PAPER

Poly(2-oxazoline) glycopolymers with tunable LCST behavior

Kristian Kempe,[a] Toni Neuwirth,[a] Justyna Czaplewska,[a] Michael Gottschaldt,[ab] Richard Hoogenboom[*c] and Ulrich S. Schubert[*ab]

A series of thermo-responsive glyco-poly(2-oxazoline)s based on 2-ethyl-2-oxazoline and 2-(dec-9-enyl)-2-oxazoline were prepared. To study the effect of the sugar content on the solution behavior in water, two sets of copolymers with constant monomer-to-initiator ratios of 20 and 50 and varying amounts of the hydrophobic alkene functionalized monomer were synthesized. The glycopolymers were obtained by the photoaddition of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glycopyranose onto the double bonds followed by deacetylation of the saccharide residues. Turbidimetry measurements of the respective glycopolymers revealed a decreasing cloud point temperature with increasing amount of sugar moieties, proposed to be caused by hydrogen bonding between the sugars and the polymer amide groups, which is enabled by the flexibility of the long decyl spacer. Due to the linear relationship between cloud point temperatures and the sugar content, the cloud points can be easily tailored for specific applications.

Introduction

Thermo-responsive polymers have received major attention in the last few years as one of the main concepts for stimuli-responsive polymers.[1,2] Their response to a temperature stimulus makes them perfect candidates to be used in applications where temperature variations occur depending on the location of action, such as in the human body where for instance healthy and cancer cells exhibit different temperatures. As a consequence, the design of new stimuli-responsive synthetic polymers is of great interest since they offer the possibility to generate reversible temperature response; not showing a denaturation of their structures upon heating as, for example, proteins do. In particular, the lower critical solution temperature (LCST) behavior of polymers represents an extensively exploited feature for the development of thermo-responsive materials. Commonly, an increase in temperature enhances the solubility of a polymer in (aqueous) solution. In contrast, the LCST behavior of aqueous polymer solutions represents the reverse effect upon heating resulting in a phase separation of the binary polymer/water mixture. Increasing the temperature above this phase transition temperature causes the disruption of hydrogen bonds between polar groups of the polymer and water. This cloud point temperature (T_{cp}) depends on the hydrophilic–hydrophobic balance of the polymer. In general, more hydrophilic polymers are better hydrated, *i.e.* a larger number of water molecules are hydrogen bonded to the polymer, which gives a favorable (negative) enthalpy contribution to the free energy of mixing ($\Delta G = \Delta H - T\Delta S$) resulting in a higher T_{cp}. However, H-bonding between polymer and solvent results in an enhanced ordering of the water molecules, thus, an unfavorable contribution to the entropy of mixing is accounted. The entropy term ($T\Delta S$) becomes predominant over the favorable enthalpy term with increasing temperature resulting in phase separation. This LCST effect can be tailored for special applications by varying the structure. The incorporation of hydrophobic groups, such as end groups or other more hydrophobic or hydrophilic comonomers can alter the T_{cp} of the system.[3] In recent years, a wide range of systems were described in literature exhibiting tunable LCST behaviors based on (co-)polymerization of monomers such as *N*-isopropylacrylamide, ethylene glycol, (meth-)acrylates and 2-oxazolines.[4–7]

Glycopolymers—synthetic polymers with pendant carbohydrate moieties—appeared to be of special interest for recognition/targeting in a variety of biological processes, like immunological recognition, interaction between bacteria or viruses with cells, as well as tissue growth and repair.[8–12] Due to their free hydroxyl groups sugar side-chains can have an influence on the solubility of the respective polymers and, thus, on the LCST behavior, in particular when copolymerized with a monomer that results in a LCST polymer.[13–18] In combination with the ability of the carbohydrate moieties to bind to a broad range of carbohydrate-binding receptors, responsible, *i.e.* for cell

[a]*Laboratory of Organic and Macromolecular Chemistry (IOMC) and Jena Center for Soft Matter (JCSM), Friedrich-Schiller-University Jena, Humboldtstr. 10, 07743 Jena, Germany*
[b]*Dutch Polymer Institute (DPI), John F. Kennedylaan 2, 5612 AB Eindhoven, The Netherlands. E-mail: ulrich.schubert@uni-jena.de; Fax: +49 3641 948 202*
[c]*Supramolecular Chemistry Group, Department of Organic Chemistry, Ghent University, Krijgslaan 281 S4, B-9000 Ghent, Belgium. E-mail: richard.hoogenboom@ugent.be; Fax: +32 9 264 4498*

proliferation and death,[19,20] it renders thermo-responsive glyco-polymers perfect candidates for biological applications, *e.g.* as targeted drug delivery systems, coating materials for surfaces and particles, for affinity separations, bioassays or biocapture analysis.[21–26]

