

Supporting Information

Gambogic Acid Deactivates Cytosolic and Mitochondrial Thioredoxins by Covalent Binding to the Functional Domain

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Supplemental Experimental Section:

The inhibitory effect of gambogic acid (**1**) on cellular TRX activity was measured by the insulin reduction assay as previously described.^{1, 2} SMMC-7721 cells were incubated with different concentrations of **1** (1.5, 3.0, and 6.0 μ M) for 2 h, respectively. The cells were harvested and lysed. The cell extracts were subjected to ultrafiltration to remove excess **1** and then added into the assay system described in EXPERIMENTAL SECTION. To correct TRX-independent thiol present in the cell extracts, the blank was made for each sample by adding the same amount of the cell extracts after the reaction had been stopped by guanidine hydrochloride. A net increase in absorbance at 412 nm for each tested sample was obtained by subtracting the blank absorbance values from sample absorbance values. TRX activity value was expressed as change in absorbance at 412 nm per minute per milligram protein (ΔA_{412} nm/min/mg). All values are expressed as means \pm SD of three independent experiments.

Legends of Supplemental Figures:

Figure S1. Inhibitory effect of gambogic acid (**1**) on cellular TRX activity. SMMC-7721 cells were exposed to different concentrations of **1** for 2 h. The TRX activity was measured by the insulin reduction assay. Values are represented as means \pm SD of three independent experiments.

Figure S2. ^1H NMR spectrum (500 MHz, $\text{DMSO-}d_6$) of gambogic acid (**1**)

Figure S3. ^{13}C NMR spectrum (500 MHz, $\text{DMSO-}d_6$) of gambogic acid (**1**)

Figure S4. MS and MS/MS spectra of gambogic acid (**1**) in ESI negative-ion mode.

Figure S5. ^1H NMR spectrum (500 MHz, $\text{DMSO-}d_6$) of gambogenic acid (**2**)

Figure S6. ^{13}C NMR spectrum (500 MHz, $\text{DMSO-}d_6$) of gambogenic acid (**2**)

Figure S7. MS and MS/MS spectra of gambogenic acid (**2**) in ESI negative-ion mode.

Figure S8. ^1H NMR spectrum (500 MHz, $\text{DMSO-}d_6$) of 9, 10-Dihydrogambogic acid (**3**)

Figure S9. MS and MS/MS spectra of 9, 10-Dihydrogambogic acid (**3**) in ESI negative-ion mode.

Figure S10. ^1H NMR spectrum (500 MHz, $\text{DMSO-}d_6$) of 10-Hydroxygambogic acid (**4**)

Figure S11. ^{13}C NMR spectrum (500 MHz, $\text{DMSO-}d_6$) of 10-Hydroxygambogic acid (**4**)

Figure S12. MS and MS/MS spectra of 10-Hydroxygambogic acid (**4**) in ESI negative-ion mode.

Figure S13. ^1H NMR spectrum (500 MHz, $\text{DMSO-}d_6$) of gambogic acid 10 α -sulfonic acid (**5**)

Figure S14. ^{13}C NMR spectrum (500 MHz, $\text{DMSO-}d_6$) of gambogic acid 10 α -sulfonic acid (**5**)

Figure S15. MS and MS/MS spectra of gambogic acid 10 α -sulfonic acid (**5**) in ESI

negative-ion mode.

Figure S16. ^1H NMR spectrum (500 MHz, $\text{DMSO-}d_6$) of gambogic acid 10 β -sulfonic acid (**6**)

Figure S17. ^{13}C NMR spectrum (500 MHz, $\text{DMSO-}d_6$) of gambogic acid 10 β -sulfonic acid (**6**)

Figure S18. MS and MS/MS spectra of gambogic acid 10 β -sulfonic acid (**6**) in ESI negative-ion mode.

Figure S19. ^1H NMR spectrum (500 MHz, $\text{DMSO-}d_6$) of biotinylated gambogic acid (**7**)

Figure S20. MS and MS/MS spectra of biotinylated gambogic acid (**7**) in ESI positive-ion mode.

Figure S21. Complete western blotting gel from Fig.4.

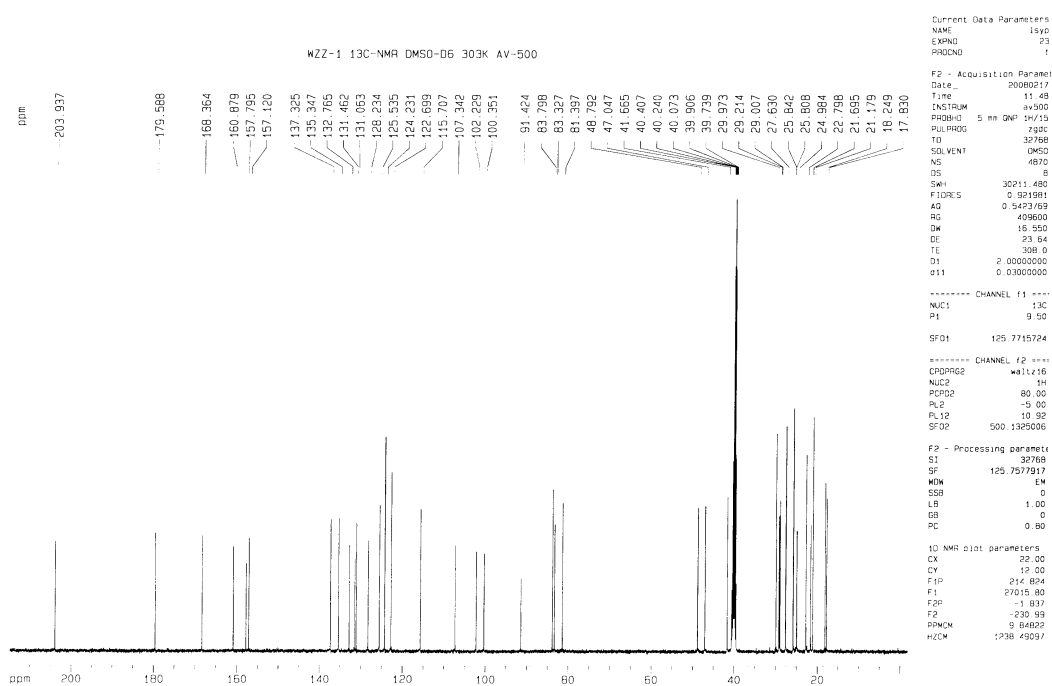


Figure S3. ^{13}C NMR spectrum (500 MHz, $\text{DMSO}-d_6$) of gambogic acid (**1**)

