Electronic Supplementary Material (ESI) for New Journal of Chemistry. This journal is © The Royal Society of Chemistry and the Centre National de la Recherche Scientifique 2017

Low Generation PAMAM-Based Nanomicelles as ROS-Responsive Gene Vector with Enhanced Transfection Efficacy and Reduced Cytotoxicity in Vitro †

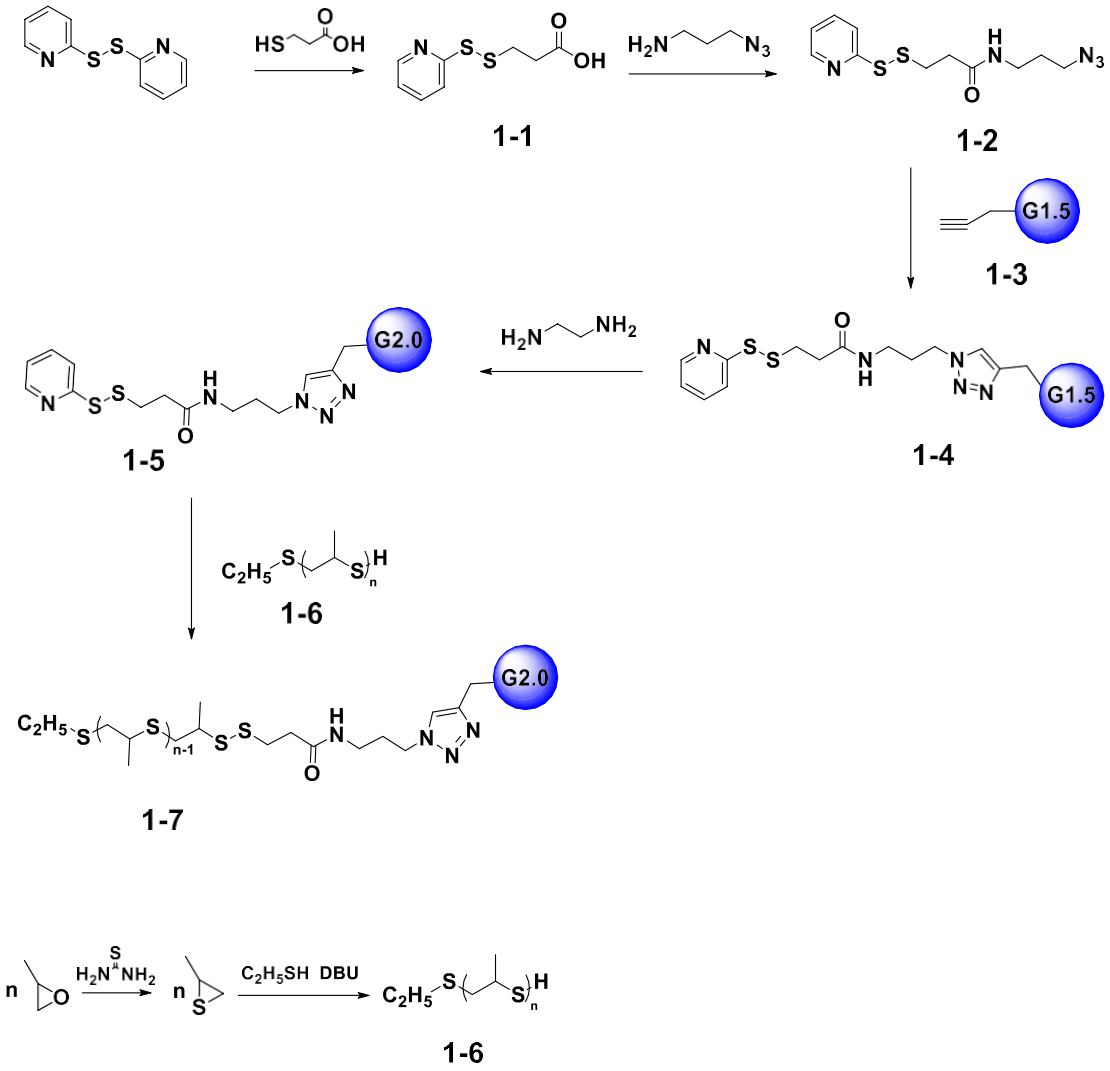
Chen-Tao Xu,[a] Guang Chen[a], Xuan Nie,[a] Long-Hai Wang,[a] Sheng-Gang Ding*[b] and Ye-Zi You*[a]

