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Effect of Sulfonamidoethylenediamine Substituents in Ru^{II} Arene Anticancer Catalysts on Transfer Hydrogenation of Coenzyme NAD⁺ by Formate

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Supplementary Information

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1. Synthesis of chelating ligands

N-(2-Aminoethyl)-4-toluensulfonamide (TsEn). This ligand was obtained by following the method described in the literature.¹⁻³ A solution of ethylenediamine (17 mL, 0.26 mol) in dichloromethane (150 mL) was placed in a round bottom flask. A solution of toluenesulfonyl chloride (5.0 g, 26 mmol) in dichloromethane (50 mL) was added slowly via a dropping funnel, and the mixture stirred vigorously for 1 h. The solution was then washed with water (3 × 25 mL), and dried over MgSO₄. Solvent was removed on a rotary evaporator to give a white solid which was purified by chromatography column (10% MeOH + 90% DCM). Yield = 3 g (53.9 %). ¹H NMR (400 MHz, CDCl₃): $\delta_{\rm H}$ 2.43 (s, 3H), 2.78 (t, *J* = 5.6 Hz, 2H), 2.95 (t, *J* = 5.6 Hz, 2H), 7.30 (d, *J* = 8.1 Hz, 2H), 7.75 (d, *J* = 8.1 Hz, 2H). ESI-MS: Calc for [C₉H₁₄N₂O₂S + H]⁺ 215.0 *m/z*, found: 215.1 *m/z*.

Methyl(2-((4-methylphenyl)sulfonamido)ethyl)carbamate. This ligand was obtained following the method described in the literature.⁴ TsEn (428 mg, 2.0 mmol) was dissolved in dichloromethane (20 mL) and then methyl chloroformate (0.185 mL, 2.4 mmol) and triethylamine (0.306 mL, 2.2 mmol) were added and the reaction mixture left stirring at ambient temperature overnight. The mixture was washed with water (20 mL), extracted with chloroform (3 × 30 mL) and then dried over anhydrous MgSO₄. The solvent was removed under rotary evaporator to give a white solid. Yield = 494 mg (89%). ¹H NMR (400 MHz, CDCl₃): $\delta_{\rm H}$ 2.43 (s, 3H), 3.06-3.10 (m, 2H), 3.26-3.29 (m, 2H), 3.65 (s, 3H), 4.97 (d, *J* = 23.4 Hz, 2H), 7.31 (d, *J* = 8.1 Hz, 2H), 7.74 (d, *J* = 8.2 Hz, 2H).

4-Methyl-N-(2-(methylamino)ethyl)benzenesulfonamide (TsEn(Me,H)).⁴ To a stirred solution of amide precursor (408 mg, 1.5 mmol) in dry THF (20 mL), a 2 M solution of LiAlH₄ (3.2 mL, 6 mmol) was added dropwise. The reaction was heated under reflux for 4 h and then 1 mL water was added and left the reaction mixture stirred for another 2 h. The product was extracted with chloroform (3 × 30 mL) and the combined organic fractions were washed with brine (2 x 30 mL) and dried over MgSO₄, concentrated by rotary evaporation to give a crude product which was further purified on a silica gel column to give a white solid. Yield = 226 mg (66%). ¹H NMR (400 MHz, CDCl₃): $\delta_{\rm H}$ 2.29 (s, 3H), 2.42 (s, 3H), 2.66 (t, *J* = 5.6 Hz, 2H), 2.99 (t, *J* = 5.4 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.75 (d, *J* = 8.1 Hz, 2H). ESI-MS: *Calc* for [C₁₀H₁₆N₂O₂S + H]⁺ 229.0 *m/z*, found: 229.1 *m/z*.

