Supplementary Material (ESI) for Chemical Communications


# Synthesis of thiol-linked neoglycopolymers and thermo-responsive glycomicelles as potential drug carrier

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# 1.0 General

## 1.1 Reagents

All reagents bought from Sigma-Aldrich except acetone, diethyl ether, ethyl acetate, *n*-hexane (Chemsupply, Crown), potassium carbonate, triethylamine (APS/AJAX, Crown) and silica gel (Scharlau, Crown). The RAFT agent cumyl dithiobenzoate (CDB) was synthesized as previously reported.[1] All chemicals were used as purchased unless otherwise specified.

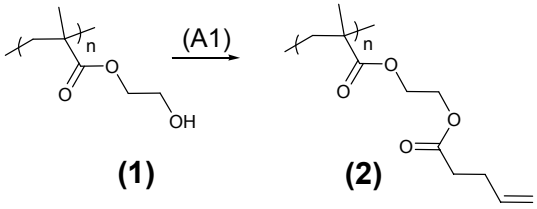
## 1.2 Analysis and equipments

Gel Permeation Chromatography (GPC) analysis of the polymers were performed in *N*,*N*-dimethylacetamide (DMAc) (0.05 %w/v LiBr, 0.05 % BHT) at 40 ˚C (1 mL $min^{-1}$ flow rate) using a Shimadzu modular system comprising a DGU-12A solvent degasser, a LC-10AT pump, a CTO-10A column oven and a RID-10A refractive index detector. The system was equipped with a 5.0 μm bead-size guard column (50 × 7.8 mm) followed by four 300 × 7.8mm linear Phenomenex columns ($10^5$, $10^4$, $10^3$ and 500 Å). The calibration curve was generated with narrow polydispersity polystyrene standards ranging from 500 to $10^6$ g/mol. NMR spectra were acquired on a Bruker 300MHz or 400MHz in deuterated DMSO. Fourier-Transform Infrared (FT-IR) measurements were performed using a Bruker IFS 66/S Fourier transform spectrometer equipped with a tungsten halogen lamp, a KBr beam splitter and a DTGS detector. Each spectrum in the spectral region of 4000-800 $cm^{-1}$ was calculated from the coadded interferograms of 32 scans with resolution of 4 $cm^{-1}$. Fourier-Transform Near-Infrared (FT-NIR) Spectroscopy was used to determine the monomer conversions by following the decrease of the vinylic stretching overtone of the monomer at ~6200 $cm^{-1}$. A Bruker IFS 66/S Fourier transform spectrometer equipped

with a tungsten halogen lamp, a CaF2 beam splitter and liquid nitrogen cooled InSb detector was used. The sample was placed in a FT-NIR quartz cuvetter (1 cm x 2 mm) and polymerised at 60 ºC. Traces were analysed with OPUS software. Each spectrum in the spectral region of 8000-4000 $cm^{-1}$ was calculated from the coadded interferograms of 64 scans with a resolution of 4 $cm^{-1}$ with measurements taken every 5 minutes. The UV irradiation experiments were carried out using a UVP CL-1000 ultraviolet crosslinker at 900 $mJ/cm^2$. Transmission Electron Microscope (TEM) Analyses were performed using a JEOL1400 TEM with a beam voltage of 100 kV and a Gatan CCD for acquisition of digital images. Samples were prepared in a preheated oven with certain temperature, by placing a droplet of a 1 mg/mL polymer solution on a formamide and graphite coated copper grid and draining the excess using filter paper after 60 seconds. To negatively stain the samples a droplet of 2 % (w/v) phosphotungstic acid solution was placed on the copper grid for 30 seconds before being drained with filter paper. Cloud points (LCSTs) of these polymers and turbidimetry assay of the ligand-protein binding were measured using Varian Cary 300 UV-Vis spectrophotometer with temperature controller. Dynamic Light Scattering (DLS) measurements were done using a Malvern Nano-ZS ZEN3600 and solutions in distilled water or HBS buffer that were filtered through 0.45 μm filters before analysis. The mean diameter was obtained from the arithmetic mean using the number distributed diameter of each particle size.

