**SUPPORTING INFORMATION**

# Physicochemical-Property Guided Design of a Highly-Sensitive Probe to Image Nitrosative Stress in the Pathology of Stroke

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## Table of Contents

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**Chemistry methods**

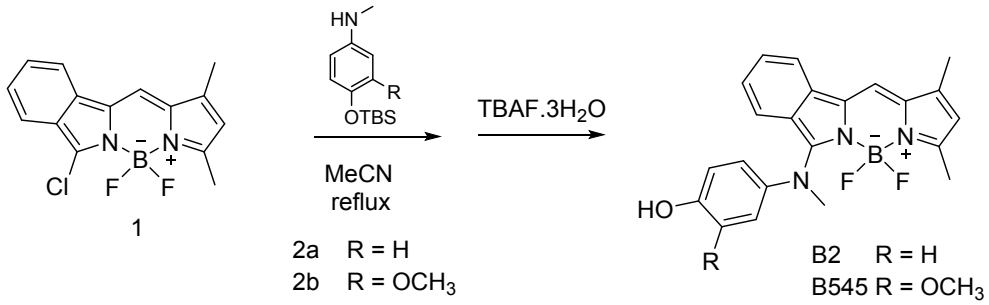
**General Experimental for Chemistry**

All reagents were purchased from commercial suppliers and used without further purification unless otherwise indicated. Acetonitrile (MeCN) was purified by distillation under vacuum. All reactions were carried out under nitrogen atmosphere. Reactions were monitored by thin-layer chromatography (TLC) carried out on Silica gel 60 F254 plates supplied by Qingdao Puke Separation Material Corporation, and UV light was used as the visualizing agent. Flash column chromatography was performed using 200-300 mesh silica gel supplied by Qingdao Marine Chemical Factory, Qingdao, China. A mixture of petroleum ether (PE, b.p. 60-90 $^{o}$C) and ethyl acetate (EtOAc) was used as the eluting solvent for both TLC analysis and column chromatography isolation. $^{1}$H NMR spectra were obtained on a Bruker Fourier transform spectrometer (500 MHz) at 25 °C. $^{13}$C NMR spectra were recorded on a Bruker Fourier transform spectrometer (125 MHz) spectrometer. All NMR spectra were calibrated using residual solvents as internal references (for $CDCl_3$: $^{1}$H NMR = 7.26, $^{13}$C NMR = 77.16; for DMSO: $^{1}$H NMR = 2.50, $^{13}$C NMR = 39.52). All chemical shifts were reported in parts per million (ppm) and coupling

constants (*J*) in Hz. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. IR spectra were taken on a Bruker Vector 22 spectrophotometer as KBr pellets. High resolution mass spectra (HRMS) were measured on an Agilent 6224 TOF LC/MS spectrometer using ESI-TOF (electrospray ionization-time of flight).

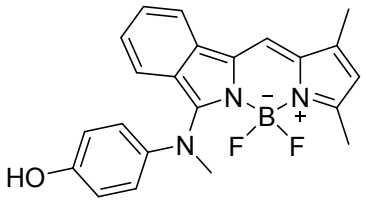
**Probe Synthesis and Structure Characterization**

**Scheme S1. Synthetic procedure of probe B545a and B545b**




**General procedure for probe synthesis**

To a stirred solution of compound **1** in dry MeCN was added the N-methyl aniline. The mixture was heated to reflux. After completion of the reaction as shown by TLC analysis which took about 2 hours, the reaction was cooled down to ambient temperature and concentrated by rotary evaporation under reduced pressure to yield the crude product. The crude product, without further purification, was directly subject to deprotection by dissolving it in THF and then treating it with $Bu_4N^+F^-•3H_2O$. After the reaction was stirred at ambient temperature for 30 minutes, the volatile organics were removed by rotary evaporation under reduced pressure and the resulting residue was purified by flash column chromatography ($SiO_2$: PE/EtOAc, 10:1) to give the probe as a purple black solid.




**Form:** purple black solid (52% yield)

**M.p.**: 230-231 °C

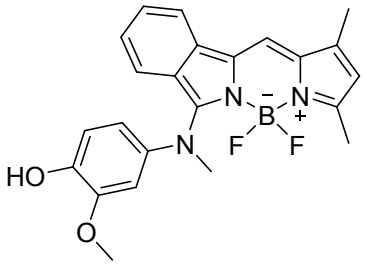
**$R_f$** = 0.40 (1:10, EtOAc:PE)

**IR ($cm^{-1}$):** 3471, 2954, 2921, 1623, 1544, 1418, 1399, 1253, 1138, 1096, 952

**$^1$H NMR (500 MHz, DMSO)** δ 9.99 (s, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 7.45 (m, 2H), 7.30 (d, *J* = 8.7 Hz, 2H), 7.03 (t, *J* = 7.7 Hz, 1H), 6.90 (d, *J* = 8.7 Hz, 2H), 6.02 (d, *J* = 8.3 Hz, 1H), 5.87 (s, 1H), 3.93 (s, 3H), 2.34 (s, 3H), 2.20 (s, 3H).

**$^{13}$C NMR (126 MHz, DMSO)** δ 157.40, 139.43, 137.29, 136.91, 130.70, 128.62, 127.68, 127.38, 126.64, 125.93, 124.93, 119.59, 116.66, 113.60, 107.50, 56.02, 54.91, 45.19, 18.56, 13.83, 10.70.

**ESI-HRMS (m/z):** $[M+H]^+$ calc'd. for $C_{22}H_{20}BF_2N_3O$ 391.1667, found 391.1670.



**Form:** purple black solid (72% yield)

**M.p.:** 180-181 °C

**$R_f$ =** 0.40 (1:8, EtOAc:PE)

**IR ($cm^{-1}$):** 3529, 2969, 2918, 1633, 1581, 1551, 1509, 1465, 1265, 1155,754

**$^1$H NMR (500 MHz, $CDCl_3$)**: δ 7.69 (d, *J* = 7.9 Hz, 1H), 7.35 (t, *J* = 7.5 Hz, 1H), 7.00 (d, *J* = 8.2 Hz, 2H), 6.95 – 6.88 (m, 2H), 6.80 (d, *J* = 2.3 Hz, 1H), 6.16 (d, *J* = 8.3 Hz, 1H), 5.93 (s, 1H), 5.76 (s, 1H), 4.05 (s, 3H), 3.82 (s, 3H), 2.50 (s, 3H), 2.25 (s, 3H).

**$^{13}$C NMR (126 MHz, $CDCl_3$):** δ 158.02, 147.52, 145.57, 139.15, 130.34, 127.33, 126.98, 125.69, 125.31, 119.46, 118.85, 115.43, 114.29, 109.61, 107.90, 56.46, 45.61, 14.14, 11.11.

**ESI-HRMS (m/z):** $[M+H]^+$ calc'd. for $C_{23}H_{22}BF_2N_3O_2$ 421.1773, found 421.1796

**Preparation of various ROS and RNS species[1]**

**$ONOO^-$**

To a vigorously stirred solution of $NaNO_2$ (0.6 M, 10 mL) and $H_2O_2$ (0.7 M, 10 mL) in deionized $H_2O$ at 0 °C was added HCl (0.6 M, 10 mL), immediately followed by the rapid addition of NaOH (1.5 M, 20 mL). Excess hydrogen peroxide was removed by passing the solution through a short column of $MnO_2$. The concentration of $ONOO^-$ was determined by UV analysis with the extinction coefficient at 302 nm (ε= 1670 $M^{-1}$ $cm^{-1}$). Aliquots of the solution were stored at -20 °C for use.

