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Hyperbranched poly(2-oxazoline)s via bisfunctional crosslinker

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ABSTRACT

Hyperbranched polymers are an interesting type of polymeric structure as they possess useful features for a range of applications. They have been used for small molecule storage and transport owing to the existence of their large number of end groups that can be used for further functionalisation. In this study, we introduce a bisfunctional 2-oxazoline based crosslinker to synthesise hyperbranched poly(2-oxazoline)s with molar mass ranging from 3.2 kDa to 22 kDa. Furthermore, to control the degree of crosslinking, an end-capping agent was added at the beginning of the polymerisation in order to prevent uncontrolled branching and subsequent gelation. Moreover, advanced viscosity gel permeation chromatography was used to compare the degree of branching present in each polymer, and the lower critical solution temperature of each branched polymer was measured, with transition temperatures ranging from 44 °C to 70 °C.

1. Introduction

Dendrimers are a unique type of polymer that are monodisperse, perfectly branched, globular structures. [1] Dendrimers have many potential applications, but due to their extremely high definition they are most useful for biomedical purposes which demand clarity of chemical structure. [2–4] Nonetheless, dendrimers can be expensive, often requiring difficult and laborious synthetic techniques. [5] Cross-linked hyperbranched polymers on the other hand are often much easier to synthesise with fewer synthetic steps, and can be synthesised in a one-pot system. [6–7] Like dendrimers, cross-linked hyperbranched polymers also exhibit valuable properties such as high solubility, low viscosity, possession of internal cavities for small molecule storage and transport, and an abundance of functional groups. [8–9].

Hyperbranched polymers have previously been synthesised with controlled polymerisation techniques using multifunctional vinyl monomers as cross-linkers and a chain transfer agent to control the degree of cross-linking. [10–12] This type of branched polymer synthesis is known as the 'Strathclyde method', and is one of the easiest and most common ways to make branched polymers. [13–14] Previous polymerisation techniques using similar approaches to the Strathclyde method include reversible addition fragmentation chain-transfer (RAFT) Polymerisation, [15] copper-mediated reversible deactivation radical polymerisation (Cu(0)-RDRP), [16] and nitroxide mediated Polymerisation (NMP). [17] However, there are very limited examples of hyperbranched poly(2-oxazoline)s in the literature, and there are none that use a bis-oxazoline cross-linker. The reason for this is because gelation is extremely prevalent and difficult to control without the use of a chain-transfer agent, of which these is no analogue in the CROP of 2-oxazolines. One notable example combined a dendrimer core with poly (2-oxazoline) arms to create a star-shaped polymer. [18] Poly(2-oxazoline) hydrogels have previously been synthesised using various methods. [19–21] These synthesised hydrogels were used for biomedical applications such as drug storage agents, [22] and DNA binding matrices. [23] The main advantage of a branched polymer compared to a hydrogel is that it is soluble, which makes polymer characterisation easier. It also means branched polymers can be used as homogenous catalysts [24] and can also be mixed with other materials as a plasticiser. [25].

Hyperbranched poly(2-oxazoline)s have previously been synthesised with therapeutic significance, albeit without the use of a cross-linker. Perrier *et al.* [26] initiated a poly(2-oxazoline) chain with propargyl tosylate and end-capped it with an ethylxanthate functionality, which could undergo reduction to a free thiol. The thiol and propargyl groups could then undergo thiol-yne reactions to yield a hyperbranched polymer. These polymers could then be partially hydrolysed to yield charged hyperbranched poly(2-oxazoline)s for gene delivery. A second example end capped poly(2-oxazoline) chains with methacrylic acid to generate a macromonomer structure that could be used to form hyperbranched polymers for comparison to PEG in a structure–activity study. [27].

The Strathclyde method is a very popular route for the synthesis of branched polymers from vinyl monomers. [10,28–29] This method

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involves the combination of a linear monomer, a bisfunctional monomer, and a chain transfer agent, which is typically a thiol. [30] The chain transfer agent is used to control the degree of branching and hence the molecular weight of the branched polymer. For poly(2-oxazoline)s, chemicals designed specifically for chain transfer do not exist, and so the molecular weight of the branched polymers can be controlled by the addition of a small amount of a sterically hindered nucleophile at the start of the polymerisation. It should be noted that chain transfer for poly(2-oxazoline)s is known to be promoted by various factors such as the solvent type used. [31] The sterically hindered nucleophile terminates living chain ends, whilst competing with the ongoing polymerisation reaction limiting the molecular weight and preventing gelation. In this work, a previously synthesised bisfunctional 2-oxazoline cross-linker (BisOx) was used to generate hyperbranched poly(2-oxazoline) structures in a one-pot system with 2-ethyl-2-oxazoline (EtOx) and an end capping agent (Scheme 1). [32] Briefly, the cross-linker was synthesised *via* thiol-ene reaction between two equivalents of 2-isopropenyl-2-oxazoline and one equivalent of 1,2-ethanedithiol.

