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^{1}H NMR Spectra of Ternary Platinum(II) Complexes with *N*-Ethyl- or *N*-Benzyl-1,2-ethanediamine and 2,2′-Bipyridine or 1,10-Phenanthroline: Intramolecular Aromatic–Aromatic Interaction in Coordination Sphere, and It's Solvent and Temperature Effects

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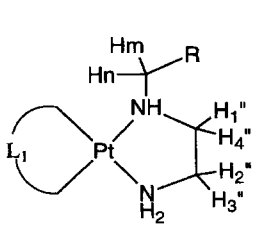
Square-planar complexes with the formula $[Pt(L_1)(L_2)]X_2$, where L_1 is di(ammine) or 2,2′-bipyridine (bpy) or 1,10-phenanthroline (phen) and L_2 is *N*-ethyl-1,2-ethanediamine (Eten) or *N*-benzyl-1,2-ethanediamine (Been), and X = NO_3^- or Cl^-, were prepared. NMR measurements of D_2O solutions of these complexes showed that the *N*-ethyl and *N*-benzyl groups are forced to take a pseudo axial disposition due to an intramolecular repulsion from hydrogen atoms of aromatic diamines for complexes where L_1 = bpy or phen and significant upfield shifts due to the ring current effect were observed for the Been complexes. An analysis of coupling with N–H and ^{195}Pt showed that the major rotamer due to rotation around CH_2–NH is (−)-*syn* for the Been with a significant intramolecular stacking between aromatic rings of L_1 and L_2, but *anti*- for the Eten complexes. The solvent and temperature dependency of the upfield shift of the Been complexes are described; the protein denaturants, guanidinium chloride, and urea, act to reduce stacking as dioxane.

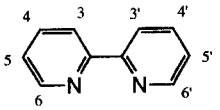
The chemical properties of Pt(II) complexes with *N*-alkyl substituted 1,2-diamines are important for the development of the Pt(II) complexes in antitumor chemotherapy.[1] Several platinum(II) complexes of 2-(aminomethyl)azetidine and 2-(aminomethyl)pyrrolidine as carrier ligands, which can be classified as *N*-alkyl-1,2-ethanediamines, have been reported to be effective as anticancer drugs when the leaving group is either dichloro or cyclobutane-1,1′-dicarboxylato ligands.[2] The stereochemistry of Pt(II) complexes with *N*-alkyl substituted 1,2-diamines has been analyzed by using methyl as an alkyl group, and it has been reported that a 1,2-diamine forms a puckered five-membered chelate ring and that an *N*-alkyl group takes either a pseudo-axial or a pseudo-equatorial disposition, depending on the combination of the absolute configuration of the coordinated secondary amine and the conformation of the five-membered chelate ring.[3—8]

In hexacoordinated metal complexes, alkyl groups bigger than methyl have a tendency to take an equatorial orientation.[9] The conformation of *N*-alkyl groups in square-planar metal complexes has been investigated for an *N*-methyl group.[3—8] In these complexes, the methyl group can take both equatorial and axial dispositions for $[Pt(NH_3)_2$(*N*-methyl-1,2-ethanediamine = Meen)$]^{2+}$, but the substitution of ammine ligands with bpy and/or phen force the *N*-methyl group to take an axial dispostion because of a strong intramolecular repulsion between the *N*-methyl group and 6,6′-hydrogens of 2,2′-bipyridine (bpy) or 2,9-hydrogens of 1,10-phenanthroline (phen) for a structure in which the *N*-methyl group takes an equatorial dispostion.[6]

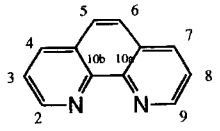
In the preceding paper we described a crystal structral determination and ^{1}H NMR spectra of three (bpy) (*N*-arylmethyl-1,2-ethanediamine)platinum(II) complexes, where aryl stands for phenyl, 1-naphthyl, and 9-anthryl.[10] In the crystalline state, intramolecular stackings of the aromatic regions were observed for 1-naphthyl and 9-anthryl, but not for phenyl as the *N*-aryl groups. However, these three complexes showed spectra characteristic of intramolecular stacking for deuterium oxide solutions.[10]

In this report, (1) the conformation of *N*-ethyl-1,2-ethanediamine (Eten) and *N*-benzyl-1,2-ethanediamine (Been) in the platinum(II) complexes (Fig. 1) was examined in solution with NMR spectroscopy to confirm the occurrence of an intramolecular stacking form for ternary complexes with aromatic diamine and Been. Intramolecular stacking causes a significant up-field shift of aromatic diamines due to a ring-current effect. (2) The factors which influence the intramolecular stacking, such as temperature and solvent composition, were explored regarding the magnitude of the up-field shift to analyze the nature of the stacking. (3) Finally, a relatively large susceptibility of the shift in the chemical shift of **3** and **5** to the nature of solvents was applied to protein denaturants, such as urea and guaidinium





bpy



phen

	L_1	L_2		L_1	L_2		L_1	L_2
1	2 NH_3	R=Ph	**3**	bpy	R=Ph	**5**	phen	R=Ph
2	2 NH_3	$R=CH_3$	**4**	bpy	$R=CH_3$	**6**	phen	$R=CH_3$

Fig. 1. Schematic representation of the prepared Pt(II) complexes.

chloride, which are known to modulate or destroy the three-dimensional structure of proteins. Though the exact mechanisms of these reagents to denature proteins have not been known,[11] the action to nonpolar groups is proposed as being one of the reasons.

Experimental

General. The absorption spectra were measured with a Shimadzu UV 2200 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL GX-400 or FX-270 spectrometer with D_2O as used a solvent and sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 (TSP) for ^{1}H and dioxane for ^{13}C as internal standards, unless otherwise stated. For ^{13}C NMR, the signals of which assignments are ambiguous are listed in a brace.

