Electronic Supplementary Material (ESI) for Dalton Transactions.

Supplementary information

Organometallic Nucleoside Analogues: Effect of the Metallocene Metal Atom on Cancer Cell Line Toxicity

Media K. Ismail, Katie A Armstrong, Samantha L Hodder, Sarah L. Horswell, Louise Male, Huy V. Nguyen, Edward A. Wilkinson, Nikolas J. Hodges, and James H. R. Tucker

Contents

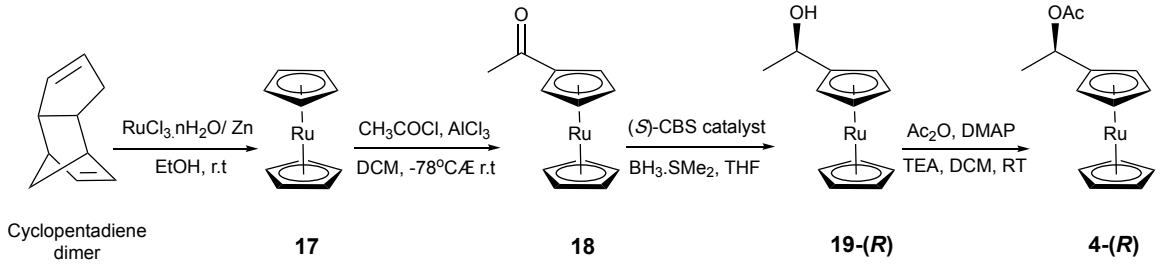
I. General information

Unless otherwise stated, solvents and reagents were obtained from commercial suppliers and organic solvents used for synthesis (e.g. THF) were dried beforehand using a SPS solvent purification system (Innovative Technology). Experimental procedure of all compounds was prepared under argon. Flash column chromatography was carried out using silica gel (Merck, grade 60). ^{1}H and ^{13}C NMR spectra were recorded on on either a Bruker AVIII300 (300 MHz ^{1}H, T = 293 K) or a Bruker AVIII400 (400 MHz ^{1}H, 101 MHz ^{13}C, T = 293 K) spectrometer. Electrospray mass spectra were measured by a Mass spectrometry (MS) and high-resolution mass spectrometry (HRMS) of molecules was performed on either a Waters Waters, XEVO, Equipped with Alliance e2695 separation module - direct injection or a Synapt G2S, Equipped with Alliance HPLC system using electrospray ionisation (ESI) or electron impact ionisation (EI) mass spectrometer.

2. Synthesis

2.1 Ugi Amine Precursor, 4-(*R*)

The immediate precursor to the ruthenocene version of Ugi's amine was synthesised using adapted literature procedures, as outlined below.



Ruthenocene, 17[1]

To a solution of $RuCl_3.nH_2O$ (1 g, 4.82 mmol, 1.0 eq) in absolute ethanol (10 mL) under an atmosphere of argon, freshly distilled cyclopentadiene obtained by fractional distillation (4.77 mL, 72.32 mmol, 15 eq) was added followed by gradual addition of zinc dust (3.15 g, 48.21 mmol, 10 eq). The reaction mixture was stirred for 2 hrs. The metallic grey solid was filtered, washing with toluene (4 x 2 mL). The filtrate was evaporated until dryness and the dark solid residue was redissolved in toluene (500 mL) and passed through a plug of silica gel. The solvent was evaporated to give **17** as a white crystalline solid product (1.45 g, 76.8%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.54 (s, 10H). ^{13}CNMR (400 MHz, $CDCl_3$) δ 70.0 (Rc).

1-Ruthenocenylethan-1-one, 18[2]

Ruthenocene **17** (6 g, 25.96 mmol, 1.0 eq) was dissolved in dry DCM (50 mL) and stirred for 5 min under an atmosphere of argon. Acetyl chloride (3.06 mL, 38.95 mmol, 1.5 eq) was added and the reaction mixture was cooled to -78 ^{o}C. $AlCl_3$ (7.1 g, 53.23 mmol, 2.05 eq) was then added gradually and the resulting mixture stirred for 15 minutes. The reaction was quenched with ice-water (40 mL), extracted with DCM and dried over Na_2SO_4. The solvent was removed *in vacuo* and the crude product purified by flash column chromatography on silica gel using 3:7 / EtOAc: hexane. The solvent was removed *in vacuo* to give the title compound as a yellow solid (6.5 g, 23.71 mmol, 91.3 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.11 – 5.06 (m, 2H), 4.79 – 4.74 (m, 2H), 4.58 (s, 5H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 200.09 (C=O), 84.1 (ipso Cp), 73.6 (CH Cp), 72.1 (CH Cp), 71.0 (CH Cp), 70.0 (CH CP), 26.8 (CH_3). HRMS (ES) (*m/z*) calcd for $C_{12}H_{13}O^{102}Ru$ 275.0010, found 275.0002.

(*R*)-1-ruthenocenylethan-1-ol, 19-(*R*)[3]

A solution of (*S*)-CBS catalyst (10.5 mL, 1M in THF, 10.6 mmol) and of $BH_3.SMe_2$ (20 %, 44 mmol) in THF was cooled to 0 °C under argon and the solution was stirred for 15 minutes. To this, a solution of 1-ruthenocenylethan-1-one **18** (4.9 g, 17.5 mmol) in THF and the rest of $BH_3.SMe_2$ was added dropwise simultaneously. After 30 minutes of stirring at 0 °C, the reaction was left to warm to room temperature. Excess $BH_3.SMe_2$ was quenched by drop-wise addition of methanol (30 mL). The crude was then poured into sat solution of NH_4Cl (100 mL), washed with ether, water, and brine and then dried with Na_2SO_4. The solvent was evaporated, and the crude was purified *via* flash column chromatography (15% Et_2O in hexane) to offer the title compound **19-(*R*)** as a light yellow solid (4.7 g, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.61 (m, 1H), 4.59 (m, 1H), 4.54 (s, 5H), 4.47 (t, *J* = 1.6 Hz, 2H), 4.24 (q, *J* = 3.8 Hz, 1H), 1.69 (s, 1H), 1.29 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 100.3 (ipso Cp), 70.5 (CH Cp), 70.3 (CH Cp), 70.2 (CH Cp), 69.7 CH Cp), 69.0 (CH Cp), 63.8 (CH*), 23.6 (CH_3). HRMS (ES) (*m/z*) calcd for $C_{12}H_{14}O\ Na^{102}Ru$ 298.9986, found 298.9983.

