

Selective and Monofunctional Guanosine 5′-Monophosphate Binding by Chloro[3-(2,3-diaminopropionylamino)propionic acid]-(dimethyl sulfoxide)platinum(II) Complex

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A new complex of chloro[3-(2,3-diaminopropionylamino)propionic acid](dimethyl sulfoxide)platinum(II) ([PtCl(dmso)(Hdapap)]$CF_3CO_2 \cdot 2H_2O$) was synthesized and characterized. Among a series of nucleic acids, the selective and 1:1 binding between [PtCl(dapap)(dmso)] and a guanosine 5′-monophosphate ion ($Hgmp^-$) in an aqueous solution at pH 4.0 (20 mmol dm^{-3}, each) was achieved by conducting ESI-MS and NMR experiments. This reaction included the initial chloride ion displacement by H_2O, followed by metal coordination to the N7-position of the guanine base. The latter step was not affected in the case of guanosine-3′,5′-cyclic monophosphate ion ($cgmp^-$), indicating that the phosphate–Pt binding can be ruled out in this system. In order to monitor the binding reaction of [PtCl(dapap)(dmso)] with several nucleic acids, time-course ^1H and ^{31}P NMR experiments were performed at 25 °C. Changes in the NMR chemical shifts of H8, H1′, and phosphate signals in $Hgmp^-$ upon the addition of [PtCl(dapap)(dmso)] revealed that two products finally remained. Since the synthetic dapap ligand could regulate the reactivity of a ligand coordinated to the Pt-center in [PtCl(dapap)(dmso)], this may arise from the presence of *cis*- and *trans*-isomers of [PtCl(dapap)-(dmso)] in solution.

Selective recognition and binding with DNA or nucleic acids by metal complexes are important subjects to note in the fields of both chemistry and biochemistry.[1–7] Noncovalent interactions, including metal coordination, play important roles to accomplish their reactions. Hydrogen bonding and hydrophobic and electrostatic interactions can increase the DNA affinity, rendering groove binding and intercalation into base pairs.[8–10] To date, many DNA binding platinum(II) or (IV) complexes have been developed to present functions at the surface of DNA. Some of these have organic amine, carboxylato, diimine, and aromatic ligands that provide several site- and base-specific binding manners. Square-planar $[Pt(bpy)(py)_2]^{2+}$ (bpy = 2,2′-bipyridine and py = pyridine),[11,12] $[Pt(en)(phen)]^{2+}$ (phen = 1,10-phenanthroline and en = ethylenediamine),[13] and $[PtL_2(dppz)]$ (dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine and L = 1-methylimidazole or 4-aminopyridine)[14] complexes, for example, are inserted into the DNA strand by utilizing π–π interactions. Since they enhance their emission intensity upon an interaction with DNA, applications of these types of complexes as DNA photo-probes are expected.[15–19] *Cisplatin* (*cis*-$[PtCl_2(NH_3)_2]$), *carboplatin* ($[Pt(cbdca)(NH_3)_2]$, $cbdca^{2-}$ = cyclobutane-1,1′-carboxylato), and their related compounds are believed to effect the cytotoxic action in cells.[20–23] Some of them actually demonstrated 1,2-intrastrand cross-linking between neighboring purine bases in DNA by using monodentate and bidentate coordination manners. At the initial stage of the coordination reaction of *cis*-$[PtCl_2(NH_3)_2]$ with DNA, the rapid releasing of chloride anions in water and the formation of hydrolytic compound *cis*-$[Pt(H_2O)_2(NH_3)_2]^{2+}$ are crucial.[24,25] The higher reactivity of H_2O therefore leads to the platination reaction of purine bases in guanine and adenine. Studies concerning the cytotoxic actions of $[PtCl(R'R''SO)(L)]^+$ (L = diamine ligand and R′R″SO = substituted sulfoxides, such as dimethyl sulfoxide) complexes, have proposed that the $[Pt(H_2O)(R'R''SO)(L)]^{2+}$ intermediate is important to form a monodentate linkage to DNA.[26] However, it is generally quite difficult to regulate such monofunctional and difunctional coordination reactions, and to control the preferences of Pt(II) complexes for guanine base in adenine- and thymine-containing DNA.[27] Initial studies of the binding of $[PtCl(terpy)]^+$ (terpy = 2,2′:6′,2″-terpyridine) to calf-thymus DNA revealed covalent binding to the bases as well as intercalation.[28] A tridentate amine ligand, such as *N*,*N*′,*N*″-trimethyldiethylenetriamine, coordinated to the platinum center formed only a monofunctional adduct with a nucleobase, and could prevent the 1,2-intrastrand cross-link reaction.[29] The structure–function relationships of Pt(II) complexes concerning base-specific and monofunctional coordination binding to a series of nucleic acids are still not fully understood. Therefore, further progress by a synthetic approach should even now be important subjects.

