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A mannosylated PEI-CPP hybrid for TRAIL gene targeting delivery for colorectal cancer therapy

ARTICLE

ε-Caprolactone Derived 2-Oxazoline Inimer for the Synthesis of Graft Copolymers

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An inimer approach is presented here for the formation of bottlebrush copolymers consisting of a poly(2-oxazoline) backbone and acrylate branches. A hydroxyl group containing 2-oxazoline (2-n-pentanol-2-oxazoline) was synthesized from ε-caprolactone, which is a sustainable starting material. 2-n-Pentanol-2-oxazoline was then further functionalized with a bromo isobutyrate group, which was used to initiate a Cu(0)-mediated Reversible-Deactivation Radical Polymerisation (RDRP) of acrylates. This compound is termed as "inimer" because it contains both a monomer (2-oxazoline) and an initiator (RDRP initiator in this study). Herein, we report the use of inimer to form 2-oxazoline based polymer backbones through cationic ring opening polymerisation and further polymerisation via Cu(0)-mediated RDRP of acrylates was achieved by initiating from the RDRP-initiator sites embedded within the poly(2-oxazoline) backbone. Furthermore, statistical copolymers of 2-ethyl 2-oxazoline and the inimer was also formed leading to statistically distributed radical initiating sites, which were then used to form graft copolymers of varying densities.

Introduction

Poly(2-oxazoline)s (POx) have been widely studied for its versatile application including its use as: an antifouling agent[1], a biomaterials[2], and a means for drug delivery[3,4]. It is becoming of increasing importance, especially in the biomedical field, displaying biocompatibility and stealth behavior.[1,4,5] Through cationic ring opening polymerisation (CROP), 2-oxazolines can be polymerized in a "living" manner and depending on the chain length and/or functional group attached to the oxazoline ring, a wide range of hydrophobic, hydrophilic, thermoresponsive polymers can be synthesized. Although 2-oxazoline based copolymers have been investigated for these various properties[6] and self-assembly behaviors[7], the combination of 2-oxazolines and other monomers, such as acrylates, is also of great interest. By combining two different types of monomers through CROP and controlled radical polymerisation techniques, multiple functionalities can be incorporated, resulting in a wider range of functional monomer combination than when using one polymerisation technique.

Graft copolymers comprise of a polymeric backbone with side chains (branches) and in the case of bottlebrush copolymers, it consists of branches very densely grafted onto the backbone. In biology, these macromolecular structures are often present, with examples including lubricin[8–10] in joint lubrication, and proteoglycans[11–13]. Bottlebrush copolymers have been used for drug delivery where it has been reported

that they display multivalency as well as extended in vivo circulation time in comparison to its linear analogues. Bottlebrush copolymers, therefore, show advantageous features and so they have been reported extensively.[8,14–18] However, the combination of 2-oxazolines and acrylates as a graft copolymer has seldom been reported, with even fewer reports on 2-oxazoline backbone and acrylate brush combination.[19–23]

Graft copolymers can be synthesized from three synthetic routes: grafting to, grafting through, and grafting from. While each method is associated with their own advantages and disadvantages,[24,25] the grafting from approach, the chosen synthetic route in this research, can give superior control over grafting density.
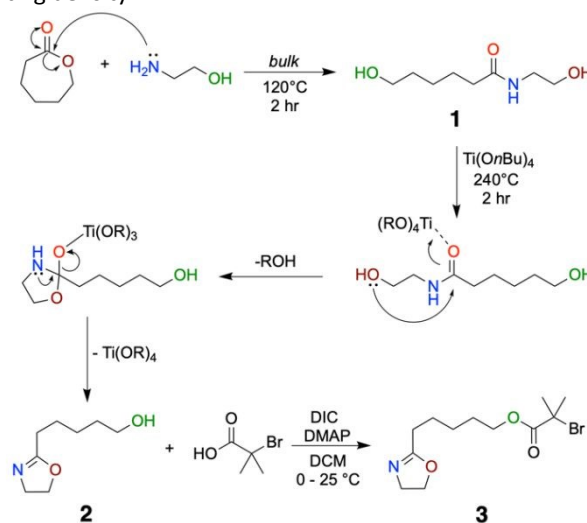
Scheme 1: Three step synthetic approach to Cu(0)-mediated RDRP inimer where: structure **1** is the amide precursor, **2** is the hydroxyl oxazoline and **3** is the desired inimer structure.

