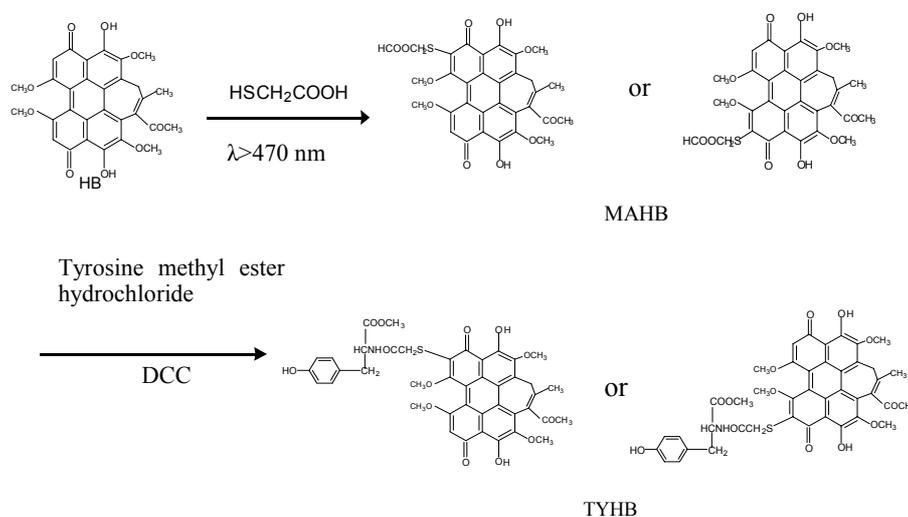


ESI

Scheme 1s. Synthesis of TYHB



MAHB 62mg (0.1mmol) was added to a stirring solution of tyrosine methyl ester hydrochloride 23mg (0.1mmol), Na_2CO_3 10.6mg (0.1mmol) and dicyclohexylcarbodiimide (DCC) 20.6mg (0.1mmol) in 20mL of CH_2Cl_2 under a nitrogen atmosphere at room temperature. After 10h reaction, the mixture was filtered, the filtrate was washed with 10% HCl, saturated NaHCO_3 aqueous solution and pure H_2O successively. The organic layer was concentrated under reduced pressure, purified by TLC with mixed solvent of CH_3Cl_3 : CH_3OH (200:5 in volume ratio) as eluant to give TYHB (30% yield). ^1H NMR (400MHz, CDCl_3) δ 15.93(s,2H), 15.32(s,1H), 6.52(d, J =8.24Hz,2H), 6.45(d, J =8.24Hz,2H), 6.04 and ?(two singlet from H on 5-and 8-carbon respectively, 1H), 5.61(d, J =8.38Hz,1H), 5.00(t,1H), 3.75–4.23(m,14H), 2.86–2.94(m,2H), 2.38(s,3H), 1.93(s,3H).

Irradiation of argon-gassed DMSO solution of TYHB (50 μM) generated an ESR signal (Fig.1, spectrum a), which has the same position and line shape as that of HB^{-1} , can be quenched efficiently by O_2 , and its formation need both irradiation and TYHB, therefore can be ascribed to the semiquinone anion radical of TYHB ($\text{TYHB}^{\cdot-}$). MAHB.

When irradiation was carried out in an oxygen-saturated DMSO solution of TYHB with 2,2,6,6-tetraethyl-4-piperidone (TEMP) as spin-trapping agent, a characteristic ESR signal of TEMPO (adduct of TEMP with $^1\text{O}_2$) was detected (three lines with identical intensity and hyperfine coupling constant of 16.0G, Fig. 1, spectrum b)². The presence of O_2 , TYHB and light are all essential for the ESR signal observation. The signal can be quenched by $^1\text{O}_2$ scavengers such as 1,4-diazabicyclo[2,2,2]octane efficiently.

When the oxygen was bubbled through the TYHB solution in DMSO in the presence of 5,5-dimethyl-1-pyrroline N-oxide (DMPO) and the TYHB solution was irradiated, the ESR signal of DMPO-superoxide anion radical adduct $\text{DMPO-O}_2^{\cdot-}$ was observed³. This signal can be characterized by three hyperfine coupling constants: $\alpha^{\text{N}} = 13.0\text{G}$, $\alpha_{\beta}^{\text{H}} = 10.1\text{G}$, and $\alpha_{\gamma}^{\text{H}} = 1.5\text{G}$ (Fig. 1, spectrum c). An efficient quenching by superoxide dismutase, a scavenger of $\text{O}_2^{\cdot-}$, supports the assignment of this signal.

If water is present in air-saturated DMSO solution of TYHB and DMPO, the ESR signal of DMPO-hydroxyl radical adduct (DMPO-OH^{\cdot}) can be readily resolved⁴, which exhibits a four-line signal with intensity ratio of 1:2:2:1 and has a hyperfine constant of 14.9 G (Fig.1, spectrum d). In aqueous solution $\text{O}_2^{\cdot-}$ undergoes rapid dismutation to form H_2O_2 which transforms to OH^{\cdot} further.⁵

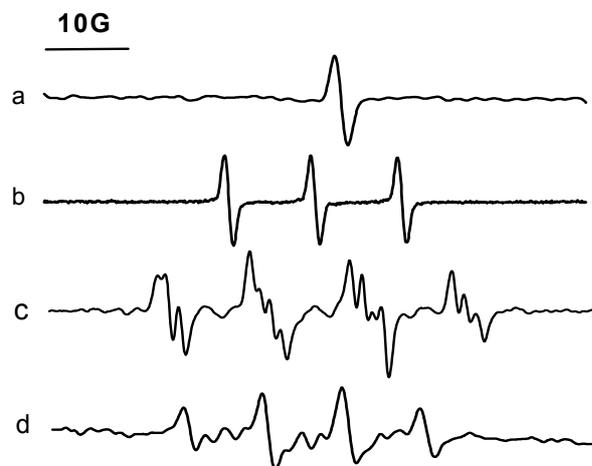


Fig. 1s Spectrum a: photoinduced ESR signal in nitrogen-saturated DMSO solution of TYHB (50 μ M), illumination was with 532nm pulsed laser for 2min. Spectrum b: similar to spectrum a, but in oxygen saturated solution and in the presence of TEMP (20mM), and irradiated for 40s. Spectrum c: similar to spectrum a, but in air-saturated solution and in the presence of DMPO (450mM), and irradiated for 1min. Spectrum d: similar to spectrum c but in the presence of a small amount of water. Spectral parameter settings: microwave bridge: X-band; sweep width: 100G; modulation amplitude: 1.0 G; receiver gain: 1×10^5 ; microwave power: 5mW.