Supporting Information

to

Cross-linking of the DNA repair protein O^6-alkylguanine DNA alkyltransferase to DNA in the presence of antitumor nitrogen mustards

Rachel Loeber, Erin Michaelson, Qingming Fang, Colin Campbell, Anthony Pegg, and Natalia Tretyakova

S-1. **Synthetic procedures.**

Guanine Half Mustards of Chlorambucil and Mechlorethamine were prepared by reacting 2'-deoxyguanosine with the corresponding mustards.

N-(2-chloroethyl)-N-[2-(guan-7-yl)ethyl]-p-aminophenylbutyric acid (*N7G-PBA-Cl*): 2'-deoxyguanosine (420 mg, 1.57 mmol) was incubated with chlorambucil (304 mg, 1.00 mmol) in triflouroethanol (25 mL) at 37 °C for 72 h under anhydrous conditions. The reaction mixture was dried under nitrogen, ~ 80% yield. UV λ_{max} = 246 nm, λ_{min} = 275 nm (pH 4.9); ESI^+-MS/MS: *m/z* 419.1 $[M + H]^+ \rightarrow$ *m/z* 268.1 $[M + H - Gua]^+$

N-(2-chloroethyl)-N-[2-(guan-7-yl)ethyl]methylamine (N7G-EMA-Cl): 2'-deoxyguanosine (502 mg, 1.88 mmol) was incubated with mechlorethamine (306 mg, 1.88 mmol) in triflouroethanol (25 mL) at 37 °C for 72 h under anhydrous conditions. The reaction mixture was dried under nitrogen, ~ 60% yield. UV: λ_{max} = 246 nm, λ_{min} = 275 nm (pH 4.9); ESI^+-MS/MS: *m/z* 271.1 $[M + H]^+ \rightarrow$ *m/z* 120.1 $[M + H - Gua]^+$.

Amino Acid-Guanine Conjugates of Chlorambucil and Mechlorethamine.

N-(2-[S-cysteinyl]ethyl)-N-(2-[guan-7-yl]ethyl)-p-aminophenylbuyric acid (Cys-N7G-PBA): N7G-PBA-Cl (160 mg, 0.382 mmol) in DMSO (3.5 mL) was combined with 0.8 equivalents of Boc-Cys-OH (70 mg, 0.316 mmol) in water (3.5 mL), and the pH of the reaction mixture was raised to ~ 9 with ammonium hydroxide. The reaction mixture was incubated at 37 °C for 18 h, filtered, and isolated by semi preparative HPLC using a Supelcosil LC-18-DB column (25 cm x 10 mm, 5 µm). The column was eluted with a linear gradient of acetonitrile (B) in 20 mM ammonium acetate, pH 4.9 (A). The solvent composition was changed from 15 to 34 % B in 22 minutes and further to 50% in 4 min. Under these conditions, Boc-protected Cys-N7G-PBA

eluted as a sharp peak at 18.6 min. ESI^+-MS/MS: *m/z* 604.3 $[M + H]^+ \rightarrow$ *m/z* 504.2 [M + H – Boc]$^+$ and *m/z* 353.2 [M + H – Boc – Gua]$^+$. HPLC-purified Boc-Cys-N7G-PBA in TFA (0.5 mL) was incubated at room temperature for 45 min. The deprotected cross-link was purified by semi-preparative HPLC as described previously. Under these conditions, Cys-N7G-PBA eluted as a sharp peak at 9.3 min (~ 1% yield). UV: λ_{max} 256 nm, λ_{min} 280 nm (pH 4.9); ESI^+-MS/MS: *m/z* 504.2 $[M + H]^+ \rightarrow$ *m/z* 353.1 [M + H – Gua]$^+$; ^{1}H NMR 600 MHz δ (DMSO-d_6): 7.87 (1H, s, H-8), 7.15 (1H, s, NH-1), 6.98 (1H, s, NH_2-8'), 7.06 (1H, s, NH_2-8'), 6.94 (2H, d, CH-2", CH-6", *J* = 8.4 Hz), 6.72 (2H, d, CH-3", CH-5", *J* = 8.4 Hz), 6.15 (2H, s, NH_2-2), 4.28 (2H, t, CH_2-1', *J* = 7.2, 13.8 Hz), 3.65 (2H, t, CH_2-2', *J* = 6.6, 13.2 Hz), 3.37 (2H, m, CH_2-4', *J* = 6.0 Hz), 2.98 (2H, m, CH_2-5', *J* = 4.2, 6.6 Hz), 2.70 (2H, t, CH_2-7', *J* = 6.0 Hz), 2.42 (2H, t, CH_2-7", *J* = 7.2 Hz), 2.15 (2H, t, CH_2-9", *J* = 7.2 Hz), 1.70 (2H, m, CH_2-8", *J* = 7.2 Hz).

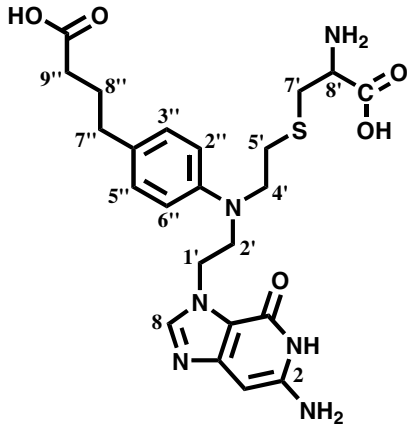
N-[2-[cysteinyl]ethyl]-N-[2-(guan-7-yl)ethyl]methylamine (Cys-N7G-EMA): Boc-Cys-OH (70 mg, 0.316 mmol) was combined with 2 equivalents N7G-EMA-Cl (282 mg, 0.731 mmol) in 7 mL DMSO/water (1:1). The pH of the reaction mixture was adjusted to ~ 9 by the addition of ammonium hydroxide, and the reaction mixture was stirred at 37 °C for 72 h. The insoluble product was isolated by filtration and further purified by semi-preparative HPLC on a Supelcosil LC-18-DB column (25 cm x 10 mm, 5 µm) eluted with a linear gradient of acetonitrile (B) in 20 mM ammonium acetate, pH 4.9 (A). The solvent composition was changed from 0 to 24% B in 24 min and further to 60% in 6 min. Under these conditions, Boc-protected Cys-N7G-EMA eluted as a sharp peak at 20.9 min. ESI^+-MS/MS: *m/z* 456.2 $[M + H]^+ \rightarrow$ *m/z* 356.1 [M + H – Boc]$^+$ and *m/z* 205.0 [M + H – Boc – Gua]$^+$. Following HPLC purification, Boc-protected Cys-N7G-EMA was dissolved in TFA (0.5 mL) and incubated at room temperature for 45 min. The

deprotected Cys-N7G-EMA was purified by HPLC using the same method described above. Under these conditions, Cys-N7G-EMA eluted as a sharp peak at 10.4 min (0.2% yield). UV: λ_{max} 280 nm, λ_{min} 246 nm (pH 4.9); ESI$^+$-MS/MS: *m/z* 356.1 $[M + H]^+ \rightarrow$ *m/z* 205.0 $[M + H - Gua]^+$; ^{1}H NMR 600 MHz δ (DMSO-d_6): 8.30 (1H, s, H-8), 4.66 (2H, d, NH_2-8'), 4.06 (2H, t, CH_2-1'), 3.69 (1H, s, NH-1), 3.54 (2H, s, NH_2-2), 3.28 (2H, t, CH_2-2'), 3.00 (2H, m, CH_2-4'), 2.90 (2H, m, CH_2-5'), 2.83 (2H, m, CH_2-7'), 2.75 (3H, s, CH_3-1''), 2.5 (1H, s, H-8').

N-[2-[N-(lysyl)ethyl]-N-[2-(guan-7-yl)ethyl]methylamine (Lys-N7G-EMA): Equimolar amounts of Boc-Lys-OMe (130 mg, 0.5 mmol) and N7G-EMA-Cl (270 mg, 0.5 mmol) were combined in 4 mL water, and ammonium hydroxide was added to increase the pH of solution to ~ 9 prior to overnight incubation at 37 °C. The Boc-protected lysine-guanine conjugate was isolated from the reaction supernatant by semi-preparative HPLC using a Supelcosil LC-18-DB column (25 cm x 10 mm, 5 μm) eluted with 20 mM ammonium acetate, pH 4.9 (A) and acetonitrile (B) at a flow rate of 3 mL/min. The solvent composition was changed linearly from 0-40% B in 30 min. Under these conditions, Boc-Lys-OMe-N7G-EMA eluted as a sharp peak at 27 min. ESI$^+$-MS/MS: *m/z* 495.0 $[M + H]^+ \rightarrow$ *m/z* 395.0 $[M + H - Boc]^+$. Removal of the Boc protecting group was achieved by incubation of Boc-Lys-OMe-N7G-EMA with TFA (10 μL) at room temperature for 45 min. ESI$^+$-MS/MS: *m/z* 395.0 $[M + H]^+ \rightarrow$ *m/z* 244.0 $[M + H - Gua]^+$. Hydrolysis of the methyl ester was achieved *via* incubation of Lys-OMe-N7G-EMA with 0.1N NaOH (20 μL) at room temperature for 3 h. The desired Lys-N7G-EMA conjugate was isolated by semi-preparative HPLC using a Supelcosil LC-18-DB column (25 cm x 10 mm, 5 μm) eluted with 20 mM NH_4OAc, pH 4.9 (A) and acetonitrile (B). The solvent composition was changed from 1-20% B in 60 min. Under these conditions, Lys-N7G-EMA eluted as a sharp peak at 18.4 min.

UV: λ_{max} 280 nm, λ_{min} 246 nm (pH 4.9); ESI^+-MS/MS: *m/z* 381.2 $[M + H]^+ \rightarrow$ *m/z* 363.1 $[M + H - H_2O]^+$ and *m/z* 230.1 $[M + H - Gua]^+$.

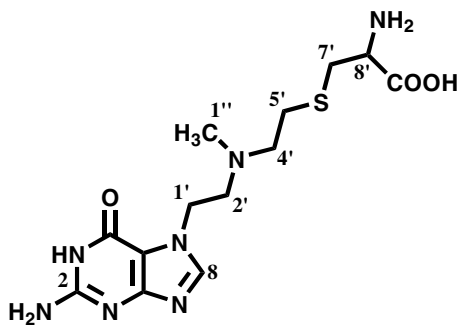
^{1}H NMR characterization of synthetic Cys-N7G-PBA in DMSO-d_6 using a 600 MHz Varian Inova NMR spectrometer.



