Supporting Information

A Novel High-capacity Immunoadsorbent with PAMAM Dendritic Spacer Arms by Click Chemistry

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General Methods

^{1}H NMR spectra were recorded on a 300 MHz NMR spectrometer (Mercury-Plus 300, Varian, American). The multiplicity of the ^{1}H NMR signal in the ^{1}H NMR spectra were expressed by the following abbreviations, respectively: s, singlet; d, doublet; t, triplet; m, multiplet. FTIR spectra were performed on a FTIR spectrophotometer (Nexus Por Euro, Nicolet, American). ^{13}C NMR spectra were proton decoupled and recorded on a 400 MHz NMR spectrometer using the carbon signal of the deuterated solvent as the internal standard. The absorbance of IgG standard solution at 280 nm, A_{280}, was determined on Ultraviolet-visible spectrophotometer (UV-2450, Shimadzu, Japan). All chemicals were obtained from commercial sources and used as received, unless otherwise mentioned.

1. Synthesis of propargyl-PAMAM Dendrons

1.1. Preparation of propargyl-PAMAM dendron G1 (G1.0 PAMAM)

G1.0 PAMAM was synthesized according to the procedures described in Fig. S4. A solution of propargyl amine (1.5 g, 27.2 mmol) in methanol (30 mL) was added dropwise to a stirred solution of MA (23.42 g, 272 mmol) in methanol (70 mL) over a

period of 1 h at ice-water bath. The resulting solution was stirred for 30 min at ice-water bath and then allowed to warm to room temperature and stirred for further 36 h. The volatiles were removed under reduced pressure using a rotary evaporator and vacuum to give the desired propargyl-PAMAM dendron G0.5 (G0.5 PAMAM) (yield: 98%).

A solution of G0.5 PAMAM (5.8 g, 25.5 mmol) in methanol (30 mL) was added dropwise to a stirred solution of 1,2-diaminoethane (30.65 g, 510 mmol) in methanol (100 mL) over a period of 1 h at ice-water bath. The resulting solution was allowed to warm to room temperature and stirred for further 72 h at room temperature at which time no methyl ester was detectable by NMR spectroscopy. The solvent was removed under reduced pressure using a rotary evaporator maintaining the temperature no higher than 40 ºC and then the excess 1,2-diaminoethane was removed using an azeotropic mixture of toluene and methanol (9:1). The remaining toluene was removed by azeotropic distillation using methanol and finally kept under vacuum to provide the amino-terminated product (yield: 98%).

G0.5 PAMAM FTIR: 3280, 2950, 2840, 2100, 1740, 1440, 1260, 1170, 1050 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 2.20 (s, 1H), 2.47 (t, 4H), 2.84 (t, 4H), 3.43 (d, 2H), 3.67 (s, 6H).

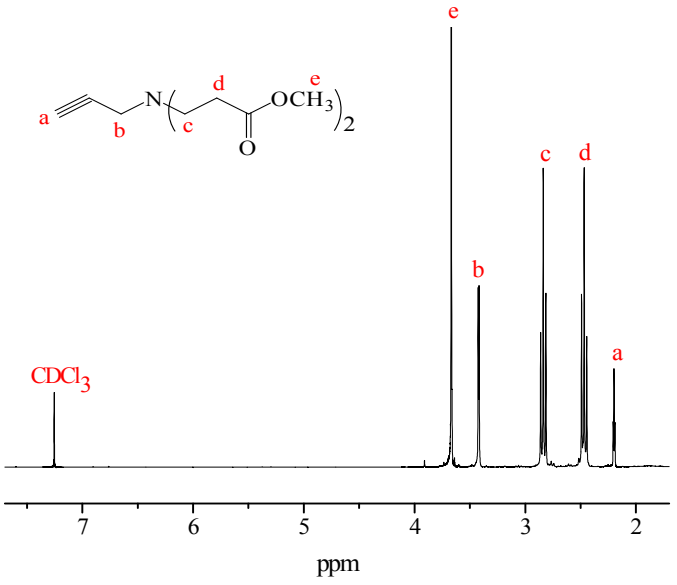

Fig. S1 The ^1H NMR spectrum of G 0.5 PAMAM.

G1.0 PAMAM FTIR: 3280, 3070, 2930, 2860, 1640, 1550, 1460, 1320, 1120,

1040 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 2.22 (s, 1H), 2.39 (t, 4H), 2.83 ~ 2.85 (q, 8H), 3.29 ~ 3.31 (q, 4H), 3.42 (s, 2H); ^{13}C NMR (100 MHz, D_2O): δ = 174.40, 77.36, 74.11, 56.99, 48.61, 41.07, 39.37, 32.89.

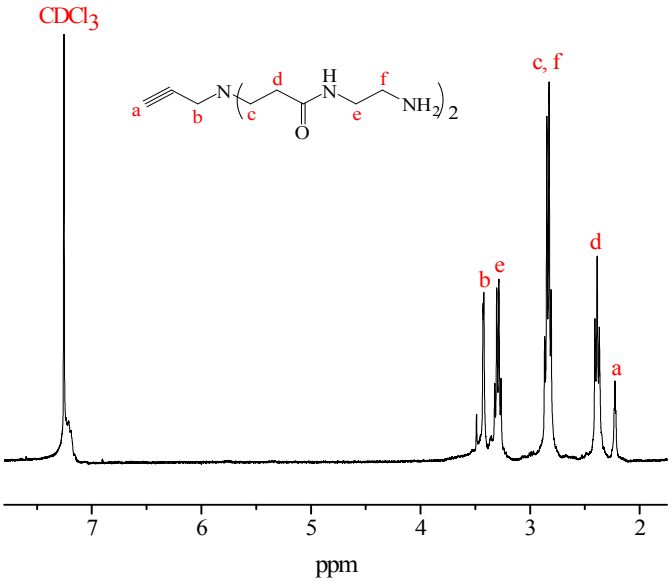

Fig. S2 The ^1H NMR spectrum of G 1.0 PAMAM.

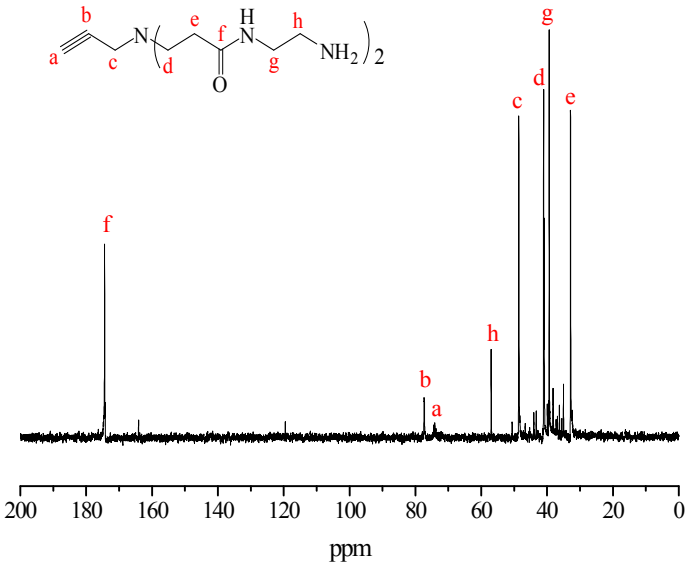

Fig. S3 The ^{13}C NMR spectrum of G 1.0 PAMAM.

