SUPPLEMENTARY MATERIAL

Protein identification

Proteins labeled with **2** were identified by mass spectrometry as described in Experimental Procedures; the results are summarised below.

Table S1. Proteins identified by mass spectrometry

Spot	Protein ID	Score	No. peptides	Coverage
Small circle, left panel, Fig. 2B	ToxoDB 27.m00003 putative protein disulfide isomerase	394	11 (8 above identity threshold)	21%
Small circle, right panel, Fig. 2B	ToxoDB 44.m00037 putative serine/ threonine protein phosphatase 2C	175	5 (all above identity threshold)	12%
Lower 2 spots, circle, Fig. 3B	ToxoDB 583.m00002 peroxiredoxin 2	133	3 (2 above identity threshold)	15%
Upper spot, circle , Fig. 3B	ToxoDB 57.m00038 Conserved hypothetical protein	60	3 (2 between homology and identity thresholds)	13%

The glutamine synthase (GS) protection assay

rTgPrxII was recombinantly expressed in *E.coli* with a His-tag and purified by Ni affinity chromatography (elution buffer: 250 mM imidazole, 50 mM sodium dihydrophosphate, 300 mM NaCl, protease inhibitors). 1 µl of commercially available GS (0.5 units), varying amounts of a rTgPrxII stock solution (prepared by dilution of a 10 mg/ml stock to 0.2 mg/ml using 100 mM Hepes, pH 7.4) and 3 µl of inactivation solution (100 mM DTT, 50 µM $FeCl_3$, 100 mM Hepes, pH 7.4) were mixed and brought up to a final volume of 20 µl using 100 mM Hepes. This mixture was incubated at 30ºC for 20 minutes. 150 µl of starting solution (100 mM Hepes, 10 mM $KAsO_4$, 20 mM NH_2OH, 0.4 mM ADP, 0.5 mM $MnCl_2$, 100 mM glutamine, pH 7.0-

7.2) was then added and the incubation was continued at 30ºC for an additional 30 minutes. Reactions were terminated by incubation with 100 µl of stopping solution (5.5% [wt/vol] $FeCl_3$•$6H_2O$, 2% [wt/vol] TCA, 2.1% [vol/vol] concentrated HCl) for 5 minutes, after which the absorption at 540 nm was measured and normalised. These assays determined that the optimum amount of rTgPrxII in the assay is 1.2 µg (Figure S1), as this quantity of rTgPrxII protects GS from inactivation to a reasonably high level while still resulting in a linear correlation between rTgPrxII activity and GS activity.

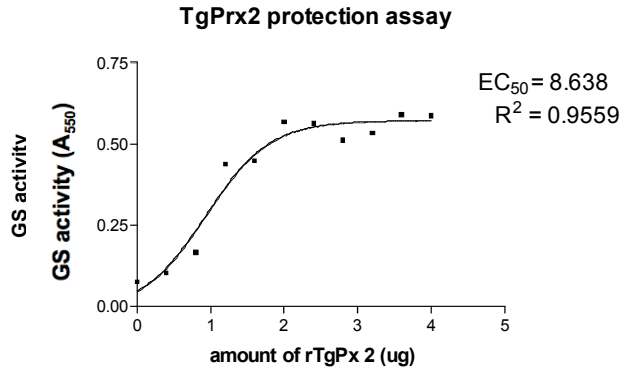


Figure S1. rTgPrxII protection assay curve.

IC_{50} Determination for 1

In initial studies, **1** and the solvent vector (acetonitrile) were tested to determine whether they inhibited GS activity directly. No inhibitory effect on GS was observed. Solutions (0.005 mM, 0.01 mM, 0.05 mM, 0.1 mM, 0.5 mM, 1 mM, 2 mM, 10 mM) of **1** were prepared in acetonitrile. 3 µl of a rTgPrxII stock solution (0.4 µg/µl) was added to 12 µl of 100 mM Hepes buffer (pH 7.4). 1 µl of the appropriate concentration of **1** was added and the assay was incubated at room temperature for 5 minutes. 1 µl of GS solution and 3 µl of inactivation solution were then added and the mixture was incubated at 30 ºC for an additional 20 minutes. 150 µl of starting solution was added and after further incubation at 30 ºC for 30 minutes, the reaction was terminated by incubation with 100 µl of stop solution for 5 minutes. The

remaining GS activity was determined by measuring the absorbance at 540 nm and the results were normalised. The IC_{50} was calculated using Prism® software .

