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Thermoresponsive copolymers with pendant D-galactosyl 1,2,3-triazole groups: Synthesis, characterization and thermal behavior

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Abstract

Galactose containing glycomonomer has been synthesized by copper catalyzed azide-alkyne cyclo-addition reaction (CuAAC) of 6-azido-6-deoxy-1,2:3,4-di-*O*-isopropylidene-*α*-D-galactopyranose with propargyl acrylate. This monomer was subjected to homopolymerization and copolymerization with *N*-isopropylacrylamide (NIPAm) in different composition by free radical polymerization using 2,2′-azobis-isobutyronitrile (AIBN) as initiator. The composition of the copolymer was determined by the ^{1}H-NMR spectroscopy. On acid hydrolysis of acetonide protected polymers, water-soluble deprotected polymers were obtained. The polymers were characterized and confirmed by NMR, IR, GPC and thermal analytical techniques. The protected and deprotected copolymers showed a sharp cloud-point temperature and a linear correlation was obtained between the lower critical solution temperatures (LCST) and the concentration of glycomonomer in the copolymers. This allows tuning the thermal response by simply playing on copolymer composition. Water contact angle experiments showed changes of hydrophilicity of copolymers with composition that supported the LCST results. The glass transition of protected copolymers followed a regular monotonic decreasing trend with increasing glycomonomer content, whereas Tg of

deprotected copolymers increased due to H-bond interaction. The attempts to develop thermoresponsive polymers having LCSTs at physiological temperature were successful.

Keywords: Thermoresponsive glycopolymers, CuAAC, PNIPAm, LCST, thermal analysis, contact angle measurement.

1. Introduction

Thermoresponsive polymers are the class of smart polymers which show a response to temperature.[1] These polymers exhibit the thermodynamic lower critical solution temperature (LCST) and show inverse solubility behaviour with an increase in temperature. The classical example is the solution of poly(*N*-isopropylacrylamide) [PNIPAm] in water which undergoes coil-to-globule transition at LCST in the range of 31-33 ^{o}C.[2] Below LCST, the polymer is completely soluble in water, while it becomes insoluble and phase-separates above its LCST. Thermally induced phase separation is attributed to the breaking of polymer-water hydrogen bonding and formation of polymer-polymer bonding at the critical temperature.[3] The balance of hydrophilic and hydrophobic constituents of the polymer chain plays a major role in determining the LCSTs of these polymers. With a LCST approaching the normal body temperature, PNIPAm is an attractive system for studies related to pharmaceutical and biomedical applications.[4] In order to develop a thermoresponsive polymer for a specific end application, amongst various approaches[5], LCST can be optimized by tailor-made approach by changing the ratios of two different monomer concentrations in the copolymerization and also by slightly varying the chemical structure of the monomers by introducing hydrophobic/hydrophilic groups in the chain.

Recent technological developments focus in great deal on the biocompatibility and biodegradability of the materials used for a variety of biological applications such as artificial

drug delivery vehicles or muscles.[6] Glycopolymers, sugar containing macromolecules, are of great interest due to their improved biocompatibility.[7] Also being biomimetic with naturally occurring glycoconjugates, synthetic glycopolymers found to play a significant role in many biomedical applications *via* multivalent binding interactions, commonly known as glyco-cluster effect.[8] With these attractive features, the pendant sugar of these polymers shows great affinity towards some specific proteins, known as lectins and forms stable complexes. For example, mannose containing polymers were strongly bound with Concanavalin A (ConA),[9] while, *N*-acetylglucosamine containing polymeric spherical brushes exhibited the selective binding to wheat germ agglutinin (WGA).[10]

Click chemistry strategy is not only used as a versatile tool for the design of complex polymer architectures, but it can be used also for the synthesis of bioactive and stable glycopolymers.[11] One of the most popular click reactions, is Cu-catalyzed Huisgen 1,3-dipolar azide and alkynes cycloaddition (CuAAC) with formation of 1,4-disubstituted 1,2,3-triazole ring.[12] The CuAAC is highly efficient and stereoselective, it requires mild reaction conditions and it is compatible with different functional groups. The CuAAC strategy has been used both to synthesize glycomonomers and to modify glycopolymer scaffolds by the post-functionalization approach. There are very few reports which follow the first protocol, while the second synthetic route was used by many researchers being more convenient to obtain glycopolymers by attaching different sugar moieties to preformed polymer scaffolds. For example, Okoth *et.al*. described CuAAC between propargyl galactoside with azide poymers with microwave assisted protocol.[13] Otman *et al*. obtained amphiphilic polymers by click chemistry of mannose azide and poly(propargyl acrylate-co-*N*-vinyl pyrrolidone).[14] Stenzel and coworkers synthesized thermoresponsive glycopolymers *via* CuAAC of galactose azide and alkyne functionalized block polymer of HEMA (2-hydroxy ethyl methacrylate) and DEGMA (diethylene glycol methyl ether methacrylate).[15] The resulting glycopolymers were

then tested for inhibition of ricin. Haddleton and coworkers reported libraries of glycopolymers using CuAAC chemistry. Their approach involved grafting of different sugar azides onto clickable polymer backbone with alkyne functionalities.[16] The same group also prepared the mannose containing glycomonomer by CuAAC of the mannose azide and propargyl methacrylate and subsequently polymerized targeting glycoproteins mimics.[17]