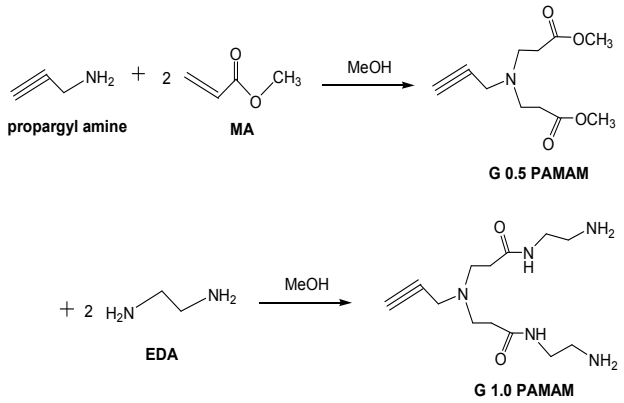

Fig. S4 The route to preparing G1.0 PAMAM.

1.2. Preparation of propargyl-PAMAM dendron G2 (G2.0 PAMAM)

Propargyl-PAMAM dendron G1.5 (G1.5 PAMAM) was synthesized from G1.0 PAMAM using the same method as Michael addition of primary amines with MA in yield of 90% and the measurement data are as follows. G2.0 PAMAM was synthesized from G1.5 PAMAM using the same method as amidation of methyl ester groups with a large excess of EDA in yield of 92% and the measurement data are as follows.

G1.5 PAMAM FTIR: 3290, 2950, 2830, 2090, 1740, 1660, 1530, 1440, 1260, 1200, 1050 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 2.19 (s, 1H), 2.43 (m, 12H), 2.55 (t, 4H), 2.76 (t, 8H), 2.85 (t, 4H), 3.30 (q, 4H), 3.43 (d, 2H), 3.67 (s, 12H).

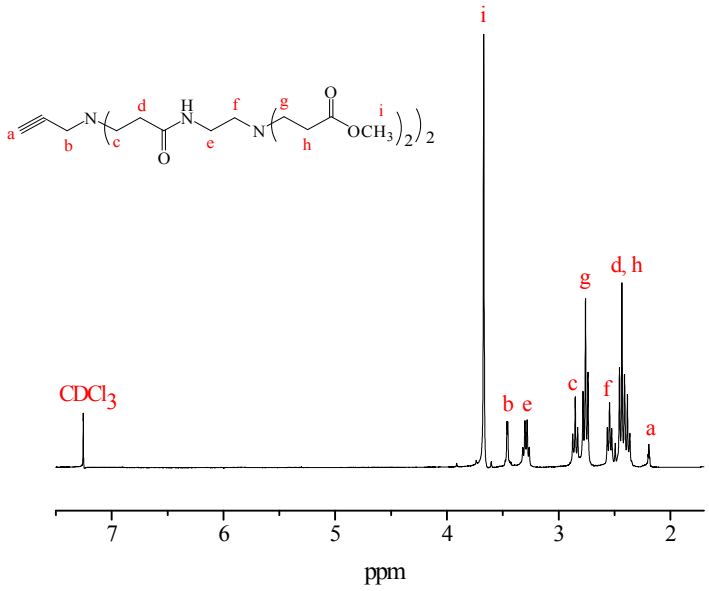

Fig. S5 The ^1H NMR spectrum of G 1.5 PAMAM.

G2.0 PAMAM FTIR: 3270, 3070, 2930, 2850, 1640, 1550, 1460, 1330, 1130,

1040 cm^{-1}; ^1H NMR (300 MHz, D_2O): δ = 2.18 (s, 1H), 2.32 (t, 12H), 2.52 (t, 4H), 2.65 (t, 8H), 2.71 (t, 12H), 3.15 (t, 12H), 3.30 (s, 2H); ^{13}C NMR (75 MHz, D_2O): δ = 174.69, 173.98, 77.25, 74.26, 52.26, 50.86, 48.71, 44.05, 41.19, 39.40, 38.23, 36.41, 32.48.

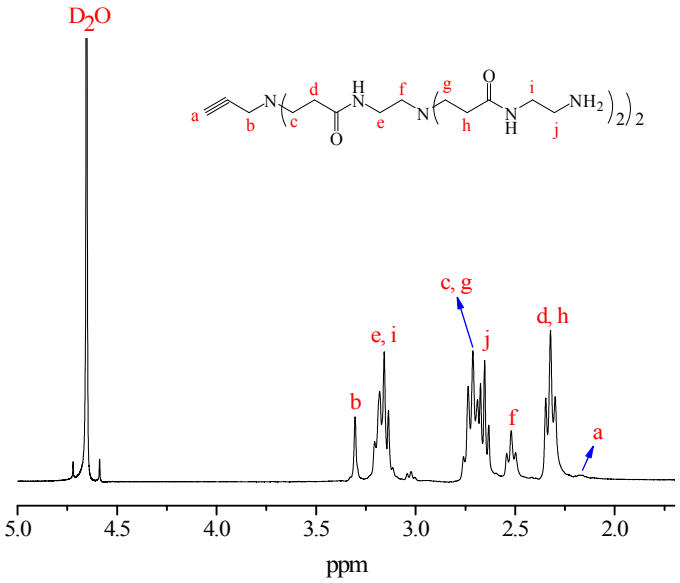

Fig. S6 The ^1H NMR spectrum of G 2.0 PAMAM.

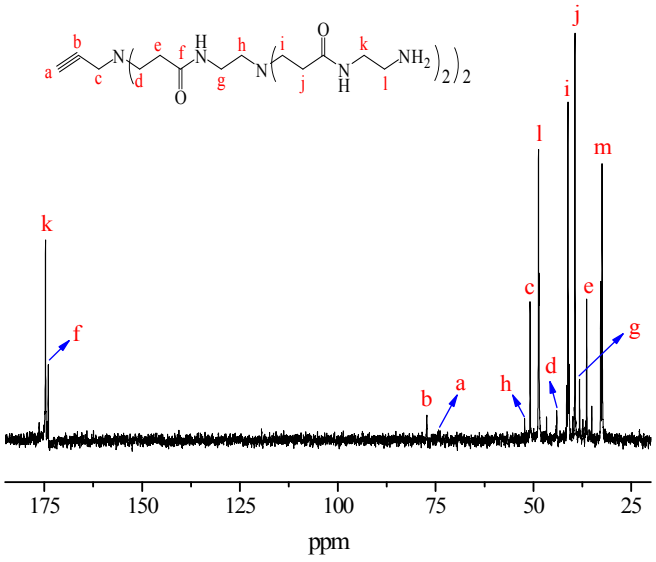

Fig. S7 The ^{13}C NMR spectrum of G 2.0 PAMAM.

