Supporting Information

Kinetically Favored Platination of Adenine in the G-Rich Human Telomeric Repeat

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Experimental Section

Materials

The DNA sequences were synthesized and desalted by IDT (Integrated DNA Technologies, Coralville, IA) and were used without further purification; DNA used in the polyacrylamide gel electrophoresis assay was purified by HPLC. Several buffer solutions were used in this study: K120 (5 mM KH_2PO_4, 1 mM Na_2EDTA, 120 mM KCl), Tris20 (20 mM Tris-HCl), K10 (20 mM Tris-HCl, 10 mM KCl), and K20 (20 mM Tris-HCl, 20 mM KCl). All buffers were adjusted to pH 7.2. The DNA sequences were dissolved in the appropriate buffer solution and stored at –20 °C. The hexamer, 5′-TTAGGG-3′ (**ss-6**) was dissolved in Tris20 buffer without annealing. Incubation of this sequence with platinum was done immediately after preparation of the solution in the absence of Na^+ and K^+ salts to avoid the (slow) formation of an intermolecular quadruplex, $[d(TTAGGG)]_4$.[1] The single-stranded 22- and 24-mers, 5′-$AGGG(TTAGGG)_3$-3′ and 5′-$TTAGGG(TTAGGG)_3$-3′ were annealed into the G-quadruplexes **G4-22** and **G4-24** by heating the samples at 90 °C for 5 min, followed by slow cooling to room temperature. The duplex **ds-22** was generated by annealing with the complementary strand, 5′-$(CCCTAA)_3CCCT$-3′. The secondary structures of the sequences **G4-22**, **G4-24**, and **ds-22** were confirmed by circular dichroism (CD) spectra and thermal melting experiments (Figures S1–S5). The concentration of DNA was calculated applying Beer's law using the extinction coefficients (260 nm) ε = 61,300 $M^{-1}\cdot cm^{-1}$, 196,479 $M^{-1}\cdot cm^{-1}$, 282,128 $M^{-1}\cdot cm^{-1}$, and 244,600 $M^{-1}\cdot cm^{-1}$ for **ds-6**, **G4-22**, **ds-22**, and **G4-24**, respectively. PT-ACRAMTU ($[Pt(en)(ACRAMTU\text{-}S)Cl](NO_3)_2$: en = ethane-1,2-diamine, ACRAMTU = 1-[2-(acridine-9-ylamino)ethyl]-1,3-dimethylthiourea, acridinium cation) was synthesized according to the published procedure.[2] Stock solutions of PT-ACRAMTU were prepared in water and stored at –20 °C in the dark. Final drug concentrations were determined spectrophotometrically (ε = 9452 $M^{-1}\cdot cm^{-1}$).[3] HPLC-grade solvents were applied in all chromatographic separations, and Millipore water was obtained from a Milli-Q A10 synthesis water purification system. All other chemicals were molecular biology grade and purchased from Aldrich or Fisher. DNase I (RNase-free) from bovine pancreas, calf intestinal alkaline phosphatase (CIP), and exonuclease I from *E. coli* were supplied by New England BioLabs (Ipswich, MA). Nuclease P_1 from penicillium citrinum was purchased from Sigma and was dissolved in nuclease P_1 buffer provided by the manufacturer (40 mM sodium acetate, 10 mM $ZnCl_2$, 100 mM NaCl and 50% glycerol, pH = 5.3). T4 polynucleotide kinase (cloned) was obtained from Epicentre Biotechnologies (Madison, WI). All enzymes were stored at –20 °C.

Thermal denaturation/renaturation of G-quadruplex DNA monitored by UV-visible spectroscopy

Thermal denaturation experiments were carried out on a Hewlett-Packard HP 8453 spectrophotometer equipped with a Peltier temperature-controlled cell holder and an external temperature probe. Stock solutions of the annealed G-quadruplex sequences **G4-22** and **G4-24** were diluted to 100 μM (nucleotides) in K10 and K20 buffer, respectively. Heating and cooling traces were recorded over the range 15 °C→70 °C→15 °C in 0.5 °C increments and a hold time of 2 min. The monitoring wavelength was 295 nm. Melting temperatures (T_m) were calculated from the first derivative of the traces using an algorithm provided in the HP thermal denaturation software.

Circular dichroism

Temperature dependent CD spectra were recorded on an AVIV Model 215 spectrophotometer equipped with a thermoelectrically controlled cell holder. The scanning wavelength ranged from 220 to 350 nm with scanning speed of 1 nm/s and response times of 2 to 5 s. CD profiles were base-line adjusted by subtracting buffer background. Samples contained 100 μM (nt) DNA.

Incubation and digestion

The DNA sequences [200 μM (nt)] were incubated with PT-ACRAMTU at a platinum-to-nucleotide ratio (r_b) of 0.2 at 37 °C for 24 h in the dark. After incubation, the samples were dialyzed against Millipore water in a 28-well dialysis apparatus at 4 °C for 24 h in the dark to remove unreacted drug. The molecular-weight cut-off (MWCO) of the dialysis membrane (regenerated cellulose, Spectrum Laboratories, Rancho Dominguez, CA) was 2000 Da. In acidic digestion assays, the pH of the samples was adjusted to 2.2–2.4 using formic acid. Acidified samples were digested at 55 °C for 16 h in the dark, centrifuged for 10 minutes at 13,400 rpm, and the supernatant collected and immediately injected into the HPLC system. In enzymatic digestions the following protocol was applied: i) addition of 70 units of DNase I and 10 μL DNase I stock buffer solution (100 mM Tris-HCl, 2.5 mM $MgCl_2$, and 0.5 mM $CaCl_2$; pH = 7.6); incubation at 37 °C for 3 h; ii) addition of 40 units of DNase I, and incubation at 37 °C for 3 h; iii) addition of 40 units of nuclease P_1 and incubation at 55 °C for 3 h; iv) addition of 30 units of nuclease P_1 and incubation at 55 °C for 18 h; v) addition of 40 units of alkaline phosphatase and 100 μL of 10 x buffer 3 provided by New England Biolabs (1 M NaCl, 500 mM Tris-HCl, and 10 mM dithiothreitol; pH 7.9) and incubation at 37 °C for 3 h; vi) addition of another 40 units of alkaline phosphatase and incubation at 37 °C for 3 h. The samples were centrifuged for 10 min at 13,400 rpm; the supernatant was collected and dialyzed for 6 h against Millipore water using a 100 Da MWCO membrane.

Kinetic assay

Three 1.5-mL samples of **G4-22** [200 μM (nt)] were prepared and equilibrated at 37 °C. PT-ACRAMTU, dissolved in a few μL of water, was added into the samples to a final r_b of 0.1, and the mixtures immediately vortexed. At each time point, 150 μL of solution were removed from the mixtures, immediately cooled on ice, and combined with 1.5 μL of 100 mM aqueous thiourea. The samples were allowed to react for 5 min on ice and were dialyzed against 5 mM Tris-HCl buffer (pH = 7.1) in the dark at 4 °C for 12 h to remove excess thiourea (MINI dialysis units, MWCO = 3500 Da; Pierce Biotechnology, Rockford, IL). (Note: Under these conditions, the highly charged, quenched drug ($[Pt(en)(ACRAMTU)(thiourea)]^{3+}$) remains strongly bound to the DNA and is not removed by dialysis; see Figure S14 for MS characterization). After lyophilization, the samples were re-dissolved in 20 μL of water and enzymatically digested using the above protocol. Data points for the calculation of reaction rates were averaged over the three experiments (vide infra).

Chromatographic separations

The mixtures resulting from acidic and enzymatic digestion assays were separated by reverse-phase HPLC using the LC module of an Agilent Technologies 1100 LC/MSD Trap system equipped with a multi-wavelength diode-array detector and autosampler. A 4.6 x 150 mm reverse-phase Agilent ZORBAX SB-C18 (5 μm) analytical column was used in all of the assays, which was maintained at 25

°C during separations. Two wavelengths, 254 nm and 413 nm, were used to monitor unmodified nucleic acid and ACRAMTU containing fragments, respectively. The following eluents were used for the separations: solvent A, degassed water/0.1% formic acid, and solvent B, acetonitrile/0.1% formic acid. The gradient for acidic digestion assays was solvent A changing from 95% to 81% over 28 min at a flow rate of 0.5 mL/min; the gradient for enzymatic digestion was 95% solvent A decreasing to 85% over 30 min at a flow rate of 0.5 mL/min. The integration of peaks was done using the LC/MSD Trap Control 4.0 data analysis software.

Mass spectrometry

Mass spectra were recorded on an Agilent 1100 LC/MSD ion trap mass spectrometer. After separation by in-line HPLC, adducts were infused into the atmospheric-pressure electrospray source. Ion evaporation was assisted by a flow of N_2 drying gas (325 °C) at a pressure of 55 psi and a flow rate of 10 L/min. For enzymatic digests, positive- and negative-ion mass spectra and tandem mass spectra (MS/MS) were recorded. During MS experiments in positive-mode, a capillary voltage of 2800 V was applied and the mass-to-charge scan range was from 200 to 2200 *m/z*; in negative-mode, the capillary voltage was 3000 V. Product-ion mass spectra in MS/MS experiments were generated by collision-induced dissociation (CID) with the assistance of helium as bath gas. The isolation mass for fragments **P2**–**P4** was 1159.0 *m/z* for positive mode and 1156.5 *m/z* for negative mode, with an isolation width of 4.0 *m/z* and a fragmentation amplitude of 1.0 V. For acidic digests, MS experiments were performed in positive-mode with a capillary voltage of 3000 V and a scan range of 100–800 *m/z*. Mass spectra were recorded in enhanced scan resolution mode (5500 *m/z* s^{-1}).

