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Facile Photocyclization Chemistry of 5-Phenylthio-2′-deoxyuridine in Duplex DNA

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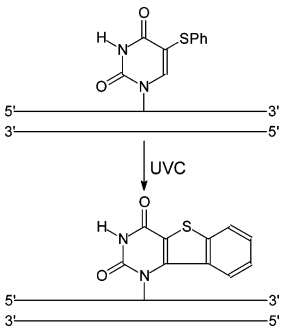
ABSTRACT



We report here the synthesis of 5-phenylthio-2′-deoxyuridine (d^{PhS}U), its incorporation into oligodeoxynucleotides (ODNs), and its photocyclization chemistry. Irradiation of dinucleoside monophosphate d(^{PhS}UG) and d^{PhS}U-bearing duplex ODNs with 254 nm light results in the facile formation of a cyclic product where the C6 of uracil is covalently bonded to the C2 of the phenyl ring. The chemistry reported here may serve as the basis for the efficient preparation of a new class of duplex DNA with an extended π system.

Phenylthio-substituted nucleosides have been successfully employed as photolabile precursors for the generation of carbon-centered radicals to mimic the deleterious effects of ionizing radiation.[1−6] In addition, it has been shown that the photoirradiation of pyrimidine nucleosides with a halogen atom being substituted at the C5 position may result in the formation of C5-centered radicals.[7−10] It remains unclear what the photochemistry is for pyrimidine nucleosides bearing a 5-thiophenyl moiety.

In this study, we set out to explore this chemistry by first synthesizing 5-phenylthio-2′-deoxyuridine (d^{PhS}U). The incorporation of a phenylthio group to the C5 of uracil was inspired from the previous procedures for the preparation of 8-benzyloxy-2′-deoxyguanosine.[11] In this regard, treatment of the commercially available 5-bromo-2′-deoxyuridine with thiophenol and sodium in DMSO renders the desired 5-phenylthio-2′-deoxyuridine in ∼47% yield (Scheme 1). We then synthesized the phosphoramidite building block of d^{PhS}U following standard procedures [12] and inserted it into dinucleoside monophosphate and ODNs by automated solid-phase synthesis (Scheme 1).

We next examined the photochemistry of d(^{PhS}UG) and d^{PhS}U-bearing ODNs. Here we began our discussion with the major product isolated from the irradiation mixture of d(^{PhS}UG) (the HPLC trace for the separation of the 254 nm

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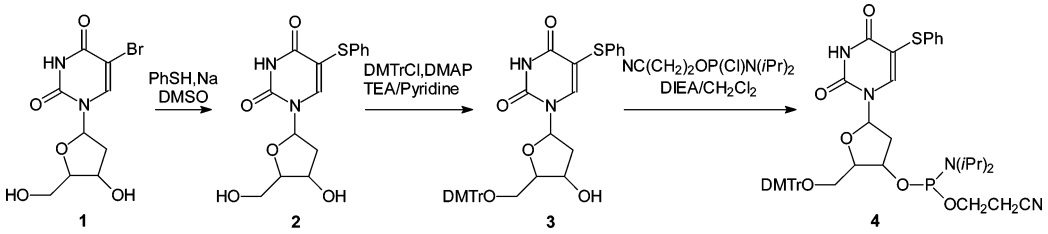
Scheme 1



irradiation mixture is shown in Figure 1a). Negative-ion ESI-MS of the major fraction eluting at 51.3 min gave an ion of *m*/*z* 661.8 (Figure 1a, inset), and we designate the product as d(^{PhS}U*G) (Scheme 2). Exact mass measurement gives *m*/*z* 662.1055 for the [M − H]$^-$ ion of d(^{PhS}U*G) (Table S1, Supporting Information), supporting that the product was initiated from the elimination of two hydrogen atoms from the starting d(^{PhS}UG). Collisional activation of the [M − H]$^-$ ion of d(^{PhS}U*G) gives rise to two abundant fragment ions of *m*/*z* 216.9 and 443.9, which are induced from the rupture of the glycosidic bond of the 5′ nucleoside (Figure 1b). The formation of these two product ions demonstrated that the two hydrogen atoms were lost from the 5′ nucleobase of d(^{PhS}UG).

