

## Studies on the Reaction of Glutathione and Formaldehyde using NMR

### Supplementary Information

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### General Procedures

NMR experiments were run using 2 mm diameter NMR tubes (Hilgenberg) in a Bruker AVIII 700 spectrometer equipped with a TCI cryoprobe, unless otherwise stated. Deuterium oxide was purchased from Acros Organics. Reduced glutathione, γ-glutamyl-cysteine, paraformaldehyde powder, acetaldehyde and 40 % methylglyoxal solution in H<sub>2</sub>O were purchased from Sigma Aldrich. High-resolution mass data (HRMS) were collected using a Bruker microTOF mass spectrometer.

## $^1\text{H}$ NMR Spectra of Glutathione and $\gamma$ -Glutamyl-Cysteine

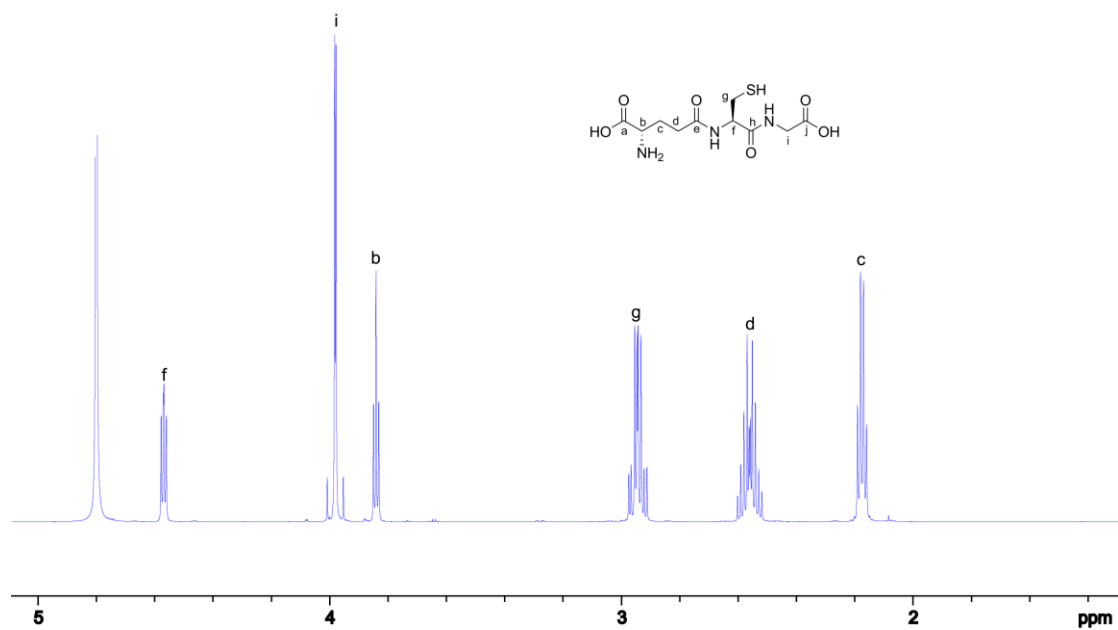


Figure S1.  $^1\text{H}$  NMR spectrum for glutathione (GSH) in  $\text{D}_2\text{O}$ .

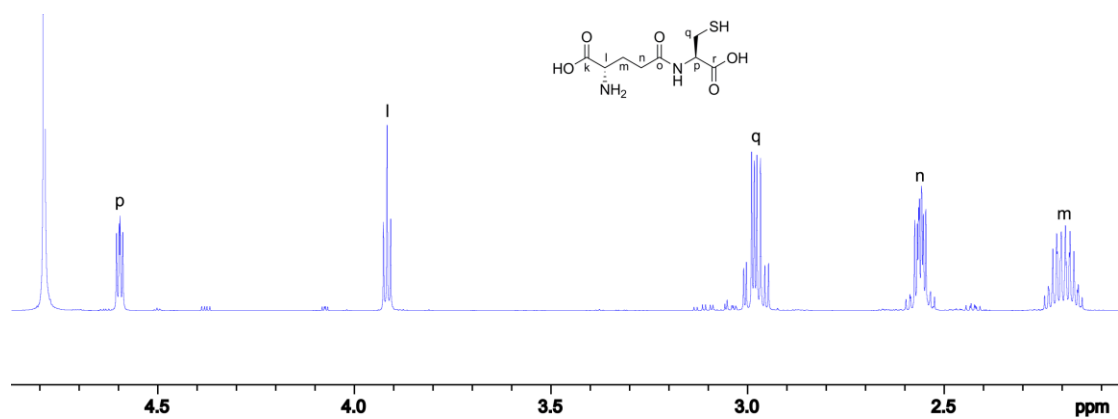


Figure S2.  $^1\text{H}$  NMR spectrum for  $\gamma$ -glutamyl-cysteine (GC) in  $\text{D}_2\text{O}$ .

## Purification of BiGF<sub>2</sub> and PGF

BiGF<sub>2</sub> and PGF were purified by HPLC from a reaction mixture containing GSH and HCHO (4 equivalents) at pD 6.5 (Na<sub>3</sub>PO<sub>4</sub> buffer). The sample was passed through a Vydac C-18 column (22 x 250 mm) using a flow rate of 4 ml min<sup>-1</sup> and a gradient scheme of: 2 - 30 % B from 0 - 20 min, 30 - 100 % B from 20 - 30 min, 100 % B from 30 - 35 min, 100 % - 2 % B from 35 - 36 min and 2 % B from 36 - 50 min, where solvent A was 99.9% H<sub>2</sub>O in 0.1% formic acid and solvent B was 99.9% MeCN in 0.1% formic acid. Elution was monitored using a Waters Quattro Micro mass spectrometer.

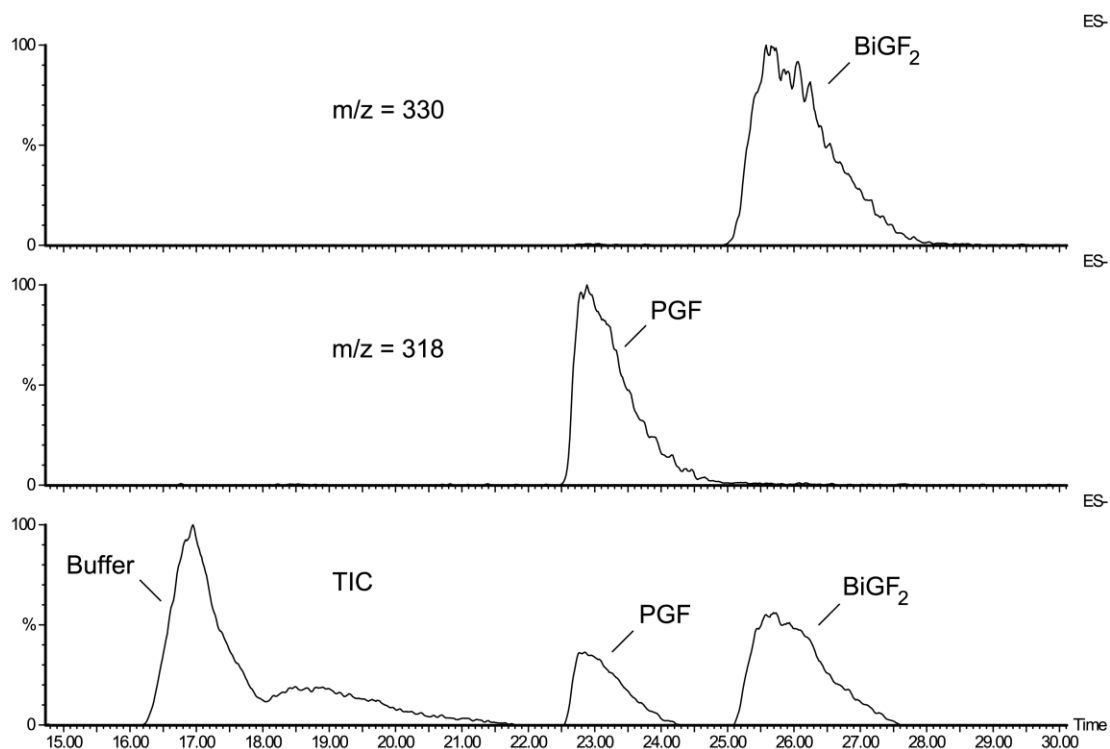


Figure S3. LC-MS Chromatogram for a reaction mixture containing GSH (13.3 mM) and HCHO (4 equivalents) at pD 6.5.

## BiGF<sub>2</sub> Characterisation

HRMS (ESI-) *m/z* calcd for C<sub>12</sub>H<sub>16</sub>N<sub>3</sub>O<sub>6</sub>S (M-H)<sup>-</sup>: 330.0765. Found 330.0763.

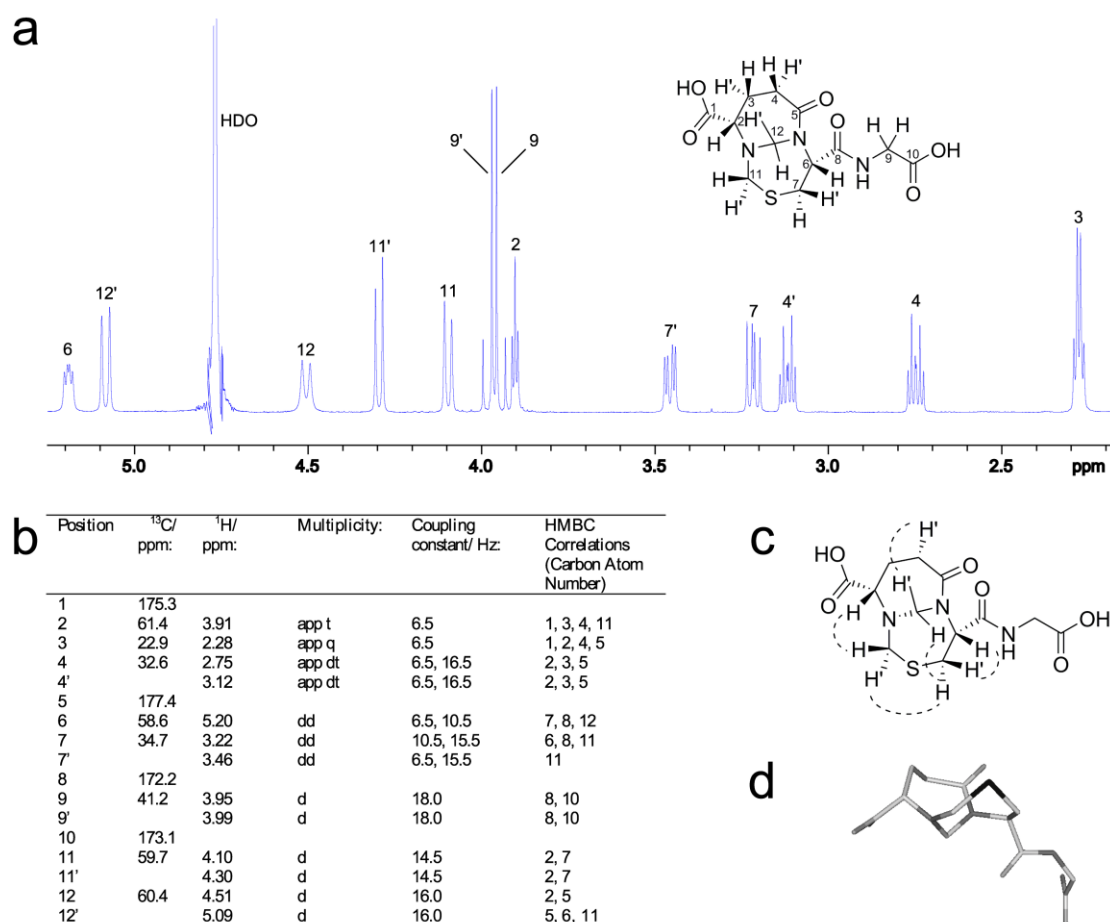


Figure S4. (a) <sup>1</sup>H NMR Spectrum of pure BiGF<sub>2</sub> in D<sub>2</sub>O. (b) Spectral assignments for BiGF<sub>2</sub>. (c) Diagram indicating some observed NOESY correlations. (d) Conformation of BiGF<sub>2</sub> when bound to CBR1 (PDB file 2PFG).<sup>1</sup> <sup>13</sup>C NMR assignments for carbons 11 and 12 (59.7 ppm and 60.4 ppm respectively) were within 2 ppm of the reported values.<sup>1</sup> However, <sup>1</sup>H-<sup>13</sup>C coupling data indicates that the higher <sup>13</sup>C chemical shift correlates to the aminal methylene.

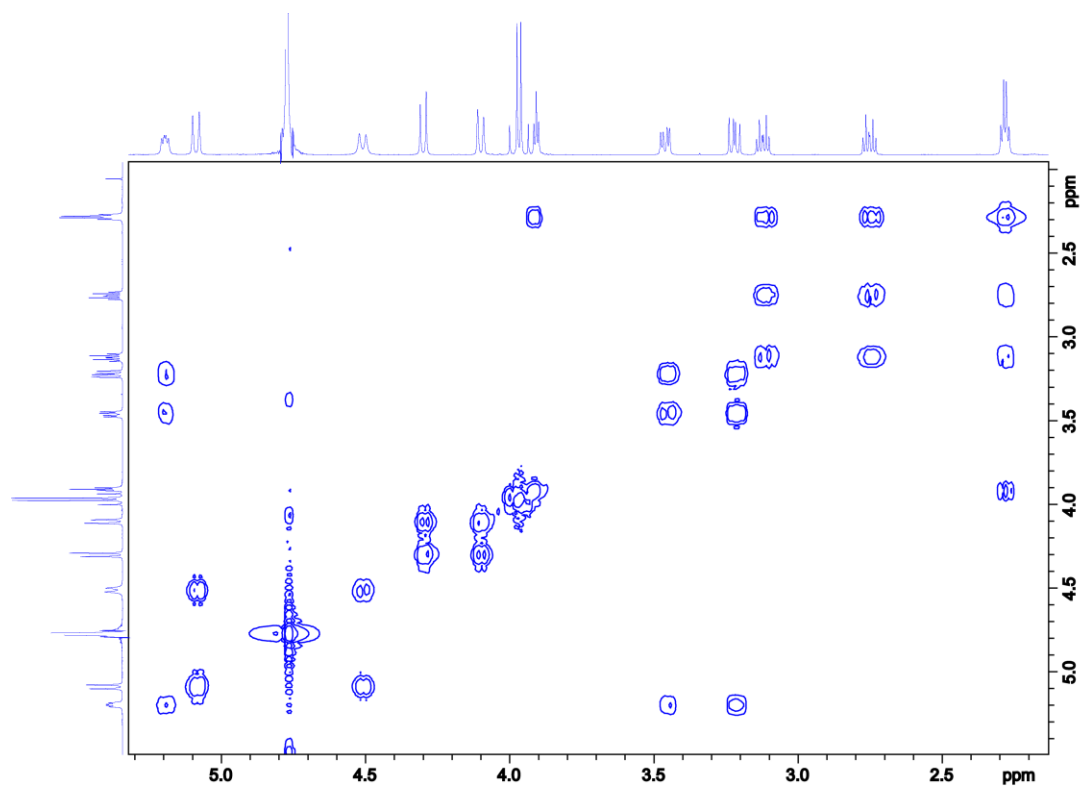


Figure S5. COSY NMR spectrum for BiGF<sub>2</sub> in D<sub>2</sub>O.

