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Supporting Information

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RNA-Selective Covalent Modification by Neo-platin (A Neomycin-Cisplatin Conjugate)

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S.1 Synthesis of 1 (scheme 1)

The synthesis of compound **3** has previously been reported (Michael, K; Wang, H.; Tor, Y. *Bioorg. Med. Chem* **1999**, *7*,1361-1371).

4: **3** (500 mg, 0.34 mmol) was dissolved in 5 mL DMF/water (10/1). Sodium azide (200 mg, 3.40 mmol) was added and the suspension was stirred at 70°C for 2 d. The reaction mixture was filtered and the filtrate was concentrated in vacuo. Flash chromatography (silica gel, 0-10% methanol in chloroform) afforded the desired product as a colorless solid (374 g, 0.30 mmol, 89%). R_f = 0.3 (chloroform/methanol 10/1).
^{1}H NMR (MeOD, 400 MHz): δ = 5.47 (br s, 1H), 5.16 (s, 1H), 4.90 (s, 1H), 4.38-4.20 (m, 2H), 4.04-3.95 (m, 1H), 3.90-3.83 (m, 2H), 3.82-3.65 (m, 3H), 3.62-3.50 (m, 9H), 3.42-3.13 (m, 6H), 2.00-1.92 (m, 1H), 1.60-1.20 (m, 55H); MS (ESI-positive): m/z = 1240.3 $[M+H]^+$, 1262.7 $[M+Na]^+$, calcd. for $C_{53}H_{93}N_9O_{24}$ 1239.63.

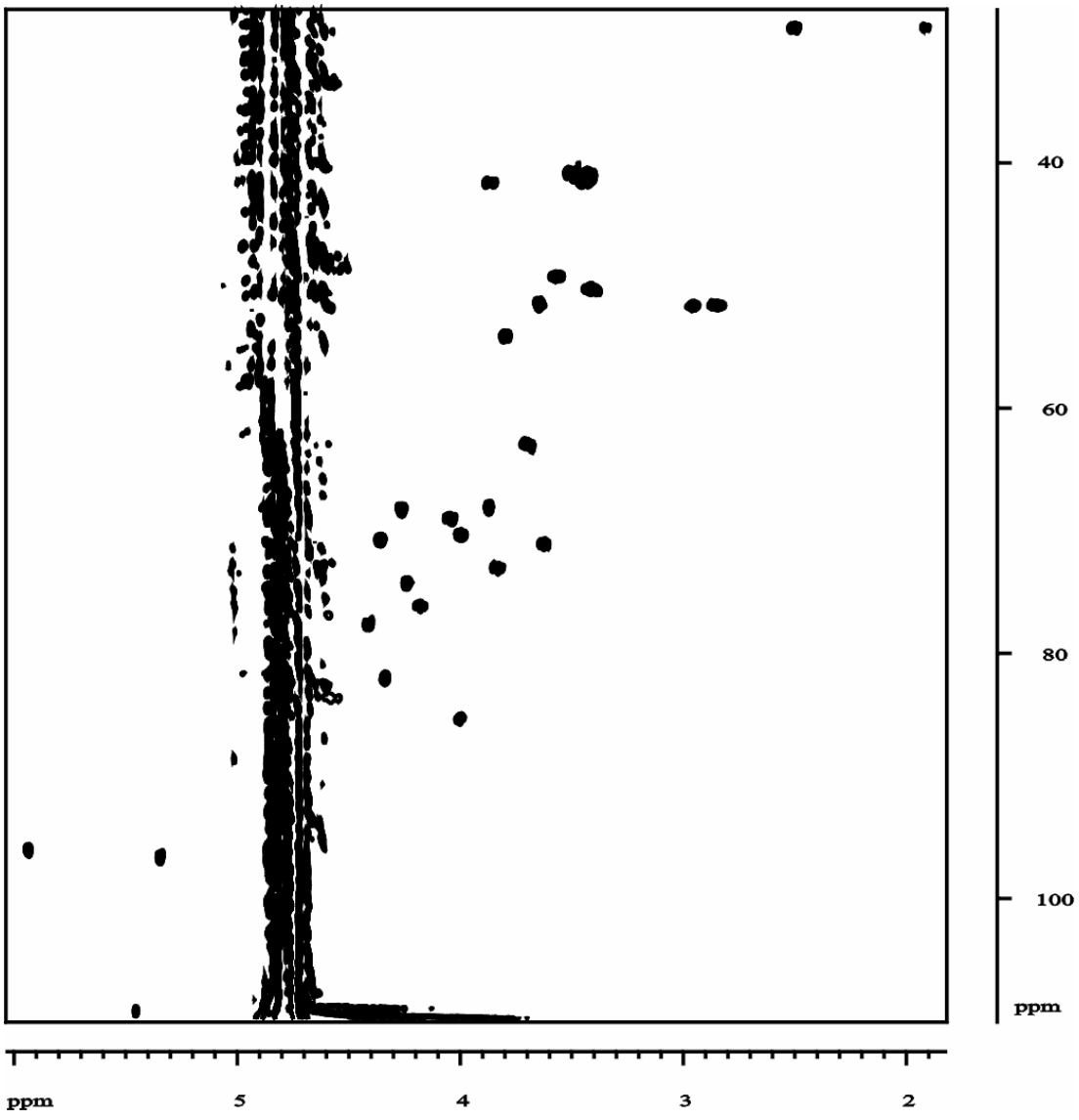
5: **4** (70.0 mg, 56.4 µmol) was dissolved in methanol (10 mL) and treated with Pd/C (10 wt % on activated carbon; 30 mg). The mixture was stirred under an atmosphere of hydrogen for 24 h at RT. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The product was used without further purification (68.0 mg, 55.8 µmol, 99%). R_f = 0.2 (chloroform/methanol 7/1).
^{1}H NMR (MeOD, 400 MHz): δ = 5.58 (br s, 1H), 5.12 (s, 1H), 4.87 (br s, water), 4.41 (br s, 1H), 4.20-4.14 (m, 1H), 4.06-3.90 (m, 1H), 3.90-3.88 (m, 2H), 3.78-3.69 (m, 3H), 3.62-3.16 (m, 13H), 2.98-2.86 (m, 1H), 2.00-1.86 (m, 1H), 1.60-1.30 (m, 55H); MS (ESI-positive): m/z = 1214.6 $[M+H]^+$, calcd. for $C_{53}H_{95}N_7O_{24}$ 1213.64.