To further elucidate the structure of the major product, we recorded its ^{1}H NMR spectrum, which showed the presence of four aromatic protons (Figure S5, Supporting Information). These protons can be assigned to the protons of the phenyl group, which is supported by 2-D NOESY measurement (Figure S6, Supporting Information). The losses of the H6 proton of uracil and an aromatic proton from the phenyl ring support the proposed structure of the major photoproduct (Scheme 2). It is worth noting that the NMR spectra are also consistent with an alternative cyclic structure where the sulfur is bonded to the C6 of the uracil component. This possibility, however, can be excluded for two reasons. First, the formation of the latter product would necessitate the cleavage of the C−S bond in d(^{PhS}UG), which is expected to lead to the formation of d(UG). We, however, were not able to detect d(UG) from the irradiation mixture. Second, we observed the facile loss of [HNCO + CO] (71 Da) upon the fragmentation of the protonated ion of the modified nucleobase, and this type of cleavage is unlikely to occur from the alternative cyclic structure (vide infra, Scheme S1, Supporting Information).

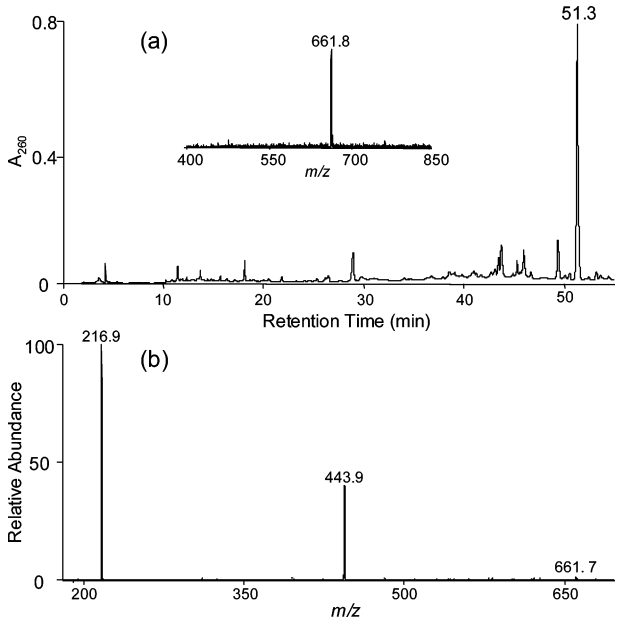
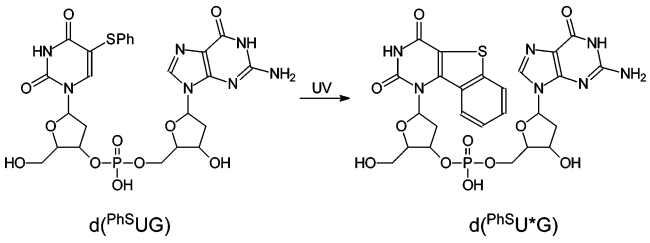


Figure 1. (a) HPLC trace for the separation of the aerobic 254 nm irradiation (30 min) mixture of d(^{PhS}UG). Shown in the inset is the negative-ion ESI-MS of the 51.3-min fraction. (b) Negative-ion ESI-MS/MS of the new cyclization product, i.e., d(^{PhS}U*G), formed from the irradiation of d(^{PhS}UG). The quantum yield for the formation of d(^{PhS}U*G) was estimated to be 0.020 (see the Supporting Information).

