Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Supporting Information

A turn-on fluorescent probe for detection of hydrogen sulfide in aqueous solution and living cells

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Contents

1. Fluorometric and MALDI-TOF Analysis

UV-visible spectra were recorded on SHIMADZU UV-2550 UV-vis spectrometer. Fluorescence spectra were recorded using a HITACHI F-4600 spectrometer. The PMT voltage was 700 V, excitation slit and emission slit were 5 nm. The path length was 1 cm with cell volume of 3.0 mL. The stock solution of FD-NO_2 was prepared in DMSO (2 mM). H_2O_2, *tert*-butylhydroperoxide (TBHP), and hypochlorite (NaOCl) were delivered from 30%, 70%, and 14.5% aqueous solutions, respectively. Nitric oxide (NO) was generated from SNP.[S1] H_2S, N_3^-, AcO^-, SCN^-, SO_3^{2-}, $S_2O_3^{2-}$, O_2^-, NO_2^-, and NO_3^- were prepared from Na_2S,[S2] NaN_3, NaOAc, KSCN, Na_2SO_3, $Na_2S_2O_3$, KO_2,[S3] $NaNO_2$, and KNO_3, respectively.

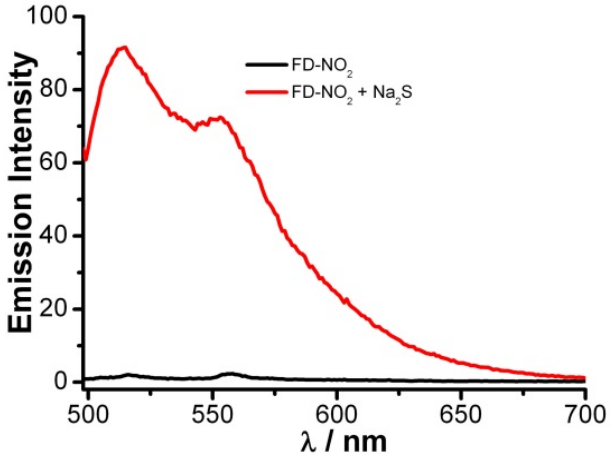

Fig. S1 Fluorescence spectra of FD-NO_2 (2μM) and their reaction solutions with excess Na_2S (100 μM) in MeOH, 25 °C.

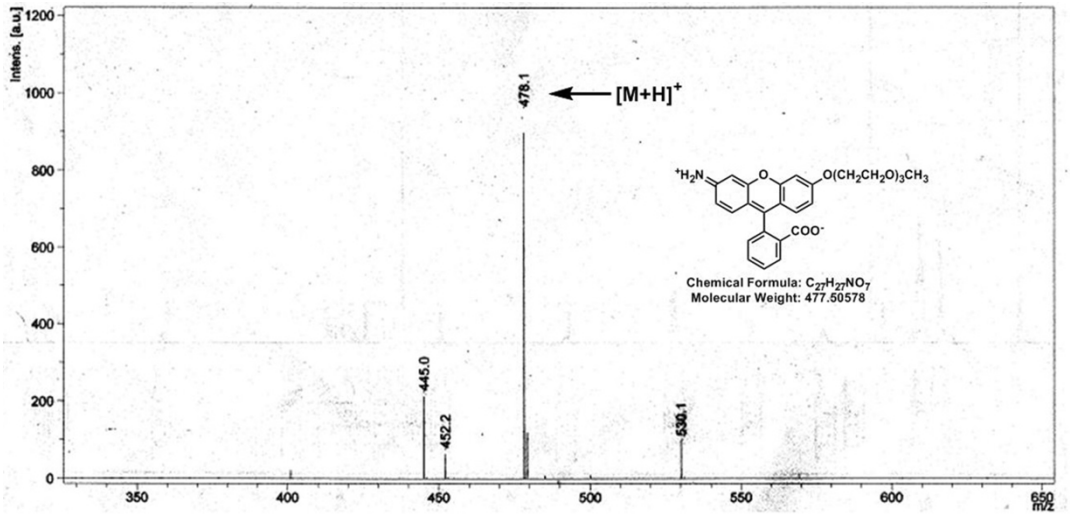

Fig. S2 MALDI-TOF spectra of FD-NO_2 after addition of 100 μM Na_2S in MeOH.

