Supporting Information

Conjugation of Butadiene Diepoxide with Glutathione Yields DNA Adducts *In Vitro* and *In Vivo*

Cho, S-H., and Guengerich, F. P. (2012) *Chem. Res. Toxicol. 25*, 000-000

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Table S3. NMR characterization of DNA adducts in D_2O

Figure S1. Calibration curve for HPLC-ESI+-MS/MS analysis of DEB-GSH conjugate using DEB-[*glycine*-$^{13}C_2$, ^{15}N]-GSH conjugate as internal standard (ISTD).

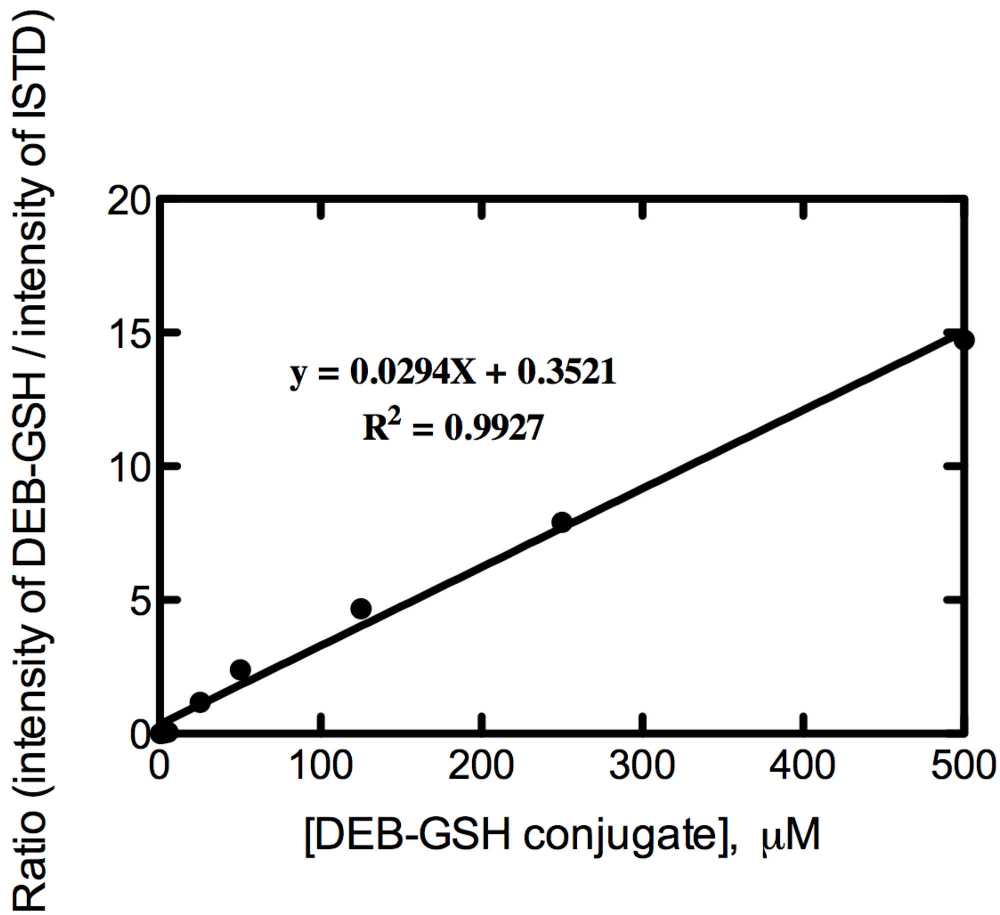


Figure S2. Time dependence of conjugation of DEB with GSH by various GSTs (rat GST 5-5 and human GST T1-1, M1-1, A1-1, A3-3, and P1-1). The concentrations of GSH and GSTs were 5 mM and 10 μg/mL, respectively.

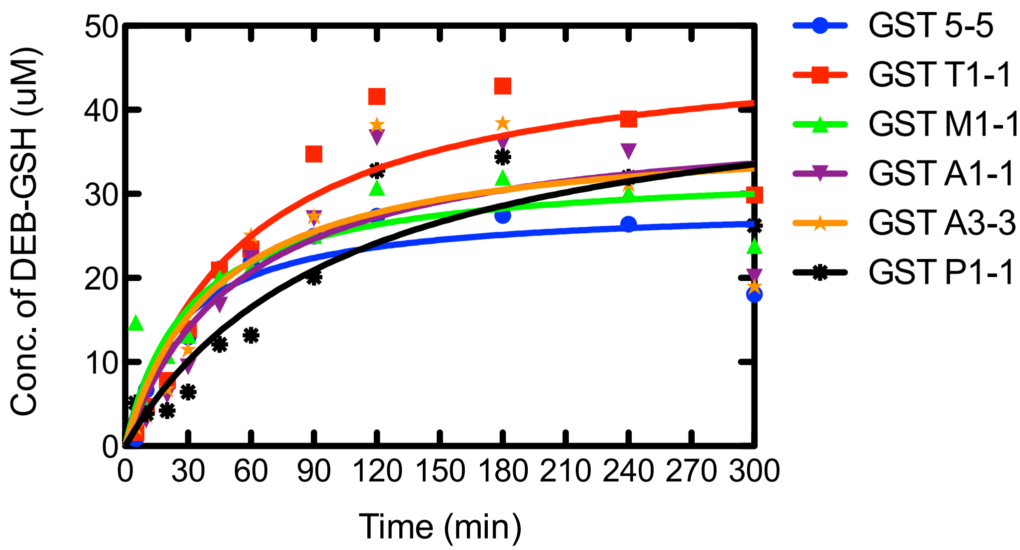


Figure S3. Lack of toxicity of butadiene-derived epoxides in *S. typhimurium* TA1535

