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PHOTOINDUCED CROSS-LINKING OF RNA BY *cis*-Rh(phen)$_2$Cl$_2^+$ AND *cis*-Rh(phen)(phi)Cl$_2^+$: A NEW FAMILY OF LIGHT ACTIVATABLE NUCLEIC ACID CROSS-LINKING AGENTS[1]

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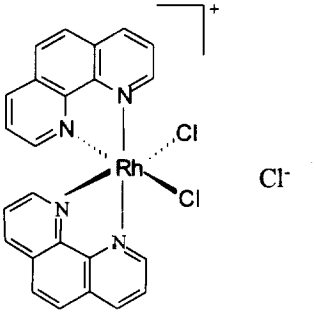
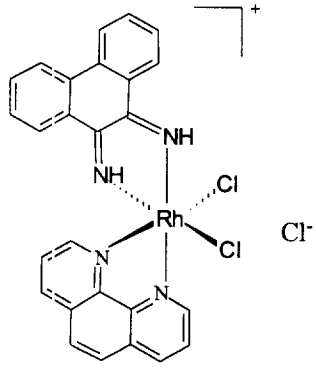
Abstract: The metal complexes, *cis*-Rh(phen)$_2$Cl$_2^+$ and its more hydrophobic analog *cis*-Rh(phen)(phi)Cl$_2^+$, have been shown to photocross-link the 120-base ϕ29-encoded pRNA. Primer extension on the *cis*-Rh(phen)(phi)Cl$_2^+$-photocross-linked RNA revealed that guanines are responsible for the interstrand cross-links.

The development of reagents that can form interstrand cross-links in DNA or RNA continues to be a subject of considerable interest in the areas of molecular biology and rational drug design.[2] The modification of the genome through the creation of such cross-links leads to the inhibition of nucleic acid replication or extension, so that in many cases such agents are potential antitumor drugs.[2a,3] Photochemical cross-linking reagents are of particular interest, since such chemicals can be selectively activated both in time and space, thus providing a "switch-like" mechanism that permits the chemical to be turned on precisely when and where a desired biological effect is intended.[4] For example, psoralens are widely employed photoactivated cross-linking agents that are clinically used in the photochemotherapy of various skin diseases[5] and, more recently, in the photodetoxification of blood.[6] Psoralens predominantly target Us and Ts in RNA and DNA, respectively,[7] and we are unaware of light-activatable reagents that can create such cross-links between purines.[2,8] We thus report here two rhodium(III) octahedral complexes that, upon UVA (320–400 nm) excitation, form cross-links in nucleic acid through the involvement of purines which are proximal within the context of the tertiary structure of the pRNA.

The metal complexes are *cis*-Rh(phen)$_2$Cl$_2^+$ ("BISPHEN", phen = 1,10-phenanthroline)[9] and *cis*-Rh(phen)(phi)Cl$_2^+$ ("PHENPHI", phi = 9,10-phenanthrenequinone diimine)[1,10] (see structures below). We have



cis-[Rh(phen)$_2$Cl$_2$]Cl ("BISPHEN")



cis-[Rh(phen)(phi)Cl$_2$]Cl ("PHENPHI")

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shown that BISPHEN is stable when mixed with DNA in the dark, but undergoes aquation and forms covalent dG adducts with calf thymus DNA upon irradiation with UVA light.[11] The complex preferentially targets purines, and of the purines does more so with guanine than with adenine analogs.[11a,11b] There is evidence that metalation occurs at N1 and O6 of deoxyguanosine, and at N3 of deoxyadenosine,[11b 12] via an electron transfer mechanism.[11d] BISPHEN photosensitizes the inactivation of infectious bacteriophage dsDNA,[11e] apparently by sensitizing pyrimidine dimerization.[11e] PHENPHI covalently binds to DNA with comparable quantum efficiency, and also preferentially targets guanine.[10] The major advantage of PHENPHI is its increased hydrophobicity by virtue of which it is taken up by tumor KB cells. It is phototoxic to both KB cells[10] and bacteriophage ϕ6 virus.[13] We now report that both metal complexes effectively form interstrand cross-links in 120-base ϕ29-encoded pRNA (see structure below) via covalent bond formation between two guanosine bases. This pRNA forms a hexameric complex to gear the DNA translocation process.[14] This is only the second class of compounds (in addition to psoralens) known to cross-link nucleic acid upon irradiation, and represents the first example of the photoinitiated cross-linking of purines.[2a,8]