# 2.0 Polymer synthesis

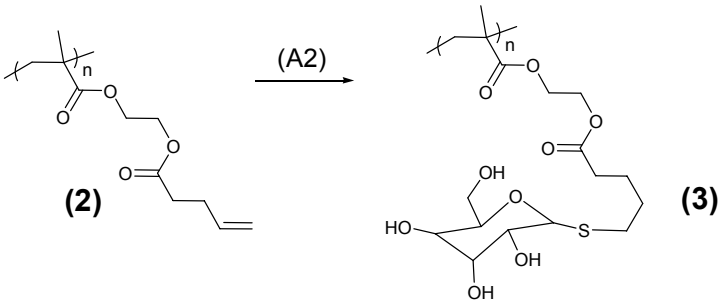
## 2.1 Synthesis of polymer (2)




**(1)** **(2)**

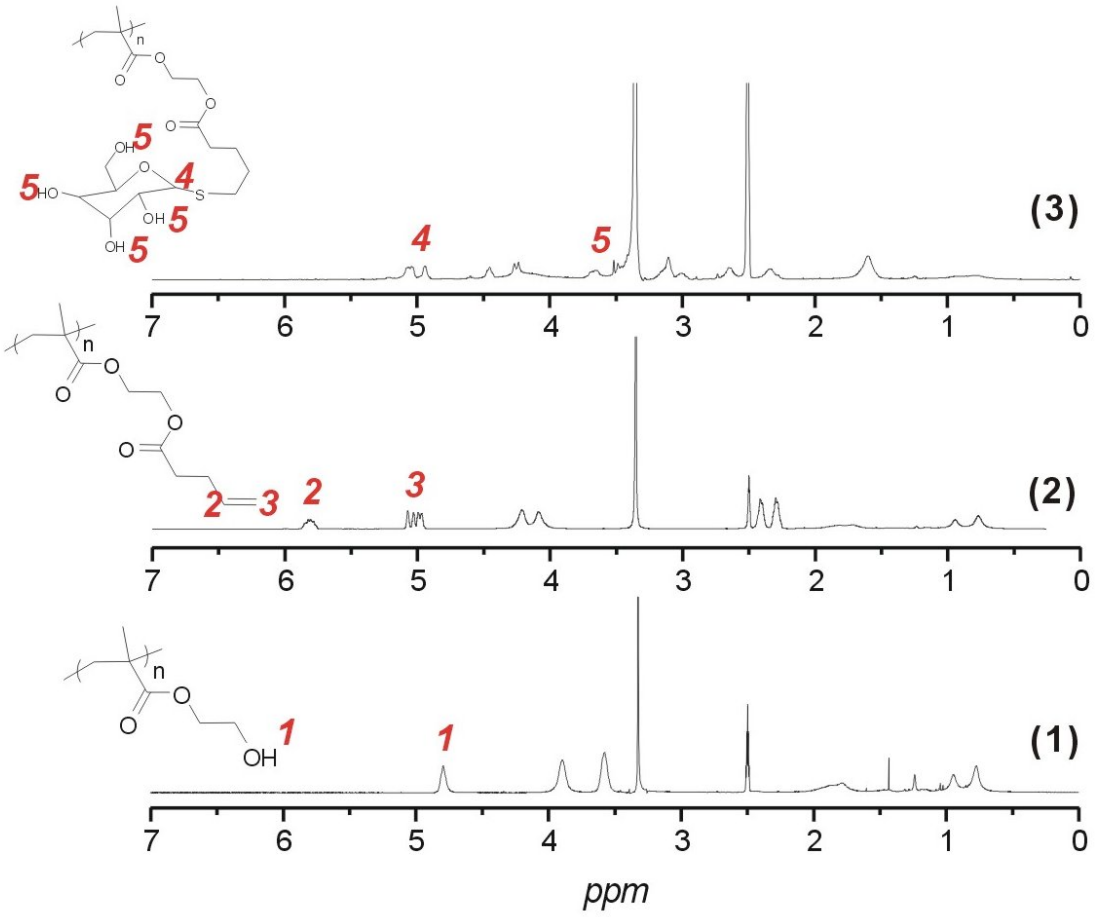
To a round bottom flask were added poly(HEMA) **(1)**, 3 equivalents (to -OH) of 4-pentenoic anhydride, 3 equivalents of pyridine, 0.15 equivalents of 4-(dimethylamino)pyridine (DMAP), and DMF. The contents of the flask were stirred overnight at 40 ºC. The mixture was precipitated in Ethanol, re-dissolve in acetone and precipitate in diethyl ether, dried under vacuum to afford polymer **(2)**.

