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Synthesis and characterization of poly(amidoamine)-platinum(II) complexes. Detailed speciation by Matrix-Assisted Laser Desorption Ionization Mass Spectrometry

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Abstract

MALDI and ESI-MS have been applied to the characterization of the reaction products between the labile *cis*-[Pt(DMSO)$_2$Cl$_2$] (**1**) and *trans*-[Pt(DMSO)$_2$Cl(CH$_3$)] (**2**) complexes with the simplest poly(amidoamine) ligand (PAMAM, G = 0, 1,2-diaminoethane as core). The comparison of the mass spectra of the starting G_0 and those of the metallo-dendrimers formed upon mixing of the reagents in an equimolecular ratio, and the analysis of the isotopic distribution in the ESI spectra, have revealed the formation of cationic and neutral mononuclear complexes with PAMAM as ligand, e.g., *cis*-[Pt(DMSO)(PAMAM)Cl]Cl or *trans*-(*C*,*N*)[Pt(DMSO)(PAMAM)Cl(CH$_3$)], together with various minor components, which have been identified as derivatives from defective structures of PAMAM. The geometry of the main products has been deduced from the values of the protons coupling constants with the isotopically abundant ^{195}Pt. The metal-to-ligand bond is restricted to the peripheral amino groups of PAMAM which shows sufficient flexibility to involve either one or two branches in the coordination bonding.

Keywords: Nitrogen ligands; Platinum (II) complexes; Dendrimers; MALDI-MS; ESI-MS

1. Introduction

Transition metal complexes containing sulfoxide as ligand are precursors in a variety of synthetic procedures [1–8]. Sulfur bonded DMSO exhibits a fairly large *trans* effect in square-planar platinum (II) complexes [9–11], exploited in inorganic syntheses [12], and a weak *trans* influence [13]. Interestingly, while compounds containing a single DMSO are quite inert [14], the presence of a second molecule increases the lability for the nucleophilic substitution [15–17]. Thus, sulfoxides spanning a mutual *cis* position display a relevant self-labilization effect, such as in the complex *cis*-[Pt(DMSO)$_2$Cl$_2$] (**1**) [12]. Organometallic complexes of the same type *cis*-[Pt(DMSO)$_2$(R)$_2$] (R = CH$_3$, aryl group) [18] are much more labile, representing the first example of a dissociative pathway for the nucleophilic substitution process in square planar platinum (II) complexes [19,20].

Only few studies have been focused on complexes having two sulfoxide ligands in *trans* configuration [21–24]. Some years ago, we reported the first investigation on such an organometallic compound, the complex *trans*-[Pt(DMSO)$_2$(CH$_3$)Cl] (**2**) [25]. This species was originally synthesized by Eaborn et al. [26] and its *trans*

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geometrical configuration has been confirmed by X-ray diffraction [25]. Multinuclear NMR experiments revealed that a chloroform solution of this complex contains four different species, depending on the adventitious water concentration in the solvent. Apart from the mixture of the parent *trans* complex and its *cis* isomer, which are the major components, a low concentration of two related *cis* and *trans*-[Pt(CH_3)Cl(DMSO)(H_2O)] aqua-species have been detected [25]. These latter compounds, which become the major species in aqueous solution [27], are responsible for the high reactivity of this system toward a variety of nucleophilic agents. On these bases, complex **2** has been extensively used as synthon for organometallic compounds containing the moieties Pt-CH_3 or Pt(DMSO)(CH_3) [28]. Reaction of **2** in chloroform or in aqueous solution with monodentate ligands L, such as amines or pyridines, leads almost quantitatively to uncharged complexes of the type [Pt(DMSO)(L)Cl(CH_3)], in which four different groups are coordinated to the platinum (II) center [29], and whose geometrical configuration depend mainly on the steric properties of the entering group. In aqueous solution, a double substitution affording cationic complex ions of the type *cis*-[Pt$(L)_2$(DMSO)(CH_3)]$^+$ has also been observed [27]. The reaction with diamines or diimines, N–N, leads to cationic complexes of the type [Pt(N–N)(DMSO)(CH_3)]Cl or neutral complexes of the type [PtCl(N–N)(CH_3)], containing 5- or 6-membered chelate rings [30]. Some of these chelated cationic compounds were used as precursors for the synthesis of functionalized porphyrins [31,32]. The reaction of **2** with long chain diamines yields dinuclear compounds of the type [{Pt(DMSO)Cl(CH_3)}$_2$(μ-N–N)]. Such complexes are able to act as multifunctional binders towards DNA [33,34]. Reaction with 2,2′:6′,2″-terpyridine leads to the complex [Pt(terpy)(CH_3)]Cl, which behaves as an intercalator towards double-stranded DNA [35–37]. Addition of sodium chloride 0.1 M leads to the formation of a unique product, the anionic complex *cis*-[Pt(DMSO)Cl_2(CH_3)]$^-$, that exhibits the same behavior towards nucleophiles of the starting mixture [27].

