New Approach for the Preparation of Efficient DNA Cleaving Agents: Ditopic Copper−Platinum Complexes Based on 3-Clip-Phen and Cisplatin

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The design and synthesis of new heterodinuclear DNA-targeting agents are described. The abilities of cisplatin and Cu(3-Clip-Phen) [Cu(1-(1,10-phenanthrolin-3-yloxy)-3-(1,10-phenanthrolin-8-yloxy)propan-2-amine)-Cl_2], an artificial DNA-cleaving agent, have been combined through their “covalent coupling”. This strategy has led to bifunctional complexes that are able to cleave the DNA in a double-stranded fashion in contrast to Cu(3-Clip-Phen) alone and have promising cytotoxicities compared to cisplatin in several cell lines.

Introduction

Among the strategies that find application in clinical anticancer chemotherapy, the cancer cells eradication induced by DNA-interacting molecules has proved its efficiency.[1,2] First of all, cisplatin is one of the most widely used anticancer agents. It is generally accepted that the distortion of DNA generated upon binding of cisplatin is largely responsible for its antitumor properties.[3] Second, the therapeutic anticancer activity of the natural antibiotic bleomycin has been attributed to the ability of its metal complexes to perform oxidative DNA cleavage via oxidative degradation of the deoxyribose units.[4] This important discovery has led to the design and preparation of synthetic models of bleomycin such as 3-Clip-Phen [1-(1,10-phenanthrolin-3-yloxy)-3-(1,10-phenanthrolin-8-yloxy)propan-2-amine)-Cl_2].[5] Copper complexes of 3-Clip-Phen mediate single-strand cleavage of DNA from the minor groove, through the oxidation of the sugar moiety, albeit without sequence specificity.[5,6]

Intrinsic or acquired drug resistance is a common problem in cisplatin chemotherapy.[7−9] Therefore, combination therapy was developed.[1] For example, bleomycin, etopside, and cisplatin are simultaneously used for testicular cancer treatment, resulting in a curing rate of >99% if applied at the early stage of the syndrome.[10] Proceeding from this success, multifunctional drugs were developed.[11] For instance, cisplatin was combined with intercalating agents known as topoisomerase blockers[12−17] and also with a photoactivated cleaving agent.[18]

The combined interest in (i) improving the DNA cleavage specificity of Cu(3-Clip-Phen)[a] complexes together with their ability to perform double-strand breaks (DSB) and (ii) circumventing drug resistance owing to the use of cisplatin[7−9,19,20] has inspired the design of bifunctional molecules (Figure 1) containing both active entities (see **13** and **14**, Scheme 1).

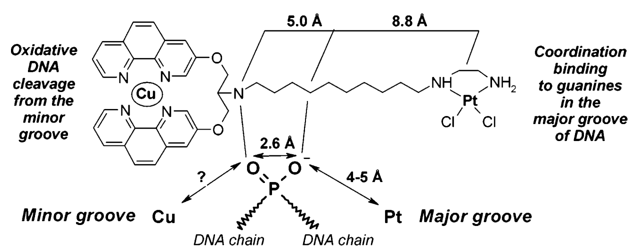

Figure 1. Strategy adopted for the synthesis of heterodinuclear minor/major groove interacting complexes.

Thus, compounds **13** and **14** have been prepared considering the intrinsic DNA-interaction characteristics of the two separate active metallic centers. Depending on their mutual degree of freedom, both moieties will or will not reach their preferential site of interaction simultaneously, namely, the nitrogen atom N7 of guanine in the major groove for the platinum component, and the minor groove for the copper unit.[6] The minimum separation distance required to achieve such concomitant minor−major groove interactions has been determined from the crystal structure of a DNA−cisplatin adduct.[21] The phosphate oxygen atom pointing toward the major groove and the platinum ion of cisplatin are 4−5 Å apart from each other (Figure 1). The distance between both oxygen atoms of the same phosphate group is about 2.6 Å.