## 2.2 Synthesis of Polymer (3)

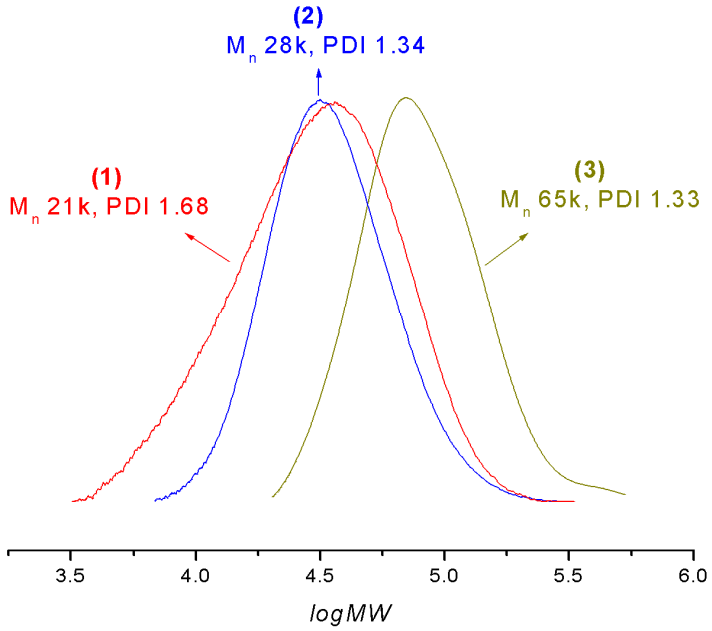



Glucothiose sodium salt (65.5 mg, 0.3 mmol) and 1 equivalent HCl in 0.2 mL DMF were added to a vial containing **(2)** (19.8 mg, 0.1 mmol alkene units) and 2,2'-dimethoxy-2-phenylacetophenone (DMPA) (1.3 mg, 0.005 mmol) in 0.3 mL DMF. The contents of the vial were thoroughly mixed and filtered through 0.45 μm filters to remove trace undissolvable sodium chloride. The mixture was then irradiated without stirring for 2 hours at room temperature. The contents were filtered using Minipore Amicon Ultra-4 centrifugal filter devices with a molecular weight cut off of 10k and then freeze-dryed.

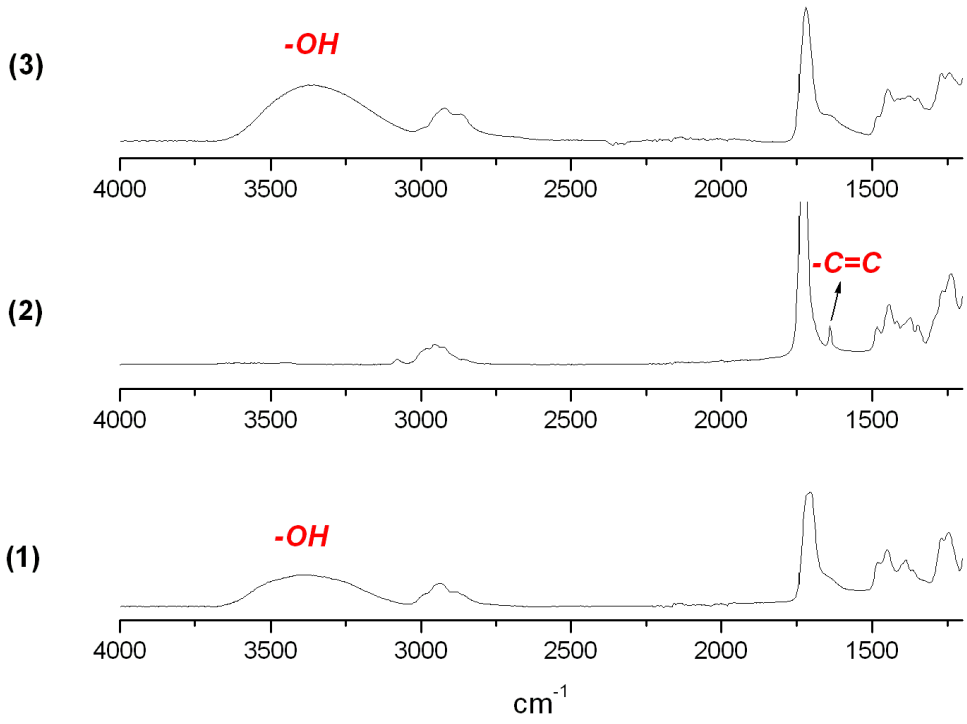
## 2.3 NMR characterisation of polymer (1), (2) and (3)



