SUPPORTING INFORMATION

Radiation Chemical Studies of Methionine in Aqueous Solution: Understanding the Role of Molecular Oxygen

Sebastián Barata-Vallejo, Carla Ferreri, Al Postigo and Chryssostomos Chatgilialoglu*

General Procedures

Materials. Solvents were purchased from Merck (HPLC grade) and used without further purification. Water was purified with a Millipore system. Commercially available starting materials were obtained from Sigma-Aldrich Co. and used as received: methionine, methionine sulfoxide, α-aminobutyric acid, 3-methylthiopropylamine, 3-methylthiopropionaldehyde, homoserine.

Continuous Radiolyses. Irradiations were performed at room temperature (22 ± 2 °C) on 1 mL samples using a ^{60}Co-Gammacell at different dose rates. The exact absorbed radiation dose was determined with the Fricke chemical dosimeter, by taking G(Fe^{3+}) = 1.61 μmol J^{-1} (*1*).

HPLC Analyses. HPLC analyses were recorded on an Agilent 1100 Liquid Chromatograph, equipped with a quaternary pump delivery system, a column thermostat and a variable-wavelenght detector. RP_{18} 5 μm columns were used as specified in each detection method. When needed, MS spectra were recorded on Esquire 3000 Plus Bruker instrument equipped with electrospray ionization source by direct sample insertion.

NMR Data on the Crude Reaction Mixture. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian VXR (^{1}H 400 MHz, ^{13}C 100.6 MHz) spectrometer; chemical shifts (δ) are reported in ppm relative to D_2O (4.79 ppm) unless otherwise specified. The commercially available compounds were used as reference for NMR (Figures S1 - S6). For 3-methylthiopropyl amine, the ^{1}H NMR spectrum was also performed (see Figure S7) adding an equimolar amount of sodium carbonate to a slightly acidic D_2O solution of the amine (pH=6) in agreement with the reported literature, and waiting some minutes for establishing the equilibrium of the protonated amine-carbamate-free amine (*2, 3*).

In the radiation experiments a reaction vessel equipped with a rubber septum was used, with a 250 mL average volume containing a 10.1-10.5 mM methionine solution). The gas mixtures were obtained by mixing the gases and controlling by a flow meter. The gas stream was flushing through a cannula inserted in the septum and the flow was adjusted in order to get a continuous bubbling during irradiation. At defined intervals, aliquots were withdrawn and the workup was carried out in order to proceed with the corresponding analysis. For NMR analysis, a sample of the crude reaction mixture was lyophilized immediately after the irradiation and then the residue was dissolved in D_2O. Figures S8-S12 show the results obtained from the reactions being the gas stream composed by pure N_2O and N_2O:O_2 (90:10, v/v), respectively.

The identification of 3-methylthiopropionaldehyde was also carried out by NMR spectroscopy. The crude reaction mixture was treated by deuterated chloroform extraction in order to separate organic soluble materials. Proton and carbon NMR spectra were recorded immediately, and the aldehyde was detected in all cases. Figures S13-S15 report representative NMR spectra of the detected aldehyde.

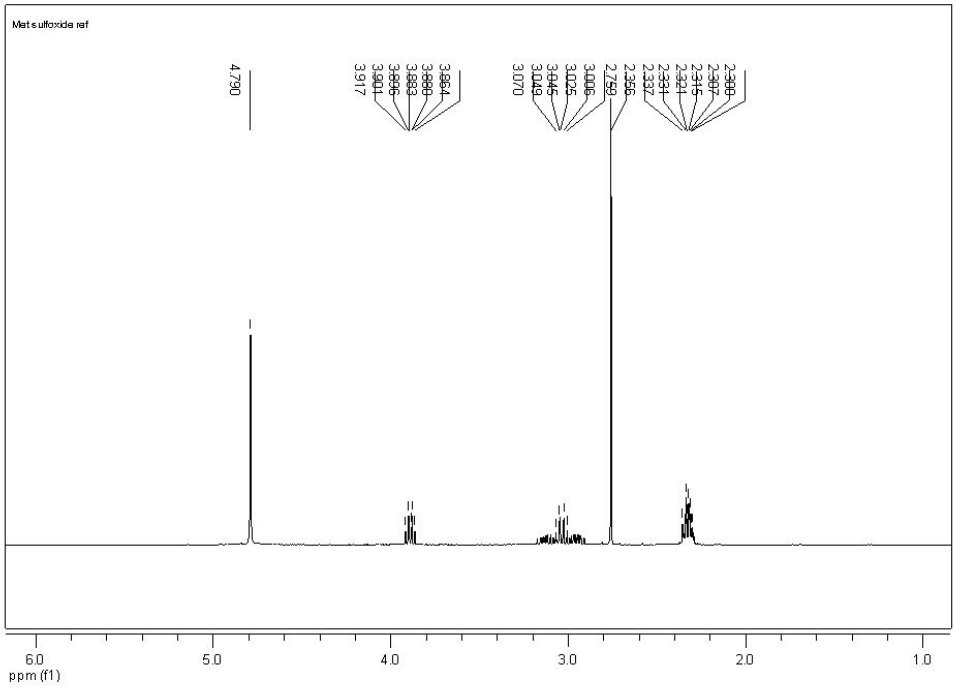

Figure S1 – ^{1}H NMR spectrum of methionine sulfoxide (D_2O)

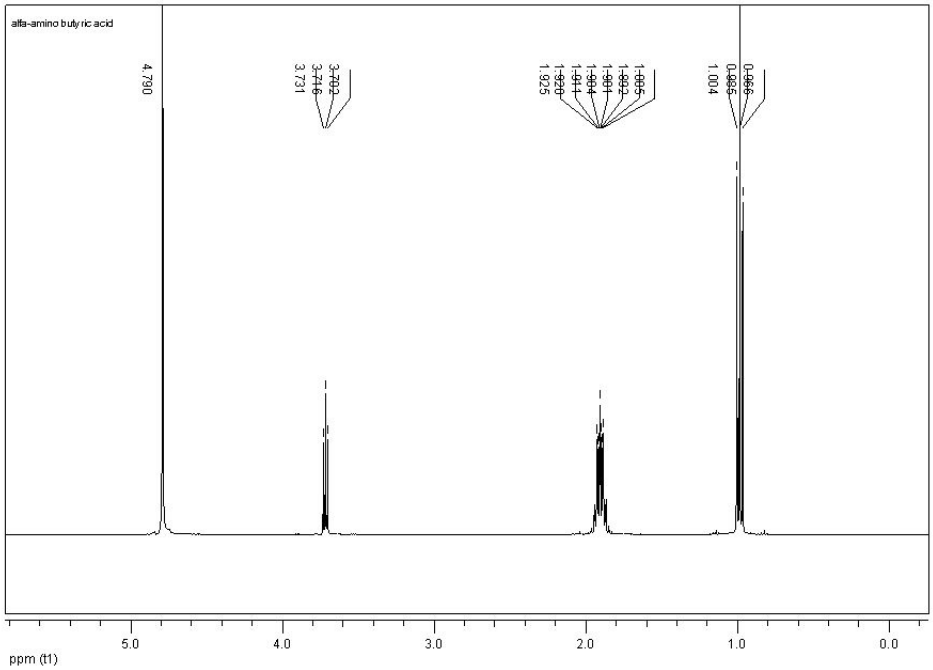

Figure S2 – ^{1}H NMR spectrum of α-aminobutyric acid (D_2O)