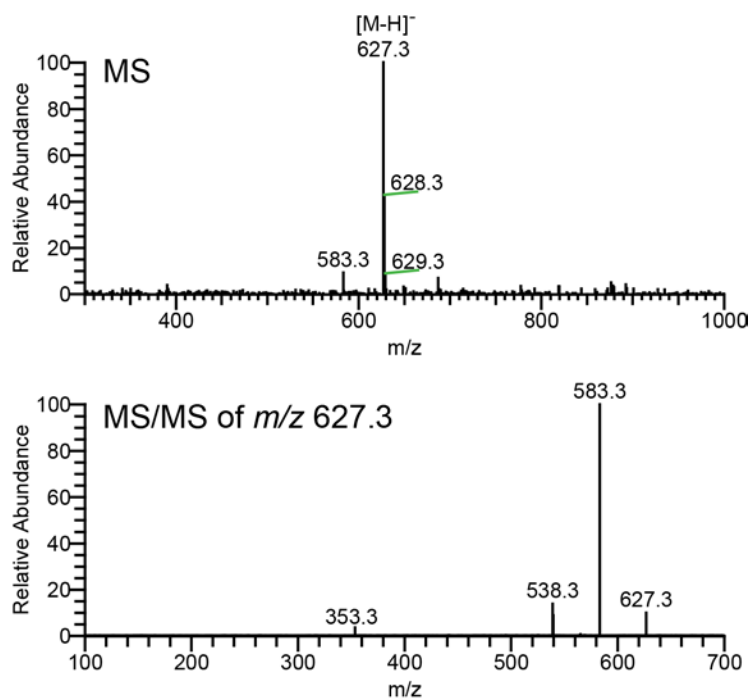


Figure S4. MS and MS/MS spectra of gambogic acid (**1**) in the ESI negative-ion mode.

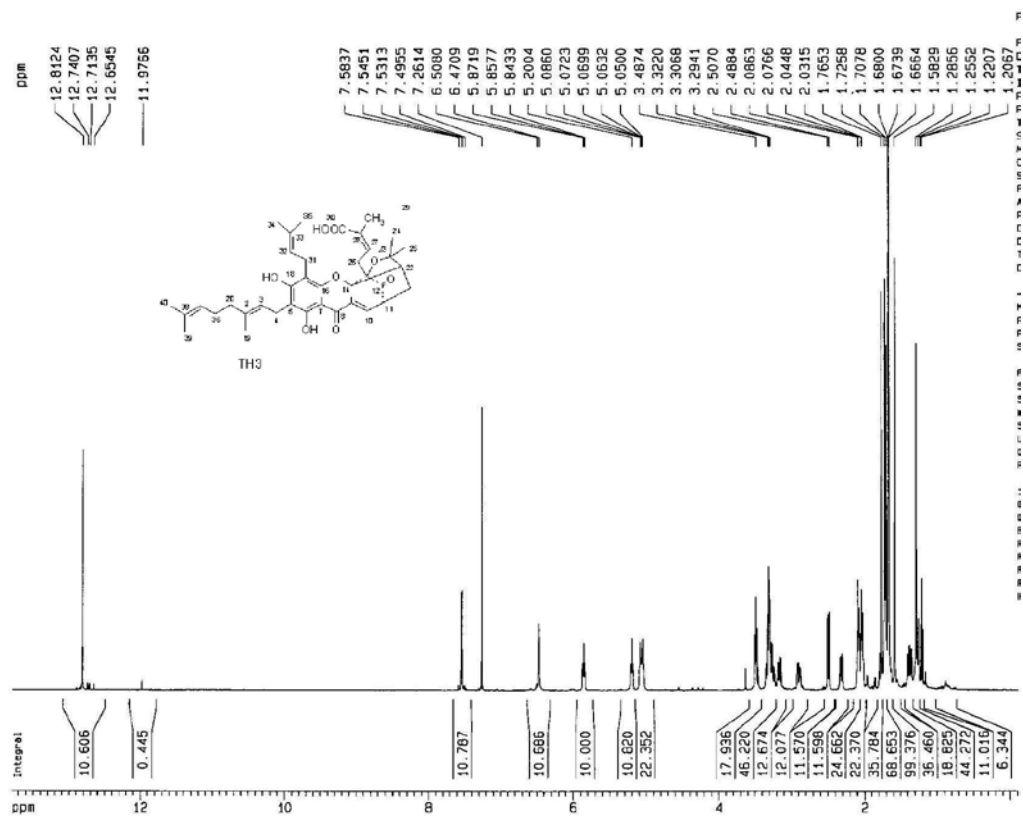


Figure S5. ^1H NMR spectrum (500 MHz, $\text{DMSO}-d_6$) of gambogenic acid (2)

