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Synthesis and DNA Interaction of Platinum Complex/Peptide Chimera as Potential Drug Candidates

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Modification and optimization of the anticancer drug cisplatin is of interest with respect to selective cell targeting and DNA binding efficiency. Attractive approaches contain both, modification of the platinum coordination sphere and design of hybrid molecules of the cisplatin binding moiety including peptide motifs. Peptides with cell penetrating, directing or recognizing properties can be implemented. In this study, positively charged peptide sequences were investigated with the potential of inducing DNA structural distortions caused by charge neutralization of the dsDNA helix. Association of charged peptides is likely to increase the flexibility of the DNA thereby facilitating platinum binding. The synthesis and DNA interaction of five new cisplatin–peptide hybrids with enhanced solubility and potential antitumor activity is presented. Propylenediamine or bisimidazole units were used as bisdentate platinum ligands and were coupled to a peptide sequence in the final elongation step of the solid-phase peptide synthesis (SPPS). Agarose and polyacrylamide gel electrophoresis, fluorescence intercalation, and thermal UV melting studies, all support the presence of covalently formed platinum DNA adducts in a reaction mediated by the positively charged peptide.

Introduction

Cisplatin [*cis*-diamminedichloroplatinum(II)], the first clinically introduced metal-based anticancer drug was discovered almost 50 years ago.[1] The compound has proven to be particularly efficient for the treatment of testicular and ovarian cancer patients. Three different platinum compounds cisplatin, carboplatin, and oxaliplatin are widely used in cancer treatment today.[2] However, the toxic side effects, combined with poor solubility, low selectivity towards tumor cells, and resistance development highlight the need for improved drug candidates.[3–5]

Detailed knowledge concerning the mechanism of cisplatin interference with cancer growth is still incomplete.[6] However, it is well established that the biological activity derives from its interactions with nuclear DNA, where predominantly adducts with the N7 atom of guanine or adenine are formed to give mainly 1,2- or 1,3-intrastrand adducts.[7,8] The biological activity of cisplatin can be linked to the disruption of the double helical DNA structure with bending of the helix and consequences on the replication or transcription machinery.[9–13] The bent structure is readily recognized by proteins, most prominently by the high mobility group (HMG) domain proteins, which shield the DNA from repair, eventually leading to induction of apoptosis.[5,14]

Although novel platinum-based anticancer drugs have been developed, their structural variation is rather limited. Typically, a cationic metal complex or metabolite is used for electrostatic interaction with the polyanionic DNA backbone.[4,15–17] Association of such complexes to the DNA backbone facilitates covalent binding[18–22] and can thus be expected to improve the therapeutic efficacy. The lower pH and chloride concentration inside the cell compared to the blood stream allow for preferential hydrolysis of cisplatin complexes in the cellular environment, providing formation of positively charged species close to its cellular target.[23,24] Thus, the mechanistic importance of the charged metal complex is indicated.

Our approach for the design of cisplatin analogs with potentially improved reactivity and selectivity is based on chimera of platinum complexes and peptides.[25–29] Platinum complexes/peptide chimera **C1**–**C5** studied in the present work contain cisplatin analogs covalently linked to peptides that vary with respect to the amount of positive charges in the amino acid side chains (Figure 1). We anticipate that the presence of charged peptides should facilitate in particular electrostatic DNA pre-association and bending of the DNA target. The newly synthesized platinum(II) complexes were based on bisimidazole or propylenediamine ligands and combined with nonapeptides containing six (**C1** and **C2**) or three lysinyl units (**C3** and **C4**) or non-charged alanyl units (**C5**). The DNA binding properties were investigated by agarose and polyacrylamide gel electrophoresis, propidium iodide intercalation and thermal denaturation

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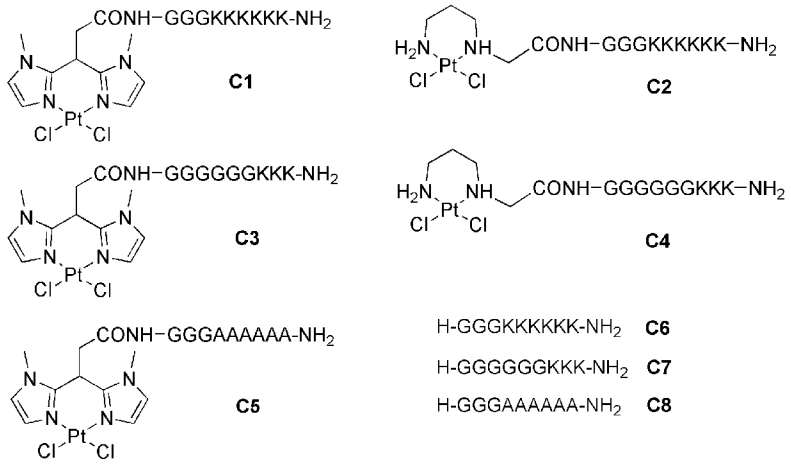


Figure 1. Platinum complex/peptide chimera **C1**–**C5** and the respective reference peptides **C6**–**C8**.

studies. Typically, enhanced adduct formation was found to take place for platinum complex/peptide chimera and DNA in the presence of positively charged peptides. The observation thus gives further support for the suggested use of platinum complex/peptide chimera as novel DNA-modifying agents.

Results and Discussion

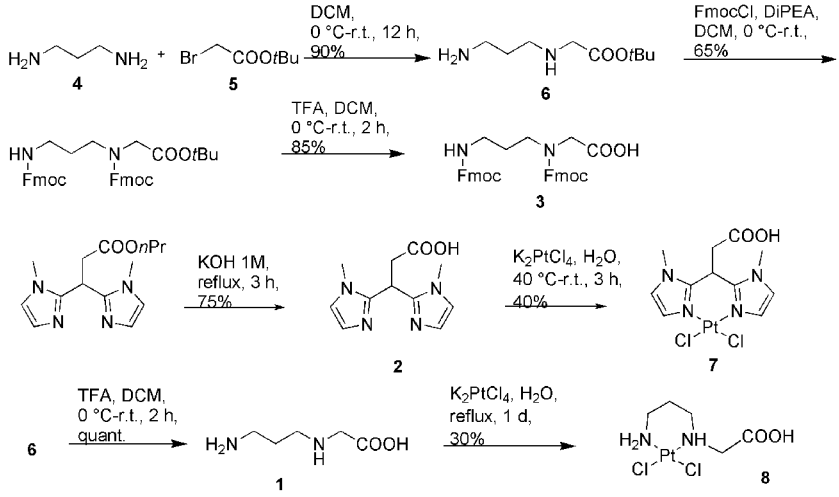
Synthesis of Platinum Complex/Peptide Chimera

Two bisdentate amine ligands for platinum coordination were prepared and attached to peptide oligomers. Aminopropyl glycine was chosen as flexible and sterically not demanding platinum binding site and the 3,3-bis(1-methylimidazol-2-yl) moiety was used to chelate the platinum moiety.[30,31] *N*-(3-Aminopropyl)glycine (**1**) and the 3,3-bis(1-methylimidazol-2-yl) moiety as propionic acid building block **2** were prepared. Both contained the carboxylic acid functionality to allow further peptide coupling with the *N*-terminal amine (Scheme 1). The peptides H-(GGGKKKKKK)-NH_2 (**C6**) H-(GGGGGGKKK)-NH_2 (**C7**), and H-(GGGAAAAAA)-NH_2 (**C8**) were obtained by automated Fmoc-SPPS on a NovaSyn® TGR resin preloaded with the first amino acid. Attachment of the platinum binding moieties generating chimera **C1**–**C5** was accomplished on solid support within the final coupling step.