6: To a solution of **5** (210 mg, 0.17 mmol) in DMF (10 mL) and 2,4,6-collidine TBTU (400 µL, 0.22 mmol), HOBt (34 mg, 0.22 mmol) and N^{α},N^{β}-Di-Fmoc-(S)-2,3-diaminopropionic acid (121 mg, 0.22 mmol) in DMF (1 mL) were added. The reaction mixture was stirred at RT for 1.5 h. The reaction was quenched with methanol and stirred for another 1 h. The solvent was removed in vacuo and the residue was redissolved in 20% piperidine in DMF (10 mL). After stirring for 30 min at RT, the reaction mixture was concentrated and dried in vacuo. Flash chromatography (silica gel, 0-10% methanol in chloroform) afforded the desired product as a colorless solid (146 mg, 0.11 mmol, 80%). R_f = 0.3 (chloroform/methanol 4/1).
^{1}H NMR (DMSO, 500 MHz): δ = 8.01 (br s, 1H), 6.92 (br s, 1H), 6.87 (s, 1H), 6.62 (d, *J* = 7 Hz, 1H), 6.47 (s, 1H), 6.25 (d, *J* = 8 Hz, 1H), 5.87 (d, *J* = 8 Hz, 1H), 5.51 (br s, -NH_2), 5.35 (br s, -NH_2), 5.01 (s, 1H), 4.93 (s, 1H), 4.79 (s, 1H), 4.22 (br s, 1H), 4.10-4.40 (m, 1H), 3.85-3.70 (m, 2H), 3.62-3.60 (m, 2H), 3.55- 3.50 (m, 5H), 3.50-3.30 (m, 6H) 3.20-3.00 (m, 6H), 2.98-2.88 (m, 1H), 2.79-2-75 (m, 1H), 1.70-1.60 (m, 1H), 1.50-1.20 (m, 55H): MS (ESI-negative): m/z = 1335 $[M+Cl]^-$, calcd. for $C_{56}H_{101}N_9O_{25}$ 1299.69.