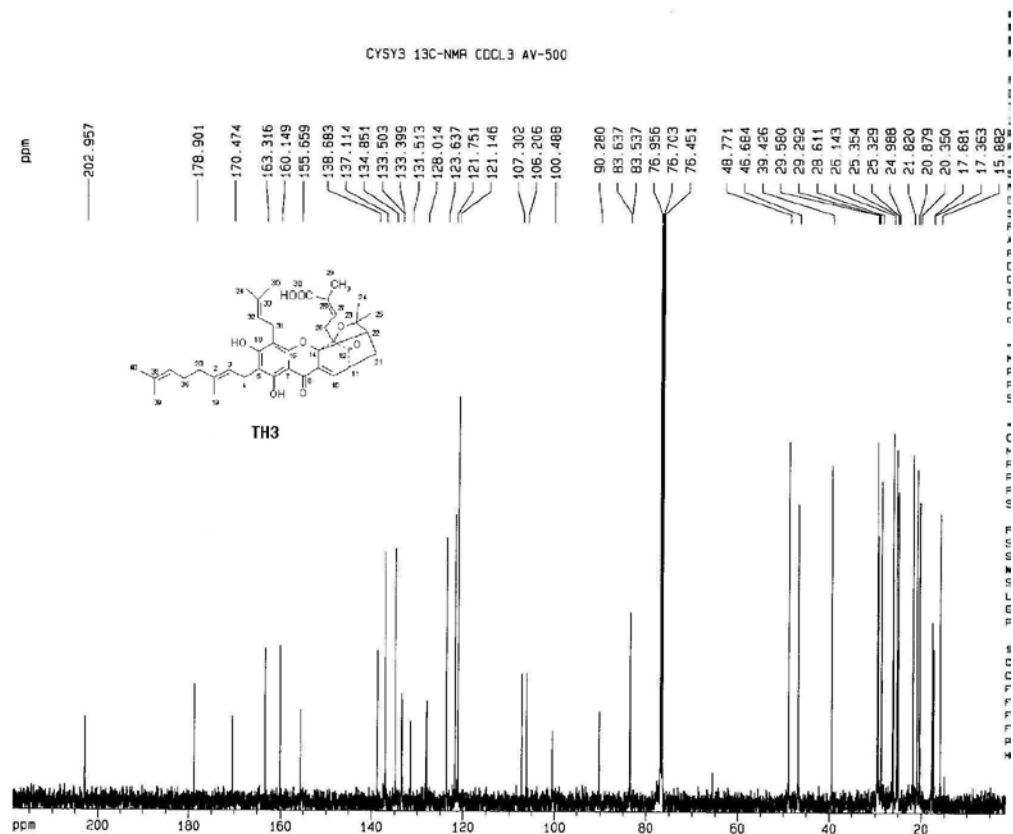


Figure S6. ^{13}C NMR spectrum (500 MHz, $\text{DMSO}-d_6$) of gambogenic acid (2)

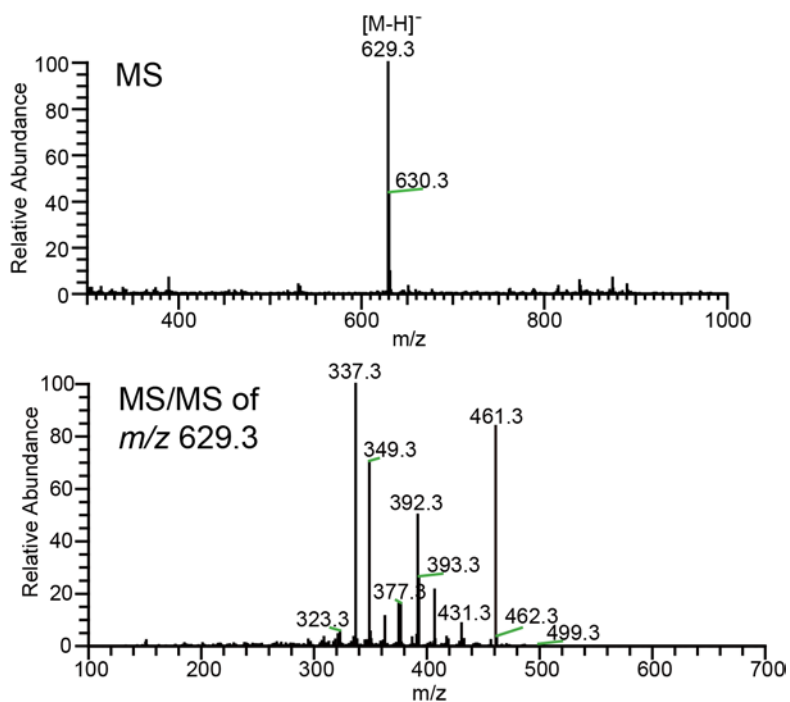


Figure S7. MS and MS/MS spectra of gambogenic acid (**2**) in ESI negative-ion mode.

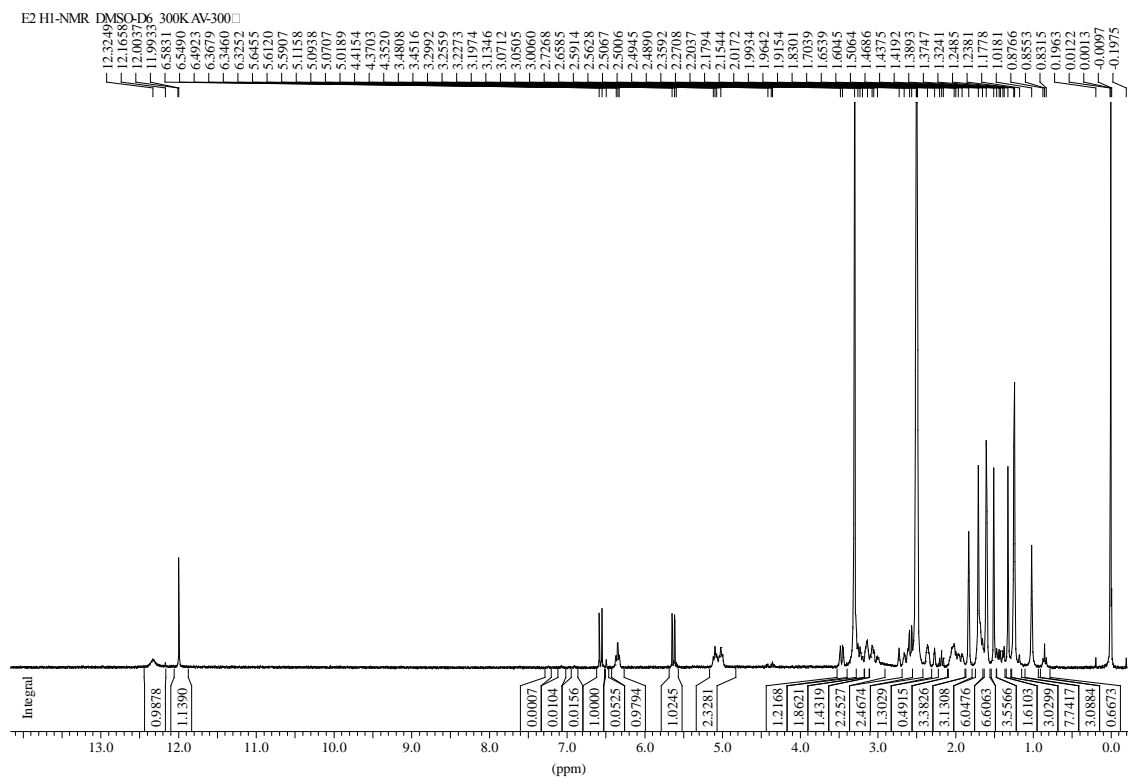


Figure S8. ¹H NMR spectrum (500 MHz, DMSO-*d*₆) of 9, 10-Dihydrogambogenic acid (**3**)