1.3. Preparation of propargyl-PAMAM dendron G3 (G3.0 PAMAM)

G3.0 PAMAM was synthesized from G2.0 PAMAM by successive Michael addition of primary amines with MA and amidation of methyl ester groups with a large excess of EDA in yield of 88 % and the measurement data are as follows.

G2.5 PAMAM FTIR: 3290, 2950, 2830, 2090, 1740, 1660, 1540, 1440, 1260, 1200, 1050 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 2.21 (s, 1H), 2.44 (m, 28H), 2.55 (t, 12H), 2.76 (t, 28H), 3.30 (d, 12H), 3.46 (d, 2H), 3.67 (s, 24H).

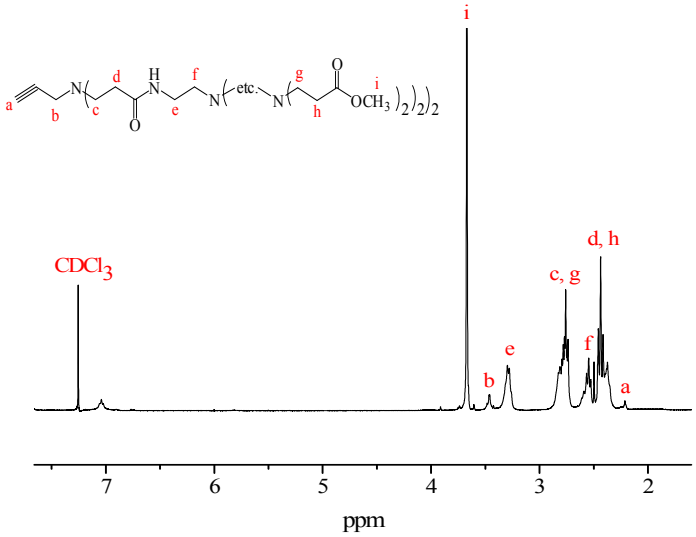

Fig. S8 The ^1H NMR spectrum of G 2.5 PAMAM.

G3.0 PAMAM FTIR: 3280, 3070, 2930, 2860, 1640, 1550, 1460, 1320, 1050 cm^{-1}; ^1H NMR (300 MHz, D_2O): δ = 2.17 (s, 1H), 2.32 (t, 28H), 2.49 (t, 12H), 2.61 (t, 16H), 2.69 (t, , 28H), 3.13 (t, 28H), 3.19 (t, 12H), 3.30 (s, 2H); ^{13}C NMR (75 MHz, D_2O): δ = 174.17, 173.84, 173.44, 77.30, 74.61, 51.01, 49.76, 48.88, 42.54, 41.37, 39.61, 38.22, 36.47, 32.48.

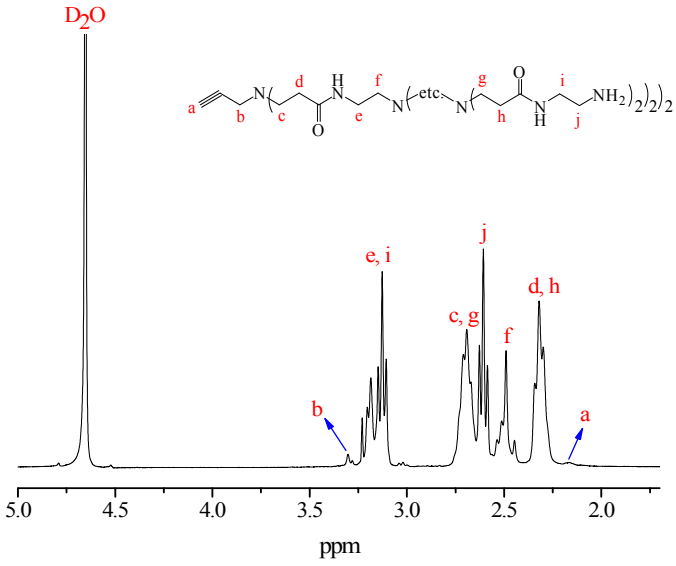

Fig. S9 The ^1H NMR spectrum of G 3.0 PAMAM.

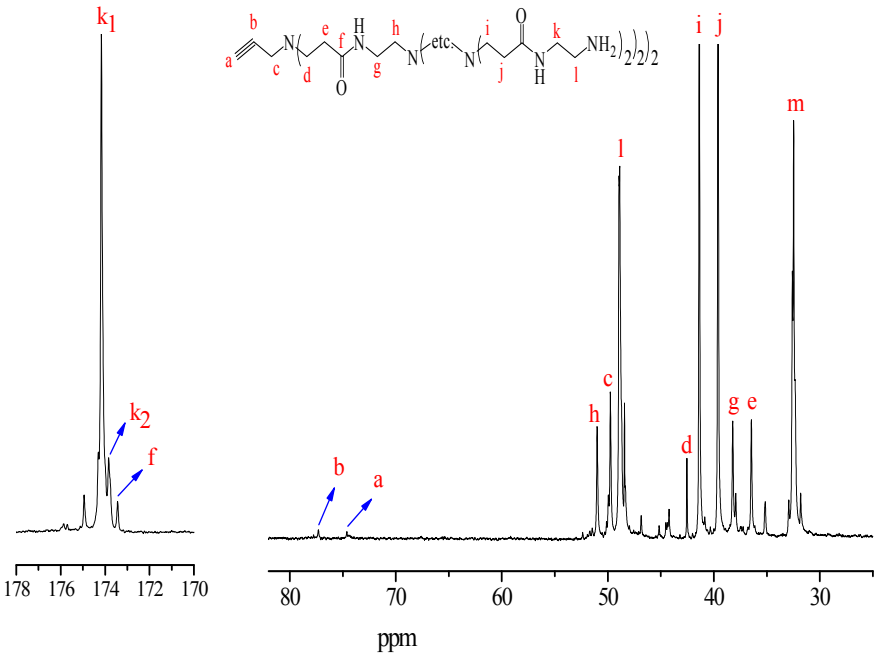

Fig. S10 The ^{13}C NMR spectrum of G 3.0 PAMAM.

1.4. Preparation of propargyl-PAMAM dendron G4 (G4.0 PAMAM)

G4.0 PAMAM was synthesized from G3.0 PAMAM also by successive Michael addition of primary amines with MA and amidation of methyl ester groups with a large excess of EDA in yield of 85 % and the measurement data are as follows.

