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Ruthenium Arene Complexes with Triphenylphosphane Ligands: Cytotoxic Activity Towards Pancreatic Cancer Cells, Interaction with Model Proteins, and Critical Effect of Ethacrynic Acid Substitution

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Supporting Information

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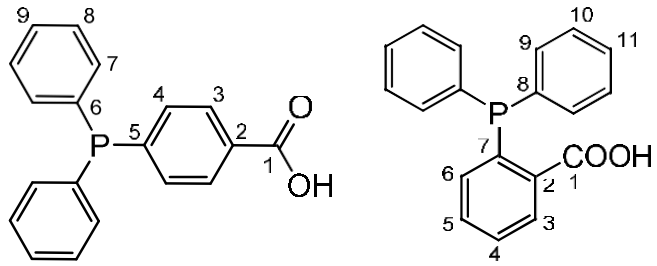
Spectroscopic characterization of $PPh_2(4\text{-}C_6H_4CO_2H)$ and $PPh_2(2\text{-}C_6H_4CO_2H)$.

Chart S1. Structures of $PPh_2(4\text{-}C_6H_4CO_2H)$ (left) and $PPh_2(2\text{-}C_6H_4CO_2H)$ (right) (numbering refers to carbon atoms).

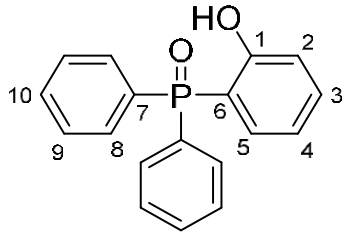


$PPh_2(4\text{-}C_6H_4CO_2H)$. Colourless crystalline solid. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3059w, 2998w, 2725w, 2551w, 1684s ($\nu_{C=O}$), 1595m, 1583m-sh, 1557m, 1478m, 1431m, 1422m, 1394m, 1317s, 1296s, 1277m-sh, 1184m, 1132w, 1115w, 1085m, 1068w, 1027w, 1017m, 999w, 939m-br, 853m, 816m, 764m, 745s, 694s. ^{1}H NMR ($CDCl_3$): δ/ppm = 8.03 (d, $^3J_{HH}$ = 7.5 Hz, 2H, C3-H), 7.39—7.32 (m, 12H, Ph_2P + C4-H), 6.78 (s, 1H, OH). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ/ppm = 171.5 (C1), 136.0 (m, C6), 134.2 (d, $^2J_{CP}$ = 19.8 Hz, C7), 133.3 (d, $^2J_{CP}$ = 18.4 Hz, C4-H), 130.0 (d, $^3J_{CP}$ = 6.3 Hz, C3-H), 129.4 (C9), 128.9 (d, $^3J_{CP}$ = 7.0 Hz, C8). $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ/ppm = —4.7.

$PPh_2(2\text{-}C_6H_4CO_2H)$. Crystalline colourless solid. IR (solid state): $\tilde{\upsilon}/cm^{-1}$ = 3068w, 3050w, 3001w, 2872w, 2823w 2732w, 2690w, 2553w, 2522w, 1685s ($\nu_{C=O}$), 1584w, 1561m, 1467m, 1434m, 1410m, 1304m, 1271s, 1179w, 1147m, 1118m, 1090m, 1069w, 1057w, 1026w, 999w, 927m, 880w, 853w, 806m, 740s, 692s. ^{1}H NMR ($CDCl_3$): δ/ppm = 8.77 (br, 1H, OH), 8.20 (m, 1H, C3-H), 7.53–7.43 (m, 2H, C4-H + C5-H), 7.43–7.30 (m, 10H, PPh_2), 7.05 (t, J = 6.0 Hz, 1H, C6-H). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ/ppm = 171.4 (C1), 141.6 (d, $^1J_{CP}$ = 19.4 Hz, C7), 137.5 (m, C8), 134.4, 134.1 (d, $^2J_{CP}$ = 20.5 Hz, C9), 133.0, 132.8, 131.8, 128.9 (C11), 128.6 (d, $^3J_{CP}$ = 7.3 Hz, C10), 128.5 (C4). $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ/ppm = —4.3.

Synthesis of O=PPh$_2$(2-C$_6$H$_4$OH).[1]

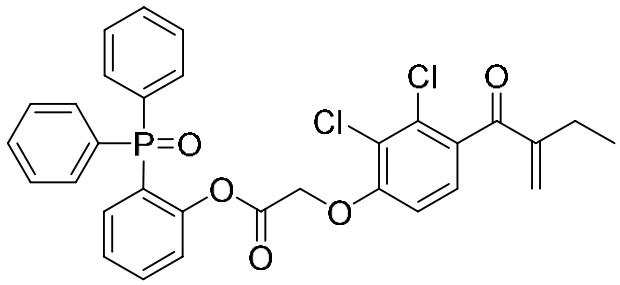
Chart S2. Structure of O=PPh$_2$(2-C$_6$H$_4$OH) (numbering refers to carbon atoms).



