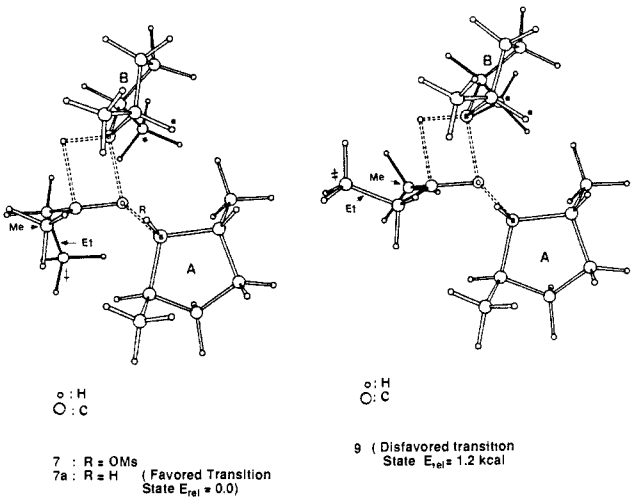

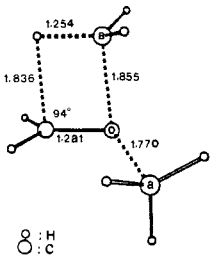

Computational Evaluation of 7. For the reaction of diborane with formaldehyde, ab initio calculations[6] were carried out to examine three different transition states,[7] one of which has one (mono)borane acting as a Lewis acid complexing with an oxygen lone-pair and the second (mono)borane undergoing a four-center addition. At the MP2/6-31G*/3-21G level this transition state (**8**) with the geometry shown is 26 kcal/mol above that of diborane plus formaldehyde.

Modified MM2 models[8,9] were devised to investigate the stereoselectivity expected in the reaction of two molecules of **1** with 2-butanone via **8**. The two models shown in **7a** and **9** lead to the formation of *R*- and *S*-2-butanol, respectively. The transition state **7a** is more stable than **9** by 1.2 kcal/mol which corresponds to 82% ee for this asymmetric reaction carried out at −9.5 °C (experimental ee 80.4%, see above).

Both **7a** and **9** have the nonreacting borane (A) coordinated on the side of the oxygen near the asterisked hydrogen projecting downward from the reacting borane (B), rather than near the asterisked methyl projecting downward. Transition states with the borane (A) on the other side of the carbonyl are 4–5 kcal/mol higher in energy. Therefore, the enantioselection of the reduction is correlated with the manner in which A is coordinated with 2-butanone. The favored transition state **7a** has the ethyl group in its preferred conformation with the methyl (dagger) anti to the forming bond.[10] The less favored transition state has the methyl (double dagger) in the disfavored "outside" conformation in order to avoid repulsion with the nonreacting borane.

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Supplementary Material Available: Detailed kinetic data including experimental procedures, tables of data, and graphs of data presented in tables (18 pages). Ordering information is given on any current masthead page.

(10) This preference is described in ref 9.

(6) Geometry optimizations were carried out with the 3-21G basis set, and energies were recomputed on these geometries at the MP2/6-31G* level. The GAUSSIAN 82 programs were used for these calculations: Binkley, J. S.; Frisch, M. J.; Defrees, D. J.; Raghavachari, K.; Whiteside, R. A.; Schlegel, H. B.; Fluder, E. M.; Pople, J. A. GAUSSIAN 82; Carnegie-Mellon University: Pittsburgh, PA.

(7) The reaction of 2-butanone with **1**-D via either one of the other two transition states exhibits no or low enantioselectivity. Details of these calculations will be described in a full paper.

(8) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127. Burkert, T.; Allinger, N. L. *Molecular Mechanics*; American Chemical Society: Washington, DC, 1982. We use ab initio calculations to determine the "normal" position of atoms and devise force constants for bond stretching, angle bending, and torsions involving unusual transition-state atoms, in order to allow movement of all atoms.

