Asymmetric transfer hydrogenation by synthetic catalysts in cancer cells

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Supplementary Information

1. Materials and methods

Potassium hydroxide, magnesium sulphate and all non-dried solvents were obtained from Fischer Scientific. (1*R*,2*R*) and (1*S*,2*S*)-1,2-diphenylethylenediamine as optically pure compounds from Arran Chemical Company Ltd (Ireland). Reduced precursors (1,4-dihydro-biphenyl and 1,4-dihydro-*m*-terphenyl) were prepared as previously reported or kindly provided by Khatija Bhayat or Dr. Russell Needham (University of Warwick, UK). All deuterated solvents were purchased from Goss Scientific. All solvents were used as received, and reagents purchased from Sigma Aldrich and used as received. We have previously reported the synthesis of [Os(η^6-*p*-cymene)(TsDPEN)] (**2**) by microwave procedures.[1]

Microwave reactor. Microwave-assisted reactions were carried out using a CEM Discovery-SP microwave reactor, using the appropriate programme as described for individual syntheses.

Nuclear magnetic resonance. Spectra for the characterisation of complexes **2**-**8** were obtained in $CDCl_3$ containing $SiMe_4$ as an internal reference. 5 mm NMR tubes were used to record spectra at 298 K on Bruker DPX-400, HD-500 or AV-600 spectrometers. Data processing was carried out using TOPSPIN version 3.2 (Bruker UK).

High resolution mass spectrometry. HRMS of complexes **2**-**8** in acetonitrile were obtained using a Bruker UHR-Q-TOF MaXis. A positive ion scan range of *m/z* 50-3000 with a spectral rate of 1 Hz was selected. Analysis was carried out through direct infusion (2 µL/min) with a syringe pump, with sodium formate (10 mM) calibration. Source conditions: ESI (+); end plate offset: -500 V; capillary: -3000 V; nebulizer gas (N_2): 0.4 bar; dry gas (N_2): 4 L/min; dry temperature: 453 K; funnel RF: 200 Vpp; multiple RF: 200Vpp; quadruple low mass: 55 *m/z*; collision energy: 5.0 eV; collision RF: 600 Vpp; ion cooler RF: 50-250 Vpp ramping; transfer time: 121 µs; pre-pulse storage time: 1 µs.

Elemental analysis. Elemental analysis (C, H, N) of complexes **2**-**8** was carried out by Warwick Analytical Services on an Exeter elemental analyser CE440.

UV-visible spectroscopy. The UV-visible spectra for complexes 3-8 in DCM (0.1 - 0.3 mM) were recorded on a Cary 300 scan spectrophotometer using a 1-cm path-length of cell, range 800-200 nm, average time 0.1 s, data interval 1 nm; scan rate 600 nm/min.

X-ray diffraction. Single crystals of ***R,R*-7** (Supplementary Figure 1) and ***S,S*-7** (Supplementary Figure 2) were grown from $CHCl_3$/hexane (Supplementary Figure 1). A suitable crystal was mounted on a glass fibre with Fromblin oil and placed on an Oxford Diffraction Gemini diffractometer with a Ruby CCD area detector. The crystal was kept at 150(2) K during data collection. Using Olex2[2], the structure was solved with the ShelXS[3] structure solution program using Direct Methods and refined with the ShelXL[3] refinement package using Least Squares minimisation. The enantiopure complexes were synthesised from starting materials of known chirality and also gave low Flack parameter values for the structural determinations (Supplementary Table 1) refined using BASF/TWIN in Shelx2014.

Asymmetric reduction of ketones.[1] 5:2 formic acid / triethylamine azeotrope (500 µL) was added to a nitrogen-purged Schlenk flask containing an osmium catalyst (5 µmol, 1 mol equiv). Acetophenone was injected (1 mmol, 200 mol equiv) and stirred for 24 h. Aliquots of reaction solution were placed into 1 mL EtOAc and 1 mL $NaHCO_3$ and the organic layer filtered through a plug of silica. Conversion and *ee* were analyzed by GC-FID using the Clarity Chromatography Suite. GC analysis was carried out using a Chrompac cyclodextrin-β-236M-19, 50 m x 0.25 mm x 0.25 µm, P = 15 psi, gas H_2.

Transfer hydrogenation kinetics. The osmium catalyst (5 µmol) was weighed into a glass vial. Deuterated benzene (100 µL) was added under an inert atmosphere of nitrogen (310 K). Formic acid / triethylamine azeotrope (5:2, 500 µL) was injected and stirred for 30 min. Acetophenone (1 mmol, 200 mol equiv) was then added and the mixture transferred to a 5 mm nitrogen-purged NMR tube. Final solution concentrations: substrate 1.67 M; Os catalyst 8.33 mmol. Final volume *ca*. 0.720 ml. Spectra were recorded every 73 s at 310.0 ± 0.5 K over a 1 h period using a Bruker AV-400 spectrometer (400 MHz), and performed in triplicate. Conversion was monitored by two integration regions, chosen to represent the starting reagent and the product. The integration of peaks at higher field (δ = 2.25-2.65 ppm) corresponds to the methyl protons (3H) in the reagent, while the lower field integration (δ = 4.55-5.00 ppm) is the quartet of the newly formed CH (Supplementary Figure 3). The

triethylamine resonance was clearly evident at 2.9 ppm (*q*), however does not overlap with the integration regions. Turnover number (TON_t defined as the moles of substrate turned over per mole of catalyst at time = *t*), was calculated for each spectrum at a specific time, *t*. TON was calculated as the product of conversion (%) and the substrate / catalyst ratio. The experiment was repeated as described using pyruvic acid (1 mmol).

Aqueous stability of complex 2. Complex **2** was dissolved in 5% v/v DMSO in 0.9% w/v saline (conditions used during biological assays). UV-visible spectra were recorded at t = 0 and t = 24 h. ^{1}H-NMR spectra were recorded (0-24 h, 100% d_6-DMSO) to detect solvent adducts.

Cell-free aqueous reduction modulation experiments. Osmium complexes ***R*,*R*-2** or ***S*,*S*-2** were incubated with sodium pyruvate in PBS, in the presence / absence of sodium formate. Final concentrations: osmium complex = 15 μM (IC_{50} concentration for A2780 human ovarian cancer cells); sodium pyruvate = 1 mM; sodium formate = 2 mM. After 24 h incubation at 310 K, concentrations of D and L-lactate were measured individually in quadruplicate using enantio-specific detection assay kits (Cayman Chemical) as described in the manufacturer's instructions. Fluorescence (λ_{ex}: 530-540 nm, λ_{em}: 585-595 nm) was read using a Promega GloMax Multi+ microplate reader. Averages and standard deviations were calculated.

^{1}H-NMR study of pyruvate reduction by *R*,*R*-2 and sodium formate. A saturated solution of catalyst ***R*,*R*-2** was prepared in PBS containing 10% v/v DMSO, and filtered before the final concentration of Os was determined by ICP-OES. Separately, stock solutions of sodium formate and sodium pyruvate (100 mM) were prepared in PBS. Solutions were mixed in a falcon tube to achieve final working concentrations: catalyst, 10 μM; pyruvate, 2 mM; formate 4 mM or 30 mM; with D_2O (10 % v/v). ^{1}H-NMR data (600 MHz, 90% H_2O / 10% D_2O, 310 K) were acquired at 660 s time intervals using water suppression (ZGGPW5). Data were processed using Topspin 3.2 (Bruker UK) and baseline-corrected using SPLINE algorithm. Integrals of pyruvate (δ = 2.40 ppm, CH_3) and lactate (δ = 1.36 ppm, CH_3) were used to determine % conversion and turnover frequency.

Inductively-coupled plasma-optical emission spectroscopy (ICP-OES). ICP-OES was used to determine Os concentrations in aqueous solutions containing sodium chloride (e.g.

drug stock solutions before administration to cells). Data were obtained using a Perkin Elmer Optima 5300 DV Optical Emission Spectrophotometer. A stock solution was prepared, containing thiourea (10 mM) and ascorbic acid (100 mg/L) in 3.6% v/v nitric acid (using freshly distilled 72% nitric acid and doubly deionized water, with additives to stabilize Os in nitric acid solution and prevent formation of OsO_4).[4] This solution was used to prepare calibration standards (0-700 ppb) and to dilute samples to within this range. The salinity of the calibration standards was adjusted to match the matrix of the samples by the addition of sodium chloride. Data were acquired and processed using WinLab32 V3.4.1 for Windows.

Inductively-coupled plasma-mass spectrometry (ICP-MS). ICP-MS was used to determine Os concentrations in digested cells. Data were obtained using an ICP-MS Agilent Technologies 7500 series in no-gas mode. Data acquisition (^{189}Os) was carried out using ICP-MS Top and processed using Offline Data Analysis (ChemStation version B.03.05, Agilent Technologies, Inc.). Standard solutions were prepared using thiourea and ascorbic acid (as described for ICP-OES) in 3.6% v/v nitric acid,[4] and samples were diluted within this range (0.1-1000 ppb). An internal standard of ^{167}Er (50 ppb) was used.

Partition coefficients (Log P). Partition coefficients were determined using the shake-flask method with 1-octanol saturated water (OSW) and water saturated 1-octanol (WSO). Saturated filtered aqueous solutions of each complex (~250 μM) were prepared and shaken with equal volumes of WSO overnight on an IKA Vibrax VXC basic shaker (1000 g/min). The osmium concentration of the aqueous layer was determined before and after shaking by ICP-MS in no-gas mode (^{189}Os). Log P values were determined as duplicates of triplicates, as part of two independent experiments, and the standard deviations were calculated. Statistical significances were determined using Welch's unpaired *t*-test at the 95% confidence level.

Flow cytometry. Flow cytometry was carried out at the School of Life Sciences (University of Warwick, UK) using a Becton Dickinson FACScan Flow Cytometer. Data were analysed using FlowJo® V10 for Windows.

Cell Culture. A2780 human ovarian carcinoma cells, MRC5 human foetal fibroblasts and PC3 human prostate carcinoma cells were obtained from the European Collection of Cell Cultures (ECACC). HOF human ovarian fibroblast cells were obtained from Caltag Medsystems (UK distributor for ScienCell Research Laboratories). Cancer cells were grown

in Roswell Park Memorial Institute medium (RPMI-1640) supplemented with 10% of foetal calf serum, 1% of 2 mM glutamine and 1% penicillin/streptomycin, and primary cell lines were grown in fibroblast growth culture medium. A2780 and PC3 cancer cells were used between passages 5 and 18, and primary cell lines (MRC5 and HOF) were used before passage 5. The cells were grown as adherent monolayers at 310 K in a 5% CO_2 humidified atmosphere and passaged at approximately 70-80% confluence. Well-plates used in biological experiments were read using a BioRad iMark plate reader fitted with a 470 nm filter for colorimetric assays, or a Promega GloMax Multi+ microplate reader for fluorescence assays.

