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The Neglected Pt–N(sulfonamido) Bond in Pt Chemistry. New Fluorophore-Containing Pt(II) Complexes Useful for Assessing Pt(II) Interactions with Biomolecules

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Treatment of *cis*-Pt(Me_2SO)$_2Cl_2$ with DNSH-tren afforded [Pt(DNSH-tren)Cl]Cl and with DNSH-dienH, under increasingly more basic conditions, led to Pt(DNSH-dienH)Cl_2, Pt(DNSH-dien)Cl, and Pt(DNS-dien). (DNSH = 5-(dimethylamino)naphthalene-1-sulfonyl, linked via a sulfonamide group to tris(2-aminoethyl)amine (DNSH-tren) and diethylenetriamine (DNSH-dienH); the H's in DNSH-dienH designate protons sometimes lost upon Pt binding, i.e., sulfonamide NH for the dienH moiety and H8 for the DNSH moiety). Respectively, the three neutral DNSH-dienH-derived complexes are difunctional, monofunctional, and nonfunctional and exhibit decreasing fluorescence in this order as the dansyl group distance to Pt decreases. 2D NMR data establish that Pt(DNS-dien) has a Pt–C8 bond and a Pt–N(sulfonamido) bond. Pt(DNSH-dien)Cl and [Pt(DNSH-tren)Cl]Cl bind to N7 of 6-oxopurines (e.g., 5′-GMP, 3′-IMP, and 9-ethylguanine) and sulfur of methionine (met). Competition and challenge reactions for Pt(II) with met and 5′-GMP typically reveal that met binding is favored kinetically but that 5′-GMP binding is favored thermodynamically. This common type of behavior was found for [Pt(DNSH-tren)Cl]Cl. In contrast, Pt(DNSH-dien)Cl had reduced kinetic selectivity for met. This unusual behavior undoubtedly arises as a consequence of the bound Pt–N(sulfonamido) group, which donates strongly to Pt (as indicated by relatively upfield dien NH signals) and which places the bulky DNSH moiety close to the monofunctional reaction site. The decrease in the relatively upfield shifts of the DNSH group signals indicates that this group stacks with the purine. This stacking could explain the unprecedented, relatively low reactivity of a Pt complex bearing a dien-type ligand toward met vs 5′-GMP.

Introduction

Platinum is the metal present in various classes of inorganic medicinal agents directed at important diseases such as cancer or virus infections.[1−4] An important feature of the Pt(II) center that may be a key property endowing Pt(II) agents with these important types of activity is the high affinity toward the N7 of purines, particularly guanine, in nucleic acids.[2,5] Platinum drugs are perhaps the best single type of anticancer drug active against a broad range of cancers.[1] Pt drugs would find wider use except for the well-known toxicity resulting from the reaction of the drugs with biological thiols and thioethers.[6] Thus, it is highly desirable to identify features that reduce S binding while maintaining N7 binding.

Reactions of the monofunctional complex, [Pt(dien)Cl]Cl (dien = diethylenetriamine), with nucleotides have been studied extensively as an aid in assessing the nature of DNA binding of difunctional Pt(II) anticancer drugs (e.g., cisplatin).[2,7] A study of the reaction rates of [Pt(dien)Cl]Cl with 5′-GMP (5′-GMP = 5′-guanosine monophosphate) and peptides [glutathione (GSH) and S-methylglutathione (GSMe) as models for cysteine and L-methionine (met) binding sites, respectively] showed that the Pt−S bond is formed much more rapidly than the Pt−N7 bond.[6] Several other studies, including competitive reactions of [Pt(dien)Cl]Cl with met

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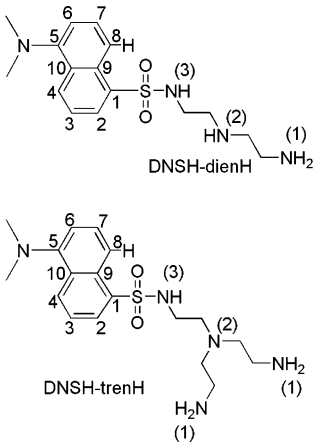

Figure 1. Fluorescent ligands used in this study.

and 5′-GMP (revealing that the kinetic product was Pt(dien)(met) and that the thermodynamic product was Pt(dien)(5′-GMP)), all indicate the same trend.[8−10]

Many Pt(II) complexes with ligands having the dien backbone have been synthesized and studied, e.g., *N*,*N*′,*N*′′-trimethyldiethylenetriamine (*N*-Me$_3$dien), *N*,*N*,*N*′,*N*′′,*N*′′-pentamethyldiethylenetriamine (*N*-Me$_5$dien), and *N*,*N*,*N*′′,*N*′′-tetramethyldiethylenetriamine (*N*-Me$_4$dien).[11−13] Preferential binding of [Pt(*N*-Me$_4$dien)Cl]Cl to the sulfur of glutathione and the absence of binding to an imidazole nitrogen of histidine was attributed to steric effects.[13]

In this study, we explore the chemistry of complexes with tridentate ligands bearing a sulfonamide and an aromatic fluorescent group. The ligands (Figure 1) used in this study are *N*-(2-((2-aminoethyl)amino)ethyl)-5-(dimethylamino)-naphthalene-1-sulfonamide (DNSH-dienH, where DNSH = 5-(dimethylamino)naphthalene-1-sulfonamide) and *N*-(2-(bis-(2-aminoethyl)amino)ethyl)-5-(dimethylamino)naphthalene-1-sulfonamide (DNSH-tren, where tren = tris(2-aminoethyl)-amine). (The H indicates protons displaceable upon Pt binding, i.e., the sulfonamide NH for the dienH moiety and the H8 for the DNSH moiety). The DNSH-dienH ligand afforded the Pt(DNSH-dienH)Cl$_2$, Pt(DNSH-dien)X (X = Cl, Br), and Pt(DNS-dien) complexes, whereas DNSH-tren gave [Pt(DNSH-tren)Cl]Cl (Figure 2). These compounds presented us with the opportunity to investigate the effect of the fluorophore on the fluorescence and the properties of complexes with small biomolecules such as 9-substituted derivatives of guanine (**G**) or of hypoxanthine (3′-inosine monophosphate, 3′-IMP).

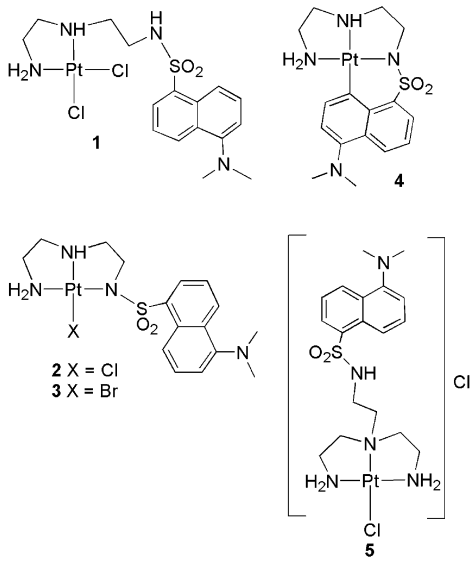

Figure 2. Pt(II) complexes synthesized in this study: Pt(DNSH-dienH)-Cl$_2$ (**1**), Pt(DNSH-dien)Cl (**2**), Pt(DNSH-dien)Br (**3**), Pt(DNS-dien) (**4**), and [Pt(DNSH-tren)Cl]Cl (**5**).

Experimental Section

Starting Materials. Tren from Strem, *N*′-Me-dien (*N*′-Me-dien = *N*-methyl-2,2′-diaminodiethylamine) from TCI America, Pt(en)-Cl$_2$, *N*-Et-en = *N*-ethylethylenediamine, and dien from Aldrich, and dansyl chloride (DNSH-Cl), 9-ethylguanine (9-EtG), and the sodium salts of 3′-guanosine monophosphate (3′-GMP), 5′-GMP, 5′-guanosine diphosphate (5′-GDP), 5′-guanosine triphosphate (5′-GTP), 3′-IMP, and met from Sigma were all used as received. The DNSH-dienH and DNSH-tren ligands were prepared by known methods,[14,15] and their ^{1}H NMR chemical shifts matched the reported values. *cis*-Pt(Me$_2$SO)$_2$Cl$_2$,[16] *cis*-Pt(Me$_2$SO)$_2$Br$_2$,[17] [Pt-(dien)Cl]Cl,[18] Pt(*N*-Et-en)Cl$_2$,[19] and [Pt(*N*′-Me-dien)Cl]Cl[20] were prepared as described in the literature.

