Supplementary Information for

Supramolecular block copolymers for gene delivery: Enhancement of transfection efficacy by charge regulation

Wumaier Yasen,[a] Ruijiao Dong,*[a,b] Linzhu Zhou,[a] Yu Huang,[a] Dongbo Guo,[a] Dong Chen,[a] Chuanlong Li,[a] Aliya Aini[c] and Xinyuan Zhu*[a]

[a]*School of Chemistry and Chemical Engineering, State Key Laboratory of Metal Matrix Composites, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, China.* [b]*Department of Chemical Engineering, Imperial College London, London SW7 2AZ, UK.* [c]*School of Foreign Languages, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, China.*

**E-mail*: drj021@163.com; xyzhu@sjtu.edu.cn; *Tel.*: +86-21-54746215

Contents

1. Materials

Poly(ethylene glycol) monomethyl ether (PEG_{113}-OH, M_n = 5.0 kDa, M_w/M_n = 1.06, Aldrich) , lipofectamine 2000 (LIP) (Life technology) , chitosan (CTS) (M_w = 50-190 kDa, Aldrich) , branched polyethyleneimine (PEI) (99%, M_w = 25 kDa, Aldrich), sodium azide (NaN_3) (99%, Aldrich), pentaethylenehexamine (PEHA) (95%, Aldrich), (98%, Aldrich), *N,N′*-carbonyldiimidazole (CDI) (Aldrich), sodium borohydride ($NaBH_4$) (≥99%, Sigma), sodium hydride (NaH) (57-63% dispersed in oil, Alfa Aesar), *N,N*-dimethylaminoethylamine (DMEDA) (99%, J&K Chemical), propargyl bromide (80% in toluene stabilized with MgO, Alfa Aesar), 1,1,4,7,7-pentamethyldiethylenetriamine (PMDETA) (98%, Alfa Aesar), pyridinium chlorochromate(PCC)(98%, Aldrich), tosyl chloride (TsCl) (99%, Shanghai Sinopharm Chemical Reagent Co. Ltd.), sodium hydrogen carbonate ($NaHCO_3$) (≥99%, Shanghai Sinopharm Chemical Reagent Co. Ltd.), 1-adamantanemethanol(AD-CH_2OH) (>99%, TCI), sodium sulfate anhydrous (Na_2SO_4) (≥99%, Shanghai Sinopharm Chemical Reagent Co. Ltd.), magnesium silicate adsorbent (synthetic) (125-250um, Aladdin), hydrochloric acid (HCl) (37%, Shanghai Sinopharm Chemical Reagent Co. Ltd.), and sodium hydroxide (NaOH) (99%, Shanghai Sinopharm Chemical Reagent Co. Ltd.) were used as received without further purification. Copper (I) bromide (CuBr) (98%, Aldrich) was firstly treated with acetic acid for several hours under stirring. After which the mixture was filtered and washed with acetic acid, diethyl ether and ethanol in succession until it turned into white. The purified CuBr was stored under nitrogen atmosphere. Methanol (MeOH) (Shanghai Sinopharm Chemical Reagent Co. Ltd.) was treated with dry molecular sieves and distilled before use. β-Cyclodextrin (β-CD) and its derivatives (Shanghai Sinopharm Chemical Reagent Co. Ltd.) were dried for 48 h at 60 ^oC in vacuum oven prior to use. Diethyl ether, dichloromethane (CH_2Cl_2), dimethyl sulfoxide (DMSO), *N,N*-Dimethylformamide (DMF) and toluene (Shanghai Sinopharm Chemical Reagent Co. Ltd.) were treated with calcium hydride and distilled before use. Ethanol, acetone and n-hexane etc. were purchased from Shanghai Sinopharm Chemical

Reagent Co. Ltd., and deionized water were used as received.

2. Instruments and measurements

Nuclear Magnetic Resonance (NMR)

^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury plus 400 NMR spectrometer. with working frequencies of 400 (^{1}H) and 101 (^{13}C) MHz in deuterated chloroform ($CDCl_3$), deuterated dimethyl sulfoxide (DMSO-d_6) and deuterium oxide (D_2O) at 293 K. The chemical shifts were referenced to residual peaks of deuterated solvents: $CDCl_3$ (7.26 ppm), DMSO-d_6 (2.48 ppm) and D_2O (4.79 ppm).

Fourier Transform Infrared Spectra (FTIR)

The KBr sample holder method was used to record FTIR spectra of these products (e.g., AD-CHO, AD-PEHA-CD, PEG-β-CD, PEG-Alkyne and β-CD-N_3) on a Paragon 1000 instrument. The samples were firstly dried for 30 min using infrared lamp before measurement in order to eliminate the residual moisture.

Ultra-Performance Liquid & Quadrupole-Time-of-Flight Mass Spectrometer (UMS) (UPLC & Q-ToF-MS)

UPLC & Q-ToF-MS measurement was performed on a Waters-ACQUITY^{TM} UPLC & Q-ToF-MS Premier (Waters Corporation, USA) at ambient temperature with methanol as the solvent.

Dynamic Light Scattering (DLS)

DLS measurement was carried out on a Malvern Zetasizer NanoS apparatus equipped with a 4.0 mW laser operating at λ = 633 nm. All samples were measured at a scattering

angle of 90^{o} at 25 ^{o}C. The sample solutions were filtered with some absorbent cotton to eliminate the dust before testing. For the sake of thermal equilibration and chemical equilibration, the sample solution (10 mM) was put into the cell for around 15 min prior to the measurement.

Gel Permeation Chromatography (GPC)

The polydispersity index (PDI) and molecular weight of the samples were determined using gel permeation chromatography/multiangle laser light scattering (GPC-MALLS). Tetrahydrofuran (THF) was utilized as the eluent at a flow rate of 1 mL/min at 30 ^{o}C. Wyatt Optilab DSP differential refractometer at 690 nm was used to determine the refractive index increment (*dn*/*dc*).

Zeta Potential Measurement

The Malvern Zetasizer NanoS was employed to measure the zeta potential (ζ) of the samples in PBS buffer at 25 ^{o}C. The cuvettes were filled with various concentrations of AD-PEHA-CD solutions in water ranged from 0.5 to 5 mM, and the measurement was performed in the ζ-model for a minimum of 10 cycles and a maximum of 100 cycles.

Atomic Force Microscopy (AFM)

The morphology of Core-Shell DNA polyplex was visualized by an atomic force microscopy (AFM) system with the Dimension 3100 model using a Nanoscope IIIa controller (Veeco, Santa Barbara, CA). 40 μL of DMEDA-g-SBC/*p*DNA complexes in deionized water (pH 7.4) containing approximate 0.08 μg of *p*DNA with N/P ratios of 5 and 20 were dropped onto freshly cleaved mica sheets for 5 minutes. Then the excess solution was absorbed by filter paper, and the samples were dried naturally in air at room temperature for 24 hours. The samples were imaged using the tapping mode with setting

of 256 × 256 pixels. Image analysis was performed using Nanoscope software after removing the background slope by flatting images.

Agarose Gel Electrophoresis

The PEHA/*P*dna, SBC/*p*DNA and DMEDA-*g*-SBC/*p*DNA complexes at various N/P ratios were prepared with the addition of different volumes of PEHA solutions or DMEDA-*g*-SBC solutions to *p*DNA solutions in PBS buffer, followed by vortexing for 6 s and incubated for 30 min at room temperature. After mixing 5 μL of 0.5 × loading buffer with polyplex solutions, the 1% agarose gel containing 0.5 μg/mL ethidium bromide was used to analyze the resulting polyplex solution. Gel electrophoresis was carried out in 0.5 × Tris-Borate-EDTA (TBE) buffer at 100 V for 1 h in a Sub-Cell system (Bio-Rad Laboratories, CA). DNA bands were visualized by a UV lamp using a Gel Doc system (Synoptics Ltd., UK).

Cell Cultures

COS-7 cells, HeLa cancer cells and MCF-7 breast cancer cells were cultured in DMEM (4.5 g/L glucose) supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL) and streptomycin (100 μg/mL) at 37 °C under a 5% CO_2 humidified atmosphere. Confluent cells were subcultured every 3 days using standard procedures.

