JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Aminyl Radical Generation via Tandem Norrish Type I Photocleavage, β-Fragmentation: Independent Generation and Reactivity of the 2′-Deoxyadenosin- *N*6-yl Radical

Liwei Zheng,[†] Markus Griesser,[‡] Derek A. Pratt,*[,‡] and Marc M. Greenberg*[,†]

[†]Department of Chemistry, Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218, United States

[‡]Department of Chemistry & Biomolecular Sciences, University of Ottawa, 10 Marie Curie Pvt., Ottawa, Canada

Supporting Information

ABSTRACT: Formal hydrogen atom abstraction from the nitrogen−hydrogen bonds in purine nucleosides produces reactive intermediates that are important in nucleic acid oxidation. Herein we describe an approach for the independent generation of the purine radical resulting from hydrogen atom abstraction from the *N*6-amine of 2′-deoxyadenosine (dA•). The method involves sequential Norrish Type I photocleavage of a ketone (**7b**) and β-fragmentation of the initially formed alkyl radical (**8b**) to form dA• and acetone. The formation of dA• was followed by laser flash photolysis, which yields a transient with $\lambda_{max} \approx 340$ nm and a broader weaker absorption centered at ∼560 nm. This transient grows in at $\geq 2 \times 10^5$ s^{-1}; however, computations and reactivity data suggest that β-fragmentation occurs much faster, implying the consumption of dA• as it is formed. Continuous photolysis of **7b** in the presence of ferrous ion or thiophenol produces good yields of dA, whereas less reactive thiols afford lower yields presumably due to a polarity mismatch. This tandem photochemical, β-fragmentation method promises to be useful for site-specific production of dA• in nucleic acid oligomers and/or polymers and also for the production of aminyl radicals, in general.

INTRODUCTION

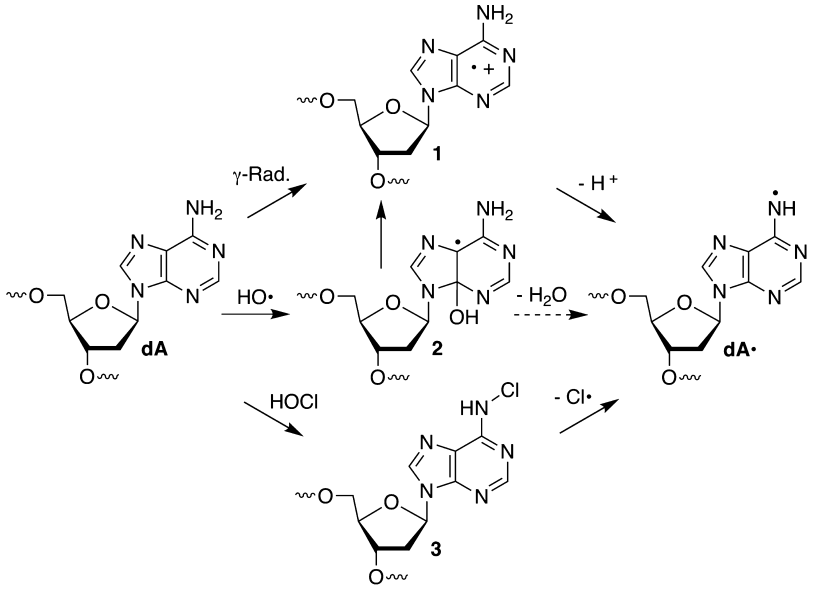
One-electron oxidation of purine nucleotides plays an important role in nucleic acid oxidation.[1] Chemists have primarily focused on the corresponding radical cations, especially that of 2′-deoxyguanosine (dG) and to a lesser extent of 2′-deoxyadenosine (dA), and their electron transfer chemistry.[2−6] Less is known about the reactivity of the formal hydrogen atom abstraction products (e.g., dG·, dA•) formed upon deprotonation of the radical cations (e.g., **1**, Scheme 1), although it seems clear that they do not react with O_2.[7] Although direct formation of neutral purine radicals by hydrogen atom abstraction by hydroxyl radicals during γ-radiolysis is generally agreed to be unfavorable, a sequential addition−elimination process (such as through **2**) can produce these species.[8,9] The neutral purine radicals are also decomposition products of chloramines (e.g., **3**) that are formed upon reaction with hypochlorite produced by myeloperoxidase as part of the inflammatory response.[10] Although independent generation of radical intermediates has been very useful for improving our understanding of nucleic acid oxidation, progress in elucidating the chemistry of purine oxidation has lagged that of other DNA and RNA components.[11] To learn more about the reactivity of the 2′-deoxyadenosin-*N*6-yl radical (dA•), we developed a photochemical method to produce this reactive species in aqueous solvent.

Purine radicals are considerably more stable than the respective one-electron oxidized pyrimidines, and the reduction potential of dG· is ∼3 kcal/mol lower than that of dA•.[12] Although the pK_a of **1** is a subject of debate, computations and spectroscopic experiments indicate that it is less than 4.2 and possibly as low as −1, indicating that the nucleoside of the radical cation exists entirely as dA• at neutral pH.[13−16] The protonation state within duplex DNA is more complicated due to delocalization of the "hole" associated with the radical cation as a result of base stacking.[5,17,18] Furthermore, deprotonation from the base-paired thymidine, which avoids dA• formation, appears to be kinetically favored when **1** is produced in duplex DNA.[19,20] Generation of dA• from **1** may quench hole migration in DNA, yet this radical has also been proposed to oxidize dG in dinucleotides and DNA.[9,13] The reactivity of dA• is underexplored. The rate constant for its reaction with molecular oxygen has been reported to be as little as 5×10^2 M^{-1} s^{-1}.[7] However, measurements of its reactivity are complicated by its rapid self-reaction ($k \sim 2 \times 10^8$ M^{-1} s^{-1}) as well as reaction with other species present under typical pulse radiolysis conditions.[21] Disproportionation between two radicals may yield 8-oxo-2′-deoxyadenosine (**4**) and dA. However, even if monomeric dA• reacts via disproportionation, it is unlikely that such a process would occur in DNA. The majority of studies on dA• have used pulse radiolysis to generate the radical, which facilitated spectroscopic detection of the radical at λ_{max} = 340 nm, and a

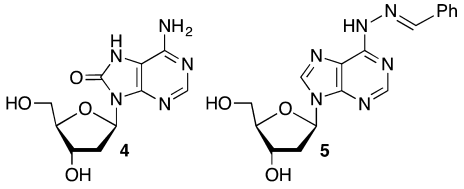
Received: January 13, 2017

DOI: 10.1021/acs.joc.7b00093
J. Org. Chem. XXXX, XXX, XXX−XXX

Scheme 1. Formation of dA•

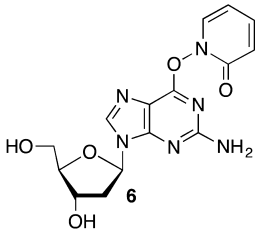


weaker broader absorption toward 600 nm.[13,16] More recently, Wagner independently generated dA• from phenylhydrazone precursors (e.g., **5**) via UV-photolysis, enabling a more thorough analysis of the radical's reactivity in solution.[22] We wish to report our own efforts in characterizing the reactivity of independently generated dA•. These studies are a necessary and important prelude to examining the corresponding radical's reactivity within biopolymers. The method employed here should also be useful for generating aminyl radicals, in general.



RESULTS AND DISCUSSION

Independent generation of nucleoside radicals and the analogous nucleotide radicals in oligonucleotides has greatly improved our understanding of nucleic acid oxidation. Several nucleoside carbon centered radicals have been independently generated photochemically.[11] However, examples of studies on the heteroatom radicals produced from purine oxidation are far more limited. The 2′-deoxyguanosine radical (dG·) is reported to have been produced via the *N*-hydroxypyrid-2(1*H*)-one (**6**) and respective thione.[23,24] As mentioned above, Wagner produced dA• from phenylhydrazone **5**, which concomitantly generates an iminyl radical in a solvent cage.[22] The formation of the radical pair consumes some dA•, and less than 10% of the precursor is converted after 1 h of photolysis.

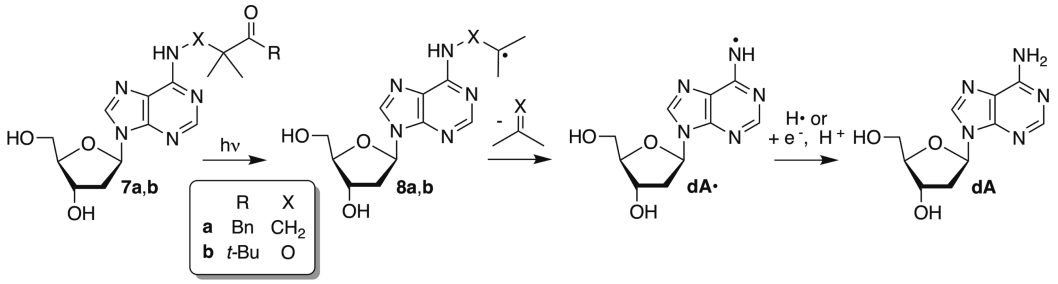


We sought an alternative method to photochemically generate dA• in high yield that would potentially be compatible with studying nucleobase radical reactivity within nucleic acids. Photochemical methods for nitrogen radical generation are less common than those for producing carbon radicals. Furthermore, our desire to carry out reactivity studies in water and the redox properties of nucleic acids place constraints on the methods that can be employed. For instance, *N*-hydroxypyridine-2-thione oxycarbonyl compounds, commonly referred to as PTOCs, are difficult to work with in water and would be unstable to the alkaline conditions commonly used for deprotecting oligonucleotides prepared by solid phase synthesis.[25] Photoinduced electron transfer processes were not pursued because of the possible secondary reactions with nucleobases in oligonucleotides. The coordination compounds frequently used are strong reducing agents in the excited state, but the ground states of the oxidized complexes are very strong oxidants that will react with nucleic acids.[26,27] Furthermore, these reactions are often carried out at higher concentrations of reactants than are typically used in nucleic acid experiments.