A solution of PPh$_2$(2-C$_6$H$_4$OH) (100 mg, 0.359 mmol) and H_2O_2 (30% *w*/*w* in H_2O, 0.10 mL, 0.98 mmol) in THF (5 mL) was stirred at room temperature for 1.5 hours. The resulting colourless suspension was filtered and the solid was washed with Et_2O then dried under vacuum (40°C). Yield: 77 mg, 73%. The compound is soluble in DMSO, less soluble in CH_2Cl_2, acetone, insoluble in Et_2O, H_2O. ^{1}H NMR (DMSO-d$_6$): δ/ppm = 10.50 (s, 1H, OH), 7.65 (dd, $^3J_{HP}$ = 12.1 Hz, $^3J_{HH}$ = 7.3 Hz, 4H, C8-H), 7.61–7.56 (m, 2H, C10-H), 7.55–7.49 (m, 5H, C5-H + C9-H), 7.45 (t, $^3J_{HH}$ = 7.6 Hz, 1H, C3-H), 6.97 (t, $^3J_{HH}$ = 6.9 Hz, 1H, C4-H), 6.88 (dd, $^3J_{HH}$ = 7.4 Hz, $^4J_{HP}$ = 6.1 Hz, 1H, C2-H). ^{31}P{^{1}H} NMR (DMSO-d$_6$): δ/ppm = 27.3.

Attempted synthesis of O=PPh$_2$(2-C$_6$H$_4$OCO-EA), O=L1.

Chart S3. Structure of **O=L1**.



Via esterification of phosphane oxide. A solution of O=PPh$_2$(2-C$_6$H$_4$OH) (52 mg, 0.18 mmol) and EA-CO$_2$H (53 mg, 0.18 mmol) in CH_2Cl_2 (6 mL) was treated with EDCI·HCl (44 mg, 0.23 mmol) and then with DMAP (6 mg, 0.05 mmol). The resulting colourless solution was stirred at room

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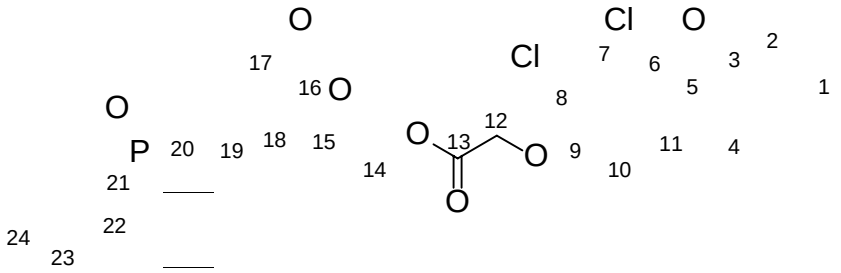
temperature for 5 hours and the progress of reaction was monitored by ^{31}P spectroscopy. Volatiles were removed under vacuum and the resulting colourless residue was dissolved in $CHCl_3$ and extracted with H_2O (x3). The organic phase was then taken to dryness under vacuum, affording a colourless solid.

Via oxidation of phosphane ester. A suspension of **L1** (163 mg, 0.29 mmol) in MeCN (10 mL) was treated with H_2O_2 (30% w/w in H_2O, 0.10 mL, 0.98 mmol) and stirred at room temperature overnight, affording a colourless solution. The progress of reaction was monitored by ^{31}P spectroscopy. Volatiles were then removed under vacuum affording a colourless solid.

During both reactions, the formation of compound **O=L1** ($^{31}P\{^1H\}$ NMR ($CDCl_3$): δ/ppm = 27.6) was observed, shortly followed by the appearance of a second product ($^{31}P\{^1H\}$ NMR ($CDCl_3$): δ/ppm = 39.4) which became predominant. The solid material isolated was composed by a mixture of both species in variable ratios and **O=L1** could not be purified by silica chromatography (decomposition in column).

Synthesis of O=PPh$_2$(4-C$_6$H$_4$CO$_2$CH$_2$CH$_2$OCO-EA), O=L2.

Chart S4. Structure of **O=L2** (numbering refers to carbon atoms).



A solution of **L2** (150 mg, 0.236 mmol) and H_2O_2 (30% *w*/*w* in H_2O, 30 µL, 0.29 mmol) in THF (5 mL) was stirred at room temperature for 4.5 hours. The progress of reaction was checked by ^{31}P NMR then volatiles were removed under vacuum. The resulting colorless oily residue was dissolved in few mL of CH_2Cl_2 and loaded on top of a silica column. The title compound was obtained as a colorless solid after elution with CH_2Cl_2:acetone 7:1 *v*/*v* and volatiles removal under vacuum (40°C). Yield: 60 mg, 39%. The compound is soluble in MeOH, acetone and chlorinated

solvents, insoluble in water. ^{1}H NMR ($CDCl_3$): δ/ppm = 8.09 (dd, $^{3}J_{HH}$ = 7.9 Hz, $^{4}J_{HP}$ = 1.8 Hz, 2H, C18-H), 7.77 (dd, $^{3}J_{HP}$ = 11.3 Hz, $^{3}J_{HH}$ = 8.2 Hz, 2H, C19-H), 7.65 (dd, $^{3}J_{HP}$ = 12.0 Hz, $^{3}J_{HH}$ = 7.4 Hz, 4H, C22-H), 7.60–7.54 (m, 2H, C24-H), 7.52–7.45 (m, 4H, C23-H), 7.09 (d, $^{3}J_{HH}$ = 8.5 Hz, 1H, C11-H), 6.79 (d, $^{3}J_{HH}$ = 8.5 Hz, 1H, C10-H), 5.91 (s, 1H, C4-H), 5.56 (s, 1H, C4-H'), 4.77 (s, 2H, C12-H), 4.56 (s, 4H, C14-H + C15-H), 2.46 (q, $^{3}J_{HH}$ = 7.1 Hz, 1H, C2-H), 1.12 (t, $^{3}J_{HH}$ = 7.4 Hz, 3H, C1-H). $^{31}P\{^{1}H\}$ NMR ($CDCl_3$): δ/ppm = 28.4.