2. Experimental

2.1. Materials

Anhydrous acetonitrile (99.9 %, Acros Organics, extra dry), triethylamine (>99 % Sigma-Aldrich), diisopropylethylamine (99 %, Thermofisher), diisopropylamine (99 %, Sigma-Aldrich), 2-propanol (99.9 %, Sigma-Aldrich), diethyl ether (99.9 %, Sigma-Aldrich), 1,2-ethanedithiol (98 %, Sigma-Aldrich), 2-ethyl-2-oxazoline (EtOx) (99 %, Sigma-Aldrich) and 2-isopropenyl-2-oxazoline (iPOx) (98 %, Sigma-Aldrich) were distilled over calcium hydride prior to use. Propargyl p-toluenesulphonate (Sigma Aldrich, >97 %) was distilled prior to use.

2.2. Methods

All GPC measurements were carried out on an Agilent Infinity II MDS instrument equipped with differential refractive index (DRI), viscometry (VS), dual angle light scatter (LS) and multiple wavelength UV detectors. The system was equipped with 2 × PLgel Mixed C columns (300 × 7.5 mm) and a PLgel 5 μm guard column. The eluent is THF with 2 % TEA (triethylamine) and 0.01 % BHT (butylated hydroxytoluene) additives. Samples were run at 1 mL/min at 30 °C. Poly(methyl methacrylate) and polystyrene standards (Agilent EasiVials) were used for calibration. Analyte samples were filtered through a GVHP membrane with 0.22 μm pore size before injection. Respectively, experimental molar mass ($M_{n,GPC}$) and dispersity (Đ) values of synthesized polymers were determined by conventional calibration using Agilent GPC/*SEC* software.

All UV measurements were measured on a Cary 3500 UV–vis Spectrophotometer. Samples were prepared at a concentration of 5 mg/mL in distilled water and experiments were run in Suprasil® quartz cuvettes (Hellman, 100-QS, light path = 10.00 mm). Samples were subjected to a heat/cool cycle from 25 °C to 85 °C and back to 25 °C at a ramp rate of 5 °C/min at a λ = 600 nm.

All ^{1}H NMR spectra were measured at 298 K on a Bruker HD400 in $CDCl_3$.

2.3. Synthesis of Bis-Oxazoline (BisOx)

The synthesis of BisOx was carried out as previously reported. [32] Briefly, 1,2-ethanedithiol (4.28 g, 44.9 mmol, 1 eq) was added to a round-bottomed flask and cooled to 0 °C. 2-isopropenyl-2-oxazoline (10.00 g, 89 mmol, 2 eq) was then added dropwise, and the reaction mixture was left, with stirring under nitrogen, for 16 h. Subsequently, the reaction mixture was dried thoroughly *in vacuo*.

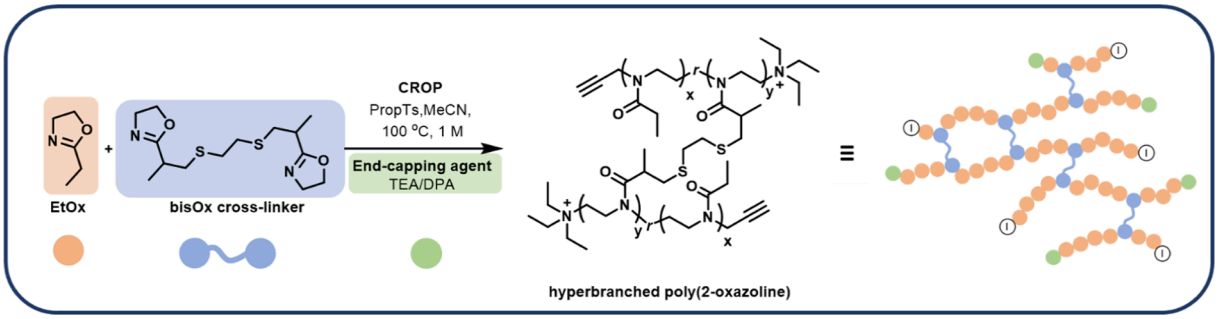
2.4. Synthesis of branched polymers

P1 - BisOx (0.10 g, 0.31 mmol, 5 eq) was added to a clean and dry microwave vial with a stirrer bar. The microwave vial was then sealed and placed under a nitrogen atmosphere. To this, EtOx (0.63 g, 6.32 mmol, 100 eq) was added. Acetonitrile (5.9 mL) was added to ensure a reaction concentration of 1 M (assuming density of BisOx and EtOx is ~ 1 g/mL). Next, PropTs (13.2 μL, 0.06 mmol, 1 eq) was added and a sample taken for T_0. The nitrogen line was removed, and the reaction flask was stirred at 100 °C in an oil bath for 16 h. A sample was taken for T_{final} before precipitating the polymer twice in diethyl ether and drying in a vacuum oven at 40 °C.