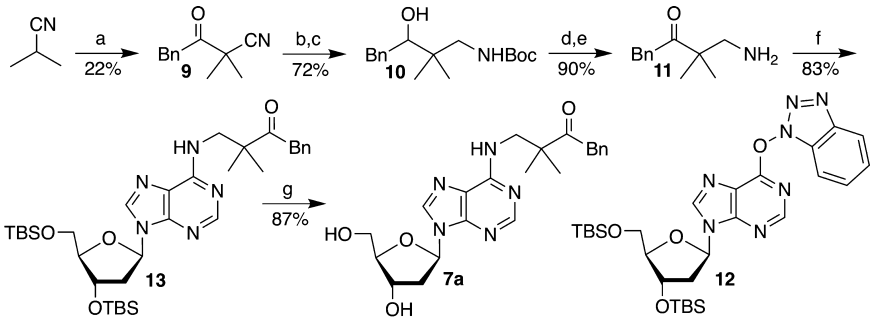
Attempted Photochemical Generation of dA• and Isobutylene via Sequential Norrish Type I Photocleavage, β-Fragmentation. Norrish Type I photochemical cleavage of ketones has been used successfully to generate a number of DNA and RNA carbon radicals in high yield.[11,28−34] We envisioned combining the Norrish Type I photocleavage of a ketone with β-fragmentation to generate dA• (Scheme 2). Amino ketone **7a** was an attractive potential precursor due to its anticipated robust stability and the reliability of the Norrish Type I photocleavage of benzyl ketones. The feasibility of β-fragmentation from **8a** to yield dA• and isobutylene was uncertain, as there were conflicting reports in the literature. Contrary to research described by Tsanaktsidis, Newcomb reported that the rate constant for generating a dialkylaminyl radical via ethylene elimination was ~4×10^4 s^{-1} at 27 °C.[35,36] However, subsequent experimental and computational experiments argued against aminyl radical formation via alkyl radical β-fragmentation.[37,38] If the β-fragmentation rate constant reported by Newcomb was correct the sequential process

DOI: 10.1021/acs.joc.7b00093
J. Org. Chem. XXXX, XXX, XXX−XXX

Scheme 2. Tandem Norrish Type I, β-Fragmenation Generation of dA•

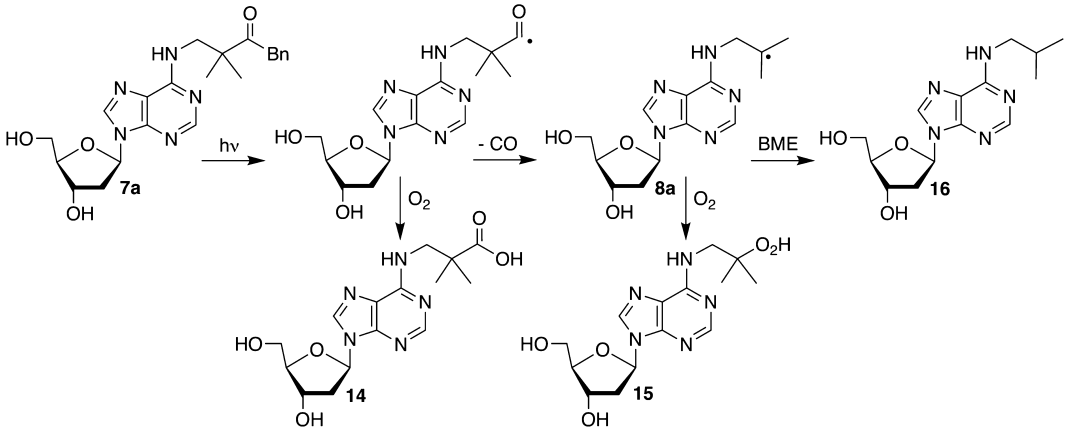


Scheme 3. Synthesis of Photochemical Precursor 7a[a]



[a]Key: (a) LiHMDS, ethyl phenylacetate, THF; (b) $LiAlH_4$, THF; (c) Boc_2O, DCM; (d) PCC, DCM; (e) TFA, DCM; (f) **12**, THF; (g) $Et_3N{\cdot}3HF$, THF.

Scheme 4. Trapping of Norrish Type I Cleavage Intermediates from 7a



(Scheme 2) would provide a source of dA• under anaerobic conditions (due to trapping of the alkyl radical by O_2).

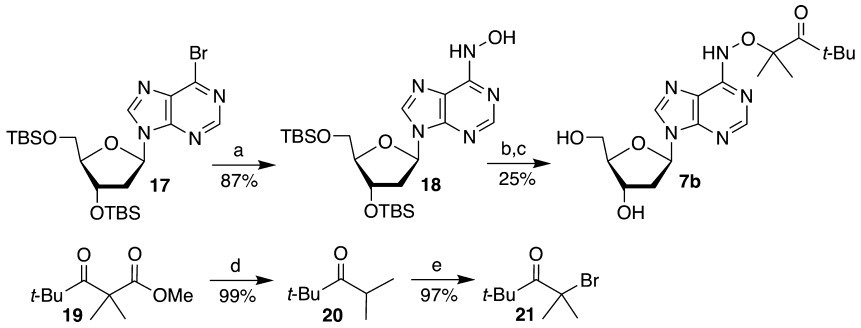
Despite the uncertainty of success of the β-fragmentation, **7a** was pursued for the aforementioned reasons. Ketone **7a** was readily prepared using the method for synthesizing *N*6-purine derivatives developed by Lakshman (**12**, Scheme 3).[39] The requisite amine (**11**) was synthesized starting from isobutyronitrile using a previously reported approach.[40] The nitrile was reduced following initial formation of the β-substituted ketone (**9**), which also reduced the ketone. After protecting the amine (**10**) the ketone was restored prior to removing the amine protecting group in **11**. Displacement of the *O*-benzotriazole group in **12** was carried out at room temperature with ~2.4 equiv of **11** to provide the protected form (**13**) of **7a**.

Photolysis of **7a** by broad-band irradiation (λ_{max} = 350 nm) did not yield detectable amounts of dA under either aerobic or anaerobic conditions. Low yields of **14** and **15** were identified by LC/MS following photolysis under aerobic conditions (Scheme 4). These products may result from O_2 interception of intermediates produced upon irradiation. Similarly, **16** was detected by LC/MS following anaerobic photolysis in the presence of β-mercaptoethanol (BME, 10 mM). As expected based upon the independent studies by Tsanaktsidis and Van Speybroeck, these results suggested that β-fragmentation from **8a** is too slow to generate dA•.

Photochemical Generation of dA• via Sequential Norrish Type I Photocleavage, β-Fragmentation of an Alkoxyamine. Replacing the methylene group in **8a** with oxygen (**8b**, Scheme 2) increases the thermodynamic driving force for β-fragmentation to dA• by producing a stronger carbon−oxygen double bond and requiring cleavage of a weaker nitrogen−oxygen bond.[41] Contrary to the above, ample experimental precedent exists for this process. For instance, Begley estimated that the rate constant for a similar β-fragmentation to form a hydroxamate radical is >2 × 10^8 s^{-1}.[42]

DOI: 10.1021/acs.joc.7b00093
J. Org. Chem. XXXX, XXX, XXX−XXX

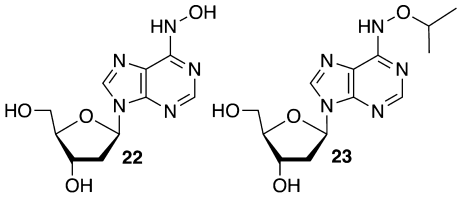
Scheme 5. Synthesis of Photochemical Precursor 7b[a]



[a]Key: (a) NH_2OH–HCl, Et_3N, THF; (b) **21**, NaH, THF; (c) $Et_3N{\cdot}3HF$, THF; (d) HOAc–HCl, Δ; (e) Br_2, HOAc, Δ.

In addition, related approaches have been used for generating nitrogen radicals under radical chain conditions.[43,44]

The approach employed for synthesizing **7a** was unsuccessful for preparing **7b** because the activated benzotriazole (**12**) was not displaced by the corresponding alkoxyamine. Consequently, *N*-hydroxylamine (**18**) was prepared from the bromide (**17**) and its conjugate base was reacted with α-bromoketone (**21**, Scheme 5).[45] The *tert*-butyl bromoketone (**21**) was prepared from β-ketoester **19** via decarboxylation (**20**) and bromination.[46] *N*-Hydroxylamine **18** was also used to prepare potential photolysis products **22** and **23**. Purification of **18** and **22** required washing with EDTA to remove unknown metals that bound the product and produced an intensely colored product.



