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Photoaddition of 5-Bromouracil to Uracil in Oligonucleotides Leading to 5,5′-Bipyrimidine-Type Adducts: Mechanism of the Photoreaction

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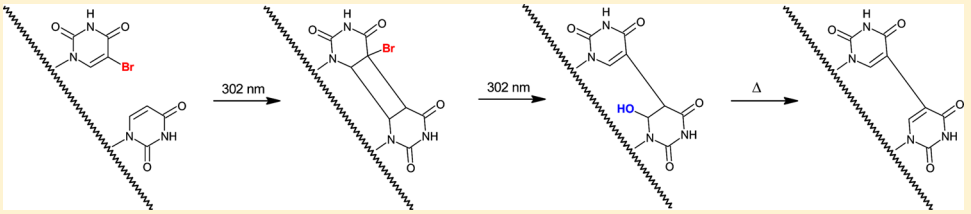
Supporting Information



ABSTRACT: 5-Bromouracil (^{Br}U) modified di- and hexanucleotides having ^{Br}U flanked on the 5′ or the 3′ side by uracil (U) have been synthesized, and their photochemical reactivity was examined under the conditions of irradiation with near UV light. The results indicate that the primary photochemical process in all of these compounds involves the formation of an intermediate cyclobutane phodoadduct composed of ^{Br}U and U, which undergoes further photochemically and thermally induced transformations to 5,5′-bipyrimidine type adducts.

It is well-known that replacement of thymine (T) in DNA with ^{Br}U significantly enhances the sensitivity of DNA to UV light.[1] The major photochemical processes of ^{Br}U in DNA involve debromination to U,[2] formation of intramolecular cross-linking products with pyrimidine[3] and purine residues,[4] as well as the generation of single and double strand breaks[5] or alkali-labile sites.[6] The generally accepted mechanisms of these processes assume either homolytic or heterolytic cleavage of the C(5)−Br bond as primary photochemical events leading to a highly reactive, intermediate C(5)-carbon-centered U radical. These processes are strongly sequence-dependent and have been studied mostly for DNA oligonucleotides having ^{Br}U adjacent to purine bases, and so far little is known about the behavior of ^{Br}U adjacent to pyrimidines in DNA. The 5,5′-diuridinyl photoadduct has been isolated from ^{Br}U modified DNA, but the mechanism of its formation is unknown.[3] Although a variety of other photoproducts of ^{Br}U in DNA have been identified,[7] the cyclobutane-type photoadducts are not included among them so far. It is therefore important to note that the efficient formation of a cyclobutane-type adduct of ^{Br}U with U without debromination of the ^{Br}U moiety was observed upon near UV light (λ > 300 nm) irradiation of a simple dinucleotide model compound in which the phosphodiester linkage was replaced by a trimethylene chain.[8] It has been demonstrated that the adduct is thermally unstable, undergoing cyclobutane ring cleavage with concomitant hydrolysis to form the 5-hydroxyuracil analogue of the starting dinucleotide model.[8] Furthermore, the formation of cyclobutane-type adducts was also observed for other 5-halogenouracil compounds, namely, in the case of thymidylyl-(3′-5′)-2′-deoxy-5-fluorouridine monophosphate[9] and 5-chlorouracil containing dinucleotide model compounds[10] and 5-bromo-2′-deoxyuridine in frozen aqueous solution.[11]

The above observations prompted us to carefully investigate the photochemical behavior of ^{Br}U modified oligonucleotides, in which the ^{Br}U residue is adjacent to a pyrimidine base. In the following, we present the results of a detailed photochemical study of dinucleotides with the sequence ^{Br}U-U or U-^{Br}U and hexanucleotides containing either of these pairs as part of their sequences (Table 1).

Solutions of the HPLC-purified dinucleotides **1** or **2** in 0.1 M phosphate buffer (pH 7, $A_{260\ nm}$ = 1.0 in a 0.2 cm UV cuvette)

Table 1. Sequences of Di- and Hexanucleotides Studied[a]

Oligonucleotide	Sequence
1	5′-^{Br}UU-3′
2	5′-U^{Br}U-3′
3	5′-TA^{Br}UUAT-3′
4	5′-AU^{Br}UATA-3′

[a]^{Br}U = 2′-*O*-methyl-5-bromouridine; U = 2′-*O*-methyluridine.