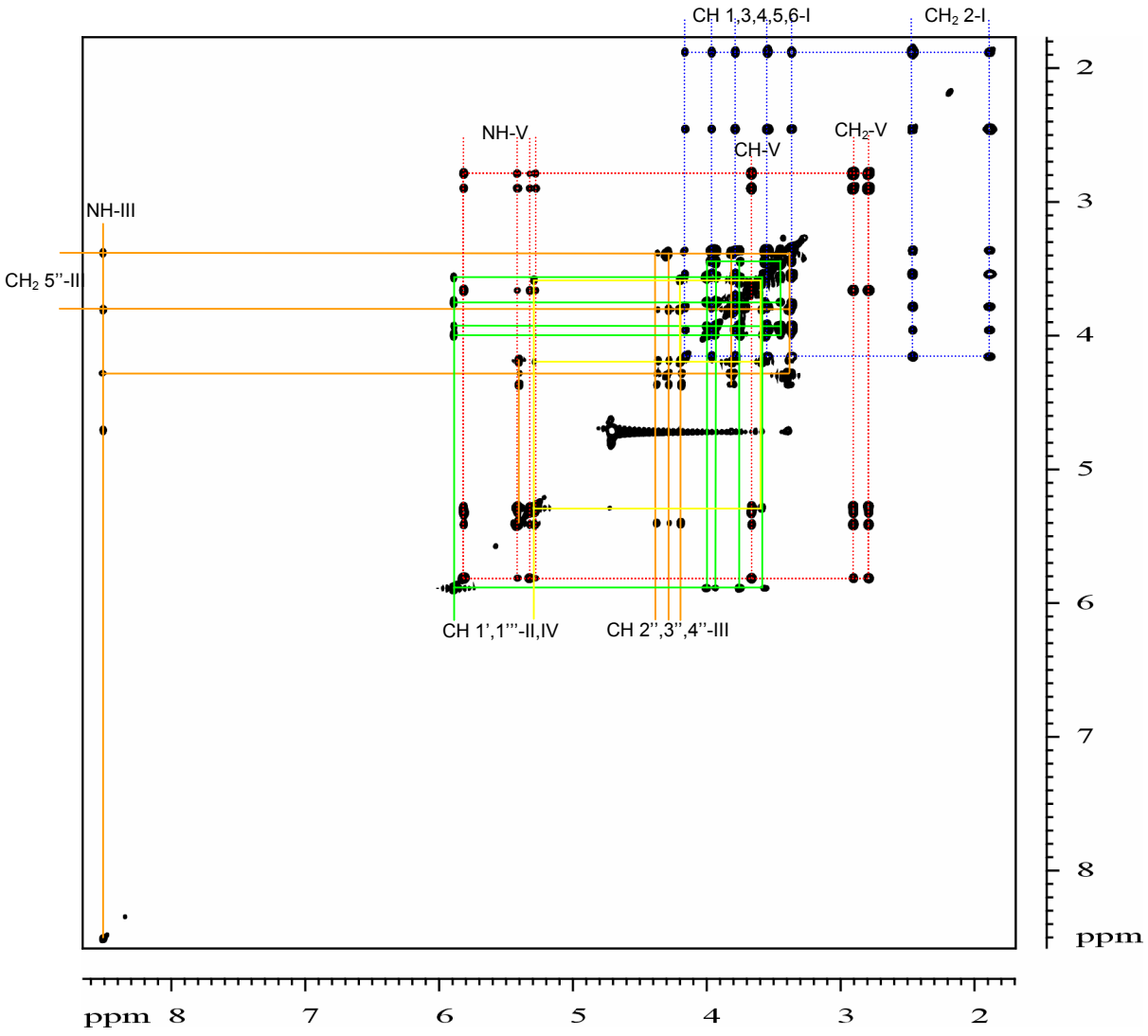
1: **6** (32 mg, 25 µmol) was dissolved in DMF (1 mL). K_2PtCl_4 (12 mg, 30 µmol) in water (600 µl) was added and stirred at RT in the dark overnight. The solvent was removed and the residue was dried in vacuo. The platinated compound (R_f = 0.8 in chloroform/methanol 7/1) was purified by flash chromatography (chloroform/methanol).

The solvent was removed and the residue was redissolved in dioxane (10 mL) and water (1 mL). The solution was treated at 0 °C with 4 M HCl in dioxane (8 mL) and allowed to come to RT. After a total of 10 min, the solvent was removed and the colorless residue was dried in vacuo. The residue was dissolved in water (2 mL), filtered and lyophilized, affording the product as the Cl-salt in form of a colorless solid (23.0 mg, 24 µmol, 95%). ^{1}H NMR (600 MHz, H_2O): δ = 8.49 (br s, 1H), 5.85 (d, *J* = 1.3 Hz, 1H), 5.83 (m, 1H), 5.41 (m, 1H), 5.38 (d, *J* = 1.7 Hz, 1H), 5.30 (m, 1H), 5.27 (s, 1H), 5.25 (m, 1H), 4.32 (m, 1H), 4.25 (m, 2H), 4.18- 4.09 (m, 3H), 3.97-3.85 (m, 3H), 3.79-3.60 (m, 4H), 3.60-3.25 (m, 9H), 2.90-2.85 (m, 1H), 2.87-2.75 (m, 1H), 2.44-2.39 (m, 1H), 1.87-1.79 (m, 1H); (150 MHz, H_2O, from HSQC): 109.00, 96.63, 96.01, 85.37, 81.98, 77.64, 76.13, 74.25, 73.08, 71.03, 70.74, 70,37, 68.94, 68,36, 68.07, 63.09, 54.24, 51.60, 51.60, 50.33, 49.26, 41.59, 41.25, 40.86, 28.99; ^{195}Pt NMR (107 MHz, D_2O): δ = -2359.7; MS (ESI-negative): m/z = 963 $[M-H]^-$, 999 $[M+Cl]^-$, calcd. for $C_{26}H_{53}N_9O_{13}PtCl_2$ 964.28.

HSQC (600MHz, H_2O) of compound **1**:



TOCSY (600MHz, H_2O) of compound **1**: Blue dashed lines: ring system I; Orange lines: ring system III; Green/yellow lines: ring systems II/IV; Red dashed lines: chelating linker V



S.2 Synthesis of 2 (scheme 2):

7: **4** (255 mg, 0.21 mmol) was treated with 50% TFA (20 mL) in dichloromethane and stirred for 30 min at RT. The solvent was removed, and the colorless residue was dissolved in MeOH (10 mL) and triethylamine (300 µL). After adding of *N,N′*-diBoc-*N″*-triflylguanidine (1.10 g, 2.81 mmol), the reaction was stirred for 2 d at 40 °C. The solution was concentrated in vacuo. Flash chromatography (silica gel, 0-10% methanol in chloroform) afforded the desired product as a colorless solid (430 g, 0.21 mmol, 99%). R_f = 0.6 (chloroform/methanol 10/1).
^{1}H NMR (MeOD, 300 MHz): δ = 5.94 (d, *J* = 5 Hz, 1H), 5.08 (d, *J* = 3 Hz, 1H), 5.02 (d, *J* = 1 Hz, 1H), 4.61-4.50 (m, 1H), 4.30-4.03 (m, 5H), 3.98-3.60 (m, 8H), 3.54-3.39 (m, 8H), 2.26-2.18 (m, 1H), 1.60-1.34 (m, 109H); MS (ESI-positive): m/z = 2093 $[M+H]^+$, calcd for $C_{89}H_{153}N_{21}O_{36}$ 2092.07.

