Supporting Information

Macrocyclic Polyamine [12]aneN_3 Modified Triphenylamine-Pyrazine Derivatives as Efficient Non-viral Gene Vectors with AIE and Two-photon Imaging Properties

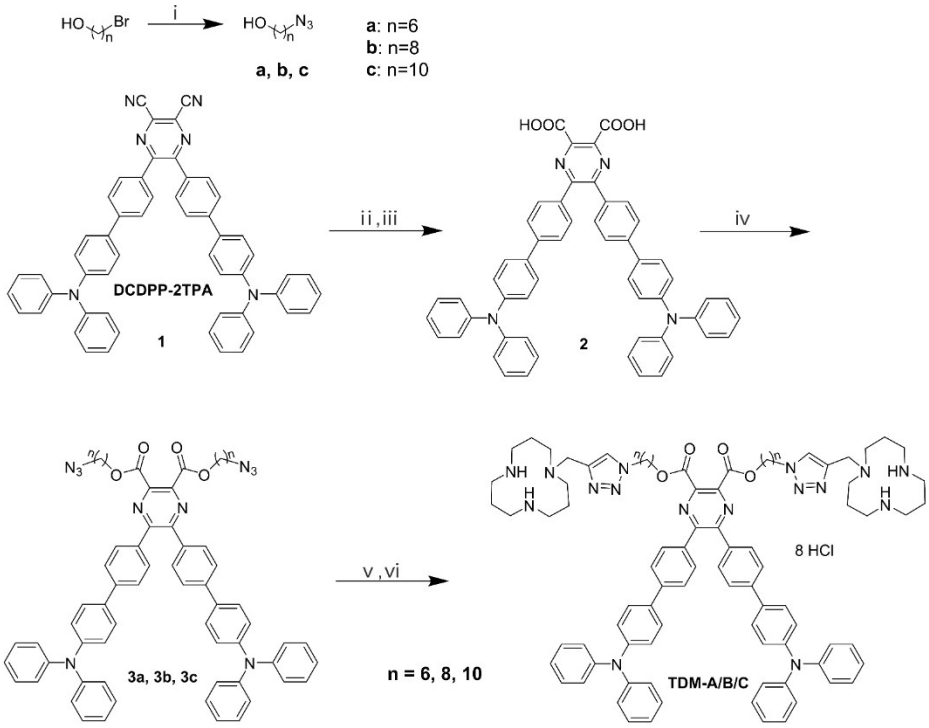
Le-Le Ma,[a] Ming-Xuan Liu,[a] Xu-Ying Liu,[a] Wan Sun,[a] Zhong-Lin Lu,[,a] Yong-Guang Gao,*[,b] and Lan He *[,c]*

[a] Key Laboratory of Radiopharmaceutics,, Ministry of Education; College of Chemistry, Beijing Normal University, Beijing 100875, P. R. China.
[b] Lab for Bone Metabolism, Key Lab for Space Biosciences and Biotechnology, School of Life Sciences, Northwestern Polytechnical University, Xi'an, Shaanxi, 710072, China.
[c] China National Institute for Food and Drug Control, Institute of Chemical Drug Control, TianTanXiLi 2, Beijing, 100050, China.

*Corresponding authors: luzl@bnu.edu.cn (Zhong-Lin Lu), helan1961@aliyun.com, (Lan He) gaoyongguang@nwpu.edu.cn (Yong-Guang Gao)

Content

1. Synthesis



Scheme S1 Schematic route for **TDM-A, TDM-B** and **TDM-C**: (i) NaN_3, DMF, 65 ºC, 8 h; (ii) NaOH (6 M), Reflux, 24 h; (iii) HCl (2 M); (iv) NMM, EDCI, HOBT, TEA, r.t., 8 h; (v) Propargyl [12]aneN_3, CuBr, $CHCl_3$, r.t., 30 min; (vi) HCl-EtOAc, DCM, r.t., 2h.

Synthesis of a, b and c. Under argon atmosphere, to the dry DMF (30 mL) solutions of 1- bromhydrin (10 mmol) were slowly added sodium azide (1.30 g, 20 mmol, 2.0 equiv.) at 0 ºC. The mixture was slowly warmed up to 65 ºC and stirred for overnight. After cooling to room temperature and filtering, saturated brine solution (50 mL) added to the system, and then the mixture solution was extracted with ethyl acetate (20 mL ×3). The collected organic layers were dried over anhydrous Na_2SO_4. After filtration and vacuum distillation, the crude product was purified by a silica gel column with

PE/EtOAc (v/v = 10:1 ~ 20:1) as eluent to give corresponding compounds **a, b and c** as colorless oil in 85 ~ 90% yields.

Compound a: 1.26 g, 8.80 mmol; Yield: 88%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.55 (t, *J* = 6.6 Hz, 2H), 3.21 (t, *J* = 6.9 Hz, 2H), 2.36 (s, 1H), 1.62 – 1.46 (m, 4H), 1.33 (dt, *J* = 7.0, 3.7 Hz, 4H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 62.57, 51.42, 32.54, 28.84, 26.57, 25.38.

Compound b: 1.54 g, 9 mmol; Yield: 90%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.48 (t, *J* = 6.7 Hz, 2H), 3.14 (t, *J* = 6.9 Hz, 2H), 2.70 (s, 1H), 1.54 – 1.37 (m, 4H), 1.27 – 1.22 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 62.55, 51.43, 32.67, 29.27, 29.10, 28.79, 26.63, 25.69.δ 62.56, 51.41, 32.53, 28.83 26.56, 25.37.

Compound c: 1.69 g, 8.5 mmol; Yield: 85%.^{1}H NMR (600 MHz, $CDCl_3$) δ 3.59 (t, *J* = 6.7 Hz, 2H), 3.22 (t, *J* = 7.0 Hz, 2H), 1.80 (s, 1H), 1.61 – 1.48 (m, 4H), 1.36 – 1.22 (m, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 63.00, 51.53, 32.80, 29.53, 29.45, 29.44, 29.18, 28.88, 26.76, 25.79.

Synthesis of compound 2. Compound **1** (1.20 g, 1.56 mmol) were prepared according to literature methods and dissolved in 30 ml of ethanol, and then 60 ml of 6M KOH solution was added. [1] The mixture was refluxed during 48 h and then cooled to room temperature. The pH was adjusted to 6–7 with 2 M HCl, a yellow color precipitate of colloidal appearance being obtained. The solid was filtered off, washed with ethanol and dried under high vacuum to obtained a pale yellow solid **2** (0.82 g, 1.01 mmol), yield: 65%.^{1}H NMR (600 MHz, DMSO-d_6): *δ*. 7.55 (dt, *J* = 20.8, 8.0 Hz, 12H), 7.24 (s, 8H), 6.97 (dt, *J* = 21.8, 11.0 Hz, 16H). ^{13}C NMR (101 MHz, $CDCl_3$): δ. 166.41, 151.81,

147.75, 147.44, 142.84, 140.76, 136.05, 133.08, 130.81, 130.13, 128.15, 126.34, 124.83, 123.93, 123.51. HRMS-ESI (m/z): calcd. $[M+H]^+$ for $[C_{54}H_{39}N_4O_4]^+$, 807.2966; found 807.2970.

Synthesis of 3a, 3b and 3c. Under argon atmosphere, compound **2** (2.02 g, 2.5 mmol), HOBT (0.85 g, 6.3 mmol), *N*-methylmorpholine (1.01 g, 10.0 mmol) and EDCI (1.44 g, 7.5 mmol) were dissolved in anhydrous DMF (10 mL). The solution was kept stirring at 0 ºC for 30 minutes, a solution of compound **a-c** (7.5 mmol, 3 equiv.) in 5 mL anhydrous DMF was added dropwise, and then allowed to warm up to room temperature and stirred for overnight, 40 mL water was added slowly to quench the reaction and a yellow solid was precipitated. After filtration and vacuum distillation, the crude product was purified by a silica gel column with PE/EtOAc (v/v = 15:1 ~ 20:1) as eluent to give corresponding compounds **3a, 3b, 3c** as yellow solid in 56 ~ 65% yields.

Compound 3a: 1.50 mg, 1.42 mmol; Yield: 56%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.63 (d, *J* = 8.4 Hz, 4H), 7.54 (d, *J* = 8.4 Hz, 4H), 7.48 (d, *J* = 8.7 Hz, 4H), 7.26 (dd, *J* = 8.4, 7.5 Hz, 12H), 7.13 – 7.10 (m, 12H), 7.03 (t, *J* = 7.3 Hz, 4H), 4.44 (t, *J* = 6.7 Hz, 4H), 3.27 (t, *J* = 6.9 Hz, 4H), 1.83 (dt, *J* = 14.3, 7.0 Hz, 4H), 1.63 (dt, *J* = 14.1, 7.0 Hz, 4H), 1.53 – 1.41 (m, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.99, 152.72, 147.88, 147.62, 141.88, 141.67, 135.46, 133.68, 130.45, 129.41, 127.78, 126.59, 124.69, 123.71, 123.25, 66.41, 51.41, 28.84, 28.45, 26.45, 25.57. HRMS-ESI (m/z): calcd. $[M+Na]^+$ for $[C_{66}H_{60}N_{10}NaO_4]^+$, 1079.4691; found 1079.4703.

Compound 3b: 1.7 g, 1.53 mmol; Yield: 61%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.63 (d, *J* = 8.4 Hz, 4H), 7.54 (d, *J* = 8.5 Hz, 4H), 7.47 (d, *J* = 8.4 Hz, 4H), , 7.27 – 7.24 (d, *J* = 7.4 Hz, 12H), 7.13 – 7.10 (m, 4H), 7.03 (t, *J* = 7.3 Hz, 4H), 4.42 (t, *J* = 6.8 Hz, 4H), 3.24 (t, *J* = 6.9 Hz, 4H), 1.81 (dd, *J* = 14.5, 7.3 Hz, 4H), 1.61 – 1.56 (m, 4H), 1.48 – 1.42 (m, 4H), 1.40 – 1.34 (m, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.00, 152.67, 147.87, 147.62, 141.85, 141.73, 135.48, 133.69, 130.46, 129.41, 127.77, 126.58, 124.68, 123.72, 123.24, 66.63, 51.52, 29.17, 29.12, 28.90, 28.53, 26.71, 25.86. HRMS-ESI (m/z): calcd. $[M+Na]^+$ for $[C_{70}H_{68}N_{10}NaO_4]^+$, 1135.5317; found 1135.5323.

Compound 3c: 1.90 g, 1.62 mmol; Yield: 65%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.64 (d, *J* = 8.4 Hz, 4H), 7.54 (d, *J* = 8.4 Hz, 4H), 7.48 (d, *J* = 8.7 Hz, 4H), 7.29 – 7.21 (m, 12H), 7.14 – 7.09 (m, 4H), 7.04 (d, *J* = 7.4 Hz, 4H), 4.43 (t, *J* = 6.8 Hz, 4H), 3.24 (t, *J* = 7.0 Hz, 4H), 1.85 – 1.73 (m, 4H), 1.64 – 1.54 (m, 4H), 1.50 – 1.41 (m, 4H), 1.36 (s), 1.31 (d, *J* = 1.0 Hz, 16H).^{13}C NMR (101 MHz, $CDCl_3$) δ 164.99, 152.64, 147.86, 147.61, 141.82, 141.74, 135.49, 129.41, 127.77, 124.68, 123.71, 123.25, 66.71, 51.56, 31.53, 29.78, 29.50, 29.48, 29.30, 29.22, 28.93, 28.57, 26.80, 25.94. HRMS-ESI (m/z): calcd. $[M+Na]^+$ for $[C_{74}H_{76}N_{10}NaO_4]^+$, 1191.5943; found 1191.5948.

Synthesis of target compound TDM-A, TDM-A, TDM-C. Under an argon atmosphere, compounds **3a-3c** (1 mmol) and propargyl [12]aneN_3 (1.02 g, 2.5 mmol)[4] were dissslved in anhydrous $CHCl_3$ (10 mL), and catalytic amount CuBr was added. The mixtures were stirred at room temperature for 6 hours, and then adding 30 mL brine, extracted with ethyl acetate (20 mL ×3), the combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated in vacuo. The obtained crudes were

purified by column chromatography on silica gel with PE/Acetone (v/v = 10:1 ~ 15:1) as the eluent to give corresponding Boc moieties as yellowish solids. The obtained Boc moieties were subsequently dissolved in a mixture solution with 5 mL anhydrous EtOAc and 1 ml anhydrous EtOH, then 5 mL saturated EtOAc solution of hydrochloric acid was added dropwise to the mixture solution. After stirring for 30 min at room temperature, a large amount of white precipitate obtained. The solid collected by filtering and then washed with aether for several times, and then the solid were dried in vacuo to give the target compounds **TDM-A/B/C** as white solid in 75–85% yields.

TDM-A: 1.10 g, 0.75 mmol; Yield: 75%. FT-IR (KBr, cm-1): 3440 (N−H stretching), 2928 (=C−H stretching), 2736 (CH_2 asymmetrical stretching), 2631 (CH_2 symmetrical stretching), 1597 (C=C and N=N stretching), 1491, 1458 (Ar stretching). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.56 (d, J = 7.7 Hz, 4H), 7.52 (d, J = 8.1 Hz, 4H), 7.47 (d, J = 7.6 Hz, 4H), 7.22 (t, J = 7.2 Hz, 8H), 6.99 (t, J = 7.1 Hz, 4H), 6.95 (d, J = 7.5 Hz, 8H), 6.91 (d, J = 7.7 Hz, 4H), 4.37 (s, 4H), 4.30 (s, 4H), 4.15 (s, 8H), 3.50 – 3.30 (m, 12H), 3.21 (s, 4H), 3.04 (s, 4H), 2.08 (d, J = 73.7 Hz, 12H), 1.81 (s, 4H), 1.66 (s, 4H), 1.43 – 1.03 (m, 12H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.80, 152.89, 147.79, 147.38, 141.60, 141.00, 135.64, 132.89, 130.85, 130.22, 128.23, 126.43, 124.87, 124.06, 123.44, 66.82, 50.42, 49.61, 47.26, 42.83, 41.79, 30.26, 28.41, 26.90, 26.11, 25.35, 23.10. HRMS-ESI (m/z): calcd. $[M+H]^+$ for $[C_{90}H_{107}N_{16}O_4]^+$, 1475.8656; found 1475.8619.

TDM-B: 1.26 g, 0.82 mmol; Yield: 82%. FT-IR (KBr, cm-1): 3443 (N−H stretching), 2933 (=C−H stretching), 2756 (CH_2 asymmetrical stretching), 2617 (CH2 symmetrical stretching), 1593 (C=C and N=N stretching), 1492, 1462 (Ar stretching). ^{1}H NMR (600

MHz, DMSO-d_6) δ 7.61 (d, J = 8.0 Hz, 4H), 7.57 (d, J = 8.3 Hz, 4H), 7.51 (d, J = 7.8 Hz, 4H), 7.26 (t, J = 7.6 Hz, 8H), 7.02 (t, J = 7.2 Hz, 4H), 6.98 (d, J = 7.8 Hz, 8H), 6.95 (d, J = 8.1 Hz, 4H), 4.35 (s, 4H), 4.31 (s, 4H), 4.19 (s, 8H), 3.55 – 3.28 (m, 12H), 3.22 (s, 4H), 3.04 (s, 4H), 2.08 (d, J = 62.2 Hz, 12H), 1.78 (s, 4H), 1.66 (s, 4H), 1.40 – 1.10 (m, 20H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.81, 152.92, 147.82, 147.42, 141.63, 141.04, 135.70, 132.95, 130.85, 130.20, 128.23, 126.42, 124.88, 124.03, 123.46, 66.77, 50.29, 49.26, 47.04, 43.24, 41.57, 30.25, 29.03, 28.88, 28.56, 28.47, 26.89, 26.36, 25.79. HRMS-ESI (m/z): calcd. $[M+H]^+$ for $[C_{94}H_{116}N_{16}O_4]^+$, 1532.9360; found 1532.9282.

TDM-C: 1.35 g, 0.85 mmol; Yield: 85%. FT-IR (KBr, cm-1): 3443 (N−H stretching), 2928 (=C−H stretching), 2754 (CH_2 asymmetrical stretching), 2623 (CH2 symmetrical stretching), 1591 (C=C and N=N stretching), 1491, 1450 (Ar stretching). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.62 (d, J = 8.0 Hz, 4H), 7.58 (d, J = 8.2 Hz, 4H), 7.51 (d, J = 7.9 Hz, 4H), 7.27 (t, J = 7.6 Hz, 8H), 7.02 (t, J = 7.5 Hz, 4H), 6.99 (d, J = 7.9 Hz, 8H), 6.95 (d, J = 8.2 Hz, 4H), 4.38 – 4.24 (m, 8H), 3.80 (s, 8H), 3.53 – 3.29 (m, 12H), 3.23 – 3.20 (m, 4H), 3.03 (s, 4H), 2.08 (d, J = 61.3 Hz, 12H), 1.75 (s, 4H), 1.65 (s, 4H), 1.36 – 1.06 (m, 28H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.81, 152.91, 147.82, 147.42, 141.64, 141.04, 135.71, 132.96, 130.84, 130.19, 128.22, 126.41, 124.87, 124.01, 123.46, 66.76, 50.17, 49.13, 47.00, 43.18, 41.45, 30.28, 30.24, 29.42, 29.37, 29.16, 28.95, 28.51, 28.49, 26.39, 25.87. HRMS-ESI (m/z): calcd. $[M+H]^+$ for $[C_{98}H_{123}N_{16}O_4]^+$, 1587.9908; found 1587.9887.