In this paper, we describe the synthesis of a platinum(II) complex, [PtCl(dmso)(Hdapap)]$CF_3CO_2 \cdot 2H_2O$, which consists of 3-(2,3-diaminopropionylamino)propionic acid (Hdapap), a chloride ion, and dimethyl sulfoxide (dmso), and its binding to nucleic acids in an aqueous solution. The binding selectivity for [PtCl(dapap)(dmso)] with a series of nucleic acids was investigated by convenient ESI-MS and ^1H and ^{31}P NMR spectra in an aqueous solution. Monofunctional and specific binding at the N7 position of the guanosine-5′-monophosphate ion ($Hgmp^-$) was observed without the formation of the gmp bisadduct. By monitoring this reaction, we

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expect to obtain more insight into the reaction mechanism, the structures of products and intermediates, and the reactivity of ligands in the [PtCl(dapap)(dmso)] complex.

Experimental

Materials. Di-*t*-butyldicarbonate ($(Boc)_2O$) was purchased from Peptide Institute, Inc. β-Alanine ethylester hydrochloride and disodium thymidine-5′-monophosphate (Na_2tmp) were purchased from Aldrich Chemical Co. and used without further purification. Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP), *N*,*N*′-diisopropylethylamine (DIEA), trifluoroacetic acid, potassium tetrachloroplatinate(II) ($K_2[PtCl_4]$), disodium guanosine-5′-monophosphate (Na_2gmp), sodium guanosine-3′,5′-cyclic monophosphate (Nacgmp), and disodium cytidine-5′-monophosphate (Na_2cmp) were purchased from Wako Chemicals and used as received. Disodium adenosine-5′-monophosphate (Na_2amp) was purchased from Calbiochem-Novabiochem Co. All other reagents and solvents were of guaranteed grade. All aqueous solutions were prepared from redistilled water and >99.9% deuterium oxide (D_2O) and the pHs of the solutions were adjusted with NaOH or NaOD. Anion-exchange Sepharose CL-6B was purchased from Amersham Pharmacia Biotech Co.

Synthesis of 2,3-Bis(*t*-butoxycarbonylamino)propionic Acid. 2,3-Diaminopropionic acid (1.00 g, 7.14 mmol) was dissolved in a 10 mL of aqueous 1.0 mol dm^{-3} NaOH and 20 mL of dioxane mixture. After the addition of $(Boc)_2O$ (7.79 g, 3.57×10^{-2} mol), the reaction mixture was stirred for 24 h at room temperature. The solvent was removed under reduced pressure and a white residue was dissolved in ethyl acetate. The solution was washed with 2.6 mmol dm^{-3} aqueous citric acid (pH 3.0), and then with water three times. The organic phase was collected and dried over Na_2SO_4. The solvent was evaporated to dryness and washed with hexane. The product was isolated by filtration and a white solid (1.34 g) was obtained in 62% yield. IR (KBr, ν/cm^{-1}) 3245 (NH), 2990 (CH), 1729, 1690 (C=O). ESI-MS (MeOH, m/z) 327.21 ($[M + Na]^+$). ^{1}H NMR (270 MHz, $CDCl_3$, 300 K, ppm from TMS) δ 1.46 (s, 18H, *t*-Bu), 3.47 (m, 1H, *methine*), 4.29 (br-s, 2H, *methylene*), 5.17 (s, 1H, amide N–*H*), 5.90 (s, 1H, amide N–*H*). Anal. Calcd for $C_{13}H_{24}N_2O_6$: C, 51.31; H, 7.95; N, 9.20%. Found: C, 51.29; H, 7.78; N, 8.90%.

Synthesis of 3-[2,3-Bis(*t*-butoxycarbonylamino)]propionic Acid Ethylester. To a solution of 2,3-bis(*t*-butoxycarbonylamino)propionic acid (1.20 g, 3.94 mmol) in 5 mL *N*,*N*-dimethylformamide (DMF), DIEA (1.03 g, 8.01 mmol), and BOP (1.72 g, 3.94 mmol) were added and stirred at room temperature for 60 min. Then, a 5 mL solution of β-alanine ethylester (607 mg, 3.95 mmol) in DMF was added dropwise and reacted for 24 h. After removal of the solvent in vacuo, the crude mixture was dissolved in CH_2Cl_2. The solution was washed with water three times and the organic solvent was evaporated to dryness. The residue was subjected to column chromatography on silica gel ($\phi 2.5 \times 20$ cm, CH_2Cl_2:MeOH = 100:1 (v/v)). A slightly yellow band was collected and evaporated to dryness. The solid product was recrystallized from ethylacetate/hexane to yield the desired compound, 871 mg (55%). IR (KBr, ν/cm^{-1}) 3245 (NH), 2990 (CH), 1730, 1700 (C=O). ESI-MS (MeOH, m/z) 426.22 ($[M + Na]^+$). ^{1}H NMR (270 MHz, $CDCl_3$, 300 K, ppm from TMS) δ 1.29 (t, 3H, $J = 7.1$ Hz, $-CH_2CH_3$), 1.45 (s, 18H, *t*-Bu), 2.52 (t, 2H, $J = 6.3$ Hz, $-CH_2CH_2CO_2Et$), 3.50 (m, 3H, $-CH_2CH_2CO_2Et$ and *methine*), 4.15 (m, 4H, $-CH_2NH_2$ and $-CH_2CH_3$), 5.07 (s, 1H, amide N–*H*), 5.74 (s, 1H, amide N–*H*), 7.01 (s, 1H, amide N–*H*). Anal. Calcd for $C_{18}H_{33}N_3O_7$: C, 53.58; H, 8.24; N, 10.41%. Found: C, 53.31; H, 8.06; N, 10.18%.