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In this study, we demonstrate a synthetic approach to a novel inimer structure from ε-caprolactone, a deviation from the conventional 2-oxazoline synthetic routes (**Scheme 1**). Lactones are a class of cyclic carboxylic esters that can also undergo ring opening polymerisation.[27,28] Poly(caprolactone)s are both biocompatible and biodegradable which has garnered much interest as a method for controlled drug delivery.[29] However, ε-caprolactone, or other members of the lactone family, has not been previously investigated for formation of inimer structures. Based on previous reports[30,31] a hydroxyl functional group containing oxazoline was designed from a commercially available and sustainable starting material ε-caprolactone. To the best of our knowledge, this is the first example of the use of a caprolactone derived hydroxyl oxazoline to be used to form a functional inimer. From this, highly dense ridged bottlebrush copolymers were formed as well as flexible brushes through the copolymerisation of the inimer with non-initiating group containing 2-oxazoline monomers.

Results and Discussion

Synthesis of inimer from ε-caprolactone

A three-step synthetic strategy was developed to prepare the inimer structure. As depicted in **Scheme 1**, the first step is amidation of ε-caprolactone through nucleophilic attack of the 2-aminoethanol which occurs in bulk conditions (Structure **1** in **Scheme 1**). In 2 hours, based on the ^{1}H NMR analysis, we observed a quantitative conversion to an amide structure (**Figure 1, top**). Subsequent addition of catalytic amounts titanium (IV) butoxide at 240 °C lead to ring closing around the amide according to the mechanism as outlined in **Scheme 1**, where coordination between the carbonyl group and titanium complex assists in the ring closure reaction. Distillation of the product yielded the purified hydroxyl oxazoline monomer (**Figure 2, middle**). This two-step synthetic route to the functional oxazoline can be done in the absence of any solvent and can be synthesized from readily available starting materials in a moderately scalable manner (>30 g). However, the multiple distillations required (in this case four) to remove residual impurities and by-products (butanol and water) significantly lowers the overall yield through the course of the purification procedure (18.2 % yield overall). Although, multiple distillations are not necessarily required, we have decided to obtain 2-n-pentanol-2-oxazoline at its highest purity as any impurities could lead to further unwanted side reactions in the third step. Compared to the thioether linked oxazoline, as previously demonstrated by our group[20], it is a less toxic and easily scalable procedure owing to the cheaper and non-toxic nature of the starting materials that is caprolactone.
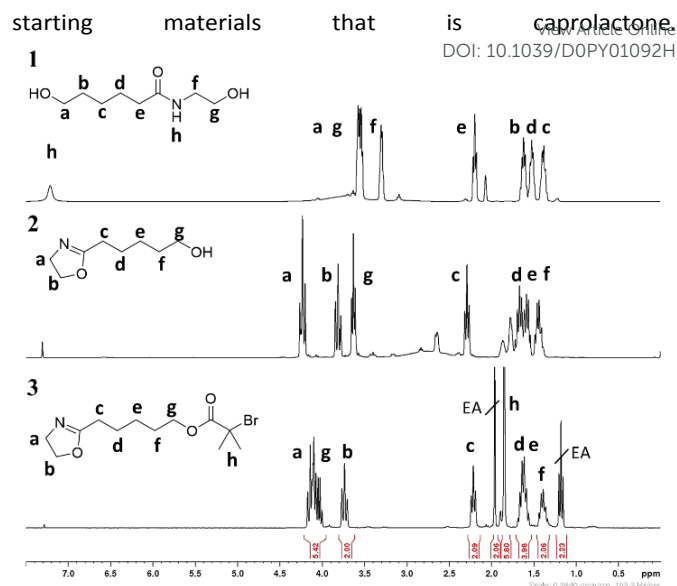

Figure 1: ^{1}H NMR of structure **1** amide intermediate (*top*), structure **2** 2-n-pentanol-2-oxazoline (*middle*), structure **3** inimer (*bottom*). EA denotes Ethyl Acetate as a solvent.

The final step of the synthesis is a Steglich esterification reaction using α-bromoisobutyric acid and 4 *N,N'*-Diisopropylcarbodiimide (DIC) as the coupling reagent. It should be noted that esterification step directly using α-bromoisobutyryl bromide causes ring opening of the 2-oxazoline as well as the ester formation. Therefore, a DMAP catalyzed acid-alcohol coupling reaction was performed in order to obtain the desired product **3,** which was finally purified by column chromatography **(Figure 1, bottom)**. This three-step synthetic route to the inimer from caprolactone was scaled up to a 20 g scale.

