Lactone monomers obtained by enzyme catalysis and their use in reversible thermoresponsive networks

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ABSTRACT: Enzyme-catalyzed transformations have a great potential in both the pharmaceutical and chemical industry to achieve complex and (stereo)selective synthesis under mild reaction conditions. Still, the implementation of biocatalysis in the prerequisite upgrading of inert synthons into activated monomers for polymer applications has not yet been fully realized. In this contribution, we show that scaled-up synthesis of bicyclic norcamphor lactone using an engineered Baeyer–Villiger monooxygenase (BVMO) is feasible to reach complete conversion of the corresponding ketone in 24 h in shake-flask. The lactone monomer obtained by enzyme catalysis was copolymerized with ε-caprolactone *via* ring-opening polymerization to study the impact of the additional ring on material properties. Moreover, four-arm star-like, homo and block copolymers were designed from ε-caprolactone, ε-decalactone, and norcamphor lactone and characterized for their structural and thermal properties. These newly explored macromolecules were functionalized with furan rings using the enzyme *Candida antarctica* lipase B which allowed the formation of thermolabile networks *via* the pericyclic reaction with bismaleimide by means of Diels–Alder chemistry. The bonding/debonding state of these star-like based materials can be tuned by a suitable selection of thermal treatment. The temperature-dependent reversibility was assessed by thermal analysis and solubility test. Our results presented here shed light on the high potential of the use of chemoenzymatic approaches in the synthesis of new functional materials with tuned physiochemical properties. © 2020 The Authors. *Journal of Applied Polymer Science* published by Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2020**, *137*, 48949.

INTRODUCTION

In the last decades, innovative green catalytic and synthetic strategies are emerging in the field of organic chemistry to overcome stringent ecological and environmental hazards.[1] In particular, biocatalysis has appeared as a promising tool for sustainable generation of agro-chemicals, medicines, and fine chemicals.[2–4] Enzymes enable stereostereo and regioselective transformations under ambient reaction conditions.[5,6] Directed evolution and enzyme engineering provide access to biocatalysts with enhanced thermostability, activity, tailored specificity, and even "new-to-nature" reactivities.[7–10] Enzyme catalysis is consequently a promising tool in synthetic

organic chemistry and has a prodigious potential in chemical and pharmaceutical processes in enabling challenging transformations in an environmentally benign fashion.[11–15]
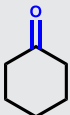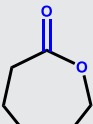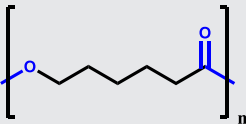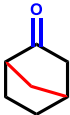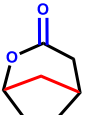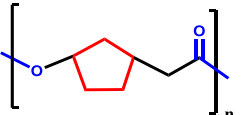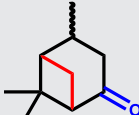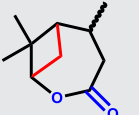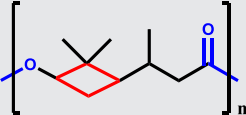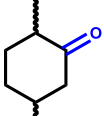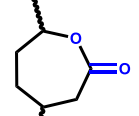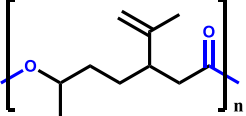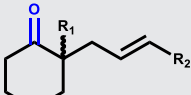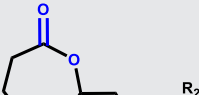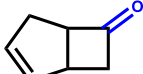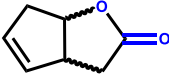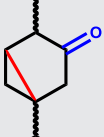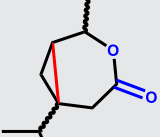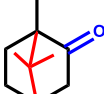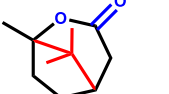
Recently, biocatalysis for polymer applications has attracted increasing interest.[16–22] Biocatalytic routes for production of monomers have been reported,[23–26] as highlighted for cyclic lactones and their corresponding ketone precursors in Table I. Schmidt *et al.* have reported an enzyme cascade based on combining an alcohol dehydrogenase with a Baeyer–Villiger monooxygenase to enable the efficient oxidation of cyclohexanol to ε-caprolactone in an aqueous one-pot

Table I. Biotransformation of Cyclic Ketones into Lactones by Baeyer–Villiger Monooxygenases and Their Polyester Forms [Color table can be viewed at wileyonlinelibrary.com]

Cyclic ketone substrate	Lactone formᵃ produced by enzyme catalysis	Polymer	References
Cyclohexanone	ε- Caprolactone	Polycaprolactone	26
Norcamphor	Norcamphor lactone	Poly(norcamphor lactone)	27-29 and this work (scale-up using engineered variant)
Verbanone	Verbanone lactone	Poly(verbanone lactone)	30
Dihydrocarvone	Dihydrocarvide	Polydihydrocarvide	25,31
Six-membered cyclic ketones bearing α-quaternary stereocenters	Seven-membered lactone bearing ε-quaternary stereocenters	n.r.	32
Bicyclo[3.2.0]hept-2-en-6-one	2-Oxabicyclo[3.3.0]oct-6-en-3-one	n.r.	33
Thujone	Thujone lactone	n.r.	34
Camphor	Camphor lactone	n.r.	34

ᵃ Only most substituted "normal" lactone product shown. n.r., to the best of our knowledge, not reported in literature.

process.[26] Furthermore, Morrill *et al.* showed the possibility of utilizing a biocatalytic venue in the synthesis of complex and bulky lactone structures.[32] The produced bulky cyclic lactones can serve as a precursor to polyesters, if the steric hindrance can be overcome by suitable selection of catalyst and reaction conditions.[25,34,35]

Polymers are essential components in textiles, cars, aeroplanes, and in sophisticated uses such as diagnostics, electronics, and tissue engineering.[36–38] For instance, lactone-derived polymers (polyesters) can be utilized in many areas, such as biodegradable materials, drug and gene delivery systems, scaffolds in tissue engineering, and regenerative medicine.[39–42] Lactones are considered as suitable starting materials for copolymerization and blending to generate biomedical devices with remarkable mechanical features and well-suited degradation kinetics.[39,40,43,44]

Star-like polymers, also referred to as star-shaped polymers, are promising in the polymer and material engineering field due to their well-defined structure and simplicity to regulate their surface functionality.[45–47] They are branched macromolecules comprising at least three linear polymer chains emanating from a central core.[45,48–50] The synthesis of star-shaped block polyesters using multifunctional initiator and different building blocks have been reported previously.[49,51,52] Block copolymers are composed of two, or more, different homopolymer segments, and can exhibit properties of each particular homopolymer from which they are originated as well as display unique features owing to the polymer structure as a whole.

