Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting information

A rhodamine-based fast and selective fluorescent probe for monitoring exogenous and endogenous nitric oxide in live cells

Qing Wang,[a] Xiaojie Jiao,[b] Chang Liu,[b] Song He,[b] Liancheng Zhao[a,b] and Xianshun Zeng*[ab]

[a] *School of Materials Science and Engineering, Harbin Institute of Technology, Harbin 150001, China.*

[b] *Tianjin Key Laboratory for Photoelectric Materials and Devices, School of Materials Science & Engineering, Tianjin University of Technology, Tianjin 300384, China*. *Email: xshzeng@tjut.edu.cn.*

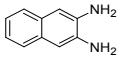
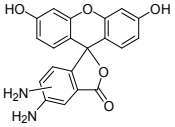
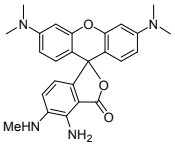
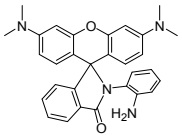
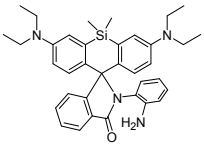
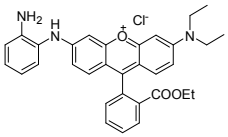
Table of Contents

General methods

DEA/NONOate (diethylamine NONOate), ascorbic acid (AA), KO_2, H_2O_2, NaClO, $NaNO_2$, $NaNO_3$, LPS and N^G-monomethyl-L-arginine (L-NMA) were obtained from commercial sources and used without additional purification. Hydroxyl radicals ($^{\cdot}OH$) were generated by reaction of Fe^{2+} with H_2O_2.[1] Peroxynitrite ($ONOO^-$) was generated from amyl nitrite and H_2O_2 following literature procedures and the concentration of the $ONOO^-$ stock solution was determined by measuring the absorbance at 302 nm (ε = 1670 M^{-1} cm^{-1}).[2]

The stock solution of NO was produced by adding H_2SO_4 (20 %) to sodium nitrite solutions and bubbling NO into water for 20 min.[3] The concentration of the NO solution was determined by the Griess method.[4] Aliquots (50 μL) of this solution were added to 1 mL of potassium phosphate buffer (0.1 mM, pH 7.4) containing sulfanilamide solution (17 mM) and N-(1-naphthyl)ethylenediamine (0.4 mM). The solution was immediately mixed by inversion and incubated at room temperature for 5 min. The colorimetric product was measured at 496 nm by use of a UV/Vis spectrophotometer. The NO concentration of the solution was calculated according to Beer's law using an extinction coefficient of 5400 M^{-1} cm^{-1} as determined from experiments using chemiluminescence standardization. Based on this method, the concentration of the NO stock solution is 1.2 mM.

Table S1 Comparison of different fluorescent probes for NO Detection

Probes	Structure	References	pH usage value	Response time	Detection limit
DAN		*Anal. Biochem.* 1993, **214**, 11.	In acidic condition	< 5 min	10 nM
DAF-n		*Angew. Chem. Int. Ed.,* 1999, **38**, 3209; *Anal. Chem.,* 1998, **70**, 2446	> 6	> 10 min (**DAF-2)**	5 nM (**DAF-2**)
DAR-n		*Anal. Chem.,* 2001, **73**, 1967	> 4	-	7 nM (DAR-4M)
RB-NO		*Org. Lett.,* 2008, **10**, 2357	> 5	30 min	3.0 nM
SiRB-NO		*Chem. Eur. J.,* 2016, **22**, 5649	Fl intensity changed with pH	> 30 min	32.6 nM
ROPD		This paper	4.0-9.3	2.5 min	68.2 nM

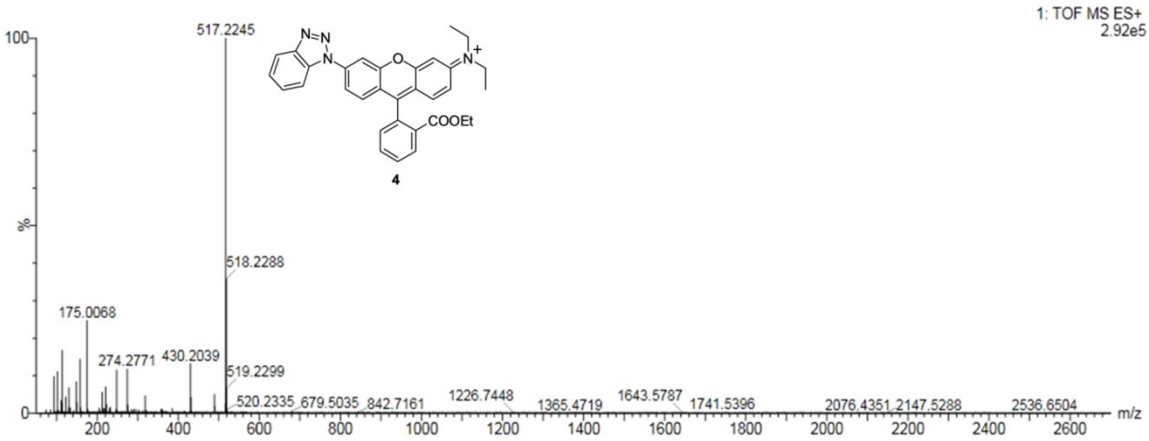


Fig. S1 HRMS spectrum of the solution of **ROPD** after the addition of 10 equiv. of NO. The peak (m/z) at 517.2245 corresponds to the triazole derivative **4** (Calcd: 517.2239).

