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# A Surprisingly Stable Macrochelate Formed from the Reaction of Cis Dinuclear Platinum Antitumor Compounds with Reduced Glutathione

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The structurally unique macrochelate [{Pt(en)}$_2$-$\mu$-{$H_2N(CH_2)_6NH_2$}-$\mu$-(SG)] (**I**) is the principal product of the reaction of the dinuclear compound [{PtCl(en)}$_2$-$\mu$-{$H_2N(CH_2)_6NH_2$}]$Cl_2$ **(1)** with reduced glutathione (GSH) in a stoichiometric 1:1 ratio in phosphate buffered saline (PBS) (pH 7.35). The macrochelate is formed through simultaneous bridging of the hexanediamine linker and glutathione thiolate. This represents a novel structure for glutathione adducts of platinum. At higher (1:4) ratios of Pt complex to GSH, an interesting interchange between bridged Pt–(SG)–Pt and terminal Pt–SG species is observed with the diamine linker still remaining intact in all cases. The integrity of **I** is further evident when reaction ratios are increased to 1:4 (Pt complex/GSH), and additional minor products are identified as [{Pt(en)SG}$_2$-$\mu$-{$NH_2(CH_2)_6NH_2$}] (**II**), which transforms to [{Pt{$NH_2(CH_2)_2NH_2$}-(SG)}$_2$-$\mu$-{$H_2N(CH_2)_6NH_2$}-$\mu$-(SG)] (**III**), where the chelate ring is broken to produce a dangling monodentate ethylenediamine. The chemical shifts of the Pt–$NH_2$ linker in all compounds are explained by consideration of the enhanced rigidity of the macrochelate (**I**) leading to shielding in comparison to the "open" monodentate structures (**II**, **III**). The remarkable stability of **I** is discussed in terms of possible biological implications.

The efficacy of platinum-based anticancer agents is a balance between target efficiency (DNA binding) and metabolism by sulfur nucleophiles. Prominent among these is the cysteine-containing tripeptide glutathione (GSH).[1−6] The principal binding modes of the Pt-glutathione (and Pt-cysteine) conjugates have been unambiguously identified as either monodentate Pt−GS or bridged Pt−GS−Pt.[2,3,6] Further reaction through N or O donor atoms of the peptide can occur through labilization of ligands ($NH_3$ or Cl) either cis or trans to the sulfur donor.[7,8] In this contribution, we describe the novel products of glutathione with [{PtCl(en)}$_2$-$\mu$-($H_2N(CH_2)_6NH_2$)]$Cl_2$ (**1**, normal and $^{15}$N-labeled at all nitrogen atoms) under physiological conditions (pH 7.35, 37 °C). The cis dinuclear geometry results in formation of a structurally unique 11-membered macrochelate in which the two Pt centers are bridged by both the diamine linker and the glutathione thiolate. (See Figure 1.)

In dinuclear or trinuclear [{*trans*-{$PtCl(NH_3)_2$}$_2$-$\mu$-Y]$^{n+}$ [Y = $H_2N(CH_2)_6NH_2$ or *trans*-$Pt(NH_3)_2(NH_2(CH_2)_6NH_2)_2$], metabolic interactions break the bridge through labilization of the Pt−$NH_2$ bond trans to glutathione.[5,6] Dinuclear platinum complexes with monofunctional coordination spheres exist as cis and trans isomers, where the geometry is dictated by the relative positions of the chloride and the diamine linker. Unlike the mononuclear compounds, both cis and trans dinuclear compounds are antitumor active. In comparative DNA-binding studies, the more sterically hindered cis compound binds to DNA at lower rates than its trans isomer and causes a higher proportion of (Pt−Pt) interstrand cross-links.[9,10]