N-(2-(Dimethylamino)ethyl)-4-methylbenzenesulfonamide (TsEn(Me,Me)). This was obtained by following the protocol described in the literature.³ A solution of N, N-dimethylethane-1, 2diamine (0.42 mL, 4.5 mmol) in dichloromethane (80 mL) was placed in a round-bottom flask. A solution of tosyl chloride (572 mg, 4.5 mmol) in DCM (50 mL) was added slowly via a dropping funnel, and the mixture was stirred vigorously for another 4 h in the ice bath. The solution was then washed with water (3 × 25 mL), and dried over MgSO₄. The solvent was concentrated by rotary evaporation to give a crude product which was further purified on a silica gel column to give a white solid. Yield = 490 mg (45%). ¹H NMR (400 MHz, CDCl₃): $\delta_{\rm H}$ 2.56 (s, 6H), 2.44 (s, 3H), 2.48 (t, *J* = 6.9 Hz, 2H), 2.97 (t, *J* = 6.8 Hz, 2H), 7.40 (d, *J* = 8.0 Hz, 2H), 7.75 (d, *J* = 8.2 Hz, 2H). ESI-MS: *Calc* for [C₁₁H₁₈N₂O₂S + H]⁺ 243.1 *m/z*, found: 243.1 *m/z*.

N-(2-(Ethylamino)ethyl)-4-methylbenzenesulfonamide (TsEn(Et,H)). This was obtained by following the method described in the literature.³ A solution of N-ethylethylenediamine (0.737 mL, 7 mmol) in dichloromethane (100 mL) was placed in a round-bottom flask. A solution of toluenesulfonyl chloride (1 g, 5.2 mmol) in DCM (50 mL) was added slowly via a dropping funnel, and the mixture was stirred vigorously for 12 h. The product was washed with brine (2 × 30 mL) and dried over MgSO₄, and concentrated by rotary evaporation to give a crude product which was further purified on a silica gel column (10% MeOH and 90% DCM) to give a white solid. Yield = 667 mg (53%). ¹H NMR (300 MHz, d₄-MeOD): $\delta_{\rm H}$ 1.29 (t, *J* = 7.2 Hz, 3H), 2.43 (s, 3H), 3.02 (q, *J* = 7.4 Hz, 14.6 Hz, 2H), 3.08 – 3.12 (m, 4H), 7.41 (d, *J* = 7.6 Hz, 2H), 7.77 (d, *J* = 7.7 Hz, 2H). ESI-MS: *Calc* for [C₁₁H₁₈N₂O₂S + H]⁺ 243.1 *m/z*, found: 243.1 *m/z*.

N-(2-(Benzylamino)ethyl)-4-methylbenzenesulfonamide (TsEn(Bz,H)). This was obtained by following the method described in the literature.⁴ A solution of N-benzylethylenediamine (0.94 mL, 6.24 mmol) in dichloromethane (100 mL) was placed in a round-bottom flask. A solution of toluenesulfonyl chloride (1 g, 5.2 mmol) in DCM (50 mL) was added slowly via a dropping funnel, and the mixture was stirred vigorously for 12 h. The product was washed with brine (2 x 30 mL) and dried over MgSO₄, and concentrated by rotary evaporation to give a crude product which was further purified on a silica gel column (10% MeOH and 90% DCM) to give a white solid. Yield = 696 mg (44%). ¹H NMR (300 MHz, d4-MeOD): $\delta_{\rm H}$ 2.44 (s, 3H), 3.07 (t, J = 5.3 Hz, 2H), 3.14 (t, J = 5.2 Hz, 2H), 4.15 (s, 3H), 7.40 – 7.47 (m, 7H), 7.77 (d, J = 7.6 Hz, 2H). ESI-MS: *Calc* for [C₁₆H₂₀N₂O₂S + H]⁺ 305.1 *m/z*, found: 305.1*m/z*.