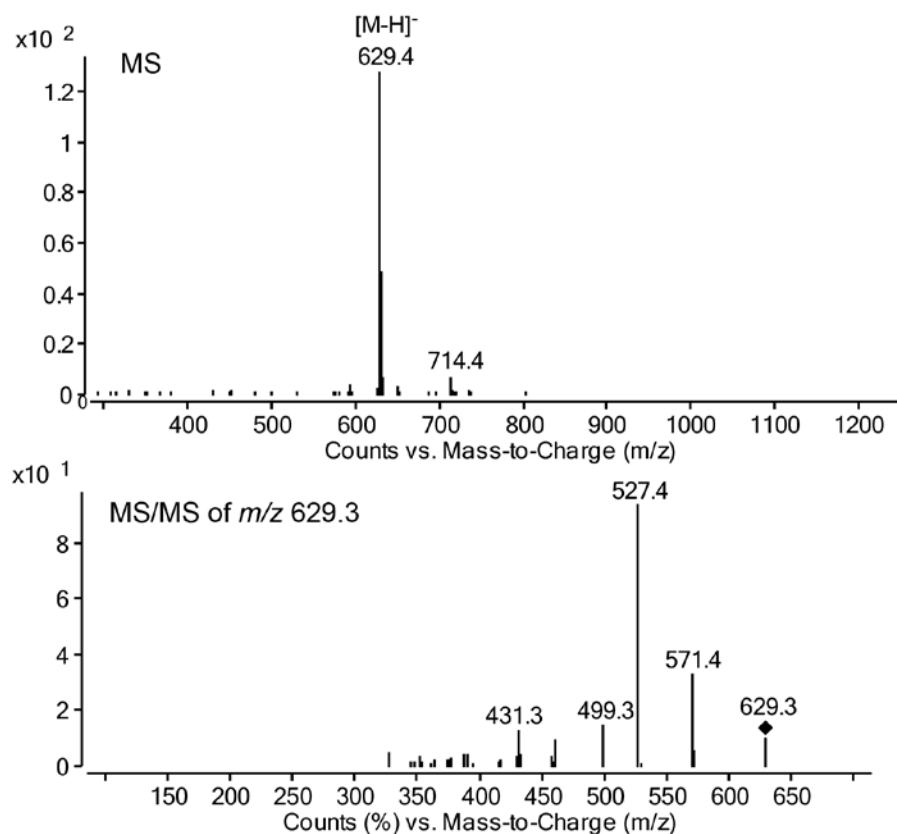


Figure S9. MS and MS/MS spectra of 9, 10-Dihydrogambogic acid (**3**) in the ESI negative-ion mode.

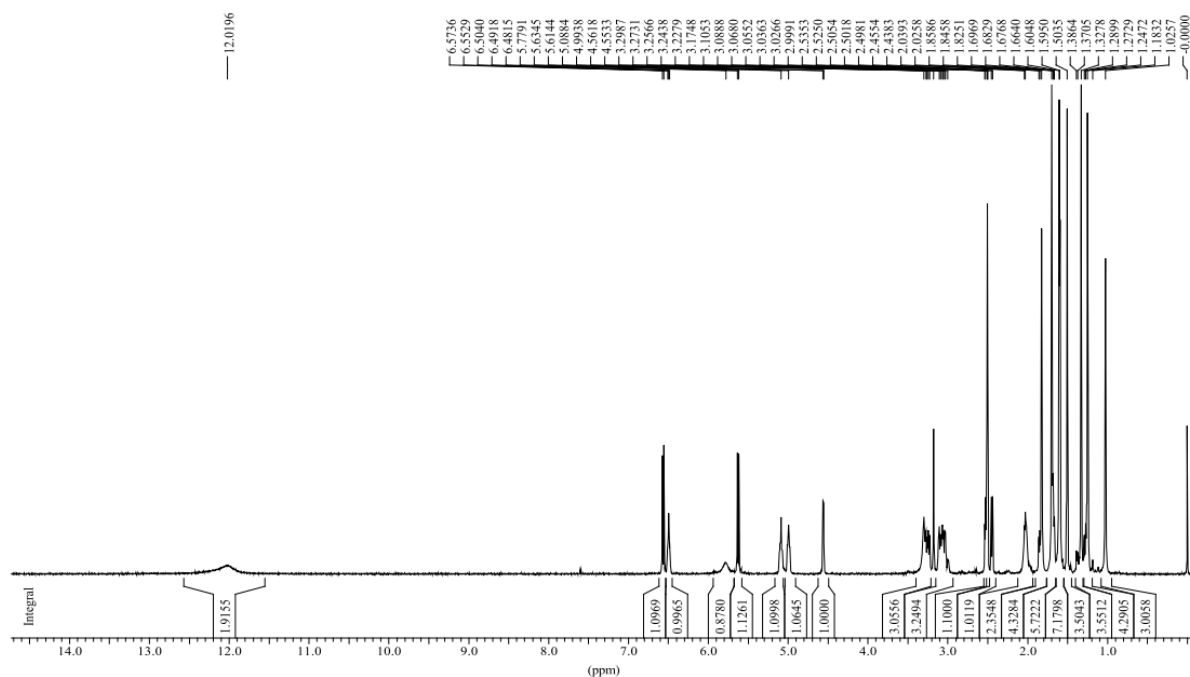


Figure S10. ¹H NMR spectrum (500 MHz, DMSO-*d*₆) of 10-Hydroxygambogic acid (**4**)

