Supporting Information for

"Quantification of N^2-Carboxymethyl-2'-deoxyguanosine in Calf-thymus DNA and Human Kidney Cells by Capillary LC Tandem Mass Spectrometry Coupled with Stable Isotope-dilution Method"

by

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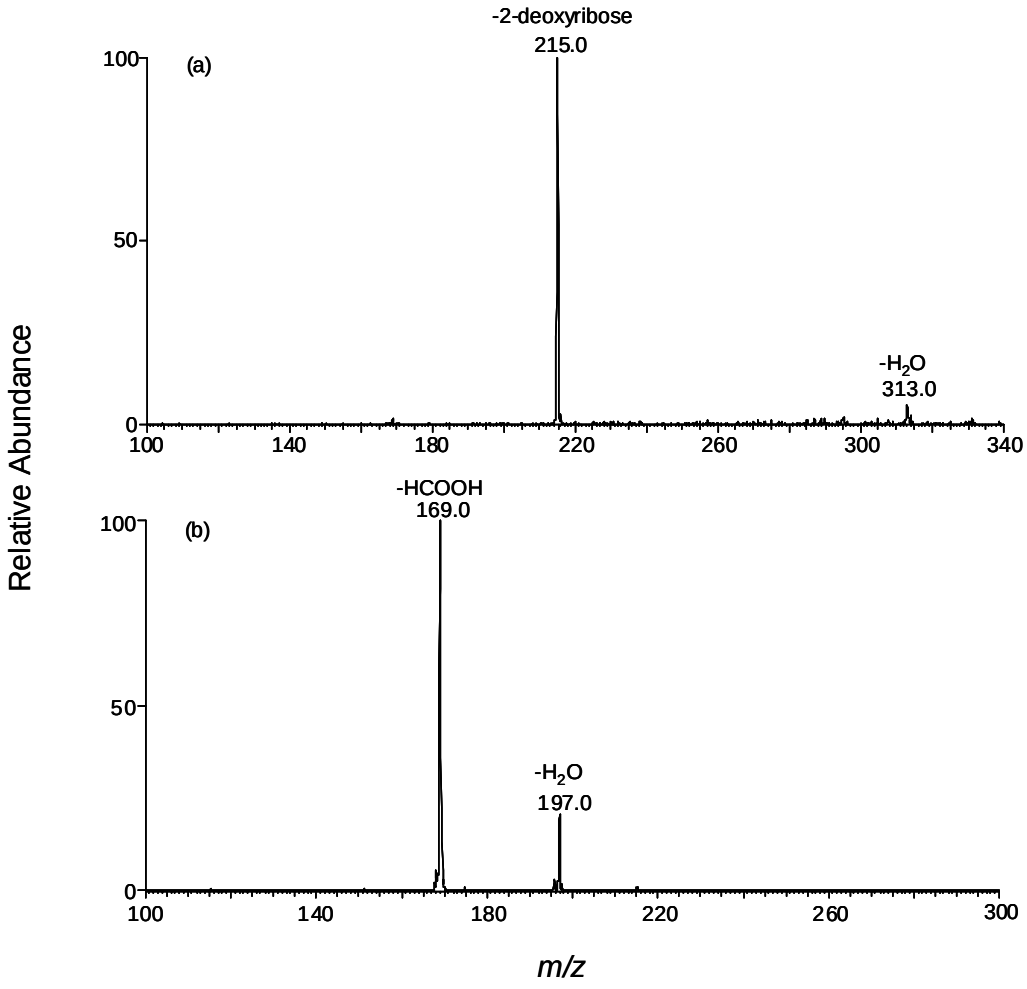

Figure S1. ESI-MS/MS (a) spectrum of the $[M+H]^+$ ion and MS^3 (b) spectrum of the ion of *m/z* 215.0 observed in MS/MS of synthetic [U-$^{15}N_5$]-N^2-CMdG.