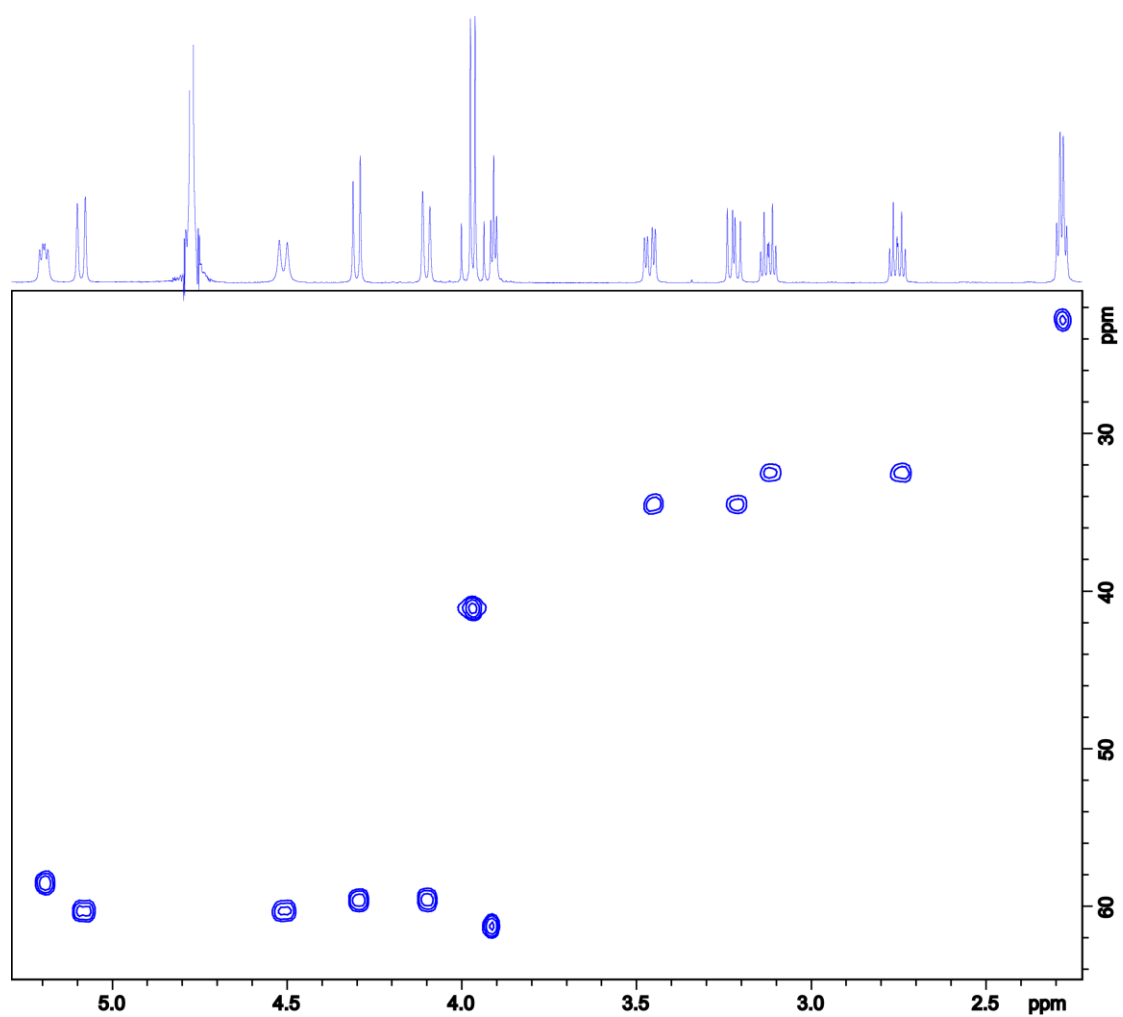


Figure S6. HSQC NMR spectrum for BiGF<sub>2</sub> in D<sub>2</sub>O.

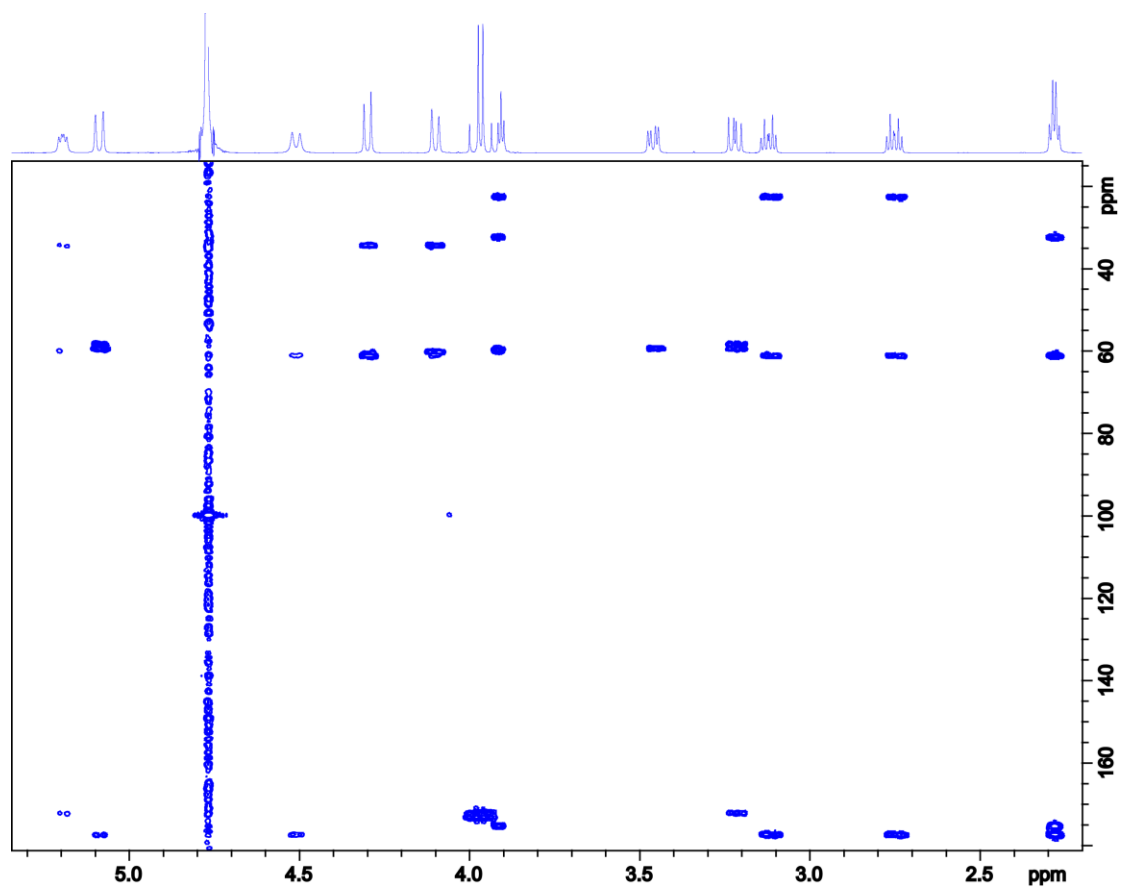


Figure S7. HMBC NMR spectrum for BiGF<sub>2</sub> in D<sub>2</sub>O.

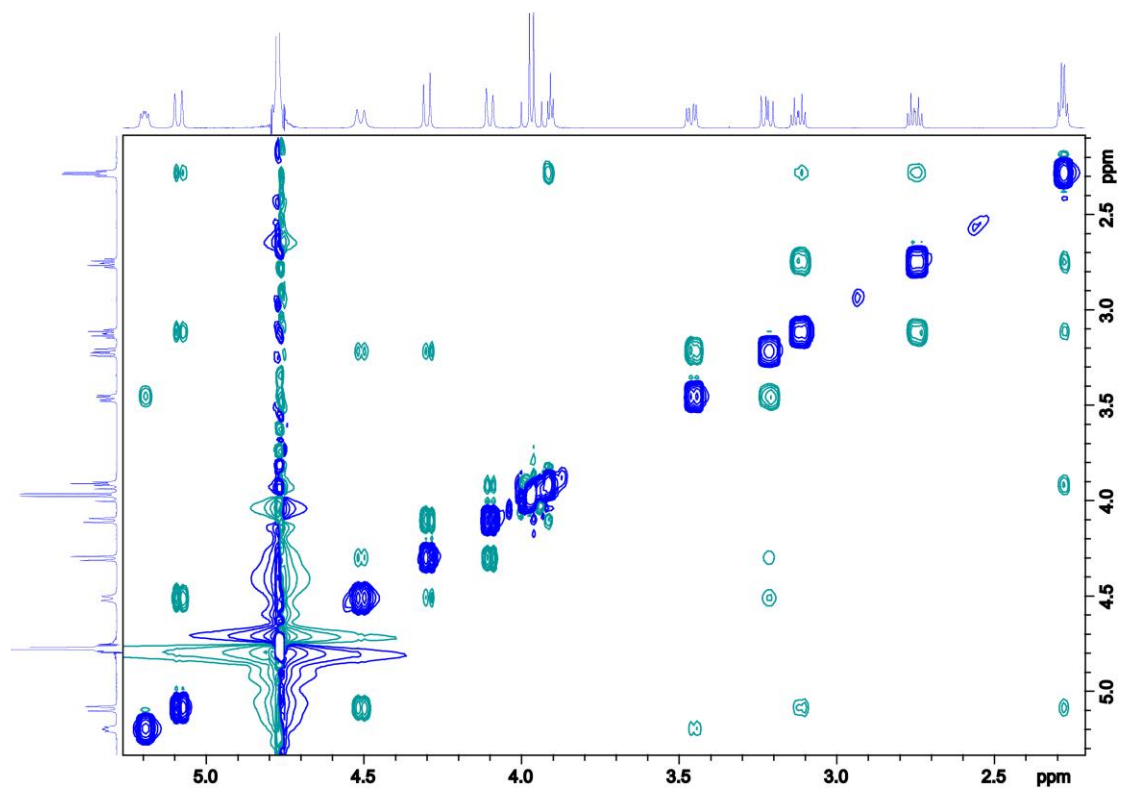


Figure S8. NOESY NMR spectrum for BiGF<sub>2</sub> in D<sub>2</sub>O (T<sub>M</sub> = 600 ms).

## PGF Spectra

HRMS (ESI-)  $m/z$  calcd for  $C_{11}H_{16}N_3O_6S$  (M-H)<sup>-</sup>: 318.0765. Found 318.0768.

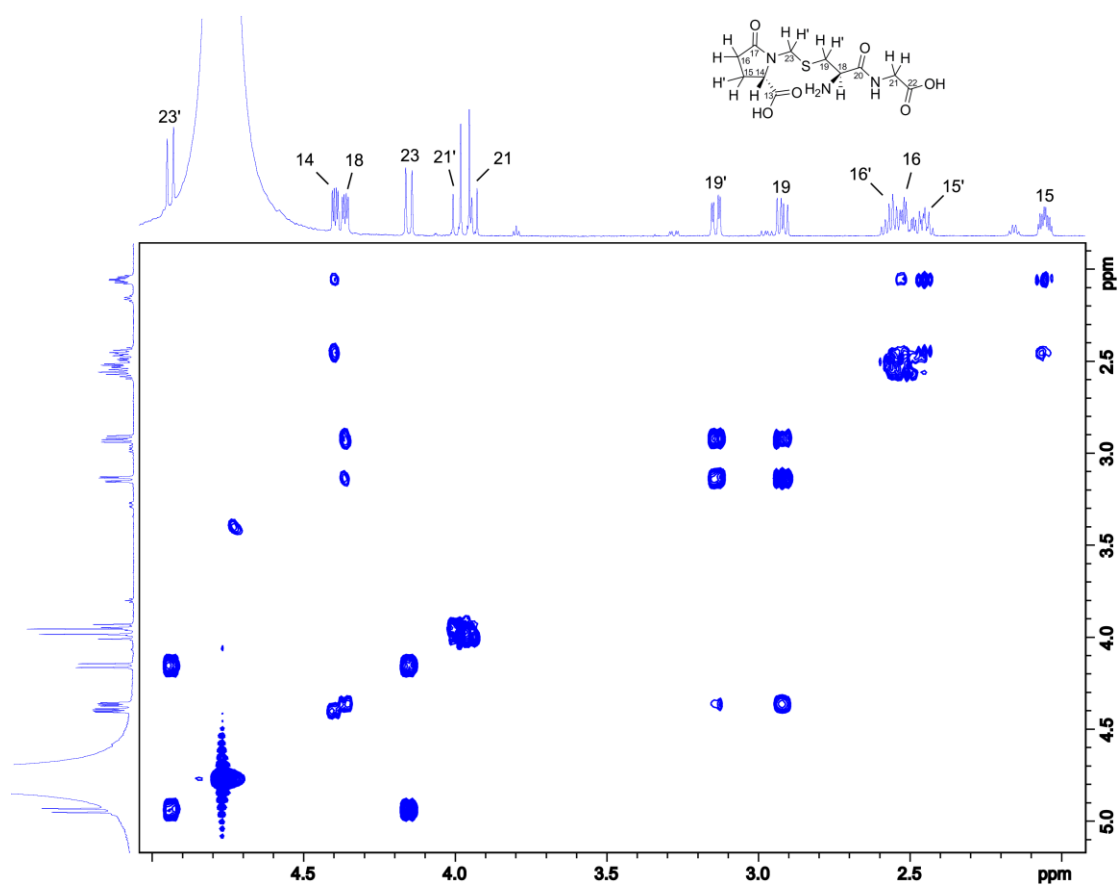


Figure S9. COSY NMR spectrum for PGF in D<sub>2</sub>O.



