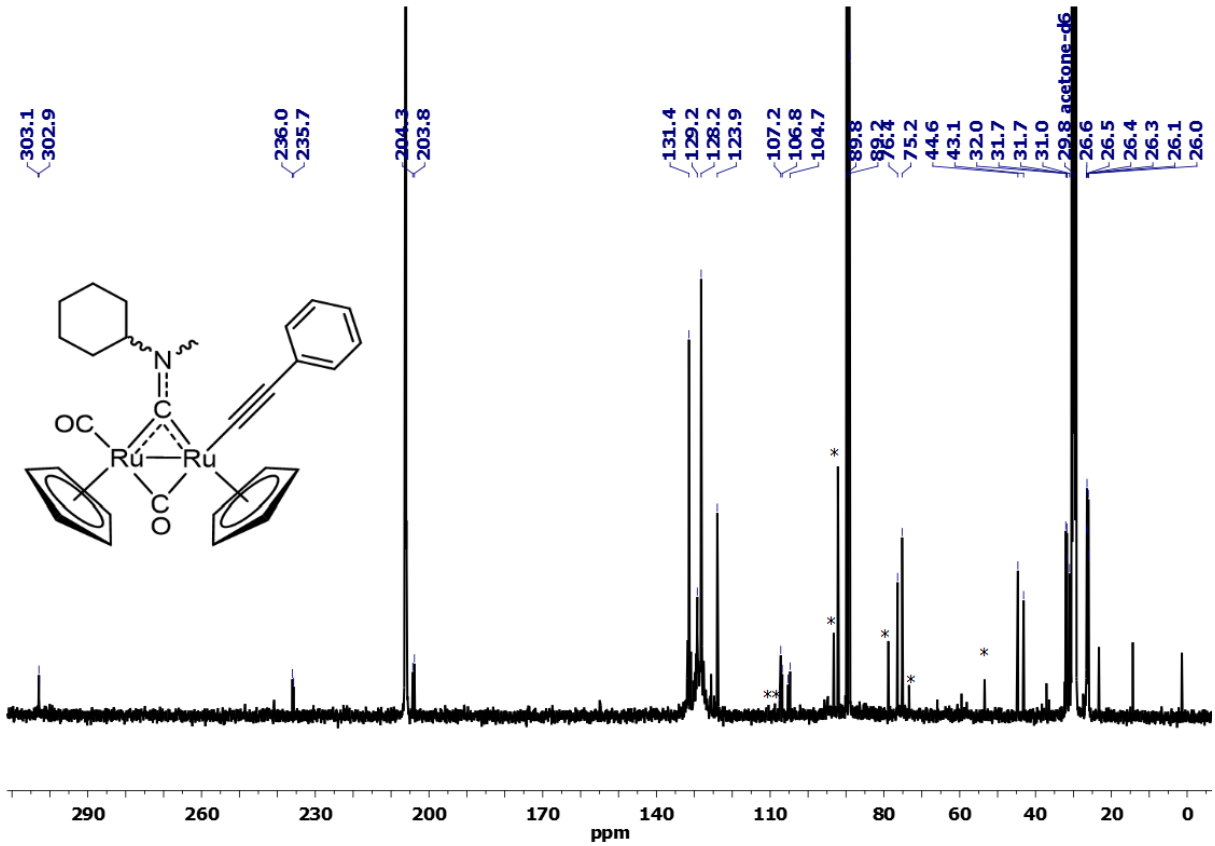


Figure S29. ^{1}H NMR spectrum (401 MHz, acetone-d_6) of [$Ru_2Cp_2(CO)(\mu\text{-}CO)\{\mu\text{-}\eta^1\text{:}\eta^3\text{-}C^3(Me)C^2(CS_2)C^1NMe(Cy)\}$], **10** (*cis-E/Z* isomers). Impurities are marked with asterisk (*).

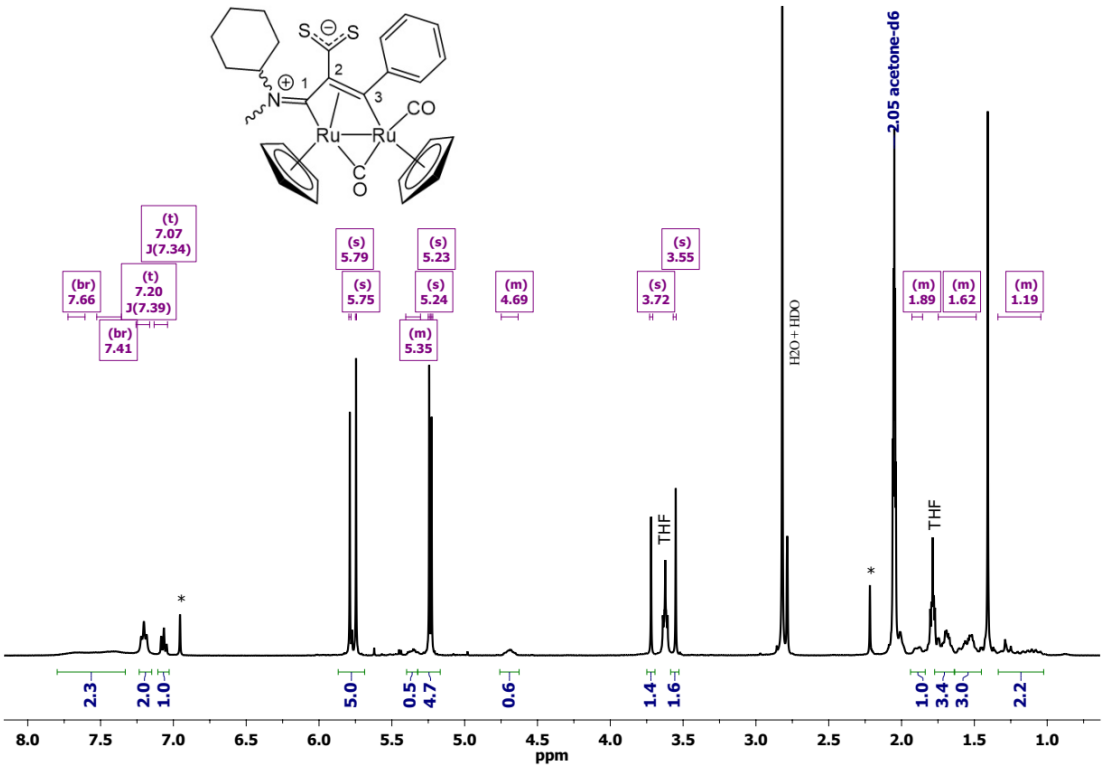


Figure S30. ^{1}H NMR spectrum (401 MHz, $CDCl_3$) of *E*-[RuCp{κ^2*C*,*S*-C(NMe(Cy))CH=C(Ph)S}(CO)], *E*-**11a**.

