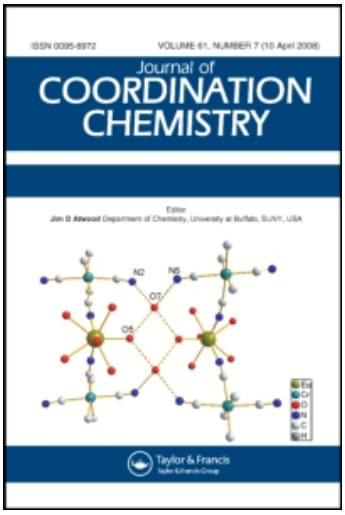

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1-Methyl-4-(methylamino)piperidine-platinum(II) adducts with DNA bases

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1-Methyl-4-(methylamino)piperidine-platinum(II) adducts with DNA bases

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A series of platinum(II) monoadducts and diadducts of the type $[Pt^{II}(mmap)LCl]NO_3$ and $[Pt^{II}(mmap)L_2](NO_3)_2$ (where mmap = 1-methyl-4-(methylamino)piperidine and L = adenine, 9-methylguanine, 7-methylguanine, cytosine, or uracil) have been synthesized and characterized by elemental analyses and by ^{1}H, ^{13}C, and ^{195}Pt nuclear magnetic resonance spectroscopy. Two adjacent corners of the platinum plane were occupied by the two amino nitrogens of 1-methyl-4-(methylamino)piperidine and the other two positions were occupied by the chloride and nitrogen atoms of the DNA base in monoadducts and two nitrogen atoms of DNA bases in diadducts.

Keywords: Platinum; Nucleobase; Adducts; Synthesis

1. Introduction

Because of the side effects of cisplatin, toxicity, cancer specificity, and especially acquired resistance, there has been a widespread search for cisplatin analogues that are structurally and functionally distinct from cisplatin and that exhibit cross-resistance in cisplatin-resistance profiles [1–5]. An understanding of which adducts are critical for killing cancer cells and might have less toxic side effects is important, because there is substantial evidence that cisplatin and many other platinum drugs are DNA-binding agents and block the DNA replication process [6–8]. For example, the intrastrand cross-link formed by cisplatin between neighboring purine bases suggests that cisplatin's toxicity originates from such lesions [9–15], but transplatin is unable to form this type of intrastrand cross-link because of geometric strain and has low antitumor activity [11, 16]. Structure activity studies support the notion that the non-leaving ligands of cisplatin analogues modulate the antitumor activity of this class of compounds. Hence the development of oxaliplatin, [oxalatoplatinum(II)], a complex with a carrier ligand (1*R*,2*R*-diaminocyclohexane, DACH) altered the spectrum of

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antitumor activity and overcame resistance [17–19]. Two other DACH-Pt complexes, L-NDDP and tetraplatin, are in phase I and phase II clinical trials [20, 21]. Therefore studies have focused on the formation of monofunctional and bifunctional platinum adducts with different DNA-binding modes [10, 15, 22–27]. We recently demonstrated that DACH-Pt adducts are cytotoxic with low cross resistance [27]. In this article we report the synthesis and characterization of a series of 1-methyl-4-(methylamino)-piperidine-Pt(II) monoadducts and diadducts with DNA bases.

2. Experimental

2.1. *Chemicals*

K_2PtCl_4 was purchased from Johnson Matthey (Seabrook, NH). Silver nitrate was purchased from Alfa Aesar (Ward Hill, MA). 1-Methyl-4-(methylamino)piperidine (mmap), adenine (ade), 9-methylguanine (9-megua), 7-methylguanine (7-megua), cytosine (cyt), uracil (ura), methylene chloride, and acetone were purchased from Aldrich Chemical Co. (Milwaukee, WI). Silver nitrate, hydrochloric acid, nitric acid, *N*,*N*-dimethylformamide (DMF), and potassium bromide were purchased from Fisher Scientific Co. (Houston, TX).

