

Supplementary Material (ESI) for Chemical Communications

This journal is (c) The Royal Society of Chemistry 2009

Title: A novel H₂O₂-triggered anti-Fenton fluorescent pro-chelator excitable with visible light

Authors: Yibin Wei and Maolin Guo

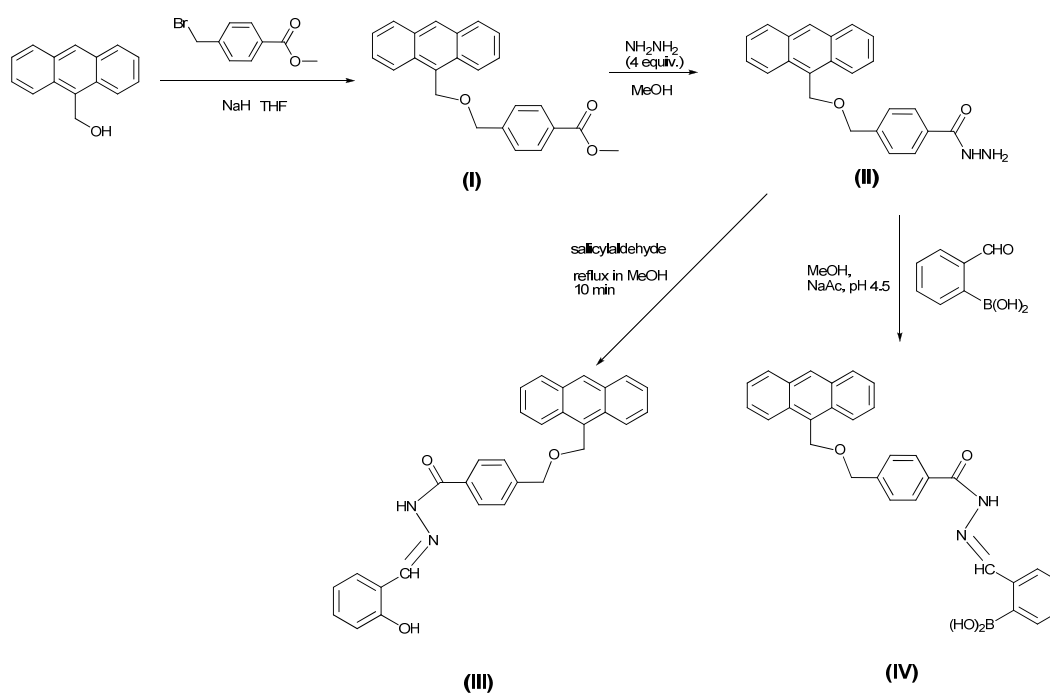
*Department of Chemistry and Biochemistry, University of Massachusetts,
Dartmouth, MA 02747 (USA)*

Supporting information

- 1. Synthesis**
- 2. Solution chemistry and spectroscopy**
- 3. Figs S1-S7.**

1. Synthesis

The fluorophore-tagged chelator SBH-AN and the prochelator SBH-B-AN were synthesized by a 3-step procedure showing in the Scheme below.^{a,b}



1.1 methyl 4-((anthracen-9-ylmethoxy)methyl)benzoate (I).

At 0°C, 5 mmol, 1.041 g 9-hydroxymethylanthracene was added in portions into a solution of 8 mmol, 0.192 g NaH (50% oil dispersion pre-washed with hexane) in 15 mL THF. The mixture was stirred for 30 min, and then to which 5 mmol, 1.145 g 4-bromomethylbenzoic acid methyl ester in 10 mL THF was dropwisely added in 30 min. The mixture was stirred overnight at room temperature. The excess of NaH was quenched by adding a few drops of water, and then the solvent was removed *in vacuo*. The residue was purified by Silica column chromatography eluting with a solvent system of Hex/AtOAc (15:1, 150 mL; 10:1, 200 mL and 5:1, 200 mL) to afford pure yellow