MTT Analysis

COS-7 cells were seeded into 96-well plates at a seeding density of 5000 cells/well in 200 μL medium. After 24 h incubation, the culture medium was removed and replaced with 200 μL of medium containing 50 μL of PEI solutions or DMEDA-*g*-SBC solutions with a series of concentrations, such as 0.001, 0.005, 0.01, 0.05, 0.1,0.25, 0.5 and 1.0 mg/mL. The cells were continuously cultured for 48 h. Then, 20 μL of 5 mg/mL MTT assays stock

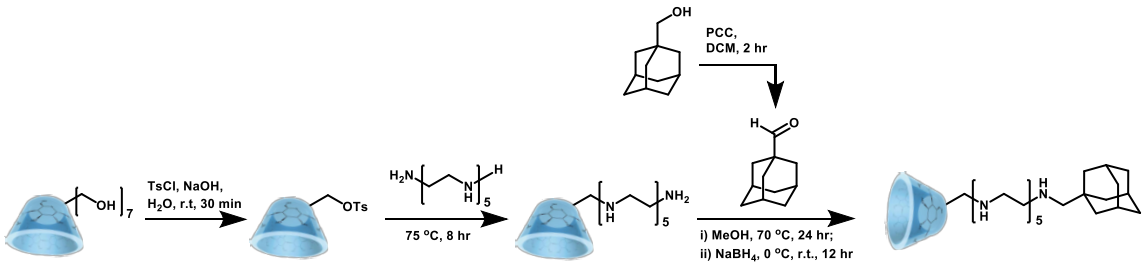
solution in PBS buffer was added to each well. After the cells being incubated for 4 h, the medium containing unreacted dye was carefully removed. The resulting blue formazan crystals were dissolved in 200 μL of DMSO, and the absorbance was measured in a Perkin-Elmer 1420 Multi-label counter at a wavelength of 490 nm.

In vitro Transfection Assay

With regard to luciferase expression studies, three different cells including COS-7 cells, HeLa cancer cells and MCF-7 cancer cells, were seeded at a density of 10^4 cells per well in 96-well plates and incubated for 16-24 h until 60-70% confluent at 37 °C and 5% CO_2. Immediately prior to transfection, the medium was removed. Then, these cells were washed and replaced with fresh and prewarmed DMEM in the absence of 10% FBS. Polyplexes were added to each well, and these cells were incubated at 37 °C for 4 h. The medium was then substituted for fresh DMEM with or without 10% FBS, and then incubated for an extra 48 h. The luciferase assay was conducted in accordance with manufacturer's protocol (Promega, Madison, WI). Relative light units (RLUs) were measured with GloMaxTM 96 microplate luminometer (Promega). The obtained RLUs were normalized with regard to protein concentration in the cell extract that was determined by the BCA protein assay kit (Beyotime, China).

3. Synthesis procedures

3.1 Synthesis of *β*-CD/adamantane-terminated pentaethylenehexamine (AD-PEHA-CD)



Scheme S1. Synthesis route of AD-PEHA-CD.

3.1.1 Synthesis of mono-(6-*O*-(*p*-tolylsulfonyl))-*β*-CD (*β*-CD-OTs)

Mono-(6-*O*-(*p*-tolylsulfonyl))-*β*-CD (*β*-CD-OTs) was prepared according to literature procedure.[1] To a solution of anhydrous *β*-CD (25.0 g, 22.0 mmol) in 0.4 M aqueous sodium hydroxide (250 mL) at 0 °C, *p*-toluenesulfonyl chloride (17.5.0 g, 92 mmol) was slowly added under vigorous stirring over 10 min. The mixture was stirred for another 30 min at 0 °C. After which, the reaction system was filtered quickly. The filtrate was then neutralized to pH 8.5 with 1M HCl solution and stirred for another 1 h. The resultant precipitate was filtered off and then washed with deionized water (30 mL × 3) and dried at 60 °C for 48 h to give *β*-CD-OTs as white solids (10.0 g, 7.75mmol, 35%).

^{1}H NMR (DMSO-d_6, 400 MHz) (***Fig. S1***): δ_H (ppm) = 2.41 (s, Ph-C***H***$_3$, 3H), 3.16-3.75 (m, ***H***-2,3,4,5,6, 42H), 4.11-4.55 (m, ***OH***-6, 6H), 4.72-4.90 (m, ***H***-1, 7H), 5.25-6.25 (br, ***OH***-2,3, 14H), 7.40 (d, J = 8.6 Hz, C***H*** on Ph, 2H), 7.75 (d, J = 7.8 Hz, C***H*** on Ph, 2H). **^{13}C NMR** (DMSO-d_6, 101 MHz): δ_C (ppm) = 21.90, 60.57, 72.70, 73.07, 73.37, 73.73, 82.15, 102.59, 128.25, 130.57, 133.28, 145.52.

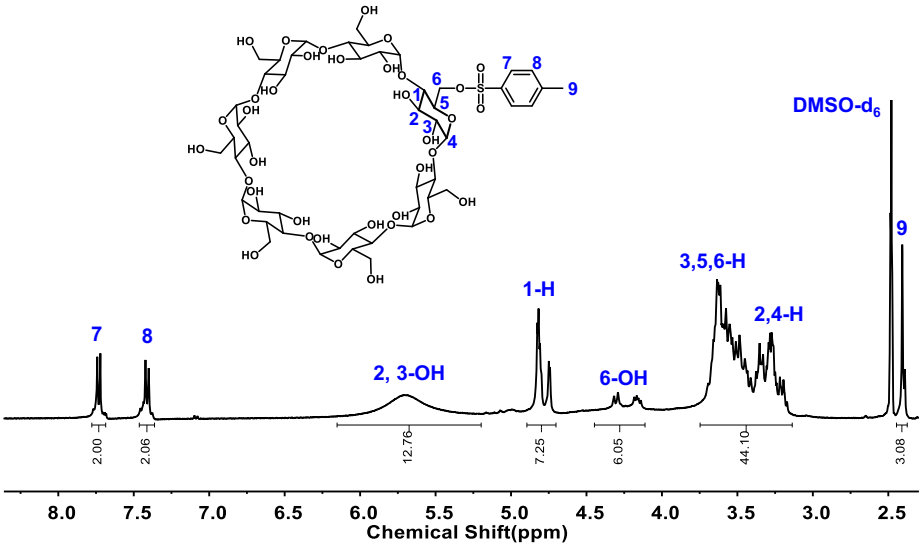


Fig. S1. ^{1}H NMR spectrum of *β*-CD-OTs in DMSO-d_6.

3.1.2 Synthesis of *β*-CD-mono-substituted PEHA (*β*-CD-PEHA)

β-CD-PEHA was prepared according to the literature.[2] Mono-(6-*O*-(*p*-tolylsulfonyl))-*β*-CD (*β*-CD-OTs) (11.6 g) was suspended in an excess amount of PEHA (66 mL) under nitrogen atmosphere, and the reaction system was stirred at 75 ^{o}C for 8 h. Then the mixture was cooled down to room temperature naturally and cold acetone (160 mL) was subsequently added into it. The precipitate was filtrated and then redissolved in in 50 mL of a water-methanol mixture, followed by re-precipitation in 100 mL acetone for several times to eliminate the unreacted PEHA. After drying at 60 ^{o}C for 48 h in a vacuum oven, a white solid (*β*-CD-PEHA) was obtained (68.5%).

^{1}H NMR (D_2O, 400 MHz) (***Fig. S2***): δ_H (ppm) = 2.74 (m, -NHC***H$_2$***C***H$_2$***NH-, 20H), 3.36-3.95 (m, ***H***-2,3,4,5,6, 42H), 4.72-4.90 (m, ***H***-1, 7H). **UPLC & Q-ToF-MS** of *β*-CD-PEHA: calculated for *m/z* = 1349.60 $[M+H]^+$, found *m/z*: 1349.6096 $[M+H]^+$.

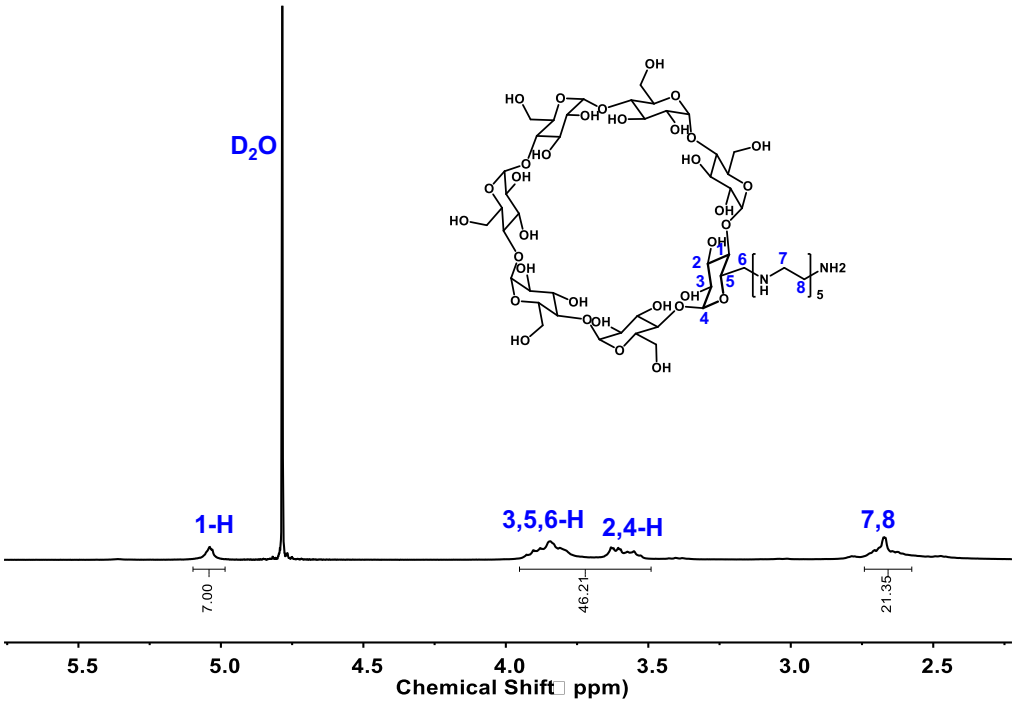


Fig. S2. [1]H NMR spectrum of *β*-CD-PEHA.

