Antiproliferative activity of a series of cisplatin-based Pt(IV)-acetylamido/carboxylato prodrugs

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ELECTRONIC SUPPLEMENTARY INFORMATION

Content:

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Table S1. Crystallographic data for complex **3a**.

Chemical formula	$C_6H_{17}Cl_2N_3O_3Pt$
M_r	445.21
Crystal system	Monoclinic
Space group	$P2_1/c$
Temperature / K	293
Wavelength / Å	0.71073
a / Å	10.400(2)
b / Å	10.093(2)
c / Å	13.361(2)
α / °	90.00
β / °	100.644(3)
γ / °	90.00
V / Å^3	1378.4(4)
Z	4
Density / Mg m^{-3}	2.145
Absorption co-efficient / mm^{-1}	1.056
Absorption correction	Multi-scan
F(000)	840
Total no. of reflections	3752
Reflections, $I > 2\sigma(I)$	3024
Max. 2θ / °	29.31
Ranges (h, k, l)	$-14 \leq h \leq 13, -13 \leq k \leq 13, -18 \leq l \leq 18$
Refinement method	Full-matrix least-squares on F^2
Goodness-of-fit on F^2	1.042
R index [$I > 2\sigma(I)$]	0.0415
R index (all data)	0.0543

Table S2. Selected bond distances (Å), angles (°) and hydrogen bonds for complex **3a**.

Pt1-N3	1.987(6)	Pt1-N2	2.036(5)
Pt1-O2	2.039(5)	Pt1-N1	2.052(7)
Pt1-Cl2	2.314(2)	Pt1-Cl1	2.325(2)
N3-C1	1.323(8)	O1-C1	1.227(9)
O2-C3	1.293(10)	C3-O3	1.206(10)
C3-C4	1.500(11)	C2-C1	1.518(10)
C4-C5	1.506(11)	C5-C6	1.548(14)
N3-Pt1-N2	92.9(2)	N3-Pt1-O2	175.6(2)
N2-Pt1-O2	91.2(2)	N3-Pt1-N1	89.8(3)
N2-Pt1-N1	89.4(2)	O2-Pt1-N1	92.0(3)
N3-Pt1-Cl2	89.8(2)	N2-Pt1-Cl2	89.22(17)
O2-Pt1-Cl2	88.53(17)	N1-Pt1-Cl2	178.58(16)
N3-Pt1-Cl1	86.89(18)	N2-Pt1-Cl1	179.65(16)
O2-Pt1-Cl1	89.05(16)	N1-Pt1-Cl1	90.30(17)
Cl2-Pt1-Cl1	91.04(7)	C1-N3-Pt1	127.6(5)
C3-O2-Pt1	123.5(5)	O3-C3-O2	125.1(8)
O3-C3-C4	121.7(9)	O2-C3-C4	113.2(9)
O1-C1-N3	123.7(7)	O1-C1-C2	120.5(7)
N3-C1-C2	115.8(7)	C3-C4-C5	116.8(10)
C4-C5-C6	109.3(14)		

Donor...Acceptor		Donor-H...Acceptor	
N1...O3 (0)	2.856(.013)	N1-H1A...O3 (0)	100.52(0.54)
N2...O3 (0)	2.885(.009)	N2-H2A...O3 (0)	131.35(0.43)
N2...O1 (0)	2.783(.009)	N2-H2C...O1 (0)	134.69(0.40)
N2...O2 (1)	3.008(.007)	N2-H2B...O2 (1)	143.27(0.37)
N1...O1 (2)	2.842(.008)	N1-H1B...O1 (2)	162.95(0.38)
N2...O1 (2)	2.963(.007)	N2-H2C...O1 (2)	131.10(0.38)

Equivalent positions: (0) x, y, z

(1) -x+1, +y+1/2, -z+1/2

(2) -x+1, -y+1, -z+1

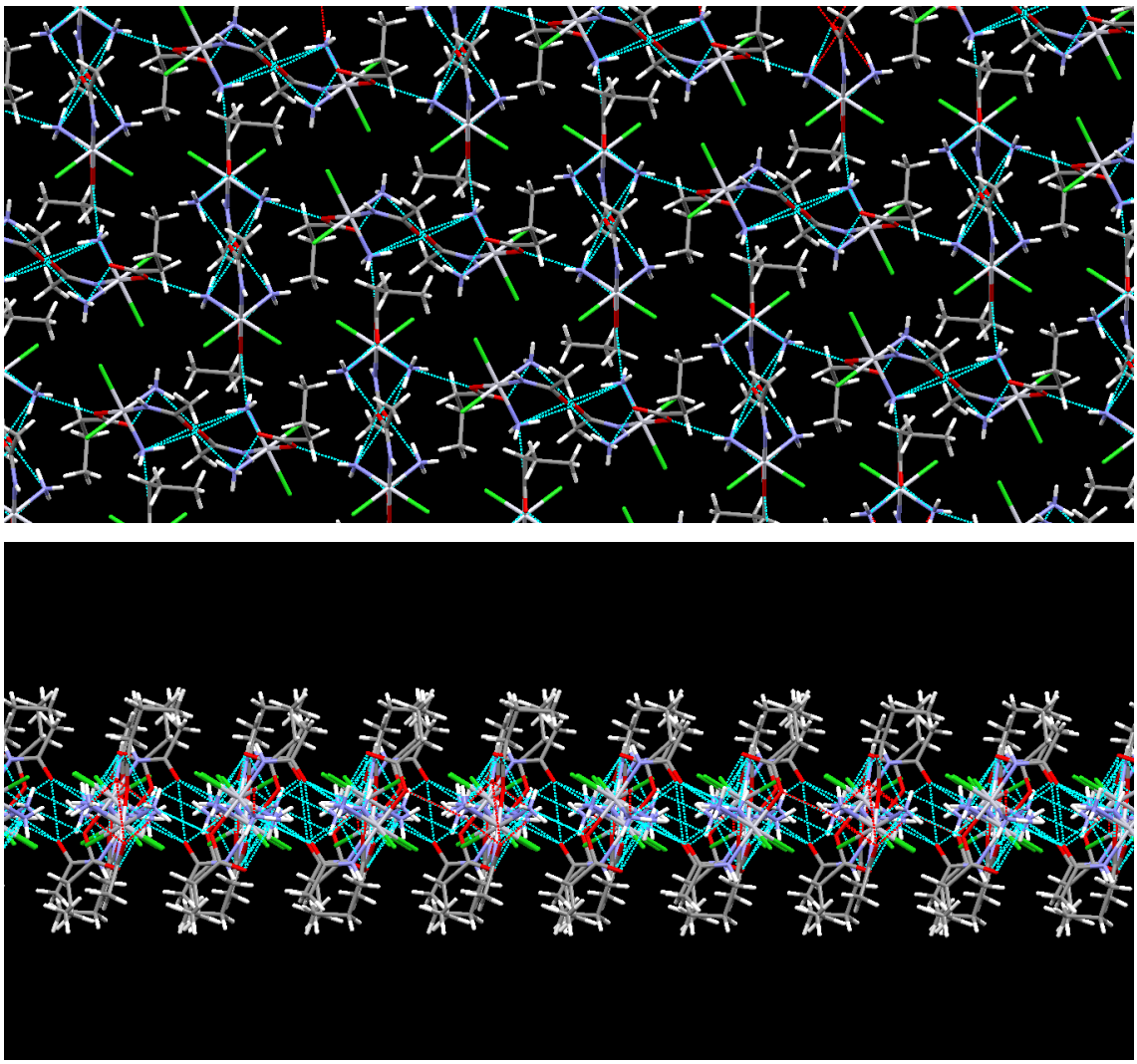

Figure S1. View of the packing looking down the *c* axis of the unit cell (above) and lateral view of the same image in which the hydrophobic tails protruding from both sides of the sheet are apparent (below).

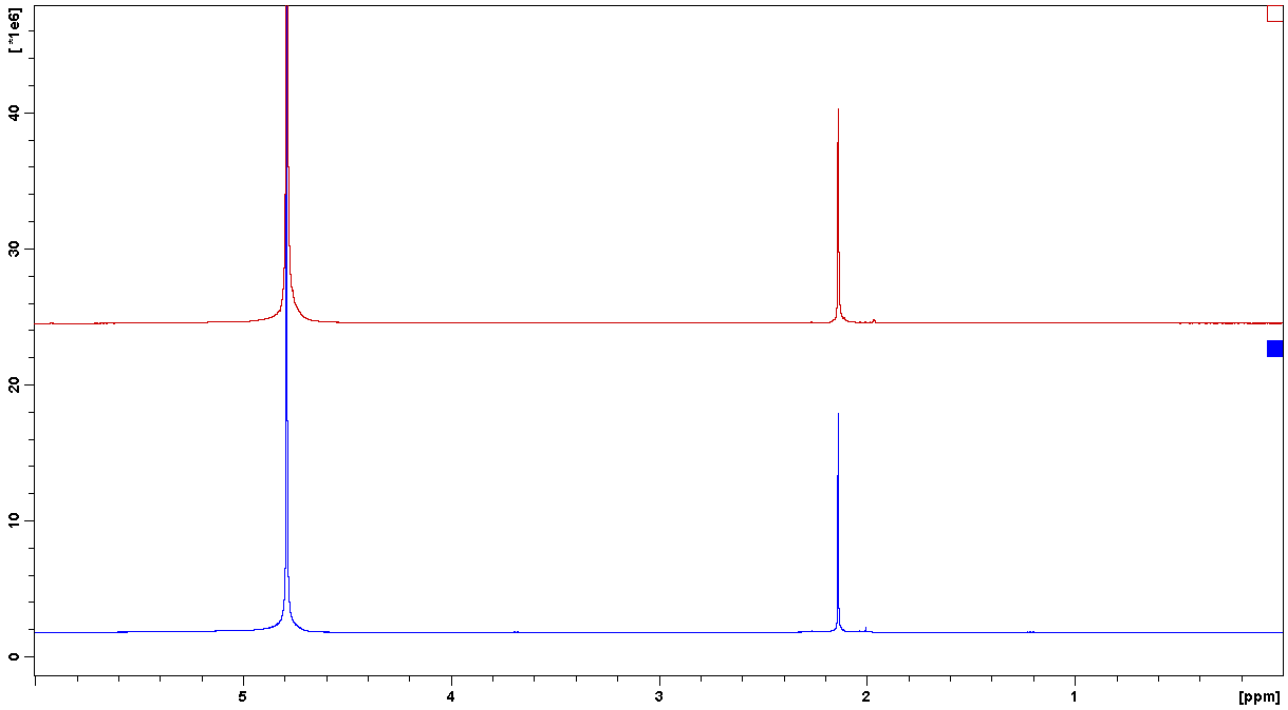

Figure S2. ^{1}H NMR spectra of **1a** (upper spectrum: fresh solution; lower spectrum: after 3 d). Solvent: phosphate buffer (PB) 100 mM in D_2O. The same results were obtained either when the samples were maintained in the dark or exposed to natural daylight cycles.

