Dalton Transactions

RSC Publishing

PAPER

Ruthenium(II) arene PTA (RAPTA) complexes: impact of enantiomerically pure chiral ligands†

Cite this: *Dalton Trans.*, 2013, **42**, 2008

Kelly J. Kilpin, Shona M. Cammack, Catherine M. Clavel and Paul J. Dyson*

Organometallic ruthenium(II) arene complexes containing the PTA ligand ([Ru(η^6-arene)Cl_2(PTA)], PTA = 1,3,5-triaza-7-phosphatricyclo-[3.3.1.1]decane, termed RAPTA) show pharmacologically relevant anti-tumour properties *in vitro*. Two new enantiomeric pairs of RAPTA compounds, containing the chiral arene (*R*)- or (*S*)-2-phenyl-*N*-(1-phenylethylene)acetamide and either dichlorido or oxalato ligands were synthesised and fully characterised. The stability of the complexes towards hydrolysis was assessed and the dichlorido complexes were found to be more stable towards hydrolysis than the prototype complex RAPTA-C, ([Ru(η^6-*p*-cymene)Cl_2(PTA)]). The cytotoxicity of the compounds towards human ovarian cancer cells is moderate to good with a degree of selectivity towards the cancer cells over healthy cells. More significantly, for the first time we were able to establish the influence of a bulky, chiral group attached to the arene on the cytotoxicity of this class of compound, with the *S*-enantiomer being more cytotoxic than the *R*-enantiomer.

Received 3rd October 2012,
Accepted 9th November 2012

DOI: 10.1039/c2dt32333h

www.rsc.org/dalton

Introduction

Following the discovery of *cisplatin* in the late 1960s, a vast amount of research has been directed towards the development of new metal-based chemotherapeutic agents.[1,2] Although thousands of new metal-based anti-cancer drugs have been proposed, with a number entering into clinical trials, only three (platinum-based) are currently in widespread clinical use.[3] This low success rate has been attributed to various problems such as high levels of *in vivo* toxicity, drug resistance and low aqueous solubility.[4]

With respect to overcoming these problematic issues, ruthenium-based complexes have been developed that show considerable promise,[5,6] in particular the Ru(III) coordination complexes NAMI-A and KP1019 (Fig. 1), which have progressed into clinical trials.[7] We are interested in organometallic Ru(II) arene complexes, typified by the prototype molecule RAPTA-C (Fig. 1), which although possesses low *in vitro* cytotoxicity, shows selectivity towards tumours *in vivo*.[8] In general, RAPTA complexes contain a face-capping arene, two labile chloride ligands and a PTA (1,3,5-triaza-7-phosphatricyclo-[3.3.1.1]-decane) ligand which imparts biologically favourable aqueous solubility to the compounds owing to its amphilic nature. Additionally, the robust nature of the RAPTA scaffold is amenable to structural modifications on the arene ring, or at the Ru centre (by substitution of either the PTA or chloride ligands).[9] Although a number of the aforementioned modifications have been carried out, and their effect on cytotoxicity determined,[10–20] a detailed study into the biological effects of chiral ancillary ligands has yet to be conducted for RAPTA-type compounds, and Ru(II) arene compounds more generally, even though examples of Ru(II) chiral–arene complexes have been reported.[21,22]

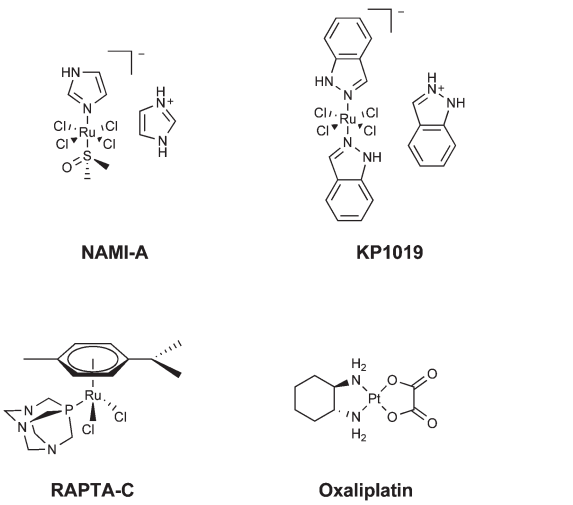


Fig. 1 Ruthenium(III) complexes currently in clinical trials, NAMI-A and KP1019, the Ru(II) arene complex, RAPTA-C and Oxaliplatin.

It is well established that different optical isomers, or enantiomers, of a compound can have markedly different biological

Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland. E-mail: paul.dyson@epfl.ch; Fax: +41 (0) 21 693 98 85; Tel: +41 (0) 21 693 98 54

†Electronic supplementary information (ESI) available: Selected NMR spectra and UV-vis traces. See DOI: 10.1039/c2dt32333h

activities, well-documented by the thalidomide tragedy of the 1980s.[23] In addition, the different enantiomers may have differing pharmacokinetics and affinities towards receptor molecules, as is the case for the opioid substitute methadone.[24] This is not only true for organic drugs – the clinically approved Pt(II) drug oxaliplatin (Fig. 1), which contains the chiral 1*R*,2*R*-cyclohexadiamine ligand is not only more active than the corresponding enantiomer (with 1*S*,2*S*-cyclohexadiamine), but also shows increased cellular uptake and DNA binding properties.[25] Only recently, chiral methyl substituted oxaliplatin derivatives have been reported, and again the stereochemistry greatly affects the biological properties of the compound.[26,27]

