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Dinuclear Platinum Complexes with Biological Relevance Based on the 1,2-Diaminocyclohexane Carrier Ligand

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The synthesis of bifunctional dinuclear platinum complexes, [{PtCl(dach)}$_2$-μ-Y]$^{n+}$Cl$_n$ (**1**–**3**; Y = $H_2N(CH_2)_3NH_2(CH_2)_4NH_2$, $H_2N(CH_2)_6NH_2(CH_2)_6NH_2$, and $H_2N(CH_2)_6NH_2(CH_2)_2NH_2(CH_2)_6NH_2$, respectively; Figure 1) is reported. There was no labilization of the polyamine linker groups of the *cis*-1,2-diaminocyclohexane complexes in the presence of sulfur-containing species at physiological pH, in contrast to previous studies preformed on trans complexes. Metabolism reactions are somewhat dependent on the nature of the polyamine: at physiological pH, the spermidine complex **1** forms an inert (tetraamine)platinum species in which one platinum is chelated by a central and terminal amino group. The stability of cis-geometry complexes may make them viable second-generation polynuclear platinum clinical candidates.

The general (di,tri)nuclear platinum structural motif offers a series of potent anticancer compounds with a rich variety of DNA binding modes.[1,2] Their chemistry and biological activity may be modulated by the geometry and number of leaving groups in the coordination sphere as well as the nature of the di/polyamine linking the platinum centers. The first of these complexes to enter clinical trials, BBR3464, [{*trans*-PtCl$(NH_3)_2$}$_2$-μ-{*trans*-Pt$(NH_3)_2(NH_2(CH_2)_6NH_2)_2$}]$(NO_3)_4$, showed some responses in cancers not usually sensitive to cisplatin treatment.[1,3] The polynuclear motif of BBR3464, and the general 1,1/t,t structure [{*trans*-PtCl$(NH_3)_2$}$_2$-μ-Y]$^{n+}$ (Y = linker such as $NH_2(CH_2)_6NH_2$, spermidine, etc.), is susceptible to decomposition, and the products observed in blood may be mimicked by reactions with sulfur-containing nucleophiles.[4−6] Briefly, substitution of the Pt−Cl bond by a trans-influencing S donor results in the loss of the di/polyamine and bridge cleavage.[6] In contrast, the 1,1/c,c cis geometry as in [{*cis*-PtCl$(NH_3)_2$}$_2$-μ-Y]$^{n+}$ does not undergo breakdown of the dinuclear structure upon reaction with sulfur nucleophiles. In the case of thiol, novel thiolate-bridged macrocycles are formed, and with the thioether methionine, slow loss of NH_3 is eventually observed.[7,8]

Second-generation polynuclear platinum clinical candidates should maintain the target (DNA) binding profile of the "parent" compound and be less susceptible to metabolic breakdown. The positively charged polyamine linker is a critical feature that contributes to cellular uptake, DNA binding, and high antitumor activity. To this extent, we now report on the synthesis and chemistry of "second generation" dinuclear polyamine-bridged complexes containing 1,2-diaminocyclohexane (dach). Use of the chelating diamine further enhances the robustness of the compounds, in combination with the high interstrand cross-linking associated with the cis (1,1/c,c) geometry.[9,10] Dinuclear platinum complexes with dach as the carrier group are also of interest in their own right as dinuclear analogues of oxaliplatin.

The compounds of this general structure, [{PtCl(dach)}$_2$-μ-Y]$^{n+}$, where Y is a variable-length spermidine (n = 3) or spermine-like (n = 4) linker, were prepared by known synthetic pathways.[11] This involves the sequential selective protection and deprotection of the terminal amines of the polyamine to give as the reactant ligand the centrally blocked (*tert*-butoxycarbonyl)polyamines such as for spermidine $H_2N(CH_2)_3NBoc(CH_2)_4NH_2$. Platination is achieved with the monoactivated *cis*-[PtCl(DMF)(dach)]$^+$, followed by deprotection of the central nitrogen to produce **1**−**3** (Figure 1).