Ketone **7b** exists as a mixture of tautomers (Figure 1A). The iminyl tautomer (**7b**-im) is slightly favored in $CDCl_3$ (Figure 1B), but dominates in CD_3CN (Figure 1C) and protic solvents (not shown). The protons in the aminyl tautomer appear downfield in the spectrum relative to the corresponding protons in **7b**-im, as expected based upon the loss in aromaticity in the latter, and consistent with trends reported in the literature.[47] The relevance of the tautomeric equilibrium to the photochemistry of **7b** is discussed below.

Laser flash photolysis of **7b** with the 308 nm emission of a nanosecond-pulsed XeCl excimer laser was carried out to obtain direct evidence for the transient formation of the aminyl radical dA•. Disappointingly, photolysis in several solvents (e.g., acetonitrile, MeOH, aqueous buffer) did not afford any detectable transients in our system (~12 mJ/pulse). We suspected that our failure to detect dA• was due to a low quantum yield for **7b** disappearance, which was measured using a structurally related ketone, 2-hydroxy-2-methylpropiophenone, as an actinometer (eqs 1, 2). We determined Φ = 0.0015 for the disappearance of **7b**, which led us to explore photosensitization conditions.

$$\text{Light flux} = \frac{n_{\text{actinometer conversion}}}{\Phi_{\text{actinometer}} t_{\text{photolysis}}} \quad (1)$$

$$\Phi_{\mathbf{7b}} = \frac{n_{\mathbf{7b}\text{ conversion}}}{1 - \frac{\%\,T}{100}(\text{Light flux}) \times t_{\text{photolysis}}} \quad (2)$$

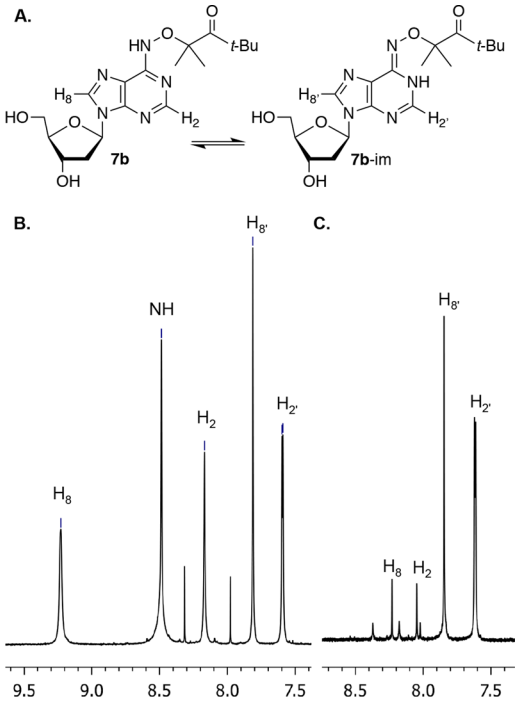


Figure 1. Solvent dependent tautomerization of **7b**. (A) Amine (**7b**) and imine (**7b**-im) tautomers. (B) ^1H NMR in $CDCl_3$. (C) ^1H NMR in CD_3CN.

Addition of low triplet energy sensitizers (e.g., benzophenone) also did not yield detectable transients. However, photolysis in the presence of acetone (200 mM in acetonitrile) yielded a prominent peak centered around 340 nm and a broader, weaker peak that stretches from 500 to 600 nm (Figure 2A). These data are consistent with previously reported spectra of dA•, which also feature a broad absorbance centered around 590 nm.[13,21] Since the alkoxyamine **7b** is in equilibrium with the oxime ether (**7b**-im), we calculated the UV–vis spectra of both the expected aminyl radical dA• and that of its iminyl tautomer (dA•-im) (Figure 2B). The calculations, using a *N*9-methyladenine analogue of dA• and dA•-im, indicate that the iminyl tautomer (24-im) is 13.0 kcal/mol higher in energy in the gas phase than the aminyl tautomer (24) (Figure 3).[48] These calculations also indicate that the observed transient is unlikely to correspond to the iminyl radical, which lacks a long wave absorption; if the iminyl radical is formed it must rapidly isomerize to dA•.

The accumulation of signal at or around 340 nm was attributed to β-fragmentation of the intermediate alkyl radical **8b** to produce dA•. Intermediate **8b** is not expected to absorb

DOI: 10.1021/acs.joc.7b00093
J. Org. Chem. XXXX, XXX, XXX–XXX

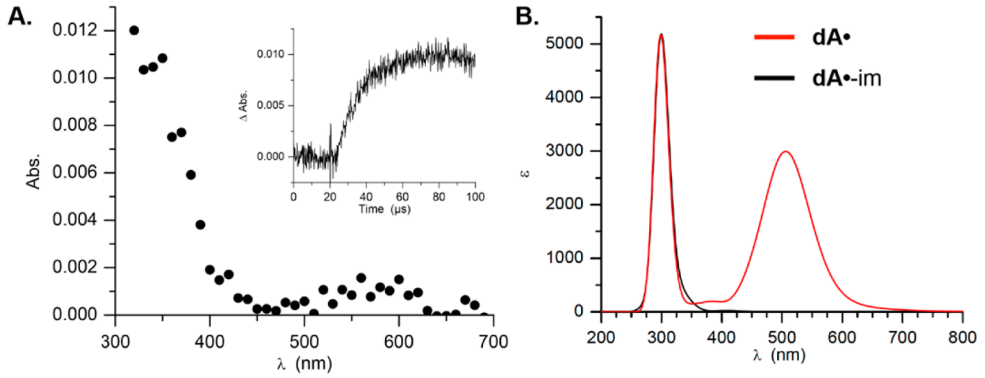


Figure 2. (A) Transient absorption spectrum obtained 40 μs after photolysis of a solution of **7b** (0.2 mM) in acetonitrile containing acetone (200 mM). Inset: growth of absorption at 340 nm. (B) Calculated absorption spectra for dA• and its iminyl tautomer (dA•-im) (TD-B3LYP/6-311++G(d,p)).

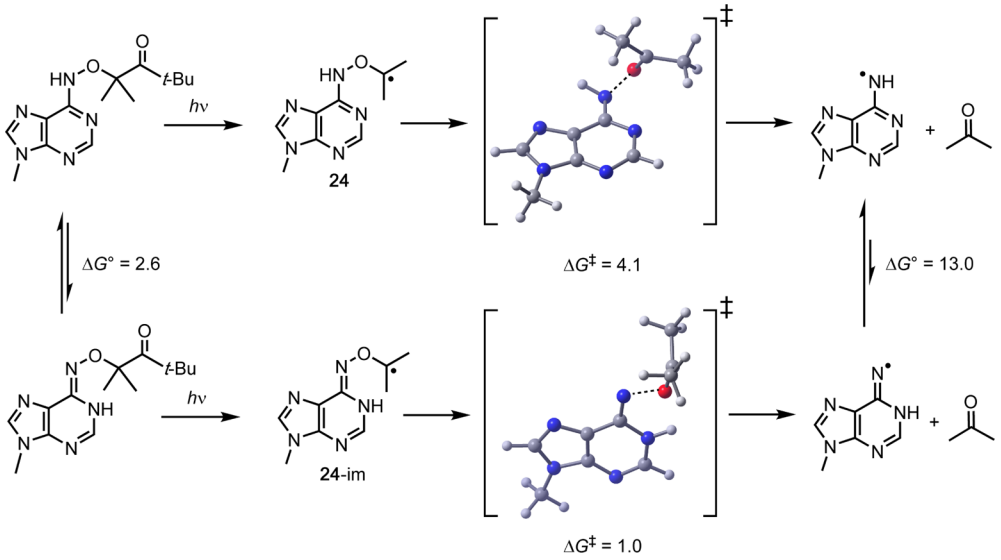
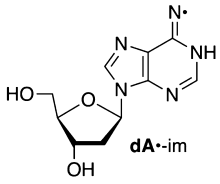


Figure 3. Calculated transition state structures for the fragmentation reactions of **24** and **24**-im (energies given in kcal/mol).



strongly above 300 nm, and its formation is presumably too fast to detect on this time scale. The rate constant for formation of dA• was fit to first-order growth kinetics, yielding a rate constant of $(2 \pm 2) \times 10^5$ s^{-1} (Figure 2A), more than 10^4- and 10^7-fold slower than that computed from either **24** or **24**-im, respectively (Figure 3). The rate constant derived from the growth rate of dA• is likely underestimated due to the relatively fast concurrent consumption that suppresses the transient signal. Indeed, if the rate constant of the β-fragmentation was only ~10^5 s^{-1}, reaction with O_2 would be competitive ($k[O_2] \approx 10^6$ s^{-1}), which does not appear to be the case (*vide infra*).[49]

Once the absorption at 340 nm reaches a maximum intensity around 80 μs, the transient slowly decays with a half-life of ca. 200 μs (Figure 4). This decay could not be fit to a second-order function, indicating that multiple decay paths are available to the radical under these conditions. Attempts to direct this along the path of reduction of dA• by H atom transfer from

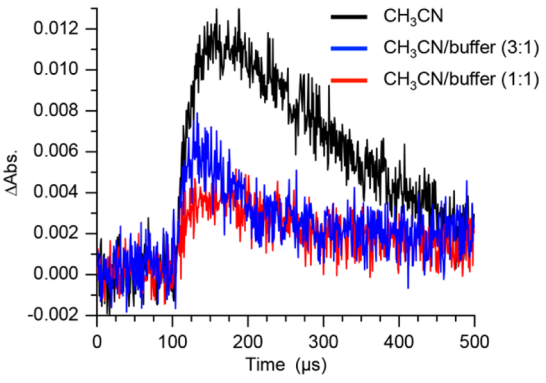


Figure 4. Transient absorption traces of a solution of **7b** (0.2 mM) in acetonitrile and different mixtures with buffer (phosphate 10 mM, pH 7.4) in the presence of acetone (200 mM) at 340 nm.

thiophenol (*vide infra*) were unsuccessful. Unfortunately, the phenylthiyl radical absorbs in this region, which may be produced in this experiment by the desired H atom transfer, H atom donation to the acetone triplet, and photolysis of residual diphenyldisulfide in the thiophenol.

