Electronic Supplementary Material (ESI) for Analyst

Electronic supplementary information

A long-lived luminescence and EPR bimodal lanthanide-based probe for free radicals

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Detailed experimental section

Calculation of the concentration of 1

The concentration of cs124-DTPA-TEMPO was determined by complexometric titration, given the 1:1 complexation of DTPA derivative to Tb^{3+}, which was confirmed by the work of Selvin *et al* (ref 45 in the text). Specifically, the nitroxide of cs124-DTPA-TEMPO was completely reduced to hydroxylamine, changing to a diamagnetic compound, by an excessive amount of ascorbate in aqueous solution at first, and then the solution was gradually titrated with a known concentration of $TbCl_3$. The Tb luminescence intensity was measured as a function of the quantity of titrant, and the concentration was calculated when the Tb luminescence reached its plateau. The relation between luminescence intensity after reduction and concentration of **1** was also established.

The concentration of purified **1** was calculated by measuring its Tb luminescence intensity after reduction by ascorbate.

Reduction of 1 by ascorbate

To 2 mL of buffer solution of 10 μM **1** in a 10 mm fluorescence cuvette, 20 μL of 10 mM ascorbate was added and the solution was shaken up quickly. The fluorescence intensity was recorded as a function of reaction time. A capillary was employed to quickly withdraw a sample after mixing for the parallel measurement of spin loss by EPR. The signals at time = 0 were recorded before ascorbate was added.

Response of 1 to carbon-centered radicals

200 μL of 10 mM AIBN (azobisisobutyronitrile) in methanol was added into 1.8 mL of the buffer solution of **1**. AIBN was purified with methanol recrystallization before use. The final solution contained 10% methanol, and no AIBN precipitation was observed in the experiment. The solution was deaerated with nitrogen prior to irradiation. A mercury arc Lamphouse HBO 50W/AC (LINOS, Germany) was used as light source, equipped with a long-pass filter UV-35 to block irradiation wavelengths shorter than 350 nm.

Response of 1 to hydroxyl radicals

2 μM of **1** in 2 ml buffer solution containing 0.1 M KNO_3 and 1 M DMSO was irradiated by the mercury arc Lamphouse HBO 50W/AC with a filter UV-30 to block irradiation wavelengths shorter than 300 nm. The solution was deaerated with nitrogen prior to irradiation.

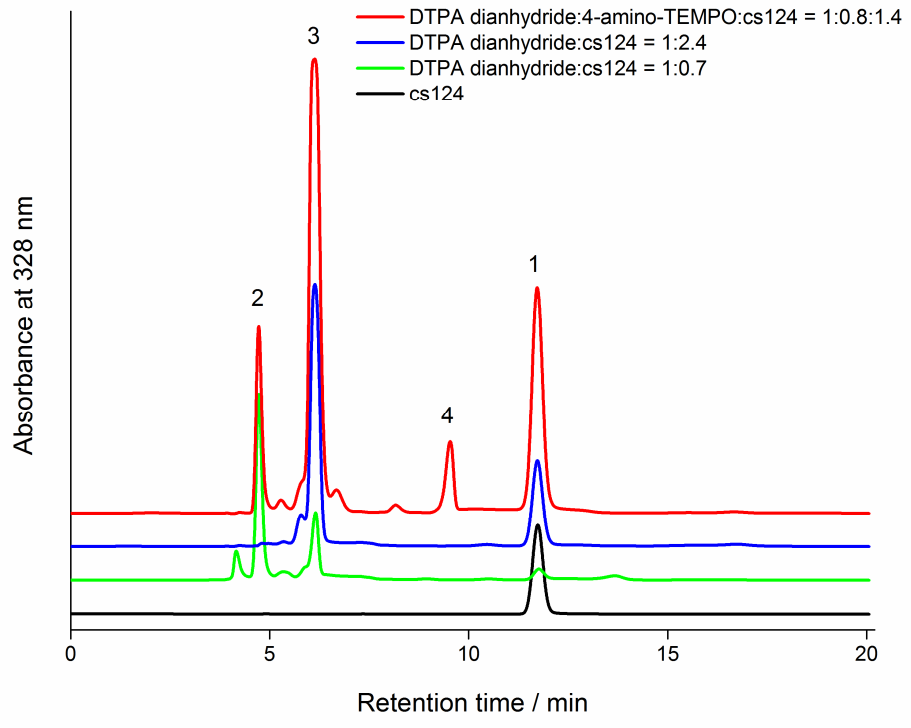
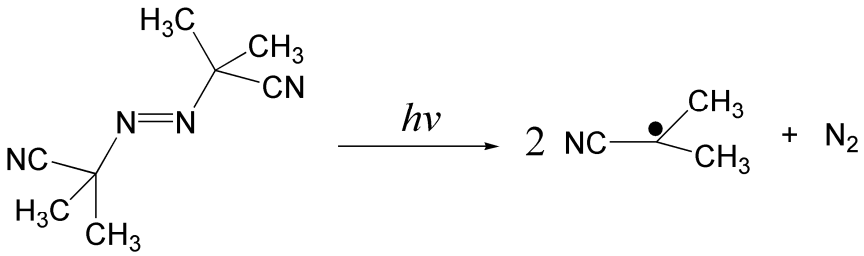


