

## *Supplementary Information*

### **Low swelling hyperbranched poly(amine-ester) hydrogels for pH-modulated differential release of anticancer drugs**

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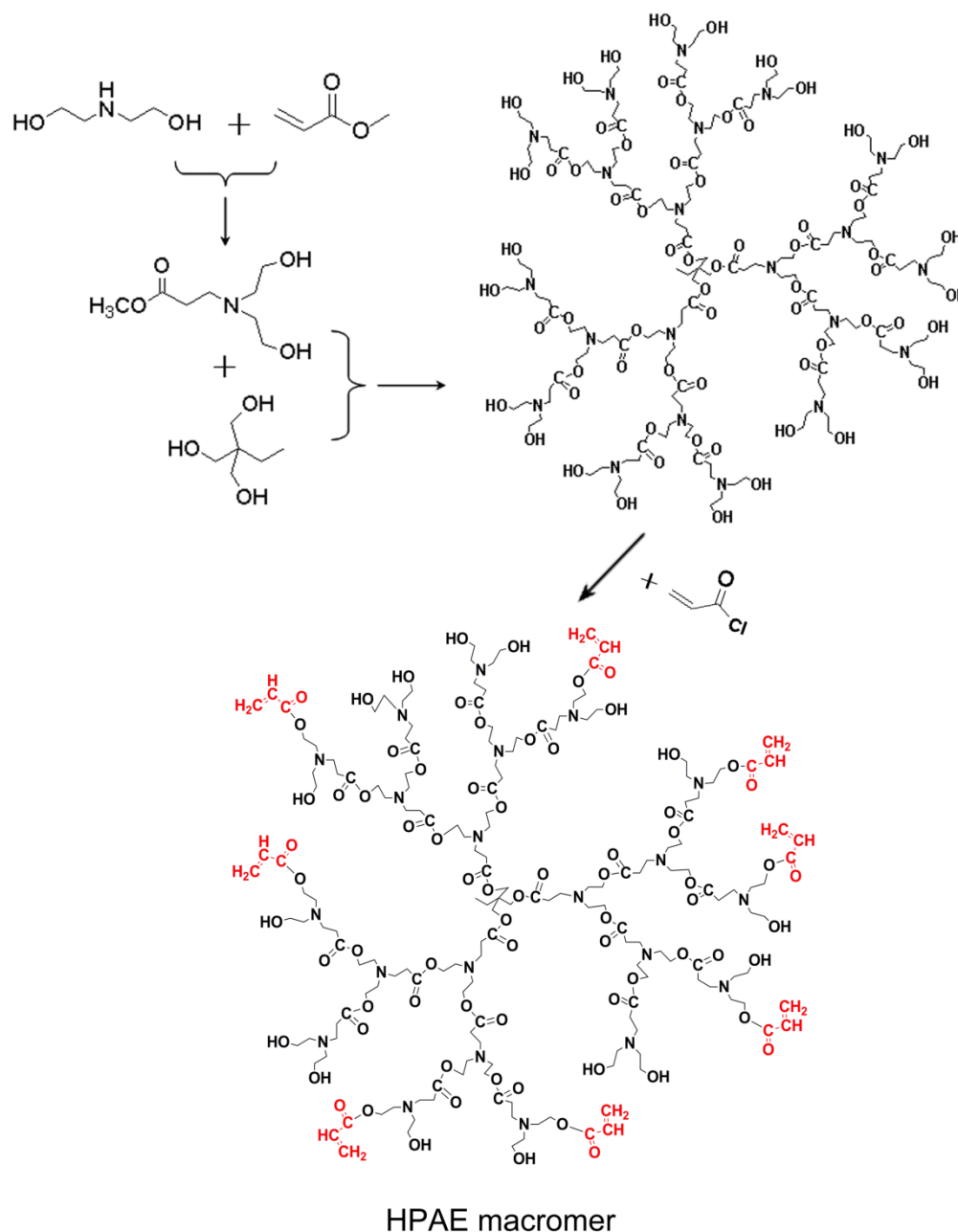
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## 1. Synthesis of C=C modified generation 5 HPAE (HPAE macromer)

The synthetic procedure was as mentioned in our previous report [1] and the synthetic process was shown in Scheme S1.



Scheme S1. Synthetic procedure of HPAE macromer

### 1.1 Synthesis of *N,N*-diethylol-3-amine methylpropionate (as an AB<sub>2</sub> monomer)

0.10 mol methyl acrylate and 0.10 mol diethanolamine were mixed with 10 mL of

methanol in a flask. The mixture was stirred for 30 min under nitrogen atmosphere, then heated to 35 °C and kept stirring for 4 h. Thereafter, methanol was removed under reduced pressure and the product was obtained as colorless oil.