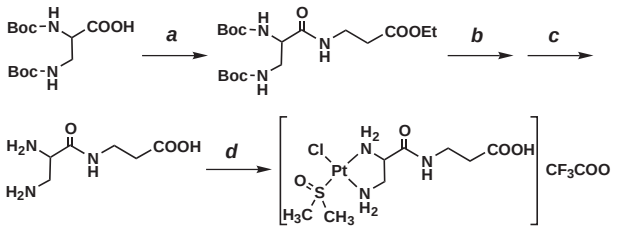
Synthesis of 3-(2,3-Diaminopropionylamino)propionic Acid (Hdapap). 3-[2,3-Bis(*t*-butoxycarbonylamino)]propionic acid ethylester (202 mg, 5.00×10^{-4} mol) was dissolved in 9 mL of MeOH:H_2O (5:4 v/v). To this solution, 1 mL of aqueous 1.0 mol dm^{-3} NaOH (2 equiv) was added and the reaction mixture was stirred for 24 h at room temperature. Then, the pH of the solution was adjusted to 3.0 by adding a few drops of 1 mol dm^{-3} HCl. Ethyl acetate was added and the product was extracted twice from the aqueous phase. The organic layer was collected and dried over Na_2SO_4. After evaporation of the solvent, white oil was obtained. This was directly used to the next step without further purification. Then, the product was dissolved in 9 mL of dry CH_2Cl_2 and reacted with trifluoroacetic acid (0.60 mL, 15 equiv) for 20 h at room temperature. After removal of the solvent and unreacted trifluoroacetic acid by evaporation several times, a white solid (85.9 mg) was obtained as a trifluoroacetate salt in 98% yield in two steps. IR (KBr, ν/cm^{-1}) 3116 (NH), 2962 (CH), 1724, 1690 (C=O). ESI-MS (MeOH, m/z) 176.32 ($[M + H]^+$). ^{1}H NMR (270 MHz, D_2O, 300 K, ppm from DSS) δ 2.50 (t, 2H, $J = 6.7$ Hz, $-CH_2CH_2CO_2H$), 3.40 (m, 3H, $-CH_2CH_2CO_2H$ and *methine*), 4.18 (m, 2H, $-CH_2NH_2$).

Synthesis of Chloro[3-(2,3-diaminopropionylamino)-propionic acid)](dimethyl sulfoxide)platinum(II) Trifuluoroacetate Dihydride, $[PtCl(dmso)(Hdapap)]CF_3CO_2 \cdot 2H_2O$. 3-(2,3-Diaminopropionylamino)propionic acid (Hdapap) (85.9 mg, 4.96×10^{-4} mol) and $[PtCl_2(dmso)_2]$ (210 mg, 5.00×10^{-4} mol, 1 equiv for Hdapap ligand) were reacted in 20 mL of DMF at 40 °C for 24 h. After cooling to room temperature, the solvent was removed in vacuo and the residue was washed with CH_2Cl_2 several times. The product was isolated by filtration and then was dried in vacuo. The target compound (228 mg) was obtained as a yellow solid in 95% yield. This was satisfactory characterized by IR, ESI-MS, NMR, and elemental analysis. IR (KBr, ν/cm^{-1}) 1687 (C=O), 1027 (S=O). ^{1}H NMR (270 MHz, D_2O, 312 K, ppm from DSS) δ 2.59 (t, 2H, $J = 6.3$ Hz, $-CH_2CH_2CO_2H$), 3.38 (m, 3H, $-CH_2CH_2CO_2H$ and *methine*), 3.45 (s, 6H, *methyl* in dmso), 3.78 (m, 2H, $-CH_2NH_2$). ^{195}Pt NMR (85.8 MHz, DMF-d_7, 312 K, ppm from $K_2[PtCl_4]$) δ −3239, −3242. Anal. Calcd for $C_{10}H_{19}N_3O_8F_3ClPtS \cdot 2H_2O$: C, 18.98; H, 3.66; N, 6.64%. Found: C, 18.53; H, 3.14; N, 6.89%.

