Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

A two-photon fluorescent probe for selective methylglyoxal detection and application in living cells

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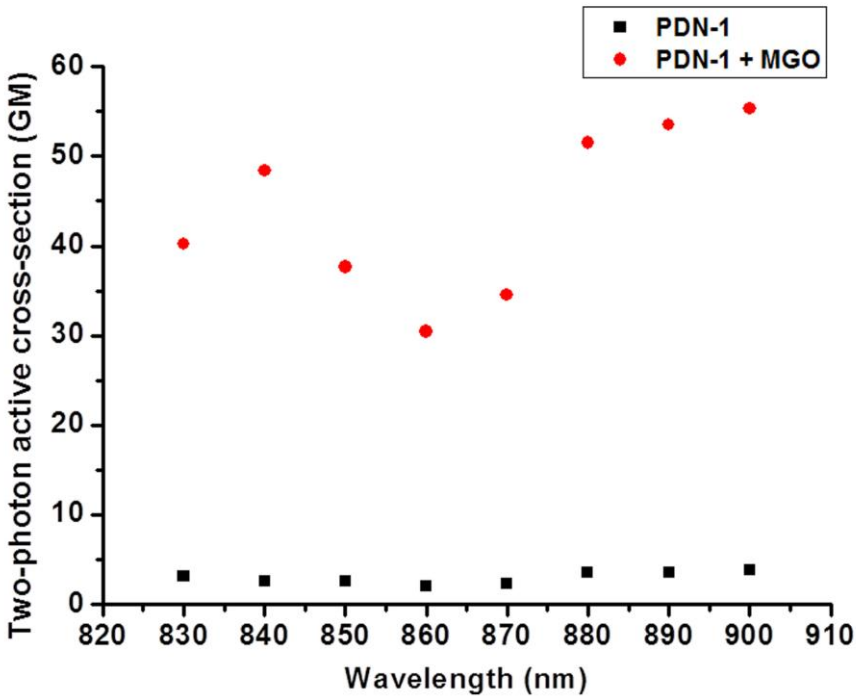


Fig. S1 The two-photon active cross-section spectra of PDN-1 (5 μM) in the absence (black dot) and presence (red dot) of MGO (50 μM). The reactions were carried out for 12 h at 37 °C in PBS buffer (10 mM, pH 7.4, containing 10% DMSO).

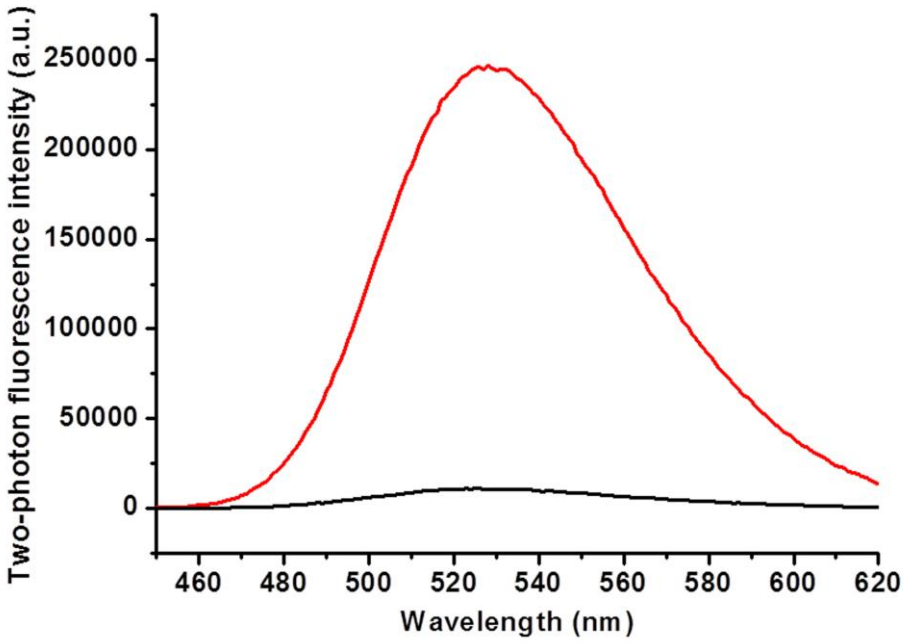


Fig. S2 The two-photon fluorescent emission changes of PDN-1 (5 μM) in the absence (black line) and presence (red line) of MGO (50 μM). The reactions were carried out for 12 h at 37 °C in PBS buffer (10 mM, pH 7.4, containing 10% DMSO). All samples were excited at 880 nm, and pulse width is 140 fs.