G3.5 PAMAM FTIR: 3300, 2950, 2830, 2090, 1740, 1660, 1530, 1440, 1260, 1200, 1050 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 2.20 (s, 1H), 2.41 (m, 60H), 2.52 (t, 28H), 2.74 (t, 60H), 3.26 (t, 28H), 3.44 (d, 2H), 3.64 (s, 48H).

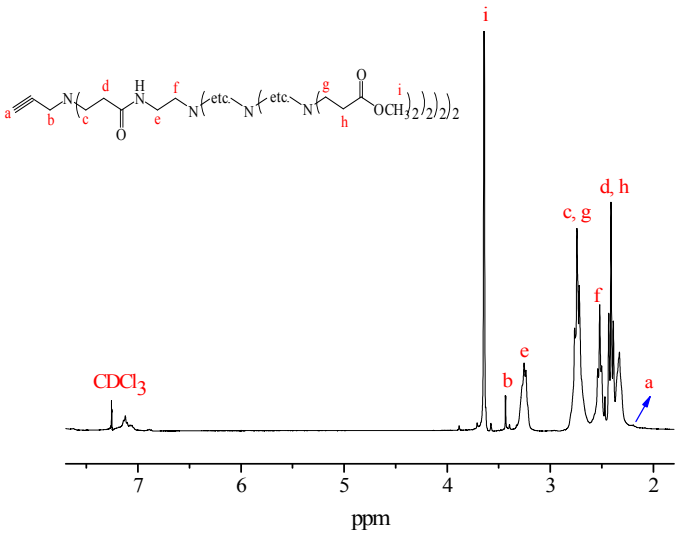

Fig. S11 The ^1H NMR spectrum of G 3.5 PAMAM.

G4.0 PAMAM FTIR: 3280, 3070, 2930, 2860, 1640, 1550, 1460, 1360, 1050 cm^{-1}; ^{1}H NMR (300 MHz, D_2O): δ = 2.16 (s, 1H), 2.32 (t, 60H), 2.49 (t, 28H), 2.60 (t, 32H), 2.71 (t, 60H), 3.12 (t, 60H), 3.30 (s, 2H); ^{13}C NMR (75 MHz, D_2O): δ = 174.23, 173.84, 173.47, 173.20, 77.23, 74.62, 50.94, 49.67, 48.80, 42.46, 41.34, 39.54, 38.19, 36.41, 32.52.

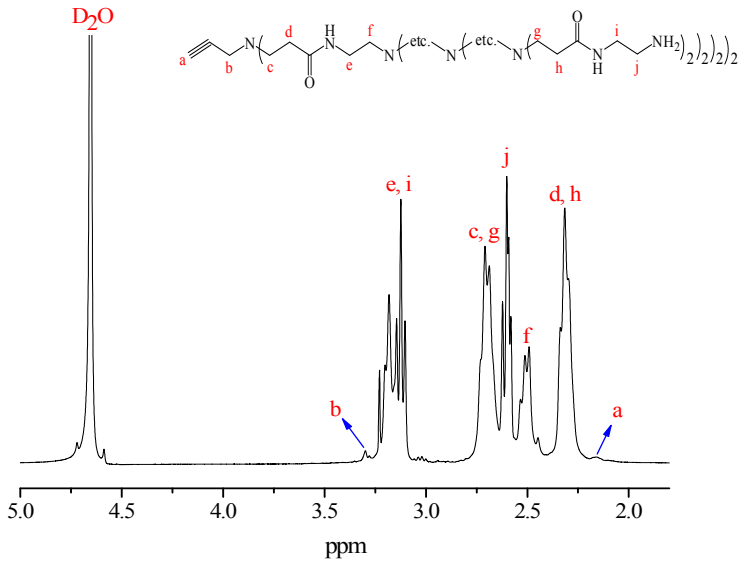

Fig. S12 The ^{1}H NMR spectrum of G 4.0 PAMAM.

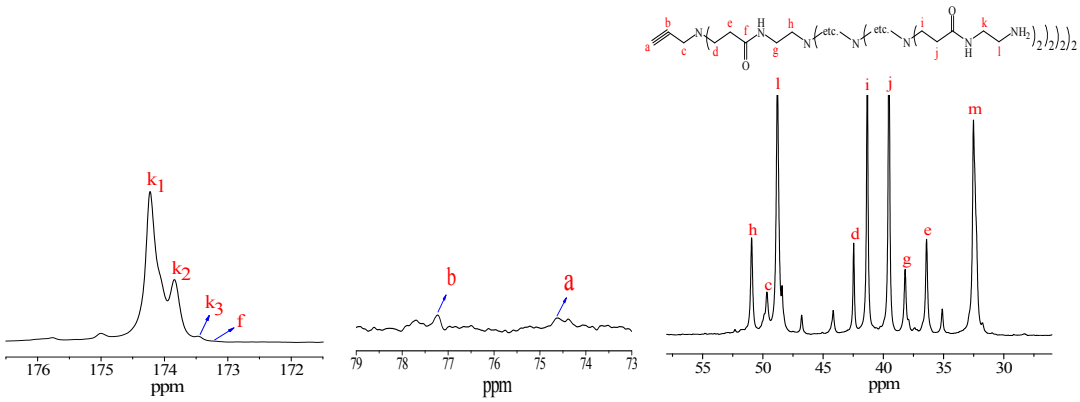

Fig. S13 The ^{13}C NMR spectrum of G 4.0 PAMAM.

2. Synthesis of Amino Acid Methyl Ester Hydrochlorides

2.1. Synthesis of His Methyl Ester Hydrochlorides

His-OMe.2HCl: FTIR: 2880, 1990, 1760, 1630, 1600, 1430, 1360, 1290, 1150; ^{1} H NMR (300 MHz, D_2O): δ = 3.28 (–CH_2–, t), 3.65 (–OCH_3, s), 4.31 (–CH–, t), 7.25 (–N=CH–, d), 8.51 (–C=CH–, d).

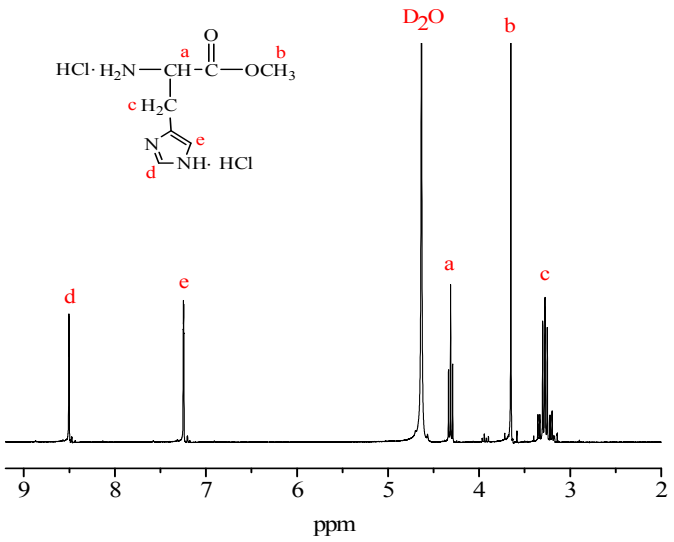

Fig. S14 The ^{1}H NMR spectrum of His-OMe.2HCl.