Sequence and M_r of purified rTgPrxII

The sequence of recombinant, His-tagged TgPrxII (M_r 26924.8 Da) is as follows:

GHHHHHHHHHHSSGHIEGRHMWVLGSTFPDVHADASGVPGDKIKLYDFLGD
SWGLLMSHPHDFTPVCTTELAQAARMAPEFAKRNCKLIGFSCDDVSSHKGW
AKDVMSVAKLSGDLPFPIIADPERKLATDLGIMDPEEKDKAGIPVTCRAAIYIG
PDRRVKGLILYPATVGRNFKEVLRALDALQLAEKYPVATPEGWFPGDKVMV
QPTLTDEEAKAKLPKGFEKKECPSGKNYLRYAPDPSA

Electrospray ionisation mass spectrometric time course analysis of the reaction of rTgPrxII with 1

19 µl of 0.5 mg/ml rTgPrxII and 1 µl of a 1 mM stock solution of **1** in acetonitrile were incubated for various times from 5 to 30 minutes and analysed by LCMS. Samples were desalted on-line through a MassPrep On-Line Desalting Cartridge 2.1 x 10 mm, eluting at 50 µl/min, with an increasing acetonitrile concentration (from 2% acetonitrile, 98% aqueous 1% formic acid to 98% acetonitrile, 2% aqueous 1% formic acid) and delivered to an electrospray ionisation mass spectrometer (LCT, Micromass, Manchester, U.K.) that had previously been calibrated using myoglobin. Only covalently bound complexes were expected to survive the chromatography and ionisation conditions used. The results (Figs. 4B[i] and S2) showed that after a 5 minute preincubation of rTgPrxII with **1,** two main species, corresponding to unreacted rTgPrxII and a complex containing both rTgPrxII and **1**, were observed. As the incubation time increased, the spectra that were obtained became more complicated, probably as a result of the signal being split between many species.

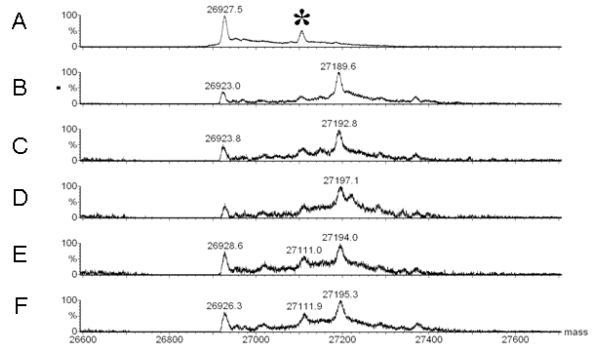


Figure S2. ESI MS analysis of the incubation of rTgPrxII with **1** as a function of time. **(A)** t=0 min, the species observed at M_r (26927.5) corresponds to rTgPrxII; * corresponds to an unidentified impurity present in the purified rTgPrxII sample; **(B)** t=5 min, the species observed at M_r (26923.0) corresponds to unreacted TgPrxII, the species at M_r (27189.6) corresponds to a rTgPrxII:**1** complex resulting from displacement of one bromine atom from **1** during the reaction. **(C-F)** A deterioration in the quality of the spectra was observed as the incubation time increased, although the main peaks remained the same as at shorter incubation times (**C** t=10 min.; **D** t=15 min.; **E** t=20 min.; **F** t=30 min.)