For other polymers using an end-capping agent e.g. **P5** the following procedure was used. Note: quantities of reagents used for **P5**-**P16** can be found in **Table S1**. BisOx (100 mg, 0.32 mmol, 6 eq) was added to a clean and dry microwave vial with a stirrer bar. The microwave vial was then sealed and placed under a nitrogen atmosphere. To this, EtOx (506 mg, 5.11 mmol, 97 eq) and TEA (5 mg, 0.05 mmol, 1 eq) were added. Acetonitrile (4.5 mL) was added to ensure a reaction concentration of 1 M (assuming density of BisOx and EtOx is ~ 1 g/mL). Next, PropTs (11 mg, 0.053 mmol, 1 eq) was added and a sample taken for T_0. The nitrogen line was removed, and the reaction flask was stirred at 100 °C in an oil bath for 16 h. A sample was taken for T_{final} before precipitating the polymer twice in diethyl ether and drying in a vacuum oven at 40 °C.

3. Results and discussion

Several sterically hindered bases have been explored in order to find the most suitable end-capping agent. An end-capping agent that is too nucleophilic or unhindered prevents polymerisation from occurring, whilst an end-capping agent that is not suitably nucleophilic or too bulky does not terminate chains effectively and results in gelation. In Table 1, the synthesised branched poly(2-oxazoline)s along with their end-capping agents, their BisOx/EtOx molar ratio as determined by NMR spectroscopy, the average degree of branching ($g'_{(n)}$), and the cloud point of each polymer can be seen.



Scheme 1. Overall reaction mechanism for the synthesis of hyperbranched poly(2-oxazoline)s.

Table 1
List of hyperbranched polymers prepared *via* CROP along with the BisOx/EtOx ratios, average $g'_{(n)}$, and cloud point onset point.

Polymer	Base (eq)	Gelation	EtOx (eq)[a]	BisOx (eq)[a]	$M_{n,GPC}$ (kDa)[b]	Đ[b]	BisOx/ EtOx ratio	$g'^{(c)}_{(n)}$	Cloud point (°C)[d]
P1	0	No	100	4	22.0	170	0.04	0.87	66
P2	0	No	100	6	19.8	106	0.06	0.75	62
P3	0	Yes	100	12	N.D.	N.D.	0.12	N.D.	N.D.
P4	0	No	200	6	11.0	17	0.03	0.94	70
P5	TEA (1)	No	66	3	4.9	12	0.05	0.90	66
P6	TEA (1)	No	53	3	3.2	3	0.06	0.83	60
P7	TEA (1)	No	60	5	3.7	8	0.08	0.80	51
P8	TEA (1)	No	57	6	4.7	52	0.11	0.82	44
P9	TEA (1)	No*	39	5	3.2	29	0.13	1.18	47
P10	TEA (0.5)	Yes	60	6	N.D.	N.D.	0.10	N.D.	N.D.
P11	TEA (5)	No	43	5	N.D.	N.D.	0.12	N.D.	N.D.
P12	DPA (1)	No	100	7	7.5	62	0.07	0.85	55
P13	DPA (1)	No	72	6	5.3	75	0.08	0.78	60
P14	DPA (1)	No	38	4	9.1	27	0.11	0.73	47
P15	IPA (1)	Yes	90	9.6	N.D.	N.D.	0.11	N.D.	N.D.
P16	DIPEA (1)	Yes	56	6	N.D.	N.D.	0.11	N.D.	N.D.

(a) as calculated by NMR spectroscopy. Note that for gelled samples, values are calculated from T_0 samples. For the others, these are the amounts incorporated into the polymer. **(b)** as calculated from refractive index from conventional GPC. (**c**) as measured by advanced viscometry GPC. (**d**) as measured by UV–vis turbidimetry. ***P9** was at the limit of solubility and was likely bordering on a hydrogel structure.

3.1. Calculation of the Zimm Branching Factor, g′

A useful value to compare the degree of branching between polymers is the Zimm Branching Factor, or g′. [33] This branching factor is the ratio of the intrinsic viscosity (IV) of a linear, non-branched reference (as calculated by viscosity GPC) with a branched polymer at each slice of the GPC chromatogram. As g′ tends towards 1, the degree of branching in the branched polymer reduces to that of a linear sample at the same molecular weight, i.e. there is no branching present. As g′ tends towards 0, the branching increases *ad infinitum*. To calculate g′, a linear reference had to be synthesised to compare to the branched polymers. It must be noted that for comparison between the linear reference and the branched polymer, a line of best fit was generated from the linear reference and extrapolated across the whole molecular weight range of the branched polymers. This is because the branched polymers synthesised here had much larger molecular weights than any linear poly(2-oxazoline) possible by conventional CROP of 2-oxazolines. There is a known chain transfer reaction that occurs for between 1 in 200 to 1 in 800 repeat units for the CROP of poly(2-oxazoline)s resulting in a branched structure, which becomes an issue at high molecular weights, and so high molecular weight poly(2-oxazoline)s that are truly linear are not achievable. [34].