Materials. Dichloro(bipyridine)platinum(II) and dichloro(phenanthroline)platinum(II) were prepared according to the method of Morgan and Burstall,[12] and Hall and Plowman,[13] respectively. *N*-Ethyl-1,2-ethanediamine and *N*-benzyl-1,2-ethanediamine were purchased from Tokyo Kasei. [Pt(bpy)(Been)]$(NO_3)_2$ (**3**) was prepared as described previously[10] (λ_{max}/nm, ε_{max}/dm^3 mol^{-1} cm^{-1}): (319, 16600); (308, 12100); (244, 20800). ^{13}C NMR δ = {157.1 (J_{Pt-C} = 32 Hz), 156.0 (J_{Pt-C} = 32 Hz) (bpy; 2,2′)}, {151.2 (J_{Pt-C} = 34 Hz), 150.8 (J_{Pt-C} = 29 Hz) (bpy; 6,6′)}, {142.9,141.7 (bpy,4,4′)}, 133.7 (J_{Pt-C} = 16 Hz) (phe, *ipso*-), 132.2 (phe, *m*-), 130.5 (phe, *p*-), 129.7 (phe, *o*-), {128.9,128.6 (bpy, 5,5′)}, {124.9, 124.4 (bpy, 3,3′)}, 58.1 ($CH_2C_6H_5$), 57.4 (CH_2NH), 42.8 (CH_2NH_2) and [Pt(bpy)-(1,2-ethanediamine = en)] $(NO_3)_2$ was prepared according to the literature.[14] Urea-d_4 and guanidinium-d_6 chloride were prepared by three cycles of dissolution on D_2O and lyophilization of urea (Nacalai) and guanidinium chloride (Wako).

[Pt(NH_3)$_2$(Been)]Cl_2·H_2O (1). This was prepared by a modified moethod of [$PtCl_2$(en)], described by Basolo et al.[14a] Into an aqueous solution of $K_2[PtCl_4]$ (416 mg, 1.0 mmol in 10 cm^3), a half volume of an aqueous solution of Been (150 mg, 1.0 mmol in 5 cm^3) was added at room temperature. The mixture was occasionally stirred, and separated yellow crystals were collected on a filter from time to time. After separation of the yellow crystals ceased, the remaining portion of the Been solution was added. The separated crystals were collected again and pooled. The collected [$PtCl_2$(Been)] was washed with small amounts of water, ethanol, and ether, successively. Yield 318 mg (76%). A 28% aqueous ammonia (10 cm^3) was added to [$PtCl_2$ (Been)] (208 mg, 0.5 mmol) in a glass tube and the tube was sealed after cooling at −78 °C, followed by heating at 70 °C in a water bath until the precipitates dissolved (ca. 3 h). The tube was opened and the reaction mixture was filtered. The filtrate was concentrated with a rotatory evaporator, and 25 cm^3 of acetone was added to cause solidification of the product. Pale-yellow precipitates were collected on a filter. Yield, 139 mg (62%) (λ_{max}/nm, ε_{max}/dm^3 mol^{-1} cm^{-1}): (268, 220); (263, 303); (256, 387); (250, 416); (204, 14800). Found: C, 23.56; H, 4.77; N, 12.16%. Calcd for [Pt(NH_3)$_2$(Been)]Cl_2·H_2O: C, 23.08; H, 4.74; N, 11.96%.

[Pt(NH_3)$_2$(Eten)]Cl_2·H_2O (2). This was prepared in a similar manner as mentioned above. The substitution reaction with aqueous ammonia completed in 30 min at 60 °C in this case. Yield of [Pt-(Eten)Cl_2], 288 mg (81%). (λ_{max}/nm, ε_{max}/dm^3 mol^{-1} cm^{-1}); (290, 48); (240, ca. 200(sh)); (223, 490); (202, 6820). Found: C,11.84; H,4.86; N,13.52%. Calcd for [Pt(NH_3)$_2$(Eten)]Cl_2·H_2O: C,11.83; H,4.96; N,13.79%.

[Pt(bpy)(Eten)]$(NO_3)_2$ (4). This was prepared by the method described for preparation of [Pt(bpy)(Been)]$(NO_3)_2$[10] using Eten (178 mg, 2.02 mmol) instead of Been. Yield, 192 mg (17%). (λ_{max}/nm, ε_{max}/dm^3 mol^{-1} cm^{-1}): (314, 13900); (307, 12800); (295, 6750(sh)); (243, 22100); (200, 40000). ^{13}C NMR δ = {157.2, 157.1 (bpy; 2,2′)}, 151.1 (bpy; 6,6′), 142.9 (bpy; 4,4′), {129.4, 129.2 (bpy; 5,5′)}, {125.1,125.0 (bpy; 3,3′)}, 53.9 (CH_2NH), 48.2 (CH_2CH_3), 53.9 (CH_2NH), 44.0 (CH_2NH_2), 13.3 (CH_3). Found: C, 29.86; H, 3.51; N, 14.95%. Calcd for [Pt(bpy)(Eten)]$(NO_3)_2$: C, 29.85; H, 3.58; N, 14.92%.

[Pt(phen)(Been)]Cl_2·$6H_2O$ (5). A suspension of [$PtCl_2$(phen)] (928 mg, 2 mmol) and Been (680 mg, 4.52 mmol) in 40 cm^3 of water was refluxed until the mixture turned to a yellow solution, which was concentrated with a rotary evaporator and washed with ether three times. To the residue, water (3 cm^3) was added, and the mixture was allowed to stand for about 2 d. Separated yellow crystals were collected on a filter and washed with two ca. 1 cm^3 portion of ethanol, three ca. 1 cm^3 ether successively. These crystals were recrystallized from 3 cm^3 of water. Yield, 481 mg (34%). (λ_{max}/nm, ε_{max}/dm^3 mol^{-1} cm^{-1}): (359, 1470); (342,1830); (278, 30300); (224, 36700). ^{13}C NMR δ = {152.0, 151.6 (phen; 2,9)}, {147.4, 146.6 (phen; 10a,10b)}, {142.0,140.8 (phen; 4,7)}, 133.8 (phe; *ipso*-), 132.3 (phe; *m*-), 131.3, 130.9 (phen; 5,6), 130.5 (phe; *p*-), 129.6 (phe; *o*-), {128.6,128.4 (phen; 3,8)}, {127.0, 126.8 (phen; 4a,6a)}, 58.4 (CH_2Ph), 57.9 (CH_2NH), 43.2 (CH_2NH_2). Found: C, 35.84; H, 4.53; N, 7.92%. Calcd for [Pt(phen)(Been)]Cl_2·$6H_2O$: C, 35.80; H, 4.86; N, 7.95%.

[Pt(phen)(Eten)]$Cl_2 \cdot 2H_2O$ (6). This was prepared by the above method using Eten in stead of Been with [$PtCl_2$(phen)] (301.5 mg, 0.0676 mmol). The crude product was recrystallized from water. Yield 91.2 mg (23.8%). (λ_{max}/nm, ε_{max}/dm^3 mol^{-1} cm^{-1}): (358, 1420); (341,1770); (323(sh), 2510); (298(sh), 8870); (277, 31600); (227, 37700). ^{13}C NMR δ = 151.9 (phen; 2,9), {147.1, 146.9 (phen; 10a,10b)}, 141.7 (phen; 4,7), 131.6 (phen; 5,6), 128.6 (phen; 3,8), {127.5, 127.3 (phen; 4a, 6a)}, 48.7 (CH_2CH_3), 54.0 (CH_2NH), 44.3 (CH_2NH_2), 13.5 (CH_3). Found: C, 33.79; H, 3.81; N, 9.94%. Calcd. for [Pt(phen)(Eten)]-$Cl_2 \cdot 2H_2O$: C, 33.69; H, 4.24; N, 9.82%.