Star-shaped polymers have been crosslinked to produce polymeric networks.[47,53] Network polymer materials have gained significant attention due to their exceptional mechanical and thermal properties.[54] Most thermosets are very problematic to recycle which is associated with ecological concern.[55] To overcome these challenges, stimuli-responsive polymers constitute one focus area in the field of polymer science and engineering.[56–60] A diverse range of chemistries has been explored to accomplish amendable polymeric networks, including disulfide metathesis, photo-dimerization, Diels–Alder (DA) and other fascinating chemistries.[57,60] Heat-induced polymer bonding/debonding can be accomplished by DA chemistry.[61–63] The DA reaction is an attractive member of the family of click chemistry, mainly due to its temperature-dependent reversibility, and its dependence on the actual diene/dienophile combination.[62,64–69]

In the current contribution, we target polymeric materials that can reorganize and reconstruct themselves by means of DA chemistry when stimulated by heat. We report a green route to convert the bicyclic ketone norcamphor ($C_7H_{10}O$) to its corresponding "normal" lactone *via* BV-oxidation under mild conditions and after 24 h, using an engineered variant of the cyclohexanone monooxygenase from *Acinetobacter calcoaceticus* (CHMO*Acineto* EC 1.14.13.22). Star-shaped poly(ε-caprolactone) (PCL) was synthesized and converted into star-shaped block copolymer using either ε-decalactone (ε-DL) or norcamphor lactone (NCL) as corepeating units. The conjunction of amorphous poly(decalactone) (PDL) or poly(norcamphor lactone) (PNCL) with the semicrystalline PCL resulted in the formation of soft and flexible materials. By utilizing the available end-functional hydroxyl groups, the four-arm star-shaped polymers (PCL-4OH, PCL-b-PDL-4OH, and PCL-b-PNCL-4OH) have been functionalized with a furan-derived building block to generate furan-capped star-shaped

polymers using *Candida antarctica* lipase B (CalB, E.C. 3.1.1.3) as biocatalyst. These materials form temperature amendable networks through the reaction with the conjugate dienophile bismaleimide (BMI) *via* a DA reaction. These newly investigated polymers showed a high potential for the design of thermolabile networks.

RESULTS AND DISCUSSION

Biocatalytic Route for Upscaled Norcamphor Lactone Synthesis

The Baeyer–Villiger reaction is a basal organic transformation to upgrade ketones into esters and lactones[70] which are important building blocks for the manufacturing of polyesters. The chemical Baeyer–Villiger process is typically dependent on peracids to generate the Criegee intermediate, which rearranges to yield the most substituted product.[71,72] In contrast to the chemically induced reaction, which is catalyzed by toxic and potentially explosive compounds, Baeyer–Villiger monooxygenases uses molecular oxygen as oxidant and produces water as side-product, thus representing a green transformation.[73,74] The substrate scope displayed by these fascinating biocatalysts can be broad; even α-substituted ketone substrates are accepted.[32,74] Efficient cofactor regeneration systems to recycle nicotinamide adenine dinucleotide phosphate ($NADP^+$) back to NADPH exist.[75]

The cyclohexanone monooxygenase from *A. calcoaceticus* (CHMO*Acineto*) is a member of the type I Baeyer–Villiger monooxygenase family.[28,76] The enzyme CHMO*Acineto* has been previously reported to accept an array of complex structures,[29,32,74,77] including norcamphor.[28,29,78] Using immobilized enzymes, Abril *et al.* reported the synthesis of norcamphor lactone on 80 mmol scale, in 81% yield and 5 days reaction time[28] which opened the door to further investigations to modulate the entire process in order to further scale it up to a better yield. We have previously reported that an engineered thermostable variant of CHMO*Acineto*, harboring four substitutions and referred to as CHMO*Acineto*_QM,[26,75] was able to fully convert the nonnatural bulky substrate norcamphor (the major product is the normal lactone, 93.4%)[27] and act on the more substituted substrate
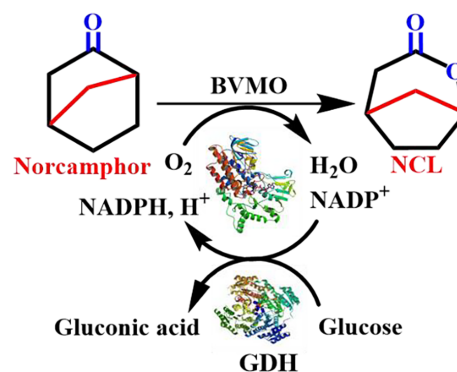
Scheme 1. Enzymatic synthesis of norcamphor lactone (NCL) using a Baeyer–Villiger monooxygenase (BVMO) enzyme (the thermostable CHMOAcineto_QM). Glucose dehydrogenase (GDH) was used for cofactor regeneration. [Color figure can be viewed at wileyonlinelibrary.com]