NMR Spectroscopy. ^{1}H, ^{13}C, and ^{195}Pt NMR spectra were recorded on either a 400 or a 500 MHz Bruker NMR spectrometer using DMSO-d_6, except for ^{1}H NMR studies of adduct formation performed in D$_2$O/DMSO-d_6 solutions. Typical concentrations were 10 mM for ^{1}H and ^{13}C NMR and 50 mM for ^{195}Pt NMR measurements. Peak positions are relative to TMS (for ^{1}H and ^{13}C in DMSO-d_6) or the residual water signal (for ^{1}H in D$_2$O/DMSO-d_6). All NMR data were processed using XWINNMR and Mestre-C software. ^{1}H−^{1}H COSY and ROESY, as well as ^{1}H−^{13}C HSQC and HMBC, NMR spectra were recorded in order to assign the signals of the products. Matrixes (512 × 2048) were collected for the ^{1}H−^{13}C HSQC and HMBC experiments. Typically, 256 scans were collected per block and an exponential apodization function with a line broadening of 0.2 Hz was used. The typical J_{H-C} in HMBC experiments was 8 Hz unless otherwise stated. The COSY experiments were performed at 25 °C with 16 scans per t_1 increment. The evolution dimension was zero-filled to 2 K prior to Fourier transformation. 2D ROESY experiments were performed

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at 25 °C by using mixing times of 300 ms (64 scans per t_1 increment). For ^{195}Pt NMR spectra, a pulse width of 20 μs, corresponding to a 30° tip angle, was used with a relaxation delay of 0.4 s. Usually, several acquisitions were recorded using a spectral window of 75 000 Hz, with 16 K data points. Typically, 10 000 scans were averaged for each acquisition and the resulting FID was multiplied by an exponential function (LB = 50 Hz) prior to Fourier transformation. All ^{195}Pt NMR chemical shifts were determined in DMSO-d_6 solutions by using *cis*-Pt$(Me_2SO)_2Cl_2$ (−3450 ppm) as an external reference, compared to a Na_2PtCl_6 standard.[21] Because the shift effect of the deprotonated sulfonamide of Pt(DNSH-dien)-Cl was not as large as expected from the literature on the shift effect of deprotonated peptides,[22] we examined a particularly wide spectral region from −1300 to −9400 ppm to ensure that the signal reported below was from this complex.

Fluorescence Spectroscopy. Spectra of Pt(II) complexes and 5′-GMP adducts (5 μM solution in H_2O/DMSO-d_6, v/v 5:1, pH = 5.5) were recorded in a 10 mm quartz fluorescence cuvette, at room temperature, in triplicate by using a Spex Fluorolog-3 spectrofluorimeter equipped with a 450 W xenon lamp and a photomultiplier tube detector; all the spectra were blank-subtracted. Emission spectra were recorded at the excitation wavelength, 360 nm.

Synthesis of Pt(DNSH-dienH)Cl$_2$ (1). A suspension of *cis*-Pt$(Me_2SO)_2Cl_2$ (150 mg, 0.355 mmol) in methanol (20 mL) was treated with DNSH-dienH (119 mg, 0.355 mmol), and the reaction mixture was stirred at room temperature for 24 h. Acetonitrile (60 mL) was added slowly to the solution, and the yellow solid that precipitated was collected on a filter, washed with water and diethyl ether, and dried under vacuum; yield, 87 mg (41%). ^{1}H NMR spectrum (DMSO-d_6) (ppm): 8.48 (d, 1H), 8.27 (d, 1H), 8.22 (t, 1H, NH), 8.13 (d, 1H), 7.61 (m, 2H), 7.25 (d, 1H), 6.98 (s, 1H, NH), 6.15 (s, 2H, NH), 3.42 (s, 2H, CH_2), 3.38 (m, 2H, CH_2), 3.15 (m, 4H, CH_2), 2.83 (s, 6H, CH_3). Anal. Calcd for $C_{16}H_{24}Cl_2N_4O_2PtS\cdot1.5H_2O\cdot CH_3OH$: C, 30.87; H, 4.72; N, 8.47. Found: C, 31.09; H, 4.57; N, 8.02.

Synthesis of Pt(DNSH-dien)Cl (2). The Pt(DNSH-dien)Cl complex was synthesized as above for complex **1**, but with triethylamine (40 μL, 0.293 mmol) added to the methanol suspension of *cis*-Pt$(Me_2SO)_2Cl_2$ immediately after addition of DNSH-dienH. The yellow solid that precipitated from the reaction mixture after 24 h of stirring was collected on a filter, washed with diethyl ether, and dried under vacuum; yield, 90 mg (45%). ^{1}H NMR spectrum (DMSO-d_6) (ppm): 8.60 (d, 1H), 8.39 (d, 1H), 8.27 (d, 1H), 7.47 (m, 2H), 7.15 (d, 1H), 6.61 (s, 1H, NH), 5.29 (s, 1 H, NH), 5.11 (s, 1H, NH), 3.18 (m, 2H, CH_2), 2.94 (m, 4H, CH_2), 2.82 (s, 6H, CH_3), 2.65 (m, 2H, CH_2). Anal. Calcd for $C_{16}H_{23}ClN_4O_2PtS\cdot0.5CH_3OH$: C, 34.08; H, 4.31; N, 9.59. Found: C, 34.47; H, 4.52; N, 9.81.

Synthesis of Pt(DNSH-dien)Br (3). A suspension of *cis*-Pt$(Me_2SO)_2Br_2$ (178 mg, 0.355 mmol) in methanol (20 mL) was treated with DNSH-dienH (119 mg, 0.355 mmol) and triethylamine (40 μL, 0.293 mmol), and the reaction mixture was stirred at room temperature for 18 h. The pale yellow solid that precipitated was collected on a filter, washed with diethyl ether, and dried under vacuum; yield, 91 mg (42%). ^{1}H NMR spectrum (DMSO-d_6) (ppm): 8.62 (d, 1H), 8.38 (d, 1H), 8.27 (d, 1H), 7,49 (t, 1H), 7.44 (t, 1H), 7.17 (d, 1H), 6.64 (s, 1H, NH), 5.32 (s, 1H, NH), 5.14 (s, 1H, NH), 3.29 (d, 2H, CH_2), 3.05 (m, 4H, CH_2), 2.81 (s, 6H, CH_3), 2.65 (m, 2H, CH_2). Anal. Calcd for $C_{16}H_{23}BrN_4O_2PtS\cdot H_2O$: C, 30.58; H, 4.01; N, 8.92. Found: C, 30.64; H, 3.81; N, 8.79.

Synthesis of Pt(DNS-dien) (4). Pt(DNS-dien) was synthesized as described for complex **2**, but with a 10-fold excess of triethylamine added (500 μL, 3.66 mmol). Yield of yellow solid, 136 mg (72%). ^{1}H NMR spectrum (DMSO-d_6) (ppm): 8.27 (d, 1H), 7.98 (d, 1H), 7.36 (t, 1H), 7.22 (d, 1H), 6.81 (d, 1H), 5.72 (s, 1H, NH), 5.53 (s, 1H, NH), 5.42 (s, 1H, NH), 3.64 (m, 2H, CH_2), 3.16 (m, 2H CH_2), 2.84 (m, 2H, CH_2), 2.71 (s, 6H, CH_3), 2.64 (m, 2H, CH_2). Anal. Calcd for $C_{16}H_{22}N_4O_2PtS\cdot0.5CH_3OH$: C, 36.41; H, 4.43; N, 10.25. Found: C, 36.41; H, 4.07; N 10.11.

Synthesis of [Pt(DNSH-tren)Cl]Cl (5). A suspension of *cis*-Pt$(Me_2SO)_2Cl_2$ (200 mg, 0.474 mmol) in methanol (20 mL) was treated with DNSH-tren (180 mg, 0.474 mmol), and the reaction mixture was heated at reflux for 1 h. The pale yellow solid that precipitated after reducing the volume to 5 mL was collected on a filter, washed with water and diethyl ether, and dried in vacuo; yield, 105 mg (35%). ^{1}H NMR spectrum (DMSO-d_6) (ppm): 8.48 (d, 1H), 8.24 (t, 1H, NH), 8.23 (d, 1H), 8.13 (d, 1H), 7.65 (m, 1H), 7.26 (d, 1H), 5.66 (s, 1H, NH), 5.37 (s, 2H, NH), 3.46 (t, 2H, CH_2), 3.07 (m, 2H, CH_2), 2.76 (m, 4H, CH_2), 2.82 (s, 6H, CH_3). Anal. Calcd for $C_{18}H_{29}Cl_2N_5O_2PtS\cdot2H_2O$: C, 31.72; H, 4.88; N, 10.28. Found: C, 31.74; H, 4.74; N, 9.99.

Formation of Biological Adducts. Solutions to study formation of adducts (5 mM in Pt) utilized D_2O/DMSO-d_6 (500 μL/100 μL) solutions. DMSO-d_6 was used to improve solubility; however, solutions of both Pt(DNSH-dien)Cl and [Pt(DNSH-tren)Cl]Cl with a higher percentage of water were readily prepared (e.g., 5 mM in 95% H_2O and 0.1 mM in 99.9% H_2O).