The living cationic ring-opening polymerization (CROP) of 2-oxazolines possesses a large potential for the preparation of thermo-responsive (co-)polymers due to the possible preparation of several hydrophilic polymers and the ease in altering the hydrophilic/hydrophobic properties of the corresponding copolymers using differently substituted monomers.[27,28] Poly(2-oxazoline)s themselves can be considered as analogues of poly (amino acid)s, *i.e.* pseudo-peptides, exhibiting in the case of 2-methyl-2-oxazoline as well as 2-ethyl-2-oxazoline a stealth behavior similar to poly(ethylene oxide) (PEO).[29] Due to its biocompatibility (co-)poly(2-oxazoline)s were used in several biomedical applications.[30,31] This biocompatibility in combination with the LCST behavior of poly(2-oxazoline)s is perfectly suited as a platform for the synthesis of glycopolymers with various tunable properties. Due to the living character of the polymerization, the composition and sequence of the copolymers can be tuned and different functionalities can be incorporated by the initiator, the termination agent or by the usage of suitable 2-substituted-2-oxazolines.[32–34] Recently, we reported the synthesis of a copolymer system exhibiting pendant alkene functionalities in the side chains based on renewable resources,[35,36] which is suitable for post-modification reactions *via* thiol–ene chemistry.[37–39] As demonstrated by Diehl and Schlaad, this approach offers the possibility for the preparation of well-defined glycopolymers from a precursor polymer using peracetylated thio-sugars.[40,41] Depending on the composition of the copolymers (2-(3-butenyl)-2-oxazoline and 2-isopropyl-2-oxazoline) the LCST behavior could be tuned showing an increase in the cloud point with increasing sugar content. Furthermore, homopolymers of glycosylated 2-(3-butenyl)-2-oxazoline revealed the formation of spherical vesicles and nanofibers upon direct dissolution in water.[42] To the best of our knowledge, up to now only one additional publication exists describing the preparation of thermo-responsive glyco-poly(2-oxazoline)s based on per-acetylated maltoheptanose end capped poly(2-oxazoline)s (POx).[43]

In this contribution, we report the systematic investigation of the LCST behavior of a series of glycopolymers derived from a precursor copolymer with a constant [*M*]/[*I*] ratio and varying ratio of 2-ethyl-2-oxazoline and 2-(dec-9-enyl)-2-oxazoline. The glycopolymers were prepared by polymer post-modification exploiting the photoaddition of thiols onto pendant alkene functionalities. The cloud points of the glycopolymers obtained showed a strong dependence on the composition of the copolymer and could be altered accordingly. Interestingly, the cloud points decreased with increasing sugar content which will be discussed in detail.

Experimental details

Materials

2-Ethyl-2-oxazoline and methyl tosylate were obtained from Acros Organics, distilled to dryness over barium oxide (BaO), and stored under argon. Acetonitrile, 2,2-dimethoxy-2-

phenylacetophenone (DMPA) and sodium methoxide were purchased from Sigma Aldrich. Dialysis bags (molar mass cut-off 3.500 g mol^{-1}) were purchased from Spectra/Por®. 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glycopyranose was prepared according to a literature procedure.[44] The 2-(dec-9-enyl)-2-oxazoline (DecEnOx) was synthesized according to a recently reported method.[35]

General methods and instrumentation

The Initiator Sixty single-mode microwave synthesizer from Biotage, equipped with a non-invasive IR sensor (accuracy: ±2%), was used for polymerizations under microwave irradiation. Microwave vials were heated to 110 °C overnight and allowed to cool to room temperature under an argon atmosphere before usage. All polymerizations were carried out with temperature control. Size exclusion chromatography measurements were performed on an Agilent system equipped with a diode array detector and a refractive index detector. Two PSS SDV (5 μm pore size) columns were placed in series. DMA with 2.1 g L^{-1} of LiCl was used as eluent at 1 mL min^{-1} flow rate, and the column oven was set to 50 °C. Molar masses were calculated against poly-styrene standards. For preparative SEC, Bio-Beads S-X1 (cross-linked polystyrene beads) from Bio-Rad were used. ^{1}H NMR spectra were recorded on a Bruker AC 300 MHz spectrometer at room temperature, with MeOH-d_4 as a solvent. The chemical shifts are given in ppm relative to the signal from residual non-deuterated solvent. Cloud points were determined in a Crystal 16 from Avantium Technologies connected to a chiller (Julabo FP 40) at a wavelength of 500 nm. The solutions were heated and cooled at a rate of 1 °C min^{-1} while stirring at 600 rpm. The cloud point was defined as the temperature where the transmittance decreased to 50% in the second heating run. Dynamic light scat-tering (DLS) measurements were carried out on a Zetasizer Nano ZS (Malvern Instrument, Malvern, UK) using a He/Ne-laser ($\lambda = 633$ nm) and a scattering angle of 173°. The mean particle size was approximated as the effective (*Z* average) diameter and the width of the distribution as the polydispersity index (PDI) obtained by the cumulant method assuming a spherical shape.

