Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Maleimide-functionalised platinum(IV) complexes as synthetic platform for targeted drug delivery

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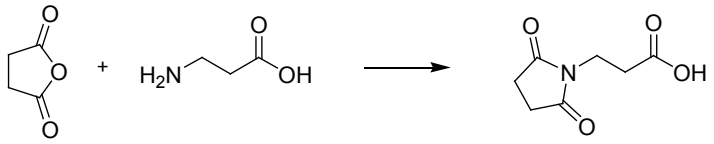
Materials and methods

K_2PtCl_4 was obtained from Johnson Matthey (Switzerland). Reverse osmosis water was distilled twice before use and absolute methanol was dried using standard procedures. All other solvents and chemicals were purchased from commercial suppliers und used without further purification. The platinum(II) starting compounds ($enPtCl_2$ and oxaliplatin) and the ligand starting material 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic acid were synthesised according to the literature.[1] Subsequently, the platinum(II) complexes were oxidised with 30% H_2O_2 due to previously published procedures.[2] ^{1}H-, ^{13}C-, ^{15}N-, and ^{195}Pt- one- and two-dimensional NMR spectra were recorded with a Bruker Avance III 500 MHz spectrometer at 500.10 (^{1}H), 125.75 (^{13}C), 50.68 (^{15}N), and 107.51 (^{195}Pt) MHz at 298 K. The solvent residual peak for ^{1}H and ^{13}C was used as internal reference, whereas ^{195}Pt chemical shifts were referenced relative to external K_2PtCl_4 and ^{15}N chemical shifts relative to external NH_4Cl. Elemental analysis measurements were carried out in the Microanalytical Laboratory of the University of Vienna on a Perkin-Elmer 2400 CHN elemental analyser. Electrospray ionization mass spectrometry (ESI-MS) was performed with a Bruker esquire$_{3000}$ instrument and preparative RP-HPLC with an Agilent 1200 Series system controlled by Chemstation software. Analytical RP-HPLC analysis was carried out with a Dionex Summit system controlled by Dionex Chromeleon 6.60 software.

Synthesis of ligands

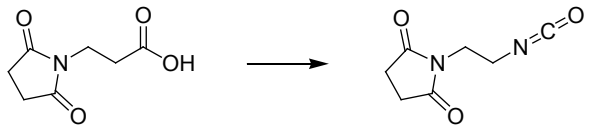
3-(2,5-Dioxopyrrolidin-1-yl)propanoic acid



3-(2,5-Dioxopyrrolidin-1-yl) propanoic acid

The compound was synthesised according to literature procedures.[3] A suspension of succinic anhydride (5.00 g, 49.96 mmol) and β-alanine (4.45 g, 49.96 mmol) in toluene (60 mL) was heated to reflux at 140°C for 3.5 h (the starting materials slowly dissolved with time). The solution was allowed to cool to RT and was stored over night at 4 °C. The solid was filtered off, washed with Et_2O, and dried under reduced pressure. The crude product was recrystallised twice in ^{i}PrOH to yield a white product. Yield: 5.86 (68%). MS (ESI^+): *m/z* 172.3 $[M + H^+]^+$. ^{1}H NMR ($CDCl_3$): δ = 3.83 (t, 2H, *J* = 7.3 Hz, NCH_2), 2.72 (s, 4H, CH_2CO), 2.68 (t, 2H, *J* =7.3 Hz, CH_2COOH) ppm.

1-(2-isocyanatoethyl)pyrrolidine-2,5-dione



3-(2,5-Dioxopyrrolidin-1-yl) propanoic acid

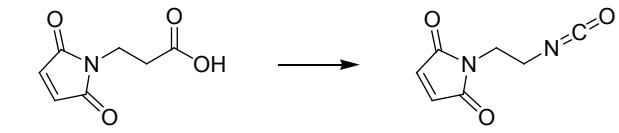
1-(2-isocyanatoethyl) pyrrolidine-2,5-dione

3-(2,5-Dioxopyrrolidin-1-yl)propanoic acid (2.68 g, 15.66 mmol) was dissolved in acetone (45 mL). The solution was cooled down with an ice/NaCl-cooling bath to approximately –5 °C. Subsequently, triethylamine (2.39 mL, 17.22 mmol) was added dropwise. Then, ethyl chloroformate (1.64 mL, 17.22 mmol) in acetone (10 mL) was added, whereby the solution gets

turbid and slightly rose-coloured. After another 10 min of stirring, NaN_3 (1.02 g, 15.66 mmol) was added and stirring was continued for 35 min. The reaction mixture was poured into H_2O (150 mL) and extracted with toluene. The combined organic layers were dried over $MgSO_4$ and heated to reflux at 140 °C for 100 min. After cooling to RT, toluene was removed under reduced pressure to yield an oily substance. The crude product was used in subsequent reactions without further purification. It was stored under argon in the fridge, where it solidified.

