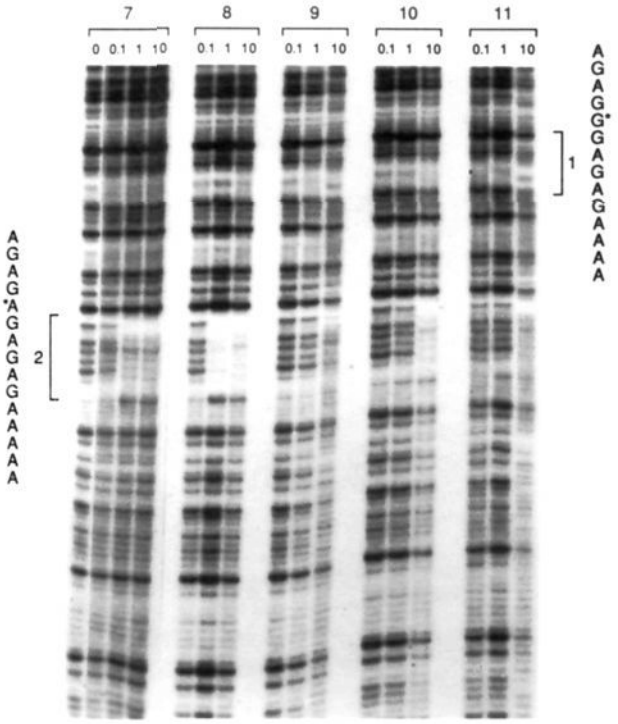

Figure 2. Footprint analysis. Conditions for footprint: Concentrations of DONs shown were in micromoles/liter, and the radiolabeled target duplex (370-bp restriction fragment derived from a PUC vector containing a synthetic DNA insert) was approximately 1 nM in 20 mM MOPS, pH 6.8, 140 mM KCl, 10 mM NaCl, 1 mM $MgCl_2$, 1 mM spermine hydrochloride. Triple-stranded hybridizations (50 μL) were done for 1 h at 37 °C followed by limited DNase I digestion (2 units for 1 min), followed by EDTA quenching, PAGE, and autoradiography.

triple-helix formation.[1b,18] The target sequence is shown in Figure 2 along with the autoradiogram resulting from the footprint experiment. The target sequence has been repeated a second time, an adenosine to guanosine base change having been introduced in the polypurine tract, creating a single triplet mismatch for the test DONs. This allows for the assessment of the ability of a given DON to discriminate a perfect match from one mismatch. The region of the mismatch target is shown by a bar labeled 1 on the autoradiogram, and the perfect match is labeled 2.

The control phosphodiester DON **7** shows protection of the target from DNase I when present at 1 μM, and binding to the single mismatched target occurs only at 100 μM. The formacetal DON **8** shows a similar specific footprint. The MP DON **10** requires a >10-fold-higher concentration to give similar protection as compared to the two previous DONs. The MEA **11** and thioformacetal **9** DONs show reduced binding relative to **10** with partial protection from DNase being observed at 10 μM.

The formacetal linkage is competent for sequence-specific triple-helix binding when placed in a 5-MeC–T context. The shorter 3′-oxygen to 5′-oxygen distance in the formacetal linkage relative to a phosphodiester is perhaps not a liability, given the shorter ribose to ribose distances in A-form helix versus B-form.[19] The reduced binding of the thioformacetal is surprising.[20] The MP **10** required a >10-fold-higher concentration for binding relative to the diester and formacetal DONs.[21] The MEA **11** is clearly inferior to MP in this context. This result parallels the two linkages' ability to form duplex structures with single-stranded DNA and RNA.[8b,9c] These results show the promise of neutral achiral formacetal DON analogues as agents capable of sequence-specific triple-helix formation.

Acknowledgment. We thank Sarah McCurdy and Terry Terhorst for oligonucleotide synthesis, Susan Hubbard and Debbie Surdel for manuscript preparation, and Dr. Heinz Moser for helpful discussions on the geometry of A- and B-form double helices.

