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Debromination of 8-bromo-2'-deoxyguanosine by methylene blue and visible light

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Abstract: Debromination of 8-bromo-2'-deoxyguanosine was accomplished in high yield under neutral conditions in aqueous methanol by irradiating with visible light in the presence of methylene blue as a sensitizer and triethylamine as an electron donor. The method can be extended for the debromination of other bromoaromatic compounds. © 1999 Elsevier Science Ltd. All rights reserved.

Dehalogenation of organohalides is of considerable importance in synthetic organic chemistry, and many approaches involving radical processes have been employed successfully for replacing a halogen atom with a hydrogen. Existing methods include catalytic (e.g., organotin[1] or titanium[2] compounds), electrocatalytic,[3] and photochemical[4] reactions. Although metal-catalyzed replacement of bromo substituents on adenosine nucleotides with deuterium or tritium has been reported,[5] there are few investigations that attempted photochemical dehalogenation of halogenated nucleic acid components. In one isolated study UV irradiation of 5-bromopyrimidine derivatives in methanol generated at least five products.[6] The yield of the debrominated compounds was highly variable and dependent on the structure of the starting material. In each case, however, the yield increased appreciably when diethylamine or triethylamine was added. Nevertheless, the maximum yield remained less than 50%. A mild debromination method of 8-bromo-2'-deoxyguanosine (8-Br-dG) would be important for the synthesis of certain modified 2'-deoxyguanosines (dG) that require protection of the C8 position. This prompted us to investigate the potential for a practical method of debromination of 8-Br-dG. Our success in causing dehalogenation of simple aryl chlorides by a relay mechanism using visible light and a photosensitizer dye suggested that debromination of 8-Br-dG might proceed as smoothly as did the dechlorination of the aromatic chlorides previously examined.[7]

Reductive cleavage of the carbon-halogen bond has been suggested to be an overall irreversible diffusion-controlled two-electron process.[3,8] The first step is a reversible one-electron transfer which generates a radical anion. The latter undergoes a cleavage of the carbon-halogen bond and yields an aryl radical. A second electron transfer (or disproportionation) and subsequent protonation gives rise to the final aromatic product. The conditions for most reductions using organometallic reagents are too harsh for the glycosidic bond of 2'-deoxypurine nucleosides. Photochemical methods with UV light are also undesirable, since UV generates multiple lesions in DNA.[9] We hoped our indirect

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method using visible light would minimize the problems of photochemical lesions that would be more pronounced with UV light. We report a mild high-yielding photochemical method of debromination of 8-Br-dG, which appears to be amenable for debromination of other bromoaromatic compounds.

Debromination of 8-Br-dG to dG occurred with visible light in the presence of a sensitizer, although several-hour irradiation was needed to obtain a reasonable yield of dG. The progress of the reaction could be easily monitored by reverse-phase HPLC,[10] which not only showed relative amounts of the starting material, 8-Br-dG, and the product, dG, but also provided the percentages of degradation products, if any. Some reports on cleavage of carbon-halogen bond indicate that in aprotic solvents the process could involve competitive reactions.[11] Several studies have also demonstrated that photo-induced dehalogenations are accelerated by the addition of triethylamine.[12] In preliminary investigations, therefore, we attempted debromination of 8-Br-dG in aqueous methanol with 0.01 M triethylamine by irradiating with visible light in the presence of one or a combination of the dyes, which included methylene blue, eosin B, methylene green, rose bengal, riboflavin and dinitromethylene blue. Since methylene blue (MB) gave the best results, all subsequent experiments were performed with this dye. Increasing the concentration of triethylamine up to 0.1 M increased the yield of dG,which remained approximately the same at 0.2 M. After screening a variety of conditions, we achieved 89% yield of dG in 9 h with 7% 8-Br-dG remaining, and further irradiation only generated additional side products without improving the yield of the desired compound.

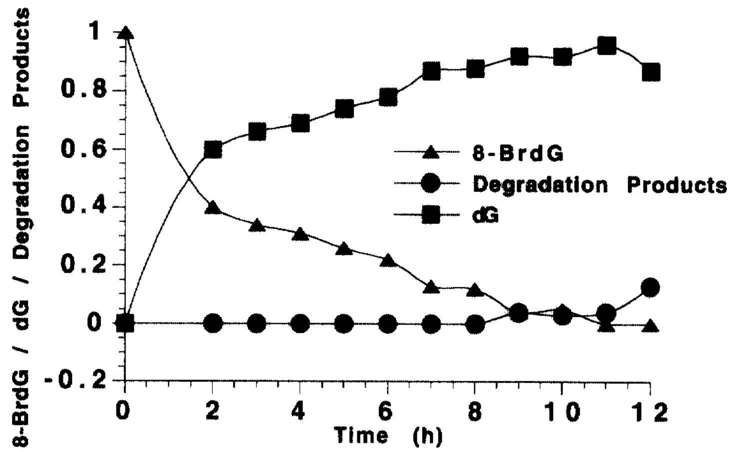


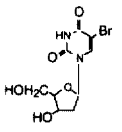
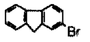
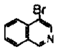
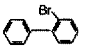
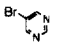
Figure 1. Time course of formation of dG and degradation products, and the mole fractions of 8-Br-dG remaining following irradiation in the cold room.