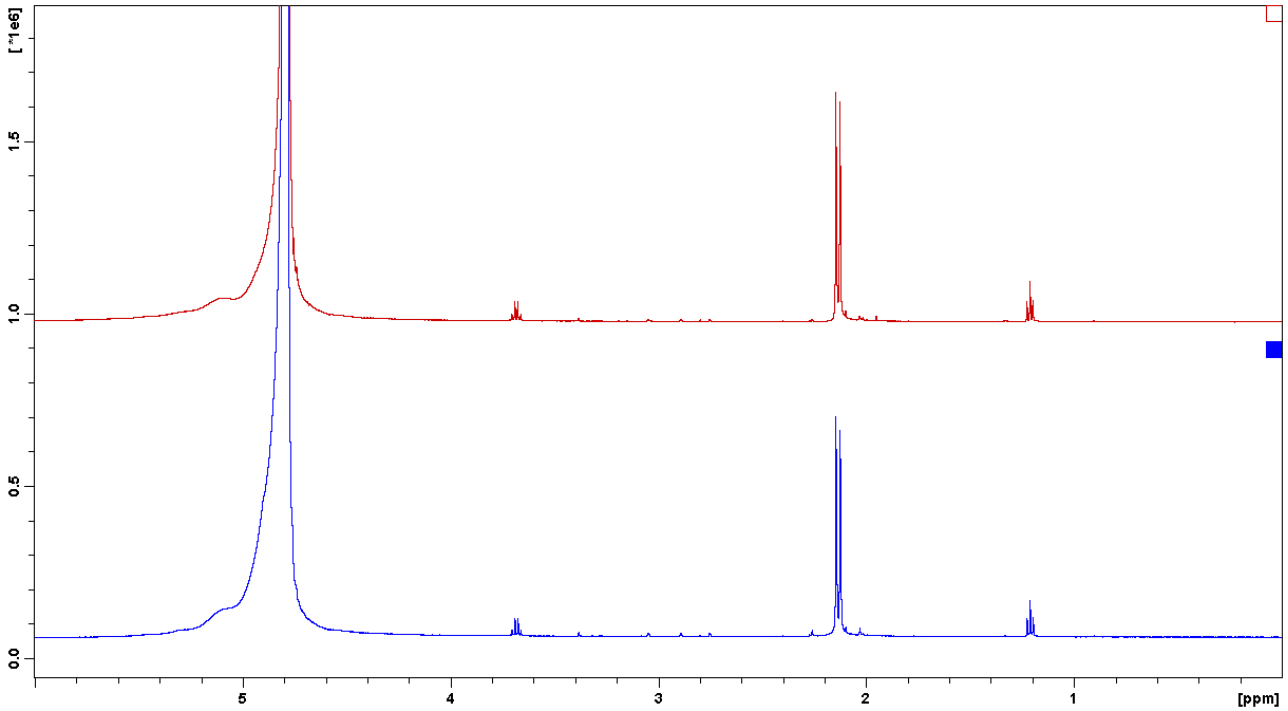

Figure S3. ^{1}H NMR spectra of **2a** (upper spectrum: fresh solution; lower spectrum: after 3 d). Solvent: PB 100 mM in D_2O. The same results were obtained either when the samples were maintained in the dark or exposed to natural daylight cycles. The signals of diethyl ether (at about 1.2 and 3.7 ppm) are also present.

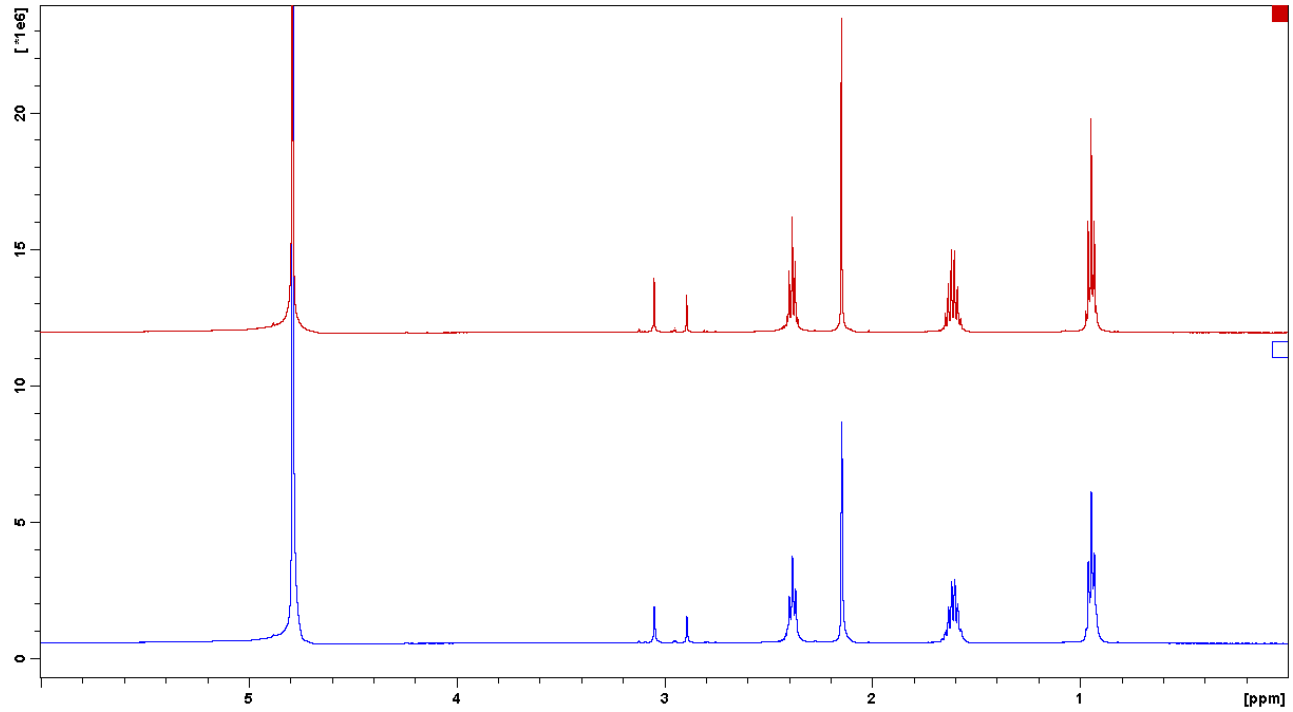

Figure S4. ^{1}H NMR spectra of **3a** (upper spectrum: fresh solution; lower spectrum: after 3 d). Solvent: PB 100 mM in D_2O. The same results were obtained either when the samples were maintained in the dark or exposed to natural daylight cycles. The signals of DMF (at about 3 ppm) are also present.

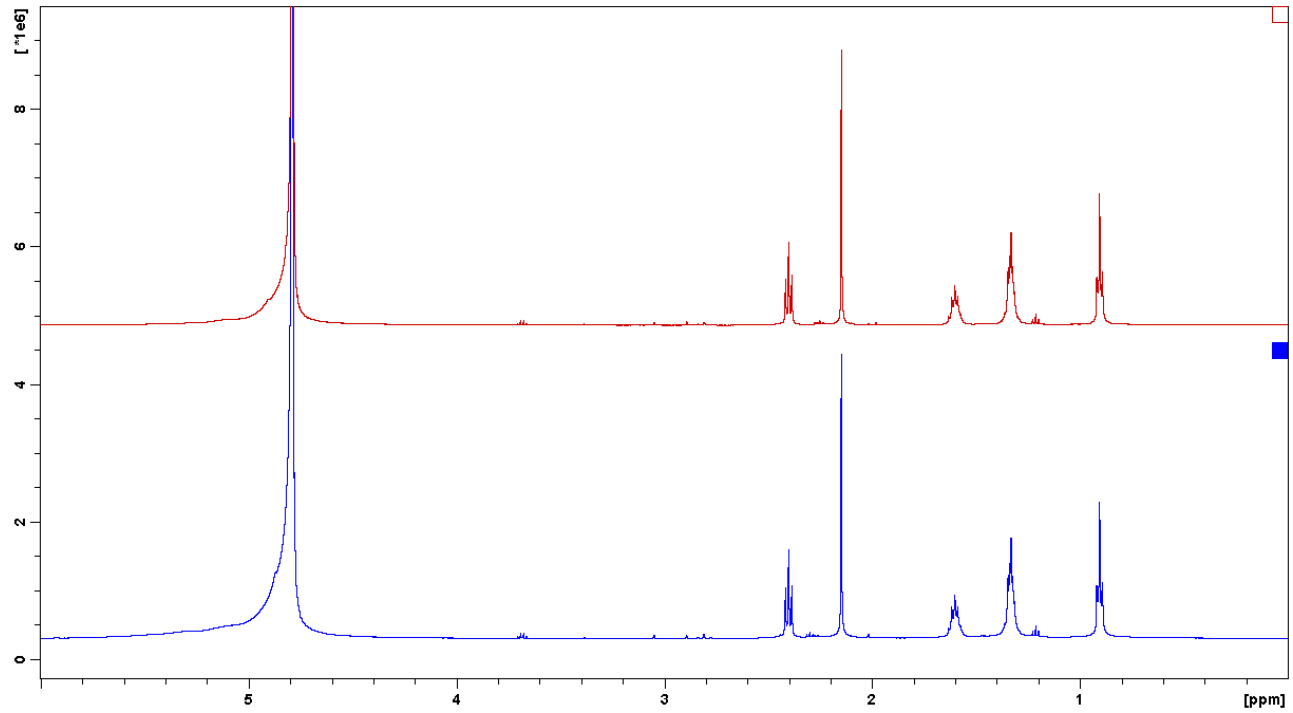

Figure S5. ^{1}H NMR spectra of **4a** (upper spectrum: fresh solution; lower spectrum: after 3 d). Solvent: PB 100 mM in D_2O. The same results were obtained either when the samples were maintained in the dark or exposed to natural daylight cycles. The signals of diethyl ether (at about 1.2 and 3.7 ppm) are also present.