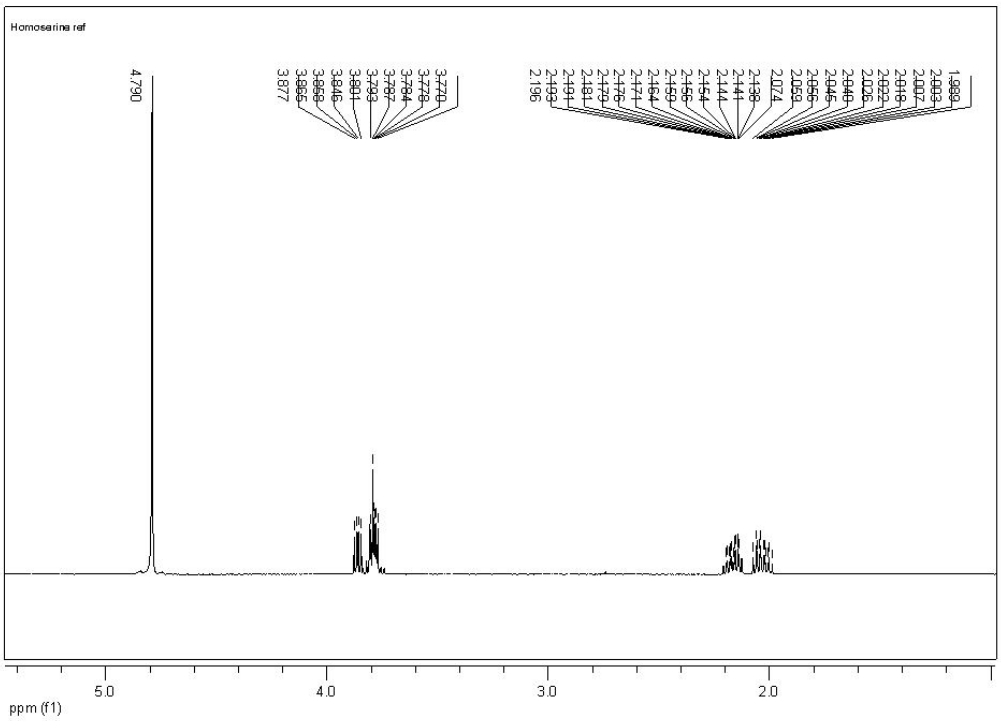

Figure S3 – ^{1}H NMR spectrum of homoserine (D_2O)

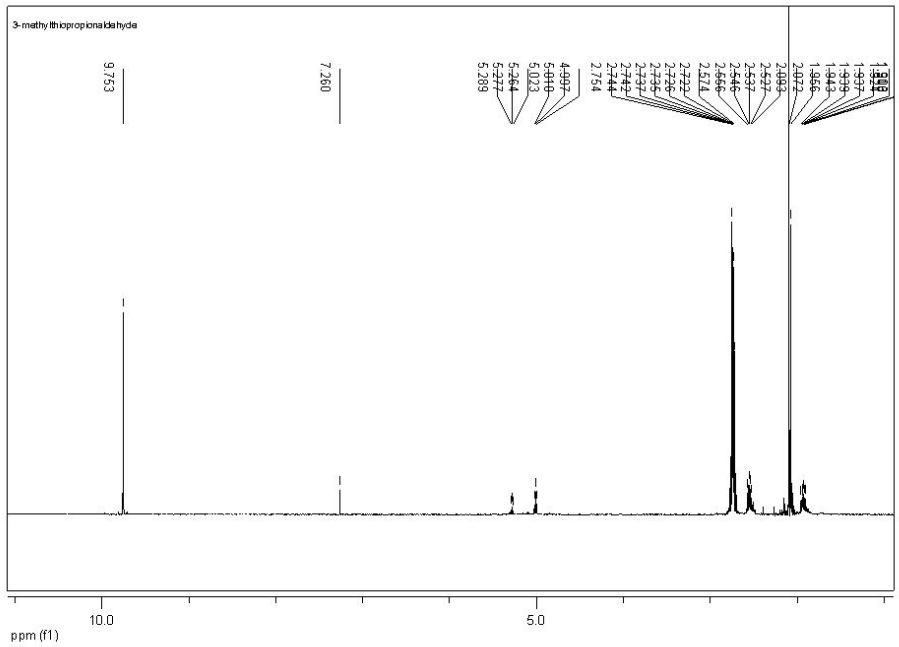

Figure S4 – ^{1}H NMR spectrum of 3-methylthiopropylaldehyde ($CDCl_3$)

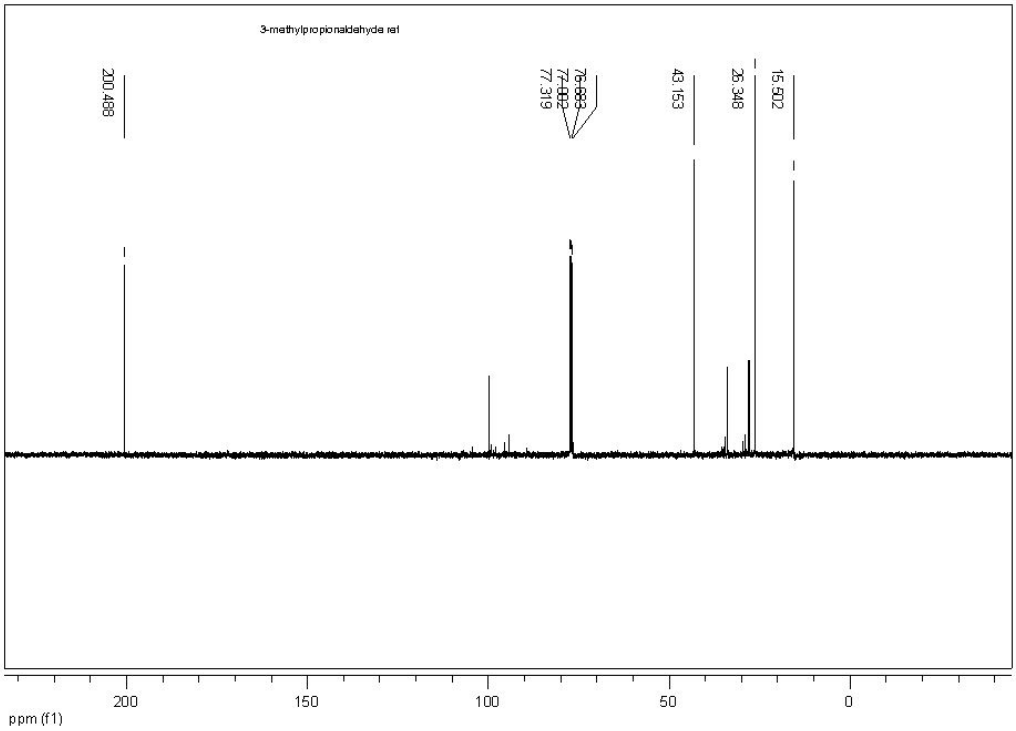

Figure S5 – ^{13}C NMR spectrum of 3-methylthiopropionaldehyde ($CDCl_3$)

