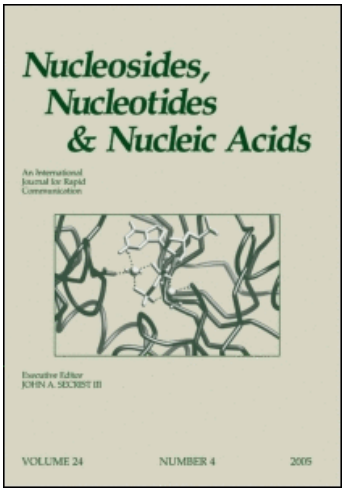


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

The Photochemistry Of 5-BrdCpdC, dCp5-BrdCpdA, and dCp5-BrdCdT in Aqueous Solution

Yuan Gao[a]; Yinsheng Wang[a]
[a] Department of Chemistry-027, University of California, Riverside, California, USA

To cite this Article Gao, Yuan and Wang, Yinsheng(2006) 'The Photochemistry Of 5-BrdCpdC, dCp5-BrdCpdA, and dCp5-BrdCdT in Aqueous Solution', Nucleosides, Nucleotides and Nucleic Acids, 25: 3, 279 — 287
To link to this Article: DOI: 10.1080/15257770500446972
URL: http://dx.doi.org/10.1080/15257770500446972

Nucleosides, Nucleotides, and Nucleic Acids, 25:279–287, 2006

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770500446972

Taylor & Francis
Taylor & Francis Group

THE PHOTOCHEMISTRY OF 5-BrdCpdC, dCp5-BrdCpdA, AND dCp5-BrdCdT IN AQUEOUS SOLUTION

Yuan Gao and Yinsheng Wang □ *Department of Chemistry-027, University of California, Riverside, California, USA*

□ *We isolated an intrastrand crosslink product from the Pyrex-filtered UV light irradiation of 5-BrdCpdC in aqueous solution. ESI-MS, MS/MS, and 2-D nuclear Overhauser effect spectroscopy (NOESY) results showed that the C5 carbon atoms of the two cytosines are covalently bonded. The same cross-link product can also be induced from the similar UV irradiation of dCp5-BrdCpdA and dCp5-BrdCpdT.*

Keywords 5-Bromocytosine; Photoproduct; Mass spectrometry; NMR

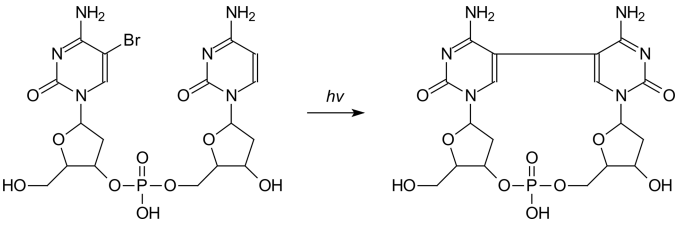
INTRODUCTION

Replacement of thymidine with 5-bromo-2′-deoxyuridine (5-BrdU) within DNA increases considerably the sensitivity of living cells and DNA to UV light and γ-ray irradiation.[1] UV irradiation of 5-BrdU-bearing DNA could lead to the formation of strand breaks and alkali labile sites.[2–5] In addition, UV irradiation of 5-bromouridine and 5-BrdU could induce the formation of a 5,5′-diuridinyl cross-link.[6] Similar irradiation of polynucleotides containing 5-bromouracil could also produce this type of photoproduct.[7] However, the photochemistry of the related 5-BrdC has not been extensively explored.[8–10]

We reported recently that the Pyrex-filtered UV light irradiation of a dinucleoside monophosphate, 5-BrdCpdG, can result in the formation of a cross-link product in which the C5 carbon atom of cytosine and the C8 carbon atom of the neighboring guanine are covalently bonded.[9] The corresponding GC cross-link can also form from the UV irradiation of 5-bromocytosine-containing duplex DNA.[9] Here we report the identification of a new intrastrand cross-link product formed between two cytosines from

Received 30 March 2005; accepted 31 October 2005.
We thank the NIH (R01 CA 96906) for supporting this research.
Address correspondence to Yinsheng Wang, Department of Chemistry-027, University of California, 900 University Avenue, Riverside, CA 92521. E-mail: yinsheng.wang@ucr.edu

the similar UV irradiation of 5-BrdCpdC (Scheme 1), dCp5-BrdCpdT, and dCp5-BrdCpdA.