Within this synthetic framework, a class of compounds with potential interest for us are the dendritic molecules, a new classes of macromolecules characterized by a self-similar structure [38,39]. They have been exploited as building blocks for supramolecular systems [40], models for biological systems [41], agents for gene transfer therapy [42] and as carrier of drugs, fragrances, cosmetics and other molecules [43–46]. We focused in particular on poly(amidoamine) *starburst* dendrimers (PAMAM), based on ammonia or 1,2-diaminoethane (en) as a core and a periphery terminated by primary amino groups. Their structural features have been largely investigated through light scattering techniques and molecular dynamics simulations [47]. PAMAM dendrimers provide multiple metal coordinating sites, either in their core or at the periphery [48,49], but, apart from very few examples [50], they contain defect structures clearly observable by MS analysis [51–57] and especially, using FAB MS [54], MALDI MS [55,56], and ESI MS [57].

In this paper, we report on the investigation of the interaction of the complexes *cis*-[Pt$(DMSO)_2Cl_2$] (**1**) and *trans*-[Pt$(DMSO)_2$Cl(CH_3)] (**2**) with the simplest PAMAM ligand (G = 0, 1,2-diaminoethane as core [58]) through MALDI and ESI-MS. Although mass spectrometry should be an effective tool in the structural analysis of coordination and organometallic complexes, the characterization poses severe problems because of the low volatility and lability of these species. The use of MALDI MS has improved the structural characterization of inorganic complexes [59] and of metallo-dendrimers [55,56].

2. Result and discussion

The interaction of **1** and **2** with PAMAM has been studied by reacting the metal complexes with the nitrogen ligand, and characterizing the resulting species directly in situ. Due to the presence, even in the lower generation here investigated, of multiple binding sites for platinum (II) [49], we have restricted the present study to an equimolar mixing of reagents. The reactions can be monitored through ^{1}H NMR and then, due to the complexity of the systems, by exploiting MALDI and ESI-MS techniques. Samples **3** and **4** contain individual species relative to both uncomplexed entire and defective ligand G_0, and to the platinum complexes. MALDI-MS spectra of these samples in water were assigned by comparison with MALDI spectrum of G_0 dendrimer starter. Table 1 collects the main observed and calculated values for the mass peaks assigned for the pure ligand and the two resulting reaction mixtures.

2.1. Reaction of *1* with Starbust PAMAM G_0

^{1}H NMR of sample **3** (spectrum not shown) in CD_3OD at room temperature was tentatively assigned by comparison with the corresponding spectra of G_0 ligand and compound **1**. The spectra evidence a general downfield shift of each set of signals for PAMAM protons. In particular, signals at δ 3.18 and 2.67 relative to –CH_2NHCO– and NH_2CH_2– in the free ligand are shifted to δ 3.54 and 2.90, respectively, as result of metal coordination to the primary amino groups. The resonance of free DMSO is evidenced at δ 2.64 ppm and is almost in 1:1 ratio with respect to the coordinated ligand that appears as a not well resolved signal at 3.34.

In Table 1, *m/z* values relative to entire G_0 and defective species $x_{br}G_0$ are reported. In the literature, it has

Table 1
Comparison of calculated and experimental mass values of G_0 and G_0/Pt^{2+} complexes (sample **3** and **4**)