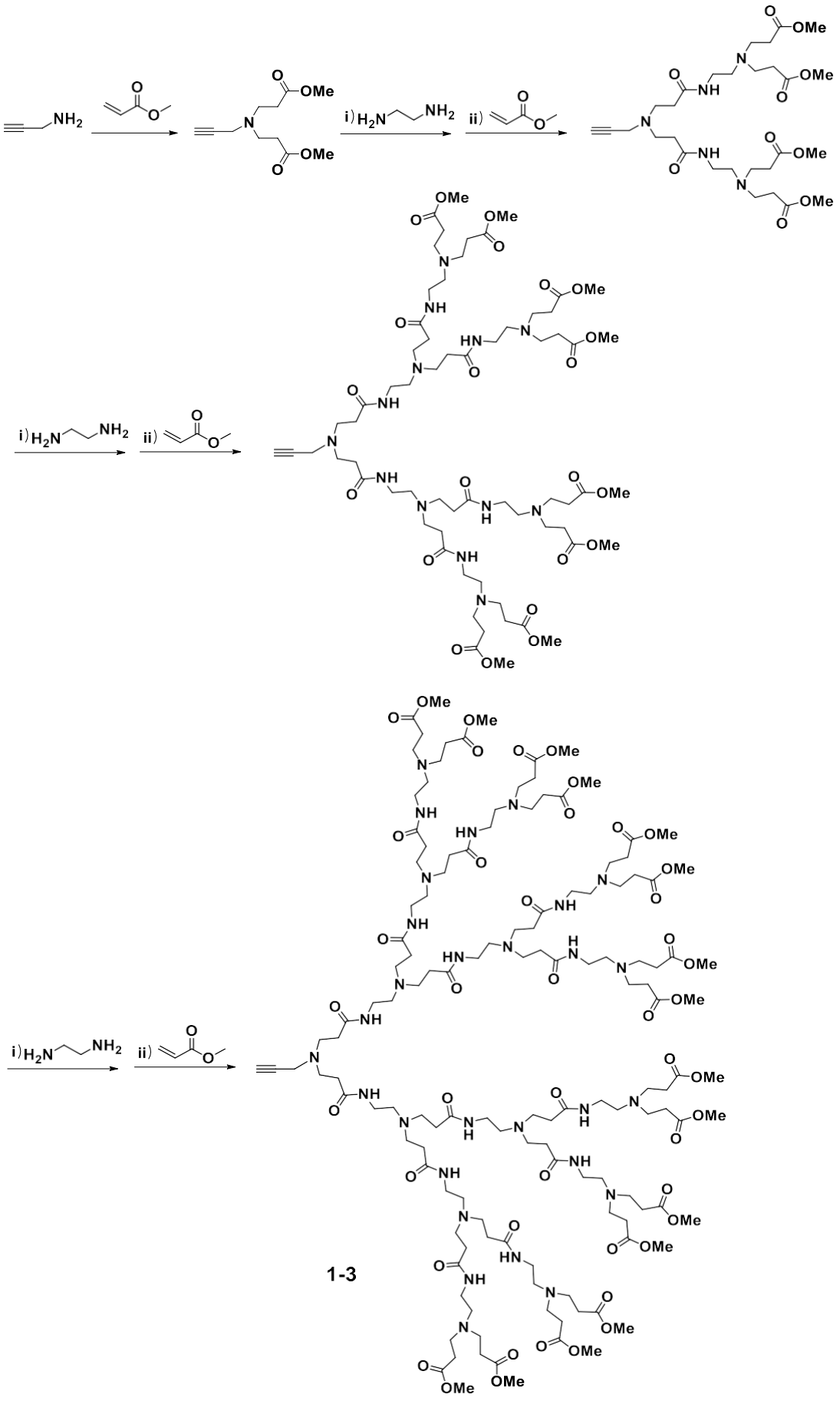
[a] Key Laboratory of Soft Matter Chemistry, Chinese Academy of Science, Department of Polymer Science and Engineering, University of Science and Technology of China, Hefei, Anhui 230026, China. E-mail: yzyou@ustc.edu.cn

[b] Department of Pediatrics, The First Affiliated Hospital of Anhui Medical University, Hefei, Anhui 230022, China. E-mail: dsg5312@163.com

