Notes

Approaches to Selective DNA Binding in Polyfunctional Dinuclear Platinum Chemistry. The Synthesis of a Trifunctional Compound and Its Interaction with the Mononucleotide 5′-Guanosine Monophosphate

Yun Qu, James A. Fitzgerald, Holger Rauter, and Nicholas Farrell*

Department of Chemistry, Virginia Commonwealth University, Richmond, Virginia 23284-2006

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Introduction

Poly(di, tri)nuclear platinum compounds are a new class of platinum anticancer agents with a unique profile of DNA binding and antitumor activity, including activity against cisplatin-resistant tumor cell lines.[1] In dinuclear chemistry, we have reported on the synthesis and biological properties of complexes which are potentially tetrafunctional (2,2/c,c [{*cis*-$PtCl_2$ (NH_3)}$_2\mu$-NH_2-R-NH_2]), trifunctional (1,2/c,c or 1,2/t,c [{$PtCl(NH_3)_2$}μ-NH_2-R-NH_2-{$PtCl_2(NH_3)$}]$^+$), and bifunctional (1,1/c,c or 1,1/t,t [{$PtCl(NH_3)_2$}μ-NH_2-R-NH_2-{$PtCl(NH_3)_2$}]$^{2+}$).[2−5] The general dinuclear platinum structure offers a rich variety of potential DNA binding modes. An interesting feature is that compounds containing two *cis*-[$PtCl_2$(amine)$_2$] coordination spheres form ternary DNA−protein crosslinks by initial production of a DNA−DNA interstrand crosslink followed by binding to the polypeptide, as exemplified by the repair protein UvrA.[6,7] The ability to form ternary DNA−protein crosslinks is shared by trifunctional compounds such as I and II, Figure 1.[8] An inherent problem in describing the exact modes of DNA−DNA and indeed ternary DNA−protein crosslinks of polyfunctional dinuclear platinum complexes is the competitive reaction between the two Pt coordination spheres.[6,8] For I and II, a plethora of interstrand crosslinks is possible depending on the two Pt−Cl bonds we chose to substitute. In this paper, we present the synthesis of a novel trifunctional dinuclear platinum complex (1,2/t-Cl,mal, III, Figure 1). It was anticipated that the differences in substitution kinetics between chloride and malonate in III would permit selective platination of the monofunctional Pt−Cl unit, which is followed by sequential reactions of malonate displacement. The reactions of the three trifunctional platinum complexes with guanosine-5′-monophosphate (GMP) as an indicator of a selective DNA crosslinking agent were followed by ^{1}H NMR spectroscopy and compared with those reported for their tetrafunctional and bifunctional analogues.[9,10]

Experimental Section

Starting Materials and Instrumentation. Reagents and guanosine 5′-monophosphate disodium salt (GMP) were purchased from Sigma or Aldrich and used without further purification. *Cis*- and *trans*-[$PtCl_2$-$(NH_3)_2$] were prepared by standard methods. K[$PtCl_3(NH_3)$], [{*cis/trans*-$PtCl(NH_3)_2$}μ-$NH_2(CH_2)_6NH_2-${*cis*-$PtCl_2(NH_3)$}]$^+$ (1,2/t,c-Cl,Cl and 1,2/c,c-Cl,Cl), and *N*-*tert*-butoxy-carbonyl-1,6-diaminohexane hydrochloride were prepared according to published procedures.[5,11] ^{1}H NMR spectra were recorded on a General Electric QE−300, or a Varian Unity 500 MHz spectrometer. Chemical shifts are referenced to internal standards Me_4Si for organic solvents and TSP (2-(trimethylsilyl)-propanesulfonate, sodium salt) for D_2O. ^{195}Pt NMR spectra were obtained on a General Electric QE−300 MHz spectrometer and referenced to K_2PtCl_4 ($\delta = -1624$ ppm) as external standard. Elemental analyses were performed by Robertson Microlit Laboratories, Madison, NJ, and FAB mass spectrometry was performed by University of Arizona, Tucson, Arizona.

