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Research Article

Oxidation of 1-N^2-etheno-2'-deoxyguanosine by singlet molecular oxygen results in 2'-deoxyguanosine: a pathway to remove exocyclic DNA damage?

Glaucia Regina Martinez[1,*], Hulyana Brum[1], Guilherme Lanzi Sassaki[1], Lauro Mera de Souza[2], Ana Paula de Melo Loureiro[3], Marisa Helena Gennari de Medeiros[4] and Paolo Di Mascio[4,*]

[1]Departamento de Bioquímica e Biologia Molecular – Setor de Ciências Biológicas – UFPR, CP 19046 CEP 81531-980 Curitiba-PR, Brazil

[2]Instituto de Pesquisa Pelé Pequeno Príncipe, Faculdades Pequeno Príncipe, Curitiba- PR, Brazil

[3]Departamento de Análises Clínicas e Toxicológicas, Faculdade de Ciências Farmacêuticas – Universidade de São Paulo (USP), CEP 05508-000 São Paulo–SP, Brazil

[4]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo (USP), CP 26077, CEP 05513-970 São Paulo-SP, Brazil

*Corresponding author

e-mail: grmartinez@ufpr.br;

pdmascio@iq.usp.br

Abstract

Exocyclic DNA adducts are considered as potential tools for the study of oxidative stress-related diseases, but an important aspect is their chemical reactivity towards oxidant species. We report here the oxidation of 1,N^2-etheno-2'-deoxyguanosine (1,N^2-εdGuo) by $^1O_2$ generated by a non-ionic water-soluble endoperoxide ($DHPNO_2$) and its corresponding [^{18}O]-oxygen isotopically labeled ($DHPN^{18}O_2$), and by photosensitization with two different photosensitizers (methylene blue and Rose Bengal). Products detection and characterization were achieved using high performance liquid chromatography coupled to ultraviolet and electrospray ionization tandem mass spectrometry, and Nuclear Magnetic Resonance analyses. We found that dGuo is regenerated via reaction of $^1O_2$ with the ε-linkage, and we propose a dioxetane as an intermediate, which cleaves and loses the aldehyde groups as formate residues, or alternatively, it generates a 1,2-ethanediol adduct. We also report herein the rate constant for $^1O_2$ to quench 1,N^2-εdGuo and other etheno modified nucleosides. The rate constant (k_t) values obtained for etheno nucleosides are comparable to the k_t of dGuo. From these results, we suggest a possible role of $^1O_2$ in the cleanup of etheno adducts by regenerating the normal base.

Keywords: alkylating damage; DNA adducts; etheno adducts; mass spectrometry; oxidation; singlet oxygen.

List of abbreviations

$^1O_2$ – singlet molecular oxygen

[^{18}O] – oxygen isotopically labeled

1,N^2-εdGuo – 1-N^2-etheno-2'-deoxyguanosine

1,N^6-εdAdo -1,N^6-etheno-2'-deoxyadenosine

3,N^4-εCyt – 3,N^4-ethenocytidine

8-oxodGuo – 8-oxo-7,8-dihydro-2'-deoxyguanosine

CID – collision-induced dissociation

dGuo – 2'-deoxyguanosine

D_2O – deuterated water

DNA – deoxyribonucleic acid

DHPN – *N,N'*-di(2,3-dihydroxypropyl)-1,4- naphthalenedipropanamide

$DHPNO_2$ – *N,N'*-di(2,3-dihydroxypropyl)-1,4- naphthalenedipropanamide endoperoxide

$DHPN^{18}O_2$ – *N,N'*-di(2,3-dihydroxypropyl)-1,4- naphthalenedipropanamide endoperoxide isotopically labeled with ^{18}O

ESI – electrospray ionization

HPLC – high performance liquid chromatography

HSQC – heteronuclear single quantum correlation

k_q – physical quenching constant

k_r – chemical quenching constant

k_t – quenching constant

MB – methylene blue

MIS – selected ion monitoring

MSn – multiple stage mass spectrometer

NL – neutral loss

NMR – Nuclear Magnetic Resonance

PDA – photodiode array detector

RB – Rose Bengal

UV – ultraviolet

Introduction

DNA adducts are products formed by the covalent reaction of electrophilic compounds with nucleophilic sites in DNA. Carcinogenic compounds may have an intrinsic reactivity with DNA or their reactivity may result from metabolic or photochemical activation of initially non-reactive substances (Jeffrey and Straub, 1997).

Among the exocyclic DNA adducts, etheno-adducts (Figure 1) have been extensively studied (Medeiros, 2009). The interest to study these lesions began with the discovery that they are induced by known occupational and environmental carcinogens, such as vinyl chloride, which is metabolized to the potent alkylating agents chloroethylene oxide and chloroacetaldehyde by microsomal monooxygenases (Barbin, 1998). The reaction of chloroacetaldehyde with guanine produces two adducts: 1,N^2-ethenoguanine e 3,N^2-ethenoguanine (Figure 1), which were first described by Sattsangi et al (1977). Another compound of interest is ethyl carbamate (urethane), which is converted to vinyl carbamate, followed by oxidation to vinyl carbamate epoxide, which is able to form the same adducts generated by the oxidation products of vinyl chloride (Guengerich *et al.*, 1991; Barbin, 2000). Furthermore, it was shown that the formation of etheno-adducts can occur endogenously via the reaction of aldehydes and epoxides resultant from the lipid peroxidation process with the DNA bases (Medeiros, 2009, Guéraud, 2017).

Along the last two decades, many studies were dedicated to elucidate the sources, the toxicological significance and consequences of these DNA lesions, and their repair pathways (Tudek *et al.*, 2017). The exogenous factors or pathophysiological conditions that lead to their increase, and whether these adducts could play a role in carcinogenesis or neurodegenerative diseases have been addressed (Bartsch *et al.*, 2011; Bartsch, 1999). The biological monitoring of etheno-adducts could also provide information about the role of oxidative stress and lipid peroxidation in pathophysiological processes and could contribute to the research of chemo preventive agents (Bartsch and Nair, 2000; Dechakhamphu *et al.*, 2010; Linhart *et al.*, 2014).

Lipid peroxidation is also a source of peroxyl radicals that can lead to the formation of singlet molecular oxygen ($^1O_2$) (Miyamoto *et al.*, 2003a,b, 2006, 2014), an oxidizing species. However, there is no information about the reactivity of $^1O_2$ towards DNA etheno-adducts. The investigation of the chemical stability of the etheno-adducts is important to better understand their biological roles.

The chemical lability of etheno-adducts is known from some studies (Yip and Tsou, 1973; Basu *et al.*, 1993; Speina *et al.*, 2001, 2003; Barbati *et al.*, 2010). The etheno-adduct 1,N^6-etheno-adenine (1,N^6-εAde) in the free deoxynucleoside form (1,N^6-εdAdo) or incorporated in oligodeoxynucleotides underwent depurination and gave ring-opened derivatives when kept in neutral and alkaline conditions (Basu *et al.*, 1993; Speina *et al.*, 2001, 2003). One of the ring-opened derivatives, 4-amino-5-(imidazol-2-yl)imidazole, was more mutagenic than the parental 1,N^6-εAde when tested in *Escherichia coli* (Basu *et al.*, 1993), and it was not repaired by several DNA glycosylases (Speina *et al.*, 2003). Instead, it was bypassed by DNA polymerases, giving mutations, or depurinated, giving apurinic sites and subsequent DNA strand breaks (Speina *et al.*, 2003). The stability of the 1,N^2-etheno-2'-deoxyguanosine (1,N^2-εdGuo) adduct was tested under oxidation reactions with hydroxyl radical and sulfate radical anion in vitro (Barbati *et al.*, 2010). Regeneration of 2'-deoxyguanosine from 1,N^2-εdGuo was observed as a result of the oxidative processes (Barbati *et al.*, 2010).

Synthetic naphthalene endoperoxides are able to release pure $^1O_2$ and also the corresponding [^{18}O]-labeled upon gentle warming (Martinez *et al.*, 2000). This approach was used before to study the oxidation of 2'-deoxyguanosine or 8-oxo-7,8-dihidro-2'-deoxyguanosine free in solution or in DNA (Duarte *et al.*, 2000; Ravanat *et al.*, 2000, 2001, 2006; Martinez *et al.*, 2002, 2007). Here we present data on the oxidation of the etheno-adduct 1,N^2-εdGuo by $^1O_2$, characterizing the products formed by spectroscopic techniques. We took advantage of the isotopic labeling using a chemical generator of [^{18}O]-labeled $^1O_2$ (DHPN$^{18}O_2$) (Martinez *et al.*, 2000). Furthermore, we also report the total quenching constant of $^1O_2$ towards 1,N^2-εdGuo and other etheno-adducts, such as 1,N^6-εdAdo and 3,N^4-ethenocytidine (3,N^4-εCyt).

Results

The analysis of the reaction of 1,N^2-εdGuo with $^1O_2$ generated by DHPNO$_2$ shows three major products (Figure 2a). Product A, with a retention time of 5.8 min, presents in the mass spectrum a molecular ion $[M + H]^+$ at *m/z* 326 and a fragment characteristic of the loss of 116 Da of 2'-deoxyribose, $[BH + H]^+$ at *m/z* = 210 (Figure 2b). The product B, with retention time of about 8.5 min, shows the mass spectrometric characteristics of dGuo, with the ion at *m/z* 268 and the fragment at *m/z* 152 (Figure 2b). The product C with retention time of about 13 min, presents the ion signal of $[M + H]^+$ at *m/z* 296, the sodium adduct $[M + Na]^+$ at

m/z 318, and the fragment resultant from the break of the *N*-glycosidic bond, $[BH + H]^+$, at *m/z* 180 (Figure 2b). The ion signal of $[M + H]^+$ at *m/z* 292, corresponding to the 1,N^2-εdGuo, was not detected after the reaction, indicating a high consumption under this condition.