MS (ESI^+): *m/z* 167.1 $[M + H]^+$. ^1H NMR ($CDCl_3$): δ = 3.74 (t, 2H, *J* = 6.0 Hz, CH_2CH_2), 3.56 (t, 2H, *J* =6.0 Hz, CH_2CH_2), 2.77 (s, 4H, CH_2CO) ppm.

1-(2-Isocyanatoethyl)-1H-pyrrole-2,5-dione



3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic acid

1-(2-Isocyanatoethyl)-1H-pyrrole-2,5-dione

3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoic acid (1 g, 5.91 mmol) was dissolved in acetone (12 mL). The solution was cooled down with an ice/NaCl-cooling bath to approximately –5 °C. Subsequently, triethylamine (902 µL, 6.50 mmol) was added dropwise. Then, ethyl chloroformate (619 µL, 6.50 mmol) in acetone (5 mL) was added, whereby the solution gets turbid and slightly rose-coloured. After another 10 min of stirring, NaN_3 (384 mg, 5.91 mmol) was added and stirring was continued for 35 min. The reaction mixture was poured into H_2O (150 mL) and extracted with toluene. The combined organic layers were dried over $MgSO_4$ and heated to reflux at 140 °C for 100 min. After cooling down to RT, toluene was removed under reduced pressure to yield an oily substance. The crude product was used in subsequent reactions without further purification. It was stored under argon in the fridge, where it solidified.

MS (ESI^+): *m/z* 167.1 $[M + H]^+$. ^1H NMR ($CDCl_3$): δ = 6.76 (s, 2H, C*H*CO), 3.74 (t, 2H, *J* = 6.0 Hz, CH_2CH_2), 3.55 (t, 2H, *J* =6.0 Hz, CH$_2$CH_2) ppm.

Synthesis of platinum(IV) complexes.

(*OC*-6-33)-Dichlorido(ethane-1,2-diamine)[bis(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethylcarbamato)platinum(IV) (3a)

A suspension of (*OC*-6-33)-dichlorido(ethane-1,2-diamine)dihydroxidoplatinum(IV) (**2a**) (300 mg, 0.83 mmol) and 1-(2-isocyanatoethyl)-1H-pyrrole-2,5-dione (554 mg, 3.33 mmol) in dry DMF (4 mL) was stirred under argon atmosphere for 4 h at RT and for another 24 h at 50 °C. After the addition of another portion of 1-(2-isocyanatoethyl)-1H-pyrrole-2,5-dione (277 mg, 1.67 mmol) stirring was continued for 24 h at 50 °C. The precipitate was filtered, washed with tridest. H_2O, EtOH as well as Et_2O, and dried under reduced pressure to yield **3a** as a white to slightly grey solid.

Yield: 389 mg (67%); Elemental analysis, found % C, 27.49; H, 3.24; N, 11.96. calcd. for $C_{16}H_{22}Cl_2N_6O_8Pt$, C, 27.76; H, 3.20; N, 12.14. MS (ESI^+): *m/z* 713.9 $[M + Na^+]^+$. ^1H NMR (DMSO-d_6): δ = 8.96 (s, 4H, NH_2), 6.98 (s, 4H, =C*H*(CO)), 6.65 (s, 2H, N*H*), 3.55–3.36 (m, 4H, NCH_2), 3.14–2.97 (m, 4H, NHCH_2), 2.77–2.58 (m, 4H, H_{en}) ppm. $^{13}C\{^1H\}$ NMR (DMSO-d_6): δ = 171.5 (=CH(*C*O)), 165.4 (NH(*C*O)), 135.0 (*C*H=*C*H), 48.8 (C_{en}), 39.3 (NH*C*H$_2$), 38.0 (N*C*H$_2$) ppm. $^{15}N\{^1H\}$ NMR (DMSO-d_6): δ = 61.5 (*N*H), –1.5 (*N*H$_2$) ppm. $^{195}Pt\{^1H\}$ NMR (DMSO-d_6): 2710 (major), 2690 (minor) ppm. Two separated platinum signals were observed due to conformational isomers of the carbamate group.[4]

(*OC*-6-13)-[(1*R*,2*R*)-Cyclohexane-1,2-diamine][bis(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethylcarbamato)oxalatoplatinum(IV) (3b)