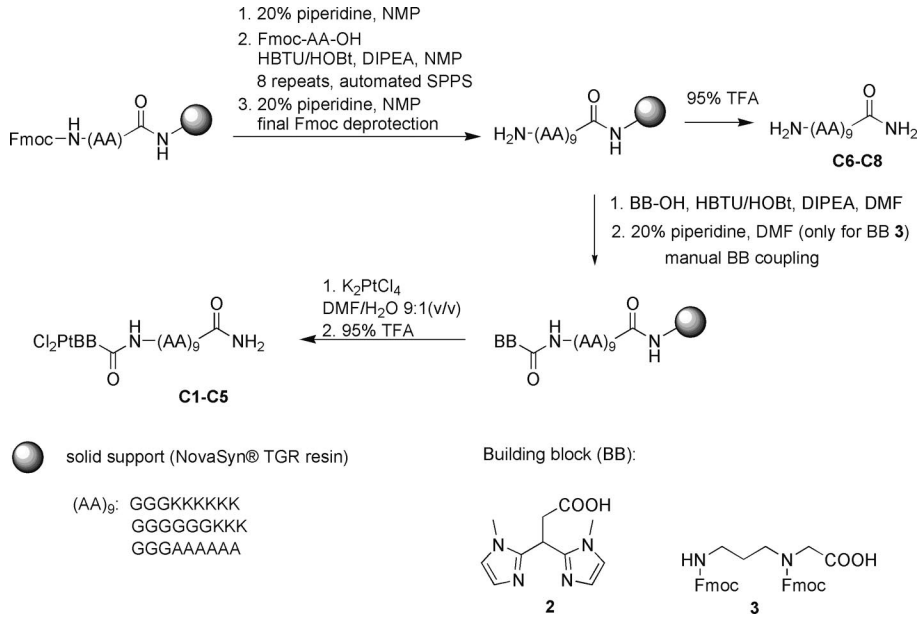
The synthesis of building block **3** was obtained in analogy to the preparation of the respective ε-bisamine[32] starting with a nucleophilic substitution of *tert*-butyl bromoacetate (**4**) by 1,3-diaminopropane (**5**) yielding amino acid **6** (Scheme 1). 9-Fluorenylmethyloxycarbonyl (Fmoc) protection of both amino groups followed by ester saponification provided building block **3** that was directly introduced in the final SPPS coupling step.

The synthesis of 3,3-bis(1-methylimidazol-2-yl)propionic acid (**2**) was provided in analogy to literature procedures by conversion of keto- and methylene-bridged bisimidazoles.[33,34] Platination of ligands **2** and **1**, respectively, was achieved by treatment with K_2PtCl_4. Cisplatin analogs **7** and **8** were characterized by high resolution mass spectrometry (HR-MS).

The required peptides and chimera of peptides and platinum complexes were obtained by automated Fmoc-SPPS



Scheme 1. Synthesis of platinum ligands **2** and **3** used for SPPS and the respective cisplatin analogs **7** and **8**.



Scheme 2. Preparation of peptides and platinum complex/peptide chimera by Fmoc-SPPS.

on a NovaSyn® TGR resin preloaded with the first amino acid (Scheme 2). Amino acid activation was performed by HBTU/HOBt. The peptide sequences prepared, H-(GGGKKKKKK)-NH_2 (**C6**), H-(GGGGGGKKK)-NH_2 (**C7**), and H-(GGGAAAAAA)-NH_2 (**C8**) (Figure 1), were chosen with a various number of positively charged side chains, thus, allowing for a gradual change of charge interaction with DNA. In addition, building blocks **2** and **3** were coupled to the *N*-terminus of the respective oligomers by manual SPPS also using HBTU/HOBt activation. The final assembly of platinum complexes was performed using resin bound oligomers followed by cleavage of the constructs from solid support by TFA treatment. After HPLC purification, the desired platinum complex/peptide chimera were obtained and characterized by HR-MS.

Plasmid Binding Studies

Agarose gel electrophoresis was used to visualize the change in electrophoretic mobility caused by platination of the DNA in a gel mobility shift assay of platinum exposed circular plasmids.[35] Considering the property of cisplatin and analogs as DNA unwinding agent,[11] we investigated

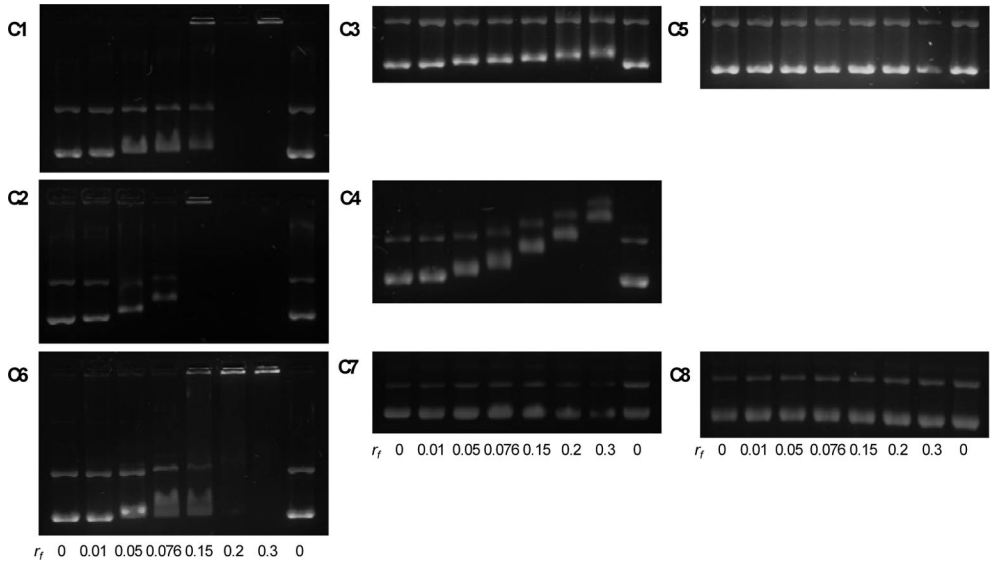


Figure 2. Gel mobility shift assay of platinum complex/peptide chimera **C1**–**C5** and peptides **C6**–**C8** with supercoiled and relaxed forms of the pUC18 plasmid (incubation for 2 h, 32 °C with r_f varying from 0 to 0.3, 10 mM Na_2HPO_4/NaH_2PO_4, pH 5.8, [pUC18] = 0.15 mM nucleobases).

the DNA binding affinity of platinum complex/peptide chimera **C1**–**C5**, peptides **C6**–**C8** and the platinated building blocks **7** and **8** (Scheme 1) with the negatively supercoiled pUC18 plasmid. After platinum binding both, supercoiled and relaxed forms, can be visualized by agarose gel electrophoresis. An increasing amount of platinum complex bound to DNA can be detected by the appearance of a slower migrating supercoiled plasmid band. For binding studies the ratio of nucleotide concentration (C_{DNA}) and concentration of platinum compounds (C_{Pt}) ($r_f = C_{Pt}/C_{DNA}$) was varied in the range of 0–0.3 (Figure 2).

The use of bisimidazole platinum complex/peptide chimera **C1**, which at pH 5.8 contains six positively charged lysinyl residues, led to unwinding of the plasmid already at low concentrations. However, smearing of the bands was observed, as well as complete retardation of the DNA migration at $r_f = 0.15$–0.2, simply based on charge neutralization (Figure 2). In line with the charge neutralization effect, a similar gel migration pattern was observed for the reference peptide **C6**. In general, for the lysinyl containing oligomers DNA precipitation was observed already at $r_f = 0.5$ (data not shown). The propylenediamine platinum complex **C2** with the same peptide sequence as **C1** provided a similar result with respect to retardation, nevertheless, unwinding of the plasmid up to $r_f = 0.15$ was more defined.

Lowering the amount of charged lysinyl side chains within the peptide to three provided an interesting difference, since addition of the peptide **C7** was not sufficient for plasmid binding. In contrast, both platinum complexes in combination with the peptide sequence G_6K_3 (**C3** and **C4**) led to distinct plasmid unwinding. For the propylenediamine platinum complex **C4** the coalescence point was almost reached at a platinum complex/DNA concentration of $r_f = 0.3$. Thus, the combination of platinum complex and positively charged peptide is indicated to provide improved DNA binding. Further, comparing the two complexes it is obvious that the propylenediamine ligand was superior over the bisimidazolyl ligand with respect to binding efficacy. Lower basicity, steric hindrance and lower conformational flexibility of the aromatic ligand system might hinder the platinum binding compared to the more site accessible and smaller aliphatic diamine. Finally, the platinum complex chimera with non-charged G_3A_6 peptide sequence **C5** as well as the peptide **C8** clearly prove the requirement of charges for interactions with the plasmid. With the use of non-charged peptides no mobility shift in the gel up to $r_f = 1.0$ was induced (data not shown). Similarly, the platinated building blocks **7** and **8** lacking the peptide chain showed no gel mobility shift up to an $r_f = 0.8$ (Supporting Information).