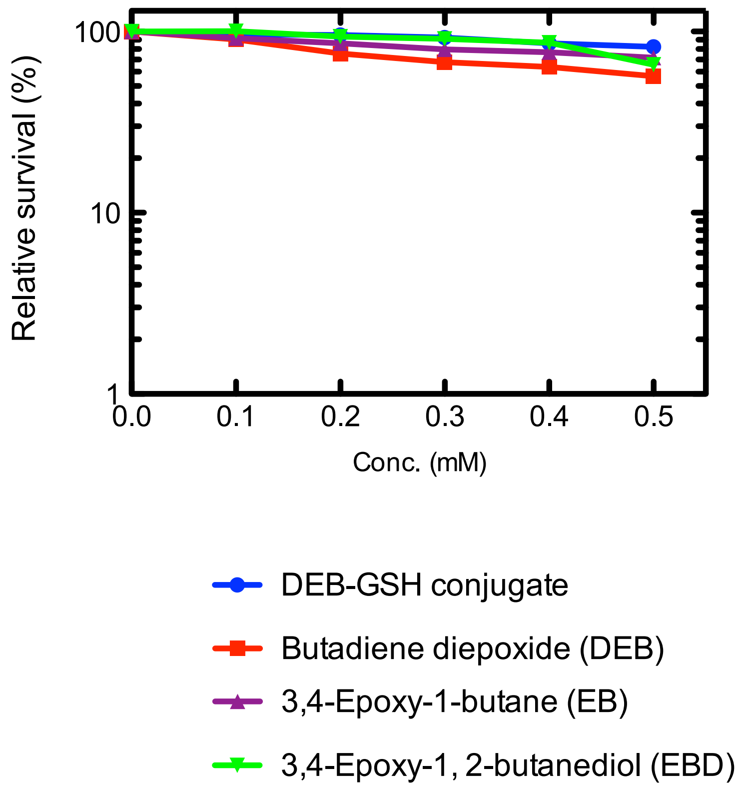


Figure S4. HPLC-MS chromatograms of DNA adducts formed by reaction of DEB-GSH conjugate with nucleosides.

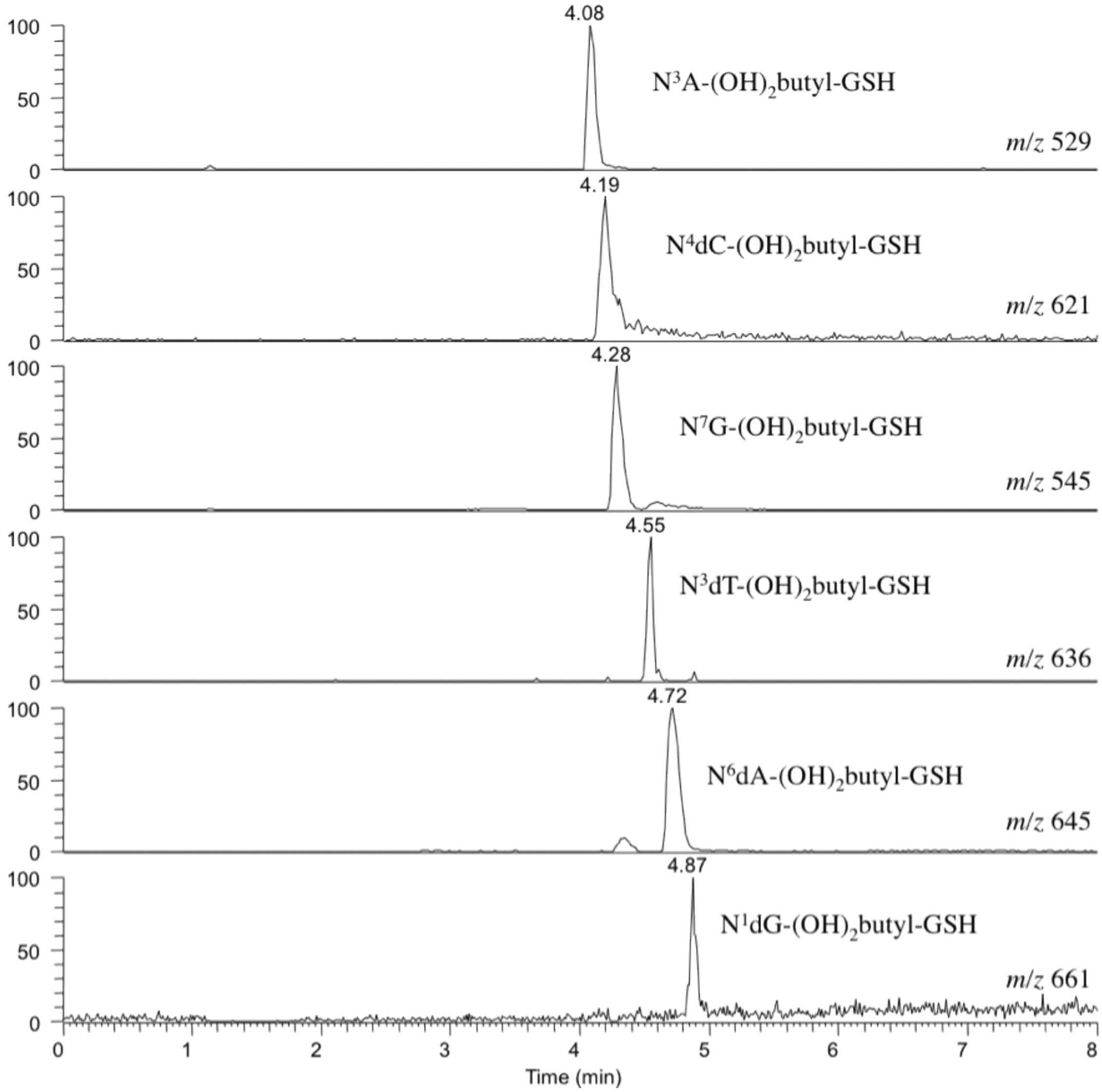


Figure S5. MS/MS spectra of N^3A-(OH)$_2$butyl-GSH (A), N^6dA-(OH)$_2$butyl-GSH (B), N^7G-(OH)$_2$butyl-GSH (C), N^1dG-(OH)$_2$butyl-GSH (D), N^4dC-(OH)$_2$butyl-GSH (E), and N^3dT-(OH)$_2$butyl-GSH (F).