2. Determination of the detection limit (LOD)

The detection limit was calculated according to the method described in reference.[S4] The emission spectrum of 2 μM FD-NO_2 in PBS buffer was collected for 30 times to determine the background noise σ. A linear regression curve was fitted according to the emission intensity at 518 nm as a function of the concentrations of H_2S in the range of 0 -

100 μM, and the slope of the curve was obtained. The detection limit (3σ/slope) was then determined to be 1 μM.

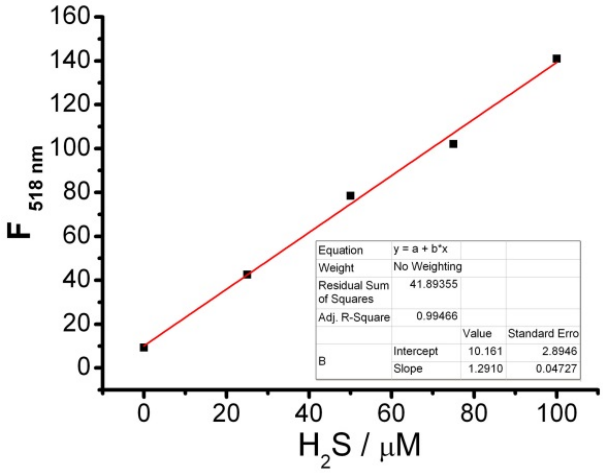

Fig. S3 Fluorescence intensities of FD-NO_2 at 518 nm as a function of the concentrations of H_2S in the range of 0 - 100 μM upon excitation at 480 nm and the calculation of the detection limit of probe FD-NO_2 for H_2S.

3. Determination of quantum yields

Fluorescence quantum yield was determined in the reference of fluorescein (Φ = 0.85) in 0.1 M aqueous NaOH.[S5] The quantum yield of FA is calculated according to following equation.

$\Phi_x=\Phi_s(A_sS_x)/(A_xS_s)$

Φ_s is the fluorescence quantum yield of fluorescein, A_x and A_s are the absorbance of FA and the standard. S_x and S_s are integrated fluorescence emission corresponding to FA and the standard.

4. pH Effect on the Fluorescence Properties

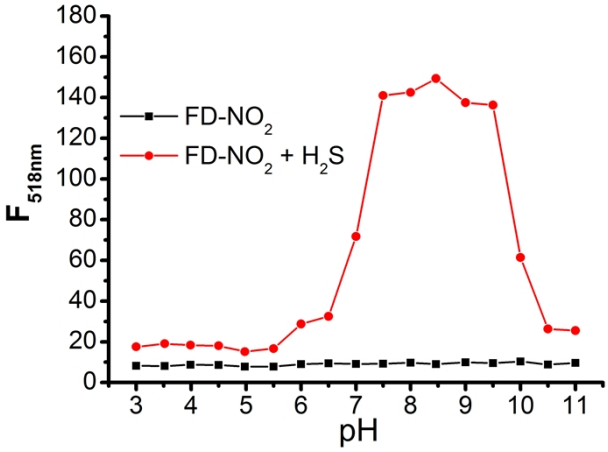

Fig. S4 Effect of pH on fluorescence intensities at 518 nm for FD-NO_2 (2 μM) and FD-NO_2 (2 μM) to H_2S (100 μM) for 50 min in PBS buffer upon excitation at 480 nm.

5. Solubility

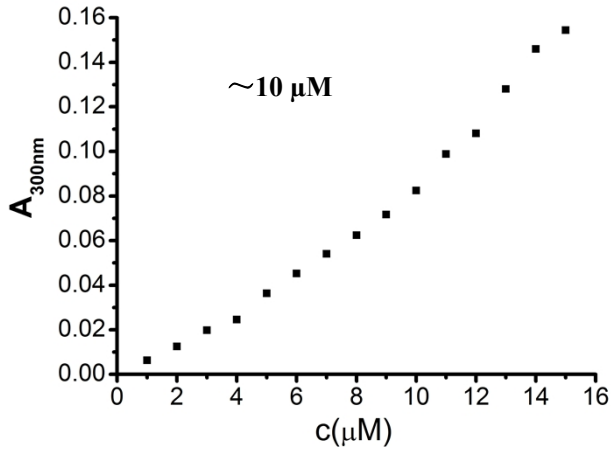

Fig. S5 Plot of absorbance at 300 nm as a function of FD-NO_2 concentration in PBS buffer (pH 7.4).

