Supplementary Information

Solid-phase synthesis and DNA binding studies of dichloroplatinum(II) conjugates of dicarba analogues of octreotide as new anticancer drugs

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1.1. Abbreviations

ACN: acetonitrile; DCM: dichloromethane; DIPC: *N,N'*-diisopropylcarbodiimide; DIPEA: *N,N*-diisopropylethylamine; DMF: *N,N*-dimethylformamide; EDC: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; ESI: electrospray ionization; Fmoc: 9-fluorenylmethyloxycarbonyl; HATU: (2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt: 1-hydroxybenzotriazole; HOSu: *N*-hydroxysuccinimide; HPLC: high performance liquid chromatography; MALDI-TOF: matrix-assisted laser desorption ionization time-of-flight; MS: mass spectrometry or mass spectrum; NMR: nuclear magnetic resonance; TIS: triisopropylsilane; TLC: thin-layer chromatography; TFA: trifluoroacetic acid.

1.2. Materials and Methods

Unless otherwise stated, common chemicals and solvents (HPLC grade or reagent grade quality) were purchased from commercial sources and used without further purification. Fmoc-protected amino acids, resins and coupling reagents for solid phase synthesis were obtained from Novabiochem, Bachem or Iris Biotech. Solid-phase syntheses were performed manually in a polypropylene syringe fitted with a polyethylene disc. Octreotide acetate was purchased from Bachem.

The synthesis of [$PtCl_2$(dap)] **2** and Fmoc-L-threoninol *p*-carboxyacetal was carried out following previously reported procedures (references 8 and 13 in the article, respectively).

TLC analyses were conducted on aluminium plates coated with a 0.2 mm thick layer of silica gel 60 F_{254} (Merck). Purification by flash column chromatography was carried out using silica gel 60 (230-400 mesh).

All compounds were fully characterized by NMR and MS:

-NMR spectra were recorded at 25ºC on a Varian Gemini 200 or 300 MHz and Varian Mercury 400 MHz spectrometers using deuterated solvents. Tetramethylsilane (TMS) was used as an internal reference (0 ppm) for ^{1}H spectra recorded in $CDCl_3$ and the residual signal of the solvent (77.16 ppm) for ^{13}C spectra. For CD_3OD, d_6-DMSO, d_7-DMF or D_2O, the residual signal of the solvent was used as a reference.

-MALDI-TOF mass spectra were recorded on a Voyager-DE^{TM}RP spectrometer (Applied Biosystems). ESI mass spectra (ESI-MS) were recorded on a Micromass

ZQ instrument with single quadrupole detector coupled to an HPLC, and high-resolution (HR) ESI-MS on an Agilent 1100 LC/MS-TOF instrument.

1.3. Synthesis of [$PtCl_2$(dap)]-octreotide conjugate 3

Octreotide acetate **1** (10 mg, 0.009 mmol), [$PtCl_2$(dap)] **2** (4.1 mg, 0.011 mmol), *N*-hydroxysuccinimide (1.6 mg, 0.013 mmol) and EDC hydrochloride (3.5 mg, 0.018 mmol) were dissolved in 0.6 mL of anhydrous DMF (previously bubbled with N_2 for 2 h) and stirred for 24 h at rt. Reversed HPLC showed the presence of a main peak (~ 70 %) which was isolated and characterized as the target conjugate **3**.

Conjugate 3: R_t= 9.1 min (gradient: 30 to 80 % in 30 min); MALDI-TOF-MS, positive mode: *m/z* 1369.1; ESI-MS, positive mode: *m/z* 1370.6 (calcd mass for $C_{52}H_{73}Cl_2N_{12}O_{11}PtS_2$ $[M+H]^+$: 1370.40).