Dependency of ^{1}H NMR Spectra of [Pt(bpy)(Been)]$(NO_3)_2$ (3) and [Pt(phen)(Been)]Cl_2 (5) on Concentration, Temperature, and Solvent. The spectra of **3** were recorded at 30 °C with concentrations of 2.66, 13.3, 26.6, 40.0, 53.3, and 66.6 mmol dm^{-3}. The temperature was varied between room temperature and 90 °C for the D_2O solution. ^{1}H NMR spectra of **3** and **5** were measured while varying the composition of dioxane/D_2O and the concentration of lithium chloride, urea-d_4, and guanidinium-d_6 chloride for D_2O solution and in CD_3OD.

Calculation of Molecular Mechanics and Anisotropic ^{1}H NMR Chemical Shift. A molecular-mechanics calculation was carried out using a modified MM2 program on an ACOS3700 computer at the Okayama University Computer Center.[15] The values of the ^{1}H NMR chemical shifts by ring-current effects of aromatic rings were calculated by a method of Abraham et al.[16] while varying the dihedral angle of the C–N (secondary) bond between 30° and 70°.

Results

Diammine complexes, **1** and **2**, were prepared by heating sealed glass tubes containing the corresponding dichloro-(L_2)-platinum(II) complex (0.5 mmol) and 28% aqueous ammonia until the materials dissolved. The addition of acetone to the concentrated residue allowed us to collect crystalline materials. The ternary platinum(II) complexes with *N*-alkyl-1,2-ethanediamines and aromatic diamines (bpy and phen), **3** to **6**, were isolated as nitrate salts for bpy complexes and chloride salts for phen complexes, respectively. The isolated platinum(II) complexes and labeling of hydrogens are shown in Fig. 1.

Diammine Complexes, 1 and 2. The methylene region of the ^{1}H NMR spectra of **2** showed resonances of the ethanediamine segment at 2.67 and 2.86 ppm, and the methylene of the ethyl substituent appeared as a quartet at 2.92 ppm with J = 7.5 Hz. However, the ethanediamine segment became more complicated for **1**: Two axial protons appeared (2.83 and 2.88 ppm) at a lower field than the equatorial protons (2.69 ppm), and the two protons of the benzyl are also magnetically nonequivalent and appeared as an AB signal; the difference in chemical shift is 0.125 ppm with a coupling constant of 13.5 Hz. The H–D exchange at the coordinated secondary amine has been extensively investigated, and is known to be catalyzed by OH^- and/or a general base.[7,8,17] In a neutral deuterium oxide solution, the H–D exchange was too fast to observe the vicinal couplings between N–H and C–H. The H–D exchange of the N–H groups was slowed down in a pD 2.0 DCl–D_2O solution. When the samples were prepared under acidic conditions, the AB pattern showed further separation due to coupling with the secondary amine proton with coupling constants of 7.5 and 5.8 Hz for the up and the downfield signals, respectively. Further, the coupling with ^{195}Pt was found for the up and downfield signals to be 36 and 20 Hz by the use of a 270 MHz spectrometer, respectively.[18]

Aromatic Diamine Complexes, 3—6. The methylene region of the ^{1}H NMR spectra of the bpy complexes, **3** and **4**, are shown in Fig. 2. The protons of the ethanediamine segment of the Eten complex, **4**, appeared as two multiplets at higher field with a separation of about 0.15 ppm and multiplets at lower field with apparently no separation. The chemical shift and coupling constants derived from LAOCN simulations[19] are listed in Table 1. These spectra resemble those of [Pt(bpy)(Meen)]$^{2+}$ reported by Humbley et al.,[3] the higher field protons ($H_{1''}$, $H_{2''}$) are assigned to the equatorial, and the lower field protons ($H_{3''}$, $H_{4''}$) are assigned to the axial protons, because large vicinal coupling constants are obtained between $H_{3''}$ and $H_{4''}$. The equatorial proton ($H_{2''}$) near to the secondary amine appears at the lower field side between $H_{1''}$ and $H_{2''}$ based on a double homo-gated experiment with N–H and –CH_3 for **4**.

Multiplets of methylene protons (H_m and H_n) of the *N*-ethyl group turned to an AB quartet upon irradiation at meth-

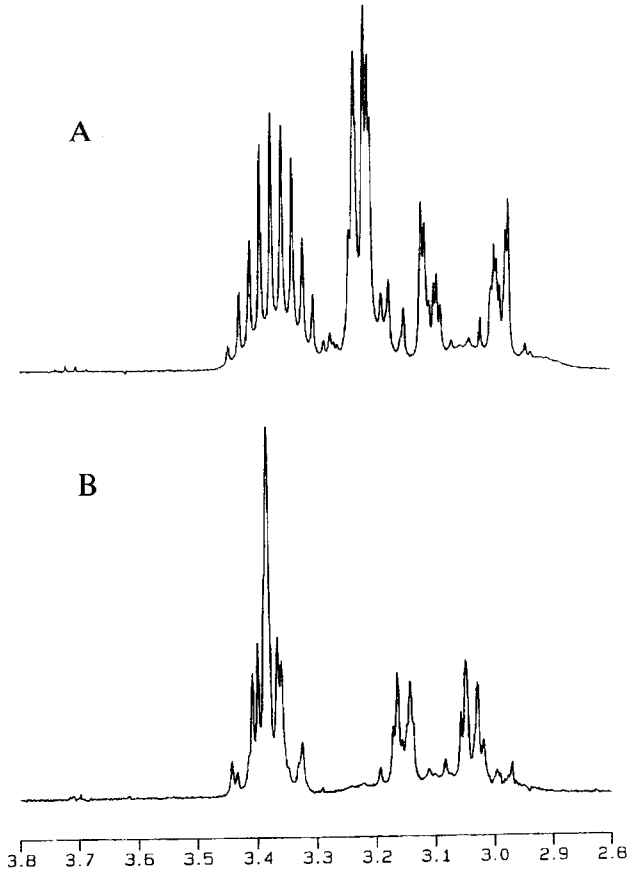


Fig. 2. Methylene region ^{1}H NMR spectra of [Pt(bpy)-(Eten)]$(NO_3)_2$ (**4**) (top) and [Pt(bpy)(Been)]$(NO_3)_2$ (**3**).