(−)-*cis*-verbanone.[30] Scaling up this transformation from 2 to 33 mM of the substrate norcamphor in shake-flask enabled the biosynthesis of norcamphor lactone in 0.5 g scale, stressing the high potential of the enzyme $CHMO_{Acineto}$_QM to be used for the production of industrially relevant products (Scheme 1). The GC-FID analysis of the extracted norcamphor lactone showed that the enzyme was able to convert 100% of the substrate norcamphor in 24 h at this reaction scale (Figure S1). Biocatalytic routes to the other lactone comonomer (ε-CL) in this study (*vide infra*) and δ-DL have recently been reported.[26,79]
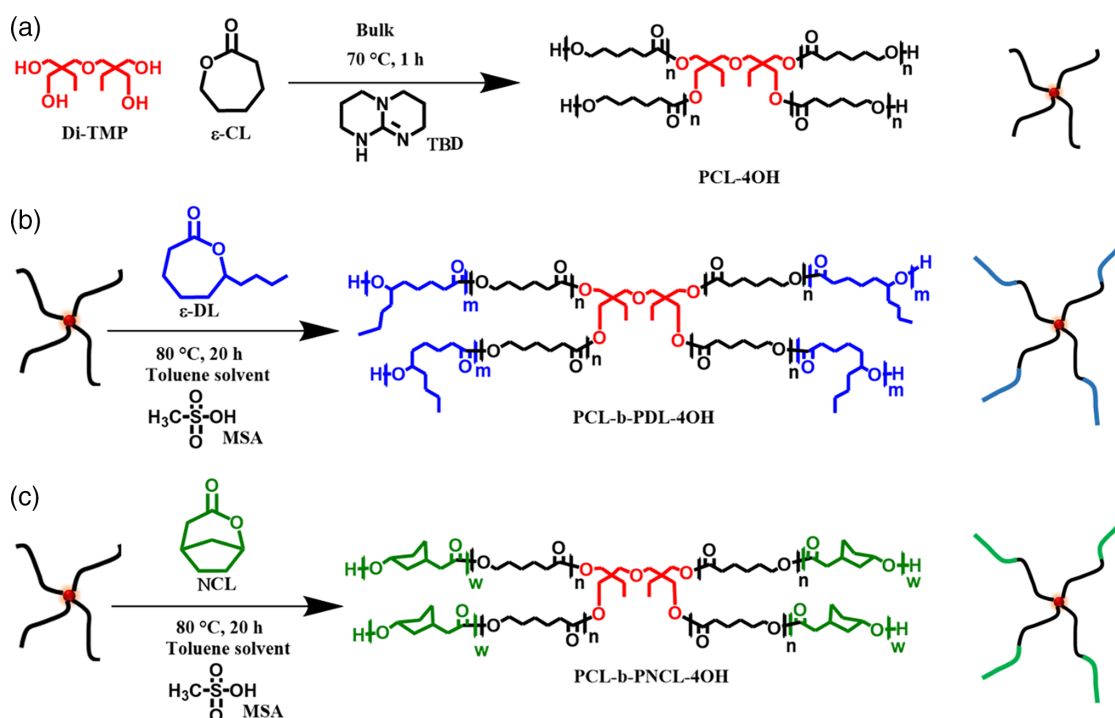
Synthesis of Star-Shaped Polymers

Star-like polymers are of interest due to their outstanding rheological and mechanical features, which are not observed in classical linear polymers.[80] The synthesis of four-arm star-like PCL was investigated by ROP of ε-CL using di-trimethylolpropane (Di-TMP) multifunctional alcohol as an initiator.[81,82] The polymerization was performed at 70 °C in bulk with 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) as an organic catalyst. This catalyst is considered to be efficient and environmentally benign to polymerize lactone monomers by ROP method [Scheme 2(a)].[83,84]

The results of the homopolymerization of ε-CL are reported in Table II and Figure 1(a). Upon polymerization, the change in the chemical shifts allows the determination of the amount of monomers that are converted into the polymeric form (Figure 1). It is indicated that ε-CL monomer can be completely consumed within 1 h of reaction at 70 °C. Herein, the polymerization of ε-CL to produce PCL-4OH is fast and proceeds to full monomer

conversion. The molecular weights (M_n) reported by SEC were similar to the NMR results (Table II, Figure 2, and Figure S2).

The synthesized star-like PCL polymer with four hydroxyl end groups can act as macroinitiator for the block copolymerization with either ε-DL or NCL in solution [Scheme 2(b,c)]. Based on our preliminary study, TBD did not show a strong catalytic activity to polymerize bulky, multicyclic lactones. Interestingly, using Methanesulfonic acid (MSA) as a catalyst readily afforded polymerization of the more sterically hindered monomers. MSA is considered as an efficient and ecofriendly catalyst.[30] In this investigation, we aim to evaluate the possibility of ROP to occur using the base → acid "*catalyst switch*" approach.[85,86] The results are reported in Table II and Figure 1(b,c). The chemical shifts in the ^1H NMR spectra revealed that the addition of either ε-DL or NCL monomer to the macroinitiator PCL-4OH resulted in a high monomeric conversion under the experimental conditions used [conversion (%) was 67 and 64% of ε-DL and NCL, respectively]. The lower conversion (%) of ε-DL and NCL in comparison with ε-CL is expected because the substituted lactones typically react slower than the corresponding unsubstituted monomers (e.g., ε-CL).[87] Also, the catalyst switch can have an impact on the conversion. The ^1H NMR spectra of the three star-like polymers show only the major resonances of the designed poly(lactones), with no evidence of transesterification side reactions[87] [Figure 2 (a) and Figures S2, S3, and S4].

In order to provide further evidence of the success of PCL-4OH to act as macroinitiator, SEC analyses were conducted to explore the change in the M_n after the copolymerization. Herein, the SEC

Scheme 2. Schematic illustration of the synthesis of star-like polymers. (a) Synthesis of four-armed PCL homopolymer in bulk using Di-TMP as initiator and TBD as a catalyst, (b,c) represent the synthesis of four-armed PCL-b-PDL and PCL-b-PNCL block copolymers, respectively, using PCL-4OH as macroinitiator and MSA as catalyst. [Color figure can be viewed at wileyonlinelibrary.com]

traces of the designed star-like block copolymers are compared with the original four-arm PCL homopolymer [Figure 2(b)]. Our results show that for each block copolymer, the SEC curve is shifted to a lower elution volume, indicating a higher molecular weight than that of the original PCL-4OH. The NMR and SEC results confirmed the block copolymerization of the star-like PCL-4OH with both ε-DL and NCL monomers using the catalyst switch strategy. The M_n of the polymeric architectures obtained by ^{1}H NMR are similar to the SEC results (Table II). Furthermore, it is worth noticing that the M_n obtained by ^{1}H NMR of these three poly(lactones) are very similar when calculated by the ratio between the protons of the repeating residues to either the proton of the terminal unit or the protons of the initiator. This provides a clear indication that the ROP was performed in a controlled manner.