Preparations. *cis*-[Pt (mal)$(NH_3)H_2N(CH_2)_6NH_3$]NO_3 (*iii*). The preparations of ***i*** and ***ii*** followed previous reports.[5] A suspension of 0.667 g (2.14 mM) of silver sulfate in a solution of 1 g (2.16 mM) of compound ***ii*** nitrate in 90 mL H_2O was stirred overnight at room temperature and protected from light. After removing silver chloride by filtration, a gelatinous slurry of barium malonate (prepared by combination of a solution of 0.223 g (2.14 mM) of malonic acid in 20 mL of H_2O with a solution of 0.675 g (2.14 mM) of barium hydroxide octahydrate in 30 mL of H_2O) was added. After stirring overnight, barium sulfate was removed by filtration, and the filtrate was evaporated to dryness to give 0.912 g of a white solid ***iii***, 85% yield. ^{1}H NMR in D_2O: δ 3.2 (s, 2H), δ 3.00 (t, 2H), δ 2.59 (t, 2H), δ 1.77 (t, 2H), δ 1.68 (t, 2H), δ 1.40 (m, 4H). Anal. Calcd for $C_9H_{22}N_4O_7Pt$: C, 21.91; H, 4.49; N, 11.36. Found: C, 21.65; H, 4.54; N, 11.12.

[{*trans*-$PtCl(NH_3)_2$}μ-$NH_2(CH_2)_6NH_2-${Pt(mal)(NH_3)}] NO_3 (III). A cold solution of *trans*-[$PtCl(NH_3)_2$(DMF)]NO_3 (−20 °C, 0.33 mM) was prepared.[5] A solution of *cis*-[Pt(mal)$(NH_3)H_2N(CH_2)_6NH_2$], prepared from compound ***iii*** (170 mg, 0.334 mM) in 5 mL of DMF neutralized with sodium methoxide (18.6 mg, 0.344 mM) in 1 mL of anhydrous methanol, was added over 15 min. Stirring was continued for 30 min at −20 °C; the solution was allowed to warm to room temperature and stirred for an additional 3 h followed by standing at 4 °C for 18 h. After filtration through activated carbon, the filtrate was evaporated to dryness. The oily residue was extracted with ether and acetone several times to yield a white solid. The white solid was extracted with 5 × 5 mL of methanol to remove the byproduct sodium nitrate and yield a white solid **III**, 58 mg, yield 23%, which is recrystallized from MeOH. ^{1}H NMR in D_2O: δ 3.33 (s, 2H), δ 2.69