G/3′-IMP Reactions. Solutions of adducts (5 mM) were prepared by treatment of Pt(DNSH-dien)Cl or [Pt(DNSH-tren)Cl]Cl in D_2O/DMSO-d_6 (500 μL/100 μL) with a stoichiometric amount of a **G** derivative or 3′-IMP at pH ≈ 5 (pH was uncorrected). Reactions were monitored by ^{1}H NMR spectroscopy until the disappearance of free **G**/3′-IMP was indicated or until a constant intensity ratio between free and complexed **G**/3′-IMP was attained. When necessary, DNO_3 and NaOD solutions (0.01 M in D_2O) were used for adjusting the pH of each sample directly in the NMR tubes. 1D and 2D NMR studies were performed at 25 °C. Methanol-d_4 was added to the samples for low-temperature experiments.

Methionine Reactions. We chose conditions, such as pH, and ratios to mimic those reported previously for the parent compound, [Pt(dien)Cl]Cl.[8]

Challenge Reactions. Solutions of Pt(DNSH-dien)Cl or [Pt(DNSH-tren)Cl]Cl in D_2O/DMSO-d_6 (500 μL/100 μL) were treated with a stoichiometric amount of met at pH ≈ 6.4. Reactions were monitored by ^{1}H NMR spectroscopy until the disappearance of free met was indicated or until a constant ratio between free and complexed met was attained. Then, 1 equiv of 5′-GMP (final ratio of Pt/met/5′-GMP = 1:1:3) was added. The complementary challenge reaction, addition of 1 equiv of met to a solution of the Pt(DNSH-dien)(5′-GMP) adduct, was also investigated (final ratio of Pt/met/5′-GMP = 1:1:3).

Competition Reactions. Solutions of Pt(DNSH-dien)Cl and [Pt(DNSH-tren)Cl]Cl in D_2O/DMSO-d_6 (500 μL/100 μL) were treated with met and 5′-GMP at pH ≈ 6.4 (Pt/met/5′-GMP = 1:1:3) and the reactions monitored by ^{1}H NMR spectroscopy.

Results

Pt(DNSH-dienH)Cl$_2$ (1). Reaction of *cis*-Pt$(Me_2SO)_2Cl_2$ with DNSH-dienH with no added base affords Pt(DNSH-dienH)Cl_2 (**1**) (Figure 2). Immediately upon dissolution of **1** in DMSO-d_6, one major and one minor set of NH signals were observed. The minor set, which disappeared after ∼5

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Table 1. ^{1}H NMR and ^{195}Pt NMR Shifts (ppm) for Selected Ligands and Complexes[a]

	ligands[b]		Pt(II) complex[c]									
	DNSH-dienH	DNSH-tren	**1**	**1-sol**	**2**	**2-sol**	**3**	**4**	**5**	**5-sol**	**6**	**6-sol**
						^{195}Pt NMR						
				−3318	−2499	−3222		−3235	−2634	−3364	−2721	−3465
						^{1}H NMR						
NMe_2	2.83	2.82	2.83	2.83	2.82	2.82	2.81	2.71	2.82	2.82		
N1H			5.23	6.15	5.11	5.97	5.14	5.42	5.37	6.31	5.43	6.55
			5.34		5.29	6.34	5.32	5.53	5.66	5.95	5.51	6.66
N2H			6.27	6.98	6.61	7.89	6.64	5.72			7.34	8.39
N3H			8.05	8.22					8.24	8.26		
H2	8.11	8.11	8.13	8.13	8.27	8.25	8.27	7.98	8.13	8.13		
H3	7.59	7.59	7.61	7.61	7.47	7.61	7.44	7.22	7.65	7.65		
H4	8.29	8.30	8.27	8.27	8.39	8.40	8.38	8.27	8.23	8.23		
H6	7.25	7.25	7.25	7.25	7.15	7.24	7.17	6.81	7.26	7.26		
H7	7.61	7.61	7.61	7.61	7.47	7.61	7.49	7.36	7.65	7.65		
H8	8.46	8.45	8.48	8.48	8.60	8.50	8.62		8.48	8.48		

[a] 10 mM, DMSO-d_6, 25 °C. [b] Chemical shifts of NH signals of protonated ligands, DNSH-dienH: 7.98 (N1H), 8.74 (N2H), 8.30 (N3H) and DNSH-tren: 8.01 (N1H), 8.37 (N3H). [c] Pt(DNSH-dienH)Cl_2 (**1**), [Pt(DNSH-dienH)(Me_2SO-d_6)Cl]$^+$ (**1-sol**), Pt(DNSH-dien)Cl (**2**), [Pt(DNSH-dien)(Me_2SO-d_6)]$^+$ (**2-sol**), Pt(DNSH-dien)Br (**3**), Pt(DNS-dien) (**4**), [Pt(DNSH-tren)Cl]Cl (**5**), [Pt(DNSH-tren)(Me_2SO-d_6)]$^{2+}$ (**5-sol**), [Pt(dien)Cl]Cl (**6**), and [Pt(dien)(Me_2SO-d_6)]$^{2+}$ (**6-sol**).

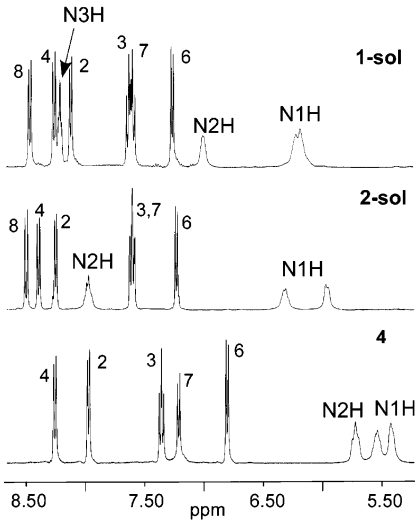

Figure 3. Aromatic ^{1}H NMR signals of the Pt(II) complexes formed by DNSH-dienH ligand; in DMSO-d_6, [Pt(DNSH-dienH)(Me_2SO-d_6)Cl]$^+$ (**1-sol**), [Pt(DNSH-dien)(Me_2SO-d_6)]$^+$ (**2-sol**), Pt(DNS-dien) (**4**).

min but reappeared on addition of $[Et_4N]Cl$ (not shown), was assigned to **1**, and the major set was assigned to the solvolysis product, [Pt(DNSH-dienH)(Me_2SO-d_6)Cl]$^+$ (**1-sol**), on the basis of its ^{195}Pt NMR chemical shift (−3318 ppm), which was very similar to that of [Pt(en)(Me_2SO-d_6)Cl]$^+$ (−3307 ppm).[23] NMR data are summarized in Table 1. The ^{1}H NMR aromatic signals of [Pt(DNSH-dienH)(Me_2SO-d_6)-Cl]$^+$ in DMSO-d_6 (Figure 3) compared to those of DNSH-dienH are not significantly affected by Pt binding to the DNSH-dienH ligand. The ^{1}H NMR NH signals of DNSH-dienH cannot be observed in DMSO-d_6 unless DNO_3 is added. We attribute this result to fast NH exchange. However, once the ligand binds to Pt(II) through N1 and N2 (for numbering, refer to Figure 1), the NH signals appear. We attribute this result to slower NH exchange for the coordinated ligand. The presence of the sulfonamide N3H signal (broad triplet, J_{H-H} = 5.3 Hz) is an indication that N1 and N2 participate in the binding to Pt(II) but N3 does not. For N3 to bind Pt(II), it must be deprotonated. Thus, the NH signals of the DNSH-dienH ligand provide very useful information for assessing the formation of the Pt-(DNSH-dienH)Cl_2 complex.

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Table 2. ^{13}C NMR Chemical Shifts (ppm) for [Pt(DNSH-dienH)(Me_2SO-d_6)Cl]$^+$ (**1-sol**), [Pt(DNSH-dien)(Me_2SO-d_6)]$^+$ (**2-sol**), and Pt(DNS-dien) (**4**) Complexes in DMSO-d_6 (10 mM, 25 °C)

	Pt(II) complex		
	1-sol	**2-sol**	**4**
NMe_2	45.1	45.1	45.3
C1	135.4	137.2	141.7
C2	127.9	127.0	121.6
C3	123.5	123.6	121.8
C4	129.0	129.1	126.3
C5	151.2	151.1	145.8
C6	115.2	114.9	114.0
C7	128.5	127.3	128.4
C8	119.0	119.9	126.8
C9	129.1	129.6	137.0
C10	129.8	128.3	128.9

The combined use of ^{1}H−^{1}H COSY and ROESY NMR experiments allowed assignment of all the aromatic signals. In particular, two sets of coupled signals have been identified from the COSY spectrum, corresponding to the DNSH H2, H3, and H4 protons and H6, H7, and H8 protons (for numbering, refer to Figure 1). The ROESY cross-peaks between the dimethylamino signal and two aromatic signals allowed identification of the H4 and H6 signals and, from these, of the other signals. ^{1}H−^{13}C HMBC and HSQC NMR experiments were used for ^{13}C NMR signal assignment (Table 2). From the ^{1}H−^{13}C HSQC NMR experiment, the signals of the carbons bearing a proton were identified easily because the proton spectrum was already assigned. The HMBC cross-peak between the proton signal of the DNSH dimethylamino group with the C5 signal was the key in assigning the rest of the peaks.