In an attempt to slow the decay of the aminyl radical (dA•) and possibly obtain cleaner decay kinetics we carried out

DOI: 10.1021/acs.joc.7b00093
J. Org. Chem. XXXX, XXX, XXX−XXX

photolyses of **7b** in acetonitrile-buffer mixtures. Unfortunately, this led to a significant reduction in signal intensity (Figure 4). This is consistent with the shorter lifetime of the acetone triplet state in aqueous solution as compared to acetonitrile, rendering photosensitization less efficient and precluding generation of radical **8b**.[50] This hypothesis is also consistent with photochemical conversion of **7b** during continuous irradiation in various solvent conditions (Table 1). We found that **7b** is converted approximately 3 times more efficiently upon broadband UV-irradiation (λ_{max} = 350 nm) in acetonitrile than in phosphate buffer. Acetone enhances the photoconversion efficiency in both solvents, notably more than 6-fold in acetonitrile, the conditions under which laser flash photolysis experiments were most successful.

Table 1. Photochemical Conversion Efficiency during Continuous UV-Irradiation

solvent	acetone[a]	hν time (min)	Conv (%)[b]	av conv rate (% min^{-1})
aq buffer[c]	–	60	30 ± 1	0.5
aq buffer[c]	+	30	51.4 ± 0.2	1.7
CH_3CN	–	30	45.2 ± 0.2	1.5
CH_3CN	+	1	10 ± 1	10

[a][Acetone] = 0.2 M. [b]Average ± std dev of 3 experiments. [c]Phosphate (10 mM, pH 7.2).

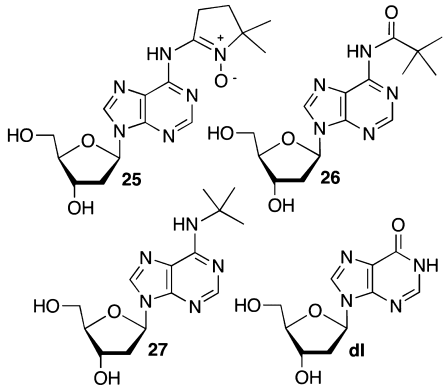
Trapping Studies of dA•. The majority of product studies involving the photolysis of **7b** were carried out in phosphate buffer to mimic the conditions under which dA• would be produced in DNA. A more limited number of experiments were carried out in acetonitrile to correlate product studies with the above LFP experiments. Inferential support for the intermediacy of dA• was gleaned from the detection of the oxidized spin trapping product **25** by LC/MS, but formation of dA served as the strongest evidence for formation of the nucleoside radical.[51] Modest yields of dA were formed following photolysis of **7b** in aqueous buffer under aerobic or anaerobic conditions in the absence of an exogenous reducing agent (Table 2).

Table 2. Effect of Reaction Conditions on 2′-Deoxyadenosine Yield from the Photolysis (4 h) of 7b in Aqueous Buffer

[**7b**] (μM)	O_2	reductant (mM)	% yield dA[a]	% mass balance
100	–	Fe^{2+} (10)	64 ± 1	76.9 ± 0.2
50	–	Fe^{2+} (10)	69 ± 6	80 ± 5
25	–	Fe^{2+} (10)	74 ± 2	86 ± 2
100	+	Fe^{2+} (10)	62 ± 1	73 ± 1
100	–	–	34 ± 4	50 ± 3
100	+	–	27 ± 2	59 ± 2
100	–	BME (100)	24 ± 2	51 ± 2
100	–	BME (500)	26 ± 2	51 ± 2
100	–	PhSH (10)	70 ± 2	84 ± 2
100	–	Cys (10)	32 ± 1	63 ± 1
100	–	GSH (10)	48 ± 3	73 ± 2

[a]Average ± std dev of 3 experiments.

The yield almost doubled under anaerobic conditions in the presence of $Fe(NH_4)_2(SO_4)_2$ (10 mM) and increased further when the concentration of **7b** was decreased to 25 μM from 100 μM. Photolysis of **7b** (0.1 mM) in acetonitrile in the absence of exogenous reductant generated dA in 54 ± 1% (3 replicates) and an identical mass balance. The decreased dA yield and mass balance in the absence of reductant could be due to a reaction between dA• and **7b**. The increased yield of dA and mass balance when the concentration of **7b** is decreased is consistent with this proposal. Under aerobic conditions, 2′-deoxyinosine (dI) was also formed in yields (11.6 ± 0.7%) comparable to those reported by Wagner upon irradiation of **5**.[22] A comparable transformation has also been detected in the reactivity of the N_4-amino radical of 2′-deoxycytidine.[52] However, computational studies do not provide a kinetically favorable pathway for this process.[53] It is similarly unclear how dI is produced from dA•. The mass balance determined by the amounts of **7b**, dA, and dI varied from 73% to 86% in the presence of a ferrous ion. Minor amounts of *tert*-butyl amide **26** and **27** were also detected under anaerobic conditions by LC/MS and may be attributed to radical recombination following Norrish Type I photocleavage.



The lack of an O_2 effect on the yield of dA from **7b** is consistent with the slow reaction of dA• with this species.[7] However, this also suggests that O_2 does not compete with β-fragmentation in **8b**. The absence of detectable levels of **22**, a likely product resulting from O_2 trapping of **8b** in aqueous solvent, is also consistent with rapid loss of acetone. Similarly, β-mercaptoethanol (BME, as high as 0.5 M) does not trap **8b** to produce **23** under anaerobic conditions. These experiments enable us to propose that β-fragmentation is significantly greater than 10^6 s^{-1}, and are consistent with the Begley's results for hydroxamate radical formation and the computational studies described above.[42] Moreover, there is no evidence for BME trapping dA•. Increasing the BME concentration from zero to 0.1 to 0.5 M has no effect on the yield of dA, and a slight decrease in the mass balance (Table 1). In contrast, cysteine (Cys) and glutathione (GSH) yield modestly higher dA yields and increased mass balances. The greatest yield and corresponding mass balance is observed when the even more reactive thiophenol (PhSH) is employed. PhSH (10 mM) was even more effective when **7b** (0.1 mM) was photolyzed in acetonitrile, where the yield of dA was 90 ± 5% and the mass balance was 94 ± 5% (3 replicates). We suggest that the correlation between increased mass balances and dA yields are a consequence of multiple roles played by the thiol(s) in the reaction, including preventing reactions between dA• and precursor **7b**, as well as other intermediates (e.g., *tert*-butyl radical) produced upon photolysis.

The lower yield of dA in reactions in the presence of thiols other than PhSH cannot be rationalized simply by consideration of the corresponding bond dissociation energies. The thiol bond strength in BME could be as low as 88 kcal/mol, and

DOI: 10.1021/acs.joc.7b00093
J. Org. Chem. XXXX, XXX, XXX−XXX

the *N*6-H BDE is greater than 97 kcal/mol in 9-methyladenine.[54−56] Hence, reduction of dA• by BME is thermodynamically favorable. The slightly more favorable reactivity of dA• with Cys and GSH could be due to a variety of factors, including polarity of the thiol bonds. We suggest that the reactions are kinetically controlled and are a consequence of a polarity mismatch between the electrophilic dA• radical and the polarity of the H donor. Nucleophilic aminyl radicals react readily with alkyl thiols or PhSH.[57] The rate constants for their reaction with thiols are comparable to those of their alkyl radical counterparts despite less favorable thermodynamics. However, the aminyl radical in dA• is conjugated to the electron-withdrawing purine ring, which increases its electrophilic character. The reactivity of dA• is more similar to an amidyl radical than an alkylamine radical. Amidyl radicals react more rapidly with electron-rich donors, such as Bu_3SnH than even PhSH.[58] Hence, the kinetic recalcitrance of dA• to react with thiols other than PhSH may be due to polarity mismatching between the electrophilic radical and the relatively electronegative hydrogen atom donor (compared to other donors such as Bu_3SnH).[57,59] Unfortunately, testing this hypothesis by examining the reaction of dA• with Bu_3SnH is not possible due to the incompatible nature of the reactants' solubilities.

