Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

The P(NIPAM-co-DABP-co-AAc) as a dual stimuli-responsive hydrogel: Temperature and pH-responsive materials for potential drug delivery applications

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General Consideration

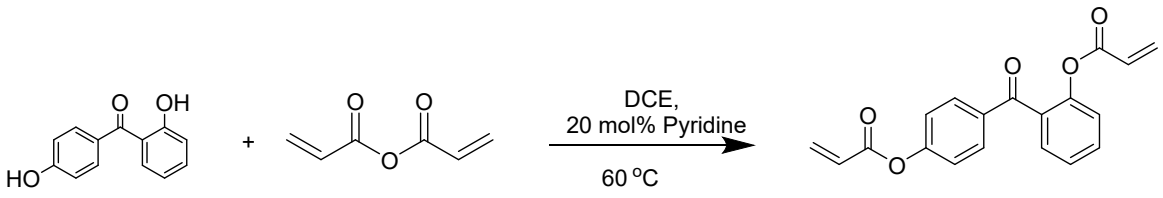
The ^{1}H and ^{13}C spectra were recorded on Bruker AVANCE NEO NMR SPECT.400 NanoBay system in $CDCl_3$ as a residual undeuterated solvent ($CDCl_3$: 7.26/77.0 ppm) for DABP, CD_3OD for PNIPAM-co-DABP (CD_3OD: 3.35-4.78 ppm), and DMSO for P(NIPAM-co-DABP-co-AAc) (CD_3SOCD_3: 2.49 ppm) using Me_4Si as an internal standard. Chemical shifts (δ) are given in ppm, and J values are given in Hz. The FT-IR spectra were recorded on neat (Solids) samples using Alpha 2, Bruker spectrometer. The mass spectra were recorded using LC/MS-MS (Agilent Technologies, USA). Column purification and Thin Layer Chromatography (TLC) were performed on silica gel (CDH silica gel 60-120 mesh, F254, Merck® silica gel respectively). Evaporation of all solvents was performed at reduced pressure, using IKA rotary evaporator. All the chemicals were purchased from Sigma Aldrich, Merck®, and TCI chemicals and used as received. Scanning electron microscopy (SEM) was utilized to observe the morphological changes of the hydrogel, which was used for the *in vitro* release studies. The hydrogel samples which were utilized for the 5-FU release in pH 1.2 and 6.8 at 37 C were lyophilized to maintain the morphological behavior before the SEM analysis. After the lyophilization, the samples were mounted on carbon tape and sputtered with gold using Quorum sputter (Q 150R S plus, Quorum, UK). The SEM

micrograph images were acquired using electron microscopy (Carl Zeiss/Gemini SEM 360) at the required magnification and voltage.

Typical synthetic scheme for triblock copolymer P(NIPAM-co-DABP-co-AAc)

A) Step-wise approach

Step 1: Typical synthetic scheme for 2,4′-diacryloyloxy benzophenone (DABP)

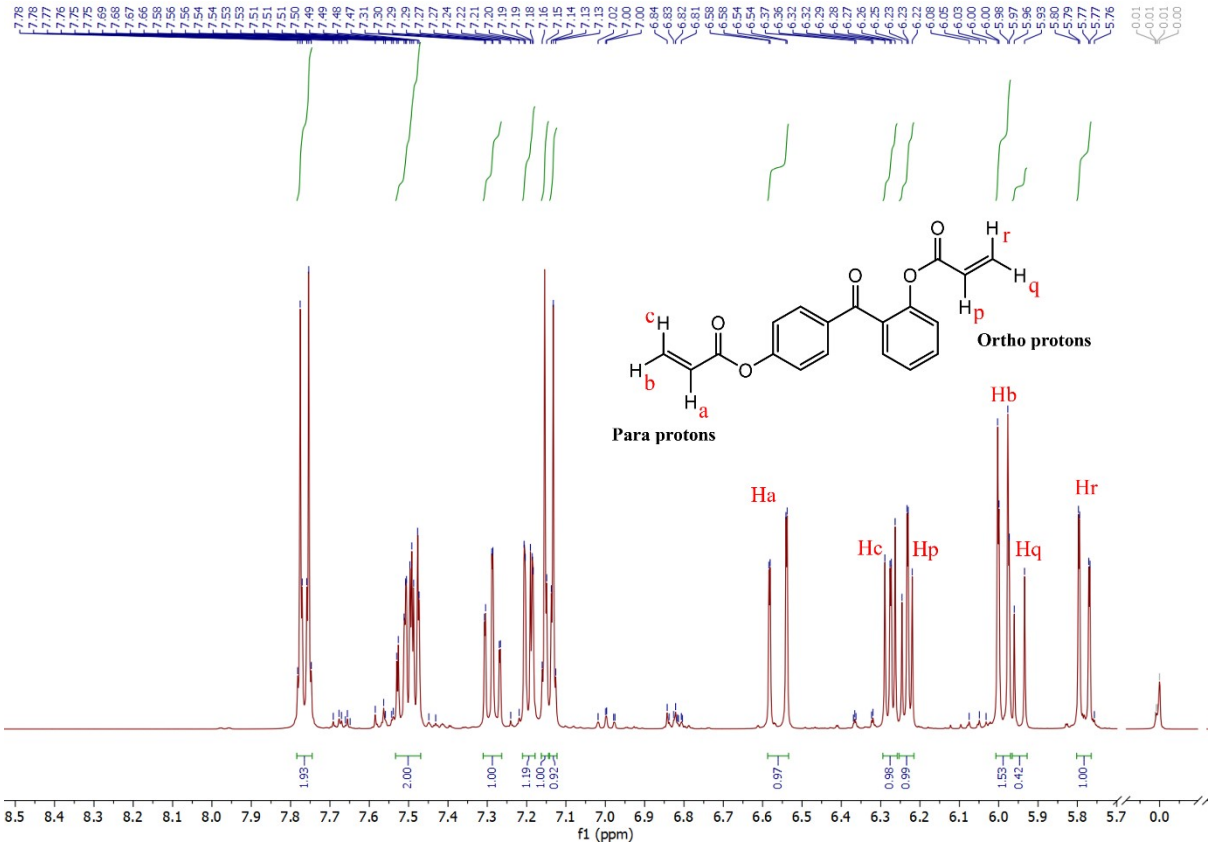


2,4'-Dihydroxybenzophenone (1)

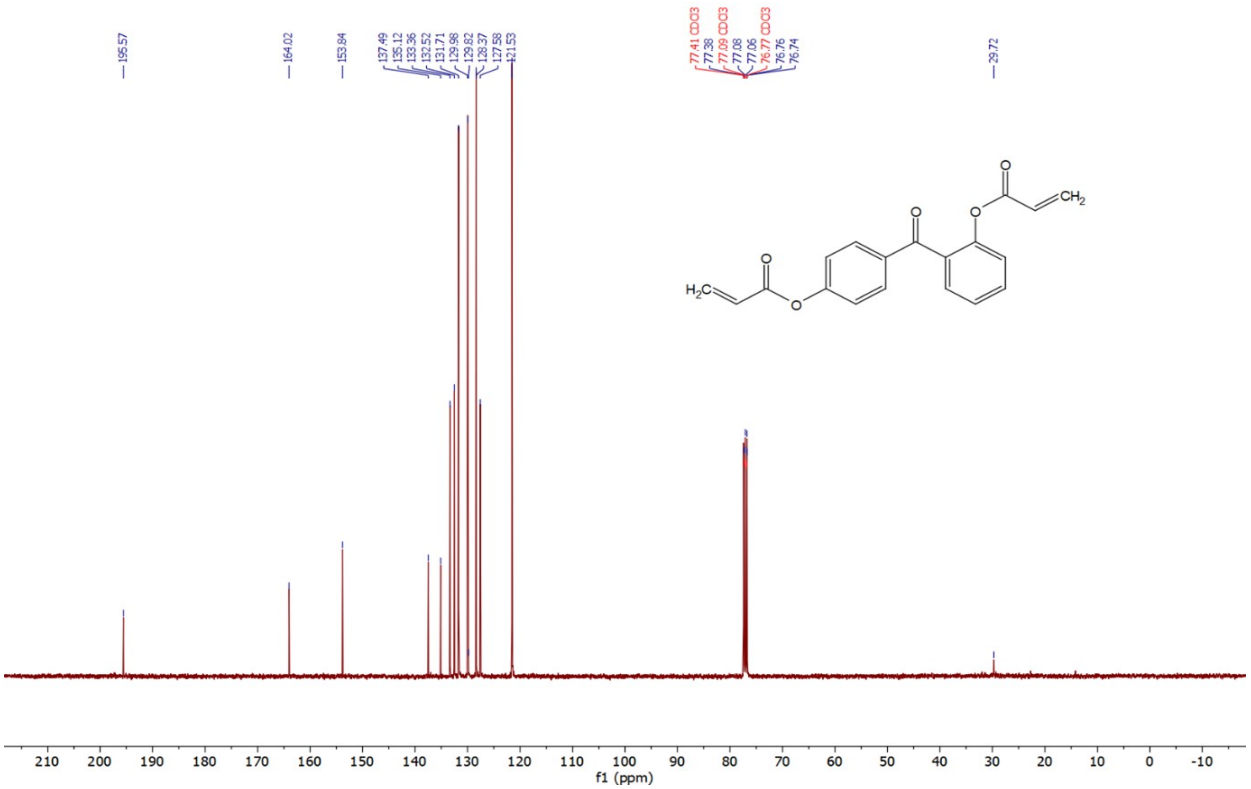
Acrylic anhydride (2)

