Electronic Supplementary information

Artificial metalloenzymes derived from bovine β-lactoglobulin for the asymmetric transfer hydrogenation of an aryl ketone – Synthesis, characterization and catalytic activity

Alice Chevalley, Mickael V. Cherrier, Juan C. Fontecilla-Camps, Mahsa Ghasemi, Michèle Salmain*

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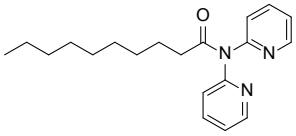
Synthesis

General methods: ^{1}H NMR spectra were recorded either on 300 or 400 MHz spectrometers (Bruker) and coupling constants (*J*) are reported in Hz to ± 0.5 Hz. The following abbreviations were utilized to describe peak patterns when appropriate: br = broad, app = apparent, s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet. Chemical shifts are quoted in parts per million (ppm) and are referenced to residual H signal of deuterochloroform (7.26 ppm), methylene chloride (5.32 ppm) or DMSO (2.50 ppm). ^{13}C NMR chemical shift are expressed in parts per million (ppm), referenced to the central peak of deuterochloroform (77.00 ppm), methylene chloride (54.00 ppm) or DMSO (39.4 ppm). When measured, DEPT signals are referred as (+) or (–) following the corresponding signals. Infrared spectra were recorded on a Tensor 27 FT-IR spectrometer (Bruker). High-resolution mass spectra were recorded by mass spectrometer in Electrospray ionization (ESI) mode (MStation JMS 700, Jeol). Analytical TLC was performed on plates pre-coated with silica gel (Merck silica gel, 60 F254), which were visualized with UV fluorescence when applicable (λ = 254 nm) and by staining with vanillin sulphuric acid solutions followed by heating. Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from sodium–benzophenone. Dichloromethane (DCM) and triethylamine (TEA) were distilled from CaH_2. All air and/or water sensitive reactions were carried out under a nitrogen atmosphere using a dual manifold high vacuum line with dry, freshly distilled solvents using standard syringe-cannula/septa and purge-and-refill techniques. Commercial 2,2-dipyridylamine was dried 2 times under azeotropic distillation with toluene before use. Other chemicals and solvents were purchased from Acros, TCI or Aldrich and were used as received. $(Cp^*Rh(\mu\text{-}Cl)Cl)_2$ dimer was prepared as previously described.[1] Compounds **2**, **4**, **2-Rh**, **2-Ru**, **4-Rh**, **4-Ru**[2] and dipyridin-2-ylmethanamide[3] were synthesized according to literature procedures. βLG isoform A, βLG isoform B and mixture of both isoforms (85% purity) were purchased from Sigma and used as received.

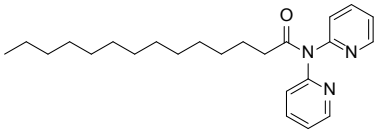
N,N-di(pyridin-2-yl)dodecanamide 1



To a solution of capric acid (251 mg, 1.5 mmol) in toluene (5 mL) was slowly added oxalyl chloride (250 µL, 2.9 mmol). The resulting reaction mixture was stirred for 2 h at room temperature followed by evaporation to dryness. The residual uncoloured oil was dissolved in DCM (5 mL) under nitrogen and 2,2-dipyridylamine (250 mg, 1.5 mmol in DCM (1mL) and TEA (265 µL, 1.9 mmol) were added. The reaction mixture was stirred overnight at room temperature. Saturated aqueous NH_4Cl was added. Aqueous phase was extracted 3 times with DCM. The combined organic phases were washed 3 times with brine, NaOH 0.1M and HCl 0.1M, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (AcOEt/PE 50:50 to 80:20) to afford compound **1** (370 mg, 78%) as light white oil.

^{1}H NMR (δ, ppm) $CDCl_3$, 300 MHz: 8.51-8.39 (m, 2H), 7.74 (tt, *J* = 6.2, 12.4 Hz, 2H), 7.50 (d, *J* = 8.1 Hz, 2H), 7.18 (ddd, *J* = 0.9, 4.9, 7.4 Hz, 2H), 2.31 (dd, *J* = 8.0, 15.3 Hz, 2H), 1.61 (m, 2H), 1.22 (s, 12H), 0.86 (t, *J* = 6.5 Hz, 3H). **^{13}C NMR** (δ, ppm) $CDCl_3$: 75 MHz. 173.8, 154.8, 149.1 (+), 138.1 (+), 122.4 (+), 121.9 (+), 36.4 (-), 31.9 (-), 29.5 (-), 29.4 (-), 29.3 (-), 29.3 (-), 25.2 (-), 22.7 (-), 14.2 (+). **IR** (υ, cm^{-1}): 3052, 2959, 2926, 2852, 1682, 1584, 1567, 1462, 1434, 1377, 1330, 1299, 1259, 1232, 1186, 1100, 1053. **HRMS** (DI, EI): Calc for $[C_{20}H_{28}N_3O, M+H]$ = 326.2226, found 326.2230.

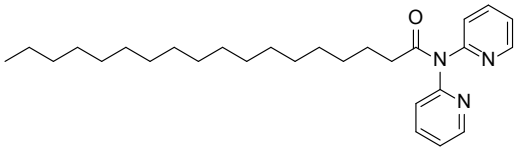
N,N-Di(pyridin-2-yl)tetradecanamide 3



To a solution containing 2,2-dipyridylamine (500 mg, 2.92 mmol) in DCM (10 mL) was added myristoyl chloride (722 µL, 2.65 mmol) under nitrogen at room temperature. To the reaction mixture was added TEA (481 µL, 3.45 mmol) and the solution was stirred at room temperature overnight. Aqueous saturated NH_4Cl and DCM were added. Aqueous phase was extracted 3 times with DCM. The combined organic phases were washed 3 times with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (AcOEt/PE 80:20) to afford compound **3** (985 mg, 97%) as a white solid.

R_f 0.7 (9:1 AcOEt/PE). **^{1}H NMR** (δ, ppm) CD_2Cl_2, 400 MHz: 8.43-8.38 (m, 2H), 7.77 (ddd, 2H, *J* = 2.0, 7.5, 8.1 Hz), 7.46 (d, 2H, *J* = 8.1 Hz), 7.19 (ddd, 2H, *J* = 0.9, 4.9, 7.4 Hz), 2.29 (t, 2H, *J* = 8.0 Hz), 1.63 (dd, *J* = 7.2, 14.5 Hz, 2H), 1.28 (m, 10H), 0.88 (t, 3H, *J* = 6.8 Hz). **^{13}C NMR** (δ, ppm) CD_2Cl_2: 101 MHz. 174.3, 155.4, 149.3 (+), 138.4 (+), 122.7 (+), 122.3 (+), 36.8 (-), 32.5 (-), 30.2 (-), 30.2 (-), 30.0 (-), 29.9 (-), 29.7 (-), 25.7 (-), 23.3 (-), 14.4 (+). **IR** (υ, cm^{-1}): 3052, 3003, 2918, 2849, 1698, 1583, 1567, 1464, 1436, 1409, 1378, 1363, 1330, 1313, 1302, 1286, 1236, 1200, 1179, 1148. **HRMS (**DI, EI): Calc for $[C_{24}H_{36}N_3O]$ = 382.2853, found 382.2861.

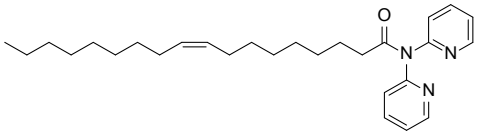
N,N-Di(pyridin-2-yl)stearamide 5



To a solution containing 2,2-dipyridylamine (500 mg, 2.92 mmol) in DCM (10 mL) was added stearoyl chloride (897 µL, 2.65 mmol) under nitrogen at room temperature. To the reaction mixture was added TEA (481 µL, 3.45 mmol) and the solution was stirred at room temperature overnight. Aqueous saturated NH_4Cl and DCM were added. Aqueous phase was extracted 3 times with DCM. The combined organic phases were washed 3 times with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (AcOEt/PE 50:50 to 70:30) to afford compound **5** (1.12 g, 97%) as a white solid.

R_f 0.7 (9:1 AcOEt/PE). **^{1}H NMR** (δ, ppm) CD_2Cl_2, 400 MHz: 8.44-8.37 (m, 2H), 7.78 (td, 2H, *J* = 2.0, 8.1 Hz), 7.46 (d, 2H, *J* = 8.1 Hz), 7.20 (ddd, 2H, *J* = 1.0, 4.9, 7.4 Hz), 2.29 (t, 2H, *J* = 7.5 Hz), 1.62 (m, 2H), 1.32-1.19 (m, 28H), 0.88 (t, 3H, *J* = 6.8 Hz). **^{13}C NMR (**δ, ppm) CD_2Cl_2: 101 MHz. 174.3, 155.4, 149.3 (+), 138.5 (+), 122.7 (+), 122.3 (+), 36.8 (-), 32.5 (-), 30.3 (-), 30.2 (-), 30.0 (-), 29.9 (-), 29.7 (-), 25.7 (-), 23.3 (-), 14.5 (+). **IR** (υ, cm^{-1}): 3052, 2917, 2849, 1699, 1584, 1568, 1470, 1464, 1436, 1378, 1349, 1315, 1306, 1287, 1257, 1248, 1237, 1178, 1149, 1100. **HRMS** (DI, EI): Calc for $[C_{28}H_{44}N_3O]$ = 438.3479, found 438.3484.

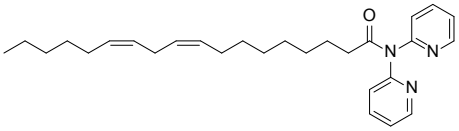
N,N-di(pyridin-2-yl)oleamide 6



To a solution of oleic acid (927 µL, 2.92 mmol) in toluene (10 mL) was slowly added oxalyl chloride (491 µL, 5.84 mmol). The resulting reaction mixture was stirred for 2 h at room temperature followed by evaporation to dryness. The residual uncoloured oil was dissolved in DCM (10 mL) under nitrogen and 2,2-dipyridylamine (500 mg, 2.92 mmol) in DCM (2mL) and TEA (529 µL, 3.8 mmol) were added. The reaction mixture was stirred overnight at room temperature. Saturated aqueous NH_4Cl was added. Aqueous phase was extracted 3 times with DCM. The combined organic phases were washed 3 times with brine, NaOH 0.1M and HCl 0.1M, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (AcOEt/PE 50:50 to 100:0) to afford compound **6** (1.05 g, 83%) as a white solid.

^{1}H NMR (δ, ppm) CD_2Cl_2, 400 MHz: 8.38 (ddd, *J* = 0.8, 2.0, 4.9 Hz, 2H), 7.73 (ddd, *J* = 2.0, 7.4, 8.1Hz, 2H), 7.44 (d, *J* = 8.1 Hz, 2H), 7.16 (ddd, *J* = 1.0, 4.9, 7.4 Hz, 2H), 5.32 (m, 2H), 2.29 (t, *J* = 7.3 Hz, 2H), 1.98 (m, 4H), 1.62 (m, 2H), 1.25 (m, 20H), 0.86 (t, *J* = 6.7 Hz, 3H). **^{13}C NMR** (δ, ppm) CD_2Cl_2: 101 MHz. 174.2, 155.5, 149.3 (-), 138.4 (-), 130.4 (-), 130.3 (-), 122.6 (-), 122.3 (-), 122.6 (-), 122.2 (-), 36.8 (+), 32.5 (+), 30.4 (+), 30.3 (+), 30.2 (+), 29.9 (+), 29.8 (+), 29.7 (+), 27.7 (+), 25.7 (+), 23.3 (+), 14.5 (-). **IR** (υ, cm^{-1}): 3052, 3003, 2920, 2851, 1696, 1585, 1567, 1464, 1436, 1376, 1336, 1313, 1285, 1253, 1236, 1180, 1150, 1102. **HRMS** (DI, EI): Calc for $[C_{28}H_{42}N_3O]$ = 436.3322, found 436.3331.

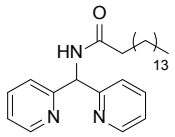
(9*Z*,12*Z*)-N,N-Di(pyridin-2-yl)octadeca-9,12-dienamide 7



To a solution of linoleic acid (393 µL, 2.92 mmol) in toluene (10 mL) was slowly added oxalyl chloride (503 µL, 5.84 mmol). The resulting reaction mixture was stirred for 2 h at room temperature followed by evaporation to dryness. The residual uncoloured oil was dissolved in DCM (12 mL) under nitrogen and 2,2-dipyridylamine (500 mg, 2.92 mmol) in DCM (2mL) and TEA (529 µL, 3.79 mmol) were added. The reaction mixture was stirred overnight at room temperature. Saturated aqueous NH_4Cl was added. Aqueous phase was extracted 3 times with DCM. The combined organic phases were washed 3 times with brine, NaOH 0.1M and HCl 0.1M, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (AcOEt/PE 50:50 to 90:10) to afford compound **7** (830 mg, 69%) as a white solid.

^{1}H NMR (δ, ppm) $CDCl_3$, 300 MHz: 8.45 (d, 2H, *J* = 3.3 Hz), 7.79 (t, 2H, *J* = 7.6 Hz), 7.53 (d, 2H, *J* = 8.0 Hz), 7.21 (dd, 2H, *J* = 5.6, 6.7 Hz), 5.45-5.25 (m, 4H), 2.75 (t, 2H, *J* = 5.7 Hz), 2.30 (t, 2H, *J* = 7.3 Hz), 2.08-1.94 (m, 4H), 1.73-1.59 (m, 2H), 1.42-1.15 (m, 14H), 0.87 (t, 3H, *J* = 6.6, Hz). **^{13}C NMR** (δ, ppm) $CDCl_3$: 75 MHz. 173.8, 154.4, 148.8 (-), 138.6 (-), 130.3 (-), 130.2 (-), 128.1 (-), 128.0 (-), 122.6 (-), 122.2 (-), 36.3 (+), 31.6 (+), 29.7 (+), 29.4 (+), 29.4 (+), 29.3 (+), 29.2 (+), 27.3 (+), 25.7 (+), 25.2 (+), 22.7 (+), 14.2 (-). **IR** (υ, cm^{-1}): 3052, 3003, 2918, 2849, 1698, 1584, 1567, 1464, 1436, 1378, 1363, 1330, 1313, 1287, 1256, 1236, 1179, 1148, 1102. **HRMS** (DI, EI): Calc for $[C_{28}H_{40}N_3O]$ = 434.3166, found 434.3176.

N-(dipyridin-2-ylmethyl)palmitamide 8



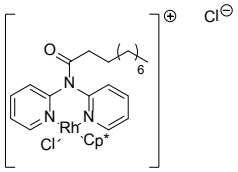
To a solution containing compound dipyridin-2-ylmethanamide (384 mg, 2.1 mmol) in DCM (10 mL) was added palmitoyl chloride (630 µL, 1.88 mmol) under nitrogen at room temperature. To the reaction mixture was added TEA (376 µL, 2.7 mmol) and the solution was stirred at room temperature overnight. Aqueous saturated NH_4Cl and DCM were added. Aqueous phase was extracted 3 times with DCM. The combined organic phases were washed 3 times with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (AcOEt/PE 90:10 to 100:0) to afford compound **10** (751 mg, 94%) as a white solid.

^{1}H NMR (δ, ppm) $CDCl_3$, 300 MHz: 8.54 (ddd, *J* = 0.8, 1.7, 4.8 Hz, 2H), 7.88 (d, *J* = 6.3 Hz, 1H), 7.63 (td, *J* = 1.8, 7.7 Hz, 2H), 7.42 (d, *J* = 7.9 Hz, 2H), 7.15 (ddd, *J* = 1.1, 4.9, 7.5 Hz, 2H), 6.22 (d, *J* = 6.7 Hz, 1H), 2.54 – 2.18 (m, 2H), 1.79 – 1.57 (m, 4H), 1.37 – 1.15 (m, 22H), 0.87 (t, *J* = 6.5 Hz, 3H). **^{13}C NMR** (δ, ppm) $CDCl_3$: 75 MHz. 159.2, 149.4 (+), 136.9 (+), 122.6 (+), 122.4 (+), 59.0 (+), 38.1 (-), 32.1 (-), 29.8 (-), 29.6 (-), 29.5 (-), 25.8 (-), 22.8 (-), 14.27 (+). **IR** (υ, cm^{-1}): 2915, 2848, 1648, 1588, 1568, 1536, 1466, 1433, 11411, 1227. **HRMS** (DI, EI): Calc for $[C_{27}H_{41}N_3O+Na]$ = 446.3142, found 446.3140.

General procedure for the synthesis of complexes

To a solution containing ligand (2.1 equiv.) in DCM (5 mL) under argon was added the dimer (1 equiv.) at room temperature. The colored reaction mixture was stirred under argon for 16 h at room temperature. DCM was evaporated *in vacuo* and Et_2O was added to the resulting colored oil. The solid residue was filtrated and washed several times with Et_2O and dried under vacuum.