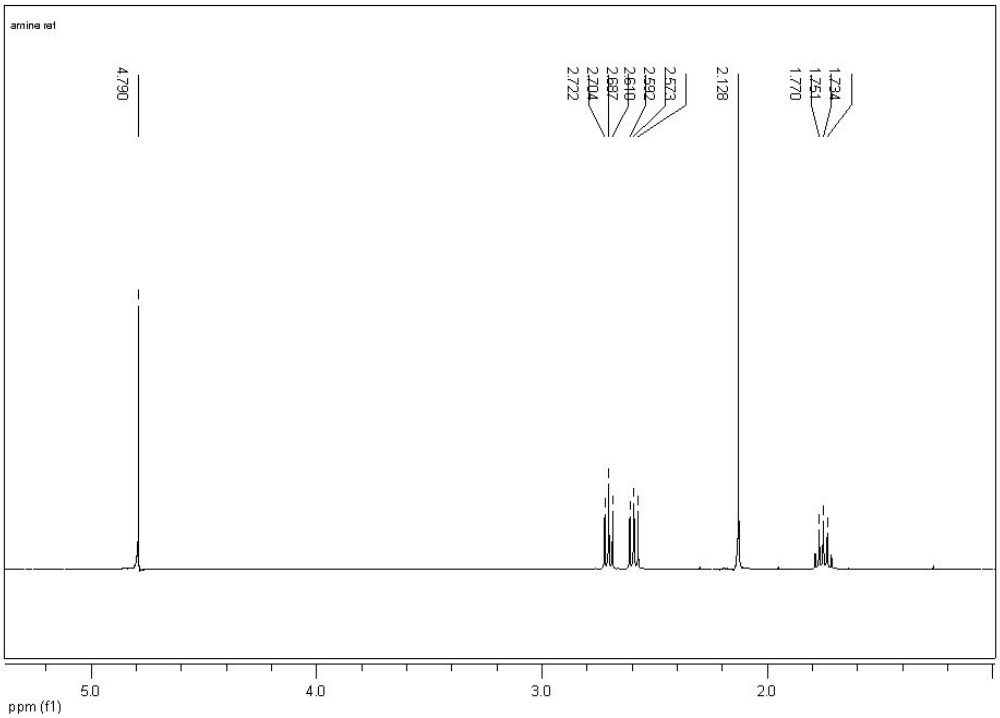

Figure S6- ^{1}H NMR spectrum of 3-methylthiopropylamine (D_2O)

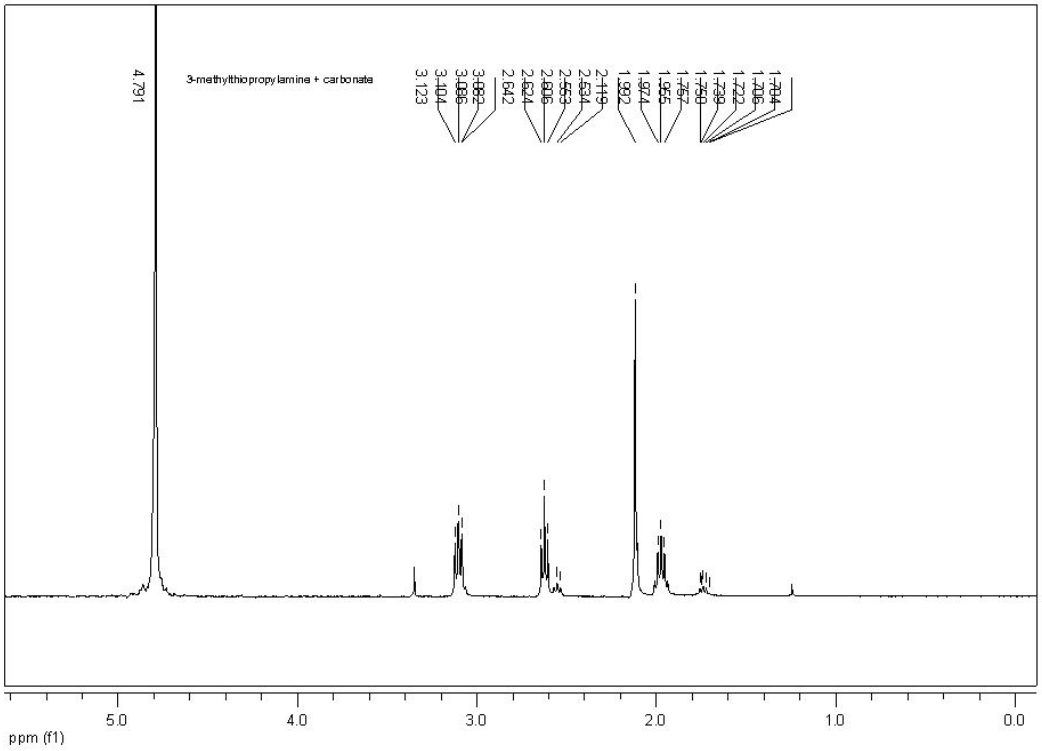

Figure S7 – ^{1}H NMR spectrum (D_2O) of 3-methylthiopropylamine adding an equimolar amount of sodium carbonate to a slightly acidic D_2O solution of the amine (pH=6)