Supplementary Material (ESI) for Chemical Communications

This journal is (c) The Royal Society of Chemistry 2009

product **I** (1.39 g, yield: 78%). $^1\text{H NMR}$ (300 MHz; CDCl_3 ; Me_4Si) δ_{H} 3.90(3H, s), 4.49(2H, s), 5.52(2H, s), 7.41-7.54(7H, m), 8.03(2H, d, $J=8.18$), 8.32(2H, d, $J=9.00$) and 8.46(1H, s). $^{13}\text{C NMR}$ (300MHz, CDCl_3 , Me_4Si) δ_{C} 165.3, 142.1, 131.4, 131.1, 129.7, 129.1, 128.6, 128.3, 127.5, 126.3, 124.9, 124.2, 71.6, 64.5 and 52.1. MS(FAB+) m/z 356.2 (M^+ , 75%) (calcd. for $\text{C}_{24}\text{H}_{20}\text{O}_3$ 356.1).

1.2 4-((anthracen-9-ylmethoxy)methyl)benzohydrazide (**II**)

To a 30 ml methanol solution of **I** (0.356 g, 1 mmol) was added 4-fold excess of hydrazine hydrate (0.245 mL, 10 mmol). The mixture was refluxed until ester disappeared, which was monitored by TLC. After cooling, a bright yellow precipitate formed and was washed by hot water, THF and diethyl ether. The product (0.268 g, yield: 75%) was not further purified and directly used in the next step of synthesis.

$^1\text{H NMR}$ (300MHz; CDCl_3 ; Me_4Si) δ_{H} 9.73(1H, s), 8.64(1H, s), 8.40(2H, d, $J=8.76$), 8.10(2H, d, $J=8.10$), 7.81(2H, s, $J=8.23$), 7.61-7.51(4H, m), 7.43(2H, D, $J=8.24$), 5.52(2H, s), 4.78(2H, s) and 4.46(2H, s). $^{13}\text{C NMR}$ (300MHz, $(\text{CD}_3)_2\text{SO}$, Me_4Si) δ_{C} 165.5, 141.5, 132.4, 130.9, 130.4, 128.8, 127.2, 126.9, 126.2, 125.1, 124.3, 71.2 and 63.9 MS(FAB+) m/z 356.2 (M^+ , 45%), 357.2($\text{M}+\text{H}^+$, 50) (calcd. for $\text{C}_{22}\text{H}_{20}\text{N}_2\text{O}_2$ 356.1).

1.3 4-((anthracen-9-ylmethoxy)methyl)- N' -(2-hydroxybenzylidene)benzohydrazide (**III**, **SBH-AN**)

A mixture of **II** (100 mg, 0.28 mmol) and salicylaldehyde (42.07mg, 0.28 mmol) in 20 mL mixture solvent of methanol and sodium acetate buffer (pH 5.0) was refluxed for 10 min. After the mixture solution was cooled to room temperature, the crude product was precipitated, collected, recrystallized from methanol and dried vacuum to afford off-white pure product (0.11g, 85%). $^1\text{H NMR}$ (300MHz; $(\text{CD}_3)_2\text{SO}$; Me_4Si) δ_{H} 12.08(1H, s), 11.30(1H, s), 8.65(1H, s), 8.64(1H, s), 8.43(2H, d, $J=8.78$), 8.13(2H, d, $J=7.67$), 7.95(2H, d, $J=8.11$), 7.62-7.50(7H, m), 7.30(1H, t, $J=7.07$), 6.95-6.90(2H, m), 5.56(2H, s) and 4.84(2H, s). $^{13}\text{C NMR}$ (300MHz, $(\text{CD}_3)_2\text{SO}$, Me_4Si) δ_{C} 162.2, 157.4, 142.6, 132.3, 131.8, 131.3, 130.9, 130.4, 129.5, 128.8, 127.4, 126.2, 125.1, 124.3, 119.2, 116.3, 71.1 and 63.9. MS(FAB+) m/z 460.2 (M^+ , 22%) (calcd. for $\text{C}_{30}\text{H}_{24}\text{N}_2\text{O}_3$ 460.5). UV/Vis, λ_{max} (DMF)/nm 288, 300, 332, 366 and 387($\epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$, 22800, 24680, 22726, 11880 and 12694).