Mixtures of BISPHEN (0.4 mM) and pRNA[15,16] (ca. 1.0 μg) were irradiated with long wavelength UV light (365 nm)[17] at 0 °C for different intervals and analyzed on an 8% polyacrylamide/8 M urea denaturing gel. A representative gel from several experiments is shown in Figure 1 (panel A). It is clear that controls of pRNA with BISPHEN/no light (lane 4) and with light/no BISPHEN (lane 2) produced no new bands. However, pRNA irradiated in the presence of BISPHEN (400 and 1000 μM) produced a new band with slower mobility than the untreated pRNA (lanes 1, 3 and 5–8). As demonstrated below, this new band was assigned to cross-linked pRNA. The video image digitized data[18] of the cross-linked bands indicated that ca. 5% of pRNA had been cross-linked in

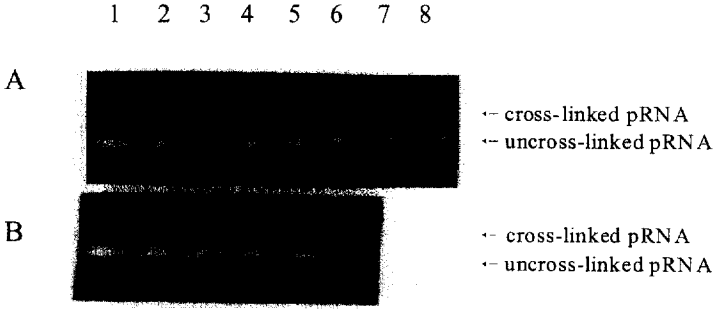

Figure 1: Denaturing 8% polyacrylamide/8 M urea gel of phototreated mixtures of pRNA with BISPHEN (panel A) and PHENPHI (panel B). **Panel A**: lane 1, 10 h irradiation; lane 2, pRNA irradiated for 31 h in the absence of BISPHEN; lane 3, 10 h irradiation with 1000 μM BISPHEN; lane 4, 15 h dark of pRNA/BISPHEN; lane 5, 5 h irradiation; lane 6, 15 h irradiation; lane 7, 25 h irradiation; lane 8, 31 h irradiation. All irradiations were in air at 0 °C with 400 μM BISPHEN except for lane 2 and 3 as noted above. **Panel B**: lane 1, pRNA irradiated in absence of PHENPHI; lane 2-6, pRNA irradiated in presence of 15, 30, 60, 120 and 250 μM PHENPHI, respectively. All irradiations were under air at 0 °C for 16 h; pRNA degradation is evident in the last lane.

in lanes 6–8. At higher concentrations of BISPHEN (ca.1.0 mM) there was also some degradation of the nucleic acid as is evident from the reduction in the density of the intact pRNA band (lane 3). The degradation was found to be a function of the absorbed UV dose.[19] It should be noted that the band labeled as "uncross-linked" is likely to be a mixture of unmodified RNA and RNA containing monoadduct(s); these are indistinguishable under the electrophoretic conditions.

pRNA (ca. 1.0 μg) was irradiated under similar conditions for 16 h with increasing amounts of PHENPHI (0–250 μM). The resultant gel is also presented in Figure 1 (panel B). One major cross-linked band was produced with slower mobility than the uncross-linked pRNA ((lanes 2–5). This band was absent when the pRNA was irradiated without PHENPHI (lane 1). Visual examination indicates that 15–120 μM PHENPHI cross-linked ca.