2,4'- diacryloyloxy benzophenone (3)

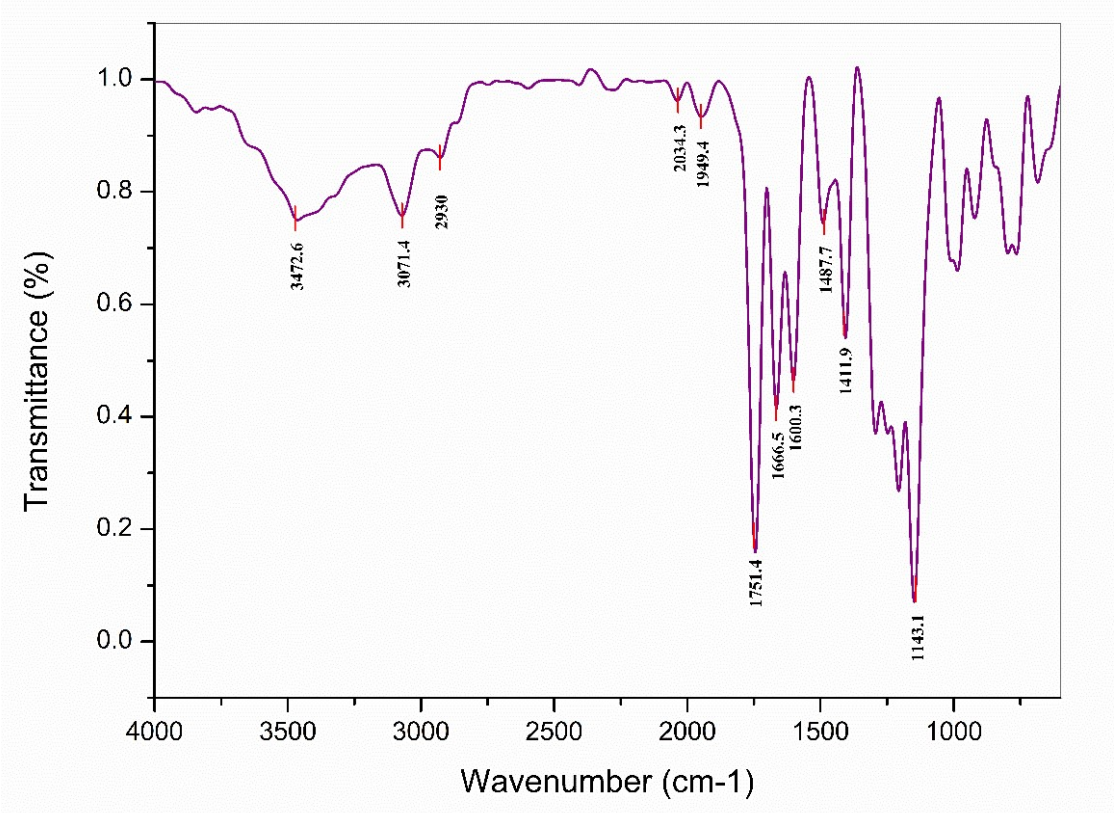
Scanned NMR, Mass, and FT-IR spectra



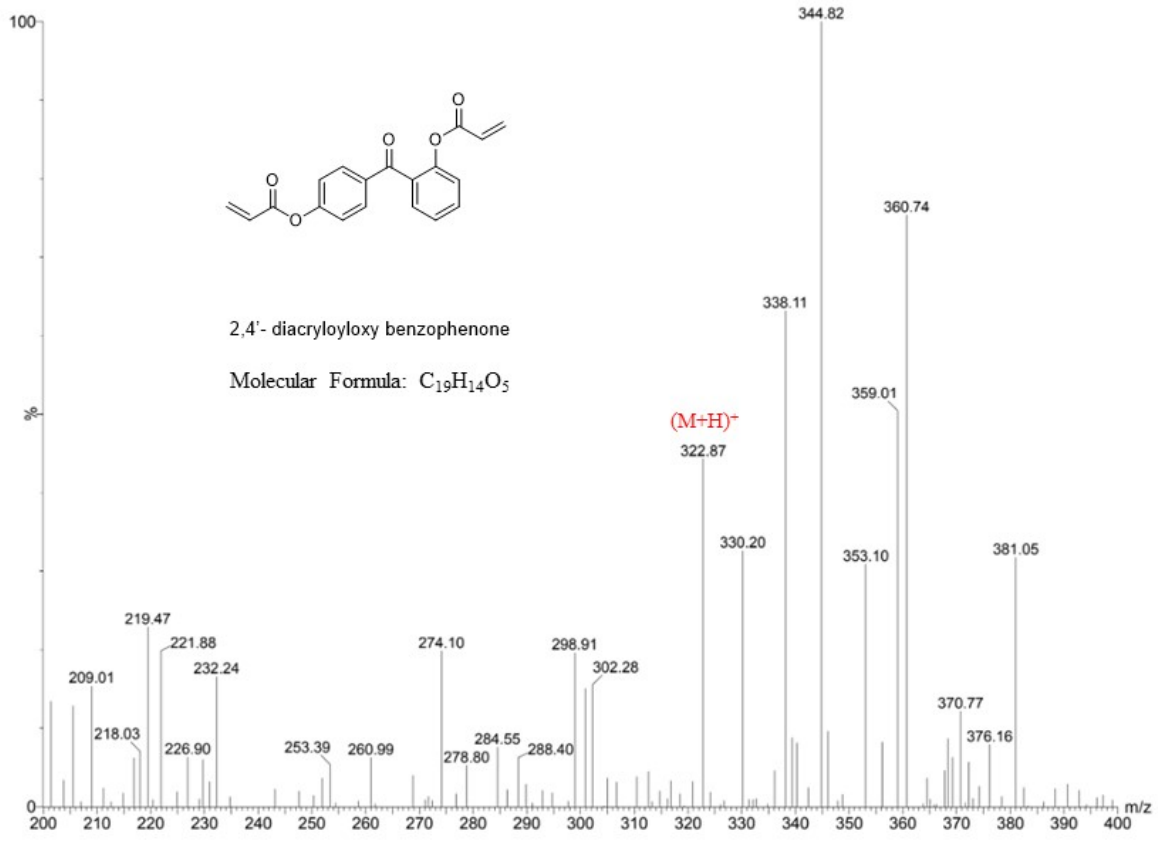
Supplementary Figure S1: H^1 NMR of 2,4′-diacryloyloxy benzophenone



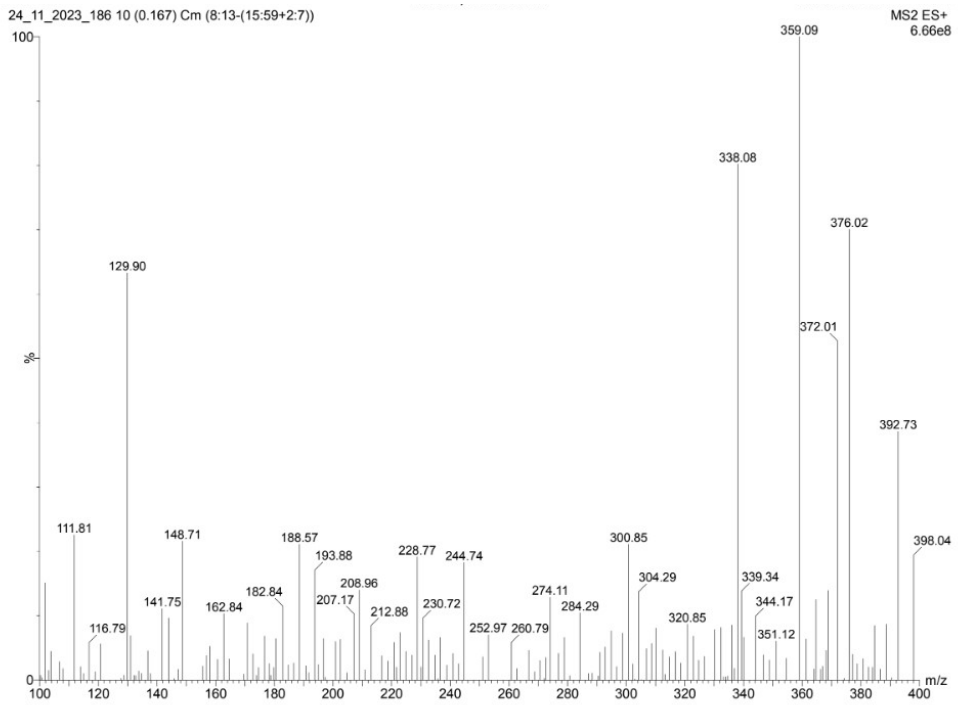
Supplementary Figure S2: C^{13} NMR of 2,4′-diacryloyloxy benzophenone