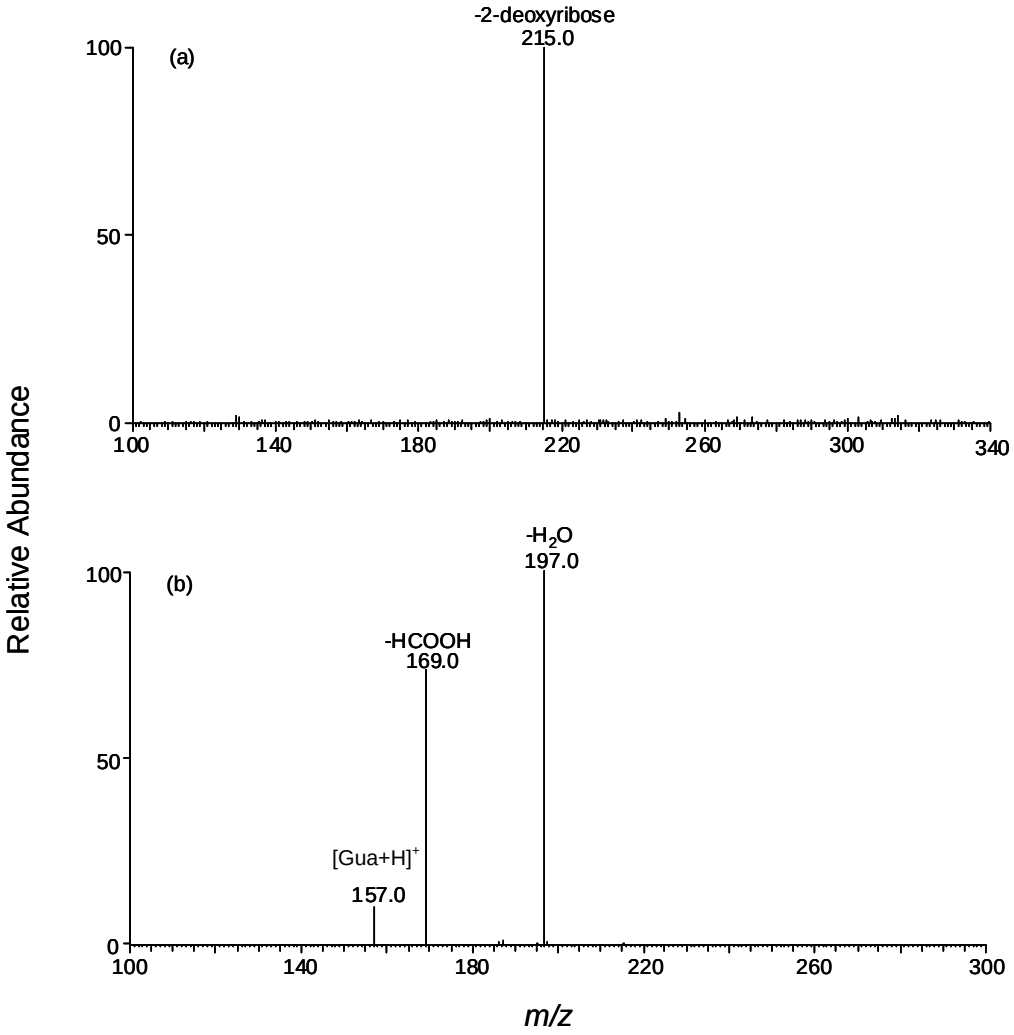

Figure S2. ESI-MS/MS of the $[M+H]^+$ ion of [U-$^{15}N_5$]-1,N^2-glyoxal-dG (a), and shown in (b) is the MS^3 of the ion of *m/z* 215.0 found in (a).

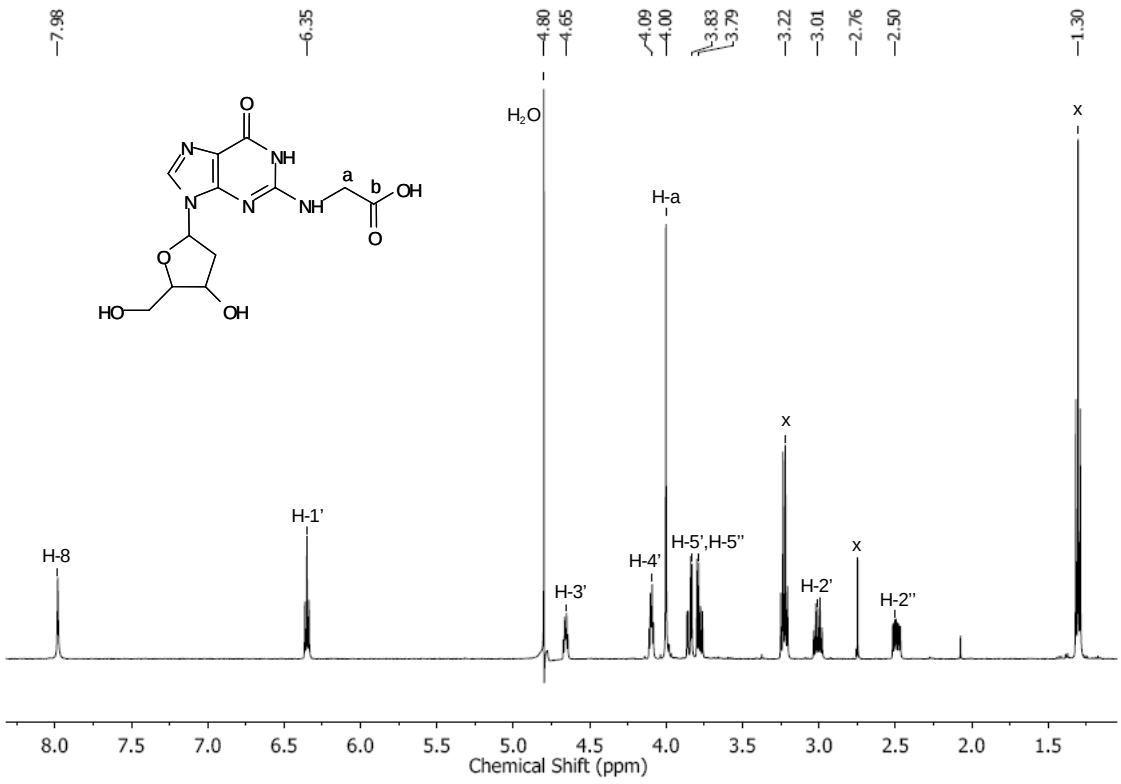

Figure S3. ^{1}H-NMR spectrum of standard N^2-CMdG (500 MHz, D_2O, 25 °C): δ 7.98 (s, 1H, H-8), 6.35 (t, 1H, H-1'), 4.65 (m, 1H, H-3'), 4.09 (m, 1H, H-4'), 4.00 (s, 2H, CH_2), 3.83 (m, 1H, H-5'), 3.79 (m, 1H, H-5''), 3.01 (m, 1H, H-2'), 2.50 (m, 1H, H-2''). "H-a" represents the carboxymethyl proton, and peaks marked with 'x' are from impurities.