Next we investigated whether similar chemistry occurs in duplex DNA. To this end, we irradiated a decameric duplex, d(ATGGCG^{PhS}UGCT)/d(AGCACGCCAT), under aerobic conditions and it turned out that this type of photocyclization chemistry can also take place in duplex DNA (the HPLC trace for the separation of the 30-min irradiation mixture is shown in Figure 2). Our conclusion is based on the following lines of evidence. First, high-resolution ESI-MS acquired on a Fourier transform ion cyclotron resonance mass spectrometer gives *m*/*z* 782.8796 for the [M − 4H]$^{4-}$ ion, which is consistent with the calculated *m*/*z* value (782.8761) for the [M − 4H]$^{4-}$ ion of the cyclization product (Table S1 (Supporting Information) and Figure 3a show the low-resolution ESI-MS of the cyclization product). Second, MS/MS of the [M − 5H]$^{5-}$ ion supports that the modification occurs on the 5-phenylthiouracil component (Figure 3b). Upon collisional activation in a mass spectrometer, an ODN undergoes cleavages at the glycosidic bond and the 3′ C−O

Scheme 2



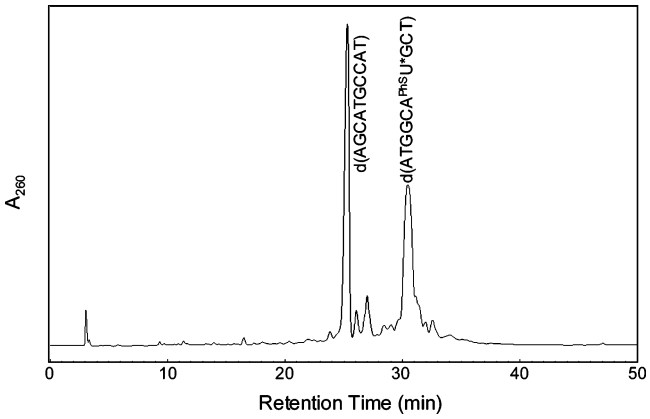


Figure 2. HPLC trace for the separation of the 254 nm irradiation (30 min) mixture of duplex DNA d(ATGGCG^{PhS}UGCT)/d(AGCACGCCAT).

bond of the same nucleotide to give [a_n − Base] and w_n ions.[13] The formation of a pair of complementary fragment ions, i.e., [a_7 − ^{PhS}U*]$^{3-}$/w_3, and the ion of *m*/*z* 217 from the modified ODN demonstrates that the rupture of the glycosidic bond of the d^{PhS}U* is the most facile cleavage site in this ODN (Figure 3b). Further collisional activation of the ion of *m*/*z* 217 (i.e., MS^3) gives a dominant fragment ion of *m*/*z* 174. Similar fragmentation of the ion of *m*/*z* 217 observed in the product-ion spectrum of the [M − H]$^-$ ion of d(^{PhS}U*G) gives the same product-ion spectrum (Figure S8, Supporting Information). These results strongly suggest that the new product formed in duplex DNA adopts the same structure as that formed in dinucleoside monophosphate.

We further digested the cyclization product-bearing ODN and d(^{PhS}U*G) with four enzymes (i.e., first with nuclease P1 and calf spleen phosphodiesterase, then with alkaline phosphatase and snake venom phosphodiesterase, see the Supporting Information) and analyzed the digestion mixture by LC-MS/MS. We chose these four enzymes because they have been routinely employed in our lab for the efficient release of oxdiatively damaged products from duplex DNA.[5,10] The LC-MS/MS results showed that the enzymatic treatment of both the d(^{PhS}U*G) and the ODN gives rise to the same mononucleoside, i.e., d^{PhS}U*. In this respect, the LC-MS/MS results showed, in the two digestion mixtures, the presence of a product eluting at the same retention time (Figure 4a&b). In addition, the product-ion spectra of the