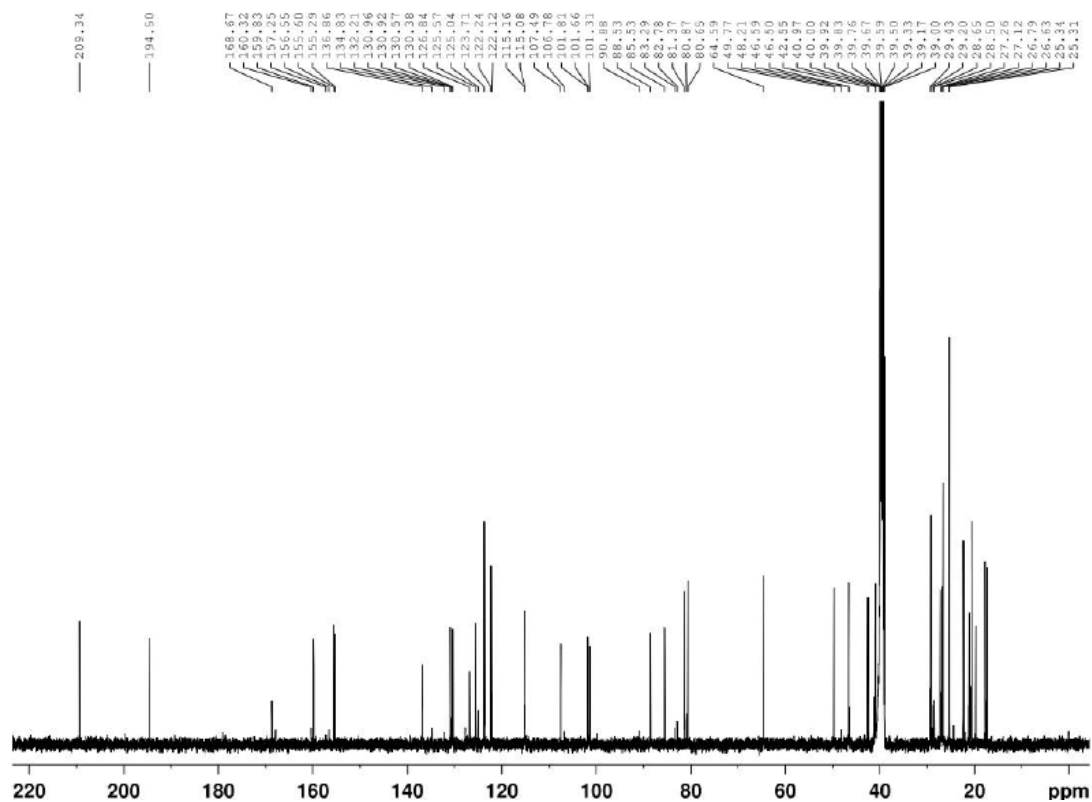


Figure S11. ¹³C NMR spectrum (500 MHz, DMSO-*d*₆) of 10-Hydroxygamboic acid (**4**)

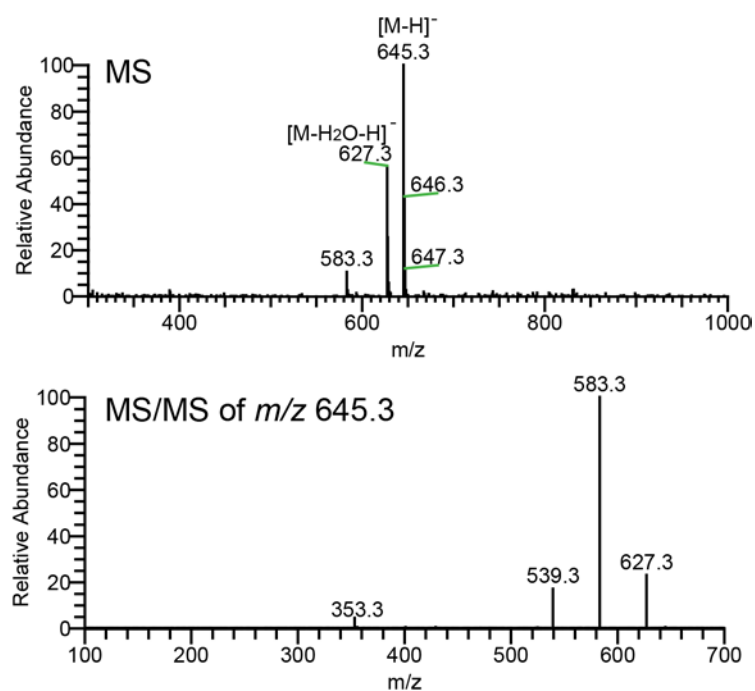


Figure S12. MS and MS/MS spectra of 10-Hydroxygamboic acid (**4**) in the ESI negative-ion mode.

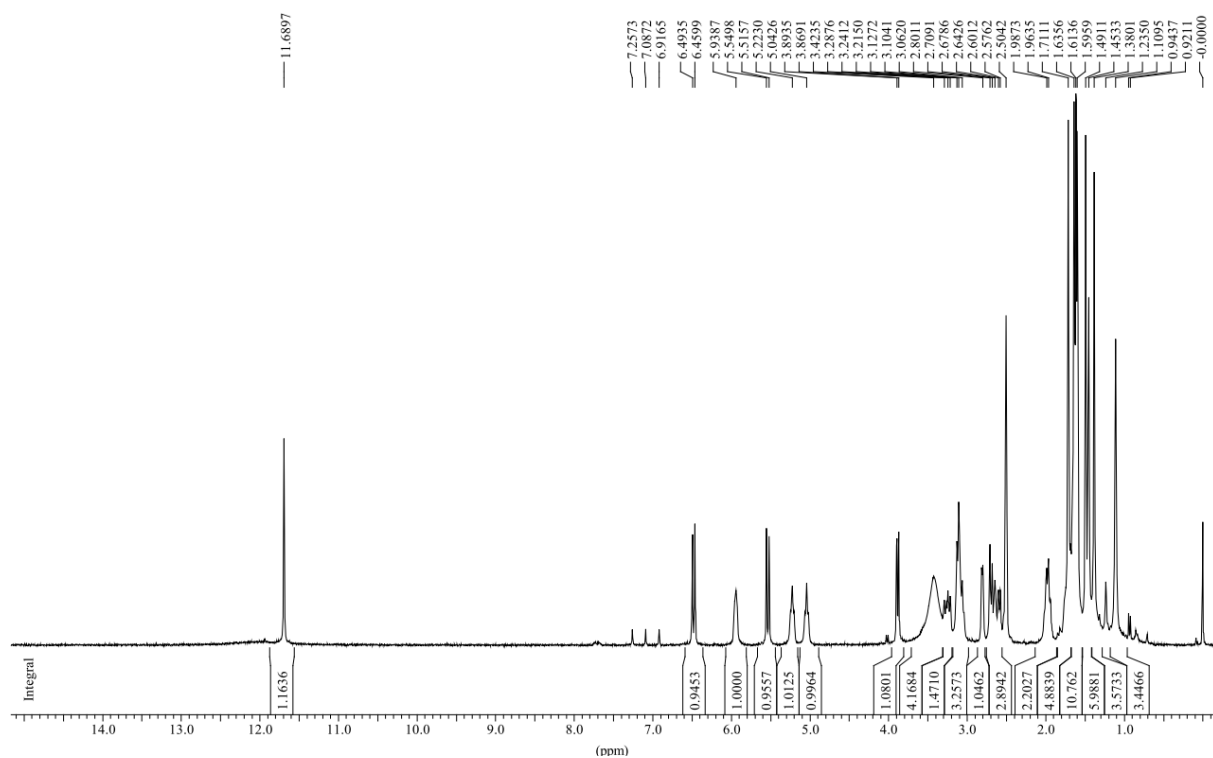


Figure S13. ^1H NMR spectrum (500 MHz, $\text{DMSO-}d_6$) of gambogic acid 10 α -sulfonic acid (**5**)