Cys-N7G-PBA

Chemical Shift	^{1}H NMR
7.87 ppm, s, 1H	**H**-8
7.15 ppm, s, 1H	N**H**-1
7.01 ppm, ss, 2H	N**H**$_2$-8'
6.94 ppm, d, 2H	C**H**-2", C**H**-6"
6.72 ppm, d, 2H	C**H**-3", C**H**-5"
6.15 ppm, s, 2H	N**H**$_2$-2
4.28 ppm, t, 2H	C**H**$_2$-1'
3.65 ppm, t, 2H	C**H**$_2$-2'
3.37 ppm, m, 2H	C**H**$_2$-4'
2.98 ppm, m, 2H	C**H**$_2$-5'
2.70 ppm, m, 2H	C**H**$_2$-7'
2.42 ppm, t, 2H	C**H**$_2$-7"
2.15 ppm, t, 2H	C**H**$_2$-9"
1.70 ppm, m, 2H	C**H**$_2$-8"

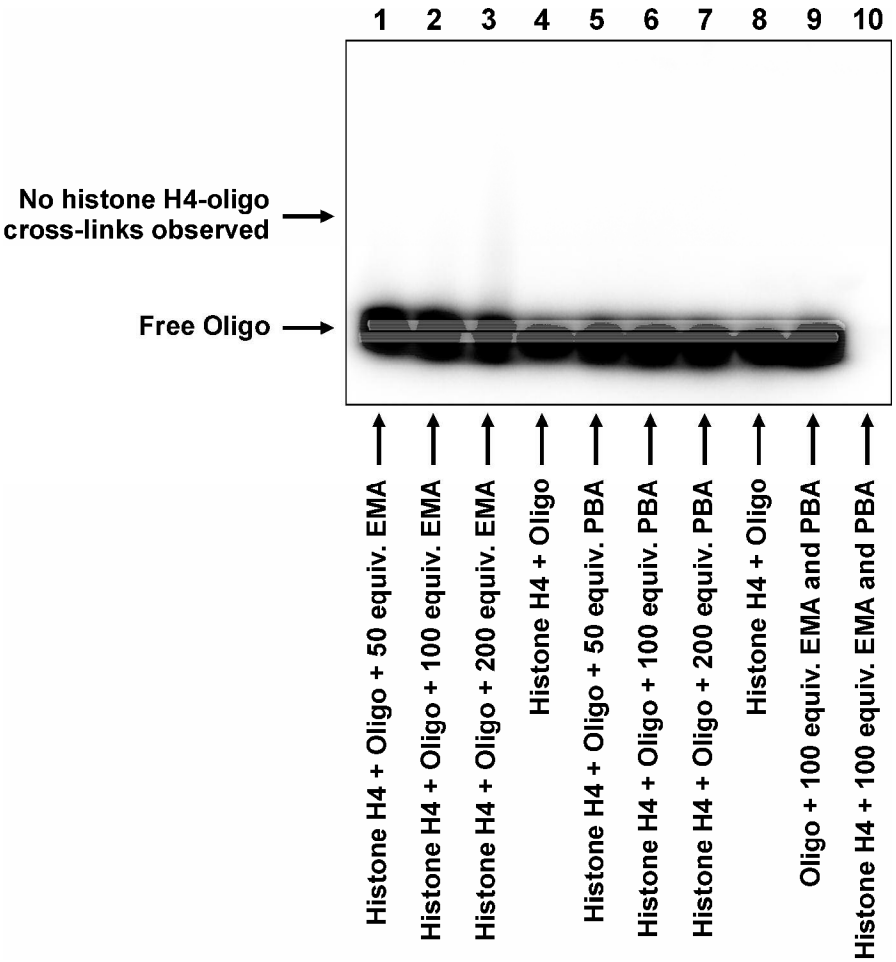
^{1}H NMR characterization of synthetic Cys-N7G-EMA in DMSO-d_6 using a 600 MHz Varian Inova NMR spectrometer.



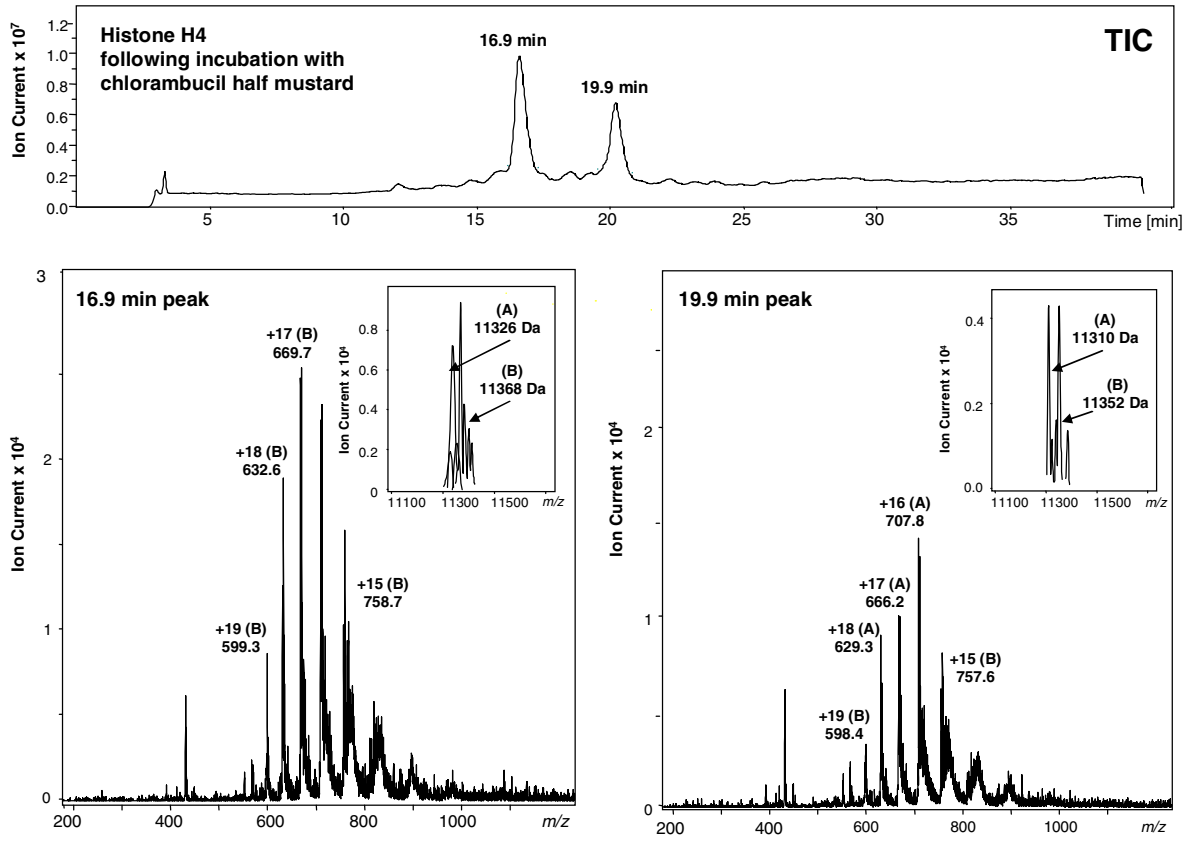
Cys-N7G-EMA

Chemical Shift	^{1}H NMR
8.30 ppm, s, 1H	H-8
4.66, ppm, d, 2H	NH$_2$-8
4.06, ppm, t, 2H	CH$_2$-1'
3.69 ppm, s, 1H	NH-1
3.54 ppm, s, 2H	NH$_2$-2
3.28 ppm, t, 2H	CH$_2$-2'
3.00 ppm, m, 2H	CH$_2$-4'
2.90 ppm, m, 2H	CH$_2$-5'
2.83 ppm, m, 2H	CH$_2$-7'
2.75 ppm, s, 3H	CH$_3$-1"
2.50 ppm, s, 1H	H-8'

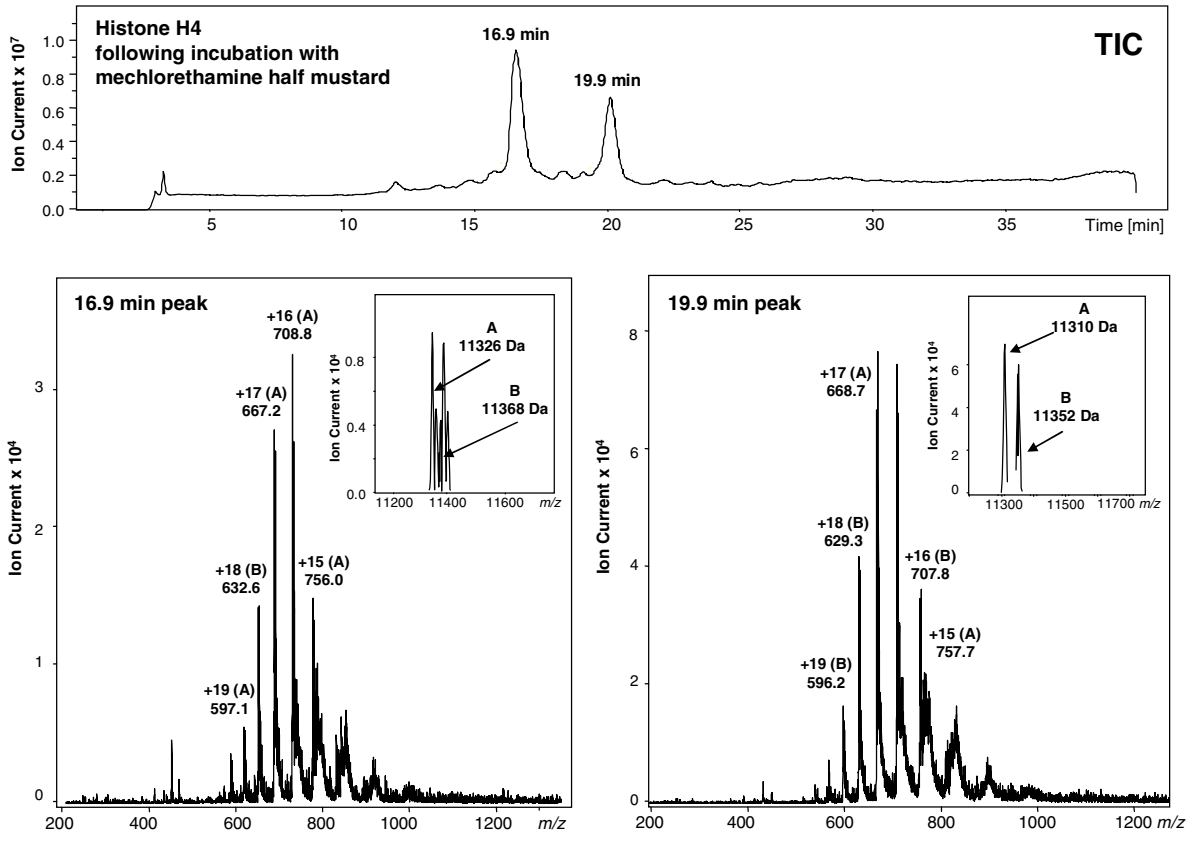
S-2. 12% SDS-PAGE analysis of ^{32}P-end labeled DNA duplexes (5'-GGA GCT GGT GGC GTA GGC, (+) strand) following incubation with increasing amounts of mechlorethamine (lanes **1**-**3**) or chlorambucil (lane **5**-**7**) in the presence of histone H4. Lanes **4** and **8** containing no nitrogen mustard serve as negative controls, as do lanes **9** and **10** lacking protein or DNA.