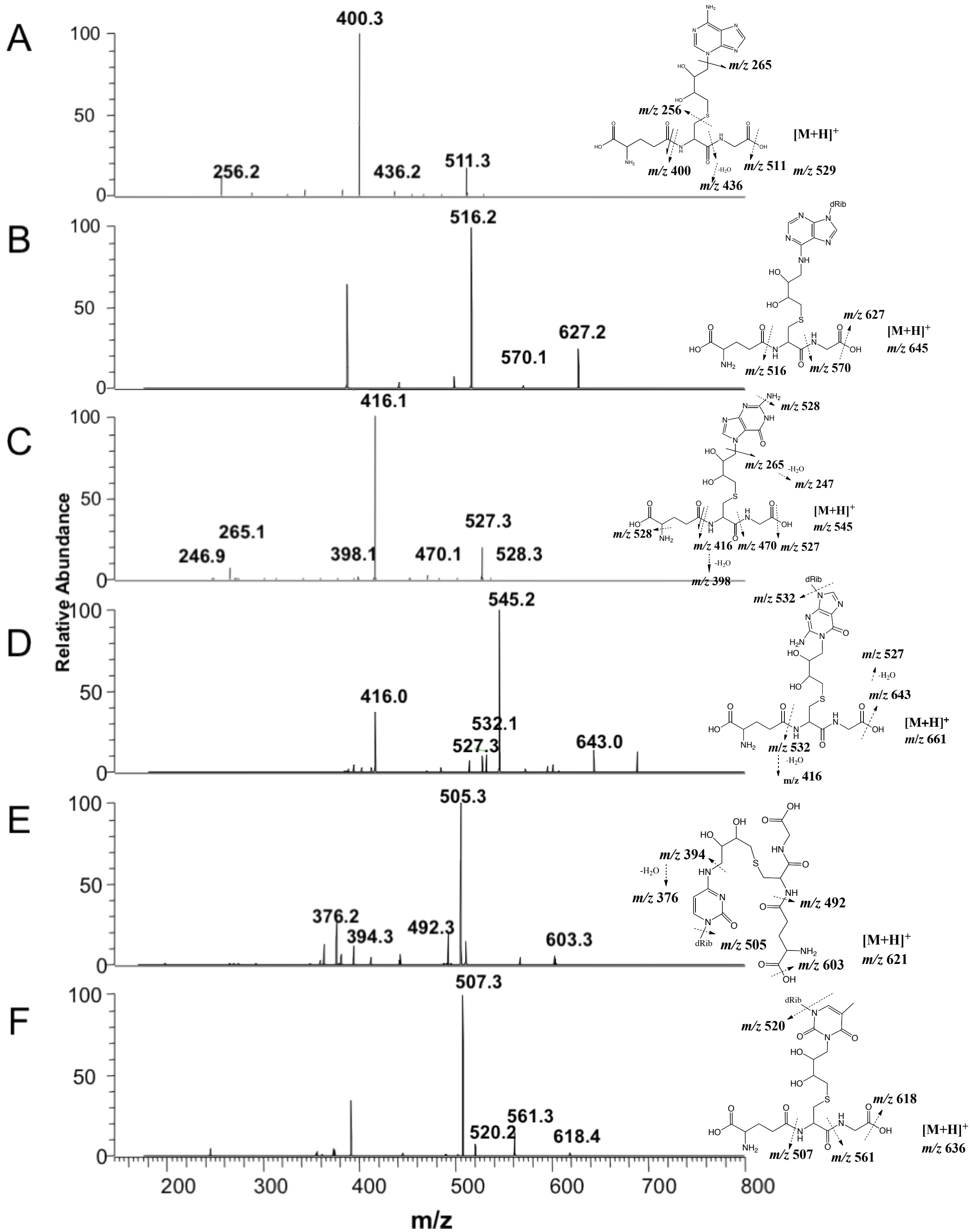


Figure S6. UV absorption spectra of N^3A-$(OH)_2$butyl-GSH (A), N^6dA-$(OH)_2$butyl-GSH (B), N^7G-$(OH)_2$butyl-GSH (C), N^1dG-$(OH)_2$butyl-GSH (D), N^4dC-$(OH)_2$butyl-GSH (E), and N^3dT-$(OH)_2$butyl-GSH (F) at acidic (0.1 M HCl; red line), neutral (H_2O; green line), and alkaline (0.1 M NaOH; blue line) conditions.