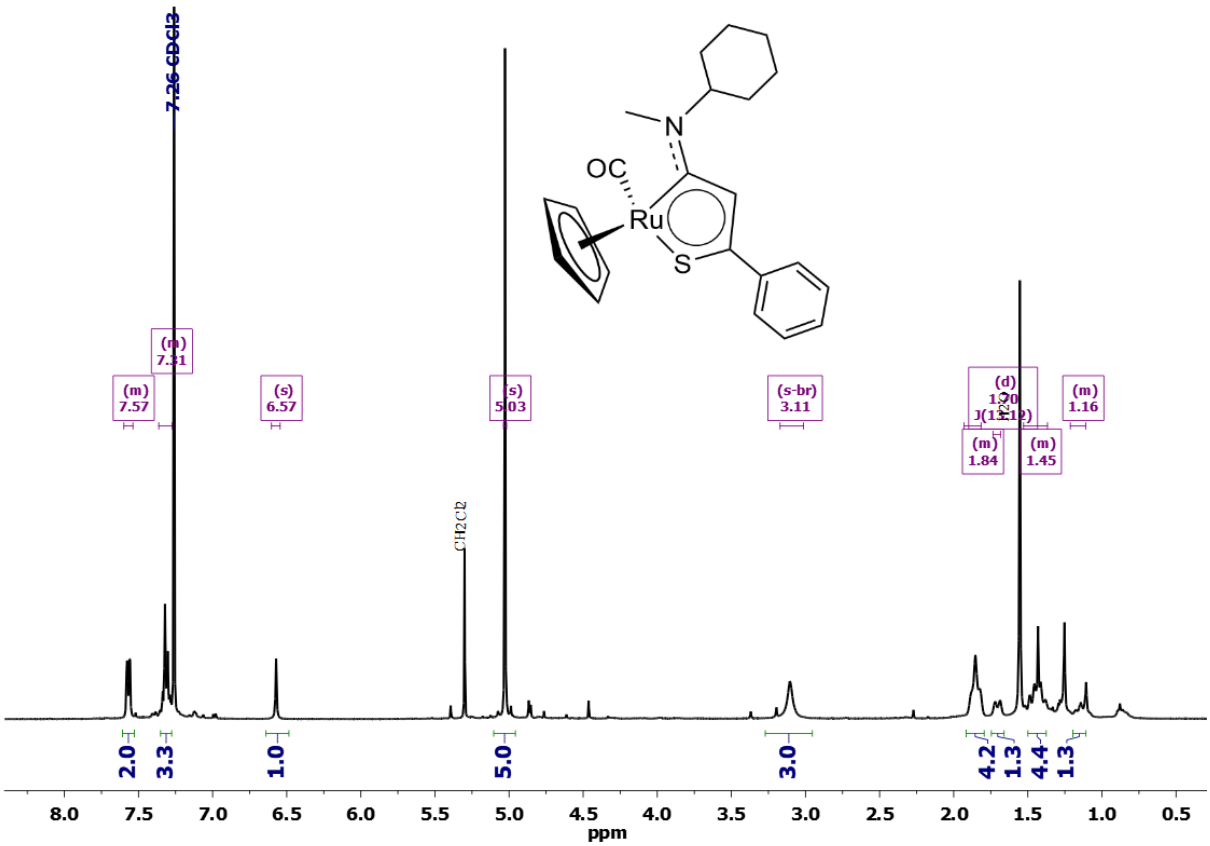


Figure S31. ^{1}H NMR spectrum (401 MHz, $CDCl_3$) of [RuCp{κ²C,S-C(Ph)=CHC(NMe(Cy))=S}(CO)], **11b** (*E*/*Z* isomers). Impurities are marked with asterisk (*).

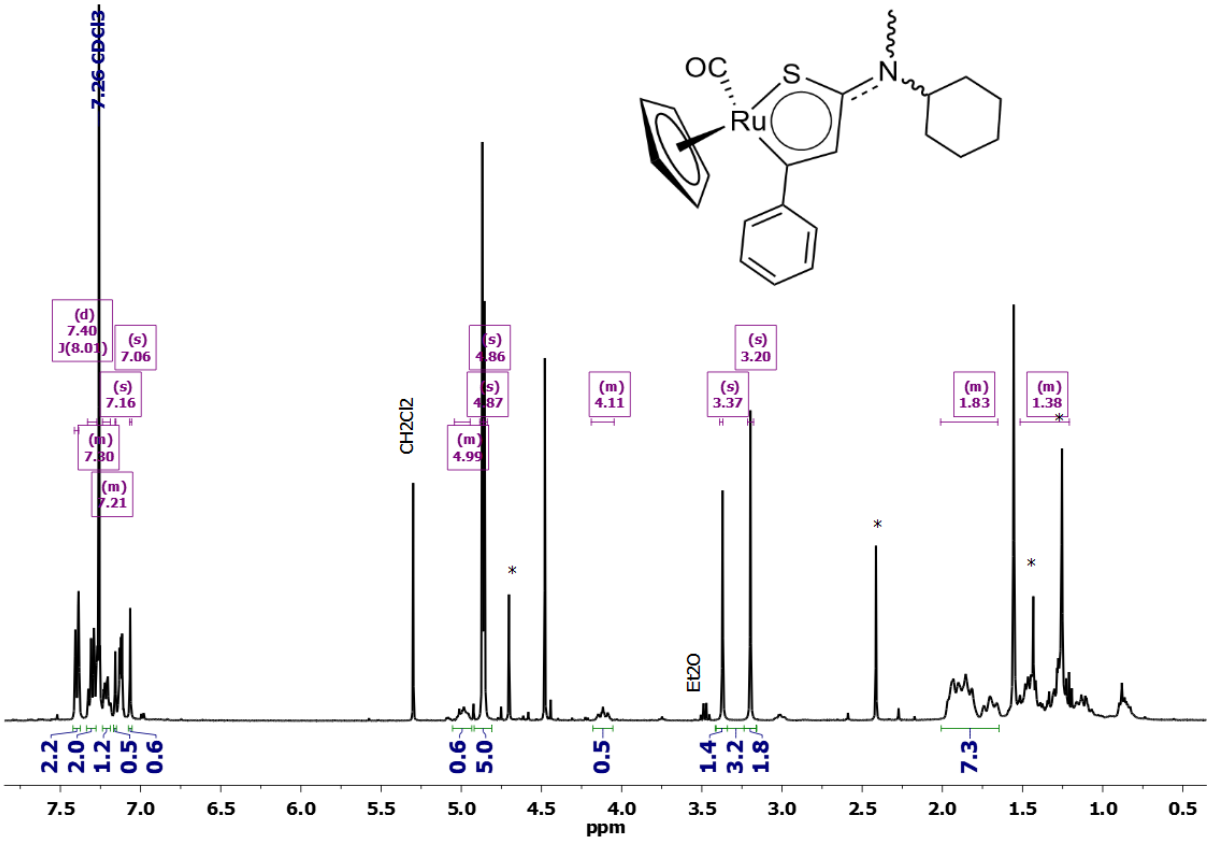
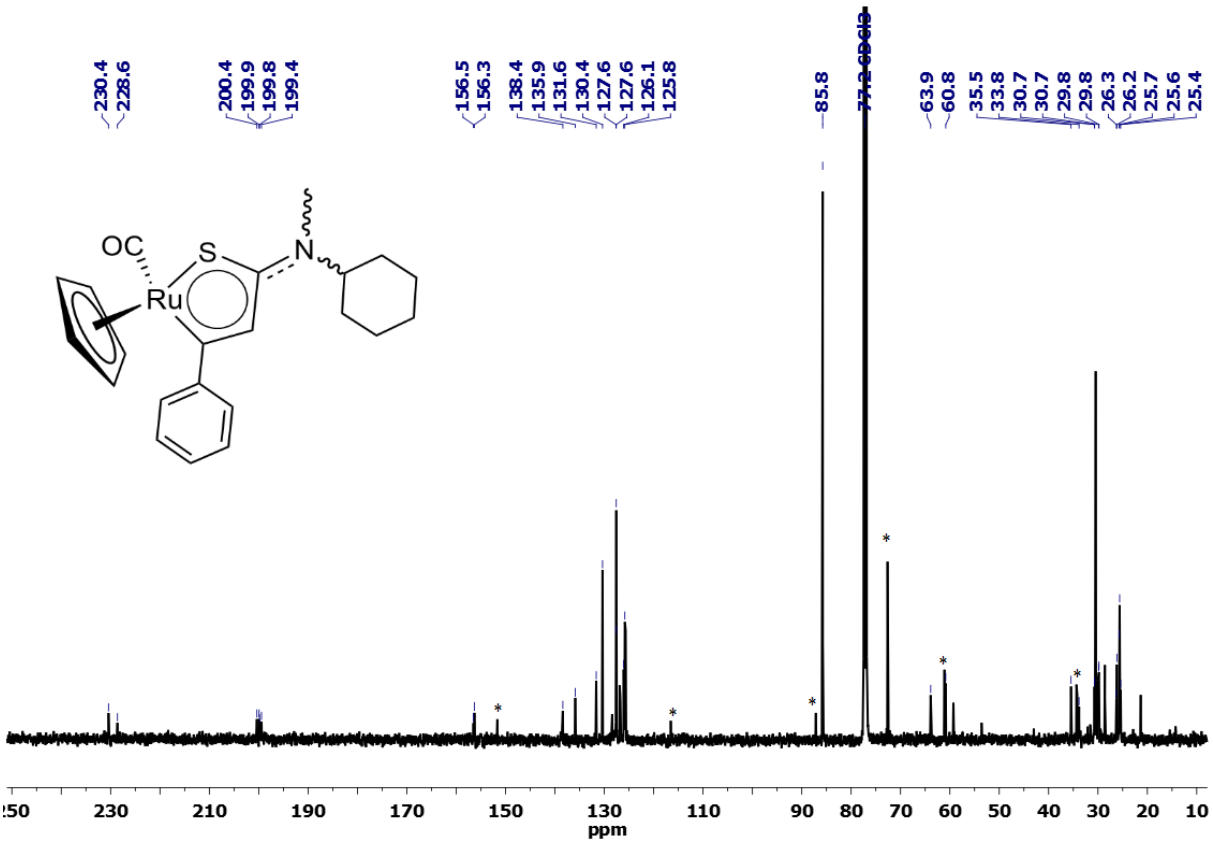