1.4 2-((2-(4-((anthracen-9-ylmethoxy)methyl)benzoyl)hydrazono)methyl)phenylboronic acid (IV, SBH-B-AN)

A mixture of **II** (50 mg, 0.14 mmol) and 2-formylphenylboronic acid (50 mg, 0.14 mmol) in 15 mL mixture solvent of methanol and sodium acetate buffer (pH 5.0) was refluxed for 10 min. After the mixture solution was cooled to room temperature, the crude product was precipitated, collected, recrystallized from methanol and dried under vacuum to afford off-white pure product (53 mg, yield 77%). ¹H NMR(300MHz; (CD₃)₂SO; Me₄Si) δ_H 11.95(1H, s), 8.76(1H, s), 8.66(1H, s), 8.50(2H, s), 8.42(1H, s), 8.39(1H, s), 8.13(2H, d, *J*=6.23), 7.94(2H, d, *J*=7.84), 7.64-7.48(8H, m), 7.44-7.37(2H, m). ¹³C NMR (300MHz, CDCl₃, Me₄Si) δ_C 162.1, 142.2, 141.5, 137.2130.9, 130.4, 128.7, 127.2, 126.2, 125.1, 124.3, 71.2, 63.9. UV/Vis, λ_{max}(DMF)/nm 305, 348, 366 and 387(ε/dm³ mol⁻¹ cm⁻¹, 24296, 8742, 11306 and 9853).

Refs:

- (a) M. A. Dam, A. Weitz, R. Helgeson, B. Ma and F. Wudl, *J Org Chem*, 2000, **65**, 3941-3946.
(b) V. A. Osyanin, P. P. Purygin and Z. P. Belousova, *Russ J Gen Chem*, 2005, **75**, 111-117.

2. Solution chemistry and spectroscopy

Solutions of FeCl₃ was prepared in methanol and FeCl₂ in diluted HCl, and used freshly. For titration experiments, metal ion solution was added to SBH-B-AN or SBH-AN (stock solutions in DMF) and the mixtures were equilibrated at 298 K for 10 min. All titrations were performed in KBP/DMF mixture solvent unless otherwise noted.

UV/Vis spectra were recorded on a Perkin-Elmer Lambda 25 spectrometer at 298 K. UV difference spectra after addition of H₂O₂ to SBH-B-AN were recorded immediately and at different time intervals. ¹H and ¹³C NMR spectra were recorded on a Bruker AC 300 spectrometer. Fluorescence spectra were recorded on a Perkin-Elmer LS55 luminescence spectrometer at 293 K. The excitation wavelengths and filters used

Supplementary Material (ESI) for Chemical Communications

This journal is (c) The Royal Society of Chemistry 2009

were indicated in the figures. MS spectrometry was carried out at University of Massachusetts Amherst Mass spectrometry facility.

The 2-deoxyribose degradation assays were performed similarly as reported previously.^[2] Typical reactions were started by adding FeCl₂ salt (0.02 mM final concentration) to a solution (0.5 mL final volume) containing 20 mM potassium phosphate buffer (pH 7.20), 0.04 mM chelator (from 1.00 mM stock solution in DMF), 10 mM 2-deoxyribose and 5 mM ascorbate. The Fenton reaction was initialized by adding varied concentration of H₂O₂ solution. Reactions were carried out for 10 min at room temperature (298 K) and were stopped by the addition of 0.5 mL 10% trichloroacetic acid (TCA) followed by 0.5 mL 1% 2-thiobarbituric acid (TBA, w/v, in 40 mM NaOH). After heated at 353 K for 15 min, the absorbance was measured at 532 nm. No chelator was added in the control reactions. In the reactions with pro-chelator, SBH-B-AN and H₂O₂ were firstly incubated at room temperature for 45 min.