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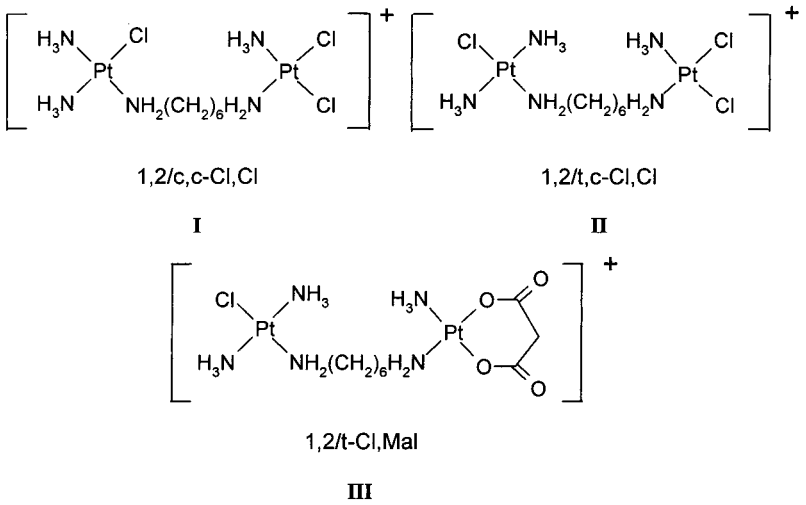
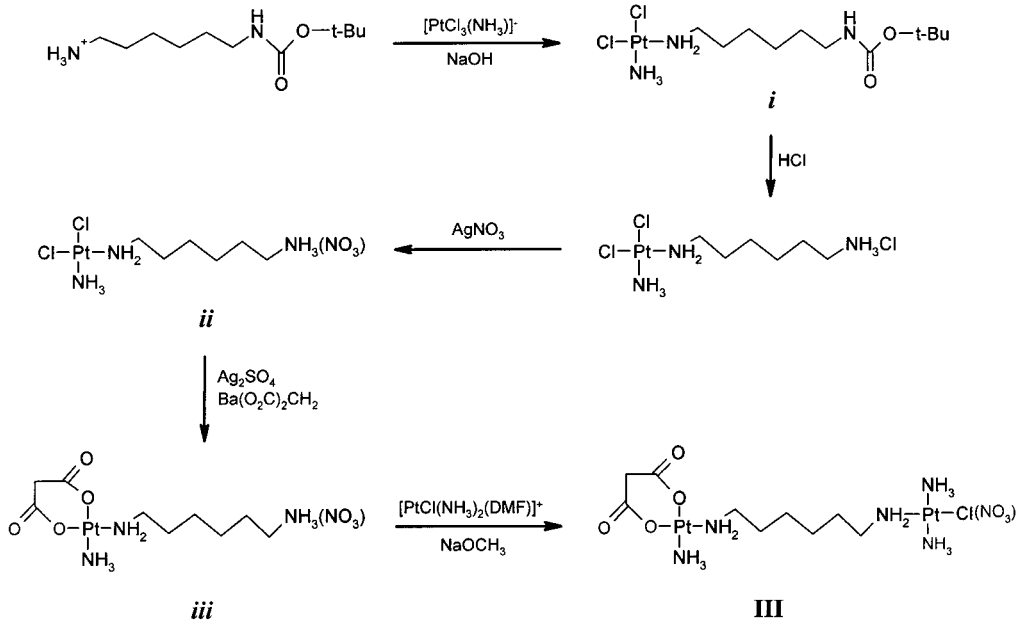


Figure 1. Chemical structure of trifunctional dinuclear platinum complexes. The system of abbreviation allows the numbers to represent the number of chloride leaving groups on each platinum atom; the lettering refers to the geometry of the leaving group with respect to the nitrogen of the bridging diamine.

Scheme 1



(t, 2H), δ 2.57 (t, 2H), δ 1.76 (t, 2H), δ 1.69 (t, 2H), δ 1.39 (m, 4H). ^{195}Pt NMR in D_2O: δ −1795, δ −2409. Anal. Calcd for $Pt_2C_9H_{27}N_6O_7Cl$: C, 14.28; H, 3.60; N, 11.10; Cl, 4.68. Found: C, 14.30; H, 3.63; N, 11.25; Cl, 4.41. FAB MS gave a peak at *m*/*z* 694.11 (calcd. 694.96), consistent with the proposed structure of **III**.

^{1}H NMR Spectroscopy. The reactions of I, II, and III (3 mM in 99.996% D_2O) with GMP (1:1 and 1:3 stoichiometry) were monitored over time at 37 °C by ^{1}H NMR spectroscopy typically using 90° pulses, a spectral width of 12 ppm, an acquisition time of 1.09 s, a relaxation delay of 2 s, and 64 scans. Reactions were quantitated by integration of H8 signals of GMP. The pD (uncorrected) of the solutions varied from 6.5 to 7.1 during the reactions.

