Functionalized polyesters based on valerolactones and [12]aneN_3 as effective non-viral gene vectors in HepG2 cells

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Supporting Information

CONTENTS

I. Materials and instruments

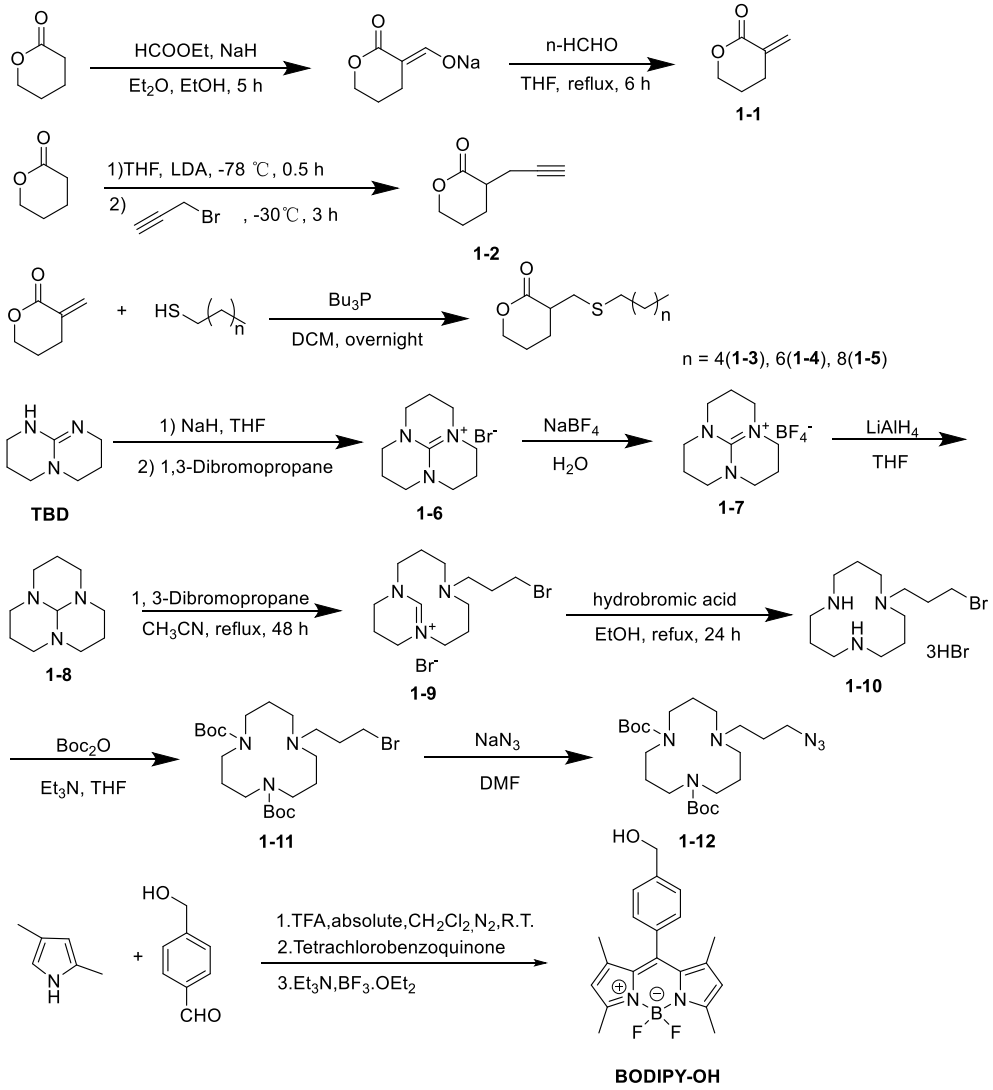
Unless otherwise specified, all the analytical pure solvents and chemicals were purchased and used without further purification. δ-Valerolactone, ethyl formate, sodium hydride, paraformaldehyde, propargyl bromide, hexamethylphosphoric triamide, 2,4-dimethylpyrrole, 4-(hydroxymethyl)benzaldehyde, trifluoroacetic acid (TFA), tetrachloro-1,4-benzoquinone, boron fluoride ethyl ether, tributylphosphine (Bu_3P), diphenyl phosphate (DPP), dioleoylphosphatidylethanolamine (DOPE), 1,3-dibromopropane, sodium tetrafluoroborate, lithium aluminum hydride, hydrobromic acid, tert-butoxycarbonyl anhydride were purchased from Macklin Reagent Co. (Shanghai, China). Lithium diisopropylamide (LDA), 1-hexanethiol, 1-decanethiol, 1-octylthiol were purchased from Energy Chemical (Anhui, China). Sodium ascorbate was purchased from Innochem Chemical (Beijing, China). Copper sulfate pentahydrate, 25 kDa PEI were purchased from Sigma-Aldrich (Shanghai, China). TBD was purchased from Raffles Pharmaceutical Technology Co., Ltd. (Guangdong, China). 50×TAE electrophoresis buffer, Tris HCl, pUC18 plasmid DNA, GoldView II, 6×DNA loading buffer, Dulbecco's modified eagle medium (DMEM), phosphate buffered saline (PBS), trypsin-EDTA solution (0.25% with phenol red), fetal bovine serum (FBS), thiazole blue (MTT), BCA reagent, Cu reagent, luciferase substrate, 5 × Cell lysis buffer, nuclear dye Hochest33342, crystal violet staining solution (1%), purchased from Solarbio Company (Beijing, China). Luciferase DNA (pLuci), red fluorescent protein DNA (pRFP), Cy5-DNA, pTRAIL plasmid DNA were purchased from Ruibiotech Co., Ltd. (Beijing, China). Agarose was purchased from Beijing Wobison Technology Co., Ltd. (Beijing, China); chlorpromazine hydrochloride (CPZ) from Tokyo Chemical Industry Co., Ltd. (Japan); methyl-β-Cyclodextrin (M-β-CD) purchased from J&K Scientific Co., Ltd. (Beijing, China); amilori (AM) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China); enzyme blue fluorescent probe purchased from Thermo Fisher Scientific Co., Ltd. (USA). pTRAIL plasmid DNA was purchased from GenePharma Co., Ltd. (Suzhou, China); The zebrafish was purchased from Shanghai Feixi Biotechnology Co., Ltd. (Shanghai,

China); the cell activity and cytotoxicity test kit was purchased from Beyotime Biotechnology (Shanghai, China).

^{1}H and ^{13}C-NMR spectra were measured on JOEL spectrometers (400 and 600 MHz) using $CDCl_3$ or CD_3OD as solvent and calibrated using tetramethyl silane (TMS) as internal reference at 25 °C. Absorption spectra were determined on a Shimadzu UV-1601PC UV-visible spectrophotometer (Japan). Fluorescence spectra were measured on Hitachi F-4600 spectrophotometer. Agarose electrophoresis was conducted using a BG-subMIDI submarine system (BayGene Biotech Company Limited, Beijing, China) and the electrophoresis images were visualized on a UVP EC3 visible imaging system using 254 nm UV light for visualization. Hitachi S-4800 (Japan) were used to obtain scanning electron microscopy (SEM) images. The average particle sizes of the polyplex were detected by dynamic light scattering (DLS) on a Brookhaven Zeta Plus Particle. Confocal fluorescence imaging was acquired with Nikon A1R MP multiphoton microscopy (Japan). Flow cytometry assays were obtained on CytoFLEX (Beckmancoulter, USA).

II. Synthesis and Characterization

Synthesis of monomer moiety



Scheme S1 Synthetic routes of monomers and functional building blocks

Compounds **1-1**, **1-2**, **1-4**, **1-12** and **BODIPY-OH** were prepared according to published procedure.[1-4]

Synthesis of **1-3** and **1-5**: To anhydrous DCM (20 mL) solution of **1-1**(0.76 g, 6.8 mmol, 6.5 equiv.), 1-hexanethiol (6.8 mmol, 6.5 equiv.) was added dropwise and then tributylphosphine (Bu_3P) (85.0 μL, 1.05 mmol, 1 equiv.) was added to a flask under an argon atmosphere. After stirring at room temperature for overnight, the solvent was evaporated, and the crude products were purified by column chromatography on silica

gel to yield compound **1-3**. Compounds **1-5** was synthesized according to a similar procedure.

1-3: yield 64%. ^{1}H NMR ($CDCl_3$, 600 MHz) δ 4.29 (m, 2H), 3.21 - 2.96 (m, 1H), 2.81 - 2.61 (m, 2H), 2.60 - 2.37 (m, 2H), 2.22 (m, 1H), 1.89 (m, 2H), 1.73 - 1.61 (m, 1H), 1.53 (m, 2H), 1.33 (m, 2H), 1.29 - 1.19 (m, 4H), 0.83 (m, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 173.22, 68.92, 40.65, 33.90, 33.27, 31.49, 29.74, 28.60, 24.37, 22.62, 22.16, 14.10.

1-5: yield 62%. ^{1}HNMR ($CDCl_3$, 600 MHz) δ 4.20 (m, 2H), 2.92 (q, *J* = 7.8 Hz, 1H), 2.61 - 2.54 (m, 2H), 2.41 (t, *J* = 7.4 Hz, 2H), 2.13 (m, 1H), 1.85-1.75 (m, 2H), 1.57 (m, 1H), 1.45 (m, 2H), 1.28 - 1.21 (m, 2H), 1.15 (m, 12H), 0.75 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 173.25, 68.93, 40.64, 33.89, 33.27, 31.98, 29.78, 29.64, 29.61, 29.40, 29.31, 28.95, 24.36, 22.77, 22.15, 14.21.

Synthesis of copolymers moiety

Polymer P2-1: In a glove box, compound **BODIPY-OH** (0.07 mmol, 1.0 equiv.), DPP (0.07 mmol, 1.0 equiv.) were dissolved in DCM (0.5 mL). After 30 minutes of reaction, **1-3** (1.40 mmol, 20.0 equiv.) were added. The reaction mixture was stirred at room temperature for 24 h (until the conversion of **1-3** was about 98% by ^{1}H NMR spectroscopy monitoring). Then **1-2** (1.40 mmol, 20.0 equiv.), DPP (0.07 mmol, 1.0 equiv.) were added into the reaction solution. The reaction mixture was stirred at room temperature for another 24 h (until the conversion of **1-2** was about 98% by ^{1}H NMR spectroscopy monitoring) then quenched with excess Et_3N. The reactant solution was dialyzed against EtOH for 24 h and then dried in vacuo to give **P2-1**.

According to the same procedure, **P2-2** and **P2-3** were obtained.

Polymer P3-1: Polymer **P2-1** (274.6 mg, 1.0 equiv.) and compound **1-12** (287.0 mg, 24.0 equiv.) were dissolved in 4 mL anhydrous THF in a double-mouth round-bottom flask. Sodium ascorbate (42.2 mg, 6.0 equiv.) and copper sulfate pentahydrate (26.6 mg, 3.0 equiv.) were dissolved in water and quickly added to the reaction system through a needle. Overnight reaction under argon at room temperature.The next day, THF was

removed by vacuum distillation, the crude product was dissolved in DCM, washed with saturated sodium chloride, and the organic phase was collected. The organic phase is dried with anhydrous sodium sulfate, filtered, and vacuum distilled to remove the solvent. After the characteristic peak of triazole was found by ^{1}H NMR, the reactant solution was dialyzed against EtOH for 24 h and then dried in vacuo to give **P3-1.**

According to the same procedure, **P3-2** and **P3-3** were obtained.

Polymer TMN1/2/3:

TMN-1: Polymer **P3-1** was added to the mixture of DCM (2.0 mL) and TFA (2.5 mL), and was stirred for 4 h at room temperature, then the solvent was removed by reduced pressure. The obtained crudes were dialyzed against EtOH for 24h and then dried in vacuo to give **TMN-1**.

According to the same procedure, **TMN-2** and **TMN-3** were obtained.

III. Experimental Sections

Fluorescence Measurements.

To prepare a 100 mg/L solution for fluorescence spectrum testing, 2.0 mg of the target polymer was weighed and dissolved in 2 mL of DMSO to obtain a 1 mg/mL stock solution. From this stock solution, 200 μL was taken and diluted with DCM, EA, H_2O, MeOH, THF, and MeCN, respectively. The total volume was adjusted to 2 mL for each dilution.

Preparation of TMN-DOPE.

TMN-1/2/3, DOPE at 1:2 molar ratios were dissolved in chloroform/methanol (3:1, v/v). The mixture was sonicated for 1 hour. Then solvent was then removed *via* vacuum distillation, and the resulting mixture was dried in a vacuum drying oven overnight. The next day, 1 mL of PBS was added, and the mixture was sonicated for 1 hour to facilitate full assembly of the molecules. The mixture was then passed through a liposome extruder (100 nm polycarbonate film) ten times and sonicated for an additional hour to obtain **TMN-DOPE**.

Preparation of TMN-3-DSPE-PEG and **TMN-3-DOPE-PEG**.