(9) These models are developed in ways analogous to the many models described in: Houk, K. N.; Paddon-Row, M. N.; Rondan, N. G.; Wu, Y.-d.; Brown, F. K.; Spellmeyer, D. C.; Metz, J. T.; Li, Y.; Loncharich, R. J. *Science (Washington, D. C.)* **1986**, *231*, 1108.

Site-Specifically Platinated DNA, a New Probe of the Biological Activity of Platinum Anticancer Drugs

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Platinum anticancer drugs, the prototype of which is *cis*-diamminedichloroplatinum(II) (*cis*-DDP), exhibit their biological activity by binding to DNA and inhibiting replication.[2] The principal adduct formed by *cis*-DDP in its reactions with DNA is an intrastrand cross-link in which the N(7) atoms of adjacent guanine bases have replaced the chloride ions in the platinum coordination sphere.[3] With the exception of short, synthetic oligonucleotides constructed to study the stereochemistry of *cis*-diammineplatinum(II) binding by NMR spectroscopy[4] and X-ray crystallography,[5] DNA platinated with *cis*-DDP has previously contained a variety of adducts, including d(GpG) and d(ApG) intrastrand cross-links and, at lower frequency, interstrand cross-links and monoadducts.[6] The heterogeneity of reaction products of these globally platinated DNAs has made it difficult to discern the effects of any one specific adduct upon the processing of DNA in vivo. In the present paper we report the design, synthesis, and preliminary characterization of a duplex bacteriophage M13 DNA containing a *cis*-[$Pt(NH_3)_2${d(GpG)}] cross-link at a unique, programmable site in the genome. The strategy used to construct this site-specifically platinated DNA should be generally applicable for building other chemically modified oligonucleotides into specific sites in DNA.

The chemically synthesized[7] dodecanucleotide d(TCTAGGCCTTCT) (9.4×10^{-4} M strand[8]) was allowed to

(1) (a) Department of Chemistry. (b) Department of Applied Biological Sciences.

(2) *Platinum Coordination Compounds in Cancer Chemotherapy*; Hacker, M. P., Douple, E. B., Krakoff, I. H., Eds.; Nijhoff: Boston, 1984.

(3) For a recent review, see: Pinto, A. L.; Lippard, S. J. *Biochim. Biophys. Acta* **1985**, *780*, 167.

(4) (a) Caradonna, J. P.; Lippard, S. J.; Gait, M. J.; Singh, M. *J. Am. Chem. Soc.* **1982**, *104*, 5793. (b) van Hemelryck, B.; Guittet, E.; Chottard, G.; Girault, J.-P.; Huynh-Dinh, T.; Lallemand, J.-Y.; Igolen, J.; Chottard, J.-C. *J. Am. Chem. Soc.* **1984**, *106*, 3037. (c) den Hartog, J. H. J.; Altona, C.; van der Marel, G. A.; Reedijk, J. *Eur. J. Biochem.* **1985**, *147*, 371 and references cited therein.

(5) Sherman, S. E.; Gibson, D.; Wang, A. H.-J.; Lippard, S. J. *Science (Washington, D.C.)* **1985**, *230*, 417.

(6) Fichtinger-Schepman, A. M. J.; van der Veer, J. L.; den Hartog, J. H. J.; Lohman, P. H. M.; Reedijk, J. *Biochemistry* **1985**, *24*, 707.

(7) Sprout, B. S.; Gait, M. J. In *Oligonucleotide Synthesis: A Practical Approach*; Gait, M. J., Ed.; IRL Press: Washington, DC, 1984.

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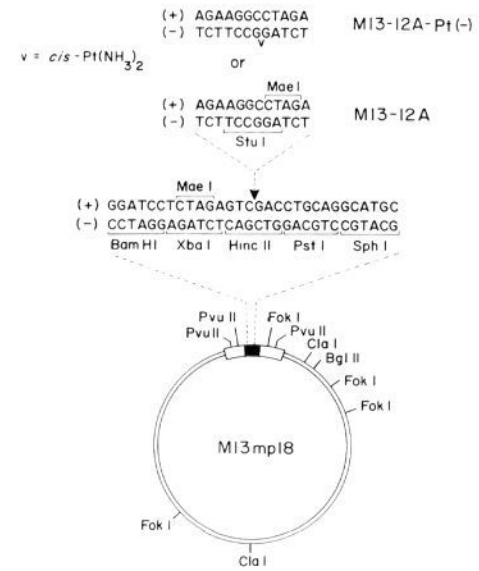