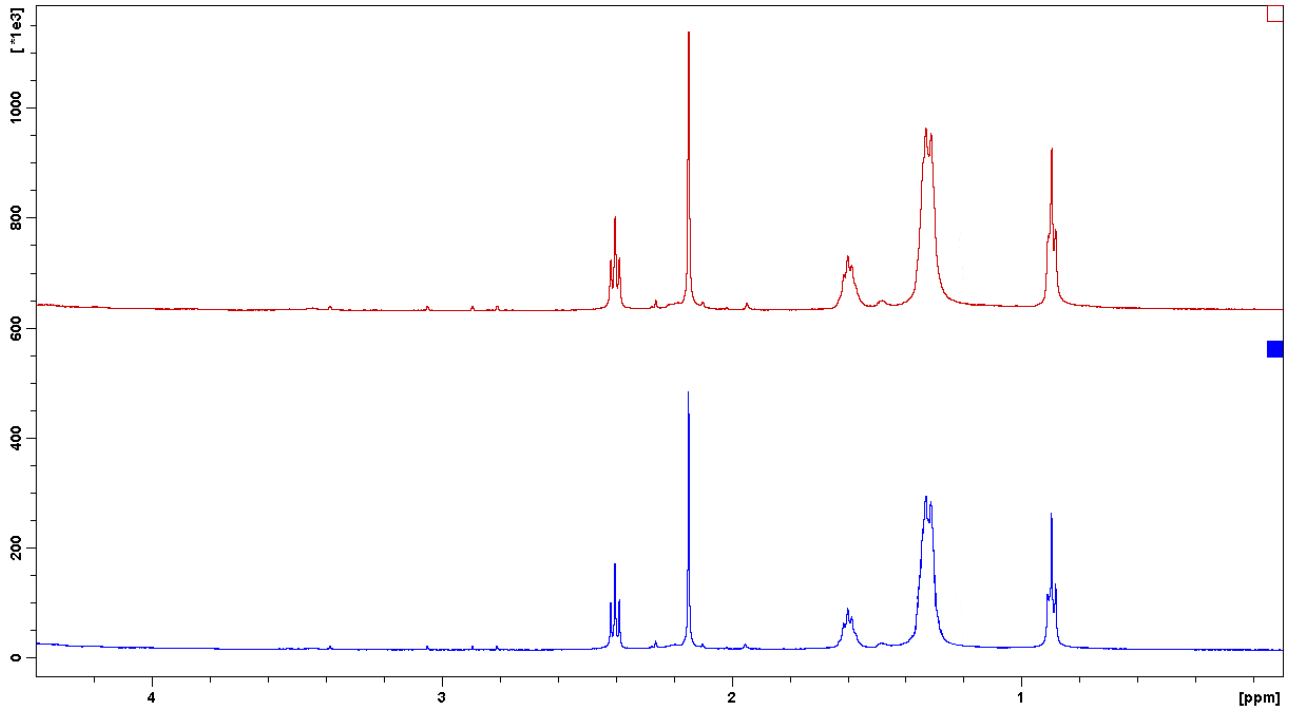

Figure S6. ^{1}H NMR spectra of **5a** (upper spectrum: fresh solution; lower spectrum: after 3 d). Solvent: PB 100 mM in D_2O. The same results were obtained either when the samples were maintained in the dark or exposed to natural daylight cycles. The signals of diethyl ether (at about 1.2 and 3.7 ppm) are also present.

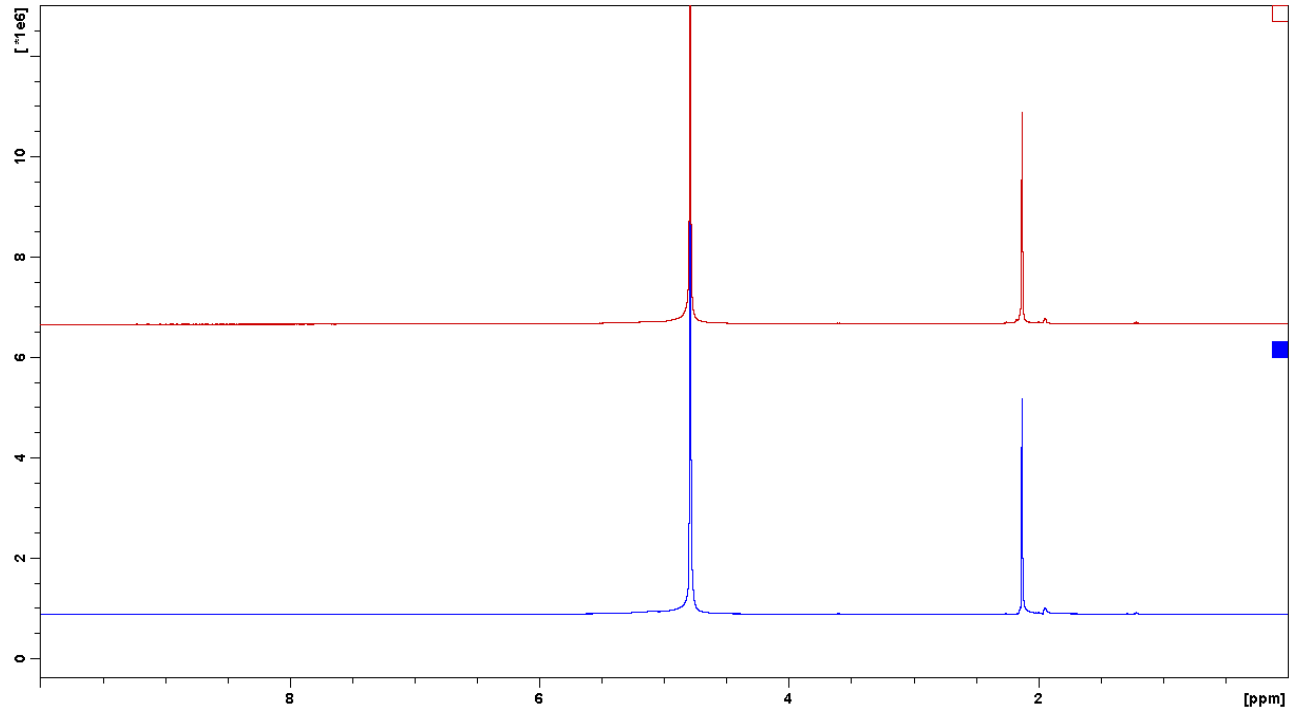

Figure S7. ^{1}H NMR spectra of **1b** maintained in the dark (upper spectrum: fresh solution; lower spectrum: after 3 d).). Solvent: PB 100 mM in D_2O.

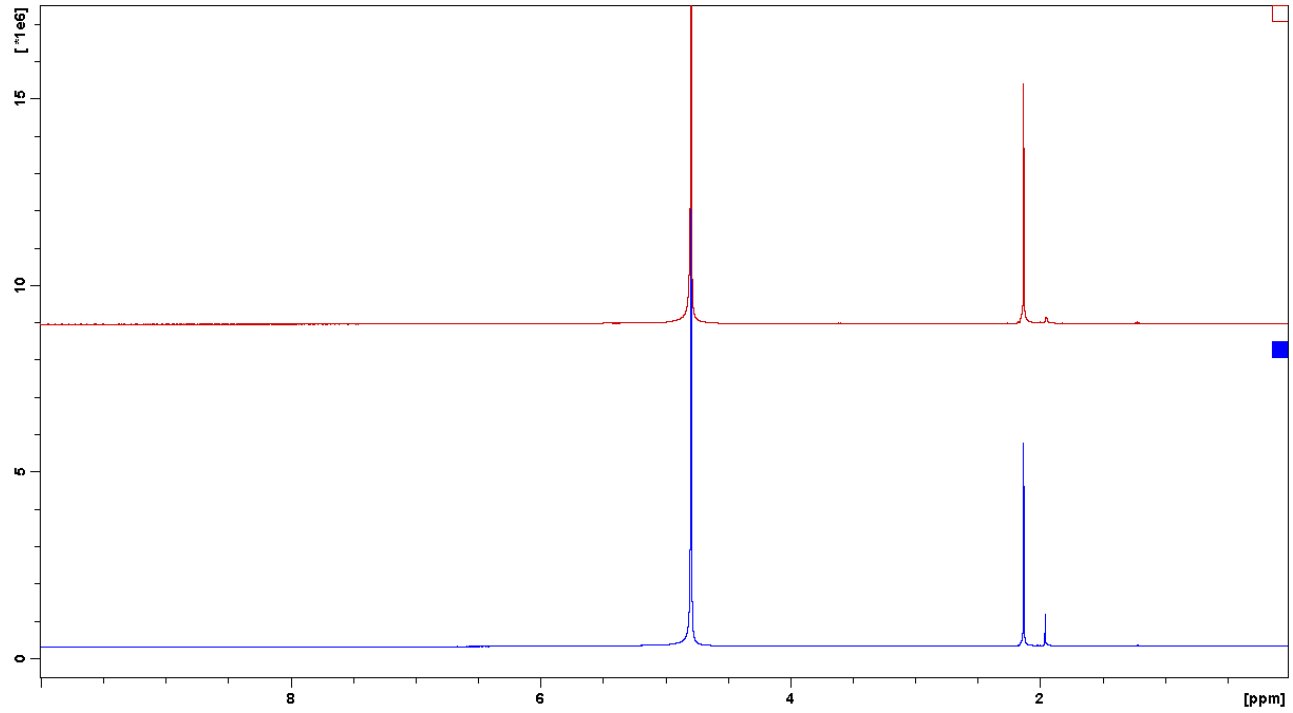

Figure S8. ^{1}H NMR spectra of **1b** exposed to natural daylight cycles (upper spectrum: fresh solution; lower spectrum: after 3 d). Solvent: PB 100 mM in D_2O.

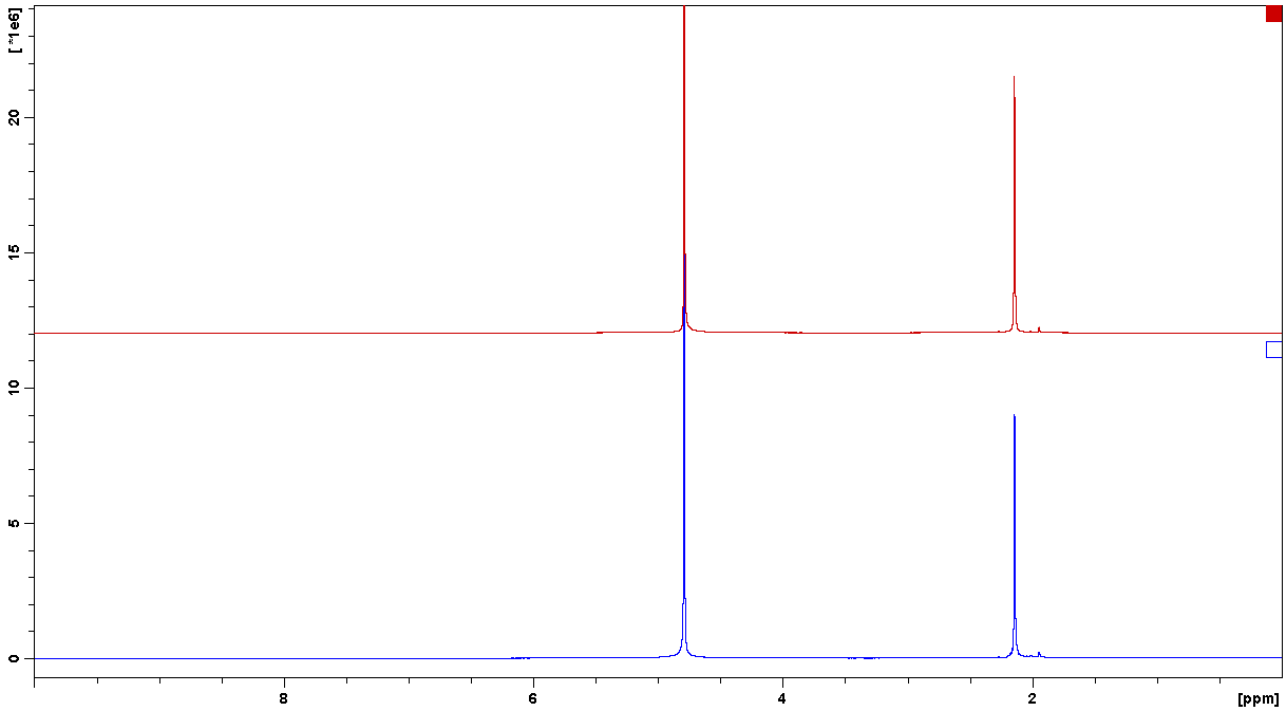

Figure S9. NMR spectra of **2b** maintained in the dark (upper spectrum: fresh solution; lower spectrum: after 3 d). Solvent: PB 100 mM in D_2O.

