Cell membrane-targeted near-infrared fluorescent probe for detecting extracellular ATP

Wan Sun[a,*], Xiangling Gu[a], Pingxuan Dong[a], Lianjun Chu[a], Zhongyu Zhang[a], Zhenyuan Cheng[a], Fan Yang[a,b,c,*]

[a]Laboratory of Novel Pharmaceutical Excipients, Sustained and Controlled Release Preparations, College of Medicine and Nursing, Dezhou University, Dezhou 253023, China

[b]Key Laboratory of Molecular and Nano Probes, Ministry of Education, Shandong Normal University, Jinan 250014, China

[c]State Key Laboratory of Analytical Chemistry for Life Science, Collaborative Innovation Center of Chemistry for Life Sciences, School of Chemistry and Chemical Engineering, Nanjing University, 210023, China

*Correspondence: sunwananhui@163.com(W. S.), yf0004032@163.com(F. Y.)

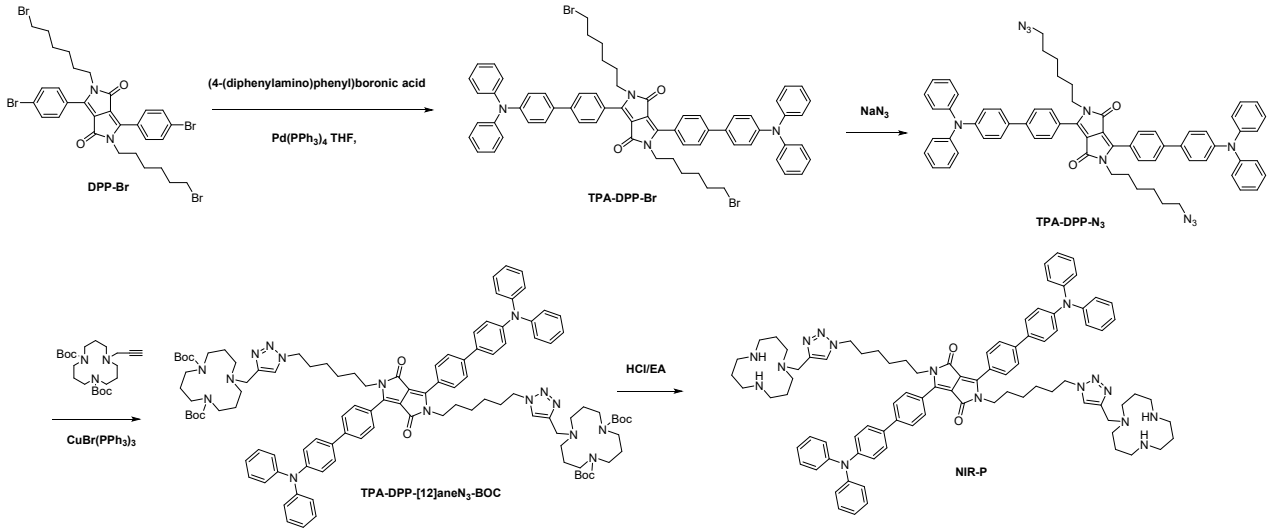


Fig S1. Synthesis of probe NIR-P

1. Synthesis of TPA-DPP-Br

Under Ar atmosphere, DPP-Br (0.0386 g, 0.05 mmol), (4-(diphenylamino)phenyl)boronic acid (0.0578 g，0.2 mmol), and $Pd(PPh_3)_4$ (0.007 g, 0.007 mmol) was dissolved in 10 mL THF. Aqueous potassium carbonate solution (1mL 2M) was added to the reaction solution and stirred at 60 °C for 24 h. purified by column chromatography (silica gel, DCM/PE = 3/1 v/v) to obtain the TPA-DPP-Br

(0.04 g 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, 4H), 7.72 (s, 4H), 7.53 (d, 4H), 7.28 (t, 9H), 3.83 (s, 4H), 3.33 (s, 4H), 1.79 (s, 4H), 1.66 (s, 4H), 1.39 (s, 4H), 1.30 (s, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.87, 148.09, 147.99, 147.45, 143.37, 133.19, 129.40, 129.23, 127.81, 126.86, 126.34, 124.83, 123.36, 123.31, 109.81, 41.90, 33.73, 32.54, 29.30, 27.65, 25.94. MS: *m/z* calcd. $[M+H]^+$ for $C_{66}H_{61}Br_2N_4O_2$, 1101.306; found, 1101.201.

2. Synthesis of TPA-DPP-N_3

A mixture of TPA-DPP-Br (0.05 g 0.045 mmol) and sodium azide (0.0318 g 0.45 mmol) was dissolved 5 mL dry DMF. The mixture was stirred under argon atmosphere at 80 °C for 24 h. After cooling to room temperature, the reaction mixture was poured into ethanol and filtered. The resulting filter cake was washed with water and dried in vacuum to give a crude product TPA-DPP-N_3 (0.033g, 72%). This crude product was used directly for the next step without further.