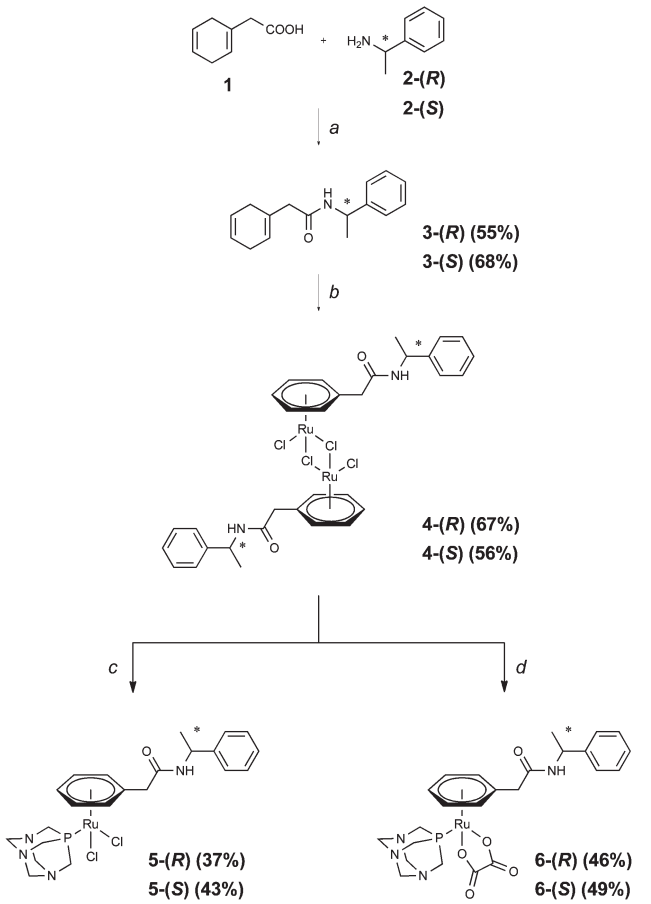
In light of these observations we thought that a comparison between the activity levels of different enantiomers of RAPTA compounds may be interesting. In this paper two new enantiomeric pairs of RAPTA-type complexes are described together with discussions of their stability towards hydrolysis and initial investigations into their biological activity.

Results and discussion

The synthesis of the complexes employed in this study was carried out using the methodology previously described to modify the RAPTA scaffold, depicted in Scheme 1.[9] Briefly, 2-(cyclohexa-1,4-dien-1-yl)acetic acid, **1**, obtained *via* a Birch reduction of phenyl acetic acid,[28] was coupled to the appropriate chiral amine, either *R*- or *S*-methylbenzylamine, **2-(*R*)** or **2-(*S*)**, with the aid of TBTU (*O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate), which suppresses racemisation.[29] An excess of the diene, **3-(*R*)** or **3-(*S*)**, was then heated to reflux in EtOH with $RuCl_3$ to give the dimeric Ru(II) complexes **4-(*R*)** or **4-(*S*)** as orange solids in good yields.[30] Reaction of **4-(*R*)** or **4-(*S*)** with 2 equivalents of PTA at room temperature affords the RAPTA complexes, **5-(*R*)** or **5-(*S*)**, also in reasonable yields as orange solids.[31] The oxalato derivatives, **6-(*R*)** or **6-(*S*)** were obtained as yellow solids by first reacting the dimer **4-(*R*)** or **4-(*S*)** with a slight excess of silver oxalate, followed by the removal of AgCl, and subsequent addition of PTA.[12]

As with other RAPTA-type complexes, both the dichlorido, **5-(*R*)** or **5-(*S*)**, and oxalato, **6-(*R*)** or **6-(*S*)**, compounds are soluble in a range of polar (H_2O, MeOH, EtOH, DMSO) and non-polar (CH_2Cl_2) solvents, with the oxalato complexes slightly more soluble than the dichlorido complexes.

All the new compounds were characterised fully and shown to be analytically pure. The ^{1}H NMR spectra of **4**, **5** and **6** (d_6-DMSO) showed peaks at 5.3–5.8 ppm, characteristic of protons on the capping arene ring. Unlike RAPTA-C (and the oxalato analogue), the ^{1}H and $^{13}C\{^1H\}$ NMR spectra of the mononuclear complexes comprise five unique proton environments, and six unique carbon environments (despite the apparent mirror plane in the molecules). However, when the spectra are acquired in D_2O this symmetry is not observed, instead the arene protons all appear to be equivalent. Such an observation suggests the presence of intramolecular (hydrogen) bonding interactions in the molecule, possibly between the amide functionality and either the dichlorido or oxalato ligands, which hold it in a favourable conformation and inhibit rotation around the Ru–arene axis. The $^{31}P\{^1H\}$ spectra of **5** and **6** each contain a singlet at −31.6 and −32.0 ppm, respectively, which agree with values reported previously for RAPTA complexes.[31] The signals characteristic of the amide functionality differ little between the diene, **3**, and complexes **4**, **5** and **6**, which suggest that it is not coordinated to the ruthenium centre. This inference was further corroborated by IR spectroscopy, which showed strong C=O stretching bands at *ca.* 1655 cm^{-1} for **3–6**. HR-ESI mass spectrometry further confirmed the formation of **5** and **6**. The dominant peaks in the spectra are those assigned to the $[M + H]^+$ ion, and in the case of **5**, less intense ions assigned to $[M - Cl]^+$ were also observed. In all cases, the assignments of the spectra were aided by comparison of the experimental and theoretical isotope patterns, arising from ruthenium (and chlorine in **5**). Analysis of the optical rotation of the complexes confirmed the optical purity of the samples.



Scheme 1 Synthetic routes to the dichlorido-**5-(*R*)**, **5-(*S*)** and oxalato-**6-(*R*)**, **6-(*S*)** Ru(II) complexes used in this study (*a*) DIEA, TBTU, CH_2Cl_2, 18 h, rt; (*b*) $RuCl_3{\cdot}3H_2O$, EtOH, 6 h, reflux; (*c*) PTA, CH_2Cl_2/MeOH (2 : 1), 3 h, rt; (*d*) 1. $Ag_2(C_2O_4)$, H_2O, 12 h, 2. PTA, MeOH, 3 h.

Like *cisplatin*, RAPTA complexes also undergo hydrolysis in aqueous solutions and at low chloride concentrations to give aquated species. At relatively high chloride concentrations this pathway is suppressed.[12,32–34] In order to suppress or reduce the rate of hydrolysis at the Ru centre, oxalato and carboxylato complexes, analogous to *oxaliplatin* and *carboplatin*, were developed, and interestingly, these modifications did not significantly alter the activity *in vitro*.[12] We decided to synthesize the oxalato analogues of **5** in order to suppress hydrolysis, which in turn would inhibit the formation of diastereoisomers arising from a chiral centre on the ligand and at the ruthenium. In this way, the chirality in the molecule is restricted to the ligand, to facilitate a direct comparison between the two enantiomers.