TMN-3, DSPE-PEG at 1:2 molar ratios were dissolved in chloroform/methanol (3:1, v/v). The mixture was sonicated for 1 hour. Then solvent was then removed via vacuum distillation, and the resulting mixture was dried in a vacuum drying oven overnight. The next day, 1 mL of PBS was added, and the mixture was sonicated for 1 hour to facilitate full assembly of the molecules. The mixture was then passed through a liposome extruder (100 nm polycarbonate film) ten times and sonicated for an additional hour to obtain **TMN-3-DSPE-PEG. TMN-3-DOPE-PEG** was prepared according to a similar procedure.

Preparation of TMN-DOPE /pDNA NPs

Separately dilute **TMN-DOPE** and pDNA into equal volumes of DMEM, then the

diluted pDNA solution is added drop by drop to the diluted **TMN-DOPE** solution. Incubate the mixture at 37°C for 30 minutes to obtain **TMN-DOPE/pDNA NPs**.

Preparation of TMN-3-DSPE-PEG /pDNA NPs and TMN-3-DOPE-PEG /pDNA NPs

Separately dilute **TMN-3-DSPE-PEG** and pDNA into equal volumes of DMEM, then the diluted pDNA solution is added drop by drop to the diluted **TMN-3-DSPE-PEG** solution. Incubate the mixture at 37°C for 30 minutes to obtain **TMN-3-DSPE-PEG /pDNA NPs. TMN-3-DOPE-PEG /pDNA NPs** were prepared according to a similar procedure.

Gel Retardation Assay.

The target polymer was dissolved in PBS to prepare a 0.2 μg/μL stock solution. The pUC18 plasmid mass concentration was 9 μg/mL. According to the plasmid mass concentration, different volumes of the mother solution were added to prepare different N/P ratio test solutions. After that, the samples were incubated at 37°C for 30 min, and 2 μL of 6× loading buffer were added to the above samples. The samples were electrophoresed at 100 V on a 0.7% agarose gel containing GelRed in Tris-acetate (TAE) running buffer for 30 min. The result was visualized under on a UVP EC3 visible imaging system.

Degradation tests.

Degradation tests were carried out on 5 mg of TMN-3. The polymer was placed in 5 mL of 2 mg/mL Lipase solutions. The sample was stirred for 24 h at room temperature and then the polymer was collected, dried and analysed through GPC.

Assembly of TMN-1/2/3-DOPE@pUC18.

The morphologies of micelles and complexes were studied by scanning electronic microscopy (SEM) and dynamic light scattering (DLS). The DLS sample were prepared

similar to gel retardation assay, the solution was diluted to 200 μL after interaction with negatively supercoiled pUC18 DNA at 37°C for 30 min. The diluted solution 10 μL dropped on the surface of wafer, then completely evaporated to obtain the SEM.

Cytotoxicity Assay.

The cytotoxicity of polyplexes was investigated in HeLa, A549, PC-3 and HepG2 cells by MTT assay. All cells were cultured in complete medium in a humidified atmosphere containing 5% CO2 at 37 ºC. After 24 h of incubation, the cells were seeded in 96-well plate (7000 cells/well) and cultured for another 24 h. The cells were incubated with different N/P ratio of polyplexes for 4 h. Then, 100 μL complete medium were added to each well and cultured for 20 h. The medium was replaced with 20 μL of MTT (5 mg/mL) and incubated for another 4 h. Finally, MTT was replaced with 120 μL of DMSO, and the plates were oscillated for 10 min to fully dissolve the formazan crystals formed by living cells in the wells. The optical density (OD) was recorded at 490 nm using a Thermo Scientific Multiskan GO. The viability of the cells was calculated using the following formula:

Cell viability (%) = (OD(Polyplex) - OD(Blank))/(OD(Control) - OD(Blank)) × 100%

OD (Polyplex), OD (Control), and OD (Blank) respectively represent the absorbance of the wells incubated with nanoparticles, only DMEM incubated with cells, and DMSO added but without cells.

In *vitro* gene transfection. Gene transfection efficiency was investigated in A549, HepG2, PC-3, and HeLa cell lines. Cells were seeded in 24-well plates (8×10^4 cells/well) and cultured in DMEM in a 5% CO_2 humidity incubator at 37 ℃ for 24 h. Before transfection, 200 μL of polyplexes in DMEM at various N/P ratio were added to a 24-well plate and incubated for 4 h. Then the medium was replaced by 600 μL of complete medium and cultured for 44 h which were performed according reported in references.

RFP Transfections in *Vitro*.

RFP transfection efficiency was investigated in HepG2 cell line. The cells were seeded in Glass Bottom Cell Culture Dishes at 1000 cells per dish and cultured for 24 h. Before transfection, 500 μL of polyplexes in DMEM were added to the dishes and incubated for 4 h. Then the medium was replaced by 1 mL of complete medium and cultured for 24 h. Finally, the cells were washed for 5 times with PBS buffer, observed using a confocal laser scanning microscope with a 10 × objective.

Cell Imaging.

Time dependence of cellular uptakes/ lysosomal escape of **TMN-3-DOPE@Cy5-DNA** were measured by confocal laser scanning microscopy (CLSM) in HepG2/A549 cell lines. The cells were seeded in Glass Bottom Cell Culture Dishes at 5×10^4 cells per dish and cultured for 24 h. After washed three times with PBS, the cells were exposure to **TMN-3-DOPE@Cy5-DNA** for predetermined durations. The Hoechst 33342 (5 μg/mL) /Lyso-Tracker Blue Commercial Stain (50 nmol/L) was also added to the cells for nuclear staining at 37 ºC for 10 min. Finally, the cells were washed for 5 times with PBS buffer, observed using a confocal laser scanning microscope with a 40 × oil-immersion objective. For the cell death staining experiment, continue to culture for 20 hours after administration for 4 hours, wash with PBS for more than three times the next day, stain with Hochest 33342 and PI, and use a laser confocal microscope to take pictures.

Flow Cytometry.

The cellular uptake of carrier/DNA complexes and cell apoptosis study were assessed by flow cytometry. For the cellular uptake study, cells were seeded in 12-well plates at a density of 1×10^5 cells per well. After initial incubation for 24 h, cells were incubated with CPZ (50 μM), MβCD (2.5 mM), or AM (75 μM) for 1 h. The culture medium was removed and fresh media containing carrier/Cy5-DNA complexes were added to the plates and incubated for another 2 h. Next, the cells were digested with trypsin,

centrifuged, and washed three times with PBS. Flow cytometry was performed on a Beckman Coulter CytoFLEX. In addition, for the cellular uptake of carrier/Cy5-DNA condensates at different temperatures, the only difference was that the cells in the experimental groups were cultured at 4 °C for 2 h and the cells of the control group were cultured at 37 °C for 2 h. For the cell apoptosis study, HepG2 cells were cultured with **TMN-3-DOPE@p53/@pTRAIL** for 4 h**,** gently washed with PBS at least three times. Then, the cells were collected and stained with Hochest 33342 and PI to detect cell apoptosis. Cells without condensate treatment were employed as blank controls.

RFP transfection in vivo. Zebrafish were purchased by the China Zebrafish Resource Center. **TMN-1/2/3-DOPE@pRFP** were prepared according to above procedure. Zebrafish were treated with 1 mL solution containing the polyplexes for 4 h, then 1 mL fresh water was added and further incubated for 24 h. The fluorescence intensity of RFP was evaluated by confocal imaging, and 25 kDa PEI was used as a control.

Tumor spheroid experiment.

A cell suspension of 4000 cells was seeded in an ultra-low adsorption 96-well plate. Within three days multicellular tumor spheroids (MCTs) were formed from the cell suspension. **TMN-3-DOPE@Cy5-DNA** were prepared according to above procedure. MCTs were treated with the polyplexes for 4 h. Then the medium was replaced by 1 mL of complete medium and cultured for 24 h. Finally, the MCTs were washed for 5 times with PBS buffer, observed using a confocal laser scanning microscope with a 10 × objective.

Cell migration study. The HepG2 cells were cultured for 4 h with **TMN-3-DOPE@p53 and TMN-3-DOPE@pTRAIL** in a 6-well chamber. Then, a wound gap on the monolayer HepG2 cells was scratched by a 10 μL tip. The scratched cells were washed away with PBS buffer, and the wound gaps were imaged by CLSM with a 10 × objection and incubated for 20 h and 44 h.

Cell cloning. Inoculate cells with 1000 cells per dish in a glass bottom cell culture dish and incubate for 24 hours. The next day, 4 hours after administration, remove the culture medium, clean with PBS, add 1 mL of pancreatic enzyme to each well for digestion, remove the pancreatic enzyme, add 1 mL of PBS and blow evenly, and take 10 μL is counted using a cell counting board. Inoculate 1000 cells per well into a 12 well plate and continue cultivation, changing the solution every three days. After 10 days, discard the old culture medium, clean with PBS, add 1 mL of paraformaldehyde for 15 minutes, clean with PBS, add 1 mL of crystal violet (0.1%) for staining, stain for 10-20 minutes, clean with PBS, take photos and record the results of cell cloning experiments, and perform quantitative analysis using ImageJ software.

IV. Supporting Figures

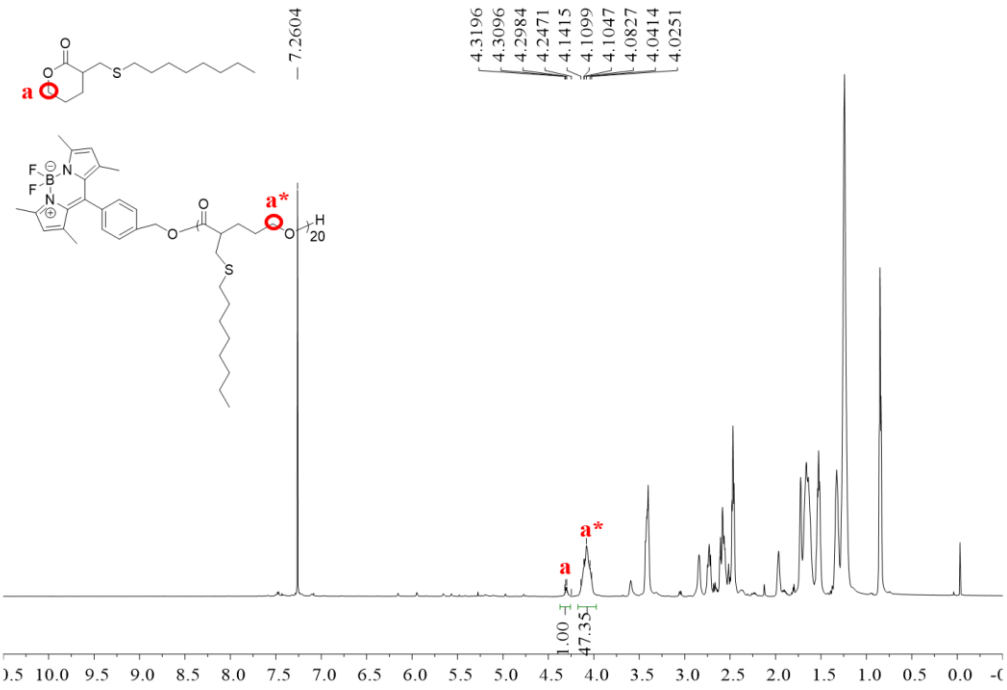


Fig. S1 ^{1}H NMR spectra of compound **1-4** after ROP reaction.

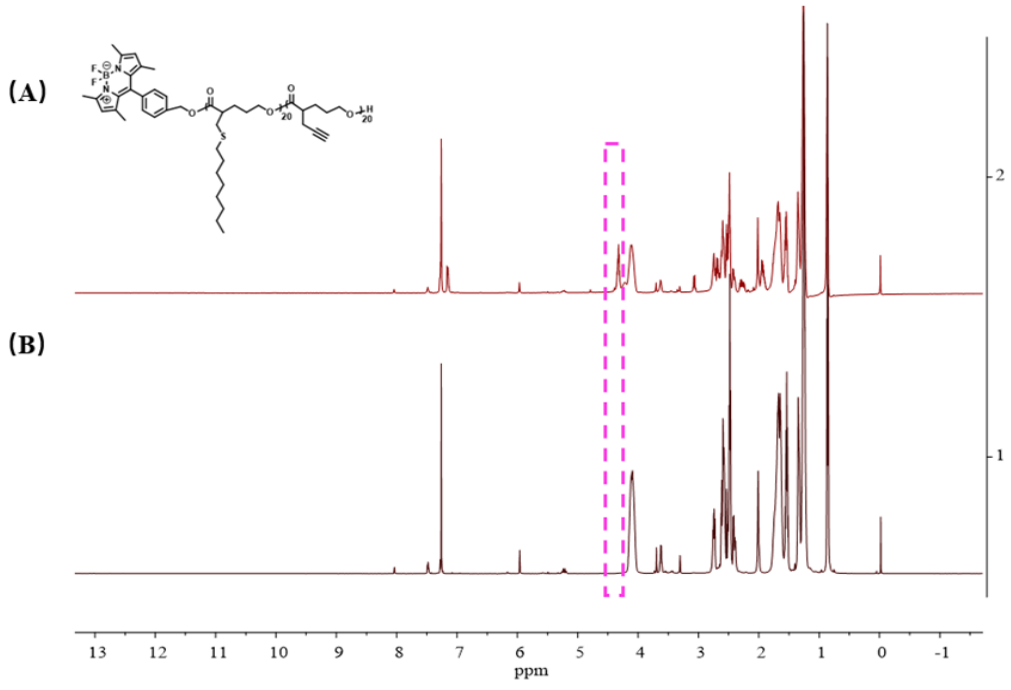


Fig. S2 ^{1}H NMR spectra of block copolyester **P2-2** before (A) and after (B) dialyzing against EtOH.

