Electronic Supplementary Information

Degradable polyesters via ring-opening polymerization of functional valerolactones for efficient gene delivery

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Content

Experiment details

1.1 Agarose gel electrophoresis

Negatively pGL-3 DNA (9 μg/mL) was treated with the obtained polymers in HEPES buffer (20 mM, pH 7.4) at room temperature with a total volume of 20 μL. After incubation for 30 min, 2 μL of 6× loading buffer were added to the mixtures. The mixtures were analyzed by electrophoresis for 40 min at 85 V on a 0.7% agarose gel in 1× TAE buffer. The gel was stained with 2 μL of 5000× Goldview II and photographed on an UVP EC3 visible imaging system.

1.2 Release of the compact DNA

The disassociation of DNA from the polyexes induced by introducing heparin sodium was investigated by monitoring the change in relative fluorescence intensity obtained with the fluorescence probe EB (ethidium bromide). Polyplexes were formulated as the above method, then aliquots of stock solutions of EB and heparin sodium were transferred into the solutions (EB 20 μM, heparin sodium 1 mg/mL). The fluorescence of the mixtures at 608 nm were recorded over different durations until reaching saturation at 37 ℃.

1.3 SEM and DLS measurements

The particles of the polyplex were prepared as the above method. Size distributions were obtained on a Brookhaven Zeta Plus Partical Size and Zeta Potential Analyzer (USA). The samples for SEM measurement were prepared by adding a certain amount of the mixtures on the silica grid, dried at 40 ℃ for 6 h. SEM images were obtained using a Hitachi S-4800 (Japan) scanning electron microscope.

1.4 Cytotoxicity

The cytotoxicity of the obtained polymers toward HepG2, HeLa, Hek293T and A549 cell lines was tested by MTT assays (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with fetal bovine serum (FBS, 10%, v/v) in a humid atmosphere containing 5% CO_2 at 37 ℃. After 48 h of incubation in the medium, the cells were seeded in 96-well plate at 5000 cells and 100 μL medium per well and cultured for another 24 h. Then the cells were treated with different amounts of polymers in 100 μL DMEM, 10 μL DMEM and 10% FBS was added to each well 4 h later. The cells were further cultured for 20 h. Then the medium was removed, 20 μL of MTT (5 mg/mL) were added to the wells, and the cells were incubated for another 4 h. Finally MTT was replaced with 200 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 absorbance of the purple formazan was recorded at 490 nm using a Thermo Scientific Multiskan GO. The relative viability of the cells was calculated based on the data of five parallel tests by using the following formula (1):

$$\text{Cell viability} = \frac{OD_{490}(Sample) - OD_{490}(Blank)}{OD_{490}(Control) - OD_{490}(Blank)} \times 100\% \qquad (1)$$

Where OD_{490} (Sample) represents the optical density of the well treated with various concentrations of the compounds, OD_{490} (Control) represents that of the wells treated with DMEM +10% FBS and OD_{490} (Blank) represents that of blank wells treated with pure DMSO. The final reported percent cell survival values are relative to the untreated control cells.

1.5 Cell transfection

To visually examine the expression of the internalized DNA (pEGFP-N1), Hek293T cells were transfected by the polymers at the optimal weight ratios. Cells were seeded in Glass Bottom Cell

Culture Dishes at 80000 cells per dish and cultured until 80% cell confluence at 37 ℃ in a humid atmosphere containing 5% CO_2. Before transfection, the medium was washed three times with DMEM, and treated with freshly prepared polyplexes and the controls (500 μL). After 4 h under standard culture conditions, the medium was replaced with 500 μL of fresh DMEM medium containing 10% FBS and cultured for another 24 h. Then, the cells were washed with PBS for 3 times, and observed under Zeiss Inverted Fluorescence Microscope with a 10× objective to examine the expression of the intracellular EGFP.

Quantitative gene transfection of the polymers condensed pGL-3 DNAs (9 μg/mL) were investigated in HepG2, A549, HeLa, and HEK293T cells. Cells were seeded in 24-well plates (80000 cells/well) and grown to reach until 80% cell confluence at 37 ℃ for 24 h in 5% CO_2. Before transfection, the medium was replaced with a serum-free DMEM culture medium containing the condensates at various concentrations. After 4 h under the standard incubation conditions, the medium was replaced with fresh medium containing 10% FBS and incubated for another 36 h. For a typical assay in a 24-well plate, 24 h post transfection as described above, cells were washed with cold PBS and lysed with 100 μL 1× Lysis reporter buffer (Promega). The luciferase activity was measured by micro plate reader (TECAN F200, Austria). Protein content of the lysed cell was determined by BCA protein assay. Gene transfection efficiency was expressed as the relative fluorescence intensity per mg protein (RLU/mg protein). All the experiments were done in triplicates to calculate the standard deviations.

1.6 Cellular uptake studies

The cellular uptakes of FITC-labeled DNA (9 μg/mL) condensates were observed by fluorescence microscopy. Hek293T cells were cultured in DMEM medium supplemented with 10% FBS in a humid atmosphere containing 5% CO_2 at 37 ℃. The cells were seeded in Glass Bottom Cell Culture Dishes at 1000 cells per dish and cultured for 24 h. After washing three times with DMEM, the cells were treated with freshly prepared polyplexes and the controls (500 μL). After incubation for 4 h, the cells were washed for 10 times with PBS (0.5 mL), the CLSM images were obtained.

Table S1. The conversion between the mass ratio (w/w) and N/P ratio.

Entry	w/w	N/P
B1	5:1	1.10
B2	4:1	0.59
B3	4:1	0.59
B4	3:1	0.50
B5	5:1	0.68
B6	4:1	0.59
C1	5:1	1.06
C2	5:1	0.92
C3	6:1	0.89
C4	5:1	0.83
P1	2:1	0.88
P2	–	–

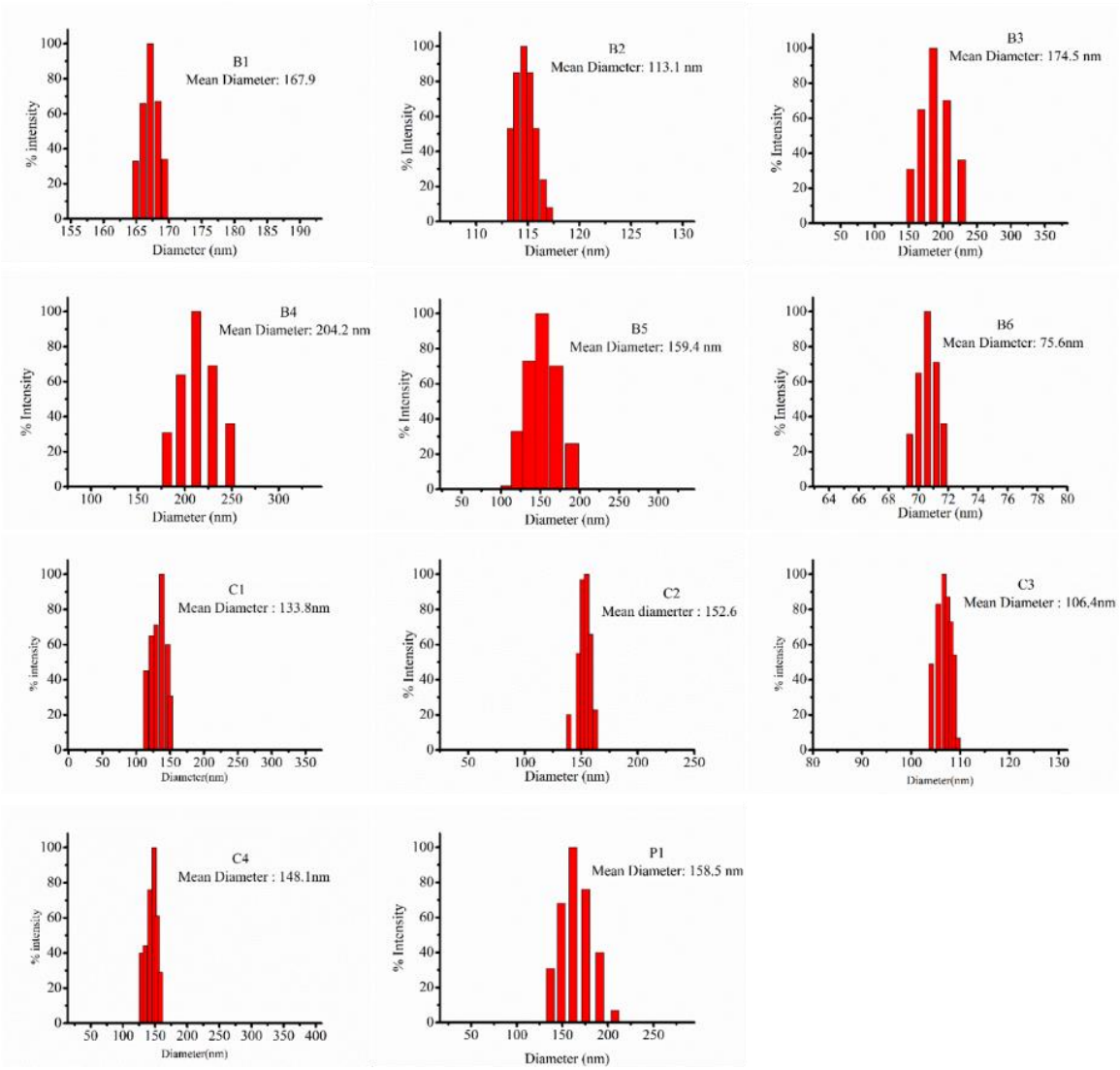

Fig. S1 Particle size distributions of polymer/DNA polyplexes at the optimal weight w/w ratios (mean ± SD, n = 3).

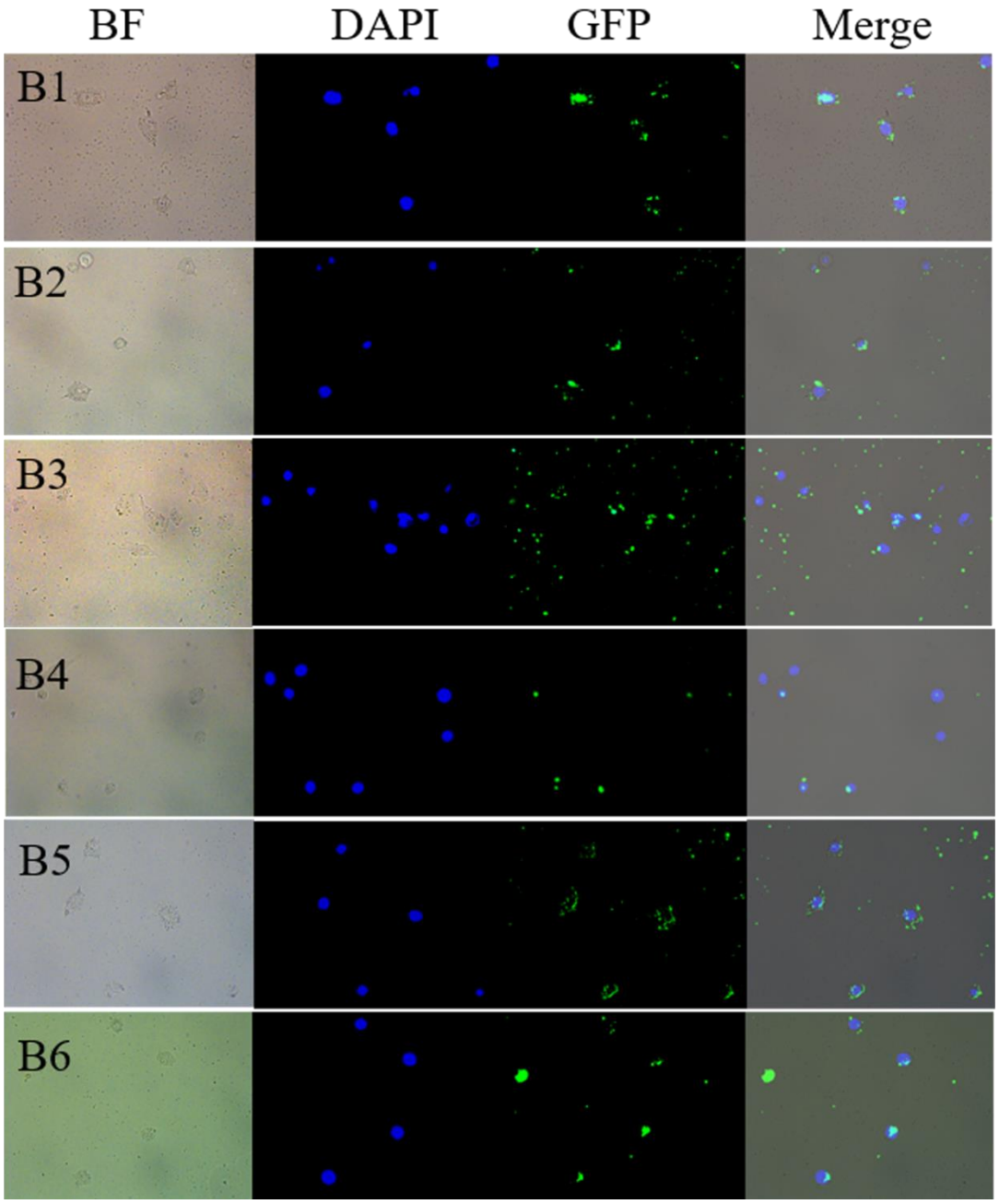

Fig. S2 Fluorescence images of Hek293T cells after 4 h co-incubation with block copolymer/DNA complexes prepared at the optimal transfection w/w ratios.