2. Uv-visible spectra of the compounds in different solvents

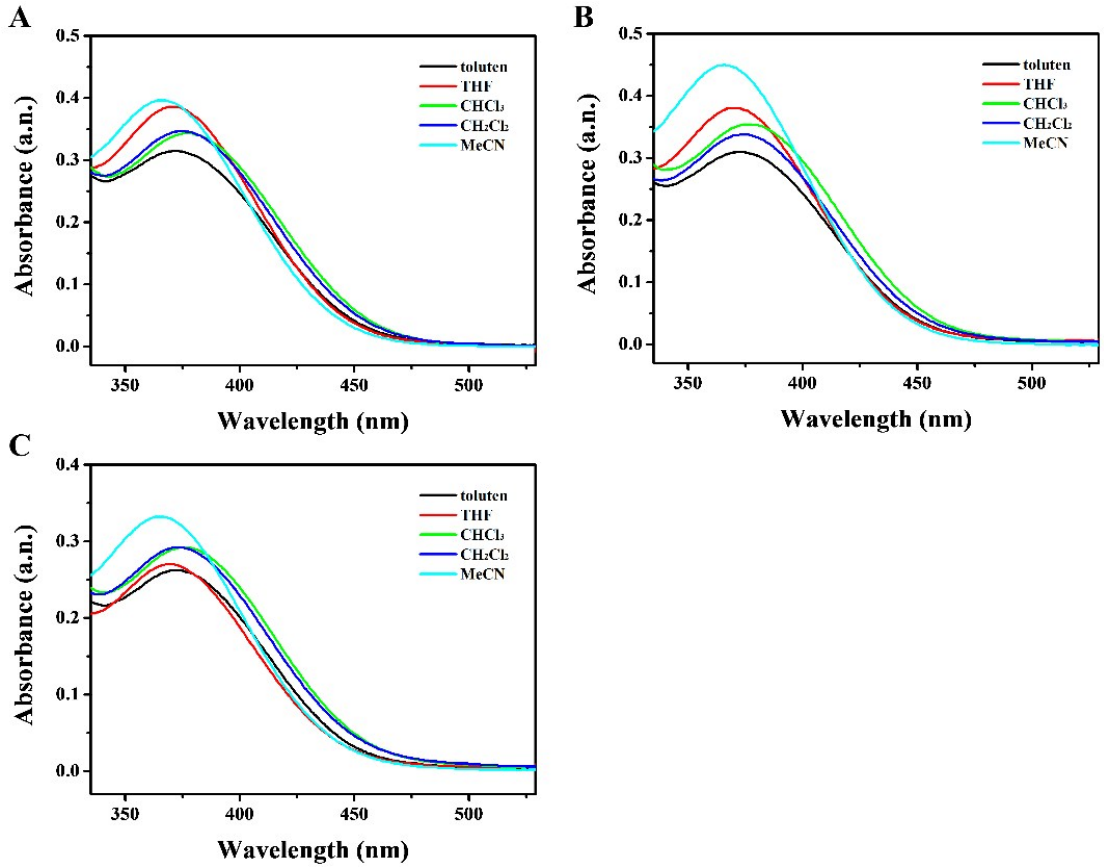


Fig. S1 UV-vis spectra of **TDM-A**(A), **TDM-B**(B) and **TDM-C** (C) in different solutions. Concentration: 10 μM.

Table S1 Summarized photophysical data of **TDM-A**, **TDM-B** and **TDM-C.**

	TDM-A				TDM-B				TDM-C			
	λ_{ab} (nm)	λ_{em} (nm)	Stoke's shift (10^3 cm^{-1})	ΦF	λ_{ab} (nm)	λ_{em} (nm)	Stoke's shift (10^3 cm^{-1})	ΦF	λ_{ab} (nm)	λ_{em} (nm)	Stoke's shift (10^3 cm^{-1})	ΦF
Toluene	370	547	8.75	0.45	371	542	8.50	0.56	370	543	8.61	0.68
THF	368	610	10.78	0.38	370	607	10.55	0.41	370	607	10.55	0.43
$CHCl_3$	380	628	10.39	0.15	377	615	10.27	0.24	380	614	10.03	0.21
CH_2Cl_2	375	655	11.40	0.14	373	657	11.59	0.19	375	649	11.26	0.2
MeCN	365	748	14.03	0.12	365	750	14.06	0.11	365	750	14.06	0.12

Abbreviation: λ_{ab} = absorption maximum, λ_{em} = emission maximum, φ_F = fluorescence quantum yield, concentration: 10 μM.

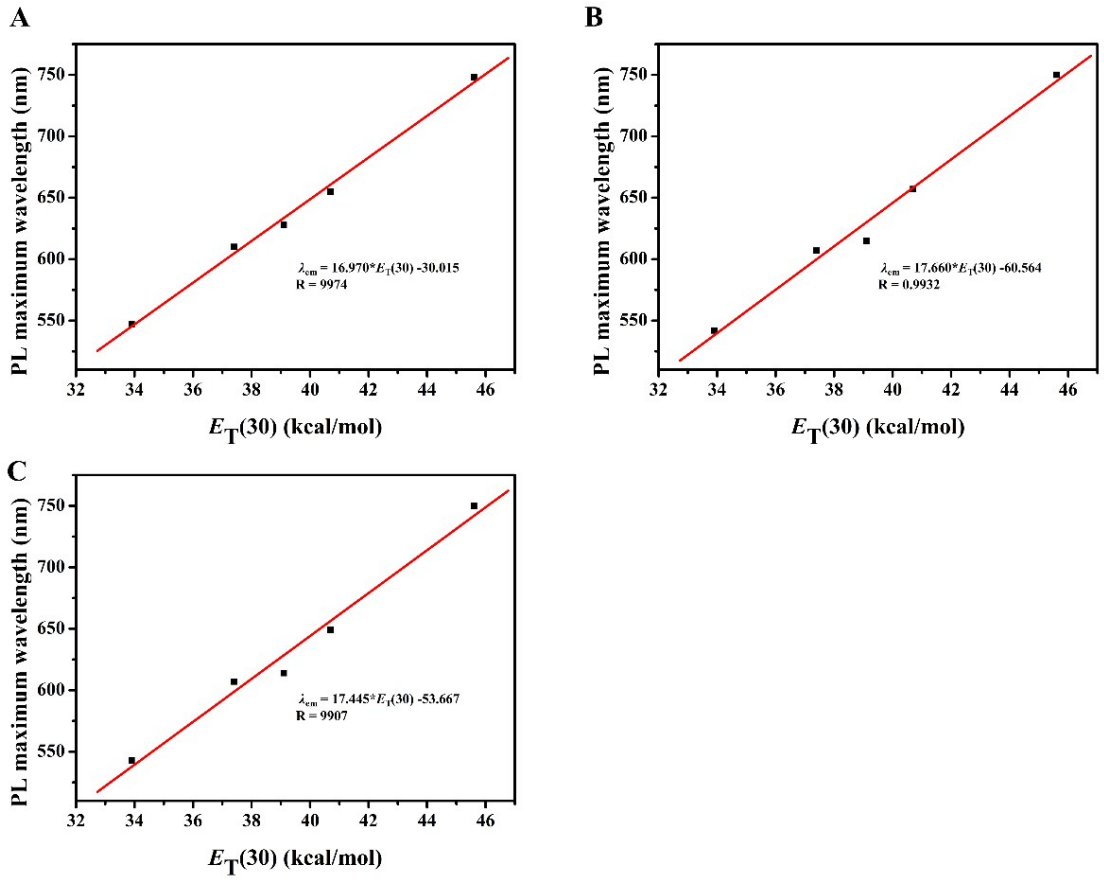


Fig. S2 Plot of the emission maximum of **TDM-A**(A), **TDM-B**(B), **TDM-C**(C) in different solvents versus $E_T(30)$, $E_T(30)$ was the empirical parameter for solvent polarity. Concentrations: 10 μM, λex = 410 nm.

3. Two-photon effect measurement

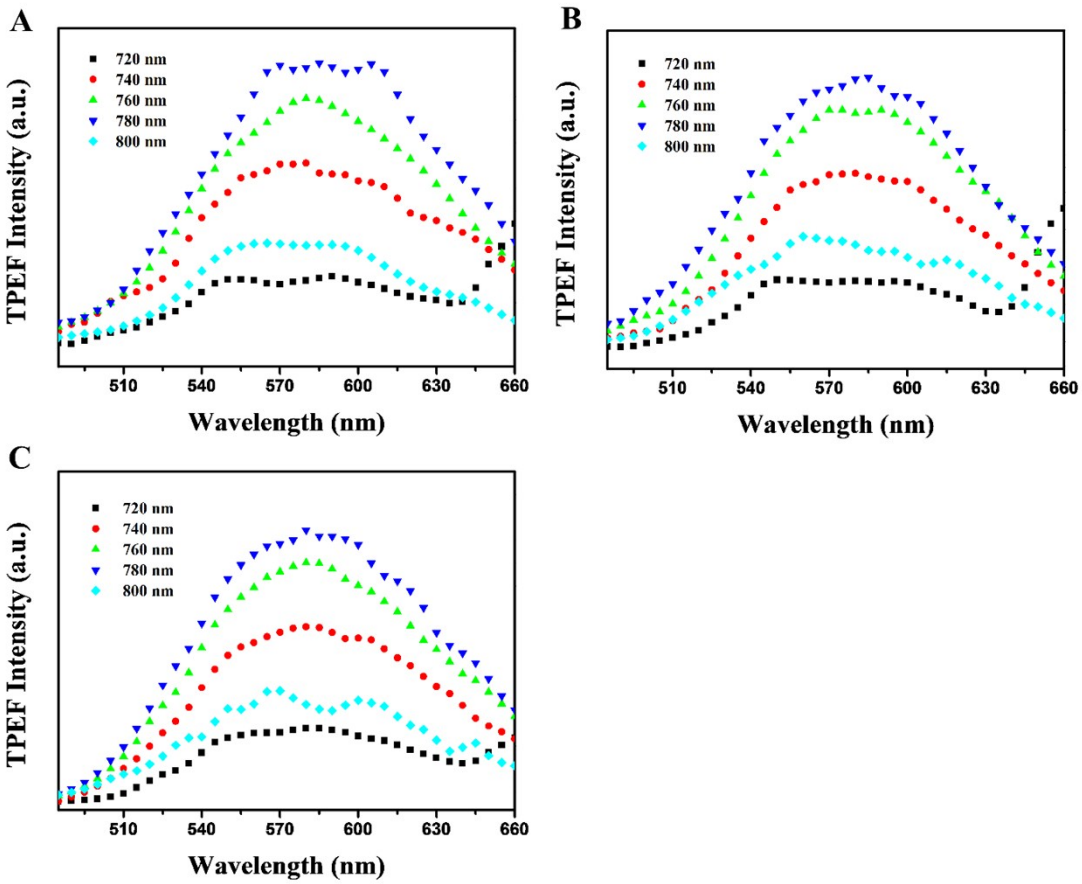


Fig. S3 TPEF spectra of **TDM-A** (A), **TDM-B** (B) and **TDM-C** (C) at different excitation wavelengths of 720-800 nm. Concentration: 10 μM.

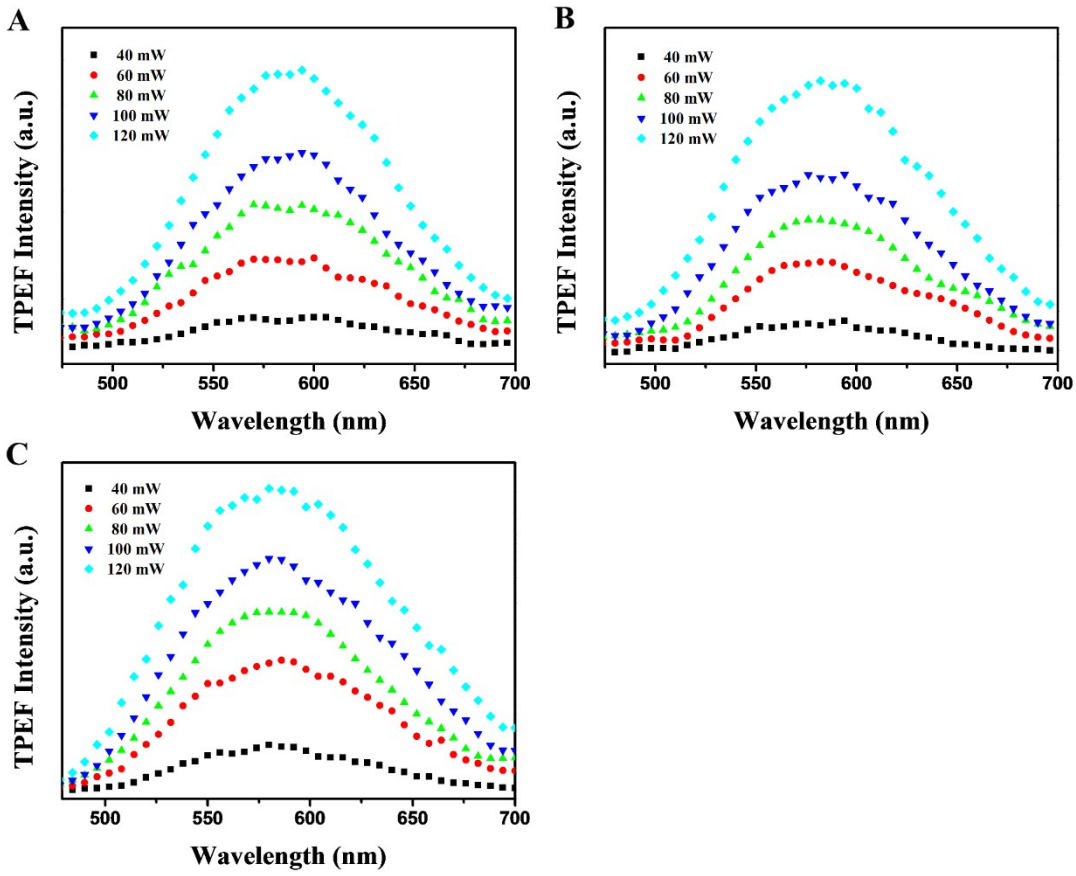


Fig. S4 TPEF spectra of **TDM-A** (A), **TDM-B** (B) and **TDM-C** (C) at different excitation energies at 780 nm. Concentration: 10 μM.

4. Figures for AIE measurements

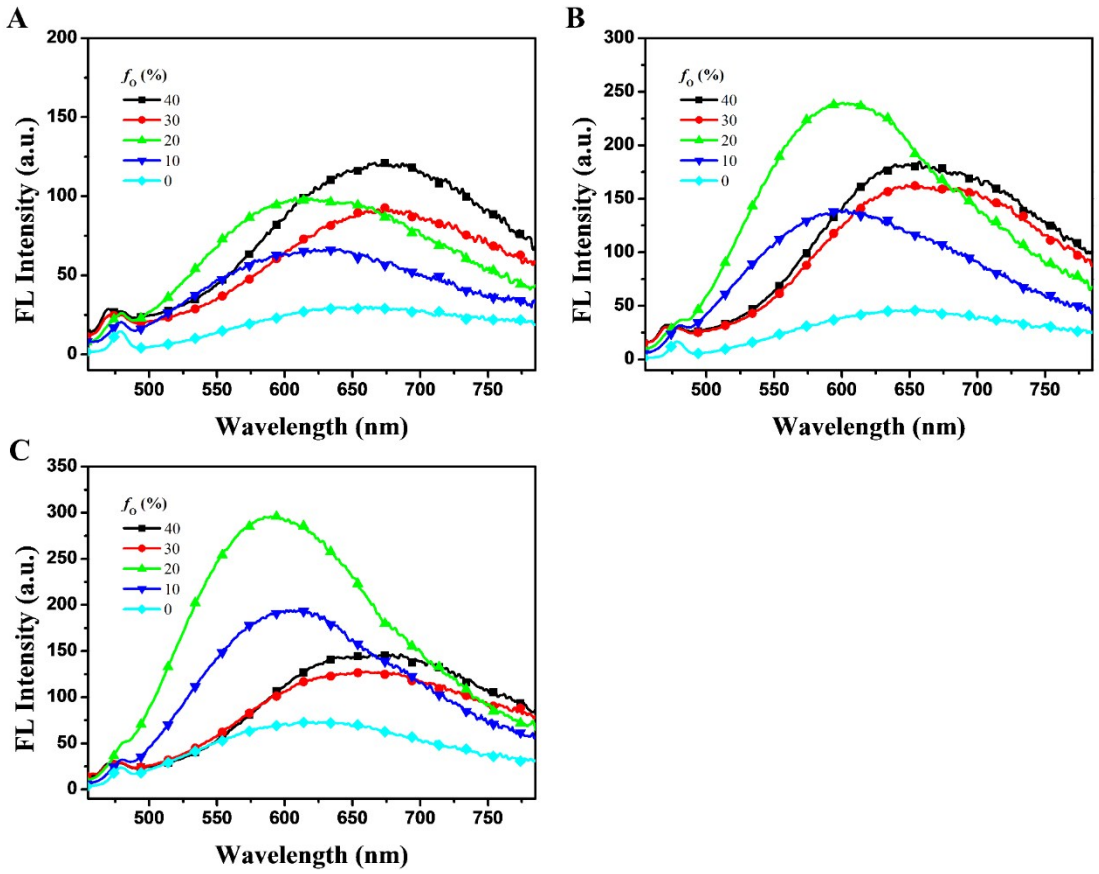


Fig. S5 Fluorescence spectra of **TDM-A** (A), **TDM-B** (B), and **TDM-C** (C) in water/THF mixtures with THF fractions (f_o) from 0 to 40%. Concentration: 10 μM; λ_{ex} = 410 nm.

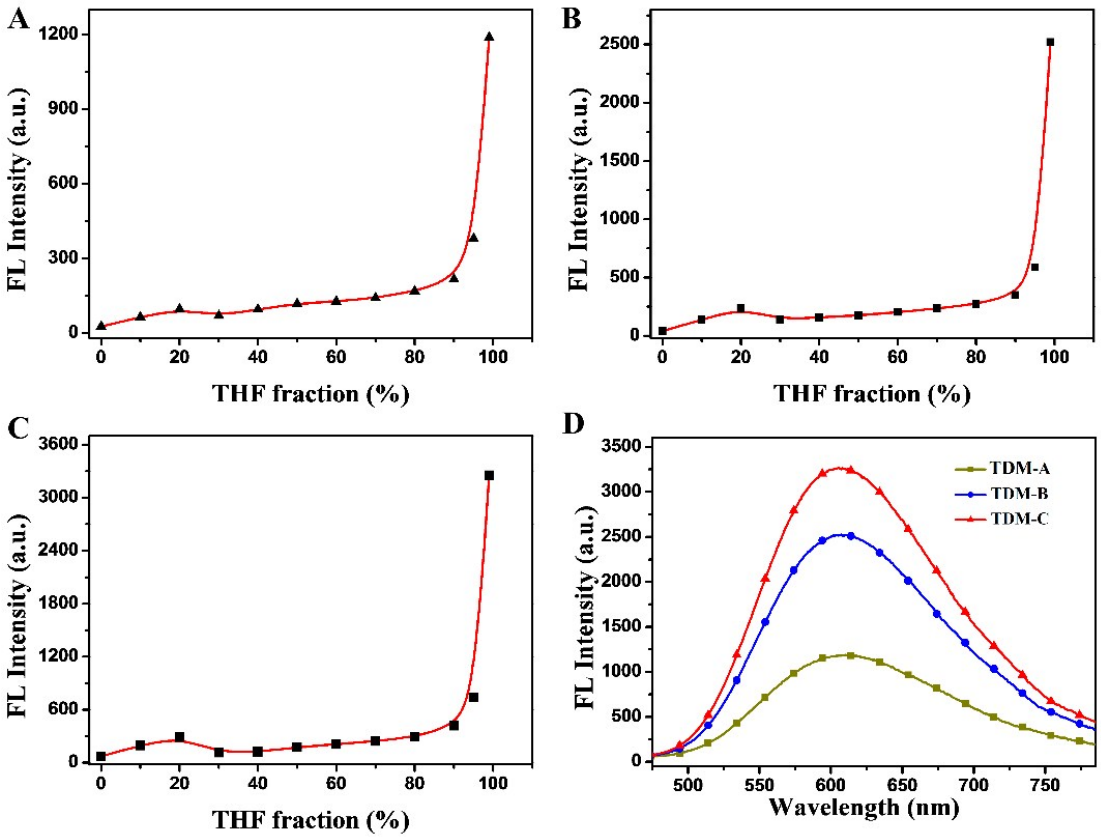


Fig. S6 Fluorescence intensity at 610 nm of **TDM-A** (A), **TDM-B** (B), and **TDM-C** (C) with different THF fractions (f_o). Fluorescence intensity of **TDM-A/B/C** with 99% THF fractions (D). Concentration: 10 μM; λ_{ex} = 410 nm.