**NO**

A solution of the $H_2SO_4$ (3.6 M) was added dropwise into a stirred solution of $NaNO_2$ (7.3 M). The emitted gas was allowed to pass through a solution of NaOH (2 M) first and then deionized $H_2O$ to make a saturated NO solution of 2.0 mM.

**$^1O_2$**

$NaMoO_4$ (10 mM) and $H_2O_2$ (10 mM) was prepared in PBS (10 mM, pH 7.4). Equal aliquots of these solutions were then mixed to yield $^1O_2$ of 5 mM**.**

**$H_2O_2$ and NaClO**

$H_2O_2$ and NaClO solution were prepared by diluting commercial $H_2O_2$ and NaClO solutions with PBS (10 mM, pH 7.4) to make 10 mM stock solutions.

**·OH**

•OH was generated by Fenton reaction. To a solution of $H_2O_2$ (1.0 mM, 1.0 mL) in PBS (10 mM, pH 7.4) was added $FeSO_4$ solution (1.0 mM, 100 μL) at ambient temperature (stock solution 0.1 mM).

**$O^{2\bullet-}$**

$KO_2$ was dissolved in dry DMSO to make a saturated solution of **$O^{2\bullet-}$** (stock solution 1

mM).

**General experimental for photophysical property characterization**

All the photophysical characterization experiments were carried out at ambient temperature. Absorption spectra were acquired using a Hitachi U-3010 spectrophotometer. Fluorescence measurements were performed on an Agilent Cary Eclipse Fluorescence Spectrophotometer with slit widths to be 5 and 5 nm for excitement and emission respectively, and the sensitivity of the detector was kept at medium.

Deionized water was used to prepare all aqueous solutions. Phosphate buffer saline (PBS, 10 mM, pH 7.4) was purged with nitrogen for 5 minutes before use. The probes were dissolved in DMSO to make 5 mM stock solutions. $ONOO^-$ and other reactive bio-relevant species were prepared as described below.

To test the fluorescence responses of the probes towards $ONOO^-$ or other reactive species, aliquots of probe stock solutions were diluted with PBS and treated with analytes to make sure both probes and analytes were kept at desired final concentrations. After quick and vigorous shaking, the mixture was allowed standing in the dark for desired time and the fluorescence spectra were then recorded under excitation at 475 nm except otherwise indicated. The emission spectra were scanned from 475 to 650 nm. All fluorometric experiments were performed in triplicate, and data shown were the average.

**Quantum yields determination**

Absorption spectra were taken in PBS (10 mM, pH 7.4, 20% ethanol). Fluorescence quantum yields were obtained by comparison with fluorescein (Φ 0.95, in 0.1 M NaOH) with the following equation where Σ[F] is the integrated fluorescence intensity, Abs is absorbance at λex 496 nm, and n represents the refractive index. For PBS and 0.1 M NaOH, we used refractive indices of 1.334 and 1.335 respectively.

$$\phi_{sample} = \phi_{standard} \cdot \frac{Abs_{standard} \cdot \sum F_{sample}}{Abs_{sample} \cdot \sum F_{standard}} \cdot \frac{n_{sample}{}^2}{n_{standard}{}^2}$$

where Φ is the quantum yield, ΣF is the integrated fluorescence intensity, Abs is absorbance at $\lambda_{ex}$ 496 nm, and $n$ represents the refractive index of the solvent.

**Biology Materials and Methods**

**Reagents**

Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco (Carlsbad, CA). Uric acid was obtained from Wako (Osaka, Japan). 5, 10, 15, 20-Tetrakis-[4-sulfonatophenyl]-porphyrinato-iron [III] (FeTPPS; Calbiochem, La Jolla, CA, USA) was used as a specific peroxynitrite decomposition catalyst. Unless otherwise stated, all reagents and chemicals were obtained from Sigma–Aldrich (St. Louis, MO).

**OGD exposure and experimental treatments of cell cultures**

To mimic a nitrosative stress condition or an ischemia-like condition in vitro, cell cultures were exposed to 3-morpholinosydnonimine (SIN-1) or OGD treatment. Cultured

cells without treatment served as controls. EA.hy926 cells were used in the present study. Briefly, for the oxygen and glucose deprivation (OGD) experiment, the culture medium was replaced and washed with glucose-free Hank's balanced salt solution (HBSS). The airtight experimental hypoxia chamber ( Billups-Rothenberg, San Diego, CA) was flushed with 95% $N_2$ / 5% $CO_2$ for 5 minutes before experiments. After that the cultures were placed in hypoxia chamber at 37 °C for incubator for the appropriate duration.

**Confocal fluorescence staining and analysis**

Confocal microscopy analysis in fixed cells was performed as previously reported. Briefly, after culture on glass cover slips overnight, cells were treated with SIN-1 or OGD for indicated time, and then stained with **B545b** probe (0.5 μM) at 37°C for 30 min before being fixed in 4% PFA. Nuclei were stained with DAPI in PBS after fixation in PFA for 10 min. Cells were then imaged by confocal microscopy (Nikon A1R).

For time-lapse confocal imaging of live cells, endothelial cells were first cultured on glass-bottom plates overnight, and then incubated with **B545b** probe (0.5 μM) at 37 °C for 30 min. The dynamic change of **B545b** fluorescence in live cells in response to SIN-1 (1 mM) stimulation was then captured by time-lapse confocal microscope for 60 min. The fluorescence density was analyzed using Image J software (NIH, Bethesda, MD, USA).

**The Cell Counting Kit-8 assay**

The Cell Counting Kit-8 (CCK-8, Beyotime, Shanghai, China) was used to analyze the effect of **B545b** on cell viability in EA.hy926 endothelial cells according to the manufacturer's protocols. Briefly, equal numbers of cultured EA.hy926 endothelial cells were incubated with or without **B545b** (0.25, 2.5, 25μM) in 96-well plate for 24 h. Then cells were incubated with CCK-8 solution (10 μL per well) at 37 $^oC$ with 5% $CO_2$ for 3 h. Absorbance at 450 nm was measured with a microplate reader (ELx800, Bio-Tek, Winooski, VT, USA). The well with medium and CCK-8 solution but without cells was used as a blank control. Cell viability of vehicle-treated control group (without exposure to **B545b**) was defined as 100%. Data are expressed as mean ± S.E.M. ns $P>0.05$ versus control.

**Animals**

Wild-type C57BL/6J mice were used in TPLSM imaging. Mice (2-3 months old, 22-25 g) were group housed on a 12-h light/dark cycle at a constant temperature of 22 ± 1 °C with 40-60% humidity. All animal studies were approved by the Committees for Animal Experiments of Zhejiang University in China and conformed to NIH guidelines (Guide for the Care and Use of Laboratory Animals. NIH publication no. 85-23, revised 1996).

**Determination of plasma and brain B545b concentrations[1]**

**B545b** (5 mg/kg) was intravenously administrated to 6-week old C57 mice through tail (18 g average body weight, female). According to approved protocols, mice were euthanized to collect blood and whole brain hemispheres 0.5, 1.0, 2.0 h after probe administration. Blood was collected into a tube containing EDTA and was subjected to centrifugation for 10 min at 6000 rpm to yield the plasma. Aliquots of plasma were mixed

with acetonitrile (1:3, v/v), centrifuged at 14500 rpm for 10 min, and the resulting supernatants were used for subsequent LC-MS analysis. Brains were homogenized in normal saline (1:3, w/v). Brain homogenates were then mixed with acetonitrile (1:3, v/v), centrifuged at 14500 rpm for 10 min, and the resulting supernatants were used for subsequent LC-MS analysis.