## 1.2 Synthesis of generation 5 HPAE macromolecules

Generation 5 HPAE macromolecules were synthesized using pseudo-one-step manner. 1, 1, 1-trimethylolpropane (0.01 mol, as a core molecule), the monomer synthesized above (0.09 mol) were mixed with the p-toluene sulphonic acid (PTSA, 0.093 g) as a catalyst in a flask. Then the mixture was stirred and kept refluxing at 120 °C for 3 h. After the removal of methanol by evaporation under reduced pressure, the generation 2 HPAE was obtained. Then the monomer and PTSA of corresponding content were added into the flask with generation 2 HPAE, and repeated above procedure to get generation 3 HPAE. Finally, with the same procedure, the product generation 5 HPAE as yellowy oil was obtained after methanol was evaporated under vacuum.

## 1.3 Modification of generation 5 HPAE macromolecules with acryloyl chloride

Generation 5 HPAE (4.66 g, [OH]  $\approx$  0.03 mol) was dissolved in 50 mL of dichloromethane with appropriate amounts of anhydrous Na<sub>2</sub>CO<sub>3</sub>, and cooled in an ice bath. Then acryloyl chloride (12 mmol) was added dropwise. The mixture was reacted at 37 °C for 6 h under magnetic stirring. Thereafter, the solution was dried with anhydrous MgSO<sub>4</sub>, and the solvent was finally evaporated under reduced pressure to obtain the product (HPAE macromers) as a yellowy viscous liquid.

## 2. Characterization of C=C modified generation 5 HPAE (HPAE macromer)

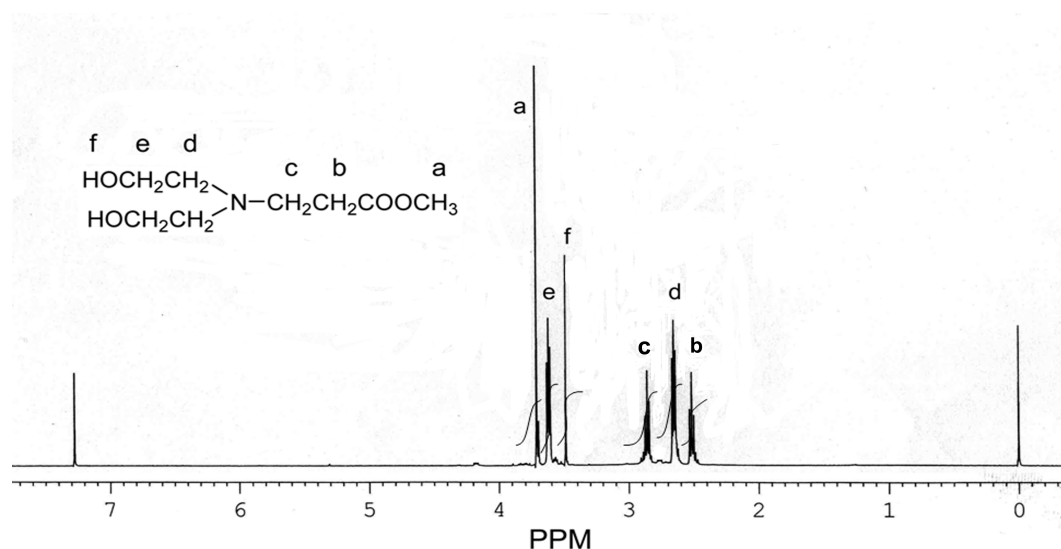
### 2.1 Characterization of the AB<sub>2</sub> monomer N, N-diethylol-3-amine methylpropionate

N, N-diethylol-3-amine methylpropionate was characterized by element analysis (CHN-O-Rapid, Foss Heraeus, Germany) (Table S1.) and <sup>1</sup>H NMR (DMX-400, Bruker, Rheinstten, Germany) (Figure S1)

<sup>1</sup>H NMR (CDCl<sub>3</sub>) [400 MHz]: 3.48 (broad, -CH<sub>2</sub>-OH, 2H);  $\delta$ (ppm)= 3.70 (s, -CH<sub>3</sub>, 3H); 3.62 (t, -CH<sub>2</sub>-OH, 4H); 2.86 (t, -N-CH<sub>2</sub>-CH<sub>2</sub>-COO-, 2H); 2.64 (t, -CH<sub>2</sub>-CH<sub>2</sub>-OH, 4H); 2.51 (t, -CH<sub>2</sub>-COO-, 2H).

