Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supporting Information

By Nanogel-loading a Triarylboron-based AIE Fluorophore to Achieve Ratiometric sensing for Hydrogen Peroxide and sequential response for pH

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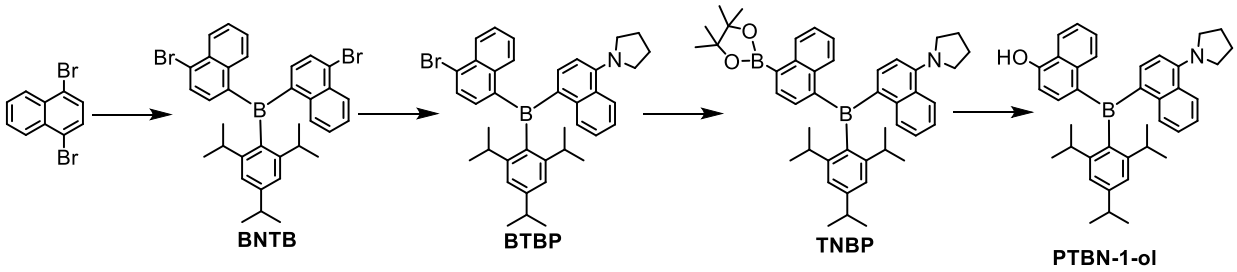
General information. All chemical reagents were purchased from J&K (Beijing, China) and used without further purification. Absorption spectra were recorded on Hitachi UV-3010. The fluorescence spectra were obtained on Hitachi F-7000. Cells were analyzed using a confocal microscope (OLYMPUS FV 1000-IX81). ^{1}H NMR spectra were obtained on BrukerAvance III 400 H (400 MHz) spectrometers.

Experimental Details

Synthsis of TNBP and PTBN-1-ol.



Scheme S1. The synthetic route to TNBP and PTBN-1-ol.

2, 4, 6-triisopropylphenylboronate (TripB$(OMe)_2$) was synthesized according to our previous reports(*Angew. Chem. Int. Ed.* **2011**, 50, 8072-8076).

Synthesis of Compound BNTB

To a solution of 1,4-dibromonaphthalene (4.72g, 16.5mmol) in anhydrous Et_2O (100mL) at -78℃ under argon, n-BuLi (7.5 mL of 2.22 M solution in n-hexane) was added. The mixture was stirred for 2 h at -78℃ and then warmed room temperature for another 2h. Then TripB$(OMe)_2$ (2.07g, 7.5mmol) was added by injection at -78℃. The mixture was stirred for 2 h at -78℃, then recovered to RT and stirred overnight. The reaction solvent was removed by a rotary evaporator, washed with saturated NaCl aqueous solution and extracted with methylene chloride. The organic layer was dried over anhydrous $MgSO_4$ and filtered. The solvent was removed by a rotary evaporator, and the crude product was purified by column chromatography (silica gel, hexane as eluent) to afford BNTB (3.12g, 65%) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, 2H), 7.77 (d, 2H), 7.58 (d, 2H), 7.46 (m, 4H), 7.09 (m, 2H), 7.01 (s, 2H), 2.94 (m, 1H), 2.65 (m, 2H), 1.31 (d, 6H), 0.94 (d, 6H), 0.80 (d, 6H); MALDI-TOF (m/z) : $[M-H]^+$ calcd for $[C_{35}H_{35}BBr_2-H]^+$ 625.1, found 625.1.

Synthesis of Compound BTBP

To a suolution of BNTB (1.26g, 2mmol), $Pd_2(dba)_3$ (0.20g, 0.2mmol), BINAP (0.26g, 0.40mmol)and sodium t-butoxide (1.20g, 12mmol) were dissolved in anhydrous toluene (20 mL). Pyrrolidine (0.122g, 2mmol) in 15 mL toluene was added dropwise in the above solution at 80℃ over 2h. Then the mixture was stirred for 12 h at 80℃. The reaction solvent was removed by a rotary evaporator, washed with saturated NaCl aqueous solution and extracted with CH_2Cl_2. The organic layer was dried over anhydrous $MgSO_4$ and filtered. The solvent was removed by a rotary evaporator, and the crude product was purified by column chromatography (silica gel, eluent: 25% Ethyl acetate in petroleum ether) to afford BTBP (0.68g, 51.2%) as a yellow powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, 1H), 8.17 (m, 1H), 7.75 (d, 2H), 7.65 (d, 1H), 7.57 (d, 1H), 7.42 (m, 2H), 7.24(s,1H), 7.09 (m, 1H), 6.98 (s, 1H), 6.95 (s, 2H), 6.71 (m, 1H), 3.59 (m, 4H), 2.91 (m, 1H),