The UV spectra of the three products were obtained by HPLC analysis with photodiode array detector, which are shown in Figure 2c. Product A shows a spectrum very similar to B, which in turn is identical to the spectrum of an authentic sample of dGuo (Figure 2d). The data obtained show that the products formed have lost the characteristic structure of the etheno-adduct, given the differences in the UV absorption spectra.

A reaction of photosensitization with Rose Bengal was performed also to confirm whether the same product profile formed by $DHPNO_2$. As can be seen in Figure 3, the analysis immediately after photosensitization clearly showed the formation of three major products, as expected. Interestingly, after incubation of the crude reaction during a week in the dark at room temperature, we observed an increase of product B (Figure 3).

Characterization of products A and C

The product A has a signal at *m/z* = 326 in the mass spectrum, this value might not match the primary cleavage product of the dioxetane with two aldehyde groups because the signal would be observed at *m/z* = 324. The compound A was observed at *m/z* 326.1113 $[M+H]^+$ in the HR-MS (Figure S4), consistent with a chemical composition of C12H16N5O6 (theoretical *m/z* 326.10951), with a mass error of 5.5 ppm. A single fragment was observed at *m/z* 210.0629, with a neutral loss (NL) of 116.0484 atomic mass units (a.m.u) consistent with the loss of the 2-deoxyribosyl residue. However, the absence of other fragments hindered the structure identification. To resolve this issue, the compound A was analyzed by multiple-stage mass spectrometry, observed in MS1 at *m/z* 326 $[M+H]^+$. Using MS2 (CID), the fragment at *m/z* 210 was produced (Figure 4), consistent with the loss of the deoxyribosyl residue. This ion (*m/z* 210) was isolated and fragmented (MS3), producing fragments at *m/z* 192 (NL of 18 a.m.u., consistent with a loss of water), *m/z* 164 (NL 46 a.m.u., loss of CH2O2) and *m/z* 152 (NL 58 a.m.u., loss of C2H2O2). The ion at *m/z* 192 was isolated and fragmented (MS4), giving rise to the fragment at *m/z* 164 (NL 28 a.m.u., loss of CO). The ion at *m/z* 164 gave, in MS5, the fragment at *m/z* 135 (NL 29 a.m.u., loss of CH3N), exposing the deaminated purine nucleobase (Figure 4).

The structural analysis of 1-N^2-εdGuo intermediates along the photooxidation was achieved using heteronuclear $^1H/^{13}C$-HSQC NMR spectroscopy. Following the literature and deduced chemical shifts it was possible to assign the hydrogen and carbon resonances of $^1H/^{13}C$ atoms of 1,2-di-OH-ethane, shown in Figure 5. As noted in the HSQC spectrum, the anomeric signals were identified at δ 4.409/83.7 and δ 4.847/82.5 namely (*a*) and (*b*), respectively. Other signals from *N-2-deoxi*-Rib*f* were also observed at δ 5.978/83.7 (84%) and δ 5.846/83.6 (16%). The signals of guanine moiety from 1-N^2-εdGuo were present at δ 7.700/138.8, δ 7.203/106.3 and δ 6.906/116.6 and oxidized at δ 7.548/137.7 and δ 7.5175/137.5.

The protons from ethane diol during the photooxidation of 1-N^2-εdGuo (*a*) at δ 5.409 and (*b*) at δ 4.847 were selected individually spins for magnetic excitation, then the spectra were collected with 20 ms and 100 ms. The proton signals represented by (*b*) was observed as the closest to the selected peak (*a*), respectively, due to the vicinal scalar coupling. Owing to this fact, it is possible to confirm the 1,2-dihydroxyethane-1-N^2-εdGuo occurrence in the reaction.

After incubation of 1,N^2-εdGuo with DHPN$^{18}O_2$, analyses were performed by HPLC with UV detection and mass spectrometry. In Figure 6, we observe the chromatograms obtained by UV detection at 260 nm and the chromatograms obtained for product C by selected ion monitoring (MIS) when the analysis was performed in full scan mode, neutral loss of 116 and daughters of 298. The product C had the incorporation of oxygen, because of an increase in molecular weight due to the incorporation of an oxygen atom-18.

Total quenching constants determination

Total deactivation constants (k_t) were determined from $^1O_2$ luminescence decay at 1270 nm using deuterated water (D_2O) as the solvent, figure S5 and table 1 show the traces and values obtained for the different compounds. The experimental values for the mean lifetime of $^1O_2$ in D_2O were around 65 μs.

We determined the quenching constants of singlet oxygen by 1,N^2-εdGuo and other etheno modified nucleosides (1,N^6-εdAdo and 3,N^4-εCyt) for comparison. The constants showed the same order of magnitude obtained for dGuo, indicating that these products can be regarded as targets of the reaction with $^1O_2$.

Discussion

Sattsangi *et al.* (1977) studied the reaction of guanosine with chloroacetaldehyde at 37°C in a pH range from 6.5 to 4.5. They reported that the relative rate of the reaction with guanosine decreases with increasing acidity of the medium and is almost negligible at pH 4.5. The formed product was characterized as 1,N^2-ethenoguanosine. In the present work, the reaction of dGuo with chloroacetaldehyde was performed at 50°C in the pH range from 6.5 to 12. We observed that at pH 8.0 the yield of 1,N^2-εdGuo is lesser than at pH 6.5. Also, at pH 12, there is no formation of the product (Figure S1), probably by the instability of etheno adducts in alkali. In addition, the chromatogram of Figure S1 shows the presence of many other products, even at pH 6.5, which required further purification. After purification of the crude extract reaction in the solid phase extraction C-18 cartridges, the fractions were pooled and lyophilized. Only the samples eluted with methanol 10 and 20% showed the presence of the product. The product was purified on a semi-preparative scale from fractions 10 and 20% CH_3OH obtained in the pre-purification step.

The analysis of the reaction of 1,N^2-εdGuo with $^1O_2$ generated by $DHPNO_2$ shows three major products with spectral data that indicate cleavage of etheno linkage (Products A and C) and regeneration of parental nucleoside dGuo (product B). The same pattern was observed to RB photosensitization. We need to emphasize that under the conditions of RB-irradiation employed here, only $^1O_2$ is generated because of the narrow wavelength from the green LED employed (Allen, 1991). A reaction of photosensitization with methylene blue (MB) was performed to verify whether the same product profile formed by $DHPNO_2$ or RB was obtained. As can be seen in Figure S2 and S3, the analysis immediately after photosensitization clearly show the formation of three major products, as expected. The HPLC analysis with detection by mass spectrometry showed the same products A, B and C obtained previously with $DHPNO_2$ and RB.

These results show that 1,N^2-εdGuo is susceptible to oxidation by $^1O_2$, whose mechanism probably involves the attack on the double bond forming a dioxetane intermediate, which after cleavage and loss of the aldehyde as a formyl group by the action of water affords the product with mass spectrum signal at m/z = 296 (Product C), this product may suffer loss of the second aldehyde group, yielding dGuo as final product (Scheme 1). Our results indicate that product A has an ethane-1,2-diol group. The presence of a diol group was not an expected product from the dioxetane decomposition. However, evidence obtained in this investigation

support this statement, since after losing the deoxyribose moiety, the precursor-fragment (*m/z* 210) undergoes a dehydration to produce the fragment at *m/z* 192, that occurs in hydroxylated molecules. Additionally, the most of other fragments were produced by losing oxygenated components, until the release of the central core of the nucleobase. These data lead to the proposal of the diol structure, which was confirmed by NMR analyses. Besides getting solid information about the chemical structure of product A, the mechanism for its generation could not be totally clarified at this moment, but our data (See Figure 3) also indicates that this compound also generates dGuo (Scheme 1).

In order to better establish the biological significance of $^1O_2$ reaction towards 1,N^2-εdGuo, the determination of the quenching constant is of fundamental importance. The deactivation of $^1O_2$ by substrates can occur by a physical or chemical mechanism. The physical pathway involves the interaction of oxygen with a (deactivator) substrate that catalyzes its conversion to the ground state without consuming oxygen or forming products. In the chemical quenching, there is oxygen consumption and product formation. The total quenching constant (k_t) is the sum of physical (k_q) and chemical (k_r) constants ($k_t = k_r + k_q$). There are two main physical mechanisms for deactivation: energy transfer and charge transfer. In both cases, it is generally accepted the formation of a transient and reversible intermediate. According to the excitation energy and the ionization potential of the deactivator, the intermediate can be described as a covalent adduct (perepoxide) intermediate, or as an exciplex or as a charge transfer complex (Sheu and Foote, 1995).

It has been reported in the literature the total deactivation constant of guanosine derivatives with hydroxyl groups of the ribose protected by t-butyldimethylsilyl guanosine substituted at position 8 with Br, CH_3, $OCH_2C_6H_5$, OCH_3 and OH (8-oxodGuo) in different deuterated solvents (dichloromethane, benzene, acetone, and acetonitrile). The results showed that k_t increased about 5 to 15 times for all derivatives in polar solvents (acetonitrile in this case), and this sensitivity to solvent polarity was more pronounced to derivatives with electron rich substituents, indicating that the transition into this state is by polar interaction with partial charge transfer (Sheu and Foote, 1995).