N-(2-((4-Fluorobenzyl)amino)ethyl)-4-methylbenzenesulfonamide (TsEn(4-F-Bz,H)). This was obtained by following the method described in the literature.⁶ A solution of N-(2-aminoethyl)-4-methylbenzenesulfonamide (TsEn) (500 mg, 2.33 mmol) and triethylamine (1.63 mL, 11 mmol) in dichloromethane (100 mL) was placed in a round-bottom flask. A solution of 1-(bromomethyl)-4-fluorobenzene (0.29 mL, 2.3 mmol) in DCM (50 mL) was added slowly via a dropping funnel, and the mixture was stirred vigorously for 12 h. The product was washed with brine (2 × 30 mL) and dried over MgSO₄, and concentrated by rotary evaporation to give a crude product which was further purified by silica gel column (10% MeOH and 90% DCM) to give a white solid. Yield = 400 mg (54%). ¹H NMR (300 MHz, d4-

MeOD): $\delta_{\rm H}$ 2.41 (s, 3H), 2.63 (t, J = 6.2 Hz, 2H), 2.97 (t, J = 6.2 Hz, 2H), 3.67 (s, 3H), 7.03 (t, J = 8.1 Hz, 2H), 7.29 (t, J = 6.7 Hz, 2H), 7.35 (d, J = 7.7 Hz, 2H), 7.71 (d, J = 7.4 Hz, 2H). ESI-MS: *Calc* for [C₁₆H₁₉FN₂O₂S + H]⁺ 323.1 m/z, found: 323.1m/z.

4-Methyl-N-(2-((naphthalen-2-ylmethyl)amino)ethyl)benzenesulfonamide (TsEn(Naph,H)). This was obtained by following the method described in the literature.⁵ A solution of TsEn (500 mg, 2.33 mmol) and triethylamine (1.63 mL, 11 mmol) in dichloromethane (100 mL) was placed in a round-bottom flask. A solution of 2-(bromomethyl)naphthalene (500 mg, 2.33 mmol) in DCM (50 mL) was added slowly via a dropping funnel, and the mixture was stirred vigorously for 12 h. The product was washed with brine (2 × 30 mL) and dried over MgSO₄, and concentrated by rotary evaporation to give a crude product which was further purified on a silica gel column (10% MeOH and 90% DCM) to give a white solid. Yield = 355 mg (43%). ¹H NMR (300 MHz, d4-MeOD): $\delta_{\rm H}$ 2.34 (s, 3H), 2.65 (t, *J* = 6.2 Hz, 2H), 3.00 (t, *J* = 6.2 Hz, 2H), 3.83 (s, 3H), 7.26 (d, *J* = 7.7 Hz, 2H), 7.40-7.47 (m, 3H), 7.67-7.71 (m, 3H), 7.79-7.84 (m, 3H). ESI-MS: *Calc* for [C₂₀H₂₂N₂O₂S + H]⁺ 355.1 m/z, found: 355.1m/z.

2. Synthesis of ruthenium complexes.

[(η⁶-*p*-cym)Ru(TsEn(Me,H))CI] (1). All Ru^{II} complexes were prepared according to related reported methods:³ [(*p*-cym)RuCl₂]₂ (100 mg, 0.163 mmol) and TsEn(Me,H) (80 mg, 0.35 mmol) were placed in a round-bottom flask to which 2-propanol (100 mL) and triethylamine (91 µL, 0.653 mmol) were added. The solution was heated under reflux in a nitrogen atmosphere (365 K) for 12 h with stirring. After which the solvent was removed on a rotary evaporator to give a dark red solid. The crude product was redissolved in dichloromethane and was washed with brine, after which the organic solvent was dried over MgSO₄ and filtered. A dark red solid was obtained after removal of DCM and recrystallized from methanol and diethyl ether. Yield = 72 mg (44.3%). ¹H NMR (400 MHz, CDCl₃): δ_H 1.26 (d, *J* = 6.9 Hz, 3H), 1.32 (d, *J* = 6.9 Hz, 3H), 2.20 (s, 3H), 2.16-2.27 (m, 2H), 2.33 (s, 3H), 2.52-2.55 (m, 1H), 2.89-2.96 (m, 1H), 3.06 (d, *J* = 6.1 Hz, 3H), 3.09 (s, 1H), 3.54 (s, 1H), 5.33 (t, *J* = 4.5 Hz, 2H), 5.56 (s, 1H), 5.85 (s, 1H), 7.14 (d, *J* = 7.9 Hz, 2H), 7.71 (d, *J* = 7.9 Hz, 2H). ¹³C NMR (125.8 MHz, CDCl₃): δ_C 19.0, 21.4, 22.0, 23.0, 30.7, 44.5, 48.3, 58.8, 127.6, 128.5, 140.1, 140.4. ESI-MS: *Calcd* for [C₂₀H₂₉N₂O₂S(Ru-Cl)]⁺ 463.0993 *m/z*, found: 463.0992 *m/z*. Anal. *Calcd* for C₂₀H₂₉CIN₂O₂SRu: C 48.23, H 5.87, N 5.62; Found: C 48.11, H 5.81, N 5.50.

