SUPPORTING INFORMATION

Novel "ruthenium cyclopentadienyl"-peptide conjugate complexes against human FGFR(+) breast cancer

João Franco Machado[a,b,c], Miguel Machuqueiro[b,d] , Fernanda Marques[c], M. Paula Robalo[e,f], M. Fátima M. Piedade[b,e], M. Helena Garcia[a,b], João D. G. Correia[c,*], Tânia S. Morais[a,b,*]

[a] Centro de Química Estrutural, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

[b] Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

[c] Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10, 2695-066 Bobadela LRS, Portugal

[d] BioISI - Biosystems & Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

[e] Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

[f] Área Departamental de Engenharia Química, Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, Rua Conselheiro Emídio Navarro 1, 1959-007, Lisboa, Portugal

Corresponding Authors:

Tânia S. Morais (tsmorais@ciencias.ulisboa.pt)

João G. Correia (jgalamba@ctn.tecnico.ulisboa.pt)

Table S1 - ^{1}H NMR data for compounds 2 – 4 and free ligands in $CDCl_3$ and $(CD_3)_2CO$.

	Compound	H1	H2	H3	H4	Cp_{α}	Cp_{β}	CH_2	CH_3
$CDCl_3$	$Ru(\eta^5\text{-}C_5H_4COOCH_2CH_3)(PPh_3)_2Cl]$ (**2**)	–	–	–	–	3.63	4.91	4.05	1.23
	2,2'-bipy	8.59	7.12	7.66	8.50	–	–	–	–
	$[Ru(\eta^5\text{-}C_5H_4COOCH_2CH_3)(PPh_3)(2,2'\text{-bipy})][CF_3SO_3]$ (**3**)	8.03	7.80	7.30*	9.12	4.74	5.32	3.93	0.94
$(CD_3)_2CO$	$[Ru(\eta^5\text{-}C_5H_4COOCH_2CH_3)(PPh_3)(2,2'\text{-bipy})][CF_3SO_3]$ (**3**)	8.24	7.97	7.43*	9.43	4.76	5.64	3.83	0.79
	$[Ru(\eta^5\text{-}C_5H_4COOH)(PPh_3)(2,2'\text{-bipy})]$ $[CF_3SO_3]$ (**4**)	8.22	7.95	7.44*	9.43	4.78	5.60	–	–

*overlapped with PPh_3

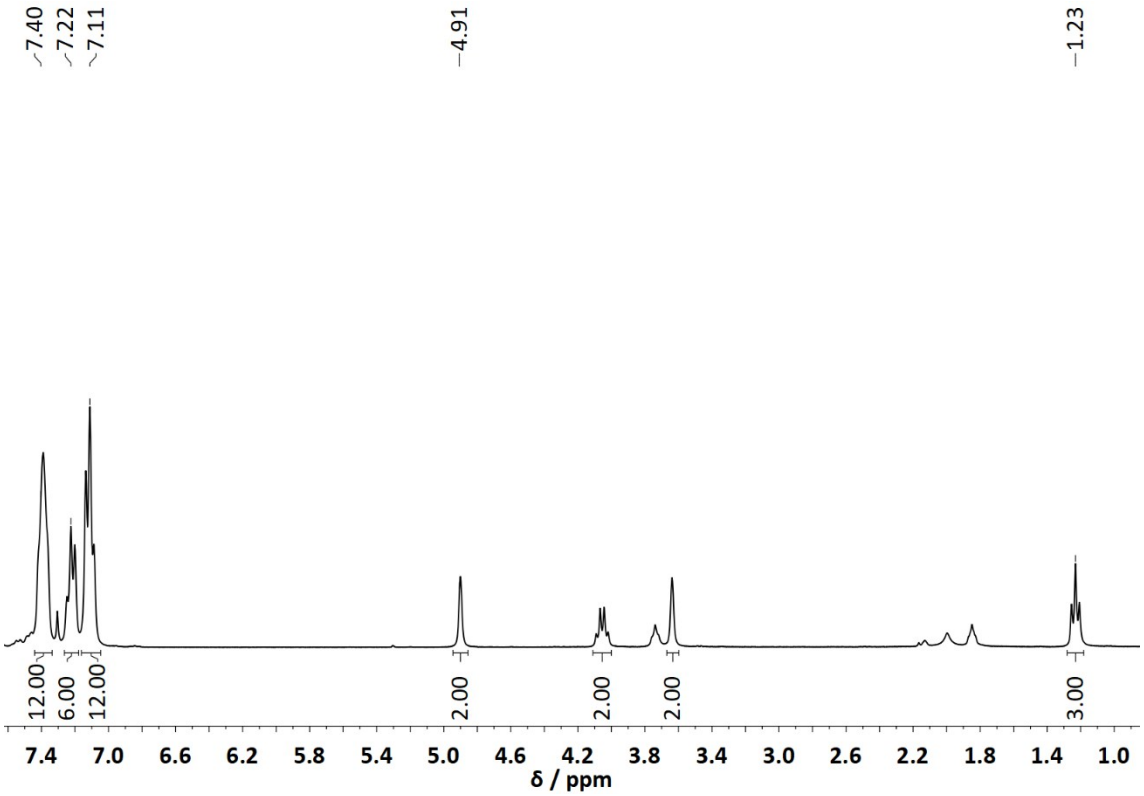

Figure S1 - ^{1}H NMR spectrum of $[Ru(\eta^5\text{-}C_5H_4COOCH_2CH_3)(PPh_3)_2Cl]$ (**2**) in $CDCl_3$.

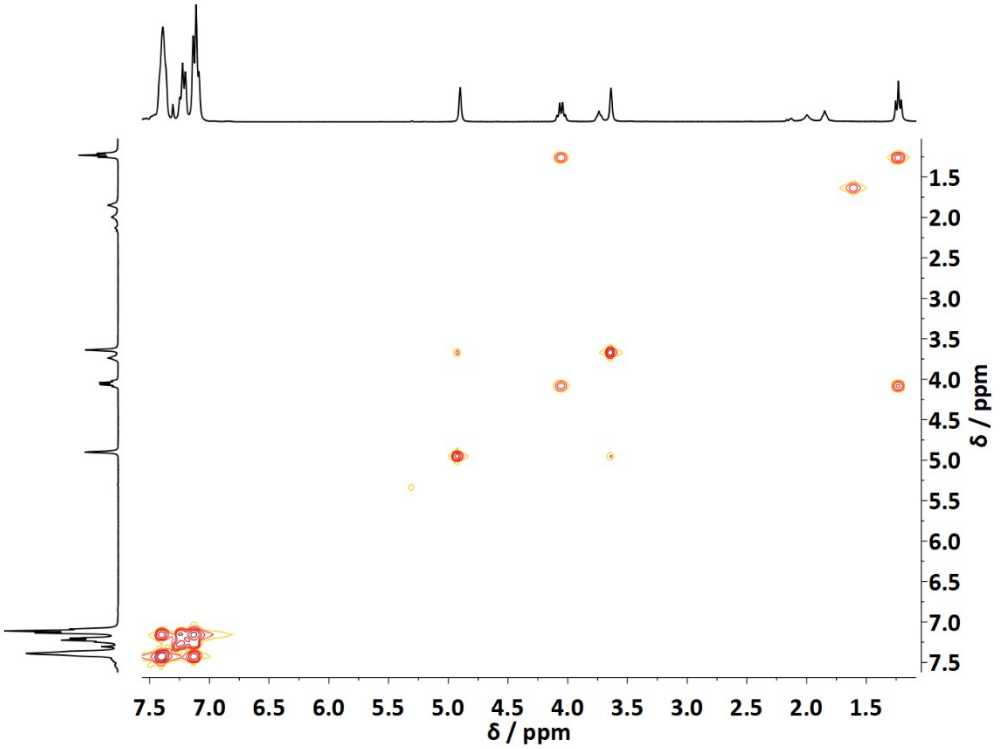

Figure S2 - COSY NMR spectrum of [Ru(η^5-$C_5H_4COOCH_2CH_3$)$(PPh_3)_2Cl$] (**2**) in $CDCl_3$.

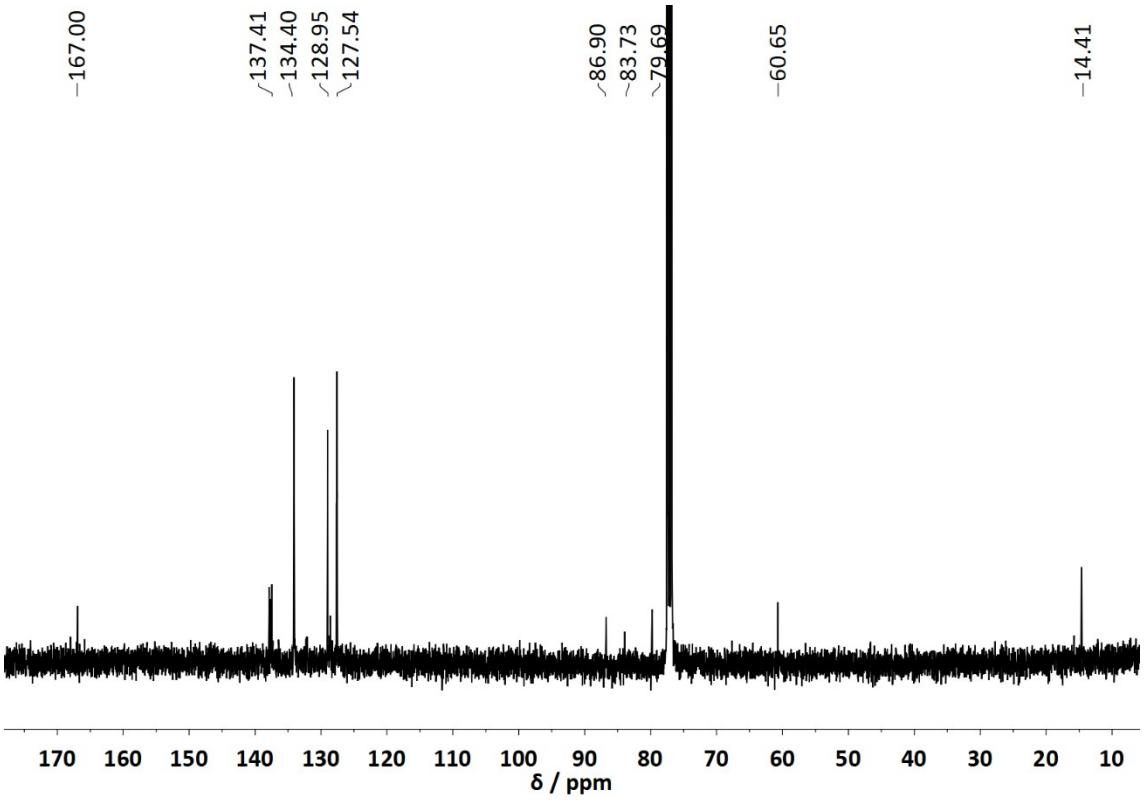

Figure S3 - ^{3}C NMR spectrum of [Ru(η^5-$C_5H_4COOCH_2CH_3$)$(PPh_3)_2Cl$] (**2**) in $CDCl_3$.