3.1.3 Synthesis of adamantylcarboxaldehyde(AD-CHO)

Pyridinium chlorochromate (PCC) (5.84 g, 27.1 mmol) was added to the solution of adamantan1ylmethano (3.0 g, 18.6 mmol) in dry dichloromethane (70 mL) slowly at room temperature under nitrogen atmosphere.[3] The reaction system was stirred for 90 min. After which the reaction mixture was diluted with dry diethyl ether (~600 mL) and vigorously stirred. The obtained mixture was filtered through a magnesium silicate adsorbent(synthetic) (125-250 μm) column and flushed with diethyl ether. The filtrate was concentrated under vacuum to obtain white solid (2.67 g, 90%).

^{1}H NMR ($CDCl_3$, 400 MHz) (***Fig. S3***): δ_H (ppm) = 1.50-2.12 (m, protons on AD, 15H), 9.30 (s, -***CH***O, 1H). **^{13}C NMR** ($CDCl_3$, 101 MHz) (***Fig. S4***): δ_C (ppm) = 27.3, 35.8, 36.4, 36.5, 206.0. **FTIR** (KBr) (***Fig. S7***): ν (cm^{-1}): 2720, 1730. **UPLC & Q-ToF-MS** of AD-CHO: calculated for *m/z* = 165.12 $[M+H]^+$, found *m/z*: 165.12 $[M+H]^+$.

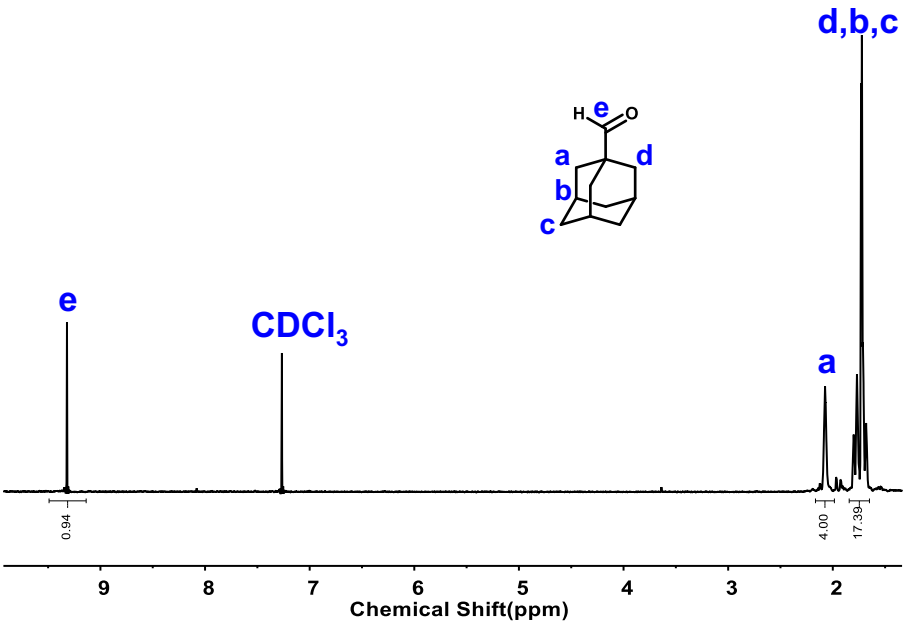


Fig. S3. ^{1}H NMR spectrum of AD-CHO in $CDCl_3$.

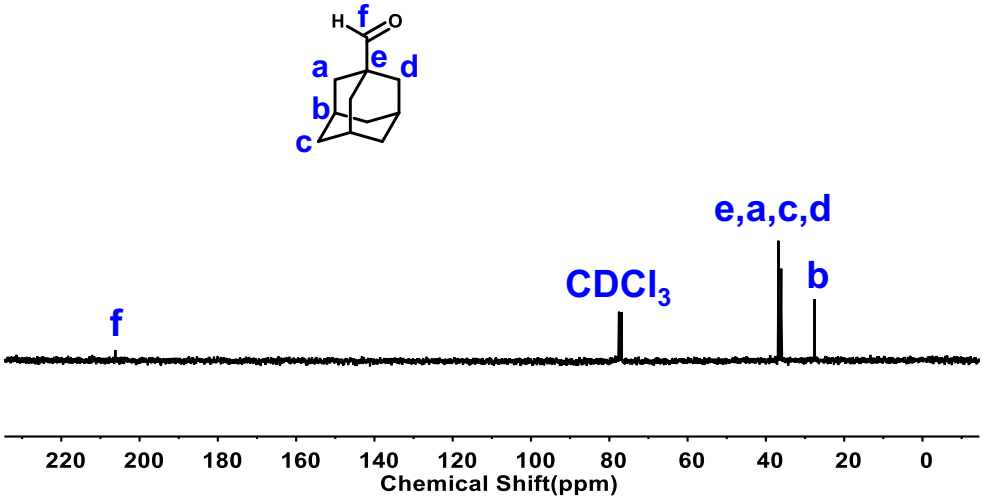


Fig. S4. ^{13}C NMR spectrum of AD-CHO in $CDCl_3$.

3.1.4 Synthesis of *β*-CD/AD-terminated PEHA (AD-PEHA-CD)

β-CD-PEHA (2.70 g, 2.0 mmol) was dissolved in a mixture of 60 mL of anhydrous methanol and 40 mL of anhydrous DMF, then adamantane-carboxaldehyde (0.657 g, 4 mmol) was added. The reaction system was heated to 70 ^{o}C. After 24 h, the reaction system was cooled to 0 ^{o}C and $NaBH_4$ (0.46 g, 12 mmol) was added slowly within 15 min.

Subsequently, the reaction system was stirred for 12 h at room temperature. Then, 3 M HCl (4 mL) was added to the mixture, and the resulting mixture was continuously stirred for another 2 h. The reaction solution was concentrated in vacuo, and the residue was purified with silica gel column chromatography (CH_2Cl_2-methanol as eluent) to provide red-brown oil (2.45 g, 82%).

^{1}H NMR (DMSO-d_6, 400 MHz) (***Fig. S5***): δ_H (ppm) = 1.50-2.12 (m, protons on AD, 15H), 2.00-3.05 (m, -NHC***H***$_2$C***H***$_2$NH-, 20H), 3.48 (m, Adamantane -C***H***$_2$NH-, 2H), 3.16-3.75 (m, ***H***-2,3,4,5,6, 42H), 4.92-5.15 (s, ***H***-1, 7H). **^{13}C NMR** (DMSO-d_6, 101 MHz) (***Fig. S6***): δ_C (ppm) = 27.3, 35.8, 36.4, 36.5, 49.0, 63.80, 72.53, 72.85, 73.51, 81.99, 102.43. **UPLC & Q-ToF-MS** of AD-PEHA-CD: calculated for *m/z* = 1497.72 $[M+H]^+$, found *m/z*: 1497.7345 $[M+H]^+$.

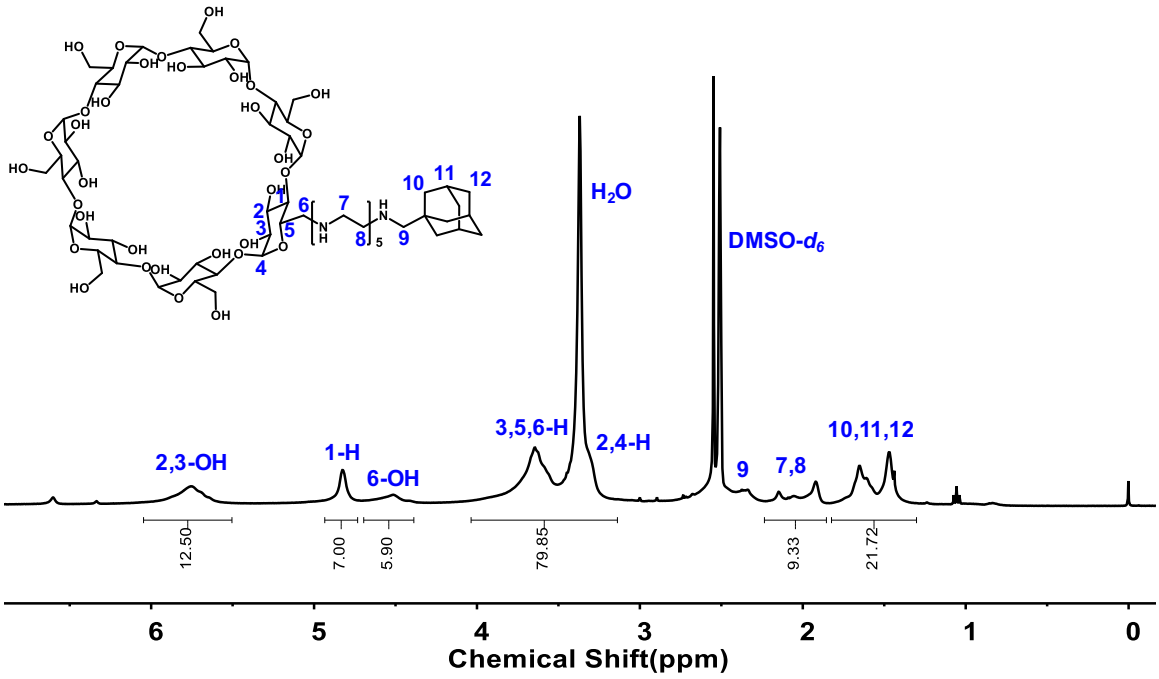


Fig. S5. ^{1}H NMR spectrum of AD-PEHA-CD in DMSO-d_6.