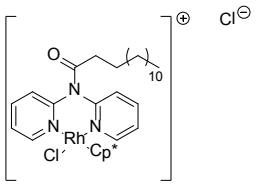
1-Rh: From **1** (0.26 mmol, 85 mg) and $[Cp^*Rh(\mu\text{-}Cl)Cl]_2$ (0.11 mmol, 67 mg). Red-orange solid (117 mg, 75 %).



^{1}H NMR (δ, ppm) DMSO, 400 MHz (mixture of Rh-Cl, Rh-H_2O and Rh-☐): 8.72 (d, 1H, J = 5.1 Hz), 8.40 (dd, 2H, *J* = 0.8, 1.9, 4.9 Hz), 8.0 (td, 1H, J = 1.2, 1.7, 7.4 Hz), 7.88 (qd, 2H, *J* = 0.6, 2.0, 7.5 Hz), 7.7 (td, 1H, J = 1.2, 5.9 Hz), 7.60 (d, 1H, J = 8.1 Hz), 7.50 (dt, 2H, *J* = 0.9, 8.1 Hz), 7.30 (qd, 2H, J = 0.9, 4.9 Hz), 2.23 (t, 2H, J = 7.3 Hz), 2.16 (t, 1H, J = 7.3), 1.62 (br s, 15H), 1.55 (br s, 7H), 1.25-1.20 (m, 20H), 0.84 (m, 5H). **^{13}C NMR** (δ, ppm) DMSO, 100 MHz, (mixture of Rh-Cl, Rh-H_2O and Rh-☐): 173.7, 155.1 (+), 152.9, 152.0 (+), 149.3 (+), 142.7 (+), 141.1 (+), 138.9 (+), 126.5 (+), 126.1 (+), 122.6 (+), 120.8 (+), 115.1 (+), 99.2, 99.1, 97.2, 97.1, 96.2, 96.1, 35.4 (-), 31.2 (-), 28.8 (-), 28.7 (-), 28.6 (-), 28.5 (-), 28.3 (-), 24.5 (-), 22.0 (-), 13.8 (+), 8.4 (+), 8.0 (+). **IR** (υ, cm^{-1}): 3406, 2924, 2854, 1699, 1637, 1599, 1468, 1378, 1250, 1186, 1028. **HRMS** (DI, EI): Calc for $[C_{30}H_{42}ClN_3ORh]$ = 598.2066, found 598.2072.

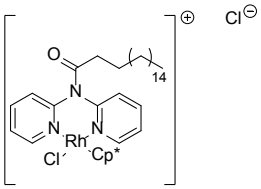
^{1}H NMR (δ, ppm) CD_2Cl_2, 400 MHz: 8.68 (d, 2H, *J* = 5.7 Hz), 8.21-8.13 (m, 4H), 7.56 (t, 2H, *J* = 5.7 Hz), 1.61 (s, 15H), 1.26 (br s, 28H), 0.87 (t, 3H, *J* = 6.7 Hz)

3-Rh: From **3** (0.26 mmol, 100 mg) and $[Cp^*Rh(\mu\text{-}Cl)Cl]_2$ (0.12 mmol, 77 mg). Red oil (108 mg, 66%).



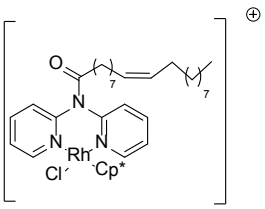
^{1}H NMR (δ, ppm) DMSO-d6, 400 MHz (mixture of Rh-Cl and Rh-H_2O): 8.74 (d, 2H, J = 6.2 Hz), 8.39 (ddd, 2H, *J* = 0.7, 1.2, 3.7 Hz), 8.2 (td, 2H, J = 1.6, 7.9 Hz), 8.0 (m, 2H), 7.88 (td, 2H, *J* = 2.0, 7.6 Hz), 7.7 (td, 2H, J = 0.9, 6.3 Hz), 7.48 (app d, 2H, *J* = 8.1 Hz), 7.30 (qd, 2H, J = 0.9, 4.9 Hz), 2.23 (t, 4H, J = 7.3 Hz), 1.62 – 1.55 (2 br s, 30H), 1.30-1.20 (m, 44H), 0.84 (m, 6H). **^{13}C NMR** (δ, ppm) DMSO-d6, 100 MHz, (mixture of Rh-Cl and Rh-H2O): 173.7, 155.1 (+), 152.9, 152.0 (+), 149.3 (+), 142.7 (+), 141.1 (+), 138.9 (+), 126.5 (+), 126.1 (+), 122.5 (+), 120.8 (+), 115.1 (+), 99.2, 99.1, 97.2, 97.1, 96.2, 96.1, 35.5 (-), 31.2 (-), 28.8 (-), 28.7 (-), 28.6 (-), 28.5 (-), 28.3 (-), 24.5 (-), 22.0 (-), 13.8 (+), 8.4 (+). **IR** (υ, cm^{-1}): 3393, 2924, 2853, 1696, 1646, 1570, 1540, 1507, 1465, 1327, 1107. **HRMS** (DI, EI): Calc for $[C_{34}H_{50}ClN_3ORh]$ = 654.2692, found 654.2701

5-Rh: From **5** (0.23 mmol, 100 mg) and $[Cp^*Rh(\mu\text{-}Cl)Cl]_2$ (0.11 mmol, 67 mg). Red oil (113 mg, 73%).



^{1}H NMR (δ, ppm) DMSO-d6, 400 MHz (mixture of Rh-Cl and Rh-H_2O): 8.71 (d, 1H, J = 4.8 Hz), 8.40 (ddd, 2H, *J* = 0.7, 1.1, 3.7 Hz), 8.3 (td, 1H, J = 1.7, 7.9 Hz), 8.1 (m, 1H), 7.87 (td, 2H, *J* = 2.0, 7.9 Hz), 7.7 (td, 1H, J = 0.7, 6.1 Hz), 7.48 (app d, 2H, *J* = 8.1 Hz), 7.29 (qd, 2H, J = 0.9, 4.9 Hz), 2.23 (t, 3H, J = 7.3 Hz), 1.62 – 1.55 (2 br s, 22H), 1.30-1.20 (m, 45H), 0.84 (m, 5H). **^{13}C NMR** (δ, ppm) DMSO-d6, 100 MHz, (mixture of Rh-Cl and Rh-H2O): 173.7, 155.1 (+), 152.9, 152.0 (+), 149.2 (+), 142.7 (+), 141.1 (+), 138.9 (+), 126.5 (+), 126.1 (+), 122.5 (+), 120.8 (+), 115.1 (+), 99.2, 99.1, 97.3, 97.2, 96.2, 96.1, 35.4 (-), 31.2 (-), 28.8 (-), 28.7 (-), 28.6 (-), 28.5 (-), 28.3 (-), 24.6 (-), 22.0 (-), 13.8 (+), 8.4 (+), 8.0 (+). **IR** (υ, cm^{-1}): 3378, 2923, 2853, 1700, 1599, 1465, 1377, 1327, 1252, 1186, 1028. **HRMS** (DI, EI): Calc for $[C_{38}H_{58}ClN_3ORh]$ = 710.3318, found 710.3331.

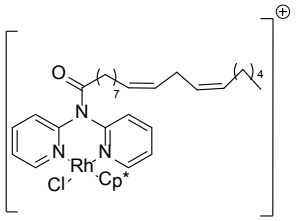
6-Rh: From **6** (0.23 mmol, 100 mg) and $[Cp^*Rh(\mu\text{-}Cl)Cl]_2$ (0.11 mmol, 67 mg). Red solid (133 mg, 83%).



^{1}H NMR (δ, ppm) DMSO-d6, 400 MHz (mixture of Rh-Cl and Rh-H_2O): 8.71 (d, 1H, J = 4.8 Hz), 8.38 (ddd, 2H, *J* = 0.7, 1.1, 3.7 Hz), 8.27 (m, 2H), 8.4 (m,2H), 7.87 (td, 2H, *J* = 2.0, 7.9 Hz), 7.71 (td, 1H, J = 0.9, 6.0 Hz), 7.48 (app d, 2H, *J* = 8.0 Hz), 7.29 (qd, 2H, J = 0.9, 4.8 Hz), 5.3 (m, 2H), 2.23 (t, 3H, J = 7.1

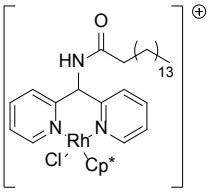
Hz), 1.96 (m, 3H), 1.62 – 1.55 (2 br s, 22H), 1.30-1.20 (m, 39H), 0.84 (m, 5H). **^{13}C NMR** (δ, ppm) DMSO-d6, 100 MHz, (mixture of Rh-Cl and Rh-H2O): 173.7, 155.1 (+), 151.3, 149.9 (+), 149.3 (+), 142.7 (+), 140.8 (+), 138.9 (+), 130.2 (+), 126.5 (+), 126.1 (+), 122.5 (+), 99.2, 99.1, 97.3, 97.2, 35.4 (-), 31.2 (-), 28.9 (-), 28.7 (-), 28.6 (-), 28.5 (-), 28.4 (-), 26.4 (-), 22.0 (-), 13.8 (+), 8.4 (+), 8.0 (+). **IR** (υ, cm^{-1}): 3382, 2925, 2854, 1700, 1636, 1559, 1377, 1327, 1250, 1189, 1028. **HRMS** (DI, EI): Calc for [$C_{38}H_{54}ClN_3ORh$] = 706.3005, found 706.3017.

7-Rh: From **7** (0.23 mmol, 100 mg) and [Cp*Rh(μ-Cl)Cl]$_2$ (0.11 mmol, 68 mg). Red oil (119 mg, 76%).



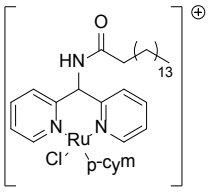
^{1}H NMR (δ, ppm) DMSO-d6, 400 MHz (mixture of Rh-Cl and Rh-H_2O): 8.73 (d, *J* = 4.9 Hz), 8.39 (d, J = 4.2 Hz), 8.28 (t, *J* = 7.5 Hz), 8.08 (br m), 7.88 (t, J = 7.7 Hz), 7.71 (t, J = 6.3 Hz), 7.49 (d, *J* = 8 Hz), 7.30 (t, J = 6.9 Hz), 5.32 (br m), 2.73 (m), 2.24 (t, J = 7.2 Hz), 1.94 (s, 3H), 2.00 (br s), 1.63 – 1.46 (3 s), 1.25 (br m), 1.43, 0.87 (br , 3H, *J* = 6). **^{13}C NMR** (δ, ppm) DMSO-d6, 100 MHz: 171.7, 154.1, 150.3, 148.3, 141.8, 138.0, 125.7, 125.3, 121.8n 98.5, 96.5, 35.2, 28.1, 24.3, 23.9, 21.7, 13.7, 8.3, 8. **IR** (υ, cm^{-1}): 3369, 3063, 2929, 2857, 1701, 1600, 1465, 1377, 1327, 1250, 1188, 1160, 1028. **HRMS** (DI, EI): Calc for [$C_{38}H_{54}ClN_3ORh$] = 706.30050, found 706.30170.

8-Rh: From **8** (0.24 mmol, 100 mg) and [Cp*Rh(μ-Cl)Cl]$_2$ (0.11 mmol, 69 mg). Orange solid (128 mg, 79%).



^{1}H NMR (δ, ppm) $CDCl_3$, 300 MHz: 11.71 (d, *J* = 9.9 Hz, 1H), 8.71 (d, *J* = 5.2 Hz, 2H), 8.61 (d, *J* = 7.7 Hz, 2H), 7.93 (t, *J* = 7.4 Hz, 2H), 7.33 (t, *J* = 6.4 Hz, 2H), 6.36 (d, *J* = 10.0 Hz, 1H), 2.88 (t, *J* = 7.6 Hz, 2H), 1.90 – 1.59 (m + s, 17H), 1.52 – 1.14 (m, 26H), 0.86 (t, *J* = 6.5 Hz, 3H). **^{13}C NMR** (δ, ppm) $CDCl_3$, 75 MHz: 176.1, 157.9, 153.4 (+), 140.8 (+), 124.8 (+), 97.2, 97.1, 59.5 (+), 36.2 (-), 32.0 (-), 29.8 (-), 29.7 (-), 29.5 (-), 25.9 (-), 22.8 (-), 14.2 (+), 9.2 (+). **IR** (υ, cm^{-1}): 3106, 2922, 2852, 1673, 1602, 1572, 1534, 1466, 1378, 1218, 1173, 1082, 1026. **HRMS** (DI, EI): Calc for [$C_{37}H_{56}ClN_3ORu$] = 696.3161, found 696.3164.

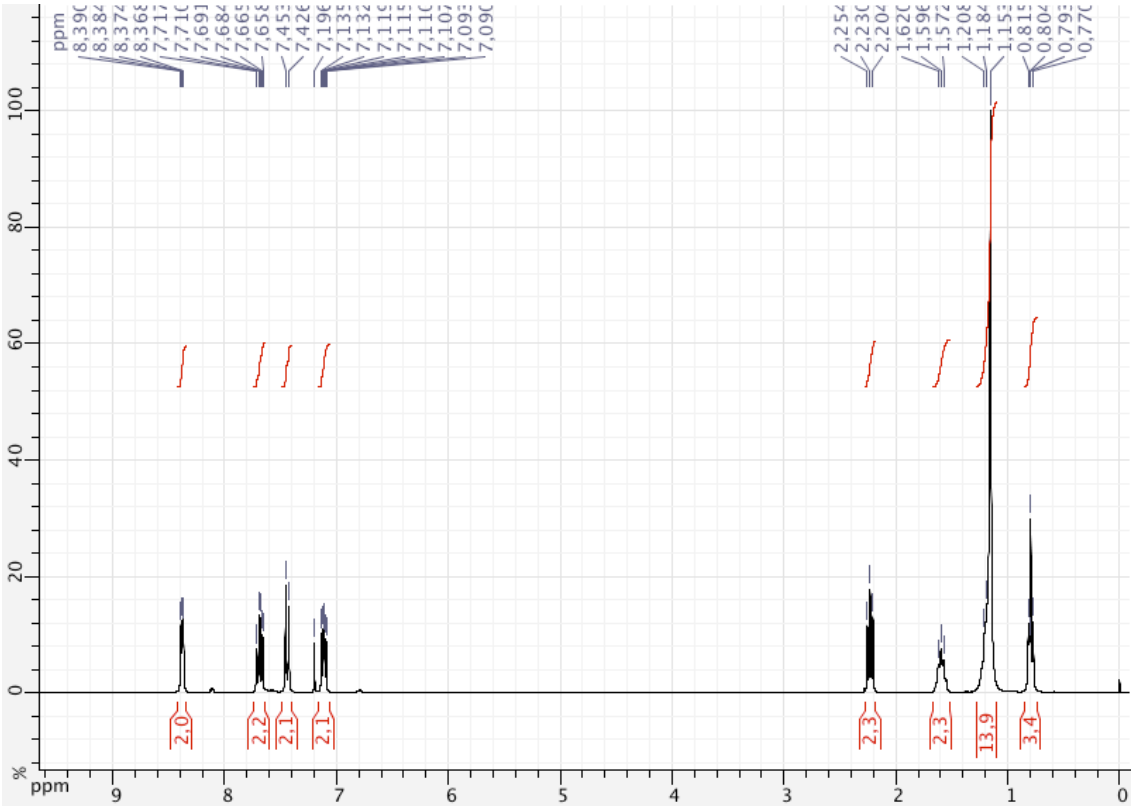
8-Ru: From **8** (0.24 mmol, 100 mg) and [(*p*-cym)Ru(μ-Cl)Cl]$_2$ (0.11 mmol, 69 mg). Red-orange solid (134 mg, 80%).