Hydrolysis studies of **5-(*S*)** and **6-(*S*)** were carried out in both aqueous and saline ([Cl^-] = 150 mM) solutions, using both $^{31}P\{^1H\}$ NMR and UV-vis spectroscopy to monitor the changes. As expected, the dichlorido species **5-(*S*)** immediately begins to undergo hydrolysis in D_2O and reaches equilibrium within 2 hours. In the $^{31}P\{^1H\}$ spectra, this process was characterised by the loss of the peak assigned to the dichlorido species **5-(*S*)** at −32.0 ppm, coupled with the appearance of two new peaks of equal intensity at −29.8 and −29.9 ppm, assigned to the two diastereoisomers represented by Form I (Scheme 2). Only upon the addition of excess $AgBF_4$ was a signal (at −28.1 ppm) attributable to Form II (Scheme 2) observed (Fig. 2). In saline D_2O ([Cl^-] = 150 mM) hydrolysis was completely suppressed, with the peak at −32 ppm, corresponding to the dichlorido form, being the only species after 48 hours.

The oxalato complexes **6-(*S*)** were completely inert to hydrolysis in D_2O solution, even after 48 hours, however in saline solution ([Cl^-] = 150 mM) a minor peak (16%) corresponding to the dichloride **5-(*S*)** appeared after 2 hours (Fig. S10, ESI†) suggesting some substitution of oxalate for chloride in relatively high chloride concentrations.

UV-Vis spectra were recorded in phosphate buffered solutions (10 mM, pH 7.4) in the absence and presence of chloride ([Cl^-] = 150 mM). The results obtained corroborate what was observed by $^{31}P\{^1H\}$ NMR spectroscopy. As an example, in the absence of chloride ions, the dichlorido complex **5-(*S*)** displayed an initial maximum at 340 nm which over time decreases with two new maxima at 296 and 380 nm appearing, indicating hydrolysis of **5-(*S*)** (Fig. 3). After 2 hours the spectra underwent no further change. In contrast, in phosphate buffered saline solutions containing 150 mM chloride the spectra of **5-(*S*)** remained unchanged over 48 hours. There were no observable changes in the spectra of the oxalato complex **6-(*S*)** over 48 hours regardless of the presence or absence of chloride. Compared to RAPTA-C under the same conditions (by both $^{31}P\{^1H\}$ NMR and UV-vis spectroscopy) hydrolysis of **5-(*S*)** is considerably slower, as RAPTA-C reached equilibrium within 15 minutes (Fig. S11, ESI†). Such a difference in exchange kinetics is not unsurprising considering the electronic differences in the arene rings.

The cytotoxicity of **5** and **6** was determined against A2780 and A2780cisR cells (cisplatin sensitive and cisplatin resistant

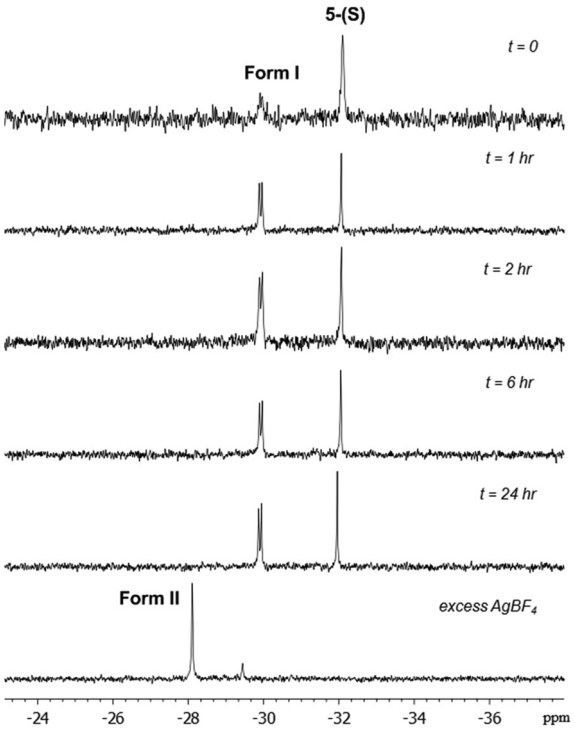


Fig. 2 Selected $^{31}P\{^1H\}$ NMR spectra of **5-(*S*)** over 24 h in D_2O, and with the addition of excess $AgBF_4$. (**5-(*S*)** = [Ru(η^6-arene)(PTA)Cl_2], Form I = [Ru(η^6-arene)-(PTA)$(OH_2)Cl]^+$, Form II = [Ru(η^6-arene)(PTA)$(OH)(H_2O)]^+$, arene = 2-phenyl-*N*-(1-phenylethylene)acetamide).

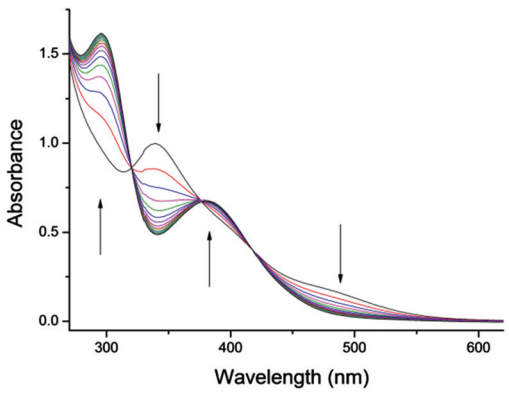
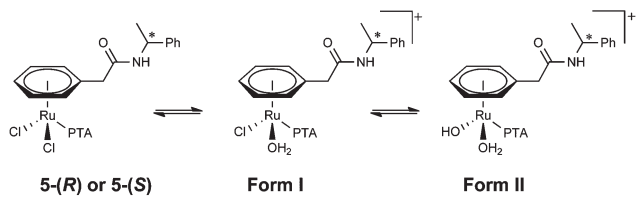


Fig. 3 UV-vis spectra (270–620 nm) of **5-(*S*)** in phosphate buffer (10 mM, pH 7.4). The arrows show the direction of the changes of the spectra. Spectra were recorded at 5 min intervals over a 2 h period.



Scheme 2 Aquation/hydrolysis of **5-(*R*)** or **5-(*S*)** in aqueous solutions.