Experimental Section

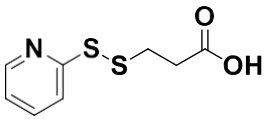
1. Synthesis Procedures





1-3

2. Synthesis and characterization of amphiphilic dendrimers (PPS-SS-PAMAM$_{G2.0}$)



1-1

Synthesis of 3-(pyridin-2-yldisulfanyl)propanoic acid (1-1): A stirring solution of 2,2'-dipyridyl disulfide (1.82 g, 8.26 mmol) in EtOH (10 mL) and acetic acid (0.40 mL) was added dropwise with 3-mercaptopropionic acid (360µL, 4.13 mmol) in EtOH (5 mL). The resulting yellow solution was further stirred for 24 h before the solvent was removed by rotary evaporation. The residual yellow syrup was dissolved in the minimum amount of CH_2Cl_2/EtOH mixture (3:2), which was then applied into an alumina (neutral alumina) column equilibrated with CH_2Cl_2/EtOH mixture (3:2). The column was flushed with a large amount of CH_2Cl_2/EtOH mixture (3:2) until the yellow fraction was totally eluted from the alumina column before flushing with a large amount of CH_2Cl_2/EtOH mixture (3:2) containing 4% acetic acid to elute the product from the column. The solvent of the fraction was removed via rotary evaporation, and the obtained material was re-dissolved in CH_2Cl_2 followed by washing it thoroughly with water to remove the acetic acid. The organic layer was then dried over anhydrous Na_2SO_4 and the solvent was removed in vacuo to obtain the product as a pale yellow solid (0.58 g, 65%).

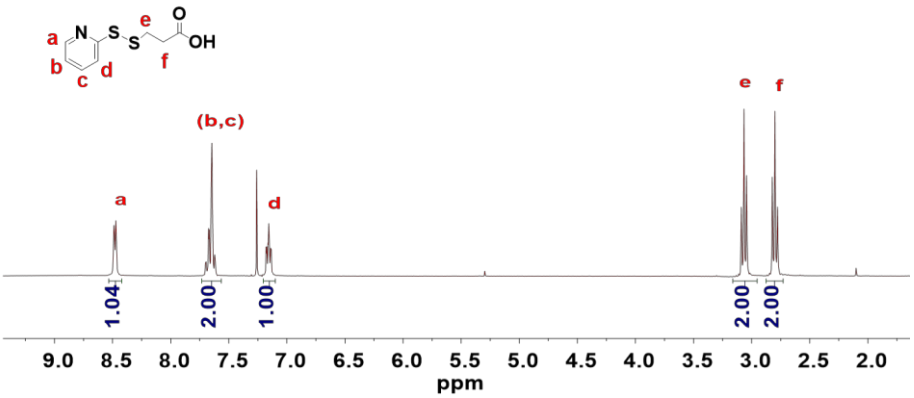
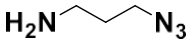


Figure S1 ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of 3-(pyridin-2-yldisulfanyl) propanoic acid.



Synthesis of 3-azidopropan-1-amine: To a solution of 3-chloropropyl-1-amine hydrochloride (4.00 g, 30.8 mmol) in water (50 mL) was added sodium azide (6.5 g, 100 mmol) and the reaction mixture was heated to 85 °C for 24 h. The solution was basified with solid sodium hydroxide and extracted with diethyl ether (3 × 70 mL), the organic layer was washed with water (20 mL) and brine (10 mL) and then dried over anhydrous Na_2SO_4 and the solvent was removed in vacuo to obtain 0.99 g (32%) of a volatile colourless oil.

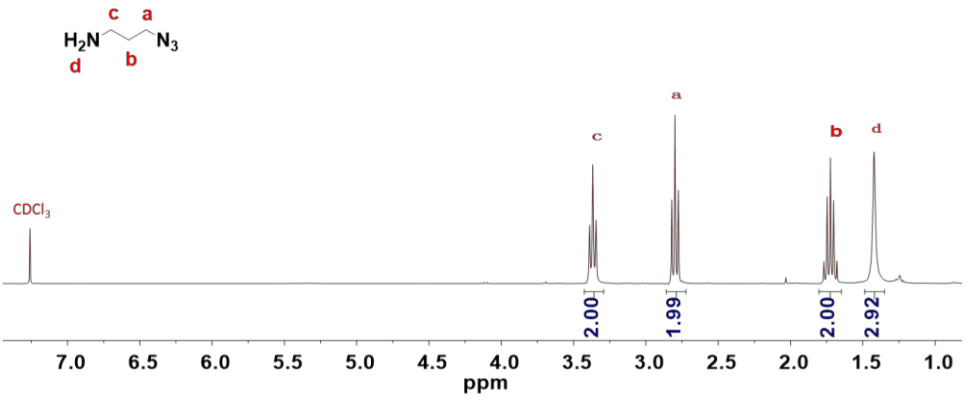
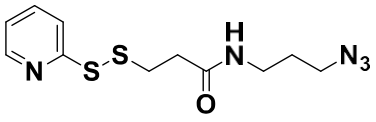


Figure S2 ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of 3-azidopropan-1-amine.