2.2. Synthesis of Phe Methyl Ester Hydrochlorides

Phe-OMe.2HCl: FTIR: 2850, 1990, 1750, 1580, 1490, 1450, 1360, 1290, 1240, 1140, 1080; ^{1}H NMR (300 MHz, D_2O): δ = 3.20 (–CH_2–, t), 3.74 (–OCH_3, s), 4.34 (–CH–, t), 7.20 (ortho-position of the benzene, d), 7.30 (meta-position and para-position of the benzene, t).

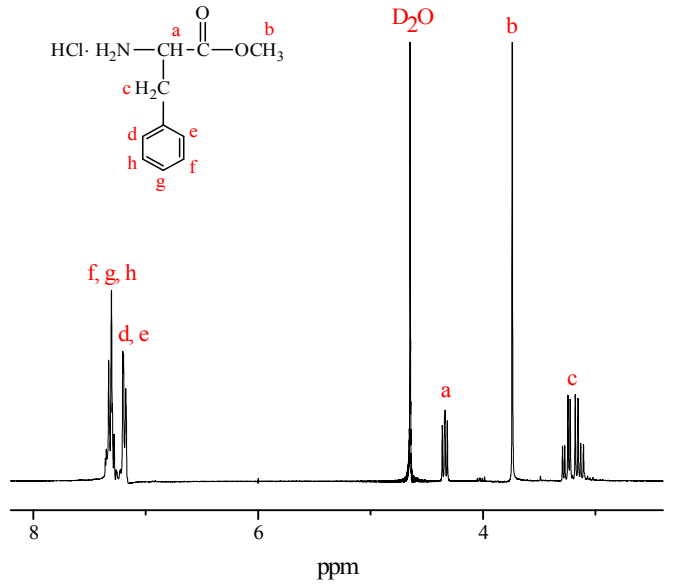

Fig. S15 The ^{1}H NMR spectrum of Phe-OMe.2HCl.

2.3. Synthesis of Trp Methyl Ester Hydrochlorides

Trp-OMe.2HCl: FTIR: 3270, 2880, 2020, 1750, 1580, 1500, 1440, 1360, 1290, 1230, 1110; ^{1}H NMR (300 MHz, D_2O): δ = 3.39 (–CH_2–, t), 3.71 (–OCH_3, s), 4.36 (–CH–,

t), 7.09 (–N=CH–, t), 7.17 (–N=CH–, t), 7.21 (–C=CH–, t), 7.42 (–N=CH–, d), 7.49 (–C=CH–, d).

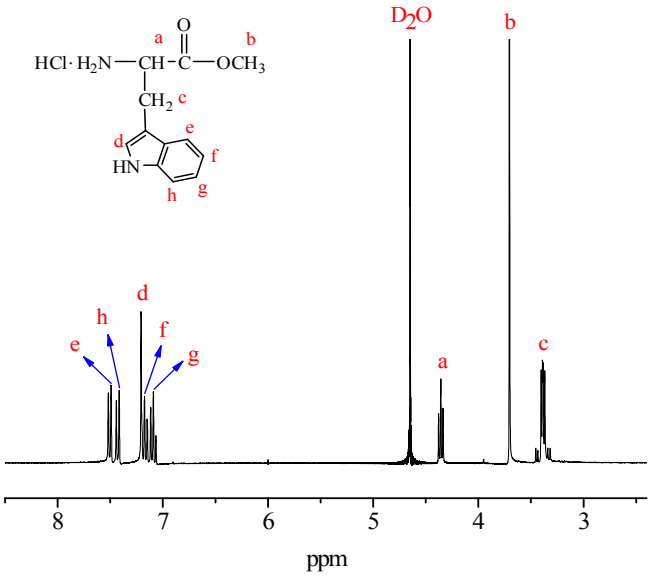

Fig. S16 The ^{1}H NMR spectrum of Trp-OMe.2HCl.

3. Synthesis of Amino Acid Modified PAMAM (PAMAM-AA)

3.1. Preparation of PAMAM-His

G1-His: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.30 (t, 1H), 2.90 ~ 3.08 (m, 16H), 3.51 (d, 2H). 3.31 (–CH_2–, t), 3.78 (–CH–, q), 6.88 (–N=CH–, s), 7.66 (–C=CH–, s).

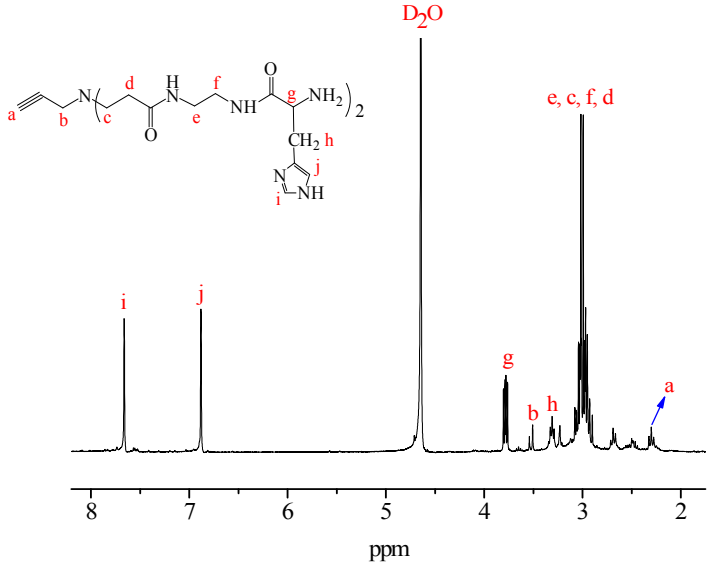

Fig. S17 The ^{1}H NMR spectrum of G1-His.

G2-His: ^{1}H NMR (300 MHz, D_2O): δ = 2.17 (s, 1H), 2.35 (m, 12H), 2.53 (t, 4H),

2.73 (t, 8H), 2.98 (m, 12H), 3.07 (m, 8H), 3.20 (s, 4H), 3.59 (s, 2H). 3.31 (–CH_2–, t), 3.81 (–CH–, q), 6.90 (–N=CH–, s), 7.60 (–C=CH–, s).

