Hyperpolarized [1-^{13}C]-Ascorbic and Dehydroascorbic Acid: Vitamin C as a probe for imaging redox status *in vivo*

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Supporting Information

General methods

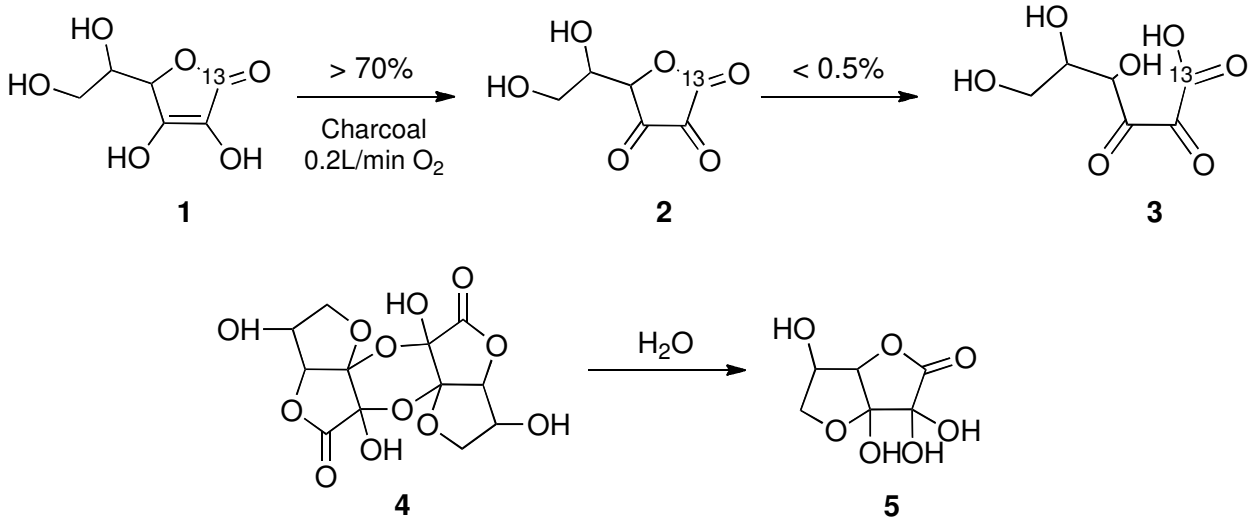
[1-^{13}C]Ascorbic acid was purchased from Omicron Biochemicals, Inc. (South Bend, IN); all other reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless stated otherwise. ^{1}H and ^{13}C NMR spectra for characterization of oxidation products were collected at 25 °C on a Bruker 500 MHz spectrometer. All chemical shifts (δ) are reported in ppm. Samples were prepared in deuterium oxide for ^{1}H NMR, with 4 mmol/L added sodium trimethylsilyl-2,2,3,3-tetradeuteroproprionate (TSP), which was used as an intensity and chemical shift reference at 0.0 ppm. Samples for ^{13}C NMR were prepared in deuterated dimethyl sulfoxide (DMSO-d_6; Cambridge Isotope Laboratories) to prevent hydrolysis of the substrate to diketogulonic acid during the longer acquisition time of this experiment and the natural abundance ^{13}C signal from DMSO-d_6 was used as an internal reference.

[1-^{13}C]Dehydroascorbic acid

[1-^{13}C]Dehydroascorbic acid was prepared by charcoal oxidation of [1-^{13}C]Ascorbic acid (Suppl Scheme 1), using a modified version of the method presented by Ohmori *et al* [1,2]. [1-^{13}C]Ascorbic acid **1** (0.5 g, 2.9 mmol) was dissolved in methanol (15 mL) and then highly purified activated charcoal Norit (0.75g) was added as a catalyst. The reaction was kept at room temperature and stirred vigorously for 90 minutes, with oxygen bubbled through at 0.2 L/minute. Spectrophotometric assay of ascorbic acid at 265 nm was performed to verify completion of the oxidation reaction. A 1 in 10 dilution of a 0.22 μm filtered reaction mixture (2 μL) was added to phosphate buffer (1 mL, 100 mM; pH 7.4) with EDTA (0.3 mM) at the start, then at 90 minutes after addition of charcoal, to ensure the absorbance at 265 nm had reduced to zero.