Exonuclease footprinting assay

The HPLC-purified sequence **G4-24** (1 μM/L, strand concentration) was 5′ end labeled using T4 polynucleotide kinase (EPICENTRE Biotechnologies, Madison, WI) and [γ-^{32}P]-ATP (Amersham Biosciences, Piscataway, NJ). Labeled **G4-24** was mixed with unlabeled sequence (200 μM, nucleotides) and annealed into the G-quadruplex. PT-ACRAMTU stock solution was titrated into the DNA to a final r_b of 0.08. After 24 h of incubation at 37 °C in the dark, aliquots were withdrawn from the mixture and digested with exonuclease I. The enzyme was added at varying concentrations, and the mixtures were allowed to digest for 1 h at 37 °C. Afterwards the enzyme was deactivated at 80 °C for 20 min. To remove PT-ACRAMTU prior to the electrophoretic separations, samples were incubated with sodium cyanide at a final concentration of 1 M at 37 °C for 15−18 h and dialyzed against water at 4 °C for 24 h to remove excess salt (Harvard Apparatus, Micro DispoDialyzer; MWCO 100 Da). Samples were heat denatured and separated on 24% polyacrylamide denaturing gels containing 8 M urea. The gels were dried and analyzed on a BioRad FX-Pro plus phosphorimager (Hercules, CA). Band intensities were analyzed using the Quantity One 1-D Analysis Software (version 4.1.1., Bio-Rad Laboratories, Hercules, CA).

NOTE:

Optical spectroscopies were used to assure that the sequences **G4-22** and **G4-24** had converted from random coil to quadruplex structures before they were incubated with platinum. The K^+ form of human telomeric DNA has been demonstrated to adopt multiple conformations characterized by parallel and antiparallel strand folding. Dynamic interconversion between different folded and unfolded conformations occurs on the order of milliseconds to several minutes, and (un)folding rates (k_u, k_f) depend on K^+ concentration.[4] At 10 mM K^+, $k_f > k_u$, and the folded quadruplexes predominate over the single-stranded conformation in solution equilibria,[5] which can be confirmed, for instance, by CD spectropolarimetry and UV melting experiments. In the present study, the adduct profiles observed in **ss-6** and **G4-22** were distinctly different, indicating that the platinum agent indeed reacted with the folded form of **G4-22**. Reaction with **G4-22** in its single-stranded form would have led to an adduct distribution similar to that found in **ss-6**, which is dominated by base content rather than DNA secondary structure. Thus, PT-ACRAMTU's adduct profile can be considered a probe of DNA secondary structure.

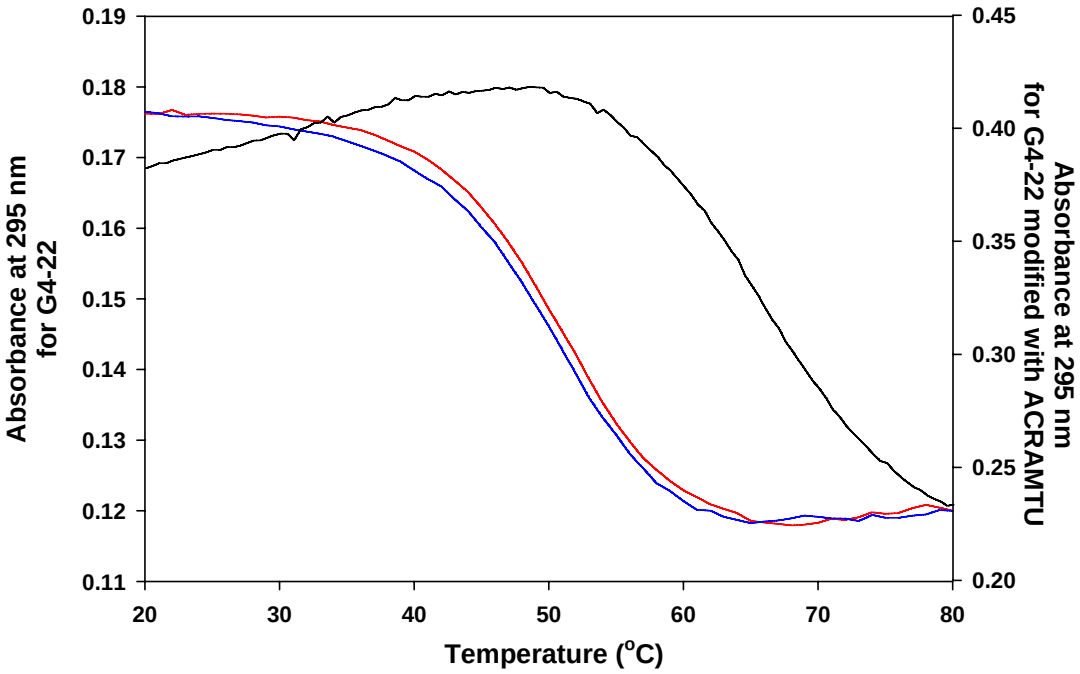


Figure S1: UV thermal melting profiles for **G4-22** in K10 buffer. The blue and red traces are the cooling and heating curves recorded for the drug-free sequence, respectively. The T_m is 49.0 °C. The black trace is the UV thermal melting profile for **G4-22** in K10 buffer in the presence of 2 equivalents of ACRAMTU ligand. The T_m is 62.4 °C.

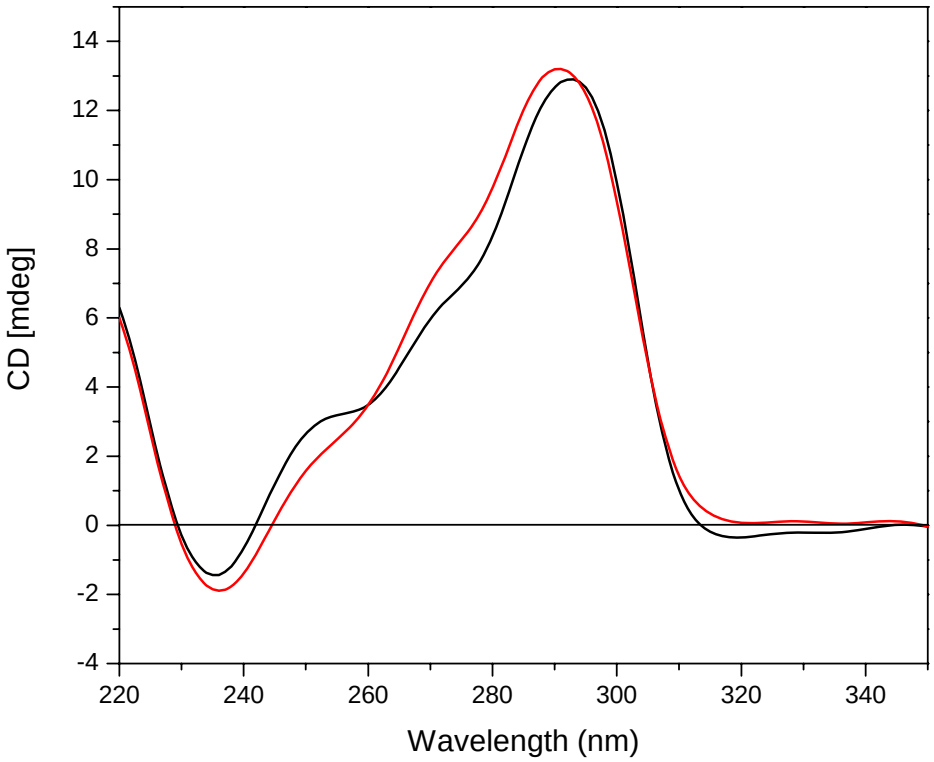


Figure S2: CD spectra for **G4-22** in K10 buffer at 20 °C (black) and 40 °C (red), indicating that at 37 °C **G4-22** exists in its G-quadruplex structure.

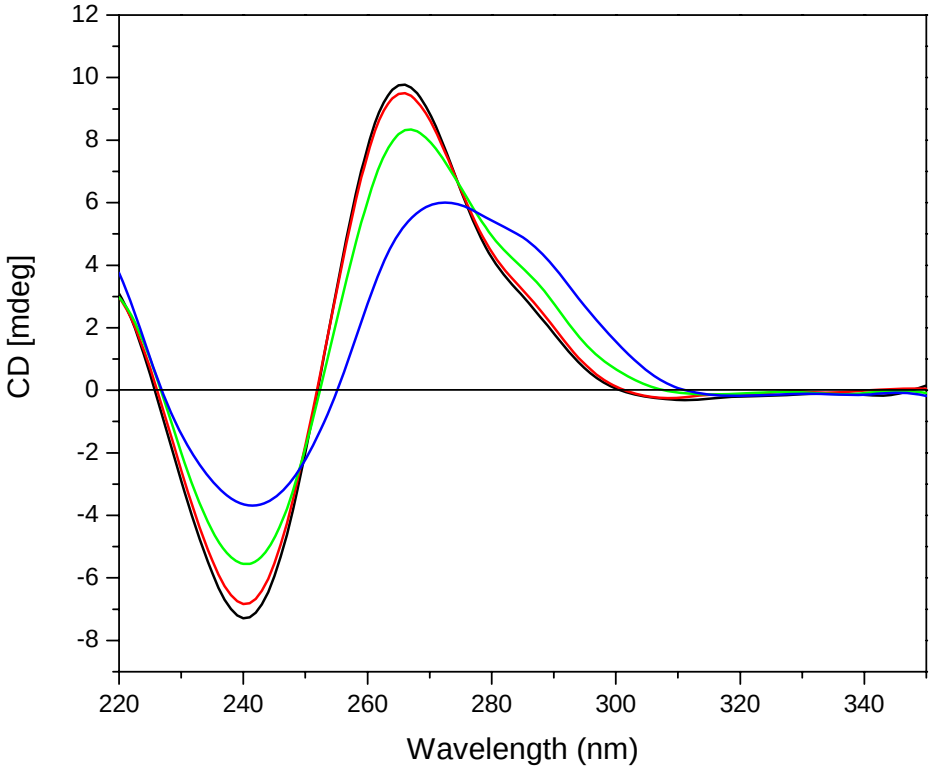


Figure S3: CD spectra for **ds-22** at varying temperatures in K120 buffer (black line: 20 °C; red line: 40 °C; green line: 60 °C; blue line: 70 °C), confirming the double-stranded form of **ds-22** under the incubation conditions.

