Supporting information

Quantitation of Lipid Peroxidation Product DNA Adducts in Human Prostate by Tandem Mass Spectrometry: A Method that Mitigates Artifacts

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Contents

Instrumentation

UV-visible spectroscopy measurements were performed on an Agilent 8453 UV spectrophotometer (Agilent Technologies) with Tray Cell (LP 1 mm, dilution factor × 10 or × 50, Hellma Analytics). HPLC-UV analyses were carried out on an Agilent 1260 Infinity HPLC-UV with auto-sampler. Vacuum centrifugation was performed on a Thermo Scientific ISS110 SpeedVac. Mass spectrometric analyses were performed on a Velos Pro Linear Ion Trap mass spectrometer (Thermo Fisher, San Jose, CA) equipped with an UltiMate™ 3000 RSLCnano System (Thermo Fisher, San Jose, CA) and an Advance CaptiveSpray source (Michrom Bioresource Inc., Auburn, CA).

Figure S1. Extracted ion chromatogram of each adduct at their LOQ and the corresponding ISTDs.

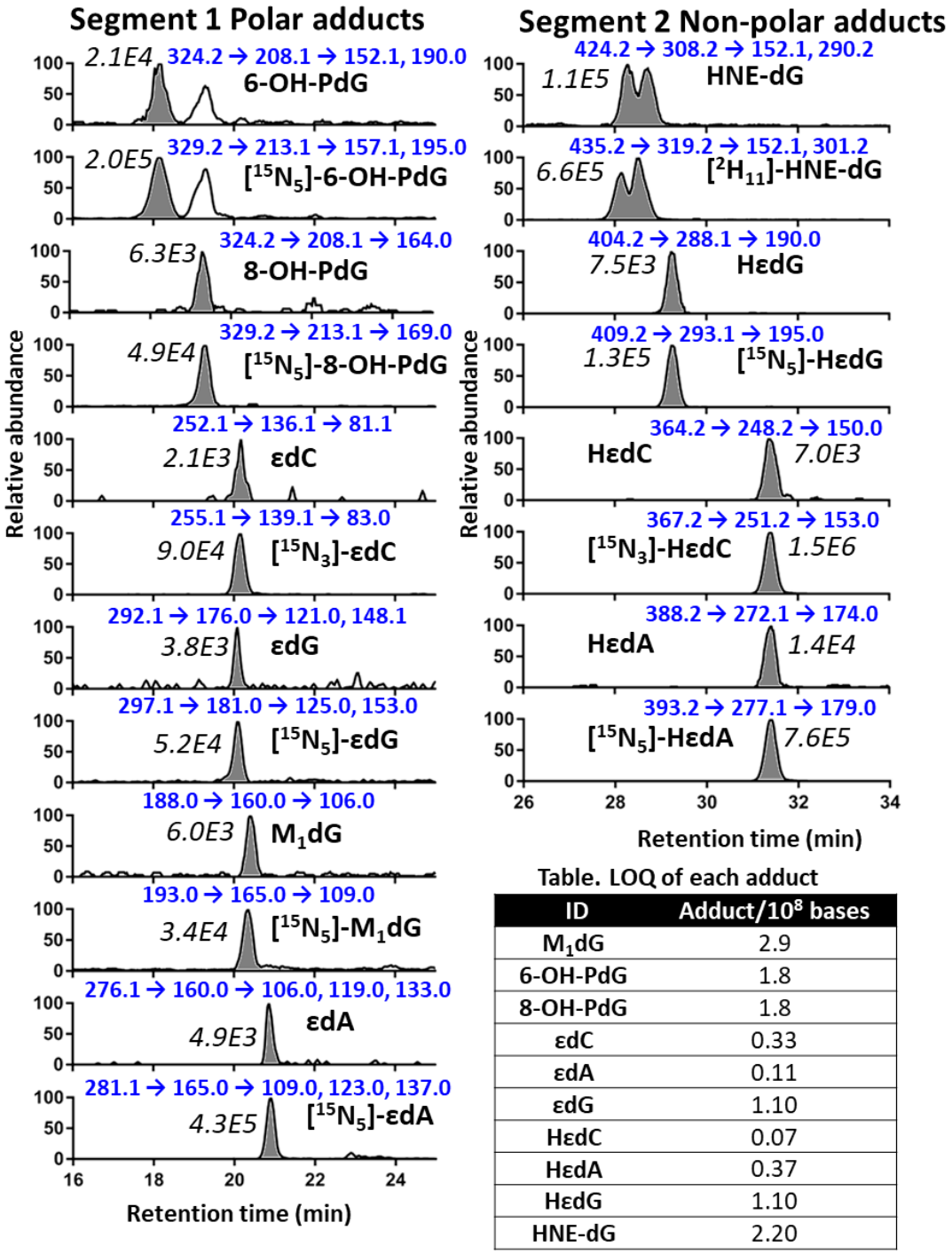


Table. LOQ of each adduct

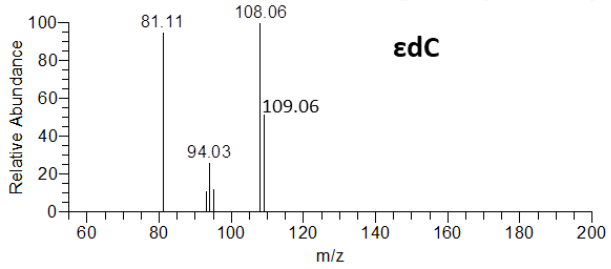
ID	Adduct/10^8 bases
M_1dG	2.9
6-OH-PdG	1.8
8-OH-PdG	1.8
εdC	0.33
εdA	0.11
εdG	1.10
HεdC	0.07
HεdA	0.37
HεdG	1.10
HNE-dG	2.20

Isotopically labeled internal standards (25 fmol/45 μg DNA, 1.83 adduct per 10^7 nts) and authentic standards at LOQ level (see table above) were spiked in liver DNA of untreated rats isolated by the **PUR/CHCl$_3$** method with BME (10 mM) and digested with **D-b** with BME (2.5 mM) as the AO. SPE-enriched sample (9 μL) was injected on to an analytical Magic C18AQ reversed-phase column (Michrom

Bioresources, Inc., 0.2 × 150 mm, 3 μm particle size, 200 Å pore size) for chromatographic separation. The mobile phases were (A) 2 mM NH_4OAc and (B) 2 mM NH_4OAc in 95% CH_3CN. The following gradient elution method was applied for method optimization: 0−9 min: 3 μL/min, 1% B; 9−10 min: 3−1 μL/min, 1% B; 10 to 30 min: 1 μL/min, 1 to 50 % B; 30−31 min: 1 μL/min, 50−90% B; 31−32 min: 1 μL/min, 90% B; 32−33 min: 1−3 μL/min, 90% B; 33−35 min: 3 μL/min, 90−1% B; 35−40min: 3 μL/min, 1% B. The sample loop was bypassed at 3.5 min. The chromatographic run was divided into two segments for monitoring the polar and non-polar adducts, respectively. The extracted ion chromatograms were obtained using the MS transitions labeled in Figure S1. Note that we used a Magic C18 AQ column for method optimization. 6-OH-PdG contains two peaks. Although the current gradient was optimized to achieve optimal peak shape for all adducts, the second peak of 6-OH-PdG coeluted with 8-OH-PdG. Therefore, unique MS^3 transitions [6-OH-PdG (324.2 → 208.1 → 152.1, 190.0, selected first peak for quantitation); 8-OH-PdG (324.2 → 208.1 → 164.0)] were used for quantitation of these two isomers. For the analysis of human prostate samples, we switched to a new Magic C18 AQ column, which we applied a slightly modified gradient that completely resolved the 6-OH-PdG and 8-OH-PdG adducts while maintaining Gaussian peak shapes of the other adducts.