Figure 1. Map of the genome created by insertion of d(TCTAGGCCTTCT)–d(AGAAGGCCTAGA) into the *Hinc* II site of M13mp18. All symbols are either self-explanatory or defined in the text.

react with 1.1 equiv of *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$[9] for 6 h in the dark at 37 °C. The initial pH of the solution was 6.0. The major reaction product was purified by semipreparative reversed-phase HPLC and was characterized by NMR spectroscopy as an intrastrand $[Pt(NH_3)_2\{d(GpG)\}]$ cross-link.[10] The yield of purified material was 38%.

In a parallel set of experiments, the synthetic duplex dodecanucleotide d(TCTAGGCCTTCT)–d(AGAAGGCCTAGA) was inserted into the *Hinc* II site of M13mp18 by blunt-ended ligation (Figure 1). This process destroyed the *Hinc* II site and created a unique *Stu* I site; the presence of the insertion was confirmed by *Stu* I sensitivity and DNA sequencing.[11] Heteroduplex DNA with a 12-base gap in the minus strand was made by dialysis of *Hinc* II linearized M13mp18 replicative form (RF) DNA and a 20-fold molar excess of M13-12A[12] viral DNA against decreasing amounts of formamide.[13] In a subsequent step, single-stranded material was separated from gapped heteroduplex DNA by chromatography on hydroxylapatite.

The modified oligonucleotide, Pt-12-T (0.02 A_{260} units), was phosphorylated with polynucleotide kinase and [γ-^{32}P]ATP. To the crude phosphorylation reaction mixture was added gapped heteroduplex DNA (1 μg), ATP (to 1 mM), and T_4 DNA ligase (800 units) in a final volume of 50 μL. The ligation reaction was allowed to proceed overnight at 16 °C. Ligated material was then separated from excess oligonucleotide and ATP by drop dialysis followed by gel filtration chromatography. The use of [γ-^{32}P]ATP allowed the introduction into the M13 genome of a radiolabel at the fifth phosphodiester bond 5′ to the site of modification.

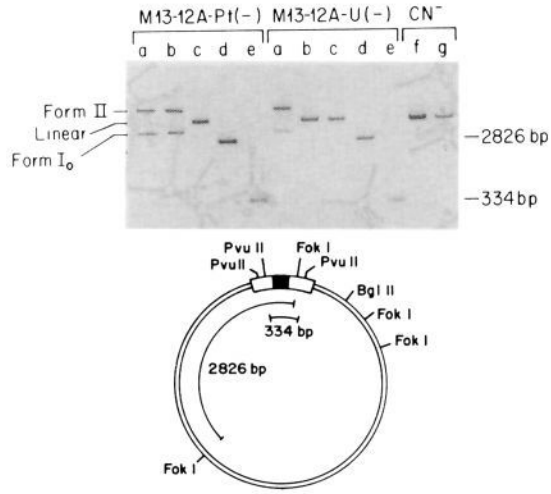

Figure 2. Characterization of the M13-12A-Pt(−) and M13-12A-u(−) genomes by restriction endonuclease digestion. Autoradiogram of a 0.8% agarose gel containing 0.5 μg/mL ethidium bromide, which adds supercoils to form I_0 DNA, increasing its mobility in the gel. Lanes a–e display material digested with nothing, *Stu* I, *Bgl* II, *Fok* I, or *Pvu* II, respectively. Lanes f and g display cyanide treated M13-12A-Pt(−) and M13-12A-u(−) followed by digestion with *Stu* I. Unlabeled M13-12A RF (0.1 μg) was added to each digestion as an internal standard. Digestion of this standard DNA was judged to be complete in all cases following visualization by ethidium bromide fluoresence. The shaded box in the circle contains the position of the ^{32}P label and, where present, the platinum atom.