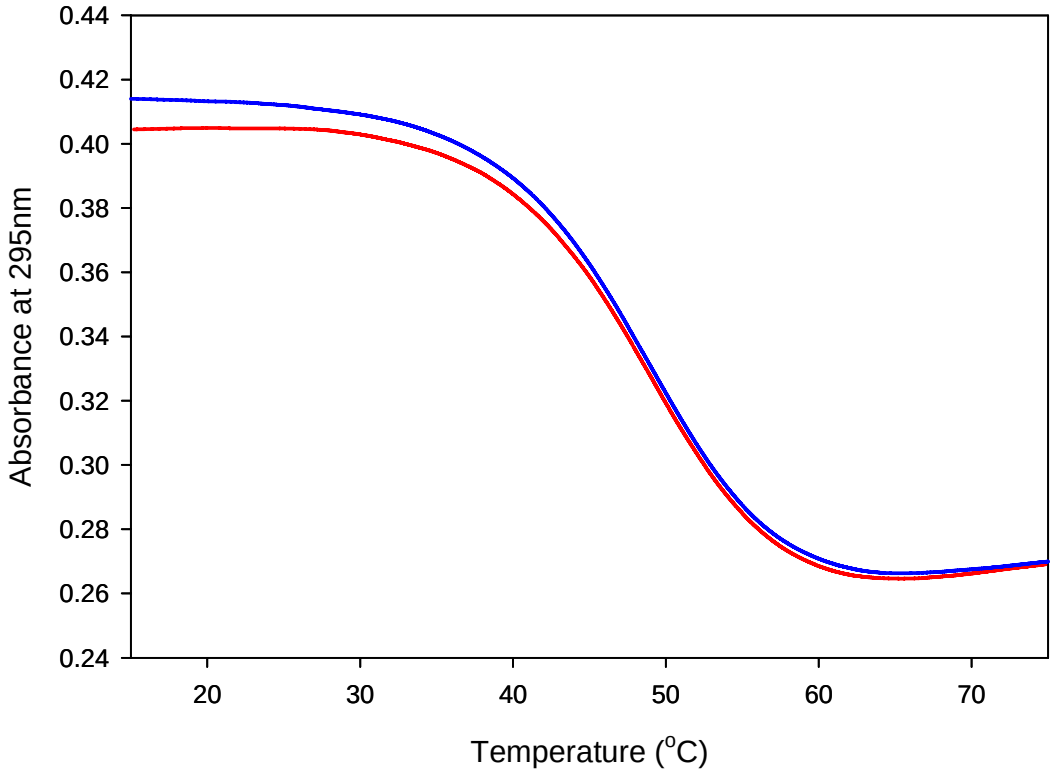


Figure S4: UV thermal melting profile for **G4-24** in K20 buffer. The blue and red lines are the cooling and heating traces, respectively. The T_m is 47.7 °C.

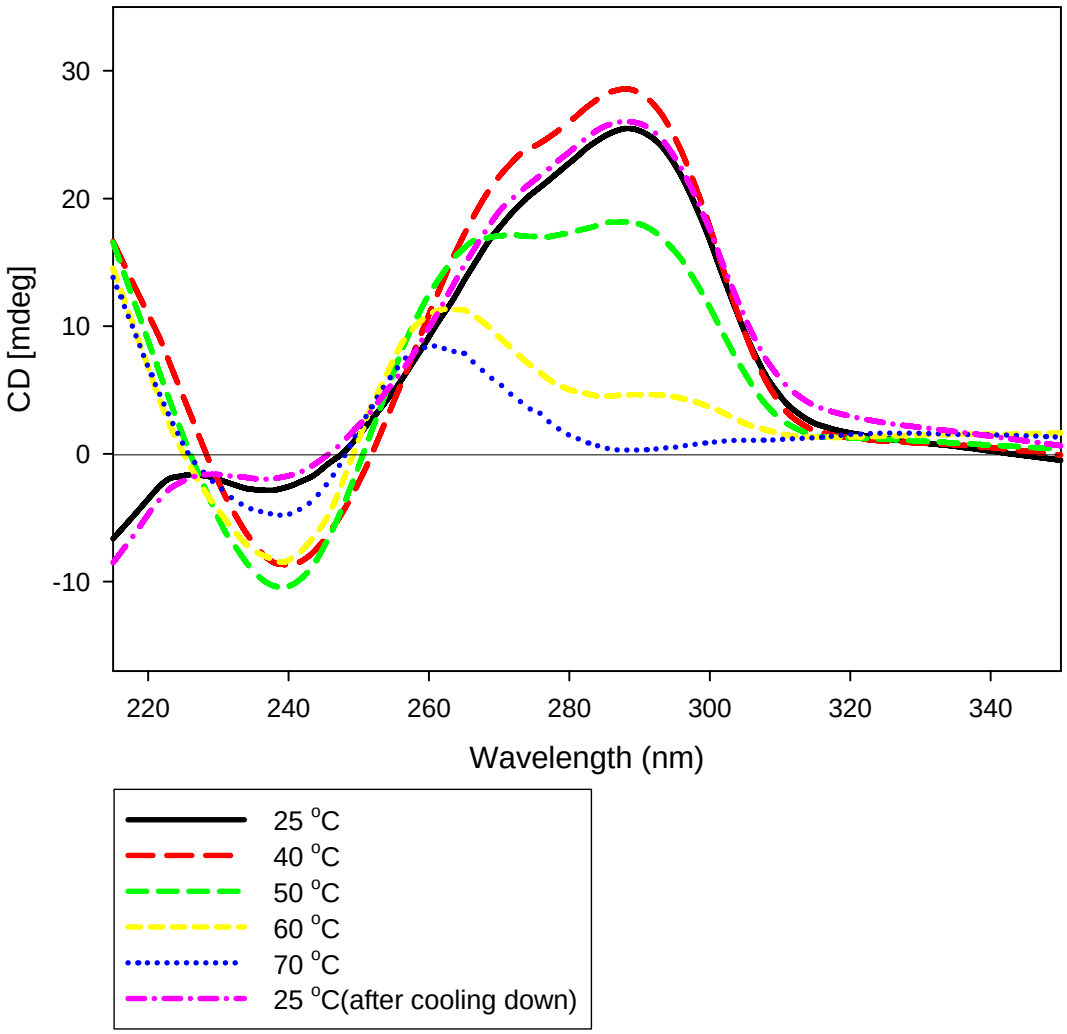
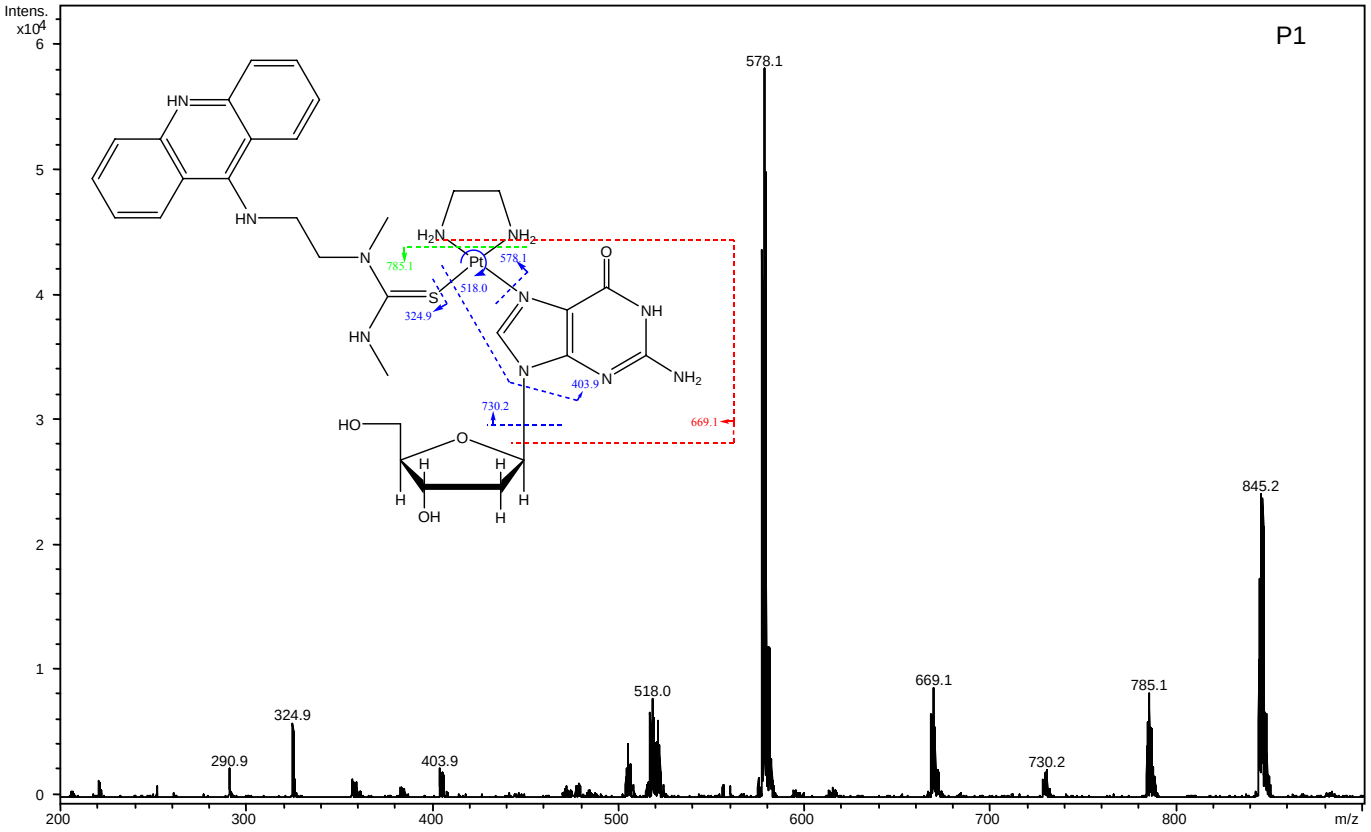
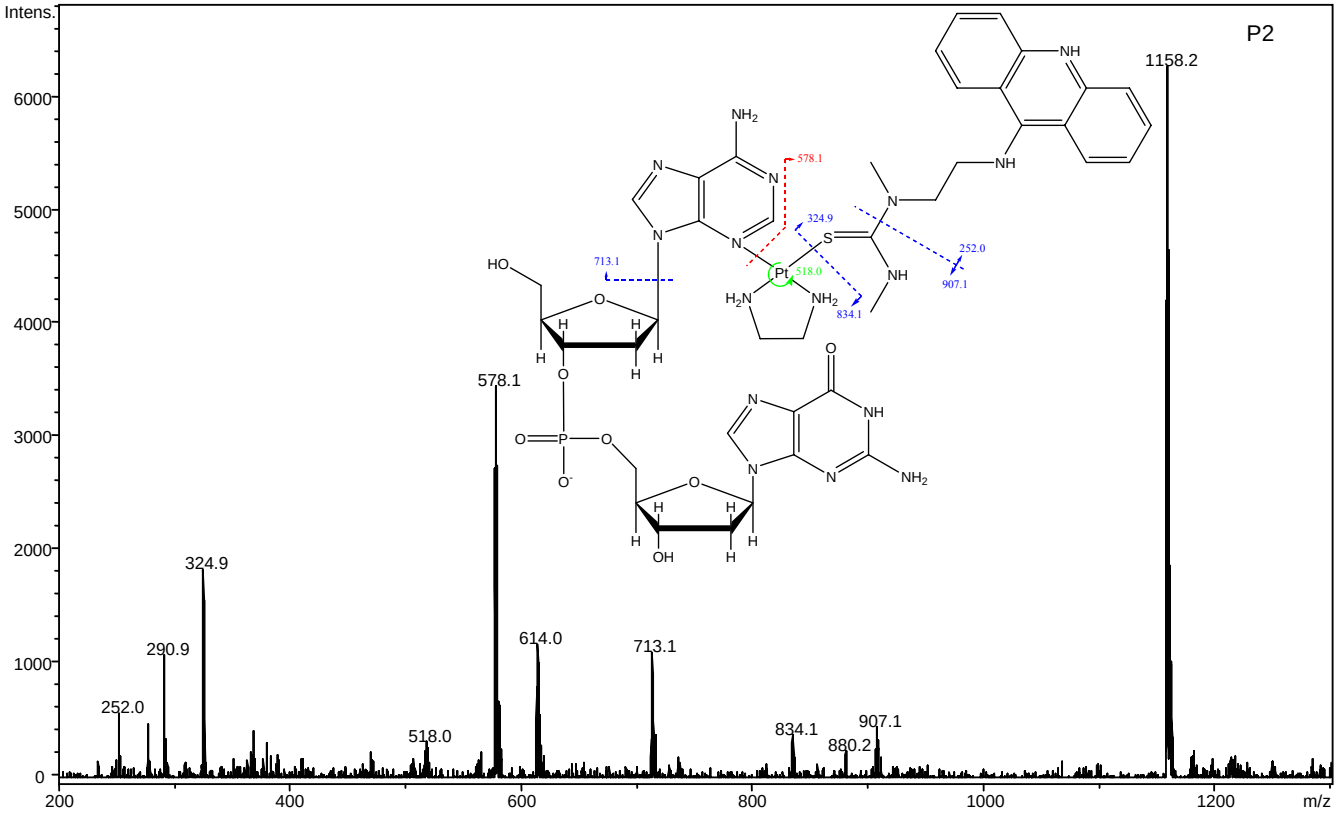