Measurements. IR, UV–vis, and ESI-MS spectra were measured with Perkin-Elmer 1740 FT-IR, Shimadzu UV-2550, and Applied Biosystems Mariner spectrometers, respectively. All ^{1}H NMR spectra were recorded on a JEOL JNM-GX270 FT-NMR spectrometer. All ^{31}P and ^{195}Pt NMR spectra were recorded on a JEOL JNM-AL400 FT-NMR spectrometer. ^{1}H NMR chemical shift values are reported in ppm as reference to the internal standard TMS in organic solvent and DSS in D_2O. ^{31}P and ^{195}Pt NMR chemical shift values are reported in ppm as reference to the external standard H_3PO_4 and $K_2[PtCl_4]$, set at 0 and −1625 ppm, respectively. The pHs of the solutions were measured on a Hitachi-Horiba F-14RS pH meter.

Results and Discussion

Synthesis and ^{195}Pt NMR of $[PtCl(dmso)(Hdapap)]$-$CF_3CO_2 \cdot 2H_2O$. The new platinum complex was synthesized according to Scheme 1. In this study, we used racemic 2,3-diaminopropionic acid as a starting material. In order to obtain a peptide-type Hdapap ligand with moderate yield, 2,3-bis(*t*-bu-



Scheme 1. *Reagents and conditions*: a, β-alanine ethylester, BOP (1 equiv), DIEA (2 equiv), dry DMF, rt, 24 h, 55%; b, NaOHaq (2 equiv), MeOH–H_2O (5:4/v:v), rt, 20 h, quant.; c, CF_3COOH (15 equiv), dry CH_2Cl_2, rt, 20 h, 98%; d, [$PtCl_2$(dmso)$_2$] (1 equiv), dry DMF, 40 °C, 24 h, 95%.

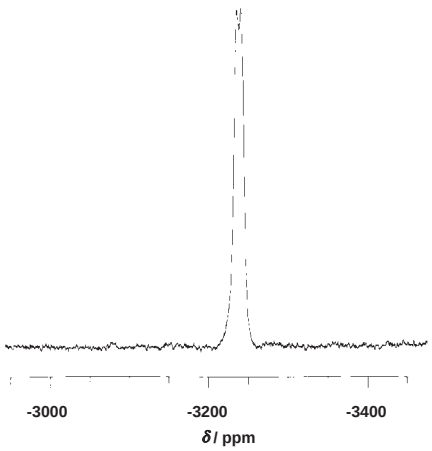


Fig. 1. 85.8 MHz ^{195}Pt NMR spectrum of [PtCl(dmso)(Hdapap)]$^+$ in DMF-d_7 (20 mmol dm^{-3}) at 25 °C.

toxycarbonylamino)propionic acid was condensed with β-alanine ethylester in the presence of BOP (yield in 55%). Removal of the protected groups in the peptide and subsequent metal complexation by using *cis*-[$PtCl_2$(dmso)$_2$][30] afforded a target compound, [PtCl(dmso)(Hdapap)]$^+$, as a trifluoroacetate form (yield in 95%). All of the compounds were satisfactorily characterized by IR, NMR, ESI-MS, and elemental analyses.

Figure 1 displays the ^{195}Pt NMR (85.8 MHz) spectrum of the target complex, [PtCl(dmso)(Hdapap)]$^+$, in DMF-d_7. It was found that the chemical shift of [PtCl(dmso)(Hdapap)]$^+$ appeared at lower frequency (δ/ppm = −3239, −3242), compared with the related types of Pt(II) complexes, for example, N_2X_2-type (*cis*-[$PtCl_2(NH_3)_2$], δ/ppm = −2149),[31] NO_2X-type ([Pt_2Cl_2(hdta)], hdta = 1,6-hexanediamine-*N*,*N*,*N*′,*N*′-tetraacetate, δ/ppm = −1330),[32] and N_2CX-type ([PtBr(N_2C)]$^+$, N_2C = 2,6-bis(dimethylaminoethyl)phenyl, δ/ppm = −2000).[33] Ongeri et al. recently reported the ^{195}Pt NMR of N_2SX-type [PtCl(L)(dmso)]Cl (L = 1,2-cyclohexanediamine) complex and its crystal structure.[34] The ^{195}Pt NMR chemical shift is at −3268 ppm in DMF-d_7 and is consistent with ours. This fact clearly indicates that the coordinated dmso molecule in [PtCl(dmso)(Hdapap)]$^+$ was bound to the Pt(II)-center by forming Pt–S bond.[26,34] A weak peak splitting at the top of the ^{195}Pt NMR signal can be observed in Fig. 1, while only a single ^{1}H NMR signal of the dmso molecule appears (see experimental section). This arises because [PtCl(dmso)(Hdapap)]$^+$ derived from the Hdapap ligand, a chloride ion, and dmso molecule appeared as a mixture of *cis*- and *trans*-isomers.