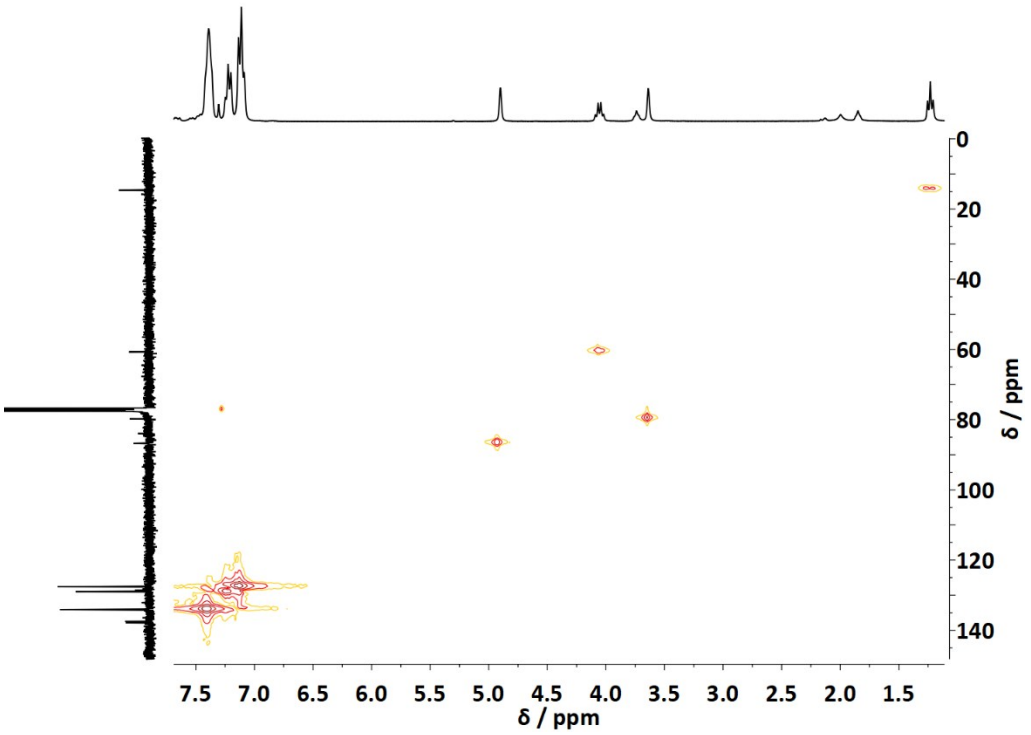

Figure S4 - HSQC NMR spectrum of [Ru(η^5-$C_5H_4COOCH_2CH_3$)$(PPh_3)_2Cl$] (**2**) in $CDCl_3$.

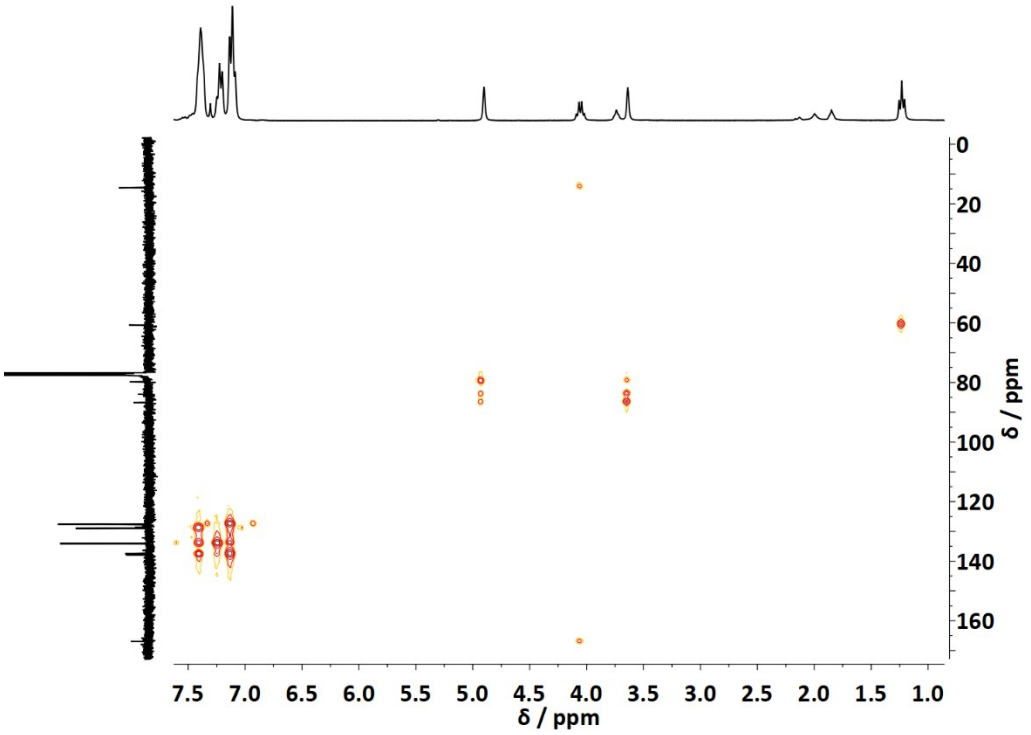

Figure S5 - HMBC NMR spectrum of [Ru(η^5-$C_5H_4COOCH_2CH_3$)$(PPh_3)_2Cl$] (**2**) in $CDCl_3$.

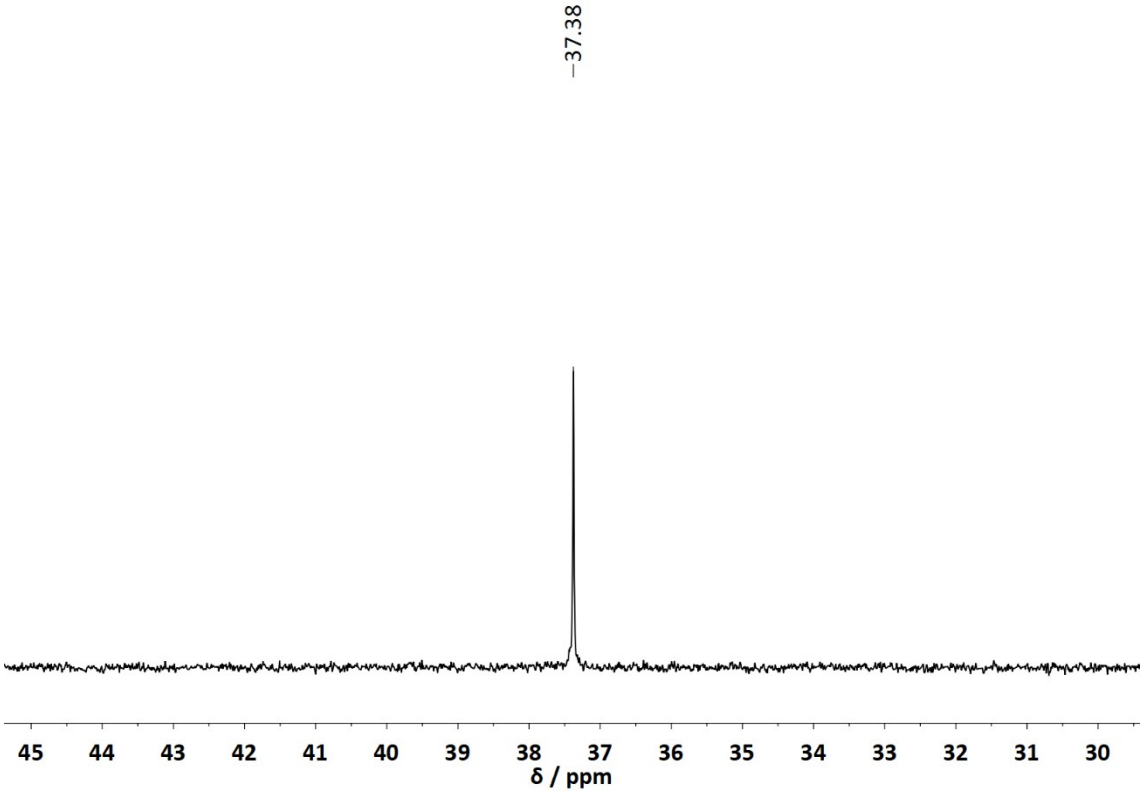

Figure S6 - ^{31}P NMR spectrum of [Ru(η^5-$C_5H_4COOCH_2CH_3$)(PPh_3)$_2$Cl] (**2**) in $CDCl_3$.

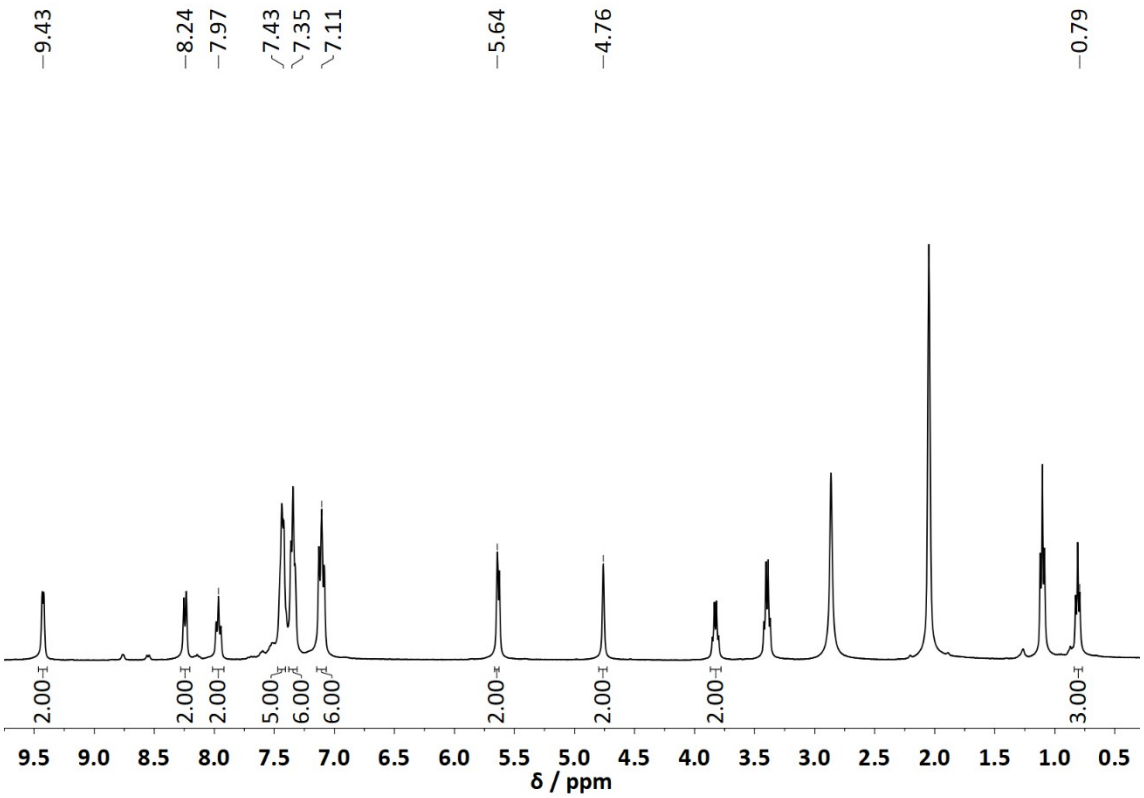

Figure S7 - ^{1}H NMR spectrum of [Ru(η^5-$C_5H_4COOCH_2CH_3$)(2,2'-bipy)][CF_3SO_3] (**3**) in $(CD_3)_2CO$.