MS^3 spectra of LPO adducts and corresponding ISTD

F: ITMS + c NSI Full ms3 252.10@cid25.00 136.00@cid42.00 [55.00-200.00]



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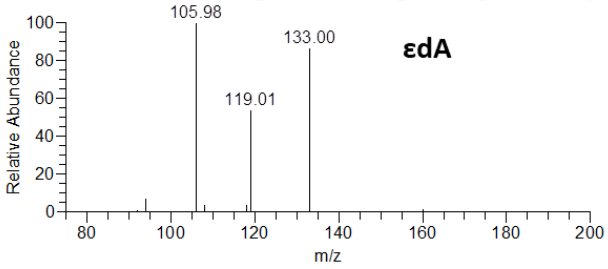

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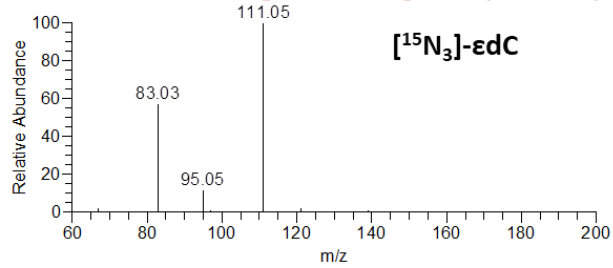

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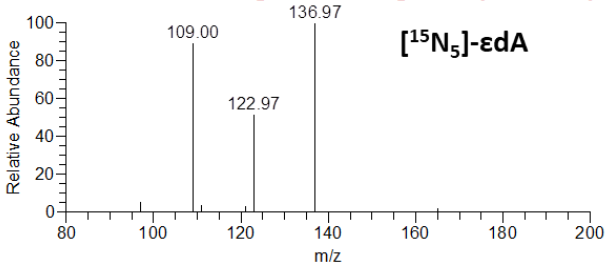

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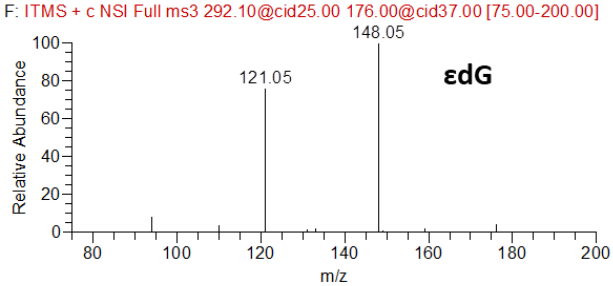

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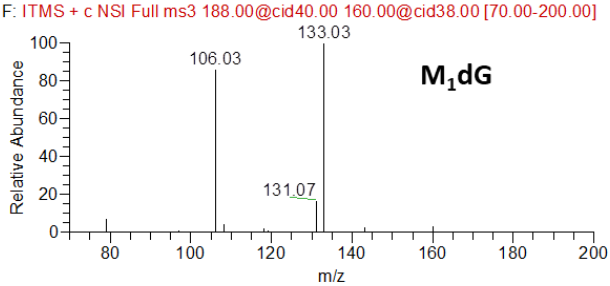

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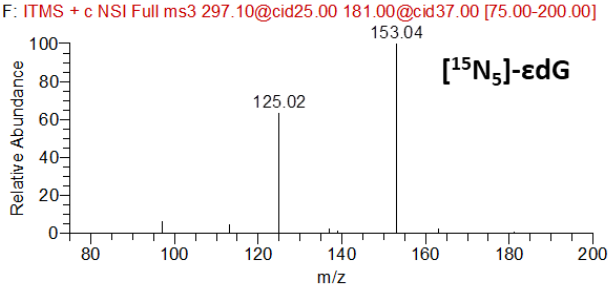

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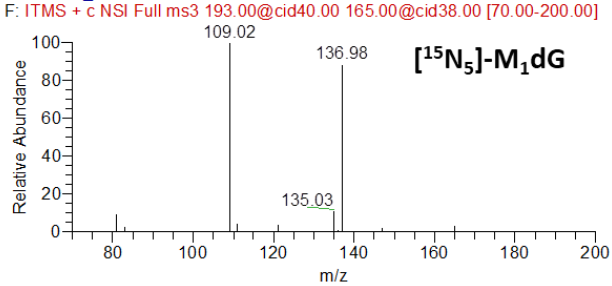