It has been proposed that the amino group of Cu(3-Clip-Phen) could be protonated, favoring its interaction with the polyanionic structure of DNA.[22] Thus, it could interact via hydrogen bonding with the oxygen atom of the phosphate, thereby pointing toward the minor groove.[23,24]

Compound **14** has, therefore, been designed with a bridge long enough to allow the interaction of both metal centers with their respective preferential target site. In the case of a shorter bridge, that is, compound **13**, both moieties will be forced to sit in the same DNA groove.

Results and Discussion

The general synthetic pathway to prepare ligands **1** and **2** is depicted in Scheme 1 (see Supporting Information for details). The selective and complete platination of the ethylenediamine unit using 1 equiv of K_2PtCl_4 was monitored by ^{195}Pt NMR and UV spectroscopy (see Supporting Information). The in situ

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[a] Abbreviations: Cu(3-Clip-Phen), Cu(1-(1,10-phenanthrolin-3-yloxy)-3-(1,10-phenanthrolin-8-yloxy)propan-2-amine)Cl_2; DSB, double-strand breaks; MPA, mercaptopropionic acid; TAQ, *thermus aquaticus*; MeOH, methanol; DMSO, dimethyl sulfoxide; DMF, dimethylformamide; PCC, pyridinium cloridotrioxidochromate; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

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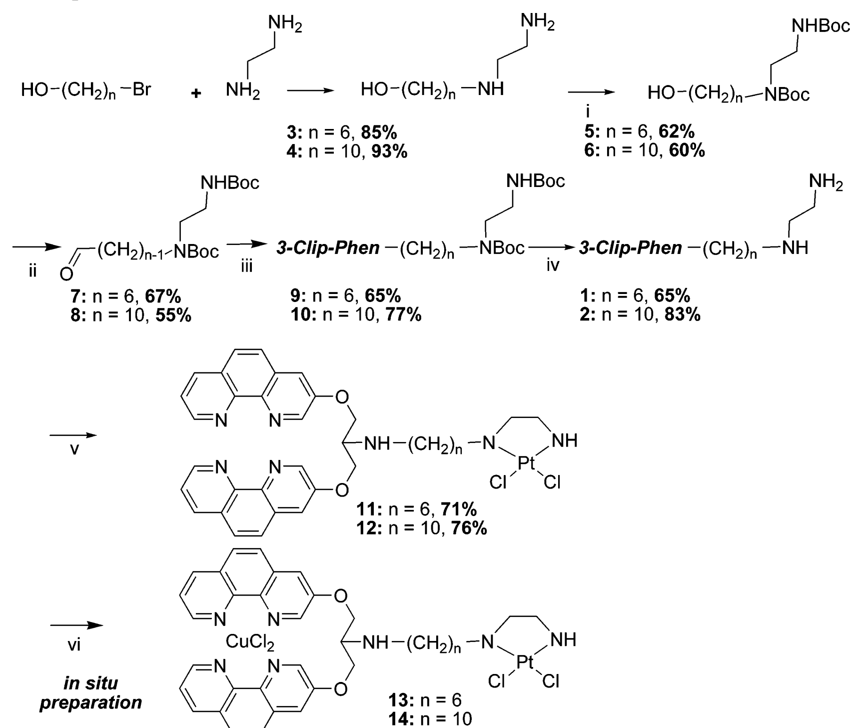
Scheme 1. Preparation of Complexes **13** and **14**[a]



[a] Reagents and conditions: (i) (1) NaH, Boc_2O, THF, overnight; (2) K_2CO_3, MeOH, reflux, 1 h; (ii) pyridinium chloridotrioxidochromate (PCC), CH_2Cl_2, 2 h; (iii) (1) 3-Clip-Phen, MeOH, reflux, 2 h; (2) $NaBH_4$, reflux, 2 h; (iv) TFA, 0 °C, 1 h; (v) K_2PtCl_4, MeOH/H_2O, 5 h; (vi) $CuCl_2$, DMF, 50 °C.