Table S1. Comparison of selected IR and NMR data for **1**-**5** and related compounds (EA-CO_2H = ethacrynic acid).

Compound IR/NMR signal[a]	**$Ph_2P(4\text{-}C_6H_4CO_2H)$**	**1**	**$Ph_2P(2\text{-}C_6H_4CO_2H)$**	**$K[Ph_2P(2\text{-}C_6H_4CO_2)]$**	**2**	**EA-CO_2H**	**$Ph_2P(2\text{-}C_6H_4OH)$**	**L1**	**3**	**EA-$CO_2(CH_2)_2OH$**	**L2** [b]	**4**	**L3**	**5**
v(C=O) / cm^{-1} C_6H_4-C(=O)O	1683s	1692s	1683s	1593s (ν_{asym}) 1375s (ν_{sym})	1604s (ν_{asym}) 1328s (ν_{sym})	-	-	-	-	-	1718s	1721m	-	-
v(C=O) / cm^{-1} EA-C(=O)O	-	-	-	-	-	1725s	-	1779m	1782s	1737s	1763m	1761m	-	-
v(C=O)[c] / cm^{-1} EA-C(=O)-C=C	-	-	-	-	-	1671s 1661s	-	1663s	1663m	1662s	1665m	1665m	-	-
v(C=C)[c] / cm^{-1} EA-C(=O)-C=C	-	-	-	-	-	1585s	-	1584s	1582m	1587m	1584m	1585m	-	-
^{1}H δ(OH) / ppm	6.78 (br)	6.30 (br)	8.77 (br)	-	-	7.75 (br)	6.35 (br)	-	-	1.83	-	-	-	-
^{13}C δ(EA-COO) / ppm	-	-	-	-	-	172.6	-	166.1	165.9	168.1	167.6	167.5	166.6	-
^{13}C δ(Ph-COO) / ppm	171.5	170.4	171.4	*n.r.*	172.3	-	-	-	-	-	166.1	165.7	167.7	-
^{31}P δ / ppm	–4.7	25.3	–4.3	–8.2	30.5	-	–28.5	–16.9	25.0	-	–4.9	25.3	-4.5	26.0 (5a) 28.9 (5b)

[a] IR spectra in the solid state; NMR spectra in $CDCl_3$ solution, except $K[PPh_2(2\text{-}C_6H_4CO_2)]$ (CD_3OD solution).

[b] IR and ^{13}C NMR from reference [12a].

[c] IR spectrum of methyl vinyl ketone in CCl_4 solution: 1707vs and 1687vs (C=O), 1618m (C=C) [Bowles, A. J.; George, W. O.; Maddams, W. F. Conformations of some αβ-unsaturated carbonyl compounds. Part I. Infrared spectra of acraldehyde, crotonaldehyde, methyl vinyl ketone, and ethylideneacetone. *J. Chem. Soc. B*, **1969**, 810-818].

Figure S1. Molecular structure of [(η^6-*p*-cymene)$RuCl_2$(κ*P*-PPh_2(4-$C_6H_4CO_2H$))], **1**. Displacement ellipsoids are at the 50% probability level. H-atoms, except H(1), have been omitted for clarity.

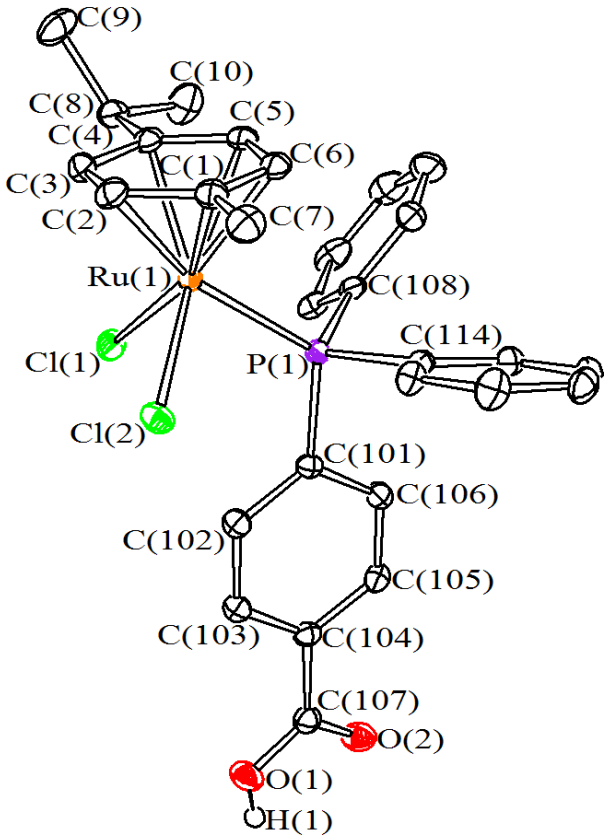


Table S2. Selected bond distances (Å) and angles (°) for **1**.

