Supporting Information

Mitochondria targeting two-photon fluorescent molecules for gene transfection and biological tracking

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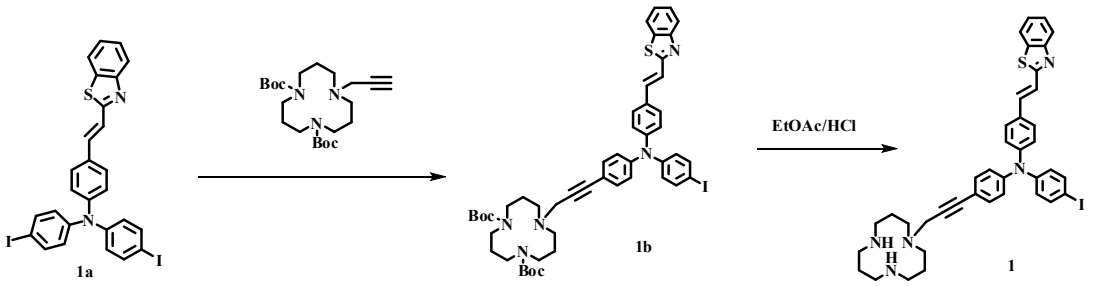
Contents

1. Syntheses

*Synthesis of **1***

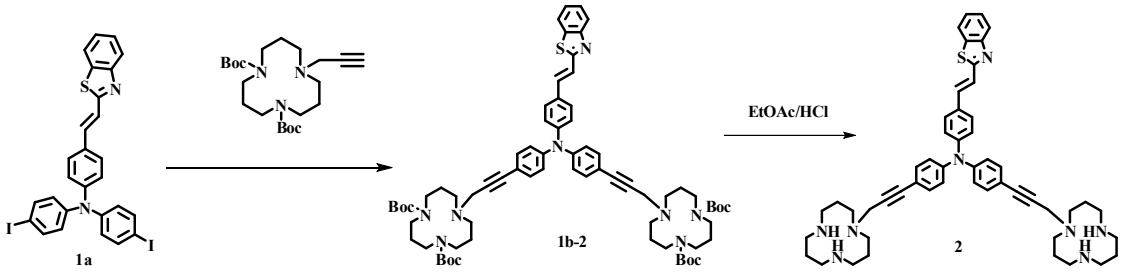


(*E*)−4−(2−(Benzo[d]thiazol−2−yl)vinyl)−*N*, *N*−bis(4−iodophenyl)aniline (**1a**) (0.3 g, 0.51 mmol), $Pd(PPh_3)_4$ (58mg，0.05mmol), CuI (10mg，0.05mmol), and propargyl−[12]aneN_3 (0.83 g, 2.0 mmol) [1] (4 equiv.) in triethylamine (15 mL) were added via a syringe under inert gas to a round−bottomed flask and the reaction mixture was stirred at 50 °C for 48 h. After removing the solvent under vacuum, the crude liquid product was then purified by flash column chromatography, eluting with petroleum ether and ethyl acetate to give yellow crystals (0.10 g, yield 21%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, *J* = 8.0 Hz, 1H), 7.84 (d, *J* = 7.7 Hz, 1H), 7.56 (d, *J* = 8.3 Hz, 2H), 7.48 – 7.41 (m, 4H), 7.32 (t, *J* = 11.4 Hz, 4H), 7.03 (dd, *J* = 17.8, 8.3 Hz, 4H), 6.86 (d, *J* = 8.3 Hz, 2H), 3.57 (s, 2H), 3.31 (d, *J* = 8.7 Hz, 8H), 2.58 (s, 4H), 1.82 (d, *J* = 8.0 Hz, 6H), 1.45 (s, 18H). ^{13}C NMR (101 MHz,) δ 167.25, 156.40, 154.00, 147.95, 146.62, 146.40, 138.59, 136.91, 134.36, 133.04, 130.77, 130.23, 128.66, 126.80, 126.64, 126.40, 126.22, 125.50, 125.32, 124.20, 123.98, 123.65, 123.17, 122.90, 121.56, 120.78, 118.31, 87.10, 85.18, 83.84, 79.32, 50.28, 46.11, 44.27, 40.74, 28.61, 26.99, 26.88, 26.10. HRMS-ESI: *m/z* calcd. $[M+H]^+$ for $C_{49}H_{58}IN_5O_4S^+$, 938.3176; found, 938.3182.

Compound **1b** (0.22 g, 0.23 mmol) was added to a saturated hydrogen chloride solution of ethyl acetate (5 mL) and the mixture was stirred for 5 h at room temperature. The resulting suspension was filtrated and the solid was washed with ethyl acetate, then dried in vacuum at 60 ^{o}C for 24 h. A red solid as compound **1** was obtained in 0.15 g (86%). 1H NMR (400 MHz,) δ 8.08 (d, *J* = 6.0 Hz, 1H), 7.96 (d, *J* = 6.2 Hz, 1H), 7.67 (dd, *J* = 26.3, 17.0 Hz, 6H), 7.47 (d, *J* = 43.0 Hz, 4H), 7.03 (s, 4H), 6.90 (s, 2H), 3.74 – 3.53 (m, 2H), 3.171 – 2.68 (m, 12H), 2.08 – 1.85(m, 6H).^{13}C NMR (126 MHz, DMSO-d_6): δ 167.35, 153.96, 147.68, 146.40, 139.04, 138.97, 137.26, 134.62, 133.63, 129.71, 128.57, 127.43, 127.02, 125.85, 124.03, 122.85, 122.66, 121.01,

88.77, 49.66, 47.52, 21.58, 19.39, 18.22. HRMS-ESI: *m/z* calcd. $[M+H]^+$ for $C_{39}H_{41}IN_5S^+$, 738.2127; found, 738.2138.

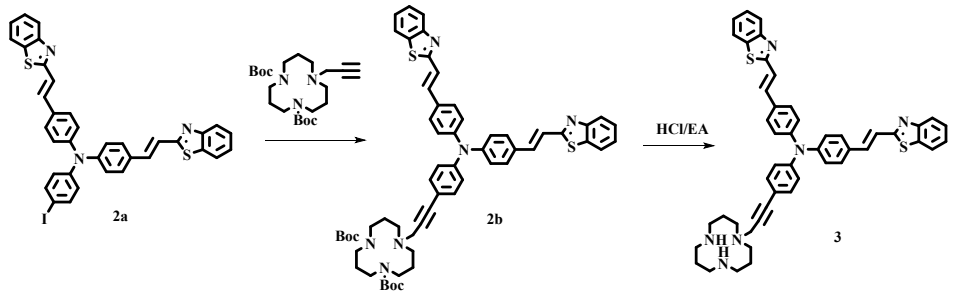
*Synthesis of **2***



According to the same procedure, 0.16g of **1b−2** was obtained, yield: 30%。^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.2 Hz, 1H), 7.86 (d, *J* = 7.8 Hz, 1H), 7.52 – 7.42 (m, 4H), 7.40 – 7.28 (m, 6H), 7.07 (d, *J* = 8.4 Hz, 2H), 7.03 (d, *J* = 8.4 Hz, 4H), 3.59 (s, 4H), 3.42 – 3.18 (m, 16H), 2.59 (s, 8H), 1.95 – 1.72 (m, 12H), 1.44 (d, *J* = 14.8 Hz, 36H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 167.23, 156.34, 153.91, 147.93, 146.38, 136.90, 134.28, 132.94, 130.93, 130.64, 130.14, 128.86, 128.57, 126.33, 125.43, 125.24, 124.27, 123.74, 122.81, 121.49, 120.66, 118.21, 85.15, 83.71, 79.26, 71.80, 50.17, 46.03, 44.19, 40.64, 29.71, 28.54, 28.50, 27.73, 26.82, 26.00, 19.18. HRMS-ESI: *m/z* calcd. $[M+H]^+$ for $C_{71}H_{95}N_8O_8S^+$, 1219.6994; found, 1219.6978.

Compound **1b-2** (0.20 g 0.17 mmol) was added to a saturated hydrogen chloride solution of ethyl acetate (5 mL) and the mixture was stirred for 5 h at room temperature. The resulting suspension was filtrated and the solid was washed with ethyl acetate, dried in vacuum at 60 °C for 24 h. A red solid as compound **2** was obtained in 0.13 g (90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (d, *J* = 7.4 Hz, 1H), 7.96 (d, *J* = 8.1 Hz, 1H), 7.76 (s, 2H), 7.64 (d, *J* = 16.5 Hz, 2H), 7.53 (d, *J* = 14.9 Hz, 2H), 7.42 (d, *J* = 6.7 Hz, 4H), 7.05 (s, 6H), 3.74 (s, 4H), 3.50 (s, 4H), 3.32 – 3.04 (m, 15H), 2.90 (s, 5H), 2.22 (s, 2H), 2.07 (s, 5H), 1.85 (s, 5H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 167.10, 153.92, 147.99, 147.57, 137.93, 137.22, 134.42, 133.63, 132.56, 132.00, 131.04, 129.74, 129.32, 128.63, 127.03, 125.87, 124.48, 122.87, 122.66, 121.14, 60.26, 53.90, 49.78, 47.49, 43.02, 41.34, 40.98, 31.79, 28.43, 21.58, 21.29, 20.60, 19.66, 19.40, 18.21, 14.60. HRMS-ESI: *m/z* calcd. $[M+H]^+$ for $C_{51}H_{63}N_8S^+$, 819.4896; found, 819.4897.