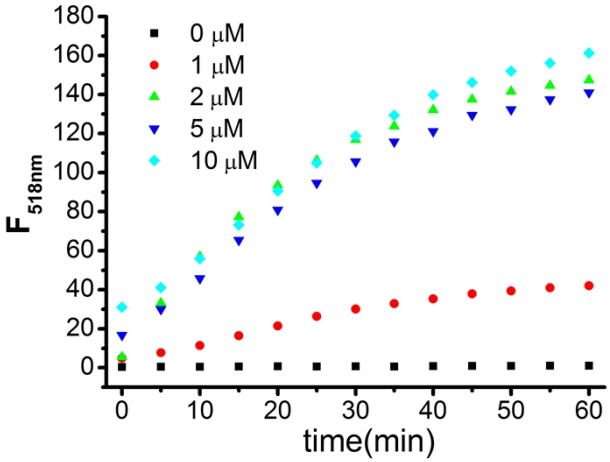

Fig. S6 Plot of fluorescence intensity at 518 nm *vs.* the reaction time in the presence of varied concentrations of FD-NO_2: 0 (control), 1, 2, 5 and 10 μM. The measurements were performed at 25 °C in PBS buffer (pH 7.4) with H_2S (100 μM) upon excitation at 480 nm.

6. Confocal Fluorescence Microscopy

Confocal fluorescence imaging experiments were performed on an Olympus FV-1000 laser scanning microscopy system, based on an IX81 (Olympus, Japan) inverted microscope. The microscope was equipped with multiple visible laser lines (405, 458, 488, 515, 543, 635 nm, CW) and UPLSAPO 60×/N.A 1.42 objective.

7. Cell Culture Methods

NIH 3T3 cells were obtained from Cell Culture Center, Institute of Basic Medical Sciences Chinese Academy of Medical Sciences; School of Basic Medicine Peking Union Medical College.

NIH 3T3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal calf serum (FCS, Gibco), 50 μg/mL penicillin/streptomycin (Hyclone) at 37°C in a 5/95 CO_2/air incubator. The cells were cultured 3 days before dye loading on a 35 mm diameter glass-bottomed coverslips. Then the cells were incubated with 10μM probe FD-NO_2 in serum-free DMEM (0.5 % DMSO, v/v) for 30 min at 37°C under 5% CO_2, and then washed with PBS three times. The FD-NO_2-loaded cells were

incubated in serum-free DMEM containing H_2S (250 μM) for another 30 min at 37 °C, the cells were then washed with PBS three times and bathed in PBS (1 mL) before imaging. As a control, the FD-NO_2-loadedcells incubated in serum-free DMEM without H_2S for 30 min at 37 °C were also imaged. To further prove that the fluorescence change of FD-NO_2in the cells arises from H_2S, another control experiment was carried out. The cells were pretreated with 500 μM $ZnCl_2$(an efficient H_2Sscavenger)[S2] for 30 min, then incubated with 10μM FD-NO_2 for 30 min, and further treated with 250 μM H_2S for 30 min at 37 °C. Excitation wavelength of laser was 488 nm. Emissions were collected at 500 - 600 nm. Images were gathered and processed with Olympus FV10-ASW software (Ver. 2.1c)

Reference

(S1) Z. N. Sun, F. Q. Liu, Y. Chen, P. K. H. Tam and D. Yang, *Org. Lett*., 2008, **10**, 2171.
(S2) H. J. Peng, Y. F. Cheng, C. F. Dai, A. L. King, B. L. Predmore, D. J. Lefer and B. H. Wang, *Angew. Chem., Int. Ed*., 2011, **50**, 9672.
(S3) S. Chen, J. Lu, C. Sun and H. Ma, *Analyst,* 2010, **135**, 577.
(S4) Y. Chen, C. Zhu, Z. Yang, J. Chen, Y. He, Y. Jiao, W. He, L.Qiu, J. Cen and Z. Guo, *Angew. Chem., Int. Ed.,* 2013, **52**, 1688.
(S5) T. Wang, Jr., E. F. Douglass, K. J. Fitzgerald and D. A. Spiegel, *J. Am. Chem. Soc.,* 2013, **135**, 12429.

