Quantitative Detection of 8-Oxo-7,8-dihydro-2′-deoxyguanosine Using Chemical Tagging and qPCR

John Ernest Vallarta Bajacan,† In Seok Hong,‡,§ Trevor W. Penning,‡ and Marc M. Greenberg†,‡

†Department of Chemistry, Johns Hopkins University, 3400 N. Charles Street, Baltimore, Maryland 21218, United States
‡Center of Excellence in Environmental Toxicology, Department of Pharmacology, Perelman School of Medicine, University of Pennsylvania, 421 Curie Boulevard, Philadelphia, Pennsylvania 19104-6061, United States

ABSTRACT: 8-Oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) is a commonly formed DNA lesion that is useful as a biomarker for oxidative stress. Although methods for selective quantification of 8-oxodGuo exist, there is room for additional methods that are sensitive and utilize instrumentation that is widely available. We previously took advantage of the reported reactivity of 8-oxodGuo to develop a method for detecting the lesion by selectively covalently tagging it with a molecule equipped with a biotin label that can be used subsequently with a reporting method (Xue, L., and Greenberg, M. M. (2007) J. Am. Chem. Soc. 129, 7010). We now report a method that can detect as little as 14 amol of 8-oxodGuo by tagging DNA with a reagent containing a disulfide that reduces background due to nonspecific binding. The reagent also contains biotin that enables capturing target DNA on streptavidin-coated magnetic beads. The captured DNA is quantified using quantitative PCR. The method is validated by comparing the amount of 8-oxodGuo detected as a function of Fe²⁺/H₂O₂/ascorbate-dose to that reported previously using mass spectrometry.

INTRODUCTION

DNA lesions can be mutagenic and have been implicated in a variety of diseases, most notably cancer, as well as aging.¹–⁶ Quantifying nucleic acid damage is a valuable exercise as modified nucleosides, nucleobases, and sugar fragments are potential biomarkers.⁷,⁸ 8-Oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) is a well-studied, mutagenic lesion that is used as a biomarker.⁹,¹⁰ This lesion is produced from the most readily oxidized of native nucleotides, dG, by a variety of damaging agents.¹¹,¹² 8-OxodGuo is more readily oxidized than dG and serves as the precursor to a number of highly mutagenic lesions that are of increasing interest to chemical toxicologists and other scientists.¹³–¹⁶ Consequently, there is significant interest in methods for its quantitation in DNA. Herein, we describe a method for detecting 8-oxodGuo that relies upon its tagging by a reagent and subsequent signal detection using quantitative PCR (qPCR).

Mass spectrometry is a sensitive and selective method for detecting a large number of DNA lesions.¹⁷–²¹ The variety of lesions detectable is expanded when the method is coupled with chemical derivatization techniques.²²,²³ Quantification is greatly facilitated by spiking samples with known quantities of isotopically labeled lesions that require costly independent synthesis. In addition, mass spectrometers are increasingly powerful, and DNA lesion detection methods employing them are proportionally more sophisticated, but the instruments are also expensive.²⁴ The comet assay is a less costly method that is particularly useful for detecting lesions in cellular DNA, but its specificity is limited by the selectivity of chemical reagents and enzymes that cleave DNA.²⁵–²⁸ Sophisticated methods that take advantage of selective DNA lesion tagging and enable the detection of the location of individual lesions in single strands of DNA are on the horizon.²⁹,³⁰ However, these methods are not yet routine.

Reagents that also take advantage of distinctive lesion reactivity but utilize more conventional reporting methods are more common.³¹,³² Aldehyde reactive probe(s) equipped with fluorophores or biotin have proven useful for quantifying abasic sites and are still being developed.³³–³⁵ Turn on sensors that exploit the formation of a fluorophore upon reaction with a functional group within a lesion have also been developed.³⁶ Molecular recognition is also a useful tool for lesion detection.³⁷ Recently, molecules that selectively recognize 8-oxodGuo and incorporate fluorescence reporting have been developed.³⁸,³⁹ There are also efforts reported that utilize aptamers to detect this damaged nucleoside or its respective free base (8-oxoGua).⁴⁰,⁴¹ It is well known that mild oxidation of 8-oxoGua produces the guanidinohydantoin (Gh) and spiropiminohydantoin (Sp)
lesions that are interesting in their own right.\(^{14,16,42,43}\) However, oxidation of 8-oxodGuo or Fapy\(\cdot\)dG in the presence of an amine nucleophile, such as a spermine (RNH\(_2\)), provides a reaction with 8-oxodGuo or Fapy\(\cdot\)dG to trap the reactive oxidized species.\(^{44,46,47}\) Fapy\(\cdot\)dG and 8-oxodGuo were distinguished from one another by using a radiometric assay and an oxidant.

Sensitivity was limited to \(\sim 10\) fmol of 8-oxodGuo. Herein, we have improved the limit of detection to 3 h by combining qPCR with a new biotinylated reagent.

### MATERIALS AND METHODS

#### General Methods.

Taq DNA polymerase, OneTaq HotStart DNA polymerase, and exonuclease I (Exo I) were from New England Biolabs. Oligonucleotides were synthesized via standard automated DNA synthesis on an Applied Biosystems model 394 instrument. Radiolabeling was carried out using standard protocols.\(^{48}\) DNA synthesis reagents, including the phosphoramidite for incorporating 8-oxodGuo were obtained from Glen Research. Radiolabeled oligonucleotides were hybridized with 1.5 equiv of complementary oligonucleotides in 10 mM potassium phosphate (pH 7.2) and 100 mM NaCl at 90 °C for 5 min and cooled to room temperature. Radiolabeled samples were counted using a Beckman Coulter LS 6500 scintillation counter. Solid-phase peptide synthesis was carried out on an Applied Biosystems model 440 peptide synthesizer. PCR primers and probes were purchased from Biosearch Technologies. qPCR was performed on a Biorad iCycler iQ instrument. Magnetic polyethyleneimine beads (SiMag-PEI) were from Chemicon. Microtiter 96-well plates were from Biorad (MLP 9601). The plasmid containing the p53 gene was obtained as previously described.\(^{49}\)

#### Preparation of DNAs by PCR.

A solution (500 \(\mu\)L) of primers (0.2 \(\mu\)M each), template DNA (20 \(\mu\)M), dNTPs (0.4 mM), and OneTaq HotStart DNA polymerase (2.5 \(\times 10^3\) U/L) in 1x OneTaq standard buffer was partitioned into thin-walled PCR tubes (50 \(\mu\)L each). The PCR cycle was performed as follows: (1) 94 °C for 30 s, (2) 94 °C for 30 s, (3) 60 °C for 30 s, (4) 68 °C for 1.5 min, (5) steps 2–4 repeated 45 times, (6) 68 °C for 5 min, and (7) held for 4 °C. The reactions were pooled, incubated with Exo I (4 \(\times 10^3\) U/L, 1 h, 37 °C), and evaporated under vacuum. The DNA was purified from the residue by silica spin column chromatography (Qiagen PCR purification kit) following the manufacturer’s protocol, except that an additional wash with 35% guanidine-\(\text{HCl}\) was performed following adsorption to the spin column. The eluent was flash-frozen and stored at \(\sim 20°\)C. DNA was quantified by TaqMan qPCR versus a UV-determined standard. Purify on a MicroSpin GFX PCR Purification Kit (GE Healthcare) following the manufacturer’s protocol, except that 1 M \(\text{HCl}\) was used instead of 0.1 M \(\text{HCl}\).

