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BODIPY-modified 2′-deoxyguanosine as a novel tool to detect DNA damages

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ABSTRACT

BODIPY-modified 2′-deoxyguanosine was synthesized for use as a detection reagent for genotoxic compounds. BODIPY-FL is a well known fluorescence reagent whose fluorescent light emission diminishes near a guanine base by a photo-induced electron transfer process. We attached BODIPY-Fl to the 5′ position of the deoxyribose moiety of 2′-deoxyguanosine. Although this compound has low fluorescence activity, when depurination by the action of alkylating reagents and dG oxidation by singlet oxygen occurred, the emission of strong fluorescence was observed. BODIPY-dG was found, therefore, to be a very useful tool for selectively detecting DNA damaging activity particularly in natural environmental extracts.

Modifications of DNA with exogenous and/or endogenous sources are now believed to be the initial step of carcinogenesis.[1–3] Such modifications would trigger DNA mutations, which, in some cases, could also be linked to several chronic diseases. Numerous DNA modifications have now been identified. These include strand breaks, the covalent modification of nucleobases with chemicals, oxidative damage, and the depurination of bases.[4] Guanine bases in DNA are also known to be the most reactive sites to genotoxic compounds.[4] The oxidation of 2′-deoxyguanosine (dG) by X-rays or activated oxygen produces several oxidative products, including 8-oxo-dG, 4,8-dihydro-4-hydroxy-8-oxo-2′-deoxyguanosine **1**, and imidazolone derivatives (Fig. 1).[5] Lipid peroxidation products can also attack dG to form etheno-dG derivatives.[4] Amino/nitro-arenes, carcinogens in foods and the atmosphere, react at the C8 position of dG in major amounts.[6,7] PAH, when metabolically activated, can react with the N^2 amino group of dG.[8] Alkylating reagents can react with *N*7 of dG to form *N*7 alkylating compound **2**, which undergoes a facile depurination reaction, releasing *N*7-alkylated guanine base (Fig. 1).[4]

To date, these DNA modifications have generally been identified by biological methods using an Ames *salmonella* reverse mutation assay to determine the mutagenicity of a compound and a *salmonella* UMU test to determine its genotoxicity. In an alternative method, 4-nitrobenzylpyridine can be used to detect DNA alkylating reagents.[9,10] Electrophilic compounds can attack the N atom of the pyridine moiety. The resulting N-alkyl compound then turns blue under alkali conditions with the loss of a benzylic proton.[9,10] The 4-NBP method can be widely used to detect alkylating compounds, including TLC analysis, and for QSAR studies to predict the mutagenic activities of compounds. Although these biological and chemical methods for detecting genotoxic compounds are all useful and widely used, they also have some drawbacks such as the need for special equipment and/or low sensitivities to active compounds. The objective of our study was to synthesis compounds that could detect mutagenic compounds in a facile and rapid manner. To enable the detection of mutagens, we focused on 4,4-difluoro-4-bora-3*a*,4*a*-diaza-*s*-indacene (BODIPY®) dyes, which are generally known to be high fluorescence compounds and are used in biological research.[11,12]

BODIPY® is a strong fluorescence dye used in the biological field. This dye is also known to have its fluorescence quenched by proximity to a guanine base through a photo-induced electron transfer mechanism.[13] When an oligonucleotide was modified with a BODIPY® derivative, the fluorescence intensity of the BODIPY® diminished by up to 95% compared to that of the original compound.[13] This property can be used to detect an oligonucleotide sequence and GTPase activities in cells.[14–16] Given the reactive nature of dG

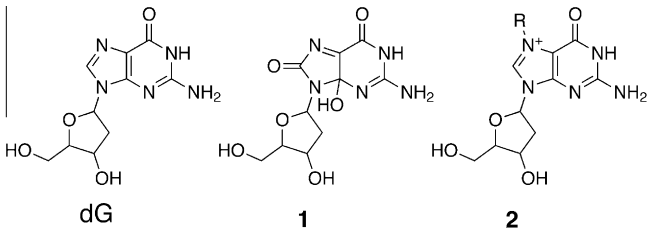

Figure 1. 2′-Deoxyguanosine (dG) and selected DNA damages, 4-OH-8-oxo-2′-deoxyguanosine **1**, and *N*7 alkylation compound **2**.