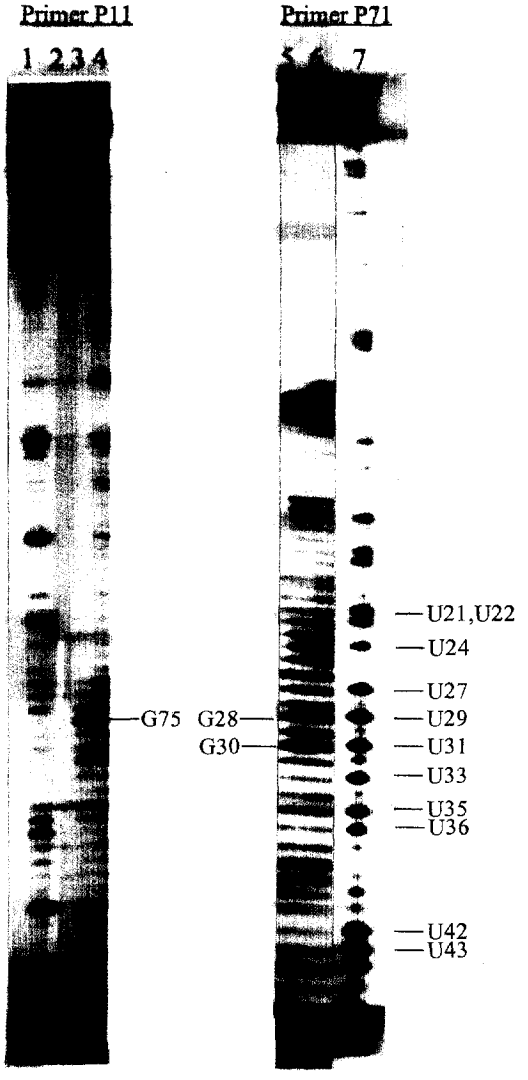

Figure 2: Autoradiogram of an 8% denaturing polyacrylamide/8 M urea sequencing gel showing termination sites of primer extension on isolated pRNA after irradiation with PHENPHI. Primer P11 was used for extension with samples in lanes 1-4, while primer P71 was used in lanes 5-7. Lane 1, untreated pRNA; lane 2, empty; lane 3 and 5, cross-linked pRNA isolated from the gel after irradiation with PHENPHI; lane 4 and 6, uncross-linked pRNA isolated from the gel after irradiation with PHENPHI; lane 7, reverse transcripts of untreated pRNA in the presence of ddATP to reveal terminations at Us for sequencing reading. Note that during primer extension, chain termination occurs one base prior to (downstream of) the cross-linking sites as marked for Gs; monoadducts account for additional terminations in lanes 3-6. Stronger bands corresponding to G75, G30 and G28 were observed with the PHENPHI-photosensitized cross-linked samples.

5–10% of the pRNA, with 100 μM giving optimal results. In a separate experiment, the mobility of the cross-linked band was found to be comparable to that observed for the cross-linked pRNA produced by irradiation of the RNA with 4'-aminomethyl-4, 5',8-trimethylpsoralen[20] (data not shown). As was the case with BISPHEN (*vide supra*), degradation of the pRNA was observed at concentrations > 120 μM (lane 6).

To identify the cross-linking sites, the cross-linked and uncross-linked bands were separately excised, and extracted overnight with 100 μL of elution buffer at 37 °C. The extracted pRNAs were precipitated with ethanol and the pellets dissolved in nuclease-free water (2.4 μL). The isolated RNA samples served as templates for primer extension. The cross-linked pRNA was annealed with 5'[γ-^{32}P]-labeled P11 or P71 primers at 75 °C for 10 min, followed by cooling to ca. 30 °C for 20 min. These two primers target the 3' end and the middle region of the pRNA sequence, respectively. Avian myeloblastosis virus (AMV) reverse transcriptase was used for chain elongation, and the cDNA was run on an 8% polyacrylamide/8 M urea sequencing gel. DNA synthesis on the photocross-linked pRNA template generated a population of DNA fragments (cf. lane 3 and 5 in Figure 2), suggesting that duplex synthesis had been terminated. To aid in the identification of the termination sites, the DNA fragments containing a U ladder were generated (lane 7) by reverse transcription in the presence of ddATP,[21] and were electrophoresed in parallel with the samples in lanes 5 and 6. Primer extension on the untreated pRNA (lane 1) served as a background control for non-specific stops. The uncross-linked pRNA in lanes 4 and 6 gave more specific chain termination points than the untreated pRNA (lane 1), with monoadducts presumably accounting for most of the stops. Analysis of the termination sites from the cross-linked pRNA template produced strong stops prior to G28 and G30 (see lane 5 in Figure 2). This is consistent with our earlier observations that the