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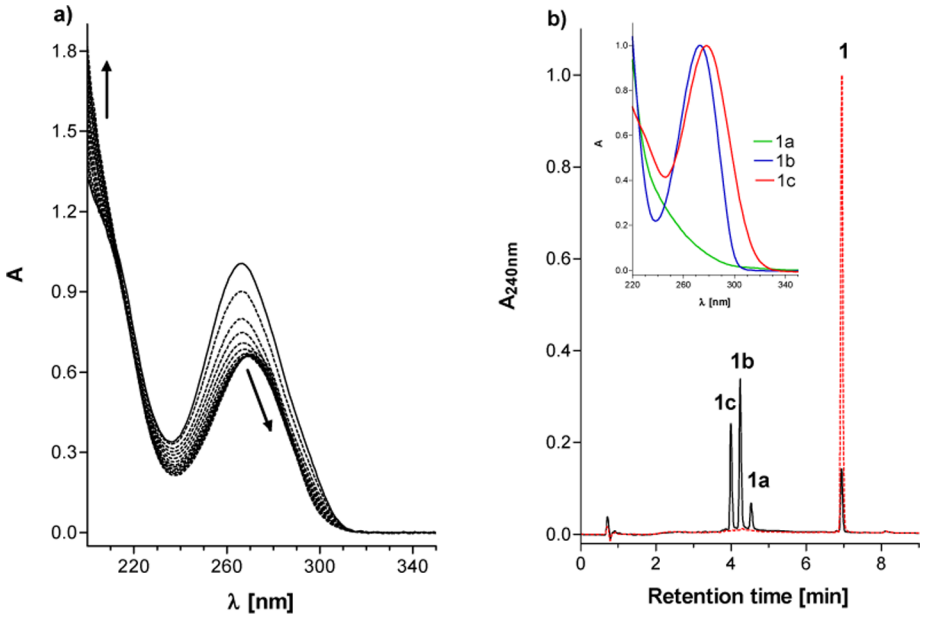
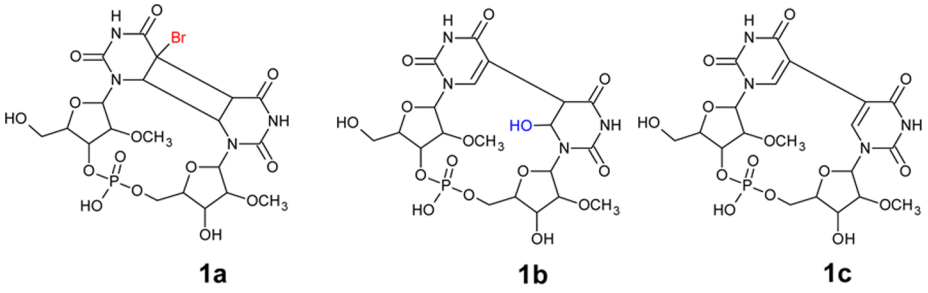


Figure 1. (a) Changes in the absorption spectrum of solution of **1** during irradiation at 302 nm under aerobic conditions. The spectra were taken with 2 min time increments. (b) HPLC analysis of the solution **1** before (red dotted line) and after 20 min of photoirradiation (black solid line). The inset shows the normalized absorption spectra of the photoproducts.

Scheme 1. Structures of Products of Irradiation of Dinucleotide 1



were irradiated at 302 nm with an argon-ion laser under aerobic conditions. The progress of the photoreaction was monitored by UV absorption spectroscopy and HPLC (Figure 1). In the case of dinucleotide **1** irradiation resulted in both a gradual decrease and a red shift of the UV absorbance at 266 nm (Figure 1a), whereas HPLC analysis of the solution irradiated to ca. 90% conversion of starting dinucleotide revealed the presence of three species, **1a**–**c** (Figure 1b).

The products **1a**–**c** were separated by semipreparative C_{18} reversed-phase HPLC and completely characterized (Scheme 1).

The structures of **1b** and **1c** (Scheme 1) were unequivocally established on the basis of ^{1}H and ^{13}C NMR spectral data. The HR ESIMS spectrum of the major product, **1b**, revealed the molecular ion at m/z 593.1126 $[M - H]^-$ consistent with the molecular formula $C_{20}H_{26}N_4O_{15}P$, indicating that its formation involves debromination of the starting dinucleotide **1** with concomitant addition of a hydroxyl group. In the case of **1c**, the HRMS spectrum gave the molecular formula $C_{20}H_{24}N_4O_{14}P$ (m/z 575.1035 $[M - H]^-$) corresponding to the loss of hydrogen bromide from the molecular formula of **1**. The presence of a hydroxyl group in **1b** was further supported by its thermal lability; it was observed that on refluxing in water (40 min), **1b** was transformed quantitatively into **1c** as a result of dehydration (Figure S1, Supporting Information).