[(η^6 -*p*-cym)Ru(TsEn(Me,Me))Cl] (2). Complex 2 was obtained by following the method described in literature using the ligand TsEn(Me,Me) (84.7 mg, 0.35 mmol).⁶ Recrystallization from methanol and diethyl ether resulted in bright wine-red solid. Yield = 75 mg (45%). ¹H NMR (400 MHz, d4-MeOD): $\delta_{\rm H}$ 1.62 (d, *J* = 6.9 Hz, 6H), 1.53 (dd, *J* = 2.7 Hz, 11.1 Hz, 1H), 2.32 (d, *J* = 5.0 Hz, 6H), 2.49 (dd, *J* = 5.4 Hz, 12.5 Hz, 1H), 2.76-2.84 (m, 1H), 2.90 (s, 3H),

2.98-3.05 (m, 1H), 3.06 (s, 3H), 3.19-3.29 (m, 1H), 5.19 (dd, J = 5.8 Hz, 44.9 Hz, 2H), 5.80 (dd, J = 5.7 Hz, 31.2 Hz, 2H), 7.14 (d, J = 8 Hz, 2H), 7.93 (d, J = 8.1 Hz, 2H). ¹³C NMR (125.8 MHz, CDCl₃): $\delta_{\rm C}$ 18.8, 21.4, 22.2, 22.6, 30.7, 47.0, 54.8, 55.9, 63.7, 128.3, 128.7, 139.9, 140.5. ESI-MS: *Calcd* for [C₂₁H₃₁N₂O₂S(Ru-Cl)]⁺ 477.1150 *m*/*z*, found: 477.1153 *m*/*z*. Anal. *Calcd* for C₂₁H₃₁ClN₂O₂SRu: C 49.26, H 6.10, N 5.47; Found: C 48.85, H 6.32, N 5.35.

[(η⁶-*p*-cym)Ru(TsEn(Et,H))Cl] (**3**). Complex **3** was obtained following the method described above for complex **1** using the ligand TsEn(Et,H) (97 mg, 0.40 mmol). Recrystallization from methanol and diethyl ether resulted in a bright wine-red solid. Yield = 108 mg (65%). ¹H NMR (400 MHz, CDCl₃): $\delta_{\rm H}$ 1.27 (d, *J* = 6.8 Hz, 3H), 1.27-1.35 (m, 6H), 2.15 (d, *J* = 7.8 Hz, 2H), 2.20 (s, 3H), 2.33 (s, 3H), 2.73 (d, *J* = 4.0 Hz, 1H), 2.87-2.94 (m, 1H), 3.12 (d, *J* = 6.9 Hz, 1H), 3.15-3.24 (m, 2H), 3.59-3.63 (m, 1H), 5.32-5.36 (m, 2H), 5.51 (s, 1H), 5.90 (s, 1H), 7.14 (d, *J* = 7.9 Hz, 2H), 7.21 (d, *J* = 7.9 Hz, 2H). ¹³C NMR (125.8 MHz, CDCl₃): $\delta_{\rm C}$ 14.9, 19.0, 21.4, 22.0, 23.0, 30.8, 48.3, 51.8, 54.9, 127.6, 128.5, 140.0, 140.4. ESI-MS: *Calcd* for [C₂₁H₃₁N₂O₂S(Ru-Cl)]⁺ 477.1150 *m/z*, found: 477.1153 *m/z*. Anal. *Calcd* for C₂₁H₃₁ClN₂O₂SRu: C 49.26, H 6.10, N 5.47; Found: C 49.15, H 6.10, N 5.38.