Ions	Experimental (MALDI-MS) (Da)[a]	Calculated mol.wt. (Da)[b]	
G_0[c]			
$M + H^+$			
$2_{br}G_0$	289.7	289.40	$C_{12}H_{29}N_6O_2$
$3_{br}G_0$	403.9	403.54	$C_{17}H_{39}N_8O_3$
$3.5_{br}G_0$	458.0	457.59	$C_{20}H_{41}N_8O_4$
G_0	518.1	517.69	$C_{22}H_{49}N_{10}O_4$
$5_{br}G_0$	632.3	631.83	$C_{27}H_{59}N_{12}O_5$
$M + Na^+$			
$3_{br}G_0$	425.9	425.5	$C_{17}H_{38}N_8NaO_3$
G_0	540.2	539.67	$C_{22}H_{48}N_{10}NaO_4$
$5_{br}G_0$	654.3	653.82	$C_{27}H_{58}N_{12}NaO_5$
Sample 3[d]			
$2_{br}G_0Pt^+$	597.4	597.05	$C_{14}H_{34}ClN_6O_3PtS$
$3_{br}G_0Pt^+$	711.6	711.20	$C_{19}H_{44}ClN_8O_4PtS$
G_0Pt^+	825.6	825.35	$C_{24}H_{54}ClN_{10}O_5PtS$
Sample 4[e]			
$M + H^+$			
$3_{br}G_0(N–N)Pt_{(–CH3–Cl)}$	598.9	598.62	$C_{17}H_{39}N_8O_3Pt$
$3_{br}G_0(N–N)Pt_{(–Cl)}$	614.7	613.66	$C_{18}H_{42}N_8O_3Pt$
$3_{br}G_0(N–N)Pt_{(–CH3)}$	634.1	634.07	$C_{17}H_{39}ClN_8O_3Pt$
$G_0(N–N)Pt_{(–CH3–Cl)}$	713.0	712.77	$C_{22}H_{49}N_{10}O_4Pt$
$G_0(N–N)Pt_{(–Cl)}$[f]	728.7	727.80	$C_{23}H_{52}N_{10}O_4Pt$
$G_0(N–N)Pt_{(–CH3)}$[f]	749.8	748.22	$C_{22}H_{49}ClN_{10}O_4Pt$
$G_0(C–N)$[f]	842.8	841.39	$C_{25}H_{58}ClN_{10}O_5PtS$
$M + Na^+$			
$G_0(C–N)$[f]	865.3	863.37	$C_{25}H_{57}ClN_{10}NaO_5PtS$

[a] External calibration was performed with a mix of ACTH fragments (see Section 4.3). Values refer to average masses unless specified otherwise.
[b] Calculated monoisotopic mass.
[c,d,e] For an exemplification refer to Chart 1,Chart 2, Chart 3, respectively. In the schemes, the structure formulas relative to the neutral species (species in G_0 alone and in sample **4**) are displayed subtracted by proton or sodium ion. In MALDI spectra of **3** and **4** we observed peaks ($M + H^+$) and ($M + Na^+$) relative to the starter ligand G_0 (not reported in table, for assignments see Fig. 1).
[f] Peak centroid is not well-defined.

been reported that a variety of defects could lead to divergence from ideality, even in the case of either dendrimers or metallo-dendrimers of low generations [39]. NMR and ESI-MS spectra have usually identified the imperfections [51]. In our case, branching ideality (4 branches) is decreased as is evidenced, in the $x_{br}G_0$ patterns, by both the entire and fractional x which correspond, respectively, to branch defective patterns and to intra-dendrimer looping, probably due to the ring closure between terminal NH_2 and a COOH of two closed branches (Chart 1). Furthermore, MALDI MS pointed out the formation of hyper-branched species. This usual behaviour has been recently discussed by Crooks and co-workers [52], who have interpreted this "missing arm" defect in dendrimer as deriving from divergent synthetic strategy and in particular from a subquantitative yield of the Michael addition of methyl acrylate to the amine-terminated dendrimer branches [39]. The defective dendrimer with "missing arm + loop" are probably due to defective propagation from the $3_{br}G_0$. Sample **3** contains abundant species assigned to platinum (II) complexes. In MALDI mass spectrum (m/z range 400–850, Fig. 1), we observed three peaks at m/z 597.4, 711.6 and 825.6 which we ascribed to $2_{br}G_0Pt^+$, $3_{br}G_0Pt^+$ and G_0Pt^+, respectively (Chart 2). Peaks assigned to the starting material were also well evident. Above $m/z = 850$, MALDI spectra do not show any intense peak. Aqueous solutions of sample **3** sprayed into ESI-MS spectrometer gave mass spectral peaks (Fig. 2(b)), which corresponds to the assignment in the MALDI spectra (Fig. 2(a)). The higher resolution of ESI mass spectra allows for assigning exhaustively all the metal complexes species. Simulated ESI mass spectra for $2_{br}G_0Pt^+$ ($PtC_{14}H_{34}N_6O_3ClS$), $3_{br}G_0Pt^+$ ($PtC_{19}H_{44}N_8O_4ClS$), and G_0Pt^+ ($PtC_{24}H_{54}N_{10}O_5ClS$) are reported in Fig. 2(b) and confirm the assignments. Since we are analyzing a complex mixture, considering that other species of lower intensity contribute to modify the isotopic distribution, calculated and experimental isotopic distribution match sufficiently. The formation of chelated species reported in Chart 2 is not surprising, on considering the flexibility of the ligand branches and the results of previous investigations on the solution behavior of complex **1**. A kinetic study on the lability of DMSO in such a complex has pointed out that the first step in the reaction of **1** with an amine (am) is the