Termination of the reaction of 1 with rTgPrxII using benzyl mercaptan

Incubation of **1** with rTgPrxII for 5 minutes gave the most reproducible result and it was clear that extended incubation times had a detrimental effect on the quality of the protein signals. It is likely that deterioration of the signal results from the initial reaction of only one of the two bromine atoms present in **1** with rTgPrxII (as judged by the observed mass shift) followed at increased time periods by reactions involving the second bromo-methylene functional group in **1** (for a schematic representation of possible processes, see Figure S3). It was therefore decided that it would be advantageous to further react the rTgPrxII:**1** complex with BnSH in order to block the second reactive site in **1** prior to trypsin digestion.. After the initial 5 minute preincubation of rTgPrxII with **1**, 1 µl of a 2 mM stock solution of BnSH in acetonitrile was added and the mixture was incubated for another 5 minutes at 30ºC. Evidence was obtained to support the direct reaction of BnSH with rTgPrxII as well as with the rTgPrxII:**1**:BnSH complex (Fig. 4B(iii)). A new species at M_r = 27050.3 Da was observed with a mass shift of 125.6 Da corresponding to the formation of a 1:1 rTgPrxII:BnSH complex (theoretical mass shift for formation = 124.2 Da). A second species at M_r = 27355.7 Da was also observed with a mass shift of 431.0 Da compared to rTgPrxII, corresponding to a 1:1:2 rTgPrxII:**1**:BnSH complex (theoretical mass shift for formation = 436.1 Da). These additional complexes presumably arise through reaction of BnSH with the protein, possibly resulting in the formation of disulfide bonds.