Table 1 Cytotoxicity (IC_{50}, μM) of selected compounds at 72 h

	A2780[a]	A2780cisR[b]	HEK[c]
5-(*R*)	44.0 ± 1.1	396.1 ± 2.6	>1000
5-(*S*)	30.1 ± 0.5	228.3 ± 15.5	254.9 ± 13.0
RAPTA-C[e]	353	252	N/A[d]
6-(*R*)	34.2 ± 0.6	33.2 ± 0.8	32.2 ± 0.9
6-(*S*)	8.7 ± 0.1	20.6 ± 2.4	32.4 ± 4.5
oxaloRAPTA-C[e]	511	656	N/A[d]

[a] Human ovarian carcinoma cells. [b] Human ovarian carcinoma cells – acquired resistance to cisplatin. [c] Human embryonic kidney cells. [d] N/A data not available. [e] Taken from ref. 35.

human ovarian cancers, respectively) and HEK cells (human embryonic kidney, a model for non-tumourigenic cells) using the MTT assay. The results, reported as IC_{50} concentrations, are presented in Table 1.

Against the A2780 cell line, all the complexes in this study are considerably more active than the prototype complexes RAPTA-C and oxaloRAPTA-C. Also, compound **5-(*R*)** has a remarkable selectivity profile with an IC_{50} of 44.0 μM in A2780 cells *versus* >1000 μM in the healthy HEK cell line. Against the A27080cisR and HEK cell lines, the dichlorido complexes **5-(*R*)** and **5-(*S*)** show similar levels of activity compared with RAPTA-C whereas the oxalato compounds **6-(*R*)** and **6-(*S*)** are far more active than oxaloRAPTA-C. The reasons for these vast differences in activity profiles are unclear although the arene ligand must play a role, possibly *via* modulation of the rate of hydrolysis at the ruthenium centre, by altering the bioavailability of the complex and/or by enhancing specific interactions with relevant targets.

It is also noteworthy that the different enantiomers also give rise to significant differences: **5-(*S*)** is much more cytotoxic toward HEK cells than **5-(*R*)** (*ca*. 254 *versus* >1000 μM, respectively) and with the oxalato complexes **6**, the *S*-enantiomer is more active than the *R*-enantiomer against the A2780 cell line (8.7 *versus* 34.2 μM, respectively). Across the other cell lines and complexes, the *S*-enantiomers were also slightly more active (with the exception of **6** against HEK). This may be in part due to the chiral centre being somewhat remote from the ruthenium centre. Moreover, racemisation and inversion of the chiral centre inside the cell cannot be discounted.

Conclusions

RAPTA complexes are a promising class of Ru(II) anti-cancer agents. In this contribution we report the synthesis, aqueous behaviour and cytotoxicity of two enantiomeric pairs of RAPTA-type compounds containing the chiral arene (*R*)- or (*S*)-2-phenyl-*N*-(1-phenylethylene)acetamide and either dichlorido or oxalato ligands. The dichlorido-complexes were significantly more stable towards hydrolysis in aqueous solution than the parent compound RAPTA-C whereas the oxalato complexes completely resisted hydrolysis. This difference can be traced to the electronic effects induced at the ruthenium(II) centre by the arene ligand.

All the complexes are significantly more cytotoxic than RAPTA-C and oxaloRAPTA-C with the dichlorido-complexes retaining *in vitro* selectivity towards cancer cell lines. There are also notable differences in cytotoxicity between the *S*- and *R*-enantiomers with the *S*-enantiomer being more cytotoxic (depending on the cell line under study). Thus, the development of chiral ruthenium(II) arene anticancer compounds should not be overlooked as the modifications described in this study result in compounds with improved cytotoxicity and selectivity profiles.

Experimental

PTA (1,3,5-triaza-7-phosphatricyclo-[3.3.1.1]decane),[36] 2-(cyclohexa-1,4-dien-1-yl)acetic acid, **1**[28] and silver oxalate[12] were synthesised according to literature procedures. All other reagents were purchased from commercial sources and used without further purification. Column chromatography was carried out on a Varian 971-FP Autocolumn using SiO_2 Luknova flash columns (40–60 μm). ^1H (400.13 MHz), $^{31}P\{^1H\}$ (161.98 MHz) and $^{13}C\{^1H\}$ (100.62 MHz) NMR spectra were recorded on a Bruker Avance II 400 spectrometer at 298 K. Chemical shifts are reported in parts per million and referenced to residual solvent peaks ($CDCl_3$: ^1H δ 7.26, ^{13}C δ 77.16; d_6-DMSO: ^1H δ 2.50, ^{13}C δ 39.52 ppm). Coupling constants (*J*) are reported in Hertz (Hz). IR spectra were recorded on a Perkin Elmer Spectrum 1 Spectrometer and UV-vis experiments were conducted using 1 cm Quartz Cells (Hellma Analytics) on a Jasco V-550 spectrometer. High Resolution Electrospray Ionization mass spectra (HR ESI-MS) were obtained on a Thermo-Finnigan LCQ Deca XP Plus quadropole ion-trap instrument operated in positive-ion mode. Specific rotation was measured on a Jasco P-2000 Polarimeter, with $[\alpha]_D$ values given in 10^{-1} deg cm^2 g^{-1}. Elemental analyses were carried out by the microanalytical laboratory at the EPFL. Melting points were determined using a SMP3 Stuart Melting Point Apparatus and are uncorrected.

Synthesis of 3-(*R*) and 3-(*S*)

2-(Cyclohexa-1,4-dien-1-yl)acetic acid, **1**, (2.00 g, 14.48 mmol, 1.0 eq.) and diisopropylethylamine (DIEA) (5.6 mL, 31.84 mmol, 2.2 eq.) were added to dry CH_2Cl_2 (30 mL) at 0 °C. With stirring, TBTU (4.64 g, 14.48 mmol, 1.0 eq.) was added and the reaction stirred for 15 min. (*R*)-1-Phenylethylamine, **2-(*R*)**, or (*S*)-1-phenylethylamine, **2-(*S*)** (1.8 mL, 14.48 mmol, 1.0 eq.) in dry CH_2Cl_2 (10 mL) was added dropwise and the reaction stirred for 18 h at room temperature. The resulting clear orange solution was washed with water (15 mL), brine (15 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo* and the product purified by flash column chromatography (9 : 1 CH_2Cl_2 : MeOH) to give **3-(*R*)** or **3-(*S*)** as white solids. (**3-(*R*)**: 1.94 g, 55%, **3-(*S*)**: 2.40 g, 68%).