Ru(1)−C(1)	2.228(3)	Ru(1)−C(2)	2.232(3)
Ru(1)−C(3)	2.228(3)	Ru(1)−C(4)	2.235(3)
Ru(1)−C(5)	2.213(3)	Ru(1)−C(6)	2.187(3)
Ru(1)−Cl(1)	2.4061(7)	Ru(1)−Cl(2)	2.4170(7)
Ru(1)−P(1)	2.3627(8)	C(104)−C(107)	1.492(4)
C(107)−O(1)	1.320(4))	C(107)−O(2)	1.217(4)
Cl(1)−Ru(1)−Cl(2)	90.17(3)	Cl(1)−Ru(1)−P(1)	83.79(3)
Cl(2)−Ru(1)−P(1)	90.42(3)	C(104)−C(107)−O(1)	114.5(3)
C(104)−C(107)−O(2)	122.8(3)	O(1)−C(107)−O(2)	122.7(3)

Figure S2. IR and UV-Vis spectral changes during monoelectronic oxidation of **1** (a, $2.0\cdot10^{-2}$ M), **3** (b, $2.0\cdot10^{-2}$ M) and **4** (c, $2.0\cdot10^{-2}$ M) in 0.2 M [$^{n}Bu_4N$][PF_6] CH_2Cl_2 solution recorded in an OTTLE cell. The initial spectrum, collected before the application of an oxidation potential, was used to calculate the differential absorbance spectra. Peak maxima/minima are referred to peak maxima in the original (↓) and final (↑) spectra.

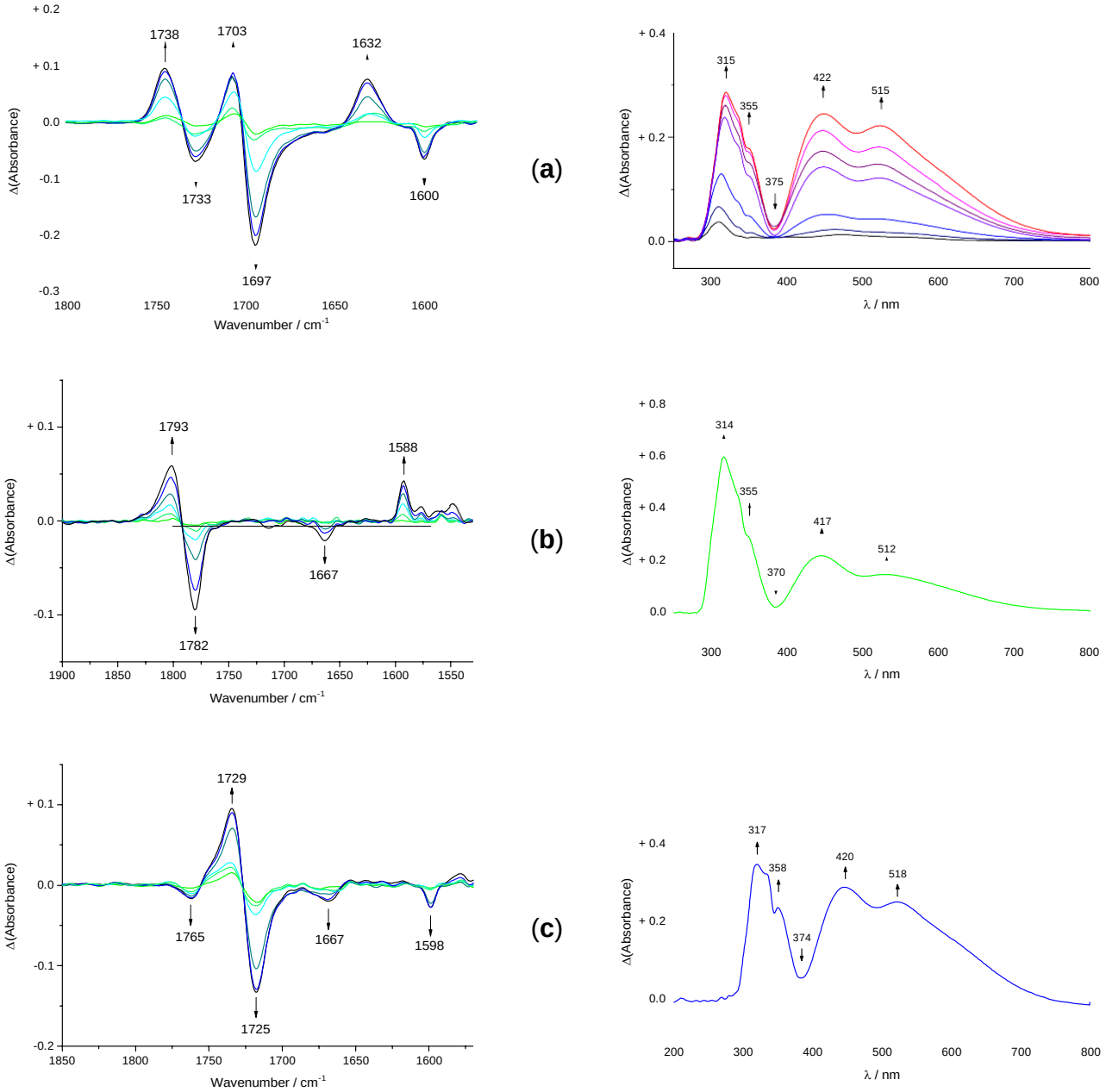


Table S3. Comparison of IR and UV-Vis absorptions for **1**,**3** and **4** and their oxidised species (EA-CO_2H = ethacrynic acid).