Figure S32. $^{13}C\{^{1}H\}$ NMR spectrum (101 MHz, $CDCl_3$) of **11b** (*E*/*Z* isomers). Impurities are marked with asterisk (*)



NMR data and spectra in aqueous solution

All data are referenced to Me_2SO_2 [δ_H = 3.12 ppm].

[**2**]CF_3SO_3. ^{1}H NMR (D_2O): δ/ppm = 5.46, 5.453, 5.448 (s, 5H, Cp^N); 5.23, 5.22, 5.20 (s, 5H, Cp); 4.68–4.56 (m, 1H, NCH^{Cy}); 3.89, 3.825, 3.819, 3.80 (s, 3H, NCH_3); 2.13–1.35 (m, 10H, CH_2^{Cy}); isomer ratios 21:38:16:25 (0 h); 15:44:11:30 (72 h). No difference in chemical shift values and isomer ratios was observed in the parallel experiment in DMEM-d solution.

[**3**]CF_3SO_3 ^{1}H NMR (D_2O/CD_3OD 6:1 *V*/*V*): δ/ppm = *8.12*, 8.08 (d, $^3J_{HH}$ = 5.2 Hz, 2H, Py/C_{ortho}), 7.72 (t, $^3J_{HH}$ = 7.7 Hz, 1H, Py/C_{para}), 7.21–7.16 (m, 2H, Py/C_{meta}); *5.51*, 5.50 (s, 5H, Cp); 5.29, *5.28* (s, 5H, Cp^N); 4.75-4.66 (m, 1H, NCH^{Cy}); 4.11, *3.89* (s, 3H, NCH_3); 2.20–1.87, 1.77 (dd, *J* = 32.4, 11.8 Hz), 1.63–1.39 (m), 1.35–1.21 (m) (10H, CH_2^{Cy}) for *cis*-*E*/*Z* isomers; 8.18 (d, $^3J_{HH}$ = 5.1 Hz, 2H, Py/C_{ortho}), 7.76 (t, $^3J_{HH}$ = 7.7 Hz, 1H, Py/C_{para}); 5.27, *5.25* (s, 5H, Cp), *5.12*, 5.08 (s, 5H, Cp^N); 4.17, *3.92* (s, 3H, NCH_3) for *trans*-*E*/*Z* isomers; *cis*-*E*/*Z* isomer ratio = 2.6 (0-72h); *cis*/*trans* isomer ratio = 15 (0 h); 21 (72 h); %*trans* isomers = 6.4 % (0 h), 4.5 % (72 h). Minimal differences in chemical shift values were observed between D_2O, D_2O/CD_3OD 6:1 *V*/*V*, D_2O/DMSO-d_6 6:1 *V*/*V* and DMEM-d/CD_3OD 6:1 *V*/*V* solutions. ^{1}H NMR (D_2O/DMSO-d_6 6:1 *V*/*V*): δ/ppm = *cis*-*E*/*Z* isomer ratio = 2.6 (0 h), 3.6 (72 h); %*trans* isomers = 3.6 % (0 h), 0 % (72 h).

Py. ^{1}H NMR (D_2O/CD_3OD 6:1 *V*/*V*): δ/ppm = 8.50 (d, $^3J_{HH}$ = 4.3 Hz, 2H), 7.87 (t, $^3J_{HH}$ = 7.7 Hz, 1H), 7.45 (dd, $^3J_{HH}$ = 7.6, 5.9 Hz, 2H). ^{1}H NMR (D_2O/DMSO-d_6 6:1 *V*/*V*): δ/ppm = 8.52 (d, $^3J_{HH}$ = 4.3 Hz, 2H), 7.87 (t, $^3J_{HH}$ = 7.7 Hz, 1H), 7.46 (dd, $^3J_{HH}$ = 7.4, 5.9 Hz, 2H).

