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Supporting Information

A mitochondria-targeted red-emitting probe for imaging of hydrogen sulfide in living cells and zebrafish

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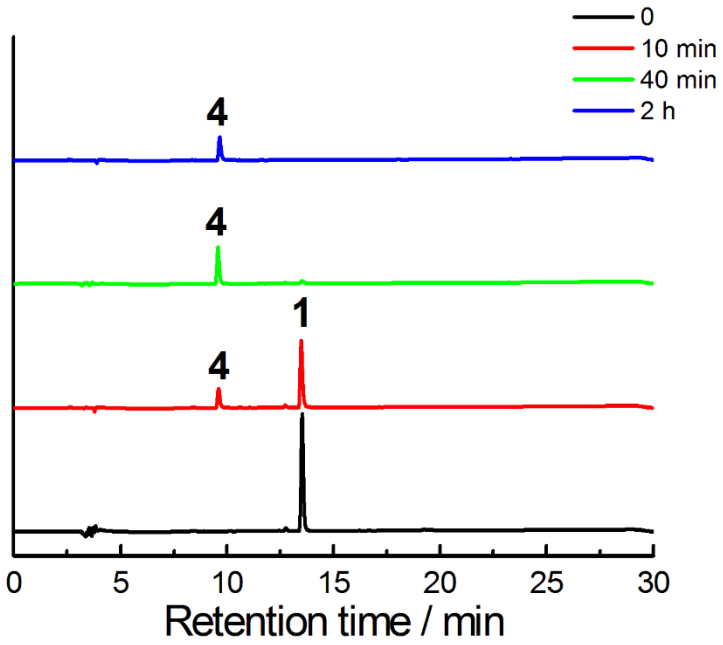


Figure S1. HPLC trances of the probe **1** (0.25 mM) and H_2S (5 mM) at different reaction time (inset). Conditions: ANGELA TECHNOLOGIES HPLC LC-10F; C18 column with 4.6 mm x 250 mm; wavelength: 273 nm; flow 1.0 mL / min; buffer A: 0.1% (v / v) trifluoroacetic acid in water; buffer B: MeOH; elution condition: 0-3 min, B: 5-50%; 3-13 min, B: 50-80%; 13-25 min, B: 80-95%; 25-30 min, B: 95-5%; 30-32min, B: 5%.

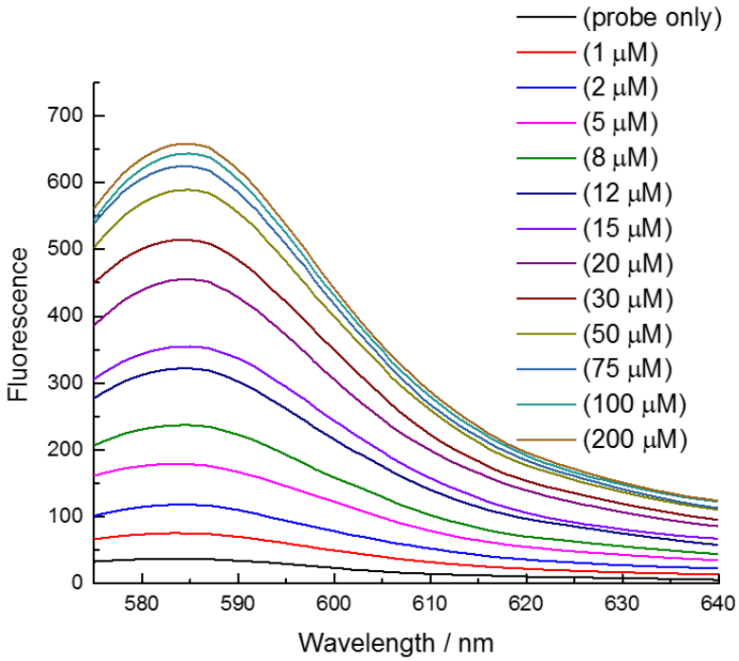


Figure S2. The titration of the probe **1** (5 μM) with variable concentrations of H_2S (0-200 μM). The emission spectra were excited with 565 nm.

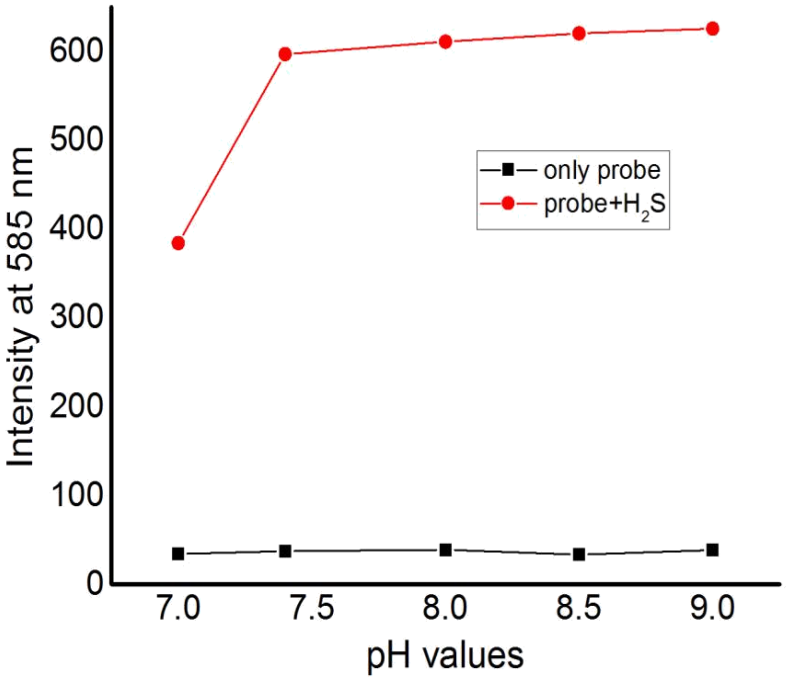
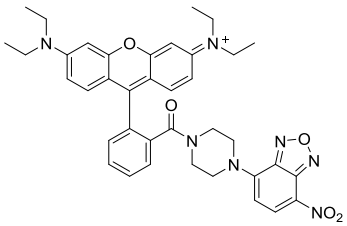
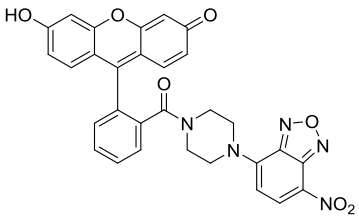
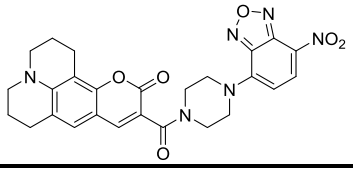
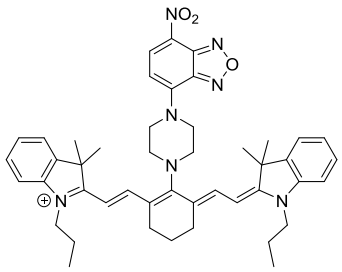
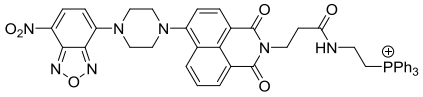
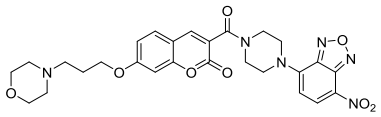
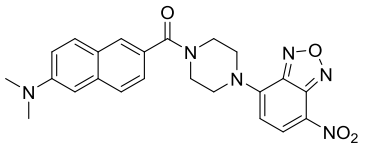