3. Synthesis of TPA-DPP-[12]aneN_3-BOC

TPA-DPP-N_3 (0.0195 mmol 0.02 g) and Propargyl-[12]aneN_3 (0.0391 mmol 0.016) in CH_2Cl_2/H_2O/tert-butanol (21 mL, 10:10:1, v/v/v) was added $CuBr(PPh_3)_3$ (0.004 mmol，0.004 g). The reaction mixture was vigorously stirred at 50°C for 12 h, and then the reaction was cooled to rt. The mixture was extracted by dichloromethane (3 × 15 mL). The combined organic layer was washed with saturated brine and dried over Na_2SO_4. After removal of the solvent, the crude product TPA-DPP-[12]aneN_3-Boc was purified by column chromatography on silica gel. (0.03 g, 80%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (s, 4H), 7.76 (s, 4H), 7.53 (s, 4H), 7.28 (d, J = 12.9 Hz, 12H), 7.15 (d, J = 7.1 Hz, 10H), 7.07 (s, 4H), 4.27 (s, 6H), 3.79 (d, J = 23.8 Hz, 6H), 3.31 (s, 14H), 2.41 (s, 6H), 2.04 (s, 3H), 1.85 (s, 10H), 1.65 (s, 8H), 1.26 (s, 36H), 0.87 (d, J = 7.6 Hz, 12H). 13C NMR (126 MHz, $CDCl_3$) 162.04, 156.39, 148.12, 147.97, 147.42, 143.38, 129.40, 129.50, 127.78, 124.84, 123.39, 123.24, 109.76, 50.14, 49.46, 45.43, 44.00, 41.83, 29.70, 29.67, 29.32, 28.50, 26.03, 14.14. MS: *m/z* calcd. $[M+2H]^{2+}$ for $C_{110}H_{140}N_{16}O_{10}$, 923.041; found, 923.152.

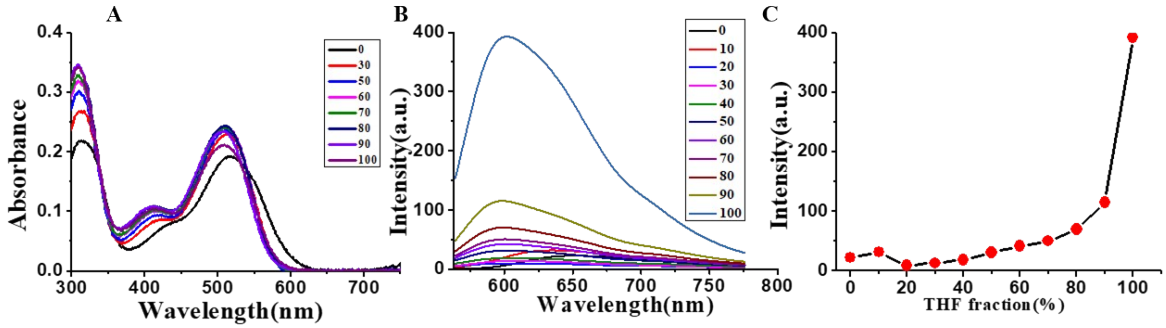


Fig. S2 Linear absorption and fluorescence spectra of **NIR-P** in different THF fractions ([**NIR-P**]= 10 μM, λ_{ex} =520 nm).