The p53 PCR fragment containing 8-oxodGuo was prepared using the forward primer \(5’-d(GCA\ GTC\ AGA\ TCC\ TAG\ CXT\ CGA\ GC)\) and the reverse primer \(5’-d(GGG\ CAG\ TGC\ TCG\ CTG\ TCC\ TGG\ CTA\ GC)\). The p53 PCR fragment containing 8-oxodGuo was prepared using the forward primer \(5’-d(bGGT\ GAT\ GAC\ GGT\ GAA\ AAC\ CTC)\), where \(b\) = biotin linked to the 3’ end of the primer via tetraethylene glycol. The reverse primer was \(5’-d(AGT\ GGT\ GTG\ TGG\ CTT\ AGT\ GC)\).

#### Synthesis of 3.

A chilled acetonitrile solution (81 mL) of Fmoc-NHS ester (18.1 g, 53.7 mmol) was added to a vigorously stirred solution of carboxylated spermine\(^{50}\) (5 g, 12.8 mmol) in 10% Na\(_2\)CO\(_3\) (81 mL) in an ice bath. The mixture was stirred vigorously and allowed to warm to room temperature overnight. A pH of 8–9 was maintained by the addition of DIPEA. Acetonitrile was removed under vacuum, and the residue was acidified with a solution of brine and 0.1 M \(\text{HCl}\) (pH 1, 200 mL). The aqueous solution was extracted with DCM (5 \(\times 200\) mL), and the organic layer was dried over Na\(_2\)SO\(_4\) and filtered. Silica gel was added prior to concentrating under vacuum. The resulting dry powder was applied over a silica gel plug and washed with copious amounts of 1:1 HEX/EA. Removal of the nonpolar species was monitored by TLC. After removing the nonpolar species, 1% formic acid was added to the eluent to elute product-containing fractions. The eluent was concentrated under vacuum and applied to a silica gel column to further purify the product using 1% formic acid in 1:1 HEX/EA. Fractions containing the product were pooled.

---

**Scheme 1**

![Scheme 1](image-url)
concentrated under vacuum, and redissolved in EA for a basic wash (5%, saturated NaHCO₃/brine), followed by an acidic wash (2×, brine 0.1 M HCl, pH 1). The organic layer was concentrated to a sticky white foam (5.4 g, 37% yield) and redissolved in minimal DCM for precipitation into vigorously stirring hexane. The supernatant was removed following centrifugation and the residue lyophilized to a white powder. ¹H NMR (CDCl₃): δ 1.10–1.48 (br, m, 9H), 1.33–1.37 (br, m, 1H), 1.41–1.43 (br, m, 4H), 1.45 (br, s, 4H), 2.14–2.24 (m, 1H), 2.51–2.61 (m, 2H), 2.72–2.79 (br, m, 9H), 2.93–2.95 (m, 1H), 3.05–3.07 (m, 1H), 3.26–3.28 (m, 2H), 3.32–3.34 (m, 1H), 3.52–3.61 (m, 1H), 3.77 (s, 6H), 4.26–4.29 (dd, 1H), 4.43–4.50 (dd, 1H), 5.02 (br, s, 1H), 5.72 (br, s, 1H), 6.49 (bd s, 1H), 6.77 (d, 4H), 7.14–7.15 (m, 4H). ¹³C NMR (CDCl₃): δ 24.9, 25.5, 25.6, 28.1, 28.2, 28.8, 33.2, 33.9, 35.9, 37.6, 39.6, 40.5, 55.6, 61.1, 61.8, 69.5, 69.9, 70.0, 70.1, 70.5, 76.7, 77.0, 77.2, 77.3, 163.8, 173.1. HRMS calc'd for C₁₄H₂₃NO₃S (M + H⁺) 279.1392; found, 279.1396.

Synthesis of 6. A mixture of biotin (770 mg, 3.2 mmol), DCC (710 mg, 3.4 mmol), and HOBt (465 mg, 3.4 mmol) was dried in vacuo for 30 min prior to suspension in 5% TEA/DMF (10 mL) under Ar at 40 °C. After 30 min, the suspension was added to 5 (1.53 g, 2.94 mmol) (predried by azotropically drying from pyridine) in 5% TEA/DMF (10 mL). After 6–8 h, the precipitate was removed by passage through Celite. DMF was evaporated in vacuo and the residue partitioned between brine and DCM. The precipitate was again removed by passage through Celite, and the filtrate was concentrated for column chromatography (5% TEA/DMC to 5% TEA/5% MeOH/DCM), affording 2.2 g (100%) of 6. ¹H NMR (CDCl₃): δ 1.02–1.05 (m, 2H), 1.42–1.76 (m, 8H), 2.16–2.19 (m, 4H), 2.80–2.84 (m, 3H), 3.10–3.15 (m, 1H), 3.32–3.34 (m, 1H), 3.52–3.61 (m, 1H), 3.77 (s, 6H), 4.26–4.29 (dd, 1H), 4.43–4.50 (dd, 1H), 5.02 (br, s, 1H), 5.72 (br, s, 1H), 6.49 (bd s, 1H), 6.77 (d, 4H), 7.14–7.15 (m, 4H). ¹³C NMR (CDCl₃): δ 24.9, 25.5, 25.6, 28.1, 28.2, 28.8, 33.2, 33.9, 35.9, 37.6, 39.6, 40.5, 55.6, 61.1, 61.8, 69.5, 69.9, 70.0, 70.1, 70.5, 76.7, 77.0, 77.2, 77.3, 163.8, 173.1. HRMS calc'd for C₁₄H₂₃NO₃S (M + H⁺) 279.1392; found, 279.1396.