In both cases the ^{1}H NMR and ^{13}C NMR spectra showed peaks characteristic of the intact sugar units of the molecules, which indicated that the photochemical and thermal transformations occurred exclusively in the nucleobases. The observed ^{1}H and ^{13}C chemical shifts for the heterocyclic parts of these compounds are in full agreement with proposed structures. The C5–C5′ interbase connectivity in both compounds was established by ^{1}H–^{13}C heteronuclear multibond correlation experiments that in the case of **1b** revealed contacts between $H6_A$ and $C5_B$ and in the case of **1c** contacts between $H6_B$ and $C5_A$ (Figures S2, S3, Supporting Information).

In the case of the minor photoproduct **1a**, the HRMS spectrum gave the molecular formula $C_{20}H_{25}BrN_4O_{14}P$ $[M - H]^-$ (655.0296 and 657.0269 for ^{79}Br and ^{81}Br, respectively) identical to that of the starting dinucleotide **1**. The relatively small amounts of this product, as well as its thermal and photochemical lability, did not allow for its full characterization. However, the molecular formula of **1a** together with its characteristic absorption spectrum without a maximum above 220 nm, displaying only a decreasing tail in the absorption going from 220 to 300 nm (Figure 1b, inset), pointed to a cyclobutane-type adduct, formed as a result of the [2 + 2] cycloaddition photoreaction of ^{Br}U with U without debromination of the former. Additional characterization of photo-

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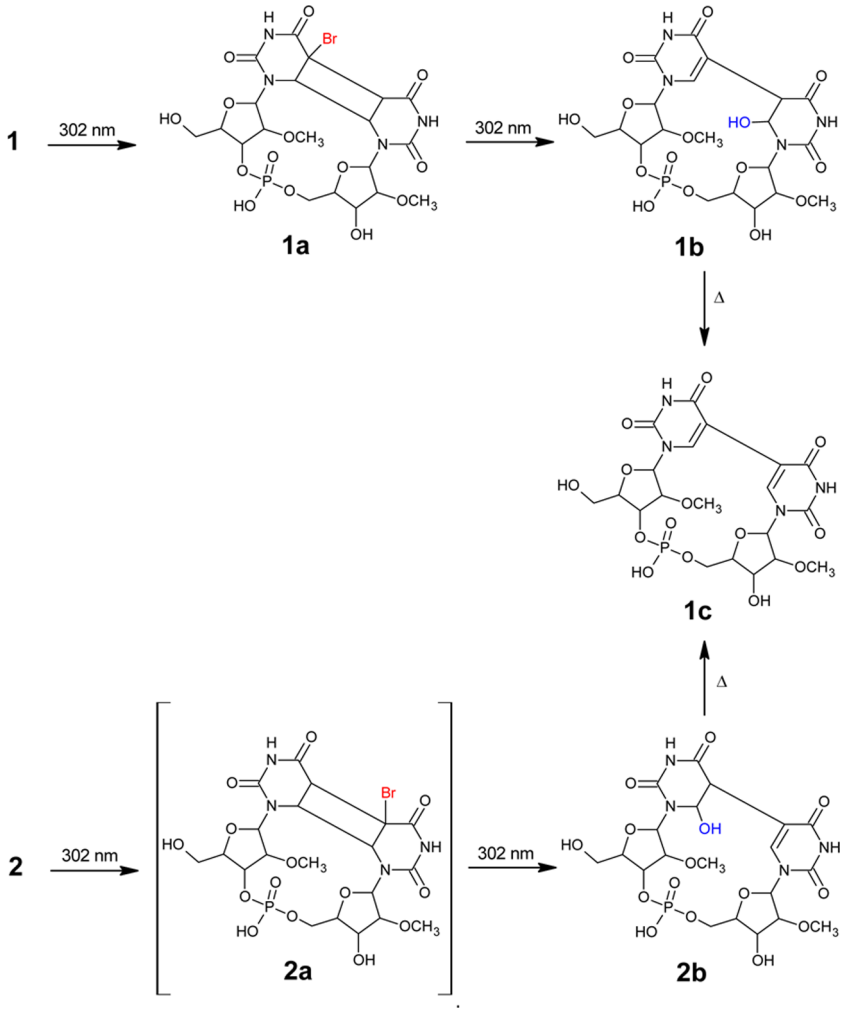
Scheme 2. Proposed Mechanism of the Formation of 5,5′-Diuridynyl Adduct 1c