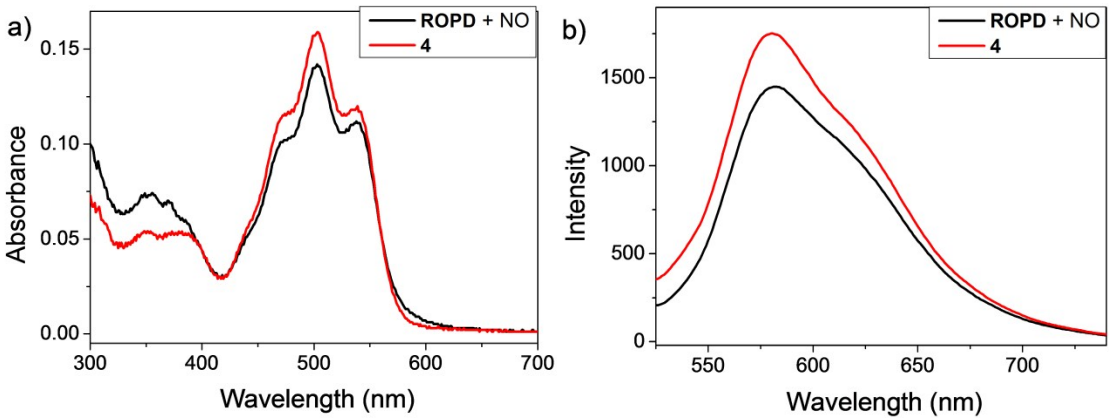


Fig. S2 The absorption a) and fluorescence emission b) spectra of **ROPD** (5 μM) in the presence of 20 equivalents of NO solution (black line) and the triazole **4** (5 μM) (red line) in PBS (100 mM, pH 7.4).

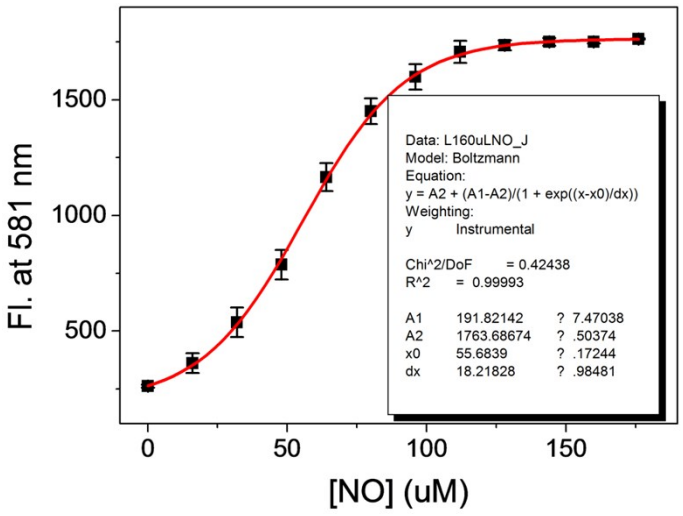


Fig. S3 Measurement of fluorescence dissociation constant (K_d) of **ROPD** with NO.

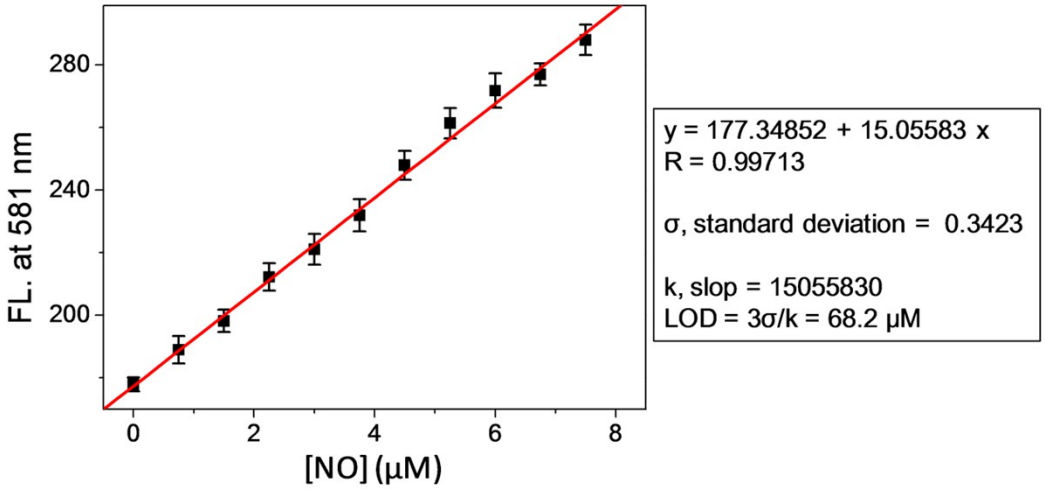


Fig. S4 The detection limit of **ROPD** (2.5 μM) towards NO by 3σ/k in PBS buffer (100 mM, pH = 7.4).

The detection limit was calculated based on the fluorescence titration. The emission intensity of the probe **ROPD** without nitric oxide was measured by 8 times, and the standard deviation of blank measurements was determined. The detection limit is then calculated with the following equation:

LOD = $3\sigma/k$

Where σ is the standard deviation of the blank solution measured by 8 times; k is the slope of the calibration curve.

From the graph we get slope (k) = 15055830, and σ value is 0.3423

Thus we get the Limit of Detection (LOD) = $3\sigma/k$ = 68.2 nM.