Microwave-assisted synthesis of the copolymers (PEtOx-*stat*-PDecEnOx)

A solution of initiator (methyl tosylate), monomers (EtOx and DecEnOx) and solvent (acetonitrile) was prepared. The total monomer concentration was adjusted to 2 M with a total [*M*]/[*I*] ratio of 20 and 50 with 10, 20, 30 and 40 mol% DecOx, respec-tively. The vial was heated to 140 °C in the microwave synthesizer. After cooling, a 15-fold excess of aqueous sodium carbonate was added and the polymerization mixture was stirred at 100 °C overnight. Subsequently, the two-phase solution was diluted with dichloromethane, the organic phase was washed three times with water, brine and dried over MgSO$_4$. The polymer was concen-trated in vacuum and precipitated into ice-cold diethyl ether or applied to preparative size exclusion chromatography.

Thiol–ene photoaddition reaction using 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glycopyranose

300 mg of the copolymer were dissolved in THF and 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glycopyranose was added in 1.3-fold

excess with respect to the double bonds. After degassing the solution for 30 min a spatula tip of 2,2-dimethoxy-2-phenyl-acetophenone (DMPA) was added and the solution was irradiated at 356 nm overnight. The resulting protected glycocopolymers were purified either by precipitation in ice-cold diethyl ether or by preparative size exclusion chromatography.

Deprotection of the protected glycocopolymers

200 mg of the protected glycocopolymer were dissolved in 8 mL dry methanol. 1 mL sodium methoxide solution was added and the copolymer solution was stirred for 2 h. Subsequently, the solvent was evaporated under reduced pressure. The polymer was re-dissolved in water, neutralized with diluted HCl and dialyzed against 500 mL distilled water for 3 days. The surrounding water was exchanged six times in a period of 12 h.

Cloud point measurements

4 mg of the copolymer were dissolved in deionized water applying a constant concentration of 4 mg mL^{-1}. The turbidity of the solutions was determined in two temperature cycles ranging from 2 to 105 °C [1 K min^{-1}]. Turbidity measurements at a wavelength of 500 nm were performed to determine the cloud point temperatures at 50% transmittance.
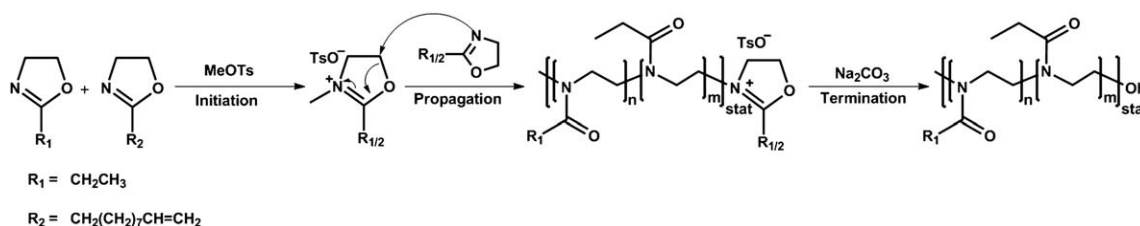
Dynamic light scattering measurements

The cloud point samples (4 mg mL^{-1}) were diluted with filtered deionized water to a concentration of 1 mg mL^{-1} and were measured 5 times at 25 °C for 150 s.

Results and discussion

Preparation of the copolymer precursors

For the systematic investigation of the LCST behavior of copoly(2-oxazoline) glycocopolymers two series of copolymers with a degree of polymerization of 20 and 50 were prepared, respectively. The copolymerization mechanism is schematically depicted in Scheme 1 showing the initiation with methyl tosylate, the incorporation of the monomers during the copolymerization and the subsequent termination reaction using a nucleophile. For the variation of the number of the sugar units different ratios of the hydrophilic monomer EtOx and the hydrophobic monomer DecEnOx, exhibiting pendant alkene groups, were adjusted using 10 mol% increments of DecEnOx (up to 40 mol% DecEnOx).