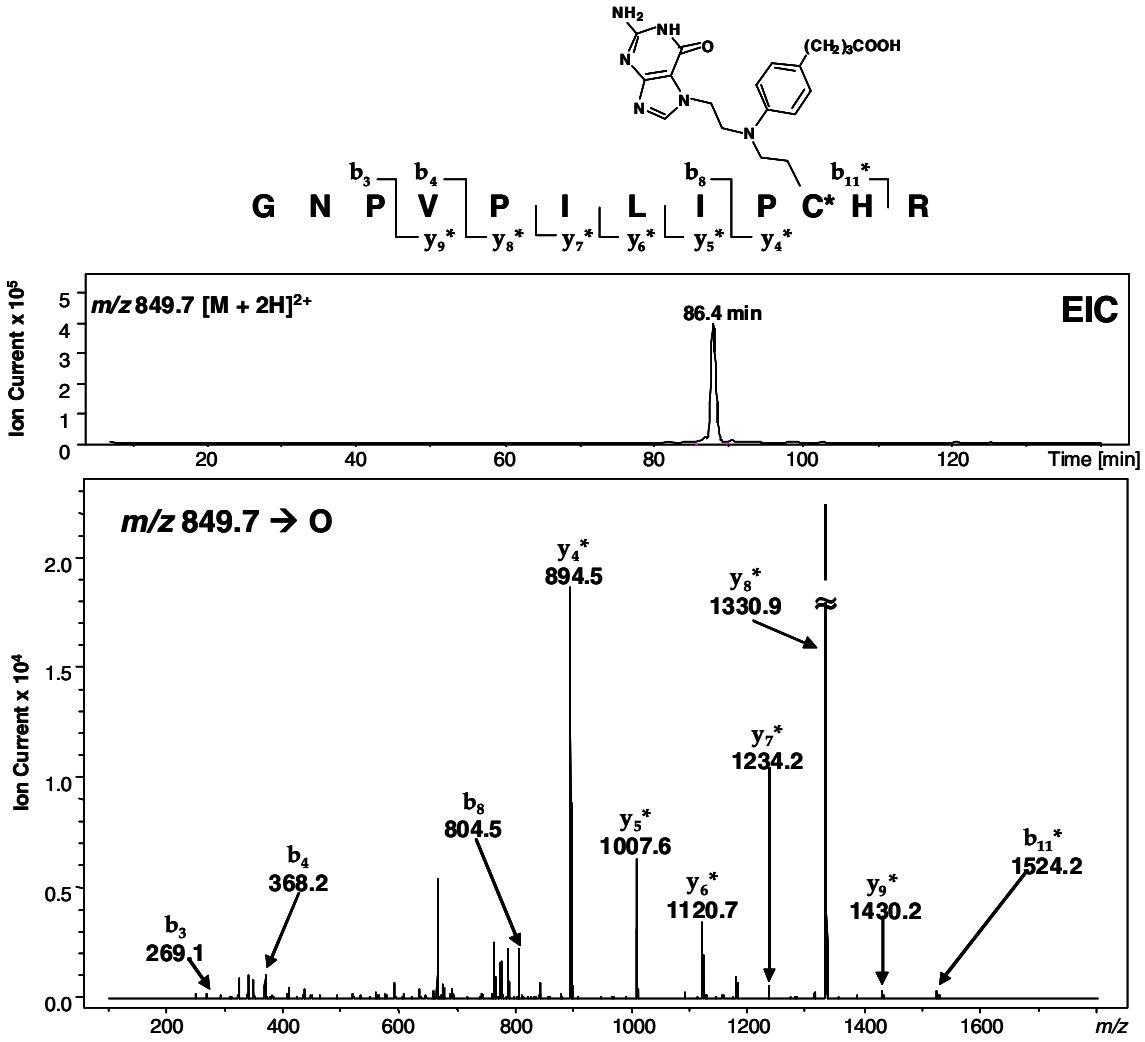
S-3. HPLC-ESI$^+$-MS analysis of histone H4 treated with chlorambucil half mustard, N7G-PBA-Cl. *Top*: Total ion chromatogram; *Bottom left*: ESI$^+$ mass spectrum of 16.9 min protein peak; *Inset:* Deconvoluted mass spectrum of the 16.9 min protein peak: *A* = histone H4 + Acet + 2Me + O (calculated *M* = 11 326 Da, observed *M* = 11 326 Da), *B* = histone H4 + 2Acet + 2Me + O (calculated *M* = 11 368 Da, observed *M* = 11 368 Da); *Bottom right*: ESI$^+$ mass spectrum of 19.9 min protein peak; *Inset*: Deconvoluted mass spectrum of the 19.9 min protein peak: *A* = histone H4 + Acet + 2Me (calculated *M* = 11 310 Da, observed *M* = 11 310 Da), *B* = histone H4 + 2Acet + 2Me (calculated *M* = 11 352 Da, observed *M* = 11 352 Da).



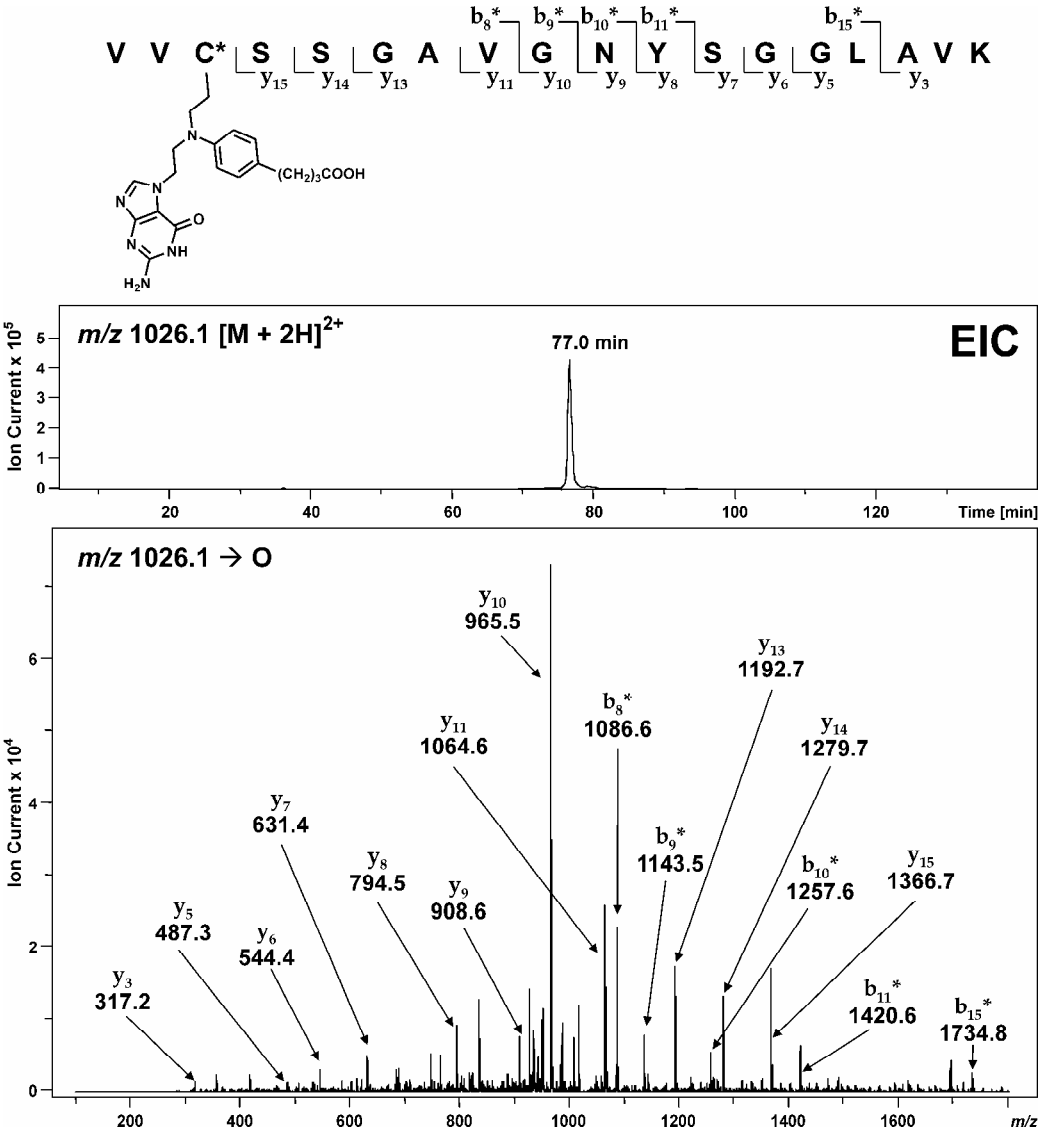
S-4. HPLC-ESI$^+$-MS analysis of histone H4 treated with mechlorethamine half mustard, N7G-EMA-Cl. *Top*: Total ion chromatogram; *Bottom left*: ESI$^+$ mass spectrum of 16.9 min protein peak; *Inset:* Deconvoluted mass spectrum of the 16.9 min protein peak: *A* = histone H4 + Acet + 2Me + O (calculated *M* = 11 326 Da, observed *M* = 11 326 Da), *B* = histone H4 + 2Acet + 2Me + O (calculated *M* = 11 368 Da, observed *M* = 11 368 Da); *Bottom right*: ESI$^+$ mass spectrum of 19.9 min protein peak; *Inset*: Deconvoluted mass spectrum of the 19.9 min protein peak: *A* = histone H4 + Acet + 2Me (calculated *M* = 11 310 Da, observed *M* = 11 310 Da), *B* = histone H4 + 2Acet + 2Me (calculated *M* = 11 352 Da, observed *M* = 11 352 Da).



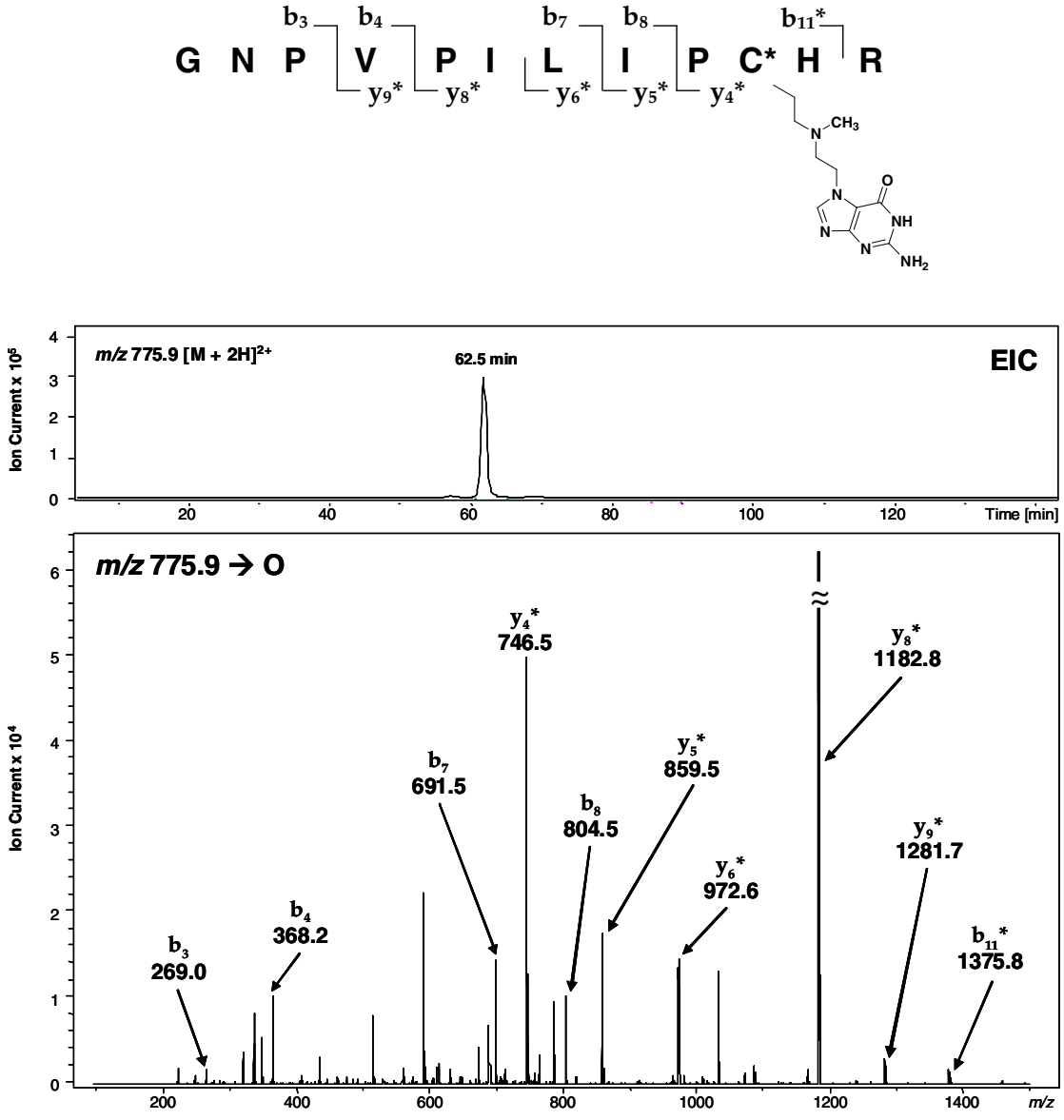
S-5. HPLC-ESI$^+$-MS/MS analysis of synthetic peptide GNPVPILIPCHR containing chlorambucil cross-link to guanine. *Top*: Extracted ion chromatogram of *m/z* 849.7 $[M + 2H]^{2+}$; *Bottom*: MS/MS spectrum of the 86.4 min peak mapping the cross-link to Cys (C*).