In our study, k_t determined for dGuo and 8-oxodGuo in D_2O were 5.39×10^6 $M^{-1}s^{-1}$ and 58.7×10^6 $M^{-1}s^{-1}$, respectively. When compared with the values determined by Sheu and Foote (1995) for the same compounds but with the deoxyribose protected by t-butyldimethylsilyl in acetonitrile the values obtained by those authors were 9.15×10^6 $M^{-1}s^{-1}$ and 65.2×10^6 $M^{-1}s^{-1}$,

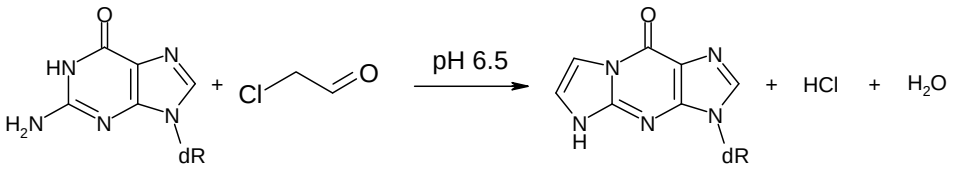
respectively. The values obtained in this work for the dGuo and 8-oxodGuo are lower because of the effect of solvent on the constant (water is more polar than acetonitrile), besides, the hydroxyl groups of 2'-deoxyribose were not protected, which could contribute to the deactivation of $^1O_2$ by the transfer of electronic excitation energy to vibrational energy levels of the OH bond. Moreover, the value of 5.3×10^6 $M^{-1}s^{-1}$ has been reported previously for dGuo in an aqueous system (Lee *et al.*, 1987), which is very close to that determined in the present work.

Also, k_t for the physical deactivator sodium azide was determined and the value obtained (5.28×10^8 $M^{-1}s^{-1}$) is in agreement with values previously reported in the literature that are in the range of 3 to 6.9×10^8 $M^{-1}s^{-1}$ (Lissi *et al.*, 1993). Interestingly, other authors also tested 1,N^2-εdGuo reactivity towards hydroxyl radical and one electron oxidation reactions, and they also concluded that the exocyclic ring of 1,N^2-εdGuo is mainly affected by oxidative processes leading to the regeneration of 2'-deoxyguanosine (Barbati *et al.,* 2010). Our results showed the same product by a different reactive oxygen species. In this way, the condition of oxidative stress may contribute to the degradation of the etheno-adducts and regeneration of the original guanine base, contributing to the maintenance of DNA or nucleotide pool.

Materials and methods

Preparation and purification of 1,N^2-εdGuo

As described by Sattsangi *et al.* (1977), the reaction of dGuo with chloroacetaldehyde generates the product, 1,N^2-εdGuo (Reaction 1). Thus, incubations were done using dGuo (Sigma-Aldrich Brasil, Brazil, 4 mM) and chloroacetaldehyde (Sigma-Aldrich Brasil, Brazil, 60 mM) in phosphate buffer pH 6.5, 8.0 and 12 at 50° C for 5 days or at 37° C for 4 days.



(Reaction 1) dR= 2-deoxyribose

Pre-purification of the adduct was performed by C-18 solid phase extraction (Supelclean-1G-6ml, Sigma-Aldrich Brasil, Brazil). The C-18 cartridge was equilibrated with 12 ml of methanol (Merck, Brazil) and then with 12 ml of water. A volume of 300 μL of the reaction solution was applied to the cartridge and the products were eluted by the following sequence of solvents: 1 ml of water, 3 ml of 2% CH_3OH, 3 ml of 5% CH_3OH, 6 ml of 10% CH_3OH, 6 ml of 20% CH_3OH, 6 ml of 80% CH_3OH and 6 ml of 100% CH_3OH. The fractions were lyophilized for further purification by HPLC-UV (Shimadzu, Japan).

The adduct 1,N^2-εdGuo was purified by semi-preparative HPLC of the 10 and 20% CH_3OH fractions from the pre-purification step. The UV detector was set for detection at 225 nm. The column used was a LC-18-Hypersil ODS (Thermo, 250×10 mm, particle size 10 μm) with a flow rate of 4.5 ml/min. The separation was performed with a linear gradient starting with 5% CH_3CN/95% water for 5 min, changing to 10% CH_3CN until 20 min, then to 60% in the next 5 minutes, staying at this condition until 30 min and returning to 5% CH_3CN until 35 min.

Incubation of 1,N^2-εdGuo with singlet molecular oxygen

Solutions were prepared in deuterated water (Sigma-Aldrich Brasil, Brazil) containing 1,N^2-εdGuo (0.5 mM) and $DHPNO_2$ or $DHPN^{18}O_2$ (10 mM) or DHPN Δ (10 mM, obtained after thermo decomposition of $DHPNO_2$), and incubated at 37°C for 2.5 h. $DHPNO_2$ or $DHPN^{18}O_2$ were prepared was previously reported (Martinez *et al.*, 2000).

Photosensitization with methylene blue: a solution of 1,N^2-εdGuo (0.5 mM) and methylene blue (Merck, Brazil, 20 μM) in deuterated water was irradiated with a 500W lamp (Philips, Brazil) under oxygen bubbling for 1.5 h at 10°C in a thermostatically controlled water bath.

Photosensitization with Rose Bengal: a solution of 1,N^2-εdGuo (0.1 mM) and Rose Bengal (Sigma-Aldrich Brasil, Brazil, 200 μM) in water was irradiated for 115 min with green LEDs (Cromatek, Brazil, 526 nm) with an irradiance of 1.67 ± 0.13 mW/cm^2.

Analysis of the products generated by the incubation of 1,N^2-εdGuo with singlet molecular oxygen

The products were analyzed using a Shimadzu HPLC equipment with a photodiode array detector (PDA) programmed to detect the wavelength range of 200-370 nm. The column was a LC-18 (250×4.6 mm, particle size 5 μm) from Supelco (Sigma-Aldrich Brasil, Brazil) with a

flow rate of 1 ml/min. The separation was performed with a linear gradient starting with 5% CH_3CN/95% water for 5 min, reaching 10% CH_3CN in 20 min, and 60% in 25 min, and returning to 5% CH_3CN until 30 min.

The analyses by mass spectrometry (Quatro II, Micromass, Waters) were performed in the positive electrospray ionization mode (ESI^+) with the following specifications: drying gas flow and nebulizer (both nitrogen) were 300 l/h and 30 l/h, source temperature: 120°C, capillary potential, and HV electrode were 3.5 and 0.3 kV, respectively. The cone potential was set at 30 V and spectra were acquired in the first analyzer in the range from 50 to 400 *m/z*.

Structural characterization of product A by mass spectrometry and nuclear magnetic resonance spectroscopy measurements

Product A was isolated by HPLC, lyophilized, and analyzed by mass spectrometry with electrospray ionization at atmospheric pressure ionization using a multiple stage mass spectrometer (MSn), LTQ-XL (Thermo Fisher Scientific™). The MSn analysis was developed in a Linear Ion Trap, by direct infusion of the sample, that was dissolved in water and infused at 10 µl/min. Nitrogen was used for sample desolvation, at flow rates of 8 and 2 arbitrary units, in the sheath and auxiliary gas, respectively, with the source temperature held at 300^{o}C. Positive ions $[M+H]^+$ were obtained applying 4.5 kV to the ion source, 40 V to the capillary and 130 V to the tube lens. The analyte was fragmented by collision-induced dissociation (CID) with 20 normalized collision energy in the MS2-MS4, in the MS5 the energy was 25. Instrument calibration is routinely performed, using Pierce™ LTQ ESI Positive Ion (Caffeine, MRFA, Ultramark-1621).

For NMR analysis, the reaction was carried out using 400 µl 1-N^2 -ƐdGuo 2.28 mM, 7.3 µl Rose bengal 5 mM and 504.7 µl D_2O totalizing 912 µl and samples loaded into a 5 mm tube. The nuclear magnetic resonance spectra were obtained using a Bruker Avance III 14.1 T spectrometer operating at 600 MHz and the data were acquired in an inverse 5-mm probe head (TXI) at 303 K. 1D ^{1}H-NMR were performed after 90 ° (p1=9.25 µs) pulse calibration. Residual HDO was pre-saturated with a pulse programme zgpr (TOPSPIN 3.2, Bruker Biospin, Rheinstetten, Germany) using a relaxation delay = 4.0 s, a number of time domain points = 65,536 and acquisition time = 5.1 s to obtain a spectrum width of 6393 Hz, using 32 data points. ^{1}H and ^{13}C chemical shifts were determined by 2D NMR analyses using

HSQC (hsqcedtgpsisp2.2), using 9014 Hz (^{1}H) and 30184 Hz (^{13}C) widths and a recycle delay of 1.160 s. The spectra were recorded for quadrature detection in the indirect dimension, using 16 scans per series of 2048×256 data points with zero filling in F1 (2048) prior to Fourier transformation. All signal assignments were performed using TopSpin program. 1D TOCSY selective excitation of compound (A), 1,N^2-ethenodiol-2'-deoxyguanosine experiments were carried out using a standard Bruker pulse program (selmlgp). A shaped pulse length of 80 ms for selective excitation was used, followed by an MLEV-17. Selective pulses of different mixing times varying from 20-100 ms using 256 scans were used.

