Electronic Supplementary Material (ESI) for RSC Advances

Supporting Information

Amino-acid derivatives as the universal molecular units endowing thermoresponsive property to water-soluble polymers with plenty of hydroxyl or amino groups

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Experimental Section

Material

Hyperbranched polyethylenimine (HPEI, M_n=1800g/mol, M_w/M_n=1.04) was purchased from Alfa Aesar, and was dried under vacuum prior to use. Hyperbranched polyglycerol (HPG, M_n=2317 g/mol^{-1}, M_w/M_n = 1.5) with an average number of hydroxyl terminal group of 33 was a gift from Professor Holger Frey (Uni-Mainz, Germany), and was dried under vacuum prior to use. Glycine (99%), L-alanine (99%) and L-valine (99%) were purchased from Tianjin Guangfu Fine Chemical Research Institute and used directly. Isobutyryl chloride (98%) was purchased from Alfa Aesar and used directly. 1,1'-Carbonyldiimidazole (CDI, 97%) was purchased from Beijing Ouhe Technology Company and used directly. Triethylamine (TEA) was obtained from Tianjin Kewei Chemical Company and purified by vacuum distillation before use. 2-Hydroxyethyl acrylate (HEA, 98%) was purchased from Tianjin Guangfu Fine Chemical Research Institute. 2,2'-Azobis(isobutyronitrile) (AIBN) was obtained from Tianjin Guangfu Fine Chemical Research Institute and purified by double recrystallization in methanol. Aspirin (99%) was purchased from Tianjin Guangfu Fine Chemical Research Institute. Hydroxyethyl cellulose (HEC, BH100K) was purchased from Tianjin Guangfu Fine Chemical Research Institute and was dried under vacuum prior to use. Poly(allylamine hydrochloride) (99%, M_w=120,000 to 200,000) was purchased from Shanghai Sinopharm Chemical Reagent Co., Ltd. Benzoylated cellulose tubing (MWCO 1,000) was purchased from Sigma and used directly. 1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC, 99%) was purchased from Suzhou Highfine Biotech Co., Ltd and used directly. *N*-Hydroxysuccinimide (NHS, 99%) was obtained from Shanghai Medpep Co., Ltd and used directly. Dibenzyl trithiocarbonate (DBTTC) was prepared according to procedure reported by Endo et al.[1] Poly(2-Hydroxyethyl acrylate) (PHEA, M_n=50000 g/mol, M_w/M_n=1.61) was prepared through the RAFT polymerization of HEA.[2] Gly-IBAm, Ala-IBAm and Val-IBAm were synthesized through the method reported by Doherty et al.[3]

General Procedure for the Syntheses of HPG-Val-IBAm, HPG-Ala-IBAm and HPG-Gly-IBAm

It was exemplified for the HPG-Val-IBAm with degree of substitution of Val-IBAm being 14.9 %. 0.187 g (1.15 mmol) of CDI was slowly added to the solution of 0.144 g (0.769 mmol) of Val-IBAm in 2 mL of anhydrous DMF. The solution was stirred at room temperature for 1 h, and then 0.300 g (4.27 mmol OH group) of HPG in 2 mL of anhydrous DMF was added. This reaction system was degassed and purged with nitrogen for three cycles. After that, under nitrogen atmosphere the mixture was stirred at 50 ^{o}C for 12 h. Most of DMF solvent was removed under vacuum and the residue was purified by dialysis against methanol using a benzoylated cellulose membrane (MWCO 1000 g/mol) for 3 days. Finally, the methanol solvent was removed under vacuum, and the product was dried at 50 ^{o}C in vacuum oven for 24 h. Yield: 67.3%; The typical ^{1}H NMR spectrum of HPG-Val-IBAm and the signal assignment were shown in Figure S1. The typical FTIR spectrum of HPG-Val-IBAm was shown in Figure S2. The typical ^{1}H NMR spectra of HPG-Ala-IBAm and HPG-Gly-IBAm were shown in Figure S3 and Figure S4, respectively.

General Procedure for the Synthesis of HPEI-Val-IBAm

It was exemplified for the HPEI-Val-IBAm with degree of substitution of Val-IBAm being 44.3%. 0.661 g (4.06 mmol) of CDI was slowly added to the solution of 0.508 g (2.71 mmol) of Val-IBAm in 2 mL of anhydrous chloroform at 0-5 ^{o}C. The solution was stirred at room temperature for 1 h, and then 0.400 g (6.70 mmol of primary and secondary amine groups) of HPEI in 3 mL of anhydrous chloroform was added. Under nitrogen atmosphere the mixture was stirred at 70 ^{o}C for 12 h. Chloroform was removed under vacuum. The residue was dissolved in 15 mL of methanol, and then purified by dialysis against methanol using a benzoylated cellulose membrane (MWCO 1000 g/mol) for 2 days. Finally, the methanol solvent was removed under vacuum, and the product was dried at 50 ^{o}C in vacuum oven for 24 h. Yield: 59.5%. The typical ^{1}H NMR spectrum of HPEI-Val-IBAm and the signal assignment were shown in Fig. S6.

General Procedure for the Synthesis of the Conjugate of HPEI-Val-IBAm and Aspirin

It was exemplified for the degree of substitution of aspirin being 2.4%. 22.9 mg of CDI was slowly added to the solution of 16.9 mg of aspirin in 2 mL of anhydrous DMF at 0-5 ^{o}C. The solution was

stirred at room temperature for 1 h, and then 30.0 mg of HPEI-Val-IBAm with the degree of substitution of Val-IBAm being 43.4% in 2 mL of anhydrous DMF was added. Under nitrogen atmosphere the mixture was stirred at 50 ^{o}C for 12 h. The mixture was purified by dialysis against methanol using a benzoylated cellulose membrane (MWCO 1000 g/mol) for 2 days. Finally, the methanol solvent was removed under vacuum, and the product was dried at 50 ^{o}C in vacuum oven for 24 h. The typical ^{1}H NMR spectrum of HPEI-Val-IBAm and the signal assignment were shown in Fig. S6.