Figure S5: CD spectra for **G4-24** at varying temperatures in K20 buffer.



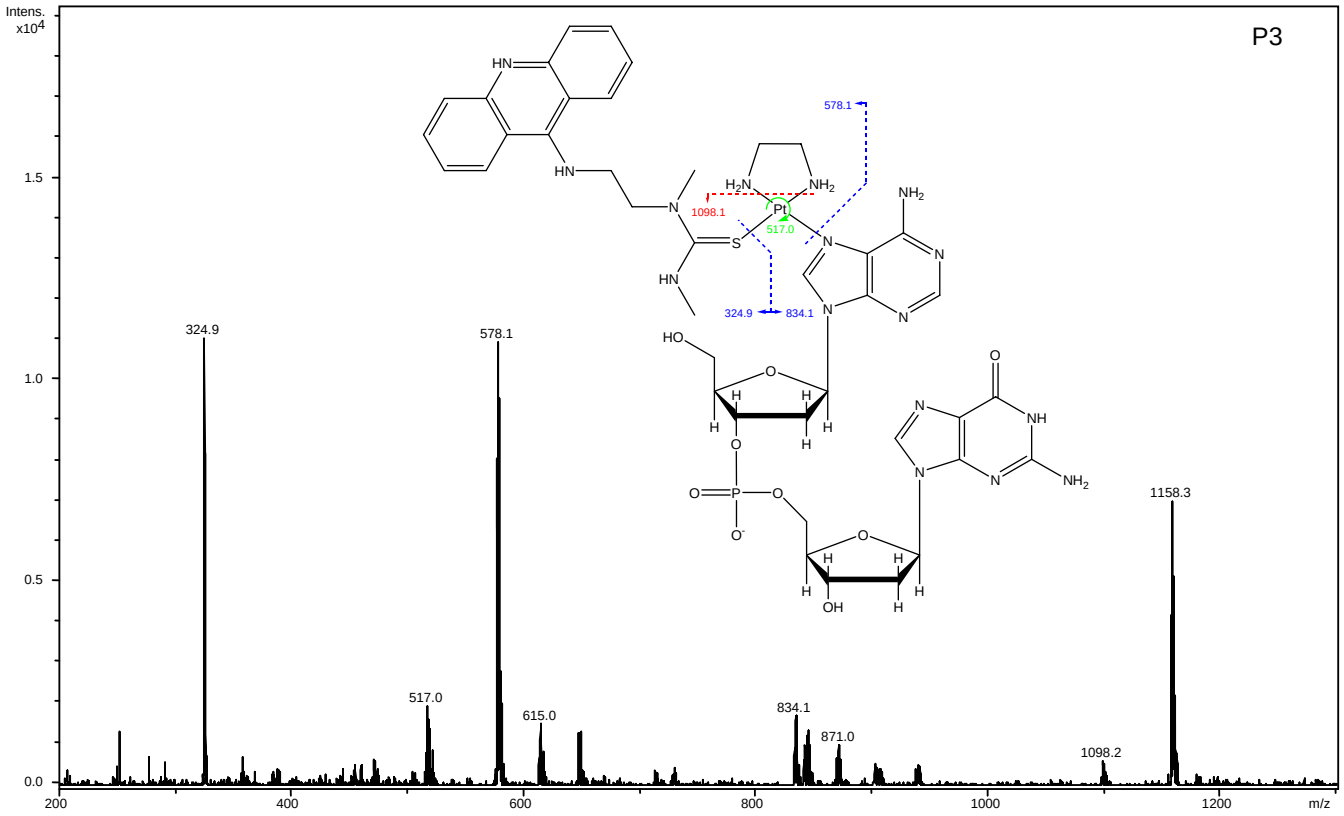
Fragment	m/z
$[P1\text{-}2H]^+$	845.2
$[Pt(ACRAMTU)(dG)\text{-}2H]^+$	785.1
$[Pt(ACRAMTU)(en)(guanine)\text{-}H]^+$	730.2
$[Pt(ACRAMTU)(guanine)\text{-}H]^+$	669.1
$[Pt(ACRAMTU)(en)\text{-}2H]^+$	578.1
$[Pt(ACRAMTU)\text{-}2H]^+$	518.0
$[Pt(en)(guanine)]^+$	403.9
$[ACRAMTU]^+$	324.9
$[N^1\text{-(acridin-9-yl)- }N^2\text{-methylethane-1,2-diamine+K}]^+$	290.9
$[N^1\text{-(acridin-9-yl)- }N^2\text{-methylethane-1,2-diamine+H}]^+$	252.0

Figure S6: Positive-mode ESMS analysis and fragmentation behavior of adduct **P1**.