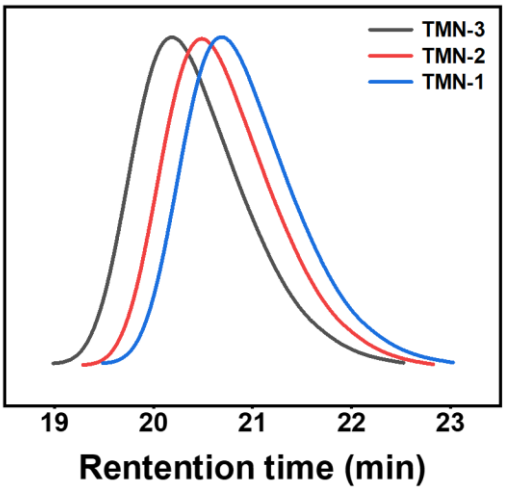


Fig. S3 GPC traces: the black lines correspond to the **TMN-3** copolymer, the red lines correspond to **TMN-2** copolymer and bule one to the **TMN-1** copolymer.

Copolymer	$M_{n,NMR}$	$M_{n,GPC}$	PDI_{GPC}
TMN-1	**19462**	**15670**	**1.21**
TMN-2	**20203**	**18383**	**1.17**
TMN-3	**20769**	**21106**	**1.12**

Table S1. Characterization of the polymers. $M_{n,\ NMR}$ was measured by ^{1}H NMR spectroscopy. $M_{n,\ GPC}$ and polydispersity index (PDI) were obtained by GPC.

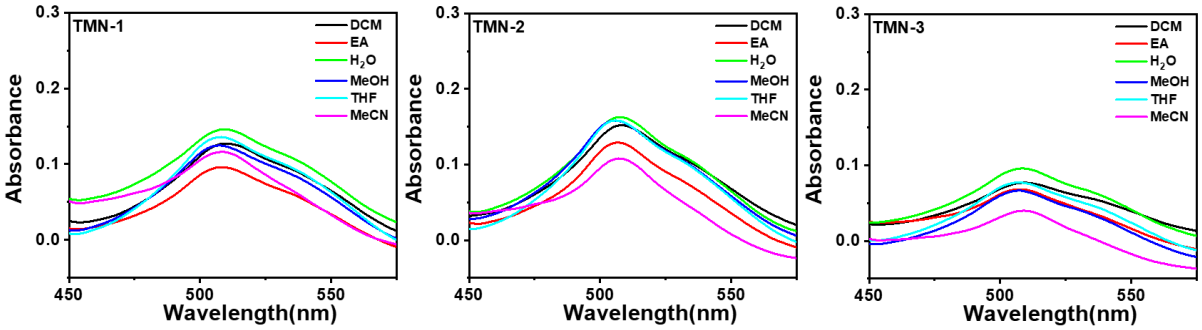


Fig. S4 UV-Vis spectra of **TMN-1/2/3** in different solutions. Concentration: 100 mg/L.

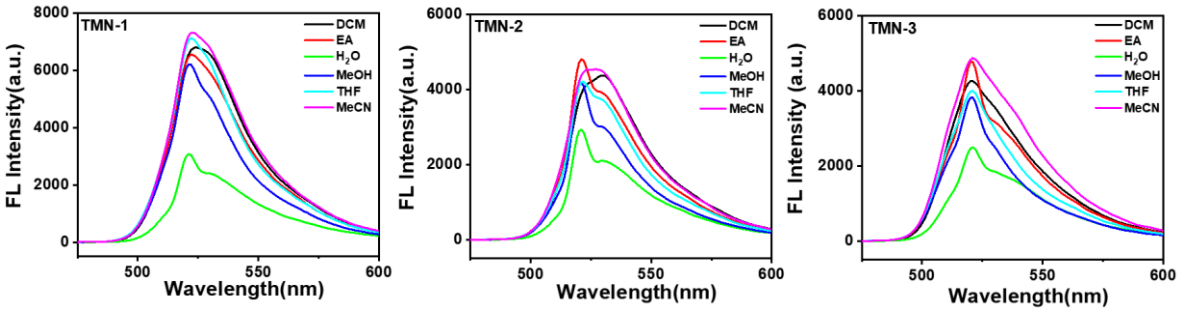
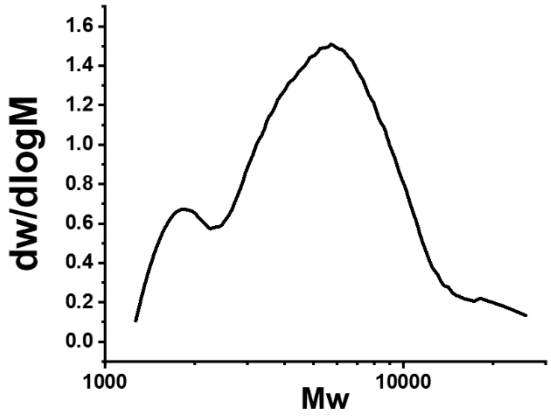


Fig. S5 Fluorescence spectra of **TMN-1/2/3** in different solutions. Concentration: 100 mg/L.



Mp	Mn	Mw	Mz	Mz + 1	Mv	PDI
6205	4089	6080	8921	12387	5728	1.48

Fig. S6 Molecular weight distribution of TMN-3 after lipase treatment.

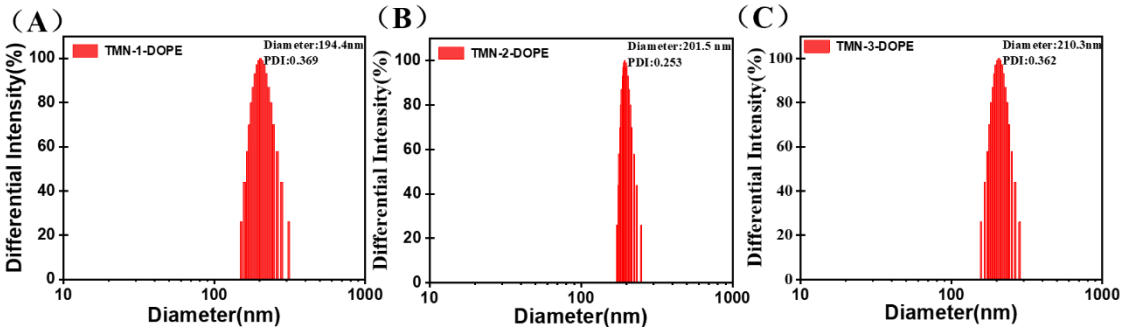


Fig. S7 DLS of **TMN-1/2/3-DOPE** condensed pUC18 DNA nanoparticles in PBS (pH = 7.4).

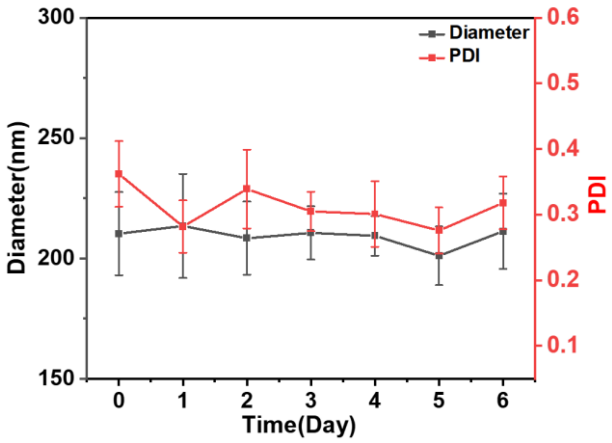


Fig. S8 Size and PDI change of the **TMN-3-DOPE@pUC18** polyplex within 6 days in PBS (pH = 7.4).

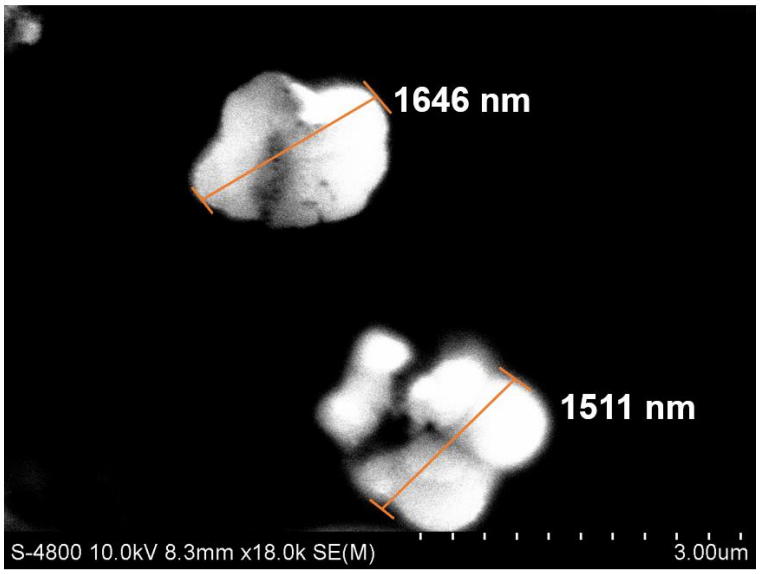


Fig. S9 The SEM image of **TMN-3-DOPE@pUC18** after adding lipase and treating for 4 hours.

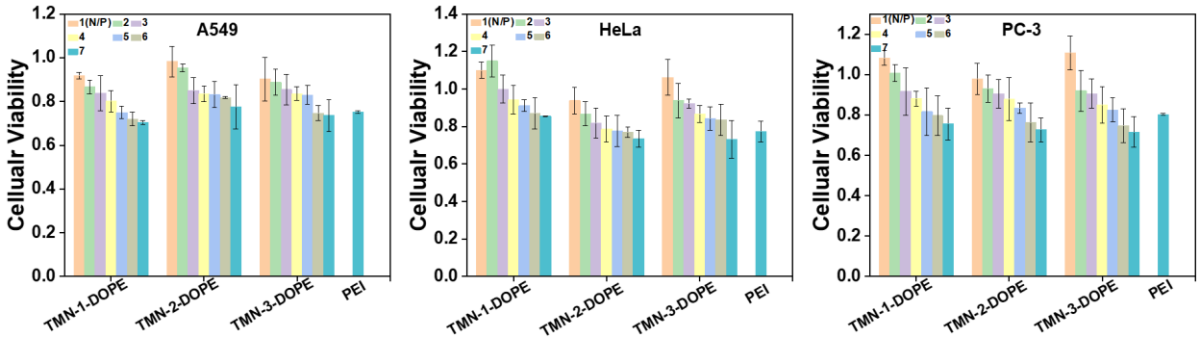


Fig. S10 Cytotoxicity of DNA complexes formed by **TMN-1/2/3-DOPE** at different N/P ratio toward A549, HeLa, PC-3 cells, and 25 kDa PEI was used as a control. [pLuci] = 10 μg/mL.

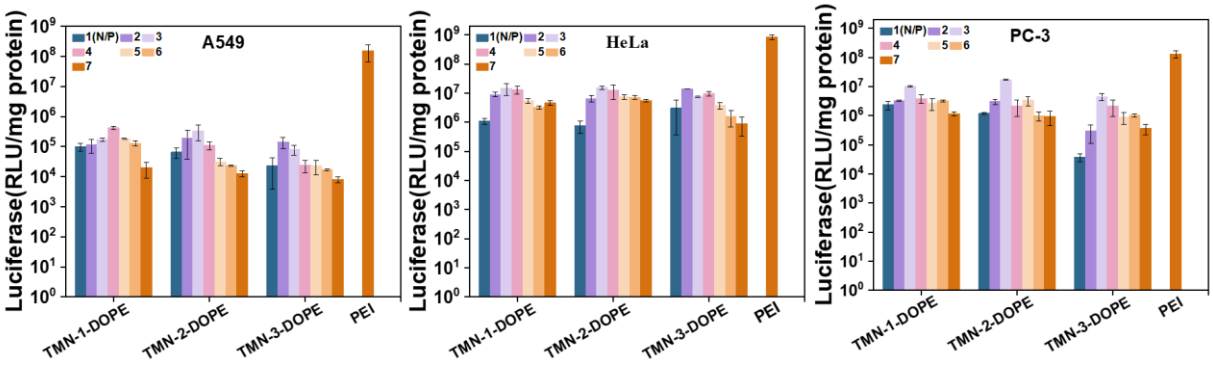


Fig S11. Luciferase expressions transfected by polyplexes at different N/P ratio in A549, HeLa and PC-3 cell lines without 10% FBS; 25 kDa PEI was used as a control, [pLuci] = 10 μg/mL.