Cis-*E*-[**4**]CF_3SO_3 ^{1}H NMR (CD_3OD/D_2O 2:1 *V*/*V*): δ/ppm = 7.45–7.41 (m, 3H), 7.38–7.33 (m, 6H), 7.25–7.21 (m, 6H) (Ph); 5.42 (s, 5H, Cp), 5.34 (s, 5H, Cp^P), 4.47 (tt, $^3J_{HH}$ = 12.1, 3.4 Hz, 1H, NCH^{Cy}), 3.59 (s, 3H, NCH_3), 2.32 (d, *J* = 13.0 Hz, 1H), 2.05 (d, *J* = 12.9 Hz, 1H), 1.94 (d, *J* = 12.3 Hz, 1H), 1.89–1.77 (m, 2H), 1.75–1.64 (m, 2H), 1.38–1.27 (m, 2H), 1.23–1.14 (m, 1H) (CH_2^{Cy}). No difference in chemical shift values and isomerization was observed in the parallel experiment in DMEM-d solution. ^{1}H NMR (DMSO-d_6/D_2O 4:3 *V*/*V*): δ/ppm = 7.62–7.57 (m, 3H), 7.52 (t, *J* = 6.7 Hz, 6H), 7.37–7.28 (m, 6H) (Ph); 5.60 (s, 5H, Cp), 5.47 (s, 5H, Cp^P), 3.58 (s, 3H, NCH_3); 2.06–1.72 (7H),

1.54–1.24 (m, 3H) (CH_2^{Cy}). $^{31}P\{^1H\}$ NMR (DMSO-d_6/D_2O 4:3 *V*/*V*): δ/ppm = 45.7. Formation of other isomers after 72 h was not observed in any experiment.

O=PPh$_3$. $^{31}P\{^1H\}$ NMR (DMSO-d_6/D_2O 4:3 *V*/*V*): δ/ppm = 33.7.

[**5**]CF_3SO_3. ^1H NMR (D_2O): δ/ppm = *5.62*, 5.61 (s, 5H, Cp); 5.46, *5.45* (s, 5H, Cp^S); *4.71–4.62*, 4.54–4.47 (m, 1H, NCH^{Cy}); 3.92, *3.79* (s, 3H, NCH_3); *3.443*, 3.38 (s, 3H, SCH_3); 3.20, *3.19* (s, 3H, SCH_3'); 2.11–1.83, 1.82–1.64, 1.58–1.37, 1.32–1.18 (m, 10H, CH_2^{Cy}); *E*/*Z* isomer ratio = 1.7 (0 h), 1.3 (72 h). Minimal differences in chemical shift values and isomer ratios were observed between D_2O, DMEM-d and DMEM-d/DMSO-d_6 6:1 *V*/*V* solutions.

DMSO. ^1H NMR (D_2O): δ/ppm = 2.70 (s).

[**6**]CF_3SO_3. ^1H NMR (D_2O): δ/ppm = 5.473, 5.469, 5.44, 5.43 (5H, Cp/Cp^S); 5.33, 5.31, 5.29 (s, 5H, Cp/Cp^S); NCH^{Cy} signals partially covered by HDO; 3.83, 3.80, 3.76, 3.72 (s, 3H, NCH_3); 2.09–2.00, 1.96–1.84, 1.80–1.66, 1.56–1.34, 1.31–1.18 (m, 10H, CH_2^{Cy}); isomer ratios = 30:23:23:24 (0 h); 26:20:27:27 (72 h). Minimal differences in the shift values and isomer ratios were observed between D_2O, D_2O/CD_3OD 6:1 *V*/*V* and DMEM-d/CD_3OD 6:1 *V*/*V* solutions.

^1H NMR (D_2O/DMSO-d_6 6:1 *V*/*V*): δ/ppm = 5.485, 5.479, 5.44, 5.43 (5H, Cp/Cp^S); 5.34, 5.32, 5.31 (s, 5H, Cp/Cp^S); 3.84, 3.80, 3.77, 3.73 (s, 3H, NCH_3); 2.11–1.66, 1.56–1.17 (m, 10H, CH_2^{Cy}); isomer ratios = 36:29:18:17 (0 h), minimal variations were observed after 72 h.

Figure S33. ^{1}H NMR spectrum (401 MHz) of a freshly-prepared solution of [**2**]CF_3SO_3 in D_2O (top, purple line) and after 72 h at 37 °C (bottom, green line); Me_2SO_2 as internal standard.

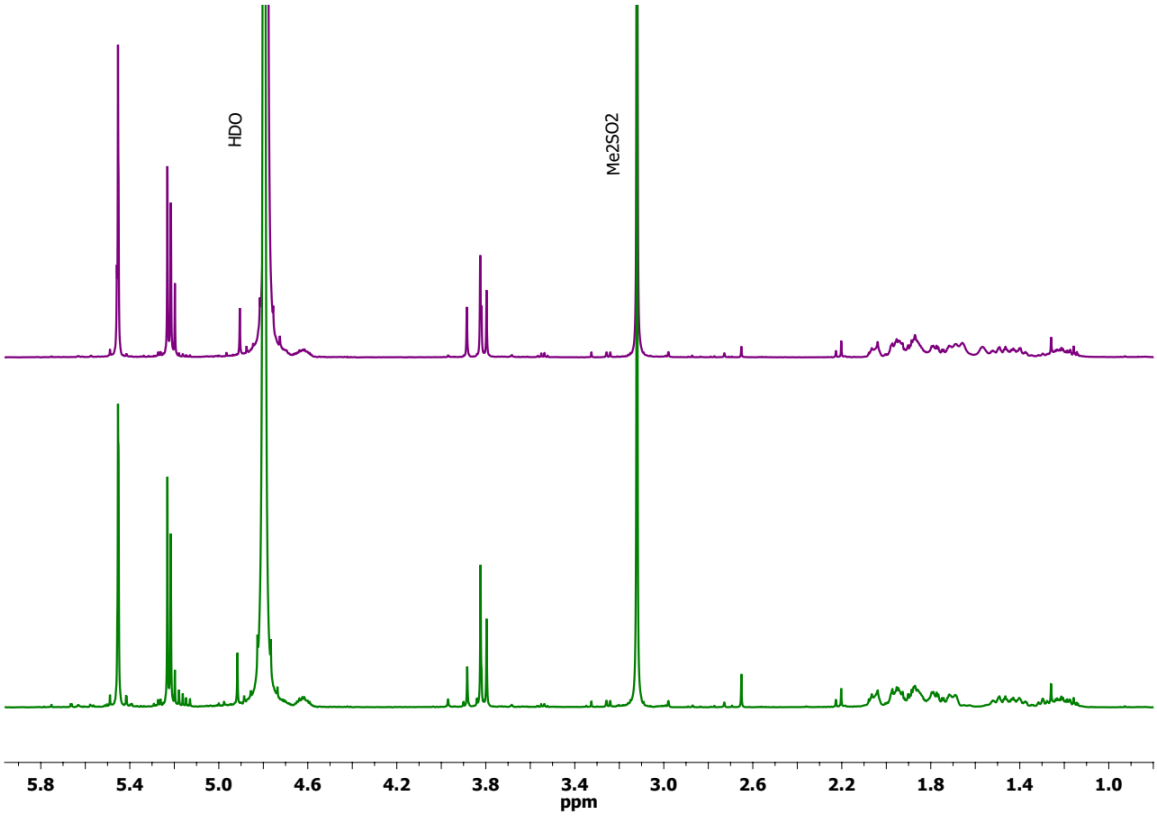


Figure S34. ^{1}H NMR spectrum (401 MHz) of a freshly-prepared solution of [**3**]CF_3SO_3 in D_2O/CD_3OD 6:1 *V*/*V* (top, purple line) and after 72 h at 37 °C (bottom, green line); Me_2SO_2 as internal standard.