CONCLUSIONS

Tandem Norrish Type I, β-fragmentation effectively produces the nucleobase radical resulting from formal H donor abstraction from the *N*6-amino position of dA (dA•). dA• is directly observed via laser flash photolysis under photosensitization conditions. Photosensitization by acetone improves the efficiency of Norrish Type I photocleavage of **7b** but is not necessary for producing dA•. β-Fragmentation of the intermediate radical formed via the photocleavage reaction (**8b**) is very rapid, and there is no evidence for trapping it even in the presence of O_2. In the presence of an appropriate reductant, high yields of dA are generated upon direct irradiation. Thiols such as β-mercaptoethanol are ineffective reductants of dA•, whereas PhSH and a ferrous ion successfully trap the radical. Inefficient trapping of electrophilic aminyl radical dA• by thiols is attributed to polarity mismatch between the reactants. Product studies carried out in the presence of an appropriate reducing agent reveal that the nonsensitized photocleavage, β-fragmentation process produces dA• with high fidelity. This method should be applicable to independent generation of neutral purine radicals derived from dG and generally useful for photochemical generation of nitrogen-centered radicals. Finally, precursor **7b** should be compatible with solid-phase oligonucleotide synthesis methods, which will facilitate determining the reactivity of dA• in DNA, of which little is known.

EXPERIMENTAL PROCEDURES

General Methods. Triethylamine, DMF, and DCM were distilled from CaH_2 under Ar or under an appropriate vacuum. THF was distilled from Na under Ar. All other reagents were purchased from commercial sources and were used without further purification unless noted otherwise. All reactions were carried out under a positive pressure of argon atmosphere and monitored by TLC on Silica Gel G-25 UV254 (0.25 mm) unless stated otherwise. Spots were detected under UV light and/or by an ethanolic solution of *p*-anisaldehyde, an aqueous solution of ammonium molybdate, ceric ammonium sulfate, or $KMnO_4$. Column flash chromatography was performed with Silicycle grade 70−230 mesh, 60−200 μm, 60 Å silica. The ratio between silica gel and crude product ranged from 100:1 to 20:1 (w/w).

Preparation of **9**. HMDS (3.2 mL, 15.0 mmol) was added to a solution of *n*-butyl lithium (14.5 mmol, 11 mL) at −78 °C in THF (7 mL), and the mixture was stirred at this temperature for 1 h. Isobutyronitrile (966 mg, 14.0 mmol) in THF (5 mL) was slowly added to the solution. The solution was stirred for 1 h at which time ethyl phenylacetate (2.228 g, 14.0 mmol) in THF (10 mL) was added. The mixture was allowed to warm up to room temperature and stirred overnight. The reaction was quenched with MeOH at room temperature, concentrated under vacuum, and purified by flash chromatography. Elution with 20% ethyl acetate in hexane gave **9** as a colorless oil (597 mg, 22%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45−7.18 (m, 5H), 4.10 (s, 2H), 1.52 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.7, 132.8, 129.7, 128.7, 127.4, 121.9, 45.2, 43.9, 23.9. IR (KBr plate) 2998, 2902, 2856, 2344, 1721, 792, 713 cm^{-1}. HRMS (FAB-double focusing magnetic sector) $C_{12}H_{14}NO^+$ $(M + H)^+$ calcd 188.1075, found 188.1072.

Preparation of **10**. A solution of **9** (168 mg, 0.9 mmol) in THF (1 mL) was added dropwise to a suspension of $LiAlH_4$ (36 mg, 0.95 mmol) in THF (1 mL) over 2 min at 0 °C. The ice bath was removed, and the reaction was stirred at room temperature for 4 h. The reaction mixture was poured into THF/H_2O (1:1, 1 mL) at 0 °C, stirred at 80 °C for 10 min, and then filtered through Celite. The filtrate was extracted with DCM (3 × 10 mL), and the organic layers were combined, dried over Na_2SO_4, and concentrated under vacuum to give 4-amino-3,3-dimethyl-1-phenylbutan-2-ol. 4-Amino-3,3-dimethyl-1-phenylbutan-2-ol was used without further purification. A solution of di-*tert*-butyl dicarbonate (207 mg, 0.95 mmol) in DCM (2 mL) was added to a solution of 4-amino-3,3-dimethyl-1-phenylbutan-2-ol in DCM (2 mL) at 0 °C and stirred at room temperature overnight. The reaction mixture was poured into water and extracted with DCM (3 × 10 mL), dried over Na_2SO_4, and concentrated under vacuum. The residue was chromatographed on silica gel and eluted with 25% ethyl acetate in hexane to give **10** as a colorless oil (188.7 mg, 72% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (m, 5H), 5.26 (t, J = 6.2 Hz, 1H), 3.53 (d, J = 9.2 Hz, 1H), 3.39 (dd, J = 14.1, 7.2 Hz, 1H), 3.26 (s, 1H), 2.81 (dd, J = 21.6, 9.1 Hz, 2H), 2.56 (dd, J = 13.8, 10.5 Hz, 1H), 1.44 (s, 9H), 0.97 (d, J = 7.5 Hz, 6H).^{13}C NMR (75 MHz, $CDCl_3$) δ 146.3, 139.6, 129.1, 122.8, 119.4, 87.9, 84.5, 72.1, 62.9, 40.6, 25.9, 25.8, 18.4, 18.0. IR (KBr plate) 3457, 3398, 2970, 2934, 2859, 1719, 1685, 792, 743, 729 cm^{-1}. HRMS (FAB-double focusing magnetic sector) $C_{17}H_{28}NO_3^+$ $(M + H)^+$ calcd 294.2069, found 294.2065.

*Preparation of t-Boc-Protected-***11**. A solution of **10** (153 mg, 0.52 mmol) in DCM (2 mL) was added to a solution of pyridine (60 mg, 0.75 mmol), PCC (162 mg, 0.75 mmol), and Celite (1.3 g) in DCM (2.5 mL). The reaction mixture was refluxed for 6.5 h. After quenching excess PCC with isopropanol (5 mL), diethyl ether (20 mL) was added to the solution. The reaction mixture was filtered through Celite and concentrated under vacuum. The residue was chromatographed on silica gel and eluted with 25% ethyl acetate in hexane to give *t*-Boc-protected-**11** as a light yellow oil (137.6 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34−7.27 (m, 2H), 7.27−7.20 (m, 1H), 7.15 (dd, J = 7.7, 0.9 Hz, 2H), 4.95 (s, 1H), 3.78 (s, 2H), 3.25 (d, J = 6.6 Hz, 2H), 1.41 (s, 9H), 1.22 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 212.7, 156.3, 134.4, 129.7, 128.5, 126.9, 79.1, 49.4, 48.0, 43.7, 28.4, 22.5. IR (KBr plate) 3387, 2975, 2935, 2849, 1760, 1697, 749, 735 cm^{-1}. HRMS (FAB-double focusing magnetic sector) $C_{17}H_{26}NO_3^+$ $(M + H)^+$ calcd 292.1913, found 292.1905.

Preparation of **11**. *t*-Boc-protected-**11** (137.6 mg, 0.47 mmol) was treated with DCM−TFA (1:1, 2 mL) for 1 h at 25 °C. The reaction mixture was then poured in sat. $NaHCO_3$ solution, extracted with DCM (3 × 10 mL), dried over Na_2SO_4, and concentrated under vacuum. The residue was chromatographed on silica gel and eluted with 40% ethyl acetate in hexane to give **11** as a colorless oil (91 mg, >99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (m, 5H), 3.80 (s, 2H), 2.81 (s, 2H), 1.47 (s, 2H), 1.19 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 212.5, 134.6, 129.6, 128.4, 126.7, 53.5, 50.8, 49.9, 44.1, 22.4. IR (KBr plate) 3391 (broad), 2963, 2925, 2854, 1700, 1043, 725, 696,

DOI: 10.1021/acs.joc.7b00093
J. Org. Chem. XXXX, XXX, XXX−XXX

667 cm^{-1}. HRMS (FAB-double focusing magnetic sector) $C_{12}H_{18}NO^+$ $(M + H)^+$ calcd 192.1388, found 192.1391.

Preparation of ***13***. Compound **12**[39] (59.8 mg, 0.1 mmol) and **11** (46 mg, 0.24 mmol) were stirred in THF (1 mL) at room temperature overnight. The reaction was concentrated under vacuum and purified by flash chromatography on a silica column. Elution with 3% MeOH in DCM gave **13** as a white solid (53.9 mg, 83%). Mp 78−79 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 8.04 (s, 1H), 7.34−7.26 (m, 2H), 7.24 (dd, J = 5.1, 3.7 Hz, 1H), 7.18−7.12 (m, 2H), 6.42 (t, J = 6.5 Hz, 1H), 4.65−4.57 (m, 1H), 4.00 (dd, J = 7.5, 3.3 Hz, 1H), 3.90−3.67 (m, 6H), 2.69−2.54 (m, 1H), 2.41 (ddd, J = 13.0, 6.1, 3.8 Hz, 1H), 1.32 (s, 6H), 0.91 (s, 9H), 0.91 (s, 9H), 0.09 (s, 6H), 0.08 (s, 3H), 0.08 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 212.2, 155.1, 152.8, 150.6, 138.2, 134.3, 129.6, 128.4, 126.8, 87.8, 84.2, 71.9, 62.8, 49.6, 43.7, 41.2, 26.0, 25.8, 22.6, 18.4, 18.0, −4.7, −4.8, −5.4, −5.5. IR (KBr plate) 2953, 2928, 2855, 1708, 1613, 1471, 1252, 835, 777, 724 cm^{-1}; HRMS (ESI-TOF) $C_{34}H_{56}N_5O_4Si_2^+$ $(M + H)^+$ calcd 654.3871, found 654.3871.