A suspension of (*OC*-6-33)-[(1*R*,2*R*)-cyclohexane-1,2-diamine]dihydroxidooxalatoplatinum(IV) **(2b)** (200 mg, 0.46 mmol) and 1-(2-isocyanatoethyl)-1H-pyrrole-2,5-dione (308 mg, 1.85 mmol) in dry DMF (3 mL) was stirred under argon atmosphere for 4 h at RT and for another 24 h at 50 °C. The slightly turbid solution was filtered and the crude product was precipitated by addition of Et_2O (200 mL). The solid was filtered, washed with Et_2O, and dried under reduced pressure. After the resolving of the greyish solid in DMSO and dilution with 3 parts of MeOH, it was purified by preparative RP-HPLC (mobile phase: aqueous 15 mM HCOOH and MeOH, solvent gradient: from 5% MeOH to 95% MeOH within 20 min, flow rate: 17 mL/min). Yield = 155 mg (44%) Elemental analysis, found % C, 33.10; H, 3.73; N, 10.27. calcd. for $C_{22}H_{28}N_6O_{12}Pt{\cdot}2H_2O$, C, 33.05; H, 4.03; N, 10.51. MS (ESI^+): *m/z* 786.1 $[M + Na^+]^+$. ^{1}H NMR (DMSO-d_6): δ = 9.53 (s, 2H, NH_2), 8.45 (s, 2H, NH_2), 6.99 (s, 4H, =C*H*(CO)), 6.80 (t, 2H, *J* = 5.3 Hz, N*H*CH_2), 3.56-3.44 (m, 2H, NHCH_2), 3.42-3.30 (m, 2H, NHCH_2), 3.19-3.06 (m, 2H, NCH_2), 3.04-2.91 (m, 2H, NCH_2), 2.72-2.57 (m, 2H, H_{chxn}), 2.26-2.11 (m, 2H, H_{chxn}), 1.62-1.47 (m, 2H, H_{chxn}), 1.47-1.32 (m, 2H, H_{chxn}), 1.32-1.17 (m, 2H, H_{chxn}) ppm. ^{13}C{^{1}H} NMR (DMSO-d_6): δ = 171.5 (=CH(*C*O)), 164.8 (NH(*C*O)), 163.6 (C_{ox}), 135.0 (*C*H=*C*H), 61.3 (*C*H_{chxn}), 39.5 (NH*C*H_2), 37.7 (N*C*H_2), 31.5 (*C*$H_{2,chxn}$), 24.0 (*C*$H_{2,chxn}$) ppm. ^{15}N{^{1}H} NMR (DMSO-d_6): δ = 60.9 (*N*H), –5.8 (*N*H_2) ppm. ^{195}Pt{^{1}H} NMR (DMSO-d_6): 3234 (major), 3225 (minor) ppm.

(*OC*-6-13)-[(1*R*,2*R*)-Cyclohexane-1,2-diamine][bis(2-(2,5-dioxopyrrolidin-1-yl)ethylcarbamato)oxalatoplatinum(IV) (4b)

A suspension of (*OC*-6-33)-[(1*R*,2*R*)-cyclohexane-1,2-diamine]dihydroxidooxalatoplatinum(IV) (**2b**) (200 mg, 0.46 mmol) and 1-(2-isocyanatoethyl)pyrrolidine-2,5-dione (312 mg, 1.85 mmol) in dry DMF (3 mL) was stirred under argon atmosphere for 24 h at RT, until the reaction mixture became clear. The crude product was precipitated by addition of Et_2O (200 mL), which was filtered, washed with Et_2O, and dried under reduced pressure. After the resolving of the solid in DMSO and dilution with 3 parts of MeOH, it was purified by preparative RP-HPLC (mobile phase: aqueous 15 mM HCOOH and MeOH, solvent gradient: from 5% MeOH to 95% MeOH within 20 min, flow rate: 17 ml/min). Yield: 187 mg (53%); Elemental analysis, found % C, 33.34; H, 4.19; N, 10.45; O, 26.13. calcd. for $C_{22}H_{32}N_6O_{12}Pt{\cdot}H_2O$, C, 33.63; H, 4.36; N, 10.70; O, 26.48. MS (ESI^+): *m/z* 790.2 $[M + Na^+]^+$. ^1H NMR (DMSO-d_6): δ = 9.62-9.23 (m, 2H, NH_2), 8.60-8.30 (m, 2H, NH_2), 6.89-6.70 (m, 2H, NHCH$_2$), 3.52-3.41 (m, 2H, NCH_2), 3.38-3.26 (m, 2H, NCH_2), 3.15-2.95 (m, 4H, NHCH_2), 2.75-2.62 (m, 2H, H_{chxn}), 2.62-2.55 (m, 8H, CH_2), 2.25-2.10 (m, 2H, H_{chxn}), 1.59-1.47 (m, 2H, H_{chxn}), 1.47-1.33 (m, 2H, H_{chxn}), 1.32-1.17 (m, 2H, H_{chxn}) ppm. $^{13}C\{^1H\}$ NMR (DMSO-d_6): δ = 178.2 (CH$_2$(*C*O)), 164.7 (NH(*C*O)), 163.6 (C_{ox}), 61.3 (*C*H$_{chxn}$), 38.6 (NH*C*H$_2$), 38.4 (N*C*H$_2$), 31.5 (*C*H$_{2,chxn}$), 28.5 ((*C*H$_2$)$_2$(CO)), 24.0 (*C*H$_{2,chxn}$) ppm. $^{15}N\{^1H\}$ NMR (DMSO-d_6): δ = 60.9 (*N*H), –5.2 (*N*H$_2$) ppm. $^{195}Pt\{^1H\}$ NMR (DMSO-d_6): 3235 (major), 3224 (minor) ppm.