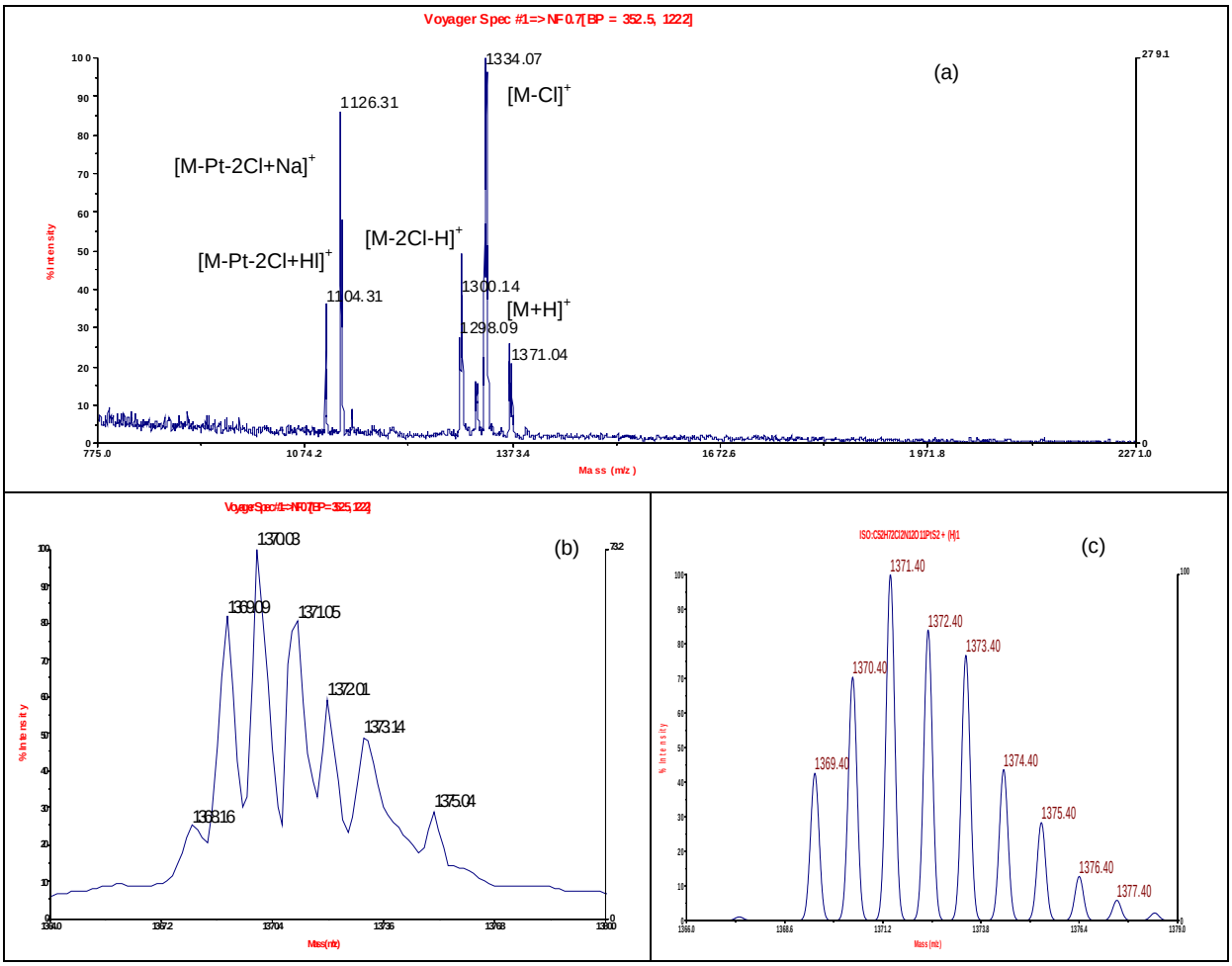

Figure S-1. MALDI-TOF MS of conjugate **3**: experimental (a, b) and calculated (c).

1.4. Solid-phase synthesis of dichloroplatinum(II) conjugates of dicarba analogues of octreotide 6 and 7

1.4.1. General procedures

Peptides were synthesized on a Rink amide resin-*p*-MBHA (f = 0.34 mmol/g, 100-200 mesh) using standard Fmoc/^{t}Bu chemistry with the following side-chain protecting groups: Boc (*N*^{i}-*tert*-butoxicarbonil, Tryptophan) and ^{t}Bu (*O*-*tert*-butyl, Threonine).
First, Fmoc-L-threoninol *p*-carboxyacetal (1.8 eq) was quantitatively incorporated using DIPC (1.8 eq) and HOBt (1.8 eq) as coupling reagents in anhydrous DMF for 1 h. The following Fmoc-protected amino acids and Fmoc-γ-Abu-OH (2.4 eq) were coupled with HATU (2.3 eq) and DIPEA (4.8 eq) in anhydrous DMF for 1 h. [$PtCl_2$(dap)] complex **2** was coupled with DIPC and HOBt in anhydrous DMF following standard procedures.

Microwave-assisted ring closing methatesis

RCM reactions were carried out in a high-pressure quartz microwave vessel fitted with self-sealing Teflon septa and equipped with a magnetic stirrer bead. Peptide-resin, second generation Grubbs catalyst (0.2 eq) and solvent (a 9:1 mixture of dry and degassed DCM/0.4 M LiCl in DMF; 0.04 mL/mg resin) were loaded into the vessel and irradiated at 100 ºC for 1 h (70 W). Once at room temperature, the resin was transferred into a polypropylene syringe fitted with a polyethylene disc and successively washed with DMF, DCM and MeOH. Finally, peptide-resin was treated with DMSO (50 eq) in DCM for 1 h at rt to remove the excess of catalyst. After washing with DMF, DCM and MeOH, the peptide-resin was dried *in vacuo*.