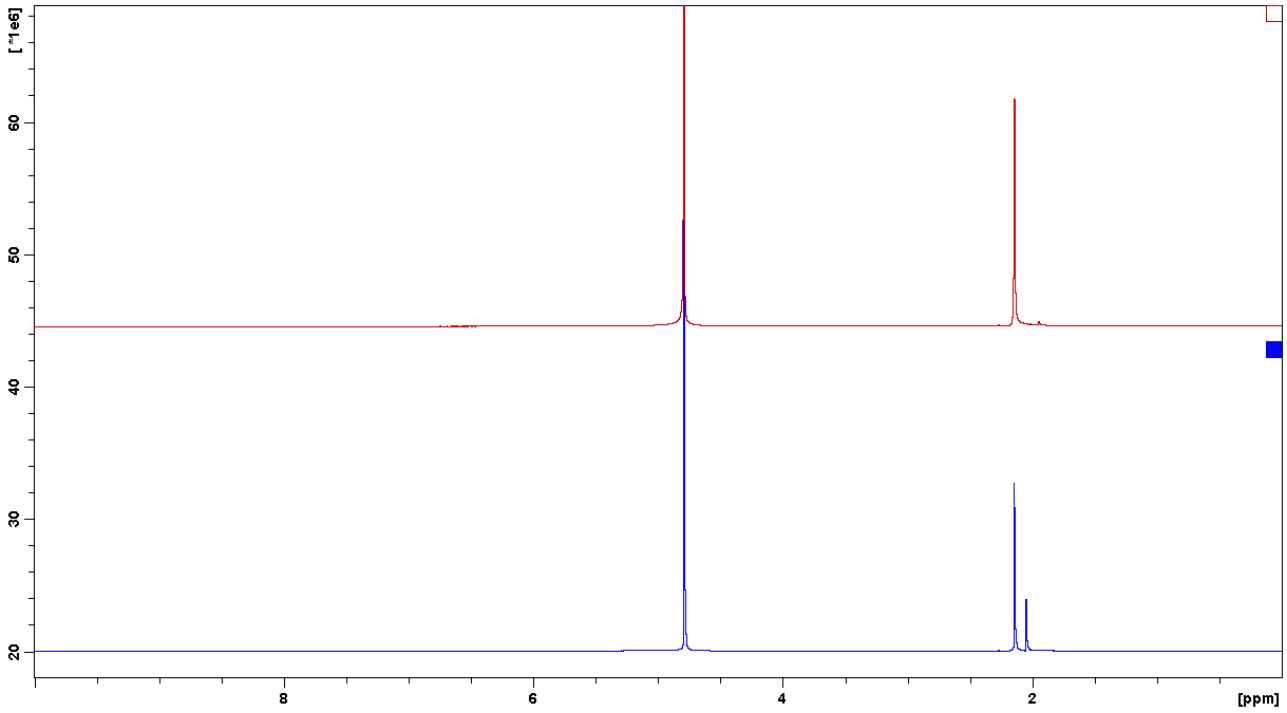

Figure S10. ^1H NMR spectra of **2b** exposed to natural daylight cycles (upper spectrum: fresh solution; lower spectrum: after 3 d). Solvent: PB 100 mM in D_2O.

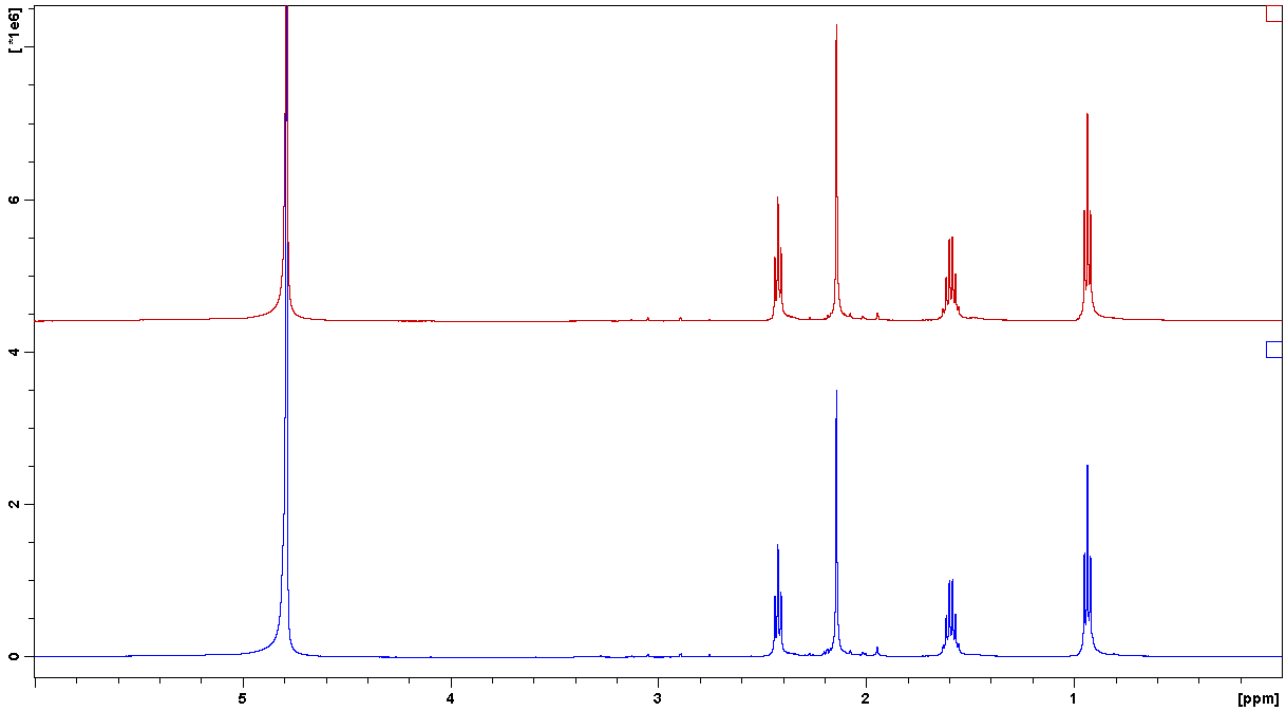

Figure S11. ^{1}H NMR spectra of **3b** (upper spectrum: fresh solution; lower spectrum: after 3 d). Solvent: PB 100 mM in D_2O. The same results were obtained either when the samples were maintained in the dark or exposed to natural daylight cycles.

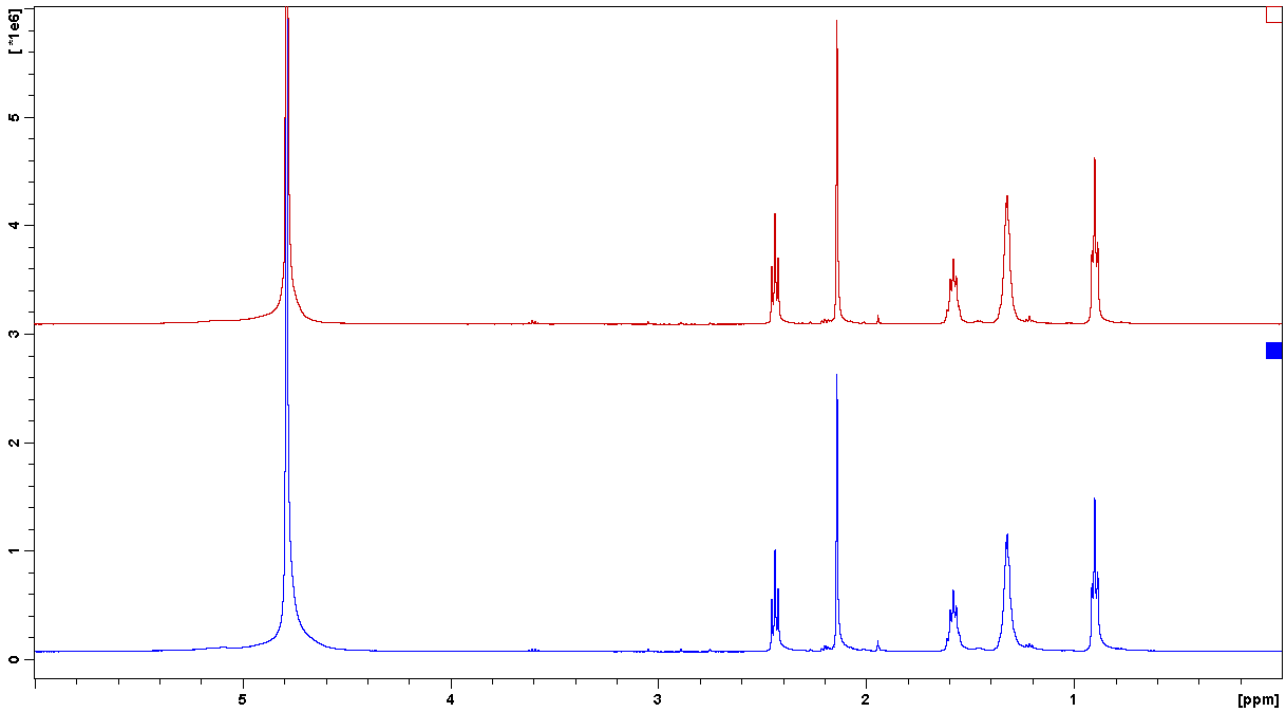

Figure S12. ^{1}H NMR spectra of **4b** (upper spectrum: fresh solution; lower spectrum: after 3 d). Solvent: PB 100 mM in D_2O. The same results were obtained either when the samples were maintained in the dark or exposed to natural daylight cycles.