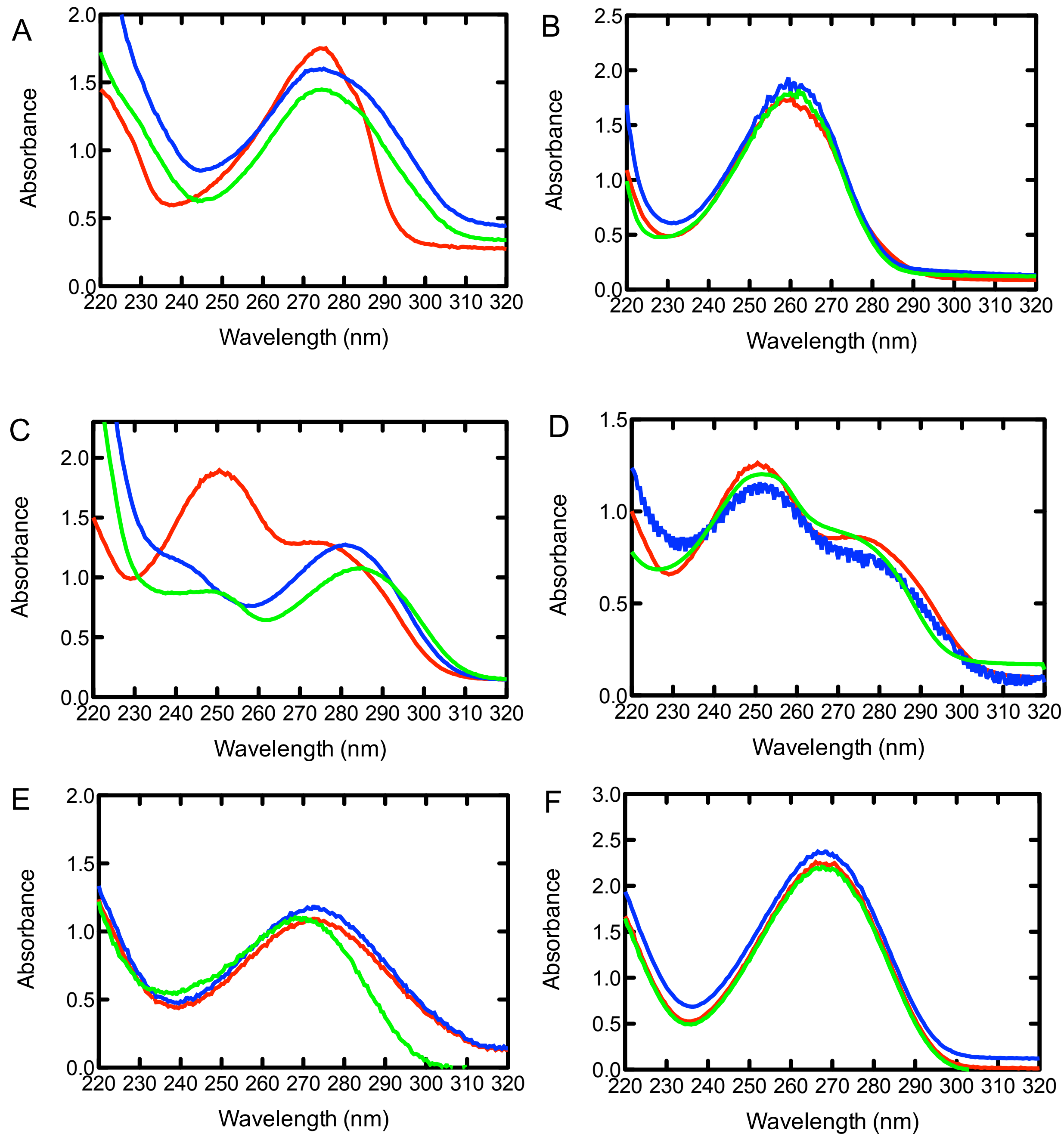


Figure S7. HPLC separation of DNA adducts from the reaction of DEB-GSH conjugate with deoxyadenosine (A), deoxyguanosine (B), deoxycytidine (C), and deoxythymidine (D) followed by neutral thermal hydrolysis.