Polymerisation of oxazoline backbone via CROP

An initial CROP of inimer **3** (In) alone using the initiator methyl tosylate was carried out. Alkyl halides are known initiators of CROP[32] and it was also reported by our group that a similar inimer structure can self-initiate even at 80 °C, significantly lower than the standard CROP temperature of 140 °C.[20] This could be associated with the electrophilicty of the tertiary alkyl halide also present in the inimer structure in this research, which could cause initiation of the oxazoline and lead to unwanted hyperbranched structures. Therefore, a lower reaction temperature of 60 °C was chosen for the polymerisation of the backbone by CROP. A short DP of 10 was initially targeted and resulted in **P1** (DP = 10) with a dispersity of Đ = 1.32 ($M_{n, SEC}$.= 2200 Da,) Detailed kinetics of this reaction showed that this polymerisation followed first order kinetics and the calculated k_{app} was determined to be 1.231 ×10^{-4} s^{-1}. Subsequent attempts at increasing the length of the backbone (**P2, P3**) resulted in larger molecular weight polymers but as higher chain lengths were targeted (in the case of **P3**, DP =100, Đ = 2.20) **(Figure S1)**, it resulted in broader dispersity, due to the extended reaction time.

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Consequently, higher molecular weight polymers were investigated through copolymerisation with a comonomer that did not contain other functionalities such as 2-ethyl-2-oxazoline (EtOx). The thioether linker containing inimer structure we previously reported showed narrow dispersity for block copolymers of EtOx and inimer, but statistical copolymers of the two led to undesirable polymerisation reactions leading poorly defined polymers.[20] In that report, it was concluded that due to the susceptibility of EtOx by the tertiary alkyl bromide of the inimer, it led to the polymerisation of the monomers both by the initiator and the tertiary alky bromide of the inimer. However, upon copolymerisation of the inimer presented in this research (In) with EtOx at a ratio of 25:25 (**P5**), well defined polymers were synthesized even at higher molecular weights (**Figure 2f**). The main difference between the two inimer structures is that of the methyl group adjacent to the oxazoline ring and the thioether linker connecting the oxazoline ring and the radical initiating group, both of which are not present in the inimer structure reported here. These results led to the conclusion that the poor polymers shown using the thioether linker containing inimer structure was due to the methyl group adjacent to the oxazoline ring, which can change the nucleophilicity of the monomer and hence change the

reactivity.[33] In addition, the sulfur could act as a nucleophile, attacking the oxazolium group, leading to unwanted termination reactions.[34] Therefore, In can be considered to be the more desirable inimer structure because of the less toxic starting materials required for its synthesis, better stability(thioether bonds are prone to oxidation[35]), and statistical copolymerisation with other 2-oxazoline monomers.