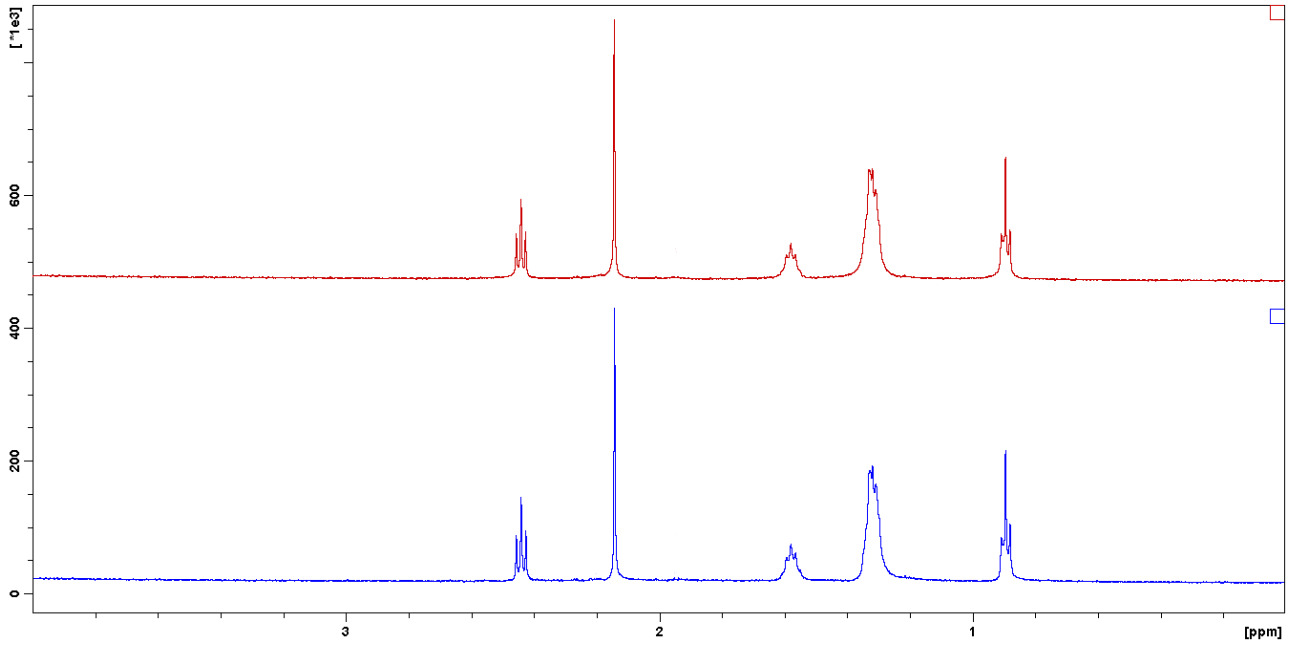

Figure S13. ^{1}H NMR spectra of **5b** (upper spectrum: fresh solution; lower spectrum: after 3 d). Solvent: PB 100 mM in D_2O. The same results were obtained either when the samples were maintained in the dark or exposed to natural daylight cycles.

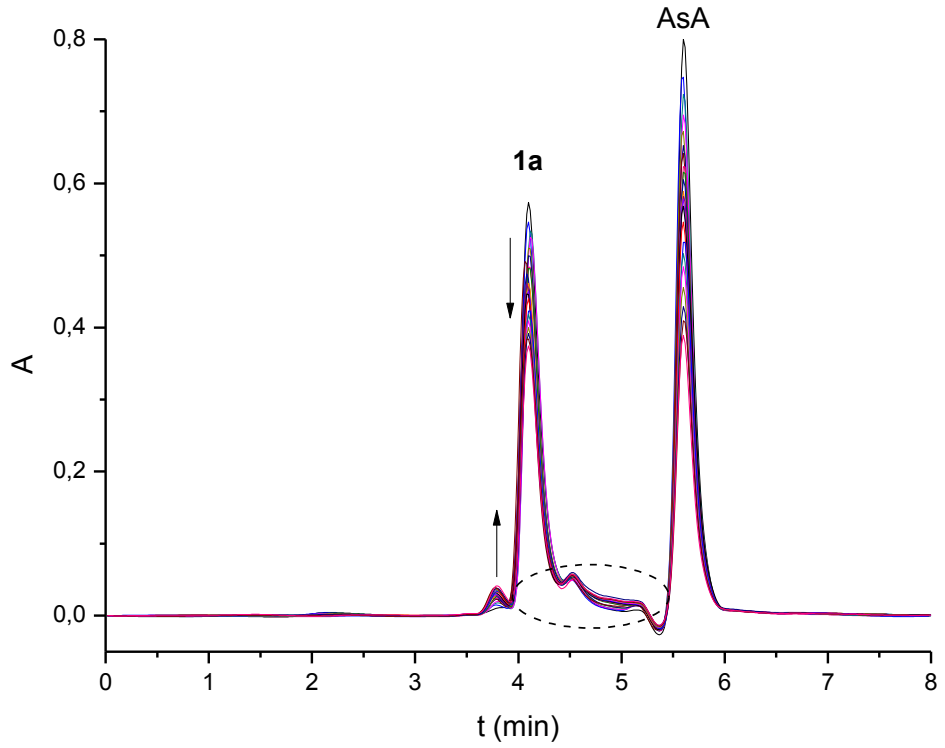

Figure S14. RP-HPLC chromatograms (eluent: 15 mM HCOOH/MeOH 70:30) of the reduction of **1a** with ascorbic acid (AsA) ([Pt] = 0.5 mM, [AsA] = 5 mM) in HEPES buffer (2 mM, pH 7.5). The peak of **1a** (4.1 min) decreases over the time, whereas a new peak at 3.8 min increases (its ESI-MS corresponds to [Pt(acetamidato-*N*)$(NH_3)_2$]$^+$ together with fragmentations of hydrolyzed cisplatin). In the region inside the circle, some different peaks overlap (HEPES buffer, cisplatin and [Pt(acetamidato-*N*)Cl$(NH_3)_2$]). The presence of this last species is confirmed by the chromatograms of the other **a** complexes, as in the case of **3a** (see Fig. S16), where this peak (4.06 min) is not overlapped to that of the original Pt(IV) complex. The ESI-MS spectrum of [Pt(acetamidato-*N*)Cl$(NH_3)_2$] is reported in Fig. S24.

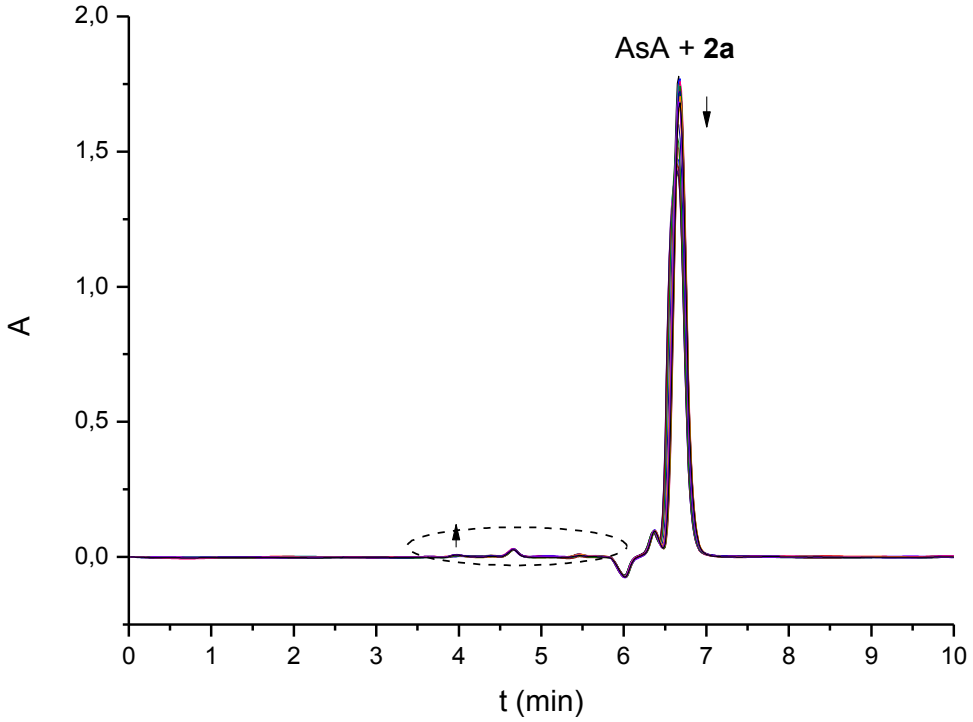

Figure S15. RP-HPLC chromatograms (eluent: 15 mM HCOOH/MeOH 90:10) of the reduction of **2a** with AsA ([Pt] = 0.5 mM, [AsA] = 5 mM) in HEPES buffer (2 mM, pH 7.5). The peak of **2a** slightly decreases but overlaps that of AsA. In the region inside the circle, some different peaks overlap (HEPES buffer, cisplatin and its hydrolyzed derivatives).

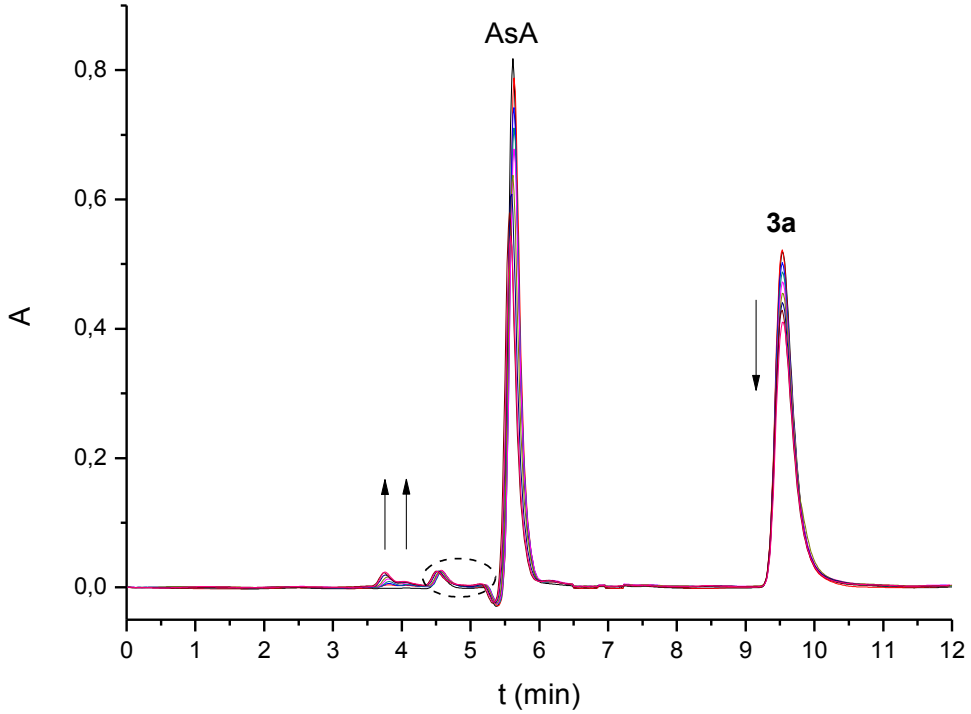

Figure S16. RP-HPLC chromatograms (eluent: 15 mM HCOOH/MeOH 70:30) of the reduction of **3a** with AsA ([Pt] = 0.5 mM, [AsA] = 5 mM) in HEPES buffer (2 mM, pH 7.5). The peak at 4.06 min, not overlapped to that of the original Pt(IV) complex (9.53 min), is compatible with [Pt(acetamidato-*N*)Cl(NH_3)$_2$], as indicated by the corresponding ESI-MS spectrum (Fig. S24). In the region inside the circle, HEPES buffer and cisplatin overlap.

