Interhelical DNA–DNA Cross-linking. Bis(monoazidomethidium)octaoxahexacosanediamine: A Probe of Packaged Nucleic Acid

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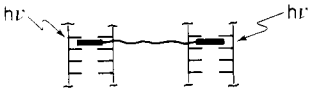
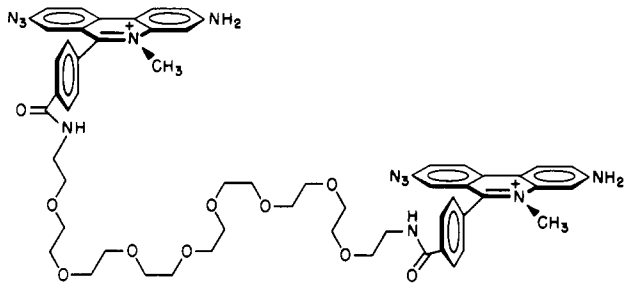
Cross-linking reagents have proven to be powerful tools for probing the higher order structure of macromolecules. Bifunctional protein–protein and protein–nucleic acid cross-linking reagents have been used to map protein–protein and protein–nucleic acid nearest neighbors in nucleosome and ribosome complexes.[2,3] This methodology has not been applied to mapping condensed nucleic acid structures such as viruses presumably because interhelical nucleic acid–nucleic acid cross-linkers have not been available. The design and syntheses of interhelical DNA–DNA cross-linkers and the development of the methodology to use them may provide a new capability to probe the structure of packaged nucleic acid.[4] Useful interhelical cross-linkers should be bifunctional, nucleic acid specific, water soluble, chemically inert, photoactivated, and efficient.



We describe the synthesis and purification of a new light-sensitive bifunctional molecule, bis(monoazidomethidium)octaoxahexacosanediamine (BAMO)[5] (**1**), which contains all the



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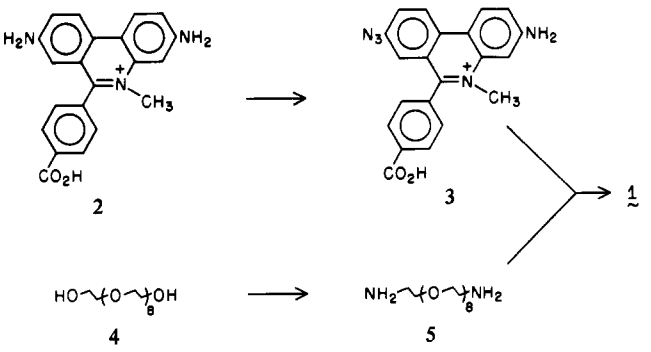
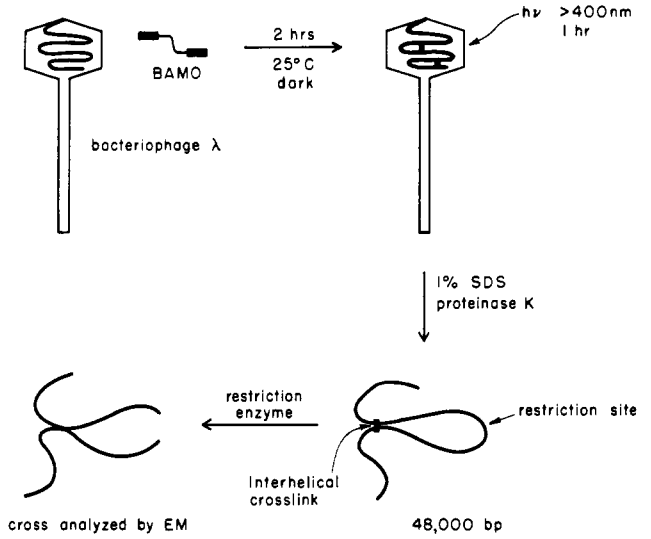
criteria presumed necessary for an interhelical DNA–DNA cross-linker: a photoreactive DNA intercalator, monoazidomethidium,[6] and a polyethyleneoxy chain sufficiently long (>25 Å) to span contiguous nucleic acid double helices.[7] We also present evidence that BAMO efficiently cross-links packaged nucleic acid in intact bacteriophage λ.