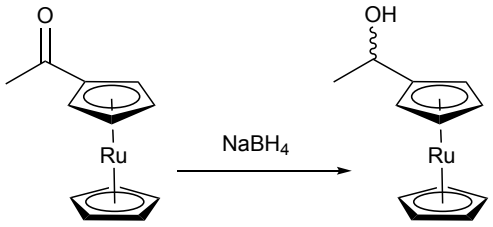
(*R*)-1-α-Acetoxyethylruthenocene, 4-(*R*)[4]

1-ruthenocenylethan-1-ol **18** (2.75 g, 9.98 mmol, 1.0 eq) was dissolved in dry DCM (20 mL) and stirred for 5 minutes under an atmosphere of argon. Ac_2O (1.42 mL, 14.98 mmol, 1.5 eq), Et_3N (2.09 mL, 14.98 mmol, 1.5 eq) and DMAP (catalytic amount) were added successively and the resulting mixture was stirred for 16 hours. The reaction was quenched with sat $NaHCO_3$ (40 mL), extracted with DCM and dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product purified by flash column chromatography on silica gel using an eluent system of 82:15:3 / hexane:ether:Et_3N. The solvent was removed *in vacuo* to give the title compound **4-(*R*)** as a yellow oil (1.0 g, 0.0032 mmol, 14%). ^{1}H NMR

(400 MHz, $CDCl_3$) δ 5.61 (q, *J* = 6.6 Hz, 1H), 4.65 (t, *J* = 2.3 Hz, 2H), 4.52 (ds, 6H), 4.50 (dd, *J* = 3.3, 1.6 Hz, 1H), 2.01 (s, 3H), 1.43 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.5 (C=O), 91.4 (ipso Cp), 71.0 (CH Cp), 70.5 (CH Cp), 70.2 (CH Cp), 68.5 (CH Cp), 68.5 (CH Cp), 21.5 (CH_3), 20.4 (α-CH_3). HRMS (ES) (*m/z*) calcd for $C_{14}H_{16}O_2Na^{102}Ru$ 341.0091, found 341.0092. IR(cm^{-1}): 3100 (=C-H), 2982 (CH_2), 2916 (CH_2), 2800 (CH_2), 1727 (C=O), 1556, 1446 (CH_2), 1368 (CH_3), 1233 (C-O), 811 (C-C), 718 (CH-Ar).

2.2 Synthesis of the racemates

All the X-ray structures of the chiral compounds reported in the text, including that of compound **1-(*S*,*R*$_p$)-Ru**, were obtained from racemates. The racemate synthesis route was the same and used the same conditions as those for the asymmetric route (see main text). The only exception was that the starting material was the racemic alcohol **19**, which was prepared from **18** as outlined below. The enantiomer of the target compound, **1-(*R*,*S*$_p$)-Ru**, was separated from its racemic mixture using chiral HPLC (Section 4). Its ^{1}H and ^{13}C NMR spectra matched that of **1-(*S*,*R*$_p$)-Ru** (optical rotation, $[\alpha]_D^{20}$, of -42 (±4), c = 0.1 in CH_3CN).



18 **19**

1-ruthenocenylethan-1-ol, 19

Sodium borohydride (0.6 g, 1.5 eq, 3.3 mmol) was added to a solution of 1-ruthenocenylethan-1-one **18** (0.13 g, 1 eq, 2.2 mmol) in dry methanol (10 mL). The reaction was stirred for 2 hrs, and then quenched with water (3mL) and extracted with DCM (15mL). The solvent was evaporated, and crude was purified *via* flash column chromatography (15% EtOAc in hexane) to give the compound **19** as a white solid (0.5 g, 83%).

[1] Kündig, E. P.; Monnier, F. R., *Advanced Synthesis & Catalysis* **2004,** *346*, 901-904.
[2] Leusmann, E.; Wagner, M.; Rosemann, N. W.; Chatterjee, S.; Dehnen, S., *Inorg. Chem.,* **2014,** *53*, 4228-4233.
[3] Schwink, L.; Knochel, P., *Chemistry-a European Journal* **1998,** *4*, 950-968.
[4] Hayashi, T.; Ohno, A.; Lu, S.-J.; Matsumoto, Y.; Fukuyo, E.; Yanagi, K., *J. Am. Chem. Soc.,* **1994,** *116*, 4221-4226.

3. NMR spectra of ruthenocene target compounds

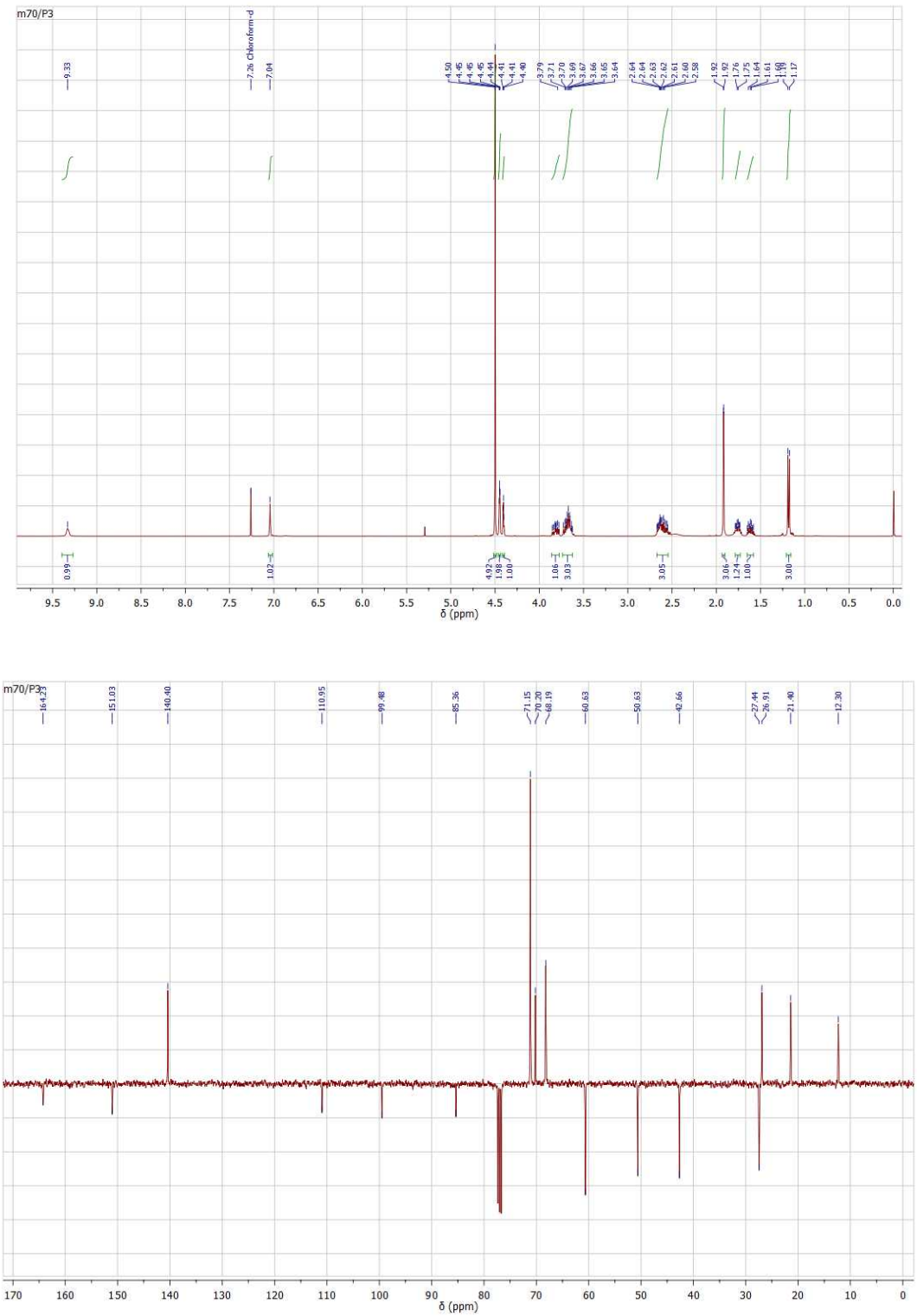
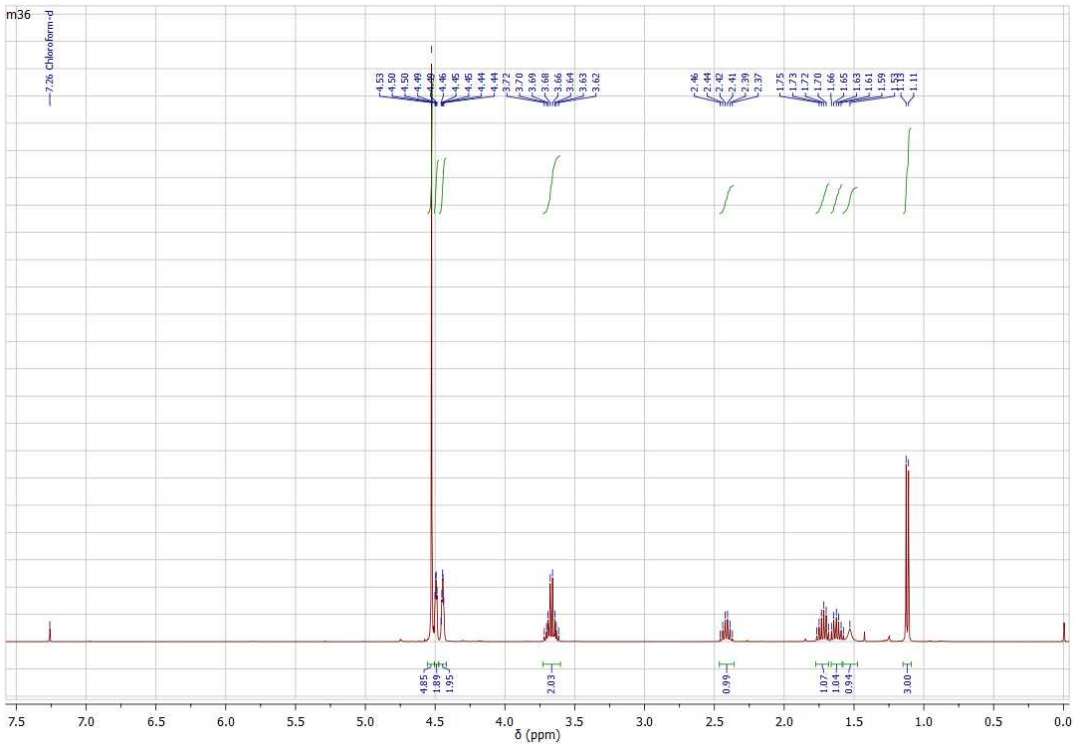