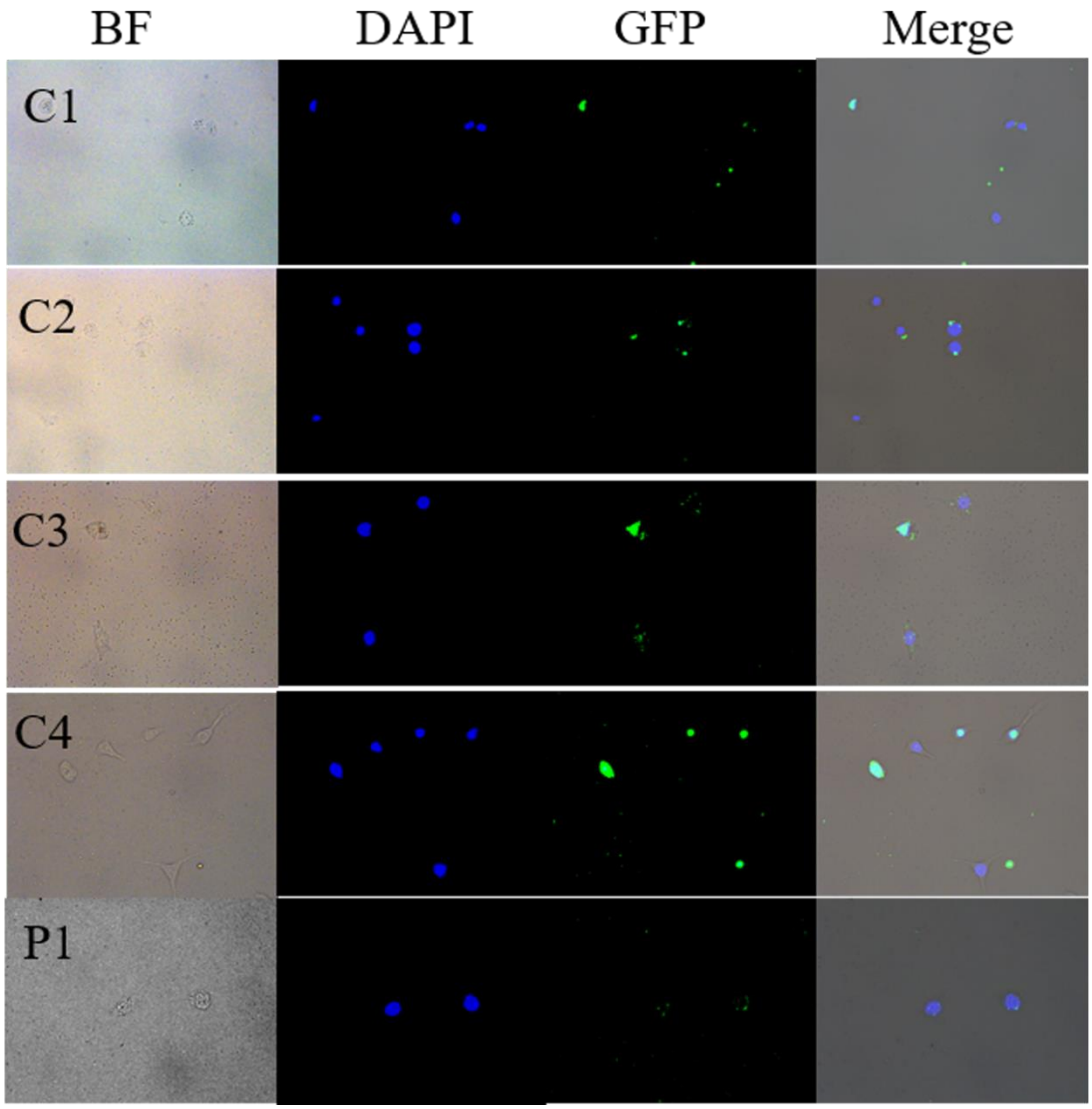

Fig. S3 Fluorescence images of Hek293T cells after 4 h co-incubation with random copolymer/DNA and **P1**/DNA complexes prepared at the optimal transfection w/w ratios.

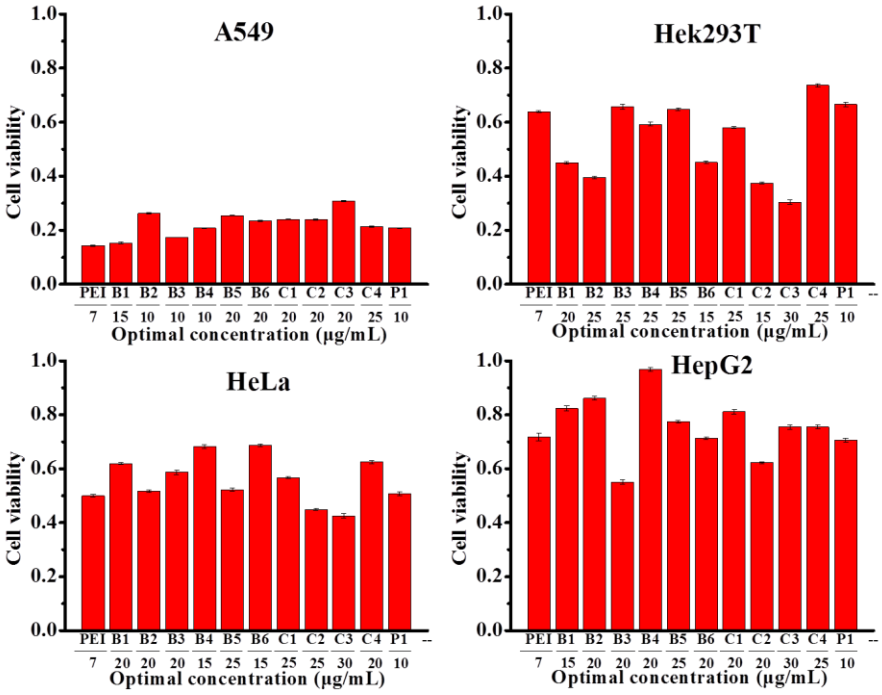

Fig. S4 Cytotoxicity of A549, Hek293T, HeLa and HepG2 cells under the optimum concentrations of the obtained polyesters and PEI 25k for gene transfections. Data represent mean ± SD (n = 3).

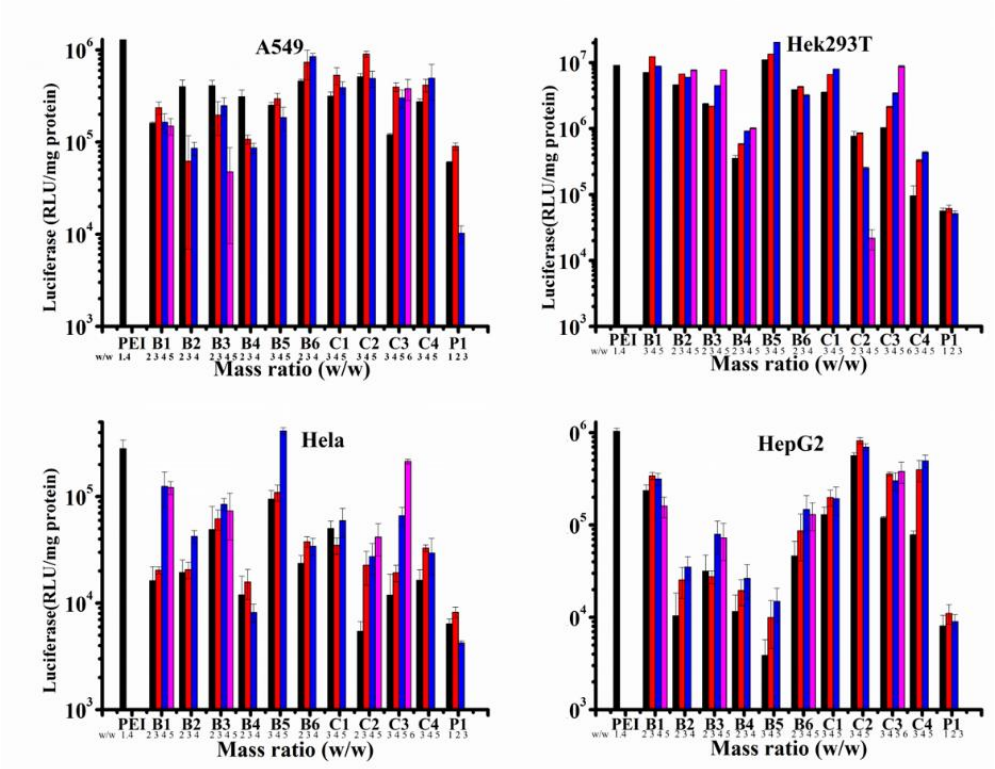

Fig. S5 Luciferase gene expressions transfected by polymer/DNA polyplexes at different weight ratios in A549, Hek293T, HeLa and HepG2 cell lines. PEI (w/w = 1.4, N/P = 10) was used as a control. Data represent mean ± SD (n = 3).

Table S2. The optimal weight ratios of polymers/pDNA for the transfections in four cell lines.

	Optimal ratio in A549 cell	Optimal ratio in Hek293T cell	Optimal ratio in Hela cell	Optimal ratio in HepG2 cell
B1	3	4	4	3
B2	2	5	4	4
B3	2	5	4	4
B4	2	5	3	4
B5	4	5	5	5
B6	4	3	3	4
C1	4	5	5	4
C2	4	3	5	4
C3	4	6	6	6
C4	5	5	4	5
P1	2	2	2	2

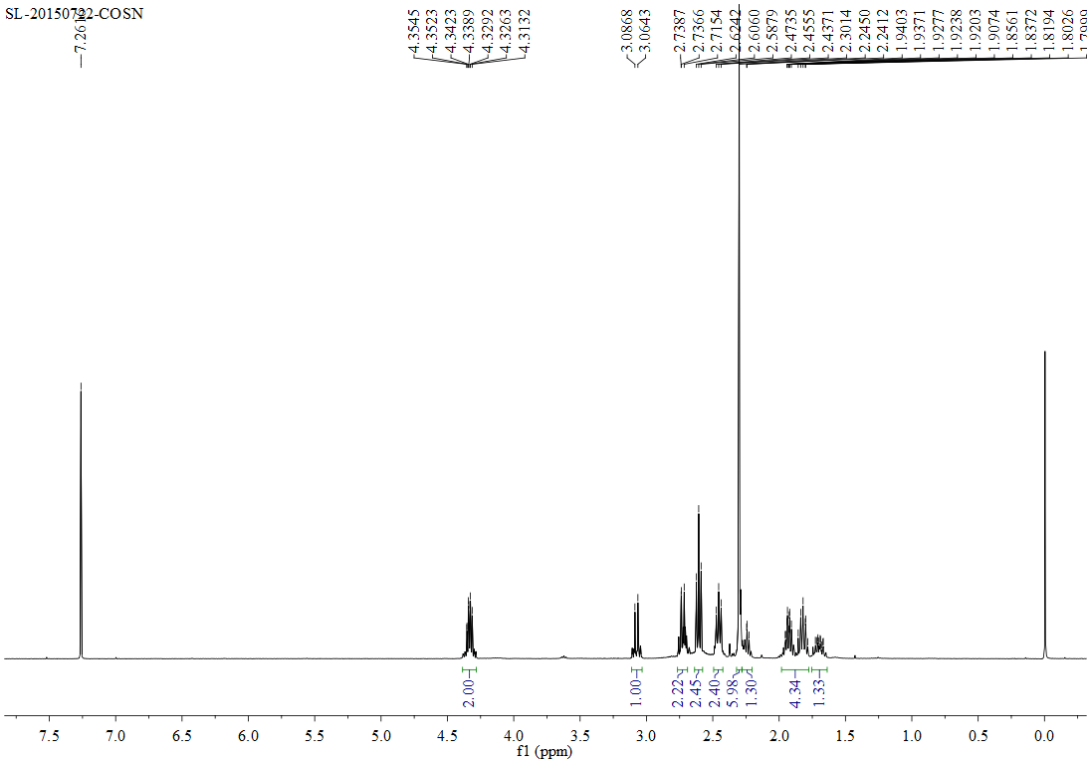

Fig. S6 ^{1}H NMR of compound **1** in $CDCl_3$.