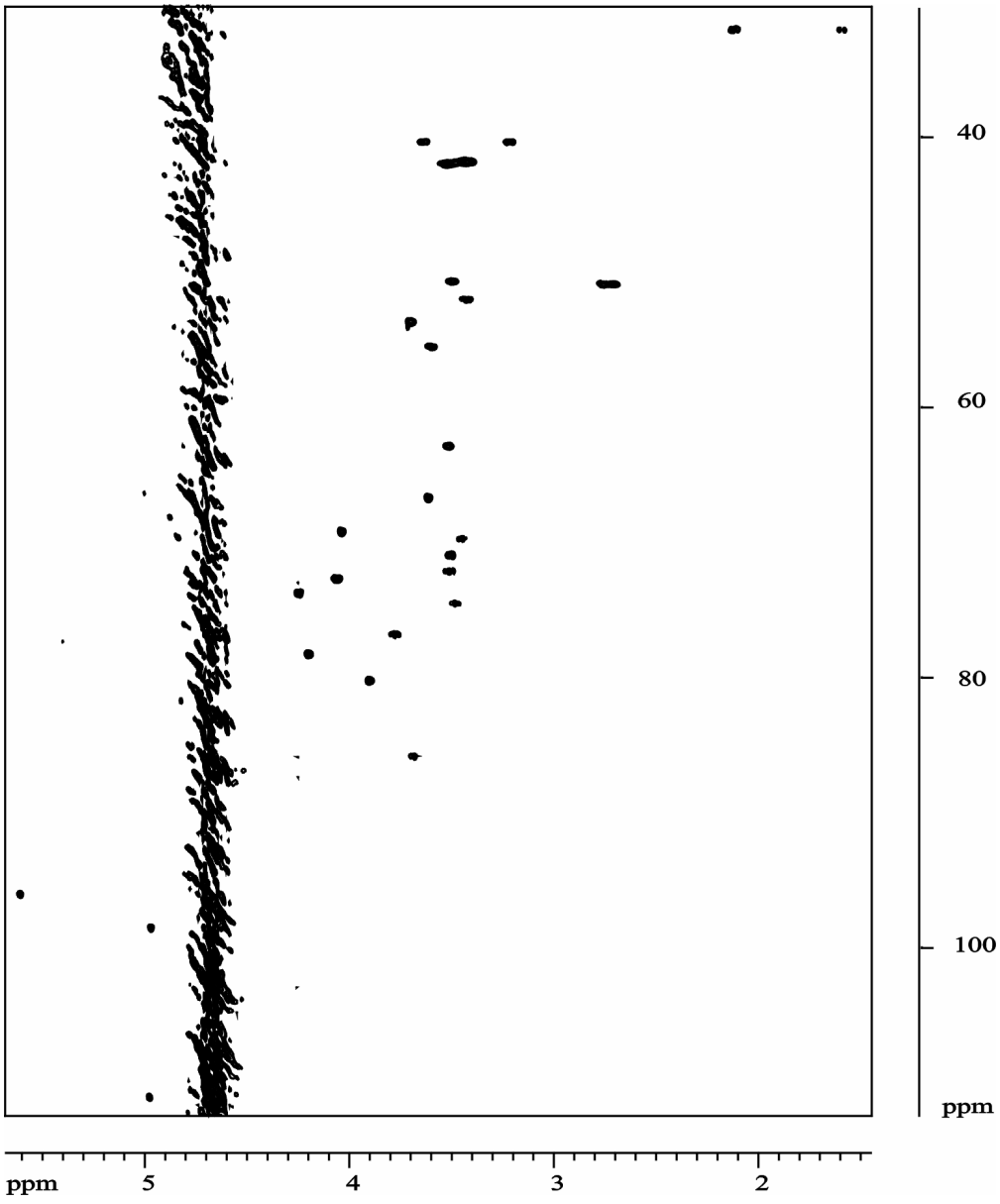
8: **7** (420 mg, 0.20 mmol) was dissolved in THF (15 mL) and treated with triphenylphosphine (350 mg, 1.34 mmol) and water (10 µL). The clear solution was stirred for 2 d at RT. The solvent was removed and the residue dried in vacuo. Flash chromatography (silica gel, 0-10% methanol in chloroform) afforded the desired product as a colorless solid (311 mg, 0.15 mmol, 75%). R_f = 0.3 (chloroform/methanol 10/1).
^{1}H NMR (MeOD, 300 MHz): δ = 5.87 (d, *J* = 4 Hz, 1H), 5.01 (br s, 2H), 4.53-4.49 (m, 1H), 4.36-4.30 (m, 2H), 4.25-4.19 (m, 3H), 4.19-4.05 (m, 1H), 4.00-3.62 (m, 8H), 3.50-3.40 (m, 3H), 3.35-3.15 (m, 2H), 2.83-2.75 (m, 2H), 2.25-2.15(m, 1H), 1.70-1.30 (m, 109H); MS (ESI-positive): m/z = 2067 $[M+H]^+$, calcd for $C_{89}H_{155}N_{19}O_{36}$ 2066.09.