SCHEME 1

MATERIALS AND METHODS

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise specified. The reagents used for solid-phase DNA synthesis were purchased from Glen Research Inc. (Sterling, VA). Dinucleoside monophosphate, 5-BrdCpdC, and trinucleoside diphosphates, dCp5-BrdCpdA and dCp5-BrdCpdT, were synthesized on a Beckman Oligo 1000S DNA synthesizer (Fullerton, CA) by using the commercially available 5-BrdC phosphoramidite building block (Glen Research Inc.) The removal of benzoyl protecting group on the amino nitrogen of cytosine and adenine as well as the cyanoethyl group on the phosphate backbone was carried out in 29% ammonia at room temperature for 48 h, and the deprotection under room temperature was necessary for minimizing the decomposition of the halogenated nucleoside.

Photoirradiation

The 5-BrdCpdC, dCp5-BrdCpdA, or dCp5-BrdCpdT (150 nmol) was dissolved in 5 mL water and transferred to a 13 × 100 mm (O.D. × length) Pyrex tube (Corning Inc., Catalog No. 99447-13). Photoirradiation was carried out for 35 min with a Hanovia 450-W medium-pressure mercury lamp. We measured the UV absorption spectrum for the Pyrex tube and the wavelength with 50% transmission was 280 nm.[10] During irradiation the Pyrex tube and lamp were immersed in an ice-water bath. The solution was exposed to air during irradiation. Irradiation was also carried out under anaerobic conditions where the solution was bubbled with argon for 30 min before irradiation and the bubbling was continued during the whole irradiation process. The resulting irradiation mixture was dried by using a Savant Speed-vac (Savant Instrument Inc., Holbrook, NY). The dried residue was re-dissolved in water for HPLC analysis.

HPLC

The HPLC separation was performed on a Surveyor system (ThermoFinnigan, San Jose, CA) with a photodiode array detector, which was set at 260 nm for monitoring the effluents. A 4.6 × 250 mm Apollo C18 column (5 μm in particle size and 300 Å in pore size, Alltech Associates Inc., Deerfield, IL) was used. A gradient of 40 min 0–12% acetonitrile in 10 mM ammonium formate (pH 6.3) was employed for the separation of the irradiation mixtures and the flow rate was 0.8 mL/min. The HPLC fractions were then dried in the Speed-vac. For NMR analysis, the products were further desalted by using a Microsorb C18 column (10 × 250 mm, 5 μm in particle size, and 300 Å in pore size, Varian, Walnut Creek, CA). After the sample was loaded, the column was washed with water for 8 min, and then the sample was eluted from the column by 50% methanol in water.

Mass Spectrometry

Electrospray ionization-mass spectrometry (ESI-MS) and tandem MS (MS/MS) experiments were conducted on an LCQ Deca XP ion-trap mass spectrometer (ThermoFinnigan, San Jose, CA). An equal-volume solvent mixture of acetonitrile and water was used as the carrier and electrospray solvent, and a 1-μL aliquot of 2 μM sample solution was injected in each run. The MS experiments were carried out in negative-ion mode, and the spray voltage was 3.0 kV. The mass width for precursor ion selection in MS/MS mode was 2 m/z units. Each spectrum was obtained by averaging approximately 50 scans, and the time for each scan was 0.1 s.

Exchangeable Proton Measurement

To measure the number of exchangeable protons, purified cross-link product was incubated with D_2O for 12 h, dried by Speed-vac, and re-dissolved in D_2O. The resulting solution was injected directly for ESI-MS and MS/MS analysis, and acetonitrile in D_2O (50/50, v/v) was used as electrospray solvent.