Table S2 Optimal luciferase expressions of **TMN-1/2/3-DOPE** in different cell lines (% of PEI).

	HeLa	HepG2	A549	PC-3
TMN-1-DOPE	<0.1	0.6	0.1	<0.1
TMN-2-DOPE	<0.1	1.6	<0.1	0.1
TMN-3-DOPE	<0.1	18.9	<0.1	<0.1

Table S3 Optimal luciferase expressions of **TMN-1/2/3-DOPE** in HepG2 cells with 10% FBS (% of PEI).

	TMN-3-DOPE	TMN-3-DOPE-PEG	TMN-3-DSPE-PEG
HepG2	**0.6**	**2.9**	**0.3**

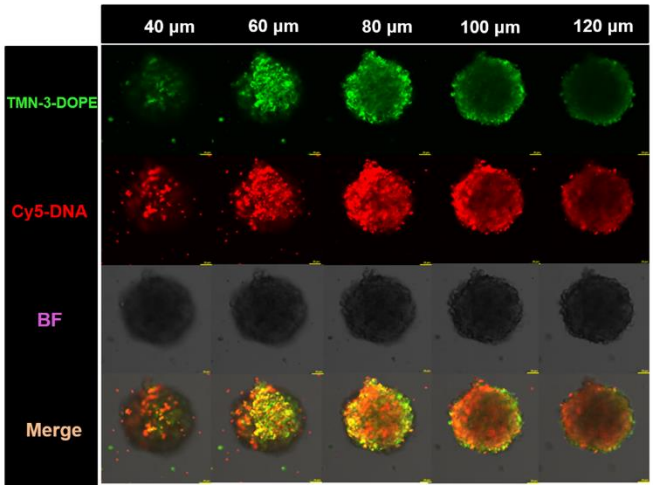


Fig S12. CLSM images of Z-superimposed scanning after 4 h incubation of **TMN-3-DOPE@Cy5-DNA** with 3D multicellular sphere (HeLa). N/P ratio = 4, [Cy5-DNA] = 10 μg/mL. Scale bar: 50 μm.

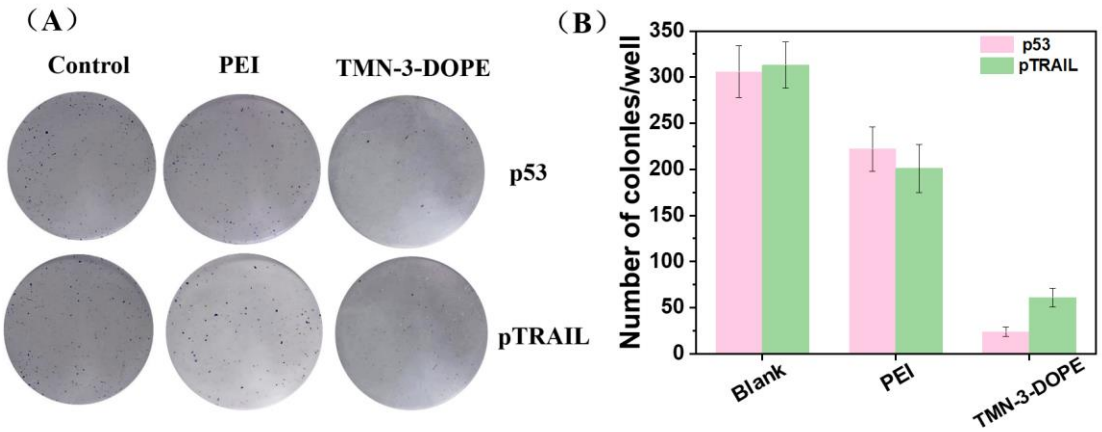


Fig S13. (A) Cell proliferation ability maps of HepG2 cells treated with **TMN-3-DOPE@p53** and **TMN-3-DOPE@pTRAIL**; (B) Comparison of cell clone numbers; N/P ratio =4, [p53] = 4 μg/mL, [pTRAIL] = 4 μg/mL.

V. Spectra of Compounds

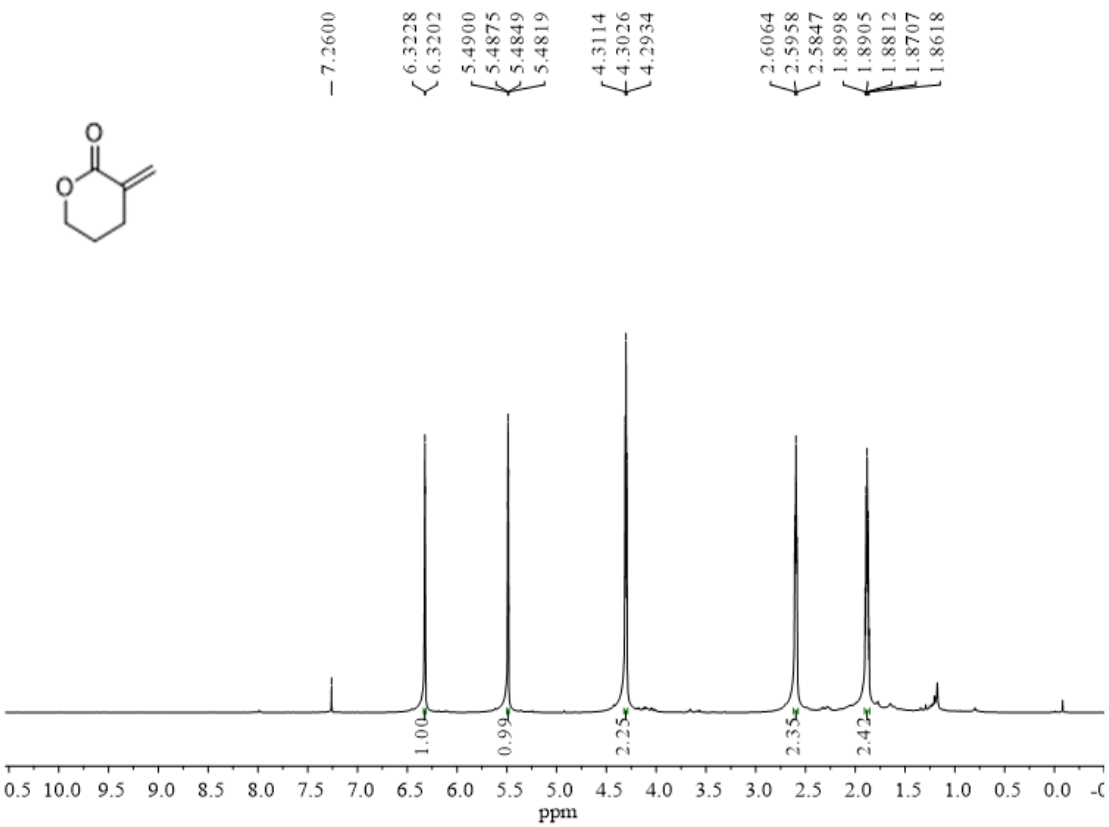


Fig. S14 ^{1}H NMR spectrum of **1-1** (600 MHz, $CDCl_3$)

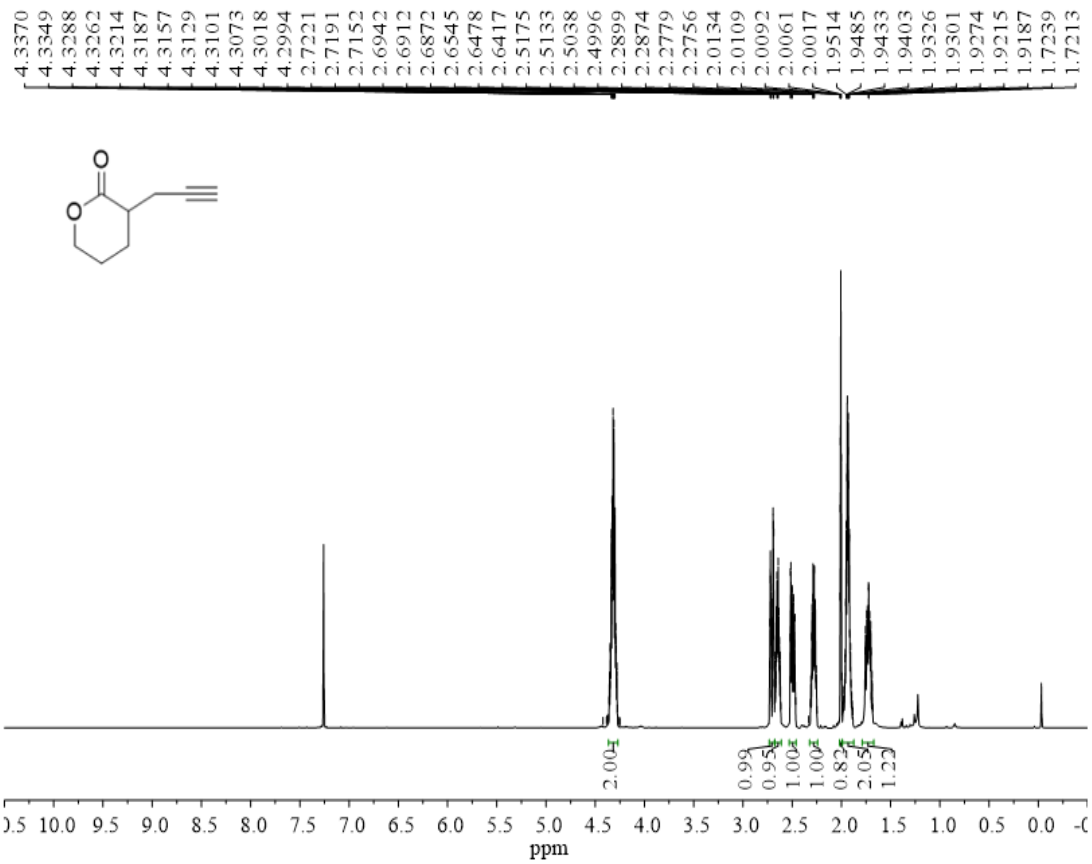


Fig. S15 ^{1}H NMR spectrum of **1-2** (600 MHz, $CDCl_3$)

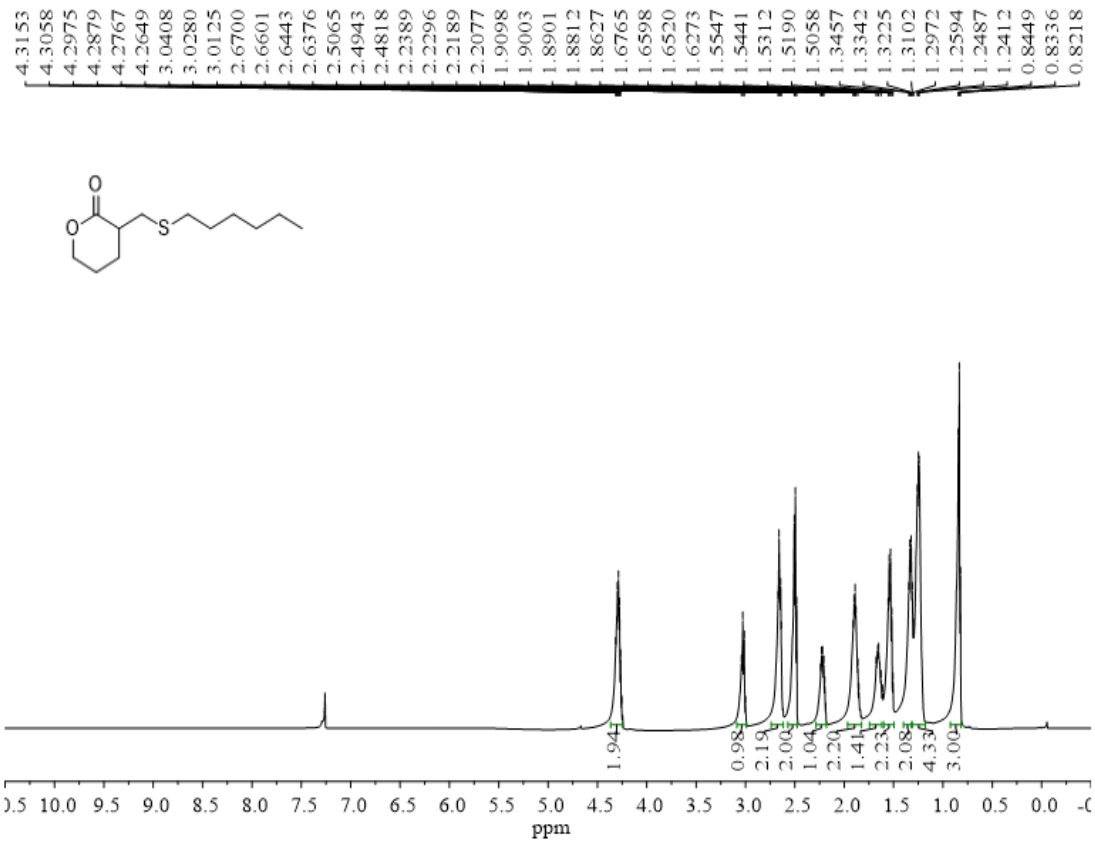


Fig. S16 ^{1}H NMR spectrum of **1-3** (600 MHz, $CDCl_3$)