Table 1. Chemical Shifts/ppm and Coupling Constants/Hz of Ethanediamine Segment ^{1}H NMR Spectra of $[Pt(L_1)(L_2)]^{2+}$

Complex	L_1	L_2	$\delta_{1''}$	$\delta_{2''}$	$\delta_{3''}$	$\delta_{4''}$	$J_{1''2''}$	$J_{1''3''}$	$J_{1''4''}$	$J_{2''3''}$	$J_{2''4''}$	$J_{3''4''}$	δ_m	δ_n	J_{mn}
3	bpy	Been	3.04	3.15	3.40	3.37	1.1	5.2	−13.0	−13.4	4.4	13.8	4.46	4.14	−12.6
4	bpy	Eten	3.06	3.13	3.24	3.25	1.9	4.4	−12.7	−13.3	4.6	13.4	3.35	3.41	−14.1
5	phen	Been	3.20	3.30	3.53	3.58	−1.2	4.3	−13.5	−13.1	4.5	14.3	4.52	4.23	−12.5
6	phen	Eten	3.11	3.24	3.35	3.37	2.0	4.8	−14.0	−13.3	4.5	13.0	3.49	3.53	−13.6
[a]	bpy	Meen	2.93	3.07	3.32	3.42	1.2	4.3	−14.5	−13.1	4.0	13.9			

a) Ref. 3.

yl for D_2O solutions of **4** and **6** while benzyl protons of the Been complexes, **3** and **5**, appeared as a distinct AB quartet in D_2O. The chemical shifts of **3** and **5** are downfield shifted compared to those of **4** and **6**, as shown in Table 1. The couplings of these protons with amino protons and ^{195}Pt were observed with a pD 2.0 DCl–D_2O solution[7,8,17] and by using a 270 MHz spectrometer with a D_2O solution,[18] respectively. In Fig. 3, a representative example of them is shown with **3**. Two protons comprising the AB signals have different coupling constants with N–H: 4.1 and 3.1 Hz for the higher field protons and 9.5 and 9.3 Hz for the lower field protons for **3** and **5**; 9.1 and 9.1 Hz for the higher field protons and 3.1 and 3.1 Hz for the lower field protons for **4** and **6** under the acidic conditions. Also, among these protons, only higher field protons of **3** and **5** showed a coupling of 61 Hz with ^{195}Pt with a 270 MHz spectrometer.

The aromatic regions of the ^{1}H NMR of **3** and **5** are shown in Figs. 4A and 5A, respectively. The chemical shifts are listed along with those of the corresponding ethanediamine complexes in Table 2. Eight signals of the bpy and phen ligands were observed separately. The ^{1}H signals of half of the

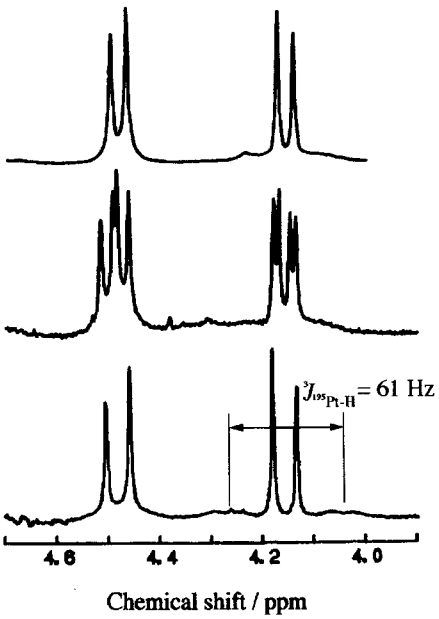


Fig. 3. Benzyl region of ^{1}H NMR spectra of [Pt(bpy)(Been)]-$(NO_3)_2$ under various experimental conditions: A, 400 MHz, D_2O solution: B, 400 MHz, pD 2.0 DCl–D_2O solution; C, 270 MHz, D_2O solution. The arrow represent $^3J_{Pt-H}$.

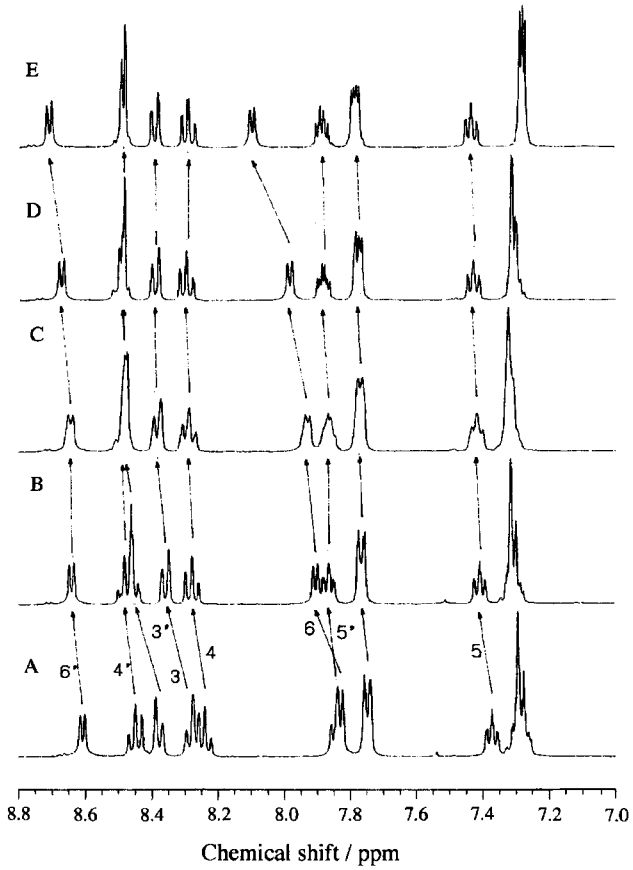


Fig. 4. Change in aromatic region of [Pt(bpy)(Been)]$(NO_3)_2$ (**3**) with varying the composition of dioxane-d_8/D_2O. A, 0/55; B, 10/55; C, 15/55; D, 20/55; E, 30/55.