Compound **IR/UV-vis signal**	**1** solid state	**1** CH_2Cl_2 solution	**1^+** CH_2Cl_2 solution	**3** solid state	**3** CH_2Cl_2 solution	**3^+** CH_2Cl_2 solution	**4** solid state	**4** CH_2Cl_2 solution	**4^+** CH_2Cl_2 solution
v(C=O) / cm^{-1} C_6H_4-C(=O)O	1692	1697	1703				1721	1725	1729
v(C=O) / cm^{-1} EA-C(=O)O				1782	1782	1793	1761	1765	1765
v(C=O) / cm^{-1} EA-C(=O)-C=C				1663	1667	1667	1665	1667	1667
v(C=C) / cm^{-1} EA-(C=O)-C=C				1582	1587	1588	1585	1587	1587
λ_{max} / nm	-	375 475	315 355 422 515	-	370 470	314 355 417 512	-	374 465	317 358 420 518

Stability studies.

General procedure. Complexes **1**−**4** were dissolved in DMSO-d_6/D_2O 9:1 *v*/*v* (1.0 mL; [Ru] = $1{\cdot}10^{-2}$ mol·L^{-1}). An aliquot of the resulting solution (0.50 mL) was transferred into a NMR tube, maintained at 37°C for 72 hours and analyzed by ^1H and $^{31}P\{^1H\}$ NMR spectroscopy as a function of time. Dimethyl sulfone ($1{\cdot}10^{-2}$ mol·L^{-1}, 1:1 mol ratio vs. Ru) was used as a reference for ^1H NMR spectra (δ/ppm = 2.97 (s, 6H) in DMSO-d_6/D_2O 9:1 *v*/*v*). The remaining solution was diluted up to 4.0 mL with DMSO/H_2O 9:1 *v*/*v* (final [Ru] = $1.2{\cdot}10^{-3}$ mol L^{-1}), maintained at 37 °C for 72 hours and analyzed by conductivity measurements and UV-Vis spectroscopy as a function of time. Parallel NMR analyses were carried out on DMSO-d_6/D_2O solutions prepared by the same procedure described above, and with the addition of NaCl (0.11 mol·L^{-1}). Molar conductivity (Λ_m) and molar absorbance (ε) values were calculated with reference to the starting material. Percent values of compounds in solution are based on ^1H NMR spectroscopy and refer to identified compounds only (indicated as "*% NMR"*) or to dimethyl sulfone as internal standard (indicated as "*% NMR vs internal standard"*).

Reference data. NMR spectra of the following compounds were recorded in DMSO-d_6/D_2O 9:1 *v*/*v* or $CDCl_3$ and used as reference for NMR assignments. ***p*-cymene**. ^1H NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 7.12–7.03 (m, 4H), 2.80 (hept, $^3J_{HH}$ = 6.9 Hz, 1H), 2.23 (s, 3H), 1.15 (d, $^3J_{HH}$ = 6.9 Hz, 6H). **EA-CO_2H**. ^1H NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 7.24 (d, $^3J_{HH}$ = 8.6 Hz, 1H), 7.02 (d, $^3J_{HH}$ = 8.7 Hz, 1H), 6.03 (s, 1H), 5.52 (s, 1H), 4.83 (s, 2H), 2.30 (q, $^3J_{HH}$ = 7.1 Hz, 2H), 1.01 (t, $^3J_{HH}$ = 7.4 Hz, 3H). **EA-$CO_2(CH_2)_2$OH**. ^1H NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 7.29 (d, $^3J_{HH}$ = 8.6 Hz, 1H), 7.13 (d, $^3J_{HH}$ = 8.6 Hz, 1H), 6.07 (s, 1H), 5.55 (s, 1H), 5.01 (s, 2H), 4.19–4.13 (m, 2H), 3.61–3.56 (m, 2H), 2.35 (q, $^3J_{HH}$ = 7.3 Hz, 2H), 1.06 (t, $^3J_{HH}$ = 7.3 Hz, 3H). **PPh_2(4-$C_6H_4CO_2$H).** ^1H NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 7.88 (d, *J* = 7.8 Hz, 2H), 7.46–7.35 (m, 6H), 7.32–7.20 (m, 6H) ppm. $^{31}P\{^1H\}$ NMR (DMSO-d_6:D_2O 9:1): δ/ppm = –6.9 ppm. **O=PPh_2(4-$C_6H_4CO_2$H).**[2] ^1H NMR (DMSO-d_6): δ/ppm = 8.13–8.08 (m, 2H), 7.81–7.74 (m, 2H), 7.69–7.61 (m, 4H), 7.61–7.55

[2] Q. Lin, S. Unal, A. R. Fornof, R. S. Armentrout, T. E. Long, *Polymer*, 2010, **47**, 4085–4093.

(m, 6H). $^{31}P\{^{1}H\}$ NMR (DMSO-d_6): δ/ppm = 26.1. **K[$PPh_2(2\text{-}C_6H_4CO_2)$].** ^{1}H NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 7.93–7.82 (m, 1H), 7.32–7.24 (m, 7H), 7.18–7.02 (m, 5H), 6.67–6.58 (m, 1H) ppm. $^{31}P\{^{1}H\}$ NMR (DMSO-d_6:D_2O 9:1): δ/ppm = –8.8. **K[$O{=}PPh_2(2\text{-}C_6H_4CO_2)$]**.[3] ^{1}H NMR ($CDCl_3$): δ/ppm = 8.41–8.33 (m, 1H), 7.17–7.10 (m, 1H). $^{31}P\{^{1}H\}$ NMR ($CDCl_3$): δ/ppm = 40.7.