Crystallographic Structure Determination

X-ray diffraction measurements of **3a** were performed with a Bruker X8 APEXII CCD diffractometer at 150 K. Single crystals were positioned at 35 mm from the detector and 565 frames were measured each for 60 s over 1° scan width. The data were processed using SAINT software.[5] Crystal data, data collection parameters, and structure refinement details are given in Table S1. The structure was solved by direct methods and refined by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters. H atoms were inserted at calculated positions and refined with a riding model. Structure solution was achieved with SHELXS-97, refinement with SHELXL-97,[6] and graphics were produced with ORTEP-3.[7] Crystallographic data for the structural analysis of **3a** has been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 916782. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

RP-HPLC studies

DMSO stocks of the test compounds **3a**, **3b,** and **4b** were serially diluted in Milli-Q water, such that the maximum DMSO content did not exceed 3%. For hydrolysis studies, solutions of complex **3a**, **3b,** and **4b** (0.32 mM) were measured for 24 h. For binding studies with cysteine (Fluka), mixtures of **3a**, **3b,** or **4b** (final concentration: 0.40 mM) and cysteine (final concentration: 2.40 mM) in H_2O were measured in a 1:6 molar ratio (Pt/cysteine = 1:3 maleimid/cysteine). For albumin-binding studies, solutions of **3a** or **3b** (0.35 mM) and albumin (0.70 mM, Sigma-Aldrich) in H_2O were measured. The experimental conditions for the chromatographic system were as follows: stationary phase: silica-based C18 gel (XBridge C18 5

µm, 4.6 mm x 150 mm); flow rate: 1.00 mL/min, injection volume: 25 µL; column temperature: 25 °C; UV-vis detection: 225 nm, 250 nm, 275 nm and 300 nm.

SEC-ICP-MS measurements

SEC-HPLC-ICP-QMS: The analysis via SEC-HPLC-ICP-QMS was carried out on a Perkin Elmer Elan DRC II with a Perkin Elmer Series 200 HPLC system. For separation by size exclusion chromatography the column Waters Biosuite 125 4 µm UHR SEC, 4.6 x 300 mm was used. The conditions were set to a flow of 300 µL min^{-1} in Tris buffer (20 mM, pH 7.3) containing 150 mM NaCl. Injection Volume was 10 µL (partial loop injection 20 µL).

Recorded Masses: SO (^{48}) Pt (^{195})

FI-ICP-QMS: Calibration of platinum and sulfur was performed by flow injection of inorganic platinum and sulfur standards. To assess the total amount of platinum and sulfur in the samples, flow injections were carried out with the same conditions as at the HPLC separation.

Sample setup: A protein/amino acid mix was prepared to assess the separation performance of the applied SEC-HPLC setup. The mix contained HSA, superoxide dismutase (SOD), GSH, and methionine. It has to be mentioned that HSA is forming dimers over time. Thus, the mass of the dimer was also taken into account.

The drugs were diluted in 150 mM NaCl and then mixed with fetal calf serum (FCS) to receive incubations with a final drug concentration of 250 µM and 50 µM. The prepared samples were then incubated at 37 °C. The samples were diluted (1:100 or 1:1000) in 150 mM NaCl prior to injection.

Result size ladder:

Compound	Time (min)	kDa
Albumin Dimer	7.9	132
Albumin	8.7	66
SOD	9.54	37
GSH	12.3	0.3
Methionine	13	0.1

In vivo investigations

Cell culture. For *in vivo* experiments, the murine colon cancer cell model CT-26 (purchased from American Type Culture Collection, Manassas, VA, USA) was used. Cells were grown in DMEM/F12 (1:1) supplemented with 10% FCS. All cell culture media and reagents were purchased from Sigma-Aldrich Austria.

Animals. Six- to eight-week-old female Balb/c mice were purchased from Harlan Laboratories (San Pietro al Natisone, Italy). The animals were kept in a pathogen-free environment and every procedure was done in a laminar airflow cabinet. Experiments were carried out according to the Austrian and FELASA guidelines for animal care and protection.

Xenograft experiments. CT-26 cells ($5x10^5$ in serum-free DMEM medium) were injected subcutaneously into the right flank. Therapy was started when tumour nodules were palpable (day 5). Animals were treated with **3a** and **3b** (18 mg/kg i.v. dissolved in 0.9% NaCl on day 5, 8, and 12 which is corresponding to an equivalent oxaliplatin dose of 9 mg/kg). Animals were controlled for distress development every day and tumour size was assessed regularly by caliper measurement. Tumour volume was calculated using the formula: (length × $width^2$) / 2.