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(1) Farrell, N. *Met. Ions Biol. Syst.* **2004**, *42*, 251−296.
(2) Farrell, N.; Qu, Y.; Bierbach, U.; Valsecchi, M.; Menta, E. In *Cisplatin*; Lippert, B., Ed.; Verlag Helvetica Chimica Acta: Zurich, Switzerland, and Wiley-VCH: New York, 1999; pp 479−496.
(3) Farrell, N. In *Platinum-Based Drugs in Cancer Therapy*; Kelland, L. R., Farrell, P., Eds.; Humana Press Inc.: Totowa, NJ, 2000; pp 321−338.
(4) Vacchina, V.; Torti, L.; Allievi, C.; Lobinski, R. *J. Anal. At. Spectrom.* **2003**, *18*, 884−890.
(5) John, T.; Ottley, C. J.; Pearson, D. G.; Nowell, G. M.; Calvert, H.; Tilby, M. J. *Int. Conf. Plasma Source Mass Spectrom., 8th* **2003**, 82−90.
(6) Oehlsen, M. E.; Qu, Y.; Farrell, N. *Inorg. Chem.* **2003**, *42*, 5498−5506.
(7) Oehlsen, M. E.; Hegmans, A.; Qu, Y.; Farrell, N. *Inorg. Chem.* **2005**, *44*, 3004−3006.
(8) Oehlsen, M. E.; Hegmans, A.; Qu, Y.; Farrell, N. *J. Biol. Inorg. Chem.* **2005**, *10*, 433−442.
(9) Farrell, N.; Appleton, T. G.; Qu, Y.; Roberts, J. D.; Fontes, A. P.; Skov, K. A.; Wu, P.; Zou, Y. *Biochemistry* **1995**, *34*, 15480−15486.
(10) Kasparkova, J.; Novakova, O.; Vrana, O.; Farrell, N.; Brabec, V. *Biochemistry* **1999**, *38*, 10997−11005.
(11) Rauter, H.; Di Domenico, R.; Menta, E.; Oliva, A.; Qu, Y.; Farrell, N. *Inorg. Chem.* **1997**, *36*, 3919−3927.

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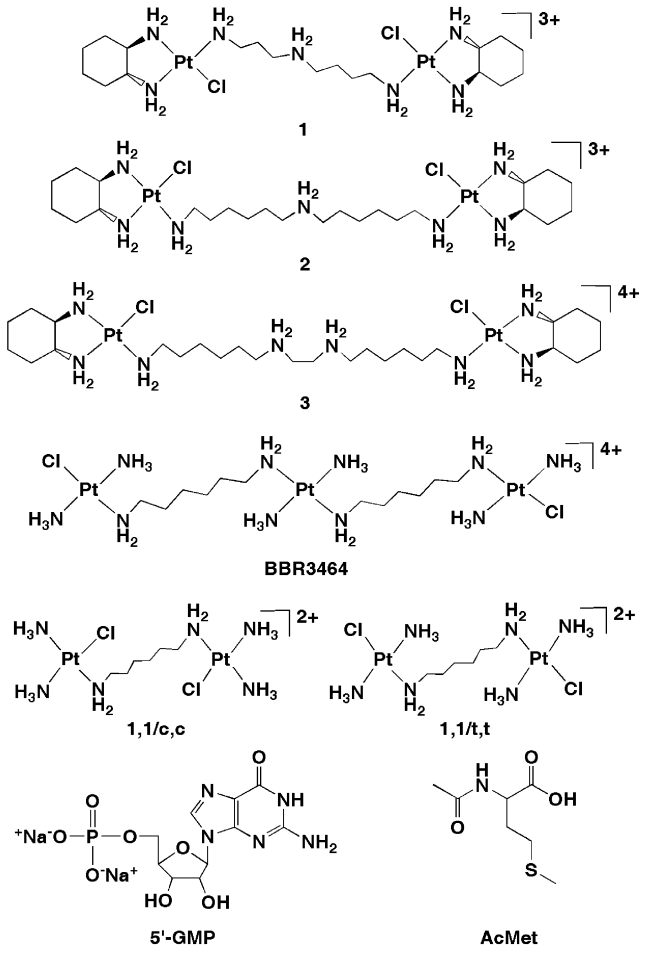