NMR Measurement

All NMR spectra were acquired on a Varian Unity Inova 500 MHz instrument (Palo Alto, CA). The residual proton signal of the solvent serves as internal reference. Two-dimensional (2-D) nuclear Overhauser effect spectroscopy (NOESY) experiments were performed at 298 K with 600 ms mixing time, and the NOE spectra were obtained from 2 × 256 × 2048 data matrices with 24 scans per t1 value.

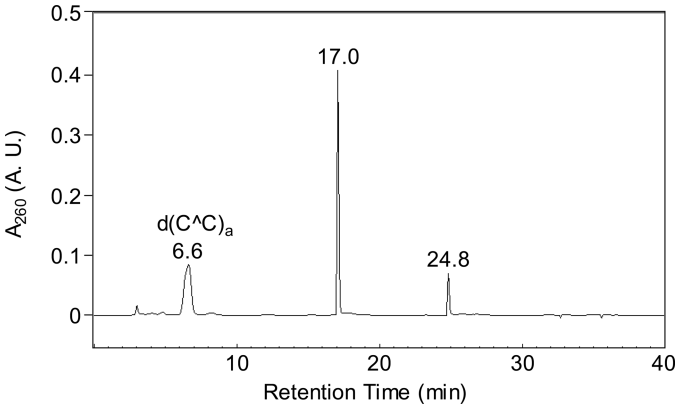


FIGURE 1 HPLC trace for the separation of a 20-min UV irradiation mixture of 5-BrdCpdC.

RESULTS AND DISCUSSION

The HPLC trace for the separation of the UV irradiation mixture of 5-BrdCpdC in aqueous solution showed that there are three major peaks eluting at 6.6, 17.0, and 24.8 min, respectively (Figure 1). MS and NMR characterizations support that the 6.6-min fraction is a photoproduct in which the C5 carbon atoms of the two neighboring cytosines are covalently bonded, and we designate the product as dC(5–5)pdC (Scheme 1, labeled as d $(C\hat{}C)_a$ in Figure 1). In this respect, negative-ion ESI-MS of this fraction gave an ion of m/z 513 (Figure 2a), indicating that the product forms from 5-BrdCpdC via the elimination of a bromine and a hydrogen. This conclusion is further supported by the absence of bromine isotope pattern in the molecular ion peak. In the product-ion spectrum (MS/MS) of the ESI-produced $[M - H]^-$ ion of dC(5-5)pdC (ion of m/z 513), we observed the fragment ions of m/z 495; 470, and 415, which result from the losses of a H_2O, HNCO, and a portion of 2-deoxyribose ($C_5H_6O_2$, 98Da), respectively (Figure 2b). In addition, we found an ion of m/z 219, which is the deprotonated ion of a moiety consisting of two cytosines with the loss of two hydrogen atoms. The presence of this ion, along with the absence of fragment ion resulting from the loss of a single cytosine, demonstrates that the photoproduct has the two cytosines being covalently bonded.

The above MS and MS/MS results show that the crosslink product arises from the elimination of a bromine and a hydrogen from the nucleobase portion of 5-BrdCpdC. To determine which atoms are involved in the cross-link, we first measured the number of acidic protons in dC(5-5)pdC by ESI-MS. In this respect, negative-ion ESI-MS gives m/z 519 for the $[M - D]^-$ ion of the cross-link product, demonstrating that there are six acidic hydrogen atoms in the $[M - H]^-$ ion of the cross-link product. Therefore, no acidic hydrogen is lost upon the formation of the cross-link product, supporting that the exocyclic amino nitrogen atom of