The current work utilizes CuAAC chemistry for synthesis of monomer containing triazole ring by Cu-catalyzed 1,3-dipolar cycloaddition of the 6-azido-6-deoxy-1,2:3,4-di-*O*-isopropylidene-*α*-D-galactopyranose with propargyl acrylate. Subsequently, glycomonomer was copolymerized with NIPAm by free radical polymerization. On acid hydrolysis, water-soluble deprotected polymer analogues were obtained. The composition of the copolymer was determined with the ^{1}H-NMR spectroscopy. The polymers properties were characterized thoroughly for their thermosensitivity by using temperature dependent UV and NMR techniques. Further, these newly synthesized thermoresponsive glycopolymers were studied by thermal properties and contact angles measurements. One of the motivations for developing these polymers was to achieve thermoresponsive polymers with tunable LCST and also to take advantage of triazole ring for its rigidity and bioactivity.[18] Such thermosensitive glycopolymers are known for controlling lectin interaction or bacterial aggregation.[19]

2. Experimental section

2.1. Materials

D-Galactose, sodium azide, tetrabutyl ammonium iodide (TBAI) were purchased from Merck. Propargyl acrylate and *N*-isopropylacrylamide (NIPAm) were procured from Sigma Aldrich and sodium ascorbate was purchased from S. D. Fine Chemicals Ltd. Dialysis bags (1000 MWCO) were purchased from Spectra/Por. 2,2′-Azobis-isobutyronitrile (AIBN),

copper sulfate pentahydrate, zinc chloride, tosyl chloride and formic acid (85%) were purchased from Spectrochem. AIBN and NIPAm were recrystallized in methanol. Thin layer chromatography was performed on pre-coated plates (0.25mm, silica gel 60 F254). Visualization was made by absorption of UV light or by thermal development after spraying with 3.5% solution of 2, 4-dinitrophenylhydrazine in methanol/H_2SO_4 or with basic aqueous potassium permanganate solution or with 10% solution of phosphomolybdic acid (PMA) in ethanol. All other chemicals were analytical-grade and were used as received.

2.2. Synthesis of the glycomonomer

A triazole containing glycomonomer **5** was synthesized from D-galactose as shown in Scheme 1. 6-Azido-6-deoxy-1,2:3,4-di-*O*-isopropylidene-*α*-D-galactopyranose (**4**) was synthesized by reported protocol.[20]

A mixture of 6-azido-6-deoxy-1,2:3,4-di-*O*-isopropylidene-*α*-D-galactopyranose (2.0 g, 5.405 mmol) and propargyl acrylate (0.596 ml, 5.405 mmol) in 10 ml of THF: water (1:1) was taken. After 10 min of stirring, sodium ascorbate (20 mg, 0.101 mmol) and copper sulfate (20 mg, 0.0801 mmol) was added. The reaction was stirred at room temperature. After completion of the reaction (as monitored by TLC), the reaction mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate. Column purification of product afforded a white crystalline solid (2.5 g, yield 95 %).

Mp: 122-123 ^{o}C; R_f =0.4 (ethyl acetate/hexane, 6:4); IR (KBr, cm^{-1}): 1728 (C=O), 1634 (C=C) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 7.81 (s, 1H, C*H* of triazole ring, $H_{5'}$), 6.44 (dd, J =1.5 and 17.1 Hz, 2H, $H_{9'a}$), 6.14 (dd, J =10.5 and 17.1 Hz , 1H, $H_{8'}$), 5.85 (dd, J =1.5 and 10.5 Hz, 2H, $H_{9'b}$), 5.53 (d, J =5.3 Hz, 1H, H_1), 5.31 (AB_q, J =12.8 and 3.9 Hz, 2H, $H_{6'a,b}$), 4.60-4.66 (m, 2H, H_{6a}, H_3), 4.5 (dd, J =6.2 and 14.3 Hz, 1H, H_{6b}), 4.34 (dd, J =2.4 and 4.8 Hz, 1H,

H$_2$), 4.18-4.20 (m, *J* =7.6 Hz, 2H, *H*$_4$, *H*$_5$),1.5 (s, 3H, *CH*$_3$), 1.4 (s, 6H, 2 x *CH*$_3$), 1.3 (s, 3H, *CH*$_3$); ^{13}C NMR: (125 MHz, $CDCl_3$): δ 165.7 (C-7′), 142.3 (C-4′), 131.3 (C-9′), 128.1 (C-8′), 125.0 (C-5′), 109.7 (C-7/C-8), 108.9 (C-8/C-7), 96.2 (C-1), 71.1 (C-4/ C-5), 70.74 (C-3), 70.3 (C-2), 67.13 (C-5/ C-4), 57.8 (C-6′), 50.5 (C-6), 24.3, 24.7, 25.7, 25.8 (4 C*H*$_3$).