Hydrogenation

In a dry quartz vessel, peptide-resin, Wilkinson's catalyst (0.3 eq) and dry deoxygenated solvent (9:1 mixture of DCM/MeOH; 0.1 mL/mg resin) were introduced. The system was purged three times using a vacuum and an argon flushing cycle before being pressurised with hydrogen gas (50 Bar). The reaction was stirred at 50ºC for 24 h. Once the hydrogen gas was vented, the peptide-resin was collected via filtration through a polypropylene syringe fitted with a polyethylene disc and successively washed with DMF, DCM and finally MeOH, before being allowed to dry *in vacuo*.

Cleavage and deprotection

Side-chain deprotection and cleavage from the resin was performed simultaneously either with TFA/TIS/H_2O 95:2.5:2.5 (peptides) or TFA/phenol/H_2O 95:2.5:2.5 (platinated peptides, **6** and **7**) for 1 h at room temperature. Most of the TFA was removed by bubbling N_2 into the solution, and the resulting residue was poured onto cold ether to precipitate the target peptide.

HPLC analysis

Analytical reversed-phase high-performance liquid chromatography (HPLC) analyses were carried out on Kromasil or Nucleosil C_{18} columns (250x4.6 mm, 5 μm, flow rate: 1 mL/min), using linear gradients of 0.045% TFA in H_2O (solvent A) and 0.036% TFA in ACN (solvent B). Small-scale purification was carried out using the same column.

MS analysis

MALDI-TOF MS analysis was carried out in the positive mode using 2,4-dihidroxybenzoic acid as a matrix.

1.4.2. MS characterization of conjugates 6 and 7

Conjugate 6 *cis*: R_t= 19.1 and 19.3 min (gradient: 20 to 60 % in 30 min); MALDI-TOF-MS, positive mode: *m/z* 1417.0; ESI-MS, positive mode: *m/z* 1417.9 (calcd mass for $C_{58}H_{82}Cl_2N_{13}O_{12}Pt$ $[M+H]^+$: 1417.52).