9: To a solution of **8** (90 mg, 44.0 µmol) in DMF/chloroform 1:1 (8 mL) and 2,4,6-collidine (124 µL, 1.14 mmol) TBTU (18.0 mg, 57.0 µmol), HOBt (8.70 mg, 57.0 µmol) and N^{α},N^{β}-Di-Fmoc-(S)-2,3-diaminopropionic acid (31.0 mg, 57.0 µmol) in DMF (1 mL) were added. The reaction mixture was stirred at RT for 4 h. After the reaction was quenched with methanol and stirred for another 1h, the solvent was removed in vacuo. The residue was redissolved in 20% piperidine in DMF (10 mL). After stirring for 30 min at RT the reaction mixture was concentrated and dried in vacuo. Flash chromatography (silica gel, 0-10% methanol in chloroform) afforded the desired product as a colorless solid (60 mg, 28.0 µmol, 63%). R_f = 0.2 (chloroform/methanol 10/1).
^{1}H NMR (MeOD, 300 MHz): δ = 5.84 (d, *J* = 3 Hz, 1H), 5.03 (s, 1H), 4.96 (d, *J* = 1.0 Hz, 1H), 4.61-4.48 (m, 1H), 4.44-4.41 (m, 1H), 4.24-4.18 (m, 2H), 4.16-4.04 (m, 1H), 3.98-3.54 (m, 10H), 3.52-3.42 (m, 4H), 3.32-3.16 (m, 4H), 2.98-2.90 (m, 1H), 2.82-2.72 (m, 1H), 2.26-2.15 (m, 1H), 1.70-1.30 (m, 109H); MS (ESI-positive): m/z = 2153 $[M+H]^+$, calcd for $C_{92}H_{161}N_{21}O_{37}$ 2152.14.

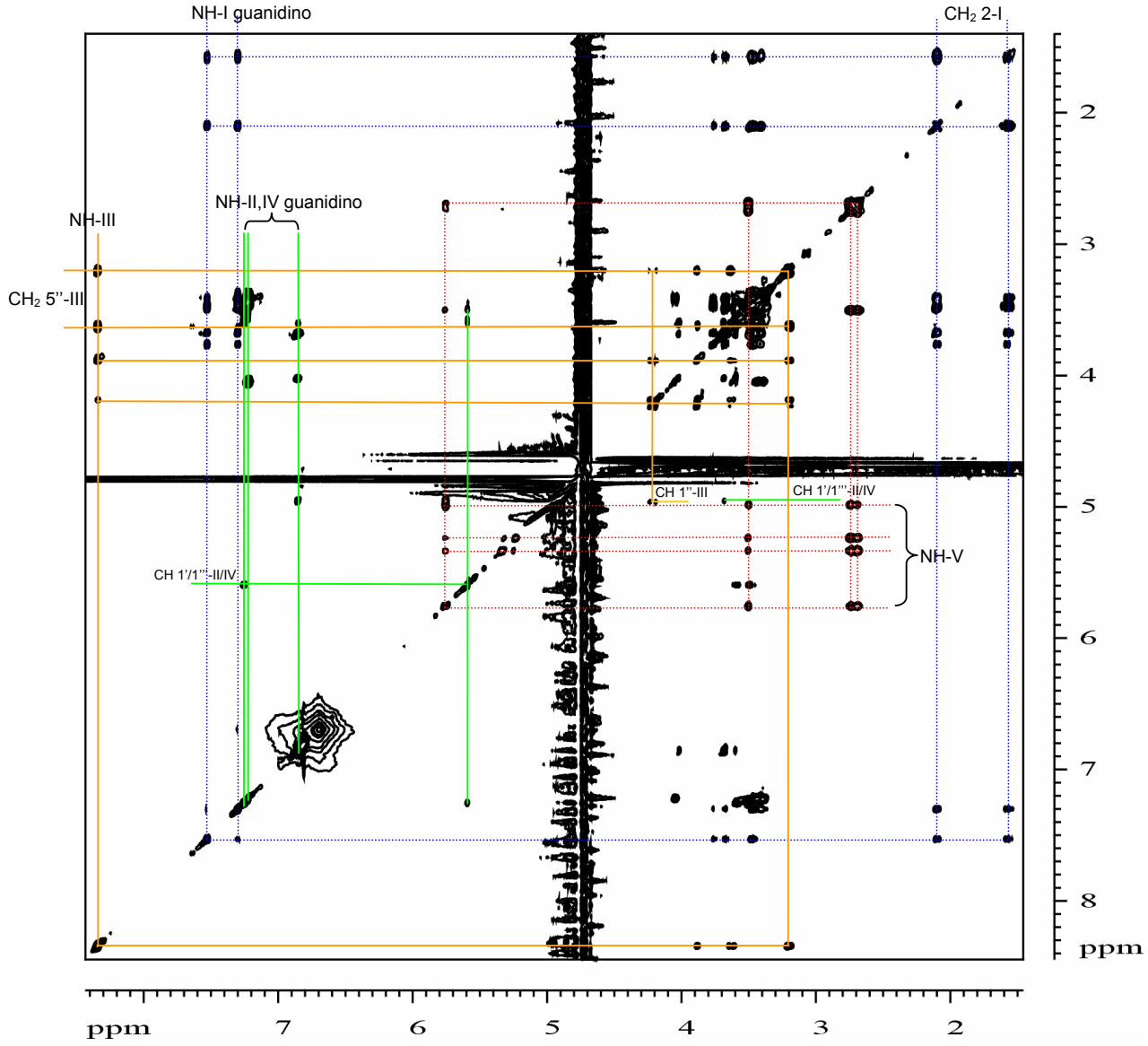
2: **9** (14.1 mg, 6.54 µmol) was dissolved in dichloromethane (5 mL) and treated with a solution of K_2PtCl_4 (50 mg, 0.12 mmol) in water (2 mL). Tetrabutylammonium chloride (20 mg) was added to the two phase system. The reaction was stirred in the dark for 6 h at RT. The organic phase was separated, dried over sodium sulfate and concentrated in vacuo. The Boc-protected (R_f = 0.8, chloroform/methanol 10/1) product was purified by