2.2. *Physical measurements*

Elemental analyses of the complexes were performed by Robertson Laboratories Inc. (Madison, NJ). ^1H, ^{13}C, and ^{195}Pt NMR spectra were recorded for complexes in solution with D_2O, DMSO-d_6 and DCl using a 300-MHz spectrometer. Shifts in the ^{195}Pt spectra were measured relative to an external standard of 0.2 M Na_2PtCl_6 in D_2O at 0.00 ppm.

2.3. *Synthesis of platinum complexes*

2.3.1. $[Pt^{II}(mmap)(ade)Cl]NO_3$ (1). $[Pt^{II}(mmap)Cl_2]$ was synthesized as described previously [22]. $[Pt^{II}(mmap)Cl_2]$ (0.4 g, 1.01 mmol) was dissolved in 100 mL of DMF, and to this solution $AgNO_3$ (0.164 g, 0.96 mmol) was added. The reaction mixture was then continuously stirred in the dark for 24 h. The AgCl precipitate was filtered off, using celite as a filter. To the filtrate $[Pt^{II}(mmap)(H_2O)Cl]NO_3$, ade (0.17 g, 1.27 mmol) was added, and the reaction mixture was then stirred for 96 h at 45°C. A pale yellow solution was obtained. A pinch of animal charcoal was added to this solution, and the solution was stirred for an additional 15 min, filtered, and evaporated to dryness. In an excess of acetone, the pale yellow product $[Pt^{II}(mmap)(ade)Cl]NO_3$ was obtained and then filtered and dried. The complex was redissolved in a minimal amount of water and precipitated with acetone. The final product was filtered, washed with acetone, and dried *in vacuo*.

Complexes **3**, **5**, **7**, and **9** were prepared by the same procedure using the corresponding bases.

2.3.2. Preparation of $[Pt^{II}(mmap)(ade)_2](NO_3)_2$(2). $[Pt^{II}(mmap)Cl_2]$ (0.50 g, 1.268 mmol) was dissolved in 200 mL of DMF, and $AgNO_3$ (0.43 g, 2.53 mmol) was added to this solution. The reaction mixture was protected from light and stirred overnight. AgCl was filtered off, using celite as a filter. To the filtrate $[Pt^{II}(mmap)(H_2O)_2](NO_3)_2$, Ade (0.34 g, 2.53 mmol) was added, and the reaction mixture was then stirred continuously for 192 h, resulting in a pale yellow solution. A pinch of animal charcoal was added to this solution, and the solution was stirred for an additional 15 min. The solution was filtered and evaporated to dryness. In an excess of acetone, the pale yellow product $[Pt^{II}(mmap)(ade)_2](NO_3)_2$ was obtained and then filtered and dried. The complex was redissolved in a minimal amount of water and precipitated with acetone. The final product was filtered, washed with acetone, and dried *in vacuo*.

Complexes **4**, **6**, **8**, and **10** were prepared by the same procedure using the corresponding bases.

3. Results and discussion

3.1. *Synthesis*

The synthesis of platinum(II) complexes is shown in scheme 1. $[Pt(mmap)Cl_2]$ was used as a precursor for both $[Pt^{II}(mmap)(L)Cl]NO_3$ and $[Pt^{II}(mmap)L_2](NO_3)_2$. The reaction of $[Pt^{II}(mmap)Cl_2]$ with 1 equivalent of $AgNO_3$ in DMF yielded $[Pt^{II}(mmap)(H_2O)Cl]NO_3$ (reaction A). The reaction of $[Pt^{II}(mmap)(H_2O)Cl]NO_3$ with 1 equivalent of the desired nucleobase in DMF produced the $[Pt^{II}(mmap)(L)Cl]NO_3$ complexes (reaction B). The reaction of $[Pt^{II}(mmap)Cl_2]$ with 2 equivalents of $AgNO_3$ and the corresponding nucleobase produced the $[Pt^{II}(mmap)L_2](NO_3)_2$ complexes (reactions C and D).