[Pt(DNSH-dien)(Me_2SO-d_6)]$^+$ (2-sol). Soon after Pt-(DNSH-dien)Cl (**2**) (Figure 2) was dissolved in DMSO-d_6, two sets of ^{1}H NMR signals were observed. After ∼2 h, one set of signals disappeared. This set reappeared when $[Et_4N]Cl$ was added (not shown). The two sets are thus

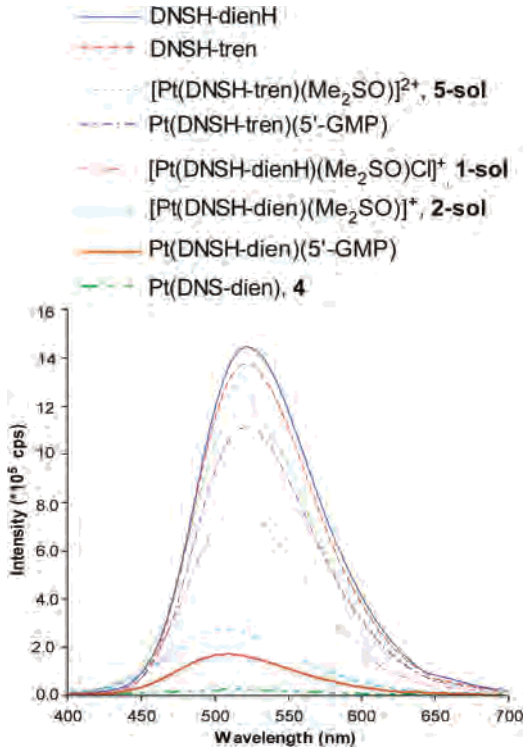

Figure 4. Fluorescence emission spectra of the DNSH-dienH and DNSH-tren ligands and their Pt(II) complexes.

clearly from the Pt(DNSH-dien)Cl complex (**2**) and the solvolysis product, [Pt(DNSH-dien)(Me_2SO-d_6)]$^+$ (**2-sol**). The latter predominates and is almost the exclusive species present in the absence of added chloride salt. The data presented here are for [Pt(DNSH-dien)(Me_2SO-d_6)]$^+$ (Figure 3). ^{1}H and ^{13}C NMR spectral assignments, summarized in Tables 1 and 2, were made in the same way as for [Pt(DNSH-dienH)(Me_2SO-d_6)Cl]$^+$ (**1-sol**). The most significant difference in the spectra of **2-sol** (with a tridentate ligand) and **1-sol** (with a bidentate ligand) is the absence of the sulfonamide N3H signal for **2-sol**. For the [Pt(DNSH-dien)(Me_2SO-d_6)]$^+$ cation, the most upfield aromatic ^{13}C NMR signal is C6 (114.9 ppm). An H8−C6 cross-peak is present in the HMBC spectra of [Pt(DNSH-dien)(Me_2SO-d_6)]$^+$ and [Pt(DNSH-dienH)(Me_2SO-d_6)Cl]$^+$ (not shown). In aqueous/DMSO-d_6 (500 μL/100 μL), **2** immediately gave evidence for a solvolysis product; careful studies varying solvent composition indicate this species, which is involved in the **G**/3′-IMP reactions and the 5′-GMP/met competition reactions, is [Pt(DNSH-dien)(Me_2SO-d_6)]$^+$ (**2-sol**). When **2-sol** was heated at 70 °C for 14 h in D_2O/DMSO-d_6 (500 μL/100 μL), the H8 DNSH signal disappeared and H7 became a doublet; an incipient AB pattern between H7 and H6 signals was observed. The fluorescence emission spectrum of **2-sol** is less intense than that of **1-sol**, Figure 4.

Pt(DNS-dien) (4). This product (**4**), formed under the most basic conditions used, has five of the six aromatic ^{1}H NMR signals expected (Figure 3), providing evidence that **4** is Pt(DNS-dien) (Figure 2). Two sets of coupled signals were identified by the COSY spectrum (Figure 5A), corresponding to the DNS protons, H2, H3, and H4 of one ring, and H6 and H7 of the ring containing the dimethylamino group. In

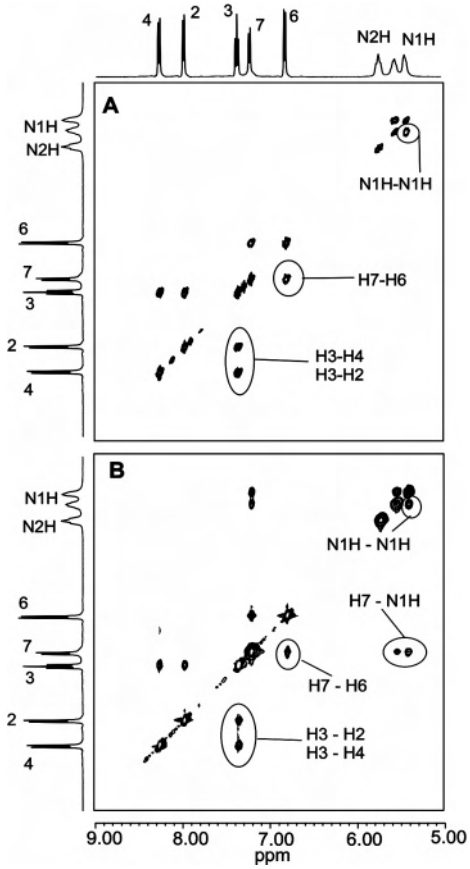

Figure 5. (A) ^{1}H−^{1}H COSY spectrum of Pt(DNS-dien) in DMSO-d_6 at 298 K. (B) ^{1}H−^{1}H ROESY spectrum of Pt(DNS-dien) in DMSO-d_6 at 298 K.

the ROESY experiment (Figure 5B), cross-peaks were observed between the H7 signal and the two N1H signals of the dien moiety. Also, the expected H6−H7, H2−H3, and H3−H4 cross-peaks were observed. All aromatic signals were upfield (Table 1) compared to the corresponding signals of [Pt(DNSH-dien)(Me_2SO-d_6)]$^+$ (**2-sol**). Of note, the H7 signal is coupled to ^{195}Pt (J_{Pt-H} = 22 Hz).

^{1}H−^{13}C HSQC and HMBC NMR experiments were used to assign the ^{13}C NMR spectrum (Table 2) and to confirm the structure of **4**. For Pt(DNS-dien), three ^{1}H−^{13}C HMBC experiments were carried out with different J_{H-C} values (6.25, 8, and 10 Hz), but none of the spectra exhibited an H8−C6 cross-peak as found in ^{1}H−^{13}C HMBC experiments (J_{H-C} = 8 Hz) with [Pt(DNSH-dien)(Me_2SO-d_6)]$^+$ and [Pt(DNSH-dienH)(Me_2SO-d_6)Cl]$^+$.

[Pt(DNSH-tren)Cl]Cl (5). [Pt(DNSH-tren)Cl]Cl (**5**) (Figure 2) was prepared by treating *cis*-Pt(Me_2SO)$_2$Cl$_2$ with DNSH-tren. In DMSO-d_6, the ^{1}H NMR aromatic signals of the DNSH group are not significantly affected by Pt binding (Table 1). Upon dissolution in DMSO-d_6, the signals of **5** decreased slightly and a downfield set of NH ^{1}H NMR signals for [Pt(DNSH-tren)(Me_2SO-d_6)]$^{2+}$ (**5-sol**) increased. However, only ~10% of [Pt(DNSH-tren)(Me_2SO-d_6)]$^{2+}$ (**5-sol**) formed. In contrast, when **5** was dissolved in a mixture of aqueous/DMSO-d_6 (500 μL/100 μL, pH = 6.3), **5-sol** formed to a much greater extent (~85% after 2 h). **5-sol** was the reactive species present in the **G**/3′-IMP reactions and the 5′-GMP/met competition reaction.