2.3. Synthesis of homopolymer and copolymers

Homopolymers (P-100 and NIPAm) and copolymers (P-5, P-10, P-20, and P-25) were prepared by free-radical polymerization with AIBN at 70 ^{o}C in 1, 4-dioxane for 24 h according to the feed ratio given in Table 1. In a typical procedure for synthesis P-5, glycomonomer **5** (0.074 g, 0.2 mmol), NIPAm (0.426 g, 3.76 mmol) and AIBN (6.6 mg, 0.0397 mmol) were taken in 1, 4-dioxane (3.0 ml) and nitrogen gas purged for 20 min (Scheme 2). The reaction mixture was stirred at 70 ^{o}C for 24 h under nitrogen atmosphere. After completion of reaction, mixture was sticky and semisolid. The content was precipitated in distilled diethyl ether and again dissolved in chloroform and re-precipitated in diethyl ether thrice. The resultant product was dried under reduced pressure at 50 ^{o}C for two days. All protected polymers (P-series) were obtained as white solids in quantitative yield.

2.4. Deprotection of polymers

The deprotection of 1,2:3,4-di-*O*-isopropylidene group of glycopolymers was done under mild acidic condition by using a formic acid solution (85%). The protected polymer (200 mg) was dissolved in 20 mL of formic acid solution and stirred for 48 h at room temperature. The resulting solution was dialyzed (Spectra/Por, MWCO: 1000) against double distilled water for two days and freeze-dried. Finally, the deprotected polymer (D-series) was obtained as solid white powder (120 mg, yield 70%).

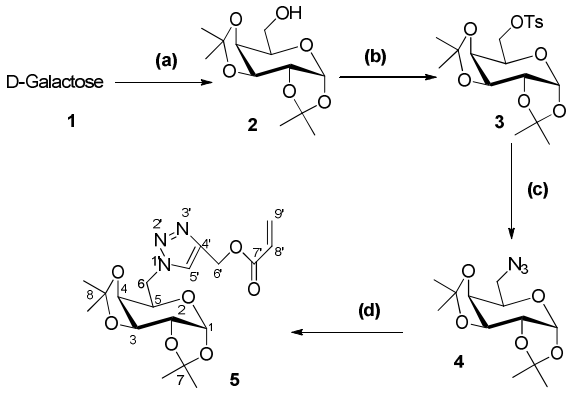
2.5. Characterization Techniques

^{1}H and ^{13}C-NMR spectra were recorded on Varian Mercury 300 and Bruker Avance AV-500 spectrometers in $CDCl_3$ and D_2O as solvents. The solvent signals were used as chemical shift markers. Temperature-dependent ^{1}H-NMR spectra of the copolymers in D_2O were recorded with a Bruker AV-500 spectrometer. FT-IR spectra were recorded on a Shimadzu FT-IR 8400 spectrophotometer. Molecular weights and molecular weight distribution of polymers were determined from gel-permeation chromatography (GPC) (Waters) by using THF as an eluent at a flow rate of 1 mL/min at 25 °C (Thermo Separation Products) equipped with spectra series UV 100 and spectra system RI 150 detectors. Sample concentration was 2 mg/ml and narrow molecular weight distribution polystyrenes were used as calibration standards. Polymeric solutions of 0.2% in double distilled water were used for determining cloud point temperatures with JASCO V-630 spectrophotometer with peltier assembly. Absorbance at 500 nm was measured with temperature scanning rate 1 ^{o}C/min. Thermogravimetric measurements were carried out using a TA-TGA 2950 instrument. The analyses were performed at 10 °C/min from room temperature to 600 °C both under nitrogen and air flow. Differential scanning calorimetry (DSC) was performed with a 2010 TA thermal analyzer at a heating rate of 20 °C/min under nitrogen flow. The temperature range explored was from room temperature to 180 °C. Quench cooling was applied between scans. The glass transition temperature (Tg) was taken as the midpoint of the stepwise increase of the specific heat associated with the transition. Static water contact angle measurements were performed on samples coated on glass slides by means of a Laurell (WS-650-23NPP) spin coater under ambient conditions. The contact angle experiments were performed with an optical contact angle and surface tension meter KSV's CAM 100 (KSV, Espoo, Finland) using Milli-Q water. The water drop profile images were collected every second in a time range of 0-60 s.

A minimum of five drops per sample were analyzed and the average value is reported together with the standard deviation.

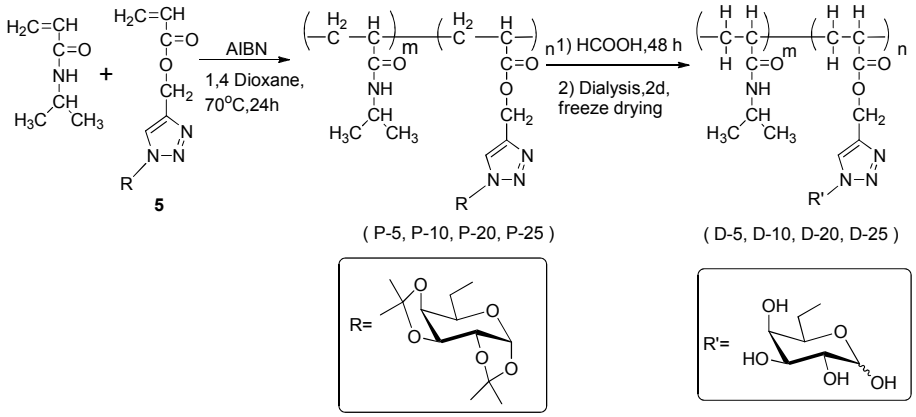
3. Results and discussion

3.1. Synthesis and characterization



Scheme 1. Synthesis of glycomonomer (a) Acetone, $ZnCl_2$, cat. H_2SO_4, rt, 6h, 81%. (b) TsCl, pyridine, cat. DMAP, 0 ^oC- rt, 8h, 75%. (c) NaN_3, TBAI, DMF, 110 ^oC, 72h, 51%. (d) Propargyl acrylate, $CuSO_4{\cdot}5H_2O$, Na ascorbate, THF: H_2O (1:1), rt, 1h, 90%.