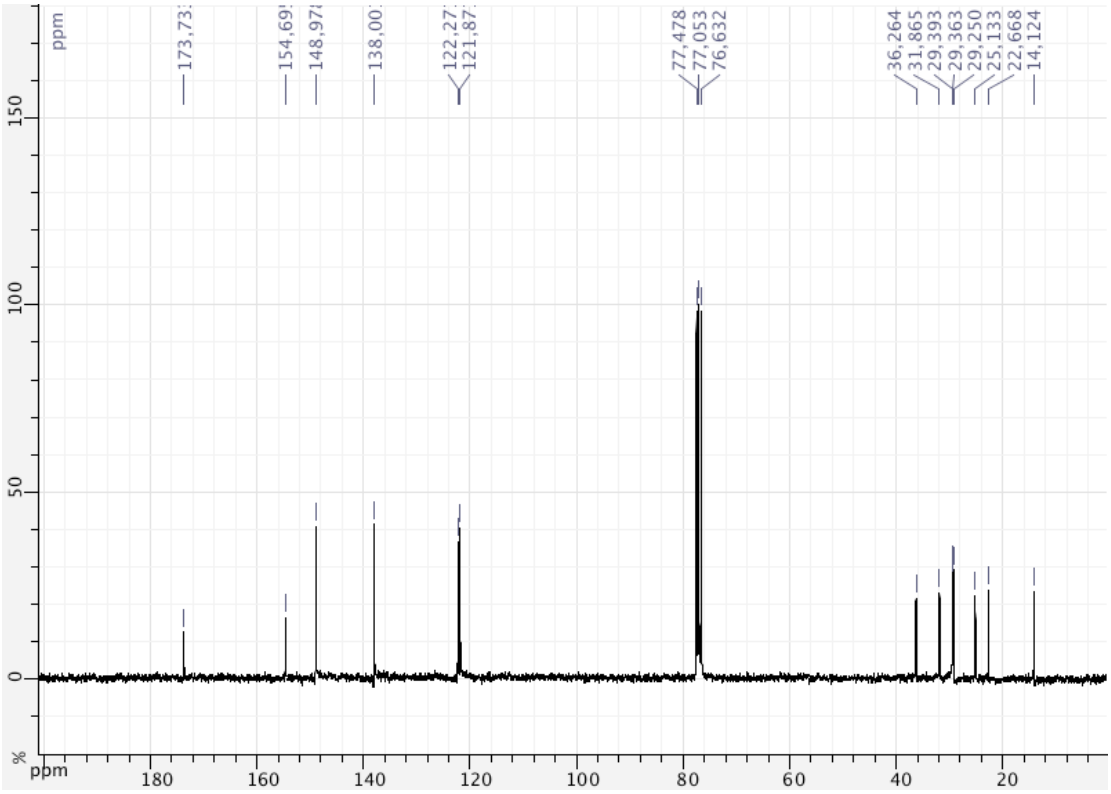


^{1}H NMR (δ, ppm) DMSO-d6, 300 MHz: 10.35 (app.s, 1H), 8.98 (d, *J* = 5.1 Hz, 2H), 8.10 (t, *J* = 7.1 Hz, 2H), 7.91 (d, *J* = 7.2 Hz, 2H), 7.58 (t, *J* = 6.2 Hz, 2H), 6.63 (d, *J* = 9.5 Hz, 1H), 6.05 (d, *J* = 5.8 Hz, 2H), 5.90 (d, *J* = 5.7 Hz, 2H), 2.95 (dt, *J* = 7.2, 14.3 Hz, 2H), 2.04 (s, 3H), 1.67 (m, 2H), 1.56 – 1.00 (m, 24H), 1.15 (d, *J* = 6.7 Hz, 6H), 0.84 (d, *J* = 6.6 Hz, 6H). **^{13}C NMR** (δ, ppm) DMSO-d6, 75 MHz: 173.4, 157.8, 157.3 (-), 140.3(-), 124.5(-), 121.7(-), 85.1(-), 84.7(-), 35.2 (+), 31.3 (+), 30.4 (-), 29.0 (+), 28.7 (+), 25.1 (+), 22.1 (+), 22.0 (-), 17.5 (-), 13.9 (-). **IR** (υ, cm^{-1}): 3112, 2923, 2853, 1671, 1605, 1571, 1529, 1468, 1438, 1378, 1220, 1164, 1114, 1086, 1057, 1032. **HRMS** (DI, EI): Calc for [$C_{37}H_{55}ClN_3ORu$] = 694.3071, found 694.3073.

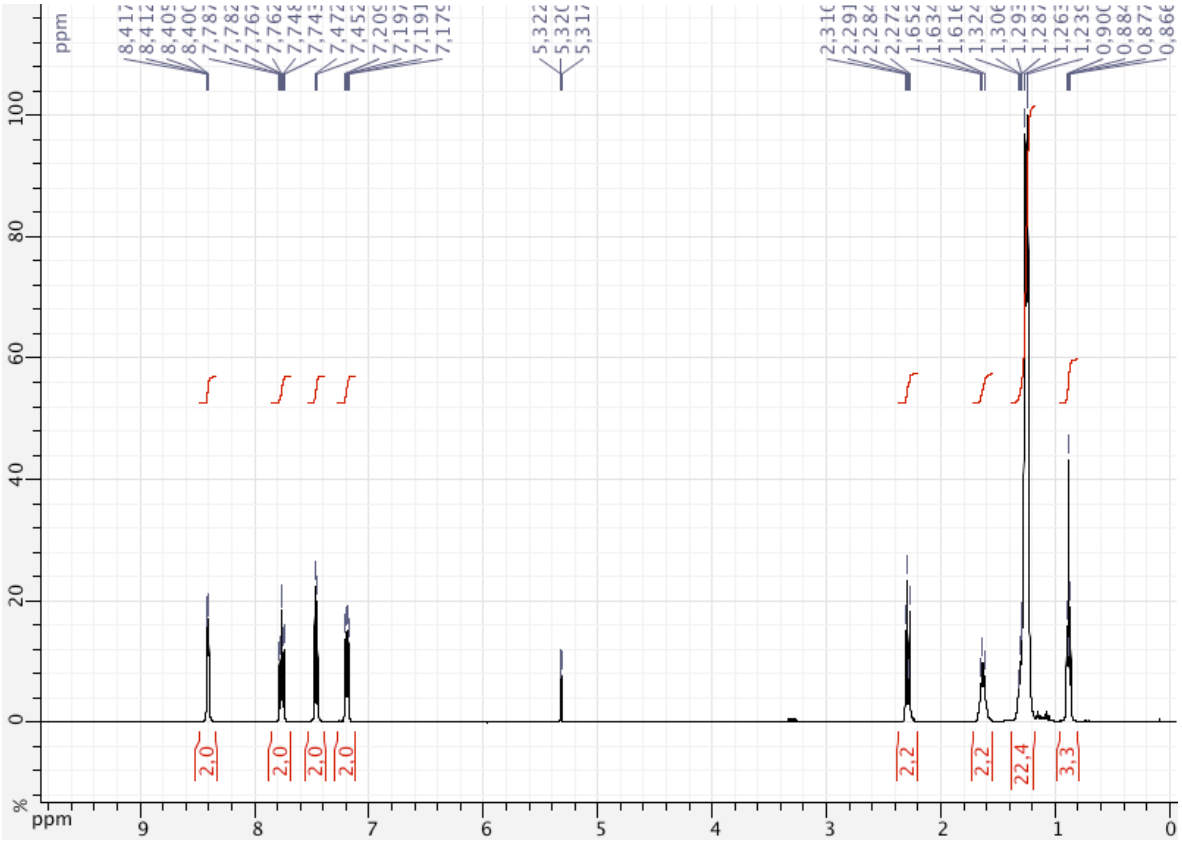
^{1}H and ^{13}C NMR spectra of ligands and complexes