Supplementary Material Available: Electrophoretic gel demonstrating chemical cleavage of **8** and **9** (1 page). Ordering information is given on any current masthead page.

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(19) (a) Triplex structures are reported to be in the A form: Rajagopal, P.; Feigon, J. *Nature (London)* **1989**, *339*, 637–640. (b) Rajagopal, P.; Feigon, J. *Biochemistry* **1989**, *28*, 7859–7870. (c) de los Santos, C.; Rosen, M.; Patel, D. *Biochemistry* **1989**, *28*, 7282–7289.

(20) Model building suggests that the 3′-carbon to 5′-carbon distance in the thioformacetal is comparable to that of a phosphodiester, yet this linkage confers reduced triplex binding properties on the DON relative to a phosphodiester and formacetal. Modelling also suggests a close contact between the 5′-oxygen of the formacetal linkage and the 6-hydrogen of the thymine ring. This contact could be disturbed by the substitution at the 5′-position with the larger, less polar sulfur (modelling performed using Biograf software by Molecular Simulations).

(21) **10** and **11** are mixtures of 16 diastereomers. One of the isomers in **10** could be the active agent, and consequently, on a molar basis, this isomer would then have an affinity comparable to that of a phosphodiester.

Mechanistic Studies on DNA Photolyase. 3. The Trapping of the One-Bond-Cleaved Intermediate from a Photodimer Radical Cation Model System

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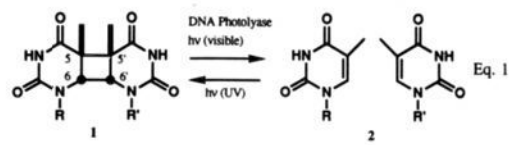
DNA photolyase[1] is the enzyme involved in the cleavage of pyrimidine photodimers in UV-damaged DNA (eq 1). Although several model systems for this reaction have been reported, their mechanisms remain poorly understood.[2] The quinone-sensitized



cleavage, for example, has been proposed[2] to proceed by electron transfer from the photodimer to the photoexcited quinone followed by sequential cleavage of the C6,C6′ and the C5,C5′ bonds of the photodimer and reduction of the uracil radical cation by the semiquinone radical (Scheme I). The only experimental evidence in support of this proposal is the observation of CIDNP in the product.[3] With the view of developing mechanistic probes for the enzymatic reaction, we have synthesized a photodimer substituted with a radical trap designed to block the quinone-sensitized photodimer fragmentation after the first CC bond cleavage.

The most frequently used trap for enzyme-generated radicals involves the rapid ring opening of the methylcyclopropyl radical.[4]

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(2) (a) Lamola, A. A. *Mol. Photochem.* **1972**, *4*, 107. (b) Roth, H. D.; Lamola, A. A. *J. Am. Chem. Soc.* **1972**, *94*, 1013. (c) Pac, C.; Kubo, J.; Majima, T.; Sakurai, H. *Photochem. Photobiol.* **1982**, *36*, 273. (d) Helene, C.; Charlier, M. *Photochem. Photobiol.* **1977**, *25*, 429. (e) Van Camp, J. R.; Young, T.; Hartmann, R. F.; Rose, S. D. *Photochem. Photobiol.* **1987**, *45*, 365. (f) Hartmann, R. F.; Van Camp, J. R.; Rose, S. D. *J. Org. Chem.* **1987**, *52*, 2684. (g) Rokita, S. E.; Walsh, C. T. *J. Am. Chem. Soc.* **1984**, *106*, 4589. (h) Jorns, M. S. *J. Am. Chem. Soc.* **1987**, *109*, 3133.