Figure S3. The emission intensity at 585 nm of **1** (5 μM) at the indicated pH values in the absence or presence of H_2S (100 μM).

Table S1. The properties of several NBD-based H_2S probes.

Probe	λex/λem (nm)	Fluor. enhancement	ϕ	LOD/μM	Rate/k_2	Ref
1	565/585	~19	0.77	0.36	27.8 $M^{-1}s^{-1}$	This work
	567/589	~4.5	0.36	0.58	113 $M^{-1}s^{-1}$	1
	502/530	~65	0.64	0.057	28 $M^{-1}s^{-1}$	1
	449/496	~200	0.81	0.9	7.6 $M^{-1}s^{-1}$	2

	730/796	~87	ND	0.04	14.9 $M^{-1}s^{-1}$	3
	394/532	~68	ND	2.46	20.4 $M^{-1}s^{-1}$	4
	415/560	~273	ND	0.43	6.8 $M^{-1}s^{-1}$	5
	330/468	~29	ND	0.024	ND	6

ND, not determined.

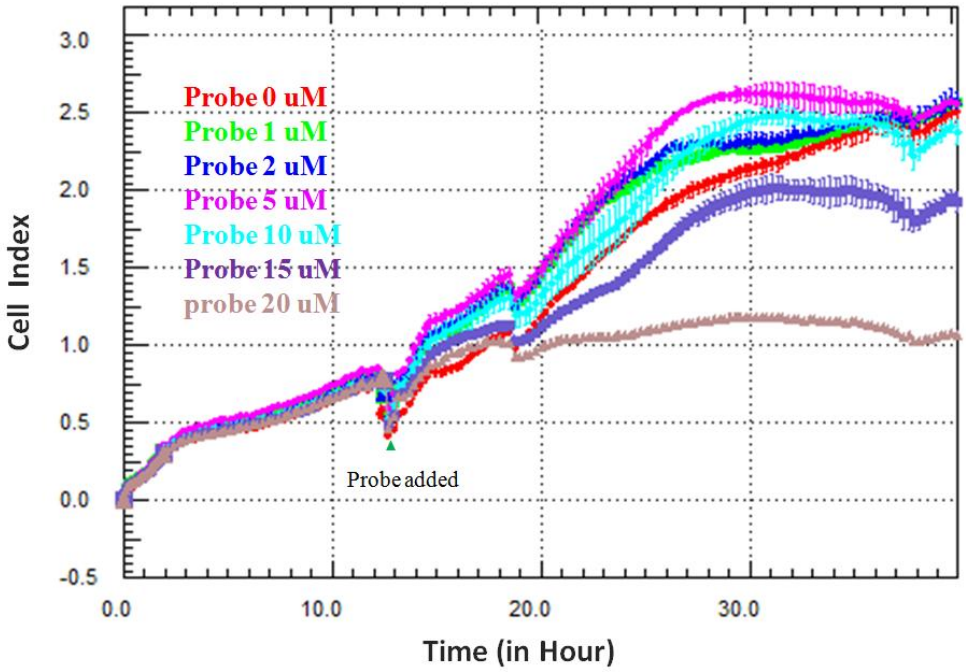


Figure S4. Evaluation on the cytotoxicity of the probe using xCELLigence RTCA system. Real-time monitoring of the density-dependent growth and proliferation of HEK293 cells with different probe concentration. The cell index values with time can reflect the adhesion number of cells inside the well.