Table 2. Chemical Shifts of bpy or phen in $[Pt(L_1)(L_2)]^{2+}$

	L_1	L_2	H_3	H_4	H_5	H_6	$H_{3'}$	$H_{4'}$	$H_{5'}$	$H_{6'}$
	bpy	en	8.44	8.45	7.82	8.66	8.44	8.45	7.82	8.66
3	bpy	Been	8.30	8.24	7.36	7.79	8.39	8.43	7.81	8.58
4	bpy	Eten	8.46	8.49	7.90	8.61	8.46	8.49	7.90	8.69
	L_1	L_2	H_2	H_3	H_4	H_5	H_6	H_7	H_8	H_9
	phen	en	8.98	8.11	8.89	8.03	8.03	8.89	8.11	8.98
5	phen	Been	8.05	7.68	8.73	8.05	8.09	8.96	8.16	9.00
6	phen	Eten	8.98	8.13	8.95	8.14	8.14	8.98	8.19	9.06

aromatic diamine rings resonated at an upper field compared to those of the other half. The up-field shifts of the signals of **3** and **5** compared to those of $[Pt(bpy)(en)]^{2+}$ and $[Pt(phen)(en)]^{2+}$ decreased in the order $H_6 > H_5 > H_4 > H_3$ for **3**, and

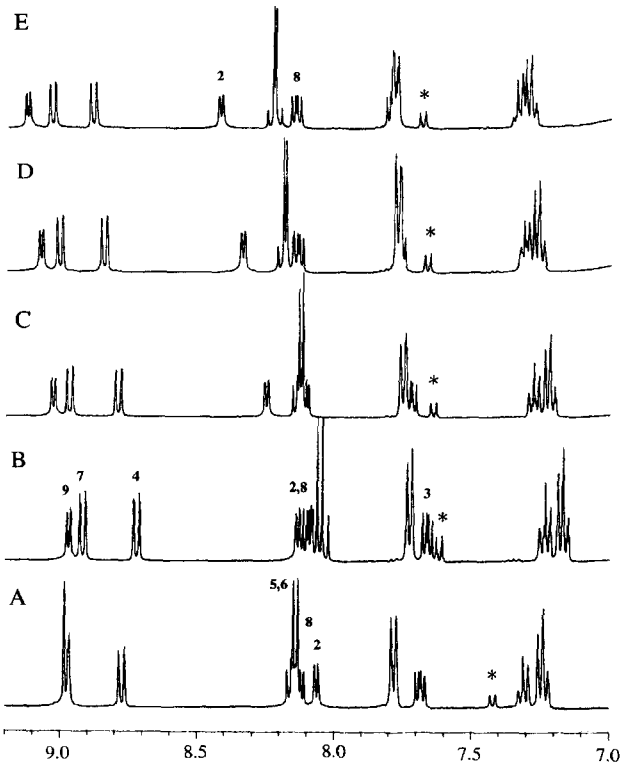


Fig. 5. Aromatic region of ^{1}H NMR of [Pt(phen)(Been)]Cl$_2$ (**5**) (A) and its change by addition of guanidine-d_6 deuterochloride: B, 2 M;C, 4 M; D, 6 M; E, 8 M. The signals indicated with * are due to sodium *p*-toluene sulfonate used as an indicator.

$H_2 > H_3 > H_4$ for **5**. The chemical shifts of other protons of **3** and **5** are almost the same as those of the corresponding en complexes.

To obtain information about intermolecular stacking, the concentration of **3** was varied from 2.66 to 66.6 mmol dm^{-3}. With increasing the concentration, linear small changes in the chemical shift were observed; all of the signals moved to a lower field, but the magnitudes were small: i.e., the largest variation was found to be 0.03 ppm between the range of concentrations.

For the Eten complexes, **4** and **6**, the symmetry of the bpy and phen part was formally lost due to the unsymmetric Eten; however, the differences in the chemical shift between the protons related by the local symmetry of the aromatic diamines were small, and the largest difference was either 0.08 ppm for the 6,6′-protons for **4** and 2,9-protons for **6**.

Upfield Shift Deduced on the Ring Current Effect. Based on an X-ray crystal structure determination on **3**,[10] the upfield shift of each proton of coordinated phen in **5** was calculated based on Abraham[16] by the rotation about the N–C bond. The results are shown in Fig. 6. The observed shifts are shown by horizontal lines. The straight line and the calculated curve cross at the torsion angle of Pt–N–C–Ph near to 50° for the differences between H_2 and H_9 and between H_3 and H_8.

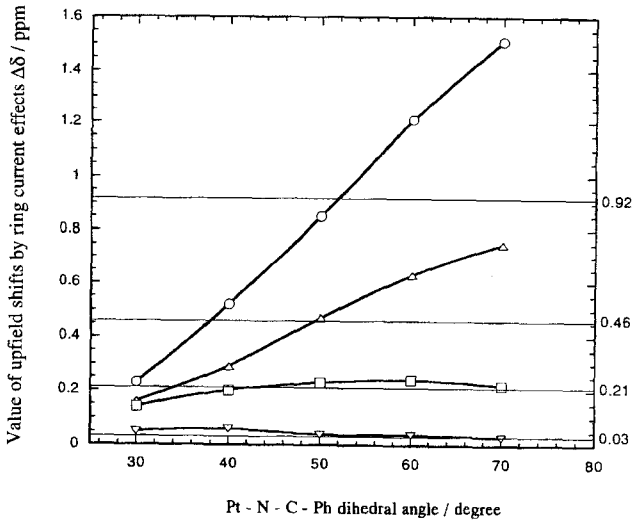


Fig. 6. Comparison of value of upfield shifts between experimental data and calculated data. Experimental data are $\Delta(H_9–H_2) = 0.92$, $\Delta(H_8–H_3) = 0.46$, $\Delta(H_7 – H_4) = 0.21$, $\Delta(H_6–H_5) = 0.03$ ppm and are represented by horizontal lines. Calculated data are shown ○, $\Delta(H_9–H_2)$;△, $\Delta(H_8–H_3)$;□, $\Delta(H_7–H_4)$;▽, $\Delta(H_6–H_5)$.

Solvent Dependency of ^{1}H Chemical Shifts of 3 in the Aromatic Region. Figure 4 shows the dependency of the aromatic region ^{1}H NMR spectra of **3** on the composition of dioxane/D_2O. The signals which had been shifted to up-field in D_2O were found to move to down-field with increasing of the content of dioxane. The most significant change was found at the the signal (H_6), which was most up-field shifted in D_2O. A similar trend was found for **5**; plots of the chemical shifts against the concentration gave straight lines, and the results are tabulated in Table 3. The solvent dependency was also evaluated with LiCl, CD_3OD, urea-d_4, and guanidinium-d_6 chloride for both Pt(II) complexes. The results are shown in Fig. 5 for **5**-guanidinium chloride, where the point without the reagent deviates from a straight line. These results for the two protein denaturants are also tabulated in Table 3. The results for LiCl and CD_3OD are given in Table 4.