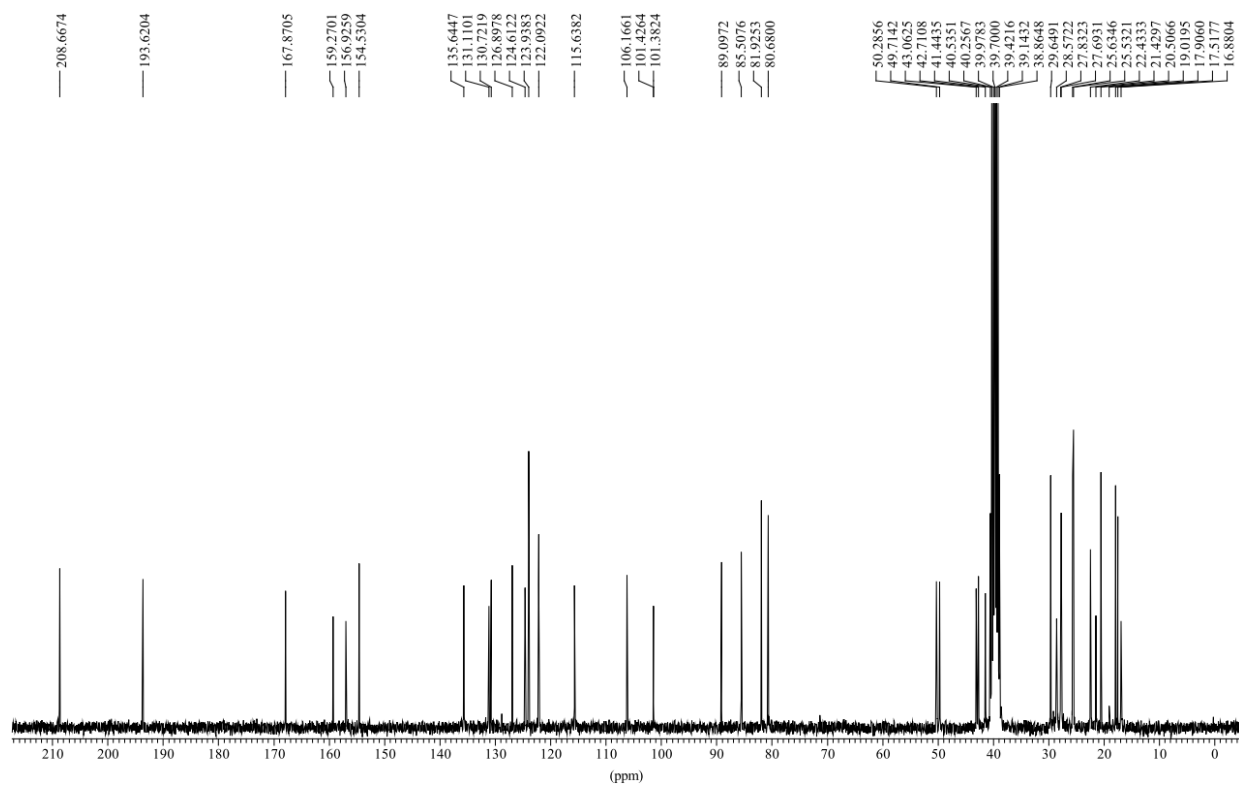


Figure S14. ^{13}C NMR spectrum (500 MHz, $\text{DMSO-}d_6$) of gambogic acid 10 α -sulfonic acid

(**5**)

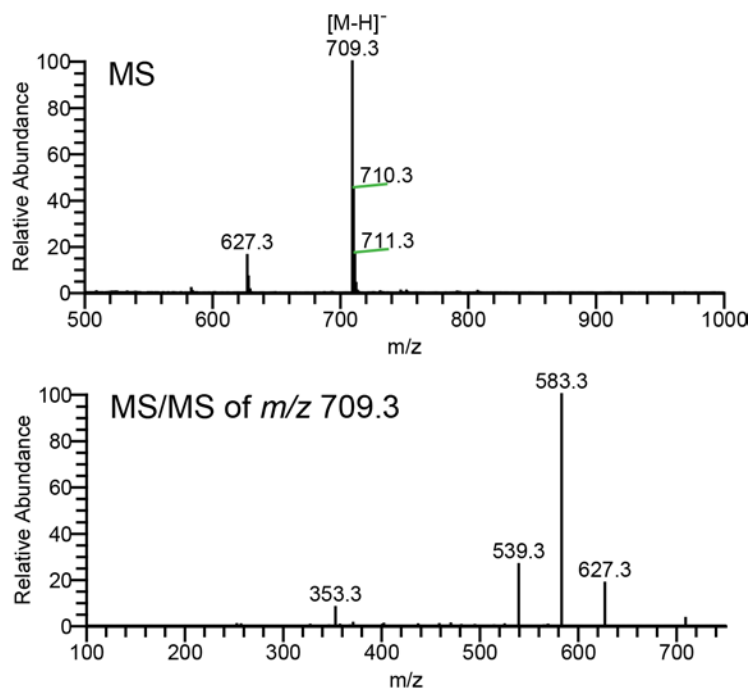


Figure S15. MS and MS/MS spectra of gambogic acid 10 α -sulfonic acid (**5**) in the ESI negative-ion mode.