As shown in Scheme 1, D-galactose was trapped in pyranose form to get 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose, **2** which on tosylation gave compound **3**. Tosylated compound **3** on treatment with sodium azide in DMF at 110 ^oC afforded azido compound **4**. In the final step, the glycomonomer **5** was prepared by Cu-catalyzed 1,3-dipolar cycloaddition of the galactose azide **4** and propargyl acrylate. The formation of galactose containing monomer **5** was confirmed by ^{1}H-NMR, ^{13}C-NMR, COSY and HSQC spectroscopic techniques (Fig. S1 - S4 in Supplementary Information).



Scheme 2. Synthesis of copolymers of NIPAm and glycomonomer **5**

Table 1- Summary of copolymers of NIPAm with glycomonomer

Sample Code	Feed ratio NIPAm: 5	Composition[a] NIPAm: 5	M_n (g/mole)	PDI[b]
P-100	0 : 100	0:100	5800	1.68
P-25	75 : 25	3.06: 1	7286	1.85
P-20	80 : 20	3.86: 1	9320	1.77
P-10	90 : 10	8.13: 1	18600	1.22
P-5	95 : 05	17.6: 1	-	-
PNIPAm	100 : 00	100:00	-	-

[a] Determined from ^{1}H NMR integrations.
[b] Determined from GPC (PS calibration).

Scheme 2 depicts the synthesis of copolymers of glycomonomer with NIPAm by conventional free radical polymerization as per feed ratio given in Table 1. The ^{1}H-NMR spectra of protected copolymers: P-5, P-10, P-20, P-25 and their comparison with the spectra of two homopolymers**:** PNIPAm and glycohomopolymer (P-100) are shown in Fig. 1. Polymer formation is confirmed by the disappearance of olefinic proton signals and appearance of –CH and –CH_2 (1.5 - 2.5 ppm) peaks for a polymer backbone and peak due to triazole proton at 7.81 ppm.

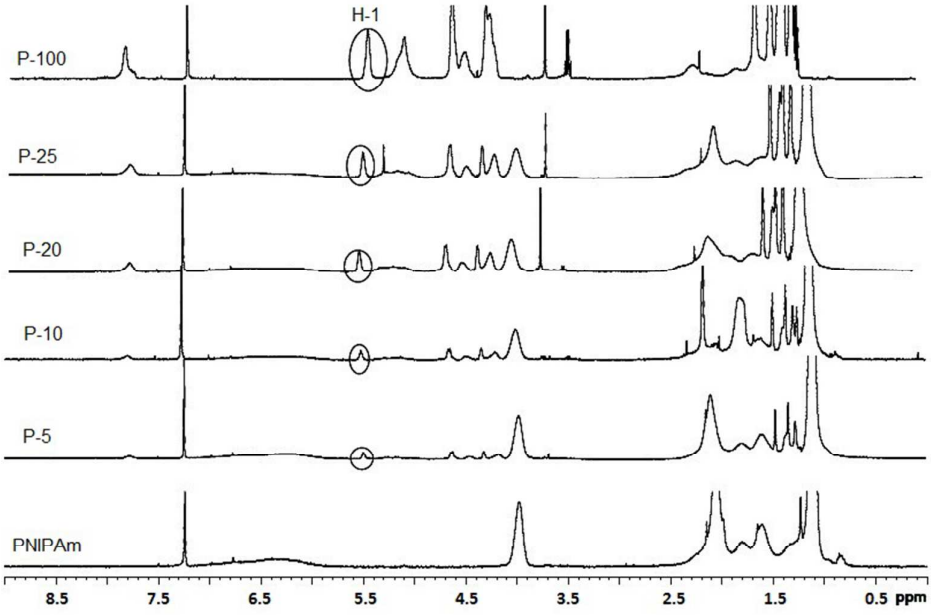


Fig. 1. Comparison of ^{1}H NMR spectra homopolymers and copolymers.

The ^{1}H-NMR spectra of P-100 shows a characteristic peak of H-1 proton (anomeric) at 5.53 ppm of galactopyranose ring which is absent in the ^{1}H NMR spectrum of PNIPAm and shows appearance of a new peak at 7.81 ppm, assigned to the triazole proton. As the percentage of glycomonomer content increases in copolymers from P-5 to P-25, the intensity of these characteristic peaks (at 5.53 and 7.81 ppm) increases distinctly. The composition of copolymers was quantitatively determined from ^{1}H-NMR spectra by referring the ratio of integrations of H-1 proton of galactopyranose ring and tertiary -CH proton of isopropyl group of NIPAm. These values were compared with the feed ratios. There is a fairly good agreement with the values of the feed ratios, showing that copolymer composition can be easily controlled by the feed ratio. The molecular weights of the polymers were determined by gel permeation GPC. As shown in Table 1, number average molecular weight (M_n) of the polymers was found to be in a range of 5.8 x 10^3 -18.6 x 10^3 g mol^{-1} with polydispersity index (PDI) of 1.22- 1.85.