[3] Additional NMR signals observed upon air exposure of K[$PPh_2(2\text{-}C_6H_4CO_2)$].

Stability studies: complex 1. The data are reported in Table S4 while the NMR detected species are shown in Scheme S1. **1a.** ^{1}H NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 7.90–7.86 (m, 2H), 7.86–7.80 (m, 2H), 7.77–7.70 (m, 4H), 7.49–7.39 (m, 6H), 5.30 (d, *J* = 6.2 Hz, 2H), 5.24 (d, *J* = 6.0 Hz, 2H), 1.75 (s, 3H), 0.93 (d, *J* = 6.9 Hz, 6H). ^{31}P{^{1}H} NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 24.8. **O=PPh$_2$(4-C$_6$H$_4$CO$_2$H)**. ^{1}H NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 8.08 (dd, *J* = 8.2, 2.3 Hz, 2H), 7.66–7.54 (m, 12H) ppm. ^{31}P{^{1}H} NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 26.7.

Scheme S1. Compounds detected in dmso/H_2O and dmso/H_2O/NaCl solutions of **1** maintained at 37°C.

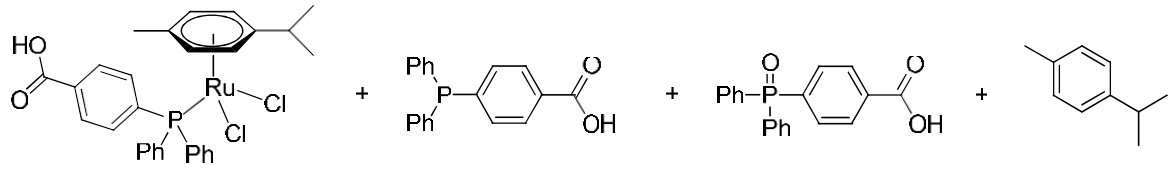


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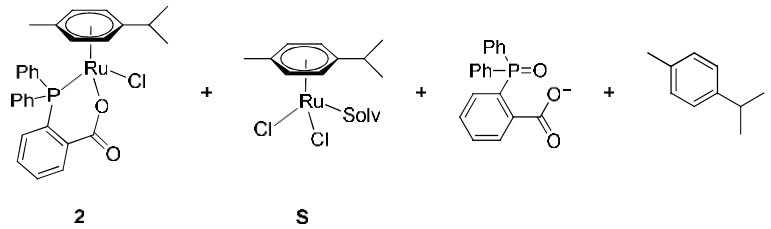
Table S4. UV-Vis absorptions, molar conductivity and NMR detected species as a function of time for DMSO/water 9:1 *v/v* solution of **1** at 37°C. Data for analogous experiments with 0.1 M NaCl are given in parentheses.

time / hours		0	4.75	17.5	25.5	48	72
ε / $M^{-1}\cdot cm^{-1}$ at λ = 290 nm		$4.40\cdot10^3$	$5.40\cdot10^3$	$6.30\cdot10^3$	$6.90\cdot10^3$	$7.60\cdot10^3$	$8.10\cdot10^3$
ε / $M^{-1}\cdot cm^{-1}$ at λ = 377 nm		$1.3\cdot10^3$	$1.2\cdot10^3$	$9.7\cdot10^2$	$7.8\cdot10^2$	$4.4\cdot10^2$	$2.3\cdot10^2$
Λ_m / $S\cdot cm^2\cdot mol^{-1}$		7	16	30	30	38	46
% **1** vs. internal standard, ^{1}H NMR		90 (95)	77 (86)	72 (85)	59 (67)	42 (53)	23 (35)
% NMR (NaCl experiment)	**1**	90 (95)	74 (78)	64 (72)	46 (51)	29 (37)	14 (21)
	PPh$_2$(4-C$_6$H$_4$CO$_2$H)	9 (4)	13 (11)	16 (14)	23 (21)	32 (29)	39 (36)
	O=PPh$_2$(4-C$_6$H$_4$CO$_2$H)	0 (0)	0 (2)	4 (3)	3 (3)	4 (4)	5 (6)
	***p*-cymene**	1 (1)	13 (9)	16 (11)	28 (25)	35 (30)	42 (37)

Stability studies: complex 2. The data are reported in Table S5 while the NMR detected species are shown in Scheme S2. **2.** ^{1}H NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 7.99–7.93 (m, 1H), 7.87–7.80 (m, 2H), 7.71–7.65 (m, 1H), 7.65–7.57 (m, 2H), 7.55–7.38 (m, 6H), 7.25 (t, *J* = 7.3 Hz, 1H), 6.68 (dd, *J* = 10.0, 8.3 Hz, 1H), 5.94 (d, *J* = 6.5 Hz, 1H), 5.70 (d, *J* = 6.3 Hz, 1H), 5.62 (d, *J* = 4.2 Hz, 1H), 5.25 (d, *J* = 5.3 Hz, 1H), 2.25 (hept, *J* = 6.8 Hz, 1H), 1.78 (s, 3H), 0.98 (d, *J* = 6.8 Hz, 3H), 0.55 (d, *J* = 6.7 Hz, 3H). ^{31}P{^{1}H} NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 30.1. **S.** ^{1}H NMR (DMSO-d_6:D_2O 9:1): δ/ppm = = 5.79 (d, *J* = 6.1 Hz, 2H), 5.74 (d, *J* = 6.1 Hz, 2H), 2.80 (hept, *J* = 6.7 Hz, 1H), 2.07 (s, 3H), 1.17 (d). O=**$PPh_2(2\text{-}C_6H_4CO_2)^-$**. ^{1}H NMR (DMSO-$d_6$:$D_2O$ 9:1): δ/ppm = 8.11–8.08 (m, 1H), 6.39 (dd, *J* = 9.8, 9.2 Hz, 1H). ^{31}P{^{1}H} NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 44.5. Minor P-containing species: ^{31}P{^{1}H} NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 22.6.