The Zimm branching factor was calculated across the whole molecular weight range and then a mean value was obtained to give an average of the amount of branching present – $g'_{(n)}$. It must be noted that small deviations in the line of best fit can drastically change the perceived amount of branching, which is a disadvantage to this approach. Nonetheless, this method is a suitable approach for relative comparison of branched polymers as long as all the polymers are analysed in the same manner. The line of best fit extrapolated from the linear reference can be seen in Fig. 1**A**, along with a branched polymer to highlight the lower overall viscosity of the branched polymer compared to the linear sample. From Fig. 1**A,** it is evident that **P1** has lower viscosity than the linear reference and thus contains more branching points across the whole polymer. In Fig. 1**B** the plot of g′ as a function of logM can be seen for **P1**, showing a decrease in g′ as logM increases. i.e. as the branched polymer gets larger, the amount of branching it contains increases. The red dashed lines indicate sections of the plots that were cut off for the $g'_{(n)}$ calculation. The reason for this cut-off was because the low polymer concentration at the extremities caused noise in the plots resulting in data that was erroneous.

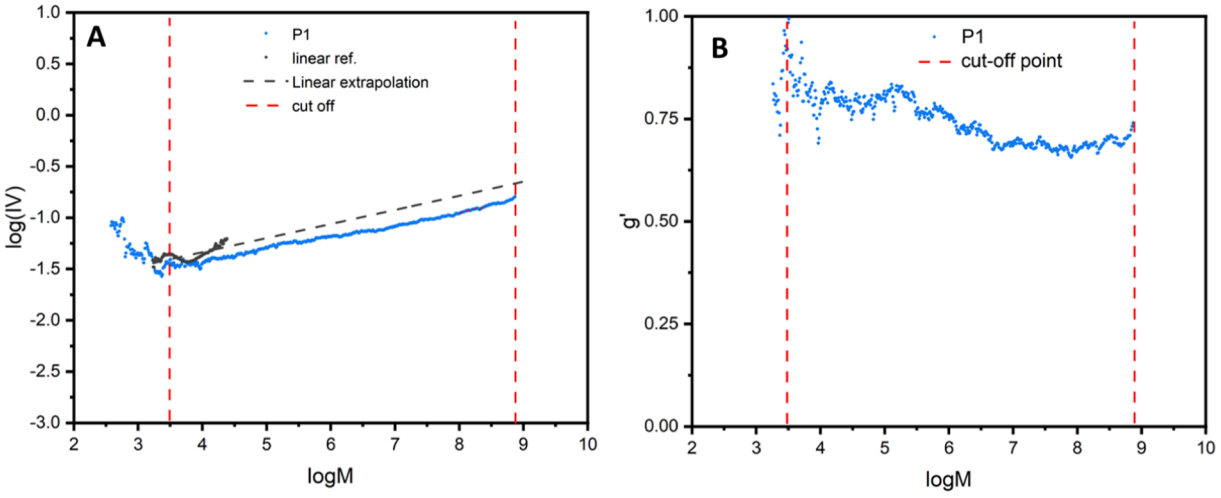


Fig. 1. (A) Mark-Houwink plot of **P1** overlaid with the linear reference. **(B)** Shows g′ as a function of logM. Red dashed lines indicate cut-offs for low concentration extremities. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Branched polymers with no end capping agent

To begin the discussion on branched polymers, a series of three polymers were synthesised with an increasing ratio of bis-oxazoline (BisOx) to 2-ethyl-2-oxazoline (EtOx) (**P1**-**P3**). The EtOx degree of polymerisation (DP) was set to 100, and the equivalents of the BisOx cross-linker were increased from 4 to 12 equivalents. **P1** had the lowest amount of cross-linker and had a $g'_{(n)}$ value of 0.87. Once the amount of cross-linker had been increased from 4 eq (**P1**) to 6 eq (**P2**) the $g'_{(n)}$ value decreased from 0.87 for **P1** to 0.75 for **P2**, corresponding to an increase in branching and associated drop in intrinsic viscosity. Next, the cross-linker amount was again increased from 6 eq to 12 eq (**P3**), increasing the BisOx/EtOx ratio from 0.06 to 0.12. This increase in cross-linker resulted in a gel forming that could not be analysed further by ^{1}H NMR spectroscopy and GPC. It should be noted that for the gelled polymers, the BisOx/EtOx ratio was determined from the NMR spectroscopy T_0. Two example gels can be seen in Fig. 2. Fig. 2**A** shows a desolvated polymer that was brittle and had poor viscoelastic properties. Fig. 2**B** shows a solvated hydrogel that had poor structural integrity and was easily broken up.

Finally, to examine how increasing the amount of EtOx to change the BisOx/EtOx ratio affected the amount of branching, the equivalents of EtOx were doubled from 100 (**P2**) to 200 (**P4**). This halved the BisOx/EtOx ratio from 0.06 to 0.03 and had the result of driving the $g'_{(n)}$ upwards to 0.94 and reducing $M_{n(GPC)}$ by approximately half. This high value of $g'_{(n)}$ is indicative of a minimal amount of branching, the lower observed $M_{n(GPC)}$ and $Đ$ are likely due to the reduced amount of branching. Nonetheless, of all the polymers investigated, **P1** and **P2** had the largest $M_{n(GPC)}$ values, and the highest values for $Đ$ indicating a lack of control when no terminating agent was used.