[(η⁶-*p*-cym)Ru(TsEn(Bz,H))Cl] (4). Complex 4 was obtained following the method described above for complex 1 using the ligand TsEn(Bz,H) (106.4 mg, 0.35 mmol). Purification by column chromatography (ethyl acetate/hexane = 2:1(v/v)) and then recrystallization from ethyl acetate and hexane by leaving the solution to evaporate slowly at ambient temperature, resulted in bright red solid. Yield = 99.2 mg (53%). ¹H NMR (400 MHz, CDCl₃): $\delta_{\rm H}$ 1.20 (d, *J* = 6.8 Hz, 3H), 1.26 (d, *J* = 6.8 Hz, 3H), 1.91-1.97 (m, 1H), 2.02-2.08 (m, 1H), 2.13 (s, 3H), 2.22 (s, 3H), 2.13 (d, *J* = 10.1 Hz 1H), 2.83-2.88 (m, 1H), 2.94 (d, *J* = 10.9 Hz, 1H), 3.70 (t, *J* = 9.5 Hz, 1H), 4.03 (t, *J* = 10.8 Hz, 2H), 4.64 (d, *J* = 13.1 Hz, 1H), 5.41 (s, 1H), 5.74 (s, 1H), 7.03 (d, *J* = 7.8 Hz, 2H), 7.17-7.20 (m, 3H), 7.59 (d, *J* = 7.9 Hz, 2H). ¹³C NMR (125.8 MHz, CDCl₃): $\delta_{\rm C}$ 19.1, 21.4, 21.9, 23.1, 30.9, 48.2, 54.7, 61.5, 127.6, 128.4, 128.5, 128.7, 129.2, 135.8, 139.9, 140.4. ESI-MS: *Calcd* for [C₂₆H₃₃N₂O₂S(Ru-Cl)]⁺ 539.1306 *m/z*, found: 539.1307 *m/z*. Anal. *Calcd* for C₂₆H₃₃ClN₂O₂SRu: C 54.39, H 5.79, N 4.88; Found: C 54.37, H 5.82, N 4.82.

[(η⁶-*p*-cymene)Ru(TsEn(4-F-Bz,H))Cl] (5). Complex **5** was obtained following the method described above for complex **1** using the ligand TsEn(4-F-Bz,H) (200 mg, 0.621 mmol) and Ru^{II} dimers [(*p*-cym)RuCl₂]₂ (184 mg, 0.3 mmol). A bright red solid was obtained by following the purification method for complex **5**. Yield = 124 mg (34%). ¹H NMR (300 MHz, CDCl₃): $\delta_{\rm H}$ 1.30 (d, *J* = 6.8 Hz, 3H), 1.36 (d, *J* = 6.9 Hz, 3H), 2.00-2.16 (m, 2H), 2.02-2.08 (m, 1H), 2.23 (s, 3H), 2.32 (s, 3H), 2.38-2.41 (m, 1H), 2.91-3.00 (m, 1H), 3.05 (d, *J* = 12.0 Hz, 1H), 3.76 (t, *J* = 9.6 Hz, 1H), 4.10 (t, *J* = 12.0 Hz, 1H), 4.72 (d, *J* = 14.2 Hz, 1H), 5.40-5.42 (m, 2H), 5.51 (s, 1H), 5.84 (s, 1H), 7.06 (t, *J* = 8.3 Hz, 2H), 7.13 (d, *J* = 7.5 Hz, 3H), 7.26-7.30 (m, 2H), 7.69 (d, *J* = 7.3 Hz, 2H). ¹³C NMR (125.8 MHz, CDCl₃): $\delta_{\rm C}$ 19.1, 21.4, 21.9, 23.1, 31.0, 48.2, 54.5, 60.6, 116.1, 116.3, 127.6, 128.5, 130.3, 130.3, 131.6, 131.7, 139.8, 140.4, 161.7, 163.7.