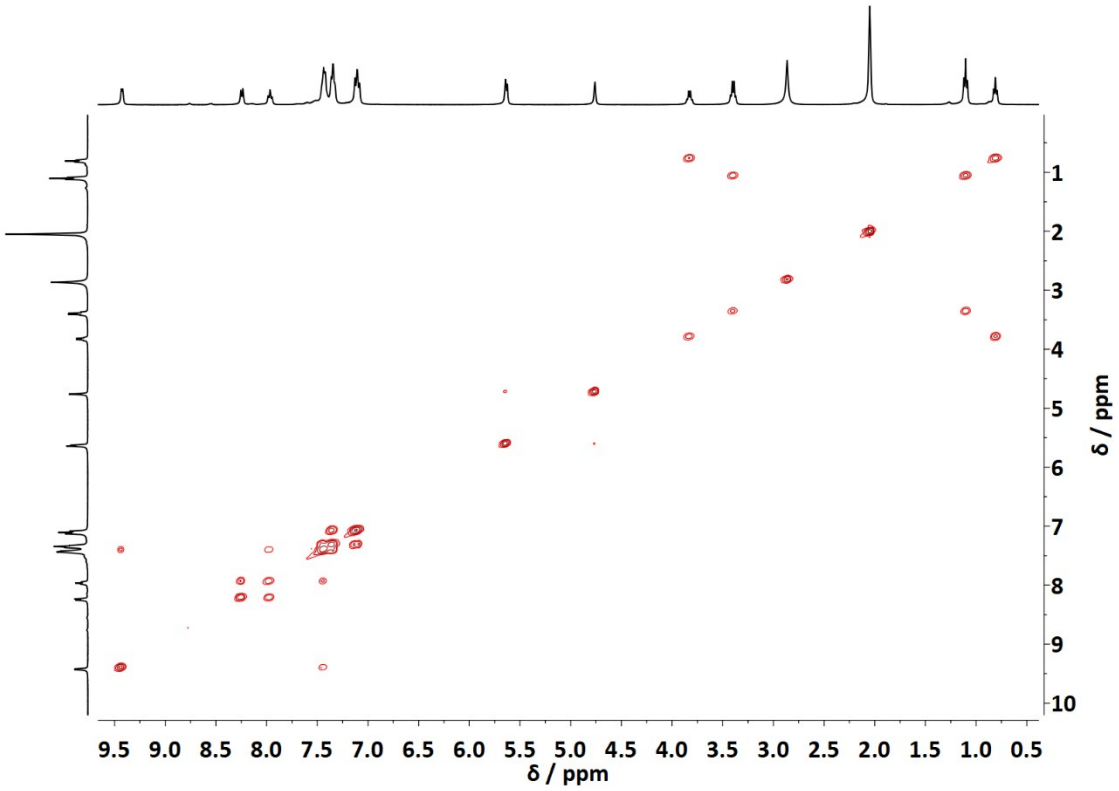

Figure S8 - COSY NMR spectrum of [Ru(η^5-$C_5H_4COOCH_2CH_3$)(2,2'-bipy)][CF_3SO_3] (**3**) in $(CD_3)_2CO$.

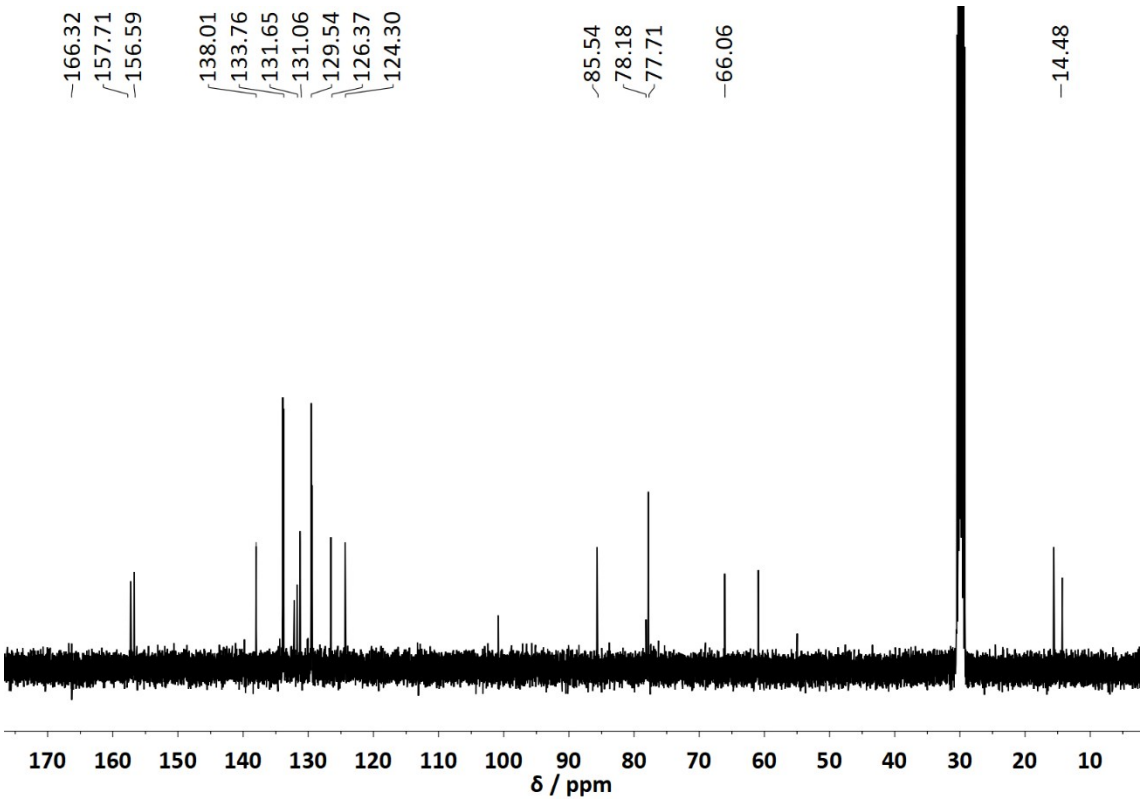

Figure S9 - ^{13}C NMR spectrum of [Ru(η^5-$C_5H_4COOCH_2CH_3$)(2,2'-bipy)][CF_3SO_3] (**3**) in $(CD_3)_2CO$.

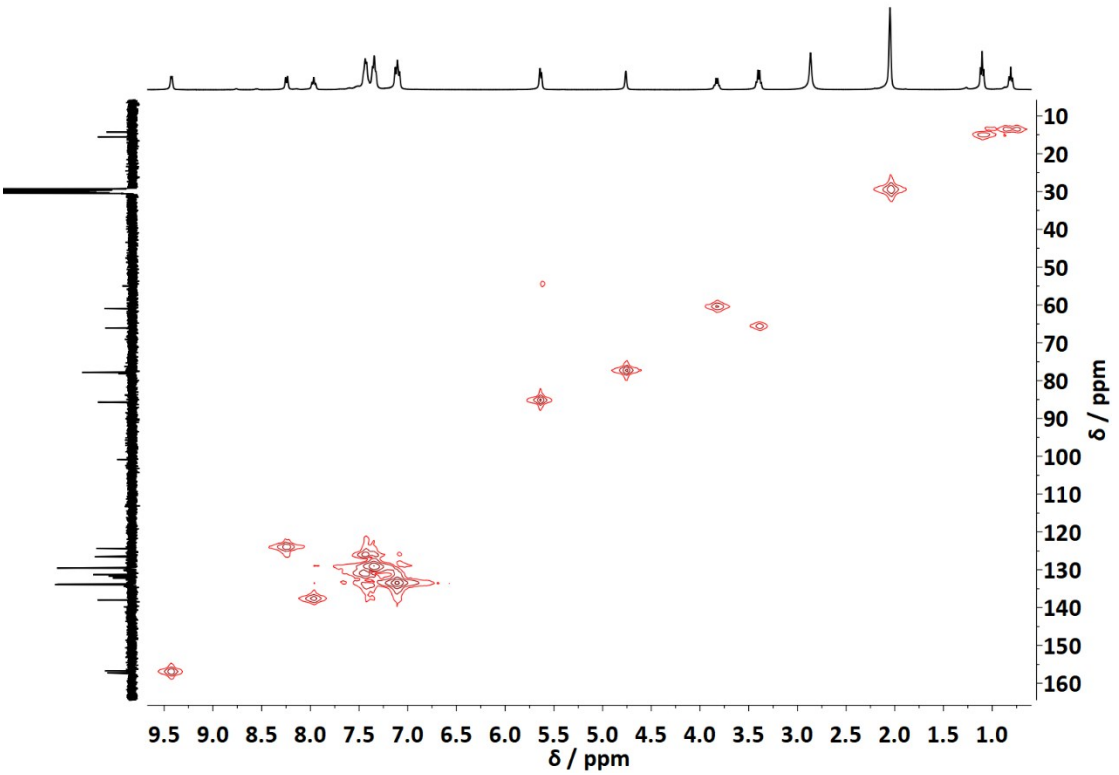

Figure S10 - HSQC NMR spectrum of [Ru(η^5-$C_5H_4COOCH_2CH_3$)(2,2'-bipy)][CF_3SO_3] (**3**) in $(CD_3)_2CO$.

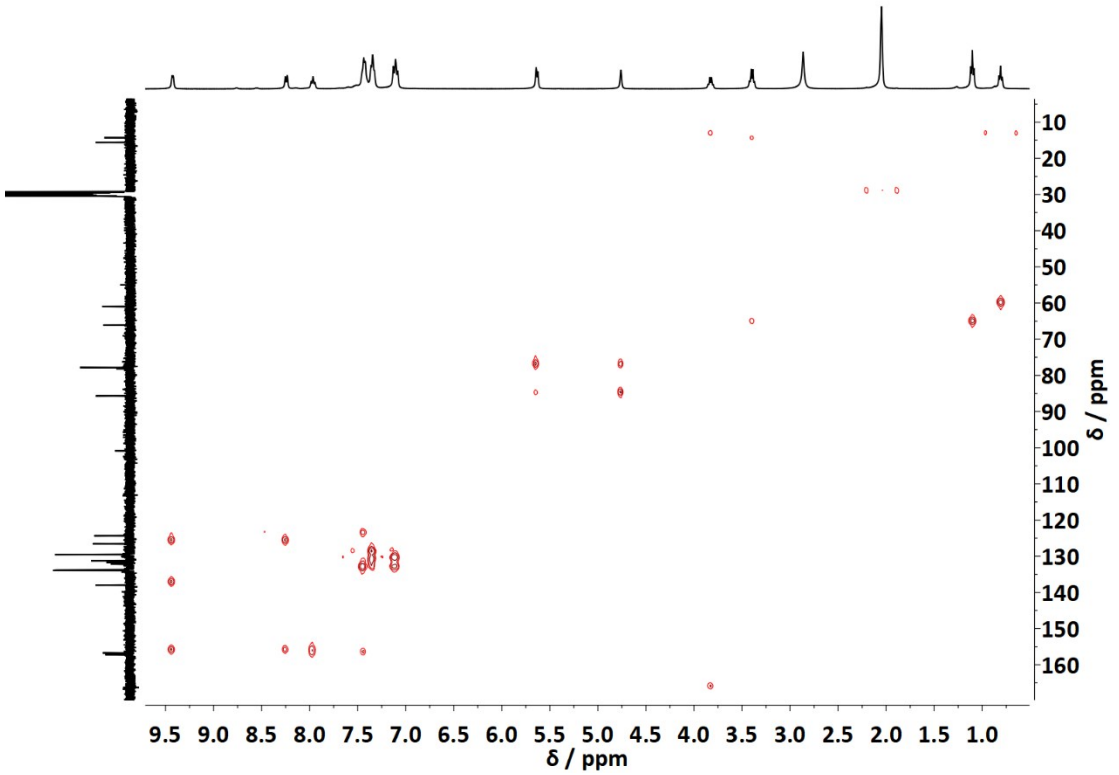

Figure S11 - HMQC NMR spectrum of [Ru(η^5-$C_5H_4COOCH_2CH_3$)(2,2'-bipy)][CF_3SO_3] (**3**) in $(CD_3)_2CO$.