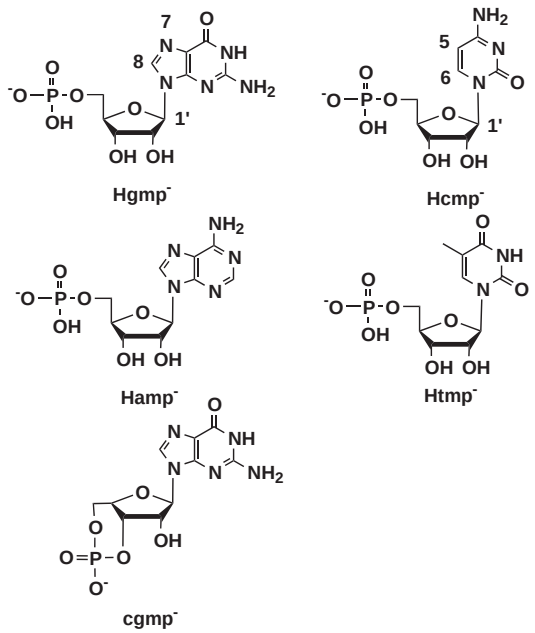


Chart 1.

Convenient Screening for the Binding Reaction by ESI-MS. In order to explore the binding property of [PtCl(dapap)(dmso)] with a series of nucleic acids, ESI-MS experiment on the positive mode was firstly carried out. We selected Hgmp$^-$, Hamp$^-$, Hcmp$^-$, and Htmp$^-$ ions as candidates (Chart 1). In an aqueous solution at pH 4.0, [PtCl(dapap)(dmso)] showed a split peak at m/z = 484.02, which corresponded to the protonated monocation form. [PtCl(dapap)(dmso)] was then reacted with the above four nucleotides (20 mmol dm^{-3}, each) for 12 h at 25 °C. Since the formation of buffer-coordinated platinum(II) species could interfere with the studied reactions,[35] we carried out all of the reactions without a buffer. The reaction pH was kept at pH 4.0 ± 0.3 during the reactions for [PtCl(dapap)(dmso)]. As shown in Fig. 2, in the presence of Hgmp$^-$ at an equivalent ratio for [PtCl(dapap)(dmso)], additional monocation peaks at m/z = 810.12, 832.33, and 854.34 were observed. They are attributable to the protonated form of the Hgmp$^-$ monoadduct complex, H[Pt(dapap)(Hgmp)(dmso)]$^+$ (calcd for $C_{18}H_{32}N_8O_{12}$PPtS), and its sodium salts of Na[Pt(dapap)(Hgmp)(dmso)]$^+$ (calcd for $C_{18}H_{31}N_8NaO_{12}$PPtS) and Na_2[Pt(dapap)(gmp)(dmso)]$^+$ (calcd for $C_{18}H_{30}N_8Na_2O_{12}$PPtS), respectively. These new signals were generated after monodentate complexation between one Hgmp$^-$ and platinum(II) complex by replacing a Cl$^-$ ion.[36] The addition of Ag$^+$ ion into the reaction mixture of [PtCl(dapap)(dmso)]/Hgmp$^-$ gave white precipitates. This indicates the presence of free Cl$^-$ released from [PtCl(dapap)(dmso)]$^+$ during the reaction. On the contrary, Hamp$^-$, Hcmp$^-$, and Htmp$^-$ gave no significant peaks under the same conditions, revealing that no Pt(II)–nucleic acid bond was formed. We also note that the appearance of H[Pt(dapap)(Hgmp)(dmso)]$^+$ and its sodium salts peaks was independent of the reaction time, and additional peaks corresponding to the Hgmp$^-$ bisadduct complexes were not observed even by

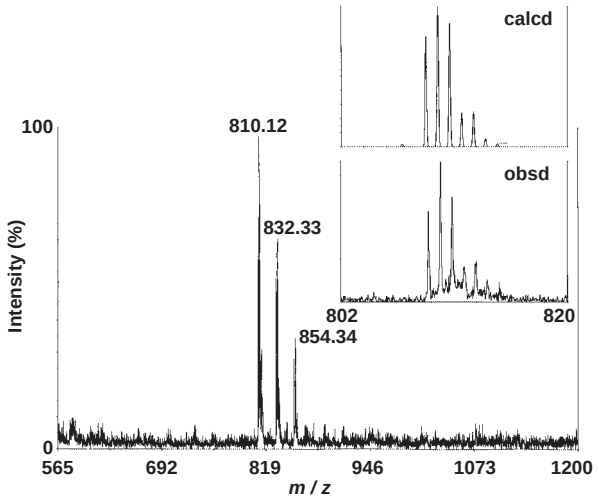


Fig. 2. ESI-MS of [PtCl(dapap)(dmso)]/Hgmp$^-$ complex in an aqueous solution (1:1, 20 mmol dm^{-3}, pH 4.0) at the measurement temperature of 140 °C (positive mode).

time-interval experiments conducted after 12 h of the reaction. From these ESI-MS results, we herein found the specific binding of [PtCl(dapap)(dmso)] to one Hgmp$^-$ ion.