To remove charcoal from the reaction mixture, coarse Celite® 545 (3 g) was suspended in methanol (8 mL), packed in filter paper (Whatman) placed inside a glass funnel and the reaction mixture was pulled through the filtration system under vacuum. Following 0.22 μm filtration, the product was dried on a rotary evaporator at 30°C then refluxed with 2-butanone (methyl ethyl ketone, MEK, 0.5mL) at 80°C for 30 minutes to remove methanol from the solution. The rotary evaporation and reflux procedure was repeated twice to minimize the

residual methanol in the final product. The final product was then lyophilized to yield (> 0.35g, 70%) a pale yellow solid. It should be noted that form **2** is for schematic purposes only[3,4]; ^{13}C NMR of the product is consistent with the bicyclic monohydrate in **5**, with the equivalent structure in the crystalline state being the dimeric arrangement in **4**. The product was stored at -20°C and was stable for at least 6 months. ^{1}H NMR (500MHz, D_2O) δ 4.60-4.58 (m, 1H), 4.29-4.26 (dd, J=10.3Hz, 5.4Hz, 1H), 4.18-4.16 (dd, J=10.3Hz, 2.5Hz, 1H), 3.84 (s, 1H) ; ^{13}C NMR (125MHz, DMSO-d_6) δ 173.3 (*-labeled), 105.9, 91.7, 88.3, 75.5, 73.6 (contaminant peaks: < 0.5% by weight starting material, δ 171.5, 168.8 DKG **3**; <0.01%, δ 36.6, 29.9, 8.3 MEK).

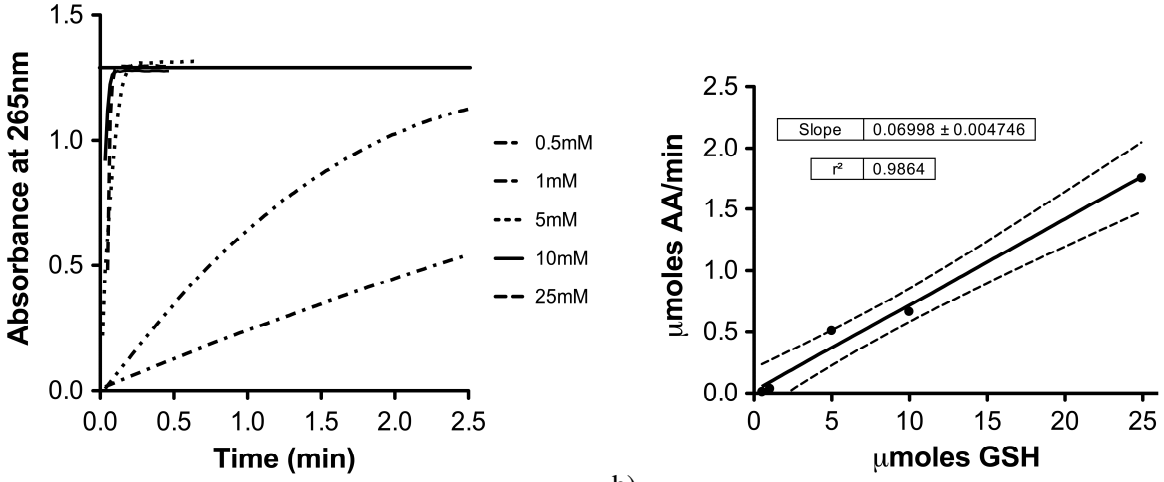


Scheme S1 Oxidation over charcoal of [1-^{13}C]-AA

UV Kinetics measurements

The rate of reaction between dehydroascorbic acid (DHA) and glutathione (GSH) was determined spectrophotometrically at 265 nm to validate the NMR measurements.[5] Phosphate buffer (0.95 mL) containing GSH (between 0.5 and 25mM) was added to the sample cuvette to provide a blank reading. Freshly prepared DHA solution (50 μL, repeated at 0.5 mM and 5 mM in the assay) in phosphate buffer was then added and the production of AA was monitored for 2.5 minutes (Figure S1a). The second order rate constant for the production

of AA was evaluated by comparison to known standards (range 0.01 mM to 1 mM) to be 0.026±0.002 $M^{-1}s^{-1}$ for 0.5 mM DHA and 0.34±0.01 $M^{-1}s^{-1}$ for 5 mM DHA (Figure S1b), in agreement with previously reported data.[5]



a) b)

Figure S1 a) Increase in absorbance at 265 nm observed upon addition of 5 mM DHA to 0.5 – 25mM GSH due to reduction of DHA to AA b) Quantification of the rate of AA production based on the initial slope of the curves in (a) and by comparison with known standards

Tumor implantation and preparation of animals for MRI

All procedures were conducted in accordance with the project and personal licenses issued under the United Kingdom Animals (Scientific Procedures) Act, 1986. Tumors were established, as described previously,[6] by subcutaneous inoculation of 5 x 10^6 EL4 murine lymphoma cells in female C57BL/6 mice and allowed to grow for up to 10 days. Animals were anaesthetized by intraperitoneal administration of 10mL/kg of a 5:4:31 mixture of Hypnorm (VetaPharma Ltd.), Hypnovel (Roche) and saline. A catheter was inserted into the tail vein and the animal was placed inside an imaging cradle with body temperature maintained using a flow of warm air.

References

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