compound 1





Compound 3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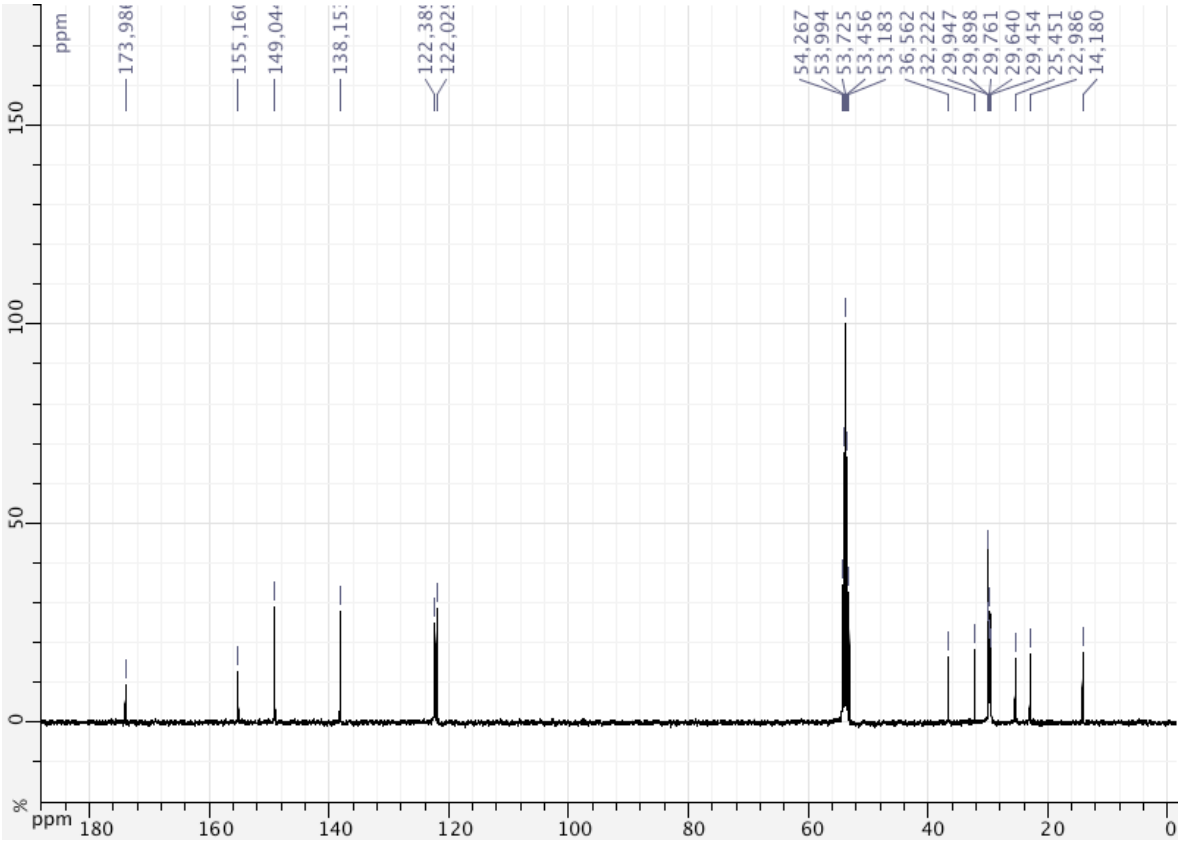
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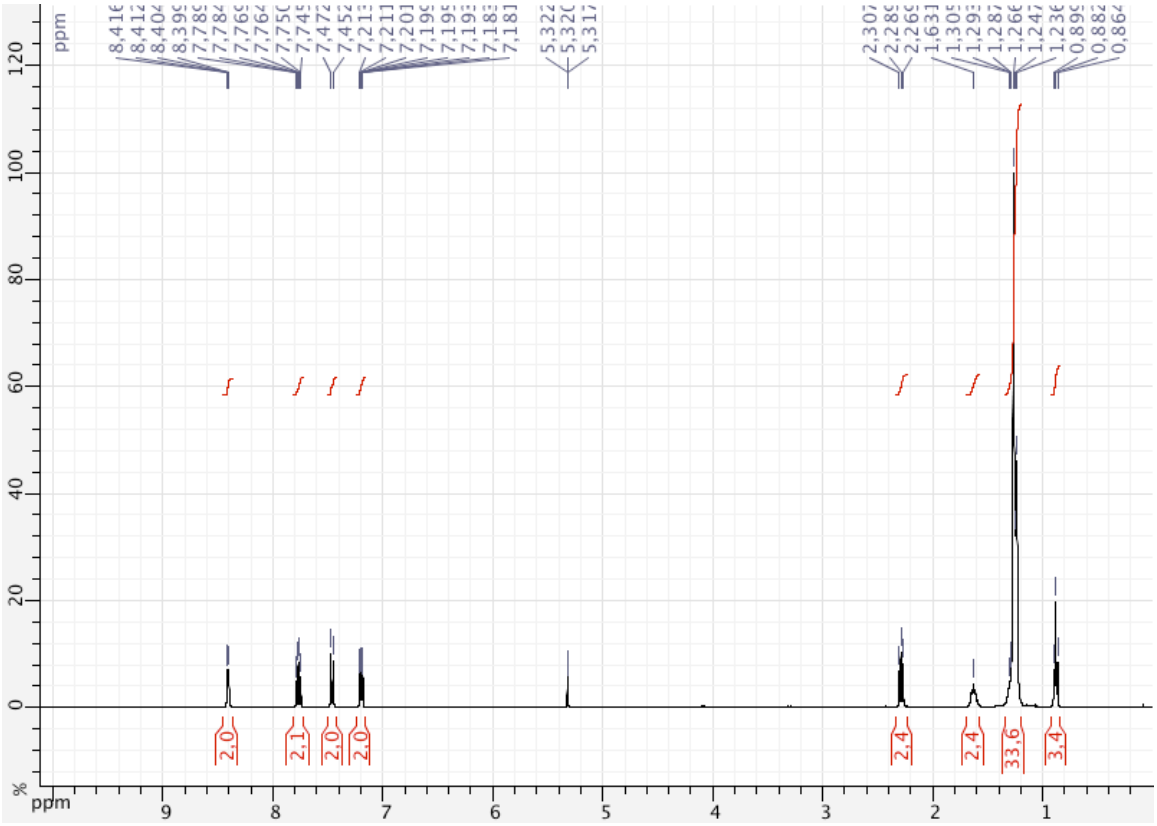
Compound 5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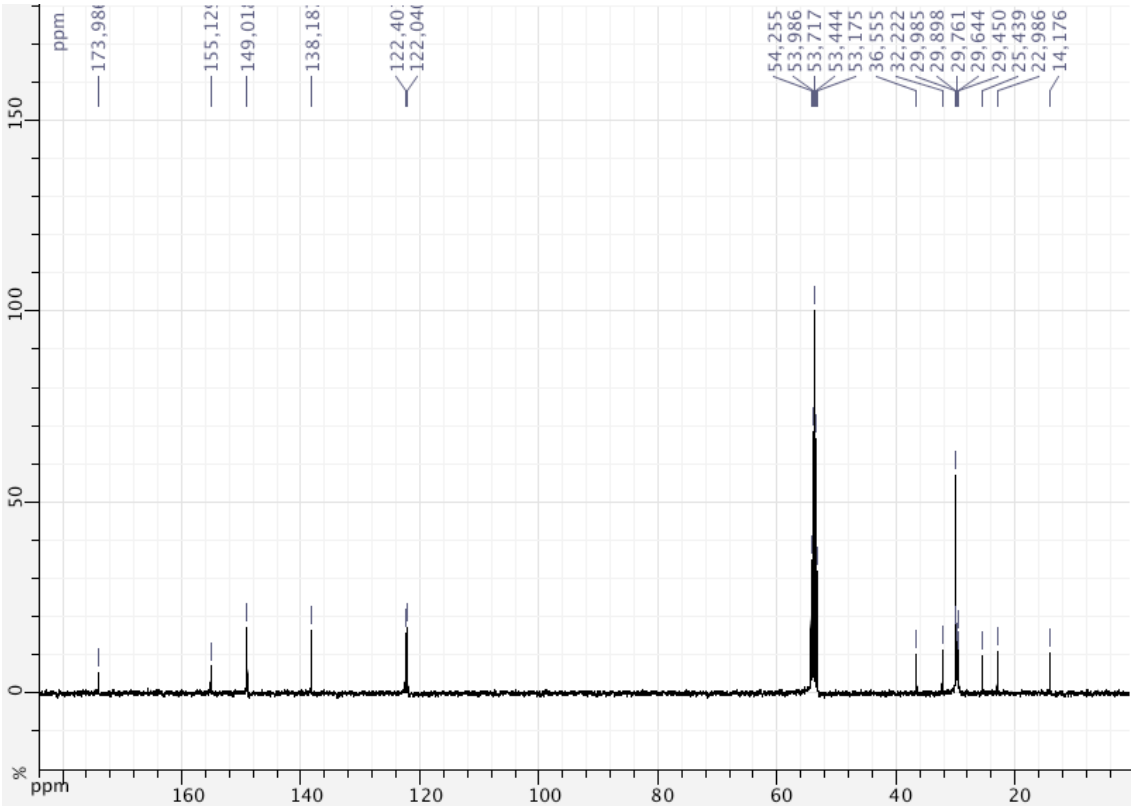
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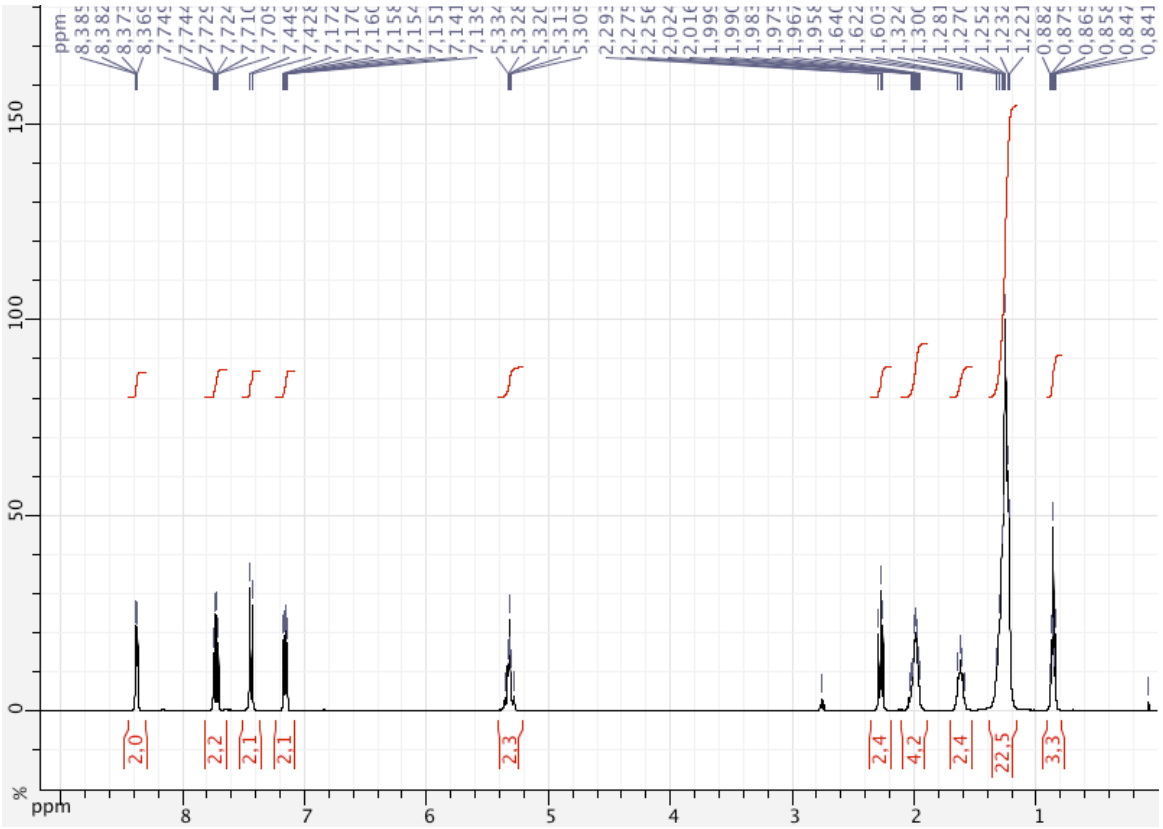
Compound 6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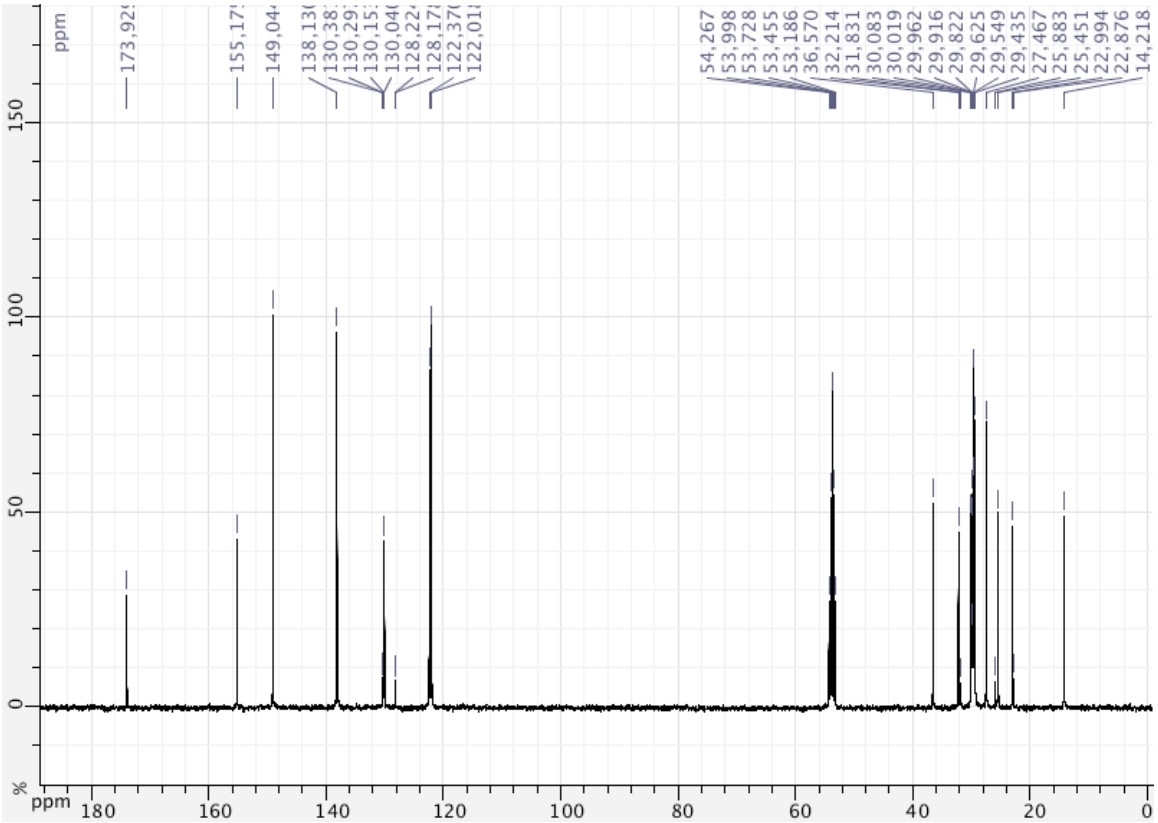
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29,962
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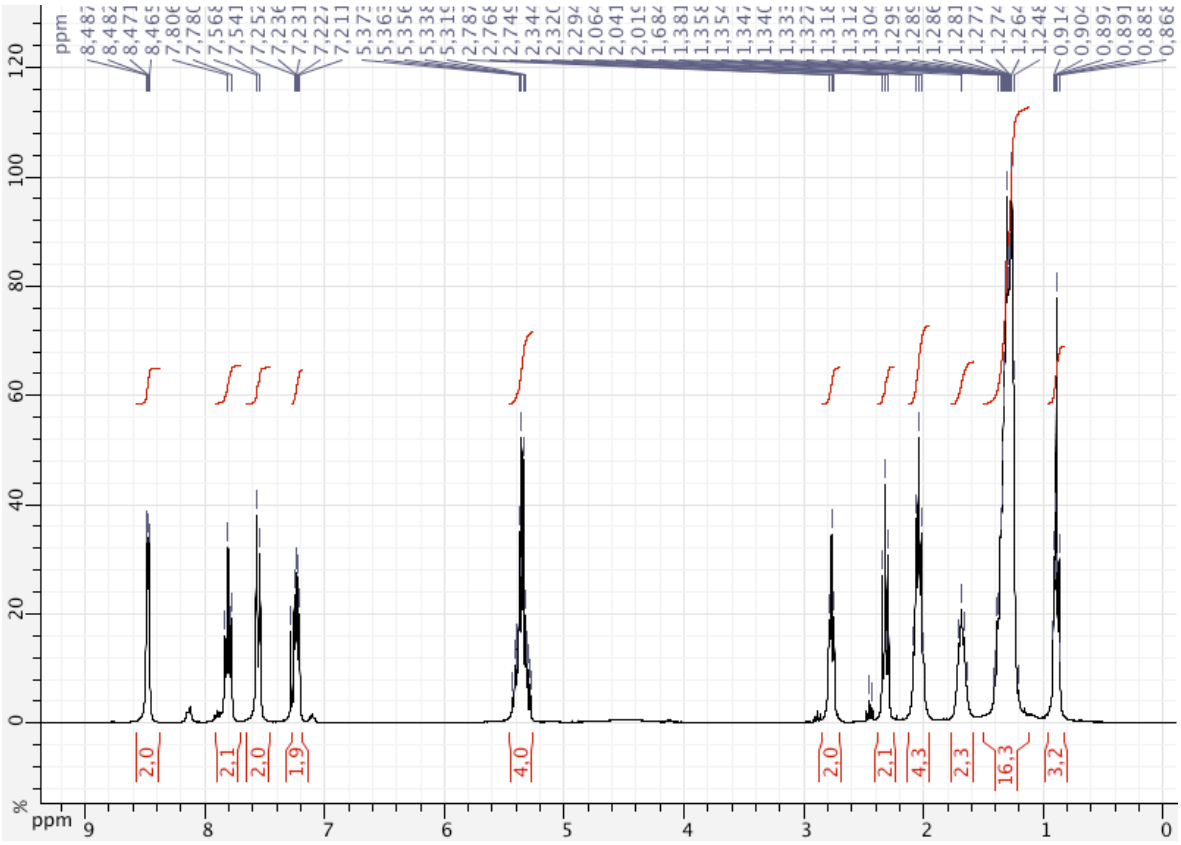