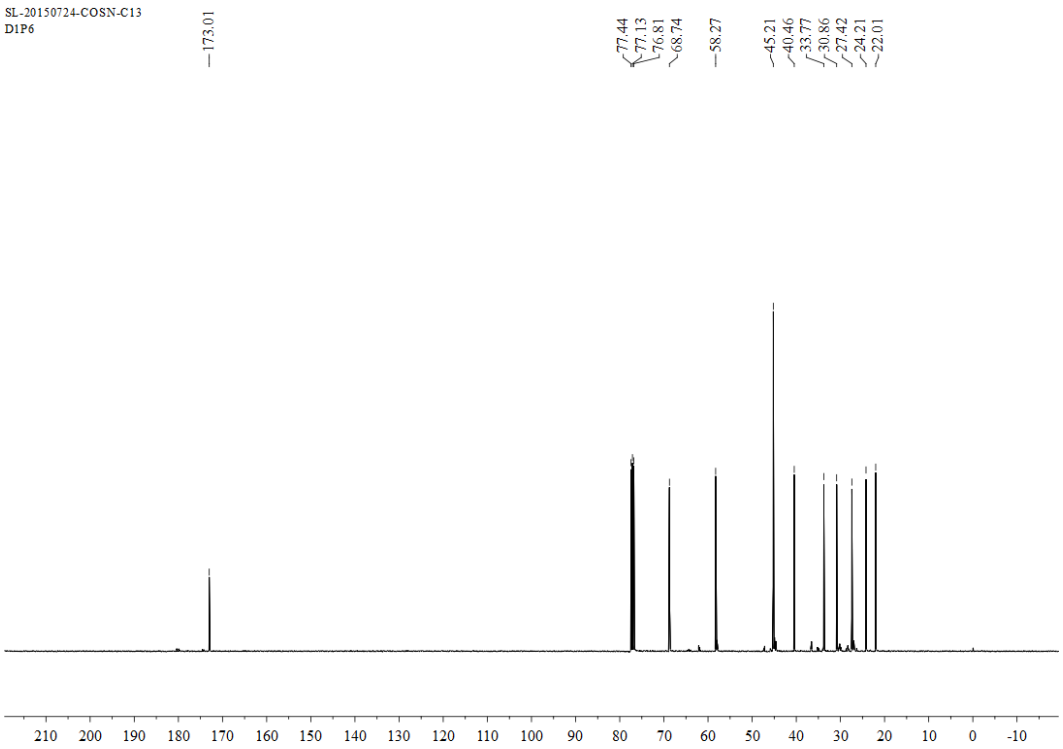

Fig. S7 ^{13}C NMR of compound **1** in $CDCl_3$.

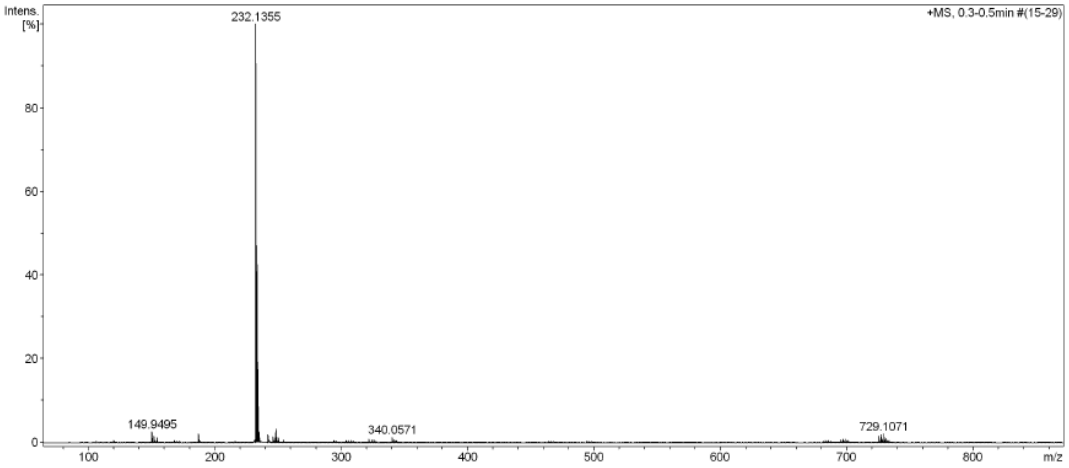

Fig. S8 ESI-MS spectrum of compound **1**.