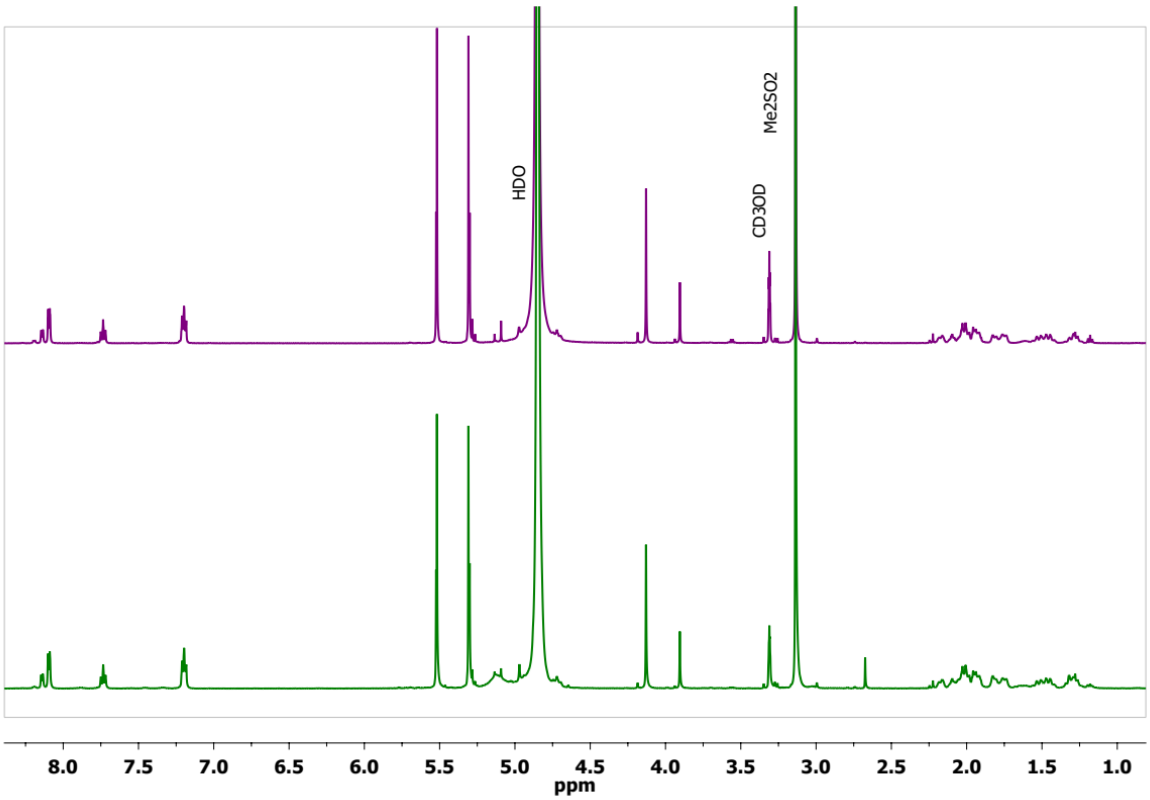


Figure S35. ^{1}H NMR spectrum (401 MHz) of a freshly-prepared solution of *cis-E*-[**4**]CF_3SO_3 in CD_3OD/D_2O 2:1 *V*/*V* (top, purple line) and after 72 h at 37 °C (bottom, green line); Me_2SO_2 as internal standard.

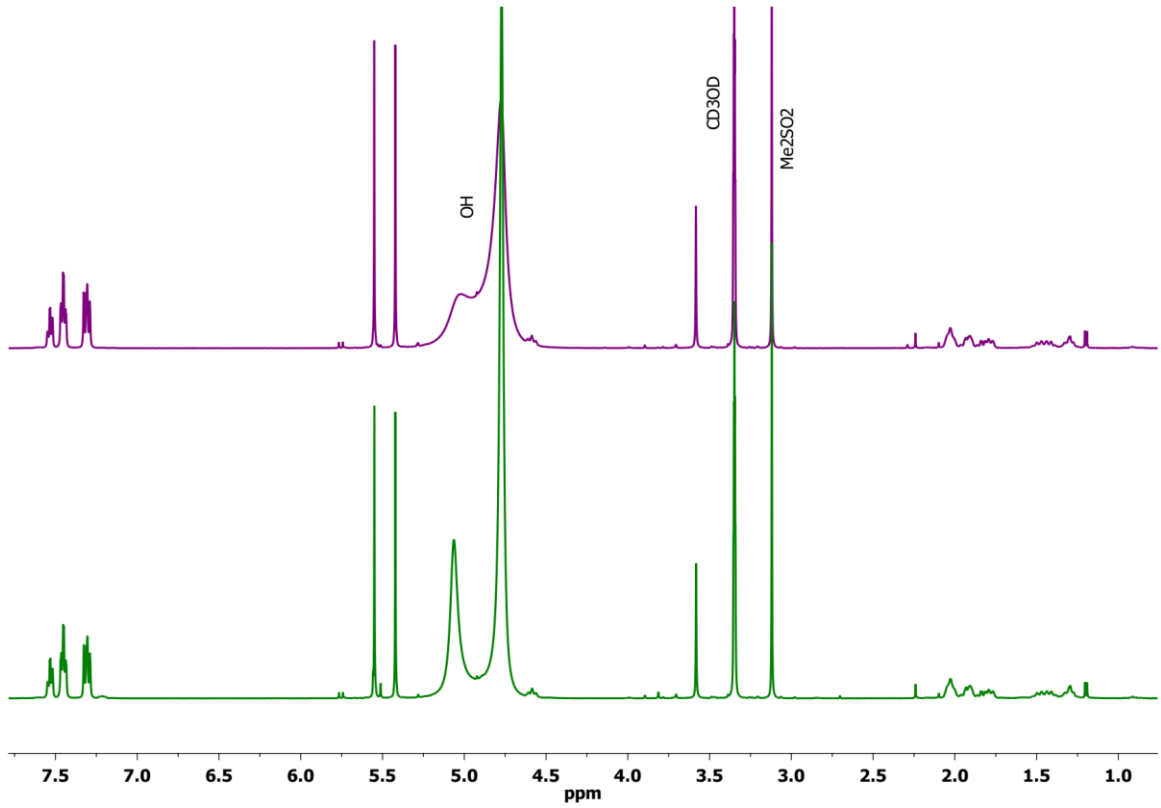


Figure S36. ^{1}H NMR spectrum (401 MHz) of a freshly-prepared solution of [**5**]CF_3SO_3 in D_2O (top, purple line) and after 72 h at 37 °C (bottom, green line); Me_2SO_2 as internal standard.