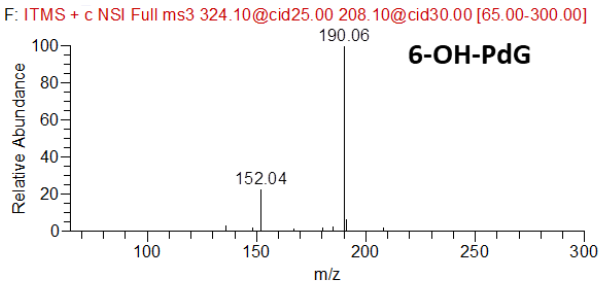
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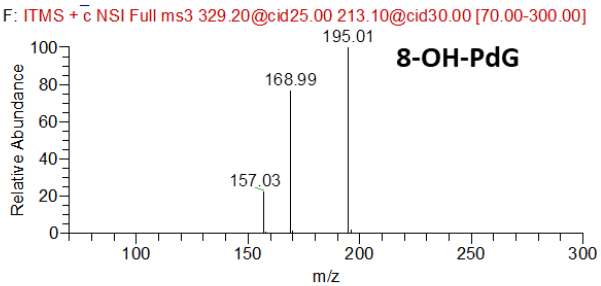
6-OH-PdG
190.06
152.04
Relative Abundance
m/z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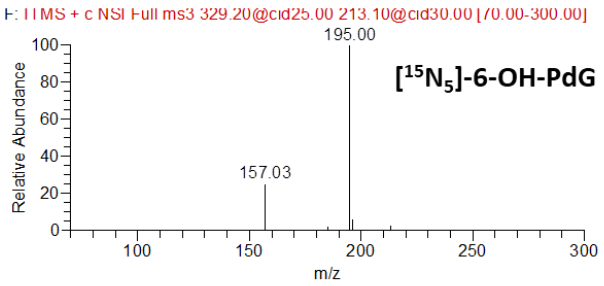
8-OH-PdG
195.01
168.99
157.03
Relative Abundance
m/z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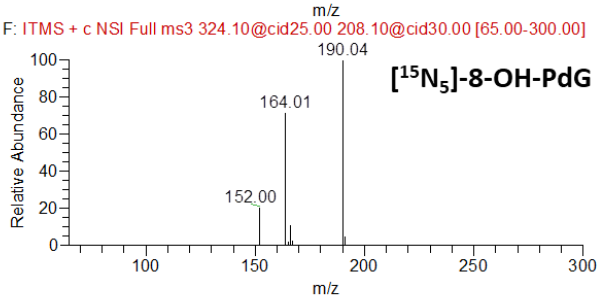
[15N5]-6-OH-PdG
195.00
157.03
Relative Abundance
m/z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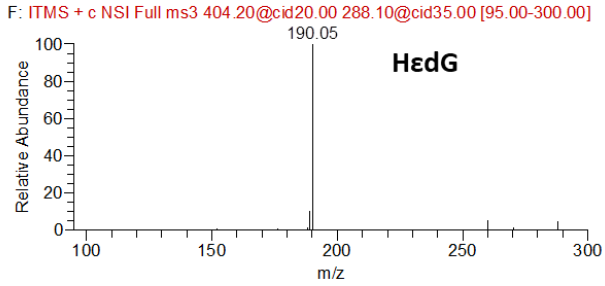
[15N5]-8-OH-PdG
190.04
164.01
152.00
Relative Abundance
m/z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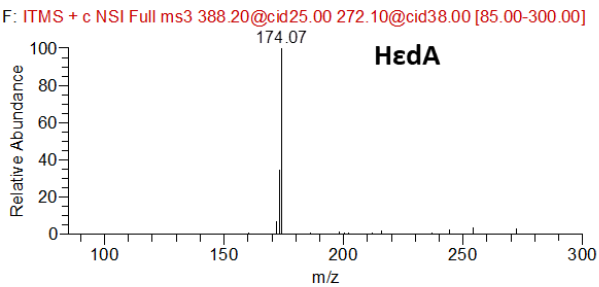
HεdG
190.05
Relative Abundance
m/z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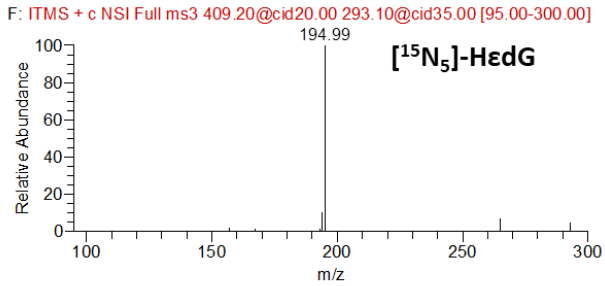
HεdA
174.07
Relative Abundance
m/z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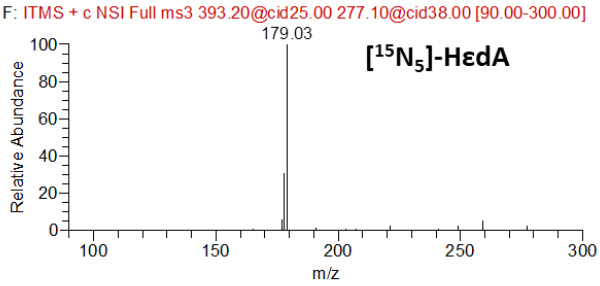
[15N5]-HεdG
194.99
Relative Abundance
m/z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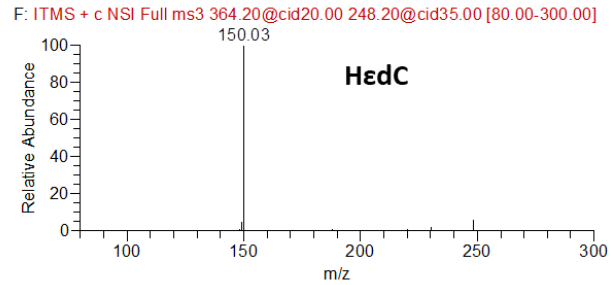
[15N5]-HεdA
179.03
Relative Abundance
m/z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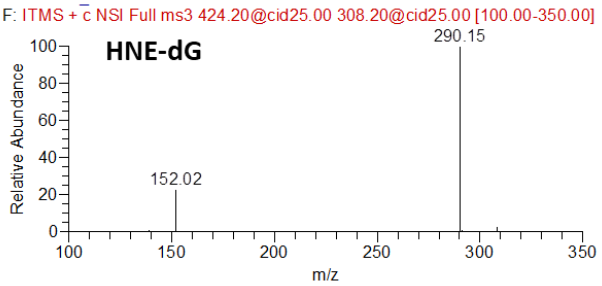
HεdC
150.03
Relative Abundance
m/z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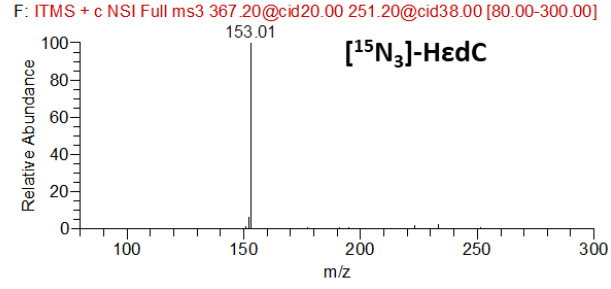
HNE-dG
290.15
152.02
Relative Abundance
m/z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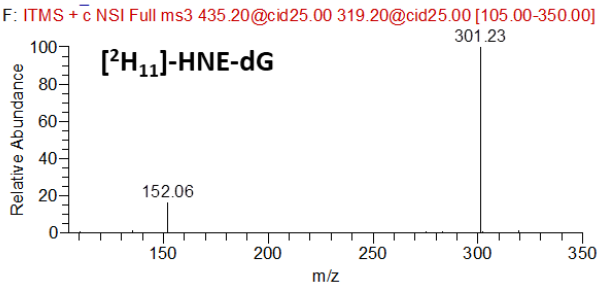
[15N3]-HεdC
153.01
Relative Abundance
m/z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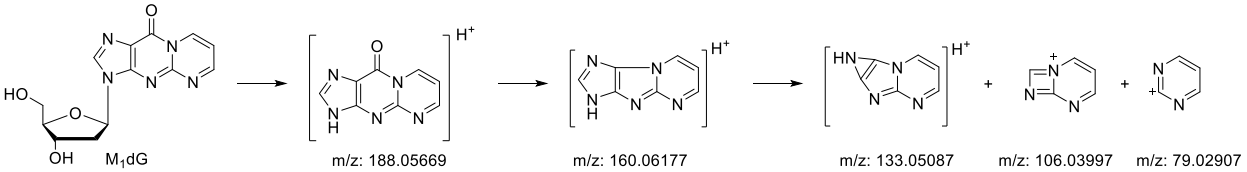
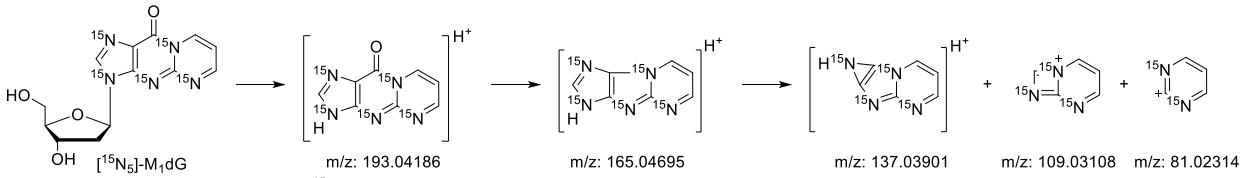
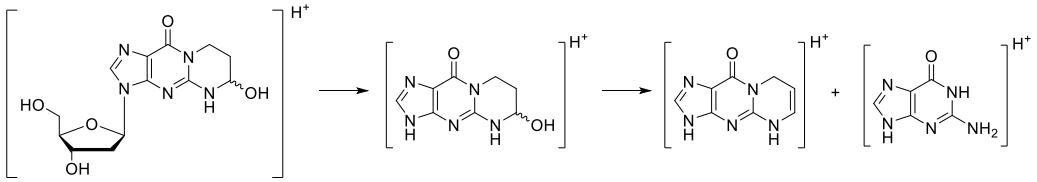
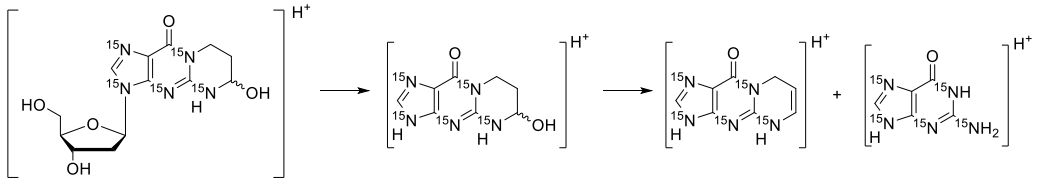
[2H11]-HNE-dG
301.23
152.06
Relative Abundance
m/z

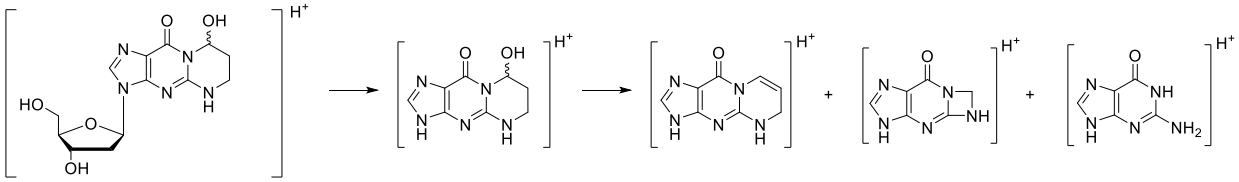
Suggested fragmentation pattern of each adduct and internal standard

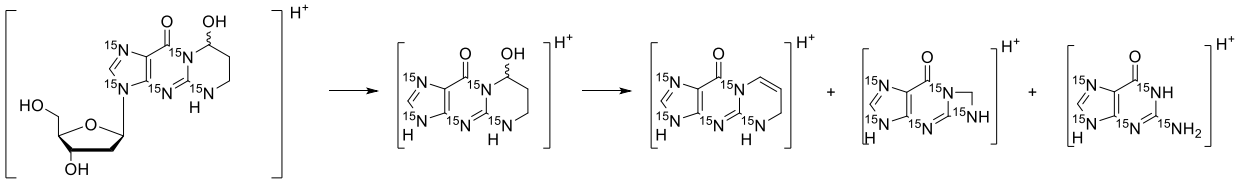


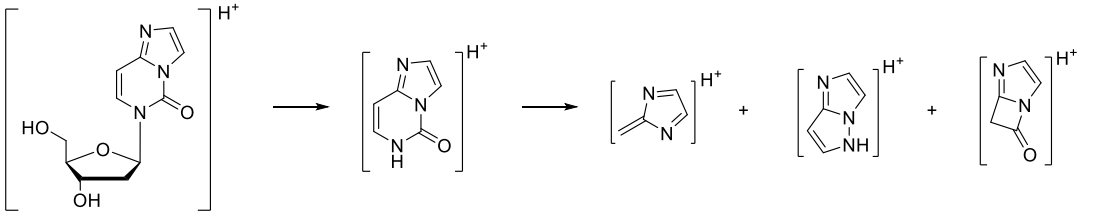












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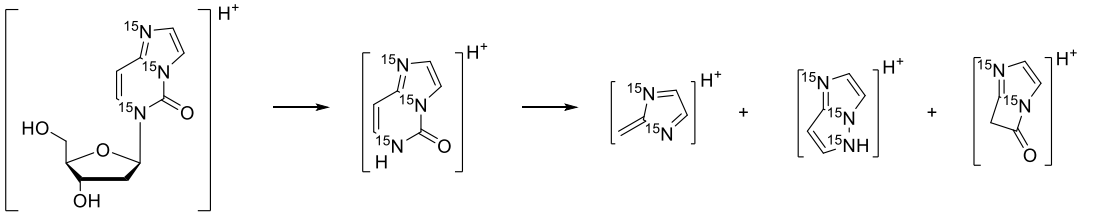
εdC