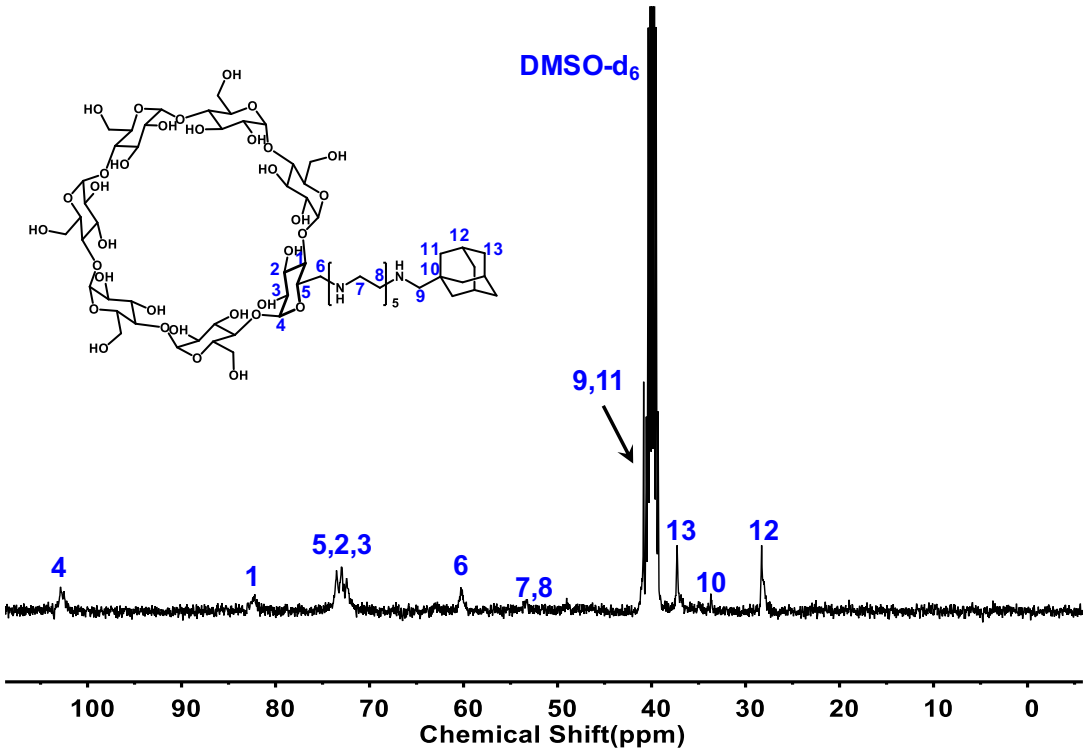


Fig. S6. ^{13}C NMR spectrum of AD-PEHA-CD in DMSO-d_6.

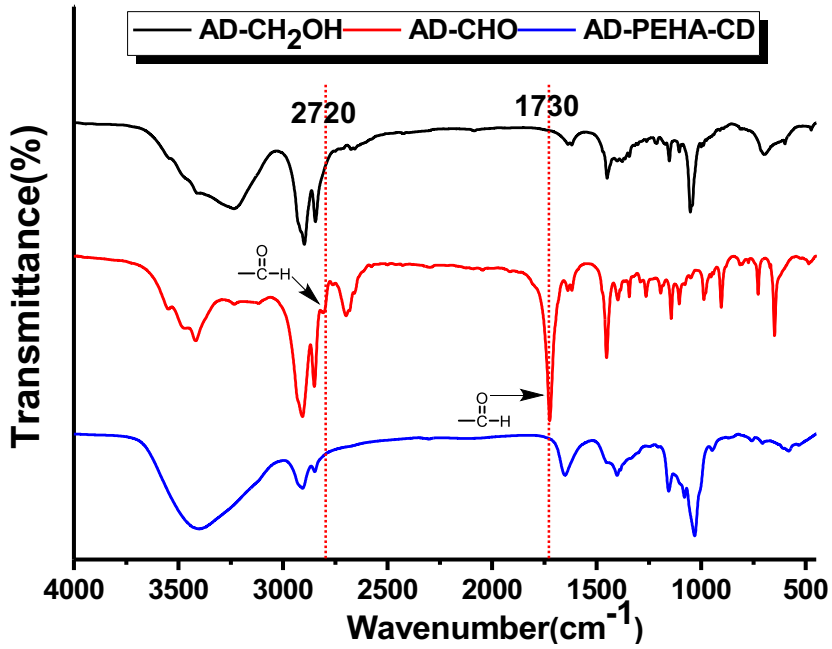
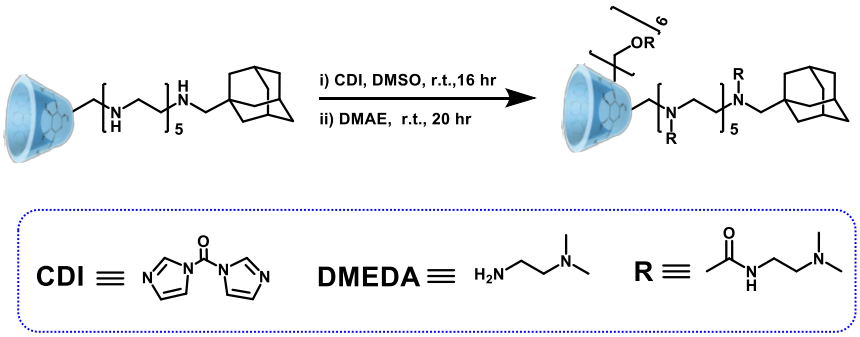


Fig. S7. FTIR spectra of AD-CH_2OH, AD-CHO, AD-PEHA-CD.

3.2 Synthesis of *N,N*-DMEDA-functionalized *β*-CD and adamantane-terminated pentaethylenehexamine (DMEDA-CD-PEHA-AD)



Scheme S2. Synthesis route of DMEDA-CD-PEHA-AD.

The synthesis procedure of DMEDA-CD-PEHA-AD was according to the ***Scheme S2***. The Dry AD-PEHA-CD (1.496 g, 1.0 mmol) in 15 mL anhydrous DMSO was added dropwise to the solution of CDI (2.9 g, 18.0 mmol) in 20 mL of anhydrous DMSO under vigorous stirring for *ca.* 2 h under nitrogen atmosphere. After which, the reaction mixture was stirred at room temperature. After 16 h, DMEDA (3.12 g, 36.0 mmol) was added slowly via a syringe to continued reacting for another 20 h with stirring. The resulting reaction solution was concentrated to about 20 mL under reduced pressure and precipitated in diethyl ether (250 mL). The resulting mixture was filtrated off. The residue was re-dissolved in methanol (15 mL) and then precipitated in diethyl ether (300 mL) for three times. The precipitates were dried at 60 ^{o}C in vacuum to yield DMEDA-CD-PEHA-AD as a white powder (1.81 g, 63%).

^{1}H NMR (DMSO-d_6, 400 MHz) (***Fig. S8***): δ_H (ppm) = 1.50-2.12 (m, protons on AD, 15H), 2.13 (br, 72H), 2.28 (br, 24H), 3.05 (br, 24H), 2.00-3.05 (m, -C***H***$_2$C***H***$_2$-, 20H), 3.48 (m, AD-C***H***$_2$-, 2H), 3.16-3.75 (m, ***H***-2,3,4,5,6, 42H), 4.92-5.15 (s, ***H***-1, 7H), 5.6-6.0 (m, ***OH***-2,3,14H), 6.94 (br, 12H). **^{13}C NMR** (DMSO-d_6, 101 MHz) (***Fig. S9***): δ_C (ppm) = 27.3, 35.8, 36.4, 36.5, 45.70, 49.0, 58.95, 63.80, 72.53, 72.85, 73.51, 81.99, 102.43, 156.90. **UPLC & Q-ToF-MS** of DMEDA-CD-PEHA-AD: calculated for *m/z* = 2866.67 $[M+H]^+$,

found *m/z*: 2866. 67 $[M+H]^+$.