Brain homogenate and plasma samples from untreated mice were prepared as above. **B545b** was added to aliquots of these samples at concentrations ranging from 20 to 1000 ng/mL. Samples were mixed with acetonitrile (1:3, v/v), centrifuged at 14500 rpm for 10 min and the supernatants were analyzed by LC-MS analysis to give the standard curves.

LC-MS analysis was conducted on a Shimadzu LCMS-2020 instrument with LCMS LabSolutions software. Samples (3 μL) were separated on a Shim-pack VP-ODS column (150L x 2.0, serial No. 4052581), eluted with $CH_3OH$:$H_2O$ (0.1% HCOOH) at a flow rate of 0.3 mL/min, first using a concentration gradient from 5:95 to 95:5 over 4 min and then being kept at 95:5 for 4 min, followed by a concentration gradient from 95:5 to 5:95 over 1 min and re-equilibration of 1 min. For MS analysis, the interface voltage was set at 4.5 kv and the detector voltage at 1.1 kv.

**Preparation of mice for TPLSM imaging**

Anesthetized adult male mice were imaged through a craniotomy window centered at stereotaxic coordinates 2.5 mm caudal to bregma and 2.5 mm lateral to the midline. The preparation of mice was similar to the surgical procedures as we previously described. Briefly, Mice were induced with 5% isoflurane for 3 minutes and then continuously anesthetized with 1.5% isoflurane. Rectal temperature was monitored throughout the surgery, and the body temperature was maintained at 37 °C ± 0.5 °C with a heating pad. After removing a flap of scalp to expose the skull surface, a bone flap was removed and replaced by a cover glass glued to skull. A 10 mm diameter metal frame was then glued and sealed with dental cement. The space between the exposed brain surface and the glass cover was filled with 1.5% (w/v) low-melting-point agarose in artificial cerebrospinal fluid. Mice were intravenously injected with 0.1 ml Dextran-Fluorescein (FITC-dextran, 70,000 MW, 10 mg/ml). The mice were then fixed to a custom-made device for imaging. Mice were injected B545b (1.5mg/ml in saline, 0.1 ml/10 g) via tail vein 1 h before imaging.

**Brain microvessel occlusion and parenchyma laser lesion**

For laser irradiation-induced microvessel occlusion, cortical brain vessels with 10-15 μm in diameter and 50-150 μm in depth below the cortical surface were selected. Laser irradiation at the two-photon wavelength of 800 nm (a Mai Tai Ti:sapphire laser light applied through a ×20 water-immersion microscope objective) was performed for the selected single microvessel region to induce vascular occlusion. Irradiation intensity ($W/cm^2$) was set depending on the vessel diameter and depth. The exact time must be determined empirically. For parenchyma laser lesion, appropriate intensity and time of laser was used to target the microvessels. The speed of blood flow was measured to ensure occlusion has occurred.

**TPLSM imaging and analysis**

The vessel imaging and analysis were performed as previously reported.[2] Briefly, For *in vivo* two-photon imaging, cortical brain vessels of 10-15 μm diameter and 50 μm below the cortical surface were selected for imaging. Single focal plane two-photon images and time-lapse images were obtained with an upright Olympus laser scanning confocal microscope (BX61W1-FV1000, Olympus, Tokyo, Japan) with an 800 nm excitation source of a Spectra-Physics MaiTai HP DeepSee femtosecond Ti:Sa laser. A long-working distance (2 mm) water-immersion objective (25×, NA = 1.05) was adopted for in vivo imaging. The images were taken with resolution of 1024 × 1024 pixels.

**Cerebral ischemia model**

The mice middle cerebral artery occlusion (MCAO) model was prepared as previously described.[3] Briefly, mice were initially anesthetized with 3% chloral hydrate. After lateral neck incision, the left common carotid artery was exposed and its external branch was ligated.

A surgical silicone suture (5-0, Doccol Corporation, USA) was inserted cranially and intraluminally 11 mm from the carotid bifurcation to occlude the middle cerebral artery (MCA). Mice were randomly assigned to four groups: MCAO 0.5 h, probe **B545b**, probe **B545b** + MCAO 0.5 h, probe **B545b** + MCAO 1 h. Mice were administered with **B545b** via tail vein (5 mg/kg) 20 min before MCAO.

**Cerebral imaging and analysis**

The anesthetized mice were transcardially perfused with PBS followed by 4 % paraformaldehyde (PFA) in PBS at indicated time after MCAO. The whole brains were immediately removed and post-fixed overnight at 4 °C. The whole brains were scanned by Maestro in vivo imaging system with a 480 nm excitation wavelength and a 560 nm filter.

**Immunofluorescence assay**

As for confocal imaging, the mice were anesthetized at the time of sacrifice and transcardially perfused with 4% paraformaldehyde in PBS after MCAO. The whole brains were immediately removed and post-fixed overnight at 4 °C. The whole brains were cut into 40-μm-thick serial sections using a vibratome. Then the brain sections were washed twice in PBS, permeabilized (in PBS with 0.1% Triton X-100), and blocked (in PBS containing 5% BSA) for 1 h at room temperature. And then the brain sections were incubated with monoclonal FasL antibody (Santa Cruz, USA) for 3 d at 4 °C. After washing, the sections were incubated with secondary antibodies (Alexa Fluor conjugated, Invitrogen, CA, USA) for 4 h at room temperature. After several rinses with PBS, sections were incubated with DAPI (1 uM) for 10 min, which indicating nuclear localization (blue, $\lambda_{ex}$ 405 nm, $\lambda_{em}$ 420-480 nm). **B545b** fluorescence was collected at 500-540 nm with $\lambda_{ex}$ 488 nm.

## Nissl staining

Nissl staining is commonly used to identify the neuronal structure in brain and performed as following described.[4] 40-μm brain sections were mounted on gelatin-coated glass slides. The samples were rehydrated with decreasing ethanol concentrations and then processed for Nissl staining (Beyotime, China) 30 min, according to the manual. A light microscope was used to visualize the Nissl stained sections.