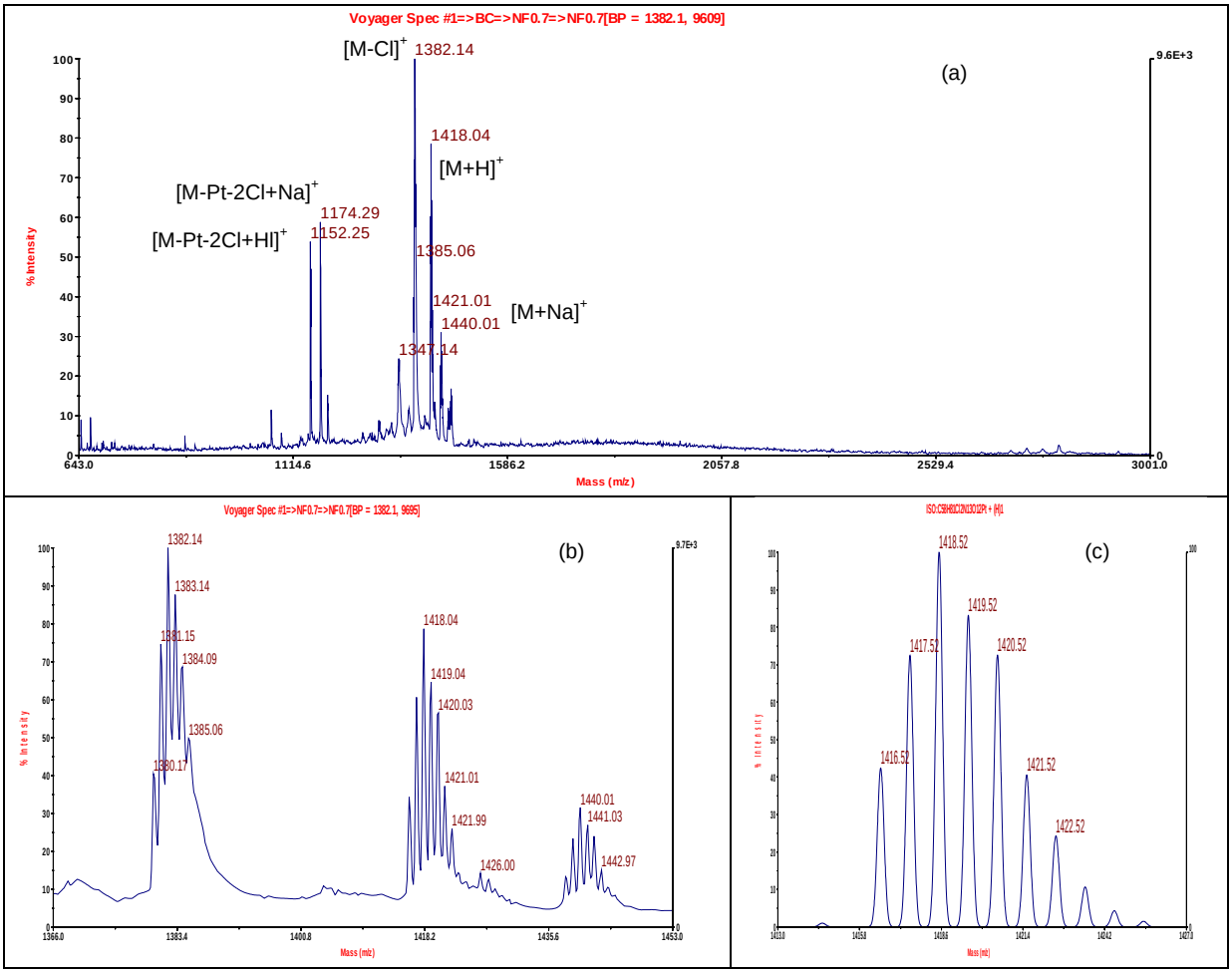

Figure S-2. MALDI-TOF MS of conjugate **6 *cis***: experimental (a, b) and calculated (c).

Conjugate 6 *trans*: R_t= 18.6 and 18.7 min (gradient: 20 to 60 % in 30 min); MALDI-TOF-MS, positive mode: *m/z* 1416.9; ESI-MS, positive mode: *m/z* 1417.9 (calcd mass for $C_{58}H_{82}Cl_2N_{13}O_{12}Pt$ $[M+H]^+$: 1417.52).

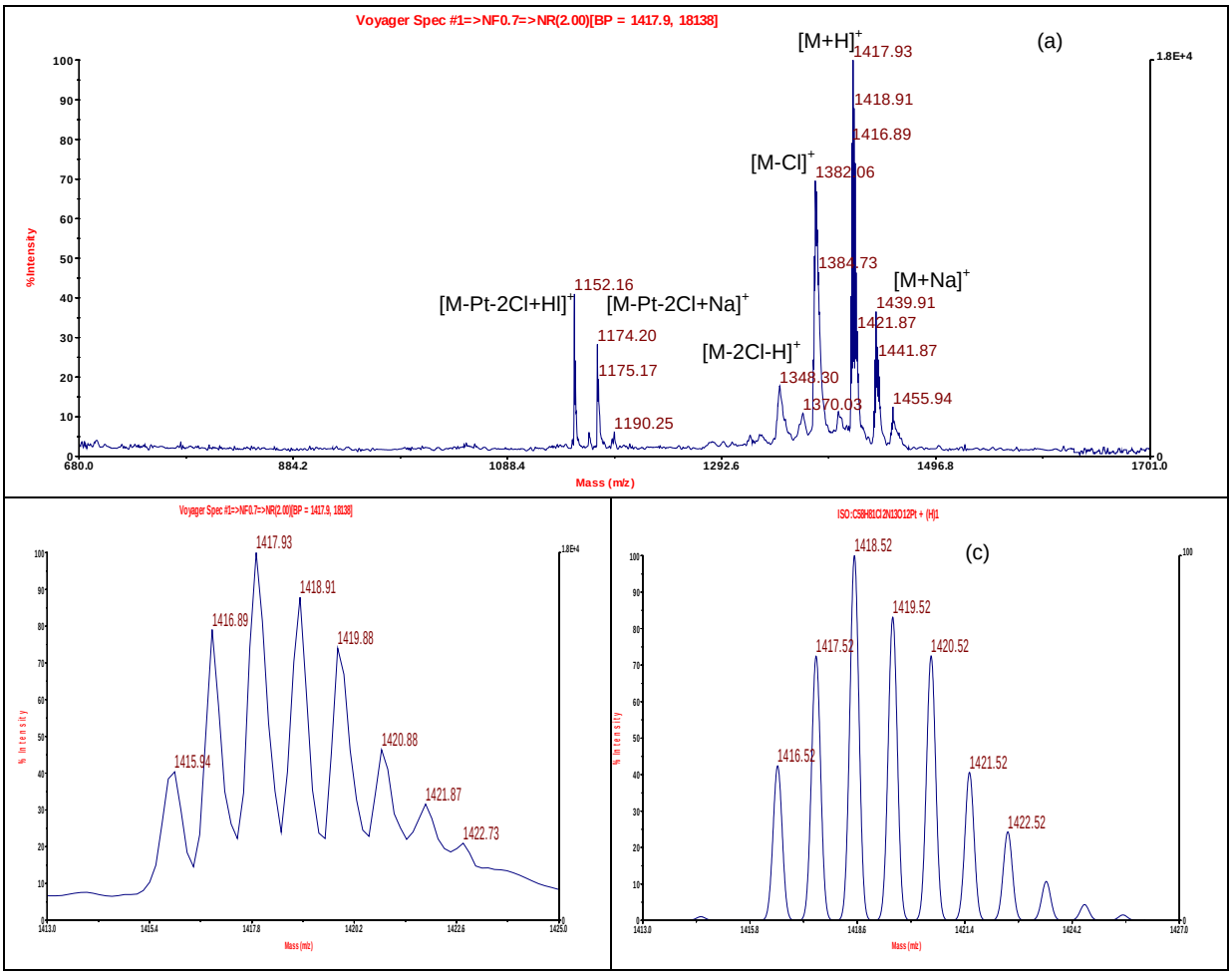

Figure S-3. MALDI-TOF MS of conjugate **6 *trans***: experimental (a, b) and calculated (c).