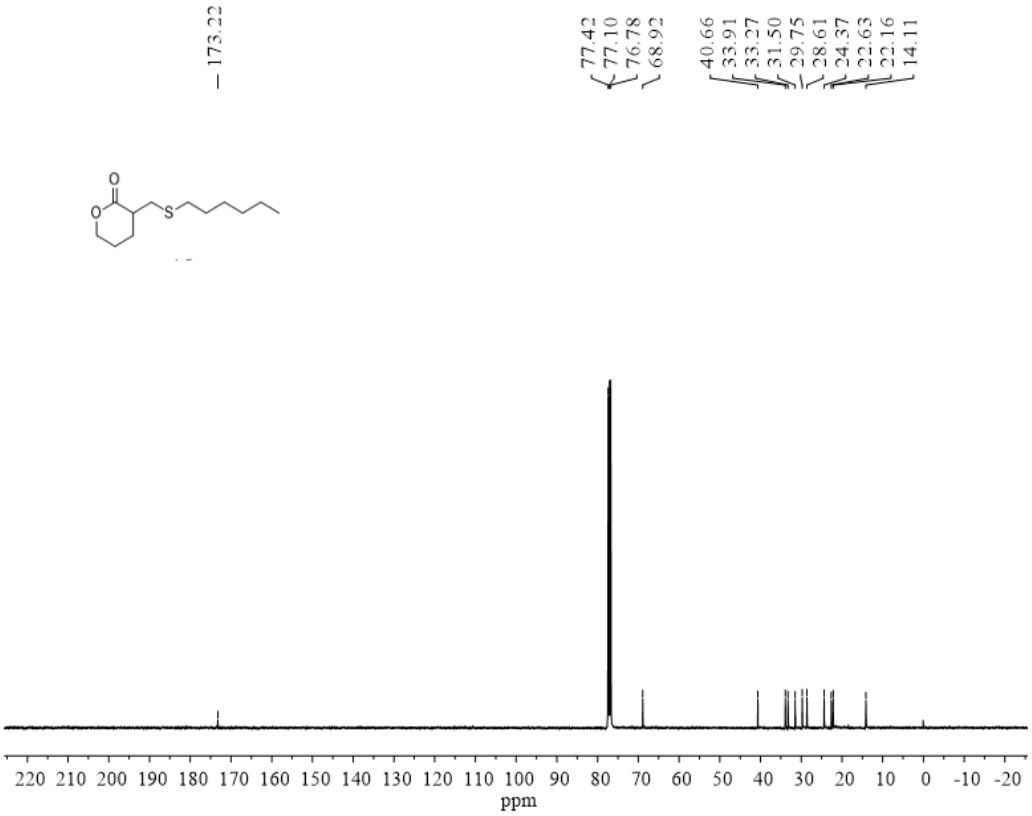


Fig. S17 ^{13}C NMR spectrum of **1-3** (101 MHz, $CDCl_3$)

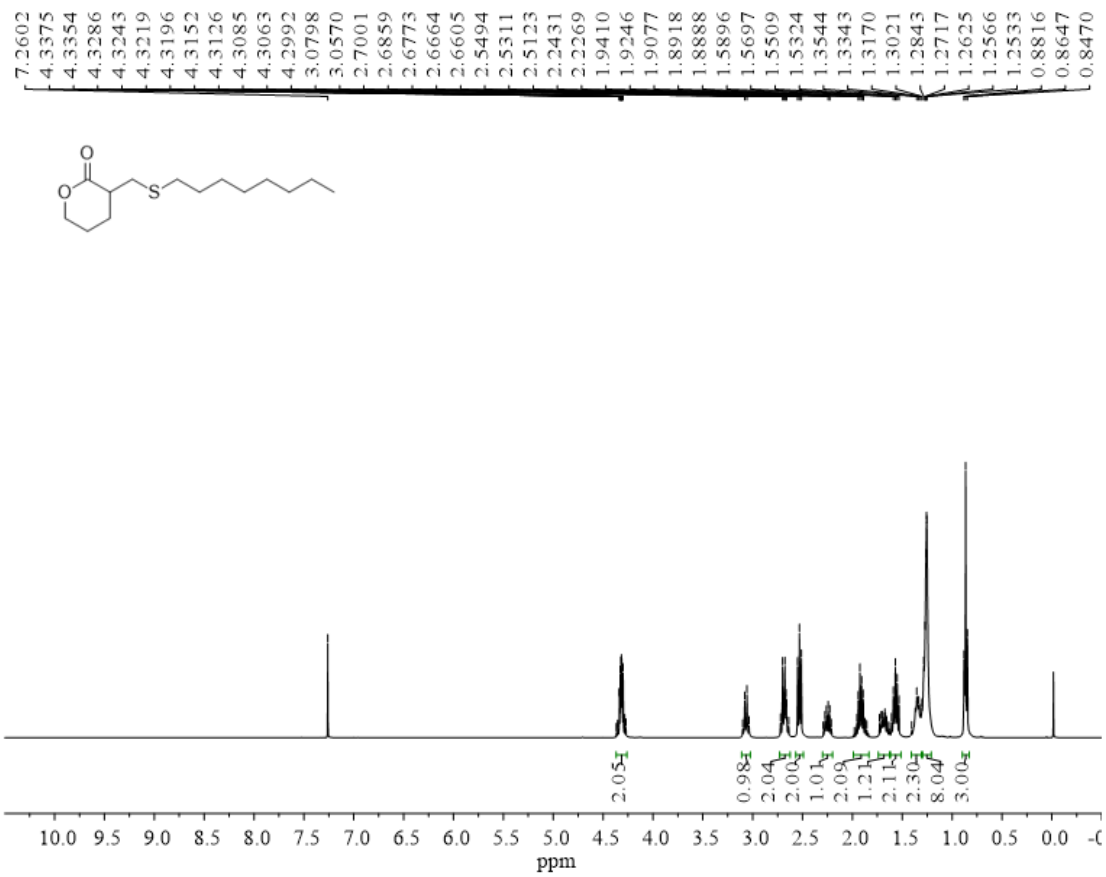


Fig. S18 ^{1}H NMR spectrum of **1-4** (400 MHz, $CDCl_3$)

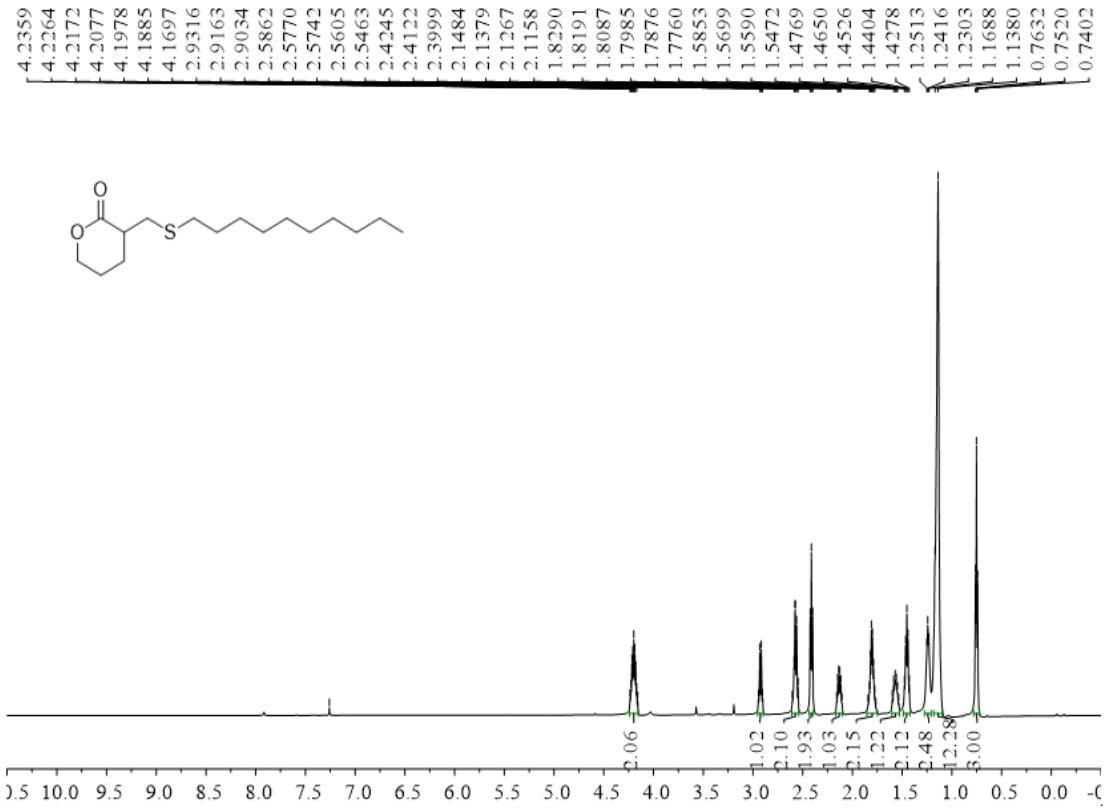


Fig. S19 ^{1}H NMR spectrum of **1-5** (600 MHz, $CDCl_3$)

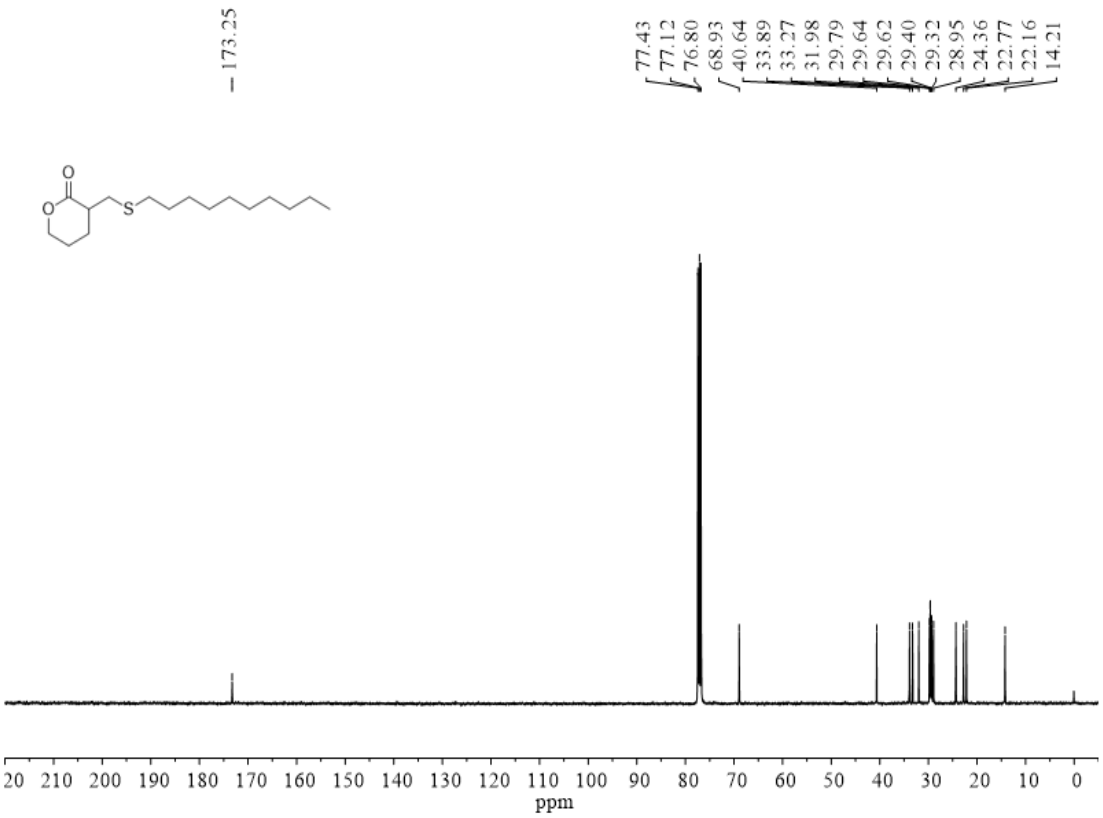


Fig. S20 ^{13}C NMR spectrum of **1-5** (101 MHz, $CDCl_3$)