## Supplementary figures

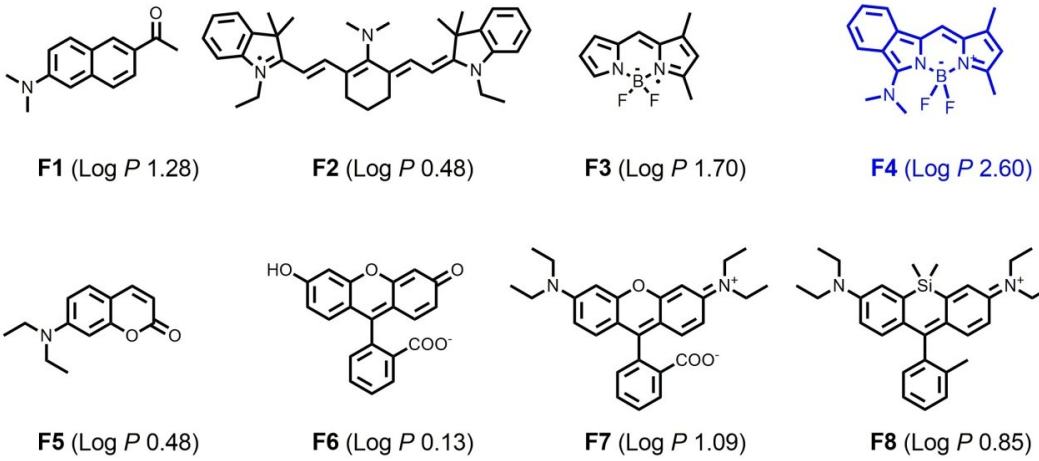



**Fig. S1** Structures of the classical fluorophores screened for potential BBB permeability and their Log *P* data.

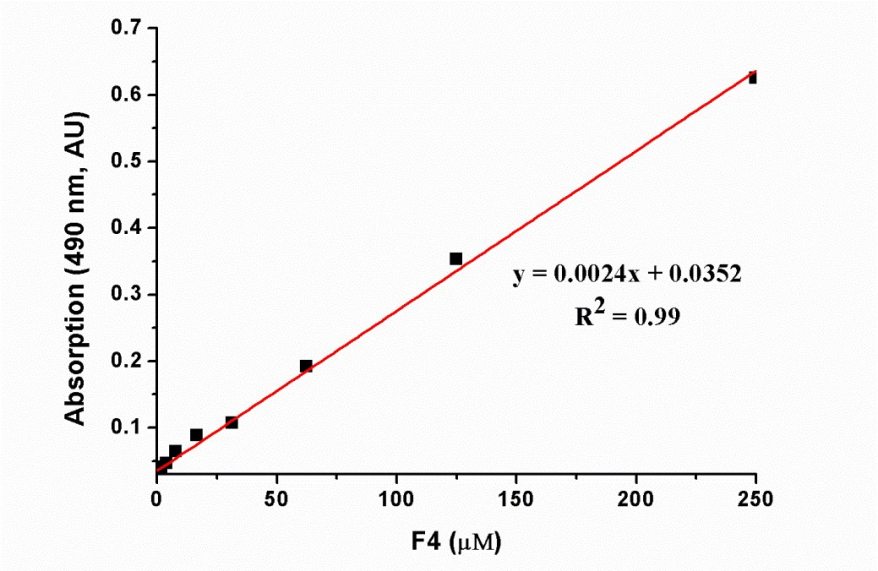



**Fig. S2** The standard curve for **F4** water solubility determination. **F4** was dissolved in DMSO to make a 100 mM stock solution. Aliquot of this solution was diluted with water to 500 μM, sonicated for 5 min, and then immediately subjected to UV absorption measurement. This 500 μM **F4** solution was kept

being diluted with water to 250 μM, sonicated and collected for the absorption spectra. The procedures were repeated till the absorption spectra of **F4** in the concentration range of 1.95 to 500 μM were measured. The intensity at maximum absorption wavelength was then plotted against concentration to yield a standard curve. Next, aliquot of the **F4** stock solution in DMSO was diluted with $H_2O$ to (500 μM). After being sonicated for 5 min, the solution was centrifuged at 14500 rpm for 10 min to remove precipitates. The supernatant was tested for the absorption spectra. The water solubility of **F4** was calculated by taking the absorption intensity of this solution at $\lambda_{max}$ into the standard curve.

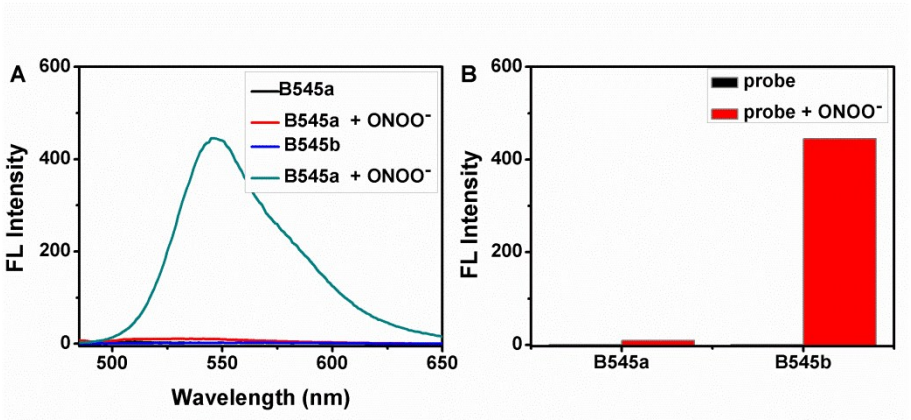



**Fig. S3 (A)** Fluorescent spectra of **B545a** and **B545b** (5.0 μM) before and after the treatment of $ONOO^-$ (10 μM, 15 min). Measurements were carried out in PBS (10 mM, pH 7.4, 20% ethanol) at ambient temperature with excitation at 475 nm. **(B)** The intensity at 545 nm of the data in Fig S3A.

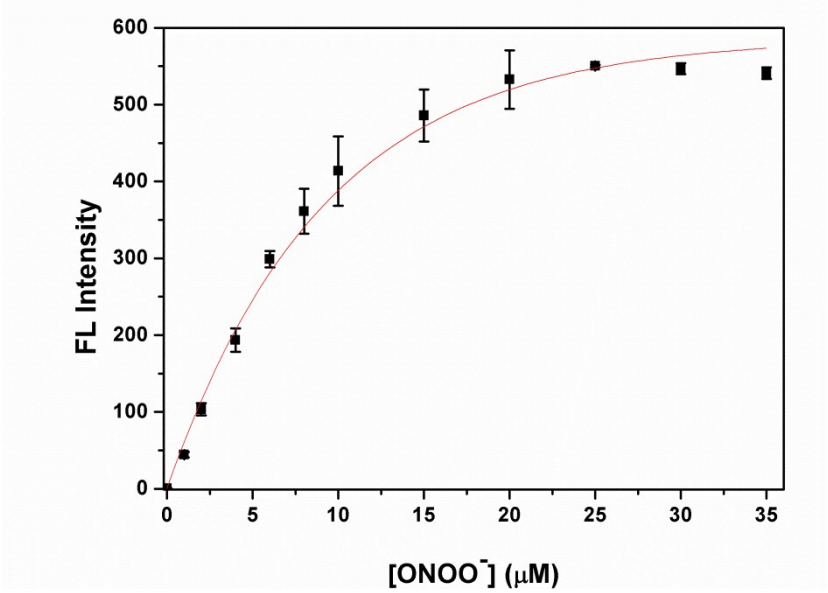



**Fig. S4** Plot of **B545b** (5 μM) fluorescence intensity at 545 nm in response to various concentrations of $ONOO^-$. Data were acquired in PBS (10 mM, pH 7.4, 20% ethanol) at ambient temperature after the treatment of $ONOO^-$ for 15 min. Excitation, 475 nm.

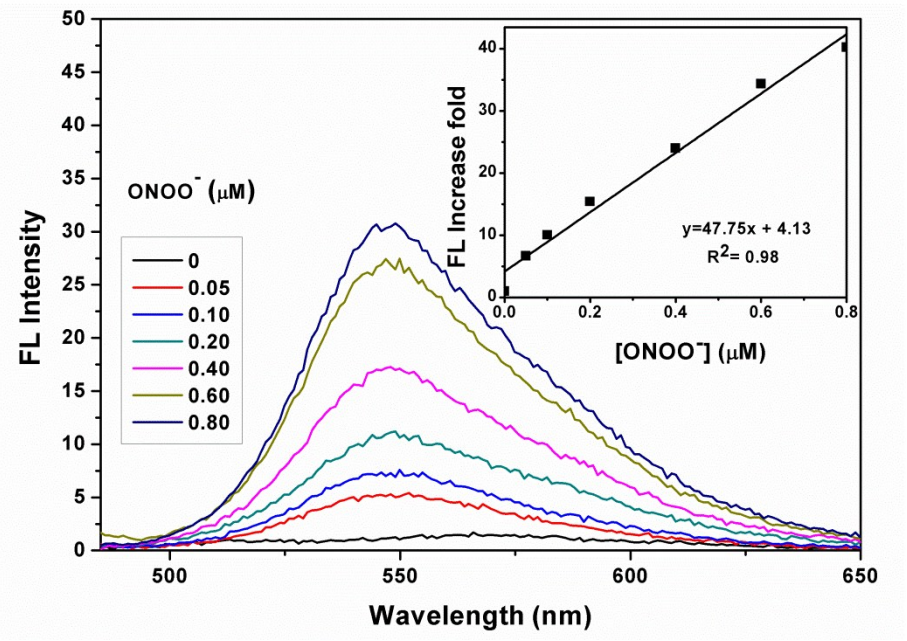



**Fig. S5** Fluorescence spectra of **B545b** (5 μM) after the treatment of low doses of $ONOO^-$. The inner panel was the plot of **B545b** fluorescence at 545 nm versus $ONOO^-$ concentration.