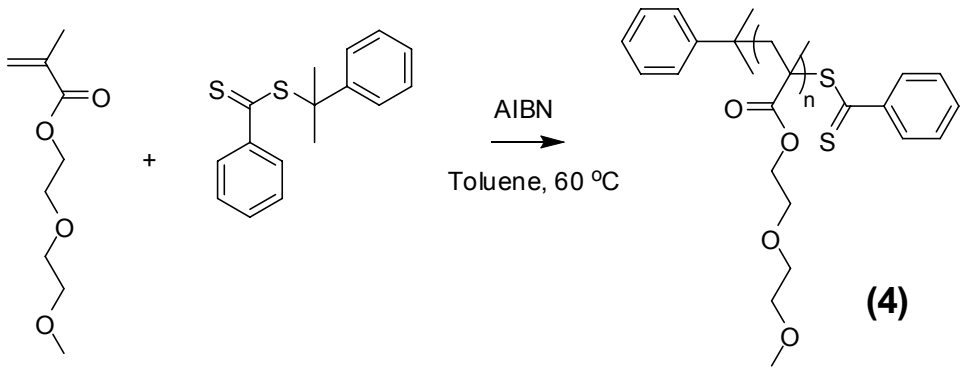
## 2.4 GPC characterisation of polymer (1), (2) and (3)



## 2.5 IR characterisation of polymer (1), (2) and (3)



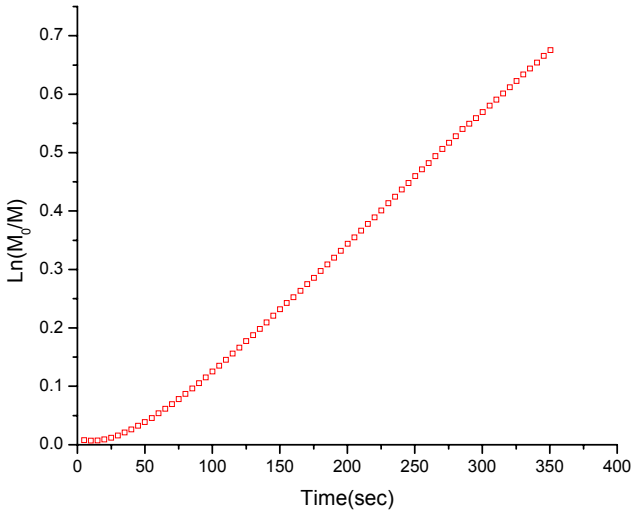

## 2.6 Polymerisation of DEGMA



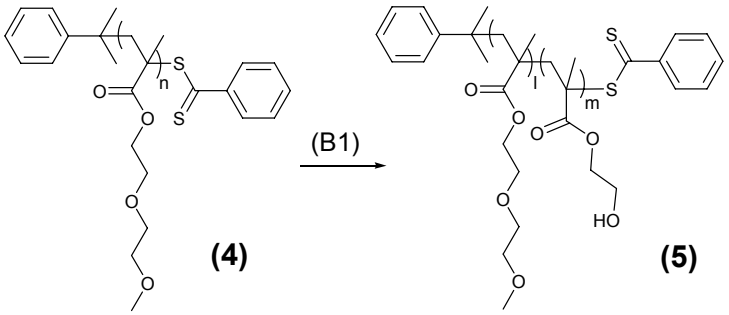

DEGMA (0.376 g, 2 mmol) were combined with CDB (5.4 mg, 0.02 mmol) and AIBN (1.6 mg, 0.01 mmol) in toluene (0.5 mL) in a dry Schlenk tube. Four freeze-pump-thaw cycles were used to remove oxygen. The mixture was then transferred via a cannula into an evacuated IR cuvette (1mm). The cuvette was heated isothermally at 60 ˚C and

monitored via on-line FT-NIR spectroscopy by following the decrease of the intensity of the vinylic stretching overtone of the monomer at 6200 $cm^{-1}$.