2.74 (m, 1H), 2.62 (m, 1H), 2.04 (m, 4H), 1.28 (d, 6H), 0.92-0.74 (m, 12H); MALDI-TOF (m/z) : [M^+] calcd for [$C_{39}H_{43}BBRN$]$^+$ 616.5, found 616.1.

Synthesis of Compound TNBP

A mixture of 0.66g (1mmol) BTBP, 0.51g(2mmol) bis(pinacolato)diboron. 0.058(8mol%) Pd(dppf)Cl_2 and 0.588g (60 mol) potassium acetate in DMSO (5mL) was stirred at 80 for 24 h under a nitrogen atmosphere. The reaction mixture was cooled to room temperature, poured into the 50mL ice water, filtrated and then purified by column chromatography on silica gel with dichloreomethane/petroleum ether (1/10) as the eluent to afford a yellow power. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, 1H), 8.16 (m, 1H), 8.00 (d, 1H), 7.71 -7.50 (m, 5H), 7.35 (m, 1H), 7.02 -6.94 (s, 4H), 6.71 (s, 1H), 3.58 (m, 4H), 2.90 (m, 1H), 2.75-2.65 (m, 2H), 2.05 (m, 4H), 1.52-1.42 (m, 12H), 1.29-1.24(m,18H); HRMS MALDI-TOF (m/z) : [M-H] $^+$ calcd for [$C_{45}H_{55}B_2NO_2$]$^+$ 663.44, found 663.44. Elemental analysis (%) calcd for $C_{45}H_{55}B_2NO_2$: C 81.45 H 8.35 N 2.11; found: C 81.25 H 8.25 N 2.07.

Synthesis of Compound PTBN-1-ol

To a solution of TNBP (200mg) in THF (5mL), 1mL 30% H_2O_2 was added. The reaction mixture was stirred at room temperature for 0.5 h. The solvent was removed by a rotary evaporator, and the crude product was purified by a flash column chromatography with dichloreomethane/petroleum ether (1/5) as the eluent to afford PTBN-1-ol. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28-8.19 (m, 2H), 7.73 (m, 3H), 7.54 (m, 3H), 7.35 (m, 1H), 7.09-7.0 (m, 3H), 6.81-6.74(m,2H), 3.58 (m, 4H), 2.93 (m, 1H), 2.82 -2.71 (m, 2H), 2.01 (m, 4H), 1.56 (m, 6H), 1.31-1.26 (m, 12H); HRMS MALDI-TOF (m/z) : [M] $^+$ calcd for [$C_{39}H_{44}BNO$]$^+$ 553.35, found 553.35. Elemental analysis (%) calcd for $C_{39}H_{44}BNO$: C 84.62 H 8.01 N 2.53; found: C 84.32 H 8.05 N 2.48.

Preparation of NG-TNPB

TNPB (0.4mg) was dissolved in 20 g of a 500 ppm solution of the polyurethane hydrogel (PU) in an ethanol/water (9:1, v/v) mixture. The resultant ratio of PU/ TNPB is 200:4 (w/w). The mixtures were thoroughly stirred for 1 h, then dialyzed against distilled water for 24 h, with an interval of 2 – 3 h to exchange the water. Finally, the aqueous dispersion of the nanogel was filtered through a 0.2 mm filter to remove large aggregates. The resultant suspension was used in further experiments。

The preparation of NG- PTBN-1-ol was according to the same process.