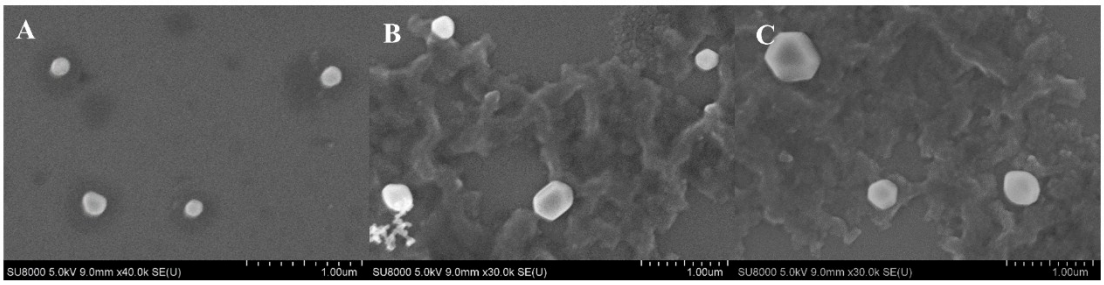


Fig. S7 SEM images of **TDM-A** (A), **TDM-B** (B) and **TDM-C** (C) in THF/water mixtures with 99% THF fraction. Scale bars: 1 μm.

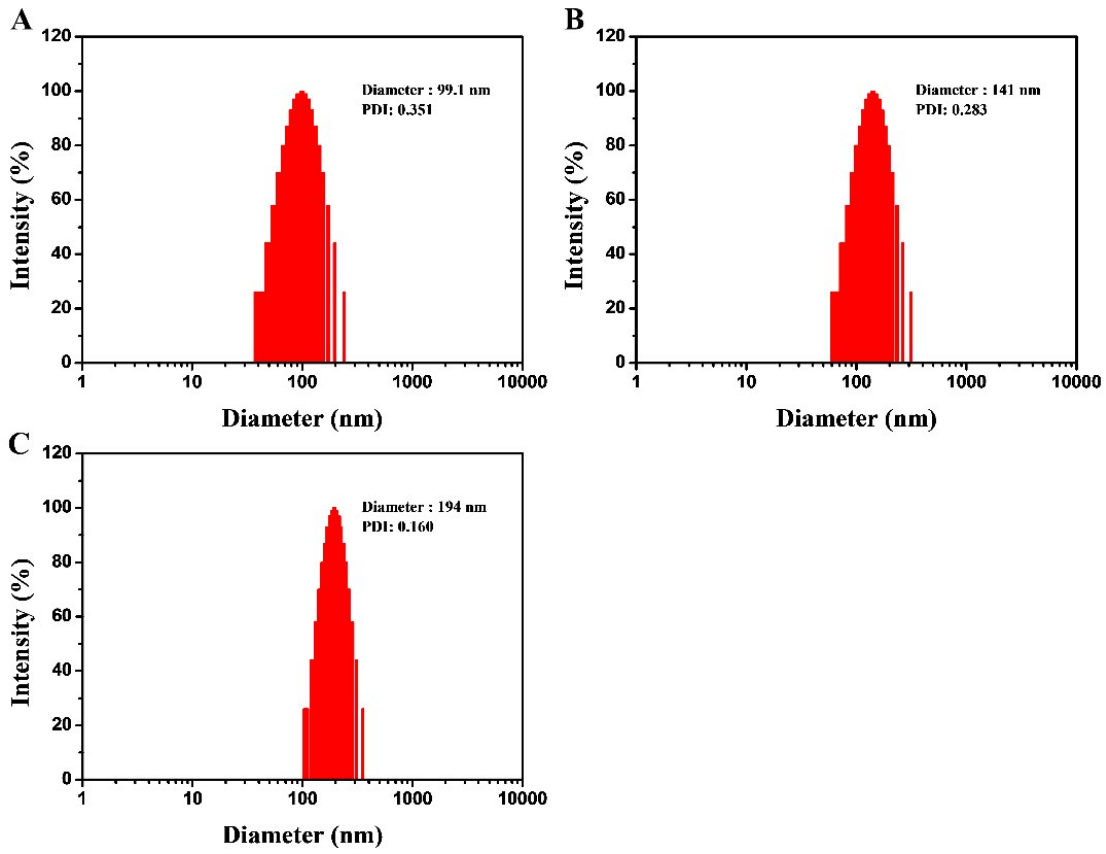


Fig. S8 Particle size distributions of **TDM-A** (A), **TDM-B** (B) and **TDM-C** (C) in THF/water mixtures with 99% THF fraction. Concentrations = 10 μM.

5. DLS and Zeta potentials

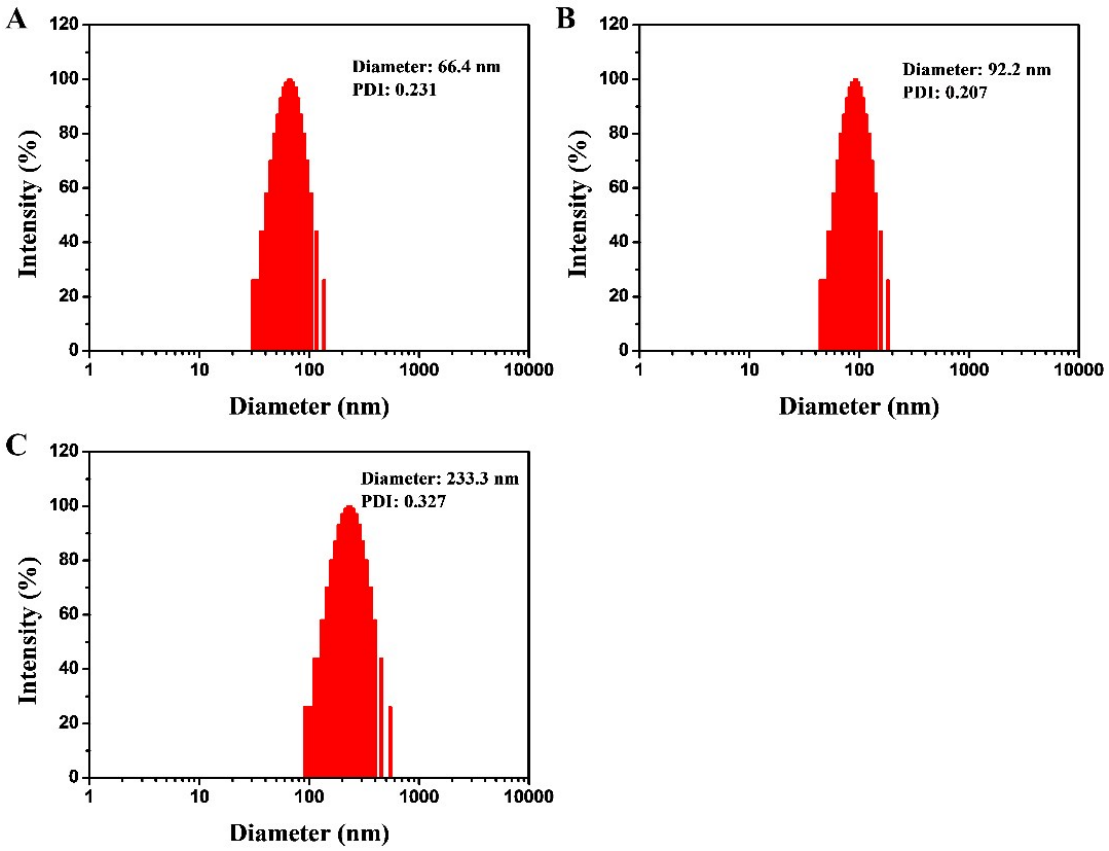


Fig. S9 Particle size distributions of condensed pUC18 DNA (9 μg/mL) by **TDM-A/B/C** in Tris-HCl buffer (5 mM, pH 7.4): **TDM-A**, 10 μM (A); **TDM-B** (B), 14 μM; **TDM-C**, 16 μM (C).

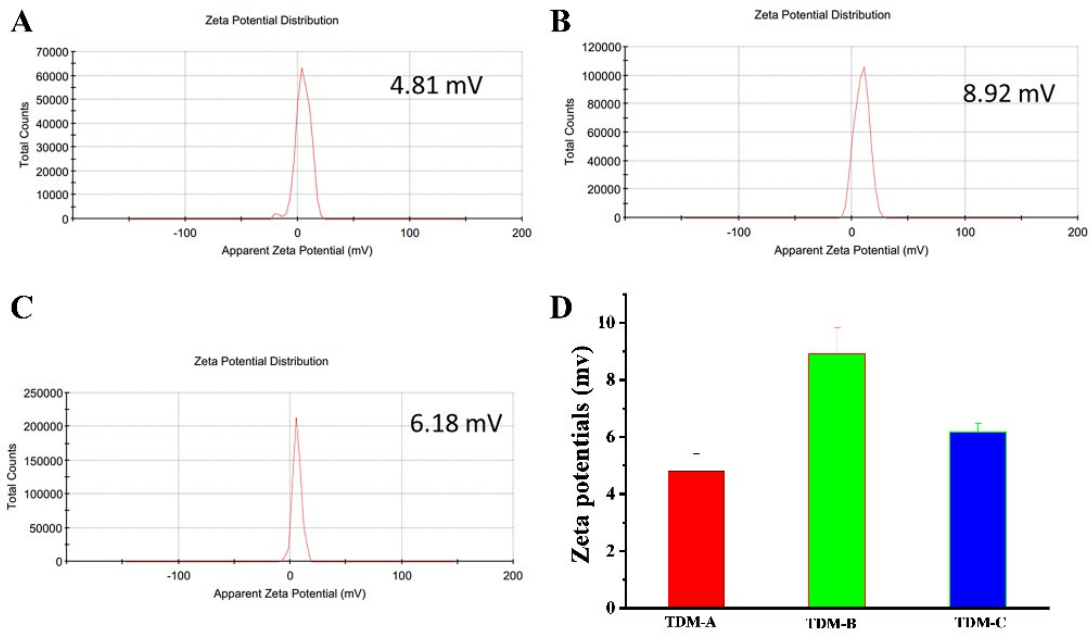


Fig. S10 Zeta potentials of pUC18 DNA complexes in 50 mM Trips-HCl (pH 7.4): **TDM-A** (A), 10 μM; **TDM-B** (B), 14 μM; and **TDM-C**, 16 μM (C). Zeta potential histogram of pUC18 DNA complex (D). [DNA] = 9 μg/mL, 37 °C.

6. Release of the compact DNA

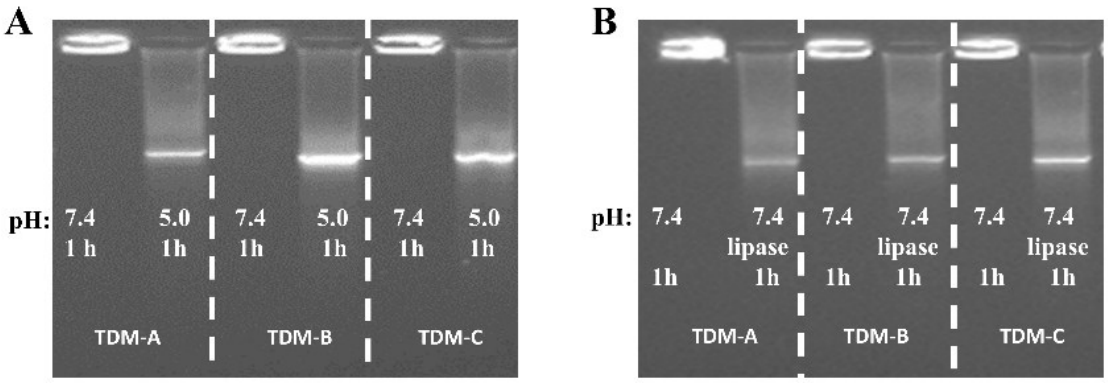


Fig. S11 Gel electrophoresis to measure the reversibility of DNA condensations caused by **TDM-A** (10 μM), **TDM-B** (14 μM) and **TDM-C** (16 μM) at different pH values (A). Gel electrophoresis experiments in the presence of lipase (B). [DNA] = 9 μg/mL, [lipase] = 10 μg/mL.

7. Interactions with ctDNAs

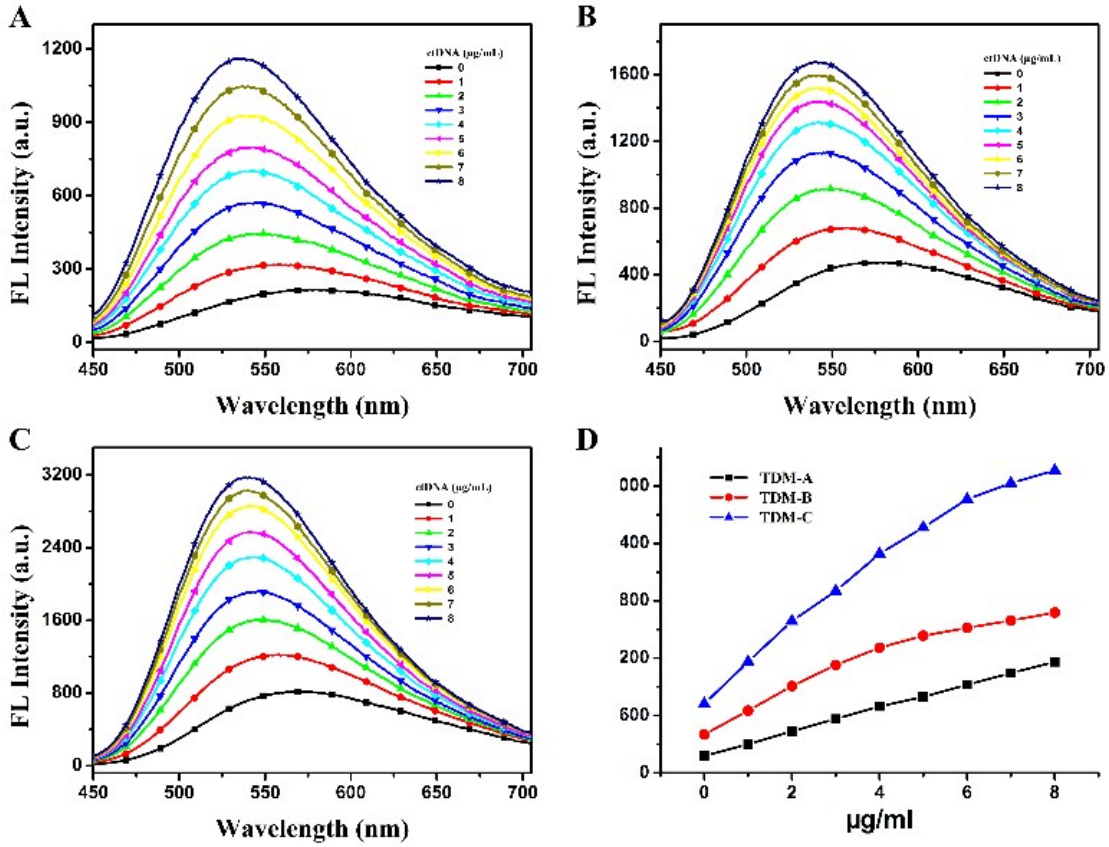


Fig. S12 Fluorescence titrations of **TDM-A** (A), **TDM-B** (B) and **TDM-C** (C) with ctDNAs. Plot of emission intensity at 535 nm vs ctDNA concentration (D). Inset: Changes of fluorescent pictures. Concentrations = 10 μM, λ_{ex} = 380 nm.

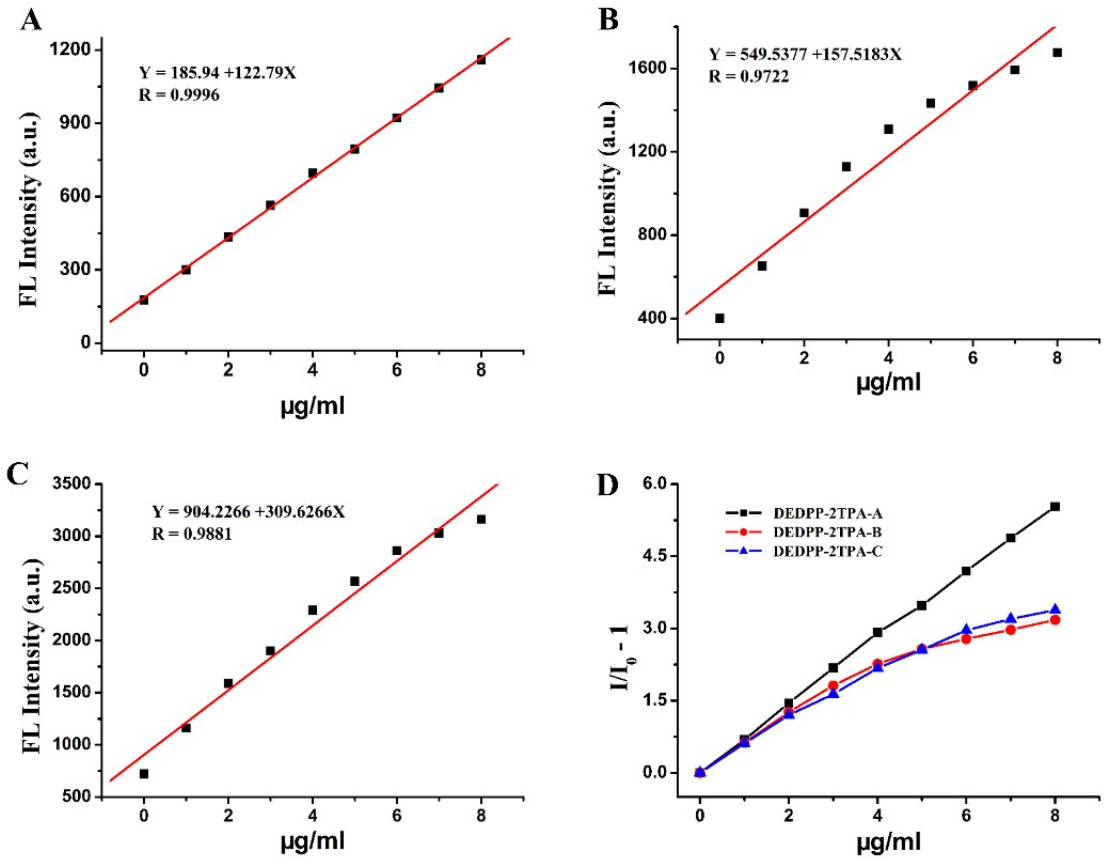


Fig. S13 Linearly fitting functions deduced from the plots of emission intensity changes at 535 nm: **TDM-A** (A), **TDM-B** (B), **TDM-C** (C). Concentrations = 10 μM, λ_{ex} = 380 nm. Plot of I/I_0 - 1 at 535 nm versus the DNA equivalents of **TDM-A/B/C** (D). I_0 = FL intensity in the absence of DNA.