The synthetic sequence is outlined in Scheme I. Diazotization of *p*-carboxymethidium chloride **2** (isopentyl nitrite, 0.2 M aq HCl, 0 °C) followed by reaction with sodium azide yields the light-sensitive monoazido-*p*-carboxymethidium chloride **3**[8,9] after chromatography on carboxymethyl cellulose (Cellex CM, pH 2.8 aq HCl eluant).[10] Successive treatment of nonaethylene glycol (**4**)[11] with tosyl chloride (pyridine, 0 °C), potassium phthalimide (DMF, 90 °C), and N_2H_4–H_2O (ethanol at reflux) affords diamine **5** upon neutralization and workup.[12] The reaction of 0.5 equiv of diamine **5** with the acyl imidazole ester of **3** (Me_2SO_4, 25 °C, 24 h) affords the light-sensitive deep red compound bis(monoazidomethidium)octaoxahexacosanediamine (BAMO), which can be purified by successive chromatography on silica gel (230–400 mesh ASTM, 0.006% methanolic HCl eluant) and amberlite (XAD-2, water and then methanol eluant), respectively.[8,13,14]

Scheme I



Scheme II



BAMO (6×10^{-6} M) is allowed to incubate with intact bacteriophage λ (6×10^{-4} M in base pairs) for 2 h at 25 °C in the

(1) Camille and Henry Dreyfus Teacher-Scholar, 1978–83.

(2) For example see: Brimacombe, R.; Stoffler, G.; Whittman, H. G. *Ann. Rev. Biochem.* **1978**, *92*, 217–249 and references cited therein.

(3) For example see: (a) Kornberg, R. D. *Ann. Rev. Biochem.* **1977**, *46*, 931–954 and references cited therein. (b) Shick, V. V.; Belyavsky, A. V.; Barykin, S. G.; Mirzabekov, A. D. *J. Mol. Biol.* **1980**, *139*, 491–517.

(4) (a) Cantor, C. R. *Ann. New York Acad. Sci.* **1980**, *346*, 319–385. (b) Haas, R.; Murphy, R. F.; Cantor, C. R. *J. Mol. Biol.*, in press.

(5) The IUPAC name for compound **1** is 6,6′-[1,26-(3,6,9,12,15,18,21,24-octaoxahexacosanediyl)bis(iminocarbonyl-4,1-phenylene)]bis(3-amino-8-azido-5-methylphenanthridinium chloride) dihydrate.

(6) (a) Graves, D. E.; Watkins, C. L.; Yielding, L. W. *Biochemistry* **1981**, *20*, 1887–1892. (b) Hixon, S. C.; White, W. E., Jr.; Yielding, K. L. *J. Mol. Biol.* **1975**, *92*, 319–329. (c) Graves, D. E.; Yielding, L. W.; Watkins, C. L.; Yielding, K. L. *Biochim. Biophys. Acta* **1977**, *479*, 98–104. (d) Sternglanz, H.; Graves, D. E.; Yielding, L. W.; Bugg, C. E., *J. Cryst. Mol. Struct.* **1978**, *8*, 93–103.

(7) (a) Earnshaw, W. C.; Harrison, S. C., *Nature (London)* **1977**, *268*, 598–602. (b) North, A. C. T.; Rich, A. *Ibid.* **1961**, *191*, 1242–1245.

(8) The synthesis, purification, and the reactions of **3** were performed under red light illumination.

(9) The monoazide **3** crystallized from 1 M HCl as the chloride monohydrate. Anal. Calcd for $C_{21}H_{18}N_4O_3Cl$: C, 59.51; H, 4.28, N, 16.52. Found: C, 59.41, H, 4.36, N, 16.46. NMR, IR (KBr, 2118 cm^{-1}), and UV–vis (λ_{max} 464 nm, ϵ 5.39×10^3/mol cm)) spectral data were consistent with the monoazide structure. The location of the azide functionality in **3** is unknown. The monoazide is more likely a mixture of isomers at C-3 and C-8. The monoazide at C-8 in **3** is drawn in analogy to the X-ray structure determination of ethidium monoazide.[6d]

(10) Cellex CM was obtained from Bio-Rad Laboratories.

(11) Bomes, B.; Heitz, W.; Kern, W. *J. Chromatogr.* **1970**, *53*, 51–54.

(12) Diamine **5**: 3,6,9,12,15,18,21,24-octaoxahexacosane-1,26 diamine. Anal. Calcd for $C_{18}H_{40}N_2O_8$: C, 52.41; H, 9.77; N, 6.79. Found: C, 52.58; H, 9.64; N, 6.79.

(13) Fieser, L. F.; Fieser, M. "Reagents for Organic Synthesis"; Wiley: New York, **1967**, Vol. 1, p 688.

(14) The yield of BAMO after chromatography was 40% based on diamine **5**. The NMR, IR (KBr, 2118 cm^{-1}), and UV-vis (λ_{max}, 481 nm, ϵ, 8730/(mol cm)) spectral data were consistent with the bis(monoazide) structure. BAMO was isolated as the dichloride dihydrate and is most likely a mixture of bis(monoazide) isomers at C-3 and C-8. Anal. Calcd for $C_{60}H_{70}N_{12}O_{12}Cl_2$: C, 58.96; H, 5.77; N, 13.75. Found: C, 58.78; H, 5.92; N, 13.53.

