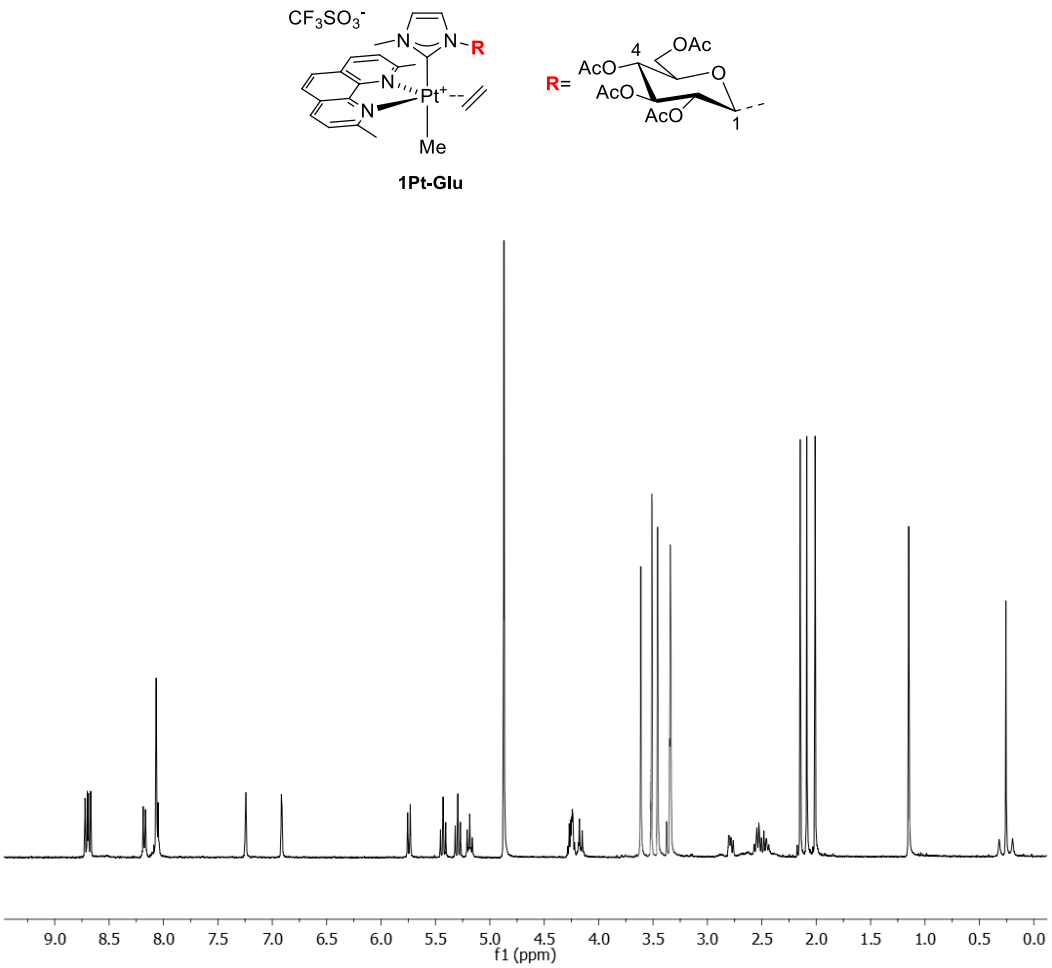
Supporting Info

A Highly Efficient and Selective Antitumor Agent Based on a Glucoconjugated Carbene Platinum(II) Complex

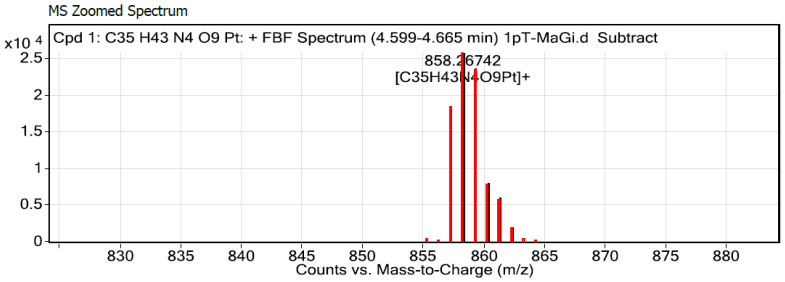
Alfonso Annunziata,[a,b] Maria Elena Cucciolito,[a,b] Roberto Esposito,[a,b] Paola Imbimbo,[a] Ganna Petruk,[a] Giarita Ferraro,[a] Valerio Pinto,[a] Angela Tuzi,[a] Daria Maria Monti,*[a] Antonello Merlino,*[a] and Francesco Ruffo *[a,b]

[a]Dipartimento di Scienze Chimiche, Università di Napoli Federico II, Complesso Universitario di Monte S. Angelo, via Cintia 21, 80126 Napoli (Italy), [b]Consorzio Interuniversitario di Reattività Chimica e Catalisi (CIRCC), via Celso Ulpiani 27, 70126 Bari (Italy).

Experimental Section



(a)

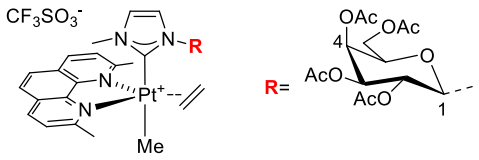


MS Spectrum Peak List

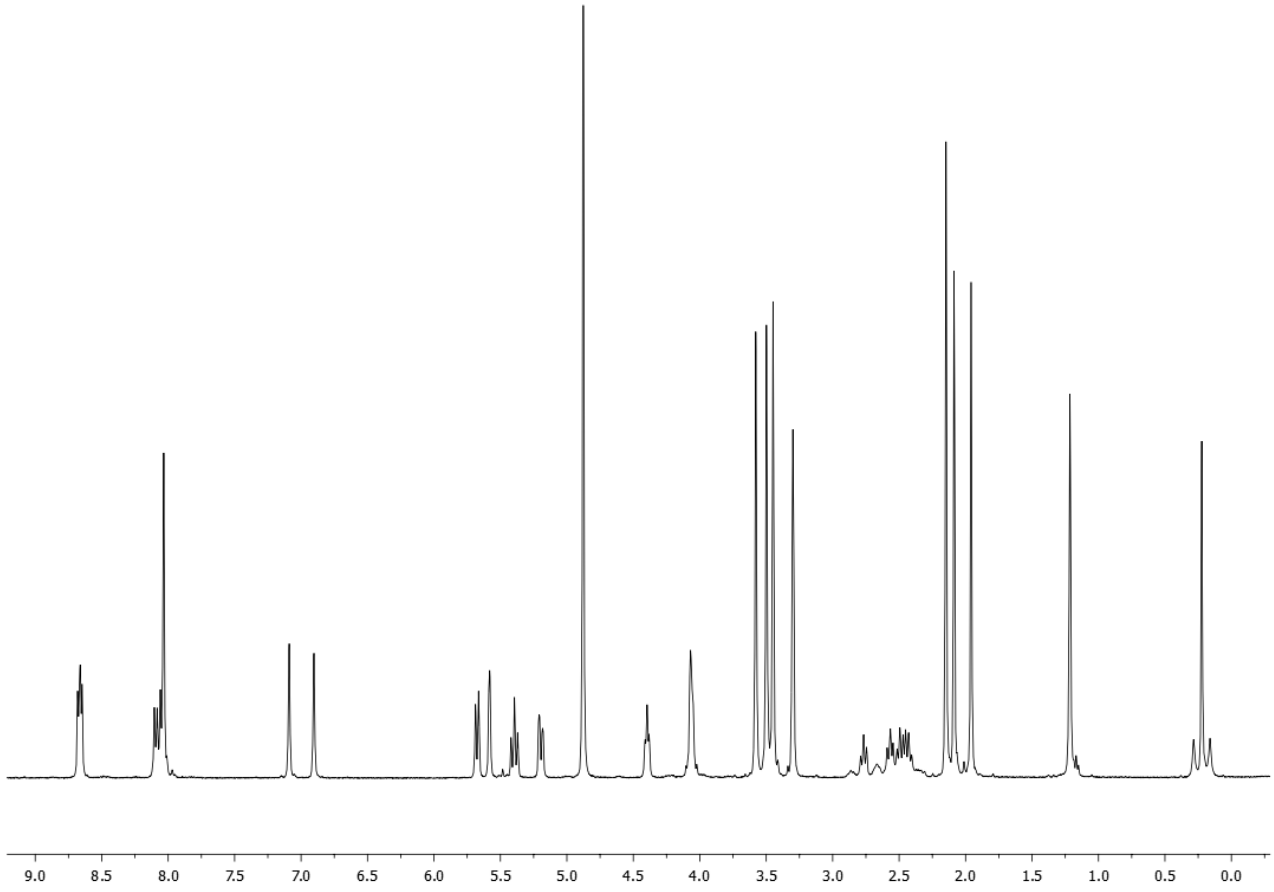
m/z	z	Abund	Formula	Ion
854.26851	1	54.58	C35H43N4O9Pt	M+
855.25643	1	323.42	C35H43N4O9Pt	M+
857.26513	1	18108.51	C35H43N4O9Pt	M+
858.26742	1	25843.42	C35H43N4O9Pt	M+
859.26867	1	23744.3	C35H43N4O9Pt	M+
860.27058	1	8090.49	C35H43N4O9Pt	M+
861.27056	1	6063.98	C35H43N4O9Pt	M+
862.27325	1	2088.52	C35H43N4O9Pt	M+
863.27535	1	339.31	C35H43N4O9Pt	M+
864.28315	1	53.49	C35H43N4O9Pt	M+