Table 1 Overview of the theoretical feed ratio of DecEnOx and percentage incorporated in the final copolymers determined by ^{1}H NMR spectroscopy

	$n + m = 20$				$n + m = 50$			
	P1a	P1b	P1c	P1d	P2a	P2b	P2c	P2d
Dec$_{feed}$/mol%	10	20	30	40	10	20	30	40
Dec$_{exp}$/mol%	9	20	30	39	10	19	30	39

As can be seen in Table 1, the theoretical feed amounts of DecEnOx are in close agreement with the DecEnOx incorporated in the final copolymers as calculated by ^{1}H NMR spectroscopy. In order to guarantee the same chemical structure of all copolymers the reaction mixture was treated with aqueous sodium carbonate at 100 °C after microwave polymerization at 140 °C to form a hydroxy end group.[45] End capping of the copolymers just with water would lead to a mixture of two different end groups (hydroxy and ester) which could have a significant influence on the properties of, in particular, the shorter copolymers.[46,47] As a representative example, the IR spectrum of **P1b** does not show the existence of an ester signal at about 1710 cm^{-1} (Fig. 1). Furthermore, a similar observation can be made by means of the corresponding ^{1}H NMR spectrum (Fig. 2). In the case of the formation of an ester end group, a peak at 4.1–4.2 ppm would appear, whereas a hydroxy end group leads to a peak at lower
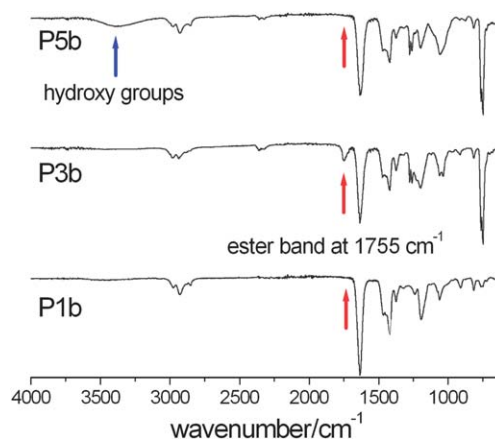

Fig. 1 IR-ATR spectra of **P1b**, **P3b** und **P5b** demonstrating the successful addition of acetyl-protected glucose units (ester band at 1755 cm^{-1}) onto the polymer precursor. The disappearance of the ester band as well as the appearance of a broad band between 3100 cm^{-1} and 3600 cm^{-1} confirms the successful deprotection of the sugar moieties.

Scheme 1 Schematic representation of the mechanism of the cationic ring-opening copolymerization of 2-ethyl-2-oxazoline and 2-(dec-9-enyl)-2-oxazoline.

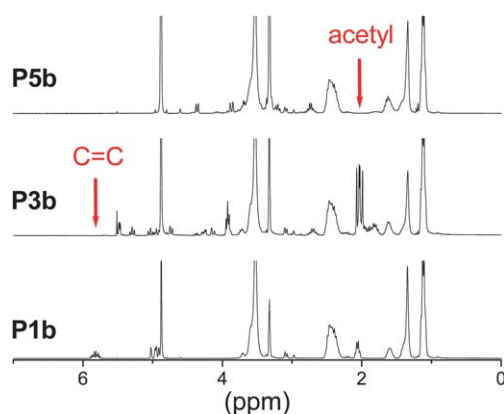**Fig. 2** ¹H NMR spectra (300 MHz; MeOH-d_4) of the copolymers **P1b**, **P3b** (thiol–ene product) and **P5b** (after deacetylation). The disappearance of the signals of the double bond protons as well as the presence of signals of the acetyl protons in **P3b** confirms the successful thiol–ene photo-addition of Ac₄GlcSH onto the copolymer **P1b**. The absence of the acetyl signals demonstrates the successful deprotection of the sugar units (**P5b**).

ppm values, just left of the signals of the polymeric backbone at about 3.8 ppm. Thus, based on the absence of the peak at 4.1 to 4.2 ppm and the appearance of the signal at 3.8 ppm it can be concluded that the copolymer possesses exclusively OH end groups.

Thiol–ene photoaddition towards glycopolymers

DecEnOx can undergo a thiol–ene reaction with diverse thiols due to its pendant alkene groups. In view of the envisioned preparation of glycopolymers, 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glycopyranose (Ac₄GlcSH) was chosen as thiol and the reactions were performed under UV irradiation at ambient temperature applying an UV-labile radical initiator (DMPA) overnight. Subsequently, the polymers bearing protected sugar residues were purified by precipitation as well as by preparative size exclusion chromatography to remove the slight excess of Ac₄GlcSH. Finally, the corresponding glycopolymers were obtained by treatment with sodium methoxide for 2 h at room temperature.