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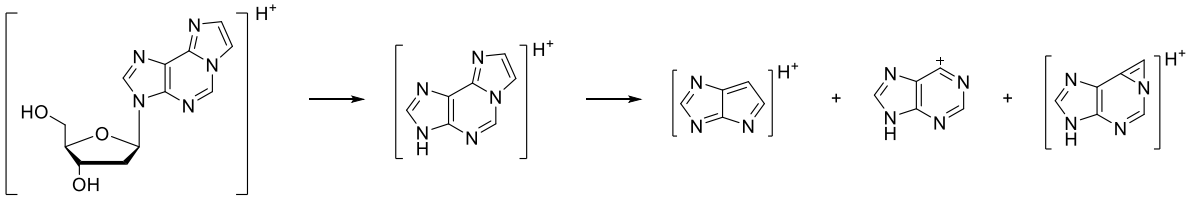
[$^{15}N_3$]-εdC



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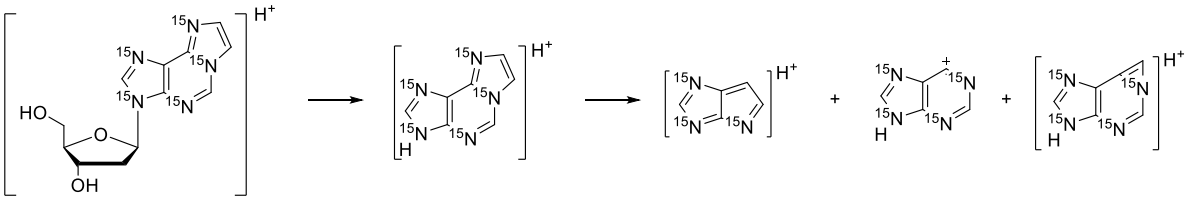
εdA



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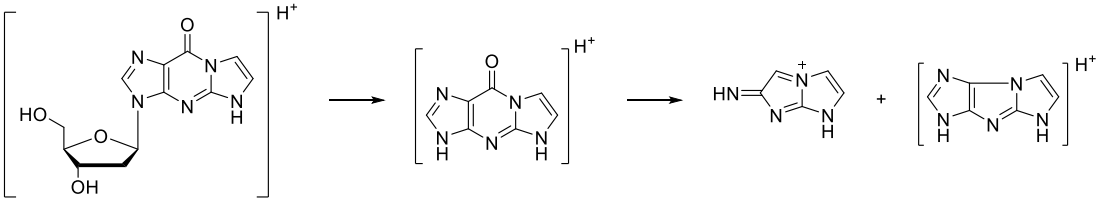
[$^{15}N_5$]-εdA



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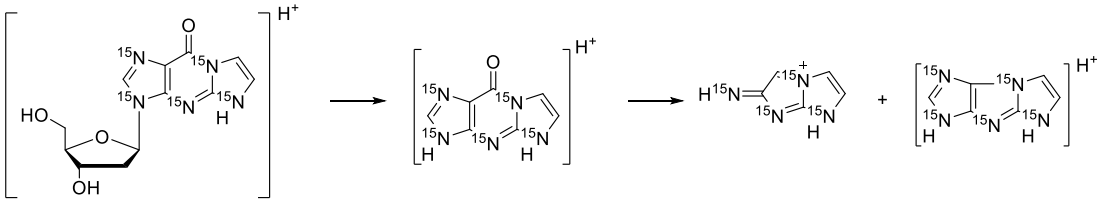
εdG



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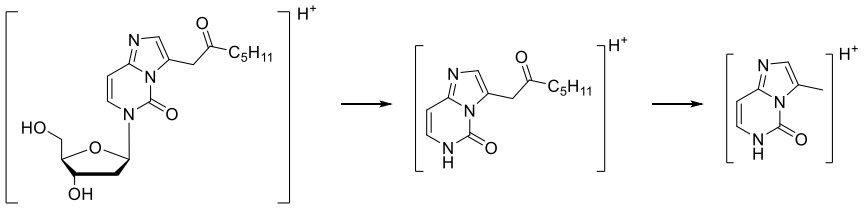
[$^{15}N_5$]-εdG



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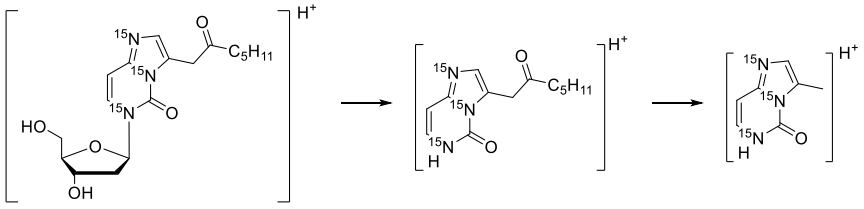
HεdC



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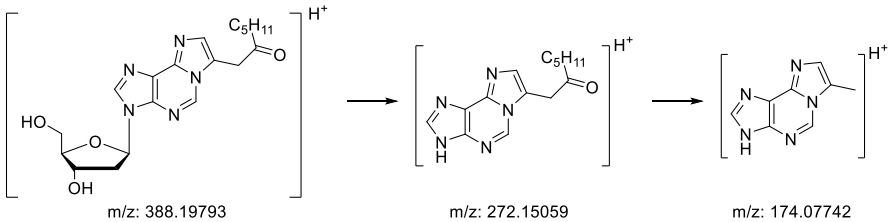

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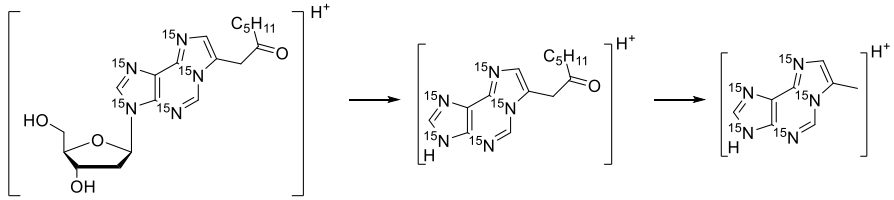
HεdA



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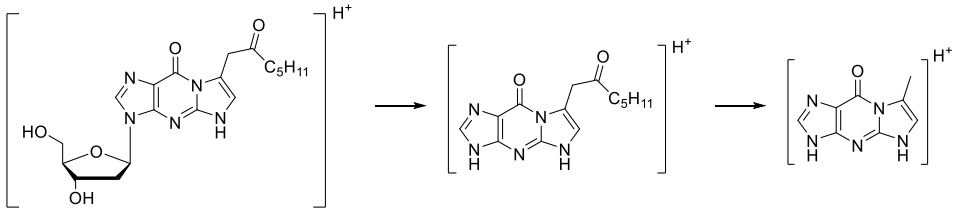
[$^{15}N_5$]-HεdA



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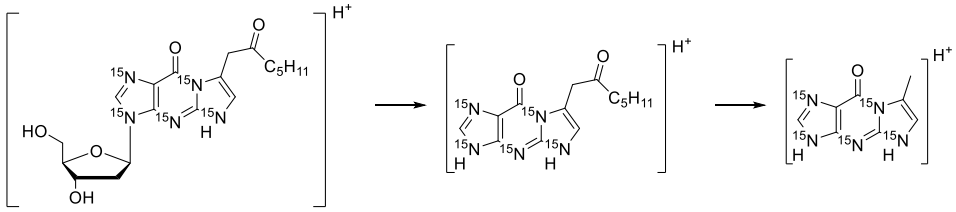
HεdG



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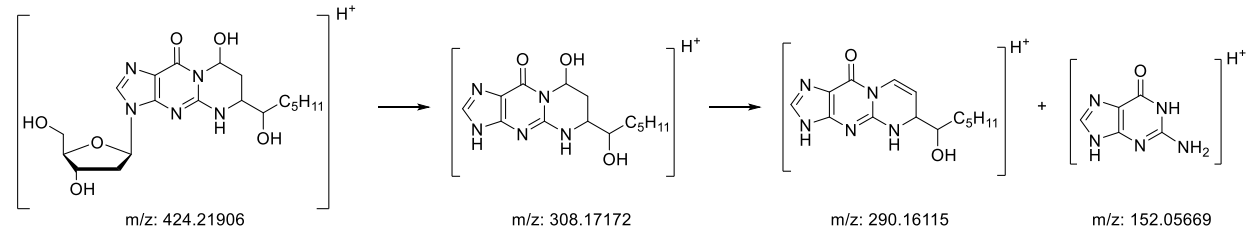
[$^{15}N_5$]-HεdG