Total quenching constants determination

Phenalenone was used as the $^1O_2$ photosensitizer and it was irradiated by a Nd:YaG laser at 355 nm. Monomol emission at 1270 nm was monitored with a special photocounting apparatus equipped with a monochromator suitable for emissions in the near-infrared region (800–1400 nm) which enabled us to follow the kinetics of formation and decay of $^1O_2$ and to determine its spectrum. The apparatus consists of a R5509 PMT photomultiplier tube from Hamamatsu Photonics (Shizuoka, Japan), cooled to -80°C with liquid nitrogen in a S600 PHOTOCOOL, PC176TSCE005 cooler from Products for Research Inc. (Danvers, MA) to reduce the dark current, and powered by a Model C3360 high voltage DC power supply from Hamamatsu Photonics with the applied potential set to -1.5 kV. The light emitted from the sample passed through a model M300 monochromator equipped with a Type G306R1U0 diffraction grating from Bentham Instruments Ltd (Reading, UK). The monochromator and the acquisition of data were controlled by F900 software version 6.22 software from Edinburgh Analytical Instruments (Livingston, UK). Experiments were conducted at room temperature in a quartz cuvette under continuous stirring with a small magnetic bar.

The rate constant for the total (physical and chemical) quenching (k_t) of $^1O_2$ was determined for each compound by employing different concentrations and Stern-Volmer plots (Wilkinson, 1993). For comparison, the k_t of sodium azide, 2'-deoxyguanosine (dGuo) and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) were also determined in the same conditions.

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Tables and figures

Table 1 Quenching constants of $^1O_2$ ($k_t = k_r + k_q$).

Compound	k_t (10^6 $M^{-1}s^{-1}$)
dGuo	5.39
8-oxodGuo	58.7
1,N^2-εdGuo	2.35
1,N^6-εdAdo	4.38
3,N^4-εCyt	2.66
NaN_3	528

The values were obtained by Stern-Volmer plots.

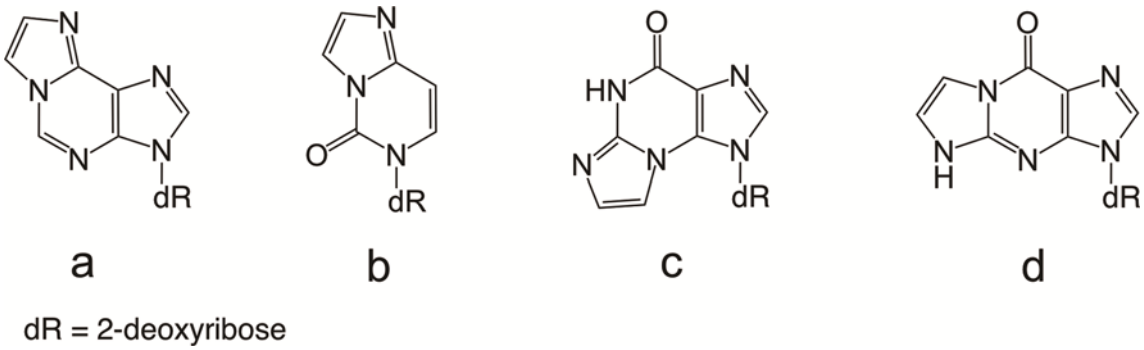
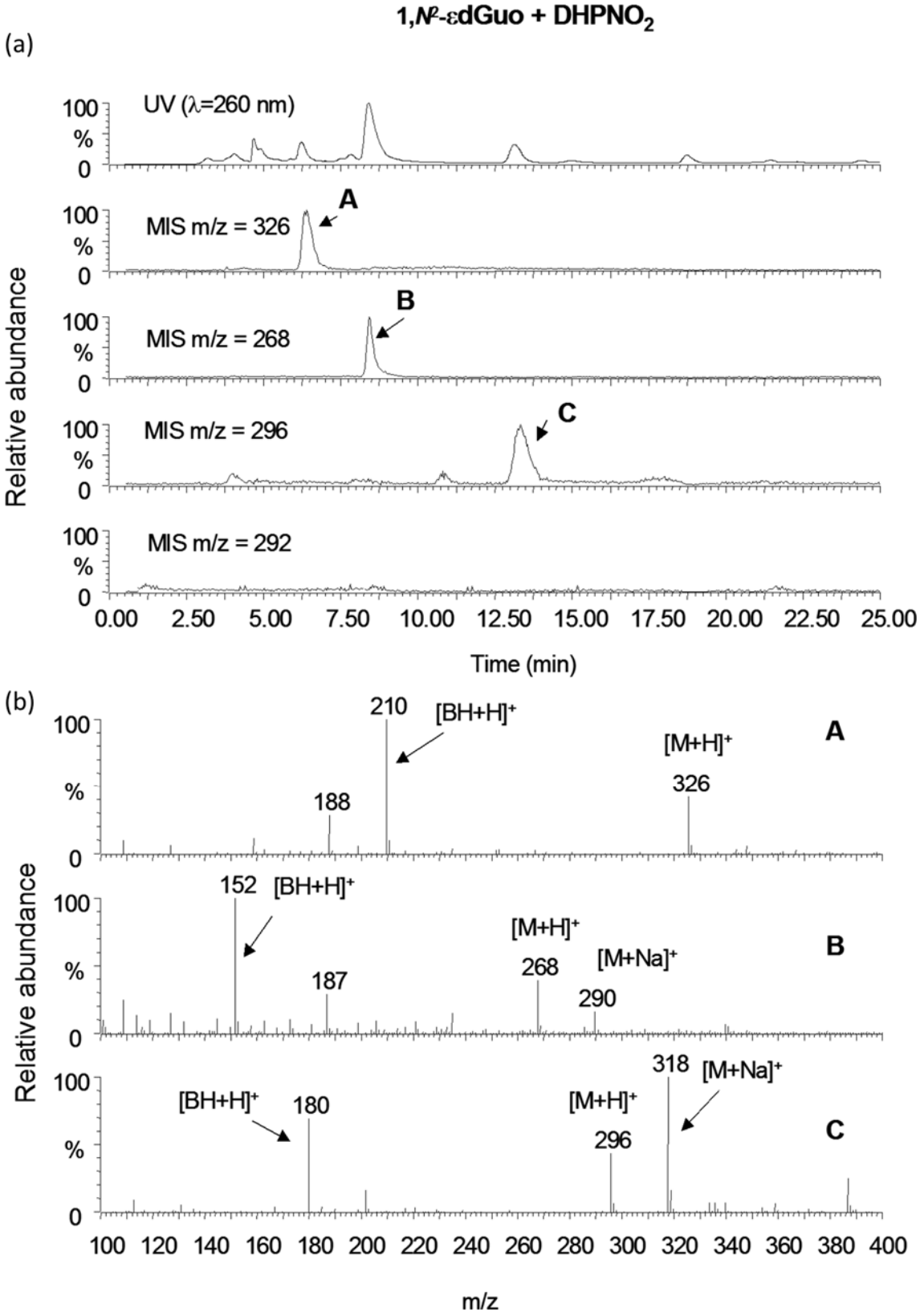


Figure 1 Chemical structure of some etheno-adducts of nucleosides, dR=2-deoxyribose. (a) 1,N^6-etheno-2'-deoxyadenosine; (b) 3,N^4-etheno-2'-deoxycytidine; (c) N^2,3-etheno-2'-deoxyguanosine; (d) 1,N^2-etheno-2'-deoxyguanosine.