**Table S1.** Element analysis values of the AB<sub>2</sub> monomer *N,N*-diethylol-3-amine methylpropionate

Element	Theoretical value (wt%)	Experimental value (wt%)
C	50.26	49.57
H	8.90	9.32
N	7.33	7.58



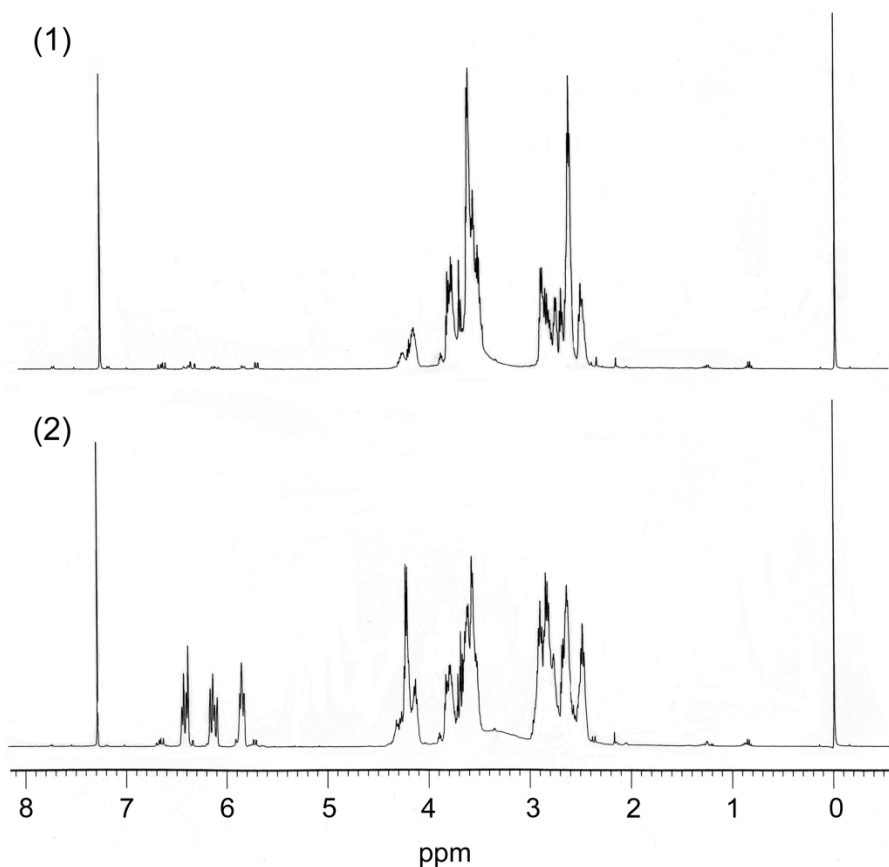
**Figure S1.** <sup>1</sup>H NMR spectrum of the AB<sub>2</sub> monomer, *N,N*-diethylol-3-amine methylpropionate.

## 2.2 <sup>1</sup>H NMR spectrum of generation 5 HPAAE and the C=C modified HPAAE molecule (HPAAE macromer)

The molecular weight distribution of generation 5 HPAAE (M<sub>w</sub>/M<sub>n</sub>=1.07) was measured by GPC (LC-20AD, Shimadzu, Japan), and the structure was characterized by <sup>1</sup>H NMR (DMX-400, Bruker, Rheinstetten, Germany) (Fig. S2-1). The hydroxyl value of generation 5 HPAAE was obtained by chemical titration, which is approximated to that of theory. The theoretical value is 361 mg KOH/g, and the experimental value is about 372 mg KOH/g.

The theoretical value of C=C modification degree is about 40 % based on the feed ratio of acryloyl chloride for reaction. The experimental value of C=C content was characterized by <sup>1</sup>H NMR (Fig. S2-2). As shown, some new signals appear at about 5.80, 6.10 and 6.40 ppm in the <sup>1</sup>H NMR spectra of HPAAE macromer comparing with

that of HPAE molecule without modification (Fig. S2-1), which can be assigned to the protons of terminal C=C. The substituted groups were identified by the signals of the C=C group at about 5.80 and 6.40 ppm (-CH=CH<sub>2</sub>), and the hydroxyl groups by the proton signals of >N-CH<sub>2</sub>-CH<sub>2</sub>-OH at about 2.55-2.80 ppm. The actual degree of C=C modification was about 36.1 % calculated from the ratios of double bond and hydroxyl according to the intensities of proton peaks.



**Figure S2.** <sup>1</sup>H NMR spectrum of the HPAE molecules: (1) before C=C modification and (2) after C=C modification.

## References

- [1] H.B. Zhang, C. Zhao, H. Cao, G.J. Wang, L. Song, G.G. Niu, H. Yang, J. Ma, S.Q. Zhu, Hyperbranched poly(amine-ester) based hydrogels for controlled multi-drug release in combination chemotherapy, *Biomaterials* 31 (2010) 5445-5454.