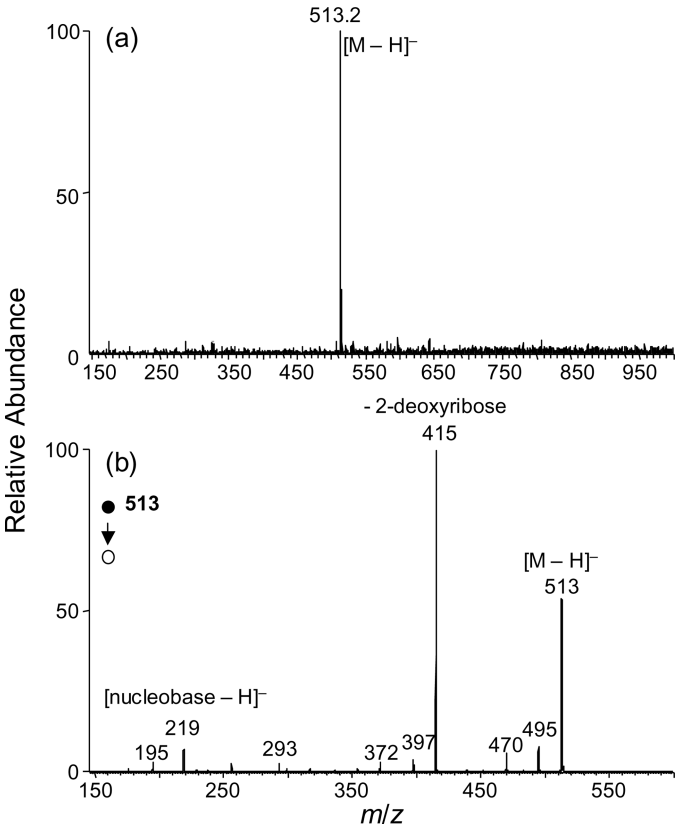


FIGURE 2 Negative-ion ESI-MS of dC(5-5)pdC (a), and the product-ion spectrum of the ESI-produced $[M - H]^-$ ion of dC(5-5)pdC (b).

neither cytosines is involved in forming the covalent bond bridging the two nucleobases.

To gain further information about the structure of the cross-link product, we acquired the ^{1}H-NMR spectrum of dC(5-5)pdC. It turned out that there are two singlet aromatic proton resonances in the spectrum (Figure 3). Moreover, 2-D NOE (nuclear Overhauser effect) spectrum of dC(5-5)pdC (Figure 4) showed a pair of correlation peaks, where one aromatic proton is correlated with one $H_{1'}$ proton and the other aromatic proton is correlated with the other H_1 proton. Therefore, the two aromatic protons can be assigned as the H6 protons of the two cytosines. These NMR results, together with the data from the MS, MS/MS, and acidic hydrogen measurements, demonstrated without ambiguity that the cross-link product has a covalent bond formed between the C5 carbon atoms of the two cytosines.

In addition to the dC(5-5)pdC, some initial evidence suggests that the two later eluting fractions (17.0- and 24.8-min fractions) might be dC(5-6)pdC and its hydration product. More detailed spectroscopic characterizations are necessary for establishing firmly their structures. We also carried out the UV