Preparation of ***7a***. $Et_3N{\cdot}3HF$ (161 mg, 1.0 mmol) was slowly added to **13** (65 mg, 0.1 mmol) in THF (1 mL). The reaction was stirred at 25 °C overnight. The reaction was concentrated under vacuum and purified by flash chromatography on a silica column. Elution with 5% MeOH in DCM gave **7a** as a white solid (37.0 mg, 87%). Mp 104−106 °C; ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.29 (s, 1H), 8.23 (s, 1H), 7.34−7.03 (m, 5H), 6.43 (dd, J = 7.8, 6.0 Hz, 1H), 4.65−4.56 (m, 1H), 4.09 (dd, J = 5.5, 2.9 Hz, 1H), 3.97 (s, 2H), 3.86 (dd, J = 12.3, 2.9 Hz, 2H), 3.75 (dd, J = 12.3, 3.3 Hz, 1H), 3.43−3.10 (m, 2H), 2.81 (ddd, J = 13.6, 8.0, 5.8 Hz, 1H), 2.41 (ddd, J = 13.4, 6.0, 2.6 Hz, 1H), 1.32 (s, 6H). ^{13}C NMR (101 MHz, MeOH-d_4) δ 211.1, 153.6, 150.5, 138.2, 133.3, 129.5, 127.9, 126.9, 126.3, 124.6, 87.0, 84.3, 70.2, 60.8, 48.0, 41.9, 38.7, 27.8, 20.0. IR (KBr plate) 3279 (broad), 2928, 1610, 1580, 1473, 1228, 1111, 1071, 836, 774, 728 cm^{-1}. HRMS (ESI-TOF) $C_{22}H_{28}N_5O_4^+$ $(M + H)^+$ calcd 426.2141, found 426.2136.

Preparation of ***20***. Acetic acid−conc. HCl (1:1, 30 mL) was added to **19**[46] (2.0 g, 10 mmol). The reaction was refluxed at 110 °C for 8 h. The cooled reaction was quenched by saturated $NaHCO_3$ solution and extracted with DCM (30 mL × 3). The organic phase was dried over Na_2SO_4. The organic phase was concentrated under vacuum to give **20** as an orange oil (1.75 g, >99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.12 (hept, J = 6.7 Hz, 1H), 1.14 (s, 9H), 1.03 (d, J = 6.7 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 220.1, 44.6, 33.8, 26.0, 20.1. IR (KBr plate) 2973, 2936, 2874, 1732 cm^{-1}.

Preparation of ***21***. Br_2 (176 mg, 1.1 mmol) was slowly added to **20** (128 mg, 1 mmol) in HOAc (2 mL). The reaction was heated at 70 °C for 5 h, poured into water, and extracted with EtOAc (5 mL × 3). The organic phase was passed through a silica plug and then concentrated under vacuum to yield **21** as a yellow oil (200 mg, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.91 (s, 6H), 1.36 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 209.2, 62.3, 45.5, 31.9, 29.3. IR (KBr plate) 2981, 2876, 1733, 1699, 1263, 1194, 1145 cm^{-1}.

Preparation of ***18***. TEA (1.01 g, 10 mmol) was added to hydroxylamine hydrochloride (345 mg, 5 mmol) in THF (10 mL). After stirring at room temperature for 30 min, **17** (270 mg, 0.5 mmol) was added, and the reaction was refluxed at 65 °C overnight. The reaction was concentrated under vacuum and purified by flash chromatography on a silica column. Elution with 5% MeOH and 1% Et_3N in DCM gave **18** as a light purple foam. The sample was dissolved in MeOH, and Na_2−EDTA was added to the solution followed by stirring at 50 °C until the purple color faded. The mixture was cooled to room temperature and filtered to remove Na_2−EDTA. The organic phase was then concentrated under vacuum give a white foam (143 mg, 58%). ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.20 (s, 1H), 8.05 (s, 1H), 6.37 (t, J = 6.4 Hz, 1H), 4.69 (dt, J = 5.6, 4.0 Hz, 1H), 3.97 (dd, J = 7.8, 3.6 Hz, 1H), 3.86 (dd, J = 11.2, 4.5 Hz, 1H), 3.76 (dd, J = 11.2, 3.6 Hz, 1H), 2.75 (dt, J = 12.5, 6.1 Hz, 1H), 2.43 (ddd, J = 13.1, 6.2, 4.2 Hz, 1H), 0.93 (s, 9H), 0.87 (s, 9H), 0.17−0.00 (m, 12H). ^{13}C NMR (75 MHz, MeOH-d_4) δ 149.7, 147.2, 145.9, 140.3, 139.5, 119.8, 89.2, 85.7, 73.4, 64.0, 41.6, 26.6, 26.5, 19.3, 19.0, 9.7, −4.2, −4.3, −5.1. IR (KBr plate) 3408 (broad), 2954, 2929, 2896, 2857, 1691, 1680, 1254, 1110, 837, 778 cm^{-1}. HRMS (ESI-TOF) $C_{22}H_{42}N_5O_4Si_2^+$ $(M + H)^+$ calcd 496.2775, found 496.2768.

Preparation of Bis-silylated ***7b***. NaH (60% in mineral oil, 28 mg, 0.7 mmol) was slowly added to **18** (335 mg, 0.7 mmol) in DMF (7 mL). The reaction was stirred at 0 °C for 1 h, at which time **21** (290 mg, 1.4 mmol) was added at 0 °C. The reaction was stirred at room temperature for 4 h, concentrated under vacuum, and purified by flash chromatography on a silica column. Elution with 50% EtOAc in hexanes gave bis-silylated **7b** as a yellow foam (320 mg, 74%). ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.94 (s, 1H), 7.62 (s, 1H), 6.28 (t, J = 6.4 Hz, 1H), 4.69 (dd, J = 9.2, 3.9 Hz, 1H), 3.95 (dd, J = 7.7, 3.7 Hz, 1H), 3.84 (dd, J = 11.2, 4.5 Hz, 1H), 3.76 (dd, J = 11.2, 3.6 Hz, 1H), 2.73 (dt, J = 12.6, 6.1 Hz, 1H), 2.40 (ddd, J = 13.2, 6.3, 4.3 Hz, 1H), 1.48 (s, 6H), 1.25 (s, 9H), 0.93 (s, 9H), 0.88 (s, 9H), 0.14 (s, 6H), 0.07 (s, 3H), 0.04 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 218.4, 144.1, 140.5, 136.5, 118.8, 87.9, 87.2, 84.2, 71.9, 62.4, 43.8, 40.2, 27.9, 25.1, 24.91, 23.8, 17.9, 17.5, −5.9, −6.0, −6.8. IR (KBr plate) 2927, 1672, 1609, 1474, 1254, 1112, 832, 782, 662 cm^{-1}. HRMS (ESI-TOF) $C_{30}H_{56}N_5O_5Si_2^+$ $(M + H)^+$ calcd 622.3820, found 622.3820.

Preparation of ***7b***. $Et_3N{\cdot}3HF$ (0.83 g, 5.2 mmol) was slowly added to bis-silylated **7b** (320 mg, 0.52 mmol), and the reaction was stirred at 25 °C overnight. The reaction was concentrated under vacuum and purified by flash chromatography on a silica column. Elution with 5% MeOH in DCM gave **7b** as a yellow foam (163 mg, 59%). ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.01 (s, 1H), 7.64 (s, 1H), 6.45−6.17 (m, 1H), 4.60−4.40 (m, 1H), 4.02 (dd, J = 6.1, 3.2 Hz, 1H), 3.80 (dd, J = 12.2, 3.2 Hz, 1H), 3.75−3.67 (m, 1H), 2.77−2.62 (m, 1H), 2.39 (ddd, J = 13.4, 6.0, 2.9 Hz, 1H), 1.48 (s, 6H), 1.24 (s, 9H). ^{13}C NMR (101 MHz, MeOH-d_4) δ 218.4, 144.2, 140.5, 140.3, 137.1, 119.2, 88.3, 87.3, 85.3, 71.4, 62.1, 43.8, 40.6, 27.9, 23.8. IR (KBr plate) 3278 (broad), 2932, 1669, 1610, 1471, 1254, 1110, 837, 778, 665 cm^{-1}. HRMS (ESI-TOF) $C_{18}H_{28}N_5O_5^+$ $(M + H)^+$ calcd 394.2090, found 394.2085.

Preparation of ***22***.[60] $Et_3N{\cdot}3HF$ (323 mg, 2.0 mmol) was slowly added to **18** (99 mg, 0.2 mmol) in THF (2 mL). The reaction was stirred at 25 °C overnight, concentrated under vacuum, and purified by flash chromatography on a silica column. Elution with 10% MeOH in DCM gave **22** as a light purple foam. The sample was dissolved in MeOH, Na_2−EDTA was added to the solution, and the reaction was stirred at 50 °C until the purple color faded. The mixture was cooled to room temperature and filtered to remove Na_2−EDTA. The organic phase was then concentrated under vacuum give a white foam (37.5 mg, 70%). ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.36 (s, 1H), 8.20 (s, 1H), 6.44 (dd, J = 7.9, 6.0 Hz, 1H), 4.58 (dt, J = 5.6, 2.7 Hz, 1H), 4.07 (dd, J = 5.8, 3.1 Hz, 1H), 3.84 (dd, J = 12.3, 3.0 Hz, 1H), 3.74 (dd, J = 12.3, 3.4 Hz, 1H), 2.80 (ddd, J = 13.6, 7.9, 5.8 Hz, 1H), 2.42 (ddd, J = 13.4, 6.0, 2.8 Hz, 1H). The spectral data match the literature.