General Procedure for the Synthesis of PHEA-Val-IBAm,

It was exemplified for the PHEA-Val-IBAm with degree of substitution of Val-IBAm being 5.8%. 0.136 g (0.836 mmol) of CDI was slowly added to the solution of 0.105 g (0.561 mmol) of Val-IBAm in 2 mL of anhydrous DMF at 0-5 ^{o}C. The solution was stirred at room temperature for 1 h, and then 0.434 g (3.74 mmol OH group) of PHEA in 3 mL of anhydrous DMF was added. This reaction system was degassed and purged with nitrogen for three cycles. After that, under nitrogen atmosphere the mixture was stirred at 50 ^{o}C for 12 h. Most of DMF solvent was removed under vacuum and the residue was purified by dialysis against methanol using a benzoylated cellulose membrane (MWCO 1000 g/mol) for 3 days. Finally, the methanol solvent was removed under vacuum, and the product was dried at 50 ^{o}C in vacuum oven for 24 h. Yield: 82.6%; The typical ^{1}H NMR spectrum of HPG-Val-IBAm and the signal assignment were shown in Fig. S7.

General Procedure for the Synthesis of HEC-Val-IBAm

It was exemplified for the HEC-Val-IBAm with degree of substitution of Val-IBAm per glucose ring being 0.133. 0.290 g (1.78 mmol) of CDI was slowly added to the solution of 0.334 g (1.79 mmol) of Val-IBAm in 5 mL of freshly-distilled DMSO. The solution was stirred at room temperature for 1 h, and then 0.300 g of HEC in 10 mL of freshly-distilled DMSO was added. This reaction system was degassed and purged with nitrogen for three cycles. After that, under nitrogen atmosphere the mixture was stirred at 80 ^{o}C for 12 h. The mixture was purified by dialysis against methanol using a benzoylated cellulose membrane (MWCO 1000 g/mol) for 3 days. Finally, the methanol solvent was removed under

vacuum, and the product was dried at 50 ^{o}C in vacuum oven for 24 h. Yield: 62.8%; The typical ^{1}H NMR spectrum of HEC-Val-IBAm and the signal assignment were shown in Fig. S8.

Preparation of polyallylamine (PAAm)

Poly(allylamine hydrochloride) was neutralized with stoichiometric amount of NaOH in water. The produced salt was removed through dialysis against water. After removing water, the pure PAAm was obtained.

General Procedure for the Synthesis of PAAm-Val-IBAm

EDC (0.201 g, 1.05 mmol) and NHS (0.121mg, 1.05 mmol) were added to the solution Val-IBAm (0.131g, 0.701mmol) in 8mL deionized water in a vial. TEA (150 μL) was added to a solution of PAAm (0.200g, 3.51mmol primary amine) in 2mL deionized water. These two solutions were mixed and stirred for 1 day at room temperature, and then was dialyzed against deionized water for 2 days. Most of water was removed under vacuum, and the product was dried in Vacuum freezing dryer for 24 h. Yield: 94.5%; The typical ^{1}H NMR spectrum of PAAm-Val-IBAm and the signal assignment were shown in Fig. S9.

Characterization

^{1}H and ^{13}C NMR spectra were recorded at 25 ^{o}C on a Varian INOVA 500MHz spectrometer, operated at 500 MHz and 125 MHz, respectively. UV-vis spectra were obtained from Purkinje General (China) T6 UV/Vis Spectrophotometer. FIIR spectrometer was recorded on a Nicolet 5DXC FTIR spectrometer. The measurement was done using KBr pellets, and the scanning range was 4000–400cm^{-1}. Elemental analysis was performed on Elementar Vario ELCUBE. Gel Permeation Chromatography (GPC) of the samples was carried out using a Viscotek triple detectors GPC270 system. This system is equipped with an online GPC degasser (VE7510), one HPLC pump (VE1122), one TSKgel guard column H_{HR}-H (column size: 6.0mm(ID)×4.0cm(L)), one GMH_{HR}-M mixed bed column (column size: 7.8mm(ID)×30.0cm(L)), one GMH_{HR}-N mixed bed column (column size: 7.8mm(ID)×30.0cm(L)) and three detectors. A refractive index detector (RI) detector (VE3580) is connected with a dual detectors

system (Model 270) in parallel way. This dual detectors system has a four-capillary differential viscometer (DV) detector and a light scattering (LS) detector in series. The wavelength of the light scattering laser is 670 nm and the scattered light is detected at right angle (RALS, 90^{o}) and low angle (LALS, 7^{o}). Dynamic light scattering (DLS) measurements were performed using the Malvern Nano ZS instrument at 25 ^{o}C with 633nm He-Ne laser light, and the light collection at 90^{o}. The particle size was calculated from COTTIN method.