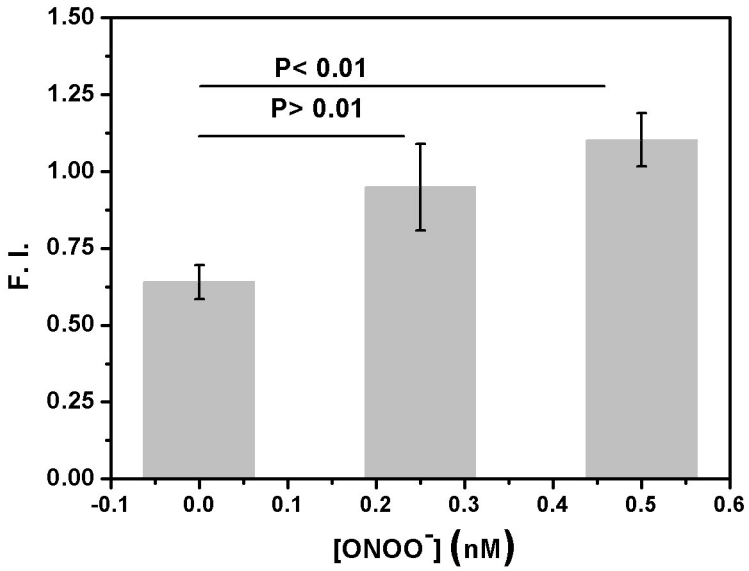



**Fig. S6** Detection limit determination of **B545b.** Results were obtained as the concentration of $ONOO^-$ that induced a statistically significant increase in **B545b** fluorescence at 545 nm compared with a blank control after 5 min of incubation (P-value < 0.01). Experiments were carried out by incubating **B545b** (5 μM) with $ONOO^-$ (0, 0.25, 0.50 nM) in PBS at ambient temperature for 5 min and then collecting the emission at 545 nm by excitation at 475 nm. Statistical analyses were performed with a two-tailed Student's t-test (n = 3). Error bars are standard deviation.

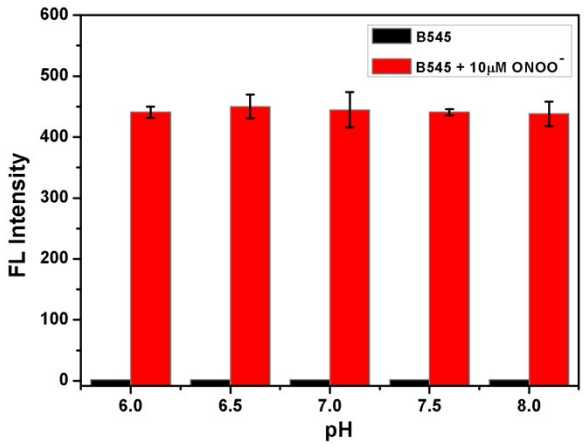



**Fig. S7** The fluorescence of **B545b** and its response towards $ONOO^-$ were insusceptible to the surrounding pH. Data were the plot of the fluorescence intensity of **B545b** (5.0 μM) at 545 nm before and after the treatment of $ONOO^-$ (10μM) to media pH.