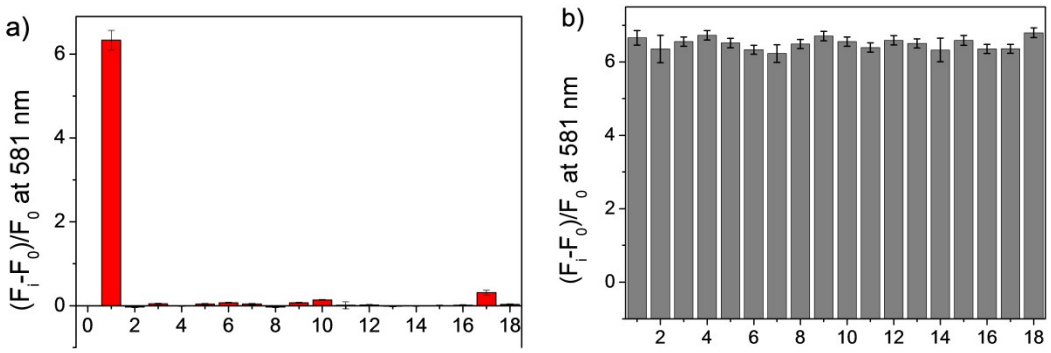


Fig. S5 a) Histogram of the fluorescence enhancement ratio $((F_i - F_0)/F_0)$ of **ROPD** (5 μM) at 581 nm in the presence of various metal ions (100 μM). 0: **ROPD**, 1: NO, 2: $AgNO_3$, 3: $Al(NO_3)_3$, 4: $Ca(NO_3)_2$, 5: $Cd(NO_3)_2$, 6: $Co(NO_3)_2$, 7: $Cr(NO_3)_3$, 8: $Cu(NO_3)_2$, 9: $FeCl_2$, 10: $FeCl_3$, 11: $Hg(ClO_4)_2$, 12: KNO_3, 13: $Mg(NO_3)_2$, 14: $MnCl_2$, 15: $NaNO_3$, 16: $Ni(NO_3)_2$, 17: $Pb(NO_3)_2$, 18: $Zn(NO_3)_2$; b) Change ratio $((F_i - F_0)/F_0)$ of fluorescence intensity (581 nm) of **ROPD** (5 μM) upon addition of each metal ions (100 μM) followed by NO (100 μM) in PBS buffer solution (100 mM, pH = 7.4). 1: probe **ROPD** after addtion of NO alone, and in the presence of 2: $AgNO_3$, 3: $Al(NO_3)_3$, 4: $Ca(NO_3)_2$, 5: $Cd(NO_3)_2$, 6: $Co(NO_3)_2$, 7: $Cr(NO_3)_3$, 8: $Cu(NO_3)_2$, 9: $FeCl_2$, 10: $FeCl_3$, 11: $Hg(ClO_4)_2$, 12: KNO_3, 13: $Mg(NO_3)_2$, 14: $MnCl_2$, 15: $NaNO_3$, 16: $Ni(NO_3)_2$, 17: $Pb(NO_3)_2$, 18: $Zn(NO_3)_2$; λ_{ex}: 505 nm. Slit: 10 nm, 10 nm.

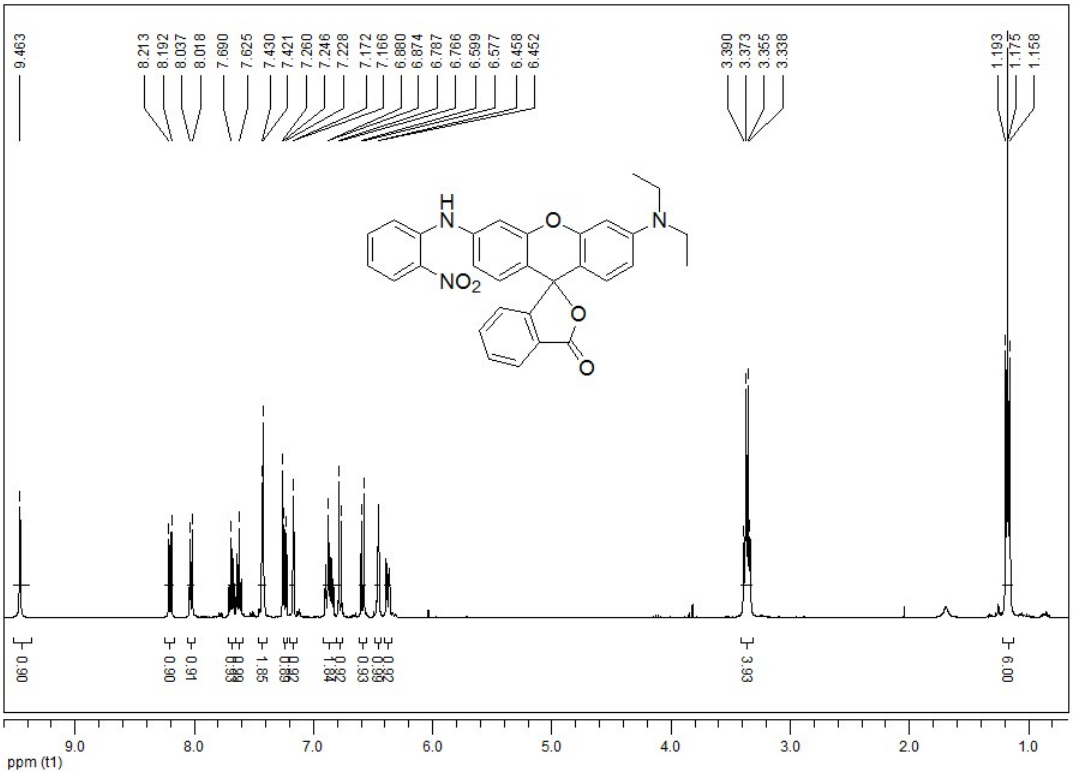


Fig. S6 ^{1}H NMR spectrum of **2** (400 MHz, $CDCl_3$).