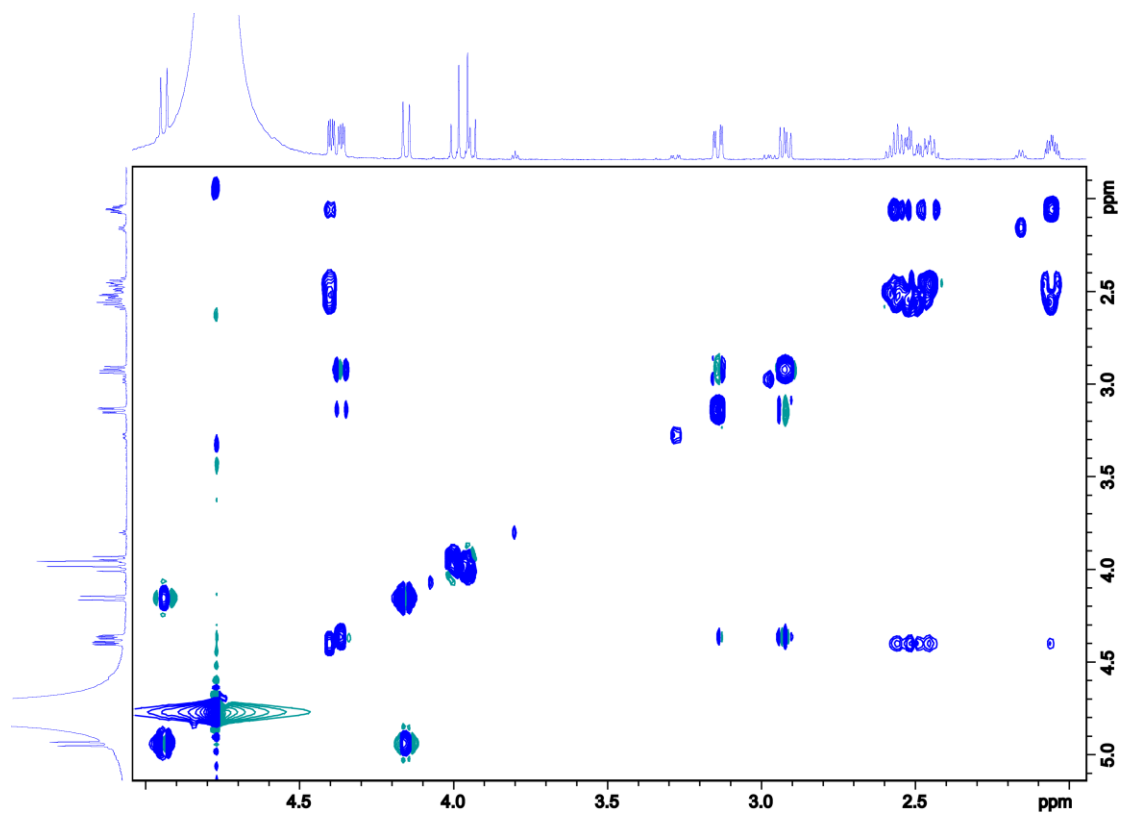
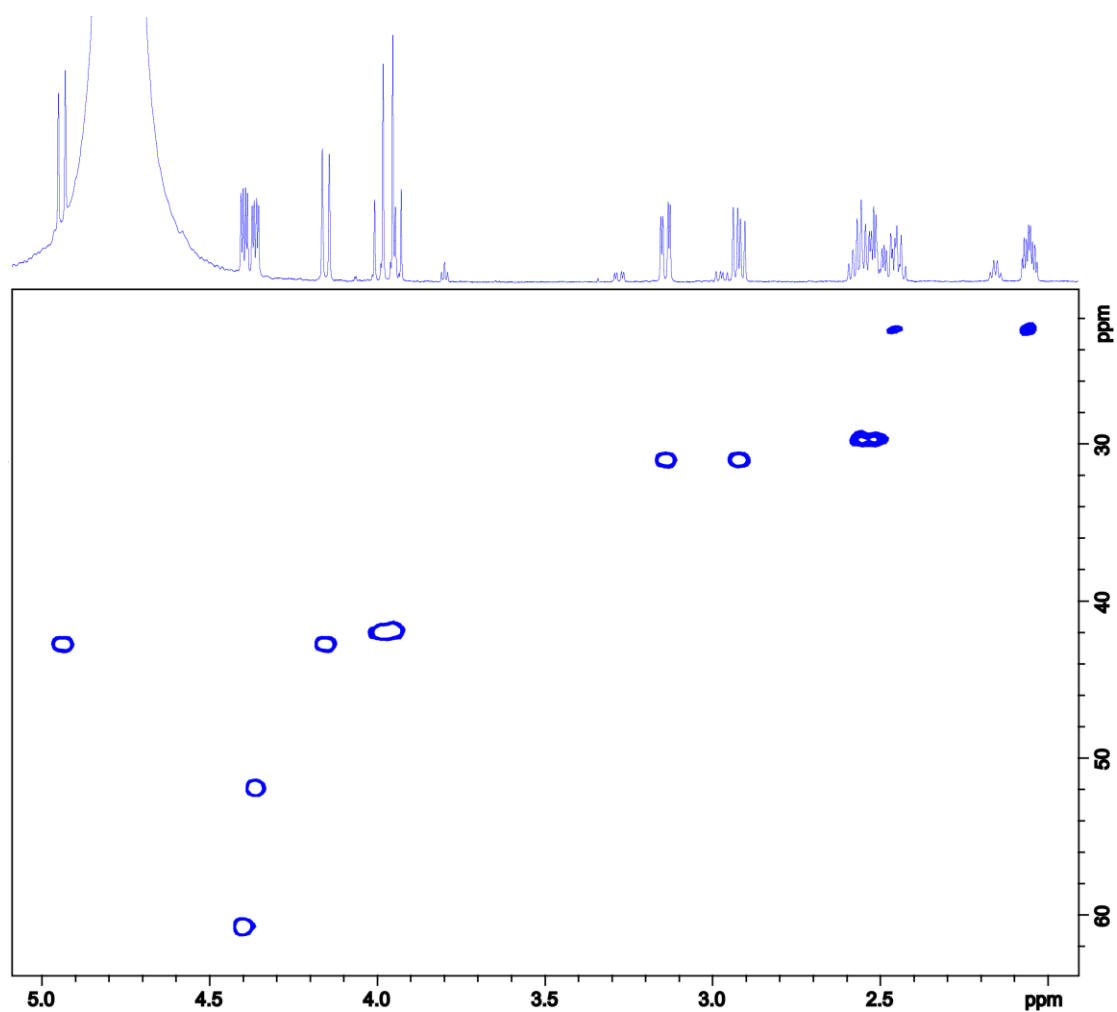


Figure S10. TOCSY NMR spectrum for PGF in D<sub>2</sub>O ( $\tau_M = 80$  ms).





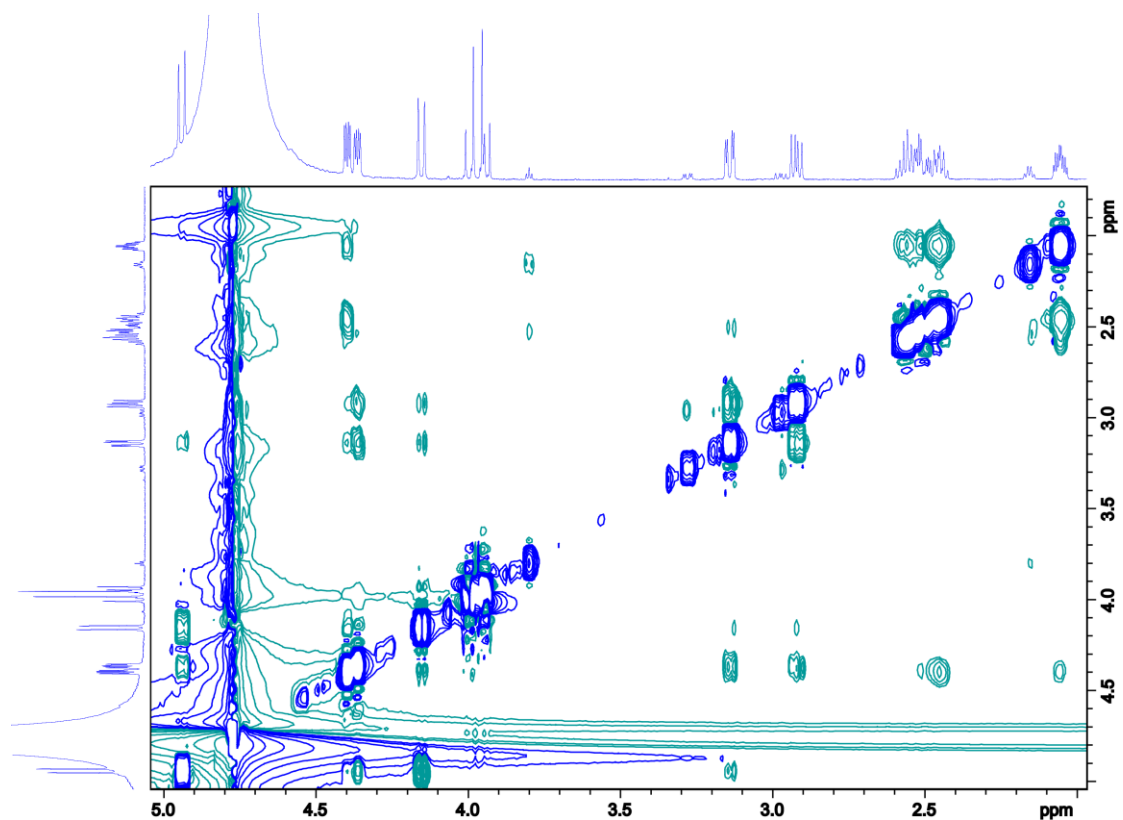


Figure S13. NOESY NMR spectrum for PGF in D<sub>2</sub>O ( $\tau_M = 600$  ms).

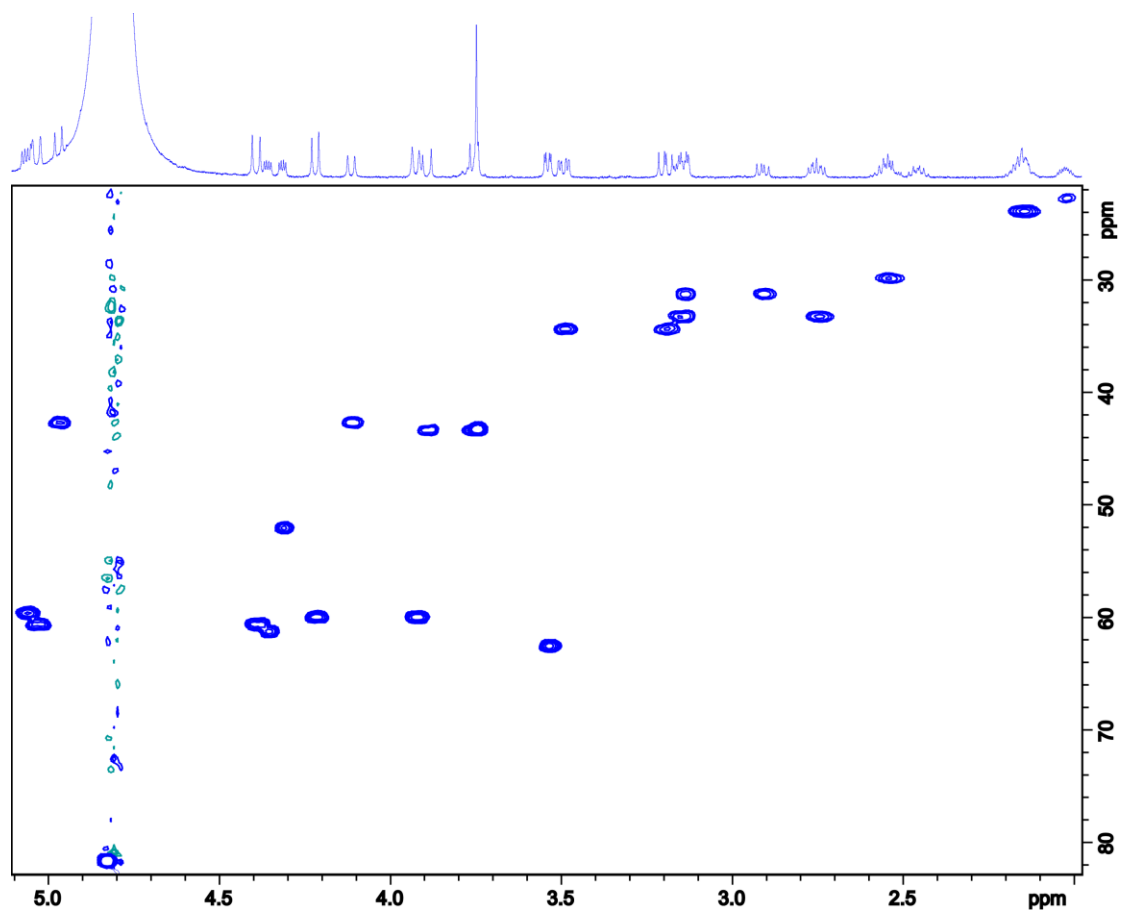


Figure S14. HSQC NMR spectrum for the reaction of GSH and 4 equivalents of HCHO at pD 6.5 ( $\text{Na}_3\text{PO}_4$  buffer) after 2 weeks.  $\text{BiGF}_2$  and PGF are present in the sample. Free formaldehyde (as  $\text{CH}_2(\text{OH})_2$ ) appears at  $\delta\text{H}$  4.83 ppm and  $\delta\text{C}$  81.6 ppm.

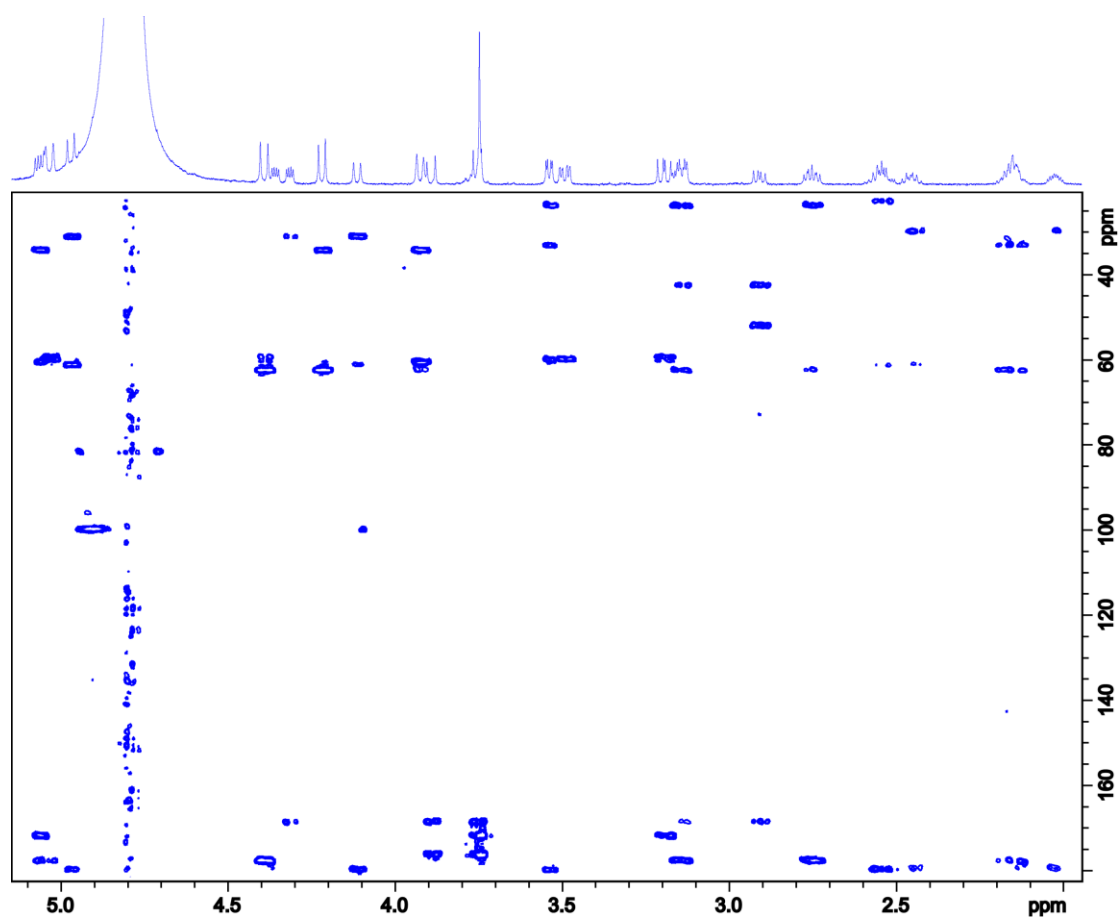


Figure S15. HMBC NMR spectrum for the reaction of GSH and 4 equivalents of HCHO at pD 6.5 ( $\text{Na}_3\text{PO}_4$  buffer) after 2 weeks. BiGF<sub>2</sub> and PGF are present in the sample.