^{1}H NMR Measurements. The ^{1}H NMR spectrum of nucleotide Hgmp$^-$ exhibits characteristic features with chemical shifts of the aromatic H8 (δ/ppm = 8.12) and sugar H1′ (δ/ppm = 5.94) proton in D_2O. The notation refers to the standard numbering assignments, as shown in Chart 1. Here, we recorded the time-course ^{1}H NMR spectra at 25 °C (pD 4.0) to elucidate the reaction process of [PtCl(dapap)(dmso)] with Hgmp$^-$.[37,38] Under these conditions, no protonation occurred on the N7 atom of the guanine base (pK_a = 2.5), and gmp was present as a monoanionic form, Hgmp$^-$, because of the pK_a values of about 6.2 and <1 for its phosphate group.[39,40] Figure 3a displays the ^{1}H NMR spectral changes of an aqueous solution containing [PtCl(dapap)(dmso)] and Hgmp$^-$ (20 mmol dm^{-3}, each) at various reaction times (0.17–12 h). New H8 and H1′ signals, marked by *open circles*, appeared within an hour at δ/ppm = 8.47 and 6.01, respectively. Interestingly, after 1 h of the reaction, other new signals at δ/ppm = 8.80 (H8) and δ/ppm = 5.92 (H1′) were observed (*closed circle*). Their signals appeared at higher frequency compared to those for the initial product, and increased in intensity with time. Unreacted free Hgmp$^-$ gradually disappeared during the reaction. Then, these three Hgmp$^-$ species would be in equilibrium after 12 h, and the signal for the coordinating dmso molecule remained on the Pt-center was still observed at δ/ppm = 3.45. Time profiles for the reaction yield (%) based on the total Hgmp$^-$ were each calculated from the integration of ^{1}H NMR signals, and are summarized in Fig. 4. This clearly shows that two products finally remained with a ratio of 47:33 after the reaction.

It is generally known that N7 is the predominant binding site for the metal coordination in the case of N9-substituted nucleotides, such as Hgmp$^-$. Reedijk et al. previously reported the ^{1}H NMR spectral changes for the 1:1 binding reaction between [PtCl(dmso)(en)]$^+$ and Hgmp$^-$ in an aqueous solution.[35] The chemical shifts of the H8 proton similarly occurred downfield by 0.7 ppm relative to free Hgmp$^-$ upon platination to the

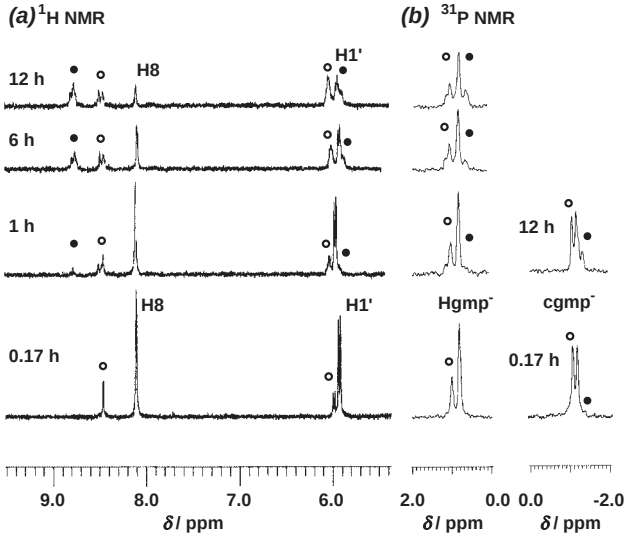


Fig. 3. (a) Partial 270 MHz ^{1}H NMR spectral change for the reaction of [PtCl(dapap)(dmso)] with Hgmp$^-$ in D_2O (1:1, 20 mmol dm^{-3}, pD 4.0, 25 °C) at various reaction times, 0.17, 1, 6, and 12 h. (b) 162 MHz ^{31}P NMR spectra of Hgmp$^-$ and cgmp$^-$ under the same conditions.

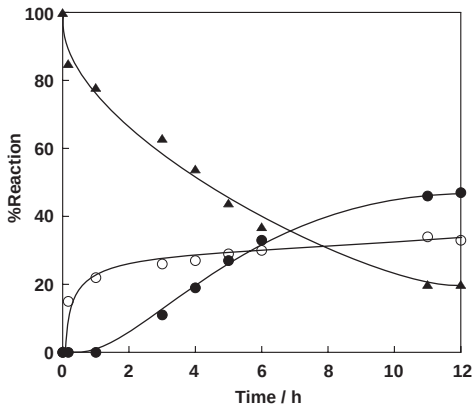
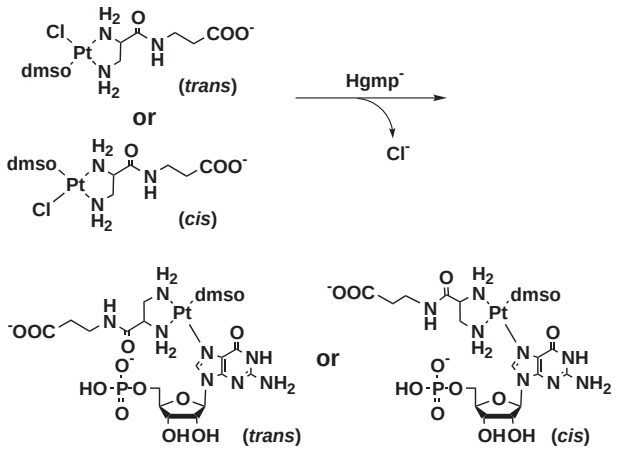