## 2.7 Kinetic investigation of the polymerization of DEGMA

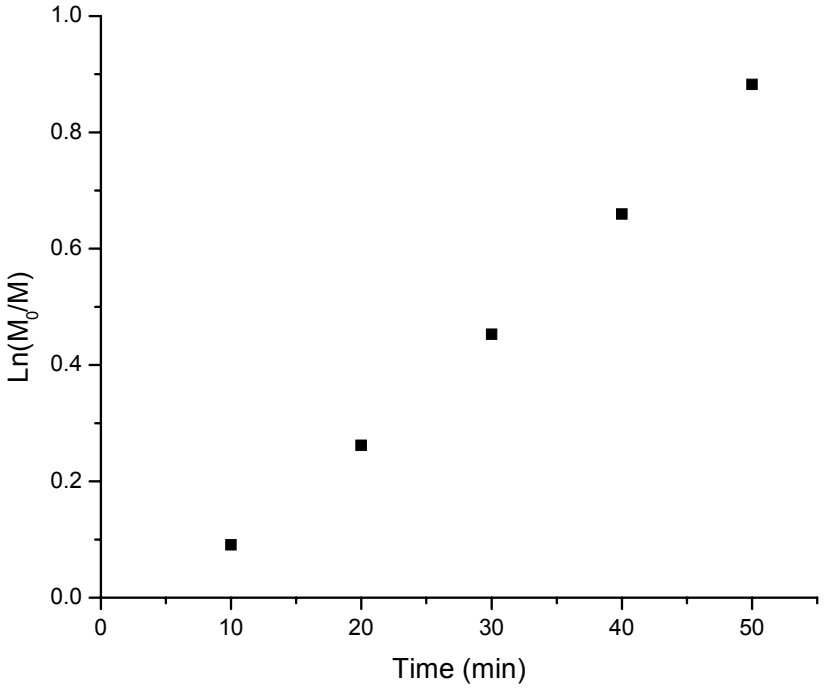



## 2.8 Synthesis of block copolymer (5)

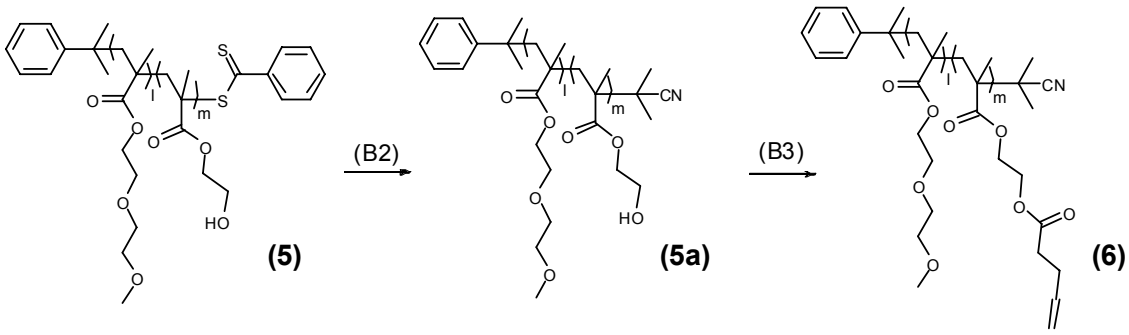



The homopolymer of DEGMA **(4)** was chosen to carry out the chain extension with HEMA. A ratio of HEMA: **(4)**: AIBN = 400:1:0.5 in DMAc (0.5 mL) were used. The polymerisation was carried out in IR cuvette (1mm). The cuvette was heated isothermally at 70 ˚C and monitored via on-line FT-NIR spectroscopy.

## 2.9 Kinetic investigation of the chain-extension of (4) with HEMA



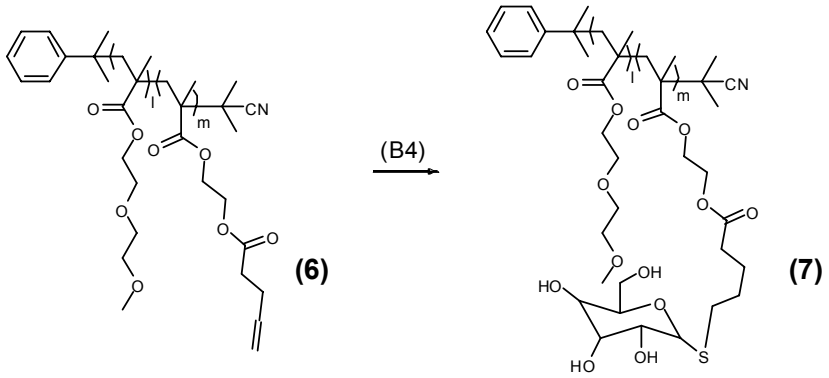

## 2.10 Synthesis of block copolymer (6)




To an ampoule was added **(5)** (110 mg, 0.002 mmol), AIBN (13.2 mg, 0.04 mmol) and toluene (1 mL). The solution was degassed with $N_2$ for 20 mins and then heated to 80 ˚C for 2.5h.[2] The solution was cooled and the polymer precipitated by dropwise addition of the solution into cold hexane to afford **(5a)**.