Table S1. Structural parameters of the obtained thermoresponsive polymers and their phase transition parameters

No.	Precursor			*DS* (%)	M_n /10^4	Phase transition	
	polymer	M_n /10^4	Amino-acid derivative			Concentration (mg/mL)	T_{cp}/°C
1	HPG	0.232	Gly-IBAm	46.9	0.429	20	48.8
2	HPG	0.232	Gly-IBAm	58.2	0.476	20	29.2
3	HPG	0.232	Gly-IBAm	63.1	0.497	20	22.6
4	HPG	0.232	Gly-IBAm	77.5	0.557	20	13.5
5	HPG	0.232	Ala-IBAm	24.0	0.344	20	40.5
6	HPG	0.232	Ala-IBAm	42.3	0.429	20	30.9
7	HPG	0.232	Ala-IBAm	49.3	0.461	20	23.9
8	HPG	0.232	Ala-IBAm	67.1	0.544	20	8.6
9	HPG	0.232	Val-IBAm	12.6	0.302	20	56.4
10	HPG	0.232	Val-IBAm	14.9	0.315	20	33.3
11	HPG	0.232	Val-IBAm	23.3	0.362	20	26.2
12	HPG	0.232	Val-IBAm	26.3	0.379	20	15.6
13	HPG	0.232	Val-IBAm	31.0	0.405	20	9.8
14	HPG	0.232	Val-IBAm	34.2	0.423	20	7.2
15	HPEI	0.180	Val-IBAm	44.3	0.405	20	33.9
16	HPEI	0.180	Val-IBAm	54.0	0.454	20	25.0
17	HPEI	0.180	Val-IBAm	61.4	0.492	20	22.7
18	HPEI	0.180	Val-IBAm	65.1	0.511	20	19.9
19	PHEA	5.00	Val-IBAm	5.8	5.76	20	32.7
20	PHEA	5.00	Val-IBAm	6.3	5.82	20	18.4
21	PHEA	5.00	Val-IBAm	10.3	6.35	20	11.5
22	HEC	10.0	Val-IBAm	7.7	10.7	10	52.2
23	HEC	10.0	Val-IBAm	13.3	11.3	10	17.5
24	PAAm	9.75	Val-IBAm	20.9	15.8	20	35.3
25	PAAm	9.75	Val-IBAm	28.1	17.9	20	28.0

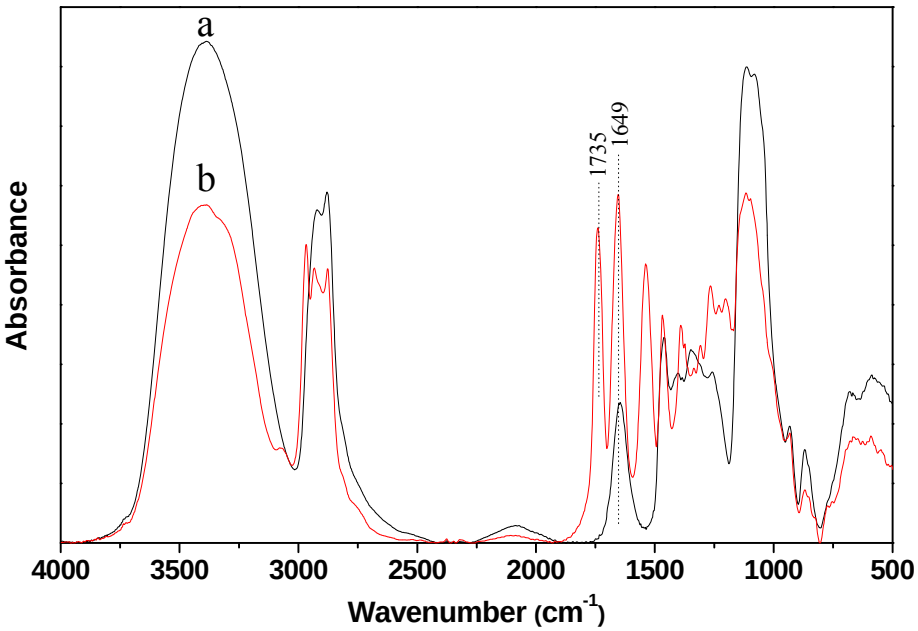

Fig. S1 Typical FTIR spectra of (a) HPG, (b) HPG-Val-IBAm.