Conjugate 7: R_t= 18.7 and 18.8 min (gradient: 20 to 60 % in 30 min); MALDI-TOF-MS, positive mode: *m/z* 1419.7; ESI-MS, positive mode: *m/z* 1419.9 (calcd mass for $C_{58}H_{84}Cl_2N_{13}O_{12}Pt$ $[M+H]^+$: 1419.54).

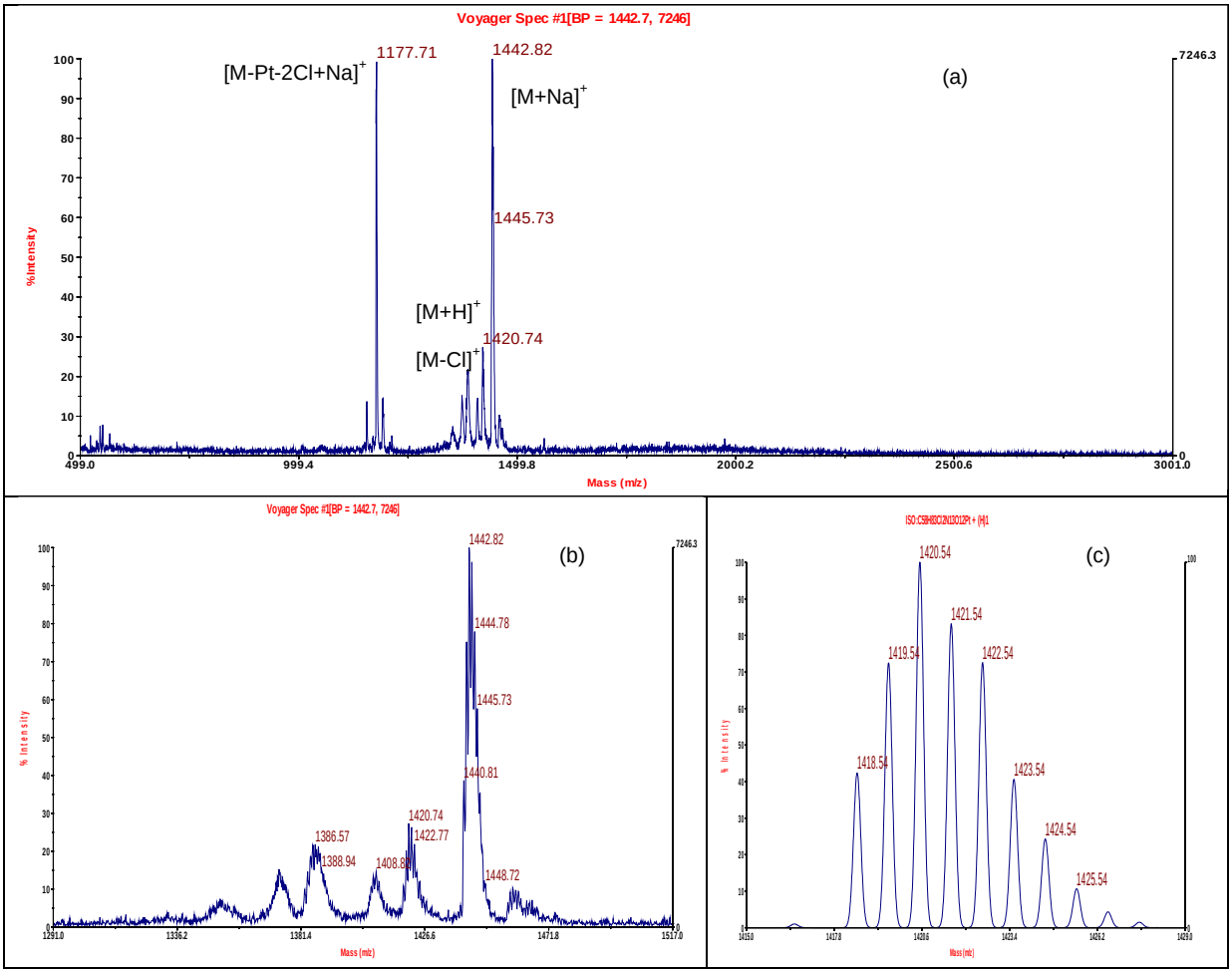

Figure S-4. MALDI-TOF MS of conjugate **7**: experimental (a, b) and calculated (c).