¹⁹F NMR (376.4 MHz, CDCl₃): -112.3. ESI-MS: *Calcd* for [C₂₆H₃₂FN₂O₂S(Ru-Cl)]⁺ 557.1212 *m/z*, found: 557.1213 *m/z*. Anal. *Calcd* for C₂₆H₃₂ClFN₂O₂SRu: C 52.74, H 5.45, N 4.73; Found: C 52.20, H 5.34, N 4.67.

[(η⁶-*p*-cymene)Ru(TsEn(Naph,H))Cl] (**6**). Complex **6** was obtained by following the method described above for complex **1** using the ligand TsEn(Naph,H) (272 mg, 0.77 mmol) and Ru^{II} dimers [(*p*-cym)RuCl₂]₂ (235.2 mg, 0.38 mmol). A dark red solid was obtained by following the purification method for complex **5**. Yield = 156 mg (33%). ¹H NMR (300 MHz, CDCl₃): $\delta_{\rm H}$ 1.32 (d, *J* = 7.1 Hz, 3H), 1.37 (d, *J* = 9.0 Hz, 3H), 2.02-2.10 (m, 1H), 2.16-2.28 (m, 3H), 2.32 (s, 3H), 2.42-2.45 (m, 1H), 2.94-3.10 (m, 2H), 3.94-4.11 (m, 1H), 4.23-4.34 (m, 1H), 4.90 (m, 1H), 4.42 (s, 2H), 5.50-5.59 (m, 1H), 5.84-5.87 (m, 1H), 7.13 (d, *J* = 8.1 Hz, 2H), 7.37 (d, *J* = 8.5 Hz, 2H), 7.51-7.53 (m, 2H), 7.69-7.71 (m, 2H), 7.81-7.86 (m, 2H). ¹³C NMR (125.8 MHz, CDCl₃): $\delta_{\rm C}$ 19.1, 21.4, 21.9, 23.2, 31.0, 48.2, 54.8, 61.6, 125.5, 126.8, 126.9, 127.6, 127.7, 127.8, 127.9, 128.5, 129.3, 129.3, 133.1, 133.2, 139.9, 140.4. ESI-MS: *Calcd* for $[C_{30}H_{35}N_2O_2S(Ru-Cl)]^+$ 589.1463 *m/z*, found: 589.1463 *m/z*. Anal. *Calcd* for $C_{30}H_{35}ClN_2O_2SRu: C$ 56.11, H 5.81, N 4.49; Found: C 56.26, H 5.51, N 4.34.

3. Crystallographic data

Crystal character	red block
Empirical formula	$C_{21}H_{31}ClN_2O_2RuS$
Formula weight	512.06
Temp (K)	150(2)
Crystal system	monoclinic
Space group	P21/c
<i>a</i> / Å	13.88136(9)
<i>b</i> / Å	10.43716(8)
<i>c</i> / Å	15.24599(11)
α/°	90
eta / °	102.1274(7)
γ / °	90
Volume / Å ³	2159.57(3)
Ζ	4
$D_{calc} (mg/cm^3)$	1.575
μ / mm ⁻¹	0.966
F (000)	1056.0
Crystal size/mm ³	$0.6 \times 0.4 \times 0.06$ orange block
Reflections collected	201186
Indep reflection	11305
$R [I \ge 2\sigma (I)]$	$R_1 = 0.0273$
Final R [all data]	$R_2 = 0.0629$

 Table S1 Crystallographic data for complex 3.

Table S2 Selected hydrogen bond lengths (Å) and angles (°) for complex 3.