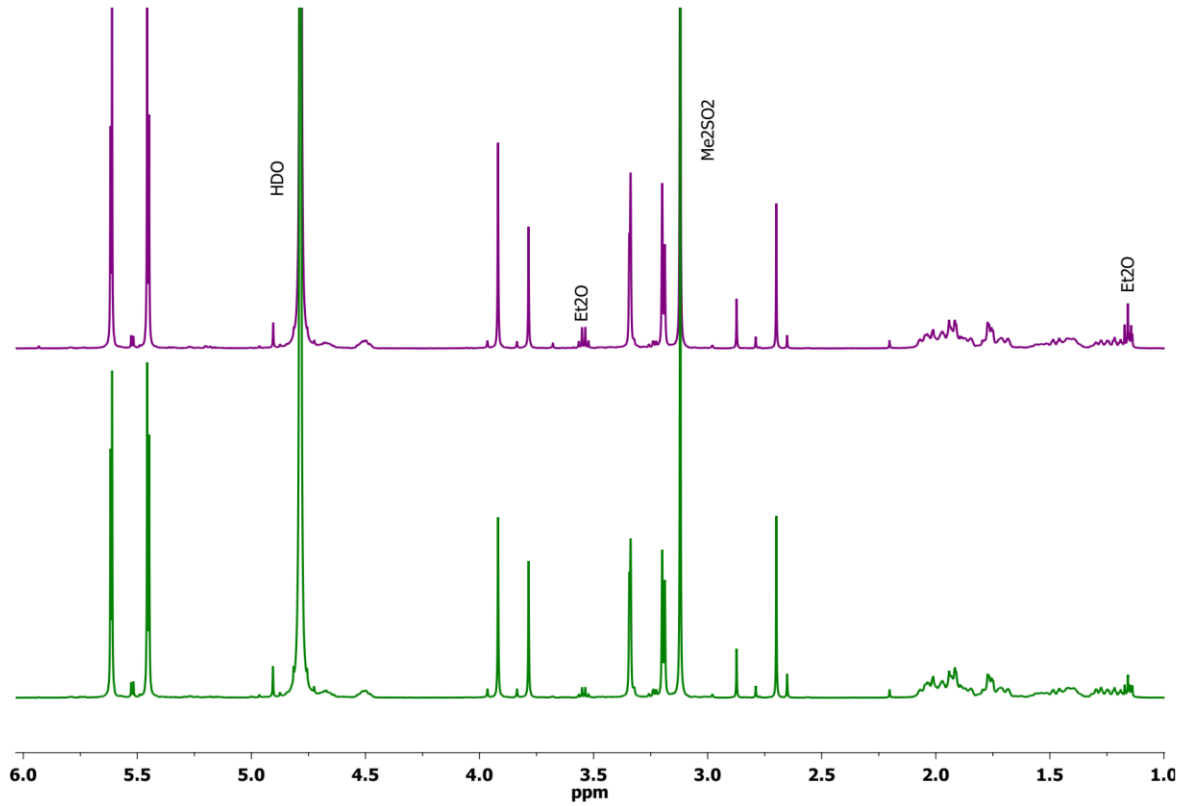


Figure S37. ^{1}H NMR spectrum (401 MHz) of a freshly-prepared solution of [**6**]CF_3SO_3 in D_2O (top, purple line) and after 72 h at 37 °C (bottom, green line); Me_2SO_2 as internal standard.

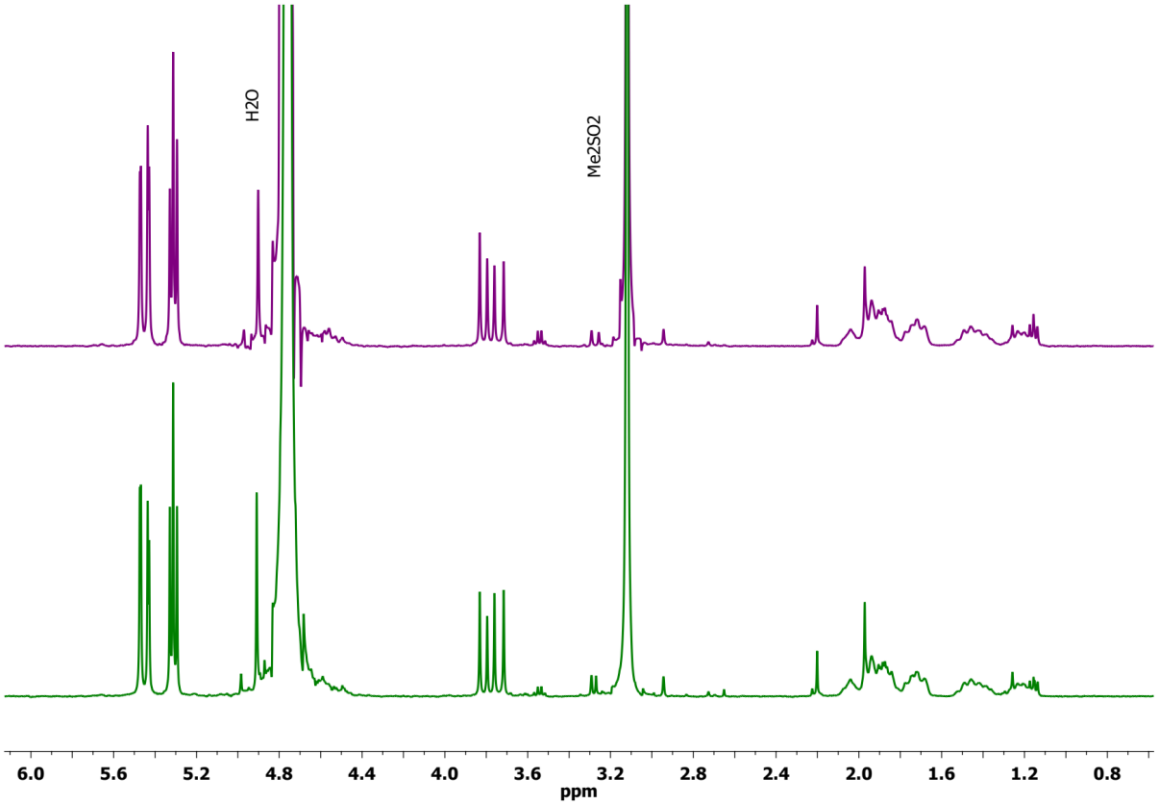


Figure S38. ^{1}H NMR spectrum (401 MHz) of a freshly-prepared solution of [**3**]CF_3SO_3 in D_2O/DMSO-d_6 6:1 *V*/*V* (top, purple line) and after 72 h at 37 °C (bottom, green line); Me_2SO_2 as internal standard.