In a 1:1 reaction of **1** and GSH (4 mM), $^{195}$Pt NMR spectroscopy showed the disappearance of starting material within 1 h and one new peak at −3140 ppm ($^{195}$Pt spectra were referenced to $Na_2[PtCl_6]$). The spectrum remained essentially unchanged up to 24 h. ESI-TOF MS of the sample (after a reaction time of 10 h) gave one signal at a mass of 930.49 amu, corresponding to the Pt−S bridged species [{Pt(en)}$_2$-$\mu$-{$H_2N(CH_2)_6NH_2$}-$\mu$-(SG)] (**I**, Scheme 1). The $^{195}$Pt NMR spectrum of a 1:4 sample acquired at $t$ = 10 h showed two peaks at −3140 and −3151 ppm. The corresponding ESI-TOF MS now showed the presence of **I** along with a new peak at 1237.08 amu assigned to [{Pt(en)SG}$_2$-$\mu$-{$H_2N(CH_2)_6NH_2$}] (**II**, Scheme 1**)**. At $t$ = 24 h, the relative ratio of these two species changed with the appearance of a third peak at $m/z$ = 1544 amu, consistent with the

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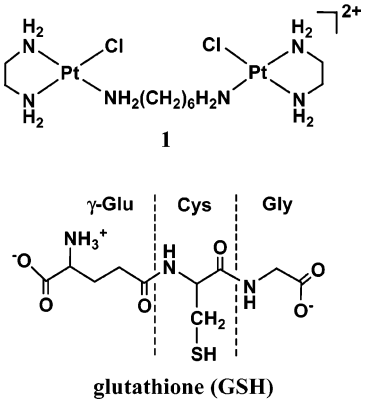
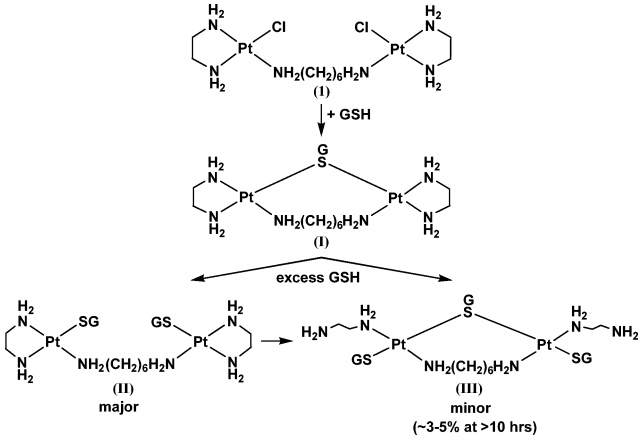



**Figure 1.** Structures of $[\{PtCl(en)\}_2\text{-}\mu\text{-}(NH_2(CH_2)_6NH_2)]^{2+}$ (**1**) and glutathione (GSH).

**Scheme 1.** Proposed Mechanism for the Reaction of **1** with Excess GSH[a]




[a] Charges omitted.

**Table 1.** $^{195}Pt$ $\{^1H-^{15}N\}$ NMR and ESI-TOF MS Values for the Products of **1** with Reduced GSH

| platinum complex | | $^1H/^{15}N$ (ppm) | $^{15}N$ trans to | $^{195}Pt$ (ppm) | $[M^+]$ (*m*/*z*) |
|---|---|---|---|---|---|
| $[\{Pt(en)Cl\}_2\text{-}\mu\text{-}\{H_2N(CH_2)_6NH_2\}]Cl_2$ | **I** | 4.44/−41.7 | en-$NH_2$ | − | − |
| | | 4.92/−30.0 | linker $NH_2$ | −2636 | N/A |
| | | 5.42/−29.6 | Cl | − | − |
| $[\{Pt(en)\}_2\text{-}\mu\text{-}\{H_2N(CH_2)_6NH_2\}\text{-}\mu\text{-}SG)]$ | **I** | 4.23/−43.1 | en-$NH_2$ | − | − |
| | | 4.93/−29.8 | linker $NH_2$ | −3140 | 930 |
| | | 4.98/−9.80 | bridged S | − | − |
| $[\{Pt(en)SG\}_2\text{-}\mu\text{-}\{H_2N(CH_2)_6NH_2\}]$ | **II** | 4.49/−40.2 | en-$NH_2$ | − | − |
| | | 4.95/−29.8 | linker $NH_2$ | −3151 | 1237 |
| | | 5.57/−9.98 | S | − | − |
| $[\{Pt[NH_2(CH_2)_2NH_2]SG\}_2\text{-}\mu\text{-}\{H_2N(CH_2)_6NH_2\}\text{-}\mu\text{-}(SG)]$ | **III** | 4.48/−39.9 | en-$NH_2$ | −3230 | 1544 |
| | | 5.49/−26.3 | linker $NH_2$ | − | − |