*Synthesis of **3***



According to the similar procedure, 0.04 g (0.058 mmol) of **2a** resulted in 0.19 g of the compound **2b** as yellow crystals (58%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.0 Hz, 2H), 7.86 (d, *J* = 7.8 Hz, 2H), 7.53 – 7.44 (m, 8H), 7.35 (d, *J* = 7.9 Hz, 6H), 7.12 (d, *J* = 8.4 Hz, 4H), 7.07 (d, *J* = 8.3 Hz, 1H), 3.59 (s, 2H), 3.33 (d, *J* = 7.2 Hz, 8H), 2.60 (s, 4H), 1.85 (d, *J* = 5.6 Hz, 7H), 1.47 (s, 18H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 167.68, 167.19, 156.35, 153.93, 147.82, 146.31, 136.86, 134.31, 133.03, 132.41, 131.35, 130.91, 130.50, 128.86, 128.76, 128.64, 126.34, 125.27, 124.63, 124.16, 122.84, 121.49, 120.85, 118.57, 85.16, 83.88, 79.28, 71.80, 68.47, 50.22, 46.07, 44.26, 40.75, 31.93, 29.70, 29.66, 29.37, 28.55, 28.47, 27.74, 26.88, 26.04, 22.70, 22.19, 19.17, 14.12. HRMS-ESI: *m/z* calcd. $[M+H]^+$ for $C_{58}H_{63}N_6O_4S_2^+$, 971.4352; found, 971.4344.

Compound **2b** (0.073 g 0.075 mmol) was added to a saturated hydrogen chloride solution of ethyl acetate (5 mL) and the mixture was stirred for 3 h at room temperature. The resulting suspension was filtrated and the solid was washed with ethyl acetate, dried in vacuum at 60 °C for 24 h. A red solid as compound **3** was obtained in 0.05 g (86%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (d, *J* = 7.7 Hz, 2H), 7.96 (d, *J* = 7.9 Hz, 2H), 7.77 (d, *J* = 8.3 Hz, 4H), 7.67 (s, 2H), 7.63 (s, 1H), 7.53 (dd, *J* = 15.9, 9.0 Hz, 4H), 7.47 – 7.39 (m, 3H), 7.11 (dt, *J* = 13.9, 7.4 Hz, 6H), 3.75 (s, 2H), 3.12 (dd, *J* = 88.1, 56.7 Hz, 12H), 1.97 (d, *J* = 84.1 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 167.10, 153.96, 147.63, 146.68, 137.23, 134.43, 133.54, 132.06, 130.94, 129.72, 129.17, 128.60, 127.00, 125.83, 124.66, 124.52, 124.40, 122.94, 122.88, 122.63, 121.07, 71.62, 49.81, 47.51, 43.02, 41.32, 29.49, 27.68, 21.60, 19.58, 19.36, 18.22. HRMS-ESI: *m/z* calcd. $[M+H]^+$ for $C_{48}H_{47}N_6S_2^+$, 771.3304; found, 771.3304.

2. UV-vis Spectra of 1, 2, 3

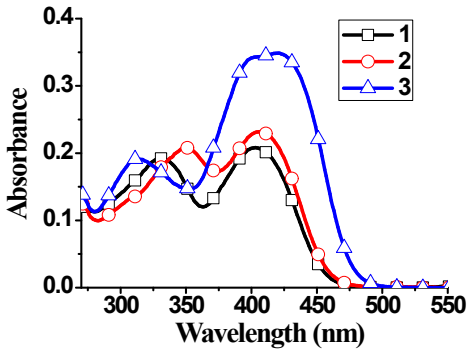


Fig. S1 Linear absorption of **1**, **2**, **3** in DMF (c = 1.0 ×10^{-5} M)

3. Fluorescence lifetime of 1, 2, 3

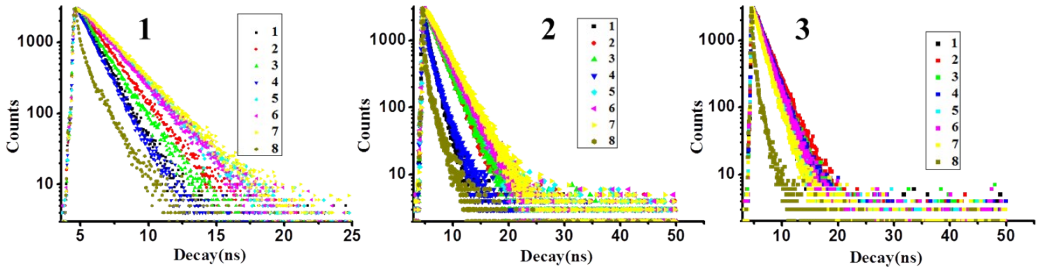


Fig. S2 Fluorescence lifetime of **1**, **2**, **3** in eight solvents (Ben, DCM, THF, EtOAc, MeCN, DMSO, DMF, H_2O)

4. Two photon absorption cross-section values of 1, 2, 3

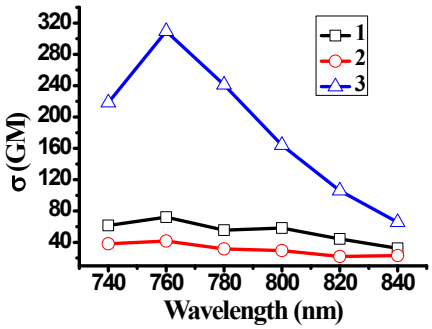


Fig. S3 Two photon absorption cross-section values of **1**, **2**, **3** in 100 μM DMSO (100 μM, Ex = 760 nm).

5. Water solubility of 1, 2, 3

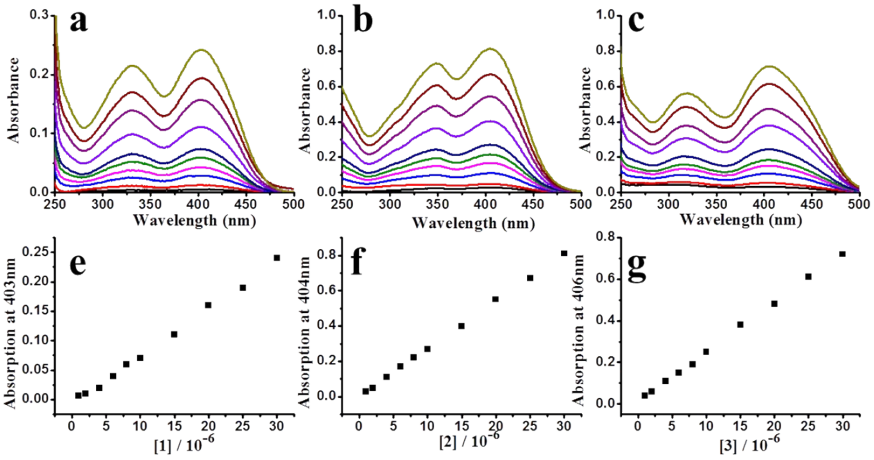


Fig. S4 Absorption spectra and plot of intensity against the concentration of **1** (a, e), **2** (b, f) and **3** (c, g) in PBS buffer (pH = 7.4), respectively. *c* = 10 μM, 25 °C.

6. Photo-stability and pH- stability of 1, 2, 3

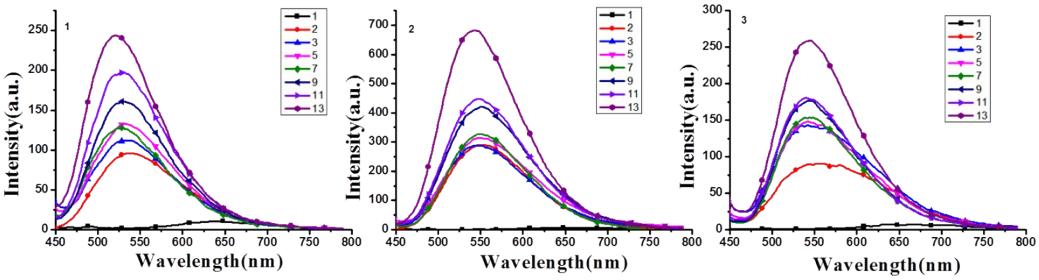


Fig. S5 The fluorescent intensities for **1, 2, 3** at varied pH values in aqueous

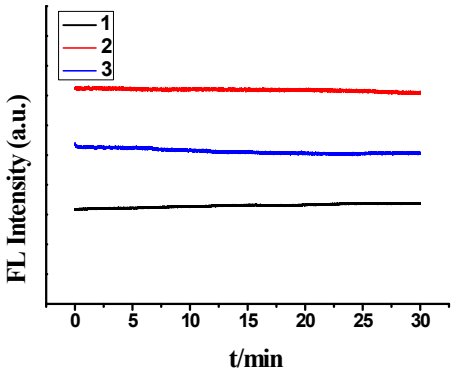


Fig. S6 Photo-stability of **1**, **2**, **3** in PBS (pH=7.2), *c* = 10 μM, 25 °C

7. Cytotoxicity of 1, 2, 3

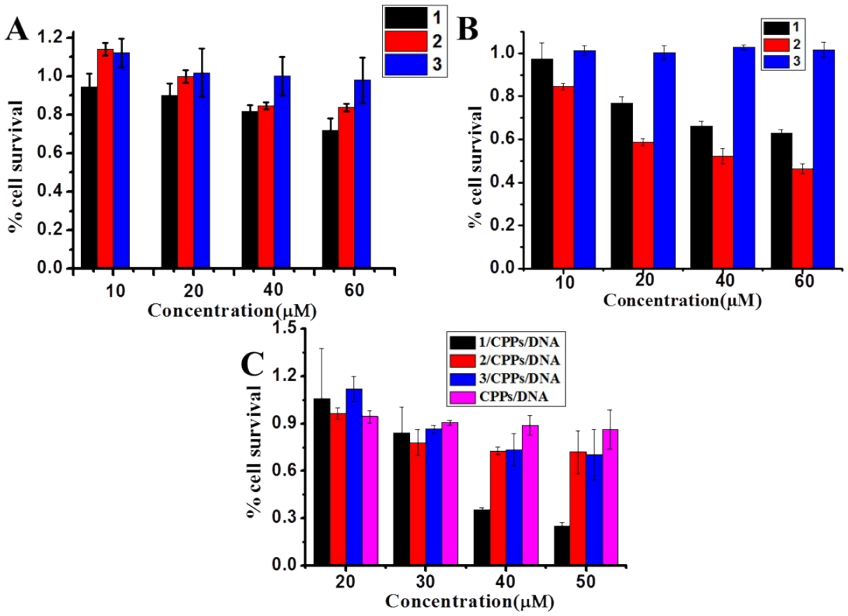


Fig. S7 Cytotoxicity data results of (A)1-3 in Hela, (B) 1-3 in A549, (C) 1-3/CPPs/DNA in Hela obtained from the MTT assay (The data are given as mean ± SD (n = 6))