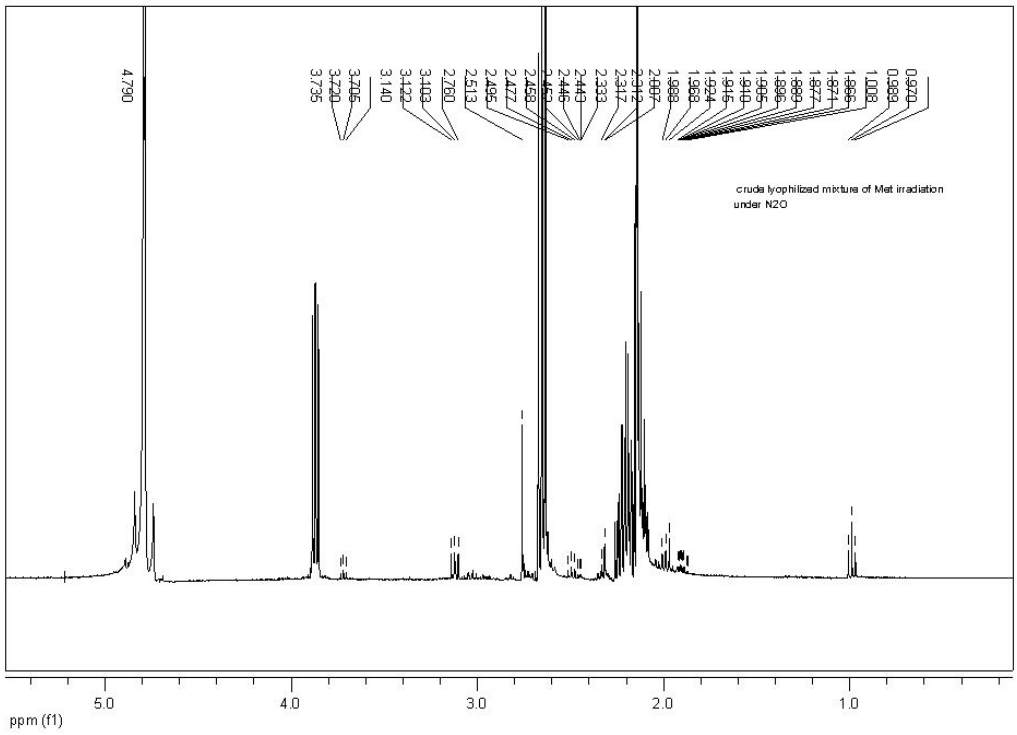

Figure S8 – ^{1}H NMR spectrum (D_2O) of the crude reaction mixture of Met irradiated with pure N_2O

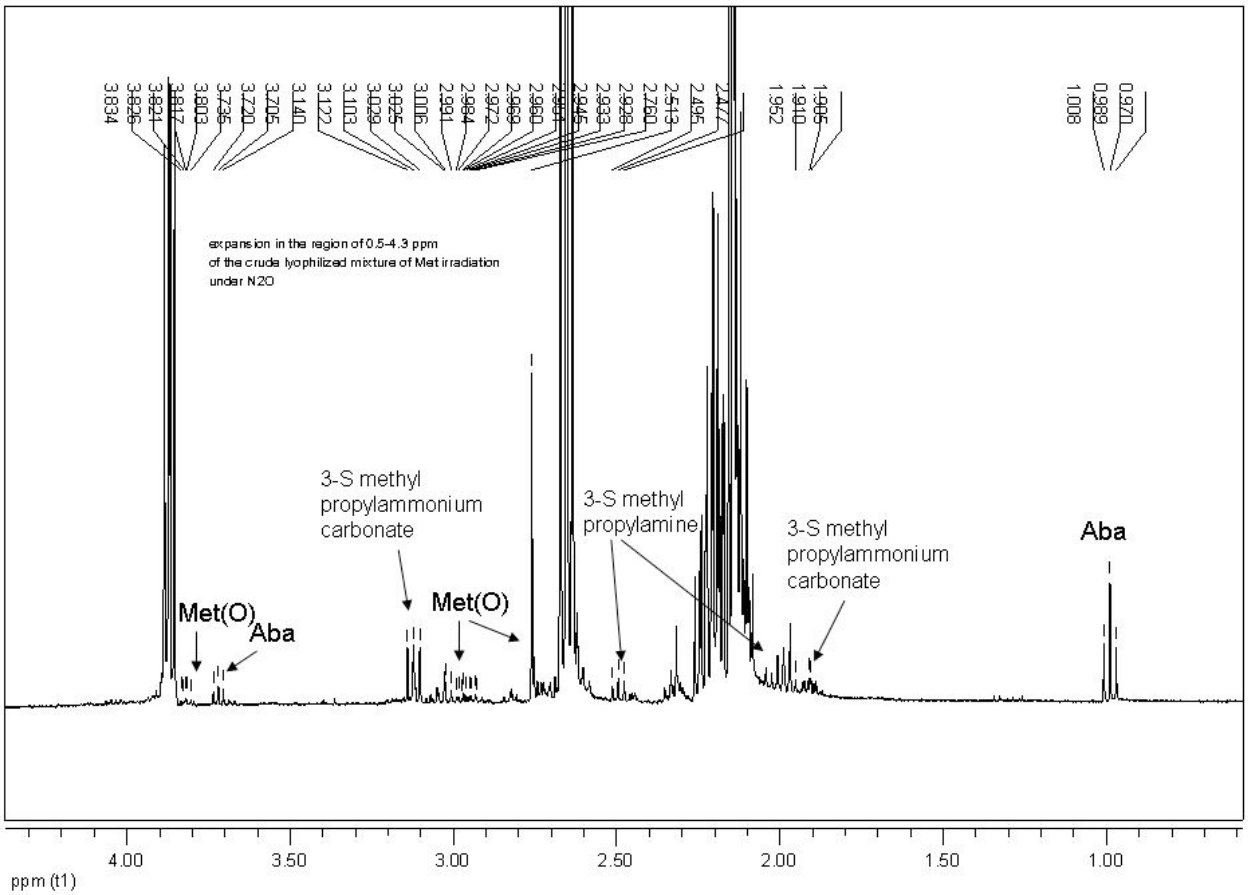

Figure S9 – Expanded region of ^{1}H NMR spectrum (D_2O) of the crude reaction mixture of Met irradiated with pure N_2O where the peaks of the reaction products are recognized (see spectra of the corresponding reference compounds).

