JOC The Journal of Organic Chemistry

Article

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Direct Time-Resolved Spectroscopic Observation of Arylnitrenium Ion Reactions with Guanine-Containing DNA Oligomers

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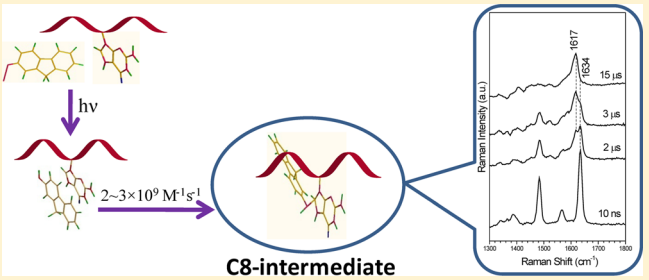
Supporting Information

ABSTRACT: The metabolic activation of a number of aromatic amine compounds to arylnitrenium ions that can react with DNA to form covalent adducts has been linked to carcinogenesis. Guanine in DNA has been shown to be the main target of N-containing carcinogens, and many monomeric guanine derivatives have been utilized as models for product analysis and spectroscopic investigations to attempt to better understand the reaction mechanisms of DNA with arylnitrenium ions. However, there are still important unresolved issues regarding how arylnitrenium ions attack guanine residues in DNA oligomers. In this article, we employed ns-TA and ns-TR^3 spectroscopies to directly observe the reaction of the 2-fluorenylnitrenium ion with selected DNA oligomers, and we detected an intermediate possessing a similar C8 structure as the intermediates produced from the reaction of monomeric guanosine derivatives with arylnitrenium ions. Our results suggest that the oligomeric structure can lead to a faster reaction rate of arylnitrenium ions with guanine residues in DNA oligomers and the reaction of arylnitrenium ions take place in a manner similar to reactions with monomeric guanosine derivatives.

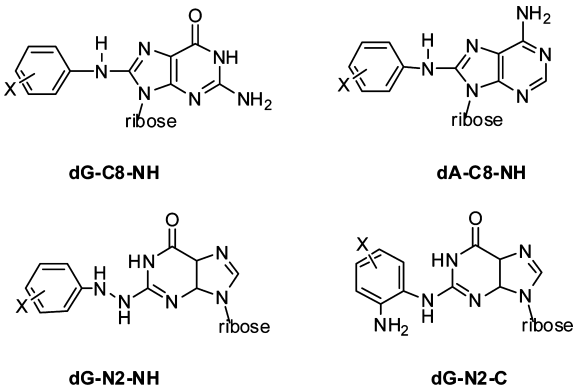


INTRODUCTION

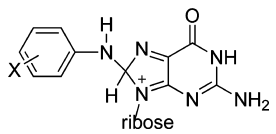
The metabolic activation of many aromatic amines and aryl nitro-substituted compounds to arylnitrenium ions that are able to react with DNA to form covalent adducts has been linked to carcinogenesis.[1−5] The most abundant adduct is dG-C8-NH, whereas dA-C8-NH, dG-N2-NH, and dG-N2-C (Scheme 1) as well as others can be found in minor amounts. The type and amount of DNA adducts generated depend on several factors including the structure and properties of the arylnitrenium ions and DNA and the reaction solution environment.[6,7] Because guanine in DNA is the main target of N-containing carcinogens, many monomeric guanine derivatives have been selected and used as models for product analysis and spectroscopic studies to attempt to elucidate the reaction mechanisms of DNA with arylnitrenium ions.[8−15] It has been reported that reactions of guanosine, 2′-deoxyguanosine-5′-phosphate, and other monomeric guanine derivatives with arylnitrenium ions occur at or near their encounter limits ($7–9 \times 10^8$ M^{-1} s^{-1}).[11−13]

Photolysis of aryl azides in aqueous solutions containing guanosine, 2′-deoxyguanosine, or some C8-substituted guanine derivatives enabled the use of nanosecond LFP[12] and nanosecond time-resolved resonance Raman (ns-TR^3) experiments[14,15] to directly observe and characterize C8 intermediates (Scheme 2) that are formed prior to the loss of the C8 proton (or substituent) to give the C8 adduct final product. These kinetic and spectroscopic studies using monomeric guanine have provided valuable characterization and insight into the reaction mechanism of guanine derivatives with arylnitrenium ions and have confirmed the existence of the C8