In vitro **Growth Inhibition Assay.** 5000 cells (A2780, HOF, MRC5 or PC3 cells) were seeded per well (150 μL) in 96-well plates. The cells were pre-incubated in drug-free media at 310 K for 48 h before adding different concentrations of the compounds to be tested. Stock solutions (*ca.* 100 μM) of the osmium complexes were prepared in DMSO (5% v/v) and medium (95% v/v), and then further diluted in culture medium until working concentrations were achieved (typically 100, 50, 25, 10, 1, 0.1 μM). drug exposure period was 24 h. After this, supernatants were removed by suction and each well was washed with PBS. A further 72 h was allowed for the cells to recover in drug-free medium at 310 K. The SRB assay was used to determine cell viability.[5] Absorbance measurements of the solubilized dye (on a BioRad iMark microplate reader using a 470 nm filter) allowed the determination of viable treated cells compared to untreated controls. Drug stock solutions were concentration-adjusted by ICP-OES. The IC_{50} values (concentrations which caused 50% of cell death), were determined as duplicates of triplicates in two independent sets of experiments and their standard deviations were calculated.

Cell viability modulation experiments. Experiments were carried out as described for the *in vitro* growth inhibition assay, however a fixed (equipotent) concentration of each osmium complex was used, corresponding to ½ × IC_{50} concentration. Stock solutions of osmium complexes were prepared *ca*. 100 μM (5% DMSO, 95% culture medium; exact Os concentrations in the drug stock solutions were determined by ICP-OES before administration to cells), and were then diluted in culture medium until working concentrations were achieved. Sodium formate was co-administered at three different concentrations (0.5, 1.0 and 2.0 mM), prepared in saline. Both solutions were added to each

well of cells independently, but within 5 min of each other. Final concentrations: Os catalyst IC_{50} / 2 (complex **2**: 7.5 μM, complex **7**: 3.3 μM); sodium formate 0-2 mM. Cell viability was determined using the SRB assay (Supplementary Tables 1 and 2). Cell viability modulation experiments were also carried out using sodium acetate (Supplementary Table 3) in place of formate (Supplementary Table 4). N-Formylmethionine modulation experiments were also carried out similarly, using three concentrations (0.25, 0.5, 1.0 mM) in PC3 human prostate cancer cells, which are known to overexpress the peptide deformylase (PDF) enzyme (Supplementary Table 5).[6] Statistical significances were determined using Welch's unpaired *t*-test at the 95% confidence level.

Metal accumulation in cancer cells. Cell accumulation studies for osmium complexes were conducted on A2780 ovarian cancer cells. Briefly, 4 x 10^6 cells were seeded on a 6-well plate. After 24 h of pre-incubation time in drug-free medium at 310 K, the complexes were added to give final concentrations equal to IC_{50} / 3 and a further 24 h of drug exposure was allowed (exact concentrations of Os in drug stock solutions were determined by ICP-OES before administration to cells). Cells were not allowed recovery time, except for efflux studies which included up to 72 h recovery in drug-free medium. After this time, cells were treated with trypsin, counted, and cell pellets were collected. Each pellet was digested overnight in concentrated nitric acid (72%) at 353 K; the resulting solutions were diluted using doubly-distilled water containing thiourea (10 mM) and ascorbic acid (100 mg/L).[4] Concentrations were adjusted to give a final acid concentration of 3.6% v/v HNO_3 and the amount of Os taken up by the cells was determined after digestion by ICP-MS in no-gas mode. Experiments did not include recovery time in drug-free media; they were carried out in triplicate and the standard deviations were calculated. Statistical significances were determined using Welch's unpaired *t*-test.

Metal distribution in cancer cells. Cell pellets, obtained in triplicate (as described for metal accumulation studies) were fractionated using the Fraction PREP kit (BioVision). Samples were digested overnight in nitric acid (200 μL, 72% v/v) at 343 K, then diluted to achieve a final working acid concentration of 3.6% v/v (taking into account the volume of the sample: cytosolic and membrane fractions = 400 μL, nuclear fraction = 200 μL). Os concentrations in digested samples were determined by ICP-MS in no-gas mode.

Reduction modulation experiments in cells. The D-lactate assay detection kit (Cayman Chemical) was stored at 255 K before use. Complexes ***R,R*-2** and ***S,S*-2** were selected for screening. 30×10^6 A2780 human ovarian cancer cells were seeded in T75 flasks with 24 h pre-incubation. After this time, solutions of Os complexes and sodium formate were added independently, but within 5 min of each other (final working concentrations: IC_{50} concentration of the osmium complex, 2 mM sodium formate) with 24 h drug exposure. The supernatant was collected for extracellular D-lactate analysis, and cells were washed, detached using trypsin / EDTA, counted and centrifuged at 1000 g for 5 min to obtain cell pellets of 40×10^6 cells for intracellular D-lactate analysis, which were processed according to the manufacturer's instructions. Fluorescence (λ_{ex}: 530-540 nm, λ_{em}: 585-595 nm) was read using a Promega GloMax Multi+ microplate reader. Samples were measured in triplicate, and standard deviations calculated. Statistical significances were determined using Welch's unpaired *t*-test.

Cell cycle analysis. Briefly, 1×10^6 A2780 cancer cells were seeded in a 6-well plate using 2 ml per well, and incubated for 24 h. The supernatant was removed by suction, and cells treated with complex **2** (IC_{50} concentration) either with or without sodium formate (2 mM) for 24 h at 310 K. After this time, the supernatant was removed by suction, cells washed with PBS and cell pellets obtained using trypsin / EDTA. After collection and centrifugation, the pellets were washed with PBS, then resuspended in ice-cold ethanol for 30 min. The ethanol was then removed and cells were washed with PBS. The pellets were resuspended in 500 µL staining buffer (50 μgmL^{-1} propidium iodide; 80 μgmL^{-1} RNAse) for 30 min. After centrifugation, the supernatant was removed and cells resuspended in PBS. Samples were analysed as instrumental triplicates by flow cytometry using a Becton Dickinson FACScan Flow Cytometer. Propidium iodide (PI) was read using the FL2 channel. Data were processed using a Watson (Pragmatic) fitting algorithm (Supplementary Figure 10) of FL2 (FlowJo V10). Statistical analysis was carried out using a two-tailed *t*-test assuming unequal variances (Welch's *t*-test).

Induction of Apoptosis. Cell pellets were obtained as described for cell cycle analysis (without fixation in ethanol). After collection and centrifugation, the pellets were washed with PBS then resuspended in 500 µL staining buffer (1% v/v Annexin V FITC conjugate; 2% v/v propidium iodide) for 30 min in the dark. Samples were analysed as instrumental

triplicates by flow cytometry using a Becton Dickinson FACScan Flow Cytometer. Data were processed using a plot of FL2 against FL1 using FlowJo V10 (see Supplementary Figure 11). Statistical analysis was carried out using a two-tailed *t*-test assuming unequal variances (Welch's *t*-test).

Membrane integrity. Treated cell pellets were obtained as described for cell cycle analysis (without fixation in ethanol). After collection and centrifugation, the pellets were washed with PBS then resuspended in 500 µL staining buffer (50 μgmL^{-1} propidium iodide; 80 μgmL^{-1} RNAse) for 30 min, protected from light. After centrifugation, the supernatant was removed and cells resuspended in PBS. Samples were analysed as instrumental triplicates by flow cytometry using a Becton Dickinson FACScan Flow Cytometer. Propidium iodide (PI) was read using the FL2 channel. Data were processed using the histogram of FL2 using FlowJo V10 (see Supplementary Figure 12). Statistical analysis was carried out using a two-tailed *t*-test assuming unequal variances (Welch's *t*-test).

2. Synthesis of sulfonamide ligands

***N*-(Methanesulfonyl)-1,2-diphenylethylenediamine (MsDPEN).** To a solution of 1,2-diphenylethylenediamine (212 mg, 1.0 mmol, 1 mol equiv) and TEA (202 mg, 2 mmol, 2 mol equiv) in dichloromethane (3.2 mL) was added a solution of methanesulfonyl chloride (138 mg, 1.2 mmol, 1.2 mol equiv) in dichloromethane (2.1 mL) drop-wise over a period of 10 min. The reaction was allowed to warm to ambient temperature for 3 h. The resulting solution was washed with water (3 x 10 mL), and the product extracted with dichloromethane (2 x 10 mL). The organic phase was washed with saturated brine solution, dried over $MgSO_4$, filtered and concentrated *in vacuo* to yield a white semi-crystalline solid, which was recrystallized from toluene and washed with diethyl ether. (113 mg, 0.39 mmol, 39%). ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS): δ=7.10-7.40 (m, 10H; ArH), 4.56 (d, ^3J(H,H)=5.2 Hz, 1H; C*H*NHTs), 4.30 (d, ^3J(H,H)=5.2 Hz, 1H; C*H*NH_2), 2.27 (s, 3H; CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, 25°C, TMS) δ 128.9, 128.7, 128.6, 128.5, 127.9, 127.8, 127.6, 126.9, 126.7, 63.4, 62.6, 60.2, 41.9, 40.7; UV/Vis: λ_{max} 227 and 259 nm; HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{15}H_{19}N_2O_2S$, 291.1162; found, 291.1164.

***N*-(4-Nitrobenzenesulfonyl)-1,2-diphenylethylenediamine (NsDPEN).** Compounds were obtained following the method described for the synthesis of MsDPEN, using 4-nitrobenzenesulfonyl chloride (266 mg, 1.2 mmol, 1.2 mol equiv) in place of 4-toluenesulfonyl chloride. The product was isolated as a white semi-crystalline solid, which was recrystallized from toluene and washed with diethyl ether. (230 mg, 0.58 mmol, 58%). ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS): δ=7.96 (d, ^3J(H,H)=8.7 Hz, 2H; ArH), 7.53 (d, ^3J(H,H)=8.7 Hz, 2H; ArH), 7.18-7.25 (m, 5H; ArH), 7.12-7.18 (m, 5H; ArH), 4.49 (d, ^3J(H,H)=4.3 Hz, 1H; C*H*NHSO_2R), 4.21 (d, ^3J(H,H)=4.3 Hz, 1H; C*H*NH_2); ^{13}C NMR (125 MHz, $CDCl_3$, 25°C, TMS) δ 128.7, 128.6, 127.9, 126.8, 126.3, 123.7, 63.2, 60.1; UV/Vis: λ_{max} 264 nm; HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{20}H_{20}N_3O_4S$, 398.1169; found, 398.1169; analysis (calcd., found for (1*R*,2*R*)-$C_{20}H_{19}N_3O_4S$): C (60.44, 60.40), H (4.82, 4.90), N (10.57, 10.41); analysis (calcd., found for (1*S*,2*S*)-$C_{20}H_{19}N_3O_4S$): C (60.44, 60.42), H (4.82, 4.87), N (10.57, 10.45).