8. HRMS-ESI Spectra

Peking University Mass Spectrometry Sample Analysis Report

Analysis Info

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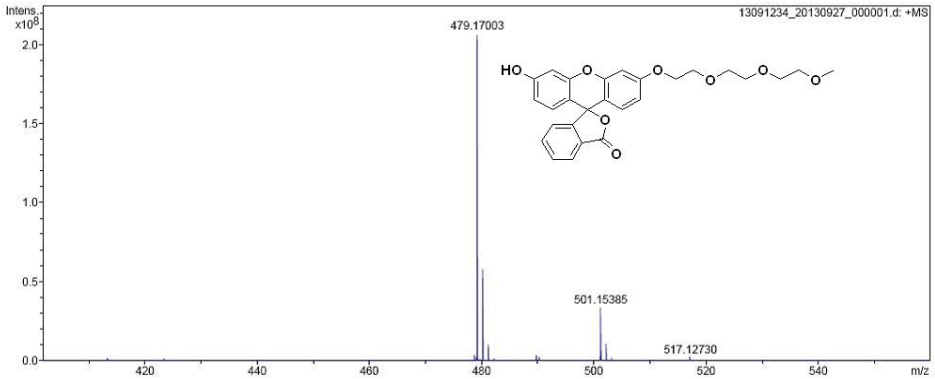
Comment	ESI Positive	Operator	Peking University



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Peking University Mass Spectrometry Sample Analysis Report

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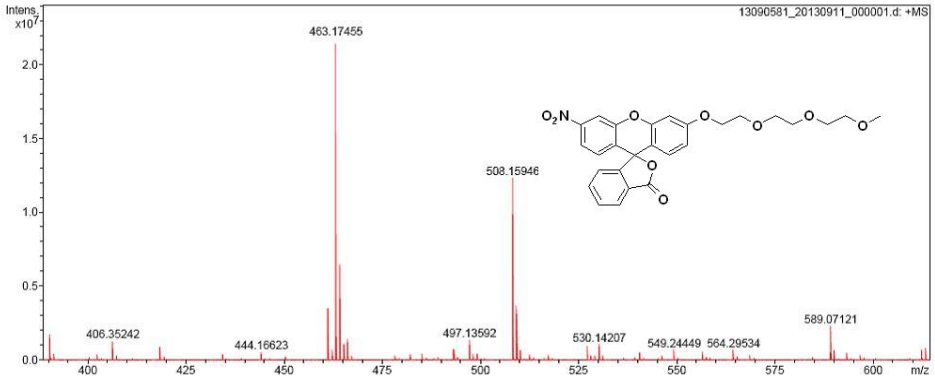