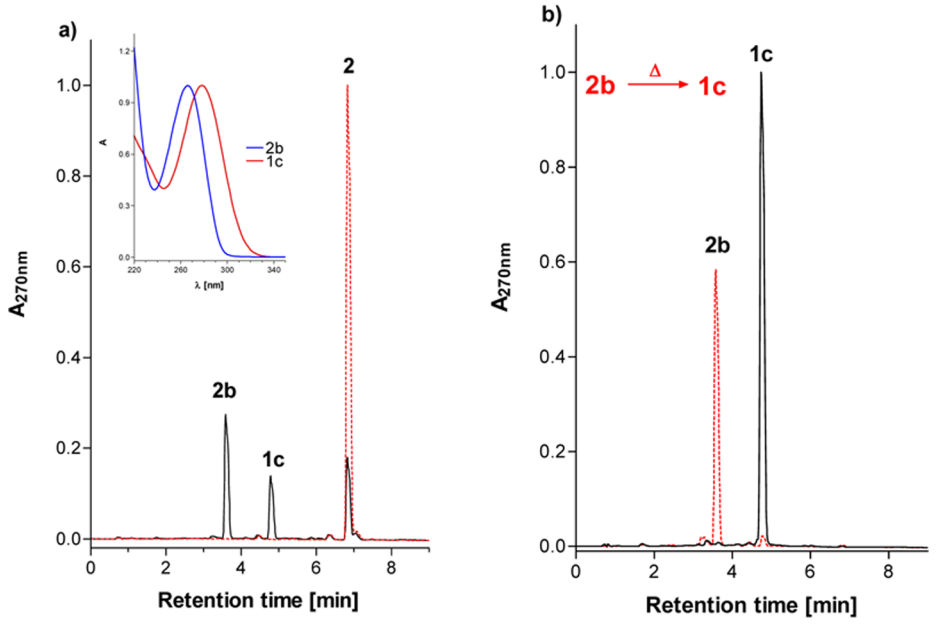


Figure 2. (a) HPLC analysis of the solution of **2** before (red dotted line) and after 45 min photoirradiation (black solid line). The inset shows the normalized absorption spectra of the photoproducts. (b) HPLC analysis of the solution of **2b** before heating (red dotted line) and after refluxing in water for 40 min (black solid line) to ca. 100% conversion.

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product **1a** as a cyclobutane adduct was provided by its partial conversion to starting dinucleotide **1** upon irradiation with 254 nm UV light, which is a typical reaction for the cyclobutane-type pyrimidine photodimers.[1] As shown in Figure S4a (Supporting Information), under these conditions **1a** undergoes transformations to two photoproducts, one of which was identified as dinucleotide **1**, and the other was found to be identical in all respects with **1b**. These observations suggested also that **1b** might be a secondary photoproduct formed from **1a**. Indeed, irradiation of **1a** at 302 nm, i.e., at the same conditions as **1**, resulted in its gradual transformation into **1b** (Figure S4b, Supporting Information). Furthermore, complete transformation of **1a** into 5,5′-diuridynyl product **1c** was observed after refluxing for 40 min in water (Figure S4c, Supporting Information).

All above observations pointed to a mechanism of near-UV-induced transformations of **1** involving formation of **1a** as a primary photoproduct, followed by its further photochemical transformation to **1b**, and thermal degradation of both photoproducts (**1a** and **1b**) to a 5,5′-diuridynyl product **1c** (Scheme 2).

Dinucleotide **2**, having the 5′-U^{Br}U-3′ sequence, appeared to be more resistant to photoirradiation compared with **1** (Figure S5, Supporting Information). The HPLC analysis of the solution of **2** irradiated to ca. 80% conversion (Figure 2a) revealed the presence of two products. The major product was identified (on the basis of HRMS and ^{1}H and ^{13}C NMR spectral data) as the 6-hydroxy-5,6-dihydro-5,5′-bipyrimidine photoadduct **2b**, which was a reversed-sequence isomer of phototoadduct **1b**. The minor product was a 5,5′-diuridynyl adduct identical in all respects to **1c**. As observed for **1b**, the photoadduct **2b** also proved to be thermally unstable, undergoing clean conversion to **1c** upon refluxing in water (Figure 2b).

Although the cyclobutane photoadduct **2a** (Scheme 2) was not detected following irradation of dinucleotide **2**, the photoproducts **2b** and **1c** were observed. On the basis of these observations it is reasonable to assume that similar photochemical pathways operate in both compounds as shown in Scheme 2. The absence of detectable amounts of **2a** in the irradiated solution of **2** can be rationalized either by a much slower rate of its formation compared to **1a** or by fast photochemical and thermal decomposition of **2a** to **2b**.