The very different structural and physicochemical features (e.g., crystallinity, flexibility, and miscibility) between the original homopolymers PCL, PDL, and PNCL would yield exciting properties of their corresponding block copolymers. Others[44,88] and in our recently reported work, we showed that PDL is a fully amorphous polymer with a glass-transition temperature (T_g) of about −57 °C.[30] In addition, PNCL homopolymer was synthesized and characterized (Figure S5). The resulting PNCL polymer had a low T_g of approximately −30 °C and showed no indication of crystallization [Figure S5(c)]. Our results revealed different properties of the star-like homo- and block copolymers [Table II, Figure 2 (c)]. Star-like PCL homopolymer has a single (T_m) of about 57 °C and has a nondiscernible T_g; this is similar to previously reported research.[46] Interestingly, the block copolymerization of PCL with either ε-DL or NCL yielded two T_ms which are lower than that of the original PCL-4OH. One possible reason of this observation is that the incorporation of a flexible terminal (either PDL or PNL) to the PCL can probably decrease the intermolecular interaction of PCL chains and disturb its crystalline rich domains, resulting in a less ordered matrix and hence, lead to a reduction in the T_m of the system. Moreover, PCL-b-PNCL-4OH polymer showed reduced T_m compared to that of PCL-b-PDL-4OH. This can be attributed to the bulky structure of PNCL that contains a ring in its backbone, which is expected to further interrupt the PCL crystalline-rich domains and induce an increased reduction in the T_m. In contrast, the PDL structure is more similar to PCL and is therefore not expected to cause a major reduction in the T_m of the system.

Crosslinking by DA Chemistry and Thermoreversibility Analyses

The terminal hydroxyl groups of the synthesized star-like polymers can be subjected to further chemical modifications and thus, enable these polymeric architectures to be utilized in networks. PCL-4OH, PCL-b-PDL-4OH, and PCL-b-PNCL-4OH were investigated for their potential to form temperature sensitive networks via DA chemistry. In the current contribution, we are interested in utilizing the furan/maleimide couple as a diene/dienophile system because of their good reactivity and more importantly because of the green origin of furan moieties as being derived from hemicellulose-based feedstock materials.[68] First, we investigated if biocatalytic generation of end-functionalized star-shape polymers is possible via transacylation, using 2-methyl

Table II. Polymerization Conditions and Macromolecular Features of the Produced Polymeric Architectures

Polymer	Reaction time (h)	Monomer	Catalyst	Initiator	[Monomer]:[Initiator]:[Catalyst] (mol/mol/mol)	Conv, NMR^a (%)	$M_{n,\,NMR}{}^b$ per arm (g/mol)	$M_{n,\,SEC}{}^c$ (g/mol)	$T_{g,\,DSC}{}^d$ (°C)	$T_{m,\,DSC}{}^d$ (°C)
PCL-4OH	1	ε-CL	TBD	Di-TMP	100:1:2	>99	2650	10 200	n.d.	57
PCL-b-PDL-4OH	24	ε-DL	MSA	PCL-4OH	100:2:2	67	4050	15 600	−58	57 and 54
PCL-b-PNCL-4OH	24	NCL	MSA	PCL-4OH	100:2:2	64	3400	13 500	−51	43 and 34

^{a} Conversion (%) of monomers to polymer determined by ^{1}H NMR.
^{b} Average molecular weight (M_n) determined by ^{1}H NMR.
^{c} Molecular weight (M_n) of the four-arm star polymers as determined by SEC.
^{d} The glass transition temperature (T_g) and the maximum melting point (T_m) are determined by DCS. n.d., not detected.
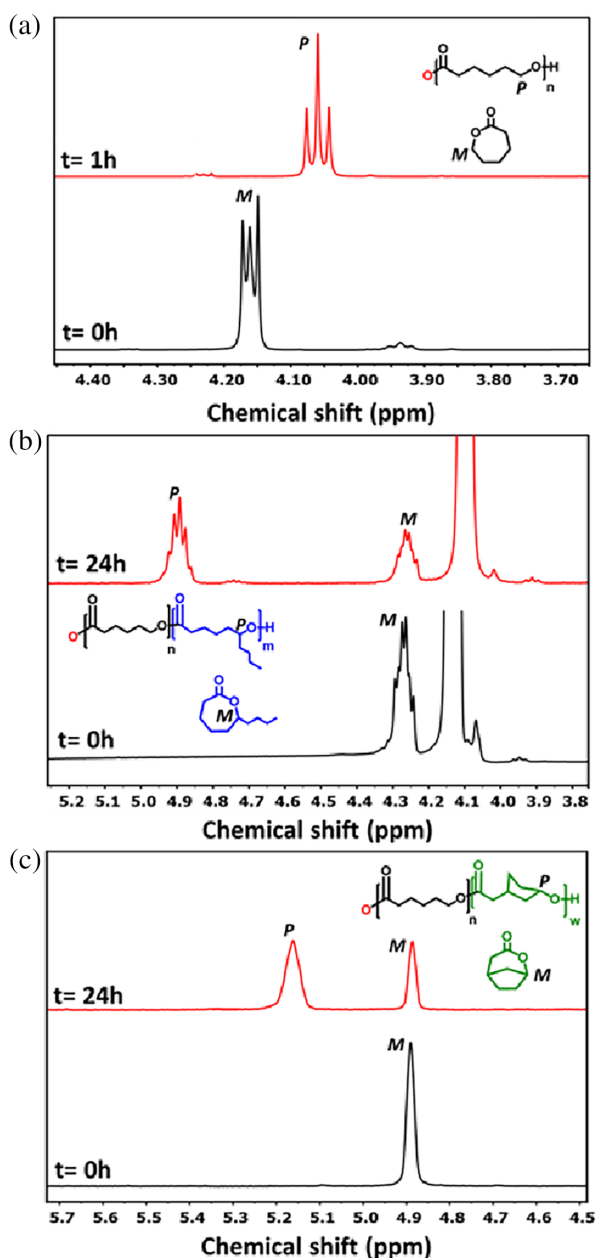
Figure 1. Efficiency of ROP in the generation of four-arm star-shaped lactone polymers using ^{1}H NMR spectra, obtained from the crude aliquots withdrawn at the start and end of the polymerization process (solvent = CDCl$_3$ at 25 °C and 400 MHz). (a–c) Results of PCL-4OH, PCL-b-PDL-4OH, and PCL-b-PNCL-4OH, respectively. M and P represent the chemical shift of the corresponding proton in the monomeric and polymeric structures, respectively. [Color figure can be viewed at wileyonlinelibrary.com]