3.3. Triethylamine as a terminating agent

In order to try to maximise the amount of branching whilst retaining solubility, a terminating agent was added at the beginning of the reaction in an attempt to prevent uncontrolled cross-linking and gel formation. Here, inspiration was provided by the Strathclyde method of branched polymer formation where a chain transfer agent is added to suppress cross-linking. In order to prevent complete termination of all polymer chains by the end-capping agent, two factors needed to be considered. Firstly, the amount of terminating agent needed to be tuned so as not to immediately terminate all living chain ends, but also sufficient amounts needed to be added to suppress gelation. Secondly, a terminating agent needed to be chosen that would react slowly enough to ensure polymer formation, but not so slowly that gelation occurred. For this reason, triethylamine (TEA) was selected as a sterically hindered base. The ^{1}H NMR spectra of T_0 and T_{Final} for **P5** can be seen in Fig. 3.

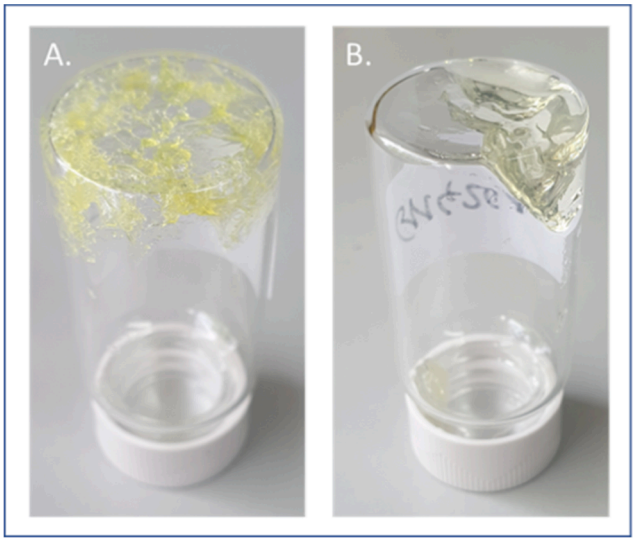


Fig. 2. (A) Hydrogel (**P3**) with solvent removed **(B)** Solvated hydrogel (**P3**).

To investigate the effect of adding TEA as a terminating agent at the start of the polymerisation, a series of polymers with increasing BisOx/EtOx were synthesised (**P5**-**P9**). For this series, the BisOx/EtOx ratio was increased from 0.05 to 0.13 whilst keeping the amount of end-capper at 1 equivalent. From **P5** to **P8**, the $g'_{(n)}$ value decreased from 0.9 to 0.82, indicative of an increase in branching. The $M_{n(GPC)}$ values are also noticeably reduced compared to **P1**-**P4** and the dispersity of the polymers is much lower. Although branched polymers clearly form, the monomer conversion is low (see **Table S2**, ESI) showing that TEA does end-cap quite effectively. Nevertheless, the branched polymers do form, with decreasing $g'_{(n)}$ values correlating with increased end-capping. The BisOx/EtOx ratio was then increased to 0.13 (**P9**), which was higher than **P3** which formed a gel. However, the solubility of **P9** in THF was extremely poor, which is reflected in the calculated $g'_{(n)}$ value of 1.18. This value is not possible, as it would mean the polymer has less branching than linear pEtOx. Interestingly, the branched polymers tended to be more easily soluble in water than THF.

As TEA was shown to be an effective terminating agent for the branched polymers described here, further reaction optimisation was continued with TEA. As previously mentioned, the amount of end-capping agent is an important factor to consider, and was the next parameter explored. Here, two polymers were synthesised with a targeted BisOx/EtOx ratio of 0.10. For **P10**, 0.5 equivalents of TEA were used, and 5 equivalents were used for **P11**. For **P10**, a gel was formed despite the BisOx/EtOx ratio of 0.10, which was lower than for **P9** (0.13) which formed a soluble sample. Therefore, the values provided are estimates given that the amount of each monomer incorporated in the gel are unknown. Clearly, 0.5 equivalents of end-capper were not enough to control end-capping in this system. Meanwhile, for **P11**, there was no monomer conversion at all as measured by ^{1}H NMR spectroscopy. The high quantity of TEA added terminates the living polymer very effectively halting any monomer conversion. Thus, there is a middle ground to be found for the amount of end-capper. Too much prevents polymerisation whereas too little results in gelation. It must be noted that the amount of terminating agent required for a specific system will likely depend on many factors, including the monomer, reaction concentration, end-capping agent used, and amount of cross-linker.