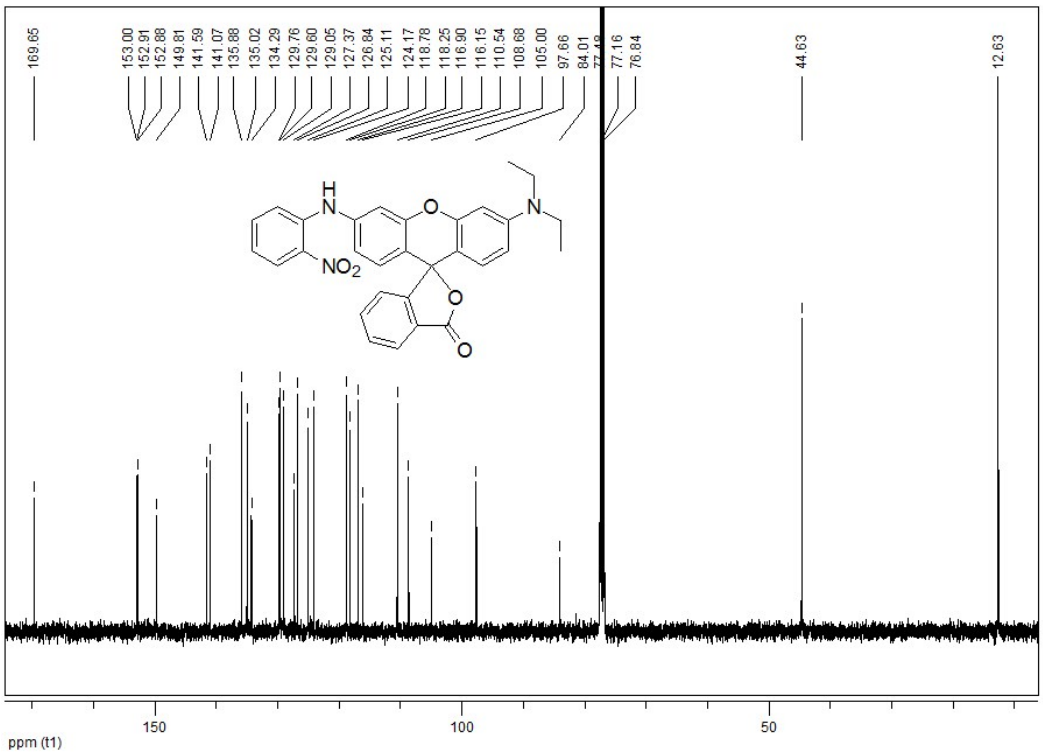


Fig. S7 ^{13}C NMR spectrum of **2** (100 MHz, $CDCl_3$).

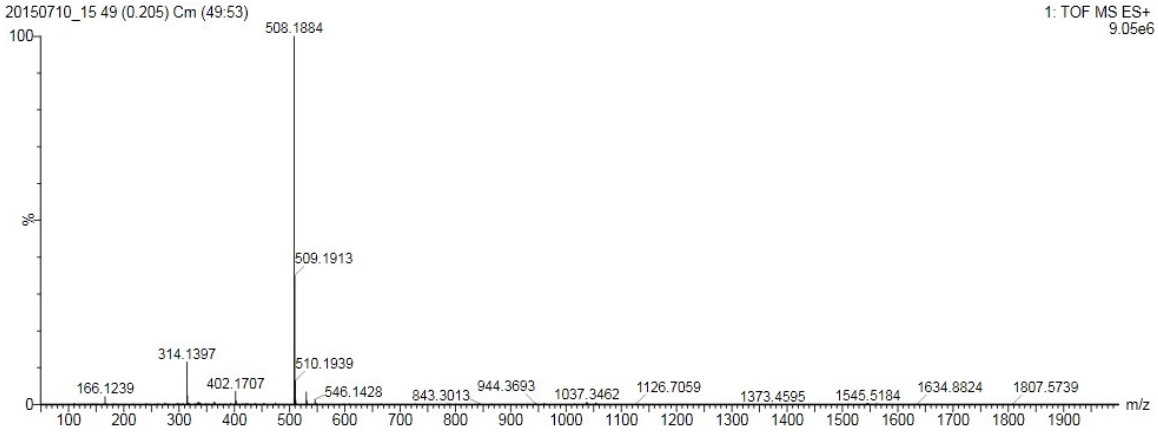


Fig. S8 HRMS of **2**. HRMS: m/z [M + H^+] = 508.1884; Calcd for [$C_{30}H_{25}N_3O_5$ + H^+]: 508.1873.