The process of the attachment of the protected sugar unit and its deprotection (Scheme 2) was monitored by IR and ¹H NMR spectroscopy as well. Upon completion of the thiol–ene reaction a signal at 1740 cm⁻¹ appeared in the IR spectrum, which

originated from the acetyl protecting groups of the sugar (Fig. 1). This signal disappears again after the deprotection of the sugar moieties yielding free hydroxyl groups which cause a broad signal at about 3500 cm⁻¹. Similar observations can be made by ¹H NMR spectroscopy. The spectrum of the starting materials shows two multiplets at about 5 and 6 ppm corresponding to the terminal alkene group of the DecEnOx. These signals vanished after the reaction with Ac₄GlcSH revealing quantitative conversion (>96%) of the double bonds. In addition, the signals from the protons of the sugar residues (4 to 6 ppm) and a strong signal at about 2 ppm belonging to the protons of the acetyl protecting groups appeared. Therefore, the successful deprotection of the sugar moieties by treatment with sodium methoxide could be proven by the disappearance of the signals of the acetyl groups in the final products. Applying these steps to all copolymers, two series of well-defined glycopolymers with a total degree of polymerization (DP) of 20 and 50 were obtained, respectively. They show narrow molar mass distributions in SEC (Fig. 3) and thus, possess polydispersity indices (PDI) between 1.1 and 1.2 (see Tables 2 and 3). As can be seen in Fig. 3, glycopolymers with higher sugar content eluted at shorter times than the ones with lower sugar content, which is attributed to the increase in the total molar mass of the glycopolymers. Furthermore, glycopolymers with a DP of 50 are shifted to lower elution
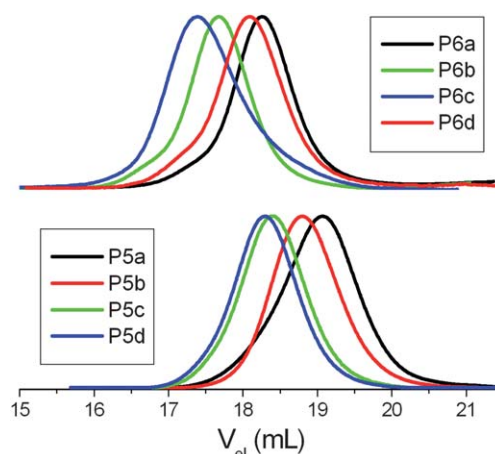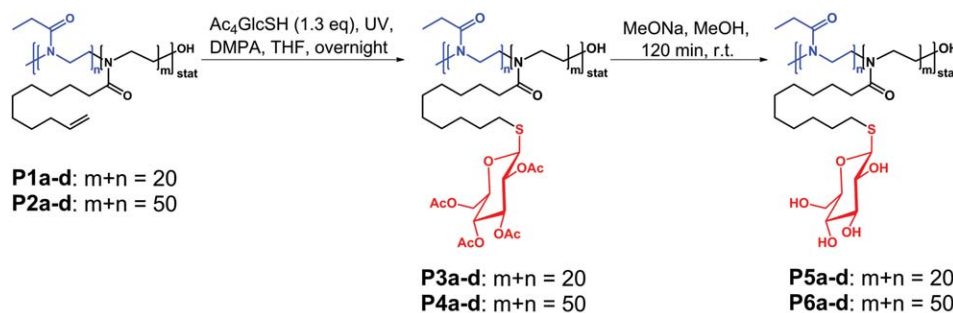

Fig. 3 SEC traces of the (deprotected) glycopolymers with a DP of 20 (**P5a–d**; bottom) as well as with a DP of 50 (**P6a–d**; top) with different amounts of Dec(Glc)Ox units (a: 10 mol%, b: 20 mol%, c: 30 mol%, d: 40%); eluent: DMA/2.1 g L⁻¹ LiCl.

Scheme 2 Schematic representation of the preparation of glycopoly(2-oxazoline)s using the thiol–ene approach encompassing the thiol–ene photo-addition as well as the deprotection of the glucose units.