Scheme S2. Compounds detected in dmso/H_2O and dmso/H_2O/NaCl solutions of **2** maintained at 37 °C.



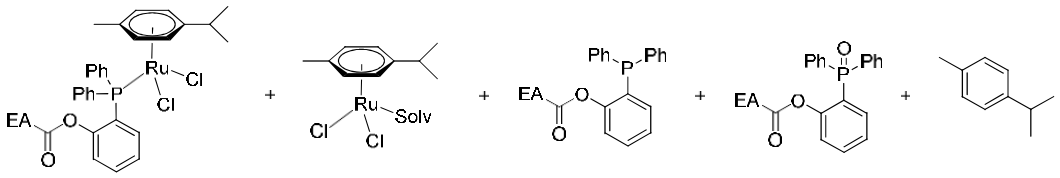
2 **S**

Table S5. UV-Vis absorptions, molar conductivity and NMR detected species as a function of time for DMSO/water 9:1 *v/v* solution of **2** at 37°C. Data for analogous experiments with 0.1 M NaCl are given in parentheses.

time / hours		0	4.75	17.5	25.5	48	72
ε / $M^{-1}\cdot cm^{-1}$ at λ = 350 nm		$1.5\cdot10^3$	$1.3\cdot10^3$	$1.2\cdot10^3$	$1.1\cdot10^3$	$9.4\cdot10^2$	$9.4\cdot10^2$
Λ_m / $S\cdot cm^2\cdot mol^{-1}$		12	20	21	17	27	29
% **2** vs. internal standard, ^{1}H NMR		96 (97)	87 (85)	76 (75)	72 (71)	55 (58)	45 (52)
% NMR (NaCl experiment)	**2**	96 (97)	88 (85)	79 (78)	72 (74)	50 (56)	41 (48)
	S	0 (0)	2 (8)	3 (9)	3 (9)	4 (8)	3 (8)
	$O=PPh_2(2\text{-}C_6H_4CO_2)^-$	0 (0)	0 (0)	0 (0)	0 (0)	13 (10)	12 (10)
	***p*-cymene**	4 (3)	10 (7)	18 (13)	25 (17)	33 (26)	44 (34)

Stability studies: complex 3. The data are reported in Table S6 while the NMR detected species are shown in Scheme S3. **3**. ^{1}H NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 7.6-7.2 (Ar), 7.12–7.03 (m + **L1**), 6.64 (d, *J* = 8.6 Hz, 1H), 6.09 (s), 5.42 (d, *J* = 5.7 Hz, 2H), 5.13 (d, *J* = 5.5 Hz, 2H), 5.53 (s + **L1**), 4.71 (s, 2H), 2.74–2.66 (m, 1H), 2.36 (q, *J* = 6.9 Hz + **L1**), 1.75 (s, 3H), 1.07 (m, 9H). ^{31}P{^{1}H} NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 24.4. **L1**. ^{1}H NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 7.6-7.2 (Ar), 7.12–7.03 (m + **3**), 6.87–6.81 (m, 1H), 6.06 (s), 5.53 (s + **3**), 5.04 (s, 2H), 2.36 (q, *J* = 6.9 Hz + **3**), 1.23–1.15 (m). ^{31}P{^{1}H} NMR (DMSO-d_6:D_2O 9:1): δ/ppm = –19.3. **O=L1**. ^{1}H NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 7.6-7.2 (Ar), 5.55 (s), 4.91 (s, 2H). ^{31}P{^{1}H} NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 51.2 ppm. Other species. ^{1}H NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 5.88 (d, *J* = 6.2 Hz, 1H), 5.63 (d, *J* = 5.7 Hz, 1H), 5.46 (d, *J* = 4.8 Hz, 1H), 5.35 (d, *J* = 7.0 Hz, 1H). ^{31}P{^{1}H} NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 18.6, 18.2 (17-72 h), 53 (25-72 h), 57 (48-72 h).

Scheme S3. Compounds detected in dmso/H_2O and dmso/H_2O/NaCl solutions of **3** maintained at 37 °C.