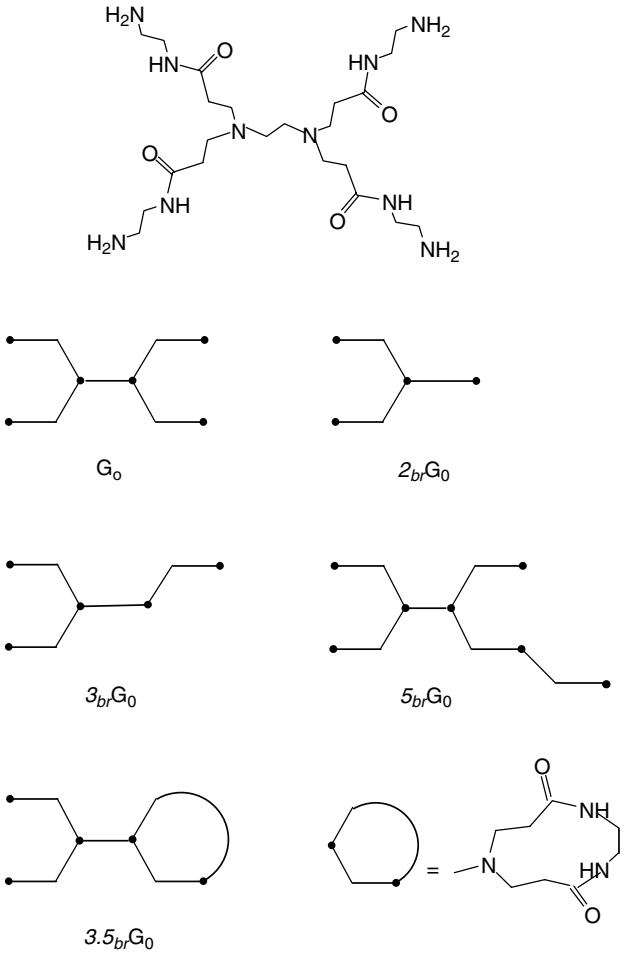

Chart 1.

facile nucleophilic substitution of a DMSO ligand to yield neutral complexes of the type *cis*-[$PtCl_2$(am)(DMSO)]. Due to the fairly high *trans* influence of DMSO, a second amine can displace the halogen anion from the coordination sphere, leading to the cationic complexes of the type *cis*-[$PtCl(DMSO)(am)_2]^+$ [12]. This second step is very fast in the reactions with chelating ligands of variable complexity [60,61].

2.2. Reaction of **2** with Starbust PAMAM G_0

Complex **2** is fairly soluble in aqueous solution up to a concentration of 0.01 M. Fig. 3 shows a comparison of ^{1}H NMR spectra relative to the free PAMAM ligand (a) and to the reaction mixture with complex **2**, in the presence of NaCl (100 mM). The preliminary formation of *cis*-[$Pt(DMSO)Cl_2(CH_3)]^-$ with the associated rapid extrusion of a molecule of DMSO is evidenced by a singlet at δ 2.50. The addition of an equivalent amount of PAMAM, the signal relative to the methyl group directly coordinated to the metal center moves upfield to δ 0.33 ($^2J_{PtH}$ = 68 Hz). The low value of the relative coupling constant indicates that the amino group of PAMAM is placed in *trans* position with respect to the methyl group [29]. Furthermore, a sensible downfield shift for critical signals such as protons in α and β position with respect to the peripheral amino groups is observed.

Previous investigations have shown that steric congestion brought about by sterically hindered entering ligands (L) reacting with **2** controls the geometrical configuration of the reaction product. The species *trans*-(*C*,*N*)[Pt(DMSO)(L)Cl(CH_3)] is preferentially formed upon displacement of a molecule of DMSO and only for small size ligands (L) the interconversion into the corresponding *cis* isomer is facile. Our MALDI data evidence that reaction of *cis*-[$Pt(DMSO)Cl_2(CH_3)]^-$ with a stoichiometric amount of PAMAM in aqueous solution leads preferentially to