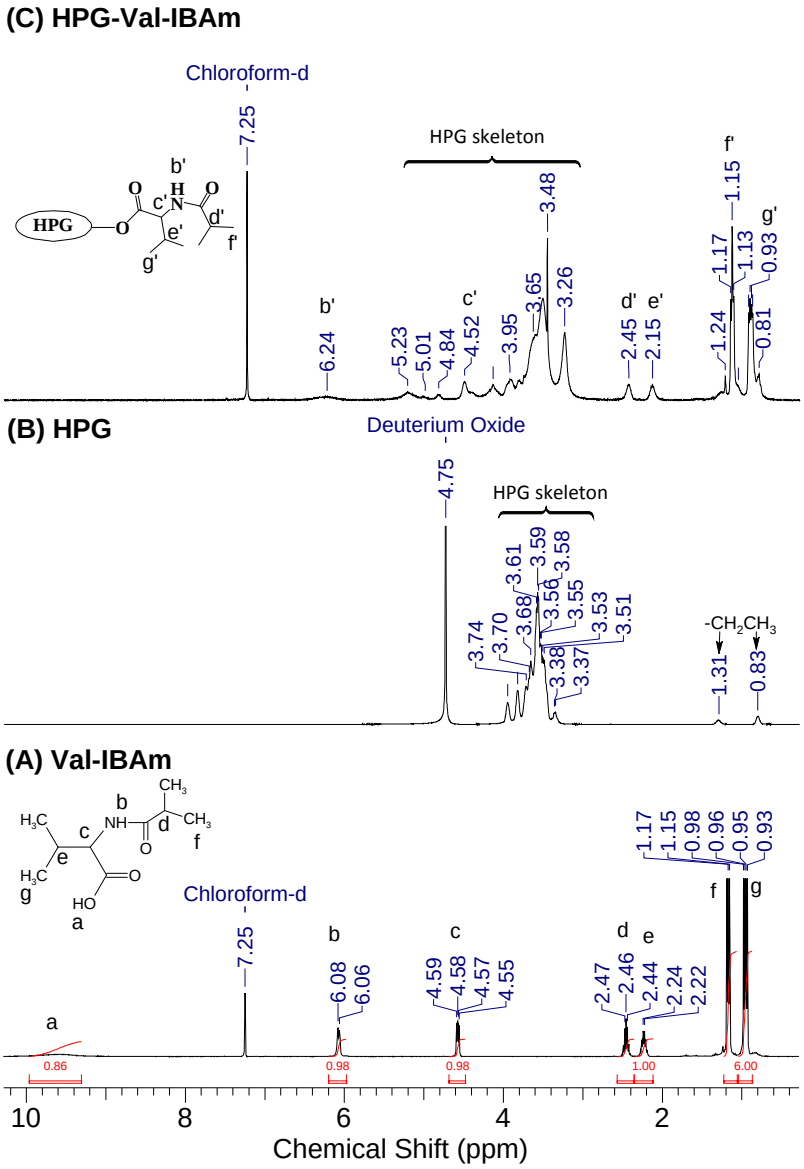

Fig. S2 Typical ^{1}H NMR spectra of (A) Val-IBAm, (B) HPG and (C) HPG-Val-IBAm.

Degree of substitution of Val-IBAm group in OH group of HPG can be calculated from Figure S2 according to eqn. 1:

$$\text{Degree of substitution} = I(d')/I(HPG)/5 \qquad (1)$$

I (d') means the integral of d' signal, which can be read off from the spectrum directly. I (HPG) means the integral of HPG skeleton, which cannot read off from the spectrum directly, but can be calculated using the integral of the signals between 3.0 and 5.5 ppm minus I (d').

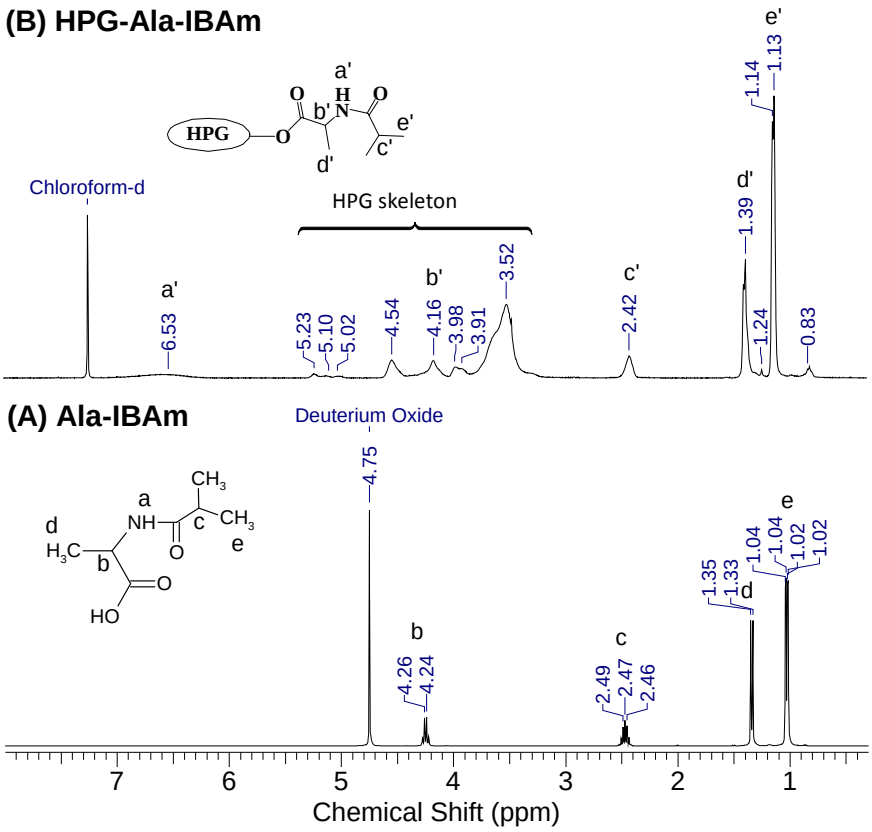

Fig. S3 Typical ^{1}H NMR spectra of (A) Ala-IBAm and (B) HPG-Ala-IBAm.

Degree of substitution of Ala-IBAm group in OH group of HPG can be calculated from Figure S3B according to eqn. 2:

$$\text{Degree of substitution} = I(c')/ I(HPG)/ 5 \qquad (2)$$

I (c') means the integral of c' signal, which can be read off from the spectrum directly. I (HPG) means the integral of HPG skeleton, which cannot read off from the spectrum directly, but can be calculated using the integral of the signals between 3.0 and 5.5 ppm minus I (c').