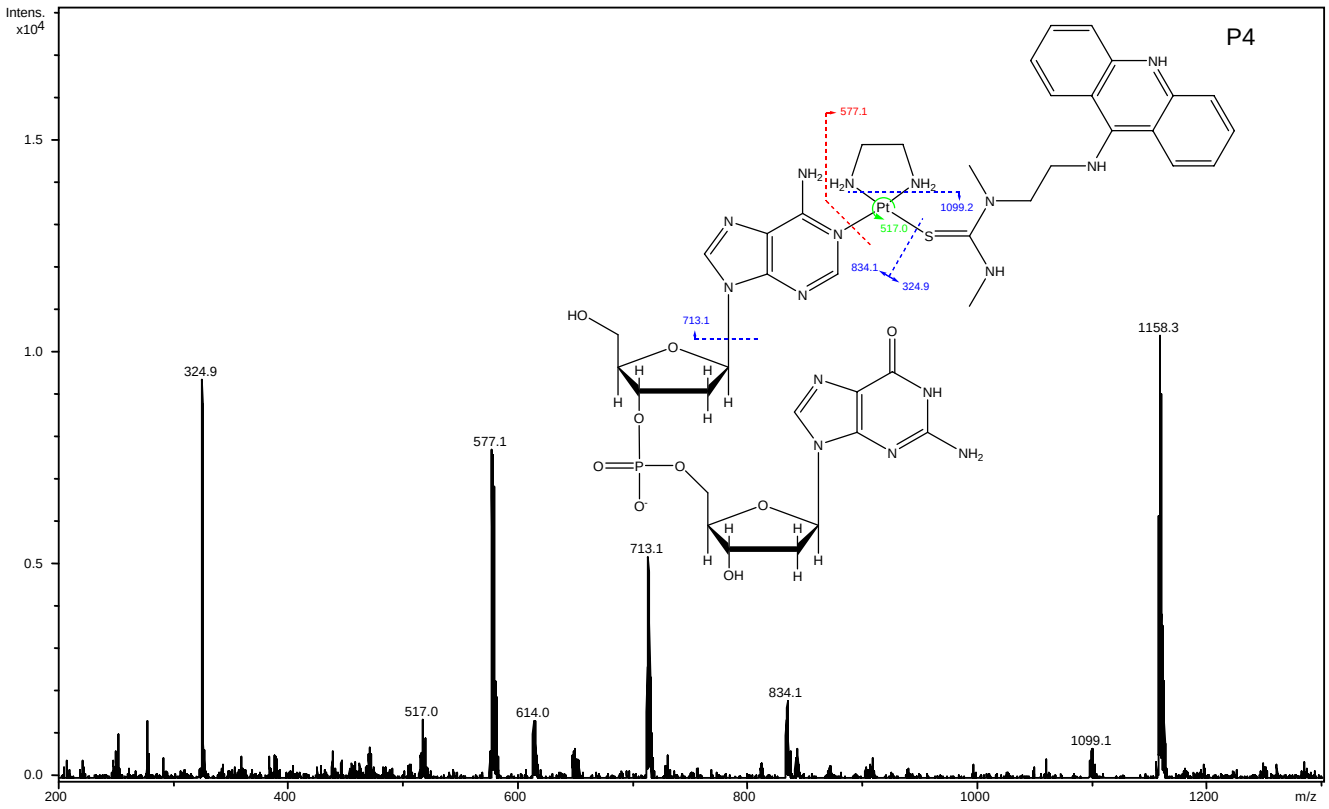
Fragment	m/z
$[P2\text{-}H]^+$	1158.2
$[F\text{-}H]^+$	907.1
$[Pt(en)ApG]^+$	834.1
$[Pt(ACRAMTU)(en)(adenine)\text{-}H]^+$	713.1
$[Pt(ACRAMTU)(en)\text{-}H+Cl]^+$	614.0
$[Pt(ACRAMTU)(en)\text{-}2H]^+$	578.1
$[Pt(ACRAMTU)\text{-}2H]^+$	518.0
$[ACRAMTU]^+$	324.9
$[N^1\text{-(acridin-9-yl)- }N^2\text{-methylethane-1,2-diamine+K}]^+$	290.9
$[N^1\text{-(acridin-9-yl)- }N^2\text{-methylethane-1,2-diamine+H}]^+$	252.0

Figure S7: Positive-mode ESMS analysis and fragmentation behavior of adduct **P2**.



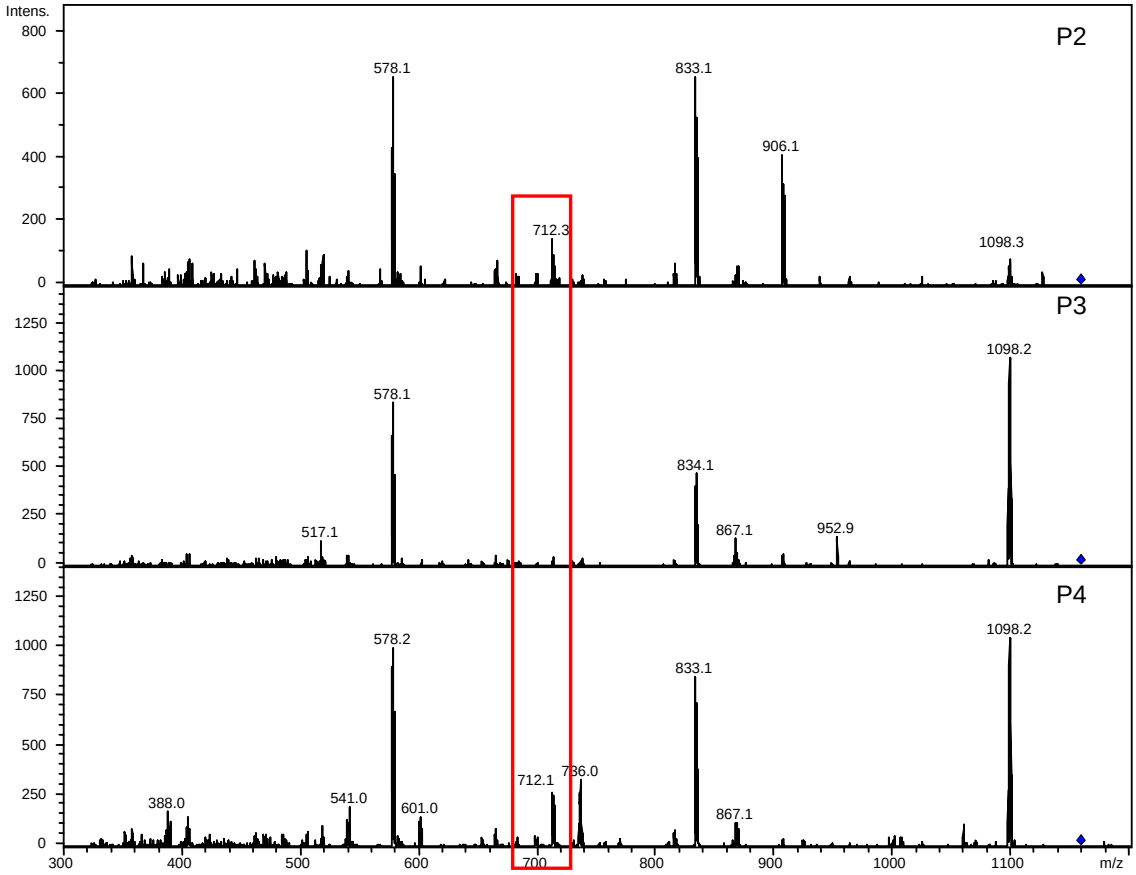
Fragment	m/z
$[P3\text{-}H]^+$	1158.3
$[Pt(ACRAMTU)(ApG)\text{-}H]^+$	1098.2
$[Pt(en)ApG]^+$	834.1
$[Pt(ACRAMTU)(en)\text{-}H+Cl]^+$	615.0
$[Pt(ACRAMTU)(en)\text{-}2H]^+$	578.1
$[Pt(ACRAMTU)\text{-}2H]^+$	517.0
$[ACRAMTU]^+$	324.9

Figure S8: Positive-mode ESMS analysis and fragmentation behavior of adduct **P3**. The absence of the fragment at *m/z* 713.0 is consistent with platination of A_{N7} ,[6] which prevents protonation of this site. Protonation of the five-membered purine ring facilitates depurination (ref [6] and literature cited threrein).



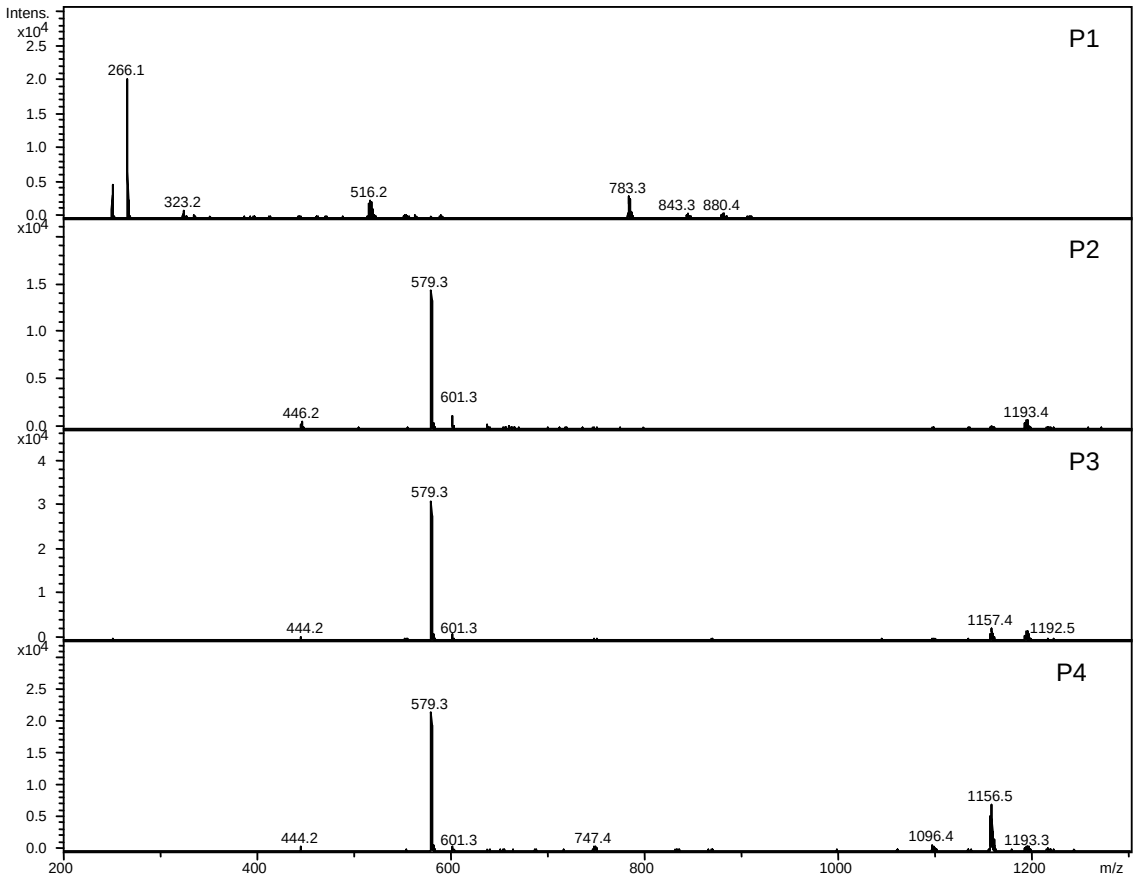
Fragment	m/z
$[P4\text{-}H]^+$	1158.3
$[Pt(ACRAMTU)(ApG)\text{-}H]^+$	1099.1
$[Pt(en)ApG]^+$	834.1
$[Pt(ACRAMTU)(en)(adenine)\text{-}H]^+$	713.1
$[Pt(ACRAMTU)(en)\text{-}H+Cl]^+$	614.0
$[Pt(ACRAMTU)(en)\text{-}2H]^+$	577.1
$[Pt(ACRAMTU)\text{-}2H]^+$	517.0
$[ACRAMTU]^+$	324.9

Figure S9: Positive-mode ESMS analysis and fragmentation behavior of adduct **P4**.