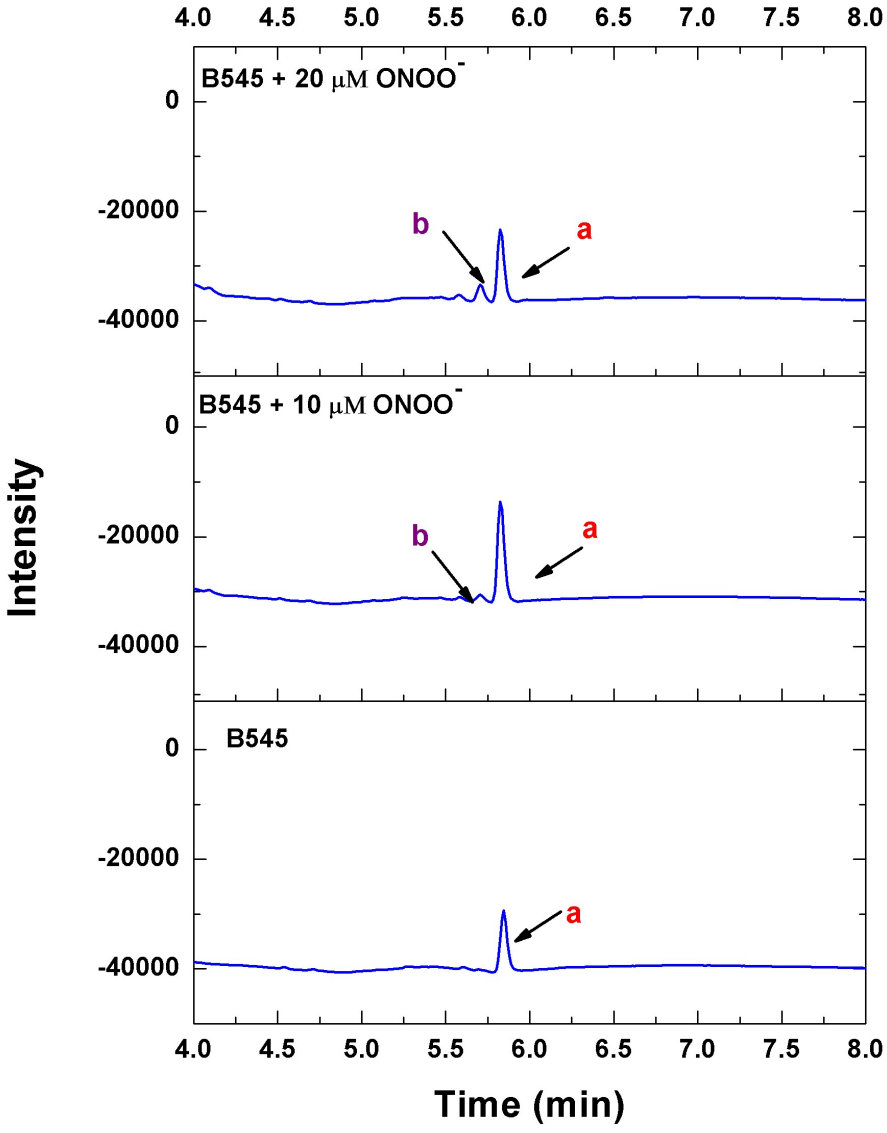



**Fig. S8** The total ion chromatogram (TIC) traces of probe **B545b** (a) and the detection product (b).

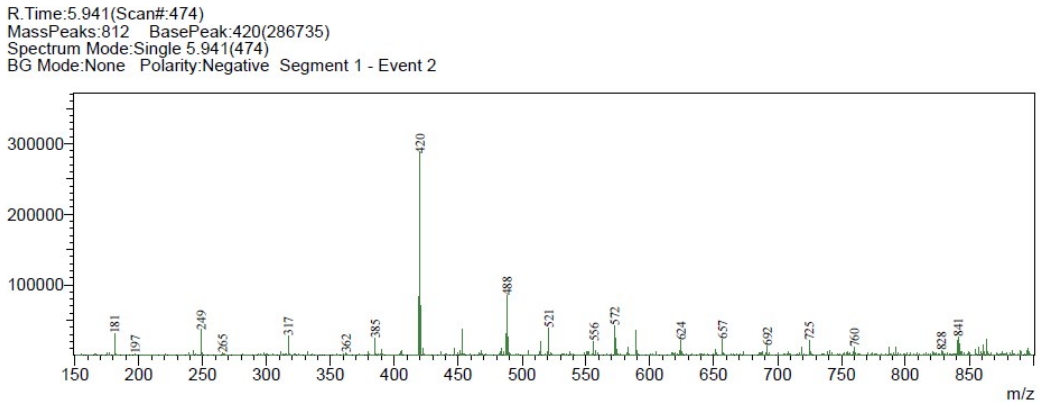



**Fig. S9** MS spectra of **B545b** (m/z 420 for $[M-1]^{-1}$) (peak a).

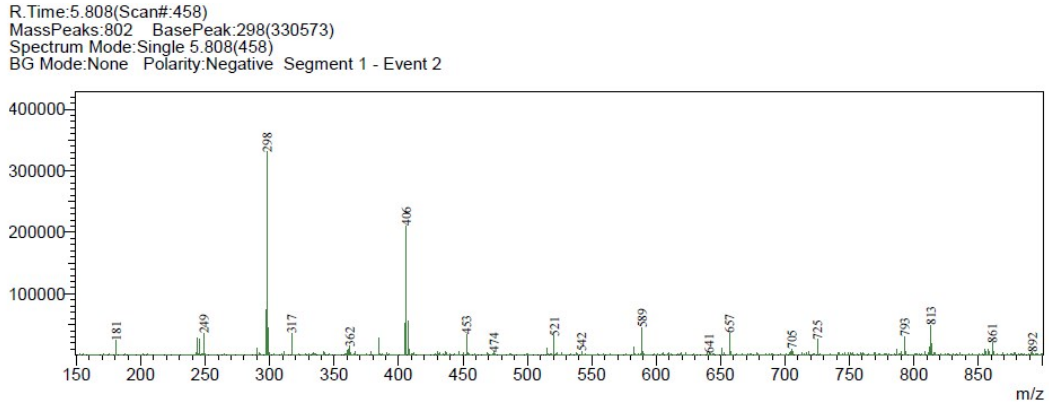



**Fig. S10** MS spectra of detection product (m/z 298 for $[M-1]^{-1}$) (peak b).

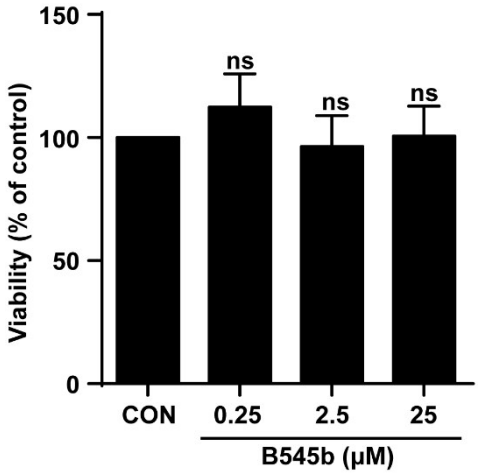



**Fig. S11** Effect of **B545b** on cell viability as determined with the CCK-8 assay. Data shown were the quantification of cell viability by CCK-8 assay. Procedures were detailed beforehand. Data are expressed as mean ± S.E.M. ns P>0.05 versus control.

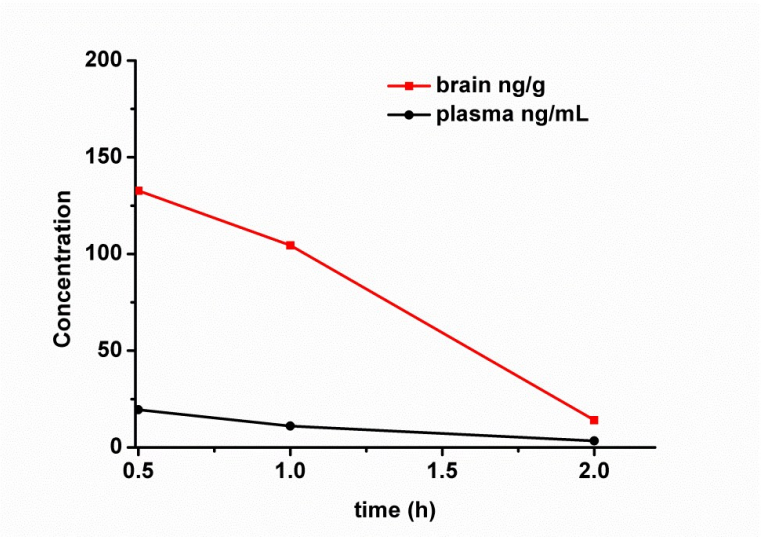



**Fig. S12** Plasma and brain **B545b** concentrations after I. V. dosing at 5 mg/kg.

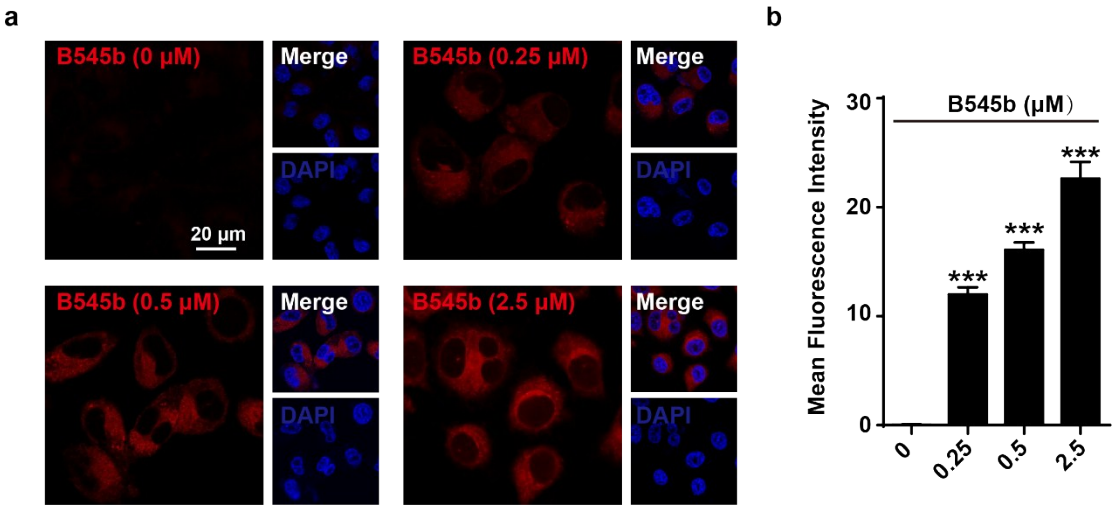



**Fig. S13** Optimizing the working concentration of **B545b** for cell imaging using a SIN-1 stimulation model. EA.hy926 endothelial cells were cultured on glass cover slips overnight, and then were incubated with SIN-1 (0.06 mM) for 1 h. Cells were then stained with **B545b** of various concentrations for 0.5 h. After fixation, cells were observed under confocal microscopy (a), and **B545b** fluorescence intensity was plotted against its concentration (b). Data are presented as a densitometric ratio change compared with control. **B545b** fluorescence was collected at 560-620 nm with $\lambda_{ex}$ 543 nm. DAPI fluorescence was collected at 420-480 nm upon excitation at 405 nm. ***P<0.001 versus control.