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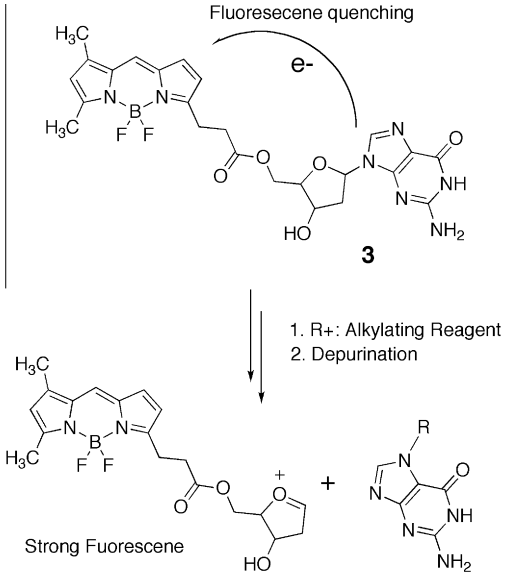

Figure 2. Concept of fluorescence detection of alkylating reagents by BODIPY-modified dG **3**.

to mutagenic compounds, we theorised that by attaching the BODIPY® to dG at the nearest position, like 5′-OH of dG, the expected compound **3** would generally show only weak fluorescence emission. However, once some modifications occur at the guanine bases, the fluorescence of BODIPY® will re-emerge (Fig. 2). This fluorescence recovery can efficiently occur when alkylating compounds attack the BODIPY®-modified dG, after which the depurination of the modified guanine bases occurs.

The target compound **3** that should be synthesized for this purpose has a very simple but compromising chemical structure, where 4,4-difluoro-5,7-dimethyl-4-bora-3*a*,4*a*-diaza-*s*-indacene-3-propionic acid (known as BODIPY®-FL) is attached to the 5′ position of dG. The synthesis of BODIPY®-FL was performed based on the published paper of Malan et al.[17] Initially, BODIPY®-FL was reacted to dG directly, under the general conditions of dicyclohexylcarbodiimide (DCC) and pyridine with 4-dimethylaminopyridine (4-DMAP), because the 5′-OH of dG can be expected to easily react prior to the hydroxy group at the 3′ position. However, this approach failed and only a crude mixture was obtained, probably because of the instability of BODIPY® under the applied reaction conditions. No direct modification of dG with BODIPY®-FL has yet been reported. We did find one paper that described how a dG adduct modified with a mutagen can be converted into a BODIPY®-FL-modified dG derivative in dichloromethane as a solvent, although both 3′-OH and 5′-OH were reacted with BODIPY®-Fl.[18] This experimental evidence encouraged us to attempt to use another protocol for synthesis. Because dG is insoluble in dichloromethane, it was necessary to protect it with appropriate protective groups. In order to enhance the solubility and protect the reactive sites, except for 5′-OH, 3′-OH was protected by TBDMS, and the N^2 of dG was protected by a phenoxyacetyl (PAC) group, which is shown as compound **4** in Figure 3. This compound was obtained by the sequence of 3′,5′-OH-bisTBDMS protection, N^2 protection with PAC anhydride,[19] followed by the selective desilylation of 5′-OH by TBAF with acetic acid. Compound **4** was further reacted with BODIPY-FL using DCC in dichloromethane with 4-DMAP. Protective groups were further de-protected with diisopropylamine in methanol and a trihydrogen fluoride–triethylamine complex in THF. After the solvent was evaporated, the compound was resuspended in acetonitrile, and the obtained precipitate was washed thoroughly, giving the desired BODIPY-modified dG **3**.[20]

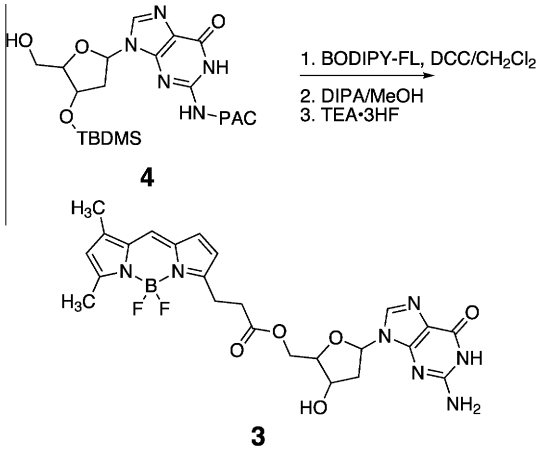

Figure 3. Synthetic procedure for BODIPY-dG **3**.