Cell culture and viability assay. Mouse fibroblast cells (NIH/3T3) were cultured in Dulbecco's Modified Eagle Medium (DMEM) with glucose (4.5 g/L), L-glutamine, sodiumpyruvate,and 10% fetal bovine serum (FBS). The cells were plated on glass bottomed dishes at 37 ℃ under 5% CO_2 atmosphere before imaging. Cell images were obtained using a confocal microscope FV1000-IX81 and were analyzed with FV10-ASW software. NIH/3T3 cells, pre-washed twice, were incubated with 10μM in cultured medium without FBS at 37 ℃ under 5% CO_2 for 30min. Then the cells were washed with PBS to remove unbounded probes for six times before *in situ* imaging by Olympus FV1000-IX81 confocal laser scanning microscopy using oil objective, with excitation by 405nm laser, and450-500 nm (cyan channels) and 530-580nm (yellow channels) emission light was collected. Cell viability was measured by MTT assay. Briefly, NIH/3T3 cells were cultured on a 96-well plate at a density of 10,000 cells in each well. After 24 h of incubation, the medium was replaced with 200 μL of fresh medium containing varied concentrations of NG-TNPB (from 0mg/mL to 0.4mg/mL) and the cells were cultured another 24h. The medium

was replaced with fresh medium (200μL) containing MTT (0.5mg/mL) and incubated for 4 h. The supernatant was removed, and 100μL of DMSO was added to each well to dissolve the formed formazan and the absorbance of the solution was measured to assess the relative viability of the cells.The absorbance values (A) were read at a wavelength of 490 nm. Relative cell viability was expressed as: $A/A_0 \times 100\%$, where A_0 is the absorbance of the experimental group and A_0 is the absorbance of the control group.

Table S1 Quantum yields Φ of TNBP in various solvents

Solvent	n-Hexane	Ether	THF	DMSO	Ethanol
Φ	0.300	0.002	0.004	0.004	0.005

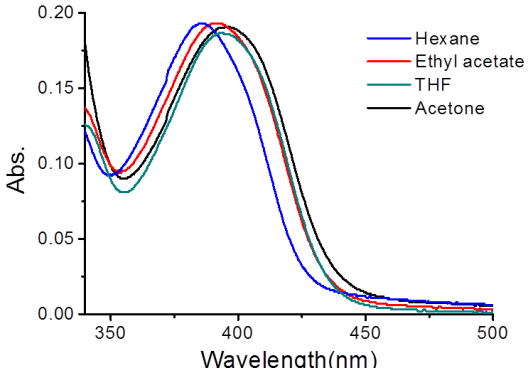


Figure S1. UV absorption spectra of PTBN-1-ol in various solvents (10μM).

Table S2 Quantum yields Φ of PTBN-1-ol in various solvents

Solvent	n-Hexane	Ether	THF	DMSO	Ethanol
Φ	0.117	0.206	0.208	0.032	0.046

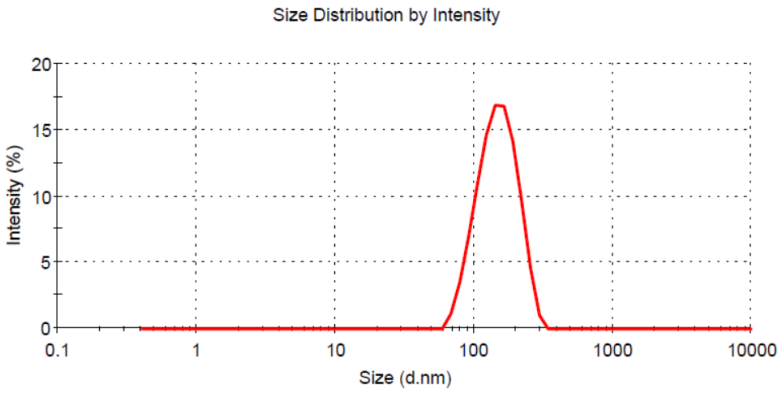


Figure S2. Size distribution of NG-TNPB measured by DLS.