(b)

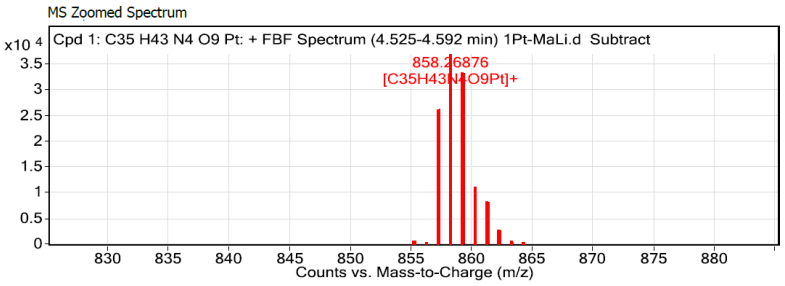
Figure S1. **1Pt-Glu:** (a) ^{1}H NMR spectrum (in CD_3OD, 400 MHz, 298 K); (b) LC/MS–QTOF.



1Pt-Gal



(a)

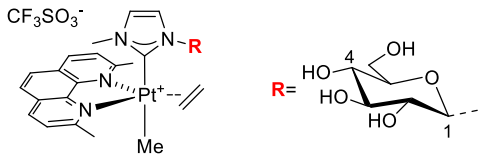


MS Spectrum Peak List

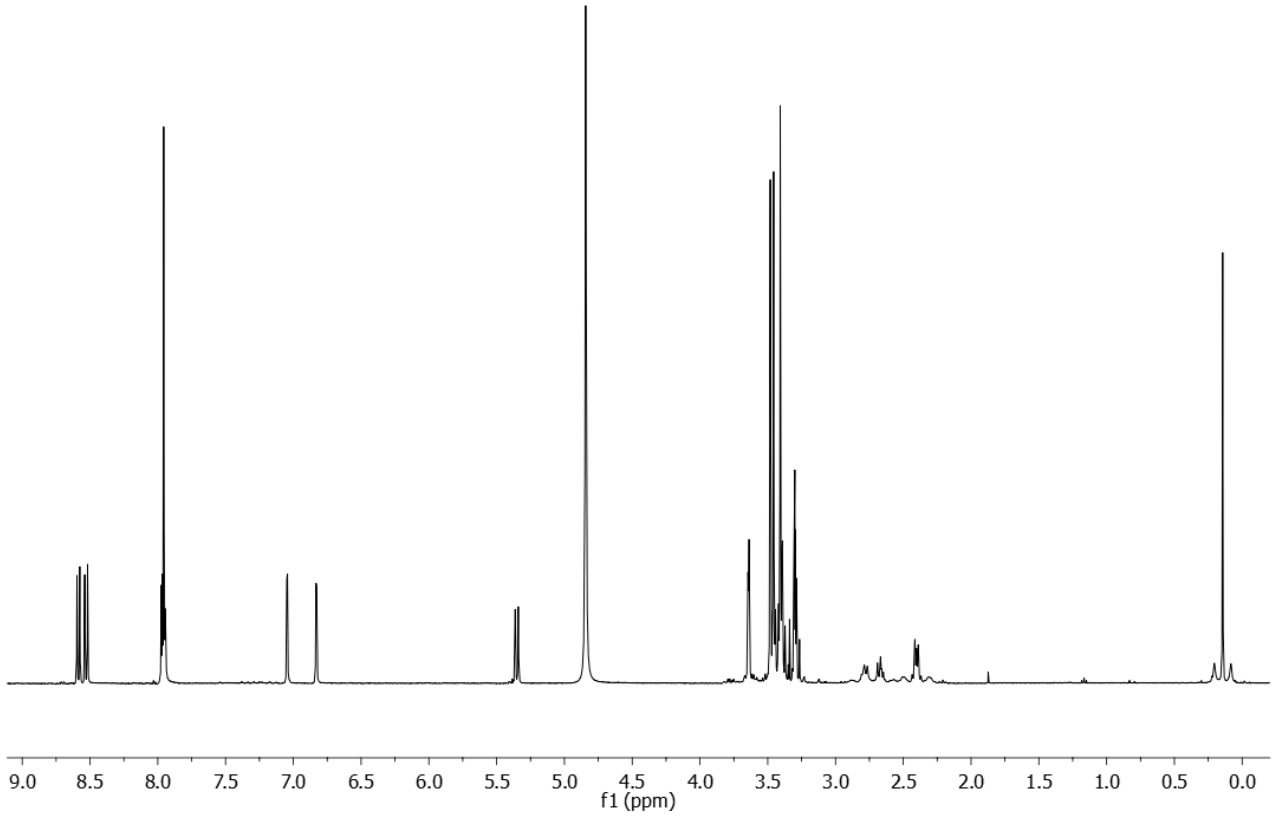
m/z	z	Abund	Formula	Ion
855.2687	1	647.23	C35H43N4O9Pt	M+
856.26376	1	94.84	C35H43N4O9Pt	M+
857.26607	1	26459.95	C35H43N4O9Pt	M+
858.26876	1	36944.34	C35H43N4O9Pt	M+
859.26987	1	33330.93	C35H43N4O9Pt	M+
860.27174	1	10788.57	C35H43N4O9Pt	M+
861.27133	1	8428.33	C35H43N4O9Pt	M+
862.27444	1	2821.26	C35H43N4O9Pt	M+
863.27653	1	597.3	C35H43N4O9Pt	M+
864.2897	1	104.96	C35H43N4O9Pt	M+

(b)

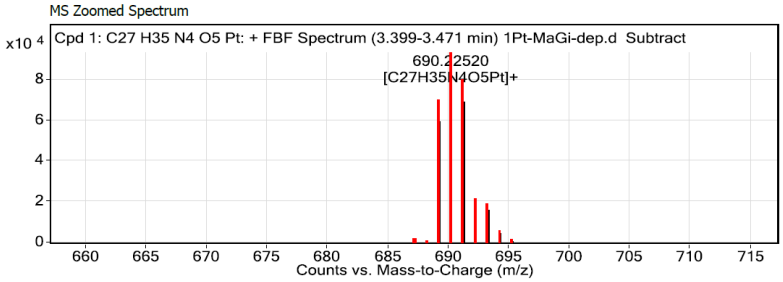
Figure S2. **1Pt-Gal:** (a) ^{1}H NMR spectrum (in CD_3OD, 400 MHz, 298 K); (b) LC/MS–QTOF.