Solid Phase Synthesis of 9 and 11. For the preparation of 11, the arginine(s) and lysine residues were added via automated SPPS from 10 (∼150 mg, 0.45 mmol/g, ∼70 pmol-scale). Fmoc cleavage was performed with 20% piperidine/NMP. All residues were coupled (2 × 30 min) with amino acid (∼0.2 M, 5 equiv), HBTP (∼0.2 M, 5 equiv), and DIPEA (∼0.4 M, 10 equiv) in NMP. The final lysine residue was coupled in its Boc protected form. Capping was performed with acetic anhydride (100%). Resin was washed with DCM, followed by NMP between each step. The remaining portions of the syntheses of 11–13 and all of 9 were carried out manually as follows. The resin was swollen in DCM with Ar bubbling for 5–10 min. The liquid was drained by aspiration and the resin washed with DCM. Alloc group cleavage was performed with Me₂NH (3 eq 24 μmol) and (Ph₃)PΔ (0.05 equiv, 4 mg, 3.4 μmol, 15 min, 3×), where the former was added first to a DCM suspension of resin with Ar bubbling. Following washing as described below, Fmoc-protected aminocaproic acid (3 equiv) was then preactivated with PyBop (3 equiv) and TEA (6 equiv) in DCM (0.1 M) for 1 h with Ar bubbling. A second coupling was repeated with 1.5× of reagents. The Fmoc group was removed by treating the preswollen resin with 20% piperidine/DCM (5 min, 3×). Biotin was then coupled in a similar manner. Unreacted amine was acetylated by treatment with 50% Ac₂O/DCM 3 times for 3, 3, and 7 min, with DCM washing in between. A methanolic solution (10 mL) of [M + H]+: calcd, 501.2; found, 501.2. IR (νmax): 3292 (br), 2927, 1703, 1644, 1553 cm⁻¹. 

Chemical Research in Toxicology
Chemical Research in Toxicology

Synthesis of 14. Manual SPPS from 10 (0.3 g, 135 μmol) was identical to that described above. Detritylation of the cysteine thiol was carried out with 2% TFA/DCM (1–2 mL, 1–2 min) and repeated until the cleavage solution ran clear (10×). After each detritylation cycle, the resin was washed with MeOH and DCM. The cysteine thiol was converted into the disulfide by treating with cystamine-2 HCl (5 equiv, 0.5–1.0 h, 3×) and DIPEA (15 equiv in DMSO (∼5 mL). The resin was washed with DMF, MeOH, DCM, and DCM following each cycle. A positive Kaiser test confirmed disulfide exchange. Manual SPPS was continued as described above. The penultimate compound was cleaved, purified, and characterized as described above. Instead of evaporating the cleavage cocktail, the product was precipitated into cold Et2O. The precipitate was lyophilized, redissolved in water, and filtered prior to HPLC purification. The following gradient (time, % B) was used at 4 mL/min: 0, 0; 5, 0; 90, 16. Ret. time: 74.5 min. MALDI-TOF-MS [M + H]+: calcld, 1414.8; obsd, 1416.1.

General Procedure for Tagging. A solution (5 μL) of DNA analyte (1 fmol), bpUC19 DNA reference (0.1 fmol), probe (0.2 μM each), TaqMan probes (0.1 μM each), NEB OneTaq HotStart DNA polymerase (2.5 μL) and water (50 μL) was added with K3Fe(CN)6 (5 μM, 2 mM) and allowed to stand for 10 min. The reaction was quenched with carrier DNA and detergent (20 μL of cDNA (50 mg/L), DTT (10 mM), and 0.05% Tween-20).

General Procedure for the Removal of Probe by Magnetic PEI-Bead. Samples were diluted further with magnetic polyethyleneimine beads (20 μL, 1 g/L in 0.05% Tween-20) and pelleted on a magnetic PCR plate. The beads were washed with wash buffer (100 μL, 8× with 10 mM DTT, 10 mM guanidine-HCl, and 0.05% Tween-20).

General Procedure for Disulfide Cleavage. PEI-beads were resuspended in fresh cleavage/elution buffer (50 μL: 0.1 M DTT, 0.1 M Tris-HCl, pH 10, and 2 M NaCl), incubated for 5 min, and pelleted magnetically for an additional 5 min.

General Procedure for the Removal of Nonbiotinylated DNA by Magnetic Streptavidin Beads. Magnetic streptavidin beads (Dynabead MyOne T1) were washed with 0.05% Tween-20 (3×). A suspension of the beads (25 μL, 1 g/L) was then incubated with PEI-bead eluent (25 μL). After 30 min, the beads were magnetically pelleted and washed sequentially with 1×, 0.5×, and 0.25× TSTBS (100 μL × 3 at each concentration; 1× TSTBS, 0.05% Tween 20, 40 mM Tris-HCl at pH 9, and 1 M NaCl). Beads were washed a final time with water and resuspended in 50 μL of 0.05% Tween-20.

General Procedure for Multiplex qPCR. Each qPCR solution (50 μL) contained 10% sample (5 μL), 0.2 mM dNTP, primers (0.2 μM each, Table 1), TaqMan probes (0.1 μM each), NEB OneTaq HotStart buffer (1×), and OneTaq HotStart DNA polymerase (2.5 × 10^4 U/L). Each qPCR experiment was prepared with a single calibration series of analyte and reference DNAs (0.25–8.5 logCN), ideally from the same working solution as the experimental samples. The 96-well plate (Biorad MLP 9601) was sealed with optical film (ABI). qPCR was performed on the iCycler iQ (filter set 4: Famm-490 and Texas Red-575) using the following program: (1) 95 °C for 15 s, (2) 66 °C for 1 min with optical measurement, (3) repeated 1–2× 50 s, and (4) held at 25 °C. The following Taqman probes were used: S′-d(FAM-TTG ATG CTG TCC CGG GAC GA-BHQ1) for p53 and S′-d(Cal-Fluor- CTG AGA GTG CAC CAT ATG CCG TGT G-BHQ2) for the pUC19 internal standard.