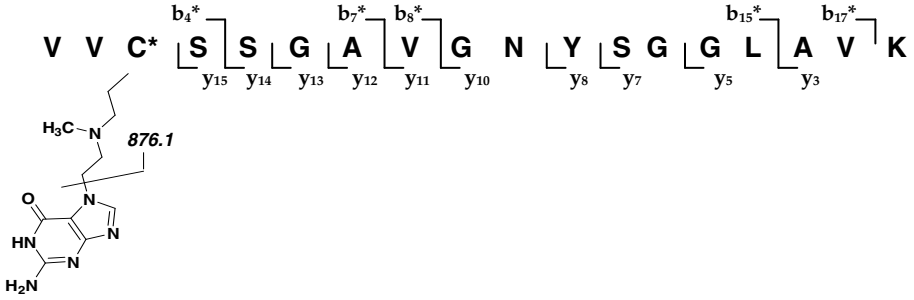
S-6. HPLC-ESI$^+$-MS/MS analysis of AGT tryptic peptide V^{148}VCSSGAVGNYSGGLAVK^{165} containing N7G-PBA-Cl-induced cross-link to guanine. *Top:* Extracted ion chromatogram of *m/z* 1026.1 $[M + 2H]^{2+}$; *Bottom:* MS/MS spectrum of adducted peptide V^{148}VCSSGAVGNYSGGLAVK^{165} mapping the cross-link to Cys^{150}.

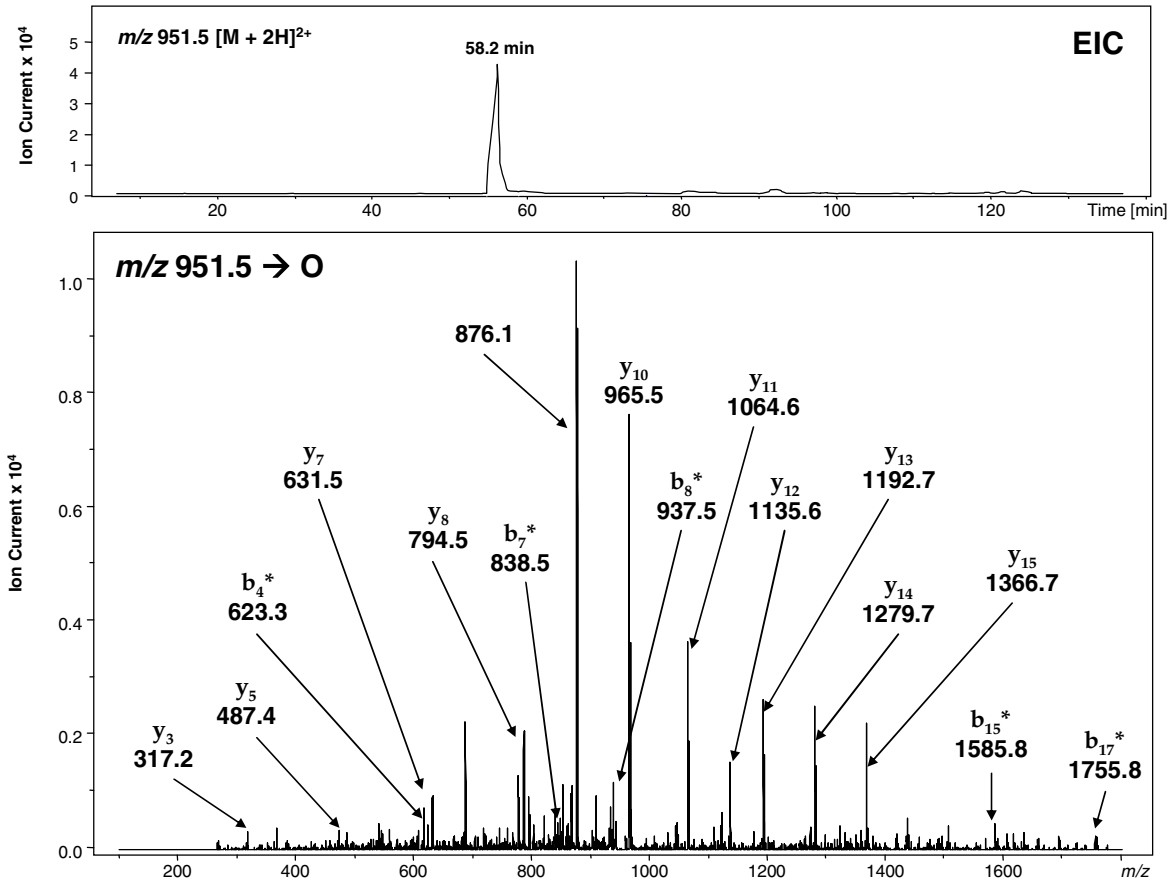


S-7. HPLC-ESI$^+$-MS/MS analysis of AGT tryptic peptide G^{136}NPVPILIPCHR^{147} containing N7G-EMA-Cl induced cross-link to guanine. *Top*: Extracted ion chromatogram of *m/z* 775.9 [M + 2H]$^{2+}$; *Bottom*: MS/MS spectrum of the 62.5 min peak mapping the cross-link to Cys^{145} (C*).

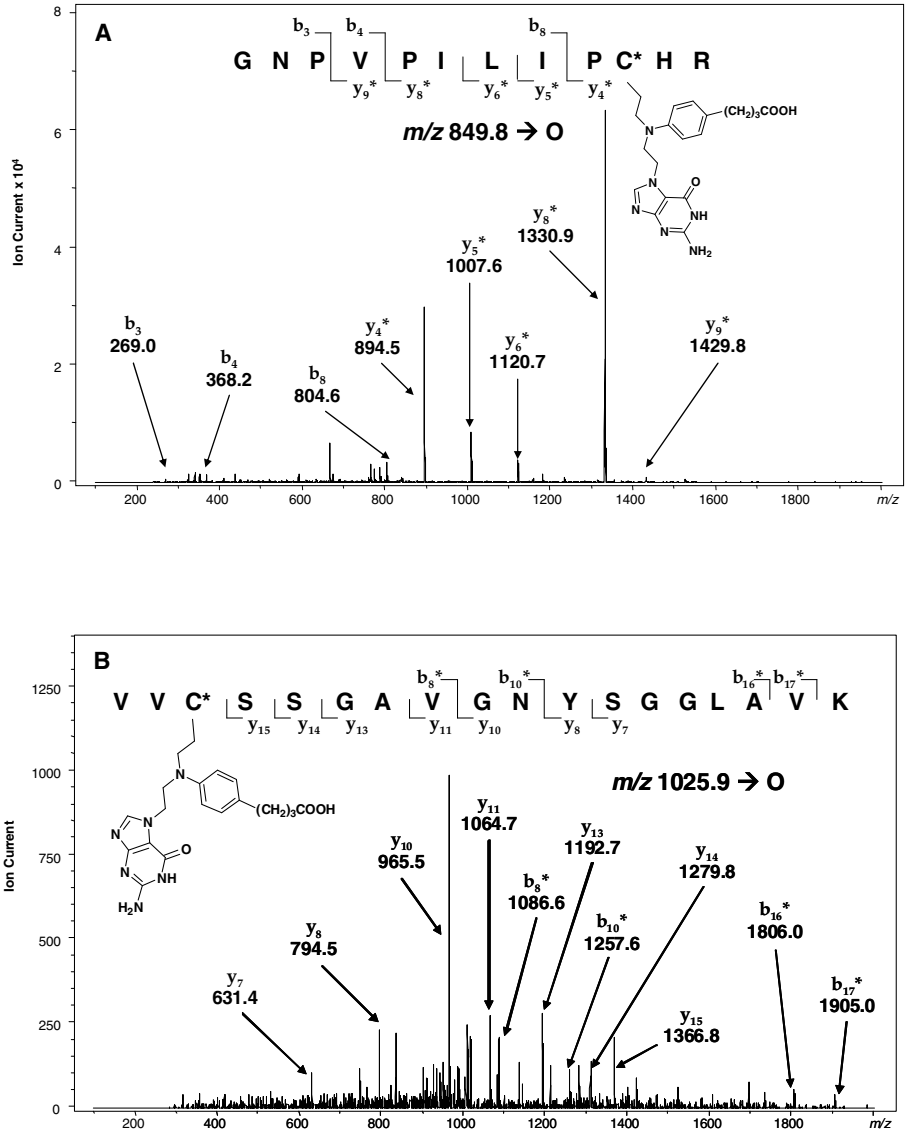


S-8. HPLC-ESI$^+$-MS/MS analysis of AGT tryptic peptide V^{148}VCSSGAVGNYSGGLAVK^{165} containing N7G-EMA-Cl induced cross-link to guanine. *Top*: Extracted ion chromatogram of *m/z* 951.5 $[M + 2H]^{2+}$; *Bottom*: MS/MS spectrum of the 58.2 min peak mapping the cross-link to Cys^{150}.