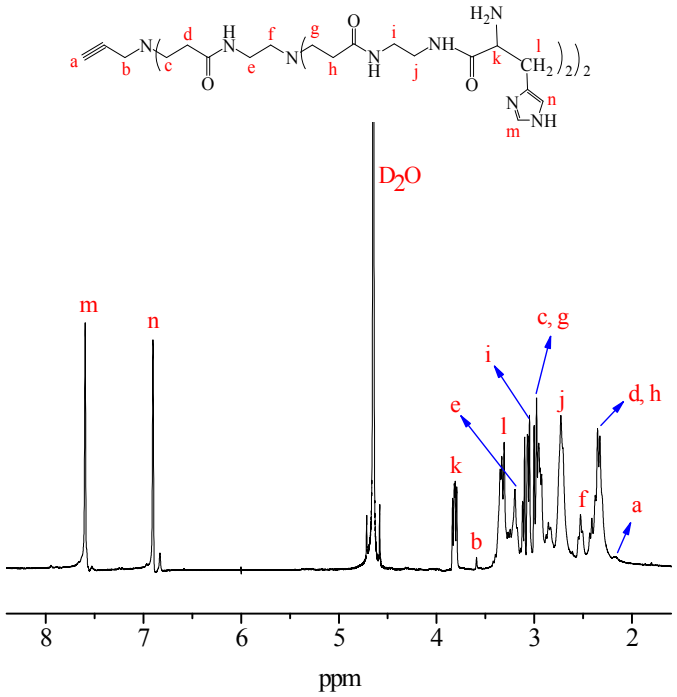

Fig. S18 The ^{1}H NMR spectrum of G2-His.

G3-His: ^{1}H NMR (300 MHz, D_2O): δ = 2.17 (s, 1H), 2.36 (t, 28H), 2.60 (t, 12H), 2.79 (t, 16H), 3.03 (t, 28H), 3.19 (t, 16H), 3.32 (t, 12H), 3.39 (s, 2H). 3.39 (–CH_2–, s), 3.87 (–CH–, s), 7.00 (–N=CH–, s), 7.81 (–C=CH–, s).

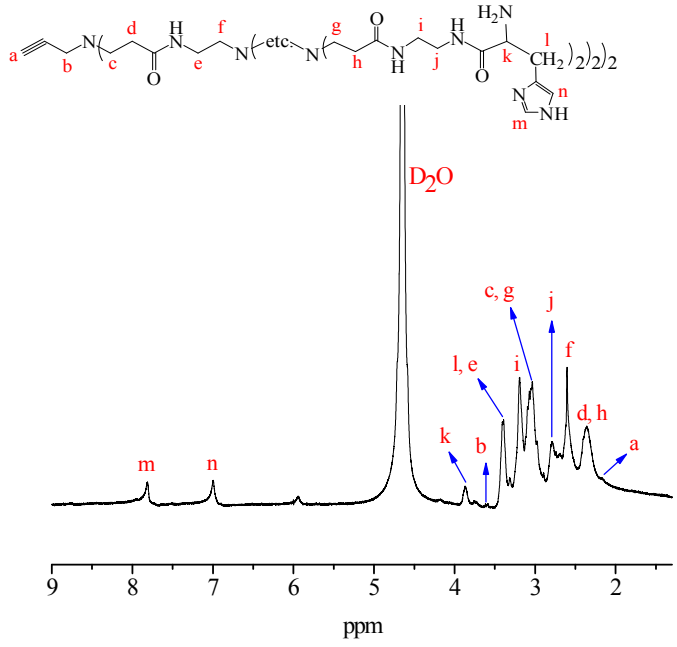

Fig. S19 The ^{1}H NMR spectrum of G3-His.

G4-His: ^{1}H NMR (300 MHz, D_2O): δ = 2.17 (s, 1H), 2.35 (m, 60H), 2.54 (t, 28H),

2.72 (t, 32H), 3.03 (m, 60H), 3.20 (t, 32H), 3.33 (m, 28H), 3.60 (s, 2H). 3.42 (–CH_2–, s), 3.86 (–CH–, q), 6.93 (–N=CH–, m), 7.64 (–C=CH–, s).

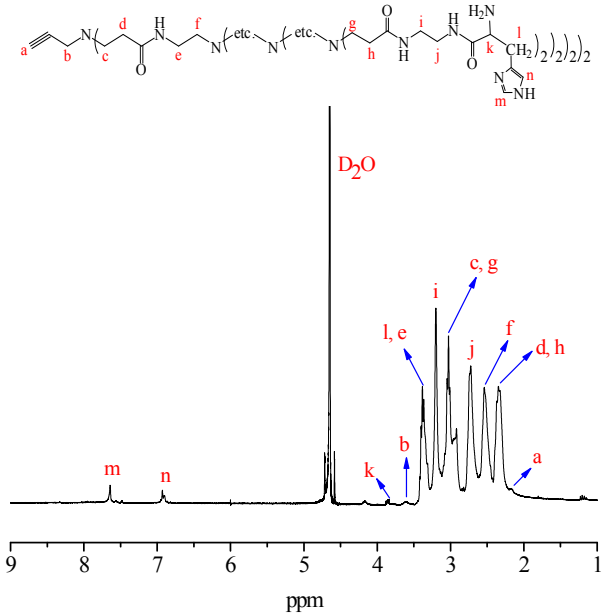

Fig. S20 The ^{1}H NMR spectrum of G4-His.

3.2. Preparation of PAMAM-Phe

G2-Phe: ^{1}H NMR (300 MHz, D_2O): δ = 2.19 (s, 1H), 2.35 (m, 12H), 2.48 (m, 4H), 2.76 (t, 8H), 3.03 (t, 12H), 3.17 (t, 8H), 3.33 (t, 4H), 3.60 (s, 2H). 3.38 (–CH_2–, t), 3.89 (–CH–, q), 7.21~7.32 (benzene).

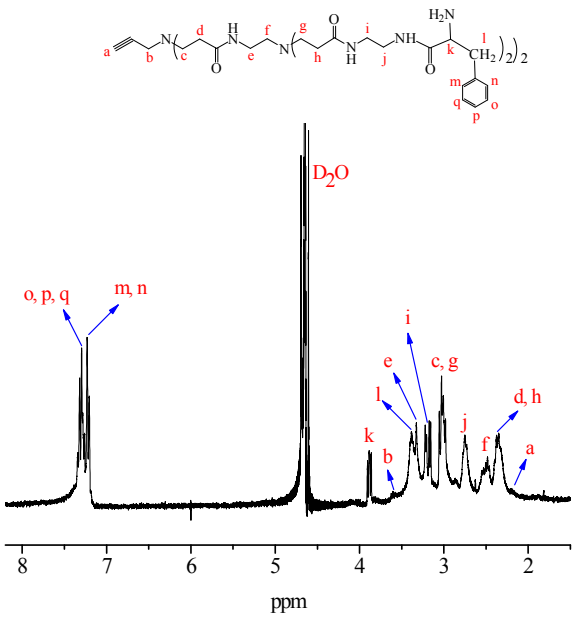

Fig. S21 The ^{1}H NMR spectrum of G2-Phe.