Concentration-dependent HSA binding studies

HSA (A1653), 2,2'-dithiodipyridine (2,2'-DTDP), cysteine (Cys), GSH, NaH_2PO_4, Na_2HPO_4, CH_3COONa, and NaCl were obtained from Sigma-Aldrich in puriss quality. Doubly distilled Milli-Q water was used for sample preparations. The HSA solution was freshly prepared before the experiments and the concentration was estimated from the UV absorption: $\varepsilon_{280\ nm}$ = 36850 $M^{-1}cm^{-1}$.[8] A Hewlett Packard 8452A spectrophotometer was used to record the UV-vis spectra in the region of 200−700 nm at 25 °C and a path length of 1 cm. Samples containing 100 μM HSA (or 50 μM Cys or 50 μM GSH) and 2,2'-DTDP (from 5 to 120 μM) in 100 mM phosphate buffer (0.10 M NaCl; pH 7.00 at 25.0 ± 0.1 °C) were incubated for 40 min (or 15 and 30 min in the case of Cys and GSH, respectively) prior to the UV-vis measurements. Thiol content of 100 μM HSA was determined in the presence of the platinum(IV) complexes (1–50 μM concentrations) in 20 mM phosphate buffer (0.10 M NaCl; pH 7.40 at 25.0 ± 0.1 °C, 2% (v/v) DMSO) and samples were incubated for 4 h prior to the addition of the 2,2'-DTDP in 100 μM concentration and the UV-Vis spectra were recorded after 40 min.

Pre-investigations for the concentration-dependent HSA binding studies:

It should be mentioned that ca. 20–30% of the cysteine (Cys)-34 thiol groups of HSA are oxidized in the human blood. Mainly disulfide bridged species are formed with small sulphydryl compounds such as Cys, GSH, and homocysteine.[9] The commercially available HSA usually differs somewhat from the native protein considering its oxidation state, since during the recovering and storage a partial oxidation of the Cys-34 thiol group can take place. Therefore, the actual oxidation state of the HSA used for the in vitro studies has to be determined prior to the characterisation of its maleimide binding properties. Reaction of the maleimide moiety of the

platinum(IV) complexes with the thiol group of Cys-34 of HSA results in formation of the thiosuccinimidyl linkage and a decrease in the free thiol content of the protein. Thus, the same method can be used in order to quantify the sulfhydryl groups of HSA in the absence or in the presence of the platinum(IV) complexes.

The concentration of the free thiol group of Cys-34 of the protein was determined via reaction with 2,2'-dithiodipyridine (2,2'-DTDP) by spectrophotometry using a slightly modified approach from that described in the literature.[10,11] Reaction of the thiols results in the stoichiometric formation of 2-thiopyridone (2-TP, Fig. Aa) after a suitable incubation time, which was found to be 40 min for complete reaction between HSA and 2,2'-DTDP. The molar ratio method was used to determine the stoichiometry of the reaction corresponding to the concentration of the thiol groups. The protein concentration was kept constant and the HSA-to-2,2'-DTDP ratio was varied. Then, the absorbance values of the generated 2-TP were measured and plotted against the concentrations of the reagent (Fig. B). The curvature exhibits a sharp break and the tangents drawn give the intersection corresponding to the stoichiometry (1:1). The intersection was found at ca. 0.24 HSA-to-2,2'-DTDP ratio representing 24 ± 2% free SH groups of the HSA used.

At first, the time-dependent 2-TP formation for the reaction of 2,2'-DTDP with Cys or GSH at two different pH-values was recorded with UV-vis spectrometry at 340 nm. In addition, the binding of Cys and GSH at constant concentration and varied 2,2'-DTDP concentrations were investigated. For each thiol (Cys or GSH) both steps of the reaction (Fig. Aa) take place within ca. 15 min at pH 7 (at pH 4 the reaction is much slower, Fig. Ab for Cys). In contrast, only the first step is completed with HSA and only the intermediate mixed-disulfide (protein-S-S-pyridine) is formed according to the slope of the absorbance-concentration plot at high excess HSA-to-2,2'-DTDP (Fig. Ac), which is just the half of the slope obtained with Cys or GSH. This

behaviour of the high molecular mass HSA reveals the hindered formation of the disulfide dimer most probably due to steric hindrance.