3.4. Diisopropylamine as a terminating agent

Once the limits of branching had been reached with TEA as the end-capper, the conditions were repeated with diisopropylamine (DPA) as the terminating agent. A branched polymer with a BisOx/EtOx ratio of 0.07 was synthesised (**P12**). This was similar to the BisOx/EtOx ratio of **P6** (0.06). These results were reflected in the similar $g'_{(n)}$ values of 0.85 (**P12**) and 0.83 (**P6**). **P12** had a much higher $M_{n(GPC)}$ of 7.5 kDa compared to 3.2 kDa for **P6** however. Observing the Mark-Houwink plot in Fig. 4, it can be seen that both polymers have similar intrinsic viscosities and thus similar amounts of branching up to around log6, which was the maximum size of **P6**. **P12** reached much larger molecular weights than **P6.** However, with some species with molecular weights above log8. The reason for this is likely due to the higher monomer conversion for **P12** compared to **P6**. **P12** used a higher DP of EtOx at the start of the reaction and this could be one possible reason for the larger size polymers. Also, the monomer conversion is generally higher for polymers using DPA as the terminating agent compared to TEA. This suggests that DPA is not as effective as end-capping as TEA although the reasons for this is not clear.

Next, the BisOx/EtOx ratio was then increased from 0.07 for **P12** to 0.08 for **P13**. This resulted in a reduction in $g'_{(n)}$ from 0.85 to 0.73 for **P12** to **P13** respectively. Next, the BisOx/EtOx ratio was increased to 0.11 for **P14** which resulted in a further decrease in $g'_{(n)}$ to 0.73, the

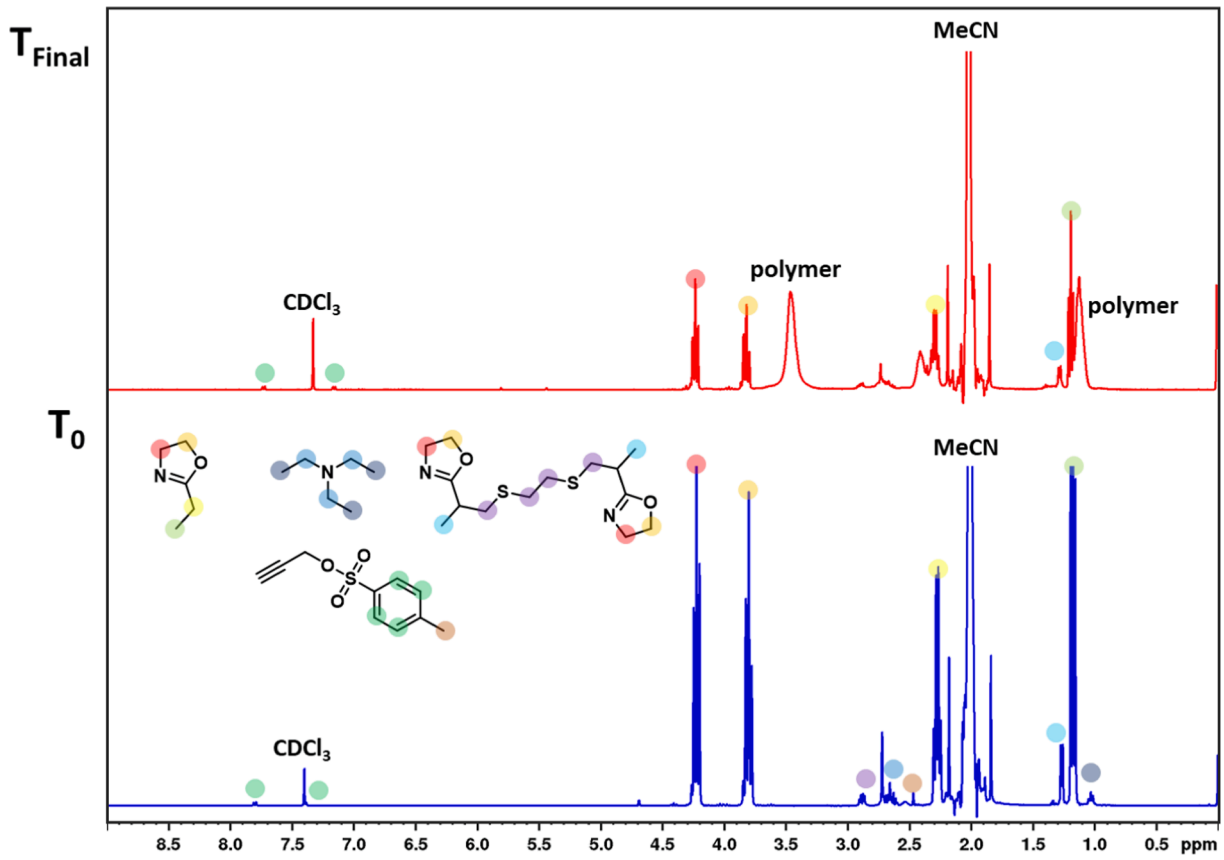


Fig. 3. ^{1}H NMR spectra of **P5**. The blue trace indicates T_0, whilst the red trace indicates T_{Final}. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