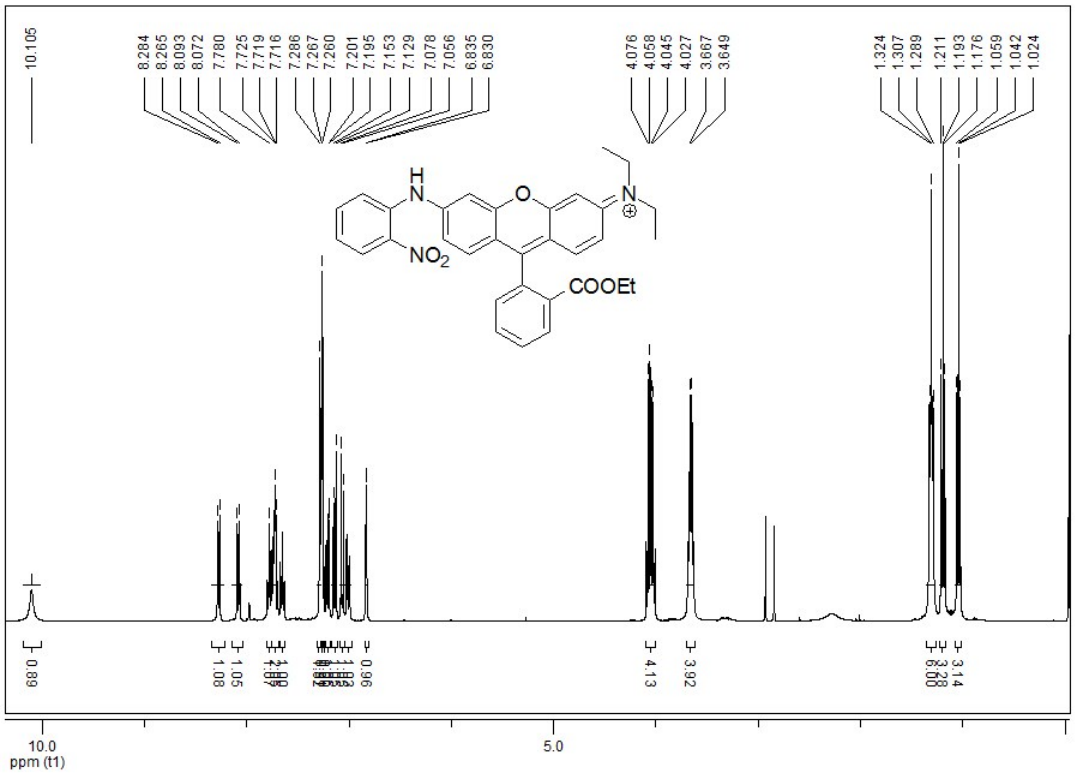


Fig. S9 ^{1}H NMR spectrum of **3** (400 MHz, $CDCl_3$).

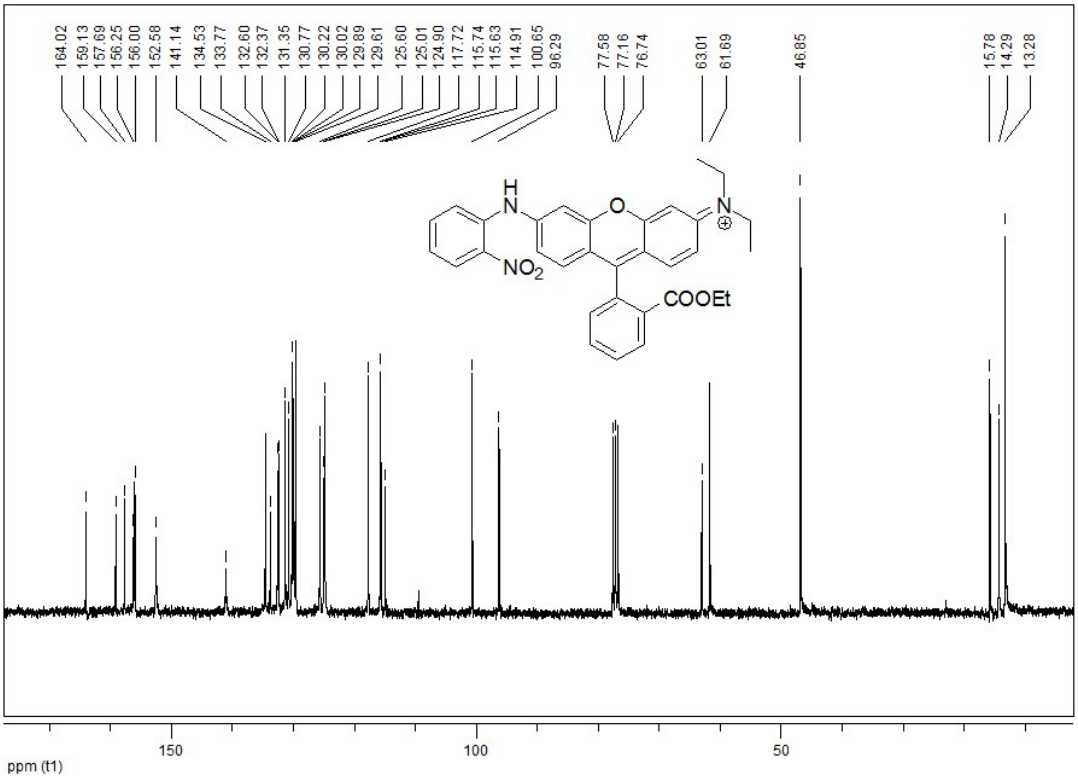


Fig. S10 ^{13}C NMR spectrum of **3** (100 MHz, $CDCl_3$).