***N*-(4-Fluorobenzenesulfonyl)-1,2-diphenylethylenediamine (FbDPEN).** The compounds were obtained following the method described for the synthesis of MsDPEN, using 4-fluorobenzenesulfonyl chloride (934 mg, 4.8 mmol, 1.2 mol equiv) in place of 4-

toluenesulfonyl chloride. The product was isolated as a white crystalline solid, which was recrystallized from toluene and washed with diethyl ether. (1076 mg, 2.90 mmol, 73%). ^{1}H NMR (400 MHz, $CDCl_3$, 25°C, TMS): δ=7.41 (dd, ^{3}J(H,H)=8.8 Hz, ^{3}J(H,H)=5.1 Hz 2H; ArH) 7.06-7.22 (m, 10H; ArH), 6.81 (ddd, ^{3}J(H,H)=9.1, ^{3}J(H,H)=8.6 Hz, ^{4}J(H,F)=5.0 Hz, 2H; ArH), 4.43 (d, ^{3}J(H,H)=5.1 Hz, 1H; CH), 4.18 (d, ^{3}J(H,H)=5.1 Hz, 1H; CH); ^{13}C NMR (125 MHz, $CDCl_3$, 25°C, TMS) δ 129.5, 129.4, 128.4, 127.7, 127.6, 127.0, 126.5, 115.7, 115.5, 63.2, 60.4; UV/Vis: λ_{max} 235 and 260 nm; HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{20}H_{20}FN_2O_2S$, 371.1224; found, 371.1230; analysis (calcd., found for (1*R*,2*R*)-$C_{20}H_{19}FN_2O_2S$): C (64.85, 64.46), H (5.17, 5.02), N (7.56, 7.48); analysis (calcd., found for (1*S*,2*S*)- $C_{20}H_{19}FN_2O_2S$): C (64.85, 64.51), H (5.17, 5.24), N (7.56, 7.51).

***N*-(Benzenesulfonyl)-1,2-diphenylethylenediamine (BsDPEN).** The compounds were obtained following the method described for the synthesis of MsDPEN, using benzenesulfonyl chloride (848 mg, 4.8 mmol, 1.2 mol equiv) in place of 4-toluenesulfonyl chloride. The product was isolated as a white semi-crystalline solid, recrystallized from toluene and washed with diethyl ether. (569 mg, 1.61 mmol, 40%). ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS): δ=7.43 (d, ^{3}J(H,H)=7.6 Hz, 2H; ArH), 7.34 (t, ^{3}J(H,H)=7.4 Hz, 1H; ArH), 7.05-7.22 (m, 12H; ArH), 4.44 (br. d, ^{3}J(H,H)=5.2 Hz, 1H; C*H*NHSO$_2$R), 4.18 (br. s, 1H; C*H*NH$_2$); ^{13}C NMR (125 MHz, $CDCl_3$, 25°C, TMS) δ 131.9, 128.5, 128.5, 128.3, 127.7, 127.4, 127.0, 126.8, 63.2, 60.4; UV/Vis: λ_{max} 231, 233 and 260 nm; HRMS (*m/z*): $[M+H]^+$ calcd. for $C_{20}H_{21}N_2O_2S$, 353.1318; found, 353.1319; analysis (calcd., found for (1*R*,2*R*)-$C_{20}H_{20}N_2O_2S$): C (68.16, 67.87), H (5.72, 5.74), N (7.95, 7.94); analysis (calcd., found for (1*S*,2*S*)- $C_{20}H_{20}N_2O_2S$): C (68.16, 67.85), H (5.72, 5.73), N (7.95, 7.93).

3. Synthesis of osmium complexes

[Os(η^6-*p*-cymene)Cl$_2$]$_2$. To a solution of $OsCl_3.3H_2O$ (1.00 g, 2.8 mmol, 2 mol equiv) in degassed methanol (10 mL) was added α-phellandrene (3.8 g, 28 mmol, 20 mol equiv). The reaction was placed in a CEM Discovery-SP microwave for 10 min (413 K, 150 W, 250 psi) after which a crystalline orange precipitate was observed. The solution was washed with *n*-pentane (3 × 10 mL) before the solid was collected, washed with additional n-pentane (3 × 10 mL) and diethyl ether, yielding orange crystals (863 mg, 1.1 mmol, 79%). The complex was characterized by NMR, with data matching those previously reported.

[Os(η^6-biphenyl)Cl$_2$]$_2$. To a solution of $OsCl_3.3H_2O$ (1.00 g, 2.8 mmol, 2 mol equiv) in degassed methanol (10 mL) was added 3-phenyl-cyclohexa-1,4-diene (1.56 g, 10 mmol, 7 mol equiv). The reaction was placed in a CEM Discovery-SP microwave for 10 min (413 K, 150 W, 250 psi) after which a dark orange precipitate was observed. The solution was washed with *n*-pentane (3 × 10 mL) before the solid was collected, washed with additional n-pentane (3 × 10 mL) and diethyl ether, yielding a dark orange amorphous solid (936 mg, 1.3 mmol, 81%). The complex was characterized by NMR, with data matching those previously reported.

[Os(η^6-*m*-terphenyl)Cl$_2$]$_2$. To a solution of $OsCl_3.3H_2O$ (287 mg, 0.82 mmol, 2 mol equiv) in freshly distilled ethanol (20 mL) was added 1,4-dihydroterphenyl (dh-*m*-terp, 570 mg, 5 mol equiv). The solution was reacted under reflux for 24 h under a nitrogen atmosphere. A brown precipitate formed upon cooling which was washed with ethanol (25 mL), diethyl ether (5 × 25 mL) and isolated as a light brown solid (173 mg, 0.18 mmol, 22%). The complex was characterized by NMR, with data matching those previously reported.

[Os(η^6-*p*-cymene)(TsDPEN)] (2). This complex was synthesised as reported previously.[1] In dichloromethane (5 ml) were stirred osmium *p*-cymene-chlorido dimer (51.4 mg, 0.065 mmol 1 mol equiv) and (1*R*,2*R*)- or (1*S*,2*S*)-(H)TsDPEN (51.3 mg, 0.14 mmol, 2.1 mol equiv) in the presence of freshly ground potassium hydroxide pellets (56.1 mg, 1 mmol, 15 mol equiv). Doubly-distilled water (5 ml) was added with vigorous stirring (10 min). The organic phase was removed, washed with water and the solvent removed under reduced pressure to yield a red oil, which was recrystallised from dichloromethane/hexane. The product isolated as a red

crystalline solid (68 mg, 0.10 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$, 25°C, TMS): δ = 7.41 (d, ^{3}J(H,H)=7.6 Hz, 2H), 7.05-7.20 (m, 10H), 6.82 (d, ^{3}J(H,H)=8.0 Hz, 2H), 6.80 (*br* s, 1H; NH), 5.79 (d, ^{3}J(H,H)=5.6 Hz, 1H; Os-ArH), 5.62 (d, ^{3}J(H,H)=5.6 Hz, 1H; Os-ArH), 5.52 (d, ^{3}J(H,H)=5.6 Hz, 1H; Os-ArH), 5.42 (d, ^{3}J(H,H)=5.6 Hz, 1H; Os-ArH), 4.42 (s, 1H; $CHCHNH_2$), 3.94 (d, ^{3}J(H,H)=4.3 Hz, 1H; TsNCH), 2.45 (sept, ^{3}J(H,H)=6.9 Hz, 1H; $CH(CH_3)_2$), 2.23 (s, 3H; CH_3), 2.22 (s, 3H; CH_3), 1.23 (d, ^{3}J(H,H)=6.9 Hz, 3H; $CH(CH_3)_2$), 1.17 (d, ^{3}J(H,H)=6.9 Hz, 3H; $CH(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C, TMS) δ = 127.4, 127.0, 126.8, 126.0, 125.9, 125.9, 125.4, 81.7, 76.2, 72.4, 70.7, 70.0, 66.2, 22.5, 22.4, 20.2; UV/Vis: λ_{max} 260, 410 and 478 nm; HRMS (ESI): *m/z* calculated for $C_{31}H_{35}N_2O_2OsS$ [M+H$^+$]: 691.2028. Found: 691.2031. Elemental analysis for **(*R*,*R*)-2:** (calculated, found for $C_{31}H_{34}N_2O_2OsS$): C (54.05, 53.66), H (4.97, 4.88), N (4.07, 3.95); Elemental analysis for **(*S*,*S*)-2:** (calculated, found for $C_{31}H_{34}N_2O_2OsS$): C (54.05, 53.71), H (4.97, 4.84), N (4.07, 4.00).

[Os(η^6-*p*-cymene)(MsDPEN)] (3). Following the method described for complex **2** using (1*R*,2*R*)- or (1*S*,2*S*)-(H)MsDPEN (41 mg, 0.14 mmol, 2.1 mol equiv.) gave an orange amorphous solid which was recrystallized from DCM / hexane. (54 mg, 0.087 mmol, 67%). ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS): δ = 7.45 (d, ^{3}J(H,H) = 7.3 Hz, 2H), 7.00-7.40 (m, 9H), 5.87 (d, ^{3}J(H,H) = 5.6 Hz, 1H, Os-ArH), 5.79 (d, ^{3}J(H,H) = 5.6 Hz, 1H, Os-ArH), 5.75 (d, ^{3}J(H,H) = 5.7 Hz, 1H, Os-ArH), 5.72 (d, ^{3}J(H,H) = 5.7 Hz, 1H, Os-ArH), 4.34 (s, 1H, TsNCH), 3.99 (d, ^{3}J(H,H) = 2.8 Hz, 1H, CH), 2.68 (sept, ^{3}J(H,H) = 6.9 Hz, 1H, C*H*$(CH_3)_2$), 2.49 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 1.36 (d, ^{3}J(H,H) = 6.9 Hz, 6H, $CH(CH_3)_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ = 128.3, 128.0, 127.4, 127.1, 126.9, 126.4, 83.0, 75.9, 72.6, 72.5, 70.3, 68.5, 40.3, 32.9, 23.7, 23.5, 21.0. UV/Vis: λ_{max} 409 and 475 nm; HRMS (ESI): *m/z* calculated for $C_{25}H_{31}N_2O_2OsS$ [M+H$^+$]: 615.1714. Found: 615.1710. Elemental analysis for **(*R*,*R*)-3:** (calculated, found for $C_{25}H_{30}N_2O_2OsS$): C (49.00, 49.06), H (4.93, 4.92), N (4.57, 4.66); Elemental analysis for **(*S*,*S*)-3:** (calculated, found for $C_{25}H_{30}N_2O_2OsS$): C (49.00, 48.72), H (4.93, 4.80), N (4.57, 4.62).

[Os(η^6-*p*-cymene)(NsDPEN)] (4). Following the method described for complex **2** using (1*R*,2*R*)- or (1*S*,2*S*)-(H)NsDPEN (56 mg, 0.14 mmol, 2.1 mol equiv) gave a dark red amorphous solid which was recrystallized from DCM / hexane. (57 mg, 0.079 mmol, 70%). ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS): δ = 7.86 (d, ^{3}J(H,H) = 8.9 Hz, 2H), 7.45 (d, ^{3}J(H,H) = 8.9 Hz, 2H), 7.00-7.25 (m, 11H), 5.95 (d, ^{3}J(H,H) = 5.6 Hz, 1H, Os-ArH), 5.80 (d,

^3J(H,H) = 5.6 Hz, 1H, Os-ArH), 5.74 (d, ^3J(H,H) = 5.5 Hz, 1H, Os-ArH), 5.68 (d, ^3J(H,H) = 5.5 Hz, 1H, Os-ArH), 4.37 (s, 1H, TsNCH), 4.02 (d, ^3J(H,H) = 4.2 Hz, 1H, CH), 2.61 (sept, ^3J(H,H) = 6.9 Hz, 1H, C*H*(CH_3)$_2$), 2.39 (s, 3H, CH_3), 1.35 (d, ^3J(H,H) = 6.9 Hz, 3H, CH(C*H*$_3$)$_2$), 1.28 (d, ^3J(H,H) = 6.9 Hz, 3H, CH(C*H*$_3$)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 128.1, 127.9, 127.4, 127.3, 126.9, 126.8, 126.3, 126.2, 123.1, 82.5, 73.3, 72.0, 71.1, 67.4, 32.9, 24.1, 23.5, 21.0; UV/Vis: λ_{max} 263 and 409 nm; HRMS (ESI): *m/z* calculated for $C_{30}H_{32}N_3O_4OsS$ [M+H$^+$]: 722.1722. Found: 722.1716. Elemental analysis for **(*R*,*R*)-4:** (calculated, found for $C_{30}H_{31}N_3O_4OsS$): C (50.05, 49.69), H (4.34, 4.47), N (5.84, 5.58); Elemental analysis for **(*S*,*S*)-4:** (calculated, found for $C_{30}H_{31}N_3O_4OsS$): C (50.05, 50.02), H (4.37, 4.22), N (5.84, 5.79).