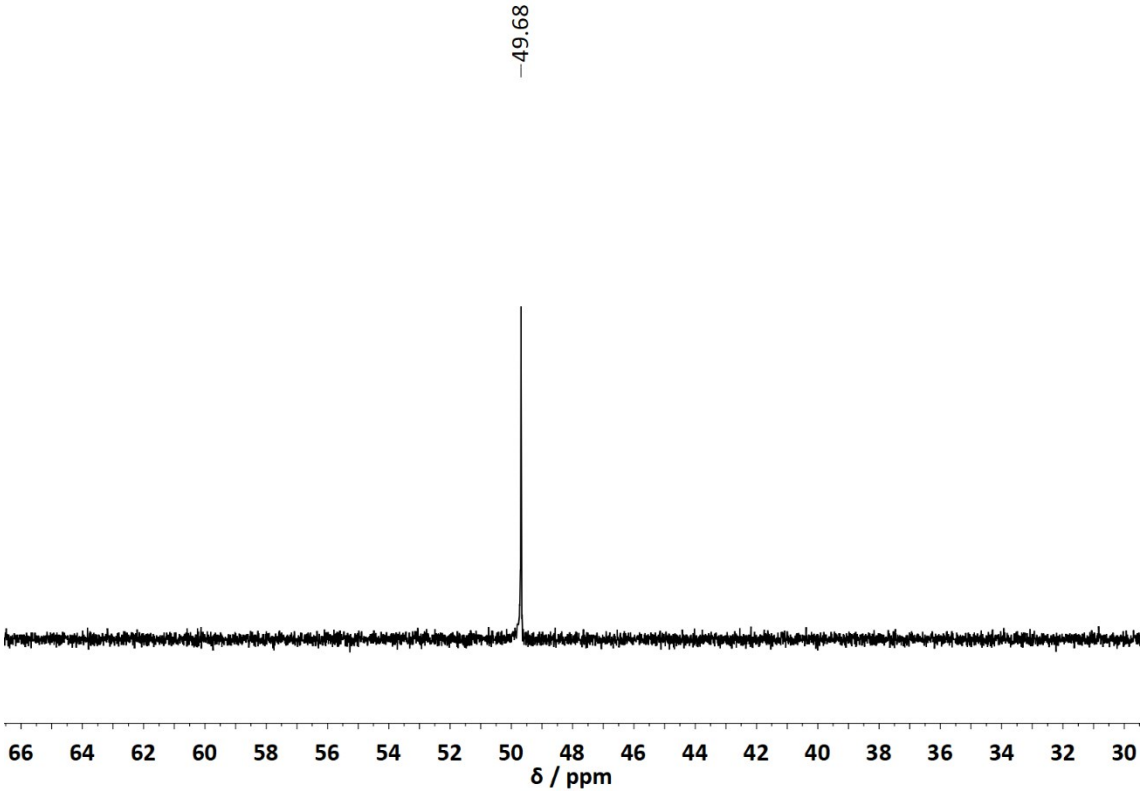

Figure S12 - ^{31}P NMR spectrum of [Ru(η^5-$C_5H_4COOCH_2CH_3$)(2,2'-bipy)][CF_3SO_3] (**3**) in $(CD_3)_2CO$.

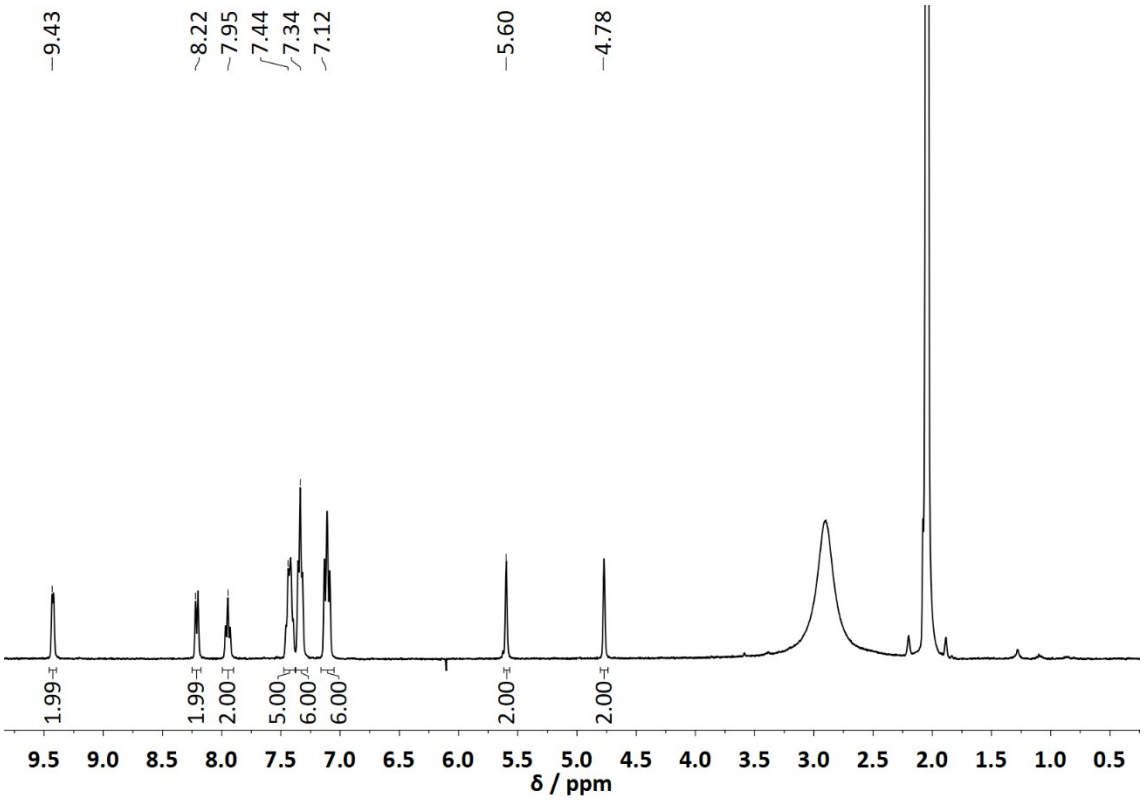

Figure S13 - ^{1}H NMR spectrum of [Ru(η^5-C_5H_4COOH)(2,2'-bipy)][CF_3SO_3] (**TM281**) in $(CD_3)_2CO$.

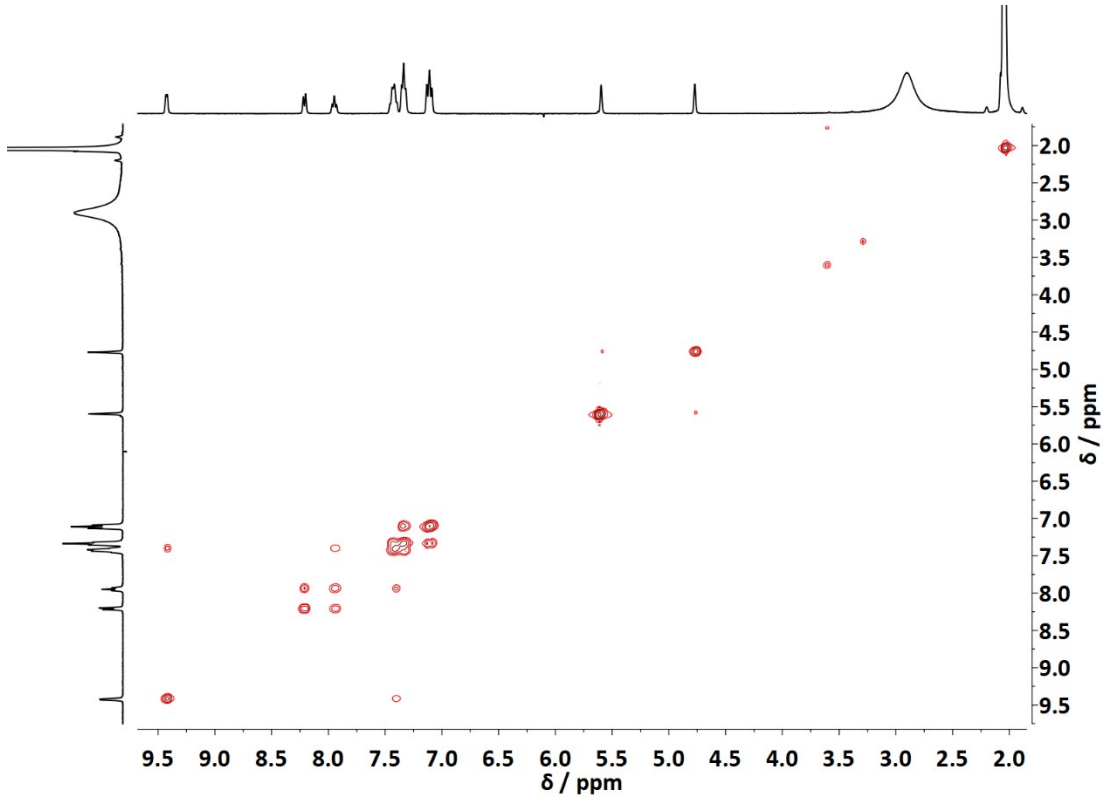

Figure S14 - COSY NMR spectrum of [Ru(η^5-C_5H_4COOH)(2,2'-bipy)][CF_3SO_3] (**TM281**) in $(CD_3)_2CO$.

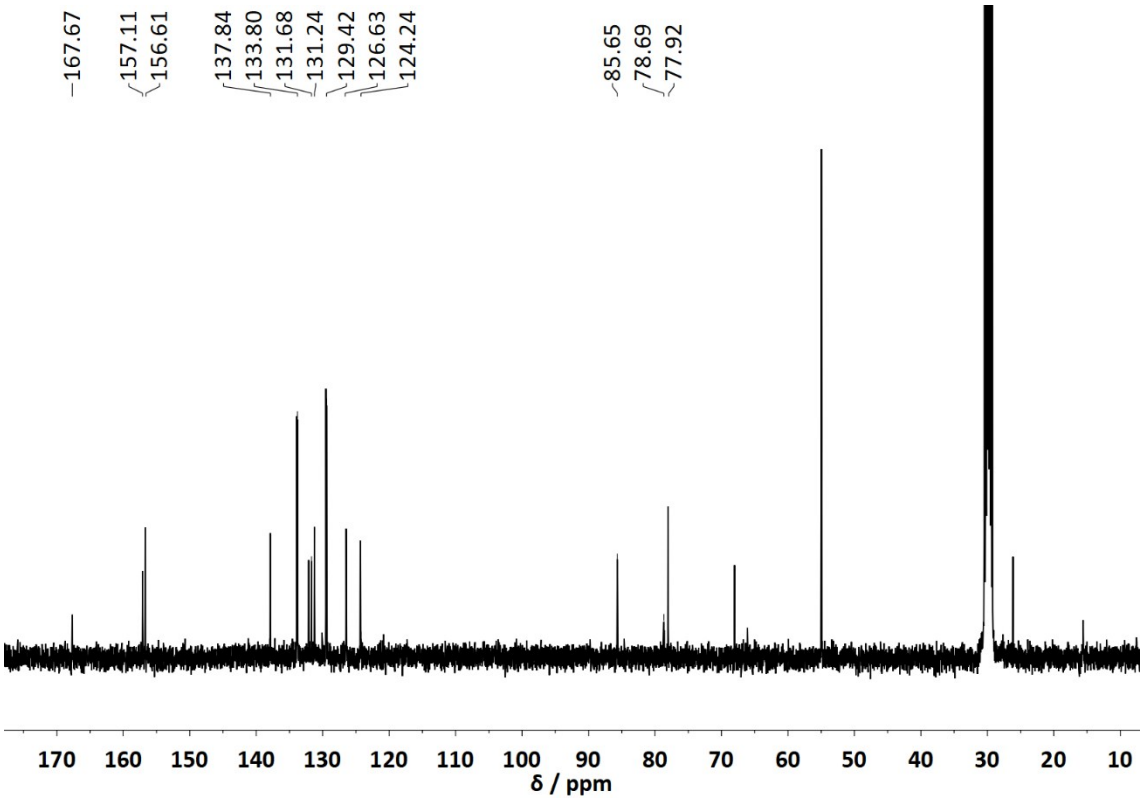

Figure S15 - ^{13}C NMR spectrum of [Ru(η^5-C_5H_4COOH)(2,2'-bipy)][CF_3SO_3] (**TM281**) in $(CD_3)_2CO$.