1.5. DNA binding studies

Complexation reactions were carried out in H_2O at 37ºC. The required volume of an aqueous solution of [$PtCl_2$(dap)] complex or dichloroplatinum(II) conjugate was added to the oligonucleotide, $^{5'}$dCATGGCT, previously liophylised in an eppendorf tube and dissolved in water, so that the relative proportion oligonucleotide/platinum(II) derivative was 1:1. The solutions were 0.1 mM in oligonucleotide. [$PtCl_2$(dap)] was solubilised and aquatised by heating for 10-15 min at 90ºC, and the required amount of the resulting solution was immediately added to the solution of the oligonucleotide. The evolution of the reactions was monitored by reversed-phase HPLC on Kromasil C18 columns (250x4.6 mm, 10 μm, flow rate: 1 mL/min), using linear gradients of aqueous triethylammonium acetate (0.05 M) (solvent A) and ACN (solvent B). Platinum adducts were isolated after several HPLC runs by using analytical separation conditions. MALDI-TOF MS analysis was carried out in the negative mode using 2,4,6-trihidroxyacetophenone matrix with ammonium citrate as an additive. Enzymatic digestions with 5'- and 3'-exonucleases (calf spleen and snake venom phosphodiesterases, respectively) were performed as previously described (references 16 and 17 on the article).

1.5.1. Reaction between $^{5'}$dCATGGCT and conjugate 6 (*cis*)

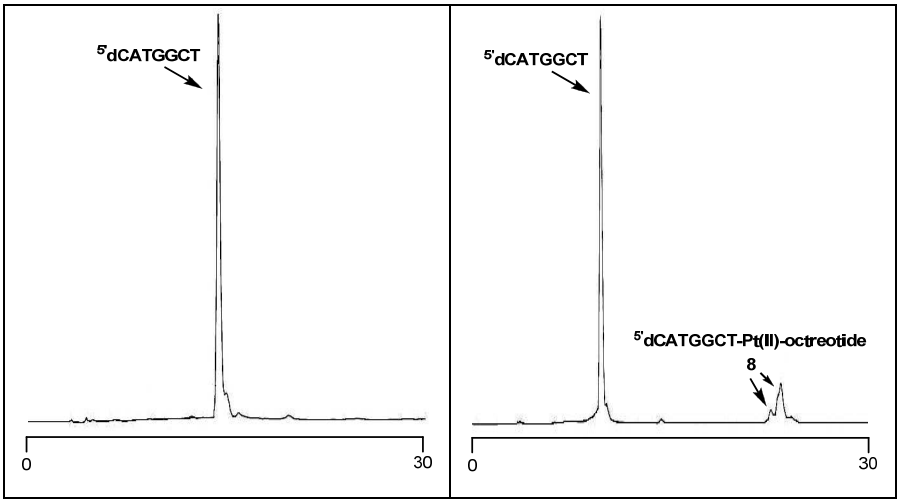

Figure S-5. HPLC traces of the oligonucleotide (left) and of the reaction mixture at 24 h (right).

Adduct $^{5'}$dCATGGCT-Pt(II)-octreotide 8: R_t= 22.4, 23.2 and 23.6 min (gradient: 5 to 35 % in 30 min); MALDI-TOF-MS, negative mode: *m/z* 3440.9; (calcd mass for $C_{126}H_{165}N_{38}O_{53}P_6Pt$ $[M-3H]^-$: 3438.95).

MALDI-TOF-MS after digestion with calf spleen phosphodiesterase: *m/z* 2534.4 (-CpApTp) and 2839.5 (-CpAp) (calcd mass for $C_{97}H_{128}N_{28}O_{35}P_3Pt$ $[M-3H]^-$: 2532.82 and for $C_{107}H_{141}N_{30}O_{42}P_4Pt$ $[M-3H]^-$: 2838.87, respectively).

MALDI-TOF-MS after digestion with snake venom phosphodiesterase: *m/z* 2847.4 (-pCpT) (calcd mass for $C_{107}H_{140}N_{33}O_{40}P_4Pt$ $[M-3H]^-$: 2847.88).