[Os(η^6-*p*-cymene)(FbDPEN)] (5). Following the method described for complex **2** using (1*R*,2*R*)- or (1*S*,2*S*)-(H)FbDPEN (52 mg, 0.14 mmol, 2.1 mol equiv) yielded an orange amorphous solid of either enantiomer. (50 mg, 0.072 mmol, 52%). ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS): δ = 7.46 (d, ^3J(H,H) = 7.5Hz, 2H), 7.29-7.34 (m, 2H), 6.90-7.26 (m, 9H), 6.75 (m, 2H), 5.90 (d, ^3J(H,H) = 5.6 Hz, 1H, Os-ArH), 5.73 (d, ^3J(H,H) = 5.6 Hz, 1H, Os-ArH), 5.64 (d, ^3J(H,H) = 5.6 Hz, 1H, Os-ArH), 5.55 (d, ^3J(H,H) = 5.6 Hz, 1H, Os-ArH), 4.47 (s, 1H, TsNCH), 4.03 (d, ^3J(H,H) = 3.6 Hz, 1H, CH), 2.55 (sept, ^3J(H,H) = 6.9 Hz, 1H, C*H*(CH_3)$_2$), 2.32 (s, 3H, CH_3), 1.32 (d, ^3J(H,H) = 6.9 Hz, 3H, CH(C*H*$_3$)$_2$), 1.25 (d, ^3J(H,H) = 6.9 Hz, 3H, CH(C*H*$_3$)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 129.2, 129.1, 128.1, 127.9, 127.2, 126.9, 126.6, 126.4, 114.8, 114.6, 82.6, 76.6, 73.5, 72.0, 71.0, 67.3, 32.8, 23.6, 23.4, 21.0. UV/Vis: λ_{max} 408 and 474 nm; HRMS (ESI): *m/z* calculated for $C_{30}H_{32}FN_2O_2OsS$ [M+H$^+$]: 695.1777. Found: 695.1780. Elemental analysis for **(*R*,*R*)-5:** (calculated, found for $C_{30}H_{31}FN_2O_2OsS$): C (52.00, 51.85), H (4.51, 4.46), N (4.04, 4.09); Elemental analysis for **(*S*,*S*)-5:** (calculated, found for $C_{30}H_{31}FN_2O_2OsS$): C (52.00, 51.70), H (4.51, 4.43), N (4.04, 4.04).

[Os(η^6-*p*-cymene)(BsDPEN)] (6). The method described for complex **2** using (1*R*,2*R*)- or (1*S*,2*S*)-(H)BsDPEN (49 mg, 0.14 mmol, 2.1 mol equiv) gave an orange amorphous solid. (44 mg, 0.065 mmol, 46%). ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS): δ = 7.48 (d, ^3J(H,H) = 7.5 Hz, 2H), 7.36 (m, 2H), 6.90-7.30 (m, 12H), 5.87 (d, ^3J(H,H) = 5.6 Hz, 1H, Os-ArH), 5.70 (d, ^3J(H,H) = 5.6 Hz, 1H, Os-ArH), 5.60 (d, ^3J(H,H) = 5.6 Hz, 1H, Os-ArH), 5.48 (d, ^3J(H,H) = 5.6 Hz, 1H, Os-ArH), 4.55 (s, 1H, TsNCH), 4.05 (d, ^3J(H,H) = 4.0 Hz, 1H, CH) 2.50 (sept, ^3J(H,H) = 6.9 Hz, 1H, C*H*(CH_3)$_2$), 2.27 (s, 3H, CH_3), 1.30 (d, ^3J(H,H) = 6.9 Hz, 3H,

CH(C*H*$_3$)$_2$), 1.22 (d, ^3J(H,H) = 6.9 Hz, 3H, CH(C*H*$_3$)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 130.4, 128.1, 127.8, 127.2, 126.9, 126.5, 82.8, 73.6, 71.9, 70.9, 67.3, 32.7, 23.6, 23.4, 20.6. UV/Vis: λ_{max} 259, 409 and 475 nm; HRMS (ESI): *m/z* calculated for $C_{30}H_{33}N_2O_2OsS$ [M+H$^+$]: 677.1871. Found: 677.1876. Elemental analysis for **(*R*,*R*)-6:** (calculated, found for $C_{30}H_{32}N_2O_2OsS$): C (53.39, 53.63), H (4.78, 4.72), N (4.15, 4.14); Elemental analysis for **(*S*,*S*)-6:** (calculated, found for $C_{30}H_{32}N_2O_2OsS$): C (53.39, 53.46), H (4.78, 4.75), N (4.15, 4.16).

[Os(η^6-biphenyl)(TsDPEN)] (7). [Os(η^6-biphenyl)Cl_2]$_2$ (67 mg, 0.081 mmol, 1.1 mol equiv) and (1*R*,2*R*)- or (1*S*,2*S*)-TsDPEN (55 mg, 0.15 mmol, 2 mol equiv), in a stirred solution in dichloromethane (2.5 ml) were reacted in a CEM Discovery-SP microwave reactor for 10 min (393K, 150 W, 250 psi). The resulting solution was filtered and combined with freshly ground potassium hydroxide pellets (56.1 mg, 1 mmol, 15 mol equiv) with stirring. Doubly-distilled water (5 mL) was added and the reaction proceeded at ambient temperature (10 min). The organic phase was isolated, washed twice with water and the solvent removed under reduced pressure to isolate the product as a dark red amorphous solid (62 mg, 0.09 mmol, 59%). ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS): δ = 7.50-6.85 (m, 19H), 6.25-5.95 (m, 4H, Os-ArH), 4.45 (s, 1H, CH), 3.95 (d, ^3J(H,H) = 3.8 Hz, 1H, C*H*NH), 2.30 (s, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 146.3, 144.1, 141.2, 140.8, 137.7, 129.2, 128.9, 128.6, 128.2, 128.0, 127.2, 127.0, 126.9, 126.6, 126.5, 83.3, 73.7, 73.3, 73.1, 70.9, 70.5, 68.3, 21.4. UV/Vis: λ_{max} 358, 416 and 486 nm; HRMS (ESI): *m/z* calculated for $C_{33}H_{31}N_2O_2OsS$ [M+H$^+$]: 711.1715. Found: 711.1712. Elemental analysis for **(*R*,*R*)-7:** (calculated, found for $C_{33}H_{30}N_2O_2OsS$): C (55.91, 55.62), H (4.27, 4.11), N (3.95, 3.88); Elemental analysis for **(*S*,*S*)-7:** (calculated, found for $C_{33}H_{30}N_2O_2OsS$): C (55.91, 55.60), H (4.27, 4.08), N (3.95, 4.00).

[Os(η^6-*m*-terp)(TsDPEN)] (8). The method described for **7**, using dimer [Os(η^6-*m*-terphenyl)Cl_2]$_2$ (40 mg, 0.04 mmol, 1 mol equiv) and ligand (1*R*,2*R*)- or (1*S*,2*S*)-(H)TsDPEN gave an orange solid (28 mg, 0.036 mmol, 43%). ^{1}H NMR (400 MHz, $CDCl_3$, 25°C, TMS): δ = 7.80-7.74 (m, 2H, ArH), 7.57-7.51 (m, 2H, ArH), 7.45-7.36 (m, 6H, ArH), 7.33-7.28 (m, 2H, ArH), 7.19-7.03 (m, 8H, ArH), 7.00-6.94 (m, 2H, ArH), 6.89 (br. d, 1H, NH), 6.80-6.75 (m, 2H, ArH), 6.70 (d, ^3J(H,H) = 5.7 Hz, 1H, ArH), 6.60 (s, 1H, ArH), 6.46 (d, ^3J(H,H) = 5.5 Hz, 1H, ArH), 6.30 (t, ^3J(H,H) = 5.7 Hz, 1H, ArH), 4.41 (s, 1H, C*H*NTs), 3.84 (d, ^3J(H,H) = 4.3 Hz, 1H, C*H*NH), 2.28 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 129.2, 129.0,

128.8, 128.7, 128.6, 128.4, 128.3, 128.0, 127.8, 127.3, 126.9, 126.7, 126.3, 83.2, 74.6, 73.9, 68.9, 66.7. UV/Vis: λ_{max} 419 and 490 nm; HRMS (ESI): *m/z* calculated for $C_{39}H_{35}N_2O_2OsS$ $[M+H^+]$: 787.2029. Found: 787.2033.

4. Supplementary Tables 1-18

Supplementary Table 1: X-ray crystallographic data for ***R,R*-7** and ***S,S*-7**

	***R,R*-7 • 2CHCl$_3$**	***S,S*-7 • 2CHCl$_3$**
Crystal character	red rod	red rod
Empirical formula	$C_{33}H_{30}N_2O_2OsS \cdot 2CHCl_3$	$C_{33}H_{30}N_2O_2OsS \cdot 2CHCl_3$
MW / gmol^{-1}	947.59	947.59
Temperature / K	150(2)	150(2)
Crystal system	orthorhombic	orthorhombic
Space group	$P2_12_12_1$	$P2_12_12_1$
***a* (Å)**	7.95564(14)	7.95923(12)
***b* (Å)**	16.3879(3)	16.3940(3)
***c* (Å)**	26.8668(5)	26.8780(5)
***α* / °**	90	90
***β* / °**	90	90
***γ* / °**	90	90
Volume / Å^3	3502.80(11)	3507.14(10)
Z	4	4
μ / mm^{-1}	4.194	4.189
F (000)	1864.0	1864.0
Crystal size / mm^3	0.35 × 0.01 × 0.01	0.40 × 0.06 × 0.02
Reflections measured	49980	50022
Independent reflections	10869	10950
R_1 [I>2σ(I)]	0.0333	0.0361
wR_2	0.0574	0.0623
ρ_{calc} / gcm^{-3}	1.797	1.795
Flack parameter	-0.023(5)	-0.0147(18)
CCDC number	1507733	1507732

Supplementary Table 2: Antiproliferative activity data (IC_{50}) for sulfonamide ligands and L or D-lactate in A2780 human ovarian cancer cells.