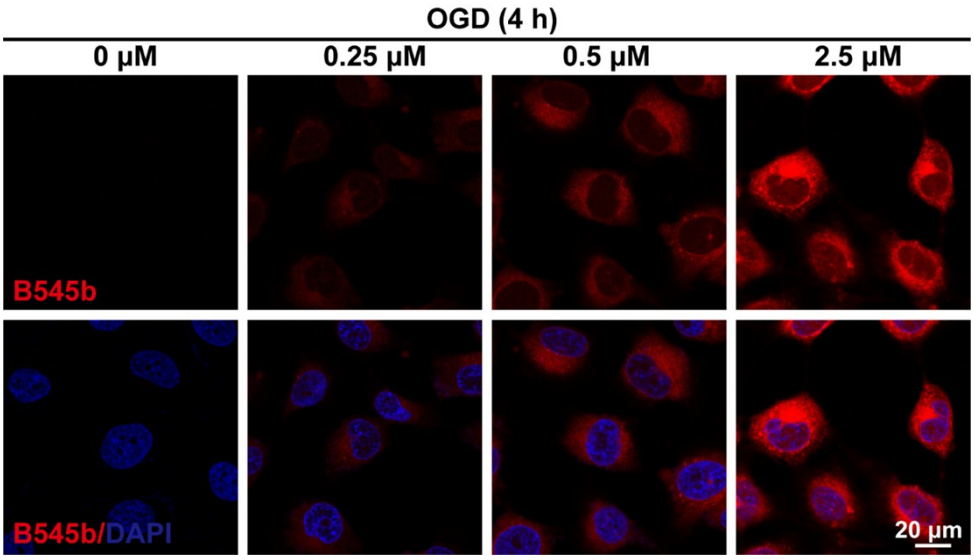
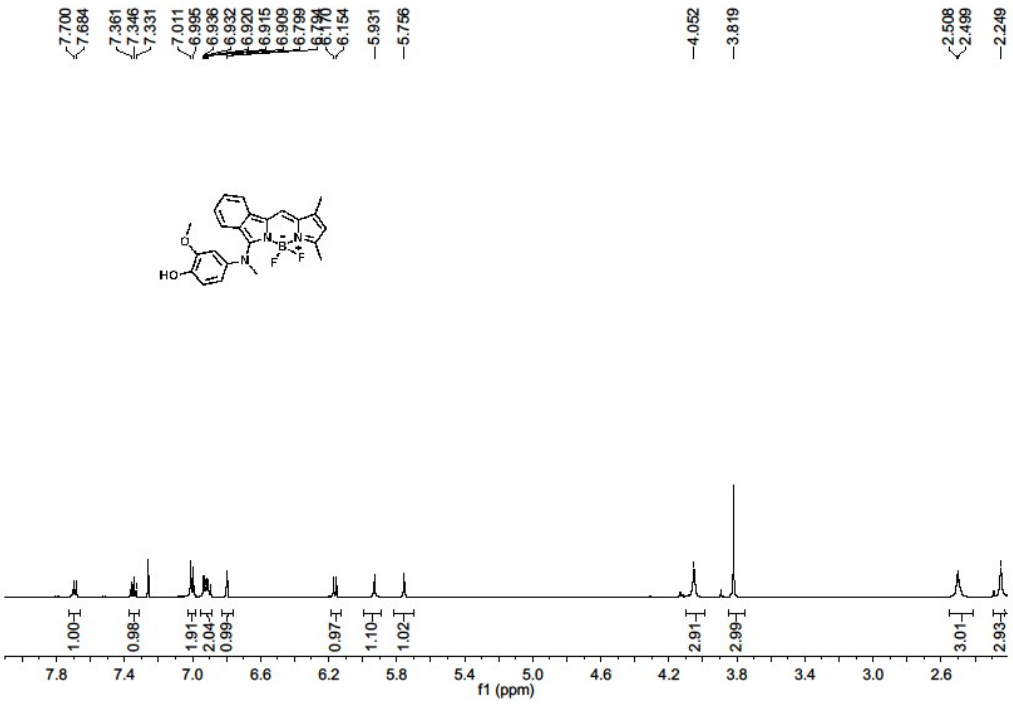
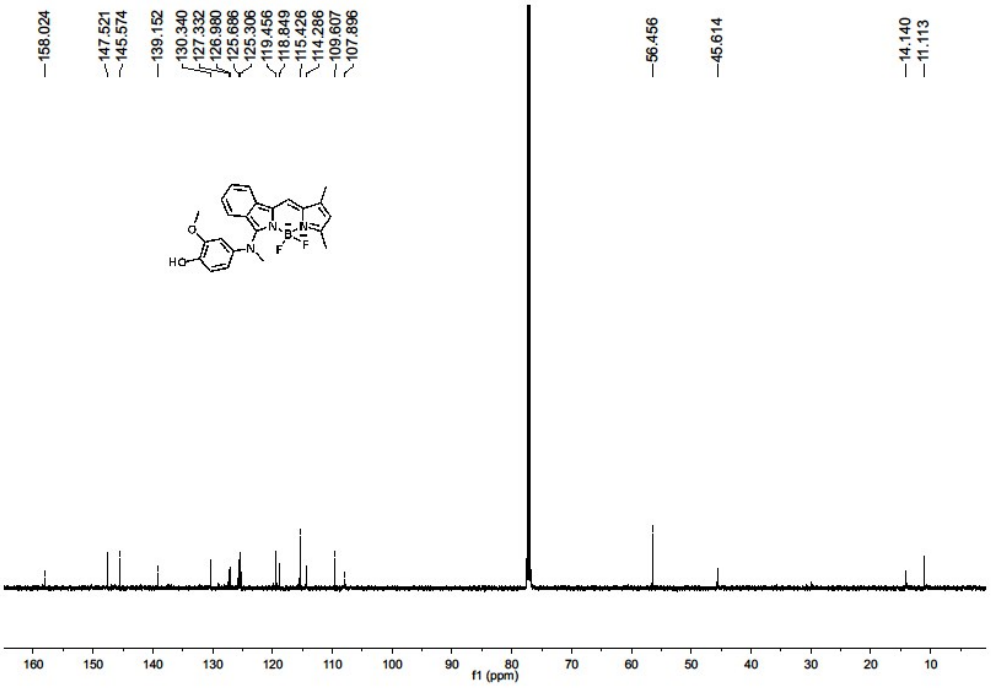
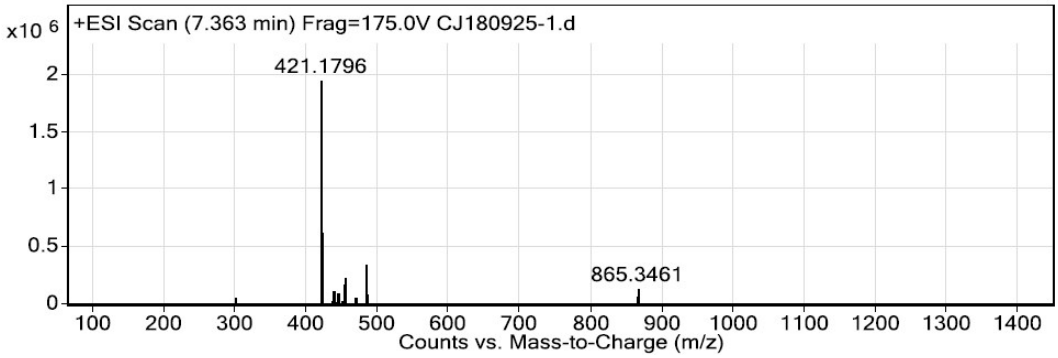