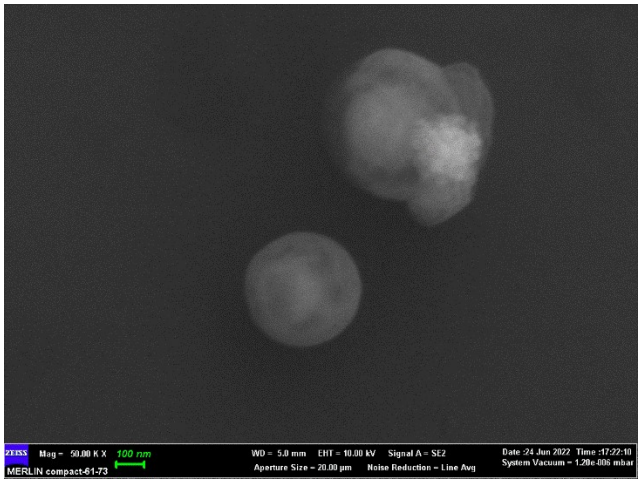


Fig. S3 SEM of liposomes

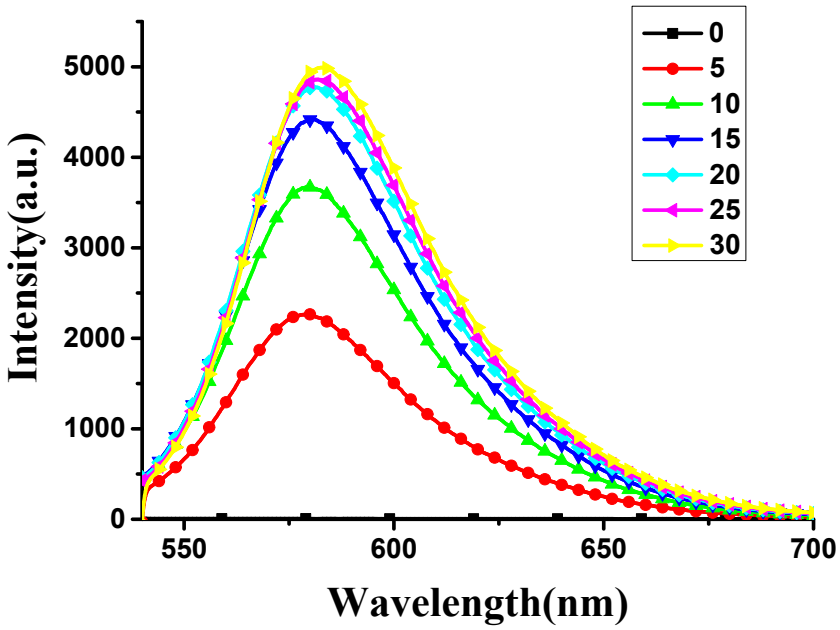


Fig. S4 Fluorescence spectra of **NIR-P** embedding into liposomes, where the concentration of **NIR-P** ranged from 5 μM to 30 μM. (λ_{ex} =520 nm)

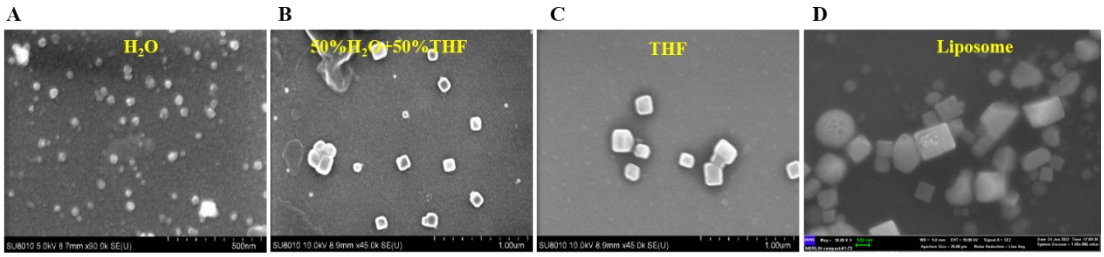


Fig. 5 SEM images of **NIR-P** in THF/H_2O mixtures with (A) 0% THF fraction, (B) 50% THF fraction, (C) 100% THF fraction, (D) liposome.

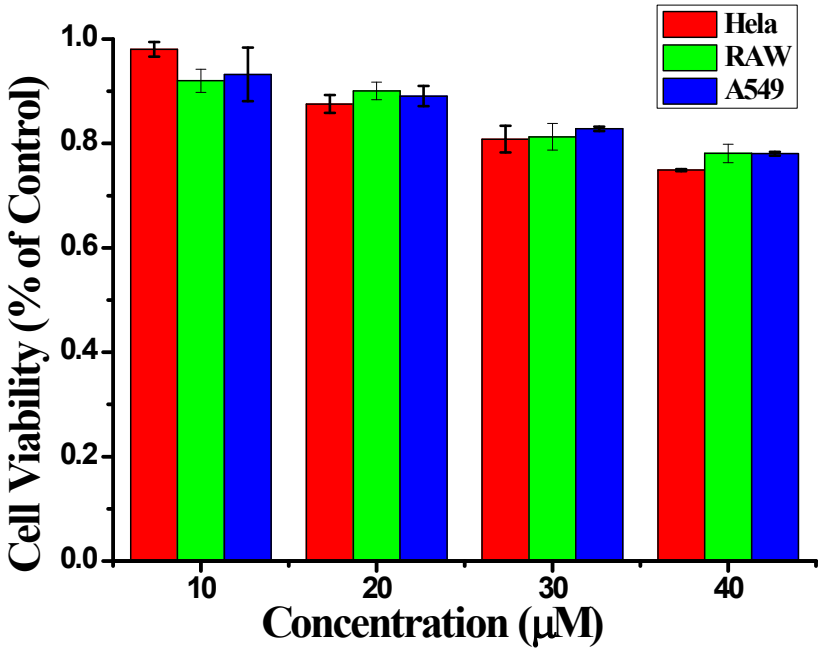


Fig. S6 In vitro cytotoxicity of **NIR-P** against Hela, RAW and A549 cells for 24 h. The viability of the cells without **NIR-P** is defined as 100%. The results are expressed as the mean ± standard deviation of six separate measurements.