The compound had a weak fluorescence nature and its emission maximum was 524 nm, which was slightly red shifted compared to that of BODIPY-FL (513 nm). In order to check our hypothesis, representative alkylating compounds, methyl methanesulfonate **5** and epichlorohydrin **6**, were added to the solution containing the BODIPY-dG derivative **3** under the slightly acidic condition of pH 5 (Fig. 4).[21] After 12 h, the solutions with the added alkylating reagents were observed to show strong green fluorescence as expected (Fig. 5, the visualization of this fluorescence enhancement with alkylating reagents is shown in the Supplementary data, Fig. 1). The green fluorescence of the solutions could be easily detected with the naked eye under a fluorescent light or a black light. The fluorescence spectra produced by the addition of the two alkylating compounds were totally equal, and there was a quantitative recovery of the fluorescence.

Alkylating reagents are generally known to attack *N*7 of dG, with the resulting *N*7-modified dG **2** being readily depurinated by a glycosidic bond cleavage. In the case of methyl methanesulfonate, the methylation of *N*7 generally occurs. The modification of the *N*7 of dG is relatively stable and the resulting methylated compound can be present for several hours under an aqueous condition. We found that 90% of starting material **3** was consumed after 4 h when it reacted with **5**. The resulting methylated adduct was depurinated within several hours at pH5 which is in accordance with the half-life of 4.4 h at pH 4.2 for *N*7 alkylated deoxyguanosine.[22] This fact well explains our experimental finding that a reaction time of 12 h was needed to enhance the fluorescence activity. We have also confirmed the final product was a ribofuranose attached with BODIPY-FL at the 5′ position and the same product had been efficiently obtained from acid treatment of BODIPY-dG (Fig. 4, Supplementary data).

In order to further elucidate the usefulness of BODIPY-dG **3**, we next attempted to check whether oxidative damages to dG could

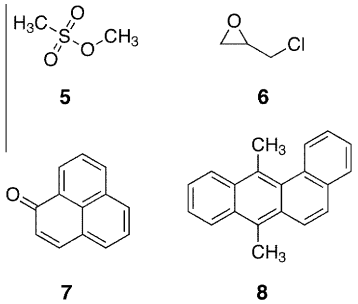

Figure 4. DNA damaging reagents tested in this study.

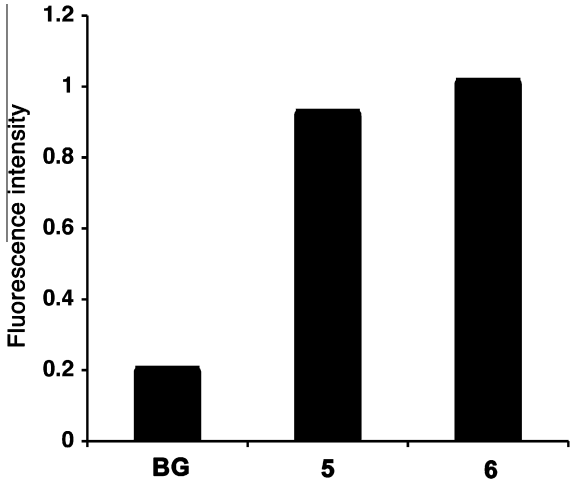

Figure 5. Fluorescence intensity of BODIPY-dG treated with alkylating reagents, **5** and **6**.

be detected in the same system. Phenalenone **7** is a known concomitant present in the atmosphere and is also known to be a singlet oxygen producer under black light irradiation (Fig. 4). Singlet oxygen, once produced, can attack dG to form a highly oxidized product, 4-OH-8-oxo-dG **1,** with a yield of around 40%.[22,23] Because the electronic properties of the oxidized compound **1** are different from those of dG, the fluorescence quenching by the electron transfer pathway observed in the original compound **3** will not be observed when the oxidation of dG occurs. Indeed, BODIPY-dG **3** with phenalenone **7** under UV light conditions had strong, dose-dependent fluorescence emission (Fig. 6).[24] Moreover, DMBA **8** can also recover the fluorescence of BODIPY-dG, probably because of the oxidative damage to the dG moiety of compound **3** (Fig. 4, Supplementary Fig. 2). Koutaka et al. showed that the on/off fluorescence of the fluorophore can be explained by the HOMO level of the donor (dG) and acceptor (BODIPY) compounds.[25] A semi-empirical chemical calculation showed that the HOMO energies of BODIPY, dG, and **1** were −8.881, −8.811, and −9.985 eV, respectively. [26] The lower HOMO value of **1** compared to that of BODIPY could explain why the highly oxidized dG moiety in compound **3** reduced its quenching property.