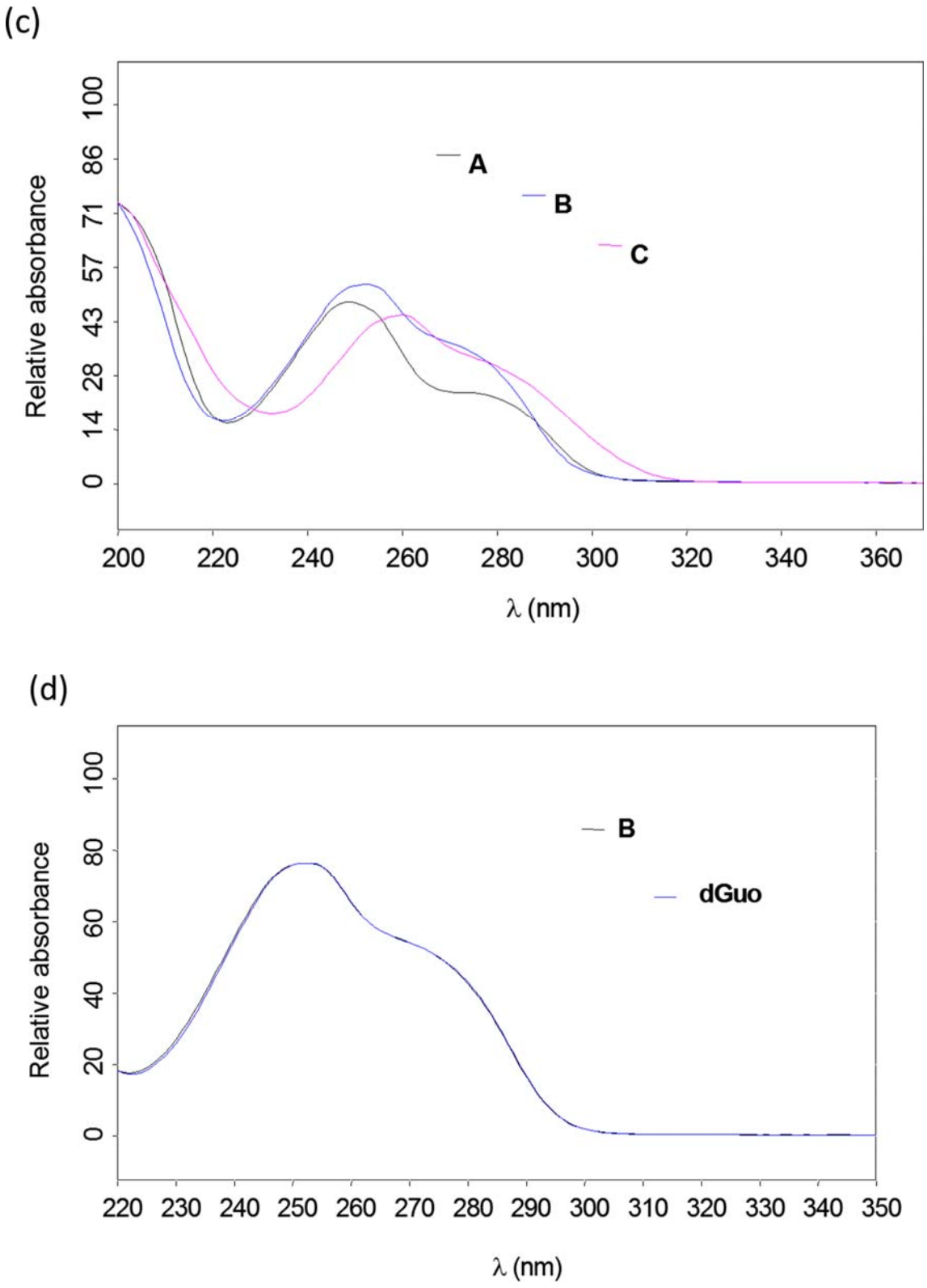


Figure 2 (a) HPLC-ESI-MS/MS Analysis of the crude mixture after incubation of 1,N^2-εdGuo with $DHPNO_2$. Chromatograms obtained by HPLC analysis of the incubation of 1,N^2-εdGuo with $^1O_2$ generated by $DHPNO_2$. Monitoring at 260 nm in a photodiode array detector and selecting the ion *m/z* 326, 268, 296 and 292 in full scan mode in the mass spectrometer

(MIS – monitoring of the ion selected). (b) Mass spectra of the products A, B and C, respectively. (c) UV spectra obtained by detection in photodiode array spectrophotometer of HPLC for the generated products A, B and C after incubation of 1,N^2-εdGuo with $DHPNO_2$. (d) UV spectra obtained by detection in photodiode array spectrophotometer of HPLC for the product B generated after incubation of 1,N^2-εdGuo with $DHPNO_2$ and an authentic sample of dGuo.

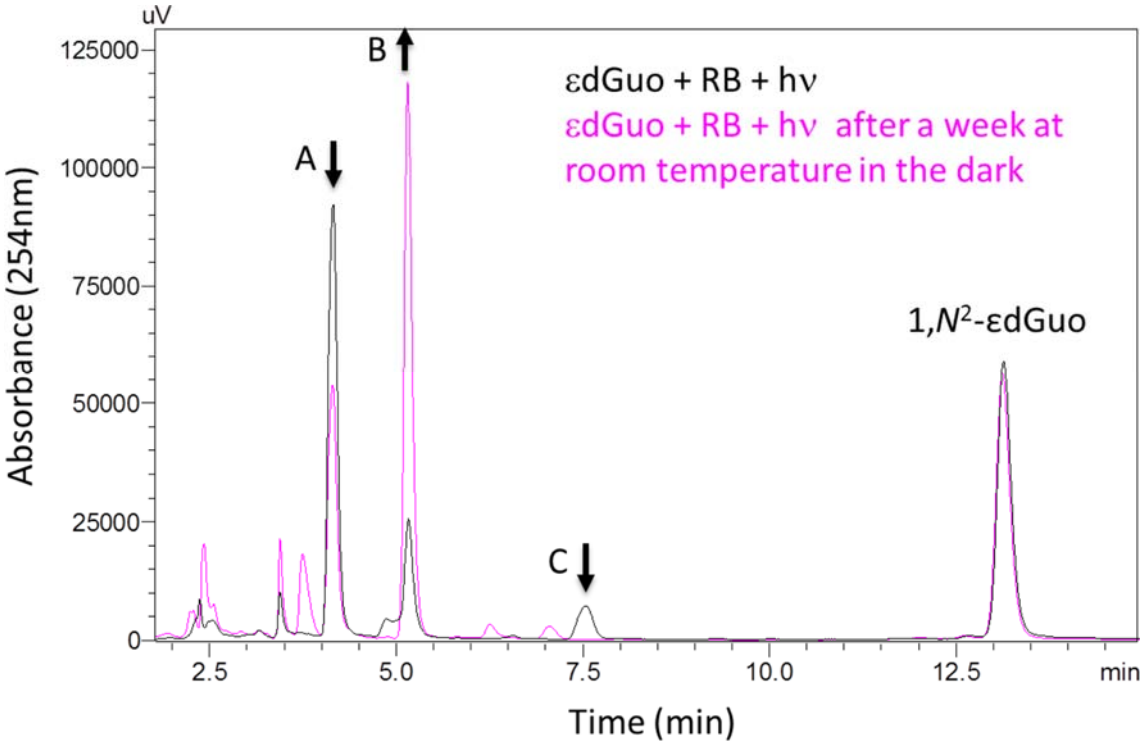


Figure 3 HPLC-PDA analysis of 1,N^2-εdGuo photooxidation.

Compared was the photooxidation of 1,N^2-εdGuo in the presence of Rose Bengal (pink trace) and the same reaction after incubation for one week at room temperature in the dark (black trace) using gradient mode separation in reverse-phase [ultrapure water (A) and acetonitrile (B): 0 – 5 min, 5% B; 20 min, 10% B; 25 min, 5% B; 30 min, 5% B].

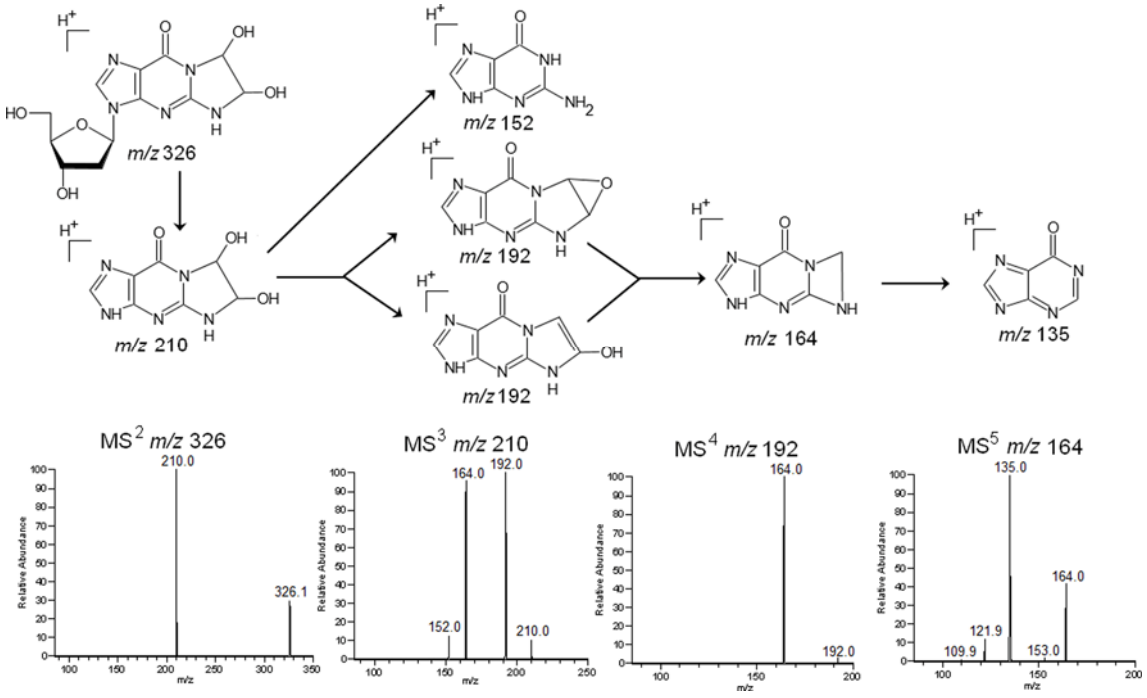


Figure 4 Multiple-stage mass spectrometry (MSn) of product A.

The compound A was isolated by HPLC and after lyophilization, it was analyzed by mass spectrometry with electrospray ionization at atmospheric pressure ionization using a multiple stage mass spectrometer.