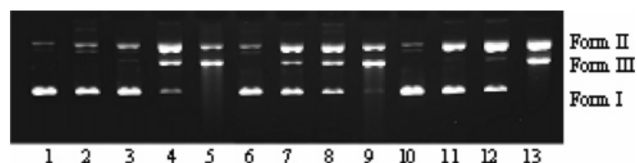


Figure 2. Comparison of the oxidative cleavage of ΦX174 plasmid DNA performed by **13**, **14**, and Cu(3-Clip-Phen) in the presence of 5 mM MPA. Lane 1: control DNA. Lane 2: 250 nM **13** without MPA. Lane 3: 100 nM **13**. Lane 4: 150 nM **13**. Lane 5: 250 nM **13**. Lane 6: 250 nM **14** without MPA. Lane 7: 100 nM **14**. Lane 8: 150 nM **14**. Lane 9: 250 nM **14**. Lane 10: 250 nM Cu(3-Clip-Phen) without MPA. Lane 11: 100 nM Cu(3-Clip-Phen). Lane 12: 150 nM Cu(3-Clip-Phen). Lane 13: 250 nM Cu(3-Clip-Phen).

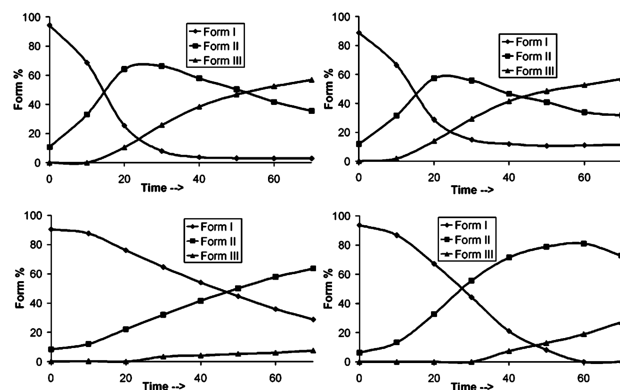


Figure 3. Time course experiments of DNA cleavage (20 μM base pairs) over a period of 70 min in the presence of 5 mM MPA and air. Before addition of the reductant, the complexes were incubated for 24 h. (a) 200 nM **13**, (b) 200 nM **14**, (c) 200 nM Cu(3-Clip-Phen), (d) 250 nM Cu(3-Clip-Phen).

reactions of the so-obtained platinum derivatives **11** and **12** with 2 equiv of $CuCl_2$ yielded the heterobimetallic complexes **13** and **14**, respectively.

The relaxation of supercoiled circular ΦX174 DNA (form I) into the relaxed (form II) and the linear (form III) conformations is monitored to compare the aerobic cleavage abilities of complexes **13**, **14**, and Cu(II)-3-Clip-Phen in the presence of a reducing agent (Figure 2). The bifunctional complexes are incubated for 20 h to allow the formation of platinum−DNA adducts. The nuclease activity is subsequently initiated by the addition of 5 mM mercaptopropionic acid (MPA) in air.

Lanes 4, 8, and 12 clearly show that the nuclease activities of **13** and **14** are higher than the one achieved with Cu(3-Clip-Phen), because more linear DNA is formed. Indeed, quantifications show that **13** and **14** cleaved the largest quantities of form I (starting material). Using the same experimental conditions (lanes 4, 8, and 12), Form III is generated by the platinum/copper complexes and not by Cu(3-Clip-Phen). Moreover, smears (resulting from multifragmented DNA) are observed when using complexes **13** or **14** at 250 nM concentration, which is not the case with Cu(3-Clip-Phen) (lanes 5, 9, and 13). The most striking feature observed for these hybrid platinum/copper complexes is that form III already appears, while form I is still present. This result obviously indicates that the heterobimetallic complexes are able to perform direct double strand cuts, whereas Cu(3-Clip-Phen) is only capable of carrying out successive single-strand cuts.[5] Time course studies of the cleavage of the complexes **13**, **14**, and Cu(3-Clip-Phen) have been performed to further investigate the direct double strand cleavage (Figure 3). From these studies, it appears that linear DNA is formed for both complexes **13** and **14** directly from the start of the reaction in contrast to Cu(3-Clip-Phen). Even after most of the supercoiled DNA has disappeared in the reaction with Cu(3-Clip-Phen), only less than 8% of linear DNA has formed. Also, these studies confirm the higher cleaving activities of complexes **13** and **14** compared to Cu(3-Clip-Phen) alone, because a fast disappearance of form I associated with the appearance of form III is observed with these bifunctional molecules. If the concentration of Cu(3-Clip-Phen) is increased to 250 nM, the