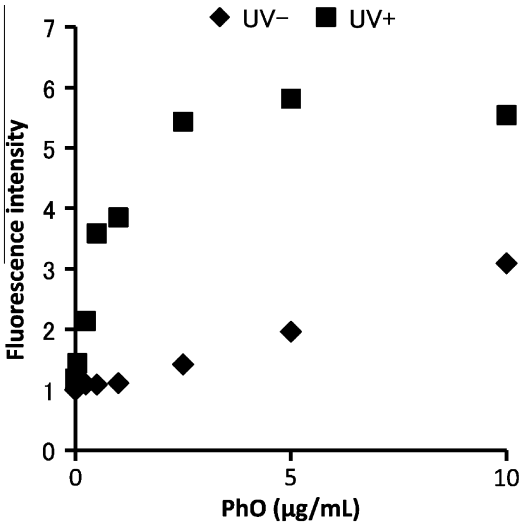

Figure 6. Fluorescence intensity of BODIPY-dG **3** treated with compound **7**, phenalenone. Phenalenone is abbreviated as PhO.

In conclusion, a simple modification of dG with BODIPY-FL could be used as a mutagen detector for oxidative DNA damages and alkylating reagents. It would be possible to use reagent **3** in an in vivo system such as for cell lines or in a test paper application to test for mutagens in the environment similar to pH test paper. Evaluation of these approaches of compound **3** and the detailed mechanism of its on/off fluorescence are now being studied in our laboratory.

Acknowledgments

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Supplementary data

Supplementary data (the visualization of the fluorescence of BODIPY-dG with alkylating reagents, fluorescence recovery of BODIPY-dG with DMBA under UVA light condition, ^{1}H- and ^{13}C-NMR of compound **3**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.05.084.

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20. *Preparation of the compound* ***3***: Into the solution of N^2-phenoxyacetyl-3′-TBDMS-2′-deoxyguanosine (0.2 mmol) and BODIPY-FL (0.4 mmol) in dichloromethane was added DCC (2 equiv) with DMAP (2 equiv). After the reaction mixture was left overnight, it was filtered and filtrate was evaporated to dryness. Residual material was subjected to a column chromatography on silica gel to give BODIPY-modified protected dG, which were further subjected to diisopropylamine in methanol for 2 h. After purified by column chromatography, resulting compounds were treated with triethylamine trihydrogen fluoride complex in THF. After 24 h, solvent was evaporated and the residual material was suspended in acetonitrile. Resulting precipitation was obtained by centrifugation and washed twice with acetonitrile to give the desired 5′-BODIPY-FL-2′-deoxyguanosine **3**. ^{1}H NMR (400 MHz, DMSO-d_6)™ = 10.50 (br s, 1H), 7.75 (s, 1H), 7.57(s, 1H), 6.93 (d, J = 4.1 Hz, 1H) 6.34 (br s, 1H), 6.23 (d, J = 3.7 Hz, 1H), 6.18(s, 1H) 6.01 (t, J = 6.9 Hz, 1H), 5.32 (br s, 1H), 4.25 (br s, 1H), 4.10 (ddd, J = 42.2, 11.8, 5.1 Hz, 2H), 3.85 (br s, 1H), 2.98 (t, J = 7.5 Hz, 2H), 2.67–2.60 (m, 2H), 2.40–2.36 8 (m, 2H), 2.34 (s, 3H), 2.13 (t, J = 7.1 Hz, 3H), ^{13}C NMR (100 MHz, DMSO-d_6)™ = 171.80, 159.77, 156.73, 156.17, 153.69, 150.99, 144.53, 135.24, 134.69, 132.99, 128.72, 125.53, 120.52, 116.78, 116.54, 83.98, 82.41, 70.65, 64.44, 32.15, 23.43, 14.62, 11.07, 10.83,

HR-HR-MS (ESI-TOF): m/z $[M+H]^+$ calcd for $C_{24}H_{27}BF_2N_7O_5$:542.2129; found 542.1989.

21. *Typical procedure for detection of alkylating compounds:* To the buffered solution (pH5) were added 10 μL of BODIPY-dG (1 mg/mL DMSO) followed by alkylating reagents (10 μL of compound **1** and 5 μL of **2**). After 12 h, the sample was diluted and the fluorescence was measured at an excitation of 490 nm. The emission spectra from 495 to 510 nm were recorded.
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24. A 96-well plate version of the fluorescence detection of singlet oxygen generating compounds: to the buffered solution (PBS, pH 7, 100 μL) containing an appropriate amounts of phenalenone **7** or DMBA **8** was added 100 μL of the BODIPY-dG solution (1 mg/mL in DMSO). After UVA irradiation for 20 min, the reaction mixture was diluted by a factor of 1000 and em 485 nm/ex 535 nm fluorescence was recorded by a fluorescence plate reader.
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26. MOPAC calculation (PM3) was performed by ChemBio 3D 12.0 with MOPAC.