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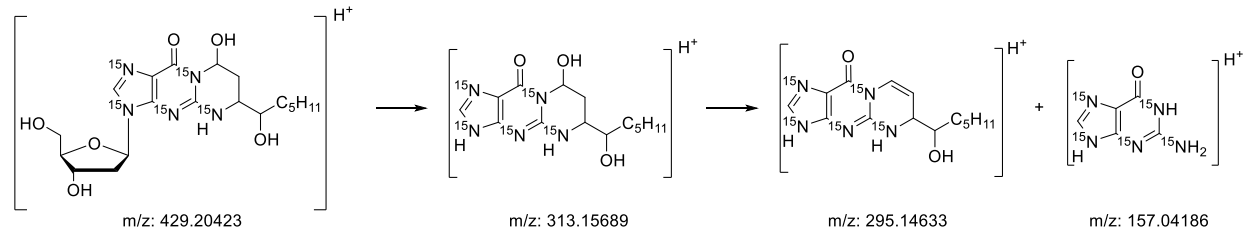
HNE-dG



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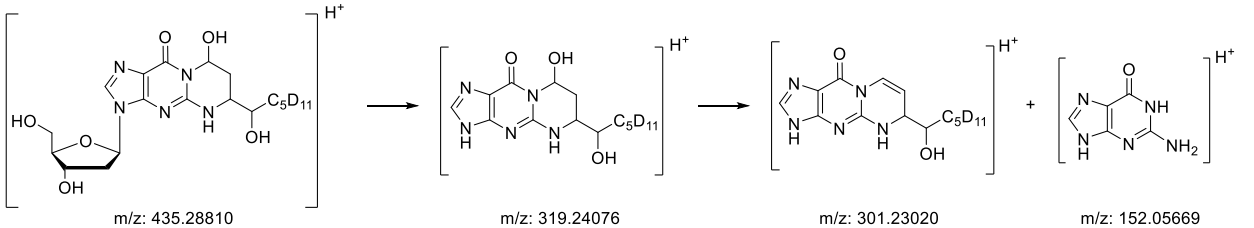
[$^{15}N_5$]-HNE-dG



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[$^{2}H_{11}$]-HNE-dG

^{15}N-labeled bacteria growth protocol

One colony of *Escherichia coli* strain (ATCC® 15224™) was cultured in ^{15}N-labeled minimal medium (5 mL) at 37 ℃ for 36 h and then transferred to ^{15}N-labeled minimal medium (250 mL), cultured at 37 ℃ for another 14 h. The OD600 was used to monitor bacterial growth until the growth curve reached a plateau. The bacteria culture was centrifuged at 3000*g* for 30 min at room temperature. The bacterial pellet was resuspended in 50% glycerol in bacterial medium solution, snap-frozen with liquid nitrogen, and stored in −80 ℃ until use.

Recipe of minimal medium

Per liter of the minimal medium contains: glycerol: 21.5 g; KH_2PO_4: 1.6 g; NaH_2PO_4: 5.3g; $(^{15}NH_4)_2SO_4$: 0.7 g; $MgSO_4.7H_2O$: 0.3 g; sodium citrate: 0.5 g; $CaCl_2{\cdot}2H_2O$: 750 μg; Na_2EDTA: 30 mg; $FeCl_3{\cdot}6H_2O$: 25 mg; $CuSO_4{\cdot}5H_2O$: 240 μg; $MnSO_4{\cdot}5H_2O$: 180 μg; $ZnSO_4{\cdot}7H_2O$: 27 μg; $CoCl_2$: 270 μg. A solution containing glycerol and the phosphate salts was made separately, the pH adjusted to 7.0, and autoclaved. The other nutrients were added as a filter-sterilized solution after the autoclaved media had cooled.

Bacterial DNA isolation method

The ^{15}N-labeled bacterial pellets were thawed on ice and centrifuged for 15 min at 3000*g* at 4 ℃. To the bacterial pellet was added TE buffer containing freshly added antioxidant cocktail (10 mM BME, 0.5 mM GSH, 0.1 mM DFO, and 0.1 mM BHT), and the mixture was pipetted several times to make a homogenous suspension. An aliquot of 2 mL bacterial cell suspension was added to a 15 mL Falcon tube and the bacteria cells were lysed using an ultrasonic processor (Sonics Vibra-Cell™ Ultrasonic Liquid Processor, VCX 130, with 3 mm OD tip). While keeping the tubes on ice, dip the ultrasonic probe into the 2-ml bacterial suspension so that the probe is about 2 to 3 mm from the bottom of the tube. Sonicate at 20 kHz over 15 seconds, with 100% amplitude and 1s on/1s off pulse mode (30 seconds for one cycle). Leave the tube on ice for 45 seconds to keep the sample cool and then sonicate again using the above parameters. After ten cycles, the solution turned clear. Subsequently, the tubes were centrifuged at 3000*g* for 20 min to remove any insoluble particles and a trace amount of unlysed cells. The supernatant was divided into aliquots of 500 μL and transferred to 1.5 mL Eppendorf tubes for DNA isolation using the **PUR** method. The same cocktail of antioxidants was used in the DNA isolation procedure.

Figure S2: Efficiency of ^{15}N-incorporation in bacterial DNA.

Isolated *E. Coli* DNA (10 μg) was completely digested to the four deoxyribonucleosides with method **D-a**. Two vol. of ethanol was added to the DNA digestion mixture containing 10 μg DNA to precipitate the enzymes. The tube was vortexed and centrifuged for 10 min at 21,000*g*, and the supernatant was dried under vacuum centrifugation. The residue was dissolved in 400 μL water and injected to an Agilent 1260 Infinity HPLC. Solvents that constituted the mobile phase were (A) 0.05% formic acid in water; (B) acetonitrile. An Eclipse Plus C18 column (4.6 × 100 mm, 3.5 μm, Agilent Technologies) was used to separate the components at a flow rate of 1 mL/min, with a 10 min linear gradient from 1% to 10% B. The signal was measured at UV wavelength 260 nm. Four peaks corresponding to the ^{15}N-labeled dC, dA, dT, and dG were collected, diluted with 0.04% formic acid in 20% acetonitrile/water, and infused into a Thermo LTQ XL™ Linear Ion Trap Mass Spectrometer at a flow rate of 3 μL/min. Source Voltage: 4.5 kV; Sheath Gas Flow Rate: 8 arb; Capillary Voltage: 9.5 V; Capillary Temp: 275 V; Tube Lens Voltage: 52V. Precursor ions of each deoxyribonucleoside, including the different numbers of ^{15}N incorporated (+n), underwent collision-induced dissociation (CID) energy. The intensity of each product ion $[M + H - 116]^+$. Isolation width (IW), activation Q (ActQ), and CID are labeled in each figure.

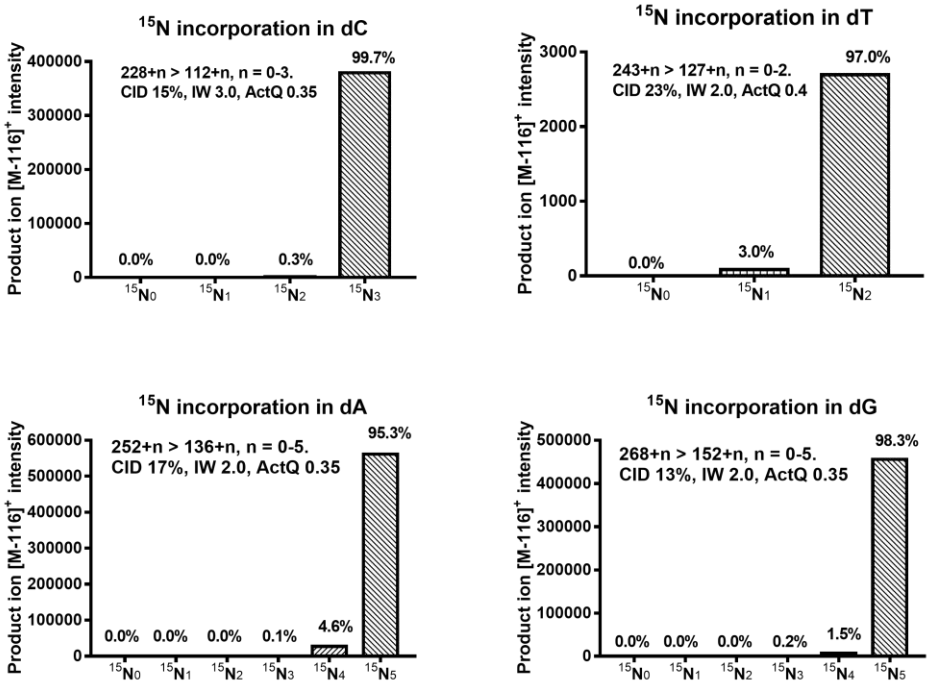

Table S1 ^{15}N-LPO adduct levels (per 10^8 nts) formed in ^{15}N-DNA incubated with or without the nuclear pellet of rat kidney tissue homogenate.