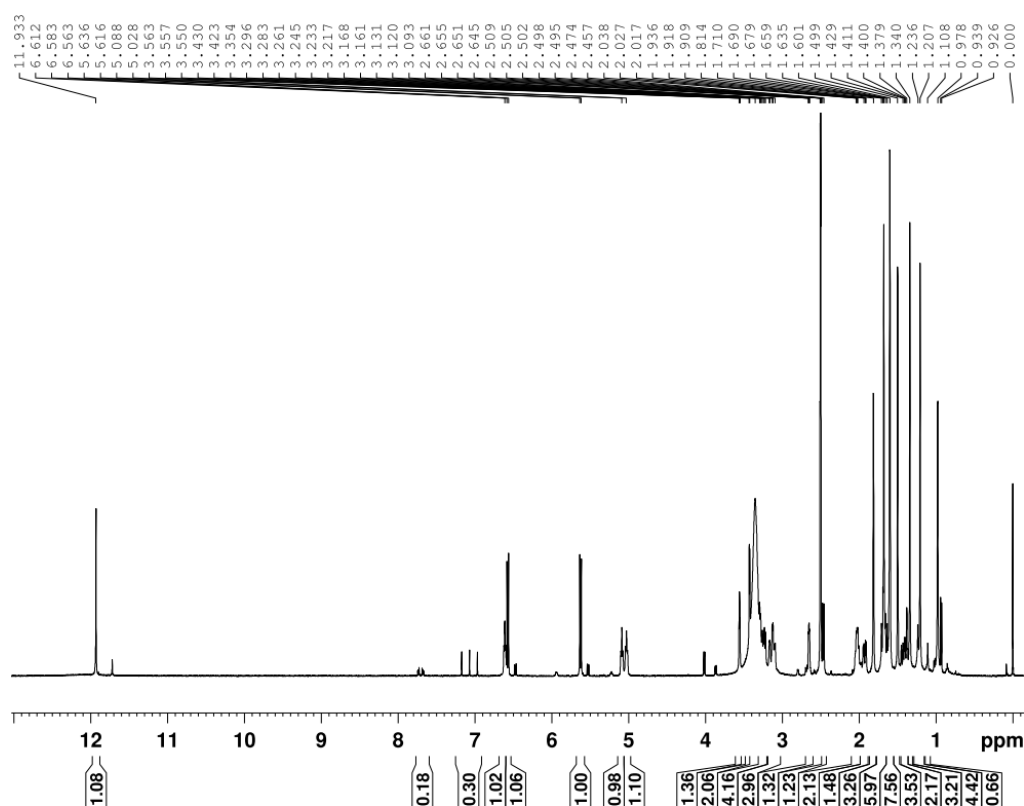


Figure S16. ¹H NMR spectrum (500 MHz, DMSO-*d*₆) of gambogic acid 10 β -sulfonic acid (**6**)

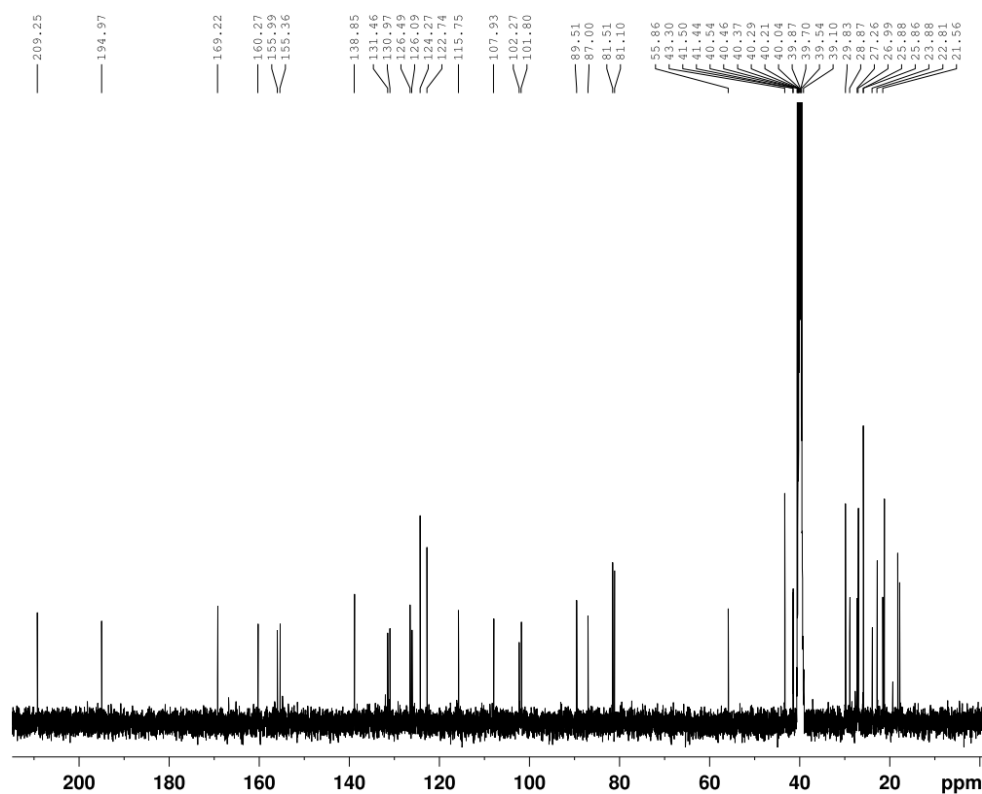


Figure S17. ^{13}C NMR spectrum (500 MHz, $\text{DMSO-}d_6$) of gambogic acid 10 β -sulfonic acid

(6)

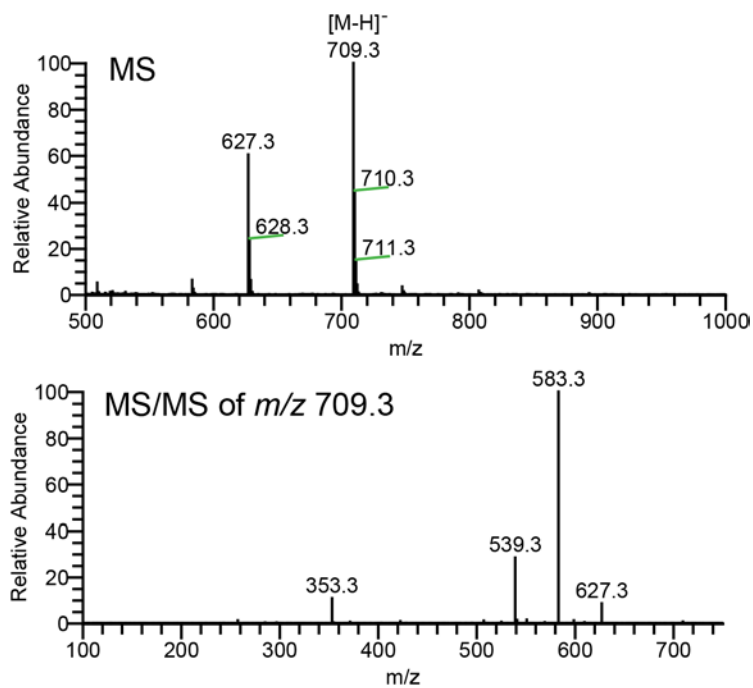


Figure S18. MS and MS/MS spectra of gambogic acid 10 β -sulfonic acid (6) in the ESI negative-ion mode.