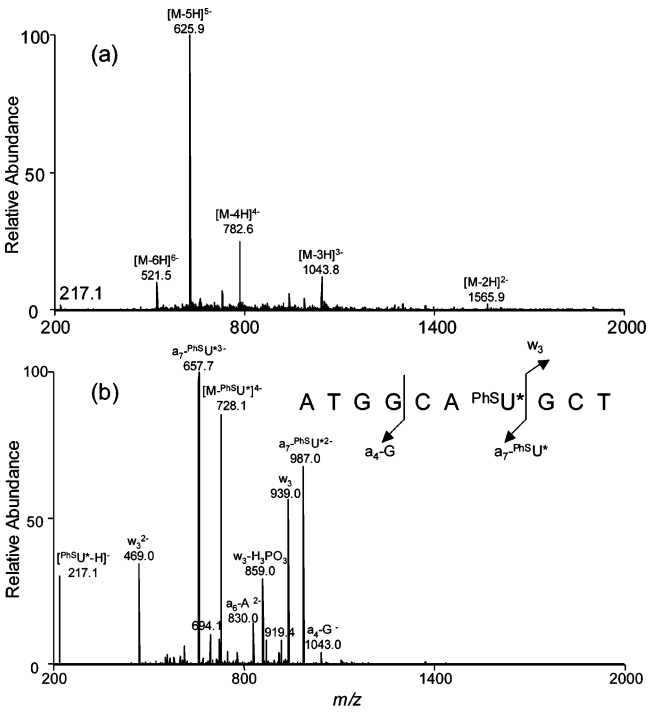


Figure 3. Negative-ion ESI-MS (a) and MS/MS (b) of d(ATGGCG^{PhS}U*GCT).

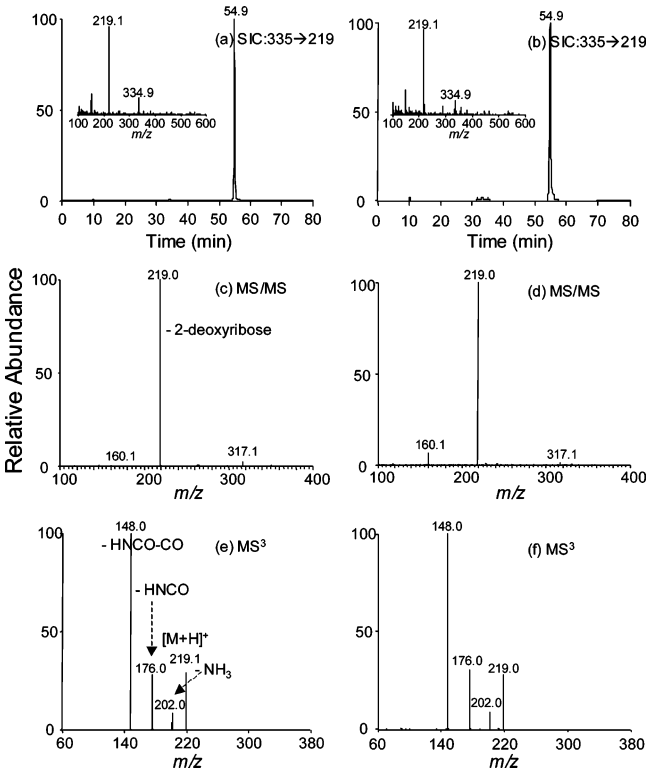


Figure 4. LC-MS/MS analysis of the enzymatic digestion products of d(^{PhS}U*G) (left) and the aerobic UV-irradiated duplex ODN d(ATGGCG^{PhS}UGCT)/d(AGCACGCCAT) (right). Panels a and b give the selected-ion chromatograms (SICs) for the monitoring of the *m*/*z* 335 → 219 transition, shown in the insets are the positive-ion ESI-MS of the 54.9-min fraction. Panels c and d show the product-ion spectra of the ions of *m*/*z* 335 observed in MS. Panels e and f illustrate the MS/MS/MS (MS^3) of the ions of *m*/*z* 219 observed in panels c and d, respectively.