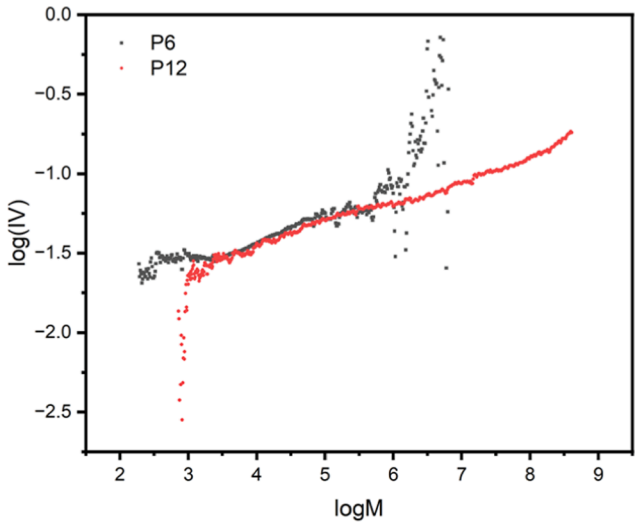


Fig. 4. Mark-Houwink plot comparing **P6** and **P12**.

lowest value of the entire set. Regarding the poor solubility of **P9**, it is likely that the highest molecular weight, most branched polymers in the sample were the least soluble and so were filtered out of the GPC sample resulting in the higher than expect $g'_{(n)}$ value. The poor solubility of the sample could result in column interactions within the GPC causing the Mark-Houwink plot to be misrepresented.

All the polymers end-capped with DPA had higher $M_{n(GPC)}$ values and dispersities when compared to TEA. Also, the monomer conversion for these polymers was much higher. These results suggest that DPA terminates less effectively than TEA, allowing for higher monomer conversion and larger polymers.

3.5. Testing other terminating agents

As well as TEA and DPA, the non-nucleophilic base diisopropylethylamine (DIPEA) was selected, and 2-propanol (IPA) was chosen as a poor nucleophile. Initially, 1 equivalent of each end-capper was tested (**P15** and **P16**). The BisOx/EtOx ratio was increased to 0.11, which was chosen to ensure gelation under normal circumstances without the presence of an end-capping agent. Nonetheless, gelation was seen for both **P15** (IPA) and **P16** (DIPEA). When TEA and DPA were used as terminating agents and the BisOx/EtOx ratio was 0.11 or higher (**P9**, TEA and **P14**, DPA) soluble branched polymers formed. This suggests that DIPEA is too sterically hindered to terminate chains. These important results suggests that TEA does end-cap polymer chains slowly whilst DIPEA does not. Furthermore, this result demonstrate that when preparing carboxylates for end-capping poly(2-oxazoline)s, DIPEA is a better choice of base than TEA. Using TEA will likely result in a mixture of chain ends that are partially terminated with the carboxylate, whilst others are terminated with TEA. This could be the reason for low end capping efficiencies where TEA and 2-bromo-2-methylpropionic acid are used as an end-capping mixture. [35].

3.6. Correlations between $g'_{(n)}$, cloud point, and BisOx/EtOx ratio

To study the relationships between the BisOx/EtOx ratio, $g'_{(n)}$ value, and cloud point, three scatter plots were constructed (Fig. 5). It must be noted that due to the poor solubility of **P9**, the measured values of $g'_{(n)}$ and the cloud point were affected. Nonetheless, it has been kept for observation in each graph, and can be seen highlighted by the red circle.