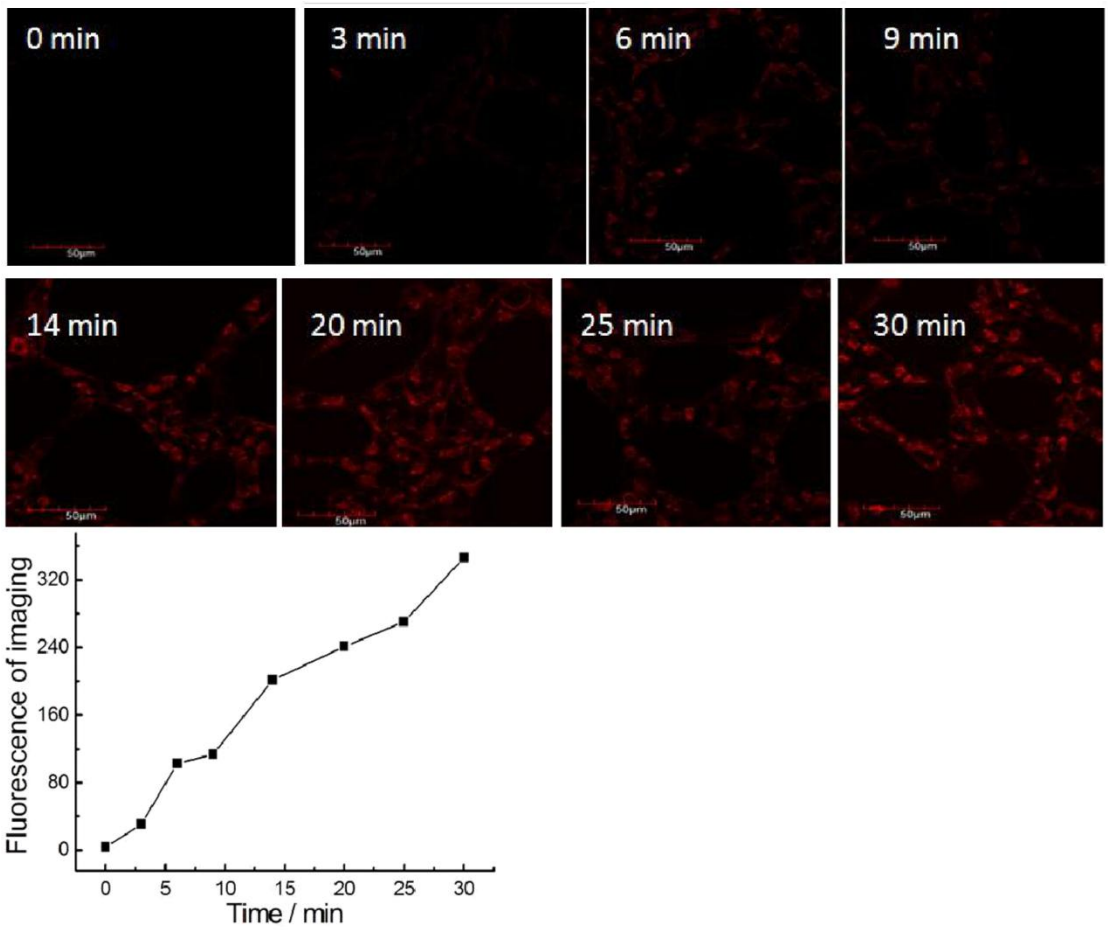


Figure S5. Fluorescence imaging for the L-Cys-induced H_2S in living cells. Cells were incubated with L-Cys (100 μM) for 30 min, then washed cells and incubated with probe **1** (5 μM) at different time intervals (0-30 min). The average fluorescence of the time-dependent images is shown below.

Supporting references:

1 R. Wang, Z. Li, C. Zhang, Y. Li, G. Xu, Q. Z. Zhang, L. Y. Li, L. Yi and Z. Xi, *ChemBioChem*., 2016, 17, 968.

2 I. Ismail, D. Wang, D. Wang, Z. Wang, C. Zhang, L. Yi and Z. Xi, *Dyes Pigments*, 2019, 163, 706.

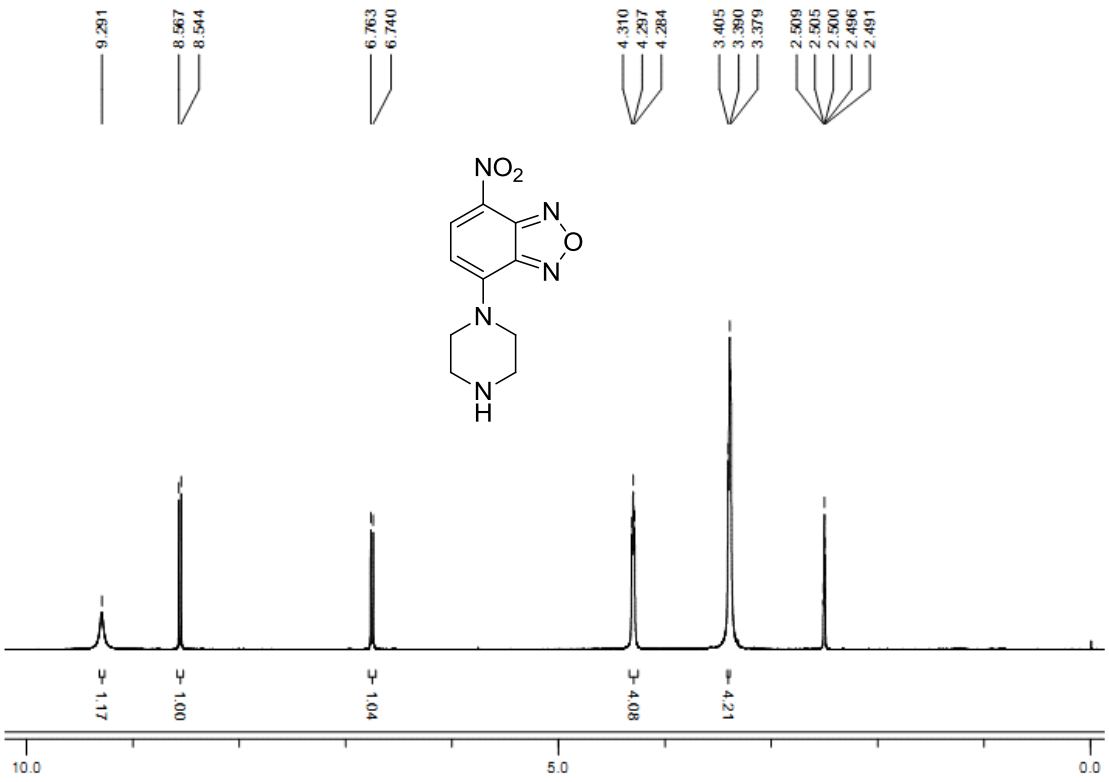
3 K. Zhang, J. Zhang, Z. Xi, L. Y. Li, X. X. Gu, Q. Z. Zhang and L. Yi, *Chem Sci*., 2017, 8, 2781.