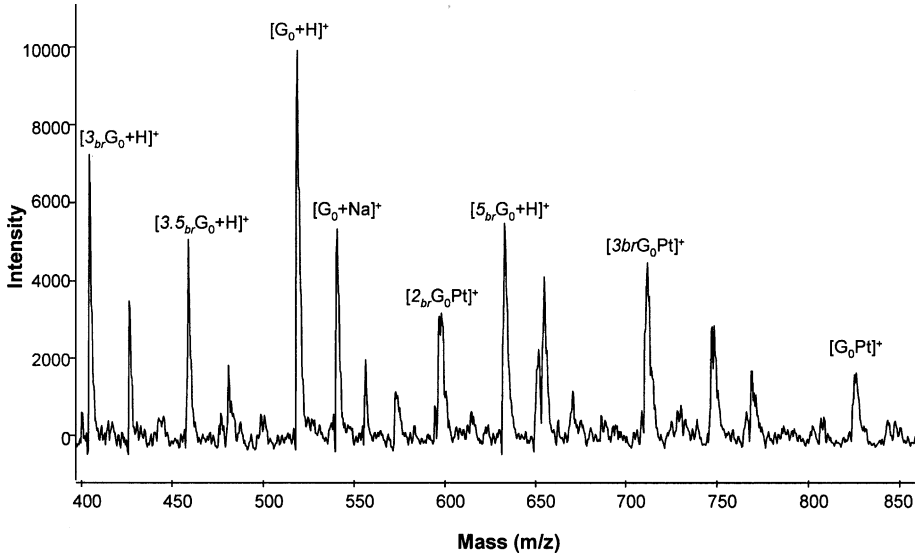

Fig. 1. Linear MALDI TOF mass spectrum of sample **3** containing the starting PAMAM G_0 and the various G_0-Pt(II) products.

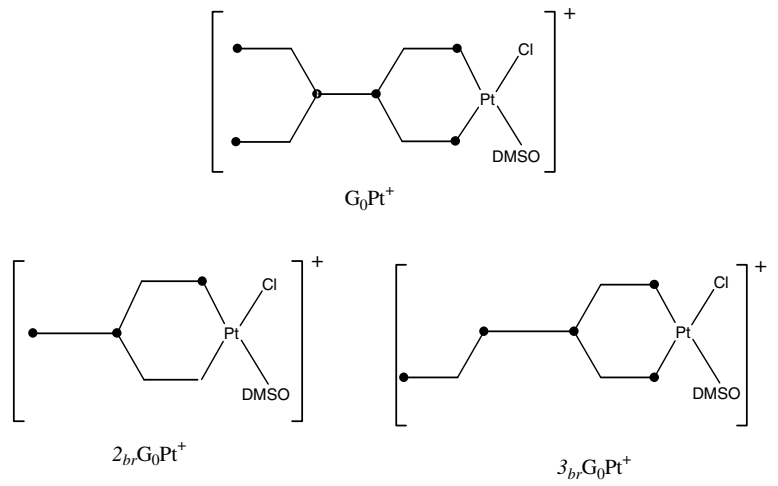

Chart 2.

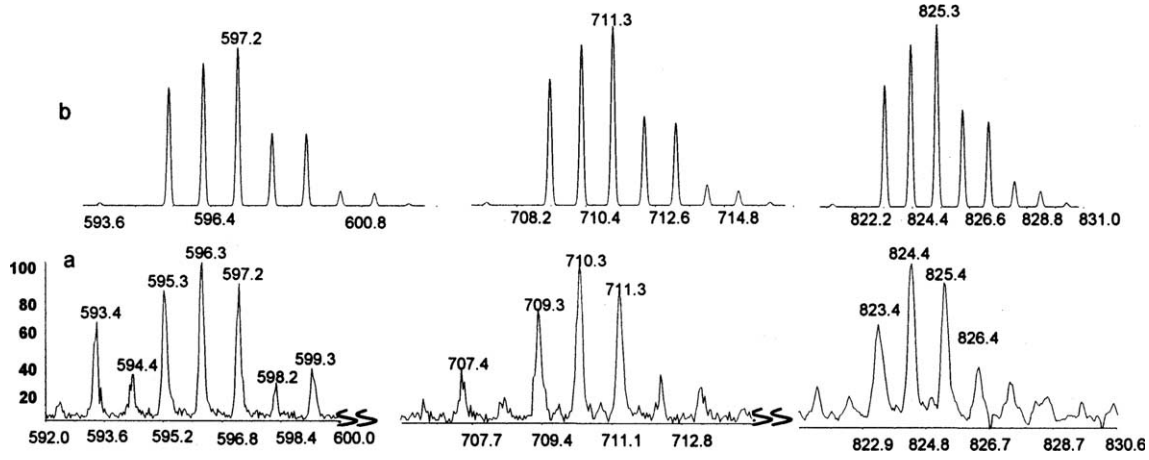

Fig. 2. Experimental (a) and calculated (b) ESI mass spectra of G_0-Pt(II) species in sample **3**. The peak centred to 597.4, 711.6 and 825.6 *m*/*z* were assigned to $2_{br}G_0Pt^+$, $3_{br}G_0Pt^+$, G_0Pt^+, respectively (see Chart 2).