Scheme 1



Scheme 2. C8 Intermediate



Received: February 27, 2014
Published: March 25, 2014

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dx.doi.org/10.1021/jo500484s | J. Org. Chem. 2014, 79, 3610−3614

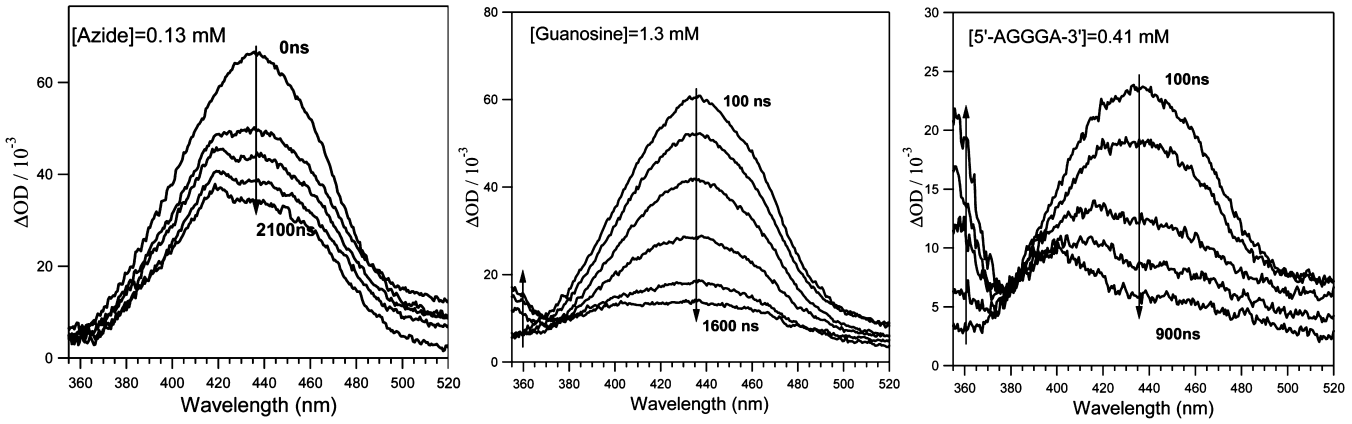

Figure 1. Transient absorption spectra recorded after a nanosecond 355 nm pump laser photolysis of 2-fluorenyl azide in mixed aqueous solution (left) as well as with guanosine (middle) or d-AGGGA DNA oligomer (right) present.

intermediate, and they suggest that the arylnitrenium-N tends to attach very fast to the guanine C8 position. However, there are still important unanswered questions regarding how arylnitrenium ions attack guanine residues in DNA oligomers. For example, are there any differences between the monomeric and polymeric (or oligomeric) guanines in their reaction with arylnitrenium ions? Does the primary structure of DNA influence the reaction of DNA with arylnitrenium ions? Here, we have employed nanosecond transient absorption (ns-TA) and ns-TR[3] spectroscopies to directly observe the reaction of the 2-fluorenylnitrenium ion with selected DNA oligomers, and we detected an intermediate possessing a similar C8 structure as the intermediates produced from the reaction of monomeric guanosine derivatives with arylnitrenium ions. Our results suggest that the oligomeric structure can lead to a faster reaction rate of arylnitrenium ions with guanine residues in DNA oligomers and the reaction of arylnitrenium ions proceeds in a manner similar to reactions with monomeric guanosine derivatives.