Amplification plots were analyzed under PCR baseline subtracted curve fit in analysis mode. The threshold position was manually set at the same position in the exponential phases of the two sets of amplification curves (one set for each fluorophore). Cq (cycle at which fluorescence from amplification exceeds the background fluorescence) data were recorded and processed using Microsoft Excel. Calibration curves (Cq vs log CN; CN = copy number) were generated by linear regression for both reference and analyte DNAs. The amount (log CN) of DNA was calculated by interpolation from the calibration curves. The tagging yield (%Yraw) was calculated by

\[
\%Y_{\text{raw}} = \frac{\text{mol tagged p53}}{\text{mol bpUC19}} \times 100\%
\]

The normalized tagging yield (%Ynorm) was obtained by

\[
\%Y_{\text{norm}} = \frac{\%Y_{\text{raw}}}{\text{mol bpUC19}}
\]

where %Yraw was the %Ynorm with 100% p53oG. The amount of 8-oxodGuo detected was calculated by

\[
8\text{-oxodGuo (mol) = } \frac{\%Y_{\text{norm}}}{100} \times (\text{initial mol p53})
\]

Iron/H2O2/Asc Oxidation of p53-PCR. DNA solutions (10 μL, 500 pM p53-G, and 50 pM bpUC19) in TES buffer (20 mM Tris HCl, pH 7.5, 2 mM EDTA, 0.2 M NaCl) in a 96-well titer plate were treated for 50 min at 37 °C with an equal volume of oxidizing agents (FeCl3, H2O2, and ascorbic acid) The oxidizing agents ranged in concentration from 50 μM FeCl3, 400 μM H2O2, and 4 mM ascorbic acid to 1.5 μM FeCl3, 1.25 μM H2O2, and 0.125 mM ascorbic acid and were changed in 2-fold increments. Two control reactions were carried out, one with p53-OG and one with p53-G as analytes. The control reactions contained H2O (10 μL) in place of the oxidizing reagents. The reactions were quenched with quencher Q (10 μL of 50 mg/mL calf thymus DNA and 30 mM L-methionine) and purified by silica spin column chromatography (Qiagen Qiapuck PCR purification kit), resulting in a final solution (50 μL) of DNA buffer in 10 mM Tris HCl at pH 7.5. 8-OxodGuo was quantified from an aliquot of the DNA sample (4 μL) mixed with 14 (1 μL, 0.5 mM), following the general procedure for tagging and qPCR described above.

### RESULTS AND DISCUSSION

We previously developed a method for quantifying 8-oxodGuo in DNA that took advantage of its lower oxidation potential than native nucleotides and many DNA lesions, in combination with nuclease trapping of a reactive oxidation product of the lesion.26 Multiple aspects of the previously reported method for detecting 8-oxodGuo were modified in the current work. The horseradish peroxidase amplification was replaced with quantitative PCR, procedures for removing excess probe were examined, and new probes were synthesized. The overall procedure (Scheme 2) involved tagging, removal of the excess probe, binding tagged DNA to streptavidin-coated magnetic beads, removal of untagged DNA (nonbiotinylated DNA), and finally PCR amplification of the bead bound DNA.

---

**Scheme 2**

![Scheme 2](image-url)
Designing Improved Probes. The ideal probe will selectively form a covalent bond to 8-oxodGuo in a large excess of dG and be readily removed from DNA when it is noncovalently bound. We postulated that incomplete removal of noncovalently bound 1 contributed to the background signal and prevented us from reaching a lower limit of detection. Consequently, we sought to design probes that would provide good yields of tagged 8-oxodGuo but be readily removed.

Triethylene glycol analogue 2 marked a modest first step in improving probe properties. The triethylene glycol group was expected to increase the probe’s water solubility, facilitating its removal from DNA. In addition, the longer linker separating the DNA tagging and reporting domains could enhance probe function. Probe 2 was synthesized by coupling the biotinylated triethylene glycol (7, Scheme 3) with the Fmoc-protected spermine derivative (3). The carboxylated spermine derivative (3) was prepared via a recently improved procedure, whereas 7 was obtained from the commercially available diamine.50,51 Diamine 4 was dimethoxytritylated prior to biotinylation to facilitate purification and handling of the advanced intermediates due to the polar nature of biotin and the lack of a convenient way of visualizing the compounds upon thin layer chromatography analysis. The Fmoc-protected probe was purified by column chromatography and deprotected with cyclohexylamine. Probe 2 was purified from this reaction by extracting it into water and washing away less polar entities via extractions. Following lyophilization, 2 was dried azeotropically and oxidant concentrations. Yields were determined using streptavidin-coated magnetic beads via liquid scintillation counting.

While an amine is necessary to trap the oxidized 8-oxodGuo, we considered the possibility that the polycationic probes (e.g.,

Scheme 3a

![Scheme 3](image)

aKey: (a) DMCl, THF; (b) biotin, DCC, HOBt; (c) HCl, MeOH; (d) EDCi, HOBt, DMF; (e) cyclohexylamine, CH2Cl2.

Using 9 as a prototype, we synthesized a series of related probes (11–13) containing between 1 and 3 arginine residues, followed by a lysine at the amino terminus. It was anticipated that the lysine would serve as the nucleophile. In addition, following Pd(0) cleavage of the alloc group, the liberated ε-amino group was coupled to aminocaproic acid prior to conjugating biotin. This assembly method increased the distance between the tagging and capture (biotin) domains of the probes. Peptide 13, containing 3 arginine residues, possessed the same overall positive charge as 1. The tagging abilities of the arginine probes (11–13) were crudely evaluated with an independently synthesized oligonucleotide containing 5'-32P-8 and liquid scintillation counting, as described above for 2. The probes were tested at 0.1 and 1 mM (Figure 1). Although the tagging efficiency of 11 was ∼3-fold greater at the higher concentration used, a much smaller difference was observed with the more highly positively charged probes (12 and 13). The concentration of K3Fe(CN)6 (1 and 10 mM) also

![Figure 1](image)
had little effect on tagging yield. Thus, the lower concentration was used in subsequent experiments. Overall, the probe containing the same overall charge as 1 (13) provided the highest tagging efficiency.

Finally, to minimize the effect of probe bound noncovalently to target DNA, we synthesized a peptide (14) in which the highly positively charged DNA binding domain was separated from biotin (used to capture tagged DNA) and the tagging domain by a cleavable disulfide linkage. We rationalized that while a positively charged DNA binding domain might assist delivering the tagging agent, it would also hinder removing it after reaction. The cleavable disulfide facilitates removing biotin that is not covalently bound to the DNA and reduces the background. Synthesis was carried out on the Wang resin containing α-Fmoc-ε-allo-lysine (10). A S-monomethoxytrityl protected cysteine, which served as the disulfide precursor was incorporated, followed by the aminocaproic acid spacer and the lysine employed as the tagging component. The biotin group was introduced as described above prior to revealing the cysteine thiol that was elaborated further to the disulfide. The thiol was condensed with cystamine by disulfide exchange, and the resulting primary amine was used to introduce the triarginine DNA binding domain. All of the peptide probes were purified by reverse-phase HPLC following cleavage from the solid phase support and characterized by mass spectrometry (see Supporting Information).