3-(*R*): Mp 114 °C; $[\alpha]_D^{20}$ = +98° (c 0.03, EtOH); Anal. calcd for $C_{16}H_{19}NO$: C 79.62, H 7.94, N 5.81. Found: C 79.35, H 7.86, N 6.04%; IR ν(cm^{-1}): 3282, 3029, 2972, 2883, 2823, 1637, 1541,

1492, 1444, 1360, 1251, 1014, 962, 743, 697, 658, 559, 501, 484, 455; NMR ($CDCl_3$) ^{1}H δ 7.34–7.26 (m, 5H, Ar*H*), 5.93 (d, J = 6 Hz, 1H, N*H*), 5.72–5.66 (m, 3H, cyclohexadiene C*H*), 5.13 (m, 1H, C*H*), 2.92 (s, 2H, CH_2), 2.74 (m, 2H, cyclohexadiene CH_2), 2.62 (m, 1H, cyclohexadiene CH_2), 1.47 (d, J = 7 Hz, 3H, CH_3); ^{13}C{^{1}H} δ 169.8 (*C*=O), 143.3 (Ar–*C*), 130.2 (diene–*C*), 128.7 (Ar–*C*), 127.3 (Ar–*C*), 126.1 (Ar–*C*), 124.0 (diene–*C*), 123.9 (diene–*C*), 123.8 (diene–*C*), 48.6 (*C*H), 45.9 (*C*H_2), 29.1 (cyclohexadiene *C*H_2), 26.9 (cyclohexadiene *C*H_2), 21.8 (*C*H_3); HR ESI-MS: m/z = 242.155 $[M + H]^+$ (calc. for $C_{16}H_{20}NO$ 242.150).

3-(*S*): Mp 114 °C; $[\alpha]_D^{20}$ = −98° (c 0.03, EtOH); Anal. calcd for $C_{16}H_{19}NO$: C 79.62, H 7.94, N 5.81. Found: C 79.65, H 7.95, N 5.73%; IR ν(cm^{-1}): 3283, 3062, 3029, 2972, 2883, 2823, 1637, 1540, 1492, 1444, 1360, 1251, 1013, 962, 743, 697, 658, 496, 467, 453; NMR ($CDCl_3$) ^{1}H δ 7.34–7.26 (m, 5H, Ar*H*), 5.92 (d, J = 6 Hz, 1H, N*H*), 5.70 (m, 2H, cyclohexadiene C*H*), 5.63 (m, 1H, cyclohexadiene C*H*), 5.14 (m, 1H, C*H*), 2.92 (s, 2H, CH_2), 2.76 (m, 2H, cyclohexadiene CH_2), 2.63 (m, 2H, cyclohexadiene CH_2) 1.47 (d, J = 7 Hz, 3H, CH_3); ^{13}C{^{1}H} δ 169.8 (*C*=O), 143.3 (Ar–*C*), 130.2 (diene–*C*), 128.7 (Ar–*C*), 127.4 (Ar–*C*), 126.1 (Ar–*C*), 124.0 (diene–*C*), 123.9 (diene–*C*), 123.8 (diene–*C*), 48.6 (*C*H), 45.9 (*C*H_2), 29.1 (cyclohexadiene *C*H_2), 26.9 (cyclohexadiene *C*H_2), 21.9 (*C*H_3); HR ESI-MS: m/z = 242.155 $[M + H]^+$ (calc. for $C_{16}H_{20}NO$ 242.150).

Synthesis of 4-(*R*) and 4-(*S*)

$RuCl_3 \cdot 3H_2O$ (100 mg, 0.42 mmol, 1.0 eq.) and either **3-(*R*)** or **3-(*S*)** (400 mg, 1.67 mmol, 4.0 eq.) were refluxed in EtOH (100 mL) under nitrogen for 6 h. While hot, the solution was filtered and the filtrate reduced in volume (to *ca.* 30 mL). Storage at −4 °C resulted in the precipitation of **4-(*R*)** or **4-(*S*)**, which was isolated by filtration, washed with Et_2O (2 × 5 mL) and dried under vacuum. (**4-(*R*)**: 105 mg, 67%, **4-(*S*)**: 88 mg, 56%).

4-(*R*): Mp 231 °C (decomp.); Anal. calcd for $C_{32}H_{34}N_2O_2Cl_4Ru_2 \cdot H_2O$: C 45.71, H 4.32, N 3.33. Found: C 45.53, H 4.07, N 3.23%; IR ν(cm^{-1}) 3276, 3036, 2973, 1642, 1542, 1493, 1444, 1342, 1254, 1145, 1018, 962, 872, 755, 695, 532, 499, 492, 476; NMR (d_6-DMSO) ^{1}H δ 8.71 (d, J = 8 Hz, 1H, N*H*), 7.30 (m, 4H, Ar*H*), 7.22 (m, 1H, Ar*H*), 6.01 (m, 2H, Ru–Ar*H*), 5.80 (m, 3H, Ru–Ar*H*), 4.91 (m, 1H, C*H*), 3.36 (s, 2H, CH_2), 1.35 (d, J = 7 Hz, 3H, CH_3).

4-(*S*): Mp 231 °C (decomp.); Anal. calcd for $C_{32}H_{34}N_2O_2Cl_4Ru_2 \cdot 3H_2O$: C 43.84, H 4.60, N 3.20. Found: C 43.74, H 3.96, N 2.98%; IR ν(cm^{-1}) 3274, 3036, 2973, 1641, 1542, 1493, 1444, 1342, 1252, 1145, 1018, 962, 871, 755, 695, 633, 564, 535, 481; NMR (d_6-DMSO) ^{1}H δ 8.72 (d, J = 7 Hz, 1H, N*H*), 7.31 (m, 4H, Ar*H*), 7.22 (m, 1H, Ar*H*), 6.01 (m, 2H, Ru–Ar*H*), 5.80 (m, 3H, Ru–Ar*H*), 4.91 (m, 1H, C*H*), 3.37 (s, 2H, CH_2), 1.34 (d, J = 7 Hz, 3H, CH_3).

Synthesis of 5-(*R*) and 5-(*S*)

4-(*R*) or **4-(*S*)** (50 mg, 0.06 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 and MeOH (60 mL, 2 : 1). PTA (19 mg, 0.12 mmol, 2.0 eq.) was added and the solution stirred at room temperature for 3 h. The solution was filtered and the solvent removed. The solid was redissolved in minimal MeOH and precipitated with Et_2O at −4 °C. The solid was isolated by filtration and washed with Et_2O (2 × 5 mL) and dried *in vacuo*. (**5-(*R*)**: 26 mg, 37%; **5-(*S*)**: 30 mg, 43%.)