Table S2 Emission enhancement calculated from the fluorescent titration of ctDNA.

ctDNA (μg/mL)	1	2	3	4	5	6	7	8
TDM-A	1.69	2.46	3.19	3.93	4.49	5.21	5.9	6.55
TDM-B	1.63	2.27	2.82	3.27	3.59	3.79	3.98	4.19
TDM-C	1.61	2.21	2.64	3.18	3.57	3.97	4.21	4.39

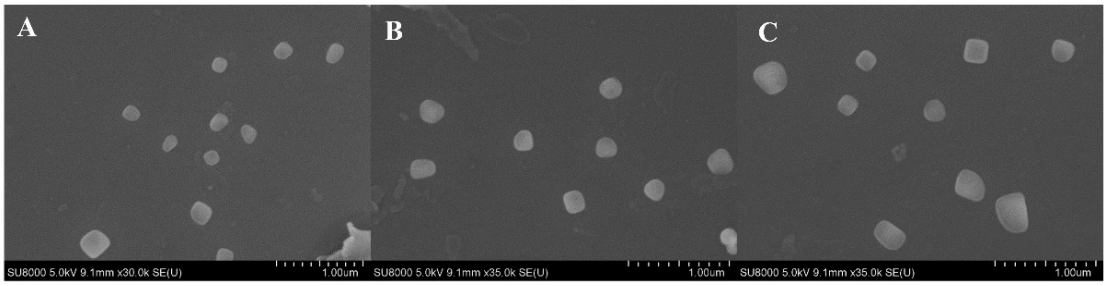


Fig. S14 SEM images of condensed ctDNA (9 μg/mL) by **DEDPP-2TPA** derivatives in Tris-HCl buffer (5 mM, pH 7.4): **TDM-A**, 10 *M*m (A); **TDM-B**, 14 μM (B); **TDM-C**, 16 μM (C). Scale bars: 1 μm.

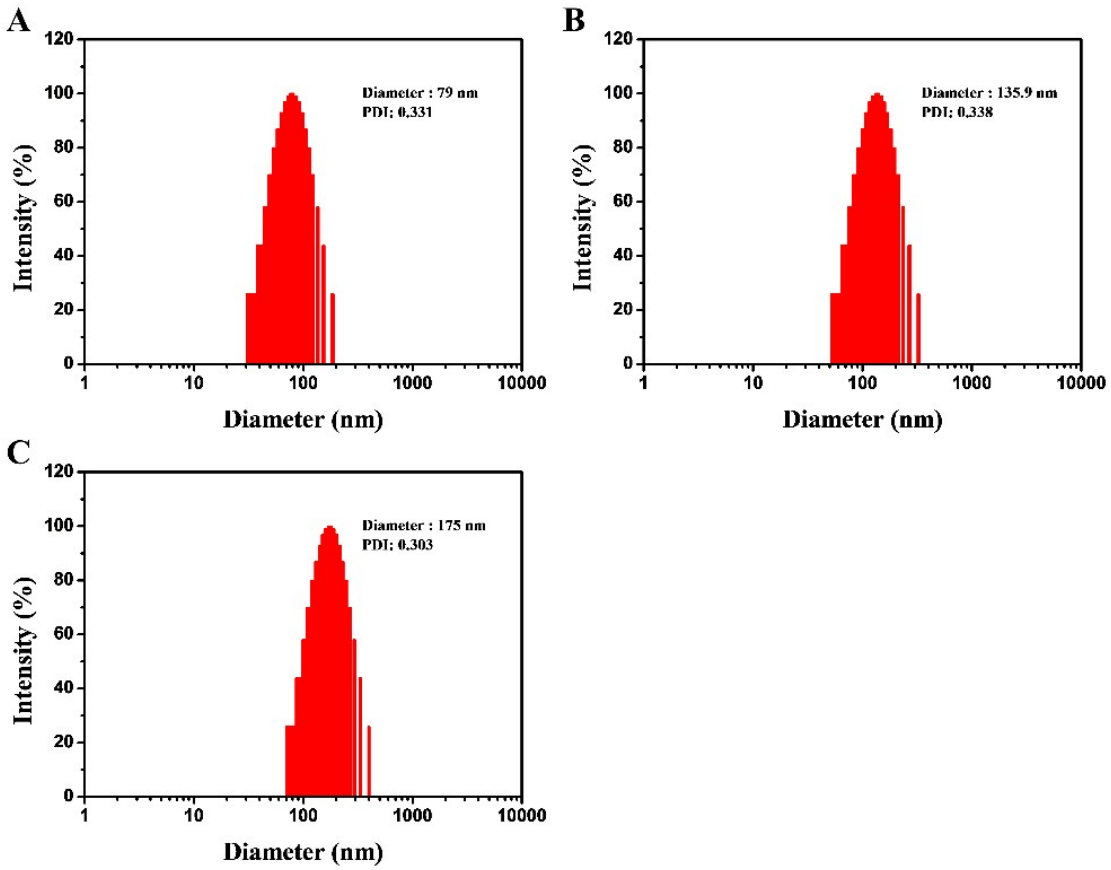


Fig. S15 Particle size distributions of **DEDPP-2TPA** derivatives condensed ctDNA nanoparticles; **TDM-A**, 10 μM (A); **TDM-B**, 14 μM (B); **TDM-C**, 16 μM (C). [pDNA]: 9 μg/mL.

8. EB assay experiment

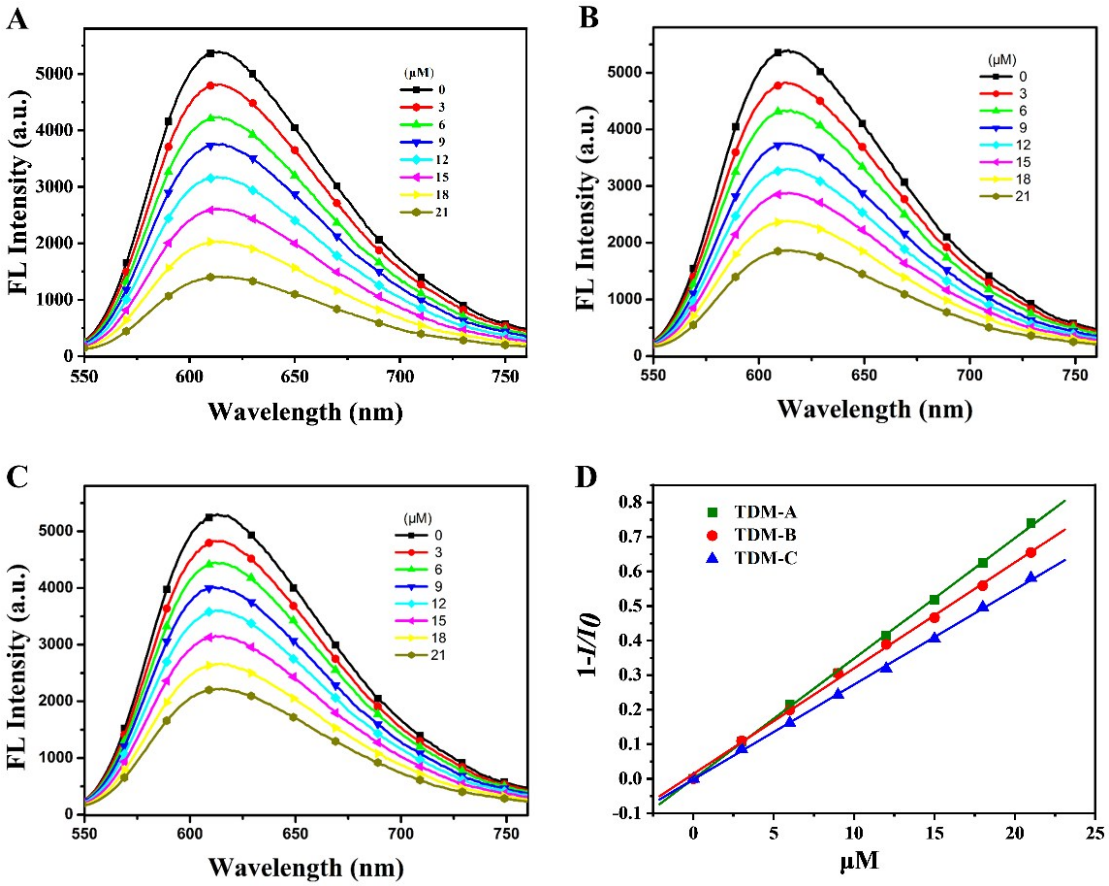


Fig. S16 Fluorescence quenching curves of EB-bounded ctDNA by **TDM-A** (A), **TDM-B** (B) ,**TDM-C** (C) and plot of 1 - I/I_0 vs concentrations of **TDM-A/B/C** (D), , λ_{ex}= 537 nm, [EB] = 20 μM, [DNA] = 100 μM, 25 °C, Tris-HCl = 5 mM , NaCl = 50 mM , pH = 7.4.

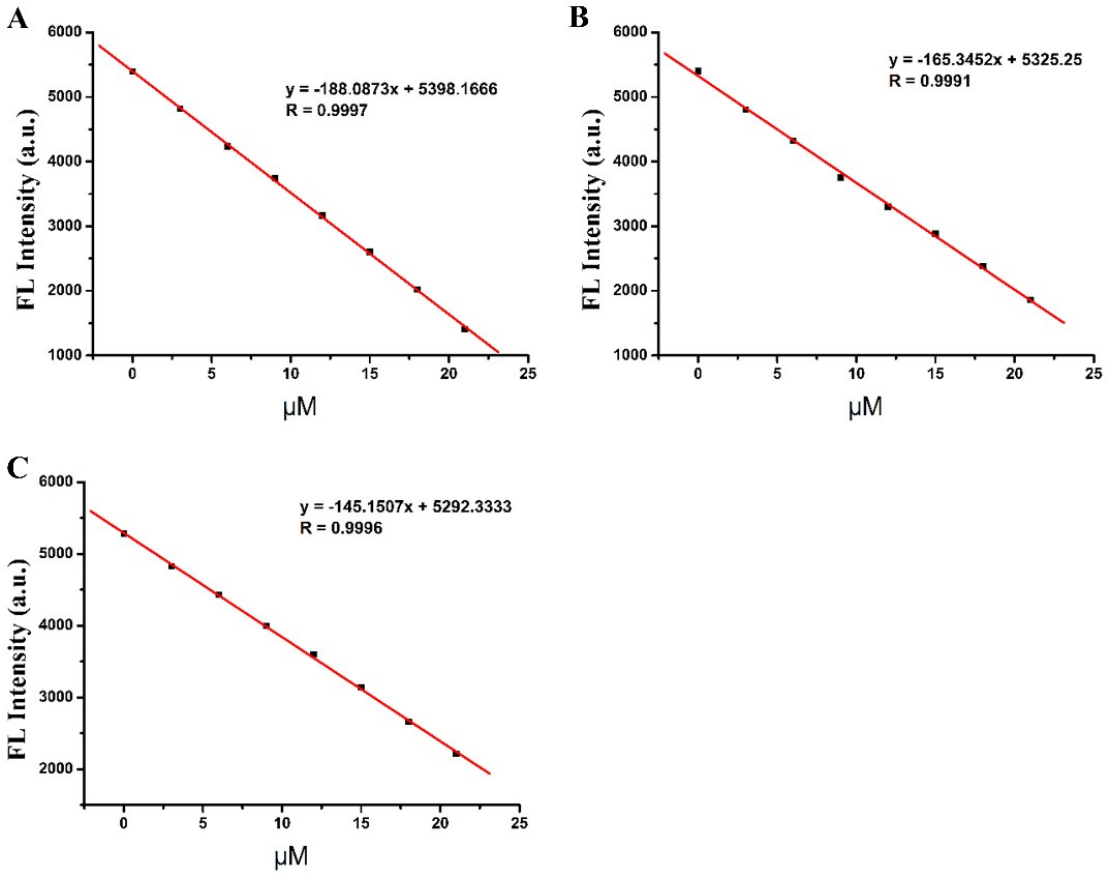


Fig. S17 Linearly fitting functions deduced from the plots of emission intensity changes at 610 nm by quenching curves of EB-bounded ctDNA: **TDM-A** (A), **TDM-B** (B), **TDM-C** (C).

9. Cellular transfection assays

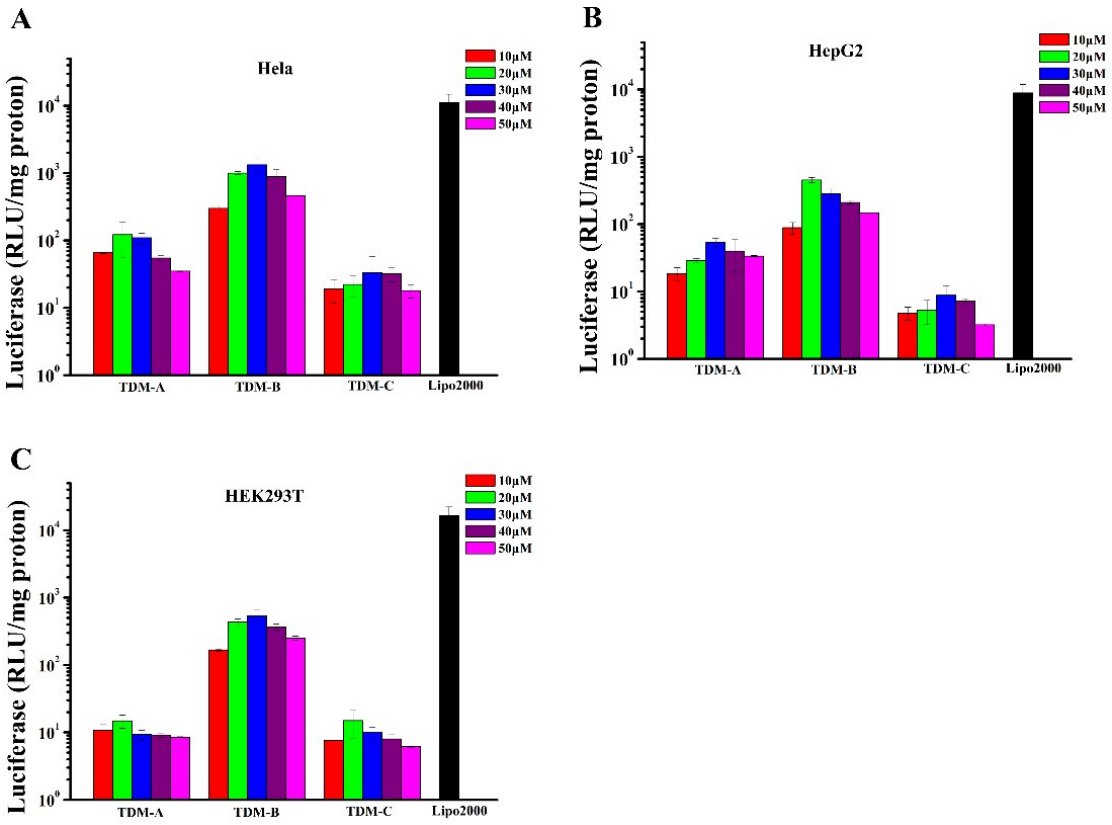


Fig. S18 Luciferase gene expressions transfected by different concentrations of **TDM-A/B/C** in three cell lines with Lipo2000 as control. Hela cell lines (A); HepG2 cell lines (B); HEK293T cell lines (C); [pGL-3] = 10 μg/mL.

Table S3 Optimal luciferase expressions of both the compounds and liposomes in different cell lines (% of lipo2000).

	TDM-A	**TDM-B**	**TDM-C**	**TDM-A** /DOPE	**TDM-B** /DOPE	**TDM-C** /DOPE
Hela	1.1	12	0.3	71	156	85
HepG2	0.6	5.1	<0.1	64	87	130
HEK293T	<0.1	3.2	<0.1	50	130	4

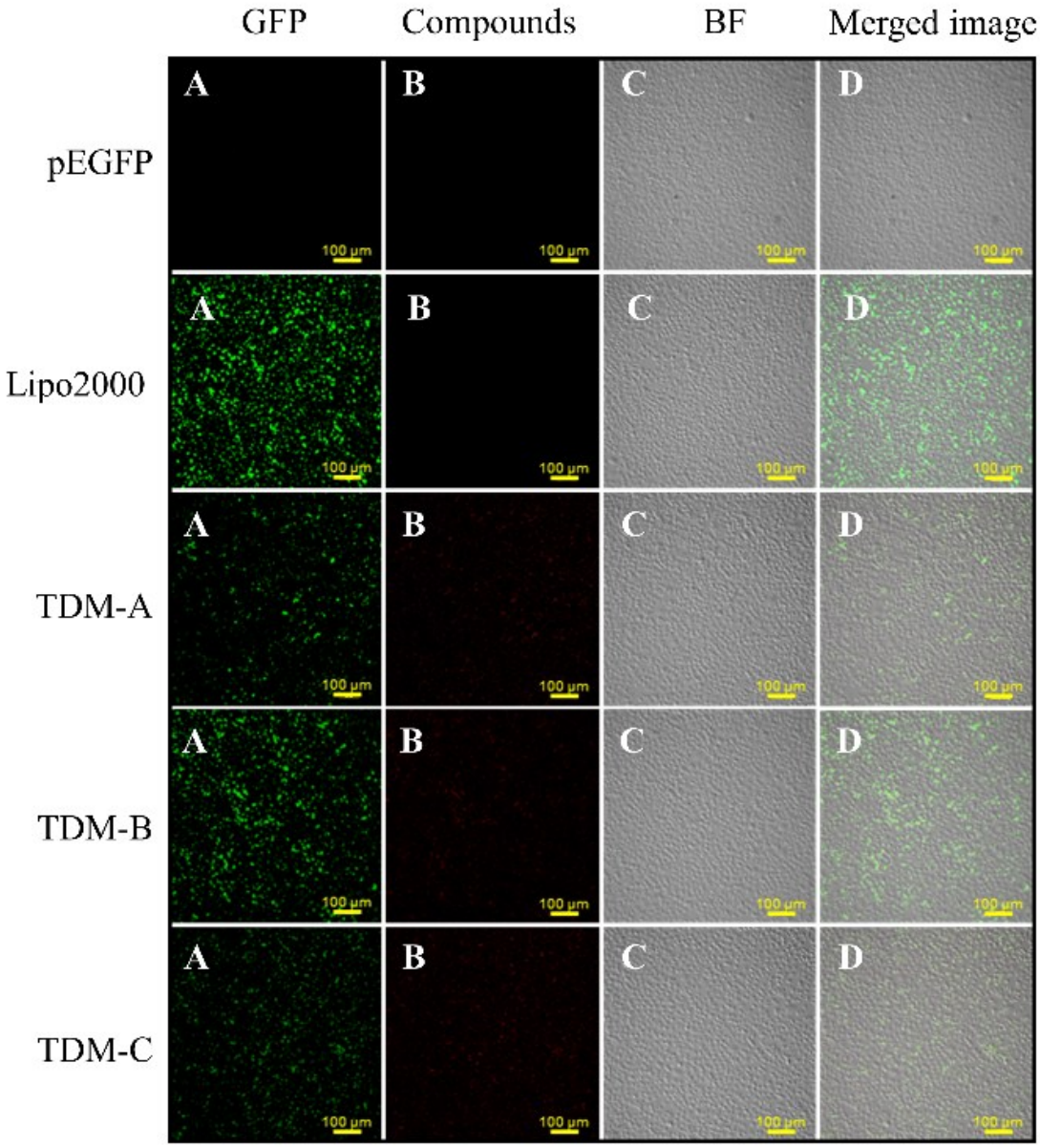


Fig. S19 Fluorescence microscope images (10×) of pEGFP-transfected in Hela cells. pEGFP was observed with FITC filter (A); The inherent red fluorescence of **TDM-A/B/C** were observed with the TRITC filter (B); Bright field (C); Merge of A, B, C (D). pEGFP; Lipo2000 and naked pEGFP were used as control. Concentration = 20 μM; [pEGFP-N1] = 10 μg/mL, scale bar: 100 μm.