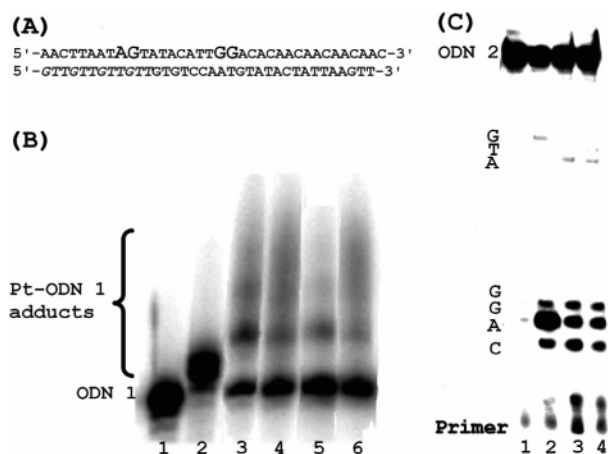


Figure 4. (a) DNA fragment (36 bp) used for these studies. The two major platination sites are shown in bold, and the primer is visualized in italic on ODN 2. (b) PAGE analysis of the platinum–ODN 1 adducts. ODN 1–ODN 2 duplex was ^{32}P-labeled on the 5′-end of ODN 1. The complexes were incubated for 24 h with the ODN 1–ODN 2 duplex before analyses. Lane 1: ODN 1. Lane 2: 3 μM cisplatin. Lane 3: 10 μM **11**. Lane 4: 10 μM **13**. Lane 5: 10 μM **12**. Lane 6: 10 μM **14**. (c) Phosphor image of a DNA sequencing gel comparing the sequence specificity of cisplatin **11** and **12** with primer extension. ODN 1–ODN 2 duplex was incubated with platinum complexes then precipitated to remove unlinked complexes. 5′-end-labeled primer was added and all the samples were extended using TAQ polymerase, starting from the 5′-end-labeled primer. Lane 1: blank experiment. Lane 2: 3 μM cisplatin. Lane 3: 10 μM **11**. Lane 4: 10 μM **12**. Note that the AG and GG sites give the sequence of the opposite strand.

full disappearance of the supercoiled DNA is observed (Figure 3d). Although, small quantities of linear DNA are still observed before the complete disappearance of supercoiled DNA, its amount is inferior to the one noticed with complexes **13** and **14**. In addition, more than 80% of circular DNA is formed, while the reaction with complexes **13** and **14** only generate a maximum of 60% of this form II.

The DSB formation was further investigated by a statistical test developed by Povirk et al., which has been used to assay other complexes such as bleomycin.[25,26] This test assumes a Poisson distribution of strand cuts and allows the calculation of the average number of DSB per molecule, n_2. The average number of DSB per molecule, n_2, is obtained from the fraction of linear DNA after cleavage, and the total average number of single- plus double-strand breaks ($n_1 + n_2$) is obtained from the fraction of remaining supercoiled DNA after reaction. To determine n_1 and n_2, both supercoiled and linear DNA should be present in the experiments. For complexes **13** and **14**, the n_1/n_2 values determined at 20 min (Figure 3) amounts to, respectively, 10.5 and 6.8 and, respectively, 5.8 and 3.1 at 30 min. The n_1/n_2 values for Cu(3-Clip-Phen) at 40 and 50 min are, respectively, 18.5 and 16.4. The DSB values of **13** and **14** are in the range of bleomycin,[26] while the cleavage occurs in a more random manner for Cu(3-Clip-Phen). These results emphasize the ability of complexes **13** and **14** to induce direct double-stranded cuts.