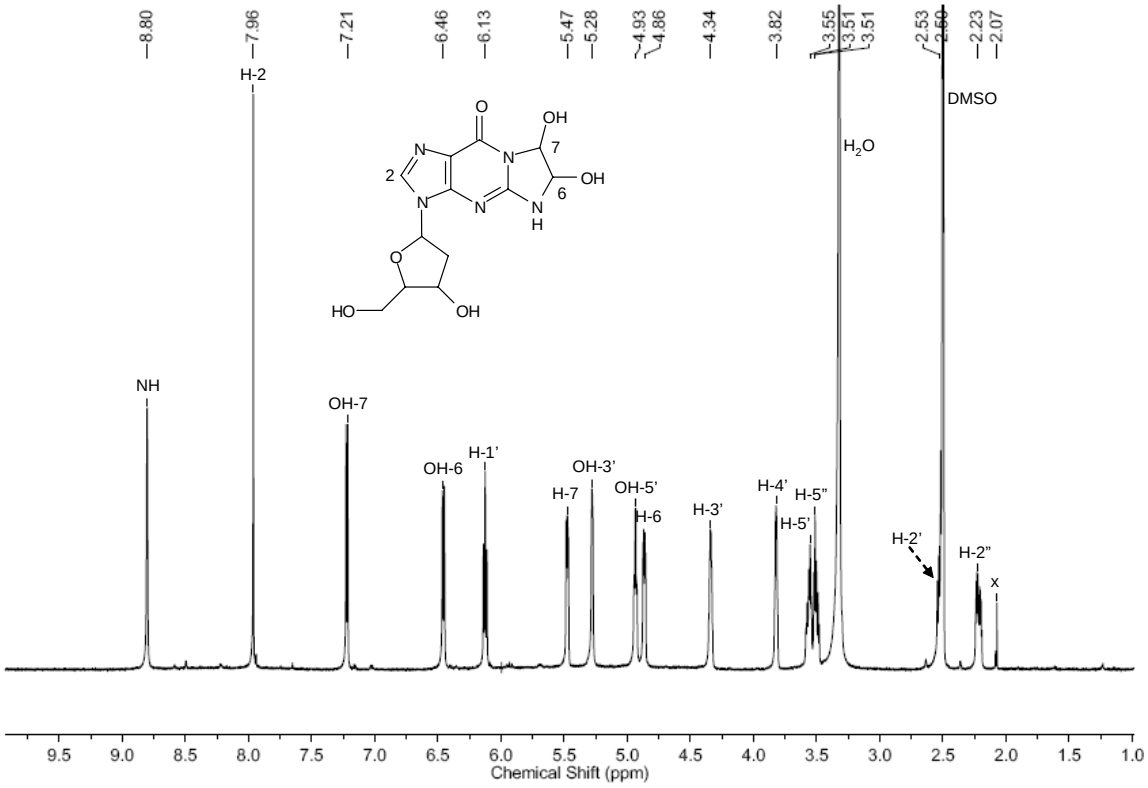

Figure S4. ^{1}H-NMR spectrum of standard 1,N^2-glyoxal-dG (600 MHz, DMSO, 25 °C): δ 8.80 (s, 1H, NH), 7.96 (s, 1H, H-2), 7.21 (d, 1H, OH-7), 6.46 (d, 1H, OH-6), 6.13 (m, 1H H-1'), 5.47 (m, 1H, H-7), 5.28 (d, 1H, OH-3'), 4.93 (t, 1H, OH-5'), 4.86 (m, 1H, H-6), 4.34 (m, 1H, H-3'), 3.82 (m, 1H, H-4'), 3.55 (m, 1H, H-5'), 3.51 (m, 1H, H-5"), 2.53 (m, 1H, H-2'), 2.23(m, 1H, H-2"). The peak marked with 'x' is from impurities.