species of the type *trans*-(*C*,*N*)[Pt(DMSO)(PAMAM)Cl(CH_3)]. MALDI mass spectrum of sample **4** (*m*/*z* range 600–900) shows significant peaks assigned to the neutral species (Table 1 and Chart 3) indicated, respectively, with $G_{0(N-N)}Pt$ and $G_{0(C-N)}Pt$, having a number entire and fractional of branches. In particular, intense peaks at *m*/*z* 842.8 and 865.3 were ascribed to *trans*-(*C*,*N*)[Pt(DMSO)(PAMAM)ClCH_3] plus a proton and a sodium ion, respectively, achieved during the MALDI ionization process. This integral species corresponds exactly to the *trans* species mono-coordinated to a primary amino group of PAMAM. Peaks at *m*/*z* 712.9, 728.7 and 749.8 were assigned, respectively, to $[G_0(N–N)Pt–CH_3–Cl + H]^+$, $[G_0(N–N)Pt–Cl + H]^+$ and to $[G_0(N–N)Pt–CH_3 + H]^+$, which derive from removal of methyl and/or chloride groups from *cis*-(*N*,*N*)[Pt(PAMAM)CH_3Cl]. Similarly mass values assigned to defective $3_{br}G_0$(N–N)Pt minus methyl or chloride or both are reported in Table 1. The observation of Pt–Cl or Pt–CH_3 fragments indicates that gas-phase reactions or rearrangements occur in the ion source [59]. On the other hand, a detailed tandem mass spectrometry analysis of the fragmentation pattern suggested that the nitrogen–metal bond is rather strong in the gas phase. In the region below *m*/*z* 900, MALDI spectra (not reported) show peaks relative to uncomplexed dendrimer as in the case of sample **3**. Other product complex species (i.e., aquo-species) were not identified, both in sample **3** and **4**, because insufficient data are available for definitive assignments.

3. Concluding remarks

MALDI MS and ESI MS have revealed to be very informative on the nature of the species present in complex reaction mixture of inorganic or organometallic complexes and ligands with multiple binding sites. On considering the application of dendrimers in gene transfer therapy [42], metal complexes containing such

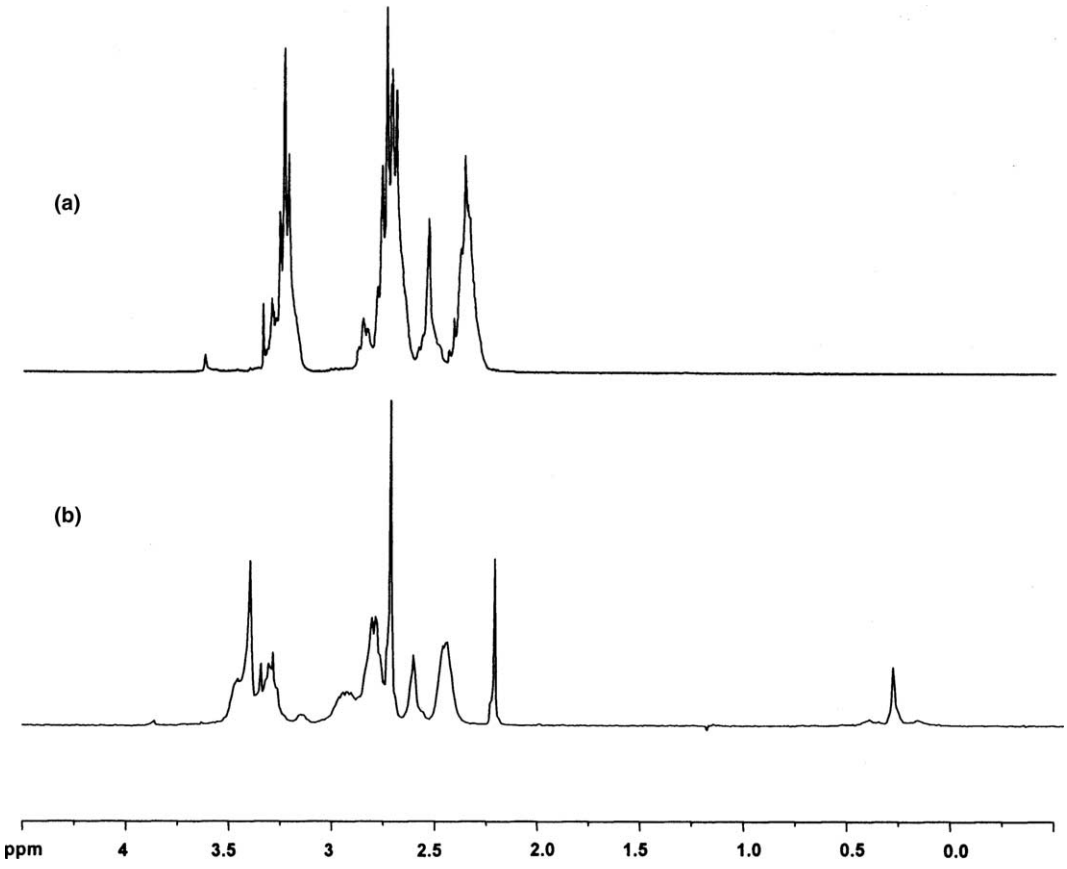

Fig. 3. ^{1}H NMR spectra of (a) G_0 alone and (b) after addition of equimolar amount of *trans*-[Pt(DMSO)$_2$Cl(CH$_3$)] in D$_2$O/NaCl (100 mM) at 298 K.