## MGF Spectra

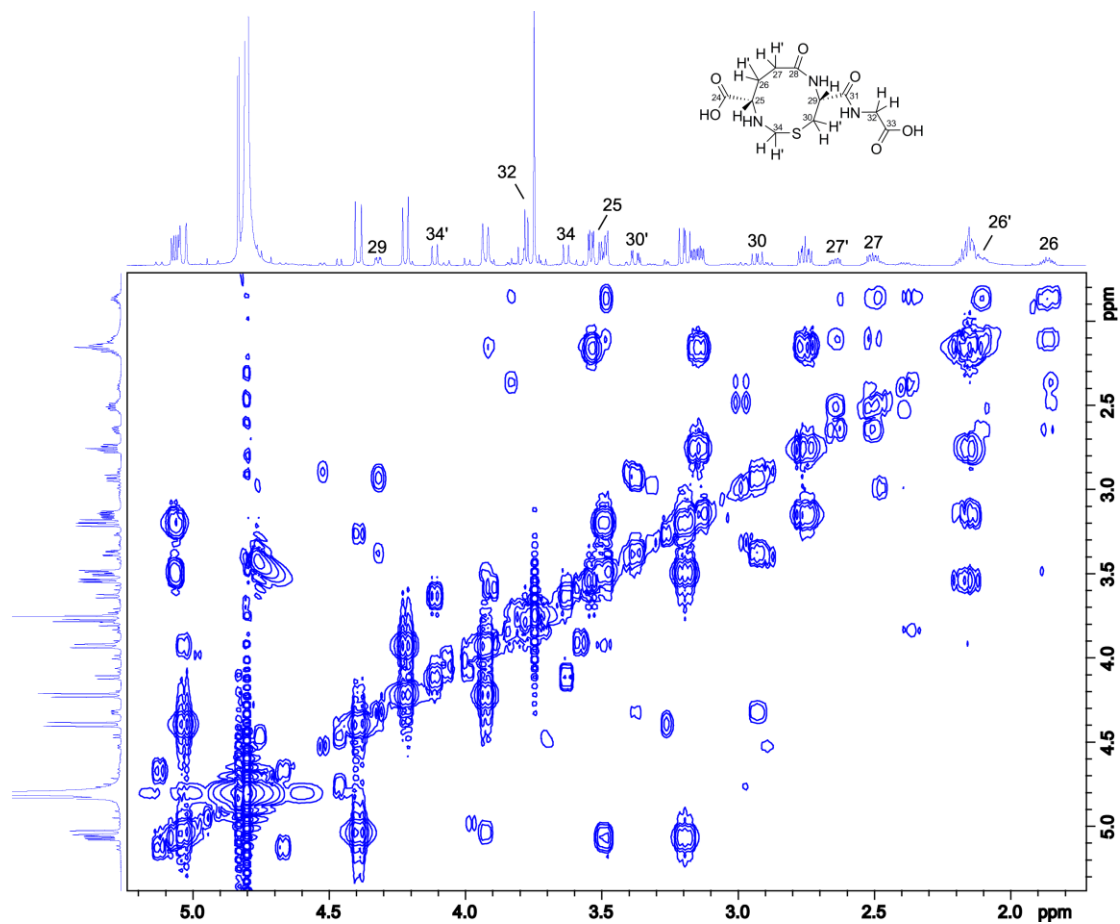


Figure S16. COSY NMR spectrum for the reaction of GSH and 4 equivalents of HCHO at pD 9.5 (Na<sub>3</sub>PO<sub>4</sub> buffer) after 2 hours. BiGF<sub>2</sub> and MGF are the major products in the sample.

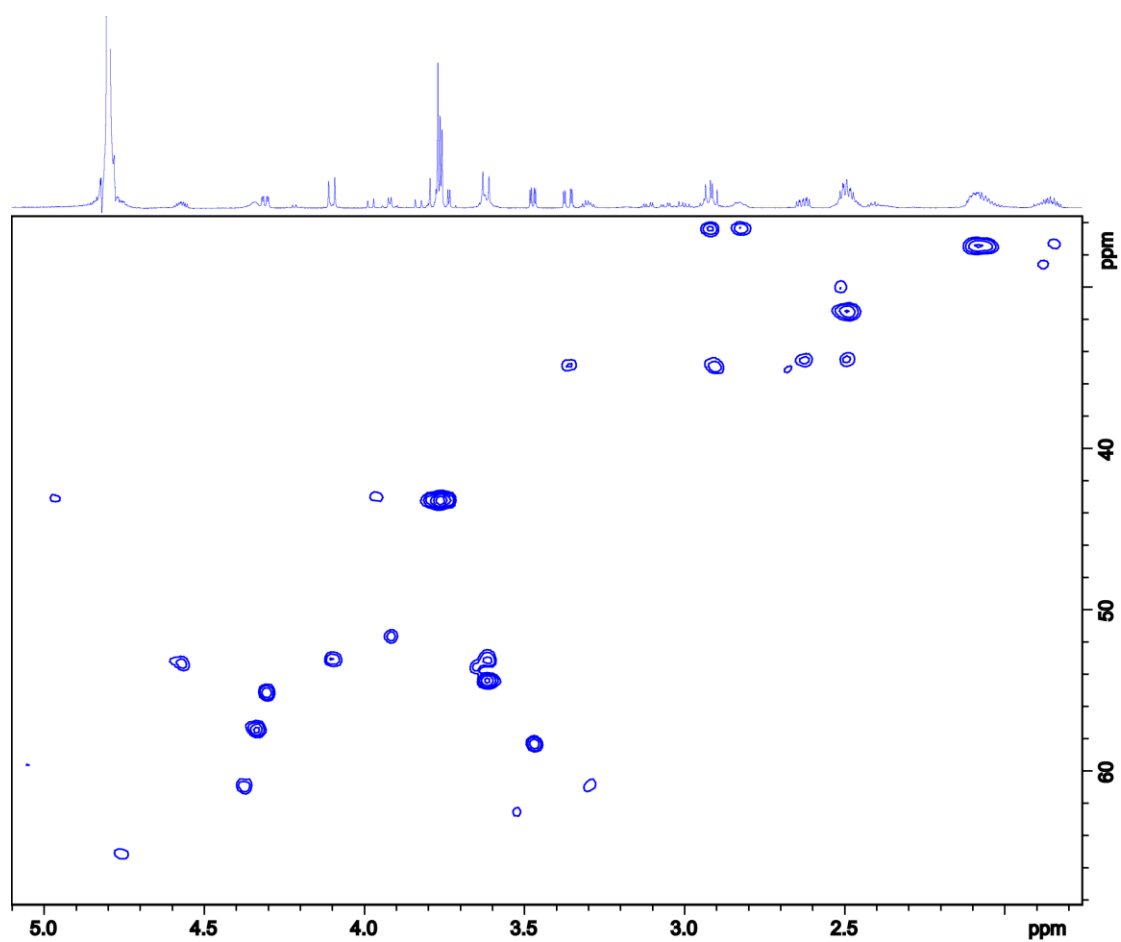


Figure S17. HSQC NMR spectrum for the reaction of GSH and 1 equivalent of HCHO at pD 9.5 ( $\text{Na}_3\text{PO}_4$  buffer) after 9 hours. GSH, PGF and MGF are the major products in the sample.



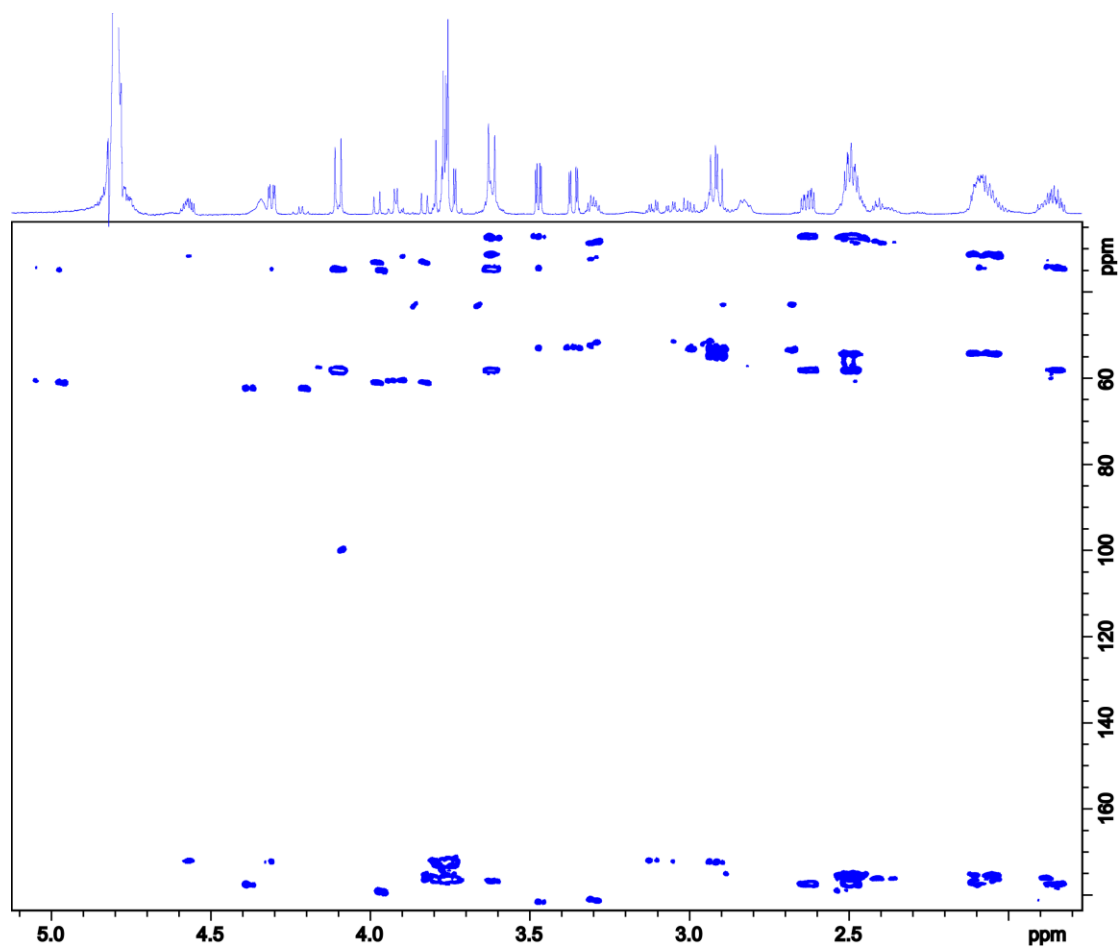


Figure S18. HMBC NMR spectrum for the reaction of GSH and 1 equivalent of HCHO at pD 9.5 ( $\text{Na}_3\text{PO}_4$  buffer). The reaction mixture was freshly prepared prior to analysis. GSH, PGF and MGF are the major products in the sample.

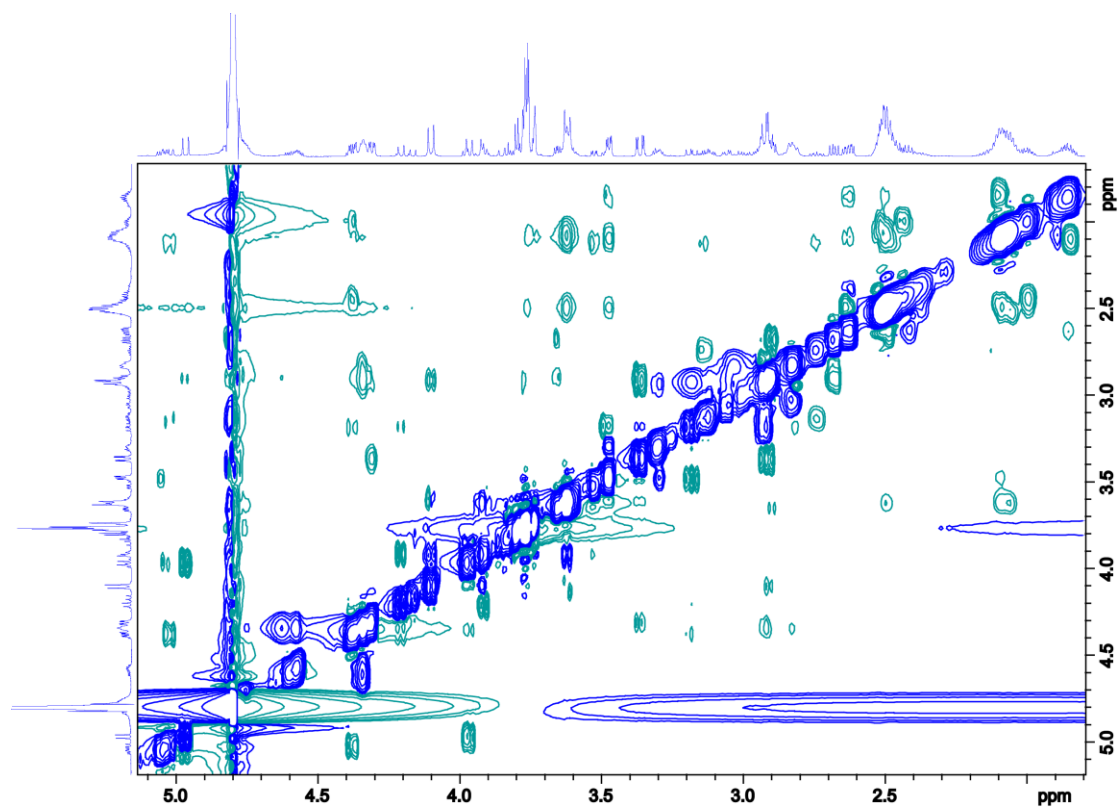


Figure S19. NOESY NMR spectrum for the reaction of GSH and 1 equivalent of HCHO at pH 9.5 ( $\text{Na}_3\text{PO}_4$  buffer) after 13 hours ( $\tau_M = 600$  ms). GSH, PGF and MGF are the major products in the sample.