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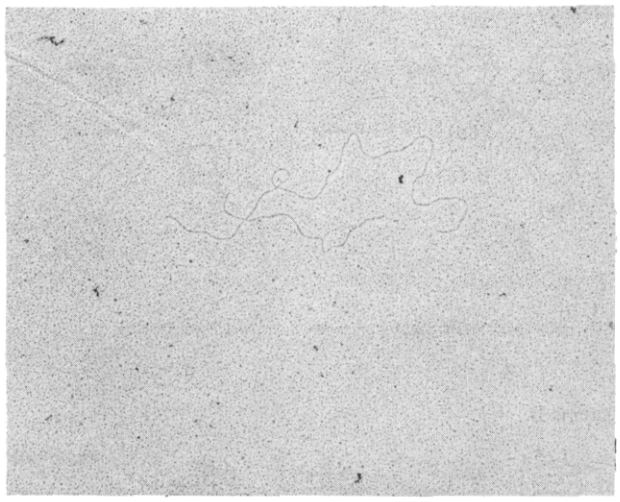

Figure 1. Cross of the 7 200 and 14 000 base pair restriction fragments from λ.[28]

dark.[15] This is irradiated in the visible (>400 nm) for 1 h.[17] The phage is burst with 1% SDS and treated with 1 mg/mL proteinase K at 37 °C for 4 h.[19] Gel electrophoresis[20] (0.3% agarose) of the BAMO/irradiated DNA (48 000 base pairs)[22] reveals higher mobility nucleic acid than the naked uncross-linked DNA. Examination of this "high mobility DNA" by electron microscopy reveals rosettes, tangled structures that may be the result of knotting.[23,25]

A protocol that affords structural data easier to interpret is shown in Scheme II. The bacteriophage λ (10^{-4} M in base pairs) is successively treated with BAMO (10^{-6} M), >400-nm light, and 1% SDS with 1 mg/mL proteinase K. The bacteriophage DNA is then purified by chromatography on Sepharose CL-2B,[26] dialyzed into an appropriate buffer,[27] treated with the restriction enzyme Mst II,[28] and spread for electron microscopy (EM).[23] Controls in the absence of cross-linker show 98% linear restriction fragments and 2% crossed structures.[30] Bacteriophage λ treated with BAMO reveal 17% crossed structures.[30] Using tritiated BAMO, we find that 6% of the reagent is covalently bound to the DNA after irradiation.[31] Because total cross-linker per DNA base pair is 0.01, an upper limit of 30 BAMO molecules/48 000 base pairs of DNA in bacteriophage λ affords the 17% "crossed" restriction fragments observed in the electron microscope (Figure 1). This suggests that BAMO is both an efficient reagent and a modest perturbation on the intact system.

One final piece of methodology will be needed before the assignment of the position of the interhelical crosslink is unique. The length of the four arms on a crossed structure can be experimentally measured from electron micrographs. Our choice of restriction enzymes and knowledge of the λ DNA restriction map, which is well documented,[29,33] allow identification of which final restriction fragments are cross-linked. However, the alignment of the crossed fragments remains to be resolved.[34] When this is accomplished, the structural data for interhelical nearest neighbors for packaged DNA will be available. Whether these "interhelical nearest neighbor" maps can be interpreted uniquely in light of conventional models for λ DNA packaging such as ball of yarn,[7,35] coaxial spool,[7,35] chain-folded structure,[7] or coil of coils[36] remains to be seen and will be reported in due course.

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Registry No. 1, 82209-34-5; **2**, 52671-19-9; **3**, 82209-35-6; **4**, 3386-18-3; **5**, 82209-36-7.

(15) Bacteriophage λ was produced by thermal induction of the lysogen w 3110 (λcI 857S7) and purified by CsCl density gradient centrifugation in 10 mM Tris HCl (pH 7.5) and 10 mM $MgSO_4$.[16] We thank B. Seed for assistance in the preparation of bacteriophage λ.

(16) Thomas, J. O. *J. Mol. Biol.* **1974**, *87*, 1–9.

(17) Photolysis was performed with a desk lamp equipped with two Westinghouse No. F15T8/CW light bulbs. The light was passed through a 5-cm 2.9 M aqueous sodium nitrite filter.[18]

(18) Bolton, P. H.; Kearns, D. R. *Nucleic Acids Res.* **1978**, *5*, 4891–4903.