Preparation of Bis-silylated ***23***. NaH (60% in mineral oil, 20 mg, 0.5 mmol) was slowly added to **18** (241 mg, 0.5 mmol) in THF (3 mL). The reaction was stirred at 0 °C for 30 min, at which time 2-bromopropane (68 mg, 0.55 mmol) was added at 0 °C. The reaction was stirred at room temperature for 2 h. The reaction was concentrated under vacuum and purified by flash chromatography on a silica column. Elution with 50% EtOAc in hexane gave bis-silylated **23** as a red oil (70 mg, 26%). ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.03 (s, 1H), 7.69 (s, 1H), 6.31 (s, 1H), 5.50 (d, J = 10.6 Hz, 1H), 4.76−4.59 (m, 1H), 4.27 (hept, J = 6.3 Hz, 1H), 3.95 (dd, J = 8.0, 3.7 Hz, 1H), 3.85 (dd, J = 11.2, 4.6 Hz, 1H), 3.76 (dd, J = 11.2, 3.7 Hz, 1H), 2.80−2.67 (m, 1H), 2.40 (ddd, J = 13.1, 6.2, 4.2 Hz, 1H), 1.29 (d, J = 6.2 Hz, 6H), 0.93 (s, 9H), 0.89 (s, 9H), 0.14 (s, 6H), 0.07 (s, 3H), 0.05 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.1, 152.9, 149.8, 142.4, 140.7, 140.1, 139.6, 136.0, 120.1, 118.8, 87.9, 84.4, 83.9, 78.0, 75.1, 71.8, 62.8, 41.5, 41.2, 26.0, 25.8, 21.8, 20.6, 18.4, 18.0, −4.6, −4.8, −5.4, −5.5. IR (KBr plate) 2975, 2920, 1670, 1581, 1412, 1223, 889, 748, 659 cm^{-1}. HRMS (ESI-TOF) $C_{25}H_{48}N_5O_4Si_2^+$ $(M + H)^+$ calcd 538.3245, found 538.3240.

Preparation of ***23***. $Et_3N{\bullet}3HF$ (122 mg, 0.76 mmol) was slowly added to bis-silylated **23** (40.5 mg, 0.076 mmol) in THF (1 mL). The reaction was stirred at 25 °C overnight. The reaction was concentrated under vacuum and purified by flash chromatography on a silica

DOI: 10.1021/acs.joc.7b00093
J. Org. Chem. XXXX, XXX, XXX−XXX

column. Elution with 5% MeOH in DCM gave **23** as a yellow foam (19 mg, 80%). ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.06 (s, 1H), 7.64 (s, 1H), 6.34 (s, 1H), 4.61−4.41 (m, 1H), 4.27 (hept, J = 6.1 Hz, 1H), 4.04 (d, J = 2.8 Hz, 1H), 3.81 (dd, J = 12.2, 3.1 Hz, 1H), 3.72 (dd, J = 12.2, 3.6 Hz, 1H), 2.72 (s, 1H), 2.39 (ddd, J = 13.4, 6.0, 2.8 Hz, 1H), 1.29 (d, J = 6.2 Hz, 6H). ^{13}C NMR (101 MHz, MeOH-d_4) δ 153.6, 145.5, 142.6, 138.6, 121.1, 89.73, 86.8, 76.1, 73.6, 63.6, 41.9, 21.9. IR (KBr plate) 3274 (broad), 2973, 2924, 1659, 1589, 1416, 1393, 1327, 1217, 1095, 1058, 967, 942, 893, 753, 653 cm^{-1}. HRMS (ESI-TOF) $C_{13}H_{20}N_5O_4^+$ $(M + H)^+$ calcd 310.1515, found 310.1513.

Photolysis of Precursors and Subsequent HPLC Analysis. Photolyses were carried out in Pyrex tubes using a Rayonet photochemical reactor (Southern New England Ultraviolet) equipped with a merry-go-round apparatus and 16 lamps having a maximum output at 350 nm. Reaction mixtures (50 μL each) containing precursor (100 μM), thymidine (100 μM), and $Fe(NH_4)_2(SO_4)_2$ (10 mM) in buffer (10 mM phosphate, pH 7.2) were photolyzed at room temperature under aerobic or anaerobic conditions. Samples for anaerobic reactions were degassed by three freeze−pump−thaw cycles at 2 mTorr and flame-sealed under vacuum. The reaction mixtures (including unphotolyzed controls) were analyzed by reversed-phase HPLC while being monitored at 260 nm. HPLC was performed on an Phenomenex Luna C-18 column (250 mm × 4.6 mm) using water and acetonitrile as eluents (1 mL·min^{-1}) from t = 0 to 1 min holding 3% ACN, from t = 1 to 10 min, from 3% to 28% ACN linearly, and then from 28% to 97% ACN linearly over 5 min. Peaks corresponding to deoxyinosine, thymidine, deoxyadenosine, **22**, **23**, and **7b** eluted at 5.4, 7.5, 9.8, 6.2, 12.3, and 15.4 min, respectively. The peaks were integrated and quantified against the internal standard thymidine.

Determination of the Quantum Yield for Photoconversion of 7b in Acetonitrile under Continuous Photolysis. The light flux of the photoreactor was determined by using 2-hydroxy-2-methylpropiophenone actinometry in acetonitrile. 2-Hydroxy-2-methylpropiophenone has a 0.38 quantum yield for Norrish type I cleavage.[61] Solutions containing the actinometer (50 μL, 320 mM) were degassed using three freeze−pump−thaw cycles and sealed under high vacuum prior to photolysis. The extent of reaction of the actinometer was measured by GC after 30 min of photolysis using dodecane as an internal standard. The conversion of the actinometer was 30.0 ± 0.7%. The optical density at 350 nm (OD_{350}) changed from 3.2 to 2.4. Therefore, the change of transmittance was negligible during photolysis. Using eq 1, light flux was calculated to be (4.2 ± 0.1) × 10^{-7} Einstein·min^{-1}. Solutions containing **7b** (50 μL, 100 μM) had an OD_{350} at 0.016. The samples were degassed and photolyzed for 60 min using the calibrated photoreactor. The extent of reaction of **7b** was determined by HPLC using thymidine as an internal standard. The conversion of **7b** was 30 ± 1%. The quantum yield for conversion of precursor **7b** was calculated using eq 2 and determined to be (1.5 ± 0.1) × 10^{-3}.

Nanosecond Transient Absorption. Experiments were performed on an LFP-112 spectrometer (Luzchem, Canada) employing an EX10 (GAM Laser, USA) XeCl Excimer laser (308 nm, ca. 12 mJ/pulse, ca. 12 ns pulse width) for excitation. The transient absorption spectra were recorded in acetonitrile or buffer mixtures (phosphate 10 mM, pH 7.4) in a quartz cuvette (1 × 1 cm^2) equipped with a septum under a nitrogen atmosphere (bubbled for 10 min before measurement).

Quantum Chemical Calculations. Calculations of thermodynamic and kinetic data were carried out using the CBS-QB3 complete basis set method as it is implemented in the Gaussian 09 software.[62,63] UV absorption were modeled using TD-B3LYP with the basis set 6-311++G(d,p) based on geometries optimized with the same methodology.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.7b00093.

NMR spectra of new compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: dpratt@uottawa.ca.

*E-mail: mgreenberg@jhu.edu.

ORCID

Markus Griesser: 0000-0002-7810-1560

Derek A. Pratt: 0000-0002-7305-745X

Marc M. Greenberg: 0000-0002-5786-6118

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful for support of this research from the National Institute of General Medical Science (GM-054996) and the Natural Sciences and Engineering Research Council of Canada.

■ REFERENCES

(1) Harris, M. A.; Mishra, A. K.; Young, R. M.; Brown, K. E.; Wasielewski, M. R.; Lewis, F. D. *J. Am. Chem. Soc.* **2016**, *138*, 5491−5494.

(2) Joseph, J.; Schuster, G. B. *Chem. Commun.* **2010**, *46*, 7872−7878.

(3) Conron, S. M. M.; Thazhathveetil, A. K.; Wasielewski, M. R.; Burin, A. L.; Lewis, F. D. *J. Am. Chem. Soc.* **2010**, *132*, 14388−14390.

(4) Genereux, J. C.; Barton, J. K. *Chem. Rev.* **2010**, *110*, 1642−1662.

(5) Renaud, N.; Berlin, Y. A.; Lewis, F. D.; Ratner, M. A. *J. Am. Chem. Soc.* **2013**, *135*, 3953−3963.

(6) Kanvah, S.; Joseph, J.; Schuster, G. B.; Barnett, R. N.; Cleveland, C. L.; Landman, U. *Acc. Chem. Res.* **2010**, *43*, 280−287.

(7) Al-Sheikhly, M. *Radiat. Phys. Chem.* **1994**, *44*, 297−301.

(8) Steenken, S. *Chem. Rev.* **1989**, *89*, 503−520.

(9) Bamatraf, M. M. M.; O'Neill, P.; Rao, B. S. M. *J. Phys. Chem. B* **2000**, *104*, 636−642.

