

Supporting information

A Two-photon Fluorescent Probe for Imaging of Endogenous Formaldehyde in HeLa Cells and Quantitative Detection of Basal Formaldehyde in Milk Samples

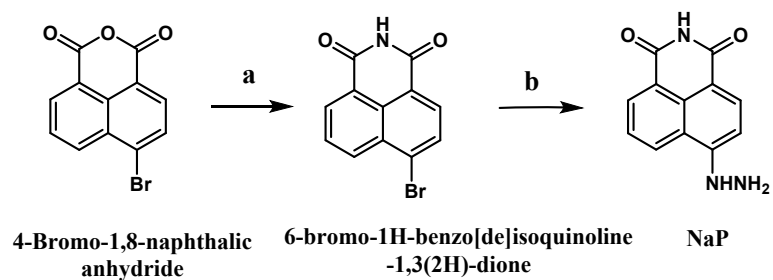
Fangyun Xin, Yong Tian, Jing Jing* and Xiaoling Zhang*

Beijing Key Laboratory of Photo-electronic/Electro- photonic Conversion Materials, Key Laboratory of Cluster Science of Ministry of Education, School of Chemistry and Chemical Engineering, Analytical and Testing Centre, Beijing Institute of Technology, Beijing 100081, P. R. China.

E-mail: zhangxl@bit.edu.cn, hellojane@bit.edu.cn

Contents

1. Scheme S1. The synthesis route of NaP -----	S3
2. Fig. S1. The two-photon fluorescence emission spectra-----	S3
3. Fig. S2. Cell viability of the HeLa Cells by MTT assay-----	S3
4. Table S1. Validation parameters for formaldehyde in milk samples-----	S4
5. Table S2. Recovery of three concentrations of spiked formaldehyde in six milk samples-----	S4
5. The calculation equation of bias-----	S4
6. Fig. S3-Fig. S10. Spectra of NMR and HRMS-----	S5-S7



Scheme S1 The synthesis route of NaP. (a) 28% ammonium hydroxide aqueous solution, 45°C, 4 h. (b) 80% hydrazine hydrate, n-butanol, N₂, reflux 3h.

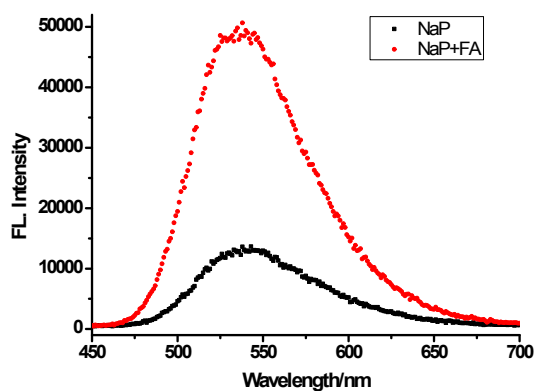


Fig. S1 Two-photon fluorescence emission of the probe NaP before and after treatment with FA. Excited at 820nm.

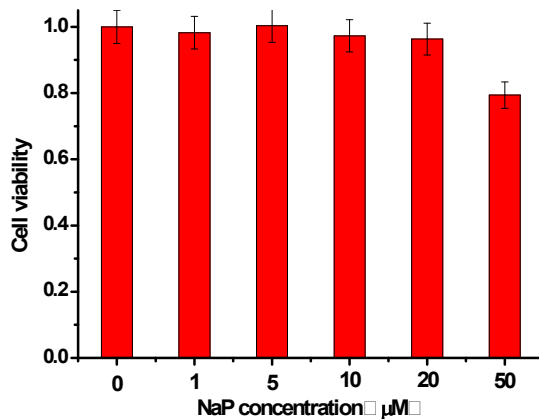


Fig. S2 Effects of probe NaP with different concentrations on the viability of the HeLa Cells. The probe with varied concentrations was incubated with the HeLa cells for 24 h. The viability of the cells in the absence of the probe was defined as 1, and the data were the mean standard deviation of five separate measurements.