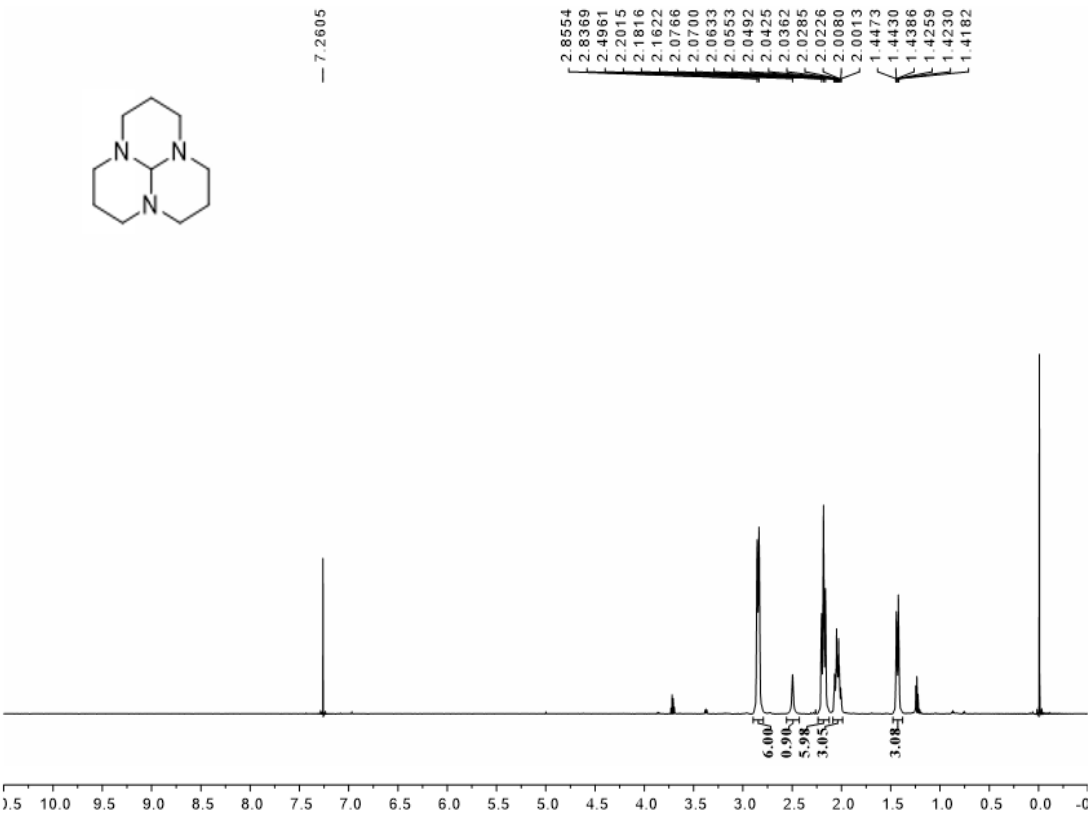


Fig. S21 ^{1}H NMR spectrum of **1-8** (600 MHz, $CDCl_3$)

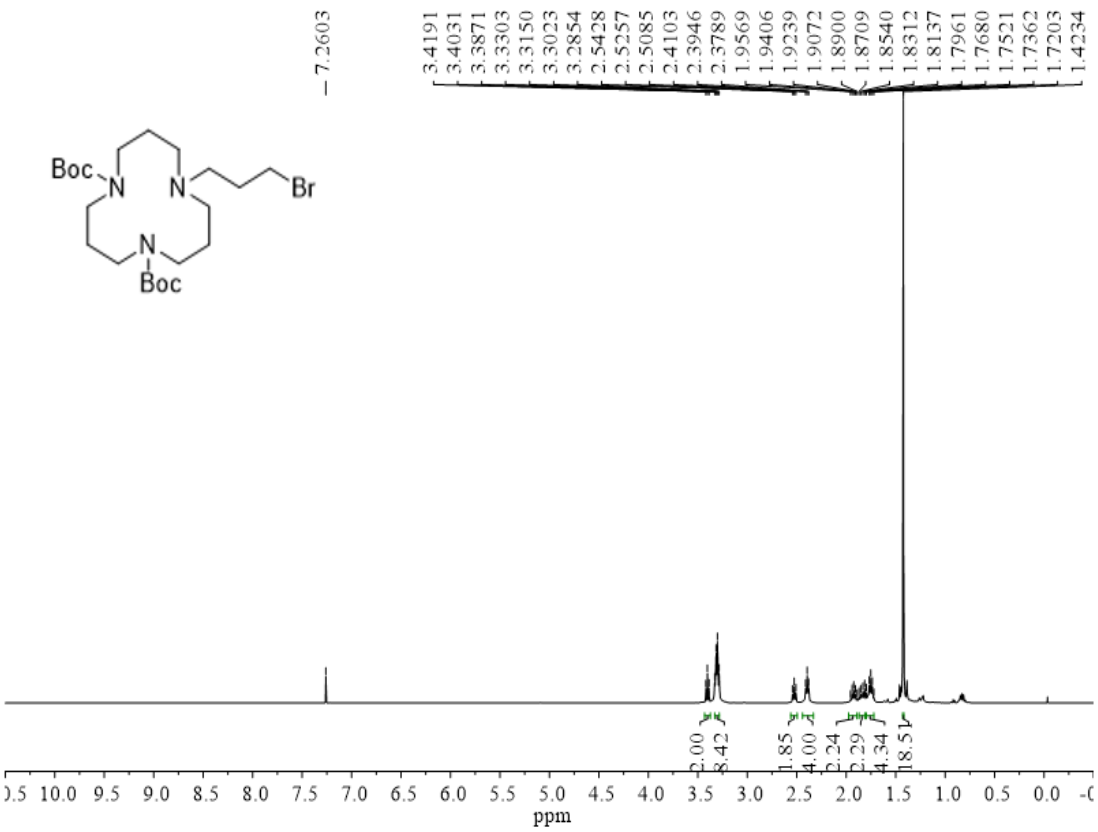


Fig. S22 ^{1}H NMR spectrum of **1-11** (400 MHz, $CDCl_3$)

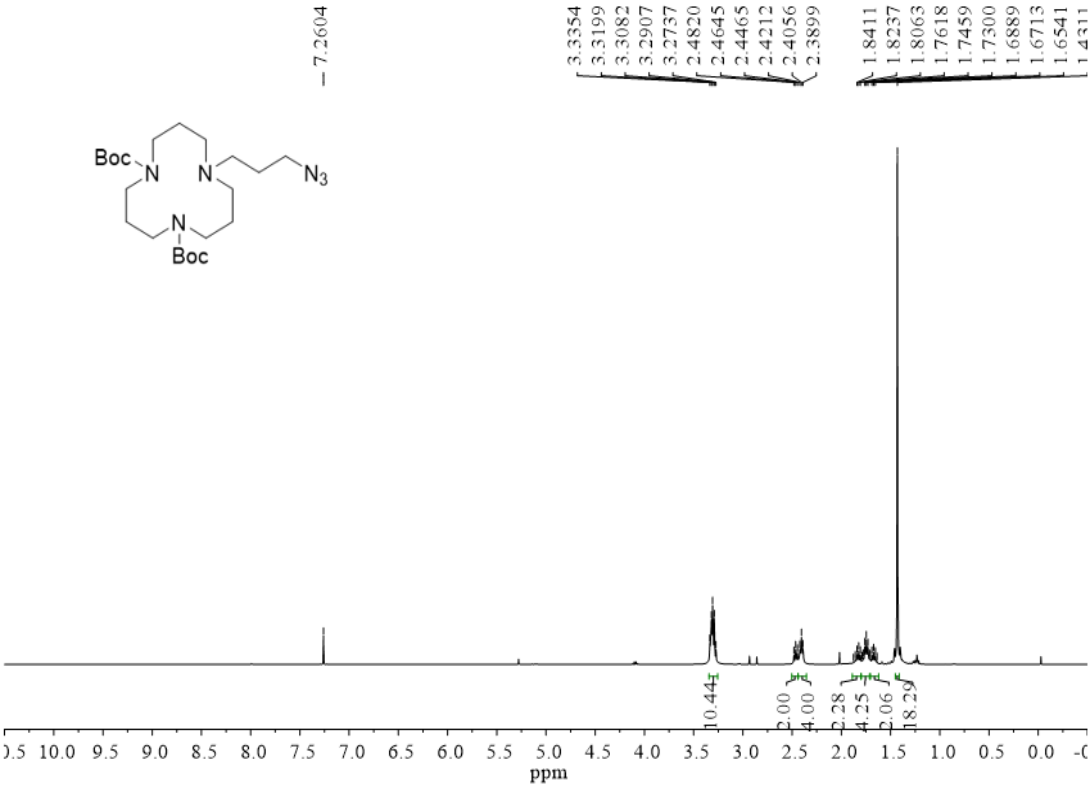


Fig. S23 ^{1}H NMR spectrum of **1-12** (400 MHz, $CDCl_3$)

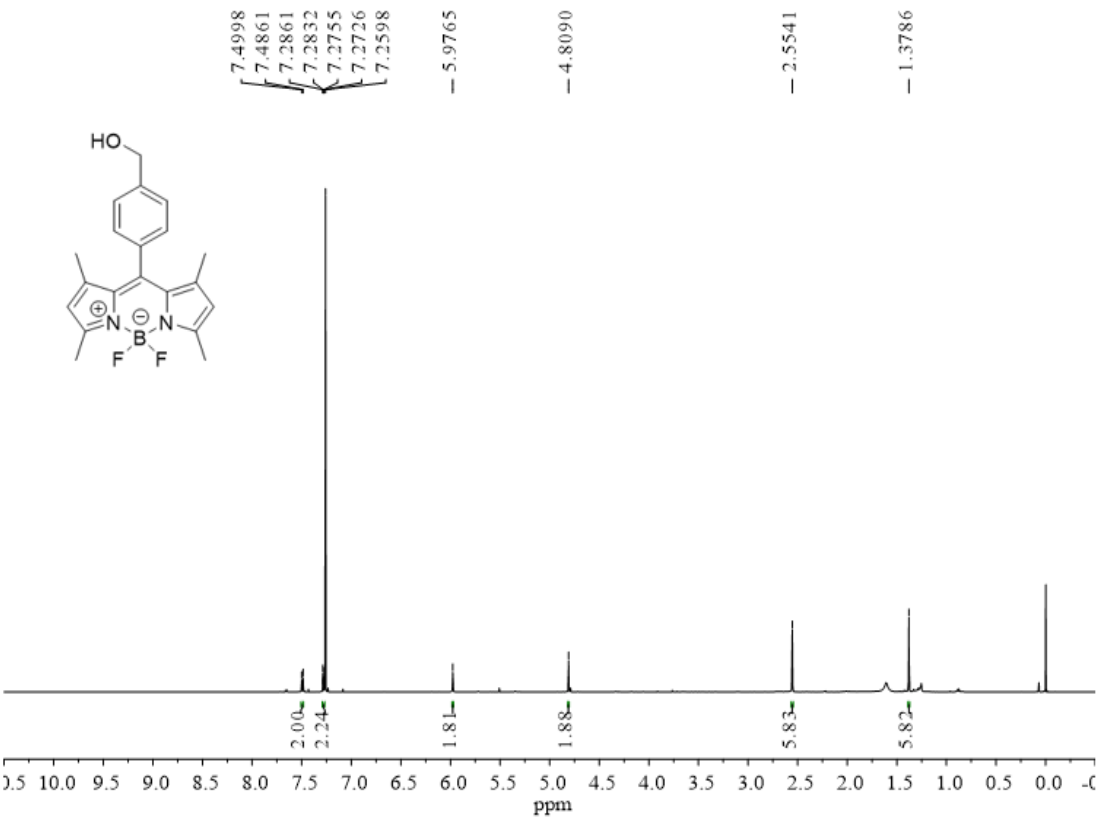


Fig. S24 ^{1}H NMR spectrum of **BODIPY-OH** (600 MHz, $CDCl_3$)