Ligand	IC_{50} (μM)
(H)TsDPEN	> 100
(H)MsDPEN	> 100
(H)NsDPEN	> 100
(H)FbDPEN	> 100
(H)BsDPEN	> 100
L-lactate	> 2000
D-lactate	> 2000

Supplementary Table 3: Antiproliferative activity (IC_{50} / μM) determinations for complexes **2**-**8** in A2780 ovarian cancer cells and PC3 prostate cancer cells (310 K, 24 h drug exposure + 72 h recovery time in drug-free medium); compared with partition coefficients (Log $P_{oct/water}$) and cellular Os accumulation (ng Os × 10^6 cells) in A2780 cancer cells exposed to complexes for 24 h at IC_{50} concentrations with no recovery time.

	Complex	IC_{50} (A2780)	IC_{50} (PC3)	Log $P_{oct/water}$	ng Os × 10^6 cells
2	[Os(*p*-cymene)(TsDPEN)]	15.5 ± 0.5	12.0 ± 0.3	1.45 ± 0.02	30 ± 2
3	[Os(*p*-cymene)(MsDPEN)]	30 ± 2	N.D.[b]	0.18 ± 0.04	4.8 ± 0.8
4	[Os(*p*-cymene)(NsDPEN)]	19.9 ± 0.5	N.D.[b]	0.71 ± 0.01	8.1 ± 0.3
5	[Os(*p*-cymene)(FbDPEN)]	17 ± 1	N.D.[b]	0.30 ± 0.03	10 ± 2
6	[Os(*p*-cymene)(BsDPEN)]	13.5 ± 0.9	N.D.[b]	0.48 ± 0.02	5.8 ± 0.7
7	[Os(*p*-cymene)(TsDPEN)]	6.5 ± 0.3	9.9 ± 0.2	1.91 ± 0.04	11 ± 1
8	[Os(*m*-terphenyl)(TsDPEN)]	4.4 ± 0.3	13.6 ± 0.2	2.3 ± 0.2	31.9 ± 0.4

[a] Inactive in the concentration range investigated. [b] N.D. = not determined.

Supplementary Table 4: Cellular distribution (population %) of Os in fractionated A2780 cells treated with **2** at IC_{50} concentration (24 h drug exposure, 310 K). Cell fractions prepared using BioVision FractionPREP cell fractionation kit. Cytosol (total cellular soluble proteins), Membrane (total cellular membrane proteins including cellular organelles and organelle membrane proteins – excluding nuclear membrane protein); nucleus (total nucleus soluble proteins and nuclear membrane proteins), Cytoskeleton (total insoluble proteins and DNA).

	Normalized population %			
	Cytosol	**Membrane**	**Nucleus**	**Cytoskeleton**
[Os(p-cymene)(TsDPEN)] **2**	47 ± 2	48 ± 3	1.6 ± 0.5	2.9 ± 0.3

Supplementary Table 5: Osmium efflux from A2780 cancer cells exposed to ***R,R*-2** for 24 h (IC_{50} concentration) with variable recovery time (up to 72 h) in drug-free medium. Data collected every 24 h, and are normalised to the total uptake with no recovery time (100%).

% Os accumulation (recovery time / h)			
0 h	24 h	48 h	72 h
100 ± 2	34 ± 4	19 ± 2	15 ± 2

Supplementary Table 6: Cell cycle analysis for A2780 cells treated with complex **2** (1 × IC_{50}, 310 K, 24 h drug exposure) in the presence / absence of sodium formate (2 mM).

	Normalized population %					
	No recovery time			**72 h recovery time**		
	G_1	**S**	**G_2/M**	**G_1**	**S**	**G_2/M**
Untreated	52 ± 2	24 ± 1	24.3 ± 0.5	62 ± 1	30.0 ± 0.8	5.0 ± 0.6
2 - formate	66.0 ± 0.9	18.1 ± 0.3	16.0 ± 0.6	66 ± 1	22.3 ± 0.4	5 ± 1
2 + formate	63 ± 3	17 ± 1	20 ± 2	71.4 ± 0.7	20.4 ± 0.4	6.3 ± 0.7

Supplementary Table 7: Induction of apoptosis in A2780 cancer cells treated with complex **2** (1 × IC_{50} concentration, 310 K, 24 h drug exposure) in the presence and absence of sodium formate (2 mM). Q1: FL1-Fl2- (viable cells). Q2: FL1+FL2- (early-apoptotic cells). Q3: FL1+FL2+ (late-apoptotic cells). Q4: FL1-FL2+ (non-viable cells).

	Normalized population %							
	No recovery time				**72 h recovery time**			
	Viable	Early-apoptotic	Late-apoptotic	Non-viable	Viable	Early-apoptotic	Late-apoptotic	Non-viable
Untreated	96.2 ± 0.2	0.03 ± 0.01	1.1 ± 0.2	2.6 ± 0.1	93.2 ± 0.3	0.01 ± 0.01	0.02 ± 0.01	6.8 ± 0.2
2 -formate	96.6 ± 0.3	0.01 ± 0.01	0.2 ± 0.1	3.3 ± 0.2	92.6 ± 0.5	0.03 ± 0.01	0.01 ± 0.01	7.4 ± 0.5
2 +formate	96.1 ± 0.1	0.02 ± 0.01	0.4 ± 0.2	3.5 ± 0.2	94.2 ± 0.5	0.01 ± 0.01	0.02 ± 0.01	5.8 ± 0.5

Supplementary Table 8: Membrane integrity assessed by flow cytometry for A2780 cancer cells treated with complex **2** (1 × IC_{50} concentration, 310 K, 24 h drug exposure) in the presence and absence of sodium formate (2 mM). Viable cell membrane (FL2-). Non-viable cell membrane (FL2+).

	Normalized population %			
	No recovery time		**72 h recovery time**	
	Viable	Non-viable	Viable	Non-viable
Untreated	97.4 ± 0.2	2.6 ± 0.1	94.4 ± 0.5	5.6 ± 0.4
2 - formate	97.8 ± 0.1	2.2 ± 0.1	95.6 ± 0.4	4.4 ± 0.3
2 + formate	98.4 ± 0.1	1.6 ± 0.1	93.1 ± 0.2	6.9 ± 0.2

Supplementary Table 9: Conversion (%) kinetics for the aqueous-phase reduction of pyruvate in phosphate-buffered saline by osmium catalyst ***R,R*-2** in the presence of sodium formate (600 MHz, 90% H_2O / 10% D_2O, 310 K. Final concentrations: Os complex = 10 μM; pyruvate = 2 mM; sodium formate = 4 mM or 30 mM).

	Final concentration / μM					
Catalyst	[catalyst]	[pyruvate]	[formate]	Ratio	TON	TOF / h^{-1}
***R,R*-2**	10	2000	4000	1: 200 : 400	21 (14 h)	1.5 ± 0.1
***R,R*-2**	10	2000	30000	1 : 200 : 3000	65 (4 h)	16.4 ± 0.7

Supplementary Table 10: 24 h aqueous-phase reduction of pyruvate at 310 K in phosphate-buffered saline by osmium catalysts ***R,R*-2** or ***S,S*-2**, in the presence of sodium formate (final concentrations: Os complex = 15 μM (1.0 × IC_{50}); pyruvate = 1 mM; sodium formate = 2 mM).

Complex	L-Lactate / μM	D-Lactate / μM	e.e. / %	Major product
[Os(*p*-cymene)((*R*,*R*)-TsDPEN)]	1 ± 2	9.2 ± 0.6	83	D-lactate
[Os(*p*-cymene)((*S*,*S*)-TsDPEN)]	12 ± 2	1 ± 0.6	84	L-lactate

Supplementary Table 11: Normalized extent of proliferation of A2780 cancer cells, MRC5 fibroblasts and HOF ovarian fibroblasts and after co-administration of an osmium catalyst (**2** or **7**; 0.5 × IC_{50}) and sodium formate (0.5, 1.0, 2.0 mM). 24 h drug exposure time + 72 h recovery in drug-free medium. Statistics calculated using a two-tailed *t*-test with unequal variances (Welch's t-test).

Cell line	Formate / mM	Complex			
		***R,R*-2**	***S,S*-2**	***S,S*-7**	**No complex**
A2780	0.0	1.00 ± 0.03	1.00 ± 0.04	1.00 ± 0.05	1.00 ± 0.09
	0.5	0.76 ± 0.05 (p = 0.0063)	0.90 ± 0.04 (p = 0.0537)	0.20 ± 0.06 (p = 0.0004)	1.00 ± 0.07 (p = 0.8908)
	1.0	0.3 ± 0.1 (p = 0.0092)	0.22 ± 0.06 (p = 0.0003)	0.2 ± 0.1 (p = 0.0098)	0.98 ± 0.07 (p = 0.7540)
	2.0	0.2 ± 0.1 (p = 0.0059)	0.17 ± 0.07 (p = 0.0004)	0.08 ± 0.09 (p = 0.0006)	0.99 ± 0.07 (p = 0.9339)
MRC5	0.0	1.00 ± 0.03	1.00 ± 0.03	1.00 ± 0.03	1.00 ± 0.09
	0.5	0.99 ± 0.04 (p = 0.6445)	0.99 ± 0.04 (p = 0.8169)	0.98 ± 0.04 (p = 0.5701)	0.96 ± 0.07 (p = 0.5674)
	1.0	0.90 ± 0.06 (p = 0.1448)	0.95 ± 0.03 (p = 0.1238)	0.98 ± 0.05 (p = 0.6001)	0.93 ± 0.08 (p = 0.3746)
	2.0	0.72 ± 0.04 (p = 0.0770)	0.85 ± 0.06 (p = 0.0530)	0.88 ± 0.08 (p = 0.1323)	0.95 ± 0.08 (p = 0.5318)
HOF	0.0	1.00 ± 0.04	1.00 ± 0.03	1.00 ± 0.03	1.00 ± 0.02
	0.5	1.03 ± 0.04 (p =0.4475)	0.94 ± 0.03 (p =0.1835)	0.94 ± 0.02 (p = 0.2558)	0.99 ± 0.03 (p = 0.7620)
	1.0	1.00 ± 0.05 (p =0.9789)	0.87± 0.03 (p =0.0493)	0.87 ± 0.02 (p =0.1223)	0.92 ± 0.02 (p = 0.0572)
	2.0	1.03 ± 0.03 (p =0.3341)	0.94 ± 0.02 (p = 0.2558)	0.94 ± 0.02 (p = 0.2558)	0.90 ± 0.03 (p = 0.1589)

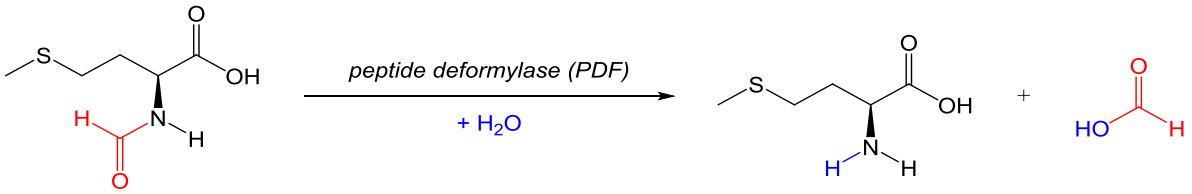
Supplementary Table 12: Normalized extent of proliferation of A2780 human cancer cells after co-administration of osmium catalyst (**2** or **7**; 0.5 × IC_{50}) together with sodium acetate (0.5, 1.0 and 2.0 mM). 24 h drug exposure time + 72 h recovery in drug-free medium. Statistics calculated using a two-tailed t-test with unequal variances (Welch's t-test).