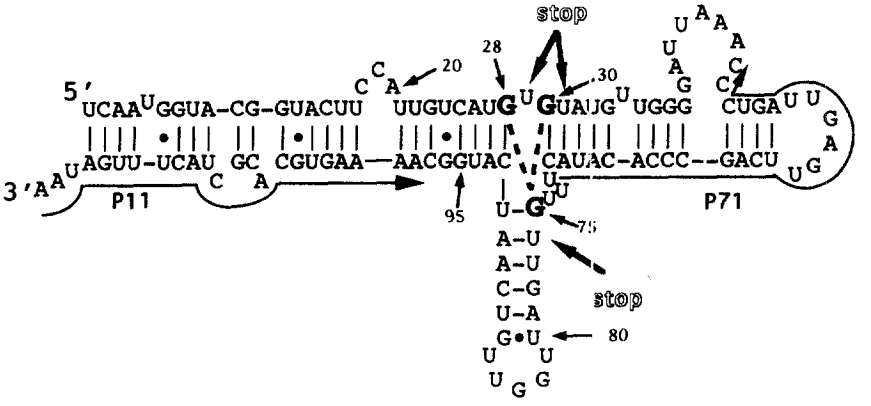

Figure 3: Schematic diagram of pRNA showing PHENPHI cross-linking sites. The pRNA sequence and its secondary structure are drawn based on sequencing, mutagenesis and ribonuclease probing.[14-16] P11 and P71, indicated with arrowhead lines, were the two primers used for primer extension. Chain elongation stops at one base prior to the cross-linked G are indicated by the arrows. The cross-linked Gs are in bold and the potential cross-linking pairs are linked with dashed lines.

metal complexes preferentially target Gs (see above).[22–24] In addition, primer 11, which is complementary to the 3' end of pRNA, produced a strong band corresponding to a stop prior to G75 (lane 3). G75 is well positioned relative to G28 and G30 in the three-helix junction area of the pRNA. From these data we conclude that RNA cross-linking results from the covalent bond formation of PHENPHI to G75 and G28 or G30. However, the data in hand do not allow us to determine the relative contribution of stops at G28 and G30 to the overall cross-linking to G75. It may be added that the possibility for a cross-link between G28 and G30 themselves was excluded because such modification is not expected to result in a significant change in the pRNA mobility.

This site selectivity exhibited by these metal complexes is reminiscent of the covalent adducts cisplatin (*cis*-diamminedichloroplatinum(II), *cis*-$Pt(NH_3)_2Cl_2$) forms with DNA in the dark, which involve predominantly intrastrand cross-links at N7 of neighboring guanines. These cross-links are believed to be responsible for the antitumor activity of cisplatin,[25] and we have noted above that both BISPHEN and PHENPHI are photocytotoxic. Further studies of these and related complexes as potential photochemotherapeutic agents, and as photoactive probes for nucleic acid structure and function, are in progress.