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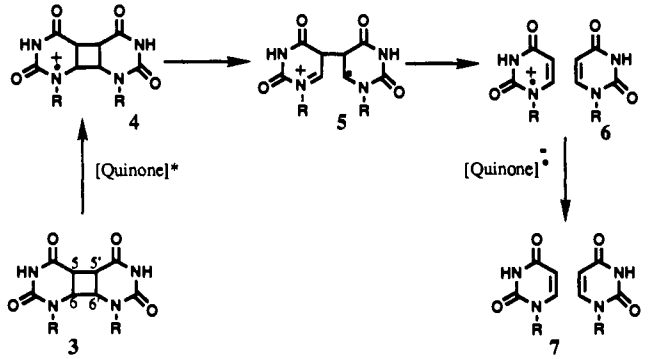
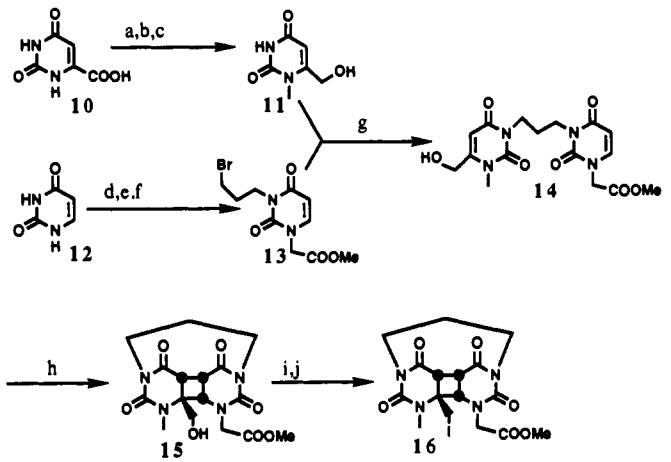
Scheme I. Proposed Mechanism[2] for the Quinone-Sensitized Cleavage of the Cyclobutane Pyrimidine Photodimer

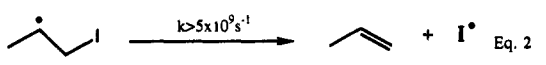


Scheme II. Synthesis of the 6-Iodomethyl Photodimer Model System[a]



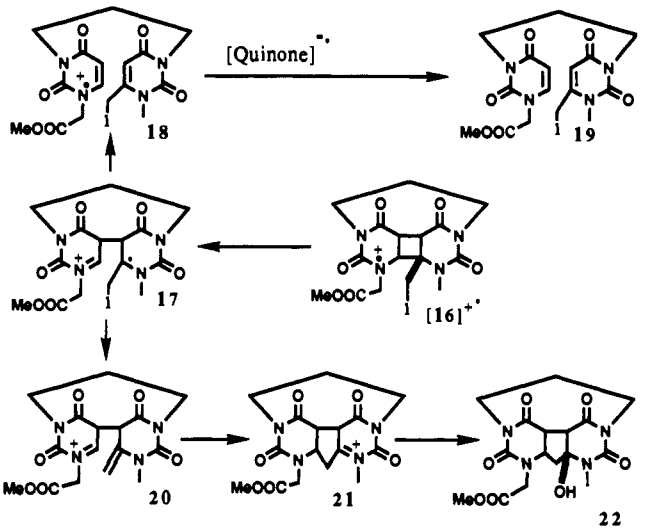
[a] (a)[8b] BuOH/H$^+$, (b)[8c] $LiAlH_4$, (c) CH_3I/K_2CO_3, (d)[8d] $ClCH_2C$-OOH, (e) MeOH/H$^+$, (f) $BrCH_2CH_2CH_2Br$, K_2CO_3/DMF, (g) K_2CO_3/DMF, (h) *hν*/acetone, (i) MsCl/pyridine, (j) LiI/2-butanone.

The large size of this substituent, coupled with the synthetic difficulty of preparing a cyclopropyl-substituted photodimer,[5] necessitated the consideration of an alternative trap for the putative intermediate **5**. Due to the rapid rate of homolysis of a carbon–iodine bond β to a radical center (>700 times faster than the rate of ring opening of the ethylcyclopropyl radical,[6] eq 2,[7]), we reasoned that the 6-iodomethyl-substituted photodimer **16** would be an attractive target. In this communication we describe the synthesis and the quinone-sensitized cleavage of this compound.