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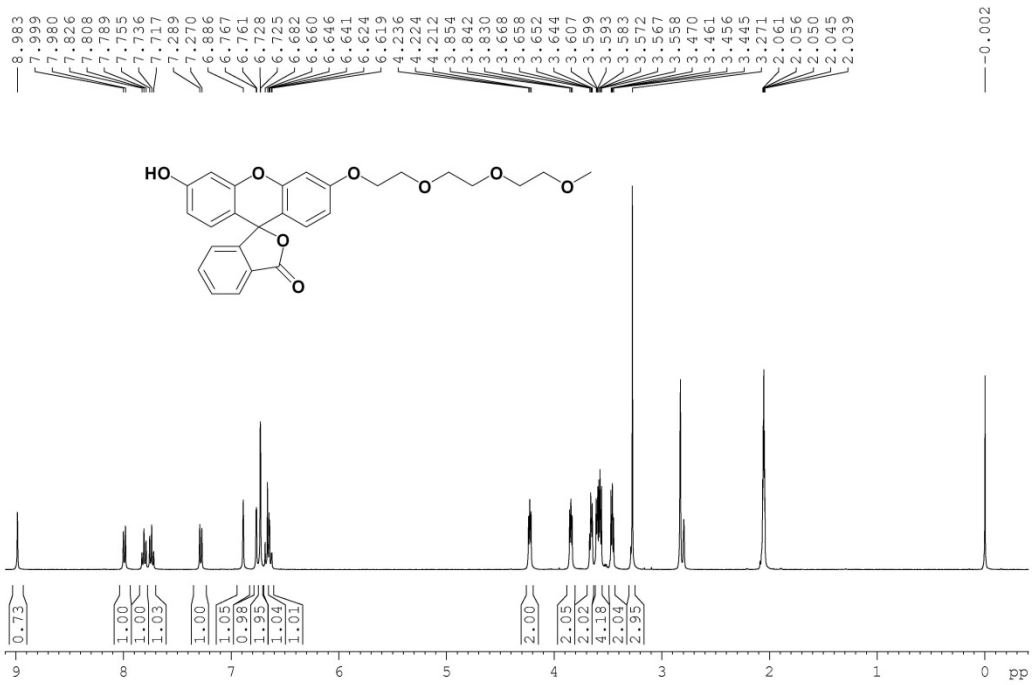
9. NMR Data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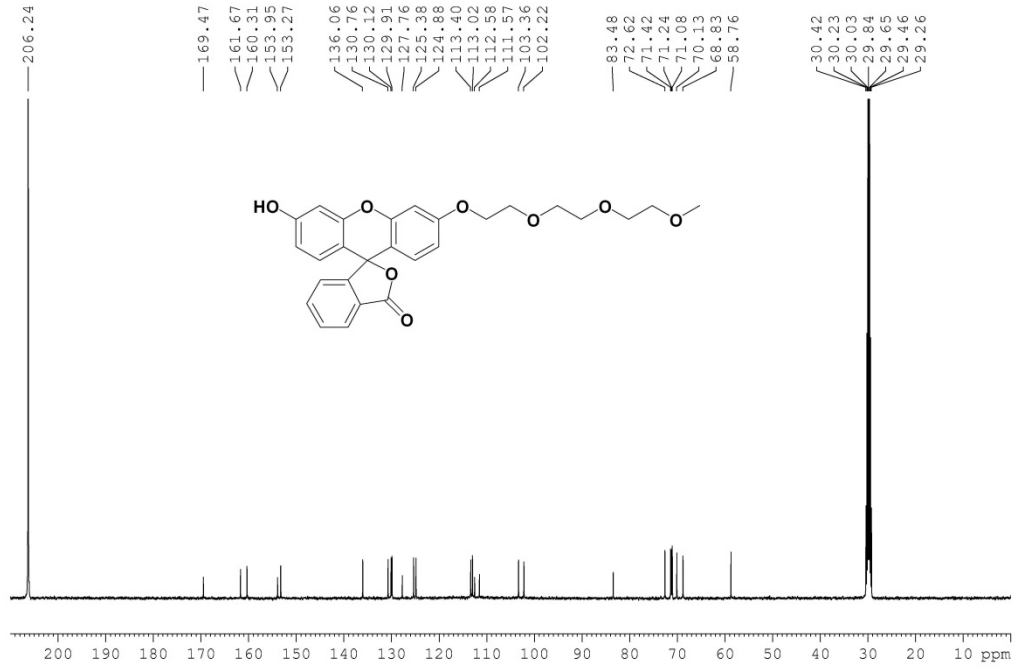