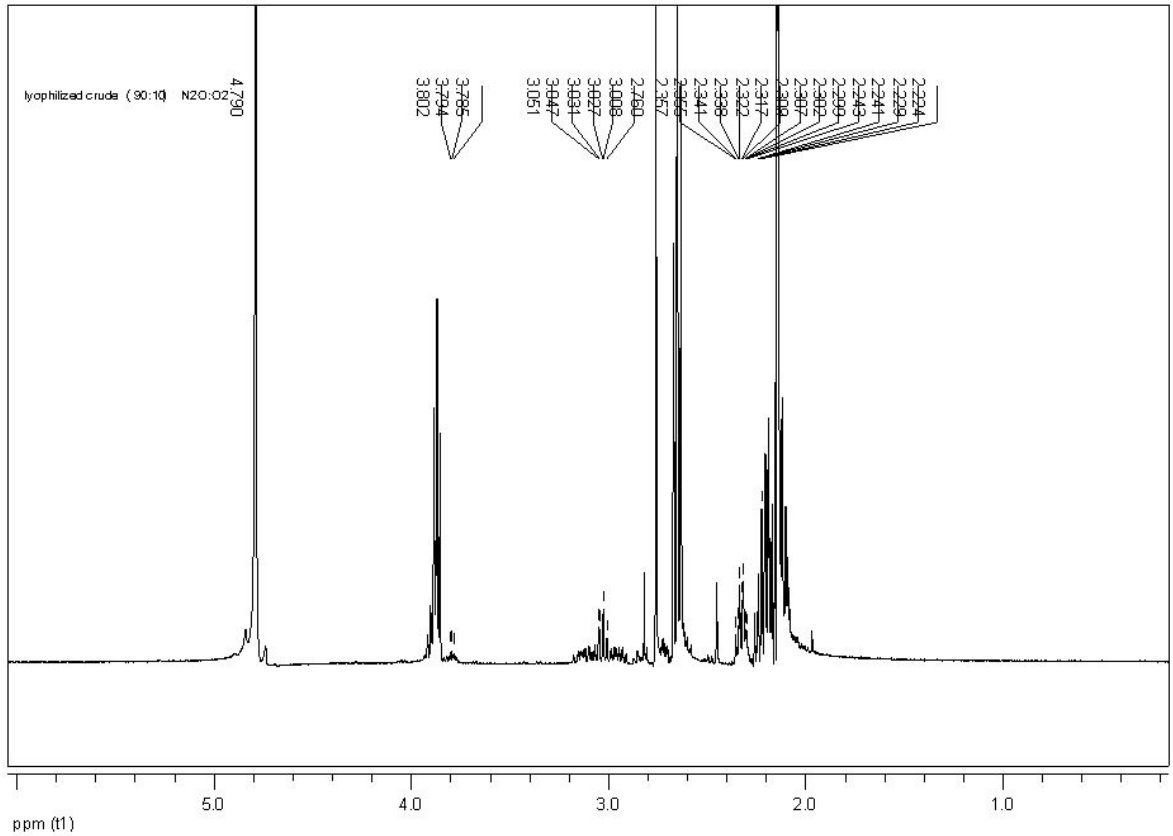

Figure S10 - ^{1}H NMR spectrum (D_2O) of the crude reaction mixture of Met irradiated with 90:10 N_2O:O_2

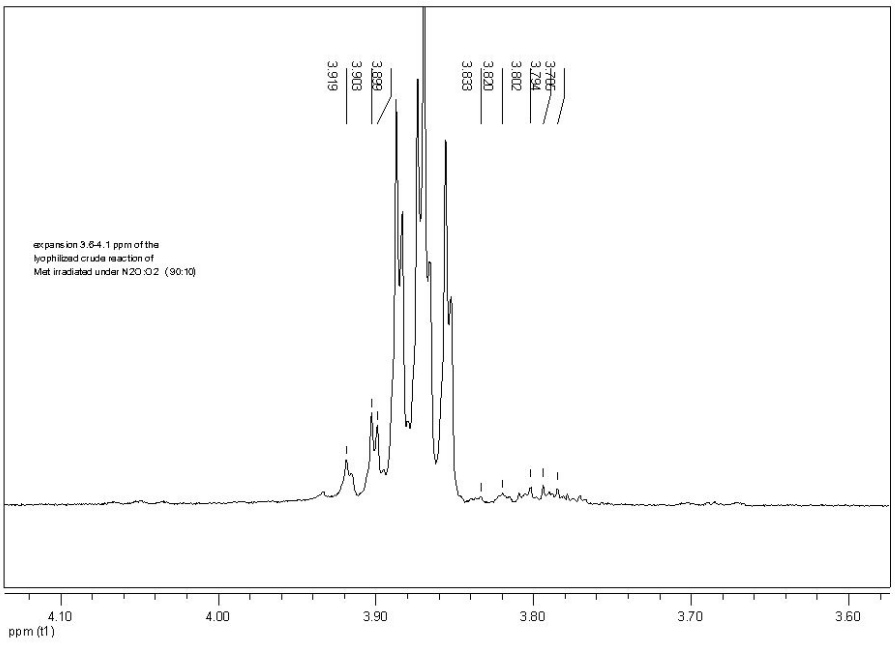

Figure S11 – Expanded region of the ^{1}H NMR spectrum of the crude reaction mixture of Met irradiated with 90:10 N_2O:O_2

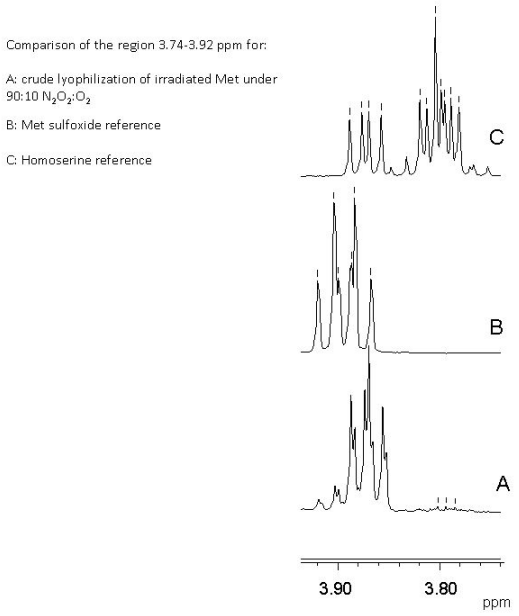

Figure S12 – Expanded region of the ^{1}H NMR spectrum (D_2O) of the crude reaction mixture of Met irradiated with 90:10 N_2O:O_2 in comparison with the standard reference compounds

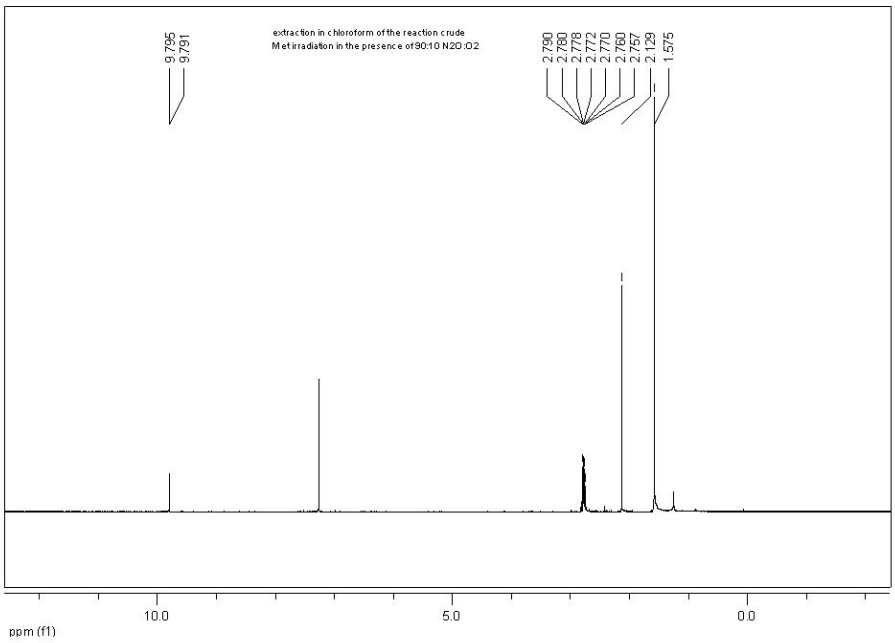

Figure S13 – ^{1}H NMR spectrum of the $CDCl_3$ extraction of the crude reaction mixture of Met irradiation at 0.5 kGy with 90:10 N_2O:O_2