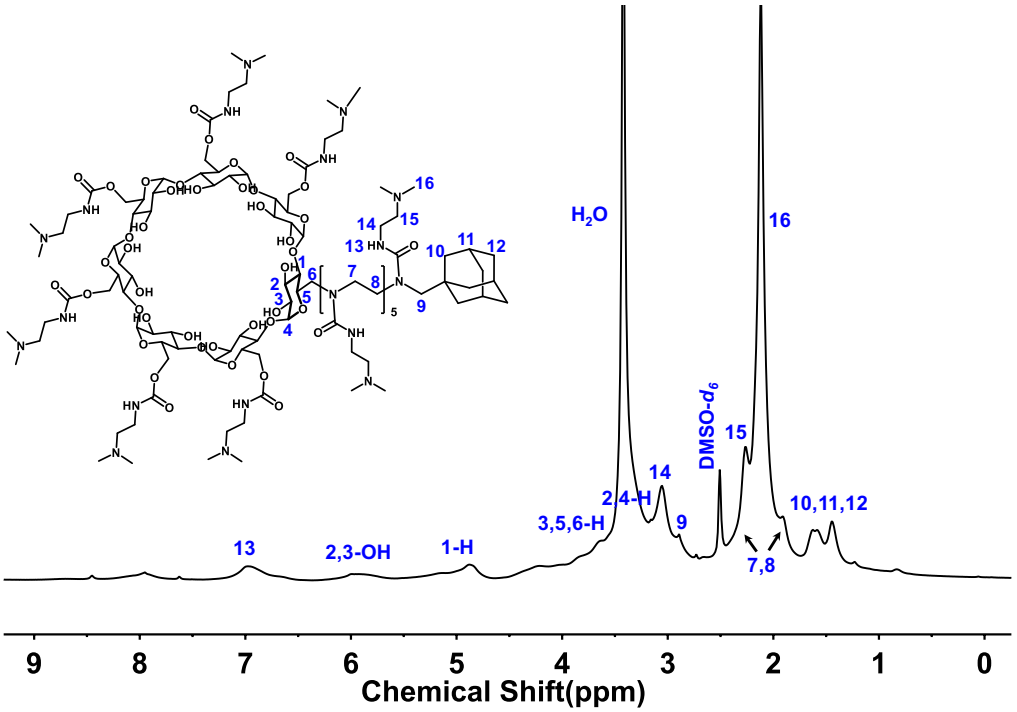


Fig. S8. ^{1}H NMR spectrum of DMEDA-CD-PEHA-AD in DMSO-d_6.

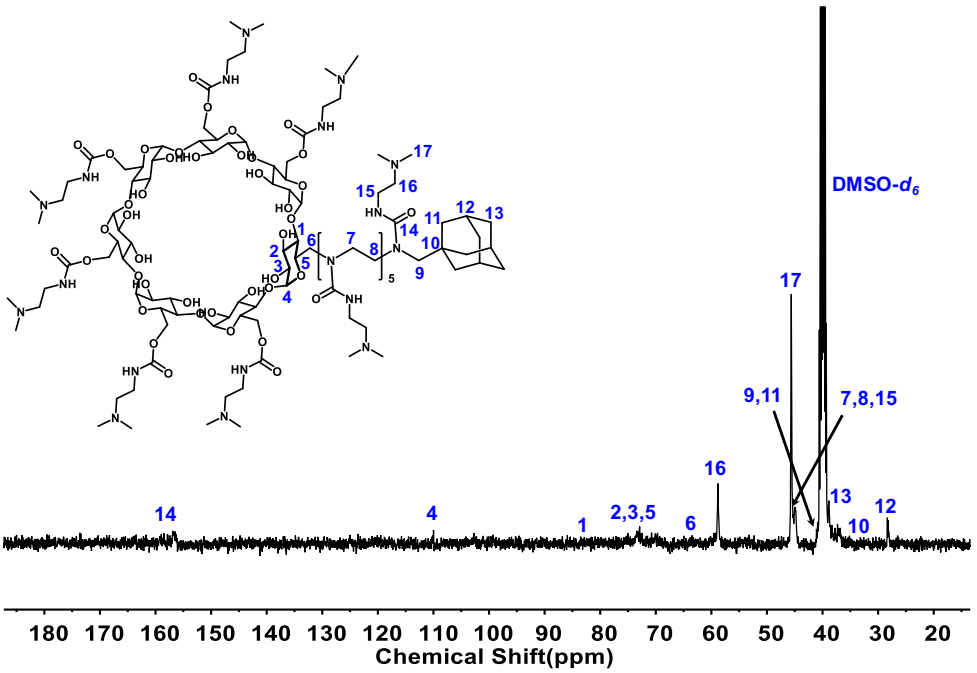
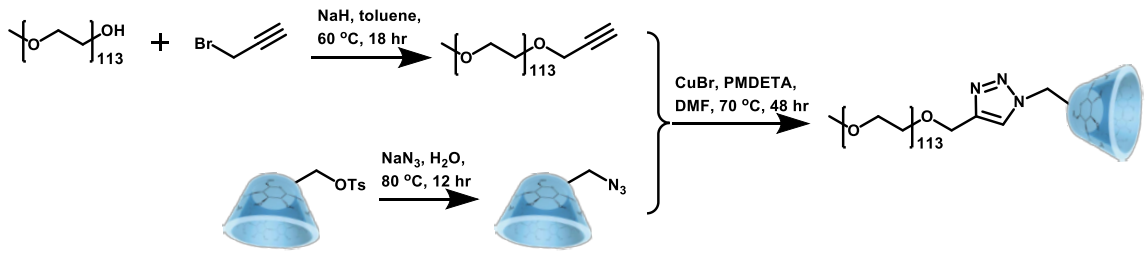


Fig. S9. ^{13}C NMR spectrum of DMEDA-CD-PEHA-AD in DMSO-d_6.

3.3 Synthesis of *β*-CD-monosubstituted poly(ethylene glycol) (PEG-CD)



Scheme S3. Synthesis route of PEG-CD.

3.3.1 Synthesis of monoalkynyl-terminated PEG (PEG-Alkyne)

The synthesis procedure of PEG-Alkyne was as follows:[4] PEG_{113}-OH (7.5 g, 1.5 mmol) was dissolved in toluene (90 mL), then the solution was allowed to warm to 60 °C and azeotropic distillation of 15-20 mL of toluene under vacuum to remove traces of water. After which sodium hydride (0.108 g, 4.5 mmol) was added to the solution under stirring. After15 min, propargyl bromide (7.5 mmol dissolved in 10 mL of toluene) was added dropwise to the reaction system then the resulting solution was allowed to stir at 60 °C for 18 h. The reaction system was allowed to cooled room temperature then filtered, the filtrates were concentrated under vacuum to get residue. The residue was dissolved in CH_2Cl_2 (50 mL). The solution was washed with saturated aqueous $NaHCO_3$ solution (15 mL × 3), dried over anhydrous Na_2SO_4 and treated with activated charcoal. The solution was precipitated into a large number of *n*-hexane after filtration. The dissolution and precipitation cycle was repeated three times. After drying in a vacuum oven overnight at room temperature, PEG-Alkyne was obtained as a white solid (6.24 g, yield 83%).

^{1}H NMR ($CDCl_3$, 400 MHz) (***Fig. S10***): δ_H (ppm) = 4.2 (2H, -OC***H***$_2$C≡CH), 3.7 (450H, -OC***H***$_2$C***H***$_2$O-), 3.4 (3H, C***H***$_3$O-), and 2.4 (1H, -OCH$_2$C≡C***H***). **^{13}C NMR** ($CDCl_3$, 101 MHz) (***Fig. S11***): δ_C (ppm) = 58.33, 58.97, 69.03, 70.52, 71.88, 74.62, 79.61. **FTIR** (KBr) (***Fig. S15***): ν (cm^{-1}) = 3256 (C≡C). **GPC** (***Fig. S16***): 5.03 kDa with PDI of 1.02, which is consistent with the value of 4.96 kDa determined by NMR.

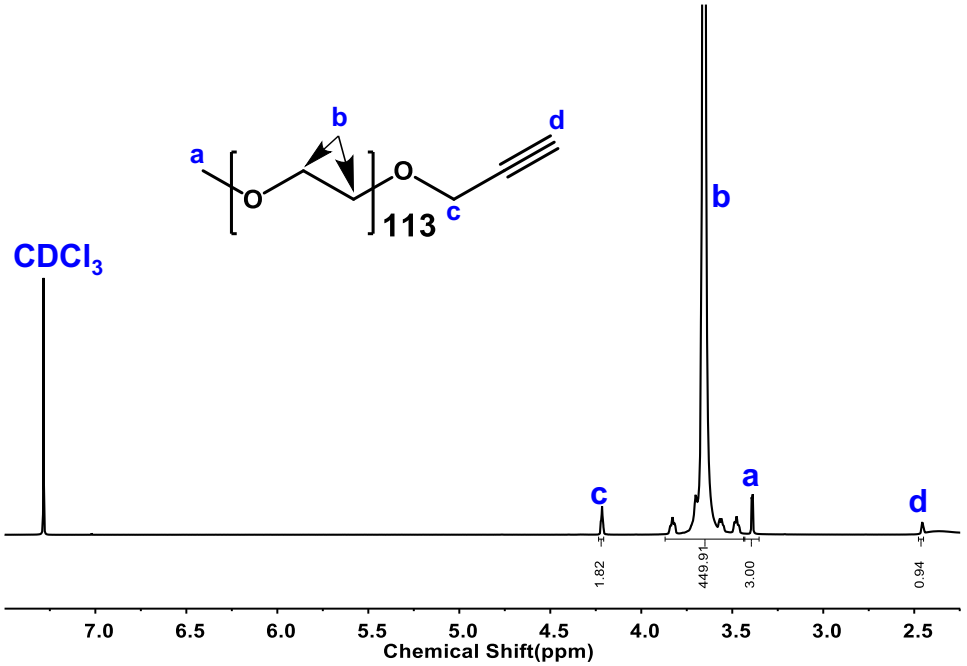


Fig. S10. ^{1}H NMR spectrum of PEG-Alkyne in $CDCl_3$.