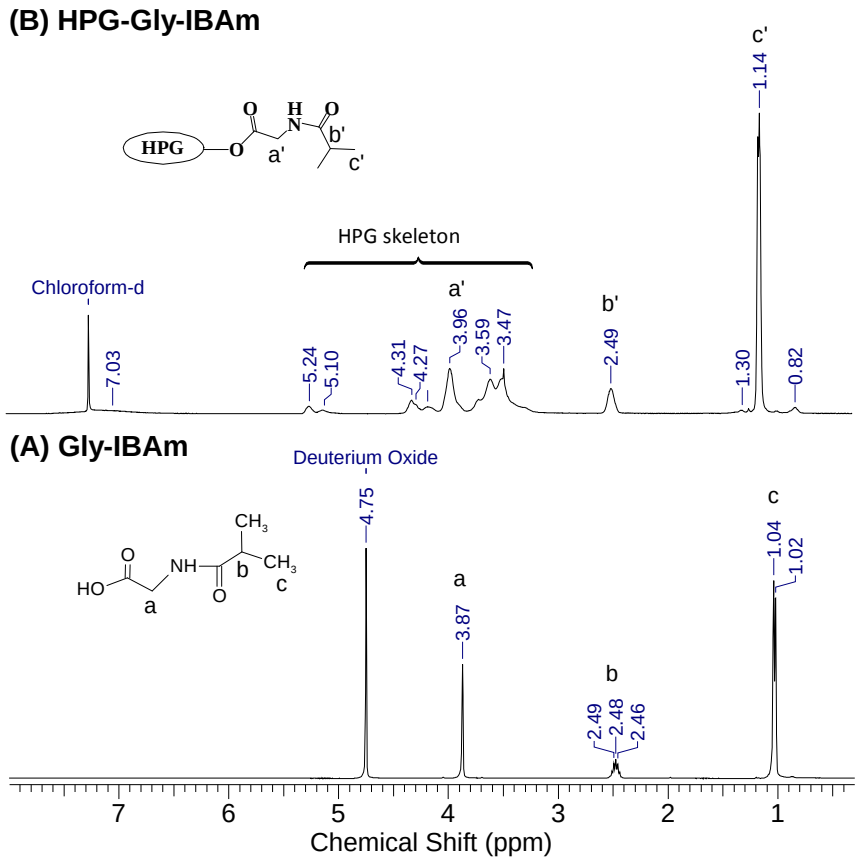

Fig. S4 Typical ^{1}H NMR spectra of (A) Gly-IBAm and (B) HPG-Gly-IBAm.

Degree of substitution of Gly-IBAm group in OH group of HPG can be calculated from Figure S4B according to eqn. 3:

$$\text{Degree of substitution} = I(b')/I(HPG)/5 \qquad (3)$$

I (b') means the integral of b' signal, which can be read off from the spectrum directly. I (HPG) means the integral of HPG skeleton, which cannot read off from the spectrum directly, but can be calculated using the integral of the signals between 3.0 and 5.5 ppm minus 2*I (b').

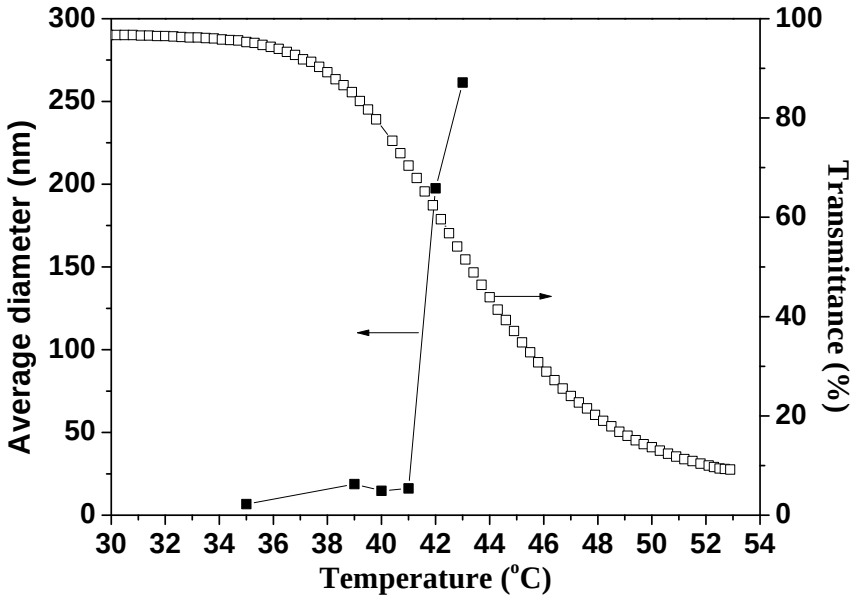

Fig. S5 Typical temperature dependent DLS and transmittance curves of HPG-Val-IBAm in deionized water (10 mg/mL)

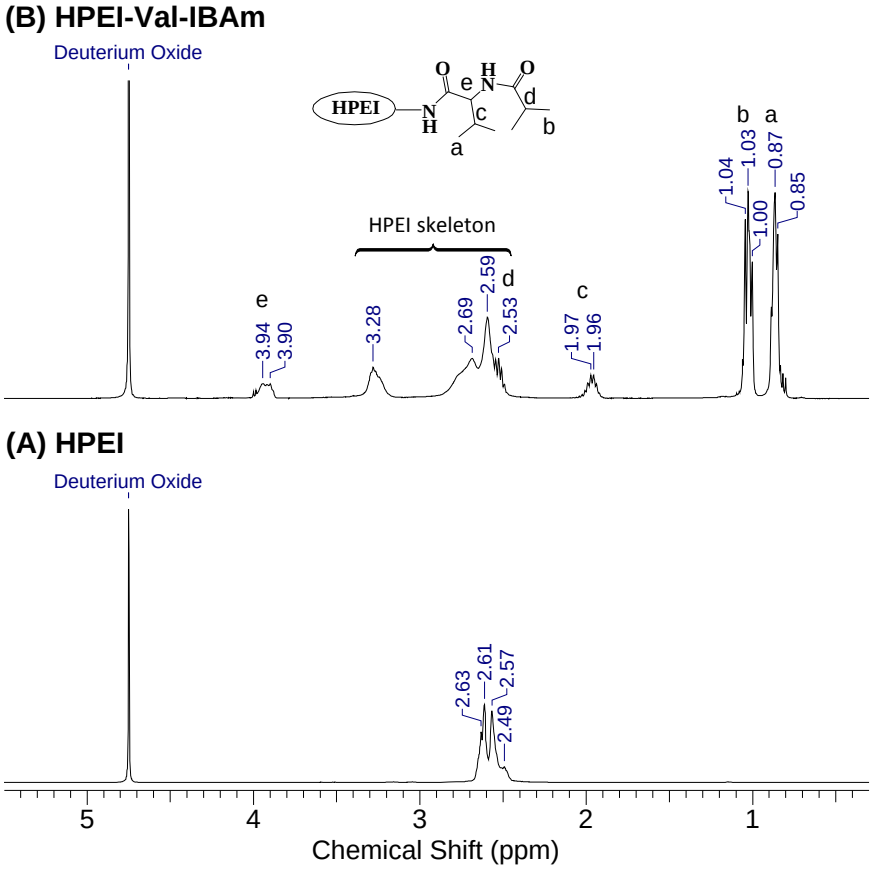

Fig. S6 Typical ^{1}H NMR spectrum of HPEI-Val-IBAm.