RESULTS AND DISCUSSION

Nanosecond 355 nm pump laser photolysis of 2-fluorenyl azide was performed in mixed aqueous solvents without or with guanine derivatives present in the solution. As shown in Figure 1, this photolysis generates the 2-fluorenylnitrenium ion within the 10 ns pump pulse duration with a characteristic absorption band at 435 nm.[12] When there are no guanine derivatives in the solution, the decay of the 2-fluorenylnitrenium ion produces an azo species carrying a sharp absorption band at 420 nm because of the equilibrium between the nitrenium ion and singlet nitrene or through an azo cation intermediate species.[16] With guanosine present in the solution, a new absorption band below 380 nm was formed during the nitrenum ion's faster decay under these conditions. This observation is consistent with reports from McClelland and co-workers[12] on arynitrenium ion reactions with monomeric guanine derivatives, and the new bands observed here below 380 nm were assigned to the C8 intermediate. In the case of DNA oligomers being present in the sample solution, the decay of the 2-fluorenylnitrenium ion also produces a new absorption band below 380 nm that displays a similar pattern with that for guanosine. Here, Figure 1 represents a typical spectrum recorded with a d-AGGGA DNA oligomer present.

To investigate how the primary structure of DNA oligomers influences their reactivity toward nitrenium ion, the kinetics at the 435 nm transient absorption band of the 2-fluorenylnitrenium ion recorded in ns-TA experiments was monitored to determine how fast the nitrenium ion is quenched by the oligomers. Under the experimental conditions employed here ($conc_{Az}$ = 0.13 mM in MeCN/H_2O 1:1 (v/v)), when no trappers (like guanosine or oligomers containing guanine) are present in aqueous solution, the 2-fluorenylnitrenium ion decays with a first-order reaction rate constant of 4.85×10^4 s^{-1}, which is a little faster than the value of 3.2×10^4 s^{-1} that was recorded with a low azide concentration and in a solution with a high water content.[17] Our ns-TA results show that oligomeric adenine, thymine, and cytosine have a negligible contribution for accelerating 2-fluorenylnitrenium ion decay, which indicates that the second-order rate constants of the 2-fluorenylnitrenium ion's reaction with oligomeric adenine, thymine, and cytosine are smaller than 1×10^7 M^{-1} s^{-1} when taking into consideration the instrument resolution and the concentration of DNA oligomers used in the experiments. This result is not strange because the monomeric adenine, thymine, and cytosine are also not very reactive with nitrenium ions, and this result is also consistent with previous product analysis studies that found that the adenine, thymine, and cytosine DNA adducts caused by aromatic amines or aryl nitro-substituted compounds are, at best, minor adducts. However, the oligomeric guanine exhibits noticeably more reactivity toward the 2-fluorenylnitrenium ion compared to monomeric guanine derivatives. Similar to the reactions with monomeric guanine derivatives, in the presence of oligomeric guanine, the decay of the 2-fluorenylnitrenium ion can be fit very well with a single exponential function, and the decay rate constants are dependent on the oligomer concentrations examined here. The second-order rate constants for the 2-fluorenylnitrenium ion with some DNA oligomers are given in Table 1. Examination of Table 1 suggests that each guanine in the oligomers is a reactive unit, with the exception of oligomers with more than three consecutive guanines present (such as d-AGGGA and d-GGGGG), and the guanine unit has a reaction rate constant toward the 2-fluorenylnitrenium ion of $2-3 \times 10^9$ M^{-1} s^{-1}, which is noticeably faster than those of monomeric guanosine derivatives ($7-9 \times 10^8$ M^{-1} s^{-1}). It is interesting to note that the self-complementary oligomer d-ATGCAT has a rate constant of 3.1×10^9 M^{-1} s^{-1}, which is much faster than a related previously published result[18] in which $k_{SS} \approx 0.27k_{d\text{-}G}$, where k_{SS} and $k_{d\text{-}G}$ are rate constants of d-ATGCAT and 2′-deoxyguanosine toward *N*-acetyl-*N*-(2-fluorenyl)nitrenium ion,

dx.doi.org/10.1021/jo500484s | J. Org. Chem. 2014, 79, 3610−3614

Table 1. Rate Constants Obtained from ns-TA Experiments of Selected DNA Oligomers with the 2-Fluorenylnitrenium Ion

oligomer	rate constant ($\times 10^9$ M^{-1} s^{-1})[a]
d-GGGGG	3.20 ± 0.18
d-AAGAA	3.69 ± 0.18
d-GAAAG	6.4 ± 0.5
d-AGGGA	6.2 ± 0.6
d-AGAGAG	8.6 ± 0.9
d-GAGAGA	8.3 ± 0.9
d-GAGAGAGAGA	12.8 ± 1.1
d-ATGCAT	3.10 ± 0.13
d-GAGAGA -TCTCTC[b]	6.79 ± 0.35