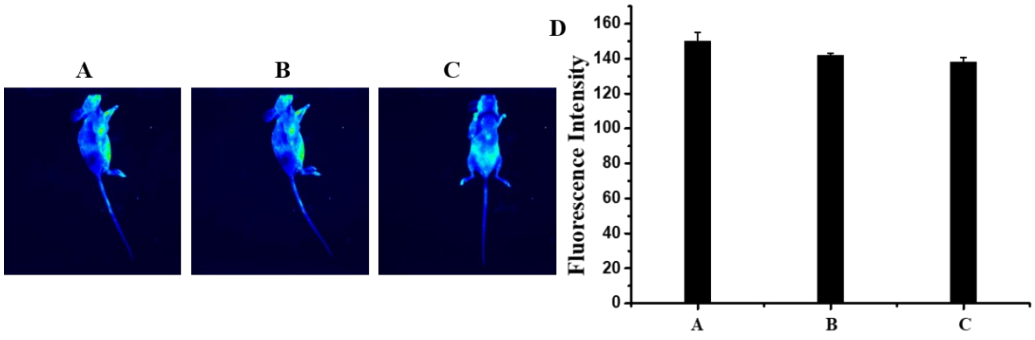


Fig. S7 In vivo imaging of **NIR-P** (40 μM) and 2-deoxyglucose (10 mM) for (A) 0, (B) 10, (C) 20 min, (D) Quantitatively calculated of images. (λ_{ex} = 532 nm, λ_{em} = 600–720 nm).

Table S1 Photophysical properties of NIR-P

Compound	Solvent	Λ_{max}[a]	A	Λ_{max}[b]	Φ
NIR-P	H_2O	514	0.22	626	0.01
	glycerin	510	0.23	554	0.25
	liposome	512	0.22	582	0.18