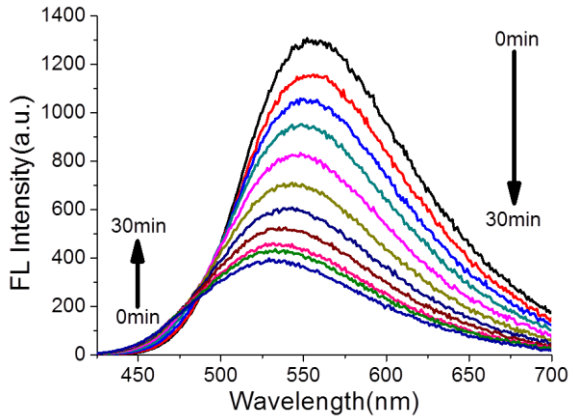


Figure S3. Fluorescence spectra changes of TNPB (10μM) with varied time in the presence of 500μM H_2O_2 in the mixed solvent of 80% water and 20% DMSO; λ_{exc} = 405nm.

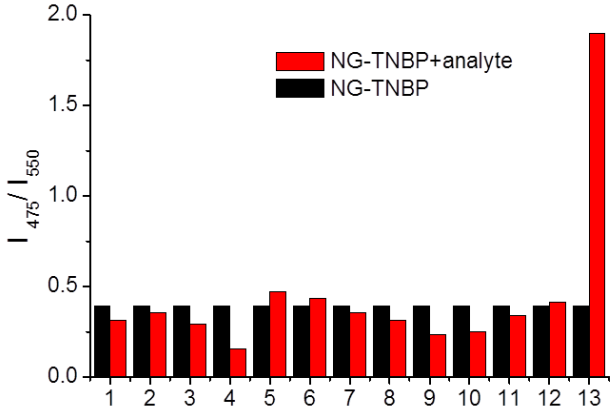


Figure S4. Fluorescence responses of NG-TNBP to various substances. 1) K^+ (1mM), 2) Mg^{2+} (1mM), 3) Ca^{2+} (1mM), 4) Fe^{3+} (1mM), 5) Fe^{2+} (1mM), 6) Cys (1mM), 7) GSH (1mM), 8) Hcy (1mM), 9) NO^{2-} (1mM), 10) $ONOO^-$ (1mM), 11) OCl^- (1mM), 12) •OH (10mM), 13) H_2O_2 (500μM).

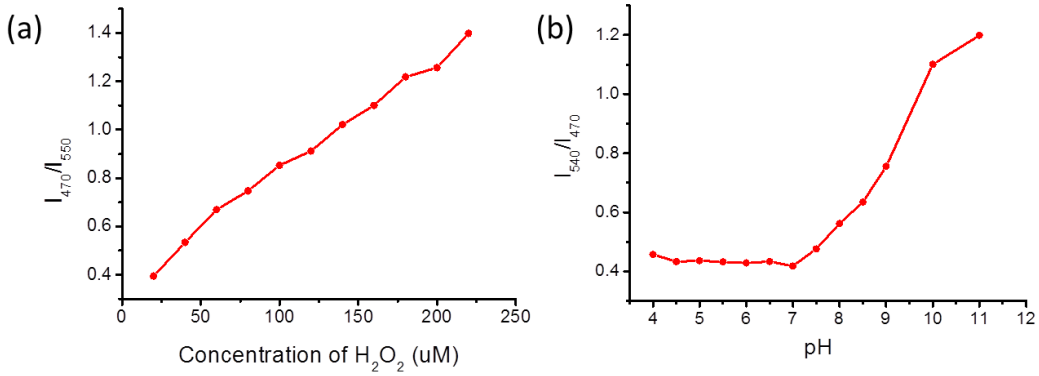


Figure S5. (a) A correlation between the ratio of fluorescence intensity (peak maximum) at 470 and 550 nm and the concentration of H_2O_2. (b) A correlation between the ratio of fluorescence intensity (peak maximum) at 540 and 470 nm and the value of pH.