Electrophoresis of the site-specifically platinated DNA through a 0.8% agarose gel containing ethidium bromide and subsequent autoradiography revealed two bands, corresponding to form I_0 (covalently closed, relaxed circular) and form *II* (nicked circular) DNA (Figure 2). The presence of form I_0 DNA indicates that ligation into the 12-base gap occurred at both the 5′- and 3′-ends of the oligonucleotide. Form *II* DNA can arise from incomplete ligation or the presence of nicks elsewhere on the genome. Digestion of M13-12A-Pt(−) with restriction enzymes *Bgl* II, *Fok* I, and *Pvu* II (Figure 2) localized the ^{32}P to the region around the former *Hinc* II site, indicating that ligation had not occurred elsewhere on the genome.

Restriction endonucleases were used to map the extent to which the *cis*-$[Pt(NH_3)_2\{d(GpG)\}]$ cross-link perturbs the DNA structure (Figure 1). The presence of the cross-link confers *Stu* I resistance upon M13-12A-Pt(−), whereas M13-12A-u(−) is completely cut by *Stu* I (Figure 2). Incubation of M13-12A-Pt(−) for 3 h at 37 °C in 0.3 M NaCN (pH 8.0) restored *Stu* I sensitivity by removing platinum from the DNA as $[Pt(CN)_4]^{2-}$.[14] The 5′-end of the *Mae* I recognition sequence overlaps the platinum cross-link by one nucleotide, which was sufficient to prevent cleavage by *Mae* I at that site. In contrast, digestion with *Bam*H I, *Xba* I, *Pst* I, or *Sph* I (Figure 1) resulted in linearized DNA (data not shown), demonstrating that enzymes with recognition sequences farther removed from the site of modification were unaffected by the presence of the platinum intrastrand cross-link. These results are consistent with an earlier study of *Bam*H I cleavage of globally platinated pBR322 DNA, in which it was estimated that endonuclease activity would be inhibited ±3 base pairs from the platinum cross-link sites.[15]

In conclusion, we have achieved the synthesis and characterization of an intact viral genome modified with *cis*-DDP at a unique, programmable site. The synthetic scheme outlined is a general one; the blunt-ended ligation step permits the construction of site-specifically modified DNAs of any sequence and length, whereas previous work has been constrained to building damage

(8) The extinction coefficient of d(TCTAGGCCTTCT) was estimated to be 1.03×10^5 M^{-1} cm^{-1} per strand by using the algorithm in: *Handbook of Biochemistry and Molecular Biology*; Fasman, G. D., Ed.; CRC Press: Cleveland, 1975.

(9) Lippert, B.; Lock, C. J. L.; Rosenberg, B.; Zvagulis, M. *Inorg. Chem.* **1977**, *16*, 1525.

(10) A pH titration of the nonexchangeable base protons of the unmodified and platinated oligonucleotide was performed to characterize the platinum adduct.[4] Spectra and titration curves will be provided in a subsequent paper.

(11) Sanger, F.; Nicklen, S.; Coulson, A. R. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5463.

(12) A systematic nomenclature has been developed for the molecules employed in this work: d(TCTAGGCCTTCT) and d(AGAAGGCCTAGA) are abbreviated 12-T and 12-A, respectively. Pt-12-T is dodecanucleotide 12-T containing the *cis*-$[Pt(NH_3)_2\{d(GpG)\}]$ cross-link. M13-12A and M13-12T are the products of the insertion of the dodecanucleotides into the *Hinc* II site of M13mp18, with 12-A and 12-T in the plus strand, respectively. M13-12A-Pt(−) and M13-12A-u(−) are M13-12A with Pt-12-T and 12-T, *respectively, built into the minus strand.*

(13) Lundquist, R. C.; Olivera, B. M. *Cell* **1982**, *31*, 53.

(14) (a) Bauer, W.; Gonias, S. L.; Kam, S. K.; Wu, K. C.; Lippard, S. J. *Biochemistry* **1978**, *17*, 1060. (b) Lippard, S. J.; Hoeschele, J. D. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 6091.