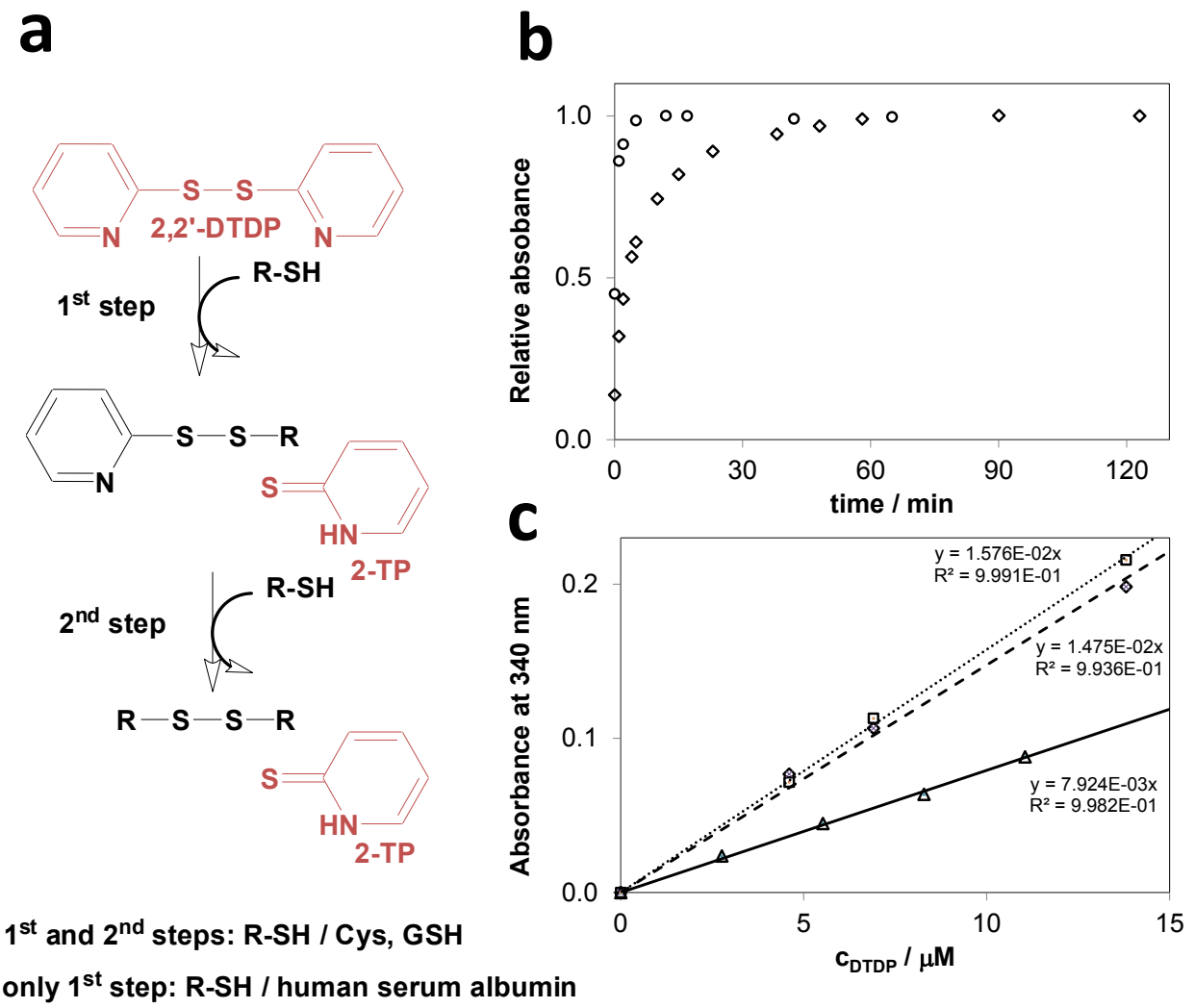


Figure A. a) Two-step reaction of 2,2'-DTDP with thiols; **b**) Time dependence of 2-TP formation of 2,2'-DTDP-Cys system at pH 4.00 (◊) and pH 7.00 (○) followed at 340 nm {c_{Cys} = 50 μM, 2,2'-DTDP : Cys = 0.5:1; in 100 mM acetate buffer (pH 4.00) or 100 mM phosphate buffer (pH 7.00); **c**) Absorbance of samples with excess substrate-to-2,2'-DTDP, where the substrates are Cys (□) or GSH (◊) or HSA (Δ) {c_{Cys} = 52.7 μM, c_{GSH} = 51.6 μM, c_{HSA} = 99.2 μM; pH = 7.00, 100 mM phosphate buffer}

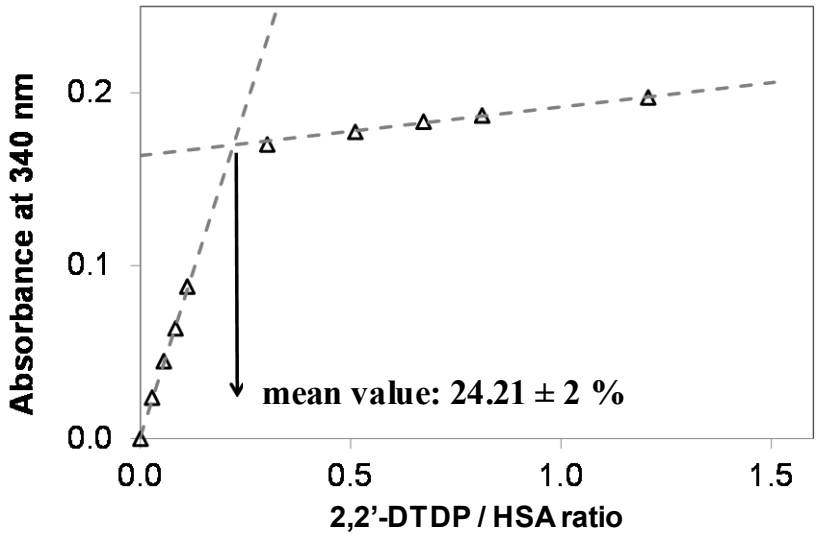


Figure B. Absorbance values at various HSA-to-2,2'-DTDP ratios. The intercept of the fitted plots on the beginning and the saturated phase gives the SH-content of HSA {c_{HSA} = 99.2 μM; pH = 7.00; 100 mM phosphate buffer}

Figures and Tables

Table S1. Crystal data and structure refinement of **3a**.