furoate as acyl donor [Figure 3(a)]. Enzyme catalysis for end-functionalization of linear polymers was previously reported[89] whereas, and to the best of our knowledge, the end-capping of star-like macromolecules via biocatalysis has been scarce. Herein, utilizing PCL-b-PDL-4OH as model system harboring secondary terminal OH-groups, the furan rings were coupled to the polymer by exploiting the biocatalytic activity of the enzyme CalB. CalB was reported for its high activity and broad substrate specificity

in transacylation reactions.[90–96] The reaction was stopped after 3 days and the coupling of furan to the polymers was confirmed by ^{1}H NMR in which the methyl ester protons were disappeared and the sp^2 protons of the furan remained intact (Figure S6). In fact, the disappearance of the methyl ester peaks and as unreacted methyl 2-furoate was removed from the reaction medium by extensive washing and purification, the residual furan rings are therefore covalently associated with the polymers. The formation of four-arm furan capped star-like polymers is confirmed by ^{1}H NMR [Figure 3(b) and Figure S7]. The resonances at 7.65, 7.31, and 6.58 δ ppm were assigned to protons x, y, and z of the furan ring, which reveals that the furan moieties are successfully coupled to the polymers. A reference reaction subjected to the same reaction conditions but without CalB showed no residual furan-derived protons, which confirms the catalytic activity of CalB. The two remaining star-like copolymers were functionalized with furoic acid by chemical catalysis. This coupling reaction will result in the formation of four-arm furan capped star polymers (PCL-4Fu and PCL-b-PNCL-4Fu starting from PCL-4OH or PCL-b-PNCL-4OH, respectively), Figure 3(b) and Figures S8 and S9. The ratio between the protons of the repeating units of the polymers to that of proton x of the furan ring showed no alteration in the M_n of the corresponding polymers, which indicates that the polymers are intact under the reaction conditions and provide further evidence of the success of the coupling reaction.

The DA reaction is well entrenched in the polymer and material engineering field because of its thermoreversibility and being performed at mild conditions without the use of catalyst.[65] The four-arm furan-capped star-shaped polymers were allowed to react with 1,1′-(methylenedi-4,1-phenylene)BMI at ambient temperature via DA reaction to form chemically crosslinked networks. In contrast, it is expected that at elevated temperature, the system will favor the retro-DA (rDA), resulting in the decrosslinking of the designed materials. It is envisaged that alternate cooling/heating will allow the bonding/debonding processes of these polymeric networks (Scheme 3).

The thermal behavior of the DA-crosslinked materials was examined by DSC analysis (DSC thermograms are given in Figure 4). At the experimental conditions (DSC heating/cooling rates), the crosslinking resulted in a complete disappearance of the T_g and crystallinity of the polymers, ascribed to either the impedance of crosslinks in the polymers or chain rearrangement.[97] Furthermore, because of the endothermic feature of the rDA reaction, it makes it feasible to explore rDA temperatures by DSC analysis. As reported in Figure 4, and for each single network architecture, a signal can be observed between 135 and 165 °C with a maximum peak transition at approximately 147 °C, which attributes to the temperature where rDA reactions predominate. Herein, the DSC results confirm the crosslinked nature of the materials at low temperature and the opening of the DA adduct at high temperature (147 °C).