flash chromatography. The solution was concentrated in vacuo. The residue was treated with 75% TFA in dichloromethane (10 mL) at RT for 2 h. Toluene (10 mL) was added and the solvent was removed in vacuo. The residue was suspended in water (2 mL) and filtered. Lyophilization yielded to the desired product as TFA-salt in form of a colorless solid (11.70 mg, 6.15 µmol, 94%).
^{1}H NMR (600 MHz, H_2O): δ = 8.32 (m, 1H), 7.51 (d, *J* = 4 Hz, 1H), 7.28 (d, *J* = 5 Hz, 1H), 7.25-7.21 (m, 4H), 6.84-6.80 (m, NH-guanidino), 6.67 (br s, NH-guanidino), 6.25 (br s, NH-guanidino), 6.15 (br s, NH-guanidino), 5.74 (br s, 1H), 5.56 (d, *J* = 1.6 Hz, 1H), 5.32 (br s, 1H), 5.22 (br s, 1H), 4.21 (br s, 1H), 4.17 (m, 1H),4.15 (m, 1H), 4.04-3.99 (m, 2H), 3.68 (m, 1H), 3.72 (m, 1H), 3.68-3.55 (m, 5H), 3.48-3.34 (m, 11H), 3.17 (m, 1H), 2.72 (m, 1H), 2.67 (m, 1H), 2.08 (m, 1H), 1.54 (m, 1H); ^{13}C NMR (150 MHz, H_2O, from HSQC): 111.41, 98.88, 96.40, 86.22, 80.54, 78.67, 77.11, 74.90, 74.16, 73.03, 72.42, 71.42, 71.21, 70.04, 69.60, 67.00, 63.18, 55.88, 54.08, 52.37, 51.29, 51.08, 42.36, 42.20, 40.71, 32.39; ^{195}Pt NMR (107 MHz, D_2O): δ = -2361.1; MS (ESI-positive): m/z = 1217.20 $[M+H]^+$, calcd for $C_{32}H_{65}N_{21}O_{13}PtCl_2$ 1216.41.

HSQC (600MHz, H_2O) of compound **2**:



TOCSY (600MHz, H_2O) of compound **2**: Blue dashed lines: ring system I; Orange lines: ring system III; Green lines: ring systems II/IV; Red dashed lines: chelating linker V



S.3 BODIPY-Neomycin Association with RRE Constructs

To probe the relative affinity of neomycin for the different RRE JW constructs used in these studies, two BODIPY-Neomycin B conjugates have been used (Figure S 3.1). BODIPY is a small, non-intercalative, neutral dye and therefore only a minor perturbation in RNA affinity is introduced by conjugation of BODIPY to neomycin.

Association experiments are conducted by titrating RNA into a solution containing 10 nM of either Amino Neo-BODIPY or Guanidino Neo-BODIPY (λ_{Ex} = 490 nm, λ_{Em} = 530 nm) at 22 °C in 100 mM sodium perchlorate, 10 mM sodium phosphate pH 7.0. At least six individual anisotropy readings are averaged for each concentration. The fluorescence anisotropy of the BODIPY-conjugate increases upon binding of the RNA (figure S 3.2), and the association curve is used to determine the C_{50} value (defined as the concentration of RNA needed to bind ½ of the BODIPY conjugate). If a 1:1 stoichiometry is assumed, the C_{50} value is approximately equal to the K_d when C_{50} is significantly higher than the concentration of the fluorescent probe (10 nM). C_{50} values that are in same order as the concentration of fluorescent probe can also be used to estimate K_d values, but a robust assessment of binding stoichiometery must be taken into consideration. Table S 3.1 summarizes the C_{50} values for Amino Neo-BODIPY and Guanidino Neo-BODIPY binding to RRE JW, 3I RRE JW, and dRRE JW.