Supplementary Figure S3: Scanned FT-IR spectrum of 2,4′-diacryloyloxy benzophenone

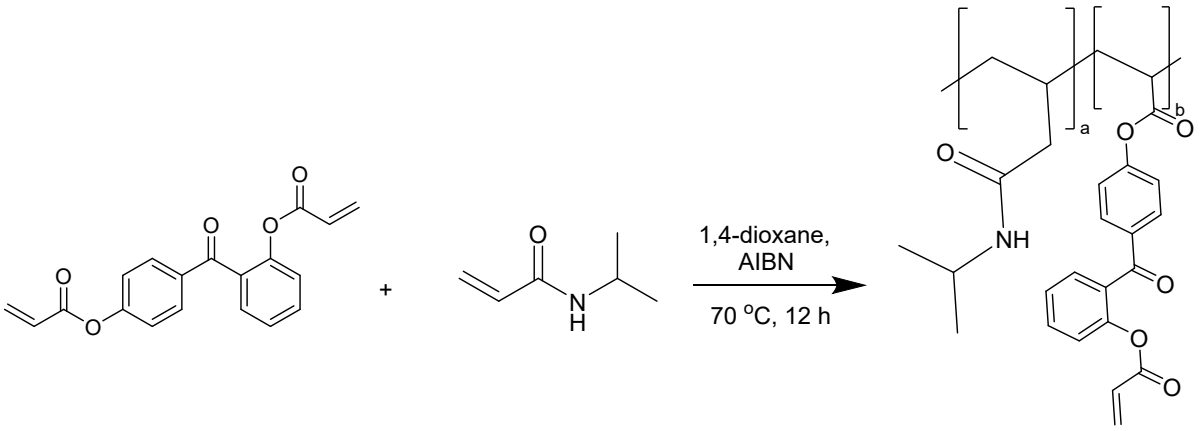


Supplementary Figure S4: Scanned mass spectrum 2,4′-diacryloyloxy benzophenone



Supplementary Figure S5: Blank mass spectrum

Step 2: Typical synthetic scheme for P(NIPAM-co-DABP)



2,4'- diacryloyloxy benzophenone

(3)

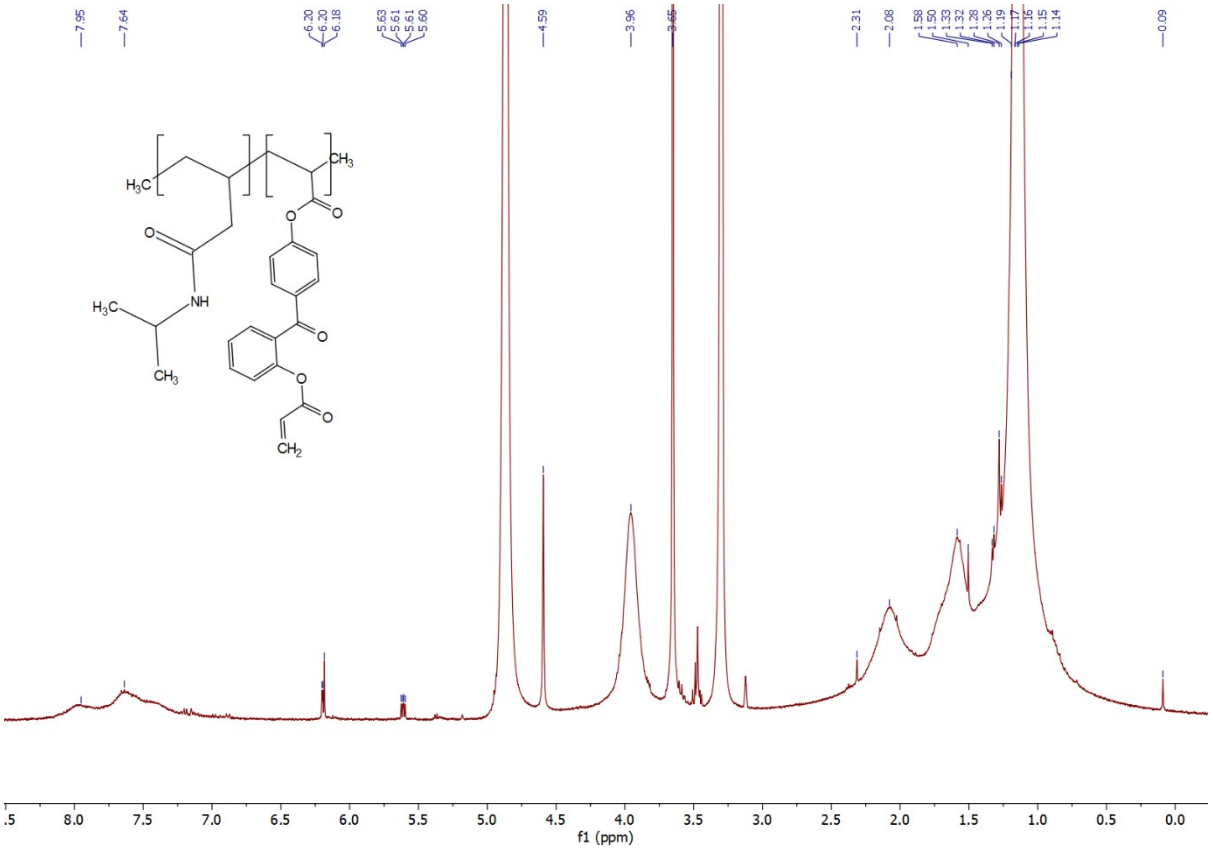
N-isopropylacrylamide

(4)

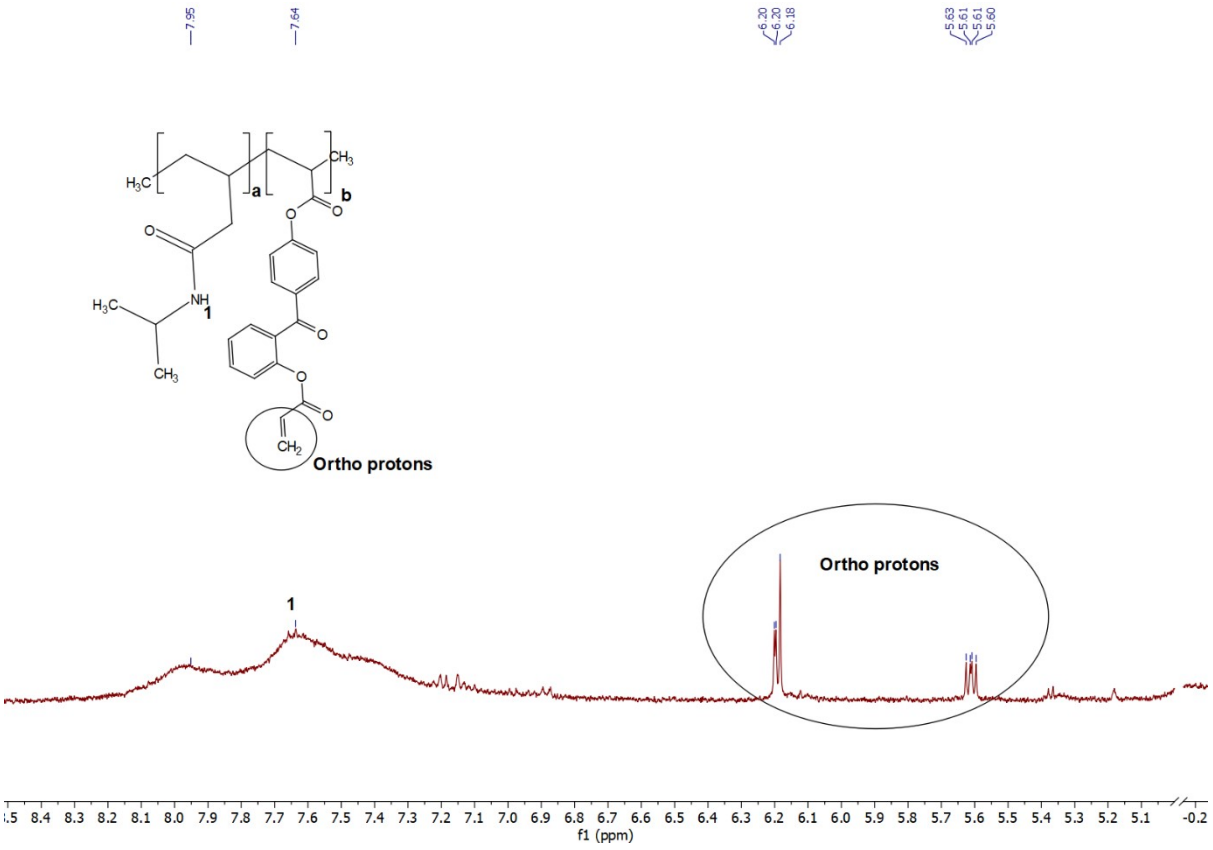
P(NIPAM-co-DABP)