Removing the Excess Probe. Large excesses of probes relative to DNA are used in the reactions to maximize the tagging, which involve trapping a reactive species. Excess probe (e.g., 13) must be removed because they inhibit the PCR. Probe concentration below 1 nM had no effect on the PCR. Considering that the probes are typically employed at 100 μM, a large majority must be removed. Several techniques to minimize the excess probe after tagging were investigated, including size-exclusion spin column chromatography, silica-spin column chromatography, NaCl/EtOH precipitation and washing, and polyethylene imine-coated magnetic bead (PEI-beads) binding and washing. The former two were extremely limited due to cost, low-throughput, and labor intensiveness. Precipitation was time-consuming and was inefficient at separating the probe(s) from DNA. In contrast, purification by PEI-beads was fast, facile, and, barring high-ionic strength media, flexible with conditions for probe removal.

\[
5\text{'-Biotin-d(\text{AAA})_3 TCT AAC ATG TGA GTT CTG AC) 15}
\]

A variety of washing solutions (10 mM) containing DTT or positively charged small molecules (guanidine, lysine, arginine, and spermidine) were assayed for removing excess probe. Their effectiveness was screened using \(^{32}\text{P}-15\) (10 pmol) and probe 14 (1 nmol). PEI-beads were added and washed with the additives. When excess probe was present, \(^{32}\text{P}-15\) could not bind to the streptavidin-coated beads. In contrast, when 14 was removed using any of the wash solutions, biotinylated DNA \(^{32}\text{P}-15\) bound the beads as effectively as that when 14 had not been added. Given the ease of preparation, both guanidine and DTT were maintained in subsequent experiments. We recognized that these experiments do not guarantee that the probe is completely removed and that any excess probe that is even noncovalently bound to DNA would contribute to the background signal. However, we were unable to directly measure low levels of probe remaining bound to the bead. It was conceivable that one could measure the bead-bound probe by radiolabeling the latter, but this was deemed impractical. After eight rounds of washes, DNA was eluted using high ionic strength buffer containing DTT to reduce the disulfide (0.1 M Tris HCl, pH 10, 2 M NaCl, 0.1% Tween-20, and 0.1 M DTT). After 5 min, the suspension was clarified by magnetic pelletization for 5 min. The eluted DNA was then bound to streptavidin beads (15 pmol binding capacity) and quantified by liquid scintillation counting.

Removing Untagged DNA. All probes contained biotin as a means for capturing tagged DNA and separating tagged and untagged material from one another. Dynabead MyOne T1 streptavidin-coated beads were employed. These beads contain a hydrophobic surface, which was desirable, as it should bind more weakly to the charged molecules (DNA and probes) employed in these studies. A 916 bp PCR fragment obtained from a plasmid containing the p53 gene was used as analyte DNA. The PCR product containing 8-oxodGuo (p53-OG) was synthesized using a forward primer that contained the lesion. An otherwise identical PCR product was prepared without 8-oxodGuo (p53-G) using a forward primer that did not contain the lesion. (The PCR fragment without 8-oxodGuo was used for optimizing conditions for removing untagged DNA.) A 1.2 kb biotinylated PCR fragment of pUC19 (bpUC19) was used as an internal standard. qPCR was used to determine the minimum background signal. Wash buffers varying in pH and ionic strength were screened, and TBS buffer (10 mM Tris, pH 9, and 1 M NaCl) was found to be the best.

Signal Amplification and Quantification Using qPCR. qPCR was chosen because of its large dynamic range, as well as its compatibility with sample preparation methods that shorten the time required to carry out the procedure. The previous procedure required adsorbing the (tagged and untagged) DNA to the surface of the microtiter plate well, followed by binding the horseradish peroxidase—streptavidin conjugate. In the current procedure, streptavidin-coated magnetic beads are used to capture the biotinylated DNA. Employing magnetic beads facilitates the removal of untagged (nonbiotinylated) DNA by washing. Removal of untagged DNA is one crucial
Chemical Research in Toxicology

Table 1. Tagging Yields of Various Probes Measured Using qPCR

| substrate | Ox. | 2 (0.1) | 9 (10) | 11 (10) | 12 (1) | 13 (0.1) | 14 (0.1) |
|-----------|-----|---------|--------|---------|--------|----------|---------|
| p53-G     | −   | 2.3 ± 0.5 | 0.1 ± 0.01 | 1.1 ± 0.1 | 0.5 ± 0.2 | 1.0 ± 0.01 | 0.02 ± 0.01 |
| p53-G     | +   | 5.6 ± 0.5 | 0.7 ± 0.1 | 1.2 ± 0.6 | 2.4 ± 0.4 | 2.0 ± 0.1 | 0.3 ± 0.03 |
| p53-OG    | +   | 17.1 ± 7.3 | 0.6 ± 0.1 | 3.4 ± 0.1 | 11.2 ± 0.5 | 12.0 ± 2.5 | 40.4 ± 6.2 |

*Tagging yields are the average ± SD of 3 independent measurements. *Tagging in the presence of K₃Fe(CN)₆ at 1 mM.

parameter for minimizing the limit of detection because untagged DNA will be amplified equally as untagged during PCR. Initially, qPCR was monitored using Sybr Green. However, greater reproducibility was achieved by adding an uncharged DNA (a 1.2 kb PCR fragment from pUC19) to the mixture prior to tagging as an internal standard. The internal standard and target DNAs were quantified by multiplex qPCR using Taqman probes containing different fluorophores for each nucleic acid substrate.

Having optimized the qPCR process and individual assay steps (Scheme 2), the performance of probes 2, 9, and 11–14 were compared (Table 1). The percent tagging of the p53-OG and p53-G fragments were measured. The background with no oxidant (K₃Fe(CN)₆) was also measured using p53-G. In general, the data were consistent with the more crude measurements described above for 11–13. Lower charged probes (e.g., 9 and 11) captured the 8-oxodGuo containing DNA less efficiently. However, the more highly charged probes yielded a higher background signal, as evidenced by the tagging of p53-G. Clevable probe 14 provided the highest overall tagging yield and greatest selectivity (~2,000-fold). Although the tagging yield for 14 was only ~40%, this is less significant than the selectivity. Increasing the tagging yield to 100% would only increase the sensitivity ~2.5-fold, assuming that the background reaction remained the same. Finally, the limit of detection using 14 was established using a mixture of p53-G and p53-OG totaling 0.9 fmol (~1 ng). The signal (% Yexo) was distinguishable from that for the background (p53-G only, 2.9 ± 0.7) when the sample contained as little as 14 amol of p53-OG (6.5 ± 1.0) in a sample containing 0.9 fmol DNA strands. This translates to 14 amol 8-oxodGuo per 1.65 pmol nt based upon 916 bp per p53-G molecule or <12 8-oxodGuo/10⁶ nt.