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References and Notes

1. Presented, in part, at the 26th Meeting of the American Society for Photobiology, Snowbird, UT, July 11-15, 1998; abstract 95S.
2. (a) Rajski, S. R.; Williams, R. M. *Chem. Rev.* **1998**, *98*, 2723. (b) Artsypovich, S. I.; Oretskaya, T. S. *Russ. Chem. Rev.* **1998**, *67*, 245.
3. (a) Hurley, L. H. *J. Med. Chem.* **1989**, *32*, 2027. (b) Shabarova, Z. A. *Biochimie* **1988**, *70*, 1323. (c) Kohn, K. W. In *Molecular Aspects of Anti-Cancer Drug Action*; Neidle, S.; Waring, M. J., Eds.; Macmillan, London, 1983, pp. 315–361.
4. Morrison, H., Ed.; *Biological Applications of Photochemical Switches*; John Wiley & Sons, Inc.; New York, NY, 1993.
5. (a) Parrish, J. A.; Fitzpatrick, T. B.; Tannenbaum, L.; Pathak, M. A. *New Engl. J. Med.* **1974**, *291*, 1207. (b) Bethea, D.; Fullmer, B.; Syed, S.; Seltzer, G.; Tiano, J.; Rischko, C.; Gillespie, L.; Brown, D.; Gasparro, F. P. *J. Dermatol. Sci.* **1999**, *19*, 78. (c) Knobler, R. M.; Hönigsmann, H.; Edelson, R. L. In *Psoralen DNA Photobiology*; Gasparro, F. P., Ed.; CRC Press, Inc., Boca Raton, FL, **1988;** Vol. II, pp. 117–133.
6. (a) Lin, L.; Cook, D. N.; Wiesehahn, G. P.; Alfonso, R.; Behrman, B.; Cimino, G. D.; Corten, L.; Damonte, P. B.; Dikeman, R.; Dupuis, K.; Fang, Y. M.; Hanson, C. V.; Hearst, J. E.; Lin, C. Y.; Londe, H. F.; Metchette, K.; Nerio, A. T.; Pu, J. T.; Reames, A. A.; Rheinschmidt, M.; Tessman, J.; Isaacs, S. T.; Wollowitz, S.; Corash, L. *Transfusion* **1997**, *37*, 423. (b) Lin, L.; Londe, H.; Hanson, C. V.; Wiesehahn, G.; Wollowitz, S.; Cimino, G.; Corash, L. *Blood* **1993**, *82*, 292. (c) Lin, L.; Wiesehahn, G. P.; Morel, P. A.; Corash, L. *Blood* **1989**, *74*, 517. (d) Wagner, S. J.; White, R.; Wolf, L.; Chapman, J.; Robinette, D.; Lawlor, T. E.; Dodd, R. Y. *Photochem. Photobiol.* **1993**, *57*, 819. (e) Margolis-Nunno, H.; Robinson, R.; Horowitz, B.; Geacintov, N. E.; Ben-Hur, E. *Photochem. Photobiol.* **1995**, *62*, 917.
7. (a) Takasugi, M.; Guendouz, A.; Chassignol, M.; Decout, J. L.; Lhomme, J.; Thuong, N. T.; Héléne, C. *Proc. Natl., Acad. Sci. U.S.A.* **1991**, *88*, 5602. (b) Meyer, Jr., R. B.; Tabone, J. C.; Hurst, G. D.; Smith, T. M.; Gamper, H. *J. Am. Chem. Soc.* **1989**, *111*, 8517. (c) Song, P. S.; Tapley, Jr., K. J. *Photochem. Photobiol.* **1979**, *29*, 1177. (d) Shi, Y.; Hearst, J. E. *Biochemistry* **1987**, *26*, 3792. (e) Thompson, J. F.; Hearst, J. E. *Cell* **1983**, 32, 1355. (f) Cimino, G. D.; Gamper, H. B.; Isaacs, S. T.; Hearst, J. E. *Ann. Rev. Biochem.* **1985**, *54*, 1151. (g) Van Houten, B.; Gamper, H.; Hearst, J. E.; Sancar, A. *J. Biol. Chem.* **1986**,

261, 14135. (h) Lee, M.; Roldan, M. C.; Haskell, M. K.; McAdam, S. R.; Hartley, J. A. *J. Med. Chem.* **1994**, *37*, 1208. (i) Chen, C.; Guo, P. *J. Virology* **1997**, *71*, 495.