(5)

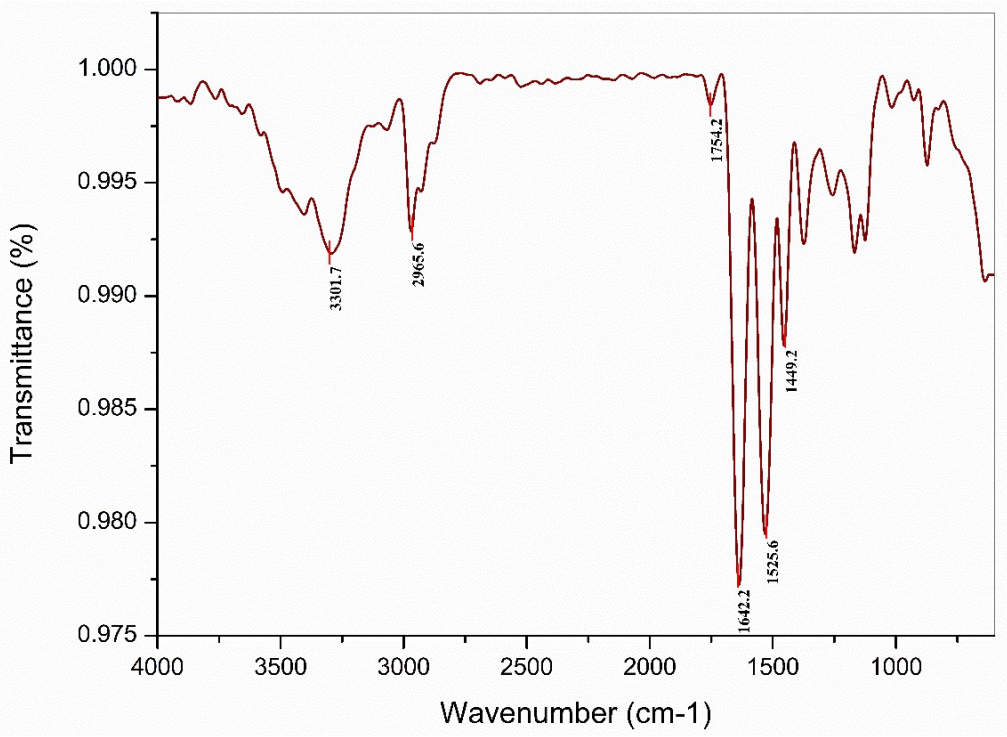
Scanned NMR and FT-IR spectra



Zoom in

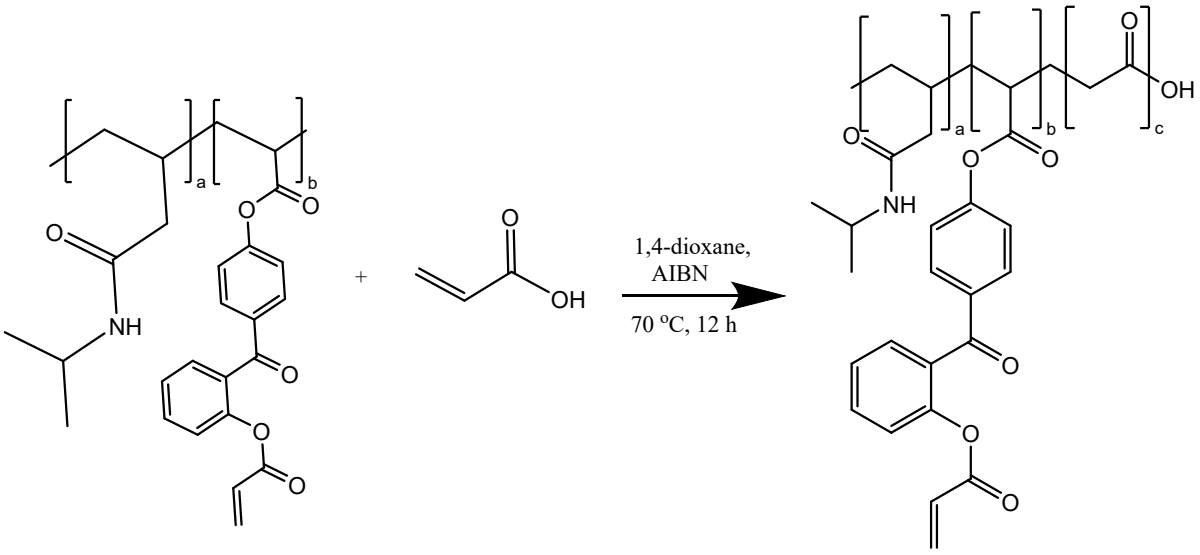


Supplementary Figure S6: Expanded H^1 NMR of P(NIPAM-co-DABP)



Supplementary Figure S7: Scanned FT-IR spectrum of P(NIPAM-co-DABP)

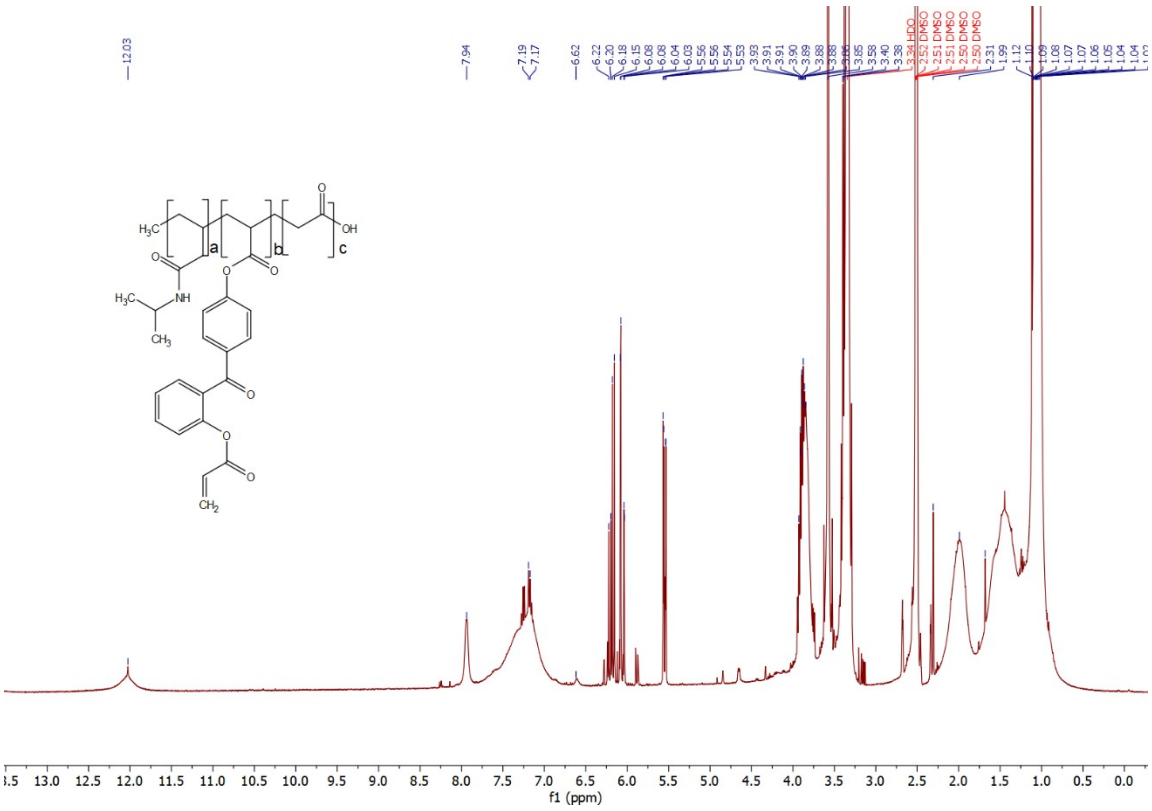
Step 3: Typical synthetic scheme for P(NIPAM-co-DABP-co-AAc)