1Pt-Glu-dep



(a)

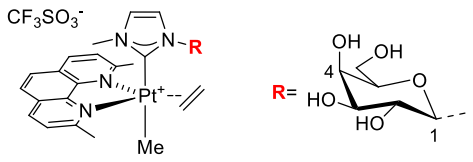


MS Spectrum Peak List

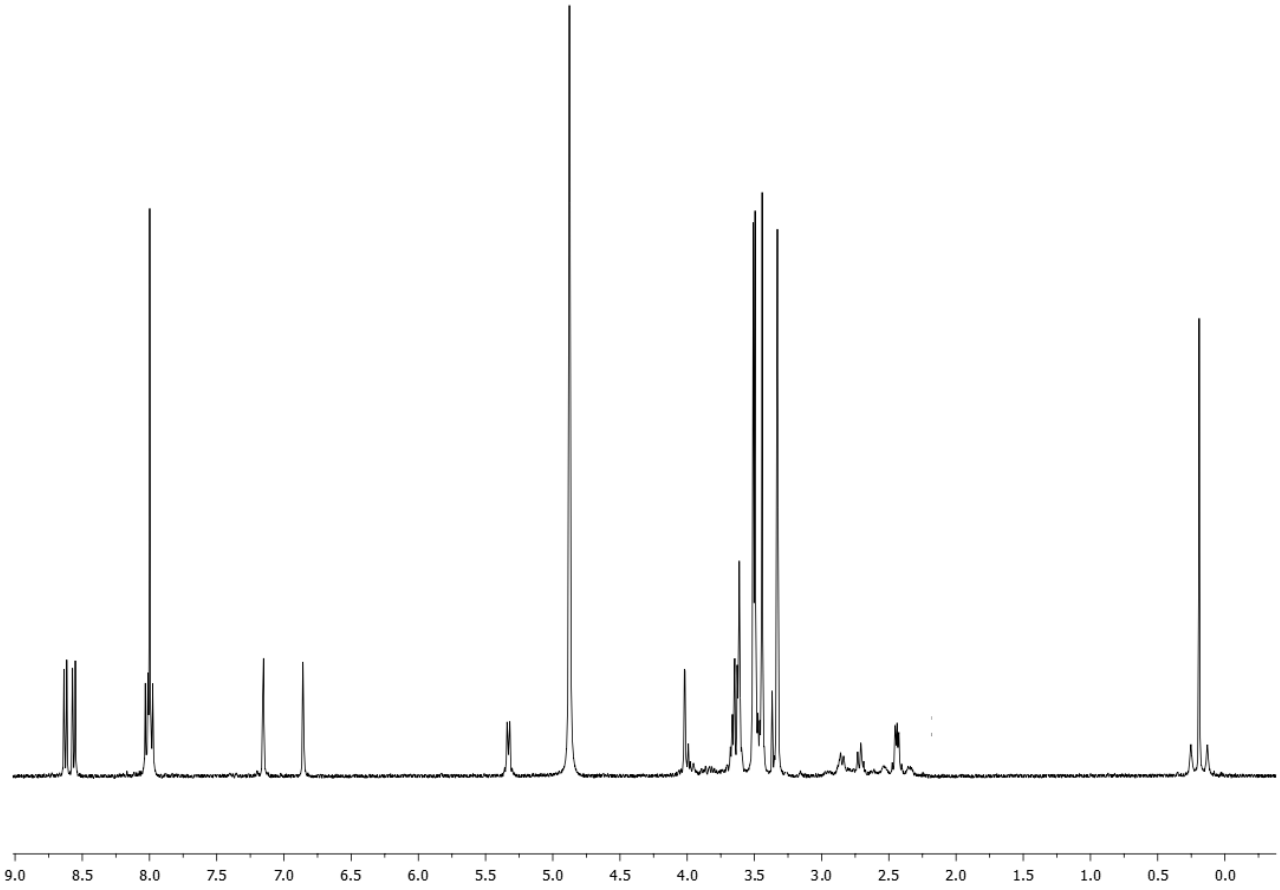
m/z	z	Abund	Formula	Ion
687.22202	1	1786.98	C27H35N4O5Pt	M+
688.22477	1	626.22	C27H35N4O5Pt	M+
689.22421	1	59434.89	C27H35N4O5Pt	M+
690.2252	1	92841.84	C27H35N4O5Pt	M+
691.22802	1	68969.88	C27H35N4O5Pt	M+
692.22987	1	19630.61	C27H35N4O5Pt	M+
693.22983	1	16181.08	C27H35N4O5Pt	M+
694.23277	1	4988.59	C27H35N4O5Pt	M+
695.2356	1	972.16	C27H35N4O5Pt	M+
696.22676	1	95.09	C27H35N4O5Pt	M+

(b)

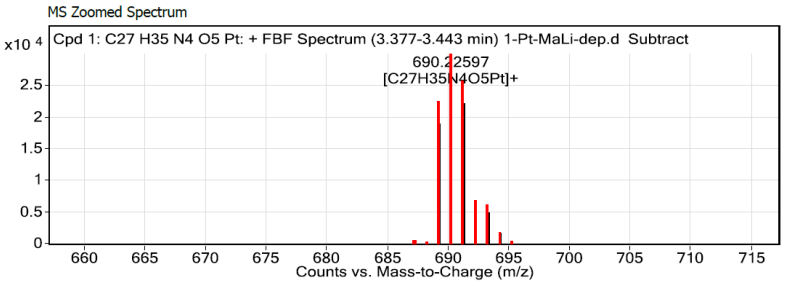
Figure S3. **1Pt-Glu-dep:** (a) ^{1}H NMR spectrum (in CD_3OD, 400 MHz, 298 K); (b) LC/MS–QTOF.



1Pt-Gal-dep



(a)