Monoadducts

$$[Pt^{II}(mmap)Cl_2] + AgNO_3 \xrightarrow{DMF} [Pt^{II}(mmap)(H_2O)Cl]NO_3 + AgCl \quad (A)$$

$$[Pt^{II}(mmap)(NO_3)Cl] + L \xrightarrow{DMF/acetone} [Pt^{II}(mmap)(L)Cl]NO_3 + H_2O \quad (B)$$

Diadducts

$$[Pt^{II}(mmap)Cl_2] + 2AgNO_3 \xrightarrow{DMF} [Pt^{II}(mmap)(H_2O)_2](NO_3)_2 + 2AgCl \quad (C)$$

$$[Pt^{II}(mmap)(H_2O)_2](NO_3)_2 + 2L \xrightarrow{DMF/acetone} [Pt^{II}(mmap)L_2](NO_3)_2 + 2H_2O \quad (D)$$

Scheme 1. (mmap) = 1-Methyl-4-(methylamino)piperidine; L = adenine, 9-methylguanine, 7-methylguanine, cytosine, or uracil.

3.2. *Characterization of platinum complexes*

Structures of the platinum complexes are shown in figure 1. Elemental analyses showed a good correlation between the theoretical and actual values. These values are shown in table 1.

Infrared spectroscopy was used to identify the functional groups of the ligands in compounds. Broad bands between 3300 and 3100 cm^{-1} were assigned to N–H stretching vibrations in the spectra. The intense bands between 1690 and 1600 cm^{-1} for mmap and between 1380 and 1200 cm^{-1} for nucleobase complexes were attributed to ν_{as}(C–O) and ν_{s}(C–O) vibrations, respectively. Pt–N and Pt–Cl stretching frequencies were observed around 580 and 360 cm^{-1}, respectively.

All complexes were further characterized by ^{1}H, ^{13}C, and ^{195}Pt NMR spectroscopy. The ^{1}H, ^{13}C, and ^{195}Pt NMR spectroscopic data were most informative with respect to the structure of the complexes (table 2). The peaks corresponding to 1-methyl-4-(methylamino)piperidine were observed between 1.23 and 2.80 ppm. In complexes **1** and **2**, C_8H- and C_2H-proton resonance peaks of the adenine were shifted downfield compared with those of the free ligand. The shift of C_8H protons to the range of 0.12–0.28 ppm, compared with that of C_2H proton (range of 0.12–0.21 ppm), suggests that the site of coordination to the Pt metal ion is N7. The downfield shift of C_8H proton to the range of 0.11–0.14 ppm observed for 9-methylguanine complexes **3** and **4** and 0.20–0.21 ppm for 7-methylguanine complexes **5** and **6** supports the coordination of N7 or N9 with the metal ion. In cytosine and uracil complexes **7**–**10**, the two doublets corresponding to the C_5H protons exhibited smaller shifts (0.5–0.18 ppm) and (0.01–0.31 ppm) than did the C_6H protons (0.46–0.47 ppm) and (0.11–0.12 ppm), respectively, which supports coordination through the N3 atom of the ligands.

The proton-decoupled ^{13}C NMR spectra of purine and pyrimidine bases were obtained from the Integrated Spectral Data Base System for Organic Compounds (SDBS).

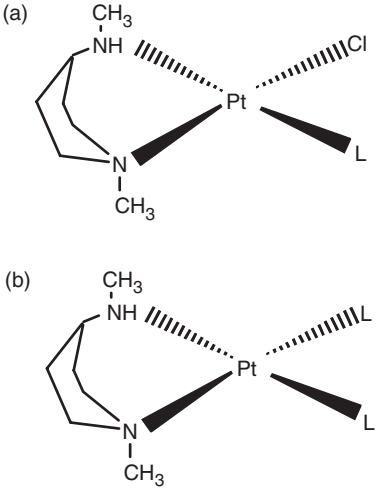

Figure 1. (a) Structure of $[Pt^{II}(mmap)LCl]NO_3$, (b) Structure of $[Pt^{II}(mmap)L_2](NO_3)_2$. mmap = 1-Methyl-4-(methylamino)piperidine; L = adenine, 9-methylguanine, 7-methylguanine, cytosine, or uracil.

Table 1. Elemental analysis of platinum complexes.