The above results suggest that similar photoreactions of ^{Br}U with U can also occur in longer oligonucleotide strands. To test this hypothesis, two hexamers, **3** and **4**, having ^{Br}U-U or U-^{Br}U sequences in the middle of the strands, were synthesized, and their photoreactivitieswere examined under conditions identical with those used for irradiation of dinucleotides. In the case of hexamer **3** the formation of thermally unstable oligonucleotide photoproduct **3a**, which undergoes conversion to another, minor product of irradiation, **3b**, upon prolonged heating, was observed (Figure S6, Supporting Information). The MALDI-TOF MS spectra of these products revealed that formation of the major product, **3a**, involves loss of bromine and addition of a hydroxyl group to the starting hexanucleotide, whereas its thermal conversion to **3b** involves loss of a H_2O molecule. These observations point to the formation of the 6-hydroxy-5,6-dihydro-5,5′-bipyrimidine photoadduct analogous to that of **1b** and its thermal conversion to a 5,5′-diuridynyl adduct analogous to **1c**. This is further supported by the appearance of a long wavelength absorption characteristic for **1c** in the UV spectrum of **3b** (Figure S7, Supporting Information).

A similar photochemical pathway involving the formation of a putative, thermally unstable 6-hydroxy-5,6-dihydro-5,5′-bipyrimidine photoadduct and its thermal conversion to a 5,5′-diuridynyl adduct was also observed for the hexamer **4** (see Supporting Information for relevant HPLC analysis, MALDI-TOF and UV spectral data). Furthermore, as in the case of dinucletides, hexamer **3** having the ^{Br}U-U sequence appeared to be more photoreactive than **4** with the U-^{Br}U sequence (Figure S8, Supporting Information).

In summary, our results indicate that ^{Br}U adjacent to a pyrimidine base in DNA oligonucleotides does not undergo cleavage of the C5−Br bond and photoreduction to uracil. Rather, its photochemical behavior resembles that of thymine, i.e., it undergoes [2 + 2] photocycloaddition typical for dipyrimidine sites leading to a cyclobutapyrimidine. The presence of the bromine atom in the structure makes the cyclobutane adduct susceptible to both photochemical and thermal degradation involving hydrolytic opening of the cyclobutane ring with concomitant debromination leading to the 6-hydroxy-5,6-dihydro-5,5′-bipyrimidine photoproduct. The latter undergoes further thermal dehydration to form a thermally stable 5,5′-diuridynyl adduct. Furthermore, on the basis of the above observations and previous reports,[8−11] the formation of cyclobutane-type adducts can now be assigned as a general feature of the photochemistry of 5-halogenouracil (^{F}U, ^{Cl}U and ^{Br}U) compounds.

EXPERIMENTAL SECTION

General Methods. HPLC analyses were performed using chromatographs equipped with diode-array UV−vis and fluorescence detectors. Photoirradiation experiments were carried out under aerobic conditions in a 0.2 cm UV cell using the 302 nm line of an argon-ion laser (equipped with a special UV grade tube and UV resonator optics). The mass spectra of oligonucleotides and products of their photoirradiation were obtained using a MALDI-TOF MS instrument equipped with a reflectron (resolution about 5000 at m/z 1000), on a MALDI metal target plate. The HRMS analysis of dinucleotides and products of their photoirradiation was performed using an ESI-TOF system. The NMR spectra (^{1}H and ^{13}C) were measured on a 700 MHz system in D_2O.

Dinucleotides 1 and 2. The synthesis, isolation and purification of dinucleotides **1** and **2** was carried out according to reported procedure.[12] A, nucleoside at 5′ site; B, nucleoside at 3′ site.