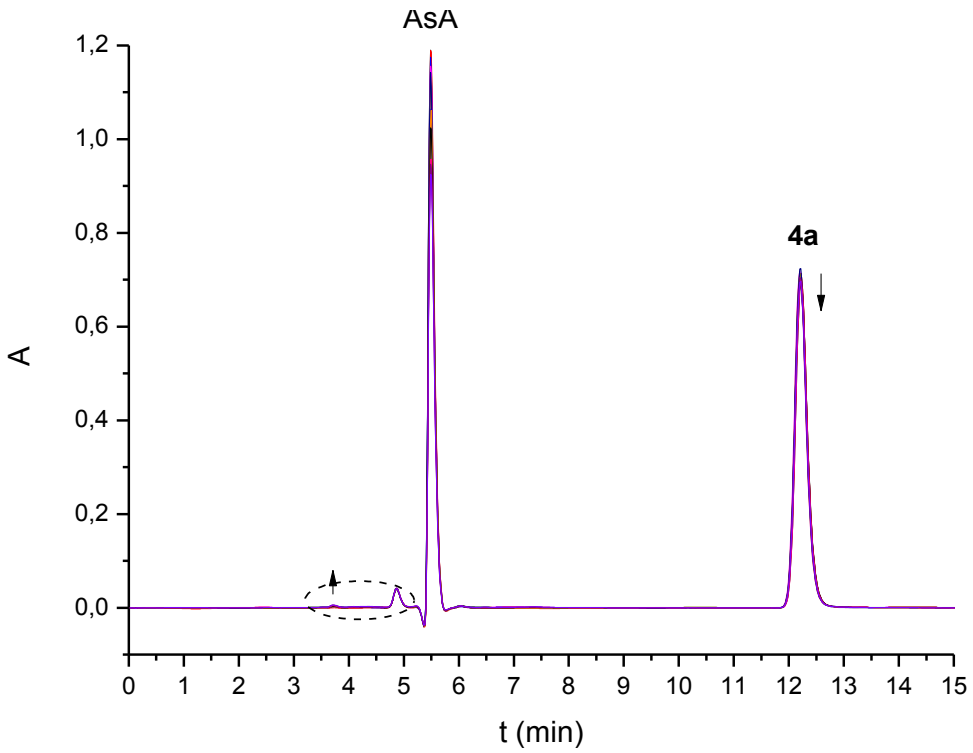

Figure S17. RP-HPLC chromatograms (eluent: 15 mM HCOOH/MeOH 50:50) of the reduction of **4a** with AsA ([Pt] = 0.5 mM, [AsA] = 5 mM) in HEPES buffer (2 mM, pH 7.5). The peak of **4a** slightly decreases over the time. In the region inside the circle, some different peaks overlap (HEPES buffer, cisplatin and its hydrolyzed derivatives).

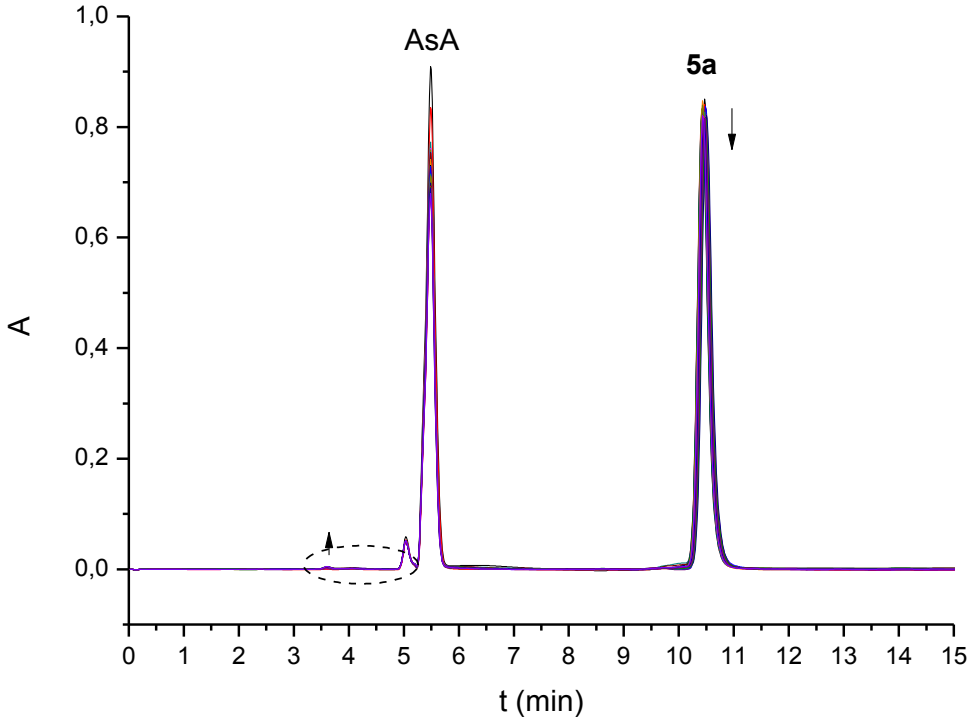

Figure S18. RP-HPLC chromatograms (eluent: 15 mM HCOOH/MeOH 30:70) of the reduction of **5a** with AsA ([Pt] = 0.5 mM, [AsA] = 5 mM) in HEPES buffer (2 mM, pH 7.5). The peak of **5a** slightly decreases over the time. In the region inside the circle, some different peaks overlap (HEPES buffer, cisplatin and its hydrolyzed derivatives).

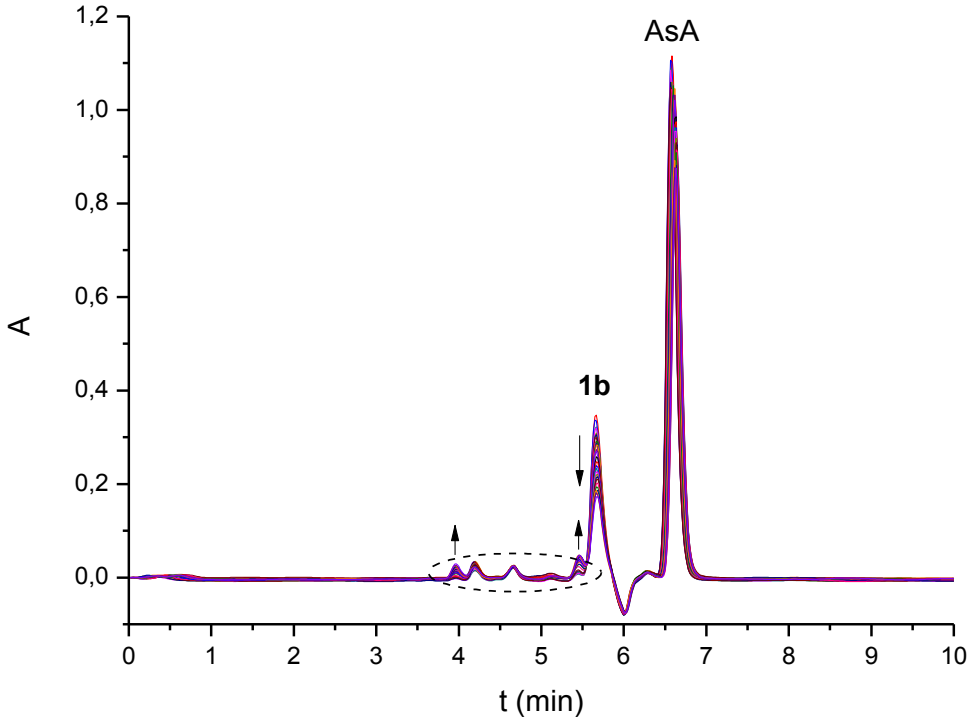

Figure S19. RP-HPLC chromatograms (eluent: 15 mM HCOOH/MeOH 90:10) of the reduction of **1b** with AsA ([Pt] = 0.5 mM, [AsA] = 5 mM) in HEPES buffer (2 mM, pH 7.5). The peak of **1b** decreases over the time, whereas new peaks increases in the region inside the circle, where some different peaks overlap (HEPES buffer, cisplatin and its hydrolyzed species).

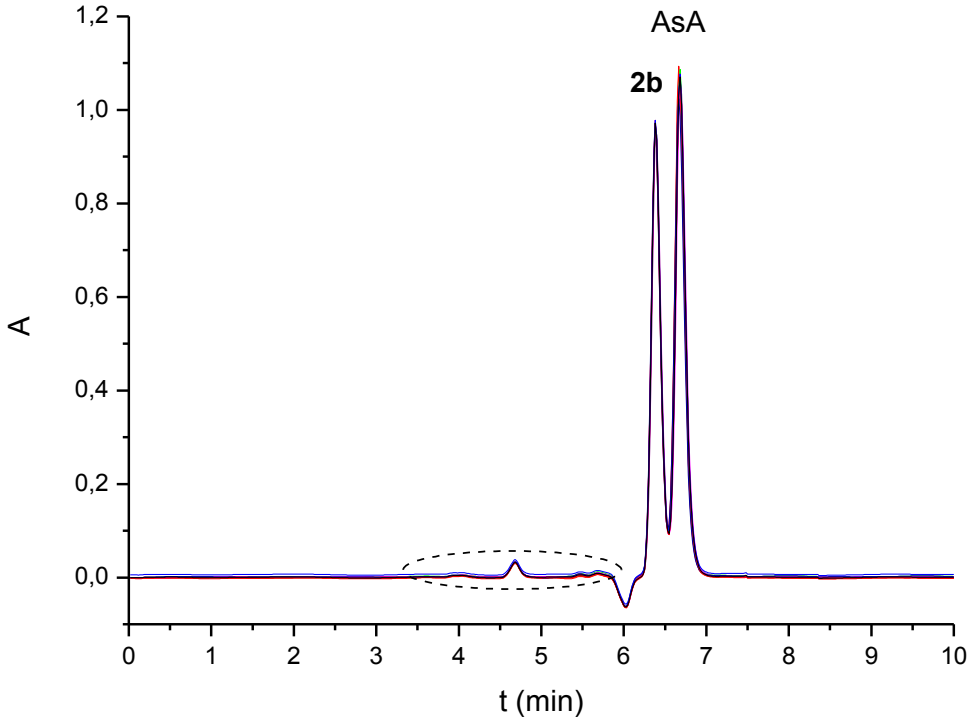

Figure S20. RP-HPLC chromatograms (eluent: 15 mM HCOOH/MeOH 90:10) of the reduction of **2b** with AsA ([Pt] = 0.5 mM, [AsA] = 5 mM) in HEPES buffer (2 mM, pH 7.5). The peak of **2b** undergoes a very little decrease over the time. In the region inside the circle, some different peaks overlap (HEPES buffer, cisplatin and its hydrolyzed derivatives).