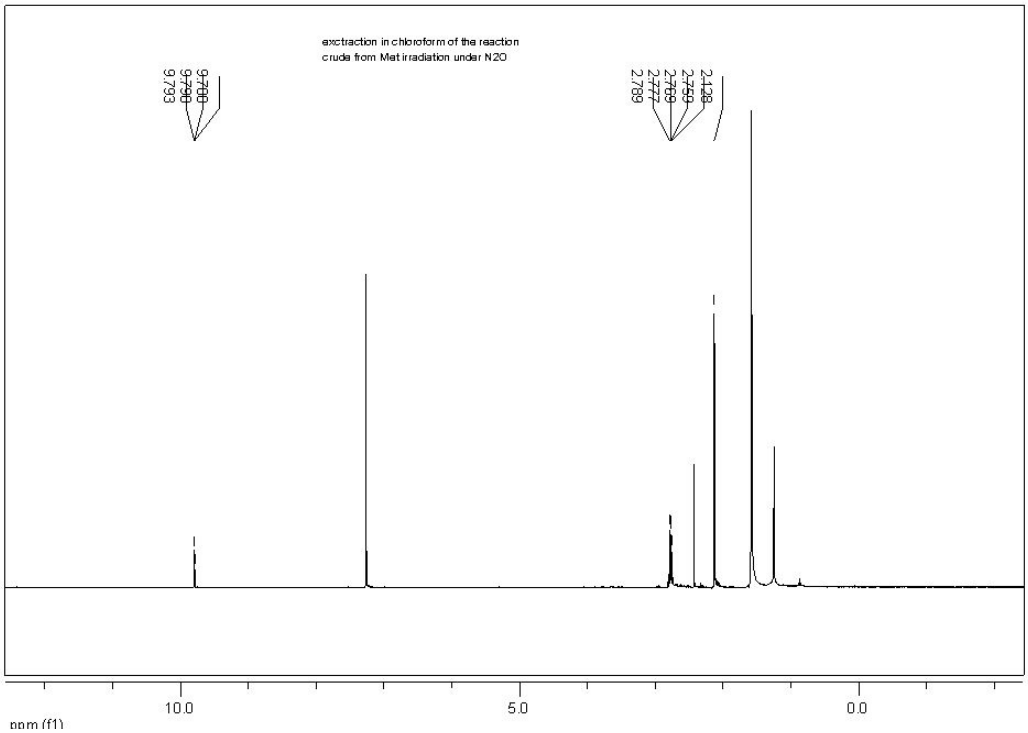

Figure S14 – ^{1}H NMR spectrum of the $CDCl_3$ extraction of the crude reaction mixture of Met irradiation at 0.5 kGy with N_2O

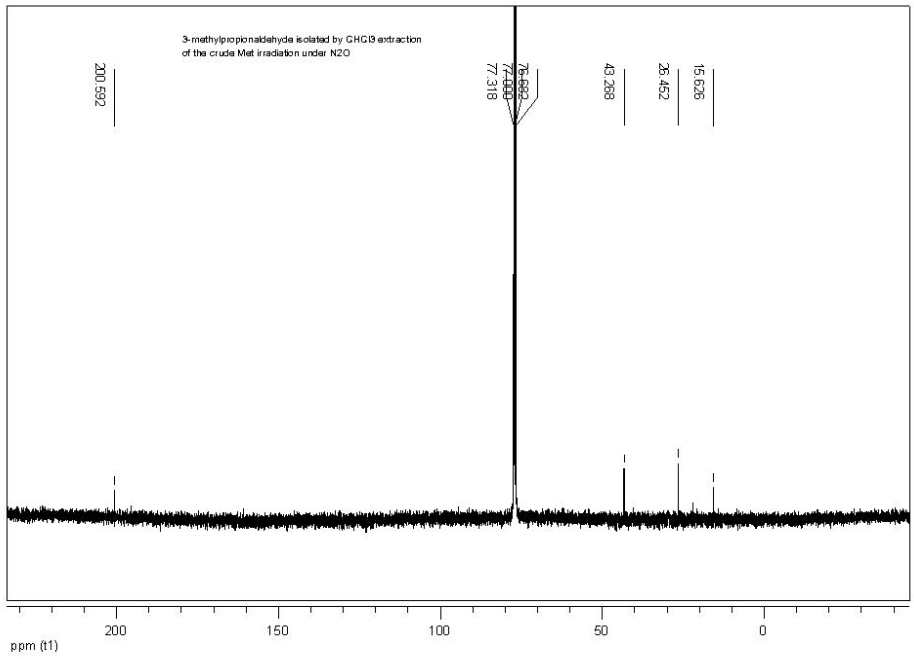

Figure S15 – Carbon NMR of the $CDCl_3$ extraction of the crude reaction mixture of Met irradiation at 0.5 kGy with N_2O

OPA derivatization and analysis of amino compounds. OPA derivatization was performed according to reported procedures that were adapted to our case (*4, 5*). Figure S16 shows a typical HPLC run containing all OPA derivatives of the amino compounds detected in our experiments, in comparison with two representative crude mixtures of Met irradiation in the presence of 90:10 N_2O:O_2 and pure N_2O. HPLC analyses were performed on a GraceSmart RP 18 5µ column (150 mm x 4.6 mm) used at 40 °C, with detection at λ = 338 nm. Mobile phase A was 10 mM NaH_2PO_4, adjusted to pH 7.5 with NaOH, while mobile phase B was acetonitrile/methanol/water (45/45/10 v/v/v). The separation was obtained at a flow rate of 1 mL/min with the following gradient program: 0.5 min at 5 % B, a 16.0 min increase of eluent B to 47 % followed by a 21.0 min step increase of eluent B to 100 %. Then washing at 100 % B and equilibration at 5 % B. Total time was 25.0 min. Calibration was done with pure standards.