Fig. 4. Time profiles for the reaction product yield (%) based on the total Hgmp$^-$ determined by integration of ^{1}H NMR; *closed triangle*: Free Hgmp$^-$, *open circle*: [Pt(dapap)(Hgmp)(dmso)] monitored at δ/ppm = 8.47, *closed circle*: [Pt(dapap)(Hgmp)(dmso)] monitored at δ/ppm = 8.80.

N7 moiety. They also found that the existence of two rotamers of [Pt(dmso)(en)(Hgmp)]$^+$ was evidenced by monitoring the H8 signal splitting generated due to the rotation of the bulky dmso molecule. The chemical shift difference between such rotamers is, however, generally very small ($\Delta\delta$/ppm < 0.05). In our case, the molecular conformation of [Pt(dapap)-(Hgmp)(dmso)] was significantly affected by the peptide dapap ligand. The peak separation of H8 signals, each at δ/ppm = 8.47 and 8.80, was apparently larger, and may be ascribable to *trans*- and *cis*-isomers of [Pt(dapap)(Hgmp)(dmso)] ions, not attributed to such rotamers (Scheme 2).

Studies for Other Nucleic Acids by ^{1}H and ^{31}P NMR. Further NMR experiments for a series of other nucleic acids will make it possible to discuss the binding mechanism and selectivity to Hgmp$^-$. We summarize the ^{1}H NMR spectral



Scheme 2. Schematic illustration for the reaction of [PtCl(dapap)(dmso)] with $Hgmp^-$ in an aqueous solution at pH 4.0.

changes of the $cgmp^-$, $Hamp^-$, $Htmp^-$, and $Hcmp^-$ ions in the presence of [PtCl(dapap)(dmso)] (see Supporting Information). The downfield chemical shifts at $\delta/\mathrm{ppm} = 8.42$ and 8.65, assignable to the H8 protons of $cgmp^-$, were observed, while the $Hamp^-$, $Htmp^-$, and $Hcmp^-$ ions did not change their signals under the same conditions. It is noteworthy that, to our knowledge, this is the first report that describes the binding specificity of the [PtCl(dmso)(en)]-type complex to a series of nucleic acids by NMR. As a result, the selective reaction between $cgmp^-$ and [PtCl(dapap)(dmso)] gave two products in the same way for $Hgmp^-$. These findings indicate that the structural difference found in the phosphate group between $Hgmp^-$ and $cgmp^-$ did not affect the cgmp(N7)-platinating reaction.

Recently, Sadler et al. pointed out an interesting phosphate effect on the binding reaction between the [RuCl(biphenyl)(en)]PF_6 complex and $Hgmp^-$.[41] They performed ^{1}H and ^{31}P NMR experiments in an aqueous solution at various reaction times. For the Ru(II)–gmp system, the formation of the $Hgmp^-$–O–PO_3–ruthenium(II) binding intermediate, followed by a rearrangement to the $Hgmp^-$(N7)-binding [Ru(Hgmp)(biphenyl)(en)]$^+$ complex, was described according to the following reaction:

$$\begin{aligned}&[\mathrm{RuCl(biphenyl)(en)}]^{2+} + \mathrm{Hgmp}^- \\ &\quad\rightarrow [\mathrm{Ru(O\text{–}PO_3\text{–}Hgmp)(biphenyl)(en)}]^+ \\ &\quad\rightarrow [\mathrm{Ru(N7\text{-}Hgmp)(biphenyl)(en)}]^+. \end{aligned} \quad (1)$$

In contrast, only the N7-binding adduct was formed for $cgmp^-$, and no phosphate-binding intermediate was detected at all. To confirm whether such phosphate effect should be mentioned in our Pt(II)–$Hgmp^-$ systems, we conducted ^{31}P NMR studies. The ^{31}P NMR chemical shift of $Hgmp^-$ and $cgmp^-$ at 25 °C (pD 4.0) appeared at $\delta/\mathrm{ppm} = 0.78$ and -1.19, respectively. Figure 3b shows the ^{31}P NMR spectral changes at various reaction times. The new signals at $\delta/\mathrm{ppm} = 0.56$ and 1.08 for $Hgmp^-$ and at $\delta/\mathrm{ppm} = -1.40$ and -1.06 for $cgmp^-$ gradually appeared and increased in intensities with time. These results explained that the above-mentioned phosphate effect could be ruled out in our systems. No spectral changes of ^{31}P NMR appeared in the case of $Hamp^-$, $Hcmp^-$, and $Htmp^-$ ions, even after 24 h.