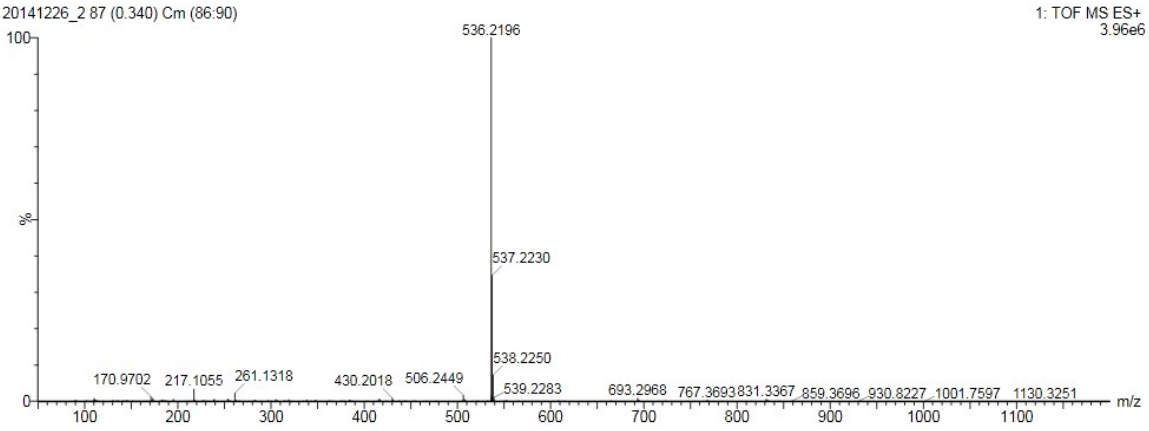


Fig. S11 HRMS of **3**. HRMS: m/z [M - Cl^-] = 536.2196; Calcd for $C_{32}H_{30}N_3O_5^+$ = 536.2186.

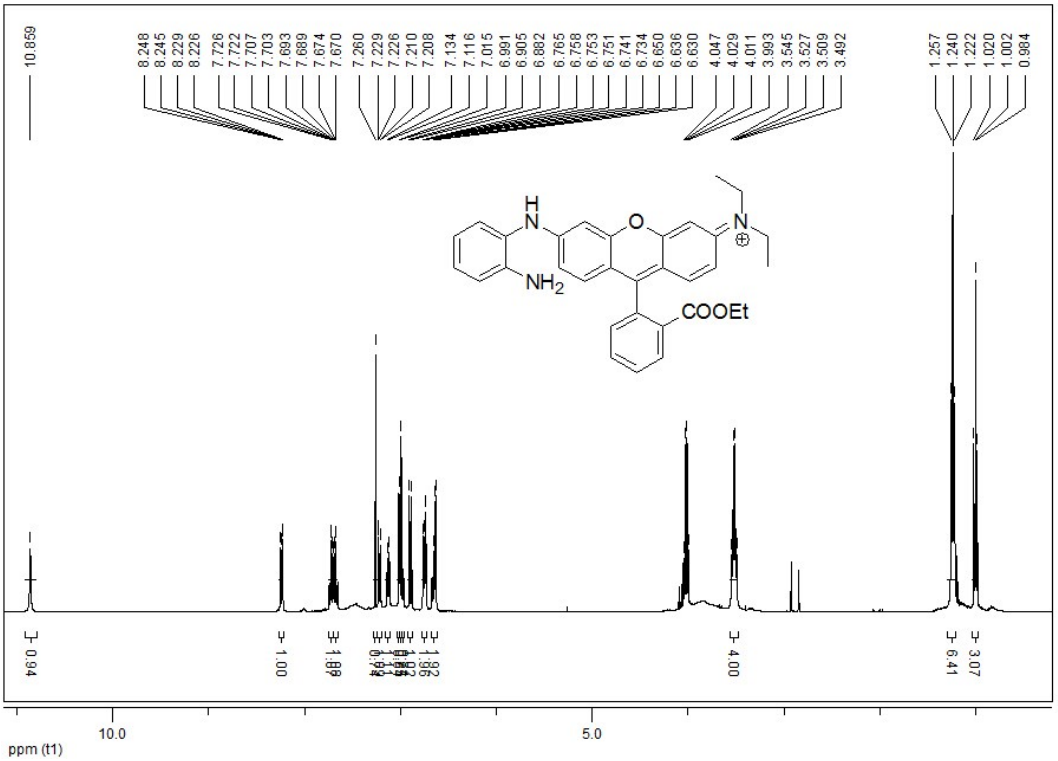


Fig. S12 ^{1}H NMR spectrum of probe **ROPD** (400 MHz, $CDCl_3$).