Compound 7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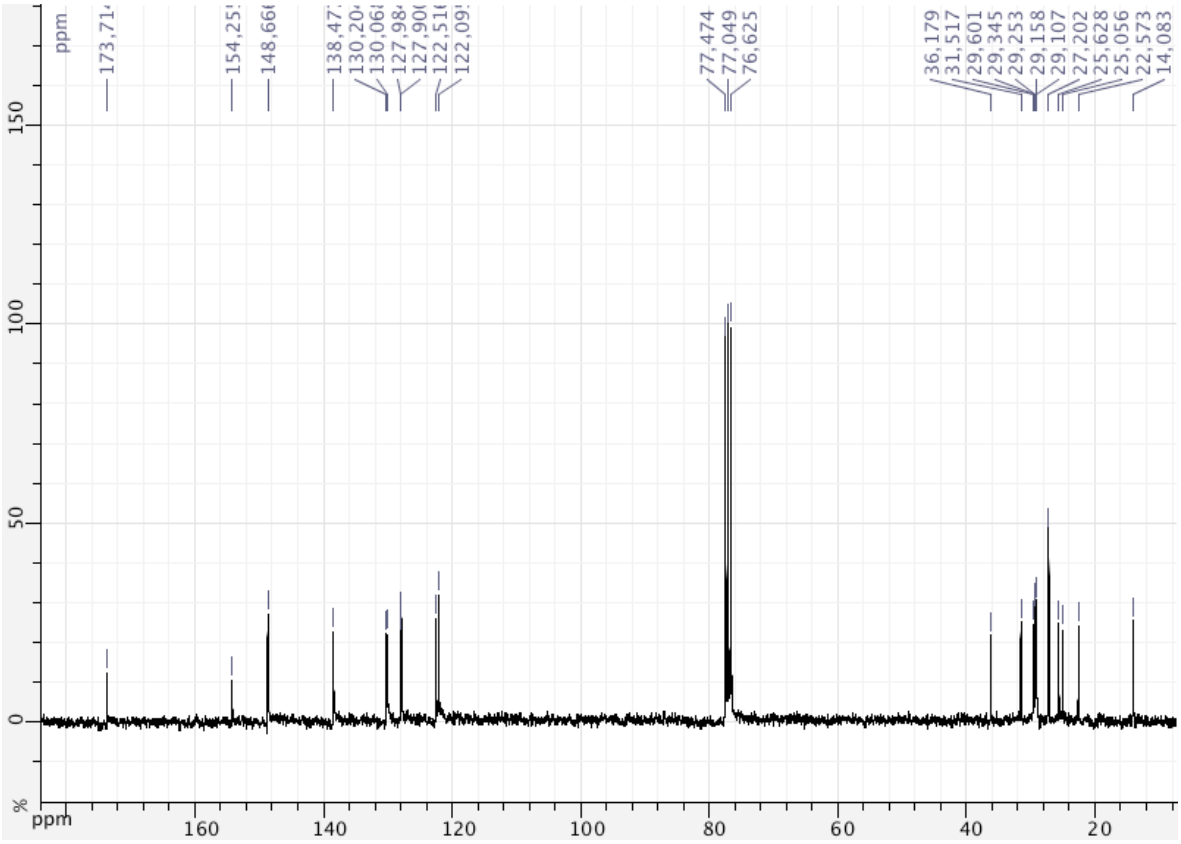
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36,179
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29,345
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29,107
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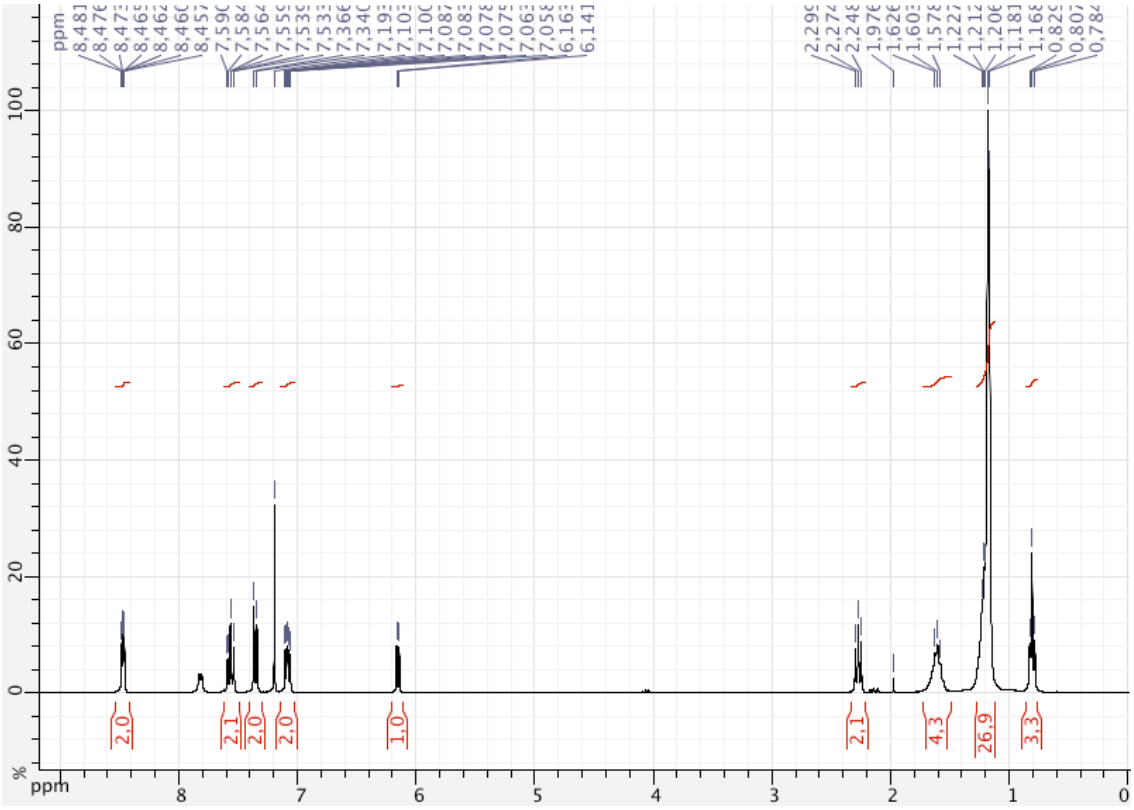
Compound 8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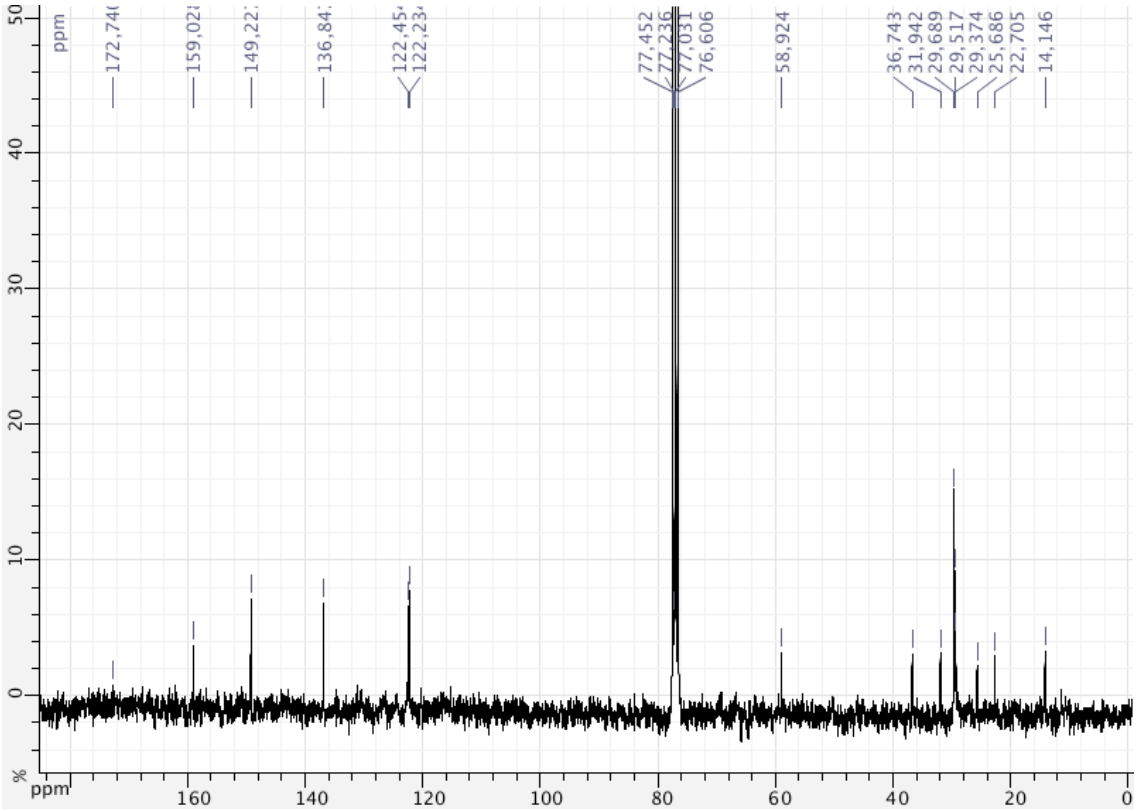
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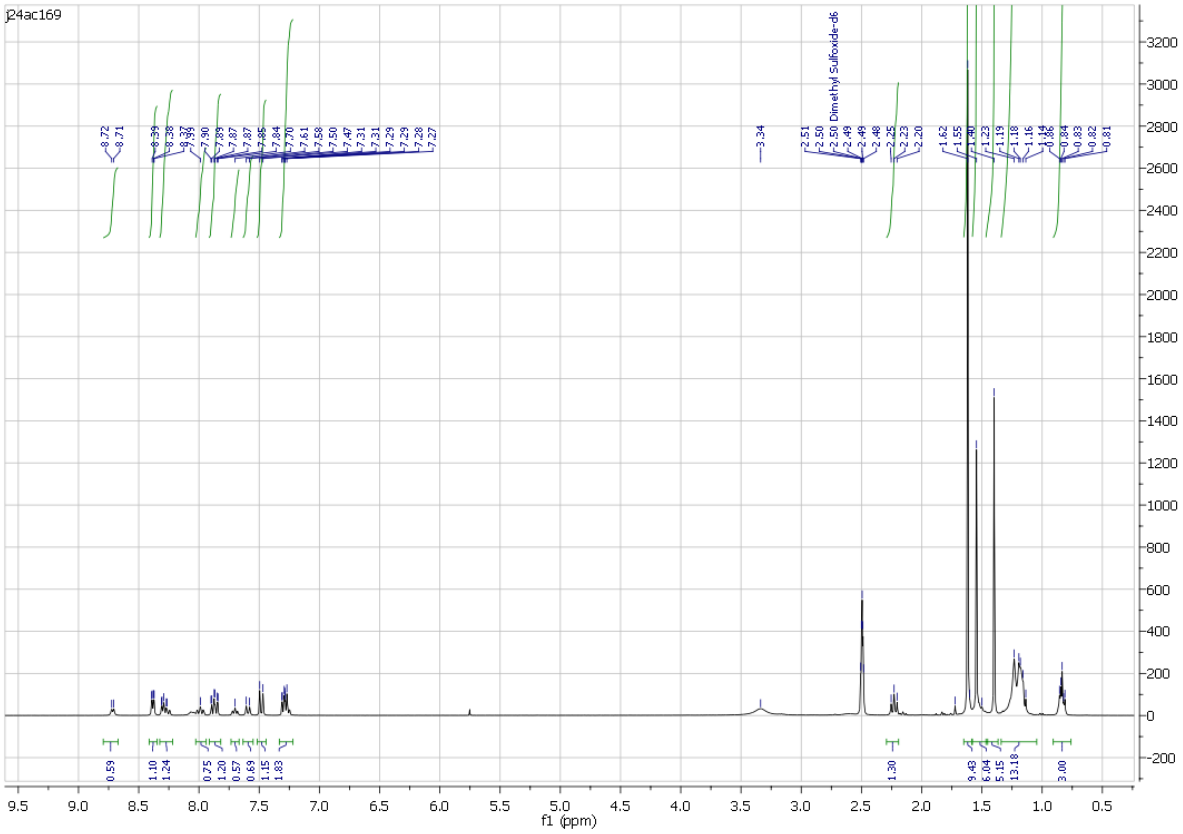
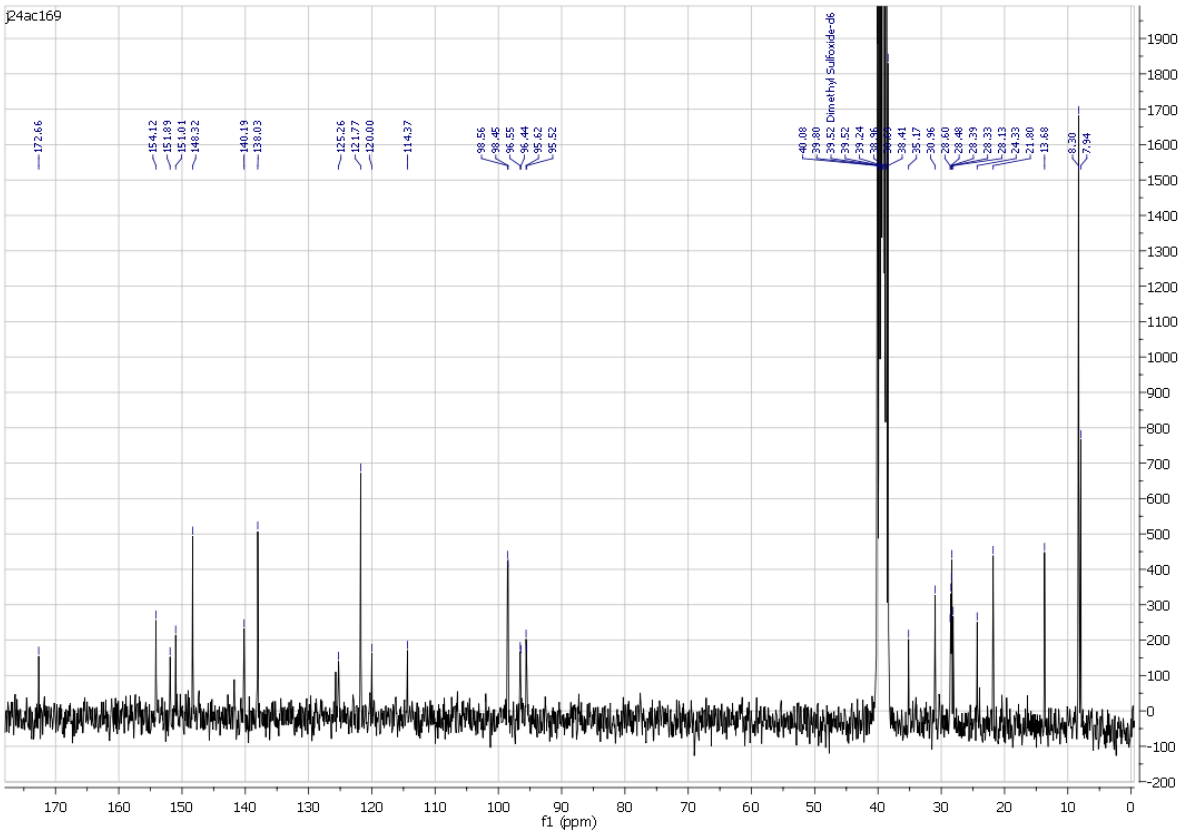
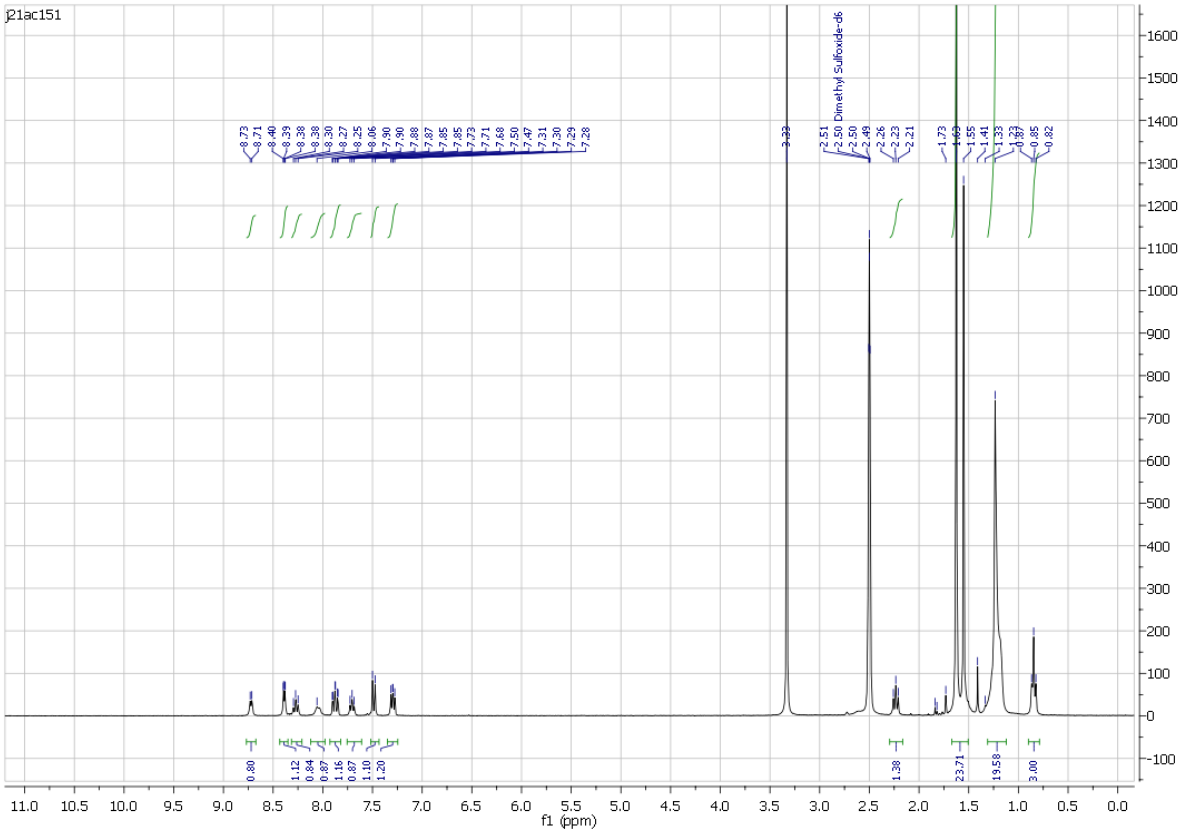
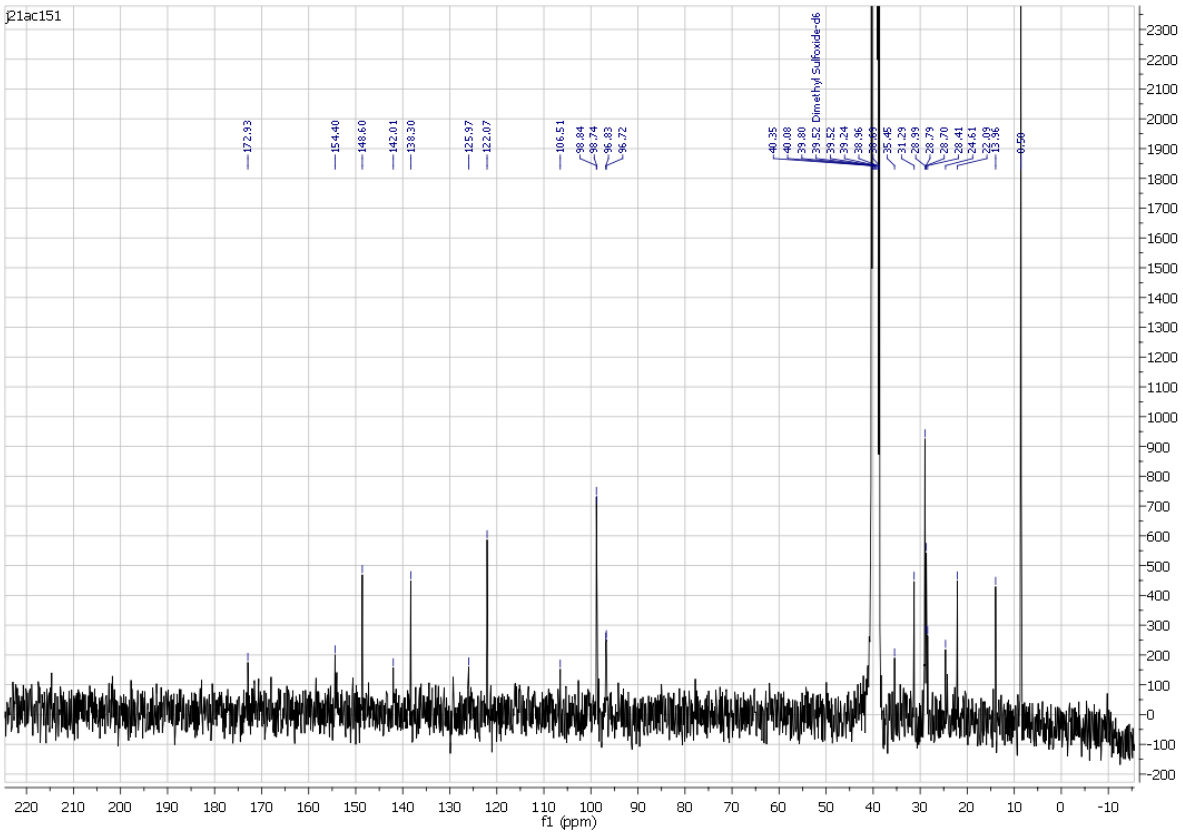
Compound 1-Rh