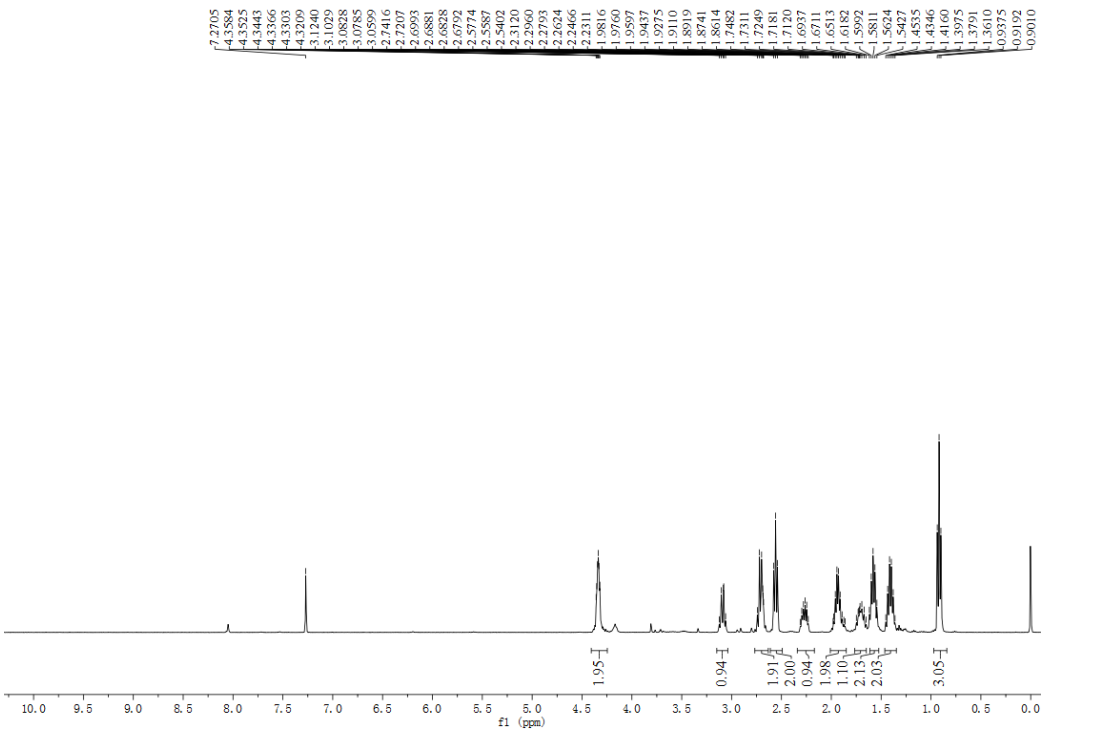

Fig. S9 ^{1}H NMR of **2** in $CDCl_3$

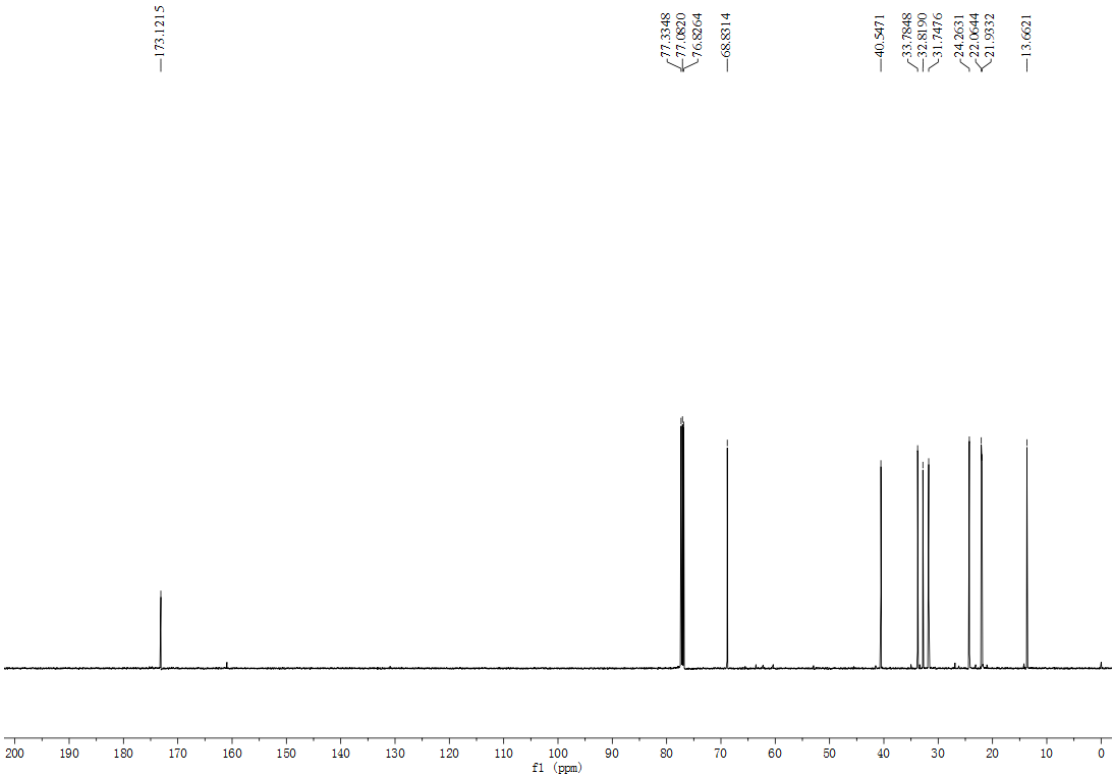

Fig. S10 ^{13}C NMR of **2** in $CDCl_3$

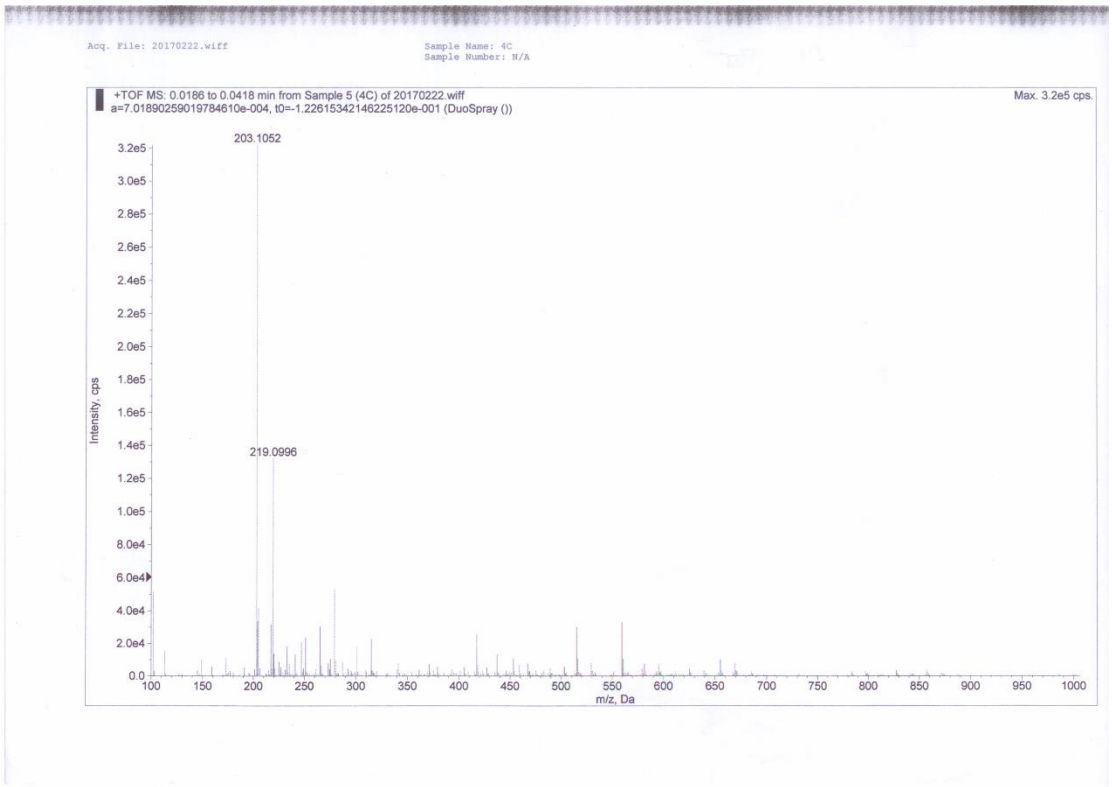

Fig. S11 ESI-MS spectrum of **2.**

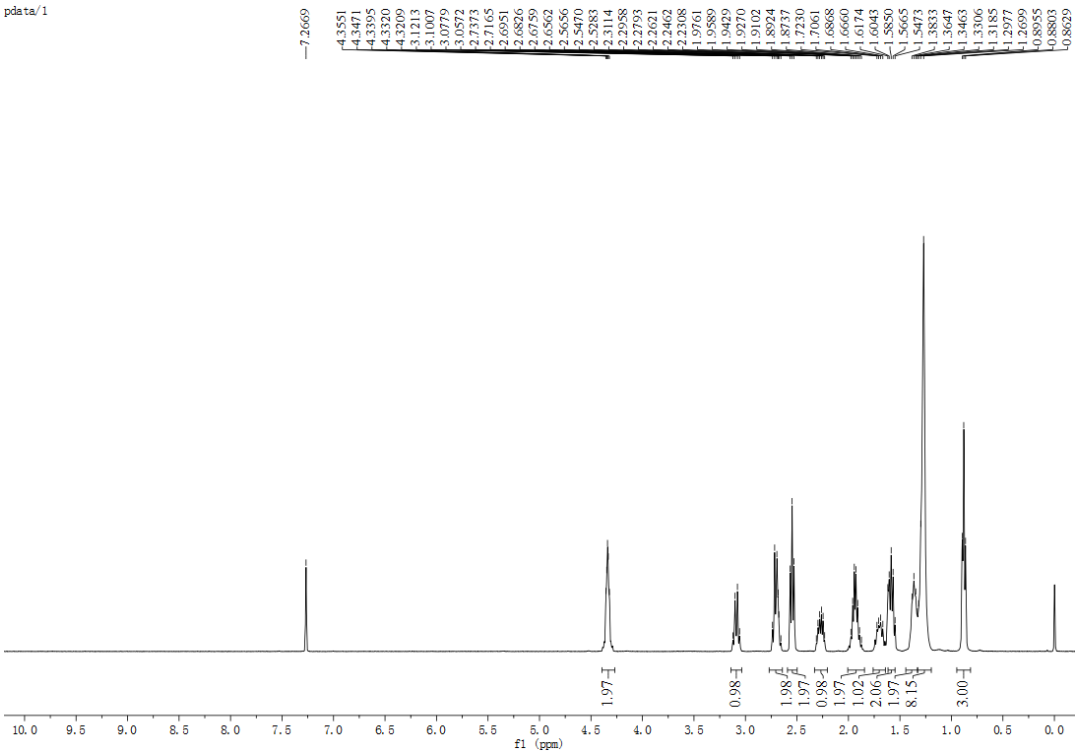

Fig. S12 ^{1}H NMR of **3** in $CDCl_3$

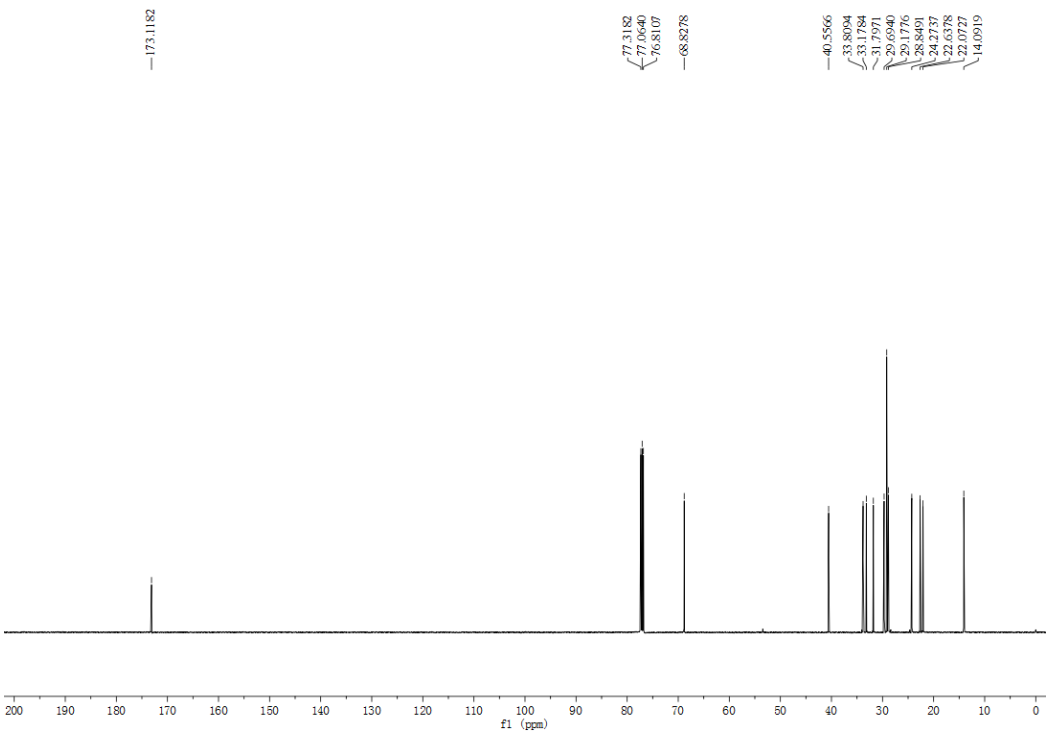

Fig. S13 ^{13}C NMR of **3** in $CDCl_3$

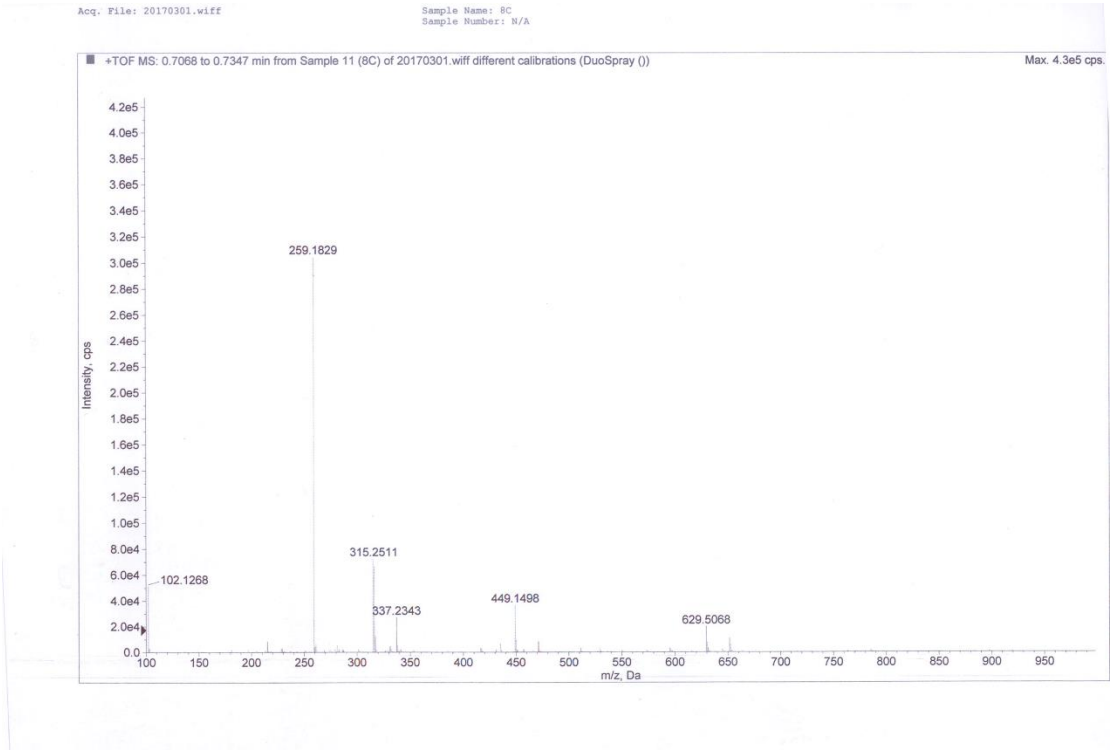

Fig. S14 ESI-MS spectrum of **3**.