8. For a recent example of the use of oligonucleotides conjugated to photoactivatable groups to form cross-links to a G on a complementary strand, see: Nakatani, K.; Shirai, J.; Sando, S.; Saito, I. *J. Am Chem. Soc.* **1997**, *119*, 7626. While our manuscript was under review, a report has appeared describing DNA cross-linking by a light-activatable progenitor of dehydromonocrotaline which then reacts with the nucleic acid in the dark. This pyrrolizidine alkaloid also targets GC-rich regions of duplex DNA preceding (photo)chemical activation, see: Tepe, J. J.; Williams, R. M. *J Am Chem. Soc.* **1999**, *121*, 2951.
9. Gidney, P. M.; Gillard, R. D.; Heaton, B. T. *J. Chem. Soc., Dalton Trans.* **1972**, 2621.
10. Terrian, D. L. *Ph. D. Dissertation* **1996,** Purdue University, West Lafayette, IN.
11. (a) Mahnken, R. E.; Bina, M; Deibel, R. M.; Luebke, K.; Morrison, H. *Photochem. Photobiol.* **1989**, *49*, 519. (b) Mahnken, R. E; Billadeau, M. A.; Nikonowicz, E. P ; Morrison, H. *J. Am. Chem. Soc.* **1992**, *114*, 9253. (c) Harmon, H. L.; Morrison, H. *Inorg. Chem.* **1995**, *34*, 4937. (d) Billadeau, M. A.; Wood, K. V.; Morrison, H. *Inorg. Chem.* **1994**, *33*, 5780. (e) Mohammad, T.; Tessman, I.; Morrison, H.; Kennedy, M. A.; Simmonds, S. W. *Photochem. Photobiol.* **1994**, *59*, 189.
12. Interestingly, when oxygen is excluded from the reaction medium, photolysis of BISPHEN and dG leads to metal coordination at the N7 of the guanine via a chain reaction.[11c]
13. Terrian, D. L.; Wagner, S. W.; Morrison, H. *Photochem. Photobiol.* **1997**, *65*, 68S.
14. (a) Guo, P.; Erickson, S.; Anderson, D. *Science* **1987**, *236*, 690. (b) Guo, P.; Zhang, C.; Chen, C.; Garver, K.; Trottier, M. *Mol. Cell* **1998**, *2*, 149. (c) Hendrix, R. W. *Cell* **1998**, *94*, 147. (d) Chen, C.; Zhang, C. L.; Guo, P. *RNA* **1999**, *5*, 1.
15. Plasmid DNA, containing coding sequences of wild-type phenotype pRNA 7/11 under T7 promoter, was digested with *EcoRI*, and the resulting DNA fragment was used as a template to synthesize pRNA by in vitro transcription with T7 polymerase.[16] The pRNA was purified by 8% polyacrylamide/8 M urea denaturing gel to homogeneity and assayed by UV absorbance at 260 and 280 nm.
16. (a) Zhang, C.; Trottier, M.; Guo, P. *Virology* **1995**, *207*, 442. (b) Zhang, C.; Lee, C.; Guo, P. *Virology* **1994**, *201*, 77.
17. The solutions, spotted on prechilled parafilm floating on ice water, were irradiated with a glass-filtered long wavelength UVGL-25 lamp (UVP, Inc.) at a distance of ca. 5 cm.
18. A Kodak Digital Science DC40 camera in combination with 1D Analysis Software Version 1.6 was used to quantify the bands.
19. This was confirmed in a time course experiment with 1.0 mM BISPHEN which showed a higher magnitude of pRNA degradation without the production of additional cross-linked pRNA.
20. Wassarman, D. A. *Mol. Biol. Rep.* **1993**, *17*, 143.
21. Sanger, F.; Nicklen, S.; Coulson, A. R. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5463.
22. See also our earlier data obtained with plasmid DNA, pBSSK$^+$.c-raf (eco), which showed that BISPHEN preferentially forms covalent adducts in the regions of contiguous Gs.[23] Such a result is in accord with the reports that a G located in a cluster of Gs is selectively oxidized relative to isolated Gs,[24] and is consistent with electron transfer from dG to the metal complex as the primary step in the binding mechanism of BISPHEN to DNA.[11c,11d]
23. Harmon, H. L. *Ph. D. Dissertation* **1995,** Purdue University, West Lafayette, IN.
24. (a) Kino, K.; Saito, I.; Sugiyama, H. *J. Am. Chem. Soc.* **1998**, *120*, 7373. (b) Sugiyama, H.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 7063. (c) Saito, I.; Takayama, M.; Sugiyama, H.; Nakatani, K.; Tsuchida, A. Yamamoto, M. *J. Am. Chem. Soc.* **1995**, *117*, 6406.
25. (a) Bruhn, S. L; Toney, J. H.; Lippard, S. J. *Prog. Inorg. Chem.* **1990**, *38*, 477. (b) Lippert, B. *Prog. Inorg. Chem.* **1989**, *37*, 1. (c) Yang, D.; van Boom, S. S. G. E.; Reedijk, J.; van Boom, J. H.; Wang, A. H. –J. *Biochemistry* **1995**, *34*, 12912. (d) Caradonna, J. P.; Lippard, S. J. *Inorg. Chem.* **1988**, *27*, 1454. (e) Pinto, A. L.; Lippard, S. J. *Biochim. Biophys. Acta* **1985**, *780*, 167.