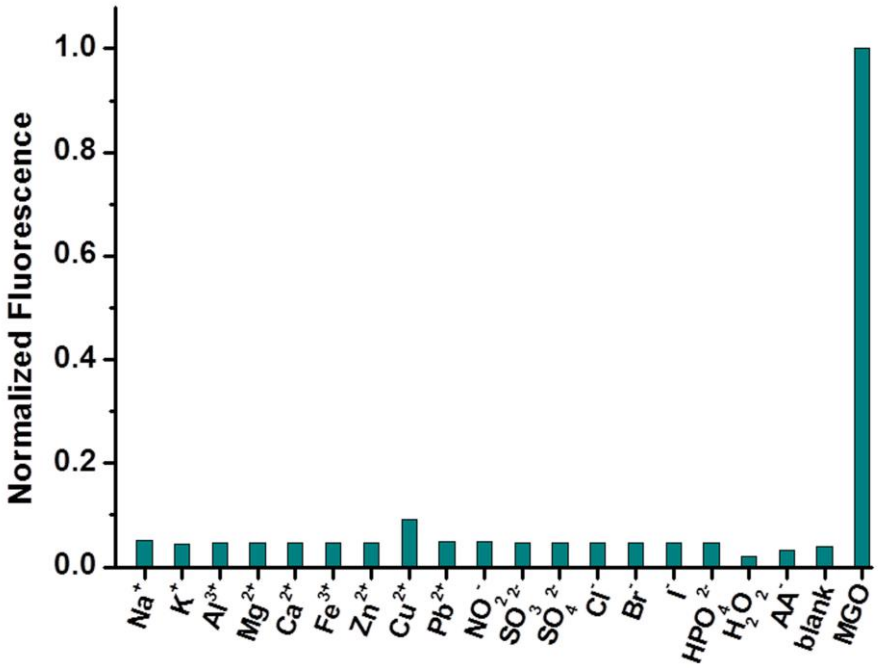


Fig. S3 Normalized fluorescent emission changes of PDN-1 (5 μM) at 528 nm, which added MGO (50 μM) in comparison with a series of metal ions, anions and peroxide at the same concentration, respectively. The reactions were carried out for 12 h at 37 °C in PBS buffer (10 mM, pH 7.4, containing 10% DMSO).

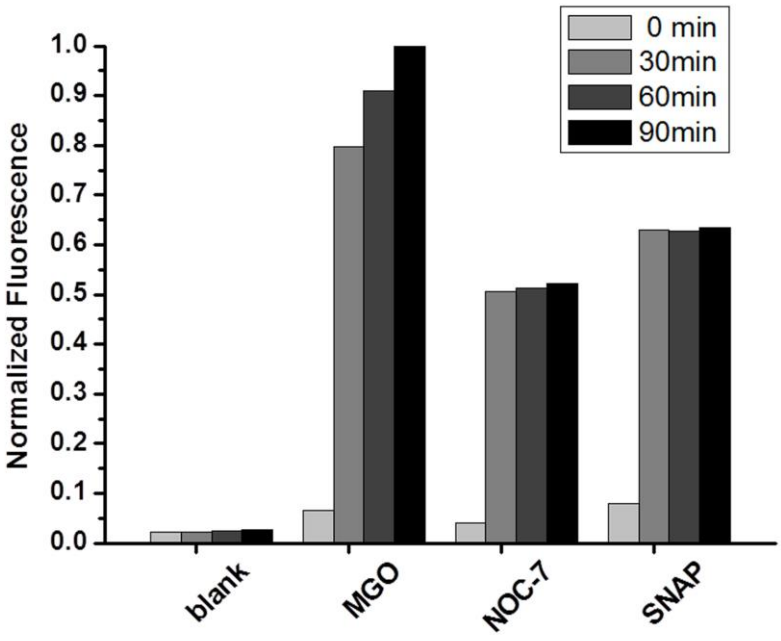


Fig. S4 Normalized fluorescent intensity of PDN-1 (5 μM) at 528 nm. The reaction solutions contained MGO (50 μM), SNAP (50 μM) and NOC-7 (25 μM, releases two equivalents of NO), respectively. Blank: PBS buffer only; MGO: methylglyoxal; NOC-7: 1-hydroxy-2-oxo-3-(N-methyl-3-aminopropyl)-3-methyl-1-triazene (a spontaneous nitric oxide

releaser); SNAP: S-nitroso-N-acetyl-DL-penicillamine (a spontaneous nitric oxide releaser). The two nitric oxide releasers were fresh prepared before detection. All solutions were incubated at 37 ℃ in PBS buffer (10 mM, pH 7.4, containing 10% DMSO).

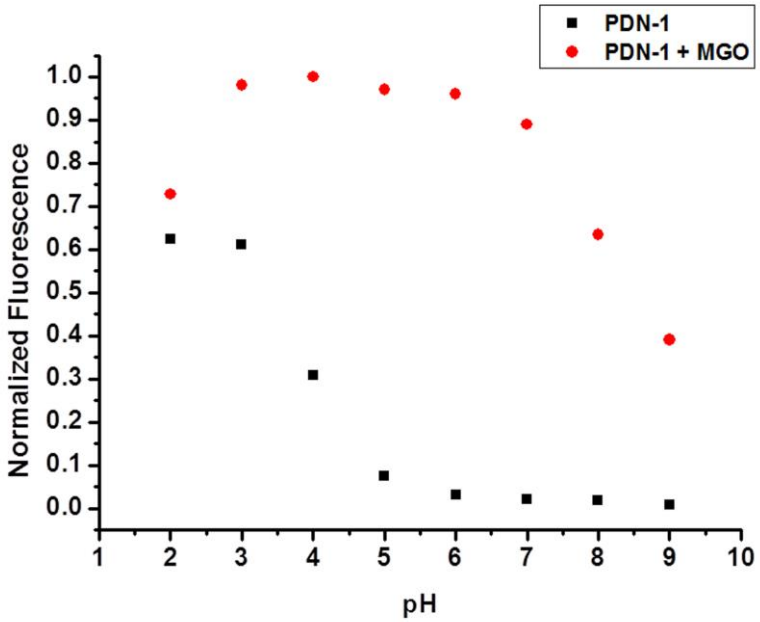


Fig. S5 Normalized fluorescent emission changes of PDN-1 (5 μM) in the absence (black dot) and presence (red dot) of MGO (50 μM). The reactions were carried out for 12 h at 37 ℃ in PBS buffer (10 mM, containing 10% DMSO) with different pH (ranging from 2.0 to 9.0). All samples were excited at 528 nm.