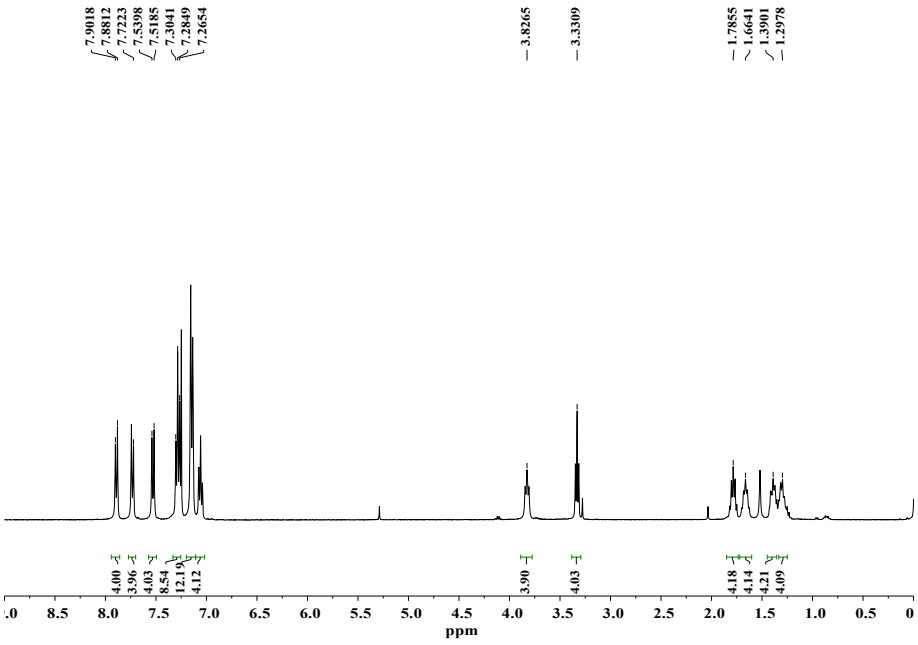


Figure S8 ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **TPA-DPP-Br**

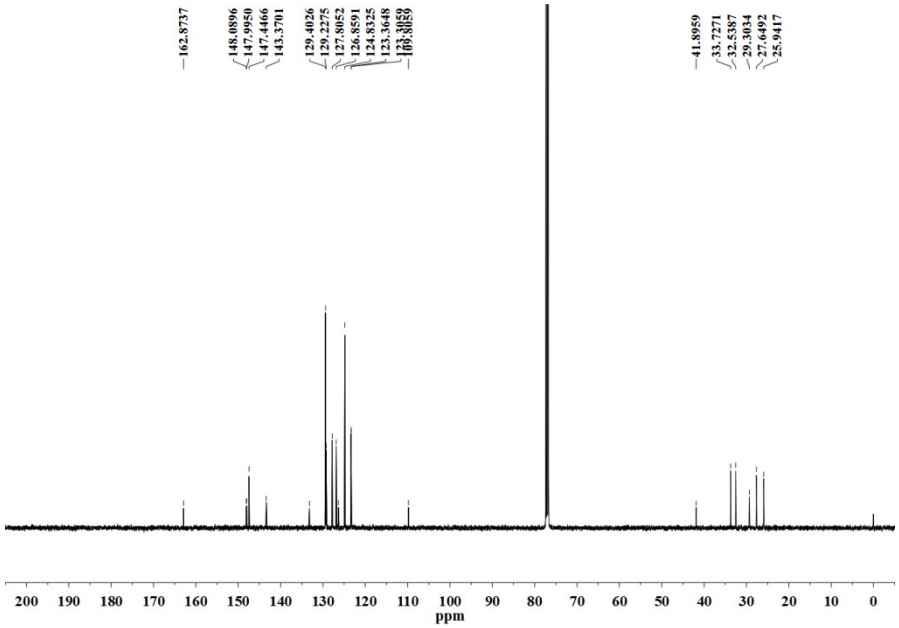


Fig. S9 ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **TPA-DPP-Br**

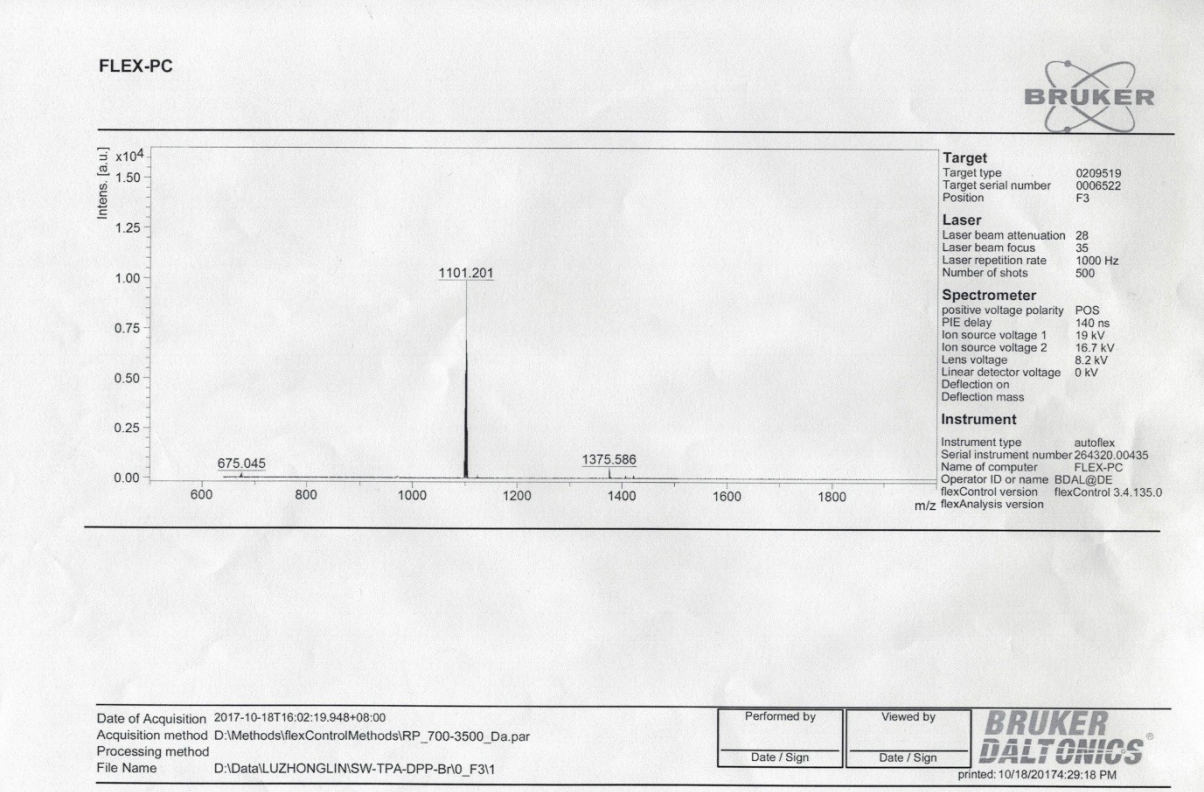