(15) Ushay, H. M.; Tullius, T. D.; Lippard, S. J. Biochemistry **1981**, *20*, 3744.

at preexisting sequences, usually the recognition sites for restriction endonucleases.[16] We are currently investigating the effects of this discrete platinum adduct upon replication, repair, and mutagenesis, in order to further our understanding of the mechanism of action of platinum anticancer drugs.

Acknowledgment. This work was supported by U.S. Public Health Service Grant CA34992 (to S.J.L.) awarded by the National Cancer Institute and by Grants CA40817 and T32ES00597 (to J.M.E.) awarded by the National Cancer Institute and the National Institute of Environmental Health Sciences, respectively. We thank Engelhard Corp. for a loan of K_2PtCl_4 from which platinum complexes were prepared.

(16) (a) Green, C. L.; Loechler, E. J.; Fowler, K. W.; Essigmann, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 13. (b) Johnson, D. L.; Reid, T. M.; Lee, M.-S.; King, C. M.; Romano, L. J. *Biochemistry* **1986**, *25*, 449. (c) Chambers, R. W.; Sledziewska-Gojska, E.; Hirani-Hojatti, S.; Borowy-Borowski, H. *Proc. Natl. Acad. U.S.A.* **1985**, *82*, 7173.

Generalized Valence Bond Description of the Bonding in [1.1.1]Propellane

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There has been considerable interest, both experimental[1] and theoretical,[2-6] in the propellane family of molecules ever since the synthesis of the first compound of this kind was carried out 18 years ago.[7] The smallest member of this series of compounds and the one generally believed to have the highest strain energy is [1.1.1]propellane. In light of some predictions that the moelcule should not even exist,[4] the recent preparation of this compound[8] and the discovery that it is, in fact, more stable than a number of its family members is particularly interesting. This has stimulated renewed activity toward the understanding of the bonding in [1.1.1]propellane and related compounds.[5,6,9] Many molecular orbital calculations have been carried out on these molecules; in this paper we report the first comprehensive valence bond calculations for a propellane. The results, we believe, offer a new perspective on the bonding between bridgehead carbon atoms and illustrate the simple way the valence bond approach accounts for crucial electronic correlation effects ignored in the molecular orbital treatments.

It recently has been shown[10,11] for multiple bonds in a number of molecules that Ω-bonds (multiple equivalent bent bonds) are energetically more favorable than the traditional σ- and π-bonds. Although this may be of some intrinsic interest, a more significant issue is whether any conceptual advantage can be derived from the description of multiple bonding in terms of Ω-bonds. That is, does this description aid in making new connections and provide fresh insight into the understanding of novel situations? In the present context, it appears that the concept of Ω-bonds provides a way to establish a natural relationship between the bonding in the C_2 molecule in its ground state and the [1.1.1]propellane molecule and thereby allows one to gain some new insight into the bonding of the latter molecule.

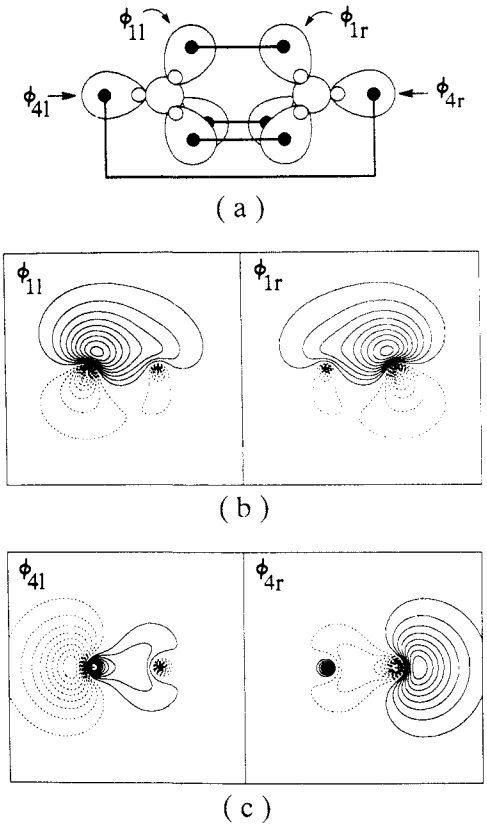

Figure 1. (a) Schematic representation of the many-electron wave function of C_2. Dots denote the electron occupancy of the orbitals and lines show which orbitals overlap to form bonds. (b) Two orbitals which form one of the three equivalent Ω-bonds. (c) The two orbitals which form the fourth pair in C_2.