Note: The number in parentheses is the millimolar concentration of AO. The effect of most antioxidants was evaluated using the same batch of ^{15}N-DNA. Due to the lack of material, the effect of TEMPO was evaluated in a separate batch of ^{15}N-DNA. The effect of DFO (0.1 mM) was evaluated a second time using the second batch of ^{15}N-DNA. N = 2.

	Adduct/10^8 nts	$^{15}N_5$-M$_1$dG		$^{15}N_3$-εdC		$^{15}N_5$-εdA		$^{15}N_5$-εdG		$^{15}N_3$-HεdC		$^{15}N_5$-HεdA		$^{15}N_5$-HεdG	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Batch 1	Ctrl 1 (^{15}N-DNA)	42.7	1.21	0.34	0.13	0.60	0.06	0.99	0.15	1.03	0.11	0.38	0.06	6.58	0.71
	No AO	34.2	3.84	0.35	0.06	0.51	0.03	2.80	0.20	15.2	1.58	5.61	0.61	45.3	10.1
	BME (10)	36.3	1.62	0.42	0.01	0.31	0.03	0.33	0.00	0.77	0.19	0.36	0.05	3.60	0.39
	BHT (0.1)	41.2	4.23	0.52	0.00	0.37	0.06	0.74	0.27	2.76	1.35	1.24	0.49	11.1	3.35
	8-HQ (0.35)	36.4	1.53	0.32	0.00	0.37	0.00	1.39	0.38	4.45	2.74	1.77	1.03	21.6	9.93
	GSH (0.5)	45.3	11.5	0.38	0.04	0.41	0.03	0.81	0.30	4.05	0.94	1.49	0.47	14.2	4.62
	GSH (5)	29.9	14.3	0.61	0.33	0.51	0.18	3.05	0.22	10.3	0.48	3.52	0.28	25.1	4.19
	DFO (0.1)	30.8	0.96	0.33	0.14	0.45	0.25	2.78	2.27	12.2	10.4	4.07	3.41	40.4	33.8
	DFO (1)	25.5	0.60	0.10	0.00	0.39	0.01	2.31	0.27	8.98	0.69	3.35	0.25	33.4	1.77
Batch 2	Ctrl 2 (^{15}N-DNA)	56.4	4.79	0.10	0.00	1.54	0.09	0.33	0.00	3.45	0.15	1.63	0.02	12.9	0.58
	DFO (0.1)	52.9	14.0	0.10	0.00	0.79	0.14	0.93	0.60	36.8	21.6	13.8	7.45	47.5	23.8
	TEMPO (15)	52.7	13.2	0.25	0.15	2.53	0.87	6.76	3.60	129	43.0	48.6	18.9	163	56.1

Table S2 ^{15}N-LPO adduct levels (per 10^8 nts) formed in ^{15}N-DNA incubated with or without the supernatant of rat kidney tissue homogenate.

Note: The number in parentheses is the millimolar concentration of AO. The effect of most antioxidants was evaluated using the same batch of ^{15}N-DNA. Due to the lack of material, the effect of TEMPO was evaluated in a separate batch of ^{15}N-DNA. The effect of DFO (0.1 mM) was evaluated a second time using the second batch of ^{15}N-DNA. N=2.

	Adduct/10^8 nts	$^{15}N_5$-M$_1$dG		$^{15}N_3$-εdC		$^{15}N_5$-εdA		$^{15}N_5$-εdG		$^{15}N_3$-HεdC		$^{15}N_5$-HεdA		$^{15}N_5$-HεdG	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Batch 1	Ctrl 1 (^{15}N-DNA)	113	4.48	0.97	0.04	3.27	0.03	1.40	0.17	29.0	2.08	113	4.48	0.97	0.04
	No AO	137	12.5	1.07	0.33	4.80	0.30	1.93	0.60	45.5	12.5	137	12.5	1.07	0.33
	BME (10)	148	6.00	0.86	0.03	2.75	0.05	1.43	0.04	18.0	0.00	148	6.00	0.86	0.03
	BHT (0.1)	98.5	8.50	0.85	0.12	2.95	0.05	1.27	0.21	20.0	0.00	98.5	8.50	0.85	0.12
	8-HQ (0.35)	127	2.00	1.01	0.09	2.70	0.60	1.40	0.03	22.5	3.50	127	2.00	1.01	0.09
	GSH (0.5)	115	0.00	0.82	0.14	2.95	0.65	1.59	0.25	17.0	1.00	115	0.08	0.82	0.14
	GSH (5)	155	0.00	0.69	0.04	1.90	0.20	0.76	0.32	12.5	1.50	155	0.22	0.69	0.04
	DFO (0.1)	129	10.0	0.82	0.07	5.00	0.60	1.96	0.25	27.0	4.00	129	10.0	0.82	0.07
	DFO (1)	137	2.00	0.55	0.05	3.40	1.40	1.45	0.23	23.5	3.50	137	2.00	0.55	0.05
Batch 2	Ctrl 2 (^{15}N-DNA)	56.4	4.79	1.54	0.09	3.45	0.15	1.63	0.02	12.9	0.58	56.4	4.79	1.54	0.09
	DFO (0.1)	91.1	5.35	0.82	0.02	5.04	0.65	2.53	0.13	21.1	0.30	91.1	5.35	0.82	0.02
	TEMPO (15)	82.6	9.96	1.42	0.05	11.5	2.42	5.17	1.02	43.6	8.39	82.6	9.96	1.42	0.05

Table S3 ^{15}N-LPO adduct levels formed in ^{15}N-dNs incubated with or without the isolated DNA of rat spleen.

Note: The number in parentheses is the millimolar concentration of AO. N=2.

	$^{15}N_5$-εdA		$^{15}N_5$-εdG		$^{15}N_3$-HεdC		$^{15}N_5$-HεdA		$^{15}N_5$-HεdG	
	Adduct/10^8 $^{15}N_5$-dA		Adduct/10^8 $^{15}N_5$-dG		Adduct/10^8 $^{15}N_3$-dC		Adduct/10^8 $^{15}N_5$-dA		Adduct/10^8 $^{15}N_5$-dG	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Ctrl (^{15}N-dNs)	0.10	0.00	0.33	0.00	0.05	0.01	0.11	0	0.29	0.08
pH 7, No AO	2.52	0.09	4.65	0.61	18.1	3.22	11.8	2.54	74.0	18.4
pH 8, No AO	2.27	0.08	6.99	1.32	18.0	0.52	11.5	0.70	68.2	1.75
pH 8, BME (0.5)	0.42	0.03	0.33	0.00	0.06	0.03	0.11	0	0.76	0.04
pH 8, BME (1)	0.30	0.07	0.33	0.00	0.05	0.03	0.11	0	0.72	0.03
pH 8, BME (2.5)	0.10	0.00	0.33	0.00	0.07	0.01	0.11	0	1.20	0.02
pH 7, BME (0.5)	0.73	0.13	0.33	0.00	0.27	0.06	0.14	0.01	0.84	0.12
pH 7, BME (1)	0.54	0.03	0.33	0.00	0.21	0.07	0.11	0.05	0.43	0.05
pH 7, BME (2.5)	0.30	0.06	0.33	0.00	0.22	0.01	0.11	0.00	0.83	0.38
pH 7, GSH (0.5)	0.74	0.00	0.33	0.00	0.49	0.04	0.27	0.00	1.19	0.05
pH 7, GSH (1)	0.64	0.03	0.33	0.00	0.56	0.32	0.35	0.09	1.38	0.20
pH 7, GSH (2.5)	0.59	0.09	0.33	0.00	1.57	0.08	0.92	0.05	1.47	0.09
pH 7, AHA (1)	2.73	0.29	4.16	0.16	20.2	4.32	12.6	3.36	75.4	16.6

Protocol for the synthesis of LPO modified DNA.

Step 1

Synthesis of MDA modified DNA. 1,1,3,3-Tetramethoxypropane (10 μL, 60.2 μmol, Sigma-Aldrich) was mixed with HCl (20 μL, 1 N) and incubated at room temperature for 1 h. The solution (10 μL) was added to potassium phosphate buffer (0.75 mL, 50 mM, pH 7.0) containing calf thymus DNA (1.5 mg, 4.5 μmol) and incubated at 37 °C for 2 days.