Figure 1. Structures of polynuclear platinum complexes [{*cis*-PtCl(dach)}$_2$-μ-H$_2$N(CH$_2$)$_3$NH$_2$(CH$_2$)$_4$NH$_2$]$^{3+}$ (**1**), [{*cis*-PtCl(dach)}$_2$-μ-H$_2$N(CH$_2$)$_6$-NH$_2$(CH$_2$)$_6$NH$_2$]$^{3+}$ (**2**), [{*cis*-PtCl(dach)}$_2$-μ-H$_2$N(CH$_2$)$_6$NH$_2$(CH$_2$)$_2$NH$_2$(CH$_2$)$_6$-NH$_2$]$^{4+}$ (**3**), [{*trans*-PtCl(NH$_3$)$_2$}$_2$-μ-{*trans*-Pt(NH$_3$)$_2$(NH$_2$(CH$_2$)$_6$NH$_2$)}]$^{4+}$ (BBR3464), [{*cis*-PtCl(NH$_3$)$_2$}$_2$-μ-H$_2$N(CH$_2$)$_6$NH$_2$]$^{2+}$ (1,1/c,c), and [{*trans*-PtCl(NH$_3$)$_2$}$_2$-μ-H$_2$N(CH$_2$)$_6$NH$_2$]$^{2+}$ (1,1/t,t) as well as guanosine 5′-monophosphate (GMP) and sulfur nucleophile *N*-acetyl-L-methionine (AcMet).

The complexes were characterized by elemental analysis and ^{1}H and ^{195}Pt NMR spectroscopy (see the Supporting Information).

Both cis- and trans-oriented dinuclear platinum complexes (1,1/c,c and 1,1/t,t) are antitumor-active.[2] In comparative DNA-binding studies, the more sterically hindered cis compound binds to DNA at slower rates and produces a higher proportion of interstrand cross-links than its trans isomer.[9,10] The reactions of the new compounds were examined with model nucleotides (5′-guanosine monophosphate (GMP)) and amino acids (L-methionine) for comparison with previous results.

In a 1:2 reaction of **2** or **3** (5 mM) and GMP (10 mM) under physiological conditions, ^{1}H and ^{195}Pt NMR spectroscopy showed the stepwise substitution

$$\mathrm{ClPt{-}PtCl} \rightarrow \mathrm{ClPt{-}Pt(GMP)} \rightarrow \mathrm{(GMP)Pt{-}Pt(GMP)}$$

The ^{1}H NMR spectrum of the GMP H(8) showed typical deshielding shifts, and the ^{195}Pt NMR spectra showed new peaks at approximately −2700 ppm indicative of the PtN$_4$ coordination sphere (Table 1).

Table 1. ^{195}Pt NMR Shifts for Polynuclear Platinum Complexes and the Products of Their Reaction with GMP and AcMet[a]

	coordination sphere			
complex	PtN$_3$Cl	PtN$_3$(GMP)	PtN$_3$(cyc)	PtN$_3$S
1-Boc	−2597	−2727		−3267
1	−2608	−2728	−2882	−3266
2	−2607	−2732		−3263
3	−2602	−2729		−3269

[a] ^{195}Pt referenced to Na$_2$[PtCl$_6$], $\delta = 0$ ppm.