1-2

Synthesis of N-(3-azidopropyl)-3-(pyridin-2-yldisulfanyl)propanami-de (1-2): To a stirring solution of 3-(pyridin-2-yldisulfanyl)propanoic acid (0.61 g, 2.837 mmol) in DCM (20 mL) was added with dicyclohexylcarbodiimide (0.922 g, 4.374 mmol) and N-hydroxysuccinimide (0.508 g, 4.374 mmol). The resulting turbid solution was stirred for a period of 2 h before 3-azidopropan-1-amine (0.2837 g, 2.837 mmol) was added. The reaction was allowed to proceed 24h before removing the precipitate by filtration. The solvent of the filtrate was removed by rotary evaporation and the residual mixture

was purified by flash column chromatography using hexane/ethyl acetate (1:2) mixture as the eluent to afford the product as a white solid (0.40 g, 48%).

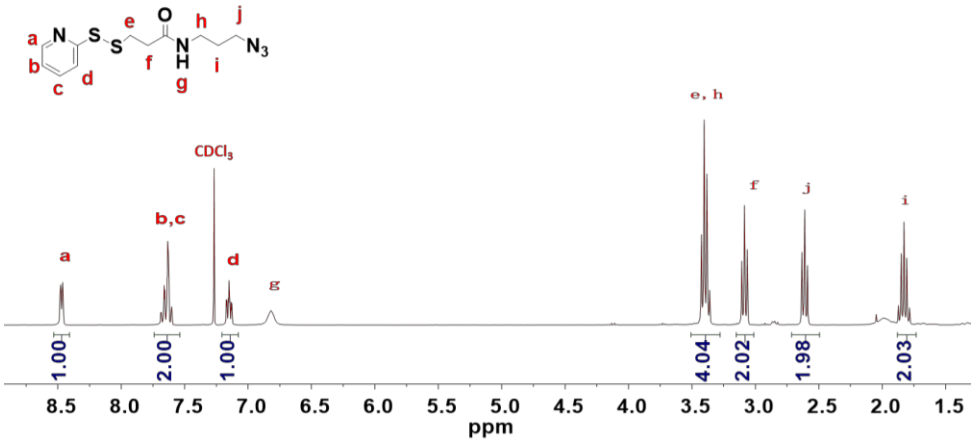
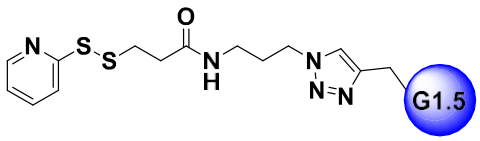


Figure S3 ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of N-(3-azidopropyl)-3-(pyridin-2-yldisulfanyl)propanamide.



1-4

Synthesis of pyridine-SS-PAMAM$_{G1.5}$ (1-4): To a solution of PAMAM G1.5 (125 mg, 0.0413mmol) (G1.5 and G2.0 PAMAMs were prepared from ethanediamine and methyl acrylate following a published procedure[1]) in DMF (7 mL) were added 1-2 (45.81 mg, 0.1384 mmol) and CuBr (3.49 mg, 0.0244 mmol) successively. The vessel was sealed and purged with argon for 5 min, and then 1.8-diazabicyclo (5,4,0)undec-7-ene (DBU) (45.91 μL, 0.3069 mmol) was added into the reaction mixture. The resulting mixture was stirred at 48 °C for 40 h. DMF was removed under reduced pressure. The obtained residue was suspended in 10 mL saturated NH_4Cl solution and extracted with CH_2Cl_2 (15.0×3). The combined organic layers were washed successively with saturated NH_4Cl solution (15.0 mL×2), saturated $NaHCO_3$ solution (15 mL×2) and brine (15 mL×2).

The organic phase was dried with Na_2SO_4 and concentrated. The crude product was purified by precipitation with CH_2Cl_2/Et_2O and the precipitate was the collected, giving the product as brown oil.