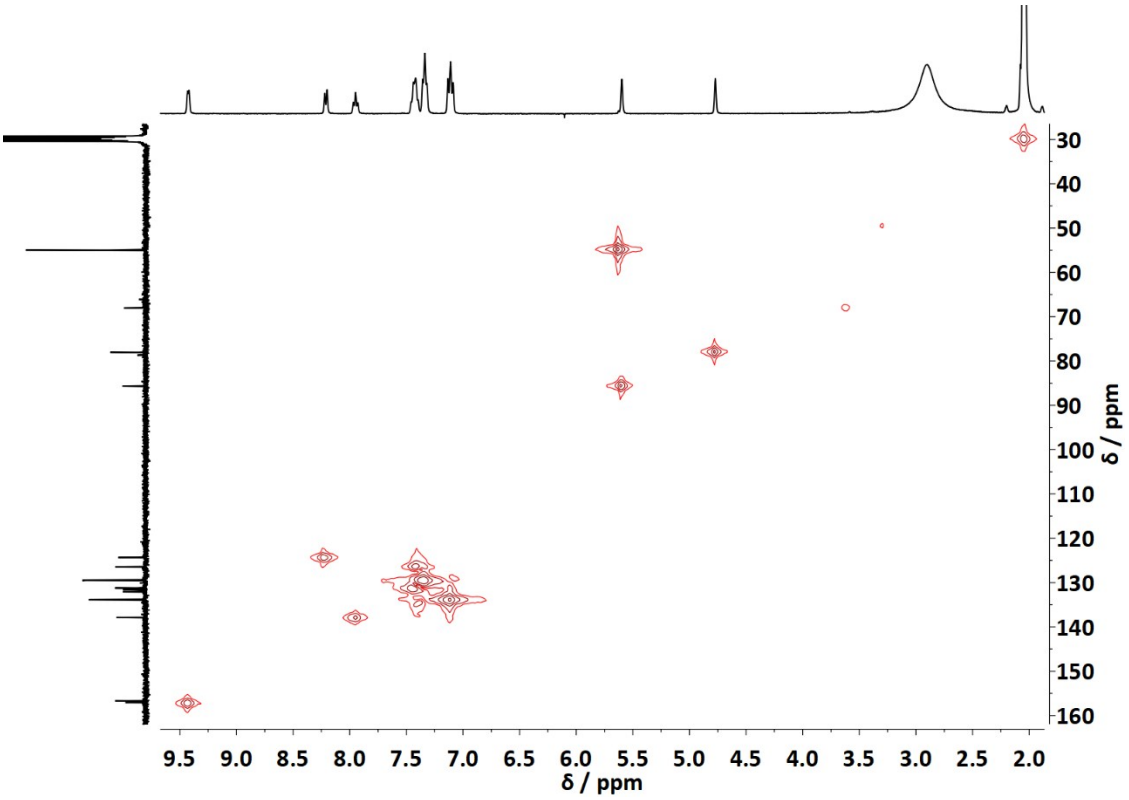

Figure S16 - HSQC NMR spectrum of [Ru(η^5-C_5H_4COOH)(2,2'-bipy)][CF_3SO_3] (**TM281**) in $(CD_3)_2CO$.

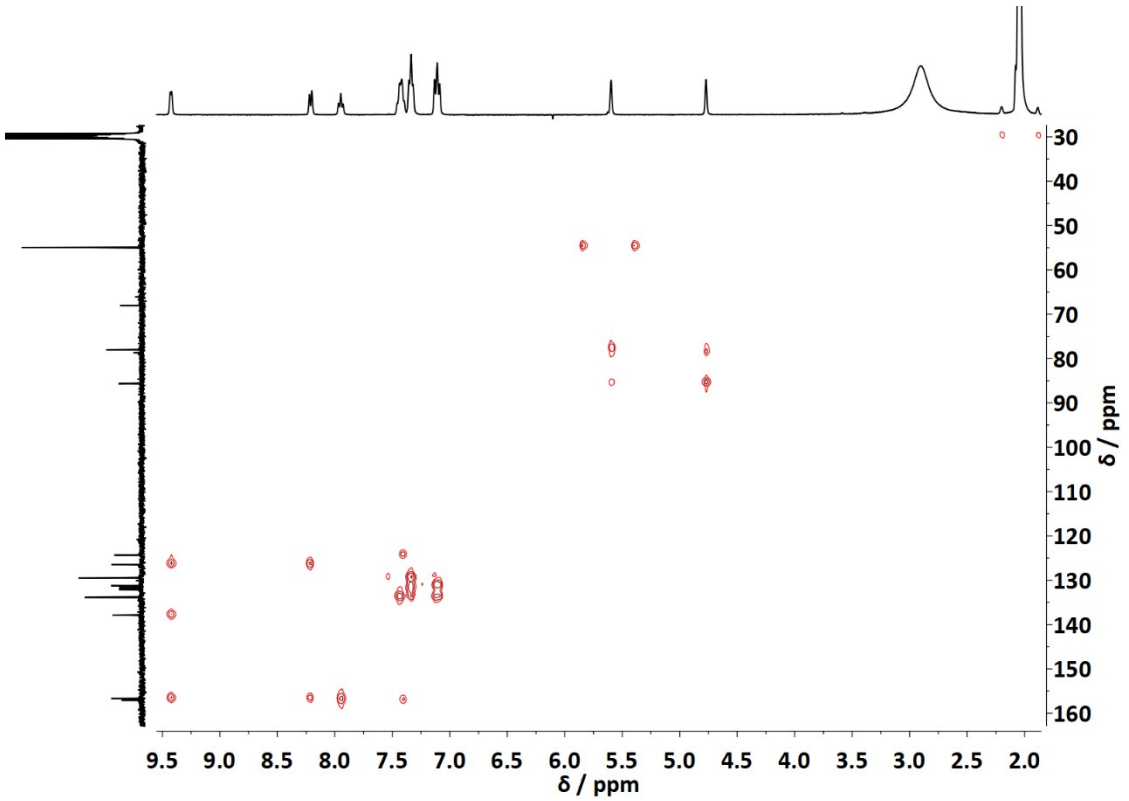

Figure S17 - HMQC NMR spectrum of [Ru(η^5-C_5H_4COOH)(2,2'-bipy)][CF_3SO_3] (**TM281**) in $(CD_3)_2CO$.

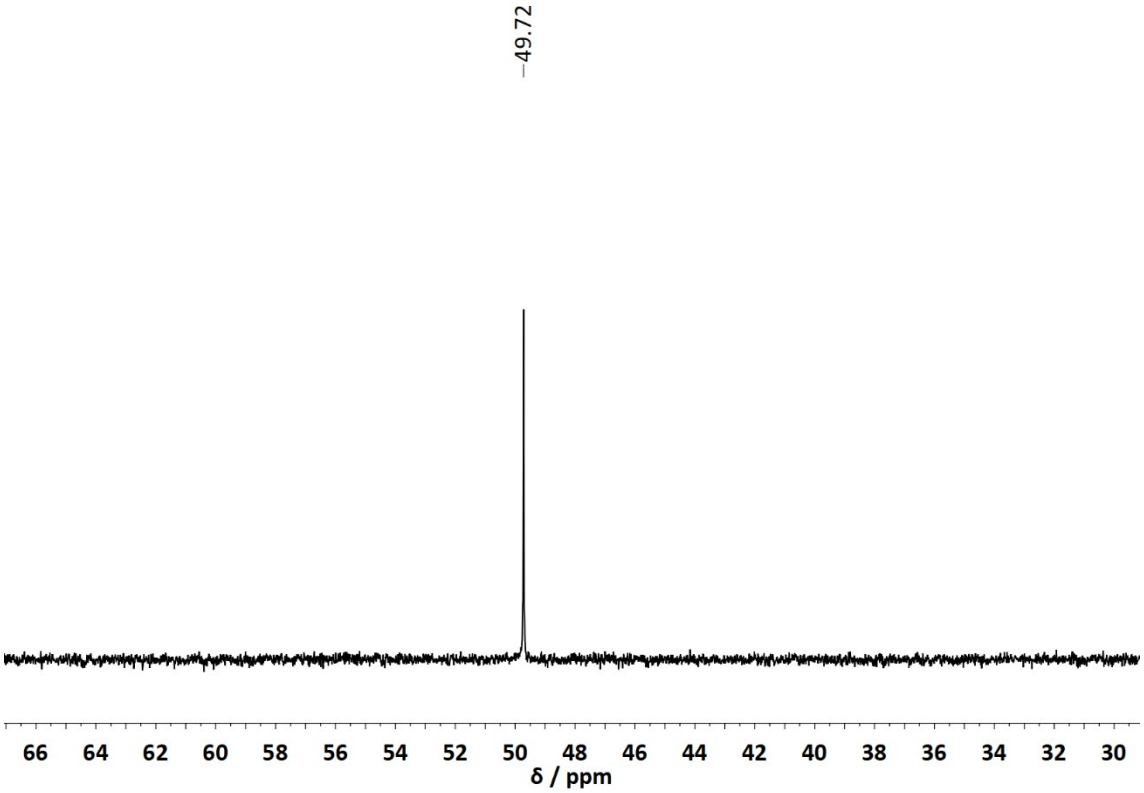

Figure S18 - ^{31}P NMR spectrum of [Ru(η^5-C_5H_4COOH)(2,2'-bipy)][CF_3SO_3] (**4**) in $(CD_3)_2CO$.

Table S2 - Electronic spectra data for complexes [Ru(η^5-$C_5H_4COOCH_2CH_3$)(PPh_3)$_2$Cl] (**2**), [Ru(η^5-$C_5H4COOCH_2CH_3$)(PPh_3)(2,2'-bipy)][CF_3SO_3] (**3**), [Ru(η^5-C_5H_4COOH)(PPh_3)(2,2'-bipy)][CF_3SO_3] (**4**) and 2,2'-bipy uncoordinated compound in dichloromethane solutions.