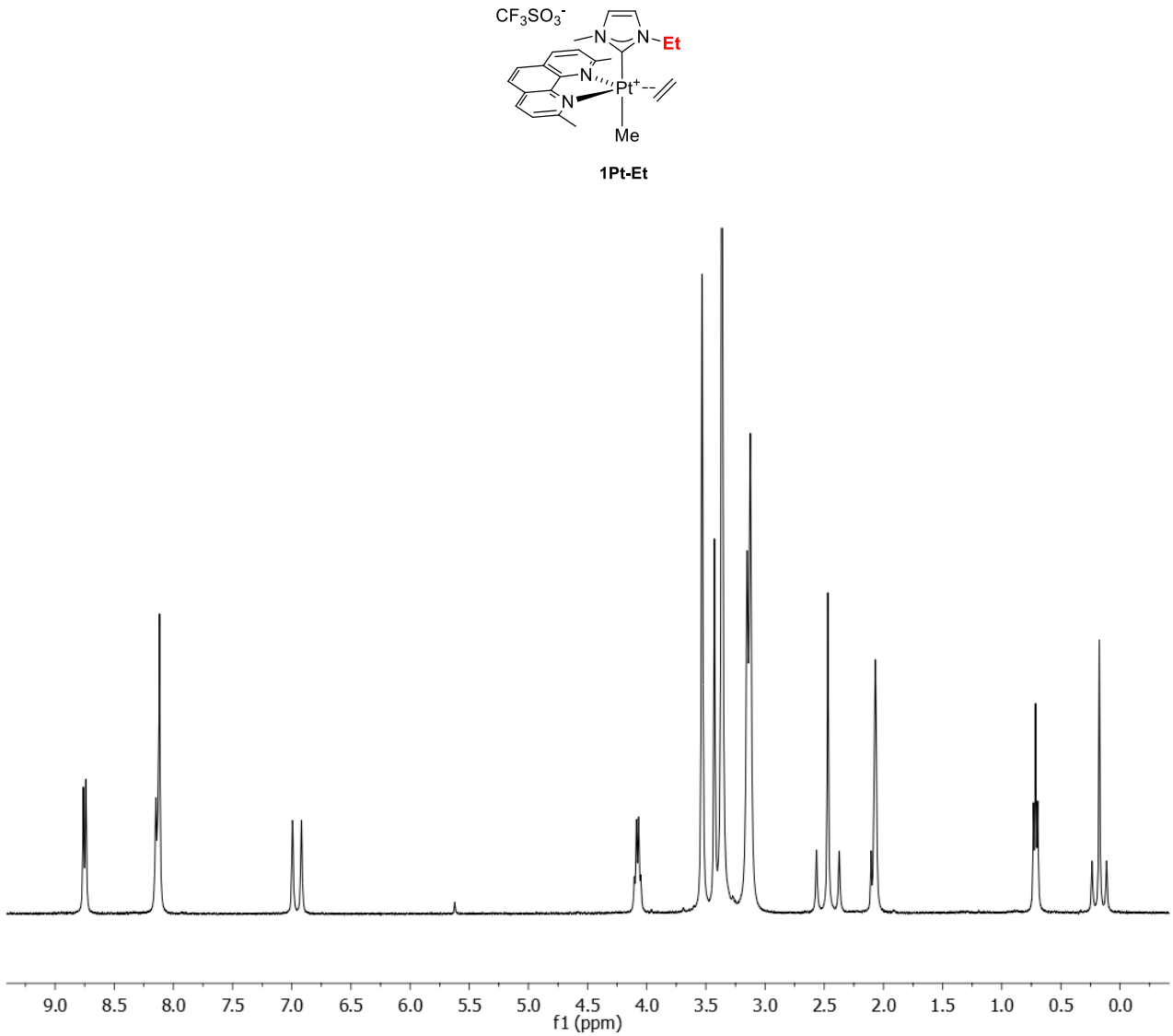


MS Spectrum Peak List

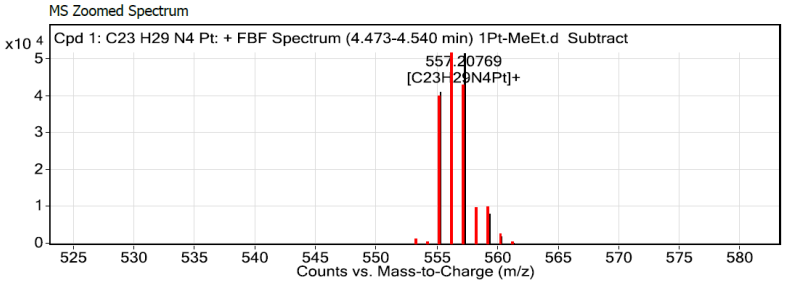
m/z	z	Abund	Formula	Ion
687.21815	1	461.67	C27H35N4O5Pt	M+
688.22332	1	85.09	C27H35N4O5Pt	M+
689.22195	1	18982.09	C27H35N4O5Pt	M+
690.22597	1	29966.2	C27H35N4O5Pt	M+
691.22516	1	22265.35	C27H35N4O5Pt	M+
692.22786	1	6076.1	C27H35N4O5Pt	M+
693.22604	1	5031.25	C27H35N4O5Pt	M+
694.2319	1	1790.82	C27H35N4O5Pt	M+
695.22783	1	100.69	C27H35N4O5Pt	M+

(b)

Figure S4. **1Pt-Gal-dep:** (a) ^{1}H NMR spectrum (in CD_3OD, 400 MHz, 298 K); (b) LC/MS–QTOF.



(a)



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
553.19699	1	692.16	C23H29N4Pt	M+
554.19828	1	134.42	C23H29N4Pt	M+
555.20393	1	41383.75	C23H29N4Pt	M+
556.19998	1	43003.9	C23H29N4Pt	M+
557.20769	1	51707.41	C23H29N4Pt	M+
558.2062	1	8223.89	C23H29N4Pt	M+
559.20585	1	8306.94	C23H29N4Pt	M+
560.20772	1	2222.31	C23H29N4Pt	M+
561.20824	1	147.34	C23H29N4Pt	M+

(b)

Figure S5. **1Pt-Et:** (a) ^{1}H NMR spectrum (in acetone-d_6, 400 MHz, 298 K); (b) LC/MS–QTOF.

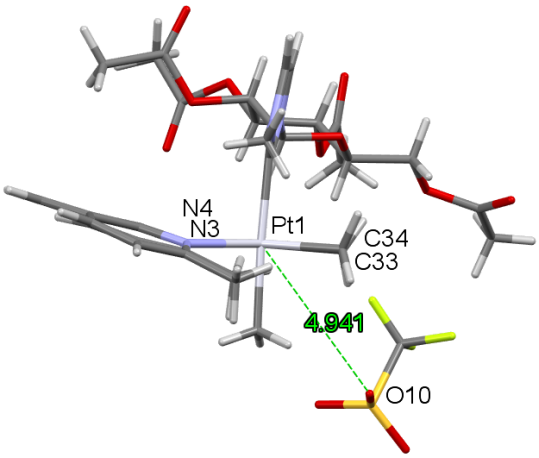


Figure S6. Projection of **1Pt-Glu** down the edge of coordination plane defined by N3/N4/C33/C34 atoms showing the flat shape of glucosyl group and the $CF_3SO_3^-$ anion nearest to Pt (shortest distance Pt···O is reported in green).

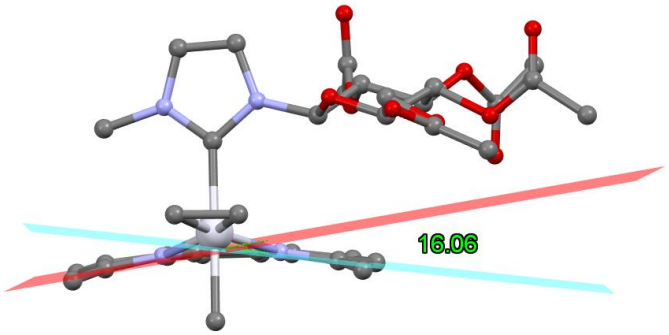


Figure S7. Bowlike distortion of dmphen ligand in **1Pt-Glu** (angle between mean planes of outer rings is reported in green).

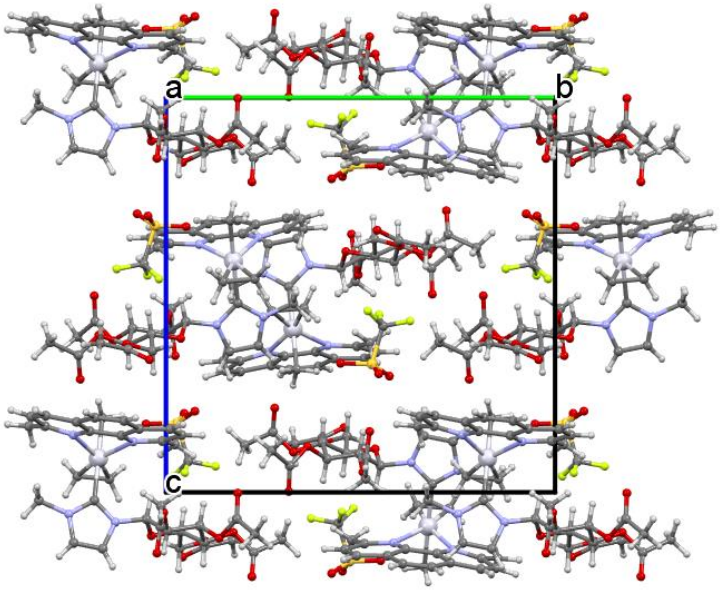


Figure S8. Crystal packing of **1Pt-Glu** viewed along **a** axis.