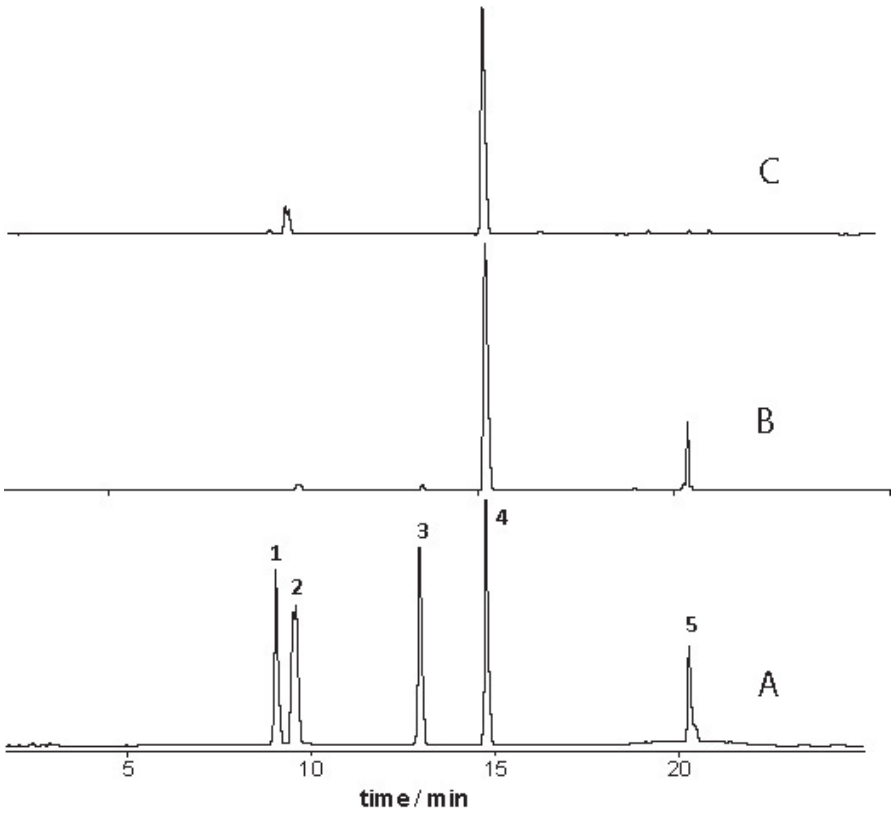

Figure S16 – HPLC traces of the OPA derivatives of amino compounds isolated from 10.2 mM Met irradiation at 4 kGy under N_2O (B) and under 80:20 N_2O:O_2 (C) in comparison with the

run containing the following amino compounds as references: 1) homoserine; 2) methionine sulfoxide (R and S epimers); 3) α-aminobutyric acid; 4) methionine; 5) 3-methylthiopropylamine.

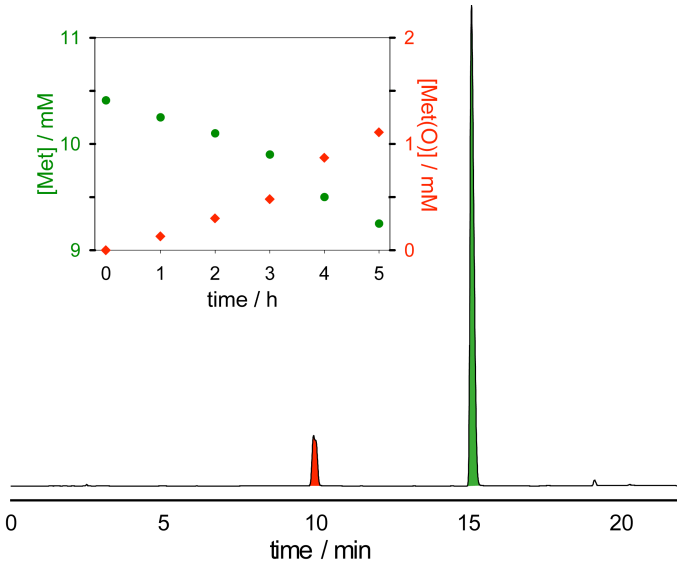

Figure S17. HPLC trace after 5 h, in which the oxidation of Met (green peak) to Met(O) (red peak) by H_2O_2 occurs quantitatively. Inset: Time-dependent of Met (green) and Met(O) (red) concentrations during the reaction course. Experimental Conditions: H_2O_2 (500 µL of a 1 M aq H_2O_2) was introduced by syringe-pump (flow rate of 100 µL/h) to an aqueous solution of methionine (250 mL, 10.41 mM, pH 5.8) under stirring during 5 h (H_2O_2 final concentration = 2 mM); Samples were withdrawn at 1 hr intervals, followed by OPA derivatization of amino compounds and HPLC analysis. The inset reports the change of concentrations of the two compounds during the reaction course.

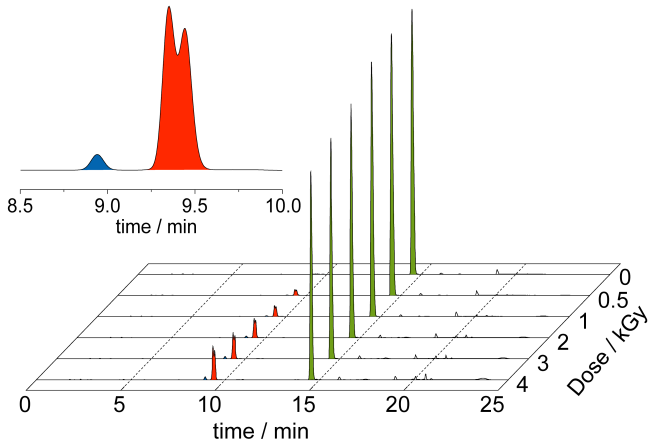

Figure S18. HPLC analyses of γ-irradiation of O_2-saturated solutions of 10.16 mM Met at natural pH (dose rate of ~7 Gy min^{-1}) after OPA derivatization of amino compounds. The consumption of Met (green peaks) led to the formation of Met(O) (red peaks) and homoserine (blue peaks). Inset: expansion of the chromatogram between 8.5-10 min of the 3.5 kGy irradiated sample; the splitting of the red peak is due to the *R* and *S* epimers of sulfoxide.

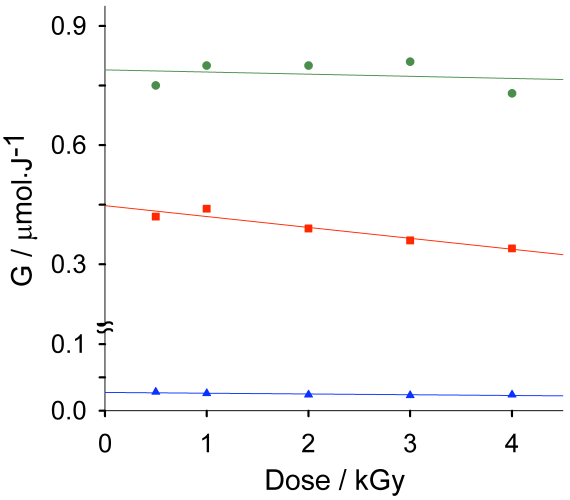

Figure S19. Radiation chemical yields (*G*) of methionine (**1**), methionine sulfoxide (**7**) and homoserine (**12**) *vs*. irradiation dose obtained from the experiment reported in Figure S18; $G(-\mathbf{1}) = 0.79$, $G(\mathbf{7}) = 0.43$ and $G(\mathbf{12}) = 0.027$ µmol J^{-1} are obtained when the lines are extrapolated to zero dose.