Table S1 Validation parameters for formaldehyde in milk samples

Sample	Formaldehyde Conc. (µg/mL)	Standard deviation	Coefficient variation
Sample 1	1.21±0.14	0.14	11.4%
Sample 2	1.60±0.34	0.34	21.3%
Sample 3	1.29±0.36	0.37	28.3%
Sample 4	1.37±0.25	0.25	18.5%
Sample 5	1.02±0.07	0.073	7.2%
Sample 6	1.49±0.45	0.45	30.0%

Table S2 Recovery of three concentrations of spiked formaldehyde in six milk samples

Sample	Formaldehyde (µM)						Recovery (%)		
	Spiked			Found					
Sample 1	10	20	30	11.4±0.3	14.3±0.3	16.0±0.2	106±4	67±1	49±3
Sample 2	10	20	30	11.9±0.4	14.0±0.4	16.7±1.6	108±3	64±2	50±7
Sample 3	10	20	30	11.5±0.1	13.1±1.3	15.6±0.2	109±5	62±9	48±5
Sample 4	10	20	30	11.8±0.7	14.0±0.3	15.4±0.4	109±8	65±2	48±3
Sample 5	10	20	30	11.9±0.6	15.1±1.2	16.3±1.1	112±5	72±6	51±5
Sample 6	10	20	30	11.6±0.2	14.7±0.8	16.7±0.7	106±1	68±4	49±6

The calculation equation of bias between the results of two methods for agreement evaluation.

$$S^2 = \frac{1}{N} \sum_{i=1}^N (X_i - \mu)^2 \quad (1)$$

Where s means standard deviation, x stands for formaldehyde concentration measured by fluorescence microscopy method, μ stands for mean value of formaldehyde concentration measured by MBTH method, N represents the number of samples.

According to the results, X_i are 1.21, 1.60, 1.29, 1.37, 1.02, 1.49 µg/mL, μ is 1.44 µg/mL and N=6. Therefore, $S^2= 0.047$, demonstrating good agreement between the results.

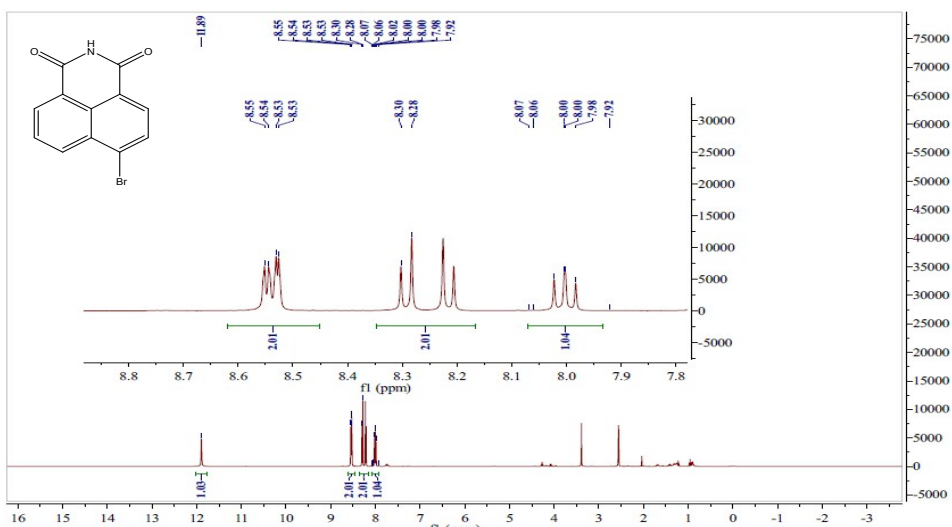


Fig. S3 ¹H NMR spectrum of the compound 2.