Oligonucleotide Binding Studies

DNA recognition and covalent binding of the cisplatin analogs was also investigated using the single-stranded 22-mer DNA 5′-TCTCCTTCTTGGTTCTCTTCTC-3′ as target. With only one d(GpG) site a defined binding stoichiometry was expected. The DNA was radio-labeled at the 5′-end using polynucleotide kinase and [γ-^{32}P]-ATP. The respective platinum complex/peptide chimera and control peptides were added to the DNA (650 cps/reaction) in 10 mM phosphate buffer, incubated at room temperature for 18 h, and analyzed under denaturing conditions. A concentration of 1 μM was used for the lysinyl containing complexes **C1**–**C4** and 10 μM for all other complexes and the peptides alone, the results are shown in Figure 3. Titration studies were initially performed to allow formation of a single adduct (data not shown).

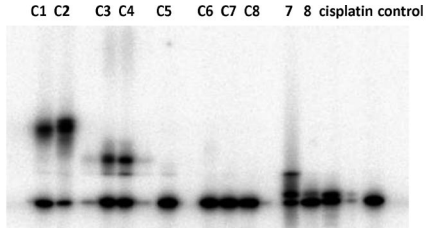


Figure 3. Autoradiogram of platinum complexes **C1**–**C5**, peptides **C6**–**C7**, platinum complexes **7** and **8** as well as cisplatin after exposure to radio-labeled DNA 5′-TCTCCTTCTTGGTTCTCTTCTC-3′. A 1.0 μM complex concentration was used for **C1**, **C2**, **C4** and **C5**; all other derivatives were added in 10 μM concentration. The samples were separated on a 0.2 mm, 20% denaturing polyacrylamide gel (PAA/8 M urea).

Both covalent binding and electrostatic association of larger molecules to DNA are known to induce changes in gel migration patterns of the resulting structurally distorted DNA. Larger molecules and structurally distorted oligomers are often found to migrate more slowly in a polyacrylamide gel.[12,36–38] The observed band shifts in polyacrylamide gel electrophoresis can be correlated to the amount of charge neutralization, but the influence of the complex size or DNA conformational change might also contribute to the observed gel shifts. Here, the platinum complex/peptide chimera **C1**–**C4** were all found to give rise to a major reaction product with retarded gel mobility indicative of efficient platinum binding. The influence of the positive charge of the lysinyl side chain was found to be reflected in stronger migration retardation for the platinum complex/peptide chimera with six lysinyl residues (**C1** and **C2**). Nevertheless, our results show that the platinum moiety is indeed required for DNA binding, since the charged peptides **C6** and **C7**, lacking the platinum complex, did not cause any significant gel shifts under these conditions (Figure 3). The requirement of charged peptides for efficient binding is further indicated by the study of complex **C5** which does not interact with the DNA as well under described reaction conditions. However, at a ten times higher concentration (100 μM) of **C5**, a slower migrating platinated product can be visualized (Supporting Information Figure S2). Even at this higher concentration, no gel retardation is seen upon addition of the peptides, **C6**–**C8**, alone (Supporting Information Figure S2). When the two different types of platinum coordination spheres are compared, it is obvious that the platinum complexes bound to the propylenediamine ligand (**C2** and **C4**) showed a significantly higher DNA bind-

ing compared to the corresponding bisimidazolyl ligand oligomers (**C1** and **C3**). The cisplatin analogs **7** and **8** lacking the peptide moiety and cisplatin alone were also shown to interact with DNA, but ten times higher concentrations were needed to obtain distinct gel retardation.[12]

In summary, the pronounced band shifts obtained for the platinum complex/peptide chimera support the assumption that attachment of charged peptides to the platinum moiety can be used to facilitate covalent nucleic acid binding. Whereas the platinum complexes alone (**7**, **8**) required ten times higher concentration compared to the platinum complex/peptide chimera for efficient DNA-binding, no DNA interaction was indicated for the charged peptides lacking the metal binding site. Therefore, we conclude that the investigated platinum complex/peptide chimera and related analogs contribute to the mechanism of covalent DNA modification by pre-association and structural distortion of the DNA.[35]

Fluorescence Intercalation Studies

DNA structural changes induced by the platinum complex/peptide chimera were studied by propidium iodide (PI) fluorescence studies. As shown previously, the PI fluorescence increases during intercalation with double-stranded DNA due to π-π stacking interactions between the cationic dye and DNA nucleobases.[39,40] The PI intercalation was monitored in the absence or presence of the different platinum complex/peptide chimera. After pre-incubation of herring DNA with platinum complexes **C1**–**C5** and peptides **C6**–**C8** (10 mM Na_2HPO_4/NaH_2PO_4, pH 5.8; $r_f = C_{complex}/C_{nucleotide}$ = 0.083; samples were pre-incubated for two hours at 32 °C), the PI was titrated into the resulting solution. The fluorescence was measured as a function of r_f (Figure 4). All reaction mixtures exhibit similar features with an initial increase of fluorescence as a result of increasing r_f, but reaching a saturation fluorescence at r_f values in the range 0.1–0.2. A slight decline in fluorescence intensity was observed in the latter interval, most likely reflecting quenching of bound PI by added, non-bound PI. A close inspection of data reveals, however, some interesting differences. First of all, the saturation fluorescence is influenced by the nature of the compounds added. The effect is particularly pronounced for compounds **C1**, **C2** and **C6**, i.e. in the presence of the hexavalent nonapeptide **C6** and in conjugation with the platinum complexes (**C1** and **C2**). Compound **C2** gives rise to the largest reduction of PI fluorescence of about 30%. The data suggest that the presence of these compounds on the DNA surface is enough to efficiently block the access of PI to intercalation sites on the DNA. The observation is in line with the suggested structural effect observed during plasmid binding studies (Figure 2). Further support for a reduction of accessible PI intercalation sites on the DNA surface is also given by the fact that the plateau value is reached earlier for the platinum compounds **C2** and **C1** with r_f saturation values at 0.14 and 0.16, respectively, as determined by first-derivative analysis. We thus conclude that whereas the non- and triply charged platinum complex/peptide chimera still allow for PI intercalation in good agreement with the neighboring exclusion principle,[41] presence of the hexavalent platinum complex/peptide chimera leads to an electrostatic contribution to surface binding that is not as readily displaced by PI. Finally, the platinum complex/peptide chimera **C3** and **C4** (three lysinyl units) caused only a minor effect, and the non-charged platinum complex/peptide chimera **C5** had no influence on PI intercalation. These data are in agreement with the previously discussed evidence obtained from the pUC18 plasmid gel shift assays of the hexavalent lysinyl unit as the moiety with the largest binding affinity and ability to induce DNA structural changes.

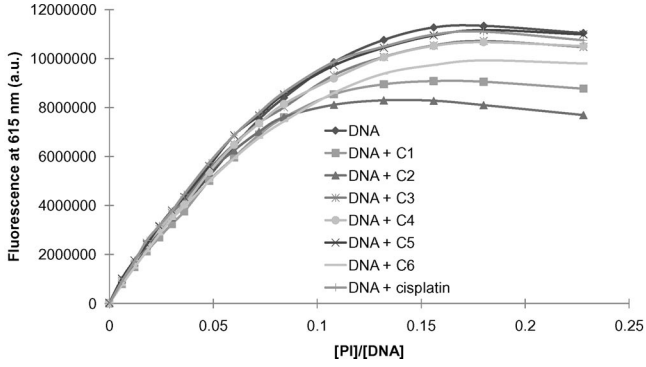


Figure 4. PI fluorescence as a function of r_f in the presence of **C1**–**C6** and cisplatin with herring DNA.

Thermal Melting Studies

Further insight into the interaction between the platinum complex/peptide chimera and DNA can be obtained by temperature dependent UV spectroscopy. Whereas covalent platinum binding can be expected to decrease the thermal stability of the duplex, its interaction with the charged peptides likely gives rise to the opposite effect.[42,43] The double strand formed by DNA oligomers 5′-TCTCCTTCTTGTGTCTCTTCTC-3′ and 3′-AGAGAG-GAAGAACACAGAGAAG-5′ (duplex 1) provided a melting temperature of t_m = 37.0 °C (1.0 μM duplex 1, 10.0 mM Na_2HPO_4/NaH_2PO_4, pH 5.8). This duplex contains a tentative GXG binding site used to kinetically favor monofunctional adduct formation over bifunctional one[44,45] thus providing insight into the initial impact of the complexes on DNA duplex stability with maintained thermodynamic stability of the central part of the oligomer. The platinum complex/peptide chimera and peptides were added in a 4 μM concentration. A comparable increase of the thermal melting temperature was observed for the polylysinyl complexes **C1** (t_m = 44.5 °C), **C2** (t_m = 50.5 °C) and the respective peptide **C6** (t_m = 49.1 °C) indicating that the stabilization caused by positively charged peptides is dominating (Figure 5). In accordance with gel electrophoresis and fluorescence results the propylenediamine platinum complex **C2** provided a stronger DNA stabilization effect com-

pared to the bisimidazolyl ligand system **C1** i.e. indicating also an influence from the platinum binding moiety. Nevertheless, as indicated by the t_m obtained for DNA stabilization in the presence of peptide **C6**, the charge neutralization can be considered as the main contribution.