Table 3. ^{1}H NMR Chemical Shifts (ppm) for the **G**/3′-IMP moeity of Pt(DNSH-dien)(**G**/3′-IMP) and Pt(DNSH-tren)(**G**/3′-IMP) Adducts in D_2O/DMSO-d_6 500 μL/100 μL, 25 °C

G/3′-IMP	pH	free H8 (H2)	H8 (H2)	free H1′	H1′
		Pt(DNSH-tren)(**G**/3′-IMP)			
5′-GMP	6.32	8.09	8.61	5.89	5.96
3′-GMP	6.32	8.00	8.40	5.90	5.94
3′-IMP	6.26	8.38 (8.23)	8.83 (8.30)	6.12	6.17
		Pt(DNSH-dien)(**G**/3′-IMP)			
9-EtG	5.23	8.01	8.11		
5′-GMP	5.21	8.11	8.27 8.25	5.87	5.69
3′-GMP	5.20	8.04	8.30 8.28	5.91	5.81
5′-GDP	5.25	8.14	8.30 8.28	5.90	5.69
5′-GTP	5.17	8.15	8.30 8.27	5.89	5.68
3′-IMP	5.23	8.39 (8.22)	8.72 (8.10) 8.69 (8.09)	6.13	6.01

Table 4. ^{1}H NMR Chemical Shifts (ppm) of the DNSH Moiety of $[Pt(DNSH\text{-}tren)(Me_2SO\text{-}d_6)]^{2+}$ (**5-sol**), $[Pt(DNSH\text{-}dien)(Me_2SO\text{-}d_6)]^{+}$ (**2-sol**), and Pt(DNSH-dien)(**G**/3′-IMP) in D_2O/DMSO-d_6 (500 μL/100 μL, 25 °C)

	5-sol	**2-sol**	Pt(DNSH-dien)(**G**/3′-IMP)			
DNSH signals			9-EtG	3′-GMP	5′-GMP	3′-IMP
pH	6.37	5.21	5.23	5.20	5.21	5.23
NMe_2	2.92	2.94	2.94	2.93	2.94	2.93
H2	8.28	8.40	8.10	8.01	8.03	8.00
H3	7.74	7.78	7.48	7.48	7.48	7.45
H4	8.57	8.52	8.22	8.19	8.20	8.19
H6	7.49	7.49	7.43	7.43	7.45	7.54
H7	7.78	7.78	7.63	7.66	7.65	7.70
H8	8.32	8.67	8.30	8.27	8.26	8.27

Pt(DNSH-dien)(G/3′-IMP) and Pt(DNSH-tren)(G/3′-IMP) Adducts. The solvent used for all adducts was D_2O/DMSO-d_6, 500 μL/100 μL, unless otherwise specified. For low-temperature experiments, the solvent mixture was D_2O/DMSO-d_6/methanol-d_4 (500 μL/100 μL/100 μL).

Pt(DNSH-tren)(G/3′-IMP) (G = 3′-GMP, 5′-GMP). The Pt(DNSH-tren)(3′-GMP) adduct (pH = 6.32) exhibited one H8 and one H1′ NMR signal downfield of the respective signal of free 3′-GMP (Table 3). The aromatic signals of the DNSH group were not shifted by 3′-GMP binding (Table 4).

Results similar to those for Pt(DNSH-tren)(3′-GMP) were observed for the Pt(DNSH-tren)(5′-GMP) adduct. For Pt(DNSH-tren)(3′-IMP), the resonances of both H8 and H2 shifted downfield of the corresponding resonances of free 3′-IMP. Selected ^{1}H NMR shifts for both free and bound **G**/3′-IMP in Pt(DNSH-tren)(**G**/3′-IMP) adducts are listed in Table 3.

Pt(DNSH-tren)(met-*S*)]$^{2+}$. We studied the spectrum of the $[Pt(DNSH\text{-}tren)(met\text{-}S)]^{2+}$ adduct (pH = 6.4, at which met has a neutral overall charge), which exhibited a sharp (fwhm = 3 Hz) S-methyl NMR signal (2.51 ppm) downfield of the S-methyl signal of free met (2.13 ppm). The α-CH signal of $[Pt(DNSH\text{-}tren)(met\text{-}S)]^{2+}$ was downfield (3.90 ppm) of that of free met (3.82 ppm). The aromatic and the dimethylamino DNSH signals were not shifted.

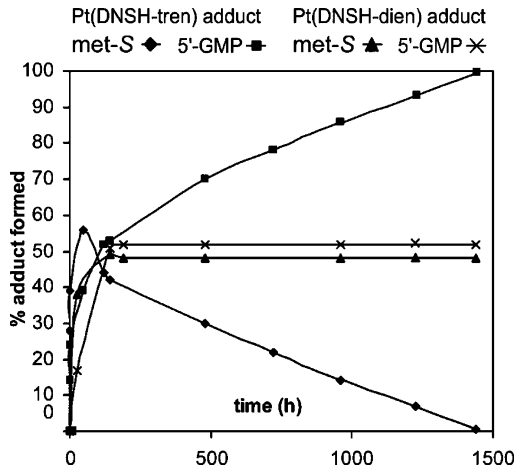

Figure 6. Time courses of competition reactions of $[Pt(DNSH\text{-}tren)(Me_2SO\text{-}d_6)]^{2+}$ (**5-sol**) and $[Pt(DNSH\text{-}dien)(Me_2SO\text{-}d_6)]+$ (**2-sol**) with met and 5′-GMP.

Challenge Reaction. A few hours after addition of 3 equiv of 5′-GMP to the solution of the $[Pt(DNSH\text{-}tren)(met\text{-}S)]^{2+}$ cation, the NMR signals of the bound met decreased and signals of the Pt(DNSH-tren)(5′-GMP) adduct appeared. After 20 and 40 days, respectively, the Pt(DNSH-tren)(5′-GMP)/ $[Pt(DNSH\text{-}tren)(met\text{-}S)]^{2+}$product distribution was 70:30 and 100:0 (no signals for the met adduct remained).

Competition Reaction. Figure 6 describes the time course of the competition reaction of **5-sol** with met and 5′-GMP (1:1:3). In the initial stages of the competition reaction, the peaks of free met decreased in size and the peaks of the $[Pt(DNSH\text{-}tren)(met\text{-}S)]^{2+}$ adduct appeared, while only a small amount of the Pt(DNSH-tren)(5′-GMP) adduct was observed (Figure 6). In later stages, the signals of $[Pt(DNSH\text{-}tren)(met\text{-}S)]^{2+}$ began decreasing and those of Pt(DNSH-tren)(5′-GMP) increased. Finally, after about two months, only Pt(DNSH-tren)(5′-GMP) signals could be detected.

[Pt(DNSH-dien)(9-EtG)]$^+$. The $[Pt(DNSH\text{-}dien)(9\text{-}EtG)]^{+}$ adduct (pH = 5.23), prepared as described above, showed one set of ^{1}H NMR signals. Compared to free 9-EtG, the H8 signal was downfield (Table 3), but the CH_3 and CH_2 signals of the ethyl group were shifted upfield to 1.17 from 1.48 ppm and to 3.92 from 4.11 ppm, respectively. The DNSH signals of the adduct were upfield of the signals of $[Pt(DNSH\text{-}dien)(Me_2SO\text{-}d_6)]^{+}$ (Table 4). A spectrum recorded at −5 °C showed no broadening of the H8 signal.

Pt(DNSH-dien)(3′-GMP). For Pt(DNSH-dien)(3′-GMP) (pH = 5.20), two aromatic singlets of similar intensity (Figure 7) integrate to a total of one proton. These singlets appear downfield of the H8 signal of free 3′-GMP and are assigned to H8 of bound 3′-GMP by two NOE cross-peaks to the H1′ signal.[24] There was one H1′ signal for the adduct, upfield of the H1′ signal of free 3′-GMP (Table 3). Also, only one set of DNSH aromatic signals was observed, upfield of the corresponding signals of the starting complex (Table

(24) Tate, S.-i.; Ono, A.; Kainosho, M. *J. Am. Chem. Soc.* **1994**, *116*, 5977−5978.

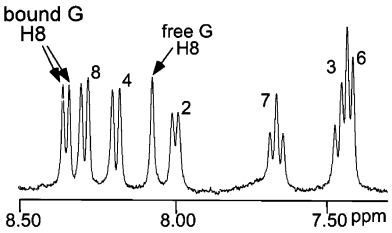

Figure 7. Aromatic region of the ^{1}H NMR spectrum of the Pt(DNSH-dien)(3′-GMP) adduct.

4). Spectra were also recorded from −5 to 65 °C in 5 °C intervals. The spectra recorded below 25 °C exhibited two well-resolved 3′-GMP H8 signals, downfield from the H8 doublet for the DNSH group. In spectra measured above 25 °C, these signals began to overlap because the DNSH H8 doublet shifted downfield. Also, the H2 doublet and H3 triplet of the DNSH group each shifted downfield and in some cases the spectra indicated that these were in fact two very closely spaced overlapping signals (of roughly equal size).

Pt(DNSH-dien)(G/3′-IMP) (G = 5′-GMP, 5′-GDP, 5′-GTP). The ^{1}H NMR spectra of the other Pt(DNSH-dien)(**G**) adducts were similar to that of Pt(DNSH-dien)(3′-GMP) (Tables 3 and 4). The Pt(DNSH-dien)(3′-IMP) adduct gave two upfield-shifted H2 signals in addition to the two downfield-shifted H8 signals and one upfield H1′signal, as observed for the **G** adducts (Table 3).

Pt(DNSH-dien)(met-*S*). The $[Pt(DNSH\text{-}dien)(met\text{-}S)]^+$ adduct in D_2O/DMSO-d_6 has a broad S-methyl signal (fwhm = 16 Hz, 2.49 ppm), which was downfield of this signal in the spectrum of free met (2.18 ppm).