(19) Proteinase K was obtained from Boehringer Mannheim Biochemicals.

(20) Agarose gels were poured and run on a horizontal gel apparatus similar to that of McDonnell et al.[21] with use of a buffer system consisting of 40 mM Tris, 5 mM sodium acetate, and 1 mM Na_2 EDTA adjusted to pH 7.8 with glacial acetic acid.

(21) McDonnell, M. W.; Simon, M. N.; Studier, F. W. *J. Mol. Biol.* **1977**, *110*, 119–146.

(22) Fiandt, M.; Honigman, A.; Rosenvold, E. C.; Szybalski, W. *Gene* **1977**, *2*, 289–293.

(23) DNA samples were spread for electron microscopy with use of the formamide modification of the Kleinschmidt technique.[24]

(24) Davis, R. W.; Simon, M. N., Davidson, N., *Meth. Enzymol. 21*, **1971**, 413–428.

(25) For examples of electron microscopy of bacteriophage λ DNA see (a) ref 16. (b) Chattoraj, D. K.; Inman, R. B. *J. Mol. Biol.* **1974**, *87*, 11–22.

(26) Sepharose CL-2B was obtained from Pharmacia Fine Chemicals.

(27) Buffer conditions for the enzyme Mst II are 6 mM Tris, 150 mM NaCl, 6 mM $MgCl_2$, (pH 7.4) with 6 mM 2-mercaptoethanol, and 100 μg/mL bovine serum albumin.

(28) Restriction endonuclease Mst II was obtained from New England Biolabs. Mst II cleaves λcI 857S7 DNA at two sites, approximately 26 400 and 33 600 bp from the left end of the genome, yielding three restriction fragments of lengths 26 400, bp, 7 200, and 14 400 bp.[29]

(29) Mst II cleavage map was obtained from New England Biolabs.

(30) A DNA species observed in the electron microscope was scored as a "linear restriction fragment" when it exhibited only two distinct ends. A DNA species was scored as a "crossed structure" when 2 N distinct ends (where $N > 1$) were continuously connected. A total of 500 DNA structures were counted for the BAMO-treated and control samples.

(31) Tritiated BAMO was synthesized according to Scheme I by using radiolabeled diamine **5**.[32] Specific activity is 1.1 Ci/mmol.

(32) Diamine **5** was custom labeled by New England Nuclear and redistilled (bulb to bulb, 230 °C at 5 μm) prior to use.

(33) Szybalski, E. H.; Szybalski, W. *Gene* **1979**, *7*, 217–270.

(34) Correct alignment of the crossed restriction fragments requires assignment of the directionality of each fragment (left to right).

(35) Richards, K.; Williams, R.; Calendar, R. *J. Mol. Biol.* **1973**, *78*, 255–259.

(36) Kilkson, R.; Maestre, M. F. *Nature (London)* **1962**, *195*, 494–495.

Cobalt Nitro Complexes as Oxygen Transfer Agents. 4.[1] Epoxidation of Olefins

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Epoxidation of propylene and higher olefins is one of the most important oxidation reactions in organic chemistry and in the chemical industry. With the exception of thallium(III),[2] all other oxidants capable of olefin epoxidation are peroxidic in nature.[3] They are obtained by reduction of molecular oxygen to hydrogen peroxide and by oxidation of alkanes to alkyl hydroperoxides or aldehydes to peracids. Under ideal conditions, only one oxygen atom of molecular oxygen is incorporated in the product epoxide while the other is utilized in the formation of side products. In the case of olefin oxidation by thallium(III) (eq 1) it is the en-

$$\mathrm{Tl(III)} + \mathrm{H_2O} + \mathrm{RCH{=}CH_2} \rightarrow \mathrm{Tl(I)} + 2\mathrm{H^+} + \mathrm{RCH(O)CH_2} \quad (1)$$

(1) (a) Part 1: Tovrog, B. S.; Diamond, S. E.; Mares, F. *J. Am. Chem. Soc.* **1979**, *101*, 270–272. (b) Part 2: Tovrog, B. S.; Mares, F.; Diamond, S. E. *Ibid.* **1980**, *102*, 6616–6618. (c) Part 3: Tovrog, B. S.; Diamond, S. E.; Mares, F.; Szalkiewicz, A. *Ibid.* **1981**, *103*, 3522–3526.

(2) Brill, W. F. U.S. Patent 4 021 453, issued May 3, 1977 to Halcon International, Inc.

(3) Sheldon, R. A.; Kochi, J. K. "Metal Catalyzed Oxidations of Organic Compounds"; Academic Press: New York, 1981; Chapters 3 and 9 and references therein.

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