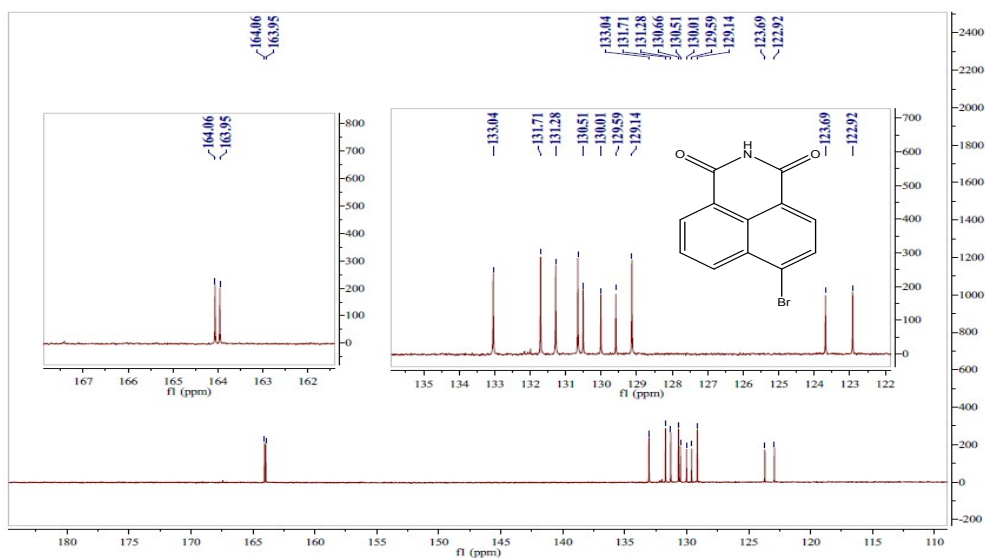


Fig. S4 ¹³C NMR spectrum of the compound 2.

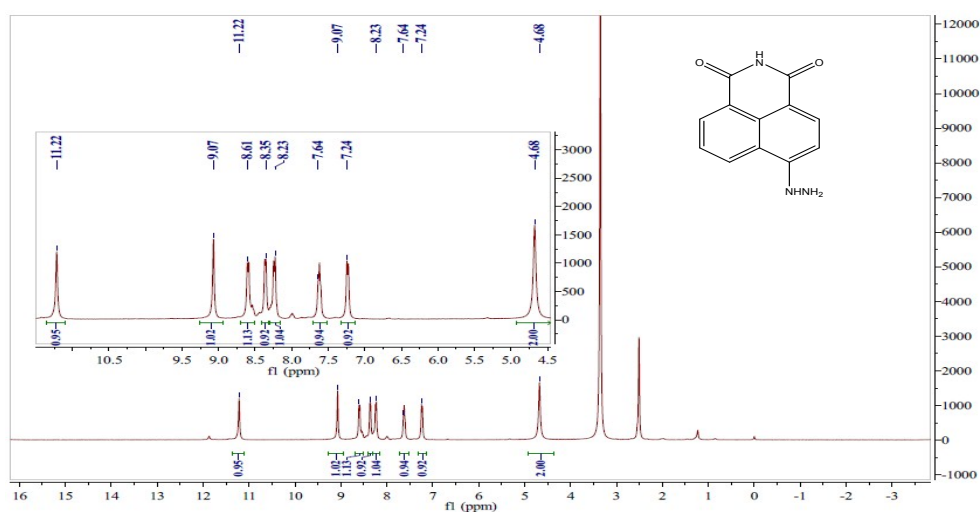


Fig. S5 ¹H NMR spectrum of the probe NaP.