Compound	**$\lambda_{máx}$ (nm)** **(ε ($M^{-1}cm^{-1}$)**
Ru(η^5-$C_5H_4COOCH_2CH_3$)(PPh_3)$_2$Cl] (**2**)	260 (18900)
	301 (sh)
	389 (2580)
2,2'-bipy	240 (17200)
	285 (22100)
[Ru(η^5-$C_5H_4COOCH_2CH_3$)(PPh_3)(2,2'-bipy)][CF_3SO_3] (**3**)	244 (40900)
	288 (33200)
	351 (sh)
	414 (6500)
	456 (sh)
[Ru(η^5-C_5H_4COOH)(PPh_3)(2,2'-bipy)][CF_3SO_3] (**4**)	239 (29900)
	292 (18200)
	359 (sh)
	419 (3210)
	458 (sh)

Table S3 - Electrochemical data for complexes **2** - **4** in CH_2Cl_2 and CH_3CN. All values *vs* SCE (v = 200 $mV.s^{-1}$).

Complex	Ep_a (V)	Ep_c (V)	$E_{1/2}$ (V)	$Ep_a - Ep_c$ (mV)	I_c/I_a
Dichloromethane					
$Ru(\eta^5\text{-}C_5H_4COOCH_2CH_3)(PPh_3)Cl]$ (**2**)	1.49	–	–	–	–
	0.65	0.57	0.61	80	1.0
$Ru(\eta^5\text{-}C_5H_4COOCH_2CH_3)(PPh_3)(2,2'\text{-bipy})][CF_3SO_3]$ (**3**)	1.31	1.17	–	140	0.6
	–	0.06	–	–	–
$Ru(\eta^5\text{-}C_5H_4COOH)(PPh_3)(2,2'\text{-bipy})][CF_3SO_3]$ (**TM281**)	1.36	1.23	–	130	0.4
	–	-0.16	–	–	–
$Ru(\eta^5\text{-}C_5H_5)(PPh_3)(2,2'\text{-bipy})][CF_3SO_3]$ (TM34)[41]	1.70	–	–	–	–
	1.53	–	–	–	–
	1.10	1.01	1.05	90	0.9
	–	-0.08[a]	–	–	–
	–	-0.56[a]	–	–	–
Acetonitrile					
$Ru(\eta^5\text{-}C_5H_4COOCH_2CH_3)(PPh_3)(2,2'\text{-bipy})][CF_3SO_3]$ (**3**)	1.26	1.13	–	130	0.5
	–	-0.16	–	–	–
$Ru(\eta^5\text{-}C_5H_4COOH)(PPh_3)(2,2'\text{-bipy})][CF_3SO_3]$ (**TM281**)	1.22	1.11	1.16	110	0.7
	-1.60	-1.69	-1.64	90	1.0[b]
$Ru(\eta^5\text{-}C_5H_5)(PPh_3)(2,2'\text{-bipy})][CF_3SO_3]$ (TM34)[41]	0.92	0.84	0.88	80	0.9

[a] Dependent of the oxidation process at 1.53 V; [b] I_a/I_c

Table S4 - Selected bond lengths and torsion angles for [Ru(η^5-$C_5H_4COOCH_2CH_3$)(PPh_3)(2,2'-bipy)][CF_3SO_3] **3**, [Ru(η^5-C_5H_4COOH) (PPh_3)(2,2'-bipy)][CF_3SO_3] **4**.

Compound	**3**	**4**
Bond lengths (Å)		
Ru-Cp[a]	1.8250(2)	1.8298(2)
Ru-P(1)	2.3203(5)	2.3212(7)
Ru-N(1)	2.0747(1)	2.078(2)
Ru-N(2)	2.0860(1)	2.080(2)
O(21)-C(26)	1.204(3)	1.213(3)
O(22)-C(26)	1.341(3)	1.332(3)
C(25)-C(26)	1.464(3)	1.469(4)
Angles (º)		
Cp[a]-Ru-P(1)	124.38(2)	125.56(2)
Cp[a]-Ru-N(1)	128.87(5)	127.65(7)
Cp[a]-Ru-N(2)	131.41(5)	128.44(6)
Cp[a]-C(25)-C(26)	177.9(2)	177.6(3)
N(1)-Ru-P(1)	90.74(5)	92.94(6)
N(2)-Ru-P(1)	89.76(5)	91.16(6)
N(1)-Ru-N(2)	76.71(7)	76.79(9)
O(21)-C(26)-O(22)	123.8(2)	124.4(3)
O(21)-C(26)-C(25)	124.9(2)	124.4(3)
O(22)-C(26)-C(25)	111.30(18)	111.2(2)
C(26)-O(22)-C(27)	116.37(19)	–
O(22)-C(27)-C(28)	106.8(2)	–

Table S5 - Hydrogen bond and Intermolecular interactions for [Ru(η^5-$C_5H_4COOCH_2CH_3$)(PPh_3)(2,2'-bipy)][CF_3SO_3] **3**, [Ru(η^5-C_5H_4COOH) (PPh_3)(2,2'-bipy)][CF_3SO_3] **4**.

Compound 3			
	H...A (Å)	**D...A (Å)**	**D - H...A (º)**
C(24) --H(24) ..O(2)	2.39	3.17	138
C(21) --H(21) ..O(3)	2.46	3.07	121
C(4) --H(4) ..O(3)	2.52	3.42	159
C(1) --H(1) ..O(1)	2.57	3.30	133
C(113) --H(113) ..π(Cent[a])	2.96	3.65	129
Compound 4			
	H...A (Å)	**D...A (Å)**	**D - H...A (º)**
O(21) --H(21O) ..O(3)	1.92	2.70	161
C(24) --H(24) ..O(1)	2.40	3.13	134
C(124)--H(124) ..O(2)	2.42	3.33	161
C(21) --H(21) ..O(2)	2.46	3.20	135
C(7) --H(7) ..O(22)	2.49	3.17	129
C(123)--H(123) ..O(21)	2.55	3.47	164
C(10) --H(10) ..O(1)	2.56	3.25	130
C(3) --H(3) ..F(1)	2.60	3.47	152
C(126)--H(126) ..F(2)	2.61	3.14	115
C(125) --H(125) ..π(Cent[b])	3.03	3.31	99

[a] Centroid of the phenyl group (C121-C126); [b] Centroid of the carboxylic group

Table S6 - Sequences and molecular weights of peptides and ruthenium-peptide conjugates (RuPC's)

Peptide/ RuPC	**Sequence**	**Formula**	**MW (Da)**	**[M-nH^{n+}]**
Pep-1	GPPDWHWKAMTH	$C_{68}H_{92}N_{20}O_{15}S_1$	1460.68	1461.8 (n=1) 731.7 (n=2)
Pep-2	SRRPASFRTARE	$C_{59}H_{101}N_{25}O_{17}$	1431.78	712.2 (n=2) 478.5 (n=3)
Pep-3	VSPPLTLGQLLS	$C_{56}H_{98}N_{14}O_{16}$	1222.72	1224.4 (n=1)
RuPC-1	Ru-PEG(3)-GPPDWHWKAMTH	$C_{111}H_{135}N_{23}O_{20}PRu$	2274.88	1138.0 (n=2) 759.1 (n=3)
RuPC-2	Ru-PEG(3)- SRRPASFRTARE	$C_{102}H_{144}N_{28}O_{22}PRu$	2245.97	749.8 (n=3) 562.3 (n=4)
RuPC-3	Ru-PEG(3)- VSPPLTLGQLLS	$C_{99}H_{141}N_{17}O_{21}PRu$	2036.93	1019.2(n=2) 680.1 (n=3)

Where Ru = [Ru(η^5-C_5H_4COOH)(PPh_3)(2,2'-bipy)][CF_3SO_3], PEG(3) = $HNCH_2CH_2(OCH_2CH_2)_3CO$

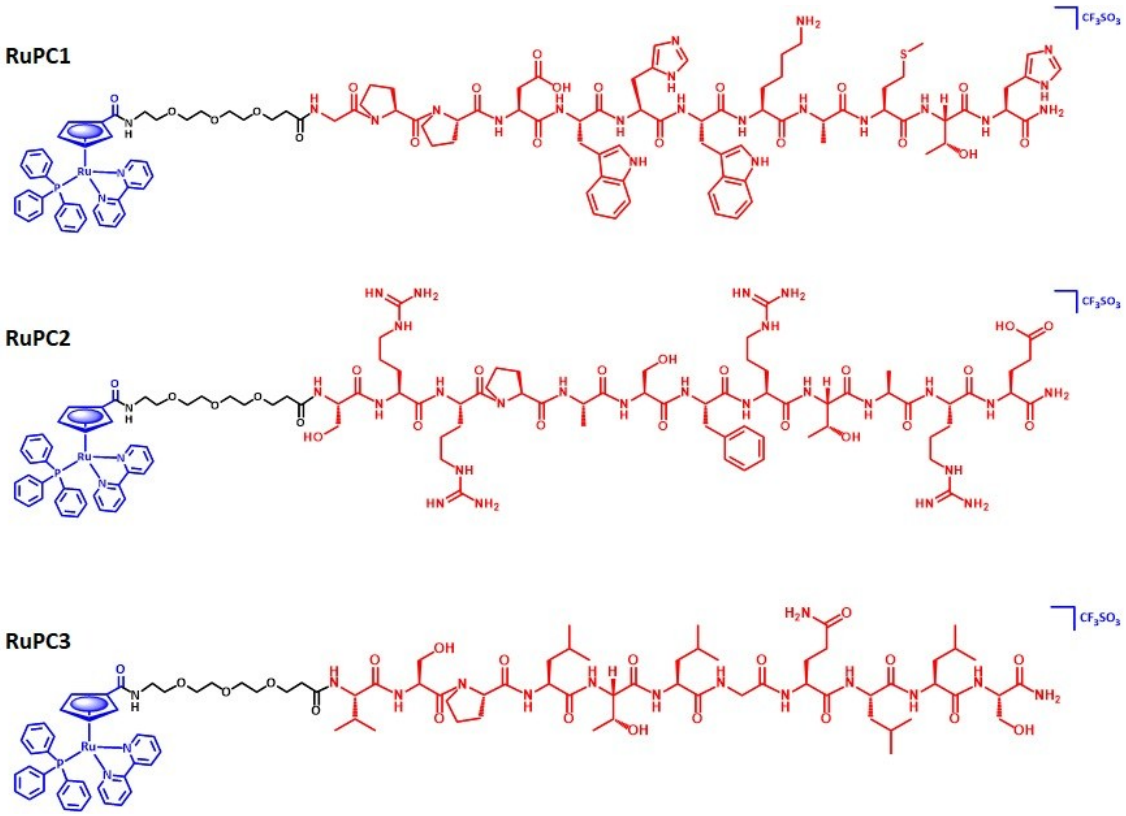

Figure S19 - Structures of **RuPC1** to **RuPC3**.