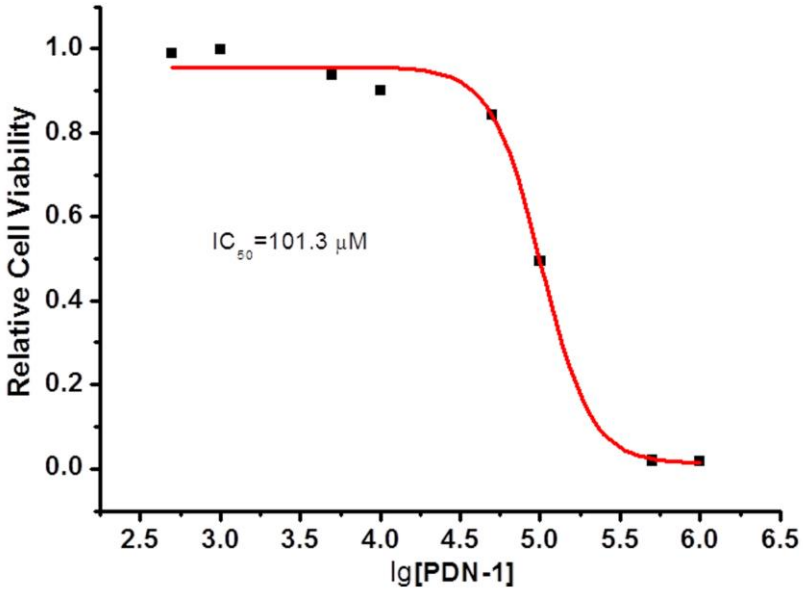


Fig. S6 Cell viability toward PDN-1 at different concentrations (500 nM , 1 μM, 5 μM, 10 μM, 50 μM, 100 μM, 500 μM, 1 mM) in MTT assay.

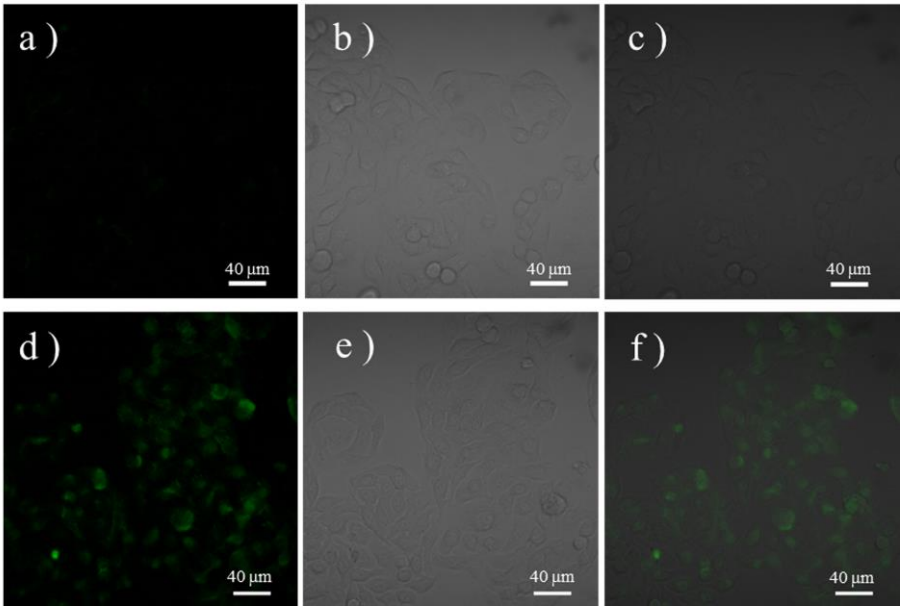
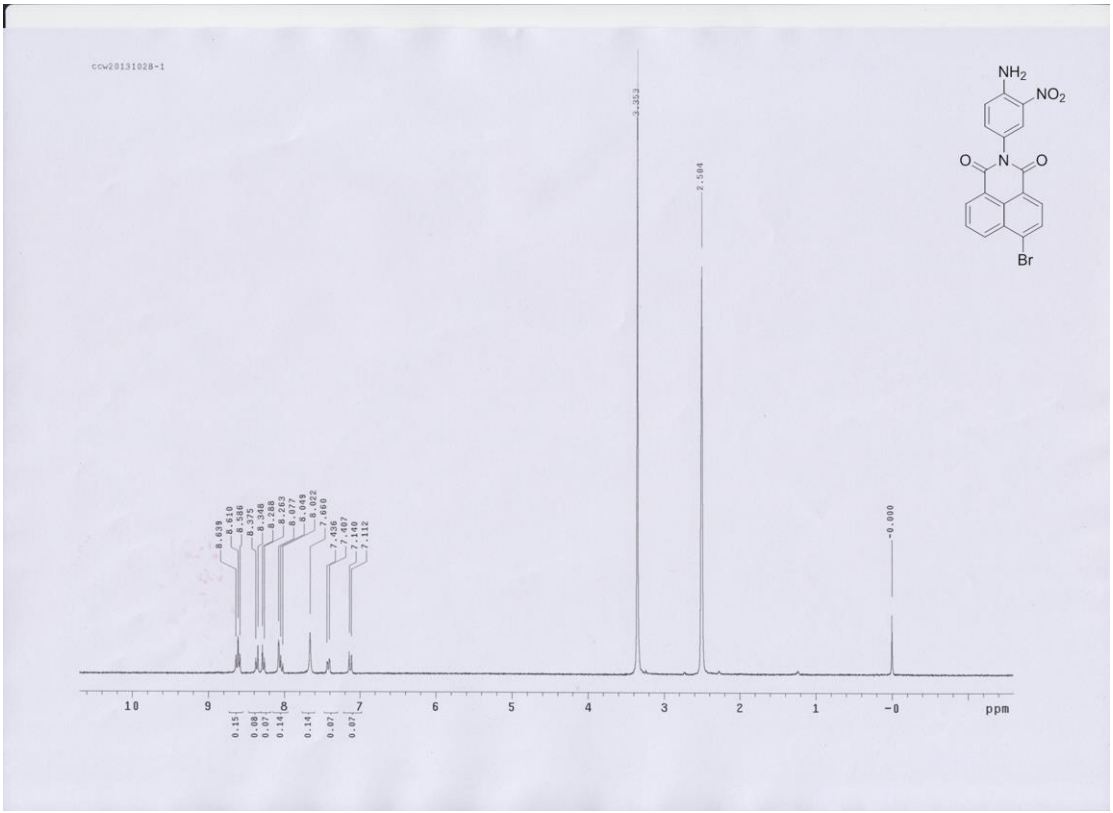
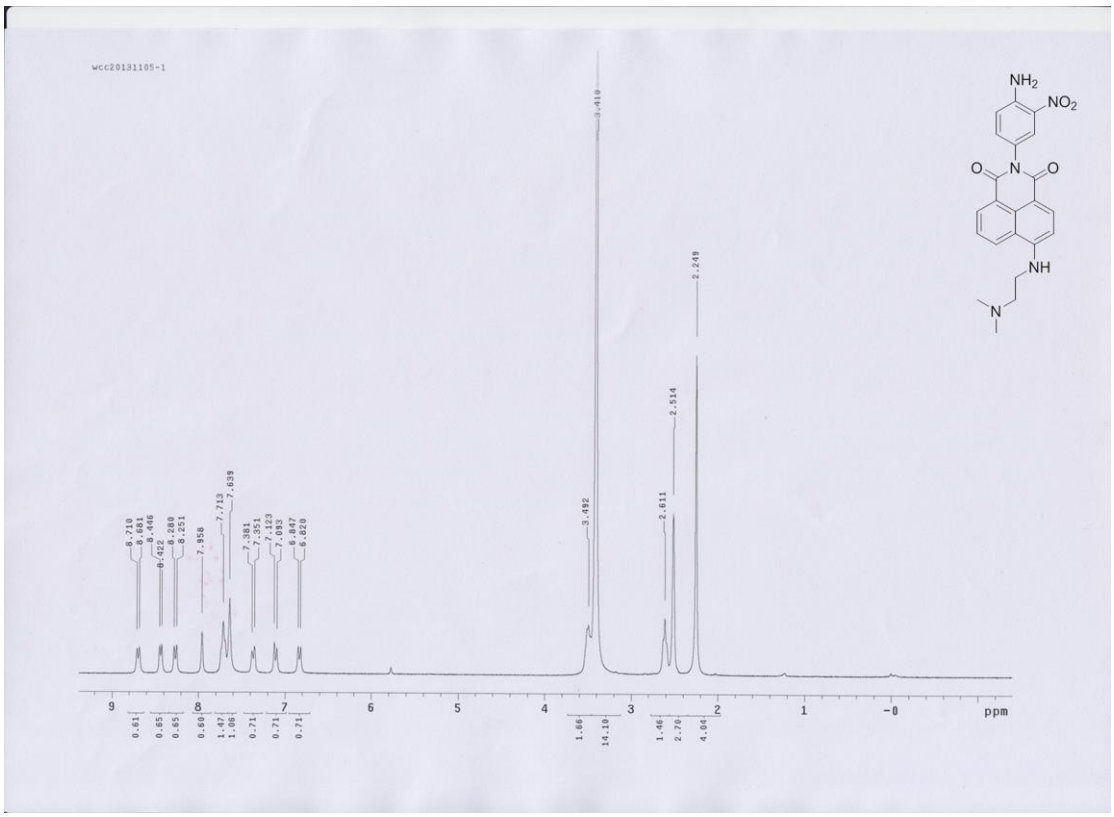