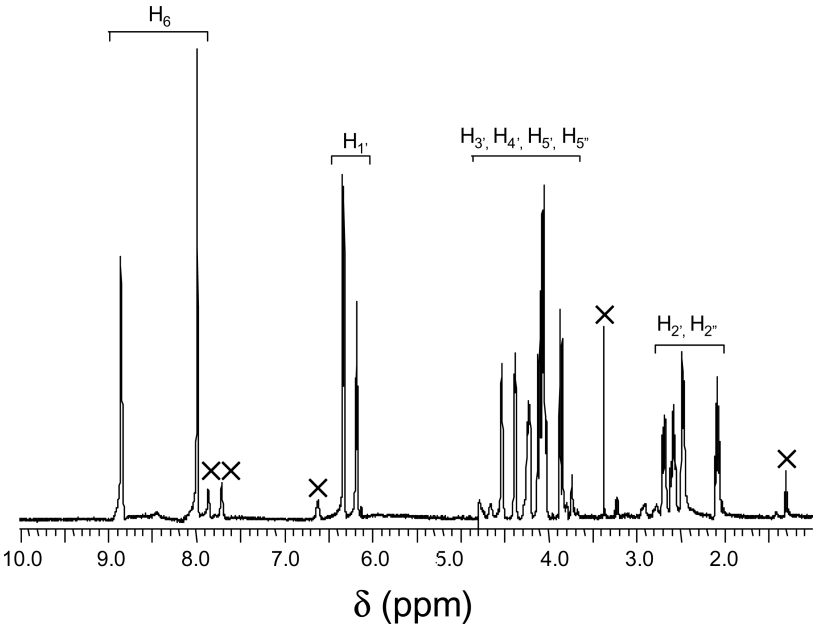


FIGURE 3 ^{1}H NMR spectrum of dC(5-5)pdC (D_2O, 500 MHz, 25°C). Impurity peaks were marked with an "X."

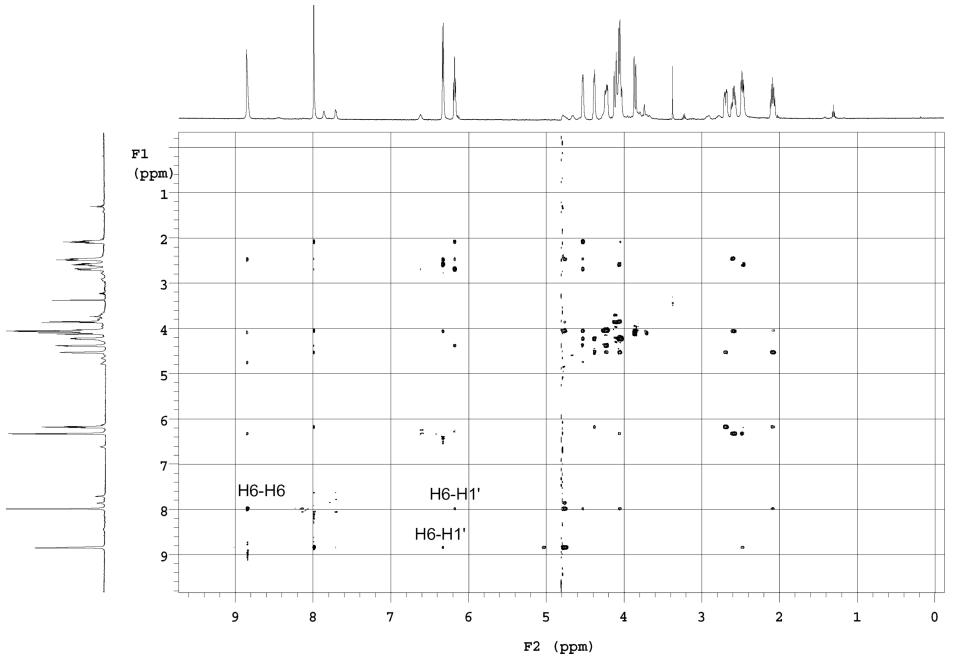


FIGURE 4 2-D NOE spectrum of dC(5-5)pdC (D_2O, 500 MHz, 25°C).

irradiation under anaerobic conditions and it turned out that it results in similar yield for the formation of dC(5-5)pdC (data not shown).

We next asked whether the same type of cross-link product could also be induced from dCp5-BrdC sequence. To this end, we irradiated dCp5-BrdCpdA and dCp5-BrdCpdT, digested the irradiation products with nuclease P1, calf spleen phosphodiesterase, calf intestinal alkaline phosphatase, and snake venom phosphodiesterase as previously described[11] and separated the resulting digestion mixtures by HPLC (Figure 5). It turned out that, from the digested irradiation mixtures of both dCp5-BrdCpdA and dCp5-BrdCpdT, we can find a fraction that exhibits very similar retention time and product-ion spectrum as dC(5-5)pdC (ESI-MS/MS are shown on the right of Figure 5).