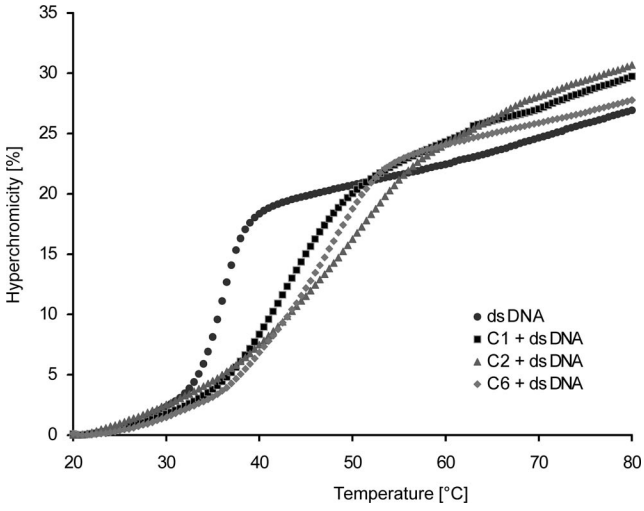


Figure 5. A representative thermal melting study of duplex 1, both alone and after pre-incubation with platinum complexes **C1**, **C2** and the polypeptide **C6** ($C_{duplex\ 1}$ = 1.0 μM, $C_{C1,C2,C6}$ = 4.0 μM, C_{Pi} = 10.0 mM, pH 5.8).

Conclusions

Targeting of polyanionic DNA by cisplatin and related compounds is known to be facilitated by the presence of positive charges on the metal center or closely related groups. In the present study, our aim has been to evaluate the potential of a novel type of extended, charged platinum complex/peptide chimera with respect to efficient DNA targeting. We were able to show that complexes with two different metal-chelating moieties, both linked to nonamer polypeptides, can successfully be made by coupling of the chelated metal complex to the polypeptide using solid phase peptide synthesis. The choice of the chelating moieties, the bisimidazolyl- and the propylenediamine-ligands, respectively, influences the reactivity of these complexes, with the propylenediamine complexes as the more reactive ones. We conclude that this observation is most likely a steric effect, where the flexible polypropylene ligand allows for more suitable location of the platinum center in close vicinity of the reactive groups on DNA. We were also able to show that an increased charge on the nonapeptide moiety – net charges studied were: 0, +3, and +6 – indeed facilitated the interaction. It seems reasonable to assume that the improved binding observed for the hexavalent compounds **C1** and **C2** is caused by a combination of i) maximized electrostatic attraction to the DNA surface and ii) increased flexibility of the DNA structure around the association site, both facilitating formation of the structurally distorted, platinated DNA structure. For the most promising compound **C2**, interactions with DNA are observed at 10-fold lower concentration compared with cisplatin. Further, for this compound the adduct formation is accompanied by about 13 °C increase of the melting temperature, consequently suggesting formation of a DNA adduct with high energetic demands during formation of the single-stranded DNA needed for efficient repair.[5] Thus, our studies are indicative of a synthetic strategy that deserves to be explored further towards the goal of providing highly efficient drug alternatives to cisplatin.

Experimental Section

General: All reagents were of analytical grade and used without further purification. Dry solvents were stored over molecular sieves (4 Å). All amino acid derivatives as well as coupling reagents and the resins for solid phase synthesis are commercially available from NovaBiochem (Darmstadt, Germany), IRIS Biotech (Marktredwitz, Germany), GL Biochem (Shanghai, China), Bachem (Bubendorf, Switzerland), Merck (Darmstadt, Germany), ABCR (Karlsruhe, Germany). Acetonitrile and methanol (HPLC-grade) were obtained from FisherScientific GmbH (Nidderau, Germany). All other chemicals were purchased from Sigma–Aldrich (Taufkirchen, Germany), Fluka (Taufkirchen, Germany), Acros Organics (Geel, Belgium), Merck (Darmstadt, Germany), Lancaster, Alfa Aesar and ABCR (the latter located in Karlsruhe, Germany) in analytical grade.

Glass equipment utilized for reactions under inert atmosphere was flame dried before use. ^1H and ^{13}C NMR spectra were recorded with a Varian Unity 300, a Varian Inova 500 or 600 or a Bruker 300 or 400 spectrometer. Chemical shifts are quoted in parts per million (ppm) downfield of TMS. Abbreviations for multiplicities are: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Coupling constants are given in Hz. ESI MS data were obtained with a Finnigan instrument (type LGC or TSQ 7000) or Bruker spectrometers (Apex-Q IV 7T and micrOTOF API). High resolution spectra were obtained with the Bruker Apex-Q IV 7T or the Bruker micrOTOF, respectively. Flash chromatography was performed using Merck silica gel 60. Thin-layer chromatography (TLC) was carried out using Merck aluminum plates of silica gel 60 F_{254}. HPLC analyses were performed on a Pharmacia Äkta Basic instrument (pump type P-900, variable wavelength detector, GE Healthcare, London, UK). UV absorption was detected at 215 nm with a linear gradient of A (0.1% TFA in H_2O) to B (0.1% TFA in MeCN/H_2O, 8:2) or to B′ (0.1% TFA in MeOH). Ultra pure water was obtained by water purification device "Simplicity" (Millipore, Bedford, UK). Peptides were analyzed using a YMC J'sphere column ODS-H80, RP-C18, 250 × 4.6 mm, 4 μm, 80 Å or a YMC J'sphere column ODS-A, RP-C18, 250 × 4.6 mm, 5 μm, 120 Å with a flow rate of 1 mL min^{-1}. Purification was performed with a YMC J'sphere column ODS-H80, RP-C18, 250 × 20 mm, 4 μm, 80 Å or with a YMC J'sphere column ODS-A, RP-C18, 250 × 20 mm, 5 μm, 120 Å with a flow rate of 10 mL min^{-1}. The fractions containing the desired product were collected and lyophilized.

Peptides were automatically synthesized via Fmoc solid-phase peptide synthesis (Fmoc-SPPS) using either the peptide synthesizer 433 A (Applied Biosystems) by applying the FastMoc 0.10 mmol (amount of resin) Fmoc standard protocol in an 8 mL reaction vessel or the "Liberty" microwave peptide synthesizer (CEM, Kamp-Lintfort, Germany) equipped with a "Discover" microwave reaction cavity (CEM), applying 0.1 mmol. Standard reagents, pro-

tocols and procedures were used for deprotection (piperidine/NMP), coupling (HBTU [= 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate]/HOBt [= *N*-hydroxybenzotriazole]/diisopropylethylamine [DIPEA]/NMP) and capping (Ac_2O/DIPEA/HOBt/NMP).

***tert*-Butyl *N*-(3-Aminopropyl)glycinate (6):** To a vigorously stirred solution of 1,3-diaminopropane (**4**, 6 mL, 71.87 mmol, 8.78 equiv.) in DCM (32 mL) *tert*-butyl bromoacetate (**5**, 1.2 mL, 8.18 mmol, 1 equiv.) in DCM (6.5 mL) was added at 0 °C over 5 h. The resulting mixture was warmed up to room temperature (approx. 3 h) and stirred overnight at room temp. The reaction was quenched with brine, then the mixture was washed with water (3 × 20 mL) and the combined aqueous phase was re-extracted with DCM (1 × 30 mL). The combined organic phase was dried with Na_2SO_4, filtered, evaporated and dried in high vacuo to yield **6** (1.390 g, 7.38 mmol, 90%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.4 [s, 9 H, $OC(CH_3)_3$], 1.54–1.63 (m, 5 H, NH, NH_2, βCH_2NH_2), 2.6 [t, ^3J(H,H) = 6.9 Hz, 2 H, γCH_2NH_2], 2.72 (t, $^3J_{H,H}$ = 6.8 Hz, 2 H, αCH_2NH_2), 3.23 (s, 2 H, CH_2COO) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 28.11 [$C(CH_3)_3$], 33.69 (βCH_2NH_2), 40.26 (αCH_2NH_2), 47.26 (γCH_2NH_2), 51.74 (CH_2CO), 81.03 [$C(CH_3)_3$], 171.63 (CO) ppm. MS (ESI): *m*/*z* = 189.2 $[M + H]^+$, 211.1 $[M + Na]^+$, 377.3 $[2M + H]^{2+}$. HRMS (ESI): $C_9H_{20}N_2O_2$ $[M + H]^+$ calcd. 189.1598; found 189.1608.