Plots of the chemical shifts of bipyridine ring protons against $1/T$ are shown in Fig. 7. A straight line was obtained for each proton with varying magnitude of negative slope. The H_6 which had been most shifted showed the steepest slope. These temperature dependency arises from the rapid equilibrium between the stacked form and the unstacked form, as described by Odani et al.[20] The observed chemical shift, δ_{obs}, is the weighted mean of the chemical shifts of the intrinsic stacked (δ_{ST}) and unstacked (δ_{US}) forms; $\delta_{obs} = x\delta_{ST} + (1-x)\delta_{US}$, where x stands for the mole fraction of the stacked form. If we make assumptions about δ_{US} and $\Delta\delta = \delta_{US} - \delta_{ST}$, the equilibrium constant, K = [stacked form]/[unstacked form], at each temperature can be calculated. With use of the values $\delta_{US} = 8.95$ ppm and $\Delta\delta = 1.0$ ppm which are derived from the dependency of the chemical shift of $H_{6'}$, plots of $\ln K$ against $1/T$ show a straight line, from which $\Delta H^{\circ\prime} = -9.2$ kJ mol^{-1} and $\Delta S^{\circ\prime} = -19$ J K^{-1} mol^{-1} are obtained through $\ln K = -\Delta G^{\circ\prime}/(RT)$.

Table 3. Dependency of Chemical Shift of bpy and phen in $[Pt(L_1)(Been)]^{2+}$ on the Composition of the D_2O–Dioxane-d_8 (weight fraction), –Guanidinium-d_6 Chloride, and –Urea-d_4 Solution

L_1 = bpy, **3**	H_3	H_4	H_5	H_6	$H_{3'}$	$H_{4'}$	$H_{5'}$	$H_{6'}$
$10^3\,\Delta\delta$/dioxane[a)]	17.8	9.5	9.3	42.6	17.1	5.0	7.3	15.2
$10^3\,\Delta\delta$/[Gdm Cl][b)]	15.8	12.5	14.9	44.4	15.0	9.1	6.7	23.1
$10^3\,\Delta\delta$/[Urea][b)]	1.5	3.5	4.1	9.8	−0.2	0.0	−1.3	−1.4
L_1 = phen, **5**	H_2	H_3	H_4	H_5	H_6	H_7	H_8	H_9
$10^3\,\Delta\delta$/dioxane[a)]	44.8	17.3	19.1	19.8	20.7	16.9	14.8	23.4
$10^3\,\Delta\delta$/[Gdm Cl][b)]	49.4	17.9	19.1	17.1	16.5	11.3	3.21	21.9
$10^3\,\Delta\delta$/[Urea][b)]	15.8	7.4	7.6	9.4	8.1	1.8	−2.2	−1.6

a) dioxane-d_8/mol kg^{-1}, b) guanidinium-d_6 chloride or urea-d_4/mol dm^{-3}.

Table 4. ^{1}H NMR Chemical Shifts of bpy Ring Protons of $[Pt(bpy)(Been)](NO_3)_2$, **3**, in D_2O, Methanol-d_4, and 4 M LiCl D_2O Solution

	H_3	H_4	H_5	H_6	$H_{3'}$	$H_{4'}$	$H_{5'}$	$H_{6'}$
D_2O	8.30	8.24	7.36	7.79	8.39	8.43	7.81	8.58
CD_3OD	8.48	8.30	7.47	8.20	8.55	8.46	7.85	8.70
4 M LiCl	8.11	8.10	7.24	7.75	8.19	8.35	7.78	8.52

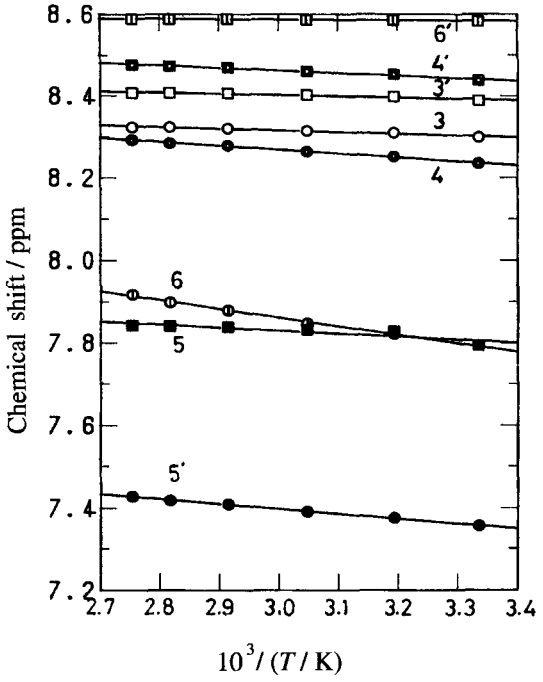


Fig. 7. Plots of chemical shifts of bipyridine ring protons against $1/T$ in D_2O solution.

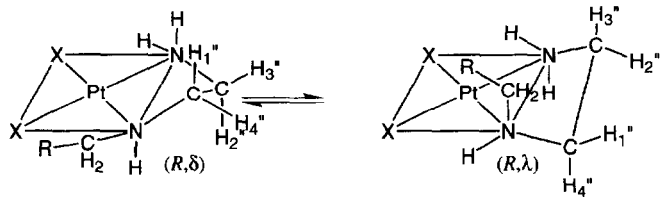
Discussion

The conformation of an *N*-alkyl-1,2-ethanediamine coordinated to an octahedral metal ion has been described earlier.[9] In square-planar complexes, the energy difference between the pseudo equatorial and the pseudo axial position is small due to the absence of the fifth and sixth ligands. The orientaion of the alkyl group and the absolute conformation of the five-membered chelate ring are linked: A pseudo equatorial substituent prefers a δ chelate ring conformation and a pseudo axial substituent prefers a λ conformation if we assume that the secondary amine has an *R*-absolute configuration upon coordination to a metal ion, for simplicity. We denote these as (R,δ) and (R,λ), respectively. When a Newman projection is drawn through the secondary nitrogen atom and the carbon atom next to the secondary amine on the substituent, three conformers emerge for each set of (R,δ) and (R,λ), as shown in Fig. 8. Two protons on the carbon atom next to the secondary amine can be differentiated as pro-*R* and pro-*S*; and the torsion angles of these protons with N–*H* and ^{195}Pt are summarized at the bottom of Fig. 8.