Challenge Reaction. No replacement of the bound met or bound 5′-GMP was observed even 60 days after addition of 3 equiv of 5′-GMP to the met adduct or 1 equiv of met to the 5′-GMP adduct, respectively.

Competition Reaction. $[Pt(DNSH\text{-}dien)(Me_2SO\text{-}d_6)]^+$ was treated with met and 5′-GMP (1:1:3). Roughly equal amounts of $[Pt(DNSH\text{-}dien)(met\text{-}S)]^+$ and Pt(DNSH-dien)(5′-GMP) were formed throughout the course of the reaction (Figure 6).

Discussion

Pt(DNSH-dienH)Cl$_2$ (1). Under the least basic synthetic conditions used, *cis*-$Pt(Me_2SO)_2Cl_2$ and DNSH-dienH formed Pt(DNSH-dienH)Cl_2, with DNSH-dienH acting as a bidentate ligand (central N2 and terminal N1) (Figure 2). One key piece of evidence for this conclusion is the presence of the sulfonamide N3H ^{1}H NMR signal at a shift similar to that of the free DNSH-dienH ligand, as expected for an unbound sulfonamide group because N3H must deprotonate prior to Pt binding. Pt(en)Cl_2 and Pt(*N*-Et-en)Cl_2 (*N*-Et-en = *N*-ethylethylenediamine), when dissolved in DMSO-d_6, give the monosolvated species, in which only one Cl ligand is replaced by DMSO.[23,25] ^{1}H NMR data indicate that DMSO replaces one of the two chlorides of Pt(*N*-Et-en)Cl_2 more readily than the other one. The ^{195}Pt NMR shifts for Pt(en)Cl_2 and Pt(*N*-Et-en)Cl_2 are −2345 and −2364 ppm, respectively, whereas these are −3307 and −3325 ppm for $[Pt(en)(Me_2SO\text{-}d_6)Cl]^+$ [23] and $[Pt(N\text{-Et-en})(Me_2SO\text{-}d_6)Cl]^+$, respectively. $[Pt(en)(Me_2SO\text{-}d_6)_2]^{2+}$ has a ^{195}Pt NMR chemical shift of −3650 ppm.[23] The ^{195}Pt NMR shifts of these monosolvated complexes are very close to the value (−3318 ppm) obtained for $[Pt(DNSH\text{-}dienH)(Me_2SO\text{-}d_6)Cl]^+$, thus indicating that in DMSO-d_6 only one Cl has been replaced to form $[Pt(DNSH\text{-}dienH)(Me_2SO\text{-}d_6)Cl]^+$ (**1-sol,** Table 1) The insignificant changes in the shifts of the DNSH ^{1}H NMR signals and in fluorescence intensity (see below and Figure 4) indicate that the DNSH moiety occupies a remote position relative to the Pt.

Pt(DNSH-dien)X (X = Cl (2) or Br (3)). The key spectroscopic evidence that the DNSH-dienH ligand acts as a tridentate ligand in Pt(DNSH-dien)X is the absence of the N3H signal indicative of a deprotonated sulfonamide N3, a requirement for a sulfonamide to bind to metals. The Cl and Br ligands are readily displaced in DMSO-d_6 to give $[Pt(DNSH\text{-}dien)(Me_2SO\text{-}d_6)]^+$ (**2-sol**). The behavior of Pt(DNSH-dien)X in DMSO-d_6 is similar to that of [Pt(dien)Cl]Cl. Immediately after dissolution in DMSO-d_6, [Pt(dien)Cl]Cl showed two sets of NH NMR signals. The upfield set, assigned to $[Pt(dien)Cl]^+$, decreased with time, whereas the downfield set, assigned to $[Pt(dien)(Me_2SO\text{-}d_6)]^{2+}$, increased with time, and a ratio of ∼1:1 was observed at equilibrium. The shifts of the central secondary NH signals were more downfield than those of the terminal primary amines in both $[Pt(dien)Cl]^+$ and $[Pt(dien)(Me_2SO\text{-}d_6)]^{2+}$.[26] The same trends were observed in the spectrum of Pt(DNSH-dien)Cl in DMSO-d_6, in which the NH signals of $[Pt(DNSH\text{-}dien)(Me_2SO\text{-}d_6)]^+$ were downfield of those of Pt(DNSH-dien)Cl, and the central N2H signal was downfield from the terminal N1H signals. Comparing the chemical shifts of the NH signals, we observed that all NH signals were shifted upfield for Pt(DNSH-dien)Cl relative to [Pt(dien)Cl]Cl (Table 1). The reason for the upfield position of the shifts is undoubtedly the stronger electron donation to Pt(II) by a sulfonamido group than by a terminal amine group.

Pt(DNS-dien) (4). The DNSH-dienH ligand can act as a quadridentate N_3C ligand (terminal N1, central N2, and sulfonamido N3 of the dien moiety and C8 of the DNS moiety), giving the chelate complex, Pt(DNS-dien) (**4**) (Figure 2). The key evidence for C8 coordination is the missing H8 ^{1}H NMR signal. The upfield-shifted positions of the aromatic and dimethylamino signals of the DNS moiety of **4**, vs the corresponding signals of $[Pt(DNSH\text{-}dien)(Me_2SO\text{-}d_6)]^+$ **(2-sol)**, are also consistent with the formation of a Pt−C8 bond. For example, the H7 signal moved upfield by 0.25 ppm. Furthermore, the coupling to ^{195}Pt of the proton (H7) on the carbon adjacent to the metalated carbon (C8) was $^3J_{Pt-H}$ = 22 Hz. These results are comparable to those observed in related Pt(II) complexes.[27−29] For example,

(25) Fanizzi, F. P.; Intini, F. P.; Maresca, L.; Natile, G. *Inorg. Chem.* **1990**, *29*, 29−33.

(26) Guo, Z. J.; Sadler, P. J.; Zang, E. *Chem. Commun. (Cambridge, U. K.)* **1997**, 27−28.

(27) Brooks, J.; Babayan, Y.; Lamansky, S.; Djurovich, P. I.; Tsyba, I.; Bau, R.; Thompson, M. E. *Inorg. Chem.* **2002**, *41*, 3055−3066.

Carlone et al.[29] found that in a Pt(II) complex with 3-nitro-9-[(2-dialkylaminoethyl)amino]acridine attached by a Pt−C bond the resonance for the proton adjacent to the Pt−C bond shifted upfield by 0.26 ppm and was coupled to ^{195}Pt ($^3J_{Pt-H}$ = 35 Hz).[29] The C8 ^{13}C NMR signal of **4** had shifted downfield from the free ligand signal after formation of the Pt−C bond, as reported for other Pt(II) complexes with a Pt−C bond. (X-ray structural analysis was also used to confirm the formation of the Pt−C bond in several cases).[27,30] The N2H signal of the N_3C complex is relatively upfield (by ∼1.5 ppm) compared to complexes having N,N,N coordination. We believe that this upfield shift is a result of the greater trans influence (compared to a Cl or Me_2SO-d_6 ligand) of the negatively charged C8 atom trans to the N2H.

[Pt(DNSH-tren)Cl]Cl (5). This complex was designed to be a control, with the DNSH group remote from Pt. The similarity of the DNSH signals to that of the free ligand and the presence of the sulfonamide NH signal confirm that the desired structure formed. An unusual feature of this compound is that it did not readily undergo solvolysis in DMSO-d_6 (∼10% [Pt(DNSH-tren)(Me_2SO-d_6)]$^{2+}$ (**5-sol**) formed). The primary amine NH signals of **5** and **5-sol** have shifts very similar to those of [Pt(dien)Cl]Cl (**6**) and [Pt(dien)-(Me_2SO-d_6)]$^{2+}$ (**6-sol**), respectively. Upon dissolution in DMSO-d_6, **6** converted to an ∼1:1 **6**/**6-sol** mixture. [Pt(*N*′-Me-dien)Cl]Cl was used as model for **5** and it was found to undergo solvolysis by only ∼15%. Thus, the presence of an alkyl group on the central N decreases DMSO solvolysis. The shielding effect of Me_2SO-d_6 compared to Cl causes ∼700 ppm upfield ^{195}Pt NMR shifts of the Me_2SO-d_6 solvolysis product relative to the starting [Pt(dien)Cl]$^+$, [Pt-(DNSH-tren)Cl]$^+$, and Pt(DNSH-dien)Cl complexes in DMSO-d_6.