Degree of substitution of Val-IBAm group in primary and secondary amino groups of HPEI can be calculated from Fig. S6B according to eqn. 4:

$$\text{Degree of substitution} = I(c)/\ I(\text{HPEI})/\ 4/\ 0.72 \qquad (4)$$

I (c) means the integral of c signal, which can be read off from the spectrum directly. I (HPEI) means the integral of HPEI skeleton, which cannot read off from the spectrum directly, but can be calculated using the integral of the signals between 2.5 and 3.5 ppm minus I (c). Since the content of primary and secondary amino groups in the total amino groups of HPEI is 0.72, thus the factor of 0.72 exists in the equation.

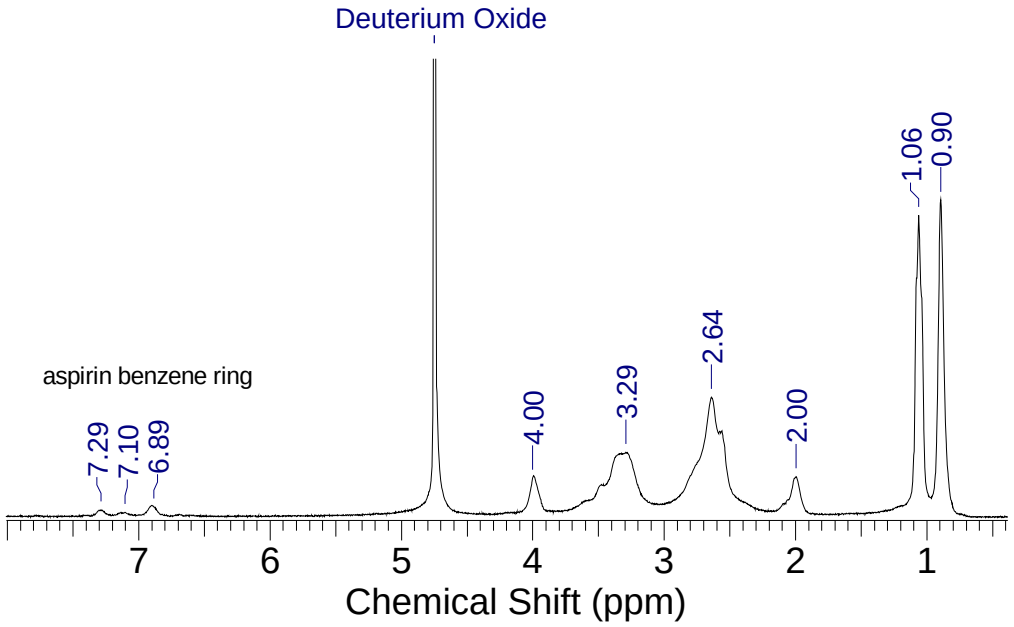

Fig. S7 Typical ^{1}H NMR spectrum of HPEI-Val-IBAm conjugated with aspirin unit.