Results and Discussion

Synthesis. The reaction sequence used to prepare [{*trans*-$PtCl(NH_3)_2$}μ-$NH_2(CH_2)_6NH_2$−{Pt(mal)(NH_3)}] NO_3, **III**, is shown in Scheme 1. The structure and purity were confirmed by ^{1}H and ^{195}Pt NMR spectroscopy, FABMS, HPLC, and elemental analysis (see Experimental Section).

Kinetics. The stepwise substitution of chloride and malonate leaving groups by GMP in the reactions of the three complexes show significant differences in reaction rates and intermediate products. The 1:3 reaction is more complicated than the 1:1 reaction in most cases, reflecting competitive reactions between the two inequivalent coordination spheres. The designation of total charges on the complexes results from allowing a 2− charge on the GMP moiety, following that of Miller and Marzilli.[10,12] Complexes II and III of Figure 1 (the 1,2/t,c-Cl,Cl and 1,2/t-Cl,mal) should produce the same final product, [{*trans*-$Pt(GMP)(NH_3)_2$}μ-$NH_2(CH_2)_6NH_2$−{*cis*-$Pt(GMP)_2(NH_3)$}]$^{2-}$, whereas I (1,2/c,c-Cl,Cl) gives a final product differing only in geometry on the monofunctional platinum unit. Previous studies showed that the H8 chemical shifts of GMP in [Pt(GMP)-$(amine)_3$], [$Pt(GMP)_2(amine)_2$], and [PtCl(GMP)$(amine)_2$] differ sufficiently, thus allowing for designation of individual species in complex mixtures.[9,10] Specifically, whereas the [$Pt(GMP)_2$-$(amine)_2$] species show shifts in the 8.5−8.6 ppm region, the monofunctional [Pt(GMP)$(amine)_3$] shifts are consistently more downfield by 0.2−0.3 ppm.

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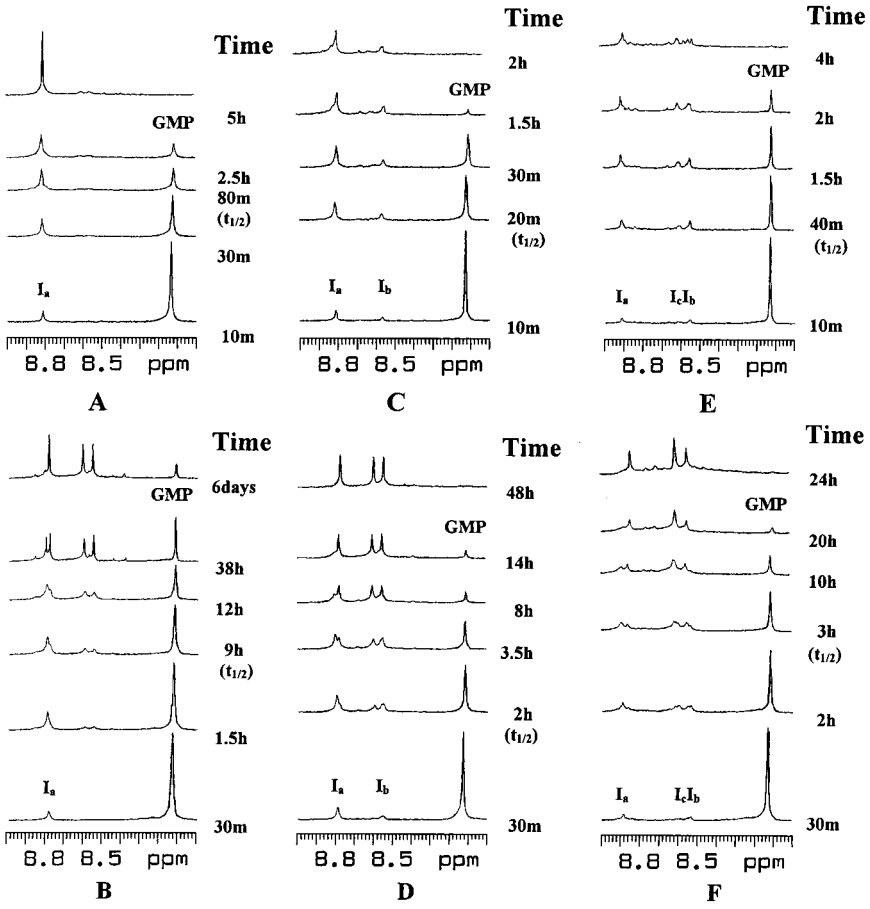