***tert*-Butyl *N*-Fmoc-(3-Fmoc-Aminopropyl)glycinate:** To a solution of *tert*-butyl *N*-(3-aminopropyl)glycinate (**6**, 1.37 g, 7.30 mmol, 1 equiv.) in DCM (37 mL) DIPEA (3.75 mL, 21.91 mmol, 3 equiv.) was added and the mixture was cooled to 0 °C. FmocCl (5.67 g, 21.91 mmol, 3 equiv.) was added and the mixture was warmed up to room temperature and stirred at room temp. for 2 d. The reaction was quenched with brine, the mixture was washed with water and the organic phase was dried with $MgSO_4$. The residue was purified by column chromatography (pentane/Et_2O, 2:1 to 1:1) to yield *tert*-butyl *N*-Fmoc-(3-Fmoc-aminopropyl)glycinate (3.00 g, 47.41 mmol, 65%) as a white solid. TLC (pentane/Et_2O, 2:1): R_f = 0.06; (pentane/Et_2O, 1:1): R_f = 0.21. ^{1}H NMR (300 MHz, $C_2D_2Cl_4$, 100 °C): δ = 1.49 [s, 9 H, $OC(CH_3)_3$], 1.58–1.74 (br. s, 2 H, βCH_2NH), 3.08–3.21 (br. s, 2 H, γCH_2NH), 3.25–3.40 (br. s, 2 H, αCH_2NH), 3.85 (s, 2 H, CH_2COO), 4.25 (m, 2 H, CH-Fmoc, CH-Fmoc′), 4.43 (d, $^3J_{H,H}$ = 6.9 Hz, 2 H, CH_2-Fmoc), 4.52 (d, $^3J_{H,H}$ = 6.2 Hz, 2 H, CH_2-Fmoc′), 7.33 (m, 4 H, H2-Fmoc, H2-Fmoc′), 7.41 (m, 4 H, H3-Fmoc, H3-Fmoc′), 7.61 (m, 4 H, H1-Fmoc, H1-Fmoc′), 7.77 (d, $^3J_{H,H}$ = 7.48 Hz, 4 H, H4-Fmoc H4-Fmoc′) ppm. ^{13}C NMR (150 MHz, $C_2D_2Cl_4$, 100 °C): δ = 27.91 (CH_3), 28.16 (βCH_2NH), 38.32 (γCH_2NH), 46.02 (αCH_2NH), 47.37 (CH-Fmoc), 47.39 (CH-Fmoc′), 49.89 ($CH_2COOtBu$), 66.48 (CH_2-Fmoc), 67.90 (CH_2-Fmoc′), 81.67 ($CtBu$), 119.70 (C4-Fmoc, Fmoc′), 124.64 (C1-Fmoc), 124.77 (C1-Fmoc′), 126.83 (C2-Fmoc), 126.91 (C2-Fmoc′), 127.41 (C3-Fmoc), 127.49 (C3-Fmoc′), 141.11 (C6-Fmoc), 141.13 (C6-Fmoc′), 143.83 (C5-Fmoc), 143.95 (C5-Fmoc′), 156.05 (CO-Fmoc), 156.11 (CO-Fmoc′), 168.33 ($COOtBu$) ppm. MS (ESI): *m*/*z* = 655.3 $[M + Na]^+$, 1287.6 $[2M + Na]^{2+}$, 1920.8 $[3M + Na]^{3+}$. HRMS (ESI): $C_{39}H_{40}N_2O_6$ $[M + Na]^+$ calcd. 655.2779; found 655.2774.

***N*-Fmoc-(3-Fmoc-aminopropyl)glycine (3):** *tert*-Butyl *N*-Fmoc-(3-Fmoc-aminopropyl)glycinate (2.82 g, 4.46 mmol) was dissolved in DCM (2.8 mL) and the solution was cooled to 0 °C, followed by dropwise addition of TFA (16.75 mL). The mixture was stirred at room temperature for 2 h. The solution was concentrated in vacuo and then co-evaporated with toluene (5 × 100 mL). The residue was solubilized in minimum amount of DCM, precipitated with pentane and dried to give **3** as a white powder (2.20 g, 3.81 mmol, 85.5%). ^{1}H NMR (300 MHz, $C_2D_2Cl_4$, 100 °C): δ = 1.53–1.68 (br. s, 2 H, βCH_2NH), 3.02–3.13 (br. s, 2 H, γCH_2NH), 3.19–3.32 (br. s, 2 H, αCH_2NH), 3.93 (s, 2 H, CH_2COO), 4.24 (t, $^3J_{H,H}$ = 6.3 Hz, 2 H, CH-Fmoc, CH-Fmoc′), 4.45 (d, $^3J_{H,H}$ = 6.7 Hz, 2 H, CH_2-Fmoc), 4.58 (d, $^3J_{H,H}$ = 5.9 Hz, 2 H, CH_2-Fmoc′), 4.85–5.25 (br. s, 1 H, NH), 7.32 (m, 4 H, H2-Fmoc, H2-Fmoc), 7.36–7.44 (m, 4 H, H3-Fmoc, H3-Fmoc′), 7.58 (t, $^3J_{H,H}$ = 7.9 Hz, 4 H, H1-Fmoc, H1-Fmoc′), 7.76 (t, $^3J_{H,H}$ = 6.8 Hz, 4 H, H4-Fmoc, H4-Fmoc′) ppm. ^{13}C NMR (150 MHz, $C_2D_2Cl_4$, 100 °C): δ = 28.09 (βCH_2NH), 38.28 (γCH_2NH), 46.12 (αCH_2NH), 47.29 (CH-Fmoc, CH-Fmoc′), 48.74 (CH_2COOH), 66.61 (CH_2-Fmoc), 67.35 (CH_2-Fmoc′), 119.70 (C4-Fmoc, C4-Fmoc′), 124.28 (C1-Fmoc), 124.69 (C1-Fmoc′), 126.83 (C2-Fmoc),126.91 (C2-Fmoc′), 127.44 (C3-Fmoc), 127.48 (C3-Fmoc′), 141.10 (C6-Fmoc), 141.14 (C6-Fmoc′), 143.63 (C5-Fmoc), 143.81 (C5-Fmoc′), 156.14 (CO-Fmoc), 156.40 (CO-Fmoc′), 171.35 (COOH) ppm. MS (ESI): *m*/*z* = 599.2 $[M + Na]^+$, 615.2 $[M + K]^+$, 1174.9 $[2M + Na]^{2+}$, 575.1 $[M - H]^-$. HRMS (ESI): $C_{35}H_{32}N_2O_6$ $[M + Na]^+$ calcd. 599.2153; found 599.2147, $[M - H]^-$ calcd. 575.2188; found 575.2182.

***N*-(3-Aminopropyl)glycine (1):** *tert*-Butyl *N*-(3-aminopropyl)glycinate (**6**, 0.17 g, 0.87 mmol) was dissolved in DCM (0.6 mL) and the solution was cooled to 0 °C, followed by addition of TFA (3.2 mL). The mixture was stirred at room temperature for 2 h. The volatiles were evaporated in vacuo and co-evaporated with toluene (6 × 100 mL). The resulting residue was lyophilized to give the TFA salt of **1** as colorless solid in quantitative yield. ^{1}H NMR (300 MHz, DMSO): δ = 1.87–1.97 (m, 2 H, βCH_2NH_2), 2.88 (t, $^3J_{H,H}$ = 7.60 Hz, 2 H, γCH_2NH_2), 3.01 (t, $^3J_{H,H}$ = 7.63 Hz, 2 H, αCH_2NH_2), 3.84 (s, 2 H, CH_2COO), 7.75–9.45 (br. s, 4 H, COOH, NH, NH_2) ppm. MS (ESI): *m*/*z* = 133.4 $[M + H]^+$, 131.1 $[M - H]^-$. HRMS (ESI): $C_5H_{12}N_2O_2$ $[M + H]^+$ calcd. 133.0972; found 133.0972, $[M - H]^-$ calcd. 131.0826; found 131.0826.