[a]All values were calculated on the basis of the oligomer concentration indicated on the sample tubes when they were purchased. [b]Double stranded.

respectively. It should be pointed out that the *N*-acetyl-*N*-(2-fluorenyl)nitrenium ion has a slightly lower reactivity toward guanine derivatives (5.6×10^8 M^{-1} s^{-1}) than the unsubstituted 2-fluorenylnitrenium ion.[11]

The 368.9 nm probe wavelength ns-TR^3 spectra obtained after 309 nm excitation of 2-fluorenyl azide in the presence of d-GTGTGT oligomer in a buffered mixed aqueous solution are shown in Figure 2. The 2-fluorenylnitrenium ion generated by protonation of singlet nitrene within 100 ps has a characteristic Raman band[19] at 1634 cm^{-1} and appears in the early time spectra (10 ns), and its decay correlates with another species that has a characteristic Raman band at 1617 cm^{-1}. The time constants for the decay of the 2-fluorenylnitrenium ion and the growth of the new intermediate are about 2 μs (see the Supporting Information for kinetic fits and full ns-TR^3 spectra). Because the decay of the 2-fluorenylnitrenium ion is due to its reactions with the oligomer and generation of the azo product, the second-order rate constant of the 2-fluorenylnitrenium ion with the d-GTGTGT oligo, k_{GT}, was estimated by using $k_{GT}[\text{oligo}] = 1/t_{ntr} - 1/t_{azo}$, where the t_{ntr} and t_{azo} represent the decay time constants of the 2-fluorenylnitrenium ion in the presence of the oligomer and without any guanine traps, respectively, recorded under the analogous experimental conditions. Our experimental results found that t_{azo}= 7 μs; therefore, $k_{GT} = 5.95 \times 10^9$ M^{-1} s^{-1}. Although there are three guanine bases present in the d-GTGTGT single-stranded DNA oligomer, the reaction rate of the 2-fluorenylnitrenium ion with a single oligomer guanine unit in it is still faster than that with the monomers of guanosine (7.2×10^8 M^{-1} s^{-1}), 2′-deoxyguanosine (7.6×10^8 M^{-1} s^{-1}), and 2′-deoxyguanosine 5′-phosphate (9.2×10^8 M^{-1} s^{-1}).[12] This result is in agreement with those obtained from the ns-TA experimental results for the selected DNA oligomers listed in Table 1.

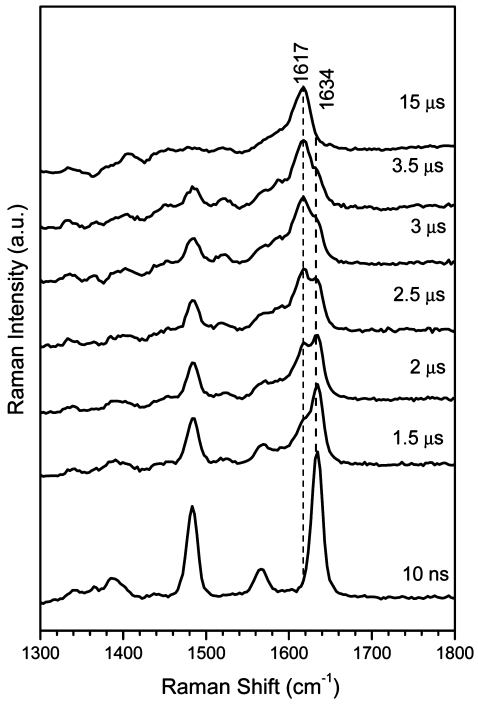

Figure 2. Selected TR^3 spectra obtained using a 368.9 nm probe wavelength after 309.1 nm photolysis of 0.3 mM 2-fluorenyl azide in the presence of 0.06 mM d-GTGTGT oligomer in a MeCN/H_2O 1:1 (v/v) mixed solvent with a 5 mM Na_2HPO_4/5 mM NaH_2PO_4 buffer. The time delays between the pump (309.1 nm) and probe (368.9 nm) laser pulses are shown to the right of each spectrum, and the Raman shifts of selected bands are presented at the top of the 15 μs spectrum.