Therefore, it appears that the platinum moiety of complexes **13** and **14** acts as an anchor to DNA through a kinetically inert coordination bond. Subsequently, the Cu(3-Clip-Phen) component can function as a cleaving agent only in the close proximity of the platinum moiety, thereby favoring double-stranded scissions.

To further investigate the platinum coordination of the complexes to DNA, high resolution analyses with a 36 bp DNA fragment were performed (Figure 4a). This fragment contains the two major platination sites on the 5′-end-labeled ODN 1 strand

Table 1. In Vitro Cytotoxicity Assays for **11**–**14**, Cu(3-Clip-Phen), and Cisplatin, against a Selection of Cancer Cell Lines

	IC$_{50}$[a] values (μM)					
cell lines	**11**	**12**	**13**	**14**	Cu(3-Clip-Phen)	cisplatin
WIDR	3.7	3.6	7.0	>10	0.58	3.2
EVSA-T	5.6	4.0	1.6	2.7	0.53	1.4
H226	3.0	4.1	5.1	>10	1.6	>10
A549	>10	7.4	>10	>10	>10	1.5
MCF-7	0.9	5.4	3	>10	>10	9
M19	6.0	5.2	0.86	0.37	0.30	1.9
IGROV	>10	>10	7.7	>10	5.4	0.56
U373	>10	6.5	7.1	>10	>10	5

[a] IC$_{50}$ = concentration of drug required to eradicate 50% of the cancer cells.

(highlighted in bold in Figure 4a). The complexes able to coordinate to DNA retard the migration rate of the complexed DNA during denaturing polyacrylamide gel electrophoresis (PAGE) as a result of the increase in molecular weight and the change of the overall charge. Therefore, complexes **11**–**14** were incubated for 24 h with the 36 bp DNA fragment, followed by PAGE to detect the platinum–DNA adducts (Figure 4b). The complexes are all able to coordinate to ODN 1, because free ODN 1 has partially disappeared and clear bands have emerged above the free ODN fragment (Figure 4b). Cisplatin has almost fully reacted, and a clear band corresponding to the adducts is observed (Figure 4b, lane 2). Complexes **11**–**14** have partially reacted with the ODN fragment with the following order of efficiency: **11** ≈ **12** > **13** = **14**. In contrast to cisplatin, the products engendered by complexes **11**–**14** give rise to a smear (Figure 4b). This is especially true for complexes **13** and **14** whose copper moieties are coordinated. No clear bands can be observed (lanes 4 and 6), most likely as the result of unselective DNA binding of the platinum part caused by the Cu(3-Clip-Phen) moiety, which preferentially coordinates in the minor groove of DNA.

Primer extension experiments are performed to investigate the sequence selective binding of platinum complexes to DNA.[27–33] TAQ polymerase has proven to effectively stop at platination sites and is, therefore, used for these studies.[34–36] However, significant quantities of reductant are needed to activate the TAQ polymerase. Consequently, the copper-containing complexes were not investigated using this method. The cisplatin-specific damage sites are the GG and AG sites, but the majority of cisplatin is detected at the GG site. Complexes **11** and **12** lead to similar results, suggesting that the platinum part is interacting with its preferred site (Figure 4c). However, the bulkiness of the complexes also affects the bases in the close proximity of the adduct. Therefore, the stops of the TAQ polymerase are also influenced by this steric issue. In the experiment with cisplatin, the polymerization is halted at the GG site, primarily on the A before the GG spot, but also at the cytosine before the adenine and at the first guanine. Near the AG site, the TAQ polymerase mainly stops at the G base. For complexes **11** and **12**, the enzyme halts at the GG site are equally distributed between the GAC nucleobases. At the AG site, the damage is mainly observed at the A base. However, no stops are detected at the G base.