(10) Hawkins, C. L.; Davies, M. J. *Chem. Res. Toxicol.* **2002**, *15*, 83−92.

(11) Greenberg, M. M. *Adv. Phys. Org. Chem.* **2016**, *50*, 119−202.

(12) Steenken, S.; Jovanovic, S. V. *J. Am. Chem. Soc.* **1997**, *119*, 617−618.

(13) Candeias, L. P.; Steenken, S. *J. Am. Chem. Soc.* **1993**, *115*, 2437−2440.

(14) Close, D. M. *J. Phys. Chem. A* **2013**, *117*, 473−480.

(15) Adhikary, A.; Kumar, A.; Khanduri, D.; Sevilla, M. D. *J. Am. Chem. Soc.* **2008**, *130*, 10282−10292.

(16) Kobayashi, K. *J. Phys. Chem. B* **2010**, *114*, 5600−5604.

(17) Xiang, L.; Palma, J. L.; Bruot, C.; Mujica, V.; Ratner, M. A.; Tao, N. *Nat. Chem.* **2015**, *7*, 221−226.

(18) Shao, F.; O'Neill, M. A.; Barton, J. K. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 17914−17919.

(19) Barnett, R. N.; Joseph, J.; Landman, U.; Schuster, G. B. *J. Am. Chem. Soc.* **2013**, *135*, 3904−3914.

(20) Ghosh, A.; Joy, A.; Schuster, G. B.; Douki, T.; Cadet, J. *Org. Biomol. Chem.* **2008**, *6*, 916−928.

(21) Ito, T.; Kuno, S.; Uchida, T.; Fujita, S.-i.; Nishimoto, S.-i. *J. Phys. Chem. B* **2009**, *113*, 389−394.

(22) Kuttappan-Nair, V.; Samson-Thibault, F.; Wagner, J. R. *Chem. Res. Toxicol.* **2010**, *23*, 48−54.

(23) Kaloudis, P.; Paris, C.; Vrantza, D.; Encinas, S.; Perez-Ruiz, R.; Miranda, M. A.; Gimisis, T. *Org. Biomol. Chem.* **2009**, *7*, 4965−4972.

(24) Vrantza, D.; Kaloudis, P.; Leondiadis, L.; Gimisis, T.; Vougioukalakis, G. C.; Orfanopoulos, M.; Gasparutto, D.; Cadet, J.; Encinas, S.; Paris, C.; Miranda, M. A. *Helv. Chim. Acta* **2006**, *89*, 2371−2386.

(25) Caruthers, M. H. *Acc. Chem. Res.* **1991**, *24*, 278−284.

(26) Chen, J.-R.; Hu, X.-Q.; Lu, L.-Q.; Xiao, W.-J. *Chem. Soc. Rev.* **2016**, *45*, 2044−2056.

(27) Prier, C. K.; Rankic, D. A.; MacMillan, D. W. C. *Chem. Rev.* **2013**, *113*, 5322−5363.

DOI: 10.1021/acs.joc.7b00093
J. Org. Chem. XXXX, XXX, XXX−XXX

(28) Meggers, E.; Michel-Beyerle, M. E.; Giese, B. *J. Am. Chem. Soc.* **1998**, *120*, 12950−12955.
(29) Meggers, E.; Kusch, D.; Spichty, M.; Wille, U.; Giese, B. *Angew. Chem., Int. Ed.* **1998**, *37*, 460−462.
(30) Shaik, R.; Ellis, M. W.; Starr, M. J.; Amato, N. J.; Bryant-Friedrich, A. C. *ChemBioChem* **2015**, *16*, 2379−2384.
(31) Paul, R.; Greenberg, M. M. *J. Am. Chem. Soc.* **2015**, *137*, 596−599.
(32) Jacobs, A. C.; Resendiz, M. J. E.; Greenberg, M. M. *J. Am. Chem. Soc.* **2011**, *133*, 5152−5159.
(33) San Pedro, J. M. N.; Greenberg, M. M. *J. Am. Chem. Soc.* **2014**, *136*, 3928−3936.
(34) Taverna Porro, M. L.; Greenberg, M. M. *J. Am. Chem. Soc.* **2013**, *135*, 16368−16371.
(35) Maxwell, B. J.; Tsanaktsidis, J. *J. Am. Chem. Soc.* **1996**, *118*, 4276−4283.
(36) Newcomb, M.; Musa, O. M.; Martinez, F. N.; Horner, J. H. *J. Am. Chem. Soc.* **1997**, *119*, 4569−4577.
(37) Maxwell, B. J.; Smith, B. J.; Tsanaktsidis, J. *J. Chem. Soc. Perkin Trans. 2* **2000**, 425−431.
(38) Van Speybroeck, V.; De Kimpe, N.; Waroquier, M. *J. Org. Chem.* **2005**, *70*, 3674−3681.
(39) Bae, S.; Lakshman, M. K. *J. Am. Chem. Soc.* **2007**, *129*, 782−789.
(40) Watanabe, N.; Sano, Y.; Suzuki, H.; Tanimura, M.; Ijuin, H. K.; Matsumoto, M. *J. Org. Chem.* **2010**, *75*, 5920−5926.
(41) The free reaction energy for the *β*-fragmentation of the C-based **8a** has been calculated to be endergonic at 8.5 kcal/mol, while the O-based **8b** is exergonic at −47.1 kcal/mol (CBS-QB3).
(42) Wu, M.; Begley, T. P. *Org. Lett.* **2000**, *2*, 1345−1348.
(43) Foo, K.; Sella, E.; Thomé, I.; Eastgate, M. D.; Baran, P. S. *J. Am. Chem. Soc.* **2014**, *136*, 5279−5282.
(44) Boivin, J.; Fouquet, E.; Schiano, A.-M.; Zard, S. Z. *Tetrahedron* **1994**, *50*, 1769−1776.
(45) Chen, J.; Guo, W.; Wang, Z.; Hu, L.; Chen, F.; Xia, Y. *J. Org. Chem.* **2016**, *81*, 5504−5512.
(46) Iida, A.; Nakazawa, S.; Okabayashi, T.; Horii, A.; Misaki, T.; Tanabe, Y. *Org. Lett.* **2006**, *8*, 5215−5218.
(47) Stolarski, R.; Kierdaszuk, B.; Hagberg, C. E.; Shugar, D. *Biochemistry* **1984**, *23*, 2906−2913.
(48) The simplified structures were used for energy calculations (CBS-QB3). For the full geometries used to calculate absorption spectra (gas phase) see the Supporting Information.
(49) We were unsuccessful in determining the kinetics in the presence of oxygen with our LFP. The signal may be suppressed because of oxygen quenching the triplet of acetone and **7b**.
(50) Porter, G.; Dogra, S. K.; Loutfy, R. O.; Sugamori, S. E.; Yip, R. W. *J. Chem. Soc., Faraday Trans. 1* **1973**, *69*, 1462−1474.
(51) Bhattacharjee, S.; Deterding, L. J.; Chatterjee, S.; Jiang, J.; Ehrenshaft, M.; Lardinois, O.; Ramirez, D. C.; Tomer, K. B.; Mason, R. P. *Free Radical Biol. Med.* **2011**, *50*, 1536−1545.
(52) Wagner, J. R.; Decarroz, C.; Berger, M.; Cadet, J. *J. Am. Chem. Soc.* **1999**, *121*, 4101−4110.
(53) Labet, V.; Grand, A.; Cadet, J.; Eriksson, L. A. *ChemPhysChem* **2008**, *9*, 1195−1203.
(54) Rauk, A.; Yu, D.; Armstrong, D. A. *J. Am. Chem. Soc.* **1998**, *120*, 8848−8855.
(55) Zierhut, M.; Roth, W.; Fischer, I. *Phys. Chem. Chem. Phys.* **2004**, *6*, 5178−5183.
(56) Lind, M. C.; Richardson, N. A.; Wheeler, S. E.; Schaefer, H. F., III *J. Phys. Chem. B* **2007**, *111*, 5525−5530.
(57) Musa, O. M.; Horner, J. H.; Shahin, H.; Newcomb, M. *J. Am. Chem. Soc.* **1996**, *118*, 3862−3868.
(58) Horner, J. H.; Musa, O. M.; Bouvier, A.; Newcomb, M. *J. Am. Chem. Soc.* **1998**, *120*, 7738−7748.
(59) Johnson, C. C.; Horner, J. H.; Tronche, C.; Newcomb, M. *J. Am. Chem. Soc.* **1995**, *117*, 1684−1687.
(60) Cappellacci, L.; Petrelli, R.; Franchetti, P.; Vita, P.; Kusumanchi, P.; Kumar, M.; Jayaram, H. N.; Zhou, B.; Yen, Y.; Grifantini, M. *Eur. J. Med. Chem.* **2011**, *46*, 1499−1508.
(61) Scaiano, J. C.; Stamplecoskie, K. G.; Hallett-Tapley, G. L. *Chem. Commun.* **2012**, *48*, 4798−4808.
(62) Montgomery, J. A.; Frisch, M. J.; Ochterski, J. W.; Petersson, G. A. *J. Chem. Phys.* **1999**, *110*, 2822−2827.
(63) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09*; Gaussian, Inc.: Wallingford, CT, 2009.

DOI: 10.1021/acs.joc.7b00093
J. Org. Chem. XXXX, XXX, XXX−XXX