Cell line	Acetate / mM	Complex			
		***R*,*R*-2**	***S*,*S*-2**	***S*,*S*-7**	**No complex**
A2780	0.0	1.00 ± 0.06	1.00 ± 0.02	1.00 ± 0.04	1.00 ± 0.06
	0.5	1.00 ± 0.06 (p = 0.9551)	0.95 ± 0.03 (p = 0.0695)	1.04 ± 0.04 (p = 0.3140)	0.99 ± 0.06 (p = 0.8383)
	1.0	1.04 ± 0.08 (p = 0.5508)	1.04 ± 0.03 (p = 0.1279)	1.08 ± 0.05 (p = 0.1055)	0.99 ± 0.09 (p = 0.8526)
	2.0	1.02 ± 0.05 (p = 0.7140)	0.98 ± 0.03 (p = 0.2665)	1.11 ± 0.04 (p = 0.0947)	0.99 ± 0.08 (p = 0.8783)

Supplementary Table 13: Cellular Os accumulation (ng Os × 10^6 cells) in A2780 cancer cells treated with either complex **2** or **7** at ⅓ × IC_{50} concentration in the presence and absence of sodium formate (2 mM). 24 h exposure, no recovery time in drug-free medium, 310 K. Statistics calculated using a two-tailed t-test with unequal variances (Welch's t-test).

	Cellular metal accumulation (ng Os × 10^6 cells)	
	0 mM formate	2 mM formate
[Os(p-cymene)(TsDPEN)] **2**	11 ± 2	10 ± 2 (p = 0.7741)
[Os(biphenyl)(TsDPEN)] **7**	7 ± 1	8 ± 2 (p = 0.7782)

Supplementary Table 14: Normalized proliferation of PC3 human prostate cancer cells after co-administration of osmium catalyst (**2** or **7**; 0.5 × IC_{50}) with either N-formyl-methionine or N-acetyl-methionine (0.25, 0.5 and 1.0 mM). 24 h drug exposure time +72 h recovery in drug-free medium. Statistics calculated using a two-tailed t-test with unequal variances (Welch's t-test).



Complex	**N-formyl-methionine (mM)**			
	0.00	0.25	0.50	1.00
[Os(*p*-cymene)((*S*,*S*)-TsDPEN)] ***S*,*S*-2**	1.00 ± 0.09	0.96 ± 0.07 (*p* = 0.4047)	0.91 ± 0.10 (*p* = 0.1465)	0.87 ± 0.06 (*p* = 0.0147)
[Os(biphenyl)((*S*,*S*)-TsDPEN)] ***S*,*S*-7**	1.00 ± 0.08	0.98 ± 0.08 (*p* = 0.6082)	0.88 ± 0.09 (*p* = 0.0424)	0.80 ± 0.06 (*p* = 0.0012)
No osmium complex Negative control	1.00 ± 0.07	1.00 ± 0.08 (*p* = 0.9760)	1.0 ± 0.1 (*p* = 0.6205)	1.0 ± 0.1 (*p* = 0.9830)

Complex	**N-acetyl-methionine (mM)**			
	0.00	0.25	0.50	1.00
[Os(*p*-cymene)((*S*,*S*)-TsDPEN)] ***S*,*S*-2**	1.00 ± 0.02	1.01 ± 0.06 (*p* = 0.7283)	1.04 ± 0.07 (*p* = 0.4130)	0.98 ± 0.02 (*p* = 0.4101)
[Os(biphenyl)((*S*,*S*)-TsDPEN)] ***S*,*S*-7**	1.00 ± 0.03	1.01 ± 0.08 (*p* = 0.8034)	1.03 ± 0.05 (*p* = 0.3956)	0.96 ± 0.09 (*p* = 0.5614)
No osmium complex Negative control	1.00 ± 0.07	1.0 ± 0.2 (*p* = 0.7406)	1.1 ± 0.2 (*p* = 0.6679)	1.1 ± 0.1 (*p* = 0.4302)

Supplementary Table 15: Normalized intracellular D-lactate concentration (μM) determined in A2780 human ovarian cancer cells treated with complex ***R,R*-2** or ***S,S*-2** (1.0 × IC_{50}) for 24 h with no recovery time, in the absence and presence of 2 mM sodium formate. Statistics calculated using a two-tailed t-test with unequal variances (Welch's t-test).

Complex	D-lactate / μM	
	0 mM formate	2 mM formate
[Os(*p*-cymene)((*R*,*R*)-TsDPEN)] ***R,R*-2**	47.6 ± 0.5	79 ± 10 (p = 0.0474)
[Os(*p*-cymene)((*S*,*S*)-TsDPEN)] ***S,S*-2**	38 ± 10	51 ± 4 (p = 0.1797)
No osmium complex Negative control	31 ± 5	29 ± 2 (p = 0.7259)

Supplementary Table 16: Statistical analysis (p values) comparing the normalized concentration of intracellular D-lactate in A2780 cancer cells that were treated with osmium catalyst ***R,R*-2** or ***S,S*-2**, with and without co-administration of sodium formate. (2 mM). Probabilities were calculated using a two-tailed t-test with unequal variances (Welch's t-test). Samples that significantly differ (95% confidence level; $p < 0.05$) are underlined and bold.

		No osmium complex		***R,R*-2**		***S,S*-2**	
	Formate (mM)	0.00	2.00	0.00	2.00	0.00	2.00
No osmium complex	0.00	-	0.7259	0.1337	**<u>0.0196</u>**	0.3981	0.1305
	2.00		-	**<u>0.0048</u>**	**<u>0.0137</u>**	0.2684	**<u>0.0028</u>**
***R,R*-2**	0.00			-	**<u>0.0474</u>**	0.2587	0.2391
	2.00				-	**<u>0.0163</u>**	**<u>0.0452</u>**
***S*,S-2**	0.00					-	0.1797
	2.00						-

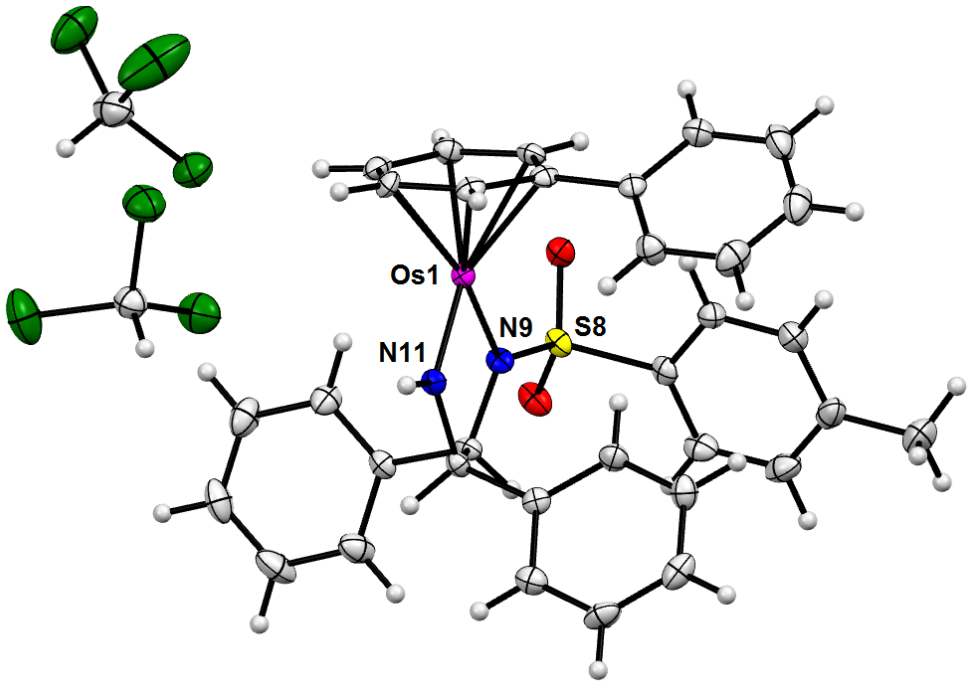
Supplementary Table 17: Normalized extracellular D-lactate concentration (μM) determined for A2780 human ovarian cancer cells treated with complex ***R,R*-2** or ***S,S*-2** ($1.0 \times IC_{50}$) for 24 h with no recovery time, in the absence and presence of 2 mM sodium formate. Statistics calculated using a two-tailed t-test with unequal variances (Welch's t-test).

Complex	D-lactate / μM	
	0 mM formate	2 mM formate
[Os(*p*-cymene)((*R*,*R*)-TsDPEN)] ***R,R*-2**	32 ± 4	32 ± 4 (p = 0.8801)
[Os(*p*-cymene)((*S*,*S*)-TsDPEN)] ***S,S*-2**	35 ± 5	36 ± 5 (p = 0.8320)
No osmium complex Negative control	38 ± 4	36 ± 6 (p = 0.6769)

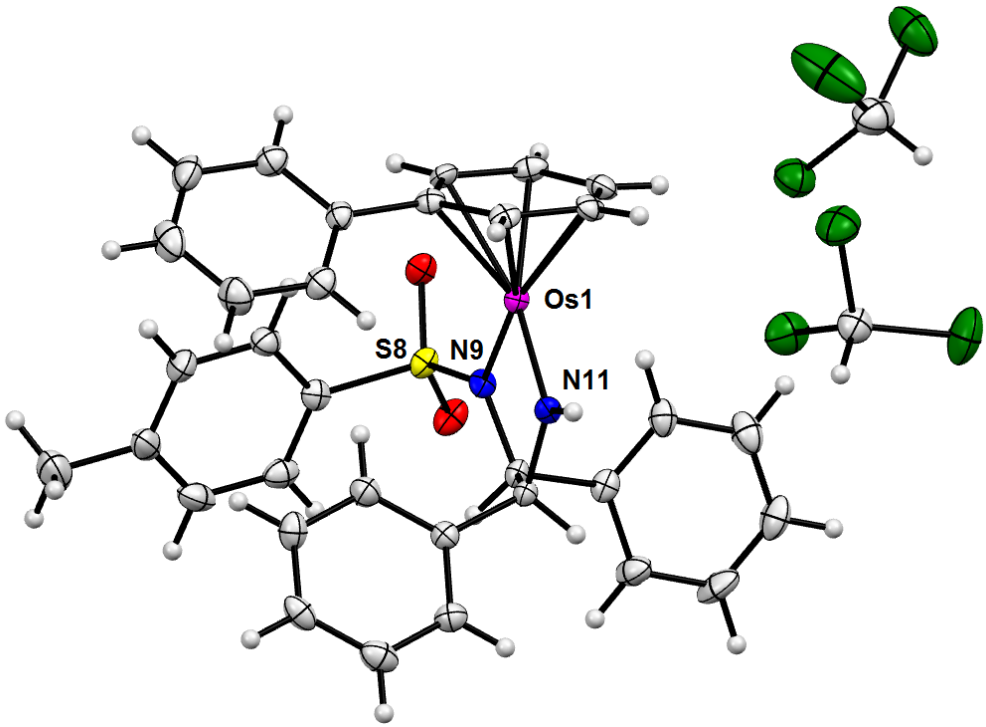
Supplementary Table 18: Statistical analysis (p values) comparing the normalized concentration of extracellular D-lactate for A2780 cancer cells treated with osmium catalyst ***R,R*-2** or ***S,S*-2**, with or without co-administration of sodium formate. (2 mM). Probabilities were calculated using a two-tailed t-test with unequal variances (Welch's t-test). Samples that significantly differ (at the 95% confidence level; $p < 0.05$) are underlined and bold.