The differing reactions rates of EtOx and In at 60 °C, with the inimer polymerizing more slowly than EtOx (**P4, Figure 2a**) suggested that copolymerisation of these two monomers could form a gradient copolymer structure. Further investigation of the reaction kinetics of each component through ^{1}H NMR of **P5** (PEtOx$_{25}$-r-PIn$_{25}$), showed similar rates of reaction during copolymerization (P(EtOx$_{25}$-r-In$_{25}$), $k_{app,EtOx}$ = 2.404 ×10^{-5} s^{-1}, $k_{app,In}$ = 2.304 ×10^{-5} s^{-1}) (**Figure 2d**). This similar reactivity rates would indicate that the inimer is statistically distributed along the backbone rather than in a gradient or block copolymer form. Different ratios of In and EtOx were polymerized **P6,** (P(EtOx$_{40}$-r-In$_{10}$), $k_{app,EtOx}$ = 2.562 ×10^{-5} s^{-1}, $k_{app,In}$ = 2.588 ×10^{-5} s^{-1}) (**Figure S6**) and have suggested at reaction rates of EtOx and In were very similar in each case.
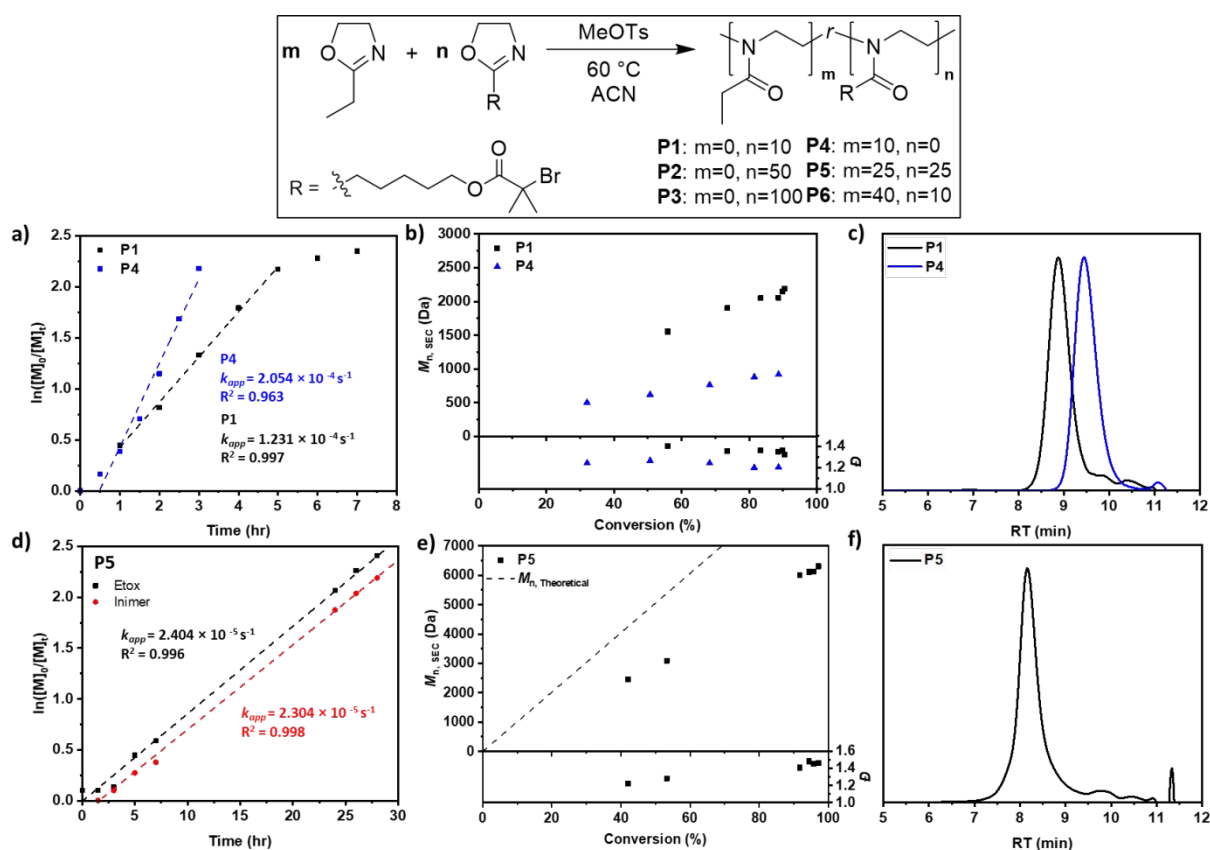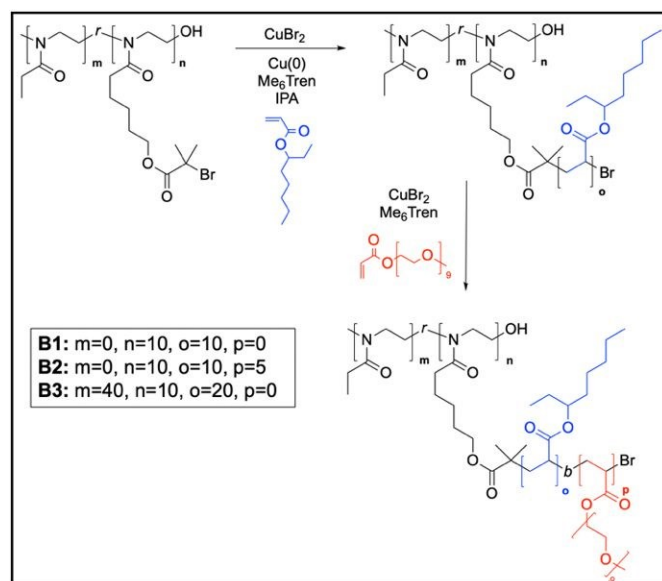
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Figure 2: CROP of In where: a) kinetics studies of **P1** and **P4** at 60 °C, b) M$_{n,SEC}$ values and corresponding dispersity values of **P1** and **P4**, c) SEC traces of **P1** (PIn$_{10}$) and **P4** (PEtOx$_{10}$) d) kinetics of each component in **P5**, e) M$_{n, SEC}$ values and corresponding dispersity values, f) SEC trace of copolymer **P5.**

Scheme 2: Polymerisation scheme of 2-ethyl hexyl acrylate using brush initiators **P1** and **P6** for **B1**-**B2** and **B3**, respectively. Where: m denotes number of non-initiating monomers, n is the number of initiating sites, o and p are brush length.