8. Laser confocal imaging of compounds 1, 2 and 3

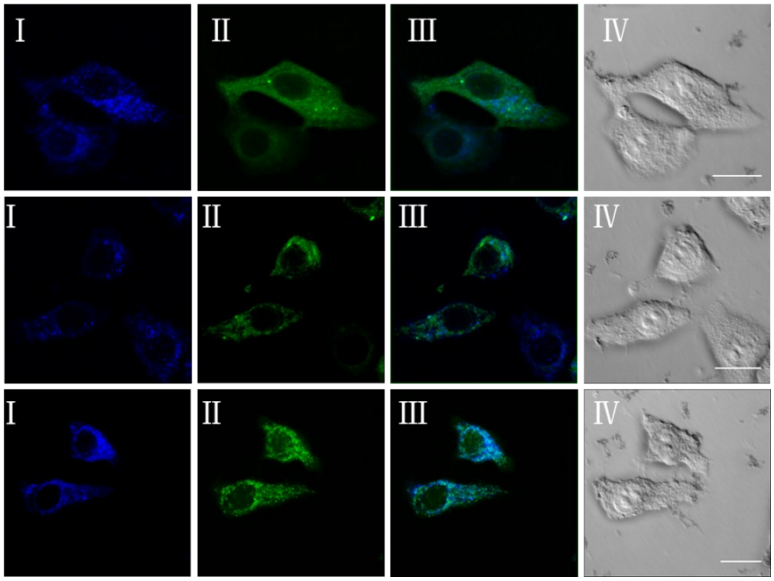


Fig. S8 Colocalization imaging of HeLa cells stained with Lyso Tracked Blue (I, λ_{ex} = 405 nm, λ_{em}= 425–475 nm**)** and with **1–3** (II, λ_{ex} = 488 nm, λ_{em}= 530–560 nm) after 30 min of incubation, (III) Merged image of (I) and (II); (IV) DIC image of HeLa cells. Scale bars = 20 μm.

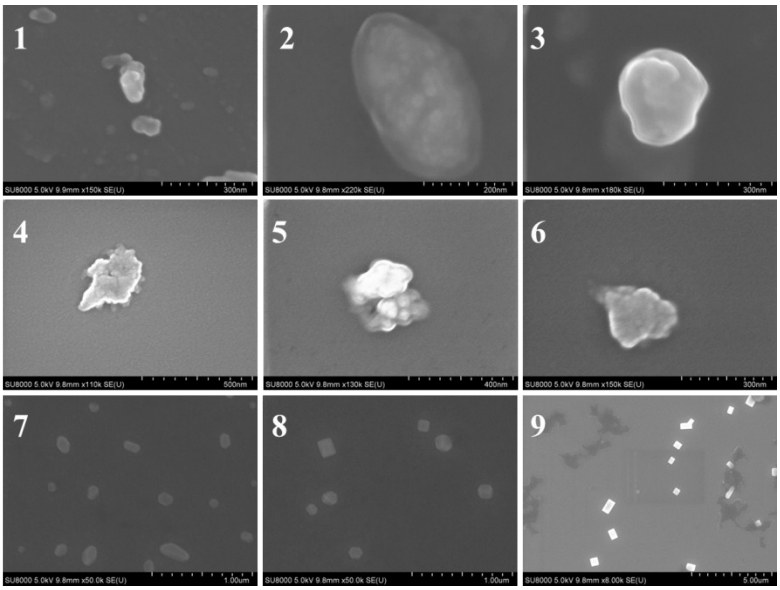


Fig. S9 SEM images of (1, 2, 3)compound 1-3; (4, 5, 6) complex 1-3/CPPs; (7, 8, 9) condensed DNA 1-3/CPPs in Tris-HCl buffer (5 mM, pH 7.4): (a) [1]= 10 μM; [2]= 10 μM;[3]= 10 μM, [DNA]= 5μM.

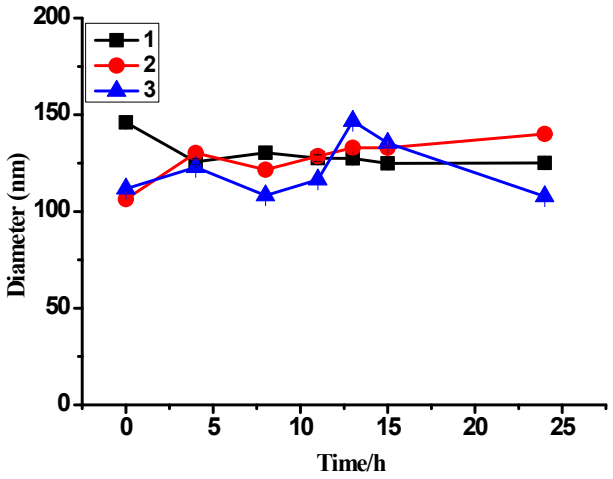


Fig. S10 The stability of **1-3/CPPs/DNA** nanoparticles in DMEM containing 10% FBS during an incubation time over 24 h. [**1-3/CPPs**] = 10 μM, [DNA] = 5 μM.

9. RFP expression of 1/CPPs; 2/CPPs; 3/CPPs, CPPs

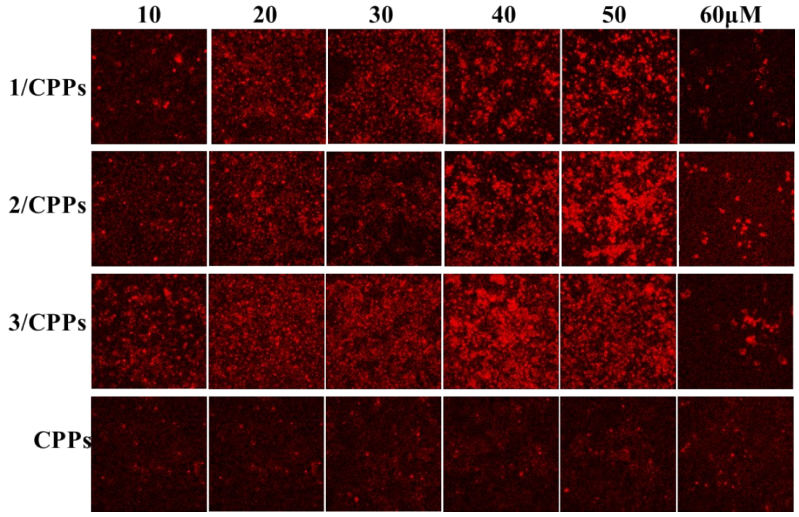


Fig. S11 Confocal microscopy images of RFP expression of 1/CPPs; 2/CPPs; 3/CPPs, CPPs with different concentration (10 - 60μM) in Hela cells. [RFP DNA]= 10μg/mL

10. Transfection efficiencies of pGL-3 DNA by 1/CPPs, 2/CPPs, 3/CPPs

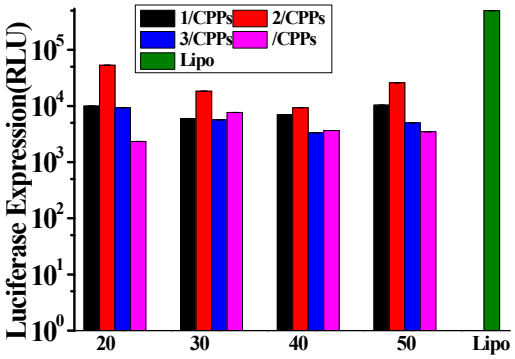


Fig. S12 Transfection efficiencies of pGL-3 DNA by **1/CPPs**, **2/CPPs**, **3/CPPs** at varied concentrations in Hela cells by luciferase assays. The [pGL-3 DNA] = 10 μg/mL. As controls, CPPs and Lipofectamine 2000 were also investigated.

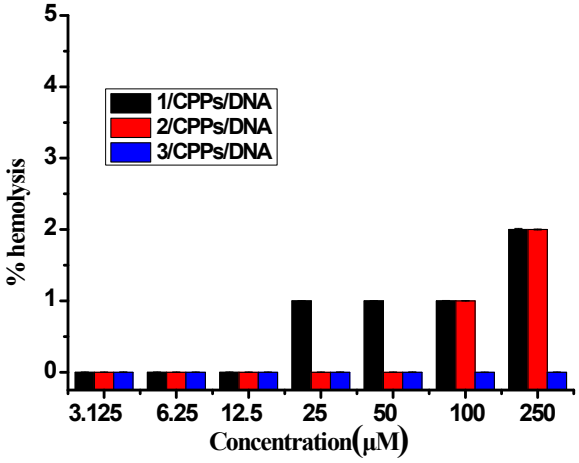


Fig. S13 Hemolytic activity of the **1-3/CPPs/DNA**

11. Photo-physical properties

Table S1 Single−photon−related photophysical properties of **1**, **2**, **3**

Compound	solvent	λ_{abs}	ε /10^4	λ_{em}	Φ/%	τ/ns
1	**B**Ben**n**	335 405	1.2 1.3	493	36.8	1.21
	DCM	334 400	1.8 1.6	509	46.5	1.68
	THF	330 401	2.4 2.5	499	50.2	1.47
	EtOAc	329 400	1.5 1.6	498	32.9	1.15
	MeCN	327 397	1.8 1.8	535	44.7	2.08
	DMF	330 402	1.9 2.1	535	40.6	2.02
	DMSO	330 407	2.0 2.0	542	37.9	2.23
	H_2O	332 404	1.4 1.4	524	20.9	0.79
2	**B**Ben**n**	359 415	1.1 1.5	491	26.3	1.18
	DCM	356 401	1.8 1.9	516	69	2.16
	THF	352 407	2.1 2.1	509	36.3	2.20
	EtOAc	353 405	1.8 1.9	503	26.8	1.37
	MeCN	348 399	1.9 2.0	539	74.5	2.60
	DMF	348 405	2.1 2.3	539	64.0	2.52
	DMSO	349 409	2.1 2.3	545	76.7	2.80
	H_2O	349 407	1.7 1.9	646	30.4	0.84
3	**B**Ben**n**	313 424	1.6 3.1	493	38.1	1.63
	DCM	313 423	1.9 3.3	521	33.9	2.12
	THF	310 420	1.7 3.0	505	41.4	1.90
	EtOAc	310 418	1.7 3.0	503	34.0	1.88
	MeCN	310 416	1.7 3.0	547	15.4	1.65
	DMF	313 422	1.7 3.1	547	17.0	1.83
	DMSO	314 423	2.0 3.3	551	14.1	1.50
	H_2O	313 414	1.5 2.1	547	−−	0.65

