

## Dioxindolylalanine as unexpected oxidation product of tryptophan by singlet molecular oxygen

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### Supporting Information

#### 1. Experimental section

**Materials.** L-Tryptophan (W), thymine, deuterium oxide (D<sub>2</sub>O), H<sub>2</sub><sup>18</sup>O (96.9 %), deuterated methanol (CD<sub>3</sub>OD), formic acid and Rose Bengal (RB) were supplied by Sigma-Aldrich (St. Louis, MO). The <sup>18</sup>O<sub>2</sub> gas cylinder came from Isotec-Sigma (St. Louis, MO). The endoperoxide of 1,4-dimethylnaphthalene (DMNO<sub>2</sub>) was synthesized and used according to a method described previously<sup>1</sup>.

**Reaction of W with <sup>16</sup>(<sup>1</sup>O<sub>2</sub>) or <sup>18</sup>(<sup>1</sup>O<sub>2</sub>) generated by photosensitization.** L-Tryptophan (20 mM) was dissolved in D<sub>2</sub>O containing 10 μM RB and irradiated using light from a tungsten lamp (500 W) filtered through a 360 nm cutoff filter. Irradiation was applied for 3 h in an ice-bath under a continuous flux of oxygen. For the photooxidation in an <sup>18</sup>O<sub>2</sub>-saturated atmosphere, oxygen contained in the system was removed by successive freezing and thawing under vacuum. This procedure was repeated at least five times to ensure complete removal of <sup>16</sup>O<sub>2</sub>. Thereafter, the whole system was connected to an <sup>18</sup>O<sub>2</sub> gas cylinder under

0.5 atm. After the irradiation, Rose Bengal was removed by passage through an Acrodisc Syringe Filter (Pall Gelman, Ann Arbor, MI) with a 0.2 $\mu$ m Supor membrane.

**Reaction of tryptophan with  $^{16}(^1\text{O}_2)$  generated by thermolysis of DMNO<sub>2</sub>.**

Tryptophan (5 mM) was allowed to react with DMNO<sub>2</sub> (20 mM) for up to 4 h at 37 °C, under agitation and in the dark. For mechanistic investigations, D<sub>2</sub>O:CD<sub>3</sub>OD (70:30) or a mixture of H<sub>2</sub><sup>18</sup>O: CD<sub>3</sub>OD (60:40) were used as solvent in the reactions. For pH-dependent studies, acetate (pH 4), and Trizma base (pH 7.4) and (pH 9) were prepared in D<sub>2</sub>O and used as buffers.

**HPLC/Mass spectrometry (MS) analysis.** Products separation was performed using an Agilent HPLC system (1200 series, Agilent Waldbronn, Germany). The column oven and auto sampler temperatures were set respectively at 25 and 10 °C. Mass spectrometry analyses were carried out in a Quattro II mass spectrometer (Micromass, Manchester, UK) with a Z-spray source. The separation of photoproducts was achieved using a 250 x 4.60 mm (particle size 5  $\mu$ m) C18 reverse-phase column (Phenomenex®). The mobile phase consisted of 4% acetonitrile in 0.05% formic acid at a flow rate of 1.0 mL/min. A small fraction of the eluent was directed into the mass spectrometer at a flow rate of 100  $\mu$ L/min. Mass spectrometry analyses were done with the electrospray ionizer in the positive ion mode. The source and desolvation temperatures were kept at 100 °C and 150°C, respectively. The cone voltage ranged from 10 to 30 V. Full-scan data were acquired over a mass range of 100-500 *m/z*. Tandem mass spectrometry (MS/MS) experiments were performed using the collision energy ranging from 5 to 20 eV. The products were quantified using selected ion monitoring (SIM) or selected reaction monitoring (SRM), using thymine as internal standard. The following *m/z* were monitored by SIM: 237, 239, 241 (diOia, hydroperoxides and FMK), 221 (alcohols),

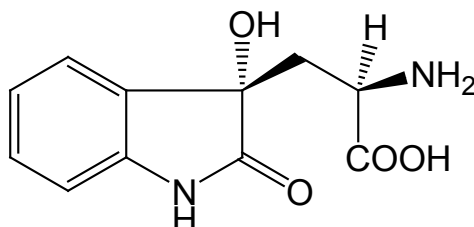
127 (thymine). For SRM experiments, the following transitions were monitored: 237>146 for diOia and hydroperoxides, 237>220, for FMK, 221>175 for alcohols.

**Accurate mass measurements.** Accurate mass measurements were performed on a micrOTOF electrospray ionization time-of-flight (ESI-TOF) mass spectrometer (Bruker Daltonics). Purified diOia were dissolved in a mixture of 0.01% formic acid:acetonitrile (1:1) and infused into the mass spectrometer at a flow rate of 180  $\mu\text{l/h}$ . The voltage of the capillary was 4 kV, the dry gas was kept at 5.0 l/min and the source temperature was maintained at 160°C. Calibration of the instrument was achieved using sodium formate. Mass spectra were acquired over a  $m/z$  range of 100-400.

**NMR spectroscopy.** NMR analyses ( $^1\text{H}$ ,  $^{13}\text{C}$ , HSQC) were performed on a Bruker Avance DRX 500 spectrometer (Bruker – Biospin, Rheinstetten, Germany), using  $\text{D}_2\text{O}$  as solvent. A small amount of *trans* and *cis* 3a-hydroxyhexahydropyrroloindoles can be visualized respectively in *R,S* diOia and *R,R* diOia samples.