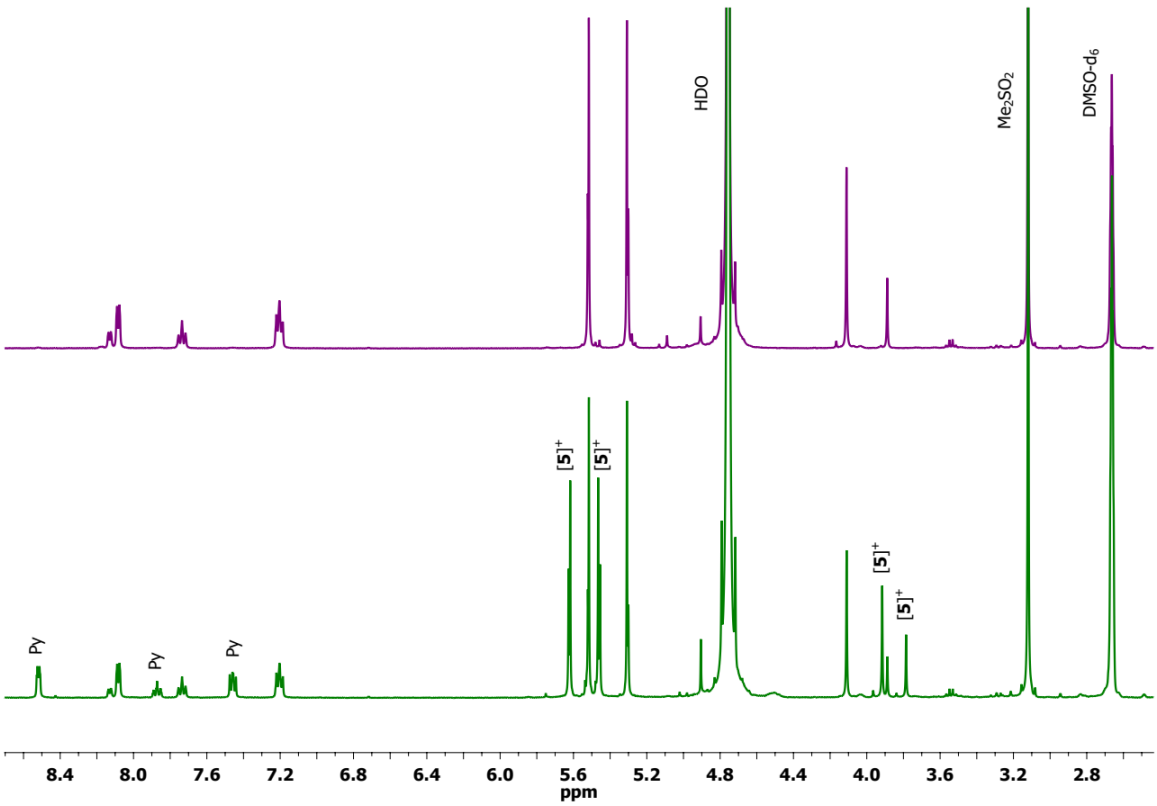


Figure S39. ^{1}H NMR spectrum (401 MHz) of a freshly-prepared solution of *cis*-*E*-[**4**]CF_3SO_3 in D_2O/DMSO-d_6 3:4 *V*/*V* (top, purple line) and after 72 h at 37 °C (bottom, green line); Me_2SO_2 as internal standard.

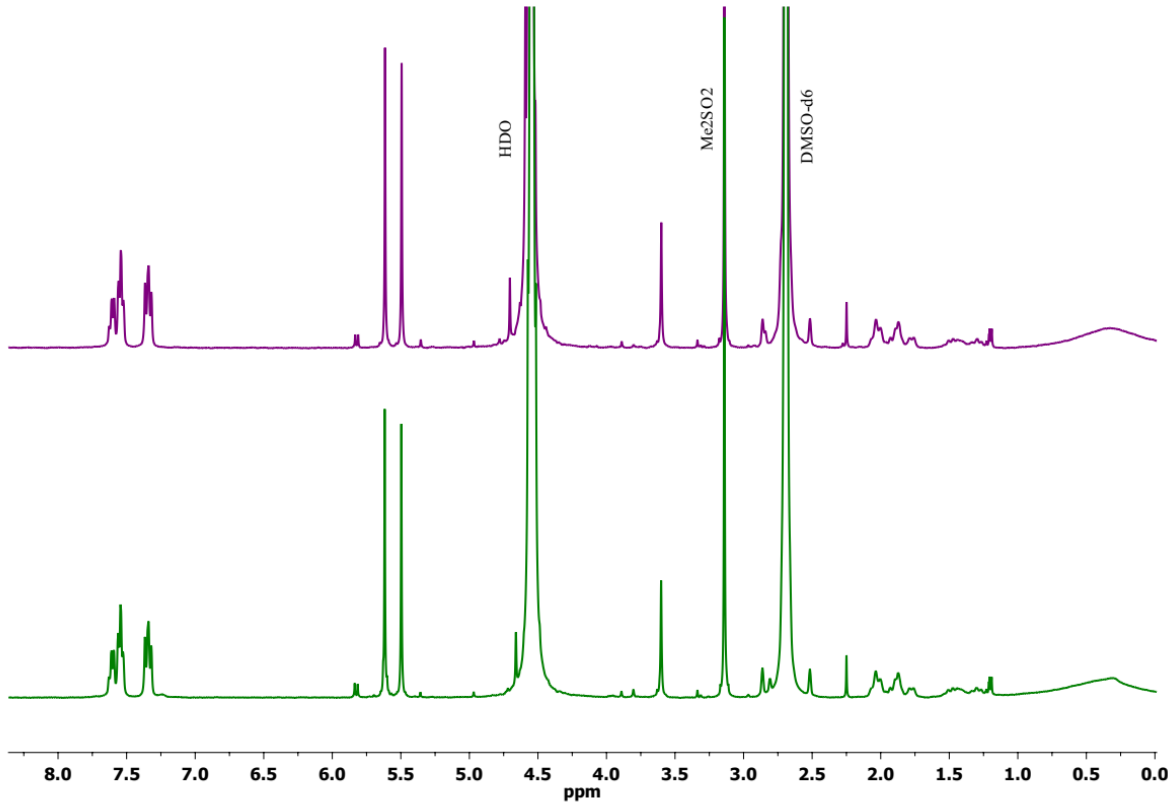


Figure S40. ^{1}H NMR spectrum (401 MHz) of a freshly-prepared solution of [**6**]CF_3SO_3 in D_2O/DMSO-d_6 6:1 *V*/*V* (top, purple line) and after 72 h at 37 °C (bottom, green line); Me_2SO_2 as internal standard.