Previously Dizdaroglu et al.[12] reported that γ irradiation of cytosine, 2′-deoxycytidine, and 2′-deoxycytidine-5′-monophosphate in N_2O-saturated aqueous solutions results in facile formation of dimers. The structures of those dimeric products, however, have not been rigorously established. In addition, we showed that Pyrex-filtered UV irradiation of duplex DNA

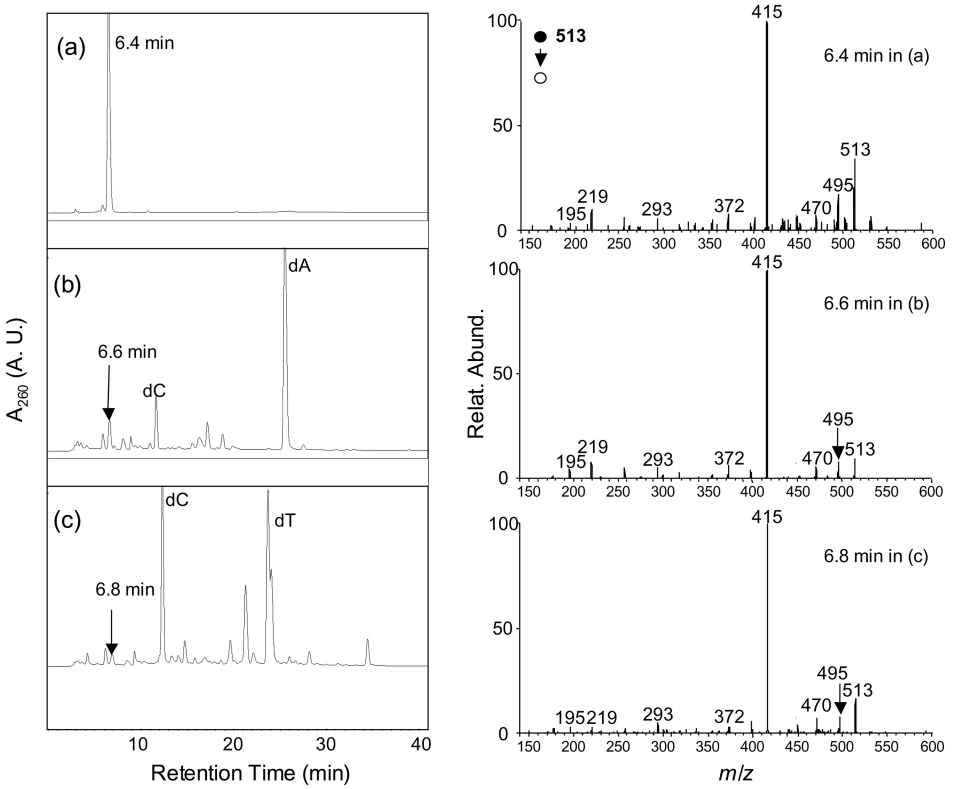


FIGURE 5 HPLC traces for the separations of standard dC(5-5)pdC (a), the digestion products of the UV irradiation mixtures of dCp5-BrdCpdA (b) and dCp5-BrdCpdT (c). The product-ion spectra of the $[M - H]^-$ ions of the crosslink-bearing fractions are shown on the right.

harboring a dGp5-BrdC sequence motif gives rise to the same type of intrastrand crosslink product as that induced from the γ irradiation of duplex DNA containing GC site.[9,13] The lesion formed from the latter pathway was thought to be initiated from the 6-hydroxy-5,6-dihydrocytosin-5-yl radical,[14] which can form upon the addition of hydroxyl radical to the C6 of cytosine. We then asked whether, under γ irradiation conditions, the 6-hydroxy-5,6-dihydrocytosin-5-yl radical can also couple with its neighboring cytosine base to give the same cross-link product as we reported here. To this end, we irradiated a self-complementary duplex oligonucleotide d(CCGGCCGGCCGGCCGG) with γ rays at a dose of 320 Gray under anaerobic conditions according to previously described procedures,[11] digested the irradiation mixture with the same four enzymes as described above, and analyzed the digestion mixture with LC-MS/MS. Our results showed that this type of cross-link lesion is not detectable from the digestion mixture of the γ irradiation products (data not shown).