Fig. S7 One-photon confocal fluorescence images of HeLa cells: fluorescence (a), brightfield (b), and overlay (c), which incubated with probe PDN-1 (10 μM) for 10 h. (d-f) are the respective fluorescence, brightfield and overlay images of HeLa cells pre-incubated with probe PDN-1 followed by MGO (100 μM) incubation for 10 h. Live cells were used for imaging. Bar = 40 μm.

Catalog of ^{1}H and ^{13}C NMR Spectra

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7.639
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6.820
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3.410
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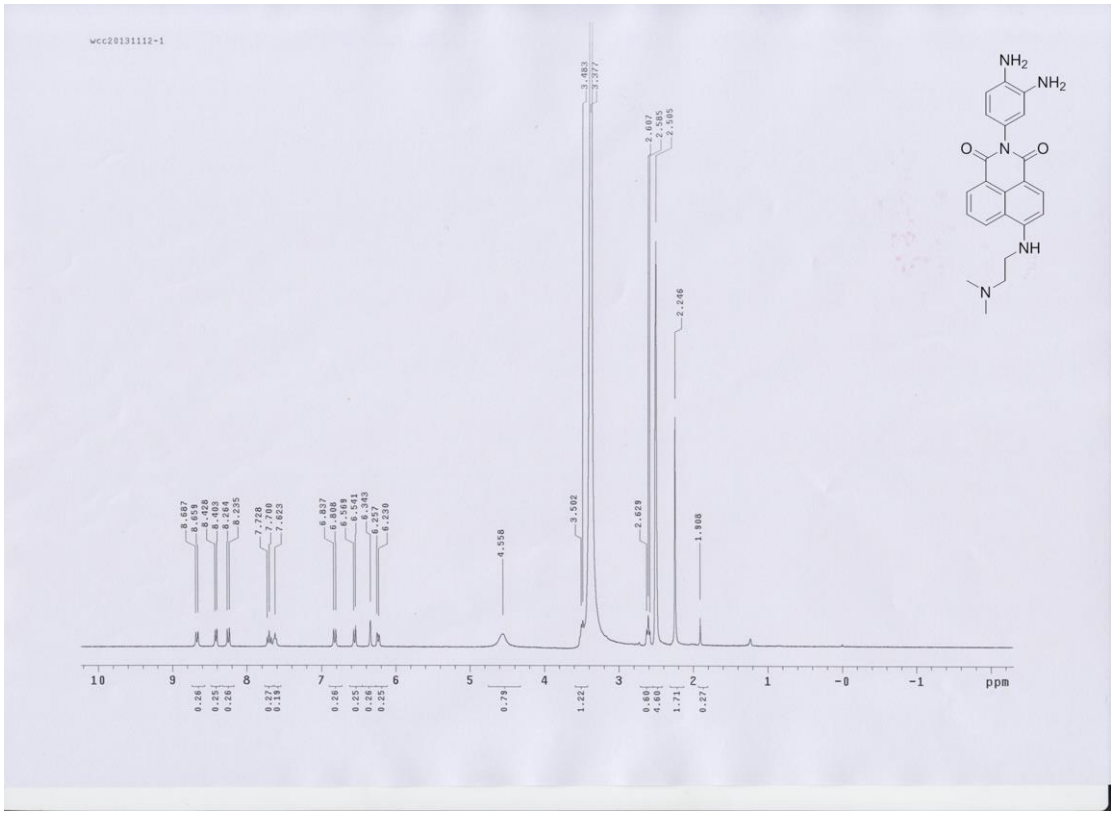
2.514
2.249
ppm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NH
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6.569
6.541
6.513
6.257
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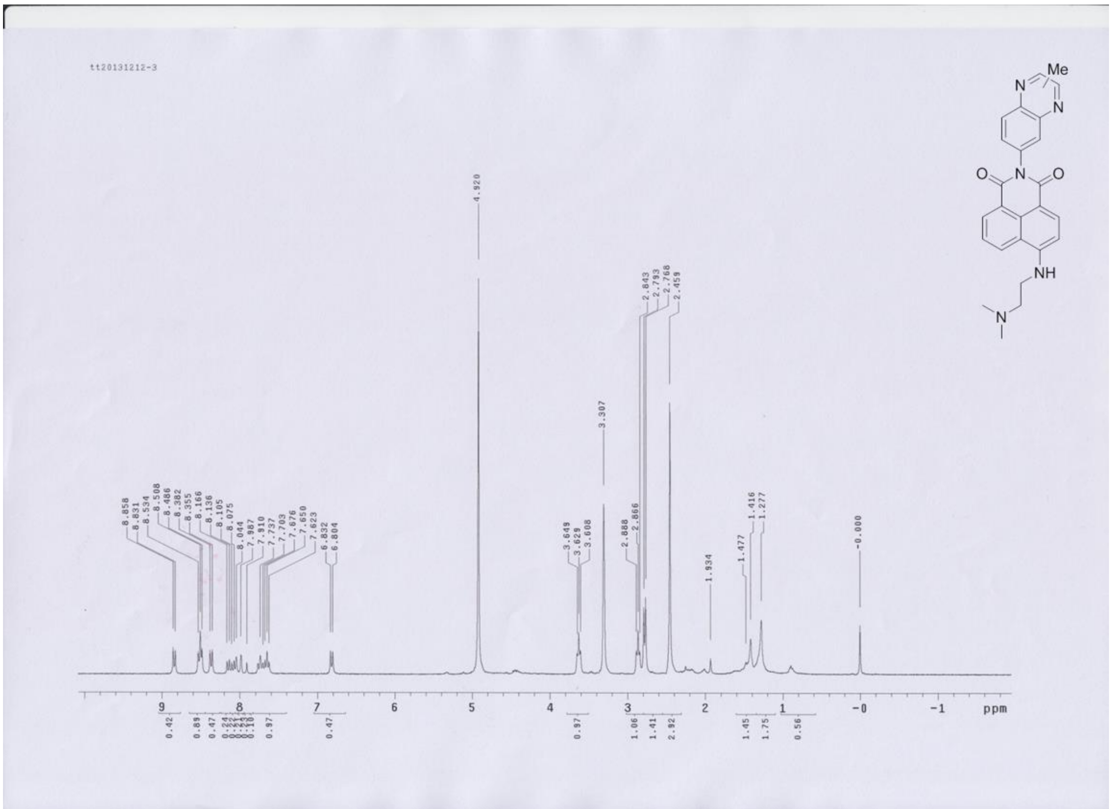
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NH