12. Spectra data of compounds synthesized

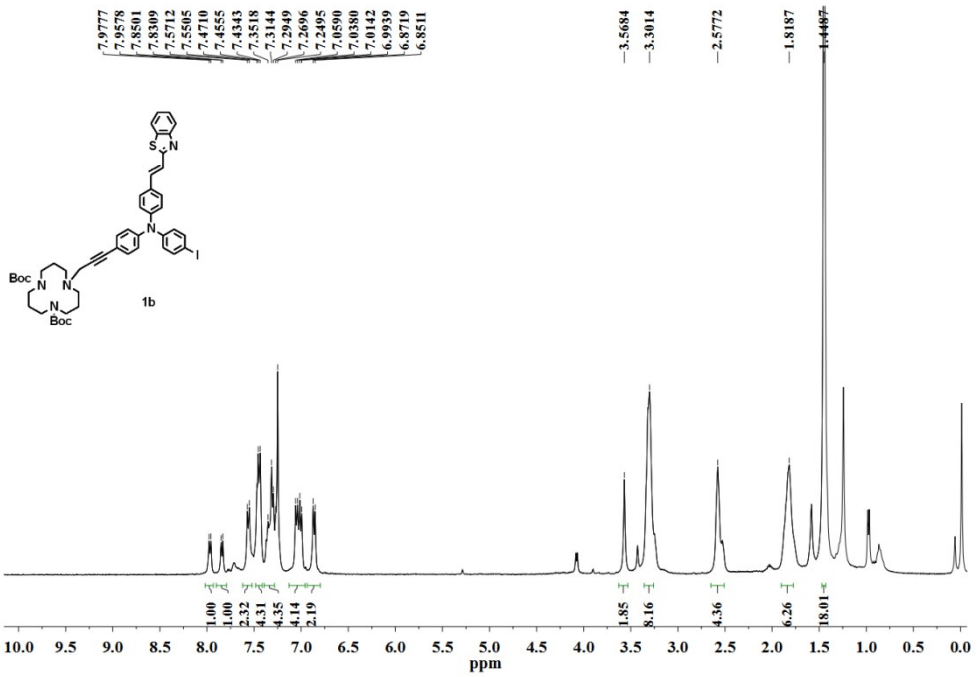


Fig. S14 ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1b**

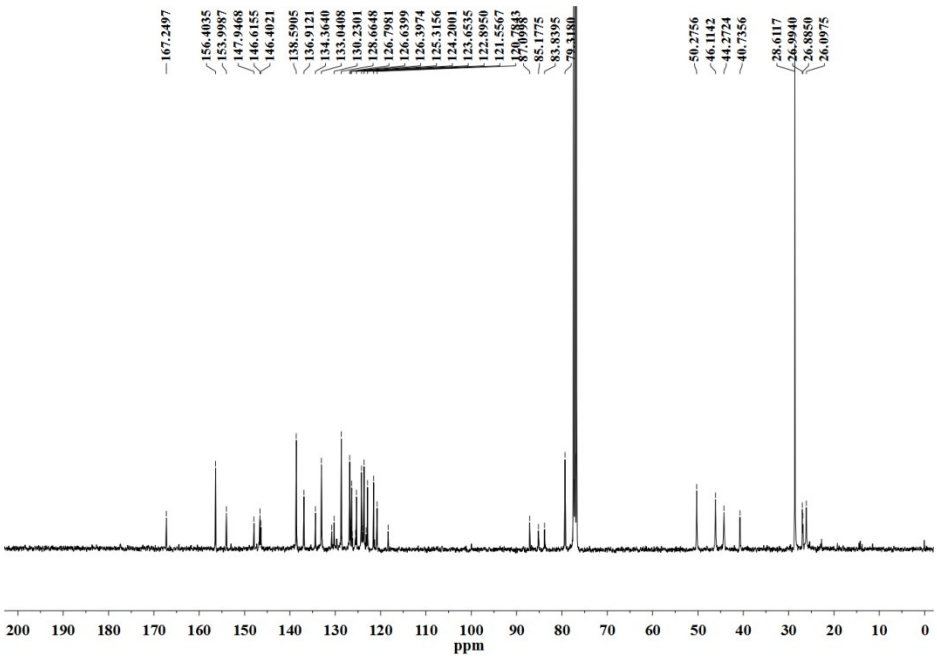
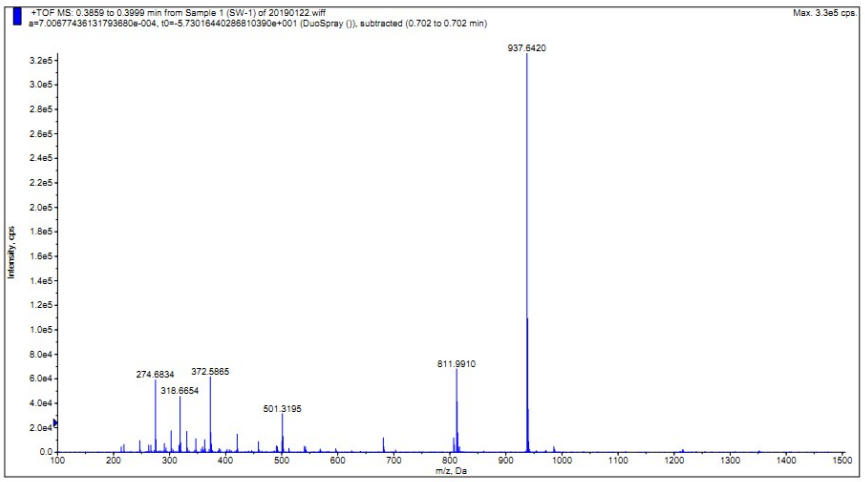
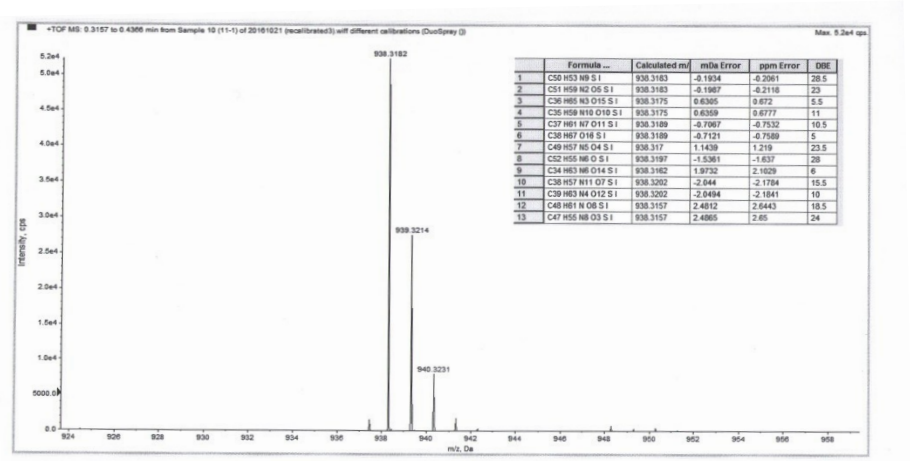


Fig. S15 ^{13}C NMR (101 MHz, $CDCl_3$) spectrum of **1b**





	Formula ...	Calculated m/	mDa Error	ppm Error	DBE
1	C50 H53 N9 S I	938.3183	-0.1934	-0.2061	28.5
2	C51 H59 N2 O6 S I	938.3183	-0.1987	-0.2118	23
3	C36 H65 N3 O15 S I	938.3175	0.6365	0.672	5.5
4	C35 H59 N10 O10 S I	938.3175	0.6359	0.6777	11
5	C37 H61 N7 O11 S I	938.3189	-0.7067	-0.7532	10.5
6	C38 H67 O16 S I	938.3189	-0.7121	-0.7589	5
7	C49 H57 N5 O4 S I	938.317	1.1439	1.219	23.5
8	C52 H55 N6 O S I	938.3197	-1.5361	-1.637	28
9	C34 H63 N6 O14 S I	938.3162	1.9732	2.1029	6
10	C38 H57 N11 O7 S I	938.3202	-2.044	-2.1784	15.5
11	C39 H63 N4 O12 S I	938.3202	-2.0494	-2.1841	10
12	C48 H61 N O8 S I	938.3157	2.4812	2.6443	18.5
13	C47 H55 N8 O3 S I	938.3157	2.4865	2.65	24