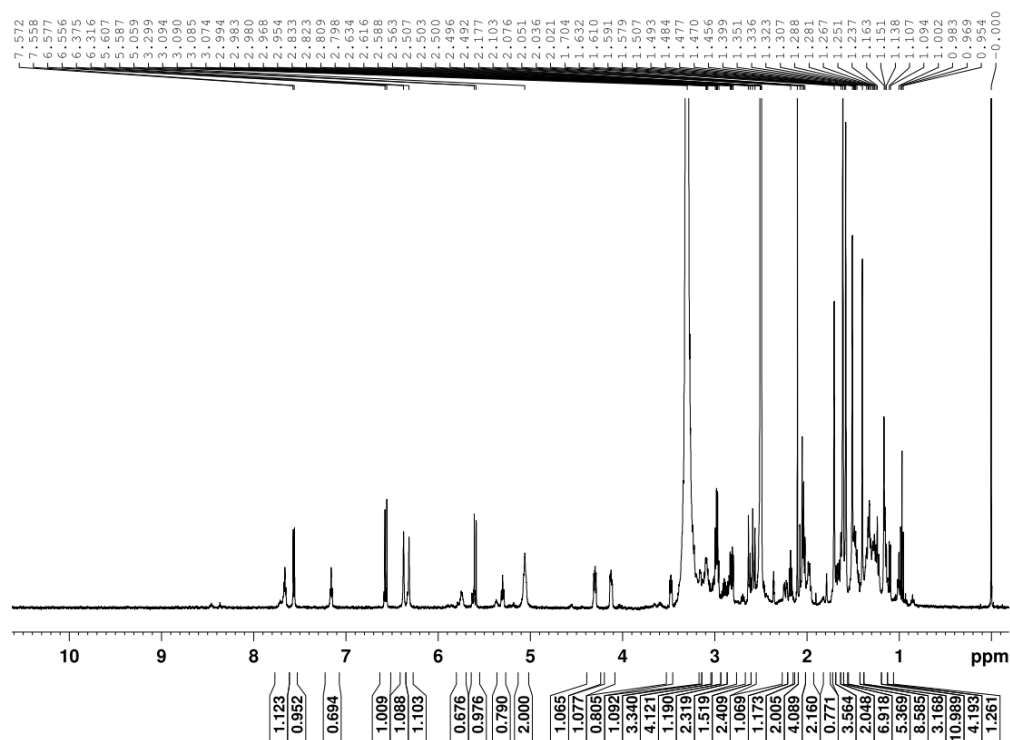


Figure S19. ^1H NMR spectrum (500 MHz, $\text{DMSO}-d_6$) of biotinylated gambogic acid (**7**)

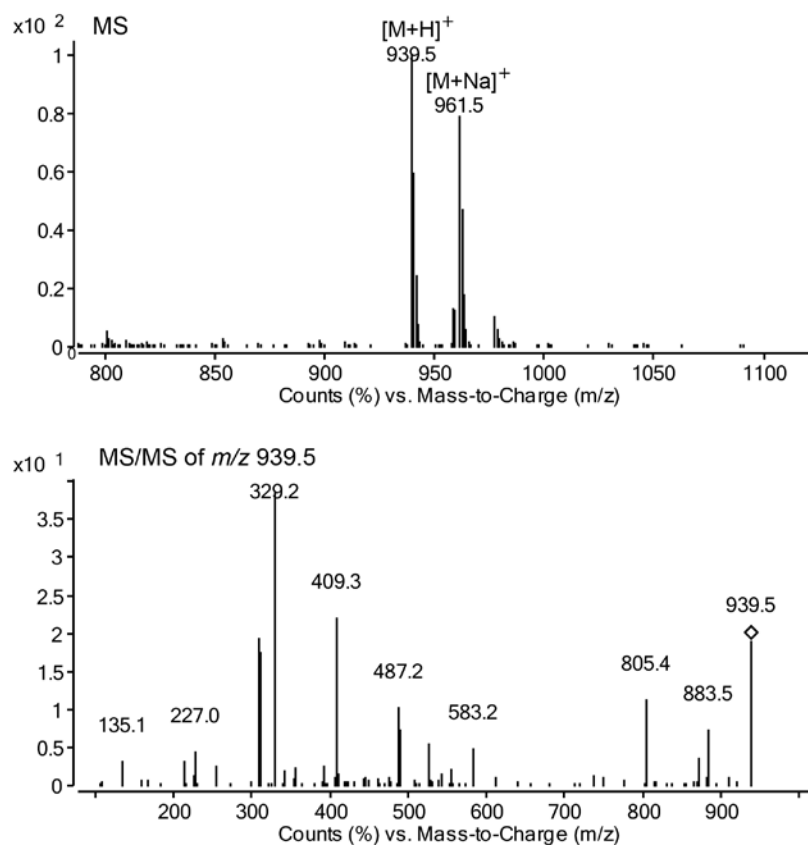


Figure S20. MS and MS/MS spectra of biotinylated gambogic acid (**7**) in the ESI positive-ion mode.

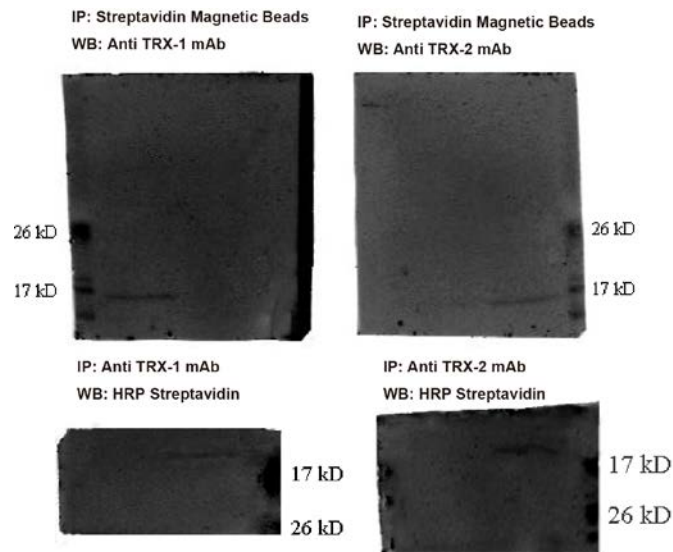


Figure S21. Complete western blotting gel from Fig.4.

References

- (1) Zhang, H.; Cao, D.; Cui, W.; Ji, M.; Qian, X.; Zhong, L. *Free. Radic. Biol. Med.* **2010**, *49*, 2010-2018.
- (2) Wakita, C.; Maeshima, T.; Yamazaki, A.; Shibata, T.; Ito, S.; Akagawa, M.; Ojika, M.; Yodoi, J.; Uchida, K. *J. Biol. Chem.* **2009**, *284*, 28810-28822.