Figure S1. ^{1}H (top) and ^{13}C NMR (bottom) spectra of compound **1-(*S*,*R*$_p$)-Ru**.



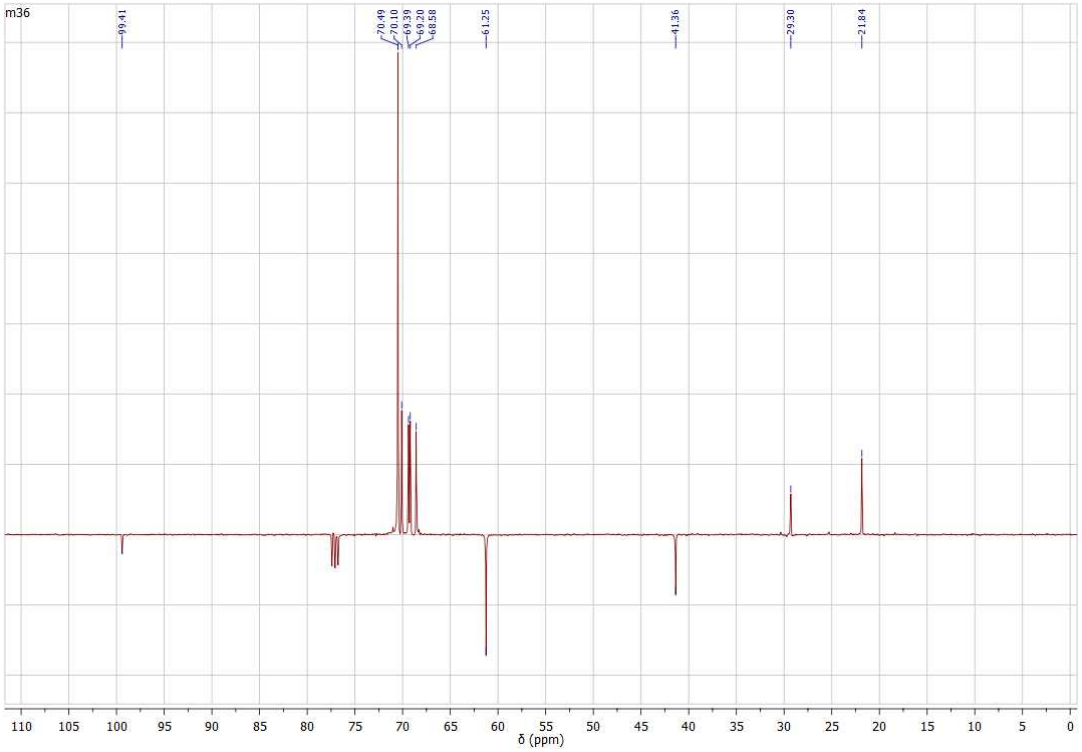
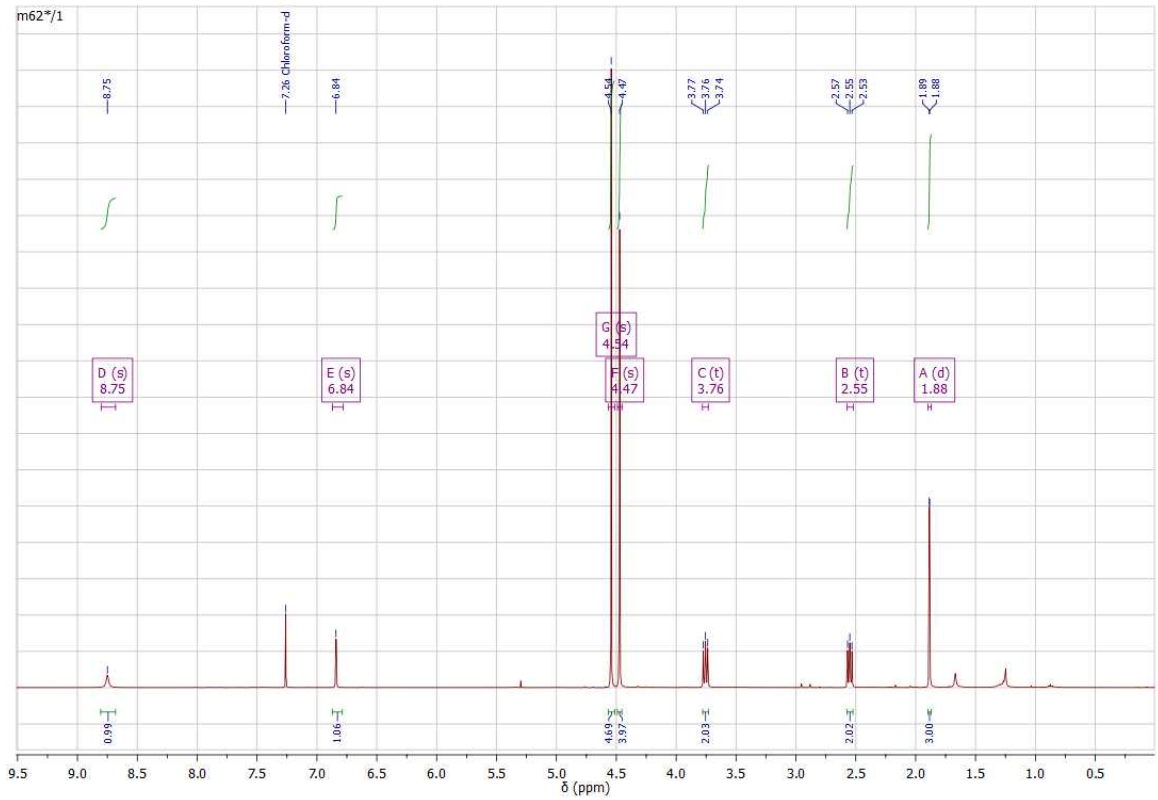