Figure 2. Reactions of trifunctional dinuclear platinum complexes with GMP followed by ^{1}H NMR spectroscopy. Reactions of 1,2/t-Cl,mal with 1:1 (A) and 1:3 GMP (B); Reactions of 1,2/t,c-Cl,Cl with 1:1 (C) and 1:3 GMP (D); Reactions of 1,2/c,c-Cl,Cl with 1:1 (E) and 1:3 GMP (F).

Complex III, 1,2/t-Cl,mal, [{*trans*-PtCl(NH$_3$)$_2$}μ-NH$_2$(CH$_2$)$_6$NH$_2$−{Pt(mal)(NH$_3$)}]$^+$. For the 1:1 reaction, the growth of only one clean signal (I_a) at 8.81 ppm is observed, indicating 100% formation of the adduct [{*trans*-Pt(**GMP**)(NH$_3$)$_2$}μ-NH$_2$(CH$_2$)$_6$NH$_2$−{Pt(mal)(NH$_3$)}], Figure 2A. The 1:3 reaction with GMP also shows initially exclusive formation of the same 1:1 adduct, [{*trans*-Pt(**GMP**)(NH$_3$)$_2$}μ-NH$_2$(CH$_2$)$_6$NH$_2$−{Pt(mal)(NH$_3$)}], δ(H8) = 8.78 ppm, Figure 2B. After approximately 1.5 h, weak signals in the 8.5−8.6 ppm region appear which grow with time. The final spectrum shows three H8 chemical shifts corresponding to the expected product [{*trans*-Pt(**GMP**)(NH$_3$)$_2$}μ-NH$_2$(CH$_2$)$_6$NH$_2$−{Pt(**GMP**)$_2$(NH$_3$)}]$^{2-}$. Thus, the peak at 8.78 ppm is assigned to the unique GMP of [{*trans*-Pt(GMP)(NH$_3$)$_2$}μ-NH$_2$(CH$_2$)$_6$NH$_2$−{*cis*-Pt(GMP)$_2$(NH$_3$)}]$^{2-}$ while the 8.60 and 8.55 ppm peaks correspond to the two inequivalent GMP, which are trans to NH$_3$ and trans to diamine in the bifunctional unit. Free GMP is still observed even after 6 days, confirming the expected slow displacement of the malonate.

A major intermediate peak appearing slightly upfield of the [Pt(GMP)(amine)$_3$] peak at 8.78 ppm, especially at the later time points, and minor peaks at around 8.5 ppm may also be observed. No attempt was made to assign these intermediates due to the complexity. Nevertheless, the profile of selective attack on the Pt−Cl unit followed by slow displacement of the malonate is consistent.

1,2/t,c-Cl,Cl, [{*trans*-PtCl(NH$_3$)$_2$}μ-NH$_2$(CH$_2$)$_6$NH$_2$−{*cis*-PtCl$_2$(NH$_3$)}]$^+$ (II). The stepwise displacement becomes more complicated when all of the leaving groups are chloride. For the 1:1 reaction, the dominant peak at early time points corresponds to substitution of the monofunctional site, with the free GMP signal decreasing in intensity and the appearance of a principal peak at 8.81 ppm, Figure 2C, I_a. A smaller intensity peak at 8.55 ppm with approximately 20% of the intensity of the downfield peak is indicative of substitution on the PtCl$_2$ unit and is seen at all time points. Thus, the 1,2/t,c-Cl,Cl shows some selectivity in its substitution reactions, with reaction being predominantly, but not exclusively, at the [PtCl(amine)$_3$] coordination sphere. Two H8 signals (I_a & I_b) are observed within 30 min, which broaden as the reaction proceeds, in ^{1}H NMR spectra of the 1:3 reaction of II with GMP, Figure 2D. It is clear that more intermediate adducts appear after two hours (the half-life of the reaction). The spectrum of the final product is identical to that of **III** above.