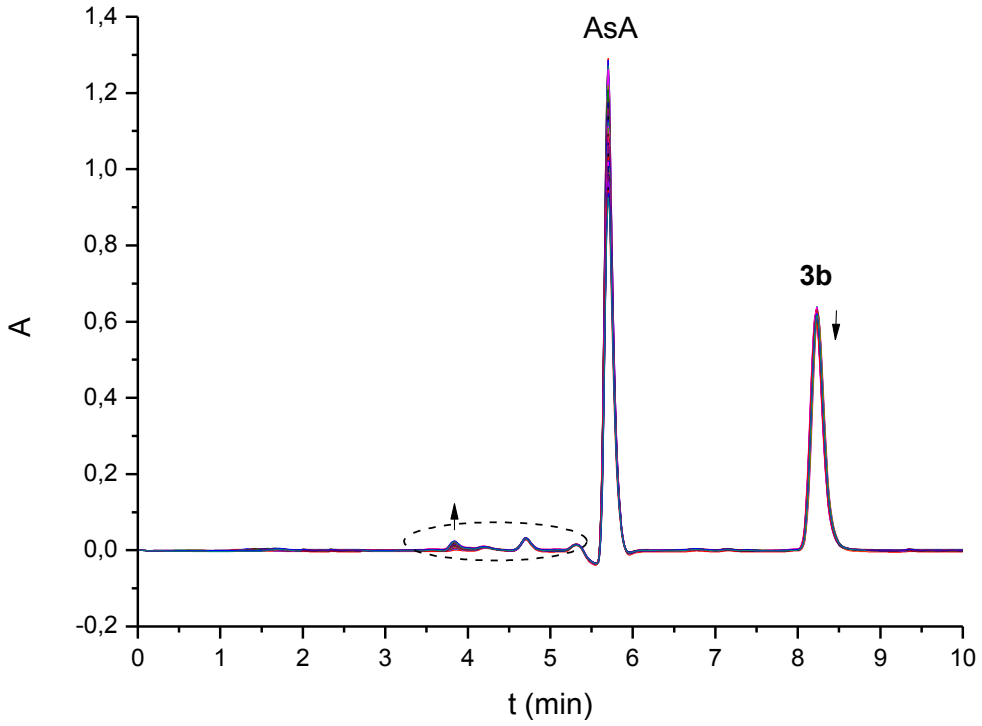

Figure S21. RP-HPLC chromatograms (eluent: 15 mM HCOOH/MeOH 70:30) of the reduction of **3b** with AsA ([Pt] = 0.5 mM, [AsA] = 5 mM) in HEPES buffer (2 mM, pH 7.5). The peak of **3b** slightly decreases over the time. In the region inside the circle, some different peaks overlap (HEPES buffer, cisplatin and its hydrolyzed derivatives).

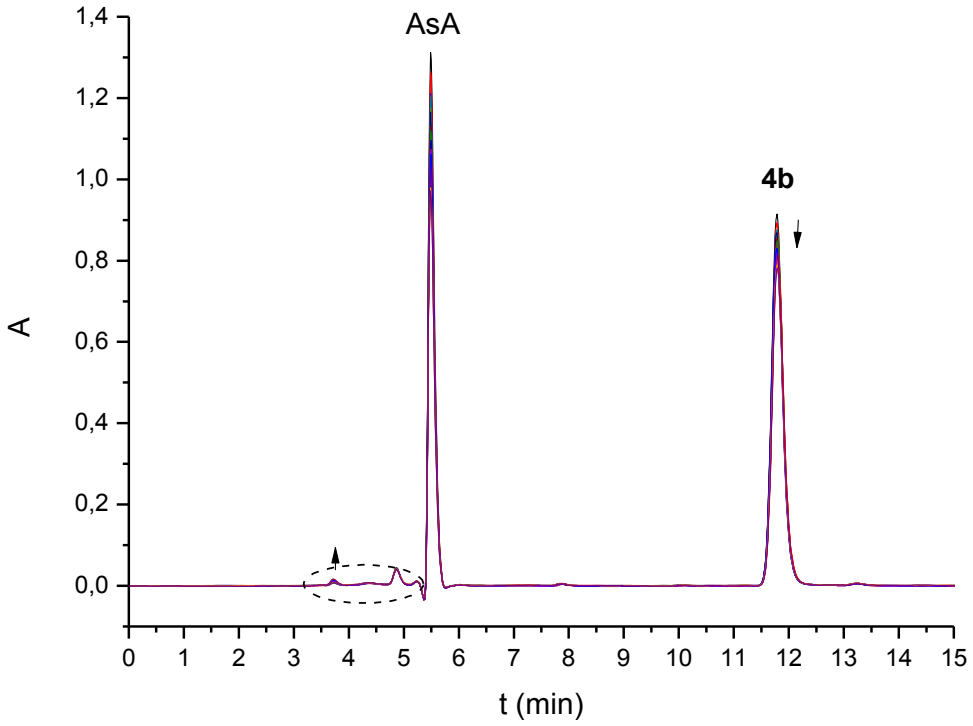

Figure S22. RP-HPLC chromatograms (eluent: 15 mM HCOOH/MeOH 50:50) of the reduction of **4b** with AsA ([Pt] = 0.5 mM, [AsA] = 5 mM) in HEPES buffer (2 mM, pH 7.5). The peak of **4b** slightly decreases over the time. In the region inside the circle, some different peaks overlap (HEPES buffer, cisplatin and its hydrolyzed derivatives).

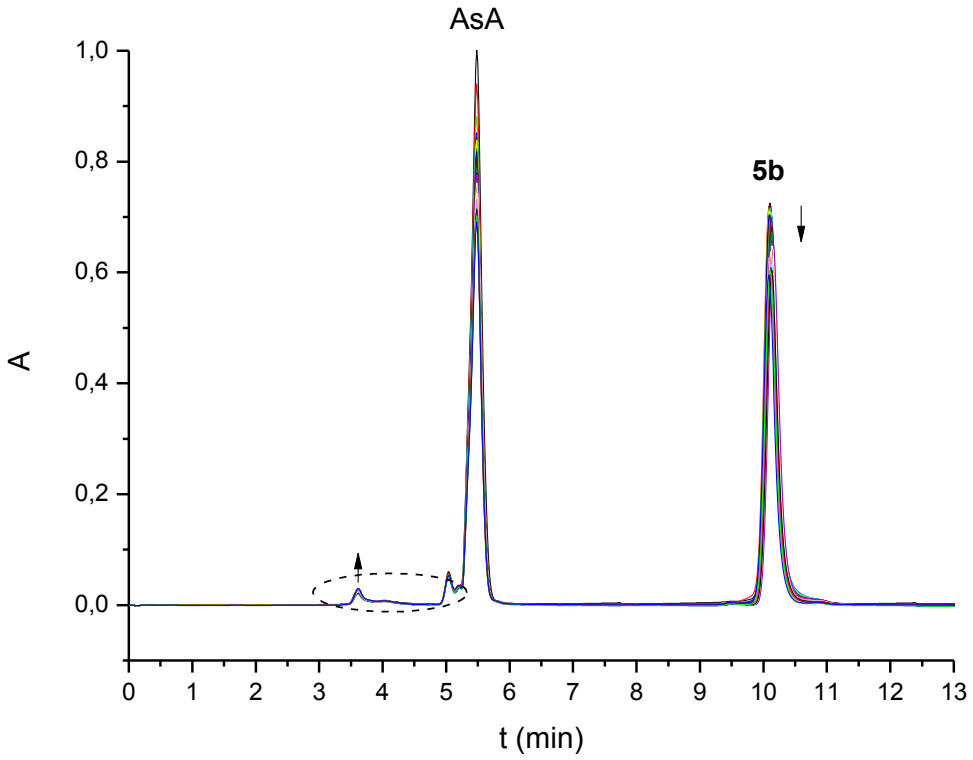

Figure S23. RP-HPLC chromatograms (eluent: 15 mM HCOOH/MeOH 30:70) of the reduction of **5b** with AsA ([Pt] = 0.5 mM, [AsA] = 5 mM) in HEPES buffer (2 mM, pH 7.5). The peak of **5b** slightly decreases over the time. In the region inside the circle, some different peaks overlap (HEPES buffer, cisplatin and its hydrolyzed derivatives).