Brush synthesis via Cu(0)-mediated RDRP

After establishing the scope of the polymerisation of In through a systematic investigation of backbone length and brush initiator density, graft copolymers were synthesized using various acrylates (**Scheme 2**). Under a well-established Cu(0)-mediated RDRP system in organic media (where: [monomer]/[In]/[Me$_6$Tren]/[CuBr$_2$] = DP/1/0.18/0.05, and 5 cm of pre-activated copper wire), 2-ethyl hexyl acrylate (EHA) was primarily used for brush formation, firstly using **P1** as the brush initiator.

With a Cu(0)-RDRP initiating group on every repeating unit of the backbone, it formed a very short, dense graft copolymer of EHA, **B1**.
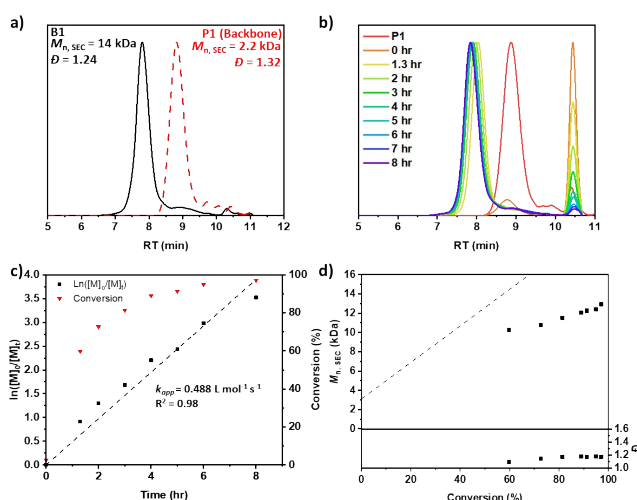

Figure 3: Analysis of bottlebrush copolymer PIn$_{10}$-*brush*-PEHA$_{10}$ (**B1**) where a) SEC traces of bottlebrush copolymer compared to linear backbone b) evolving SEC traces of **B1** over time c) kinetics of **B1** d) $M_{n, SEC}$ and corresponding dispersity values.
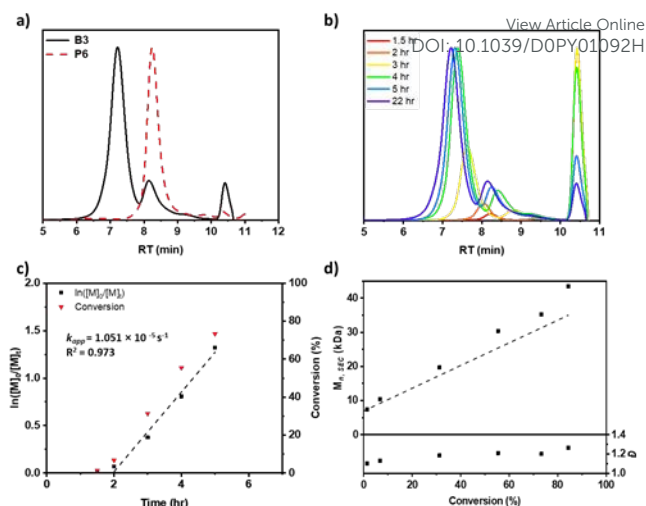

Figure 4: Analysis of bottlebrush copolymer **B3** where a) SEC traces of bottlebrush copolymer compared to linear backbone b) evolving SEC traces of **B3** over time c) kinetics of **B3** d) $M_{n, SEC}$ and corresponding dispersity values.