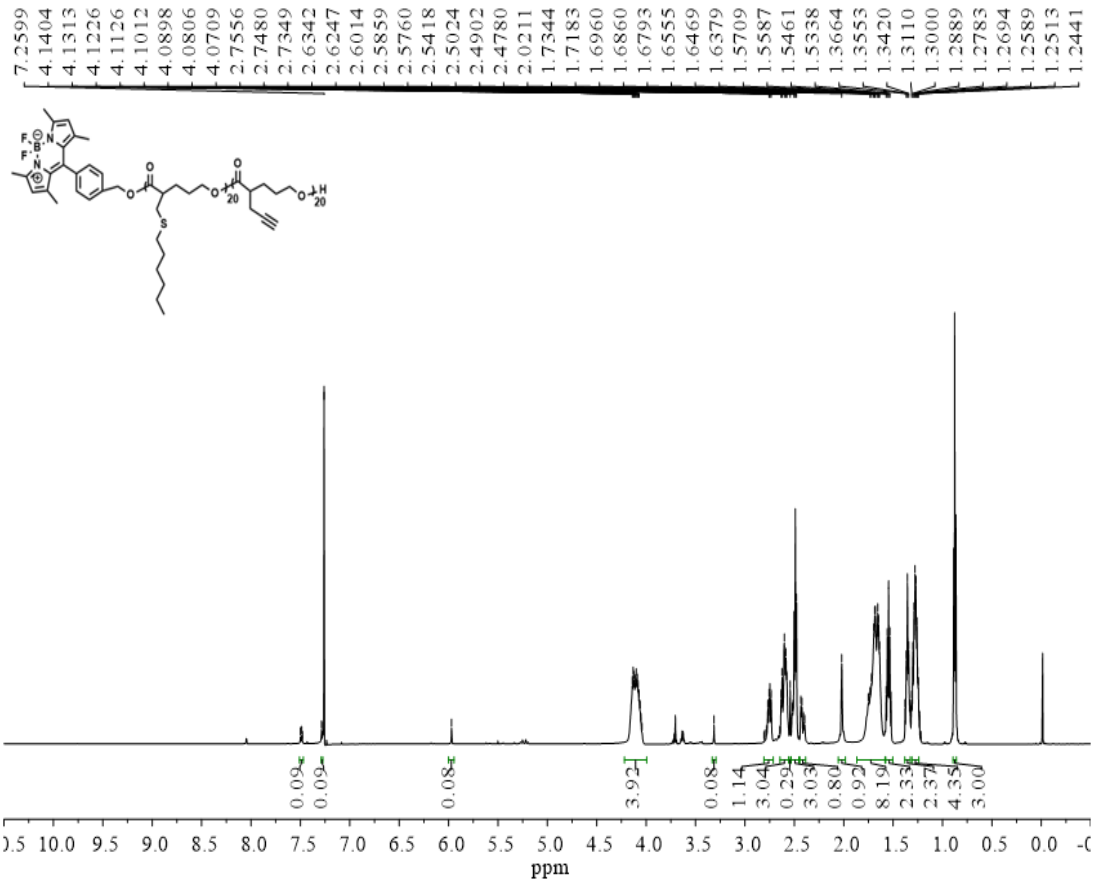


Fig. S25 ^{1}H NMR spectrum of **P2-1** (600 MHz, $CDCl_3$)

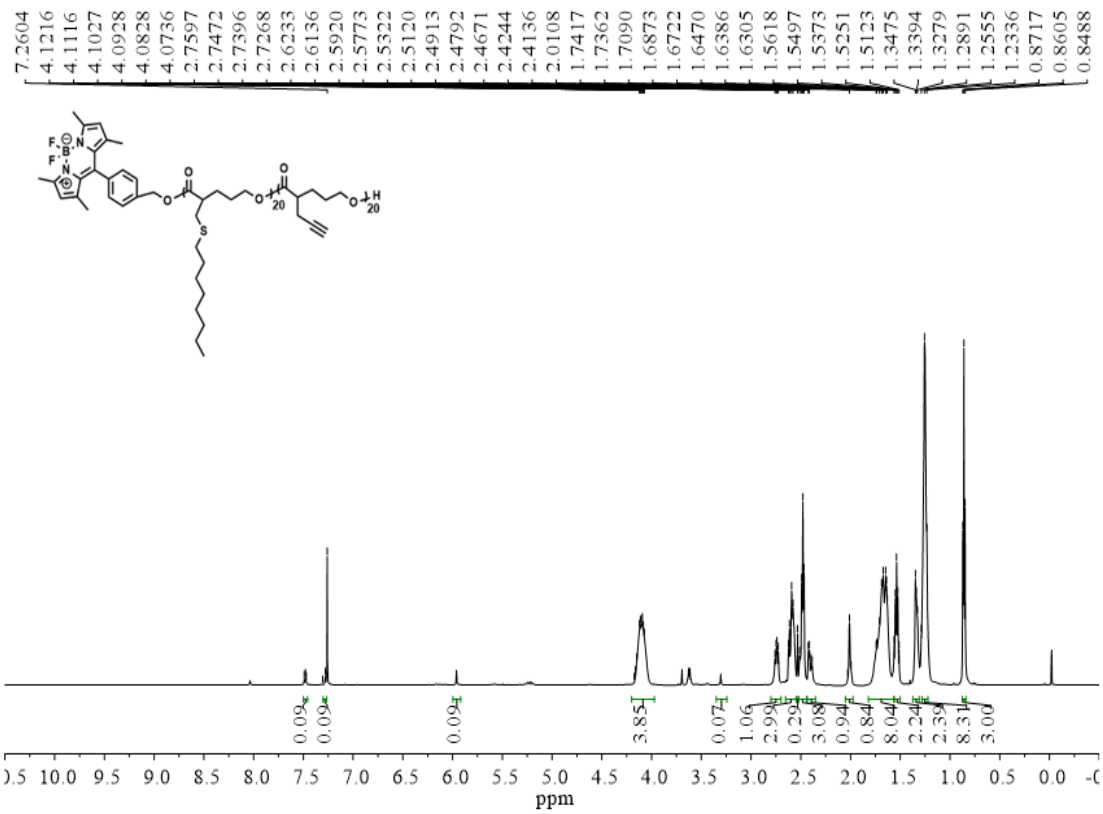


Fig. S26 ^{1}H NMR spectrum of **P2-2** (600 MHz, $CDCl_3$)

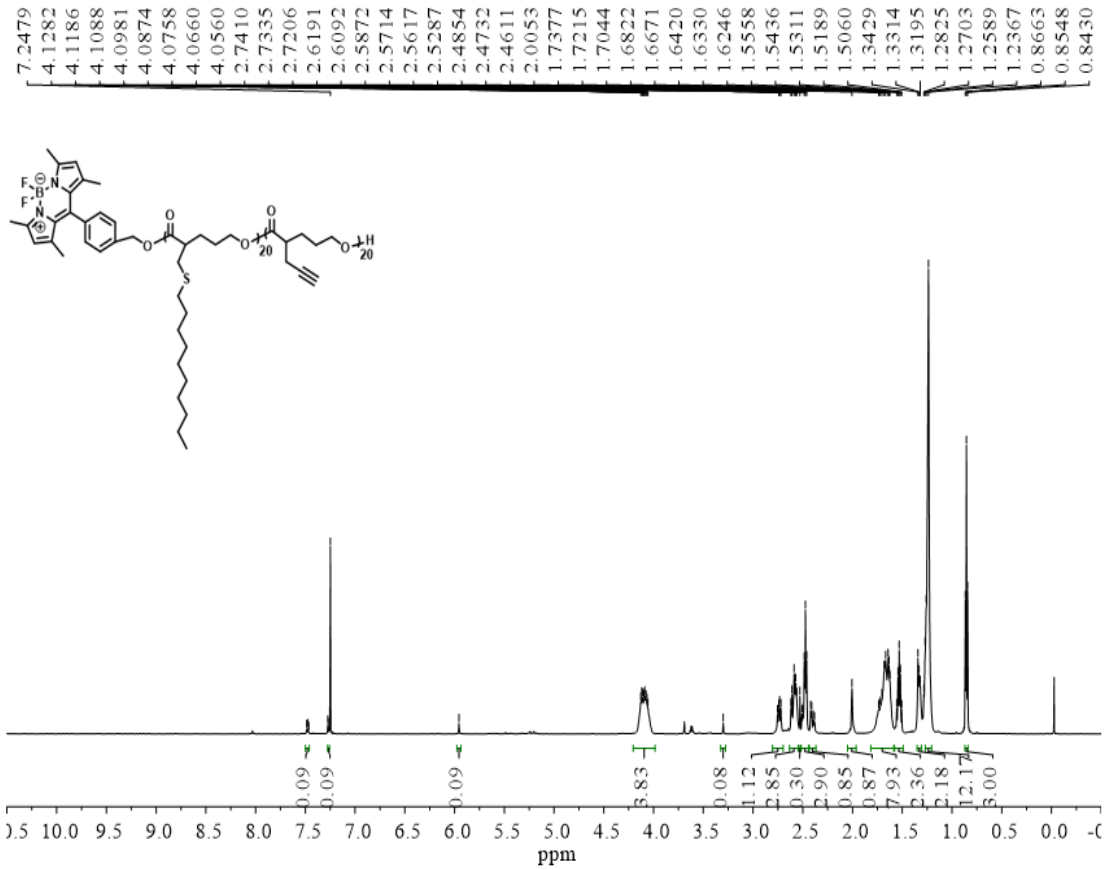


Fig. S27 ^{1}H NMR spectrum of **P2-3** (600 MHz, $CDCl_3$)

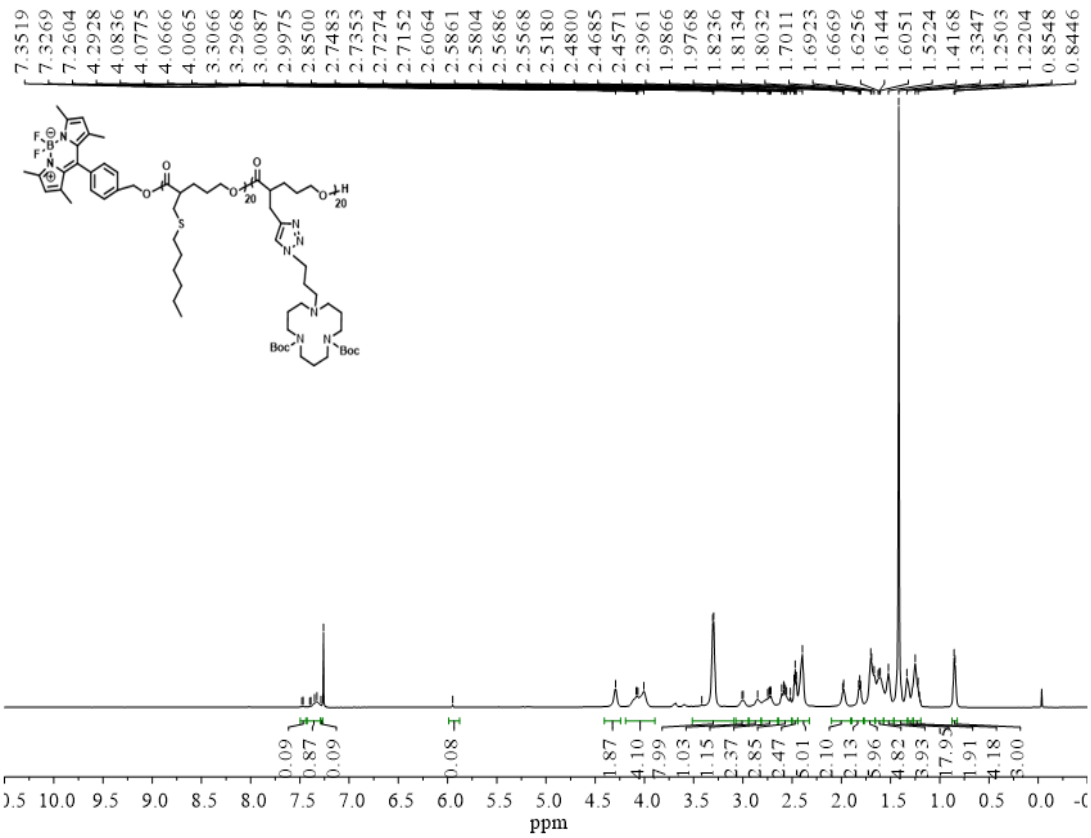


Fig. S28 ^{1}H NMR spectrum of **P3-1** (600 MHz, $CDCl_3$)