## Characterisation of HMGC, BiGCF<sub>2</sub> and PGCF

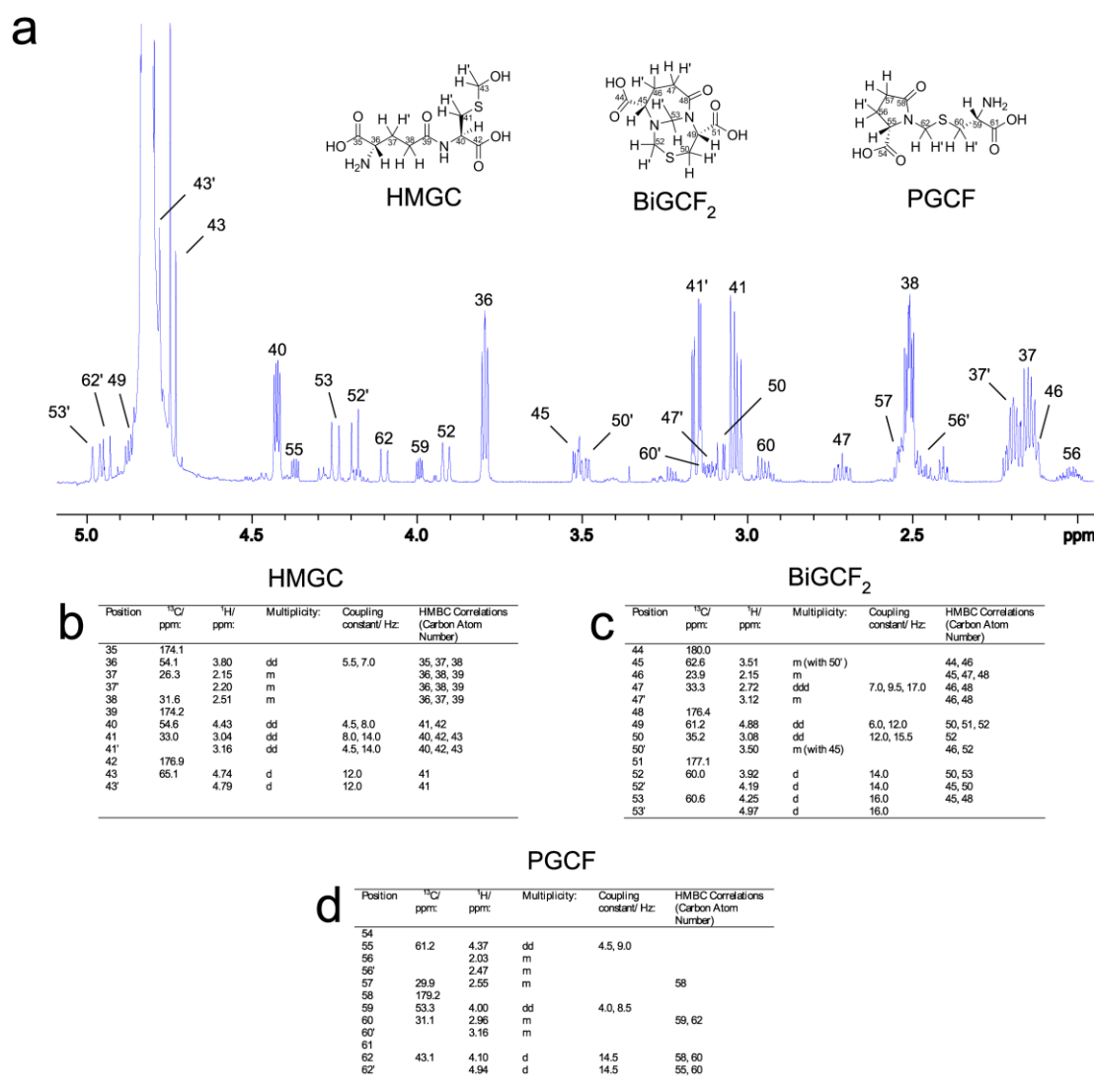


Figure S20. (a) <sup>1</sup>H NMR spectrum for the reaction of  $\gamma$ -glutamyl-cysteine and 4 equivalents of HCHO at pD 5.5 (Na<sub>3</sub>PO<sub>4</sub> buffer) after 2 days. HMGC, BiGCF<sub>2</sub> and PGCF are all present in the sample. (b) Spectral assignments for HMGC. (c) Spectral assignments for BiGCF<sub>2</sub>. (d) Spectral assignments for PGCF.

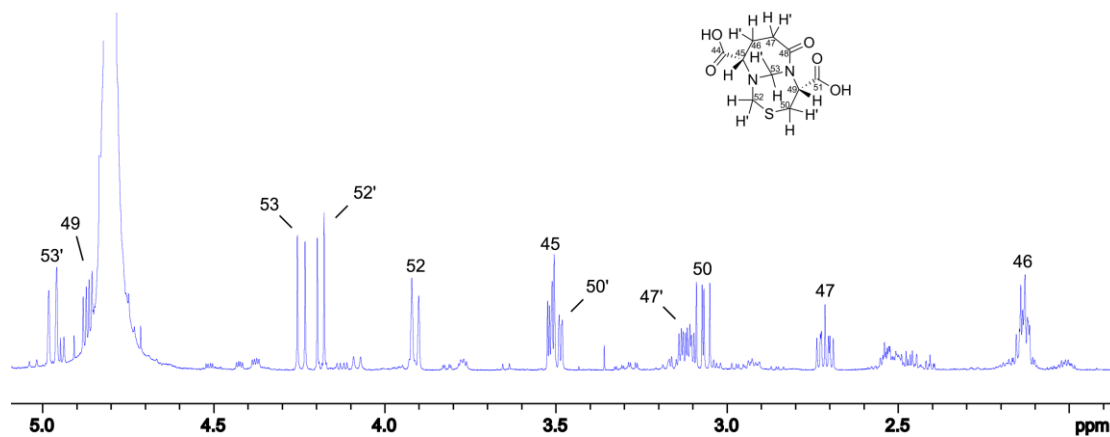


Figure S21.  $^1\text{H}$  NMR spectrum for the reaction of  $\gamma$ -glutamyl-cysteine and 4 equivalent of  $\text{HCHO}$  at pD 9.5 ( $\text{Na}_3\text{PO}_4$  buffer) after 2 days. Resonances corresponding to  $\text{BiGCF}_2$  are highlighted.

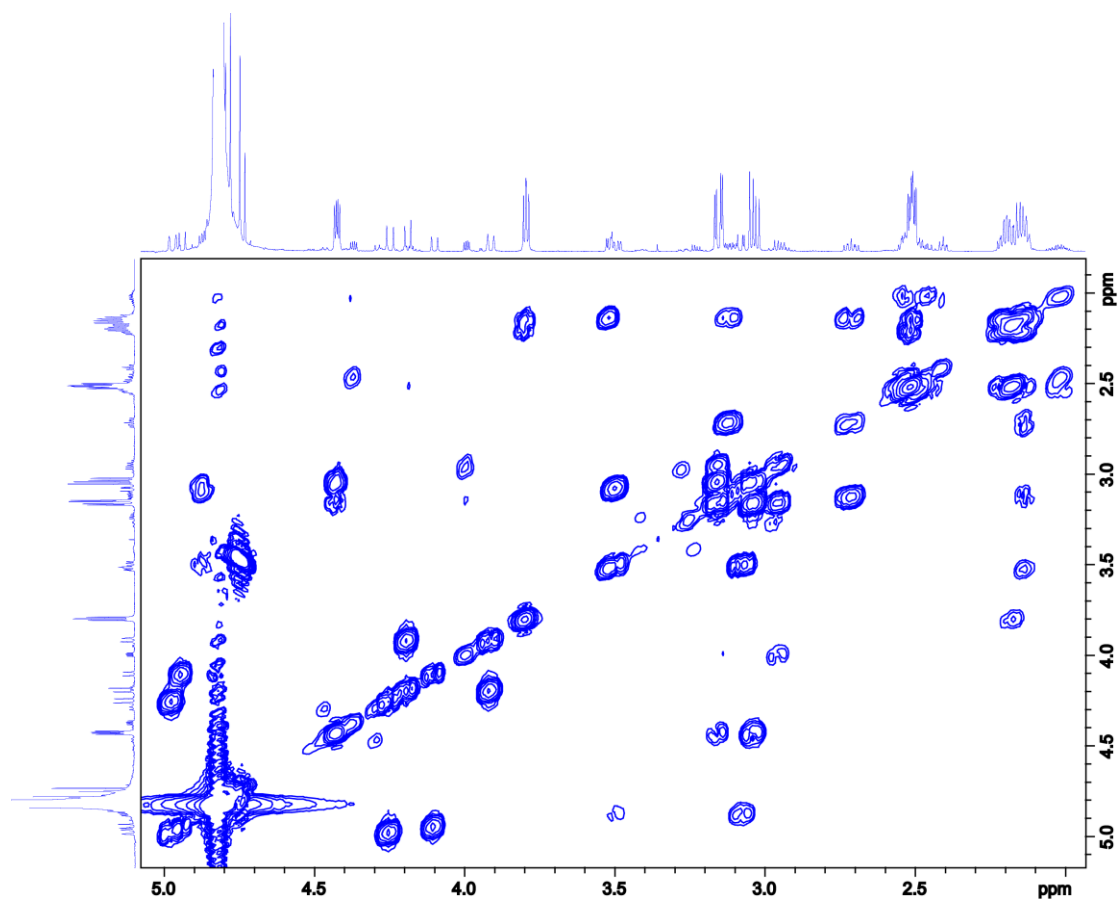


Figure S22. COSY NMR spectrum for the reaction of  $\gamma$ -glutamyl-cysteine and 4 equivalents of  $\text{HCHO}$  at pD 5.5 ( $\text{Na}_3\text{PO}_4$  buffer) after 2 days. HMGC,  $\text{BiGCF}_2$  and PGCF are present in the sample.

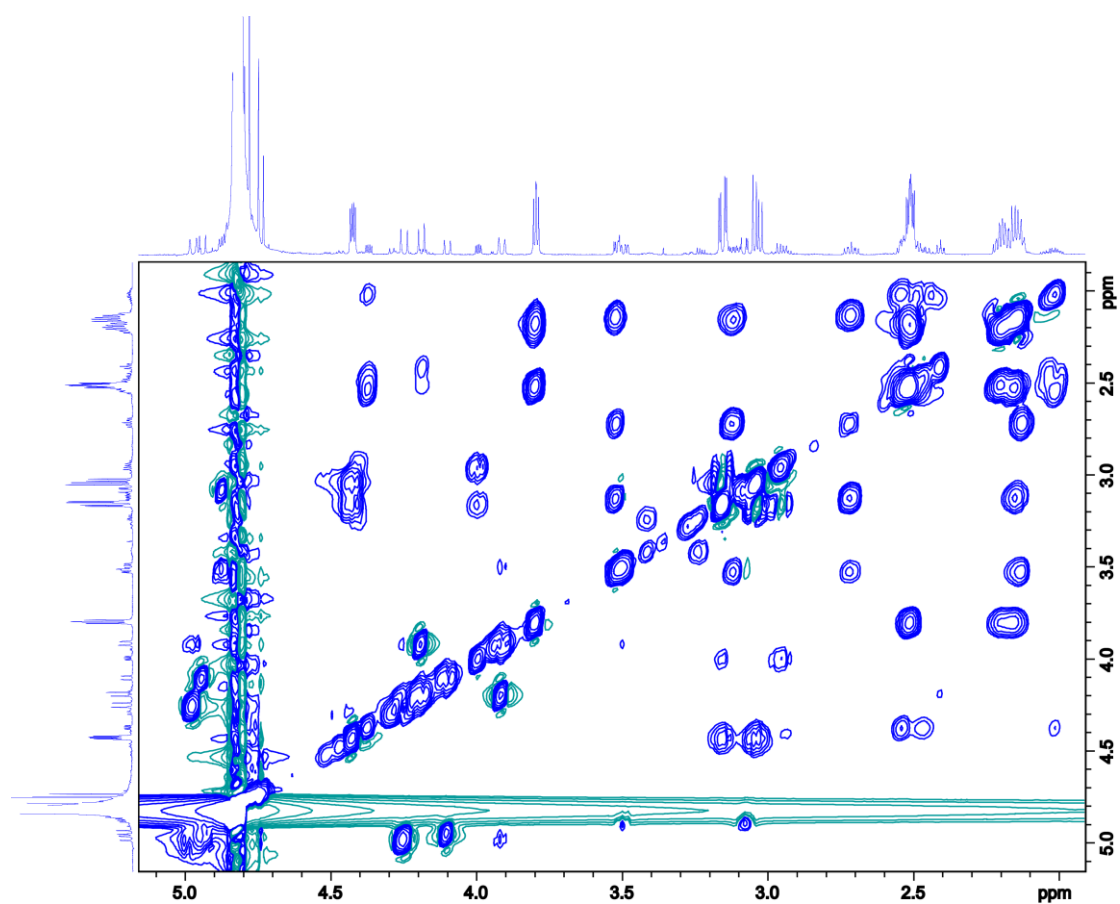


Figure S23. TOCSY NMR spectrum for the reaction of  $\gamma$ -glutamyl-cysteine and 4 equivalents of HCHO at pD 5.5 ( $\text{Na}_3\text{PO}_4$  buffer) after 2 days ( $\tau_M = 80$  ms). HMGC, BiGCF<sub>2</sub> and PGCF are present in the sample.

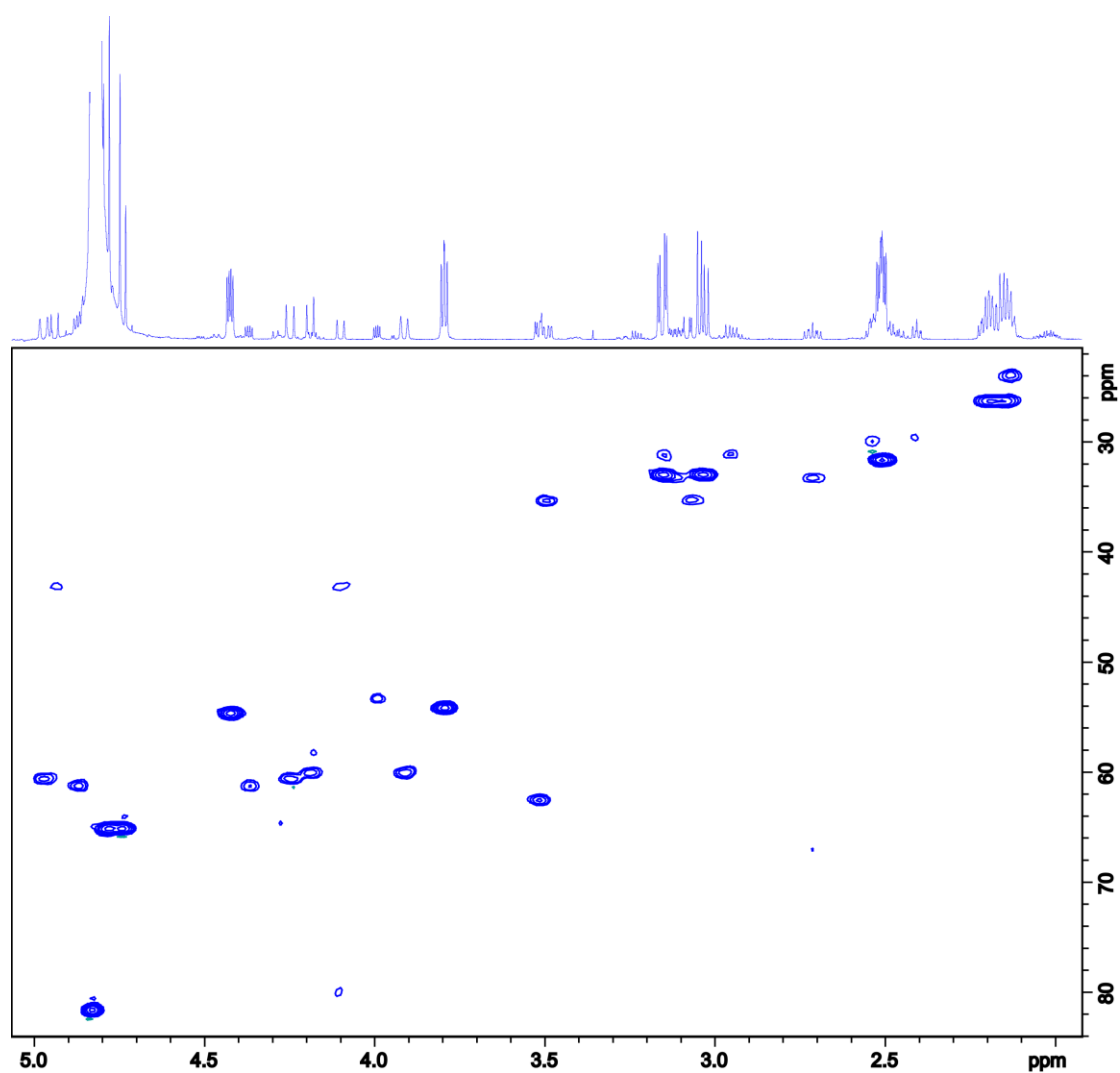


Figure S24. HSQC NMR spectrum for the reaction of  $\gamma$ -glutamyl-cysteine and 4 equivalents of HCHO at pD 5.5 ( $\text{Na}_3\text{PO}_4$  buffer) after 2 days. HMGC, BiGCF<sub>2</sub> and PGCF are present in the sample. Free formaldehyde (as  $\text{CH}_2(\text{OH})_2$ ) appears at  $\delta\text{H}$  4.83 ppm and  $\delta\text{C}$  81.6 ppm.