Synthesis of HNE modified DNA. (*E*)-4-Hydroxynonenal-dimethylacetal (200 μL, 1 mg, 5 μmol in hexane, Sigma-Aldrich) was dried with nitrogen gas and dissolved in ethanol (45 μL). The solution was mixed with HCl (5 μL, 1 N) and incubated at room temperature for 1 h. Ethanol (50 μL) and potassium phosphate buffer (0.5 mL, 100 mM, pH 7.0) containing calf thymus DNA (1 mg, 3 μmol) was added to the solution and incubated at 37 °C for 3 days.[1]

Synthesis of ONE modified DNA. ONE (provided by Dr. Ian Blair, (University of Pennsylvania, Philadelphia, PA) (4 mg, 26 μmol) was dissolved in ethanol (40 μL) and added to potassium phosphate buffer (0.5 mL, 10 mM, pH 7.0) containing calf thymus DNA (1 mg, 3 μmol). The mixture was incubated at 37 °C for 3 days.

Synthesis of chloroacetaldehyde modified DNA (etheno adducts). Chloroacetaldehyde solution (5 μL, 39 μmol, 50 wt. % in H_2O, Sigma-Aldrich) was added to potassium phosphate buffer (1 mL, 50 mM, pH 7.0) containing calf thymus DNA (1 mg, 3 μmol). The mixture was incubated at 37 °C for 2 days.

Synthesis of acrolein modified DNA. Acrolein dimethyl acetal (10 μL, 84 μmol, Sigma-Aldrich) was mixed with HCl (10 μL, 1 N) and incubated at room temperature for 1 hour. The solution (5 μL) was added to potassium phosphate buffer (1 mL, 50 mM, pH 7.0) containing calf thymus DNA (1 mg, 3 μmol). The mixture was incubated at 37 °C for 36 h.

Step 2

After the above reactions, the excess reagents were extracted with chloroform (700 μL × 2) and the organic phase was discarded. The DNA was precipitated by adding 0.1 vol. of NaCl (5 M) and 1 vol. of chilled isopropanol. The DNA pellet was washed with 70% cold ethanol × 2 and dried under vacuum centrifugation. The pellet was reconstituted in LC-MS water.

The different types of LPO-modified DNA were added to calf thymus DNA (1.14 μg/μL in water). The final DNA mixture contains different types of LPO adducts that were diluted to a quantifiable range. The dilution factors were as follows: MDA modified DNA: 400; HNE modified DNA: 50; ONE modified DNA: 10000; acrolein modified DNA: 300; chloroacetaldehyde modified DNA: 4000.

Table S4 ^{15}N-adduct levels in ^{15}N-DNA incubated with or without the nuclear pellet of rat liver tissue homogenate.

Note: ^{15}N-adduct levels in ^{15}N-DNA incubated with and isolated from the nuclear pellet of rat liver tissue using PUR/$CHCl_3$ or Phe/$CHCl_3$ method with (+) or without (−) BME (10 mM). Directly digested ^{15}N-DNA was used as the control. BME (2.5 mM) was added in the digestion buffer of each condition. N = 3.

Adduct/10^8 nts	$^{15}N_5$-M_1dG		$^{15}N_5$-εdA		$^{15}N_3$-HεdC	
	Mean	SD	Mean	SD	Mean	SD
Ctrl (^{15}N-DNA)	134	9.6	1.584	0.195	0.051	0.012
PUR/$CHCl_3$ + BME	163	13.5	0.682	0.094	0.066	0.001
Phe/$CHCl_3$ + BME	157	6.2	1.186	0.240	0.057	0.004
PUR/$CHCl_3$ - BME	245	101	2.167	0.952	0.120	0.054
Phe/$CHCl_3$ - BME	161	64.9	1.786	0.583	0.138	0.058

Table S5 ^{15}N-adduct levels in ^{15}N-dNs incubated with or without the isolated DNA from rat liver.

Note: ^{15}N-adduct levels in ^{15}N-dNs before (Ctrl) and after incubation with the digestion matrix containing rat DNA (45 μg DNA) with (+) or without (−) BME (2.5 mM). DNA was isolated using **PUR/CHCl$_3$** or **Phe/CHCl$_3$** method with BME (10 mM). N = 3.

Adduct/10^8 nts	$^{15}N_5$-εdA		$^{15}N_3$-HεdC		$^{15}N_5$-HεdA		$^{15}N_5$-HεdG	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Ctrl (^{15}N-dNs)	0.690	0.163	0.043	0.015	0.041	0.004	0.330	0.000
PUR/$CHCl_3$ + BME	0.270	0.038	0.041	0.022	0.062	0.013	0.330	0.000
Phe/$CHCl_3$ + BME	0.496	0.167	0.035	0.006	0.062	0.011	0.330	0.000
PUR/$CHCl_3$ - BME	0.474	0.131	0.085	0.013	0.106	0.004	1.137	0.245
Phe/$CHCl_3$ - BME	1.747	0.201	0.127	0.009	0.250	0.044	2.869	0.464

Figure S3. Artifact formation during DNA storage.

Rat liver DNA was isolated by **PUR**, **PUR/CHCl$_3$**, or **Phe/CHCl$_3$** method with BME (10 mM). DNA pellet was washed with 70% ethanol in HEPES buffer (5 mM, pH 8.0) containing freshly added BME (2.5 mM). The dried DNA pellet was stored in the dark at room temperature for 0 h, 69 h, or 144 h, and then immediately stored in −80 ºC. At 144 h, the DNA was digested using method **D-b** and quantitated with ^{15}N-labeled ISTD.

n = 3. Dashed lines show the limit of quantitation for each method.

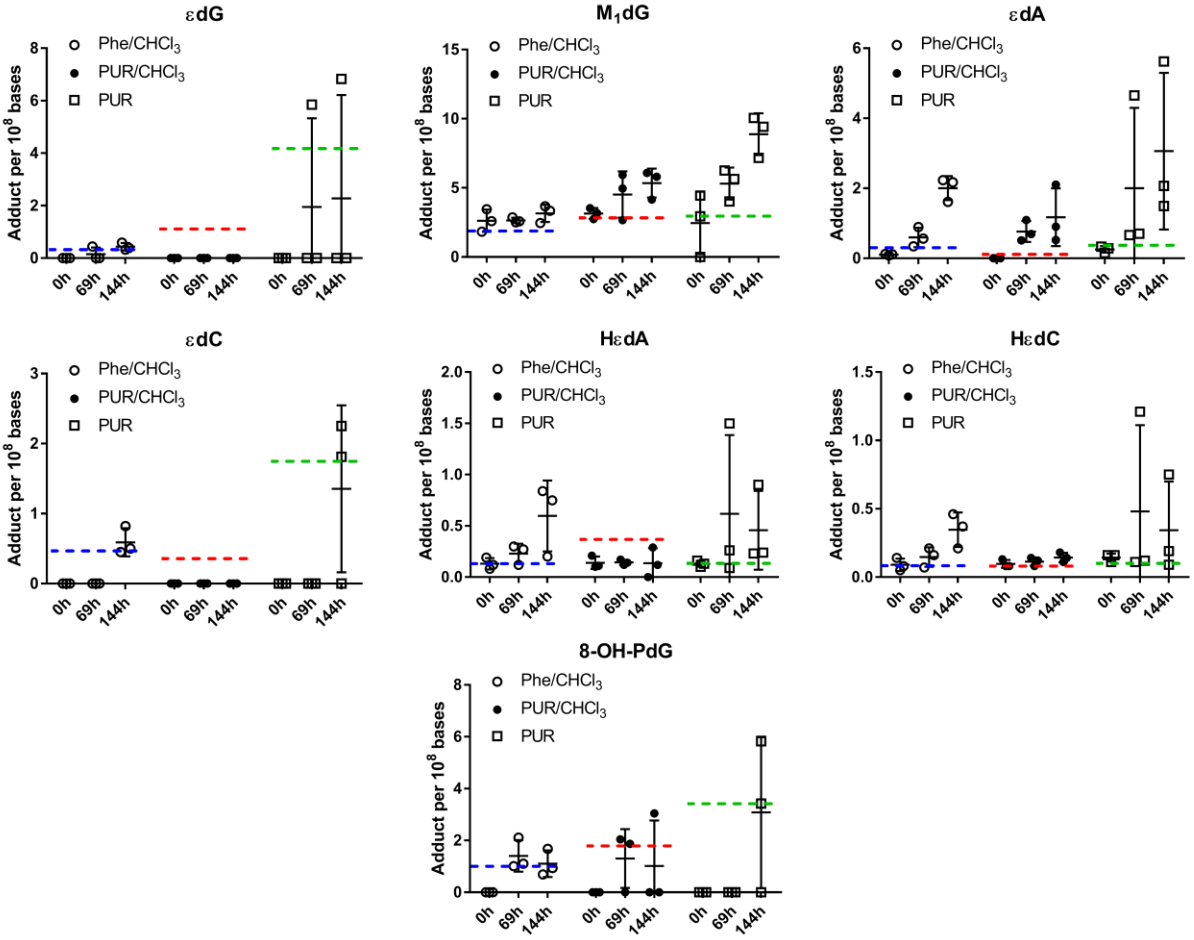

Figure S4. LPO adduct levels in commercial CT DNA before or after incubating in water at 37 ºC for 24 h.