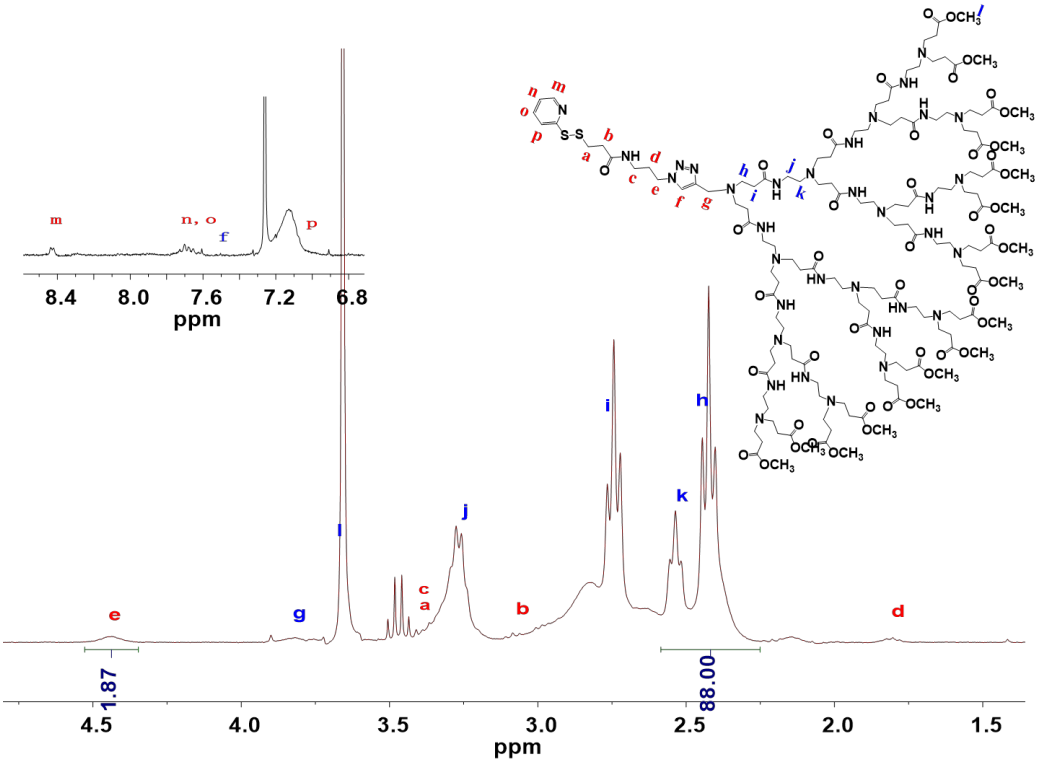
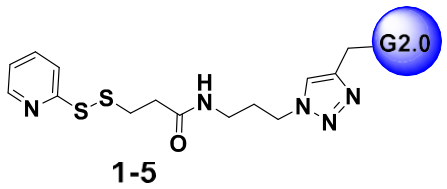


Figure S4 ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of pyridine-SS-PAMAM$_{G1.5}$.



Synthesis of pyridine-SS-PAMAM$_{G2.0}$ (1-5): To a solution of pyridine-SS-PAMAM$_{G1.5}$ (53.8 mg, 0.0155 mmol) in methanol was added ethylenediamine (2.32 mL, 34.66 mmol). The reaction mixture was stirred for 80 h at 30 °C under nitrogen. When the reaction was completed, the reaction solution was evaporated, the obtained residue was dissolved in a small amount of methanol and then precipitated in Et_2O for three times, yielding amine terminated product as a brown solid.