A higher yield was obtained, however, by carrying out the reaction in a cold room (at 4°C), when the yield of dG increased to 96% with the formation of only 4% degradation products[13] (Figure 1). This suggests that some of the side products were formed due to the elevated temperature of the reaction mixture. The yield of dG remained the same with increasing concentration of 8-Br-dG up to a point when the solution was nearly saturated with it. The use of methanol was crucial for this reaction, because when methanol was replaced with acetonitrile the yield of dG dropped dramatically with extensive degradation of both 8-Br-dG and dG. It is noteworthy that irradiation in the absence of MB led to no debromination, while irradiation with MB but in the absence of triethylamine resulted in extensive degradation of the starting material.

Can this debromination reaction be applied to other bromoaromatic compounds? To explore this question, we attempted debromination of a series of compounds under the same set of conditions used earlier. As shown in Table 1, most compounds gave acceptable yields suggesting that this approach can be extended to other bromoaromatic compounds. However, the method was not applicable to 2-bromoadamantane in which bromine was attached to a tetrahedral carbon.

Table 1

Photosensitized debromination using visible light

Starting Material	% Yield	Time
 5-Bromo-2'-deoxyuridine	96	12 h
 1-Bromopyrene	98	15 min
 2-Bromofluorene	91	17.5 h
 4-Bromoisoquinoline	67	24 h
 2-Bromobiphenyl	77	12 h
 5-Bromopyrimidine	52	24 h
 2-Bromoadamantane	<1	18 h

Because of these generally encouraging synthetic results, some thought was given to the possible mechanism of reaction. It appears that the initial role of triethylamine is to interact via a charge transfer process with MB*. Fluorescence of MB is readily quenched by triethylamine; 20% of the MB fluorescence was quenched with only 10^{-5} M triethylamine, with linear Stern Volmer kinetics observed (kSV=2.1 x 10^{4} M^{-1}; r^2=0.98). This interaction would yield the MB radical anion whenever the ion pairs initially formed diffuse apart. To probe the mechanistic role of the solvent, an examination of debromination of 1-bromopyrene in CH_3OD was undertaken. The strategy of Bunce,[4b] when he probed the triethylamine-promoted photodechlorination of aryl chlorides, was that radical intermediates would remove methyl hydrogens from CH_3OD whereas an anion would remove the more acidic deuterium atom. Like Bunce's results in his dechlorination study,[4b] we found the debrominated pyrene did not incorporate any deuterium. This is consistent with a mechanism that progresses from an electron transfer to the bromo compound from the MB radical anion, followed by breakdown of the aryl bromide to give an aryl radical and a bromide ion. Some evidence for the role of MB as a chemical catalyst (and the involvement of radicals and radical ion intermediates) rather than merely as an energy transfer agent is inferred by its partial bleaching during photodebromination. The amount of bleaching varied somewhat with solvent composition and the substrate, but generally a loss of about half of the MB was observed.

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10. **HPLC**. HPLC separations were performed using a reverse-phase column (Phenomenex Ultramex C-18, 4.6 x 250 mm). The debromination of 8-Br-dG was monitored by using a gradient of 0-12% acetonitrile in water (1 mL/min flow rate). The elution times of dG and 8-Br-dG were 13.0 and 21.0 min, respectively.
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12. Davidson, R.S.; Goodin, J.W. *Tetrahedron Lett.* **1981**, *22*, 163 and references cited therein.
13. **Typical method of irradiation**. Methylene blue (0.376 mg) was added to a solution of 8-Br-dG (80 mg) dissolved in 1 mL methanol:water (20:80). The mixture was transferred to a 1 mL quartz cuvette and purged with nitrogen for 5 min. Et3N (1.4 mL) was added and the solution was purged with nitrogen for an additional 5 min. Subsequently, the cuvette was stoppered and irradiated with two 90 W halogen lamps (placed10 inches apart with the cuvette in the middle).

   Purification. Methylene blue was removed from the reaction mixture by using an ion exchange column. A Bakerbond spe column (inner diameter 0.5 inch) was dry packed 1.5 inches with Bakerbond carboxylic acid ion exchange resin (40 µm with a pore size of 60 A°). The reaction mixture was loaded onto the column and eluted with water until the nucleosides eluted (5-7 mL). Subsequently, the dye was eluted with ~ 5 M NaCl, and the column was ready to be reused.