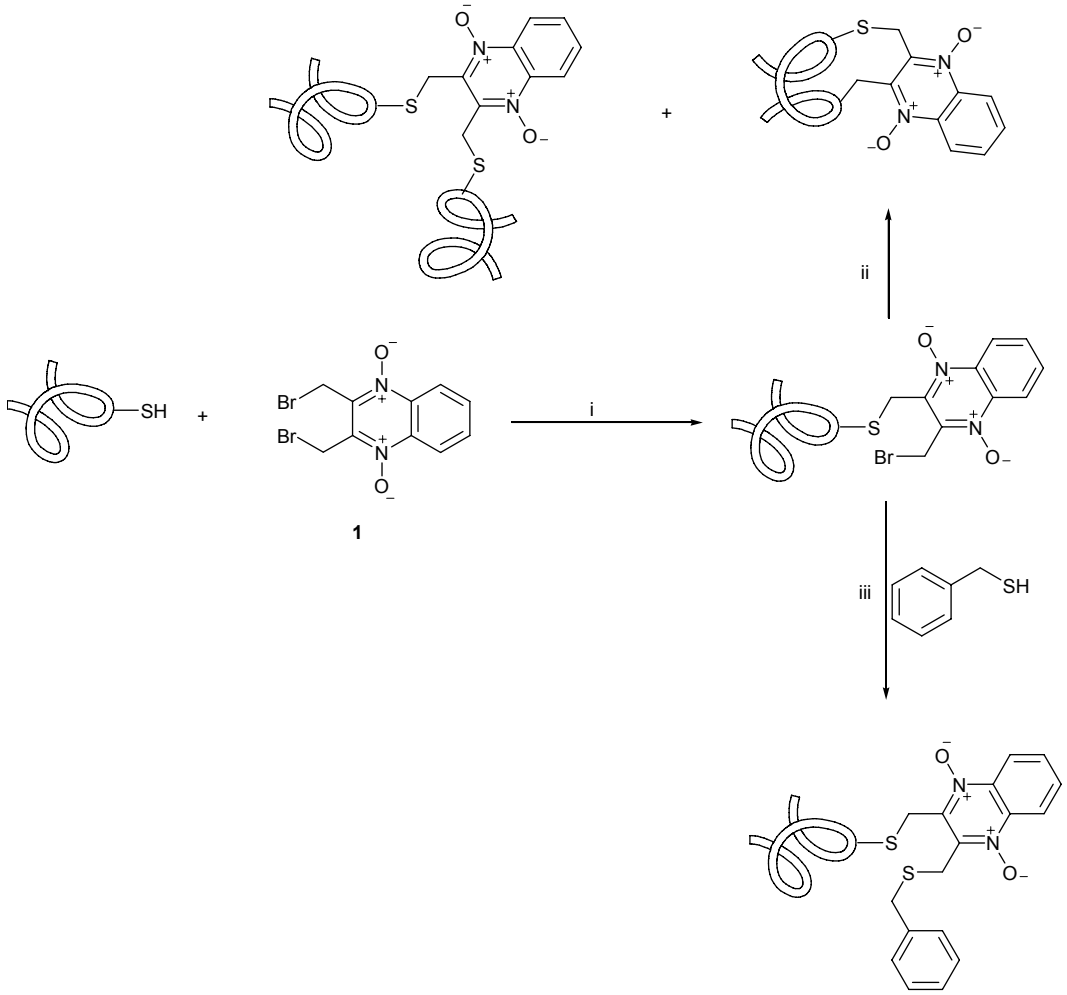


Figure S3. Schematic representation of the chemical steps proposed to occur during the course of the reaction of rTgPrxII with **1** and BnSH. i. 30 ºC 5 min; rTgPrxII reacts with **1** to displace one bromine atom only. ii. 30 ºC at extended preincubation times; TgPrxII reacts with **1** displacing both bromine atoms or dimer formation occurs. iii. 30 ºC 5 min. The unreacted bromine group in the rTgPrxII:**1** complex is capped by reaction with BnSH.

Mapping the site of rTgPrxII modification by 1

rTgPrxII and the rTgPrxII:**1**:BnSH complex, generated as described above, were digested with trypsin (0.3 µl, 60 ng) at 37 °C overnight. The resultant peptides were acidified prior to desalting and concentration using a micro C18 column (0.2 µl ZipTip, Millipore, Gloucestershire, U.K.) according to the manufacturer's instructions. The peptides were eluted directly from the tip onto the target in 1.5 µl

alpha-cyano-4-hydroxycinnamic acid (10 mg/ml in 50:50 acetonitrile:0.1% TFA). MALDI-MS and MS/MS was acquired using a 4800 MALDI-TOF/TOF Analyzer (Applied Biosystems, Foster City, CA) equipped with a Nd:YAG 355 nm laser and calibrated using a mixture of peptides.

Theoretical trypsin digest map of rTgPrxII showing the cysteine-containing tryptic peptides

The cysteine-containing tryptic peptides expected from MALDI-MS analysis are highlighted in blue. The predicted tryptic peptides containing Cys66 and Cys209 are too small for observation using this technique and are highlighted in red.

GHHHHHHHHH HSSGHIEGRH MWVLGSTFPD VHADASGVPG DKIKLYDFLG
DSWGLLMSHP HDFTPVCTTE LAQAARMAPE FAKRNCKLIG FSCDDVSSHK
GWAKDVMSVA KLSGDLPFPI IADPERKLAT DLGIMDPEEK DKAGIPVTCR
AAIYIGPDRR VKGLILYPAT VGRNFKEVLR ALDALQLAEK YPVATPEGWF
PGDKVMVQPT LTDEEAKAKL PKGFEKKECP SGKNYLRYAP DPSA

Detailed analysis of the Cys47-containing peptide

In MALDI-MS analysis of the rTgPrxII:**1**:BnSH complex the tryptic peptide containing Cys47 was observed in both a modified and unmodified form (Figure S5A and B). The majority of the modified peptide signal occurred at 3869.9 Da, 278.2 Da higher than the unmodified peptide (3591.7 Da), with smaller peaks at 3885.9 Da (mass shift = 294.2 Da) and 3901.8 Da (mass shift = 310.1 Da, Figure S5B). The latter species (mass 3901.8 Da) corresponded to the predicted mass expected upon reaction of a cysteine thiol group with **1** followed by BnSH in the context of this particular peptide (Figure S3). The species corresponding to the masses at 3885.9 and 3869.9 Da are consistent with the loss of 1 and 2 oxygen atoms from **1** from the rTgPrxII:**1**:BnSH complex in the context of the Cys47 peptide. Due to the very low intensity of the 3901.8 Da peptide, the MS/MS associated with this peak was less reliable and is not shown.