10. Cellular uptake studies

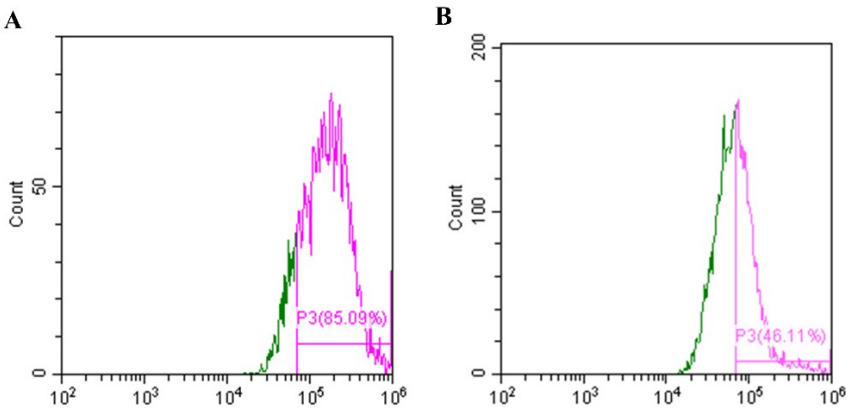


Fig. S20 Cellular uptake of **TDM-B**/DOPE-DNA complexes incubated at 37 °C (A) and 4 °C (B) in Hela cell lines.

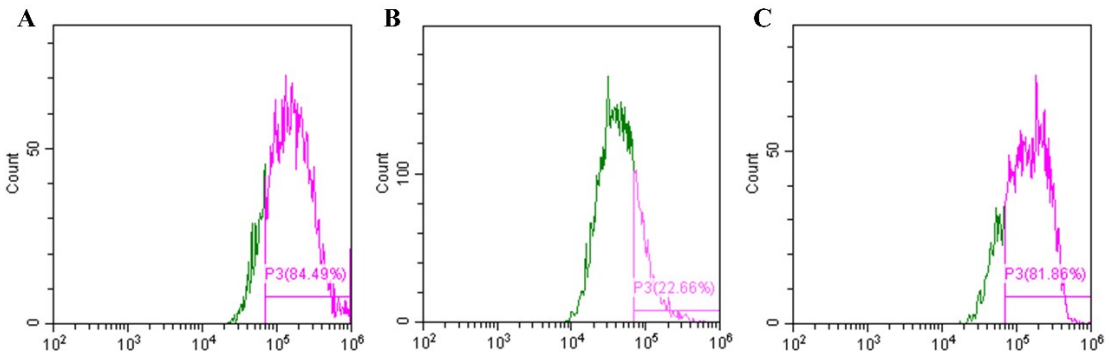


Fig. S21 Cellular uptake of **TDM-B**/DOPE-DNA complexes in the presence of chlorpromazine (A), methyl-β-cyclodextrin (B) and amiloride hydrochloride (C) in Hela cell lines.

11. Elemental analysis of TDM-A, TDM-B and TDM-C

Table S4 Elemental analysis of the obtained **TDM-A, TDM-B** and **TDM-C** for their hydrochloride salts.

Name	Nitrogen [%]		Carbon [%]		Hydrogen [%]	
	calcd.	found	calcd.	found	calcd.	found
TDM-A	12.68	12.15	61.16	61.15	6.5	6.46
TDM-B	12.29	11.88	61.91	62.86	6.74	6.81
TDM-C	11.92	10.35	62.62	61.56	6.97	6.89

12. ^{1}H, ^{13}C-NMR Spectra and HR-MS of synthesized compounds

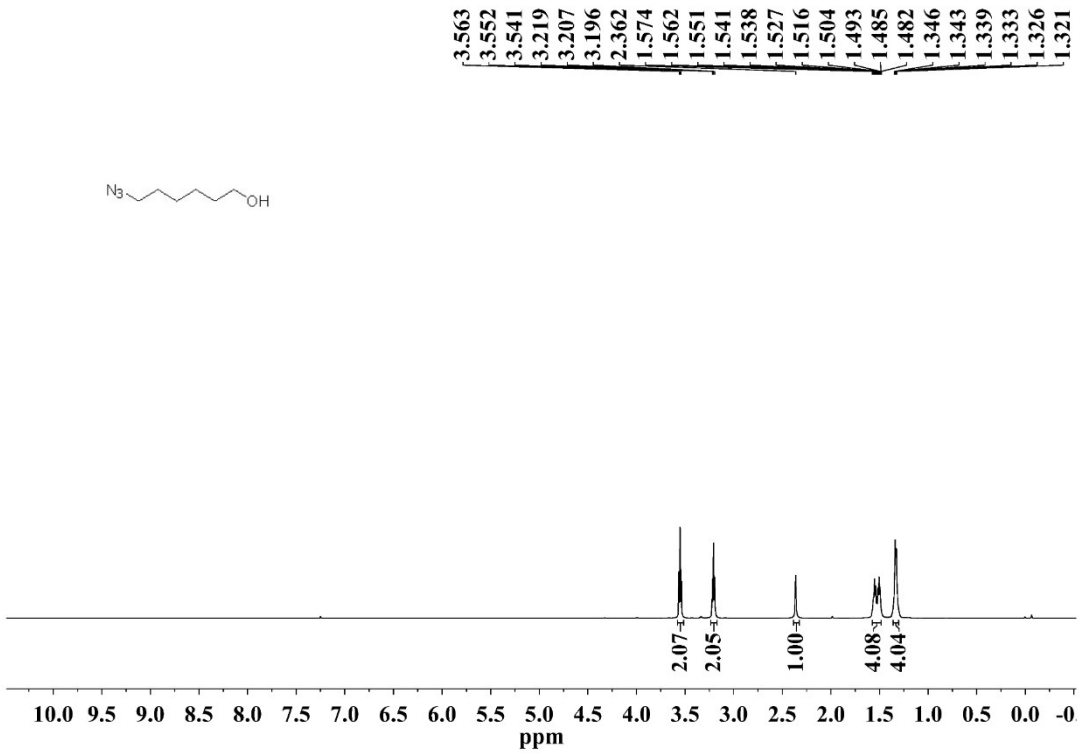


Fig. S22 ^{1}H NMR spectrum of **a** (400 MHz, $CDCl_3$).

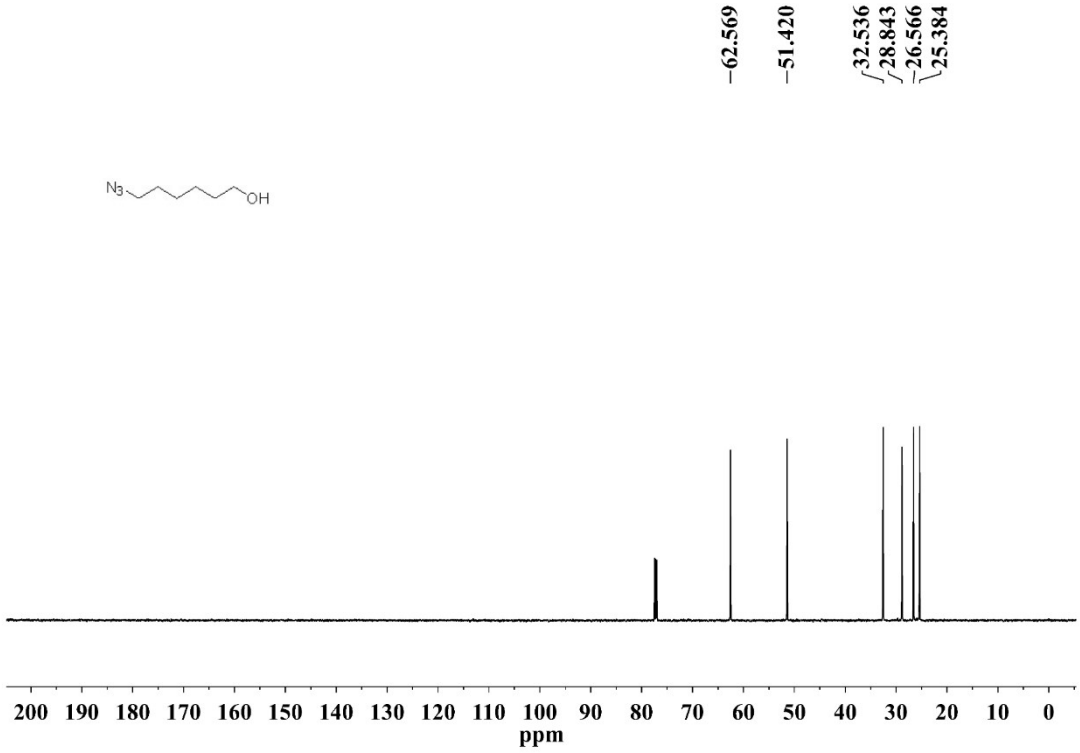


Fig. S23 ^{13}C NMR spectrum of **a** in $CDCl_3$ (100 MHz, $CDCl_3$).

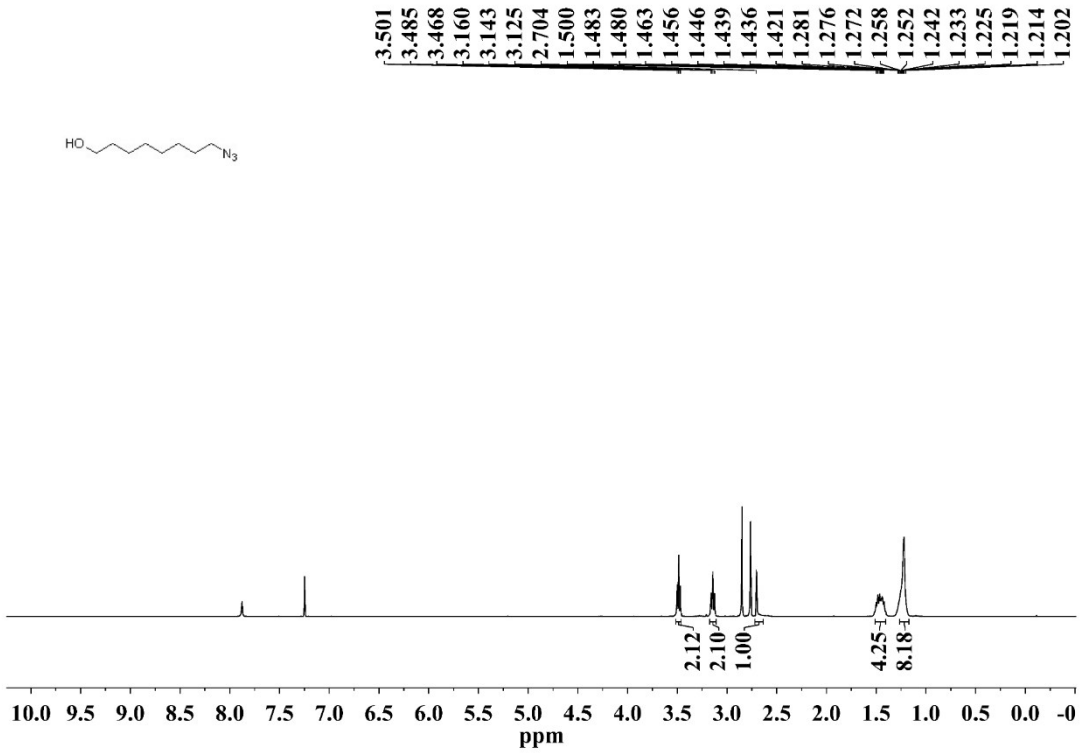


Fig. S24 ^{1}H NMR spectrum of **b** (400 MHz, $CDCl_3$).

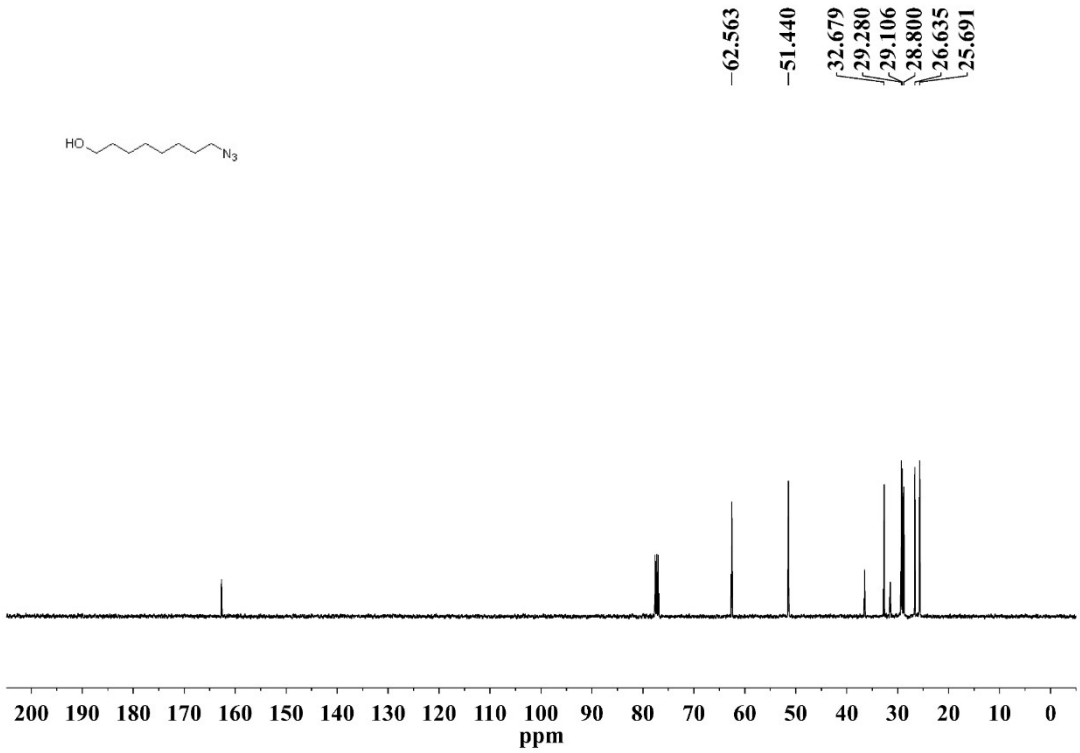


Fig. S25 ^{13}C NMR spectrum of **b** in $CDCl_3$ (100 MHz, $CDCl_3$).

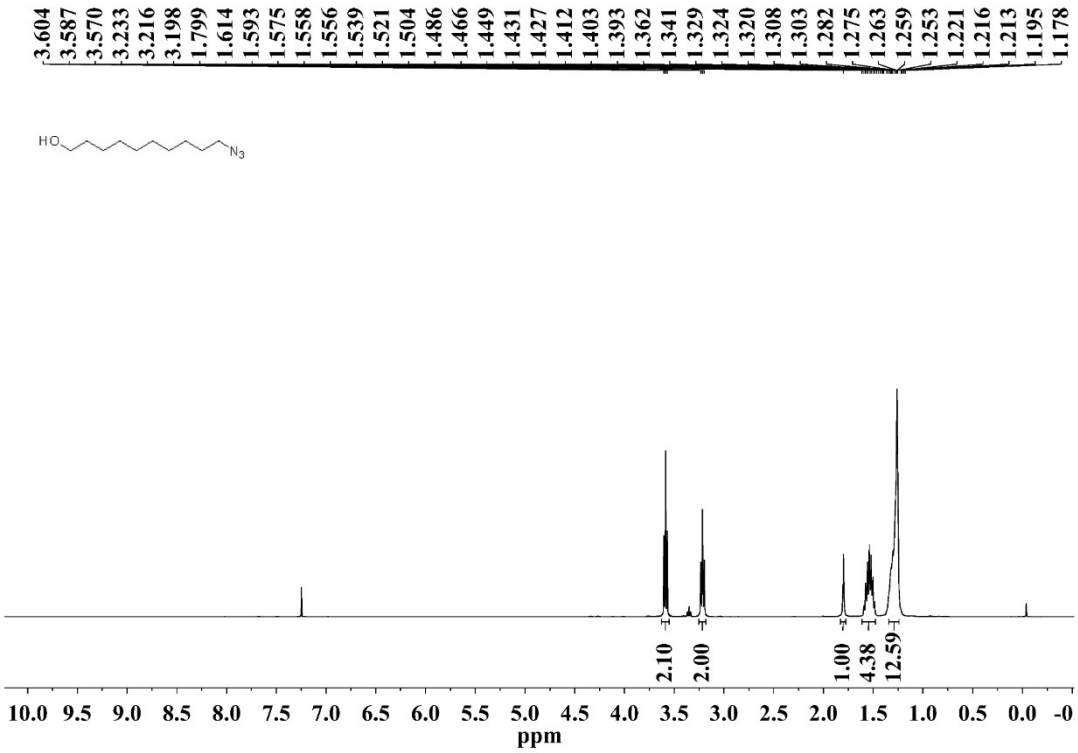


Fig. S26 ^{1}H NMR spectrum of **c** (400 MHz, $CDCl_3$).