In order to promote an additional evidence of the bonding/debonding behavior of the polymeric networks in response to heating/cooling, the materials were subjected to solubility tests at different temperatures. It is expected that at elevated temperature the networked materials will debond by means of cycloreversion,
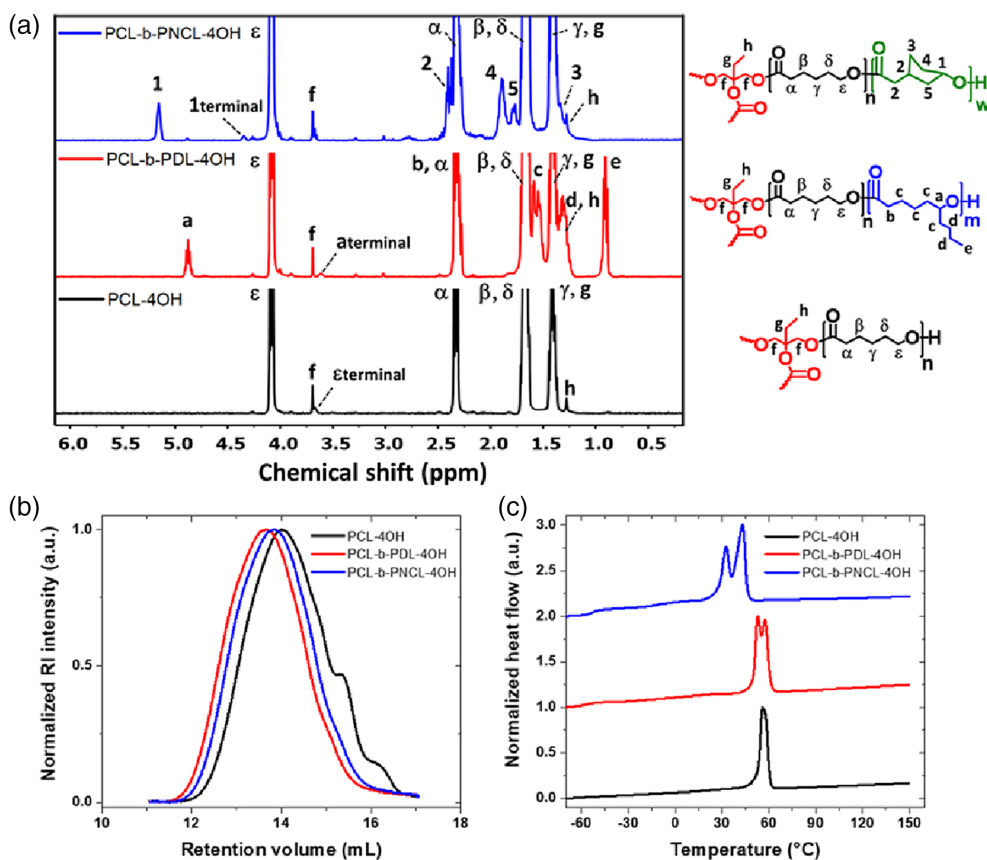
Figure 2. Properties of the designed star-like polymers. (a–c) ^{1}H NMR spectra (solvent = CDCl$_3$ at 25 °C and 400 MHz), SEC traces, and DSC thermograms (endo-up, obtained from second heating), respectively. [Color figure can be viewed at wileyonlinelibrary.com]
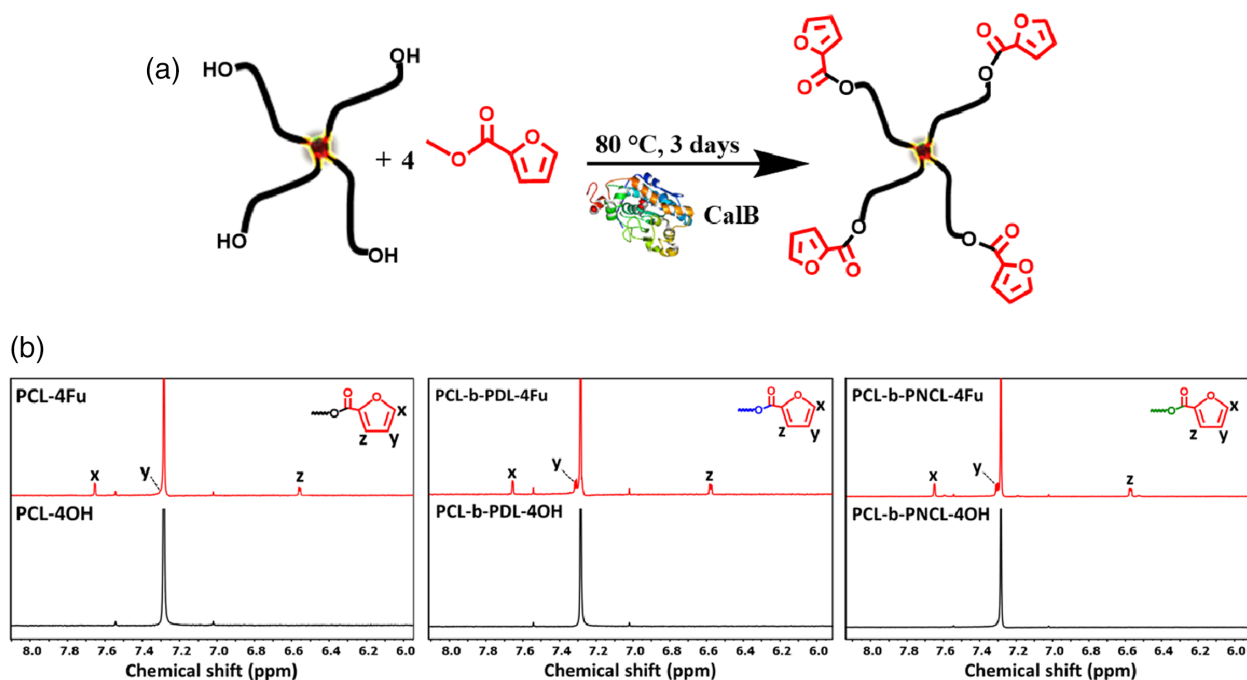
Figure 3. Functionalization of star-like polymers. (a) Schematic illustration of the biocatalytic synthesis of four-arm furan capped star-like polymers represented by formation of PCL-4Fu from PCL-4OH, using methyl 2-furoate as acyl donor. (b) ^{1}H NMR spectra of the furan capped polymers (PCL-4Fu, PCL-b-PDL-4Fu, or PCL-b-PNCL-4Fu) and their corresponding original star polymer (solvent = CDCl$_3$ at 25 °C and 400 MHz). [Color figure can be viewed at wileyonlinelibrary.com]
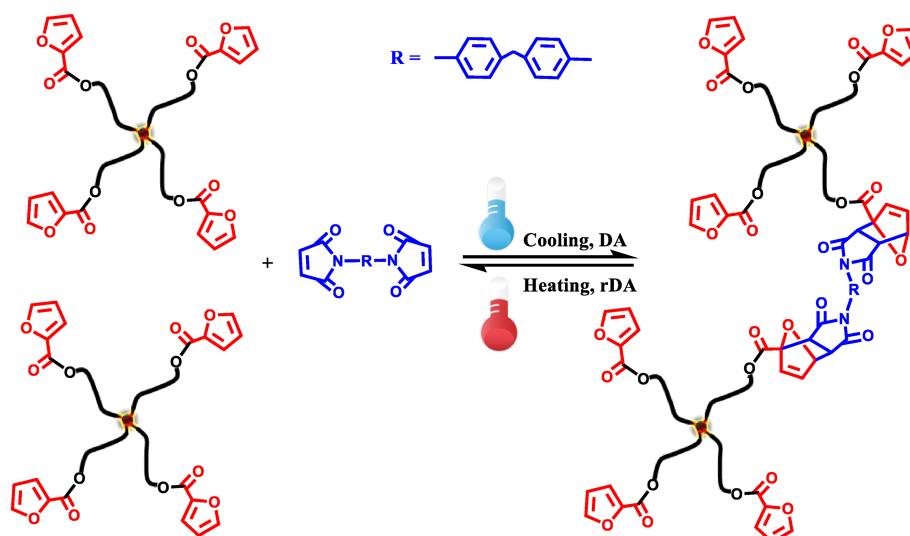
Scheme 3. Schematic illustration of DA/rDA reaction between the four-arm furan capped star-like polymers (PCL-4Fu, PCL-b-PDL-4Fu, or PCL-b-PNCL-4Fu) and bismaleimide (BMI). The polymeric networks debond at high temperatures *via* cycloreversion and recrosslink by DA reaction at inferior temperatures. [Color figure can be viewed at wileyonlinelibrary.com]
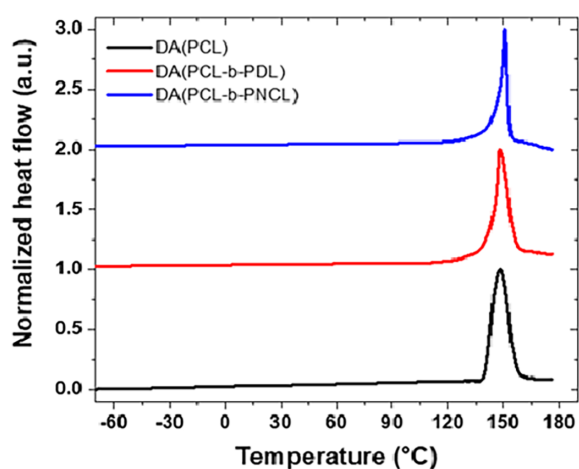
Figure 4. DSC thermograms (endo-up) of the designed networks. Peak transition refers to the rDA of the system. [Color figure can be viewed at wileyonlinelibrary.com]

which can be observed at the macromolecular scale by a change in the solubility.[65,98]