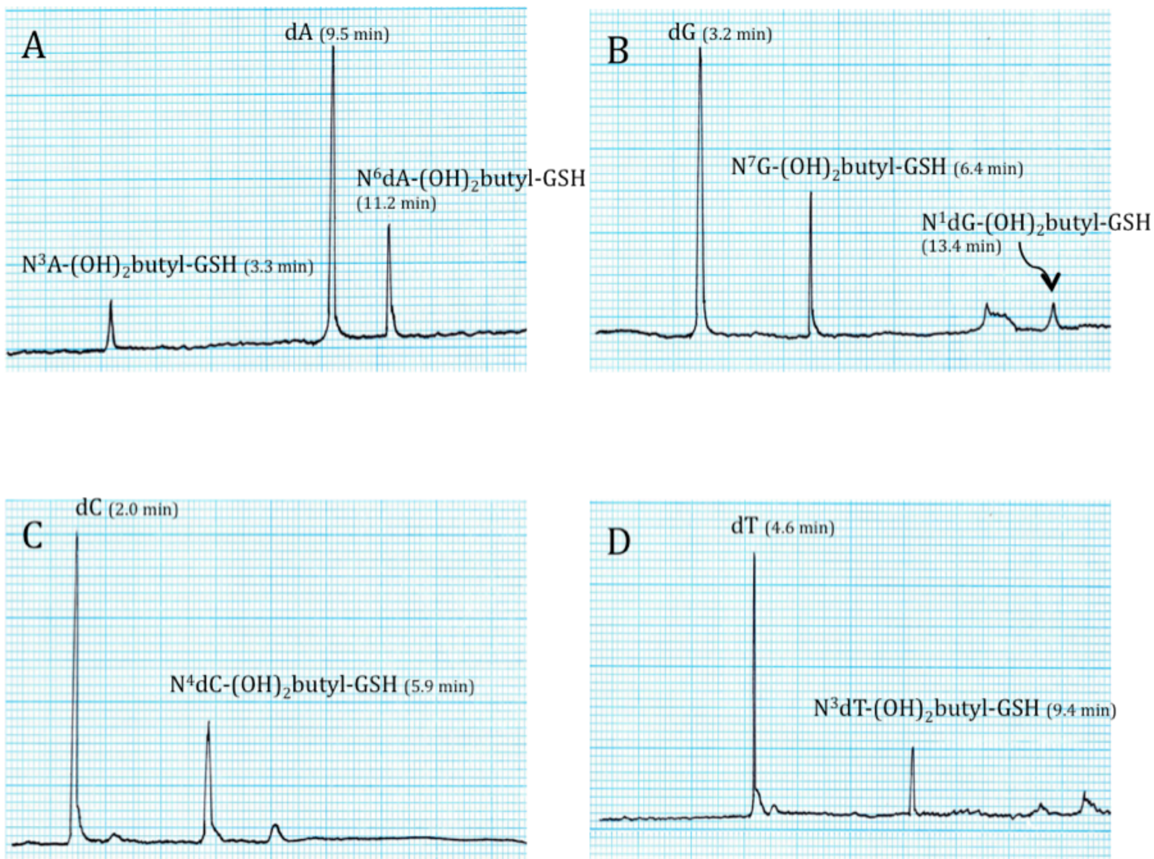


Figure S8. Calibration curves for HPLC-ESI$^+$-MS/MS analysis of N^3A-$(OH)_2$butyl-GSH (A), N^6dA-$(OH)_2$butyl-GSH (B), N^7G-$(OH)_2$butyl-GSH (C), N^1dG-$(OH)_2$butyl-GSH (D), N^4dC-$(OH)_2$butyl-GSH (E), N^3dT-$(OH)_2$butyl-GSH (F) using N^6dA-$(OH)_2$butyl-[*glycine*-$^{13}C_2$, ^{15}N]-GSH and N^7G-$(OH)_2$butyl-[*glycine*-$^{13}C_2$, ^{15}N]-GSH as internal standards (ISTDs).

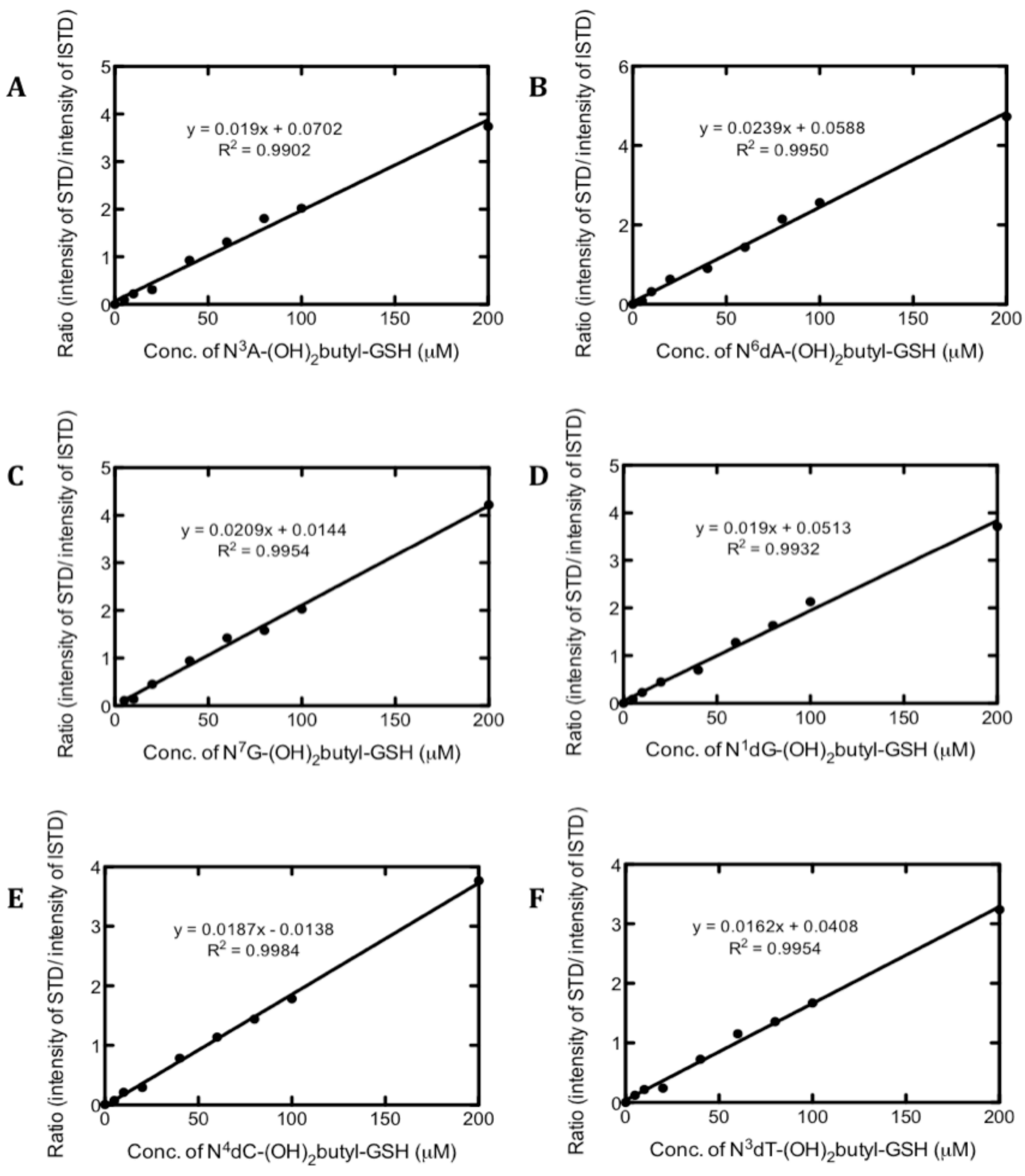


Figure S9. Synthesis of [^{18}O]-N^{7}G-(OH)$_3$butane from the reaction of DEB with guanine (A) and extracted ion chromatogram (B) and MS/MS spectrum of [^{18}O]-N^{7}G-(OH)$_3$butane (C).