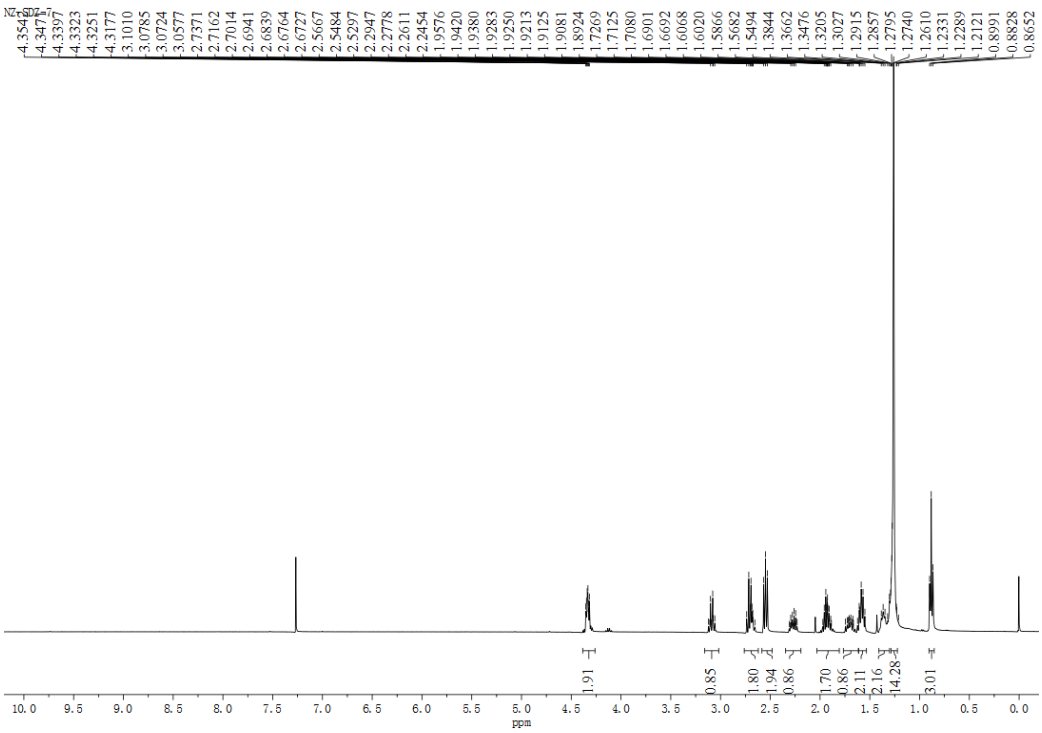

Fig. S15 ^{1}H NMR of **4** in $CDCl_3$

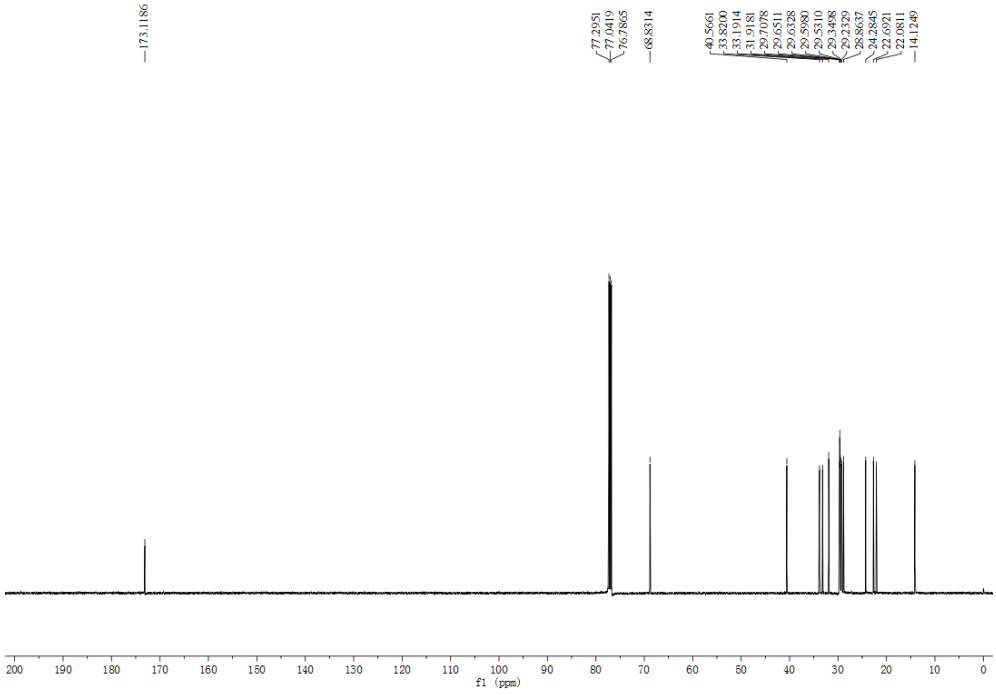

Fig. S16 ^{13}C NMR of **4** in $CDCl_3$

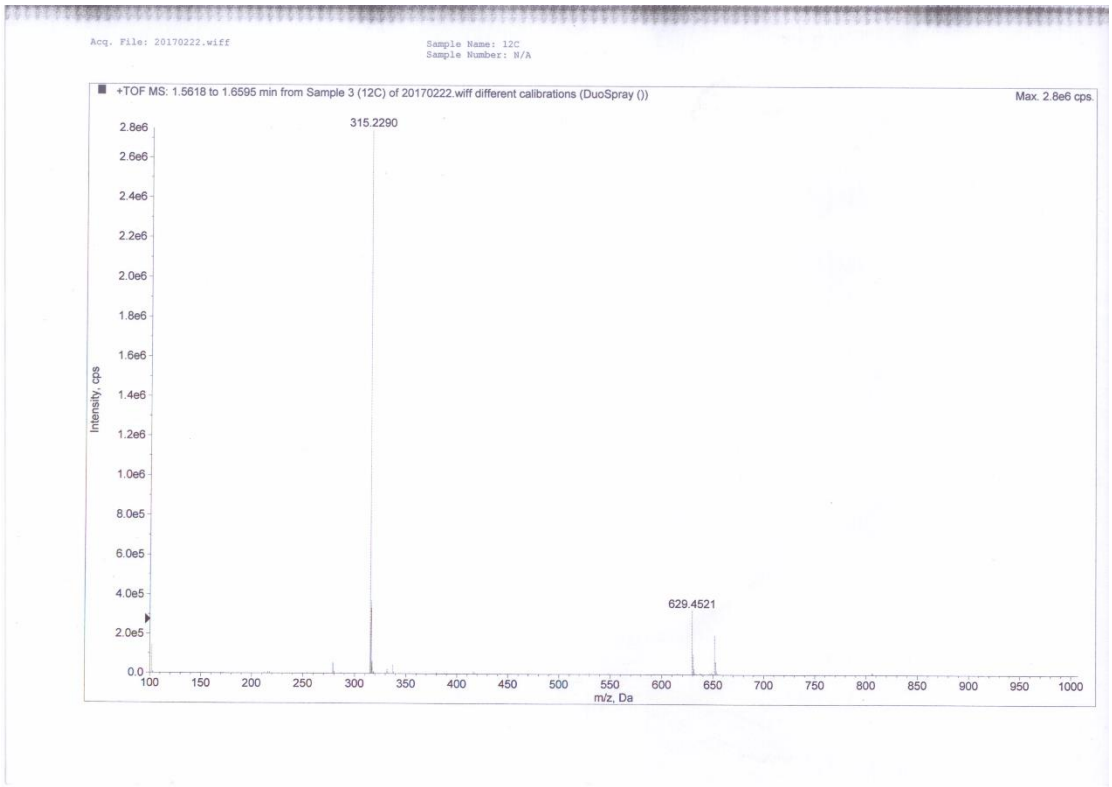

Fig. S17 ESI-MS spectrum of **4**.

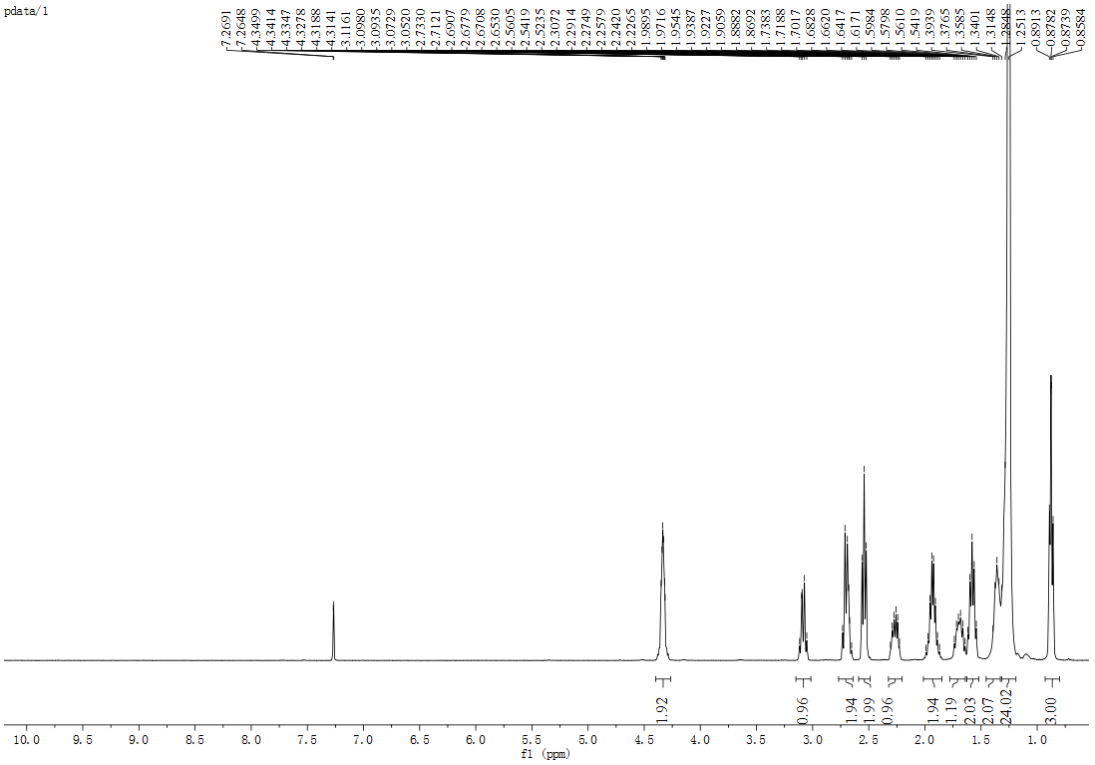

Fig. S18 ^{1}H NMR of **5** in $CDCl_3$

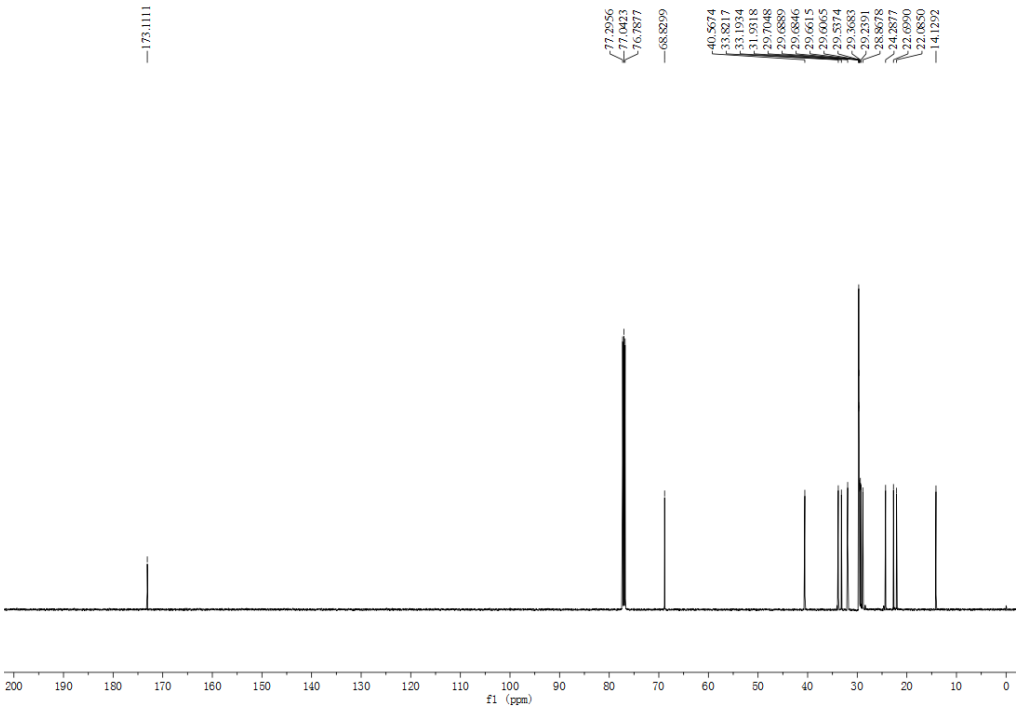

Fig. S19 ^{13}C NMR of **5** in $CDCl_3$

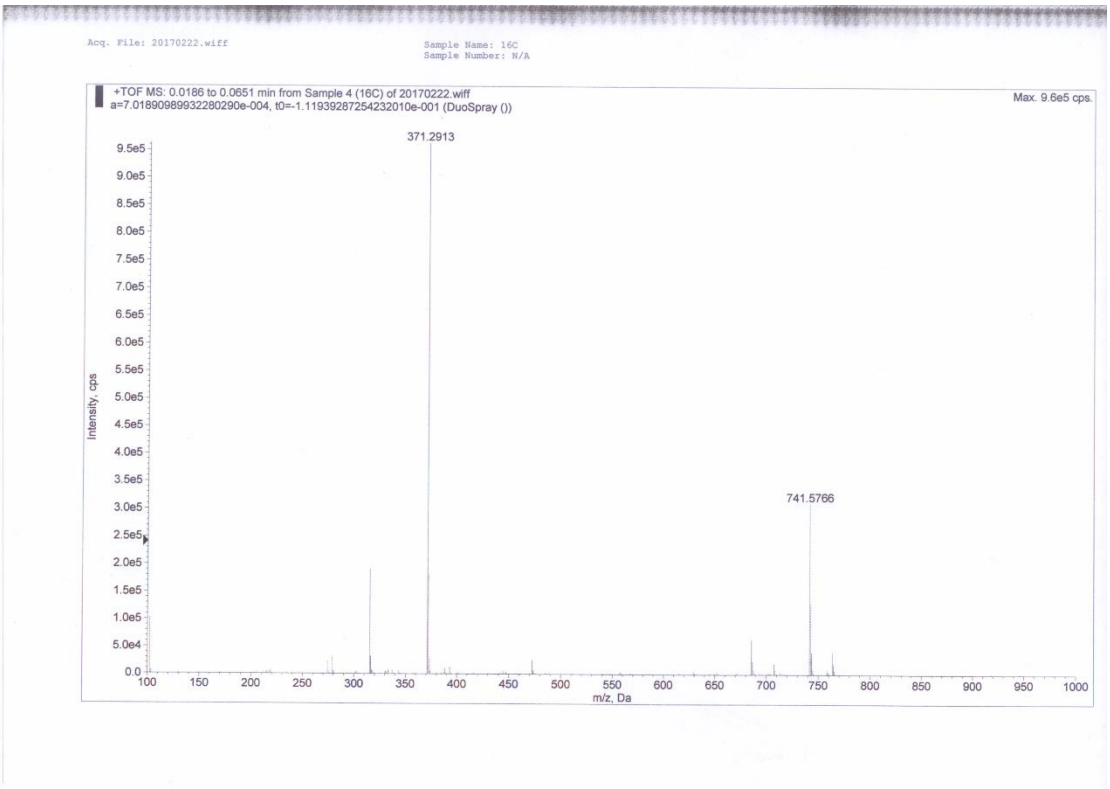

Fig. S20 ESI-MS spectrum of **5**.