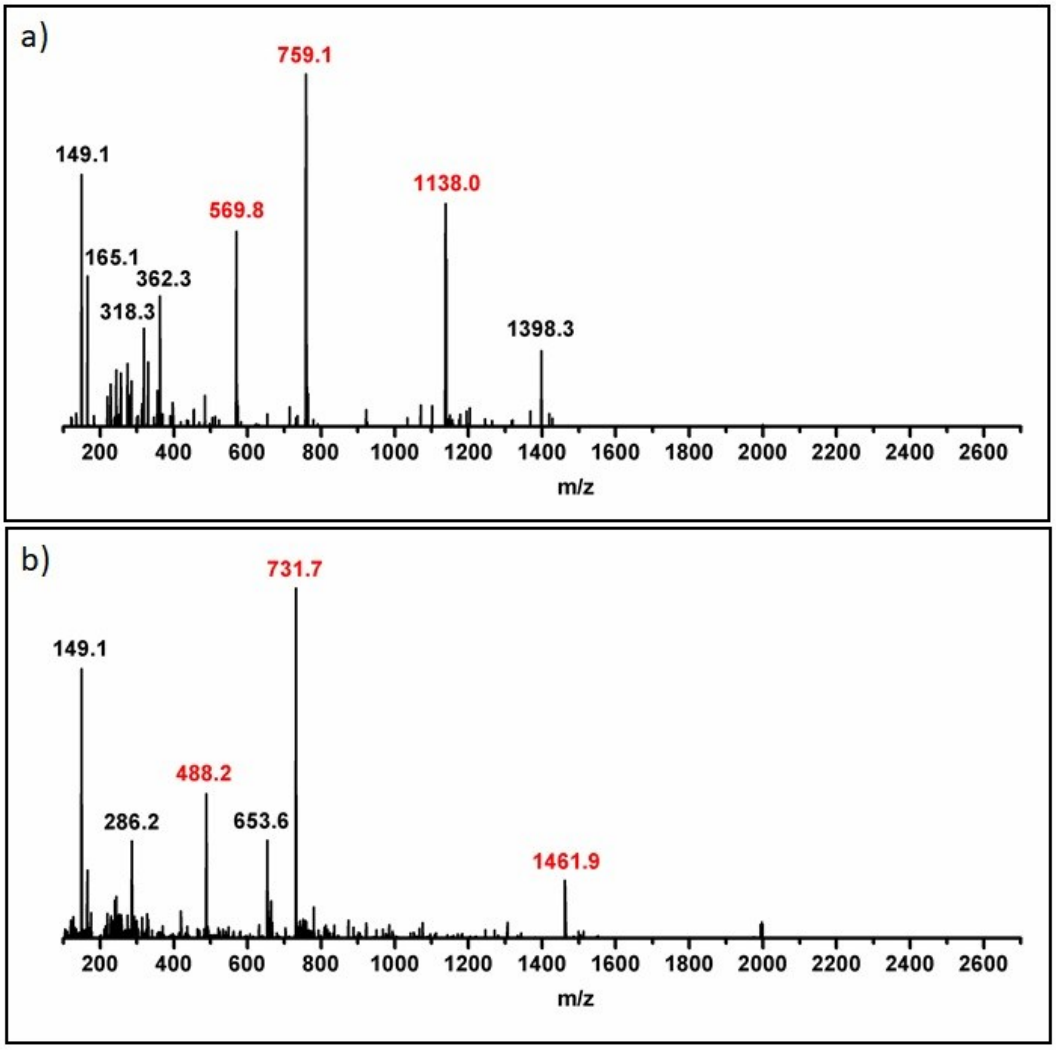

Figure S20 - ESI-MS spectrum of pure a) RuPC-1 (Ru-PEG(3)-GPPDWHWKAMTH) and b) Pep-1 (GPPDWHWKAMTH)

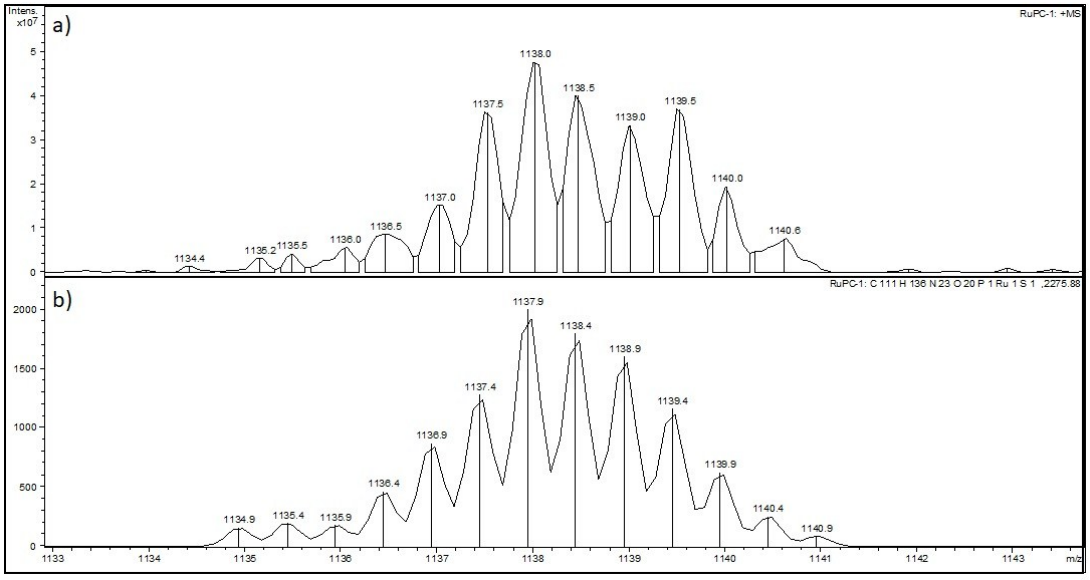

Figure S21 – Observed (a) and simulated (b) ESI-MS Spectra of RuPC-1 (Ru-PEG(3)-GPPDWHWKAMTH).

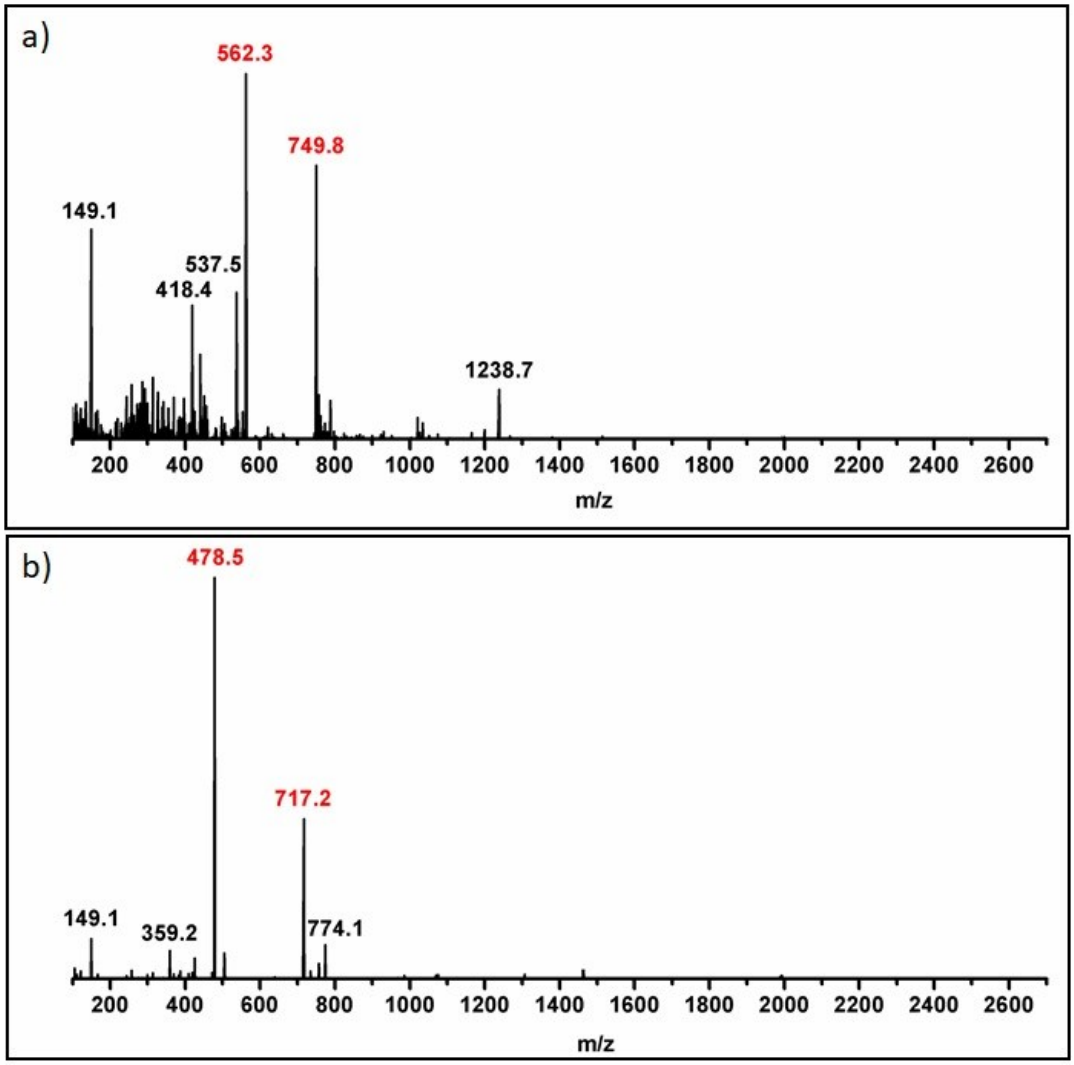

Figure S22 - ESI-MS spectrum of pure a) RuPC-2 (Ru-PEG(3)- SRRPASFRTARE) and b) Pep-2 (SRRPASFRTARE)

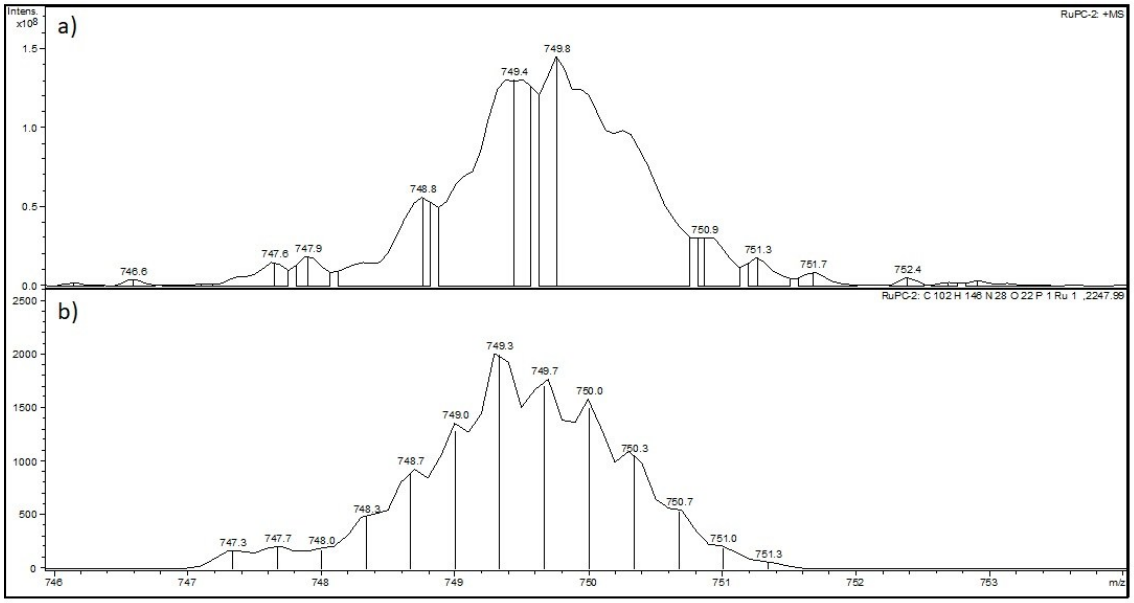

Figure S23 - Observed (a) and simulated (b) ESI-MS Spectra of RuPC-2 (Ru-PEG(3)- SRRPASFRTARE)