In the ns-TR^3 spectra, the 1617 cm^{-1} species that correlated with the 2-fluorenylnitrenium ion decay was identified to have a C8-intermediate structure in which the 2-fluorenylnitrenium-N attaches to the C8 atom of the oligomer guanine unit and with the C8 hydrogen still present in the intermediate. There are three reasons for this assignment: (1) this intermediate is generated because of the d-GTGTGT oligomer rather than monomeric guanosine, as was illustrated by the reaction rate constant in the prior paragraph, (2) the formation of this intermediate is correlated with the decay of the 2-fluorenylnitrenium ion (Supporting Information), and (3) the Raman spectrum of this intermediate appears very similar, although not identical, to those other authentic C8 intermediates[15] formed from reactions of the 2-fluorenylnitrenium ion with guanosine and 2′-deoxyguanosine, as shown in Figure 3.

Our ns-TA results show that with the oligomeric guanine present (1) the decays of the 2-fluorenylnitrenium ion are

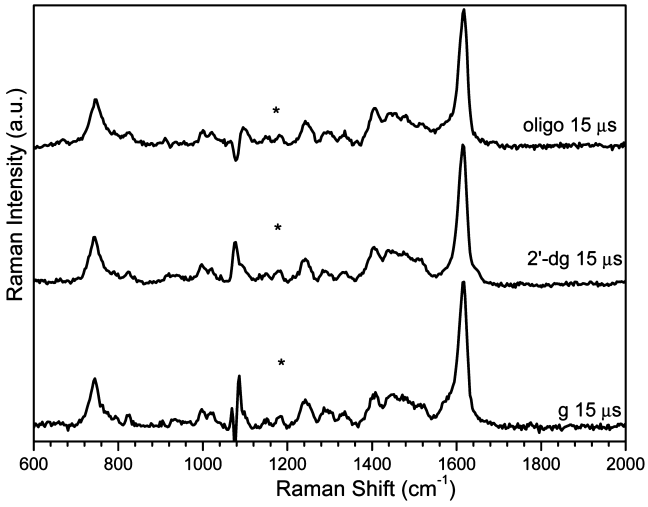

Figure 3. Comparison of the transient resonance Raman spectra obtained at 15 μs with guanosine (bottom), 2′-deoxyguanosine (middle), and d-GTGTGT oligomer (top) present, respectively. All of these spectra were recorded with a 368.9 nm probe wavelength after 309 nm laser photolysis of 0.3 mM 2-fluorenyl azide in MeCN/H_2O 1:1 (v/v) (5 mM Na_2HPO_4/5 mM NaH_2PO_4 buffer).

dx.doi.org/10.1021/jo500484s | J. Org. Chem. 2014, 79, 3610−3614

strongly dependent on the oligomer concentration in all of the solutions regardless of whether there is an excess of azide precursor or oligomers and (2) the decay time constants versus the oligomer concentration can be fit very well with a linear function. The $2-3 \times 10^9$ M^{-1} s^{-1} reaction rate constant for each oligomeric guanine unit suggests that the oligomeric guanine appears to be more reactive toward the 2-fluorenylnitrenium ion than monomeric guanine derivatives. That the reaction rate of oligomeric guanine with the nitrenium ion is so fast and close to a diffusion rate may be due to two possible reasons: the oligomeric structure could decrease the reaction energy barrier of the guanine unit with the 2-fluorenylnitrenium ion or there could be some weak preinteraction between the oligomer and the azide precursor and/or the nitrenium ion. However, if the reaction proceeds via a σ-stacked or π-stacked structure, then the oligomeric structure may not influence the reaction barriers significantly when considering results from computational studies in the literature.[20,21] In the σ-stacked interaction, hydrogen bonding plays an important role, and it decreases the C8-channel transition state energy much more than that of the N7 channel.[20] The hydrogen bonding induced by water solvent molecules may be similar for monomeric and oligomeric guanine, and strong hydrogen bonding between the DNA base pairs probably has a small impact on this reaction, as similar reaction rates were observed for oligomers containing G-C segments as well as for others with different sequences. In the π-stacked interaction, the stability of the transition state is determined by the LUMO of the arylnitrenium ion and the HOMO of guanine,[21] and the latter is similar for oligomeric or monomeric guanines, as the adjacent saturated moieties have little influence on it. It is interesting to note that in our ns-TA experiments the DNA oligomers containing three or more consecutive guanines, such as d-AGGGA and d-GGGGG, exhibit only one guanine unit reactivity, namely, these oligomers have a reaction rate constant equivalent to the oligomer possessing only one guanine unit. This might be due to the presence of a guanine quadruplex structure,[22] which prevents the arylnitrenium ion molecule from approaching parallel to the guanine ring plane. This inference may be indirect evidence that the reaction of DNA guanine with arylnitrenium ions occurs in a π-stacked manner because the guanine C8 atoms are exposed to the solvent in the guanine quadruplex.