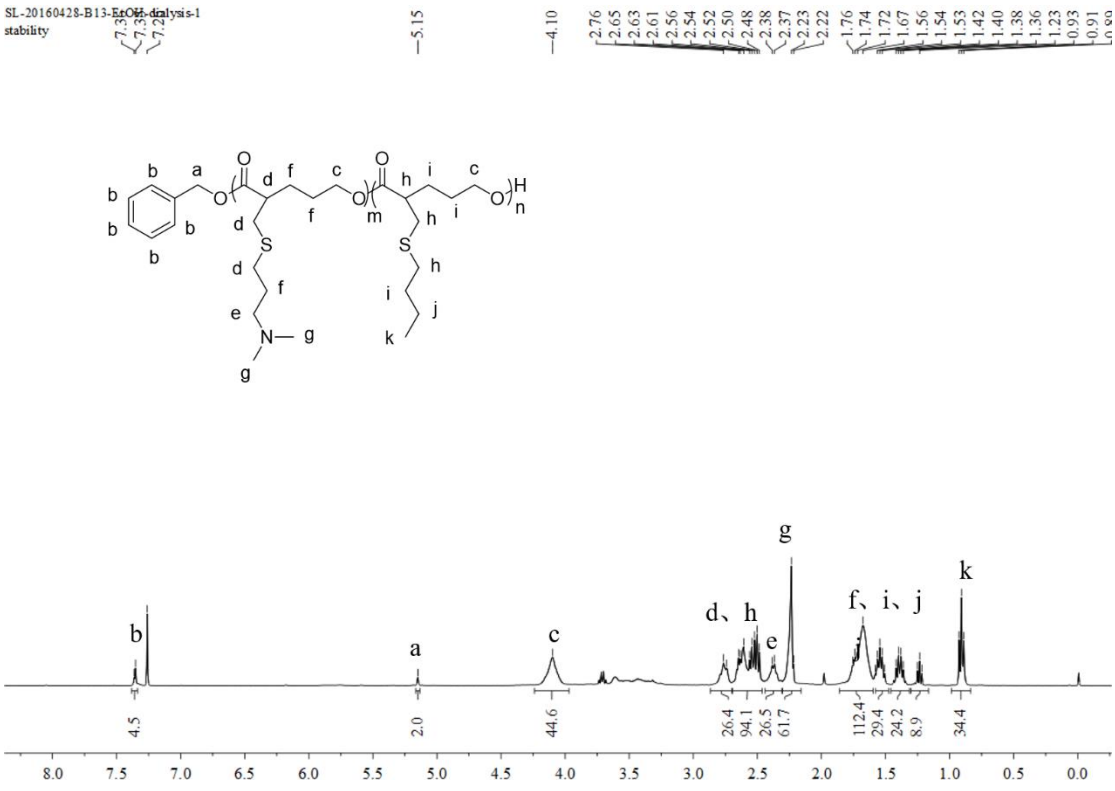

Fig. S21 ^{1}H NMR of **B1** in $CDCl_3$.

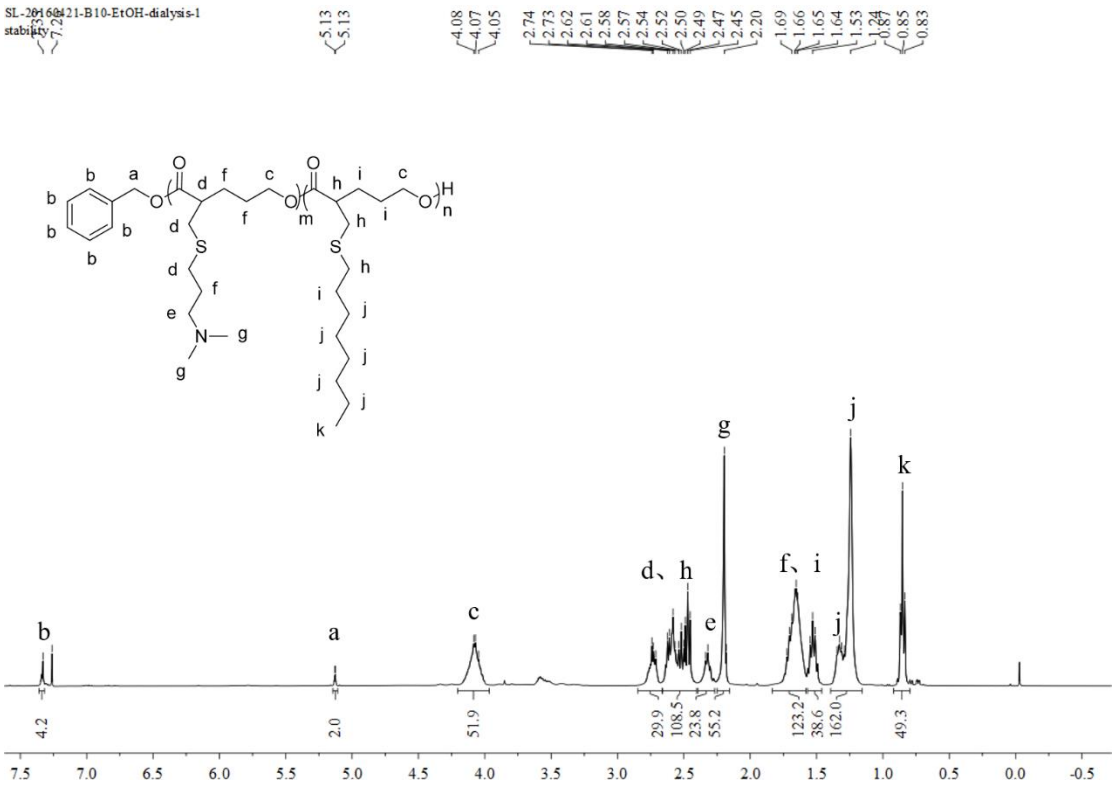

Fig. S22 ^{1}H NMR of **B2** in $CDCl_3$.

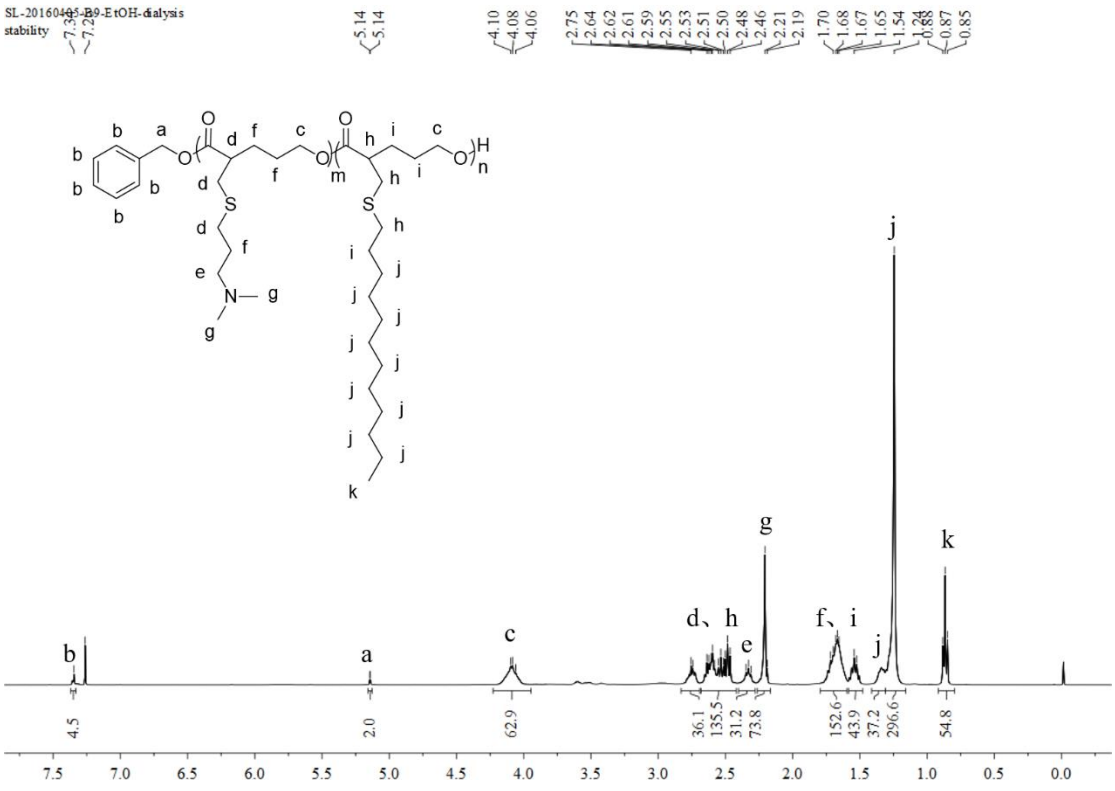

Fig. S23 ^{1}H NMR of **B3** in $CDCl_3$.

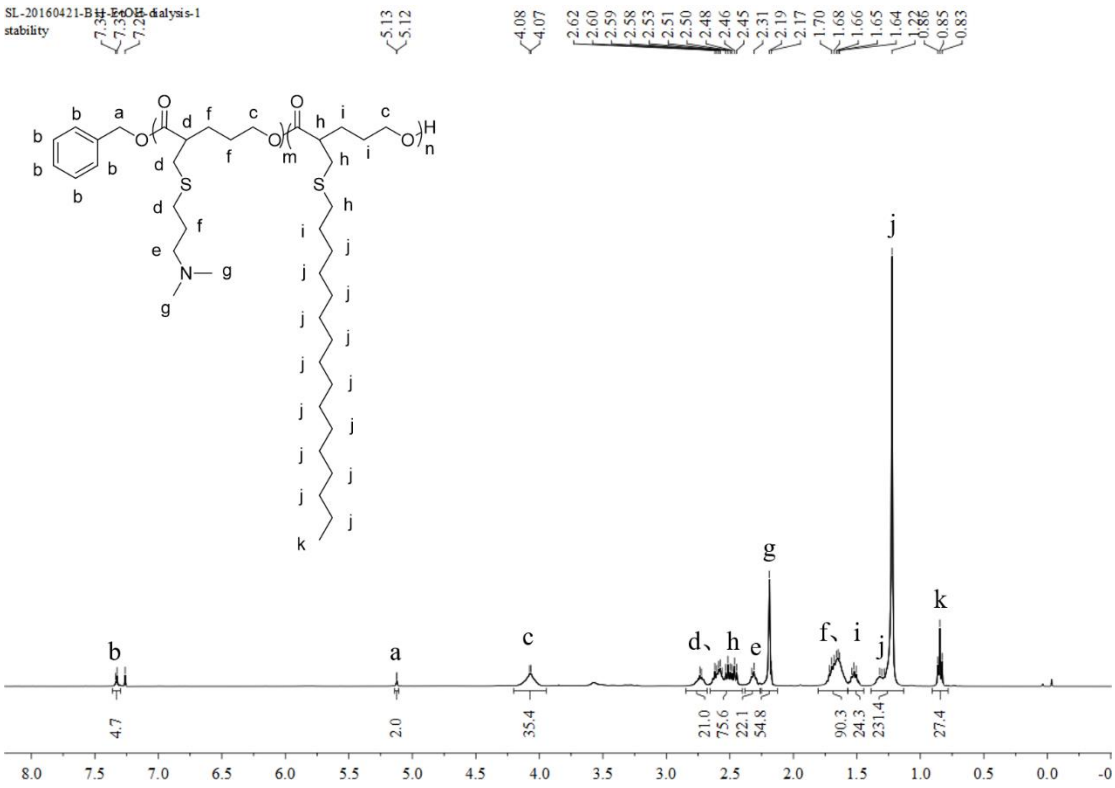

Fig. S24 ^{1}H NMR of **B4** in $CDCl_3$.