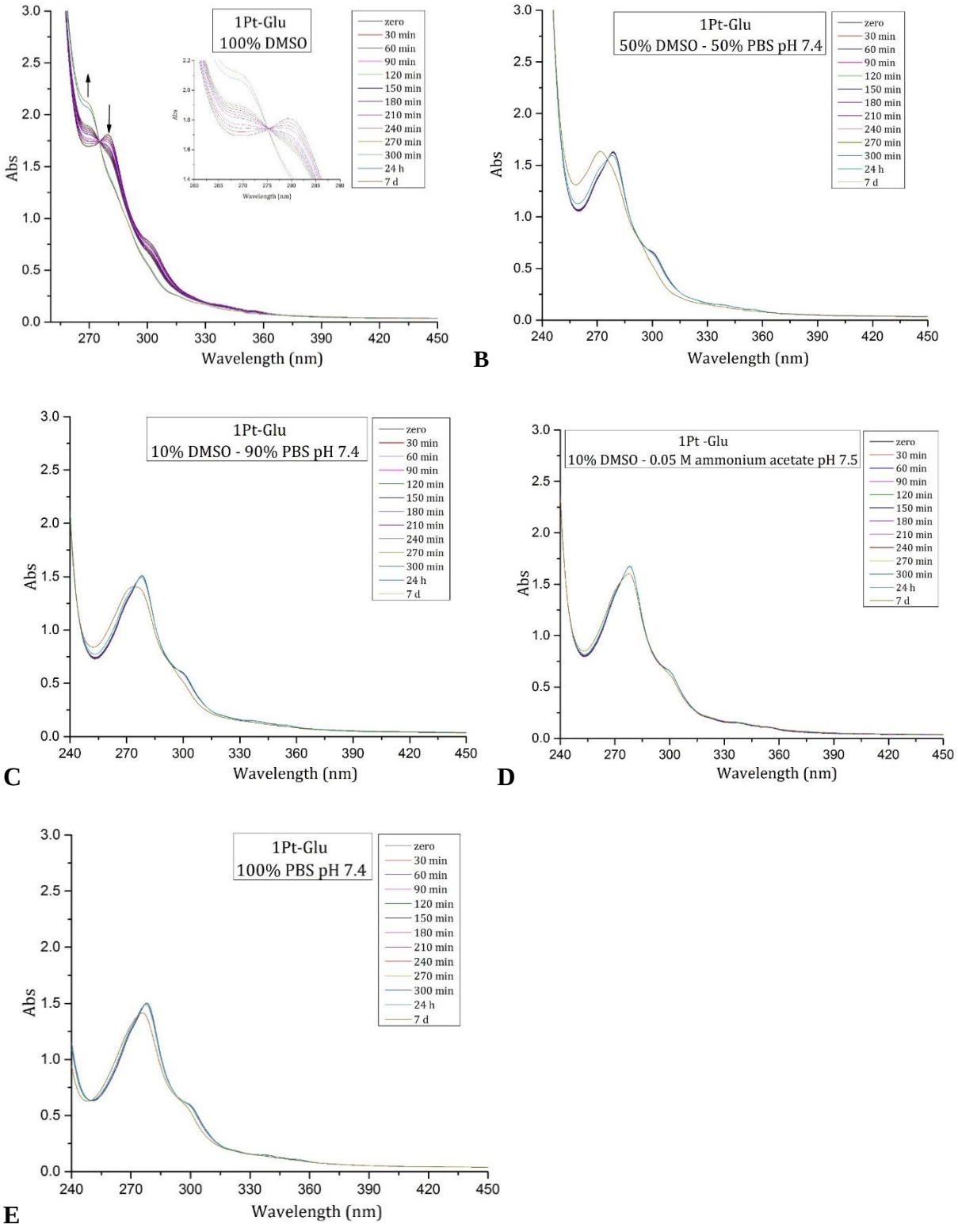


Figure S9. Time course UV-vis spectra of 5×10^{-5} M **1Pt-Glu** in 100% DMSO (A), 50% DMSO – 50% PBS pH 7.4 (B), 10% DMSO - 90% PBS pH 7.4 (C), 10% DMSO - 0.05 M ammonium acetate pH 7.5 (D), 100% PBS pH 7.4 (0.5% DMSO; E).

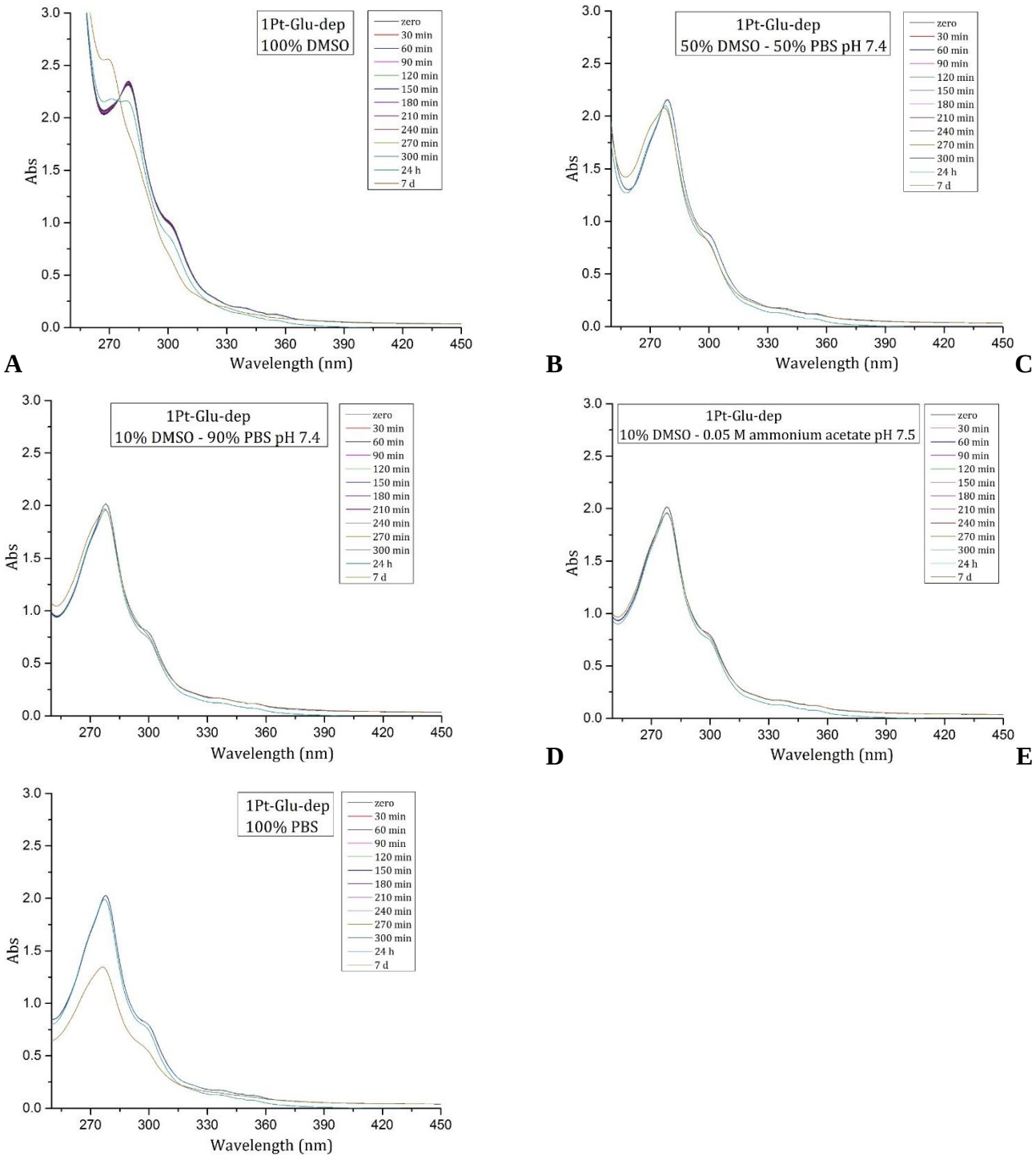


Figure S10. Time course UV-vis spectra of 5×10^{-5} M **1Pt-Glu-dep** in 100% DMSO (A), 50% DMSO – 50% PBS pH 7.4 (B), 10% DMSO - 90% PBS pH 7.4 (C), 10% DMSO - 0.05 M ammonium acetate pH 7.5 (D), 100% PBS pH 7.4 (0.5% DMSO; E).