The synthesis[8] of the model photodimer **16** is based on Leonard's use of a trimethylene linker to control the stereochemistry of the photodimerization reaction and is outlined in Scheme II. Irradiation of **16** in the presence of DDQ[9,10] resulted in the formation

Scheme III. Mechanistic Proposal for the Cleavage of the 6-Iodomethyl Photodimer



of two major photoproducts, **19** and **22**,[11] in a 1.2:1 ratio.[12] We propose that these are formed as outlined in Scheme III. Cleavage of the 5,5 bond of **17** followed by reduction would give the iodomethyl-substituted bis(uracil) **19**. Loss of an iodine atom from **17** followed by cyclization and hydration of the resulting acyliminium ion[13] should give the ring-expanded dimer **22**.

Quasi-concerted [2 + 1] cycloadditions of a variety of alkene radical cations have been proposed based on stereochemical[14] and theoretical[15] considerations. The trapping experiment described here argues against a concerted mechanism for the fragmentation of the photodimer radical cation. This study supports the mechanism for the quinone-sensitized cleavage of pyrimidine photodimers outlined in Scheme I and demonstrates that the iodomethyl substituent is a viable radical trap for the one-bond-cleaved intermediate **5**. It is surprising that this small, accessible, fast radical trap has not been used previously in mechanistic enzymology. Our observation[16] that the 5-ethyl-2′-deoxyuridine dinucleotide photodimer is a substrate for the *Escherichia coli* DNA photolyase suggests that the corresponding 5-iodomethyl-substituted photodimer will serve as a useful mechanistic probe for the enzymatic reaction.

Acknowledgment. We thank Greg VanDuyne and Jorge Rios Steiner for the Cornell X-ray Facility for assistance with the structure determination of **16**. This research was supported by a grant from the National Institutes of Health (GM40498).

Registry No. 10, 65-86-1; **10** butyl ester, 22754-37-6; **11**, 2476-13-3; **11** 1-demethyl derivative, 22126-44-9; **12**, 66-22-8; **12** 1-acetic acid de-

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(9) In a typical experiment, a solution of 7.4 mg (0.015 mM) of the iodomethyl photodimer **16** and 3.4 mg (0.015 mM) of DDQ in 3 mL of acetonitrile was irradiated, under argon purge, for 30 min (50% conversion) in a Rayonet reactor. The blacklight lamps had a λ_{max} at 350 nm, and a 313-nm cutoff filter was used to remove short-wavelength UV irradiation. ^1H NMR analysis of the reaction mixture demonstrated the clean conversion of **16** to **19** and **22**.

(10) Sasson, S.; Elad, E. *J. Org. Chem.* **1972**, *37*, 3164.

(11) Compound **22** was characterized by its ^1H, ^{13}C, and 2D COSY NMR spectra and by FAB MS.

(12) When **16** was irradiated in the absence of DDQ, no reaction occurred, demonstrating the photochemical and thermal stability of this photodimer. Allyl iodide was also stable under the reaction conditions, indicating that **22** was not formed by direct oxidation/ionization of the C–I bond by the photoexcited DDQ.

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(15) (a) Pabon, R. A.; Bauld, N. L. *J. Am. Chem. Soc.* **1984**, *106*, 1145. (b) Bauld, N. L.; Bellville, D. J.; Pabon, R.; Chelsky, R.; Green, G. *J. Am. Chem. Soc.* **1983**, *105*, 2378.

(16) Burdi, D.; Begley, T., unpublished observations.

rivative, 4113-97-7; **12** 1-methyl acetate derivative, 5236-60-2; **13**, 135790-03-3; **14**, 135790-04-4; **15**, 135790-05-5; **15** mesylate derivative, 135790-12-4; **16**, 135790-06-6; **17**, 135790-07-7; **18**, 135790-08-8; **19**, 135790-09-9; **20**, 135822-29-6; **21**, 135790-10-2; **22**, 135790-11-3; DDQ, 84-58-2; allyl iodide, 556-56-9.

Supplementary Material Available: Spectroscopic data for **16**, **19**, and **22** and X-ray structural parameters for **16** (11 pages). Ordering information is given on any current masthead page.