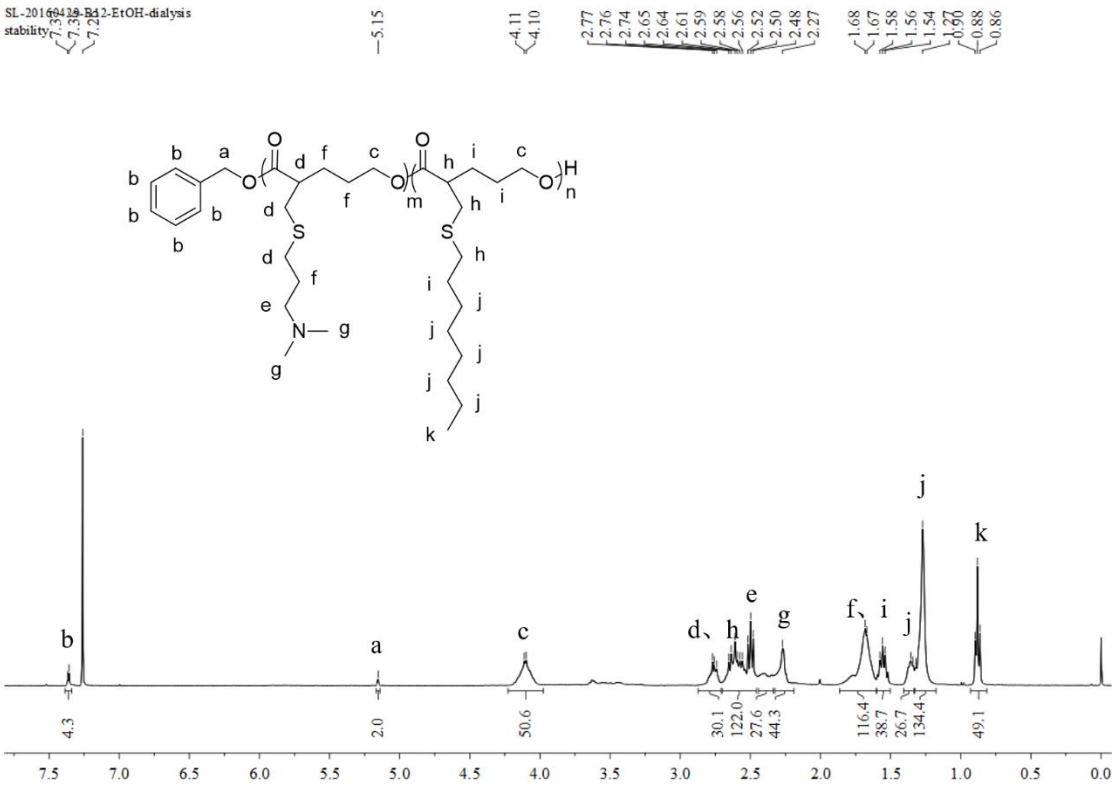

Fig. S25 ^{1}H NMR of **B5** in $CDCl_3$.

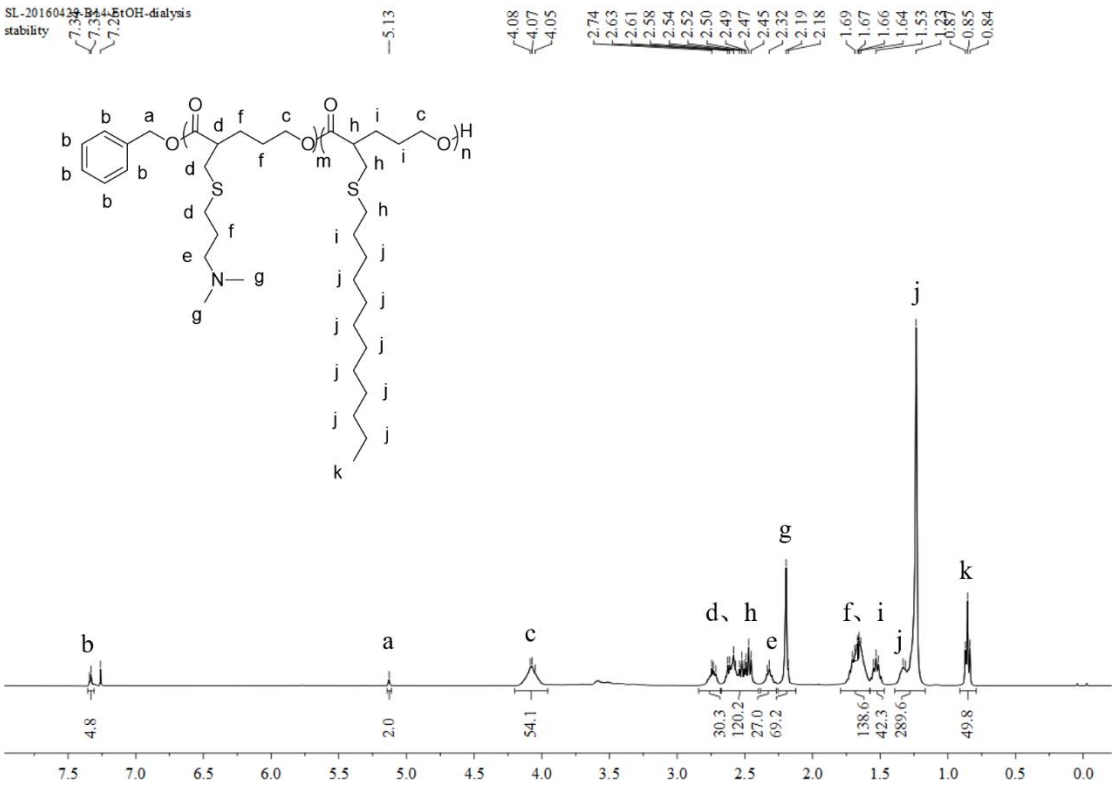

Fig. S26 ^{1}H NMR of **B6** in $CDCl_3$.

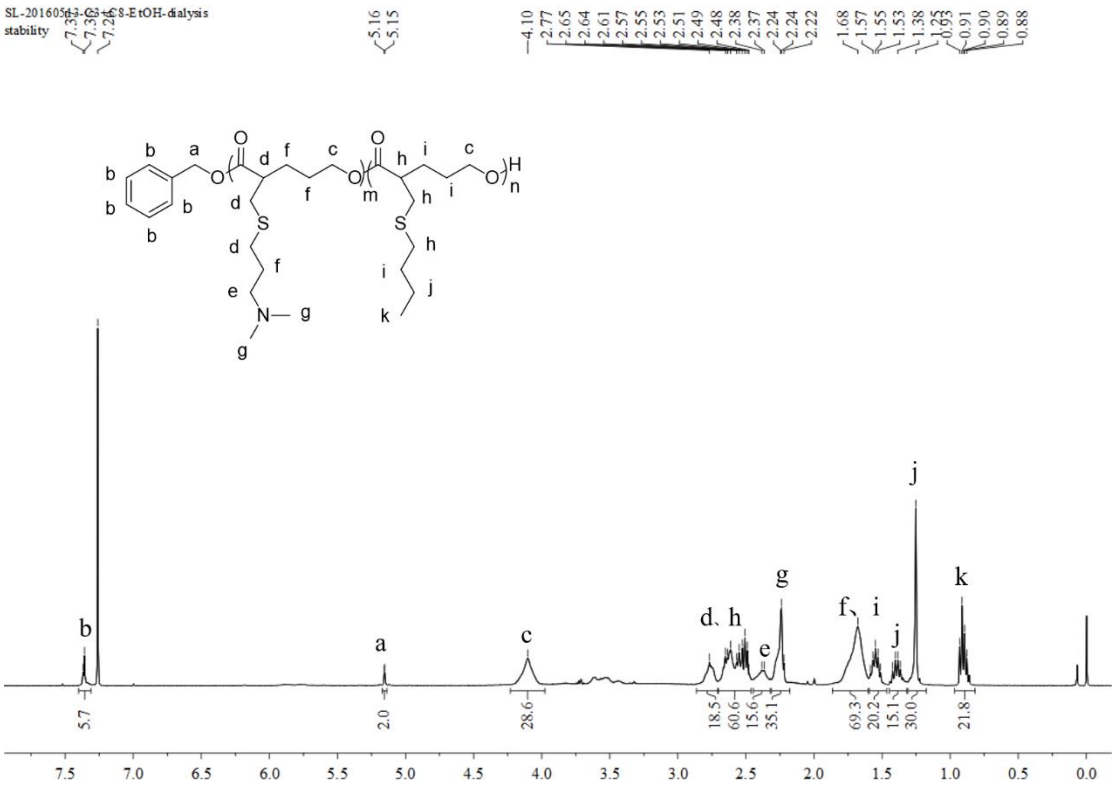

Fig. S27 ^{1}H NMR of **C1** in $CDCl_3$.

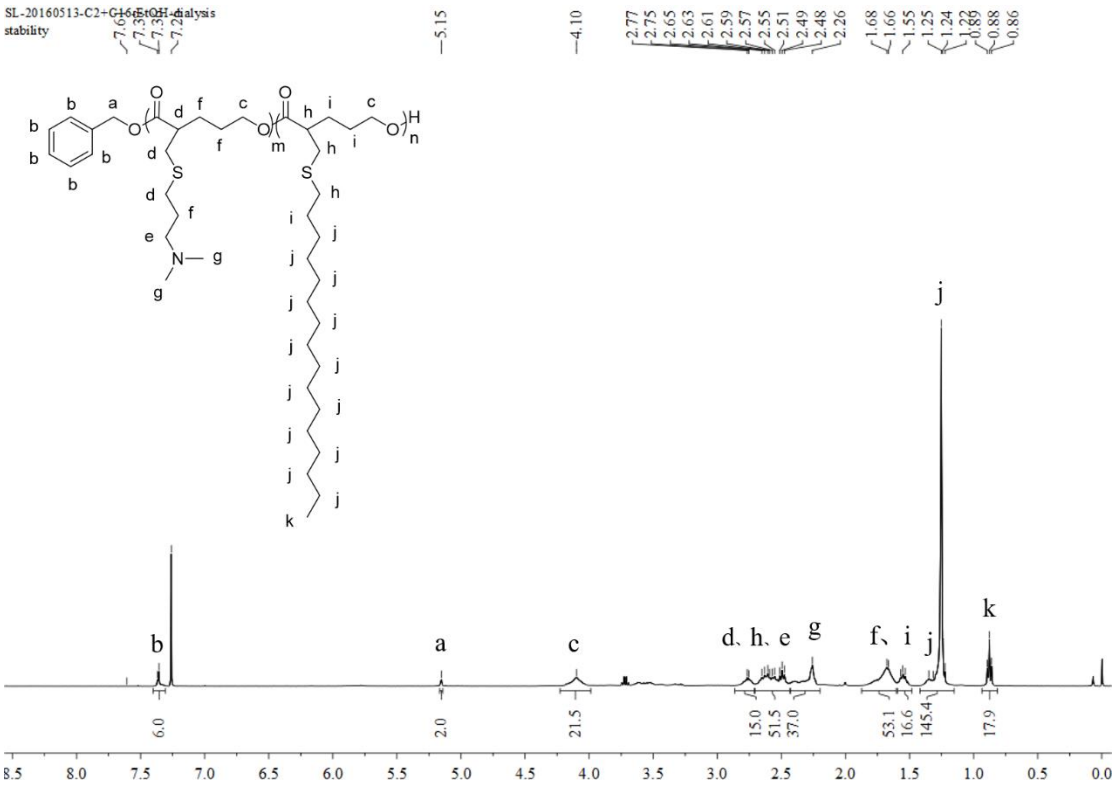

Fig. S28 ^{1}H NMR of **C2** in $CDCl_3$.