Adduct	Fragment	m/z
P2 [Pt(ACRAMTU)(en)-A(N3)pG]	$[Pt(ACRAMTU)(ApG)-H]^+$	1098.3
	$[Pt(en)ApG]^+$	833.1
	$[Pt(ACRAMTU)(en)(adenine)-H]^+$	712.3
	$[Pt(ACRAMTU)(en)-2H]^+$	578.1
P3 [Pt(ACRAMTU)(en)-A(N7)pG]	$[Pt(ACRAMTU)(ApG)-H]^+$	1098.2
	$[Pt(en)ApG]^+$	834.1
	$[Pt(ACRAMTU)(en)-2H]^+$	578.1
	$[Pt(ACRAMTU)-2H]^+$	517.1
P4 [Pt(ACRAMTU)(en)-A(N1)pG]	$[Pt(ACRAMTU)(ApG)-H]^+$	1098.2
	$[Pt(en)ApG]^+$	833.1
	$[Pt(ACRAMTU)(en)(adenine)-2H+Na]^+$	736.0
	$[Pt(ACRAMTU)(en)(adenine)-H]^+$	712.1
	$[Pt(ACRAMTU)(en)-3H+Na]^+$	601.0
	$[Pt(ACRAMTU)(en)-2H]^+$	578.2
	$[Pt(ACRAMTU)-3H+Na]^+$	541.0
	$[Pt(en)(adenine)]^+$	388.0

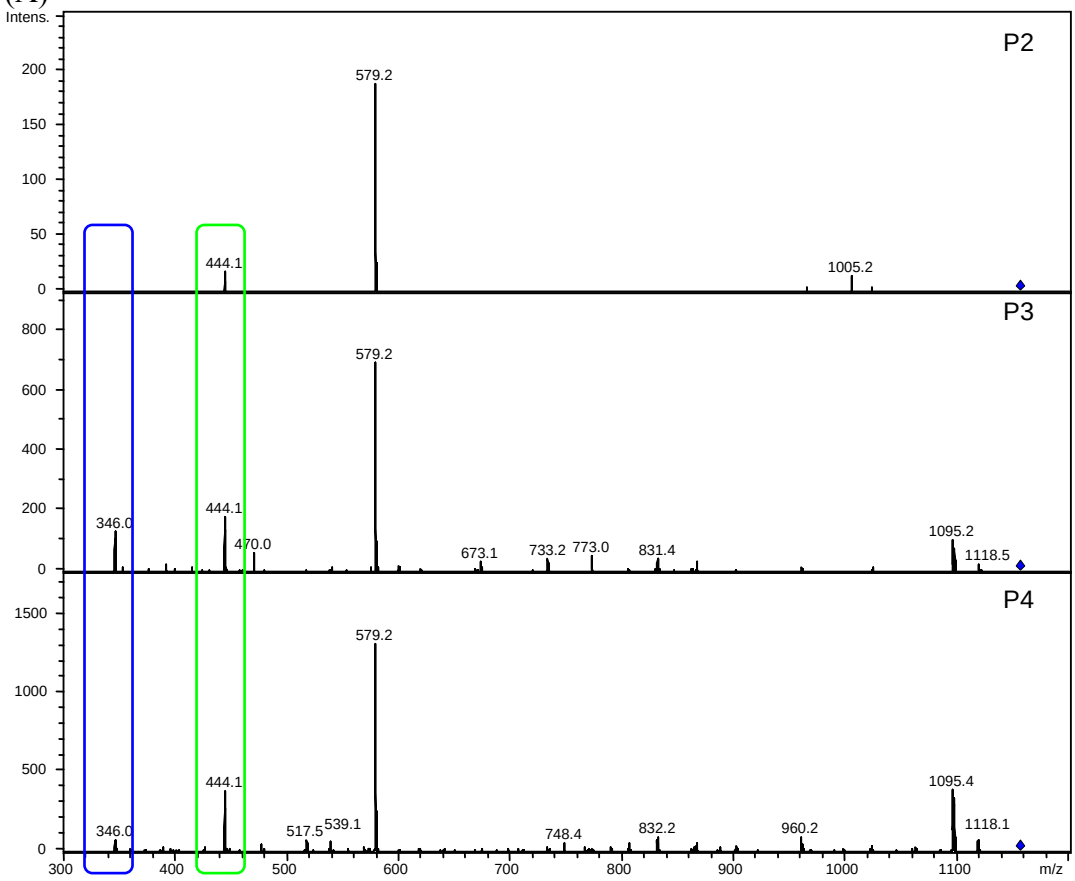
Figure S10: Positive-mode MS/MS spectra and summary of fragments for **P2**, **P3** and **P4** resulting from collision-induced dissociation (isolated mass: 1159.0 *m/z*, blue diamond). The fragment expected for depurination (red box; 712 *m/z*) is absent for **P3**, which is consistent with platination of N7.



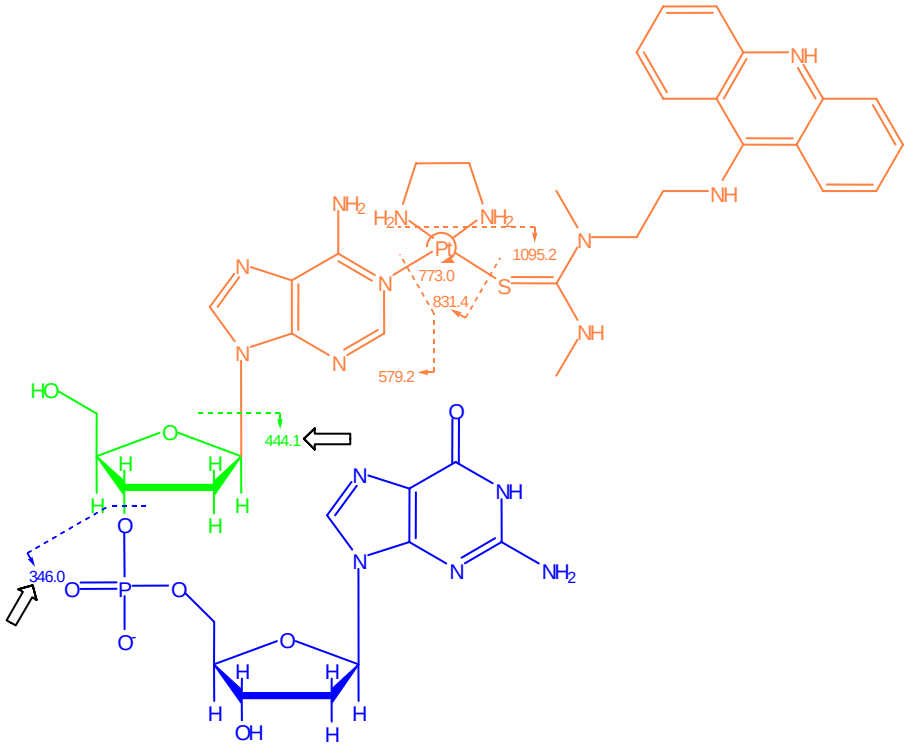
Adduct	Fragment	m/z
P1 [Pt(ACRAMTU)(en)-dG]	[P1-3H+Cl]$^-$	880.4
	[P1-4H]$^-$	843.3
	[Pt(ACRAMTU)(dG)-4H]$^-$	783.3
	[Pt(ACRAMTU)-4H]$^-$	516.2
	[ACRAMTU-2H]$^-$	323.2
	[dG-H]$^-$	266.1
P2 [Pt(ACRAMTU)(en)-A(N3)pG]	[P2-2H+Cl]$^-$	1193.4
	[ApG-H+Na]$^-$	601.3
	[ApG]$^-$	579.3
	[ApG-adenine-H]$^-$	446.2
P3 [Pt(ACRAMTU)(en)-A(N7)pG]	[P3-2H+Cl]$^-$	1192.5
	[P3-3H]$^-$	1157.4
	[ApG-H+Na]$^-$	601.3
	[ApG]$^-$	579.3
	[ApG-adenine-H]$^-$	444.2
P4 [Pt(ACRAMTU)(en)-A(N1)pG]	[P4-2H+Cl]$^-$	1193.3
	[P4-3H]$^-$	1156.5
	[P4-(en)-3H]$^-$	1096.4
	[Pt(ACRAMTU)(en)(adenine)-2H+Cl]$^-$	747.4
	[ApG-H+Na]$^-$	601.4
	[ApG]$^-$	579.3
	[ApG-adenine-H]$^-$	444.2

Figure S11: Negative-mode ESMS analysis and summary of fragments for **P1**, **P2**, **P3** and **P4**.