Figure S2. ^{1}H (top) and ^{13} NMR (bottom) spectra of compound **2-(*S*)**.



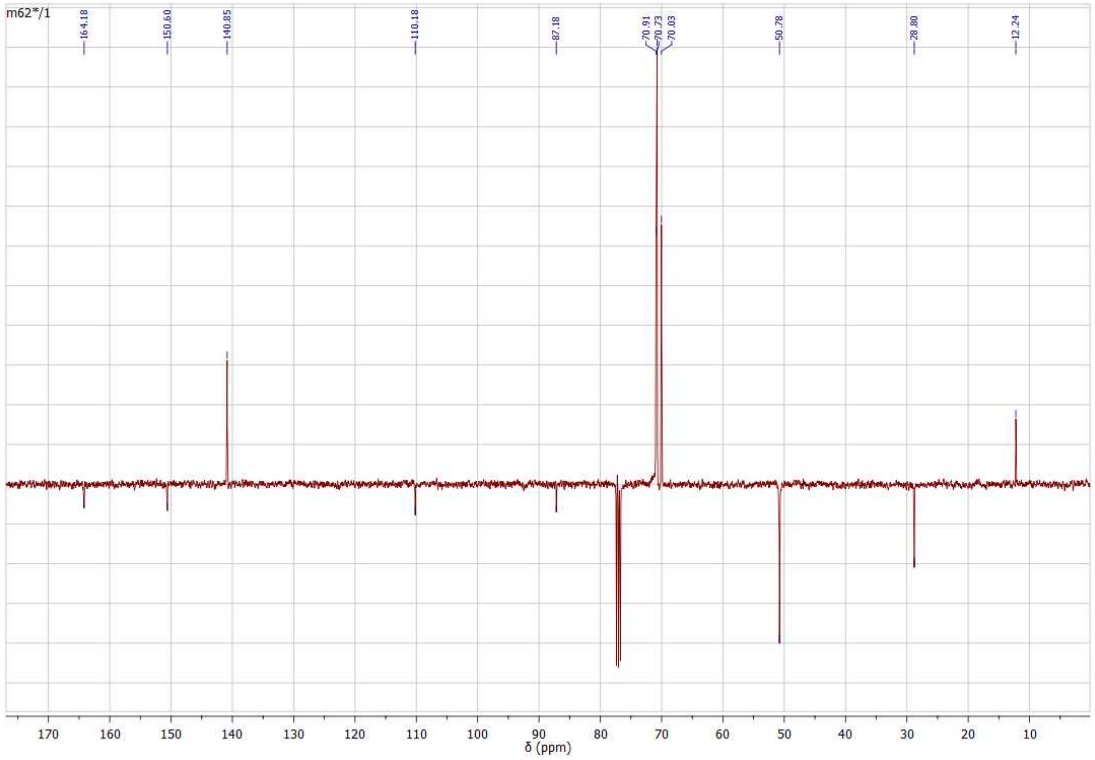
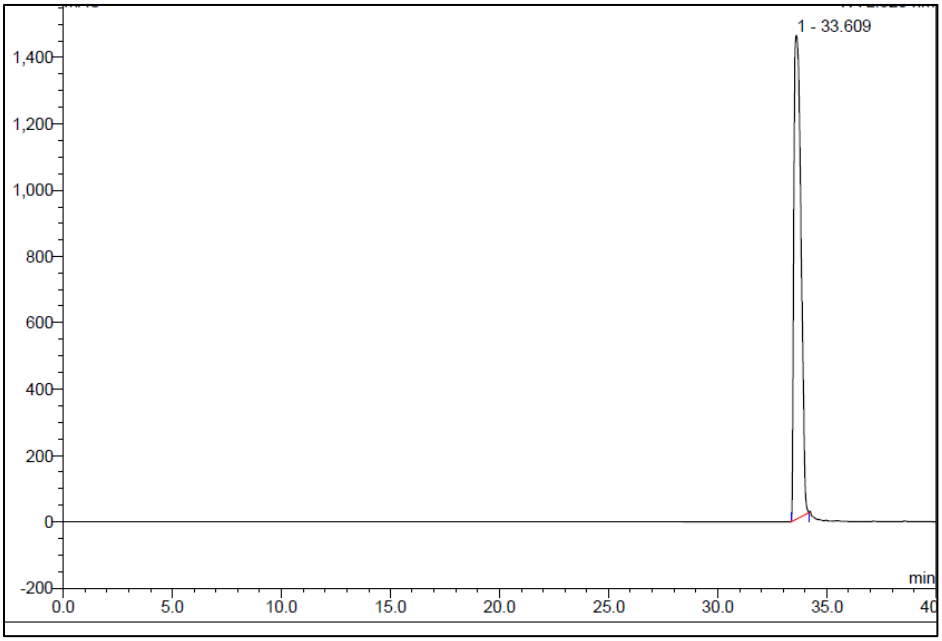


Figure S3. ^{1}H (top) and ^{13}C NMR (bottom) spectra of compound **3**.

4. HPLC characterisation

4.1 Ruthenocene version of the Ugi amine 5-(*S*)

To analyse the chiral purity of the ruthenocene version of the Ugi amine **5-(*S*)**, a chiral column Cellulose-1 was used under reversed phase conditions with an isocratic solvent method of 10% acetonitrile in water. A retention time at 33.6 min was observed as shown below, with chiral purity judged to be >98%.