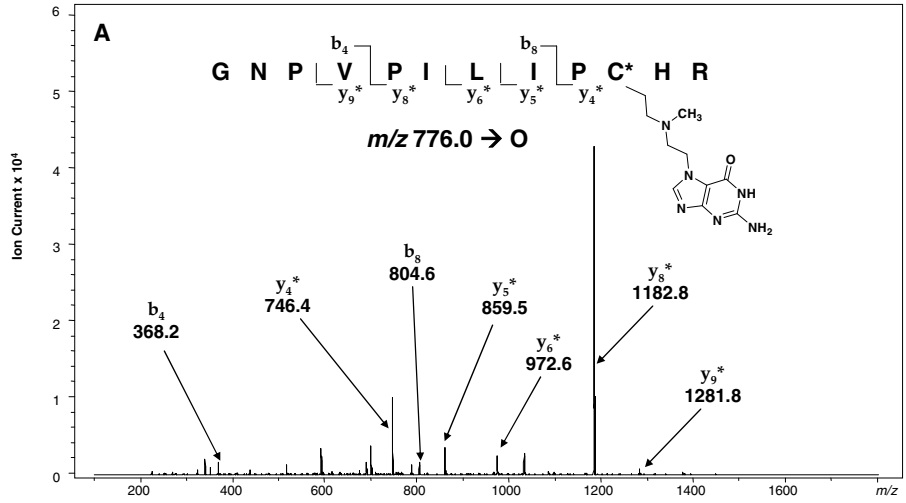


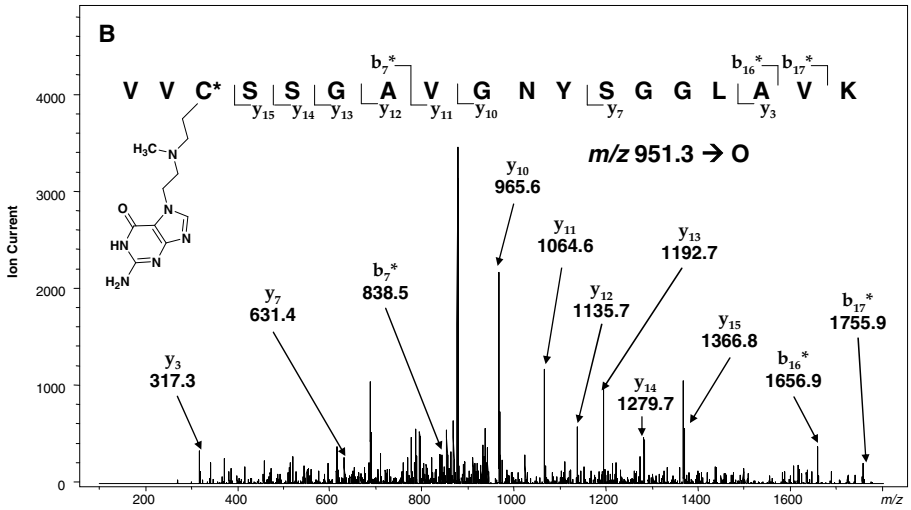


S-9. HPLC-ESI$^+$-MS/MS analysis of AGT tryptic peptides G^{136}NPVPILIPCHR^{147} (**A**) and V^{148}VCSSGAVGNYSGGLAVK^{165} (**B**) containing chlorambucil cross-links to guanine obtained from chlorambucil treatments of AGT-DNA mixtures.

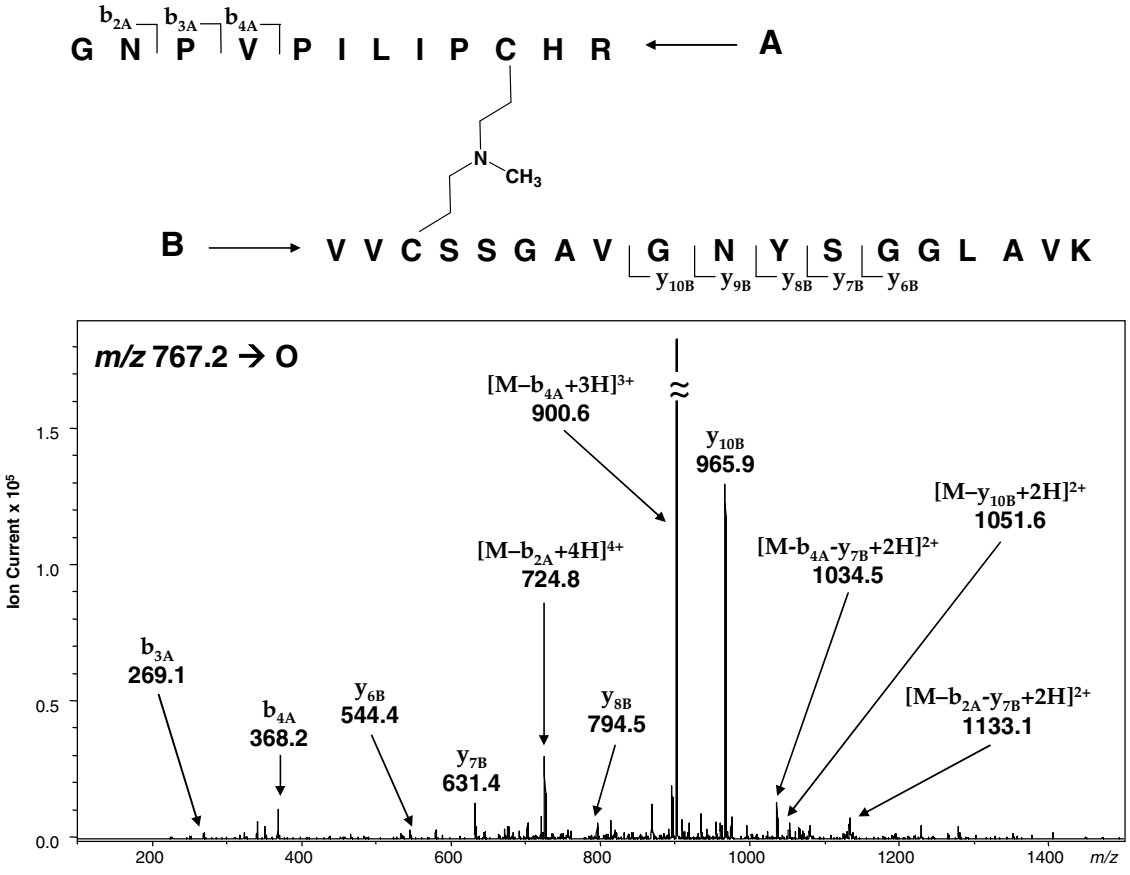


S-10. HPLC-ESI$^+$-MS/MS analysis of AGT tryptic peptides G^{136}NPVPI LIPCHR^{147} (**A**) and V^{148}VCSSGAVGNYSGGLAVK^{165} (**B**) containing mechlorethamine cross-links to guanine obtained from mechlorethamine treatment of AGT-DNA mixtures.

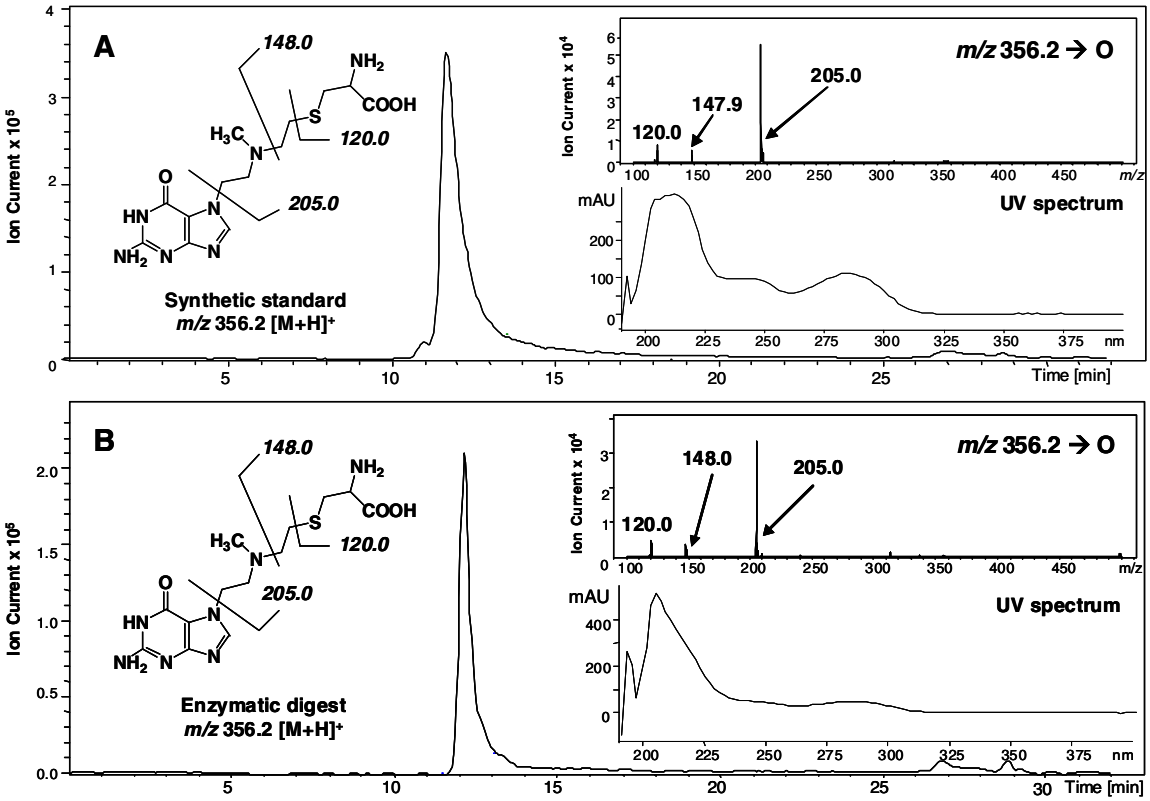




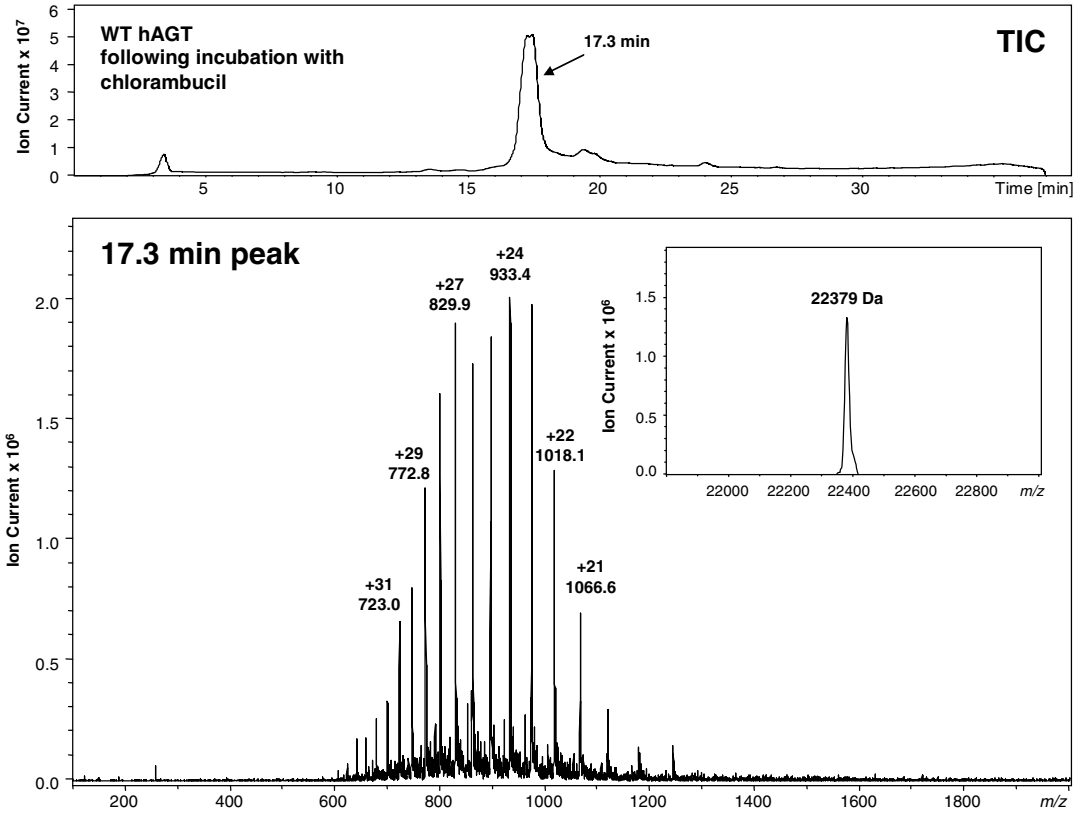
S-11. MS/MS analysis of AGT tryptic peptides G[136]NPVPILIPCHR[147] and V[148]VCSSGAVGNYSGGLAVK[165] cross-linked by mechlorethamine.