(A)


Intens.
P2
579.2
444.1
1005.2
P3
579.2
346.0
444.1
470.0
673.1
733.2
773.0
831.4
1095.2
1118.5
P4
579.2
444.1
346.0
517.5
539.1
748.4
832.2
960.2
1095.4
1118.1
m/z

(B)


NH
NH
NH2
H2N
NH2
Pt
1095.2
773.0
831.4
579.2
N
N
N
N
S
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HO
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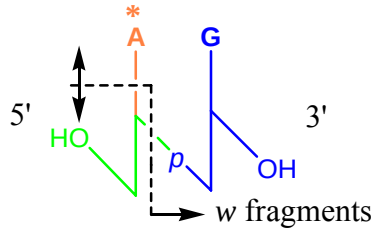
O
O
N
N
NH
N
NH2
OH

(C)

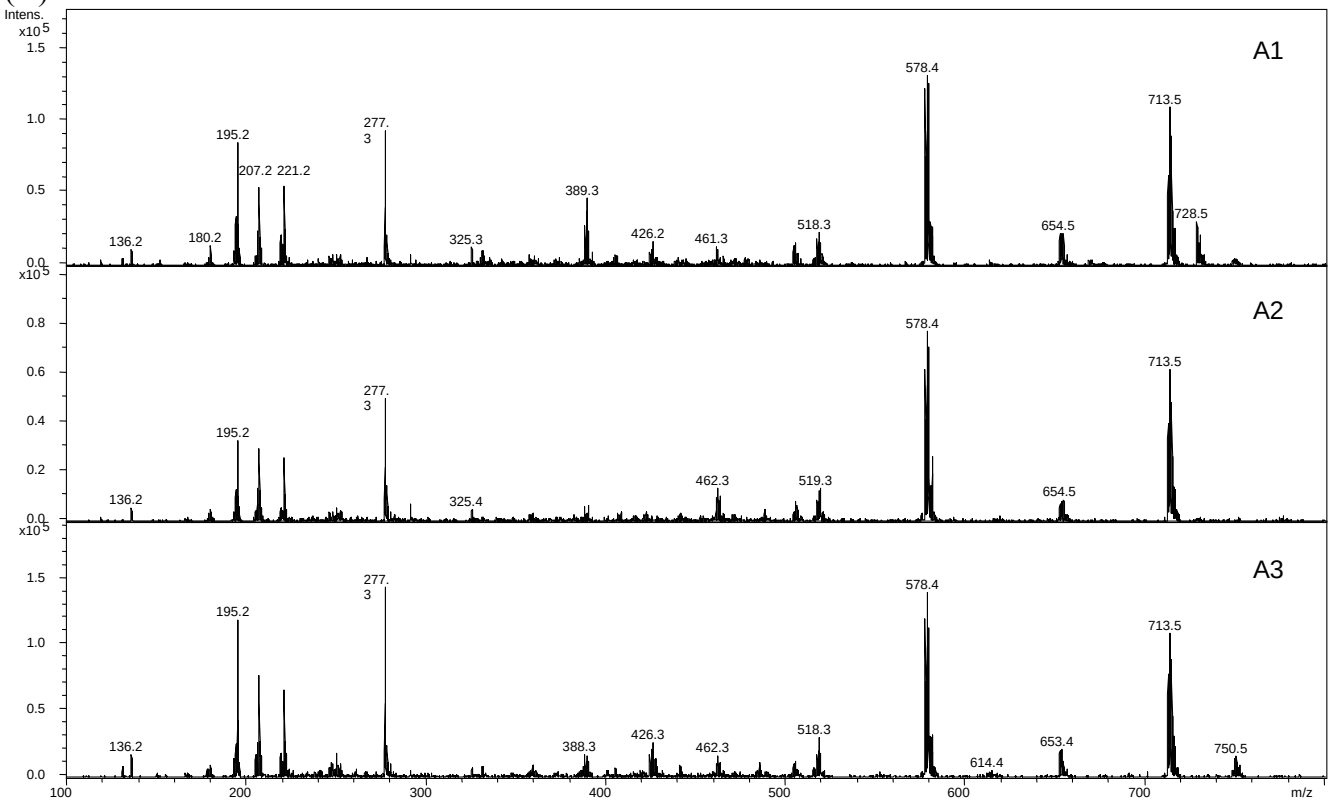


(D)

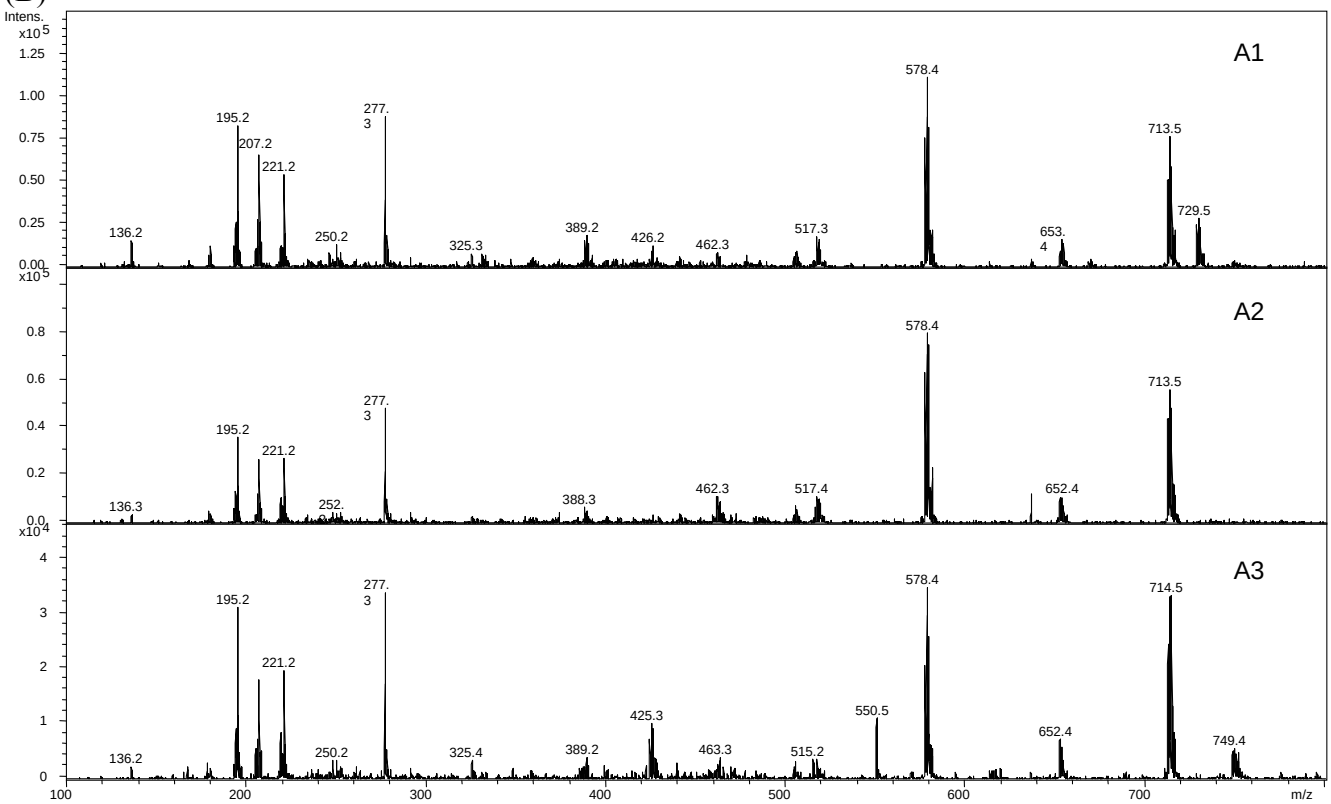
Adduct	Fragment	m/z
P2 [Pt(ACRAMTU)(en)-A(N3)pG]	[P2-guanine-4H]$^-$	1005.2
	[ApG]$^-$	579.2
	[ApG-adenine-H]$^-$	444.1
P3 [Pt(ACRAMTU)(en)-A(N7)pG]	[P3-(en)-4H+Na]$^-$	1118.5
	[P3-(en)-3H]$^-$	1095.2
	[Pt(en)ApG-2H]$^-$	831.4
	[Pt(ApG)-2H]$^-$	773.0
	[Pt(ACRAMTU)(en)(adenine)-4H+Na]$^-$	733.2
	[Pt(ACRAMTU)(adenine)-4H+Na]$^-$	673.1
	[ApG]$^-$	579.2
	[ApG-adenine-H]$^-$	444.1
	[dG(PO_3)+H]$^-$	346.0
P4 [Pt(ACRAMTU)(en)-A(N1)pG]	[P4-(en)-4H+Na]$^-$	1181.1
	[P4-(en)-3H]$^-$	1095.4
	[Pt(en)ApG-2H]$^-$	832.2
	[Pt(ACRAMTU)(en)(adenine)-2H+Cl]$^-$	748.4
	[ApG]$^-$	579.2
	[Pt(ACRAMTU)-5H+Na]$^-$	539.1
	[Pt(ACRAMTU)-4H]$^-$	517.5
	[ApG-adenine-H]$^-$	444.1
	[dG(PO_3)+H]$^-$	346.0

Figure S12: (A) Negative-mode MS/MS spectra for **P2**, **P3** and **P4**. The *w* fragment for **P2** is not observed due to the low abundance of the adduct. (B) Fragmentation pattern for **P4**. (C) Formation of *w* fragments. When the 5′ nucleobase is modified in dinucleotide sequences, the C3′-O bond is weakened and becomes susceptible to collision-induced cleavage. This reactivity feature can be used to confirm that A, and not G, is platinated in d(ApG).[6] The peaks assigned to fragments resulting from cleavage of the C3′-O bond are highlighted with black arrows in (B) and rectangular boxes in (A)). (D) Summary of fragments resulting from collision-induced dissociation (isolated mass: 1156.5 *m/z*, blue diamonds in (A)) for **P4**.