[*N*-(3-Aminopropyl)glycine]dichloroplatinum (8): The TFA salt of *N*-(3-aminopropyl)glycine (**1**, 0.87 mmol) was dissolved in water (20 mL), added to a solution of K_2PtCl_4 (0.36 g, 0.87 mmol) in water (20 mL) and refluxed for 40 h in the dark. The solution was concentrated at reduced pressure and the resulted precipitate was filtered and recrystallized from water to give **8** as a yellowish solid (0.10 g, 0.26 mmol, 30.1%). ^{1}H NMR (400 MHz, DMSO): δ = 1.83–1.98 (m, 1 H, H_A, βCH_2NH_2), 2.28–2.40 (m, 1 H, H_B, βCH_2NH_2), 2.57–2.74 (m, 2 H, γCH_2NH_2), 2.81–2.93 (m, 2 H, αCH_2NH_2), 3.01 (dd, $^3J_{H,H}$ = 2.67, $^2J_{H,H}$ = 16.3 Hz, 1 H, H_A, CH_2COO), 3.40 (dd, $^3J_{H,H}$ = 7.0, $^2J_{H,H}$ = 16.3 Hz, 1 H, H_B, CH_2COO), 6.41 (s, 1 H, NH), 7.78 (s, 1 H, NH_2) ppm. ^{195}Pt NMR (64.5 MHz, DMSO): δ = –2995.63 ppm. MS (ESI): *m*/*z* = 397.0 $[M - H]^-$, 817.0 $[2M + Na - 2H]^-$, 1236.9 $[3M + 2Na - 3H]^-$. HRMS (ESI): $C_5H_{12}Cl_2N_2O_2Pt$ $[M - H]^-$ calcd. 396.9847; found 396.9835. $C_5H_{12}Cl_2N_2O_2Pt$: calcd. C 15.08, H 3.04, N 7.04; found C 14.89, H 3.11, N 6.59.

3,3-Bis(1-methylimidazol-2-yl)propionic Acid (2): To a solution of propyl-3,3-bis(1-methylimidazol-2-yl)propionate (1.74 g, 6.29 mmol, 1 equiv.) in THF (16 mL) a solution of KOH (1 M, 6.29 mL, 6.29 mmol, 1 equiv.) was added, the solution was refluxed for 3 h and allowed to cool to room temperature over a period of 1 h. The mixture was neutralized with HCl (1 M, 6.29 mL, 6.29 mmol, 1 equiv.), the organic phase was evaporated, the aqueous phase was diluted with water (10 mL) and washed with DCM (2 × 20 mL) in order to remove traces of adduct. The water phase was collected and evaporated, the residue was extracted three times with cold MeOH and the precipitated white KCl was removed by filtration. The organic phase was evaporated and the product was recrystallized from ethanol/water (19:1) to give **2** as white crystals (1.10 g,

4.69 mmol, 74.6%). ^{1}H NMR (300 MHz, DMSO): δ = 3.12 (d, $^3J_{H,H}$ = 7.3 Hz, 2 H, $CHCH_2$), 3.50 (s, 6 H, NCH_3), 4.82 (t, $^3J_{H,H}$ = 7.3 Hz, 1 H, $CHCH_2$), 6.84 (d, $^3J_{H,H}$ = 1.2 Hz, 2 H, $CH_{imid}NMe$), 7.08 (d, $^3J_{H,H}$ = 1.2 Hz, 2 H, $CH_{imid}N{=}C$) ppm. ^{13}C NMR (125 MHz, DMSO): δ = 31.83 ($CHCH_2$), 32.36 (NCH_3), 36.71 ($CHCH_2$), 121.99 ($CH_{imid}N{=}C$), 125.21 ($CH_{imid}NMe$), 145.22 ($N{=}C{-}NCH_3$), 171.81 (C=O) ppm. MS (ESI): *m*/*z* = 233.1 $[M - H]^-$. HRMS (ESI): $C_{11}H_{14}N_4O_2$ $[M - H]^-$ calcd. 233.1044; found 233.1043.

[3,3-Bis(1-methylimidazol-2-yl)propionic acid]dichloroplatinum (7): To a solution of K_2PtCl_4 (0.18 g, 0.43 mmol) in water (10 mL), a solution of 3,3-bis(1-methylimidazol-2-yl)propionic acid (**2**, 0.10 g, 0.43 mmol) in water (10 mL) was added at 30 °C and the resulting mixture was heated up to 40 °C and stirred at 40 °C for 3 h in the dark, allowed to cool to room temperature over 1 h. The suspension was kept at 0 °C for 1 h and the formed precipitate was filtered and washed with water to give **7** as a yellow solid (85 mg, 0.17 mmol, 40%). ^{1}H NMR (300 MHz, DMSO): δ = 3.55 (d, $^3J_{H,H}$ = 7.4 Hz, 2 H, $CHCH_2$), 3.84 (s, 6 H, NCH_3), 4.85 (t, $^3J_{H,H}$ = 7.4 Hz, 1 H, $CHCH_2$), 7.26 (d, $^3J_{H,H}$ = 1.7 Hz, 2 H, $CH_{imid}NMe$), 7.32 (d, $^3J_{H,H}$ = 1.9 Hz, 2 H, $CH_{imid}N{=}C$), 12.00–12.36 (br. s, 1 H, COOH) ppm. ^{15}N NMR (40.5 MHz, DMSO): δ = –215.5 (NMe), –225.3 (N=C) ppm. ^{195}Pt NMR (64.5 MHz, DMSO): δ = –2955.47 ppm. MS (ESI): *m*/*z* = 523.0 $[M + Na]^+$, 545.0 $[M - H + 2Na]^+$, 1067.0 $[2M - 2H + 3Na]^+$, 1236.9 $[3M + 2Na - 3H]^-$, 465.0 $[M - Cl]^+$, 499.0 [M – H], 999.0 $[2M - H]^-$. HRMS (ESI): $C_{11}H_{14}Cl_2N_4O_2Pt$ $[M + Na]^+$ calcd. 523.0031; found 523.0027, $[M - H]^-$ calcd. 499.0066; found 499.0052. $C_{11}H_{14}Cl_2N_4O_2Pt$: calcd. C 26.41, H 2.82, N 11.20; found C 26.63, H 3.01, N 11.27.

Peptide Synthesis: The synthesis was performed on a NovaSyn® TGR resin (0.2–0.3 mmol/g resin loading capacity). The loading with the first amino acid [Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH] was carried out manually in a syringe equipped with a polyethylene frit; the resin was pre-swollen in DCM (1.5 h) and NMP (3 h). DIC (5 equiv.) was added to a solution of the amino acid derivative (5 equiv.) and HOBt (5 equiv.) in NMP (2 mL) and allowed to react at room temperature for 5 min. The resulting suspension was added to the resin and the reaction mixture was shaken at room temperature for 1.5 h. After washing, the resin with NMP, DCM, NMP, the loading procedure was repeated using a solution of amino acid derivative (5 equiv.), HOAt (5 equiv.), DIPEA (5 equiv.) in NMP (2 mL) at room temperature for 2 h. Finally, the resin was thoroughly washed with NMP, DCM and dried with KOH in an exsiccator.

All peptides were synthesized by automated SPPS on a self loaded NovaSyn® TGR resin according to standard Fmoc chemistry protocols on a 0.10 mmol scale. Double coupling was performed for Fmoc-Lys(Boc)-OH and Fmoc-Ala-OH. After automated synthesis, the resin was placed in a syringe equipped with a polyethylene frit and dried with KOH in an exsiccator. All further reactions were performed in the syringe.

For **C6**, **C7** and **C8**, the peptides were cleaved from the resin with a TFA/TES/H_2O (95:2.5:2.5, v/v/v) solution (100 mg resin/1.0–2.0 mL) at room temperature. All solvents were evaporated and the crude product was precipitated in cold diethyl ether (5 mL) and purified by reverse phase HPLC to give the desired products as white or colorless solids.