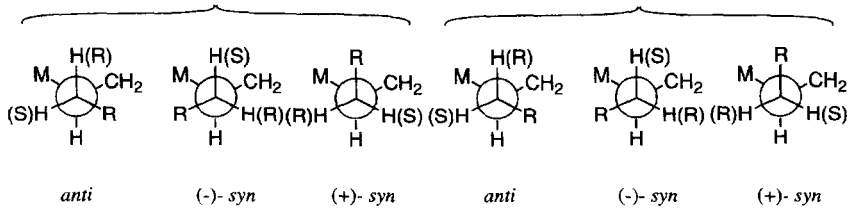
The ^{1}H NMR spectra of **2** showed a quartet for the methylene protons, and the ethanediamine segment also exhibited a simple coupling pattern. In this complex the ethyl substituent undergoes rapid interconversion between the pseudo-axial and pseudo-equatorial positions, because the vicinal couplings ($^3J_{H-C-N-H}$) between protons has the same magnitude. On the other hand, **1** showed the two protons of the benzyl with a separation of 0.13 ppm. The coupling with ^{195}Pt was larger for the upper field protons and the coupling constant between these protons and N–H are 7.5 and 5.8 Hz, respectively. In a hexacoordinated $[Co(CN)_4(Been)]^-$, the corresponding AB signal showed a difference of 0.52 ppm, and the coupling constants for the upper field signal and the lower signal are distinctly different: 10.7 and ca. 2 Hz, respectively.[9] These findings are consistent with the structural fluctuation for diammine complexes, **1** and **2**. The coupling of these protons with N–H and ^{195}Pt for **1** is in agreement with structure alternating an *anti* and a (−)-*syn* rotamers, because the only H(*R*) takes the *anti* to Pt with (−)-*syn* conformer, while the H(*R*) and H(*S*) locate alternately at the position *anti* to N–H on the interconversion between the *anti* and (−)-*syn* conformer.

In the crystal of **3** grown in water–ethanol, the complex cation obviously takes an (R,λ) and (S,δ) conformation with the phenyl group being *anti* to Pt.[10] However, a close examination of the crystal structure showed that the phenyl group of the Been was stacked with the bpy ring of the adjacent comlex cation, and that this packing is likely to be the major factor in determining the crystal structure. Thus, the conformation of the solution has to be examined by other experimental methods.

The benzyl protons of **3** or **5** resonated at 4.46 and 4.14 ppm or 4.52 and 4.23 ppm, respectively, and their differences are



Perspective view of $[MX_2(N(R)\text{-}RCH_2CH_2NH_2))]^{n+}$



B Newman projections through RCH_2-NH bond

Relationship between conformers and their torsion angles

		$R(\delta)$			$R(\lambda)$		
		anti	(-)-*syn*	(+)-*syn*	*anti*	(-)-*syn*	(+)-*syn*
Pro-R	N	180°	60°	60°	180°	60°	60°
	Pt	60°	180°	60°	60°	180°	60°
Pro-S	N	60°	180°	60°	60°	180°	60°
	Pt	60°	60°	180°	60°	60°	180°

Fig. 8. Schematic representation of structure of coordinated diamines with the secondary amine site being *R* configuration and the relationship of methylene protons on the carbon atom next to the secondary amine and the torsion angles with NMR active nucleus.

about 0.3 ppm. The proton appeared at higher field couples weakly, but that at lower field couples strongly with N–H for the pD 2.0 DCl–D_2O solution. A strong coupling of 61 Hz with ^{195}Pt was observed for the upper field proton resonance with a 270 MHz spectrometer.

Based on these results, the Been and Eten ligands of **3** through **6** are found to be inhibited from taking (R,δ) and (S,λ) sets and the *N*-alkyl substituents take pseudo axial positions for the complexes with bpy and phen as L_1. The coupling of protons on the carbon atom next to the secondary amine provides a clue about the conformation: a small coupling with N–H and a large coupling with ^{195} Pt for higher field proton and large coupling with N–H for the lower field for **3** and **5** and large coupling with N–H and for higher field proton and small coupling with N–H for the lower field for **4** and **6**. An inspection of Fig. 8 with these results suggests that we assign the higher and lower field protons of **3** and **5** to pro-*R* and pro-*S* in the (−)*syn* - and those of **4** and **6** to pro-*R* and pro-*S* in the *anti*-conformers, respectively, based on the Karplus-type vicinal coupling dependency on the dihedral angle.[3b] These lead to the conclusion that the most populated rotamer is *anti*- for the Eten and is (−)-*syn* rotamer for the Been complexes.

The insensitivity of the chemical shifts of **3** to its concentration and the direction of each shift on increasing the concentration has clearly shown that the unsymmetrical shift shown in Table 2 was not due to intermolecular stacking.

A large change in the chemical shift of the aromatic diamines occurred for the Been complexes (**3** and **5**), while a small change was observed for the Eten complexes (**4** and **6**). The large change is the consequence of intramolecular stacking of the aromatic rings, and is confirmed by a calculation of the ring-current effect.

Comparison of the Conformation of the Ligand between a Crystal and an Aqueous Solution. The ternary complexes, **3** and **5**, showed a significant up-field shift for half of the aromatic diamines. The up-field shift of the protons on the aromatic diamine part can be evaluated based on the ring-current effect from the phenyl ring of Been. A model of **5** was constructed based on the crystal structure of **3**. The phenyl ring was rotated around the C–N_{sec} bond and the change in the chemical shift for each phen protons was calculated based on the ring-current effect; the results are shown in Fig. 6. The most populated conformation of **3**

and **5** in solution is (−)-*syn*, because the upfield shift of the protons is in agreement with the expected upfield shift for the (−)-*syn* conformer. Thus, the conformation of the ternary complexes containing Been and aromatic diamine takes a (−)-*syn* conformer in a deuterium oxide solution, while the conformation of the complex cation is *anti* in crystals.

A MM2 calculation also showed that the most stabilized form is a (−)-*syn* rotamer with a torsion angle of 63° for Pt–N–C–Phe for **5**, though the *anti* rotamer has near stabilization. This shows that a van der Waals interaction is important for the stacking of **3** and **5**. The energy-minimized structure for **5** is shown in Fig. 9, along with the structure deduced based on the NMR results.