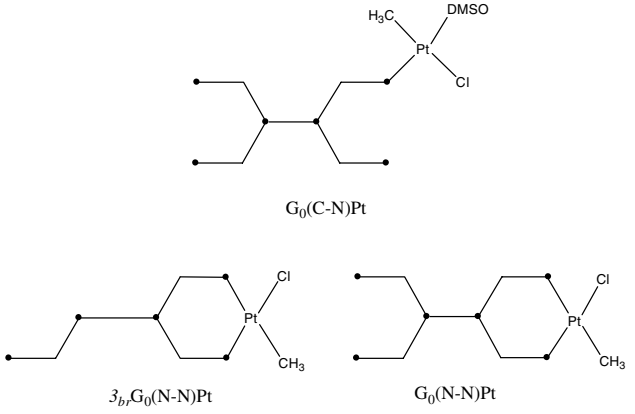

Chart 3.

macromolecules can be of interest in metal drug delivery [62]. Preliminary investigations have shown that mononuclear complexes with PAMAM as ligand, e.g., *trans*-(*C*,*N*)[Pt(DMSO)(PAMAM)Cl(CH$_3$)] or *cis*-[Pt(DMSO)(PAMAM)Cl]Cl (Fig. 4), can act as multifunctional binders with nucleic acids. These species are high flexible and possess primary and tertiary amino groups, which can be protonated at physiological pH values. Such property can be conveniently exploited to direct the metal fragment towards the negatively charged phosphate groups of the DNA backbone, and facilitating in such a way the interaction between platinum (II) and the nucleobases.

4. Experimental

4.1. Materials

K_2PtCl_4 (Strem Chemical Co.) was purified from metallic Pt and K_2PtCl_6 by dissolving it in water and filtering. *cis*-[PtCl$_2$(DMSO)$_2$] (**1**) [22] and *trans*-[Pt(DMSO)$_2$Cl(CH$_3$)] (**2**) [28] were prepared according to the literature methods. The solvents used were purified and dried by standard techniques. All the other reagents were of the highest commercial grade available and were used as received or were purified by distillation or recrystallization when necessary. Starburst PAMAM dendrimer (G = 0, 1,2-diaminoethane core) was received from Aldrich as methanol solution (20% w/w) and its purity was checked through NMR spectroscopy and MALDI spectrometry (sample solution 0.02% in methanol). This latter technique pointed out the presence, together with the expected compound, of various minor components, which have been identified as defective structures of PAMAM. The entire and the defective

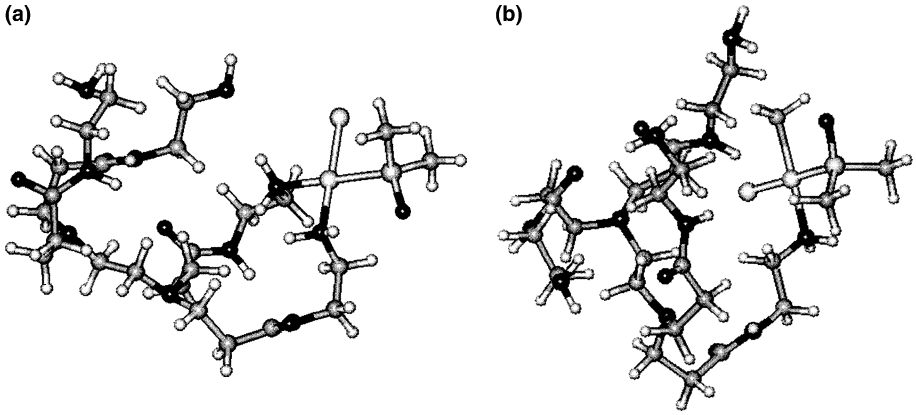

Fig. 4. MM2 minimized (HYPERCHEM v5.0) molecular structures of (a) *cis*-[Pt(DMSO)(PAMAM)Cl] Cl and (b) *trans*-(*C*,*N*)[Pt(DMSO)-(PAMAM)Cl(CH_3)].

structures are abbreviated G_0 and $x_{br}G_0$ with $x = 2$, 3, 3.5, 5, ... branches (br), respectively. The semi-integer x number refers to systems having interlooping defects (for an exemplification see Chart 1). For convention, we have indicated with a full dot the nitrogen atoms of the ligand.