G3-Phe: ^{1}H NMR (300 MHz, D_2O): δ = 2.19 (s, 1H), 2.34 (t, 28H), 2.54 (m, 12H),

2.73 (t, 16H), 3.00 (m, 28H), 3.21 (m, 16H), 3.32 (t, 12H), 3.63 (s, 2H). 3.37 (–CH_2–, t), 3.87 (–CH–, q), 7.20 ~ 7.32 (benzene).

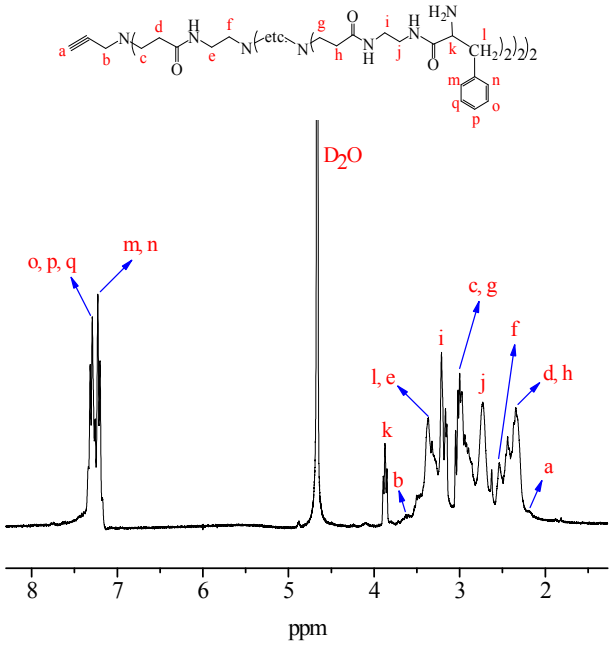

Fig. S22 The ^{1}H NMR spectrum of G3-Phe.

G4-Phe: ^{1}H NMR (300 MHz, D_2O): δ = 2.14 (s, 1H), 2.31 (m, 60H), 2.64 (t, 60H), 2.77 (m, 60H), 3.17 (t, 60H), 3.61 (s, 2H). 3.50 (–CH_2–, t), 3.71 (–CH–, q), 7.11 ~ 7.21 (benzene).

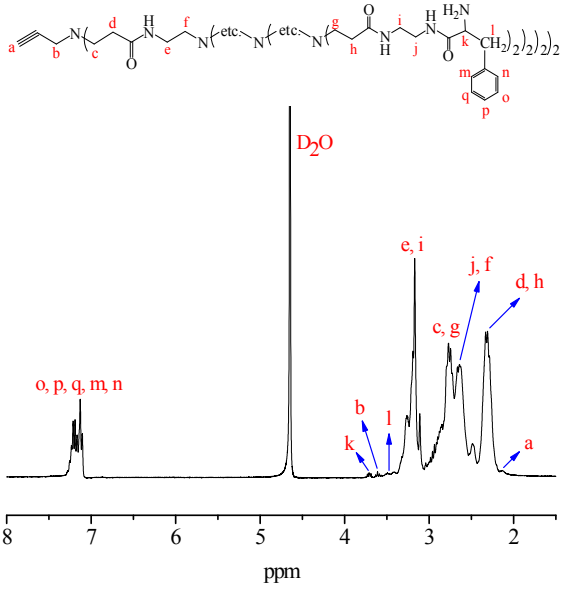

Fig. S23 The ^{1}H NMR spectrum of G4-Phe.

3.3. Preparation of PAMAM-Trp

G2-Trp: ^{1}H NMR (300 MHz, D_2O): δ = 2.18 (s, 1H), 2.37 (m, 12H), 2.53 (t, 4H),

2.74 (t, 8H), 3.03 (m, 12H), 3.21 (t, 8H), 3.32 (t, 4H), 3.60 (s, 2H). 3.37 (–CH_2–, t), 3.94 (–CH–, q), 7.08 ~ 7.60 (heterocycle).

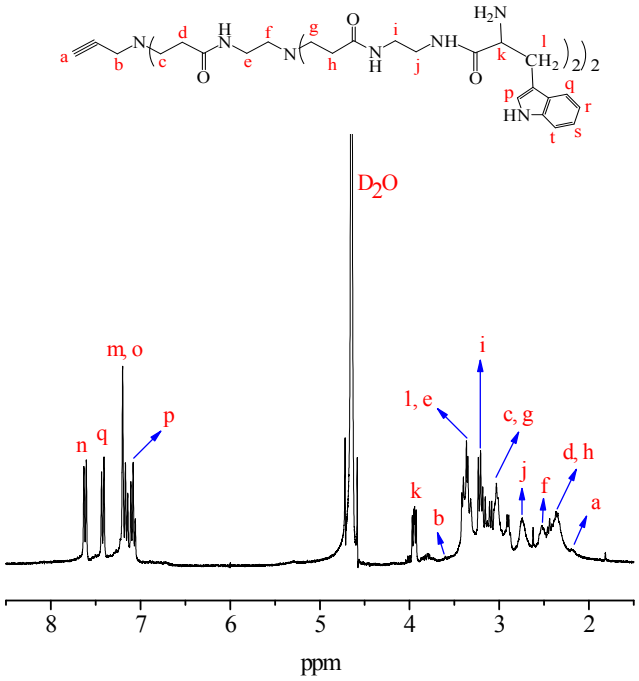

Fig. S24 The ^{1}H NMR spectrum of G2-Trp.

G3-Trp: ^{1}H NMR (300 MHz, D_2O): δ = 2.16 (s, 1H), 2.26 (m, 28H), 2.66 (m, 28H), 2.72 (m, 28H), 3.16 (m, 28H), 3.69 (s, 2H). 3.41 (–CH_2–, t), 3.82 (–CH–, q), 7.03 ~ 7.56 (heterocycle).

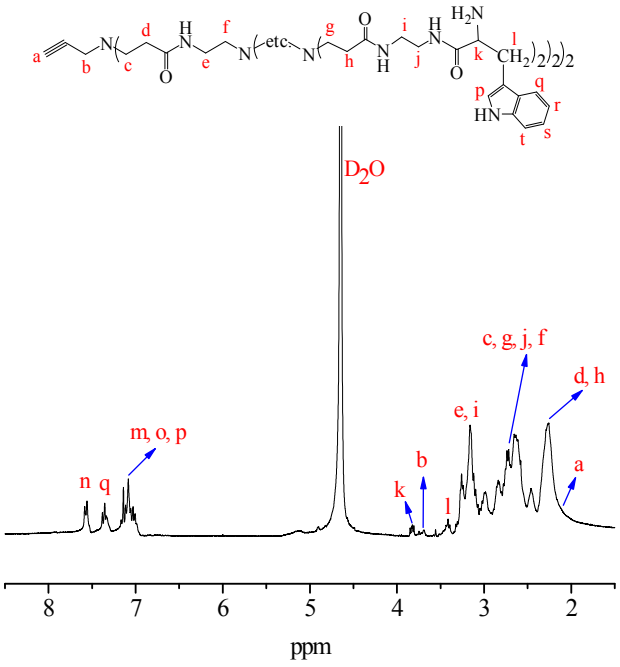

Fig. S25 The ^{1}H NMR spectrum of G3-Trp.