To conclude, we isolated a novel cross-link product from the Pyrex-filtered UV light irradiation mixture of 5-BrdCpdC. Proton NMR and mass spectrometric characterizations demonstrate that the two adjacent cytosines are cross-linked via the formation of a covalent bond between their C5 carbon atoms. The same type of cross-link product can be induced from the similar UV irradiation of dCp5-BrdCpdA and dCp5-BrdCpdT.

REFERENCES

1. Cadet, J.; Vigny, P. The Photochemistry of Nucleic Acids. In *Bioorganic Photochemistry*; Morrison, H., Ed.; John Wiley, New York, 1990, 1–272.
2. Sugiyama, H.; Tsutsumi, Y.; Saito, I. Highly sequence-selective photoreaction of 5-bromouracil-containing deoxyhexanucleotides. J. Am. Chem. Soc. **1990**, 112, 6720–6721.
3. Cook, G.P.; Chen, T.; Koppisch, A.T.; Greenberg, M.M. The effects of secondary structure and O2 on the formation of direct strand breaks upon UV irradiation of 5-bromodeoxyuridine-containing oligonucleotides. Chem. Biol. **1999**, 6, 451–459.
4. Cook, G.P.; Greenberg, M.M. A novel mechanism for the formation of direct strand breaks upon anaerobic photolysis of duplex DNA containing 5-bromodeoxyuridine. J. Am. Chem. Soc. **1996**, 118, 10025–10030.
5. Chen, T.; Cook, G.P.; Koppisch, A.T.; Greenberg, M.M. Investigation of the origin of the sequence selectivity for the 5-halo-2′-deoxyuridine sensitization of DNA to damage by UV-irradiation. J. Am. Chem. Soc. **2000**, 122, 3861–3866.
6. Sasson, S.; Wang, S.Y. Photochemistry of 5-bromouridine and 5-bromo-2′-deoxyuridine in ice and in "puddles." Photochem. Photobiol. **1977**, 26, 357–361.
7. Sasson, S.; Wang, S.Y.; Ehrlich, M. 5,5′-Diuridinyl, a major photoproduct from UV-irradiation of polynucleotides containing bromouracil. Photochem. Photobiol. **1977**, 25, 11–13.
8. Celewicz, L. Photochemical reactions of 5-bromocytosine and its N-1-substituted derivatives with Na-acetyl-L-tryptophan N-ethylamide in aqueous solution. J. Photochem. Photobiol. B: Biol. **1995**, 30, 129–137.
9. Zeng, Y.; Wang, Y. Facile formation of an intrastrand cross-link lesion between cytosine and guanine upon Pyrex-filtered UV light irradiation of d(^{Br}CG) and duplex DNA containing 5-bromocytosine. J. Am. Chem. Soc. **2004**, 126, 6552–6553.
10. Hong, H.; Wang, Y. Formation of intrastrand cross-link products between cytosine and adenine from UV irradiation of d(^{Br}CA) and duplex DNA containing a 5-bromocytosine. J. Am. Chem. Soc. **2005**, 127, 13969–13977.

11. Zhang, Q.; Wang, Y. Generation of 5-(2′-deoxycytidyl)methyl radical and the formation of intrastrand cross-link lesions in oligodeoxyribonucleotides. Nucleic Acids Res. **2005**, 33, 1593–1603.
12. Dizdaroglu, M.; Simic, M.G. Radiation-induced cross-linking of cytosine. Radiat. Res. **1984**, 100, 41–46.
13. Gu, C.; Wang, Y. LC-MS/MS identification and yeast polymerase eta bypass of a novel gamma-irradiation-induced intrastrand cross-link lesion G[8-5]C. Biochemistry **2004**, 43, 6745–6750.
14. Box, H.C.; Budzinski, E.E.; Dawidzik, J.B.; Wallace, J.C.; Iijima, H. Tandem lesions and other products in X-irradiated DNA oligomers. Radiat. Res. **1998**, 149, 433–439.