**Validating the 8-OxodGuo Measurement by 14.** The amount of 8-oxodGuo formed in the 53-PCR fragment upon treatment with FeCl₂, H₂O₂, and ascorbate was measured using 14 and the above-described process. The 8-oxodGuo dependence on the level of oxidative stress was determined over a range of ferrous ion concentrations and compared to the amount determined by Cadet using LC/MS to analyze DNA exposed to the same conditions (Figure 2). The concentrations of H₂O₂ and ascorbate were increased proportionally with FeCl₂ concentration. The agreement in the amount of 8-oxodGuo formed as measured by the two methods was excellent over the range of FeCl₂ between 0.8 and 12.5 μM (Figure 2). However, the methods yielded very different results at 25 μM FeCl₂, where we measured 477 ± 113 8-oxodGuo nt/10⁶ nt, but one would expect a value of ~250 using Cadet’s LC/MS method. Nonetheless, the two methods agree well with one another over a 15-fold range.

**CONCLUSIONS**

Probe 14 enables us to detect as little as 14 amol of 8-oxodGuo in 3–4 h. Typical experiments used 1 ng of DNA substrate, and the quantitative analysis agrees well with data obtained using enzyme digestion and LC/MS analysis. The amount of DNA substrate used in the assay is considerably less than that in a typical experiment using LC/MS. Furthermore, the small quantity of substrate needed will be useful for analyzing valuable nucleic acids that are in short supply, such as telomeric DNA. The sensitivity of the experiment is enhanced by the use of qPCR, which can in principle be used to detect very small numbers of molecules, provided the background tagging reaction can be reduced further. Furthermore, the qPCR method only requires that a portion of the target DNA sequence be known. Hence, it is not limited to specific sequences. Because DNA is not digested during the analysis, the method is potentially useful for analyzing 8-oxodGuo at specific sites by taking advantage of known methods for recognizing specific nucleic acid sequences. The advent of a PEI-bead washing procedure and a probe (14) that allows us to reductively cleave the highly positively charged DNA binding domain from the reporter group reduces the likelihood that the background signal is due to the noncovalently bound probe. Nonspecific tagging of dG by the probe(s) under the oxidative conditions of the reaction is a possible source of background signal. This may result from undesired oxidation of the dG ultimately to 8-oxodGuo. Future improvements will address this potential problem by examining the oxidation conditions in the tagging reaction. Given the large dynamic range of qPCR and its sensitivity, reducing the background signal will decrease the method’s limit of detection and increase its value to the scientific community.

**ASSOCIATED CONTENT**

**Supporting Information**

Mass spectra of 9 and 11–14. This material is available free of charge via the Internet at http://pubs.acs.org.
Chemical Research in Toxicology

■ AUTHOR INFORMATION

Corresponding Author
*Phone: 410-516-8095. Fax: 410-516-7044. E-mail: mgreenberg@jhu.edu.

Present Address
3(L.S.H.) Department of Chemistry, Kongju National University, 182, Shinkwan-dong, Kongju, Chungnam 314-701, Korea.

Funding
We are grateful for the support of this research by the National Science Foundation (CHE-0956466 to M.M.G.) and the Center of Excellence in Environmental Toxicology, supported in part by the NIEHS (P30-ES013508 to T.M.P.).

Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Professor James Stivers (JHU School of Medicine) and his research group for providing access to and assistance with the qPCR instrument.

■ ABBREVIATIONS

8-oxoGuo, 8-oxo-7,8-dihydro-2′-deoxyguanosine; 8-oxoGu, 8-oxo-7,8-dihydroguanine; amol, attomole; qPCR, quantitative polymerase chain reaction; Fapy, 8-oxo-7,8-dihydro-2′-deoxyguanosine; Fmoc, 9-fluorenylethoxycarbonyl; HOBt, hydroxybenzotriazole; DMF, dimethylformamide; DMTrCl, dimethoxytrityl chloride; TEA, triethylamine; NHS ester, N-hydroxysuccinimide ester; DIPEA, N,N-diisopropylethylamine; EtBr, ethidium bromide; G+C, guanosine and cytosine DNA components by hydroxyl radical and one-electron oxidants in aerated aqueous solutions.