Table 2 SEC summary (M_n and PDI values; PS calibration) of the statistical copolymers (**P1**), the acetylated glycopolymers (**P3**) and the corresponding glycopolymers (**P5**) with a total $[M]/[I]$ ratio of 20 with different DecEnOx increments (**a–d**)

	M_n/g mol^{-1}	PDI		M_n/g mol^{-1}	PDI		M_n/g mol^{-1}	PDI
P1a	4100	1.15	**P3a**	5700	1.13	**P5a**	8400	1.20
P1b	4700	1.12	**P3b**	6700	1.10	**P5b**	9200	1.14
P1c	5200	1.12	**P3c**	7400	1.14	**P5c**	13 600	1.12
P1d	5200	1.12	**P3d**	7800	1.11	**P5d**	14 600	1.12

Table 3 SEC summary (M_n and PDI values; PS calibration) of the statistical copolymers (**P2**), the acetylated glycopolymers (**P4**) and the corresponding glycopolymers (**P6**) with a total $[M]/[I]$ ratio of 50 with different DecEnOx increments (**a–d**)

	M_n/g mol^{-1}	PDI		M_n/g mol^{-1}	PDI		M_n/g mol^{-1}	PDI
P2a	9300	1.10	**P4a**	11 200	1.12	**P6a**	14 200	1.13
P2b	10 500	1.10	**P4b**	12 400	1.11	**P6b**	15 800	1.15
P2c	9600	1.16	**P4c**	13 100	1.17	**P6c**	19 400	1.15
P2d	11 200	1.13	**P4d**	14 300	1.15	**P6d**	19 900	1.20

volumes (Fig. 3, top) compared to the glycopolymers with a DP of 20 (Fig. 3, bottom).

Solubility of the copolymers/glycopolymers in aqueous media

To study the effect of the sugar content on the thermo-responsive behavior of the glycopolymers in aqueous solution, turbidimetric measurements were performed at a constant copolymer concentration of 4 mg mL^{-1}. The water solubility of the starting copolymers with 10 to 40 mol% DecEnOx in steps of 10 mol% depends on the amount of DecEnOx incorporated in the copolymer and the total degree of polymerization (Tables 4 and 5). For a total DP of 20, copolymers with 10 mol% and 20 mol% DecEnOx revealed good water solubility, even if the cloud point of **P1b** is already close to room temperature. The copolymers with a higher degree of DecEnOx were insoluble in water in the investigated temperature range (2 to 105 °C). As expected, the higher the amount of the hydrophobic monomer the lower the cloud point of the copolymer. By increasing the total DP to 50, only the copolymer with the lowest DecEnOx content was still soluble in water under these conditions. A decrease in the cloud point temperature and the lower solubility upon increasing the molar mass are common observations following the Flory–Huggins model.

Upon the photoaddition of Ac$_4$GlcSH onto the pendant alkene groups the copolymers with 30 mol% and 40 mol% DecEnOx remained water insoluble, whereas the other ones

showed a different somewhat unexpected behavior as observed for the protected glycopolymers **P3a** and **P3b** (**P4a** and **P4b** with DP of 50 were not studied in detail). Instead of showing a lower cloud point temperature as one would expect due to the increase in hydrophobicity of the complete system, the cloud point increased compared to the starting copolymers. This observation can be attributed to the formation of aggregates as revealed by dynamic light scattering (DLS) measurements of copolymer **P3a** (Fig. 4). The starting copolymer **P1a** is solubilized as individual polymer chains in aqueous solution while the attachment of the larger (hydrophobic) protected sugar moieties results in a self-assembly of the polymer chains under the formation of aggregates with a diameter of 220 nm and a low PDI$_{particle}$ value. This aggregation causes a change in the thermo-responsiveness of the system, since the hydrophobic domains are shielded from the aqueous solution, thereby increasing the solubility of the hydrophilic parts that stabilize the aggregates. A similar behavior was observed for copolymer **P3b** possessing 20 mol% DecEnOx units. The cloud point is again slightly higher for **P3b**. DLS measurements revealed the existence of large ill-defined aggregates (330 nm; PDI$_{particle}$ = 0.6) in the case of copolymer **P3b**. Similar to **P3a**, the formation of aggregates renders the copolymer soluble at a temperature lower than 21 °C, which would not be possible without the existence of these aggregates due to the higher hydrophobicity of the copolymer caused by the incorporation of the hydrophobic Ac$_4$GlcSH residues. The turbidity measurements revealed a sharp decrease in transmittance consistent with a sharp phase transition for **P3b** in contrast to **P3a** (Fig. 5). The lower number of hydrophobic Dec(Ac$_4$Glc)Ox repeating units in **P3a** results in a smaller hydrophobic attraction between the chains while at the same time the larger amount of hydrophilic EtOx units will stabilize initially formed aggregates by suppressing further aggregation. As a result, **P3a** shows a gradual collapse and assembly upon increasing the temperature resulting in a broad phase transition. Nonetheless, both polymers reveal a reversible phase transition with a similarly small hysteresis.