Empirical formula	$C_{16}H_{22}Cl_2N_6O_8Pt$
Fw	692.39
Space group	monoclinic, C2/c
a [Å]	26.9636(16)
b [Å]	7.7770(4)
c [Å]	11.3395(6)
α [°]	
β [°]	106.782(2)
γ [°]	
V [Å^3]	2276.6(2)
Z	4
λ [Å]	0.71073
ρ_{calcd} [g cm^{-3}]	2020
crystal size [mm^3]	0.12 x 0.10 x 0.02 mm
T [K]	150(2)
μ [mm^{-1}]	6454
R_1[a]	0.0181
wR_2[b]	0.0438
GOF[c]	1.007

[a] $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. [b] $wR_2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$. [c] GOF $= \{\Sigma[w(F_o^2 - F_c^2)^2]/(n - p)\}^{1/2}$, where n is the number of reflections and p is the total number of parameters refined.

Table S2. Selected bond lengths (Å) and angles (°) for **3a**.

Pt-O(1)	2.0002 (19)
Pt-O(2)	2.0003 (19)
Pt-N(1)	2.037 (2)
Pt-N(2)	2.037 (2)
Pt-Cl(1)	2.3196 (6)
Pt-Cl(2)	2.3196 (6)
O(1)-Pt-O(2)	177.67 (9)
N(1)-Pt-N(2)	83.64 (12)
Cl(1)-Pt-Cl(2)	92.06 (3)

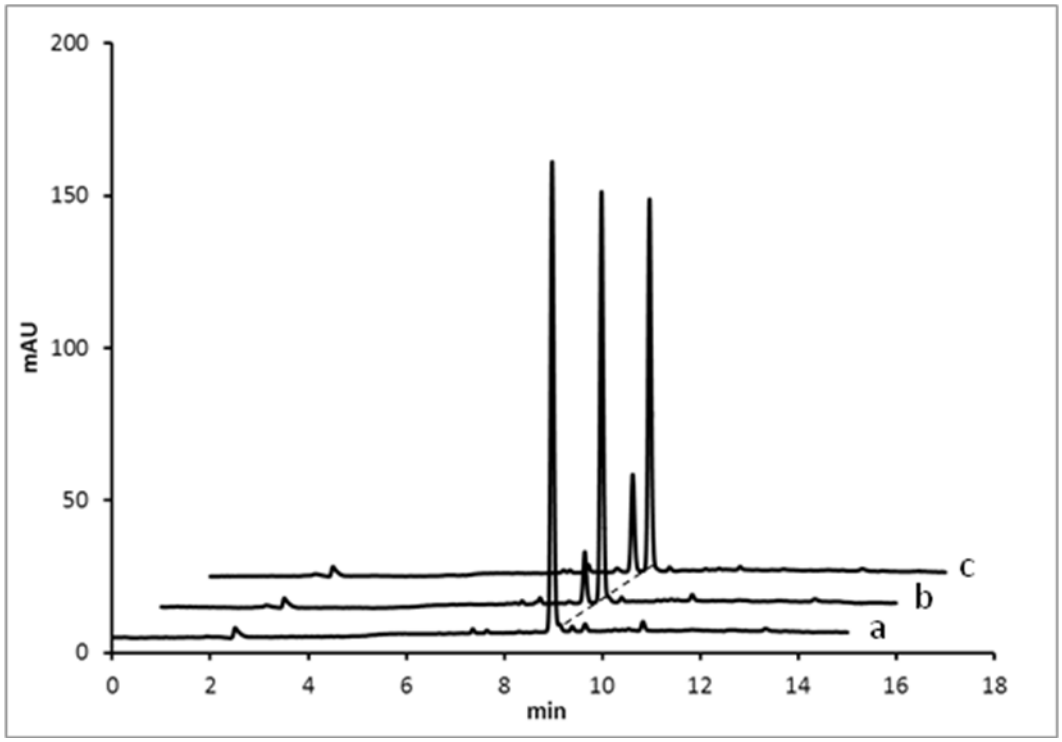


Figure S1. Time course of hydrolysis of **3a** in H_2O measured by RP-HPLC after (a) 5 min, (b) 155 min, and (c) 20 h 45 min.