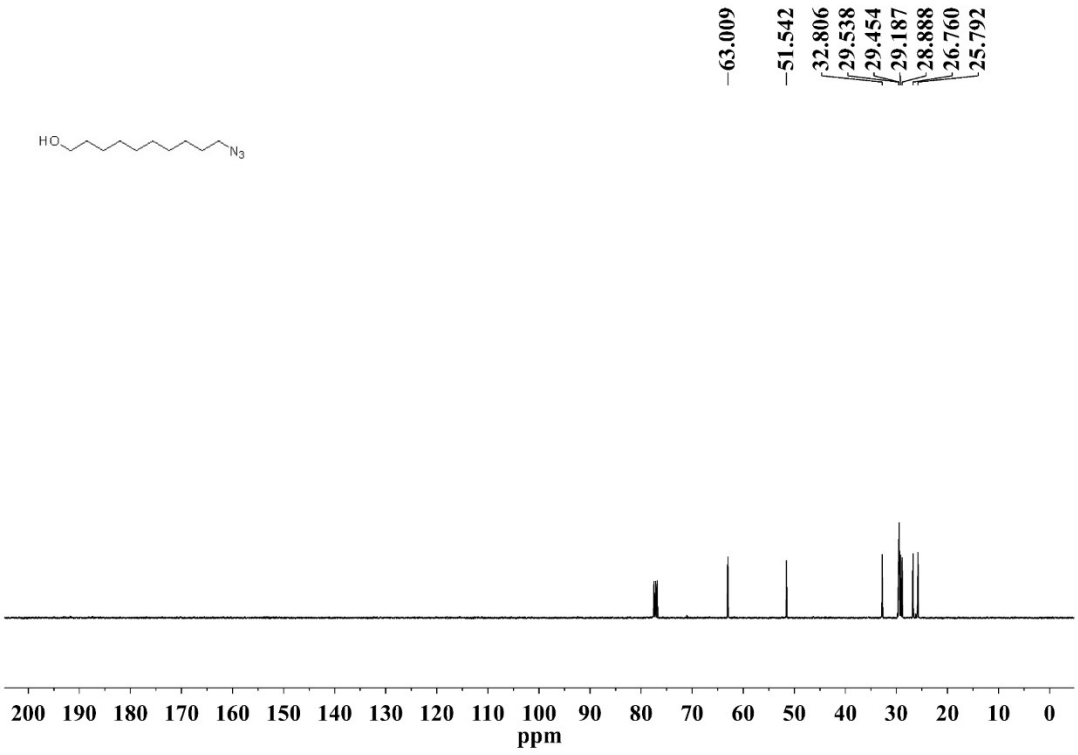


Fig. S27 ^{13}C NMR spectrum of **c** in $CDCl_3$ (100 MHz, $CDCl_3$).

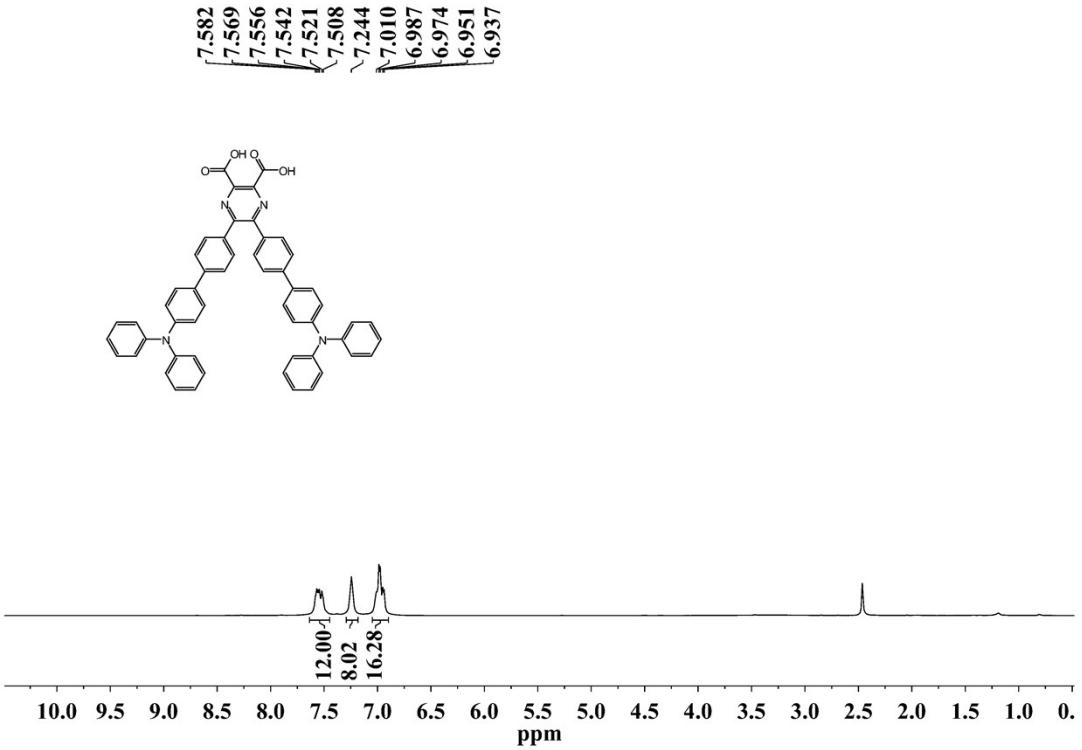


Fig. S28 ^{1}H NMR spectrum of **2** (400 MHz, $CDCl_3$).

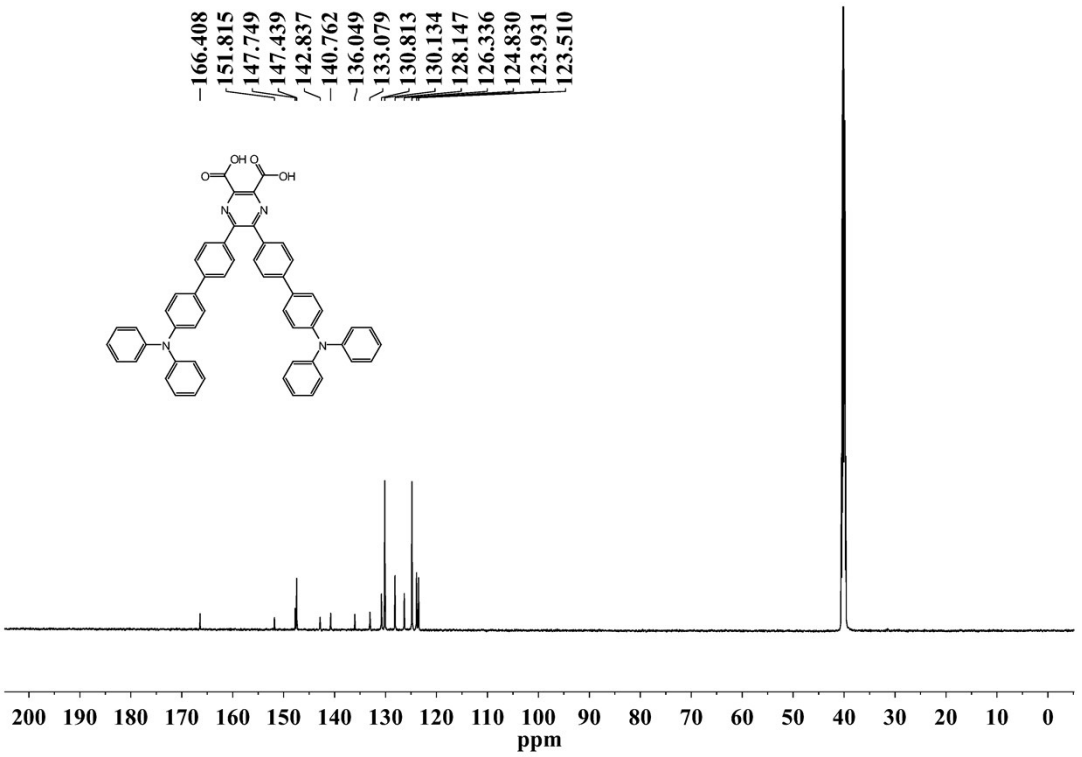


Fig. S29 ^{13}C NMR spectrum of **2** in $CDCl_3$ (100 MHz, $CDCl_3$).

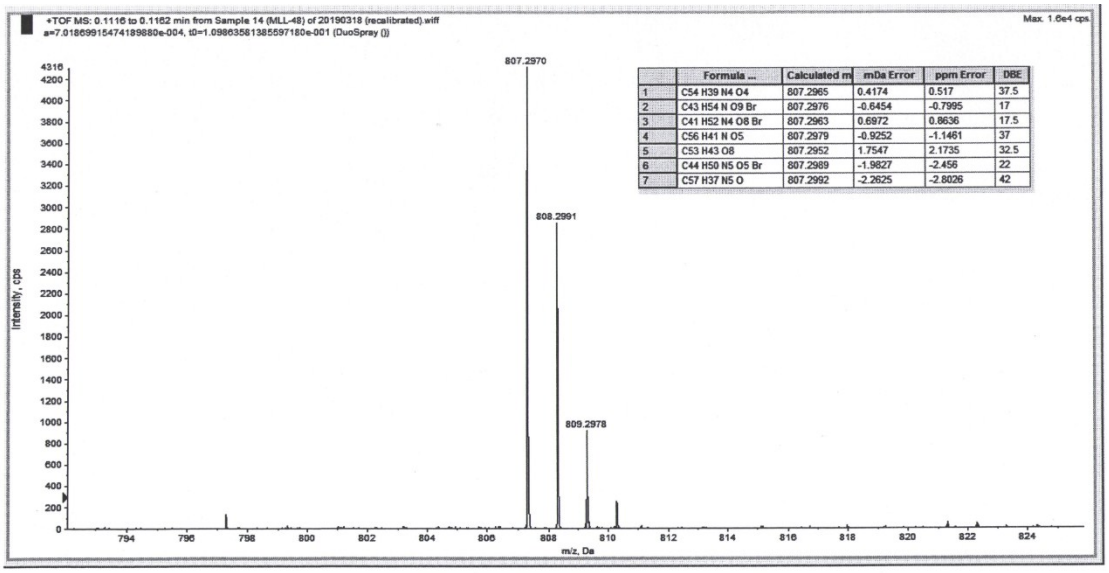


Fig. S30 HR-MS spectrum of **2.**

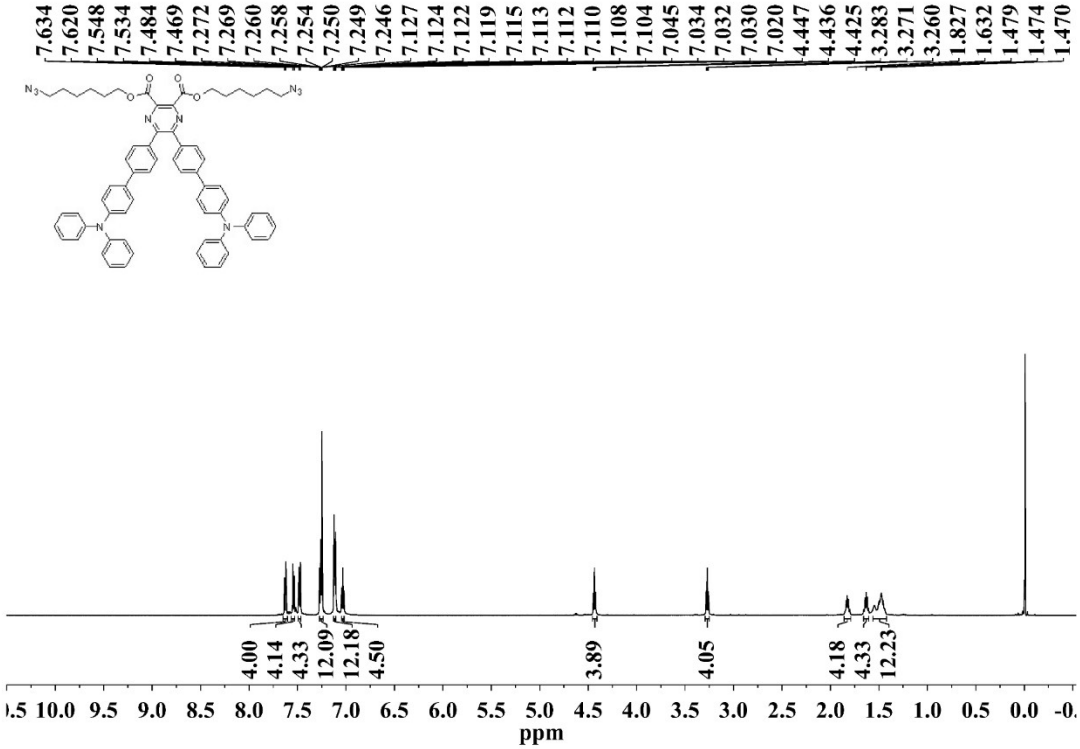


Fig. S31 ^{1}H NMR spectrum of **3a** (400 MHz, $CDCl_3$).

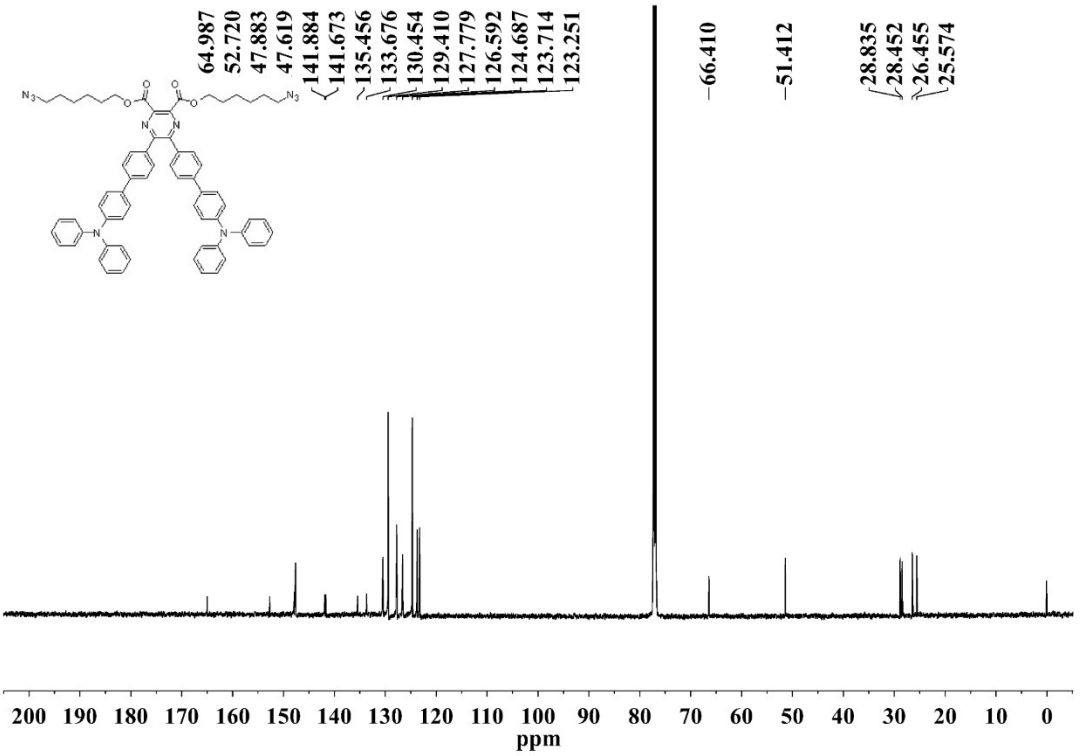


Fig. S32[13]C NMR spectrum of **3a** in $CDCl_3$ (100 MHz, $CDCl_3$).

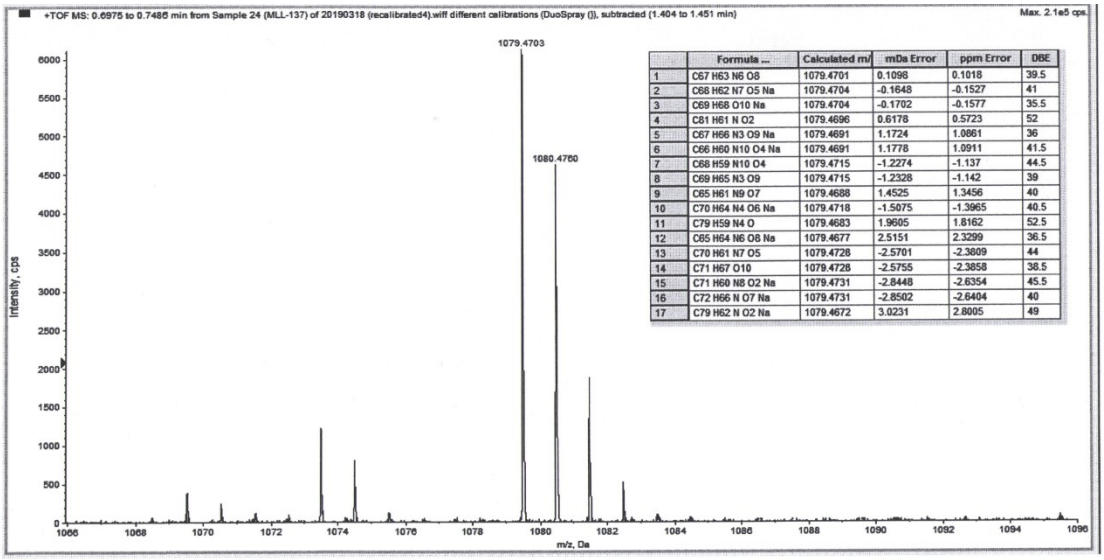


Fig. S33 HR-MS spectrum of **3a.**

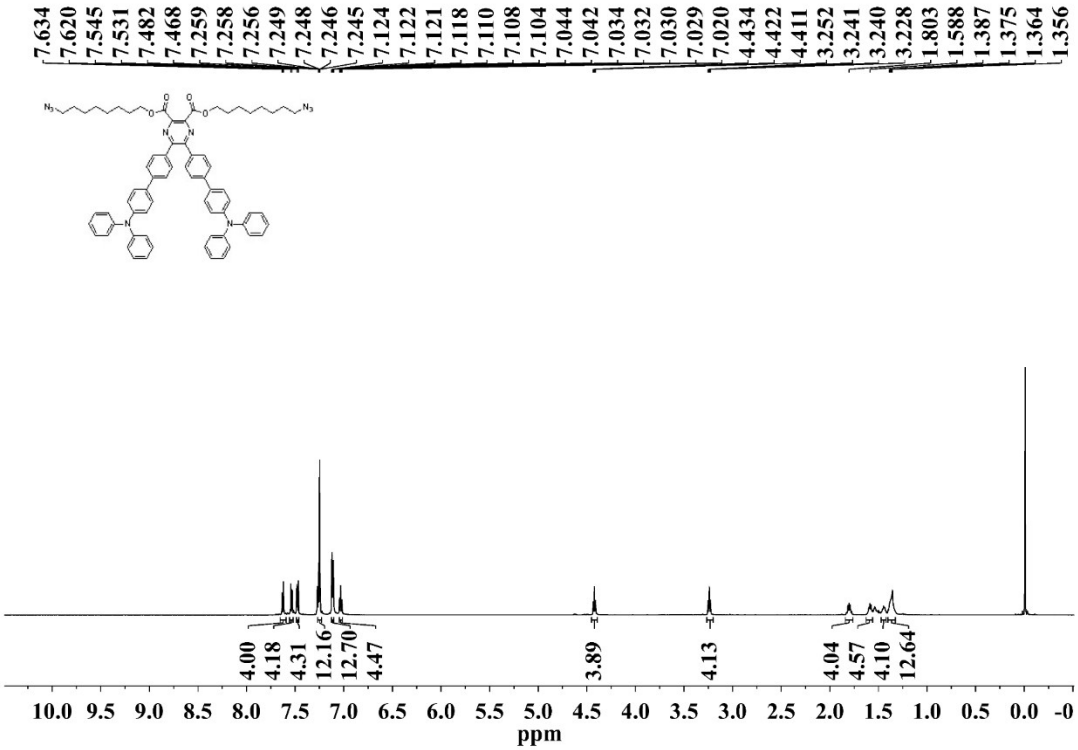


Fig. S34 ^{1}H NMR spectrum of **3b** (400 MHz, $CDCl_3$).