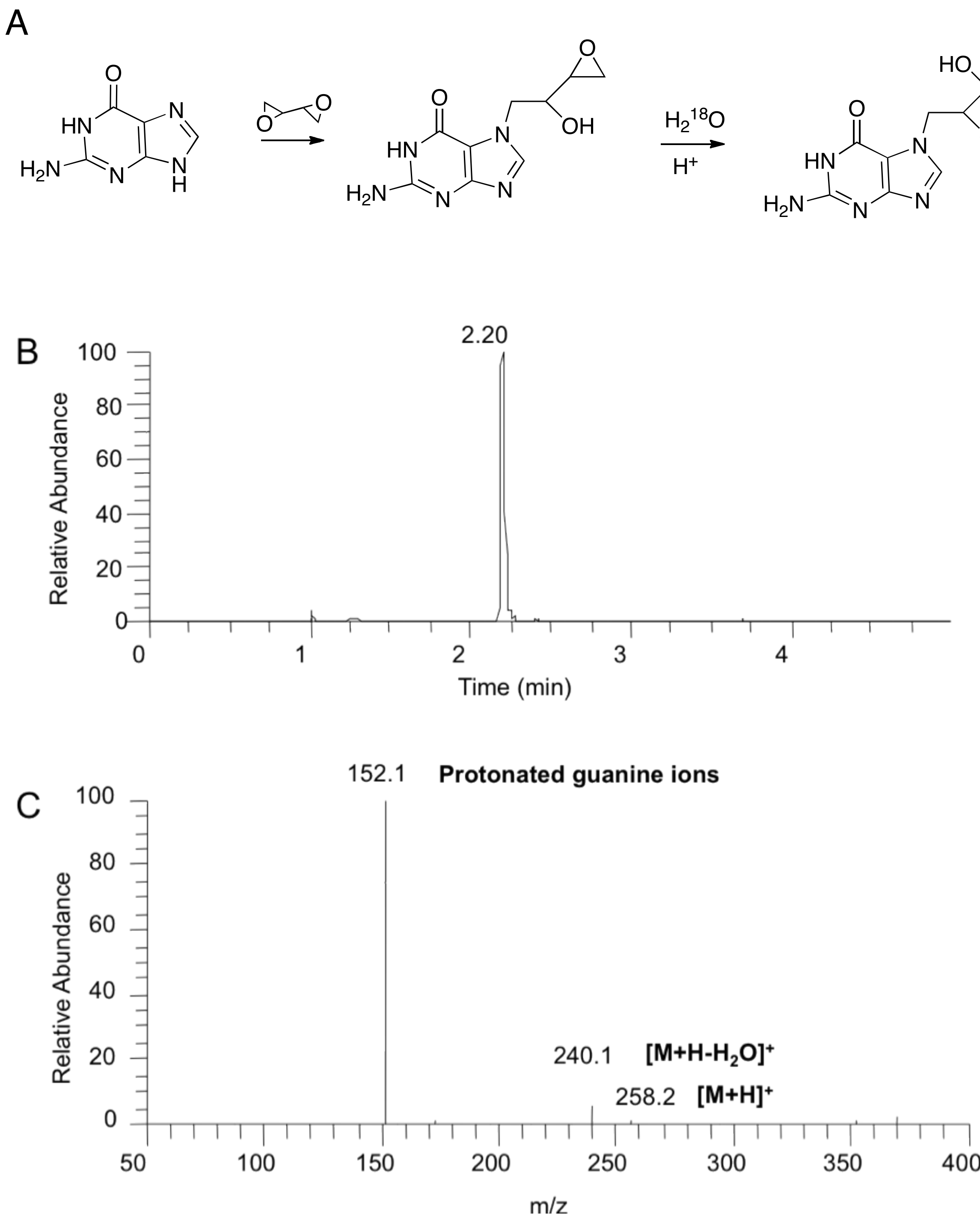


Table S1. NMR characterization of *S,S*-, *R,R*- and *meso*-DEB in $CDCl_3$ (at room temperature)

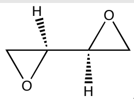
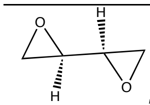
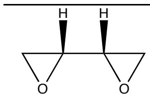
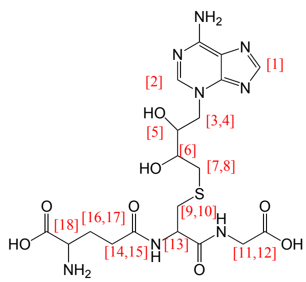
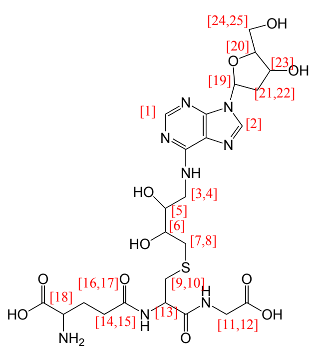
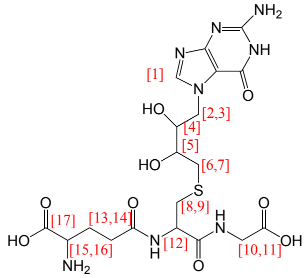
	Chemical shift (ppm)	Carbon chemical shift (ppm)
 *R,R*-DEB	2.71 (2H, m)	43.3
	2.82 (2H, m)	51.2
	2.88 (2H, m)	
 *S,S*-DEB	2.71 (2H, m)	43.3
	2.82 (2H, m)	51.2
	2.88 (2H, m)	
 *meso*-DEB	2.68 (2H, m)	44.3
	2.78 (2H, m)	50.3
	2.93 (2H, m)	

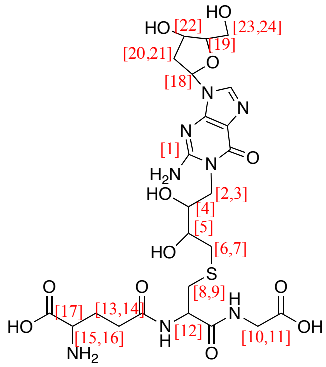
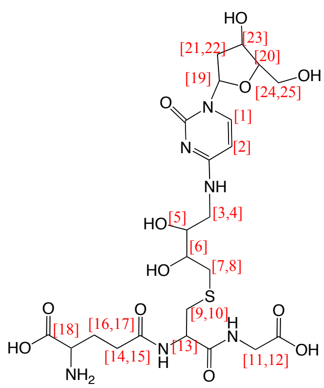
Table S2. Mutagenicity of DEB-GSH conjugate and other butadiene epoxides in *S. typhimurium* TA1537.