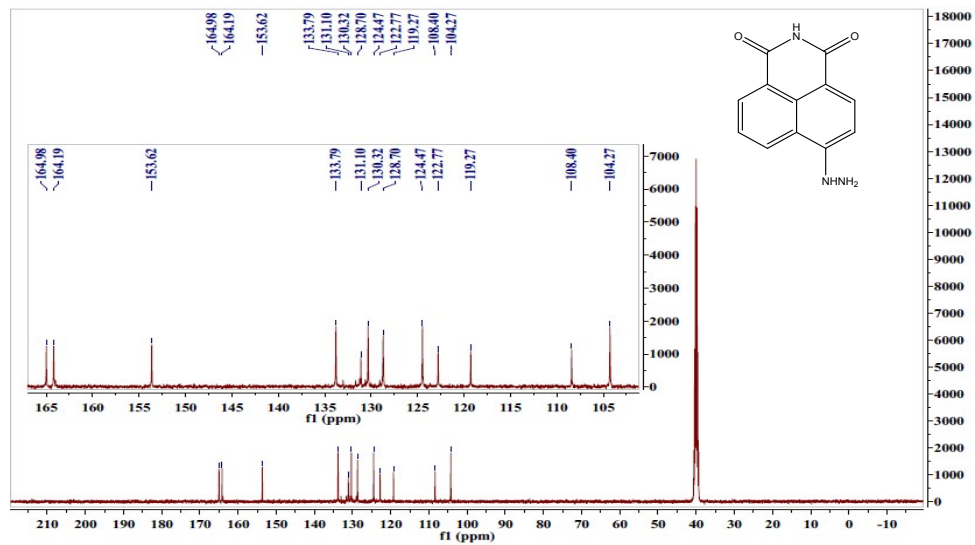


Fig. S6 ^{13}C NMR spectrum of the probe NaP.

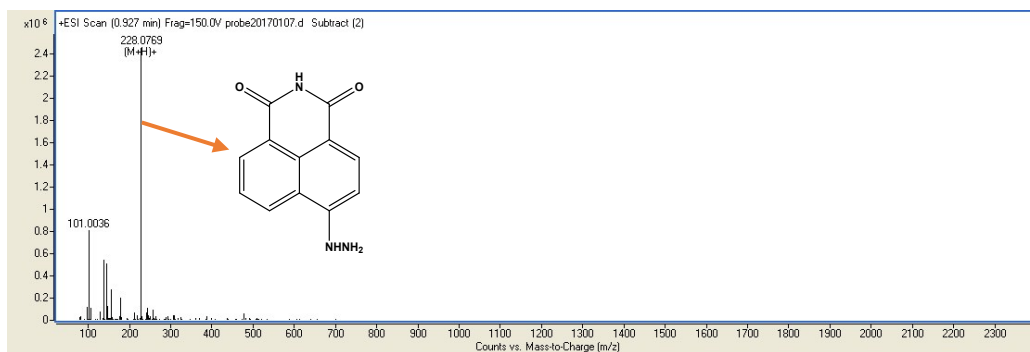


Fig. S7 HR-MS spectrum of the probe NaP.