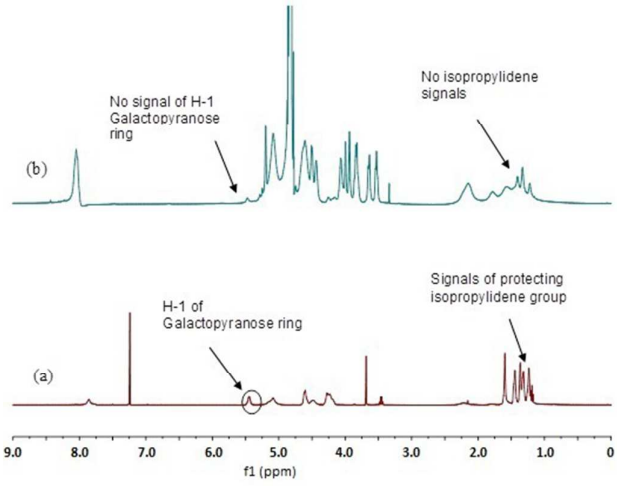


Fig. 2. ^{1}H-NMR spectra of (a) P-100 (in $CDCl_3$) (b) D-100 (in D_2O).

On the acid hydrolysis, glycohomopolymer and its copolymers were converted into hydrophilic polymers. These polymers were dialyzed against double distilled water to remove byproducts of hydrolysis and impurities. Resultant deprotected hydrophilic polymers (D-series) were characterized by ^{1}H-NMR spectroscopy. The absence of isopropylidene proton peaks at 1.3-1.5 ppm confirmed the deprotection as seen in Fig. 2. FTIR spectroscopy also offered the evidence of deprotection. Fig. 3 shows the FTIR spectra of both protected and deprotected glycohomopolymer. The deprotected polymers showed broad absorption peak around 3400 cm^{-1} due to free hydroxyl groups of deprotected sugar.

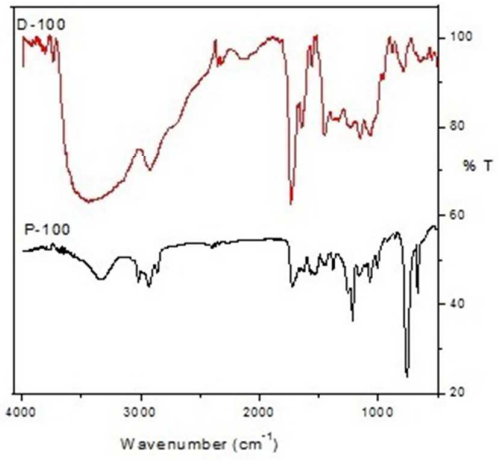


Fig. 3. FTIR spectra of P-100 and D-100.

3.2. Thermosensitivity studies

Fig. 4 shows temperature dependent absorbance at 500 nm of synthesized polymers. PNIPAm homopolymer clearly showed LCST (cloud point temperature) at 34.3 °C. The LCSTs of protected copolymers were found to be less than that of PNIPAm homopolymer due to increase in overall hydrophobic content of copolymers. It was observed that as the amount of glycomonomer increases, the LCST of copolymer decreases and also solubility of protected polymer in water decreases. The LCSTs of two protected polymers (P-20, P-25) were not recorded as they were insoluble in water. The LCSTs of deprotected polymers were higher than that of PNIPAm and of protected copolymers. This interesting observation was attributed to increase in hydrophilicity of polymers after the deprotection. All hydroxyl groups of the sugar moiety become free after deprotection and can easily form hydrogen bonding with water even at temperatures higher than LCST of PNIPAm. These observations also support the fact that copolymerizing NIPAm with a hydrophobic comonomer resulted in a lowering of LCST, whereas copolymerizing NIPAm with a hydrophilic comonomer resulted in a higher LCST.[5b] It is worth mentioning that we were successful to tailor the LCST of polymer D-5 at ~ 37 °C which is the physiological temperature of human body. This

interesting result provides the strong impetus towards the biological utility of the developed glycopolymer. The linear correlation between LCST and concentration of glycomonomer was obtained for deprotected copolymers (Fig. 5) which could be helpful for designing tailor-made polymers with desired LCST.

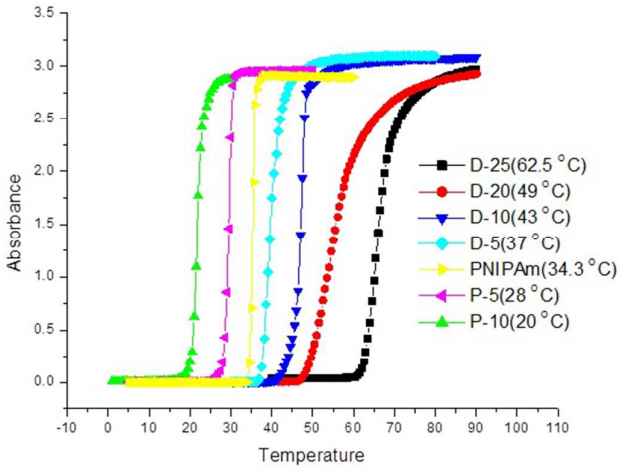


Fig. 4. Cloud points of PNIPAm, protected and deprotected copolymers.