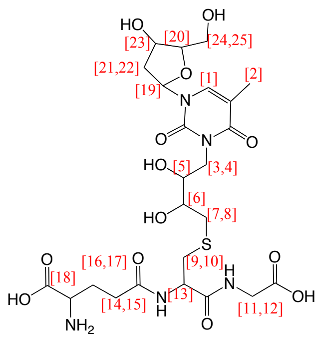
Conc. (mM)	TA1537 revertants/plate			
	DEB-GSH conjugate	DEB	3,4-Epoxy-1-butane	3,4-Epoxy-1,2-butanediol
0	11	9	8	10
0.1	8	5	5	9
0.2	15	5	6	5
0.3	6	10	7	7
0.4	8	15	10	12
0.5	7	8	9	13

Spontaneous revertant control values for *S. typhimurium* TA1537: 5-20; positive control was 9-aminoacridine, which produced 2-3 × 10^8 mutants/μmol.

Table S3. NMR characterization of DNA adducts in D_2O (at room temperature)

DNA adducts	chemical shift (ppm)	assignment	COSY
	7.99 (s, 1H)	CH [1]	ND
N[3]A-(OH)$_2$butyl-GSH	7.91 (s, 1H)	CH [2]	
	4.26 (m, 1H)	CH$_2$ [3]	
	4.38 (dd, 1H)	CH$_2$ [4]	
	4.19 (m, 1H)	CHOH [5]	
	3.41 (m, 1H)	CHOH [6]	
	2.62 (m, 1H)	CH$_2$ [7]	
	2.81 (m, 1H)	CH$_2$ [8]	
	2.84 (m, 1H)	CH$_2$ [9]	
	3.00 (m, 1H)	CH$_2$ [10]	
	3.74 (m, 2H)	CH$_2$ [11,12]	
	4.44 (m, 1H)	CH [13]	
	2.31 (m, 2H)	CH$_2$ [14,15]	
	1.92 (m, 1H)	CH$_2$ [16]	
	2.11 (m, 1H)	CH$_2$ [17]	
	3.78 (t, 1H)	CH [18]	
	8.18 (s, 1H)	CH [1]	
N[6]dA-(OH)$_2$butyl-GSH	8.34 (s, 1H)	CH [2]	
	3.70 (m, 1H)	CH$_2$ [3]	3.82 [4]; 4.12[5]
	3.82 (dd, 1H)	CH$_2$ [4]	3.70 [3]; 4.12[5]
	4.12 (m, 1H)	CHOH [5]	3.70 [3]; 3.82 [4]; 3.64 [6]
	3.64 (m, 1H)	CHOH [6]	4.12[5]
	2.62 (m, 1H)	CH$_2$ [7]	
	2.81 (m, 1H)	CH$_2$ [8]	
	2.79 (m, 1H)	CH$_2$ [9]	2.95 [10]; 4.44 [13]
	2.95 (m, 1H)	CH$_2$ [10]	2.79 [9]; 4.44 [13]
	3.74 (m, 2H)	CH$_2$ [11,12]	
	4.44 (m, 1H)	CH [13]	2.79 [9]; 2.95 [10]
	2.31 (m, 2H)	CH$_2$ [14,15]	1.92 [16]; 2.11 [17]
	1.92 (m, 1H)	CH$_2$ [16]	2.11 [17]; 2.31 [14,15]
	2.11 (m, 1H)	CH$_2$ [17]	1.92 [16]; 2.31 [14,15]
	3.78 (t, 1H)	CH [18]	
	6.24 (m, 1H)	CH [19]	2.39 [21]; 2.80 [22]
	4.07 (m, 1H)	CH [20]	4.52 [23]; 3.69 [24]
	2.39 (m, 1H)	CH$_2$ [21]	6.24 [19]; 2.80 [22]
	2.80 (m, 1H)	CH$_2$ [22]	6.24 [19]; 2.39 [21]; 4.52 [23]
	4.52 (m, 1H)	CHOH [23]	4.07 [20]; 2.80 [22]
	3.69 (m, 1H)	CH$_2$OH [24]	4.07 [20]
	3.77 (m, 1H)	CH$_2$OH [25]	
	8.05 (s, 1H)	CH [1]	
N[7]G-(OH)$_2$butyl-GSH	4.24 (m, 1H)	CH$_2$ [2]	4.44 [3]; 3.81 [4]
	4.44 (dd, 1H)	CH$_2$ [3]	4.24 [2]; 3.81 [4]
	3.81 (m, 1H)	CHOH [4]	4.24 [2]; 4.44 [3]; 3.39 [5]
	3.39 (m, 1H)	CHOH [5]	3.81 [4]
	2.69 (m, 1H)	CH$_2$ [6]	
	2.88 (m, 1H)	CH$_2$ [7]	
	2.81 (m, 1H)	CH$_2$ [8]	2.96 [9]; 4.44 [12]
	2.96 (m, 1H)	CH$_2$ [9]	2.81 [8]; 4.44 [12]
	3.74 (m, 2H)	CH$_2$ [10,11]	
	4.44 (m, 1H)	CH [12]	2.81 [8]; 2.96 [9]
	2.31 (m, 2H)	CH$_2$ [13,14]	1.92 [15]; 2.11 [16]