As a showcase, DA (crosslinked) (PCL-b-PNCL) is presented in Figure 5. The samples were immersed in DMF at 50 °C under continuous magnetic stirring. After 1 h of mixing, the viscosity was very high and the polymers were not able to flow, forming a gel that did not flow even after the inversion of the vial. This observation is ascribed to the crosslinked nature of the materials at 50 °C. The gels dissolve and got converted into a homogenous and low viscosity mixture upon heating at 150 °C for 5 min (temperature chosen from data in Figure 4). This correlates well with the decrosslinking of the networks because of the opening of the DA adduct by retro DA at this elevated temperature. Conversely, the viscosity increased slowly and the materials showed a

high tendency to return to the original nonflowing gel upon cooling to 50 °C for 72 h. In fact, the materials were able to crosslink again *via* DA reaction which favors the reformation of the DA adduct at this lower temperature. The solubility analysis confirms the temperature responsivity of these newly reported polymers.

Furthermore, to have a profound understanding of molecular-level rDA reactions between the furan capped star-like polymers and BMI, samples of the networks that were dissolved in DMF at 150 °C in the previous solubility tests were subjected to ^{1}H NMR analysis. These spectra were compared with the original furan capped polymers and BMI (Figure S10). The ^{1}H NMR spectrum of the dissolved networks are evidently a superposition of the initial reactants. In fact, the furan and maleimide moieties are retained intact as evidenced by the recovery of their characteristic proton resonances after being dissolved in DMF. This observation reveals that the thermal treatment of the designed networks will favor the rDA reaction and eventually maintain the integrity of the materials without altering the furan/maleimide functional groups, enabling this diene/dienophile system to crosslink over again once kept at ambient temperatures, in agreement with the results from the solubility tests (Figure 5).

CONCLUSIONS

In this research, we described a scaled-up biocatalytic synthesis of monomeric lactones and their use to design previously unreported polymers for applications as thermosensitive gels. Norcamphor lactone was synthesized by biocatalysis in the 33 mM scale (corresponding to 0.5 g) in shake flask, and with 100% conversion after 24 h by an engineered variant of cyclohexanone monooxygenase from *A. calcoaceticus* (CHMO$_{Acineto}$_QM). This activated monomeric residue was incorporated in the synthesis of homo- and block star-like copolymers. PCL-4OH, PCL-b-PDL-4OH, and PCL-b-PNCL-4OH were successfully
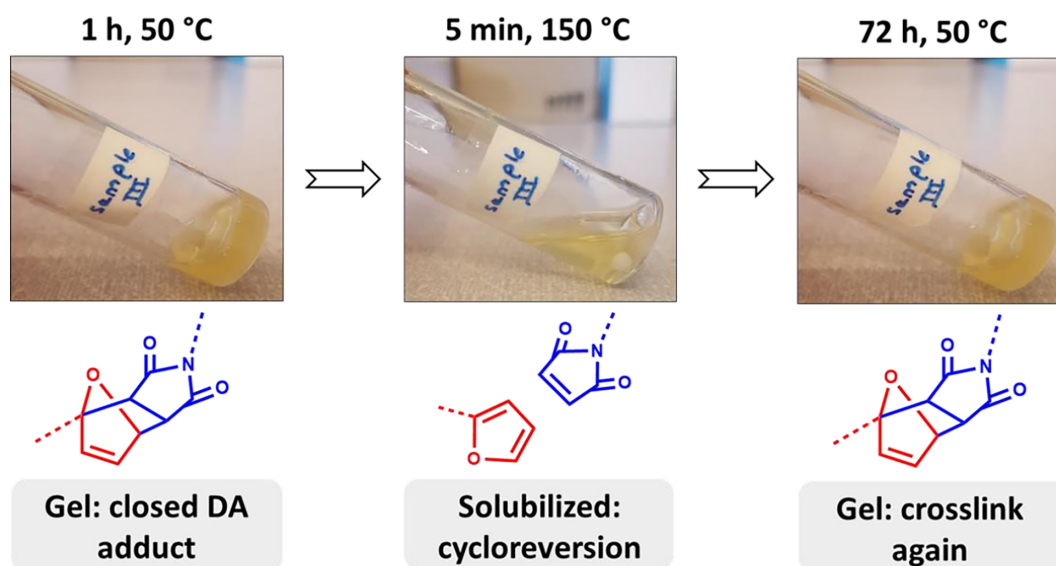
Figure 5. Evidence of temperature induced bonding/debonding shown here for DA(PCL-b-PNCL) by solubility test in DMF at 50 and 150 °C. [Color figure can be viewed at wileyonlinelibrary.com]

produced by means of ROP enabled by the catalyst MSA for the more bulky structures. These polymeric architectures were analyzed for their physicochemical features and showed interesting properties. For instance, the copolymerization resulted in a significant reduction in the T_m of the system and induced the formation of flexible materials. In order to extend their use in the field of material engineering for applications as temperature amendable gels, the obtained polymers were functionalized with furan moieties; a reaction that was found to be catalyzed by *C. antarctica* lipase B. Thermoresponsive networks were generated *via* the reaction with BMI by DA chemistry. The bonding/debonding state of the designed gels can be modulated in response to a specific thermal treatment. It is envisioned that the findings herein can be applicable to other bulky cyclic building blocks such as the natural products Verbenone, Dihydrocarvone, Thujone, and camphor shown in Table I. We strongly believe that the incorporation of "green" biocatalytic routes in the synthesis of polymers is a very promising approach and could permit the production of new polymer types to enable their use as advanced functional materials.