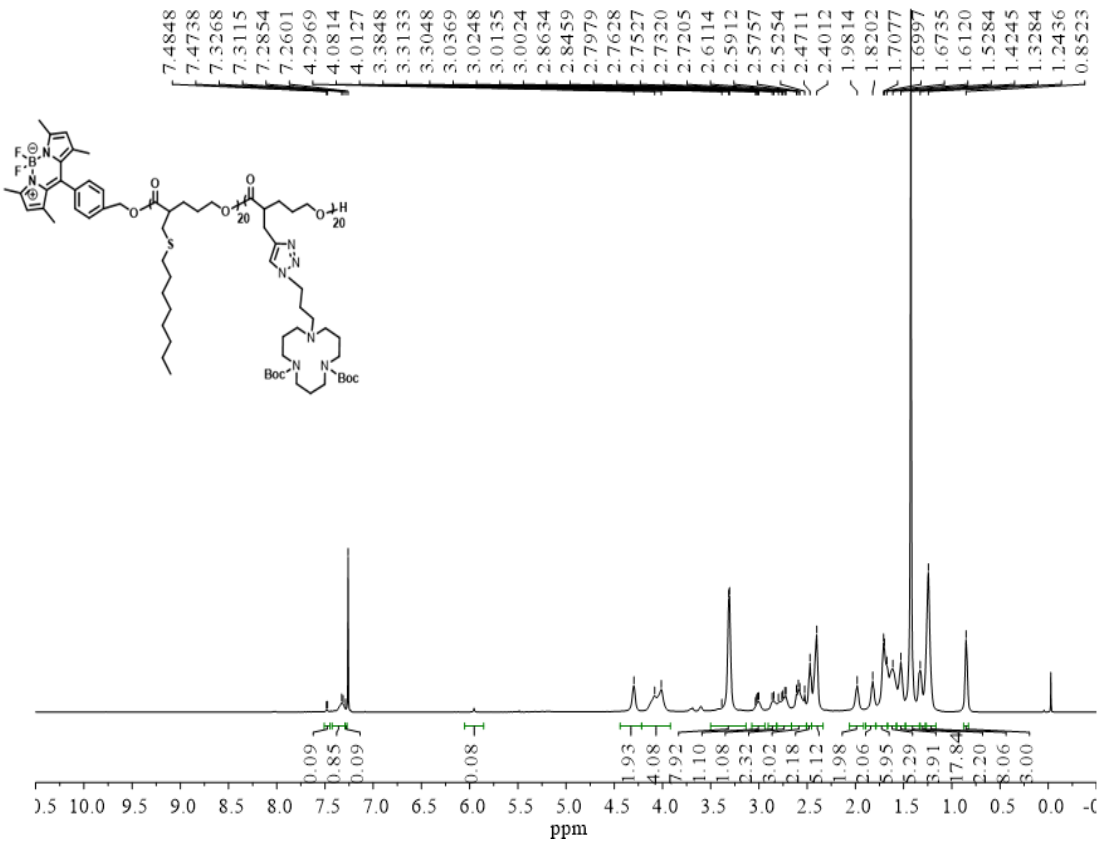


Fig. S29 ^{1}H NMR spectrum of **P3-2** (600 MHz, $CDCl_3$)

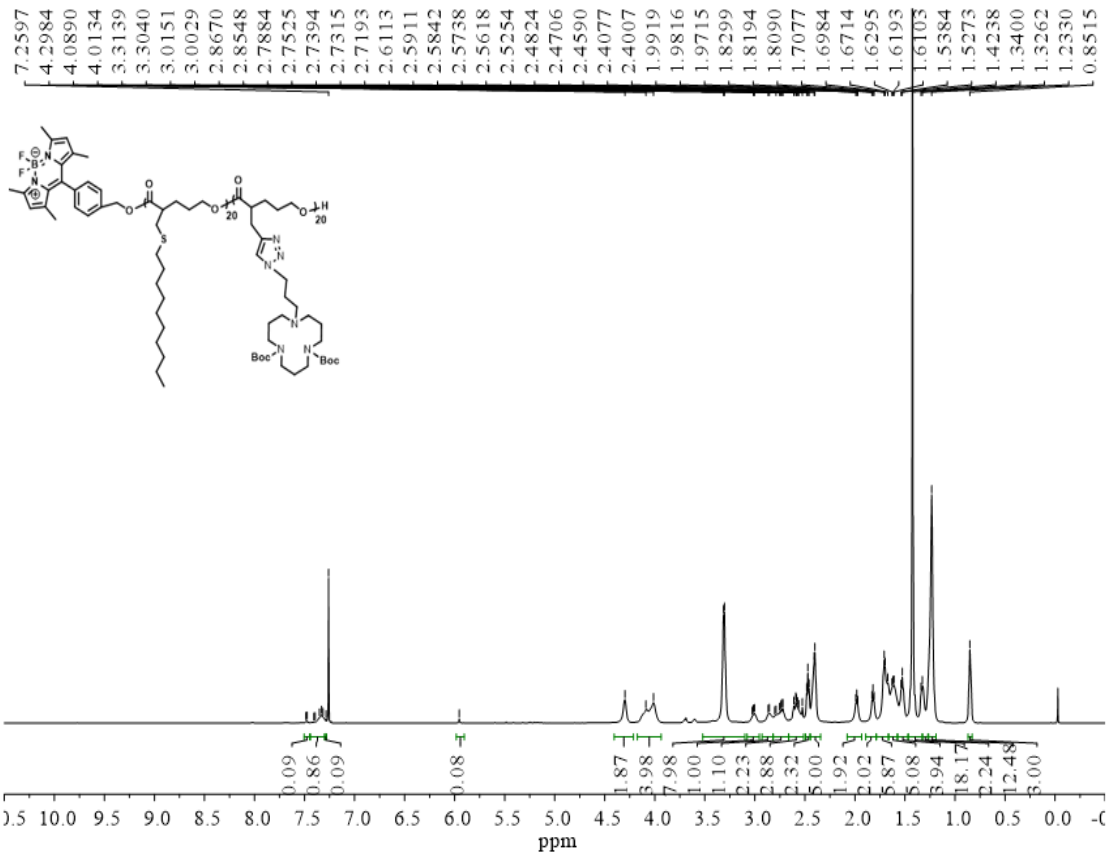


Fig. S30 ^{1}H NMR spectrum of **P3-3** (600 MHz, $CDCl_3$)

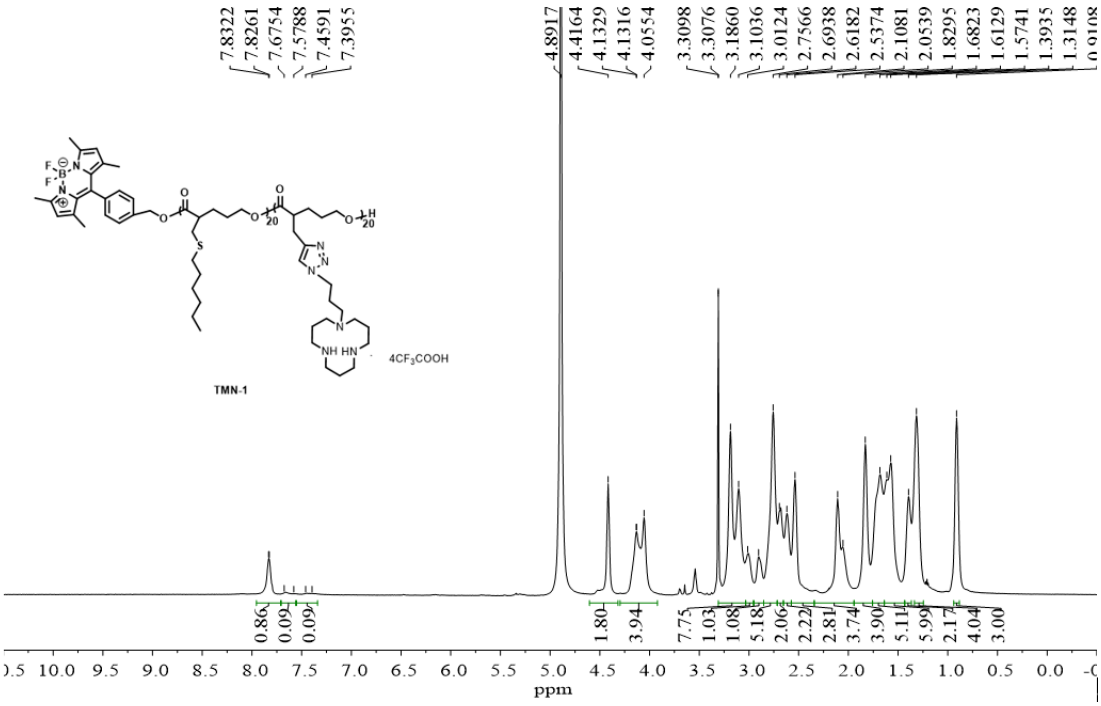


Fig. S31 ^{1}H NMR spectrum of **TMN-1** (600 MHz, CD_3OD)

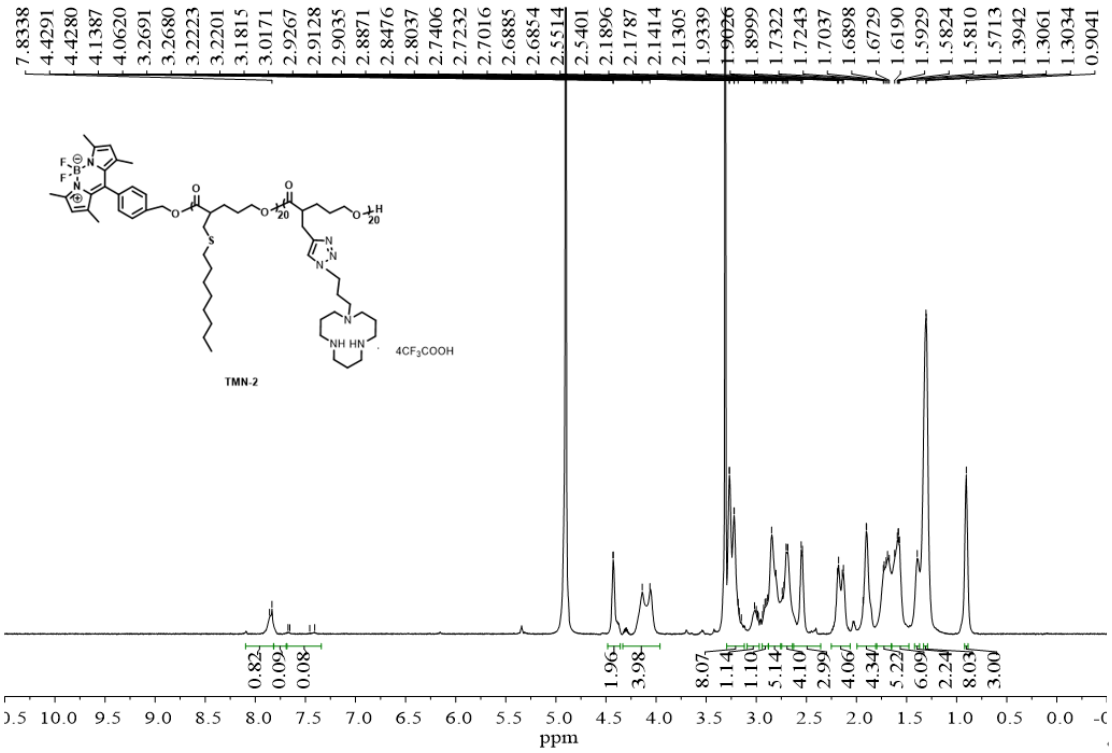


Fig. S32 ^{1}H NMR spectrum of **TMN-2** (600 MHz, CD_3OD)

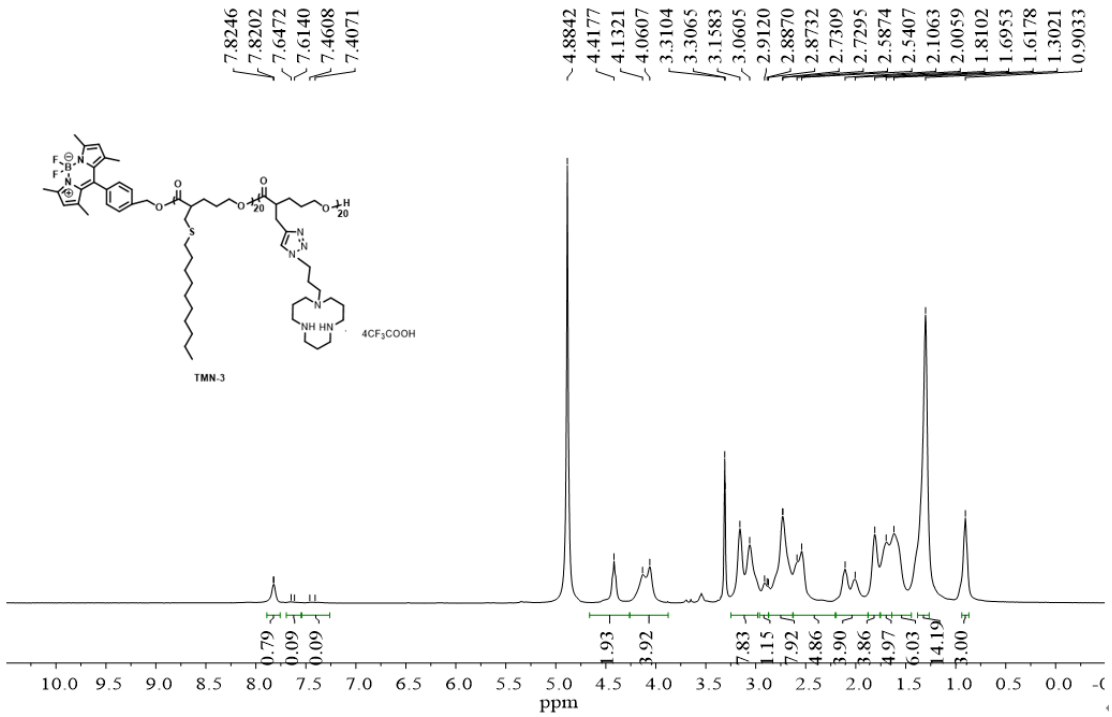


Fig. S33 ^{1}H NMR spectrum of **TMN-3** (400 MHz, CD_3OD)

VI. Reference

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