3 **S** **L1** **O=L1**

Table S6. UV-Vis absorptions, molar conductivity and NMR detected species as a function of time for DMSO/water 9:1 *v/v* solution of **3** at 37°C. Data for analogous experiments with 0.1 M NaCl are given in parentheses.

time / hours		0	4.75	17.5	25.5	48	72
ε / $M^{-1}\cdot cm^{-1}$ (λ_{max} / nm)		$1.5\cdot10^3$ (348)	$1.9\cdot10^3$ (340)	$2.3\cdot10^3$ (337)	$2.5\cdot10^3$ (335)	$2.7\cdot10^3$ (333)	$2.8\cdot10^3$ (333)
ε / $M^{-1}\cdot cm^{-1}$ at λ = 410 nm		$9.4\cdot10^2$	$8.1\cdot10^2$	$7.2\cdot10^2$	$6.5\cdot10^2$	$5.2\cdot10^2$	$3.9\cdot10^2$
Λ_m / $S\cdot cm^2\cdot mol^{-1}$		12	20	24	20	30	32
% **3** vs. internal standard, ^{1}H NMR		64 (62)	47 (61)	44 (62)	39 (62)	30 (57)	24 (49)
% NMR (NaCl experiment)	**3**	47 (45)	34 (39)	33 (39)	31 (38)	23 (35)	17 (29)
	S	27 (28)	32 (30)	32 (29)	31 (27)	29 (25)	27 (20)
	L1	26 (27)	25 (26)	21 (22)	18 (22)	14 (20)	12 (20)
	O=L1	0 (0)	6 (3)	11 (7)	12 (7)	17 (9)	21 (12)
	***p*-cymene**	0 (0)	3 (2)	3 (3)	8 (6)	17 (11)	23 (19)

Stability studies: complex 4. The data are reported in Table S7 while the NMR detected species are shown in Scheme S4. **4**. ^{1}H NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 7.88–7.82 (m, 4H), 7.77–7.69 (m, 4H), 7.51–7.45 (m, 2H), 7.45–7.38 (m, 4H), 7.20 (d, *J* = 8.5 Hz, 1H), 7.12 (d, *J* = 8.6 Hz, 1H), 6.02 (s, 1H), 5.49 (s, 1H), 5.31 (d, *J* = 6.1 Hz, 2H), 5.23 (d, *J* = 5.9 Hz, 2H), 5.02 (s, 2H), 4.47 (s, 4H), 2.32 (q, *J* = 6.8 Hz, 2H), 1.74 (s, 3H), 1.03 (t, *J* = 7.4 Hz, 3H), 0.93 (d, *J* = 6.8 Hz, 6H). ^{31}P{^{1}H} NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 25.0. **L2**. ^{1}H NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 7.68–7.53 (m, 4H), 7.51–7.45 (m + **4**), 7.33–7.23 (m, 4H), 7.19 (d, *J* = 8.6 Hz), 7.14 (d, *J* = 8.5 Hz), 6.00 (s), 5.46 (d, *J* = 2.5 Hz), 5.03 (s), 4.49 (s), {2.32 (q, *J* = 6.8 Hz + **4**)}, 1.03 (t, *J* = 7.4 Hz). ^{31}P{^{1}H} NMR (DMSO-d_6:D_2O 9:1): δ/ppm = -6.3. **O=L2**. ^{1}H NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 8.04 (dd, *J* = 8.5, 2.3 Hz, 2H), 7.89–7.86 (m, 2H), 7.77 (d, *J* = 8.4 Hz, 2H), 7.68–7.53 (m + **L2**). ^{31}P{^{1}H} NMR (DMSO-d_6:D_2O 9:1): δ/ppm = 26.5.

Scheme S4. Compounds detected in dmso/H_2O and dmso/H_2O/NaCl solutions of **4** maintained at 37 °C.

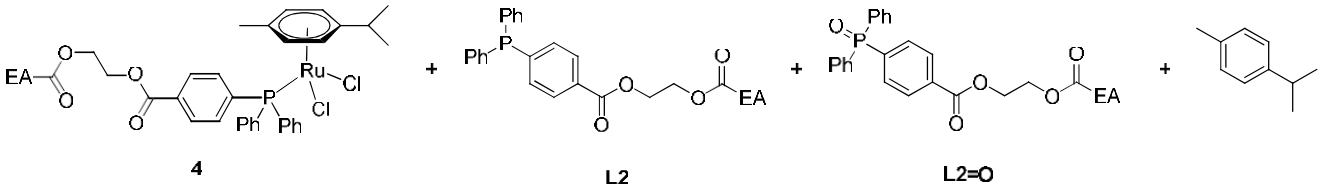


Table S7. UV-Vis absorptions, molar conductivity and NMR detected species as a function of time for DMSO/water 9:1 *v*/*v* solution of **4** at 37°C. Data for analogous experiments with 0.1 M NaCl are given in parentheses.

time / hours		0	4.75	17.5	25.5	48	72
ε / $M^{-1}\cdot cm^{-1}$ at λ = 375 nm		$1.5\cdot10^3$	$1.3\cdot10^3$	$1.2\cdot10^3$	$1.1\cdot10^3$	$7.0\cdot10^2$	$5.4\cdot10^2$
Λ_m / $S\cdot cm^2\cdot mol^{-1}$		8	16	20	16	25	29
% **4** vs. internal standard, ^{1}H NMR		82 (82)	77 (78)	77 (78)	72 (66)	57 (53)	45 (39)
% NMR (NaCl experiment)	**4**	82 (82)	72 (70)	69 (70)	58 (54)	40 (38)	27 (24)
	L2	13 (12)	15 (16)	15 (16)	19 (23)	28 (32)	37 (41)
	O=L2	4 (4)	7 (7)	9 (7)	8 (6)	9 (7)	8 (7)
	***p*-cymene**	1 (2)	6 (7)	7 (7)	15 (17)	23 (23)	28 (28)

Figure S3. Deconvoluted ESI–MS spectra of lysozyme in ammonium acetate buffer (pH 6.8), incubated with complexes **1**, **3** and **4**, respectively, after 72 h at 37 °C (protein concentration = 10^{-4} M; Ru/protein molar ratio = 3).