EXPERIMENTAL SECTION

Chemicals and Reagents
Buffer components, bovine serum albumin (BSA), isopropyl-β-D-thiogalactopyranoside (IPTG), kanamycin sulfate, immobilized CalB, norcamphor, (racemate), ε-caprolactone, ε-decalactone, TBD, p-toluenesulfonic acid monohydrate (PTSA), 2-furoic acid, and 1,1′-(methylenedi-4,1-phenylene) BMI were purchased from Sigma-Aldrich (USA). Di-TMP was purchased from Perstorp (Sweden).

Recombinant Expression of CHMO$_{Acineto}$_QM
The enzyme CHMO$_{Acineto}$_QM was expressed as previously described.[30] Briefly, freshly transformed *E. coli* cells were cultivated in 30 mL 2xYT medium containing 40 µg mL^{-1} kanamycin sulfate overnight at 37 °C and 200 rpm. The overnight culture

was used to inoculate 200 mL of fresh medium to OD$_{600}$ of 0.1. The culture was incubated at 37 °C and 200 rpm until OD$_{600}$ of 0.5–0.7 was reached. To induce the protein expression, 0.05 mM of IPTG was added to the culture and the induction was carried out at 25 °C and 180 rpm. The cells were harvested after 20 h at 2276 × *g* for 15 min at 4 °C. One gram wet cell pellet was resuspended in Tris buffer pH 8.5 (5 mL 50 mM) and sonicated for 3 min, 60% duty cycle, under ice cooling using a Misonix sonifier cell disruptor ultrasonic S-4000 probe (Misonix Inc., Farmingdale, NY). The suspension was centrifuged at 40 000 × *g* and 4 °C for 20 min in order to remove cell debris.

Protein Analysis
Protein concentration was measured by Bradford method[99] using Bio-Rad Protein assay kit (Bio-Rad, Hercules, CA). BSA was used as protein standard. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli,[100] using 4% stacking and 15% separating gels, purchased from Bio-Rad with the prestained protein marker SeeBlue Plus2 (Thermo Fisher Scientific, Sweden). Proteins were stained with InstantBlue (Expedeon, Cambridge, UK).

Synthesis of Star-like Polymers
Prior to conducting the polymerizations, all glassware was flame-dried and then handled under dry nitrogen atmosphere. Aliquots for ^{1}H NMR analysis were withdrawn from the reaction mixture at regular time intervals using disposable syringes.

PCL-4OH homopolymer synthesis: The polymerization was performed in bulk. The reactants, caprolactone (ε-CL) monomer (8.1 g), TBD catalyst (0.2 g), and Di-TMP initiator (0.046 g), ([CL]/[TBD] = 50; [CL]/[Di-TMP] = 100) were added to a 25 mL three-neck, round-bottom flask equipped with a stir bar under a nitrogen atmosphere and immersed into a preheated oil bath at 70 °C and kept for 1 h under magnetic stirring. The reaction mixture was then cooled to room temperature. The product was dissolved in chloroform and precipitated three times by

adding it dropwise into excess cold methanol (75 mL). The purified PCL was dried at 50 °C under vacuum for 3 days.

Block copolymerization: The polymerizations were performed in solution. A 1.6 mL of dry toluene solvent was added to a three-necked, round-bottom flask. The reactants, monomer [ε-DL (0.6 g) or NCL (0.44 g)], PCL-4OH initiator (0.8 g), and Methanesulfonic acid (MSA) (4.4 μL) catalyst, ([M]/[MSA] = 50; [Monomer]/[PCL-4OH] = 50) were transferred to the reaction medium. The polymerization reactions were performed under nitrogen atmosphere at 80 °C and kept for 24 h under magnetic stirring. The reaction mixtures were cooled to room temperature and the polymers were recovered by precipitation in an excess volume of cold methanol. The products were then subjected to three consecutive dissolving/precipitation cycles using alternatively chloroform and cold methanol. The polymers were dried at 50 °C under vacuum for 3 days.

Synthesis of Methyl 2-Furoate

2-Furoic acid (1 eq, 0.5 g) and PTSA catalyst (0.05 eq, 0.042 g) were transferred to a 50 mL three-neck, round-bottom flask containing 30 mL dry methanol. The reaction was kept for 24 h at 80 °C under magnetic stirring and reflux. Thin layer chromatography was used to analyze the progression of the reaction by UV irradiation. A repeated liquid–liquid extraction was used to purify the product, using dichloromethane (DCM) as the organic phase and saturated $NaHCO_3$ as the aqueous phase. The mixture was then dried over magnesium sulfate. The methyl 2-furoate was finally recovered from DCM using a rotary evaporator and dried further at 50 °C under vacuum for 1 day prior to use. The isolated product was a yellowish liquid, and its ^1H NMR spectra o showed resonances at δ ppm 7.6 (1H), 7.2 (1H), 6.5 (1H), and 3.9 (3H) (Figure S11).

Synthesis of Furan Capped Four-Arm Star-like Polymers

The corresponding four-arm star-like polymer (1 eq, 300 mg), 2-methyl furoate (6 eq, 15 mg) and CalB (15 mg, equal weight of carrier with immobilized enzyme as 2-methyl furoate) were transferred to a round-bottom flask and diluted into a desired volume of dry toluene (1 mL). The reaction was launched by placing the flask into a preheated oil bath at 60 °C. After 3 days, the reaction was stopped and the materials were recovered by precipitation in an excess volume of cold methanol. The products were dissolved in chloroform and precipitated in methanol cooled by dry-ice. This procedure was repeated three times. The products were dried at 50 °C under vacuum for 3 days.

Synthesis of the Networks by DA Reaction

The furan capped star polymers were crosslinked *via* DA chemistry using 1,1′-(methylenedi-4,1-phenylene)BMI as a crosslinker. Stoichiometric amounts of furan and maleimide functions were used (furan/maleimide mol ratio is 1:1). The corresponding furan capped four-arm star-like polymer and BMI were transferred to a 25 mL three-neck, round-bottom flask containing dry chloroform. The mixture was subjected to magnetic stirring at 50 °C for 3 days under nitrogen flow and reflux. The obtained products were dried at 50 °C under vacuum for 3 days.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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