	1.92 (m, 1H)	C$\underline{H}_2$ [15]	2.11 [16]; 2.31 [13,14]
	2.11 (m, 1H)	C$\underline{H}_2$ [16]	1.92 [15]; 2.31 [13,14]
	3.78 (t, 1H)	CH [17]	
	7.85 (s, 1H)	C$\underline{H}$ [1]	ND
N[1]dG-$(OH)_2$butyl-GSH	4.23 (m, 1H)	C$\underline{H}_2$ [2]	
	4.34 (dd, 1H)	C$\underline{H}_2$ [3]	
	4.21 (m, 1H)	C$\underline{H}$OH [4]	
	3.66 (m, 1H)	C$\underline{H}$OH [5]	
	2.58 (m, 1H)	C$\underline{H}_2$ [6]	
	2.77 (m, 1H)	C$\underline{H}_2$ [7]	
	2.84 (m, 1H)	C$\underline{H}_2$ [8]	
	3.00 (m, 1H)	C$\underline{H}_2$ [9]	
	3.74 (m, 2H)	C$\underline{H}_2$ [10,11]	
	4.44 (m, 1H)	C$\underline{H}$ [12]	
	2.31 (m, 2H)	C$\underline{H}_2$ [13,14]	
	1.92 (m, 1H)	C$\underline{H}_2$ [15]	
	2.11 (m, 1H)	C$\underline{H}_2$ [16]	
	3.78 (t, 1H)	CH [17]	
	6.06 (m, 1H)	CH [18]	
	4.02 (m, 1H)	CH [19]	
	2.25 (m, 1H)	C$\underline{H}_2$ [20]	
	2.64 (m, 1H)	C$\underline{H}_2$ [21]	
	4.02 (m, 1H)	C$\underline{H}$OH [22]	
	3.70 (m, 1H)	C$\underline{H}_2$OH [23]	
	3.73 (m, 1H)	C$\underline{H}_2$OH [24]	
	7.78 (s, 1H)	C$\underline{H}$ [1]	
N[4]dC-$(OH)_2$butyl-GSH	5.76 (s, 1H)	C$\underline{H}$ [2]	
	3.53 (m, 1H)	C$\underline{H}_2$ [3]	3.82 [4]; 3.77 [5]
	3.82 (dd, 1H)	C$\underline{H}_2$ [4]	3.53 [3]; 3.77 [5]
	3.77 (m, 1H)	C$\underline{H}$OH [5]	3.53 [3]; 3.82 [4]; 3.65 [6]
	3.65 (m, 1H)	C$\underline{H}$OH [6]	3.77 [5]
	2.61 (m, 1H)	C$\underline{H}_2$ [7]	
	2.71 (m, 1H)	C$\underline{H}_2$ [8]	
	2.74 (m, 1H)	C$\underline{H}_2$ [9]	2.86 [10]; 4.44 [13]
	2.86 (m, 1H)	C$\underline{H}_2$ [10]	2.74 [9]; 4.44 [13]
	3.74 (m, 2H)	C$\underline{H}_2$ [11,12]	
	4.44 (m, 1H)	C$\underline{H}$ [13]	2.74 [9]; 2.86 [10]
	2.31 (m, 2H)	C$\underline{H}_2$ [14,15]	1.92 [16]; 2.11 [17]
	1.92 (m, 1H)	C$\underline{H}_2$ [16]	2.11 [17]; 2.31 [14,15]
	2.11 (m, 1H)	C$\underline{H}_2$ [17]	1.92 [16]; 2.31 [14,15]
	3.78 (t, 1H)	CH [18]	
	6.25 (m, 1H)	CH [19]	2.16 [21]; 2.38 [22]
	4.06 (m, 1H)	CH [20]	4.50 [23]; 3.90 [24]
	2.16 (m, 1H)	C$\underline{H}_2$ [21]	6.25 [19]; 2.38 [22]
	2.38 (m, 1H)	C$\underline{H}_2$ [22]	6.25 [19]; 2.16 [21]; 4.50 [23]
	4.50 (m, 1H)	C$\underline{H}$OH [23]	4.06 [20]; 2.38 [22]
	3.90 (m, 1H)	C$\underline{H}_2$OH [24]	4.06 [20]
	4.04 (m, 1H)	C$\underline{H}_2$OH [25]	
	7.54 (s, 1H)	C$\underline{H}$ [1]	
N[3]dT-$(OH)_2$butyl-GSH	1.90 (s, 3H)	C$\underline{H}_3$ [2]	
	3.79 (m, 1H)	C$\underline{H}_2$ [3]	3.88 [4]; 3.92 [5]
	3.88 (dd, 1H)	C$\underline{H}_2$ [4]	3.79 [3]; 3.92 [5]
	3.92 (m, 1H)	C$\underline{H}$OH [5]	3.79 [3]; 3.88 [4]; 3.72 [6]



3.72 (m, 1H)	CHOH [6]	3.92 [5]
2.68 (m, 1H)	CH$_2$ [7]	
2.78 (m, 1H)	CH$_2$ [8]	
2.79 (m, 1H)	CH$_2$ [9]	2.92 [10]; 4.44 [13]
2.92 (m, 1H)	CH$_2$ [10]	2.79 [9]; 4.44 [13]
3.74 (m, 2H)	CH$_2$ [11,12]	
4.44 (m, 1H)	CH [13]	2.79 [9]; 2.92 [10]
2.31 (m, 2H)	CH$_2$ [14,15]	1.92 [16]; 2.11 [17]
1.92 (m, 1H)	CH$_2$ [16]	2.11 [17]; 2.31 [14,15]
2.11 (m, 1H)	CH$_2$ [17]	1.92 [16]; 2.31 [14,15]
3.78 (t, 1H)	CH [18]	
6.30 (m, 1H)	CH [19]	2.26 [21]; 2.45 [22]
3.95 (m, 1H)	CH [20]	4.44 [23]; 3.79 [24]
2.26 (m, 1H)	CH$_2$ [21]	6.30 [19]; 2.45 [22]
2.45 (m, 1H)	CH$_2$ [22]	6.30 [19]; 2.26 [21]; 4.44 [23]
4.44 (m, 1H)	CHOH [23]	3.95 [20]; 2.45 [22]
3.79 (m, 1H)	CH$_2$OH [24]	3.95 [20]
3.80 (m, 1H)	CH$_2$OH [25]	

ND: not determined