1,2/c,c-Cl,Cl, [{*cis*-PtCl(NH$_3$)$_2$}μ-NH$_2$(CH$_2$)$_6$NH$_2$−{*cis*-PtCl$_2$(NH$_3$)}]$^+$ (I). In contrast to the 1,2/t,c isomer, the 1,2/c,c isomer is less selective in its binding to GMP. For either 1:1 or 1:3 reactions, immediately upon mixing, three H8 signals (I_a, I_b, and I_c, Figure 2E) appear, with approximately equal intensities at 8.91, 8.66, and 8.55 ppm, indicating simultaneous reaction on both platinum centers and thus little or no selectivity in substitution reactions in this case. The three H8 chemical shifts of the final adduct are at 8.84, 8.61, and 8.55 ppm; these are consistent with the formation of [{*cis*-Pt(GMP)(NH$_3$)$_2$}μ-NH$_2$(CH$_2$)$_6$NH$_2$−{*cis*-Pt(GMP)$_2$(NH$_3$)}]$^{2-}$. These results agree with previous work on bifunctional compounds. In dinuclear compounds, the steric hindrance of the Cl groups cis to the diamine bridge results in a slower rate of substitution with both model mononucleotides such as GMP and DNA itself.[4,9,13] The

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two-step reactions of the [{*cis/trans*-$PtCl(NH_3)_2$}$_2\mu$-$NH_2(CH_2)_n$-NH_2]$^{2+}$ may be depicted as:

$$Cl-Pt-Pt-Cl \rightarrow Cl-Pt-Pt(GMP) \rightarrow (GMP)Pt-Pt(GMP)$$

Presumably, the decreased accessibility in **I** allows the competitive displacement on the $[PtCl_2(amine)_2]$ side:

$$Cl-Pt-Pt-Cl_2 \rightarrow (GMP)Pt-Pt-Cl_2 \textit{ or } Cl-Pt-PtCl(GMP)$$

Ternary DNA−protein crosslinks by [{*cis*-$PtCl_2(NH_3)$}$_2\mu$-$NH_2(CH_2)_4NH_2$] are formed by peptide binding to a presumably (Pt,Pt) interstrand crosslink.[6] The selectivity of this process is of considerable interest as it may allow for the in vivo study of how these interstrand crosslinks (even formed by bifunctional substitution of 1,1/t,t compounds) are recognized and processed by repair proteins. Recently, the conformational flexibility of the 1,4-crosslink has been shown by NMR spectroscopy.[14] In contrast to the well-documented case of HMG protein recognition of the cisplatin adduct of DNA,[15,16] the (Pt,Pt) 1,4-crosslink is only weakly recognized.[17] This work was undertaken to examine whether the pathways of reaction of three trifunctional dinuclear platinum complexes might be controlled on DNA. In principle, reaction of **III** with DNA would produce a monofunctional intermediate which will crosslink by ring-opening of the malonate chelate. Attack of the third site by a suitable amino acid will be competitive with further DNA substitution because the crosslink inherently limits rotation of the platinum coordination spheres around the DNA backbone. Such DNA−protein crosslinking by trifunctional dinuclear platinum complexes containing a 1,4-interstrand crosslink may allow for details of the molecular features of protein recognition of this biologically important conformation of DNA. Furthermore, the dinuclear platinum−DNA adducts may be capable of trapping other proteins and be useful in the isolation of specific DNA−proteins from crude cell extracts.

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