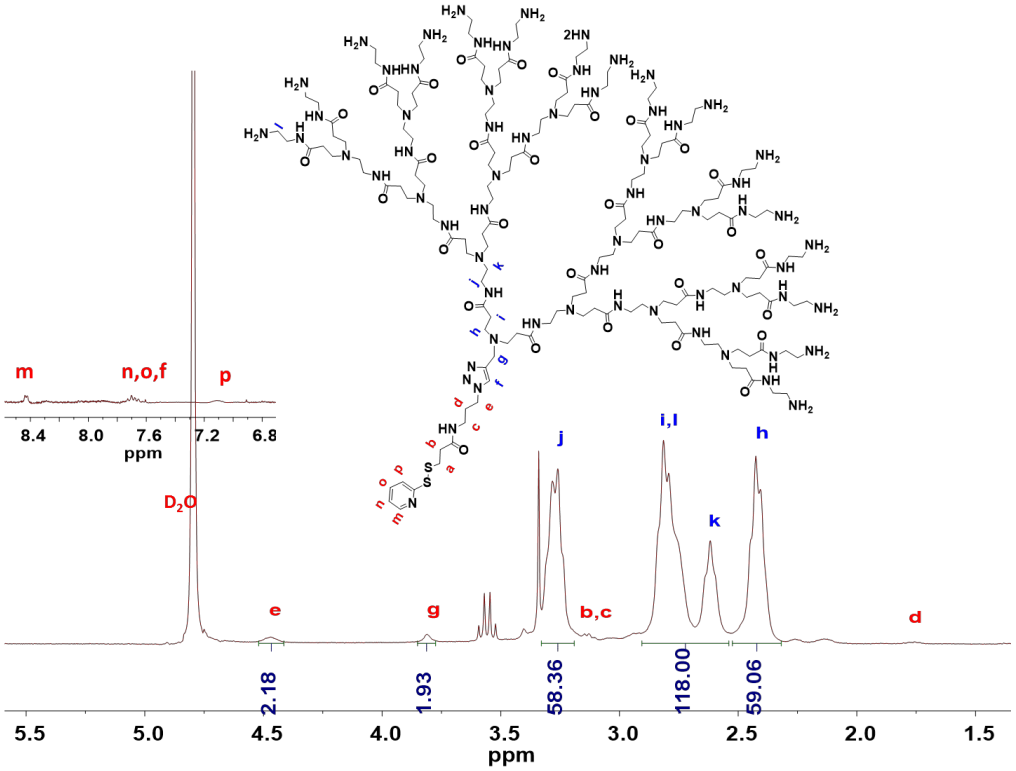


Figure S5 ^{1}H NMR (300 MHz, D_2O) spectrum of the pyridine-SS-PAMAM$_{G2.0}$.

Synthesis of 2-methylthiirane : In a dried 250 mL RB flask which was equiped with condensate recovery unit, propylene oxide (0.75 mol, 43.56 g) and thiourea (0.5 mol, 38.06 g) were added at 0℃ for 2 h, the resulting mixture was stirred at 25℃ for 12 h before removing the precipitate by filtration. The obtained residue was washed with water for 3 times, then the organic phase was dried with anhydrous Na_2SO_4. Finally, the propylene sulfide was purified by distillation.

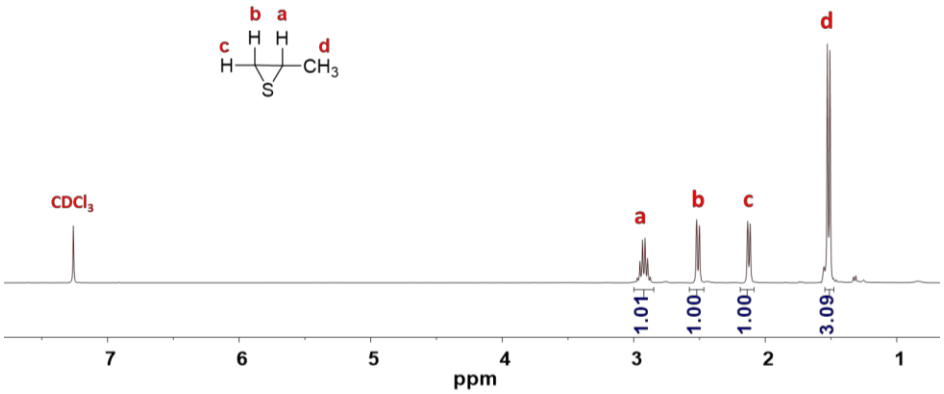
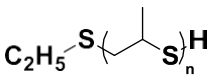


Figure S6 ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of 2-methylthiirane.



1-6

Synthesis of poly (propylene sulfide). 1,8-Diazabicyclo[5.4.0]-undec-7-ene (DBU) (3 mmol, 0.448 mL) and dry THF (15 mL) was degassed for 30 minutes in a flask at 0℃. The degassed solution of ethanethiol (3 mmol, 0.216 mL) in THF (10 mL) was added drop wise and allowed to react for 30 minutes. Then freshly distilled and degassed propylene sulfide (60 mmol, 4.68 mL) was added to the reaction mixture, and the temperature was maintained at 0℃ for 2 h. The reaction was quenched by addition of acetic acid (6 mmol, 0.342 mL) and stirred overnight at room tempetature. The polymer solution was filtered to remove precipitated salt and further purified by three precipitations into cold methanol before vacuum-drying to yield a colorless viscous polymer.