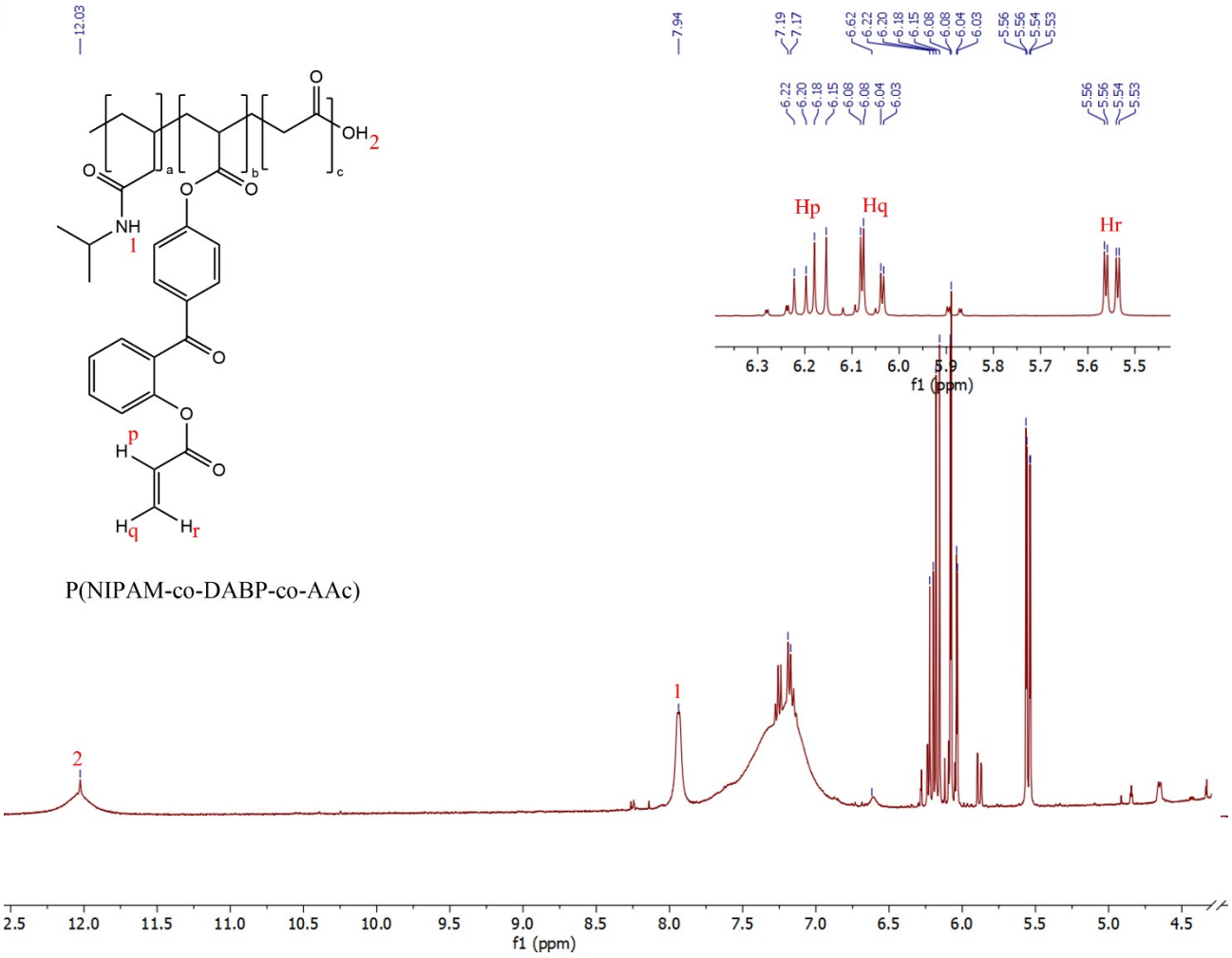
P(NIPAM-co-DABP)
(5)

Acrylic acid
(6)

P(NIPAM-co-DABP-co-AAc)
(7)

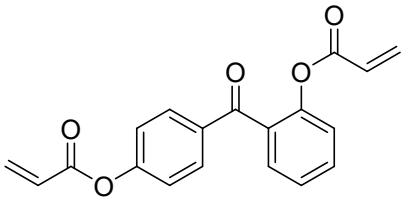


Zoom in



Supplementary Figure S8: Expanded H^1 NMR of P(NIPAM-co-DABP-co-AAc)

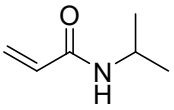
B) One-pot multicomponent synthesis of P(NIPAM-co-DABP-co-AAc)



2,4'- diacryloyloxy benzophenone

(3)

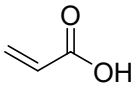
+



N-isopropylacrylamide

(4)

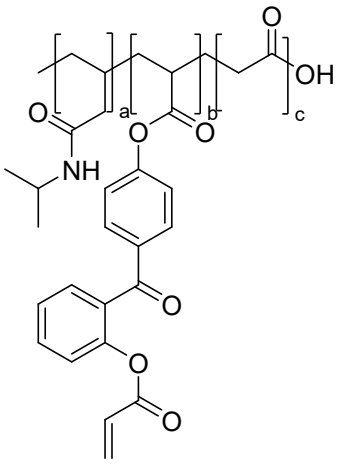
+



Acrylic acid

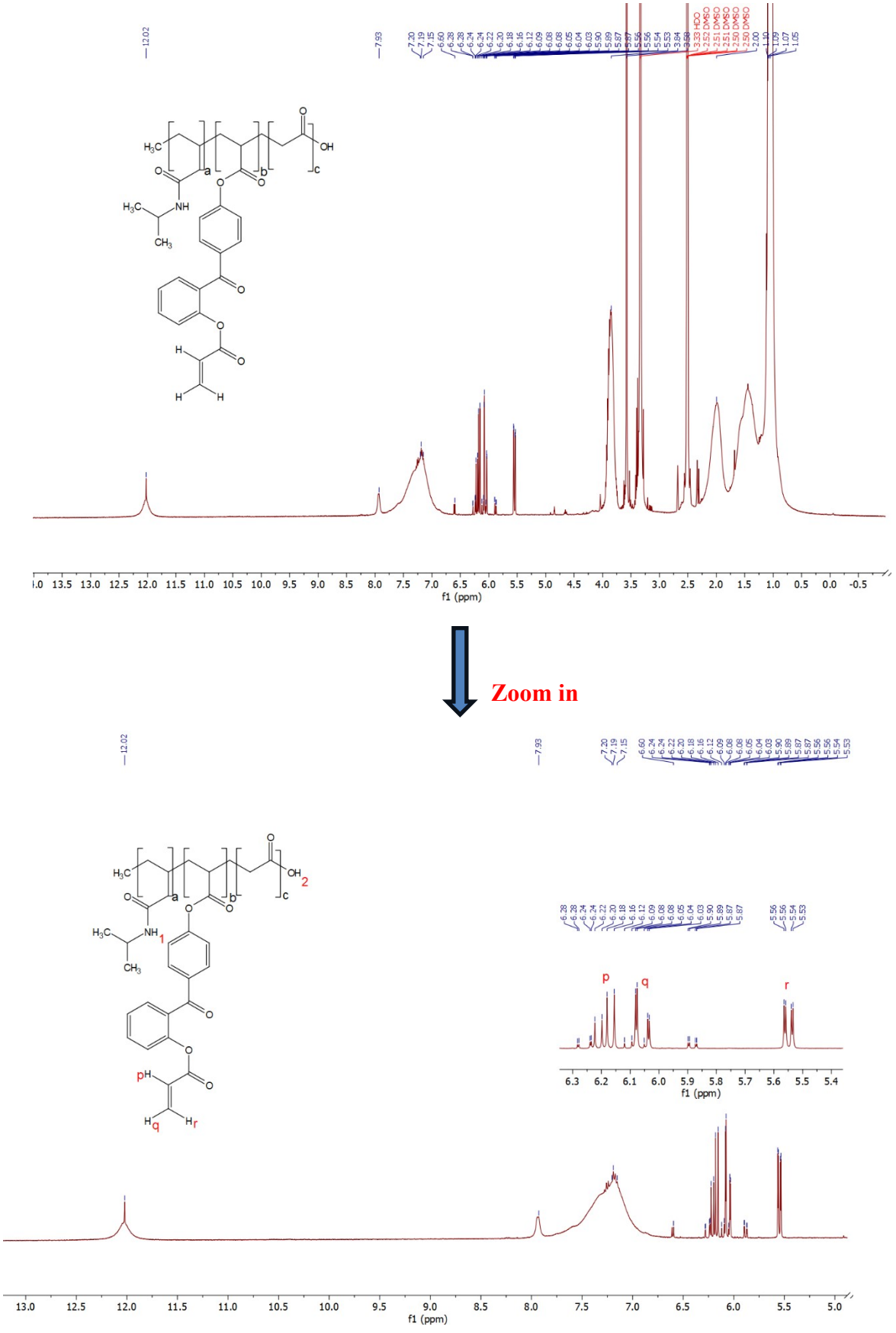
(6)