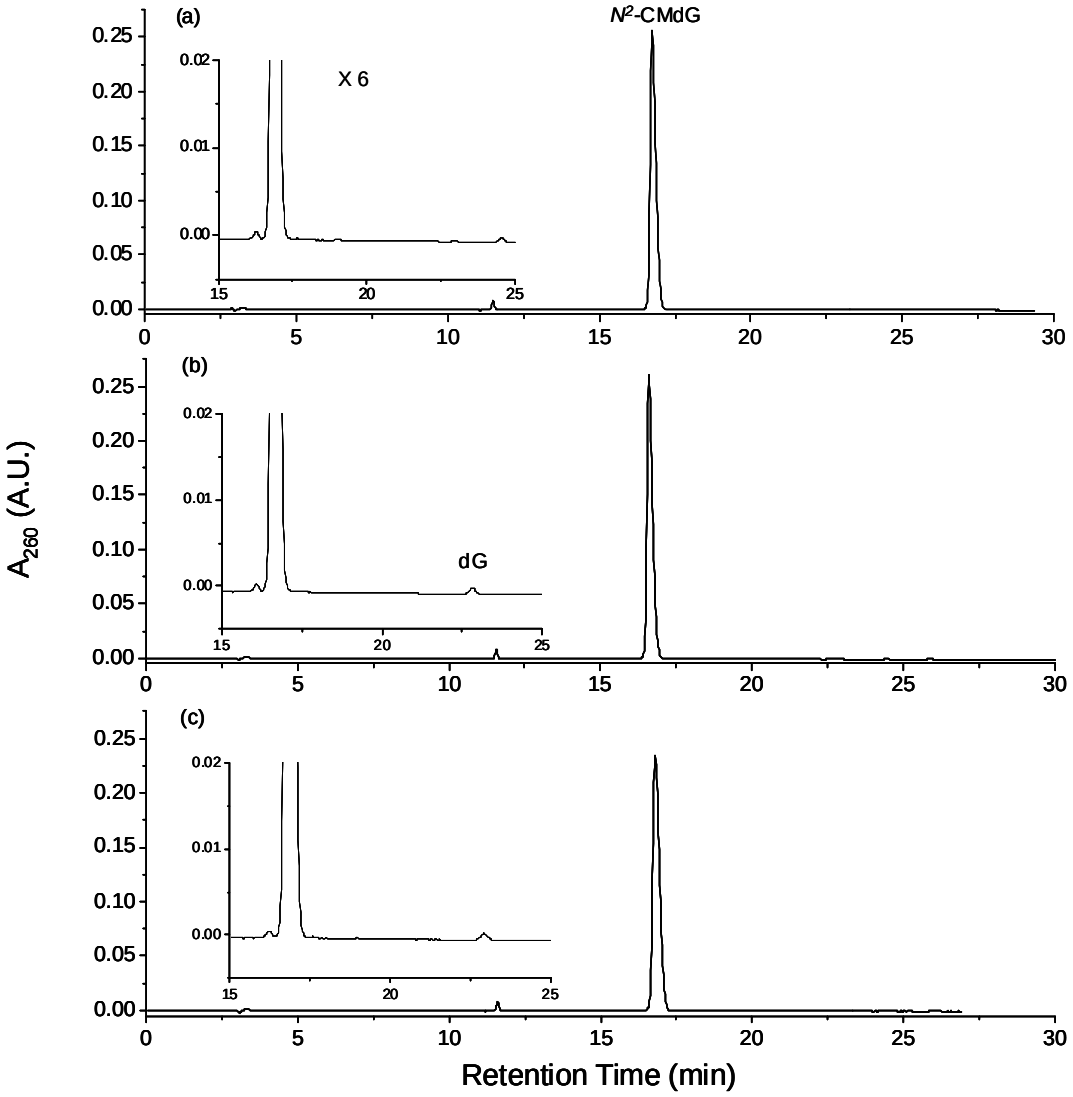

Figure S5. HPLC traces for the separation of aliquots removed from the N^2-CMdG solution after incubation at 37 °C in PBS buffer for 0 (a), 2 (b) and 7 (c) days, respectively. Shown in the insets are the expanded chromatograms to visualize better the dG peak.

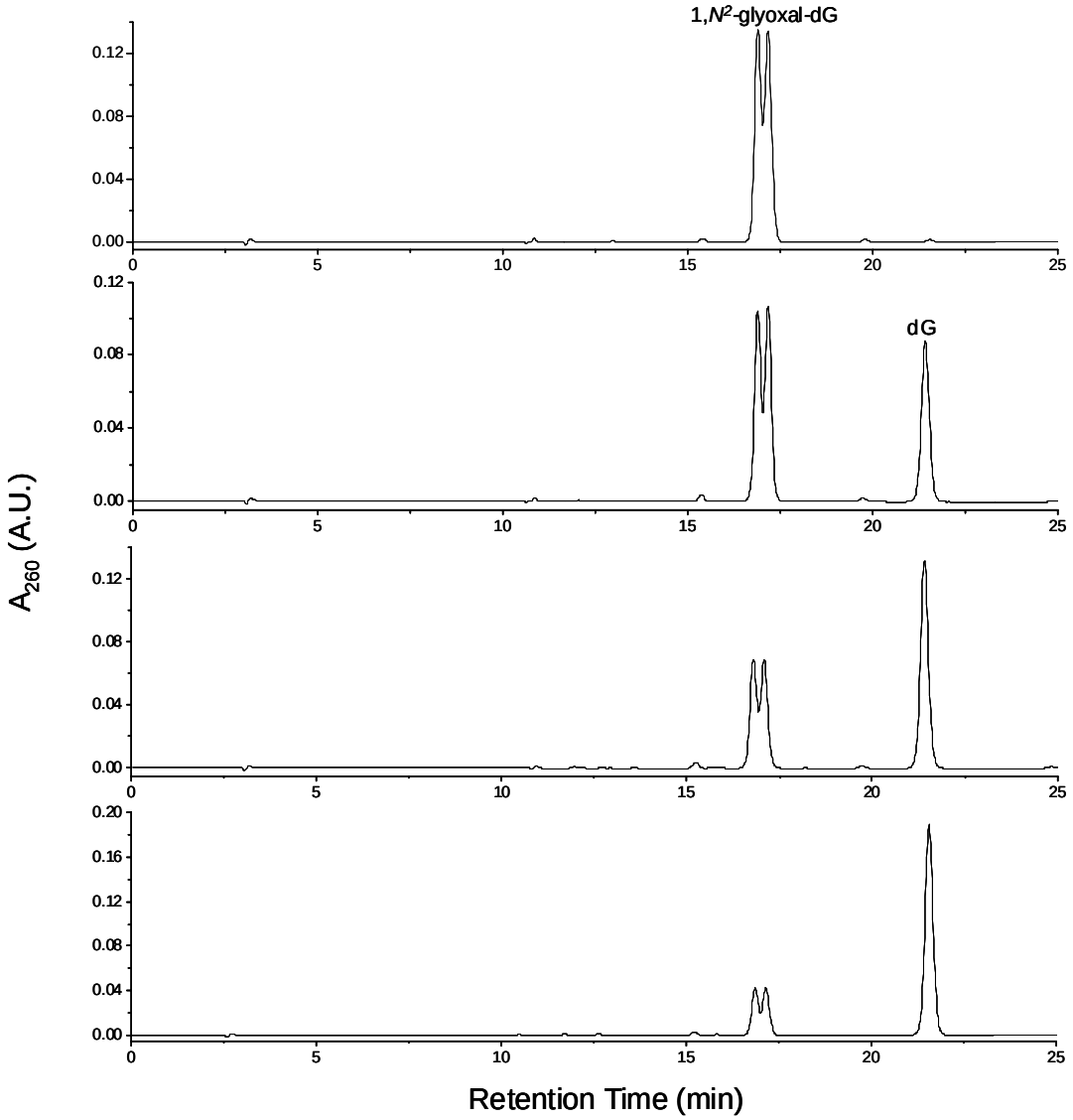

Figure S6. HPLC traces for the separation of aliquots removed from the 1,N^2-glyoxal-dG solution after incubation at 37 °C in PBS buffer for 0 hr (a), 4 hr (b), 8 hr (c) and 2 days (d), respectively. The doublet peaks with the retention time at 16.9 and 17.1 min correspond to the two diastereomers of 1,N^2-glyoxal-dG.