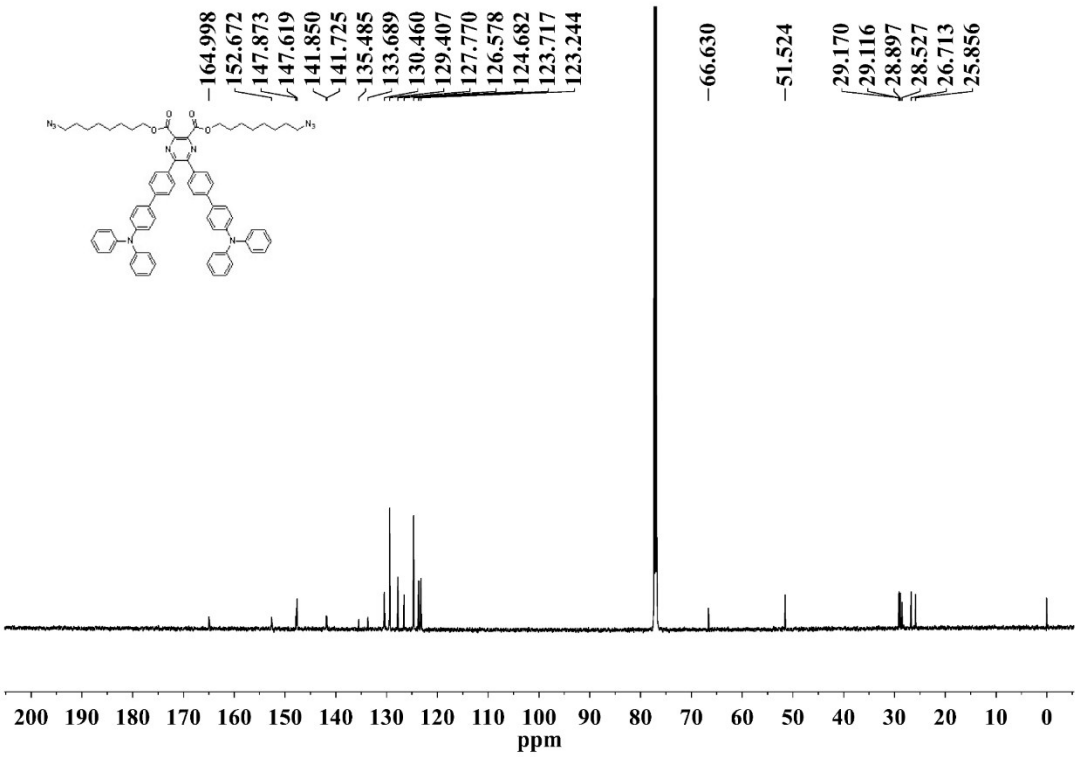
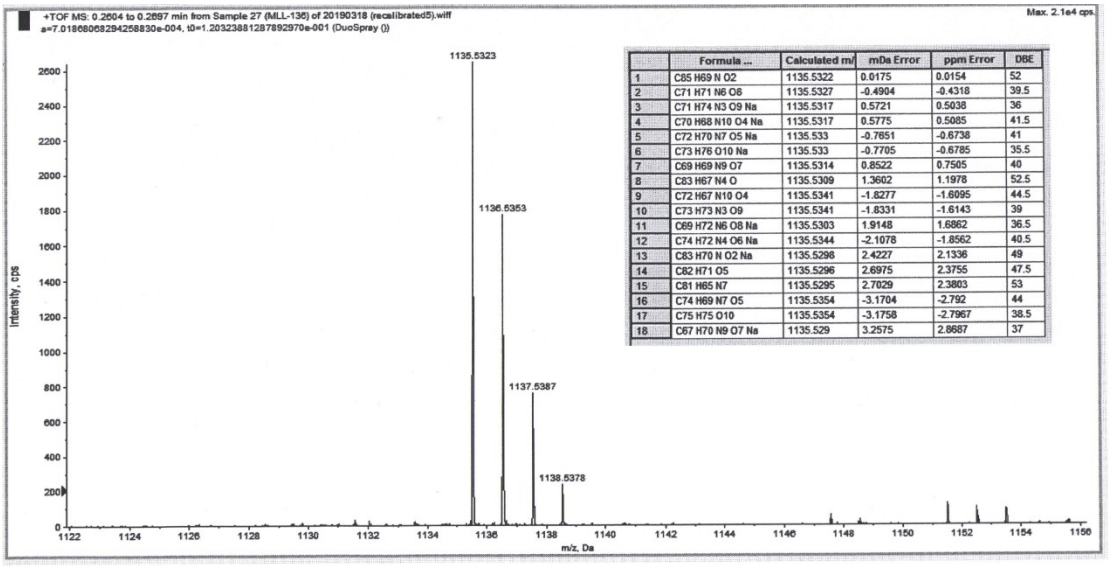


Fig. S35 ^{13}C NMR spectrum of **3b** in $CDCl_3$ (100 MHz, $CDCl_3$).



	Formula ...	Calculated m/	mDa Error	ppm Error	DBE
1	C85 H69 N O2	1135.5322	0.0175	0.0154	52
2	C71 H71 N6 O8	1135.5327	-0.4904	-0.4318	39.5
3	C71 H74 N3 O9 Na	1135.5317	0.5721	0.5038	36
4	C70 H68 N10 O4 Na	1135.5317	0.5775	0.5085	41.5
5	C72 H70 N7 O5 Na	1135.533	-0.7651	-0.6738	41
6	C73 H76 O10 Na	1135.533	-0.7705	-0.6785	35.5
7	C69 H69 N9 O7	1135.5314	0.8522	0.7505	40
8	C83 H67 N4 O	1135.5309	1.3602	1.1978	52.5
9	C72 H67 N10 O4	1135.5341	-1.8277	-1.6095	44.5
10	C73 H73 N3 O9	1135.5341	-1.8331	-1.6143	39
11	C69 H72 N6 O8 Na	1135.5303	1.9148	1.6862	36.5
12	C74 H72 N4 O6 Na	1135.5344	-2.1078	-1.8562	40.5
13	C83 H70 N O2 Na	1135.5298	2.4227	2.1336	49
14	C82 H71 O5	1135.5296	2.6975	2.3755	47.5
15	C81 H65 N7	1135.5295	2.7029	2.3803	53
16	C74 H69 N7 O5	1135.5354	-3.1704	-2.792	44
17	C75 H75 O10	1135.5354	-3.1758	-2.7967	38.5
18	C67 H70 N9 O7 Na	1135.529	3.2575	2.8687	37

Fig. S36 HR-MS spectrum of **3b.**

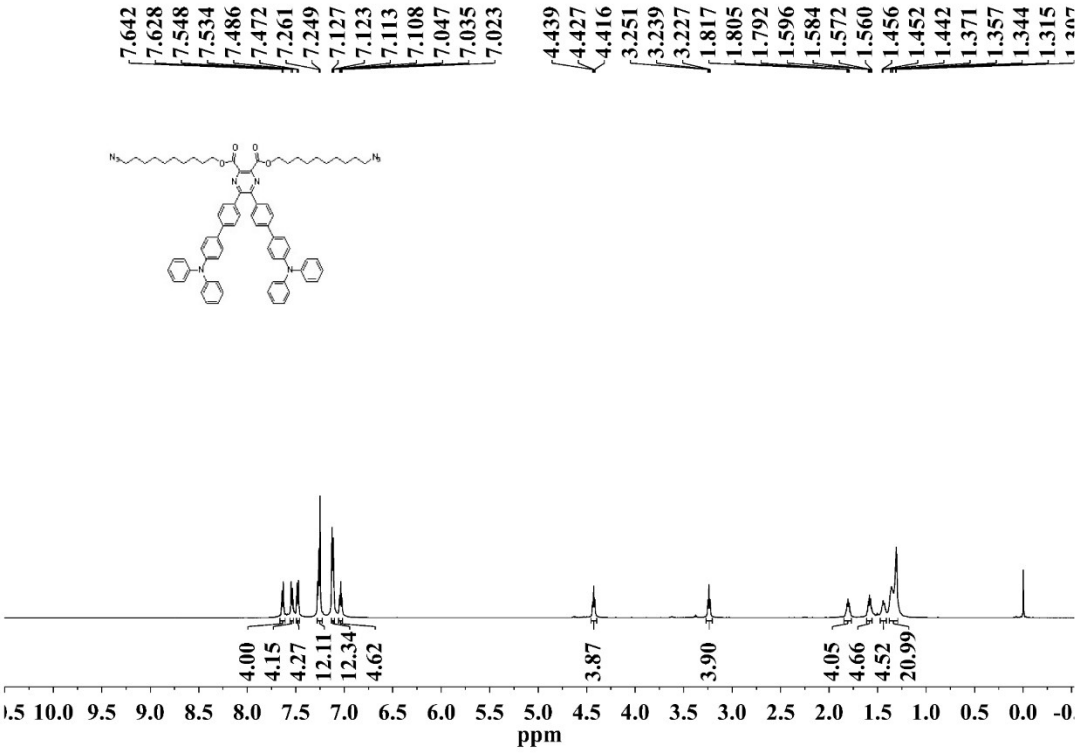


Fig. S37 ^{1}H NMR spectrum of **3c** (400 MHz, DMSO).

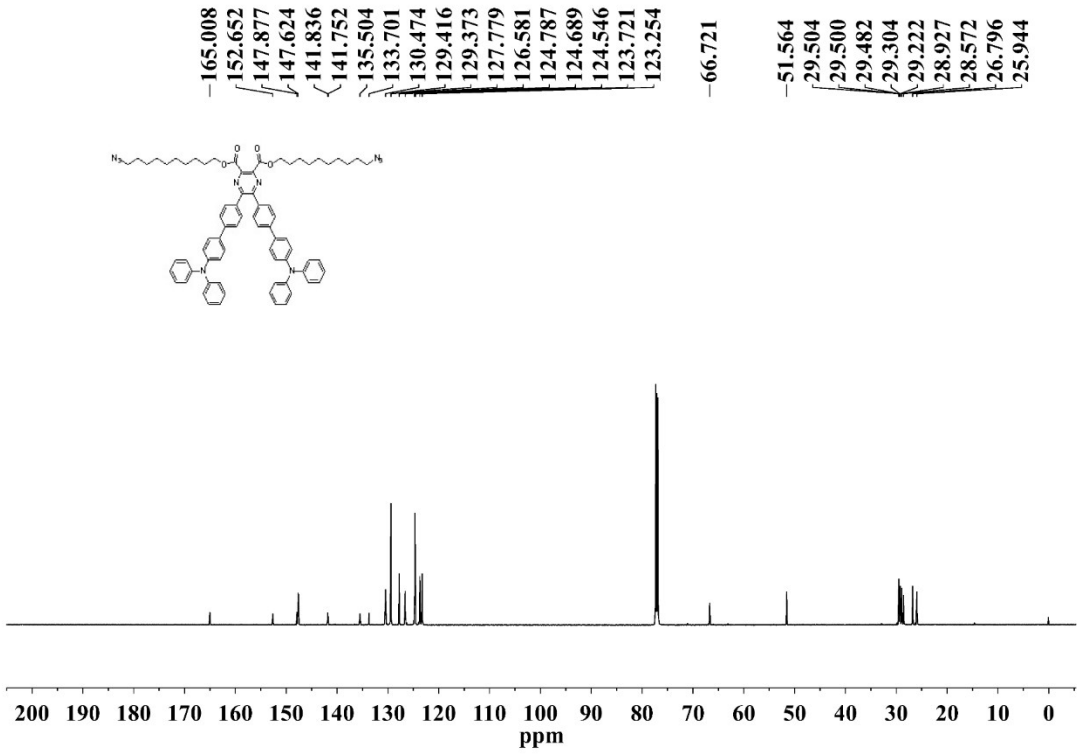
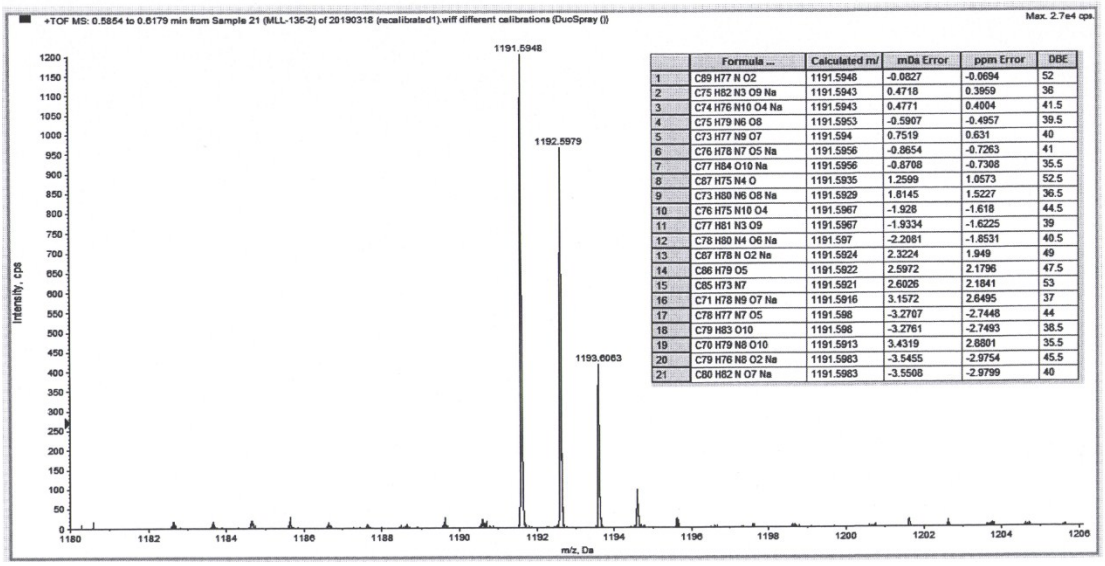


Fig. S38 ^{13}C NMR spectrum of **3c** in DMSO (100 MHz, DMSO).



	Formula ...	Calculated m/	mDa Error	ppm Error	DBE
1	C89 H77 N O2	1191.5948	-0.0827	-0.0694	52
2	C75 H82 N3 O9 Na	1191.5943	0.4718	0.3959	36
3	C74 H76 N10 O4 Na	1191.5943	0.4771	0.4004	41.5
4	C75 H79 N6 O8	1191.5953	-0.5907	-0.4957	39.5
5	C73 H77 N9 O7	1191.594	0.7519	0.631	40
6	C76 H78 N7 O5 Na	1191.5956	-0.8654	-0.7263	41
7	C77 H84 O10 Na	1191.5956	-0.8708	-0.7308	35.5
8	C87 H75 N4 O	1191.5935	1.2599	1.0573	52.5
9	C73 H80 N6 O6 Na	1191.5929	1.8145	1.5227	36.5
10	C76 H75 N10 O4	1191.5967	-1.928	-1.618	44.5
11	C77 H81 N3 O9	1191.5967	-1.9334	-1.6225	39
12	C78 H80 N4 O6 Na	1191.597	-2.2081	-1.8531	40.5
13	C87 H78 N O2 Na	1191.5924	2.3224	1.949	49
14	C86 H79 O5	1191.5922	2.5972	2.1796	47.5
15	C85 H73 N7	1191.5921	2.6026	2.1841	53
16	C71 H78 N9 O7 Na	1191.5916	3.1572	2.6495	37
17	C78 H77 N7 O5	1191.598	-3.2707	-2.7448	44
18	C79 H83 O10	1191.598	-3.2761	-2.7493	38.5
19	C70 H79 N8 O10	1191.5913	3.4319	2.8801	35.5
20	C79 H76 N8 O2 Na	1191.5983	-3.5455	-2.9754	45.5
21	C80 H82 N O7 Na	1191.5983	-3.5508	-2.9799	40

Fig. S39 HR-MS spectrum of **3c.**

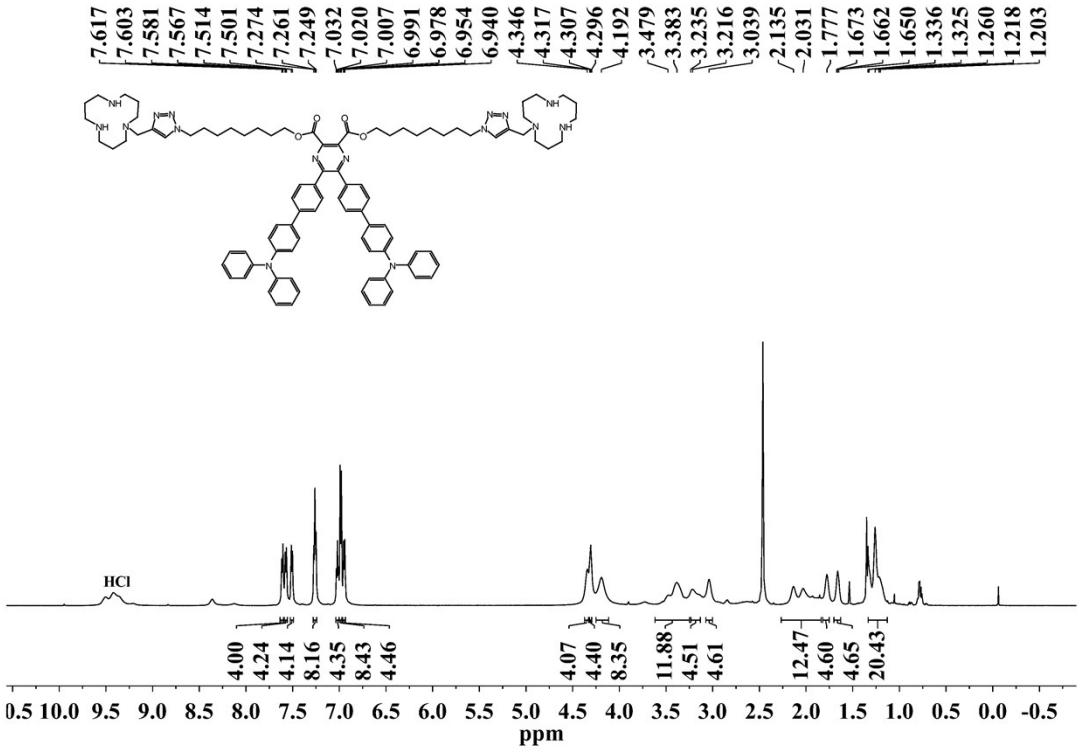


Fig. S40 ^{1}H NMR spectrum of **TDM-A** (400 MHz, DMSO).