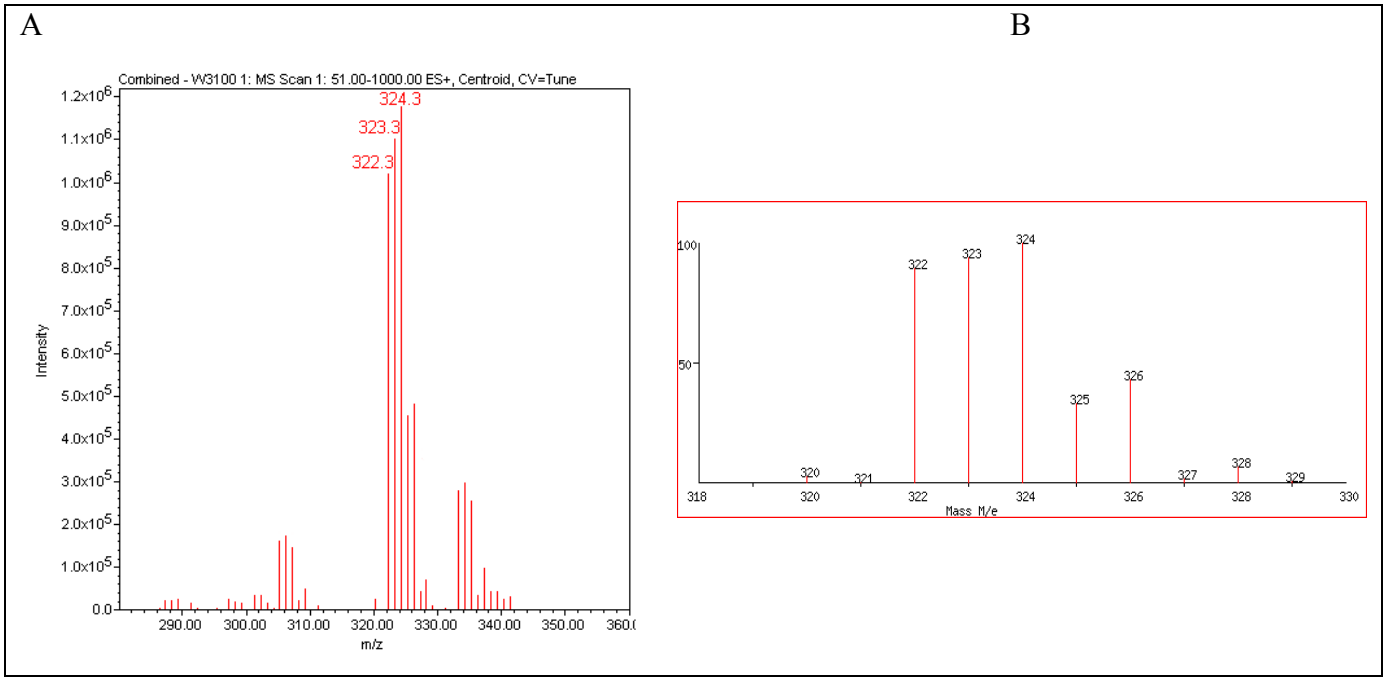

Figure S24. A) ESI-MS spectrum of [Pt(acetamidato-*N*)Cl(NH_3)$_2$] obtained as byproduct of the reduction of the **a** complexes (see Figs. S2 and S3). The spectrum shows the peak corresponding to $[M+H]^+$ at 324.3 m/z, and its fragmentations $[M-Cl+H_2O]^+$ at 306.2 m/z and $[M-Cl+HCOOH]^+$ at 334.3 m/z. B) MS simulation for $C_2H_{11}ClN_3OPt$ as $[M+H]^+$.

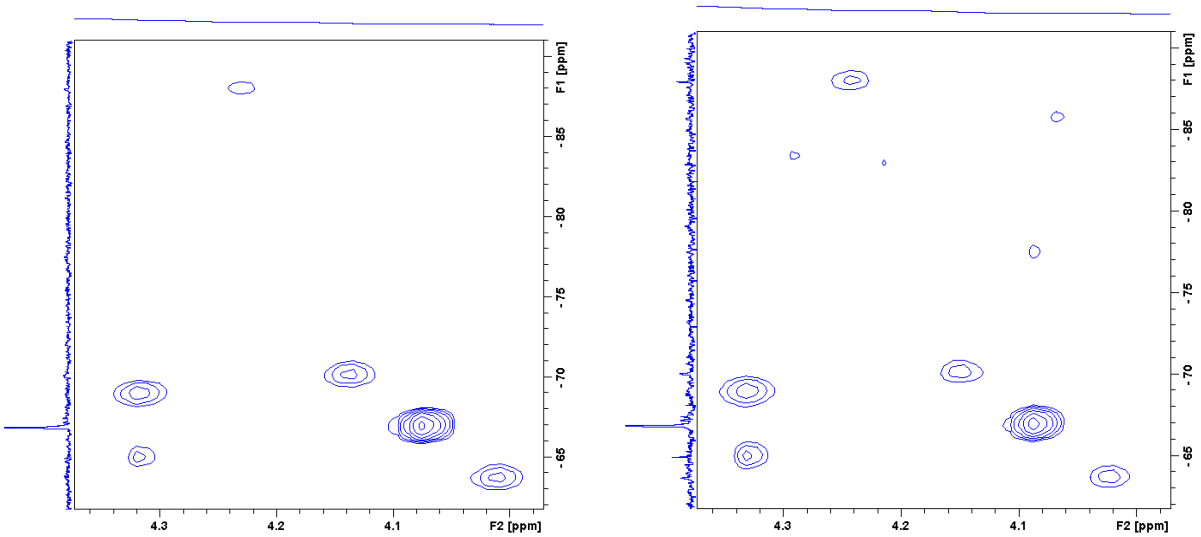

Figure S25. [^{1}H, ^{15}N] HSQC spectrum of the reduction of **1a** (20 mM) in the presence of AsA (40 mM) in 80 mM HEPES with 10% v/v D_2O and 5 mM [Cl^-] after 1 h (left) and 4 h (right) reaction time, respectively.

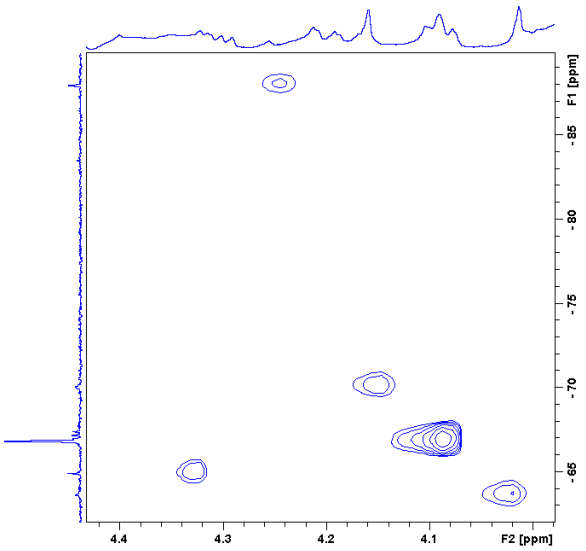

Figure S26. [^{1}H, ^{15}N] HSQC spectrum of the reduction of **1b** (20 mM) in the presence of AsA (40 mM) in 80 mM HEPES with 10% v/v D_2O and 5 mM [Cl^-] after 4 h reaction time.

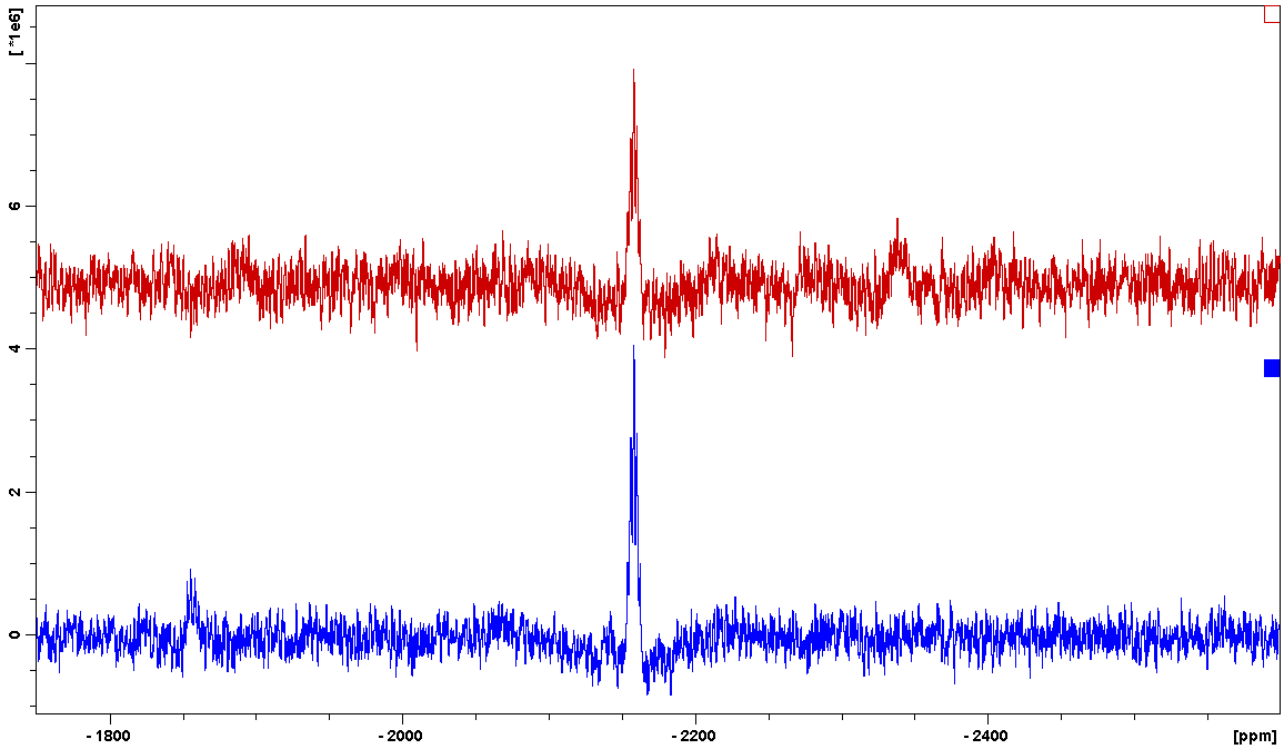

Figure S27. ^{195}Pt NMR spectra of reduction of **1a** (20 mM) in the presence of AsA (40 mM) in 80 mM HEPES with 10% v/v D_2O and 5 mM [Cl^-] after 6 h (lower spectrum) and 18 h (upper spectrum) reaction time.

Table S3. Miscellaneous experimental chemical and biological data of the complexes under investigation.

Compound	solubility [mM][a]	E_p [V][b]	t_R [min][c]	IC_{50} [μM][d]	AR[d]
cisplatin	-	-	5.2	0.48±0.11	1.40±0.57
2a	108±9	-0.660	5.4	33.1±4.7	0.77±0.21
3a	37.9±0.1	-0.688	6.8	11.8±1.9	0.63±0.15
4a	9.6±0.1	-0.681	14.4	0.20±0.02	4.44±0.11
5a	1.8±0.1	-0.702	56.8	0.04±0.01	8.47±0.94
2b	105±10	-0.486	5.5	12.1±5.2[e]	0.26±0.13
3b	27.0±0.4	-0.512	6.7	2.85±0.36	0.77±0.13
4b	8.1±0.1	-0.547	14.1	0.31±0.15	5.08±0.81
5b	3.8±0.2	-0.526	54.4	0.11±0.05	7.80±1.69

[a] The water solubility data were determined from saturated solutions of the Pt(IV) complexes in milliQ water. After 24 h stirring in the dark at 25 °C, the solid residue was filtered off (0.20 μm regenerated cellulose filters) and the Pt content of the solutions was determined by means of ICP-OES.

[b] Reduction peak potentials (E_p) were measured at a glassy carbon working electrode in ethanol solutions containing 0.1 M $[NBu_4][ClO_4]$ as supporting electrolyte. Scan rate = 0.2 V s^{-1}. All potentials are reported in V *vs.* Ag/AgCl, 3 M KCl.

[c] HPLC retention times (t_R) were measured on a C18 column, by using a mobile phase containing 15 mM aqueous HCOOH and CH_3OH in 1:1 ratio.

[d] Half-inhibitory concentrations (IC_{50}) and accumulation ratios (AR) were measured on A2780 ovarian cancer cell lines, after 72 h and 4 h of treatment, respectively.

[e] data from I. Zanellato, I. Bonarrigo, D. Colangelo, E. Gabano, M. Ravera, M. Alessio and D. Osella, *J. Inorg. Biochem.*, 2014, **140**, 219-227.

See Experimental section for more details.