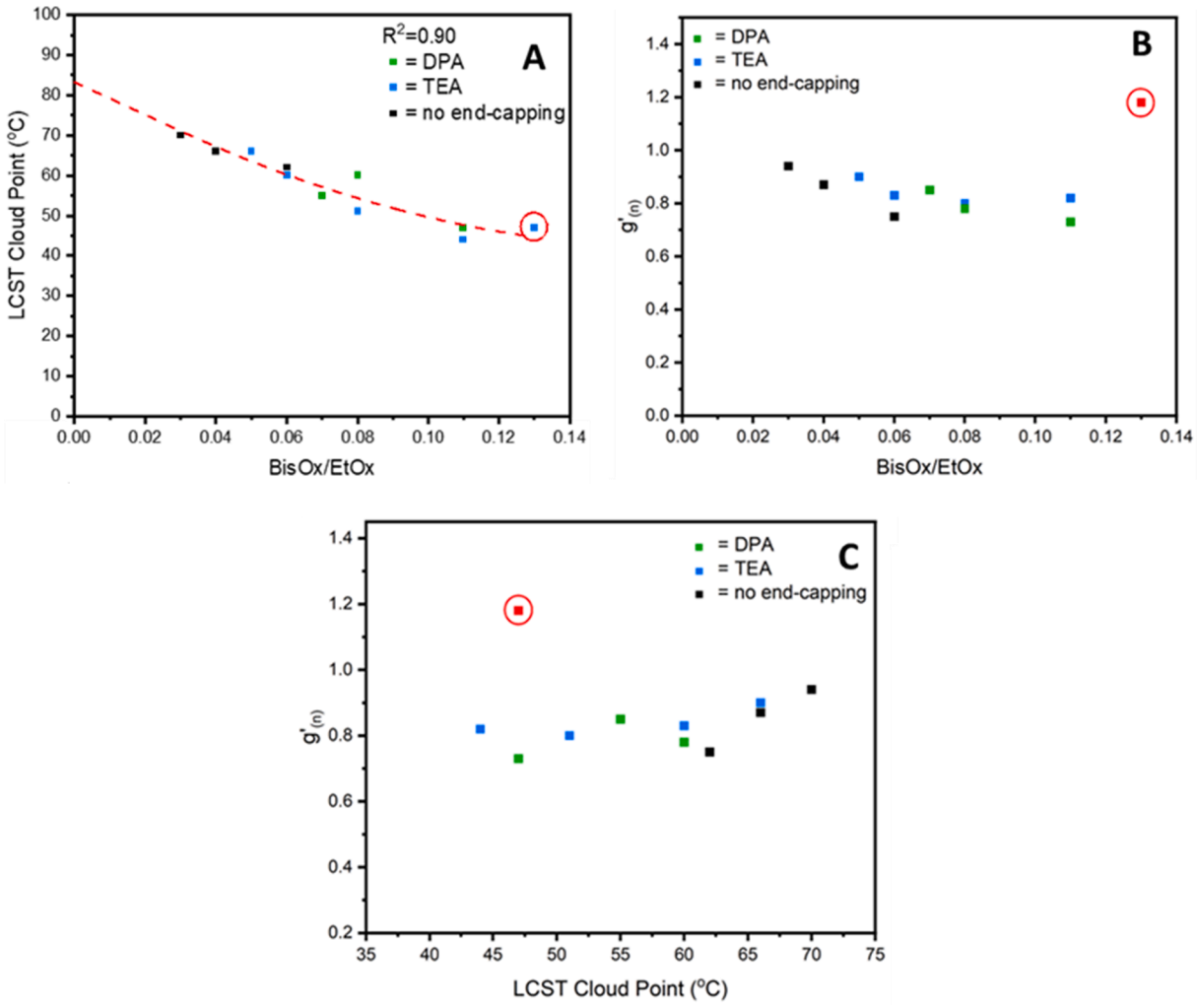


Fig. 5. Scatter plots of **(A)** cloud point vs BisOx/EtOx ratio **(B)** BisOx/EtOx ratio vs $g'_{(n)}$. **(C)** g' value vs cloud point.

Furthermore, each point has been coloured depending on the type of terminating agent used.

In Fig. 5**A**, the scatter plot of BisOx/EtOx ratio vs cloud point has been plotted. A polynomial line of best fit has been derived that shows reasonable correlation between the two variables, with an R^2 value of 0.90. Interestingly, the cloud point appears to begin to plateau at around 50 °C once the BisOx/EtOx ratio reaches above 0.1, suggesting that further addition of cross-linker will not reduce the cloud point further. Extrapolating the fit to the y axis allows for prediction of the cloud point for linear pEtOx, suggesting that it is around 85–90 °C, which is an excellent fit for DP 100–150 pEtOx according to literature data. [36] It should be noted that it is not clear as to whether the decrease in cloud point is due to branching, the addition of the more hydrophobic BisOx monomer, an increase in molecular weight, or a combination thereof.

In addition, the BisOx/EtOx ratio has been plotted against $g'_{(n)}$. As can be seen in Fig. 5**B** the branched polymers with no end-capping have the highest $g'_{(n)}$ values and lowest BisOx/EtOx values, indicating that they have the least amount of branching of the set, despite having the highest $M_{n(GPC)}$ values. There is no apparent difference between the amount of branching between polymers end-capped with DPA and those with TEA, however. There is a good trend with decreasing $g'_{(n)}$ and increasing the BisOx/EtOx ratio for the polymers with no added terminating agent, however when a terminating agent is added the trend is not as clear.

Fig. 5**C** shows the relationship between $g'_{(n)}$ and cloud point. As $g'_{(n)}$ increases, the cloud point increases alongside it. It may be possible to synthesise a hyperbranched poly(2-oxazoline) that has an cloud point of around body temperature. Hyperbranched polymers can be used for drug delivery, [37–38] and so a hyperbranched polymer with thermoresponsivity at around body temperature is an exciting proposition because it could be used for targeted drug delivery.

4. Conclusion

In conclusion, hyperbranched poly(2-oxazoline)s have been synthesised for the first time using a bis-oxazoline cross-linker. Furthermore, a novel approach was taken as demonstrated by the addition of end-capping agents at the beginning of the polymerisation in order to control the degree of cross-linking. Various parameters were explored including the type of end-capping agent, amount of end-capping agent, and variation in the mono-functional monomer and bis-functional cross-linker ratio. The amount of cross-linking was shown to have an effect on the cloud point of the polymers, with more cross-linking reducing the cloud point until a minimum at around 45 °C was reached. Advanced GPC was used with great effect to probe the branched structure of the polymers, and correlations were drawn between the BisOx/EtOx ratio, average Zimm branching factor $g'_{(n)}$, and the cloud point onset. Future work would be to explore different end-capping groups and attempt to reduce the cloud point further to around body temperature. Drug encapsulation potential would be a useful application for these polymers and so analysing this would be beneficial.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eurpolymj.2022.111678.

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