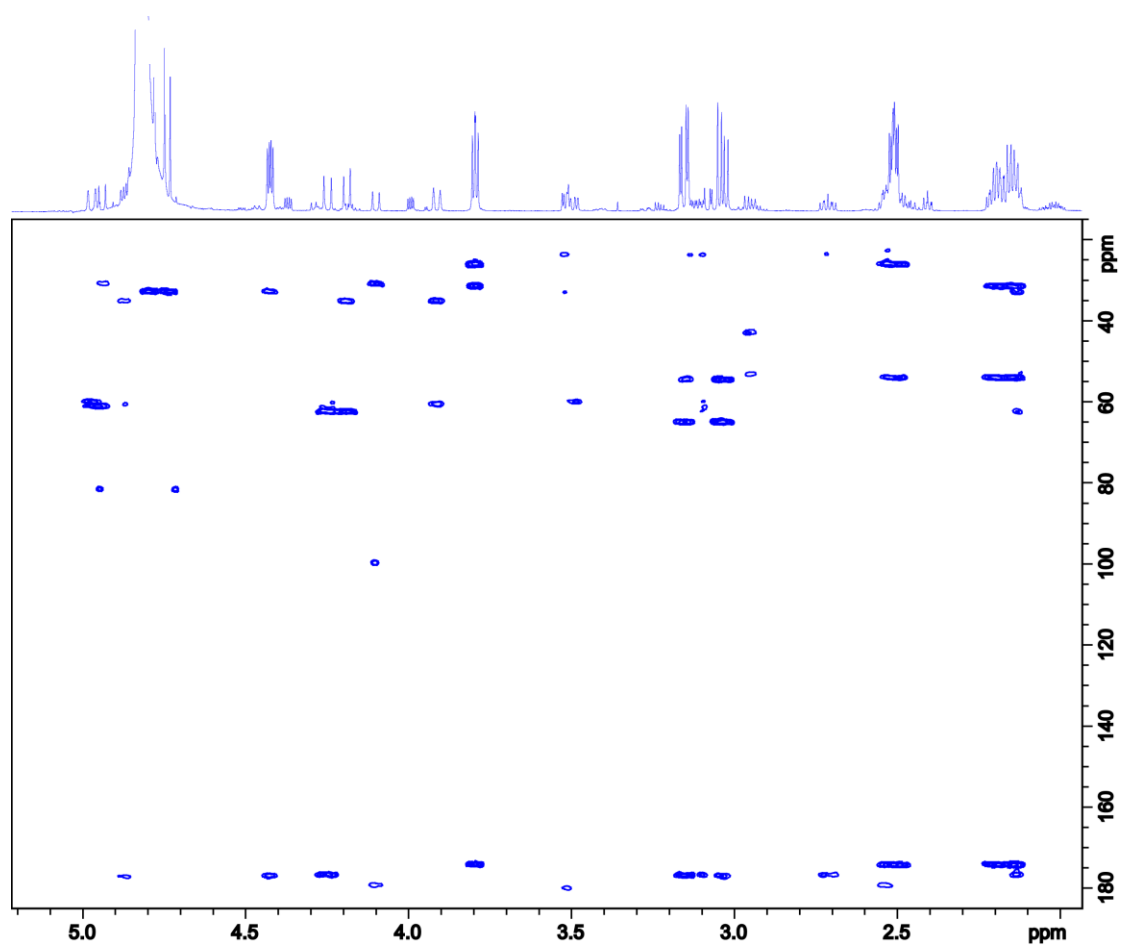


Figure S25. HMBC NMR spectrum for the reaction of  $\gamma$ -glutamyl-cysteine and 4 equivalents of HCHO at pD 5.5 ( $\text{Na}_3\text{PO}_4$  buffer) after 2 days. HMGC, BiGCF<sub>2</sub> and PGCF are present in the sample.

## MGCF Characterisation

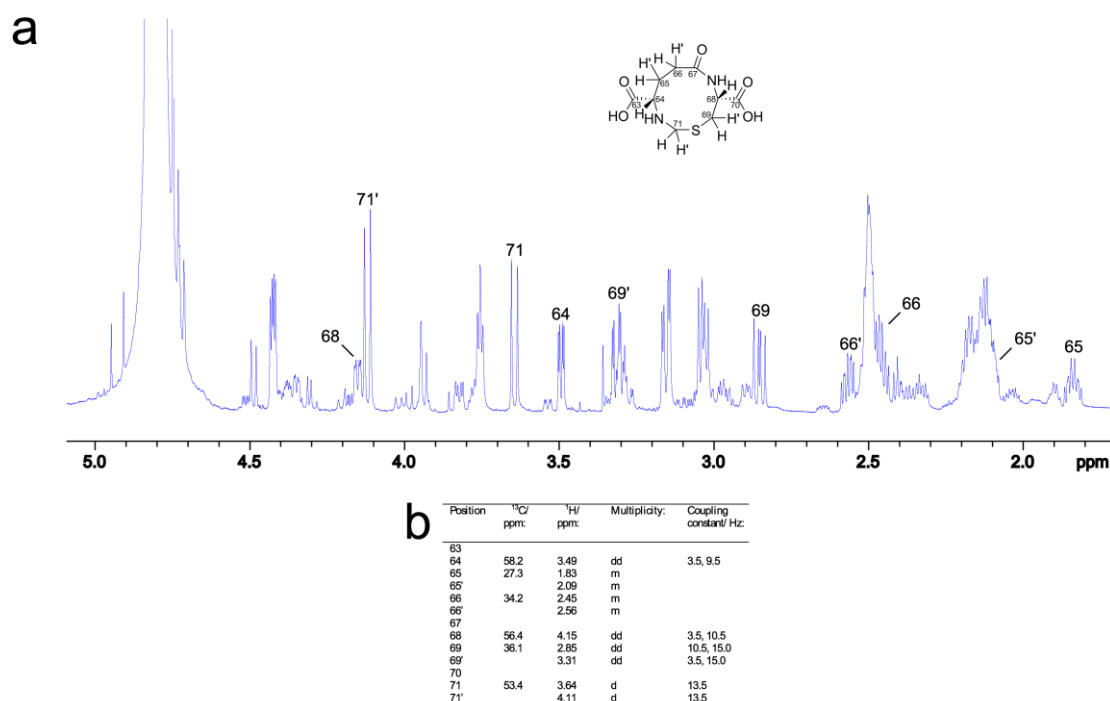


Figure S26. (a) <sup>1</sup>H NMR spectrum for the reaction of  $\gamma$ -glutamyl-cysteine and 4 equivalent of HCHO at pD 9.5 ( $\text{Na}_3\text{PO}_4$  buffer) after 8 minutes. Resonances corresponding to MGCF are highlighted. (b) Spectral assignments for MGCF.



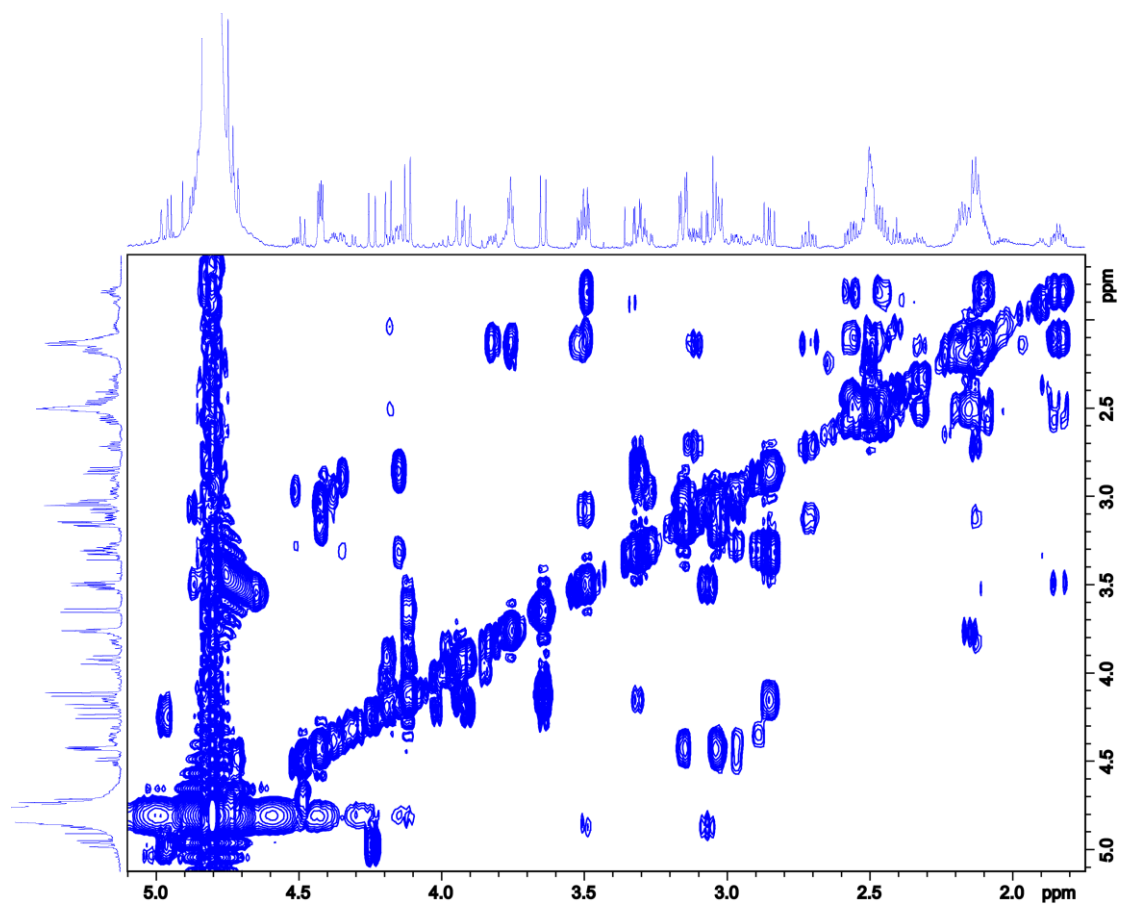


Figure S27. COSY NMR spectrum for the reaction of  $\gamma$ -glutamyl-cysteine and 4 equivalents of HCHO at pD 9.5 ( $\text{Na}_3\text{PO}_4$  buffer) after 3 hours.

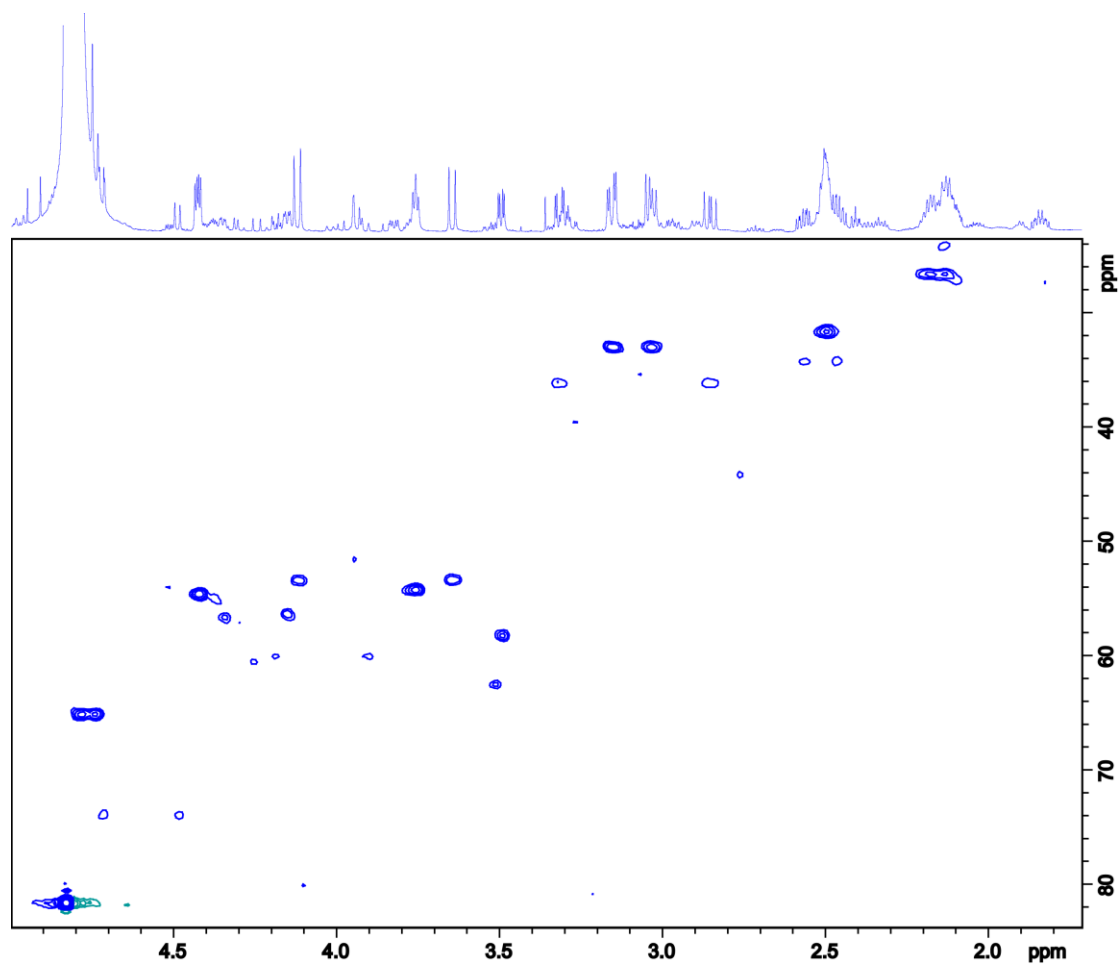


Figure S28. HSQC NMR spectrum for the reaction of  $\gamma$ -glutamyl-cysteine and 4 equivalents of HCHO at pD 9.5 ( $\text{Na}_3\text{PO}_4$  buffer) after 3 hours. Free formaldehyde (as  $\text{CH}_2(\text{OH})_2$ ) appears at  $\delta\text{H}$  4.83 ppm and  $\delta\text{C}$  81.6 ppm.