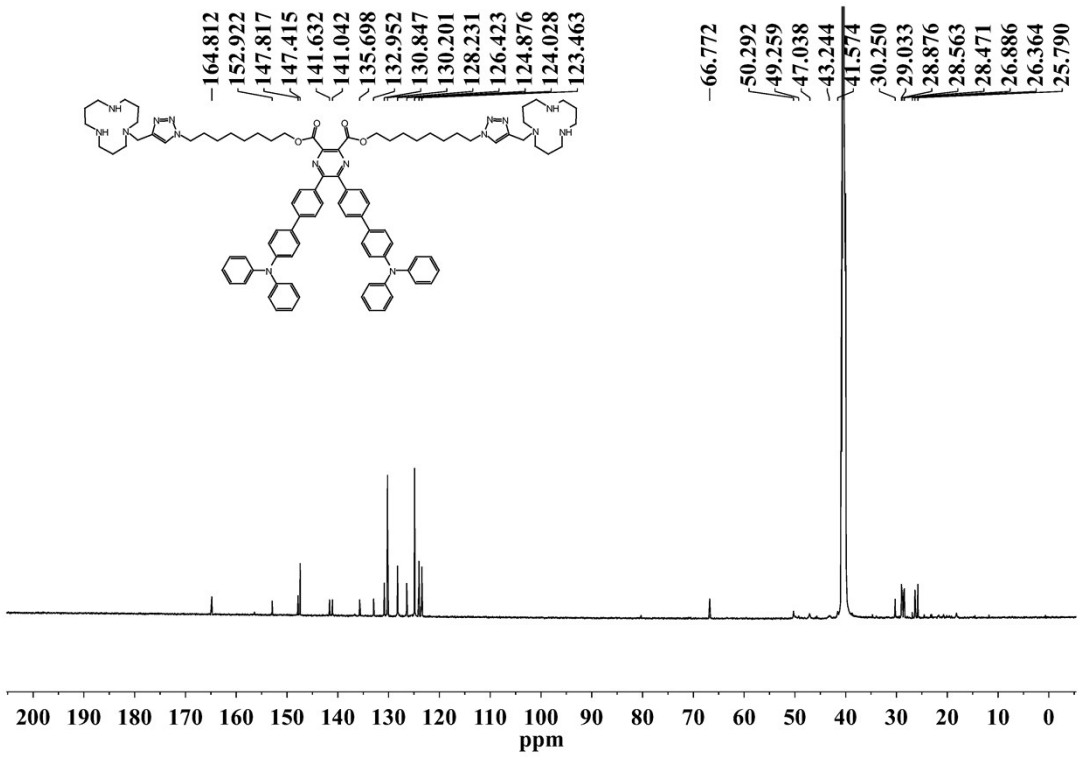


Fig. S41 ^{13}C NMR spectrum of **TDM-A** in DMSO (100 MHz, DMSO).

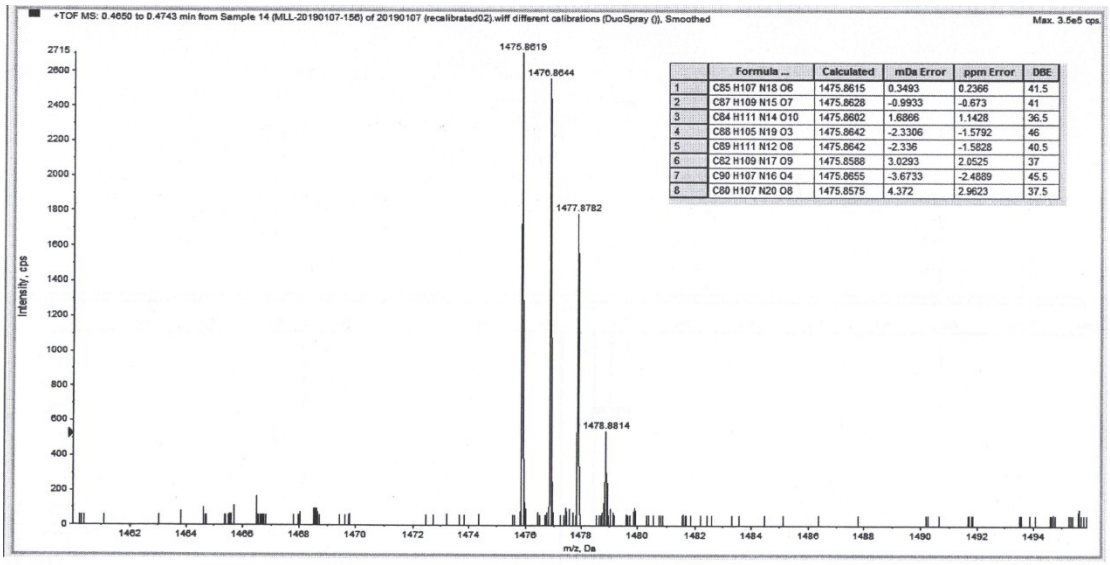


Fig. S42 HR-MS spectrum of **TDM-A.**

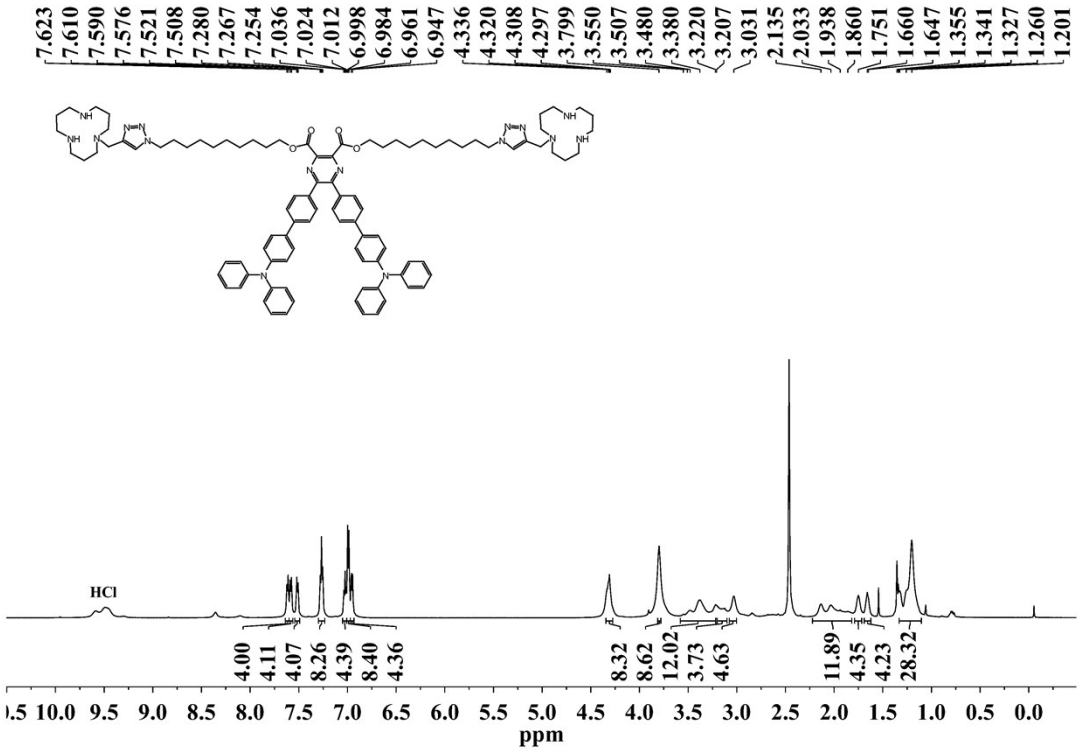


Fig. S43 ^{1}H NMR spectrum of **TDM-B** (400 MHz, DMSO).

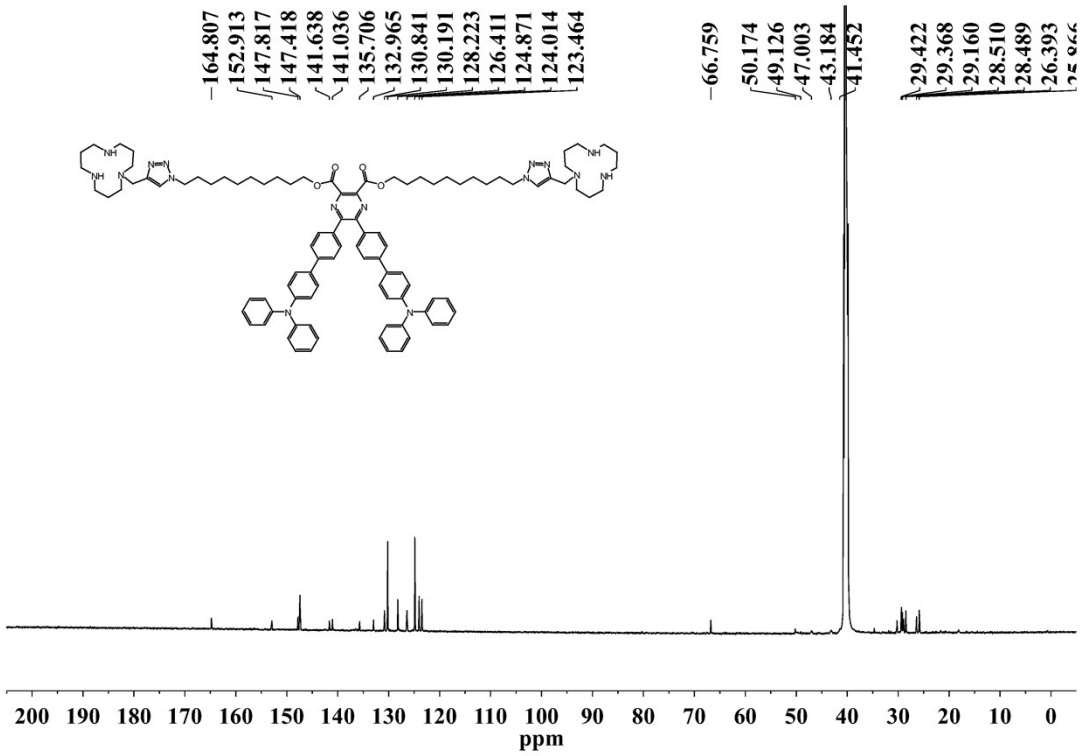


Fig. S44 ^{13}C NMR spectrum of **TDM-B** in DMSO (100 MHz, DMSO).

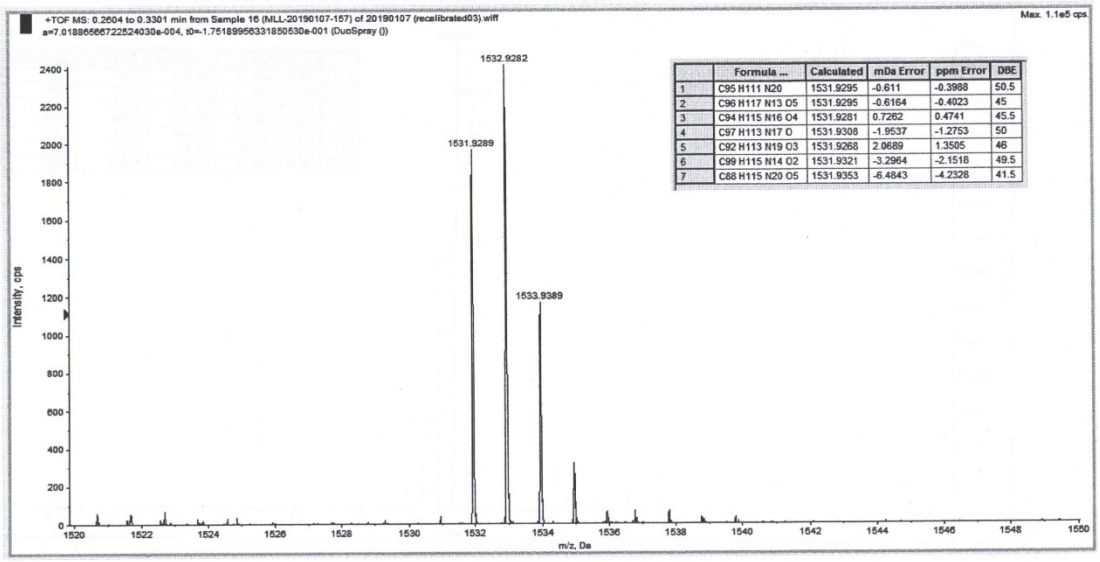


Fig. S45 HR-MS spectrum of **TDM-B.**

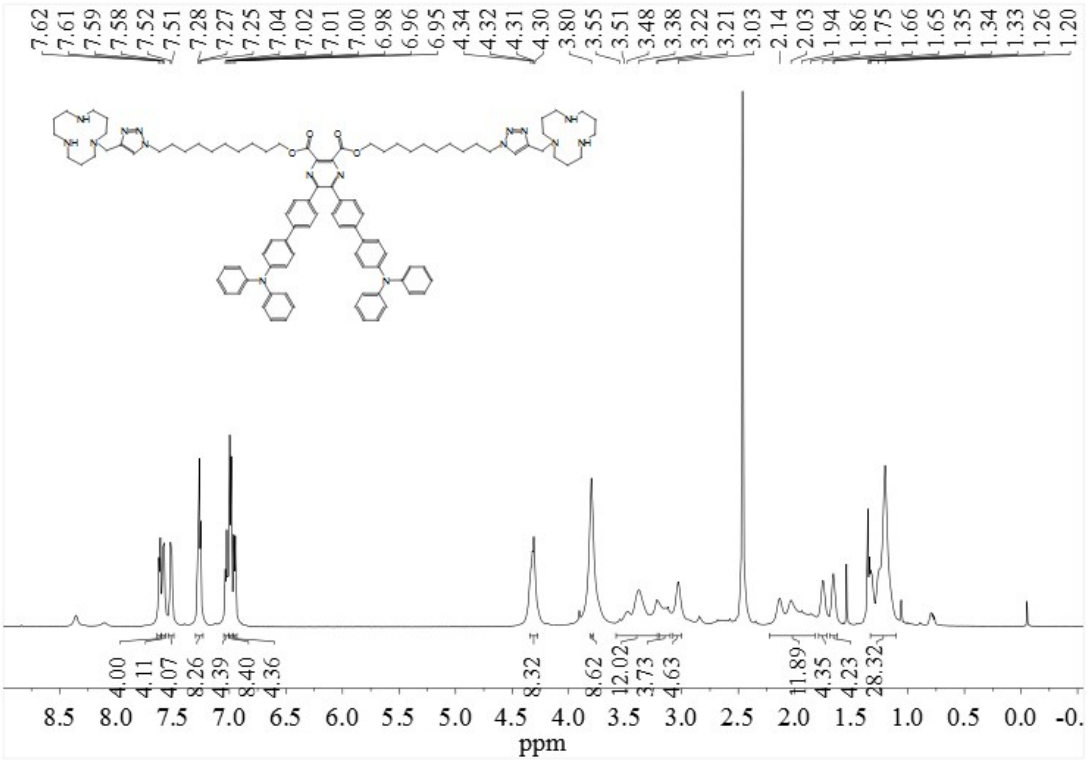


Fig. S46 ^{1}H NMR spectrum of **TDM-C** (400 MHz, $CDCl_3$).

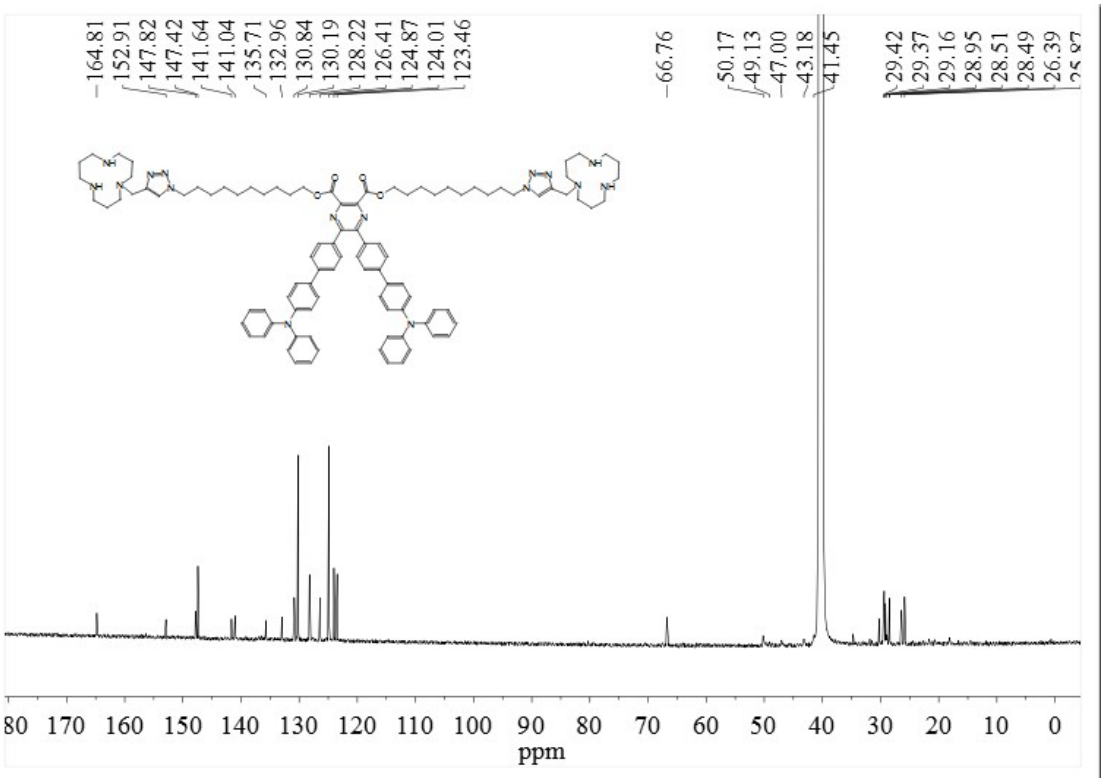


Fig. S47 ^{13}C NMR spectrum of **TDM-C** in $CDCl_3$ (100 MHz, $CDCl_3$).

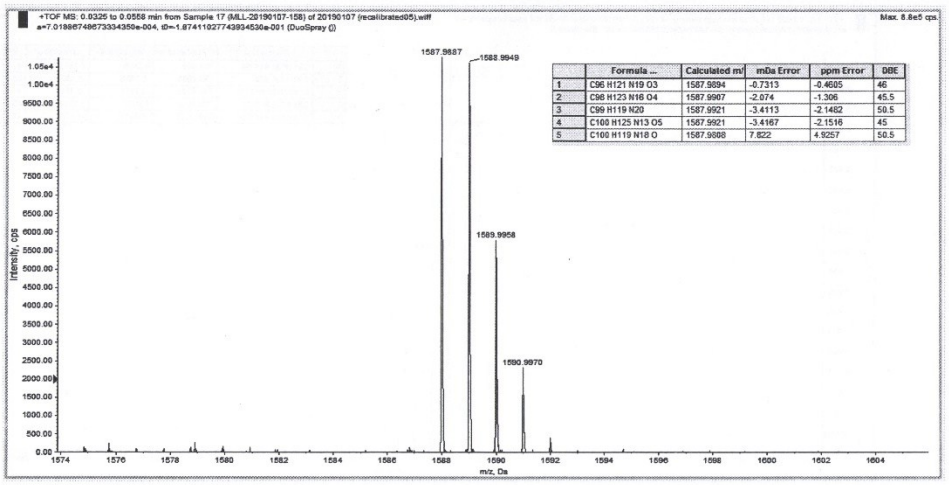


Fig. S48 HR-MS spectrum of **TDM-C.**

References:

1. Y. Wang, M. Chen, N. Alifu, S. Li, W. Qin, A. Qin, B. Z. Tang, J. Qian, *ACS Nano*, 2017, **11**, 10452-10461.