70 °C | 1,4-dioxane, AIBN



P(NIPAM-co-DABP-co-AAc)

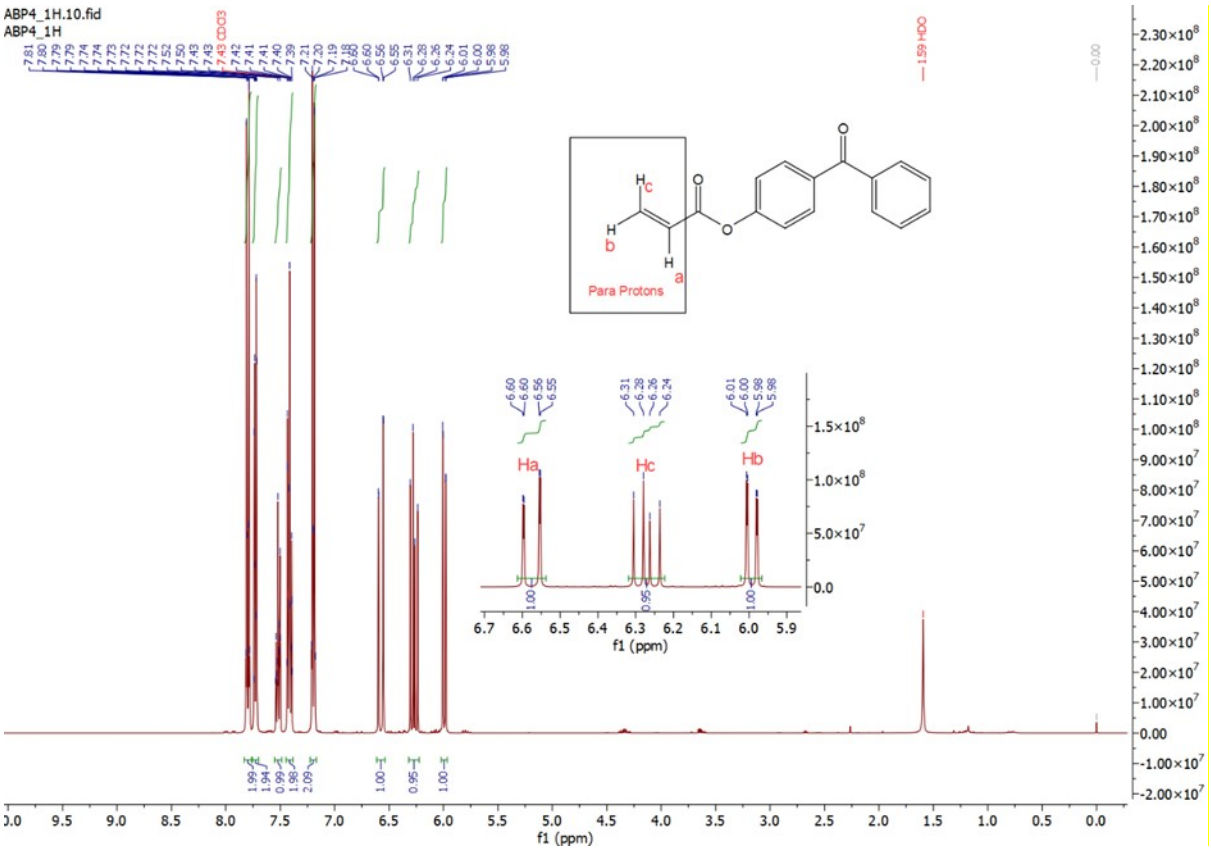
(7)



Supplementary Figure S9: Expanded H^1 NMR of P(NIPAM-co-DABP-co-AAc)

The detailed strategy to confirm regio-selective copolymerization of the PNIPAM to the para-acryloyl unit of DABP is given in supplementary information.

To confirm the regio-selective copolymerization of the PNIPAM to the para-acryloyl unit of DABP, we synthesized 4-acroryloxybenzophenone using the procedure reported in our previous published work [1]. In 4-acroryloxybenzophenone, the acryloyl protons are present at the para position, and we confirmed the same by taking the ^{1}H NMR spectra of 4-acroryloxybenzophenone (As shown in **Supplementary Figure S10**).



Supplementary Figure S10: ^{1}H NMR of 4-acroryloxybenzophenone

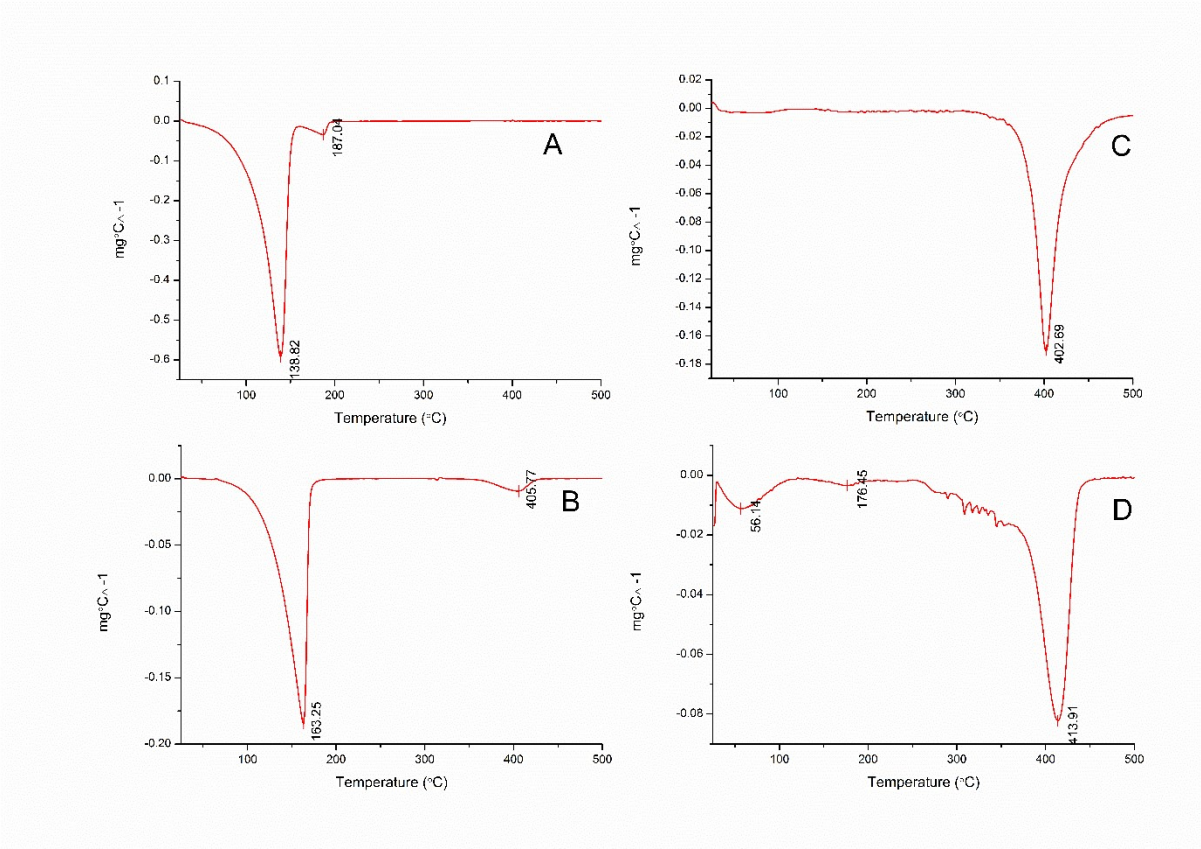
In ^{1}H NMR spectra of 4-acroryloxybenzophenone, we got the para proton shift in the range of 5.98-6.60 ppm. In detail, 1H NMR (400 MHz, Chloroform-d) δ 7.88 – 7.66 (m, 4H), 7.59 – 7.46 (m, 1H), 7.46 – 7.35 (m, 2H), 7.26 – 7.13 (m, 2H), 6.58 (dd, J = 17.3, 1.2 Hz, 1H), 6.27 (dd, J = 17.3, 10.4 Hz, 1H), 5.99 (dd, J = 10.4, 1.2 Hz, 1H). In the next step, we analyzed the ^{1}H NMR spectra of DABP and compared them with the ^{1}H NMR of 4-acroryloxybenzophenone. From the ^{1}H NMR spectra of DABP (**Supplementary Figure S1**), we confirmed the presence of para and ortho proton and matched with the para shift of 4-