The cytotoxic activities of complexes **11**–**14**, Cu(3-Clip-Phen) and cisplatin have been determined for breast (MCF7 and EVSA-T), colon (WIDR), ovarian (IGROV), melanoma (M19), renal (A498), non-small lung (H226), two glioblastomas (Hs683 and U373), two colorectal (HCT-15 and LoVo), and lung (A549) cancer cell lines. The results of the most relevant activities are summarized in Table 1. The cytotoxicity of 3-Clip-Phen for several cell lines is included in the Supporting Information. The cytotoxicities of all complexes are insignificant

for the cell lines HCT-15, Hs683, LoVo, and A498. Therefore, the corresponding values have been excluded from Table 1. Indeed, a complex with an IC_{50} value higher than 10 μM (>10) is considered to be inactive.[12]

The IC_{50} values observed for Cu(3-Clip-Phen) are in some cases superior to those achieved with cisplatin (Table 1, WIDR, EVSA-T, H226, and M19). The significant antiproliferative activity of 3-Clip-Phen and the corresponding copper complex has been previously measured on the L1210 murine leukemia cell line.[28] These good activities confirm the high potential of Cu(3-Clip-Phen) as an antitumor agent and strengthen the potential of our strategy to prepare hybrid Cu(3-Clip-Phen)/*cis*-Pt derivatives. The IC_{50} values obtained with the copper-free complexes (**11** and **12**) are similar or higher compared to the ones achieved with cisplatin, for the cell lines WIDR, H226, and MCF-7. Complex **11** is even 10 times more active than cisplatin for the cell line MCF-7, where Cu(3-Clip-Phen) has been found to be inactive. Interestingly, in some cell lines, the cytotoxicities of **11** or **12** are even higher than those reached with **13** or **14**. However, for the EVSA-T and M19 cell lines, the hybrid platinum/copper complexes are more active, which is in agreement with the known activity of Cu(3-Clip-Phen). However, Cu(3-Clip-Phen) is equally good or better in both cell lines. In most cases, compound **12** is more active than **11**, and surprisingly, **13** is more efficient than **14**.

The new synthetic approach to produce highly efficient DNA-cleaving agents herein reported now also offers the prospect to improve the nuclease activity of well-known effective drugs through their synergistic combination within a single molecule. The resulting ditopic (or even multitopic) bio-active molecules thus expand the mechanism of action of its well-established antitumor-active components. Fine tuning of the cleaving ability of such Cu−Pt complexes is currently in progress by further varying the nature and the length of the bridge between the platinum and the Cu(3-Clip-Phen) moieties. Investigations on the cleavage mechanism with these potential anticancer drugs are currently carried out.