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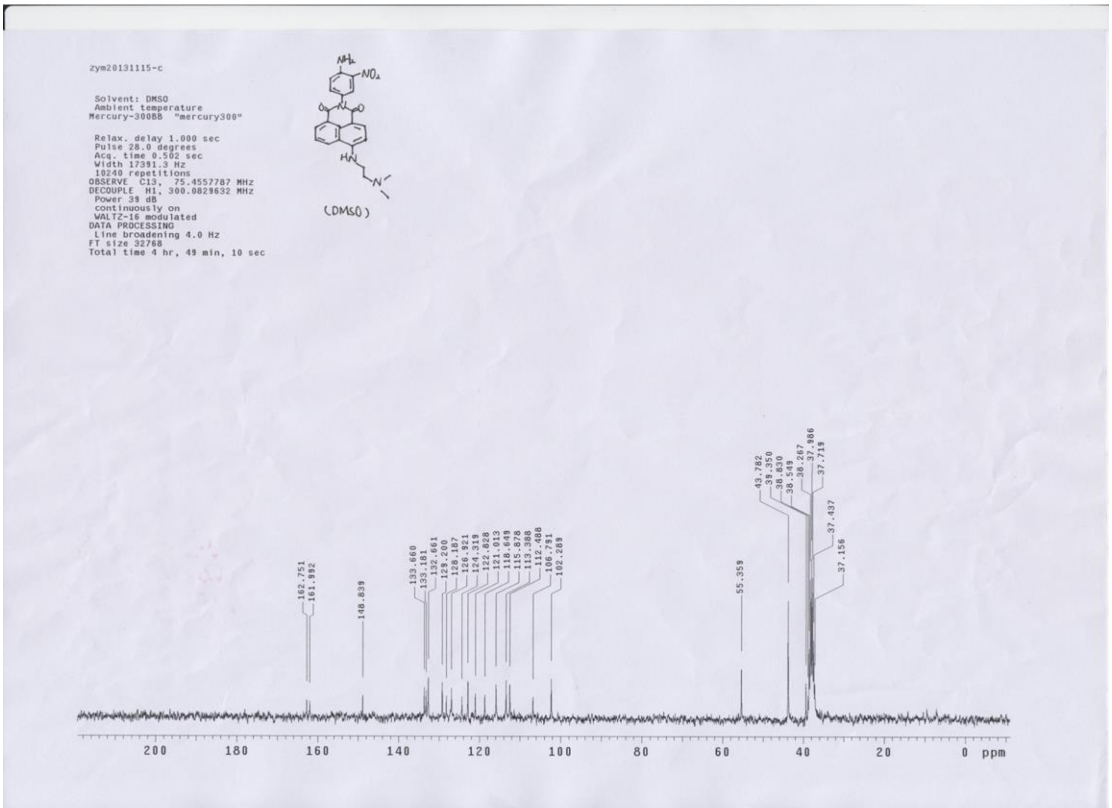
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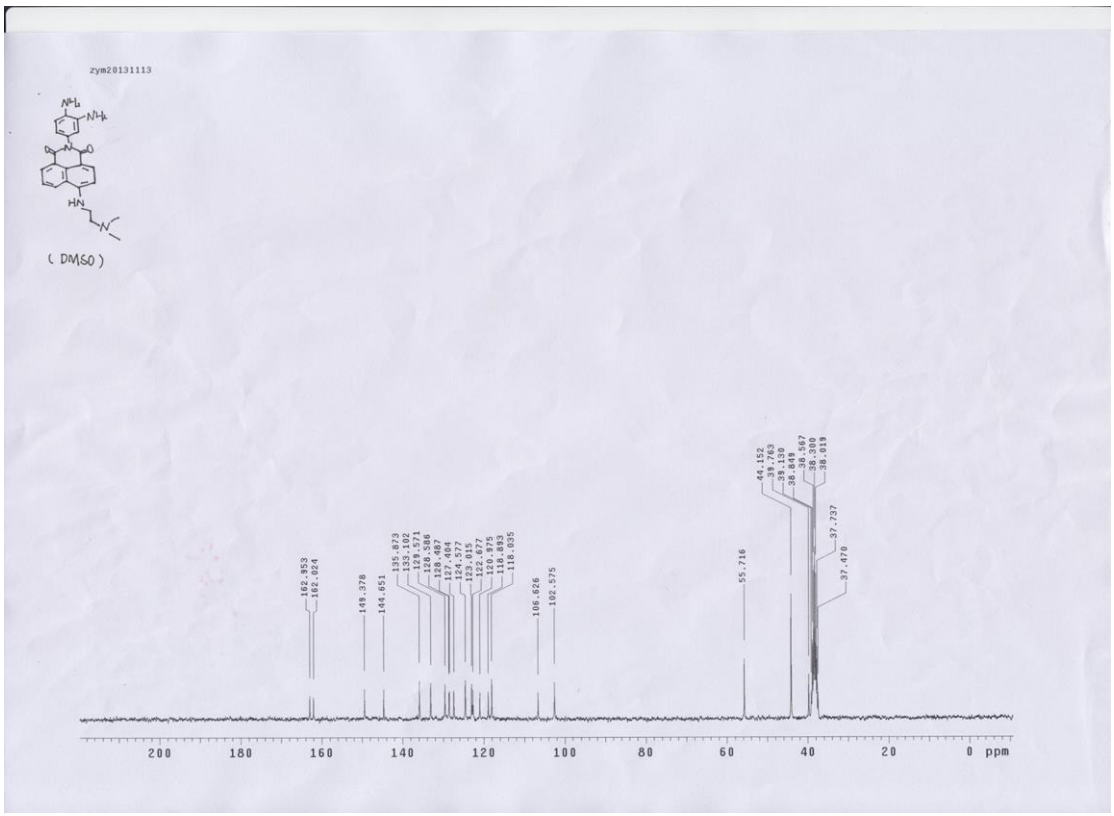
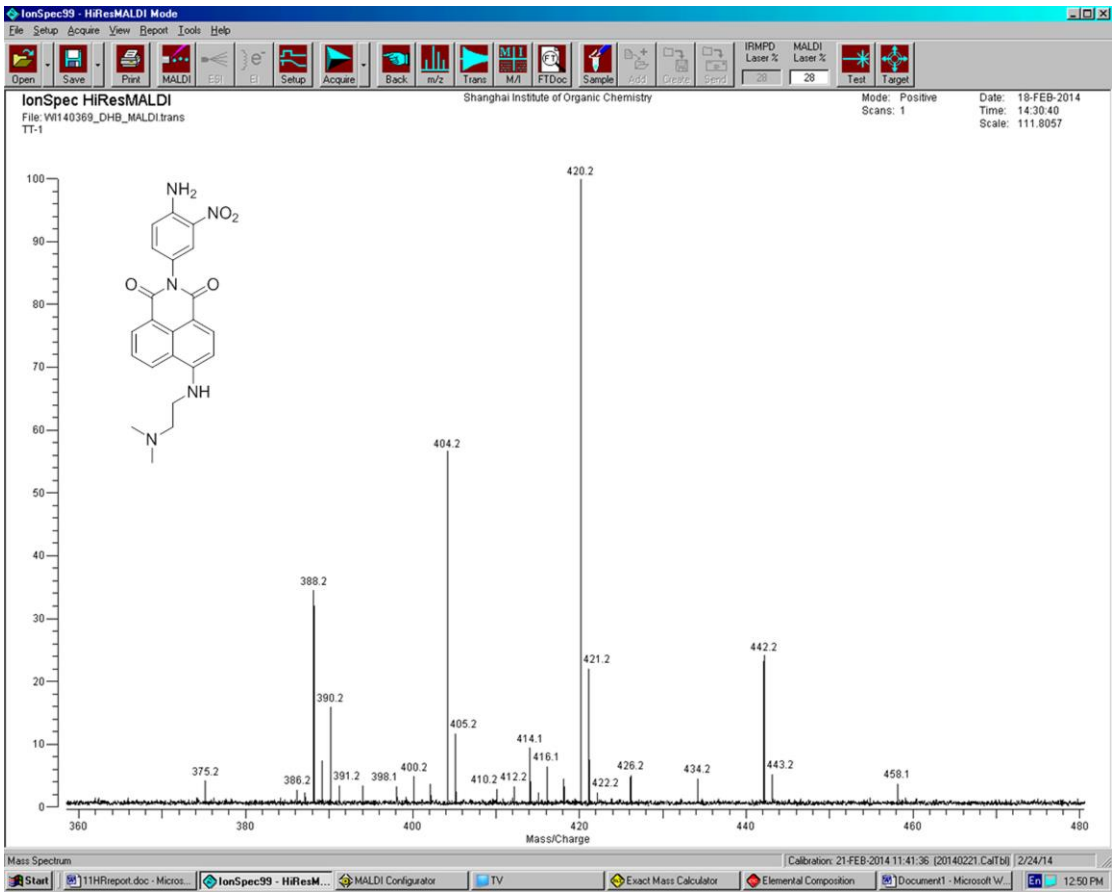
Pulse 28.0 degrees
Acq. time 0.502 sec
Width 17391.3 Hz
10240 repetitions
OBSERVE C13, 75.4557787 MHz
DECOUPLE H1, 300.0829632 MHz
Power 39 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 4.0 Hz
FT size 32768
Total time 4 hr, 49 min, 10 sec
(DMSO)
ppm