Compound **1**. Yield: 44%. ^{1}H NMR: (D_2O) δ 8.37 (s, 1H, H6$_A$), 7.85−7.84 (d, J = 8.1 Hz, 1H, H6$_B$), 5.88−5.87 (d, J = 3.1 Hz, 1H, H1′$_B$), 5.80−5.79 (d, J = 2.3 Hz, 1H, H1′$_A$), 5.71−5.70 (d, J = 8.0 Hz, 1H, H5$_B$), 4.48−4.45 (m, 1H, H3′$_A$), 4.29−4.28 (m, 1H, H3′$_B$), 4.15−4.14 (m, 1H, H4′$_A$), 4.11−4.10 (m, 1H, H4′$_B$), 4.10−4.09 (m, 1H, H5′$_B$), 4.05−4.04 (m, 1H, H2′$_A$), 3.99−3.98 (m, 1H, H5″$_B$), 3.88−3.87 (m, 1H, H2′$_B$), 3.85 (m, 1H, H5′$_A$), 3.74−3.72 (m, 1H, H5″$_A$), 3.45 (s, 3H, OCH$_{3B}$), 3.40 (s, 3H, OCH$_{3A}$). ^{13}C NMR: (D_2O) δ 165.6 (C4$_B$), 161.5 (C4$_A$), 151.2 (C2$_B$), 150.5 (C2$_A$), 140.9 (C6$_B$), 140.6 (C6$_A$), 102.0 (C5$_B$), 96.2 (C5$_A$), 87.4 (C1′$_A$), 87.2 (C1′$_B$), 82.9 (C4′$_A$), 82.8 (C2′$_B$), 82.0 (C4′$_B$), 81.5 (C2′$_A$), 70.4 (C3′$_A$), 67.6 (C3′$_B$), 63.7 (C5′$_B$), 58.8 (C5′$_A$), 58.1 (OCH$_{3A}$), 57.7 (OCH$_{3B}$). HR MS (ESI) calcd for $C_{20}H_{25}Br^{79}N_4O_{14}P - H^+$ and $C_{20}H_{25}Br^{81}N_4O_{14}P - H^+$: 655.0289 and 657.0269, found 655.0296 and 657.0267. UV (H_2O): λ_{max} = 268 nm.

Compound **2**. Yield: 40%. ^{1}H NMR: (D_2O) δ 8.24 (s, 1H, H6$_B$), 8.04−8.03 (d, J = 8.2 Hz, 1H, H6$_A$), 6.00−5.99 (d, J = 2.8 Hz, 1H, H1′$_B$), 5.87 (s, 1H, H1′$_A$), 5.80−5.78 (d, J = 8.1 Hz, 1H, H5$_A$), 4.66−4.63 (m, 1H, H3′$_A$), 4.41−4.39 (t, J = 7.8 Hz, 1H, H3′$_B$), 4.25−4.24 (m, 1H, H4′$_B$), 4.24−4.22 (m, 1H, H5′$_B$), 4.22−4.21 (m, 1H, H4′$_A$), 4.12−4.10 (m, 1H, H2′$_A$), 4.09−4.08 (m, 1H, H5″$_B$), 3.98−3.96 (m, 1H, H2′$_B$), 3.95 (m, 1H, H5′$_A$), 3.84−3.81 (m, 1H, H5″$_A$), 3.57 (s, 3H, OCH$_{3A}$), 3.51 (s, 3H, OCH$_{3B}$). ^{13}C NMR: (D_2O) δ 165.9 (C4$_A$),

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161.4 ($C4_B$), 151.1 ($C2_A$), 150.7 ($C2_B$), 141.2 ($C6_A$), 139.8 ($C6_B$), 101.8 ($C5_A$), 96.7 ($C5_B$), 87.5 ($C1'_A$), 87.3 ($C1'_B$), 83.0 ($C2'_B$), 82.8 ($C4'_B$), 82.2 ($C4'_A$), 81.4 ($C2'_A$), 70.4 ($C3'_A$), 67.3 ($C3'_B$), 63.2 ($C5'_B$), 59.0 ($C5'_A$), 58.1 (OCH_{3B}), 57.3 (OCH_{3A}). HR MS (ESI) calcd for $C_{20}H_{25}Br^{79}N_4O_{14}P - H^+$ and $C_{20}H_{25}Br^{81}N_4O_{14}P - H^+$: 655.0289 and 657.0269, found 655.0288 and 657.0255. UV (H_2O): λ_{max} = 268 nm.

Hexanucleotides 3 and 4. Automated solid phase synthesis of the ^{Br}U containing hexanucleotides was carried out on a DNA synthesizer using the standard phosphoramidite method. The coupling efficiency was monitored with a trityl monitor. Deprotection and purification of the hexanucleotides were carried out according to reported procedures.[13]

Photochemistry of ^{Br}U Modified Dinucleotides 1 and 2. General Procedure for UV Irradiation. Solutions of the dinucleotide samples in 0.1 M phosphate buffer, pH 7.0 ($A_{268\ nm}$ = 1.0 in a 0.2 cm path length cuvette) were irradiated with an argon-ion laser (λ = 302 nm) under aerobic conditions at room temperature. The progress of the photoreaction was monitored by HPLC using a C_{18} column (1.8 μm, 4.6 mm × 50 mm), eluted with A/B using a linear gradient of 0–20% of B over 10 min at a flow rate of 0.6 mL/min (A, 0.1 M CH_3COONH_4; B, 0.1 M CH_3COONH_4/CH_3CN (50/50). Photoproducts were isolated by preparative HPLC on a RP_{18} 7.0 μm, 19.0 mm × 150 mm column, using elution with a linear gradient of acetonitrile (0–20%, 30 min) in 0.1 M CH_3COONH_4 buffer. A, nucleoside at 5′ site; B, nucleoside at 3′ site.