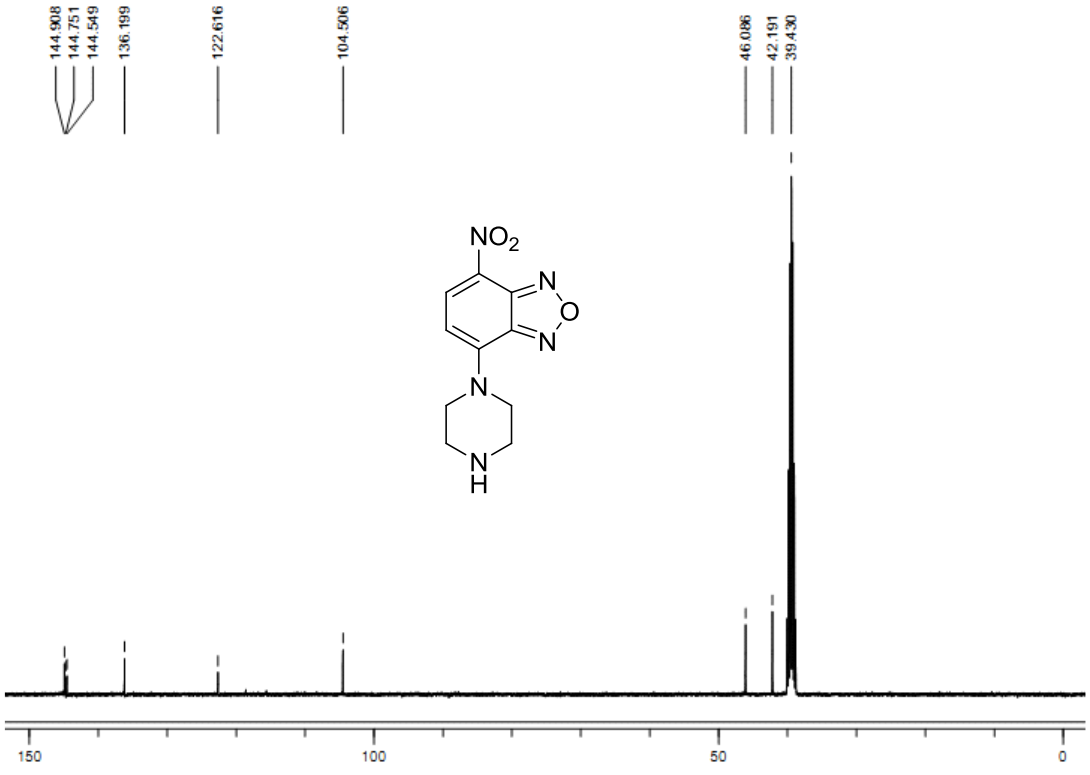
4 Y. L. Pak, J. Li, K. C. Ko, G. Kim, J. Y. Lee and J. Yoon, *Anal Chem*., 2016, 88, 5481.

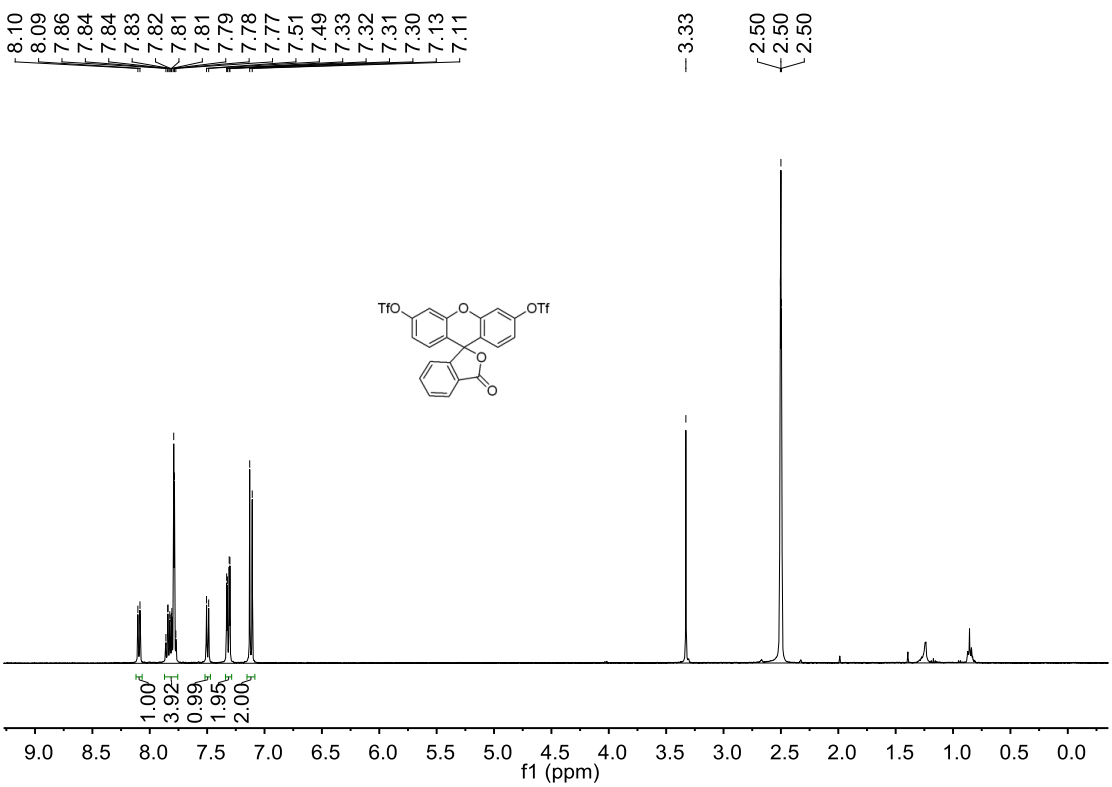
5 J. Zhang, R. Y. Wang, Z. T. Zhu, L. Yi and Z. Xi, *Tetrahedron*., 2015, 71, 8576.