Comparing the SEC traces of the backbone (**P1**) to the graft copolymer there was a significant shift from **P1** and **B1**, showing a molecular weight of 14 kDa (**Figure 3**). The end group fidelity, or "livingness" of chain end, was investigated through the synthesis of bottlebrush block copolymerisation with oligo(ethylene glycol)acrylate (OEGA, M_n=480 Da) (**B2**). **P1** was used as a brush initiator and as was in the case for **B1** (PIn$_{10}$-*brush*-PEHA$_{10}$). EHA was polymerized under standard Cu(0)-mediated RDRP conditions in IPA. Once near-full conversion of EHA was confirmed, the second brush block was polymerized using OEGA. In order to maintain high conversion of the second block[36], an additional portion of deactivator (Cu$_2$Br) and ligand (Me$_6$Tren) was added. This yielded in near full conversion of the second monomer (**B2**, $M_{n, SEC}$ = 28 kDa, Đ = 1.25) (**Figure S9**)

The inimer backbone structure was further investigated by grafting from a copolymer of EtOx and In. We have established that copolymerisation of EtOx and In results in well-defined copolymers with the radical initiating sites distributed along the polymer backbone. Hence polymer **P6** was used to form graft copolymers, again by Cu(0)-mediated RDRP in organic media. This resulted in longer, less dense brush-like structures compared to when using the homopolymer of inimer as the brush initiator. Study of the reaction kinetics, however, showed an induction period of around 2 hours before the reaction showed any conversion. Although Cu(0)-mediated RDRP is known to have some induction period[37], the much longer induction period observed in **B3** compared to **B1** or **B2** could be due to relatively lower solubility of **P6** in the solvent, IPA (**Figure S10**). Nevertheless, the polymerisation reached to 77 % acrylate monomer conversion after 5 hours and SEC traces of the resulting graft copolymer showed residual oxazoline backbone polymer (**Figure 4**). Based on the GPC traces in Figure 4b, there is an indication that some polymer chains cannot initiate the brush polymerisation. For **B3**, only 20 % of the backbone has brush initiating sites, whereas in **B1**, 100 % of the backbone was brush initiating. Therefore in **B3**, where **P6** was used as the backbone (DP = 50, EtOx =40, In = 10), it is statistically possible

that there might be some chains where there are less than 10 initiating sites on the backbone, or in some cases, none. Although we showed that because of the similar rates of reaction between EtOx and In, the brush initiating sites were statistically distributed along the backbone, there is still a limit to precisely control over monomer location within the polymer.[38] Because of this statistical distribution of brush initiating and non-brush initiating monomers, it is difficult to have a uniform distribution of the brush polymer since the backbone itself is a distribution of linear polymers containing ten or less brush initiating sites. There is another possibility that may result in the formation of the low molar mass peak, which is the presence of small initiating groups such as 2-bromo isobutyrate. This compound may form upon hydrolysis of ester group in the inimer structure or might be left during the synthesis of the inimer, which the latter is not possible as that would have stopped the cationic ring opening polymerisation. We have looked into the 3 to 4 ppm region in the ^{1}H NMR of **P6** where if hydrolysis took place would show two different shifts of the $-CH_2$ peak adjacent to the ester group; one associated with the inimer structure, and one associated with that of the 2-n-pentanol-2-oxazoline. However, we could not identify a second peak that may belong to a hydrolyzed 2-bromo-isobutyrate compound.

These demonstrations of graft copolymer synthesis using In has shown that the Cu(0)-RDRP initiating group on the monomer does not interfere with the backbone polymerisation and in the case of **B1** can produce well defined graft copolymers. However, further structural elucidation through microscopy will be needed to confirm their brush-like structure.

Conclusion

To conclude, a synthetic route to a 2-oxazoline inimer was established whereby ε-caprolactone, a commercially available, non-toxic starting material, was used to from the hydroxyl 2-oxazoline precursor to form the inimer in a scalable manner. The synthesis of the monomer led to the formation of brush copolymers using the grafting from approach, first using CROP for the backbone and Cu(0)-mediated RDRP for the brushes. Kinetic studies were carried out on the CROP of the new monomer where it showed that polymerisation on its own or with a comonomer occurs is a "living" manner. Based on the monitoring of monomer consumption throughout the reaction, both EtOx and inimer showed similar rates of reaction indicating incorporation of In in a statistical fashion. Cu(0)-mediated RDRP using the inimer containing polymers with various acrylates resulted in well-defined graft copolymers.

Author Contributions

J.K. has performed all the experimental work. The project was designed, discussed, and written by the contributions of all authors.

Conflicts of interest

The authors declare no competing financial interests.

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