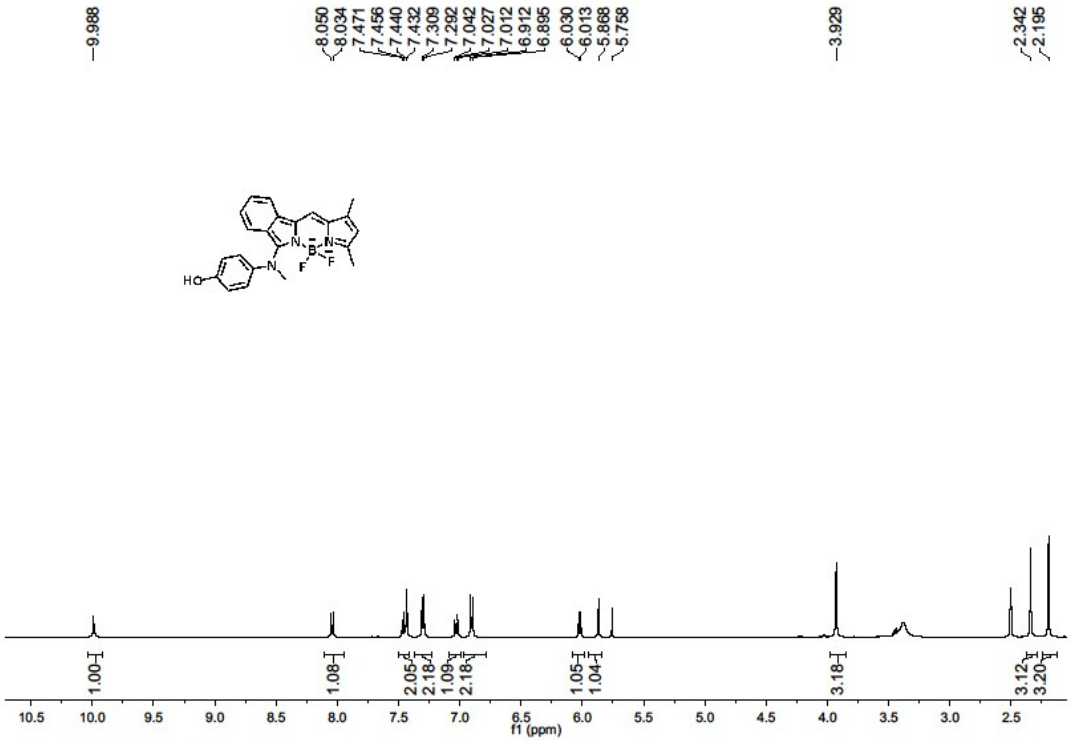

**Fig. S14** Optimizing the working concentration of **B545b** for cell imaging using an OGD model. EA.hy926 endothelial cells were cultured on glass cover slips overnight, then exposed to OGD for 4 h. Cells were then stained with **B545b** of various concentrations for 0.5 h, and imaged after fixation. **B545b** fluorescence was collected at 560-620 nm with $\lambda_{ex}$ 543 nm. DAPI fluorescence was collected at 420-480 nm upon excitation at 405 nm.






**$^{1}$H NMR, $^{13}$C NMR and HRMS spectra of B545b**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8.034
7.471
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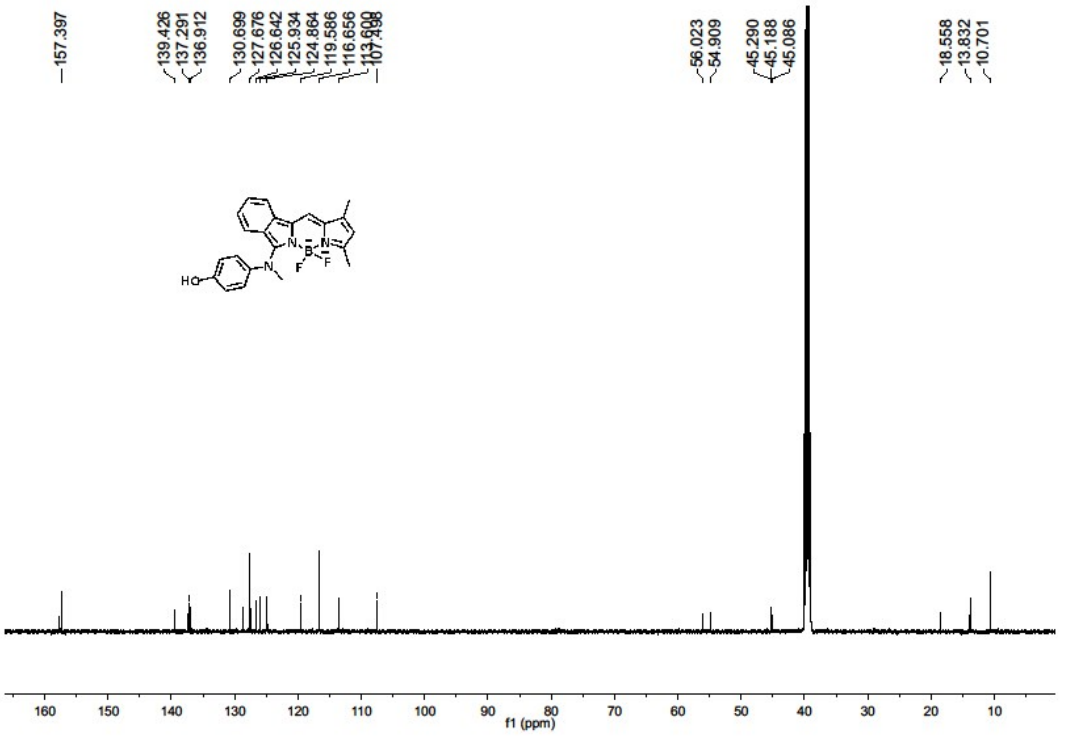
7.440
7.432
7.309
7.292
7.042
7.027
7.012
6.912
6.895
6.030
6.013
5.868
5.758
3.929
2.342
2.195
HO
N
B
F
F
1.00
1.08
2.05
2.18
1.09
2.18
1.05
1.04
3.18
3.12
3.20
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
f1 (ppm)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139.426
137.291
136.912
130.699
127.676
126.642
125.934
124.864
119.586
116.656
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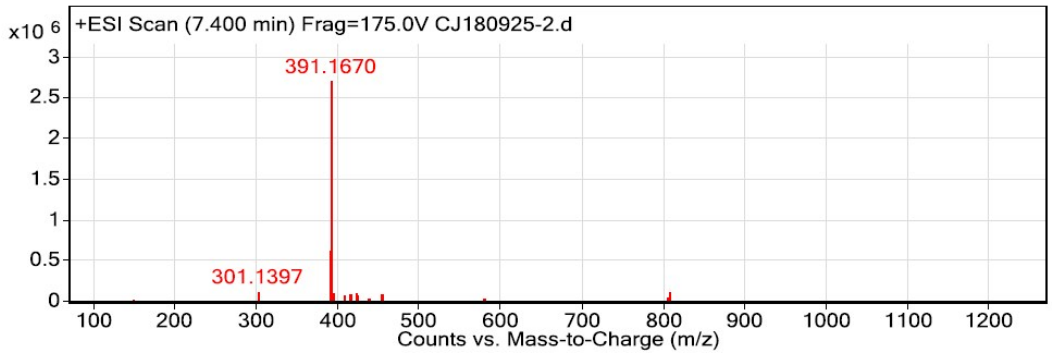
56.023
54.909
45.290
45.188
45.086
18.558
13.832
10.701
HO
N
B
F
F
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)




**$^{1}$H NMR, $^{13}$C NMR and HRMS spectra of B545a**

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