6 Y. Tang and G. F. Jiang, *New J Chem*., 2017, 41, 6774.

2. Supporting spectra



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TfO
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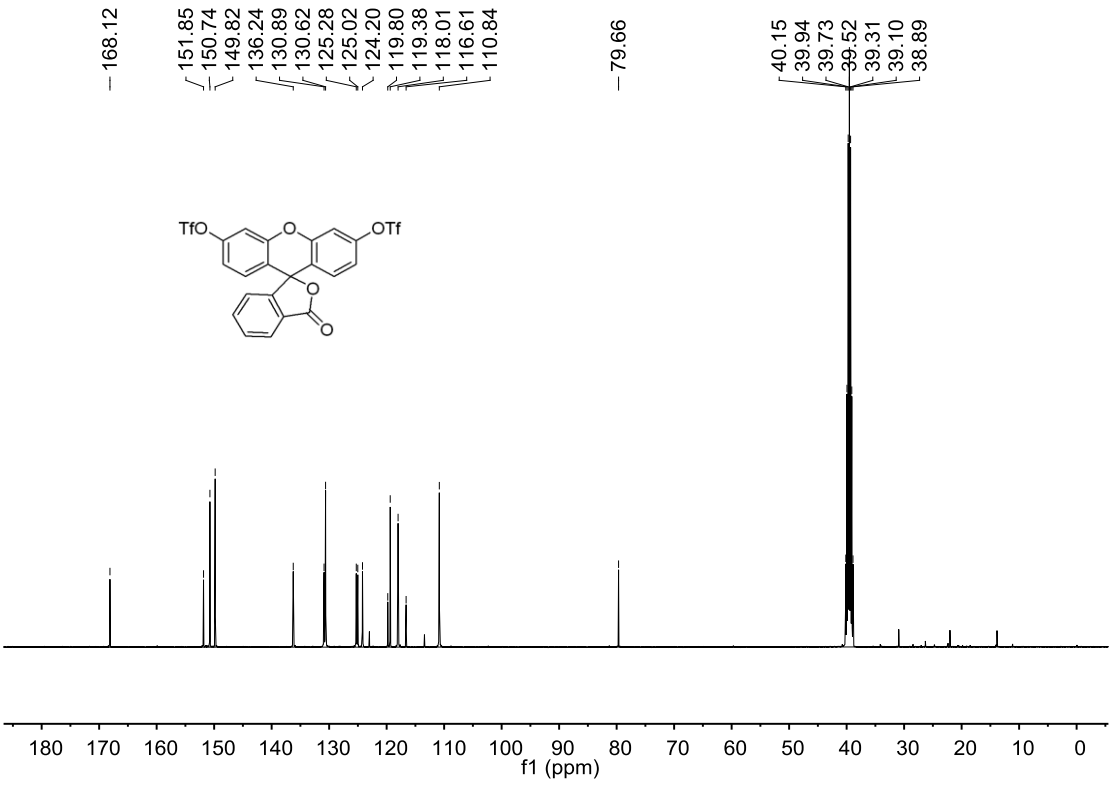