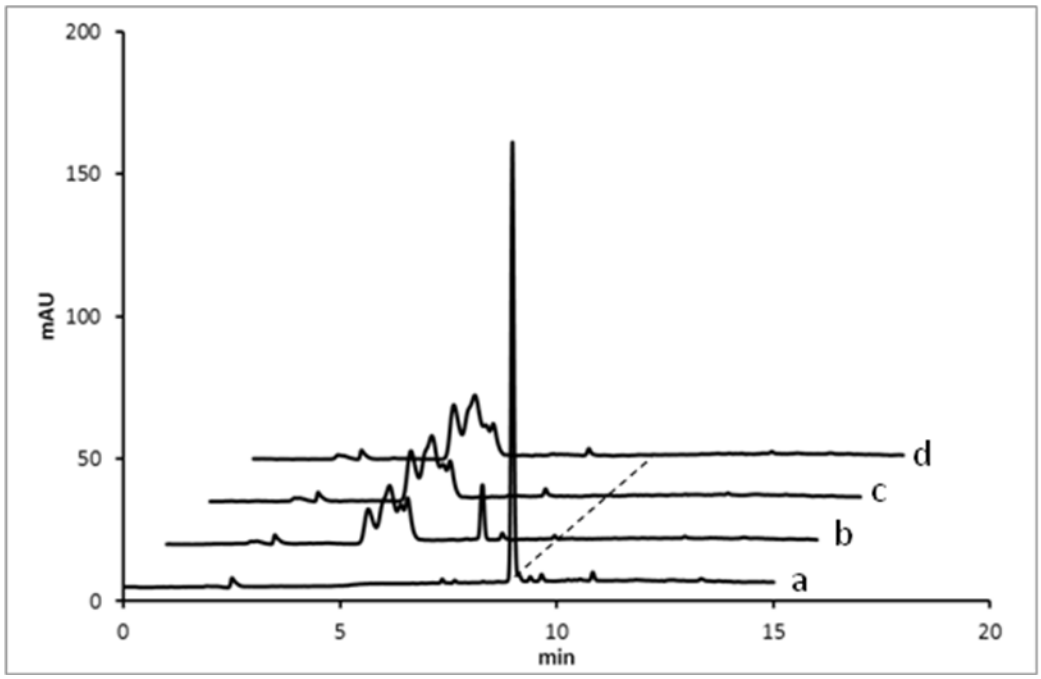


Figure S2. Time course of reaction of **3a** with Cys in H_2O measured by RP-HPLC, (a) without Cys, after (b) 5 min with Cys, (c) 30 min, and (d) 55 min.

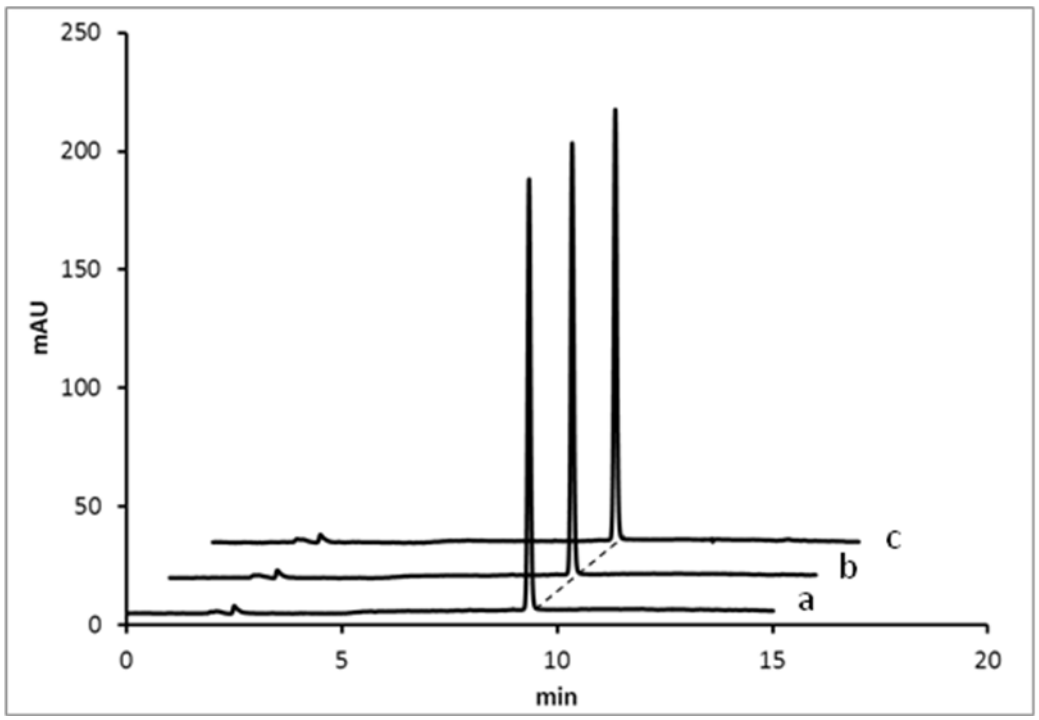


Figure S3. Time course of reaction of **4b** with Cys in H_2O measured by RP-HPLC after (a) 5 min, (b) 30 min, and (c) 55 min.

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