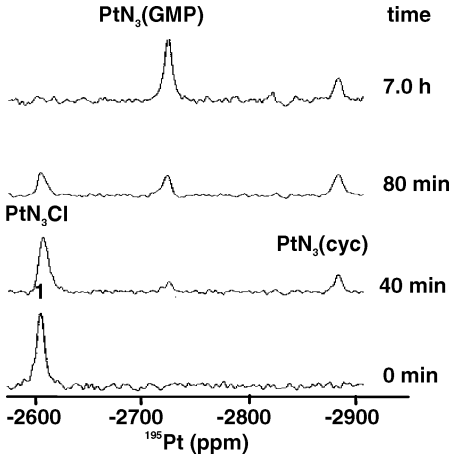
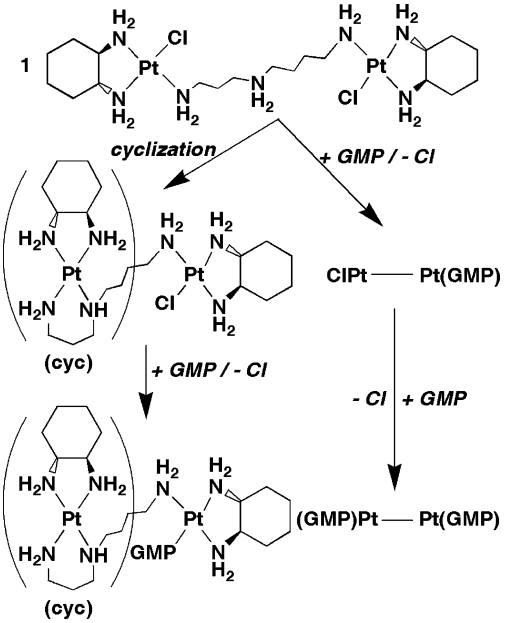


Figure 2. ^{195}Pt NMR spectra of the 1:2 (**1**/GMP) reaction at $t = 40$ min, 80 min, and 7.0 h. Compound **1** showed signs of cyclization at 40 min.

Scheme 1



In the case of **1**, ^{195}Pt NMR spectroscopy showed two new peaks at −2727 and −2884 ppm within 40 min. The former peak increased in intensity as the reaction proceeded, while the latter remained relatively constant. Control experiments at pH 7.4 in the absence of the mononucleotide gave ^{195}Pt NMR signals at −2883 and −2610 ppm, consistent with competitive chelation of one platinum unit from the central spermidine nitrogen producing an inert six-membered (tetraamine)platinum species, PtN$_4$(cyc) (Figure 2 and Scheme 1). The percentage of chelation increased upon an increase in the pH of the solution. At pH > 10, a new peak appeared

at −2323 ppm and was assigned to a PtN_3O coordination sphere, attributed to the possible formation of intramolecular hydroxo bridges.[12] When the precursor 1-Boc was allowed to react with GMP, only one product peak at −2730 ppm was observed with no evidence of chelation. The chelate formation of small-chain polyamines is consistent with previous observations; indeed, attempts to prepare the spermine-linked compound invariably gave mixtures, and this particular ligand was not pursued further.[13−15]

The ^{195}Pt NMR spectra of the reaction between complexes **1**−**3** and AcMet (both 1:2 and 1:4 complex/ligand ratios) gave final products corresponding to PtN_3S coordination spheres. Similar reactions have been observed for the "simple" 1,1/c,c compound, but in this case, products containing PtN_2S_2 coordination spheres ($\delta_{Pt} = -3410$ ppm) resulting from slow loss of one NH_3 group and further substitution by methionine are observed. In contrast, the current results confirm that the presence of the bidentate amine prevents such trans labilization: the bidentate carrier group remains intact, and no evidence for ring opening was observed.[8] For **1**, a very small amount of the PtN_4(cyc) species is observed, even after the disappearance of all of the starting material. This suggests that chelate formation may displace the Pt−S bond, but the extent of chelation is significantly less than that in the 5′-GMP case.