acroryloxybenzophenone. As shown in **Supplementary Figure S1**, the para-proton shifts were observed in the range of 6.0-6.58 ppm, slightly shifted as compared to the 4-acroryloxybenzophenone, and the ortho-proton shift was observed in the range of 5.8-6.25 ppm. Finally, the P(NIPAM-co-DABP) ^{1}H NMR spectra were recorded and compared with the ^{1}H NMR spectra of 4-acroryloxybenzophenone and DABP and analyzed the regio-selective copolymerization of the PNIPAM to the para-acryloyl unit of DABP. As shown in **Supplementary Figure S6,** indicating the absence of NMR signals in the region of 6.0-6.58 ppm, which was assigned as a para proton shift. On the other hand, the shift for the ortho protons is visible in the range of 5.60-6.20 ppm, which is aligned with the ortho proton shift in DABP, confirming the copolymerization of PNIPAM at the para position of the acryloyl unit of DABP.

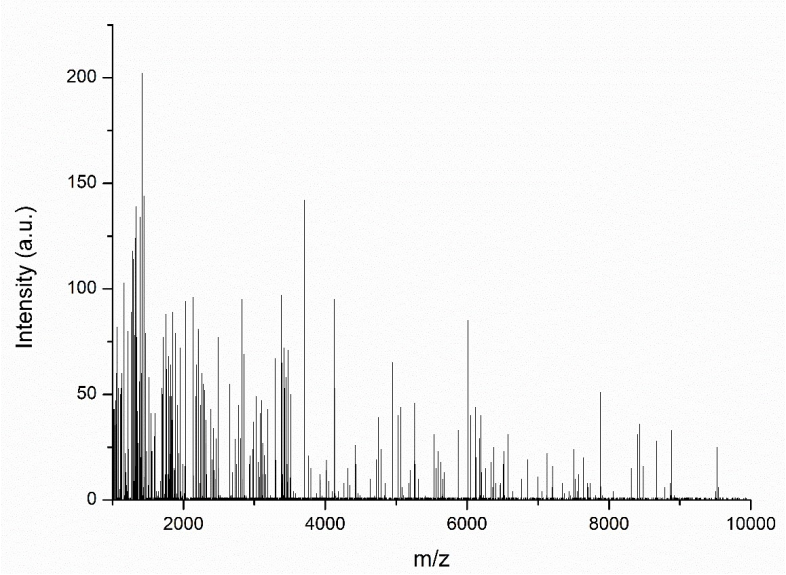
Reference:

1) P. Suryavanshi, S. Kawre, M. Maniruzzaman, K. Seth and S. Banerjee, *Chemical Papers*, 2023, 1-10.

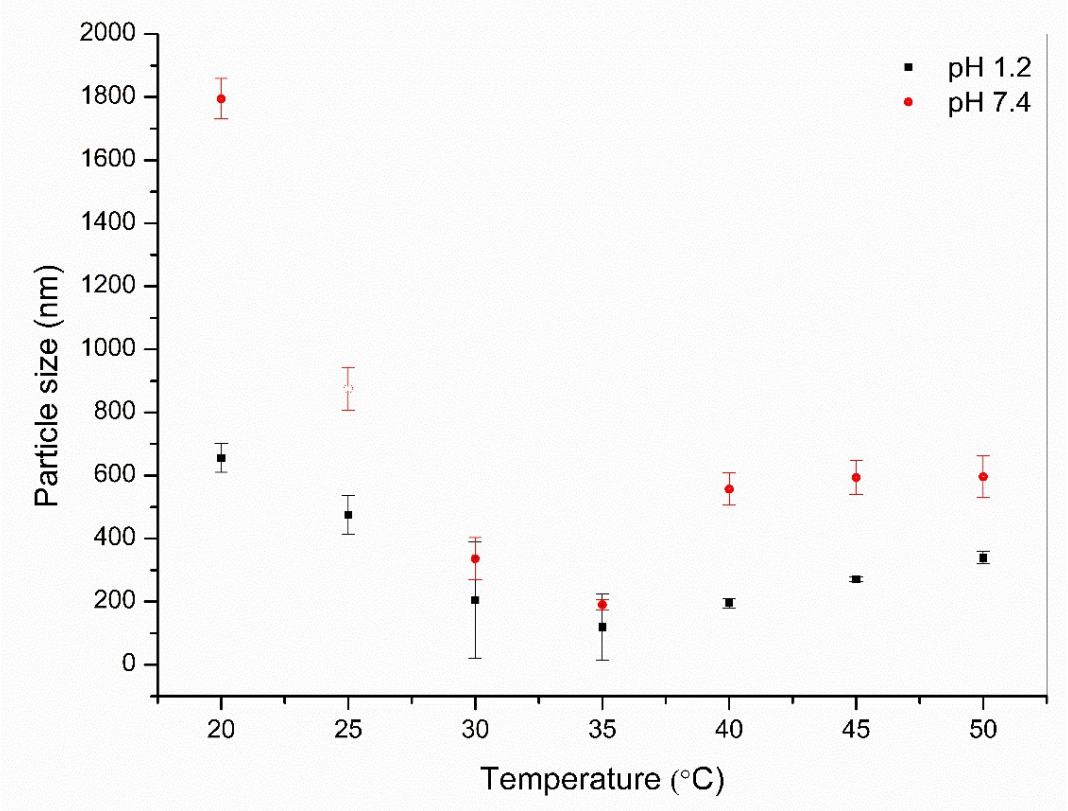
Supplementary Figures



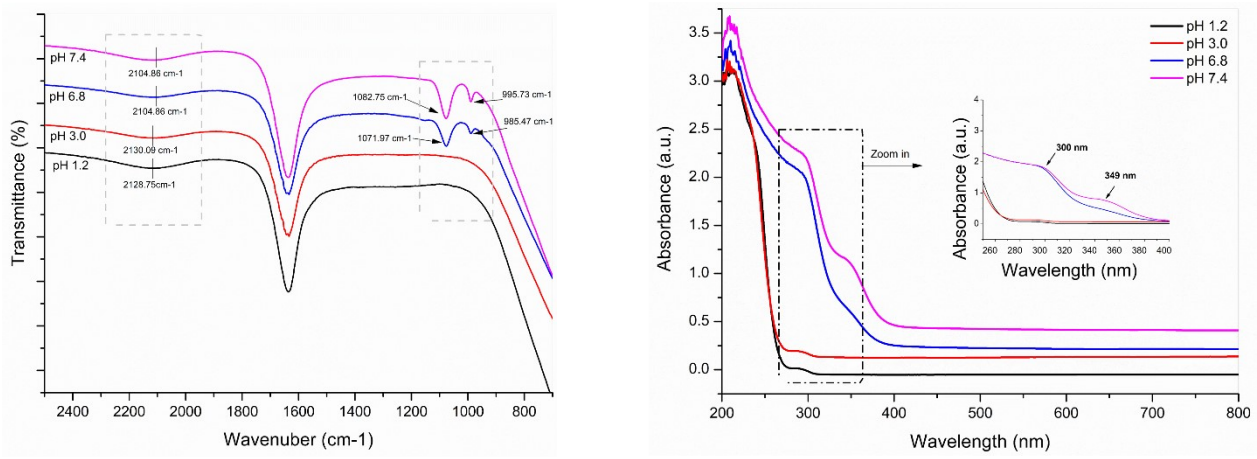
Supplementary Figure S11: First derivative TGA graph of (A) AAc, (B) NIPAM, (C) DABP, and (D) P(NIPAM-co-DABP-co-AAc).