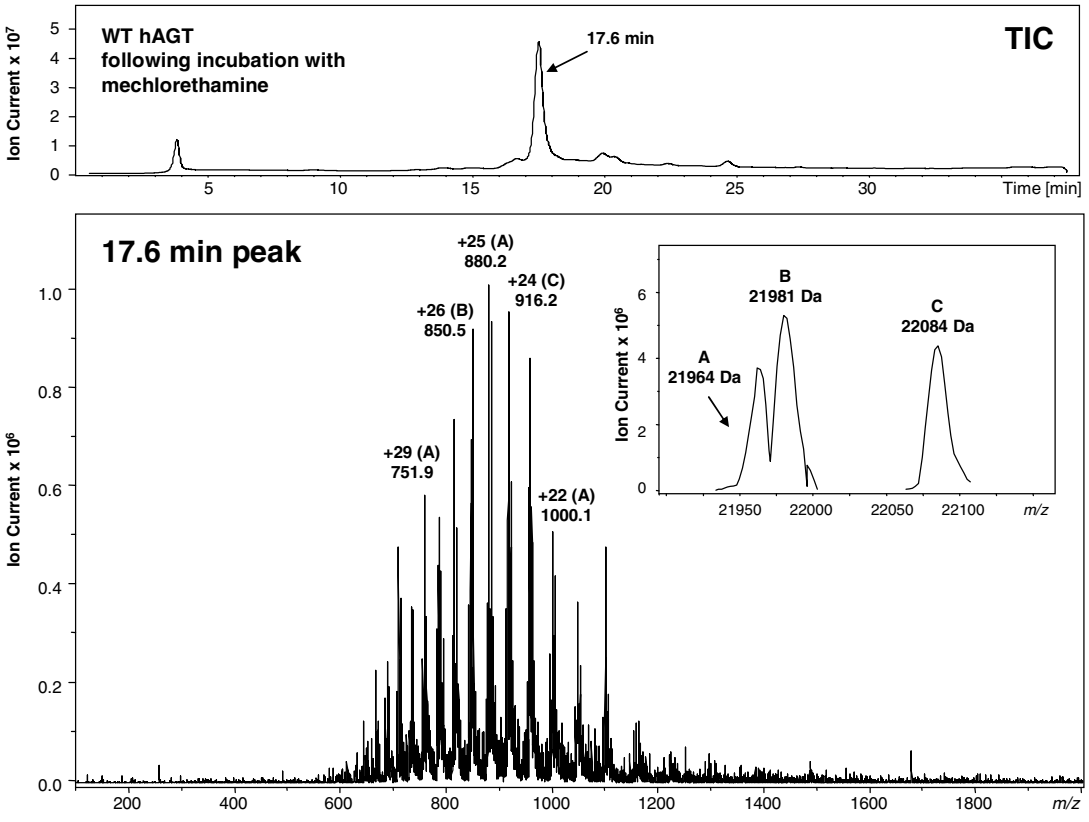
S-12. HPLC-ESI$^+$-MS/MS analysis of Cys-N7G-EMA conjugates: (**A**) Synthetic Cys-N7G-EMA (*m/z* 356.2 $[M + H]^+$) *Inset*: MS/MS fragmentation and UV spectrum of the authentic standard; (**B**) Cys-N7G-EMA present in enzymatic digests of wild type AGT treated with mechlorethamine half mustard (*m/z* 356.2 $[M + H]^+$) *Inset*: MS/MS fragmentation and UV spectrum of AGT-derived Cys-N7G-EMA.



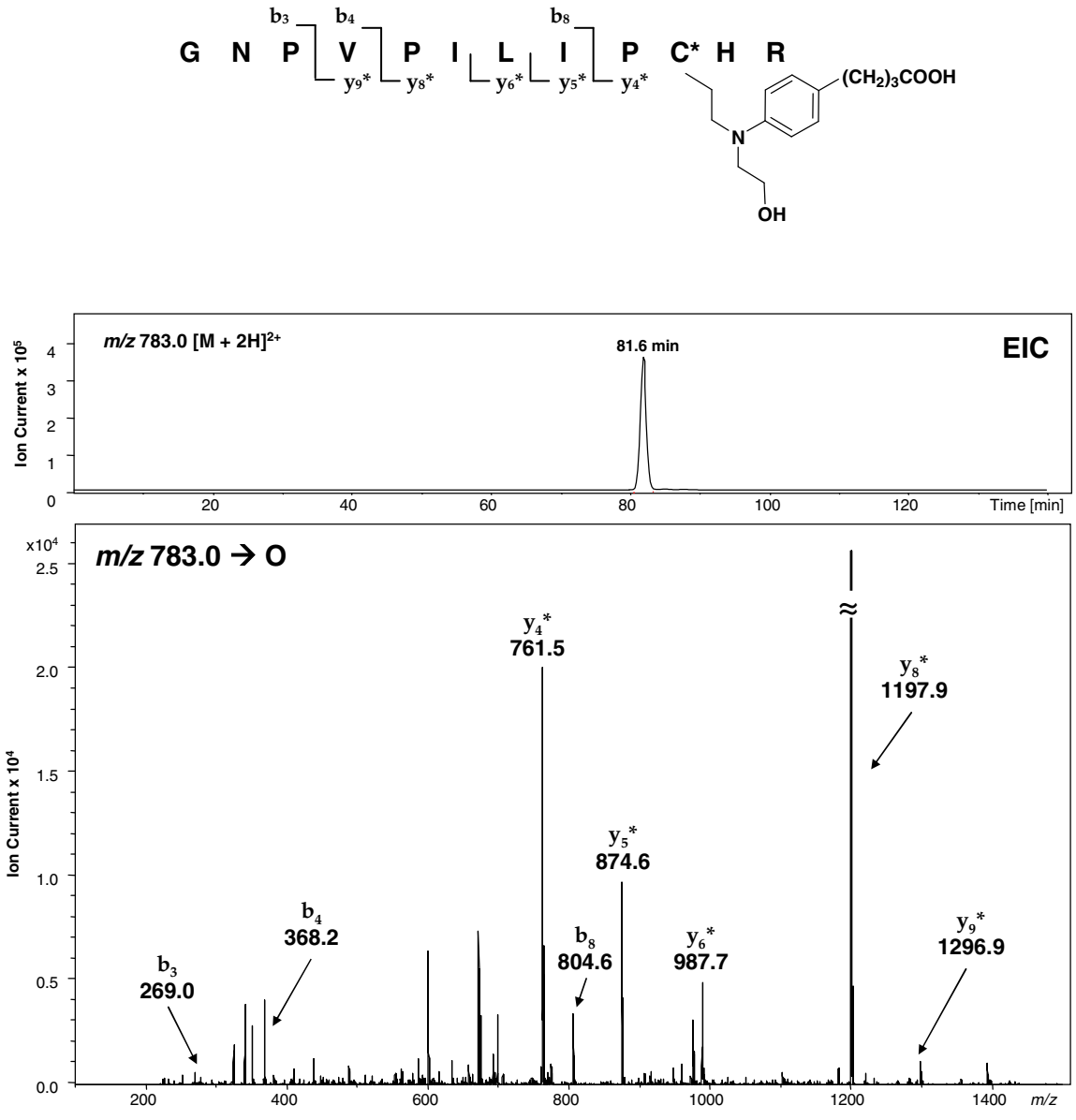
S-13. HPLC-ESI$^+$-MS analysis of AGT protein following incubation with chlorambucil. *Top*: Total ion chromatogram; *Bottom:* ESI$^+$ mass spectrum of 17.3 min protein peak; *Inset:* Deconvoluted mass spectrum of the 17.3 min peak: The observed mass of 22 379 Da corresponds to WT AGT containing two hydrolyzed chlorambucil adducts (calculated M = 22 376 Da).



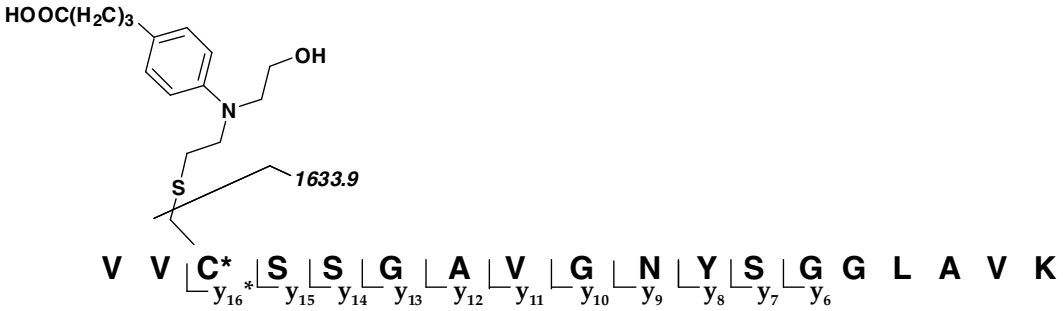
S-14. HPLC-ESI$^+$-MS analysis of AGT protein following incubation with mechlorethamine. *Top*: Total ion chromatogram; *Bottom:* ESI$^+$ mass spectrum of 17.6 min protein peak; *Inset:* Deconvoluted mass spectrum of the 17.6 min peak: *A* = WT AGT containing an intramolecular mechlorethamine cross-link (calculated *M* = 21 959 Da, observed *M* = 21 964 Da), *B* = WT AGT containing a single hydrolyzed mechlorethamine adduct (calculated *M* = 21 978 Da, observed *M* = 21 981 Da), *C* = AGT containing two hydrolyzed mechlorethamine adducts (calculated *M* = 22 080 Da, observed *M* = 22 084 Da).

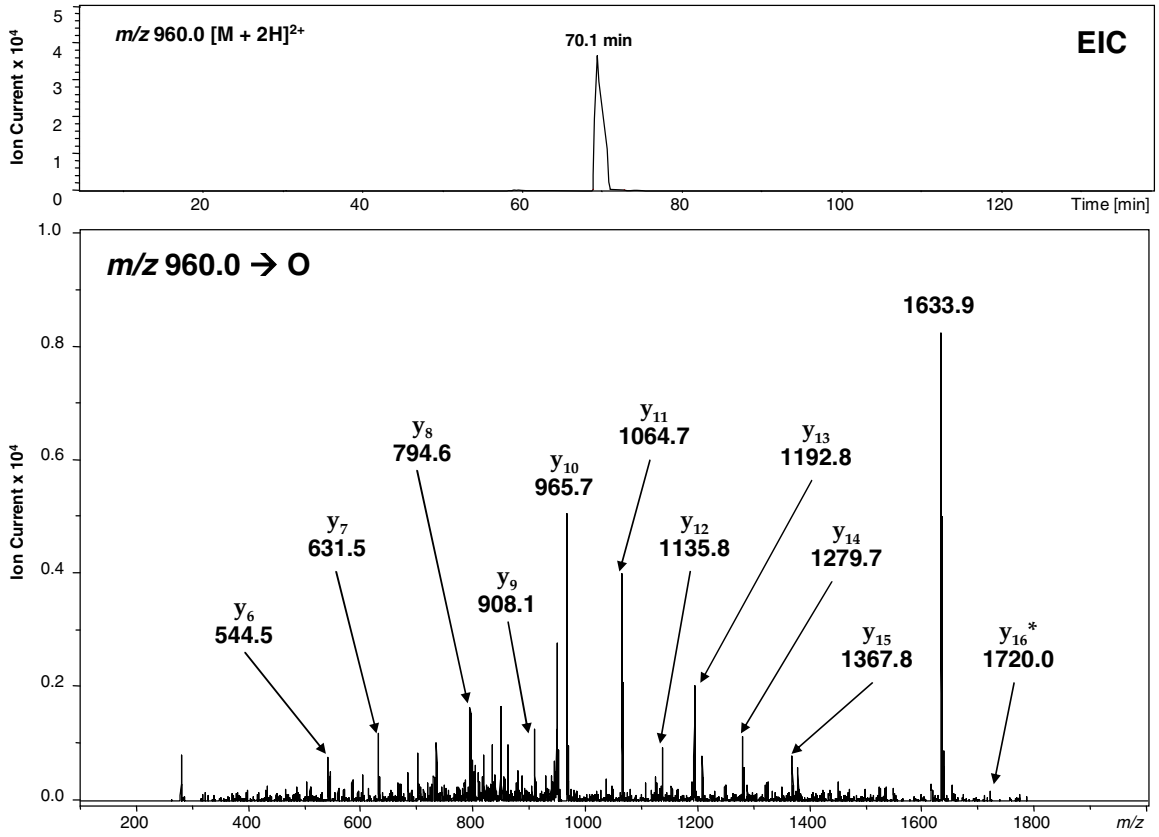


S-15. HPLC-ESI$^+$-MS/MS analysis of AGT tryptic peptide G^{136}NPVPILIPCHR^{147} containing hydrolyzed chlorambucil monoadduct. *Top*: Extracted ion chromatogram of *m/z* 783.0 [M + 2H]$^{2+}$; *Bottom*: MS/MS spectrum of the 81.6 min peak mapping the adduct to Cys^{145} (C*).