D	Н	А	d (D-H)/Å	d (H-A)/Å	d (D-A)/Å D) -H-A/°	Symmetry Operator
N12	H12	O8B	1.00	2.11	3.1078(17)	173.3	1-X, -1/2+Y, 1/2-Z
N12A	H12A	Cl1	1.00	2.25	2.744(9)	109.3	

4. Effect of formate on cell proliferation

		[Formate]			
Complex	Cell Viability Decrease (%)				
	0.5 mM	1.0 mM	2.0 mM		
1	6.1 ± 3.8	14.4 ± 5.4	20.4 ± 3.2		
2	6.3 ± 2.4	8.8 ± 2.4	20.8 ± 3.1		
3	2.6 ± 1.7	11.2 ± 1.8	20.3 ± 2.3		
4	17.3 ± 3.4	26.2 ± 4.8	32.0 ± 3.6		
5	11.7 ± 4.8	16.6 ± 3.1	32.0 ± 3.2		
6	28.3 ± 2.6	29.2 ± 1.9	36.1 ± 2.3		

Table S3 Percentage decrease of cell viability (error propagation) induced by complexes **1-6** in the presence of various concentrations of formate.

5. ROS populations

Table S4 Induction of ROS and superoxide determined by flow cytometry experiments on A2780 human ovarian cancer cells. The FL1 channel (Ex/Em: 490/525 nm) detects ROS and FL2 channel (Ex/Em: 550/620 nm) detects superoxide.

Complex	Population (%)					
Complex	FL-1-/FL-2-	FL-1+/FL-2-	FL-1-/FL-2+	FL-1+/FL-2+		
1	0.13±0.1 ***	83.3±1.3 ***	0.02 ± 0.01	16.5±1.0 **		
4	0 ***	68.7±0.7 ***	0	31.3±0.3 ***		
Negative control	99.89 ± 0.04	0.09 ± 0.03	$0.04{\pm}0.01$	0		

All values compared to the untreated controls. In all cases, independent two-sample *t*-tests with unequal variances, Welch's tests, were carried out to establish statistical significance of the variations (p < 0.001 for ***, p < 0.01 for **, and p < 0.05 for *).

6. Energy barriers from DFT calculations

Table S5 Computed DF	Γ energy barriers ΔE	(kJ/ mol) related to	o cycle of hydride transfer.
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AE(ltI/mol)	Ru ^{II} Complex						
$\Delta E(KJ/III0I)$	1	2	3	4	5	6	
$\Delta E1$	-61	-63	-61	-51	-31	-54	
$\Delta E2$	-6	+6	+8	-10	-10	-12	
$\Delta E3$	+130	+146	+121	+140	+138	+137	
$\Delta E4$	-3	-63	-7	-21	-21	-14	
$\Delta E5$	-55	-43	-55	-40	-40	-41	
$\Delta E6$	+144	+146	+146	+131	+136	+127	

7. Figures



Fig. S1 Monitoring of kinetics of TH of NAD⁺ to NADH catalysed by complex **4** using sodium formate as hydride source followed by UV-vis spectroscopy, and plot of increase in absorbance at 340 nm with time (inset).



Fig. S2 Dependence of turnover frequency on pH* for the reduction of NAD⁺ by complex 4 using formate as a hydride source (molar ratio of NAD⁺: complex 4: sodium formate = 4:1:25, respectively, 310 K in d₄-MeOD/D₂O (2:8 v/v)).



Fig. S3 Dependence of the reaction rate on the concentration of sodium formate for the transfer reduction of NAD⁺ catalysed by complex 4 (molar ratio of NAD⁺: complex 4: sodium formate = 4:1:X, respectively, where X = 5, 10, 25, 50 and 100, at 310 K in d₄-MeOD/D₂O (2:8, v/v)).