■ REFERENCES

(1) Lombard, D. B., Chu, K. F., Mostoslavsky, R., Franco, S., Gostissa, M., and Alt, F. W. (2005) DNA repair, genome stability, and aging. Cell 120, 497–512.
(2) DePinho, R. A. (2000) The age of cancer. Nature 408, 248–254.
(3) Moreira, P. I., Nunomura, A., Nakamura, M., Takeda, A., Shenk, J. C., Aliev, G., Smith, M. A., and Perry, G. (2008) Nucleic acid oxidation in Alzheimer disease. Cell 120, 497–512.
(4) Hasty, P., Campisi, J., Hoeijmakers, J. H. J., van Steeg, H., and McFaline, J. L., and Pang, B. (2007) Challenges in developing DNA repair assays for molecular epidemiology studies: Distribution of OGG activity in healthy individuals. DNA Repair 6, 45–60.
(5) Steenken, S., and Jovanovic, S. V. (1997) How easily oxidizable is DNA? One-electron reduction potentials of adenosine and guanosine radicals in aqueous solution. J. Am. Chem. Soc. 119, 617–618.
(6) Steenken, S., Jovanovic, S. V., Bietti, M., and Bernhard, K. (2000) The trap depth (in DNA) of 8-oxo-7,8-dihydro-2′-deoxyguanosine as derived from electron-transfer equilibria in aqueous solution. J. Am. Chem. Soc. 122, 2373–2374.
(7) Fleming, A. M., Muller, J. G., Dlouhy, A. C., and Burrows, C. J. (2012) Structural context effects in the oxidation of 8-oxo-7,8-dihydro-2′-deoxyguanosine to hydantoin products: Electrostatics, base stacking, and base pairing. J. Am. Chem. Soc. 134, 15091–15102.
(8) Fleming, A. M., Orendt, A. M., He, Y., Zhu, J., Dokor, R. K., and Burrows, C. J. (2013) Reconciliation of chemical, enzymatic, spectroscopic, and computational data to assign the absolute configuration of the DNA base lesion spiroiminodihydropyrimidone. J. Am. Chem. Soc. 135, 18191–18204.
(9) Steenken, S., Jovanovic, S. V., Bietti, M., and Bernhard, K. (2000) The trap depth (in DNA) of 8-oxo-7,8-dihydro-2′-deoxyguanosine as derived from electron-transfer equilibria in aqueous solution. J. Am. Chem. Soc. 122, 2373–2374.
(10) Paz-Elizur, T., Elinger, D., Leitner-Dagan, Y., Blumenstein, S., Krupsky, M., Berrebi, A., Schecktmann, E., Freedman, L., and Livneh, Z. (2006) Reduced repair of the oxidative 8-oxoguanine DNA damage and risk of head and neck cancer. Cancer Res. 66, 11683–11689.
(11) Paz-Elizur, T., Elinger, D., Leitner-Dagan, Y., Blumenstein, S., Krupsky, M., Berrebi, A., Schecktmann, E., and Livneh, Z. (2007) Development of an enzymatic DNA repair assay for molecular epidemiology studies: Distribution of OGG activity in healthy individuals. DNA Repair 6, 45–60.
(12) Steenken, S., Jovanovic, S. V., Bietti, M., and Bernhard, K. (2000) The trap depth (in DNA) of 8-oxo-7,8-dihydro-2′-deoxyguanosine as derived from electron-transfer equilibria in aqueous solution. J. Am. Chem. Soc. 122, 2373–2374.
(13) Fleming, A. M., Muller, J. G., Dlouhy, A. C., and Burrows, C. J. (2012) Structural context effects in the oxidation of 8-oxo-7,8-dihydro-2′-deoxyguanosine to hydantoin products: Electrostatics, base stacking, and base pairing. J. Am. Chem. Soc. 134, 15091–15102.
(14) Fleming, A. M., Orendt, A. M., He, Y., Zhu, J., Dokor, R. K., and Burrows, C. J. (2013) Reconciliation of chemical, enzymatic, spectroscopic, and computational data to assign the absolute configuration of the DNA base lesion spiroiminodihydropyrimidone. J. Am. Chem. Soc. 135, 18191–18204.
(15) Henderson, P. T., Delaney, J. C., Muller, J. G., Neeley, W. L., Tannenbaum, S. R., Burrows, C. J., and Essigmann, J. M. (2003) The hydantoin lesions formed from oxidation of 7,8-dihydro-8-oxoguanine are potent sources of replication errors in vivo. Biochemistry 42, 9257–9262.
(16) Fleming, A. M., and Burrows, C. J. (2013) G-quadruplex folds of the human telomere sequence alter the site reactivity and reaction pathway of guanine oxidation compared to duplex DNA. Chem. Res. Toxicol. 26, 593–607.
(17) Cadet, J., Loft, S., Olniski, R., Evans, M. D., Bialkowski, K., Richard Wagner, J., Dedon, P. C., Muller, P., Greenberg, M. M., and Cooke, M. S. (2012) Biologically relevant oxidants and terminology, classification and nomenclature of oxidatively generated damage to nucleobases and 2-deoxyribosyl radicals. Acc. Chem. Res. 45, 381–391.
(18) Wagner, J. R., and Cadet, J. (2010) Oxidation reactions of cytosine DNA components by hydroxyl radical and one-electron oxidants in aerated aqueous solutions. Acc. Chem. Res. 43, 564–571.
(19) Cadet, J., Douki, T., and Ravanat, J.-L. (2008) Oxidatively generated damage to the guanine moiety of DNA: Mechanistic aspects and formation in cells. Acc. Chem. Res. 41, 1075–1083.
(20) Dedon, P. C., and Jaruga, P. (2012) Mechanisms of free radical-induced damage to DNA. Free Radical Res. 46, 382–419.
(21) Jaruga, P., Kirkali, G., and Dedon, P. C. (2008) Measurement of formamidopyrimidines in DNA. Free Radical Biol. Med. 45, 1601–1609.
(22) Chan, W., Chen, B., Wang, L., Taghizadeh, K., Demott, M. S., and Dedon, P. C. (2010) Quantification of the 2-deoxyribonolactone and nucleoside 5′'-aldehyde products of 2-deoxyribose oxidation in DNA and cells by isotope-dilution gas chromatography mass spectrometry: Differential effects of α-radiation and Fe6+-EDTA. J. Am. Chem. Soc. 132, 6145–6153.
(23) Chen, B., Zhou, X., Taghizadeh, K., Chen, J., Stubbe, J., and Dedon, P. C. (2007) GC/MS methods to quantify the 2-deoxypentos-4-ulose and 3′-phosphoglycolate pathways of 4′-oxidation of 2-deoxyribose in DNA: Application to DNA damage produced by α-radiation and bleomycin. Chem. Res. Toxicol. 20, 1701–1708.
(24) Chowdhury, G., and Guengerich, F. P. (2008) Direct detection and mapping of sites of base modification in DNA fragments by tandem mass spectrometry. Angew. Chem., Int. Ed. 47, 381–384.
(25) Viswesh, V., Gates, K., and Sun, D. (2010) Characterization of DNA damage induced by a natural product antitumor antibiotic leinamycin in human cancer cells. Chem. Res. Toxicol. 23, 99–107.

(26) Mangal, D., Vudathala, D., Park, J.-H., Lee, S. H., Penning, T. M., and Blair, I. A. (2009) Analysis of 7,8-dihydro-8-oxo-2'-deoxyguanosine in cellular DNA during oxidative stress. Chem. Res. Toxicol. 22, 788–797.

(27) McKenna, D. J., Gallus, M., McKeown, S. R., Downes, C. S., and McKevoy-Martin, V. J. (2003) Modification of the alkaline comet assay to allow simultaneous evaluation of mitomycin C-induced DNA cross-link damage and repair of specific DNA sequences. DNA Repair 2, 879–890.

(28) Sauvaigo, S., Petic-Calin, C., Caillat, S., Odin, F., and Cadet, J. (2002) Comet assay coupled to repair enzymes for the detection of oxidative damage to DNA induced by low doses of gamma-radiation: Use of YOYO-1, low-background slides, and optimized electrophoresis conditions. Anal. Biochem. 303, 107–109.

(29) An, N., Fleming, A. M., White, H. S., and Burrows, C. J. (2012) Crown ether-electrolyte interactions permit nanopore detection of individual DNA abasic sites in single molecules. Proc. Natl. Acad. Sci. U.S.A. 109, 11504–11509.