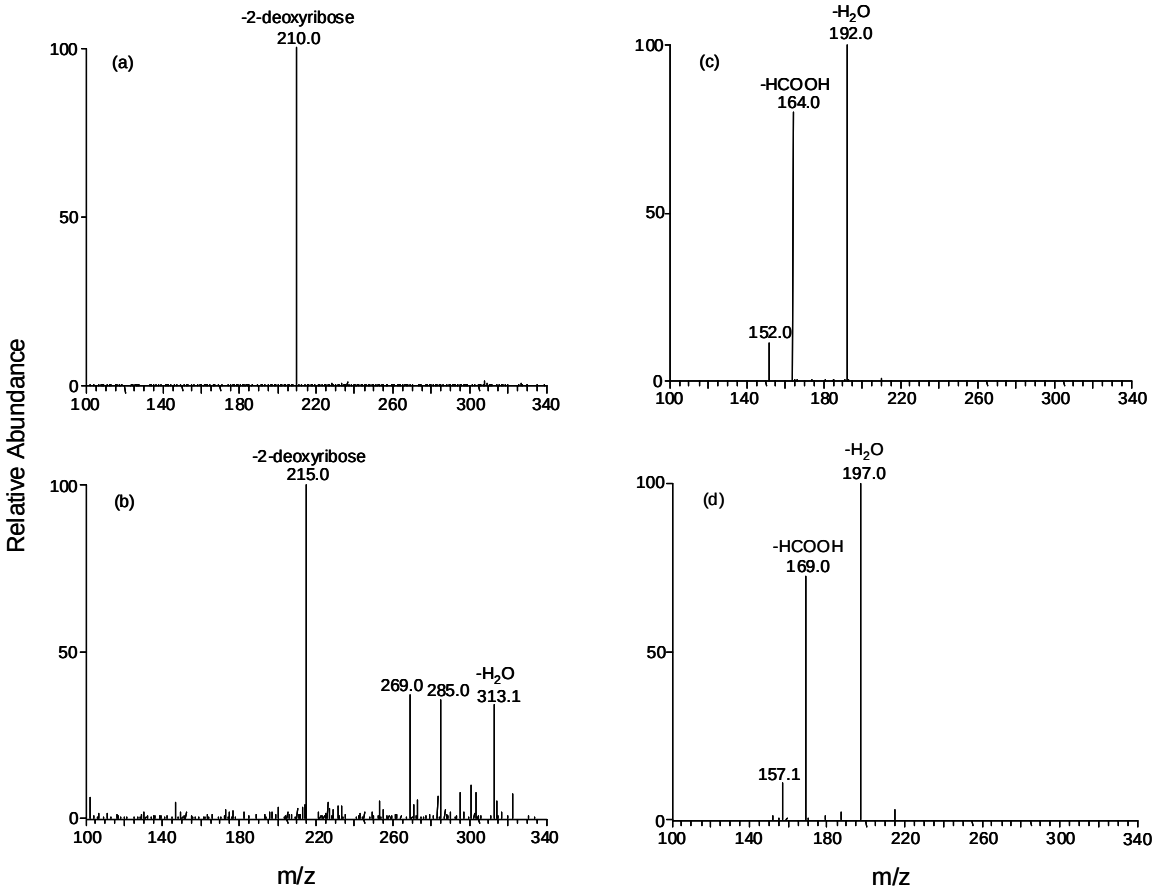

Figure S7. Product-ion spectra of the ion *m/z* 326 (a), *m/z* 331 (b) and MS^3 spectra of *m/z* 210 (c), *m/z* 215 (d). Panels (a) and (c) are for unlabeled, and (b) and (d) are for the [U-$^{15}N_5$]-1,N^2-glyoxal-dG in the digestion mixture of calf thymus DNA treated with 250 μM of glyoxal.