## HEG Characterisation

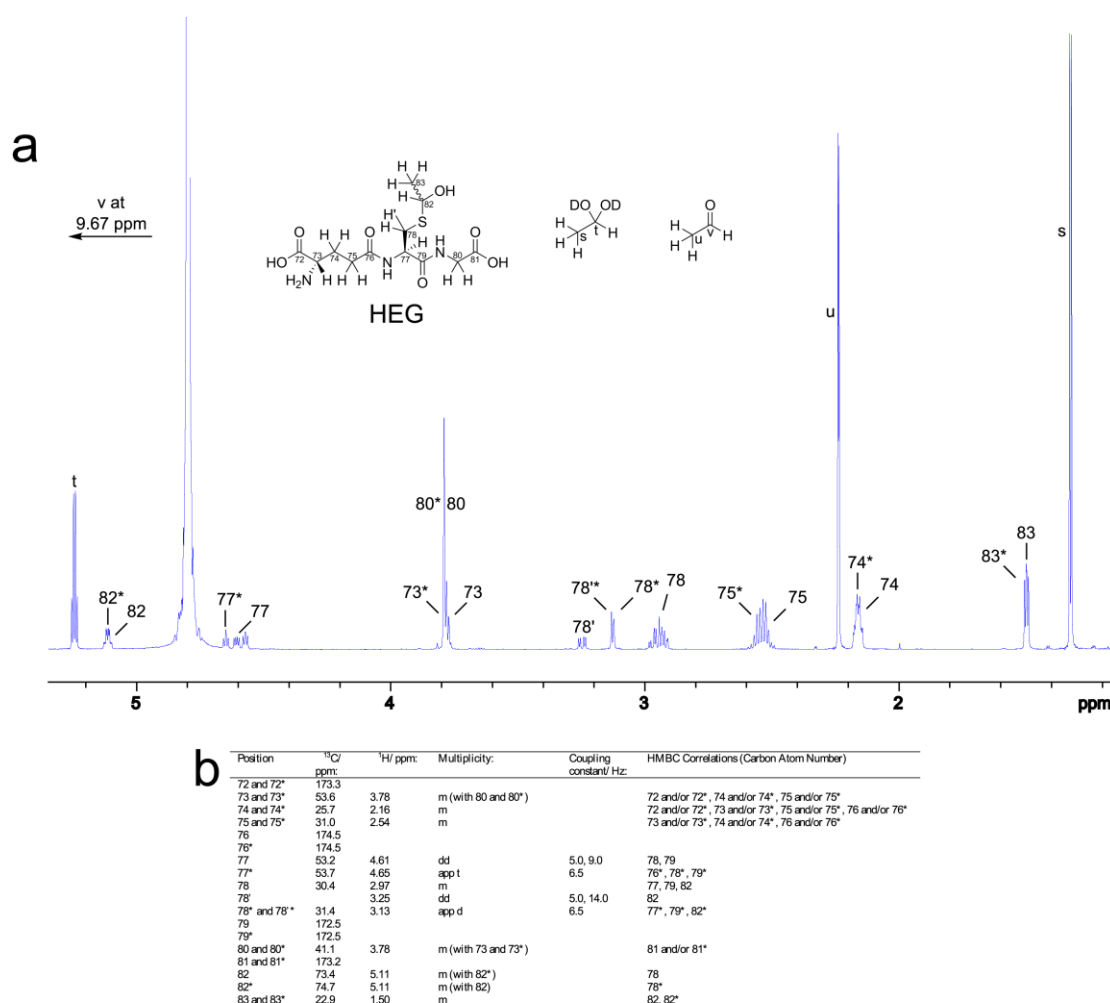


Figure S29. (a) <sup>1</sup>H NMR spectrum for the reaction of GSH and 4 equivalents of acetaldehyde at pD 5.5 (Na<sub>3</sub>PO<sub>4</sub> buffer) after 8 minutes. Both diastereomers of HEG, as well as GSH are present in the sample. Asterisks indicate resonances corresponding to the same diastereomer. (b) Spectral assignments for HEG.

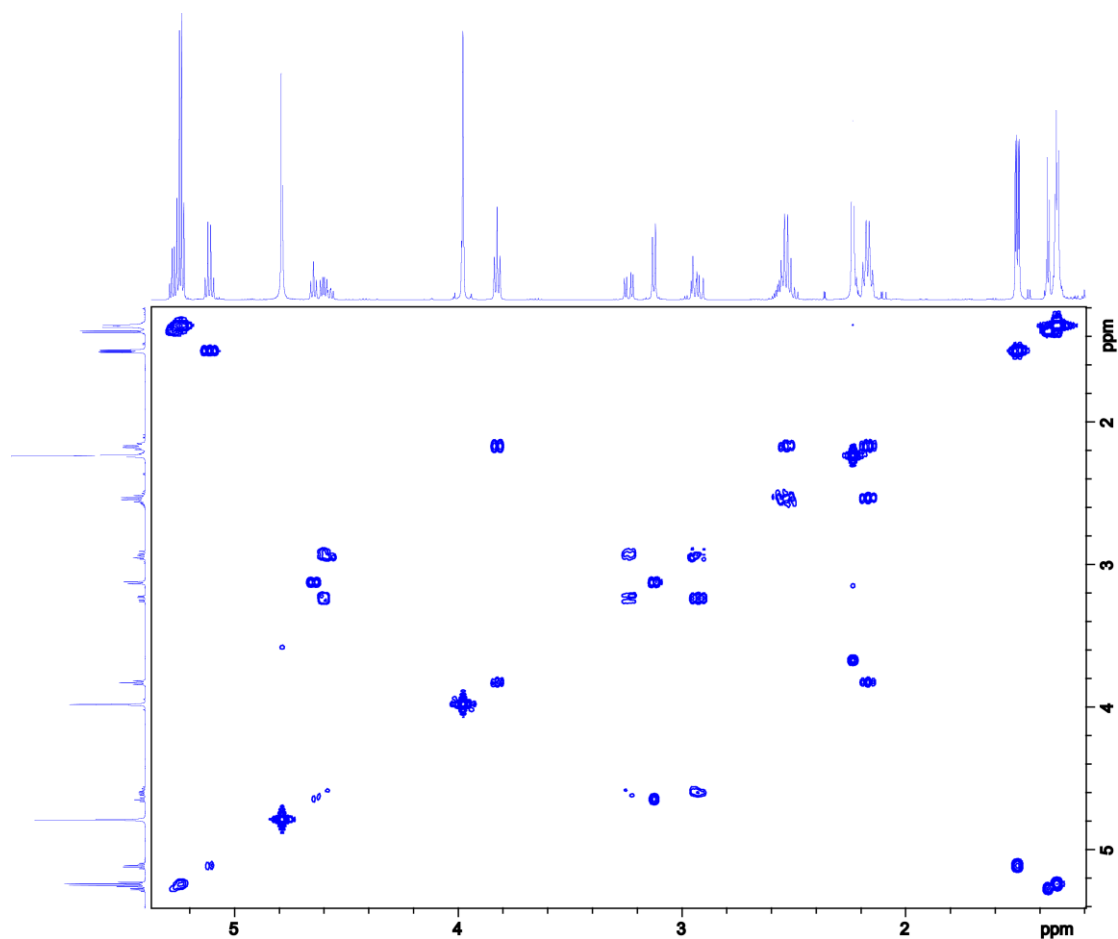


Figure S30. COSY NMR spectrum for the reaction of GSH and 8 equivalents of acetaldehyde at pD 6.0 in D<sub>2</sub>O after 5 hours. The spectrum was collected on a Bruker AVII500 NMR spectrometer. The pD was altered to the correct pD directly after mixing, using deuterated solutions of DCl and NaOD. Note the shift of the resonance corresponding to 73 and 73\* to 3.83 ppm.

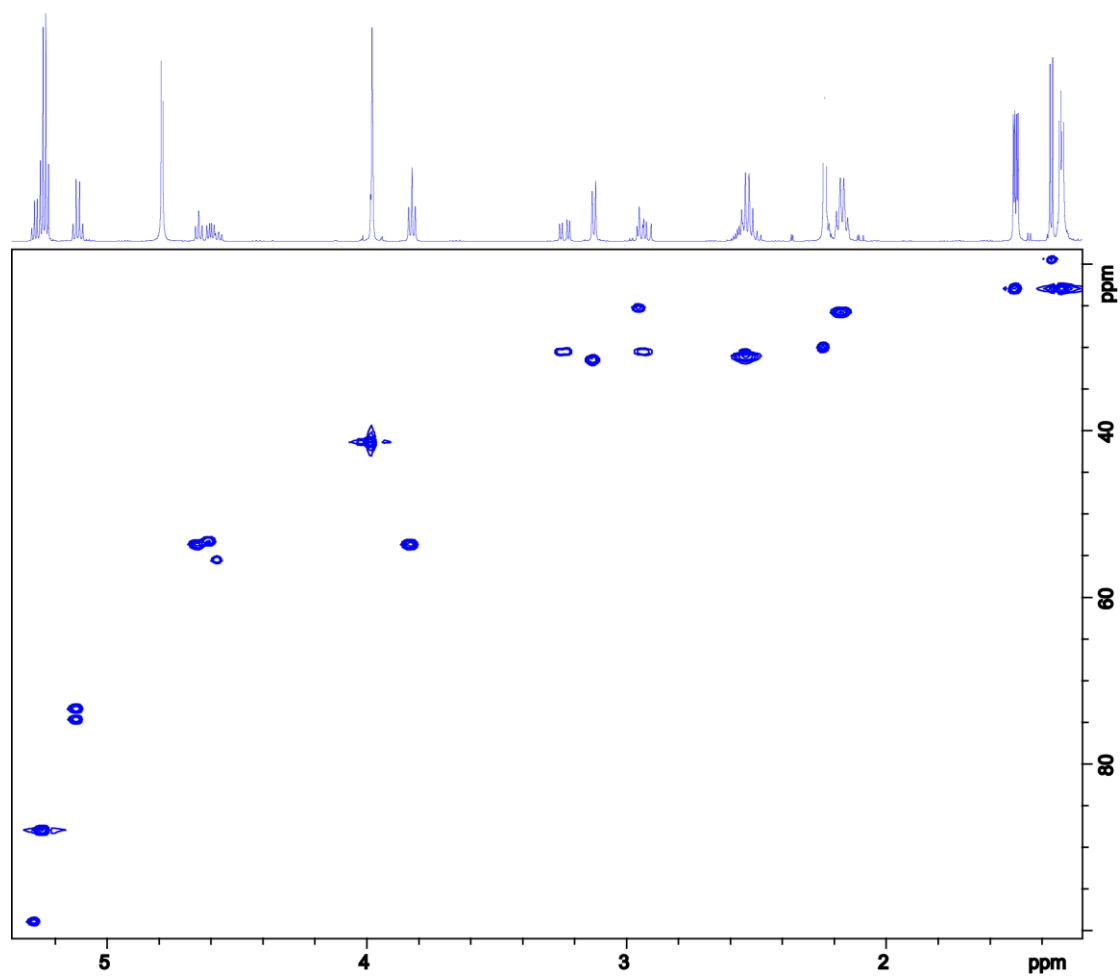


Figure S31. HSQC NMR spectrum for the reaction of GSH and 8 equivalents of acetaldehyde at pD 6.0 in  $\text{D}_2\text{O}$  after 5 hours. The spectrum was collected on a Bruker AVII 500 NMR spectrometer. The pD was altered to the correct pD directly after mixing, using deuterated solutions of DCl and NaOD. Free acetaldehyde is also present in the spectrum (resonances at  $\delta_{\text{C}}$  22.9 ppm, 29.9 ppm and 87.9 ppm).

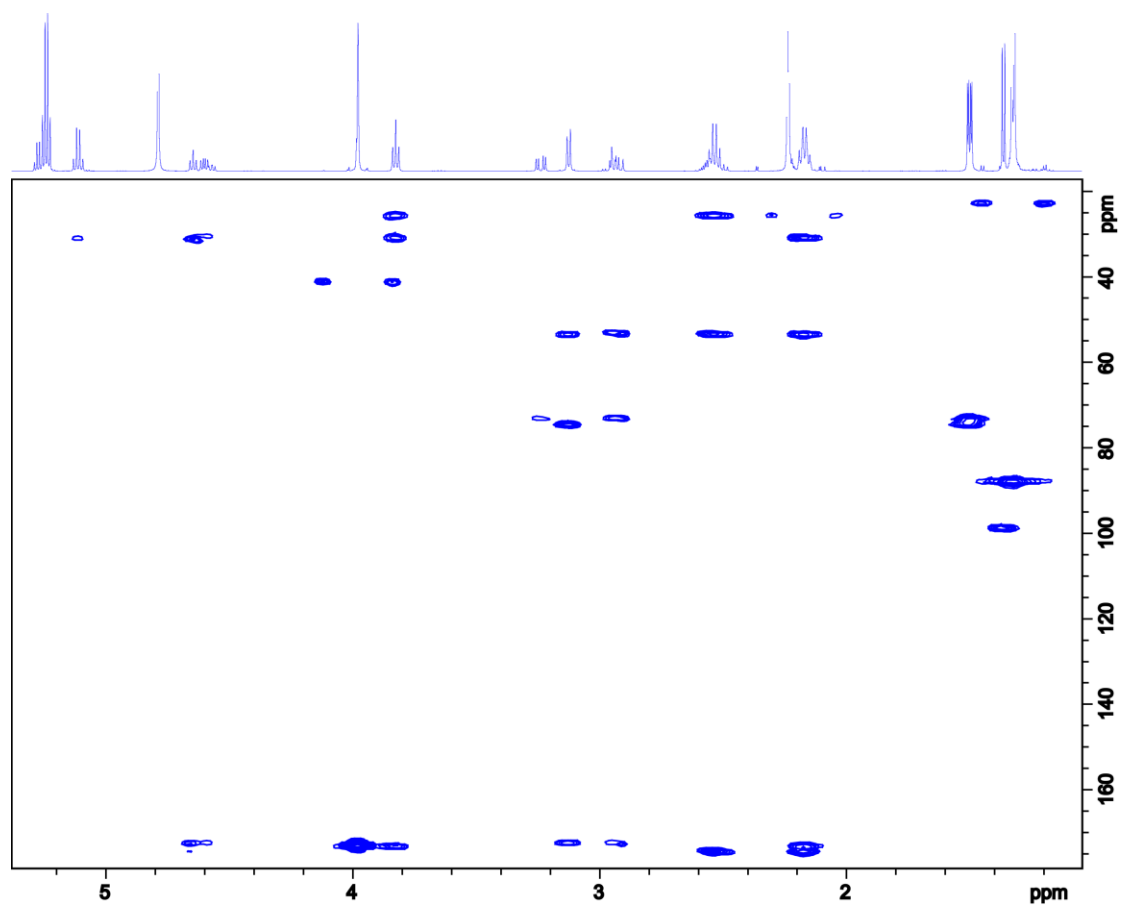


Figure S32. HMBC NMR spectrum for the reaction of GSH and 8 equivalents of acetaldehyde at pD 6.0 in D<sub>2</sub>O after 5 hours. The spectrum was collected on a Bruker AVII 500 NMR spectrometer. The pD was altered to the correct pD directly after mixing, using deuterated solutions of DCl and NaOD.