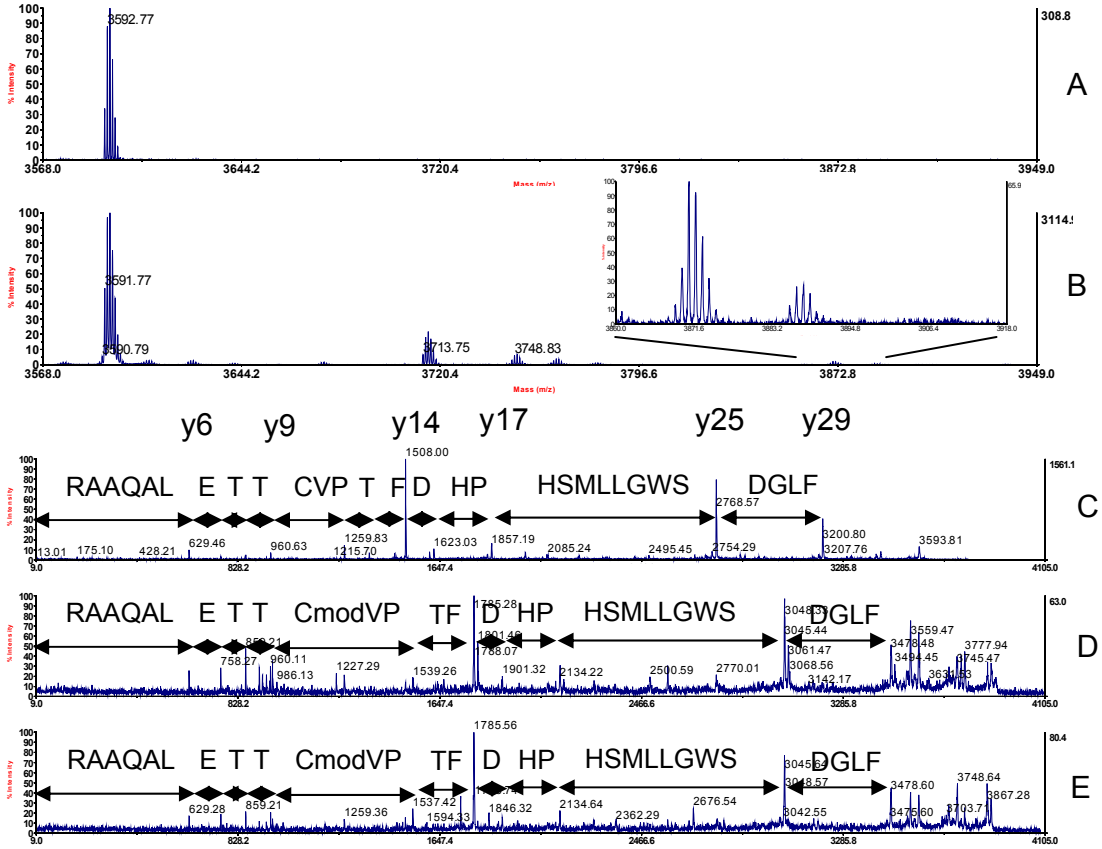
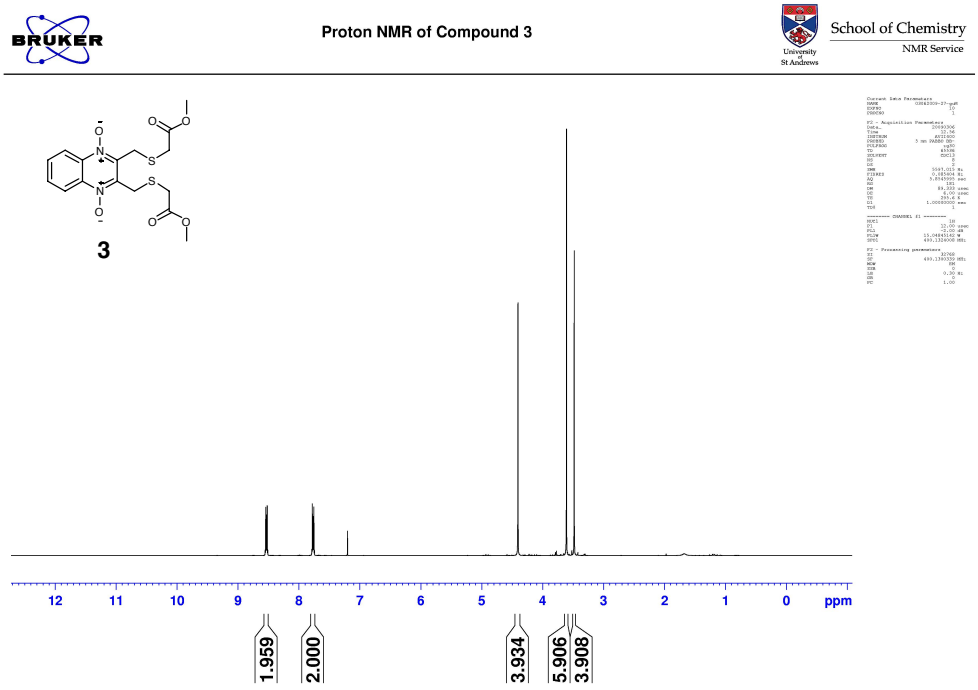