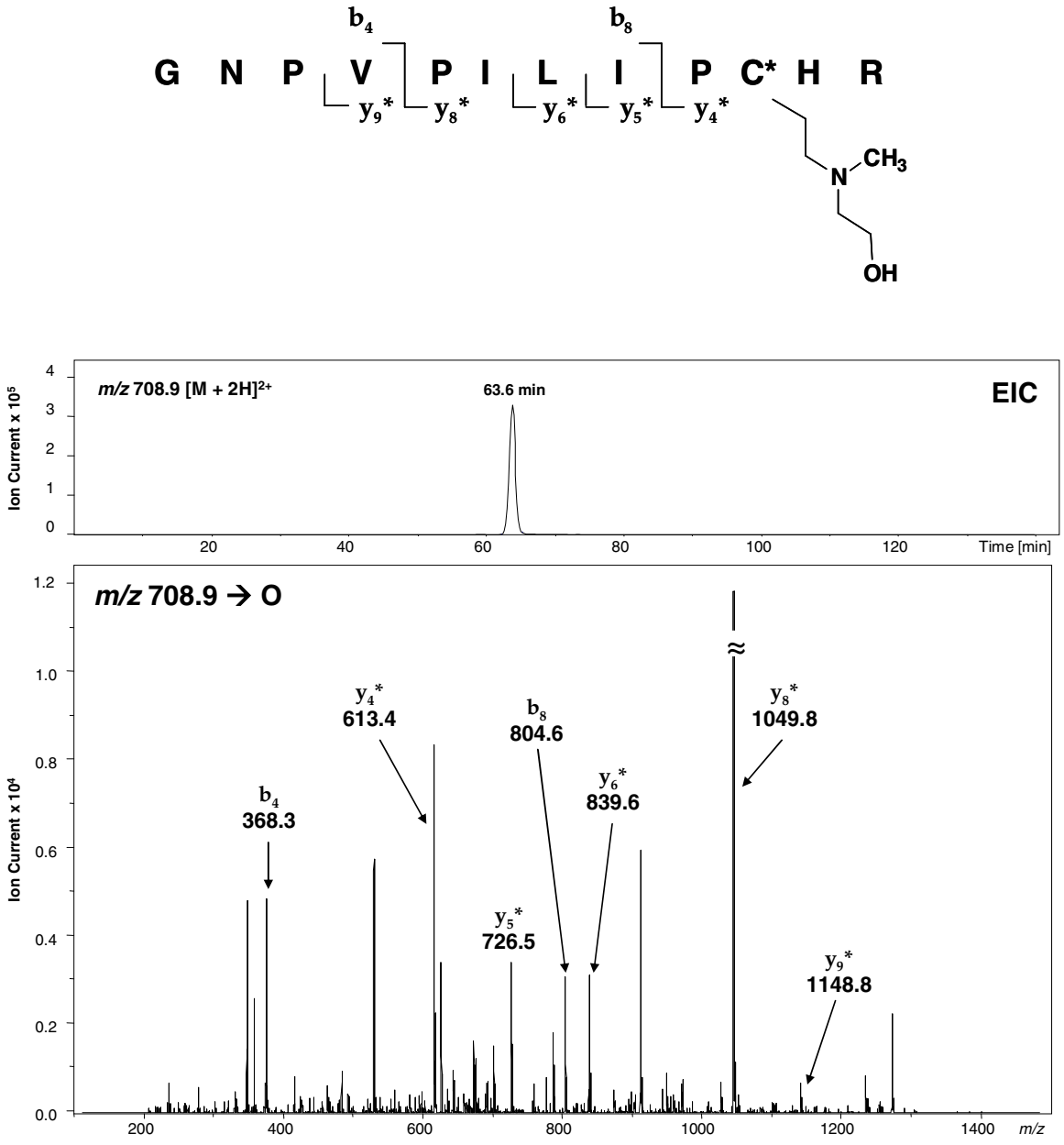


S-16. HPLC-ESI$^+$-MS/MS analysis of AGT tryptic peptide V^{148}VCSSGAVGNYSGGLAVK^{165} containing hydrolyzed chlorambucil monoadduct. *Top*: Extracted ion chromatogram of *m/z* 960.0 $[M + 2H]^{2+}$; *Bottom*: MS/MS spectrum of the 70.1 min peak mapping the adduct to Cys^{150} (C*).

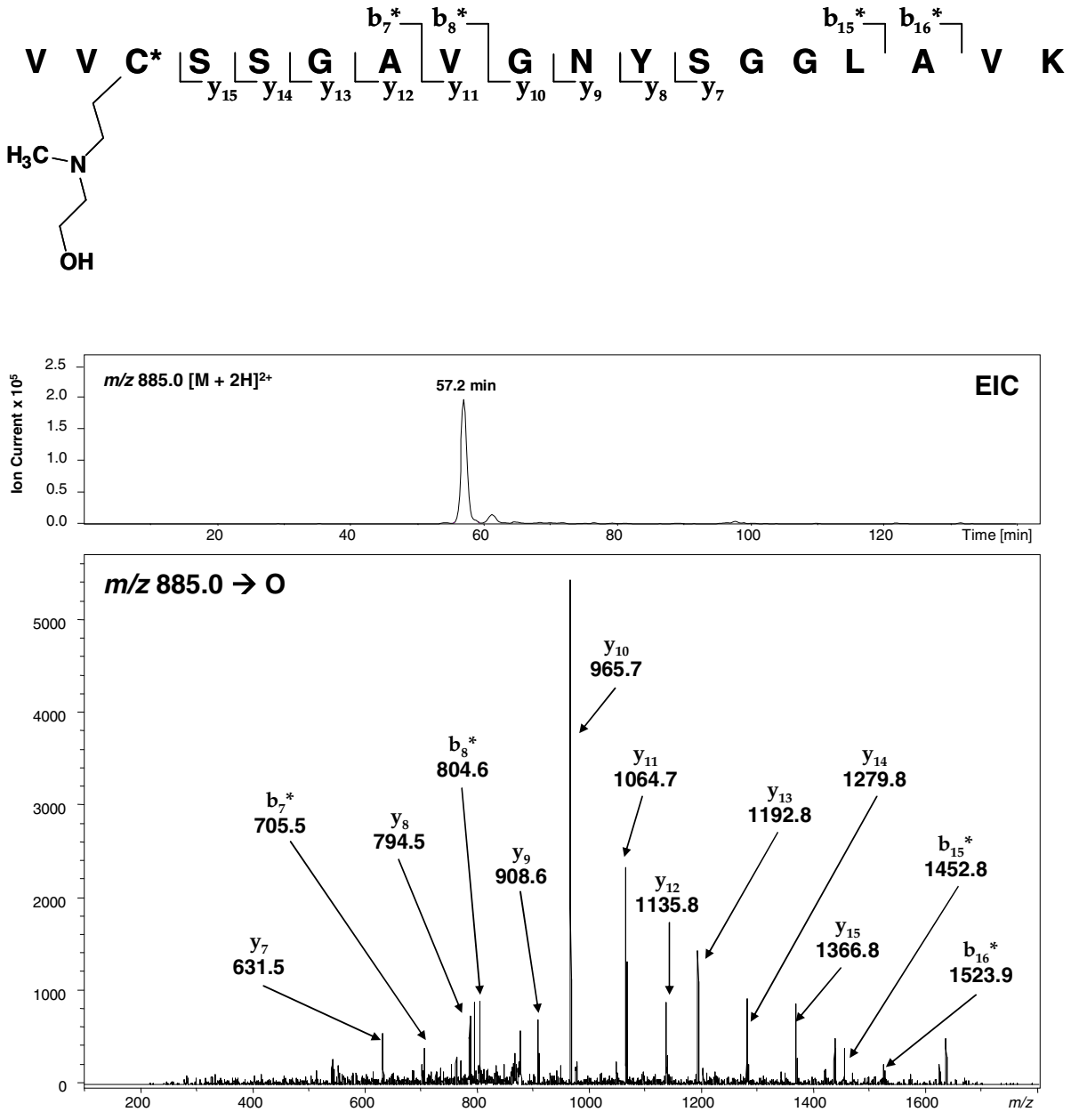




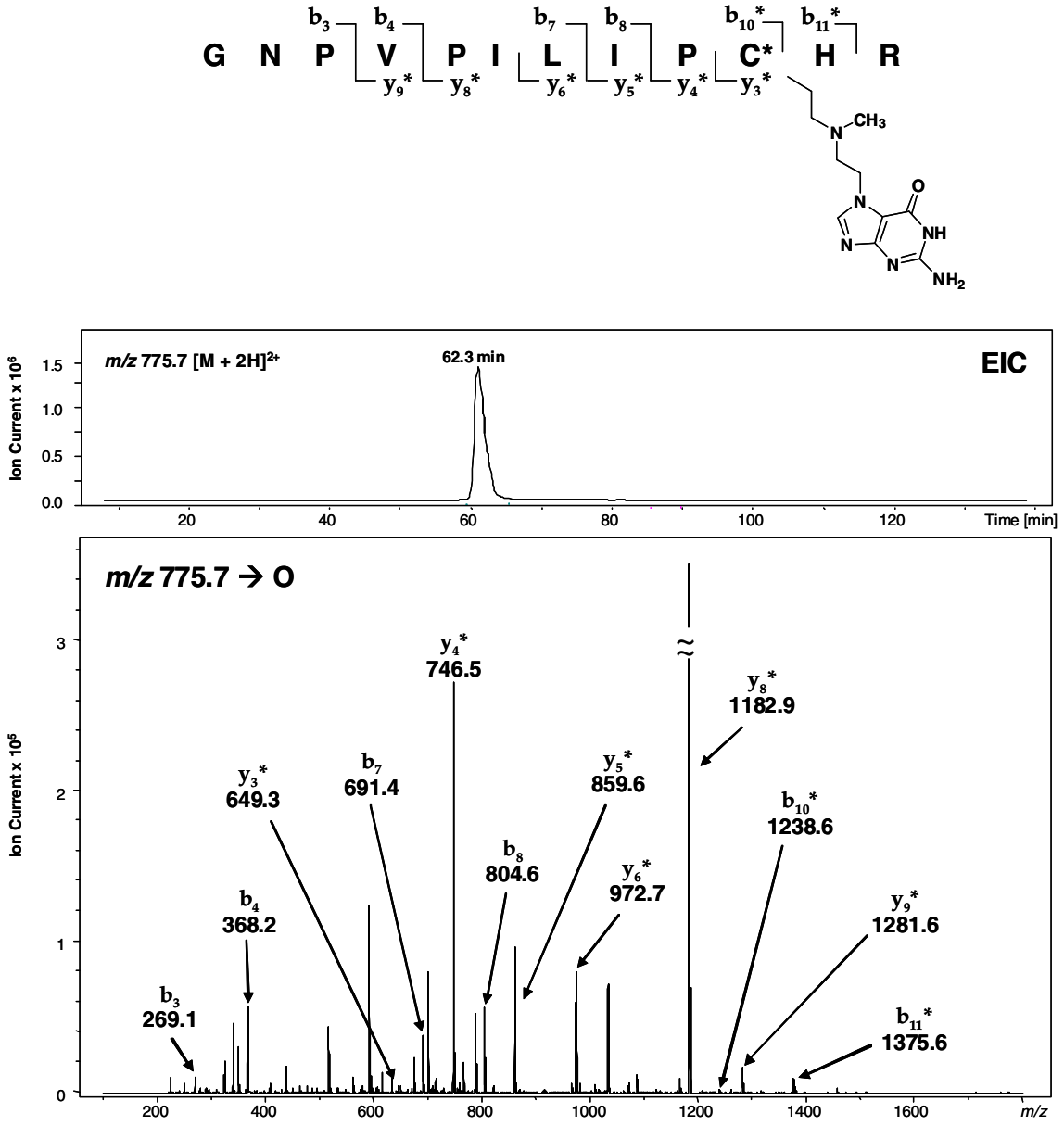
S-17. HPLC-ESI$^+$-MS/MS analysis of AGT tryptic peptide G^{136}NPVPILIPCHR^{147} containing hydrolyzed mechlorethamine monoadduct. *Top*: Extracted ion chromatogram of *m/z* 708.9 [M + 2H]$^{2+}$; *Bottom*: MS/MS spectrum of the 63.6 min peak mapping the cross-link to Cys^{145}.