## MGG Characterisation

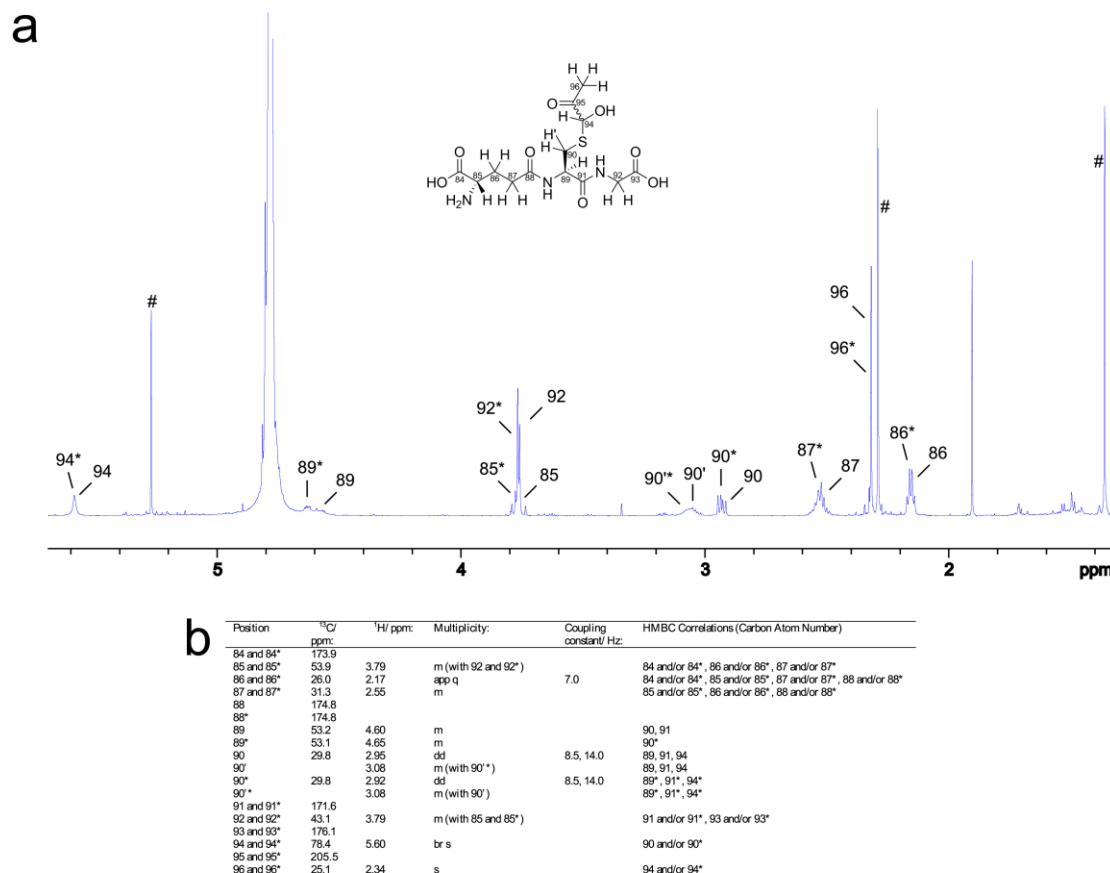


Figure S33. (a) <sup>1</sup>H NMR spectrum for the reaction of GSH and 4 equivalents of methylglyoxal at pD 7.5 (Na<sub>3</sub>PO<sub>4</sub> buffer) after 8 minutes. Both diastereomers of MGG are present in the spectrum. Asterisks indicate resonances corresponding to the same diastereomer. Resonances marked with a hash correspond to methylglyoxal. (b) Spectral assignments for MGG.

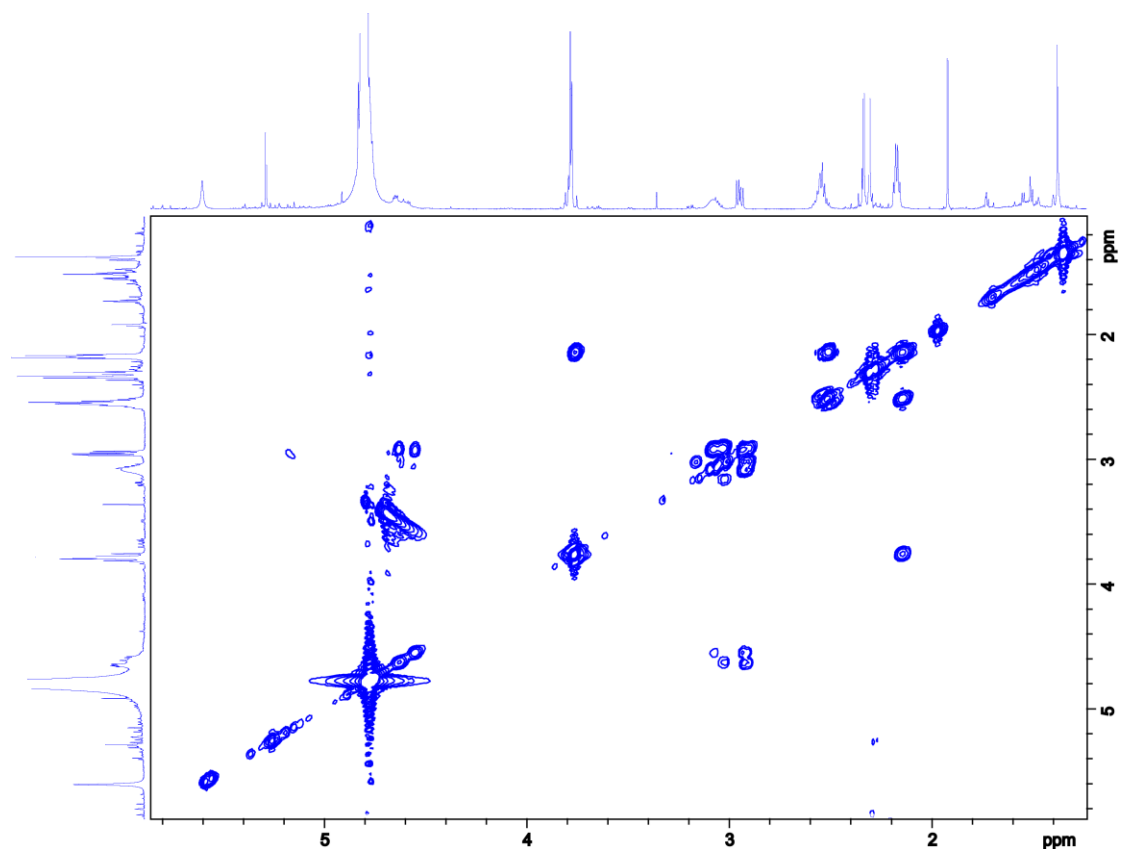


Figure S34. COSY NMR spectrum for the reaction of GSH and 4 equivalents of methylglyoxal at pD 5.5 ( $\text{Na}_3\text{PO}_4$  buffer) after 3 hours. The  $^1\text{H}$  NMR spectrum from Figure S33a is shown on the F1 and F2 axes.



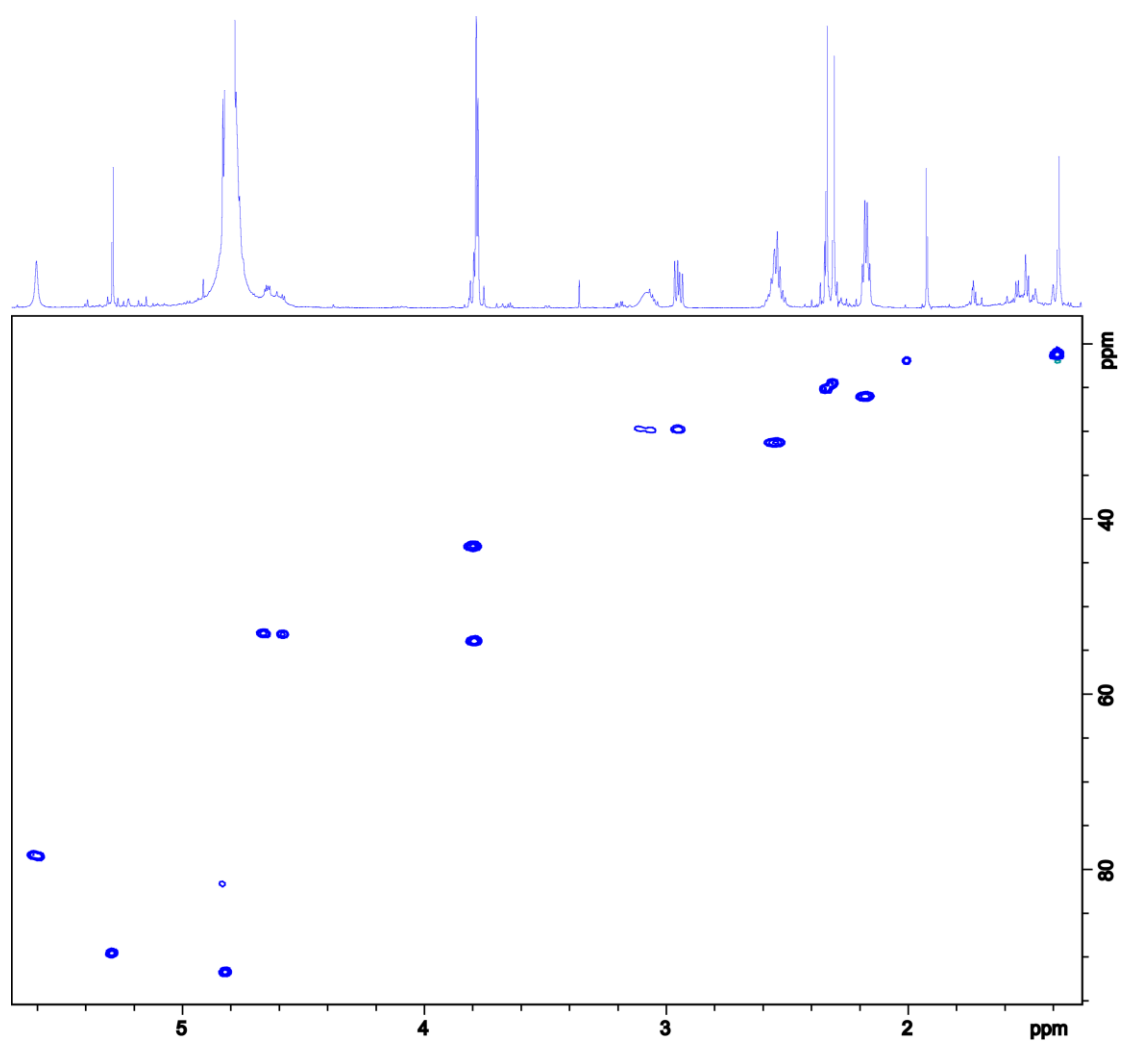


Figure S35. HSQC NMR spectrum for the reaction of GSH and 4 equivalents of methylglyoxal at pD 5.5 ( $\text{Na}_3\text{PO}_4$  buffer) after 6 hours. The  $^1\text{H}$  NMR spectrum from Figure S33a is shown on F2 axis. Free methylglyoxal is also present in the spectrum (resonances at  $\delta\text{C}$  21.3 ppm, 24.5 ppm and 89.6 ppm).

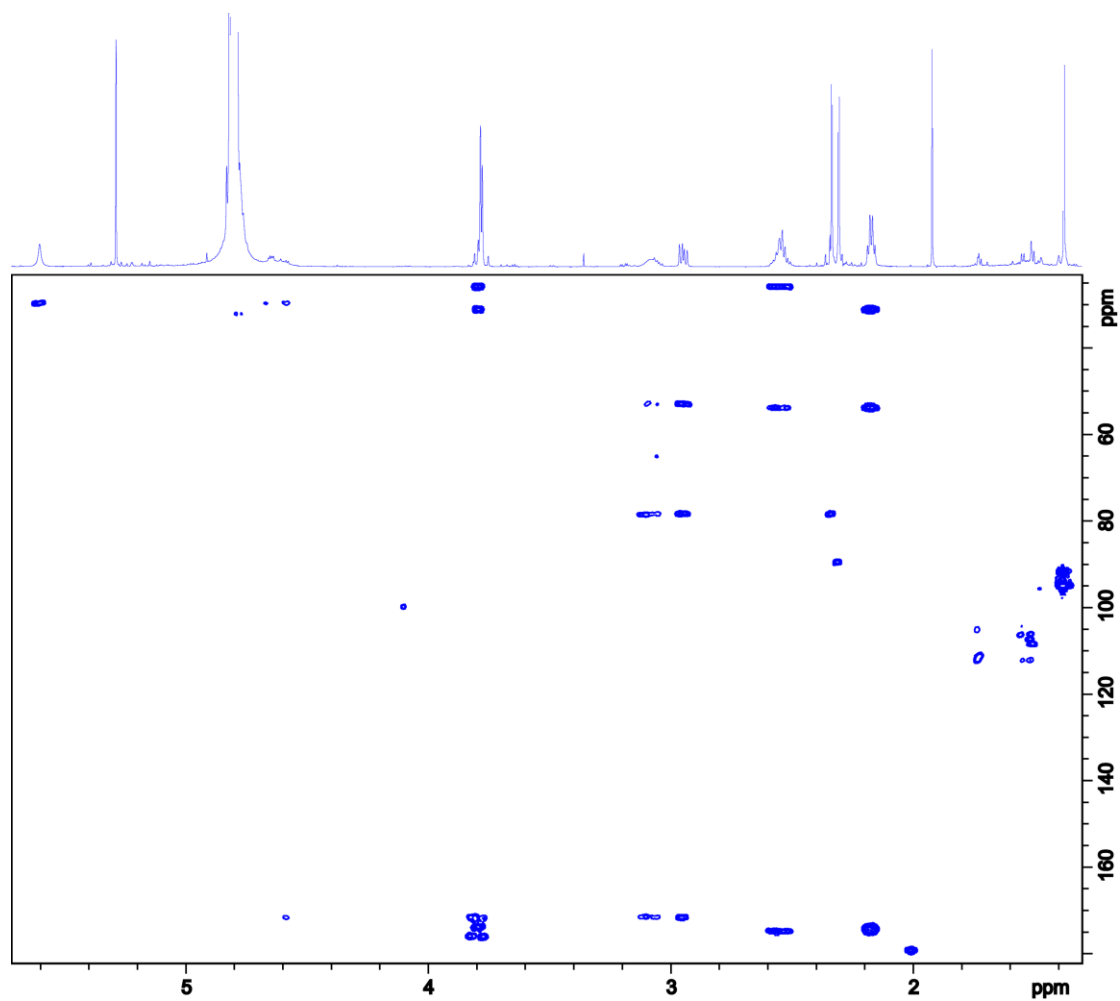


Figure S36. HMBC NMR spectrum for the reaction of GSH and 4 equivalents of methylglyoxal at pD 5.5 ( $\text{Na}_3\text{PO}_4$  buffer) after 6 hours. The  $^1\text{H}$  NMR spectrum from Figure S33a is shown on the F2 axis.

## Reference

1. R. Bateman, D. Rauh and K. M. Shokat, *Org. Biomol. Chem.*, 2007, **5**, 3363-3367.