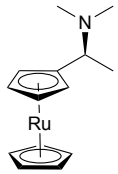


Figure S4. Chiral HPLC trace of **5-(*S*)**

4.2 Compound 1-(*S*,R_p)-Ru and its enantiomer 1-(*R*,S_p)-Ru

The racemic mixture of **1-(*S*,R_p)-Ru** and **1-(*R*,S_p)-Ru** was separated on the chiral column Cellulose 1. A mixture of water and acetonitrile (gradient of 25-70%, 1 mL/min) was used, giving retention times of 18.96 and 14.14 respectively, with final chiral purities of >99%. The chiral purity of **1-(*S*,R_p)-Ru** synthesised via the asymmetric route was found to be greater than 98% (chiral AD column).

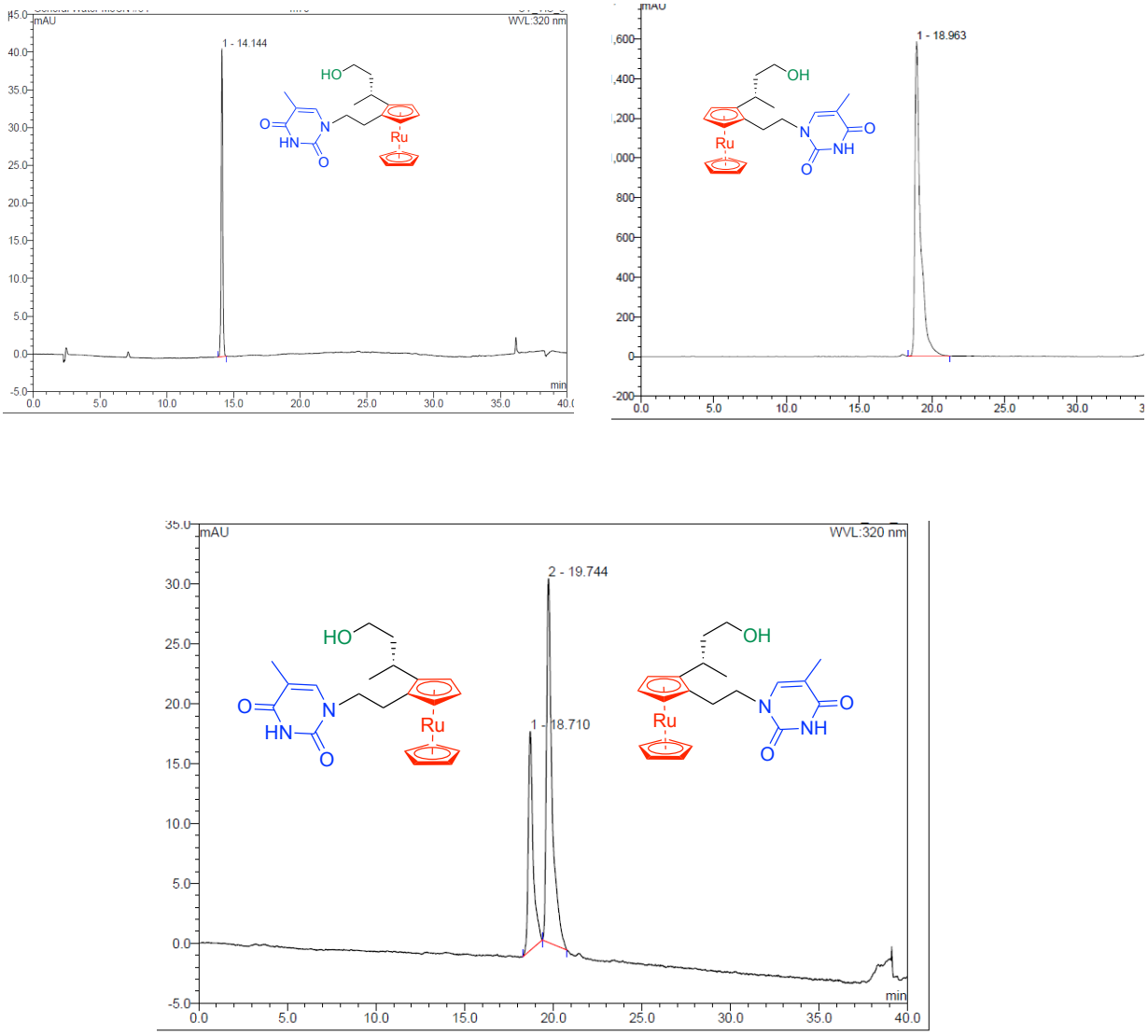


Figure S5. Chiral HPLC traces of compounds **1-(*R*,S_p)-Ru** and **1-(*S*,R_p)-Ru** (top), and their racemic mixture (bottom).

5. Electrochemistry

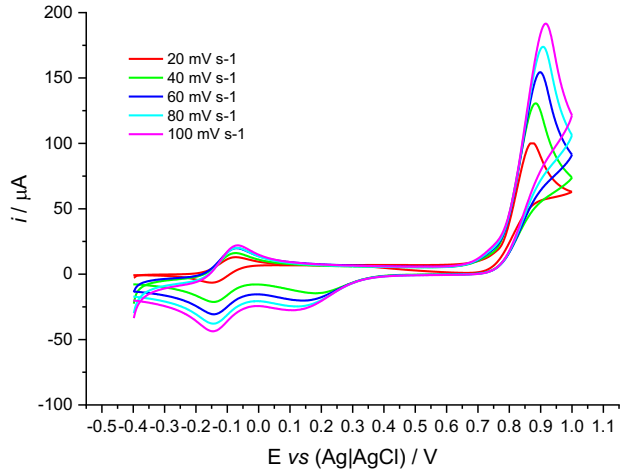


Figure S6. Cyclic voltammograms of ruthenocene [$RuCp_2$], **17**, (1.0 mM) at various scan rates in dry acetonitrile with an electrolyte of [NBu_4][PF_6] (0.1 M) and dmfc (1.0 mM) as an internal reference.

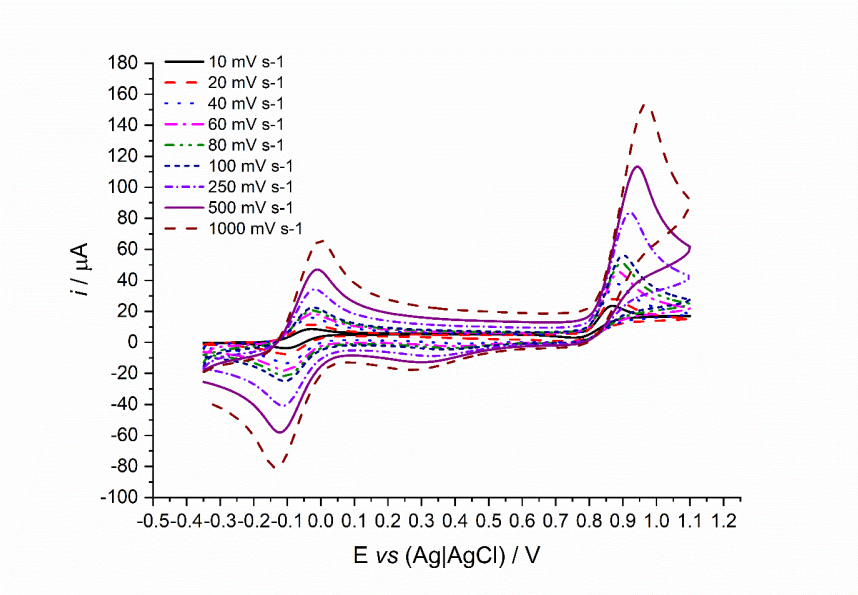


Figure S7. Cyclic voltammograms of **2-(*S*)** (1.0 mM) at various scan rates in dry acetonitrile with an electrolyte of [NBu_4][PF_6] (0.1 M) and dmfc (1.0 mM) as internal reference.