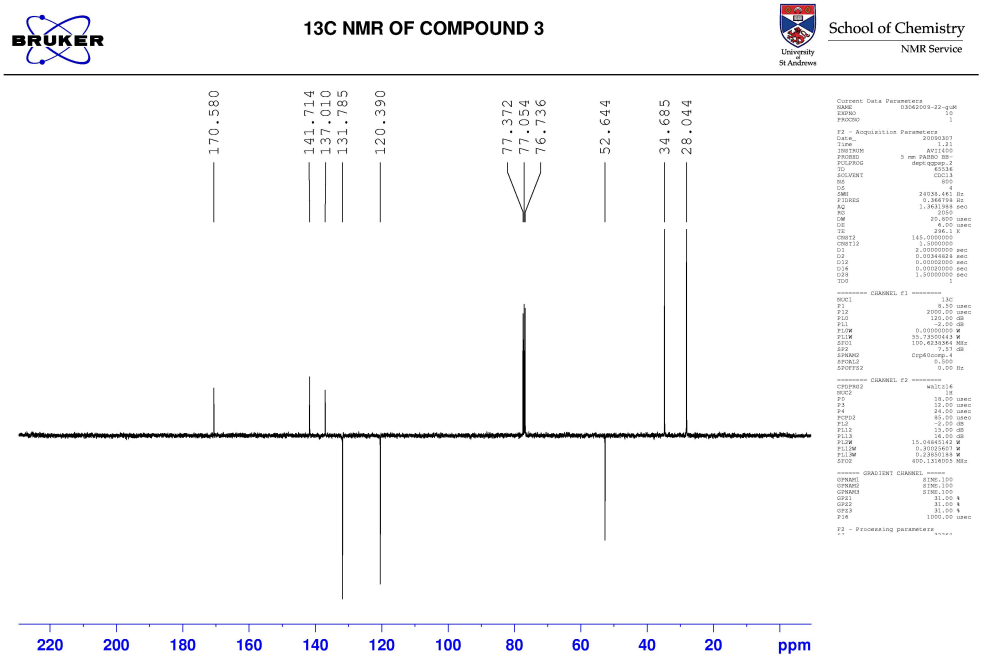
Figure S5. (A,B) MALDI-MS analysis of the unmodified and modified Cys47-containing peptide and **(C-E)** MS/MS analysis of (C) unmodified, (D) modified with loss of 16, (E) modified with loss of 32 Cys47-containing peptide.

MS/MS analysis of the unmodified peptide and two of the three modified peptide signals at 3869.9 and 3885.9 Da gave a series of similar spectra (Figure S5C, D and E and data not shown (3901.8 Da)). A partial y ion series showing the same masses in all spectra until y9 was identified, implying that there was no modification on residues TTELAQAAR. However, between y9 and y12 a discontinuity was observed between the spectra, with subsequent y ions in the unmodified peptide appearing lower in mass when compared to the modified peptides. This is consistent with the reaction of **1** and BnSH with Cys47 (as outlined in Fig. S3), Val46 or Pro45. However, reaction of the thiol functionality in Cys47 with **1** makes the best chemical sense. It should also be noted that a proportion of this tryptic peptide is not modified in this analysis, in contrast to the apparently complete modification seen in the

electrospray mass data (Fig.4Biii). It may be that the modified peptide is more sterically hindered for tryptic digestion and therefore remains incompletely digested, such that the unmodified peptide is overrepresented in the MALDI-MS analysis. Furthermore, the relative ionisation efficiency of the modified peptide compared to the unmodified peptide is unknown, so the relative signal intensities cannot be compared quantitatively.

^{1}H and ^{13}C NMR Spectra for 3




13C NMR OF COMPOUND 3
School of Chemistry
NMR Service
University
St Andrews
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137.010
131.785
120.390
77.372
77.054
76.736
52.644
34.685
28.044
220
200
180
160
140
120
100
80
60
40
20
ppm