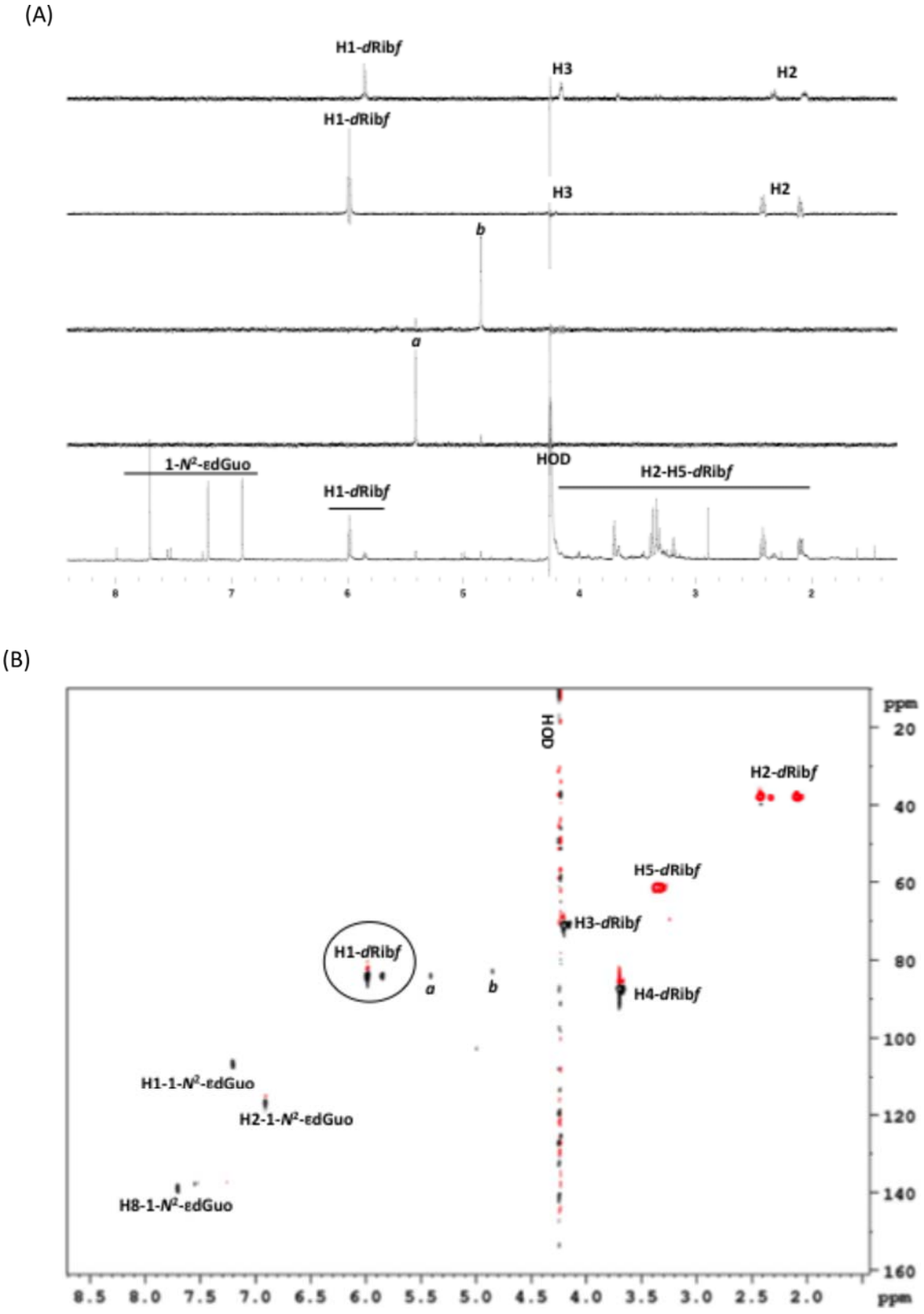


Figure 5 (A) 1D selective TOCSY (A and B) and full ^{1}H-NMR (C) spectra. The key signals from the etheno diol hydrogens (*a*) and (*b*) formed along photooxidation of 1-N^2-

εdGuo in the presence of Rose Bengal, corresponding to compound A. Proton signal selected to magnetic excitation (a) and closest correlated signal (b) 1D TOCSY spectra were collected at 100 ms of mixing time. (B) Edited HSQC NMR spectrum; the positive phase (black) correspond to CH, and the negative phase (red) correspond to CH_2. $^1H/^{13}C$ NMR signals of the crude mixture after photooxidation of 1-N^2-εdGuo in the presence of Rose Bengal.

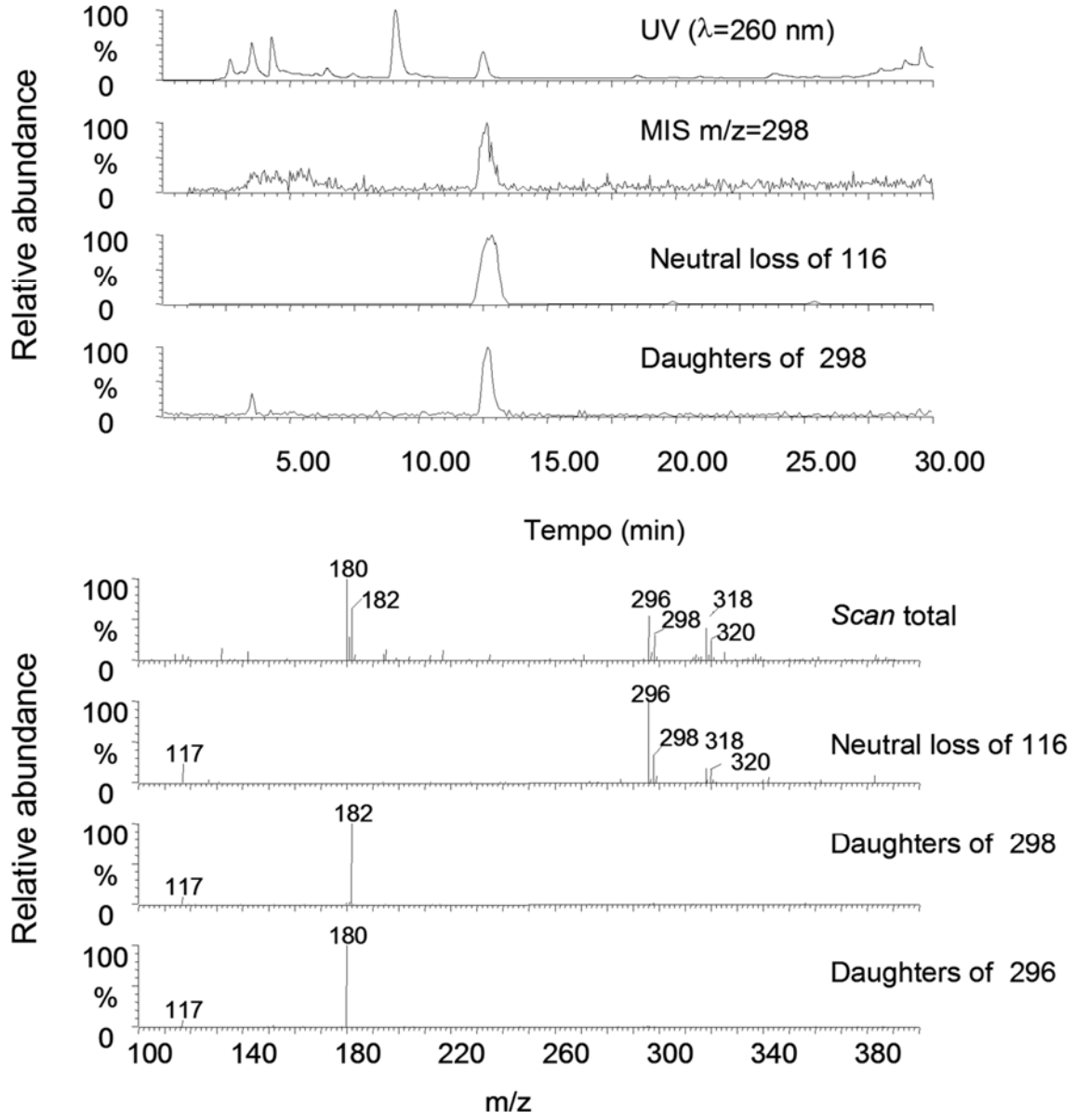
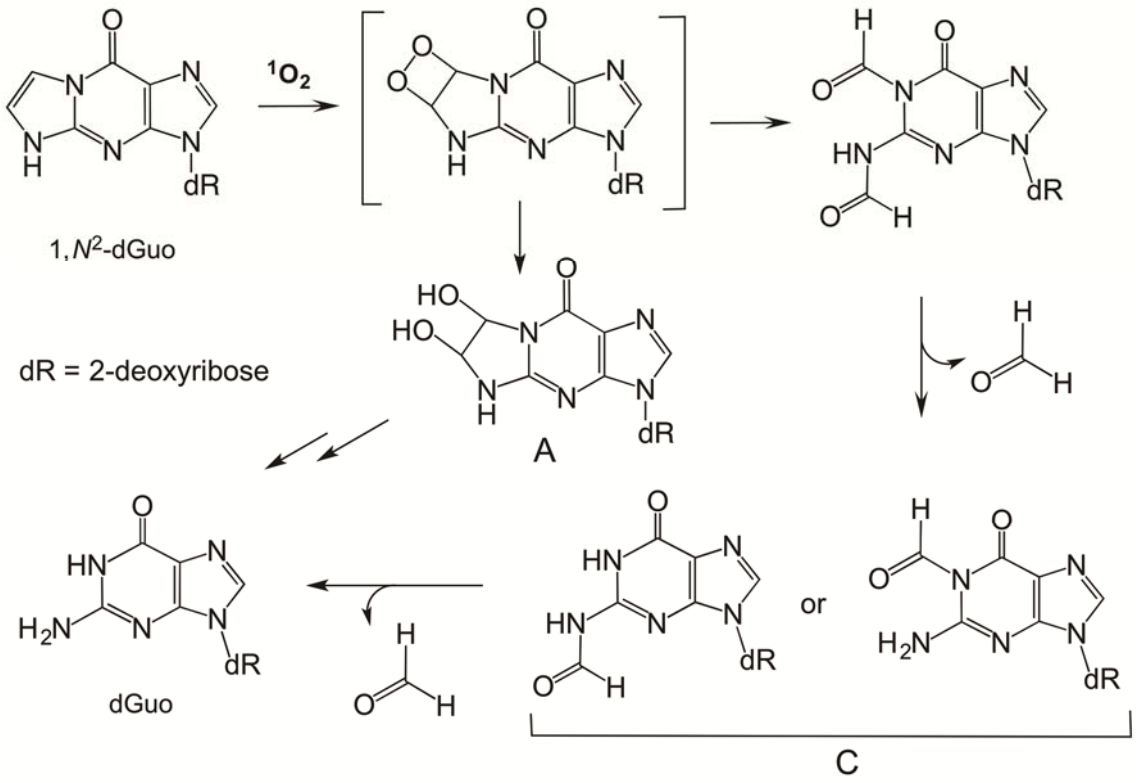


Figure 6 HPLC-ESI-MS/MS analysis of the crude mixture after incubation of 1,N^2-εdGuo with DHPN$^{18}O_2$.