(A)


Intens.
x10^5
A1
578.4
713.5
277.3
195.2
207.2
221.2
389.3
728.5
136.2
180.2
325.3
426.2
461.3
518.3
654.5
A2
578.4
713.5
277.3
195.2
462.3
519.3
136.2
325.4
654.5
A3
578.4
277.3
195.2
713.5
136.2
388.3
426.3
462.3
518.3
653.4
614.4
750.5
100
200
300
400
500
600
700
m/z

(B)


Intens.
x10^5
A1
578.4
277.3
195.2
207.2
221.2
713.5
729.5
136.2
250.2
325.3
389.2
426.2
462.3
517.3
653.4
A2
578.4
713.5
277.3
195.2
221.2
136.3
252.
388.3
462.3
517.4
652.4
x10^4
A3
578.4
714.5
277.3
195.2
221.2
550.5
425.3
652.4
136.2
250.2
325.4
389.2
463.3
515.2
749.4
100
200
300
400
500
600
700
m/z

(C)

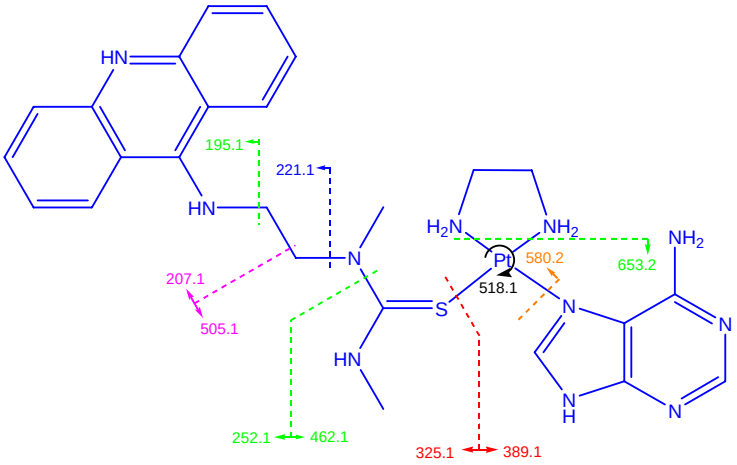


Figure S13: Fragmentation pattern in MS positive mode for depurinated adducts **A1**, **A2**, **A3** after acidic digestion of drug-modified **G4-22** (A) and **ds-22** (B). (C) Fragmentation pattern for adduct **A1**, which is similar for all the adducts.

Modeling and Calculation of the Kinetics Data

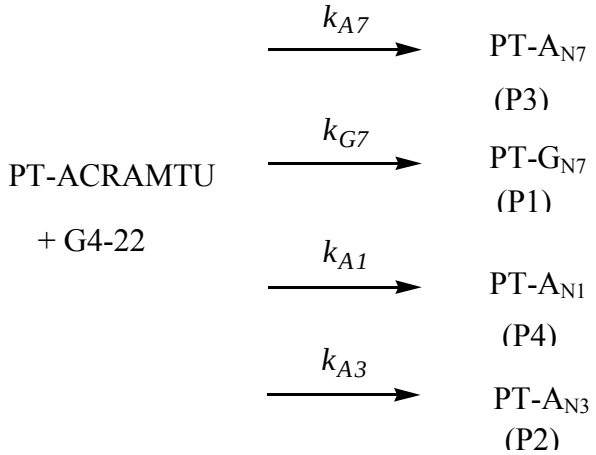
The calculations below are based on the following assumptions:

1) The total amount of free (non-DNA-bound) PT-ACRAMTU at each time point is detected in the form of $[Pt(en)(ACRAMTU)(thiourea)]^{3+}$ by LC-MS (see also Figure 14). This species forms rapidly and quantitatively by quenching with thiourea and does not platinate DNA because it lacks a suitable leaving group. Incubation with thiourea was done under conditions, which have been demonstrated to not reverse existing PT-ACRAMTU–DNA adducts.[7, 8]

2) The reaction of PT-ACRAMTU was treated as a system of parallel, independent pseudo-first order reactions, in which either pseudo-first-order hydrolysis becomes rate-limiting, or the low r_b of 0.1 produces pseudo-first order conditions.

As stated in the Experimental Section, after enzymatic digestion of modified **G4-22**, samples were injected into the LC-ESMS system. Five peaks were monitored in the HPLC profiles: $[Pt(en)(ACRAMTU)(thiourea)]^{3+}$, **P1**, **P2**, **P3**, and **P4**. Peak areas were used to represent the concentrations of each species, which is reasonable because each fragment contains *one* acridine chromophore and the monitoring wavelength is acridine-specific (413 nm).

In order to calculate the pseudo-first-order rate constants (k) for the parallel reactions, it was necessary to extract k_{obs} for PT-ACRAMTU consumption.



To calculate k_{obs} for PT-ACRAMTU, we used the first-order kinetic equation (eq 1), where [PT-ACRAMTU] = [Pt(en)(ACRAMTU)(thiourea)]:

$$\frac{d[PT.ACRAMTU]}{dt} = -k_{obs}[PT.ACRAMTU] \quad \text{(eq 1)}$$

$$\ln\frac{[PT.ACRAMTU]_0}{[PT.ACRAMTU]} = k_{obs}t \quad \text{(eq 2)}$$

According to eq 2, the data set for PT-ACRAMTU was fitted with the equation $y = ae^{-bx}$, where y represents the percentage of free PT-ACRAMTU ($\frac{[PT.ACRAMTU]}{[PT.ACRAMTU]_0} = \frac{[PT.ACRAMTU.thiourea]}{[PT.ACRAMTU]_0}$), and x the time (min). Since this reaction is considered pseudo-first-order, we restricted $a = 1$. After

regression, the value of k_{obs} is given by the slope *b*.

The average of the three determinations gave: $\boxed{k_{obs} = 1.58 \times 10^{-4}\ s^{-1};\ t_{1/2} = 1.2\ h}$

Based on this k_{obs} value, we calculated the *k* values for **P1**, **P2**, **P3**, and **P4** formation as follows:

$$-\frac{d[PT.ACRAMTU]}{dt} = (k_{A7} + k_{G7} + k_{A1} + k_{A3})[PT.ACRAMTU]$$

$$(k_{obs} = k_{A7} + k_{G7} + k_{A1} + k_{A3})$$

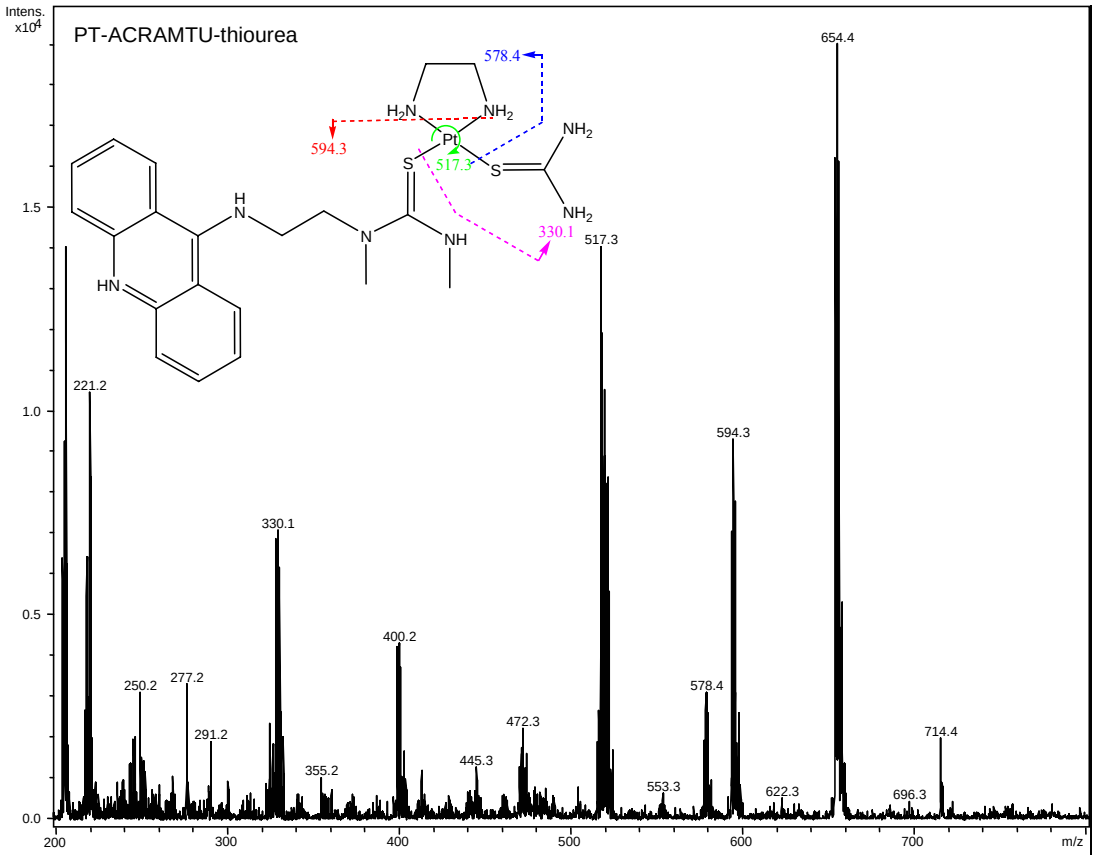
$$\frac{d[PT.AN7]}{dt} = k_{A7}[PT.ACRAMTU] = k_{A7}[PT.ACRAMTU]_0 e^{-k_{obs}t}$$

$$\int_0^{[PT-AN7]} d[PT.AN7] = \int_0^t k_{A7}[PT.ACRAMTU]_0 e^{-k_{obs}t} dt$$

$$\frac{[PT.AN7]}{[PT.ACRAMTU]_0} = \frac{k_{A7}}{k_{obs}}(1 - e^{-k_{obs}t}) \quad \text{(eq. 3)}$$

We have used eq 3 for **P3** (k_{N7}) as an example. The peak areas for **P3** were fitted with the equation $y = a(1-e^{-bx})$, where *y* is the percentage of **P3** and *x* the time (min); *b* is restricted to k_{obs} and *a* is the ratio ($\frac{k_{A7}}{k_{obs}}$). The resulting individual rate constants were extracted from the curve fits and are summarized below.

	Value (× 10^{-5} s^{-1})
k_{obs}	15.8
k_{A7}	7.68
k_{G7}	4.88
k_{A1}	2.31
k_{A3}	0.96



Fragment	m/z
[PT_ACRAMTU_thiourea-2H]$^+$	654.4
[PT_ACRAMTU_thiourea-en-2H]$^+$	594.3
[PT_ACRAMTU-2H]$^+$	578.4
[Pt_ACRAMTU-2H]$^+$	517.3
[PT-ACRAMTU-acridine]$^+$	400.2
[Pt_en_thiourea-H]$^+$	330.1

Figure S14: Positive-mode ESMS analysis of the quenched drug, Pt(en)(ACRAMTU)(thiourea), and summary of observed fragmentation.

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