(30) Schibel, A. E. P., An, N., Jin, Q., Fleming, A. M., Burrows, C. J., and White, H. S. (2010) Nanopore detection of 8-oxo-7,8-dihydródeoxyguanosine in deoxyribonucleic acid via adduct formation to the DNA damage site. J. Am. Chem. Soc. 132, 17992–17995.

(31) Sato, K., and Greenberg, M. M. (2005) Selective detection of 2'-deoxyribononolactone in DNA. J. Am. Chem. Soc. 127, 2806–2807.

(32) Dhar, S., Kodama, T., and Greenberg, M. M. (2007) Selective detection and quantification of oxidized abasic lesions in DNA. J. Am. Chem. Soc. 129, 8702–8703.

(33) Ide, H., Akamatsu, K., Kimura, Y., Michiue, K., Makino, K., Asaeda, A., Takamori, Y., and Kubo, K. (1993) Synthesis and damage specificity of a novel probe for the detection of abasic sites in DNA. Biochemistry 32, 8276–8283.

(34) Asaeda, A., Ide, H., Terato, H., Takamori, Y., and Kubo, K. (1998) Highly sensitive assay of DNA abasic sites in mammalian cells—optimization of the aldehyde reactive assay DNA method. Anal. Chim. Acta 365, 35–41.

(35) Kojima, N., Takebayashi, T., Mikami, A., Ohtsuka, E., and Komatsu, Y. (2009) Construction of highly reactive probes for abasic site detection by introduction of an aromatic and a guanidine residue into an aminoxy group. J. Am. Chem. Soc. 131, 13208–13209.

(36) Hirose, W., Sato, K., and Matsuda, A. (2010) Selective detection of 5-formyl-2'-deoxyuridine, an oxidative lesion of thymidine, in DNA by a fluorogenic reagent. Angew. Chem., Int. Ed. 49, 8392–8394.

(37) Gong, J., and Sturla, S. J. (2007) A synthetic nucleoside probe that discerns a DNA adduct from unmodified DNA. J. Am. Chem. Soc. 129, 4882–4883.

(38) Taniguchi, Y., Kawaguchi, R., and Sasaki, S. (2011) Adenosine-1,3-diazaphenoxazine derivative for selective base pair formation with 8-oxo-2'-deoxyguanosine in DNA. J. Am. Chem. Soc. 133, 7272–7275.

(39) Nakagawa, O., Ono, S., Li, Z., Tsujimoto, A., and Sasaki, S. (2007) Specific fluorescent probe for 8-oxoguanosine. Angew. Chem., Int. Ed. 46, 4500–4503.

(40) Roy, J., Chirapia, P., Ganguly, S., and Huang, H. (2012) A DNA aptamer sensor for 8-oxo-7,8-dihydrowguna. Bioorg. Med. Chem. Lett. 22, 863–867.

(41) Zhang, Q., Wang, Y., Meng, X., Dhar, R., and Huang, H. (2013) Triple-stranded DNA containing 8-oxo-7,8-dihydró-2'-deoxyguanosine: Implication in the design of selective aptamer sensors for 8-oxo-7,8-dihydrodeoxyguanosine. Anal. Chem. 85, 201–207.

(42) Muller, J. G., Duarte, V., Hickson, R. P., and Burrows, C. J. (1998) Gel electrophoretic detection of 7,8-dihydró-8-oxoguanine and 7,8-dihydró-8-oxoadenine via oxidation by Ir(IV). Nucleic Acids Res. 26, 2247–2249.

(43) Munk, B. H., Burrows, C. J., and Schlegel, H. B. (2008) An exploration of mechanisms for the transformation of 8-oxoguanine to guanidinohydantoin and spiroiminodihydantoin by density functional theory. J. Am. Chem. Soc. 130, 5245–5256.

(44) Hosford, M. E., Muller, J. G., and Burrows, C. J. (2004) Spermocyte participates in oxidative damage of guanosine and 8-oxoguanosine leading to deoxyribosylurua formation. J. Am. Chem. Soc. 126, 9540–9541.

(45) Hickerson, R. P., Chepanoske, C. L., Williams, S. D., David, S. S., and Burrows, C. J. (1999) Mechanism-based DNA-protein cross-linking of MutY via oxidation of 8-oxoguanosine. J. Am. Chem. Soc. 121, 9901–9902.

(46) Xue, L., and Greenberg, M. M. (2007) Facile quantification of lesions derived from 2'-deoxyguanosine in DNA. J. Am. Chem. Soc. 129, 7010–7011.

(47) Greenberg, M. M. (2012) The foraminidopyrimidines: Purine lesions formed in competition with 8-oxopurines from oxidative stress. Acc. Chem. Res. 45, 588–597.

(48) Maniatis, T., Fritzsch, E. F., and Sambrook, J. (1982) Molecular Cloning. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

(49) Yu, D., Berlin, J. A., Penning, T. M., and Field, J. M. (2002) Reactive oxygen species generated by PAH o-quinones cause change-in-function mutations in p53. Chem. Res. Toxicol. 15, 832–842.

(50) Ewert, K. K., Evans, H. M., Zidovska, A., Bouxsein, N. F., Ahmad, A., and Safinya, C. R. (2006) A columnar phase of dendirtic lipid,abised caticon liposome, DNA complexes for gene delivery: Hexagonally ordered cylindrical micelles embedded in a DNA honeycomb lattice. J. Am. Chem. Soc. 128, 3998–4006.

(51) Huber, M., Pelleties, J. G., Torossian, K., Donne, P., Gamache, L., Charest-Gaudreault, R., Audette, M., and Poulin, R. (1996) 2,2',2'-dithiobis(N-ethyl-spermine-5-carboxamide) is a high affinity, membrane-impermeant antagonist of the mammalian polyamine transport system. J. Biol. Chem. 271, 27556–27563.

(52) Samson-Thibault, F., Madagundu, G. S., Gao, S., Cadet, J., and Wagner, J. R. (2012) Profiling cytokine oxidation in DNA by LC-MS/MS. Chem. Res. Toxicol. 25, 1902–1911.

(53) Balbo, S., Turesky, R. J., and Villalta, P. W. (2014) DNA adductomics. Chem. Res. Toxicol. 27, 356–366.

(54) Xu, X., Muller, J. G., Ye, Y., and Burrows, C. J. (2008) DNA-protein cross-links between guanine and lysine depend on the mechanism of oxidation for formation of C5 versus C8 guanosine adducts. J. Am. Chem. Soc. 130, 703–709.