Compound **1a**. HR MS (ESI-TOF) calcd for $C_{20}H_{25}Br^{79}N_4O_{14}P - H^+$ and $C_{20}H_{25}Br^{81}N_4O_{14}P - H^+$: 655.0289 and 657.0269, found 655.0296 and 657.0269.

Compound **1b**. ^1H NMR: (D_2O) δ 8.50 (s, 1H, $H6_A$), 5.88 (s, 1H, $H1'_A$), 5.71–5.70 (d, J = 6.7 Hz, 1H, $H1'_B$), 4.92 (s, 1H, $H6_B$), 4.25–4.21 (m, 1H, $H3'_A$), 4.18–4.15 (m, 1H, $H4'_A$), 4.14–4.12 (m, 1H, $H3'_B$), 4.07–4.04 (m, 1H, $H5'_A$), 4.00–3.97 (m, 1H, $H4'_B$), 3.91–3.89 (m, 1H, $H2'_A$), 3.88–3.86 (m, 1H, $H5''_A$), 3.85–3.83 (m, 1H, $H5'_B$), 3.83–3.81 (m, 1H, $H5''_B$), 3.77–3.77 (m, 1H, $H2'_B$), 3.71 (s, 1H, $H5_B$), 3.48 (s, 3H, OCH_{3A}), 3.34 (s, 3H, OCH_{3B}). ^{13}C NMR: (D_2O) δ 171.8 ($C4_B$), 163.4 ($C4_A$), 152.8 ($C2_B$), 150.3 ($C2_A$), 141.6 ($C6_A$), 106.5 ($C5_A$), 86.5 ($C1'_A$), 84.6 ($C1'_B$), 82.5 ($C2'_A$), 82.2 ($C4'_B$), 80.9 ($C4'_A$), 80.3 ($C2'_B$), 78.2 ($C6_B$), 68.3 ($C3'_A$), 67.5 ($C3'_B$), 65.6 ($C5'_B$), 58.1 (OCH_{3B}), 57.9 (OCH_{3A}), 57.7 ($C5'_A$), 47.9 ($C5_B$). HR MS (ESI-TOF) calcd for $C_{20}H_{26}N_4O_{15}P - H^+$: 593.1138, found 593.1126. UV (H_2O): λ_{max} = 274 nm.

Compound **1c**. ^1H NMR: (D_2O) δ 8.66 (s, 1H, $H6_A$), 7.51 (s, 1H, $H6_B$), 6,00 (s, 1H, $H1'_A$), 5,86–5,85 (d, J = 4.3 Hz, 1H, $H1'_B$), 4.60–4.57 (m, 1H, $H3'_A$), 4.49–4.47 (t, J = 5.6 Hz, 1H, $H3'_B$), 4.15–4.14 (m, 1H, $H4'_B$), 4.10–4.10 (m, 1H, $H5'_B$), 4.08–4.07 (m, 1H, $H4'_A$), 4.06–4.05 (m, 1H, $H5''_B$), 4.00–3.99 (m, 1H, $H5'_A$), 3.90–3.88 (m, 1H, $H2'_B$), 3.87–3.83 (m, 1H, $H2'_A$), 3.72–3.69 (m, 1H, $H5''_A$), 3.49 (s, 3H, OCH_{3A}), 3.41 (s, 3H, OCH_{3B}). ^{13}C NMR: (D_2O) δ 163.9 ($C4_B$), 163.5 ($C4_A$), 151.0 ($C2_{A,B}$), 140.7 ($C6_B$), 140.3 ($C6_A$),108.4 ($C5_B$), 107.9 ($C5_A$), 87.2 ($C1'_B$), 86.3 ($C1'_A$), 83.3 ($C2'_B$), 82.6 ($C2'_A$), 82.4 ($C4'_B$), 80.7 ($C4'_A$), 68.6 ($C3'_A$), 66.9 ($C3'_B$), 62.6 ($C5'_B$), 58.2 ($OCH_{3A,B}$), 57.0 ($C5'_A$). HR MS (ESI-TOF) calcd for $C_{20}H_{24}N_4O_{14}P - H^+$: 575.1032, found 575.1035. UV (H_2O): λ_{max} = 278 nm.