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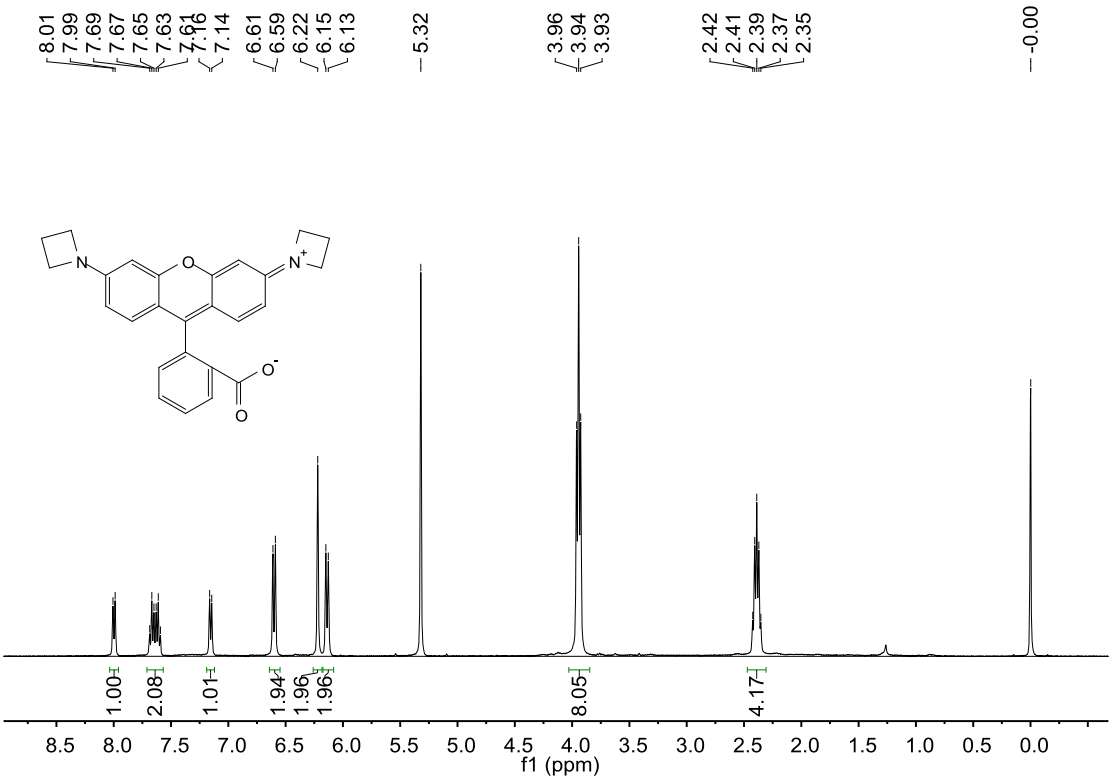
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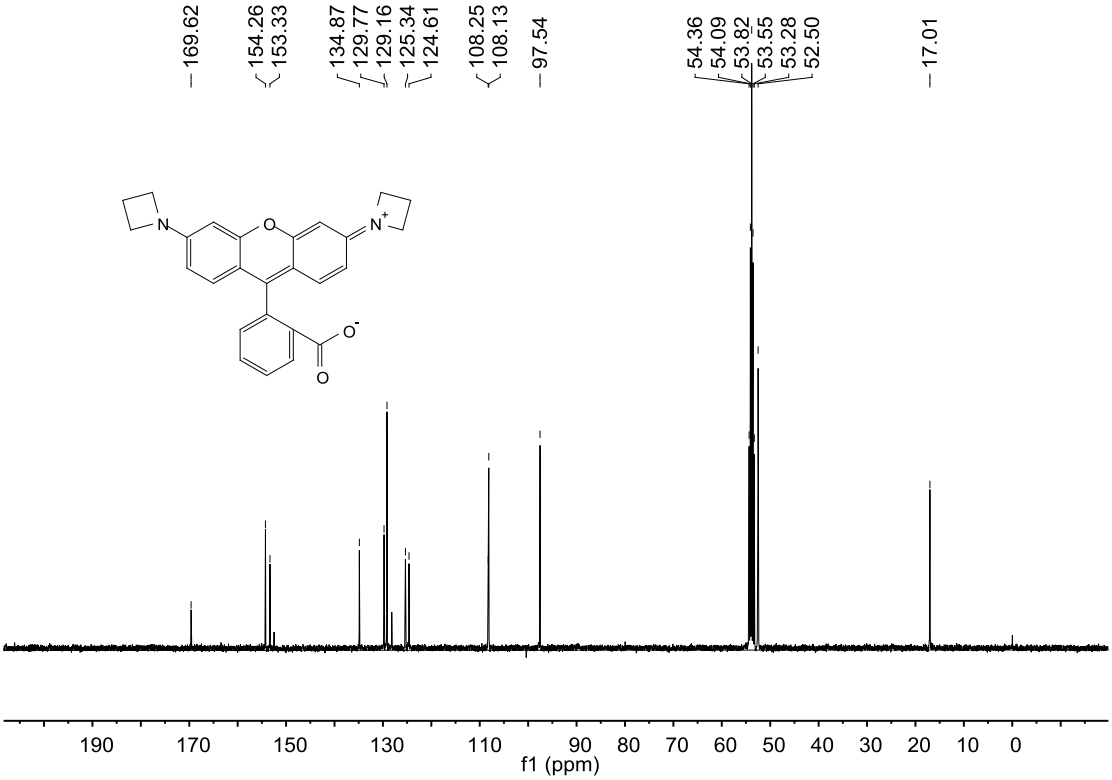
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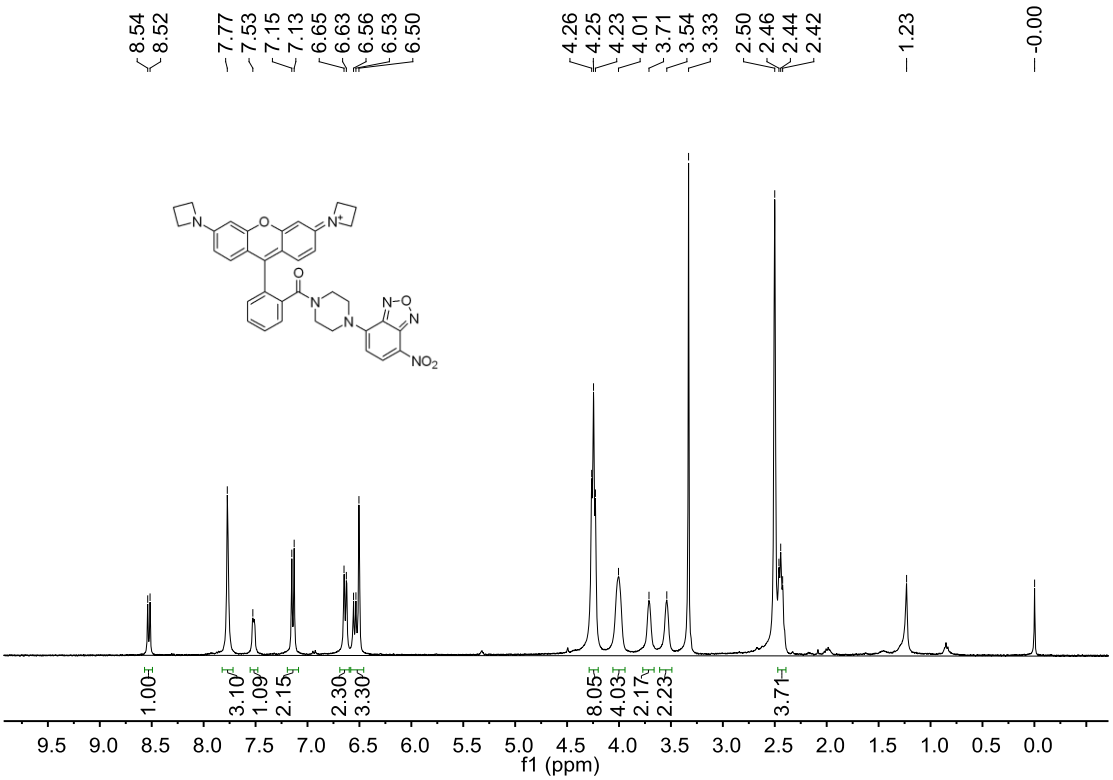