Nature of π–π Stacking. The stacking of aromatic compounds in aqueous solution is now well documented.[20,21] However, there are several proposals about the nature of the π–π stacking: van der Waals interaction, coulombic interaction, charge transfer, and quadrupole–quadrupole interaction. The π–π stacking in metal complexes was studied earlier by equilibrium measurements for the formation of ternary complexes of copper(II) where the components had aromatic substituents.[22] The best- studied systems are those between a transition metal ion, α-amino acids (derivatives) with an aromatic side chain such as tryptophan, tyrosine, and phenylalanine, and aromatic diamines such as bpy and phen. These systems were investigated by potentiometric, X-ray crystal structural, and NMR methods.[22] Up-field shifts due to ring-current effects have been reported for several of their palladium(II) complexes: $[Pd(bpy)(dipeptide)]^+$ where dipeptides contain tryptophan, tyrosine, and phenylalanine residue.[20] For these dipeptides the largest up-field shifts were observed at the aromatic protons residing on the amino acid side chain.[20] Though the aromatic substituents are bonded to the β carbons of amino acid residues, face-to-face stacking occurred with axially oriented β carbons.[20] For the *N*-benzyl-1,2-ethanediamine complex, the largest up-field shifts were observed at one of the 6-protons for bpy and either one of 2- or 9-proton for phen; the chemical-shift changes in the phenyl group of Been are −0.22 and −0.32 ppm at *m*-, −0.22 and −0.25 ppm at *p*-, and 0.11 and 0.12 ppm at *o*-protons for **3** and **5**, respectively compared to **1**. The up-field shifts for *m*- and *p*-protons are expected for a stacked structure, and the down-field shift of *o*-protons is a consequence of the proximity of the d_z2 orbital of Pt(II).[23] These results are consistent with the structure depicted in Fig. 9 for **5**. The planes of the aromatic rings of Been and bpy and phen are not parallel to each other, but the separation between them is about 3.6 Å.[10] Thus, the *N*-aryl group has a strong tendency to form intramolecular stacking if the metal ion has bpy or phen derivatives in the same coordination sphere.

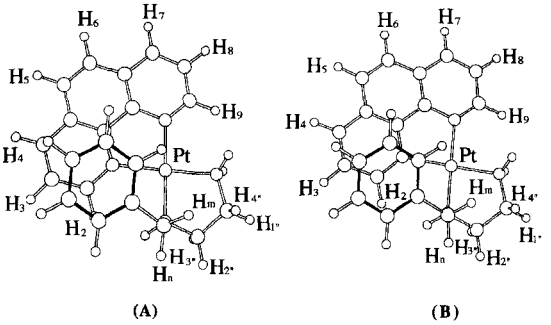


Fig. 9. Structures of $[Pt(phen)(Been)]^{2+}$ viewed along secondary amine nitrogen–benzyl carbon bonds. (A) the predicted structure obtained by the calculation of ring current effect. (B) the stable structure obtained by MM2 calculation.

Solvent Dependency and Simple Method for Evaluating Protein Structure-Breaking Reagents. The dependency of intramolecular stacking and hydrophobic interactions on the solvent composition has been explored by Sigel for the system of $[M(phen)(2\text{-phenylacetate})]^+$ and $[M(phen)(3\text{-phenylpropionate})]^+$ (M = Cu and Zn): The addition of dioxane or ethanol to water retards the formation of the closed ternary system where the phenyl and phen cause stacking.[24] Although the present platinum(II) complexes are different from the system of Sigel et al. in that the ligands do not have a tendency to dissociate, the addition of dioxane caused a decrease of the up-field shift of the aromatic diamines, as shown in Fig. 4. Thus, the decrease in the polarity of the solvent causes a destabilization of the stacked form of **3** and **5**.

The effect of the addition of organic solvent to an aqueous solution on the stability of molecular complexes has been evaluated for many systems.[25] The temperature dependency of bipyridine ring protons also shows that the rotamers other than (−)-*syn*-conformer are populated at high temperature. However, the most populated rotamer should still be the (−)-*syn*-conformer even at 90 °C, because the chemical shift of H_6 is 7.92 ppm at this temperature. The magnitude of $\Delta H^{\circ\prime}$ and $\Delta S^{\circ\prime}$ are $-9.2\ kJ\ mol^{-1}$ and $-19\ J\ mol^{-1}\ K^{-1}$; these values are larger in absolute value than $[Pd(bpy)(AA\text{–}Gly)]^{2+}$ (AA = Tyr or Phe) and $[Pd(bpy)(AA\text{–}Glu)]^{2+}$ (AA = Trp, Tyr or Phe),[20] reflecting the ease to form the stacked form, because the benzyl group is forced to take an axial orientation prior to stacking in $[Pt(Been)(bpy)]^{2+}$. Both the enthalpy and the entropy have negative values; the main driving force for the stacking is enthalpy change, but the desolvation of water from the aromatic ring tends to compensate any decrease in the entropy so as to give $\Delta G^{\circ\prime} = -3.4\ kJ\ mol^{-1}$ at 25 °C in water.

These results led us to measure the dependency of the up-field shift of the aromatic diamines of **3** and **5** on the concentration of urea and guanidinium chloride. These reagents are well known to act as denaturants of the three-dimensional structure of the protein, and are widely used for the dissolution of the inclusion body of proteins produced by genetic engineering.[26] An exact reasoning of these effects of urea and guanidinium chloride has not been reached,[11] but several explanations are proposed: water-structure breaker, hydrogen bond from the denaturant to peptide backbone, and the easier exposure of the hydrophobic side change with an unfolding of the protein structure.[26] The hydrogen-bond network of aqueous urea and guanidinium chloride has recently been explored by a Monte-Carlo method, and the hydrogen-

bond close to these molecules are similar to hydrogen-bonds close to non-ionic solutes, although these compounds are polarized.[11] These compounds are highly soluble and the denaturation of proteins are accomplished by the dual nature of the polar and nonpolar characteristics. These denaturants are expected to influence the structure of the present intramolecular stacking, or relative population of rotamers because the stacking is stabilized by mainly van der Waals interactions between the aromatic rings. The results show that the guanidinium chloride and urea cause down-field shifts, as shown in Fig. 5 and Table 3. The most striking point is that guanidinium chloride is approximately three-times more effective than urea. This is close to the relative effectiveness of these denaturants in the denaturation of most small proteins; i.e. 2.3-times more effective with guanidinium chloride to urea.[26] The present results have shown that the main function of urea and guanidinium chloride on even these small complex cations is a weakening of the interaction of nonpolar groups, because these break the intramolecular stacking of aromatic rings. The small differences found in the magnitudes of the shifts of those protons could be ascribed to the polar effect of these denaturants through the coordinated amine's NH groups.

On the other hand, LiCl, salting-out reagent of proteins,[27] has an effect on the structure, denaturation, dissociation to subunits, and the activity of enzymes, and has been reported to enhance the denaturation and refolding to native proteins in conjunction with urea.[28] 4 M (1 M = 1 mol dm^{-3}) LiCl enhances the upfield shift of protons of aromatic diamines of **3** and **5**. This shows that LiCl acts differently from urea and guanidiunium chloride.

These observations suggest that the ^{1}H NMR spectrum of **3** or **5** can be used as a simple criterion in an evaluation of the nature of various solvents.

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