Compound **2b**. ^1H NMR: (D_2O) δ 7.47 (s, 1H, $H6_B$), 5.71 (s, 1H, $H1'_A$), 5.55–5.54 (d, J = 2.3 Hz, 1H, $H1'_B$), 5.27–5.26 (d, J = 2.1 Hz, 1H, $H6_A$), 4.74–4.72 (t, J = 6.08 Hz, 1H, $H3'_B$), 4.53–4.51 (m, 1H, $H2'_B$), 4.01–3.98 (m, 1H, $H3'_A$), 3.98–3.96 (m, 1H, $H4'_B$), 3.93–3.90 (m, 1H, $H4'_A$), 3.87 (s, 1H, $H5_A$), 3.85–3.84 (m, 1H, $H5'_B$), 3.83–3.82 (m, 1H, $H5''_B$), 3.77–3.74 (m, 1H, $H5'_A$), 3.64–3.61 (m, 1H, $H5''_A$), 3.52–3.50 (m, 1H, $H2'_A$), 3.39 (s, 3H, OCH_{3A}), 3.38 (s, 3H, OCH_{3B}). ^{13}C NMR: (D_2O) δ 170.9 ($C4_A$), 163.1 ($C4_B$), 152.2 ($C2_A$), 149.9 ($C2_B$), 142.3 ($C6_B$), 107.9 ($C5_B$), 91.4 ($C1'_B$), 86.6 ($C1'_A$), 83.5 ($C2'_A$), 81.9 ($C4'_B$), 81.2 ($C2'_B$), 80.8 ($C4'_A$), 76.0 ($C6_A$), 70.1 ($C3'_A$), 68.4 ($C3'_B$), 63.3 ($C5'_B$), 58.2 ($C5'_A$), 57.8 ($OCH_{3A,B}$), 45.2 ($C5_A$). HR MS (ESI-TOF) calcd for $C_{20}H_{26}N_4O_{15}P - H^+$: 593.1138, found 593.1104. UV (H_2O): λ_{max} = 266 nm.

Photochemistry of ^{Br}U modified hexanucleotides 3 and 4. Solutions of the hexanucleotide samples in 0.1 M phosphate buffer, pH = 7.0 ($A_{260\ nm}$ = 1.0 in a 0.2 cm path length cuvette) were irradiated with an argon-ion laser λ = 302 nm) under aerobic conditions at room temperature. The progress of photoreaction was monitored by HPLC using a C_{18} column (2.5 μm, 4.8 mm × 50 mm), eluted with A/B using a linear gradient of 14–30% of B over 15 min at a flow rate of 0.6 mL/min (A, 0.1 M CH_3COONH_4; B, 0.1 M CH_3COONH_4/CH_3CN (50/50). Photoproducts were isolated by preparative HPLC on a C_{18} column (2.5 μm, 10.0 mm × 50 mm), eluted with A/B using a linear gradient of 14–35% of B over 15 min at a flow rate of 1.0 mL/min (A, 0.1 M CH_3COONH_4; B, 0.1 M CH_3COONH_4/CH_3CN (50/50). Duplexes **3**/**5** and **4**/**6** were obtained by mixing of ^{Br}U modified strands with unmodified complementary strands in a ratio of 1:1 ($A_{260\ nm}$ = 1.0 in 0.1 M phosphate buffer, pH 7). The estimated melting temperature of duplexes was about 8 °C. The solutions were irradiated with an argon-ion laser (λ = 302 nm) in a 10/2 mm quartz cuvette under aerobic conditions at 2 °C.

Compound **3a**. MALDI-TOF MS calcd for $C_{60}H_{75}N_{18}O_{39}P_5 - 2H^+$: 1827.219, found 1827.250. UV (H_2O): λ_{max} = 262 nm.

Compound **3b**. MALDI-TOF MS calcd for $C_{60}H_{75}N_{18}O_{38}P_5^+$: 1811.204, found 1811.880. UV (H_2O): λ_{max} = 262 nm.

Compound **4a**. MALDI-TOF MS calcd for $C_{60}H_{76}N_{21}O_{37}P_5^+$: 1838.232, found 1838.694. UV (H_2O): λ_{max} = 262 nm.

Compound **4b**. MALDI-TOF MS calcd for $C_{60}H_{74}N_{21}O_{36}P_5^+$: 1820.217, found 1820.014. UV (H_2O): λ_{max} = 262 nm.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The MS, NMR and UV spectra of starting compounds and photoproducts. The HPLC analyses of photoirradiated solutions and tests of thermal and photochemical stability of the photoproducts. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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