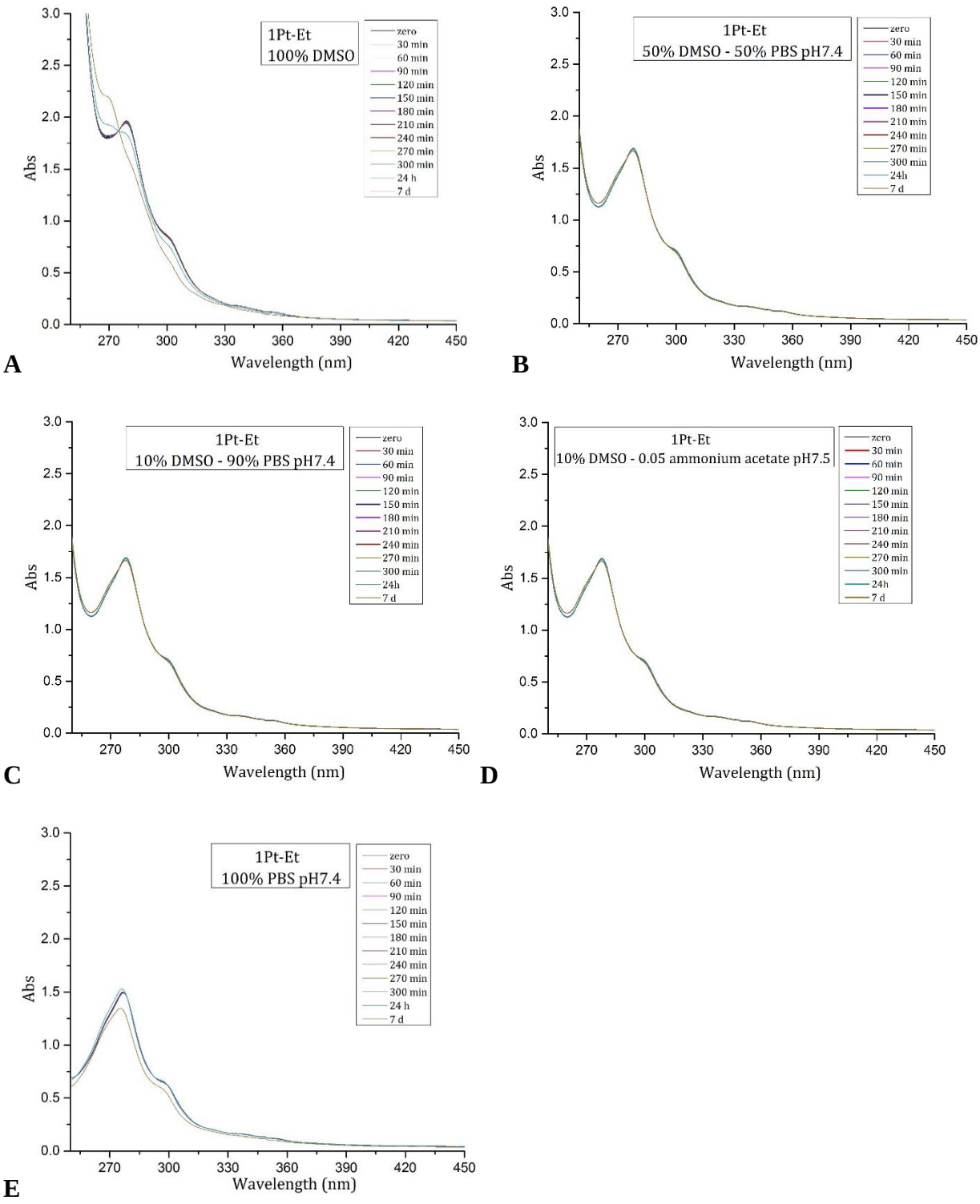


Figure S11. Time course UV-vis spectra of 5×10^{-5} M **1Pt-Et** in 100% DMSO (A), 50% DMSO – 50% PBS pH 7.4 (B), 10% DMSO - 90% PBS pH 7.4 (C), 10% DMSO - 0.05 M ammonium acetate pH 7.5 (D), 100% PBS pH 7.4 (0.5% DMSO; E).

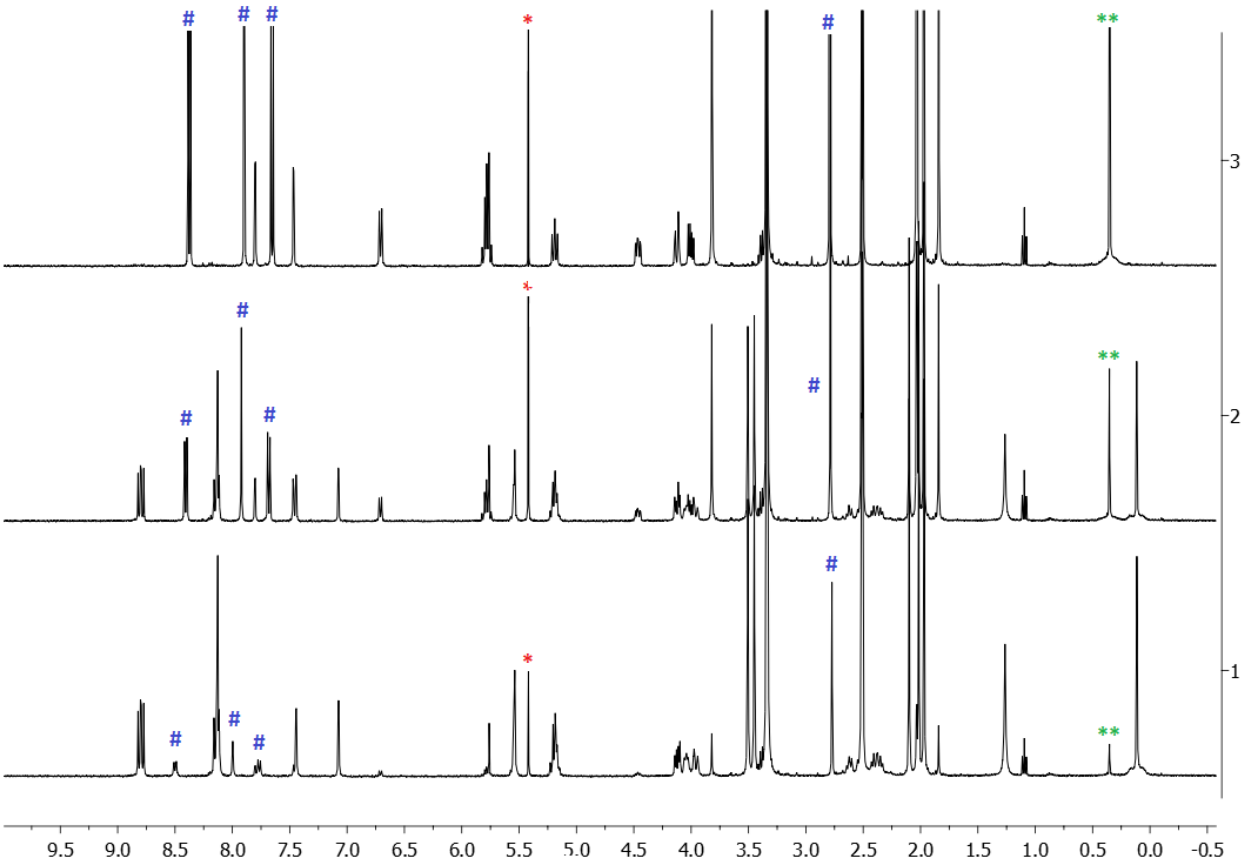


Figure S12. ^{1}H NMR spectra at 400 MHz in DMSO-d_6 of **1Pt-Glu** after 1 hour (trace 1), after 3 hours (trace 2), after 60 hours (trace 3). Legend= *: free ethene; #: free 2,9-dimethyl-1,10-phenanthroline; **: Pt-Me in the square-planar product.

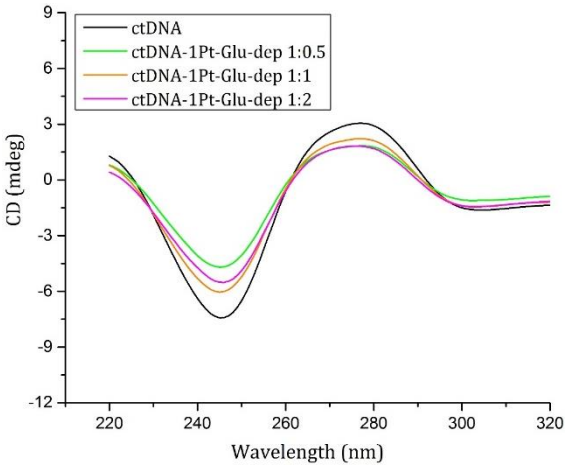


Figure S13. CD spectra of calf thymus DNA (200 μM in 0.01 M ammonium acetate buffer, pH 7.5) in the absence and in the presence of **1Pt-Glu-dep** in 1:0.5, 1:1 and 1:2 DNA to metal molar ratios.