Commercial CT DNA was incubated in water at 37 ºC for 24 h, then digested using method **D-b** with BME (n= 3 independent assays). Freshly prepared CT DNA was digested using the same method as the control group. The level of adducts after incubation was compared to the control group with a two-tailed T Test. ***: $p < 0.001$.

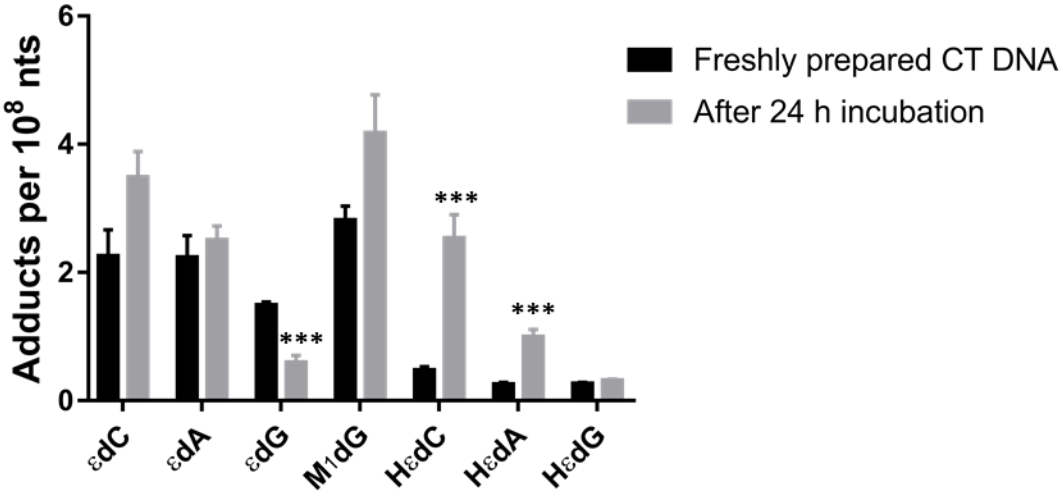

Figure S5. Calibration curves of LPO adducts.

Seven-point calibration curves were generated by spiking ^{15}N-labeled or ^{2}H-labeled internal standards (25 fmol/45 μg DNA, 1.83 adduct per 10^7 nts) and authentic standards (n = 3) in liver DNA of unttreated rats isolated by optimized **PUR/CHCl$_3$** method with BME (10 mM) and digested with **D-b** with BME (2.5 mM) as the AO. The level of spiked authentic standards, peak area ratio to internal standards and CV% at each calibration level are reported in Table S6. The background level of adducts (Table S7) was subtracted from each calibration level. The least-squares linear regression was used to fit the experimental data with a weighting factor of 1/y.

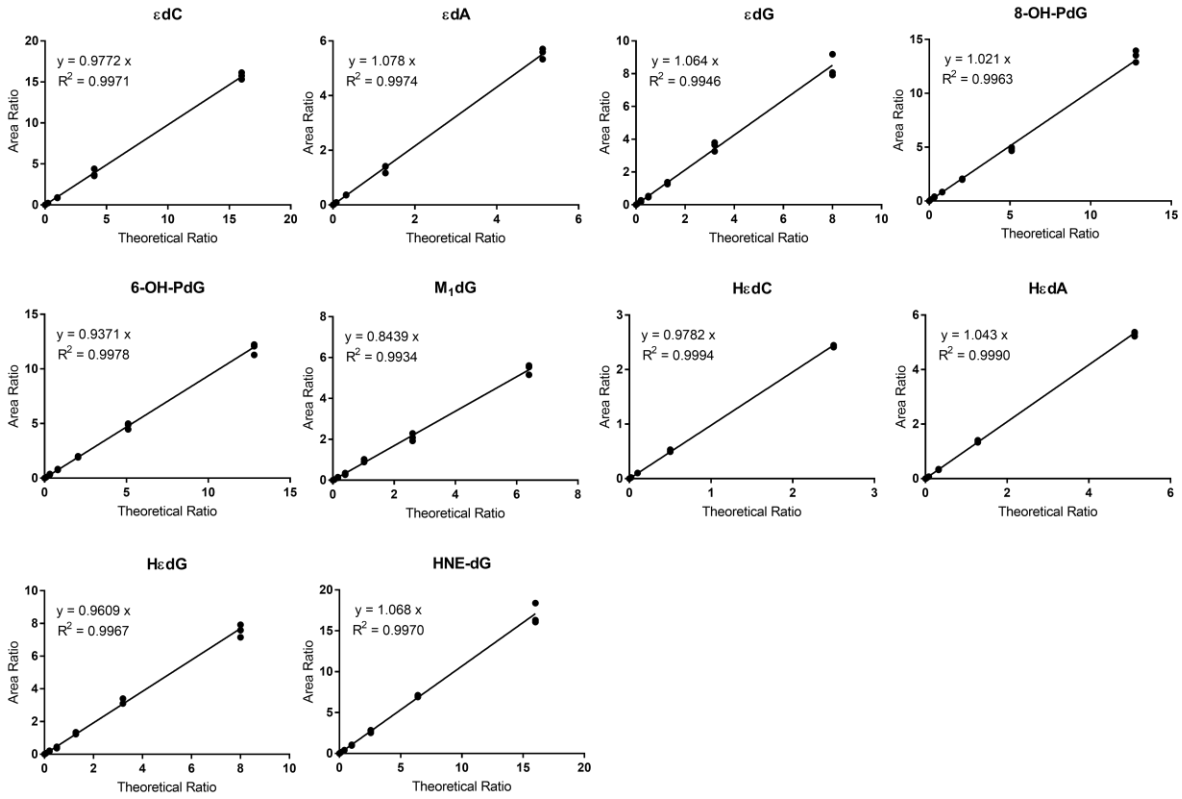

Table S6. Detected area ratio of each calibration point with CV% (n=3 data points per calibrant level).

LOQ levels are marked in **bold**.

	Adduct/10^8 nts	Area Ratio	CV%		Adduct/10^8 nts	Area Ratio	CV%
8-OH-PdG	0	0	0.0	HεdC	0	0	0.0
	1.76	0.13	12.7		0.02	0.0010	7.5
	5.86	0.36	10.9		**0.07**	0.0041	11.3
	14.6	0.85	3.6		0.4	0.021	6.3
	37.5	2.00	2.9		1.8	0.10	2.2
	93.8	4.83	3.3		9.2	0.50	4.6
	234	13.45	4.0		46	2.42	0.8
6-OH-PdG	0	0	0.0	HεdA	0	0	0.0
	1.76	0.10	18.7		0.11	0.0053	13.0
	5.86	0.34	7.8		**0.37**	0.017	5.1
	14.6	0.78	5.5		1.5	0.070	3.0
	37.5	1.96	2.8		5.9	0.34	3.8
	93.8	4.78	5.7		23.5	1.38	3.2
	234	11.86	4.3		94	5.31	1.4
εdC	0	0	0.0	HεdG	0	0	0.0
	0.33	0.024	31.0		**1.10**	0.058	10.3
	1.1	0.055	34.0		3.67	0.19	11.3
	4.4	0.23	4.0		9.2	0.43	11.7
	18.3	0.91	5.7		23.5	1.27	5.1
	73.3	3.87	11.9		58.7	3.22	5.1
	293	15.74	2.6		147	7.56	5.1
εdA	0	0	0.0	M_1dG	0	0	0.0
	0.11	0.0063	38.8		0.88	0.020	42.3
	0.37	0.021	12.5		**2.94**	0.13	12.9
	1.5	0.092	9.9		7.36	0.31	13.2
	5.9	0.36	1.7		18.8	0.97	7.8
	23.5	1.33	10.6		47.0	2.10	8.7
	94	5.55	3.5		118	5.43	4.5
εdG	0	0	0.0	HNE-dG	0	0	0.0
	1.10	0.073	1.9		**2.20**	0.14	2.1
	3.67	0.22	20.0		7.33	0.43	5.5
	9.2	0.50	6.7		18.3	1.04	5.8
	23.5	1.33	4.5		46.9	2.71	6.2
	58.7	3.57	7.8		117	7.03	1.5
	147	8.39	8.3		293	16.92	7.5

Table S7 Background LPO adduct level in rat liver

Background level of LPO adduct was observed in the rat liver used for constructing the calibration curve (n=3). Although these adducts are below the LOQ, we subtracted these values from each calibration point to increase the accuracy of the calibration curve. Other adducts are below the limit of detection.

	Detected Adduct/10^8 nts
M_1dG	1.0 ± 0.35
εdA	0.082 ± 0.02
HεdA	0.024 ± 0.01
HNE-dG	0.37 ± 0.05

References

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