Fluorescence Spectroscopy. The fluorescence intensity of *N*-dansyl-*N*′-ethylthiourea was quenched by 87% after addition of Pt(II).[31] This significant decrease was attributed to the "heavy atom effect."[31] The presence of Pt(II) enhances the rate of the spin-forbidden process (S1 → T1).[32−34] The fluorescence intensity of the DNSH-tren ligand is decreased by only 20% on formation of [Pt(DNSH-tren)(Me_2SO)]$^{2+}$ (Figure 4). We attribute the small degree of the decrease to the remote position of the fluorophore from the Pt. Fluorescence spectra of [Pt(DNSH-dienH)(Me_2SO)Cl]$^+$, [Pt(DNSH-dien)(Me_2SO)]$^+$, and Pt(DNS-dien) showed progressively more significant quenching of the fluorescence intensity compared to that of the DNSH-dienH ligand (Figure 4), probably due to the heavy atom effect[33] of Pt(II). The greater quenching of the fluorescence intensity as the hapticity of the DNSH-dienH ligand is associated with the increased proximity of the dansyl group to the Pt. The high level of quenching of Pt(DNS-dien) relative to [Pt(DNSH-dienH)-(Me_2SO)Cl]$^+$ and [Pt(DNSH-dien)(Me_2SO)]$^+$ may also be attributed to the Pt−C8 bond, which has changed the electronic properties of the fluorophore, in addition to the heavy-atom effect.[33,34] In the Pt(DNSH-dien)(5′-GMP) adduct, the observed additional quenching is attributed to efficient energy transfer from the naphthalene ring to the guanine base.[35] The insignificant additional quenching of the fluorescence intensity of Pt(DNSH-tren)(5′-GMP) relative to that of [Pt(DNSH-tren)(Me_2SO)]$^{2+}$ (**5-sol**) is explained by the remote position of the nucleotide relative to the fluorophore.

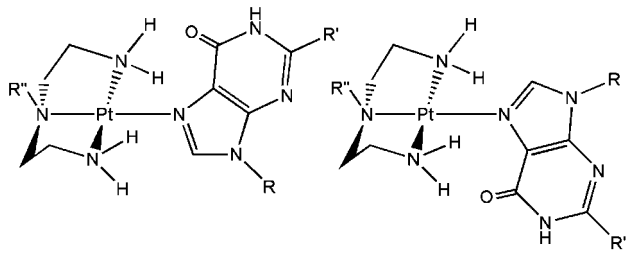

Figure 8. Two 6-oxopurine orientations for Pt complexes with dien-type ligands symmetric about a plane perpendicular to the coordination plane and bisecting the ligand. For the two possible rotamers of Pt(DNSH-tren)-(**G**/3′-IMP) adducts, R is a sugar−phosphate; R′ = H (IMP) or NH_2 (GMP); R″ = $CH_2CH_2NHDNSH$. For dien analogues, R″ = H.

Pt(DNSH-tren)(G/3′-IMP) (G = 3′-GMP, 5′-GMP). The 6-oxopurine nucleobase of **G**/3′-IMP lies with its plane roughly perpendicular to the Pt coordination plane. In a Pt(II) complex possessing a carrier ligand unsymmetrical with respect to the coordination plane but symmetrical about a plane perpendicular to the coordination plane, two 6-oxopurine orientations are possible (Figure 8), and hence, two magnetically inequivalent sets of signals are expected for slow rotation of the 6-oxopurine about the Pt−N7 bond. For Pt(DNSH-tren)(**G**/3′-IMP) adducts, O6 can be on either the same or the opposite side of the coordination plane as the $CH_2CH_2NHDNSH$ substituent. In related cases with tridentate dien-type ligands with both terminal nitrogens substituted, two downfield-shifted H8 signals indicating slow rotation of the two rotamers have been observed.[11,12] The observation of only one downfield-shifted H8 (and one H2 for 3′-IMP) signal and only one H1′ signal for each Pt(DNSH-tren)(**G**/3′-IMP) adduct is attributed to nucleobase binding to Pt via N7 and to rapid rotation around the Pt−N7 bond preventing the observation of separate signals for the two rotamers. For Pt(dien)(**G**) adducts, fast rotation around the Pt−N7 bond is expected because both terminal nitrogens are primary amino groups. Only one H8 signal was observed,[36] as expected. The aromatic signals of the DNSH group were unaffected by **G**/3′-IMP binding to [Pt(DNSH-

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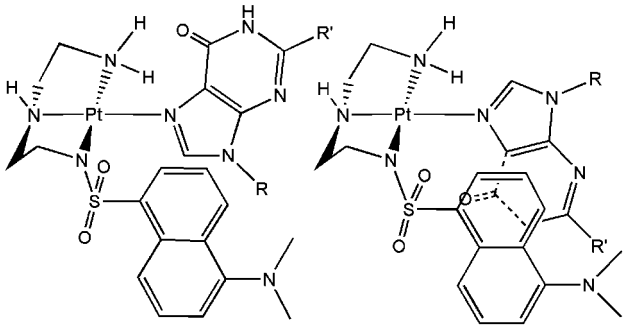

Figure 9. Two of the conceivable rotamers of Pt(DNSH-dien)(**G**/3′−IMP) complexes. Rotamers illustrated have one of the conceivable DNSH orientations and two base orientations with O6 on the same and the opposite side of the proton on the central N2. For 9-EtG (R = ethyl; R′ = NH_2), the mirror image of the adduct is an enantiomer. For nucleotides (R is a sugar−phosphate and R′ = H (IMP) or NH_2 (GMP)) the adducts with the mirror image of the Pt(DNSH-dien) moiety are diastereoisomers of the rotamers shown.

tren)(Me_2SO-d_6)]$^{2+}$ (**5-sol**), most likely because the DNSH group is not close to the bound 6-oxopurine base.

[Pt(DNSH-tren)(met-*S*)]$^{2+}$. The downfield shifts of the S-methyl and CH signals of met on binding to [Pt(DNSH-tren)(Me_2SO-d_6)]$^{2+}$ (**5-sol**) reported above are attributed to S binding to Pt. Similar shifts were reported for [Pt(dien)(met-*S*)]$^{2+}$.[8,37] Thus, the adduct is [Pt(DNSH-tren)(met-*S*)]$^{2+}$.

Challenge Reaction. After addition of 5′-GMP to a [Pt(DNSH-tren)(met-*S*)]$^{2+}$ solution, the met-*S* adduct converted completely to the 5′-GMP adduct. This result was expected because the 5′-GMP adduct is expected to be the thermodynamic product on the basis of challenge studies reported for the [Pt(dien)(met-*S*)]$^{2+}$ adduct.[37]

Competition Reaction. Initially, the [Pt(DNSH-tren)(met-*S*)]$^{2+}$ adduct formed to a much greater extent than the Pt(DNSH-tren)(5′-GMP) adduct in a competition reaction of met and 5′-GMP for [Pt(DNSH-tren)(Me_2SO-d_6)]$^{2+}$ (**5-sol**), indicating that [Pt(DNSH-tren)(met-*S*)]$^{2+}$ was the preferred kinetic product. With time, the N7-bound 5′-GMP (thermodynamic) product formed (Figure 6), as found previously for the [Pt(dien)(met-*S*)]$^{2+}$ and [Pt(dien)(GSMe-*S*)]$^{2+}$ adducts.[6,8,37] At 40 h at the same pH (6.3) and same Pt(II)/met/5′-GMP ratio (1:1:3), **5-sol** formed 55% of the met adduct, while [Pt(dien)(H_2O)]$^{2+}$ formed 42% of the met adduct.[8] These results of intermolecular displacement support the previous findings that the Pt−S (thioether) bond is the kinetically favored bond type, whereas the Pt−N7 bond is the thermodynamically favored bond type.[8] Because the dansyl fluorophore of **5-sol** is positioned away from the Pt(II) binding site, its presence did not affect the reactivity or selectivity toward N-bearing (GMP) or S-bearing (met) biological targets of Pt(II).

Possible Rotamers for the Pt(DNSH-dien)(G/3′-IMP) Adducts. Although the central N2 is not substituted as in **5**, the central N2H atom breaks the symmetry of the Pt coordination plane in Pt(DNSH-dien)(**G**/3′-IMP) adducts. Therefore, the nucleobase, which is perpendicular to the plane, has two orientations (Figure 9). Also, the DNSH group has two possible orientations, with the dimethylamino group on the same or the opposite side of the coordination plane as the central N2H. Four rotamers are conceivable for the Pt(DNSH-dien)(**G**/3′-IMP) adduct illustrated. However, the DNSH group normally exhibits fast rotation around the C−sulfonamide bond,[38] leading to averaging of the signals of the different rotamers involving the DNSH group. Thus, for slow Pt−N7 rotation, two rotamers at most can be detected on the NMR time scale for each of the two Pt(DNSH-dien) chiralities.

[Pt(DNSH-dien)(9-EtG)]$^{+}$. [Pt(DNSH-dien)(9-EtG)]$^{+}$ has only one 9-EtG H8 signal, which showed no broadening even in a spectrum recorded at −5 °C, indicating that rotation around the Pt−N7 bond is too fast to allow observation of separate signals for the two rotamers.[39] Usually, when 9-EtG binds to Pt(II), the Et group signals move downfield;[40] the upfield-shifted Et signals of [Pt(DNSH-dien)(9-EtG)]$^{+}$ are undoubtedly due to the proximity of the 9-EtG to the anisotropic DNSH moiety.