Monitored was the absorbance at 260 nm in a photodiode array detector, selecting the ion *m/z* 298 (MIS) in full scan mode, the neutral loss of 116, and the ion *m/z* 182 (MIS) from the daughters of 298 in the mass spectrometer.



Scheme 1 A possible mechanism of the reaction between 1,N^2-εdGuo and $^1O_2$.

Supplementary table and figures

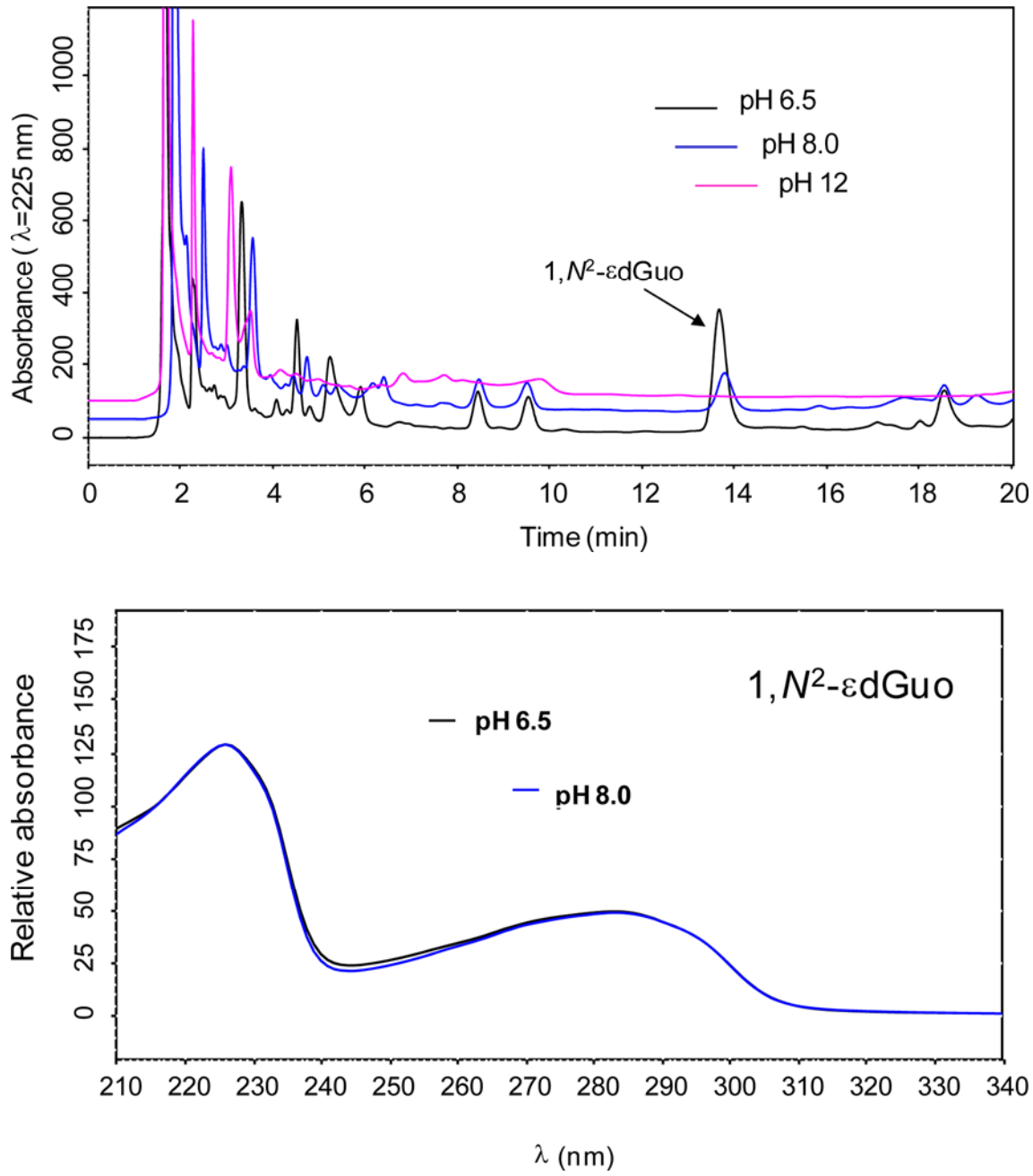


Figure S1 (A) HPLC elution profile on a C-18 column of the crude mixture after incubation of dGuo with chloroacetaldehyde at different pH. (B) UV spectra obtained for the product formed at pH 6.5 and 8.0 and with retention time of 13.8 min on HPLC separation.

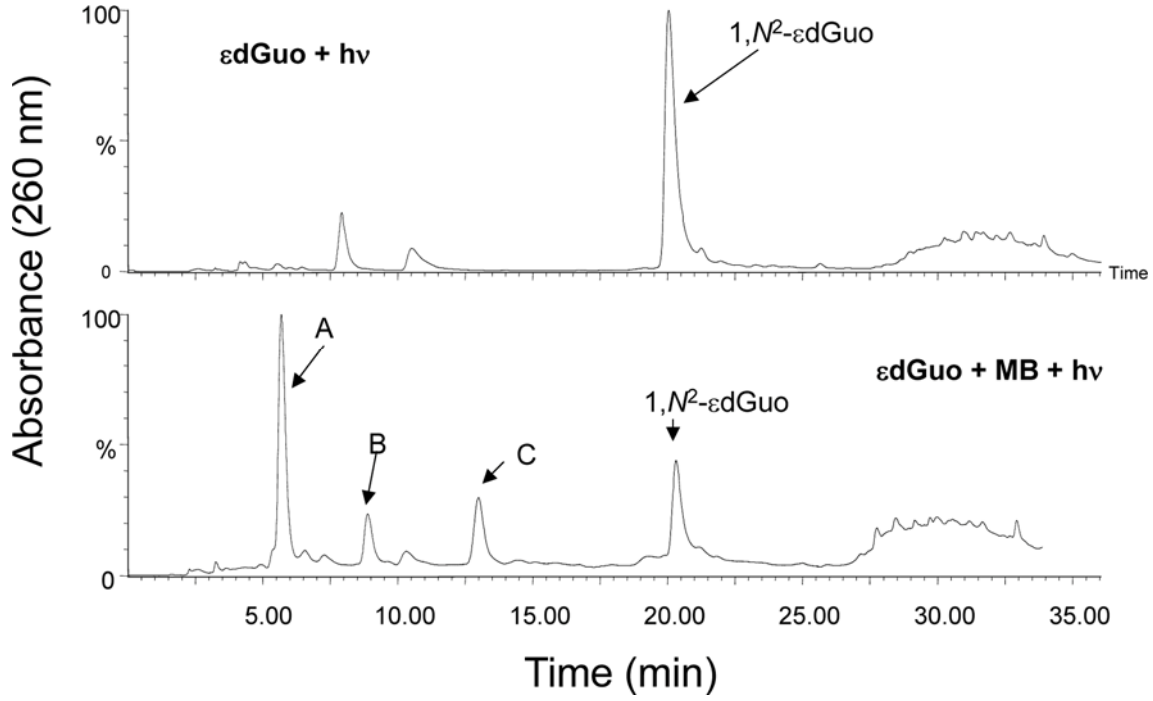


Figure S2 Chromatograms obtained by HPLC analysis of the control sample and after incubating 1, N^2-εdGuo with $^1O_2$ generated by photosensitization with MB.