Fig. S16 The MS and HRMS-ESI spectrum of **1b**

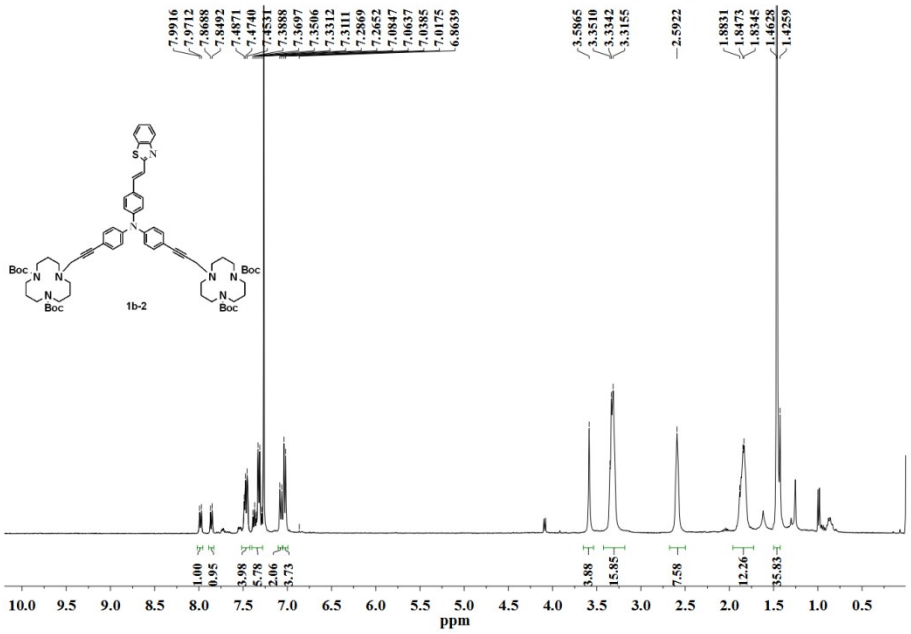


Fig. S17 ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1b-2**

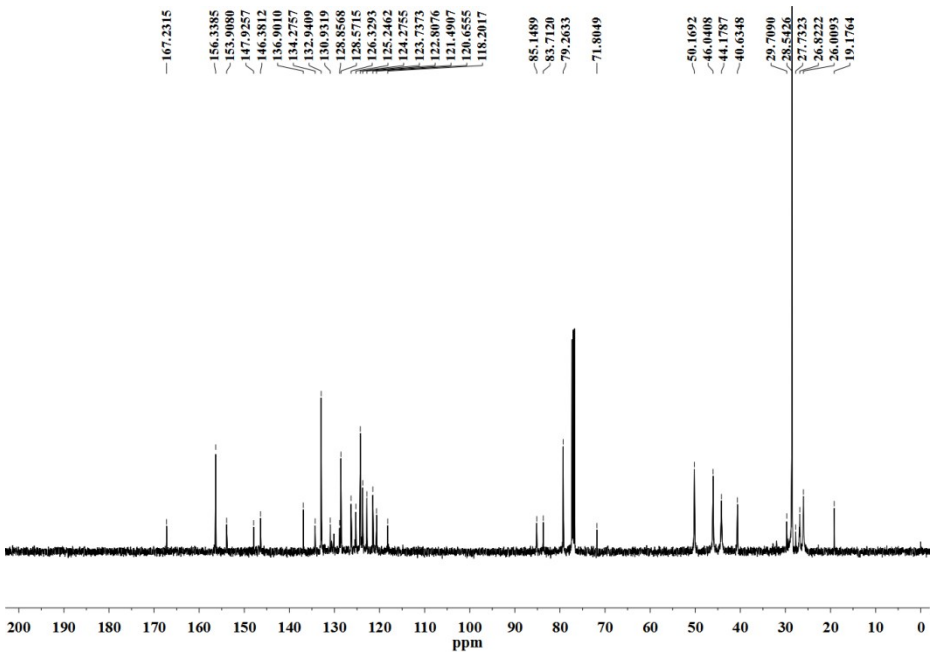
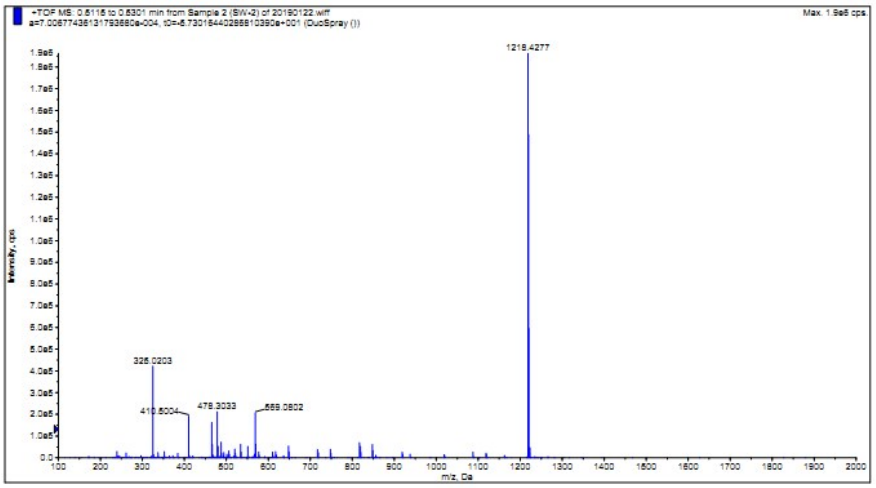
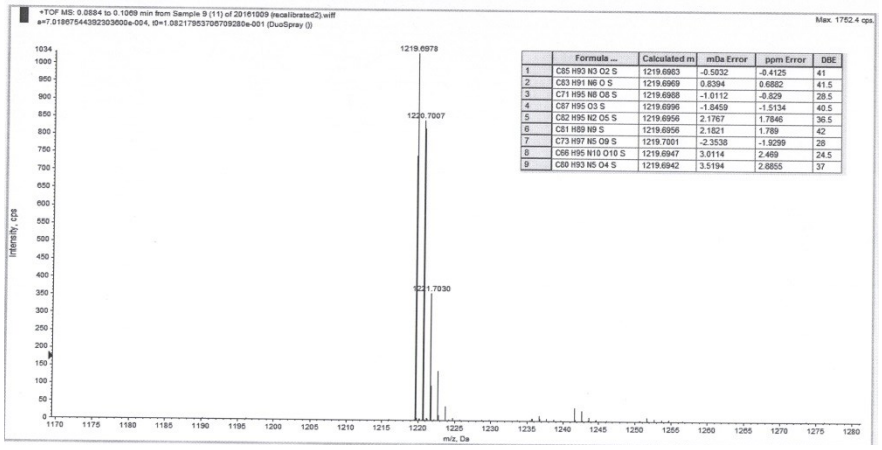


Fig. S18 ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **1b-2**





	Formula ...	Calculated m	mDa Error	ppm Error	DBE
1	C85 H93 N3 O2 S	1219.6983	-0.5932	-0.4125	41
2	C83 H91 N6 O S	1219.6969	0.8394	0.6882	41.5
3	C71 H95 N8 O8 S	1219.6968	-1.0112	-0.829	28.5
4	C67 H95 O3 S	1219.6996	-1.8459	-1.5134	40.5
5	C82 H95 N2 O5 S	1219.6956	2.1767	1.7846	36.5
6	C81 H89 N9 S	1219.6956	2.1821	1.789	42
7	C73 H97 N5 O9 S	1219.7001	-2.3538	-1.9299	28
8	C66 H95 N10 O10 S	1219.6947	3.0114	2.469	24.5
9	C80 H93 N5 O4 S	1219.6942	3.5194	2.8855	37