Upon the deprotection of the sugar moieties the corresponding glycopolymers **P5a–d** and **P6a–d** were obtained, which all exhibit cloud point temperatures. In general it can be stated that the amount of sugar moieties has a significant influence on the cloud point of the glycopolymers albeit a contrary effect, *i.e.* an increasing cloud point with increasing hydrophilic sugar content, was expected as observed by Schlaad and co-workers using a shorter spacer between the polymer backbone and the sugar moiety. Nevertheless, it was found in the current study that increasing the sugar content results in a decrease in the cloud point temperature of the copolymers (Fig. 6), which is in contrast to an increase in hydrophilicity of the overall system. This

Table 4 Cloud point temperatures (°C; heating and cooling cycles) obtained for the statistical copolymers (**P1**), the acetylated glycopolymers (**P3**) and the corresponding glycopolymers (**P5**) with a total DP of 20 with different DecEnOx increments (**a–d**)

	Heating	Cooling		Heating	Cooling		Heating	Cooling
P1a	53.3	52.5	**P3a**	66.2	61.2	**P5a**	39.4	38.1
P1b	18.5	17.8	**P3b**	21.5	18.1	**P5b**	30.8	31
P1c	Insoluble	Insoluble	**P3c**	Insoluble	Insoluble	**P5c**	17.5	16.4
P1d	Insoluble	Insoluble	**P3d**	Insoluble	Insoluble	**P5d**	12.4	13.1

Table 5 Cloud point temperatures (°C; heating and cooling cycles) obtained for the statistical copolymers (**P2**), the acetylated glycopolymers (**P4**) and the corresponding glycopolymers (**P5**) with a total DP of 50 with different DecEnOx increments (**a–d**) [not det. = not determined]

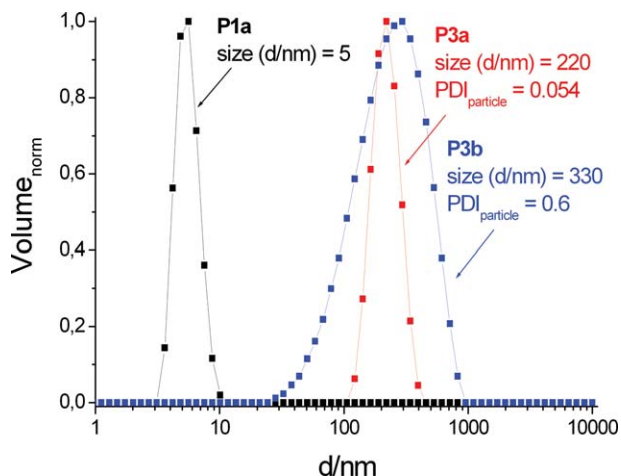|	Heating	Cooling		Heating	Cooling		Heating	Cooling
P2a	33.7	32.6	**P4a**	Not det.	Not det.	**P6a**	44.6	42.5
P2b	Insoluble	Insoluble	**P4b**	Not det.	Not det.	**P6b**	34.5	36.8
P2c	Insoluble	Insoluble	**P4c**	Not det.	Not det.	**P6c**	18.3	21.8
P2d	Insoluble	Insoluble	**P4d**	Not det.	Not det.	**P6d**	14.5	15.3

Fig. 4 DLS analysis of **P3a** and **P3b** in water (4 mg mL^{-1}) revealing the formation of large aggregates after the thiol–ene modification of the precursor copolymer **P1a** with Ac$_4$GlcSH.

unexpected phenomenon is proposed to be related to the high flexibility of the DecEnOx chains in combination with hydrogen bonding. Due to the long alkyl chains acting as flexible spacers between the polymer backbone and the sugar residues, the hydroxyl groups of the sugar moieties can fold back to interact with the polymer amide groups by intramolecular hydrogen bonding. These intramolecular hydrogen bonds compete with the water hydration, thereby decreasing the enthalpy gain of the
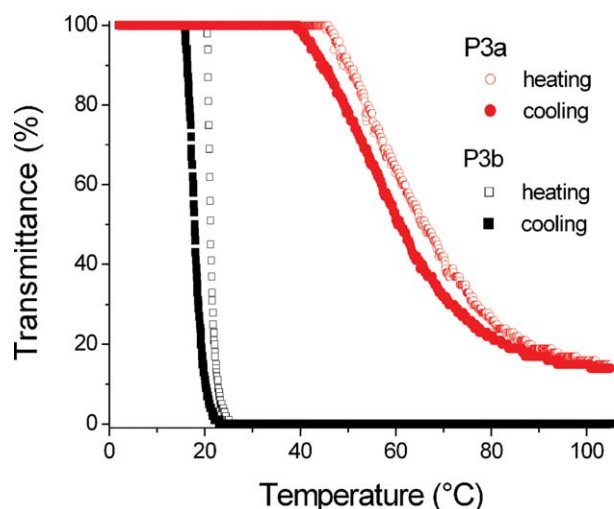