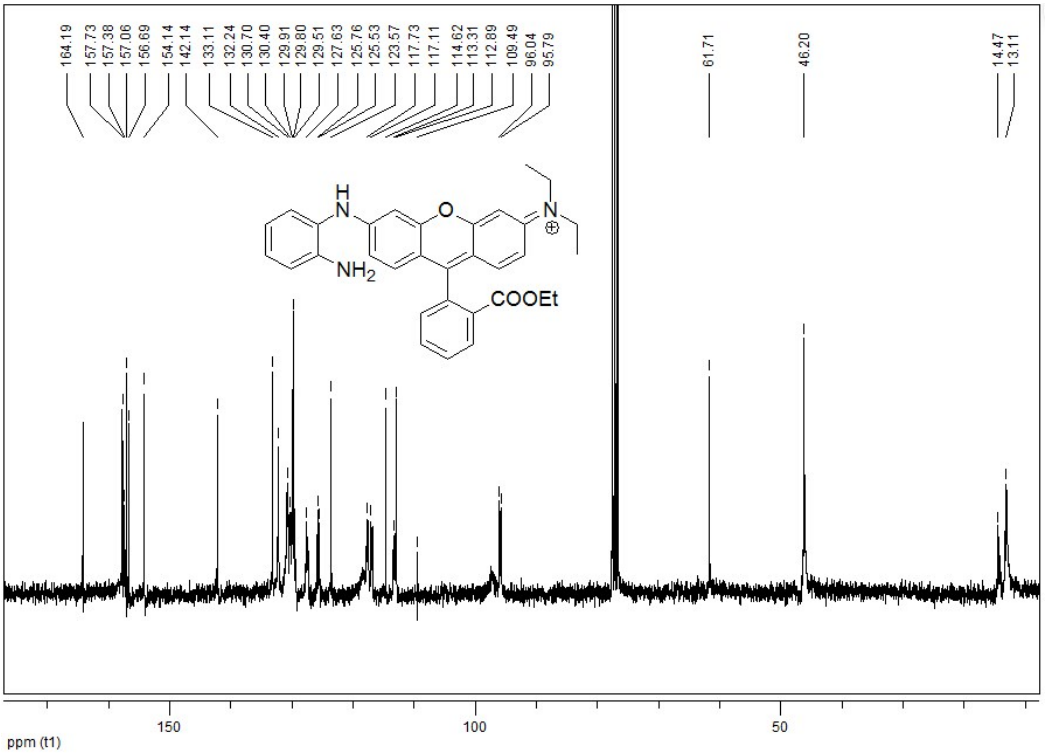


Fig. S13 ^{13}C NMR spectrum of probe **ROPD** (100 MHz, $CDCl_3$).

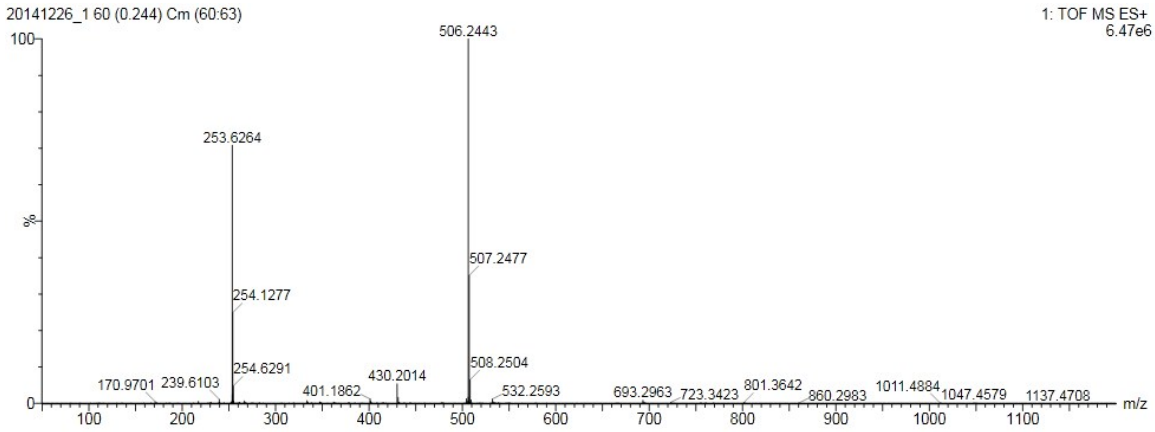


Fig. S14 HRMS of probe **ROPD**. HRMS: m/z [M - Cl^- + H^+]/2 = 253.6264, [M - Cl^-] = 506.2443; Calcd for [$C_{32}H_{32}N_3O_3^+$ + H^+]/2 = 253.6261; [$C_{32}H_{32}N_3O_3^+$] = 506.2444.

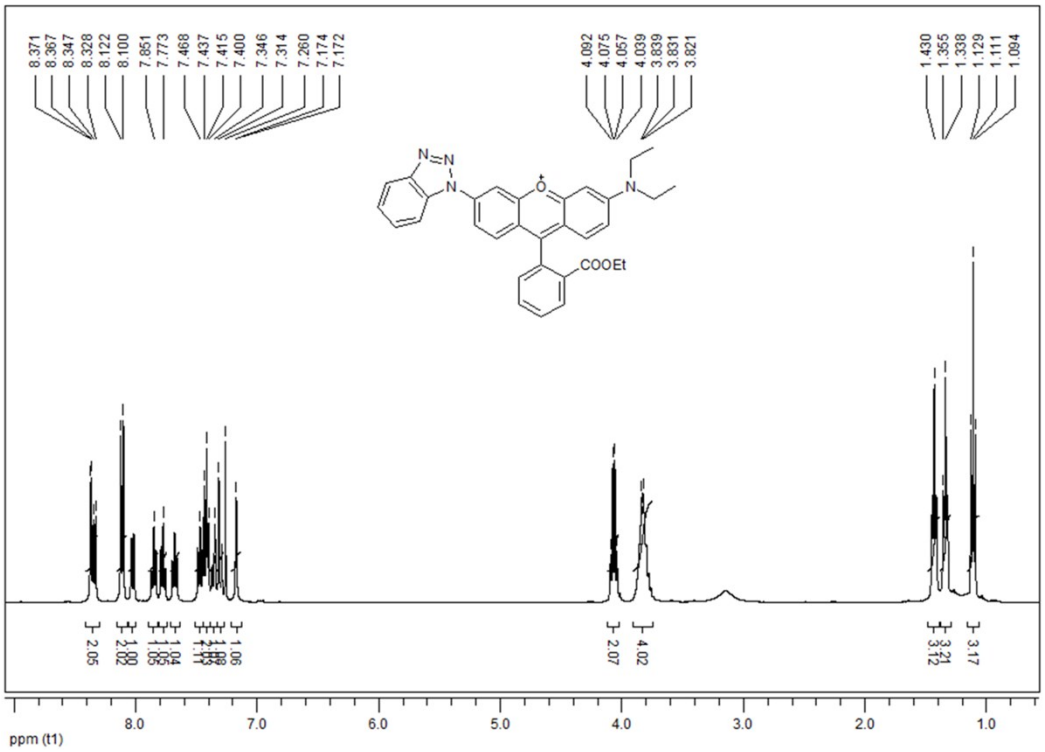


Fig. S15 ^{1}H NMR spectrum of the triazole **4** (400 MHz, $CDCl_3$).