Fig. S19 The MS and HRMS-ESI spectrum of **1b-2**

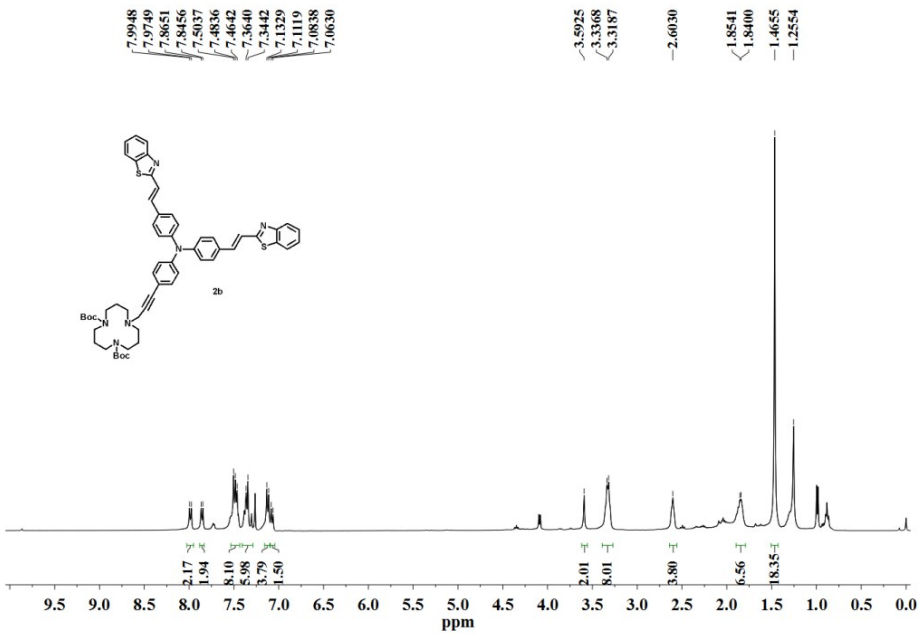


Fig. S20 ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **2b**

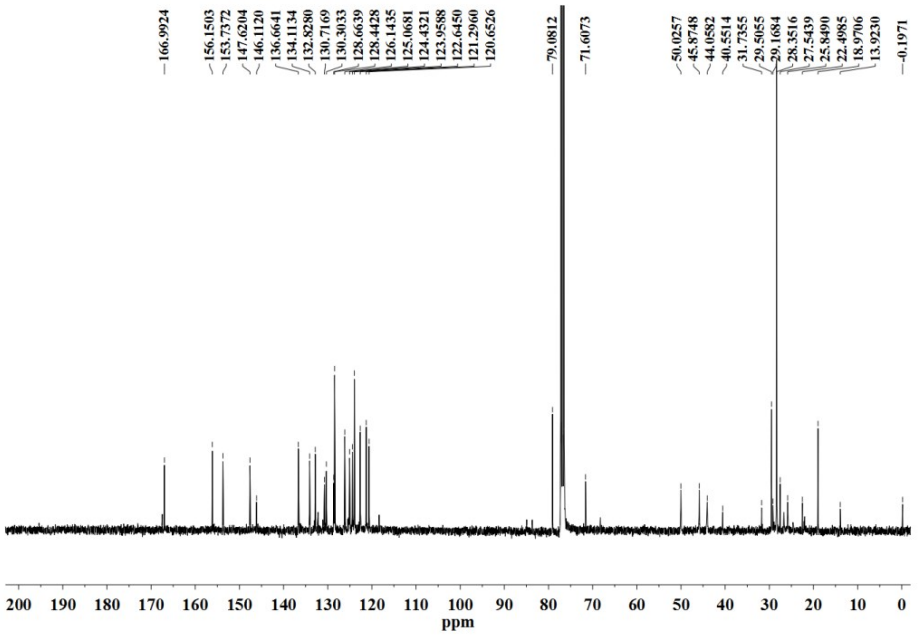
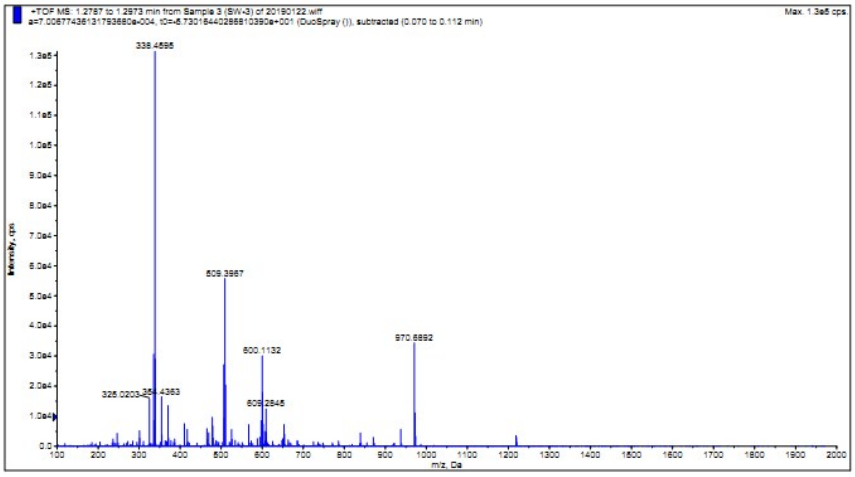
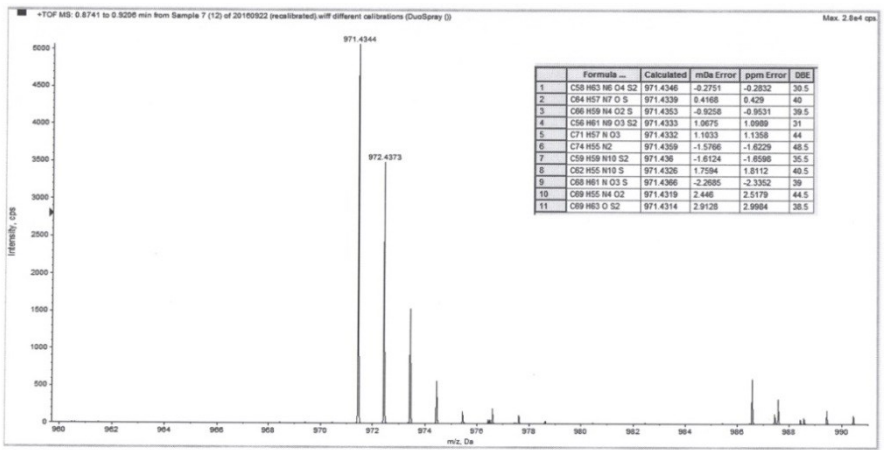


Fig. S21 ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of **2b**





	Formula ...	Calculated	mDa Error	ppm Error	DBE
1	C58 H63 N6 O4 S2	971.4346	-0.2751	-0.2832	30.5
2	C64 H57 N7 O S	971.4339	0.4168	0.429	40
3	C66 H59 N4 O2 S	971.4353	-0.9258	-0.9531	39.5
4	C56 H61 N9 O3 S2	971.4333	1.9675	1.9989	31
5	C71 H57 N O3	971.4332	1.1633	1.1358	44
6	C74 H55 N2	971.4359	-1.5766	-1.6229	48.5
7	C59 H59 N10 S2	971.436	-1.6124	-1.6598	35.5
8	C62 H55 N10 S	971.4326	1.7594	1.8112	40.5
9	C68 H61 N O3 S	971.4366	-2.2685	-2.3352	39
10	C69 H55 N4 O2	971.4319	2.446	2.5179	44.5
11	C69 H63 O S2	971.4314	2.9128	2.9984	38.5

Fig. S22 The MS and HRMS-ESI spectrum of **2b**

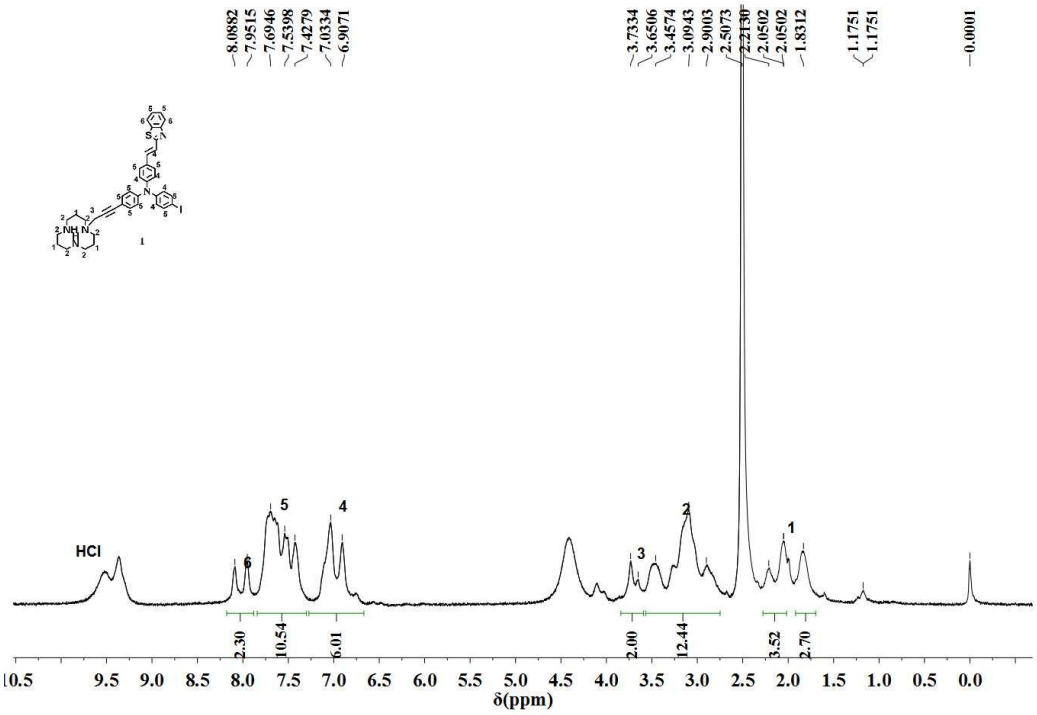


Fig. S23 ^{1}H NMR (400 MHz, DMSO-*d6*) spectrum of **1**

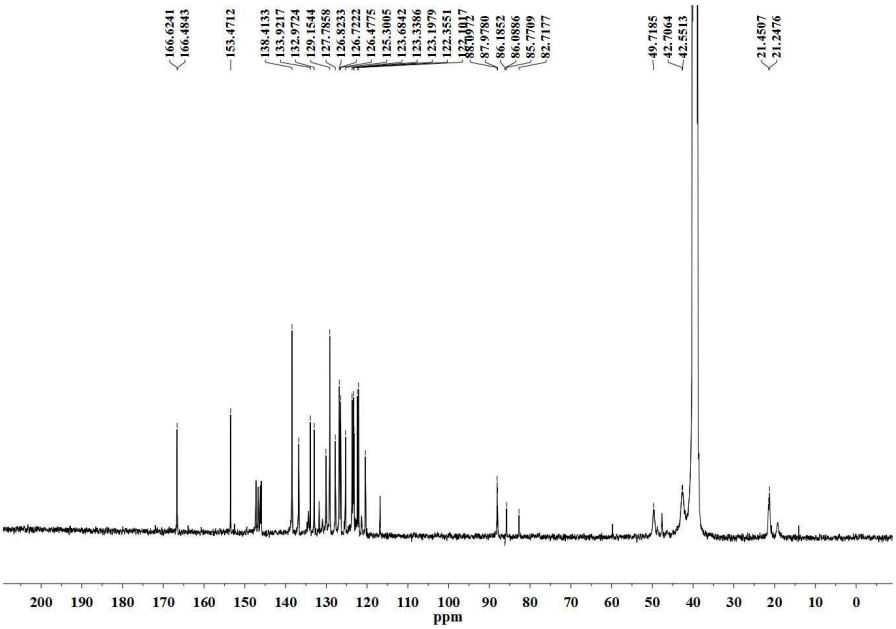
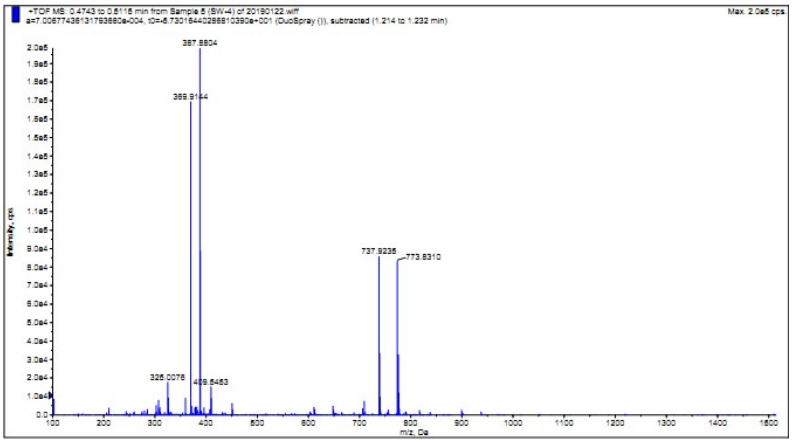