Fig. 5 Transmittance curves during heating and cooling scans (polymer concentration = 4 mg mL^{-1}, heating/cooling rate = 1 °C min^{-1}).
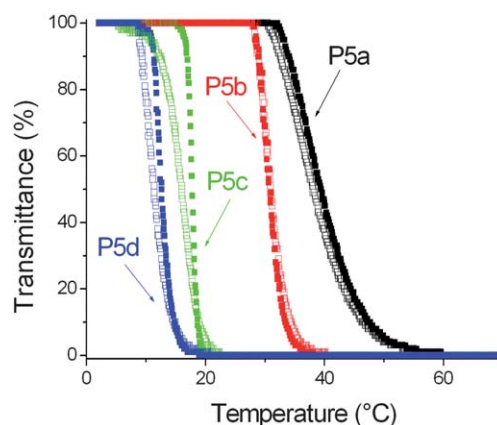

Fig. 6 Representative transmittance curves of the glycopolymers (**P5a–d**) in aqueous solution during heating (■) and cooling (□) scans (polymer concentration = 4 mg mL^{-1}, heating/cooling rate = 1 °C min^{-1}).
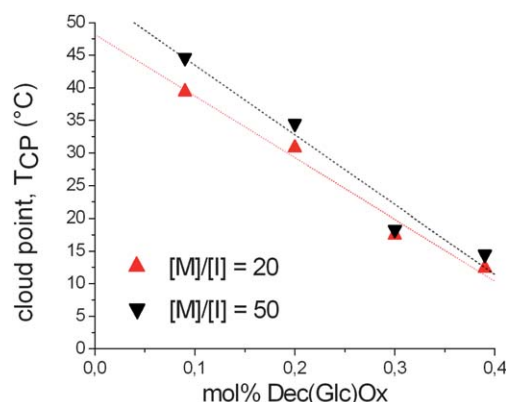

Fig. 7 Cloud point temperatures in aqueous solution of the (deprotected) glycopolymers with a total DP of 20 (**P5a–d**) and 50 (**P6a–d**) as a function of the Dec(Glc)Ox content in the copolymer.

latter. As a result, the entropy term will become dominant at lower temperatures resulting in a lower cloud point. A small hysteresis is observed, in particular for the glycopolymers with a higher sugar content **P5c** and **P5d**, which might be caused by the higher number of intramolecular hydrogen bonds, which need to be disrupted during the cooling run again.[1]

A similar trend could be observed for the copolymers with a total DP of 50 as shown in Fig. 7. Surprisingly, the cloud points of the longer glycopolymers are in the same range as the ones for the shorter glycopolymers. This uncommon observation might indicate that the cloud point behavior is predominantly governed by the intramolecular hydrogen bonding suppressing the

Flory–Huggins chain length effect. Nevertheless, the linear dependence of the cloud point on the amount of sugar moieties allows straightforward tuning of the cloud points enabling a perfect control of the LCST behavior for specific applications.

Conclusions

A systematical study of the cloud points of copoly(2-oxazoline) bearing glycosylated side chains is reported based on two copolymer series of 2-ethyl-2-oxazoline and 2-(dec-9-enyl)-2-oxazoline with a total degree of polymerization of 20 and 50, respectively. All polymers were synthesized by a microwave-assisted living cationic ring-opening polymerization showing a perfect consistency between theoretical and experimentally determined molar ratios. In order to obtain the corresponding glyco-poly(2-oxazoline)s 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glycopyranose was "clicked" by thiol–ene photoaddition reactions onto the pendant double bonds of the precursor copolymers followed by the deacetylation of the sugar moieties with sodium methoxide. Turbidity measurements of the glycopolymers in aqueous solution revealed a decrease in the cloud points with increasing number of sugar moieties. This unexpected behavior is proposed to be caused by hydrogen bonding between the hydroxy sugar groups and the polymer backbone facilitated by the large flexible decyl spacer. The linear dependency of the cloud point temperatures in this systematic study allows straightforward tuning of the LCST behavior for specific applications. In the future these results will be exploited for the synthesis of more complex polymer structures to study the controlled binding of lectins by varying the type and amount of sugar moieties.

Acknowledgements

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