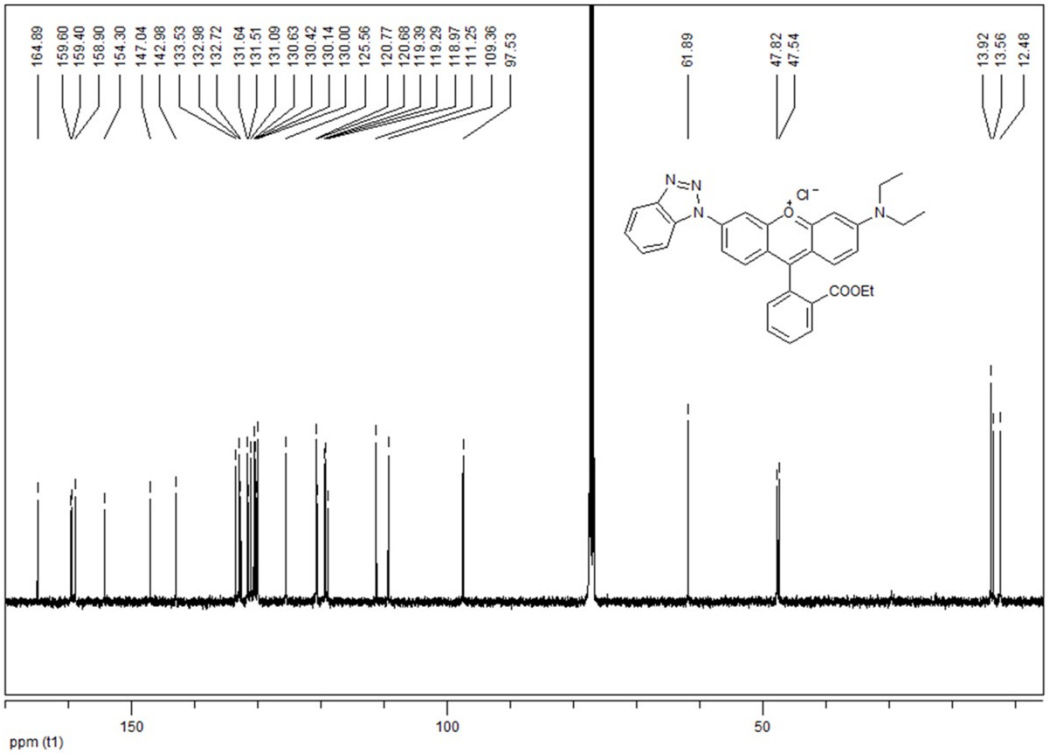


Fig. S16 ^{13}C NMR spectrum of triazole **4** (100 MHz, $CDCl_3$).

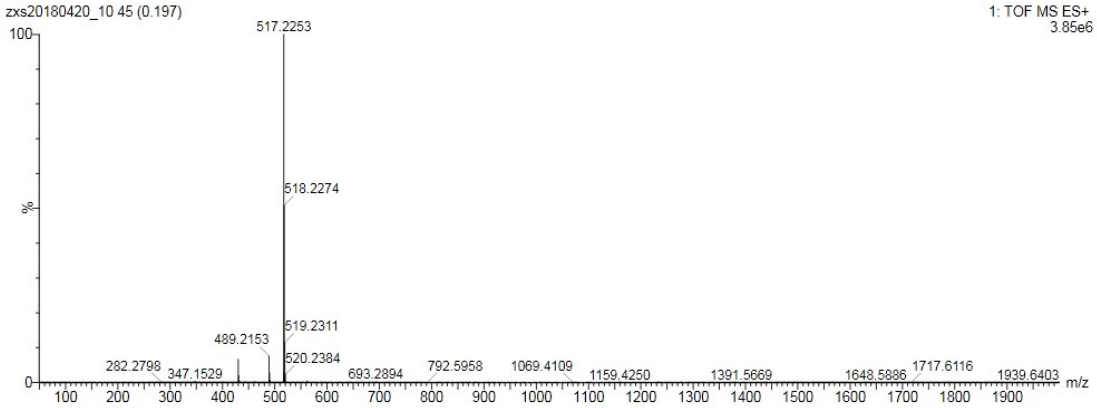


Fig. S17 HRMS of triazole **4**. HRMS: m/z [M - Cl^-] = 517.2253; Calcd for [$C_{32}H_{29}N_4O_3^+$] = 517.2240.

References

[1] (a) H. H. Fenton, *Chem. News*, 1876, **33**, 190; (b) A. E.Albers, B. C. Dickinson, E. W. Miller and C. J. Chang, *Bioorg. Med. Chem. Lett*., 2008, **18**, 5948.

[2] R. M. Uppu and W. A. Pryor, *Anal. Biochem.*, 1996, **236**, 242.

[3] J. Ouyang, H, Hong, C. Shen, Y. Zhao, C. Ouyang, L. Dong, J. Zhu, Z. Guo, K. Zeng, J. Chen, C. Zhang and J. Zhang, *Free Radic. Biol. Med.,* 2008, **45**, 1426.

[4] L. A. Ridnour, J. E. Sim, M. A. Hayward, D. A. Wink, S. M. Martin, G. R. Buettner and D. R. Spitz, *Anal. Biochem.,* 2000, **281**, 223.