3. Figs S1-S7

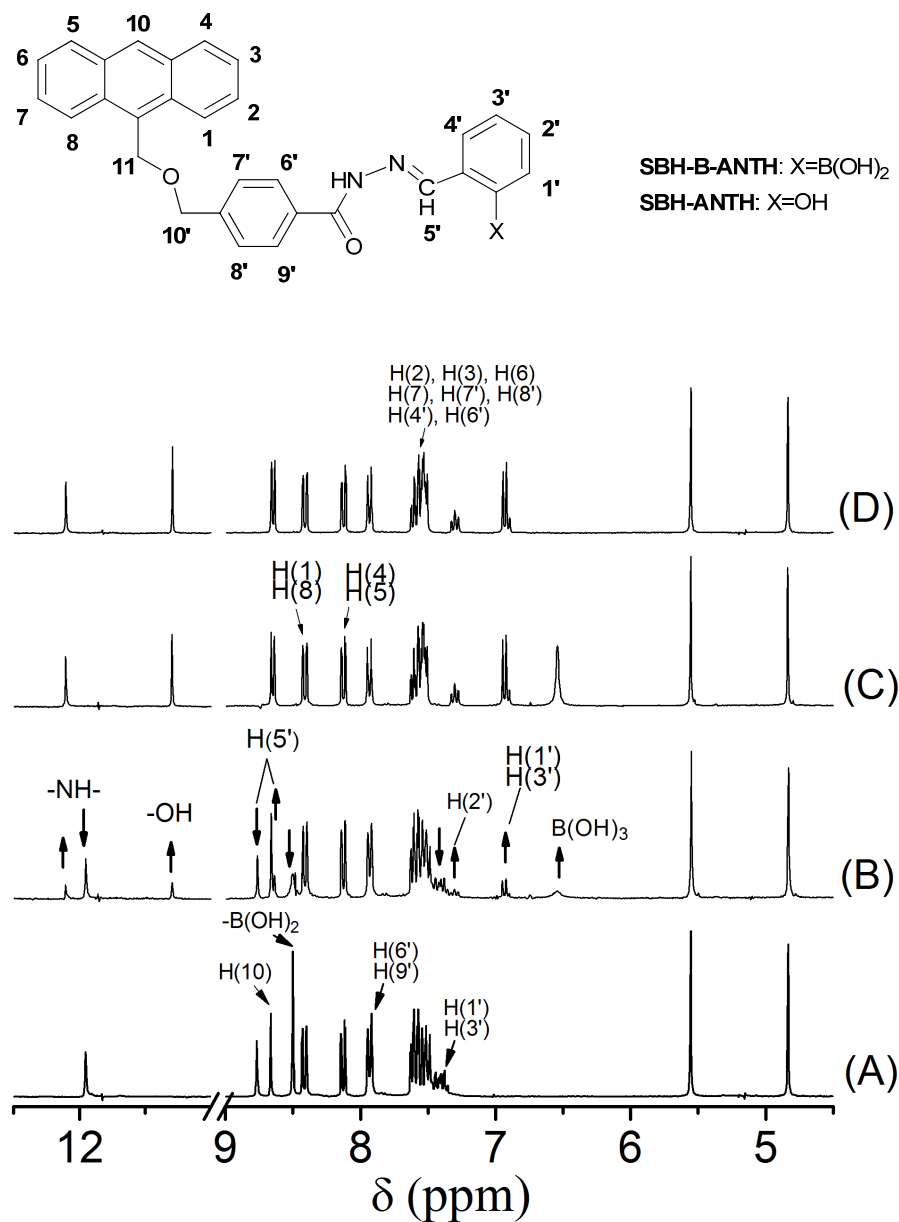


Fig. S1 ^1H NMR spectra (in 6d -DMSO) of (A) SBH-B-AN (5 mM), the reaction of SBH-B-AN (5 mM) with H₂O₂ (50 mM) after (B) 1 h and (C) 4 h at 293 K, and (D) SBH-AN (5 mM). The bulky H₂O₂ peaks at 10.22 ppm were omitted for clarity.