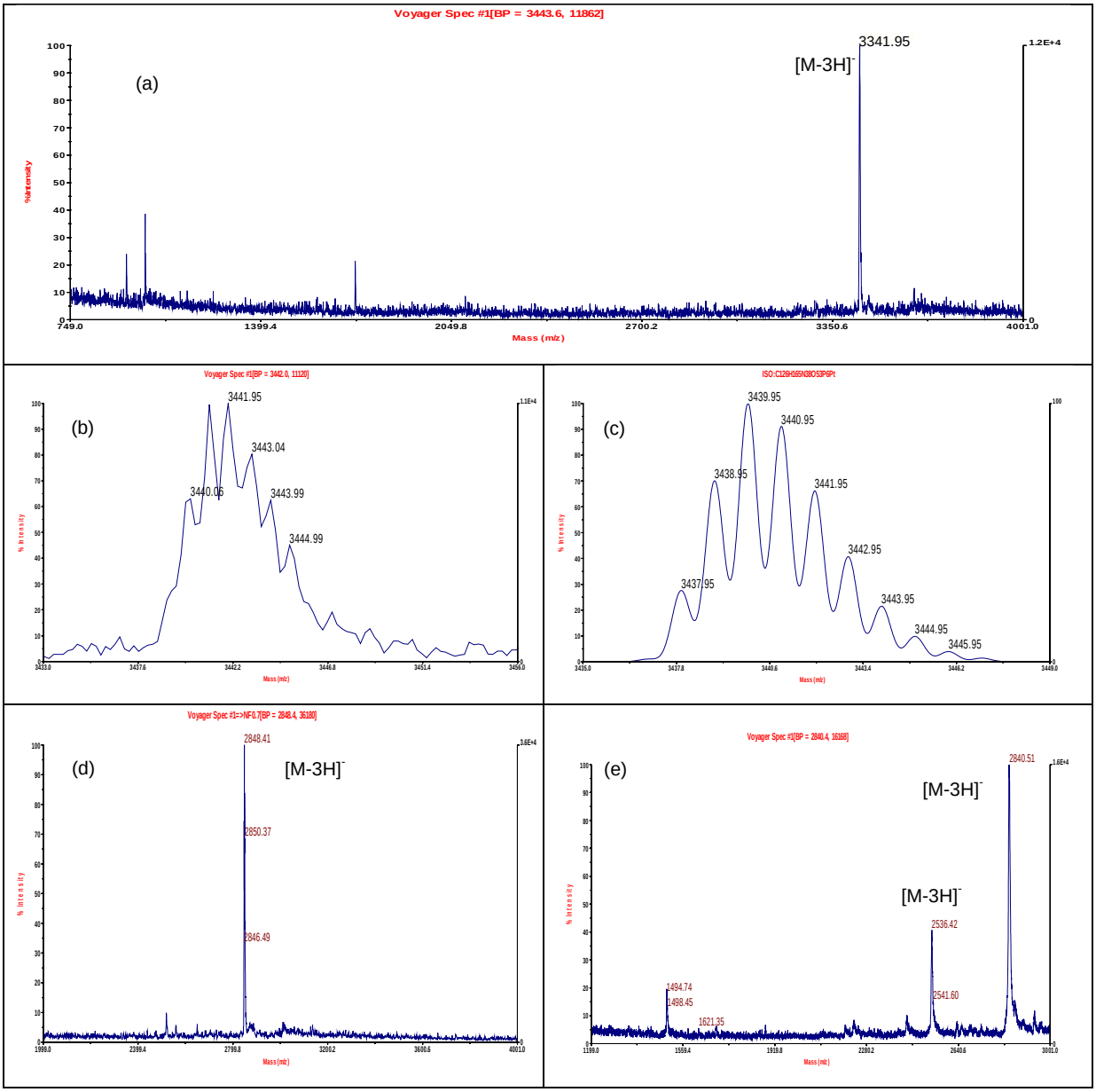

Figure S-6. MALDI-TOF MS of adduct **8** (peak R_t 22.4 min): experimental (a, b), calculated (c) and after digestion with snake venom (d) or calf spleen phosphodiesterase (e).

1.5.2. Reaction between 5'dCATGGCT and [$PtCl_2$(dap)] 2

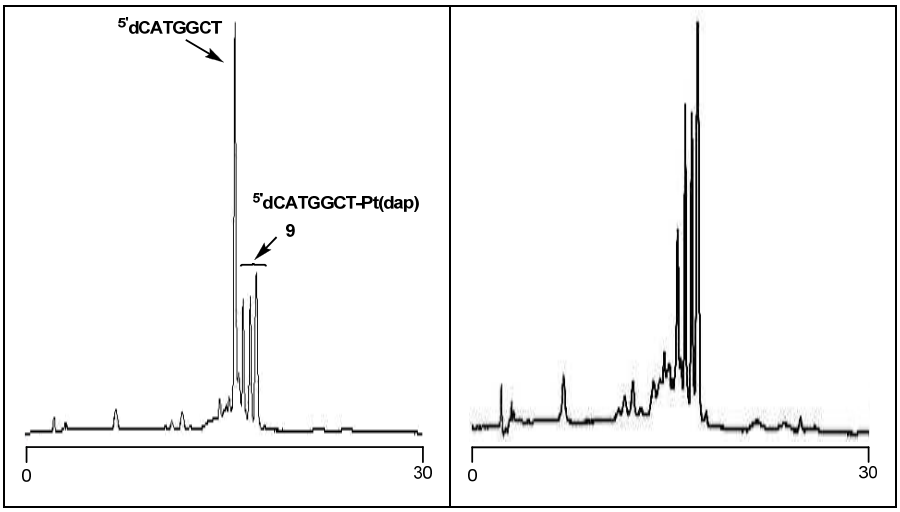

Figure S-5. HPLC traces of the reaction mixture at 20 h (left) and 48 h (right).

Adduct 5'dCATGGCT-[$PtCl_2$(dap)] 9: R_t= 16.6, 17.1 and 17.6 min (gradient: 5 to 35 % in 30 min); MALDI-TOF-MS, negative mode: *m/z* 2392.0; (calcd mass for $C_{71}H_{92}N_{27}O_{43}P_6Pt$ $[M-3H]^-$: 2391.42).