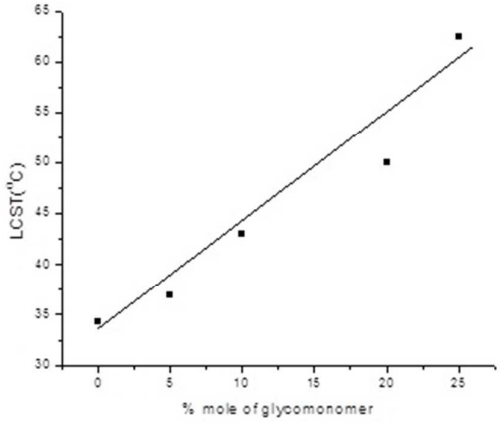


Fig. 5. Linear correlation between LCST and % mole glycomonomer.

We have successfully demonstrated the mechanism of phase transition by temperature dependent ^{1}H-NMR experiment. The deprotected copolymer (D-5 with cloud point 37 ^{o}C)

was dissolved at a concentration of 1.0 mg/mL in D_2O solvent. The sample was heated to the measuring temperature and left 30 min to reach thermal equilibrium. The ^{1}H-NMR spectrum was confirmed with 100 s relaxation delay and 64 scans. As shown in Fig. 6, when temperature was less than the LCST, all proton signals were well resolved and distinct. However, on increasing temperature, peaks were shifted to downfield. At 45 ^oC, peaks a, b, and c (due to methyl protons of PNIPAm, backbone protons and tertiary proton of isopropyl group of PNIPAm respectively) broadened and disappeared subsequently. This could be attributed to phase separation of polymer chains above LCST due to breakdown of hydrogen bonding at elevated temperature.

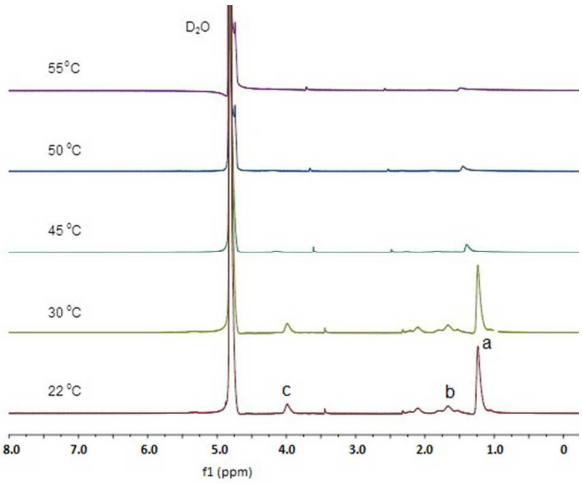


Fig. 6. ^{1}H NMR spectra of D-5 polymer in D_2O at different temperature.

3.3. Contact angle measurements

To demonstrate the changes in hydrophilicity, water contact angle (WCA, θ) measurements were performed on the homopolymers and on selected copolymers. The contact angle values taken 30 sec after drop deposition are listed in Table 2. Compared with PNIPAm (θ = 43°), the glycohomopolymer (P-100) in its protected form is much more hydrophobic (θ = 69.6°), whereas after deprotection (D-100), it becomes more hydrophilic than PNIPAm (θ = 27.6°).

Hydrophilicity changes are also highlighted by the different trend of the θ vs. t curves (see Fig. 7), where strong water/polymer interaction causes a faster contact angle decrease with time. The curves of protected copolymers lay between those of PNIPAm and P-100, according to expected composition-dependent hydrophilicity changes. Analogously, deprotected copolymer D-20 has a behaviour intermediate between those of PNIPAm and D-100. The WCA results confirm that differences of hydrophobicity underpin the observed changes of LCST.

Table 2 Water contact angle of protected and deprotected samples

Sample	Water contact angle [a] (°)	Standard deviation
PNIPAm	43.0	1.4
P-5	60.4	0.9
P-20	68.2	0.5
P-100	69.6	1.6
D-20	37.2	1.5
D-100	27.6	1.9

[a] Value taken 30 sec after drop deposition.

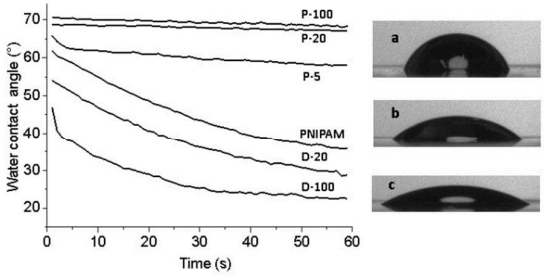


Fig. 7. Water contact angle as a function of time. Exemplary drops taken after 30 s from drop deposition on samples: a) P-100, b) PNIPAm c) D-100.

3.4. Thermal analysis

Fig. 8a shows the thermogravimetric (TGA) curves of protected polymer samples together

with that of PNIPAm, while Fig. 8b reports the derivative TGA curves in the T-range of the main degradation. After an initial weight loss possibly associated with some humidity absorbed by the environment, a main weight loss occurs on a rather broad temperature range between 300 °C and 450 °C (Fig. 8a). The derivative curves clearly reveal that the degradation behaviour of the copolymers includes two weight loss processes, whose sharpness and intensity gradually change with composition. The temperature location of such phenomena (T_{max}), taken at the peak of the derivative curves, is reported in Table 3 together with the T_{max} values of the two homopolymers. The latter show a difference of about 60 °C, with PNIPAm being more thermally stable. Comparison of T_{max} values of copolymers with those of PNIPAm and P-100 (Table 3) and analysis of the relative intensities of the weight drops (Fig. 8) that change accordingly to the change of copolymer composition, suggests to correlate the first and second weight loss steps in copolymers to the content of glycomonomer and NIPAm respectively.