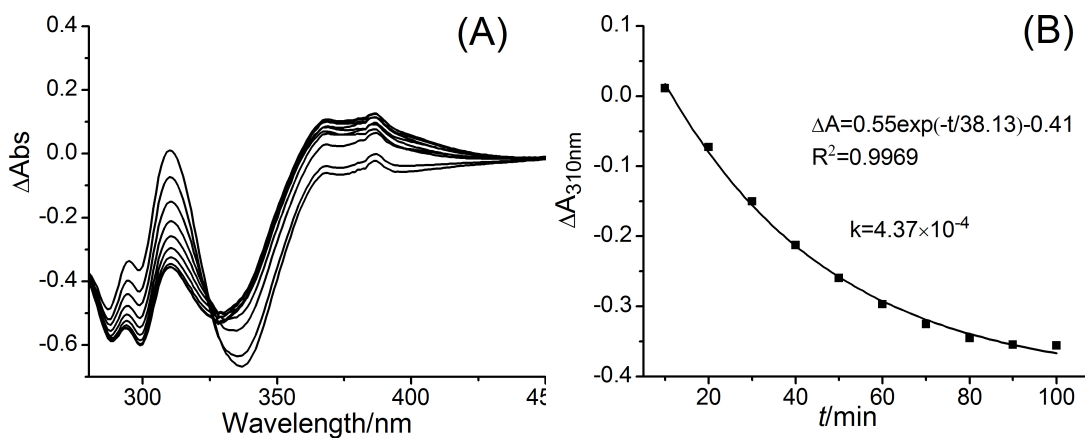


Fig. S2 (A) UV/Vis spectra of 50 μM SBH-ANTH (a) and 50 μM SBH-B-ANTH(b) solution in DMF/KPB (1:1), pH 7.30. (B) The UV/Vis difference spectra of the time courses of a 50 μM SBH-B-Fluoro solution after 0.5 mM H_2O_2 was added. The H_2O_2 -triggered reactions were performed in DMF/KPB (1:1), pH 7.30 at 298 K with stirring and measured with 10 min intervals.

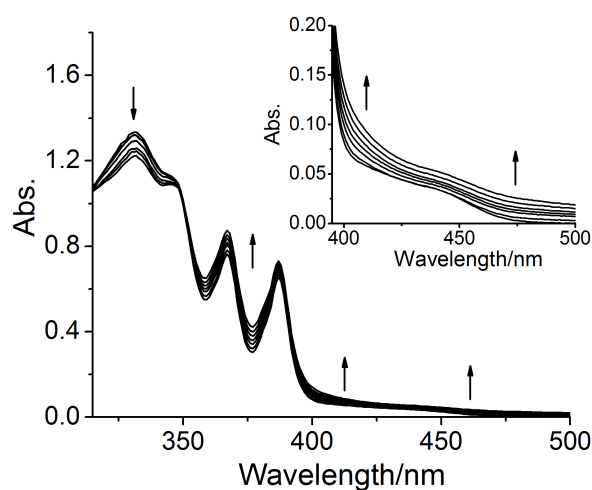


Fig. S3 UV/Vis spectra of 50 μM SBH-AN titrated by Fe^{III} in potassium phosphate buffer, pH 7.30. Fe^{III} was added with 5- μM increment in the range of 5 μM to 50 μM . The solutions were incubated with stirring at 298 K for 15 min to accomplish binding, and then the spectra were measured.

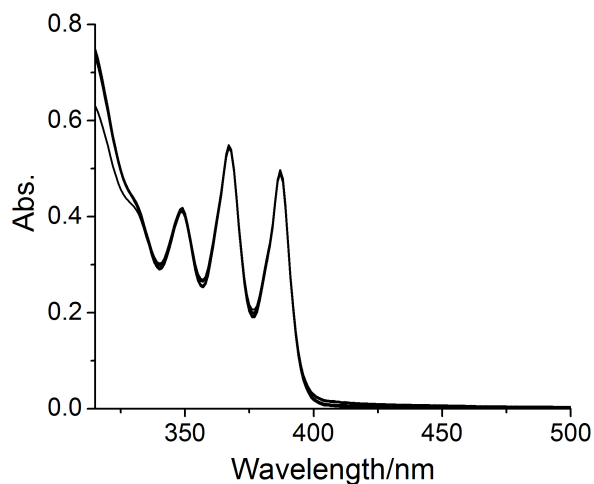


Fig. S4 UV/Vis spectra for SBH-B-AN (50 μM) titrated by Fe^{III} in the absence of H_2O_2 . All titrations were conducted in DMF/KPB (1:1), pH 7.30, 298 K.