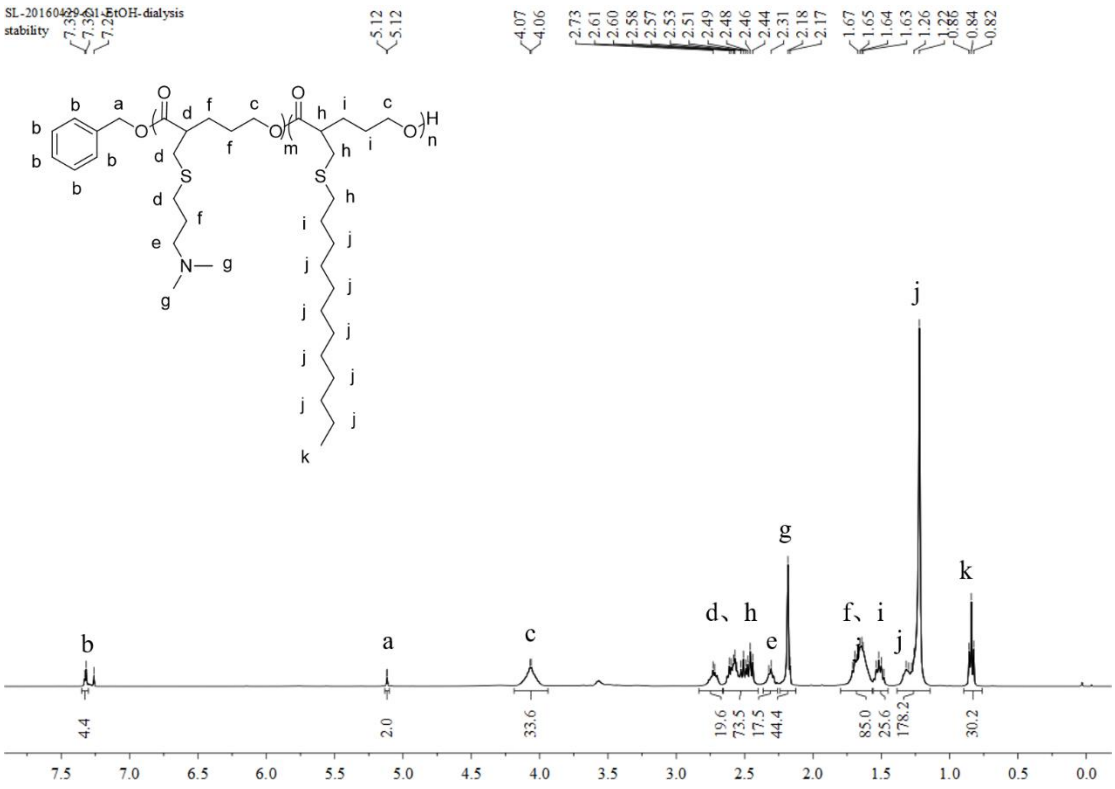

Fig. S29 ^{1}H NMR of **C3** in $CDCl_3$.

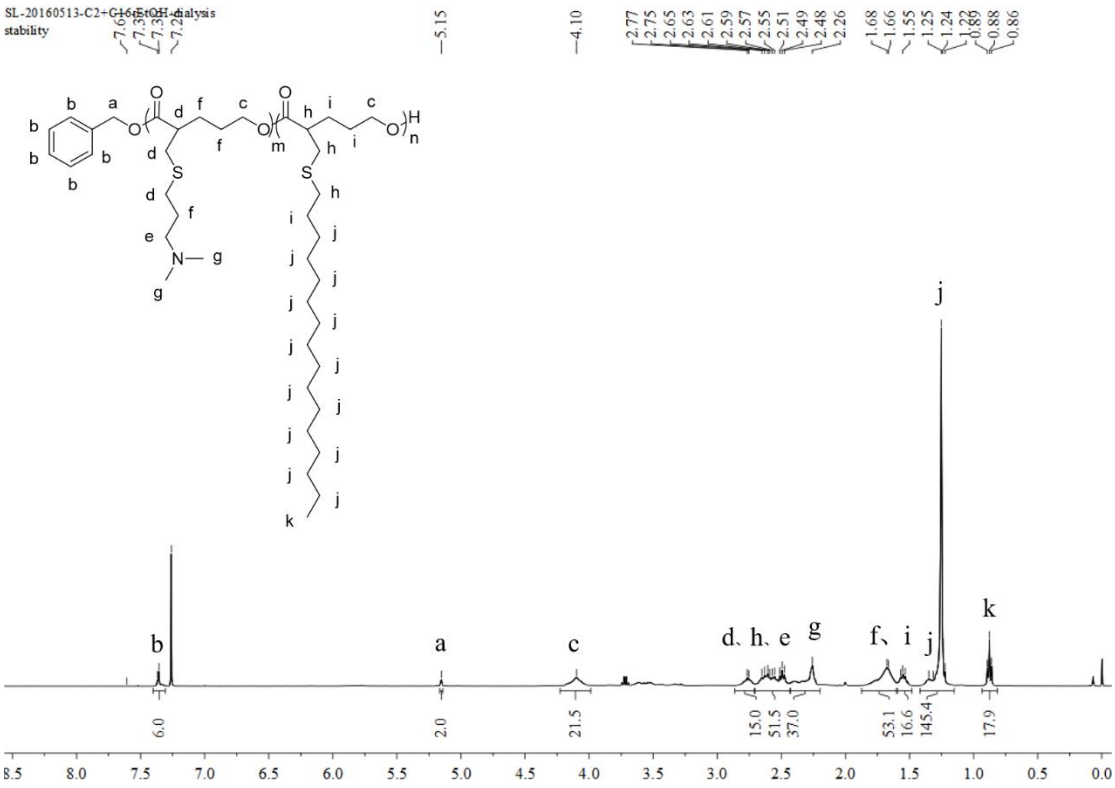

Fig. S30 ^{1}H NMR of **C4** in $CDCl_3$.

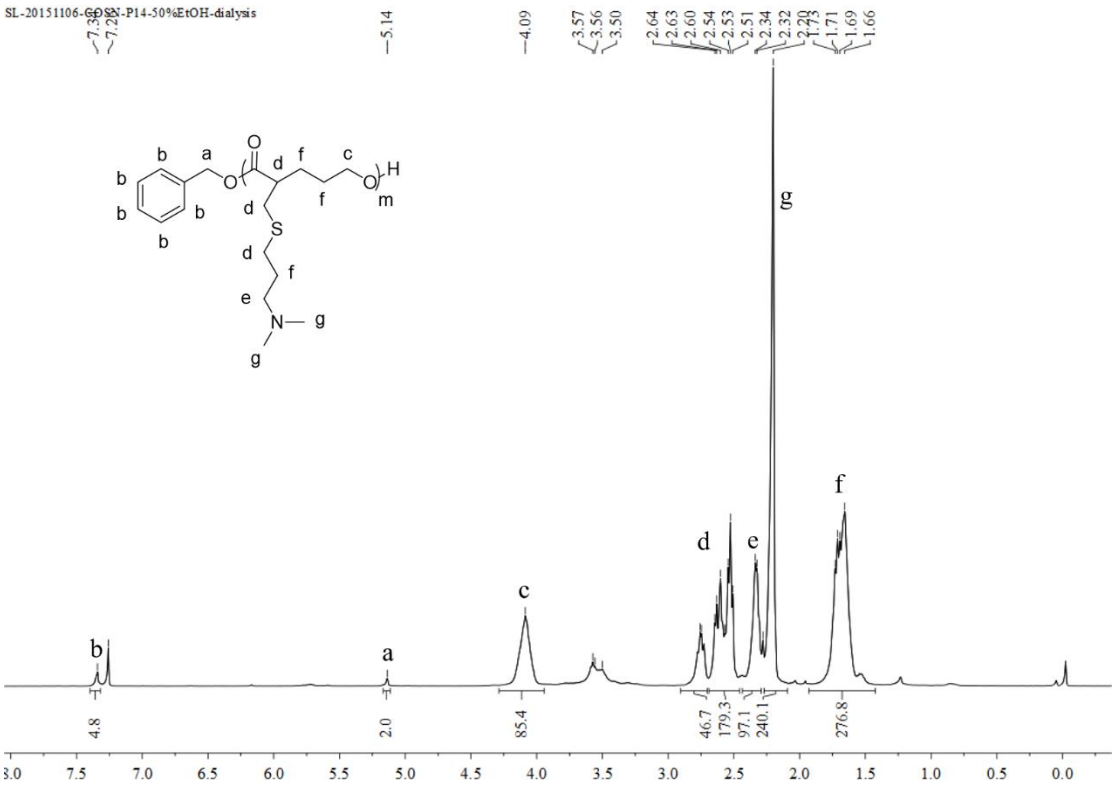

Fig. S31 ^{1}H NMR of **P1** in $CDCl_3$.

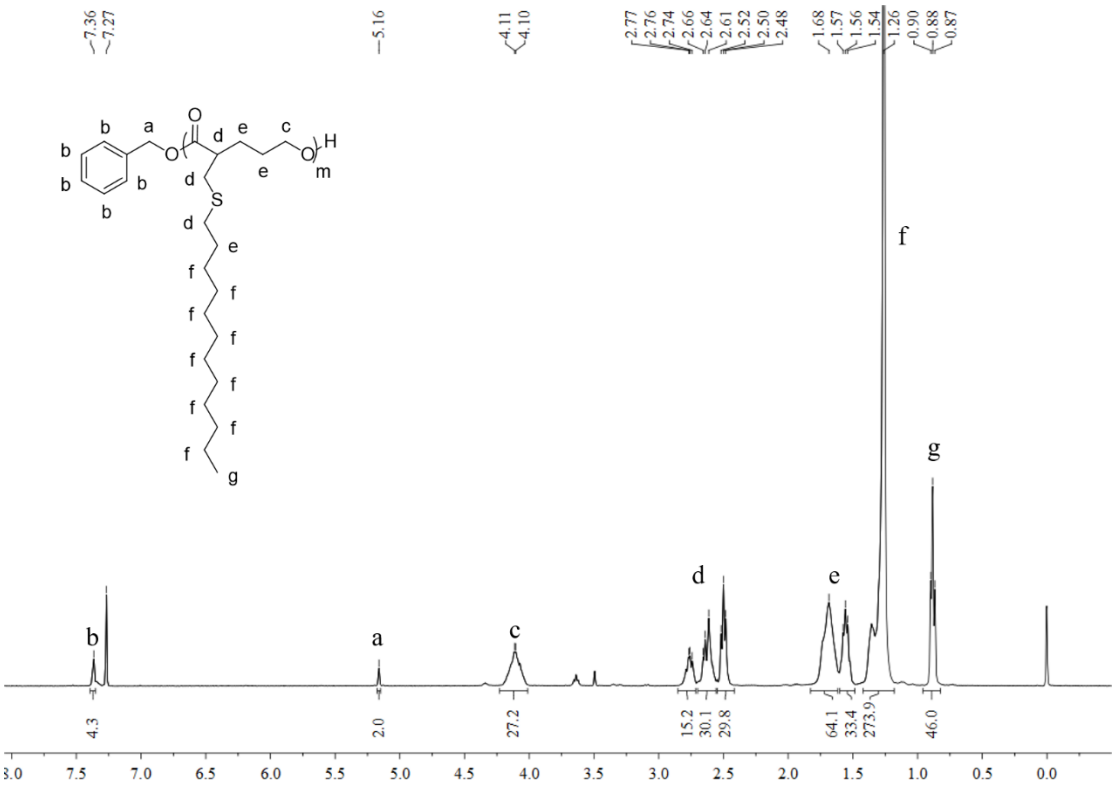

Fig. S32 ^{1}H NMR of **P2** in $CDCl_3$.