4.2. Reaction of complexes *1* and *2* with PAMAM

4.2.1. Reaction of cis-dichlorobis(dimethylsulfoxide)platinum (II) (*1*) with Starbust PAMAM G_0

Complex **1** (0.03 g, 0.071 mmol) was suspended by stirring in methanol (30 mL) and lightly sonicated. PAMAM (0.063 g, 0.12 mmol) was diluted in methanol (30 mL) and added dropwise. The reaction mixture was stirred for about 1 day at room temperature, until the starting complex was completely solubilized. After removing the solvent under vacuum at 30 °C, the pale-yellow oil was re-dissolved in the minimum volume of CD_3OD. ^{1}H NMR (CD_3OD) δ/ppm: 3.64–3.45 (br. m, CH_2CH_2NHCO, 8H), 3.34 (br.s, DMSO, 6H), 2.97–2.82 (br.m, $(CH_2)_2$-en-$(CH_2)_2$, NH_2CH_2, 16H), 2.66–2.70 (br.s, NCH_2CH_2N, 4H), 2.52–2.31 (br.m, CH_2CH_2CONH, 8H).

The main product *cis*-(dimethylsulfoxide)(*Starburst*-PAMAM-G_0)chloroplatinum (II) chloride, *cis*-Pt(DMSO)(PAMAM)Cl]Cl (**3**), and its related species are abbreviated G_0Pt^+ and $x_{br}G_0Pt^+$, and reported in Chart 2.

4.2.2. Reaction of trans-chloromethylbis(dimethylsulfoxide)platinum (II) (*2*) with Starbust PAMAM G_0

Complex **4** and related species were prepared by reacting complex **2** (1.7 mg, 0.0041 mmol) with a slight excess of pure PAMAM ligand (0.0043 mmol) in D_2O and in the presence of sodium chloride (0.5 mL, 100 mM NaCl). ^{1}H NMR (D_2O) δ/ppm: 3.60–3.19 (br.m, CH_2CH_2NHCO, CH_3 of Pt-DMSO, 14H), 3.07–2.67 (br.m, $(CH_2)_2$-en-$(CH_2)_2$, NH_2CH_2, 16H), 2.62 (br.s, NCH_2CH_2N, 4H), 2.54–2.36 (br m, CH_2CH_2CONH, 8H), 0.33 (s, Pt–CH_3, $^2J_{Pt-H}$ = 68 Hz, 3H).

The main product *trans*-(*C*,*N*)-methylchloride(dimethylsulfoxide)(Starbust-PAMAM- G_0)platinum(II), *trans*-(*C*,*N*)[Pt(DMSO)(PAMAM)Cl(CH_3)] (**4**), and its related species are abbreviated $G_{0(C-N)}Pt$, while the entire and defective species of the type *cis*-(*N*,*N*)-[Pt-(PAMAM)CH_3Cl] are indicated $G_{0(N-N)}Pt$ and $x_{br}G_{0(N-N)}Pt$, respectively, and reported in Chart 3.

4.3. Instruments

MALDI-MS spectra were acquired using a Perseptive BioSystems Voyager-DE STR (Framingham, MA, USA) equipped with delayed extraction technology operating in linear mode. Resolution was below 2000 m/Δm. Positive ions were accelerated through 20 kV. The dried analytes **3** and **4** (1 mg) were dissolved in H_2O (20 mL). 2,5-Dihydroxybenzoic acid (30 mg/mL in H_2O-CH_3CN 3:1 v/v containing 0.1% trifluoroacetic acid) was used as the matrix. The sample-matrix solution (1:3 v/v) was deposited (1 μL) onto the probe tip and dried at room temperature. In order to obtain a stronger signals, re-crystallisation from methanol (0.2 μL) was performed according to a published procedure [63]. External calibration was achieved by using a mix of adrenocorticotropic hormone (ACTH fragment 1-17 and ACTH fragment 7-38, 1:1 mol/mol).

Samples, dissolved in 200 μL of H_2O, were also analyzed by using Perseptive BioSystems ESI Mariner^{TM} mass spectrometer (flow rate 7 μL/min). Nozzle potential was set at 90V, detector voltage at 2100 V and spray tip potential at 3500 V. Resolution was about 5000 m/Δm.

^{1}H NMR spectra were obtained on a Bruker AMX-R 300 spectrometer equipped with a broadband probe operating at 300.13 MHz. Sample solutions in D_2O

(99.8%) were referenced to 3-(trimethylsilyl)propionate (TSP). In each case, chemical shift (δ) are reported in p.p.m. downfield from TMS and coupling constants in Hz.

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