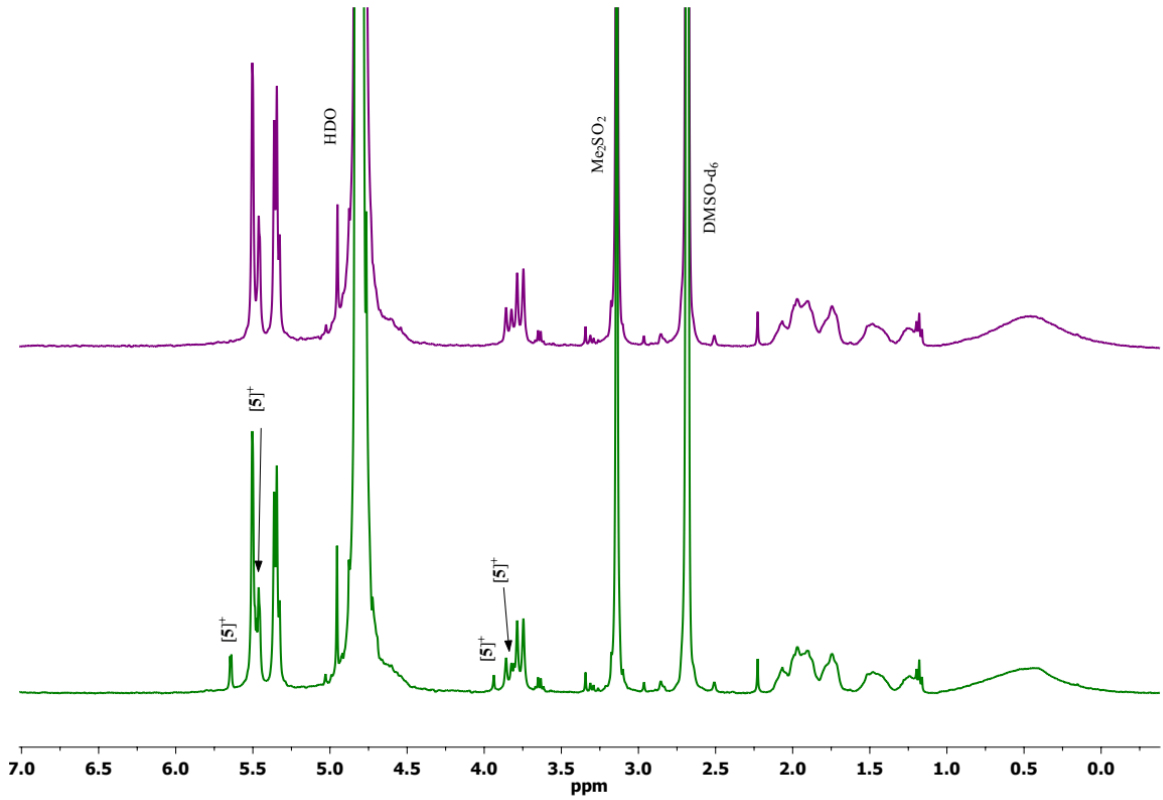


Figure S41. ^{1}H NMR spectrum (401 MHz) of a freshly-prepared solution of [**5**]CF_3SO_3 in DMEM-d/DMSO-d_6 6:1 *V*/*V* (top, purple line) and after 72 h at 37 °C (bottom, green line); Me_2SO_2 as internal standard. Signals marked with asterisk (*) are due to components of the cell culture medium (DMEM).

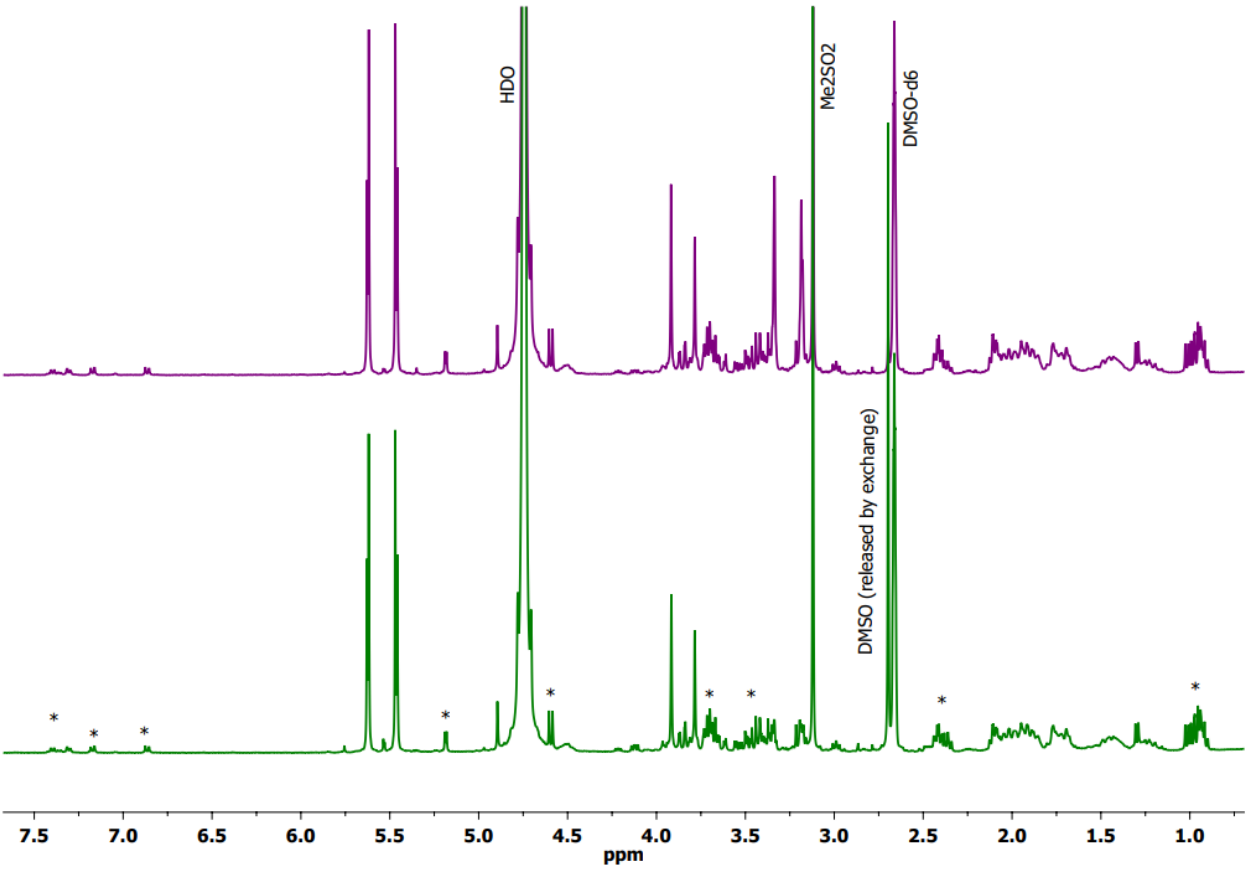


Table S1. ^{1}H NMR monitoring of a D_2O/DMSO-d_6 6:1 *V*/*V* solution of [**3**]CF_3SO_3 kept at 37 °C for > 92 h. Relative % amounts of species in solution are referred to Me_2SO_2 as internal standard.

time (h)	% amount in solution		
	[3]$^+$	**[5-d_6]$^+$**	**Pyridine**
0	98	0	2
3.5	94	2	7
23	82	14	19
42.5	70	28	26
67	62	39	36
72	54	45	39
92.5	48	51	40

Figure S42. Plot of octanol-water partition coefficients (Log_{10} P_{ow}; *y* axis) *vs*. IC_{50} values (μM; *x* axis) on A2780, A2780cis and HEK 293T cells determined for the diruthenium compounds [**1**,**3**-**6**]CF_3SO_3. The dotted light blue curve represents a logarithmic fitting of the data for A2780 cells (R^2 = 0.92).