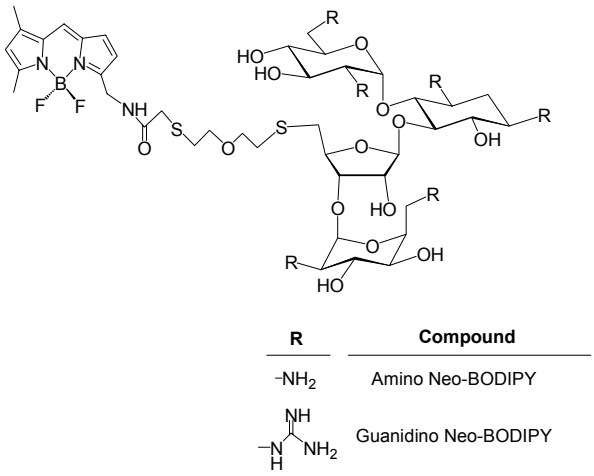


Figure S3.1 Structures of the neomycin B probes "Amino Neo-BODIPY" and "Guanidino Neo-BODIPY" (synthesis to be reported elsewhere).

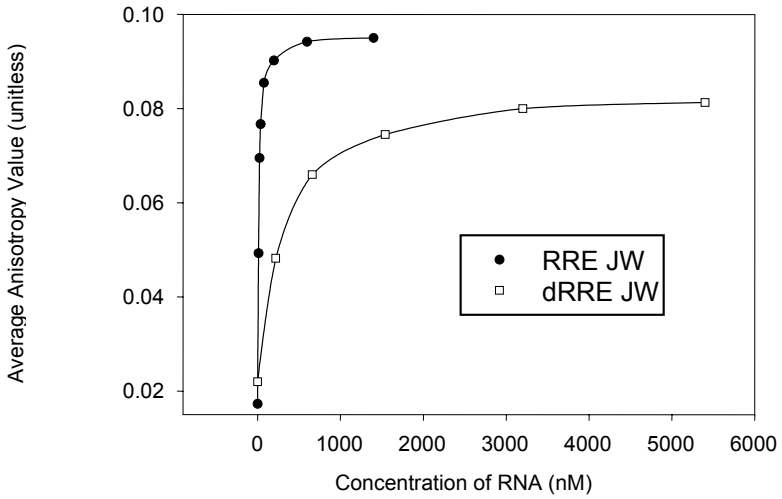


Figure S3.2 Examples of the changes in fluorescence anisotropy of Guanidino Neo-BODIPY as a function of RNA concentration.

Table S3.1 C_{50} values (nM) according to fluorescence anisotropy.*

	Amino Neo-BODIPY	Guanidino Neo-BODIPY
RRE JW	60	15
3 I RRE JW	800	17
dRRE JW	12,000	250

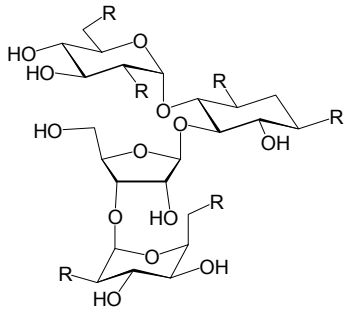
*Estimated error +/- 30% of reported C_{50} value.

S.4 BODIPY-Neomycin Displacement from RRE JW

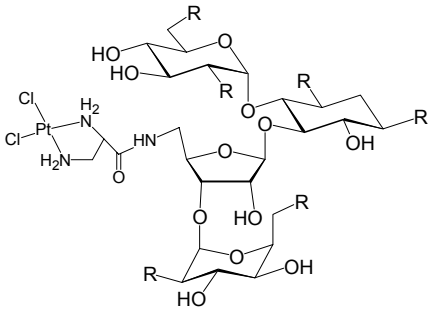
To determine what effect the Pt(II) conjugating linker has on the RRE affinity of neomycin B and guanidino neomycin B, the displacement of BODIPY-Neomycin from the RRE JW has been conducted. The covalent crosslinking reactivities of **1** and **2** (figure S 4.1) were deactivated by treatment of 10 mM stocks of these compounds with 40 mM KCN. KCN has been shown to effectively deactivate cis-platin, leaving just the amino ligands. (REF). DPAGE experiments confirm that these stocks are not capable of forming covalent cross-links with the RRE.

Displacement experiments were conducted by mixing 10 nM of either Amino Neo-BODIPY or Guanidino Neo-BODIPY (figure S 3.1) with 50 nM of RRE JW. By monitoring the fluorescence anisotopy of the BODIPY conjugate (as described in S.3), the displacement of the BODIPY conjugate is apparent (figure S 4.2). The concentration of compound needed to displace ½ of the BODIPY conjugate from the RRE is the IC_{50} value

for that compound. These values are summarized in Table S 4.1. Since multiple equivalents of neomycin can bind the RRE, the K_i values estimated in Table S 4.1 are for comparison purposes only. These values suggest that the addition of BODIPY to Neomycin introduces a small (2 – 7 fold) increase in affinity for RRE JW; likewise, the conjugation of Pt to Neomycin introduces a small (2 – 4 fold) increase in affinity for the RRE JW.