2D Nuclear Magnetic Resonance Analysis of Osmylated C_{60}

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Buckminsterfullerene (C_{60}) presents a novel array of pyramidalized tricoordinate carbons with a spherical closed-shell topology. Derivatives of C_{60} where added atoms make specific carbons tetracoordinate generate new topologies. For example, our 1:1 and 2:1 adducts of OsO_4 with C_{60} correspond to cup- and band-shaped arrays of unsaturated carbons.[1] With our crystal structure of the 1:1 adduct $C_{60}(OsO_4)$(4-*tert*-butylpyridine)$_2$ (**1**) we proved the soccer ball shaped carbon framework of C_{60} and provided structural information for the cup-shaped π-system.[1] Here we report a 2D NMR analysis of **1** whereby we establish regioselective osmylation of C_{60}, assign chemical shifts for the carbons in **1**, determine C–C coupling constants corresponding to the two types of C–C bonds in C_{60}, and provide the first correlation of quaternary–quaternary carbon bond lengths to $^1J_{CC}$.

Carbon-13-enriched C_{60} was prepared from cored natural abundance carbon rods packed with ^{13}C powder[2] and converted to **1**.[1] The 1D ^{13}C NMR spectrum of enriched **1** showed 22 peaks. Five of the peaks were assigned to coordinated 4-*tert*-butylpyridine.[3] Of the 17 remaining peaks, four displayed approximately half the intensity of the other 13 peaks. This pattern agrees with the structure of **1**, considering that it has two approximate mirror planes, one containing carbons 1, 2, 59, and 60, and one containing carbons 26, 36, 31, and 41 (Figure 1a). Accordingly, the C_{60} segment of **1** has 17 types of carbons, 13 represented four times, and four that lie on a mirror plane and are represented two times (Figure 1b).

The resolution of multiple cluster carbons indicates that the C–O bonding is not fluxional on the NMR time scale. Signals corresponding to the other possible 1:1 adduct where OsO_4 has added across the junction of a five- and a six-membered ring were not detectable. The isomer observed in the crystal thus represents the whole, and the observed regioselectivity agrees with theory.[1]

The 17 types of carbons were assigned on the basis of the connectivities derived from a 2D NMR INADEQUATE experiment (Figure 2, Table I).[2,4,5] The half-intensity peaks (a, g, n, and p) were assigned to the carbons on the approximate mirror planes (types 1, 8, 13, and 17). Peaks a and p were assigned to types 1 and 17 because they each couple with only one carbon. Of the pair, the remote upfield peak, a, was assigned to the tetracoordinate O-bonded carbon, type 1.[6,7] Starting from carbon

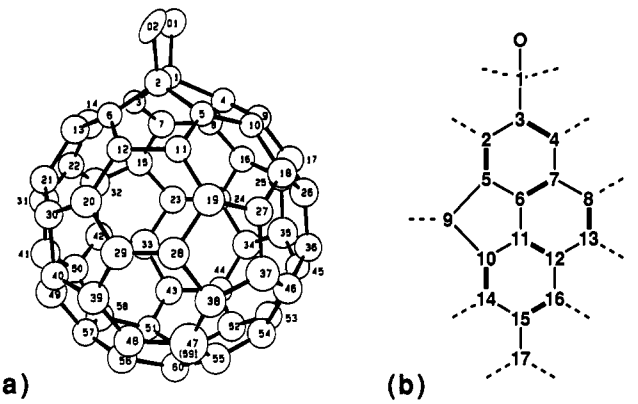

Figure 1. $C_{60}O_2$ unit of crystal structure of $C_{60}(OsO_4)$(4-*tert*-butylpyridine)$_2$ (**1**) showing cluster carbon numbering scheme (a), and fragment showing the connectivities of the 17 types of carbons in the cluster (b). Narrow lines indicate six–five ring fusions, bold lines indicate six–six ring fusions, and dashed lines indicate bonds between symmetry-related carbons or nonindependent couplings.