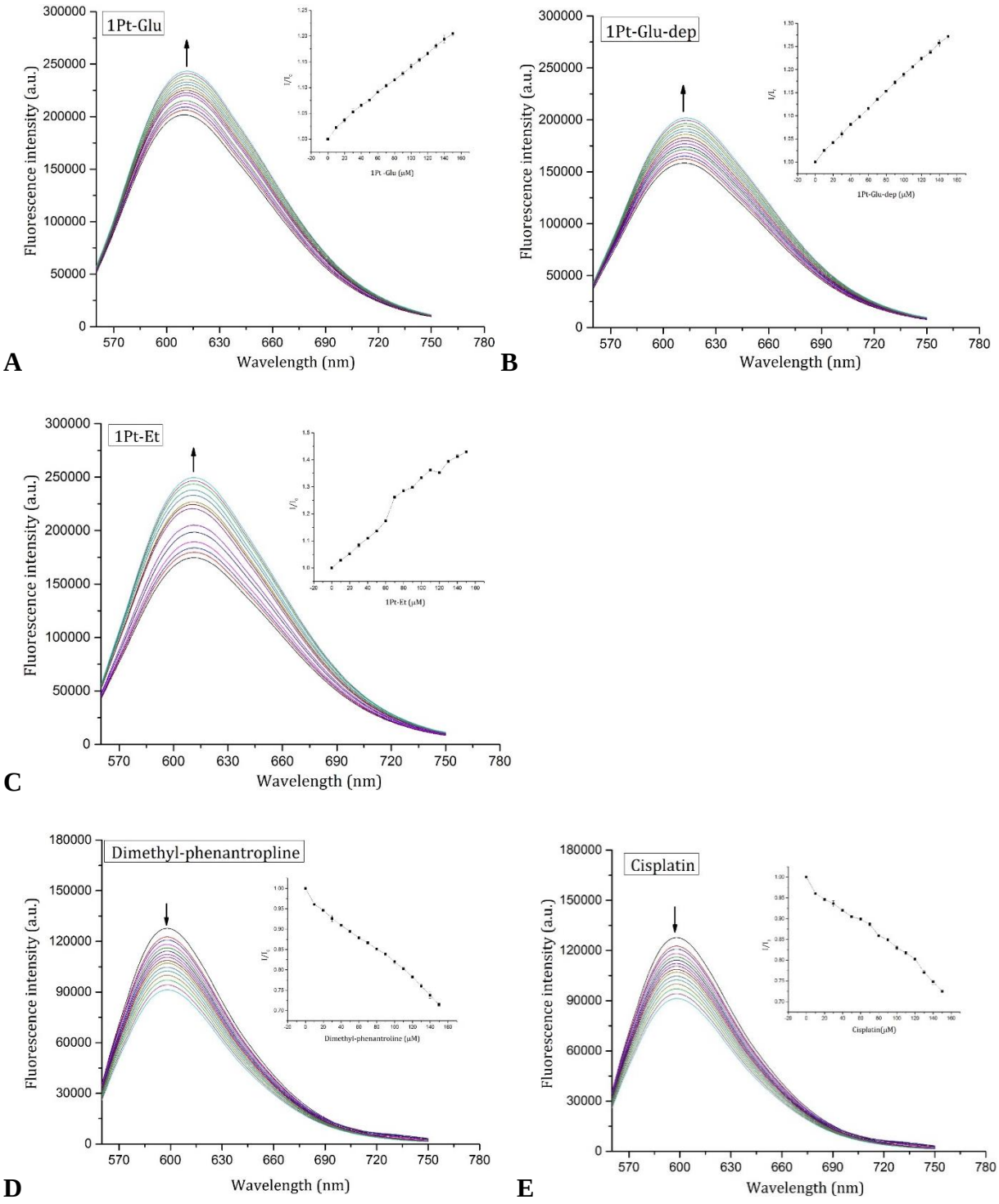


Figure S14. Emission spectra of ethidium bromide bound to DNA in the presence of **1Pt-Glu** (A), **1Pt-Glu-dep** (B), **1Pt-Et** (C). 0 − 150 μM. In the inset, the fluorescence quenching as a function of the Pt compound concentration is also reported. Emission spectra of ethidium bromide bound to DNA in the presence of the dmphen ligand (D) and in the presence of cisplatin (E) are also reported as an internal control.

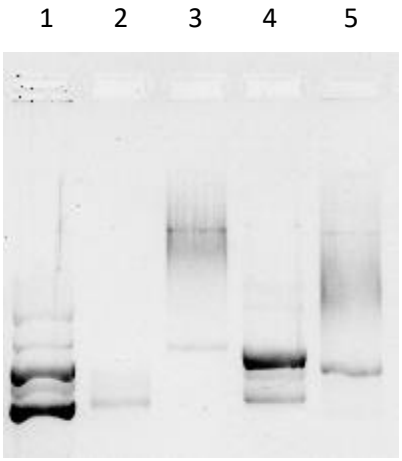
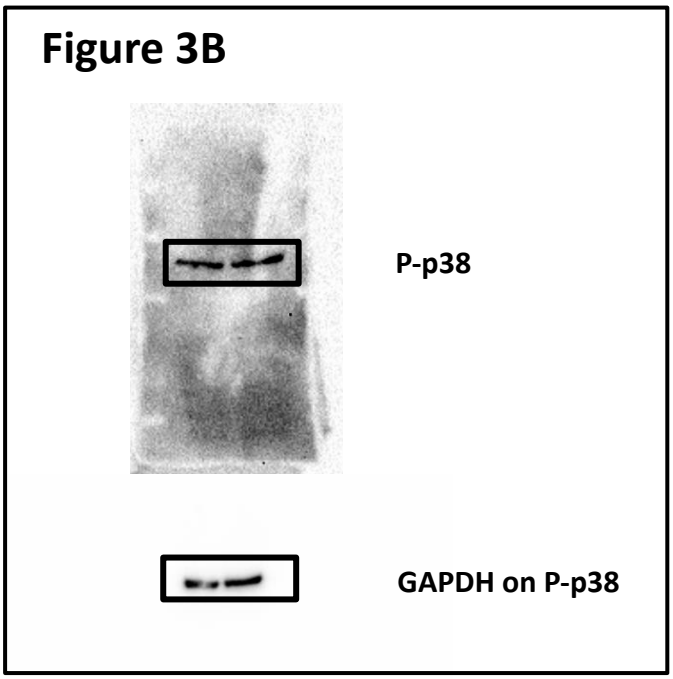


Figure S15. Agarose gel mobility shift assay. 200 ng of pGEX plasmid DNA were incubated with the Pt compounds at 1:50 ratio at 37°C for 90 minutes. At the end of the incubation, 100 ng of DNA were loaded onto a 1% agarose gel. The gel electrophoresis was performed applying a potential of 100 V for 45 minutes. The image was obtained with Chemidoc imaging system (Biorad). Lane 1: untreated pGEX plasmid DNA; lane 2: cisplatin; lane 3: **1Pt-Glu**; lane 4: **1Pt-Glu-dep**; lane 5: **1Pt-Et**.

Table S1. Crystal data and structure refinement details for **1Pt-Glu**

Empirical formula	$C_{35}\ H_{43}\ N_4\ O_9\ Pt \cdot C\ F_3\ S$
Formula weight	1007.89
Temperature	298(2)K
Wavelength	0.71073 Å
Crystal system, space group	Orthorhombic, $P\ 2_1 2_1 2_1$
Unit cell dimensions	a = 13.170(2) Å, α = 90°
	b = 17.362(5) Å, β = 90°
	c = 17.639(6) Å, γ = 90°
Volume	4033.3(19) $Å^3$
Z, Calculated density	4, 1.660 Mg/m^3
Absorption coefficient	3.610 mm^{-1}
F(000)	2016
Crystal size	0.50 x 0.40 x 0.08 mm
Theta range for data collection	2.590 to 27.656 °
Reflections collected / unique	23099 / 8502 [R(int) = 0.0391]
Data / restraints / parameters	8502 / 0 / 523
Goodness-of-fit on F2	1.093
Final R indices [I>2sigma(I)]	*R1* = 0.0374, *wR2* = 0.0811
R indices (all data)	*R1* = 0.0503, *wR2* = 0.0879
Absolute structural parameter	0.046(11)
Largest diff. peak and hole	1.371 and -0.787 $e \cdot Å^{-3}$

Figure S16. Uncropped Western blots of Figure 3.