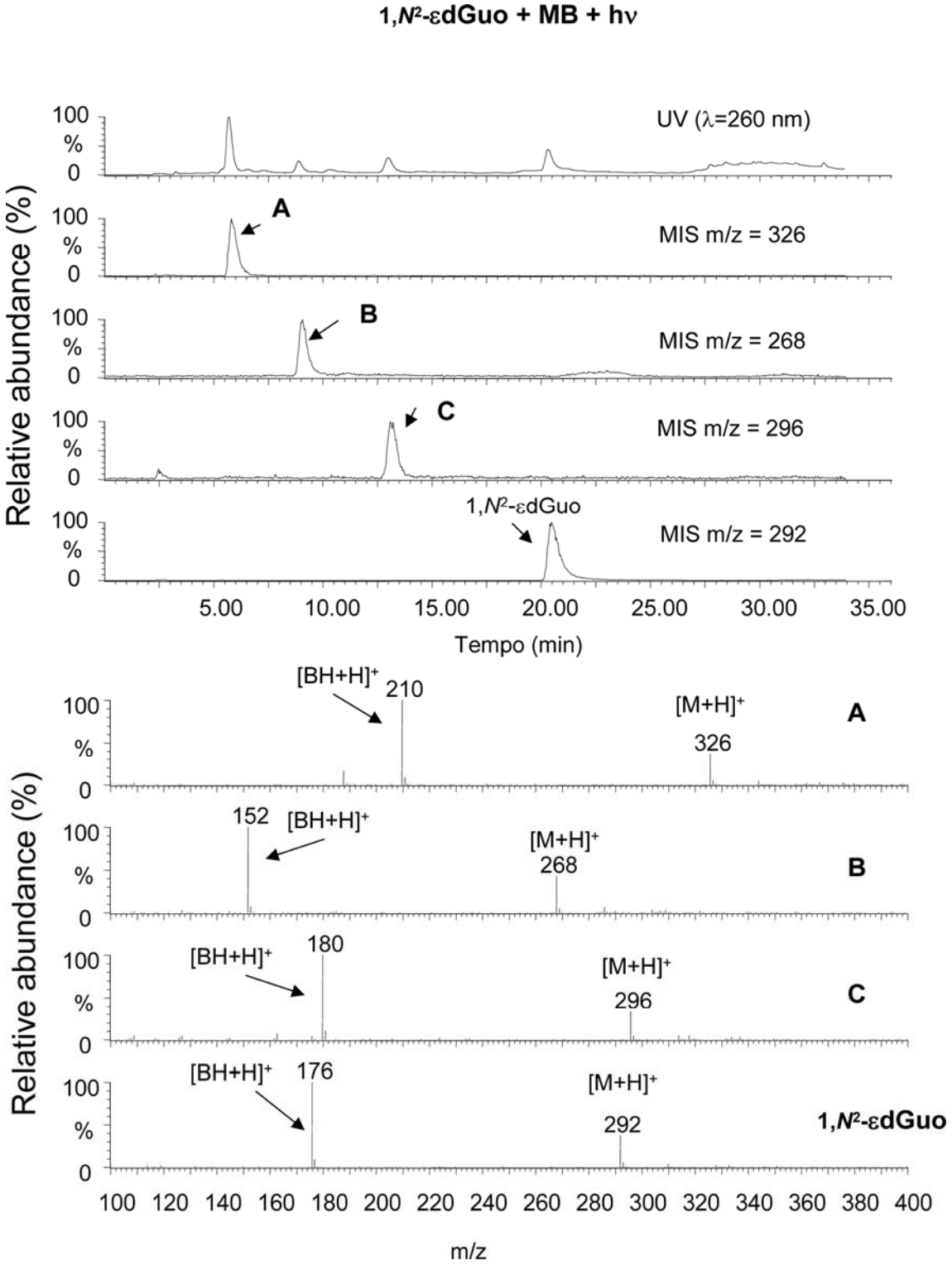


Figure S3 Chromatograms obtained by HPLC analysis of the products resultant from the incubation of 1,N^2-εdGuo with $^1O_2$ generated by photosensitization with MB. Monitoring at 260 nm and by mass spectrometry selecting the ion *m/z* 326, 268, 296 and 292 (MIS) in full scan mode. Mass spectra of the products A, B, C and 1,N^2-εdGuo.

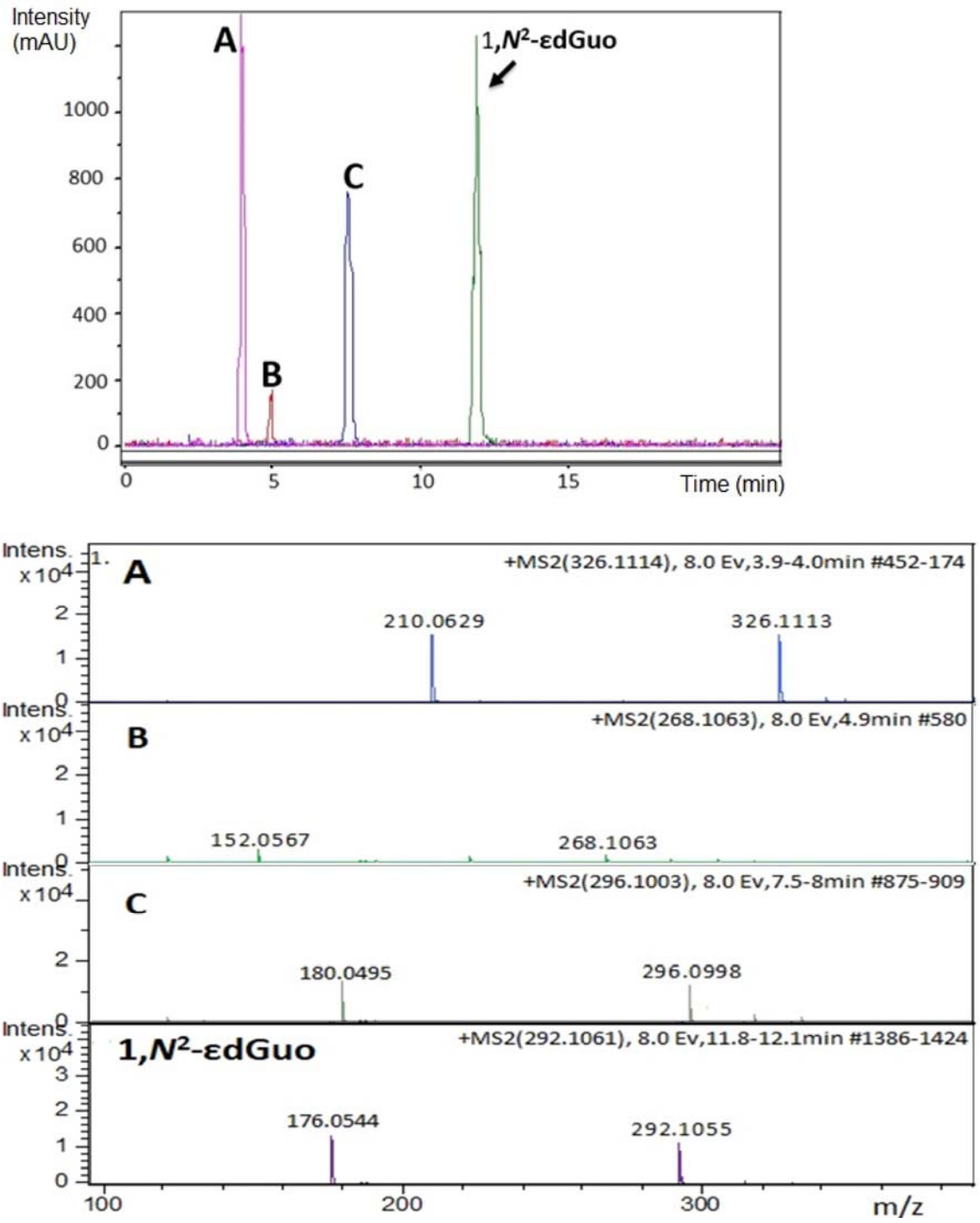


Figure S4 HR-MS of RB-photosensitization of 1,N^2-εdGuo. HPLC-ESI-MS/MS analysis of photooxidation of 1-N^2-εdGuo in the presence of Rose Bengal using MIS mode selecting the parent ions m/z 326 (A), m/z 268 (B), m/z 296 (C), and m/z 292 (1- N^2-εdGuo) with fragments in m/z 210, m/z 152, m/z 180 and m/z 176, respectively. It was used gradient mode separation condition in reverse-phase (ultrapure water (A) and ACN (B) : 0 – 5 min, 5% B; 20 min, 10% B; 25 min, 5% B; 30 min, 5% B.

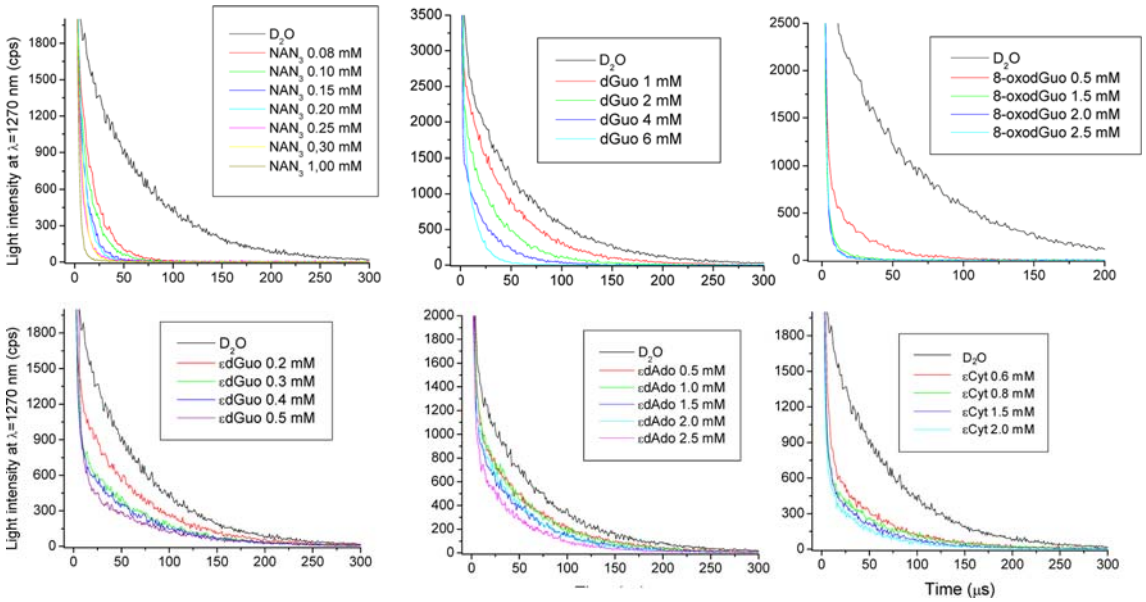


Figure S5 Decay of luminescence at 1270 nm and quenching effect on the lifetime of $^1O_2$.