Catalog of mass spectra and high resolution MS data report



High resolution MS data report of compound 3:

Target Mass:

Target m/z = 420.1676 ±0.002

Charge = +1

Possible Elements:

Element:	Exact Mass:	Min:	Max:
C	12.000000	0	100
H	1.007825	0	100
N	14.003074	0	5
O	15.994915	0	5

Additional Search Restrictions:

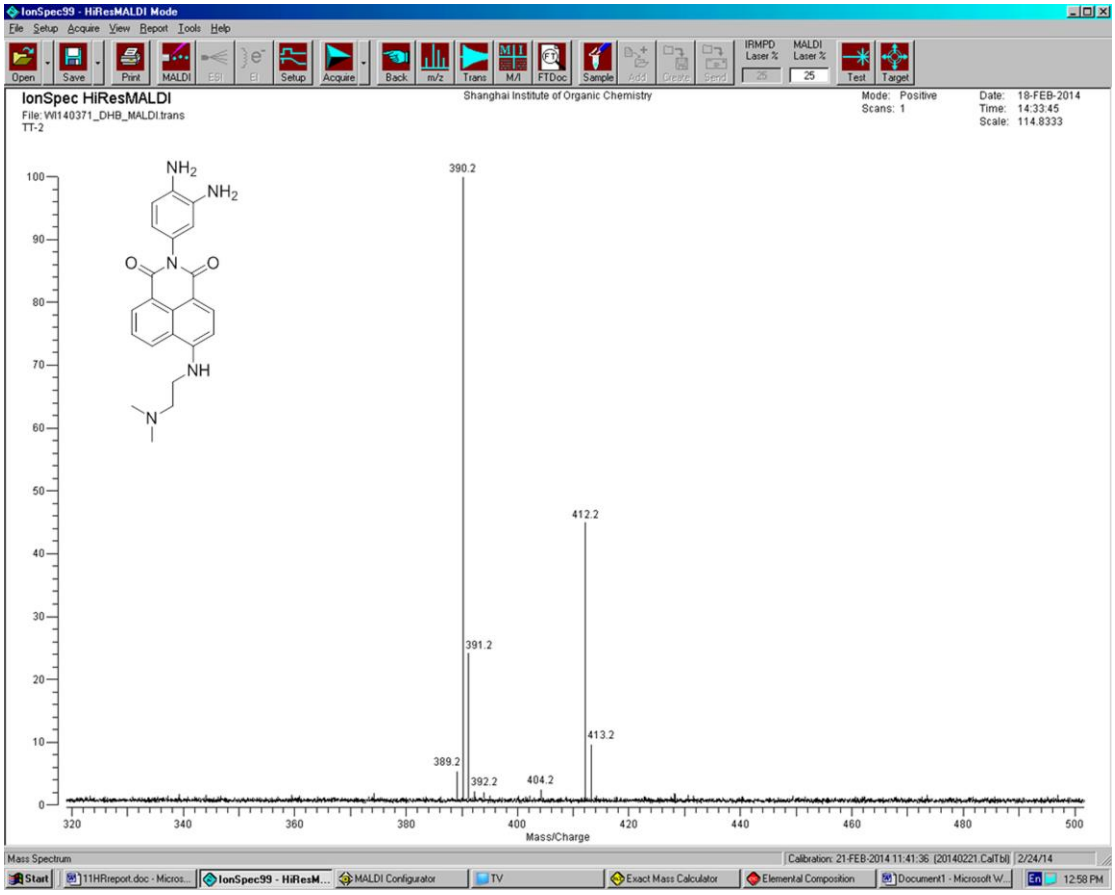
DBE Limit Mode = Both Integer and Half-Integer