No detailed kinetic analyses have been performed on the reactions with small-molecule nucleotides and amino acids, but some reasonable estimates may be gauged by comparing the approximate half-time to reaction, based on integration of nonexchangeable ^{1}H and ^{195}Pt NMR signals. The rate of reaction with GMP for the spermidine-linked compound is not possible to estimate because of the competing cyclization reaction, but the half-lives for **2** and **3** are approximately 80 min. These are significantly faster compared with those of 1,1/t,t (185 min) and 1,1/c,c (250 min).[16] Possible explanations are the enhanced charge on the platinum complex (3+ and 4+ for **2** and **3**, respectively) and the smaller bite angle of the five-membered ethylenediamine-based chelate compared to those of the *cis*-Pt$(NH_3)_2$ group. In agreement with the former possibility, the approximate half-time for the reaction of I-Boc (2+ charge, central nitrogen blocked) with 5′-GMP is 225 min. The reaction with N-AcMet is faster with approximately half-times of 20−30 min in all three cases; in this case, N-AcMet binding to **1** is faster than chelation.

The introduction of biologically relevant polyamines as linker groups into the polynuclear platinum complexes has a significant impact on their DNA-binding properties (binding ratio and conformational changes), cellular uptake, and cytotoxicity.[2,17] Long-chain polyamines avoid the cis-complexation reactions of spermidine and spermine. The current series of dach-based dinuclear compounds will be expected to mimic the DNA-binding profile of BBR3464, while bridge-deactivating reactions are minimized. The biological activity of all new complexes is undergoing investigation. Dinuclear 1,1/t,t polyamine-bridged compounds are themselves very potent; nanomolar cytotoxicity is obtained for the long-chain compound BR3610, [{*trans*-PtCl$(NH_3)_2$}$_2$-μ-{*trans*-$(H_2N(CH_2)_6NH_2(CH_2)_2NH_2(CH_2)_6NH_2$}]$(NO_3)_4$, designed to mimic the same distance between the two Pt−Cl units in BBR3464.[2,18] The compounds described here, such as **3**, should contain the main features of BBR3464 and BBR3610 but with enhanced stability to metabolic deactivation.

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Supporting Information Available: Synthesis and characterization of polyamine ligands and their dach-based dinuclear platinum complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Zhang, J.; Thomas, D. S.; Davies, M. S.; Berners-Price, S. J.; Farrell, N. *J. Biol. Inorg. Chem.* **2005**, *10*, 652−666.
(13) Navarro-Ranninger, C.; Ochoa, P. A.; Perez, J. M.; Gonzalez, V. M.; Masaguer, J. R.; Alonso, C. *J. Inorg. Biochem.* **1994**, *53*, 177−190.
(14) Amo-Ochoa, P.; Gonzalez, V. M.; Perez, J. M.; Masaguer, J. R.; Alonso, C.; Navarro-Ranninger, C. *J. Inorg. Biochem.* **1996**, *64*, 287−299.
(15) Teixeira, L. J.; Seabra, M.; Reis, E.; da Cruz, M. T.; de Lima, M. C.; Pereira, E.; Miranda, M. A.; Marques, M. P. *J. Med. Chem.* **2004**, *47*, 2917−2925.
(16) Mellish, K. J.; Qu, Y.; Scarsdale, J. N.; Farrell, N. **1997**, *25*, 1265−1271.
(17) McGregor, T. D.; Kasparkova, J.; Neplechova, K.; Novakova, O.; Penazova, H.; Vrana, O.; Brabec, V.; Farrell, N. *J. Biol. Inorg. Chem.* **2006**, *7*, 397−404.
(18) Billecke, C.; Finniss, S.; Tahash, L.; Miller, C.; Mikkelsen, T.; Farrell, N. P.; Bogler, O. *Neuro-Oncology (Durham, NC, U.S.)* **2006**, *8*, 215−226.