In the present work, the generalized valence bond (GVB) approach with the perfect pairing (PP) restriction is used.[12] Although GVB-PP finds Ω-bonds as the energetically favored description of bonding in many cases,[10,11] restrictions in this wave function actually bias it against the Ω-description.[11] Rather than attempting to obtain a more sophisticated wave function that removes this bias toward the σ,π-description in C_2, we adopt the expedient of starting with the Ω-bond description at the perfect pairing level of approximation. This allows us to make a simple connection to [1.1.1]propellane, which is the molecule of chief interest here.

In Figure 1, some results of a GVB-PP (four GVB pairs) calculation[13] for C_2 are presented. Panel a gives a schematic representation of the many-electron wave function of the molecule, illustrating the approximately tetrahedral hybrid orbitals on each of the carbon atoms. Three of these orbitals on the left atom

(1) Wiberg, K. B. *Acc. Chem. Res.* **1984**, *17*, 379. Also references contained therein.
(2) Newton, M. D.; Schulman, J. M. *J. Am. Chem. Soc.* **1972**, *94*, 773.
(3) Stohrer, W.-D.; Hoffmann, R. *J. Am. Chem. Soc.* **1972**, *94*, 779.
(4) Liebman, J. F.; Greenberg, A. *Strained Organic Molecules*; Academic Press: New York, 1978.
(5) Wiberg, K. B. *J. Am. Chem. Soc.* **1983**, *105*, 1227.
(6) Jackson, J. E.; Allen, L. C. *J. Am. Chem. Soc.* **1984**, *106*, 591.
(7) Wiberg, K. B.; Hiatt, J. E.; Burgmaier, G. J. *Tetrahedron Lett.* **1968**, 5855.
(8) Wiberg, K. B.; Walker, F. H. *J. Am. Chem. Soc.* **1982**, *104*, 5239.
(9) Wiberg, K. B.; Dailey, W. P.; Walker, F. H.; Waddell, S. T.; Crocker, L. S.; Newton, M. D. *J. Am. Chem. Soc.* **1985**, *107*, 7247.
(10) Messmer, R. P.; Schultz, P. A.; Tatar, R. C.; Freund, H.-J. *Chem. Phys. Lett.* **1986**, *126*, 176.
(11) Messmer, R. P.; Schultz, P. A., submitted for publication in *Phys. Rev. Lett.*

(12) Bair, R. A.; Goddard, W. A., III; Voter, A. F.; Rappé, A. K.; Yaffe, L. G.; Bobrowicz, F. W.; Wadt, W. R.; Hay, P. J.; Hunt, W. J. *GVB2P5* Program, unpublished. Bair, R. A. *Ph.D. Thesis*, Caltech, 1980. Hunt, W. J.; Hay, P. J.; Goddard, W. A., III *J. Chem. Phys.* **1972**, *57*, 738. Bobrowicz, F. W.; Goddard, W. A., III In *Modern Theoretical Chemistry*; Schaefer, H. F., III, Ed.; Plenum Press: New York, 1977; Vol. 3, Chapter 4.
(13) The calculations employed a standard valence double-ζ plus polarization basis set (Dunning, T. H., Jr.; Hay, P. J. In *Modern Theoretical Chemistry*; Schaefer, H. F., III, Ed.; Plenum Press: New York, 1977; Vol. 3, Chapter 1). The experimental geometry was used.[14]
(14) Huber, K. P.; Herzberg, G. *Molecular Spectra and Molecular Structure IV. Constants of Diatomic Molecules*; Van Nostrand Reinhold: New York, 1979.

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