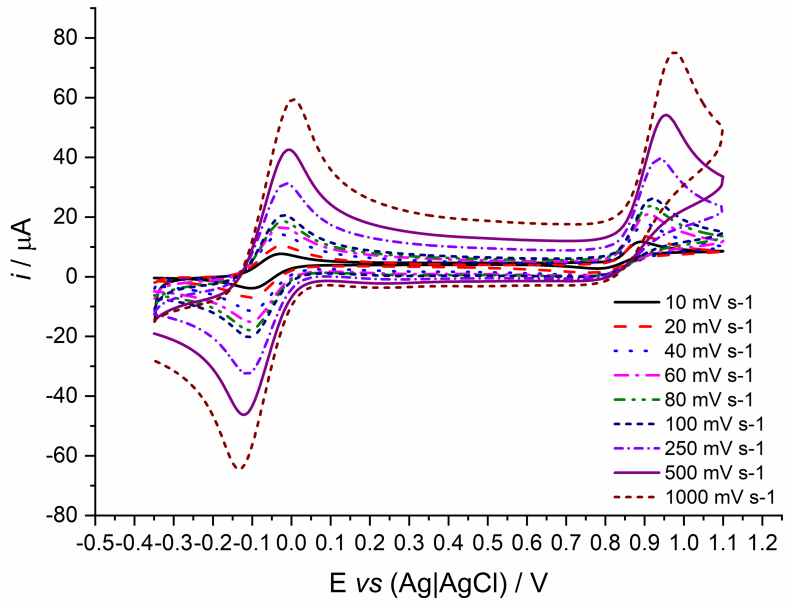


Figure S8. Cyclic voltammograms of **3** (1.0 mM) at various scan rates in dry acetonitrile with an electrolyte of $[NBu_4][PF_6]$ (0.1 M) and dmfc (1.0 mM) as an internal reference.

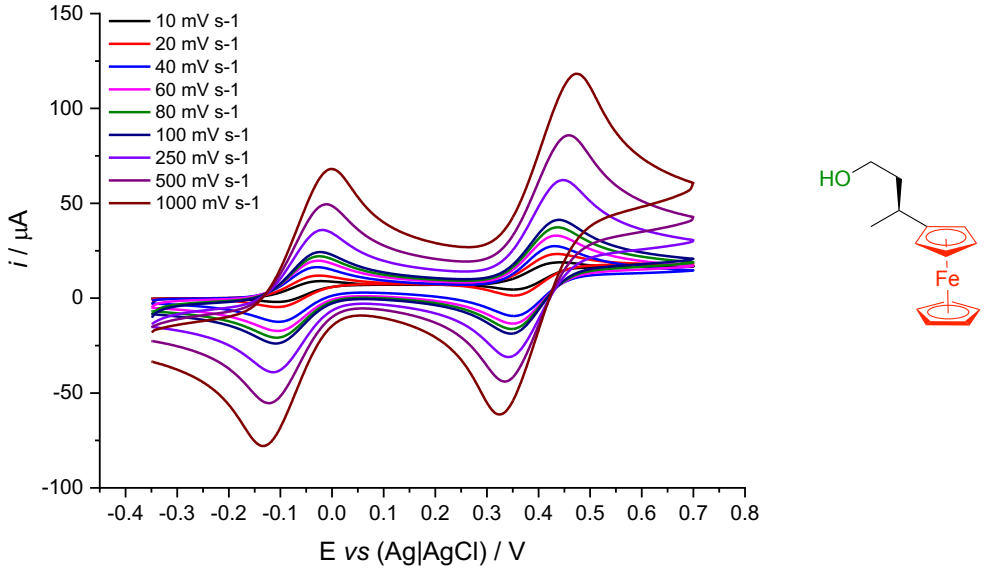


Figure S9. Cyclic voltammograms of the ferrocene analogue of **2-(*S*)** (1.0 mM) at various scan rates in dry acetonitrile with an electrolyte of $[NBu_4][PF_6]$ (0.1 M) and dmfc (1.0 mM) as an internal reference ($E_{1/2}$ vs dmfc = 0.463 V, E_{pa} vs dmfc = 497 V)

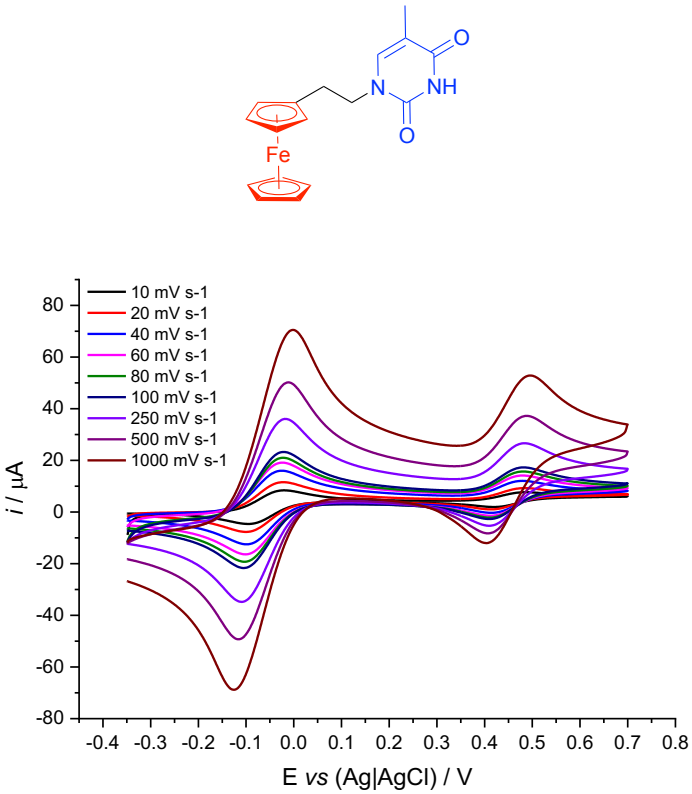


Figure S10. Cyclic voltammograms of the ferrocene analogue of **3** (1.0 mM) at various scan rates in dry acetonitrile with an electrolyte of $[NBu_4][PF_6]$ (0.1 M)) and dmfc (1.0 mM) as an internal reference ($E_{1/2}$ vs dmfc = 0.511 V, E_{pa} vs dmfc = 540 V)