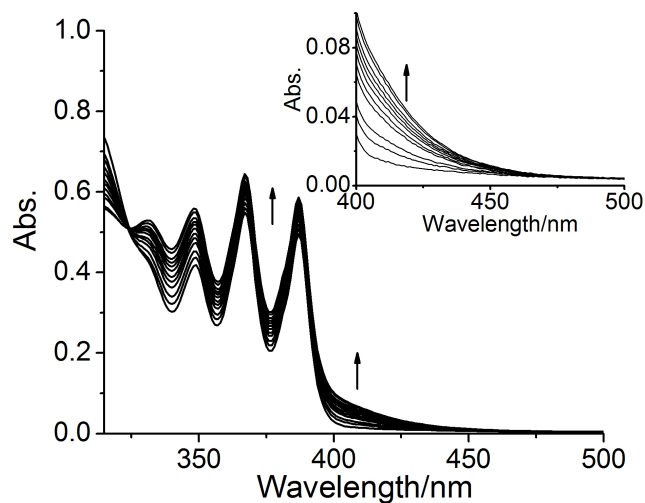


Fig. S5 UV/Vis spectra of the time course after the addition of 0.5 mM H_2O_2 into a mixture of SBH-B-AN (50 μM) and FeCl_3 (25 μM). The H_2O_2 -triggered reactions were incubated in DMF/KPB (1:1), pH 7.30 at 298 K with stirring and measured with 10 min intervals.

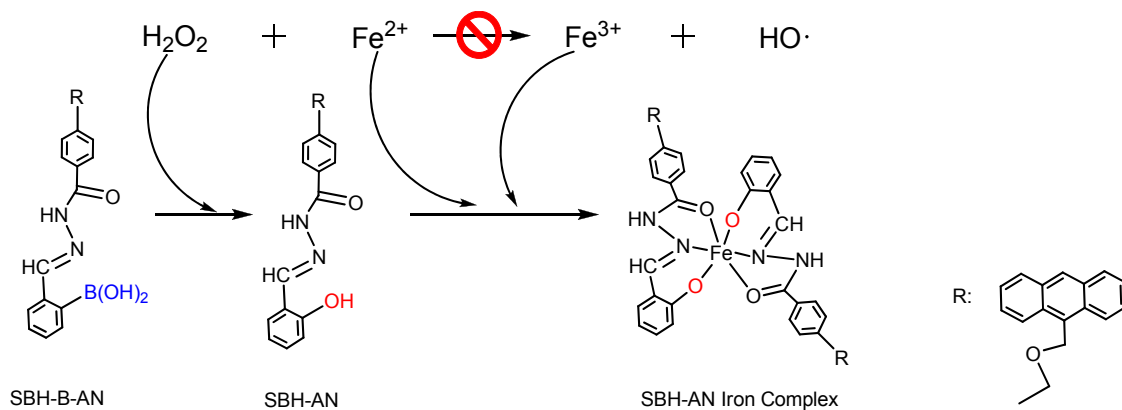


Fig. S6 The proposed mechanism. The prochelator SBH-B-AN reacts with H_2O_2 to yield the active chelator SBH-AN, which sequesters iron. This process attenuates Fe-promoted Fenton reactions under physiological relevant conditions.

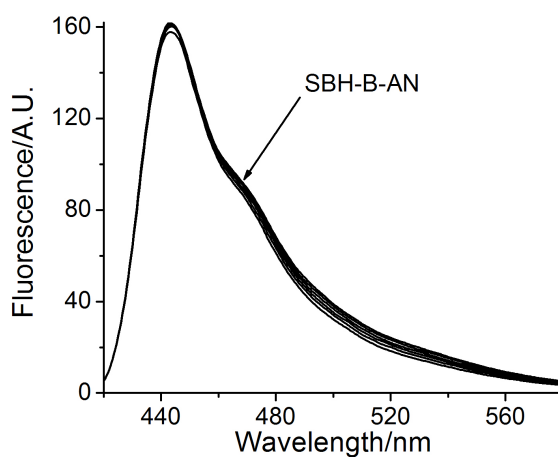


Fig. S7 Fluorescence ($\lambda_{\text{ex}} = 400 \text{ nm}$) titration of $10 \mu\text{M}$ SBH-B-AN with Fe^{III} in $1 \mu\text{M}$ increments in DMF/KPB (1:1), pH 7.30, at 298 K.

Supplementary Material (ESI) for Chemical Communications

This journal is (c) The Royal Society of Chemistry 2009