G4-Trp: ^{1}H NMR (300 MHz, D_2O): δ = 2.16 (s, 1H), 2.31 (m, 60H), 2.59 (m, 60H), 2.72 (m, 60H), 3.17 (m, 60H), 3.71 (s, 2H). 3.37 (–CH_2–, t), 3.78 (–CH–, q), 7.03 ~ 7.57 (heterocycle).

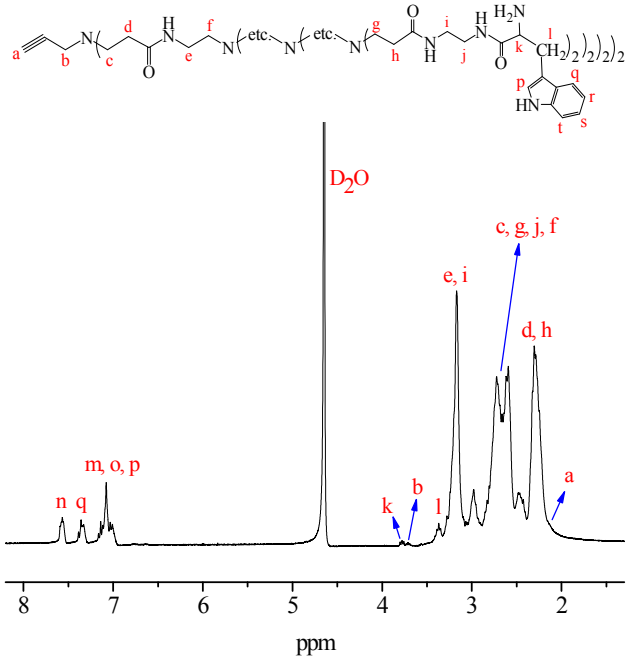

Fig. S26 The ^{1}H NMR spectrum of G4-Trp.

4. Preparation of azidated sepharose (Sep-N_3)

Sepharose (Sep) as a support matrix was activated by epichlorohydrin followed by reacting with sodium azide to obtain azidated sepharose, Sep-N_3, as depicted in Fig. S27. Sep-N_3 can be prepared *via* two reaction steps. Firstly, Sep was activated through its hydroxy with epichlorohydrin to prepare epoxidized sepharose, Sep-Epoxy; and then, nucleophilic displacement reaction was carried out in which azide ion attacks epoxide resulting in the opening of the ring to obtain azidated sepharose.

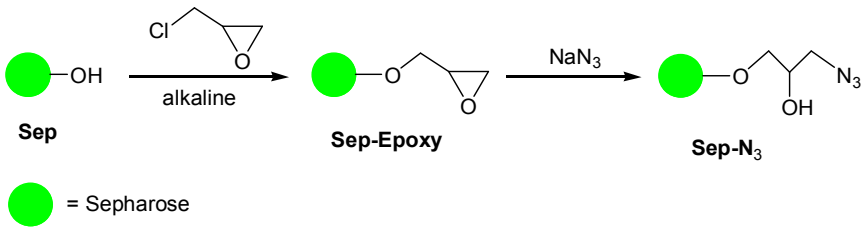

Fig. S27 The route to synthesizing azidated sepharose (Sep-N_3).

4.1. Preparation of epoxidized sepharose (Sep-Epoxy)

Suction-dried sepharose 6FF (2.0 g) was mixed with epichlorohydrin (4.0 mL) and

2.0 M sodium hydroxide solution (4.0 mL) in a 50.0 mL flask. The reaction slurry was shaken at 30 °C for 4 h in a rocking incubator and then the reaction was stopped by washing the gel with large volumes of water until added phenolphthalein did not turn red in the elute containing sodium thiosulphate. The epoxy group content available in the prepared epoxidized sepharose, Sep-Epoxy, was determined by means of acid-base titration.[1] Briefly, 3.0 mL of 1.3 M $Na_2S_2O_3$ was added to the Erlenmeyer flask containing 1.0 g of the Sep-Epoxy and shaken well. Upon being at room temperature for 15 min, the solution was titrated by 0.01 M HCl using bromothymol blue as an indicator. A blank titration was also carried out under the control conditions.

4.2. Azidation of epoxidized sepharose

Sep-N_3 was prepared by the azidation of Sep-Epoxy, which was done according to the procedure as described previously.[2, 3] Sodium azide (2.45 g) was dissolved in 40.0 mL of water containing tetraethylammonium chloride (0.32 g) as the phase transfer catalyst, and then Sep-Epoxy (4.0 g) was added. The mixture was kept away from light and stirred overnight at room temperature, followed by washing with water. The degree of azidation of the prepared Sep-N_3 was measured by an elemental analysis method.

5. Determination of the calibration curve for IgG detection

Standard IgG solutions with different concentrations (c) were first prepared using a 0.02 M citric acid buffer solution at pH 2.5 as a solvent. The absorbance at 280 nm (A_{280}) for each IgG solution of known concentration was measured with an Ultraviolet-visible spectrophotometer. The calibration curves of the standard IgG solutions were then generated by plotting A_{280} as a function of c, as depicted in Fig. S28. The IgG concentration of the eluate to be detected could be conveniently obtained by means of the corresponding calibration curve after its A_{280} value was measured.

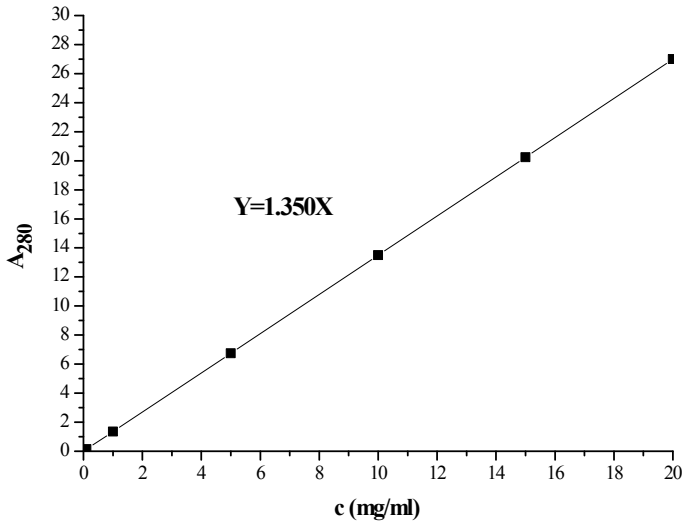

Fig. S28 The calibration curve of the standard IgG solutions.

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[2] S. R. Jameela, S. Lakshmi, N. R. James and A. Jayakrishnan, *J. Appl. Polym. Sci.*, 2002, **86**, 1873–1877.

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