6. X-Ray Crystallography

The datasets for **1-(*S*,R_p)-Ru**, **3**, **5-(*R*)** and **16** were measured on an Agilent SuperNova diffractometer using an Atlas detector. The data collections were driven and processed and numerical absorption corrections based on gaussian integration over a multifaceted crystal model were applied using CrysAlisPro.[5] The dataset for **15** was measured by the UK National Crystallography Service[6] on a Rigaku AFC12 goniometer equipped with an enhanced sensitivity (HG) Saturn724+ detector mounted at the window of an FR-E+ SuperBright molybdenum rotating anode generator with HF Varimax optics. The data collection was driven and processed and an empirical absorption correction using spherical harmonics was implemented in CrysAlisPro.[5] The structures of **5-(*R*)**, **15** and **16** were solved using ShelXS,[7] the structure of **3** was solved using ShelXT [8] and that of **1-(*S*,R_p)-Ru** was solved using olex2.solve.[9] All structures were refined by a full-matrix least-squares procedure on F^2 in ShelXL.[10] Figures and reports were produced using OLEX2.[11] All non-hydrogen atoms in both structures were refined with anisotropic displacement parameters Unless otherwise stated, in all structures, the hydrogen atoms were fixed as riding models and the U_{iso} of all hydrogen atoms were based on the U_{eq} of the parent atoms.

5. CrysAlisPro, Rigaku Oxford Diffraction, 2015.

6. S. J. Coles and P. A. Gale, *Chem. Sci.*, 2012, **3**, 683-689

7. G. M. Sheldrick, *Acta Cryst.* 2008, **A64**, 112-122.

8. G. M. Sheldrick, *Acta Cryst.* 2015, **A71**, 3-8.

9. L. J. Bourhis, O. V. Dolomanov, R. J. Gildea, J. A. K. Howard, H. Puschmann, *Acta Cryst.* 2015, **A71**, 59-75.

10. G. M. Sheldrick, *Acta Cryst.* 2015, **C71**, 3-8.

11. O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard, H. Puschmann, *J. Appl. Crystallogr.* 2009, **42**, 339-341.

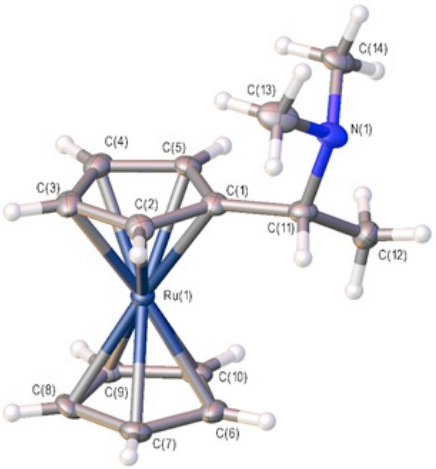


Figure S11. Crystal structure of **5-(*R*)** with ellipsoids drawn at the 50% probability level.

Notes: All hydrogen atoms were fixed as riding models. The structure is in a centrosymmetric space group such that in two molecules of the unit cell, C(11) is *R* and in the other two molecules, C(11) is *S*. The (*R*) isomer is shown above.

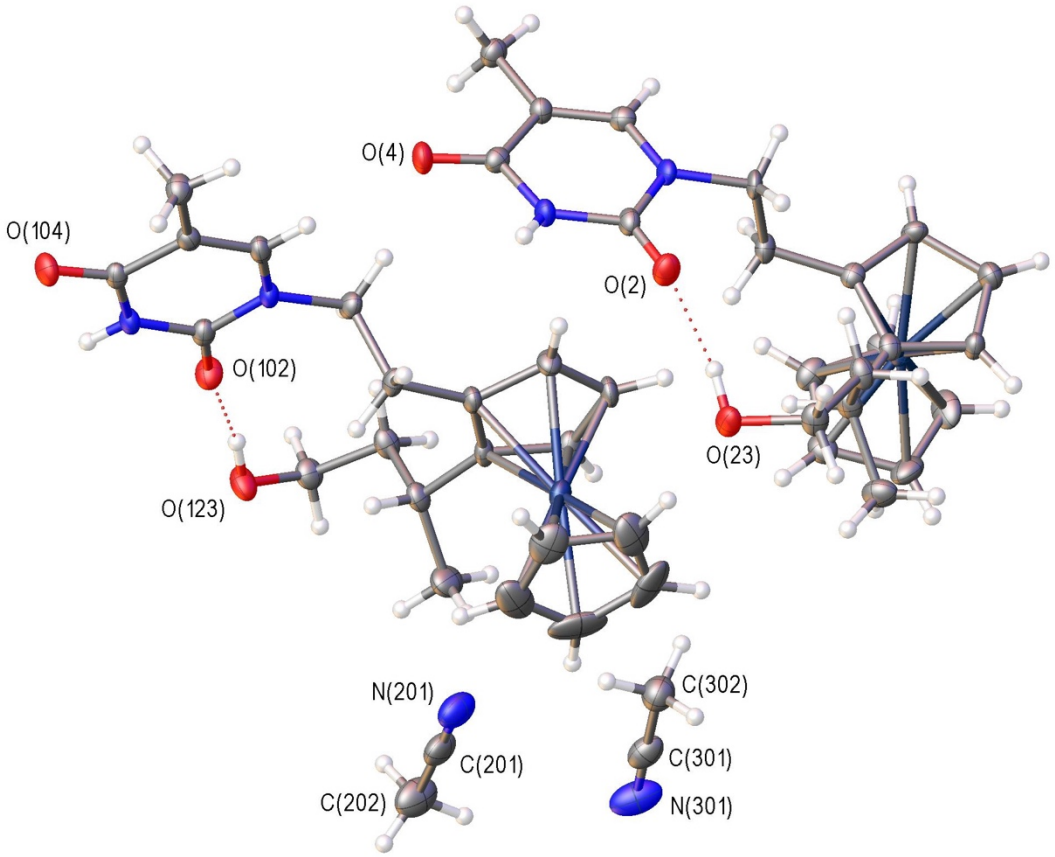


Figure S12 Crystal structure of **1-(*R,S*$_p$)-Ru** and **1-(*S,R*$_p$)-Ru** with ellipsoids drawn at the 50 % probability level. The structure contains two crystallographically-independent molecules and also two independent molecules of acetonitrile. Intramolecular hydrogen bonding is shown using a dotted line.

Notes: The structure is in a centrosymmetric space group such that in one half of the unit cell C(20) is *S* and C(120) is *R* and in the other half C(20) is *R* and C(120) is *S* (the enantiomer **1-(*S,R*$_p$)-Ru** is depicted in Fig. 2 of the main text). The hydrogen atoms bonded to N(3), O(23), N(103) and O(123) were located in the electron density and their positions refined, with N(3)-H(3) subject to a bond distance restraint. All remaining hydrogen atoms were fixed as riding models and the isotropic thermal parameter (U_{iso}) of all hydrogen atoms were based on the U_{eq} of the parent atom.