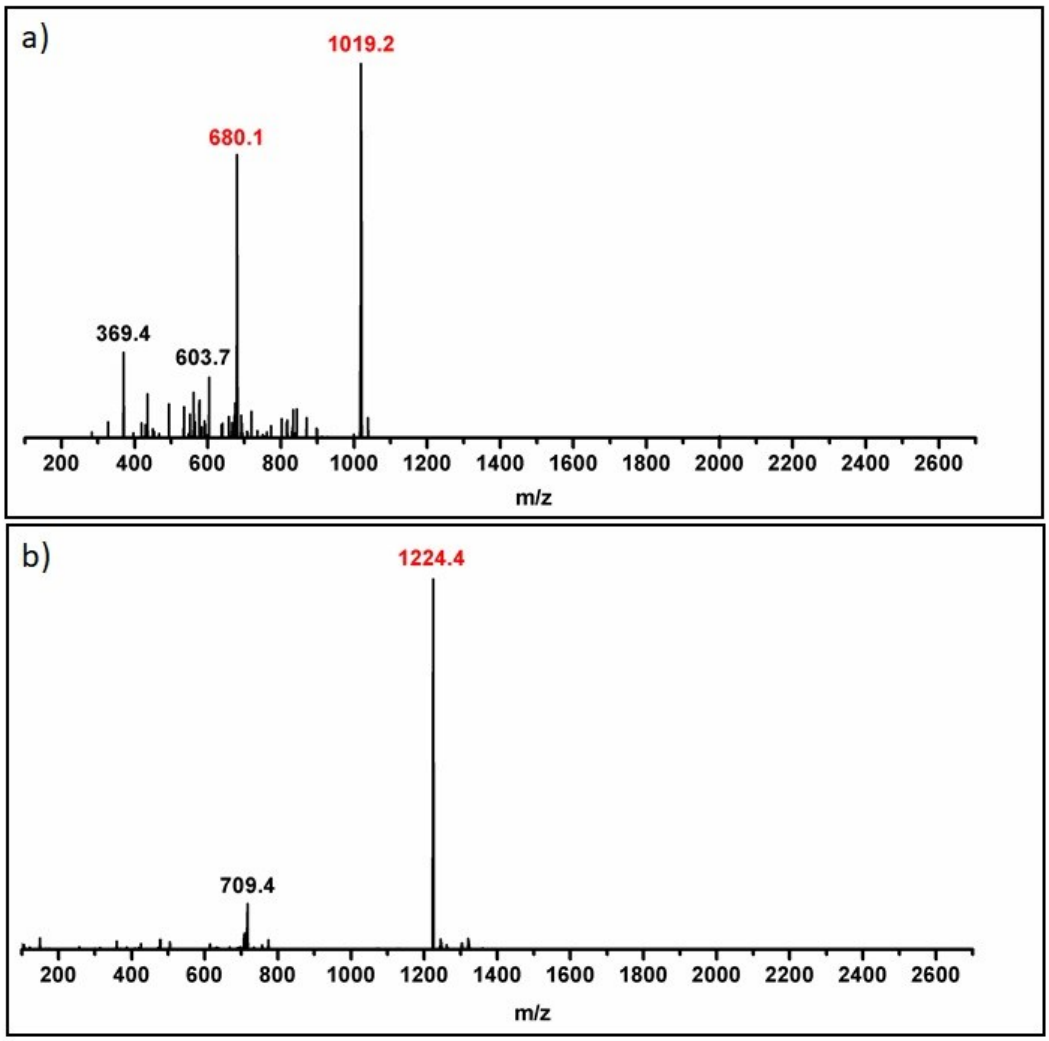

Figure S24 - ESI-MS spectrum of pure a) RuPC-3 (Ru-PEG(3)- VSPPLTLGQLLS) and b) Pep-3 (VSPPLTLGQLLS)

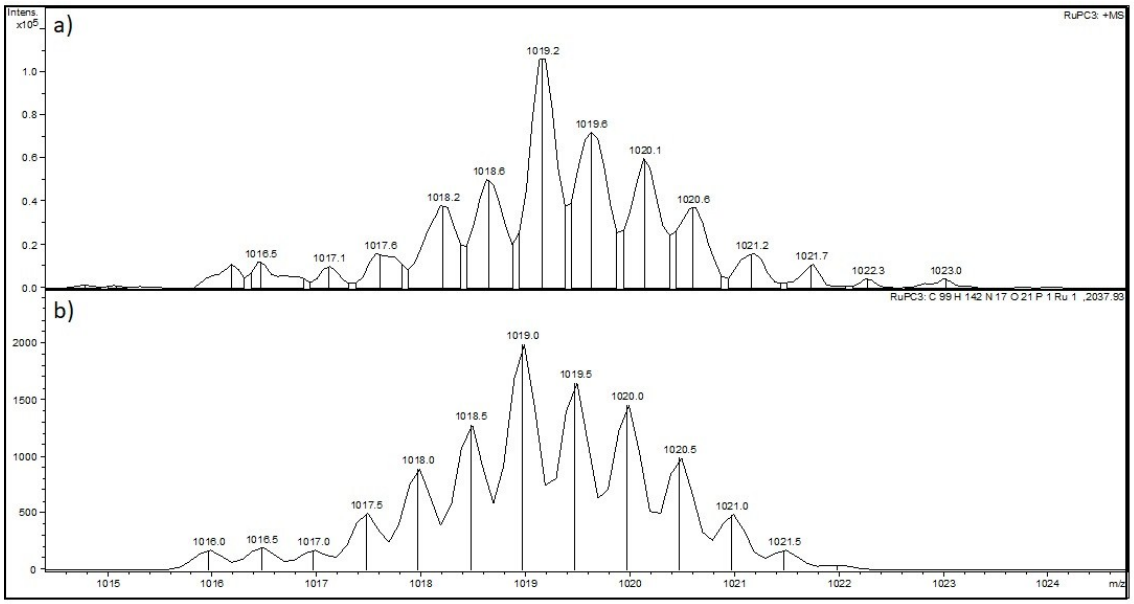

Figure S25 - Observed (a) and simulated (b) ESI-MS Spectra of RuPC-3 (Ru-PEG(3)- VSPPLTLGQLLS)

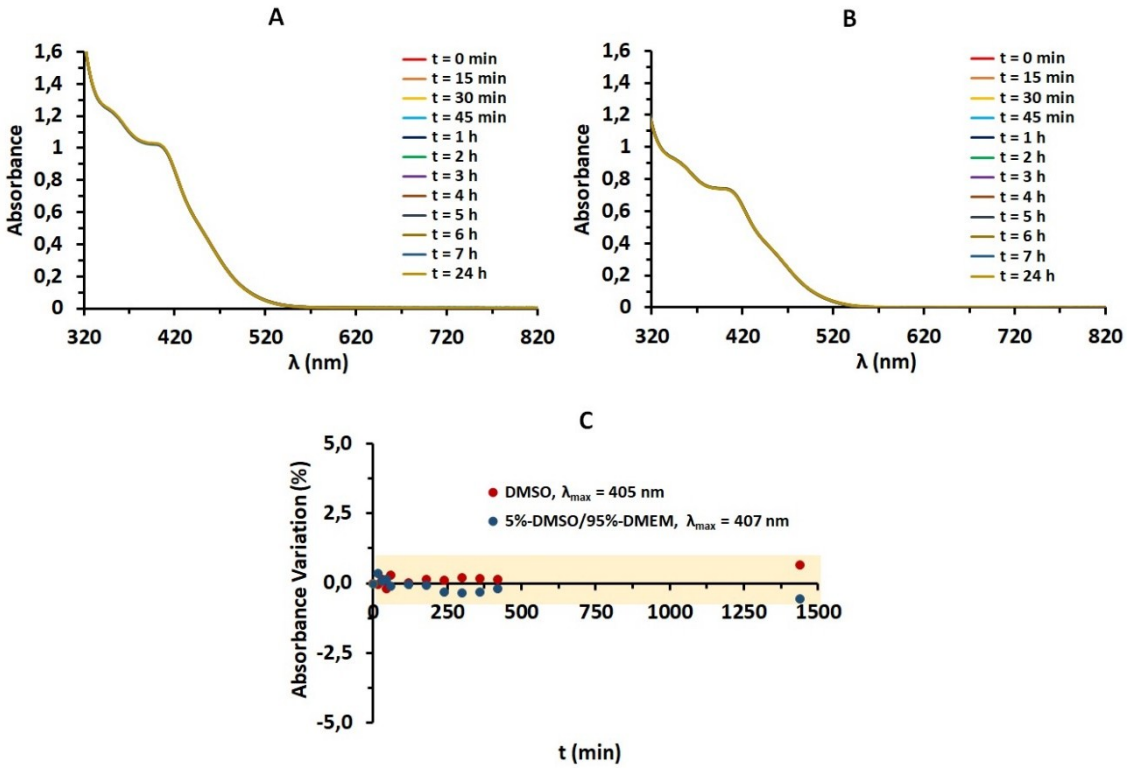

Figure S26 - Stability of [Ru(η^5-C_5H_4COOH)(PPh_3)(2,2'-bipy)][CF_3SO_3] (**TM281**) in solution over 24 hours: UV-visible spectra acquired from 0 min up to 24 h in DMSO at 280 µM (**A**) and in 5%-DMSO/95%-DMEM+GlutaMAX-I™ at 200 µM (**B**); variation of the maximum absorption observed at 405 nm in DMSO (•) and at 407 nm 5%-DMSO/95%-DMEM+GlutaMAX-I™ (•) along time (0 min to 1440 min = 24 h).

Table S7 - Data collection and structure refinement parameters for [Ru(η^5-$C_5H_4COOCH_2CH_3$)(PPh_3)(2,2'-bipy)][CF_3SO_3] **3**, [Ru(η^5-C_5H_4COOH)(PPh_3)(2,2'-bipy)][CF_3SO_3] **4**.

Compound	**3**	**4**
Empirical formula	$C_{37}H_{32}F_3N_2O_5PRuS$	$C_{35}H_{28}F_3N_2O_5PRuS$
Formula Weight	805.74	777.69
T (K)	150(2)	150(2)
Wavelength (Å)	0.71073	0.71073
Crystal System	Monoclinic	Monoclinic
Space Group	P 21/n	P 21/n
Unit cell dimensions		
a (Å)	9.8575(5)	10.5328(8)
b (Å)	18.2757(8)	12.3220(8)
c (Å)	19.2762(9)	24.8165(18)
α (°)	90	90
β (°)	96.230(2)	95.282(4)
γ (°)	90	90
Volume ($Å^3$)	3452.2(3)	3207.1(4)
Z	4	4
Calculated density (Mgm^{-3})	1.550	1.611
Absorption Coefficient (mm^{-1})	0.624	0.668
F (000)	1640	1576
θ Range for data collection (°)	2.126 to 26.757	2.178 to 26.456
Limiting indices	$-12 \le h \le 12$, $-19 \le k \le 23$, $-23 \le l \le 24$	$-13 \le h \le 11$, $-15 \le k \le 15$, $-29 \le l \le 31$
Reflections collected/ unique	29214 / 7346 [R(int) = 0.0452]	25471 / 6594 [R(int) = 0.0672]
Completeness to Refinement method	θ = 25.242 [100%]	θ = 25.242 [99.8%]
Refinement method	Full-matrix least-squares on F^2	
Data/ restraints/ parameters	7346 / 0 / 451	6594 / 0 / 437
Goodness-on-fit on F^2	1.015	1.012
Final R indices [$I>2\sigma(I)$]	R1 = 0.0296 wR2 = 0.0642	R1 = 0.0347 wR2 = 0.0735
R indices (all data)	R1 = 0.0423 wR2 = 0.0689	R1 = 0.0498 wR2 = 0.0799
Largest diff. peak and hole ($eÅ)^{-3}$	0.363 and -0.381	0.416 and -0.474