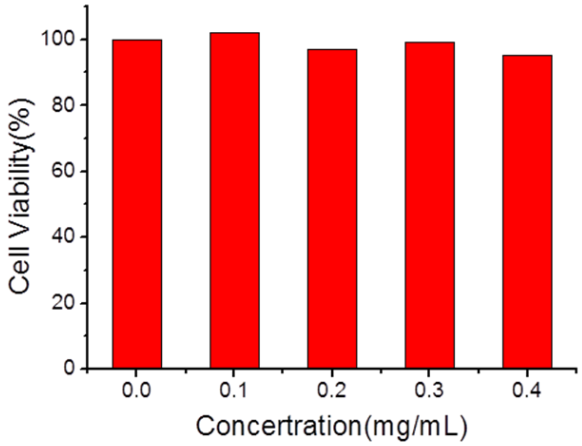
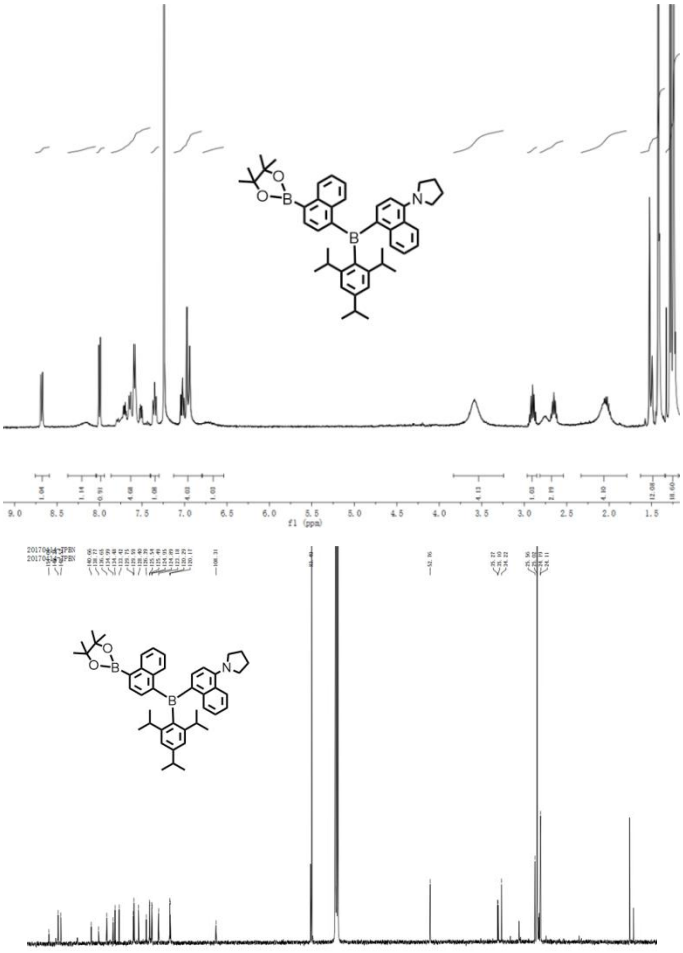
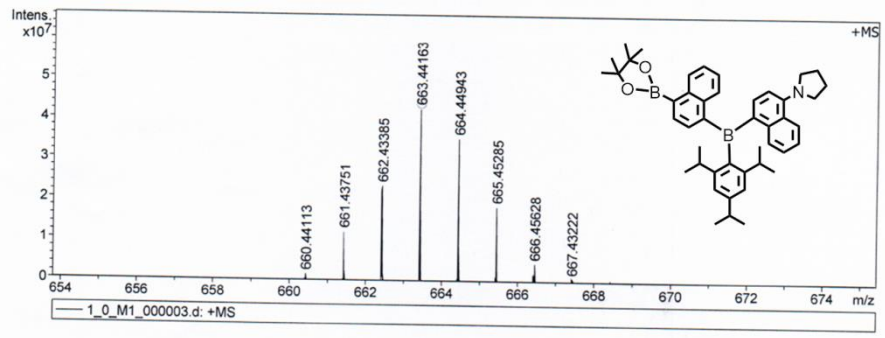


Figure S6. Cytotoxicity of NG-TNBP on NIH/3T3 cells determined by MTT assay.