Fig. S24 ^{13}C NMR (126 MHz, DMSO-*d6*) spectrum of **1**



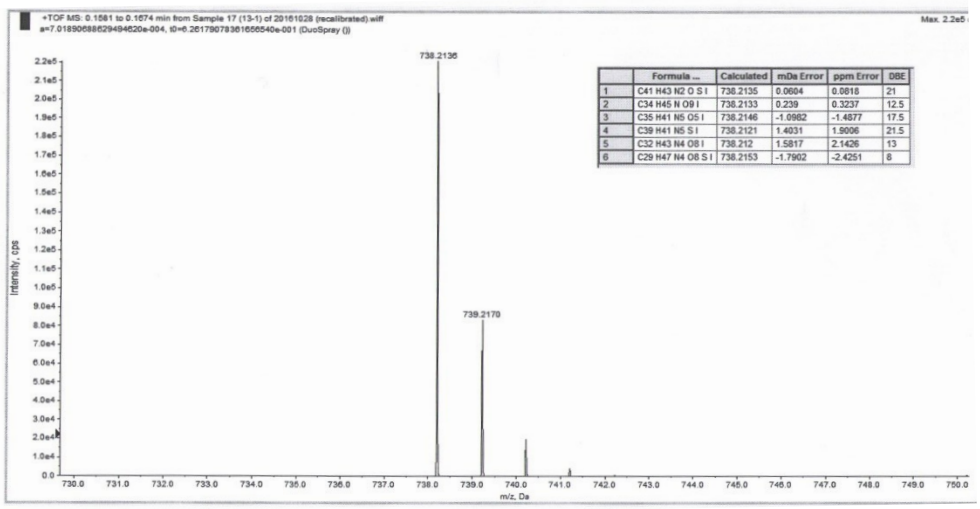


Fig. S25 The MS and HRMS-ESI spectrum of **1**

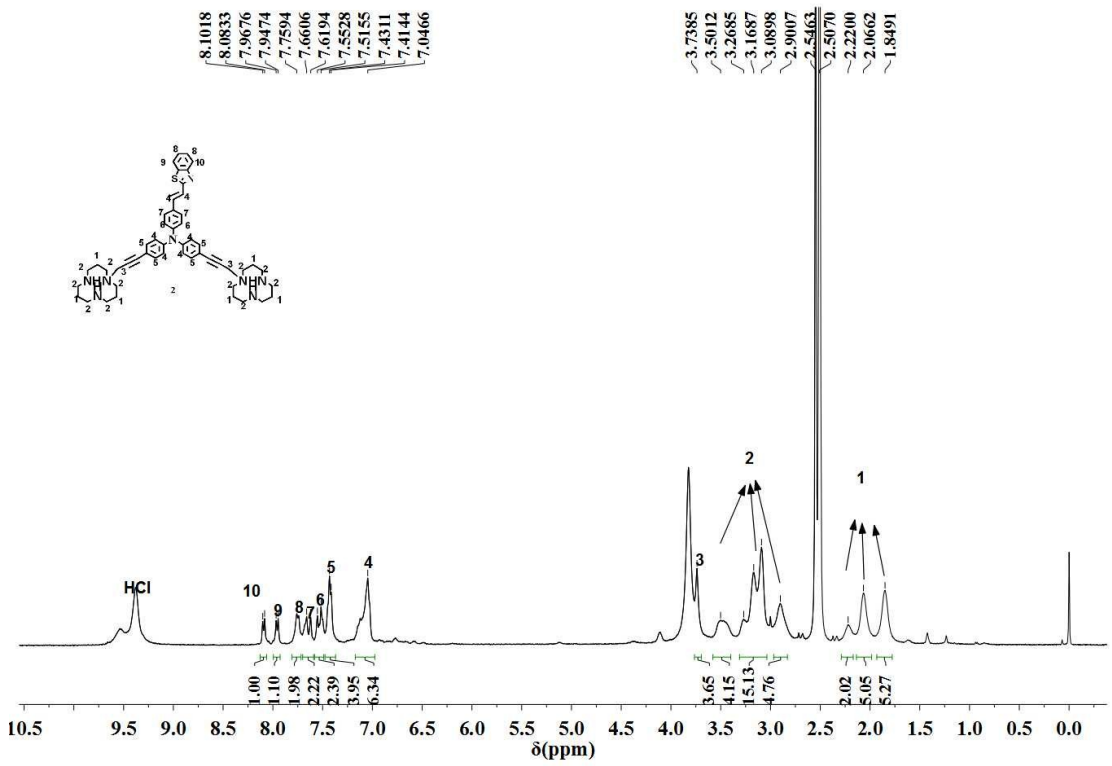


Fig. S26 ^{1}H NMR (400 MHz, DMSO-*d6*) spectrum of **2**

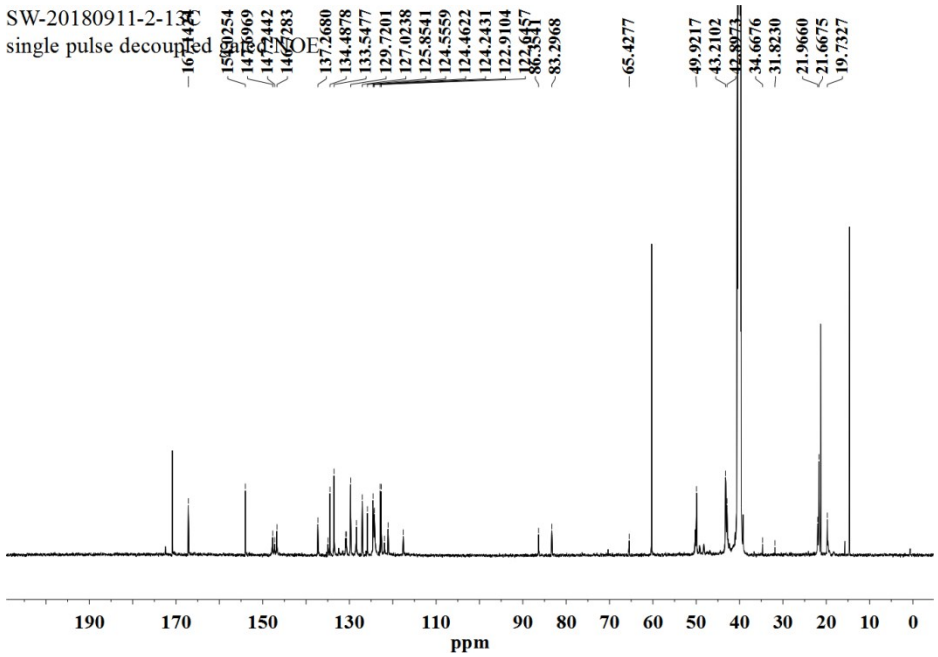
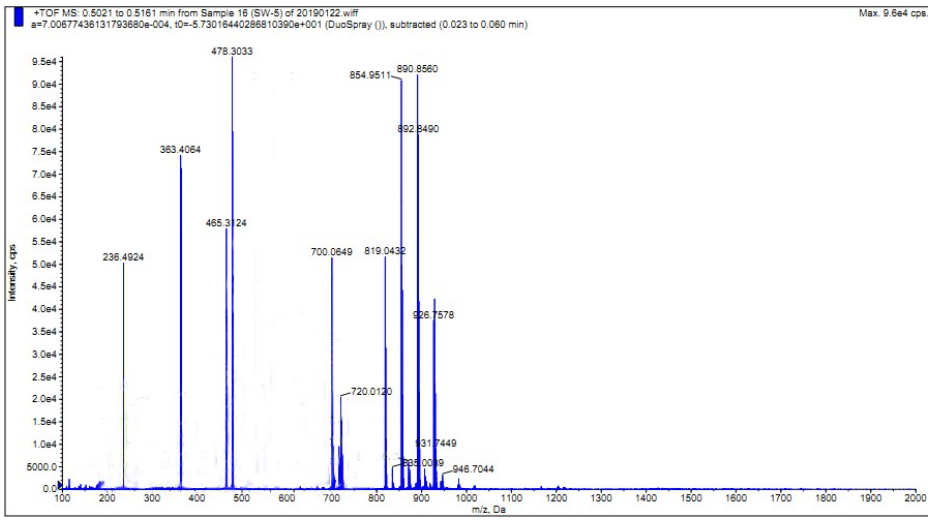
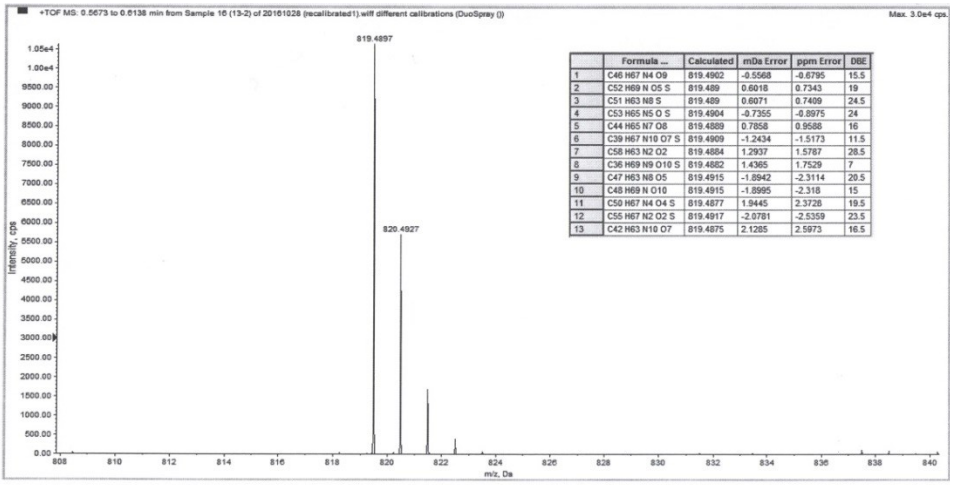