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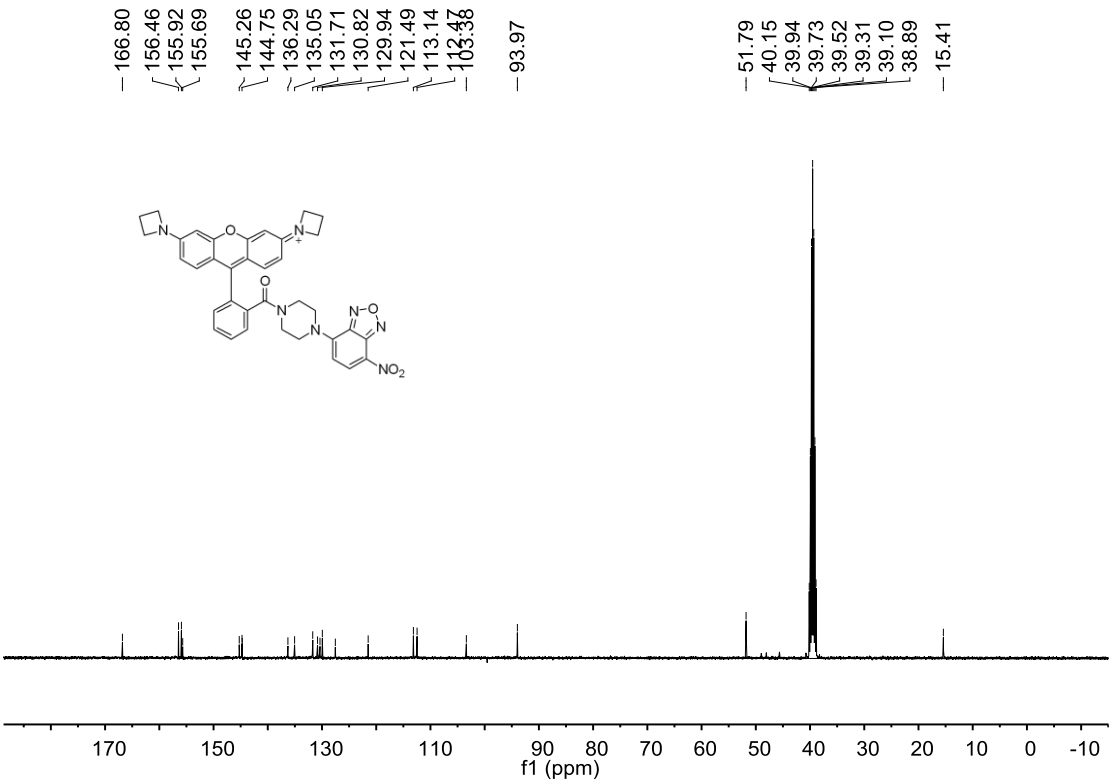
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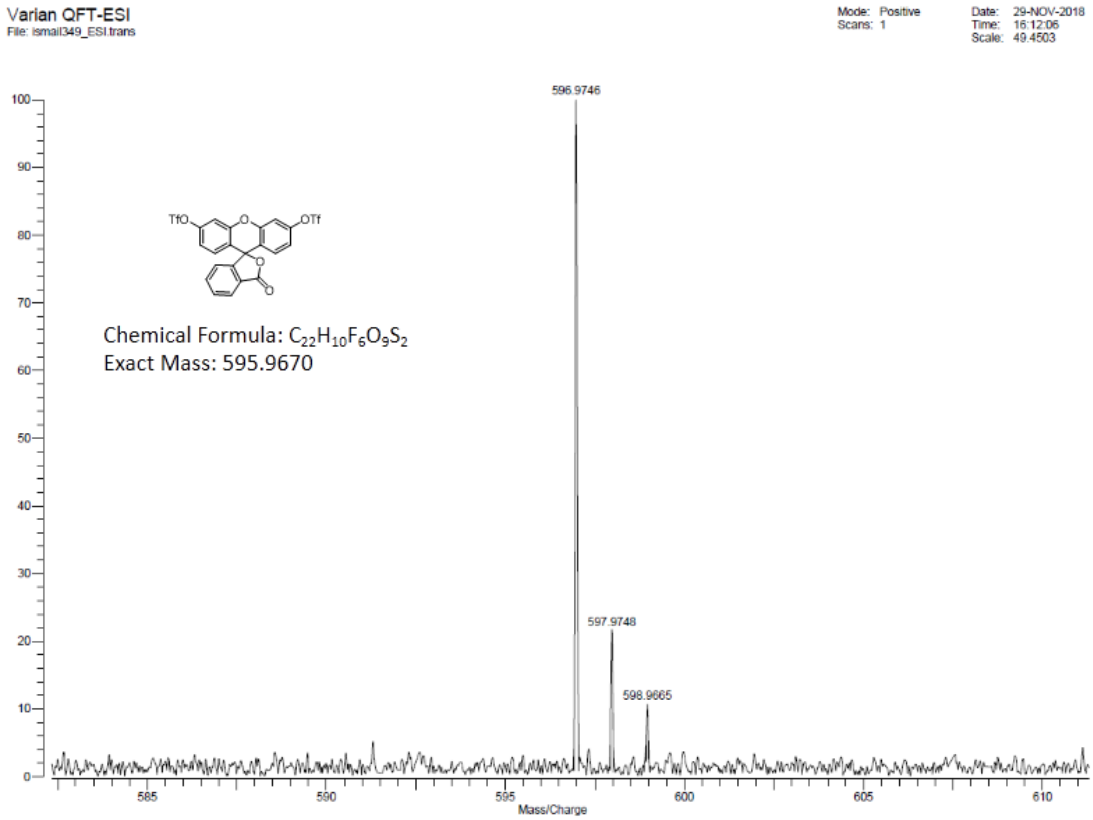
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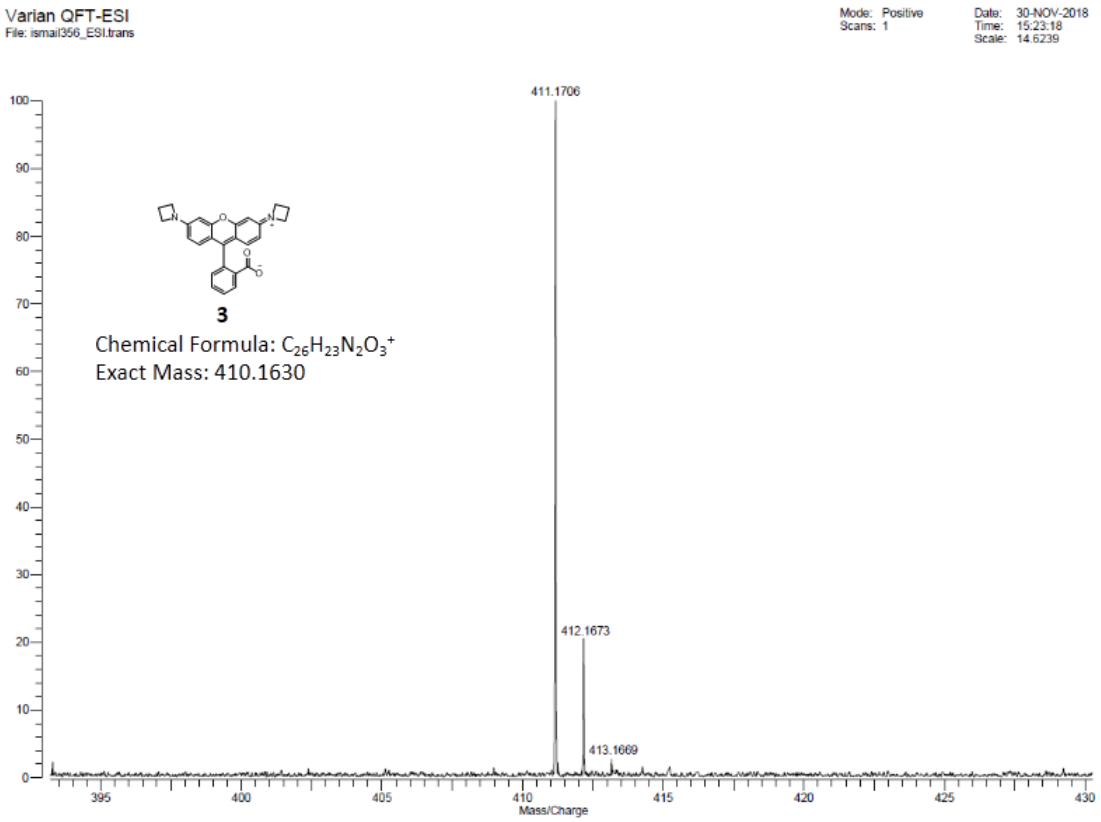