Fig. S10 MS spctrum of **TPA-DPP-Br**

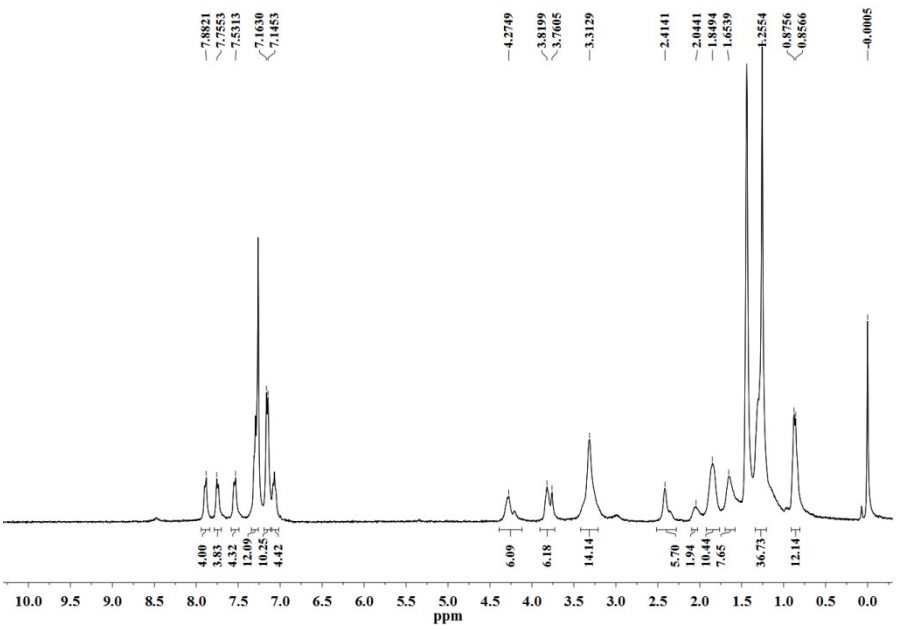


Fig. S11 ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **TPA-DPP-[12]aneN_3-Boc**

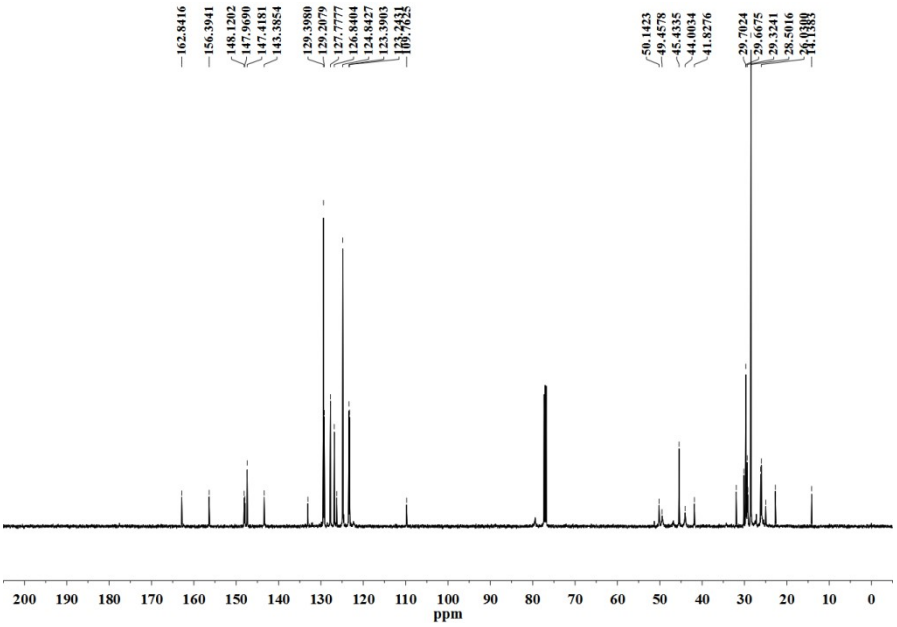


Fig. S12 ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **TPA-DPP-[12]aneN_3-Boc**