Further coupling for **C1**–**C5** with the desired building block [3,3-bis(1-methylimidazol-2-yl)propionic acid for **C1**, **C3**, **C5**] or *tert*-butyl *N*-Fmoc-(3-Fmoc-aminopropyl)glycine for **C2**, **C4** was carried out manually. The resin was swollen for 2 h in DMF, a mixture of the building block (5 equiv.), HBTU (4.5 equiv.), HOBt (4.5 equiv.), DIPEA (10 equiv.) in DMF (2.0–3.0 mL) was added to the resin and shaken at room temperature for 40 min. The resin was thoroughly washed with DMF, DCM and dried with KOH in an exsiccator. A test cleavage was accomplished before further proceeding.

In the next step, platination was performed on the resin. A solution of K_2PtCl_4 (10 equiv., 0.05 M) in DMF/H_2O (9:1, v/v) was added to the resin and shaken in the dark for 30 h. Cleavage from the resin was carried out with a TFA/H_2O (95:5, v/v) solution (100 mg resin/1.0–2.0 mL) for 2 h, followed by evaporation, precipitation of the crude products with cold diethyl ether. Purification by reverse-phase HPLC resulted in complexes **C1**–**C5** as white solids.

H-GGGKKKKKK-NH_2 (C6): Yield 38.0 mg (40%); HPLC (0→50% B in 30 min), t_R: 10.41 min. MS (ESI): *m*/*z* = 319.9 $[M + 3H]^{3+}$, 479.3 $[M + 2H]^{2+}$, 955.8 $[M - H]^-$. HRMS (ESI): $C_{42}H_{84}N_{16}O_9$ $[M + 2H]^{2+}$ calcd. 479.3376; found 479.3379.

H-GGGGGGKKK-NH_2 (C7): Yield 7.5 mg (10%) HPLC (0→5% B′ in 30 min), t_R: 11.10 min. MS (ESI): *m*/*z* = 372.7 $[M + 2H]^{2+}$, 744.5 $[M + H]^+$, 742.4 $[M - H]^-$. HRMS (ESI): $C_{30}H_{57}N_{13}O_9$ $[M + H]^+$ calcd. 744.44750; found 744.44717, $[M + Na]^+$ calcd. 766.4294; found 766.4294, $[M - H]^-$ calcd. 742.4329; found 742.4328.

H-GGGAAAAAA-NH_2 (C8): Yield 10 mg (20%, synthesis on 0.08 mmol resin); HPLC (0→50% B in 15 min), t_R: 10.64 min. MS (ESI): *m*/*z* = 615.3 $[M + H]^+$, 637.3 $[M + Na]^+$, 613.4 $[M - H]^-$. HRMS (ESI): $C_{24}H_{42}N_{10}O_9$ $[M + H]^+$ calcd. 615.3209; found 615.3202.

[3,3-Bis(1-methylimidazol-2-yl)propionyl]-GGGKKKKKK-NH_2-dichloroplatinum (C1): Yield 22 mg (15%); HPLC (5→45% B in 30 min) t_R: 17.35 min or (0→60% B′ in 30 min) t_R: 18.58 min. MS (ESI): *m*/*z* = 1438.6 $[M + H]^+$, 719.8 $[M + 2H]^{2+}$, 480.2 $[M + 3H]^{3+}$. HRMS (ESI): $C_{53}H_{96}N_{20}O_{10}PtCl_2$ $[M + 2H]^{2+}$ calcd. 719.3384; found 719.3384.

***N*-(3-Aminopropyl)glycinyl-GGGKKKKKK-NH_2-dichloroplatinum (C2):** Yield 10 mg (8%); HPLC (5→30% B in 17 min) t_R: 10.52 min or (5→10% B′ in 30 min) t_R: 10.37 min. MS (ESI): *m*/*z* = 1336.6 $[M + H]^+$, 668.3 $[M + 2H]^{2+}$, 446.2 $[M + 3H]^{3+}$. HRMS (ESI): $C_{47}H_{94}N_{18}O_{10}PtCl_2$ $[M–Cl]^+$ calcd. 1299.6710; found 1299.6721.

[3,3-Bis(1-methylimidazol-2-yl)propionyl]-GGGGGGKKK-NH_2-dichloroplatinum (C3): Yield 35.6 mg (29%); HPLC (0→50% B in 30 min) t_R: 21.14 min. MS (ESI): *m*/*z* = 1226.5 $[M + H]^+$, 613.8 $[M + 2H]^{2+}$. HRMS (ESI): $C_{41}H_{69}N_{17}O_{10}PtCl_2$ $[M + 2H]^{2+}$ calcd. 612.7281; found 612.7281.

***N*-(3-Aminopropyl)glycinyl-GGGGGGKKK-NH_2-dichloroplatinum (C4):** Yield 16 mg (14%); HPLC (0→40% B in 30 min) t_R: 12.43 min. MS (ESI): *m*/*z* = 1123.4 $[M + H]^+$, 562.2 $[M + 2H]^{2+}$. HRMS (ESI): $C_{35}H_{67}N_{15}O_{10}PtCl_2$ $[M + H]^+$ calcd. 1122.4271; found 1122.4261, $[M–Cl]^+$ calcd. 1086.4505; found 1086.4500.

[3,3-Bis(1-methylimidazol-2-yl)propionyl]-GGGAAAAAA-NH_2-dichloroplatinum (C5): Yield 7 mg, 13% (synthesis based on 0.05 mmol resin); HPLC (15→75% B in 30 min) t_R: 13.58 min. MS (ESI): *m*/*z* = 1096.4 $[M + H]^+$. HRMS (ESI): $C_{35}H_{54}N_{14}O_{10}PtCl_2$ $[M + H]^+$ calcd. 1095.3223; found 1095.3229.

Agarose Gel Mobility Shift Assay: All experiments were performed in 10 mM phosphate buffer pH 5.8. Platination of plasmid DNA (pUC18 plasmid, purified by use of a midi-prep kit from Sigma–Aldrich) was performed at different r_f ratios; $r_f = C_{complex}/C_{nucleotide}$ from 0 to 0.3, with constant concentration of DNA, 0.15 mM as determined by a Nanodrop ND-1000 spectrophotometer. All sam-

ples were incubated at 32 °C for 2 h. The reactions were quenched by addition of 5 μL loading dye with NaCl [1.1 M urea, TBE, 33% (v/v) formamide, 0.025% (w/v) bromophenol blue, 0.025% (w/v) xylene cyanol, 500 mM NaCl].

The gel mobility shift assays were analyzed on 1% agarose-TAE gel containing 0.6 μg/mL ethidium bromide. The gels were run for 2 h at 6 V/cm. The gel image was visualized by UV light and a photo was taken using a MP-4 Polaroid camera and analyzed by DiMAGE Capture A2.

Gel analysis with 5′-^{32}P-end-labeled DNA: 5′-DNA radio-labeling was performed in T4 polynucleotide kinase (PNK) buffer (50 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 5 mM DTT, 0.1 mM spermidine, 0.1 mM EDTA) (Fermentas) with 10 U PNK (Fermentas) 1.11 MBq [γ-^{32}P]-ATP (Perkin–Elmer), 200 pmol DNA (single-stranded 22-mer 5′-TCTCCTTCTTGGTTCTCTTCTC-3′ of HPLC grade quality, IBA GmbH, Göttingen, Germany) in a total reaction volume of 20 μL at 37 °C. After incubation for 30 min, another 10 U PNK was added and the reaction proceeded for 30 min. The labeled DNA was purified on denaturing PAGE [20% acrylamide Mbis 24/1 (GERBU, Germany)/8 M urea]. The bands were visualized by lumino-radiography using a Bio-Image Analyzer (Fujifilm), excised and eluted at 4 °C overnight in 1.0 M NaOAc, pH 5.5 and recovered by ethanol precipitation.

Gelshift Studies: Radio-labeled DNA, typically 650 cps/reaction or 950 cps/reaction when using large excess conditions, was mixed with different platinum-peptide complexes in 10 mM phosphate buffer (Na_2HPO_4/NaH_2PO_4, pH 5.8). The platinum-peptide complexes or peptides alone were added to give a final concentration of 1 μM or 10 μM, which corresponds to at least equimolar concentrations of the complex and labeled DNA or 100 μM the latter corresponding to at least a 100-fold excess of the complex over nucleotide concentration. The samples were incubated at room temperature for 18 h and analyzed on denaturing PAGE (20% polyacrylamide/8 M urea, TBE buffer). The bands were visualized by lumino-radiography using a Bio-Image Analyzer (Fujifilm).