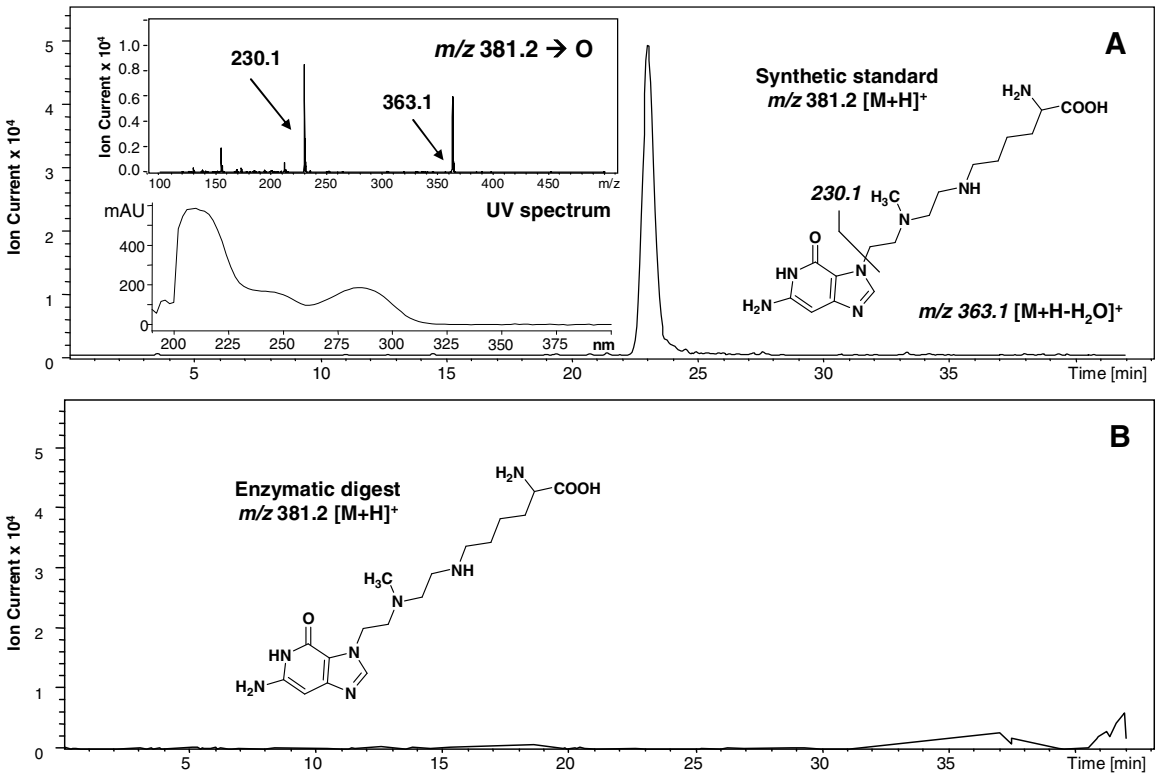
S-18. HPLC-ESI$^+$-MS/MS analysis of AGT tryptic peptide V^{148}VCSSGAVGNYSGGLAVK^{165} containing hydrolyzed mechlorethamine monoadduct. *Top*: Extracted ion chromatogram of *m/z* 885.0 $[M + 2H]^{2+}$; *Bottom*: MS/MS spectrum of the 57.2 min peak mapping the cross-link to Cys^{150}.



S-19. HPLC-ESI$^+$-MS/MS analysis of synthetic peptide GNPVPILIPCHR containing mechlorethamine cross-link to guanine. *Top*: Extracted ion chromatogram of *m/z* 775.7 [M + 2H]$^{2+}$; *Bottom*: MS/MS spectrum of the 62.3 min peak mapping the cross-link to Cys (C*).



S-20. HPLC-ESI$^+$-MS/MS analysis of Lys-N7G-EMA (*m/z* 381.2 $[M + H]^+$): (**A**) Synthetic standard; *Inset*: MS/MS fragmentation and UV spectrum of Lys-N7G-EMA. (**B**) HPLC-ESI$^+$-MS/MS analysis of the total digests of AGT treated with mechlorethamine in the presence of DNA.



S-21. Amino acid sequences of the AGT protein variants employed in this study.

Wild type AGT with C-Terminal His Tag

MDKDCEMKRT TLDSPLGKLE LSGCEQGLHE IKLLGKGTSA ADAVEVPAPA AVLGGPEPLM QCTAWLNAYF HQPEAIEEFP VPALHHPVFQ QESFTRQVLW KLLKVVKFGE VISYQQLAAL AGNPKAARAV GGAMRGNPVP ILIPCHRVVC SSGAVGNYSG GLAVKEWLLA HEGHRLGKPG LGGSSGLAGA WLKGAGATSG SHHHHHH

MW (average mass): 21876 Da / MW (monoisotopic mass): 21862 Da

C145A AGT with N-Terminal His Tag

MRGSHHHHHH GSMDKDCEMK RTTLDSPLGK LELSGCEQGL HEIKLLGKGT SAADAVEVPA PAAVLGGPEP LMQCTAWLNA YFHQPEAIEE FPVPALHHPV FQQESFTRQV LWKLLKVVKF GEVISYQQLA ALAGNPKAAR AVGGAMRGNP VPILIPAHRV VCSSGAVGNY SGGLAVKEWL LAHEGHRLGK PGLGGSSGLA GAWLKGAGAT SGSPPAGRN

MW (average mass): 23012.40 Da / MW (monoisotopic mass): 22997.79 Da

C145A, C150S AGT with N-Terminal His Tag

MRGSHHHHHH GSMDKDCEMK RTTLDSPLGK LELSGCEQGL HEIKLLGKGT SAADAVEVPA PAAVLGGPEP LMQCTAWLNA YFHQPEAIEE FPVPALHHPV FQQESFTRQV LWKLLKVVKF GEVISYQQLA ALAGNPKAAR AVGGAMRGNP VPILIPAHRV VSSSGAVGNY SGGLAVKEWL LAHEGHRLGK PGLGGSSGLA GAWLKGAGAT SGSPPAGRN

MW (average mass): 22996.34 Da / MW (monoisotopic mass): 22981.81 Da

S-22. AGT tryptic peptides

Position	Peptide	$[M+H]^{+}_{calculated}$	$[M+2H]^{2+}_{calculated}$	Observed Ions
1-9	MDKDCEMKR	1156.4	578.7	1156.7, 578.9
10-18	TTLDSPLGK	931.5	466.3	931.7, 466.3
19-32	LELSGCEQGLHEIK	1555.8	778.4	778.8
33-36	LLGK	430.3	215.7	430.4, 215.6
37-96	GTSAADAVEVPAPAAVLG GPEPLMQCTAWLNAYFH QPEAIEEFPVPALHHPVFQ QESFTR	6469.2	3235.1	ND
97-101	QVLWK	673.4	337.2	673.5
102-104	LLK	373.3	187.1	373.3, 187.1
105-107	VVK	345.3	173.1	ND
108-125	FGEVISYQQLAALAGNPK	1906.0	953.5	953.5
126-128	AAR	317.2	159.0	317.2
129-135	AVGGAMR	661.4	331.2	661.4, 331.2
136-147	GNPVPILIPCHR	1315.7	658.4	1315.7, 658.6
148-165	VVCSSGAVGNYSGGLAVK	1667.8	834.4	834.6
166-175	EWLLAHEGHR	1247.6	624.3	1247.7, 624.5
176-193	LGKPGLGGSSGLAGAWLK	1668.9	835.0	1669.0, 835.2
194-207	GAGATSGSHHHHHH	1429.6	715.3	1429.6, 715.4

S-23. Amino acid sequence of bovine histone H4

Histone H4
SGRGKGGKGL GKGGAKRHRK VLRDNIQGIT KPAIRRLARR GGVKRISGLI YEETRGVLKV FLENVIRDAV TYTEHAKRKT VTAMDVVYAL KRQGRTLYGF GG
MW (average mass): 11236.2 Da / MW (monoisotopic mass): 11229.4 Da
Observed Masses: 11310 Da (MW + Acet + 2Me); 11352 Da (MW + 2Acet + 2Me); 11326 Da (MW + Acet + 2Me + O); and 11368 Da (MW + 2 Acet + 2 Me + O)

S-24. Histone H4 tryptic peptides

Position	Peptide	$[M+H]^{+}_{calculated}$	$[M+2H]^{2+}_{calculated}$	Observed Ions
1-8	SGRGKGGK	746.43	373.72	746.8, 374.3
4-16 + 2Me	GKGG^{Me}KGLG^{Me}KGGAK	1142.6	571.8	1142.6, 571.8
6-16 + 2Acet	GG^{Acet}KGLG^{Acet}KGGAK	1014.55	507.28	1014.7
17-19	RHR	468.3	234.65	ND
20-23	KVLR	515.4	257.7	515.4, 258.1
24-35	DNIQGITKPAIR	1325.75	663.38	1325.8, 663.6
36-39	RLAR	515.64	258.32	ND
40-45	RGGVKR	672.43	336.72	672.5, 336.8
46-55	ISGLIYEETR	1180.62	590.81	1180.6, 590.9
56-59	GVLK	416.54	208.77	416.3
60-67	VFLENVIR	989.58	495.29	989.6, 495.3
68-77	DAVTYTEHAK	1134.54	567.77	1134.5, 567.9
78-79	RK	303.39	170.2	ND
80-91 + Oxygen	TVTAMDVVYALK	1326.7	663.85	1326.0, 663.7
92-95	RQGR	516.3	258.65	ND
96-102	TLYGFGG	714.35	357.68	714.5