Fig. S1 HPLC chromatograms of reactant cs124 and synthetic systems with different molars ratios of DTPA dianhydride to 4-amino-TEMPO to cs124. There are only four fractions that can be detected by the UV detector at 328 nm: cs124, cs124-DTPA-TEMPO, and byproducts DTPA-cs124 and DTPA-$(cs124)_2$. As control, DTPA-cs124 and DTPA-$(cs124)_2$ were also synthesized by controlling the molar ratios of DTPA dianhydride to cs124. The peaks of 1~3 were identified to be cs124, DTPA-cs124 and DTPA-$(cs124)_2$, respectively. The rest fourth peak was attributed to cs124-DTPA-TEMPO. The product was further confirmed by other methods in the text.



AIBN

Scheme S1 The production of carbon-centered radicals from the photolysis of AIBN.

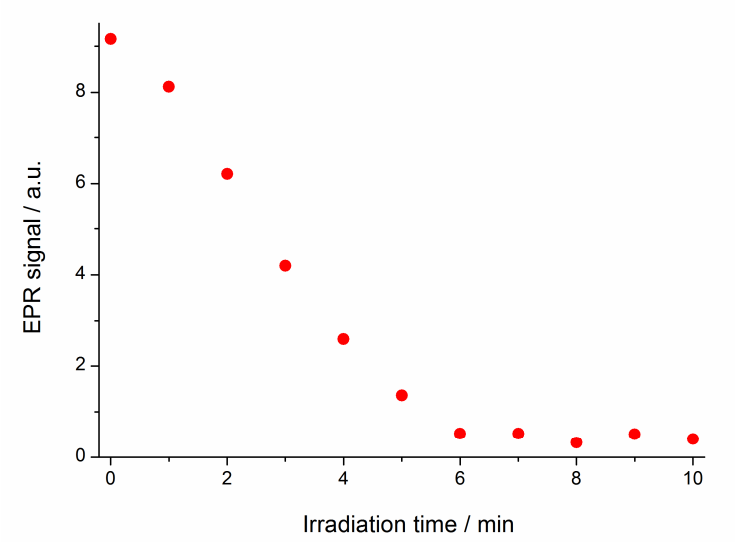


Fig. S2 Carbon-centered radical-trapping induced EPR decrease (g = 2.006) of **1**. The concentration of **1** was 10 μM, and for AIBN 1 mM, irradiated with UV light (λ > 350 nm). The solution was 10 mM Tris-HCl buffer at pH 7.4, containing 10 % methanol.

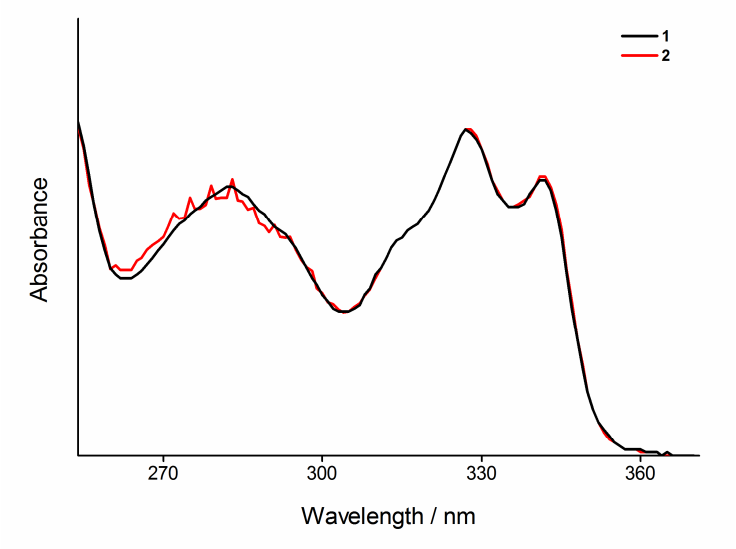


Fig. S3 Absorption spectra of **1** and **2**. The absorbance of **2** was corrected by subtracting absorbance of ascorbate of the same concentration.