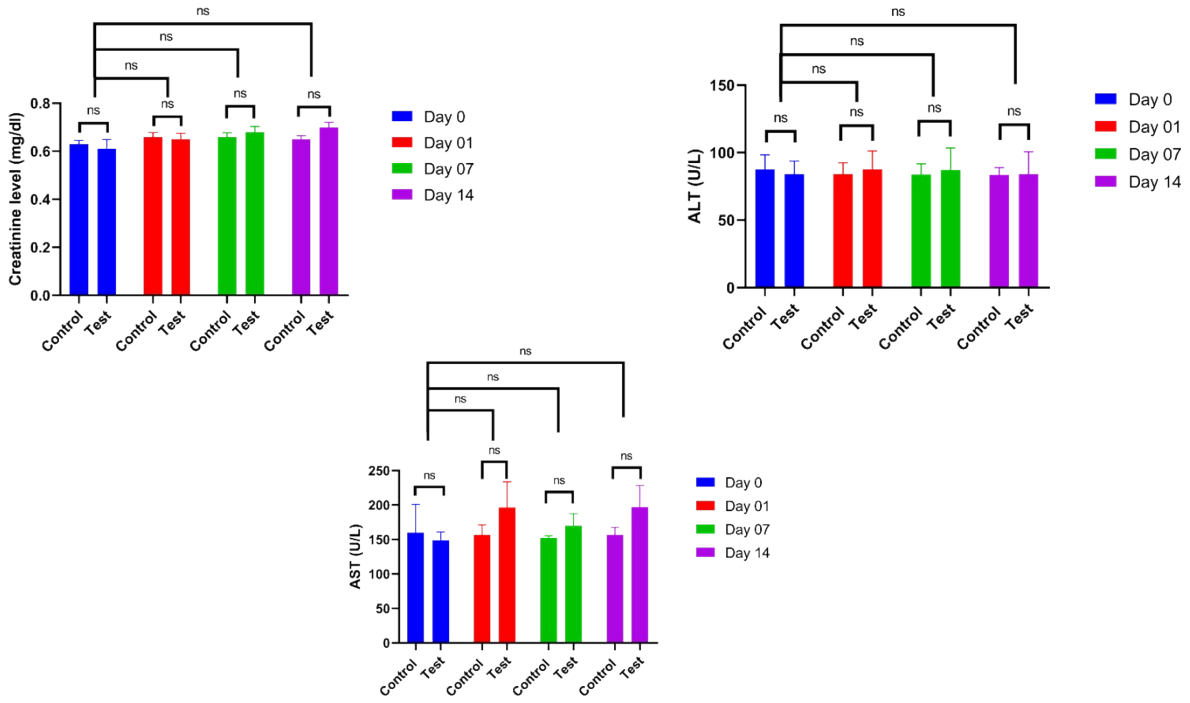
Supplementary Figure S12: Full recorded MALDI-ToF spectrum of P(NIPAM-co-DABP-co-AAc)



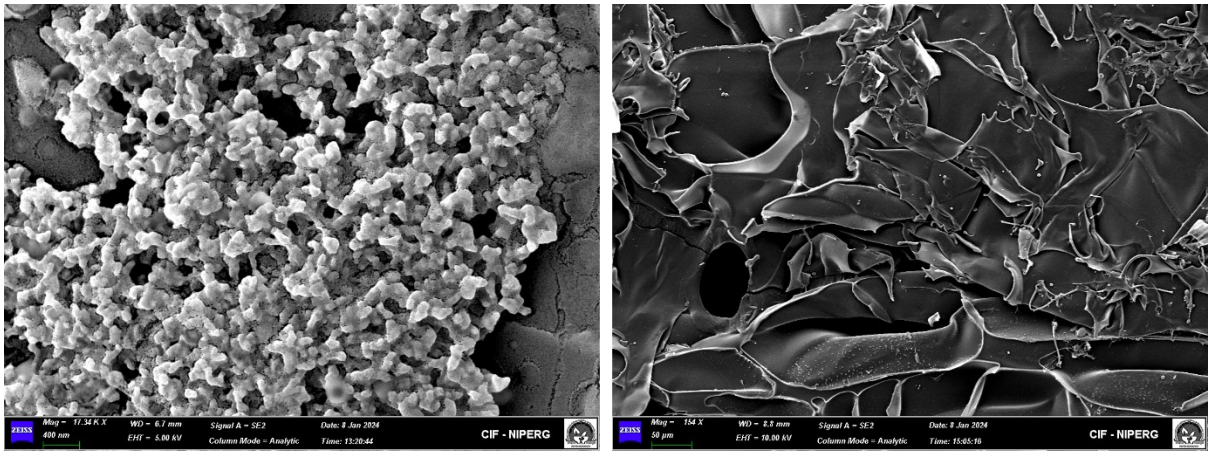
Supplementary Figure S13: P(NIPAM-co-DABP-co-AAc) hydrogel diameter as a function of temperature at indicated pH values.



Supplementary Figure S14: pH-responsive FT-IR (a) and UV-visible spectroscopy (b).



Supplementary Figure S15: Biochemical parameters of all experimental rats at indicated days (ns = nonsignificant)



(a) **(b)**

Supplementary Figure S16: SEM images of hydrogel samples which were used for the 5-FU release in (a) pH 1.2 solution and (b) pH 6.8 solution

Supplementary Tables

Table 1: General and body weight observations of all experimental rats as a preliminary evaluation for any sign of toxicity of P(NIPAM-co-DABP-co-AAc)

Parameters to be observed	Control Group (n = 3) Mean±SD	Test Group (n = 3) Mean±SD
Body weight (gm)		
Before treatment	187.5±12.21	173.5±3.39
04 hr	185.0±14.95	170.0±2.75
Day 01	189.83±14.74	179.33±6.59
Day 07	191.33±15.30	180.33±5.75
Day 14	191.83±14.21	189.33±8.47
General Appearance (Dehydration, diarrhea, Body temperature, Alopecia, Lacrimation, Eye opacity, etc.)		
Before treatment	Normal	Normal
04 hr		
Day 01		

	Day 07 Day 14	
Mortality	No	No

Table 2: Total haematological parameters of SD rats before and after oral administration of synthesized P(NIPAM-co-DABP-co-AAc)

Hematological parameters	**Control Group (n = 3) Mean±SD**				**Test Group (n = 3) Mean±SD**			
	Before dosing	01 Day	07 Day	14 Day	Before dosing	01 Day	07 Day	14 Day
WBC (10^3/µL)	15.86±5.24	13.26±3.01	12.26±3.58	12.06±2.31	13.66±3.36	10.1±1.64	10.21±2.55	11.23±2.93
RBC (10^3/µL)	8.63±0.49	8.78±0.23	8.11±0.33	8.81±4.87	9.77±0.96	6.08±0.56	8.37±2.06	10.36±1.82
HGB (g/dL)	15.83±1.80	13.98±1.20	14.83±1.50	18.33±7.49	17.96±2.02	11.28±0.77	16.05±4.18	16.89±1.38
HCT (%)	44.06±4.75	47.56±3.27	46.56±4.81	51.13±27.28	50.73±5.37	32.5±2.49	50.3±11.75	51.24±8.78
MCV (fL)	50.93±2.60	54.55±4.61	57.26±3.61	58.3±3.02	51.9±1.32	53.46±1.39	55.21±1.63	58.93±2.13
MCH (pg)	18.3±1.03	17.85±1.32	18.26±1.12	22.43±4.60	18.36±0.50	18.56±0.60	19.11±0.44	21.55±2.37
MCHC (g/dL)	35.93±1.07	31.54±0.06	31.86±0.05	38.43±7.40	35.36±0.25	34.70±0.37	32.76±0.77	36.51±3.19
PLT (10^3/µL)	680.0±95.68	754.66±60.30	798.66±52.30	815.12±56.21	717.66±74.31	848.8±76.21	859.21±68.21	865±58.12
RDWCV (%)	13.33±0.92	14.3±0.84	14.6±0.87	14.7±0.84	12.76±0.73	13.08±0.51	13.54±0.72	13.65±0.54
RDWSD (fL)	27.13±2.00	29.3±3.87	33.5±3.91	35.6±4.21	26.5±2.21	28.0±1.64	30.23±1.96	29.54±1.65
PCT (%)	0.38±0.06	0.49±0.05	0.53±0.06	0.49±0.06	0.39±0.03	0.41±0.16	0.45±0.13	0.51±0.07
MPV (fL)	5.7±0.26	5.98±0.40	6.16±0.35	6.12±0.32	5.53±0.15	5.54±0.27	6.11±0.17	5.95±0.40
PDW (%)	15.86±0.20	14.98±0.45	15.63±0.55	18.2±0.17	15.7±0.40	14.98±0.33	15.02±0.49	16.12±0.35