Experimental Section

Synthesis of Pt[N*1*-{6-[3-Clip-Phen]-hexyl}-ethane-1,2-diamine]Cl$_2$ (11) and Pt[N*1*-{10-[3-Clip-Phen]-decyl}-ethane-1,2-diamine]Cl$_2$ (12). A solution of K_2PtCl_4 (0.1 mmol) in 3 mL of H_2O was added dropwise to a solution of **1** or **2** (0.1 mmol) in 6 mL of MeOH over a period of 2 min. The resulting off-white precipitate was filtered after a reaction time of 5 h. The product was washed consecutively with 3 × 10 mL of H_2O, 3 × 10 mL of MeOH, and 2 × 20 mL of diethyl ether. The coordination compounds were dried overnight at 50 °C under reduced pressure. The integrals of the ^{1}H NMR peaks around 4.60 ppm may be inaccurate due to the suppression of the water signal by the program used during the scanning of the sample. Also, the signal owing to DMSO, used to dissolve the samples, is partially overlapping the peaks in the 2.70 ppm area. Data for **11**: Off-white powder (yield = 71%); ^{1}H NMR (DMSO-d_6 and D_2O, 300 MHz) δ 8.82 (br, 2H), 8.64 (br, 2H), 8.42 (br, 2H), 7.78 (br, 2H), 7.64−7.54 (br, 6 H), 4.84 (br, 1H), 4.57−4.53 (br, 2H) 4.15 (br, 1H), 3.07−2.69 (m, 6H), 1.80 (br, 2H), 1.67 (br, 2H), 1.41 (br, 4H) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz) δ 153.7, 148.2, 142.4, 139.3, 137.4, 129.9, 127.5, 127.1, 123.0, 116.4, 65.2, 55.3, 46.7, 46.0, 44.2, 35.4, 25.3 ppm; ^{195}Pt NMR (DMSO-d_6, 300 MHz) δ −2308 (complex) and −2949 (complex with coordinated DMSO) ppm. MS (MALDI-TOF) *m/z* 898.6, 897.6, 899.6, 900.6, 901.6,902.6 [(M + DMSO-(solvent) − Cl$^-$)$^+$; calcd for $C_{37}H_{45}ClN_7O_3PtS^+$: 898.3, 897.3, 899.3, 900.3, 901.3,902.3], 784.4, 785.4, 783.4, 786.4, 787.5, 788.4 [(M − HCl − Cl$^-$)$^+$; calcd for $C_{35}H_{38}N_7O_2Pt^+$: 784.3, 785.3, 783.3, 786.3, 787.3, 788.3]. Data for **12**: Off-white powder (yield = 76%); ^{1}H NMR (DMSO-d_6 and D_2O, 300 MHz) δ 8.78 (br, 2H), 8.68 (br, 2H), 8.23 (br, 2H), 7.67−7.49 (m, 4H), 4.81 (br, 1H), 4.58 (br, 3H), 4.11 (br, 2H), 2.82−2.69 (br, 7H), 1.76 (br, 16H) ppm; ^{13}C NMR (DMSO-d_6, 75 MHz) δ 154.5, 150.7, 145.5, 143.3, 138.5, 130.8, 128.5, 127.8, 123.9, 117.4, 66.4, 56.7, 53.2, 48.0, 47.4, 36.6, 29.9, 27.4−26.8 ppm; ^{195}Pt NMR (DMF-d_7, 300 MHz) δ −2329 ppm. MS (MALDI-TOF) *m/z* 839.1, 840.1, 838.1, 841.1, 842.1, 843.1 [(M − HCl − Cl$^-$)$^+$; calcd for $C_{39}H_{46}N_7O_2Pt^+$: 839.3, 840.3, 838.3, 841.3, 842.3, 843.3]. ICP-AES Pt to K ratio = 1:0.37. Anal. Calcd for $C_{39}H_{47}Cl_2N_7O_2Pt{\cdot}0.37KCl{\cdot}6H_2O$: C, 44.72; H, 5.68; N, 9.36. Found: C, 44.25; H, 5.04; N, 9.19.

Synthesis of CuPt[N*1*-{6-[3-Clip-Phen]-hexyl}-ethane-1,2-diamine]Cl$_4$ (13) and CuPt[N*1*-{10-[3-Clip-Phen]-decyl}-ethane-1,2-diamine]Cl$_4$ (14). $CuCl_2$ (0.1 mmol) was added as a solid to a suspension of **11** or **12** (0.05 mmol) in DMF (25 mL). The reaction was stirred overnight at 50 °C. The DMF was partially evaporated under reduced pressure, and the crude was precipitated in 100 mL of diethyl ether. The solid material was filtered and washed with 3 × 20 mL of diethyl ether and dried overnight at 50 °C under reduced pressure. The resolution of the mass measurements was not sufficient enough, because only broadened peaks were observed. Data for **13**: MS (MALDI-TOF) *m/z* 954.1 [(M − Cl$^-$)$^+$; calcd for $C_{35}H_{39}Cl_3N_7O_2PtCu^+$: 954.7], UV−vis (DMSO/$H_2O$ 10/90) λ_{max}/nm (ϵ/dm^3 mol^{-1} cm^{-1}): 281 (41 500), 318 (14 400), 346 (5700). Data for **14**: MS (MALDI-TOF) *m/z* 1055.1 [(M + DMSO −2Cl$^-$)$^+$; calcd for $C_{41}H_{53}Cl_2N_7O_3SPtCu^+$: 1053.5], 1077.1 [(M + DMSO − 2Cl$^-$)$^+$; calcd for $C_{41}H_{53}Cl_2N_7O_3SNaPtCu^+$: 1076.5], UV−vis (DMSO/$H_2O$ 10/90) λ_{max}/nm (ϵ/dm^3 mol^{-1} cm^{-1}): 282 (43 600), 319 (17 500), 347 (8000)