5-(*R*): Mp 197 °C (decomp.); $[\alpha]_D^{20}$ = −20° (c 0.08, 100 mM saline); Anal. calcd for $C_{22}H_{29}N_4OPCl_2Ru \cdot 0.5H_2O$: C 45.75, H 5.24, N 9.71. Found: C 45.87, H 5.05, N 9.57%; IR ν(cm^{-1}) 3277, 2926, 1655, 1500, 1443, 1406, 1335, 1277, 1239, 1099, 1010, 970, 944, 866, 799, 757, 742, 698, 577, 540, 524, 455, 516; NMR (d_6-DMSO) ^{1}H δ 8.67 (d, J = 8 Hz, 1H, N*H*), 7.31 (m, 4H, Ar*H*), 7.21 (m, 4H, Ar*H*), 5.80 (m, 2H, Ru–Ar*H*), 5.64 (dd, J = 6, 16 Hz, 2H, Ru–Ar*H*), 5.33 (t, J = 6 Hz, 1H, Ru–Ar*H*), 4.89 (m, 1H, C*H*), 4.43 (s, 6H, N–CH_2–N), 4.19 (s, 6H, P–CH_2–N), 3.18 (s, 2H, CH_2), 1.35 (d, J = 6 Hz, 3H, CH_3); ^{13}C{^{1}H} δ 167.6 (*C*=O), 144.4 (Ar–*C*), 128.2 (Ar–*C*), 126.6 (Ar–*C*), 125.9 (Ar–*C*), 101.7 (d, J = 4 Hz, Ru–*C*), 88.1 (d, J = 5 Hz, Ru–*C*), 87.7 (d, J = 5 Hz, Ru–*C*), 86.0 (Ru–*C*), 85.6 (Ru–*C*), 78.7 (Ru–*C*), 72.2 (d, J = 7 Hz, N–*C*H_2–N), 51.9 (d, J = 18 Hz, P–*C*H_2–N), 48.2 (*C*H), 22.5 (*C*H_3) (CH_2 under solvent, *ca.* 39.5); ^{31}P{^{1}H} δ −31.6; HR ESI-MS m/z = 533.083 (15%, $[M - Cl]^+$, calc. for $C_{22}H_{30}ClN_4OPRu$ 533.081), 569.057 $[M + H]^+$ (calc. for $C_{22}H_{30}Cl_2N_4OPRu$ 569.058).

5-(*S*): Mp 197 °C (decomp.); $[\alpha]_D^{20}$ = +20° (c 0.08, 100 mM saline); Anal. calcd for $C_{22}H_{29}N_4OPCl_2Ru \cdot 1.5H_2O$: C 44.36, H 5.42, N 9.41. Found C 44.36, H 5.25, N 9.94%; IR ν(cm^{-1}) 3277, 3053, 1657, 1504, 1443, 1406, 1336, 1277, 1240, 1198, 1099, 1010, 971, 944, 896, 866, 810, 799, 757, 742, 699, 576, 532, 516, 493; NMR (d_6-DMSO) ^{1}H δ 8.67 (d, J = 8 Hz, 1H, NH), 7.29 (m, 4H, ArH), 7.21 (m, 1H, ArH) 5.81 (m, 1H, Ru–ArH), 5.67 (dd, J = 17, 6 Hz, 1H, Ar–RuH), 5.63 (dd, J = 17, 6 Hz, 1H, Ar–RuH), 5.34 (t, J = 6 Hz, Ru–ArH), 4.88 (m, 1H, CH), 4.43 (s, 6H, N–CH_2–N), 4.18 (s, 6H, P–CH_2–N), 3.18 (s, 2H, CH_2), 1.35 (d, J = 7 Hz, 3H, CH_3); ^{13}C{^{1}H} δ 167.6 (*C*=O), 144.4 (Ar–*C*), 128.2 (Ar–*C*), 126.6 (Ar–*C*), 125.9 (Ar–*C*), 101.7 (d, J = 4 Hz, Ru–*C*), 88.3 (d, J = 5 Hz, Ru–*C*), 87.9 (d, J = 5 Hz, Ru–*C*), 86.0 (Ru–*C*), 85.5 (Ru–*C*), 78.7 (Ru–*C*), 72.2 (d, J = 7 Hz, N–*C*H_2–N), 51.9 (d, J = 18 Hz, P–*C*H_2–N), 48.2 (*C*H), 22.5 (*C*H_3) (CH_2 under solvent, *ca.* 39.5); ^{31}P{^{1}H} δ −31.5; HR ESI-MS m/z = 533.088 (12%, $[M - Cl]^+$, calc. for $C_{22}H_{30}ClN_4OPRu$ 533.081), 569.058 (100%, $[M + H]^+$, calc. for $C_{22}H_{30}Cl_2N_4OPRu$ 569.058).

Synthesis of 6-(*R*) and 6-(*S*)

4-(*R*) or **4-(*S*)** (50 mg, 0.06 mmol, 1.0 eq.) was dissolved in water (100 mL) and silver oxalate (46 mg, 0.15 mmol, 2.5 eq.) added. The reaction was stirred for 12 h under a nitrogen atmosphere in a foil-covered flask. The silver chloride was removed by filtration through celite and the solvent removed. The solid was redissolved in MeOH (25 mL) and PTA (23 mg, 0.14 mmol, 2.4 eq.) was added and the solution stirred for a further 2 h. The solvent was reduced to ∼5% of the original volume and Et_2O (25 mL) was added. The solution was stored at −4 °C and the yellow precipitate isolated by filtration, washed with Et_2O (2 × 5 mL) and dried *in vacuo*. (**6-(*R*)**: 35 mg, 49%, **6-(*S*)**: 33 mg, 46%.)