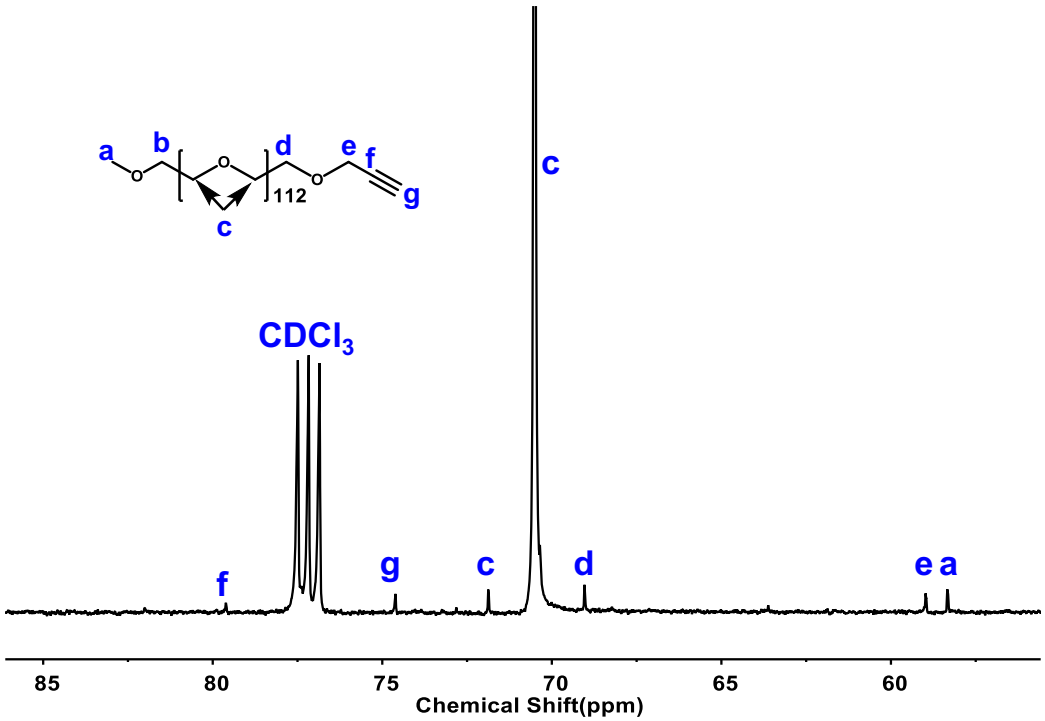


Fig. S11. ^{13}C NMR spectrum of PEG-Alkyne in $CDCl_3$.

3.3.2 Synthesis of mono-(6-azido-6-desoxy)-*β*-CD (*β*-CD-N_3)

Mono-(6-azido-6-desoxy)-*β*-CD (*β*-CD-N_3) was prepared according to literature procedure.[1] Sodium azide (5.06 g, 77.0 mmol) was added slowly to a solution of nono-(6-*O*-(*p*-tolylsulfonyl))-*β*-CD (20.0 g, 15.52 mmol) in deionized water (150mL) at 80 °C. The reaction system was allowed to stir 12 h until it became transparent. Acetione (1200mL) was poured into the system and the resulting mixture was filtered. The white solid was dissolved in water (60 mL) and precipitated in acetone (600 mL) again. The collected white powder was then dried in vacuum oven at 60 °C for 48 h (16.38 g, 14.12 mmol, 91.1%).

^{1}H NMR (DMSO-d_6, 400 MHz) (***Fig.12***): δ_H (ppm) = 3.12-3.42 (m, ***H***-2,4, 14H), 3.49-3.82 (m, ***H***-3,5,6, 28H), 4.40-4.58 (m, ***OH***-6, 6H), 4.75-4.92 (m, ***H***-1, 7H), 5.52-5.92 (m, ***OH***-2,3, 14H). **^{13}C NMR** (DMSO-d_6, 101 MHz): δ_c (ppm) = 51.73, 60.57, 70.85, 72.68, 73.03, 73.71, 82.16, 83.62, 102.58. **FTIR** (KBr) (***Fig. S15***): ν (cm^{-1}) = 2106 (N_3).

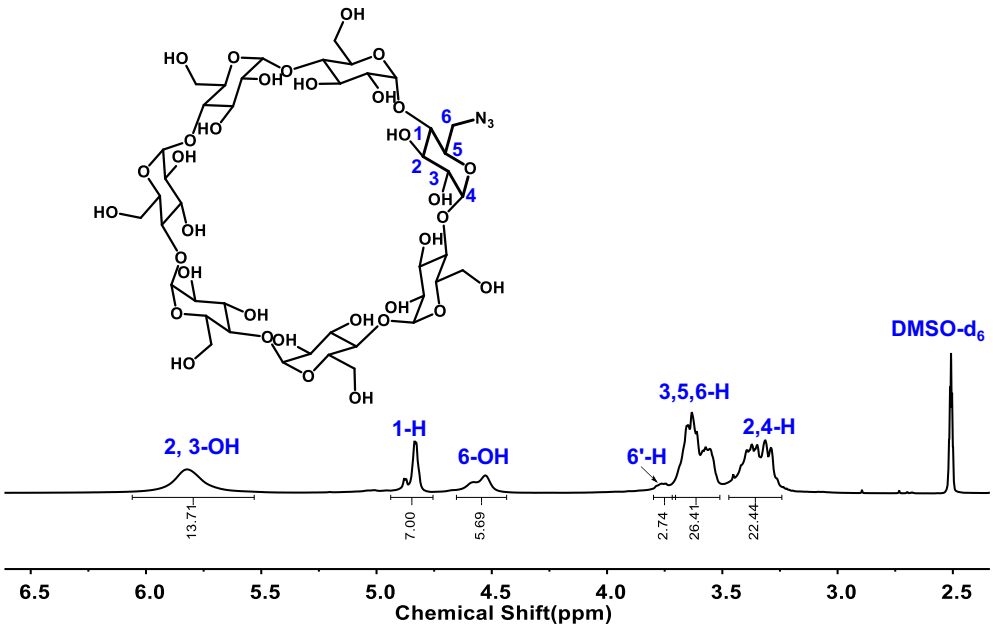


Fig. S12. ^{1}H NMR spectrum of *β*-CD-N_3 in DMSO-d_6.

3.3.3 Synthesis of PEG-CD

Catalyst CuBr (72mg, 4.31 mmol, 1.0 *equiv.*) and PMDETA (87 mg, 4.31 mmol, *1.0 equiv.*) were added to the solution of PEG-Alkyne (2.50 g, 0.5mmol, 1.0 *equiv.*) and *β*-CD-N_3 (0.70 g, 0.6mmol, 1.2 *equiv.*) in dry DMF (60 mL) under nitrogen atmosphere. The mixture was allowed to warm to 70 ^{o}C with vigorous stirring for 48 h. After which the reaction system was exposed to air under stirring for 30 min to terminate the reaction, and the solvent was removed in vacuum, followed by dilution with chloroform (200 mL) and passing through neutral alumina to get rid of the copper salt. The filtrate was concentrated and then purified by dialyzing against deionized water for 48 h using a dialysis tube with molecular-weight cut-off (MWCO) of 2 kDa, to remove excess *β*-CD-N_3 and other impurities. After removal of the water by freeze-drying, a white powder was obtained (2.23 g, 72%).

^{1}H NMR (DMSO-d_6, 400 MHz) (***Fig. S13***): δ_H (ppm) = 3.2 (m, C***H***$_3$O-, 3H), 3.40-3.75 (m, ***H***-2,3,4,5,6, -OC***H***$_2$C***H***$_2$O-, 492H), 4.43-4.62 (m, 6-***OH***, C***H***$_2$-triazole, 8H), 4.75-4.92 (m, ***H***-1, 7H), 5.52-5.92 (m, ***OH***-2,3, 14H), 8.05 (s, C***H*** on triazole, 1H). **^{13}C NMR** (DMSO-d_6, 101 MHz) (***Fig. S14***): δ_C (ppm) = 58.51, 60.42, 60.69, 63.80, 70.26, 71.75, 72.53, 72.85, 73.51, 81.99, 102.43, 124.43, 145.21. **FTIR** (KBr) (***Fig. S15***): ν (cm^{-1}) = 1601 (C=C), 1518/1499 (N=N). **GPC** (***Fig. S16***): 6.73 kDa with PDI of 1.20, in good agreement of the value of 6.16 kDa calculated by NMR.