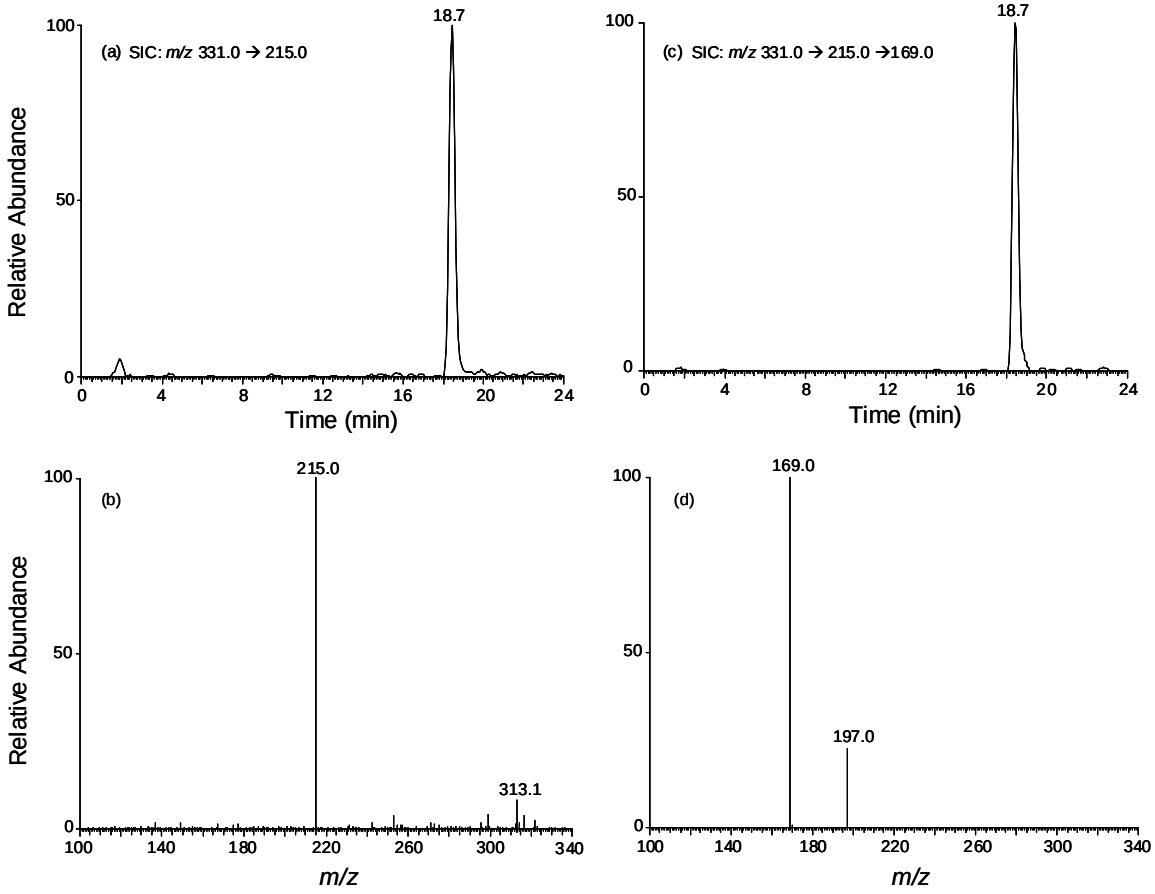

Figure S8. Selected-ion chromatograms (SICs) for monitoring *m/z* 331→ 215 (a) and *m/z* 331→ 215→ 169 (c) transitions for [U-$^{15}N_5$]-N^2-CMdG, and the product-ion spectra of the ion of *m/z* 331 (b) and MS^3 spectra of the ion of *m/z* 215 (d).

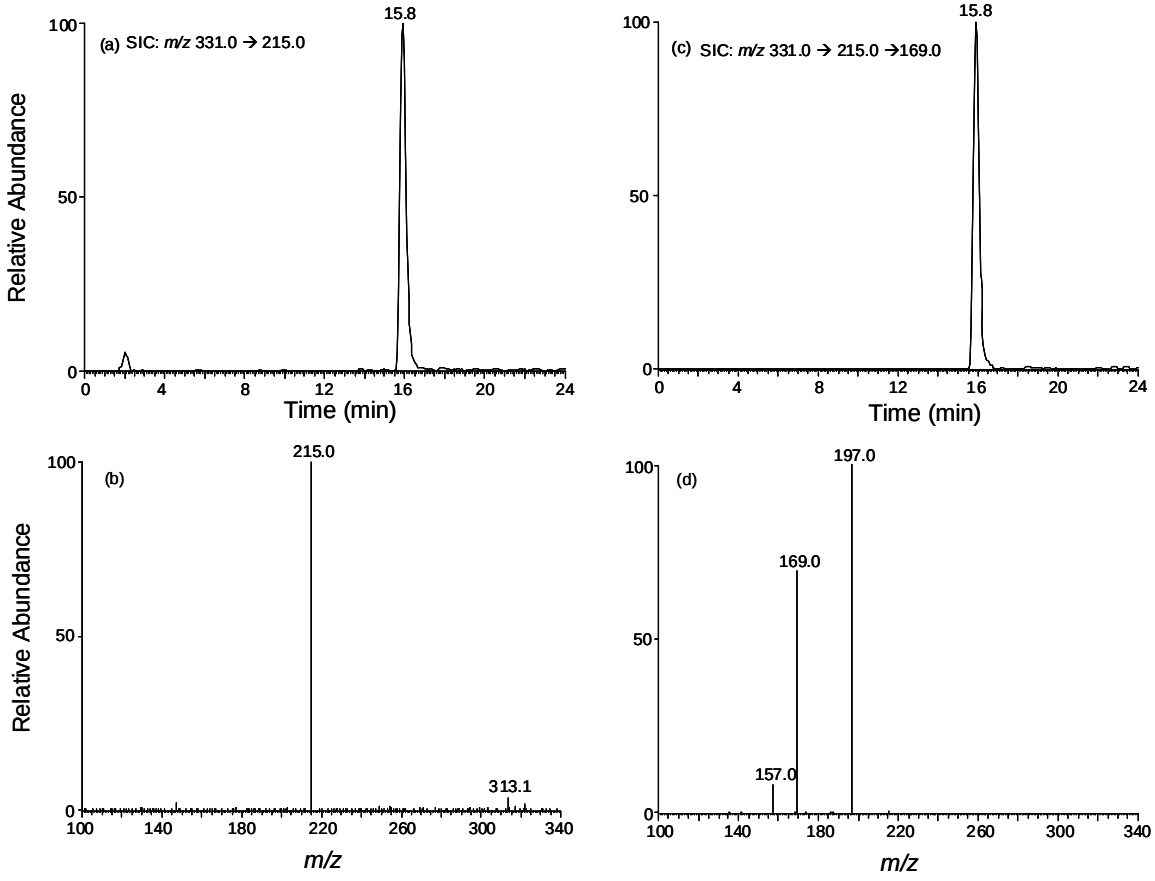

Figure S9. Selected-ion chromatograms (SICs) for monitoring the *m/z* 331→ 215 (a) and *m/z* 331→ 215→ 169 (c) transitions for [U-$^{15}N_5$]-1,N^2-glyoxal-dG and the product-ion spectra of the ion of *m/z* 331 (b) and MS^3 spectra of the ion of *m/z* 215 (d).