[M + H]$^+$ ions (*m*/*z* 335) are identical (Figure 4c,d). Moreover, further fragmentation of the ion of *m*/*z* 219

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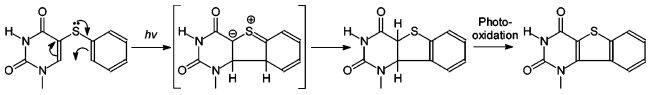
observed in the MS/MS for the 54.9-min fractions gives the same product-ion spectra (MS^3, Figure 4e,f). In this regard, we observed facile losses of NH_3 and HNCO from the modified nucleobase component. These two neutral losses have been previously found for the fragmentation of protonated uracil.[14] The most abundant fragment ion observed in MS^3 is, however, due to the loss of a 71-Da neutral fragment, which can be attributed to the elimination of both CO and HNCO. This type of neutral loss was shown to be a very minor pathway for protonated uracil,[14] and this loss from the cyclic product can be explained from its unique structure (Scheme S1, Supporting Information).

It is worth noting that similar photochemistry was observed previously for diphenyl thioether, which, upon UV irradiation in I_2-containing cyclohexane solution under N_2 atmosphere, results in the efficient production of dibenzothiophene.[15] Following the mechanism proposed for the formation of dibenzothiophene,[15] we proposed a mechanism for the formation of the cyclic product (Scheme 3). In this respect,

Scheme 3



photoirradiation can result in the covalent coupling of the C2 carbon of the phenyl component to the C6 of the uracil moiety. The resulting coupling product may undergo photooxidation to give the benzothiophene analogue of the nucleobase component (Scheme 3).

In support of our proposed mechanism, we were able to isolate two intermediates from the mixture emanating from shorter periods of irradiation (HPLC traces for the time-course study are shown in Figure S9, Supporting Information). These two intermediates are very likely the dihydrouracil derivatives of the dinucleoside monophosphate based on ESI-MS and MS/MS results (Figure S10, Supporting Information). In this respect, ESI-MS showed that the two intermediates shared the same molecular weights as d(^{PhS}UG) (Figure S10, Supporting Information). Product-ion spectra of the $[M - H]^-$ ions of these two products showed the facile cleavage of the glycosidic bond of the 5′ nucleoside to give an ion of *m*/*z* 444. Moreover, comparison of the MS/MS of the two intermediates with that of d(^{PhS}UG) revealed a markedly decreased formation of the ion of *m*/*z* 219, which is the deprotonated ion of the modified uracil component, for the two intermediates (Figure S10, Supporting Information). These results are in accordance with the saturation of the uracil moiety in the two intermediates thereby supporting that the these two intermediates are the two isomeric dihydrouracil derivatives. As the irradiation time increases, we observed a gradual increase of d(^{PhS}U*G) with the concomitant decrease of the two dihydrouracil derivatives (Figure S9, Supporting Information). This, together with the fact that 254 nm irradiation of both intermediates can lead to the formation of d(^{PhS}U*G) (data not shown), supports that d(^{PhS}U*G) is the secondary product formed from the two dihydrouracil derivatives (Scheme 3).

To summarize, we synthesized 5-phenylthio-2′-deoxyuridine, incorporated it into ODNs, and showed that the irradiation of d^{PhS}U-bearing dinucleoside monophosphates and double-stranded ODNs with 254 nm light can result in the facile formation of a product with benzothiophene being fused to the uracil ring. Recent work by Kool and coworkers[16−22] showed that duplex DNA containing a thymine analogue with increased base-pair size can lead to more stable duplexes. When compared with the previous synthesis of the thymidine analogues, the chemistry reported here offered a more convenient preparation of another class of duplex DNA with an enlarged π system. In this respect, the improved π stacking offered by the modified nucleoside may lead to the formation of more stable duplexes than natural double-stranded DNA, which may be employed for the sensitive detection of nucleic acids in vivo.

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Supporting Information Available: NMR spectra of synthetic compounds, LC traces, MS data, and LC-MS/MS results. This material is available free of charge via the Internet at http://pubs.acs.org.

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