		No osmium complex		***R,R*-2**		***S,S*-2**	
	Formate (mM)	0.00	2.00	0.00	2.00	0.00	2.00
No osmium complex	0.00	-	0.6769	0.1270	0.1125	0.4136	0.5365
	2.00		-	0.6335	0.5942	0.9287	0.9600
***R,R*-2**	0.00			-	0.8801	0.5157	0.3823
	2.00				-	0.4535	0.3353
***S*,S-2**	0.00					-	0.8320
	2.00						-

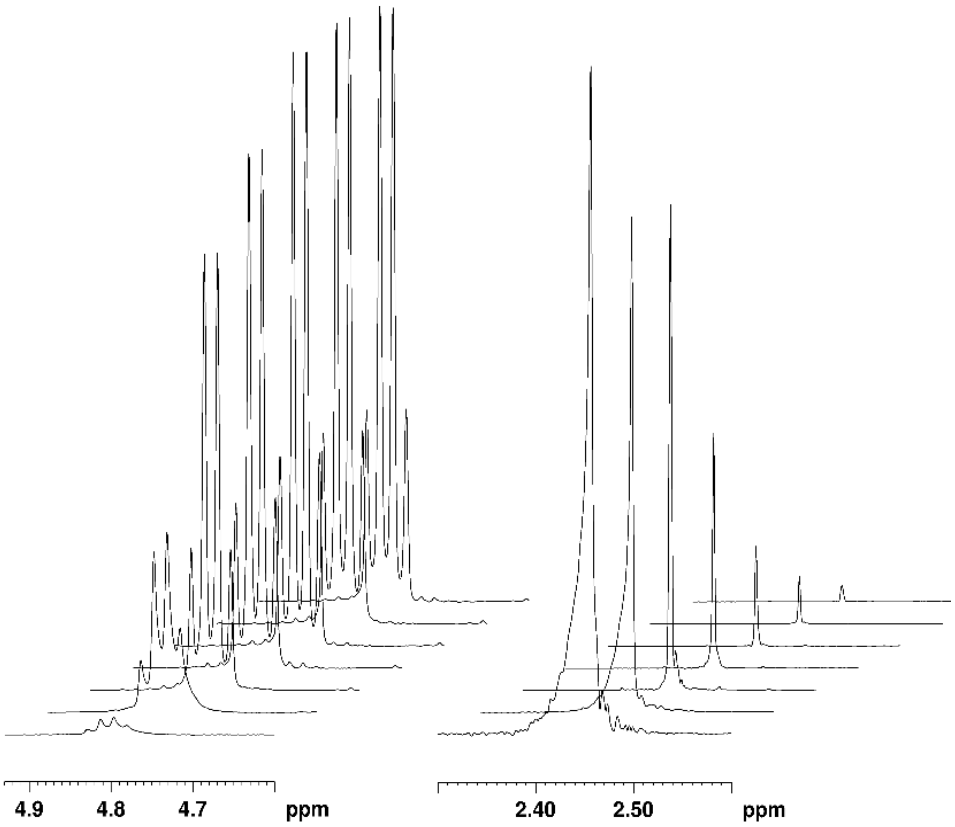
Supplementary Figures 1-12



Supplementary Figure 1: ORTEP diagram for **(*R*,*R*)-7 • 2CHCl$_3$**. Thermal ellipsoids shown at 50% probability level.



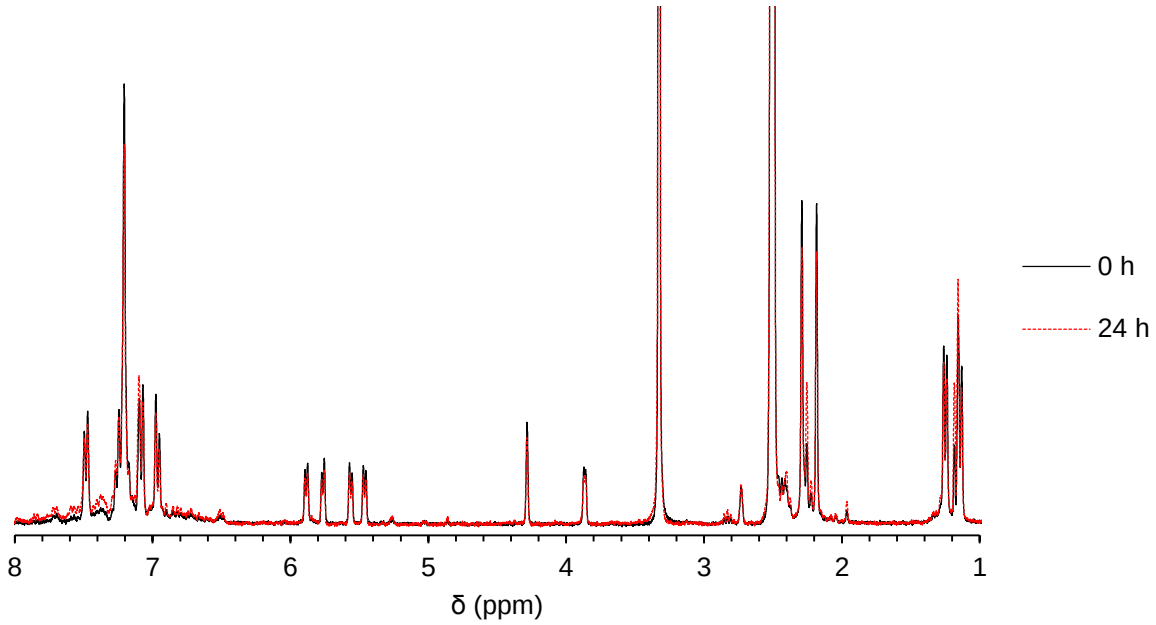
Supplementary Figure 2: ORTEP diagram for **(*S*,*S*)-7 • 2CHCl$_3$.** Thermal ellipsoids shown at 50% probability level.



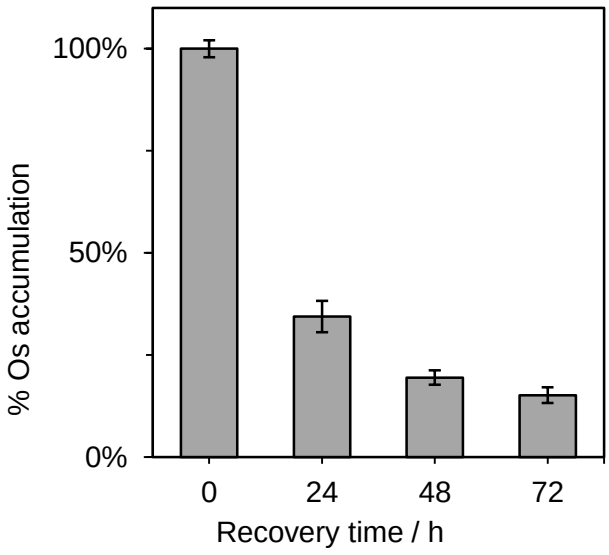
$$\text{conversion} = 100 - \left(\frac{\frac{I_{2.25-2.65}}{3}}{I_{4.55-5.00} + \frac{I_{2.25-2.65}}{3}} \times 100\% \right)$$

$$\text{TON}_t = \frac{[\text{conversion}]_\text{t}}{100} \cdot \frac{[\text{substrate}]_0}{[\text{catalyst}]} = \frac{\delta[\text{TON}]_\text{t}}{\delta \text{t}}$$

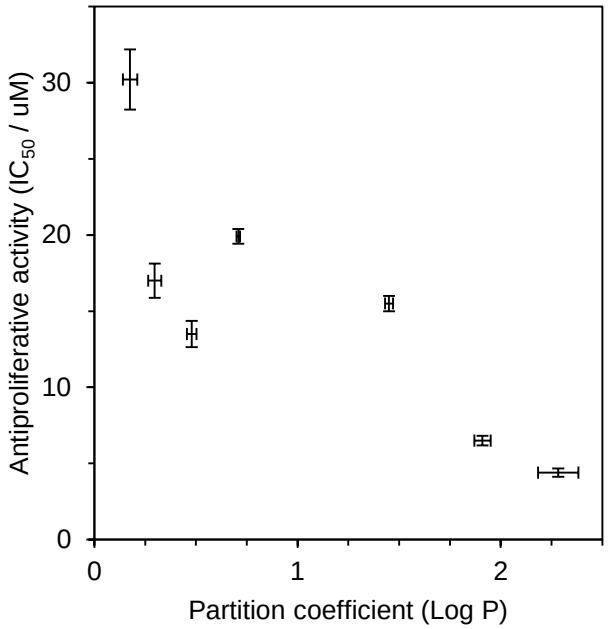
Supplementary Figure 3: Catalytic reduction of acetophenone, monitored by ^{1}H-NMR allows facile determination of reaction kinetics.



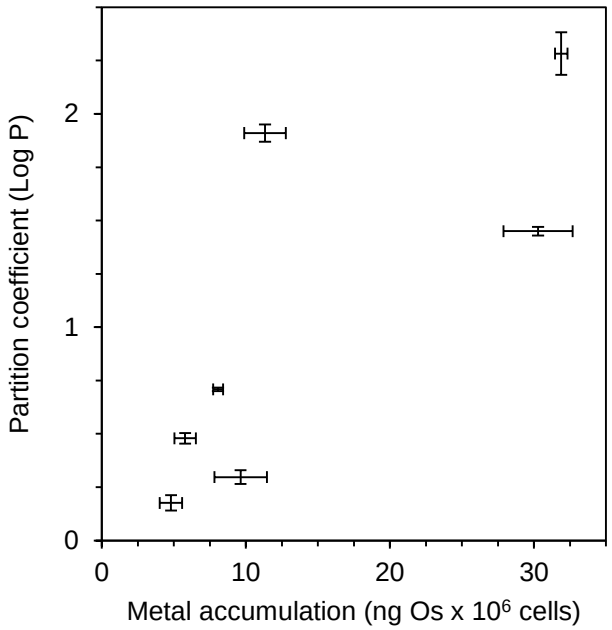
Supplementary Figure 4: Stability of complex **2** studied over a 24 h period at 310 K by ^{1}H-NMR spectroscopy in d^{6}-DMSO.