Minimum DBE = 0

Search Results:

Number of Hits = 2

m/z	Delta m/z	DBE	Formula
420.16797	-0.00037	14.0	$C_{24}H_{24}N_2O_5^{+1}$
420.16663	0.00097	14.5	$C_{22}H_{22}N_5O_4^{+1}$



High resolution MS data report of PDN-1:

Target Mass:

Target m/z = 390.1932 ±0.002

Charge = +1

Possible Elements:

Element:	Exact Mass:	Min:	Max:
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H	1.007825	0	100
N	14.003074	0	5
O	15.994915	0	5

Additional Search Restrictions:

DBE Limit Mode = Both Integer and Half-Integer

Minimum DBE = 0

Search Results:

Number of Hits = 2

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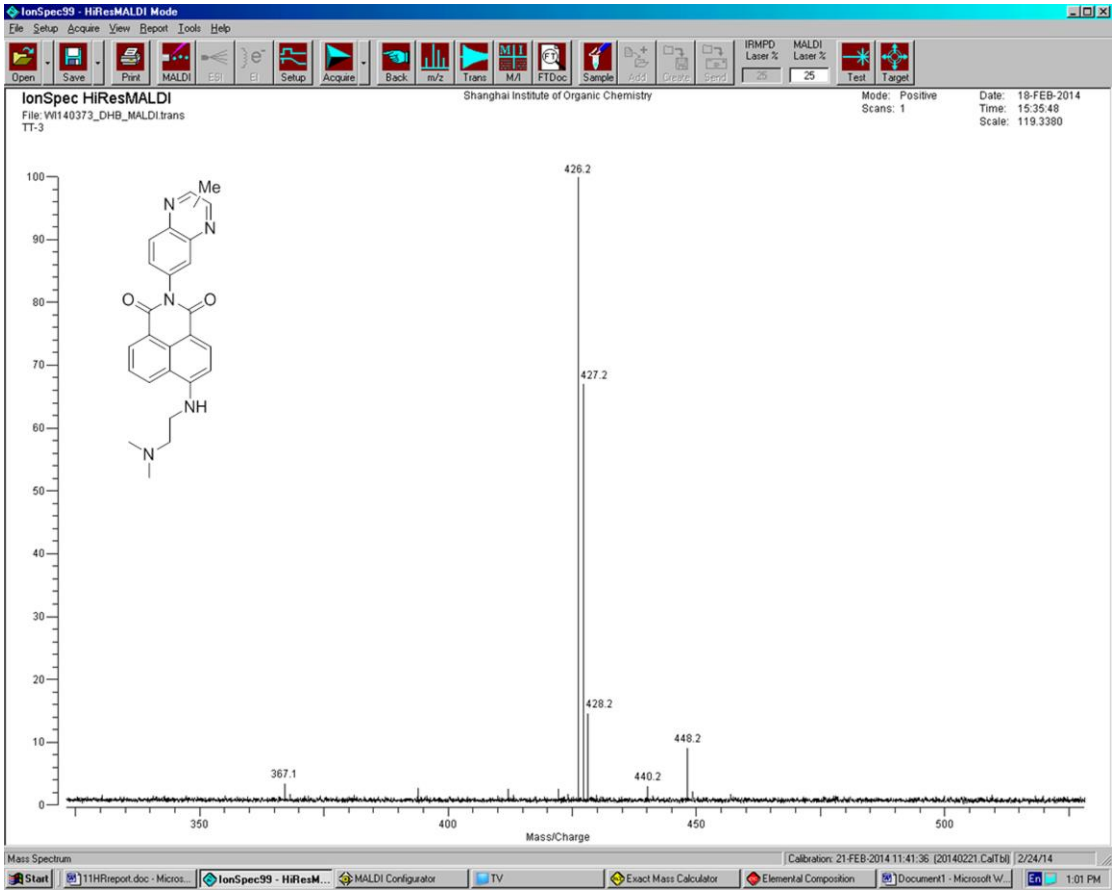
390.19379	-0.00059	13.0	$C_{24}H_{26}N_2O_3^{+1}$
390.19245	0.00075	13.5	$C_{22}H_{24}N_5O_2^{+1}$



High resolution MS data report of compound 4:

Target Mass:

Target m/z = 426.1935 ± 0.002

Charge = +1

Possible Elements:

Element:	Exact Mass:	Min:	Max:
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H	1.007825	0	100
N	14.003074	0	5
O	15.994915	0	5

Additional Search Restrictions:

DBE Limit Mode = Both Integer and Half-Integer

Minimum DBE = 0

Search Results:

Number of Hits = 2

m/z	Delta m/z	DBE	Formula
426.19379	-0.00029	16.0	$C_{27}H_{26}N_2O_3^{+1}$
426.19245	0.00105	16.5	$C_{25}H_{24}N_5O_2^{+1}$