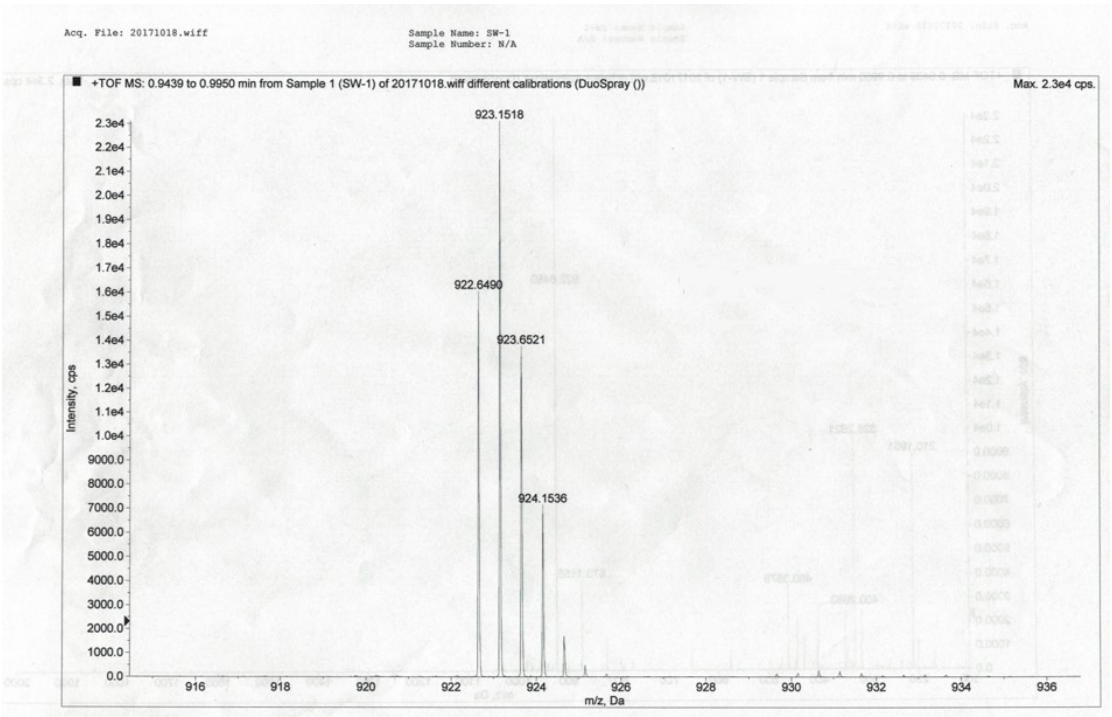


Fig. S13 MS spctrum of **TPA-DPP-[12]aneN$_3$-Boc**

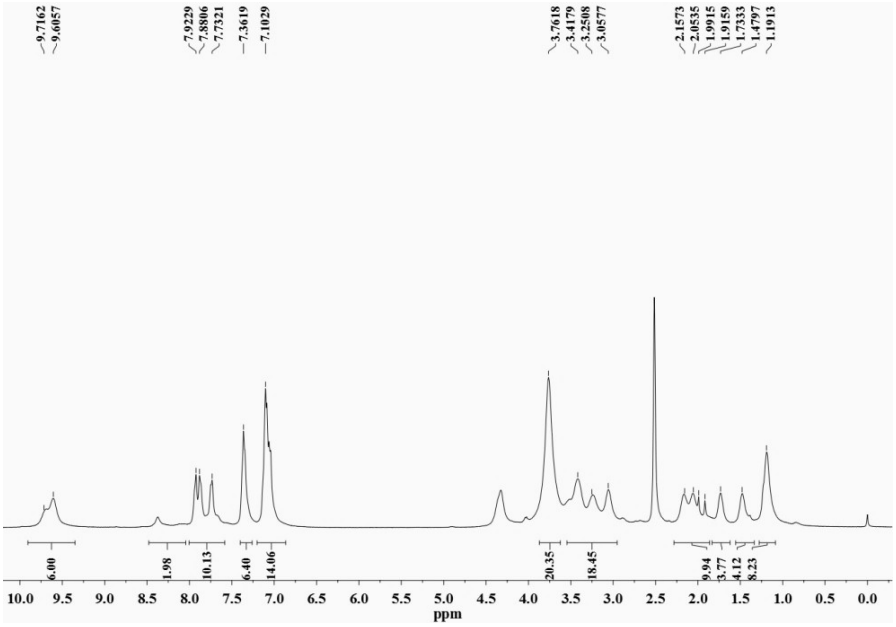


Fig. S14 ^{1}H NMR (400 MHz, DMSO) spectrum of **NIR-P**

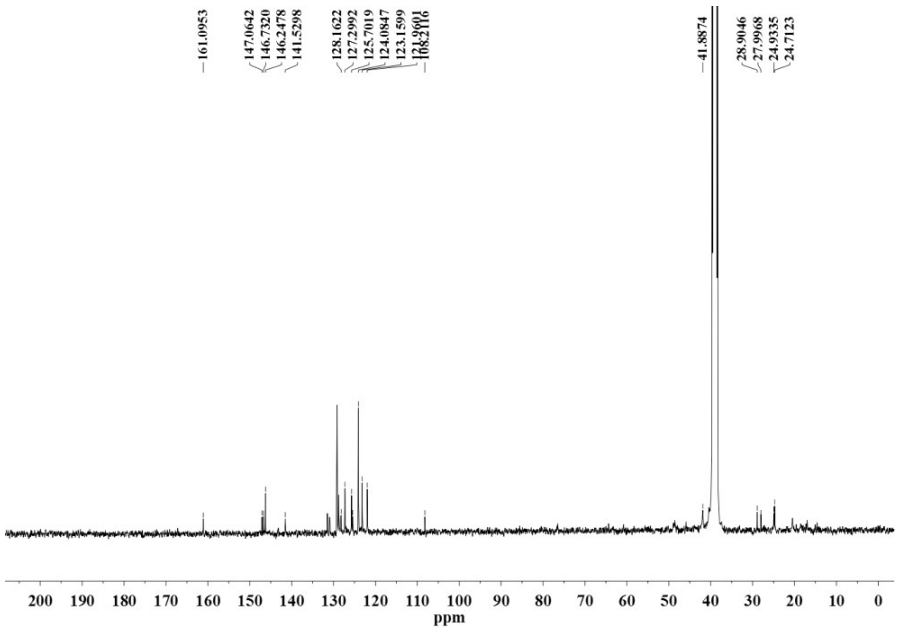
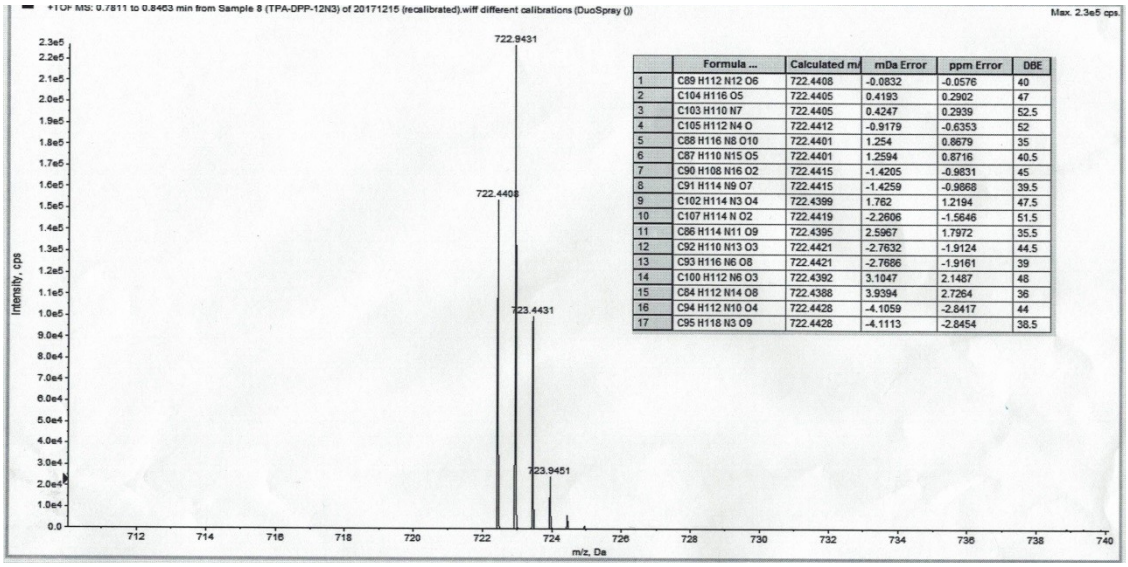


Fig. S15 ^{13}H NMR (126 MHz, DMSO) spectrum of **NIR-P**



	Formula ...	Calculated m/	mDa Error	ppm Error	DBE
1	C89 H112 N12 O6	722.4408	-0.0832	-0.0576	40
2	C104 H116 O5	722.4405	0.4193	0.2902	47
3	C103 H110 N7	722.4405	0.4247	0.2939	52.5
4	C105 H112 N4 O	722.4412	-0.9179	-0.6353	52
5	C88 H116 N8 O10	722.4401	1.254	0.8679	35
6	C87 H110 N15 O5	722.4401	1.2594	0.8716	40.5
7	C90 H108 N16 O2	722.4415	-1.4205	-0.9831	45
8	C91 H114 N9 O7	722.4415	-1.4259	-0.9868	39.5
9	C102 H114 N3 O4	722.4399	1.762	1.2194	47.5
10	C107 H114 N O2	722.4419	-2.2606	-1.5646	51.5
11	C86 H114 N11 O9	722.4395	2.5967	1.7972	35.5
12	C92 H110 N13 O3	722.4421	-2.7632	-1.9124	44.5
13	C93 H116 N6 O8	722.4421	-2.7686	-1.9161	39
14	C100 H112 N6 O3	722.4392	3.1047	2.1487	48
15	C84 H112 N14 O8	722.4388	3.9394	2.7264	36
16	C94 H112 N10 O4	722.4428	-4.1059	-2.8417	44
17	C95 H118 N3 O9	722.4428	-4.1113	-2.8454	38.5

Fig. S16 HR-MS spctrum of **NIR-P**