ring-opened $[\{Pt\{NH_2(CH_2)_2NH_2\}(SG)\}_2\text{-}\mu\text{-}\{H_2N(CH_2)_6\text{-}NH_2\}\text{-}\mu\text{-}(SG)]$ (**III**, Scheme 1). The corresponding $^{195}Pt$ NMR shift is −3230 ppm. The chemical shifts are consistent with the slight differences in $PtSN_3$ and $PtS_2N_2$ coordination spheres.[2,3,6,11] Characterization data for all structures reported here are listed in Table 1 and shown in the Supporting Information (Figures S1 and S2).

The $\{^1H-^{15}N\}$ HSQC NMR spectrum of **1** ($^{15}N$-labeled at all nitrogen atoms) showed three peaks assigned as the linker $^{15}NH_2$ ($\delta$ $^1H$, 4.44)/($\delta$ $^{15}N$, −41.7) and the two

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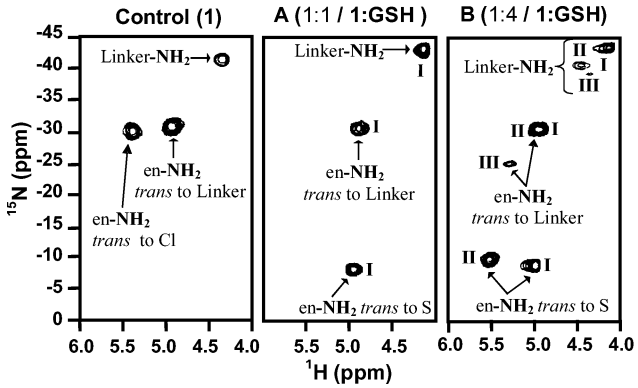


**Figure 2.** 2D $\{^1H-^{15}N\}$ NMR spectroscopy of the reaction of **1** and GSH in 150 mM PBS at 37 °C. (PBS buffer was prepared in 92:8 $H_2O/D_2O$ phosphate buffered saline [phosphate] = 150 mM, pH 7.35, [NaCl] = 120 mM, [KCl] = 2.7 mM.) $^1H-^{15}N$ spectra were referenced to sodium 3-(trimethylsilyl)propionate (TSP) and $^{15}NH_4NO_3$ at $\delta = 0$ ppm. The panel labeled "control" is the spectrum of 4 mM **1**. (A) Spectrum of the 1:1 reaction mixture at 10 h. (B) Spectrum of the 1:4 reaction after 24 h. The concentration of complex **1** was 4 mM in all reactions. Three distinct products (**I**−**III**) can be assigned; see Table 1, Figure S2, and text.

ethylenediamine nitrogen atoms (4.92/−30.0 and 5.42/−29.6) (Figure 2, control) The en-$^{15}NH_2$ trans to the Cl was assigned to the peak farther downfield (5.42 ppm) on the proton axis. The 1:1 spectrum after 10 h showed that the linker remained relatively unaffected, with chemical shifts at 4.23/−43.1, while the en-$^{15}NH_2$ trans linker sustained shifts to 4.94/−29.8. In contrast, the third peak detected at 4.98/−9.8 is in the chemical shift range for an en-$^{15}NH_2$ trans to a sulfur atom[11] and is thus assigned to the en-$^{15}NH_2$ trans to the coordinated S (Figure 2A). The $\{^1H-^{15}N\}$ shifts are in agreement with the proposed structure of **I**. At a ratio of 1:4, some overlap occurs, but distinct peaks due to **II** were detected at 5.57/−9.98 (en-$^{15}NH_2$ trans to S) and 4.49/−40.2 (linker $^{15}NH_2$) (Figure 2B).