Fluorescence Measurements: DNA (DNA sodium salt from herring testis, Sigma–Aldrich) at a final concentration of 150 μM nucleotide was mixed with the different complexes at a final complex concentration of 12.5 μM giving a complex/nucleotide ratio (r_f) of 0.08 in 10 mM phosphate buffer (Na_2HPO_4/NaH_2PO_4, pH 5.8). All complex samples were incubated at 32 °C for 2 h before being transferred to a 1 cm cuvette and titrated with propidium iodide. The reactions were studied on a SPEX FluoroMax-2 (Jobin Yvon) spectrofluorimeter. The excitation wavelength was fixed to 535 nm and the emission was monitored between 550–650 nm.

Thermal Melting Studies: Two complementary strands of 22-mer DNA oligomers 5′-TCTCCTTCTTGTGTCTCTTCTC-3′ and 3′-AGAGAGGAAGAACACAGAGAAG-5′, named duplex 1, (IBA GmbH, Göttingen, Germany) were mixed in 10.0 mM phosphate buffer (Na_2HPO_4/NaH_2PO_4, pH 5.8) and annealed by heating to 100 °C for 2 min and slowly cooled to room temperature over 20 min. After the two strands had annealed the complexes **C1**–**C8** were directly added into the cuvettes. A Cary 4000 UV/Vis spectrophotometer (Varian) was used equipped with a temperature control unit and 1 cm cuvettes. All melting studies were performed at a duplex concentration of 1.0 μM and a complex concentration of 4.0 μM. Data were collected every 0.5 °C from 20 °C to 95 °C with an increasing temperature of 0.2 °C per minute. The thermal melting temperature t_m was determined by the first derivative method. All experiments were performed in triplicates at three independent occasions.

Supporting Information (see also the footnote on the first page of this article): Additional gel mobility shift assay using plasmid pUC18, autoradiogram of all complexes with radio-labeled DNA at high r_f values, and thermal melting studies of DNA platinum complexes.

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[1] B. Rosenberg, L. VanCamp, J. E. Trosko, V. H. Mansour, *Nature* **1969**, *222*, 385–386.
[2] T. Boulikas, M. Vougiouka, *Oncol. Rep.* **2004**, *11*, 559–595.
[3] L. Kelland, *Nat. Rev. Cancer* **2007**, *7*, 573–584.
[4] M. A. Jakupec, M. Galanski, V. B. Arion, C. G. Hartinger, B. K. Keppler, *Dalton Trans.* **2008**, *2*, 183–194.
[5] Y. Jung, Y. S. J. Lippard, *Chem. Rev.* **2007**, *107*, 1387–1407.
[6] D. Gibson, *Dalton Trans.* **2009**, *48*, 10681–10689.
[7] A. M. J. Fichtinger-Schepman, J. L. van der Veer, J. H. J. den Hartog, P. H. M. Lohman, J. Reedijk, *Biochemistry* **1985**, *24*, 707–713.
[8] A. Eastman, *Biochemistry* **1986**, *25*, 3912–3915.
[9] R. C. Todd, S. J. Lippard, *Metallomics* **2009**, *1*, 280–291.
[10] P. M. Takahara, C. A. Frederick, S. J. Lippard, *J. Am. Chem. Soc.* **1996**, *118*, 12309–12321.
[11] S. F. Bellon, J. H. Coleman, S. J. Lippard, *Biochemistry* **1991**, *30*, 8026–8035.
[12] S. F. Bellon, S. J. Lippard, *Biophys. Chem.* **1990**, *35*, 179–188.
[13] D. Yang, S. S. G. E. van Boom, J. Reedijk, J. H. van Boom, A. H. J. Wang, *Biochemistry* **1995**, *34*, 12912–12920.
[14] J. C. Huang, D. B. Zamble, J. T. Reardon, S. J. Lippard, A. Sancar, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 10394–10398.
[15] F. R. Keene, J. A. Smith, J. G. Collins, *Coord. Chem. Rev.* **2009**, *253*, 2021–2035.
[16] K. S. Lovejoy, S. J. Lippard, *Dalton Trans.* **2009**, *48*, 10651–10659.
[17] D. Lebwohl, R. Canetta, *Eur. J. Cancer* **1998**, *10*, 1522–1534.
[18] S. K. C. Elmroth, S. J. Lippard, *J. Am. Chem. Soc.* **1994**, *116*, 3633–3634.
[19] S. K. C. Elmroth, S. J. Lippard, *Inorg. Chem.* **1995**, *34*, 5234–5243.
[20] J. Kjellstrom, S. K. C. Elmroth, *Chem. Commun.* **1997**, 1701–1702.
[21] A. Sykfont, A. Ericson, S. K. C. Elmroth, *Chem. Commun.* **2001**, 1190–1191.
[22] A. S. Snygg, M. Brindell, G. Stochel, S. K. C. Elmroth, *Dalton Trans.* **2005**, 1221–1227.
[23] M. S. Davies, S. J. Berners-Price, T. W. Hambley, *Inorg. Chem.* **2000**, *39*, 5603–5613.
[24] S. Hanlon, L. Wong, G. R. Pack, *Biophys. J.* **1997**, *72*, 291–300.
[25] F. Noor, R. Kinscherf, G. A. Bonaterra, S. Walczak, S. Wölfl, N. Metzler-Nolte, *ChemBioChem* **2009**, *10*, 493–502.
[26] F. Noor, A. Wüstholz, R. Kinscherf, N. Metzler-Nolte, *Angew. Chem. Int. Ed.* **2005**, *44*, 2429–2432.
[27] S. I. Kirin, P. Dübon, T. Weyhermüller, E. Bill, N. Metzler-Nolte, *Inorg. Chem.* **2005**, *44*, 5405–5415.
[28] G. Dirscherl, R. Knape, P. Hanson, B. König, *Tetrahedron* **2007**, *63*, 4918–4928.
[29] G. Dirscherl, B. König, *Eur. J. Org. Chem.* **2008**, 597–634.
[30] M. Grehl, B. Krebs, *Inorg. Chem.* **1994**, *33*, 3877–3885.
[31] M. J. Bloemink, H. Engelking, S. Karentzopoulos, B. Krebs, J. Reedijk, *Inorg. Chem.* **1996**, *35*, 619–627.

[32] M. S. Robillard, A. R. P. M. Valentijn, N. J. Meeuwenoord, G. A. van der Marel, J. H. van Boom, J. Reedijk, *Angew. Chem.* **2000**, *112*, 3226–3229.
[33] N. Braussaud, T. Rüther, K. J. Cavell, B. W. Skelton, A. H. White, *Synthesis* **2001**, 626–632.
[34] P. C. A. Bruijnincx, M. Lutz, A. L. Spek, E. E. van Faassen, B. M. Weckhuysen, G. van Koten, R. J. M. Klein Gebbnik, *Eur. J. Inorg. Chem.* **2005**, 779–787.
[35] G. L. Cohen, W. R. Bauer, J. K. Barton, S. J. Lippard, *Science* **1979**, *203*, 1014–1016.
[36] E. K. Liebler, U. Diederichsen, *Org. Lett.* **2004**, *6*, 2893–2896.
[37] L. S. Lerman, H. L. Frisch, *Biopolymers* **1982**, *21*, 995–997.
[38] M. Leng, *Biophys. Chem.* **1990**, *35*, 155–163.
[39] M. J. Waring, *J. Mol. Biol.* **1965**, *13*, 269–282.
[40] T. Suzuki, K. Fujikura, T. Higashiyama, K. Takata, *J. Histochem. Cytochem.* **1997**, *45*, 49–53.
[41] M. Blackburn, J. M. Gait, D. Loakes, D. M. Williams, in: *Nucleic Acids in Chemistry and Biology*, RSC publishing, 3rd edition, **2006**, pp. 353–354.
[42] N. Poklar, D. S. Pitch, S. J. Lippard, E. A. Redding, S. U. Dunham, K. J. Breslauer, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 7606–7611.
[43] M. C. Olmsted, J. P. Bond, C. F. Anderson, M. T. Record, *Biophys. J.* **1995**, *68*, 634–647.
[44] M. S. Davies, S. J. Berners-Price, T. W. Hambley, *J. Am. Chem. Soc.* **1998**, *120*, 11380–11390.
[45] J. Kozelka, *Inorg. Chim. Acta* **2009**, *362*, 651–668.

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