We would expect to observe an interaction between the azide precursor and the oligomers, but both the UV−vis spectra and the resonance Raman spectra showed no difference for azide/oligomer coexistence or their individual existence. Comparison of the transient Raman spectra of the 2-fluorenylnitrenium ion recorded in a mixed aqueous solution without and with 2′-deoxyguanosine or the d-GTGTGT oligomer shows that the 1382 cm^{-1} Raman band of the 2-fluorenylnitrenium ion blue shifts to 1387 cm^{-1} and appears to be a little narrower when the oligomer is present in the solution, as shown in the inset of Figure 4. Results from DFT calculations for the 2-fluorenylnitrenium ion suggests that the 1372 cm^{-1} vibration corresponds to N−H and C−N bending in the plane motions;[23] hence, the narrower and blue-shifted Raman band implies that there could be some interactions between the oligomer and the 2-fuorenylnitrenium ion that modestly affects its structure. One would expect that any prebound azide/nitrenium ion could react in a first-order fashion with the oligomeric guanine. However, as aforementioned, our experimental results show that the nitrenium ion reacts in a second-order fashion with the oligomeric guanine. One would further expect the reaction of a prebound species to be invisible to the nanosecond measurements, or, more accurately, that the observation would be a combination of immediate formation of the C8 intermediate (from the prebound azide/nitrenium ion) and a slow diffusion-controlled reaction starting from an unbound species.

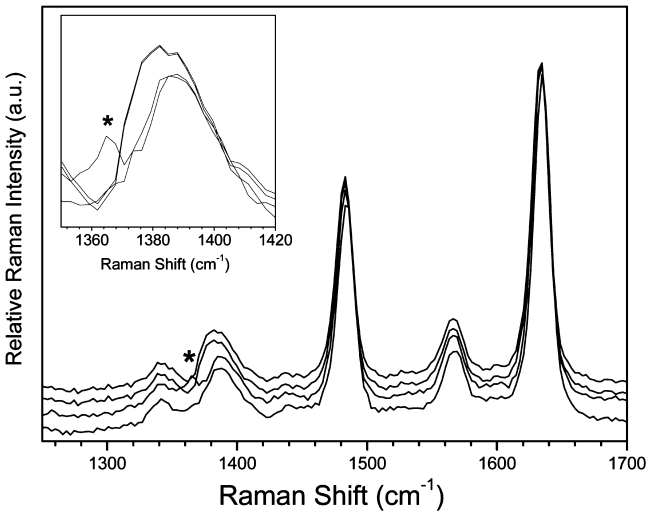

Figure 4. Transient Raman spectra of the 2-fluorenylnitrenium ion recorded with a 368.9 nm probe wavelength after 309 nm excitation in a mixed aqueous solution with 2′-deoxyguanosine at 10 ns, without any guanine derivatives at 10 ns, and with d-GTGTGT at 10 and 300 ns, respectively, from top to bottom. The inset displays a magnified view of the 1382 cm^{-1} Raman band that appears narrowed in the spectra for d-GTGTGT at 10 and 300 ns. The star indicates the regions affected by solvent subtraction artifacts.