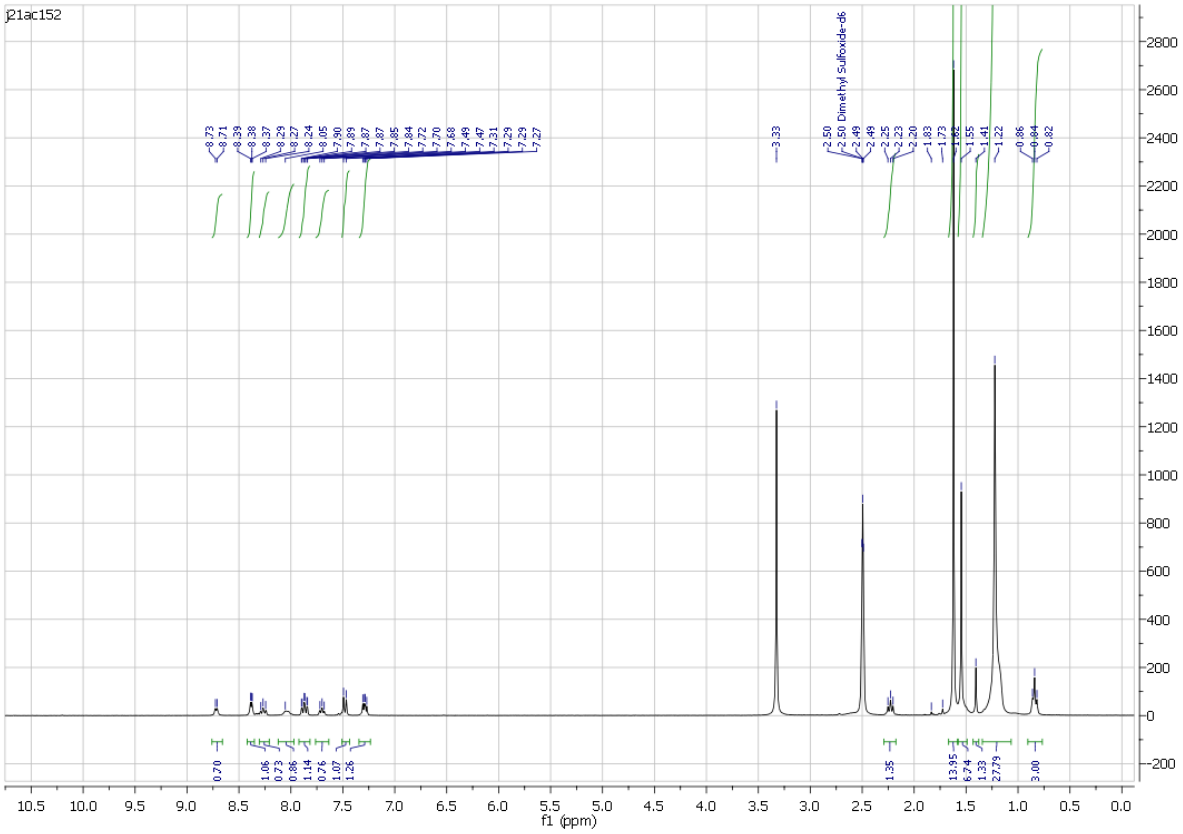


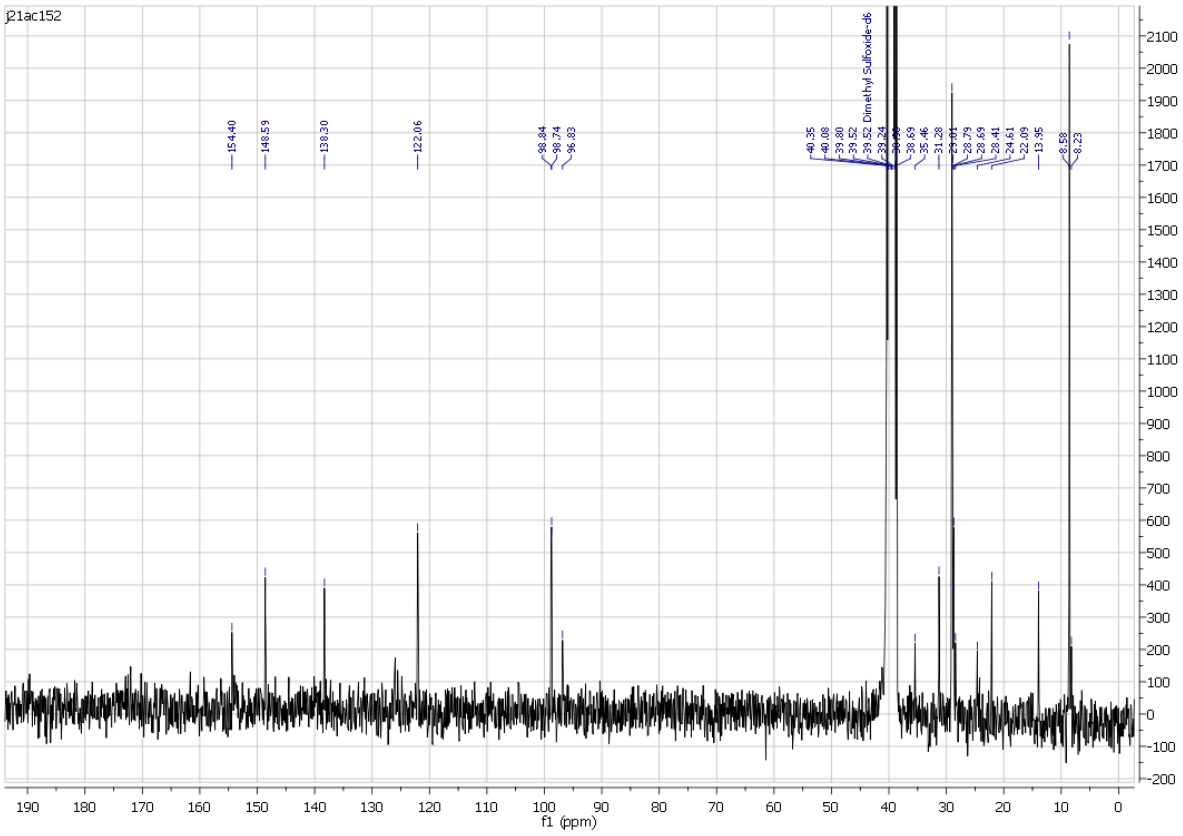
Compound 3-Rh



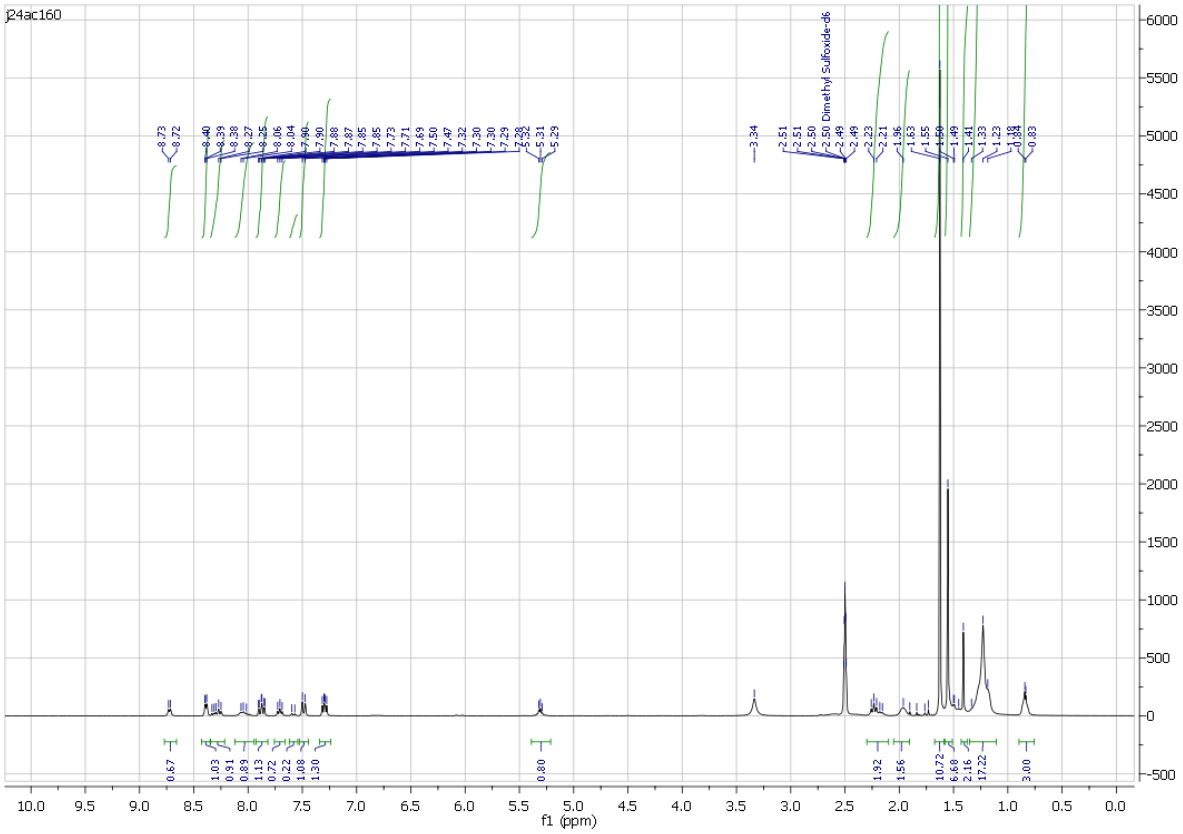


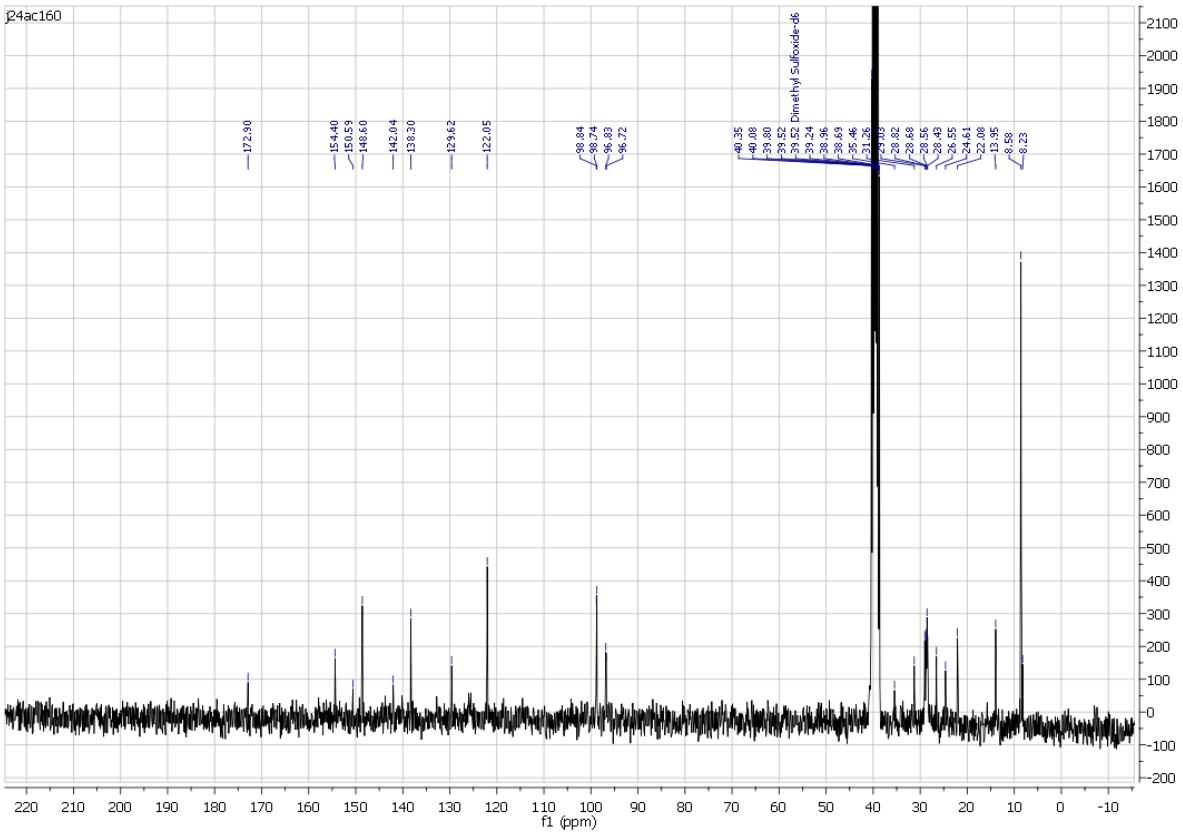
Compound 5-Rh



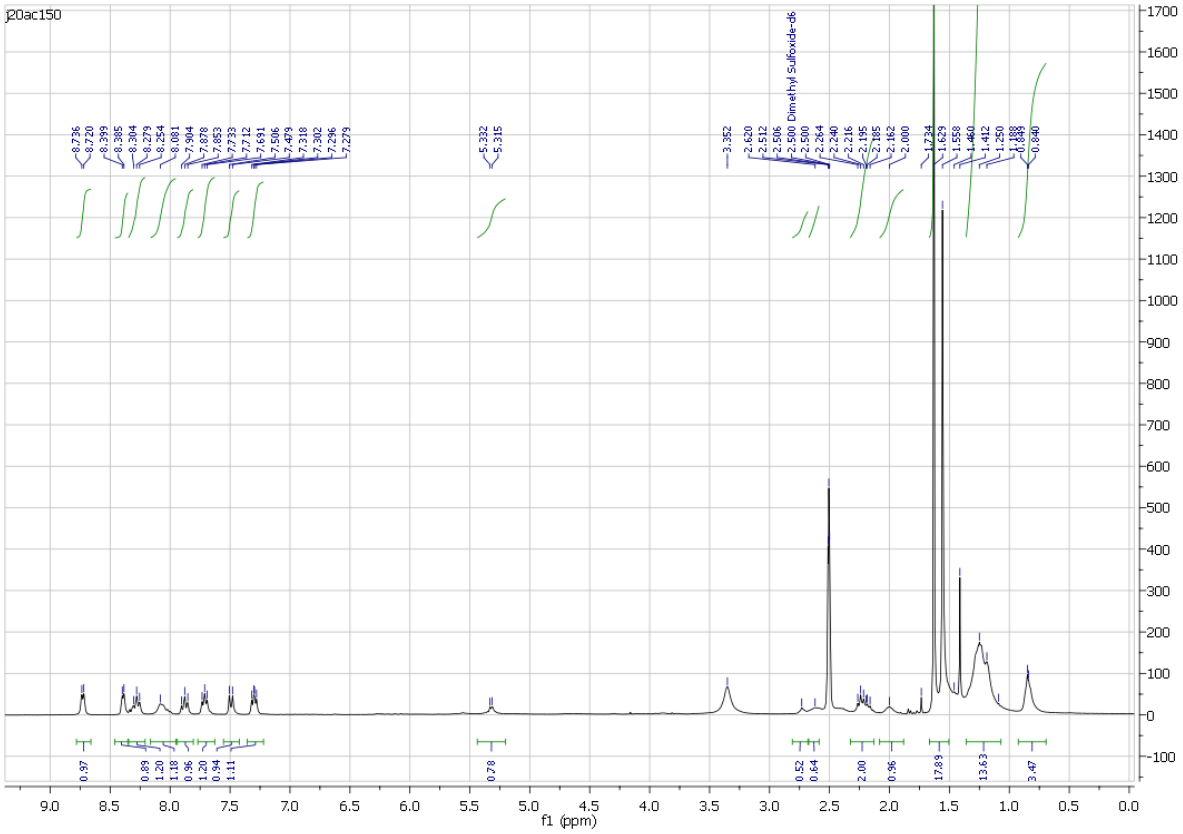


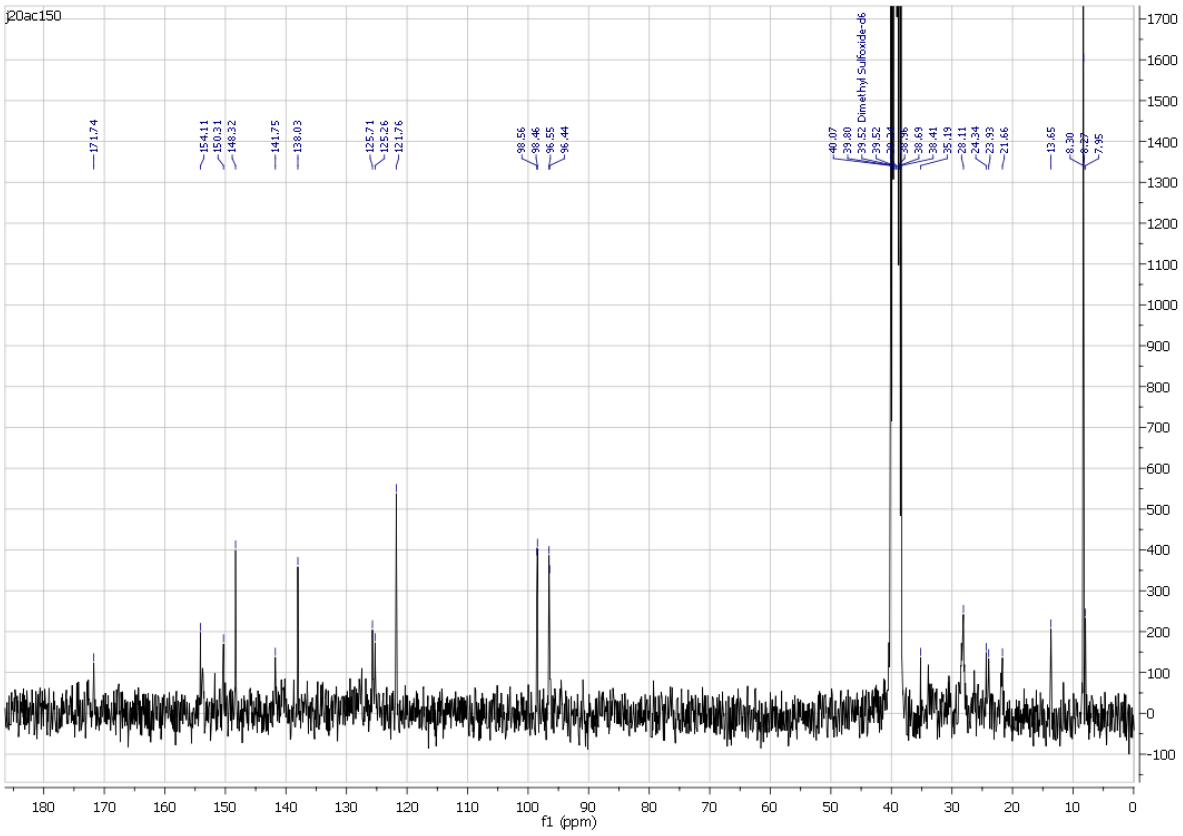
Compound 6-Rh



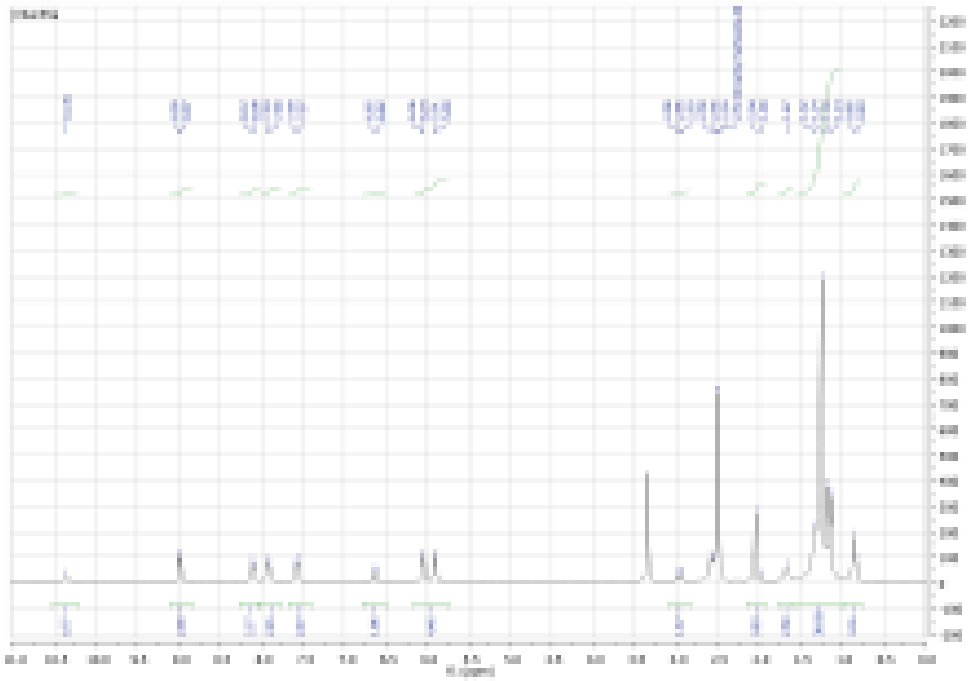


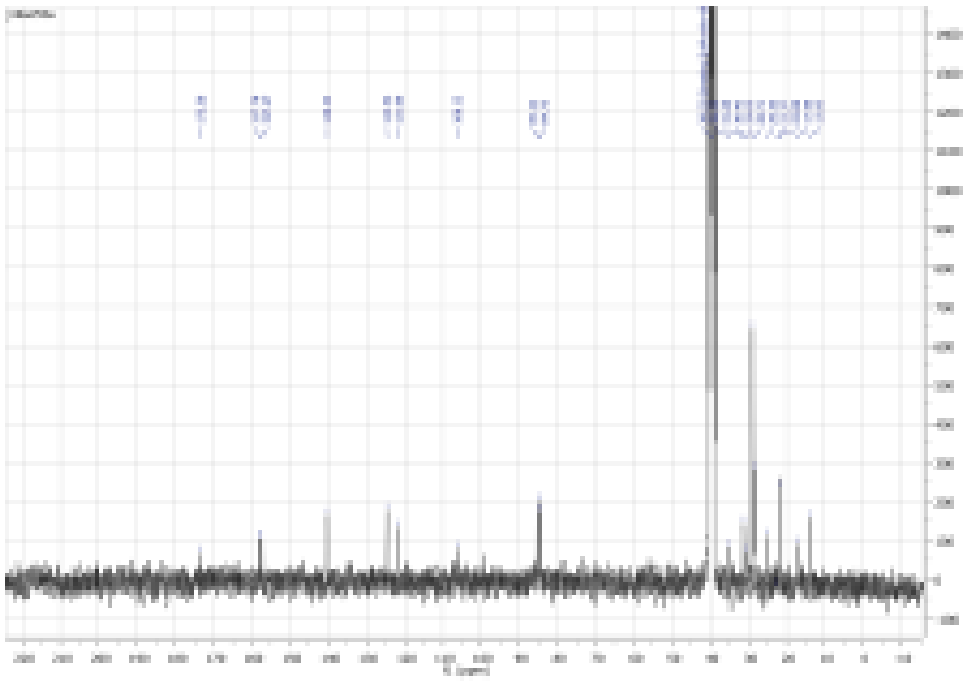
Compound 7-Rh



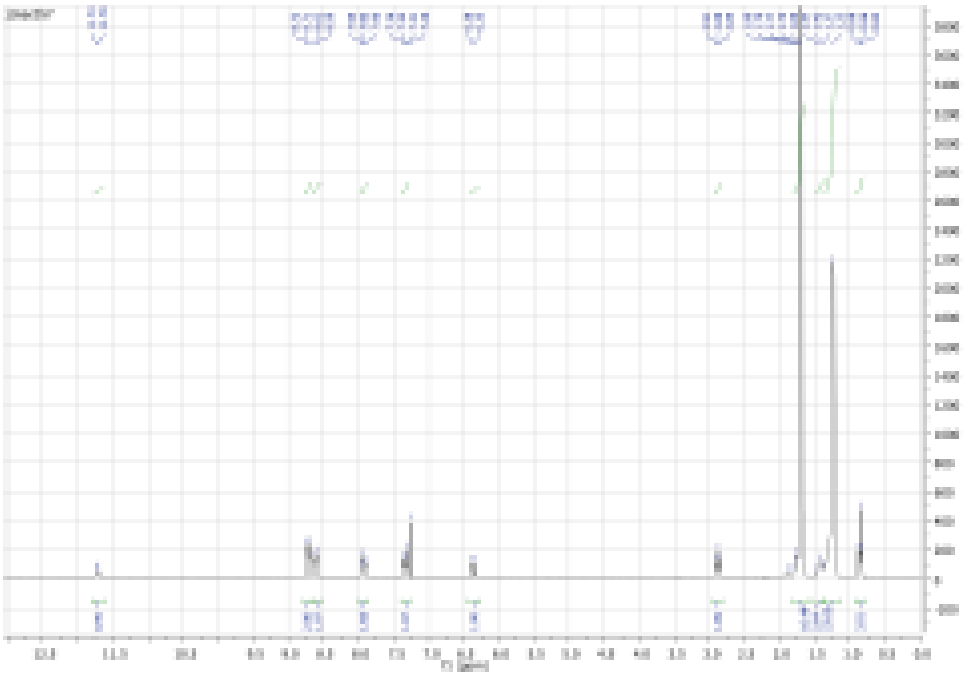


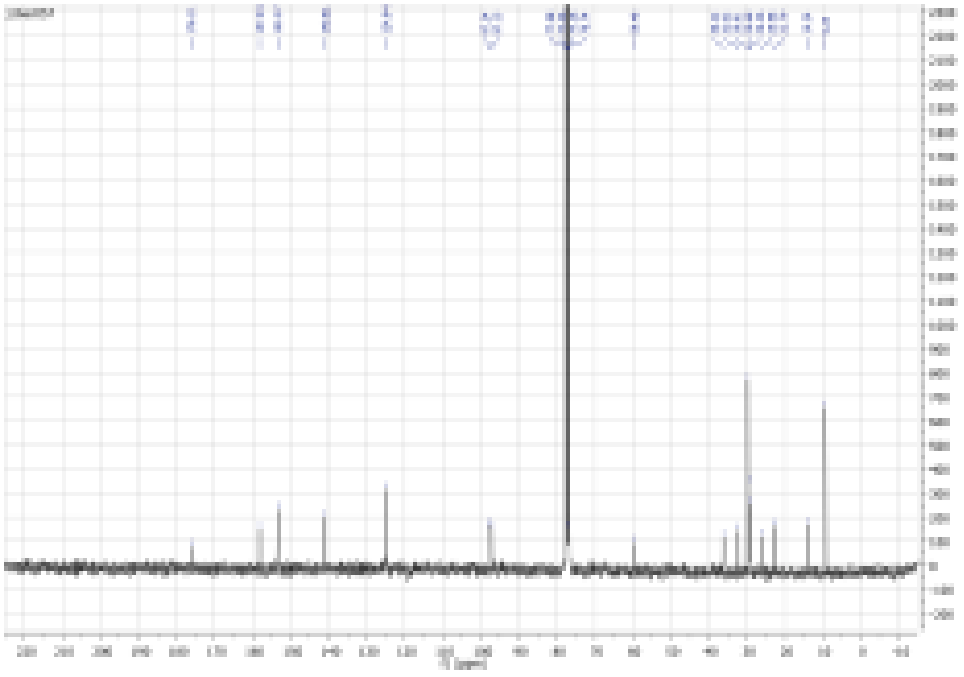
Compound 8-Ru





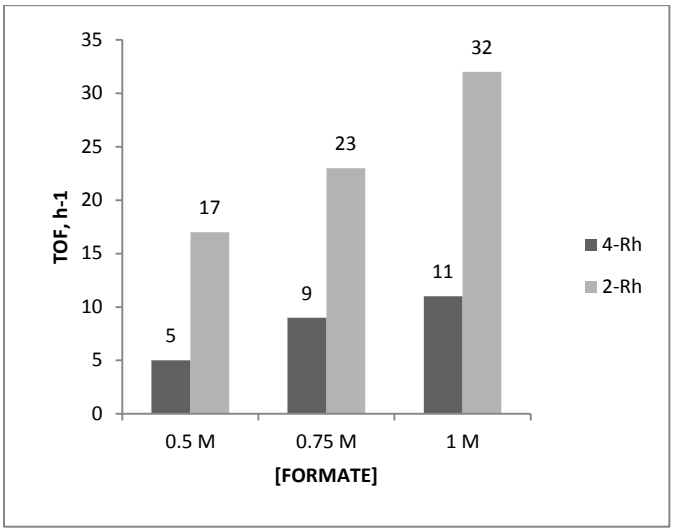
Compound 8-Rh





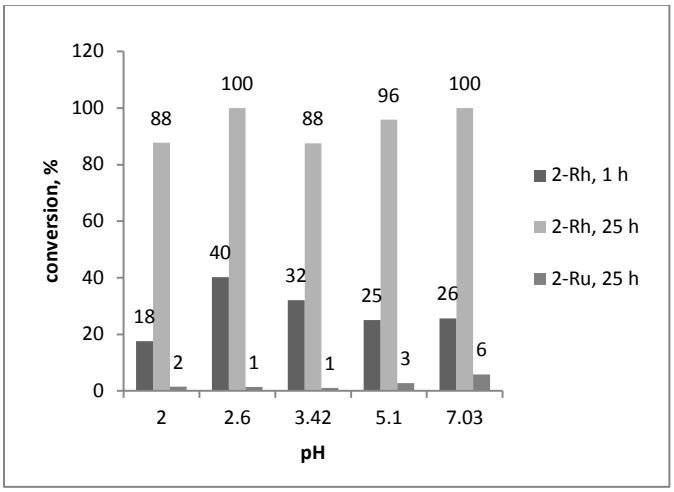
Effect of [formate] on the transfer hydrogenation of TFACP catalysed by 2-Rh or 4-Rh

Fig. S1 Turnover frequency versus [formate] for the TH of TFACP using **4-Rh** or **2-Rh**. Conditions: [S] = 10 mM, [cat] = 0.2 mM, pH 7.4, 40°C (v=1 mL).



Effect of initial pH on the transfer hydrogenation of TFACP catalysed by 2-Ru or 2-Rh

Fig. S2 Conversion versus initial solution pH for the aqueous TH of TFACP using **2-Rh** or **2-Ru**. Conditions: [S] = 5 mM, [cat] = 0.1 mM, [formate] = 1 M, 40°C (v=1 mL).



Circular dichroism measurements

Circular dichroism spectra were recorded on a J-815 UD spectrometer (JASCO) at 20°C in 1- or 0.2 cm-pathlength quartz cuvettes between 250 and 310 nm for ligands and ruthenium compounds and between 250 and 450 nm for rhodium compounds. Temperature control was provided by a Peltier thermostat. All spectra were accumulated 3 times (2 times for Rh compounds) with bandwidth of 1.0 nm and a resolution of 0.1 nm at a scan speed of 50 nm/min.

Preparation of βLG solutions: a solution of βLG (A+B) was prepared in 20 mM phosphate buffer (pH 7.5). Its concentration was determined using absorbance at 280 nm with ε = 17600 $M^{-1}cm^{-1}$.[4] Protein solutions were kept for up to 1 week at -20°C.