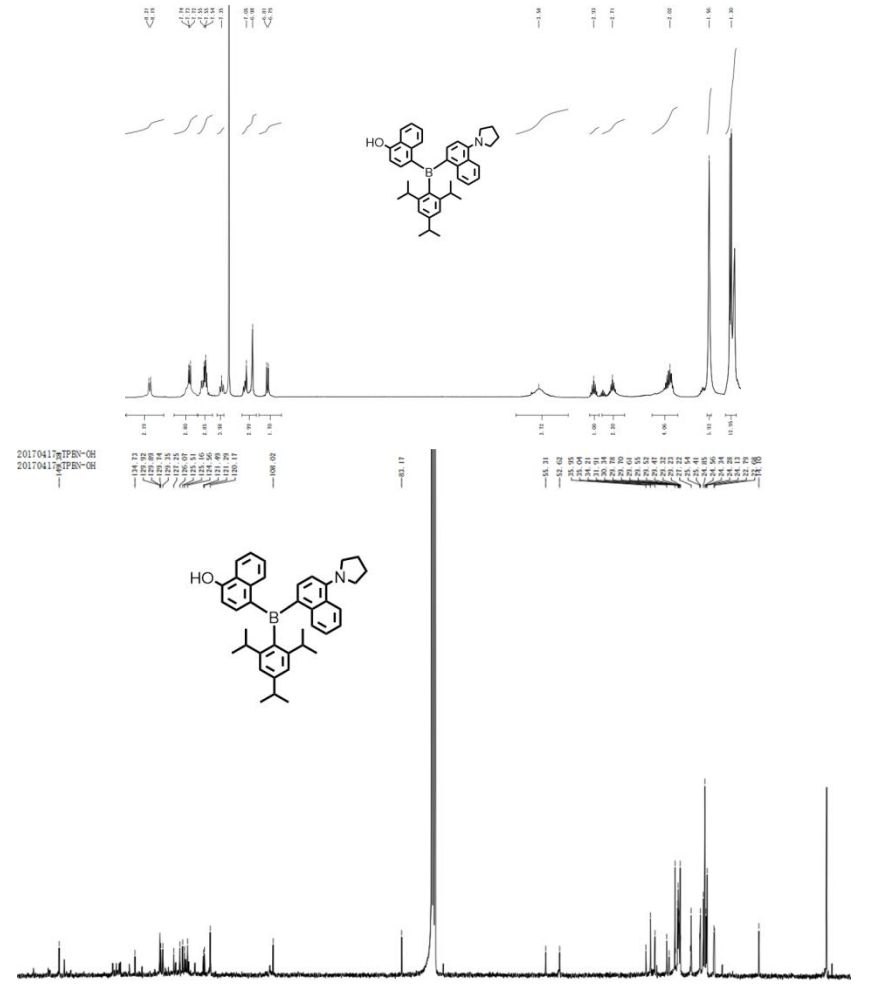
Considering biocompatibility is always one of the foremost property for a probe to be practically used in living cells, we first evaluate the cytotoxicity of NG-TNPB on NIH/3T3 cells by a standard 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyltetrazolium bromide (MTT) assay.(Figure S4) NG-TNPB shows no apparent effects on the cell viability, even at a high concentration of 0.4mg/mL demonstrating a good biocompatibility of NG-TNPB with NIH/3T3 cells.

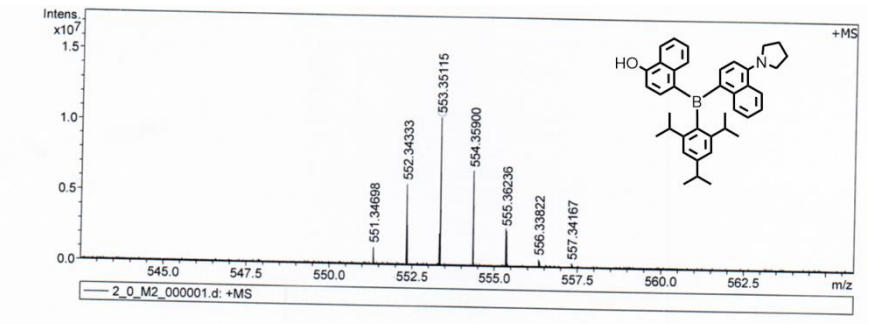
^{1}HNMR, ^{13}CNMR and HRMS spectra of TNPB and PTBN-1-ol





Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
663.441626	1	C45H55B2NO2	100.00	663.442802	-0.4	1.2	19.1	20.0	odd	ok





Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
553.351152	1	C39H44BNO	100.00	553.351721	-1.0	0.7	25.6	19.0	odd	ok