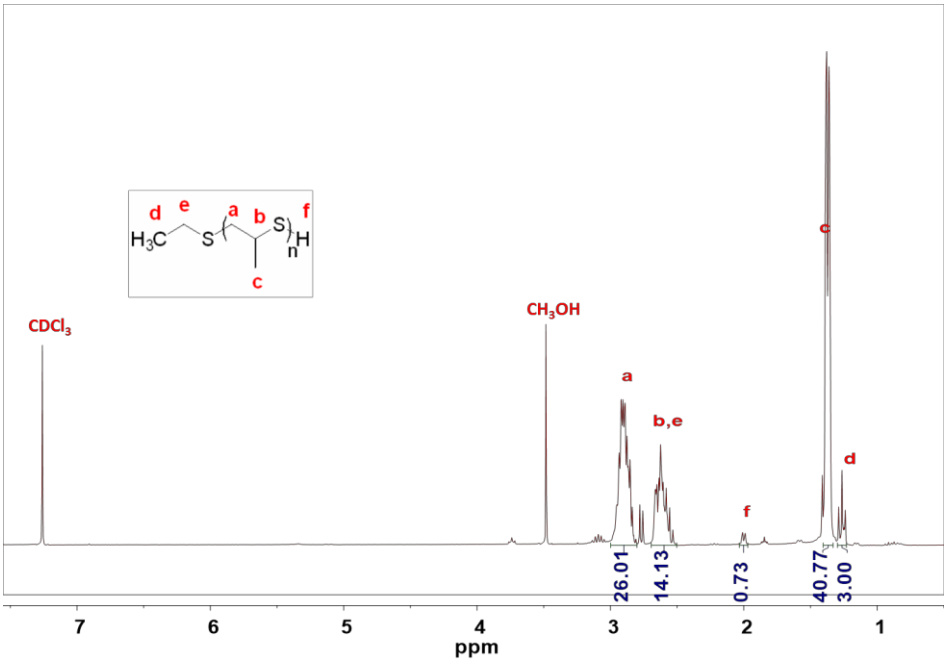


Figure S7 ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of poly (propylene sulfide).

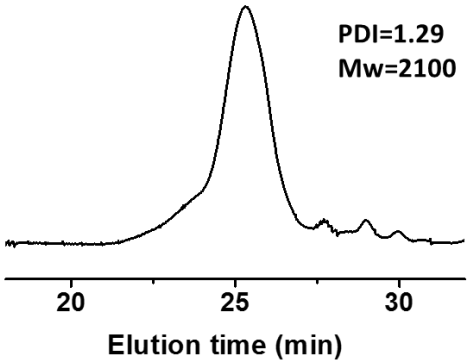
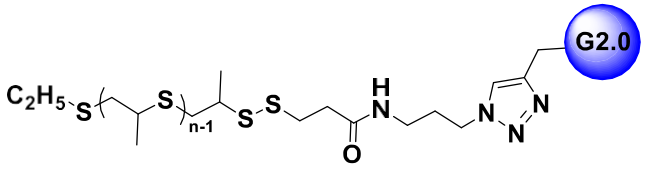


Figure S8 GPC refractive index detector traces of poly (propylene sulfide).



1-7

Synthesis of PPS-SS-PAMAM$_{G2.0}$ (1-7): To a solution of 1-5 (330 mg, 0.0841 mmol) in DMSO was added PPS (86 μL, 0.0840 mmol). The reaction mixture was stirred for 80 h at 30℃ under nitrogen. When the reaction was completed, the reaction solution was precipitated in a large amounts of Et_2O. And then the crude product was further washed with THF to give the product as a brown solid.