R	Compound
$-NH_2$	Neomycin B
	Guanidino Neomycin B



R	Compound
$-NH_2$	**1**
	**2**

Figure S4.1 Structures of Neomycin B, Guanidino Neomycin B and the platinated versions of these glycosides.

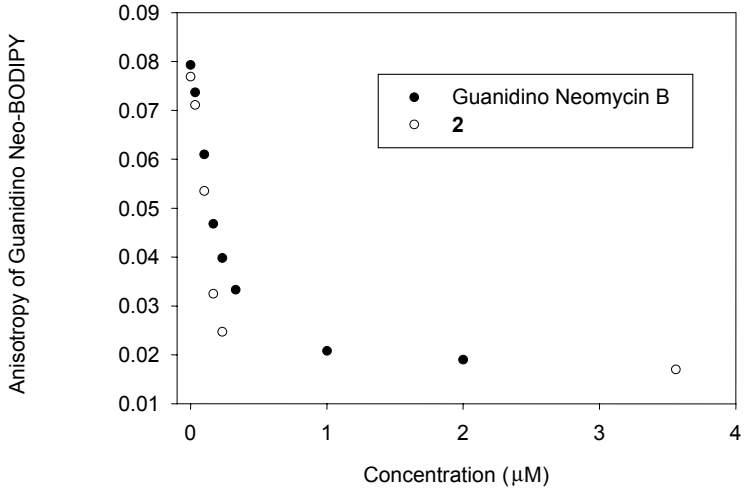


Figure S4.2 Displacement of Guanidino Neo-BODIPY from RRE JW by **2** and Guanidino Neomycin B.

Table S4.1 Summary of IC_{50} values (nM) for displacement from RRE JW

Compound	Compound Displaced	IC_{50}*
Neomycin B	Amino Neo-BODIPY	400
1**	Amino Neo-BODIPY	120
Guanidino Neomycin B	Guanidino-Neo-BODIPY	155
2**	Guanidino-Neo-BODIPY	120

* Estimated error +/- 30% of reported value.

*** CN^- treated (see above).

S.5 Displacement of a Rev peptide from RRE JW by native PAGE

Native gel shift experiments were conducted in order to evaluate the ability of **1** to inhibit the normal function of the RRE (i.e. its ability to bind Rev). Reaction conditions and electrophoresis were carried out as described in section S.3 except that no urea was contained within the gel and 2 μM of the Rev 35-50 peptide "Rev-IA" was included. RRE JW, under native conditions, consistently appears as two bands, suggesting two alternate folds for this RNA (Figure S 6.1). Each band, however, exhibits similar behavior. The peptide Rev-IA has the sequence $_{suc}$TRQARRNRRRRWRERQRAAAAC$_{am}$ and was synthesized as described (*Angew. Chem. Intl. Ed.* **2000**, *39*, 1788-1790).

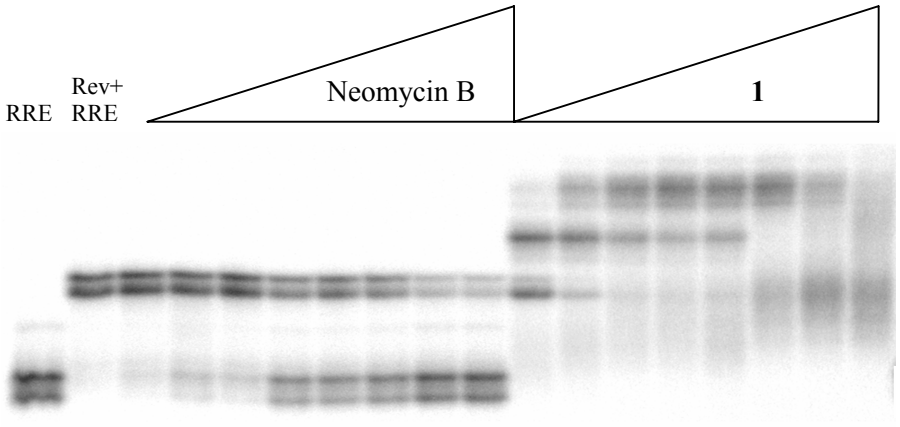


Figure S5.1 Native gel-shift. 25 nM of RRE JW is mixed with 2 mM of "Rev-IA", then Neomycin B is titrated (0.5 μM, 1.0 μM, 1.25 μM, 2.5 μM, 3.75 μM, 5.0 μM, 7.5 μM, 10 μM) or **1** is titrated (0.25 μM, 0.5 μM, 0.75 μM, 1.0 μM, 1.25 μM, 2.5 μM, 5.0 μM, 10 μM).

Interestingly, at low concentrations, **1** can cross-link the Rev-RRE complex without displacing the peptide. It appears that three equivalents of **1** are necessary for peptide displacement. This may be true with Neomycin B as well, but neomycin may not form a kinetically stable tertiary complex with Rev-RRE that can be observed by electrophoresis.