Only one H8 signal was observed for the [Pt(DNSH-dien)(9-EtG)]$^{+}$ adduct. For **G** adducts, the presence of the sugar−phosphate group is known to slow rotation.[12,39] Because the rotation around the Pt−N7 bond in [Pt(DNSH-dien)(9-EtG)]$^{+}$ could be faster than in nucleotide adducts, we investigated Pt(DNSH-dien)(nucleotide) adducts to determine if the presence of the bulky DNSH moiety on one side of Pt(II) binding site could slow the rotation enough to allow the observation of H8 signals of more than one rotamer.

Pt(DNSH-dien)(G/3′-IMP) (G = 5′-GMP, 5′-GDP, 5′-GTP). The results for these Pt(DNSH-dien) 6-oxopurine nucleotide adducts were similar, and we shall refer mainly to the results obtained for the 3′-GMP adduct. Two H8 signals of approximately equal intensity were observed at 25 °C, as expected if the adducts for each Pt(DNSH-dien) chirality are formed to a comparable extent and if for each Pt(DNSH-dien) chirality the two 6-oxopurine rotamers rotate rapidly on the NMR time scale about the Pt−N7 bond. A previous study of the Pt(*N*-Me_3dien)(**G**) (**G** = 3′-GMP, 5′-GMP) adducts showed broad peaks at room temperature, and low temperature was necessary for the rotation to be slow enough to give sharp NMR signals for the two rotamers.[12] Apparently, the presence of the bulkier DNSH moiety on one side of Pt(II) binding site cannot slow the rotation enough to allow the observation of more than one rotamer (for a 6-oxopurine nucleotide) at room temperature.

The aromatic DNSH and H1′ signals of the Pt(DNSH-dien)(3′-GMP) adduct shifted upfield when compared to those of the starting [Pt(DNSH-dien)(Me_2SO-d_6)]$^{+}$ complex and free 3′-GMP, respectively (Tables 3 and 4). These upfield shifts of the aromatic signals of the DNSH group and of the H1′ signal upon adduct formation are consistent with the proximity of the 3′-GMP to the DNSH group. On binding of the

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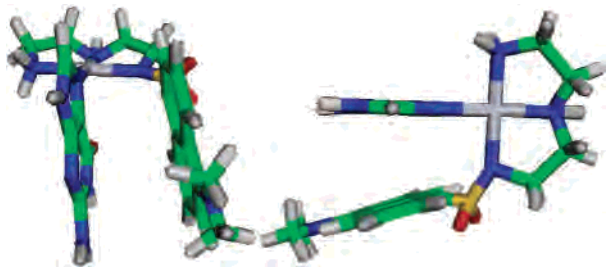

Figure 10. Proposed stacking interaction between the **G**/3′-IMP base and the DNSH moiety viewed along (left) and from the top (right) of the coordination plane. The average separation of the dansyl and the guanine planes is 3.6 Å.

6-oxopurine derivatives to Pt, the 3′-IMP H2 signals are shifted upfield (in contrast to the typical slight downfield shift) and the purine H8 signals are shifted downfield by about 0.3 ppm (an amount that is less than that for Pt(DNSH-tren)(**G**/3′-IMP) (Table 3) and less than the typical 0.5−0.7 ppm).[36,37]

The DNSH aromatic group is attached to the Pt(II) through a sulfonamido group, which keeps the naphthalene ring in proximity to the nucleobase. The observation of the relatively upfield shifts does not necessarily establish a stacking interaction (Figure 10). However, there is evidence for purine−aromatic group stacking in Pt(DNSH-dien)(**G**) adducts. As the temperature was increased from below room temperature to 65 °C, the DNSH moiety signals shifted downfield. This result is expected if the upfield shifting at low temperature is in part due to stacking because the average proximity of the two groups, which is fixed by the Pt−N bonds, would not change with temperature in the absence of stacking at low temperature. When Heetebrij et al.[41] treated [Pt(en)($NH_2(CH_2)_6$NH-dinitrophenyl)Cl]Cl (a monofunctional complex) with 5′-GMP, they observed that the dinitrophenyl group signals shifted upfield, which they attributed to a stacking interaction between the guanine base of 5′-GMP and the dinitrophenyl group. Stacking is possible because of the long, flexible $(CH_2)_6$ chain. Also, the 5′-GMP adduct exhibited only one downfield 5′-GMP H8 signal, indicating rapid rotation about the Pt−N7 bond. In this regard, this adduct is similar to the **G** adducts formed by [Pt(dien)Cl]Cl[6,36,37] and by Pt(DNSH-dien)Cl. The 5′-GMP H8 signal of the Pt(en)($NH_2(CH_2)_6$NH-dinitrophenyl)(5′-GMP) adduct had a typical downfield shift. The aromatic group is attached by a flexible $(CH_2)_6$ chain and apparently may not approach the purine base as closely as the dansyl group does in the adducts reported here.

[Pt(DNSH-dien)(met-*S*)]$^+$. The downfield shifts of the S-methyl signal of the [Pt(DNSH-dien)(met-*S*)]$^+$ adduct are attributed to binding of Pt(II) to the S of met.[37] When met binds both through S and N, the rate of inversion at S decreased enough that broadening can be observed in NMR spectra.[42] A slightly later report concluded that, in the [Pt(dien)(met-*S*)]$^{2+}$ adduct, where met is bound only through S, the inversion is fast on the NMR scale and therefore broadening of the S-methyl signal was not observed.[37] Our finding that the S-methyl signal for the [Pt(DNSH-tren)(met-*S*)]$^{2+}$ adduct is sharp appears to support this suggestion. However, if the S of the monodentate met of the [Pt(DNSH-dien)(met-*S*)]$^+$ adduct inverted rapidly, we should observe two sharp S-methyl signals, one for each Pt(DNSH-dien) chirality. If these signals overlapped, the overlapped signal should be sharp. The broad S-methyl signal of the [Pt(DNSH-dien)(met-*S*)]$^+$ adduct suggests a possible alternative explanation for the sharp S-methyl signal of [Pt(dien)(met-*S*)]$^{2+}$: the difference in shifts for the two [Pt(dien)(met-*S*)]$^{2+}$ diastereoisomers is too small to cause broadening of the S-methyl signal. If this explanation holds, then the rate of inversion at S cannot be assessed by ^{1}H NMR methods when the two cis coordination environments are too similar.

Challenge Reactions. No replacement of the bound monodentate ligand was observed after addition of 5′-GMP or met to a solution of the [Pt(DNSH-dien)(met-*S*)]$^+$ or the Pt(DNSH-dien)(5′-GMP) adduct, respectively. The presence of the Pt−sulfonamido bulky group close to the binding site affected its selectivity toward N7 over sulfur binding.

Competition Reaction. No kinetic selectivity of [Pt(DNSH-dien)(Me_2SO-d_6)]$^+$ **(2-sol)** toward met over 5′-GMP was observed, and the ratio of the two adducts throughout the formation reaction was approximately 1:1 (Figure 6). In contrast, the competition reactions of [Pt(DNSH-tren)(Me_2SO-d_6)]$^{2+}$ (**5-sol**) (Figure 6) and [Pt(dien)Cl]Cl[6,9,10] show preferential kinetic binding by met followed by thermodynamic binding by 5′-GMP. The presence of a bulky group very close to the Pt(II) binding site in **2-sol** significantly affected the selectivity of Pt(II) toward met and 5′-GMP.

Conclusions

The DNSH-dienH ligand can act as a bidentate, tridentate, or quadridentate ligand. The observation of only two H8 ^{1}H NMR signals for the Pt(DNSH-dien)(6-oxopurine nucleotide) adducts indicates rapid rotation around the Pt−N7 bond. Thus, the addition of a bulky substituent on just one terminal N of a dien-type ligand has been shown to be insufficient to slow the rotation around the Pt−N7 bond. The upfield shifts of the signals of the DNSH group of all **G**/3′-IMP adducts, the ethyl group of the 9-EtG adduct, and the H2 and H1′ of nucleotide adducts all indicate that the dansyl naphthalene group and the 6-oxopurine base are close and parallel. The additional quenching found for the Pt(DNSH-dien)(5′-IMP) adduct can be attributed to energy transfer, which is effective because of the close proximity of the dansyl group to the guanine base.[35] This quenching is avoided when the aromatic fluorophore is positioned remotely from Pt(II), as exemplified by [Pt(DNSH-tren)Cl]Cl. This latter compound and [Pt(dien)Cl]Cl, which have no substituents on the terminal amino groups to influence the approach of the incoming monodentate ligand, have similar reactivity patterns toward 5′-GMP

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and met. The resulting adducts and those derived from Pt(DNSH-dien)Cl exhibit similar dynamic properties. However, the bulky dansyl group near the reaction site of Pt(DNSH-dien)Cl increases the reactivity toward 5′-GMP relative to that toward met. This effect could be explained by stacking between the dansyl group and the guanine base of 5′-GMP.

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