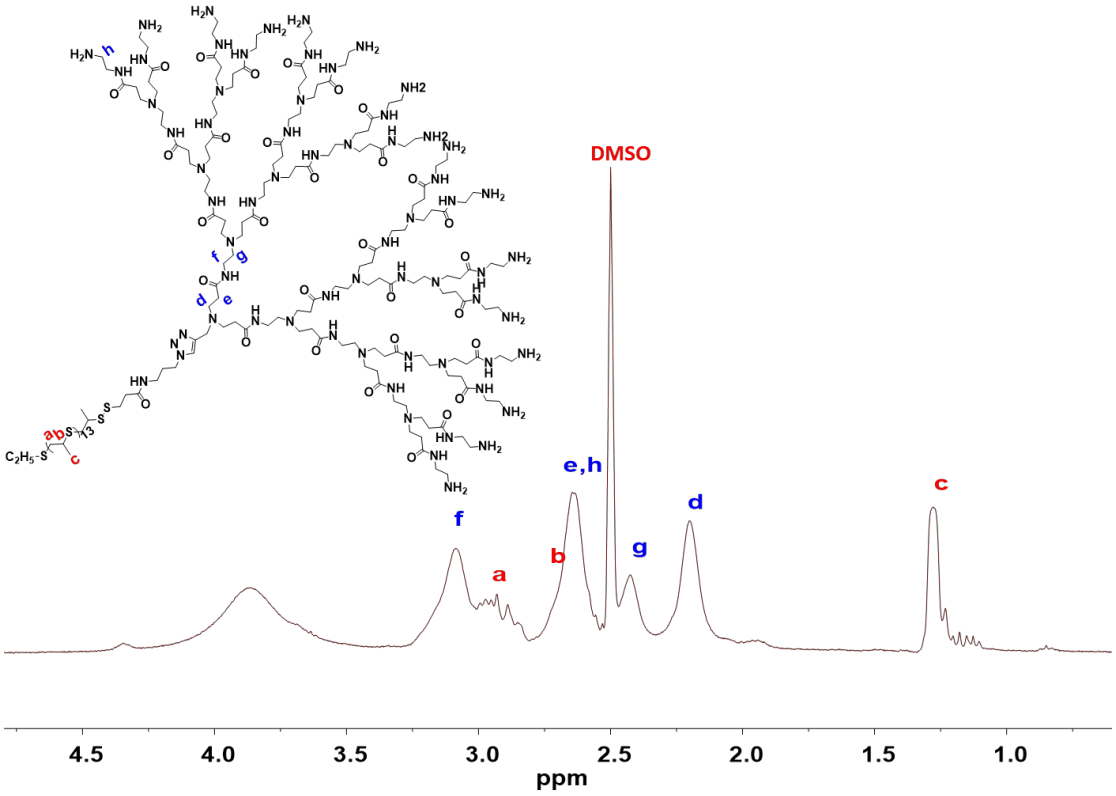


Figure S9 ^{1}H NMR (300 MHz, DMSO-d6) spectrum of the PPS-SS-PAMAM$_{G2.0}$.

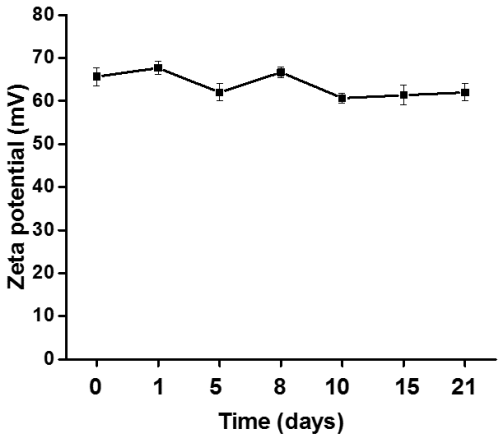


Figure S10 Zeta potential stability of PPS-SS-PAMAM$_{G2.0}$ nanomicelles.

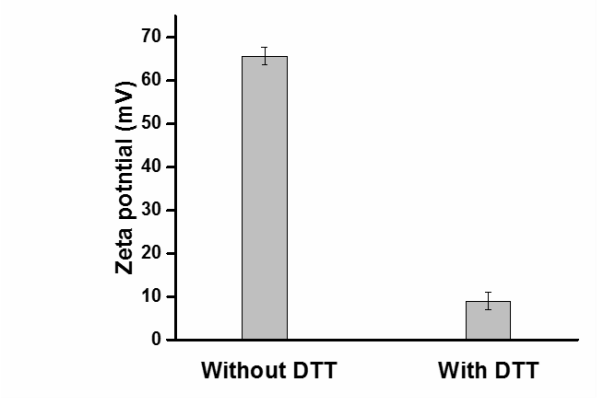


Figure S11 Zeta potential of PPS-SS-PAMAM$_{G2.0}$ nanomicelles treated with/without DTT (20 mM for 24 h).

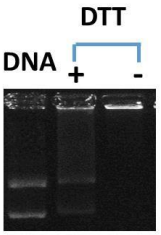


Figure S12 Agarose gel electrophoresis of the nanomicelles/DNA polyplexes treated with/without DTT (10 mM for 24 h) at the N/P ratio of 2.

References

1 T. Yu, X. Liu, A. L. Bolcato-Bellemin, Y. Wang, C. Liu, P. Erbacher, F. Qu, P. Rocchi, J. P. Behr and L. Peng, *Angew. Chem., Int. Ed.,* 2012, **51**, 8478-8484.