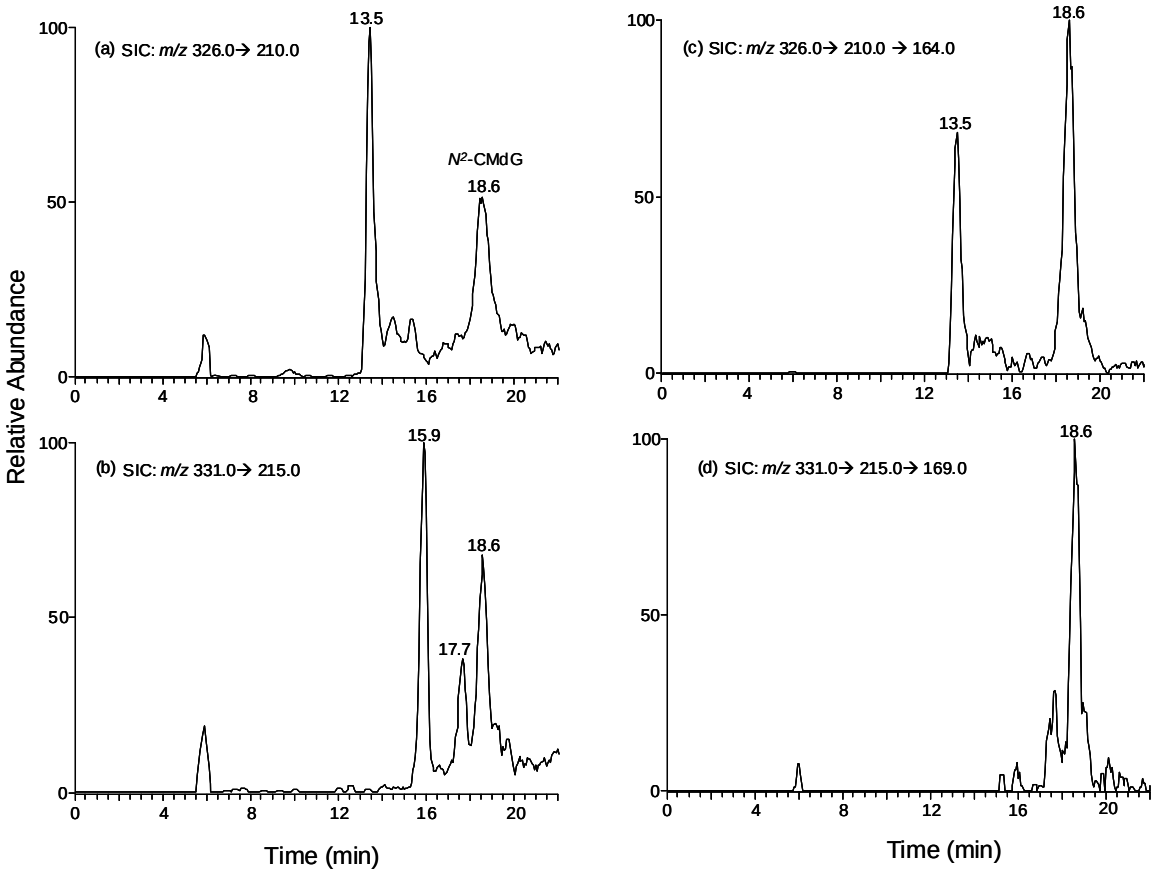

Figure S10. Selected-ion chromatograms for monitoring the *m/z* 326→ 210 (a) and *m/z* 326→ 210→ 164 (c) (for unlabeled N^2-CMdG), *m/z* 331→ 215 (b) and *m/z* 331→ 215→ 169 (d) (for [U-$^{15}N_5$]-N^2-CMdG) transitions of the digestion mixtures of the nuclear DNA extracted from 293T cells, which were treated with 50 μM of glyoxal.