Analysis of Platinum Adducts by High-Resolution Polyacrylamide Gel Electrophoresis. The ODNs 1 and 2 and the primer were purchased from Eurogentec and purified on a 15% polyacrylamide gel. Concentrations of single-stranded ODNs were determined by UV titration at 260 nm.[37] The ODNs were end-labeled with ^{32}P using standard procedures with T_4 polynucleotide kinase (New England BioLabs) and [γ-^{32}P]ATP for the 5′-end, before being purified on a MicroSpin G25 column (Pharmacia).[38]

Analysis of the platinum−DNA adducts: The 36mer ODN 1−ODN 2 duplex target (2 μM, 5′-end labeled on ODN 1) was annealed in 1100 μL of Tris-HCl (20 mM, pH 7.2) by heating to 90 °C for 5 min, followed by slow cooling to room temperature. Samples were divided in shares of 60 μL before addition of 60 μL of complex solution (6 μM cisplatin or 20 μM of complexes **11**−**14**). Samples were incubated for 20 h at 37 °C and subsequently precipitated with 100 μL of sodium acetate buffer (3 M, pH 5.2) and 1300 μL of cold ethanol. The resulting pellets were rinsed twice with 200 μL of cold ethanol and then lyophilized. The samples dissolved in 5 μL of a bromophenol blue/xylene cylenol/formamide solution were separated by denaturing 20% polyacrylamide gel electrophoresis.

Primer extension experiments: The 36mer ODN 1−ODN 2 duplex target (2 μM) was annealed in 1100 μL of Tris-HCl (20 mM, pH 7.2) by heating to 90 °C for 5 min, followed by slow cooling to room temperature. Samples were divided in shares of 60 μL before the addition of 60 μL of complex solution (6 μM cisplatin or 20 μM complex **11** and **12**). Samples were incubated for 20 h at 37 °C and then precipitated with 100 μL of sodium acetate buffer (3M, pH 5.2) and 1300 μL of cold ethanol. The resulting pellets were rinsed twice with 200 μL of cold ethanol and then were lyophilized. The samples were dissolved in MilliQ H_2O to a 1.25 μM concentration of ODN 1/ODN 2. To 2 μL of this solution was added 1 μL of enzyme 10 × buffer, 2 μL of primer/ODN 1 solution (1 equiv of 5′-end labeled primer + 1 equiv of ODN 1), and 2 μL of MilliQ H_2O. The samples were hybridized by heating to 90 °C for 5 min, followed by slow cooling to room temperature. To this solution, 2 μL of a 250 μM dGTP, dCTP, dATP, and dTTP solution were added and 1 μL of enzyme (2.5 units TAQ polymerase) was added. The reaction time with the TAQ polymerase was 30 min at 37 °C. TAQ polymerase is a thermally stable enzyme; therefore, the samples were directly frozen in liquid nitrogen. A total of 5 μL of a bromophenol blue/xylene cylenol/formamide solution were added to a 5 μL sample, followed by

separation by denaturing 20% polyacrylamide gel electrophoresis. Maxam and Gilbert sequencing scale, where the 3′-phosphate ends of the resulting fragments were removed with polynucleotide kinase, was used to analyze DNA fragments.[32]

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Supporting Information Available: Detailed experimental procedures, including spectroscopic and analytical data, UV-spectra of selective coordination, gel electrophoreses details, and cytotoxicity studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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