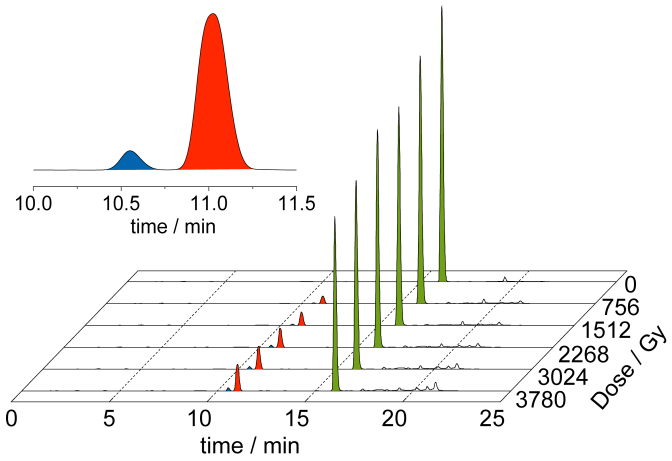

Figure S20. HPLC analyses of γ-irradiation of N_2O/O_2 (90:10, v/v)-saturated solutions of 10.38 mM Met at natural pH (dose rate of ~7 Gy min^{-1}) after OPA derivatization of amino compounds. The consumption of Met (green peaks) led to the formation of Met(O) (red paeks) and homoserine (blue peaks). Inset: expansion of the chromatogram between 8.5-10 min of the 3.78 kGy irradiated sample.

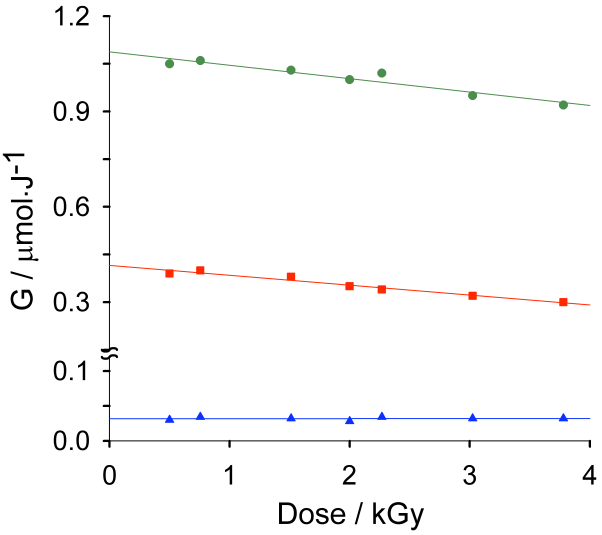

Figure S21. Radiation chemical yields (G) of methionine (**1**), methionine sulfoxide (**7**) and homoserine (**12**) *vs*. irradiation dose obtained from the experiment reported in Figure S20; $G(-\mathbf{1})$ = 1.10, $G(\mathbf{7})$ = 0.42 and $G(\mathbf{12})$ = 0.034 µmol J^{-1} are obtained when the lines are extrapolated to zero dose.

Quantitation of 3-methylthiopropionaldehyde. The quantitation of this aldehyde was performed as the corresponding 2,4-dinitrophenyhydrazone derivative, following a published protocol adapted to our case (*6*). 1 mL of the irradiated methionine sample (0.5 kGy) was diluted with 500 µL 0.4 % v/v conc. H_3PO_4 (in 9/1 acetonitrile/water, v/v). 500 µL of 2,4-dinitrophenylhydrazine 40 mM in acetonitrile is added and the solution was vortexed for 5 min. After derivatization, the reaction mixture was diluted to the 1/10 ratio in acetonitrile/water (1/1 v/v) and 20 µL were injected for HPLC analysis using a LiChroCART RP 18 5µ column (250 mm x 4 mm), at 30 °C, with detection at λ = 360 nm. Mobile phase A was water and mobile phase B was acetonitrile. The separation was obtained at a flow rate of 1 mL/min with a gradient program as follows: 10 min at 40 % B, followed by a 25.0 min step to increase eluent B to100 %. Then washing at 100 % B and equilibration at 40 % B. Total time was 35.0 min.

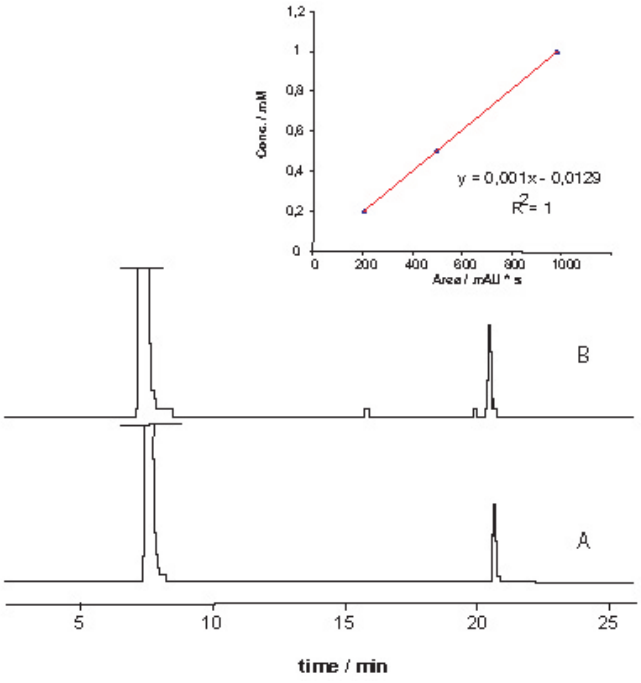

Figure S23 – HPLC traces of 2,4-diphenylhydrazine derivatization of 3-methylthiopropionaldehyde (A) and a representative sample of the analogous derivatization

carried out on a chloroform extract of Met irradiated samples (0.5 kGy). (B) The major peak is 2,4-dinitrophenylhydrazine remaining in the sample. The inset shows the calibration curve with an authentic sample from the commercially available aldehyde, which was derivatized to the corresponding hydrazone.

References

(1) Spinks, J.W.T., and Woods, R.J. (1990) *An Introduction to Radiation Chemistry*, 3rd Ed., Wiley, New York, p 100.

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(6) Cardoso, D. R., Bettin, S. M., Reche, R. V., Lima-Neto, B. S., and Franco, D. W. (2003) *J. Food. Compos. Anal. 16*, 563–573.