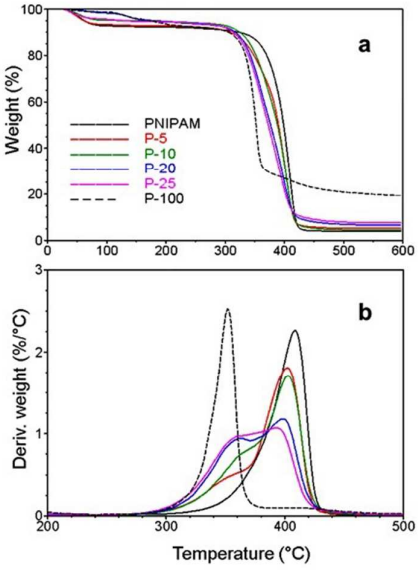


Fig. 8. TGA weight loss curves (a) derivative curves (b) under N_2 flow of PNIPAm, P-100 and protected copolymers.

Worth noticing is the large solid residue at 600 °C of homopolymer P-100 in the TGA experiments run under nitrogen flow (Fig. 8b). In analogous TGA analyses run under air flow, the residue at 600 °C is found to approach zero in all samples, including P-100 (curve shown as Fig. S5 in Supplementary Information). This result indicates that, in the presence of oxygen, the substances forming the solid residue under N_2 at 600 °C oxidize and yield volatile products.

Table 3 Thermal properties of protected polymers and of PNIPAm.

	TGA		DSC	
Sample	T_{max} (^oC)	Residue at 600^oC (%)	T_g (°C)	Δc_p (J/g deg)
PNIPAm	409	4.04	140	0.61
P-5	351, 402	5.30	134	0.55
P-10	365, 402	4.55	126	0.59
P-20	363, 399	6.73	121	0.50
P-25	360, 392	7.70	118	0.54
P-100	351	19.31	94	0.46

After deprotection, all copolymers as well as the glycohomopolymer were subjected to TGA analysis. Fig. 9 compares the TGA curves before and after deprotection (under N_2 flow) of the glycohomopolymer and two copolymers containing 5 mol % and 25 mol % of glycomonomer. Upon deprotection, the TGA curve of the glycohomopolymer (Fig. 9c) remarkably changes, denoting a decrease of thermal stability and a very consistent increase of the solid residue at 600 °C (from ca. 20% in P-100 to 35% in D-100). This behaviour of lower thermal stability and larger solid residue is also apparent in the TGA curves of the copolymers in Fig. 9, being more marked, as expected, in the copolymer with the higher glycomonomer content (25 mol%, Fig. 9b).

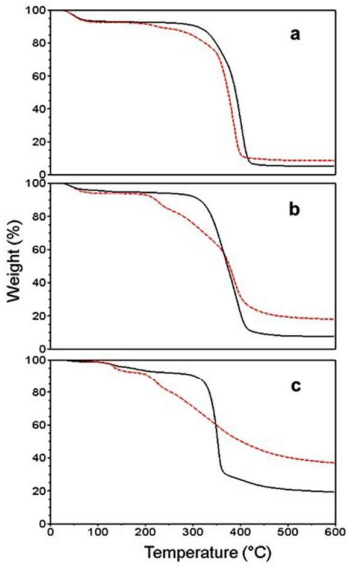


Fig. 9. TGA curves under N_2 flow of protected (solid lines) and deprotected polymers (dotted lines): (a) P-5 and D-5; (b) P-25 and D-25; (c) P-100 and D-100.

As already described for the protected polymers, also in the case of deprotected samples when the purge gas in TGA experiments is switched from N_2 to air, the curves show an additional high temperature weight loss that brings the solid residue close to zero (Fig. S6 in Supplementary Information).

The calorimetric behaviour of all samples, both protected and deprotected, was studied by means of differential scanning calorimetry (DSC). Fig. 10 shows the DSC curves of protected polymers and of PNIPAm. The only thermal event observed in the DSC curves is an endothermal baseline shift associated with the glass transition, whose temperature and specific heat increment are listed in Table 3. The absence of any crystallization/melting phenomena indicates that all analyzed samples are totally amorphous. This result reflects the absence of regular chirality along the chain, the presence of bulky and complex side chains and randomness of comonomer distribution. In addition, as commonly expected in random copolymers, the glass transition changes with composition and is intermediate between those of the two homopolymers.

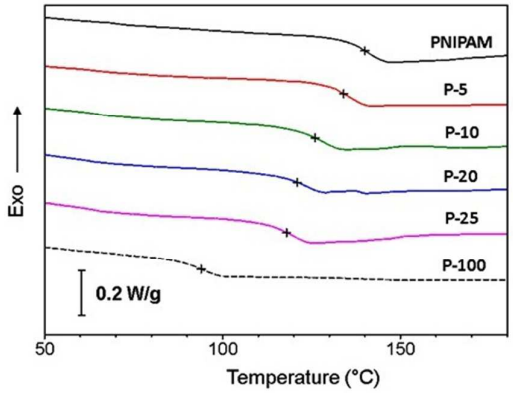


Fig. 10. DSC curves of PNIPAm and protected samples: P-100 and copolymers. The glass transition temperature (Tg) is marked on the curves.