Complexes	Observed (Calcd) C	H	N	Cl	% Yield
$[Pt^{II}(mmap)(ade)Cl]NO_3$ (**1**)	26.09	4.06	19.29	5.78	
	(26.67)	(3.93)	(19.14)	(5.94)	72.5
$[Pt^{II}(mmap)(ade)_2](NO_3)_2$ (**2**)	28.63	3.80	27.55	–	
	(28.53)	(3.62)	(27.41)	–	78.3
$[Pt^{II}(mmap)(9\text{-megua})Cl]NO_3$ (**3**)	25.99	3.67	18.23	5.87	
	(26.64)	(3.90)	(19.12)	(6.06)	80.4
$[Pt^{II}(mmap)(9\text{-megua})_2](NO_3)_2$ (**4**)	27.65	3.60	23.38	–	
	(28.67)	(4.02)	(24.65)	–	85.8
$[Pt^{II}(mmap)(7\text{-megua})Cl]NO_3 \cdot H_2O$ (**5**)	25.79	3.97	18.58	5.51	
	(25.84)	(4.14)	(18.50)	(5.88)	79.2
$[Pt^{II}(mmap)(7\text{-megua})_2](NO_3)_2 \cdot 2H_2O$ (**6**)	27.96	4.01	23.86	–	
	(28.28)	(4.21)	(24.31)	–	82.39
$[Pt^{II}(mmap)(cyt)Cl]NO_3$ (**7**)	25.07	3.68	15.55	6.96	
	(24.83)	(3.95)	(15.80)	(6.67)	85.1
$[Pt^{II}(mmap)(cyt)_2](NO_3)_2$ (**8**)	26.85	3.94	19.02	–	
	(26.90)	(3.88)	(20.90)	–	83.6
$[Pt^{II}(mmap)(ura)Cl]NO_3$ (**9**)	24.93	3.88	13.20	6.80	
	(24.73)	(3.75)	(13.14)	(6.66)	77.9
$[Pt^{II}(mmap)(ura)_2](NO_3)_2 \cdot H_2O$ (**10**)	25.96	4.36	16.09		
	(25.82)	(4.01)	(16.01)		82.0

(mamp) = 1-Methyl-4-(methylamino)piperidine; (ade) = adenine, (9-megua) = 9-methylguanine, (7-megua) = 7-methyguanine, (cyt) = cytosine, or = (ura) uracil.

In complexes **1** and **2**, shifts of 1.47–3.27 ppm and 1.00–2.14 ppm were observed at C_4, C_5, and C_8, respectively, compared with adenine free ligand. In complexes **3** and **4**, shifts of 1.33–2.34 ppm at C_4, 3.59–3.86 ppm at C_5, and 2.36–2.56 ppm at C_8 were observed as compared with 9-megua. The identical shifts in adenine complexes **1** and **2** and in (N9-substituted) 9-methylguanine complexes **3** and **4** support the notion that N7 serves as a binding site. In complexes **5** and **6**, the C_2, C_6, and C_8 carbons showed shifts of 1.00–2.63, 1.90–4.50, and 4.46–4.98 ppm as compared with the 7-methylguanine, suggesting that coordination site is N9 and not N7 because of the methyl substituent. In both monoadducts and diadducts of cytosine and uracil (**7**–**10**), C2 showed a shift range of 3.29–3.86 ppm and C4 a shift range of 1.87–3.07 ppm compared with free ligand, suggesting N3 as a site of coordination. X-ray diffraction and NMR studies of the complexes formed between platinum and N9- and N7-alkylated purines, N1-alkylated pyrimidines, nucleosides, and nucleotides have been used to elucidate the platinum binding sites. The preferred binding sites are N7 for adenine and 9-methylguanine, N9 for 7-methylguanine purine complexes, and N3 for pyrimidine complexes [10, 15, 22–27]. NH_2 is apparently not involved in coordination, because there was only a negligible shift in NH_2 protons in DMF-d_7. This is further supported by the results of ^{195}Pt NMR spectroscopy.

In the ^{195}Pt NMR spectra, the Pt(II) complexes **1**, **3**, **5**, **7**, and **9** showed a signal in the range of −2367 through −2464 ppm for monoadducts and a signal in the range of −2531 to −2687 ppm for diadducts **2**, **4**, **6**, **8**, and **10**. Such chemical shifts are typical for square-planar Pt(II) complexes that contain three nitrogen and one chlorine

Table 2. ^{1}H, ^{13}C, and ^{195}Pt NMR spectroscopic data for platinum complexes.