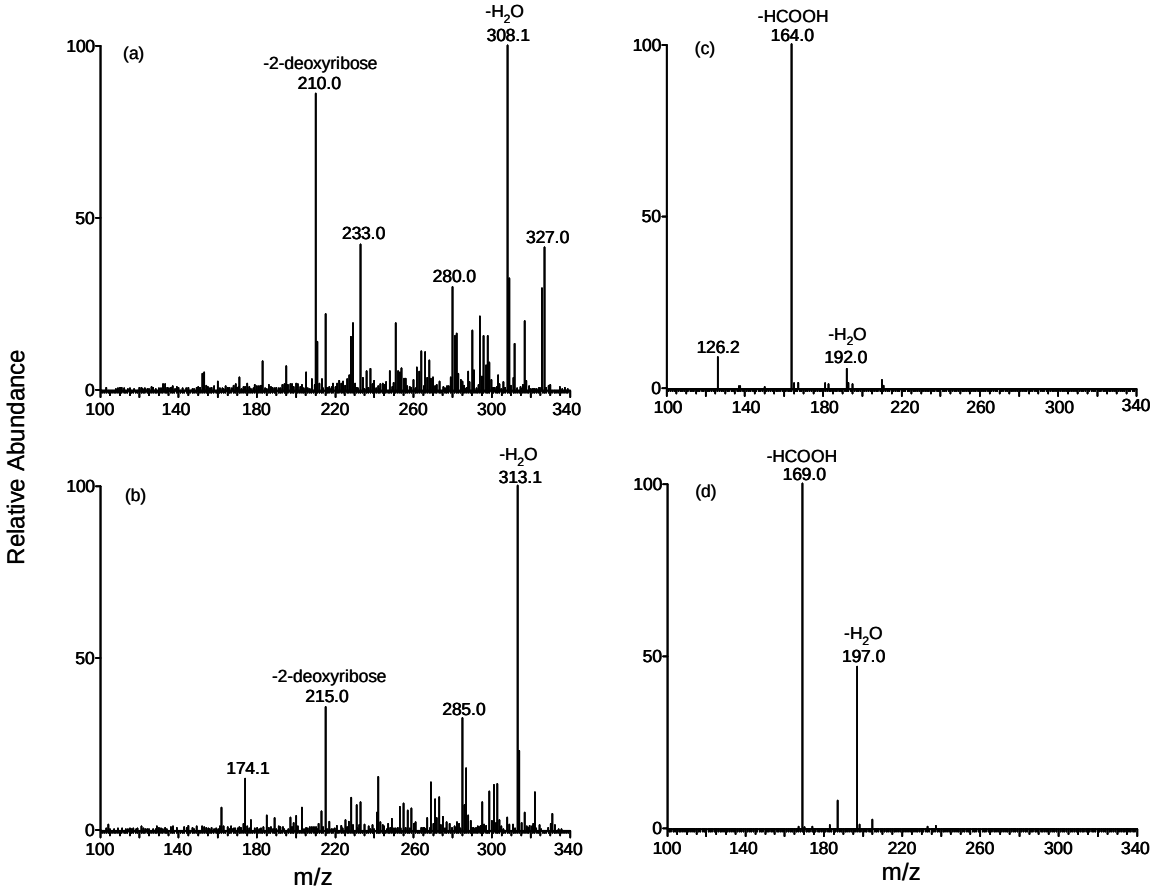

Figure S11. Product-ion spectra of the ion *m/z* 326 (a), *m/z* 331 (b) and MS^3 spectra of *m/z* 210 (c), *m/z* 215 (d). Panels (a) and (c) are for unlabeled, and (b) and (d) are for [U-$^{15}N_5$]-N^2-CMdG in the nuclear DNA from 293T cells, respectively.

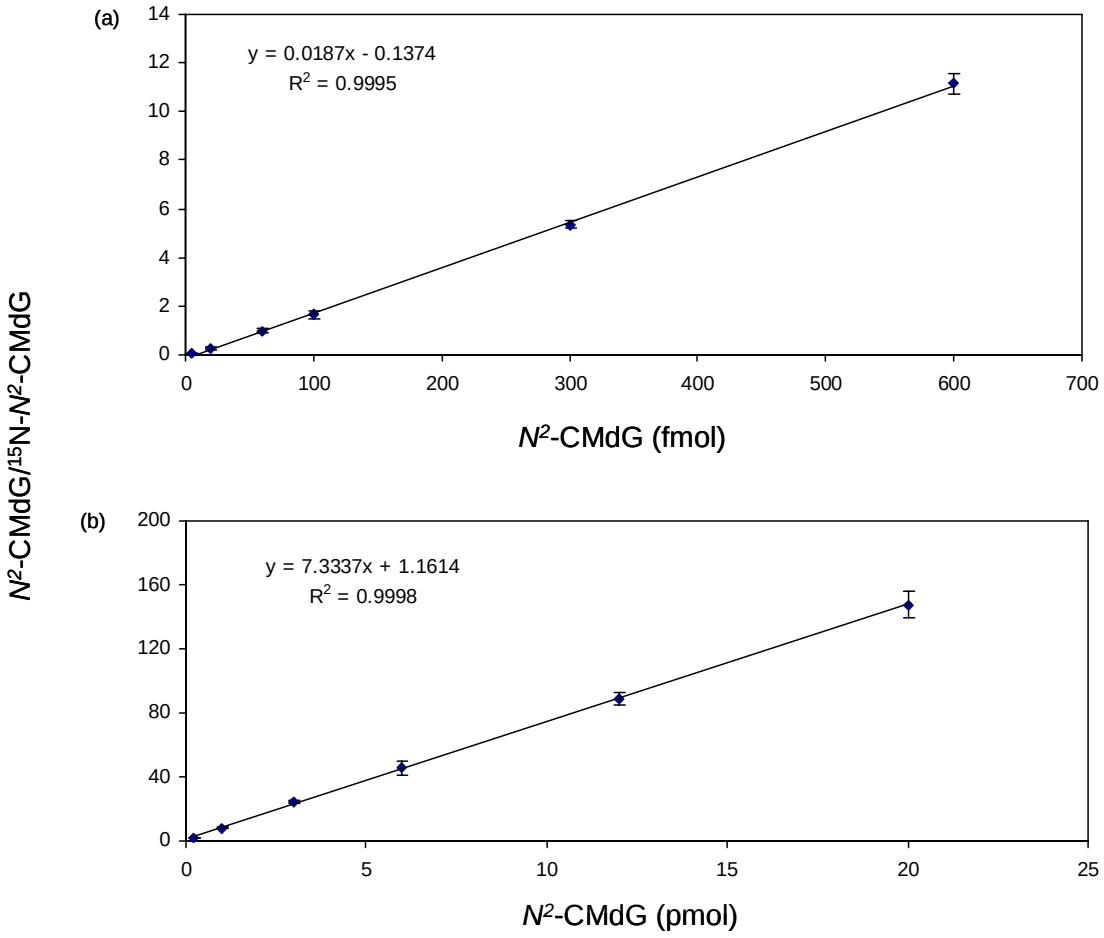

Figure S12. Calibration curves for the quantification of N^2-CMdG. The amounts of [U-$^{15}N_5$]-N^2-CMdG were 55 fmol (a) and 100 fmol (b), respectively.