Fig. S27 ^{13}C NMR (126 MHz, DMSO-*d6*) spectrum of **2**





	Formula ...	Calculated	mDa Error	ppm Error	DBE
1	C46 H67 N4 O9	819.4902	-0.5568	-0.6795	15.5
2	C52 H69 N O5 S	819.489	0.6018	0.7343	19
3	C51 H63 N8 S	819.489	0.6071	0.7409	24.5
4	C53 H65 N5 O S	819.4904	-0.7355	-0.8975	24
5	C44 H65 N7 O6	819.4889	0.7858	0.9588	16
6	C39 H67 N10 O7 S	819.4909	-1.2434	-1.5173	11.5
7	C58 H63 N2 O2	819.4884	1.2937	1.5787	28.5
8	C36 H69 N9 O10 S	819.4882	1.4365	1.7529	7
9	C47 H63 N8 O5	819.4915	-1.8942	-2.3114	20.5
10	C48 H69 N O10	819.4915	-1.8995	-2.318	15
11	C50 H67 N4 O4 S	819.4877	1.9445	2.3728	19.5
12	C55 H67 N2 O2 S	819.4917	-2.0781	-2.5359	23.5
13	C42 H63 N10 O7	819.4875	2.1285	2.5973	16.5

Fig. S28 The HRMS-ESI spectrum of **2**

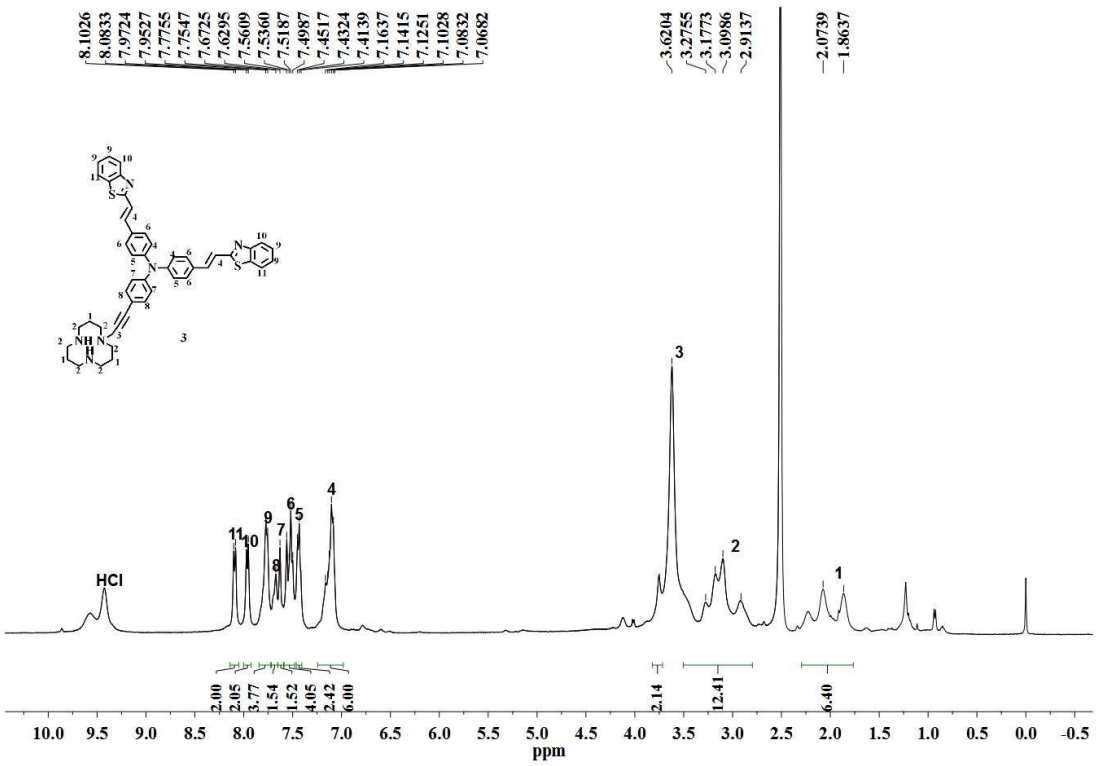


Fig. S29 ^{1}H NMR (400 MHz, DMSO-*d6*) spectrum of **3**

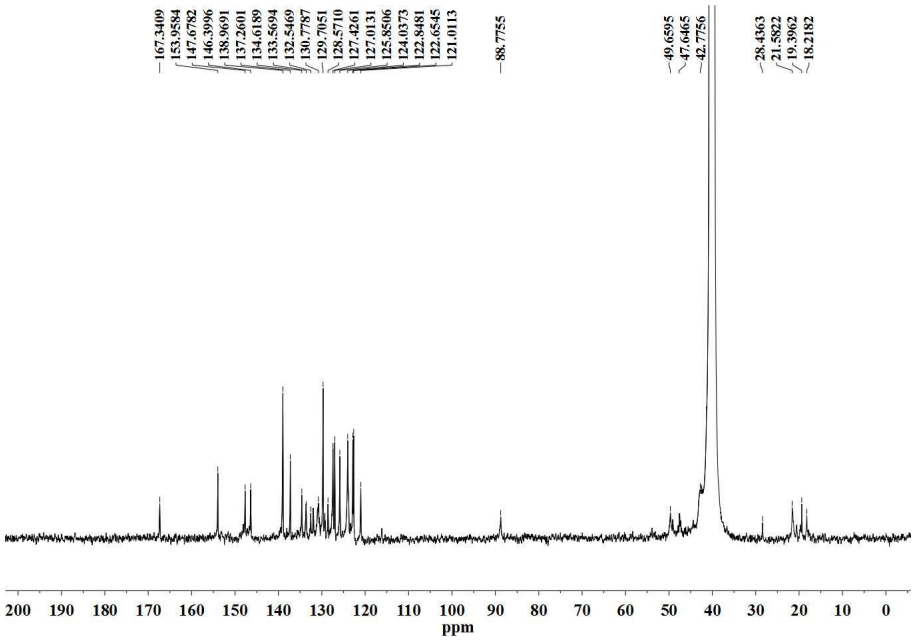
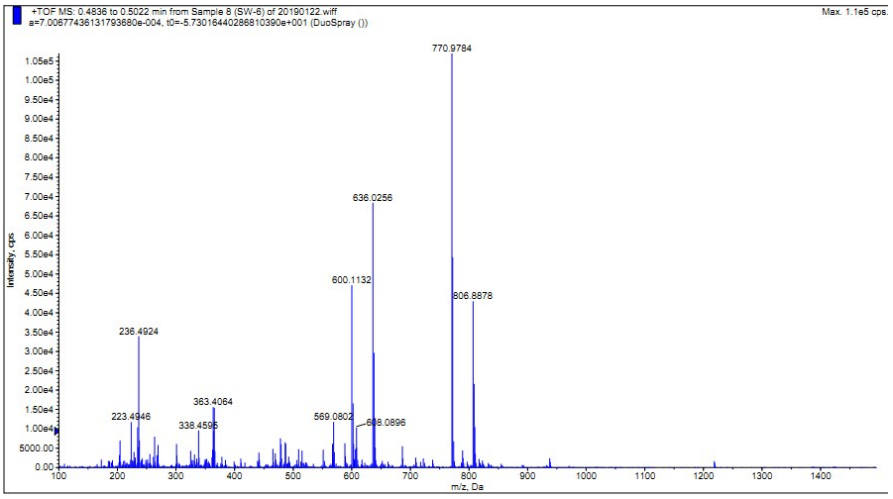
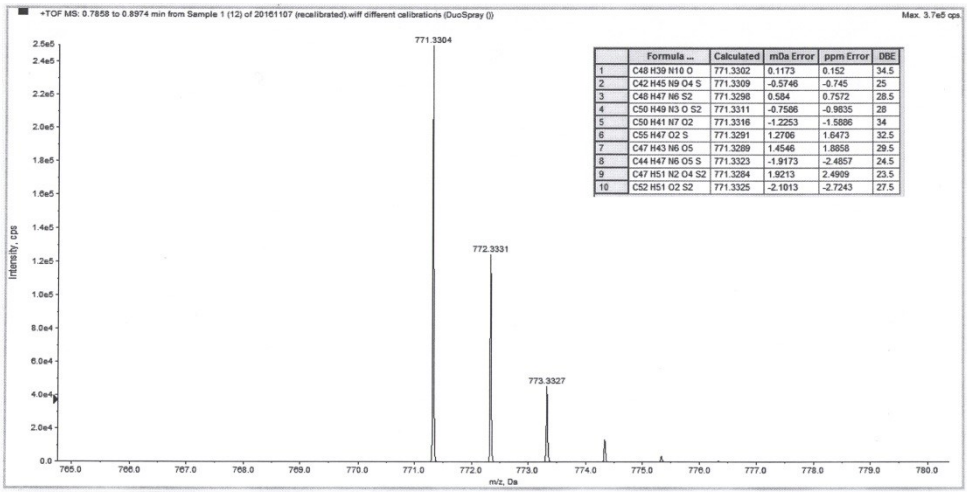


Fig. S30 ^{13}C NMR (126 MHz, DMSO-*d6*) spectrum of **3**





	Formula ...	Calculated	mDa Error	ppm Error	DBE
1	C48 H39 N10 O	771.3302	0.1173	0.152	34.5
2	C42 H45 N9 O4 S	771.3309	-0.5746	-0.745	25
3	C48 H47 N6 S2	771.3298	0.584	0.7572	28.5
4	C50 H49 N3 O S2	771.3311	-0.7586	-0.9835	28
5	C50 H41 N7 O2	771.3316	-1.2253	-1.5886	34
6	C55 H47 O2 S	771.3291	1.2706	1.6473	32.5
7	C47 H43 N6 O5	771.3289	1.4546	1.8858	29.5
8	C44 H47 N6 O5 S	771.3323	-1.9173	-2.4857	24.5
9	C47 H51 N2 O4 S2	771.3284	1.9213	2.4909	23.5
10	C52 H51 O2 S2	771.3325	-2.1013	-2.7243	27.5

Fig. S31 The MS and HRMS-ESI spectrum of **3**

13. References

1. Z. F. Guo, H. Yan, Z.-F. Li, Z. L. Lu, Org. Bio. Chem., 2011, **9**, 6788-6796.