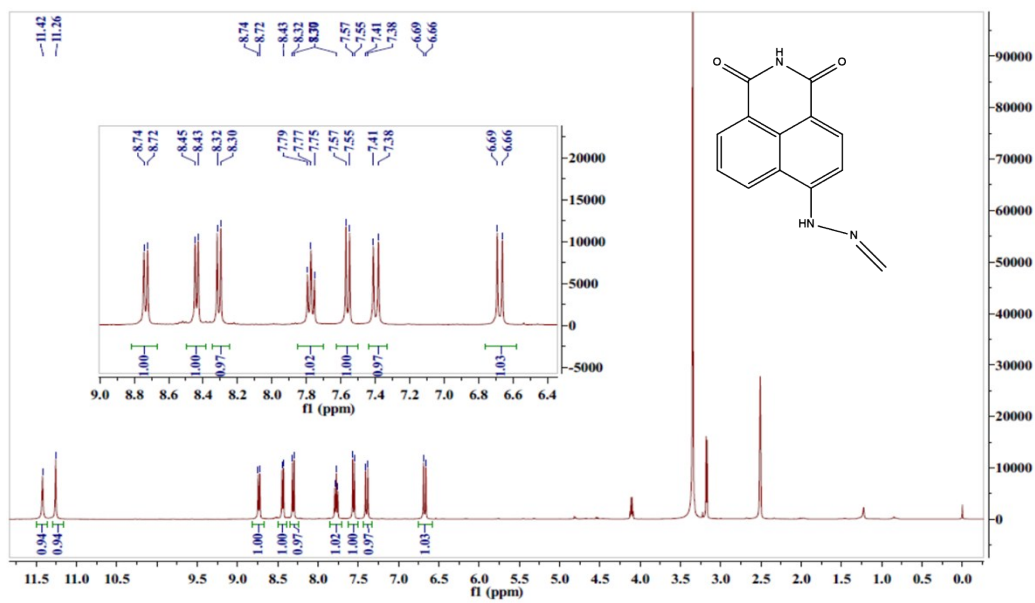


Fig. S8 ^1H NMR spectrum of NaPFA.

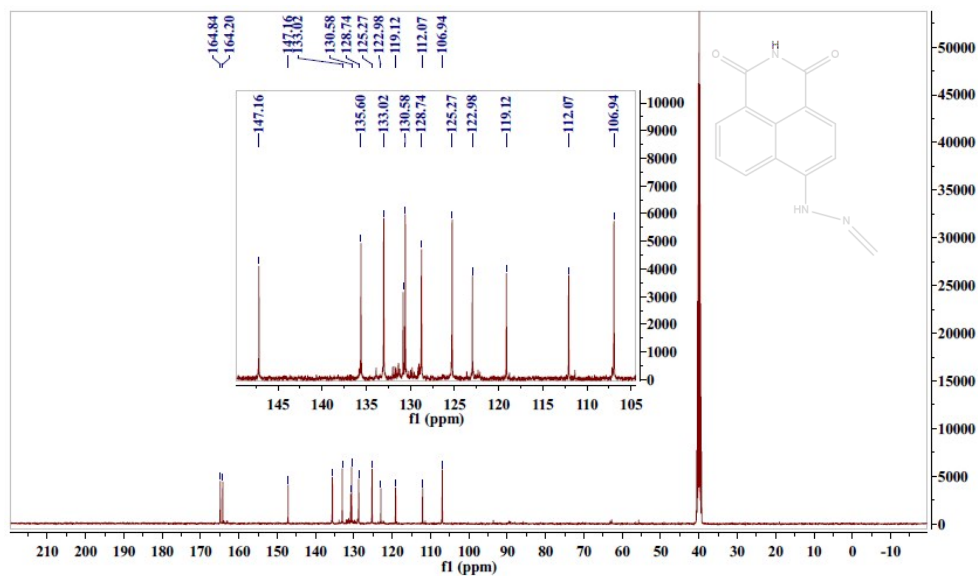


Fig. S9 ^{13}C NMR spectrum of the product of NaPFA.

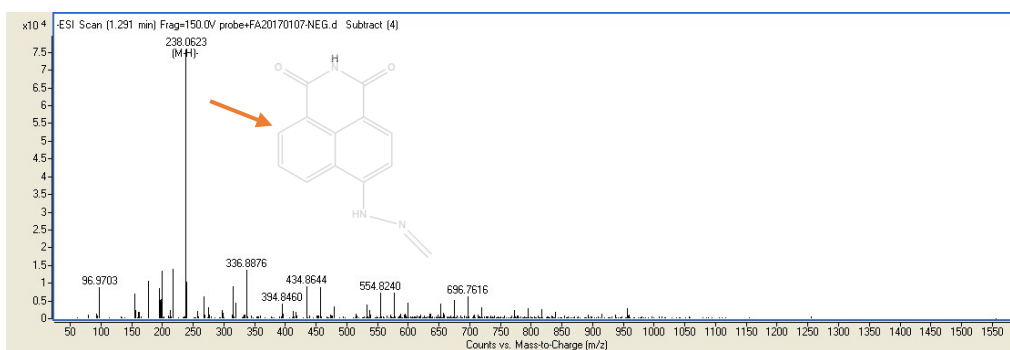


Fig. S10 HR-MS spectrum of the product of NaPFA.