## 2. Results

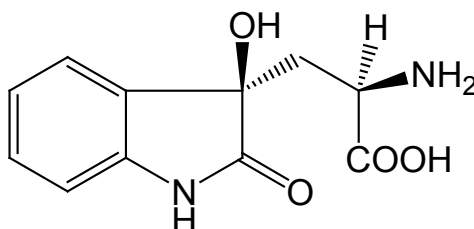
### *R,S* $\alpha$ -amino-2,3-dihydro-3-hydroxy-2-oxo-1H-indole-3-propanoic acid



*R,S* diOia

<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O)  $\delta$  7.55 (d,  $J$  = 7.51 Hz, 1H), 7.45 (t,  $J$  = 7.76 Hz, 7.76 Hz, 1H), 7.25 (t,  $J$  = 7.60 Hz, 7.60 Hz, 1H), 7.10 (d,  $J$  = 7.81 Hz, 1H), 4.26 (dd,  $J$  = 9.63 Hz, 3.66 Hz, 1H), 2.54 (dd,  $J$  = 15.45 Hz, 3.68 Hz, 1H), 2.32 (dd,  $J$  = 15.45 Hz, 9.65 Hz, 1H). <sup>13</sup>C NMR (126 MHz, D<sub>2</sub>O)  $\delta$  180.86, 173.68, 139.97, 130.50, 130.09, 123.98, 123.63, 111.38, 75.17, 51.00, 37.04. HRMS-ESI ( $m/z$ ): calculated for C<sub>11</sub>H<sub>13</sub>N<sub>2</sub>O<sub>4</sub> [M+H]<sup>+</sup>: 237.0876; observed: 237.0881.

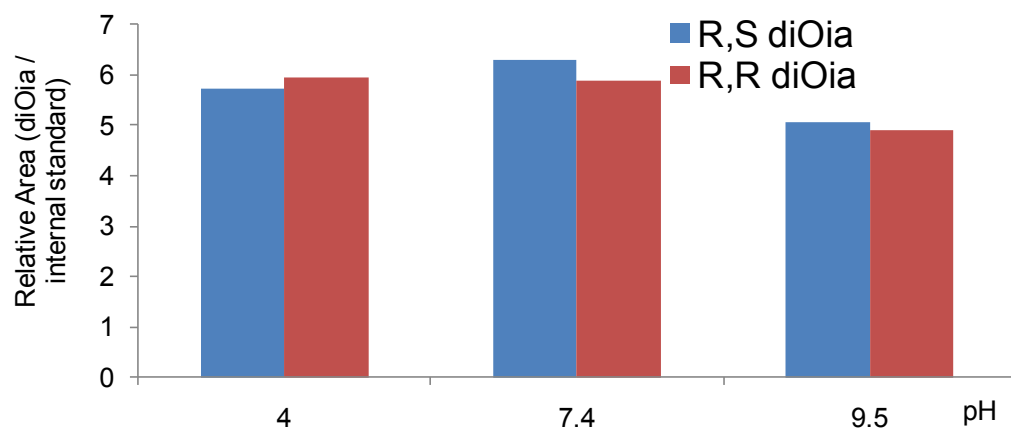
### *R,R* $\alpha$ -amino-2,3-dihydro-3-hydroxy-2-oxo-1H-indole-3-propanoic acid



*R,R* diOia

<sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O)  $\delta$  7.49 (d,  $J$  = 7.53 Hz, 1H), 7.42 (t,  $J$  = 7.77 Hz, 7.77 Hz, 1H), 7.22 (t,  $J$  = 7.58 Hz, 7.58 Hz, 1H), 7.08 (d,  $J$  = 7.83 Hz, 1H), 4.36 (dd,  $J$  = 9.99 Hz, 3.48 Hz, 1H), 2.51 (dd,  $J$  = 15.65 Hz, 9.99 Hz, 1H), 2.38 (dd,  $J$  = 15.63 Hz, 3.49 Hz, 1H). <sup>13</sup>C NMR (126 MHz, D<sub>2</sub>O)  $\delta$  180.33, 173.81, 139.62, 130.72, 130.30, 123.69, 123.54, 111.09, 75.02, 50.68, 37.17. HRMS-ESI ( $m/z$ ): calculated for C<sub>11</sub>H<sub>13</sub>N<sub>2</sub>O<sub>4</sub> [M+H]<sup>+</sup>: 237.0876; observed: 237.0883.

Yields of *R,S* and *R,R* diOia obtained by oxidation of W by  $^1\text{O}_2$  in three different pHs



**Fig.S1** Yields of *R,S* and *R,R* diOia obtained by oxidation of W by  $^1\text{O}_2$  in three different pHs (4, 7.4 and 9.5). DMNO<sub>2</sub> was used as  $^1\text{O}_2$  source and diOia were quantified by SIM MS, relating their areas with that obtained for the internal standard.

## References

1. P. Di Mascio, E. J. H. Bechara and J. C. Rubim, Dioxygen NIR FT-Emission ( $1\Delta_g\ 3\Sigma^-g$ ) and Raman Spectra of 1, 4-Dimethylnaphthalene Endoperoxide: A Source of Singlet Molecular Oxygen, *Appl. Spectrosc.*, 1992, **46**, 236-239.