6-(*R*): Mp 171 °C (decomp.); $[\alpha]_D^{20}$ = −29° (c 0.05, 100 mM saline); Anal. calcd for $C_{24}H_{29}N_4O_5PRu \cdot 3H_2O$: C 44.99, H 5.51, N 8.75. Found: C 44.75, H 4.93, 10.54%; IR ν(cm^{-1}) 3272, 3054,

1696, 1645, 1541, 1494, 1445, 1364, 1284, 1240, 1097, 1012, 968, 945, 899, 782, 741, 699, 576, 538, 526, 517, 471; NMR (d_6-DMSO) ^{1}H δ 8.72 (d, J = 8 Hz, 1H, N*H*), 7.30 (m, 4H, Ar*H*), 7.23 (m, 1H, Ar*H*), 5.96 (d, J = 2 Hz, 2H, Ru–Ar*H*), 5.79 (dd, J = 6, 19 Hz, 2H, Ru–Ar*H*), 5.48 (t, J = 5 Hz, 1H, Ru–Ar*H*), 4.89 (m, 1H, C*H*), 4.42 (s, 6H, N–CH_2–N), 4.03 (s, 6H, P–CH_2–N), 3.20 (s, 2H, CH_2), 1.36 (d, J = 7 Hz, 3H, CH_3); ^{13}C{^{1}H} δ 167.0 (C═O), 164.0 (2 × C═O, oxalato), 144.4 (Ar–C), 128.3 (Ar–C), 126.7 (Ar–C), 125.9 (Ar–C), 102.4 (d, J = 4 Hz, Ru–*C*), 87.2 (Ru–*C*), 87.0 (Ru–*C*), 86.9 (d, J = 5 Hz, Ru–*C*), 86.6 (d, J = 4 Hz, Ru–*C*), 76.4 (Ru–*C*), 71.8 (d, J = 6 Hz, N–*C*H_2–N), 50.1 (d, J = 18 Hz, P–*C*H_2–N), 48.2 (*C*H), 38.5 (*C*H_2), 22.6 (*C*H_3); ^{31}P{^{1}H} δ −32.0; HR ESI-MS m/z = 587.100 (100%, [M + H]$^+$, calc. for $C_{24}H_{30}N_4O_5PRu$ 587.100).

6-(*S*): Mp 171 °C (decomp.), $[\alpha]_D^{20}$ = +29° (c 0.05, 100 mM saline); Anal. calcd for $C_{24}H_{29}N_4O_5PRu{\cdot}2H_2O$: C 46.29, H 5.35, N 9.00. Found: C 46.54, H 4.76, N 8.95%; IR ν(cm^{-1}) 3276, 3060, 1696, 1670, 1654, 1541, 1445, 1364, 1285, 1240, 1098, 1012, 967, 946, 899, 781, 742, 698, 576, 518, 511, 532, 492, 478; NMR (d_6-DMSO) ^{1}H δ 8.71 (d, J = 8 Hz, 1H, N*H*), 7.30 (m, 4H, Ar*H*), 7.23 (m, 1H, Ar*H*), 5.95 (m, 2H, Ru–Ar*H*), 5.79 (dd, J = 6, 18 Hz, 2H, Ru–Ar*H*), 5.48 (t, J = 6 Hz, 1H, Ru–Ar*H*), 4.88 (m, 1H, C*H*), 4.42 (s, 6H, N–CH_2–N), 4.02 (s, 6H, P–CH_2–N), 3.20 (s, 2H, CH_2), 1.35 (d, J = 7 Hz, 3H, CH_3); ^{13}C{^{1}H} δ 166.9 (C═O), 164.0 (2 × C═O, oxalato), 144.4 (Ar–C), 128.2 (Ar–C), 126.6 (Ar–C), 125.9 (Ar–C), 102.4 (d, J = 4 Hz, Ru–*C*), 87.2 (Ru–*C*), 87.0 (Ru–*C*), 86.9 (d, J = 5 Hz, Ru–*C*), 86.6 (d, J = 5 Hz, Ru–*C*), 76.4 (Ru–*C*), 71.8 (d, J = 7 Hz, N–*C*H_2–N), 50.1 (d, J = 14 Hz, P–*C*H_2–N), 48.2 (*C*H), 38.5 (*C*H_2), 22.6 (*C*H_3); ^{31}P{^{1}H} δ −32.0; HR ESI-MS m/z = 587.101 (100%, [M + H]$^+$, calc. for $C_{24}H_{30}N_4O_5PRu$ 587.100).

Cell culture conditions and cytotoxicity assay

The human A2780 and A2780cisR ovarian carcinoma and HEK (human embryonic kidney) cells were obtained from the European Collection of Cell Cultures (Salisbury, UK). A2780 and A2780cisR cells were grown routinely in RPMI-1640 medium, while HEK cells were grown with DMEM medium, with 10% foetal calf serum (FCS) and antibiotics at 37 °C and 5% CO_2. Cytotoxicity was determined using the MTT assay (MTT = 3(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide). Cells were seeded in 96-well plates as monolayers with 100 μL of cell solution (approximately 20 000 cells) per well and pre-incubated for 24 h in medium supplemented with 10% FCS. Compounds were prepared as DMSO solutions then dissolved in the culture medium and serially diluted to the appropriate concentration, to give a final DMSO concentration of 0.5%. 100 μL of drug solution was added to each well and the plates were incubated for another 72 h. Subsequently, MTT (5 mg mL^{-1} solution) was added to the cells and the plates were incubated for a further 2 h. The culture medium was aspirated, and the purple formazan crystals formed by the mitochondrial dehydrogenase activity of vital cells were dissolved in DMSO. The optical density, directly proportional to the number of surviving cells, was quantified at 590 nm using a multiwell plate reader and the fraction of surviving cells was calculated from the absorbance of untreated control cells. Evaluation is based on means from two independent experiments, each comprising three micro-cultures per concentration level.

Acknowledgements

This work was supported by the New Zealand Foundation of Research, Science and Technology (Postdoctoral Fellowship, EPFL1001, to KJK), an Erasmus Grant (Commission of the European Communities within the framework of the LLP Erasmus Programme – SMC), and the Swiss National Science Foundation (CMC). Ms. S. Crot is gratefully acknowledged for technical assistance and Dr B. S. Murray for helpful discussions.