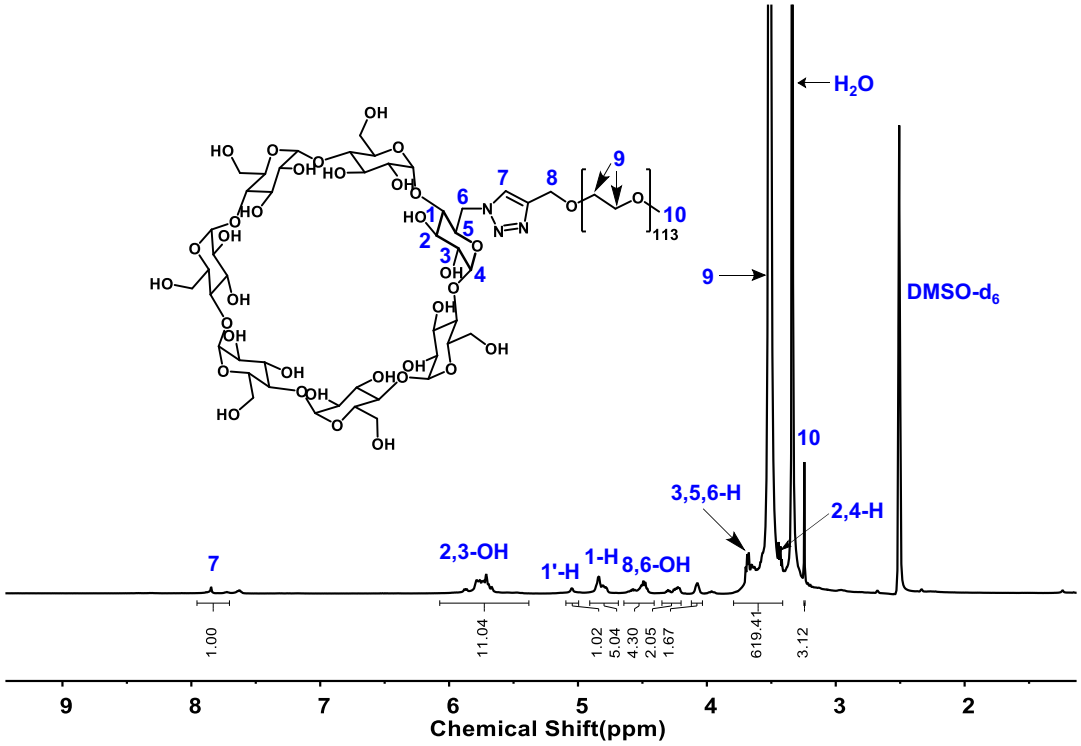


Fig. S13. ^{1}H NMR spectrum of PEG-CD in DMSO-d_6.

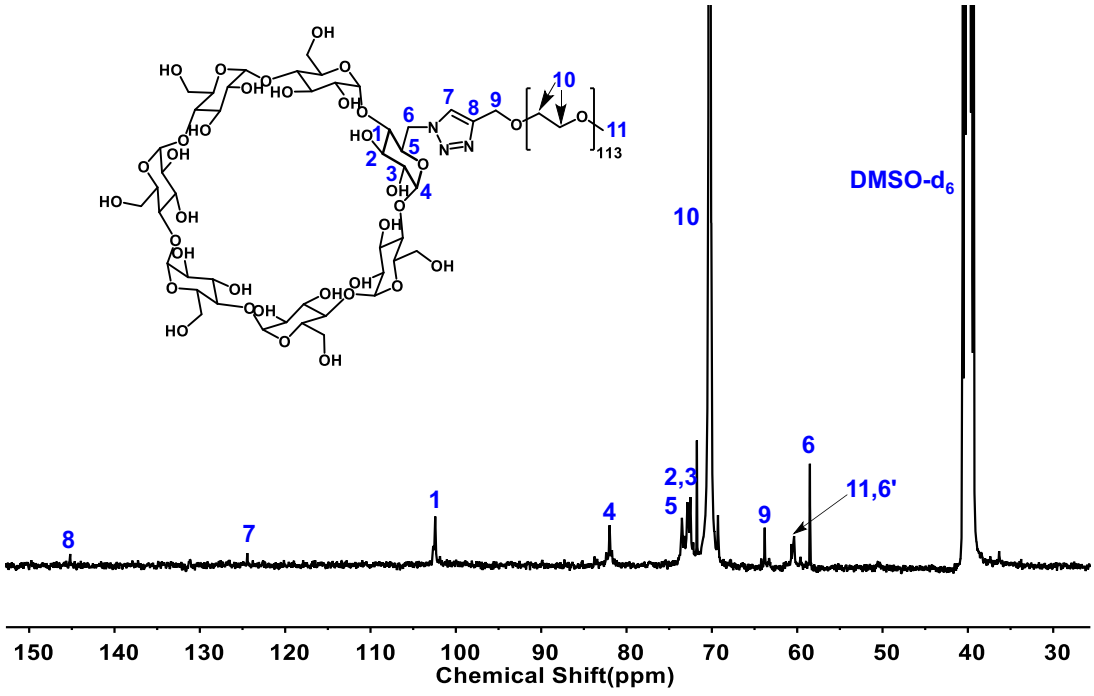


Fig. S14. ^{13}C NMR spectrum of PEG-CD in DMSO-d_6.

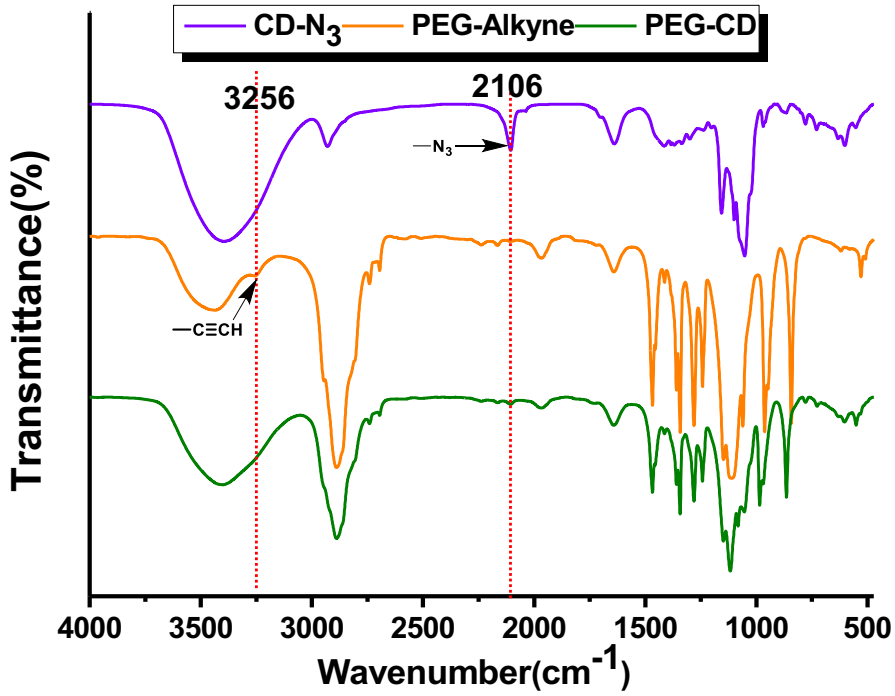


Fig. S15. FTIR spectra of β-CD-N_3, PEG-Alkyne and PEG-CD.

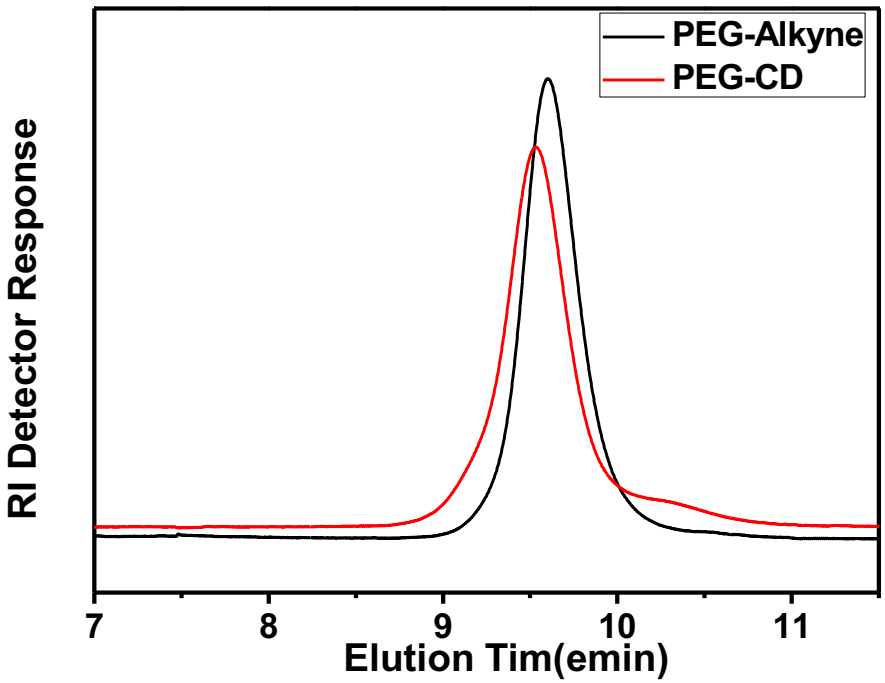


Fig. S16. GPC spectra of PEG-Alkyne and PEG-CD.