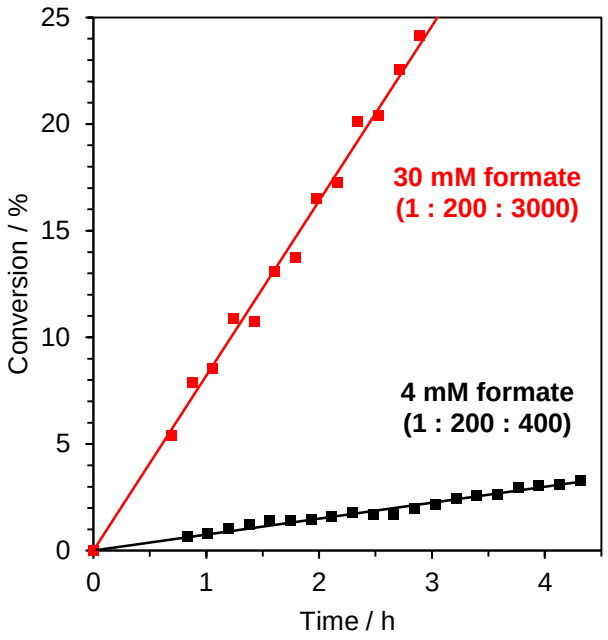
Supplementary Figure 5: Efflux of complex ***R,R*-2** from A2780 cancer cells exposed to complex **2** for 24 h (1.0 × IC_{50}, 15 µM) then allowed to recover in drug-free culture medium at variable time points (up to 72 h). Data were collected in triplicate and are shown normalized to the total accumulation after 24 h drug exposure with no recovery time (100%).



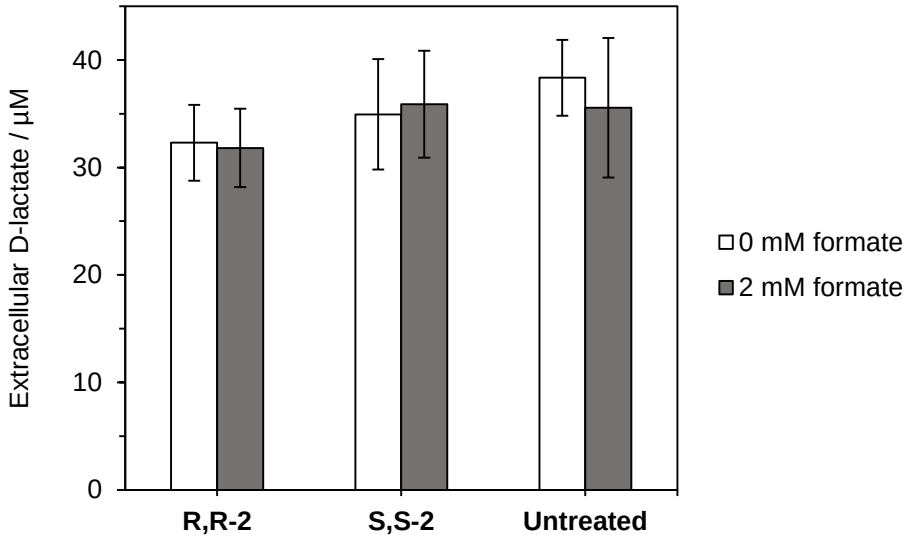
Supplementary Figure 6: Correlation of octanol-water partition coefficient (Log P data are in Supplementary Table 3) with antiproliferative activity (IC_{50} / μM – data in Table 1) for complexes **2**-**8** in A2780 human cancer cells (Pearson's r = -0.92).



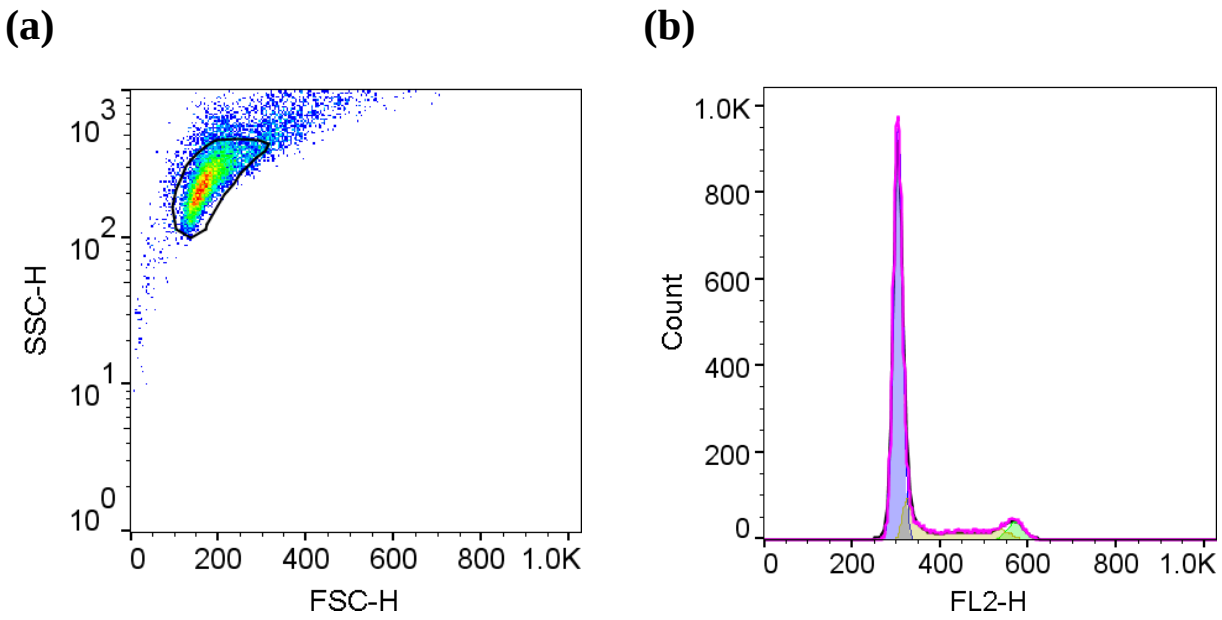
Supplementary Figure 7: Correlation of octanol-water partition coefficient (Log P) with cellular osmium accumulation (ng Os × 10^6 cells) for complexes **2**-**8** in A2780 human cancer cells (Pearson's r = 0.77). Numerical data are in Supplementary Table 3.



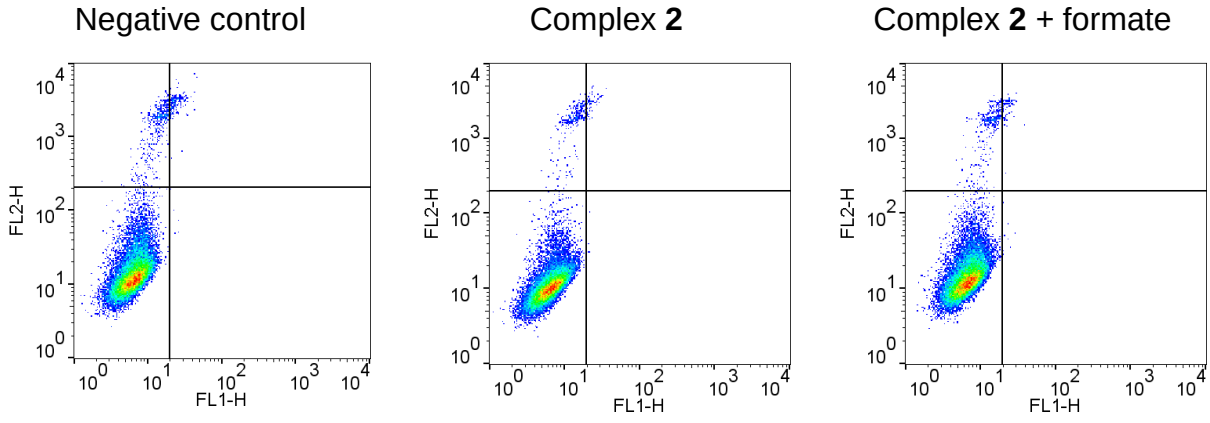
Supplementary Figure 8: The rate of conversion of pyruvate to lactate using catalyst ***R,R*-2** is highly dependent on formate concentration. (600 MHz, 90% H_2O / 10% D_2O, 310 K. Catalyst, 10 μM; pyruvate, 2 mM; formate 4 mM or 30 mM).



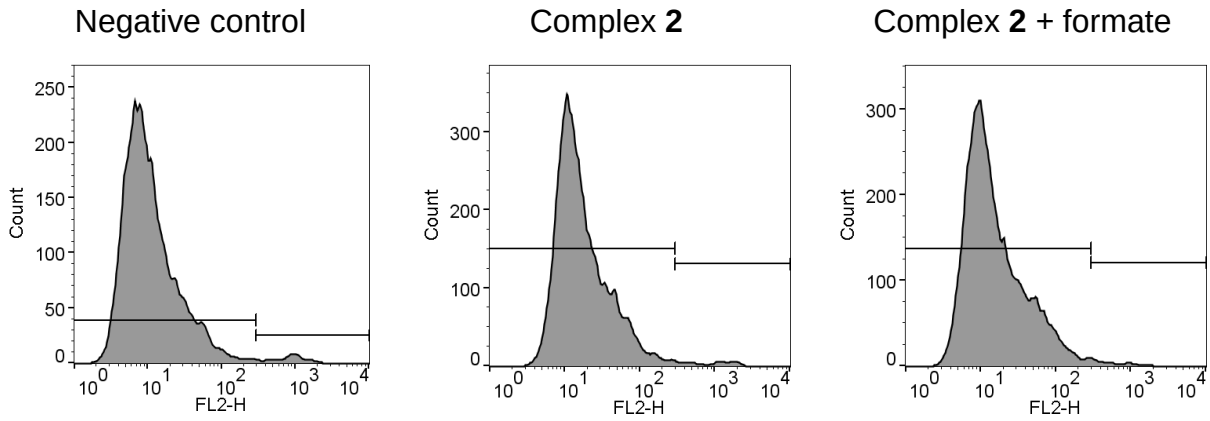
Supplementary Figure 9: Extracellular D-lactate (μM) determined after 24 h exposure of A2780 cancer cells to complex **2** at IC_{50} concentration (15 μM). Neither enantiomer of complex **2**, with or without sodium formate, affects the concentration of D-lactate relative to the catalyst-free control. Error bars shown as +/- one standard deviation from the mean.



Supplementary Figure 10: (a) Example population gating for flow cytometry analysis of A2780 cancer cells. (b) Example population fitting model (Watson Pragmatic) for the cell cycle analysis of A2780 cancer cells. G1 population (●); S-phase population (●); G2 population (●). Experimental data (●); fitted parameters (●).



Supplementary Figure 11: Detection of apoptosis by dual staining A2780 cancer cells with Annexin V-FITC (detected using FL1 channel, green) and propidium iodide (detected using FL2 channel, red). Data were analysed using FlowJo V10. Quadrants: viable (FL1-FL2-), early-apoptotic (FL1+ FL2-), late-apoptotic (FL1+ FL2+) and non-viable (FL1- FL2+) cells.



Supplementary Figure 12: Membrane integrity of A2780 ovarian cancer cells was determined using propidium iodide staining, detected using FL2 channel (red). Data were analysed (FL2-, viable; FL2+, non-viable) using FlowJo V10. Statistical analysis was carried out using a two-tailed *t*-test assuming unequal variances (Welch's *t*-test).

5. References

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