Fig. S4 Plot of the reciprocal of the TOF against sodium formate concentration for the reduction of NAD⁺ in the presence of various molar equiv of formate, catalyzed by complex **4**. For a reaction following Michaelis-type kinetics, $TOF = TOF_{max}[S]/(K_M + [S])$, where TOFmax is the turnover frequency at infinite substrate (formate) concentration, [S] is the substrate concentration, and K_M is the Michaelis constant. Hence, $TOF^{-1} = (K_M/TOF_{max})(1/[S]) + (1/TOF_{max})$, and Km and TOF_{max} can be obtained from the gradient and y intercept, respectively, of the double-reciprocal plot.



Fig. S5 Relative hydrophobicity measurements by HPLC using aqueous 50 mM NaCl (solvent A), and aqueous 50 mM NaCl /CH₃CN 1:1 v/v (solvent B).



Fig. S6 Low field region of the ¹H NMR spectrum of complex 4 (2 mM) on reaction with 1.5 mol equiv (3 mM) (a) 9-ethylguanine (9-EtG), (b) adenosine 5'-monophosphate (5'-AMP), in 10% d₄-MeOD /90% D₂O, pH* 7.2, 310 K, showing formation of the 9-EtG, but not 5'-AMP adduct.



Fig. S7 Effect of complex **4** on the electrophoretic mobilities of supercoiled (sc) and open circular (oc) forms of plasmid DNA.



Fig. S8 Optimized structures of complex **2** in the catalytic cycle described in Fig. 8, as determined by DFT calculations, involving initial aquation, followed by formate binding, hydride transfer to Ru and on to NAD⁺. State 1: $[(\eta^6-p-cym)Ru(TsEn(Me,Me))\bullet(OH_2)]^+$ and formate (isolated NAD⁺); State 2: $[(\eta^6-p-cym)Ru(TsEn(Me,Me))\bullet(OH_2)]^+$ interacting with NAD⁺ and formate, with T-shaped stacking of NH₂ of adenosine with tosyl ring; State 3: $[(\eta^6-p-cym)Ru(TsEn(Me,Me))\bullet(OH_2)]^+$

p-cym)Ru(TsEn(Me,Me))•(HCOO⁻)]•NAD and associated water, with T-shaped stacking of NH₂ of adenosine with tosyl ring; State 4: $[(\eta^6-p-cym)Ru(TsEn(Me,Me))•H]•NAD^+$ with associated water and CO₂; State 5: $[(\eta^6-p-cym)Ru(TsEn(Me,Me))•(OH_2)•(NADH)]$ and CO₂, with η^2 -*p*-cym coordination to Ru; State 6: $[(\eta^6-p-cym)Ru(TsEn(Me,Me))•(OH_2)]$ •NADH and CO₂; State 7: $[(\eta^6-p-cym)Ru(TsEn(Me,Me))•(OH_2)]^+$, water and CO₂ (and isolated NADH). The T-shaped stacking of adenosine NH₂ with the tosyl ring is also present for states 5 and 6. The attached pdb files show the hydrogen bond patterns displayed by coordinated water and phosphate oxygen.

8. References

- X. Li, L. Li, Y. Tang, L. Zhong, Cun L, J. Zhu, J. Liao and J. Deng, J. Org. Chem., 2010, 75, 2981-2988.
- 2. X. Wu, J. Liu, X. Li, A. Zanotti-Gerosa, F. Hancock, D. Vinci, J. Ruan and J. Xiao, *Angew. Chem. Int. Ed.*, 2006, **45**, 6718-6722.
- 3. J. J. Soldevila-Barreda, P. C. A. Bruijnincx, A. Habtemariam, G. J. Clarkson, R. J. Deeth and P. J. Sadler, *Organometallics*, 2012, **31**, 5958-5967.
- 4. J. E. D. Martins, G. J. Clarkson and M. Wills, Org. Lett., 2009, 11, 847-850.
- 5. E. Gabano, C. Cassino, S. Bonetti, C. Prandi, D. Colangelo, A. Ghiglia and D. Osella, *Org. Biomol. Chem.*, 2015, **3**, 3531-3539.
- 6. T. Koike and T. Ikariya, Organometallics, 2005, 24, 724-730.