MALDI-TOF-MS after digestion with calf spleen phosphodiesterase: *m/z* 1488.1 (-CpApTp) and 1792.1 (-CpAp) (calcd mass for $C_{42}H_{55}N_{17}O_{25}P_3Pt$ $[M-3H]^-$: 1486.27 and for $C_{52}H_{68}N_{19}O_{32}P_4Pt$ $[M-3H]^-$: 1790.31, respectively).

MALDI-TOF-MS after digestion with snake venom phosphodiesterase: *m/z* 1799.5 (-pCpT) (calcd mass for $C_{52}H_{67}N_{22}O_{30}P_4Pt$ $[M-3H]^-$: 1797.3).

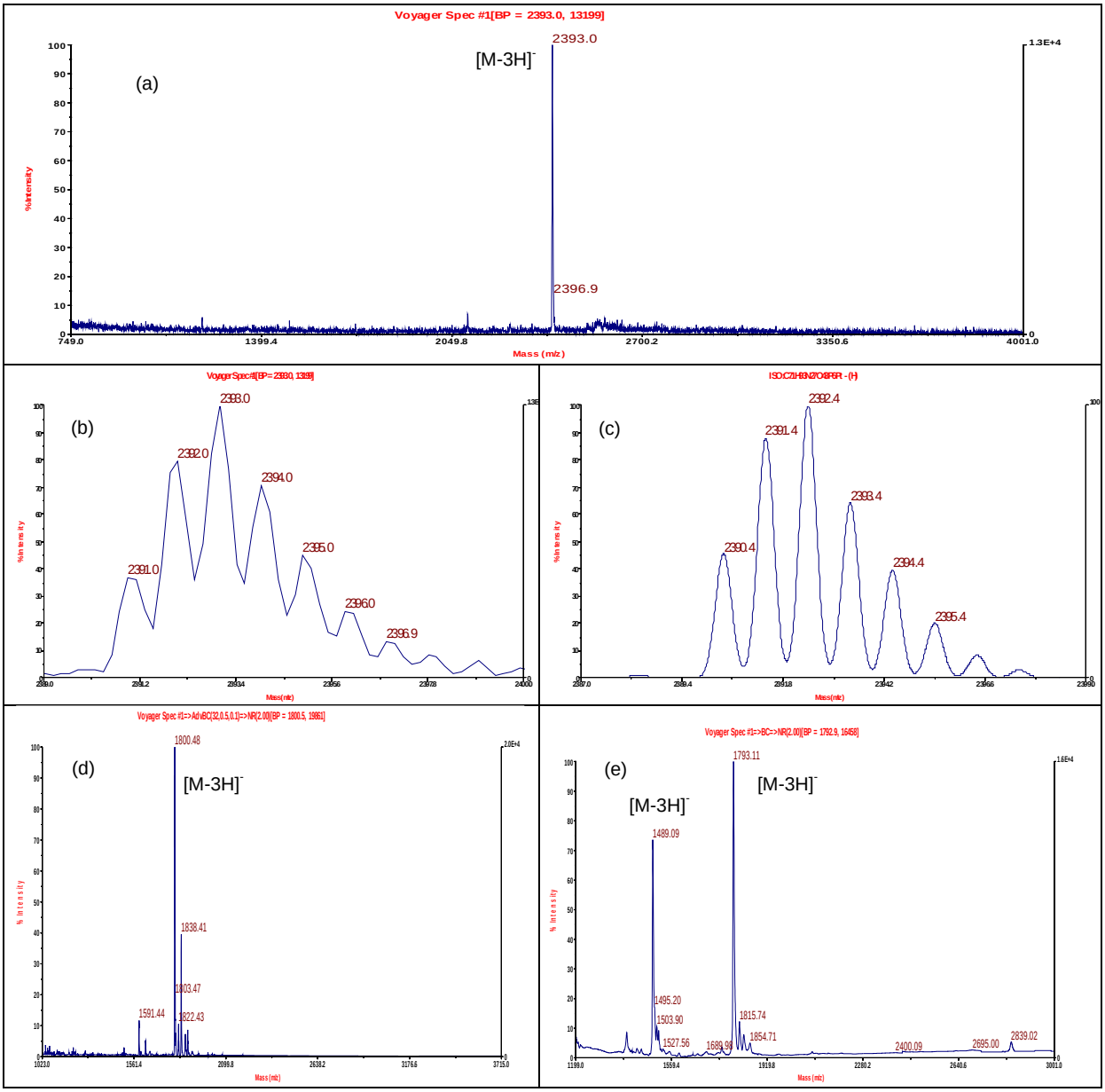

Figure S-7. MALDI-TOF MS of adduct **9** (peak R_t 16.6 min): experimental (a, b), calculated (c) and after digestion with snake venom (d) or calf spleen phosphodiesterase (e).