To a round bottom flask were added **(5a)**, 3 equivalents (to -OH) of 4-pentenoic anhydride, 3 equivalents of pyridine, 0.15 equivalents of 4-(dimethylamino)pyridine (DMAP), and DMF. The contents of the flask were stirred at room temperature for 72 h. The mixture was precipitated in diether ether, re-dissolve in toluene and reprecipitate in diether ether, and then dried under vacumm to afford polymer **(6).**

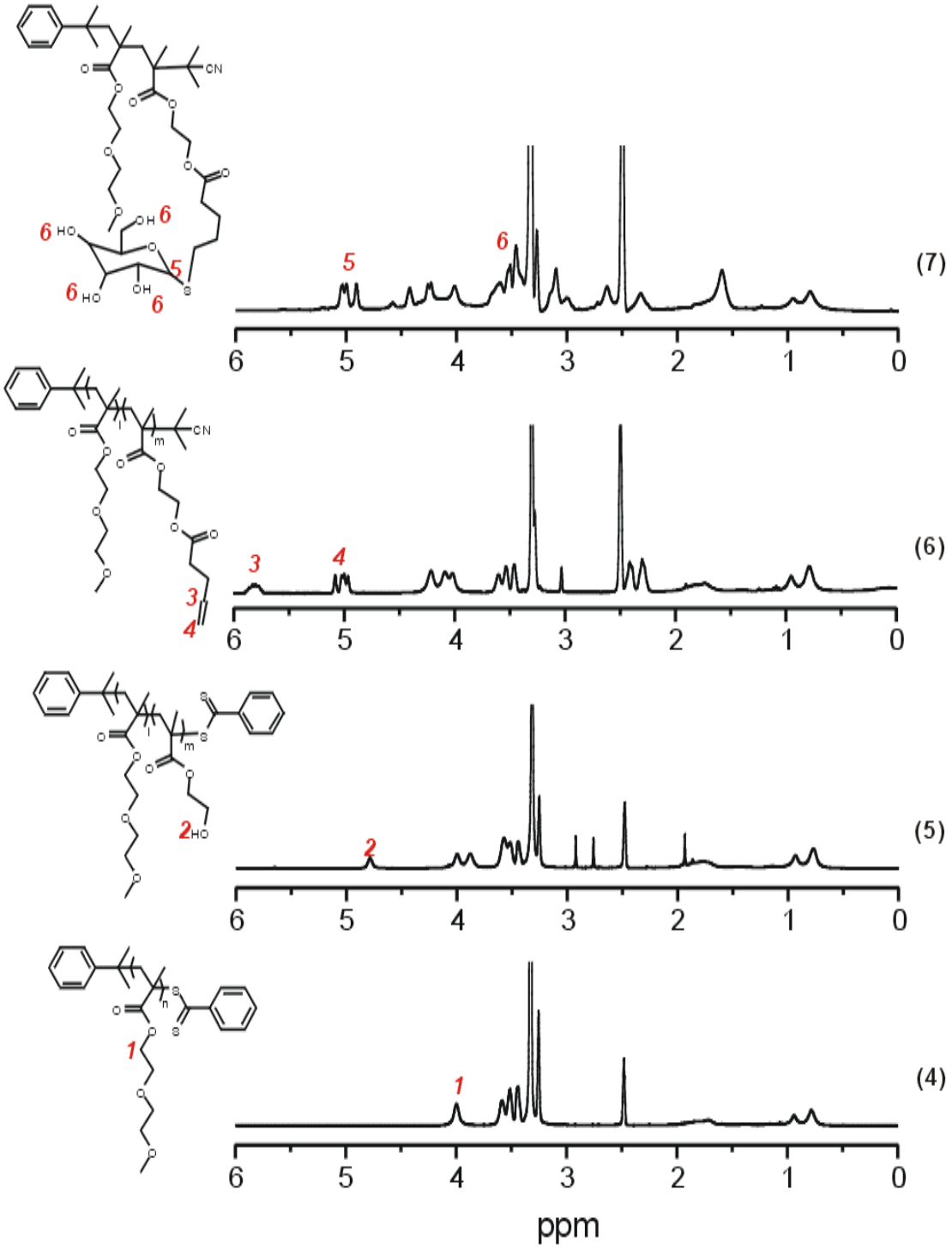
## 2.11 Synthesis of Glycopolymer (7)