The Tgs of both protected and deprotected polymers are plotted in Fig. 11 as a function of the weight fraction (w) content of glycomonomer in copolymers. The dependence of Tg on composition of protected copolymers is compared with that predicted for random copolymers by Fox equation:[21]

$$1/Tg_{copo} = w_1/Tg_1 + w_2/Tg_2 \quad [1]$$

where Tg_1 and Tg_2 are the Tgs of the reference homopolymers. A rather good agreement is observed between the curve and the experimental data of the protected samples. DSC experiments were also run after deprotection of polymers and the resulting Tg data are plotted in Fig. 11. Deprotection causes a 21 °C increase of the glycohomopolymer Tg value, likely to be due to hydrogen bonds involving the free hydroxyls on the deprotected sugar rings. Opposite to the common random copolymer behaviour shown by the protected samples, the glass transition of deprotected copolymers does not follow a monotonic decreasing trend from that of PNIPAm to that of D-100, but it rather increases with respect to that of PNIPAm in the composition range explored. It is known that such behaviour may be found when strong bonds, such as H-bonds, are formed between monomer units in

copolymers.[22] In order to activate the cooperative motions that set up at Tg, such bonds must be broken and this causes an up-shift of the transition.

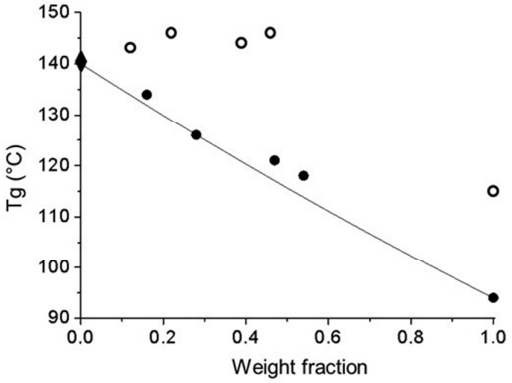


Fig. 11. Tg as a function of glycomonomer weight fraction of protected (•), deprotected (o) polymers and PNIPAm (♦). The line represents Fox equation [1] applied to protected copolymers.

4. Conclusions

In conclusion, we have explored the copper catalyzed azide-alkyne click reaction (CuAAC) for synthesis of triazole containing glycomonomer from cheaply available D-galactose as starting materials. The synthetic protocol is simple, highly efficient, functional group tolerant and in aqueous medium. The homopolymerization as well as copolymerization of glycomonomer with *N*-isopropylacrylamide (NIPAm) at different compositions afforded well defined thermoresponsive glycopolymers. Protected polymers on acid hydrolysis yielded hydrophilic polymers. Thermosensitivity of synthesized glycopolymers has been evaluated by cloud point method and strongly supported by temperature dependent NMR study. The protected and deprotected copolymers exhibited the lower and higher cloud point temperature respectively, when compared with that of PNIPAm. This observation was attributed to change in hydrophilicity as well predicted by water contact angle results. A peculiar Tg-

composition behavior associated with H-bond interaction in deprotected copolymers was evidenced by DSC.

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Electronic Supplementary Information

Contained in this are ^{1}H and ^{13}C-NMR, COSY and HSQC spectra of compound **5**, TGA curves of P-100 and D-100 under N_2 and air flow. Supplementary data related to this article can be found online at

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Thermoresponsive copolymers with pendant D-galactosyl 1,2,3-triazole groups: Synthesis, characterization and thermal behavior

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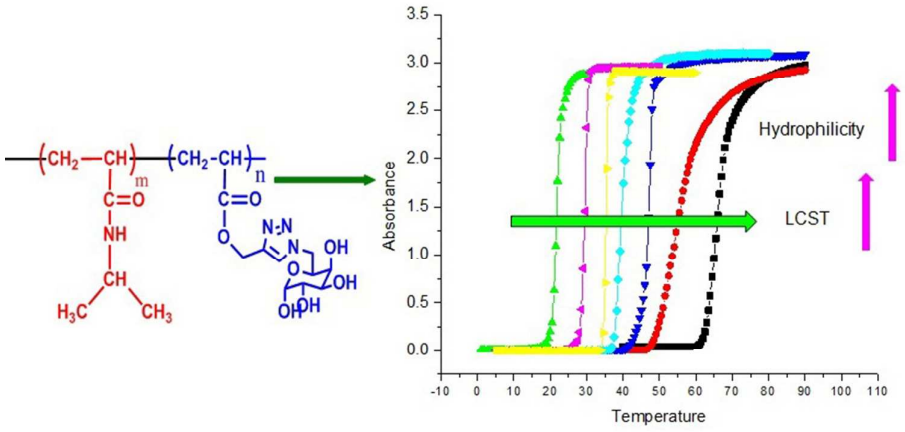

A series of glycopolymers containing D-galactosyl 1,2,3-triazole groups were synthesized which exhibited thermosensitivity properties.