4. Preparation of supramolecular block copolymers

For supramolecular block copolymers, the constant molar concentration of PEG-CD (1 mM) was mixed with different amounts of AD-PEHA-CD or DMEDA-CD-PEHA-AD macromolecular building blocks in water at the molar ratios from 1:0 to 1:40, followed by continuous stirring at ambient temperature for 6 h to ensure that the supramolecular polymerization is complete, and finally charge-variable supramolecular block copolymers with various polymerization degrees will be acquired.

5. Determination of mobility and conductivity of SHP and SBC

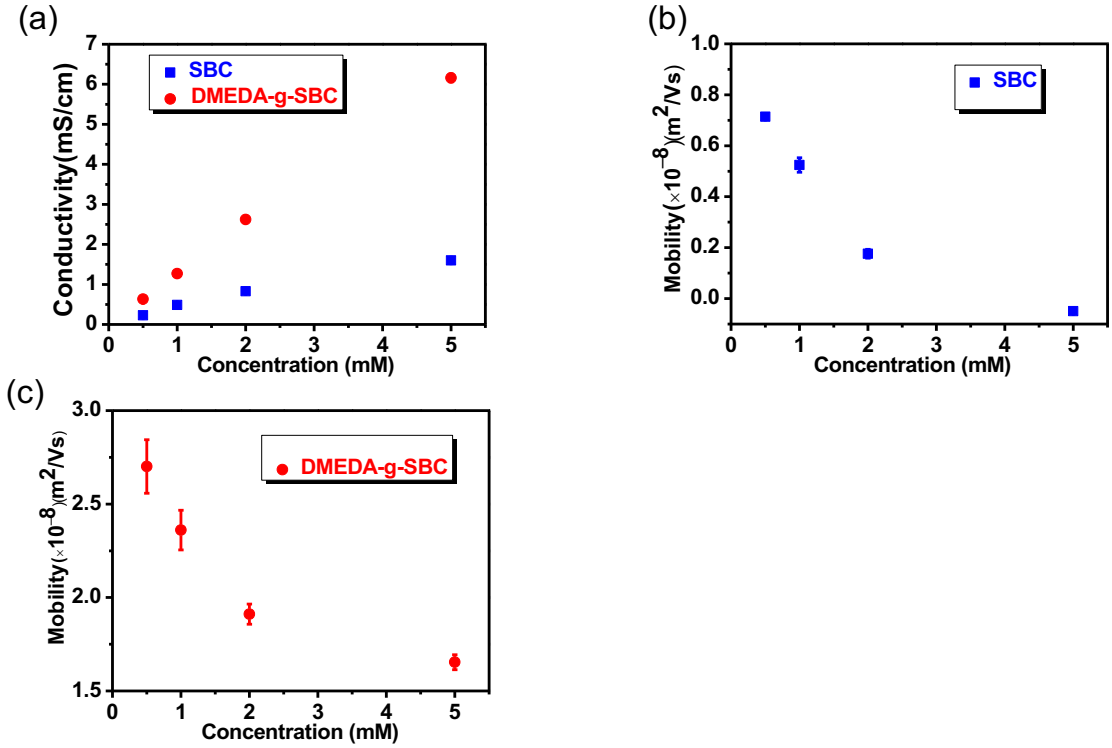


Fig. S17. (a) conductivity and (b,c) mobility of supramolecular block copolymer (SBC) and its DMEDA-functionalized supramolecular block copolymer (DMEDA-*g*-SBC) in PBS buffer (pH 7.4) as a function of molar concentration.

6. Degree of polymerization and molecular weight of SBCs

Table S1. The degree of polymerization (DP) and molecular weight of SBC and DMEDA-*g*-SBC in water at different concentrations.

Molar ratios	Concentration(M)	DP	$M_{n\text{-}SBC}$(kDa)[a]	$M_{n\text{-}DMEDA\text{-}g\text{-}SBC}$(kDa)[a]
1:00	0	0	6.20	6.20
1:0.5	0.0005	4	12.18	17.66
1:1	0.001	6	15.17	23.39
1:2	0.002	8	18.17	29.12
1:5	0.005	13	25.65	43.45
1:10	0.01	18	33.13	57.77
1:20	0.02	25	43.60	77.83
1:30	0.03	31	52.57	95.02
1:40	0.04	36	60.05	109.4

[a] $M_{n\text{-}SBC}=M_{PEG\text{-}CD}+DP \times M_{AD\text{-}PEAH\text{-}CD}$ ($DP=(K_a[\text{Conc.}])^{1/2}$; $K_a=3.236 \times 10^4 M^{-1}$)

[a] $M_{n\text{-}DMEDA\text{-}g\text{-}SBC}=M_{PEG\text{-}CD}+DP \times M_{DMEDA\text{-}CD\text{-}PEHA\text{-}AD}$ ($DP=(K_a[\text{Conc.}])^{1/2}$; $K_a=3.236 \times 10^4 M^{-1}$)

7. Agarose gel electrophoresis retardation by PEHA

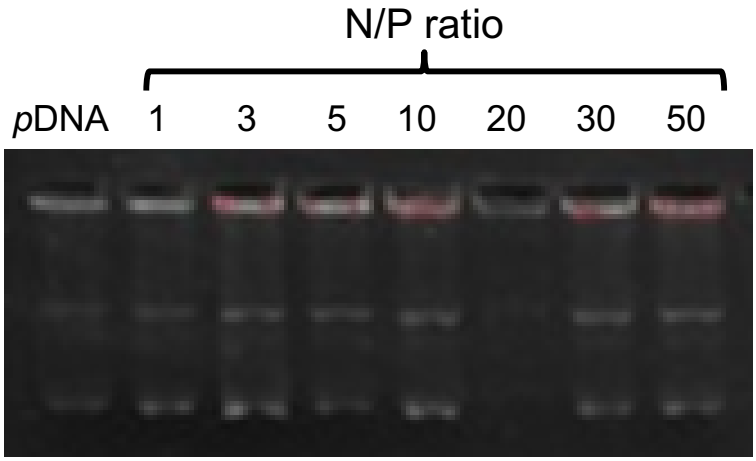


Fig. S18. Agarose gel electrophoresis retardation of *p*DNA by PEHA at various N/P ratios of 1, 3, 5, 10, 20, 30 and 50.

In ***Fig. S18***, even though the N/P ratio of PEHA/*p*DNA is up to 20, *p*DNA can migrate through agarose gel at roughly the same rate as naked *p*DNA. This result indicates that *p*DNA cannot be retarded by the PEHA molecule.

8. Physiological stability of SBC or DMEDA-*g*-SBC/pDNA polyplexes

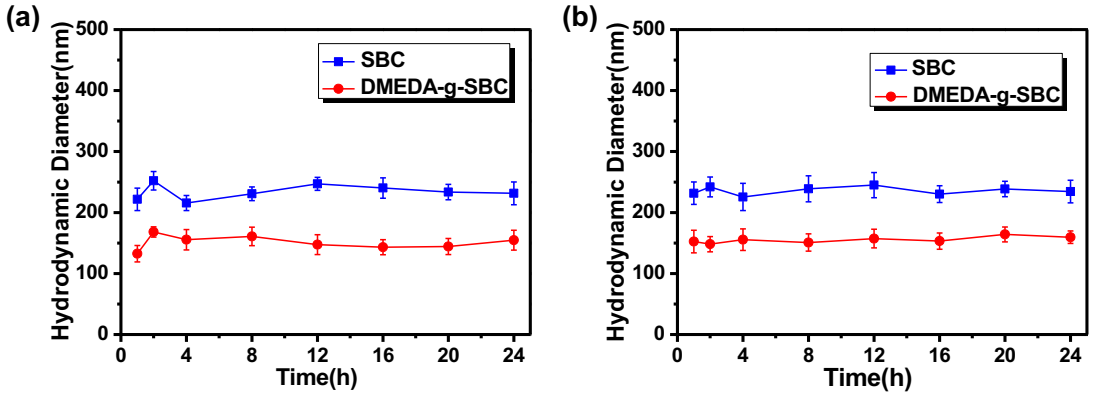


Fig. S19. Size variation of SBC/pDNA and DMEDA-*g*-SBC/pDNA polyplexes at N/P ratio of 30 in (a) PBS buffer (pH 7.4) and (b) serum at 37 ^{o}C for 24 h monitored by DLS.

9. References

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