Binding Mechanism and Column Chromatography. This was proved that the platinum(II) complex, [PtCl(dapap)(dmso)], forms a selective and monofunctional 1:1 complex with $Hgmp^-$ in an aqueous solution. This reaction includes two processes, such as an initial chloride ion replacement by H_2O, and a following metal coordination to the N7-position of the guanine base. Since ^{1}H and ^{31}P NMR results support the presence of two species of reaction products, the $Hgmp^-$ binding may proceed as shown in Scheme 2.[42] We herein expect that the reactivity of the *cis*-complex of [PtCl(dapap)(dmso)] in Scheme 2 may be lower than that of the *trans*-isomer, because the electron-withdrawing effect of the carbonyl group in the dapap ligand would strengthen the Pt–Cl bond for the *cis*-isomer. Such an electron-withdrawing effect may also be influential for the Pt–N7 bond of the reaction product, the *cis*-[Pt(dapap)(Hgmp)(dmso)] complex. The electron densities of both the N7- and its neighboring H8-atoms of the guanine base in the *cis*-complex are decreased, which causes the NMR chemical shift difference between two isomers. Then, the open and closed symbol in Figs. 3 and 4 would correspond to the *trans*-[Pt(dapap)(Hgmp)(dmso)] and *cis*-[Pt(dapap)(Hgmp)(dmso)], respectively. At the same time, the structural difference between *cis*- and *trans*-[Pt(dapap)(Hgmp)(dmso)] may lead to a structural rearrangement to produce a thermodynamically stable isomer. This resulted in giving the final ratio of the product yields as 47(*cis*):33(*trans*) after the reaction.

The reaction products formed after the 1:1 binding between [PtCl(dapap)(dmso)] and $Hgmp^-$ would be isolated by column chromatography, including HPLC.[43] Such experiments should help to determine the structure of [Pt(dapap)(Hgmp)(dmso)]. We then carried out the following experiments by using anion-exchange chromatography on a Sepharose CL-6B at 4 °C. In order to first check the elution property of gmp, only a Na_2gmp solution containing sodium phosphate buffer was loaded on the column at pH 7.0. This procedure proved that the Na_2gmp was successfully eluted with a 20 mmol dm^{-3} phosphate buffer by monitoring the absorption at 253 nm (data not shown). Next, after 10 h of the reaction between [PtCl(dapap)(dmso)] and $Hgmp^-$ (20 mmol dm^{-3}, each), the reaction solution was similarly loaded on a Sepharose CL-6B. Since gmp is present as a dianionic form, gmp^{2-}, at pH 7.0, the reaction product written as [Pt(dapap)(gmp)(dmso)]$^-$ may also be isolated by anion-exchange chromatography. As a result, three fractions were eluted with a 0–80 mmol dm^{-3} sodium phosphate buffer at pH 7.0. One of these is clearly attributed to unreacted gmp^{2-}. Another two components, being ascribable to the *cis*- and *trans*-isomers of [Pt(dapap)(gmp)(dmso)]$^-$, were then collected and were tried to identify by spectroscopic measurements. Due to the difficulty of complete separation of each fraction, we unfortunately did not succeed in the characterization until now by ESI-MS and NMR measurements. Some of the other reasons may be their low stabilities and separation yields (<1%, each); therefore, further experiments are necessary to investigate the situation in detail.

In conclusion, we have prepared a new platinum(II) complex, [PtCl(dmso)(Hdapap)]$CF_3CO_2 \cdot 2H_2O$, that can specifi-

cally recognize one $Hgmp^-$ in an aqueous solution. The ^{195}Pt NMR signal of $[PtCl(dmso)(Hdapap)]^+$ clearly showed the presence of the Pt–S bond in this complex. The specific 1:1 binding reaction between [PtCl(dapap)(dmso)] and $Hgmp^-$ was proved to be monitored by the ESI-MS and NMR spectra. Time-course experiments revealed that two products finally remained after the $Hgmp^-$ platinating reaction. ^{31}P NMR results also suggested that this reaction did not include the phosphate effect. Since the synthetic dapap could regulate the reactivity of another ligand coordinated to the Pt-center, these reaction products may be attributable to the *cis*- and *trans*-isomers of [PtCl(dapap)(dmso)] in solution.

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Supporting Information

Table S1 and Figures S1–S5 in PDF format. These materials are available free of charge on the web at http://www.csj.jp/journals/bcsj/.

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42 The use of racemic ligand of dapap would also give diastereomers for the reaction between [PtCl(dapap)(dmso)] complex and one $Hgmp^-$ in solution. However, each diastereomer was not discriminative by ^1H and ^{31}P NMR, we discuss about the formation processes of *cis*- and *trans*-[Pt(dapap)(Hgmp)(dmso)] from these NMR results.

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