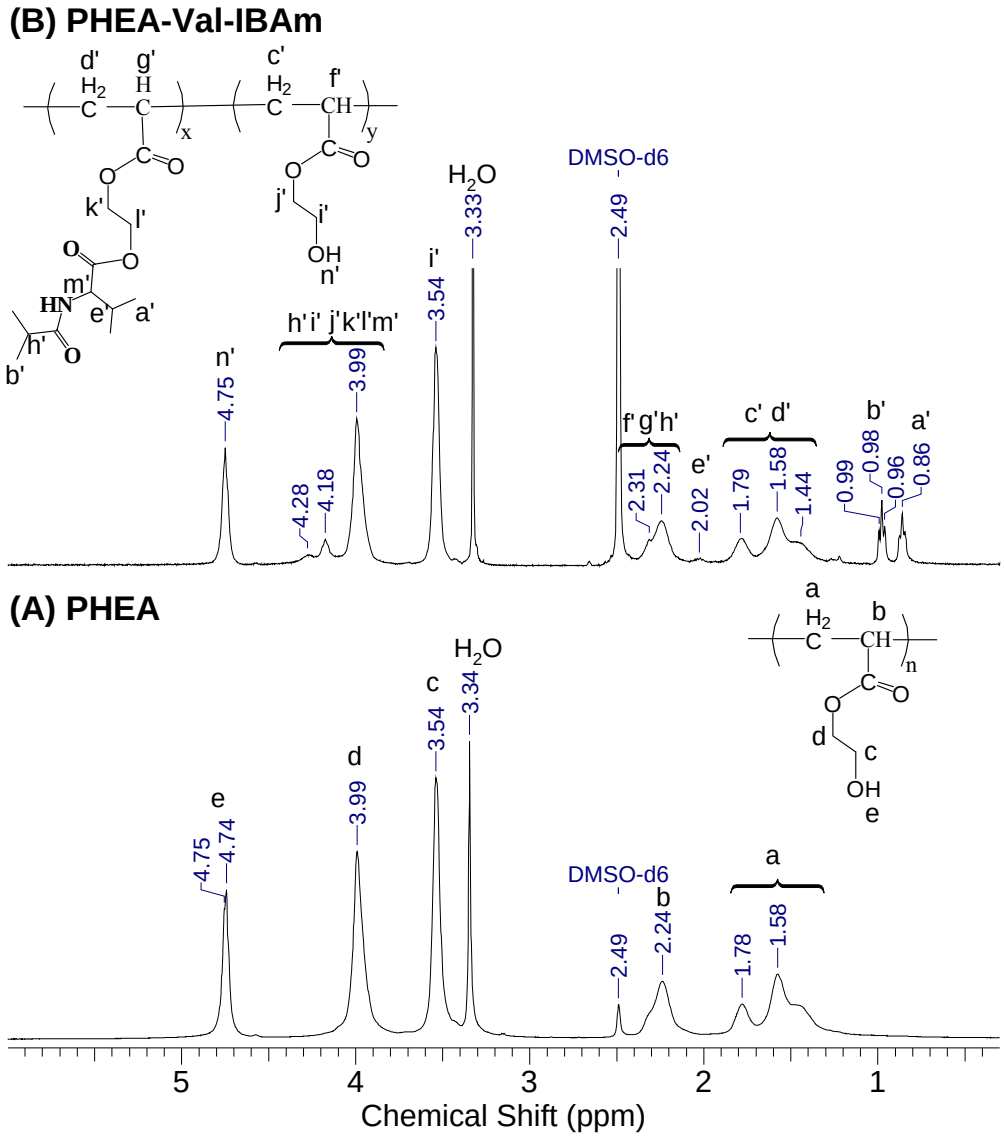

Fig. S8 Typical ^{1}H NMR spectra of (A) PHEA, (B) PHEA-Val-IBAm.

Degree of substitution of Val-IBAm group in OH groups of PHEA can be calculated from Fig. S8B according to eqn. 5:

$$\text{Degree of substitution} = I(a'+b')/12 \,/\, [I(i')/2 + I(a'+b')/12] \qquad (5)$$

I (a'+b') means the integrals of a' and b' signals, which can be read off from the spectrum directly. I (i') means the integral of I' signal, which can be read off from the spectrum directly, too.

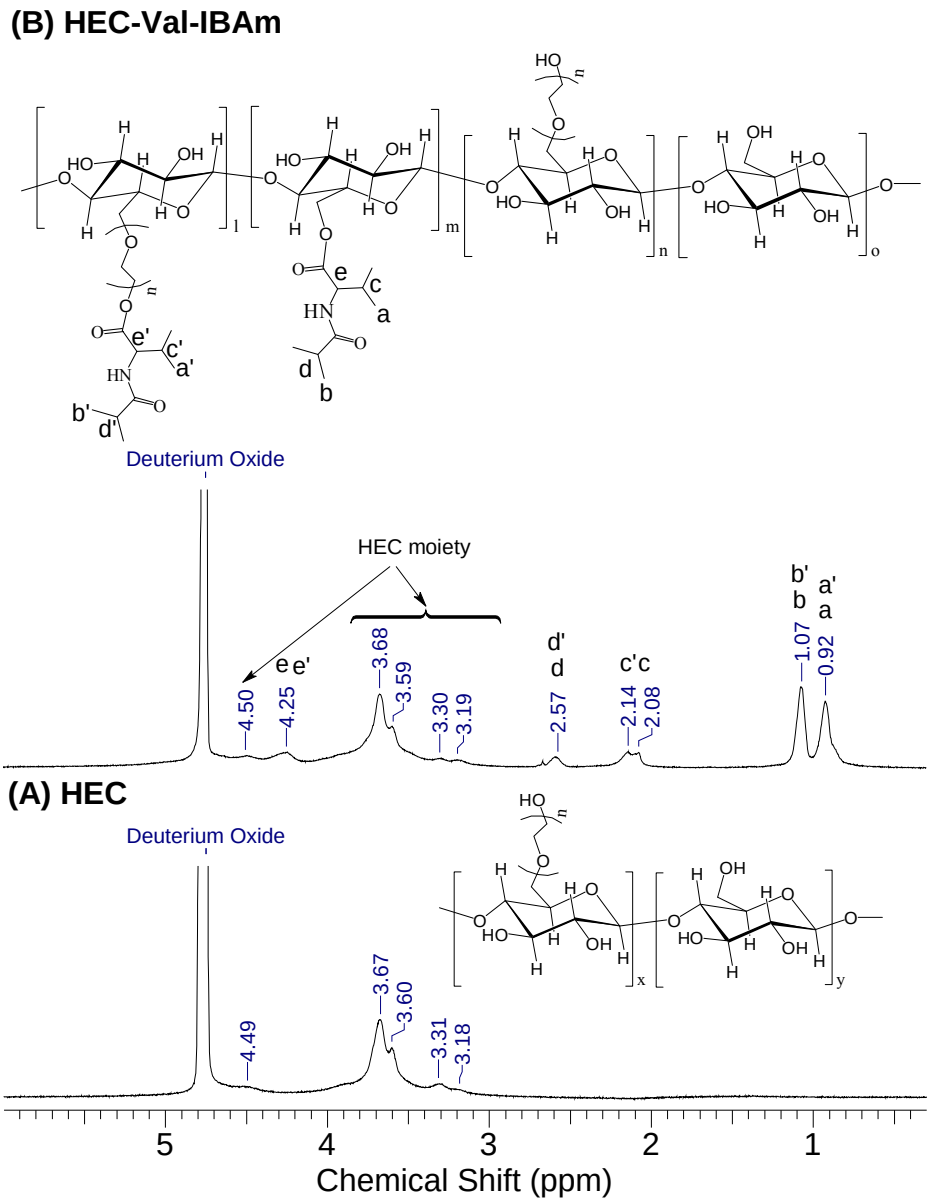

Fig. S9 Typical ^{1}H NMR spectra of (A) HEC, (B) HEC-Val-IBAm.