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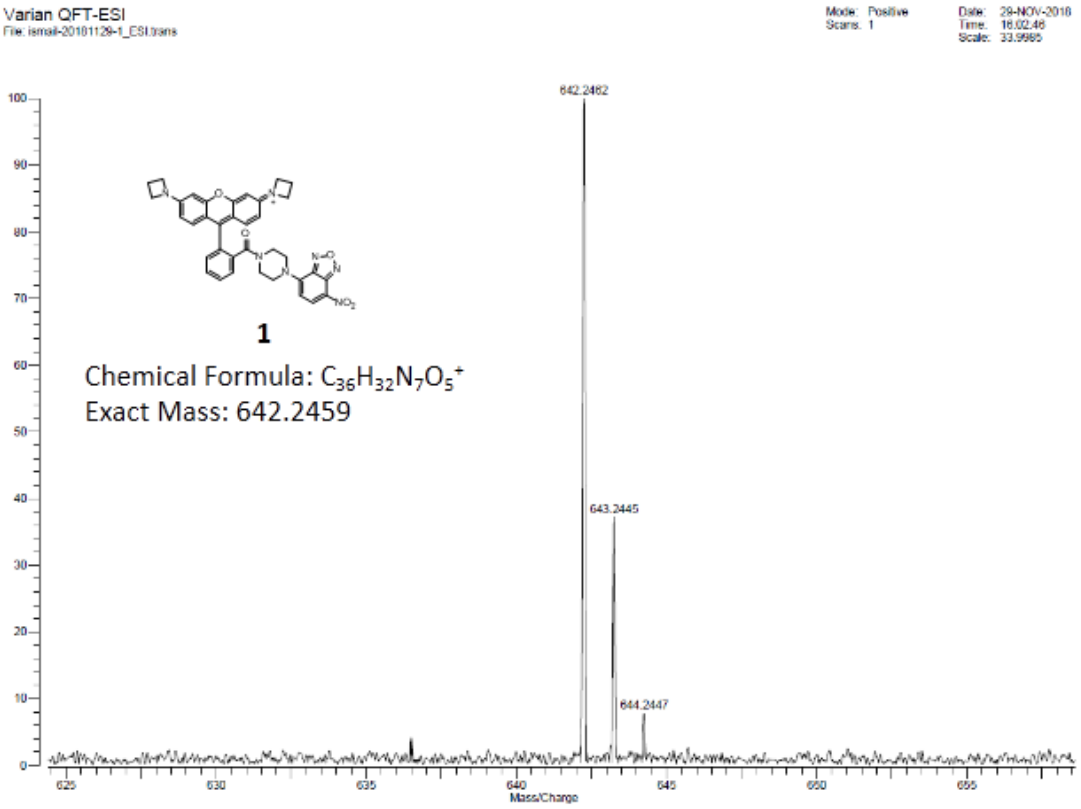
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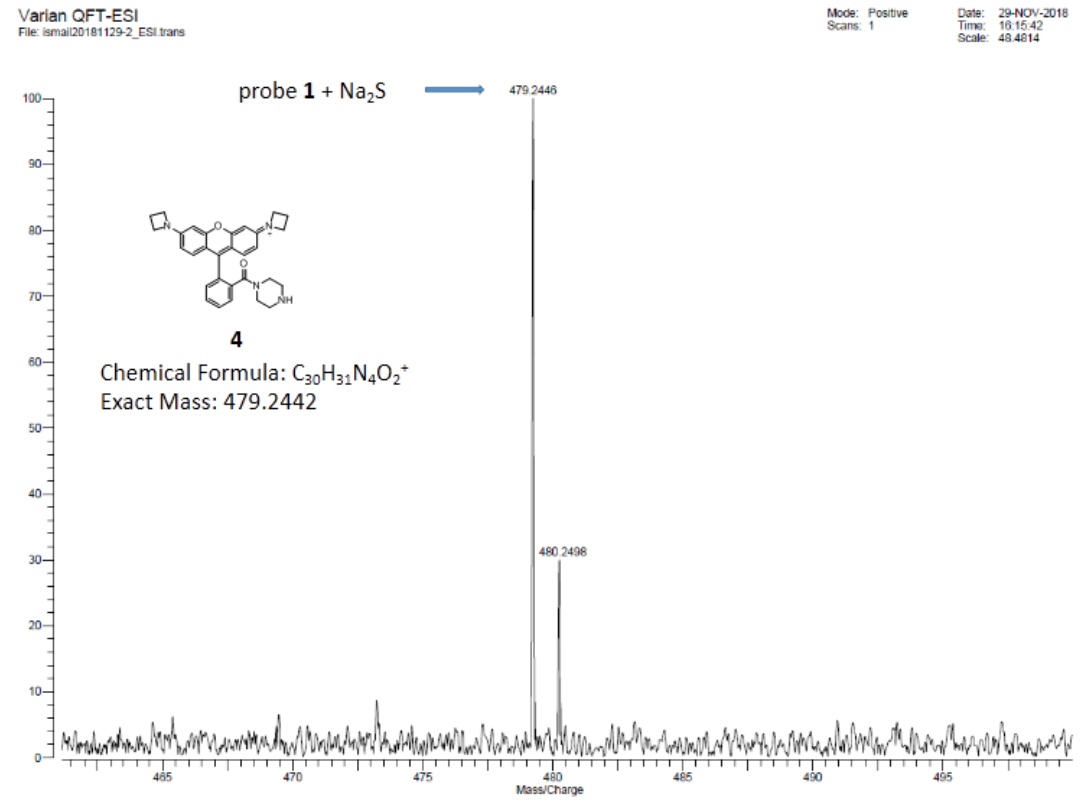
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