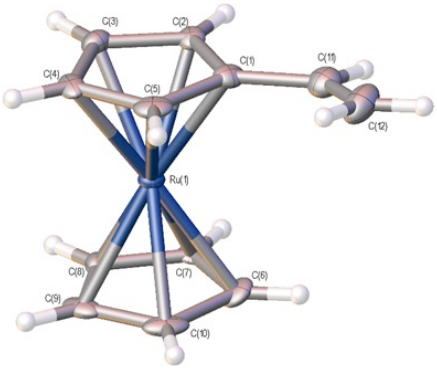


Figure S13. Crystal structure of vinylruthenocene **15** with ellipsoids drawn at the 50 % probability level.

Notes: The crystal was a non-merohedral twin. The two components are related by 180° about the [1 0 0] reciprocal direction and the refined percentage ratio of the two components is 54.2(3) : 45.8(3). All hydrogen atoms were fixed as riding models.

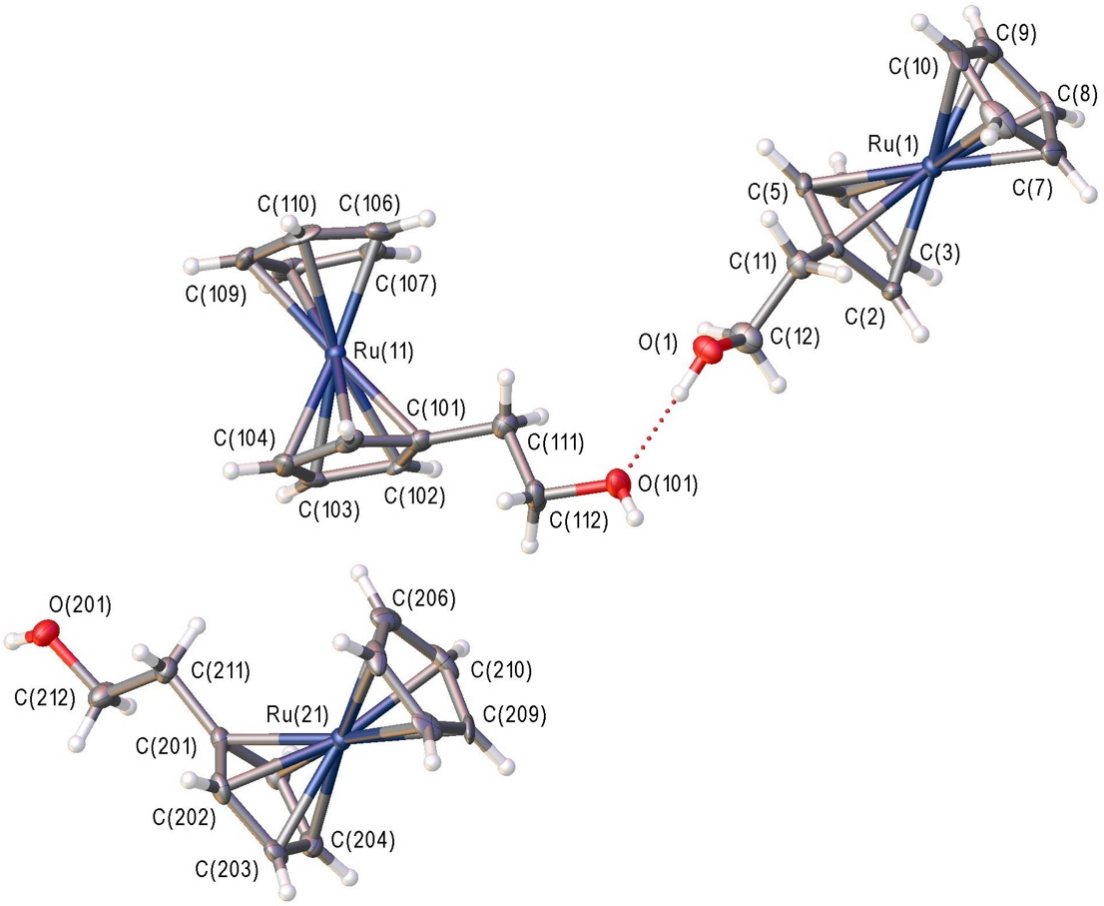


Figure S14. Crystal structure of **16** with ellipsoids drawn at the 50 % probability level. The structure contains three crystallographically-independent molecules. Hydrogen bonding is shown using a dotted line.

Notes: The structure contains three crystallographically-independent molecules. The hydrogen atoms bonded to O(1), O(101) and O(201) were located in the electron density and their positions and thermal parameters refined. All remaining hydrogen atoms were fixed as riding models and the isotropic thermal parameters (U_{iso}) were based on the U_{eq} of the parent atom.

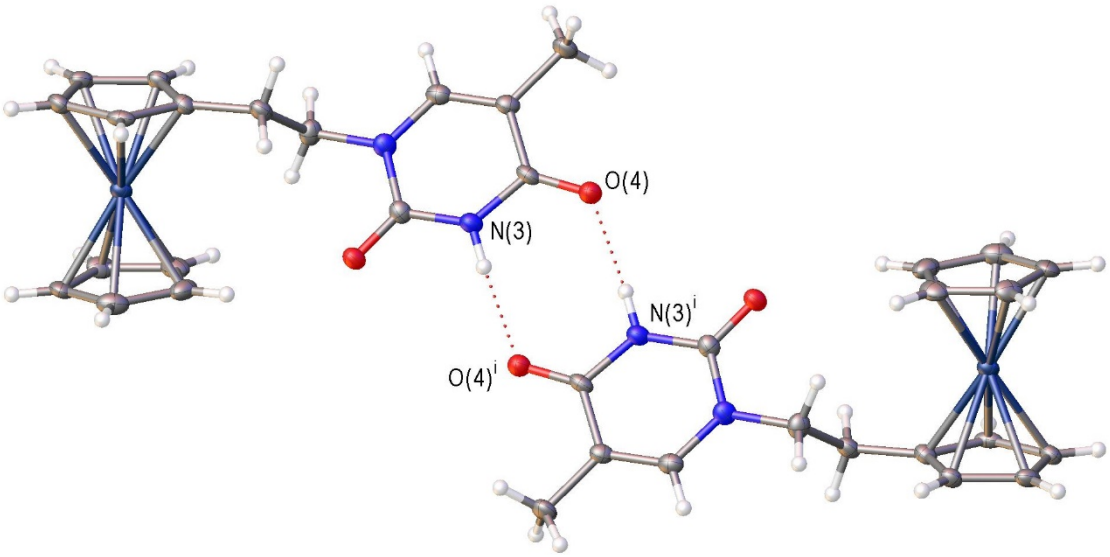


Figure S15. Crystal structure of **3** with ellipsoids drawn at the 50 % probability level. Dimers are formed through hydrogen bonding. Symmetry codes used to generate equivalent positions: (i): 2-x, 1-y, 1-z.

Notes: The crystal was twinned with the two domains related by 180° about the reciprocal vector [0 0 1] with the refined percentage ratio 59.4 (1) : 40.6 (1). The hydrogen atom bonded to N(3) was located in the electron density and its position refined. All remaining hydrogen atoms were fixed as riding models and the isotropic thermal parameters (Uiso) of all hydrogen atoms were based on the Ueq of the parent atom.