Preparation of ligand or complex solutions: compounds **4**, **2, 4-Rh** and palmitic acid were dissolved in absolute ethanol at a concentration of 10 mM. The other compounds were dissolved in DMSO to reach a 10 mM concentration. All these compounds are stable in solution when kept at 4°C.

CD measurements for association between ligands (or complexes) and βLG: The βLG solution (50 µM) was transferred in a 1 cm optical pathlength cuvette and small aliquots of ligand / complex solution (2 µL) were added sequentially to achieve more than 1 equiv. of ligand. Alternatively, a βLG solution (100 µM) was transferred in a 0.2 cm optical pathlength cuvette and small aliquots of ligand solutions (2 µL) were added sequentially to achieve more than 1 equiv. of ligand/complex. In each case, the solutions were homogenized after each addition for 1 min before acquisition.

CD measurements for displacement studies: A βLG solution (100 µM in phosphate buffer, pH 7.5) was transferred in a 0.2 cm optical pathlength cuvette and 0.8 equiv. of the first compound was added. The solution was homogenized for 1 min before acquisition. To this reaction mixture was then added sequentially small aliquots (2 µL) of the second compound. The reaction mixture was homogenized for 1 min before each acquisition.

Fig. S3 Competitive titration of mixture of βLG (126 µM in 20 mM phosphate pH 7.5) and **4** (0.8 mole eq.) by palmitic acid (PA) by CD spectroscopy

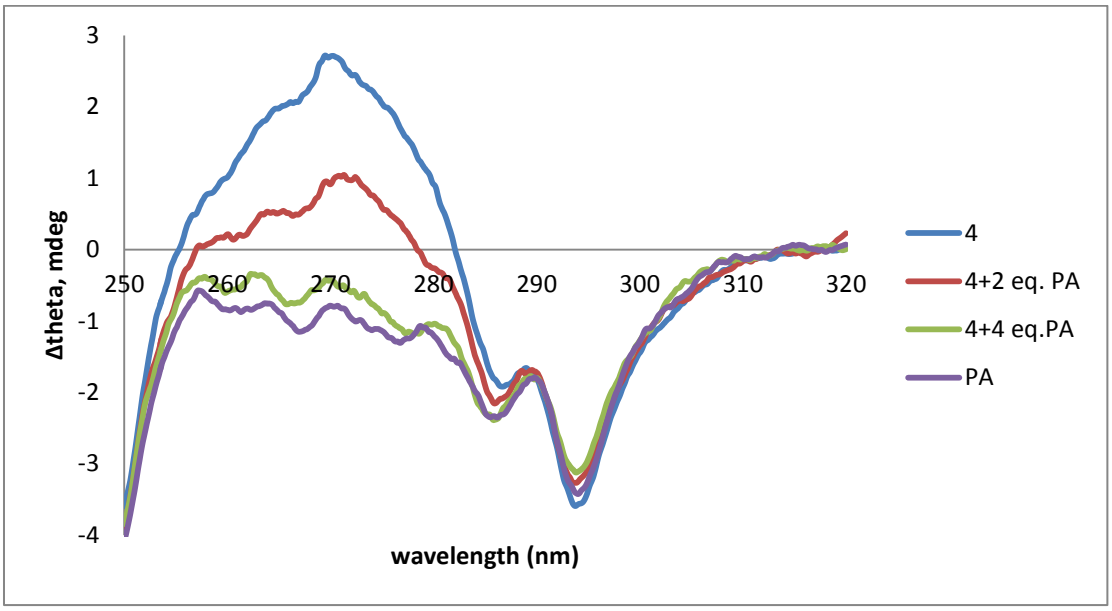


Fig S4 pH-dependence of CD spectrum of mixture of βLG (126 µM in 20 mM phosphate pH 7.5) and **4** (1 mole eq.). Progressive acidification by gradual addition of HCl.