References

1 G. Gasser, I. Ott and N. Metzler-Nolte, *J. Med. Chem.*, 2010, **54**, 3–25.
2 G. Sava, A. Bergamo and P. J. Dyson, *Dalton Trans.*, 2011, 9069–9075.
3 N. J. Wheate, S. Walker, G. E. Craig and R. Oun, *Dalton Trans.*, 2010, 8113–8127.
4 S. Dhar and S. J. Lippard, in *Bioinorganic Medicinal Chemistry*, ed. E. Alessio, Wiley-VCH Verlag GmbH & Co., 2011, pp. 79–95.
5 G. S. Smith and B. Therrien, *Dalton Trans.*, 2011, 10793–10800.
6 W. H. Ang and P. J. Dyson, *Eur. J. Inorg. Chem.*, 2006, **2006**, 3993–3993.
7 A. Bergamo, C. Gaiddon, J. H. M. Schellens, J. H. Beijnen and G. Sava, *J. Inorg. Biochem.*, 2012, **106**, 90–99.
8 C. Scolaro, A. Bergamo, L. Brescacin, R. Delfino, M. Cocchietto, G. Laurenczy, T. J. Geldbach, G. Sava and P. J. Dyson, *J. Med. Chem.*, 2005, **48**, 4161–4171.
9 W. H. Ang, A. Casini, G. Sava and P. J. Dyson, *J. Organomet. Chem.*, 2011, **696**, 989–998.
10 A. A. Nazarov, J. Risse, W. H. Ang, F. Schmitt, O. Zava, A. Ruggi, M. Groessl, R. Scopelitti, L. Juillerat-Jeanneret, C. G. Hartinger and P. J. Dyson, *Inorg. Chem.*, 2012, **51**, 3633–3639.
11 D. J. M. Snelders, A. Casini, F. Edafe, G. van Koten, R. J. M. Klein Gebbink and P. J. Dyson, *J. Organomet. Chem.*, 2011, **696**, 1108–1116.
12 W. H. Ang, E. Daldini, C. Scolaro, R. Scopelliti, L. Juillerat-Jeannerat and P. J. Dyson, *Inorg. Chem.*, 2006, **45**, 9006–9013.
13 A. K. Renfrew, R. Scopelliti and P. J. Dyson, *Inorg. Chem.*, 2010, **49**, 2239–2246.
14 C. Scolaro, A. B. Chaplin, C. G. Hartinger, A. Bergamo, M. Cocchietto, B. K. Keppler, G. Sava and P. J. Dyson, *Dalton Trans.*, 2007, 5065–5072.

15 A. K. Renfrew, L. Juillerat-Jeanneret and P. J. Dyson, *J. Organomet. Chem.*, 2011, **696**, 772–779.
16 C. A. Vock, C. Scolaro, A. D. Phillips, R. Scopelliti, G. Sava and P. J. Dyson, *J. Med. Chem.*, 2006, **49**, 5552–5561.
17 K. J. Kilpin, C. M. Clavel, F. Edafe and P. J. Dyson, *Organometallics*, 2012, **31**, 7031–7039.
18 A. Kurzwernhart, W. Kandioller, C. Bartel, S. Bachler, R. Trondl, G. Muhlgassner, M. A. Jakupec, V. B. Arion, D. Marko, B. K. Keppler and C. G. Hartinger, *Chem. Commun.*, 2012, 4839–4841.
19 M. Hanif, A. A. Nazarov, A. Legin, M. Groessl, V. B. Arion, M. A. Jakupec, Y. O. Tsybin, P. J. Dyson, B. K. Keppler and C. G. Hartinger, *Chem. Commun.*, 2012, 1475–1477.
20 H. Henke, W. Kandioller, M. Hanif, B. K. Keppler and C. G. Hartinger, *Chem. Biodiversity*, 2012, **9**, 1718–1727.
21 H. Dialer, P. Mayer, K. Polborn and W. Beck, *Eur. J. Inorg. Chem.*, 2001, 1051–1055.
22 L. Vieille-Petit, B. Therrien and G. Süss-Fink, *Eur. J. Inorg. Chem.*, 2003, 3707–3711.
23 M. E. Franks, G. R. Macpherson and W. D. Figg, *Lancet*, 2004, **363**, 1802–1811.
24 B. Kasprzyk-Hordern, *Chem. Soc. Rev.*, 2010, **39**, 4466–4503.
25 L. Pendyala, Y. Kidani, R. Perez, J. Wilkes, R. J. Bernacki and P. J. Creaven, *Cancer Lett.*, 1995, **97**, 177–184.
26 S. A. Abramkin, U. Jungwirth, S. M. Valiahdi, C. Dworak, L. Habala, K. Meelich, W. Berger, M. A. Jakupec, C. G. Hartinger, A. A. Nazarov, M. Galanski and B. K. Keppler, *J. Med. Chem.*, 2010, **53**, 7356–7364.
27 U. Jungwirth, D. N. Xanthos, J. Gojo, A. K. Bytzek, W. Körner, P. Heffeter, S. A. Abramkin, M. A. Jakupec, C. G. Hartinger, U. Windberger, M. Galanski, B. K. Keppler and W. Berger, *Mol. Pharmacol.*, 2012, **81**, 719–728.
28 H. S. I. Chao and G. A. Berchtold, *J. Org. Chem.*, 1981, **46**, 1191–1194.
29 R. Knorr, A. Trzeciak, W. Bannwarth and D. Gillessen, *Tetrahedron Lett.*, 1989, **30**, 1927–1930.
30 M. A. Bennett and A. K. Smith, *J. Chem. Soc., Dalton Trans.*, 1974, 233–241.
31 C. S. Allardyce, P. J. Dyson, D. J. Ellis and S. L. Heath, *Chem. Commun.*, 2001, 1396–1397.
32 A. Dorcier, W. H. Ang, S. Bolaño, L. Gonsalvi, L. Juillerat-Jeannerat, G. Laurenczy, M. Peruzzini, A. D. Phillips, F. Zanobini and P. J. Dyson, *Organometallics*, 2006, **25**, 4090–4096.
33 C. Gossens, A. Dorcier, P. J. Dyson and U. Rothlisberger, *Organometallics*, 2007, **26**, 3969–3975.
34 C. Scolaro, C. G. Hartinger, C. S. Allardyce, B. K. Keppler and P. J. Dyson, *J. Inorg. Biochem.*, 2008, **102**, 1743–1748.
35 W. H. Ang, *PhD thesis, École Polytechnique Fédérale de Lausanne*, 2007.
36 D. J. Daigle, T. J. Decuir, J. B. Robertson and D. J. Darensbourg, in *Inorganic Syntheses*, ed. M. Y. Darensbourg, John Wiley & Sons, Inc., 1998, vol. 32, pp. 40–45.