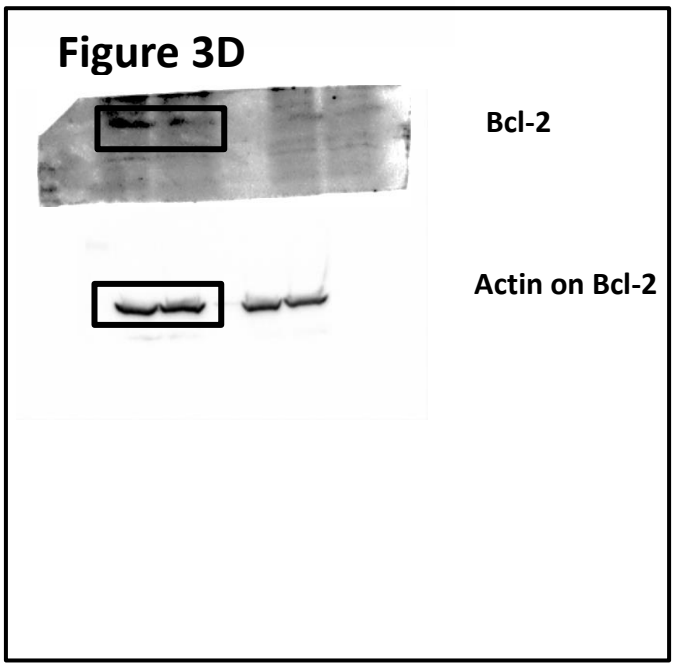
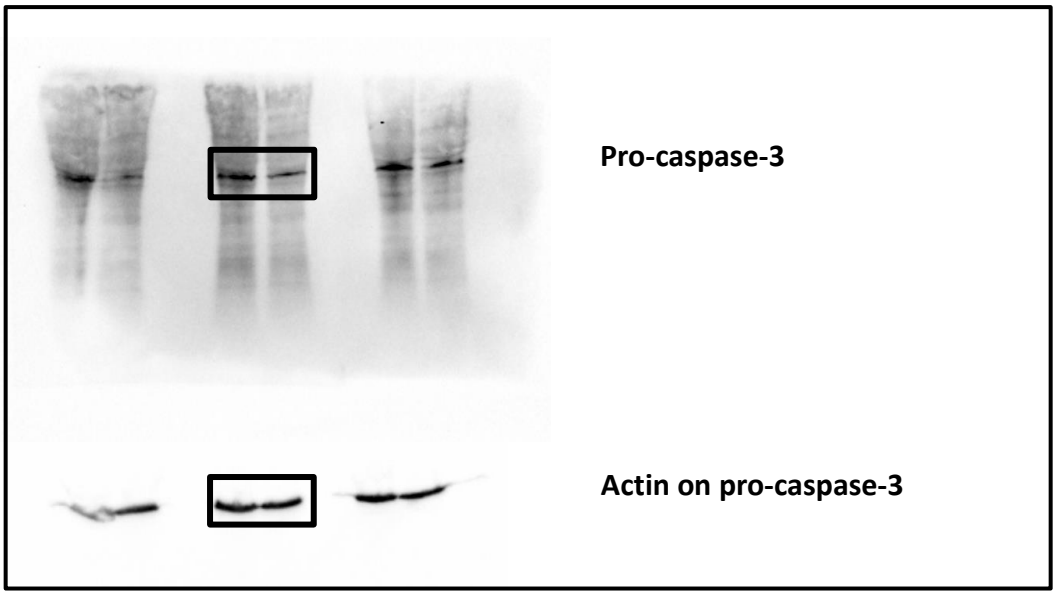
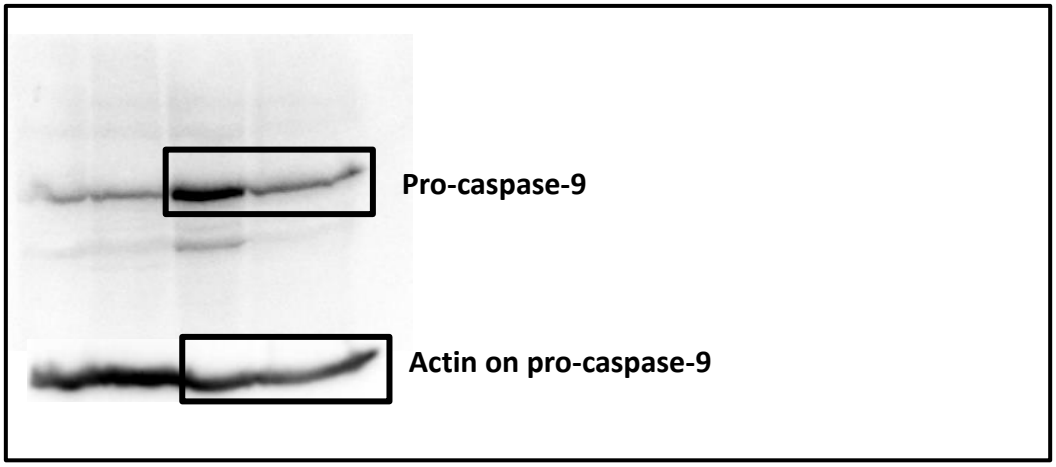
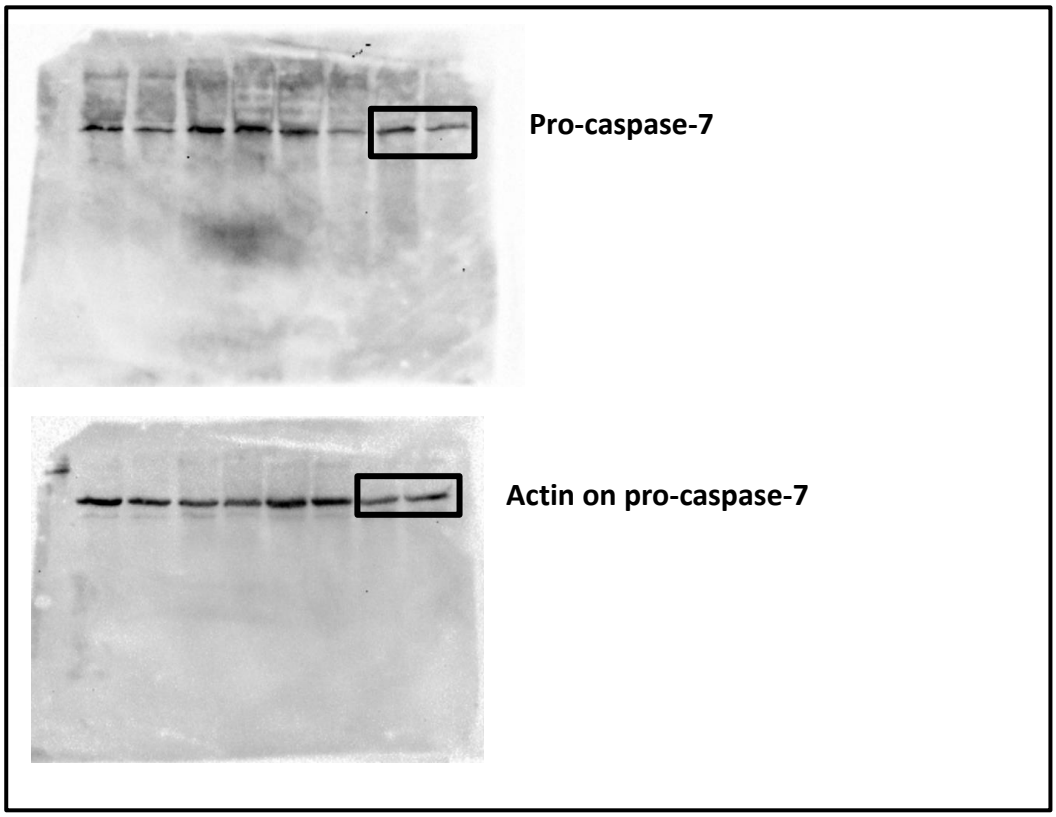


Figure 3E


Pro-caspase-3
Actin on pro-caspase-3


Pro-caspase-9
Actin on pro-caspase-9


Pro-caspase-7
Actin on pro-caspase-7