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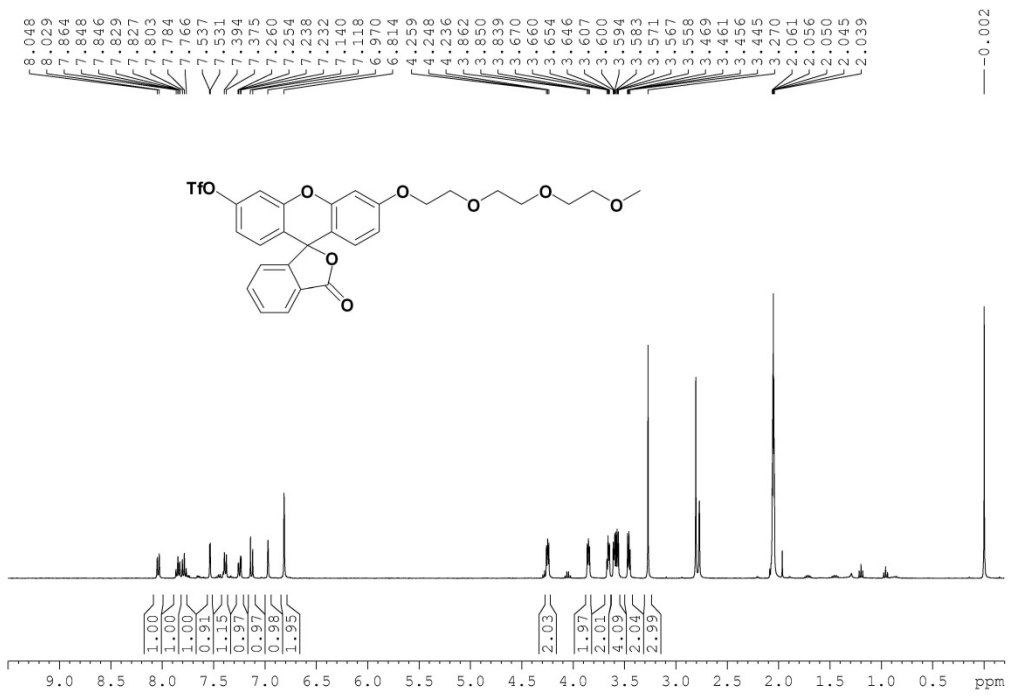
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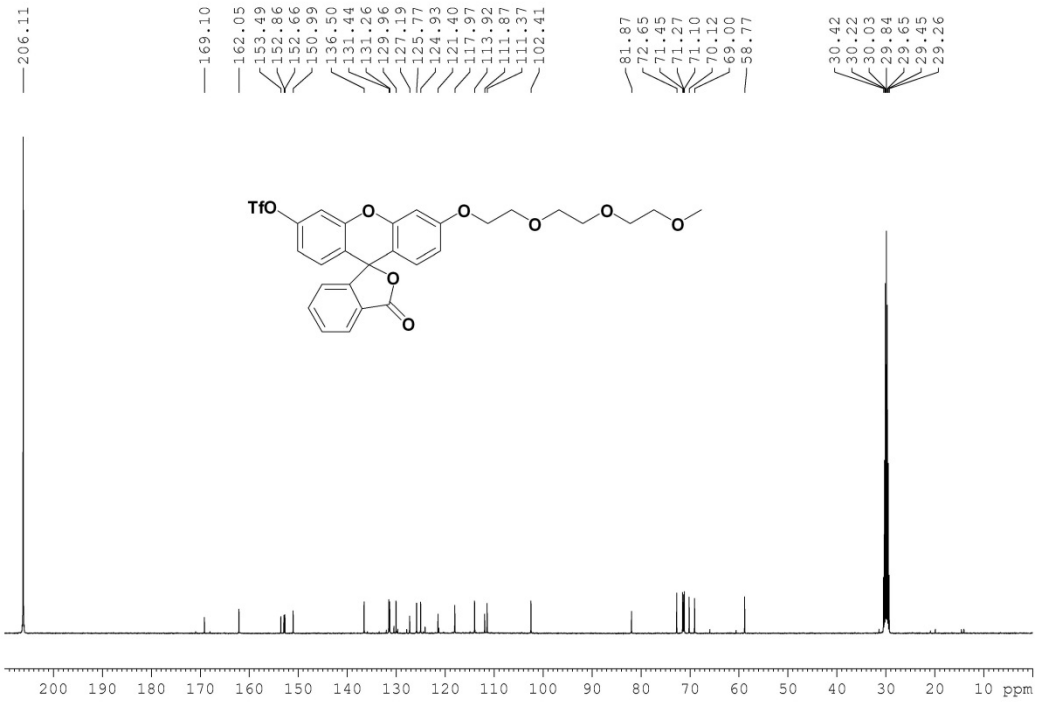
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