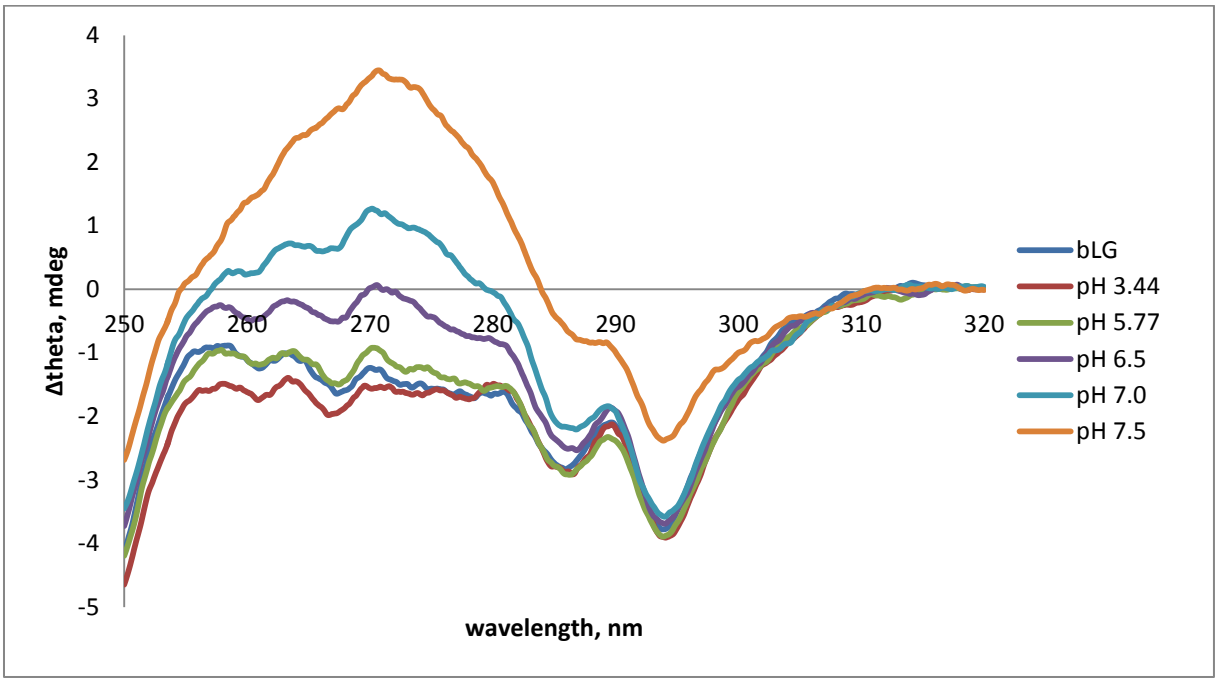
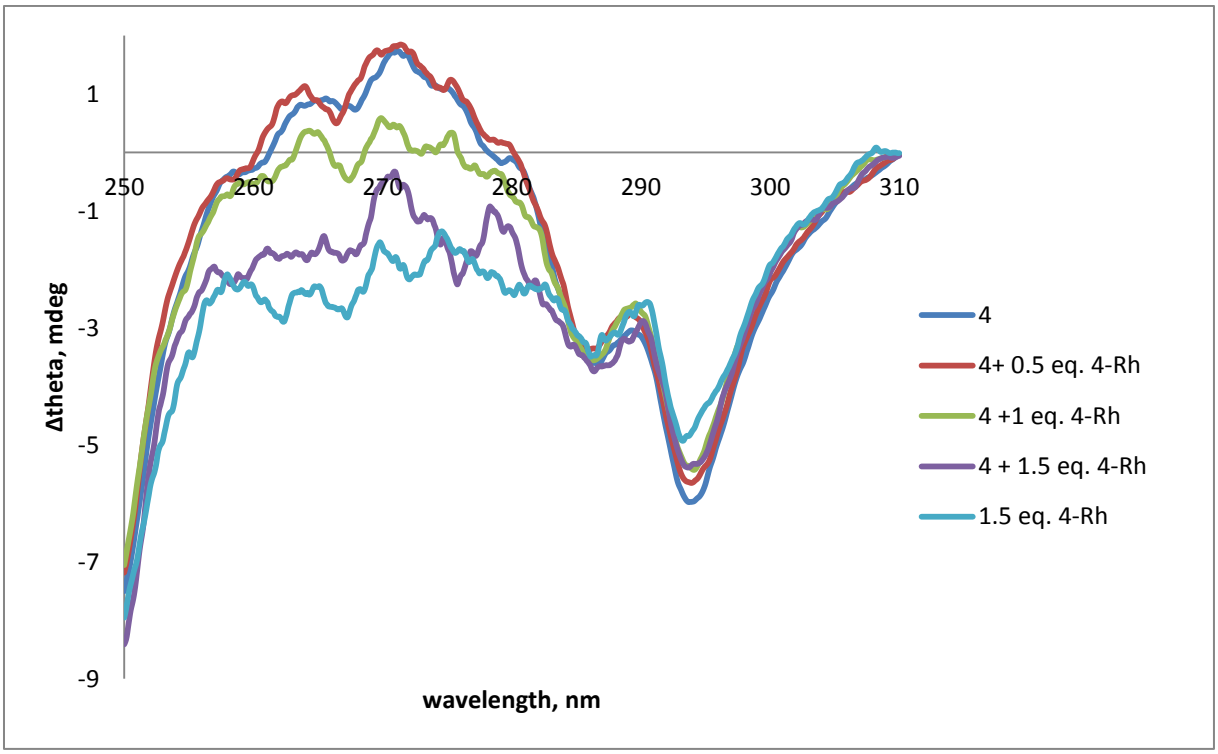


Fig. S5 Competitive titration of mixture of βLG (184 µM in 20 mM phosphate pH 7.5) and **4** (0.7 mole eq.) by **4-Ru** by CD spectroscopy



Fluorescence spectroscopy measurements

Fluorescence spectra were recorded on a F-6200 spectrofluorimeter (JASCO) at 20°C in a 1 cm-pathlength quartz cuvette between 300 and 430 nm (λ_{ex} = 290 nm) with excitation and emission bandwidth 5 nm, data pitch 1 nm, scanning rate 125 nm/min. A solution of βLG (9.5 µM in 20 mM phosphate pH 7.5) was transferred to the quartz cuvette and small aliquots of **2-Rh** or **4-Rh** (2 mM in DMSO, 2 µL) were added sequentially to achieve up to ca. 3 - 4 equiv. of complex. The blank experiment was performed with a solution of N-acetyl-L-tryptophanamide NALTA (9.5 µM) instead of βLG. The apparent dissociation constant K'_d and apparent molar ratio of complex / βLG n were determined according to ref. [5].

The equation of mass law can be expressed as eqn (1)

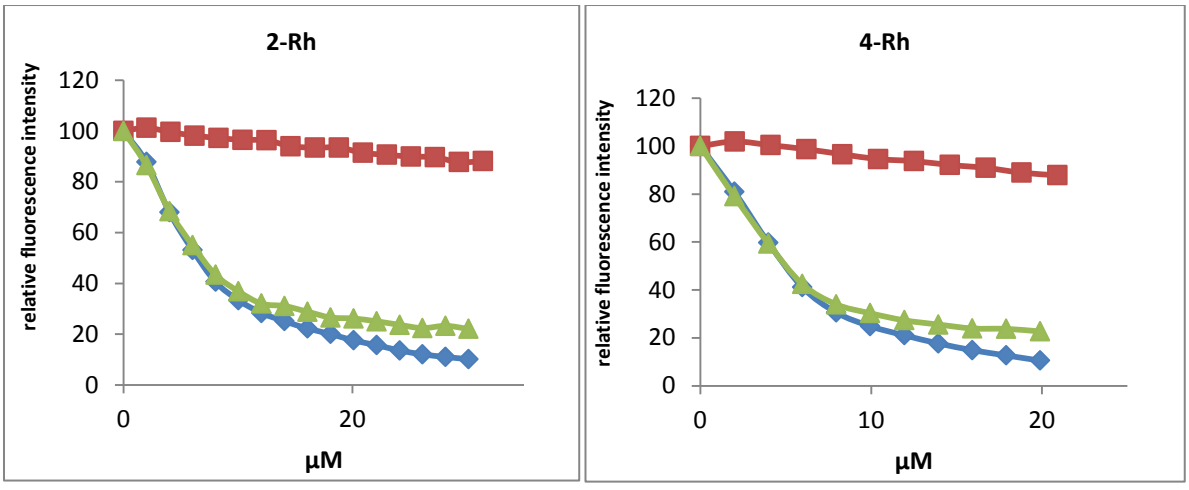
$$(1)\ P_0 \times a = \frac{1}{n} \times \frac{R_0 a}{1-a} - \frac{K'_d}{n}$$

with P_0 the total protein concentration and R_0 the total ligand concentration. The a parameter is defined as the fraction of free binding sites; it can be calculated for each data point by applying equation (2)

$$(2)\ a = \frac{F - F_{min}}{F_0 - F_{min}}$$

where F is the fluorescence intensity at a certain R_0, F_{min} the fluorescence intensity at saturation of the protein binding sites and F_0 is the initial fluorescence intensity.

Fig. S6 Titration of βLG (9.5 µM in 20 mM phosphate pH 7.5) with **2-Rh** and **4-Rh** followed by the quenching of protein fluorescence. Excitation wavelength 290 nm; emission wavelength 335 nm (βLG) or 355 nm (NALTA). Observed fluorescence intensity (υ); fluorescence intensity of NALTA (ν); corrected fluorescence intensity (σ)



By plotting P_0a vs $R_0a/(1-a)$, a straight line is obtained with an intercept of K'_d/n and a slope of 1/n.

Fig. S7 Linear least-square plot of Eqn (1) for the titration of βLG with **2-Rh** or **4-Rh**

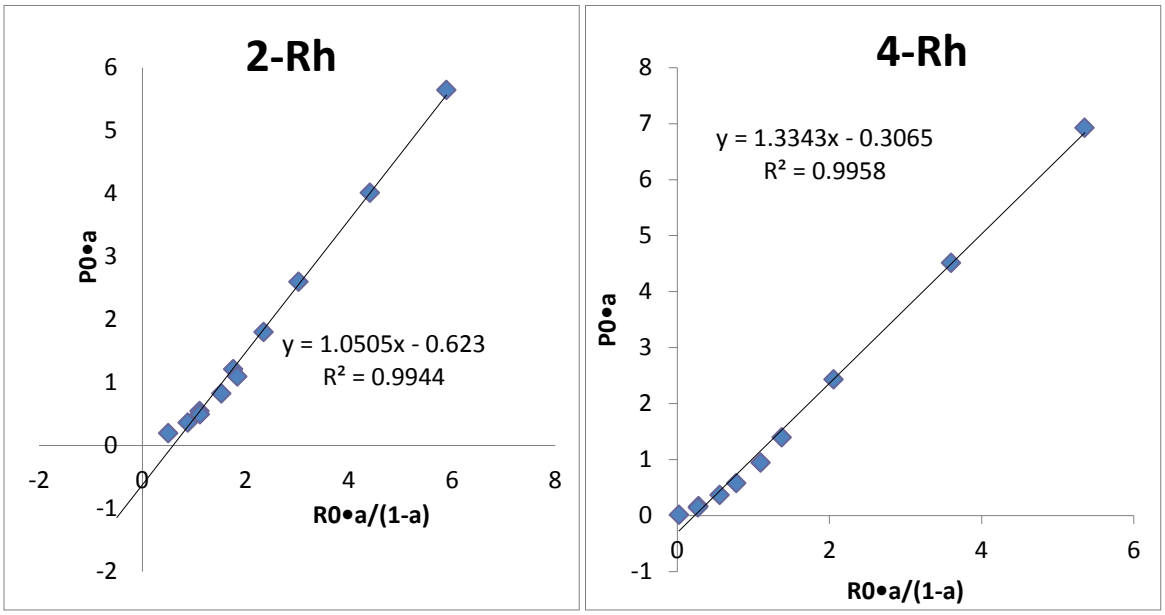
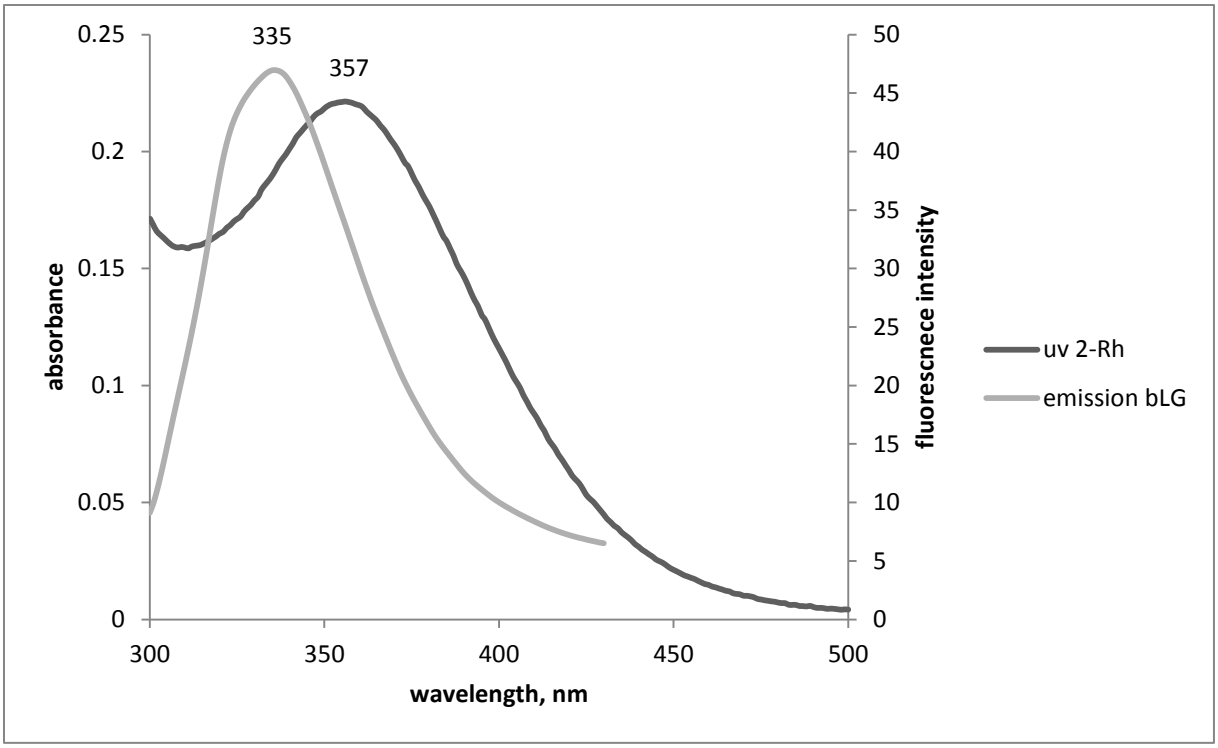


Fig. S8 Absorption spectrum of **2-Rh** in water and fluorescence emission spectrum of βLG in phosphate buffer pH 7.5 (λ_{ex} = 290 nm)



Catalytic runs with complexes and TFACP – determination of conversion rates by RP-HPLC

Analytical reverse phase HPLC was performed on Beckman System Gold instrument using Jupiter Proteo C18 column, 4µm, 150 x 2 mm, 90Â (Phenomenex) using a isocratic eluent containing 50% H_2O, 50% ACN for 10 min with a flow rate of 0.2 mL/min and detection set at 254 nm. Volume of

injection: 20µL of a 1 mM solution in water. Retention time for trifluoroacetophenone: 3.9 min; (+/-)-α-(trifluoromethyl) benzyl alcohol: 6 min.

Catalytic runs with 2-Rh and other aryl ketones – determination of conversion rates by GC

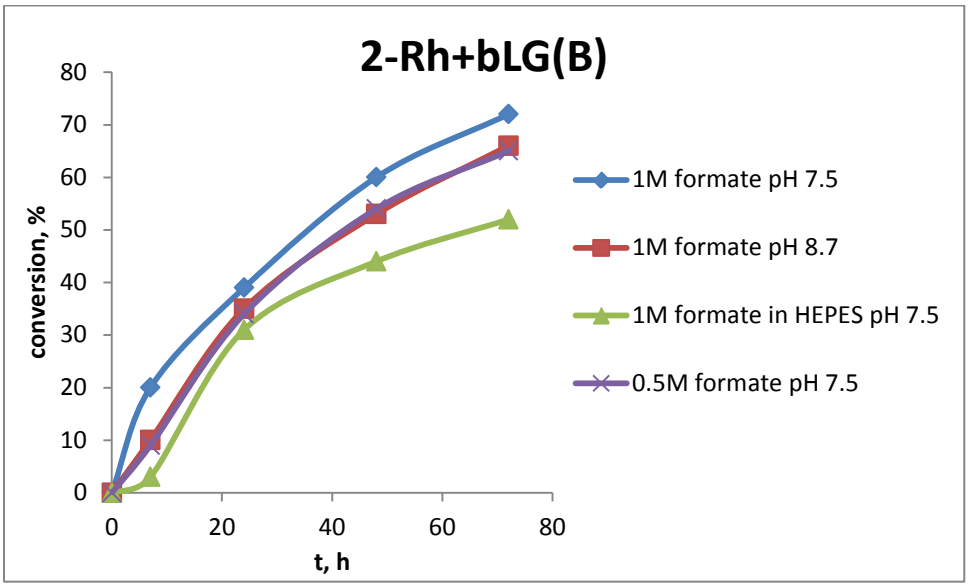
Analytical GC was performed on CP-3380 instrument (Varian) using equipped with a split/splitless injector (T = 250 °C), a 25 m x 0.25 mm x 0.25 µm CP-Chirasil-DEX CB capillary column (Chrompack) and a FID detector (T = 250°C). Injection volume: 5 µL. The following programme was used for analysis: 50 °C (2 min), 50-110 °C (10 °C/min), 110 °C (1 min), 110-140 °C (1.5 °C/min), 140 °C (3 min), 140-200 °C (10 °C/min), 200 °C (2 min).

Ketone	Retention time (min)	
	Ketone	Alcohol
Acetophenone	12.9	16.8, 17.3
4-methylacetophenone	16.9	20.4, 21.05
4-bromoacetophenone	24.4	28.0, 28.25
2-methoxyacetophone	22	25.1, 25.4

Catalytic runs with metalloproteins – determination of conversion rates and enantiomeric excesses by chiral HPLC

Analytical chiral HPLC was performed on Beckman System Gold instrument using Nucleodex β-PM column, 5 µm, 200 x 4 mm (Macherey-Nagel) using a isocratic eluent containing 60% H_2O, 40% ACN at a flow rate of 0.4 mL/min and detection set at 254 nm. Injection volume: 20 µL. Retention time for trifluoroacetophenone: 10.0 min. Retention time for (*S*)-α-(trifluoromethyl)benzyl alcohol: 12.9 min. (*R*)-α-(trifluoromethyl)benzyl alcohol: 13.8 min.

Fig. S9 Kinetic curves of ATH of TFACP in the presence of **2-Rh**⊂ bLG(B) in various reaction conditions



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