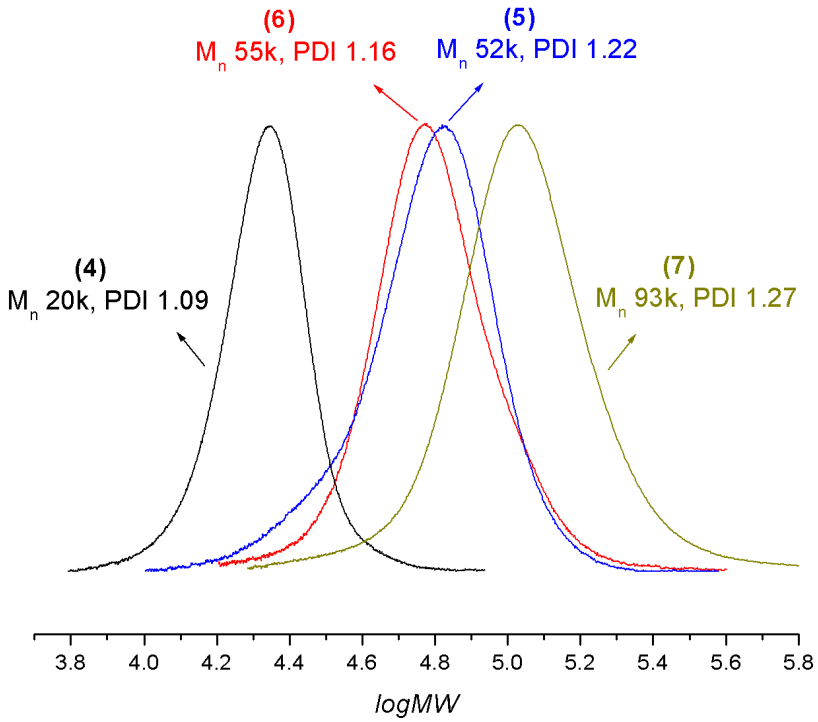

**(6)** **(7)**

The same procedure as the synthesis of **(3)** was used, except that **(6)**, instead of **(2)**, was used for the modification.

## 2.12 NMR characterisation of polymer (4), (5), (6) and (7)



### 2.13 GPC characterisation of polymer (4), (5), (6) and (7)




## 3.0 lectin-binding test

### 3.1 Turbidimetry assay

1 μM Con A in pH 7.4 HEPES-buffered saline (HBS) was made fresh before the assay.[3] Turbidity measurements were performed by adding 500 μL of the Con A solution to a dry quartz micro-cuvette and put into the holder of the spectrometer at certain temperature for 1 min. A solution of the ligand (**(3)** or **(7)**) in HBS buffer was then added (50 μL at 500 μM per glucose residue). Upon addition, the solution was mixed vigorously for 5 s using a pipet. Absorbance data were recorded at 420 nm for 10 min at 1.2 Hz.

### 3.2 DLS analysis

DLS experiments were performed to study the lectin-binding properties of the glycopolymers synthesized on a Malvern Nano-Zetasizer. 1.5 mL freshly made 1 μM Con A in pH 7.4 HBS buffer was added to a cuvette and put into the holder of the Zetasizer at certain temperature for 5 min. 150 μL of the ligand in HBS buffer (500 μM per glucose residue) was then added and mixed vigorously for 5 s and incubated in the holder for 10 min before the instrument start to measure the particle size.

## 4.0 References

1. S. Oae, T. Yagihara and T. Okabe, *Tetrahedron*, 1972, **28**, 3203-3216.
2. S. Perrier, P. Takolpuckdee and C. A. Mars, *Macromolecules*, 2005, **38**, 2033-2036.
3. C. W. Cairo, J. E. Gestwicki, M. Kanai and L. L. Kiessling, *J. Am. Chem. Soc.*, 2002, **124**, 1615-1619.