	^{1}H			^{13}C						^{195}Pt
Complexes	C2	C8	CH_3	C2	C4	C5	C6	C8	CH_3	
Adenine	8.11	8.14	–	153.41	156.36	119.07	151.71	141.30	–	
Pt^{II}(mmap)(ade)Cl]NO_3 (**1**)	8.32	8.42	–	154.85	156.91	117.62	148.98	143.54	–	−2460
[Pt^{II}(mmap)(ade)$_2$](NO_3)$_2$ (**2**)	7.99	8.02	–	152.07	154.00	115.80	148.13	142.22	–	−2687
9-Methylguanine	–	7.62[a]	3.39	154.35[a]	157.73	117.24	152.40	138.94	30.12	
[Pt^{II}(mmap)(9-megua)Cl]NO_3 (**3**)	–	7.73[a]	3.73	154.13[a]	155.39	113.65	151.18	141.30	30.80	−2367
[Pt^{II}(mmap)(9-megua)$_2$](NO_3)$_2$ (**4**)		7.76[a]	3.46	154.88[a]	156.40	113.38	151.35	141.50	31.01	−2531
7-Methylguanine	–	8.56[b]	3.03	154.91[b]	155.13	108.51	149.77	139.15	35.58	
[Pt^{II}(mmap)(7-megua)Cl]NO_3 (**5**)	–	8.35[b]	3.85	152.28[b]	153.75	109.72	147.87	144.13	34.75	−2444
[Pt^{II}(mmap)(7-megua)$_2$](NO_3)$_2$ (**6**)	–	8.30[b]	3.80	155.90[b]	156.39	109.08	154.27	143.61	34.61	−2598
		C_5H	C_6H							
Cytosine		5.97	7.94	159.91	168.95	95.79	144.05			–
[Pt^{II}(mmap)(cyt)Cl]NO_3 (**7**)		6.15	7.58	156.05	165.88	95.01	143.91			−2464
[Pt^{II}(mmap)(cyt)$_2$](NO_3)$_2$ (**8**)		5.92	7.47	156.62	167.08	95.24	144.51			−2682
Uracil		5.47[a]	7.40	152.27[a]	165.0	101.01	142.21			
[Pt^{II}(mmap)(ura)Cl]NO_3 (**9**)		5.74[a]	7.32	148.77[a]	162.17	100.55	142.21			−2432[a]
[Pt^{II}(mmap)(ura)$_2$](NO_3)$_2$ (**10**)		5.74[a]	7.41	149.08[a]	164.12	100.52	142.05			−2641[a]

(mamp) = 1-Methyl-4-(methylamino)piperidine; (ade) = adenine, (9-megua) = 9-methylguanine, (7-megua) = 7-methyguanine, (cyt) = cytosine, or (ura) = uracil; a = DMSO-d_6; b = DCl.

donor for monoadducts and four nitrogen donors for diadducts. Figure 1 shows the proposed structure of such complexes.

4. Biological relevence and conclusions

Recently we demonstrated that *trans*-1*R*,2*R*-diaminocyclohexane-platinum(II) (DACH-Pt) and *cis*-diamine-platinum(II) adducts with adenine and guanine nucleobases were 4- to 29-fold less potent than *cis*-diaminedichloroplatinum(II) (cisplatin) against A2780 cells except for DACH-Pt adduct with 9-ethylguanine, which was about 6-fold more potent. Resistance factors for DACH-Pt- nucleobase adducts were also substantially three-fold lower than cisplatin, but nine-fold higher for diamine-Pt-nucleobase adducts [27]. The low potency of DACH-Pt-nucleobase adducts compared to cisplatin, is likely due to the presence of three nitrogen atoms attached to the platinum and this will presumably result in monofunctional interaction with DNA. Some similar monofunctional platinum analogues have also been reported as less potent cytotoxic agents [28–30]. As a part of long-term goal of studying the biological activity of platinum complexes with antitumor activity, we have synthesized and characterized a series 1-methyl-4-(methylamino)piperidine-Pt(II) monoadducts and diadducts by elemental analyses and NMR spectroscopic technique.

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