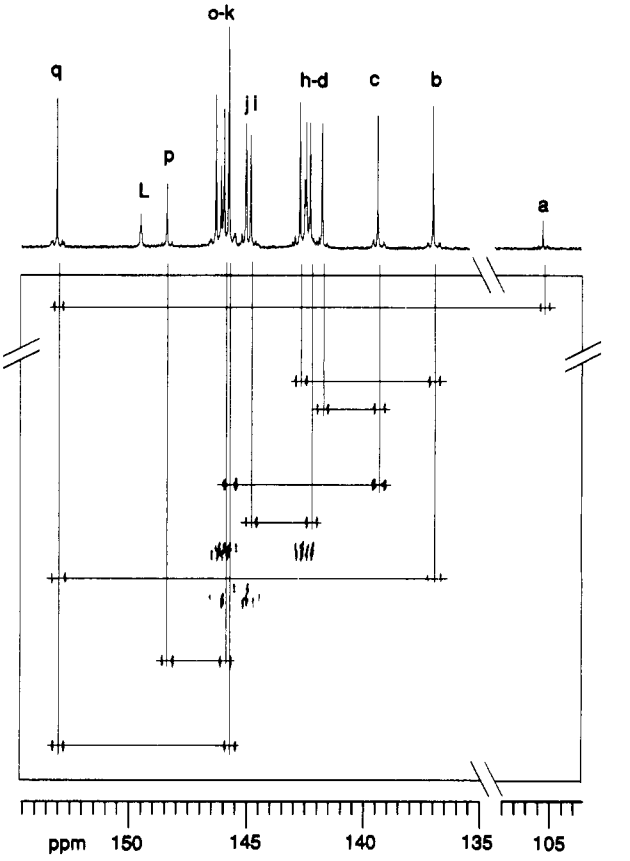

Figure 2. Upper spectrum: 1D ^{13}C NMR spectrum of **1**. Cluster carbons are labeled a–q as assigned in Table I; L indicates 4-*tert*-butylpyridine. Lower spectrum: 2D NMR INADEQUATE spectrum of **1**.[4,5] Vertical and horizontal lines delineate couplings: a–q, b–h, b–q, c–d, c–k, c–l, e–i, k–q, and m–p. Other couplings are not marked for clarity.

(1) Hawkins, J. M.; Meyer, A.; Lewis, T. A.; Loren, S.; Hollander, F. J. *Science* **1991**, *252*, 312.

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(3) Coordinated 4-*tert*-butylpyridine carbons: δ 165.95, 149.51, 122.85, 35.16, 30.52 ppm.

(4) A 2D ^{13}C–^{13}C chemical shift correlation spectrum of 40 mg of **1** (C1–C60 5.0% ^{13}C) in 3.3 mL of $CDCl_3$ at 26 °C was acquired at 125.276 MHz on a Bruker AM-500 instrument using a 10-mm probe. The INADEQUATE experiment[5a] was used with a modified phase cycling using 45° phase shifts.[5b] Proton decoupling simplified 4-*tert*-butylpyridine signals. The spectral width was set to 8196 Hz in F_2 and 16 392 Hz in F_1; 8K points were sampled in F_2; 400 increments of 96 scans were acquired in F_1. The refocusing delay was set to 5 ms. The repetition time was set to 5.5 s (T_1 for C(type 2)–C(type 17) < 4 s). The resulting matrix was processed with zero-filling in F_1 for a final size of 4K × 2K points. The F_1 dimension was processed with a real transform; a magnitude calculation was not applied. Coupling constants were measured from individual rows.

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(6) Peaks b–p fall within the range observed for C_{70}, 130.28–150.07 ppm, and average 143.8 ppm, close to the resonance of C_{60} at 142.68 ppm. Taylor, R.; Hare, J. P.; Abdul-Sada, K. A.; Kroto, H. W. *J. Chem. Soc., Chem. Commun.* **1990**, 1423. Johnson, R. D.; Meijer, G.; Bethune, D. S. *J. Am. Chem. Soc.* **1990**, *112*, 8983.

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