Degree of substitution of Val-IBAm group in OH groups of HEC cannot be calculated from Figure S9B due to the uncertainty of the amount of EO units in HEC and the overlapping of the signals of EO units, glucose rings and the solvents. Thus, the degree of substitution of Val-IBAm group in OH groups of HEC was determined from the elemental analysis.

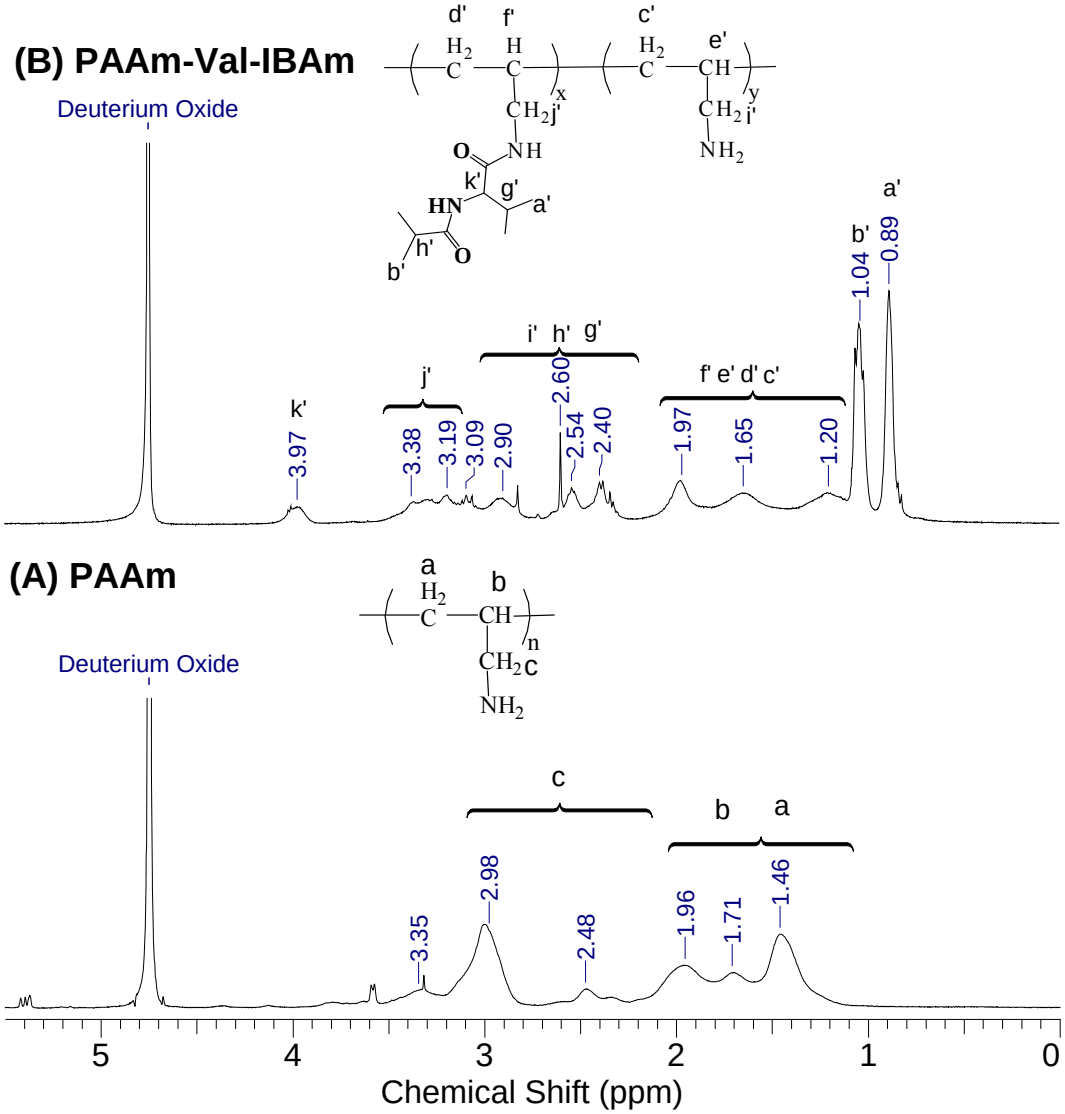

Fig. S10 Typical ^{1}H NMR spectra of (A) PAAm, (B) PAAm-Val-IBAm.

Degree of substitution of Val-IBAm group in amino groups of PAAm can be calculated from Fig. S10B according to eqn. 6:

$$\text{Degree of substitution} = I(k') / [I(a' + b' + c' + d' + e' + f') - 12\, I(k')] / 3 \qquad (6)$$

I (a' +b' +c' +d' +e' +f') means the integrals of a', b', c', d', e' and f' signals, which can be read off from the spectrum directly. I (k') means the integral of k' signal, which can be read off from the spectrum directly, too.

1. Aoyagi, N. & Endo, T. Functional RAFT Agents for Radical-Controlled Polymerization: Quantitative Synthesis of Trithiocarbonates Containing Functional Groups as RAFT Agents Using Equivalent Amount of CS_2. *J. Polym. Sci. Part A: Polym. Chem.* **47**, 3702–3709 (2009).
2. Steinhauer, W., Hoogenboom, R., Keul, H. & Moeller, M. Copolymerization of 2-Hydroxyethyl Acrylate and 2-Methoxyethyl Acrylate via RAFT: Kinetics and Thermoresponsive Properties. *Macromolecules* **43**, 7041-7047 (2010).
3. Doherty, D. G. & Popenoe, E. A. The Resolution of Amino Acids by Asymmetric Enzymatic Synthesis. *J. Biol. Chem.* **189**, 447-460 (1951).