Thus, the single generated product of the 1:1 reaction is the sulfur-bridged **I**, a structurally unique 11-membered macrochelate in which the two Pt units are bridged by two dissimilar groups: cysteine thiol and the $-NH_2$ groups of the linking diamine. **I** is remarkably stable and does not undergo exchange until the addition of excess GSH (Scheme 1). Its conversion to **II** is slow and represents a novel molecular conversion: ring opening and the entry of a second glutathione moiety producing the "open" $Pt(GS)_2N_2$ structure. In no case were any chelation products through O or N binding sites of glutathione observed up to 24 h, in distinct contrast to *cis*-$[PtCl_2(amine)_2]$.[1−3,7,8] The later, but slow formation of the en-ring-opened complex **III** is due to the trans influence of S.

Structural information on **I** and **II** can be obtained from analysis of the NMR chemical shifts aided by molecular modeling (Figure 3). Geometry-optimized molecular modeling gave for **I** a Pt−Pt distance of 3.05 Å and a $NH_2-NH_2$ distance of 4.17 Å, with the two platinum planes making an approximate dihedral angle of 70°. In contrast, the equivalent parameters for **II** are 8.04 Å (Pt−Pt) and 7.20 Å ($NH_2-NH_2$). The S−Pt−S angle for **I** (82.6°) was less than that for $[Pt_2(bipy)_2\text{-}\mu\text{-}(N-AcCys\text{-}S)_2]$ (95.1°).[12] The proximity of the two platinum atoms can result in mutual shielding,

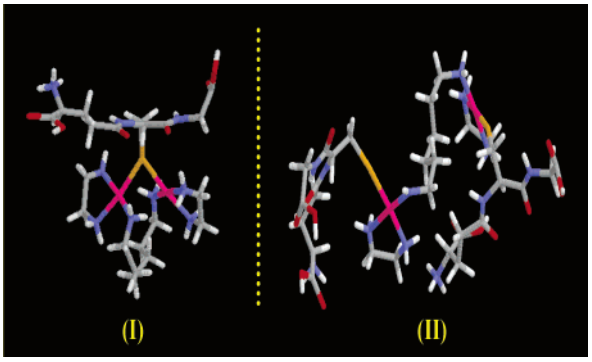


**Figure 3.** Geometry-optimized molecular modeling of the sulfur-bridged complex **I** and the disubstituted platinum complex **II**.

reflecting the relative chemical shifts of **I** and **II**. The linker $NH_2$ of **I** is shielded significantly in comparison to that of **II**. The relatively rigid macrochelate structure is likely to limit solvent access with subsequent shielding, analogous to similar observations in Pt−DNA adducts of $[\{trans\text{-}\{PtCl(NH_3)_2\}_2\text{-}\mu\text{-}(H_2N(CH_2)_6NH_2)]^{2+}$.[13]

A critical feature of di(tri)nuclear compounds is their formation of long-range (Pt−Pt) interstrand cross-links. These adducts are not recognized by HMG-domain proteins, which recognize cisplatin-bound DNA and are poor substrates for nucleotide excision repair.[13,14] The results described herein emphasize the distinct difference between the isomers in dinuclear chemistry, not just in target reactions, but also in metabolism. The trans dinuclear compounds suffer degradation and thus lose their ability to form (Pt−Pt) interstrand cross-links,[7] whereas the dinuclear structure is preserved in the present case. **I** is stable in aqueous solution but undergoes interesting rearrangements in the presence of additional nucleophiles, and it will be of considerable interest to study its reactivity with other biological nucleophiles, including DNA. The dynamic behavior of the macrochelate can be controlled by chain length. The trinuclear compound BBR3464 is the first polynuclear drug to undergo human clinical trials. Responses were observed in cisplatin-relapsed ovarian cancer in Phase II clinical trials.[15] Second-generation analogues can be developed by building on chemical and clinical experience. With respect to the chemistry of anticancer drugs, the present contribution shows that a pharmacokinetic profile of a dinuclear compound can be manipulated through structure from both target (DNA) and metabolism (such as reactivity with GSH) aspects. The cis dinuclear geometry thus represents an intriguing template for further study.

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**Supporting Information Available:** Experimental conditions, $^{195}$Pt NMR spectra of reaction between **1** and GSH (1:1 and 1:4; Figure S1) and ESI-TOF MS of the 1:1 and 1:4 (**1**/GSH) reactions (Figure S2). The 1:4 reaction data were acquired at $t = 10$ and 24 h. This material is available free of charge via the Internet at http://pubs.acs.org.

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