CONCLUSIONS

Our TR^3 results show that the reaction of oligomeric guanine with the 2-fluorenylnitrenium ion produces C8 intermediates similar to those formed from the reaction of monomeric guanine derivatives, consistent with product analysis and biological experimental observations in vivo that showed that the C8 guanine adducts are the major reaction products. The oligomeric structure does not greatly influence the reaction intermediate structure but does accelerate the reaction rate of the guanine unit toward the arylnitrenium ion and may modestly affect the structure of the intermediates that lead to the C8 adduct. Comparison of the transient Raman results indicates that this acceleration may be due (at least partially) to a preinteraction between the arylnitrenium ion and the DNA oligomer.

EXPERIMENTAL SECTION

ns-TA. The ns-TA measurements were performed on a LP-920 Laser flash photolysis setup (Edinburgh Instruments). The 355 nm pump laser pulse was obtained from the third harmonic output of an Nd:YAG Q-switched laser, and the probe light was provided by a 450 W Xe arc lamp. These two light beams vertically overlap onto a 1 cm quartz cell. The signals were analyzed by a symmetrical Czerny−Turner monochromator, detected by a Hamamatsu R928 photomultiplier, and processed via an interfaced computer and analytical software.

ns-TR^3. A homemade ns-TR^3 spectroscopy apparatus described previously[23−25] was used in this work to acquire the ns-TR^3 spectra.

dx.doi.org/10.1021/jo500484s | J. Org. Chem. 2014, 79, 3610−3614

The 309.1 nm pump and 368.9 nm probe laser pulses were obtained from the anti-Stokes Raman scattering of hydrogen Raman-shifted lines from the third and second harmonics of a Nd:YAG Q-switched laser, respectively. Two lasers were electronically synchronized by a pulse delay generator, and the relative timing of the pump and probe laser pulses were displayed on a 500 MHz oscilloscope. The time resolution of the experiments was about 5 ns. The pump and probe laser beams were focused onto a flowing liquid stream of sample using a near-collinear geometry. The Raman-scattered light was collected in a backscattering geometry and detected by a liquid nitrogen-cooled charge-coupled device detector (CCD). The Raman signal was read by an interfaced PC, and the Raman spectra were obtained from subtraction of an appropriately scaled probe-before-pump spectrum from the corresponding pump−probe spectrum. The TR^3 spectra were calibrated by utilizing the known wavenumbers of the acetonitrile (MeCN) Raman bands. The areas of the Raman bands were determined by fittings using Lorentzian functions.

The preparation of the 2-fluorenyl azide precursor for the 2-fluorenylnitrenium ion was done following literature methods[26,27] and has been described in detail in ref 14. The 2-fluorenyl azide concentration was prepared to be 0.13 mM for ns-TA and 0.3 mM for ns-TR^3 experiments. All of the oligomers were purchased from IDT and were used as received; oligomer lengths of 5 to 6 bases were chosen mainly because of their cost and the convenience of obtaining samples. Spectroscopic grade MeCN and deionized water were used in preparing samples in a MeCN/H_2O 1:1 (v/v) solvent with a 0.005 M Na_2HPO_4/0.005 M NaH_2PO_4 buffer.

■ ASSOCIATED CONTENT

Supporting Information

TR^3 spectra after 309 nm laser photolysis of 0.3 mM 2-fluorenyl azide in the presence of 0.06 mM d-GTGTGT oligo in MeCN/H_2O 1:1 (v/v); plot of the area of the Raman bands at 1634 and 1617 cm^{-1} versus delay time fitted with an exponential function; plots of the decay rate constants of the 2-fluorenylnitrenium ion as a function of the DNA oligomers' concentration in a mixed aqueous solvent fitted to a linear function; decay traces of the 2-fluorenylnitrenium ion monitored at 435 nm in the presence of DNA oligomers in a mixed aqueous solvent fitted to an exponential function. 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.

■ ACKNOWLEDGMENTS

This work was supported by grants from The University of Hong Kong (201109176157) and the Natural Science Foundation of China (21202032) to J.X. and from the Research Grants Council of Hong Kong (HKU 7035/13P) to D.L.P. Partial support from the Areas of Excellence Scheme (AoE/P-03/08) and the Special Equipment Grant (SEG HKU/07) are also acknowledged.

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